Disseny i síntesi de nous compostos multipotents per al tractament de la malaltia d'Alzheimer

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Programa de doctorat:
Química Orgànica Experimental i Industrial

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Memòria presentada per Maria Elisabet Viayna Gaza
Per a optar al títol de doctora per la Universitat de Barcelona

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Als meus pares

En primer lloc vull agrair el suport incondicional del meu director de tesi, Diego Muñoz-Torrero. *Quiero agradecer, la confianza que has depositado en mí, tu dedicación, tu interés porque este proyecto saliera adelante y el haberme inculcado la importancia del trabajo bien hecho. Eres para mí un ejemplo a seguir en el ámbito profesional y personal. Muchas gracias.* També vull agrair al professor Pelayo Camps l'oportunitat que fa una quants anys em va brindar de formar part del seu grup de recerca i la seva ajuda i consell en molts moments.

També vull agrair a tots aquells investigadors que han col·laborat en fer possible aquest treball. En primer lloc, vull expressar un especial agraïment als *del pis de dalt* no només per la realització dels estudis de modelatge molecular dels compostos sintetitzats en aquesta tesi sinó també pels bons moments compartits: Axel, Jordi, Salo, Javi, Xavi, Curu, Flavio, Assumpta i Ramon. També vull agrair al grup del Dr. Rodolfo Lavilla i especiament a l'Esther per haver-nos introduït al món de les reaccions multicomponent. També vull donar les gràcies a tots els grups que han dut a terme l'avaluació farmacològica i assajos bioquímics dels compostos sintetitzats en aquest treball de tesi: al grup de recerca dels Drs. Badia i Clos de la Universitat Autònoma de Barcelona, al grup de les Dres. Andrisano, Bartolini i Mancini de la Universitat de Bologna, al Dr. Raimon Sabater, al grup dels Drs. Pallàs i Camins de la Universitat de Barcelona, al grup de la Dra. Rodríguez-Franco del CSIC de Madrid, al grup del Dr. Luciano Saso de la Universitat Sapienza de Roma, a les Dres. Jud i Hutter-Paier de *JSW Life Sciences Gmbh* i al grup del Dr. Salmona del Institut *Mario Negri* de Milà. Finalment, vull agrair a tot el personal dels Centres Científics i Tecnològics de la UB, especialment a l'Ana Linares, l'haver fet possible la caracterització de tots els compostos preparats en aquest treball de tesi.

També vull donar les gràcies a Santi i a Carmen: *por amenizarnos a la hora de comer con vuestras historias (3 hijos dan para muchas), por vuestros consejos y ayuda, en el ámbito profesional y personal y por haber tenido siempre la puerta del despacho abierta para mí*.

També vull agrair a tota la gent amb qui he coincidit al laboratori des del dia que vaig arribar al departament. Tots heu contribuït a fer possible aquesta tesi. Als que hi éreu quan vaig arribar i em veu donar un cop de mà en tantíssims moments: la Loli, l'Ayats, el Jordi, el Sabin, la Sílvia, l'Aina i molt especialment a la Rosa i el Jose. També a la meva companya de màster, Roser, i els companys de químiques, especialment a la Núria. Vull expressar un especial agraïment als que heu estat els meus *companys de tesi*, amb qui més moments he compartit i a qui més trobaré a faltar quan marxi (o marxeu). A l'Eva per haver-me escoltat i ajudat en incomptables moments espero de tot cor que em mantinguis *update*, a la Tània, per haver deixat el que estiguessis fent per a donar-me un cop de mà i per recordar-te sempre d'on està tot! i al Matias, pel teu sentit de l'humor, veritablement ets únic! Vull agrair a tots els *treballs dirigits*, *erasmus* i *leonardos* que he supervisat per haver contribuït amb el vostre *granet de sorra* a fer possible aquesta tesi: Natalia (*triazoles and I miss you so much!*), Mattia, Anna, Marisa, Marc, Andrés, Edu, Gemma, Helena, Francesca, Magda, i molt especialment a les que us *heu quedat* i ara

recolliu el testimoni del *huprina's team*, la Irene, trobarem molt a faltar la nostra filla adoptiva, i l'Ornella, *espero por tu bien que Italia gane el próximo mundial...benne*: ha estat un plaer haver coincidit amb vosaltres i haver col·laborat en la vostra formació, us desitjo moltíssima sort en el vostre futur. També vull agrair als *últims fitxatges* per haver omplert el laboratori de bon rotllo i de bons moments: a l'Elena, per haver estat una gran companya de vitrina i de pàdel (visca les Melis!), al David, perquè no hi ha ningú millor per a parlar de política ni de música (recorda't de nosaltres quan siguis president de la Generalitat!), a l'Ane, per tenir sempre un somriure preparat i a Marta *1*, pel teu riure contagiós; Ane i Marta: espero que Santi en sàpiga trobar un també per a vosaltres! Us desitjo a tots el millor en aquesta etapa que comenceu, heu estat uns companys de feina excepcionals, us trobaré molt a faltar! També vull agrair a tots els estudiants que han passat pel laboratori durant aquests anys: a l'Arnau, perquè *escolta*...no hi ha ningú millor per cotillejar, la Marta *2* i la Rosana us desitjo que tingueu unes bones estades i una bona *reentrée* quan torneu al *lab*, l'Aleix, Stefania, Ilaria, Matteo, Toni, Marga, les *Sílvies*, Daniela, Albert, Jordi, Aurore, Claudio, Emma, Salva, Stephanie, Jordi, Maria, Alexia, Anna, Andrea i molt especialment a *la* Euge: *sería relindo que volviéramos a coincidir algún día*. A tots els de LIDQO: Raül, trobem a faltar el teu *català* a l'hora de dinar, Maria, Yolanda, Andrés, i sobretot a la *Paye*, *porque fuiste una compañera genial el tiempo que coincidimos, aquí se te echa de menos…ea!* També als de Salvat que m'heu donat un cop de mà en molts moments, Juanlo i Jaume. També vull agrair a tots *els d'Orgànica* per haver-me ajudat en moltes ocasions. I finalment, vull donar les gràcies a la Rita, la Pilar, la Maite, la Manolita i Javier.

I would like to thank everyone I met at GSK specially my supervisor, Seb, for your patience, for everything you taught me and for being a good boss and friend. I would also like to thank Alex, Ben and Sisco, for being the best UK-family I could have had. Finally, I would like to thank everyone I met at VT, specially Paul and Dawn for your kind help. También me gustaría agradecer a los nuevos compañeros de Carles: Atila, Esther y Silvia, por vuestra compañía y buenos momentos durante las larguísimas horas de escritura de tesis y, sobre todo, gracias por cuidar tan bien de Carles.

Vull dedicar aquest a tesi a tota la meva família, especialment als meus pares i al meu germà: perquè sempre heu estat al meu costat, per tot el que m'heu ensenyat i ajudat però sobretot, pel vostre suport incondicional. També vull agrair a Cinta, Pere i Jordi els sopars i dinars, la vostra hospitalitat i per com *xalem* quan baixem a Tortosa. També vull dedicar aquesta tesi a tots els meus amics: a la *chupipandi*, als *mo*'s i a les *capsuletes* per haver compartit sopars, dinars, escapades, viatges i grans moments.

Finalment, vull dedicar aquesta tesi molt especialment al Carles. No sé per on començar-te a agrair tot el que has fet per a mi tots aquests anys, la teva ajuda, suport, paciència i tot el que m'has ensenyat. Però sobretot vull agrair-te que hagis volgut començar amb mi el *nostre propi projecte*. De tot cor, *gràcies*.

El treball experimental recollit en aquesta memòria s'ha realitzat al Laboratori de Química Farmacèutica de la Facultat de Farmàcia de la Universitat de Barcelona sota la direcció del Dr. Diego Muñoz-Torrero.

Aquesta tesi ha estat possible gràcies a la concessió d'una Beca d'Iniciació de Tesi Doctoral (BIT 2010) atorgada per la Facultat de Farmàcia de la Universitat de Barcelona, d'una Beca per a la Formació de Personal Investigador (FI-2011) atorgada per l'Agència de Gestió d'Ajuts Universitaris i de Recerca de la Generalitat de Catalunya i d'una beca de *Formación de Profesorado Universitario* (FPU2010) atorgada pel *Ministerio de Ciencia e Innovación*.

El present treball ha estat finançat pel *Ministerio de Ciencia e Innovación* (projectes CTQ2008-03768/PPQ i CTQ2011-22433/PPQ), per l'Agència de Gestió d'Ajuts Universitaris i de Recerca de la Generalitat de Catalunya (2009SGR1396) i per la Fundación para el Desarrollo de la Investigación en Genómica y Proteómica, Genoma España (FGB 306351).

Abbreviations

Aβ	β-amyloid peptide
$A\beta_{42}$	42 amino acid β-amyloid peptide
ACh	acetylcholine
AChE	acetylcholinesterase
AChEI	AChE inhibitor
AD	Alzheimer's disease
ApoE	apolipoprotein E
APP	amyloid precursor protein
aq.	aqueous
BACE-1	β-secretase
bAChE	bovine AChE
BBB	blood brain barrier
BChE	butyrylcholinesterase
BChEI	BChE inhibitor
Bn	benzyl
Boc	*tert*-butoxycarbonyl
CNS	central nervous system
COX	cyclooxygenase
CSF	cerebrospinal fluid
DDQ	dichlorodicyanobenzoquinone
DEA	diethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
eeAChE	*Electrophorus electricus* AChE
FAD	flavin adenine dinucleotide
GFP	green fluorescence protein
hAChE	human AChE
HMBC	heteronuclear multiple bond correlation
HPLC	high pressure liquid chromatography
HSQC	heteronuclear single quantum correlation
IC_{50}	concentration that inhibits 50% of the enzymatic activity
iNOS	inducible nitric oxide synthase
LDA	lithium diisopropylamide
mAChE	mouse AChE
MAO	monoamine oxidase
MCR	multicomponent reaction
MD	molecular dynamics
min	minutes

MPLC	medium pressure liquid chromatography
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
Ms	mesylate
NAC	non β-amyloid component
NMR	nuclear magnetic resonance
PDB	Protein Data Bank
PET	positron emission tomography
PHF	paired helical filaments
PKC	protein kinase C
PMB	*p*-methoxybenzyl
*p*TsOH	*p*-toluenesulfonic acid
r. t.	room temperature
RNS	reactive nitrogen species
ROS	reactive oxygen species
sat.	saturated
sol.	solution
TcAChE	*Torpedo californica* AChE
THF	tetrahydrofuran
TIPS	triisopropylsilyl

Sumary

1. INTRODUCTION

1.1 Alzheimer's disease.

In 1901, Dr. Alois Alzheimer observed a patient at the Frankfurt Asylum named Auguste Deter. The 51-year-old patient had strange behavioural symptoms, including a loss of short-term memory. In April 1906, Mrs. Deter died and Alzheimer used staining techniques to identify amyloid plaques and neurofibrillary tangles. A speech given on the 3rd of November of 1906 at the 37th Meeting of South-West German Psychiatrists in Tübingen was the first time the pathology and the clinical symptoms of this novel class of dementia were presented together.[1]

Nowadays, Alzheimer's disease (AD) is the main cause of dementia and the third cause of death in elderly people in developed countries, approaching epidemic proportions and affecting millions of people worldwide.[2,3] It is estimated that AD currently affects 36 million people worldwide, its prevalence raising steadily with age. After 65, the likelihood of developing AD doubles every 5 years. The prevalence of AD is expected to increase, affecting 66 million people in 2030 and 115 million people in 2050, along with the increase of average life expectancy and the aging of the population, becoming a major social and sanitary concern in developed countries.[4,5]

AD develops slowly, and it is believed that pathological events begin decades before measurable symptoms appear. It is clinically characterized by deterioration of cognitive function, dementia, memory loss, and altered behaviour. The earliest symptoms are often manifested as subtle, intermittent deficits in the remembrance of minor events of everyday life, referred to as loss of episodic memory. After many months of gradually progressive memory loss, other cognitive symptoms appear and slowly advance. Over a further period of years, profound dementia develops that affects multiple cognitive and behavioural spheres. Death usually comes by way of minor respiratory complications, such as aspiration or pneumonia.[6,7]

AD is related to age, genetic predisposition to suffering the disease is only related to 3% of all cases.[8] Three main mutations on genes encoding for transmembrane proteins capable of driving an early appearance of the disease have been described, namely the gene coding for the β-amyloid precursor protein (APP) on chromosome 21, preseniline-1 on chromosome 14, and preseniline-2 on chromosome 1.[9,10] Nevertheless, it is important to point out that most of

[1]Alzheimer, A. Allgemeine Zeitschrift für Psychiatrie and Psychisch-Gerichtliche Medizin **1907**, 64, 146. [2]Mount, C.; Dowton, C. *Nat. Med.* **2006**, 12, 780. [3]Muñoz-Torrero, D. *Curr. Med. Chem.* **2008**, 15, 2433. [4](a) *Alzheimer's Disease International. World Alzheimer Report: The benefits of early diagnosis and intervention* **2011**. (b) *Alzheimer's Disease International. World Alzheimer Report: The Global Economic Impact of Dementia from Alzheimers Association* **2010**. [5]Walsh, D.M.; Selkoe, D.J. *Neuron*. **2004**, 44, 181. [6]Cummings, J.L.; Askin-Edgar, S. *CNS Drugs* **2000**, *13*, 385. [7]Leonard, B.E. *Hum. Psycopharmacol*. **1998**, 13, 83. [8]Lahiri, D.K.; Farlow, M.R., Sambamurti, K.; Greig, N.H; Giacobini, E.; Schneider, L.S. *Curr. Drug. Targets* **2003**, 4, 97. [9]Goate, A.; Chartier-Harling, N.C.; Mullan, M.; Brown, J.; Crawford, F.; Fidani, L.; Giuffra, L.; Haynes, A.; Irving, N.; James, L.; Mant, R.; Newton, P; Rooke, K.; Roques, P.; Talbot, C.; Pericak-Vance, M.; Roses, A.; Williamson, R.; Rossor, M.; Owen, M.; Hardy, J. *Nature* **1991**, *349*, 704. [10]Sherrington, R.; Rogaev, E.I.; Liang, Y.; Rogaeva, E.A.; Levesque, G.; Ikeda, M.; Chi, H.; Lin C.; Li, G.; Holman, K.; Tsuda, T.; Mar, L.; Foncin, J.-F.; Bruni, A.C.; Montesi, M.P.; Sorbi, S.; Rainero, I.; Pinessi, L.; Nee, L.; Chumakov, I.; Pollen D.; Brookes, A.; Sanseau P.; Polinsky, R.J.; Wasco, W.; Da Silva, H.A.R.; Haines, J.L.; Perikac-Vance, M.A.; Tanzi, R.E.; Roses, A.D.; Fraser, P.E.; Rommens, J.M.; St. George-Hyslop, P.H. *Nature* **1995**, *375*, 754.

the patients suffering AD do not have familiar precedents or show any of the previously described genetic mutations.[11]

Although the cost of coping with AD is already substantial in terms of loss of life quality and productivity as well as in the economical efforts that are being made in order to, on the one hand, take care of AD patients, and on the other hand, decipher and learn how to treat AD, near-term predictions indicate that the expense will soon be unaffordable for our society. The total worldwide estimated cost associated with AD currently accounts for approximately 1% of the world's gross domestic product. The lack of effective therapies, a large aging population, and increasing life expectancy worldwide are rapidly converging into an impending crisis for health care providers.[6,12]

1.2 Pathogenesis of Alzheimer's disease.

The etiology of AD is still incompletely understood. However, two characteristic neuropathological key events are now clearly defined, namely senile plaques and neurofibrillary tangles, which are mainly composed of β-amyloid peptide (Aβ) and hyperphosphorylated tau protein, respectively. These two proteins seem to be at the root of the pathogenesis of the disease. Some debate exists about which of them occurs first and which one is the consequence or even if they are both consequences of the disease.[3,13–15] However, so far, the most prevailing hypothesis on the cause of AD is the so-called amyloid cascade hypothesis, which points to the aggregation of the Aβ into oligomers or fibrils as the key process associated with progression of AD. This hypothesis establishes that increased production, aggregation and accumulation of Aβ in the brain triggers a cascade of neurotoxic events, which eventually leads to a widespread neuronal degeneration and to the clinical symptomatology of the dementia (Figure 1.1).[16–19]

Aβ is a 39–43 amino acid peptide generated by proteolytic cleavage from the transmembrane glycoprotein APP through the sequential action of β-secretase (BACE-1) and γ-secretase. In healthy people, APP is processed by α- and γ-secretase to give non-amyloid products that in fact, show neurotrophic and neuroprotective effects, but in people suffering AD Aβ synthesis is increased, especially the one leading to the most insoluble form, called $A\beta_{42}$, which is also the one that shows the strongest tendency to aggregate (Figure 1.2).[16,20]

[3]Muñoz-Torrero, D. *Curr. Med. Chem.* **2008**, 15, 2433. [6]Cummings, J.L.; Askin-Edgar, S. *CNS Drugs* **2000**, *13*, 385. [11]Levy-Lehad, E.; Wijsman, E.M.; Nemens, E.; Anderson, A.L.; Goddard, K.A.B.; Wiltfang, J. *Brain* **2006**, *129*, 743.[12]Gray, L. *Cell* **2012**, *18*, 6. [13]Klafki, H.-W.; Staufenbiel, M.; Kornhuber, J.; Wiltfang, J. *Brain* **2006**, *129*, 2840. [14]Schönheit, B.; Zarski, R.; Ohm, T.G. *Neurobiol. Aging* **2004**, *25*, 743. [15]Castellani, R.J.; Zhu, X.; Lee, H-G.; Moreira, P.I.; Perry, G.; Smith, M.A. *Expert Rev. Neurother.* **2007**, 7, 473. [16]Hamley, I.W. *Chem. Rev.* **2012**, *112*, 5147. [17]Goedert, M.; Spillantini, M.G. Science **2006**, *314*, 777. [18]Hardy, J.; Allsop, D. *Trends Pharm. Sci.* **1991**, *12*, 383. [19]Hardy, J. *Curr. Alzheimer Res*. **2006**, *3*, 71. [20]Coughlan, C.M.; Breen, K.C. *Pharmacol. Ther.* **2000**, *86*, 111.

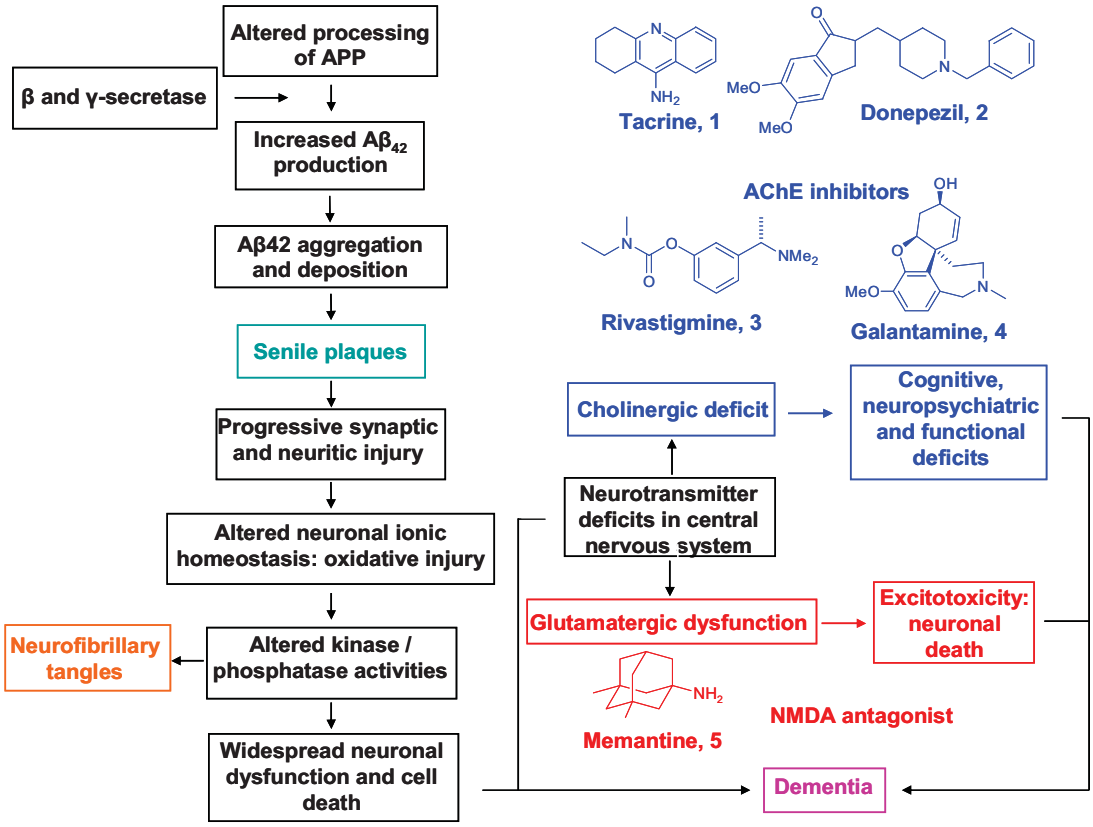

Figure 1.1 Neurotoxic cascade of AD and current available treatment.

$A\beta_{42}$, the variant preferentially implicated in AD, nucleates more rapidly, is more fibrillogenic and shows higher toxicity than $A\beta_{40}$. Early and selective deposition of $A\beta_{42}$ as well as a reduction of its clearance is observed in AD patients.[16] Fibrillar aggregated Aβ is deposited in amyloid plaques (senile plaques) in the brain of AD patients, constituting a prominent hallmark of the disease. Plaque-derived Aβ fibrils enhance oxidative stress and inflammation, leading to kinases and phospatases alteration and neuronal toxicity (Figure 1.1 and 1.2).[21,22] Particularly, cholinergic and glutamatergic neurones are the most commonly affected by Aβ peptide neurotoxicity.

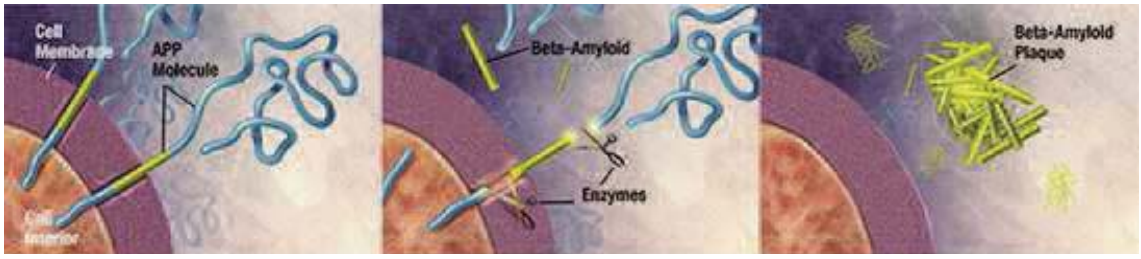

Figure 1.2 Scheme of the pathological cleavage of APP followed by aggregation into senile plaques formation. (Image source: BioEd Online)

Senile plaques are extracellular deposits of amyloid in the gray matter of the brain. They are variable in shape and size, but are on the average 50 μm in size. In AD they are primarily

[16]Hamley, I.W. *Chem. Rev.* **2012**, *112*, 5147. [21]Bartolini, M.; Andrisano, V. *ChemBioChem* **2010**, *11*, 1018. [22]Citron, M. *Nat. Rev. Drug Discovery* **2010**, *9*, 387.

composed of Aβ, however, non β-amyloid component (NAC) is also co-deposited along with Aβ in plaques.[16,23] NAC comprises different proteins or peptidic fragments that may act as chaperones which in some cases may facilitate aggregation, deposition and neurotoxicity of Aβ.[20] Some of these NAC are common to all kinds of amyloid, such as, proteoglycans. Typical extracellular matrix proteins are also observed, such as, type V collagen, entactin and laminin. Furthermore, in the central part of the plaque other proteins such as, α_1-antichymotrypsin, ubiquitin, lysosomal proteins, acetylcholinesterase (AChE), apolipoprotein E (apoE) and residues 61−95 of *α*-synuclein can be found.[16,24]

Neurofibrillary tangles are aggregates of hyperphosphorylated tau protein commonly known as a primary marker of AD. They are produced inside the cell by hyperphosphorylation of a microtubule-associated protein known as tau, by different kinases such as Cdk5 and GSK-3β, causing it to aggregate, first as oligomers, namely, paired helical filaments (PHF) and later on in an insoluble form, namely the neurofibrillary tangles (Figure 1.3).[25] Although the protein tau has been considered to have an important role in AD progression, recent discoveries point out that its processing occurs downstream of Aβ accumulation (Figure 1.1).[16]

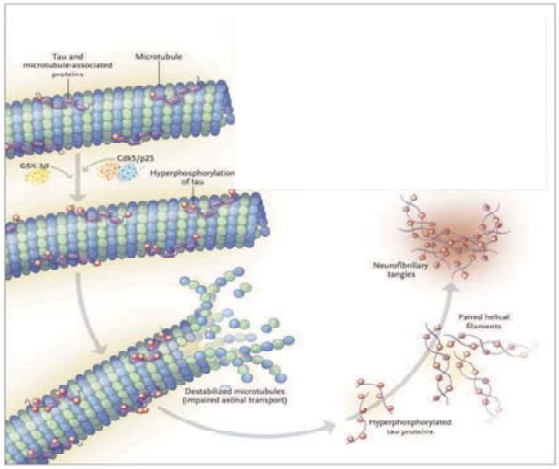

Figure 1.3 Scheme of the pathological hyperphosphorylation of tau followed by aggregation into neurofibrillary tangles. (Image source: OSU Cell Biology of Exercise website)

1.3 Currently commercialized anti-Alzheimer drugs.

The current therapeutic arsenal for AD is dominated by a group of drugs which were conceptually developed to treat the very late symptoms of dementia, which, in turn arise from the deficit in central nervous system (CNS) of different neurotransmitters, particularly acetylcholine (ACh), which are being biosynthesized and released from the neurons which are dying due to the neurodegeneration. This strategy is based upon the so called “cholinergic

[16]Hamley, I.W. *Chem. Rev*. **2012**, *112*, 5147. [20]Coughlan, C.M.; Breen, K.C. *Pharmacol. Ther.* **2000**, *86*, 111. [23]Bodles, A.M.; Guthrie, D.J.S.; Greer, B.; Irvine, G.B. *J. Neurochem.* **2001**, *78,* 384. [24]Inestrosa, N.C. *Las incomunicaciones del Alzheimer, Atenea Impresores Ltda*. **2007**. [25]Goedert, M. *Trends Neurosci.* **1993**, *16*, 460.

hypothesis" that connects cognitive and functional decline in AD to a lack of ACh in the CNS caused by the degeneration of the cholinergic neurons of basal nuclei, which project their axons towards the hippocampus. Consequently, the use of cholinomimetic agents, able to compensate for this deficit should allow the alleviation of the symptomatology of the disease.[26]

AChE is an enzyme located at the synapse of the cholinergic neurons and it is responsible for the hydrolysis of ACh. The X-ray tridimensional structure of the *Torpedo californica* AChE (*Tc*AChE) was elucidated in 1991 by Drs. Sussman and Silman at the Weizmann Institute of Science of Israel.[27] The active site of the enzyme is constituted by a catalytic triad (Ser220, His440 and Glu327) responsible for the hydrolysis of the neurotransmitter and an anionic hydrophobic site located near the active site (Trp84, Tyr130, Tyr330 and Phe330) that stabilizes the positive charge of the quaternary ammonium group of ACh and it allows to position the ester group of ACh in order to face the active site (Figure 1.4).

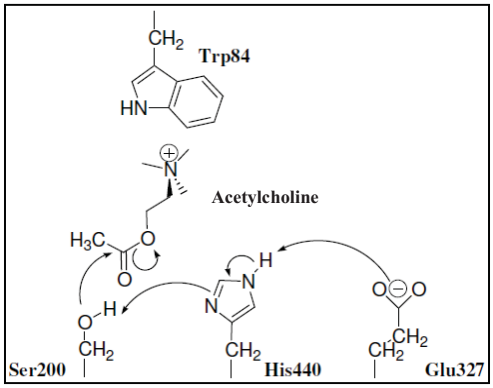

Figure 1.4 Active site of AChE. Scheme of the residues involved in the hydrolysis of ACh. (Image source: Introducció a la Química Terapèutica, Delgado, A.; Minguillón, C.; Joglar, J. Ediciones Díaz de Santos, Madrid, 2004)

The active site, where hydrolysis of ACh takes place, is located at the bottom of a narrow gorge, about 20 Å deep, which mainly consists of aromatic residues (Phe288 and Phe290). At the mouth of this narrow gorge the peripheral site of the enzyme is located (Tyr70, Asp72, Tyr121, Trp279 and Tyr334) which is responsible of the early binding of ACh to the enzyme and directs the neurotransmitter towards the active site located at the bottom of the gorge (Figure 1.5). [27]

As it has been reported above, in healthy brains AChE hydrolizes the majority of ACh while the second cholinesterase present in human blood: butyrylcholinesterase (BChE) plays a secondary role. However, it is important to note that as AD progresses, the activity of AChE decreases, while that of BChE increases in the hypocampus and temporal cortex.[28,29] Consequently, in recent years both selective and non-selective BChE inhibitors (BChEIs) have

[26]Camps, P.; Muñoz-Torrero, D. *Mini-Rev. Med. Chem.* **2002**, *2*, 11. [27]Sussman, J.L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872. [28]Fernández-Bachiller, M.I.; Pérez, C.; Monjas, L.; Rademann, J.; Rodríguez-Franco, M.I. *J. Med. Chem.* **2012**, *55,* 1303. [29]Perry, E.K.; Perry, R.H.; Blessed, G.; Tomlinson, B.E. *Neuropathol. Appl. Neurobiol*. **1978**, *4*, 273.

received increasing attention.[30,31] In this light, recent clinical trials have demonstrated that patients treated with inhibitors of both AChE and BChE showed minor cortical atrophic changes and attenuated loss of brain volumes.[32–34] These findings are consistent with the hypothesis that inhibition of both enzymes may have neuroprotective and disease-modifying effects.[35]

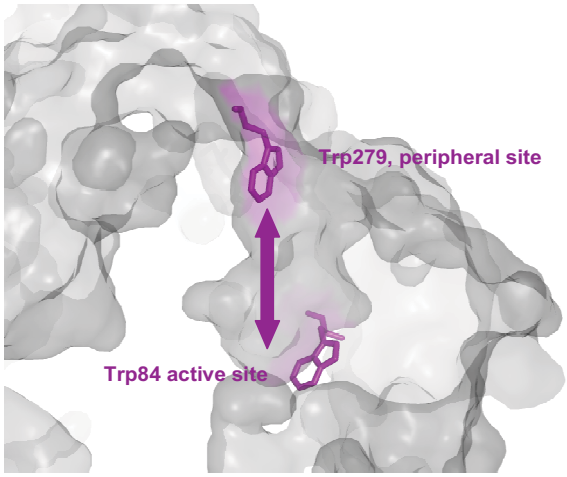

Figure 1.5 Catalytic gorge of AChE with the characteristic active and peripheral sites residues.

1.3.1 Acetylcholinesterase inhibitors (AChEIs).

With the sole exception of the glutamate NMDA receptor antagonist memantine, **5** (marketed as Auxura® in 2002, figure 1.1)[36], the other four commercialized anti-Alzheimer drugs, namely tacrine **1** (marketed by Parke-Davis as Cognex® in 1993),[37,38] donepezil, **2** (marketed by Pfizer as Aricept® in 1996),[39,40] rivastigmine, **3** (marketed by Novartis as Exelon® in 2000),[41] and galantamine, **4**, (marketed as Reminyl® in 2001, figure 1.1 and 1.6),[42] are cholinomimetic agents with the pharmacological profile of AChEIs, which lead to an increase in the levels of ACh at the synapse. Tacrine, donepezil and galantamine are reversible AChEIs and rivastigmine is considered a pseudoirreversible AChEI as it contains a carbamate group that reacts with residue Ser200, carbamoylating the enzyme, whose activity is slowly recovered after hydrolysis of the serine carbamate group.

In principle, the four commercialized AChEIs were developed to inhibit the hydrolysis of ACh, and, therefore they were expected to interact with the active site of the enzyme. In the event, tacrine, rivastigmine and galantamine do clearly interact within the active site of AChE as

[30]Lane, R.M.; Potkin, S.G.; Enz, A. *Int. J. Neuropsychopharmacol*. **2006**, *9*, 101. [31]Greig, N.; Utsuki, T.; Ingram, D.; Wang, Y.; Pepeu, G.; Scali, C.; Yu, Q.; Mamczarz, J.; Holloway, H.; Giordano, T.; Chen, D.; Furukawa, K.; Sambamurti, K.; Brossi, A.; Lahiri, D. *Proc. Natl. Acad. Sci. U.S.A*. **2005**, *102*, 17213.[32]Venneri, A.; Shanks, M.F.; Staff, R.T.; Pestell, S.J.; Forbes, K.E.; Gemmell, H.G.; Murray, A.D. *Neuroreport* **2002**, *13*, 83. [33]Venneri, A.; McGeown, W.J.; Shanks, M.F. *Neuroreport* **2005**, *16*, 107. [34]Veneri, A.; Lane, R. *Neuroreport* **2009**, *20*, 285. [35]Shanks, M.; Kivipelto, M.; Bullok, R.; Lane, R. *Curr. Med. Res. Opin*. **2009**, *25*, 2439. [36]Lipton, S.A. *Nature* **2004**, *428*, 473. [37]Gualtieri, F.; Deu, S.; Manetti, D.; Romanelli, M.N. *Farmaco* **1995**, *50*, 489. [38]Davis, K.L.; Thal, L.J.; Gamzu, E.R.; Davis, C.S.; Woolson, R.F.; Gracon, S.I.; Drachman, D.A.; Schneider, L.S.; Whitehouse, P.J.; Hoover, T.M. *New Engl. J. Med.* **1992**, *327*, 1253. [39]Marx, J. *Science* **1996**, *273*, 50. [40]Sugimoto, H.; Iimura, Y.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1995**, *38*, 4821. [41]Polinsky, R. J. *Clin. Therap.* **1998**, *20*, 634-647.[42]Sramek, J.J.; Frackiewicz, E.J.; Cutler, N.R. *Expert Opin. Invest. Drugs* **2000**, *9*, 2393.

shown in the X-ray tridimensional structures of their complexes with *Tc*AChE (PDB ID's: 1ACJ, 1GQR and 1DTI, respectively) solved by Sussman and Silman,[43–46] while a completely different mode of interaction is observed for donepezil (PDB ID: 1EVE), whose molecule spans the whole length of the active site gorge, simultaneously interacting with Trp84 and Trp279, the two characteristic tryptophan residues of the catalytic and peripheral site, respectively.[47] Thus, donepezil is the sole marketed AChEI exhibiting dual site binding mode of interaction (Figure 1.6).

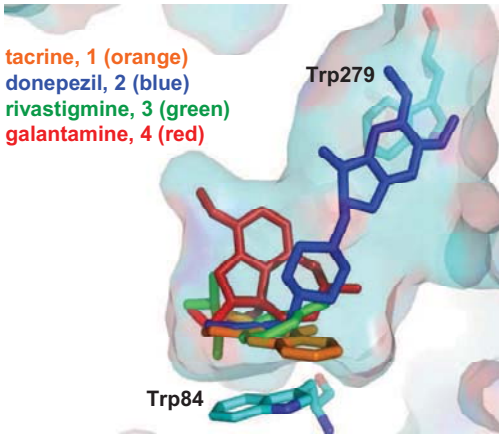

Figure 1.6 Binding mode of the four marketed AChEIs with *Tc*AChE.

1.3.2 Neuroprotective effects of AChEIs.

AChEIs currently represent the most effective treatment to achieve a short-term (6 to 12 months) cognitive and functional improvement.[7] However, AChEIs have been criticized because they are considered as a merely symptomatic treatment, showing modest overall results and being effective in only a third part of the patients.[47] Nevertheless, over the past few years numerous preclinical, radiologic and clinical studies have evidenced a plethora of neuroprotective effects associated to the use of these compounds that challenge the prevailing view of these compounds just as palliative drugs, and suggest that these drugs could have an impact on the natural history of AD. Thus, apart form the inhibition of the hydrolysis of ACh, currently commercialized AChEIs have shown to exhibit a number of biological effects which are clearly beyond the symptomatic improvement of cognition and function and are suggestive of the fact that these compounds could positively modify AD progression.[48–51]

A large body of evidence coming from preclinical studies has shown that the four marketed anti-Alzheimer AChEIs and particularly donepezil, with which most studies have been carried out, exhibit neuroprotective effect both *in vitro* and *in vivo* against a variety of neurotoxic insults.

[7]Leonard, B.E. *Hum. Psycopharmacol.* **1998**, 13, 83. [43]Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P.; Silman, I.; Sussman, J.L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9031. [44]Bar-On, P.; Millard, CB.; Harel, M.; Dvir, H.; Enz, A.; Sussman, J.L.; Silman, I. *Biochemistry* **2002**, *41*, 3555. [45]Greenblatt, H.M.; Kryger, G.; Lewis, T.; Silman, I.; Sussman, J. *FEBS Lett,* **1999**, *463*, 321. [46]Kryger, G.; Silman, I.; Sussman, J.L. *Structure* **1999**, *7*, 297. [47]Giacobini, E. *Neurochem. Res.* **2000**, *25*, 1185. [48]Francis, P.T.; Nordberg, A.; Arnold, S.E. *Trends Pharmacol. Sci.* **2005**, *26*, 104. [49]Riepe, M.W. *Eur. J. Neurol.* **2005**, *12*, 3. [50]Geerts, H. *Brain Res. Bull.* **2005**, *64*, 519. [51]Muñoz-Torrero, D. *Neuroprotección en la enfermedad de Alzheimer*; Edicomplet **2007**, Madrid.

These compounds display neuroprotection against glutamate-[50,52] or NMDA-[53] induced toxicity normally in primary cultures of rat cortical neurons, against Aβ-induced toxicity in primary cultures of rat septal cholinergic neurons,[54,55] SH-SY5Y cells,[56]or mice[57] and against other neurotoxic agents such as hydrogen peroxide,[58] or carbon monoxide.[59] Some neuroprotective effects of these drugs in oxygen-glucose deprivation models of neuronal injury have been also reported.[49,52,60,61]

Analogously, there is also growing evidence from clinical trials suggestive of a positive disease-modifying role for AChEIs. Usually, the longer the patient preserves cognition and function, the longer the delay to nursing home placement, the most clinically relevant end point associated with AD progression. Indeed, AChEIs may afford sustained benefits over time, a considerable number of AD patients showing long-lasting cognitive stabilization after AChEIs treatment, which cannot be solely explained on the basis of a merely symptomatic effect.[62]

A recent study on available long-term clinical data from patients remaining on treatment with rivastigmine or donepezil for up to 5 years or with galantamine for up to 4 years has suggested that AChEIs may delay, albeit not stop, cognitive and functional decline.[63] Also, specifically designed clinical studies with AChEIs have shown that these drugs can positively alter the course of AD, as indicated by the delay in admission to a nursing home, by the preservation of the benefits after withdrawal of the AChEI, resulting in less deterioration in cognitive function relative to placebo-treated patients, and by the preserved advantage in AChEI-treated AD patients relative to a group of patients initially randomized to placebo and taking the AChEI in an extension phase of the study.[64]

Neuroradiological techniques have also contributed to the demonstration of the neuroprotective effect of AChEIs, such as, magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) and positron emission tomography (PET).[65] MRI-detectable volumetric changes in the medial temporal lobe or in the whole brain due to the progressive brain atrophy in AD patients constitute valid biomarkers of pathologic progression of AD.[66] Thus, quantification of the rate of hippocampus atrophy over time has been used to test the disease-modifying effects of AChEIs.[67] Indeed, the most rigorous evidence for the neuroprotective role of AChEIs come from MRI studies which showed that two different groups of AD patients

[49]Riepe, M.W. *Eur. J. Neurol*. **2005**, *12*, 3. [50]Geerts, H. *Brain Res. Bull*. **2005**, *64*, 519. [51]Muñoz-Torrero, D. *Neuroprotección en la enfermedad de Alzheimer*; Edicomplet **2007**, Madrid. [52]Akaike, A. *Alzheimer Dis. Assoc. Disord*. **2006**, *20 (Suppl. 1)*, S8. [53]Akasofu, S.; Kimura, M.; Kosasa, T.; Ogura, H.; Sawada, K. *Eur. J. Pharmacol*. **2006**, *530*, 215. [54]Kimura, M.; Akasofu, S.; Ogura, H.; Sawada, K. *Brain Res*. **2005**, *1047*, 72. [55]Kimura, M.; Komatsu, H.; Ogura, H.; Sawada, K. *Neurosci. Lett*. **2005**, *391*, 17. [56]Arias, E.; Gallego-Sandín, S.; Villarrolla, M.; García, A.G.; López, M.G. *J. Pharmacol. Exp. Ther*. 2005, 315, 1346. [57]Meunier, J.; Ieni, J.; Maurice, T. *Br. J. Pharmacol*. **2006**, *149*, 998. [58]Zhang, H.Y.; Tang, X.C. *Neurosci. Lett*. **2000**, *292*, 41 [59]Meunier, J.; Ieni, J.; Maurice, T. *J. Pharmacol. Exp. Ther*. **2006**, *306*, 53. [60]Zhou, J.; Fu, Y.; Tang, X.C. *Neurosci. Lett*. **2001**, *306*, 53. [61]Sobrado, M.; Roda, J.M:; López, M.G.; Egea, J.; García, A.G. *Neurosci. Lett*. **2004**, *365*, 132. [62]Sabbagh, M.N.; Farlow, M.R. ; Relkin, N.; Beach, T.G. *Alzheimer's Dementia* **2006**, *2*, 118. [63]Bullok, R.; Dengiz, A. *Int. J. Clin. Pract*. **2005**, *59*, 817. [64]Mori, E.; Hashimoto, M.; Krishnan, K.R.; Doraiswamy, P.M. *Alzheimer Dis. Assoc. Disord*. **2006**, *20 (Suppl. 1)*, S19. [65]Modrego, P.J. *Curr. Med. Chem*. **2006**, *13*, 3417. [66]Silbert, L.C.; Quinn, J.F.; Moore, M.M.; Corbridge, E.; Ball, M.J.; Murdoch, G.; Sexton, G.; Kaye, J.A. *Neurology* **2003**, *61*, 487. [67]Mori, E.; Lee, K.; Yasuda, M.; Hashimoto, M.; Kazui, H.; Hirono, N.; Matsui, M. *Ann. Neurol.* **2002**, *51*, 209.

receiving donepezil underwent a significantly lower degree of hippocampus atrophy than the untreated group.[68,69]

Different mechanisms have been proposed to explain the neuroprotective effect of AChEIs as neither are all of them active in the same assays nor their potencies as neuroprotectants correlate with their potencies as AChEIs. Thus, the knowledge of the mechanism of action responsible for the neuroprotective effects of AChEIs is of great interest, especially for the design and development of new drugs with the same mode of action and improved potency.[51]

Some of the different mechanisms proposed to explain this neuroprotective activity of AChEIs include blockade of the glutamate-induced excitotoxicity through antagonism of NMDA receptors,[70] overexpression of the antiapoptotic protein bcl-2 following stimulation of α4β2 nicotinic receptors,[50,56,71,72] mobilization of calcium pools from the intracellular reticulum following activation of σ_1 receptors or,[57,59] upregulation of the neuroprotective splice variants of AChE.[48] However, the most interesting way to modify AD progression would involve an interference in the earliest events of the neurotoxic cascade, namely in Aβ formation and aggregation. Indeed, this kind of mechanisms has been demonstrated for AChEIs.[51,73–80]

Some interactions between the cholinergic system and APP metabolism have been established. It is known that carbacol, a non selective muscarinic agonist, increases the secretion of the non-amyloidogenic fragment sAPPα in cells expressing M_1 and M_3 muscarinic receptors, but not in cells expressing M_2 and M_4 receptors.[80] This effect is particularly interesting taking into account that formation of sAPPα involves the proteolytic cleavage of APP by α-secretase. Because the APP processing via α- or β-secretase are mutually exclusive, the activation of the α-secretase pathway involved in this effect precludes formation of Aβ. Direct activation of muscarinic M_1 and M_3 receptors by selective muscarinic agonists or indirect activation thereof by AChEIs seems to trigger a downstream signal transduction cascade involving protein kinase C (PKC), which in turn can stimulate the non-amyloidogenic processing of APP. The effects of AChEIs on the levels of sAPPα differ among cell types and depend on the specific drug, the duration of the treatment and tested dose.[75]

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[48]Francis, P.T.; Nordberg, A.; Arnold, S.E. *Trends Pharmacol. Sci.* **2005**, *26*, 104. [50]Geerts, H. *Brain Res. Bull*. **2005**, *64*, 519. [51]Muñoz-Torrero, D. *Neuroprotección en la enfermedad de Alzheimer*; Edicomplet **2007**, Madrid. [56]Arias, E.; Gallego-Sandín, S.; Villarrolla, M.; García, A.G.; López, M.G. *J. Pharmacol. Exp. Ther*. 2005, 315, 1346. [57]Meunier, J.; Ieni, J.; Maurice, T. *Br. J. Pharmacol*. **2006**, *149*, 998. [59]Meunier, J.; Ieni, J.; Maurice, T. *J. Pharmacol. Exp. Ther*. **2006**, *306*, 53. [68]Hashimoto, M.; Kazui, H.; Matsumoto, K.; Nakano, Y.; Yasuda, M.; Mori, E. *Am. J. Psychiatry* **2005**, *162*, 676. [69]Krishman, K.P.R.; Charles, H.C.; Doraiswamy, P.M.; Mintzer, J.; Weisler, R.; Yu, X.; Perdomo, C.; Ieni, J.R.; Rogers, S. *Am. J. Psychiatry* **2003**, *160*, 2003. [70]Wang, X.-D.; Chen, X.-Q.; Yang, H.-H.; Hu, G.-Y. *Neurosci. Lett*. **1999**, *272*, 21. [71]Takada-Takatori, Y.; Kume, T.; Sugimoto, M.; Katsui, H.; Sugimoto, H.; Akaike, A. *Neuropharmacology* **2006**, *51*, 474. [72]Kihara, T.; Sawada, H.; Nakamizo, T.; Kanki, R.; Yamashita, H.; Maelicke, A.; Shimohama, S. *Biochem. Biophys. Res. Commun*. **2004**, *325*, 976. [73]Maurice, T.; Meunier, J.; Feng, B.; Ieni, J.; Monaghan, D.T. *J. Pharmacol. Exp. Ther*. **2006**, *317*, 606. [74]Nordberg, A. *Alzheimer Dis. Assoc. Disord.* **2006**, *20 (Suppl. 1)*, S12. [75]Lahiri, D.K.; Rogers, J.T.; Greig, N.H.; Sambamurti, K. *Curr. Pharm. Des*. **2004**, *10*, 3111. [76]Colombres, M., Sagal, J.P.; Inestrosa, N.C. *Curr. Pharm. Des*. **2004**, *10*, 3121. [77]Racchi, M.; Mazzucchelli, M.; Porrello, E.; Lanni, C.; Govoni, S. *Pharmacol. Res*. **2004**, *50*, 441. [78]Bandyopadhyay, S.; Goldstein, L.E.; Lahiri, D.K.; Rogers, J.T. *Curr. Med. Chem*. **2007**, *14*, 2848. [79]Verhoeff, N.P.L.G. *Expert Rev. Neurother*. **2005**, *5*, 277. [80]Nitsch, R.M.; Slack, B.E.; Wurtman, R.J.; Growdon, J.H. *Science*, **1992**, *258*, 304.

The increase on the levels of sAPPα induced by tacrine and donepezil has been demonstrated in different studies *in vitro*.[81,82] Moreover, some changes in APP processing have been observed in AD patients short-term treated with donepezil, which seems to be able to restore the balance between α- and β-secretase activities altered in AD pathology.[83,84]

In addition to PKC, other pathways can be activated downstream of the cholinergic receptor that can modulate APP processing, such as tyrosine kinase, and MAP kinase.[77] Moreover, AChEI's could interfere APP processing through other mechanisms such as regulation of its synthesis or turnover acting at a transcriptional or post-transcriptional stages, maturation and sorting acting at post-translational stages, or even inhibition of APP processing enzymes such as BACE-1 and γ-secretase.[75] Indeed, donepezil has been reported to exhibit a potent inhibitory activity of BACE-1, with an IC_{50} of 170 nM, while tacrine, rivastigmine and galantamine were inactive at 3−5 μM concentrations.[85,86]

1.4 Connection between cholinergic and amyloid hypotheses.

Overall, most of the above-mentioned mechanisms of neuroprotection by AChEIs depend on their action on biological targets other than the enzyme AChE, and therefore are very difficult to rationalize from a structural point of view, which would be necessary for the design of novel drugs hitting these specific targets.[3] The situation is completely different when an additional mechanism of neuroprotection by AChEIs is considered, namely their interference with Aβ aggregation. This consideration derives from some findings of Dr. Nibaldo Inestrosa from *Pontificia Universidad Católica de Chile.* Inestrosa found that the enzyme AChE, which colocalizes with Aβ in the senile plaques, can bind Aβ, accelerating its aggregation and increasing its neurotoxicity. Thus, AChE would act as a "pathological chaperone" inducing a conformational change from a non-amyloidogenic form of Aβ towards a β-sheet rich conformer with an increased tendency to form aggregates (Figure 1.7).[87,88]

The Aβ proaggregating effect of AChE was found to be independent of the source and structural polymorphism of the enzyme. Conversely, other proteins which also colocalize with Aβ in the senile plaques, such as BChE were found not to be able to significantly affect Aβ aggregation.[87] AChE not only accelerates Aβ aggregation but it also increases Aβ neurotoxicity. The stable macromolecular complex formed upon binding of AChE to Aβ is more neurotoxic than Aβ fibrils alone both *in vitro* (PC12 cells and primary retina cells)[88,89] and *in vivo* (rat

[3]Muñoz-Torrero, D. *Curr. Med. Chem.* **2008**, 15, 2433. [75]Lahiri, D.K.; Rogers, J.T.; Greig, N.H.; Sambamurti, K. *Curr. Pharm. Des*. **2004**, *10*, 3111. [77]Racchi, M.; Mazzucchelli, M.; Porrello, E.; Lanni, C.; Govoni, S. *Pharmacol. Res*. **2004**, *50*, 441. [81]Chong, Y.H.; Suh, Y.H. *Life Sci.* **1996**, *59*, 545. [82]Zimmermann, M.; Gardoni, F.; Marcello, E.; Colciaghi, F.; Borroni, B.; Padovani, A.; Cattabeni, F.; Di Luca, M. *J. Neurochem*. **2004**, *90*, 1489. [83]Borroni, B.; Colciaghi, F.; Pastorino, L.; Pettenati, C.; Cottini, E.; Rozzini, L.; Monastero, R.; Lenzi, G.L.; Cattabeni, F.; Di Luca, M.; Padovani, A. *Arch. Neurol*. **2001**, *58*, 442. [84]Zimmermann, M.; Borroni, B.; Cattabeni, F.; Padovani, A.; Di Luca, M. *Neurobiol. Dis.* **2005**, *19*, 237.[85]Mancini, F.; Naldi, M.; Cavrini, V.; Andrisano, V. *Anal. Bioanal. Chem*. **2007**, *388*, 1175. [86]Rosini, M.; Andrisano, V.; Bartolini, M.; Melchiorre, C. WO2006/080043 A2. [87]Inestrosa, N.C.; Alvarez, A.; Pérez, C.A.; Moreno, R.D.; Vicente, M.; Linker, C.; Casanueva, O.I.; Soto, C.; Garrido, C. *Neuron* **1996**, *16*, 81. [88]Alvarez, A.; Alarcón, R.; Opazo, C.; Campos, E.O.; Muñoz, F.J.; Calderón, F.H.; Dajas, F.; Gentry, M.K.; Doctor, B.P.; De Mello, F.G.; Inestrosa, N.C. *J. Neurosci.* **1998**, *18*, 3213. [89]Muñoz, F. Inestrosa, N.C. *FEBS Lett*. **1999**, *450*, 205.

hippocampus).[90] In the latter studies, the hippocampus injection of AChE-Aβ complexes resulted in the appearance of some features reminiscent of AD-like lesions in rat brain. The animals injected with the AChE-Aβ complexes formed bigger amyloid deposits than those formed in animals injected with Aβ alone, which led to a stronger neuronal loss.[90]

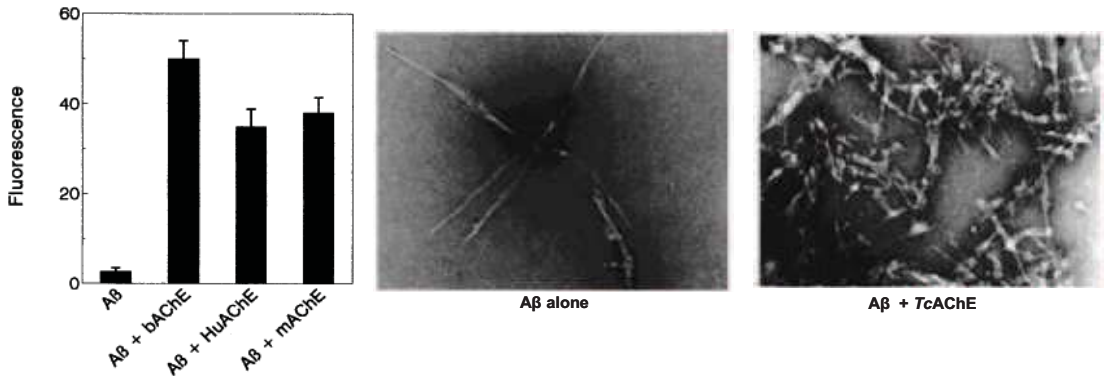


Figure 1.7 Left: Aβ aggregation in presence of bovine AChE (bAChE), human AChE (hAChE) or mouse AChE (mAChE), quantified according to the increase of fluorescence due to Thioflavin T, a compound that binds the β-sheets of Aβ when it is forming aggregates (Aβ 240 μM, AChE 2.4−9.6 μM, r. t., 48 h.) Right: Electronic micrographies of Aβ fibrils alone or in the presence of *Tc*AChE.

The results obtained by Dr. Inestrosa were independently confirmed by Dr. Stephen Brimijoin from Mayo Clinic in the USA in *in vivo* studies with doubly transgenic mice expressing human APP and hAChE in brain. Simultaneous expression of human APP (and therefore Aβ) and hAChE resulted in clear earlier onset of amyloid plaque formation in cerebral cortex relative to singly transgenic animals expressing only human APP (30−50% sooner).[91] Moreover, amyloid burden rose with age, remaining always higher in the doubly transgenic animals than in singly transgenic littermates. Even more interestingly, Dr. Brimijoin and Dr. Hermona Soreq from the Hebrew University of Jerusalem in Israel found that the doubly transgenic mice exhibited increased AD-like pathology, the increase of brain amyloid burden closely correlating with a decline of working memory in behavioural studies.[92,93]

According to the results obtained by Inestrosa and Brimijoin, AChE would occupy a place upstream in the neurotoxic cascade of AD (Figure 1.1). Consequently, those AChEIs capable of interfering the AChE-Aβ interaction could affect the basic disease process of AD.[93] More interestingly, Inestrosa found that AChE binds to Aβ through a hydrophobic environment close to the so-called peripheral site of the enzyme (Figure 1.5).[94] Indeed, propidium, **6** (Figure 1.8) a specific inhibitor known to block the peripheral site of AChE, at 50 μM inhibited by 75% the AChE-induced-Aβ aggregation while the active-site inhibitor edrophonium, **7**, had no effect on Aβ aggregation at 100 μM concentration of inhibitor (Figure 1.8).[87] In an independent work, Dr. Andrissano form *Università di Bologna* in Italy obtained similar results: at 100 μM, propidium

[87]Inestrosa, N.C.; Alvarez, A.; Pérez, C.A.; Moreno, R.D.; Vicente, M.; Linker, C.; Casanueva, O.I.; Soto, C.; Garrido, C. *Neuron* **1996**, *16*, 81. [90]Reyes, A.E.; Chacón, M.A.; Dinamarca, M.C.; Cepa, W.; Morgan, C.; Inestrosa, N.C. *Am. J. Pathol*. **2004**, *164*, 2163. [91]Rees, T.; Hammond, P.I.; Soreq, H.; Younkin, S.; Brimijoin, S. *Neurobiol. Aging* **2003**, *24*, 777. [92]Rees, T.M.; Berson, A.; Sklan, E.H.; Younkin, S.; Brimiojin, S.; Soreq, H. *Curr. Alzheimer Res.* **2005**, *2*, 291 [93]Rees, T.M.; Brimijoin, S. *Drugs Today* **2005**, *39*, 75. [94]De Ferrari, G.V.; Canales, M.A.; Shin, I.; Weiner, L.M.; Silman, I.; Inestrosa, N.C. *Biochemistry* **2001**, *40*, 10447.

was found to inhibit by 82% the AChE-induced Aβ aggregation, while edrophonium was inactive.[95]

Considering Inestrosa and Brimijoin's results, inhibitors capable of blocking the recognition zone of Aβ within AChE, i.e. the peripheral site, would be expected to block the AChE-induced Aβ aggregation, and therefore the cascade of neurodegeneration from the beginning. Indeed, as previously mentioned, the peripheral site AChEI propidium, **6** (Figure 1.8), exhibits an outstanding Aβ antiaggregating effect *in vitro*. However, its cationic character precludes its entrance in the CNS, and therefore its use as an anti-Alzheimer drug.

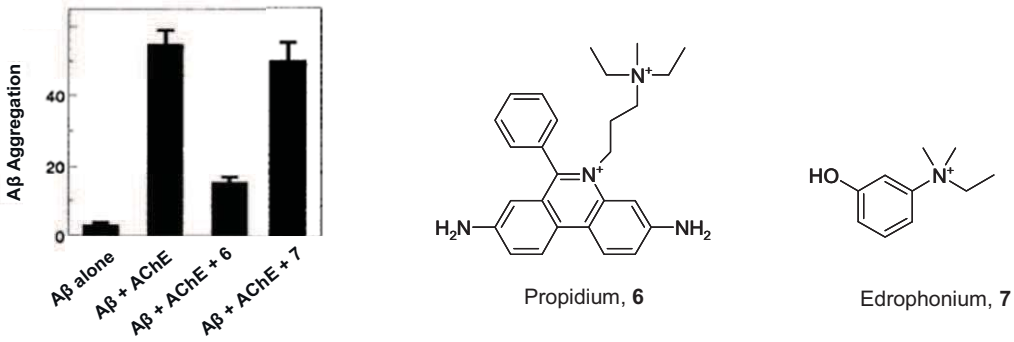


Figure 1.8 Left: Aβ aggregation alone and in the presence of AChE, propidium and edrophonium quantified according to the increase of fluorescence due to thioflavin T (Aβ 240 μM, AChE 2.4−9.6 μM, r. t., 48h). Right: Structure of propidium, **6**, and edrophonium, **7**.

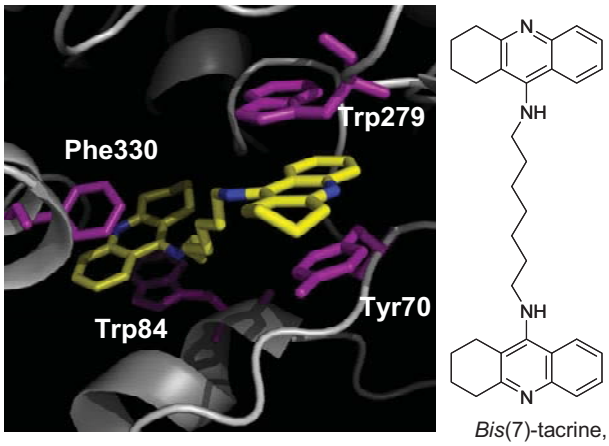
Taking into account the particular architecture of AChE in which the active site and the peripheral site, where Aβ is recognized by AChE, are close enough as to be simultaneously spanned by a single not too large molecule, such as donepezil, **2** (Figure 1.1 and 1.6), an alternative to the design of peripheral site AChEIs would be the development of dual binding site AChEIs capable of interacting simultaneously with both the active and the peripheral site. These dual binding site AChEIs would be expected, not only to have a much higher affinity towards the enzyme than selective active or peripheral site AChEIs, as the recognition points between the inhibitor and the enzyme would be increased, but also, they should exhibit an AChE-induced Aβ antiaggregating effect due to blockade of the peripheral site of the enzyme, thereby being able to block the neurodegererative cascade of AD at the very beginning.[96]

1.5. Dual binding site AChEIs.

The findings of Inestrosa and Brimijoin have driven the rational design of dual binding site AChEIs in the past decade. In 1996, the first family of purposely designed dual binding site AChEIs was developed by Dr. Pang at the Mayo Cancer Centre in the USA and Dr. Carlier at the Hong Kong University of Science and Technology in China. The pioneering work of Pang, which was contemporaneous to the findings of Inestrosa, focussed on dimers of tacrine, the

[95]Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. *Biochem. Pharmacol.* **2003**, *65*, 407. [96]Muñoz-Torrero. D.; Camps, P. *Curr. Med. Chem.* **2006**, *13*, 399.

most potent of which was *bis*(7)-tacrine, **8** (Figure 1.9), [97,98] which turned out to be 150-fold more active than parent tacrine, **1**, as inhibitor of rat brain AChE and 500-fold more potent as inhibitor of hAChE. Moreover, *bis*(7)-tacrine inhibits AChE-induced Aβ aggregation (68% of inhibition at 100 μM concentration of inhibitor).[97,98,99] This AChE-induced Aβ antiaggregating activity might be responsible for the multiple neuroprotective effects shown by this compound, as *bis*(7)-tacrine protects against apoptosis induced by ischemia in primary cultured cortical mouse astrocytes,[100] by hydrogen peroxide in rat pheochromocytoma PC12 cells,[101] by glutamate in primary cultured rat cortical neurons,[102] and by Aβ in primary cultured rat cortical neurons.[103]



Bis(7)-tacrine, **8**

Figure 1.9 Left: Binding mode of *bis*(7)-tacrine to *Tc*AChE (PDB ID: 2CKM).
Right: Structure of *bis*(7)-tacrine, **8**.

The above mentioned mechanisms of neuroprotection by *bis*(7)tacrine have not been related to its dual site binding character, that otherwise has been clearly established by resolution of the X-ray crystal structure of its *Tc*AChE complex (Figure 1.9, PDB ID: 2CKM).[104] Not only can *bis*(7)tacrine interfere upstream in the neurotoxic cascade of AD by inhibiting Aβ aggregation, but this compound is also able to reduce Aβ formation *in vitro* by directly inhibiting BACE-1, exhibiting an IC_{50} value of 7.5 μM.[105] Interestingly, in Neuro2a APPswe neuroblastoma cells the BACE-1 inhibition activity was accompanied by a slightly activation of α-secretase.[105]

Following the design of *bis*(7)tacrine, many novel structural families of dual binding site AChEIs have been developed (Figure 1.10). These compounds are promising disease-modifying AD drug candidates, because they may simultaneously improve cognition and slow

[97]Pang. Y.-P.; Quiram. P.; Jelacic. T.; Hong. F.; Brimijoin. S. *J. Biol. Chem.* **1996**, *271*, 23646. [98]Carlier, P.R.; Han, Y.F.; Chow, E.S.-H.; Li, C.P.-L.; Wang, H.; Lieu, T.X.; Wong, H.S.; Pang, Y.-P. *Bioorg. Med. Chem.* **1999**, *7*, 351. [99]Bolognesi, M.L. ; Cavalli, A. ; Valgimigli, L.; Bartolini, M.; Rosini, M.; Andrisano, V.; Recanatini, M.; Melchiorre, C. *J. Med. Chem.* **2007**, *50*, 6446. [100]Han, Y.-F.; Wu, D.-C.; Xiao, X.-Q.; Chen, P.M.Y.; Chung, W.; Lee, N.T.K.; Pang, Y.-P.; Carlier, P.R. *Neurosci. Lett.* **2000**, *288*, 95. [101]Xiao, X.-Q.; Lee, N.T.K.; Carlier, P.R.; Pang, Y.-P.; Han, Y.-F. *Neurosci. Lett*. **2000**, *290*, 197. [102]Li, W.; Pi, R.; Chan, H.H.N.; Fu, H.; Lee, N.T. K.; Tsang, H.W.; Pu, Y.; Chang, D.C.; Li, C.; Luo, J.; Xiong, K.; Li, Z.; Xue, H.; Carlier, P.R.; Pang, Y.; Tsim, K.W.K.; Li, M.; Han, Y. *J. Biol. Chem*. **2005**, *280*, 18179. [103]Fu, H.; Li, W.; Lao, Y.; Luo, J.; Lee, N.T.K.; Kan, K.K.W.; Tsahg, H.W.; Tsim, K.W.K.; Pang, Y.; Li, Z.; Chang, D.C.; Li, M.; Han, Y. *J. Neurochem*. **2006**, *98,* 1400. [104]Rydberg, E.H.; Brumshtein, B.; Greenblatt, H.M.; Harry, M.; Wong, D.M.; Shaya, D.; Williams, L.D.; Carlier, P.R.; Pang, Y.-P.; Silman, I.; Sussman, J.L. *J. Med. Chem.* **2006**, *49*, 5491. [105]Fu, H.; Li, W.; Luo, J.; Lee, N.T.K.; Li, M.; Tsim, K.W.K. Pang, Y.; Youdim, M.B.H.; Han, Y. *Biochem. Biophys. Res. Commun*. **2008**, *366*, 631.

down the rate of Aβ-induced degeneration. These compounds have proved to be able to bind AChE simultaneously interacting with the active and peripheral site, which results in high AChE inhibitory activity, presenting IC_{50} values in the low nanomolar or even picomolar range. Most interestingly, some of these compounds have proved to inhibit AChE-induced Aβ aggregation *in vitro* showing IC_{50} values in the low micromolar range.[96,106–114]

The firstly developed classes of dual binding site AChEIs, i.e. bis-tacrines, were designed to improve AChE inhibitory activity. AP2238, **9** (Figure 1.10), was developed by Drs. Valenti and Recanatini from the *Università di Bologna* in Italy, and it was the first direct evidence of the Aβ antiaggregating effect of a purposely designed dual binding site AChEI.[115] Thus, on the basis of docking simulations AP2238, **9** (Figure 1.10) was rationally designed by combination of three structural motifs suited for interaction with the active and peripheral sites and also with the aromatic residues which are lining the wall of the active-site gorge as a third recognition zone within the enzyme. For the interaction of the AChE active and peripheral sites the *N*-benzylamino moiety present in many AChEIs such as donepezil, and a synthetically accessible coumarin system were chosen, respectively, while a *p*-phenylene moiety was selected for the interaction with mid-gorge aromatic residues.[115] This compound exhibited a hAChE IC_{50} value of 45 nM and a moderate AChE-induced Aβ antiaggregating effect (35% of inhibition at 100 μM).[115] Moreover, its *N*-ethyl analogue presents a high BACE-1 inhibitory activity (IC_{50} = 238 nM).[116]

Dra. Ana Martínez at Neuropharma S.A. (currently called Noscira) developed the tacrine-based dual binding site AChEI **10**, which is the most potent AChE-induced Aβ aggregation inhibitor and among the most potent hAChE inhibitors reported so far.[117] On the basis of its pharmacological, structural, and electronic properties an indol system was chosen as the peripheral site interacting unit, which was linked to the active site interacting unit through an amide-containing oligomethylene linker. Compound **10** exhibited a highly potent inhibitory activity of hAChE (IC_{50} = 20 pM) and an almost complete inhibition of the AChE-induced Aβ aggregation at a 100 μM concentration of inhibitor (96% of inhibition, IC_{50} = 6 μM).[117]

Memoquine, **11** (Figure 1.10) was developed by Drs. Melchiorre and Bolognesi from the *Università di Bologna* in Italy and it is one of the most promising anti-Alzheimer drug candidates. This compound combines a highly hAChE inhibitory activity (IC_{50} = 1.55 nM), a highly AChE-

[96]Muñoz-Torrero. D.; Camps, P. *Curr. Med. Chem.* **2006**, *13*, 399. [106]Castro, A.; Martínez, A. *Mini-Rev. Med. Chem.* **2001**, *1*, 267. [107]Du, D.-M.; Carlier, P.R. *Curr. Pharm. Des*. **2004**, *10*, 1341. [108]Li, W.M.; Kan, K.K.W.; Carlier, P.R.; Pang, Y.-P.; Han. Y.F. *Curr. Alzheimer Res*. **2007**, *4*, 386. [109]Recanatini, M.; Valenti, P. *Curr. Pharm. Des*. **2004**, *10*, 3157. [110]Castro, A.; Martínez, A. *Curr. Pharm. Des*. **2006**, *12*, 4377. [111]Holzgrabe, U.; Kapková, P.; Alptüzün, V.; Scheiber, J.; Kugelmann, E. *Expert Opin. Ther. Targets* **2007**, *11*, 161. [112]Musial, A.; Bajda, M.; Malawska, B. *Curr. Med. Chem.* **2007**, *14*, 2654. [113]Haviv, H.; Wong, D.M.; Silman, I.; Sussman, J.L. *Curr. Top. Med. Chem.* **2007**, *7*, 375. [114]Cavalli, A.; Bolognesi, M.L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. *J. Med. Chem*. **2008**, *51*, 347.[115]Piazzi, L.; Rampa, A.; Bisi, A.; Gobbi, S.; Belluti, F.; Cavalli, A.; Bartolini, M.; Andrisano, V.; Valenti, P.; Recanatini, M. *J. Med. Chem.* **2003**, *46*, 2279. [116]Piazzi, L.; Cavalli, A.; Collizi, F.; Belluti, F.; Bartollini, M.; Mancini, F.; Recanatini, M.; Andrisano, V.; Rampa, A. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 423. [117]Muñoz-Ruiz, P.; Rubio, L.; García-Palomero, E.; Dorronsoro, I.; del Monte-Millán, M.; Valenzuela, R.; Usán, P.; de Austria, C.; Bartolini, M.; Andrisano, V.; Bidon-Chanal, A.; Orozco, M.; Luque, F.J.; Medina, M.; Martínez, A. *J. Med. Chem.* **2005**, *48*, 7223.

induced Aβ antiaggregating effect (87% of inhibition at a 100 μM concentration of inhibitor, IC_{50} = 28 μM), a highly spontaneous Aβ antiaggregating (96% of inhibition at a 100 μM concentration of inhibitor, IC_{50} = 6 μM) and a highly BACE-1 inhibitory activity (IC_{50} = 108 nM). Moreover it has exhibited neuroprotective effects in SH-SY5Y cells which express high levels of NAD(P)H:quinine oxidoreductase against oxidative stress.[118,119]

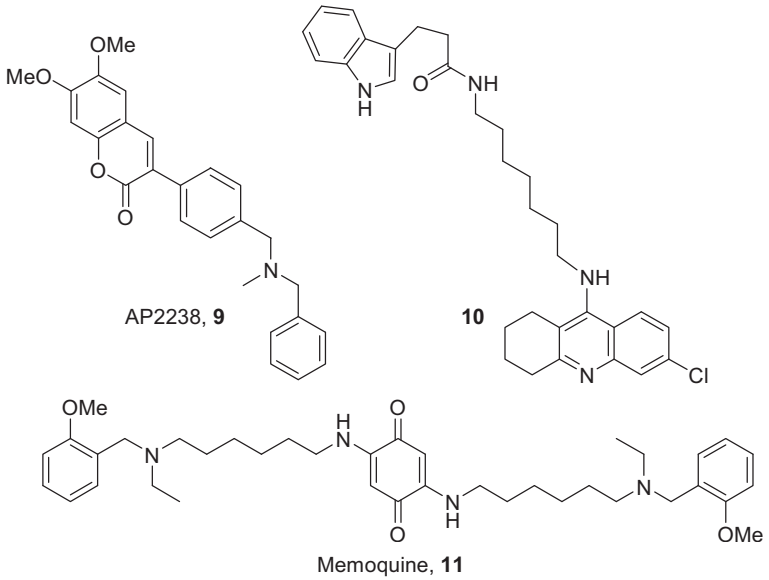


Figure 1.10 Structure of some rationally designed dual binding site AChEIs.

Memoquine and NP-61, a dual binding site AChEI developed by Neuropharma S.A. have recently been tested *in vivo*. Thus, proof-of-concept performed in animal models with these two AChEIs and the fact that NP-61 has already entered clinical trials are a clear evidence of the promising role of these compounds as disease-modifying agents for AD treatment.[120]

1.6 Precedents in the design of AChEIs in our research group.

1.6.1 The huprines.

About 15 years ago, Drs. Pelayo Camps and Diego Muñoz-Torrero from the *Univestat de Barcelona* in Spain developed a novel class of potent active site AChEIs called huprines, which turned out to be among the most potent reversible AChEIs reported so far (Figure 1.11). [121–128]

[118]Cavalli, A; Bolognesi, M.L.; Capsoni, S.; Andrisano, V.; Bartolini, M.; Margotti, E.; Cattaneo, A.; Recanatini, M.; Melchiorre, C. *Angew. Chem. Int. Ed.* **2007**, *46*, 3689. [119]Bolognesi, M.L.; Banzi, R.; Bartolini, M.; Cavalli, A.; Tarozzi, A.; Andrisano, V.; Minarini, A.; Rosini, M.; Tumiatti, V.; Bergamini, C.; Fato, R.; Lenaz, G.; Hrelia, P.; Cattaneo, A.; Recanatini, M.; Melchiorre, C. *J. Med. Chem.* **2007**, *50*, 4882.[120]http://www.noscira.es. [121]Badia, A.; Baños, J.E.; Camps, P.; Contreras, J.; Görbig, D.M.; Muñoz-Torrero, D.; Simon, M.; Vivas, N.M. *Bioorg. Med. Chem.* **1998**, *6*, 427. [122]Camps, P.; Contreras, J.; Font-Bardia, M.; Morral, J. ; Muñoz-Torrero, D.; Solans, X. *Tetrahedron: Asymmetry* **1998**, *9*, 835. [123]Camps, P.; El Achab, R.; Görbig, D.M.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.* **1999**, *42*, 3227. [124]Camps, P.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X. Orozco, M.; Luque, F.J. *J. Med. Chem.* **2000**, *43*, 4657. [125]Camps, P.; Gómez, E.; Muñoz-Torrero, D.; Badia, A.; Vivas, N.M.; Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.* **2001**, *44*, 4733. [126]Camps, P.; Cusack, B.; Mallender, W.D.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Rosenberry, T.L. *Mol. Pharmacol.* **2000**, *57*, 409. [127]Dvir, H.; Wong, D.M.; Harel, M.; Barril, X.; Orozco, M.; Luque, F.J.; Muñoz-Torrero, D.; Camps, P.; Rosenberry, T.L.; Silman, I.; Sussman, J.L. *Biochemistry* **2002**, *41*, 2970. [128]Muñoz-Torrero, D.; Camps, P. *Expert Opin. Drug Discovery* **2008**, *3*, 65.

They were designed by combination of the 4-aminoquinoline moiety of tacrine, **1**, with the carbobicyclic substructure of (–)-huperzine A, **12** (Figure 1.11), an alkaloid isolated from *Huperzia serrata* that is a potent AChEI with an increased interest as a potential treatment for AD, it is commercialized as a nutraceutical in the USA (Cerebra®) and it has been recently approved in China as a palliative treatment for AD.

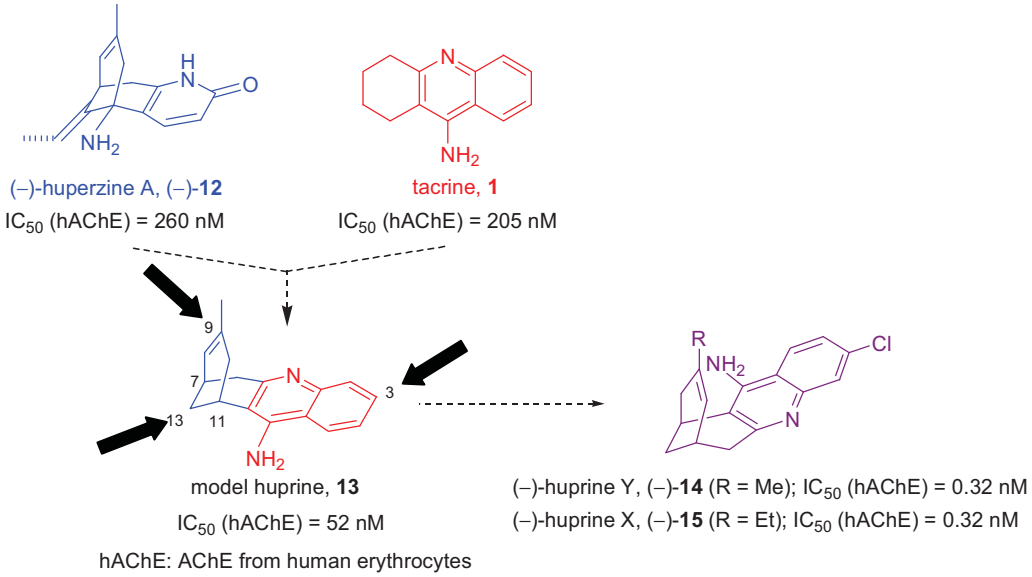


Figure 1.11 Design and pharmacomodulation of huprines.

More than 30 different huprines, designed by introduction of structural modifications on the methylene bridge between the 7 and 11 positions, the substituents on the benzene ring of the aminoquinoline system and the substituents at position 9, were synthesized and pharmacologically tested. The most active huprines prepared to date are the so-called (−)-huprine Y, (−)-**14** (Figure 1.11) and its 9-ethyl analogue, (−)-huprine X, (−)-**15**, which are, in racemic form, up to 640- and 810-fold more potent human AChEIs than the parent compounds tacrine and (–)-huperzine A, respectively.

The X-ray crystallographic structure of the complex between *Tc*AChE and (–)-huprine X, (–)-**15**, (Figure 1.12, PDB ID 1E66),[127] obtained by Dr. Joel L. Sussman at Weizmann Institute of Science in Israel, showed that huprines tightly bind to the active site of AChE with one of the highest affinities ever reported for a reversible inhibitor: the 4-aminoquinoline functional group of (–)-huprine X, (–)-**15**, binds to the same aromatic system where tacrine, **1**, usually sits by π-stacking interactions with Trp84 and Phe330 residues and by hydrogen-bonding between the pyridinic nitrogen atom, protonated at physiological pH, and the carbonyl oxygen atom of His440. Moreover, other hydrogen-bonding interactions take place, mediated by water molecules, between the aniline nitrogen atom and Asp72, Tyr121 and Tyr334 residues;

[127]Dvir, H.; Wong, D.M.; Harel, M.; Barril, X.; Orozco, M.; Luque, F.J.; Muñoz-Torrero, D.; Camps, P.; Rosenberry, T.L.; Silman, I.; Sussman, J.L. *Biochemistry* **2002**, *41*, 2970.

additionally, the carbocyclic portion of (–)-huprine X, (–)-**15**, fits in the same hydrophobic portion of the enzyme where (–)-huperzine A, (–)-**12**, usually sits (PDB ID: 1VOT, Figure 1.12).

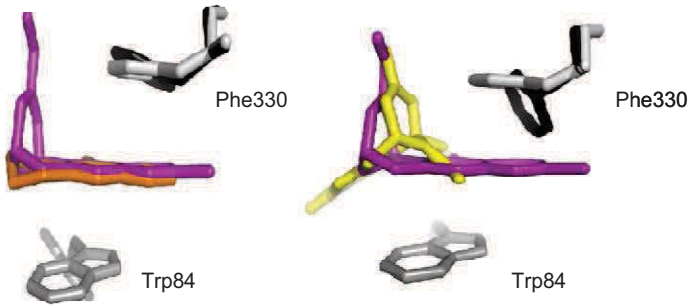


Figure 1.12 Left: Comparison between the dispositions of tacrine, **1**, (orange) and (–)-huprine X, (–)-**15**, (purple) in the active site of *Tc*AChE. Right: Overlapping between the disposition of (–)-huperzine A, (–)-**12**, (yellow) and (–)-**15** (purple) in the active site of *Tc*AChE.

Apart from keeping the interactions corresponding to the substructure of tacrine, **1**, and some of the interactions of (–)-huperzine A, (–)-**12**, (–)-huprine X, (–)-**15**, also presents additional interactions through the chlorine atom that locates at an hydrophobic pocket where it establishes hydrophobic interactions with aromatic residues Trp432 and Phe330 and with the methyl group of Met436 and Ile439 (Figure 1.13).

Huprine X, **15**, has proved to be able to decrease the levels of Aβ in *3xTg*-AD mice brains, and to clearly increase the levels of synaptophysin.[129] This neuroprotective activity could be due to the activation of different receptors involved in AD, such as, the M_1 muscarinic receptor or the antagonistic effect exhibited towards the NMDA glutamate receptor. However, when treating *APPswe* mice with huprine X this neuroprotective effect was not observed.

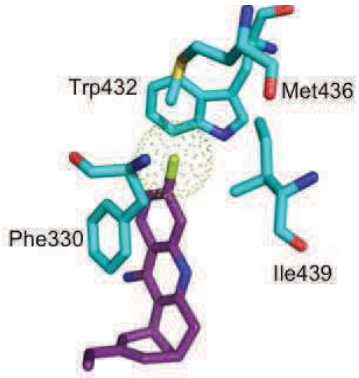


Figure 1.13 Interactions between the chlorine atom of (–)-huprine X, **15**, (purple) with the active site of AChE (residues in light blue).

[129]Hedberg, M.M.; Clos, M.V.; Ratia, M.; Gonzalez, D.; Unger Lithner, C.; Camps, P.; Muñoz-Torrero, D.; Badia, A.; Giménez-Llort, L.; Nordberg, A. *Neurodegener. Dis.* **2010**, *7*, 379.

1.6.2 Dual binding site AChEIs developed in our research group.

Given the fact that, as previously mentioned, the enzyme AChE, besides its important role in the cholinergic transmission, also participates in other functions related to neuronal development such as, promoting the Aβ aggregation mediated by an interaction between Aβ and the peripheral site of the enzyme, in the last decade, dual binding site AChEIs have been regarded as very promising anti-Alzheimer disease-modifying drug candidates.[3]

In this context, in order to increase even more the affinity and inhibitory potency towards AChE and to inhibit Aβ aggregation, a few years ago, Drs. Pelayo Camps and Diego Muñoz-Torrero, in the frame of the PhD thesis work of Drs. Xavier Formosa and Carles Galdeano, developed a novel series of dual binding site AChE inhibitors namely the huprine−tacrine hybrids (Figure 1.14).[130−133] These novel compounds consisted of a unit of racemic or enantiopure hupine Y, **14**, as the active site interacting unit, and a moiety of tacrine, **1**, or 6-chlorotacrine, **16**, as the peripheral site interacting unit, connecting both units with oligomethylene linkers of different lengths, and, for compounds **17c** and **18c**, incorporating a protonatable methylamino group in order to enable cation−π interactions with midgorge aromatic residues (Figure 1.14).

Huprine−tacrine hybrids dual site binding, supported by kinetic and molecular modelling studies,[132,133] resulted in a highly potent inhibition of the catalytic activity of hAChE (IC_{50} = 0.31−11.5 nM) and, more importantly, in the *in vitro* neutralization of the pathological chaperoning effect of AChE toward the aggregation of Aβ (23−67% of inhibition at 100 μM concentration of inhibitor). Moreover, these compounds also exhibited a potent inhibitory activity towards hBChE (IC_{50} = 24.6−139 nM), a significant inhibitory activity of the self-induced Aβ aggregation (28−61% of inhibition at 10 μM concentration of inhibitor) and BACE-1 (IC_{50} = 4.9−7.3 μM) which should reinforce their symptomatic and disease-modifying effects, positioning huprine−tacrine hybrids as promising lead compounds for AD.

[3]Muñoz-Torrero, D. *Curr. Med. Chem.* **2008**, 15, 2433. [130]Formosa X. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2006**. [131]Camps, P.; Formosa, X.; Muñoz-Torrero, D.; Petrignet, J.; Badia, A.; Clos, M.V. *J. Med. Chem.* **2005**, *48*, 1701. [132]Galdeano C. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2012** [133]Galdeano, C.; Viayna, E.; Sola I.; Formosa, X.; Camps, P.; Badia, A.; Clos, M.V.; Relat, J.; Ratia, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Salmona, M.; Minguillón C.; González-Muñoz, G.C.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Muñoz-Torrero, D. *J. Med. Chem.* **2012**, *55*, 661.

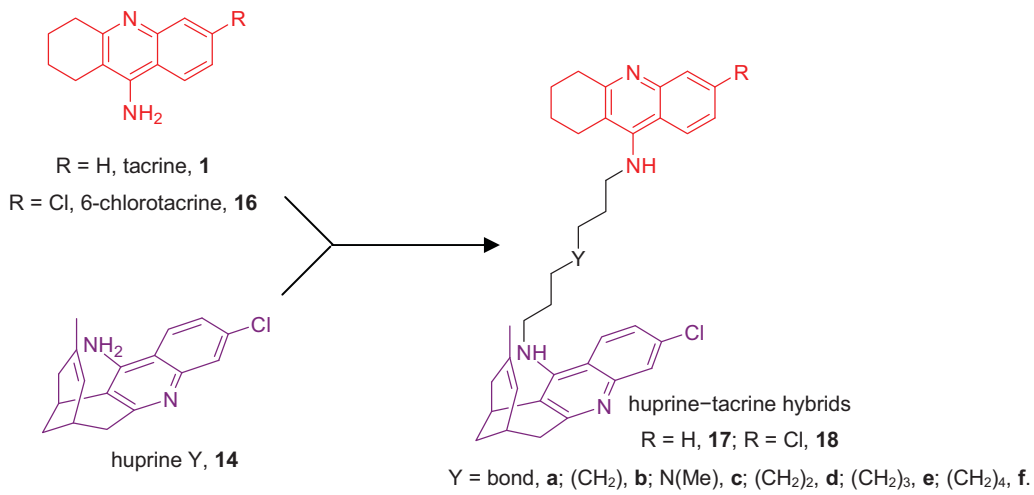


Figure 1.14 Design of huprine−tacrine hybrids **17**−**18**

In 2008, Drs. Camps and Muñoz-Torrero, in the frame of Drs. Xavier Formosa and Carles Galdeano PhD thesis works and Michele Scarpellini and Tània Gómez Experimental Master work, developed a novel series of donepezil−tacrine hybrids designed to simultaneously interact with the active, peripheral and midgorge binding sites of AChE. These compounds consisted of a unit of tacrine, **1**, or 6-chlorotacrine, **16**, to occupy the same position as tacrine at the AChE active site, and the 5,6-dimethoxy-2-[4-piperidinyl)methyl]-1-indanone moiety of donepezil (or the indane derivative thereof), whose position along the enzyme gorge and the peripheral site can be modulated by a suitable tether that connects tacrine and donepezil fragments (Figure 1.15).[130,132, 134−137]

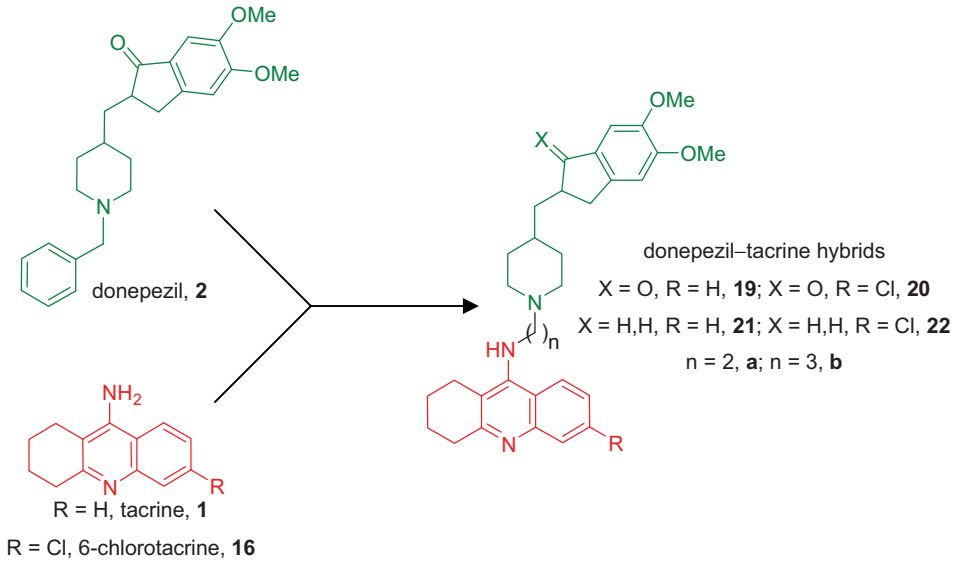
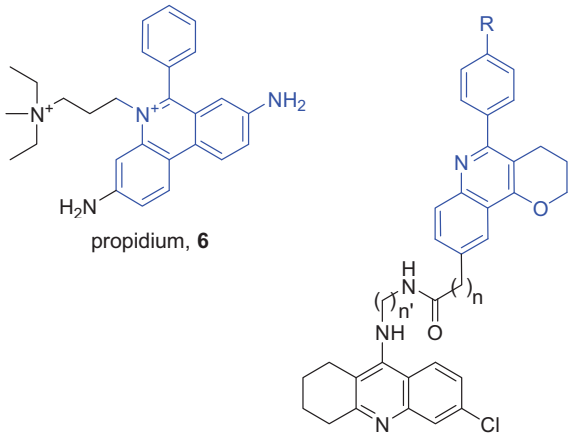


Figure 1.15 Design of donepezil–tacrine hybrids **19**−**22**.

[130]Formosa X. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2006**. [132]Galdeano C. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2012**. [134]Scarpellini, M. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2006**. [135]Gómez, T. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2007**. [136]Camps, P.; Muñoz-Torrero, D.; Formosa, X.; Scarpellini, M. WO 2007/122274A1. [137]Camps, P.; Formosa, X.; Galdeano, C.; Gómez, T.; Muñoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia A.; Clos, M.V.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F.J. *J. Med. Chem*. **2008**, 51, 3558.

All donepezil–tacrine hybrids were highly potent inhibitors of hAChE (IC_{50} = 0.27−5.13 nM) and hBChE (IC_{50} = 7.25−88.7 nM). Moreover, six out of the eight hybrids of the series, particularly those bearing the indane moiety, exhibited a significant AChE-induced Aβ aggregation inhibitory activity (38−66% of inhibition at 100 μM concentration of inhibitor).[137]

In 2009, Drs. Camps and Muñoz-Torrero established a collaboration with Dr. Rodolfo Lavilla of the *Universitat de Barcelona* in Spain, in the context of Dr. Carles Galdeano PhD thesis work, and developed two series of dual binding site AChE inhibitors which were consistent of a unit of 6-chlorotacrine, **16** as the active site interacting unit and a Povarov-type multicomponent reaction-derived pyrano[3,2,*c*]-quinoline scaffold, reminiscent of the peripheral site inhibitor propidium, **6** (Figure 1.8), to block the peripheral site (Figure 1.16).[130,132,138−140]



propidium, **6**

	n	n'	R
23	0	6	Cl
24	0	7	Cl
25	0	8	Cl
26	0	9	Cl
27	0	10	Cl
28	2	4	H
29	2	5	H
30	2	6	H
31	2	7	H
32	2	8	H

Figure 1.16 Structure of propidium, **6** and pyrano[3,2-*c*]quinoline–6-chlorotacrine hybrids **23**–**32**.

These novel compounds, and particularly, **32**, retained the AChE inhibitory activity of the parent 6-chlorotacrine, **14**, (IC_{50} = 7.03−50.0 nM), while exhibiting a significant *in vitro* inhibitory activity toward the AChE-induced (23−46% inhibition at 100 μM concentration of inhibitor) and self-induced Aβ aggregation (12−49% inhibition at 50 μM concentration of inhibitor) and toward BACE-1 (14−78% inhibition at 2.5 μM concentration of inhibitor) which makes them promising anti-Alzheimer lead compounds.[140]

[130]Formosa X. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2006**. [132]Galdeano C. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2012**. [137]Camps, P.; Formosa, X.; Galdeano, C.; Gómez, T.; Muñoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia A.; Clos, M.V.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F.J. *J. Med. Chem.* **2008**, *51*, 3558. [138]Galdeano, C. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2006**. [139]Ramírez, L. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2008**. [140]Camps, P.; Formosa, X.; Galdeano, C.; Muñoz-Torrero, D.; Ramírez, L.; Gómez, E.; Isambert, N.; Lavilla, R.; Badia, A.; Clos, M.V.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Huertas, O.; Dafni, T.; Luque, F.J. *J. Med. Chem.* **2009**, *52*, 5365.

1.7 What's next in anti-Alzheimer drug discovery.

1.7.1 Aβ-directed strategies.

Aβ seems to be the main target in order to prevent the neurodegenerative process of AD. Thus, in the last years important efforts in order to develop anti-Alzheimer drugs capable of positively modifying the progression of the disease by inhibiting the formation and aggregation of Aβ have been made.[141,142]

However, clinical trials carried out with different Aβ-directed agents have been quite disappointing. Clinical trials carried out with vaccine AN1792 and tramiprostat, an Aβ aggregation inhibitor, were discontinued due to safety and efficacy issues. In spite of the enormous difficulty that finding an anti-Alzheimer disease-modifying drug is representing, some other drug candidates are advancing in clinical trials, such as second generation Aβ-directed vaccines with a better safety profile compared to AN1792, namely CAD-106 and V-950 (phase I clinical trials), anti-Aβ monoclonal antibodies such as bapineuzumab (phase III clinical trials) or LY2062430 (phase II clinical trials),[143] BACE-1 inhibitors such as CTS-21166 (phase I clinical trials),[144] and α-secretase modulators such as LY450139 and MK0752 (phase II clinical trials) and E2012 (phase I clinical trials).[145]

BACE-1 constitutes a prime therapeutic target for disease-modifying anti-Alzheimer drugs, as it is involved in the initial and rate-limiting step of the proteolytic cleavage of APP to Aβ; its inhibition should decrease all forms of Aβ. Indeed, Aβ generation, amyloid pathology, and cognitive deficits are abrogated in BACE-1-deficient bigenic mice overexpressing APP.[146] However, BACE-1 has other substrates in addition to APP, and so its complete inhibition might lead to serious side effects. Thus, although initial studies on BACE-1-deficient mice indicated no adverse phenotype,[147] some subtle effects on both peripheral and central myelin formation have recently been found.[148] This mechanism-based toxicity, more importantly present in inhibitors of the more promiscuous γ-secretase, requires some selectivity or a balanced inhibition, which could be reached by mild inhibition or by simultaneously addressing multiple targets in order to minimize harmful side effects.[21] Indeed, the degree of decrease in Aβ synthesis that secretase inhibitors have to achieve to affect the progression of AD is a matter of debate,[149,150] and partial inhibition of BACE-1 has been proposed to provide therapeutic benefits with limited mechanism-

[21]Bartolini, M.; Andrisano, V. *ChemBioChem* **2010**, *11*, 1018. [141]Melnikova, I. *Nat. Rev. Drug Discovery* **2007**, 6, 341. [142]Skovronsky, D.M.; Lee, V.M.-Y.; Trojanowski, J.Q. *Annu. Rev. Pathol. Mech. Dis*. **2006**, *1*, 151. [143]Yamin, G.; Ono, K.; Inayathullah, M.; Teplow, D.B. *Curr. Pharm. Des.* **2008**, *14*, 3231. [144]Ghosh, A.K.; Gemma, S.; Tang, J. *Neurotherapeutics* **2008**, *5*, 399. [145]Silvestri, R. *Med. Res. Rev*. **2009**, *29*, 295. [146]De Strooper, B.; Vassar, R.; Golde, T. *Nat. Rev. Neurol*. **2010**, *6*, 99. [147]Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M. J.; Branstetter, D. G.; Chen, K. S.; Freedman, S. B.; Frigon, N. L.; Games, D.; Hu, K.; Johnson-Wood, K.; Kappenman, K. E.; Kawabe, T. T.; Kola, I.; Kuehn, R.; Lee, M.; Liu, W.; Motter, R.; Nichols, N. F.; Power, M.; Robertson, D. W.; Schenk, D.; Schoor, M.; Shopp, G. M.; Shuck, M. E.; Sinha, S.; Svensson, K. A.; Tatsuno, G.; Tintrup, H.; Wijsman, J.; Wright, S.; McConlogue, L. *Hum. Mol. Genet*. **2001**, *10*, 1317. [148]Willem, M.; Garratt, A. N.; Novak, B.; Citron, M.; Kaufmann, S.; Rittger, A.; DeStrooper, B.; Saftig, P.; Birchmeier, C.; Haass, C. *Science* **2006**, *314*, 664. [149]De Strooper, B.; Vassar, R.; Golde, T. *Nat. Rev. Neurol.* **2010**, *6*, 99. [150]McConlogue, L.; Buttini, M.; Anderson, J. P.; Brigham, E. F.; Chen, K. S.; Freedman, S. B.; Games, D.; Johnson-Wood, K.; Lee, M.; Zeller, M.; Liu, W.; Motter, R.; Sinha, S. *J. Biol. Chem.* **2007**, *282*, 26326.

based adverse effects.[149] Thus, heterozygous BACE-1 knockout APP transgenic mice with only 15% reduction of brain Aβ show a dramatic decrease in brain amyloid burden at old age.[150]

Although highly active *in vitro* peptide inhibitors with poor pharmacokinetics are known, development of potent brain permeable inhibitors is turning out to be a difficult task. Indeed, so far only one BACE-1 inhibitor, namely CTS-21166, has reached clinical trials. Therefore, there is still an increasing need for small organic BACE-1 inhibitors that are able to cross the blood–brain barrier (BBB).[151] As previously mentioned, some dual binding site AChEIs such as *bis*(7)-tacrine[105] and some derivatives thereof,[152] lipocrine,[86] memoquin,[118] AP2243,[116] and some pyrano[3,2-c]quinoline-6-chlorotacrine heterodimers[140] exhibit significant BACE-1 inhibitory activity, which is independent of their action on the primary target, AChE.

1.7.2 Neurofibrillary tangles-directed strategies.

Even though it appears that the formation of the neurofibrillary tangles takes place downstream in the neurotoxic cascade of AD compared to Aβ aggregation, another possible strategy would be to find a compound capable of blocking their formation and aggregation. As previously mentioned, neurofibrillary tangles are formed by hyperphosphorylation of tau protein due to deregulation of different kinases, namely Cdk5 and GSK-3β to form PHFs which later on aggregate to constitute the neurofibrillary tangles.[153] Thus, another possible strategy for AD treatment consists of the development of compounds capable of inhibiting these kinases and also to block either the aggregation of hyperphosphorylated tau protein into PHFs or, later on, into neurofibrillary tangles.

1.7.3 Monoamine oxidase-directed strategies.

Monoamine oxidase (MAO) is a flavoprotein located at the external mitochondrial membrane of neurones and glia cells. It exists in two different isoforms, MAO-A and MAO-B. It has been reported that MAO-B activity increases with age and high concentrations of this protein have been observed at the senile plaques.[154,155] Moreover, MAO-B produces hydrogen peroxide when carrying out oxidative desamination of endogenous amines and generating reactive oxygen species (ROS), which suggests a possible implication with the oxidative stress

[86]Rosini, M.; Andrisano, V.; Bartolini, M.; Melchiorre, C. WO2006/080043 A2. [105]Fu, H.; Li, W.; Luo, J.; Lee, N.T.K.; Li, M.; Tsim, K.W.K. Pang, Y.; Youdim, M.B.H.; Han, Y. *Biochem. Biophys. Res. Commun*. **2008**, *366*, 631. [116]Piazzi, L.; Cavalli, A.; Collizi, F.; Belluti, F.; Bartollini, M.; Mancini, F.; Recanatini, M.; Andrisano, V.; Rampa, A. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 423. [118]Cavalli, A; Bolognesi, M.L.; Capsoni, S.; Andrisano, V.; Bartolini, M.; Margotti, E.; Cattaneo, A.; Recanatini, M.; Melchiorre, C. *Angew. Chem. Int. Ed.* **2007**, *46*, 3689. [140]Camps, P.; Formosa, X.; Galdeano, C.; Muñoz-Torrero, D.; Ramírez, L.; Gómez, E.; Isambert, N.; Lavilla, R.; Badia, A.; Clos, M.V.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Huertas, O.; Dafni, T.; Luque, F.J. *J. Med. Chem*. **2009**, *52*, 5365.[149]De Strooper, B.; Vassar, R.; Golde, T. *Nat. Rev. Neurol.* **2010**, *6*, 99. [150]McConlogue, L.; Buttini, M.; Anderson, J. P.; Brigham, E. F.; Chen, K. S.; Freedman, S. B.; Games, D.; Johnson-Wood, K.; Lee, M.; Zeller, M.; Liu, W.; Motter, R.; Sinha, S. *J. Biol. Chem.* **2007**, *282*, 26326.[151]Jakob-Roetne R.; Jacobsen, H. *Angew. Chem.* **2009**, *121*, 3074; *Angew. Chem. Int. Ed.* **2009**, *48*, 3030. [152]Bolognesi, M. L.; Bartolini, M.; Mancini, F.; Chiriano, G.; Ceccarini, L.; Rosini, M.; Milelli, A.; Tumiatti, V.; Andrisano, V.; Melchiorre, C. *ChemMedChem* **2010**, *5*, 1215. [153]Buee, L.; Bussiere, T.; Buee-Scherrer, V.; Delacourte, A.; Hof, P.R. *Brain Res. Rev*. **2000**, *33*, 95. [154]Carreiras, M.C.; Marco, J.L. *Curr. Pharm. Des.* **2004**, *10*, 3167. [155]Saura, J.; Luque, J.L.; Cesura, A.M.; Da Prada, M.; Chan-Palay, V.; Huber, G.; Loffer, J.; Richards, J.G. *Neurosci*. **1994**, *62,* 15.

associated to AD.[156] For these reasons selective MAO-B inhibitors, such as, selegiline, **33**, or rasagiline, **34** (Figure 1.17), have been proposed as potential anti-Alzheimer candidates.[157]

Selegiline, **33** Rasagiline, **34**

Figure 1.17. Structure of MAO-B inhibitors selegiline, **33**, and rasagiline, **34.**

1.7.4 Oxidative stress-directed strategies.

During aging, the endogenous antioxidant system progressively decays, and an increasing body of evidence supports the involvement of oxidative stress in different pathologies, such as cancer, cardiovascular, and neurodegenerative diseases. In the case of AD, it has been reported that oxidative damage in cellular structures is an event that precedes the appearance of the other pathological hallmarks of AD, namely, senile plaques and neurofibrillary tangles,[158,159] pointing out the early involvement of oxidative stress in the pathogenesis and progression of the disease.[160,161]

Furthermore, plaque-derived Aβ fibrils can also enhance extensive oxidative stress and inflammation, leading to neuronal toxicity. As a matter of fact, Aβ plays a very significant role in the pathogenesis of AD by inducing important events of the neurodegenerative cascade such as a massive increase in ROS production. There are ions, such as Cu^{2+} and Zn^{+}, that can promote Aβ aggregation, whereas some of them, especially Cu^{2+} and Fe^{3+}, can also generate ROS such as hydrogen peroxide.[21,22,162]

Furthermore, a recent statistical study involving 23 developed countries suggests that higher consumption of dietary antioxidants such as flavonoids is associated with lower population rates of dementia.[163] In this light, a considerable research has been conducted on the use of antioxidants to slow down or reverse the pathology and course of AD. In particular the antioxidants capsaicin, **35**, melatonin, **36**, lipoic acid, **37**, various flavones, and some polyphenols, such as curcumin, **38,** and, vitamin E, **39** (Figure 1.18), showed protection against Aβ-induced oxidative stress and neurotoxicity.[164]

[21]Bartolini, M.; Andrisano, V. *ChemBioChem* **2010**, *11*, 1018. [22]Citron, M. *Nat. Rev. Drug Discovery* **2010**, *9*, 387. [156]Good, P.F.; Werner, P.; Hsu, A.; Olanow, C.W.; Perl, D.P. *Am. J. Pathol.* **1996**, *149*, 21. [157]Ebadi, M.; Sharma, S.; Shavadi, S.; El Refakey, K. *J. Neurosci. Res.* **2002**, *67*, 285.[158]Gu, F.; Zhu, M.; Shi, J.; Hu, Y.; Zhao, Z. *Neurosci. Lett.* **2008**, *440*, 44. [159]Moreira, P.I.; Santos, M.S.; Oliveira, C.R.; Shenk, J.C.; Nunomura, A.; Smith, M.A.; Zhu, X.; Perry, G. *CNS Neurol. Disord. Drug Targets* **2008**, *7*, 3. [160]Ansari, M.A.; Scheff, S.W. J. *Neuropathol. Exp. Neurol.* **2010**, *69*, 155. [161]Reddy, V.P.; Zhu, X.; Perry, G.; Smith, M.A. *J. Alzheimers Dis.* **2009**, *16*, 763. [162](a) Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M.V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855. (b) Muñoz-Torrero, D.; Camps, P.; Gómez, T.; Viayna, E.; Galdeano, C. *P201000016*, *PCT/ES2010/070862*, **2010**. [163]Beking, K.; Vieira, A. *Public Health Nutr.* **2010**, *13*, 1403. [164]Butterfield D. A.; Castegna A.; Pcernich C.; Drake J.; Scapagnini G.; Calabrese V. *J. Nutr. Biochem.* **2002**, *13*, 444.

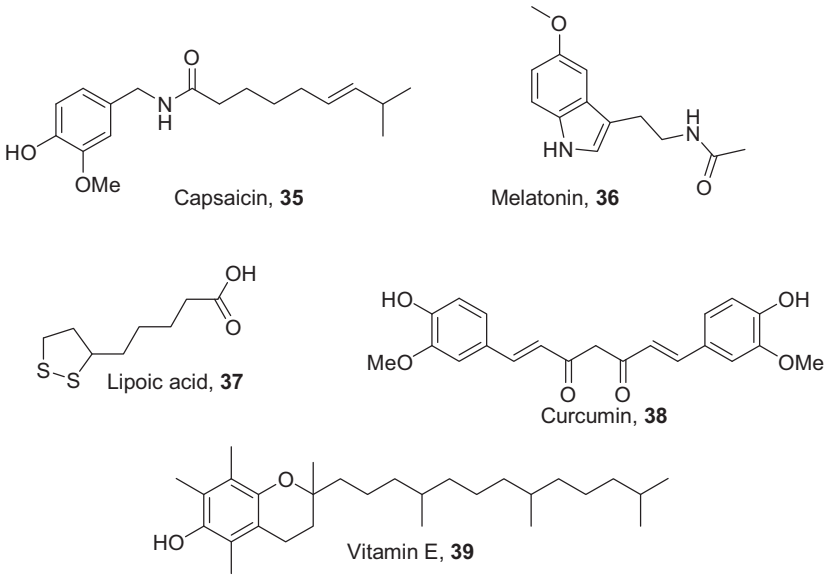


Figure 1.18 Structure of natural antioxidants **35**−**39**.

1.7.5 Use of multipotent compounds in AD.

In living organisms, viability and functionality is accomplished through a constant flow of information transmitted through interactions between the basic building blocks RNA, DNA, proteins and small molecules, which is represented as a biological network that, although inherent in its complexity, is bound with stability and equilibrium. Any change that irreversibly distorts the equilibrium in the network could result in pathological conditions and hence confer disease. It is increasingly becoming clear that for a drug to combat a disease effectively, it should target not a single but several building blocks in this biological network. Thus, there is growing increasing interest in the concept of network pharmacology, as opposed to the "one target-one disease" approach, which has dominated the drug discovery process in the lasts years, as an alternative drug discovery paradigm aimed at developing drugs or therapeutic interventions able to hit different biological targets (polypharmacology).[165−169]

Indeed, it is becoming increasingly apparent that diseases, in general, and complex diseases as AD, in particular, are not linear processes but rather complex networks of interconnected protein targets, with alternative, redundant, compensatory signaling pathways that render ineffective to the modulation of a single target of the pathological network by compounds as those anti-Alzheimer drug candidates that are recently failing in clinical trials.

There are three possible approaches to polypharmacology (Figure 1.19). Traditionally, clinicians have treated unresponsive patients by combining therapeutic mechanisms with cocktails of drugs (Scenario A, Figure 1.19). However the benefits of this approach are often

[165]Hopkins, A.L. *Nat. Chem. Biol*. **2008**, *4*, 682. [166]Hopkins, A.L. *Nat. Biotechnol*. **2007**, *25*, 1110. [167]Janga, S.C.; Tzakos, A. *Mol. BioSyst*. **2009**, *5*, 1536. [168]Bianchi, M.T. *Medical Hypotheses* **2010**, *74*, 297. [169]a) Morphy, R.; Rankovic, Z. *J. Med. Chem*. **2005**, *48*, 6523. b) Viayna, E.; Sola, I.; Di Pietro, O.; Muñoz-Torrero, D. *Curr. Med. Chem.* **2013**, *20*, in press.

compromised by poor patient compliance. Recently, there has been a move toward multicomponent drugs or fixed−dose combinations whereby two or more agents are coformulated in a single tablet to make dosing regimens simpler and thereby to improve patient compliance (Scenario B, Figure 1.19). Nevertheless, there are significant risks involved in the development of multicomponent drugs, such as high likelihood of drug−drug interactions, differences in the relative rates of metabolism between patients, which can produce highly complex pharmacokinetic / pharmacodynamic relationships, as well as possible formulation issues.[169]

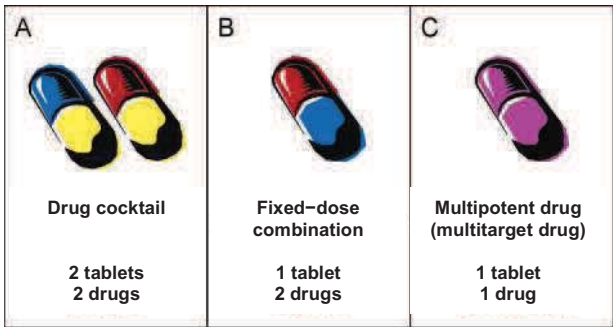


Figure 1.19 Three main scenarios for multitarget therapy.[169a]

An alternative strategy is to develop a single chemical entity that is able to modulate multiple targets simultaneously (Scenario C, Figure 1.19). Compared to multicomponent drugs, the multipotent drug approach has a profound risk-benefit profile as it is devoid of drug−drug interaction issues and benefits from the simplicity of development, patent and regulatory issues and clinical trial design of any new clinical entity. The main challenge associated to this approach is achieving properly balanced activities at the different targets. However, the increased complexity in the design and optimization of activity is shifted toward the earliest and therefore least expensive stages of the drug discovery process.[169]

In the particular case of anti-Alzheimer drug discovery, the polyetiological origin of AD renders untenable the notion that it can be efficiently managed through single-target drugs. In an attempt to fulfil the acute need for effective disease-modifying anti-Alzheimer therapies, research efforts are gradually shifting toward the rational design of drug candidates aimed at hitting multiple biological targets, preferentially those involved in the mechanisms that drive the progression of the disease.

[169] a)Morphy, R.; Rankovic, Z. *J. Med. Chem*. **2005**, *48*, 6523. b) Viayna, E.; Sola, I.; Di Pietro, O.; Muñoz-Torrero, D. *Curr. Med. Chem.* **2013**, *20*, in press.

2. OBJECTIVES

2.1 Preparation of racemic and enantiopure huprine Y and X.

Taking into account the excellent pharmacological profile exhibited by huprines Y and X and given the importance that dual binding site AChEIs have acquired in the last decade as a possible disease-modifying strategy for the treatment of AD, the main objective of this PhD Thesis work consisted in the preparation of different huprine-based dual binding site AChEIs. With this purpose, the first objective of this PhD Thesis work consisted of the preparation of racemic and enantiopure huprines Y and X in sufficient amount to synthesize the planned hybrids (Figure 2.1).

Figure 2.1 Structure of enantiopure huprine Y and X.

For the preparation of racemic huprine Y, (±)-**14**, the synthetic route that had been developed in our research group more than 10 years ago was to be used but including a few modifications in order to make its preparation more easy to scale up. Furthermore, the research group of Dr. Pierre-Yves Renard at the University of Rouen in France recently developed a shorter methodology for the preparation of huprines that might also be applied.[170]

For the syntheis of the planned huprine-based dual binding site AChEIs, preparation of enantiopure huprine Y and X was also required. The optimal procedure for the obtention of enantiopure huprines involves the chromatographic resolution of their racemic mixtures through medium pressure liquid chromatography (MPLC) using microcrystalline cellulose triacetate (15–25 μm) as the chiral stationary phase and 96% ethanol as the eluent.

2.2 Preparation of racemic and enantiopure donepezil−huprine hybrids.

Given the excellent pharmacological profile exhibited by the donepezil−tacrine hybrids **19**−**22a**,**b**, particularly those bearing an indane moiety, which showed a highly Aβ antiaggregating activity, making them promising disease-modifying anti-Alzheimer drug candidates, an analogous huprine-derived series of dual binding site AChEIs was envisioned, i.e. the donepezil−huprine hybrids (general structure **I**, Figure 2.2). These novel hybrids would consist of a unit of racemic or enantiopure huprine Y or X as the active site interacting unit and a donepezil-related 5,6-dimethoxy-2-[(4-piperidinyl)methyl]indane moiety as the peripheral site to mid-gorge interacting unit, connected through a short oligomethylene linker.

[170]Ronco, C.; Jean, L.; Renard, P.- Y. *Tetrahedron* **2010**, *66*, 7399.

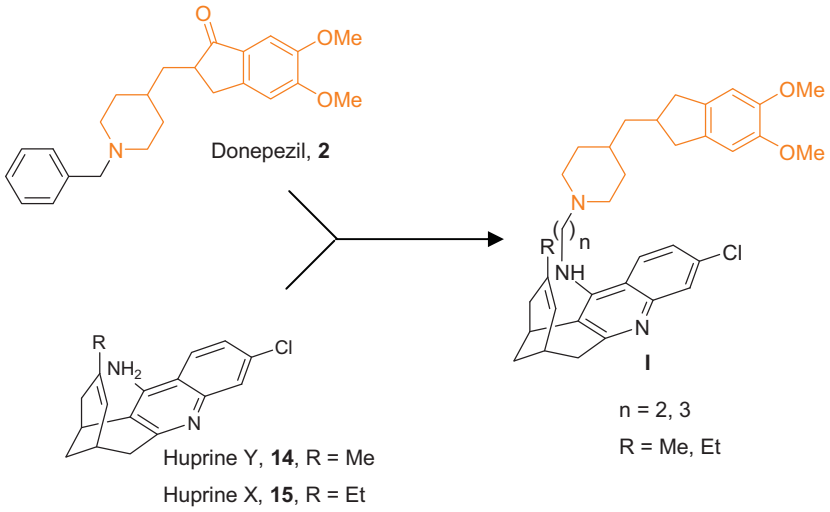


Figure 2.2 Design and general structure of the novel donepezil−huprine hybrids (**I**).

The synthesis of the donepezil−tacrine hybrids **19**−**22a**,**b** had been carried out by amination of the 4-chloroquinoline with the skeleton of tacrine with 2-aminoethanol or 3-amino-1-propanol, followed by mesylation and alkylation of the donepezil-derived piperidine.[137] In the frame of the PhD Thesis work of Dr. Carles Galdeano and the Experimental Master of Tània Gómez, an alternative synthetic pathway was set up, involving the alkylation of the donepezil-derived piperidine with 2-bromoethanol or 3-chloro-1-propanol, followed by transformation of the resulting alcohols to the corresponding chloroderivatives and final alkylation of tacrine. This new methodology was preferred for the preparation of donepezil−huprine hybrids as it did not require the tedious preparation of the 4-chloroquinoline with the skeleton of huprine.[162]

2.3 Preparation of racemic rhein−huprine hybrids.

Taking into account the complexity and polyetiological origin of AD and the increasingly idea that for a drug to combat such a complex disease effectively, it should simultaneously modulate not a single but several targets involved in the pathological network, a novel multipotent huprine-based series of compounds was envisioned. Dr. Eckhard Mandelkow from the Max-Plank Unit for Structural Molecular Biology in Hamburg recently reported the ability of different anthraquinones to inhibit tau protein aggregation and dissolve its filaments *in vitro* and in cells.[171] With this in mind, a family of rhein−huprine hybrids was designed. These compounds would consist of a unit of racemic huprine Y as the AChE active site interacting unit, a unit of the anthraquinone compound rhein, **40**, as the peripheral site interacting and putative tau antiaggregating unit connected through

[137] Camps, P.; Formosa, X.; Galdeano, C.; Gómez, T.; Muñoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia A.; Clos, M.V.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F.J. *J. Med. Chem.* **2008**, *51*, 3558. [162](a) Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M.V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855. (b) Muñoz-Torrero, D.; Camps, P.; Gómez, T.; Viayna, E.; Galdeano, C. *P201000016*, *PCT/ES2010/070862,* **2010**. [171]Pickhardt, M.; Gazova, M.; von Bergen, M.; Khlistunova, I.; Wang, Y.; Hascher, A.; Mandelkow, E.-M.; Biernat, J.; Mandelkow, E. *J. Biol. Chem.* **2005**, *5*, 3628.

an amide-containing oligomethylene or aromatic linker (general structure **II**, Figure 2.3).

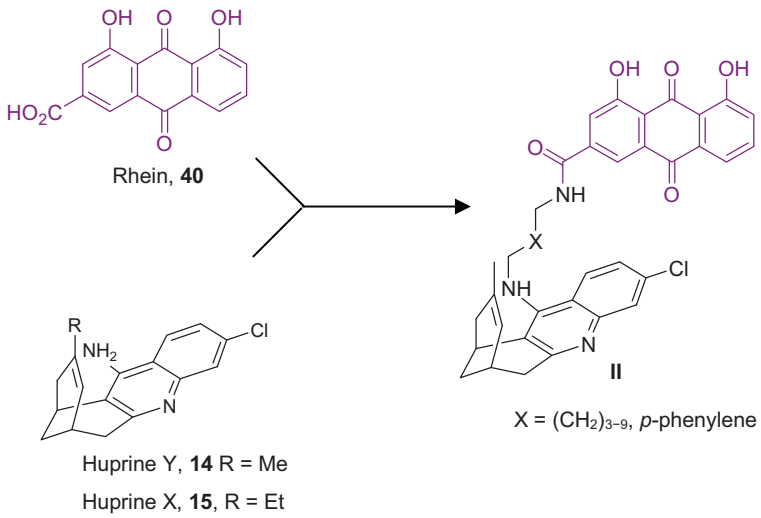


Figure 2.3 Design and general structure of the rhein−huprine hybrids (**II**).

For the preparation of the desired rhein−huprine hybrids, a new methodology was envisioned based on the alkylation of racemic huprine Y with an appropriate ω-bromoalkanenitrile or by reductive alkylation with *p*-cyanobenzaldehyde followed by reduction of the resulting cyanoalkylhuprines to the corresponding aminoalkylhuprines and final amide formation with rhein.

2.4 Preparation of racemic capsaicin−huprine hybrids.

In the context of the use of multipotent compounds for the treatment of AD, and given the considerable research that has been conducted on the use of antioxidants to slow down or reverse the pathology and course of AD, a series of capsaicin−huprine hybrids was designed as a novel family of multipotent dual binding site AChEIs. This new series would consist of a unit of racemic huprine Y, for the interaction with the active site of the enzyme, and a capsaicin-derived unit for the interaction with the peripheral site of AChE and as a putative antioxidant unit, connected through an amide-containing saturated or unsaturated oligomethylene or aromatic linker (general structure, **III**, Figure 2.4).

For the preparation of the saturated- and aromatic-tether containing capsaicin−huprine hybrids a similar methodology to the previously mentioned for the preparation of rhein−huprine hybrids was envisaged, consisting of the alkylation of racemic huprine Y with an appropriate ω-bromoalkanenitrile or by reductive alkylation with *p*-cyanobenzaldehyde but in this case followed by the hydrolysis of the cyanoalkylhuprines to the corresponding carboxylic acids, that were to be coupled with 4-hydroxy-3-methoxybenzylamine for the amide bond formation.

For the preparation of the unsaturated-tether containing hybrids a different synthetic approach

was envisioned based on a cross metathesis reaction between huprine- and capsaicin-derived olefins using different Grubbs and Grubbs-Hoveyda catalysts.

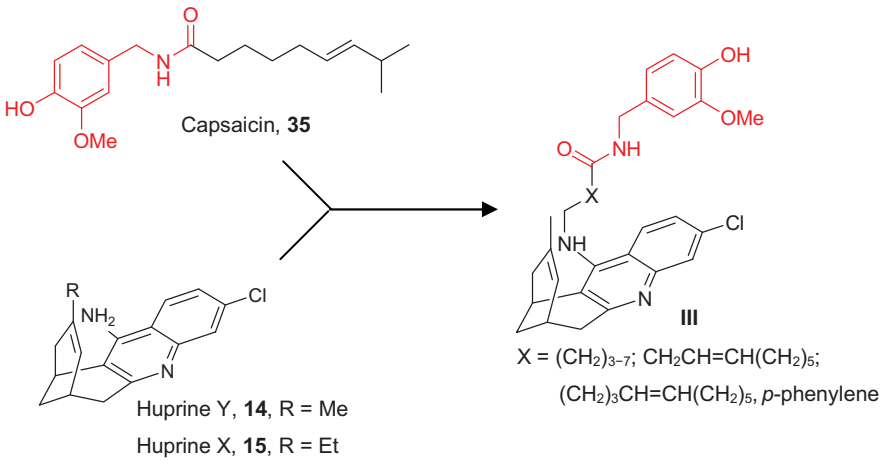


Figure 2.4 Structure of capsaicin, **35**, and general structure of the capsaicin−huprine hybrids (**III**).

2.5 Preparation of pyrido[3,2-c]quinolines as a novel family of peripheral site AChEIs.

Taking into account the excellent pharmacological profile exhibited by the pyrano[3,2-*c*]quinoline–6-chlorotacrine hybrids **23**–**32** (Figure 1.16), and given the potent Aβ-antiaggregating effect of the peripheral site AChEI propidium, **6** (Figure 2.5), a collaboration with Dr. Rodolfo Lavilla from the *Universitat de Barcelona* was established in order to design and synthesize a novel series of peripheral site AChEIs structurally related to propidium,**6**, but devoid of its permanent positive charge, in order to increase their ability to enter the CNS (general structure **IV**, Figure 2.5). The greater basicity of the quinoline N atom in the novel pyrido[3,2-*c*]quinolines relative to that of the pyrano[3,2-*c*]quinoline moiety of compounds **23**–**32** should enable the novel compounds to be developed on this PhD Thesis work to establish cation–π interactions, apart from π–π stacking interactions, with the tryptophan residue at peripheral site of the enzyme, thereby reinforcing its affinity (general structure **IV**, Figure 2.5).

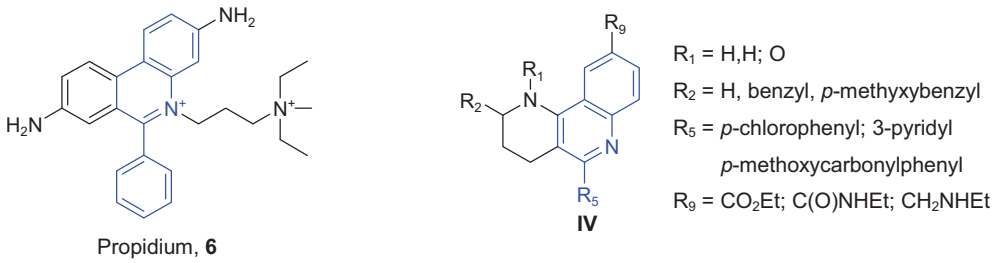
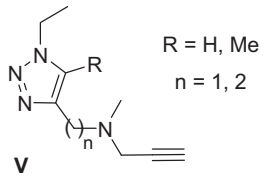


Figure 2.5 Structure of propidium, **6**, and general structure of the novel pyrido[3,2-*c*]quinolines (**IV**).

For the preparation of this novel series a Povarov-type multicomponenet reaction (MCR) followed by oxidation of the resulting diastereomeric mixture was envisioned. A second basic centre in the side chain at position 9 would be introduced by hydrolysis of the ethyl ester followed by amide formation by reaction with ethylamine and final reduction of the carbonyl group to afford the desired ethylaminomethyl group.

2.6 Preparation of MAO-B inhibitors.

Given the increasing interest in MAO-B inhibitors as a potential treatment for AD, the preparation of a novel series of triazoles containing a propargylamine group expected to selectively inhibit MAO-B was envisioned (general structure **V**, Figure 2.6). The novel compounds were rationally designed under the guidance of molecular modelling studies carried out by Daniel Botelho Silva of the group of Dr. F. Javier Luques.



V

Figure 2.6 General structure of the novel triazoles (V).

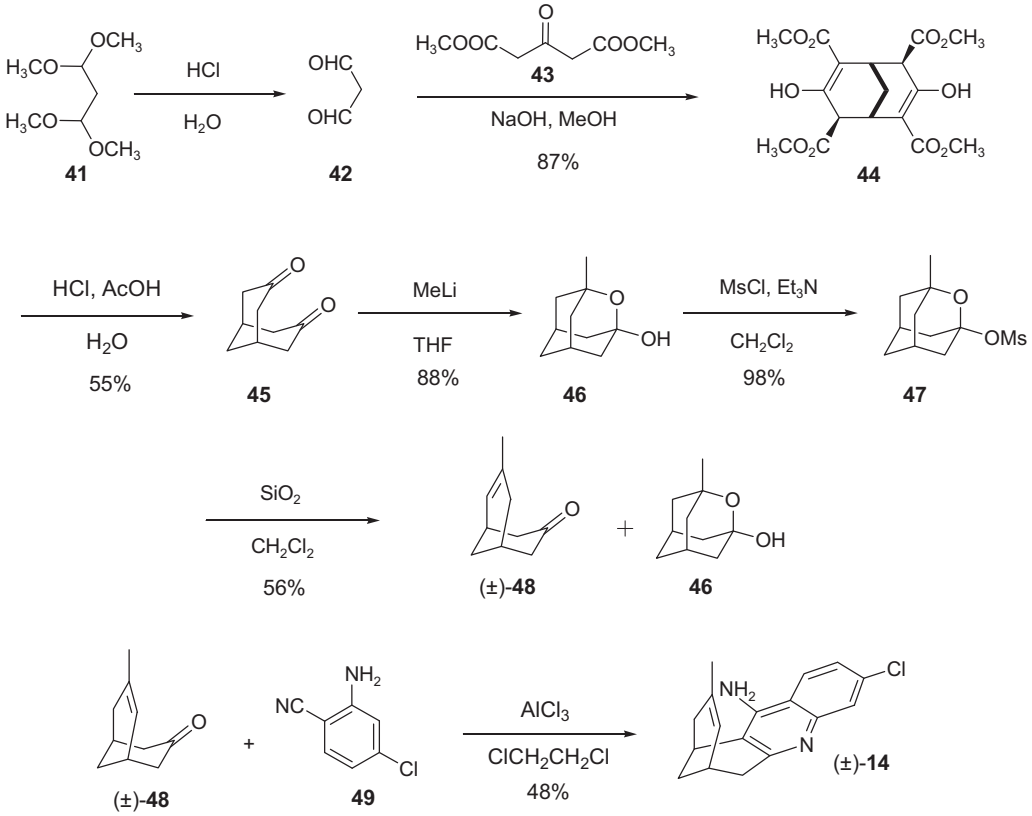
The preparation of the desired triazoles was envisioned through the prototypical cycloaddition click−reaction between an alkyne and an azide, followed by a number of standard organic synthesis transformations. The methyl substituent at position 5 of the triazole system, that according to the modelling studies should provide MAO-B versus MAO-A selectivity, would be introduced by lithiation of position 5 with *n*-BuLi followed by alkylation with MeI.

3. RESULTS AND DISCUSSION

3.1 Preparation of racemic and enantiopure huprine Y and X

3.1.1 Preparation of (±)-huprine Y, (±)-14.

As it has been already mentioned in the introduction, some years ago, Drs. Camps and Muñoz-Torrero developed a novel class of potent active site AChEIs called huprines, which turned out to be among the most potent reversible AChEIs. At the moment of starting this PhD thesis experimental work, there was enough (±)-huprine X, (±)-**15**, available in the laboratory, however, (±)-huprine Y, (±)-**14**, needed to be prepared in order to proceed with the synthesis of the planned huprine-based hybrids. For the preparation of (±)-**14** the synthetic route that had been developed some years ago in our group was followed (Scheme 3.1.1).[23] Nevertheless, a few changes were introduced in order to make the synthetic pathway easier to be scaled up.



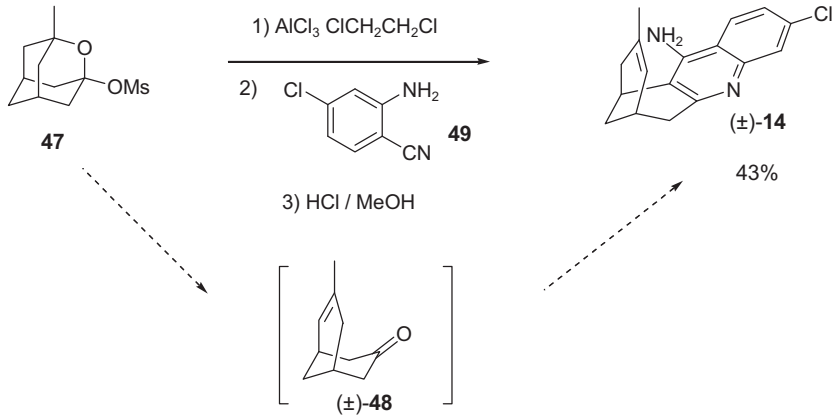
Scheme 3.1.1

The synthetic route consisted of an initial acidic hydrolysis of the diacetal protected form of malonaldehyde **41** in the presence of aqueous HCl followed by reaction with two equivalents of dimethyl acetonedicarboxylate, **43**, in the presence of NaOH in MeOH, to give tetraester **44** in 87% overall yield. Acidic hydrolysis followed by decarboxylation of tetraester **44** with HCl and AcOH, afforded diketone **45** in 55% yield. Addition of MeLi to diketone **45** afforded oxaadamantanol **46** in 88% yield, which was transformed into the corresponding mesylate, **47**, in 98% yield by treatment with MsCl in the presence of Et_3N.

[23]Camps, P.; El Achab, R.; Görbig, D.M.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.* **1999**, *42*, 3227.

When Drs. Camps and Muñoz-Torrero first reported the preparation of huprines,[121–124] the original synthetic procedure involved the fragmentation of mesylate **47** in the presence of SiO_2 to give a mixture of enone (±)-**48** and oxaadamantanol **46**, from which pure enone (±)-**48** could be isolated in 56% yield, after a tedious chromatographic separation. Final Friedländer condensation of (±)-**48** with 2-amino-4-chlorobenzonitrile, **49**, in the presence of $AlCl_3$, followed by a tedious silica gel column chromatographic purification, afforded (±)-huprine Y, (±)-**14**, in 48% yield (Scheme 3.1.1).

In 2010, the research group of Dr. Pierre-Yves Renard at the University of Rouen in France, developed a shorter methodology for the preparation of huprines that has been also applied in this PhD Thesis work.[170] This new methodology involved the one-pot transformation of mesylate **47** into (±)-**14**, without isolating or purifying enone (±)-**48**, thus avoiding one of the tedious column chromatography purifications, which was an important drawback when the synthesis had to be scaled up (Scheme 3.1.2). However, this methodology still required the final column chromatography purification of (±)-**14**. In order to eliminate any chromatographic purification and make this synthetic pathway even more easily scalable, in this PhD Thesis work a number of different conditions were tested to crystallize huprine Y. Nevertheless, all the conditions assayed, either did not work or afforded (±)-**14** in a very low yield. Finally, pure (±)-**14** could be isolated from the reaction crude by treatment of the crude with HCl / MeOH, in order to form (±)-**14**·HCl, followed by evaporation, treatment with hot EtOAc, filtration and washing of the resulting solid with an excess of hot EtOAc. These conditions afforded pure (±)-**14**·HCl in 43% yield and, for the first time, with no need of column chromatography purification procedures all along the synthetic pathway. Using this methodology, more than 100 g of racemic huprine Y have been synthesized in different batches in this PhD Thesis work.

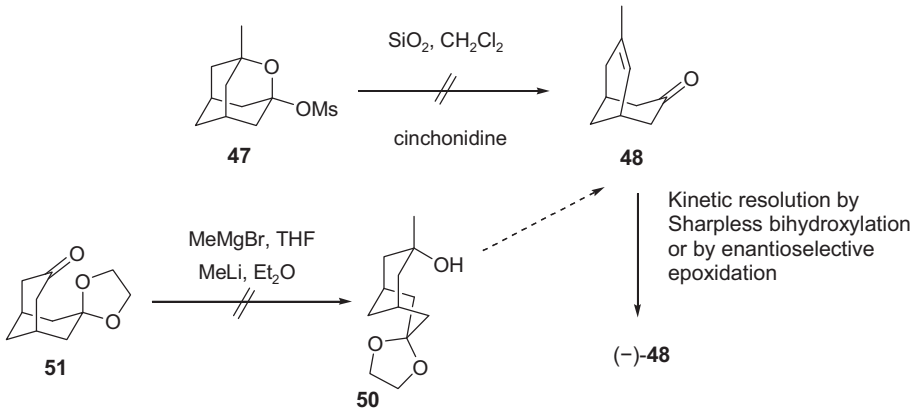


Scheme 3.1.2

[121]Badia, A.; Baños, J.E.; Camps, P.; Contreras, J.; Görbig, D.M.; Muñoz-Torrero, D.; Simon, M.; Vivas, N.M. *Bioorg. Med. Chem.* **1998**, *6*, 427. [122]Camps, P.; Contreras, J.; Font-Bardia, M.; Morral, J. ; Muñoz-Torrero, D.; Solans, X. *Tetrahedron: Asymmetry* **1998**, *9*, 835. [123]Camps, P.; El Achab, R.; Görbig, D.M.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.* **1999**, *42*, 3227. [124]Camps, P.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X. Orozco, M.; Luque, F.J. *J. Med. Chem.* **2000**, *43*, 4657. [170]Ronco, C.; Jean, L.; Renard, P.- Y. *Tetrahedron* **2010**, *66*, 7399.

3.1.2 Precedents in the preparation of enantiopure huprines.

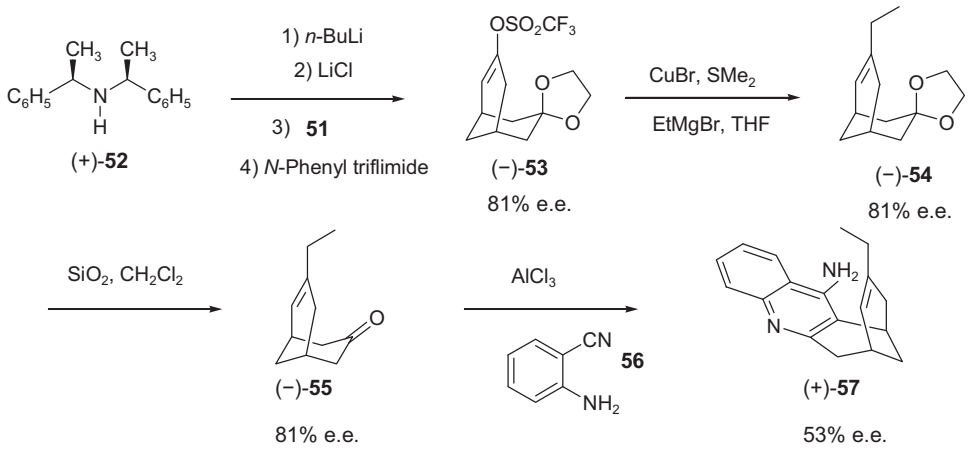
When Drs. Camps and Muñoz-Torrero first attempted the synthesis of huprine Y in enantiopure form, it was envisaged from the corresponding enantiopure enone. To this end, the enantioselective fragmentation of the corresponding mesylate, by adding cinchonidine to the reaction medium was attempted. However, fragmentation of **47** in the presence of cinchonidine did not take place. Another potentially enantioselective approach to enantiopure enone **48**, based on the enantioselective dehydration of alcohol **50**, failed because the addition of MeMgBr or MeLi to the monoketal **51** always gave unreacted starting material. Attempts to carry out the kinetic resolution of (±)-**48** by enantioselective bihydroxylation using the Sharpless procedure[172,173] or enantioselective epoxidation using the method of Jacobsen[174,175] were discouraging, recovering the starting ketone with very low specific rotation (Scheme 3.1.3).[122]



Scheme 3.1.3

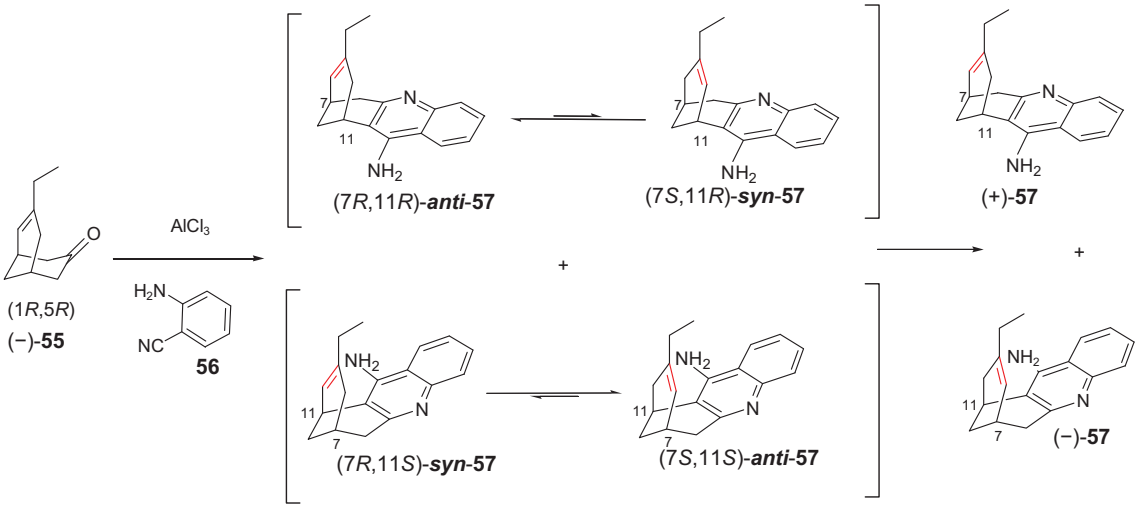
Finally, a new methodology was developed that allowed the preparation of enone **55** in enantiopure form. Monoketal **51** was reacted with the amide of (+)-bis[(*R*)-1-phenylethyl]amine, (+)-**52**, generated in situ by reaction of (+)-**52** with *n*-BuLi in the presence of LiCl, to give the desired enantiopure enolate that was captured with *N*-phenyl triflimide to give the corresponding enol triflate, (−)-**53**, in 64% yield and 81% enantiomeric excess (e.e.). Reaction of (−)-**53** with the cuprate generated *in situ* from EtMgBr gave (−)-**54** in 67% yield and 81% e.e. Hydrolysis of (−)-**54** to (−)-**55** was successfully carried out without racemization by reaction of (−)-**54** with SiO_2. Finally, (−)-**55** was reacted with 2-aminobenzonitrile, **56**, catalyzed by $AlCl_3$ in 1,2-dichloroethane under reflux giving the corresponding huprine, (+)-**57**, which was transformed into the corresponding hydrochloride and crystallized from MeOH / EtOAc to give (+)-**57**·HCl in 75% yield and 53% e.e., which on crystallization again from MeOH / EtOAc gave (+)-**57**·HCl in 26% yield and 99% e.e. (Scheme 3.1.4).

[122]Camps, P.; Contreras, J.; Font-Bardia, M.; Morral, J. ; Muñoz-Torrero, D.; Solans, X. *Tetrahedron: Asymmetry* **1998**, *9*, 835. [172]Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem*. **1992**, *57*, 2768. [173]Amberg, W.; Bennani, Y. L.; Chadha, R. K.; Crispino, G. A.; Davis, W. D.; Hartung, J.; Jeong, K.-S.; Ogino, Y.; Shibata, T.; Sharpless, K. B. *J. Org. Chem*. **1993**, *58*, 844. [174]Palucki, M.; McCormick, G.J.; Jacobsen, E.N. *Tetrahedron Lett*. **1995**, *36*, 5457. [175]Vander Velde S.L.; Jacobsen, E.N. *J. Org. Chem*. **1995**, *60*, 5380.



Scheme 3.1.4

As it can be seen from these data, partial racemization took place during the Friedländer reaction. This fact may be explained taking into account the reaction mechanism (Scheme 3.1.5). Reaction of enone (−)-**55** and 2-aminobenzonitrile, **56**, would give a mixture of two regioisomeric huprines: (7*R*,11*R*)-***anti*-57** and (7*R*,11*S*)-***syn*-57** which would be in equilibrium with the corresponding regioisomers (7*S*,11*R*)-***syn*-57** and (7*S*,11*S*)-***anti*-57**, respectively, the anti regioisomers being reasonably thermodynamically more stable.[123] Thus, formation of a little amount of *syn*-huprine (7*R*,11*S*)-***syn*-57**, together with the major *anti*-huprine (7*R*,11*R*)-***anti*-57**, would lead to the final formation of the *anti*-huprine (7*S*,11*S*)-***anti*-57**, thereby lowering the e.e. of the major (7*R*,11*R*)-***anti*-57**. (Scheme 3.1.5).[125]



Scheme 3.1.5

Although the successful preparation of pure (−)- and (+)-**57** samples had been achieved, in order to make more easily available these and related compounds, the resolution of the racemic mixture (±)-**57** by several procedures was attempted. Several attempts to selectively crystallize one or the other enantiomer from a MeOH / EtOAc solution of the racemic mixture, by seeding

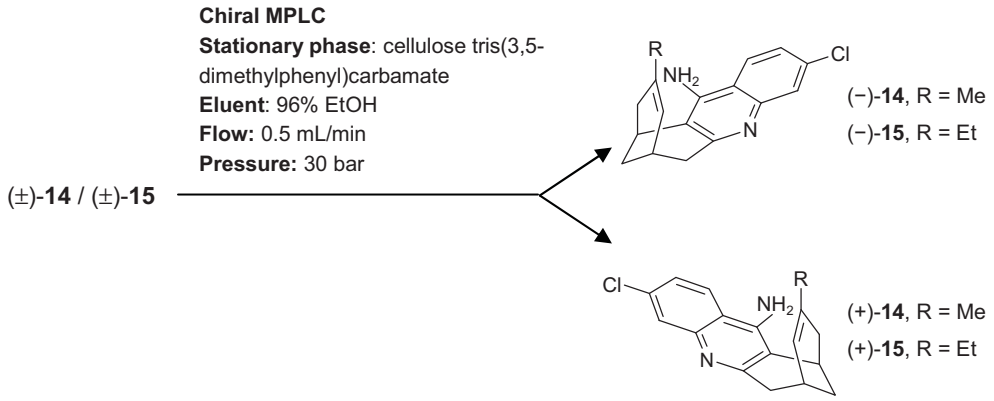
[123]Camps, P.; El Achab, R.; Görbig, D.M.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.* **1999**, *42*, 3227. [125] Camps, P.; Gómez, E.; Muñoz-Torrero, D.; Badia, A.; Vivas, N.M.; Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.* **2001**, *44*, 4733.

the crystallization by addition of crystals of one of the enantiomers were fruitless. Also, several attempts to carry out the resolution of (±)-**57** by crystallization of the salts derived from several enantiopure acids, such as (+)-camphorsulfonic and (+)-tartaric acid as well as (*S*)-naproxen in different solvents, gave products from which the liberated bases showed very low specific rotations.

An attempt to separate (±)-**57** by column chromatography using the chiral stationary phase cellulose tris(3,5-dimethylphenyl)carbamate supported on silica gel (SDS 40, 40–60 μm), was also fruitless. Fortunately, resolution of (±)-**57** on a preparative scale by MPLC using microcrystalline cellulose triacetate (15–25 μm) as the chiral stationary phase and 96% ethanol as the eluent, followed by analysis of the fractions by chiral HPLC, worked efficiently.[122]

3.1.3 Preparation of enantiopure huprine Y and X.

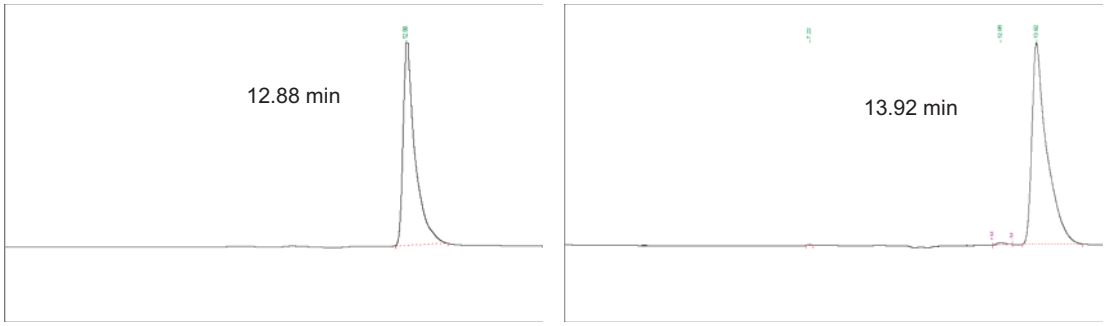
Given the fact that the most efficient methodology so far for the preparation of huprines in enantiopure form consisted of the chromatographic resolution of their racemic mixtures on a preparative scale by MPLC using microcrystalline cellulose triacetate (15–25 μm) as the chiral stationary phase, this was the methodology of choice in this PhD Thesis work (Scheme 3.1.6).



Scheme 3.1.6 Preparation of enantiopure huprine Y and X.

In order to determine the e.e. of the different fractions collected in the MPLC a new and faster methodology was developed consistent of the use of a Perkin Elmer *series 200* HPLC provided with a λ variable detector and a *Chiralcel OD* column containing cellulose tris(3,5-dimethylphenylcarbamate) as the chiral stationary phase, instead of the *Chiralcel OD-H* that had been previously used in our group.[122] The optimal HPLC conditions for the analysis of the MPLC fractions involved the use of a mixture of hexane/ EtOH / diethylamine (DEA) in a ratio of 75:25:0.5, a flow of 0.3 mL / min and a λ of 235 nm (Scheme 3.1.7).

[122]Camps, P.; Contreras, J.; Font-Bardia, M.; Morral, J. ; Muñoz-Torrero, D.; Solans, X. *Tetrahedron: Asymmetry* **1998**, *9*, 835.



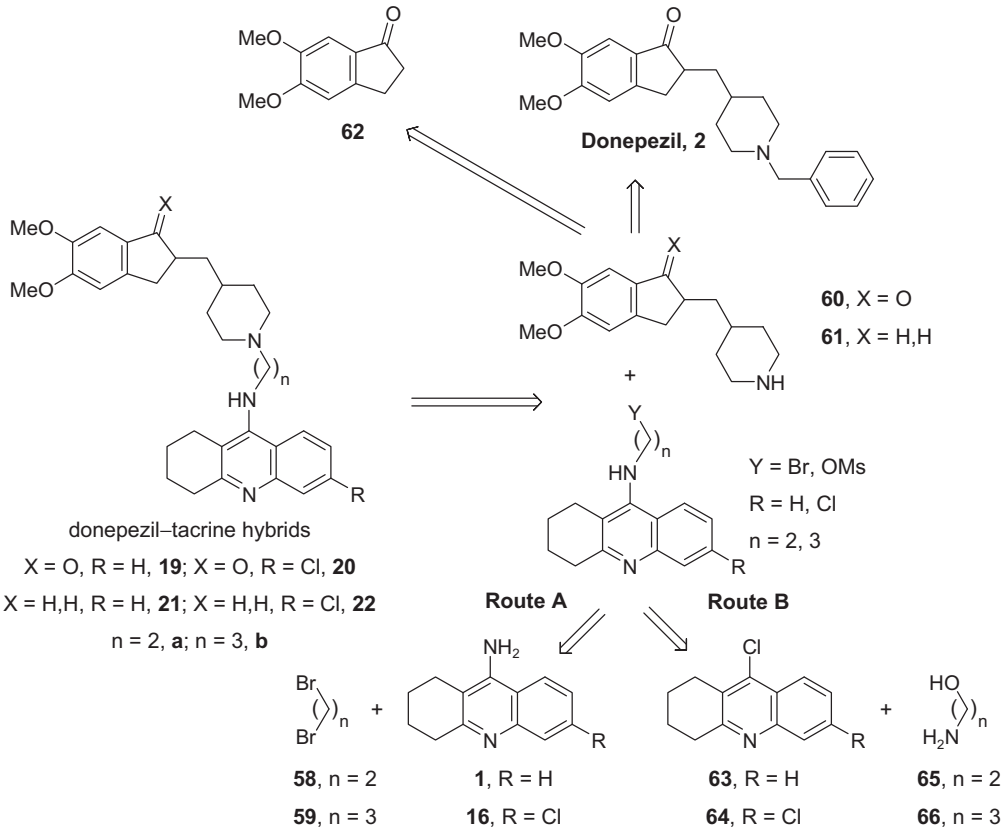
Scheme 3.1.7 Chromatograms of (–)-huprine Y, (–)-**14** (left) and (+)-huprine Y, (+)-**14** (right).

Using this methodology more than 2 g of each enantiomer of huprines Y and X were obtained and analyzed in this PhD Thesis work (300 mg of racemic huprine Y or X were loaded in each MPLC separation, affording approximately 110 mg of each enantiomer).

3. 2 Preparation of racemic and enantiopure donepezil−huprine hybrids

3.2.1 Precedents in the preparation of donepezil- and huprine-based hybrids in our research group.

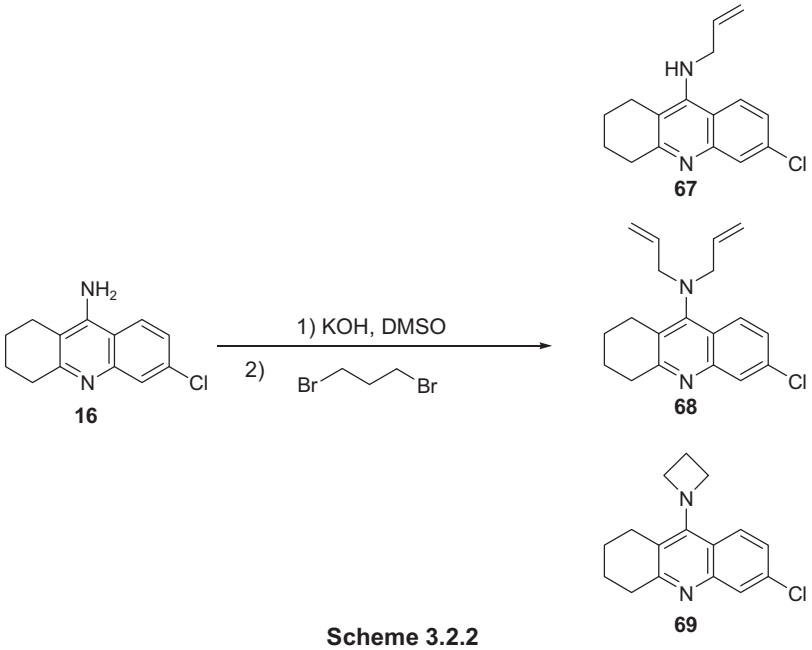
As it has been mentioned in the introduction, in 2008, Drs. Camps and Muñoz-Torrero developed a novel family of dual binding site AChEIs, namely the donepezil−tacrine hybrids **19**−**22a**,**b** (Figure 1.15), which exhibited an excellent pharmacological profile making them potential disease-modifying anti-Alzheimer drug candidates.[130,132,134−137] In the context of the PhD Thesis work of Dr. Xavier Formosa and the Experimental Master work of Michele Scarpellini, the preparation of donepezil−tacrine hybrids, **19**−**22a**,**b** had been envisioned following two different synthetic strategies (**routes A** and **B**, Scheme 3.2.1). **Route A** involved the alkylation of tacrine, **1**, or 6-chlorotacrine, **16**, with 1,2-dibromoethane, **58**, or 1,3-dibromopropane, **59**, followed by reaction of the resulting bromoalkyltacrine, or bromoalkylchlorotacrine, with piperidines **60** or **61** that could be easily accessed from donepezil, **2** or indanone, **62**. **Route B** involved an amination of 4-chloroquinolines **63** or **64**, with 2-aminoethanol, **65**, or 3-amino-1-propanol, **66**, followed by conversion of the alcohol into a mesylate and final reaction with piperidines **60** or **61** (Scheme 3.2.1).



Scheme 3.2.1

[130]Formosa X. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2006**. [132]Galdeano C. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2012**. [134]Scarpellini, M. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2006**. [135]Gómez, T. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2007**. [136]Camps, P.; Muñoz-Torrero, D.; Formosa, X.; Scarpellini, M. WO 2007/122274A1. [137]Camps, P.; Formosa, X.; Galdeano, C.; Gómez, T.; Muñoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia A.; Clos, M.V.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F.J. *J. Med. Chem.* **2008**, *51*, 3558.

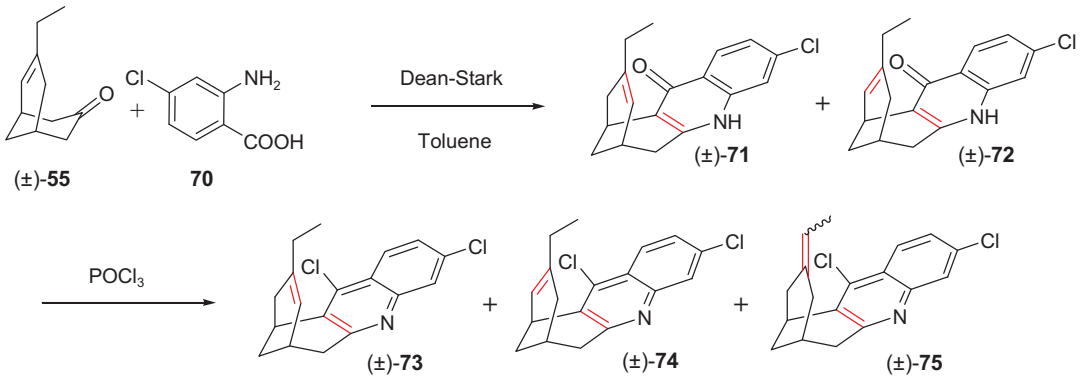
When **route A** was attempted in the context of the Experimental Master work of Michele Scarpellini, the initial alkylation of 6-chlorotacrine, **16**, with 1,3-dibromopropane provided a mixture of *N*-allychlorotacrine, **67**, *N*,*N*-diallylchlorotacrine, **68**, azetidine **69** and unreacted 6-chlorotacrine, **16**, in an approximate ratio 44:8:22:26 (determined by ^{1}H-NMR) (Scheme 3.2.2). When the alkylating agent was changed to 1,3-dichloropropane almost identical results were observed and when 3-chloro-1-propanol was assayed only unreacted 6-chlorotacine was recovered. Given these results, the preparation of donepezil−tacrine hybrids, **19**−**22a**,**b** was finally carried out following **route B**, which was based on the amination of the 4-chloroquinolines with the core of tacrine and 6-chlorotacrine **63** and **64** (Scheme 3.2.1).[134]



Scheme 3.2.2

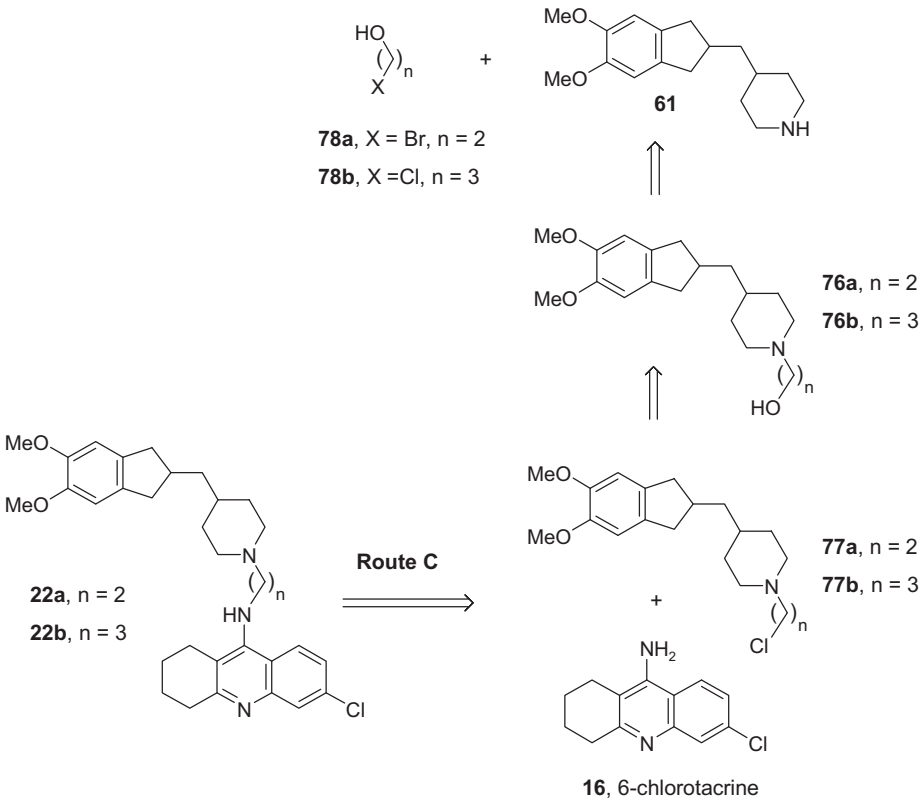
Preparation of the donepezil−huprine hybrids planned in this PhD Thesis work following **route B** required the synthesis of the 4-chloroquinolines with the skeleton of (±)-huprine Y and X followed by amination with 2-aminoethanol or 1-amino-3-propanol. Both reactions had been assayed in the context of the PhD Thesis work of Dr. Xavier Formosa for the preparation of huprine−tacrine hybrids, **17**−**18a**−**f** (Figure 1.14).[130] Unfortunately both reactions proceeded in very poor yields, especially the preparation of the 4-chloroquinoline with the core of (±)-huprine X, (±)-**73**, due to the formation of significant amounts of regioisomers and other isomeric byproducts, which constituted an inconvenience for the synthesis of the donepezil−huprine hybrids planned in the present PhD Thesis work (Scheme 3.2.3).

[130]Formosa X. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2006**. [134]Scarpellini, M. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2006**



Scheme 3.2.3

For this reason, and in order to improve the yields previously described for the preparation of donepezil−tacrine hybrids, in the context of the Experimental Master work of Tània Gómez a new methodology was developed (**route C**, Scheme 3.2.4), which consisted of the alkylation of piperidine **61** with an ω-haloalcohol to provide alcohols **76a**,**b** which were converted into the corresponding chloroderivatives **77a**,**b** that were reacted with 6-chlorotacrine, **16**, to give the desired hybrids **22a**,**b** (Scheme 3.2.4).[135]



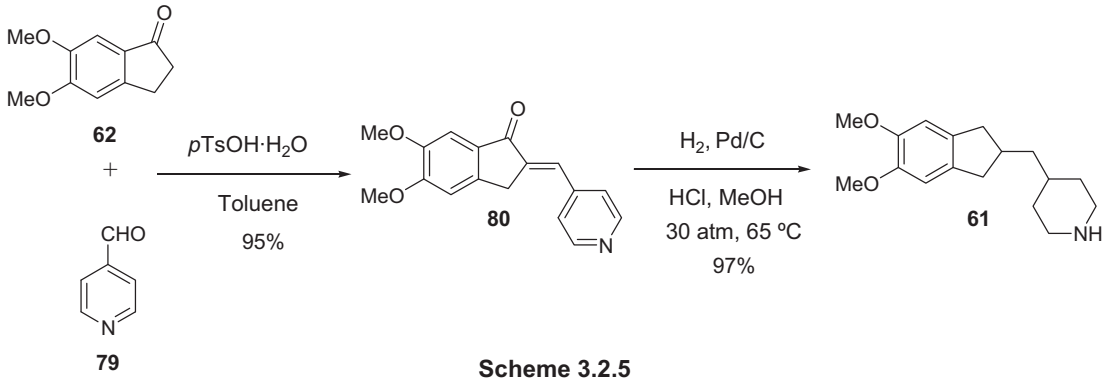
Scheme 3.2.4

[135]Gómez, T. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2007**.

3.2.2 Preparation of donepezil−huprine hybrids.

The main difference in the pharmacological profile of donepezil−tacrine hybrids bearing an indanone or an indane system lies in the fact that the former are more potent hAChE inhibitors (up to 4-fold), whereas the latter are more potent inhibitors of hAChE-induced Aβ aggregation (1.5-fold).[137] The most valuable activity of dual binding site AChEIs is the interference with Aβ, as this could endow the compound with a potential disease-modifying effect, while inhibition of the catalytic activity of AChE would be responsible for a symptomatic action. Therefore, in the present PhD Thesis work, the preparation of donepezil−huprine hybrids **81**–**82a**,**b** (Scheme 3.2.8), which consisted of a unit of racemic or enantiopure huprine X or Y as the active site interacting unit and a donepezil-related indane moiety for its interaction with the peripheral site, was envisioned with the hope of attaining a greater Aβ anti-aggregating effect, albeit perhaps at the expense of a somewhat lower inhibitory potency toward AChE. Moreover, the lack of stereogenic centers in the indane moiety avoids problems related to the formation and separation of diastereomeric mixtures when combined with the chiral huprine moiety; such problems would have been present if the chiral indanone had been used instead.

For the preparation of **81**–**82a**,**b** (Scheme 3.2.8), the synthetic pathway that had been already reported in the Experimental Master work of Tània Gómez for the preparation of **22a**,**b** (Scheme 3.2.4),[135] was applied. With this purpose, piperidine **61** was accessed by an aldolic condensation of 4-pyridinecarboxaldehyde, **79**, with 5,6-dimethoxy-1-indanone, **62**, under *p*-TsOH catalysis in toluene, heating under reflux, using a Dean-Stark equipment for 6 h, followed by hydrogenation of the resulting α,β-unsaturated ketone, **80**, in the presence of HCl and 5% Pd / C in MeOH at 30 atm and 65 ºC for 7 days (Scheme 3.2.5).

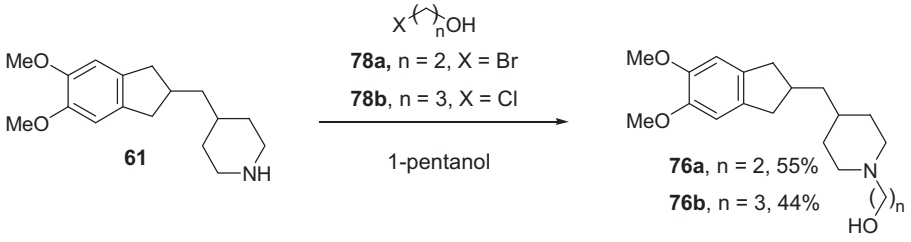


Scheme 3.2.5

Piperidine **61** was alkylated with 2-bromoethanol, **78a**, and 3-chloro-1-propanol, **78b**, in 1-pentanol, heating under reflux for 48 h to provide, after an acid−base extraction procedure to remove the excess of 1-pentanol and alkylating agent, followed by purification through silica gel

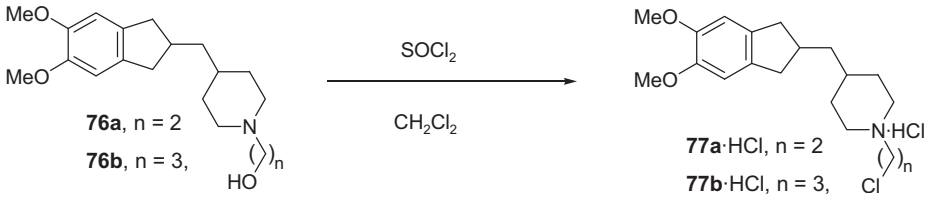
[135]Gómez, T. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2007**. [137]Camps, P.; Formosa, X.; Galdeano, C.; Gómez, T.; Muñoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia A.; Clos, M.V.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F.J. *J. Med. Chem.* **2008**, 51, 3558.

column chromatography, alcohols **76a** and **76b** in 55% and 44% yield, respectively (Scheme 3.2.6).



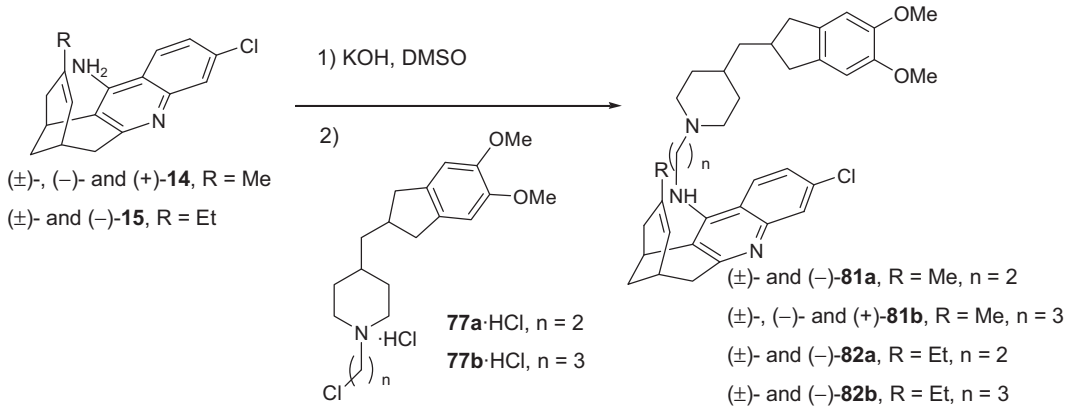
Scheme 3.2.6

Alcohols **76a**,**b** were transformed into the corresponding chloroderivatives **77a**,**b**·HCl by reaction with an excess of $SOCl_2$ in CH_2Cl_2 heating under reflux for 4 h, without alkaline aqueous workup to avoid the subsequent formation of cyclization by-products arising from intramolecular alkylation of the piperidine nitrogen atom (Scheme 3.2.7). Chloroderivatives **77a**,**b**·HCl were used as a crude directly in the next step.



Scheme 3.2.7

Finally, racemic or enantiopure huprines Y and X were alkylated with the crude chloroderivatives **77a** and **77b** using an excess of KOH in DMSO at room temperature (r. t.) for 5 days to afford racemic or enantiopure donepezil–huprine hybrids **81**–**82a**,**b** in moderate yields, after a tedious silica gel column chromatography purification. The novel hybrids **81**–**82a**,**b** were fully characterized as dihydrochlorides (Scheme 3.2.8).[162]



Scheme 3.2.8

[162](a) Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M.V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855. (b) Muñoz-Torrero, D.; Camps, P.; Gómez, T.; Viayna, E.; Galdeano, C. *P201000016*, *PCT/ES2010/070862*, **2010**.

3.2.3 Pharmacological evaluation of donepezil−huprine hybrids.

3.2.3.1 Activity toward cholinesterases.

Drs. Albert Badia and M. Victòria Clos from the *Universitat Autònoma de Barcelona* determined the AChE inhibitory activity of hybrids **81**–**82a**,**b** using the method of Ellman *et al.*[176] on recombinant hAChE (Table 3.2.1). The novel donepezil–huprine hybrids turned out to be potent inhibitors of hAChE, exhibiting IC_{50} values in the low nanomolar range in most cases. Some clear structure–activity relationship trends are evident from the data in Table 3.2.1.

As it was expected, the levorotatory enantiomers of these hybrids, bearing a (7*S*,11*S*)-huprine moiety, are the eutomers, they being roughly 2-fold more potent than the corresponding racemic compounds and much more potent than the dextrorotatory enantiomers [(−)-**81b** is 19-fold more potent than (+)-**81b**]. Moreover, the hAChE inhibitory potency of the hybrids increases with the presence of a trimethylene linker, as the corresponding compounds are 3–8-fold more potent than their ethylene-linked counterparts. Finally, the inhibitory activity also increases with the presence of a methyl substituent at position 9 of the huprine moiety (huprine Y derivatives are ~1.5–3-fold more potent than their huprine X-based counterparts). This latter trend was a priori unexpected, because the reverse trend is found for the parent compounds [(−)-huprine Y is 1.6-fold less potent than (−)-huprine X in the inhibition of the human recombinant AChE; they are equipotent as inhibitors of human erythrocytes AChE]. Overall, the most potent hAChEI of the series is (−)-**81b**, which turned out to be 8-fold more potent than donepezil but, contrary to our expectations, 6-fold less potent than the parent (−)-huprine Y. Also, the most potent huprine X-based hybrid, namely (−)-**82b**, turned out to be 14-fold less potent than the parent (−)-huprine X, (−)-**15**.

The inhibitory activity of the most potent hybrids (−)-**81b** and (−)-**82b** was in the same range as that found for the most potent compounds of the structurally related indane-bearing donepezil–tacrine hybrids **21**−**22a**,**b** (Scheme 3.2.1). Nevertheless, caution should be taken in making a strict comparison because the AChE source was different, as donepezil−tacrine hybrids were evaluated using human erythrocyte AChE instead of human recombinant AChE.[162]

Recent evidence has shown that ihibition of BChE might be valuable in the search for anti-Alzheimer agents.[32−35] Consequently, Drs. Badia and Clos also determined the inhibitory activity toward serum hBChE by the method of Ellman *et al.*[176] (Table 3.2.1). Huprines are selective for

[32]Venneri, A.; Shanks, M.F.; Staff, R.T.; Pestell, S.J.; Forbes, K.E.; Gemmell, H.G.; Murray, A.D. *Neuroreport* **2002**, *13*, 83. [33]Venneri, A.; McGeown, W.J.; Shanks, M.F. *Neuroreport* **2005**, *16*, 107. [34]Veneri, A.; Lane, R. *Neuroreport* **2009**, *20*, 285. [35]Shanks, M.; Kivipelto, M.; Bullok, R.; Lane, R. *Curr. Med. Res. Opin*. **2009**, *25*, 2439. [162](a) Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M.V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855. (b) Muñoz-Torrero, D.; Camps, P.; Gómez, T.; Viayna, E.; Galdeano, C. *P201000016*, *PCT/ES2010/070862*, **2010**. [176]Ellman, G.L.; Courtney, K.D.; Andres, B. Jr.; Featherstone, R.M. *Biochem. Pharmacol.* **1961**, *7*, 88.

hAChE versus hBChE inhibition. The presence of the chlorine atom at the huprine unit, which is partly responsible for the high hAChE inhibitory activity of 4-aminoquinoline derivatives,[127] becomes detrimental for hBChE inhibition. Steric hindrance due to the proximity of the chlorine atom to the terminal methyl group of Met437 in the hBChE active site seems to account for the detrimental influence of this substituent on the hBChE inhibitory activity relative to unsubstituted 4-aminoquinolines such as tacrine,[177] which is a 5-fold more potent inhibitor toward hBChE than hAChE.[137] Nevertheless, as huprines themselves, donepezil−huprine hybrids can be considered moderately potent inhibitors of hBChE, with IC_{50} values in the nanomolar range.

Table 3.2.1 AChE and BChE inhibitory activities of the hydrochlorides of donepezil, racemic and enantiopure huprines Y and X, and the dihydrochlorides of racemic and enantiopure donepezil−huprine hybrids **81a**,**b** and **82a**,**b**.[a]

Compound	IC_{50} (nM) hAChE	IC_{50} (nM) hBChE
(±)-**81a**·2HCl	18.1 ± 1.2	336 ± 21
(−)-**81a**·2HCl	13.0 ± 0.1	303 ± 12
(±)-**81b**·2HCl	5.37 ± 0.3	88 ± 2.3
(−)-**81b**·2HCl	2.61 ± 0.2	349 ± 20
(+)-**81b**·2HCl	49.9 ± 5.1	95 ± 5.3
(±)-**82a**·2HCl	48.6 ± 5.2	174 ± 9.4
(−)-**82a**·2HCl	28.6 ± 2.3	419 ± 17
(±)-**82b**·2HCl	6.32 ± 0.1	61 ± 3.2
(−)-**82b**·2HCl	3.85 ± 0.2	194 ± 9.3
(±)-**donepezil**·HCl	21.4 ± 2.3	7273 ± 621
(±)-**huprine Y**·HCl	0.61 ± 0.03	236 ± 44
(−)-**huprine Y**·HCl	0.43 ± 0.03	247 ± 18
(+)-**huprine Y**·HCl	13.6 ± 1.5	153 ± 31
(±)-**huprine X**·HCl	0.67 ± 0.02	15.8 ± 2.4
(−)-**huprine X**·HCl	0.27 ± 0.02	159 ± 10
(+)-**huprine X**·HCl	6.30 ± 0.5	58.3 ± 5.9

[a]Values are expressed as the mean ± SEM of at least four experiments; IC_{50}: 50% inhibitory concentration of human recombinant or human serum BChE activity.

Following the same trend found in the parent huprines, the hBChE inhibitory activity of the novel hybrids increases with the presence of an ethyl substituent at position 9 of the huprine

[127]Dvir, H.; Wong, D.M.; Harel, M.; Barril, X.; Orozco, M.; Luque, F.J.; Muñoz-Torrero, D.; Camps, P.; Rosenberry, T.L.; Silman, I.; Sussman, J.L. *Biochemistry* **2002**, *41*, 2970.[137]Camps, P.; Formosa, X.; Galdeano, C.; Gómez, T.; Muñoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia A.; Clos, M.V.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F.J. *J. Med. Chem.* **2008**, *51*, 3558. [177]Muñoz-Ruiz, P.; Rubio, L.; García-Palomero, E.; Dorronsoro, I.; Del Monte-Millán, M.; Valenzuela, R.; Usán, P.; de Austria, C.; Bartolini, M.; Andrisano, V.; Bidon-Chanal, A.; Orozco, M.; Luque, F.J.; Medina, M.; Martínez, A. *J. Med. Chem.* **2005**, *48*, 7223.

moiety (huprine X derivatives being 1.5–2-fold more potent than their huprine Y based counterparts) and with the presence of a dextrorotatory huprine moiety, with the (7*R*,11*R*) configuration. The tether length leading to higher activity turned out to be that corresponding to a trimethylene linker, the corresponding hybrids being 1.2–4-fold more potent than their ethylene-linked counterparts. Overall, the most potent hBChEI among these hybrids was (±)-**82b**, which is 119-fold more potent than donepezil, but 4-fold less potent than the parent (±)-huprine X, (±)-**15**. Slightly lower inhibitory potencies were found for (±)-**81b** and (+)-**81b**.[162]

3.2.3.2 Activity toward Aβ aggregation.

Regarding the potential interference of the new hybrids with Aβ, Drs. Vincenza Andrisano and Manuela Bartolini from the *Università di Bologna* tested their ability to inhibit AChE-induced aggregation of $A\beta_{1-40}$, by using a thioflavin T fluorescence method.[95] The Aβ anti-aggregating effect of racemic and enantiopure huprines Y and X was also determined, while that of donepezil was already described.[95] The new hybrids significantly inhibit, at a concentration of 100 μM, hAChE-induced Aβ aggregation, with percentages of inhibition ranging from 25% to 50% (Table 3.2.2). In all cases, the inhibitory activity was higher than that of the dual binding site AChEI donepezil (22%), likely as a result of a better dual site binding to AChE, and in most cases also higher than those of the parent huprines, which exhibited remarkable inhibitory activity (12–37%).

Although (−)-huprine X has been shown to bind tightly to the AChE active site[127] kinetic studies have demonstrated that this compound interferes with the binding of the peripheral site AChEI propidium to AChE.[126] The binding geometry and added molecular volume of huprines, when bound to the active site, could explain the decreased affinity of peripheral site ligands such as propidium,[126] as well as Aβ. The AChE-induced Aβ aggregation inhibitory activity of the novel hybrids **81**–**82a**,**b** slightly increased with the presence of a trimethylene linker (up to 1.6-fold relative to the ethylene-linked counterparts) and a unit of racemic huprine, while the presence of a methyl or ethyl substituent at position 9 of the huprine moiety had essentially no effect on this activity. Overall, the most potent compounds were the trimethylene linked hybrids (±)-**81b**, (−)-**81b**, (±)-**82b**, and (−)-**82b**, as well as the ethylene-linked hybrid (±)-**81a**, all of which showed percentages of inhibition between 40% and 50%, which are in the same range as those of the structurally related donepezil−tacrine hybrids **21**–**22a**,**b** (Scheme 3.2.1 and Figure 1.15).

[95]Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. *Biochem. Pharmacol.* **2003**, *65*, 407. [126]Camps, P.; Cusack, B.; Mallender, W.D.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Rosenberry, T.L. *Mol. Pharmacol.* **2000**, *57*, 409. [127]Dvir, H.; Wong, D.M.; Harel, M.; Barril, X.; Orozco, M.; Luque, F.J.; Muñoz-Torrero, D.; Camps, P.; Rosenberry, T.L.; Silman, I.; Sussman, J.L. *Biochemistry* **2002**, *41*, 2970. [162](a) Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M.V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855. (b) Muñoz-Torrero, D.; Camps, P.; Gómez, T.; Viayna, E.; Galdeano, C. *P201000016*, *PCT/ES2010/070862*, **2010**.

Besides, given the fact that a number of dual binding site AChE inhibitors have been found to exhibit significant inhibitory activity toward Aβ self-aggregation,[133,137,140] Drs. Bartolini and Andrisano also determined the inhibitory activity of these hybrids toward the self-induced Aβ aggregation. The new donepezil–huprine hybrids significantly inhibit the self-induced Aβ aggregation when tested at a concentration 5-fold lower than that of Aβ, with percentages of inhibition ranging from 16% to 30% (Table 3.2.2). In all cases, this effect was higher than that of donepezil (<5%) and (−)- and (+)-huprine Y (10% and 13%, respectively), while similar to that of racemic and enantiopure huprine X (23–25%). Contrary to the trend found for the parent huprines, the Aβ anti-aggregating action of the novel hybrids slightly increased with the presence of a methyl group at position 9 of the huprine moiety (1.2–1.6-fold relative to the ethyl-substituted counterparts). Also, this activity slightly increased with the presence of a trimethylene linker (1.1–1.8-fold relative to the ethylene-linked counterparts), while it generally remained insensitive to the configuration of the huprine moiety.

The strongest inhibitors of spontaneous Aβ aggregation among known dual binding site AChEIs show potencies in the low micromolar range. The expected IC_{50} values for most of the hybrids of this family must be clearly below 50 μM, so that they could be considered moderate inhibitors of $A\beta_{1-42}$ self-aggregation (Table 3.2.2).

3.2.3.3 Activity toward Aβ formation.

BACE-1 constitutes one of the main therapeutic targets for the design of disease-modifying anti-Alzheimer drug candidates, as its inhibition would reduce the formation of Aβ, thus, blocking the neurodegenerative cascade associated to AD from the beginning. For this reason, and given the fact that some dual binding site AChEIs had exhibited ability to inhibit BACE-1,[140] in a collaboration with Dr. Francesca Mancini from the *Università di Bologa*, BACE-1 inhibitory activity for donepezil−huprine hybrids was screened at a single concentration (5 μM) by a fluorimetric assay, which is considered predictive of activity in cell culture.[178] All of the hybrids exhibited significant BACE-1 inhibition (12–31%). The substitution pattern leading to greater BACE-1 inhibition involved the presence of a methyl substituent at position 9 of the huprine moiety (1.3–2.1-fold more potent than the ethyl-substituted counterparts) and a trimethylene linker (1.1–2-fold more potent than the ethylene-linked counterparts), while a clear trend regarding the configuration of the huprine moiety was not found. The most potent BACE-1

[133]Galdeano, C.; Viayna, E.; Sola I.; Formosa, X.; Camps, P.; Badia, A.; Clos, M.V.; Relat, J.; Ratia, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Salmona, M.; Minguillón C.; González-Muñoz, G.C.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Muñoz-Torrero, D. *J. Med. Chem.* **2012**, *55*, 661. [137]Camps, P.; Formosa, X.; Galdeano, C.; Gómez, T.; Muñoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia A.; Clos, M.V.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F.J. *J. Med. Chem.* **2008**, *51*, 3558. [140]Camps, P.; Formosa, X.; Galdeano, C.; Muñoz-Torrero, D.; Ramírez, L.; Gómez, E.; Isambert, N.; Lavilla, R.; Badia, A.; Clos, M.V.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Huertas, O.; Dafni, T.; Luque, F.J. *J. Med. Chem.* **2009**, *52*, 5365. [178]Hanessian, S.; Yun, H.; Hou, Y.; Yang, G.; Bayrakdarian, M.; Therrien, E.; Moitessier, N.; Roggo, S.: Veenstra, S.; Tintelnot-Blomley, M.; Rondeau, J. M.; Ostermeier, C.; Strauss, A.; Ramage, P.; Paganetti, P.; Neumann, U.; Betschart, C. *J. Med. Chem.* **2005**, *48*, 5175.

inhibitors, i.e. hybrid (±)-**81b**, (−)-**81b**, and (−)-**81a**, turned out to be more potent inhibitors than the parent huprines, and roughly equipotent to donepezil (IC_{50} = 11.3 ± 0.9 μM) when evaluated under the same assay conditions. Indeed, hybrid (−)-**81b** exhibited an IC_{50} value of 11.0 ± 0.59 μM, thus constituting a moderate BACE-1 inhibitor (Table 3.2.2).[162]

Table 3.2.2 Aβ aggregation and BACE-1 inhibitory activities of the hydrochlorides of donepezil, racemic and enantiopure huprines Y and X, and the dihydrochlorides of racemic and enantiopure donepezil−huprine hybrids **81a**,**b** and **82a**,**b**.[a]

Compound	AChE-induced $A\beta_{1-40}$ aggregation (%)[b]	$A\beta_{1-42}$ self induecced aggregation (%)[c]	BACE-1 (%)[d]
(±)-**81a**·2HCl	44.2 ± 1.0	26.0 ± 2.0	14.3 ± 7.6[e]
(−)-**81a**·2HCl	31.1 ± 0.4	16.3 ± 0.5	24.6 ± 3.0[e]
(±)-**81b**·2HCl	44.5 ± 1.8	28.5 ± 0.4	29.1 ± 2.9[e]
(−)-**81b**·2HCl	41.5 ± 2.4	29.0 ± 2.0	30.8 ± 4.1[e,f]
(+)-**81b**·2HCl	24.9 ± 2.4	29.9 ± 1.3	13.1 ± 4.7[e]
(±)-**82a**·2HCl	35.7 ± 3.2	16.3 ± 0.5	19.2 ± 2.2[e]
(−)-**82a**·2HCl	27.3 ± 2.6	20.9 ± 0.3	12.5 ± 0.7[e]
(±)-**82b**·2HCl	49.8 ± 0.4	20.9 ± 0.3	18.0 ± 9.0[e]
(−)-**82b**·2HCl	43.8 ± 0.8	23.6 ± 4.0	14.9 ± 3.9[e]
(±)-**donepezil**·HCl	22[g]	<5[h]	[i]
(±)-**huprine Y**·HCl	37.5 ± 0.5	nd[j]	nd[j]
(−)-**huprine Y**·HCl	25.1 ± 4.9	10.2 ± 6.5	14.0 ± 0.1[k]
(+)-**huprine Y**·HCl	11.6 ± 1.7	13.2 ± 1.9	13.6 ± 2.3[k]
(±)-**huprine X**·HCl	34.1 ± 0.2	25.5 ± 2.1	15.7 ± 3.8[k]
(−)-**huprine X**·HCl	28.9 ± 0.2	25.4 ± 1.2	21.8 ± 7.2[k]
(+)-**huprine X**·HCl	21.5 ± 1.4	22.9 ± 0.4	16.7 ± 0.6[k]

[a]Values are expressed as the mean ± SEM from two independent measurements, each performed in duplicate. [b]Inhibitor concentration: 100 μM. [c]Inhibitor concentration: 10 μM ([Aβ] / [I] = 5:1). [d]Inhibitor concentration: 5 μM. [e]Human recombinant BACE-1 (Sigma), substrate M-2420 (Bachem). [f]IC_{50} = 11.0 ±0.59 μM. [g]Data from Ref.[95] [h]Data from Ref.[99] [i]IC_{50} = 11.3 ± 0.9 μM. [j]Not determined. [k]Human recombinant BACE-1 (Invitrogen), substrate Panvera Peptide (Invitrogen).

3.2.3.4 In vitro BBB permeation assay.

Successful CNS drugs must overcome the biological hurdle of BBB penetration. To predict the brain penetration of the donepezil–huprine hybrids described herein, in a collaboration with

[95]Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. *Biochem. Pharmacol.* **2003**, *65*, 407. [99]Bolognesi, M.L. ; Cavalli, A. ; Valgimigli, L.; Bartolini, M.; Rosini, M.; Andrisano, V.; Recanatini, M.; Melchiorre, C. *J. Med. Chem*. **2007**, *50*, 6446. [162](a) Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M.V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855. (b) Muñoz-Torrero, D.; Camps, P.; Gómez, T.; Viayna, E.; Galdeano, C. *P201000016*, *PCT/ES2010/070862*, **2010**.

Dr. Maria Isabel Rodríguez-Franco from Instituto de Química Médica (CSIC) in Madrid, the widely known PAMPA-BBB assay[179] was successfully applied to our compounds.[162] The *in vitro* permeability (P_e) of racemic **81**–**82a**,**b**, and of the parent huprines Y and X and donepezil through a lipid extract of porcine brain were determined. At each solvent mixture, assay validation was made by comparing the experimental permeability with the reported values of 15 commercial drugs, which gave a good linear correlation. Taking into account the limits established by Di *et al.* for BBB permeation,[179] it was established that compounds with permeability values greater than 7.8×10^{-6} cms^{-1} (when PBS / EtOH 80:20 was used as solvent) or 9.7×10^{-6} cms^{-1} (when PBS / EtOH 70:30 was used as solvent) should cross the BBB. All tested hybrids, as well as the parent huprines and donepezil, showed permeability values over the above limits (Table 3.2.3), pointing out that they could cross the BBB and reach their pharmacological targets located in the CNS, as had been already confirmed for huprines in *in vivo* and *ex vivo* studies,[124] and, of course, for donepezil during clinical use.

Table 3.2.3 Permeability results from the PAMPA-BBB assay for the racemic donepezil–huprine hybrids, huprines Y and X, and donepezil, with their predicted penetration into the CNS.

Compound	P_e (10^{-6} cm s^{-1})[a]	Prediction
(±)-**81a**·2HCl[b]	15.2 ± 0.1	CNS+
(±)-**81b**·2HCl[b]	11.4 ± 0.2	CNS+
(±)-**82a**·2HCl[b]	21.3 ± 0.4	CNS+
(±)-**82b**·2HCl[c]	17.1 ± 0.4	CNS+
(±)-**huprine Y**·HCl[c]	18.2 ± 0.1	CNS+
(±)-**huprine X**·HCl[c]	17.8 ± 0.1	CNS+
(±)-**donepezil**·HCl[c]	25.2 ± 0.2	CNS+

[a]Values are expressed as the mean ± SD of three independent experiments. [b]Compound dissolved in PBS / EtOH 70:30. [c]Compound dissolved in PBS / EtOH 80:20.

3.2.3.5 Ex vivo pharmacological evaluation.

Given the excellent pharmacological profile exhibited *in vitro* by donepezil−huprine hybrids, especially by (−)-**81b**, which showed an IC_{50} value of 2.61 nM for hAChE inhibition, a 41.5% inhibition of the AChE-induced Aβ aggregation effect at 100 μM concentration of inhibitor and an IC_{50} value of 11.0 μM toward BACE-1, among other activities, this novel family of multipotent compounds was protected in a patent of the *Universitat de Barcelona,* ES P201000016, *Compuestos multifuncionales modificadores de la enfermedad de Alzheimer para el tratamiento*

[124]Camps, P.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X. Orozco, M.; Luque, F.J. *J. Med. Chem.* **2000**, *43*, 4657. [162](a) Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M.V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855. (b) Muñoz-Torrero, D.; Camps, P.; Gómez, T.; Viayna, E.; Galdeano, C. *P201000016*, *PCT/ES2010/070862*, **2010**. [179]Di, L.; Kerns, E.H.; Fan, K.; McConnell, O.J.; Carter, G.T. *Eur. J. Med. Chem.* **2003**, *38*, 223.

de esta enfermedad (PCT/ES2010/070862). The project related to the development of these donepezil−huprine hybrids was selected in the programme *Fons Prova de Concepte* of the *Fundació Bosch i Gimpera-Barcelona Knowledge Campus* and the *Vicerectorat d'Innovació i Transferència del Coneixement* of the *Universitat de Barcelona*. This programme, whose aim was funding research activities to add value to technological projects, allowed us to carry out an *ex vivo* assay of (±)-**81b** in order to prove the ability of these compounds to cross the BBB and access the CNS in mice (Table 3.2.4 and Figure 3.2.1).

For this reason, in the PhD Thesis work of Dr. Carles Galdeano in a collaboration with Dr. M. V. Clos of *Universitat Autònoma de Barcelona*, the anticholinesterasic effect of (±)-**81b** in mouse brain was assessed. With this purpose, groups of 9 OF1 mice were treated intraperitoneally (i. p.) with (±)-**81b** (10 μmol / Kg), donepezil (10 μmol / Kg) and saline solution (0.1 mL / 10 g). The animals were sacrificed at several stages after drug administration, and brains were taken and AChE activity in brain homogenates was determined by the method of Ellman.[176] Both, (±)-**81b** and donepezil crossed BBB very fast. In fact, 5 min after administration, a marked inhibition of brain AChE activity was observed for (±)-**81b** (59%) and donepezil (73%) (Table 3.2.4 and Figure 3.2.1).

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Table 3.2.4 Percentages of inhibition of mouse brain AChE. Each value represents the average ± SM of at least three independent experiments.

	(±)-**81b**	Donepezil
Time	% of AChE inhibition over the control ± SD	
5 min	59.38 ± 5.52	73.07 ± 1.81
10 min	54.14 ± 5.61	69.67 ± 0.90
20 min	46.21 ± 2.78	68.10 ± 6.92
30 min	50.28 ± 3.45	75.78 ± 4.25
40 min	31.02 ± 11.47	53.12 ± 11.57
60 min	20.49 ± 6.34	45.93 ± 9.97
120 min	16.17 ± 5.1	45.78 ± 5.67
6 h	8.22 ± 2.02	7.91 ± 4.01
24 h	-14.56 ± 8.61	-21.42 ± 9.80

The AChE inhibition progressively decreases for both compounds and almost completely disappears 6 h after administration. Moreover, after administration of donepezil a noticeable reduction of motor activity was observed as a side effect, whereas animals treated with (±)-**81b** did not show any behavioural alteration. The inhibitory effect exhibited by (±)-**81b** on mouse brain AChE activity clearly demonstrates its ability to cross BBB and enter the CNS.

[176]Ellman, G. L.; Courtney, K.D.; Andres, B. Jr.; Featherstone, R.M. *Biochem. Pharmacol.* **1961**, *7*, 88.

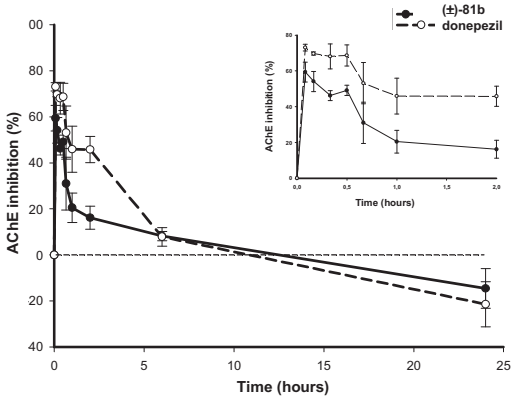


Figure 3.2.1

3.2.3.6 In vivo pharmacological evaluation.

After proving the ability of donepezil−huprine hybrids to cross BBB and access CNS, two different *in vivo* assays were carried out. First, Drs. Mario Salmona and Luisa Diomede from the *Istituto di Ricerche Farmacologiche Mario Negri* in Milan examined the effects of the lead compound of the series, (−)-**81b**, on the sequence of events leading to Aβ toxicity, using transgenic worms, namely transgenic *Caenorhabditis elegans*, as a simplified invertebrate model of AD.[180−182] To investigate the drugs' ability to counteract the Aβ deposition and toxicity *in vivo,* two different transgenic *C. elegans* strains were employed. On the one hand, they used the CL4176 transgenic *C. elegans* strain, engineered to inducibly express human $A\beta_{1-42}$ peptide in muscle when temperature is raised. These nematodes, when shifted to non-permissive conditions, become rapidly paralyzed and it was suggested that the acute induction of the transgene caused the production of Aβ oligomers which are responsible for the toxicity[181] offering an original *in vivo* model for investigating the toxicity specifically related to small oligomeric forms of Aβ. On the other hand, the CL2006 *C. elegans* strain constitutively expressing cytoplasmic human $A\beta_{3-42}$ in the body wall muscle cells, in which the age-related progressive reduction of muscle-specific motility correlates with the accumulation of both fibrils and oligomers of $A\beta_{3-42}$, was also employed. Both strains had already been employed as *in vivo* models of AD and used to demonstrate the effect of *Gingko biloba* extract EGb761, gingkolides and other compounds, in counteracting Aβ toxicity.[182−184]

Thus, CL4176 *C. elegans* were treated with vehicle or drugs (25−50 μM, 100 mL/plate) and Aβ-induced paralysis was scored at 24 h. Worms that did not move or only moved the head when gently touched with a platinum loop were scored as paralyzed. The percentage of the number of paralyzed worms on the total number of worms was calculated (Figure 3.2.2). (−)-

[180]Link, C.D. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 9368. [181]Link, C.D. *Genes Brain Behav*. **2005**, *4*, 147. [182]Diomede, L.; Cassata, G.; Fiordaliso, F.; Salio, M.; Ami, D.; Natalello, A.; Doglia, S.M.; De Luigi, A.; Salmona, M. *Neurobiol. Dis.*, **2010**, *40*, 424. [183]Wu, Y.; Wu, Z.; Butko, P.; Christen, Y.; Lambert, M.P.; Klein, W.L.; Link, C.D.; Luo, Y. *J. Neurosci.* **2006**, *26*, 13102. [184]McColl, G.; Roberts, B.R.; Gunn, A.P.; Perez, K.A.; Tew, D.J.; Masters, C.L.; Barnham, K.J.; Cherny, R.A.; Bush, A.I. *J. Biol. Chem.* **2009**, *284*, 22697.

81b (in this study refered to as AVCRI104) exhibited a significant protection from the Aβ-induced paralysis in this *C. elegans* model, which was even superior to that of the positive control tetracycline. However, it was not able to reduce amyloid deposits in *C. elegans* (strain CL2006) relative to control animals. Drs. Salmona and Diomede are currently trying to unravel the mechanism of underlying the protective effect of (−)-**81b** in the first *C. elegans* model.

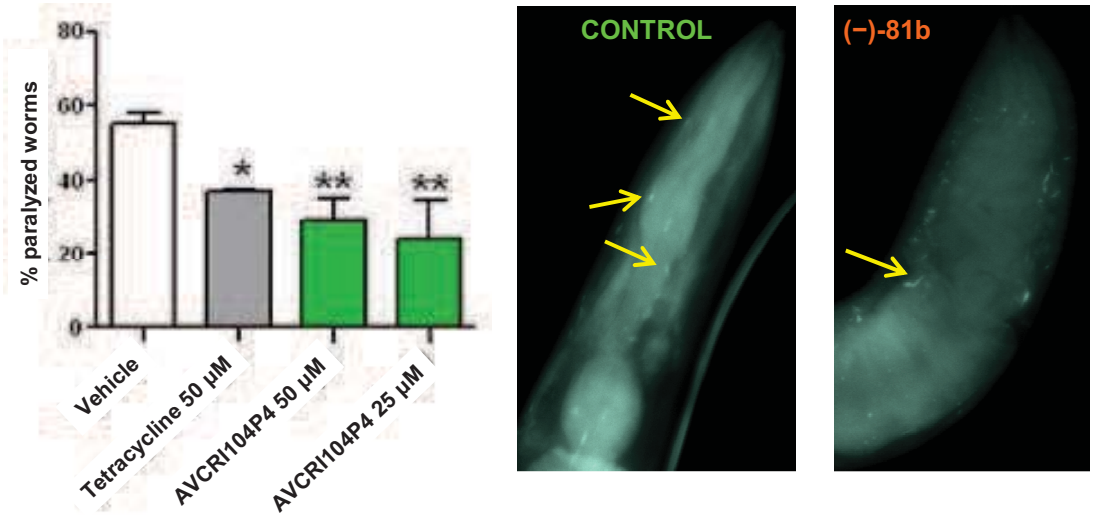


Figure 3.2.2

Furthermore, a collaboration was established with *Fundación Genoma España* which provided fundings for preliminary preclinical studies, particularly, the proof of concept in an AD mouse model, in order to obtain data on the efficacy and safety of the selected hybrid (–)-**81b**. With this aim, in the PhD Thesis work of Irene Sola 10 g of (–)-**81b** (an amount 135-fold greater than that previously prepared in this PhD Thesis work) were synthesized. Then, Drs. Daniela Jud and Birgit Hutter-Paier, from *JSW Life Sciences Gmbh,* tested (–)-**81b** in APP_{SL} transgenic mice using the Morris water maze test,[185] one of the most widely used tasks in behavioral neuroscience for studying the psychological processes and neural mechanisms of spatial learning and memory, and determined its effect on Aβ levels. In the Morris water maze test, animals, usually rats or mice, are placed in a large circular pool of water and required to escape from water onto a hidden platform whose location can normally be identified only using spatial memory (Figure 3.2.3).

(–)-**81b** proved to be able to significantly improve cognition after 3-month oral administration to APP_{SL} transgenic mice, in terms of escape latency, swim length, and swim speed relative to vehicle treated animals in the Morris water maze. Unfortunately, (−)-**81b** did not alter $Aβ_{1-38}$, $Aβ_{1-40}$ or $Aβ_{1-42}$ neither in the cerebrospinal fluid (CSF) nor in brain homogenate preparations, nor altered cortical and hippocampal amyloid plaque load compared to vehicle treated animals.

[185]Brandeis, R.; Brandys, Y.; Yehuda, S. *Int. J. Neurosci.* **1989**, *48*, 29.

Despite the protective effect and cognition enhancing effects displayed *in vivo* by (−)-**81b**, its lack of efficacy regarding the amyloid burden led us to discontinue its preclinical development.

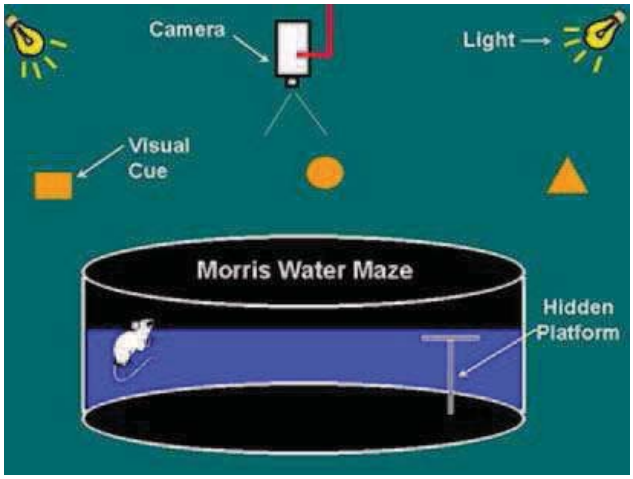


Figure 3.2.3

3.2.4 Molecular modelling studies of donepezil−huprine hybrids.

In the PhD Thesis work of Dr. Carles Galdeano, the binding mode of compounds (−)-**81a** and (−)-**81b** to hAChE was explored by means of 30 ns molecular dynamics (MD) simulations.[162]

In the modelled (−)-**81a**–hAChE complex, binding of the huprine Y moiety at the catalytic site nearly matches the position of huprine X in the X-ray crystallographic structure of the complex with TcAChE (PDB ID: 1E66) (Figure 3.2.4).[127] Thus, the heteroaromatic ring system of the huprine Y unit of (−)-**81a** stacks against the indole ring of Trp86 (hAChE residue numbering), and the protonated quinoline nitrogen atom establishes an hydrogen bond with the carbonyl group of His447. However, the stacking of the quinoline system of huprines against the benzene ring of Phe330 (*Tc*AChE residue numbering) found in the X-ray structure is not maintained in the interaction of (−)-**81a** with hAChE (note that Phe330 in TcAChE is replaced by Tyr337 in hAChE). The loss of this interaction can be ascribed to the formation of a hydrogen bond between the hydroxy group of Tyr337 and the protonated piperidine nitrogen atom of (−)-**81a**, which in turn precludes the proper arrangement for the stacking of the phenol ring of Tyr337 and the quinoline system of the huprine Y unit. In the peripheral site, the indane moiety of (−)-**81a** establishes a *π*-stacking interaction with Trp286 (hAChE residue numbering, equivalent to Trp279 in TcAChE)), thus mimicking the interaction observed in the X-ray structure of donepezil bound to TcAChE (PDB ID: 1EVE).[46] There is, however, a sizable displacement in the relative position of the indane and indanone systems of (−)-**81a** and donepezil, as the five-

[46]Kryger, G.; Silman, I.; Sussman, J.L. *Structure* **1999**, *7*, 297. [127]Dvir, H.; Wong, D.M.; Harel, M.; Barril, X.; Orozco, M.; Luque, F.J.; Muñoz-Torrero, D.; Camps, P.; Rosenberry, T.L.; Silman, I.; Sussman, J.L. *Biochemistry* **2002**, *41*, 2970. [162](a) Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M.V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855. (b) Muñoz-Torrero, D.; Camps, P.; Gómez, T.; Viayna, E.; Galdeano, C. *P201000016*, *PCT/ES2010/070862*, **2010**.

membered ring of the indane moiety of (−)-**81a** occupies roughly the same position as the aromatic ring of the indanone moiety of donepezil, thus leading to a better stacking between the indane system of (−)-**81a** and Trp286 relative to that of donepezil.

In the mid-gorge region the presence of the piperidine ring gives rise to a remarkable structural rearrangement of specific residues. For example, in the complex between donepezil and TcAChE, Tyr121 forms a water-mediated interaction with the piperidine nitrogen atom of donepezil. In the modelled (−)-**81a**–hAChE complex, this interaction is lost due to the new orientation adopted by the piperidine ring to form a hydrogen bond with Tyr337. In turn, this new orientation would cause the piperidine ring to collide with Tyr124 and Phe338 (Tyr121 and Phe331 in TcAChE). However, the steric clash is minimized upon displacement of the side chain of those residues, which in turn affects the spatial arrangement of Phe297 and Phe295 (Phe290 and Phe288 in TcAChE). Finally, the piperidine ring also forces the distortion of Tyr341 (Tyr334 in TcAChE), which in turn affects the spatial orientation of Trp439 and Met443 (Trp432 and Met 436 in TcAChE).

The dual site binding of (−)-**81b** at the catalytic and peripheral sites of hAChE retains the essential features noted for (−)-**81a**, such as stacking against Trp86 and Trp286, and the hydrogen bond with His447. However, replacement of the ethylene linker in (−)-**81a** by a trimethylene linker in (−)-**81b** influences the positioning of the piperidine ring in the mid-gorge site and leads to different interactions with the neighboring residues. Thus, the hydrogen bond formed between (−)-**81a** and Tyr337 is lost in (−)-**81b**, whereas the additional methylene unit in the linker permits the approach of the piperidine ring to the carboxylate group of Asp74, leading to a salt bridge interaction. Moreover, the increased length of the linker minimizes the steric hindrance of the piperidine ring with both Tyr124 and Tyr341, which occupy positions similar to those observed in the X-ray structures 1E66 and 1EVE.

The preceding findings suggest that the decrease in inhibitory potency of the novel hybrids (−)-**81a** and (−)-**81b** relative to the parent huprine Y can be related to the structural distortion generated by the presence of the piperidine ring in the tether that links huprine and indane moieties. In particular, such distortion affects the pocket filled by the methyl / ethyl substituent bound at position 9 in the huprine moiety, and could thus explain the different influence exerted by the replacement of methyl by ethyl in the inhibitory potency of the hybrids and the parent compounds (Table 3.2.1). Thus, whereas replacement of methyl by ethyl in (−)-huprine increases the inhibitory potency by 1.6-fold, the reverse trend is observed for the hybrids, with potency decreasing by ~1.5–3-fold (Table 3.2.1). To check the preceding hypothesis, thermodynamic integration (TI) calculations coupled with MD simulations were performed to rationalize the difference in binding affinity between methyl- and ethyl-substituted hybrids.

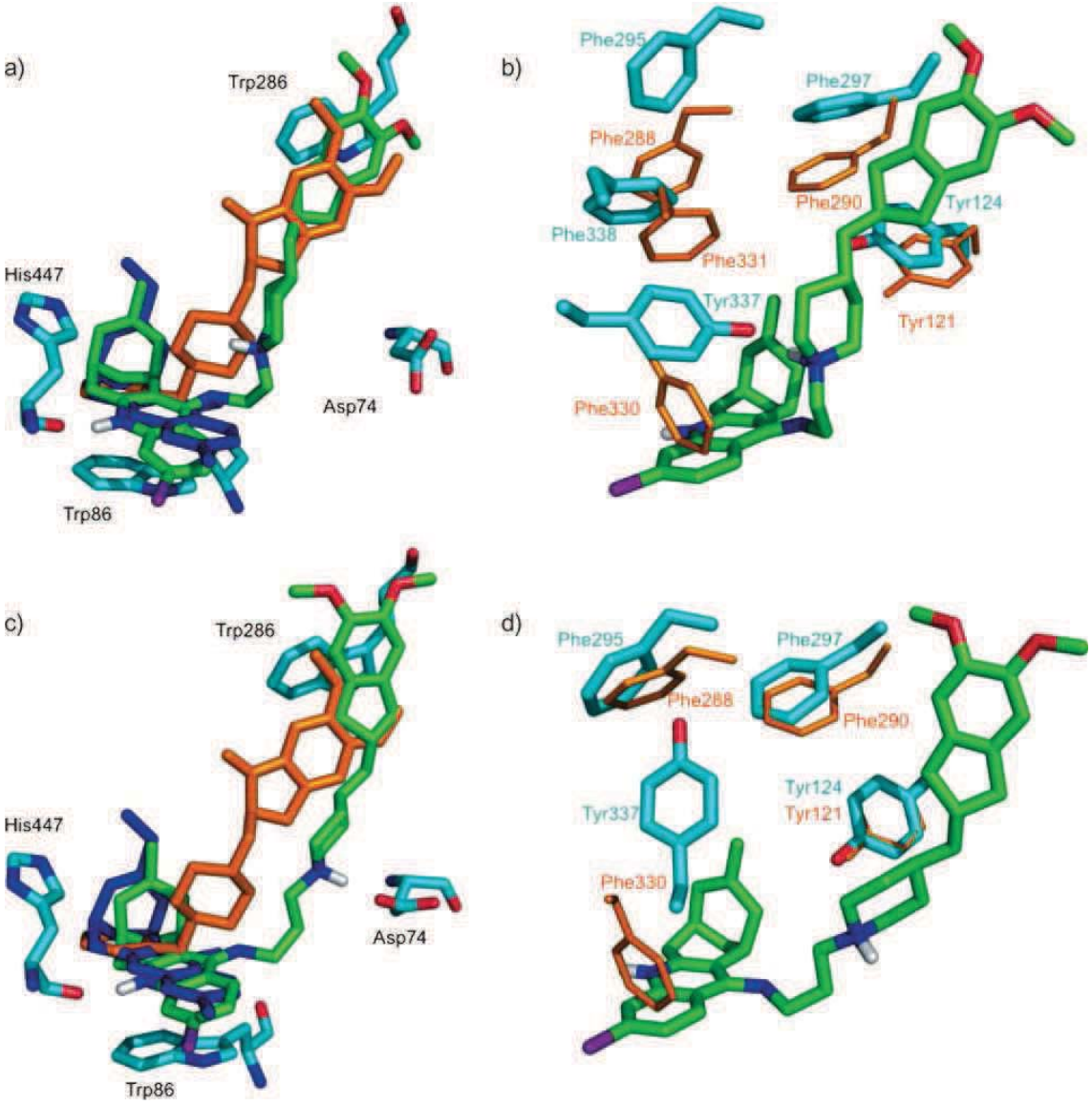


Figure 3.2.4 Superposition of the structures (green) collected at the end of the 30 ns MD simulations of compounds a) (−)-**81a** and c) (−)-**81b** bound to hAChE, and the X-ray crystallographic structures of TcAChE with donepezil (orange, PDB ID: 1EVE) and (−)-huprine X (dark blue, PDB ID: 1E66). Comparison of the structures (green) and relevant residues (cyan) collected at the end of the 30 ns MD simulations of compounds b) (−)-**81a** and d) (−)-**81b** with the X-ray crystallographic structure of TcAChE with donepezil (orange, PDB ID: 1EVE).

The change in binding free energy was determined from the thermodynamic cycle shown in Figure 3.2.5. The results predict that the conversion from methyl [(−)-**81a**,**b**] to ethyl [(−)-**82a**,**b**] decreases the binding affinity for the enzyme by 0.4 and 0.3 kcalmol^{-1}, respectively, which is in agreement with the experimental data. In contrast, similar calculations performed for the parent compound, (−)-huprine, predicted huprine X to be more potent than huprine Y by 0.7 kcalmol^{-1}. As a whole, these results reflect the changes in the available experimental data originated upon replacement of methyl by ethyl at position 9, thus lending support to the structural models derived from the extended MD simulations. Moreover, these results highlight the importance of the mid-gorge site in modulating the binding affinity of dual binding site inhibitors in AChE.[128]

[128]Muñoz-Torrero, D.; Camps, P. *Expert Opin. Drug Discovery* **2008**, *3*, 65.

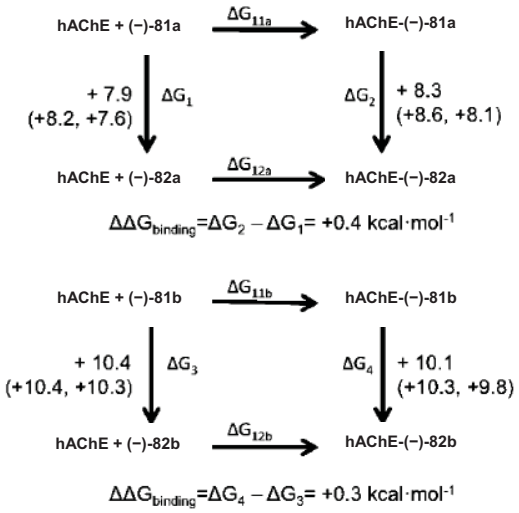


Figure 3.2.5 Thermodynamic cycle used in free energy calculations in water and in the hAChE enzyme to determine relative binding. The results for the forward and reverse mutations are given in parentheses. Values are in kcal mol^{-1}.

3.2.5 Kinetic analysis of AChE inhibition of donepezil−huprine hybrids.

To gain further insight into the mechanism of action of this family of compounds on human recombinant AChE, a kinetic study was carried out by Dr. Manuela Bartolini of the *Università di Bologna* with the most potent compound of the series: hybrid (−)-**81b**. Graphical analysis of the overlaid reciprocal Lineweaver–Burk plots (Figure 3.2.6) showed both increased slopes (decreased V_{max}) and intercepts (higher K_M) with increasing inhibitor concentrations. This pattern indicates mixed-type inhibition, and therefore supports the dual site binding to AChE suggested by MD simulations. Re-plots of the slope versus concentration of hybrid (−)-**81b** gave an estimate of the competitive inhibition constant, K_i, of 0.53 nM.

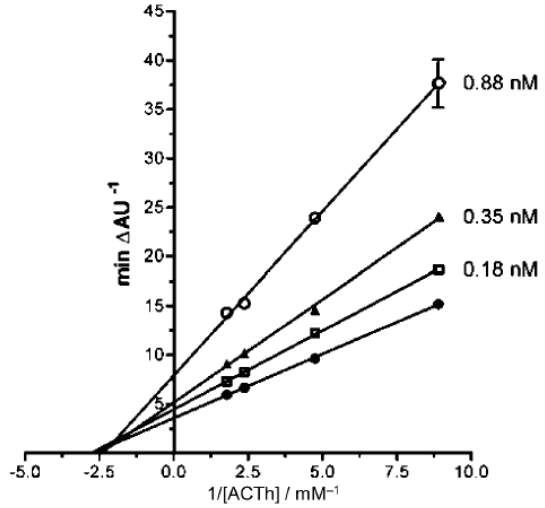
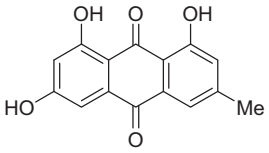


Figure 3.2.6 Kinetic study on the mechanism of AChE inhibition by hybrid (−)-**81b**. Overlaid Lineweaver–Burk reciprocal plots of AChE initial velocity at increasing substrate concentration (ATCh, 0.56–0.11 mM) in the absence of inhibitor and in the presence of (−)-**81b** (0.18–0.88 nM) are shown. Lines were derived from a weighted least-squares analysis of the data points.

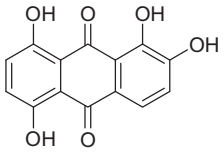
3. 3 Preparation of racemic rhein−huprine hybrids

3.3.1 Anthraquinones as tau protein aggregation inhibitors.

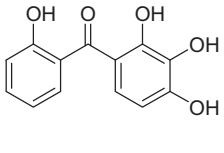
Given the fact that the abnormal aggregation of tau protein into PHFs and, later on, into neurofibrillary tangles, is one of the hallmarks of AD and because the aggregation of tau in AD correlates with the clinical progression of the disease, it seems likely that inhibition or reversal of tau aggregation could protect the affected neurons. To test this idea Dr. Mandelkow, from the Max-Plank Unit for Structural Molecular Biology, began a search for inhibitors of tau aggregation and screened a library of 200,000 compounds and found out that compounds sharing a core structure of hydroxyanthraquinone, **83**−**87** (Figure 3.3.1) inhibit PHF assembly.[171]



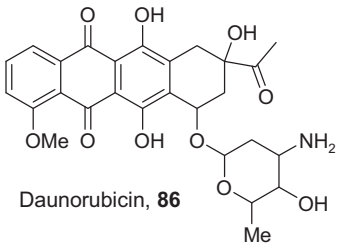
Emodin, **83**



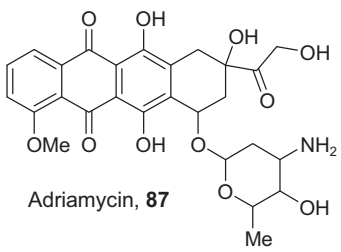
PHF016, **84**



PHF005, **85**



Daunorubicin, **86**



Adriamycin, **87**

Figure 3.3.1 Structure of tau protein aggregation inhibitors.

Compounds capable of interfering with the aggregation of tau protein all shared a polycyclic aromatic ring system derived from hydroxyanthraquinone, hydroxybenzophenone or hydroxynaphthacendione. These five compounds **83**−**87** were all able to inhibit the transition from soluble to aggregated PHFs,[186,187] using fixed protein concentrations, and measuring the extent of aggregation via the fluorescence of thioflavine S.[188] These compounds exhibited IC_{50} values in the sub-to-low micromolar range. More interestingly, the ability of compounds **83**−**87** to dissolve preformed PHFs was also tested by Dr. Mandelkow again using the thioflavine S assay to find that they exhibited DC_{50} values again in the low micromolar range.

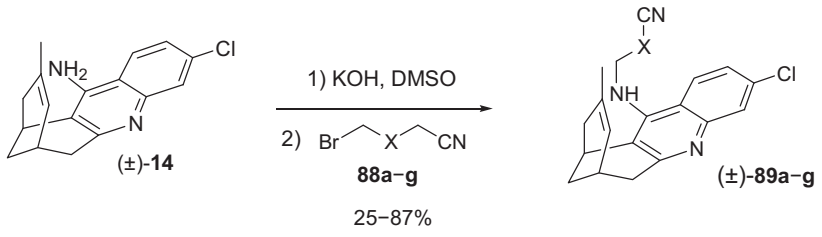
With this results in mind, and taken into account the complexity and polyetiological origin of AD, which makes simultaneous modulation of several targets involved in the pathological network a more efficient therapeutic approach, in the present PhD Thesis work, a novel family of multipotent dual binding site AChEIs containing a racemic huprine Y, as the active site interacting unit and the anthraquinone moiety of rhein, **40** (Figure 2.3 and Scheme 3.3.4), to

[171]Pickhardt, M.; Gazova, M.; von Bergen, M.; Khlistunova, I.; Wang, Y.; Hascher, A.; Mandelkow, E.-M.; Biernat, J.; Mandelkow, E. *J. Biol. Chem.* **2005**, *5*, 3628. [186]von Bergen, M.; Friedhoff, P.; Biernat, J.; Heberle, J.; Mandelkow, E.-M.; Mandelkow, E. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 5129. [187]von Bergen, M.; Barghorn, S.; Li, L.; Marx, A.; Biernat, J.; Mandelkow, E.-M.; Mandelkow, E. *J. Biol. Chem.* **2001**, *276*, 48165. [188]Friedhoff, P.; Schneider, A.; Mandelkow, E. M.; Mandelkow, E. *Biochemistry* **1998**, *37*, 10223.

enable both π−π interactions with Trp286 at the AChE peripheral site and a potential tau antiaggregating effect, linked through an amide-containing oligomethylene or aromatic linker, was envisioned. This novel family, namely rhein−huprine hybrids should be able to simultaneously hit three different targets of the pathological network associated to AD, namely, cholinesterases, and Aβ and tau protein aggregation.

3.3.2 Preparation of rhein−huprine hybrids.

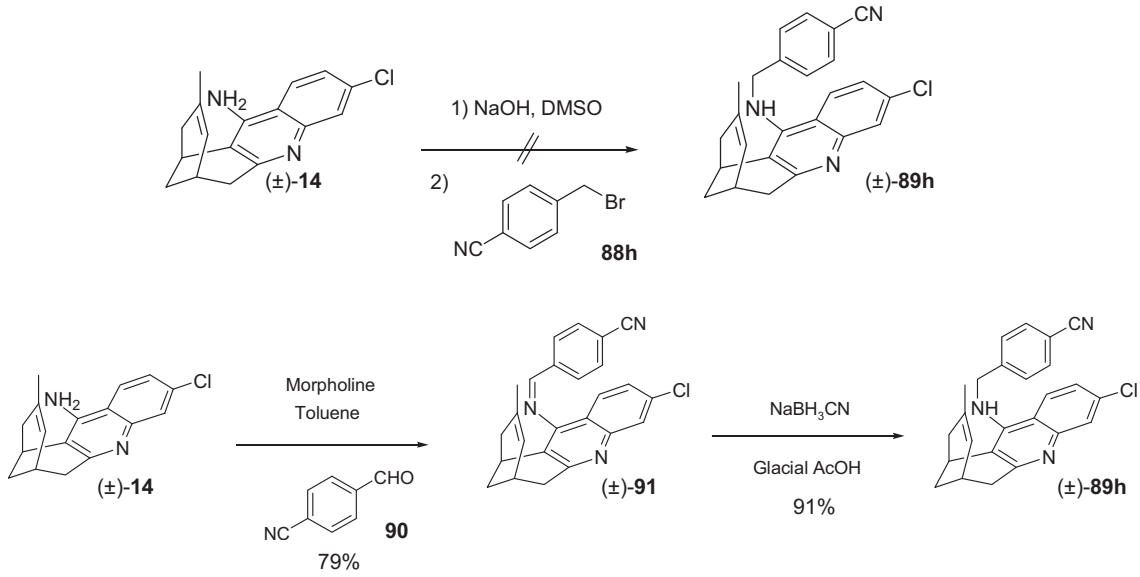
For the preparation of the desired rhein−huprine hybrids, a new methodology was developed which required a first alkylation of racemic huprine Y, (±)-**14**, with an ω-bromoalkanenitrile (±)-**88a−g** in the presence of NaOH in DMSO at r. t. overnight that gave mixtures of the desired cyanoalkylhuprines, unreacted (±)-huprine Y, (±)-**14**, and dialkylation byproducts which were purified through silica gel column chromatography to provide cyanoalkylhuprines (±)-**89a−g** (Scheme 3.3.1) in moderate to good yields (25−87%). It is important to note that ω-bromoalkanenitriles (±)-**88a−c** were commercially available, however, (±)-**88d−g** had to be prepared by reaction of the appropriate dibromoalkanes with NaCN, followed by microdistillation of the reaction crudes in order to separate the desired ω-bromoalkanenitrile from the unreacted dibromoalkane and direaction byproducts.



	(±)-88,89a	(±)-88,89b	(±)-88,89c	(±)-88,89d	(±)-88,89e	(±)-88,89f	(±)-88,89g
X	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_5$	$(CH_2)_6$	$(CH_2)_7$	$(CH_2)_8$	$(CH_2)_9$

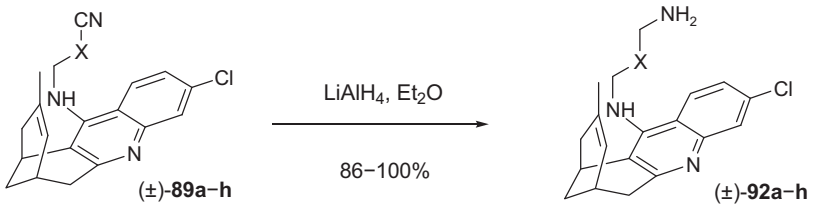
Scheme 3.3.1

The rhein−huprine hybrid (±)-**93h**, containing a *p*-phenylene moiety in the linker (Scheme 3.3.4), was designed in order to gain additional π−π stacking interactions with AChE mid-gorge residues. Using the same methodology than that used for the synthesis of cyanoalkylhuprines (±)-**89a−g**, (±)-huprine Y, (±)-**14**, was reacted with 4-(bromomethyl)benzonitrile, but only unreacted starting material was recovered. Alternatively, a reductive alkylation reaction was assayed. Thus, reaction of (±)-huprine Y, (±)-**14**, with *p*-cyanobenzaldehyde, **90**, in the presence of freshly distilled morpholine, in toluene, heating under reflux for 48 h, provided, after purification through silica gel column chromatography, (±)-**91**, in 79% yield. Imine (±)-**91** was reduced with $NaBH_3CN$ in glacial AcOH at r. t. for 3 h to give (±)-**89h** in 91% yield (Scheme 3.3.2).



Scheme 3.3.2

Cyanoalkyl- and cyanoarylalkyl-huprines (±)-**89a−h**, were reduced with $LiAlH_4$ in Et_2O at r. t. overnight to afford the corresponding aminoalkylhuprines (±)-**92a−h** in excellent yields and with no need of purification (Scheme 3.3.3).

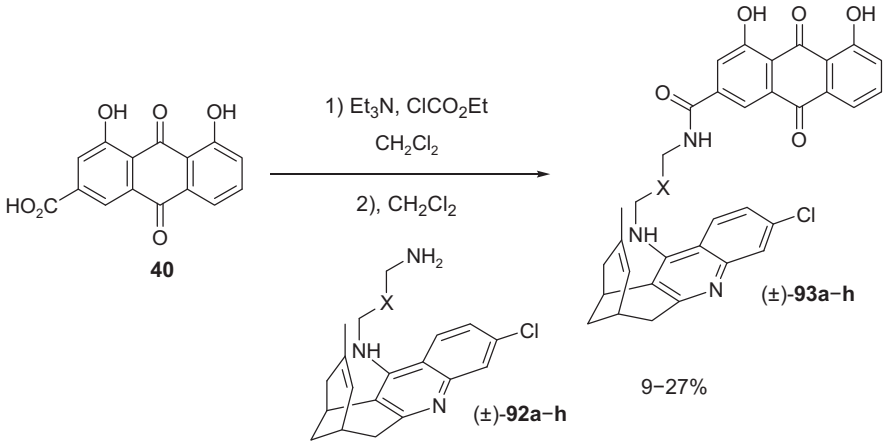


	(±)-**89**,**92a**	(±)-**89**,**92b**	(±)-**89**,**92c**	(±)-**89**,**92d**	(±)-**89**,**92e**	(±)-**89**,**92f**	(±)-**89**,**92g**	(±)-**89**,**92h**
X	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_5$	$(CH_2)_6$	$(CH_2)_7$	$(CH_2)_8$	$(CH_2)_9$	*p*-phenylene

Scheme 3.3.3

Finally, rhein, **40**, was treated with freshly distilled Et_3N and $ClCO_2Et$ in CH_2Cl_2 at 0 ºC for 30 min and the resulting mixed anhydrides were reacted with aminoalkylhuprines (±)-**92a−h** at r. t. for three days, to afford the desired hybrids, (±)-**93a−h**, in low to moderate yields after a tedious silica gel column chromatography purification (Scheme 3.3.4).[189] (±)-**93a−h** were transformed into the corresponding hydrochlorides. Worthy of note, when the reaction was carried out using the diacetylated form of rhein, known as diacerin, similar yields were observed.

[189]Muñoz-Torrero, D.; Viayna, E.; Sola, I.; Vázquez, S. P201230706, **2012**.



	(±)-**92,93a**	(±)-**92,93b**	(±)-**92,93c**	(±)-**92,93d**	(±)-**92,93e**	(±)-**92,93f**	(±)-**92,93g**	(±)-**92,93h**
X	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_5$	$(CH_2)_6$	$(CH_2)_7$	$(CH_2)_8$	$(CH_2)_9$	*p*-phenylene

Scheme 3.3.4

3.3.3 Pharmacological evaluation of rhein−huprine hybrids.

3.3.3.1 Activity toward cholinesterases.

Drs. Albert Badia and M. Victòria Clos determined the inhibitory activity of rhein−huprine hybrids(±)-**93a−h** toward, *Electrophorus electricus* AChE (eeAChE), which is a much more economic source of AChE, as usual, using the method of Ellman *et al.*[176] (Table 3.3.1). The novel rhein–huprine hybrids turned out to be potent inhibitors of eeAChE, exhibiting IC_{50} values in the nanomolar range. Later on, Drs. Vincenza Andrisano and Manuela Bartolini, also using the method of Ellman *et al.*[176] determined the hAChE inhibitory activity of hybrids (±)-**93a−h**, to find that these hybrids exhibited an even higher inhibitory activity toward hAChE than for eeAChE. All the hybrids exhibited IC_{50} values in the low nanomolar range. Some structure–activity relationship trends could be extracted from the data in Table 3.3.1. For instance, contrary to the results observed eeAChE inhibition, the inhibitory activity of (±)-**93a−h** toward hAChE generally decreases with longer tethers, the pentamethylene linked (±)-**93a** being the most potent hAChE inhibitor of the series, with an IC_{50} value of 1.07 nM. Contrary to our expectations, the introduction of a phenyl ring in the linker in order to establish extra $\pi-\pi$ stacking interactions with the mid-gorge aromatic residues is detrimental for the hAChE inhibition. Overall, the most potent hAChEI of the series is (±)-**93a**, which turned out to be much more potent than rhein which did not show any activity against hAChE, but as we had also observed in the donepezil−huprine hybrids, (±)-**93a** turned out to be 13-fold less potent than the parent (±)-huprine Y. The lower hAChE inhibitory activity relative to the parent huprine might

[176]Ellman, G.L.; Courtney, K.D.; Andres, B. Jr.; Featherstone, R.M. *Biochem. Pharmacol.* **1961**, *7*, 88.

indicate a difference in the binding mode of (±)-huprine Y alone and when it is linked to the anthraquinone moiety.[189]

Table 3.3.1 hAChE, eeAChE and hBChE inhibitory activities of rhein and the hydrochlorides of (±)-huprine Y and (±)-**93a−h**.[a]

Compound	IC_{50} (nM) hAChE[a]	IC_{50} (nM) eeAChE[a]	IC_{50} (nM) hBChE[a]
(±)-**93a**·HCl	1.07 ± 0.05	62.9 ± 4.8	950 ± 30
(±)-**93b**·HCl	1.52 ± 0.08	71.7 ± 7.9	107 ± 40
(±)-**93c**·HCl	3.18 ± 0.16	379.4 ± 70.9	146 ± 16
(±)-**93d**·HCl	4.36 ± 0.22	88.6 ± 6.7	350 ± 17
(±)-**93e**·HCl	3.60 ± 0.21	32.6 ± 1.7	620 ± 24
(±)-**93f**·HCl	7.61 ± 0.45	46.7 ± 2.4	110 ± 40
(±)-**93g**·HCl	17.4 ± 2.2	16.3 ± 2.3	645 ± 67
(±)-**93h**·HCl	18.2 ± 2.2	60.0 ± 7.5	510 ± 20
(±)-**huprine Y**·HCl	0.08 ± 0.01	0.08 ± 0.01	175 ± 6.3
Rhein	> 10000	637 ± 87	*nd*

[a]Values are expressed as the mean ± SEM of at least four experiments; IC_{50}: inhibitory concentration (nM) of recombinant human AChE, *Electrophorus electricus* AChE or human serum BChE activity. *nd* means not determined.

Drs. Badia and Clos also determined the inhibitory activity toward serum hBChE by the method of Ellman *et al.*[176] (Table 3.3.1). As it has been already mentioned, huprines are selective for hAChE versus hBChE inhibition due to the presence of the chlorine atom in the quinoline system.[127,137,177] Analogously, rhein−huprine hybrids are also selective for hAChE vs hBChE inhibition, but they can still be considered moderately potent inhibitors of hBChE, with IC_{50} values in the nanomolar range.

3.3.3.2 Activity toward Aβ aggregation.

The potential ability of the new hybrids to inhibit Aβ aggregation through their interaction with the peripheral site of AChE was assessed by Drs. Vincenza Andrisano and Manuela Bartolini using a thioflavin T fluorescence method.[95] The new hybrids (±)-**93a–h** were found to significantly inhibit hAChE-induced $A\beta_{1-40}$ aggregation, with percentages of inhibition ranging

[95]Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. *Biochem. Pharmacol.* **2003**, *65*, 407. [127]Dvir, H.; Wong, D.M.; Harel, M.; Barril, X.; Orozco, M.; Luque, F. J.; Muñoz-Torrero, D.; Camps, P.; Rosenberry, T.L.; Silman, I.; Sussman, J.L. *Biochemistry* **2002**, *41*, 2970. [137]Camps, P.; Formosa, X.; Galdeano, C.; Gómez, T.; Muñoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia A.; Clos, M.V.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F.J. *J. Med. Chem.* **2008**, *51*, 3558. [176]Ellman, G.L.; Courtney, K.D.; Andres, B. Jr.; Featherstone, R.M. *Biochem. Pharmacol.* **1961**, *7*, 88. [177]Muñoz-Ruiz, P.; Rubio, L.; García-Palomero, E.; Dorronsoro, I.; Del Monte-Millán, M.; Valenzuela, R.; Usán, P.; de Austria, C.; Bartolini, M.; Andrisano, V.; Bidon-Chanal, A.; Orozco, M.; Luque, F.J.; Medina, M.; Martínez, A. *J. Med. Chem.* **2005**, *48*, 7223. [189]Muñoz-Torreo, D.; Viayna, E.; Sola, I.; Vázquez, S. P201230706, **2012**.

from 29% to 52% at 100 μM concentration, in all cases higher than that of the parent huprine Y (Table 3.3.2).

The AChE-induced Aβ aggregation inhibitory activity of the novel hybrids (±)-**93a**–**h** increases with longer tethers exhibiting a maximum inhibitory activity when bearing a heptamethylene linker, (±)-**93c**, and again decreases when the tethers length is further increased. The introduction of a phenyl ring into the linker also decreases the AChE-induced Aβ aggregation inhibitory activity. Overall, the most potent compounds were (±)-**93b** and (±)-**93c**, which at a 100 μM concentration of inhibitor showed percentages of inhibition above 50% (Table 3.3.2).

Table 3.3.2 Aβ aggregation and BACE-1 inhibitory activities of the hydrochloride of racemic huprine Y and hybrids (±)-**93a**−**h**.[a]

Compound	AChE-induced $A\beta_{1-40}$ aggregation (%)[b]	Self-induced $A\beta_{1-42}$ aggregation (%)[c]	BACE-1 IC_{50} (μM)[d,e]
(±)-**93a**·HCl	45.0 ± 7.5	38.7 ± 5.0	*na*
(±)-**93b**·HCl	50.5 ± 8.7	40.8 ± 5.7	*na*
(±)-**93c**·HCl	52.5 ± 2.9	40.6 ± 1.9	*na*
(±)-**93d**·HCl	44.7 ± 8.4	33.1 ± 5.4	0.98 ± 0.17
(±)-**93e**·HCl	48.7 ± 8.4	38.0 ± 4.6	0.12 ± 0.09
(±)-**93f**·HCl	29.2 ± 2.4	40.9 ± 4.4	1.19 ± 0.18
(±)-**93g**·HCl	38.2 ± 2.6	35.3 ± 4.0	1.20 ± 0.15
(±)-**93h**·HCl	35.2 ± 1.8	32.4 ± 3.6	2.02 ± 0.44
(±)-**huprine Y**·HCl	17.1 ± 4.5	*nd*	*nd*
Rhein	*nd*	*nd*	*na*

[a]Values are expressed as the mean ± SEM from two independent measurements, each performed in duplicate. [b]Inhibitor concentration: 100 μM. [c]Inhibitor concentration: 10 μM ([Aβ] / [I] = 5:1). [d]The values are expressed as mean ± standard error of the mean of at least four experiments. IC_{50}. [e]Human recombinant BACE-1 (Sigma), substrate M-2420 (Bachem). *nd* means not determined. *na* means not active.

Drs. Bartolini and Andrisano also determined the inhibitory activity of (±)-**93a**–**h** toward the self-induced $A\beta_{1-42}$ aggregation. The new rhein–huprine hybrids significantly inhibit the self-induced Aβ aggregation when tested at a 10 μM concentration of inhibitor, with percentages of inhibition ranging from 32% to 41% (Table 3.3.2). Thus, they can be considered moderate inhibitors of the self-induced Aβ aggregation.

Very interestingly, Dr. Raimon Sabaté from *Universitat de Barcelona* tested the ability of our coumpounds to inhibit Aβ aggregation, in a simplified model *in vivo* model of Aβ aggregation namely an *Escherichia coli* strain that overexpresses Aβ forming a kind of aggregates called inclusion bodies.[190,191] (±)-**93a**–**h** exhibited percentages of inhibition ranging from 63% to 76% at

[190]Martson, F.O.A. *Biochem. J.* **1986**, *240*, 1. [191]Waldo, G.S.; Standish, B.M.; Berendzen, J.; Terwilliger, T.C. *Nat. Biotech*. **1999**, *17*, 691.

10 μM concentration of inhibitor (Figure 3.3.2). The percentages of Aβ inhibition were slightly higher than those found *in vitro*.

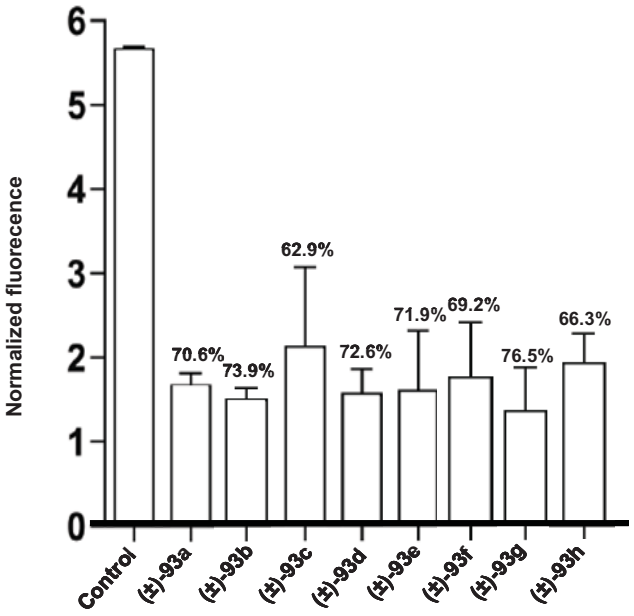


Figure 3.3.2 Graphical representation of inhibition of Aβ aggregation and percentages of inhibition of rhein−huprine hybrids, (±)-**93a–h** using 10 μM concentration of inhibitor in *Escherichia coli* BL21 competent cells which were transformed with pET28 vector (Novagen, Inc., Madison, WI, USA) encoding the sequence for $A\beta_{1-42}$.

3.3.3.3 Activity toward Aβ formation.

Given our previous experience on dual binding site AChEIs exhibiting BACE-1 inhibitory activity,[162] Drs. Angela De Simone and Vincenza Andrisano also tested the inhibitory activity of (±)-**93a**–**h** against BACE-1 (Table 3.3.2). Rhein−huprine hybrids (±)-**93d**–**h** can be considered potent BACE-1 inhibitors with IC_{50} values in the nanomolar and low micromolar range. Very interestingly, hybrid (±)-**93e**, bearing a nonamethylene linker, is the most active compound with an IC_{50} value of 120 nM.

3.3.3.4 Activity toward tau protein aggregation.

As it has been already mentioned, not only was introduced the anthraquinone−derived rhein moiety of the novel hybrids to allow an interaction with the peripheral site of AChE, and thus with the AChE-induced Aβ-aggregation, but also to endow these compounds with the ability to inhibit tau-protein aggregation as it had been reported for some structurally related compounds (Figure 3.3.1).[171] Using an *Escherichia coli in vivo* model of tau aggregation, Dr.

[162](a) Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M.V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855. (b) Muñoz-Torrero, D.; Camps, P.; Gómez, T.; Viayna, E.; Galdeano, C. *P201000016*, **2010**. [171]Pickhardt, M.; Gazova, M.; von Bergen, M.; Khlistunova, I.; Wang, Y.; Hascher, A.; Mandelkow, E.-M.; Biernat, J.; Mandelkow, E. *J. Biol. Chem.* **2005**, *5*, 3628.

Raimon Sabaté found that the novel rhein−huprine hybrids significantly inhibit tau protein aggregation at 10 μM concentration of inhibitor with percentages of inhibition from 24% to 58%. Overall, the most active compound turned out to be the hybrid bearing a decamethylene linker, (±)-**93f**, which at a 10 μM concentration was able to inhibit protein tau aggregation in 58%. Thus this compound must have an IC_{50} value for this activity in the low micromolar range.

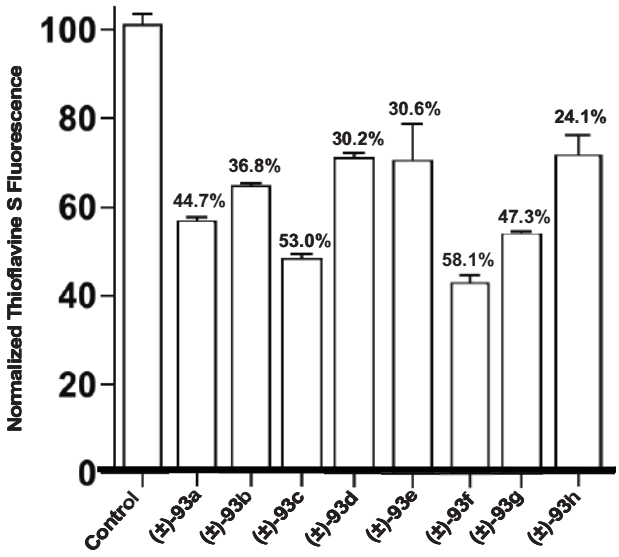


Figure 3.3.2 Graphical representation of inhibition of tau protein aggregation and percentatges of inhibition of rhein−huprine hybrids, (±)-**93a**–**h** using 10 μ concentration of inhibitor in *Escherichia coli* BL21 (DE3) with pTARA competent cells were transformed with pRKT42 vector encoding four repetitions of tau protein in two insert.

3.3.3.5 In vitro BBB permeation assay.

In order to demonstrate the ability of rhein−huprine hybrids to cross the BBB and access the CNS, which is an essential property for a compound to become a lead candidate for the treatment of AD, Dr. Belén Pérez from the *Universitat Autònoma de Barcelona* applied the PAMPA-BBB assay[179] to our compounds. The *in vitro* permeability, P_e, of (±)-**93a**,**c**–**h**, through a lipid extract of porcine brain were determined. At each solvent mixture, assay validation was made by comparing the experimental permeability with the reported values of 15 commercial drugs that gave a good linear correlation. The ability of these compouns to cross BBB was determined, taking into account the following limits: compounds having high permeability to access CNS should show a P_e value above 4.98, those compounds showing a P_e value between 4.98 and 2.2 would have an uncertain CNS permeability and those compounds showing P_e values below 2.2 a priori should not be able to cross the BBB, thus, would be discarded as lead compounds for the treatment of AD. All rhein−huprine hybrids exhibited P_e above 4.98, and therefore, were predicted to be able to cross the BBB and enter the CNS (Table 3.3.3).

[179]Di, L.; Kerns, E.H.; Fan, K.; McConnell, O.J.; Carter, G.T. *Eur. J. Med. Chem.* **2003**, *38*, 223.

Table 3.3.3 Permeability results from the PAMPA-BBB assay for the rhein–huprine hybrids, with their predicted penetration into the CNS.

Compound	P_e (10^{-6} cm s^{-1})[a]	Prediction
(±)-**93a**·HCl	20.0 ± 1.0	CNS+
(±)-**93c**·HCl	27.5 ± 0.7	CNS+
(±)-**93d**·HCl	22.4 ± 1.3	CNS+
(±)-**93e**·HCl	21.5 ± 0.7	CNS+
(±)-**93f**·HCl	18.1 ± 0.7	CNS+
(±)-**93g**·HCl	16.4 ± 1.0	CNS+
(±)-**93h**·HCl	16.3 ± 2.5	CNS+

[a]Values are expressed as the mean ± SD of three independent experiments.

In the light of the outstanding multipotent pharmacological profile of these rhein−huprine hybrids, encompassing inhibitory activity against Aβ and tau aggregation, Aβ formation and cholinesterases, an *in vivo* study in a transgenic mouse model of AD has been indicated in collaboration with the group of Prof. Nibaldo Inestrosa at the *Pontificia Universidad Católica de Chile*.

3.4 Preparation of racemic capsaicin−huprine hybrids.

3.4.1 Interest of antioxidants for the treatment of AD.

The causes of AD remain unclear; however, it has long been proposed that abnormal metabolic oxidative reactions in the CNS might have a pathological role. Thus, besides the pathological hallmarks of the disease, namely, Aβ plaques and neurofibrillary tangles, AD brains exhibit constant evidence of ROS- and reactive nitrogen species (RNS)-mediated injury. These reactive species are formed during normal metabolic reactions, are usually instable from a chemical point of view and are highly reactive, for this reason, their levels are kept low by efficient antioxidant systems. Nevertheless, in some circumstances the production of oxidant species can exceed the endogenous antioxidant ability to destroy them and an oxidative imbalance occurs. This event results in cellular oxidative stress and subsequent molecular oxidative damage, which can translate into altered cellular functions and, as final result, cell death.[192,193]

Several lines of evidence have clearly demonstrated the importance of neuroinflammation and oxidative stress in the pathogenesis of AD. Among the major players involved in neuroinflammation are: Aβ, which is responsible for the generation of superoxide anion and α -carbon-centred radicals; cyclooxigenase (COX), which during its catalytic cycle produces both free radicals and prostaglandins; and inducible nitric oxide synthase (iNOS), which is responsible for the formation of nitric oxide (NO) RNS.[194,195] Furthermore, abundant data has implicated the role of biometals such as copper, iron and zinc in the Aβ aggregate deposition and neurotoxicity including the formation of ROS.[196] All the mentioned pro-oxidant species contribute to the massive destruction of some brain areas, in particular the entorhinal cortex and hippocampus in the early stage of AD. After several years, the damage spreads to the temporal, frontal and parietal cortex.[197]

In the past several years, on the basis of *in vitro* and *in vivo* studies in laboratory animals, natural antioxidants, such as capsaicin, **35**, curcumin, **38**, resveratrol, **94**, L-carnitine, **95**, and S-allylcysteine, **96** (Figure 3.4.1), have been proposed as alternative therapeutic agents for AD. An increasing number of studies demonstrate the efficacy of primary antioxidants, such as polyphenols, or secondary antioxidants, such as acetylcarnitine, to reduce or to block neuronal death occurring in the pathophysiology of this disorder. These studies revealed that other mechanisms than the antioxidant activities could be involved in the neuroprotective effect of these compounds.[194,198]

[192]Domenico, P. *Trends Pharmacol. Sci*. **2008**, *29*, 609. [193]Polidori, C.M. *J. Alzheimer's Dis.* **2004**, *6*, 185. [194]Mancuso, C.; Bates, T.E.; Butterfield, D.A.; Calafato, S.; Cornelius, C.; De Lorenzo, A.; Kostova, A.T.D.; Calabrese, V. *Expert Opin. Investig. Drugs* **2007**, *16*, 1921. [195]Mancuso, C.; Scapagnini, G.; Currò, D. *Front. Biosci*. **2007**, *12*, 1107. [196]Chen, S.-Y.; Chen Y.; Li, Y.-P.; Chen, S.-H.; Tan, J.-H.; Ou, T.-M.; Gu, L.-Q.; Huang, Z.-S. *Bioorg. Med. Chem*. **2011**, *19*, 5596. [197]Thompson, P.M.; Hayashi, K.M.; Dutton, R.A. *Ann. NY Acad. Sci.* **2006**, *8*, 1975. [198]Dairam, A.; Fogel, R.; Daya, S.; Limson, J.L. *J. Agric. Food Chem*. **2008**, *56*, 3350.

Indeed, it has been demonstrated that antioxidants and metal chelators may be of beneficial use in the treatment of these neurodegenerative disorders. Statistics confirm that there is a reduced incidence of AD among the inhabitants of Indian villages compared to the Western world.[199,200] It has been thought that this is due to the various antioxidant and neuroprotective components in the spices commonly consumed in the diet of this population group. These potential neuroprotective benefits conferred by some dietary components such as capsaicin, **35**, curcumin, **38**, or *S*-allylcysteine, **96**, which are the most active agents present in the Indian spices chilli, turmeric, and garlic, respectively, have been further investigated by studying their antioxidant and iron-binding properties. These natural antioxidants exhibited a high ability to scavenge free radicals, to reduce the production of superoxide anion in rat brain homogenate and also to chelate Fe^{2+} which diminishes the available Fe^{2+} that reduces H_2O_2 to the potent hydroxyl radical.[198]

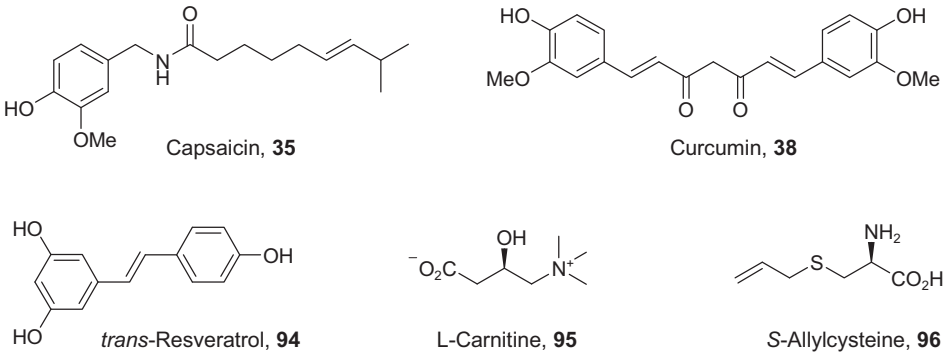


Figure 3.4.1 Chemical structure of natural antioxidants: capsaicin, **35**, curcumin, **38**, *trans*-resveratrol, **94**, carnitine, **95**, and *S*-allylcysteine, **96**.

3.4.2 Preparation of racemic capsaicin−huprine hybrids.

Taking into account the increasingly interest in the use of antioxidants as a potential treatment for AD, a novel series of multipotent dual binding site AChEIs, bearing a unit of racemic huprine Y as the active site interacting unit and a fragment derived from the natural antioxidant capsaicin, **35**, to interact with the peripheral site of AChE and to endow the hybrids with antioxidant properties, was envisaged. Overall, this novel family of compounds, namely capsaicin−huprine hybrids **97a−h** (Figure 3.4.2), should be endowed with a very interesting multipotent pharmacological profile, as they should be able to block the neurodegenerative cascade associated to AD at three different levels through the following mechanisms: inhibition of cholinesterases, blockade of Aβ aggregation and antioxidant activity.

[198]Dairam, A.; FOgel, R.; Daya, S.; Limson, J.L. *J. Agric. Food. Chem.* **2008**, *56*, 3350. [199]Ganguli, M.; Chandra, V.; Kamboh, M.I.; Johnston, J.M.; Dodge, H.H.; Thelma, B.K.; Juyal, R.C.; Pandav, R.; Belle, S.H.; DeKosty, S.T.; *Arch. Neurol.* **2000**, *57*, 824. [200]Lim, G.P.; Chu, T.; Yang, F.; Beech, W.; Fratschy, S. A.; Cole, G.M. *J. Neurosci.* **2001**, *21*, 8370.

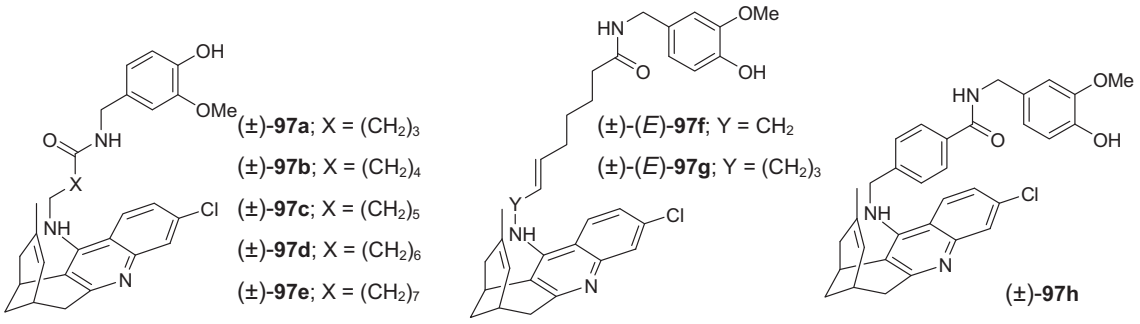
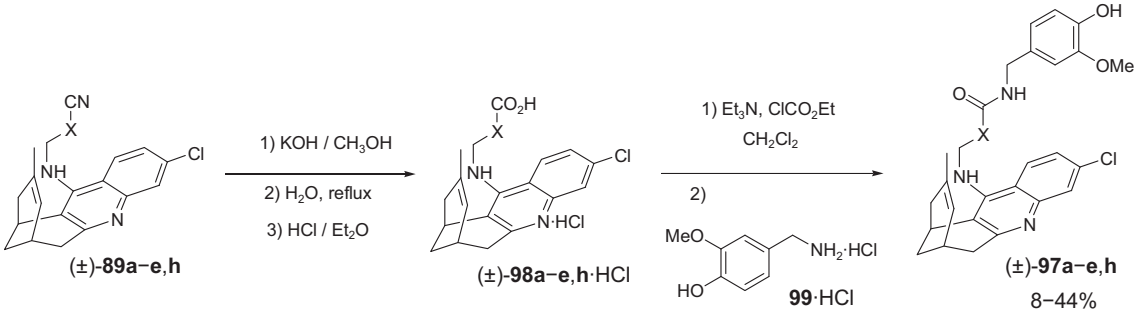


Figure 3.4.2 Structure of the novel capsaicin−huprine hybrids, **97a−h**.

Capsaicin−huprine hybrids bearing a saturated oligomethylene linker, (±)-**97a−e**, and an aromatic linker, (±)-**97h**, could be accessed by hydrolysis of nitriles (±)-**89a−e**,**h**, whose preparation had been set up for the synthesis of the rhein−huprine hybrids (Schemes 3.3.1, 3.3.2 and 3.4.1), followed by amide formation reaction of the resulting carboxylic acids, (±)-**98a−e**,**h**·HCl with commercially available 4-hydroxy-3-methoxybenzylamine hydrochloride, **99**·HCl. Thus, nitriles (±)-**89a−e**,**h** were treated with a 40% KOH MeOH solution and H_2O, heating under reflux overnight, followed by evaporation of the reaction mixtures and treatment of the residues with HCl / Et_2O to give the corresponding carboxylic acids (±)-**98a−e**,**h**·HCl that were used as crudes in the following step. Finally, (±)-**98a−e**,**h**·HCl were treated with freshly distilled Et_3N and $ClCO_2Et$ in CH_2Cl_2 at 0 ºC for 30 min and the resulting mixed anhydrides were reacted with 4-hydroxy-3-methoxybenzylamine hydrochloride, **99**·HCl, at r. t. for three days, to afford the desired hybrids, (±)-**97a−e**,**h**, in low to moderate overall yields, after a tedious silica gel column chromatography purification (Scheme 3.4.1).

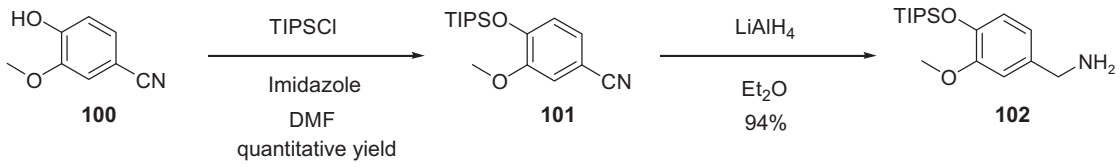


	(±)-**89**,**97**,**98a**	(±)-**89**,**97**,**98b**	(±)-**89**,**97**,**98c**	(±)-**89**,**97**,**98d**	(±)-**89**,**97**,**98e**	(±)-**89**,**97**,**98h**
X	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_5$	$(CH_2)_6$	$(CH_2)_7$	*p*-phenylene

Scheme 3.4.1

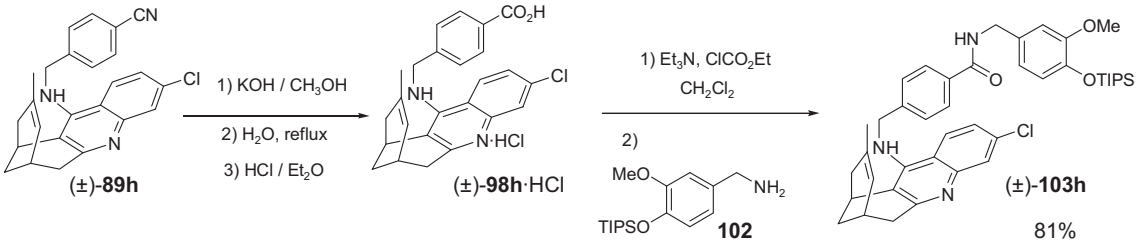
In order to improve the yield for the preparation of hybrids (±)-**97a−e**,**h**, to make the procedure more efficient in case the preparation of any of these compounds needed to be scaled up in a future, a protected form of the 4-hydroxy-3-methoxybenzylamine was prepared and coupled with the aromatic carboxylic acid (±)-**98h**·HCl. Thus, following a methodology

previously reported by Carr *et. al*.[201] 4-hydroxy-3-methoxybenzonitrile, **100**, was treated with TIPSCl in the presence of imidazole in DMF at r. t. overnight to give the silylether **101**, in quantitative yield, after purification of the reaction crude through silica gel column chromatography. Nitrile **101** was reduced with $LiAlH_4$ in Et_2O at r. t. for 2 h to give the corresponding amine, **102**, after filtering through Celite®, in 94% yield (Scheme 3.4.2).



Scheme 3.4.2

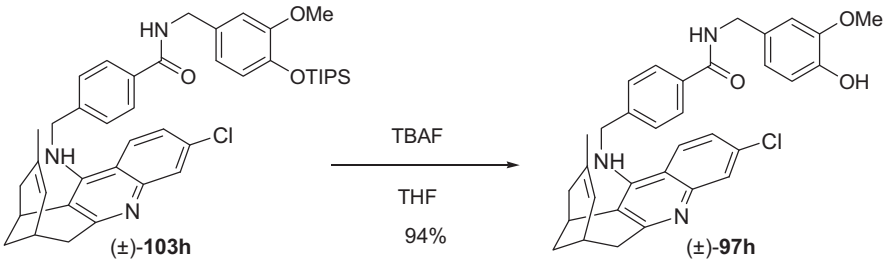
Following the same procedure as the previously described for the preparation of the unprotected hybrids, (±)-**97a**−**e**,**h**, the aromatic nitrile (±)-**89h** was hydrolyzed to the corresponding carboxylic acid (±)-**98h**·HCl which was treated with freshly distilled Et_3N and $ClCO_2Et$ in CH_2Cl_2 at 0 ºC for 30 min and the resulting mixed anhydride was reacted with **102** at r. t. for three days, to afford, after silica gel column chromatography purification, the protected hybrid (±)-**103h**, in 81% overall yield from nitrile (±)-**89h**, much higher than when the reaction had been carried out with the unprotected phenol **99** (33% yield from nitrile (±)-**89h**) (Scheme 3.4.1 and 3.4.3).



Scheme 3.4.3

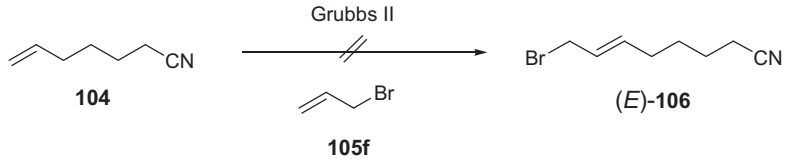
(±)-**103h** was finally deprotected with TBAF in THF at r. t. for 4 h to give hybrid (±)-**97h** in 94% yield and with no need of further purification. The overall yield for the preparation of (±)-**97h** following the synthetic route incorporating protection and deprotection of the capsaicin-related phenol was 72%, more than 2-fold higher than when (±)-**97h** was prepared straight from unprotected benzylamine **99** (Scheme 3.4.4).

[201]Carr, J.L.; Wease, K.N.; Van Ryssen, M.P.; Paterson, S.; Agate, B.; Gallaguer, K.A.; Brown, C.T.A.; Roderick, Scott, R.H.; Conway, S.J. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 208.



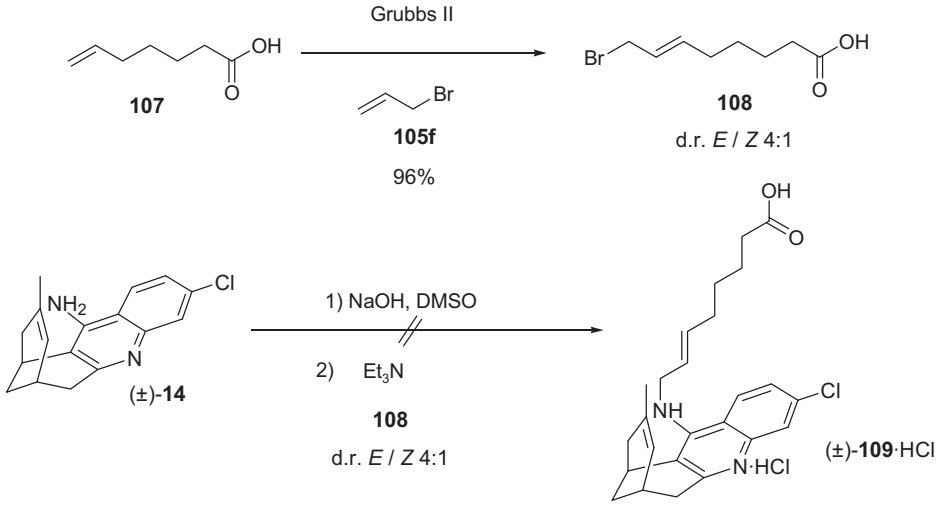
Scheme 3.4.4

For the preparation of capsaicin−huprine hybrids (±)-**97f**,**g**, bearing an unsaturated oligomethylene linker, a first attempt to prepare unsaturated ω-bromoalkenenitrile (*E*)-**106** from a cross metathesis reaction between 6-heptenenitrile, **104**, and allyl bromide, **105f**, in the presence of 10 mol % of Grubbs 2nd generation catalyst heating at 70 ºC overnight failed, giving only unreacted starting material and homo-reaction byproduct 1,4-dibromobutene (Scheme 3.4.5). [202,203]



Scheme 3.4.5

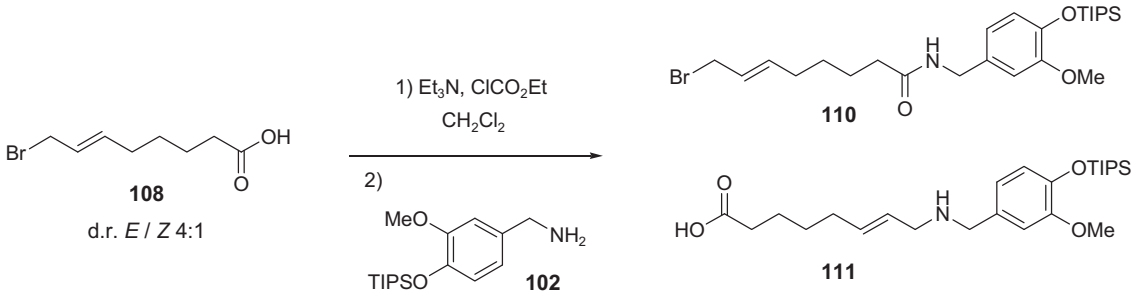
When the same reaction conditions were applied to 6-heptenoic acid, **107**, the desired cross metathesis reaction product, **108**, was obtained in 96% yield in a diastereomeric ratio (d.r.) *E* / *Z* 4:1, after silica gel column chromatography purification. However, treatment of racemic huprine Y, (±)-**14**, with unsaturated bromo acid **108**, in the presence of NaOH and Et_3N in DMSO overnight gave only unreacted starting materials (Scheme 3.4.6).



Scheme 3.4.6

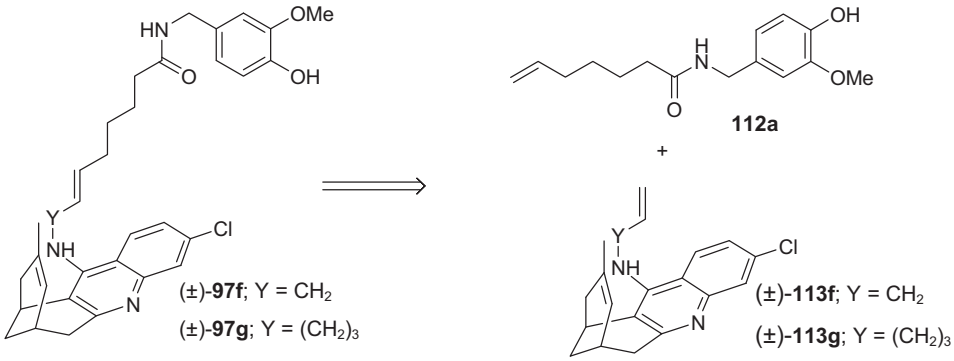
[202]Grubbs, R.H. *Tetrahedron* **2004**, *60*, 7117. [203]Chatterjee, A.K.; Choi, T.-L.; Sanders, D.; Grubbs, R.H. *J. Am. Chem. Soc.* **2002**, *125*, 11630.

An attempt to access the capsaicin-related alkylating agent **110** through amide formation reaction between bromo acid **108** and protected benzylamine **102** gave mainly impure acid **111**, formed by reaction at the more electrophylic allylic position of **108** (Scheme 3.4.7).



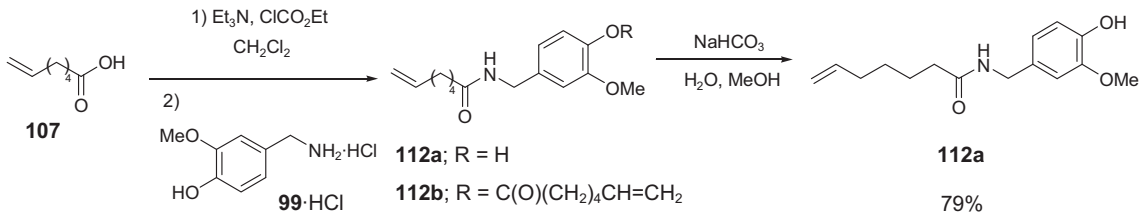
Scheme 3.4.7

Finally, a direct cross metathesis reaction between olefins (±)-**113f** or (±)-**113g** with **112a** catalyzed by different Grubbs or Grubbs-Hoveyda catalysts was envisaged (Scheme 3.4.8).



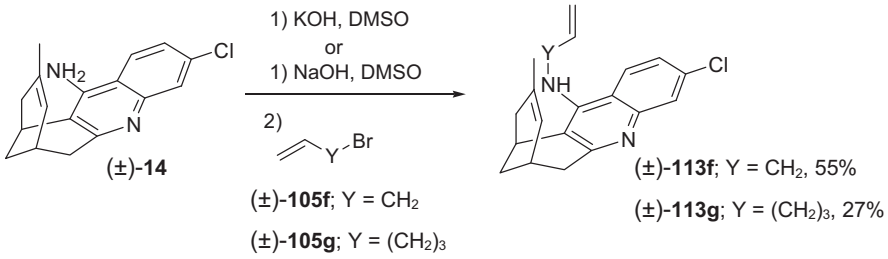
Scheme 3.4.8

6-Heptenoic acid, **107**, was treated with freshly distilled Et_3N and $ClCO_2Et$ in CH_2Cl_2 at 0 ºC for 30 min and the resulting mixed anhydride was reacted with **99**·HCl at r. t. for three days, to afford a mixture of the desired amide **112a** and approximately a 10% of the amide-ester byproduct **112b** (determined by ^{1}H-NMR). The ester group of byproduct **112b** was selectively hydrolyzed by treatment of the mixture **112a** / **112b** with a saturated $NaHCO_3$ aqueous solution, in MeOH at r. t. overnight to provide the desired amide **112a** in 79% overall yield (Scheme 3.4.9).



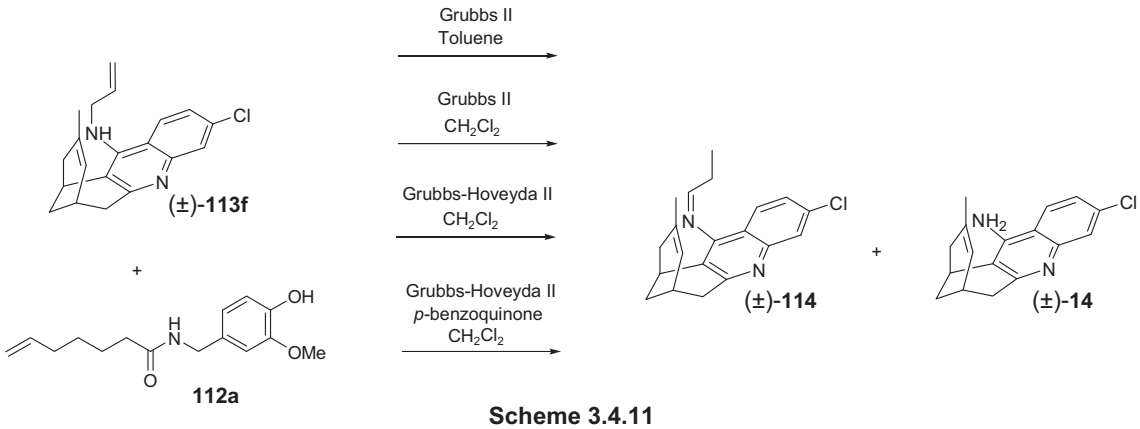
Scheme 3.4.9

(±)-Huprine Y, (±)-**14**, was separately reacted with allyl bromide, **105f**, and 5-bromo-1-pentene, **105g**, in the presence of KOH and NaOH, respectively, at r. t. in DMSO overnight to give, after purification through silica gel column chromatography of the corresponding crudes, (±)-**113f** and (±)-**113g**, in 55% and 27% isolated yield, respectively (Scheme 3.4.10).



Scheme 3.4.10

Cross metathesis reaction between olefins **112a** and (±)-**113f** turned out to be a difficult task. Different attempts using a variety of conditions (Scheme 3.4.11) that had been reported in the literature,[204−206] afforded only the isomerisation byproduct (±)-**114** and (±)-huprine Y, (±)-**14**, likely formed by hydrolysis of the imine (±)-**114** (Scheme 3.4.11).

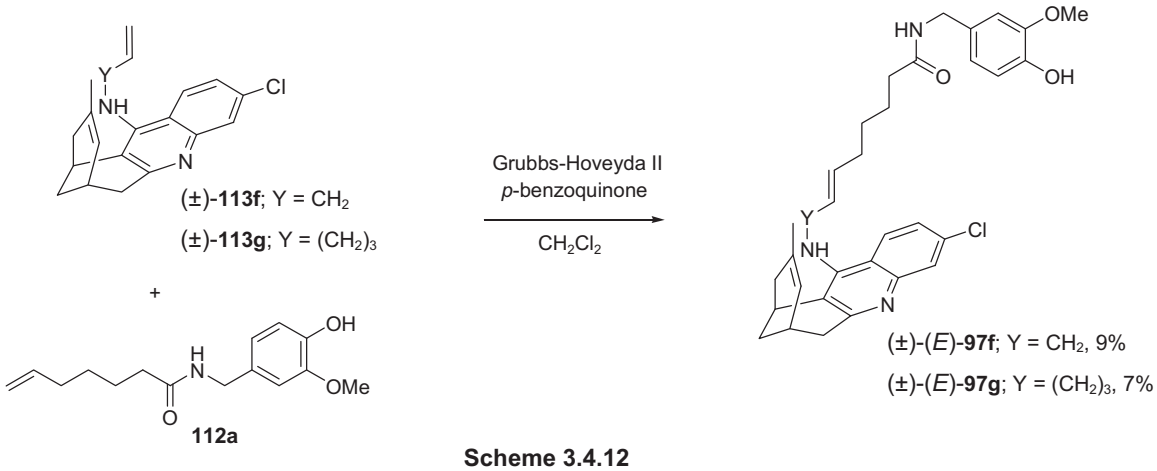


Scheme 3.4.11

In order to prevent undesirable isomerisation during olefin metathesis, following a methodology recently described by Grubbs *et al*.[207] 10 mol % *p*-benzoquinone was incorporated to the reaction mixture. However, when the reaction was carried out using 3 mol % Grubbs 2nd generation catalyst and 10 mol % *p*-benzoquinone, in CH_2Cl_2, heating under reflux overnight, again isomerisation byproduct (±)-**114** and (±)-huprine Y, (±)-**14**, were recovered in 51% and 23% yield, respectively (Scheme 3.4.11). Finally, olefins (±)-**113f** and (±)-**113g** were separately reacted with **112a** in the presence of 5 mol % Grubbs-Hoveyda 2nd generation catalyst and 10 mol % *p*-benzoquinone, heating under reflux overnight to provide, after a tedious purification through silica gel column chromatography, (±)-(*E*)-**97f** and (±)-(*E*)-**97g**, albeit in very low yields, 9% and 7% yield, respectively (Scheme 3.4.12). Only the *E* diastereoisomer was isolated, as

[204]Roychowdhury, A.; Wolfert, M.A.; Boons, G.-J. *ChemBioChem* **2005**, *6*, 2088. [205]Ghera, B.B.; Fache, F.; Parrot-López, H. *Tetrahedron* **2006**, *62*, 4807. [206]Bradshaw, B.; Etxebarria-Jardí, G.; Bonjoch, J. *J. Am Chem Soc.* **2010**, *132*, 5966. [207]Hong, S.H.; Sanders, D.P.; Lee, C.W.; Grubbs, R.H. *J. Am. Chem. Soc.* **2005**, *127*, 17160.

evidenced by the vicinal coupling constant of 15.6 Hz between the two olefin protons. In order to improve the yields, the reaction of (±)-**113f** and **112a** was also assayed in toluene under reflux overnight, however, an inseparable mixture of (±)-(*E*)-**97f** / (±)-**14** in an aproximate ratio 72:28 (determined by ^{1}H-NMR) was obtained, indicating again partial isomerisation of (±)-**113f** followed by hydrolysis of the resulting imine.



Scheme 3.4.12

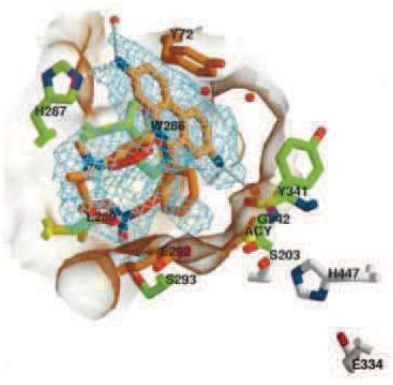
Capsaicin−huprine hybrids (±)-**97a−h** were transformed to the corresponding hydrochlorides and have been fully characterized and are being pharmacologicaly evaluated. Drs. Andrisano and Bartolini from the *Università di Bologna* are determinig IC_{50} values toward hAChE and also the percentages of inhibition toward AChE-induced and self-induced Aβ aggregation, whereas Dr. Luciano Saso at the *Università di Roma La Sapienza* is determining the antioxidant activity of hybrids (±)-**97a−h**.

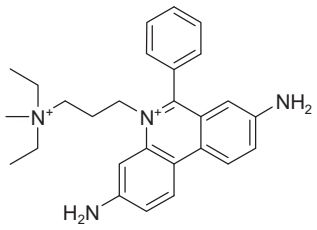
3.5 Preparation of pyrido[3,2-*c*]quinolines as a novel family of peripheral site AChEIs.

3.5.1 Precedents in the preparation of propidium-related dual binding site AChEIs in our research group.

As previously mentioned, in the past decade, the design of novel classes of AChEIs as therapeutic interventions for AD has been mostly driven by the pivotal finding that AChE can bind the Aβ, thereby promoting Aβ aggregation as an early event in the neurodegenerative cascade of AD.[87,88] Blockade of the peripheral site of AChE, the Aβ recognition zone within the enzyme,[94] was therefore expected to affect the AChE-induced Aβ aggregation and could be a potential strategy to modulate the progression of AD. On the basis of these premises, novel classes of dual binding site AChEIs have emerged as promising disease-modifying anti-Alzheimer drug candidates.[3]

The design of this kind of inhibitors is usually carried out by linking through a tether of suitable length an active site interacting unit, usually derived from a known active site AChEI, with a peripheral site interacting unit suited to interact with Trp286 (hAChE residue numbering). With a few exceptions in which a peripheral-site interacting unit containing an aliphatic amine, protonatable at physiological pH, or a quaternary ammonium group which establishes cation−π interactions with Trp286, in most cases the peripheral-site interacting unit contains aromatic moieties able to establish π−π stacking interactions with Trp286, in some cases reinforced by concomitant cation−π interactions due to the presence of protonatable or quaternary nitrogen atoms in the aromatic system. The prototype of peripheral site AChEI is propidium, **6** (Figure 3.5.1) which binds the AChE peripheral site in two orientations related by a flip of 180º around the phenanthridinium pseudosymmetry axis (Figure 3.5.1).[208,209] The driving force for the binding of propidium to the peripheral site is the π−π stacking, reinforced by cation−π interactions, between the phenanthridinium moiety and Trp286, which is supplemented by a hydrogen bond between one of the aromatic amino groups and His287.



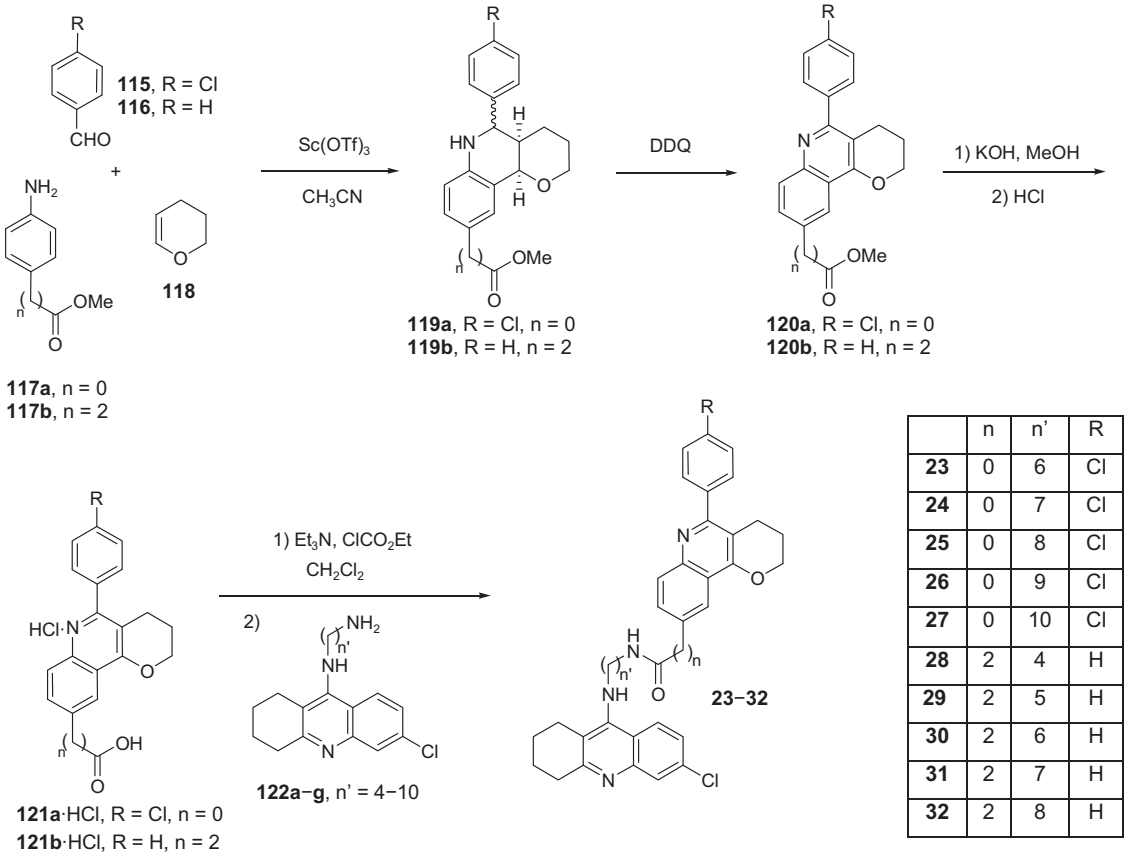


propidium, **6**

Figure 3.5.1 Binding mode of propidium to the peripheral site of mAChE and structure of propidium, **6**.

[3]Muñoz-Torrero, D. *Curr. Med. Chem.* **2008**, 15, 2433. [87]Inestrosa, N.C.; Alvarez, A.; Pérez, C.A.; Moreno, R.D.; Vicente, M.; Linker, C.; Casanueva, O.I.; Soto, C.; Garrido, C. *Neuron* **1996**, *16*, 81. [88]Alvarez, A.; Alarcón, R.; Opazo, C.; Campos, E.O.; Muñoz, F.J.; Calderón, F.H.; Dajas, F.; Gentry, M.K.; Doctor, B.P.; De Mello, F.G.; Inestrosa, N.C. *J. Neurosci.* **1998**, *18*, 3213. [94]De Ferrari, G.V.; Canales, M.A.; Shin, I.; Weiner, L.M.; Silman, I.; Inestrosa, N.C. *Biochemistry* **2001**, *40*, 10447. [208]Bourne, Y.; Taylor, P.; Radic, Z.; Marchot, P. *EMBO J.* **2003**, *22*, 1. [209]Cavalli, A.; Bottegoni, G.; Raco, C.; De Vivo, M.; Recanatini, M. A. *J. Med. Chem.* **2004**, 47, 3991.

In 2009, in the context of the PhD Thesis work of Dr. Carles Galdeano and the Experimental Master of Lorena Ramírez, in collaboration with Dr. Rodolfo Lavilla at the *Universitat de Barcelona*, a novel series of dual binding site AChEIs was developed, namely the pyrano[3,2,c] quinoline−6-chlorotacrine hybrids, **23−32** (Figure 1.16 and Scheme 3.5.1). The preparation of these compounds was accomplished following the synthetic pathway depicted in Scheme 3.5.1, which consisted of the coupling of tricyclic esters **120a** and **120b** with aminoalkyltacrines **122a−g**. Straightforward synthesis of **120a**,**b** was carried out through a Povarov-type multicomponent reaction (MCR).[210] Thus, reaction of 3,4-dihydro-2H-pyran, **118**, with anilines **117a**,**b**, and aldehydes **115** or **116** under $Sc(OTf)_3$ catalysis in CH_3CN afforded a diastereomeric mixture of pyranotetrahydroquinolines **119a**,**b**, whose DDQ oxidation yielded the desired tricyclic esters **120a**,**b** (Scheme 3.5.1). Aminoalkyltacrines **122a−g** were synthesized following a procedure that involves amination of the corresponding 6,9-dichloroacridine with α,ω-diamines in refluxing 1-pentanol. Hydrolysis of esters **120a**,**b** followed by treatment of the corresponding carboxylic acids **121a**,**b** with Et_3N and $ClCO_2Et$ in CH_2Cl_2, and reaction of the resulting mixed anhydrides with 1 equiv of aminoalkyltacrines **122a−g** afforded hybrids **23−32**.[132,139−140]



	n	n'	R
23	0	6	Cl
24	0	7	Cl
25	0	8	Cl
26	0	9	Cl
27	0	10	Cl
28	2	4	H
29	2	5	H
30	2	6	H
31	2	7	H
32	2	8	H

Scheme 3.5.1

[132]Galdeano C. PhD thesis. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2012**. [139]Ramírez, L. Experimental Master. *Unitat de Química Farmacèutica, Departament de Farmacologia i Química Terapèutica. Universitat de Barcelona*, **2008**. [140]Camps, P.; Formosa, X.; Galdeano, C.; Muñoz-Torrero, D.; Ramírez, L.; Gómez, E.; Isambert, N.; Lavilla, R.; Badia, A.; Clos, M.V.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Huertas, O.; Dafni, T.; Luque, F.J. *J. Med. Chem.* **2009**, *52*, 5365. [210]Povarov, L. S. *Russ. Chem. Rev*. **1967**, *36*, 656.

These novel hybrids were fully pharmacologically evaluated toward cholinesterases and Aβ aggregation and formation and turned out to be potent AChE inhibitors with IC_{50} values in the low nanomolar range (IC_{50} = 7−50 nM), they also exhibited a significant *in vitro* inhibitory activity toward the AChE-induced Aβ aggregation (23−46% inhibition at 100 μM concentration of inhibitor) and also proved to be able to inhibit self-induced Aβ aggregation (12−49% inhibition at 50 μM concentration of inhibitor). Moreover, they also inhibited BACE-1 (14−78% inhibition at 2.5 μM concentration of inhibitor).[140]

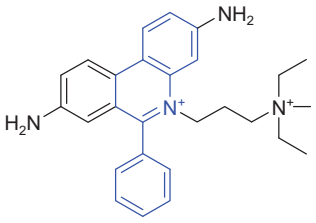
Molecular dynamic simulations carried out by Dr. F. Javier Luque confirmed the dual binding site character of these compounds, and, particularly the ability of the pyrano[3,2-*c*]quinoline moiety, non protonated at physiological pH, to establish *π*−*π* stacking interactions with the peripheral site Trp286 residue.

3.5.2 Preparation of peripheral site AChEIs.

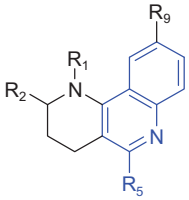
Given the excellent pharmacological profile exhibited by pyrano[3,2-*c*]quinoline−6-chlorotacrine hybrids **23**−**32** (Scheme 3.5.1) and taking into account the increasing interest in the use of MCR as a new methodology for the synthesis of complex and structurally diverse compound libraries which can be suitable for medicinal chemistry purposes,[211] during the course of the present PhD Thesis work a collaboration was again established with Dr. Rodolfo Lavilla of the *Universitat de Barcelona*, in order to design and synthesize a series of peripheral site AChEIs, structurally related to the pyrano[3,2-*c*]quinoline scaffold of **23**−**32** but with a better interaction at the peripheral site of AChE, which could be accessed through a Povarov-type MCR.[212−214]

As it has been mentioned in the previous section, peripheral site AChEIs usually interact with Trp286, through *π*−*π* stacking interactions that can be reinforced through cation−*π* interactions established between a positively charged nitrogen atom and the indole moiety of Trp286, as it is the case of propidium, **6**. With this in mind, and in order to increase the basicity of the quinolinic nitrogen atom present in the pyrano[3,2,c]quinoline moiety of hybrids **23**−**32**, a new series of pyrido[3,2-*c*]quinolines, **123**−**132** (Figure 3.5.2), that could be also accessed through a Povarov-type MRC followed by oxidation of the resulting diastereomeric mixture, was envisaged. (Scheme 3.5.3). It is important to note that in the results and discussion section of the present PhD Thesis, the novel series of peripheral site inhibitors will be named pyrido[3,2-*c*]quinoline instead of tetrahydrobenzo[*h*][1,6]naphthyridine to keep the analogy with the previous series pyrano[3,2-*c*]quinoline.

[140]Camps, P.; Formosa, X.; Galdeano, C.; Muñoz-Torrero, D.; Ramírez, L.; Gómez, E.; Isambert, N.; Lavilla, R.; Badia, A.; Clos, M.V.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Huertas, O.; Dafni, T.; Luque, F.J. *J. Med. Chem.* **2009**, *52*, 5365. [211]Orru, R.V.A.; Ruijter, E. (Eds.). Series *Topics in Heterocyclic Chemistry*, vol. *25*, Springer, **2010**. [212]Vicente-García, E.; Catti, F.; Ramón, R.; Lavilla, R. *Org. Lett*. **2010**, *12*, 860. [213]Vicente-García, E.; Ramón, R.; Lavilla, R. *Synthesis* **2011**, *14*, 2237. [214]Vicente-García, E.; Ramón, R.; Preciado, S.; Lavilla, R. *Beilstein J. Org. Chem*. **2011**, *7*, 980.



Propidium, **6**

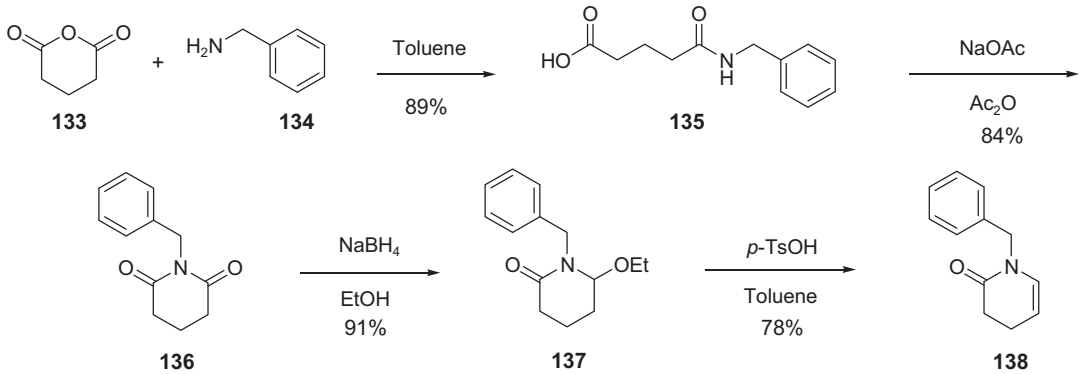


123–132

	R_1	R_2	R_5	R_9		R_1	R_2	R_5	R_9
123	Bn	O	*p*-chlorophenyl	CO_2Et	**128**	Bn	H,H	3-pyridyl	CO_2Et
124	Bn	O	*p*-methoxycarbonylphenyl	CO_2Et	**129**	Bn	H,H	*p*-chlorophenyl	C(O)NHEt
125	Bn	O	3-pyridyl	CO_2Et	**130**	Bn	H,H	*p*-chlorophenyl	CH_2NHEt
126	Bn	H,H	*p*-chlorophenyl	CO_2Et	**131**	H	H,H	*p*-chlorophenyl	CO_2Et
127	Bn	H,H	*p*-methoxycarbonylphenyl	CO_2Et	**132**	PMB	H,H	*p*-chlorophenyl	CO_2Et

Figure 3.5.2 Structure of prototype peripheral site AChEI propidium, **6**, and novel pyrido[3,2-*c*]quinolines, **123–132**

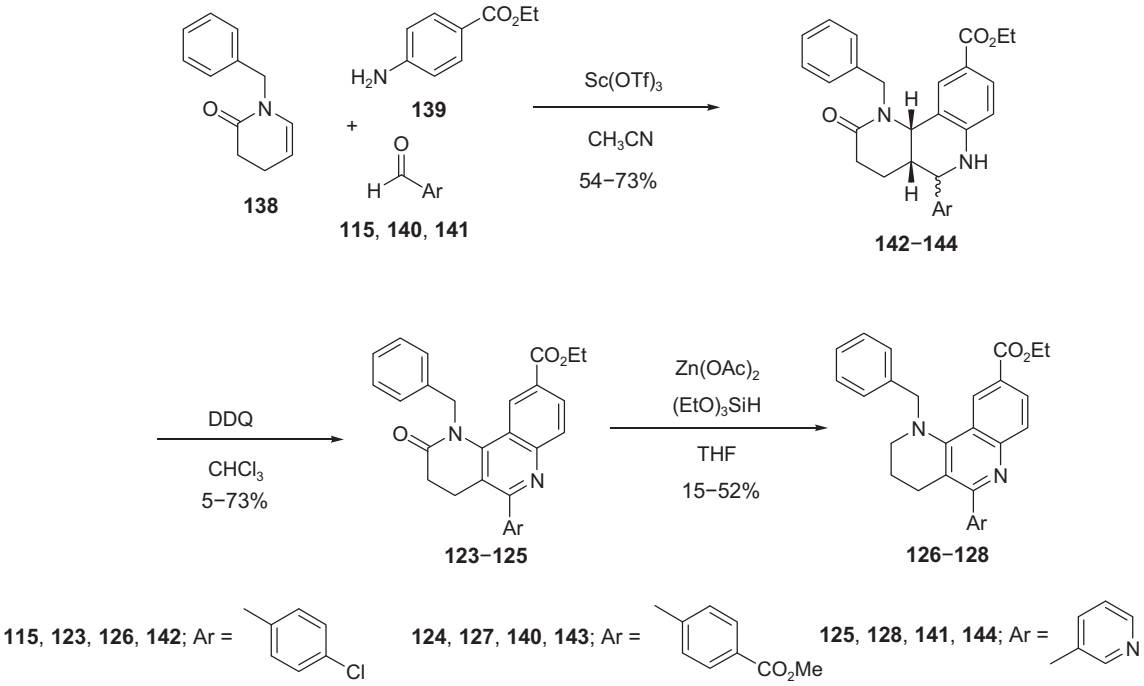
Compounds **123–132** were accessed following the synthetic pathway depicted in Scheme 3.5.3, which first required preparation of unsaturated lactam **138** as starting material for the Povarov-type MCR. Lactam **138** was successfully prepared following a described procedure.[215–219] Thus, reaction between glutaric anhydride, **133**, and freshly distilled benzylamine, **134**, in refluxing toluene overnight, gave the amido carboxylic acid **135** in 89% yield with no need of purification. Imide **136** was prepared by intramolecular cyclization from **135** in the presence of NaOAc and Ac_2O at 80 ºC overnight, in 84% yield after silica gel column chromatography purification of the resulting crude. Imide **136** was subjected to $NaBH_4$ reduction in EtOH at –20 ºC for 4 h which afforded **137** in 91% yield with no need of purification. Subsequent dehydration of **137** with *p*-TsOH in refluxing toluene, using a Dean-Stark equipment, gave the unsaturated lactam **138** in 78% yield, again with no need of purification (Scheme 3.5.2).



Scheme 3.5.2

[215]Yu, J.; Truc, V.; Riebel, P.; Hierl, E.; Mudryk, B. *Tetrahedron* **2005**, *45*, 4011. [216] Galbo, F.L.; Cochiato, E.G.; Guarna, A.; Faggi, C. *J. Org. Chem*. **2003**, *68*, 6360. [217]Khan, M.M.; Melmon, K.L.; Egli, M.; Lok, S.; Goodman, M. *J. Med. Chem.* **1987**, *30*, 2115. [218]Yoshifuji, S.; Arakawa, Y.; Nitta, Y. *Chem. Pharm. Bull*. **1987**, *35*, 357. [219]Padwa, A.; Rashatasakhon, P.; Rose, M. *J. Org. Chem*. **2003**, *68*, 5139.

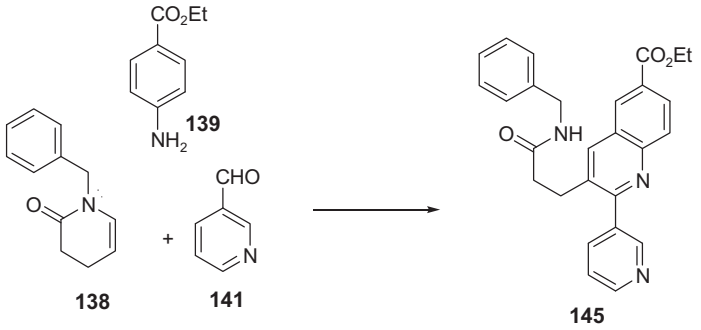
The Povarov reaction of aniline **139** with **138** and aromatic aldehydes **115**, **140** and **141**, under $Sc(OTf)_3$ catalysis in CH_3CN at r. t. for 3 days afforded, after silica gel column chromatography purification of the resulting reaction crudes, a diastereomeric mixture of tetrahydroquinolines **142**−**144**, in moderate to good yields. DDQ oxidation of the diastereomeric mixtures of tetrahydroquinolines **142** and **143** allowed preparation of quinolines **123** and **124** in 73% and 36% yields, respectively, after silica gel column chromatography purification of the corresponding reaction crudes. However DDQ oxidation of tetrahydroquinoline **144**, bearing a 3-pyridyl substituent at position 5, gave quinoline **125**, after a tedious chromatography purification of the reaction crude in only 5% yield, providing mainly byproduct **145**. When the reaction was assayed using MnO_2 as the oxidising agent only starting material and byproduct **145** were observed (Scheme 3.5.4)



Scheme 3.5.3

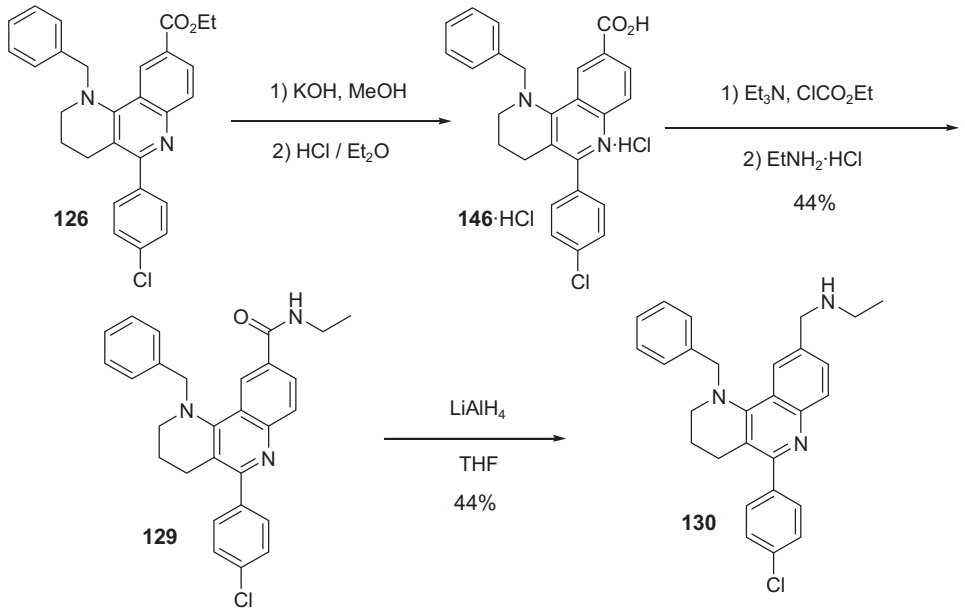
Finally, reduction of lactames **123**−**125** to the corresponding desired pyridooquinolines **126**−**128** was carried out using a methodology that selectively reduced the amide carbonyl group without affecting the ester,[220] which required treatment with $(EtO)_3SiH$ under $Zn(OAc)_2$ catalysis, in THF at 65 ºC in a sealed vessel for two days, and provided **126**−**128** in low to moderate yields, after silica gel column chromatography purification of the reaction crudes (Scheme 3.5.3).

[220]Das, S.; Addis, D.; Zhou, S.; Junge, K.; Beller, M. *J. Am. Chem. Soc*. **2010**, *132*, 1770.



Scheme 3.5.4

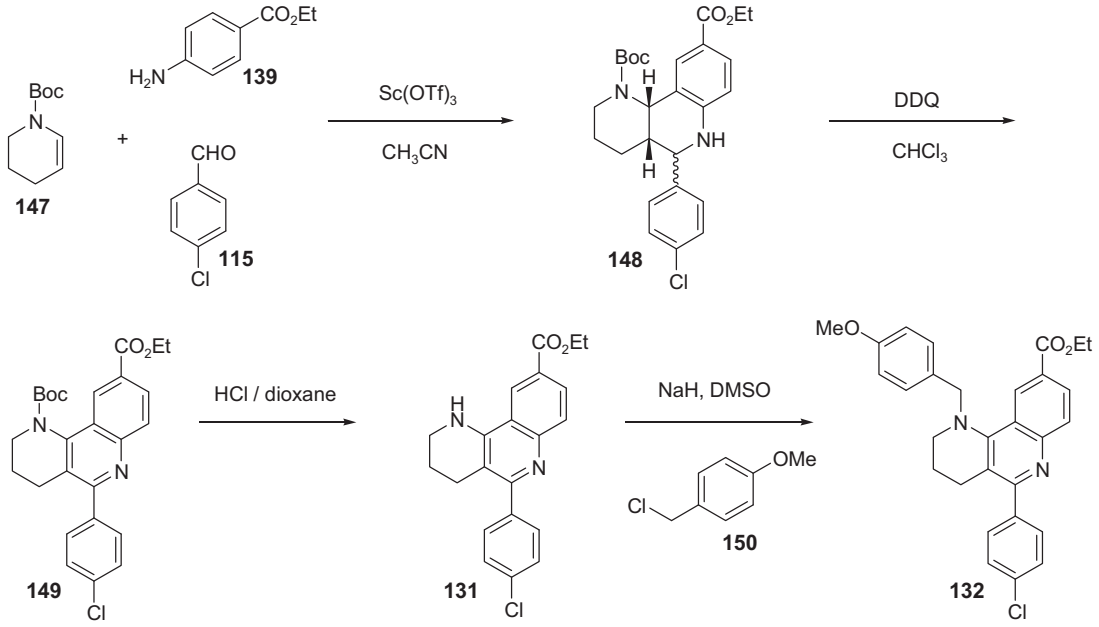
Pyrido[3,2-*c*]quinolines **129** and **130**, bearing a *N*-ethylcarbamoyl and *N*-ethylaminomethyl substituents at position 9, were accessed from pyridoquinoline **126**. Thus, ester **126** was treated with a 40% solution of KOH in MeOH and H_2O, heating under reflux overnight, followed by evaporation of the reaction mixture and treatment of the residue with HCl / Et_2O to give the corresponding carboxylic acid, **146**·HCl, that was used as a crude in the next step. **146**·HCl was treated with freshly distilled Et_3N and $ClCO_2Et$ in CH_2Cl_2 at 0 ºC for 30 min and the resulting mixed anhydride was reacted with ethylamine hydrochloride, at r. t. for three days, to afford the desired amide **129**, in 44% overall yield from **126**, after a tedious silica gel column chromatography purification (Scheme 3.5.5). Finally, **129** was reduced with $LiAlH_4$ in THF, heating under reflux overnight to provide, after column chromatography purification of the reaction crude, amine **130** in 44% yield (Scheme 3.5.5).



Scheme 3.5.5

Worthy of note, in the context of the PhD Thesis work of Dr. Esther Vicente of the group of Dr. Rodolfo Lavilla, compounds **131** and **132** were also prepared, following a similar synthetic route to that used for the preparation of **123**−**128** (Scheme 3.5.3) but starting from commercially available *N*-Boc-tetrahydropyridine, **147**, which was reacted with aniline **139** and *p*-

chlorobenzaldehyde, **115**, followed by DDQ oxidation of the diastereomeric mixture of tetrahydroquinolines **148**, afforded **149**. **149** was deprotected with HCl in dioxane to give the desired pyridoquinoline **131**. Finally, the anilinic nitrogen of **131** was alkylated with *p*-methoxybenzyl chloride in the presence of NaH and DMSO to give quinoline **132** (Scheme 3.5.6.).



Scheme 3.5.6

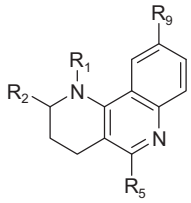
3.5.3 Pharmacological evaluation and optimization of pyrido[3,2-c]quinolines 123−132.

3.5.3.1 Activity toward cholinesterases.

The AChE inhibitory activity of compounds **123−132** toward eeAChE was determined by Drs. Badia and Clos using the method of Ellman *et al.*[176] (Table 3.5.1). Considering that **123−132** were designed to interact with the peripheral site of AChE, they were expected to show rather low AChE inhibitory activities, with IC_{50} values in the same range as that of prototype peripheral site inhibitor propidium, **6** [IC_{50}(hAChE) = 32.3 μM].[221] However, **123−132** turned out to be potent eeAChE inhibitors with IC_{50} values in the low micromolar, and, in some cases, nanomolar range. Nevertheless, caution should be taken in making a strict comparison with the hAChE IC_{50} value reported for propidium as the AChE source was different.

[176]Ellman, G.L.; Courtney, K.D.; Andres, B. Jr.; Featherstone, R.M. *Biochem. Pharmacol.* **1961**, *7*, 88. [221]Bolognesi, M. L.; Andrisano, V.; Bartolini, M.; Banzi, R.; Melchiorre, C. *J. Med. Chem.* **2005**, *48*, 24.

Table 3.5.1 eeAChE, hBChE and self-induced Aβ aggregation inhibitory activities of **123**−**132**.[a,b]



Compound	R_1	R_2	R_5	R_9	IC_{50} (μM) eeAChE[a]	IC_{50} (nM) hBChE[a]	Self-induecced $A\beta_{1-42}$ aggregation (%)[b]
123	Bn	O	*p*-chlorophenyl	CO_2Et	5.21 ± 0.33	>100.	5.0
124	Bn	O	*p*-CO_2MePh	CO_2Et	13.61 ± 1.85	>100	4.2
125	Bn	O	3-pyridyl	CO_2Et	>100	>100	5.2
126	Bn	H,H	*p*-chlorophenyl	CO_2Et	6.33 ± 1.85	>100	0.5
127	Bn	H,H	*p*-CO_2MePh	CO_2Et	6.62 ± 0.62	>100	10.5
128	Bn	H,H	3-pyridyl	CO_2Et	1.97 ± 0.17	*nd*	8.0
129	Bn	H,H	*p*-chlorophenyl	C(O)NHEt	5.48 ± 0.512	>100.	10.5
130	Bn	H,H	*p*-chlorophenyl	CH_2NHEt	0.147 ± 0.01	*n. d.*	3.6
131	H	H,H	*p*-chlorophenyl	CO_2Et	0.281 ± 0.03	>100	5.7
132	PMB	H,H	*p*-chlorophenyl	CO_2Et	>100	>100	1.9

[a]Values are expressed as the mean ± SEM of at least four experiments; IC_{50}: inhibitory concentration (μM) of *Electrophorus electricus* AChE or human serum BChE activity. *na* means not active. *nd* means not determined. [b]Inhibitor concentration: 10 μM ([Aβ] / [I] = 5:1).

Some clear structure–activity relationship trends could be extracted from the data in Table 3.5.1. Aminoquinolines **126**−**128** were more potent than their oxidized analogues, amidoquinolines **123**−**125**, which, except for **126** which was roughly equipotent to its oxidized analogue **123**, **127** and **128** were 2-fold and 50-fold more potent than the corresponding oxidized analogues **124** and **125**, respectively. This trend was already expected due to the increase in the basicity of the pyridinic nitrogen in the aminoquinoline system in comparison to the amidoquinoline moiety, which should facilitate the cation−π interaction with the peripheral site. Moreover, introduction of a second protonatable at physiological pH nitrogen atom was favourable for the eeAChE inhibitory activity. Thus, **128**, bearing a 3-pyridyl substituent at position 5 was 3-fold more potent than its *p*-chlorophenyl analogue, **126**; and **130**, incorporating a *N*-ethylaminomethyl substituent at position 9 was 45-fold more potent than its ethoxycarbonyl analogue, **126**.

Furthermore, debenzylation of the anilinic nitrogen also increased AChE inhibitory activity, **131** being 22-fold more potent than its benzylated analogue, **126**. Introduction of a *p*-methoxybenzyl substituent at the anilicic nitrogen was detrimental, **132** being more than 15-fold less potent than its *N*-benzyl analogue **126** and 350-fold less potent than its debenzylated analogue **131**.

Overall, the most active compound of the series was **130**, bearing a *N*-ethylaminomethyl substituent at position 9, a *p*-chlorophenyl at position 5 and a benzyl substituent on the anilinic nitrogen. However, it seemed likely that the structural characteristics that should confer the highest AChE inhibitory activity should be a *N*-ethylaminomethyl substituent at position 9, a 3-pyridyl substituent at position 5 and a debenzylated anilinic nitrogen. With this in mind, a second generation of aminoquinolines was designed and synthesized in the context of the PhD Thesis works of Ornella Di Pietro and Dr. Esther Vicente and will also be reviewed in section 3.5.2.3.

Finally, compounds **123−132** did not exhibit activity toward hBChE. This was *a priori* unexpected as propidium, **6**, is 2-fold more potent inhibitor of hBChE than hAChE [IC_{50}(hBChE) = 13.2 μM].[221]

3.5.3.2 Activity toward Aβ aggregation.

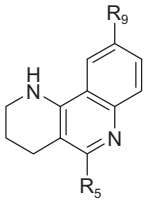
Taking into account that compounds **123−132** were designed to interact with the peripheral site of AChE, they should, therefore, be endowed with an AChE-induced Aβ anti-aggregating effect. With this in mind, Drs. Bartolini and Andrisano determined the ability of compounds **123−132** ability to inhibit self-induced Aβ aggregation and will soon be determining AChE-induced Aβ aggregation.

Compounds **123−132** inhibited self-induced Aβ aggregation with low percentages of inhibition ranging from 0.5% to 10.5% using 10 μM concentration of inhibitor, in all cases lower than that of propidium, **6**, which exhibited a 90% of inhibition of the self-induced Aβ aggregation using 10 μM concentration of inhibitor.[140]

3.5.3.3 Optimization of pyrido[3,2-c]quinolines 123−132 as AChEIs.

Taking into account the unexpected by high eeAChE inhibitory activity of compounds **123−132**, in the context of the PhD Thesis works of Ornella Di Pietro and Dr. Esther Vicente a second generation of pyrido[3,2-*c*]quinolines was envisaged, which would combine the structural features that conferred the highest activities in the previous series. Ornella Di Pietro and Dr. Esther Vicente prepared compounds **151−154** (Figure 3.5.3) using the same methodology depicted in Schemes 3.5.5 and 3.5.6. These second generation of Povarov-type MCR compounds would incorporate a *N*-ethylaminomethyl substituent at position 9, a 3-pyridyl substituent at position 5 and a debenzylated anilinic nitrogen.

[140]Camps, P.; Formosa, X.; Galdeano, C.; Muñoz-Torrero, D.; Ramírez, L.; Gómez, E.; Isambert, N.; Lavilla, R.; Badia, A.; Clos, M.V.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M.P.; Rodríguez-Franco, M.I.; Huertas, O.; Dafni, T.; Luque, F.J. *J. Med. Chem*. **2009**, *52*, 5365. [221]Bolognesi, M. L.; Andrisano, V.; Bartolini, M.; Banzi, R.; Melchiorre, C. *J. Med. Chem*. **2005**, *48*, 24



	R_9	Ar
151	CO_2Et	3-pyridyl
152	C(O)NHEt	*p*-chlorophenyl
153	CH_2NHEt	*p*-chlorophenyl
154	CH_2NHEt	3-pyridyl

Figure 3.5.3 Structure of second generation pyrido[3,2-*c*]quinolines **151**−**154**

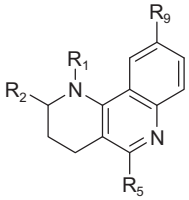
Drs. Badia and Clos evaluated compounds **151**−**154** toward eeAChE and hBChE. Second generation pyrido[3,2-*c*]quinolines were, in general more potent eeAChEIs than the first generation compounds (Table 3.5.2), all exhibiting IC_{50} values in the nanomolar range. Thus, they could be considered potent eeAChEIs. In general, debenzylation of the anilinic nitrogen increased the activity in all cases, **151** and **152** being 13- and 119-fold more potent than their benzylated counterparts **128** and **129**, however in the case of **153**, bearing a *N*-ethylaminomethyl substituent at position 9 and a *p*-chlorophenyl at position 5 the activity slightly decreased when the anilinic nitrogen was debenzylated, **130** being 4-fold more potent than its debenzylated analogue **153**.

Drs. Badia and Clos also determined hBChE IC_{50} values for this second generation of peripheral site AChEIs, **151**−**154** (Table 3.5.2). Surprisingly, three of these compounds, **152**, **153** and **154** exhibited IC_{50} values in the low micromolar range, **152** being the most active compound of the series against hBChE, with an IC_{50} value of 916 nM.

Recently, Drs. Andrisano and Bartolini determined the hAChE inhibitory activity of the whole series of pyrido[3,2-*c*]quinolines, **123**−**132** and **151**−**154**, using the method of Ellman *et al.*,[176] which again exhibited an unexpected high inhibitory activity compared to that of prototype peripheral site inhibitor propidium, **6** [IC_{50}(hAChE) = 32.3 μM],[221] with IC_{50} values in the low micromolar, and, in some cases, nanomolar range. Based on the data included in Table 3.5.2 it can be concluded that the most favourable structural characteristics that confer the highest hAChE inhibitory activity were the incorporation of a *N*-ethylcarbamoyl or a *N*-ethylaminomethyl group at position 9, a *p*-chlorophenyl at position 5 and a debenzylated anilinic nitrogen, **152** and **153** being the most active compounds of the whole series with hAChE IC_{50} values of 147 nM and 556 nM, 4-fold and 1.7-fold more potent than their benzylated analogues **129** and **130**, respectively, and more than 170-fold and 45-fold more potent than the 9-ethoxycarbonyl-substituted derivative, **131** (Table 3.5.2). The preceding findings suggest that the increase in inhibitory potency of the novel compounds **123**−**132** and **151**−**154** relative to that of propidium, **6**, might be related to differences in the binding mode that is being studied in the context of the PhD Thesis work of Ornella Di Pietro.

[176]Ellman, G.L.; Courtney, K.D.; Andres, B. Jr.; Featherstone, R.M. *Biochem. Pharmacol.* **1961**, *7*, 88. [221]Bolognesi, M. L.; Andrisano, V.; Bartolini, M.; Banzi, R.; Melchiorre, C. *J. Med. Chem*. **2005**, *48*, 24.

Table 3.5.2 eeAChE, hBChE and self-induced Aβ aggregation inhibitory activities of **120**−**123**.[a,b]



	R_1	R_2	R_5	R_9	IC_{50} (μM) hAChE[a]	IC_{50} (μM) eeAChE[a]	IC_{50} (nM) hBChE[a]	Self-induced $Aβ_{1-42}$ aggregation (%)[b]
123	Bn	O	*p*-chlorophenyl	CO_2Et	4.15 ± 0.16	5.21 ± 0.33	>100	5.0
124	Bn	O	*p*-CO_2MePh	CO_2Et	> 25	13.61 ± 1.85	>100	4.2
125	Bn	O	3-pyridyl	CO_2Et	13.0 ± 0.8	>100	>100	5.2
126	Bn	H,H	*p*-chlorophenyl	CO_2Et	*na*	6.33 ± 1.85	>100	0.5
127	Bn	H,H	*p*-CO_2MePh	CO_2Et	*na*	6.62 ± 0.62	>100	10.5
128	Bn	H,H	3-pyridyl	CO_2Et	1.06 ± 0.09	1.97 ± 0.17	*n. d.*	8.0
129	Bn	H,H	*p*-chlorophenyl	C(O)NHEt	0.596 ± 0.05	5.48 ± 0.512	>100	10.5
130	Bn	H,H	*p*-chlorophenyl	CH_2NHEt	0.942 ± 0.04	0.147 ± 0.014	*n. d.*	3.6
131	H	H,H	*p*-chlorophenyl	CO_2Et	> 25	0.281 ± 0.031	>100	5.7
132	PMB	H,H	*p*-chlorophenyl	CO_2Et	*na*	>100	>100	1.9
151	H	H,H	3-pyridyl	CO_2Et	22.8 ± 1.6	0.148 ± 0.016	>100	12.4
152	H	H,H	*p*-chlorophenyl	C(O)NHEt	0.147 ± 0.007	0.046 ± 0.063	0.92 ± 0.03	15.4
153	H	H,H	*p*-chlorophenyl	CH_2NHEt	0.556 ± 0.024	0.569 ± 0.04	1.37 ± 0.07	15.6
154	H	H,H	3-pyridyl	CH_2NHEt	15.8 ± 0.9	2.55 ± 0.3	2.59 ± 0.14	15.9

[a]Values are expressed as the mean ± SEM of at least four experiments; IC_{50}: inhibitory concentration (μM) of recombinant human AChE, *Electrophorus electricus* AChE or human serum BChE activity. *na* means not active. *nd* means not determined. [b]Inhibitor concentration: 10 μM ([Aβ] / [I] = 5:1).

Drs. Bartolini and Andrisano also determined the ability of compounds second generation pyrido[3,2-*c*]quinolines to inhibit Aβ self-inducedaggregation **151**−**154** exhibited percentages of inhibition of 12−16% using 10 μM concentration of inhibitor, in all cases higher than the first generation of compounds, albeit still lower than propidium, **6**, which exhibited a 90% of inhibition of the self-induced Aβ aggregation using 10 μM concentration of inhibitor. Drs. Bartolini and Andrisano will be soon determining the AChE-induced Aβ aggregation inhibitory activity of both first and second generation of pyrido[3,2-*c*]quinolines.

3.5.4 Molecular modelling studies of 152.

To gain insight into the molecular determinants that modulate the hAChE inhibitory activity of the novel compounds, in the context of the PhD Thesis work of Ornella Di Pietro, the binding mode of the most active product **152** was investigated and compared to that of its benzylated analogue **129** by means of docking calculations on recombinant hAChE (PDB entry: 3LII). The conformation of the active site and the gorge appears to be highly conserved in different X-ray crystallographic structures, whereas slight modifications were found in the peripheral site residues, mainly affecting Trp286. For this reason, three different docking calculations changing the orientation of the peripheral site Trp286 residue were carried out. Specifically, the orientations that Trp286 adopts in the *Mus musculus* AChE (mmAChE)−propidium crystal complex (PDB entry: 1N5R), in the TcAChE−*bis*(7)-tacrine complex (PDB entry: 2CKM) and in the tacrine−propidium (TZS)−AChE crystal (PDB entry: 1Q83). Due to the chemical similarity with propidium, **6**, the binding mode of the aminoquinoline unit of our compounds could be inferred form the X-ray crystallographic structure of the enzyme complex with propidium. After removing the propidium unit from the complex, quinoline **152** and **129** were docked on it and the most stable binding modes were determined (Figure 3.5.2). The main difference in the binding mode between benzylated and debenzylated compounds seems to be that, debenzylated **152** occupies the peripheral site and mid-gorge while the bulkier **129**, bearing a benzyl substituent in the anilinic nitrogen placed at the peripheral site only.

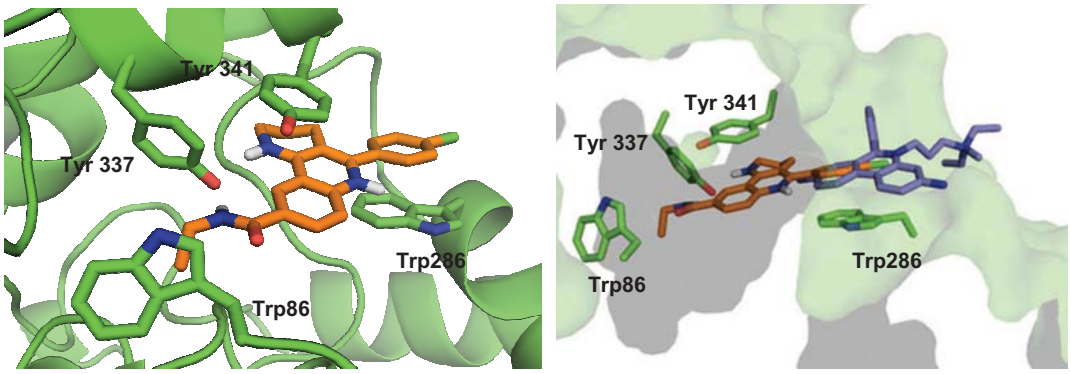


Figure 3.5.2 Left: Representation of the most favorable binding mode of quinoline **152** with hAChE. Right: Comparison between the most favorable binding modes of quinoline **152** (colored) and propidium (blue) within the hAChE gorge.

Inspection of the model suggests that $\pi-\pi$ stacking interactions are established between the *p*-chlorophenyl system of **152** and Trp286 at the peripheral site and between the quinoline system of **152** and Tyr341 at the calatytic gorge, together with H-bonding of the lateral amide with Tyr337 (Figure 3.5.2). The balance of these attractive interactions can account for the high affinity of this compound for its receptor site. On the other hand, compared to propidium, **6**, which fills the peripheral site, compound **152** is more deeply inserted into the gorge, which likely contributes to explain its better inhibitory activity (Figure 3.5.2). Therefore, **152** interacts, not

only with the peripheral site of the enzyme, but also with some mid-gorge residues. This would explain why **152** is more than 700-fold more potent than the prototype propidium, **6**. Currently, molecular dynamic simulations are being carried out to confirm the docking results.

2.6 Preparation of MAO-B inhibitors.

3.6.1 Interest in the use of MAO inhibitors in AD.

The multifactorial nature of AD supports the currently most innovative therapeutic approach based on the "one molecule, multiple targets" paradigm. In this context, besides cholinergic deficit, alterations in other neurotransmitter systems, especially serotoninergic and dopaminergic, are also thought to be responsible for the behavioural disturbances observed in patients with AD. Thus, MAO, the enzyme that catalyzes the oxidative deamination of a variety of biogenic and xenobiotic amines, is also an important target to be considered for the treatment of specific features of this multifactorial disease. In addition, selective inhibitors for MAO-A have shown to be effective antidepressants while MAO-B inhibitors, although apparently devoid of antidepressant action, have shown to be useful in the treatment of Parkinson's disease. MAO-B activity increases with age and is particularly high in the hypothalamus, hippocampus and around the seline plaques in the brains of AD patients leading to a decrease in the levels of dopamine, norepinephrine and serotonine, compared with normal ageing brain.[221–223]

Furthermore, as it has already been mentioned in section 1.7.3, MAO-B produces hydrogen peroxide when carrying out oxidative desamination of endogenous amines generating ROS, which is one of the main pro-oxidant species which contribute to the massive destruction of some brain areas, in particular the entorhinal cortex and hippocampus in the early stage of AD.[156] Thus, MAO-B inhibitors may act by both reducing the formation of oxygen radicals and by elevating the levels of monoamines.[224] Besides, the development of selective MAO-B inhibitors is advantageous in order to minimize side effects related to the potentiation of the cardiovascular effect of tyramine, a limited side effect of nonselective MAO inhibitors which inhibit MAO-A.

3.6.2 Design of novel selective MAO-B inhibitors.

The aim of this PhD Thesis work was to develop a scaffold that could selectively inhibit MAO-B which, in a future work, might be structurally modified incorporating different fragments to become a multi-target-directed ligand able to act as both MAO-B and dual binding site AChE inhibitor in an innovative drug discovery approach for the treatment of neurodegenerative disorders. These compounds would be expected to block the neurodegenerative cascade at three different levels, namely, by cholinesterases, Aβ-aggregation and MAO-B inhibition, which consequently should also reduce the oxidative stress associated to AD.

Previous studies concerning a series of multitarget hybrid compounds combining the benzylpiperidine moiety of donepezil, **2**, and the indolylpropargylamino moiety of PFN9601, **155**, (a known inhibitor of MAO enzymes), linked through a short methylene tether had been reported

[156]Good, P.F.; Werner, P.; Hsu, A.; Olanow, C.W.; Perl, D.P. *Am. J. Pathol*. **1996**, *149*, 21.[221]Bolognesi, M. L.; Andrisano, V.; Bartolini, M.; Banzi, R.; Melchiorre, C. *J. Med. Chem*. **2005**, *48*, 24. [222]Coyle, J.T.; Puttfarcken P. *Science* **1993**, *262*, 689. [223]Yu, P.H. *Gen. Pharm*. **1994**, *25*, 1527.

in the literature.[225] This hybrid, **156**, although active against AChE and MAO, presented non-balanced inhibitory potencies as well as selectivity toward MAO-A rather than MAO-B.

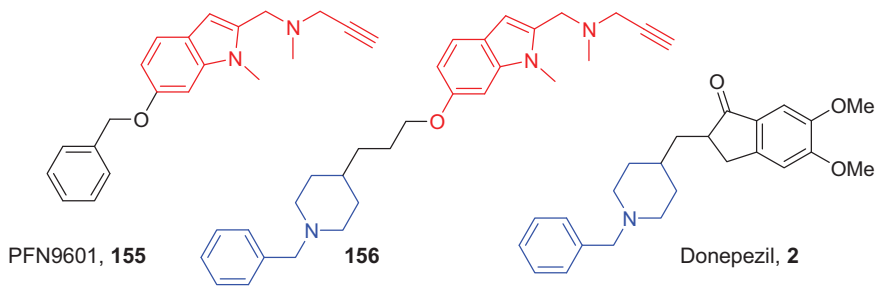
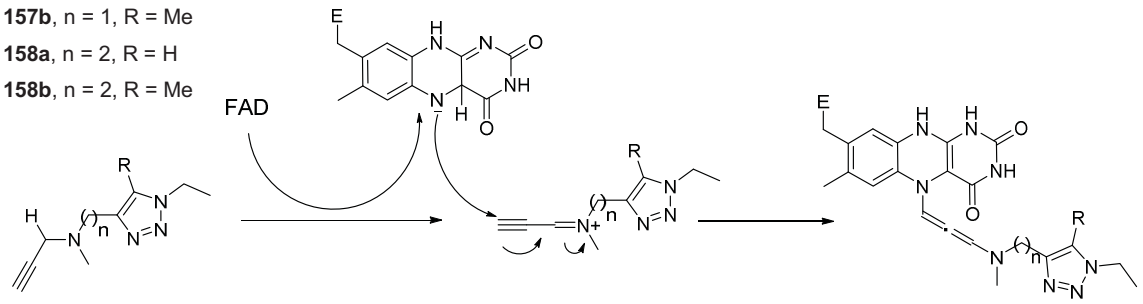


Figure 3.6.1 Structure of MAO inhibitor PFN9601, **155**, AChE inhibitor donepezil, **2** and multi-target compound **156**.

In this PhD Thesis work we decided to explore novel structural fragments retaining MAO-B inhibition but reducing MAO-A inhibitory activity and maintaining AChE inhibitory activity. In this context, a collaboration was established with Dr. Luque research group, which, based on docking and molecular dynamic simulation studies, led to the design of a series of 1,2,3-triazole derivatives structurally suited to fit the binding pocket of MAO-B, bearing (*N*-methyl)propargylaminoalkyl groups which should be able to form a covalent bond with the flavin adenine dinucleotide (FAD) cofactor present in both enzyme isoforms, MAO-B and MAO-A (Scheme 3.6.1).[226,227] According to the computational studies, the suitable tether length to link the 1,2,3-triazole and the propargylamino group corresponded to a chain of one or two methylenes. Moreover, the introduction of a methyl substituent at position 5 of the triazole should confer these compounds selectivity toward MAO-B.

157a, n = 1, R = H
157b, n = 1, R = Me
158a, n = 2, R = H
158b, n = 2, R = Me



Scheme 3.6.1 Putative mechanism of action of the novel triazoles.

It is important to point out that one of the main reasons why a 1,2,3-triazole core was chosen is due to the fact that some prominent examples of dual binding site AChEIs containing a 1,2,3-triazole fragment in the linker have been reported in the literature,[228–230] thus, the 1,2,3-

[225]Bolea, I.; Juárez-Jiménez, J.; de los Rïos, C.; Pouplana, R.; Luque, F.J.; Unzeta, M:; Marco-Contelles, J.; Samadi, A. *J. Med. Chem.* **2011**, *54*, 8251. [226]Szewczuk, L.M.; Culhane, C.; Yang, M.; Majumdar, A.; Yu, H.; Cole, P.A. *Biochemistry* **2007**, *46*, 6892. [227]Yang, M.; Culhane, J.C.; Szewczuk, L.M.; Gocke, C.B.; Brautingam, C.A.; Tomchick, D.R.; Machius, M.; Cole, P.A.; Yu, Hongtao. *Nat. Struct. Mol. Biol.* **2007**, *14*, 535. [228]Manetsch, R.; Krasinski, A.; Radic, Z.; Raushel, J.; Taylor, P.; Sharpless, K.B.; Kolb, H.C. *J. Am. Chem. Soc.* **2004**, *126*, 12809. [229]Shi, A.; Huang, L.; Lu, C.; He, F.; Li, X. *Bioorg. Med. Chem.* **2011**, *19*, 2298. [230]Radic, Z.; Kalisiak, J.; Fokin, V.V.; Sharpless, K.B.; Taylor, P. *Chem. Biol. Interact.* **2010**, *187*, 163.

triazole core would be also suitable, once the structural features conferring selective MAO-B inhibition were identified, to design huprine-based dual binding site AChEIs. One of the most interesting examples of the use of 1,2,3-triazoles to interact with AChE was that reported by Sharpless *et al.*[228] who carried out target-guided synthesis, which uses the target enzyme for assembling its owns inhibitors. Sharpless and co-workers used *in situ* click chemistry approach for assembling the 1,2,3-triazole inside the enzyme. They prepared a battery of azide and alkyne building blocks capable of interacting with either the peripheral or the active site of AChE and then incubated them with the enzyme so that the click Huisgen reaction between the alkyne and the azide took place, thus resulting in the covalent linkage of the peripheral and the active site interacting fragments through a 1,2,3-triazole ring. Detailed binding studies of their most active inhibitor, *syn*-TZ2PA6, **159**, to AChE revealed that the 1,2,3-triazole ring established interactions with other residues of the catalytic gorge (Figure 3.6.2).[228]

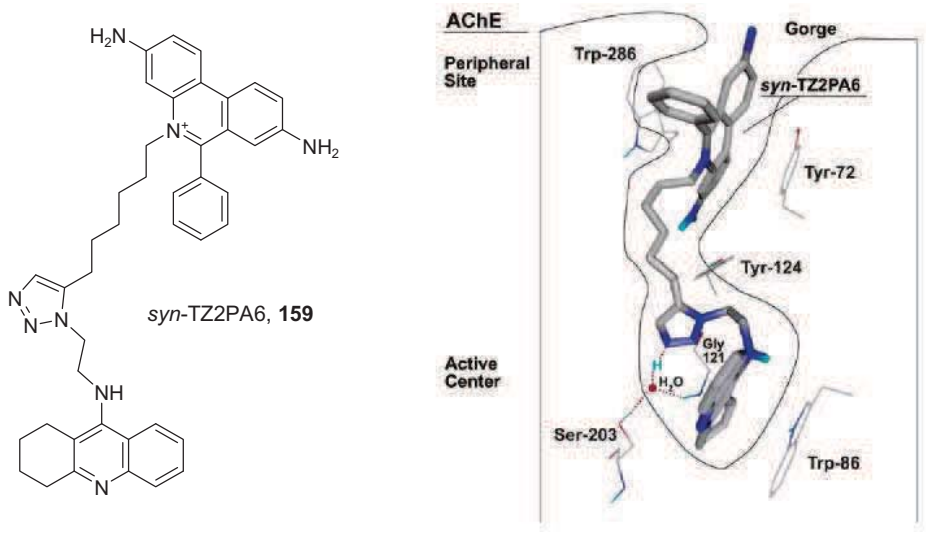


Figure 3.6.2 Left: Structure of *in situ* generated dual binding site AChEI *syn*-TZ2PA6, **159**. Right: Schematic representation of the binding interactions between *syn*-TZ2PA6, **159** and mAChE.[228]

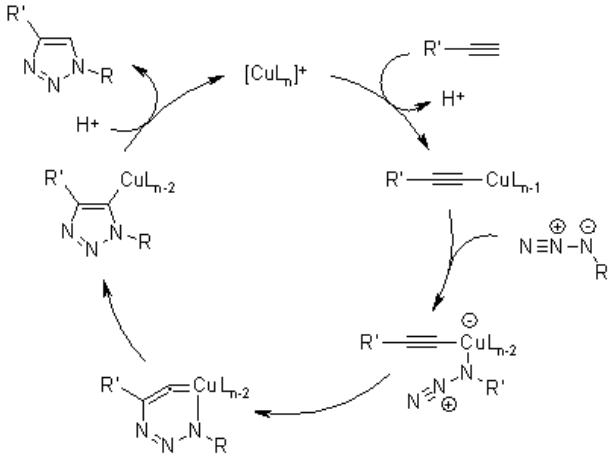
3.6.3 Preparation of a novel series of 1,2,3-triazole derivatives using a click-chemistry approach.

Click-chemistry is a term that was introduced by K. B. Sharpless in 2001 to describe reactions that are high yielding, wide in scope, create only byproducts that can be removed without chromatography, are stereospecific, simple to perform, and can be conducted in easily removable or benign solvents.[231] This concept was developed in parallel with the interest within the pharmaceutical, materials, and other industries in capabilities for generating large libraries of compounds for screening in discovery research. Several types of reactions have been identified that fulfil these criteria; thermodynamically-favoured reactions that lead specifically to one product,[232] such as nucleophilic ring opening reactions of epoxides,[233] and aziridines,[234]

[228]Manetsch, R.; Krasinski, A.; Radic, Z.; Raushel, J.; Taylor, P.; Sharpless, K.B.; Kolb, H.C. *J. Am. Chem. Soc*. **2004**, *126*, 12809. [231]Kolb, H.C.; Finn, M.G.; Sharpless, K.B. *Angew. Chem. Int. Ed*. **2001**, *40*, 2004. . [232]Gil, M.V.; Arévalo, M.J.; López, O. *Synthesis* **2007**, *11*, 1589. [233]Fringuelli, F.; Pizzo, F.; Tortoioli, S.; Vaccaro, L. *J. Org. Chem*. **2004**, *69*, 7745. [234]Chuang, T.-S.; Sharpless, K.B. *Org. Lett*. **2000**, *2*, 3555.

non-aldol type carbonyl reactions, such as formation of hydrazones[235] and heterocycles,[232] additions to carbon−carbon multiple bonds, such oxidative formation of epoxides[232] and Michael additions,[236] and cycloaddition reactions.[232,236]

One of the most popular click chemistry reactions is the Huisgen 1,3-dipolar cycloaddition of alkynes to azides,[237] however, it requires elevated temperatures and often produces mixtures of the two regioisomers when using asymmetric alkynes. A Cu-catalyzed variant, that follows a different mechanism can be conducted under aqueous conditions, even at room temperature and allows the synthesis of the 1,4-disubstituted regioisomers specifically.[238,239] By contrast, a later developed Rh-catalyzed reaction gives the opposite regioselectivity with the formation of 1,5-disubstituted 1,2,3-triazoles.[240] Thus, these catalyzed reactions fully comply with the definition of click chemistry. This Cu-catalyzed Huisgen 1,3-cycloaddition was discovered concurrently and independently by the groups of Valery V. Fokin and Barry K. Sharpless at the Scripts Institute, California,[238] and Morten Meldal in the Carlsberg Laboratory, Denmark.[239] Mechanistically, a copper acetylide forms, after which the azide displaces another ligand and binds to the copper. Then, an unusual six-membered copper(III) metallacycle is formed. The barrier for this process has been calculated to be considerably lower than the one for the uncatalyzed reaction. Ring contraction to a triazolyl-copper derivative is followed by protonolysis that delivers the triazole product and closes the catalytic cycle (Scheme 3.6.2).

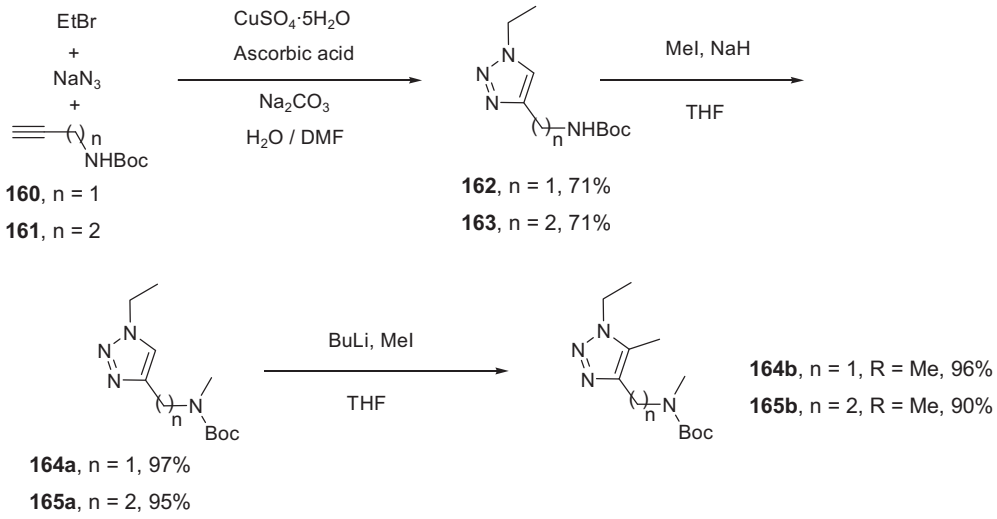


Scheme 3.6.2

For the preparation of triazoles **157**,**158a**,**b**, a click chemistry approach was envisioned. Thus, reaction of ethyl azide, generated *in situ* through reaction of ethyl bromide with NaN_3, and *N*-Boc-propargylamine, **160**, or *N*-Boc-butinylamine, **161**, in the presence of an excess of Na_2CO_3 and catalytic $CuSO_4 \cdot 5H_2O$ and ascorbic acid in H_2O and DMF at r. t. overnight[241]

[232]Gil, M.V.; Arévalo, M.J.; López, O. *Synthesis* **2007**, *11*, 1589. [235]Ganguly, T.; Kasten, B.R.; Bucar, D.-K.; MacGillivray, L.R.; Berkman, C.E.; Benny, P.D. *Chem. Commun*. **2011**, *47*, 12846. [236]Ramachary, D.B.; Barbas, C.F. *Chem. Eur. J.* **2004**, *10*, 5323. [337]Huisgen, P., Padwa, A.*1,3-Dipolar Cycloaddition Chemistry. Ed. Wiley*: New York, **1984**, *1*. [238]Rostovtsev, V.V.; Green, L.G; Fokin, V.V.; Sharpless, K.B. *Angew. Chem. Int. Ed*. **2002**, *41*, 2596. [239]Tornoe, C.W.; Christensen, C.; Meldal, M. *J. Org. Chem*. **2002**, *67*, 3057. [240]Zhang, L.; Chen, X.; Xue, P.; Sun, H.H.Y.; Williams, I.D.; Sharpless, K.B.; Fokin, V.V.; Jia, G.J. *J. Am. Chem. Soc.* **2005**, *127*, 15998. [241]Crowley, J.D.; Bandeen, P.H. *Dalton Trans*. **2010**, *39*, 612.

afforded triazoles **161** and **162** in 71% yield and with no need of purification (Scheme 3.6.3). As expected, only the *anti*-1,2,3-triazole was observed, which was corroborated in the HMBC spectra that showed a H−C correlation between C5 and N1-CH_2 (Figure 3.6.3).



Scheme 3.6.3.

Triazoles **162** and **163** were treated with an excess of NaH and MeI in THF at r. t. for 3 h to afford **164a** and **165a** in 97% and 95% yield, respectively, and with no need of column chromatography purification. Finally, a methyl substituent was introduced at position 5 of the triazoles **164a** and **165a** by reaction of those with BuLi at -78 ºC followed by treatment with MeI, at -78 ºC for 1 h and at r. t. for 2 h to give **164b** and **165b** in 96% and 90% yield, respectively, and again with no need of purification (Scheme 3.6.3).

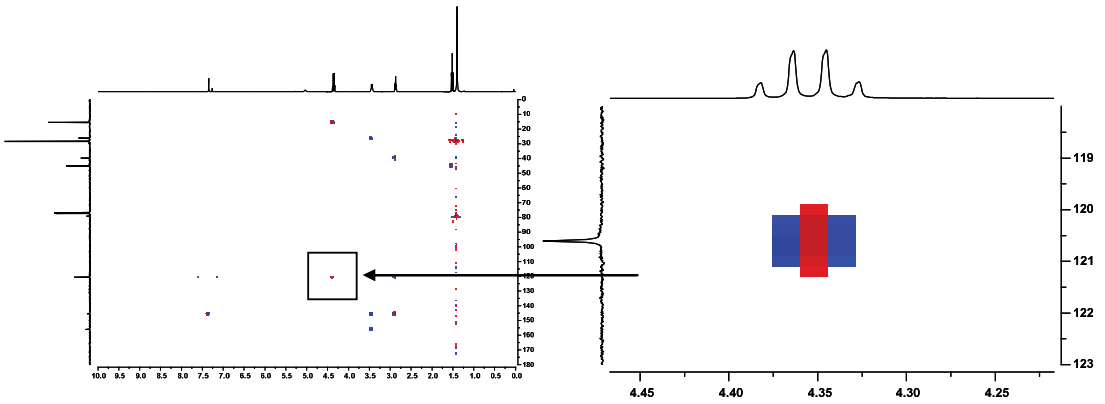
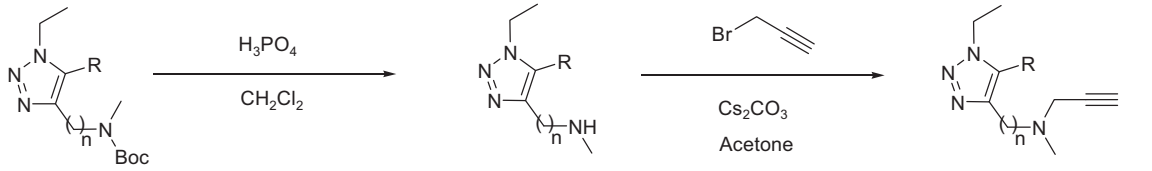


Figure 3.6.3 Left: HMBC spectrum of **164a** carried in a Mercury 400 MHz equipment in $CDCl_3$. Right: enlargement of the H−C correlation between 5-*C* and 1-N-C*H*$_2$.

164,**165a**,**b** were deprotected by treatment with an excess of 85% H_3PO_4 in CH_2Cl_2 at r. t. for 1.5 to 4 h to give the desired unprotected triazoles **166**,**167a**,**b** in 85% to 92% yield and again with no need of purification processes. Finally, **166**,**167a**,**b** were treated with Cs_2CO_3 and 1 equivalent of propargyl bromide in acetone at 0 ºC from 2 to 24 h to give the desired propargylamines **157**,**158a**,**b** in moderate to good yield (Scheme 3.6.4). It is important to point

out that dipropargylated byproduct was observed, specially, when the reaction was carried out at r. t.



164a, n = 1, R = H
164b, n = 1, R = Me
165a, n = 2, R = H
165b, n = 2, R = Me

166a, n = 1, R = H, 86%
166b, n = 1, R = Me, 92%
167a, n = 2, R = H, 90%
167b, n = 2, R = Me, 85%

157a, n = 1, R = H, 89%
157b, n = 1, R = Me, 60%
158a, n = 2, R = H, 59%
158b, n = 2, R = Me, 53%

Scheme 3.6.4

3.6.4 Pharmacological evaluation of novel triazoles toward MAO-A and MAO-B.

Dr. Mercedes Unzeta from the *Universitat Autònoma de Barcelona* evaluated the novel triazoles toward human MAO-A (hMAO-A) and human MAO-B (hMAO-B). As we expected, the introduction of the methyl substituent at position 5 of the 1,2,3-triazole increased the inhibitory activity toward hMAO-B, **157b** and **158b** being 2-fold and 1.5-fold more potent hMAO-B inhibitors than their non-methylated analogues **157a** and **158a**. Also **157b** showed the expected MAO-B selectivity it being 2.5-fold more potent inhibitor toward hMAO-B than hMAO-A. However, contrary to our expectations, **157**,**158a**,**b** exhibited in general low inhibitory activities toward both enzyme isoforms, with IC_{50} values in the high micromolar range (Table 3.6.1).

Table 3.6.1 hMAO-A and h-MAO-B inhibitory activities of triazoles **157**,**158a,b**.[a]

Compound	IC_{50} (μM) hMAO-A [a]	IC_{50} (μM) hMAO-B[a]
157a·HCl	135 ± 22	249 ± 94
157b·HCl	267 ± 31	105 ± 27
158a·HCl	553 ± 84	374 ± 119
158b·HCl	91 ± 14	238 ± 52

[a]Values are expressed as the mean ± SEM of at least three experiments in quadruplicate; IC_{50}: inhibitory concentration (μM) of hMAO-A and hMAO-B.

A possible explanation of the low activity could be the high polarity of the triazole group which might prevent our compounds to access the FAD group with which the propargyl group of **157**,**158a**,**b** should react, as it is located at the end of a hydrophobic gorge. Moreover, the high polarity of the triazole should increase the desolvation penalty which would also explain the low activities. In order to improve the activity and selectivity for MAO-B inhibition, a second generation of triazoles has been designed which either incorporate a benzyl or phenethyl substituent at position 1 of the triazole, a butyl at position 4 of the triazole or a phenylene linker

between the triazole and the propargylamine group. These compounds are being prepared by Internship Erasmus PhD student Natalia Guizor and will soon be pharmacologically evaluated.

4. CONCLUSIONS

The first objective of this PhD Thesis work consisted of the preparation of racemic huprine Y and enantiopure huprines Y and X in sufficient amount as to allow to synthesize the huprine-based hybrids that had been envisioned in this PhD project. Thus, the synthetic pathway developed in our research group more than 10 years ago was successfully applied, introducing a few changes that made preparation of racemic huprine Y more easily scalable, namely i) direct transformation of mesylate **47** into the desired huprine Y, (±)-**14**, without isolating enone (±)-**48**, thus avoiding one of the tedious column chromatography purification procedures, which represented an inconvenience when the synthetic pathway had to be highly scaled up; and ii) substitution of the final column chromatography purification of the crude huprine by treatment of the reaction crude with HCl / MeOH, in order to convert (±)-**14** into the corresponding hydrochloride followed by washing with hot EtOAc to remove unreacted 2-amino-4-chlorobenzonitrile and other non-basic byproducts. For the preparation of enantiopure huprines Y and X, the most favourable methodology, which consisted of the chromatographic resolution of the corresponding racemic mixtures on a preparative scale by MPLC using microcrystalline cellulose triacetate (15–25 μm) as the chiral stationary phase and 96% ethanol as the eluent, worked efficiently. Previous to the separation, a novel more efficient methodology for the HPLC analysis of the MPLC fractions was successfully developed, which involved the use of a *Chiralcel OD* column containing cellulose tris(3,5-dimethylphenylcarbamate) as the chiral stationary phase, a mixture of hexane / EtOH / DEA 75:25:0.5 as the eluent, a flow of 0.3 mL / min and a λ of 235 nm.

In the present PhD Thesis work, a new family of donepezil–huprine hybrids was designed and synthesized to simultaneously interact with the peripheral, mid-gorge, and active sites of hAChE, with the aim of attaining high hAChE inhibitory activity and more importantly of interfering with AChE-induced Aβ aggregation; the former effect is of interest for the management of the symptomatology of AD, and the latter for the modification of AD progression. For the preparation of the desired hybrids, the donepezil-based indane moiety was prepared through an aldolic condensation of 4-pyridinecarboxaldehyde, **79**, with 5,6-dimethoxy-1-indanone, **62**, followed by hydrogenation of the resulting pyridyl-α,β-unsaturated indanone, **80**, reaction of the resulting piperidine **61** with 2-bromoethanol or 3-chloro-1-propanol and transformation of the obtained alcohols, **76a**,**b** to the corresponding chloroderivates, **77a**,**b**. Finally, chloroderivates **77a**,**b**, were coupled to racemic and enantiopure huprine Y and X to afford the desired hybrids, (±)-, (–)- and (+)-**81**,**82a**,**b**, which were fully characterized and pharmacologically evaluated. The novel donepezil–huprine hybrids turned out to be potent inhibitors of hAChE and moderately potent inhibitors of hAChE-induced Aβ aggregation, they being clearly superior to the parent donepezil in both activities. However, they exhibited lower inhibitory potency toward hAChE than the parent huprine. This fact was studied by MD simulations and could be ascribed to the structural distortion within hAChE generated by the presence of the piperidine ring in the linker of the hybrids. Interestingly, the novel hybrids also exhibited significant inhibitory activity toward hBChE and Aβ self-aggregation, were able to block Aβ formation through inhibition of BACE-1, and have been found to be able to cross the

BBB and enter the CNS in both PAMPA-BBB and *ex vivo* assays. Moreover, two different *in vivo* assays were carried out with the compound exhibiting the best pharmacological profile: (−)-**81b**. This compound protected *Caenorhabditis elegans* from the Aβ-induced paralysis, but it was not able to reduce amyloid burden. (−)-**81b** was also tested in APP_{SL} transgenic mice using the Morris water maze test and proved to be able to significantly improve cognition after 3-month oral administration. However, (−)-**81b** did not alter $Aβ_{1-38}$, $Aβ_{1-40}$ or $Aβ_{1-42}$ levels neither in the CSF, nor in brain homogenate preparations, nor alter cortical and hippocampal amyloid plaque load.

A series of rhein−huprine hybrids, containing a unit of racemic huprine Y as the AChE active site interacting unit and an anthraquinone fragment derived from rhein, **40**, expected to inhibit tau protein aggregation and to interact with the peripheral site of AChE, connected through an amide-containing oligomethylene or aromatic linker, were also envisioned as multi-target directed anti-Alzheimer drug candidates. A synthetic pathway for the preparation of the desired hybrids was developed, which involved an initial alkylation of racemic huprine Y, with an ω-bromoalkanenitrile to give cyanoalkylhuprines (±)-**89a−g**. For the preparation of (±)-**93h**, containing an aromatic linker, a reductive alkylation with *p*-cyanobenzaldehyde was successfully applied. Finally, nitriles (±)-**89a−h** were reduced with $LiAlH_4$ to the corresponding amines which were coupled with rhein, **40**, by amide formation. Then novel rhein−huprine hybrids, (±)-**93a−h**, were fully characterized and pharmacologically evaluated. (±)-**93a−h**, albeit being less potent than the parent huprine Y, turned out to be potent hAChEIs, with IC_{50} values in the low nanomolar range, and moderately potent inhibitors of hBChE, with IC_{50} values in the nanomolar range. The novel rhein−huprine hybrids showed to significantly inhibit both AChE-induced and self-induced Aβ aggregation, the latter both *in vitro* and in *E. coli* overexpressing the peptide. Finally, some of these hybrids also turned out to be potent inhibitors of Aβ formation, with IC_{50} values toward BACE-1 in the nanomolar or low micromolar range, also to significantly inhibit tau protein aggregation in *E. coli* overexpressing the peptide, and to be able to cross the BBB and enter the CNS in PAMPA-BBB studies, making them promising anti-Alzheimer drug candidates.

A third huprine-based multipotent dual binding site AChEIs series was designed and synthesized in the present PhD Thesis work, namely the capsaicin−huprine hybrids, which consisted of a unit of racemic huprine Y, as the active site interacting unit, and a fragment derived from the natural antioxidant capsaicin to interact with the peripheral site and to afford antioxidant properties, connected through an amide containing saturated or unsaturated oligomethylene or aromatic linker. For the preparation of capsaicin−huprine hybrids bearing a saturated oligomethylene linker, (±)-**97a−e**, and an aromatic linker, (±)-**97h**, nitriles (±)-**89a−e**,**h** were again prepared, but this time they were hydrolysed to afford the corresponding carboxylic acids which were coupled to the capsaicin derived commercially available 4-hydroxy-3-methoxybenzylamine moiety through an amide formation reaction which afforded (±)-**97a−e**,**h**. An alternative route, which included protection and deprotection steps of the phenol as a

triisipropylsilyl ether was also successfully applied. Capsaicin−huprine hybrids bearing an unsaturated oligomethylene linker, (±)-**97f**,**g**, were accessed through a methodology that involved as a key step an olefin cross metathesis reaction using a Grubbs-Hoveyda 2nd generation catalyst between previously synthesized huprine- and capsaicin-derived olefins. All capsaicin−huprine hybrids were chemically characterized and are being pharmacologically evaluated toward cholinesterases, Aβ aggregation and oxidative stress.

The fifth objective of this PhD Thesis work, which consisted of the preparation of pyrido[3,2-*c*]quinolines structurally related to the peripheral site AChEI propidium, but devoid of any permanent positive charge was tacked in the frame of a collaboration with Dr. Rodolfo Lavilla's research group. Thus, a series of amidoquinolines and aminoquinolines were successfully accessed for further pharmacological evaluation, though a Povarove-type MCR, followed by oxidation of the resulting diastereomeric mixtures of tetrahydroquinolines and reduction of the lactames to the desired pyridoquinolines. Moreover, a synthetic route for accessing pyridoquinolines **129**, **130** and **152**−**154**, bearing either a *N*-ethylcarbamoyl or *N*-ethylaminomethyl substituent at position 9, was successfully developed in the present PhD Thesis work, which consisted of the basic hydrolysis of the ethyl ester precursor, followed by amide formation reaction with ethylamine hydrochloride and reduction to the amine with $LiAlH_4$. Quinolines **123**−**132** and **151**−**154** were fully characterized and pharmacologically evaluated toward hAChE, eeAChE, hBChE and self-induced Aβ aggregation and are being evaluated toward hAChE and AChE-induced Aβ aggregation. All quinolines were moderalety potent hAChE inhibitors, with IC_{50} values in the low micromolar and nanomolar range, clearly more potent than parent propidium. This surprisingly high hAChE inhibitory activity was studied by docking calculations of the most potent inhibitor of the series, **152**, and might be ascribed to the different binding mode of our compounds to hAChE, compared to that of propidium, as they seem to interact, not only with residues of the peripheral site, namely Trp286, but also with mid-gorge residues. Quinolines **123**−**132** and **151**−**154** were either not able to inhibit, or show a moderately potent inhibitory activity of hBChE, with IC_{50} values in the low micromolar range and they turned out to be poor to moderate inhibitors of the self-induced Aβ aggregation. Once the pharmacological evaluation of the novel pyridoquinolines is completed, and that exhibiting the best pharmacological profile is identified, it will be coupled to an active site AChEI to prepare a dual binding site AChEI.

Finally, the synthesis of a series of 1,2,3-triazoles, bearing a propargylamino group that should be able to covalently interact with the FAD group of MAO-B enzyme, was also planned. MAO-B inhibition is of high interest for the treatment of AD, as MAO-B activity has proved to be increased in elderly people, to be present around the seline plaques in the brains of AD patients and to produce hydrogen peroxide, generating ROS, and contributing to the massive destruction of some brain areas in early stages of AD. Thus, on the basis of previous docking and MD simulation studies carried by Dr. Javier Luque's research group, 4 novel triazoles were

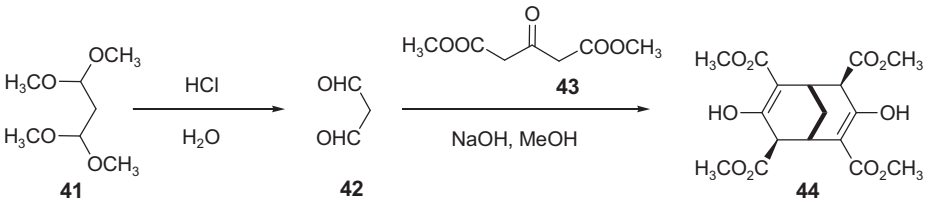
successfully accessed using a click-chemistry approach followed by simple organic synthesis transformations, in excellent yields and with no need of chromatographic purification. Novel triazoles **157**,**158a**,**b** were fully characterized and pharmacologically evaluated toward MAO-A and MAO-B. As we expected the introduction of the methyl substituent at position 5 of the triazole increased the affinity toward MAO-B, moreover **157b** did show selectivity toward MAO-B inhibition. Unfortunately, **157**,**158a**,**b** exhibited a poor inhibition of both enzymes, with IC_{50} values in the high micromolar range. This poor MAO-A and MAO-B inhibitory activity could be explained due to the high polarity that the triazole group confers to the molecule, thus, a second generation of triazoles bearing more lipophilic substituents, namely benzyl, phenethyl and butyl groups has been envisioned and is being prepared by Internship Erasmus PhD student Natalia Guizor. Once an active and selective MAO-B inhibitor scaffold is identified it might be combined with another pharmacophoric unit to prepare a multipotent dual binding site AChEI.

5. EXPERIMENTAL PART

Material and methods.

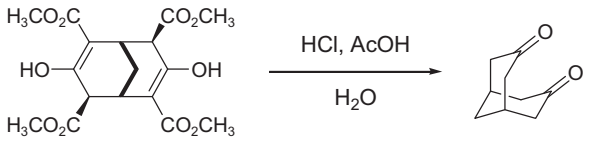
Melting points were determined in open capillary tubes with MFB 595010M Gallenkamp or a Büchi B-540 menting point apparatus. 300 MHz ^{1}H / 75.4 MHz ^{13}C NMR spectra, 400 MHz ^{1}H / 100.6 ^{13}C NMR spectra and 500 MHz / 125.7 MHz ^{13}C NMR spectra were recorded on Varian Gemini 300, Varian Mercury 400, and Varian Inova 500 spectrometers, respectively. The chemical shifts are reported in ppm (δ scale) relative to internal tetramethylsilane, and coupling constants are reported in Hertz (Hz). Assignments given for the NMR spectra of the new compounds have been carried out on the basis of DEPT, COSY ^{1}H / ^{1}H (standard procedures), and COSY ^{1}H / ^{13}C (gHSQC and gHMBC sequences) experiments. IR spectra were run on a Perkin-Elmer Spectrum RX spectophotometer. Absortion values are expressed as wave-numbers (cm^{-1}); only significant absortion bands are given. Column chromatography was performed on silica gel 60 Å (35–70 mesh, SDS, ref 2000027) or with a Biotage FLASH 40M Silica equipment (KP-Sil^{TM} 40−63 μm, 40 × 150 mm cartridges). Thin layer chromatography was performed with aluminum-backed sheets with silica gel 60 F_{254} (Merck, ref 1.05554), and spots were visualized with UV light and 1% aqueous solution of $KMnO_4$. NMR spectra have been carried out at the Centres Científics i Tecnològics de la Universitat de Barcelona (CCiTUB), elemental analysis and high resolution mass spectra were carried out at the Servei de Microanàlisi from the IIQAB (CSIC, Barcelona) with a Carlo Erba 1106 analyzer and at the CCiTUB and with a LC/MSD TOF Agilent Technologics spectrometer respectively. Analytical samples of all the compounds prepared in this PhD Thesis work were dried at 65 ºC / 2 Torr for at least 48 h (standard conditions). All the new compounds which were subjected to pharmacological evaluation possessed a purity ≥ 95% as evidenced by their elemental analyses.

Preparation of tetramethyl 3,7-dihydroxybicyclo[3.3.1]nona-2,6-diene-2,4,6,8-tetracarboxylate, 44.



In a triple neck 2 L round-bottomed flask equipped with magnetic stirrer and a pressure-equalizing dropping funnel a suspension of 1,1,3,3-tetramethoxypropane (113 mL, 113 g, 688 mmol) in 2 N HCl (345 mL, 690 mmol) was prepared and stirred at r. t. for 1.5 h, then cooled to 5 ºC with an ice / H_2O bath and treated dropwise with 5 N NaOH (240 mL, 1.20 mol). The resulting alkaline solution was diluted with MeOH (335 mL), treated with dimethyl acetonedicarboxylate (193 mL, 233 g, 1.34 mol) and again diluted with MeOH (230 mL). The reaction mixture was stirred at r. t. for 3 days, cooled to 5 ºC with an ice / H_2O bath, acidified with concentrated HCl (42 mL), filtered under vacuum and the solid washed with H_2O (2 x 50 mL), to afford the desired tetraester **44** (230 g, 87% yield) as a beige solid.

Preparation of bicyclo[3.3.1]nonane-3,7-dione, 45.



44 **45**

In a triple neck 2 L round-bottomed flask equipped with magnetic stirrer and a condenser, a supension of the tetraester **44** (120 g, 312 mmol) in H_2O (320 mL) was prepared and succesively treated with concentrated HCl (320 mL) and AcOH (640 mL). The reaction mixture was stirred under reflux for 24 h, then cooled to r. t. and extracted with CH_2Cl_2 (5 x 350 mL). The combined organic extracts were washed with 5 N NaOH (1 x 1200 mL), dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the desired diketone **45** (26.0 g, 55% yield) as a beige solid.

R_f = 0.14 (silica gel, 10 cm, hexane / EtOAc 1:1).

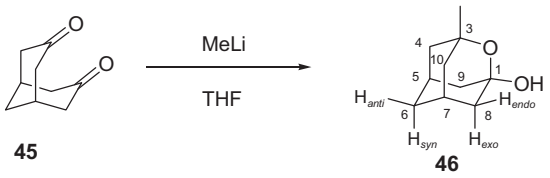
Melting point: 240−242 ºC (sublimated at 140 ºC and 0.8 Torr).

IR (KBr) v: 1706 (C=O st) cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.23 (s, 2H, 9-H), 2.38 [d, *J* = 15,4 Hz, 4H, 2(4,6,8)-H_{endo}], 2.62 [dd, *J* = 15.4 Hz, *J'* = 5.4 Hz, 4H, 2(4,6,8)-H_{exo}], 2.88 [broad s, 2H, 1(5)-H].

^{13}C NMR (50.4 MHz, $CDCl_3$) δ: 31.0 (CH_2, C9), 32.3 [CH, C1(5)], 47.5 [CH_2, C2(4,6,8)], 208.9 [C, C3(7)].

Preparation of 3-methyl-2-oxa-1-adamantanol, 46.



In a triple neck 1 L round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a pressure-equalizing dropping funnel, MeLi (1.6 N in Et_2O, 100 mL, 160 mmol) was placed, cooled to 0 ºC with an ice bath and treated dropwise with a solution of diketone **45** (16.2 g, 106 mmol) in anhydrous THF (320 mL). The reaction mixture was stirred at 0 ºC for 35 min, then treated with sat. aq. NH_4Cl (250 mL) and extracted with Et_2O (3 x 200 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give the desired oxaadamantanol **46** (15.7 g, 88% yield) as a yellow solid.

R_f = 0.36 (silica gel, 10 cm, hexane / EtOAc 1:1).

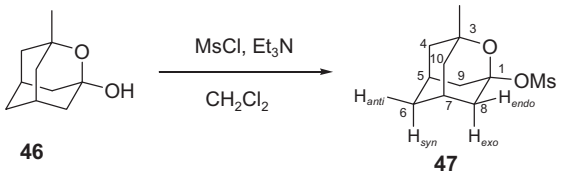
Melting point: 87−90 ºC (sublimated at 80 ºC / 0.5 Torr).

IR (KBr) v: 3327 (OH st) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.15 (s, 3H, 3-CH_3), 1.47 [ddd, *J* = 12.0 Hz, *J'* = 3.5 Hz, *J''* = 2.0 Hz, 2H, 4(10)-H_{exo}], 1.62 [broad d, *J* = 12.0 Hz, 2H, 4(10)-H_{endo}], 1.65 (m, 2H, 6-H_{syn} and 6-H_{anti}), 1.68 [broad d, *J* = 12.0 Hz, 2 H, 8(9)-H_{endo}], 1.75 [ddd, *J* = 12.0 Hz, *J'* = 3.5 Hz, *J''* = 2.0 Hz, 2H, 8(9)-H_{exo}], 2.30 [m, 2 H, 5(7)-H]. OH was not observed.

^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 28.5 (CH_3, 3-CH_3), 29.2 [CH, C5(7)], 33.5 (CH_2, C6), 40.2 [CH_2, C4(10)], 40.7 [CH_2, C8(9)], 74.6 (C, C3), 94.7 (C, C1).

Preparation of 3-methyl-2-oxa-1-adamantyl methanesulfonate, 47.



In a triple neck 1L round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of oxaadamantanol **46** (20.0 g, 119 mmol) and freshly distilled Et_3N (24.0 mL, 17.4 g, 172 mmol) in anhydrous CH_2Cl_2 (560 mL) was prepared, cooled to −10 ºC with an ice / NaCl bath and treated dropwise with MsCl (15.0 mL, 22.2 g, 193 mmol). The reaction mixture was stirred at −10 ºC for 30 min, then poured onto a mixture of 10% aq. HCl (320 mL) and ice (80 mL) and extracted with CH_2Cl_2 (2 x 150 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$ (250 mL) and brine (250 mL), dried with anydrous Na_2SO_4, filtered and evaporated under reduced pressure to give desired mesylate **47** (28.7 g, 98% yield) as a brown solid.

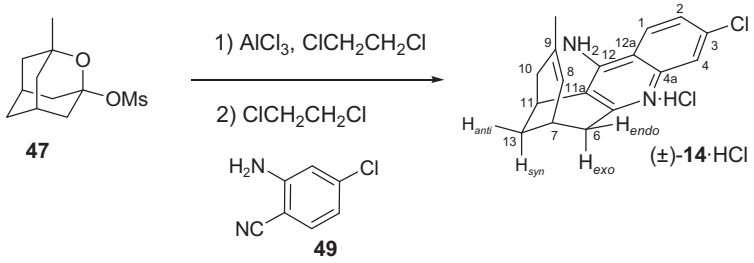
Melting point: 79−81 ºC (CH_2Cl_2).

IR (KBr) v: 1345 (SO_2 st as), 1175 (SO_2 st si) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.19 (s, 3H, 3-CH_3), 1.50 [broad d, *J* = 1.5 Hz, 2H, 4(10)-H_{exo}] 1.62−1.72 (complex signal, 2H, 6-H_{syn} and 6-H_{anti}), 1.69 [broad d, *J* = 12.5 Hz, 2H 4(10)-H_{endo}], 1.92 [broad d, *J* = 11.5 Hz, 2H, 8(9)-H_{exo}], 2.18 [broad d, *J* = 11.5 Hz, 2H, 8(9)-H_{endo}], 2.36 [broad s, 2H, 5(7)-H], 3.11 (s, 3H, CH_3SO_3).

^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 28.3 (CH_3, 3-CH_3), 30.0 [CH, C5(7)], 33.2 (CH_2, C6), 39.7 [CH_2, C8(9)], 40.0 [CH_2, C4(10)], 42.0 (CH_3, CH_3SO_3), 77.4 (C, C3), 108.0 (C, C1).

Preparation of 12-amino-3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinoline hydrochloride, (±)-14·HCl.



In a triple neck 2 L round-bottomed flask equipped with an inert atmosphere, magnetic stirrer, a pressure-equalizing dropping funnel and a condenser, mesylate **47** (85.0 g, 345 mmol) was dissolved in anhydrous 1,2-dichloroethane (400 mL), and treated with $AlCl_3$ (56.0 g, 421 mmol). The resulting suspension was stirred under reflux for 5 min, cooled to r. t. and treated dropwise with a suspension of 2-amino-4-chlorobenzonitrile (36.6 g, 381 mmol) in anhydrous 1,2-dichloroethane (600 mL). The reaction mixture was stirred under reflux overnight, allowed to cool to r. t., diluted with H_2O (800 mL) and THF (600 mL), alkalinized with 5 N NaOH (400 mL) and again stirred at r.t. for 30 min. The phases were separated and the aqueous layer was extracted with EtOAc (6 x 300 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a solid (110 g) that proved to be consistent with a mixture of **49** / (±)-**14** in an approximate ratio 59:41 (^{1}H NMR) that was dissolved in a CH_2Cl_2 / MeOH mixture (5:3, 800 mL), treated with HCl (1.4 N in MeOH, 116 mL) and dried under reduced pressure to give a solid (47 g) that was suspended in EtOAc (670 mL), heated under reflux, filtered hot and washed with excess of EtOAc to give a yellowish solid that was again suspended in EtOAc (430 mL), heated under reflux and filtered hot to give the pure (±)-huprine Y·HCl, (±)-**14**·HCl (47 g, 43% yield).

Melting point: 251−252 ºC (EtOAc).

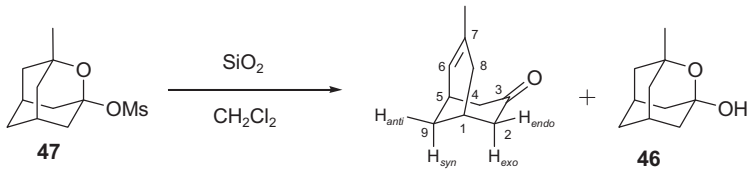
IR (KBr) ν: 3700-2300 (max. at 3337, 3145, 3013, 2988, 2924, 2838, 2700, NH_2, N^+H and CH st), 1669, 1636,1589 (Ar–C–C and Ar–C–N st) cm^{-1}.

^{1}H NMR (500 MHz, CD_3OD) δ: 1.57 (s, 3H, 9-CH_3) 1.94 (dddd, *J* = 12.5 Hz, *J'* = 5.5 Hz, *J''* ≈ *J'''* ≈ 2.0 Hz, 1H, 13-H_{syn}), 1.97 (d, *J* = 18.0 Hz, 10-H_{endo}), 2.06 (dddd, *J* = 12.5 Hz, *J'* = 4.0 Hz, *J''* ≈ *J'''* ≈ 2.0 Hz, 1H, 13-H_{anti}), 2.51 (ddm, *J* = 18.0 Hz, *J'* = 5.5 Hz, 1H, 10-H_{exo}), 2.77 (m, 1H, 7-H), 2.88 (ddd, *J* = 18.0 Hz, *J'* ≈ *J''* ≈ 2.0 Hz, 1H, 6-H_{endo}), 3.20 (dd, *J* = 18.0 Hz, *J'* = 5.5 Hz, 1H, 6-H_{exo}), 3.37 (m, 1H, 11-H), 4.83 (broad s, NH_2 and NH^+), 5.56 (dm, *J* = 5.5 Hz, 1H, 8-H), 7.55 (dd, *J* = 9.0 Hz, *J'* ≈ 1,5 Hz, 1H, 2-H), 7.75 (d, *J* = 1.5 Hz, 1H, 4-H), 8.35 (d, *J* = 9.0 Hz, 1H, 1-H).

^{13}C NMR (75.4 MHz, CD_3OD) δ: 23.5 (CH_3, 9-CH_3), 27.5 (CH, C11), 28.1 (CH, C7), 29.2 (CH_2, C13), 35.8 (CH_2, C10), 35.9 (CH_2, C6), 115.2 (C) and 115.5 (C) (C11a and C12a), 199.1 (CH,

C4), 125.1 (CH, C8), 126.4 (CH, C1), 127.6 (CH, C2), 134.9 (C, C9), 139.4 (C, C4a), 140.3 (C, C3), 153.0 (C) and 156.6 (C) (C5a and C12).

Preparation of 7-methylbicyclo[3.3.1]non-6-en-3-one (±)-48.



In a 1 L round-bottomed flask equipped with magnetic stirrer, a suspension of mesylate **47** (32.6 g, 132 mmol) and SiO_2 (33 g) in CH_2Cl_2 (350 mL) was prepared. The reaction mixture was stirred at r. t. for 3 h evaporated under reduced pressure and the residue purified through column chromatography (silica gel, 35–70 μm, 330 g, Ø = 8 cm; #1–12, 1000 mL, hexane; #13–17, 500 mL, hexane / EtOAc 95:5; #18–22, 500 mL, hexane / EtOAc 90:10; #23–28, 500 ml, hexane / EtOAc 85:15; #29–54, 2500 mL, hexane / EtOAc 80:20; #55–59, 500 mL, hexane / EtOAc 75:25; #60–69, 1000 L, hexane / EtOAc 70:30; #70–81, 2000 mL, hexane / EtOAc 50:50), providing the enone (±)-**48** (#30–43, 11.0 g, 56% yield) as a colourless oil and oxaadamantanol **46** (#67–78, 2.50 g, 11% yield) as a yellow solid.

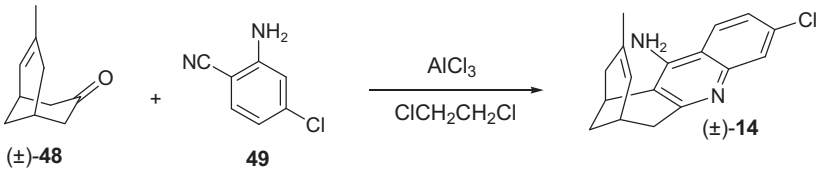
R_f = 0.61 (silica gel, 10 cm, hexane / EtOAc 1:1).

IR (NaCl) v: 1709 (C=O st) cm^{-1}.

^{1}H RMN (500 MHz, $CDCl_3$) δ: 1.57 (s, 3H, 7-CH_3), 1.78 (broad d, *J* = 18.0 Hz, 1H, 8-H_{endo}), 1.90 (dm, *J* = 12.5 Hz, 1H, 9-H_{anti}), 1.97 (dm *J* = 12.5 Hz, 1H, 9-H_{syn}), 2.24 (dddd, *J* = 15.5 Hz, *J'* = *J''* = *J'''* = 2.0 Hz, 1H, 2-H_{endo}) 2.28 (dddd, *J* = 14.5 Hz, *J'* = *J''* = *J'''* = 2.0 Hz, 1H, 4-H_{endo}), 2.33 (broad dd, *J* = 18.0 Hz, *J'* = 6.0 Hz, 1H, 8-H_{exo}), 2.40 (dd, *J* = 14.5 Hz, *J'* = 4.5 Hz, 1H, 4-H_{exo}), 2.48 (broad dd, *J* = 15.5 Hz, *J'* = 6.5 Hz, 1H, 2-H_{exo}), 2.55 (m, 1H, 1-H), 2.63 (broad s, 1H, 5-H), 5.40 (dm, *J* = 6.0 Hz, 1H, 6-H).

^{13}C RMN (50,3 MHz, $CDCl_3$) δ: 23.1 (CH_3, 7-CH_3), 30.1 (CH, C1), 30.1 (CH_2, C9), 31.0 (CH, C5), 37.3 (CH_2, C8), 46.4 (CH_2, C4), 49.0 (CH_2, C2), 124.5 (CH, C6), 132.7 (C, C7), 212.2 (C, C3).

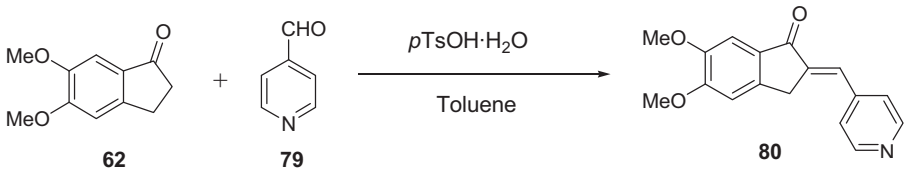
Preparation of (±)-huprine Y, (±)-14, from enone, (±)-48.



(±)-**48** **49** (±)-**14**

In a triple neck 2 L round-bottomed flask equipped with an inert atmosphere, magnetic stirrer, a pressure-equalizing dropping funnel and a condenser, a suspension of 2-amino-4-chlorobenzonitrile, **49** (10.8 g, 71.0 mmol) and $AlCl_3$ (9.50 g, 71.4 mmol) in 1,2-dichloroethane (100 mL), was prepared and treated with a solution of enone (±)-**48** (7.10 g, 47.3 mmol) in 1,2-dichloroethane (435 mL). The reaction mixture was stirred under reflux overnight, then allowed to cool to r. t., diluted with H_2O (250 mL) and THF (250 mL), alkalinized with 5 N NaOH (34 mL), again stirred at r. t. for 50 min and concentrated under vacuum. The resulting suspension was filtered and the yellow solid was recrystallized from EOAc / hexane (9:1, 300 mL). The beige solid was suspended in 2 N NaOH and extracted with EtOAc (3 x 200 mL) and CH_2Cl_2 (3 x 200 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give the desired (±)-huprine Y, (±)-**14** (6.50 g, 48% yield) as a beige solid.

Preparation of (*E*)-5,6-dimethoxy-2-[(4-pyridyl)methylene]-1-indanone, 80.



In a 250 mL round-bottomed flask equipped with magnetic stirrer, a Dean-Stark and a condenser, a solution of 5,6-dimethoxy-1-indanone, **62** (12.8 g, 66.7 mmol) in toluene (160 mL) was prepared and treated with 4-pyridinecarboxaldehyde, **79** (8.90 mL, 10.1 g, 94.6 mmol) and *p*-TsOH·H_2O (19.6 g, 103 mmol). The reaction mixture was stirred under reflux for 6 h, then warmed to r. t., filtered and the solid washed with 10% aq. $NaHCO_3$ (160 mL) and H_2O (130 mL), to give, after drying at 50 ºC / 20 Torr, the desired **80** (17.8 g, 95% yield) as a yellow solid.

R_f = 0.35 (silica gel, 10 cm, EtOAc / hexane 9:1).

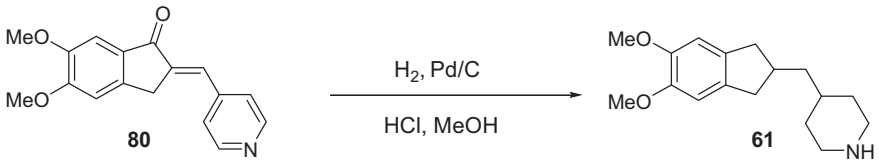
Melting point: 207–208 ºC (MeOH).

IR (KBr) v: 1689 (C=O st) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.91 (s, 3H, 6-OCH_3), 3.93 (d, *J* = 1.5 Hz, 2H, 3-H_2), 3.98 (s, 3H, 5-OCH_3), 6.94 (s, 1H, 4-H), 7.27 (s, 1H, 7-H), 7.40–7.44 [complex signal, 3H, 3(5)-H 4-pyridyl and 2=CH], 8.66 [dm, *J* = 4.8 Hz, 2H, 2(6)-H 4-pyridyl].

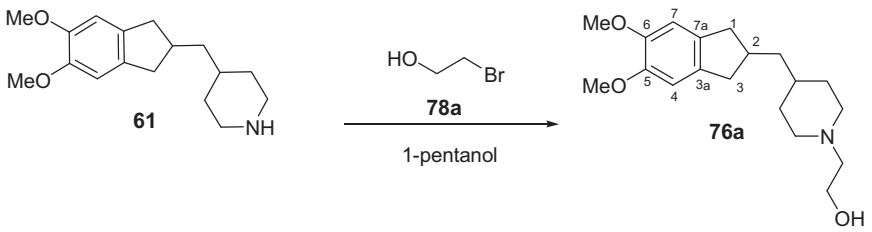
^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 31.9 (CH_2, C3), 56.1 (CH_3, 6-OCH_3), 56.3 (CH_3, 5-OCH_3), 105.0 (CH, C7), 107.1 (CH, C4), 123.8 [2 CH, C3(5) 4-pyridyl], 129.0 (CH, 2=CH), 130.6 (C, C7a), 139.7 (C) and 142.6 (C) (C2 and C4 4-pyridyl), 144.7 (C, C3a), 149.8 (C, C6), 150.4 [2 CH, C2(6) 4-pyridyl], 155.9 (C, C5), 192.1 (C, C1).

Preparation of 5,6-dimethoxy-2-[(4-piperidinyl)methyl]indane, 61.



In an hydrogenation flask, a suspension of **80** (28.5 g, 101 mmol), concentrated HCl (17 mL, 172 mmol) and 5% Pd/C (50% of H_2O, 19.0 g) in MeOH (480 mL) was placed, and hydrogenated at 30 atm and 65 ºC for 7 days. The resulting suspension was filtered and evaporated under reduced pressure. The residue was suspended in 5 N NaOH (250 mL) and extracted with CH_2Cl_2 (2 x 250 mL) and EtOAc (2 x 250 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give the desired indane **61**, (26.9 g, 97% yield) as a beige solid.

Preparation of 2-{[1-(2-hydroxyethyl)piperidin-4-yl]methyl}-5,6-dimethoxyindane, 76a.



In a 25 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a condenser, a solution of piperidine **61** (970 mg, 3.53 mmol) and 2-bromoethanol, **78a** (0.5 mL, 0.88 g, 7.05 mmol) in 1-pentanol (14 mL) was prepared. The reaction mixture was stirred under reflux for 48 h, cooled to r. t., diluted with EtOAc (60 mL) and extracted with 1 N HCl (3 x 20 mL). The combined aqueous extracts were washed with EtOAc (4 x 20 mL), alkanilized with solid NaOH to pH = 14 and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were dried with anydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a grey solid (861 mg), which was purified through column chromatography (silica gel, 35–70 μm, 43 g, Ø = 3.5 cm; #1–40, 900 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 92:8:0.5; #41–45, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 90:10:0.5; #46–68, 500 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 80:20:0.5), to provide the desired alcohol **76a** (#8–27, 616 mg, 55% yield) and unreacted piperidine **61** (#41–68, 193 mg).

R_f = 0.27 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 8.5:1.5:0.05).

Melting point: 78–79 ºC (CH_2Cl_2 / MeOH / 50% aq. NH_4OH 92:8:0.5).

IR (KBr) v: 3514 (OH st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24 [dddd, $J \approx J' \approx J'' \approx$ 12.4 Hz, J''' = 3.2 Hz, 2H, 3(5)-H_{ax} of piperidine], partially overlapped 1.30−1.44 (m, 1H, 4-H of piperidine), 1.44 (dd, $J = J'$ = 6.8 Hz, 2H, 2-CH_2), 1.72 [broad d, J = 12.0 Hz, 2H, 3(5)-H_{eq} of piperidine], 2.05 [ddd, $J = J'$ = 11.6 Hz, J'' = 1.6 Hz, 2H, 2(6)-H_{ax} of piperidine], partially overlapped 2.46−2.61 [complex signal, 3H, 1(3)-H_a of indane and 2-H of indane], 2.51 (t, J = 5.2 Hz, 2H, NC$H_2$$CH_2$OH], 2.90 [broad d, J = 11.6 Hz, 2H, 2(6)-H_{eq} of piperidine], 2.96 [dd, J = 14.0 Hz, J' = 7.2 Hz, 2H, 1(3)-H_b of indane], 3.60 (t, J = 5.2 Hz, 2H, NCH_2CH_2OH), 3.84 [s, 6H, 5(6)-OCH_3], 6.73 [s, 2H, 4(7)-H of indane]. OH was not observed.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 32.7 [CH_2, C3(5) of piperidine], 34.5 (CH, C4 of piperidine), 37.7 (CH, C2 of indane), 39.5 [CH_2, C1(3) of indane], 42.8 (CH_2, 2-CH_2), 53.8 [CH_2, C2(6) of piperidine], 56.0 [CH_3, 5(6)-OCH_3], 57.8 (CH_2, NCH_2CH_2OH), 59.5 (CH_2, N$CH_2$$CH_2$OH), 107.9 [CH, C4(7) of indane], 134.9 [C, C3a(7a) of indane], 147.8 [C, C5(6) of indane].

Elemental analysis: Calculated for $C_{19}H_{29}NO_3$: C: 71.44%; H: 9.15%; N: 4.38%.

Observed: C: 71.41%; H: 9.09%; N: 4.36%.

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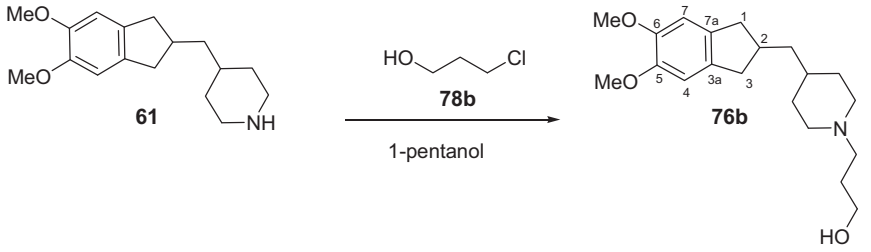
Preparation of 2-{[1-(3-hydroxypropyl)piperidin-4-yl]methyl}-5,6-dimethoxyindane, 76b.



In a 25 mL round-bottomed flask equipped with an inert atmposphere, magnetic stirrer and a condenser, a solution of piperidine **61** (1.09 g, 3.96 mmol) and 3-chloro-1-propanol, **78b** (0.70 mL, 0.79 g, 8.37 mmol) in 1-pentanol (15 mL) was prepared. The reaction mixture was stirred under reflux for 48 h, cooled to r. t., diluted with EtOAc (60 mL) and extracted with 1 N HCl (3 x 20 mL). The combined aqueous extracts were washed with EtOAc (4 x 20 mL), alkalinized with solid NaOH to pH = 14 and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, and evaporated under reduced pressure to give a grey solid (1,02 g) which was purified through column chromatography (silica gel, 35–70 μm, 51 g, Ø = 3.5 cm; #1–40, 900 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 92:8:0.5; #41–45, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 90:10:0.5; #46–58, 500 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 85:15:0.5), to provide the desired alcohol **76b** (#8–16, 577 mg, 44% yield) and unreacted piperidine **61** (#44–55, 78 mg).

R_f = 0.29 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Melting point = 85–86 ºC (CH_2Cl_2 / MeOH / 50% aq. NH_4OH 92:8:0.5).

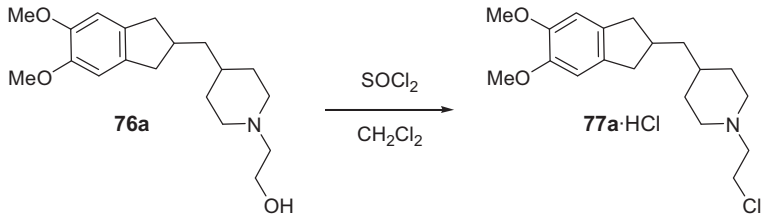
IR (KBr) v: 3172 (OH st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 [dddd, $J \approx J' \approx J'' \approx$ 12.4 Hz, J''' = 3.6 Hz, 2H, 3(5)-H_{ax} of piperidine], partially overlapped 1.30–1.41 (m, 1H, 4-H of piperidine), 1.42 (dd, $J = J'$ = 6.4 Hz, 2H, 2-CH_2), 1.67–1.76 [complex signal, 4H, $NCH_2CH_2CH_2OH$ and 3(5)-H_{eq} of piperidine], 1.92 [broad dd, $J \approx J' \approx$ 11.2 Hz, 2H, 2(6)-H_{ax} of piperidine], 2.45–2.55 [complex signal, 3H, 1(3)-H_a of indane and 2-H of indane], 2.58 (t, J = 5.6 Hz, 2H, $NCH_2CH_2CH_2OH$), 2.95 [dd, J = 14.0 Hz, J' = 7.6 Hz, 2H, 1(3)-H_b of indane], 3.05 [broad d, J = 11.2 Hz, 2H, 2(6)-H_{eq} of piperidine], 3.79 (t, J = 5.6 Hz, 2H, $NCH_2CH_2CH_2OH$), 3.83 [s, 6H, 5(6)-OCH_3], 6.72 [s, 2H, 4(7)-H of indane]. OH was not observed.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 27.1 (CH_2, $NCH_2CH_2CH_2OH$), 32.6 [CH_2, C3(5) of piperidine], 34.4 (CH, C4 of piperidine), 37.7 (CH, C2 of indane), 39.4 [CH_2, C1(3) of indane], 42.6 (CH_2, 2-CH_2), 54.2 [CH_2, C2(6) of piperidine], 56.0 [CH_3, 5(6)-OCH_3], 59.3 (CH_2, $NCH_2CH_2CH_2OH$), 64.8 (CH_2, $NCH_2CH_2CH_2OH$), 107.8 [CH, C4(7) of indane], 134.9 [C, C3a(7a) of indane], 147.8 [C, C5(6) of indane].

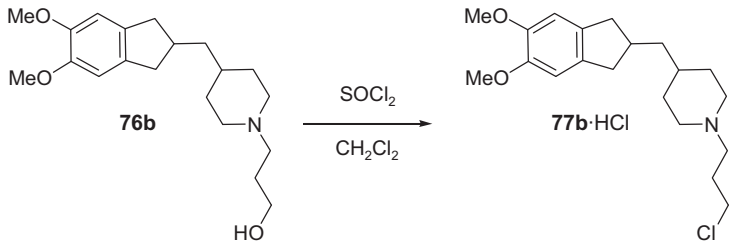
Elemental analysis: Calculated for $C_{20}H_{31}NO_3$: C: 72.04%; H: 9.37%; N: 4.20%.
Observed: C: 72.06%; H: 9.25%; N: 4.16%.

Preparation of 2-{[1-(2-chloroethyl)piperidin-4-yl]methyl}-5,6-dimethoxyindane, 77a·HCl.



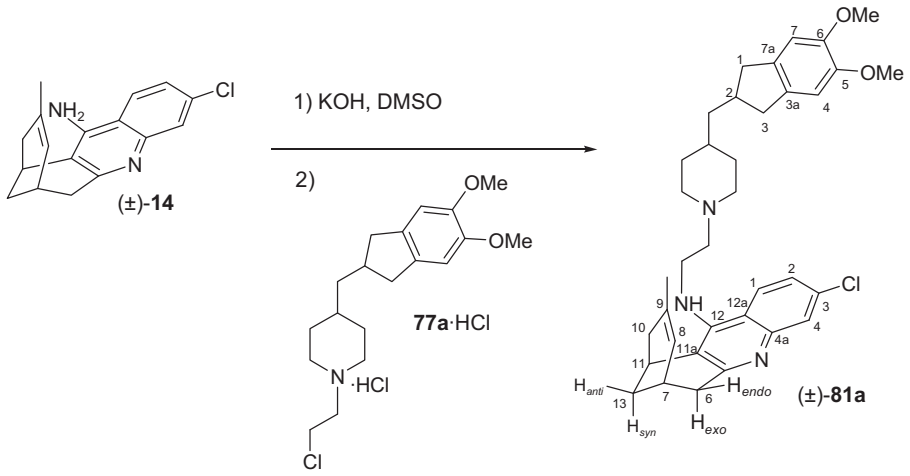
In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a condenser, a solution of alcohol **76a** (566 mg, 1.77 mmol) in anhydrous CH_2Cl_2 (25 mL) was prepared, cooled to 5 ºC with an ice / H_2O bath and treated dropwise with $SOCl_2$ (6.43 mL, 10.5 g, 88.5 mmol). The reaction mixture was stirred under reflux for 4 h, then cooled to r. t. and evaporated under reduced pressure. The resulting brown oil was taken in CH_2Cl_2 (5 x 10 mL) and evaporated under reduced pressure to give a beige solid (671 mg) that proved to be consistent with desired **77a**·HCl and that was used as a crude in the next step without further purification.

Preparation of 2-{[1-(3-chloropropyl)piperidin-4-yl]methyl}-5,6-dimethoxyindane, 77b·HCl.



In a double neck 50 mL round-bottomed flask equipped with a inert atmosphere, magnetic stirrer and a condenser, a solution of alcohol **76b** (694 mg, 2.08 mmol) in anhydrous CH_2Cl_2 (30 mL) was prepared, cooled to 5 ºC with an ice / H_2O bath and treated dropwise with $SOCl_2$ (7.6 mL, 12.4 g, 105 mmol). The reaction mixture was stirred under reflux for 4 h, then cooled to r. t. and evaporated under reduced pressure. The resulting brown oil was taken in CH_2Cl_2 (5 x 15 mL) and evaporated under reduced pressure to give a beige solid (825 mg) that proved to be consistent with the desired **77b**·HCl that was used as a crude in the next step without further purification.

Preparation of (±)-3-chloro-12-[(2-{4-[(5,6-dimethoxyindan-2-yl)methyl]piperidin-1-yl}ethyl) amino]-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinoline, (±)-81a.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (235 mg, 0.83 mmol) and finely powdered KOH (85% purity, 241 mg, 3.65 mmol) were placed and suspended in anhydrous DMSO (4 mL). The resulting suspension was stirred at r. t. for 2 h, then treated with a solution of **77a**·HCl (371 mg of a crude that could contain a maximum of 0.97 mmol) in anhydrous DMSO (1.5 mL) (previously warmed at 70 ºC in a water bath). The reaction mixture was stirred at r. t. for 72 h, then diluted with H_2O (25 mL) and extracted with CH_2Cl_2 (4 x 15 mL). The combined organic extracts were washed with H_2O (3 x 25 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure to give a brownish solid (384 mg), which was purified through column chromatography (silica gel, 35–70 μm, 50 g, Ø = 3 cm; #1–90, 1400 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.4), to provide the desired hybrid (±)-**81a** (#34–51, 103 mg, 21% isolated yield) as a beige oil, a mixture of (±)-**81a** / (±)-**14** in an approximate ratio 64:36 (^{1}H NMR) (#52–62, 114 mg, 36% total yield of (±)-**81a**), and unreacted huprine Y (±)-**14** (#63–84, 47 mg).

R_f = 0.64 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-81a· HCl.

In a 10 mL round-bottomed flask, (±)-**81a** (90 mg, 0.15 mmol) was dissolved in CH_2Cl_2 (3 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.40 N, 3.50 mL), evaporated under reduced pressure and washed with Et_2O (3 x 2 mL) to give, after drying under standard conditions (±)-**81a**·2HCl (96 mg) as a beige solid.

Melting point: 200–202 ºC (CH_2Cl_2 / MeOH 6:7).

IR (KBr) ν: 3500–2500 (max. at 3417, 3256, 3055, 3001, 2928, 2835, NH, N^+H and CH st), 1630, 1581, 1504 (Ar–C–C and Ar–C–N st) cm^{-1}.

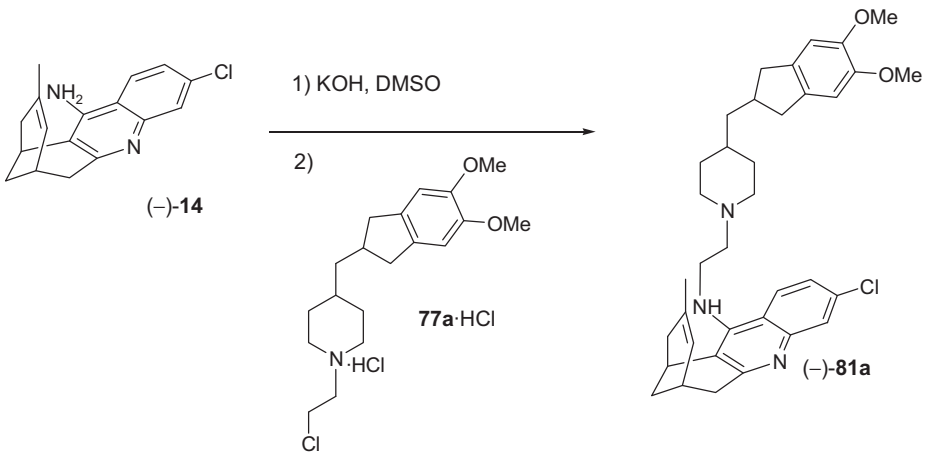
^{1}H NMR (500 MHz, CD_3OD) δ: 1.53 (t, *J* = 7.0 Hz, 2H, 2-CH_2 of indane), 1.58 (2, 3H, 9-CH_3), 1.66 [m, 2H, 3(5)-H_{ax} of piperidine], 1.72–1.80 (broad signal, 1H, 4-H of piperidine), 1.93 (d, *J* = 18.5 Hz, 1H, 10-H_{endo}), partially overlapped 1.97 (dm, *J* ≈ 13.0 Hz, 1H, 13-H_{syn}), 2.06 [broad d, *J* = 14.5 Hz, 2H, 3(5)-H_{eq} of piperidine], partially overlapped 2.10 (dm, *J* = 13.0 Hz, 1H, 13-H_{anti}), 2.53 [broad d, *J* = 14.0 Hz, 2H, 1(3)-H_{cis} of indane], partially oevrlapped 2.48–2.60 (m, 1H, 2-H of indane), partially overlapped 2.60 (broad dd, *J* ≈ 18.5 Hz, *J'* = 5.0 Hz, 1H, 10-H_{exo}), 2.78 (m, 1H, 7-H), 2.92 (dm, *J* = 18.0 Hz, 1H, 6-H_{endo}), 3.00 [dd, *J* ≈ 14.0 Hz, *J'* ≈ 7.0 Hz, 2 H, 1(3)-H_{trans} of indane], 3.11 [broad t, *J* = 11.5 Hz, 2H, 2(6)-H_{ax} of piperidine], 3.24 (dd, *J* = 18.0 Hz, *J'* = 5.5 Hz, 1H, 6-H_{exo}), 3.60 (m, 1H, 11-H), partially overlapped 3.62 (m, 2H, $NHCH_2C\mathit{H}_2N$), 3.68–3.76 [broad signal, 2H, 2(6)-H_{eq} of piperidine], 3.77 [s, 6H, 5(6)-OCH_3 of indane], 4.42 (t, *J* = 6.5 Hz, 2H, $NH\mathit{CH}_2CH_2N$), 4.84 (s, NH and $^{+}$NH), 5.58 (broad d, *J* = 4.5 Hz, 1H, 8-H), 6.78 [s, 2H, 4(7)-H of indane], 7.64 (dd, *J* = 9.0 Hz, *J'* = 2.0 Hz, 1 H, 2-H), 7.81 (d, *J* ≈ 2.0 Hz, 1H, 4-H), 8.35 (d, *J* = 9.0 Hz, 1H, 1-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.4 (CH_3, 9-CH_3), 27.5 (CH, C11), 27.8 (CH, C7), 29.1 (CH_2, C13), 30.9 [CH_2, C3(5) of piperidine], 33.4 (CH, C4 of piperidine), 36.2 (CH_2, C10), 36.6 (CH_2, C6), 38.9 (CH, C2 of indane), 40.3 [CH_2, C1(3) of indane], 43.0 (CH_2, $NH\mathit{C}H_2CH_2N$), 43.9 (CH_2, 2-CH_2 of indane), 54.9 [CH_2, C2(6) of piperidine], 56.7 [CH_3, 5(6)-OCH_3 of indane], 57.2 (CH_2, $NHCH_2\mathit{C}H_2N$), 109.7 [CH, C4(7) of indane], 116.0 (C) and 119.3 (C) (C11a and C12a), 119.4 (CH, C4), 125.1 (CH, C8), 127.5 (CH, C2), 129.0 (CH, C1), 134.7 (C, C9), 136.3 [C, C3a(7a) of indane], 140.4 (C, C3), 140.8 (C, C4a), 149.4 [C, C5(6) of indane], 152.5 (C, C5a), 157.1 (C, C12).

HRMS, ESI:
Calculated for ($C_{36}H_{44}{}^{35}ClN_3O_2$ + H^{+}): 586.3194.
Observed: 586.3207.

Elemental analysis: Calculated for $C_{36}H_{44}ClN_3O_2 \cdot 2HCl \cdot 3H_2O$:
C: 60.63%; H: 7.35%; N: 5.89%; Cl: 14.91%.
Observed: C: 60.90%; H: 7.05%; N: 5.93%; Cl: 15.06%.

Preparation of (–)-(7*S*,11*S*)-3-chloro-12-[(2-{4-[5,6-dimethoxyindan-2-yl)methyl]piperidin-1-yl}ethyl)amino]-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinoline, (–)-81a.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (–)-huprine Y, (–)-**14** (>99% ee, 295 mg, 1.04 mmol) and finely powdered KOH (85% purity, 380 mg, 5.76 mmol) were placed and suspended in anhydrous DMSO (3 mL). The resulting suspension was stirred at r. t. for 2 h, then treated with a solution of **77a**·HCl (473 mg of a crude that could contain a maximum of 1.30 mmol) in anhydrous DMSO (5 mL) (previously warmed at 70 ºC in a water bath). The reaction mixture was stirred at r. t. for 72 h, then diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were washed with H_2O (3 x 25 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure to give a brownish solid (686 mg), which was purified through column chromatography (silica gel, 35–70 μm, 68 g, Ø = 3 cm; #1–13, 400 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #14–127, 3500 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #128–148, 500 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2), to provide the desired hybrid (–)-**81a** (#72–108, 106 mg, 17% yield) as a colourless oil and a mixture of (–)-**81a** / (–)-**14** in an approximate ratio 55:45 (^{1}H NMR) [#109–142, 210 mg, 36% total yield of (–)-**81a**).

R_f = 0.64 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (–)-81a·2HCl:

In a 25 mL round-bottomed flask, (–)-**81a** (106 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.65 N, 2.5 mL) evaporated under reduced pressure and washed with pentane (2 x 5 mL) to give, after drying under standard conditions (–)-**81a**·2HCl (104 mg) as a white solid.

Melting point: 228–230 ºC (CH_2Cl_2 / MeOH 4:1).

$[\alpha]^{20}_D$ = –139 (*c* 0.96, MeOH).

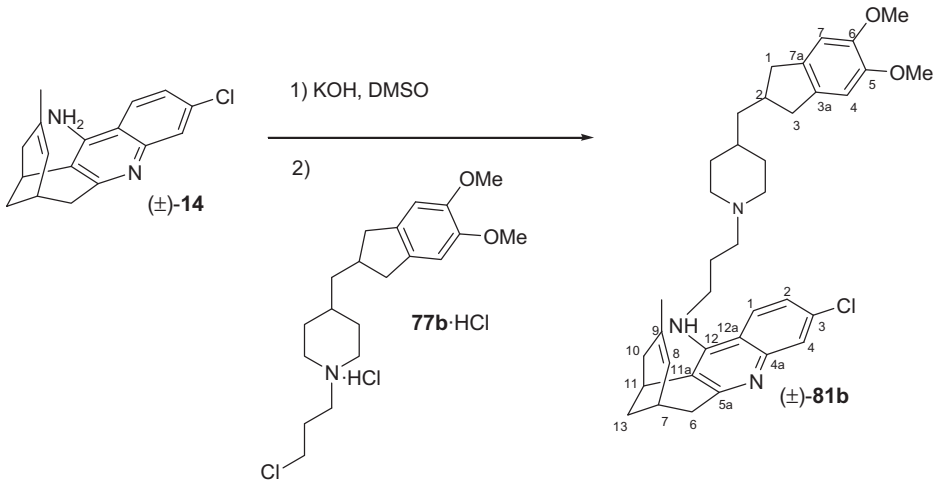
IR (KBr) v: 3600–2500 (max at 3551, 3388, 3342, 3229, 3056, 2928, 2842, 2701, NH, N^+H and CH st), 1629, 1582, 1505 (Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR and ^{13}C NMR spectra were identical to those previously reported for (±)-**81a**.

HRMS, ESI:
Calculated for ($C_{36}H_{44}{}^{35}ClN_3O_2 + H^+$): 586.3194.
Observed: 586.3193.

Elemental analysis: Calculated for $C_{36}H_{44}ClN_3O_2 \cdot 2HCl \cdot 2H_2O$:
C: 62.20%; H: 7.25%; N: 6.04%; Cl: 15.30%.
Observed: C: 62.45%; H: 7.20%; N: 6.19%; Cl: 15.13%.

Preparation of (±)-3-chloro-12-[(3-{4-[(5,6-dimethoxyindan-2-yl)methyl]piperidin-1-yl}propyl)amino]-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinoline, (±)-81b.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (311 mg, 1.09 mmol) and finely powdered KOH (85% purity, 374 mg, 5.67 mmol) were placed and suspended in anhydrous DMSO (5 mL). The resulting suspension was stirred at r. t. for 2 h, then treated with a solution of **77b**·HCl (508 mg of a crude that could contain a maximum of 1.28 mmol) in anhydrous DMSO (1.6 mL) (previously warmed at 70 ºC in a water bath). The reaction mixture was stirred at r. t. for 72 h, then diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (5 x 15 mL). The combined organic extracts were washed with H_2O (3 x 25 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure to give a brownish solid (641 mg), which was purified through column chromatography (silica gel, 35–70 μm, 65 g, Ø = 3 cm; #1–102, 1800 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #103–113, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 95:5:0.2; #114–130, 400 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 92:8:0.2), to provide huprine Y, (±)-**14** (#112–127, 155 mg) and a mixture of unreacted (±)-**14** and protonated hybrid (±)-**81b**·HCl (#92–111, 472 mg) which was dissolved in CH_2Cl_2 (15 mL), and washed with 2 N NaOH (3 x 15 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellowish solid (388 mg), which was purified through column chromatography (Biotage Flash 40M, 40 x 150 mm; #1–108, 2000 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2), to afford the desired hybrid (±)-**81b** (#1–24, 62 mg), and a mixture of (±)-**81b** and unreacted (±)-**14** (#25–102, 296 mg) which was again purified through column chromatography (Biotage Flash 40M, 40 x 150 mm; #1–68, 1000 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #69–136, 1000 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2; #137–209, 1000 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #210–250, 1000 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2; #251–273, 600 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 95:5:0.2), to provide hybrid (±)-**81b** (#223–233, 74 mg, 21% yield) as a yellow solid and a mixture of (±)-**81b** / (±)-**14** in an approximate ratio 62:38 (^{1}H NMR) (#71–113, 152 mg, 35% total yield of (±)-**81b**).

R_f = 0.59 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-81b·2HCl.

In a 25 mL round-bottomed flask, (±)-**81b** (136 mg, 0.23 mmol) was dissolved in CH_2Cl_2 (3 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.4 N, 5.1 mL) and evaporated under reduced pressure. The residue was dissolved in MeOH (0.5 mL) and precipitated with EtOAc (4 mL), the solvent was decanted and the residue was washed with Et_2O (2 x 5 mL) to give, after drying under standard conditions, (±)-**81b**·2HCl (131 mg) as a white solid.

Melting point: 184–186 ºC (MeOH / EtOAc 1:8).

IR (KBr) ν: 3600–2500 (max at 3417, 3252, 3060, 2995, 2928, 2835, NH, N^+H and CH st), 1630, 1581, 1504 (Ar–C–C and Ar–C–N st) cm^{-1}.

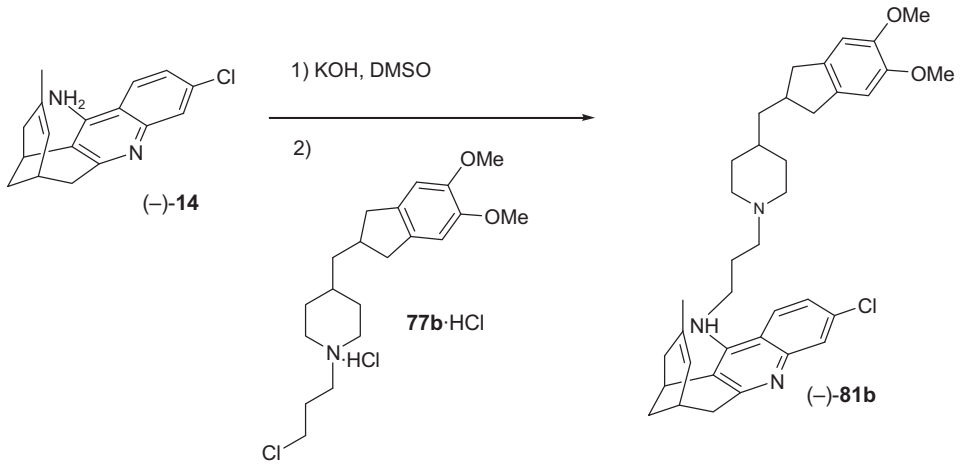
^1H NMR (500 MHz, CD_3OD) δ: 1.51 (m, 2H, 2-CH_2 of indane), partially overlapped 1.55 [m, 2H, 3(5)-H_{ax} of piperidine], 1.58 (s, 3H, 9-CH_3), 1.70–1.80 (broad signal, 1H, 4-H of piperidine), 1.92 (d, *J* = 17.5 Hz, 1H, 10-H_{endo}), partially overlapped 1.97 (dm, *J* ≈ 13.0 Hz, 1H, 13-H_{syn}), 2.04 [broad d, *J* = 14.5 Hz, 2H, 3(5)-H_{eq} of piperidine], 2.09 (dm, *J* = 13.0 Hz, 1H, 13-H_{anti}), 2.37 (m, 2H, $NHCH_2CH_2CH_2N$), 2.52 [broad d, *J* = 13.0 Hz, 2H, 1(3)-H_{cis} of indane], partially overlapped 2.48–2.59 (m, 1H, 2-H of indane), partially overlapped 2.62 (broad d, *J* ≈ 17.5 Hz, *J'* = 4.0 Hz, 1H, 10-H_{exo}), 2.77 (m, 1H, 7-H), 2.88 (d, *J* = 18.0 Hz, 1H, 6-H_{endo}), 2.99 [dd, *J* ≈ 13.0 Hz, *J'* = 8.0 Hz, 2H, 1(3)-H_{trans} of indane], partially overlapped 3.00 [m, 2H, piperidine 2(6)-H_{ax} of piperidine], 3.22 (dd, *J* = 18.0 Hz, *J'* = 5.5 Hz, 1H, 6-H_{exo}), 3.27 (m, 2H, $NHCH_2CH_2CH_2N$), 3.54 (m, 1H, 11-H), 3.58–3.66 [broad signal, 2H, 2(6)-H_{eq} of piperidine], 3.77 [s, 6H, 5(6)-OCH_3 of indane], 4.08 (t, *J* = 7.0 Hz, 2H, $NHCH_2CH_2CH_2N$), 4.85 (s, NH and ^+NH), 5.58 (broad d, *J* = 5,0 Hz, 1H, 8-H), 6.78 [s, 2H, 4(7)-H of indane], 7.60 (dd, *J* = 9.5 Hz, *J'* = 2.0 Hz, 1H, 2-H), 7.78 (d, *J* = 2.0 Hz, 1H, 4-H), 8.41 (d, *J* = 9.5 Hz, 1H, 1-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9-CH_3), 26.2 (CH_2, $NHCH_2CH_2CH_2N$), 27.4 (CH, C11), 27.9 (CH, C7), 29.3 (CH_2, C13), 30.9 [CH_2, C3(5) of piperidine], 33.6 (CH, C4 of piperidine), 36.1 (CH_2, C10), 36.4 (CH_2, C6), 38.8 (CH, C2 of indane), 40.3 [CH_2, C1(3) of indane], 43.0 (CH_2, 2-CH_2 of indane), 46.6 (CH_2, $NHCH_2CH_2CH_2N$), 54.3 [CH_2, C2(6) of piperidine], 55.3 (CH_2, $NHCH_2CH_2CH_2N$), 56.7 [CH_3, 5(6)-OCH_3 of indane], 109.7 [CH, C4(7) of indane], 115.8 (C) and 118.3 (C) (C11a and C12a), 119.2 (CH, C4), 125.1 (CH, C8), 127.2 (CH, C2), 129.4 (CH, C1), 134.7 (C, C9), 136.3 [C, C3a(7a) of indane], 140.3 (C, C3), 140.9 (C, C4a), 149.4 [C, C5(6) of indane], 151.8 (C, C5a), 156.9 (C, C12).

HRMS, ESI:
Calculated for ($C_{37}H_{46}{}^{35}ClN_3O_2 + H^+$): 600.3351.
Observed: 600.3358.

Elemental analysis: Calculated for $C_{37}H_{46}ClN_3O_2 \cdot 2HCl \cdot 2H_2O$:
C: 62.66%; H: 7.39%; N: 5.93%; Cl: 15.00%.
Observed: C: 62.29%; H: 7.19%; N: 5.71%; Cl: 15.05%.

Preparation of (–)-(7*S*,11*S*)-3-chloro-12-[(3-{4-[5,6-dimethoxyindan-2-yl)methyl]piperidin-1-yl}propyl)amino]-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinoline, (–)-81b.



In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (–)-huprine Y, (–)-**14** (>99% ee, 739 mg, 2.60 mmol) and finely powdered KOH (85% purity, 964 mmg, 14.6 mmol) were placed and suspended in anhydrous DMSO (10 mL). The resulting suspension was stirred at r. t. for 2 h, then treated with a solution of **77b**·HCl (1.20 g of a crude that could contain a maximum of 3.08 mmol) in anhydrous DMSO (9 mL) (previously warmed at 70 ºC in a water bath). The reaction mixture was stirred at r. t. for 72 h, then diluted with H_2O (200 mL) and extracted with CH_2Cl_2 (2 x 150 mL). The combined organic extracts were washed with H_2O (3 x 200 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure to give a brownish solid (1.40 g), which was purified through column chromatography (silica gel 35–70 µm, 98 g, Ø = 5 cm; #1-34, 3500 mL, hexane / EtOAc / Et_3N 50:50:0.2), to provide unreacted (–)-**14** (#9–17, 256 mg) and the desired hybrid (–)-**81b** (#18–34, 597 mg, 38% yield) as a colourless oil.

R_f = 0.62 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (–)-81b·2HCl.

In a 10 mL round-bottomed flask, (–)-**81b** (64 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (7 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.61 N, 1.68 mL), evaporated under reduced pressure and washed with pentane (2 x 5 mL) to give, after drying under standard conditions, (–)-**81b**·2HCl (72 mg) as a white solid.

Melting point: 206–208 ºC (CH_2Cl_2 / MeOH 5:1).

$[\alpha]^{20}_D$ = –139 (*c* 0.11, MeOH)

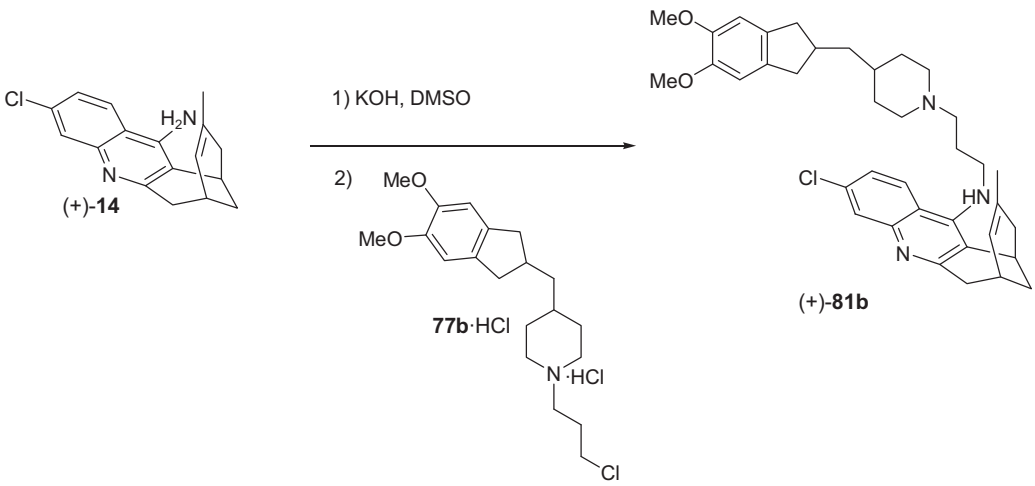
IR (KBr) v: 3600–2500 (max. at 3371, 3232, 3119, 3055, 2924, 2856, 2646, NH, N^+H and CH st), 1630, 1582, 1502 (Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR and ^{13}C NMR spectra were identical to those previously reported for (±)-**81b**.

HRMS, ESI:
Calculated for ($C_{37}H_{46}{}^{35}ClN_3O_2 + H^+$): 600.3351.
Observed: 600.3344.

Elemental analysis: Calculated for $C_{37}H_{46}ClN_3O_2 \cdot 2HCl \cdot 3.5H_2O$:
C: 60.36%; H: 7.53%; N: 5.71%; Cl: 14.45%.
Observed: C: 60.07%; H: 7.29%; N: 5.34%; Cl: 14.79%.

Preparation of (+)-(7*R*,11*R*)-3-chloro-12-[(3-{4-[5,6-dimethoxyindan-2-yl)metil]piperidin-1-yl}propyl)amino]-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinoline, (+)-81b.



In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (+)-huprine Y, (+)-**14** (>99% ee, 283 mg, 0.99 mmol) and finely powdered KOH (85% purity, 396 mg, 6.00 mmol) were placed and suspended in anhydrous DMSO (3 mL). The resulting suspension was stirred at r. t. for 2 h, then treated with a solution of **77b**·HCl (461 mg of a crude that could contain a maximum of 1.39 mmol) in anhydrous DMSO (3.5 mL) (previously warmed at 70 ºC in a water bath). The reaction mixture was stirred at r. t. for 72 h, then diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (6 x 50 mL). The combined organic extracts were washed with H_2O (5 x 100 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure to give a brownish solid (590 mg), which was purified through column chromatography (silica gel, 35–70 µm, 59 g, Ø = 3 cm; #1–21, 5000 mL, hexane / EtOAc / Et_3N 50:50:0.2), to provide unreacted (+)-**14** (#4–7, 41 mg), a mixture of (+)-**14** / (+)-**81b** in an approximate ratio 35:65 (^{1}H NMR) (#8, 20 mg) and the desired hybrid (+)-**81b** (#9–21, 279 mg, 47% yield) as a yellow oil.

R_f = 0.58 (CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (+)-81b·2HCl.

In a 50 mL round-bottomed flask, (+)-**81b** (279 mg, 0.47 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.68 N, 6.40 mL) and evaporated under reduced pressure. The residue was dissolved in MeOH (1.5 mL) and precipitated with EtOAc (9.6 mL), the solvent was decanted and the residue was dried and washed with pentane (2 x 5 mL) to give, after drying under standard conditions, (+)-**81b**·2HCl (235 mg) as a beige solid.

Melting point: 198–199 ºC (MeOH / EtOAc 2:13).

$[\alpha]^{20}_D$ = +140 (*c* 0.82, MeOH).

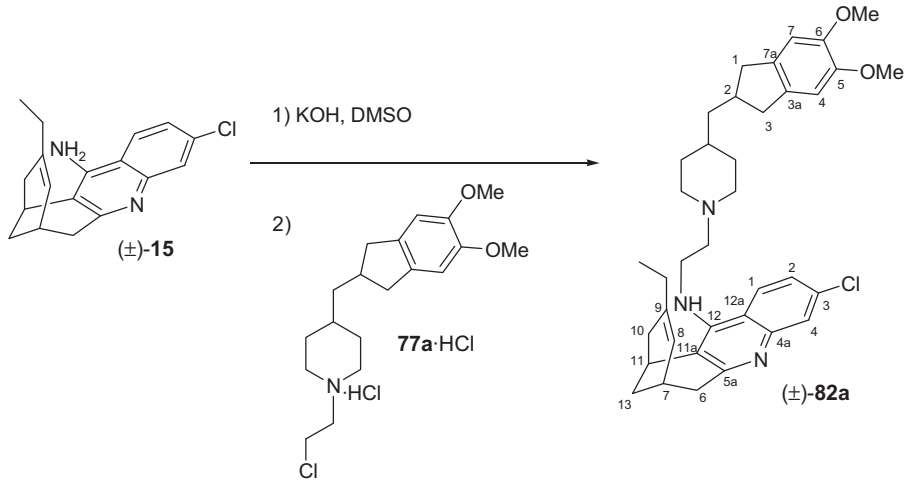
IR (KBr) v: 3600–2500 (max. at 3400, 3220, 3119, 3061, 3014, 2920, 2897, 2833, 2628, 2552, NH, N^+H and CH st), 1630, 1605, 1583, 1504 (Ar−C−C and Ar−C−N st) cm^{-1}.

^1H NMR and ^{13}C NMR spectra were identical to those previously reported for (±)-**81b**.

HRMS, ESI:
Calculated for ($C_{37}H_{46}{}^{35}ClN_3O_2 + H^+$): 600.3351.
Observed: 600.3352.

Elemental analysis: Calculated for $C_{37}H_{46}ClN_3O_2 \cdot 2HCl \cdot 2.5H_2O$:
C: 61.88%; H: 7.44%; N: 5.85%; Cl: 14.81%.
Observed: C: 62.14%; H: 7.22%; N: 5.81%; Cl: 14.38%.

Preparation of (±)-3-chloro-12-[(2-{4-[(5,6-dimethoxyindan-2-yl)methyl]piperidin-1-yl}ethyl)amino]-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline, (±)-82a.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine X, (±)-**15** (322 mg, 1.08 mmol) and finely powdered KOH (85% purity, 354 mg, 5.36 mmol) were placed and suspended in anhydrous DMSO (4.5 mL). The resulting suspension was stirred at r. t. for 2 h, then treated with a solution of **77a**·HCl (464 mg of a crude that could contain a maximum of 1.14 mmol) in anhydrous DMSO (1.5 mL) (previously warmed at 70 ºC in a water bath). The reaction mixture was stirred at r. t. for 72 h, then diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (4 x 15 mL). The combined organic extracts were washed with H_2O (3 x 30 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure to give a brownish solid (549 mg), which was purified through column chromatography (silica gel, 35–70 µm, 55 g, Ø = 3 cm; #1–120, 2000 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #121–127, 100 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2; #128–140, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 95:5:0.2), to provide the desired hybrid (±)-**82a** (#57–111, 181 mg, 28% yield) as a yellowish solid.

R_f = 0.58 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-82a·2HCl.

In a 10 mL round-bottomed flask, (±)-**82a** (181 mg, 0.30 mmol) was dissolved in CH_2Cl_2 (3 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.4 N, 6.80 mL) and evaporated under reduced pressure. The residue was dissolved in MeOH (0.4 mL) and precipitated with EtOAc (1.8 mL), the solvent was decanted and the residue was dried and washed with Et_2O (2 x 5 mL) to give, after drying under standard conditions, (±)-**82a**·2HCl (116 mg) as a beige solid.

Melting point: 222–225 ºC (MeOH / EtOAc 2:9).

IR (KBr) ν: 3600–2500 (max. at 3402, 3256, 3226, 3056, 2925, 2851, 2707, 2651, NH, N^+H and CH st), 1629, 1582, 1557, 1505 (Ar–C–C and Ar–C–N st) cm^{-1}.

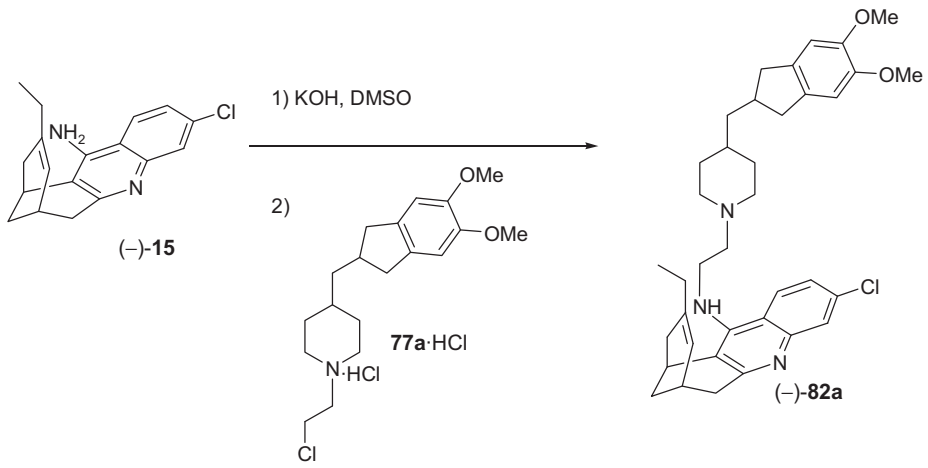
^{1}H NMR (500 MHz, CD_3OD) δ: 0.91 (t, *J* = 7.5 Hz, 3H, 9-CH_2CH_3), 1.52 (m, 2H, 2-CH_2 of indane), 1.55–1.68 [broad signal, 2H, 3(5)-H_{ax} of piperidine], 1.70–1.80 (broad signal, 1H, 4-H of piperidine), 1.86 (dq, *J* = 15.0 Hz, *J'* = 7.5 Hz, 1 H) and 1.89 (dq, *J* = 15.0 Hz, *J'* = 7.5 Hz, 1 H) (9-CH_2CH_3), 1.93 (d, *J* = 18.0 Hz, 1H, 10-H_{endo}), 1.99 (dm, *J* = 13.0 Hz, 1H, 13-H_{syn}), 2.05 [broad d, *J* = 13.5 Hz, 2H, 3(5)-H_{eq} of piperidine], 2.11 (dm, *J* ≈ 13.0 Hz, 1H, 13-H_{anti}), 2.52 [broad d, *J* = 13.0 Hz, 2H, 1(3)-H_{cis} of indane], partially overlapped 2.48–2.60 (m, 1H, 2-H of indane), 2.63 (broad dd, *J* ≈ 18.0 Hz, *J'* = 4.5 Hz, 1H, 10-H_{exo}), 2.81 (m, 1H, 7-H), 2.92 (broad d, *J* = 18.0 Hz, 1H, 6-H_{endo}), 2.99 [dd, *J* = 13.0 Hz, *J'* = 7.0 Hz, 2H, 1(3)-H_{trans} of indane], 3.04–3.15 [broad signal, 2H, 2(6)-H_{ax} of piperidine], 3.25 (dd, *J* = 18.0 Hz, *J'* = 5.5 Hz, 1 H, 6-H_{exo}), 3.57 (m, 1 H, 11-H), partially overlapped 3.61 (m, 2H, $NHCH_2CH_2N$), 3.64–3.74 [broad signal, 2H, 2(6)-H_{eq} of piperidine], 3.77 [s, 6H, 5(6)-OCH_3 of indane], 4.41 (t, *J* = 6.0 Hz, 2H, $NHCH_2CH_2N$), 4.84 (s, NH and $^{+}$NH), 5.59 (broad d, *J* = 5.5 Hz, 1H, 8-H), 6.78 [s, 2H, 4(7)-H of indane], 7.63 (dd, *J* = 9.0 Hz, *J'* = 2.0 Hz, 1H, 2-H), 7.81 (d, *J* = 2.0 Hz, 1H, 4-H), 8.35 (d, *J* = 9.0 Hz, 1H, 1-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 12.5 (CH_3, 9-CH_2CH_3), 27.5 (CH, C11), 27.8 (CH, C7), 29.4 (CH_2, C13), 30.9 (CH_2, 9-CH_2CH_3), 31.0 [CH_2, C3(5) of piperidine], 33.5 (CH, C4 of piperidine), 35.1 (CH_2, C10), 36.3 (CH_2, C6), 38.9 (CH, C2 of indane), 40.3 [CH_2, C1(3) of indane], 43.1 (CH_2, $NHCH_2CH_2N$), 43.9 (CH_2, 2-CH_2 of indane), 54.9 [CH_2, C2(6) of piperidine], 56.7 [CH_3, 5(6)-OCH_3 of indane], 57.1 (CH_2, $NHCH_2CH_2N$), 109.7 [CH, C4(7) of indane], 116.0 (C) and 119.3 (C) (C11a and C12a), 119.5 (CH, C4), 123.4 (CH, C8), 127.5 (CH, C2), 128.9 (CH, C1), 136.3 [C, C3a(7a) of indane], 140.2 (C, C9), 140.4 (C, C3), 140.8 (C, C4a), 149.4 [C, C5(6) of indane], 152.6 (C, C5a), 157.1 (C, C12).

HRMS, ESI:
Calculated for ($C_{37}H_{46}{}^{35}ClN_3O_2 + H^+$): 600.3351.
Observed: 600.3358.

Elemental analysis: Calculated for $C_{37}H_{46}ClN_3O_2 \cdot 2HCl \cdot 1.5H_2O$:
C: 63.47%; H: 7.34%; N: 6.00%; Cl: 15.19%.
Observed: C: 63.51%; H: 7.18%; N: 5.94%; Cl: 15.21%.

Preparation of (–)-(7*S*,11*S*)-3-chloro-12-[(2-{4-[5,6-dimethoxyindan-2-yl)methyl]piperidin-1-yl}ethyl)amino]-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline, (–)-82a.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (–)-huprine X, (–)-**15** (>99% ee, 303 mg, 1.02 mmol) and finely powdered KOH (85% purity, 349 mg, 5.29 mmol) were placed and suspended in anhydrous DMSO (4.5 mL). The resulting suspension was stirred at r. t. for 2 h, then treated with a solution of **77a**·HCl (413 mg of a crude that could contain a maximum of 1.14 mmol) in anhydrous DMSO (5 mL) (previously warmed at 70 ºC in a water bath). The reaction mixture was stirred at r. t. for 72 h, then diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were washed with H_2O (3 x 100 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure to give a brownish solid (555 mg), which was purified through column chromatography (silica gel, 35–70 µm, 55 g, Ø = 3 cm; #1–22, 700 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #23–123, 3000 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #124–141, 500 mL, CH_2Cl_2 / MeOH / 50 aq. NH_4OH 98:2:0.2), to provide the desired hybrid (–)-**82a** (#75–105, 110 mg, 18% yield) as a colourless oil and a mixture of (–)-**82a** / (–)-**15** in an approximate ratio 68:32 (^{1}H NMR) (#106–135, 156 mg, 35% total yield of (–)-**82a**).

R_f = 0.58 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (–)-82a·2HCl.

In a 25 mL round-bottomed flask, (–)-**82a** (110 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.65 N, 2.5 mL), evaporated under reduced pressure and washed with pentane (3 x 4 mL) to give, after drying under standard conditions, (–)-**82a**·2HCl (105 mg) as a beige solid.

$[\alpha]^{20}_D$ = –140 (*c* 1.39, MeOH).

Melting point: 251–252 ºC (CH_2Cl_2 / MeOH 4:1).

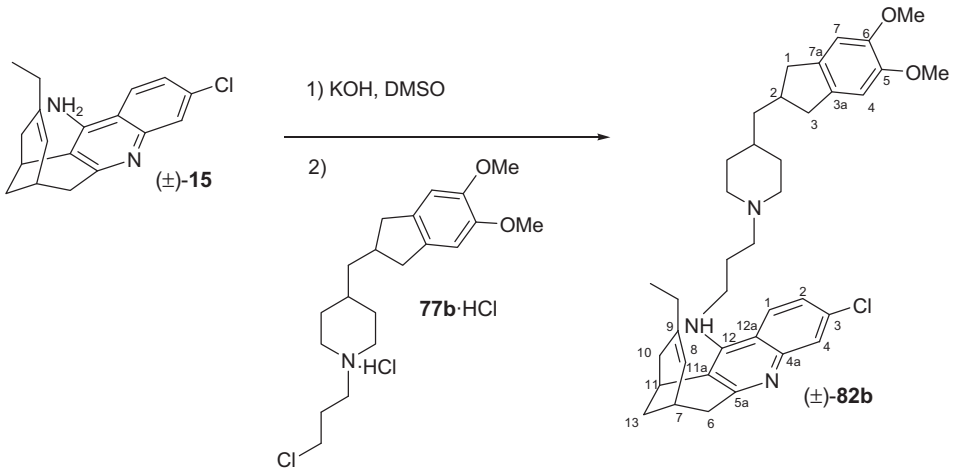
IR (KBr) v: 3500–2500 (max. at 3402, 3220, 3117, 3047, 2927, 2904, 2837, 2718, 2677, 2642, 2505, NH, N^+H and CH st), 1631, 1601, 1582,1502 (Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR and ^{13}C NMR were identical to those previously reported for (±)-**82a**.

HRMS, ESI:
Calculated for ($C_{37}H_{46}{}^{35}ClN_3O_2 + H^+$): 600.3351.
Observed: 600.3353.

Elemental analysis: Calculated for $C_{37}H_{46}ClN_3O_2 \cdot 2HCl \cdot 1.75H_2O$:
C: 63.06%; H: 7.37%; N: 5.96%; Cl: 15.09%.
Observed: C: 63.03%; H: 7.27%; N: 5.94%; Cl: 14.50%.

Preparation of (±)-3-chloro-12-[(3-{4-[(5,6-dimethoxyindan-2-yl)methyl]piperidin-1-yl}propyl)amino]-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline, (±)-82b.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine X, (±)-**15** (230 mg, 0.77 mmol) and finely powdered KOH (85% purity, 225 mg, 3.41 mmol) were placed and suspended in anhydrous DMSO (3.5 mL). The resulting suspension was stirred at r. t. for 2 h, then treated with a solution of **77b**·HCl (358 mg of a crude that could contain a maximum of 0.84 mmol) in anhydrous DMSO (2.2 mL) (previously warmed at 70 ºC in a water bath). The reaction mixture was stirred at r. t. for 72 h, then diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (4 x 15 mL). The combined organic extracts were washed with H_2O (4 x 30 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure to give a brownish solid (412 mg), which was purified through column chromatography (Biotage Flash 40M, 40 x 150 mm; #1–30, 1 L, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #31–63, 1 L, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2; #64–93, 1 L, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #94–123, 1 L, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 97:3:0.2; #124–153, 1 L, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 90:10:0.2), to provide a mixture of (±)-**15** / (±)-**82b** in an approximate ratio 10:90 (^{1}H NMR) (#71–113, 138 mg) and the desired hybrid (±)-**82b** (#114–126, 97 mg, 21% isolated yield, 47% total yield) as a yellow oil.

R_f = 0.59 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-82b·2HCl.

In a 10 mL round-bottomed flask, (±)-**82b** (97 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (3 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (3.5 N, 0.4 mL) and evaporated under reduced pressure. The residue was dissolved in MeOH (0.1 mL) and precipitated with EtOAc (0.8 mL), the solvent was decanted and the residue was washed with Et_2O (3 x 4 mL) to give, after drying under standard conditions, (±)-**82a**·2HCl (105 mg) as a beige solid.

Melting point: 210–211 ºC (MeOH / EtOAc 1:8).

IR (KBr) v: 3600–2500 (max. at 3372, 3254, 3120, 3055, 2926, 2837, 2723, NH, N^+H and CH st), 1630, 1583, 1503 (Ar–C–C and Ar–C–N st) cm^{-1}.

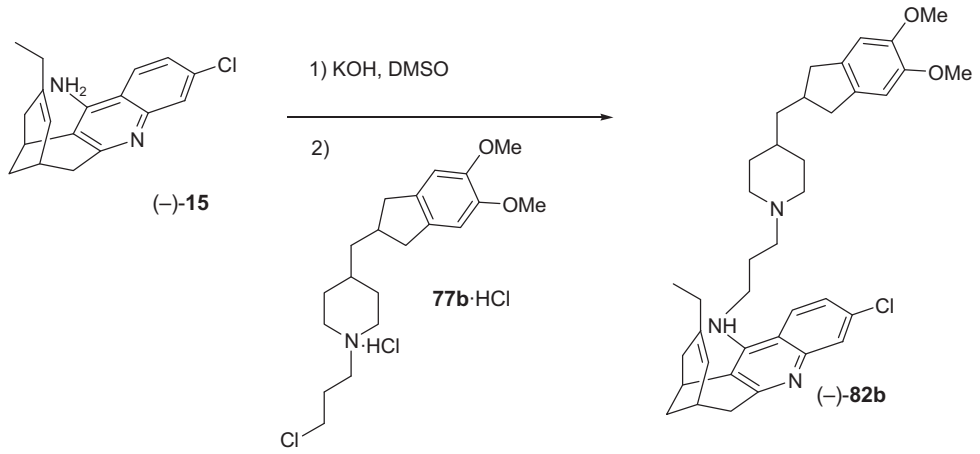
^1H NMR (500 MHz, CD_3OD) δ: 0.91 (t, *J* = 7.5 Hz, 3H, 9-CH_2CH_3), 1.46–1.60 [complex signal, 4H, 2-CH_2 of indane and 3(5)-H_{ax} of piperidine], 1.70–1.78 (broad signal, 1H, 4-H of piperidine), 1.84–1.92 (complex signal, 2H, 9-CH_2CH_3), 1.94 (d, *J* = 18.0 Hz, 1H, 10-H_{endo}), partially overlapped 1.97 (broad d, *J* ≈ 12.5 Hz, 1H, 13-H_{syn}), 2.04 [broad d, *J* = 13.5 Hz, 2H, 3(5)-H_{eq} of piperidine], 2.11 (dm, *J* = 12.5 Hz, 1H, 13-H_{anti}), 2.36 (m, 2H, $NHCH_2CH_2CH_2N$), 2.52 [broad d, *J* = 13.0 Hz, 2H, 1(3)-H_{cis} of indane], partially overlapped 2.48–2.58 (m, 1H, 2-H of indane), 2.62 (broad dd, *J* ≈ 18.0 Hz, *J'* ≈ 4.0 Hz, 1H, 10-H_{exo}), 2.80 (m, 1H, 7-H), 2.88 (d, *J* ≈ 18.0 Hz, 1H, 6-H_{endo}), 2.99 [dd, *J* ≈ 13.0 Hz, *J'* ≈ 7.0 Hz, 2H, 1(3)-H_{trans} of indane], partially overlapped 3.04 [m, 2H, 2(6)-H_{ax} of piperidine], 3.23 (dd, *J* = 18.0 Hz, *J'* = 5.5 Hz, 1H, 6-H_{exo}), partially overlapped 3.27 (m, 2H, $NHCH_2CH_2CH_2N$), 3.54 (m, 1H, 11-H), 3.58–3.66 [m, 2H, 2(6)-H_{eq} of piperidine], 3.77 [s, 6H, 5(6)-OCH_3 of indane], 4.08 (t, *J* = 7.0 Hz, 2H, $NHCH_2CH_2CH_2N$), 4.84 (s, NH and ^+NH), 5.59 (broad d, *J* = 5.0 Hz, 1H, 8-H), 6.78 [s, 2H, 4(7)-H of indane], 7.60 (dd, *J* ≈ 9.5 Hz, *J'* = 1.5 Hz, 1H, 2-H), 7.78 (d, *J* = 1.5 Hz, 1H, 4-H), 8.40 (d, *J* = 9.5 Hz, 1H, 1-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 12.5 (CH_3, 9-CH_2CH_3), 26.2 (CH_2, $NHCH_2CH_2CH_2N$), 27.3 (CH, C11), 27.8 (CH, C7), 29.5 (CH_2, C13), 30.87 (CH_2) and 30.92 (CH_2) [9-CH_2CH_3 and C3(5) of piperidine], 33.6 (CH, C4 of piperidine), 34.9 (CH_2, C10), 36.2 (CH_2, C6), 38.8 (CH, C2 of indane), 40.3 [CH_2, C1(3) of indane], 43.0 (CH_2, 2-CH_2 of indane), 46.5 (CH_2, $NHCH_2CH_2CH_2N$), 54.3 [CH_2, C2(6) of piperidine], 55.3 (CH_2, $NHCH_2CH_2CH_2N$), 56.7 [CH_3, 5(6)-OCH_3 of indane], 109.7 [CH, C4(7) of indane], 115.8 (C) and 118.3 (C) (C11a and C12a), 119.2 (CH, C4), 123.3 (CH, C8), 120.2 (CH, C2), 129.3 (CH, C1), 136.3 [C, C3a(7a) of indane], 140.2 (C, C9), 140.3 (C, C3), 140.8 (C, C4a), 149.4 [C, C5(6) of indane], 151.8 (C, C5a), 156.8 (C, C12).

HRMS, ESI:
Calculated for ($C_{38}H_{48}{}^{35}ClN_3O_2 + H^+$): 614.3507.
Observed: 614.3515.

Elemental analysis: Calculated for $C_{38}H_{48}ClN_3O_2 \cdot 2HCl \cdot 2.5H_2O$:
C: 62.33%; H: 7.57%; N: 5.74%; Cl: 14.53%.
Observed: C: 62.11%; H: 7.19%; N: 5.59%; Cl: 14.79%.

Preparation of (–)-(7*S*,11*S*)-3-chloro-12-[(3-{4-[5,6-dimethoxyindan-2-yl)methyl]piperidin-1-yl}propyl)amino]-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline, (–)-82b.



In a double neck 10 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (–)-huprine X, (–)-**15** (>99% ee, 311 mg, 1.09 mmol) and finely powdered KOH (85% purity, 357 mg, 5.41 mmol) were placed and suspended in anhydrous DMSO (4.7 mL). The resulting suspension was stirred at r. t. for 2 h, then treated with a solution of **77b**·HCl (408 mg of a crude that could contain a maximum of 1.23 mmol) in anhydrous DMSO (1.2 mL) (previously warmed at 70 ºC in a water bath). The reaction mixture was stirred at r. t. for 72 h, then diluted with H_2O (40 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were washed with H_2O (8 x 30 mL), dried with anhydrous Na_2SO_4 and evaporated under reduced pressure to give a brownish solid (610 mg), which was purified through column chromatography (silica gel, 35–70 µm, 76 g, Ø = 3 cm; #1–480, 8400 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2) to provide a mixture of (–)-**82b** / (–)-**15** in an approximate ratio 65:35 (determined by ^1H NMR) (#290–432, 362 mg) that was again purified through column chromatography (Silica gel, 35–70 µm, 45 g, Ø = 3 cm; #1–130, 1200 mL, hexane / EtOAc / Et_3N 50:50:0.2), to provide unreacted (–)-**15** (#7–62, 143 mg) and the desired hybrid (–)-**82b** (#73–87, 151 mg, 23% yield) as a yellow oil.

R_f = 0.61 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (–)-82b·2HCl.

In a 25 mL round-bottomed flask, (–)-**82b** (143 mg, 0.23 mmol) was dissolved in CH_2Cl_2 (8 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.68 N, 3.25 mL), washed with pentane (3 x 4 mL) to give, after drying under standard conditions, (–)-**82b**·2HCl (154 mg) as a yellow solid.

Melting point: 234–236 ºC (CH_2Cl_2 / MeOH 5:2).

$[\alpha]^{20}_D$ = –136 (*c* = 0.10, MeOH).

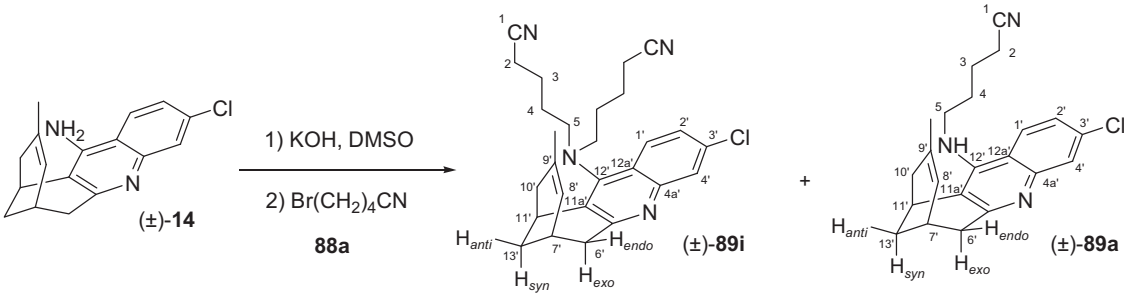
IR (KBr) v: 3600–2500 (max. at 3419, 3129, 3064, 2925, 2852, NH, N^+H and CH st), 1629, 1583, 1505 (Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR and ^{13}C NMR spectra were identical to those previously reported for (±)-**82b**.

HRMS, ESI:
Calculated for ($C_{38}H_{48}{}^{35}ClN_3O_2 + H^+$): 614.3507.
Observed: 614.3501.

Elemental analysis: Calculated for $C_{38}H_{48}ClN_3O_2 \cdot 2HCl \cdot 3H_2O$:
C: 61.58%; H: 7.61%; N: 5.67%; Cl: 14.35%.
Observed: C: 61.42%; H: 7.55%; N: 5.40%; Cl: 14.10%.

Preparation of (±)-5-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]pentanenitrile, (±)-89a.



In a double neck 10 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (300 mg, 1.06 mmol) and finely powdered KOH (85% purity, 139 g, 2.11 mmol) were placed and suspended in anhydrous DMSO (6 mL). The resulting suspension was stirred, heating every 10 min approximately with a heat gun for 1 h and at r. t. one more hour, then treated with 5-bromovaleronitrile, **88a** (0.15 mL, 208 mg, 1.24 mmol). The reaction mixture was stirred at r. t. overnight, then diluted with 5 N NaOH (25 mL) and extracted with EtOAc (3 x 40 mL). The combined organic extracts were washed with H_2O (3 x 50 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (469 mg), which was purified by column chromatography (silica gel, 40–60 μm, 32 g, Ø = 2.5 cm; #1–13, 1200 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #14–26, 1200 mL, CH_2C_2 / MeOH / 50% aq. NH_4OH 99:1:0.2), to provide dialkylated byproduct (±)-**89i** (#6–10, 78 mg, 16% yield) as a yellow oil and the desired nitrile (±)-**89a** (#15–17, 273 mg, 71% yield) as a beige solid.

$R_{f[(\pm)\text{-}\mathbf{89a}]}$ = 0.50 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

$R_{f[(\pm)\text{-}\mathbf{89i}]}$ = 0.77 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-89a· HCl.

In a 25 mL round-bottomed flask, (±)-**89a** (52 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (4.50 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (1.40 N, 0.30 mL), evaporated under reduced pressure, and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**89a**·HCl (58.0 mg) as a yellow solid.

Melting point: 181–183 ℃ (CH_2Cl_2 / MeOH 94:6).

IR (KBr) v: 3500–2500 (max. at 3364, 3254, 3049, 3014, 2926, 2885, 2651, NH, N^+H and CH st), 2243 (CN st), 1635, 1629, 1602, 1582, 1570, 1562, (Ar–C–C and Ar–C–N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.59 (s, 3H, 9'-CH_3); 1'79 (tt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, 3-H_2), 1.93 (d, *J* = 17.6 Hz, 1H, 10'-H_{endo}), overlapped 1.92 –1.98 (m, 1H, 13'-H_{syn}), 2.01 (tt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, 4-H_2), 2.09 (dm, *J* = 12.8 Hz, 1H, 13'-H_{anti}), 2.56 (t, *J* = 7.2 Hz, 2H, 2-H_2), partially overlapped 2.53–2.60 (m, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.88 (ddd, *J* = 18.0 Hz, *J'* = *J''* = 1.6 Hz, 1H, 6'-H_{endo}), 3.22 (dd, *J* = 18.0 Hz, *J'* = 5.2 Hz, 1H, 6'-H_{exo}), 3.47 (m, 1H, 11'-H), 4.03 (t, *J* = 7'6 Hz, 2H, 5-H_2), 4.85 (s, NH and $^{+}$NH), 5.59 (broad d, *J* = 4.4 Hz, 1H, 8'-H), 7.57 (dd, *J* = 9.2 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.78 (d, *J* ≈ 2.0 Hz, 1H, 4'-H), 8.40 (d, *J* ≈ 9.2 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 17.1 (CH_2, C2), 23.4 (CH_3, 9'-CH_3), 23.9 (CH_2, C3), 27.3 (CH, C11'), 27.9 (CH, C7'), 29.3 (CH_2, C13'), 30.3 (CH_2, C4), 36.1 (CH_2), 36.2 (CH_2) (C6', C10'), 48.8 (CH_2, C5), 115.8 (C, C12'a), 117.9 (C, C11'a), 119.2 (CH, C4'), 120.9 (C, CN), 125.1 (CH, C8'), 126.8 (CH, C2'), 129.4 (CH, C1'), 134.6 (C, C9'), 140.3 (C, C3'), 141.0 (C, C4a'), 151.5 (C, C5a'), 157.0 (C, C12').

HRMS, ESI:
Calculated for ($C_{22}H_{24}{}^{35}ClN_3$ + H^+): 366.1732.
Observed: 366.1729.

Analytical sample of (±)-89i·HCl.

In a 25 mL round-bottomed flask, (±)-**89i** (78 mg, 0.17 mmol) was dissolved in CH_2Cl_2 (4.50 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.65 N, 0.64 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**89i**·HCl (80 mg) as a yellow solid.

Melting point: 196–198 ºC (CH_2Cl_2 / MeOH 87:13).

IR (KBr) v: 3500-2450 (max. at 3430, 3044, 2919, 2875, 2464, N^+H and CH st), 2243 (CN st), 1632, 1604, 1574 (Ar–C–C and Ar–C–N st) cm^{-1}.

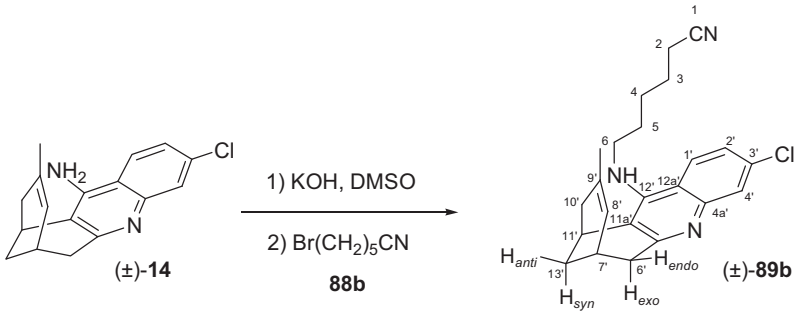
^{1}H NMR (400 MHz, CD_3OD) δ: 1.59 (s, 3H, 9'-CH_3), 1.65 (tt, *J* ≈ *J'* ≈ 7.2 Hz, 4H, 3-H_2), 1.72–1.98 (complex signal, 4H, 4-H_2), 2.00 (broad d, *J* = 17.6 Hz, 1H, 10'-H_{endo}), partially overlapped 2.06 (dm, *J* ≈ 12.8 Hz, 1H, 13'-H_{syn}), 2.12 (dm, *J* = 12.8 Hz, 1H, 13'-H_{anti}), 2.48 (t, *J* = 7.2 Hz, 4H, 2-H_2), 2.69 (dd, *J* = 17.6 Hz, *J'* = 5.6 Hz, 1H, 10'-H_{exo}), 2.82 (m, 1H, 7'-H), 3.17 (ddd, *J* = 18.0 Hz, *J'* ≈ *J'* ≈ 1.8 Hz, 1H, 6'-H_{endo}), 3.39 (dd, *J* = 18.0 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.69–3.84 (complex signal, 2H, 5-H_2), overlapped 3.73 (m, 1H, 11'-H), 3.73 (ddd, *J* = 13.6 Hz, *J'* = 9.6 Hz, *J''* = 5.6 Hz, 2H) and 3.80 (ddd, *J* = 13.6 Hz, *J'* = 9.2 Hz, *J''* = 6.0 Hz, 2H) (5-H_2), 4.84 (s, $^{+}$NH), 5.68 (broad d, *J* = 4.4 Hz, 1H, 8'-H), 7.72 (dd, *J* = 9.2 Hz, *J'* = 2.4 Hz, 1H, 2'-H), 7.99 (d, *J* = 2.4 Hz, 1H, 4'-H), 8.26 (d, *J* = 9.2 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 17.1 (CH_2, C2), 23.3 (CH_3, 9'-CH_3), 23.9 (CH_2, C3), 27.6 (CH, C7'), 28.6 (CH_2, C13'), 28.9 (CH_2, C4), 29.7 (CH, C11'), 37.7 (CH_2, C6'), 38.7 (CH_2, C10'), 55.1 (CH_2, C5),

120.0 (CH, C4'), 120.9 (C, CN), 125.5 (C, C12a'), 125.7 (CH, C8'), 192.2 (CH, C2'), 130.1 (CH, C1'), 132.5 (C, C11a'), 134.3 (C, C9'), 140.36 (C), 140.43 (C) (C3', C4a'), 157.6 (C, C5a'), 163.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{27}H_{31}{}^{35}ClN_4 + H^+$): 447.2310.
Observed: 447.2309.

Preparation of (±)-6-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]hexanenitrile, (±)-89b.



In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (1.50 g, 5.28 mmol) and finely powdered KOH (85% purity, 697 mg, 10.6 mmol) were placed and suspended in anhydrous DMSO (15 mL). The resulting suspension was stirred, heating every 10 min approximately with a heat gun for 1 h and at r. t. one more hour, then treated with 6-bromohexanenitrile, **88b** (0.77 mL, 1.02 g, 5.80 mmol). The reaction mixture was stirred at r. t. overnight, then diluted with 5 N NaOH (170 mL) and extracted with EtOAc (3 x 100 mL). The combined organic extracts were washed with H_2O (3 x 100 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (2.88 g), which was purified by column chromatography (silica gel, 40–60 μm, 46 g, Ø = 3 cm; #1–140, 13.5 L, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2), to provide (±)-**89b** (#53–84, 1.26 g, 63% yield) as a yellow solid.

R_f = 0.77 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-89b·HCl.

In a 25 mL round-bottomed flask, (±)-**89b** (90 mg, 0.24 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (1.40 N, 0.50 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**89b**·HCl (96 mg) as a yellow solid.

Melting point: 143–145 ℃ (CH_2Cl_2 / MeOH 92:8).

IR (KBr) ν: 3500–2500 (max. at 3398, 3245, 3048, 2924, 2853, 2734 NH, N^+H and CH st), 2242 (CN st), 1724, 1618, 1572 (Ar–C–C and Ar–C–N st) cm^{-1}.

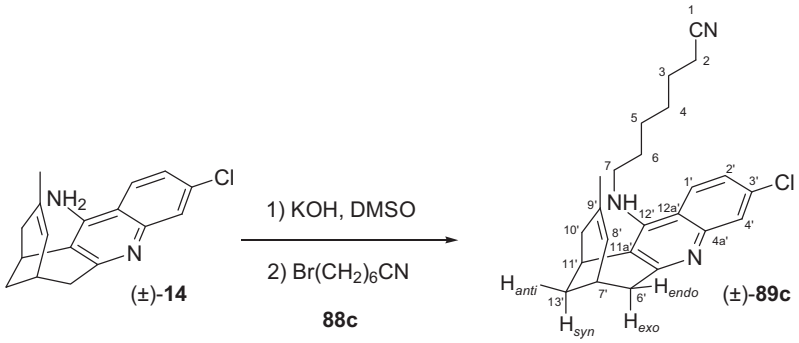
^1H NMR (400 MHz, CD_3OD) δ: 1.59 (s, 3H, 9'-CH_3), partially overlapped 1.54–1.62 (m, 2H, 4-H_2), 1.71 (m, 2H, 3-H_2), partially overlapped 1.91 (tt, $J \approx J' \approx$ 7.6 Hz, 2H, 5-H_2), overlapped 1.90–1.95 (m, 1H, 10'-H_{endo}), partially overlapped 1.92–1.98 (dm, $J \approx$ 12.8 Hz, 1H, 13'-H_{syn}), 2.09 (dm, $J \approx$ 12.8 Hz, 1H, 13'-H_{anti}), 2.48 (t, $J \approx$ 7.2 Hz, 2H, 2-H_2), 2.56 (dm, $J \approx$ 17.2 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.87 (ddd, J = 17.6 Hz, $J' = J'' =$ 1.6 Hz, 1H, 6'-H_{endo}), 3.21 (dd, J = 17.6 Hz, $J' \approx$ 5.6 Hz, 1H, 6'-H_{exo}), 3.46

(m, 1H, 11'-H), 4.01 (t, *J* ≈ 7.6 Hz, 2H, 6-H_2), 4.85 (s, NH and $^{+}$NH), 5.59 (broad d, *J* = 4.4 Hz, 1H, 8'-H), 7.57 (dd, *J* = 9.2 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.77 (d, J = 2.0 Hz, 1H, 4'-H), 8.40 (d, *J* ≈ 9.2 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 17.2 (CH_2, C2), 23.4 (CH_3, 9'-CH_3), 26.0 (CH_2, C3), 26.8 (CH_2, C4), 27.3 (CH, C11'), 27.9 (CH, C7'), 29.3 (CH_2, C13'), 30.5 (CH_2, C5), 36.0 (CH_2), 36.2 (CH_2) (C6', C10'), 49.4 (CH_2, C6), 115.7 (C, C12a'), 117.8 (C, C11a'), 119.2 (CH, C4'), 121.0 (C, CN), 125.1 (CH, C8'), 126.8 (CH, C2'), 129.4 (CH, C1'), 134.6 (C, C9'), 140.3 (C, C3'), 141.0 (C, C4a'), 151.4 (C, C5a'), 157.0 (C, C12').

HRMS, ESI:
Calculated for ($C_{23}H_{26}{}^{35}ClN_3$ + H^+): 380.1888.
Observed: 380.1889.

Preparation of (±)-7-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]heptanenitrile, (±)-89c.



In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (2.00 g, 7.04 mmol) and finely powdered KOH (85% purity, 931 mg, 14.1 mmol) were placed and suspended in anhydrous DMSO (20 mL). The resulting suspension was stirred, heating every 10 min approximately with a heat gun for 1 h and at r. t. one more hour, then treated with 7-bromoheptanenitrile, **88c** (1.21 mL, 1.53 g, 8.02 mmol). The reaction mixture was stirred at r. t. overnight, then diluted with 5 N NaOH (190 mL) and extracted with EtOAc (3 x 150 mL). The combined organic extracts were washed with H_2O (4 x 150 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (3.50 g), which was purified by column chromatography (silica gel, 40–60 µm, 105 g, Ø = 5 cm; #1–94, 8.4 L, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2), to provide (±)-**89c** (#32–76, 2.40 g, 87% yield) as a yellow solid.

R_f = 0.66 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-89c·HCl.

In a 25 mL round-bottomed flask, (±)-**89c** (122 mg, 0.31 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (1.40 N, 0.66 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**89c**·HCl (122 mg) as a yellow solid.

Melting point: 125–126 ℃ (CH_2Cl_2 / MeOH 94:6).

IR (KBr) ν: 3500–2500 (max. at 3219, 3049, 2927, 2852, 2733, 2651, NH, N^+H and CH st), 2242 (CN st), 1620, 1571 (Ar–C–C and Ar–C–N st) cm^{-1}.

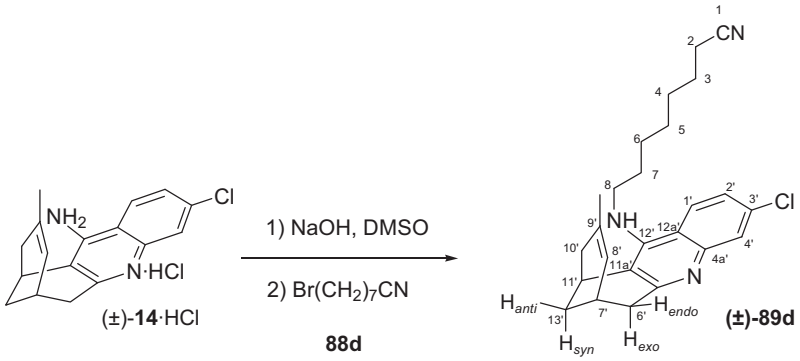
^1H NMR (400 MHz, CD_3OD) δ: 1.44–1.56 (complex signal, 4H, 4-H_2 and 5-H_2), 1.59 (s, 3H, 9'-CH_3), 1.62–1.70 (m, 2H, 3-H_2), 1.91 (tt, *J* = *J'* = 7.2 Hz, 2H, 6-H_2), overlapped 1.86–1.98 (complex signal, 2H, 10'-H_{endo}, 13'-H_{syn}), 2.09 (dm, *J* = 13.2 Hz, 1H, 13'-H_{anti}), 2.46 (t, *J* = 7.2 Hz, 2H, 2-H_2), 2.56 (dm, *J* = 17.6 Hz, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.87 (dm, J ≈ 18.0 Hz, 1H, 6'-H_{endo}), 3.21 (dd, J = 18.0 Hz, *J'* =

5.6 Hz, 1H, 6'-H_{exo}), 3.46 (m, 1H, 11'-H), 3.99 (t, J ≈ 7.2 Hz, 2H, 7-H_2), 4.85 (s, NH and $^{+}$NH), 5.59 (broad d, *J* = 4.4 Hz, 1H, 8'-H), 7.56 (dd, *J* = 9.6 Hz, *J'* = 2.0 Hz, 2'-H), 7.77 (d, *J* = 2.0 Hz, 1H, 4'-H), 8.40 (d, *J* = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 17.2 (CH_2, C2), 23.4 (CH_3, 9'-CH_3), 26.3 (CH_2, C3), 27.0 (CH_2, C5), 27.3 (CH, C11'), 27.9 (CH, C7'), 29.3 (2CH_2, C13' and C4), 31.0 (CH_2, C6), 36.0 (CH_2), 36.1 (CH_2), (C6', C10'), 49.6 (CH_2, C7), 115.7 (C, C12a'), 117.7 (C, C11a'), 119.2 (CH, C4'), 121.1 (C, CN), 125.1 (CH, C8'), 126.7 (CH, C2'), 129.5 (CH, C1'), 134.6 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 151.3 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{24}H_{28}{}^{35}ClN_3$ + H^+): 394.2045.
Observed: 394.2049.

Preparation of (±)-8-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]octanenitrile, (±)-89d.



In a triple neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y·HCl, (±)-**14**·HCl (1.86 g, 5.81 mmol) and finely powdered NaOH (1.17 g, 29.2 mmol) were placed and suspended in anhydrous DMSO (17 mL). The resulting suspension was stirred, heating every 10 min approximately with a heat gun for 1 h and at r. t. one more hour, then treated with a solution of 8-bromooctanenitrile, **88d** (1.39 g, 6.81 mmol) in DMSO (5 mL). The reaction mixture was stirred at r. t. overnight, then diluted with 5 N NaOH (150 mL) and extracted with EtOAc (3 x 120 mL). The combined organic extracts were washed with H_2O (4 x 150 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (2.30 g) which was purified by column chromatography (silica gel, 40–60 µm, 100 g, Ø = 5 cm; #1–82, 7.3 L, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2), to provide (±)-**89d** (#26–76, 1.00 g, 42% yield) as a yellow solid.

R_f = 0.65 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-89d·HCl.

In a 25 mL round-bottomed flask, (±)-**89d** (41.0 mg, 0.10 mmol) was dissolved in CH_2Cl_2 (3 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.43 N, 0.7 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**89d**·HCl (41.6 mg) as a yellow solid.

Melting point: 130–131 ℃ (CH_2Cl_2 / MeOH 81:19).

IR (KBr) v: 3500–2500 (max. at 3397, 3244, 3108, 3048, 3007, 2925, 2852, NH, N^+H and CH st), 2242 (CN st), 1701, 1624, 1562 (Ar–C–C and Ar–C–N st) cm^{-1}.

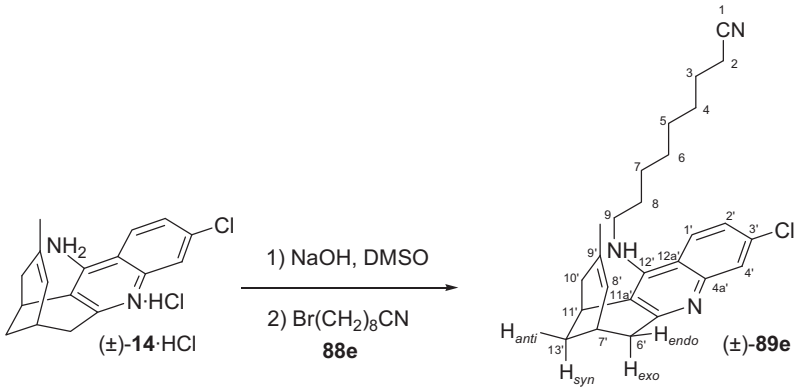
^1H NMR (400 MHz, CD_3OD) δ: 1.40–1'52 (complex signal, 6H, 6-H_2, 5-H_2 and 4-H_2), 1.58 (s, 3H, 9'-CH_3), 1.64 (tt, $J \approx J' \approx$ 7.6 Hz, 2H, 3-H_2), partially overlapped 1.88 (tt, $J \approx J' \approx$ 7.2 Hz, 2H, 7-H_2), partially overlapped 1.94 (broad d, J = 17.2 Hz, 1H, 10'-H_{endo}), overlapped 1.90–1.98 (m, 1H, 13'-H_{syn}), 2.08 (dm, J = 12.4 Hz, 1H, 13'-H_{anti}), 2.44 (t, $J \approx$ 7.2 Hz, 2H, 2-H_2), 2.56 (broad dd, J = 17.6 Hz, J' =

4.4 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.89 (broad d, *J* = 18.0 Hz, 1H, 6'-H_{endo}), 3.21 (dd, *J* = 18.0 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.47 (m, 1H, 11'-H), 3.99 (t, *J* = 7.2 Hz, 2H, 8-H_2), 4.85 (s, NH and $^{+}$NH), 5.58 (broad d, *J* = 5.2 Hz, 1H, 8'-H), 7.54 (dd, *J* = 9.6 Hz, *J'* = 1.6 Hz, 1H, 2'-H), 7.79 (d, *J* = 1.6 Hz, 1H, 4'-H), 8.40 (d, *J* = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 17.3 (CH_2, C2), 23.5 (CH_3, 9'-CH_3), 26.3 (CH_2, C3), 27.2 (CH, C11), 27.5 (CH_2, C6), 27.8 (CH, C7'), 29.3 (CH_2, C13'), 29.4 (CH_2), 29.6 (CH_2) (C4, C5), 31.1 (CH_2, C7), 36.0 (CH_2), 36.1 (CH_2), (C6', C10'), 49.6 (CH_2, C8), 115.6 (C, C12a'), 117.6 (C, C11a'), 119.1 (CH, C4'), 121.2 (C, CN), 125.1 (CH, C8'), 126.6 (CH, C2'), 129.5 (CH, C1'), 134.5 (C, C9'), 140.1 (C, C3'), 140.9 (C, C4a'), 151.2 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{25}H_{30}{}^{35}ClN_3 + H^+$): 408.2201.
Observed: 408.2203.

Preparation of (±)-9-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]nonanenitrile, (±)-89e.



In a triple neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y·HCl, (±)-**14**·HCl (1.89 g, 5.91 mmol) and finely powdered NaOH (1.21 g, 30.3 mmol) were placed and suspended with anhydrous DMSO (18 mL). The resulting suspension was stirred, heating every 10 min approximately with a heat gun for 1 h and at r. t. one more hour, then treated with a solution of 9-bromononanenitrile, **88e** (1.56 g, 7.19 mmol) in anhydrous DMSO (4 mL). The reaction mixture was stirred at r. t. overnight, then diluted with 5 N NaOH (150 mL) and extracted with EtOAc (3 x 120 mL). The combined organic extracts were washed with H_2O (4 x 150 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (2.86 g), which was purified by column chromatography (silica gel, 40–60 μm, 100 g, Ø = 5 cm; #1–62, 6.5 L, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2), to provide (±)-**89e** (#35–50, 1.65 g, 66% yield) as a yellow solid.

R_f = 0.56 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-89e·HCl.

In a 25 mL round-bottomed flask, (±)-**89e** (91 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (6.6 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.43 N, 1.5 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**89e**·HCl (101 mg) as a yellow solid.

Melting point: 121–123 ℃ (CH_2Cl_2 / MeOH 81:19).

IR (KBr) ν: 3500–2500 (max. at 3228, 3108, 3048, 3001, 2926, 2854, 2745, NH, N^+H and CH st), 2242 (CN st), 1718, 1630, 1582, 1569, 1501 (Ar–C–C and Ar–C–N st) cm^{-1}.

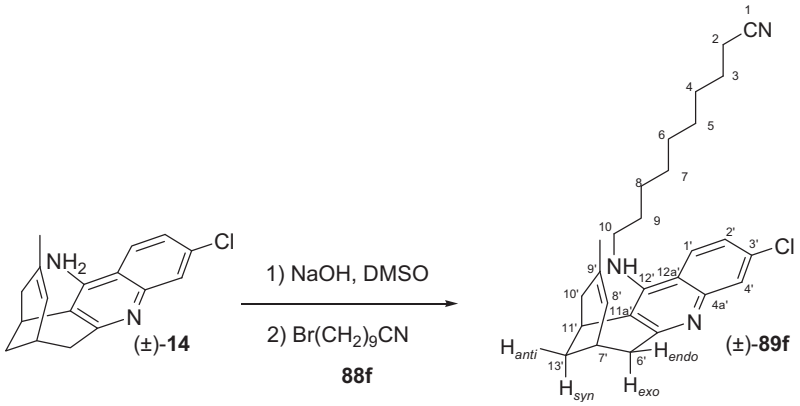
^1H NMR (400 MHz, CD_3OD) δ: 1.32–1.50 (complex signal, 8H, 4-H_2, 5-H_2, 6-H_2 and 7-H_2), 1.59 (s, 3H, 9'-CH_3), partially overlapped 1.62 (tt, $J \approx J' \approx$ 7.2 Hz, 2H, 3-H_2), partially overlapped 1.87 (tt, $J \approx J' \approx$ 7.2 Hz, 2H, 8-H_2), partially overlapped 1.93 (broad d, J = 18.0 Hz, 1H, 10'-H_{endo}), overlapped

1.90–1.96 (m, 1H, 13'-H_{syn}), 2.08 (dm, *J* = 12.4 Hz, 1H, 13'-H_{anti}), 2.43 (t, *J* = 7.2 Hz, 2H, 2-H_2), 2.56 (dm, *J* = 17.6 Hz, *J'* = 4.8 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.88 (broad d, *J* = 17.6 Hz, 1H, 6'-H_{endo}), 3.21 (dd, *J* ≈ 17.6 Hz, *J'* ≈ 5.6 Hz, 1H, 6'-H_{exo}), 3.46 (m, 1H, 11'-H), 3.99 (t, *J* = 7.2 Hz, 2H, 9-H_2), 4.85 (s, NH and $^{+}$NH), 5.59 (d, *J* = 4.4 Hz, 1H, 8'-H), 7.55 (dd, *J* = 9.6 Hz, *J'* = 2.4 Hz, 1H, 2'-H), 7.78 (d, *J* = 2.4 Hz, 1H, 4'-H), 8.40 (d, J = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 17.3 (CH_2, C2), 23.5 (CH_3, 9'-CH_3), 26.4 (CH_2, C3), 27.3 (CH, C11), 27.7 (CH_2, C7), 27.8 (CH, C7'), 29.3 (CH_2, C13'), 29.6 (CH_2), 29.7 (CH_2), 30.0 (CH_2), (C4, C5, C6), 31.2 (CH_2, C8), 36.0 (CH_2), 36.1 (CH_2), (C6', C10'), 49.6 (CH_2, C9), 115.6 (C, C12a'), 117.6 (C, C11a'), 119.1 (CH, C4'), 121.2 (C, CN), 125.1 (CH, C8'), 126.6 (CH, C2'), 129.5 (CH, C1'), 134.5 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 151.2 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{26}H_{32}{}^{35}ClN_3 + H^+$): 422.2358.
Observed: 422.2363.

***P*reparation of (±)-10-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]decanenitrile, (±)-89f.**



In a triple neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (2.00 g, 7.04 mmol) and finely powdered NaOH (927 mg, 23.2 mmol) were placed and suspended in anhydrous DMSO (18 mL). The resulting suspension was stirred, heating every 10 min approximately with a heat gun for 1 h and at r. t. one more hour, then treated with a solution of 10-bromodecanenitrile, **88f** (87% purity, 2.15 g, 8.06 mmol) in DMSO (4 mL). The reaction mixture was stirred at r. t. overnight, then diluted with 5 N NaOH (300 mL) and H_2O (100 mL), and extracted with EtOAc (3 x 150 mL). The combined organic extracts were washed with H_2O (5 x 100 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (2.84 g), which was purified by column chromatography (silica gel, 40–60 μm, 120 g, Ø = 5 cm; #1–24, 1250 mL CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #25–42, 2 L, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2), to provide (±)-**89f** (#14–20, 756 mg, 25% yield) as a yellow solid and starting material (±)-**14** (#28–40, 382 mg).

R_f = 0.66 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-89f·HCl.

In a 25 mL round-bottomed flask, (±)-**89f** (107 mg, 0.25 mmol) was dissolved in CH_2Cl_2 (8 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.71 N, 1.00 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**89f**·HCl (103 mg) as a yellow solid.

Melting point: 129–130 ℃ (CH_2Cl_2 / MeOH 88:12)

IR (KBr) ν: 3500–2500 (max. at 3229, 3105, 3047, 3007, 2926, 2853, 2740, 2640, NH, N^+H and CH st), 2242 (CN st), 1630, 1582, 1549, 1511 (Ar–C–C and Ar–C–N st) cm^{-1}.

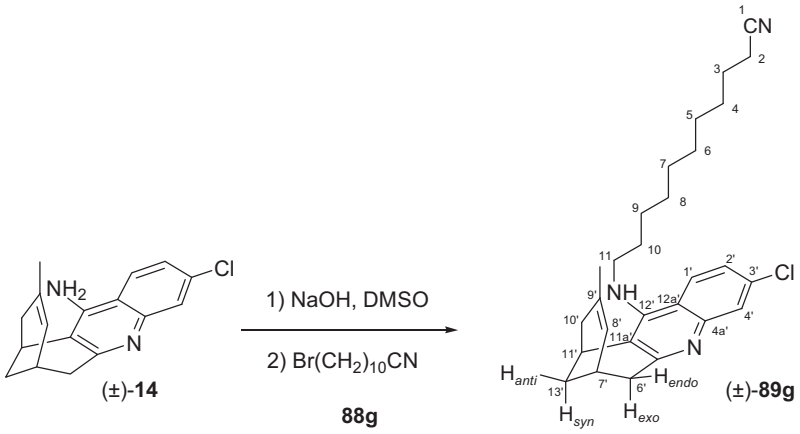
^1H NMR (400 MHz, CD_3OD) δ: 1.35–1.45 (complex signal, 10H, 4-H_2, 5-H_2, 6-H_2, 7-H_2 and 8-H_2), 1.59 (s, 3H, 9'-CH_3), partially overlapped 1.61 (tt, $J \approx J' \approx$ 7'2 Hz, 2H, 3-H_2), partially overlapped 1.87 (tt, $J \approx$

J' ≈ 7.2 Hz, 2H, 9-H_2), overlapped 1.85–1.96 (complex signal, 1H, 13'-H_{syn}), partially overlapped 1.94 (d, *J* ≈ 16.8 Hz, 1H, 10'-H_{endo}), 2.09 (dm, *J* = 12.8 Hz, 1H, 13'-H_{anti}), 2.43 (t, *J* ≈ 7.0 Hz, 2H, 2-H_2), 2.55 (dd, *J* = 17.6 Hz, *J'* = 4.4 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.88 (broad d, *J* = 17.6 Hz, 1H, 6'-H_{endo}), 3.21 (dd, *J* = 17.6 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.46 (m, 1H, 11'-H), 3.98 (t, *J* ≈ 6.8 Hz, 2H, 10-H_2), 4.85 (s, NH and $^{+}$NH), 5.59 (d, *J* = 4.8 Hz, 1H, 8'-H), 7.55 (dd, *J* = 9.6 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.77 (d, *J* = 2.0 Hz, 1H, 4'-H), 8.40 (d, J = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 17.3 (CH_2, C2), 23.5 (CH_3, 9'-CH_3), 26.4 (CH_2, C3), 27.3 (CH, C11'), 27.7 (CH_2, C8), 27.8 (CH, C7'), 29.3 (CH_2, C13'), 29.6 (CH_2), 29.7 (CH_2), 30.1 (CH_2), 30.3 (CH_2) (C4, C5, C6, C7), 31.2 (CH_2, C9), 36.0 (CH_2), 36.1 (CH_2) (C6', C10'), 49.7 (CH_2, C10), 115.6 (C, C12a'), 117.6 (C, C11a'), 119.1 (CH, C4'), 121.2 (C, CN), 125.1 (CH, C8'), 126.6 (CH, C2'), 129.5 (CH, C1'), 134.5 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 151.2 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{27}H_{34}{}^{35}ClN_3 + H^+$): 436.2514.
Observed: 436.2508.

Preparation of (±)-11-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]undecanenitrile, (±)-89g.



In a triple neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (1.70 g, 5.99 mmol) and finely powdered NaOH (791 mg, 19.8 mmol) were placed and suspended in anhydrous DMSO (15 mL). The resulting suspension was stirred, heating every 10 min approximately with a heat gun for 1 h and at r. t. one more hour, then treated with a solution of 11-bromoundecanenitrile, **88g** (80% purity, 2.11 g, 6.86 mmol) in DMSO (4 mL). The reaction mixture was stirred at r. t. overnight, then diluted with 5 N NaOH (70 mL) and H_2O (50 mL), and extracted with EtOAc (3 x 80 mL). The combined organic extracts were washed with H_2O (5 x 80 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (2.20 g), which was purified by column chromatography (silica gel, 40–60 μm, 90 g, Ø = 5 cm; #1–87, 8800 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2), to provide (±)-**89g** (#41–61, 1.09 mg, 40%) as a yellow solid.

R_f = 0.62 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-89g·HCl.

In a 25 mL round-bottomed flask, (±)-**89g** (50 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.75 N, 0.44 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**89g**·HCl (103 mg) as a yellow solid.

Melting point: 131–133 ℃ (CH_2Cl_2 / MeOH 93:7).

IR (KBr) ν: 3500–2500 (max. at 3395, 3231, 3111, 3048, 3012, 2925, 2853, 2742, 2645, NH, N^+H and CH st), 2242 (CN st), 1628, 1582, 1559, 1542, 1521, 1507, (Ar–C–C and Ar–C–N st) cm^{-1}.

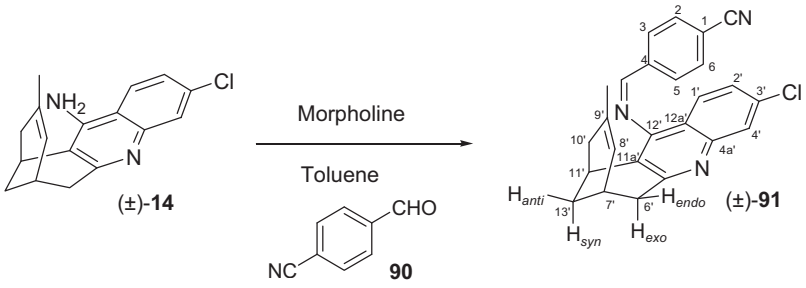
^1H NMR (400 MHz, CD_3OD) δ: 1.27–1.44 (complex signal, 12H, 4-H_2, 5-H_2, 6-H_2, 7-H_2, 8-H_2 and 9-H_2), 1.59 (s, 3H, 9'-CH_3), partially overlapped 1.62 (tt, $J \approx J' \approx$ 7.2 Hz, 2H, 3-H_2), partially overlapped 1.87

(tt, $J \approx J' \approx 7.2$ Hz, 2H, 10-H_2), overlapped 1.86–1.96 (complex signal, 1H, 13'-H_{syn}), partially overlapped 1.94 (d, $J \approx 17.2$ Hz, 1H, 10'-H_{endo}), 2.08 (dm, $J = 12.4$ Hz, 1H, 13'-H_{anti}), 2.43 (t, $J \approx 7.0$ Hz, 2H, 2-H_2), 2.56 (dd, $J = 17.6$ Hz, $J' = 4.4$ Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.88 (d, $J = 18.0$ Hz, 1H, 6'-H_{endo}), 3.21 (dd, $J = 17.6$ Hz, $J' = 5.6$ Hz, 1H, 6'-H_{exo}), 3.46 (m, 1H, 11'-H), 3.99 (t, $J \approx 6.6$ Hz, 2H, 11-H_2), 4.85 (s, NH and $^{+}$NH), 5.59 (broad d, $J = 4.8$ Hz, 1H, 8'-H), 7.54 (dd, $J = 9.6$ Hz, $J' = 2.0$ Hz, 1H, 2'-H), 7.79 (d, $J = 2.0$ Hz, 1H, 4'-H), 8.40 (d, $J = 9.6$ Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 17.3 (CH_2, C2), 23.5 (CH_3, 9'-CH_3), 26.4 (CH_2, C3), 27.3 (CH, C11'), 27.76 (CH_2, C9), 27.83 (CH, C7'), 29.3 (CH_2, C13'), 29.7 (CH_2), 29.8 (CH_2), 30.2 (CH_2), 30.3 (CH_2), 30.4 (CH_2) (C4, C5, C6, C7, C8), 31.2 (CH_2, C10), 36.0 (CH_2), 36.1 (CH_2) (C6', C10'), 49.6 (CH_2, C11), 115.6 (C, C12a'), 117.6 (C, C11a'), 119.1 (CH, C4'), 121.2 (C, CN), 125.1 (CH, C8'), 126.6 (CH, C2'), 129.5 (CH, C1'), 134.5 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 151.2 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{28}H_{36}{}^{35}ClN_3 + H^+$): 450.2671.
Observed: 450.2664.

Preparation of (±)-4-{[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)imino]methyl}benzonitrile, (±)-91.



In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer, a condenser and 4 Å molecular sieves, a suspension of (±)-huprine Y, (±)-**14** (2.00 g, 7.04 mmol) in anhydrous toluene (19 mL) was treated with freshly distilled morpholine (0.67 mL, 675 mg, 7.75 mmol) and *p*-cyanobenzaldehyde, **90** (1.80 g, 13.7 mmol). The reaction mixture was stirred under reflux for 48 h, then concentrated under reduced preasure and the residue purified through column chromatography (silica gel, 40–60 μm, 120 g, Ø = 5 cm; #1–64, 6.1 L, CH_2Cl_2), to provide (±)-**91** (#15–63, 2.20 g, 79% yield) as a yellow solid.

R_f = 0.17 (silica gel, 10 cm, CH_2Cl_2).

Analytical sample of (±)-91.

In a 25 mL round-bottomed flask, (±)-**91** (100 mg, 0.25 mmol) was dissolved in CH_2Cl_2 (6.6 mL), filtered with a PTFE filter (0.2 μm), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**91** (94 mg) as a yellow solid.

Melting point: 208–210 ℃ (CH_2Cl_2).

IR (KBr) v: 2225 (CN st), 1721, 1639, 1600, 1570, 1553, 1500, 1479, 1455 (Ar–C–C and Ar–C–N st) cm^{-1}.

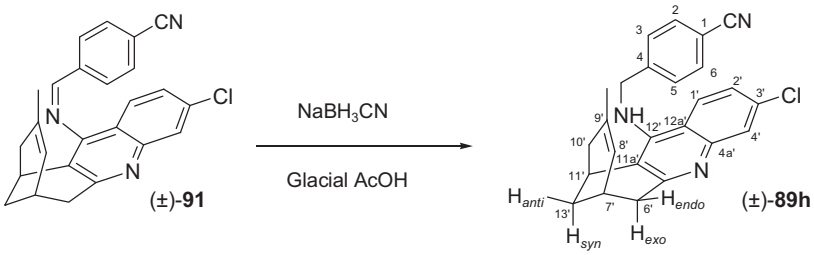
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.52 (s, 3H, 9'-CH_3), 1.85 (broad d, *J* = 17.2 Hz, 1H, 10'-H_{endo}), 1.95–1.97 (complex signal, 2H, 13'-H_{syn} and 13'-H_{anti}), 2.35 (dd, *J* = 17.2 Hz, *J'* = 4.6 Hz, 1H, 10'-H_{exo}), 2.78 (m, 1H, 7'-H), 3.12 (broad d, *J* = 18.0 Hz, 1H, 6'-H_{endo}), 3.21 (dd, *J* = 17.6 Hz, *J'* = 5.2 Hz, 1H, 6'-H_{exo}), 3.35 (m, 1H, 11'-H), 5.56 (broad d, *J* = 5.2 Hz, 1H, 8'-H), 7.31, (dd, *J* = 9.2 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.47 (d, *J* = 9.2 Hz, 1H, 1'-H), 7.86 [dt, *J* = 8.0 Hz, *J'* = 1.6 Hz, 2H, 2(6)-H], 8.00 (d, *J* = 2.0 Hz, 2H, 4'-H), 8.12 [d, *J* = 8.4 Hz, 2H, 3(5)-H], 8.42 (s, 1H, C*H*N).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 23.4 (CH_3, 9'-CH_3), 28.1 (CH, C11'), 28.3 (CH, C7'), 28.6 (CH_2, C13'), 37.1 (CH_2, C10'), 40.0 (CH_2, C6'), 115.8 (C C1), 117.8 (C, C12a'), 118.1 (C, CN), 122.9 (C, C11a'), 123.9 (CH, C1'), 125.3 (CH, C8'), 126.4 (CH, C2'), 127.3 (CH, C4'), 129.4 [CH, C3(5)], 132.3 (C, C9'),

132.8 [CH, C2(6)], 134.9 (C, C3'), 138.5 (C, C4), 147.2 (C, C4a'), 153.4 (C, C12'), 159.8 (C, C5a'), 162.1 (CH, CHN).

HRMS, ESI:
Calculated for ($C_{25}H_{20}{}^{35}ClN_3 + H^+$): 398.1419.
Observed: 398.1426.

Preparation of (±)-4-{[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]methyl}benzonitrile (±)-89h.



In a 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of (±)-**91** (2.30 g, 5.79 mmol) in glacial AcOH (40 mL) was treated with solid $NaBH_3CN$ (764 mg, 12.3 mmol) portionwise for 1 h. The reaction mixture was stirred at r. t. for 3 h, then transferred to a conical flask, cooled to 0 ºC with an ice bath, treated with 10 N NaOH to pH = 14 and extracted with EtOAc (2 x 200 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the desired amine (±)-**89h** (2.10 g, 91% yield) as a yellow solid.

R_f = 0.75 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-89h.

In a 25 mL round-bottomed flask, (±)-**89h** (60 mg, 0.15 mmol) was dissolved in CH_2Cl_2 (5 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 0.85 mL) evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**89h**·HCl (62 mg) as a yellow solid.

Melting point: 217–218 ℃ (CH_2Cl_2 / MeOH 85:15).

IR (KBr) v: 3500–2500 (max. at 3229, 3101, 3050, 2999, 2901, 2722, NH, N^+H and CH st), 2226 (CN st), 1718, 1631, 1582, 1555, 1507 (Ar–C–C and Ar–C–N st) cm^{-1}.

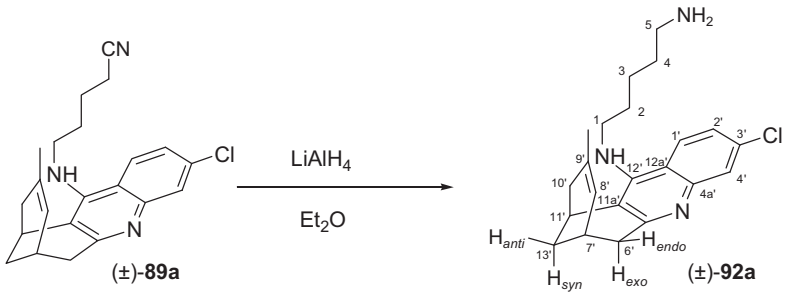
^1H NMR (400 MHz, CD_3OD) δ: 1.61 (s, 3H, 9'-CH_3), 1.98 (broad d, *J* = 17.6 Hz, 1H, 10-H_{endo}), overlapped 2.00 (m, 1H, 13'-H_{syn}), 2.10 (dm, *J* = 12.4 Hz, 1H, 13'-H_{anti}), 2.57 (dd, *J* = 18.0 Hz, *J'* = 4.8 Hz, 1H, 10'-H_{exo}), 2.80 (m, 1H, 7'-H), 2.94 (broad d, *J* = 18.0 Hz, 1H, 6'-H_{endo}), 3.26 (dd, *J* = 18.0 Hz, *J'* = 5.4 Hz, 1H, 6'-H_{exo}), 3.53 (m, 1H, 11'-H), 4.85 (s, NH and $^+$NH), 5.28 (s, 2H, CH_2N), 5.61 (broad d, *J* = 4.8 Hz, 1H, 8'-H), 7.39, (dd, *J* = 9.2 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.63 [d, *J* = 8.8 Hz, 2H, 3(5)-H], 7.79 [complex signal, 3H, 4'-H, 2(6)-H], 8.10 (d, *J* = 9.2 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.4 (CH_3, 9'-CH_3), 27.6 (CH, C11'), 27.8 (CH, C7'), 29.2 (CH_2, C13'), 36.2 (CH_2), 36.4 (CH_2) (C6', C10'), 112.8 (C, C1), 115.7 (C, C12a'), 118.7 (C, C11a'), 119.4 (CH, C4'), 125.1 (CH, C8'), 127.0 (CH, C2'), 128.7 [CH, C3(5)], 128.9 (CH, C1'), 134.0 [CH, C2(6)],

134.6 (2C, C9' and CN), 140.4 (C, C3'), 140.8 (C, C4a'), 144.6 (C, C4), 152.2 (C, C5a'), 157.4 (C, C12').

HRMS, ESI:
Calculated for ($C_{25}H_{22}{}^{35}ClN_3 + H^+$): 400.1575.
Observed: 400.1582.

Preparation of (±)-*N*-(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)pentane-1,5-diamine, (±)-92a.



(±)-**89a** (±)-**92a**

In a triple neck 250 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a suspension of $LiAlH_4$ (0.17 g, 4.47 mmol) in anhydrous Et_2O (63 mL) was prepared, then (±)-**89a** (0.76 g, 2.08 mmol) was added. The reaction mixture was stirred at r. t. overnight, then treated with wet Et_2O (200 mL) and evaporated under reduced pressure. The solid was suspended in CH_2Cl_2, filtered through Celite® and evaporated under reduced pressure, to give amine (±)-**92a** (750 mg, 98% yield) as a yellow oil.

R_f = 0.03 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-92a·2HCl.

In a 25 mL round-bottomed flask, (±)-**92a** (50 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (3.50 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (1.4 N, 0.87 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**92a**·2HCl (54 mg) as a yellow solid.

Melting point: 193–194 ºC (CH_2Cl_2 / MeOH 80:20).

IR (KBr) v: 3500-2500 (max. at 3396, 3253, 2925, 2856, NH, N^+H and CH st), 1628, 1583, 1570, 1558, (Ar–C–C and Ar–C–N st) cm^{-1}.

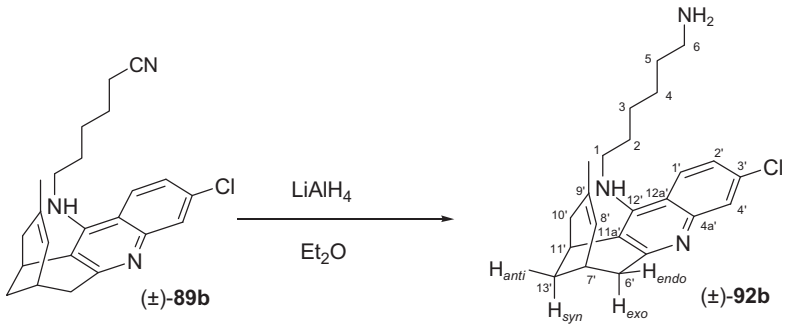
^1H NMR (400 MHz, CD_3OD) δ: 1.55 (tt, $J \approx J' \approx 7.64$ Hz, 2H, 3-H_2), 1.59 (s, 3H, 9'-CH_3), 1.76 (m, 2H, 4-H_2), 1.90–2.02 (complex signal, 4H, 10'-H_{endo}, 13'-H_{syn}, 2-H_2), 2.09 (dm, J = 12.8 Hz, 1H, 13'-H_{anti}), 2.57 (ddm, J = 17.6 Hz, J' = 6.0 Hz, 1H, 10'-H_{exo}), 2.78 (m, 1H, 7'-H), 2.87 (broad d, J = 18.0 Hz, 1H, 6'-H_{endo}), 2.97 (t, J = 7.6 Hz, 2H, 5-H_2), 3.21 (dd, J = 18.0 Hz, J' = 5.6 Hz, 1H, 6'-H_{exo}), 3.49 (m, 1H, 11'-H), 4.01 (t, $J \approx 7.6$ Hz, 2H, 1-H_2), 4.85 (s, NH and $^+$NH), 5.59 (broad d, J = 4.4 Hz, 1H, 8'-H), 7.57 (dd, J = 9.2 Hz, J' = 2.0 Hz, 1H, 2'-H), 7.78 (d, J = 2.0 Hz, 1H, 4'-H), 8.41 (d, J = 9.2 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 24.7 (CH_2, C3), 27.3 (CH, C11'), 27.9 (CH, C7'), 28.2 (CH_2, C4), 29.3 (CH_2, C13'), 30.8 (CH_2, C2), 36.0 (CH_2), 36.2 (CH_2) (C6', C10'), 40.5 (CH_2, C5),

49.4 (CH_2, C1), 115.7 (C, C12a'), 117.8 (C, C11a'), 119.2 (CH, C4'), 125.1 (CH, C8'), 126.8 (CH, C2'), 129.4 (CH, C1'), 134.6 (C, C9'), 140.3 (C, C3'), 141.0 (C, C4a'), 151.4 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{22}H_{28}{}^{35}ClN_3 + H^+$): 370.2045.
Observed: 370.2041.

Preparation of (±)-*N*-(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)hexane-1,6-diamine, (±)-92b.



(±)-**89b** (±)-**92b**

In a 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**89b** (1.26 g, 3.32 mmol) was suspended in anhydrous Et_2O (50 mL), cooled to 0 ºC with an ice bath and treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (2.5 mL 10.0 mmol). The reaction mixture was stirred at r. t. overnight, then cooled again to 0 ºC with an ice bath, diluted dropwise with 1 N NaOH (100 mL) and H_2O (50 mL) and extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the desired amine (±)-**92b** (1.23 g, 97% yield) as a yellow oil.

R_f = 0.02 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-92b·2HCl.

In a 25 mL round-bottomed flask, (±)-**92b** (55 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (3.50 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (1.4 N, 0.92 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**92b**·2HCl (61 mg) as a yellow solid.

Melting point: 180–181 ºC (CH_2Cl_2 / MeOH 80:20).

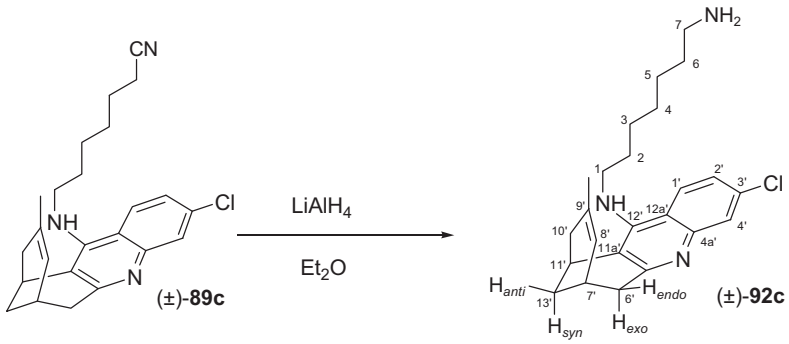
IR (KBr) v: 3500-2500 (max. at 3403, 3240, 2926, 2852, NH, N^+H and CH st), 1728, 1624, 1578, (Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR (400 MHz, CD_3OD) δ: 1.46–1.51 (complex signal, 4H, 3-H_2, 4-H_2), 1.59 (s, 3H, 9'-CH_3), 1.70 (tt, $J \approx J' \approx$ 7.4 Hz 2H, 5-H_2), 1.85–1.97 (complex signal, 4H, 10'-H_{endo}, 13'-H_{syn}, 2-H_2), 2.09 (dm, $J \approx$ 12.6 Hz, 1H, 13'-H_{anti}), 2.56 (dd, J = 17.6 Hz, J' = 4.8 Hz, 1H, 10'-H_{exo}), 2.78 (m, 1H, 7'-H), 2.87 (dm, $J \approx$ 18.0 Hz, 1H, 6'-H_{endo}), 2.94 (t, J = 7.6 Hz, 2H, 6-H_2), 3.21 (dd, J = 18.0 Hz, J' = 5.6 Hz, 1H, 6'-H_{exo}), 3.47 (m, 1H, 11'-H), 4.00 (t, J = 7.6 Hz, 2H, 1-H_2), 4.85 (s, NH and $^+$NH), 5.59 (broad d, J = 4.4 Hz, 1H, 8'-H), 7.57 (dd, J = 9.2 Hz, J' = 2.4 Hz, 1H, 2'-H), 7.77 (d, J = 2.4 Hz, 1H, 4'-H), 8.41 (d, J = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 27.1 (CH_2, C3), 27.3 (CH, C11'), 27.4 (CH_2, C4), 27.9 (CH, C7'), 28.5 (CH_2, C5), 29.3 (CH_2, C13'), 31.1 (CH_2, C2), 36.08 (CH_2), 36.15 (CH_2) (C6', C10'), 40.6 (CH_2, C6), 49.5 (CH_2, C1), 115.7 (C, C12a'), 117.7 (C, C11a'), 119.2 (CH, C4'), 125.1 (CH, C8'), 126.7 (CH, C2'), 129.5 (CH, C1'), 134.6 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 151.3 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{23}H_{30}{}^{35}ClN_3 + H^+$): 384.2201.
Observed: 384.2197.

Preparation of (±)-*N*-(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)heptane-1,7di-amine, (±)-92c.



In a 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**89c** (2.20 g, 5.60 mmol) was suspended in anhydrous Et_2O (100 mL), cooled to 0 ºC with an ice bath and treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (4.3 mL 17.2 mmol). The reaction mixture was stirred at r. t. overnight, then cooled again to 0 ºC with an ice bath, diluted dropwise with 1 N NaOH (150 mL) and H_2O (50 mL) and extracted with EtOAc (3 x 200 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the desired amine (±)-**92c** (1.98 g, 89% yield) as a yellow oil.

R_f = 0.07 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-92c·2HCl.

In a 25 mL round-bottomed flask, (±)-**92c** (104 mg, 0.26 mmol) was dissolved in CH_2Cl_2 (5.0 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (1.4 N, 1.70 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**92c**·2HCl (108 mg) as a yellow solid.

Melting point: 181–183 ºC (CH_2Cl_2 / MeOH 75:25).

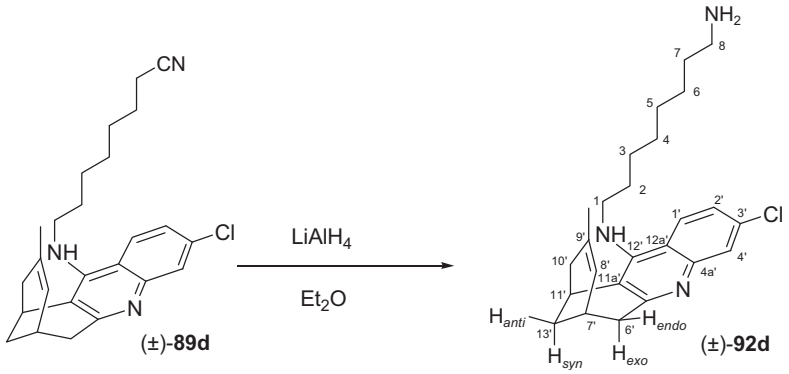
IR (KBr) v: 3500-2500 (max. at 3233, 2927, 2877, 2849, 2782, NH, N^+H and CH st), 1628, 1582, 1566, 1550, 1524, 1508, 1492, 1476 (Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR (400 MHz, CD_3OD) δ: 1.41–1.52 (complex signal, 6H, 3-H_2, 4-H_2, 5-H_2), 1.59 (s, 3H, 9'-CH_3), 1.68 (tt, $J \approx J' \approx$ 7.2 Hz 2H, 6-H_2), 1.84–1.95 (complex signal, 4H, 10'-H_{endo}, 13'-H_{syn}, 2-H_2), 2.09 (dm, J = 13.2 Hz, J' = 2.0 Hz, 1H, 13'-H_{anti}), 2.56 (dd, J = 17.6 Hz, J' = 4.8 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.87 (ddd, J = 18.0 Hz, $J' \approx J'' \approx$ 1.6 Hz, 1H, 6'-H_{endo}), partially overlapped 2.93 (t, J = 7.6 Hz, 2H, 7-H_2), 3.21 (dd, J = 18.0 Hz, J' = 5.6 Hz, 1H, 6'-H_{exo}), 3.47 (m, 1H, 11'-H), 3.99 (t, $J \approx$ 7.6 Hz, 2H, 1-H_2), 4.85 (s, NH and $^+$NH), 5.59 (broad d, J = 4.4 Hz, 1H, 8'-H), 7.56 (dd, J = 9.6 Hz, J' = 2.0 Hz, 1H, 2'-H), 7.78 (d, J = 2.0 Hz, 1H, 4'-H), 8.41 (d, J = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 27.3 (CH, C11'), 27.4 (CH_2, C5), 27.7 (CH_2, C4), 27.8 (CH, C7'), 28.5 (CH_2, C3), 29.3 (CH_2, C13'), 29.8 (CH_2, C2), 31.2 (CH_2, C6), 36.0 (CH_2), 36.1 (CH_2) (C6', C10'), 40.7 (CH_2, C7), 49.6 (CH_2, C1), 115.6 (C, C12a'), 117.6 (C, C11a'), 119.1 (CH, C4'), 125.1 (CH, C8'), 126.7 (CH, C2'), 129.5 (CH, C1'), 134.6 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 151.3 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{24}H_{32}{}^{35}ClN_3 + H^+$): 398.2358.
Observed: 398.2357.

Preparation of (±)-*N*-(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)octane-1,8-diamine, (±)-92d.



In a 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**89d** (1.09 g, 2.68 mmol) was suspended in anhydrous Et_2O (40 mL), cooled to 0 ºC with an ice bath and treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (2.01 mL 8.04 mmol). The reaction mixture was stirred at r. t. overnight. As TLC revealed the presence of starting material, the reaction mixture was again cooled to 0 ºC with an ice bath and treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (0.80 mL 3.20 mmol) and stirred at r. t. for 3 h, when TLC revealed total consumption of the starting material. The reation mixture was then cooled to 0 ºC with an ice bath, diluted dropwise with 1 N NaOH (50 mL) and H_2O (120 mL) and extracted with EtOAc (3 x 120 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the desired amine (±)-**92d** (1.14 g, quantitative yield) as a yellow oil.

R_f = 0.07 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-92d·2HCl.

In a 25 mL round-bottomed flask, (±)-**92d** (73 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 3.00 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**92d**·2HCl (53 mg) as a yellow solid.

Melting point: 209–211 ℃ (CH_2Cl_2 / MeOH 76:24).

IR (KBr) ν: 3500–2500 (max. at 3395, 3245, 2927, 2854, 2780, NH, N^+H and CH st), 1628, 1583, 1524, 1508, 1501 (Ar–C–C and Ar–C–N st) cm^{-1}.

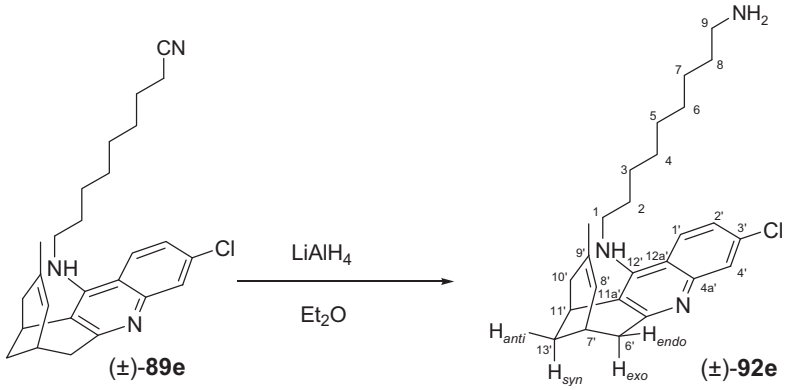
^1H NMR (400 MHz, CD_3OD) δ: 1.40–1.48 (complex signal, 8H, 3-H_2, 4-H_2, 5-H_2, 6-H_2), 1.59 (s, 3H, 9'-CH_3), 1.66 (tt, $J \approx J' \approx$ 7.2 Hz 2H, 7-H_2), overlapped 1.84–1.95 (m, 1H, 13'-H_{syn}), partially overlapped 1.84–1.93 (tt, $J \approx J' \approx$ 7.2 Hz, 2H, 2-H_2), partially overlapped 1.93 (broad d, J = 17.6 Hz, 1H, 10'-H_{endo}), 2.10 (dm, $J \approx$ 10.4 Hz, 1H, 13'-H_{anti}), 2.56 (dd, J = 17.6 Hz, J' = 4.8 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), partially overlapped 2.87 (ddd, $J \approx$ 17.6 Hz, $J' \approx J'' \approx$ 1.6 Hz, 1H, 6'-H_{endo}), 2.92 (tt, $J \approx J' \approx$ 7.6 Hz,

2H, 8-H_2), 3.21 (dd, *J* = 17.6 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.46 (m, 1H, 11'-H), 3.99 (tt, *J* ≈ *J'* ≈ 7.6 Hz, 2H, 1-H_2), 4.85 (s, NH and $^{+}$NH), 5.59 (broad d, *J* = 4.8 Hz, 1H, 8'-H), 7.56 (dd, *J* = 9.6 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.78 (d, *J* = 2.0 Hz, 1H, 4'-H), 8.40 (d, *J* ≈ 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 27.3 (CH, C11'), 27.4 (CH_2, C3), 27.8 (CH_2, C4), 27.9 (CH, C7'), 28.5 (CH_2, C5), 29.2 (CH_2, C13'), 30.1 (2CH_2, C6, C7), 31.3 (CH_2, C2), 36.0 (CH_2), 36.1 (CH_2) (C6', C10'), 40.7 (CH_2, C8), 49.7 (CH_2, C1), 115.6 (C, C12a'), 117.6 (C, C11a'), 119.1 (CH, C4'), 125.1 (CH, C8'), 126.7 (CH, C2'), 129.5 (CH, C1'), 134.5 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 151.2 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{25}H_{34}{}^{35}ClN_3$ + H^+): 412.2514.
Observed: 412.2509.

Preparation of (±)-*N*-(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)nonane-1,9-diamine, (±)-92e.



(±)-**89e** (±)-**92e**

In a 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**89e** (1.60 g, 3.80 mmol) was suspended in anhydrous Et_2O (67 mL), cooled to 0 ºC with an ice bath and treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (2.85 mL 11.4 mmol). The reaction mixture was stirred at r. t. overnight, then cooled to 0 ºC with an ice bath, diluted dropwise with 1 N NaOH (60 mL) and H_2O (150 mL) and extracted with EtOAc (3 x 150 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the desired amine (±)-**92e** (1.39 g, 86% yield) as a yellow oil.

R_f = 0.10 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-92e·2HCl.

In a 25 mL round-bottomed flask, (±)-**92e** (58.0 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 2.30 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**92e**·2HCl (56 mg) as a yellow solid.

Melting point: 169–171 ℃ (CH_2Cl_2 / MeOH 82:18).

IR (KBr) ν: 3500–2500 (max. at 3397, 3245, 2925, 2853, 2795, NH, N^+H and CH st), 1629, 1582, 1508, (Ar–C–C and Ar–C–N st) cm^{-1}.

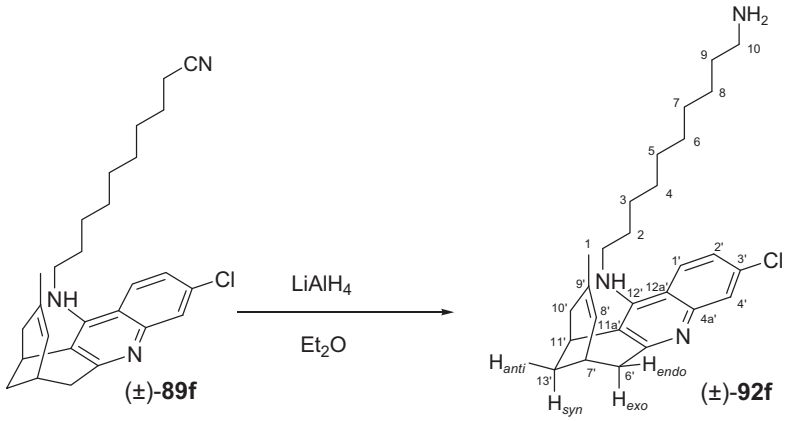
^1H NMR (400 MHz, CD_3OD) δ: 1.34–1.48 (complex signal, 10H, 3-H_2, 4-H_2, 5-H_2, 6-H_2, 7-H_2), 1.59 (s, 3H, 9'-CH_3), 1.66 (tt, $J \approx J' \approx$ 6.8 Hz, 2H, 8-H_2), partially overlapped 1.87 (tt, $J \approx J' \approx$ 7.6 Hz, 2H, 2-H_2), overlapped 1.83–1.96 (m, 1H, 13'-H_{syn}), partially overlapped 1.93 (broad d, J = 17.6 Hz, 1H, 10'-H_{endo}), 2.09 (dm, J = 14.8 Hz, 1H, 13'-H_{anti}), 2.56 (dd, J = 18.0 Hz, J' = 4.8 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), partially overlapped 2.87 (ddd, J = 17.6 Hz, $J' \approx J'' \approx$ 1.6 Hz, 1H, 6'-H_{endo}), 2.91 (tt, J = 7.2 Hz, 2H, 9-H_2), 3.21 (dd, J = 17.6 Hz, J' = 5.6 Hz, 1H, 6'-H_{exo}), 3.46 (m, 1H, 11'-H), 3.98 (tt, $J \approx J' \approx$ 7.2 Hz, 2H,

1-H_2), 4.85 (s, NH and $^{+}$NH), 5.59 (broad d, *J* = 4.8 Hz, 1H, 8'-H), 7.56 (dd, *J* = 9.6 Hz, *J'* = 2.0 Hz, 1H, 2-H), 7.78 (d, *J* = 2.0 Hz, 1H, 4'-H), 8.40 (d, *J* = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 27.3 (CH, C11'), 27.5 (CH_2, C3), 27.8 (CH + CH_2, C7', C4), 28.6 (CH_2, C5), 29.3 (CH_2, C13'), 30.1 (CH_2), 30.2 (CH_2), 30.4 (CH_2) (C6, C7, C8), 31.3 (CH_2, C2), 36.0 (CH_2), 36.1 (CH_2), (C6', C10'), 40.8 (CH_2, C9), 49.7 (CH_2, C1), 115.6 (C, C12a'), 117.6 (C, C11a'), 119.1 (CH, C4'), 125.1 (CH, C8'), 126.6 (CH, C2'), 129.5 (CH, C1'), 134.5 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 151.2 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{26}H_{36}{}^{35}ClN_3$ + H^+): 426.2671.
Observed: 426.2664.

Preparation of (±)-*N*-(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-ethanocycloocta[*b*]quinolin-12-yl)decane-1,10-diamine, (±)-92f.



(±)-**89f** (±)-**92f**

In a 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**89f** (645 mg, 1.48 mmol) was suspended in anhydrous Et_2O (30 mL), cooled to 0 ºC with an ice bath and treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (1.15 mL, 4.60 mmol). The reaction mixture was stirred at r. t. overnight. As TLC revealed the presence of starting material, the reaction mixture was again cooled to 0 ºC with an ice bath and treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (1.15 mL, 4.60 mmol), and stirred at r. t. overnight, when TLC revealed total consumption of the starting material. The reaction mixture was then cooled to 0 ºC with an ice bath, diluted dropwise with 1 N NaOH (35 mL) and H_2O (90 mL) and extracted with EtOAc (3 x 100 mL). The combined organic extracts were washed with H_2O (3 x 150 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the desired amine (±)-**92f** (581 mg, 90% yield) as a yellow oil.

R_f = 0.06 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-92f·2HCl.

In a 25 mL round-bottomed flask, (±)-**92f** (50 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 1.32 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**92f**·2HCl (53 mg) as a yellow solid.

Melting point: 179–180 ℃ (CH_2Cl_2 / MeOH 89:11).

IR (KBr) ν: 3500–2500 (max. at 3382, 3235, 2997, 2926, 2853, 2790, NH N^+H and CH st), 1630, 1582, 1512 (Ar–C–C and Ar–C–N st) cm^{-1}.

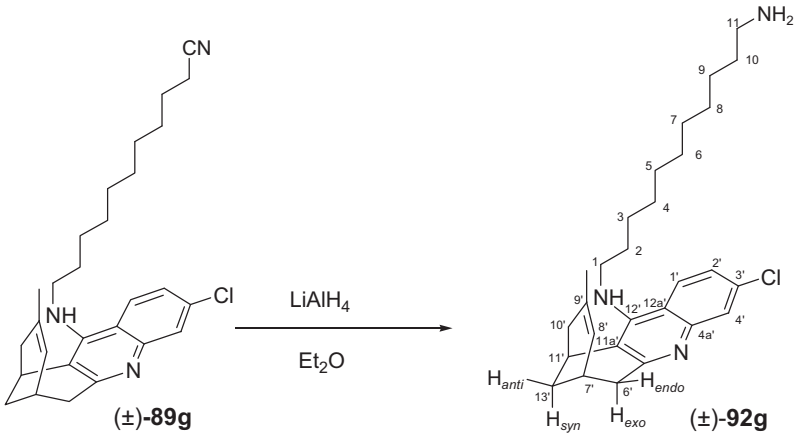
^1H NMR (400 MHz, CD_3OD) δ: 1.30–1.46 (complex signal, 12H, 3-H_2, 4-H_2, 5-H_2, 6-H_2, 7-H_2, 8-H_2), 1.58 (s, 3H, 9'-CH_3), 1.66 (tt, $J \approx J' \approx$ 7.2 Hz 2H, 9-H_2), partially overlapped 1.87 (tt, $J \approx J' \approx$ 7.6 Hz, 2H, 2-H_2), overlapped 1.83–1.96 (m, 1H, 13'-H_{syn}), partially overlapped 1.94 (broad d, J = 17.2 Hz, 1H, 10'-H_{endo}), 2.08 (dm, $J \approx$ 12.4 Hz, 1H, 13'-H_{anti}), 2.56 (dd, J = 17.6 Hz, J' = 4.4 Hz, 1H, 10'-H_{exo}), 2.77 (m,

1H, 7'-H), partially overlapped 2.88 (d, *J* = 18.0 Hz, 6'-H_{endo}), partially overlapped 2.91 (tt, *J* ≈ *J'* ≈ 7.8 Hz, 2H, 10-H_2), 3.21 (dd, *J* = 18.0 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.47 (m, 1H, 11'-H), 3.99 (tt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, 1-H_2), 4.85 (s, NH and $^{+}$NH), 5.59 (broad d, *J* = 4.8 Hz, 1H, 8'-H), 7.55 (dd, *J* = 9.6 Hz, *J'* ≈ 2.0 Hz, 1H, 2'-H), 7.80 (d, *J* = 2.0 Hz, 1H, 4'-H), 8.40 (d, *J* = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 27.3 (CH, C11'), 27.5 (CH_2, C3), 27.8 (CH + CH_2, C7', C4), 28.6 (CH_2, C5), 29.3 (CH_2, C13'), 30.2 (CH_2), 30.3 (CH_2), 30.4 (CH_2), 30.5 (CH_2) (C6, C7, C8, C9), 31.3 (CH_2, C2), 36.0 (CH_2), 36.1 (CH_2) (C6', C10'), 40.8 (CH_2, C10), 49.7 (CH_2, C1), 115.6 (C, C12a'), 117.6 (C, C11a'), 119.1 (CH, C4'), 125.1 (CH, C8'), 126.6 (CH, C2'), 129.5 (CH, C1'), 134.5 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 151.2 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{27}H_{38}{}^{35}ClN_3$ + H^+): 440.2827.
Observed: 440.2820.

Preparation of (±)-*N*-(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-ethanocycloocta[*b*]quinolin-12-yl)undecane-1,11-diamine, (±)-92g.



(±)-**89g** (±)-**92g**

In a 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**89g** (1.05 g, 2.33 mmol) was suspended in anhydrous Et_2O (40 mL), cooled to 0 ºC with an ice bath and treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (1.75 mL, 7.00 mmol). The reaction mixture was stirred r. t. for 4 h. As TLC revealed the presence of starting material, the reaction mixture was again cooled to 0 ºC with an ice bath, treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (1.75 mL, 7.00 mmol), and again stirred at r. t. overnight, when TLC revealed total consumption of the starting material. The reaction mixture was then cooled to 0 ºC with an ice bath, diluted dropwise with 1 N NaOH (50 mL) and H_2O (50 mL) and extracted with EtOAc (2 x 100 mL). The combined organic extracts were washed with H_2O (100 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the desired amine (±)-**92g** (849 mg, 81% yield) as a yellow oil.

R_f = 0.05 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-92g·2HCl.

In a 25 mL round-bottomed flask, (±)-**92g** (62 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 1.64 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**92g**·2HCl (60.0 mg) as a yellow solid.

Melting point: 164–165 ℃ (CH_2Cl_2 / MeOH 78:22).

IR (KBr) ν: 3500–2500 (max. at 3385, 3232, 3043, 2924, 2852, 2790, 2651, NH, N^+H and CH st), 1630, 1582, 1512 (Ar–C–C and Ar–C–N st) cm^{-1}.

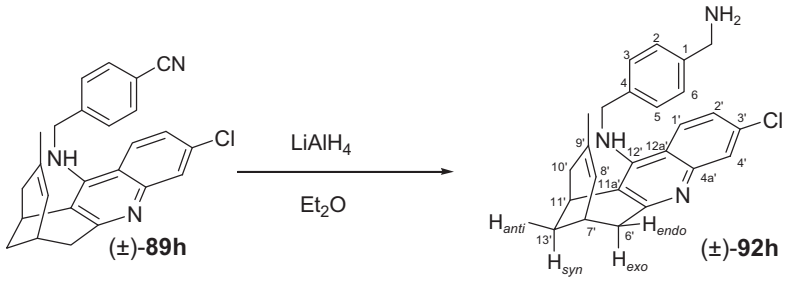
^1H NMR (400 MHz, CD_3OD) δ: 1.26–1.44 (complex signal, 14H, 3-H_2, 4-H_2, 5-H_2, 6-H_2, 7-H_2, 8-H_2, 9-H_2), 1.58 (s, 3H, 9'-CH_3), 1.67 (tt, $J \approx J' \approx$ 6.8 Hz 2H, 10-H_2), partially overlapped 1.87 (tt, $J \approx J' \approx$ 7.2 Hz, 2H, 2-H_2), overlapped 1.81–1.93 (m, 1H, 13-H'$_{syn}$), partially overlapped 1.94 (broad d, J = 17.2 Hz,

1H, 10'-H_{endo}), 2.08 (dm, *J* = 12.4 Hz, 1H, 13'-H_{anti}), 2.56 (dd, *J* = 17.2 Hz, *J'* = 4.4 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), partially overlapped 2.91 (d, *J* = 17.6, Hz, 6'-H_{endo}), partially overlapped 2.93 (tt, *J* ≈ *J'* ≈ 7.6 Hz, 2H, 11-H_2), 3.21 (dd, *J* = 17.6 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.49 (m, 1H, 11'-H), 3.99 (tt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, 1-H_2), 4.85 (s, NH and $^{+}$NH), 5.58 (broad d, *J* = 5.2 Hz, 1H, 8'-H), 7.53 (dd, *J* ≈ 9.6 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.82 (d, *J* = 2.0 Hz, 1H, 4'-H), 8.40 (d, *J* = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 27.3 (CH, C11'), 27.5 (CH_2, C3), 27.8 (CH + CH_2, C7', C4), 28.5 (CH_2, C5), 29.3 (CH_2, C13'), 30.2 (CH_2), 30.3 (CH_2), 30.47 (CH_2), 30.51 (CH_2), 30.6 (CH_2) (C6, C7, C8, C9, C10), 31.2 (CH_2, C2), 36.0 (CH_2), 36.1 (CH_2) (C6', C10'), 40.8 (CH_2, C11), 49.6 (CH_2, C1), 115.6 (C, C12a'), 117.6 (C, C11a'), 119.1 (CH, C4'), 125.1 (CH, C8'), 126.6 (CH, C2'), 129.5 (CH, C1'), 134.5 (C, C9'), 140.1 (C, C3'), 140.9 (C, C4a'), 151.2 (C, C5a'), 156.9 (C, C12').

HRMS, ESI:
Calculated for ($C_{28}H_{40}{}^{35}ClN_3$ + H^+): 454.2984.
Observed: 454.2969.

Preparation of (±)-4-{[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]methyl}benzylamine, (±)-92h.



In a 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**89h** (370 mg, 0.93 mmol) was suspended in anhydrous Et_2O (15 mL), cooled to 0 ºC with an ice bath and treated dropwise with a 4 M solution of $LiAlH_4$ in Et_2O (0.7 mL 2.80 mmol). The reaction mixture was stirred at r. t. overnight, then cooled to 0 ºC with an ice bath, diluted dropwise with 1 N NaOH (20 mL) and H_2O (20 mL) and extracted with EtOAc (3 x 30 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give the desired amine (±)-**92h** (352 mg, 94% yield) as a yellow oil.

R_f = 0.11 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-92h·2HCl.

In a 25 mL round-bottomed flask, (±)-**92h** (40 mg, 0.10 mmol) was dissolved in CH_2Cl_2 (5 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 1.70 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**92h**·2HCl (42 mg) as a yellow solid.

Melting point: 151–152 ℃ (CH_2Cl_2 / MeOH 82:18).

IR (KBr) ν: 3500–2500 (max. at 3390, 3245, 3043, 3002, 2899, 2795, 2609, NH, N^+H and CH st), 1715, 1700, 1631, 1583, 1517 (Ar–C–C and Ar–C–N st) cm^{-1}.

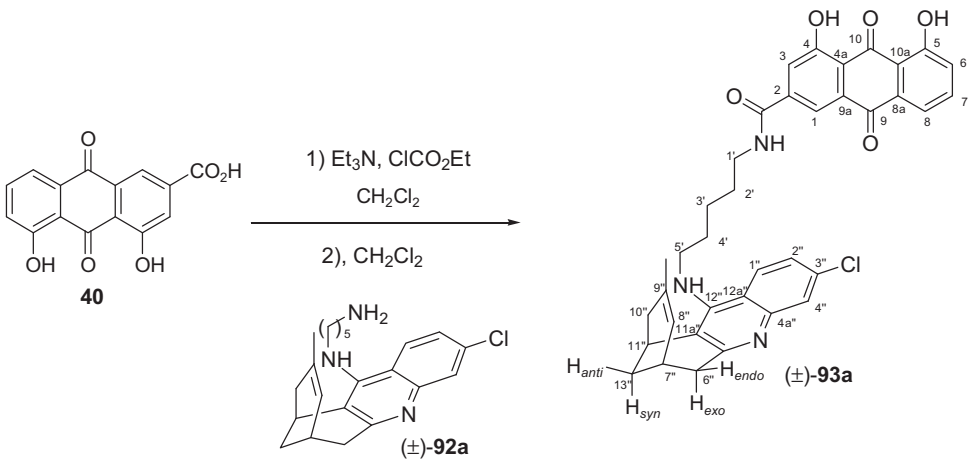
^{1}H NMR (400 MHz, CD_3OD) δ: 1.61 (s, 3H, 9'-CH_3), partially overlapped 2.00 (broad d, *J* = 18.4 Hz, 1H, 10'-H_{endo}), overlapped 1.96–2.00 (m, 1H, 13'-H_{syn}), 2.10 (dm, *J* = 12.4 Hz, 1H, 13'-H_{anti}), 2.57 (dd, *J* ≈ 18.4 Hz, *J'* ≈ 4.8 Hz, 1H, 10'-H_{exo}), 2.80 (m, 1H, 7'-H), 2.82 (dm, *J* = 18.0, Hz, 6'-H_{endo}), 3.25 (dd, *J* = 18.0 Hz, *J'* = 5.8 Hz, 1H, 6'-H_{exo}), 3.54 (m, 1H, 11'-H), 4.14 (s, 2H, CH_2NH_2), 4.85 (s, NH and $^+$NH), 5.25 (s, 2H, CH_2NH), 5.61 (broad d, *J* = 4.8 Hz, 1H, 8'-H), 7.34 (dd, *J* = 9.2 Hz, *J'* = 2.4 Hz, 1H, 2'-H), 7.50–7.57 [complex signal, 4H, 2(6)-H_2 and 3(5)-H_2), 7.78 (d, *J* = 2.4 Hz, 1H, 4'-H), 8.21 (d, *J* = 9.2 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 27.6 (CH, C11'), 27.9 (CH, C7'), 29.3 (CH_2, C13'), 36.1 (CH_2), 36.3 (CH_2) (C6', C10'), 43.9 (CH_2, CH_2NH), 51.7 (CH_2, CH_2NH_2), 115.6 (C, C12a'),

118.4 (C, C11a'), 119.3 (CH, C4'), 125.1 (CH, C8'), 126.7 (CH, C2'), 128.5 [CH, C2(6)], 129.3 (CH, C1'), 130.9 [CH, C3(5)], 134.2 (C), 134.6 (C), 140.0 (C) (C1, C4, C9'), 140.3 (C, C3'), 140.9 (C, C4a'), 151.9 (C, C5a'), 157.3 (C, C12').

HRMS, ESI:
Calculated for ($C_{25}H_{26}{}^{35}ClN_3 + H^+$): 403.1575.
Observed: 403.1578.

Preparation of (±)-*N*-{5-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]pentyl}-9,10-dihydro-4,5-dihydroxy-9,10-dioxoanthracene-2-carboxamide, (±)-93a.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, **40** (477 mg, 1.68 mmol) was suspended in anhydrous CH_2Cl_2 (6 mL) and cooled to 0 ºC with an ice bath, then treated dropwise with freshly distilled Et_3N (0.47 mL, 341 mg, 3.38 mmol) and $ClCO_2Et$ (0.16 mL, 182 mg, 1.68 mmol). The resulting solution was stirred at 0 ºC for 30 min and treated with a solution of (±)-**92a** (621 mg, 1.68 mmol) in anhydrous CH_2Cl_2 (6 mL). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (50 mL), the phases were separated and the aqueous phase was again extracted with CH_2Cl_2 (3 x 35 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a red solid (770 mg), which was purified by column chromatography (silica gel, 54 g, 40–60 μm, Ø = 3 cm; #1–32, 1100 mL, CH_2Cl_2 / 50 aq. NH_4OH 100:0.2; #33–44, 500 mL, CH_2Cl_2 / MeOH / 50 aq. NH_4OH 99.5:0.5:0.2; #45–99, 2500 mL, CH_2Cl_2 / MeOH / 50 aq. NH_4OH 99:1:0.2), to provide the hybrid (±)-**93a** (#69–81, 140 mg, 13% yield) as a red solid.

R_f = 0.45 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-93a·HCl.

In a 25 mL round-bottomed flask, (±)-**93a** (79 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (4 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (1.4 N, 0.26 mL) and evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**93a**·HCl (80 mg) as a yellow solid.

Melting point: 213–215 ℃ (CH_2Cl_2 / MeOH 94:6).

IR (KBr) ν: 3500–2500 (max. at 3224, 3047, 3005, 2923, 2852, OH, NH, N^+H and CH st), 1669, 1628, 1582, 1565 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

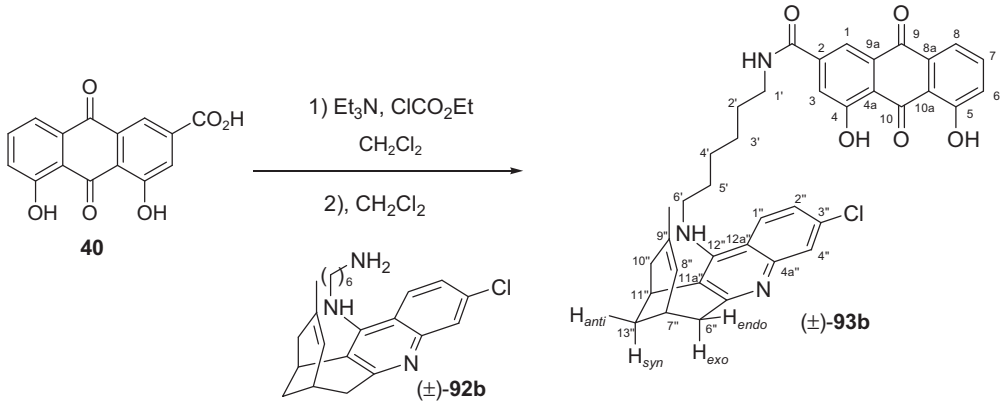
^{1}H NMR (500 MHz, CD_3OD) δ: 1.54–1.64 (complex signal, 2H, 3'-H_2), partially overlapped 1.59 (s, 3H, 9''-CH_3), 1.77 (tt, $J \approx J' \approx 7.0$ Hz, 2H, 2'-H_2), partially overlapped 1.90–2.00 (complex signal, 3H, 13''-H_{syn}, 4'-H_2), 1.94 (d, $J \approx 17.0$ Hz, 1H, 10''-H_{endo}), 2.08 (broad d, $J = 12.5$ Hz, 1H, 13''-H_{anti}), 2.56 (broad dd, $J = 17.0$ Hz, $J' = 4.0$ Hz, 1H, 10''-H_{exo}), 2.77 (m, 1H, 7''-H), 2.84 (d, $J = 17.5$ Hz, 1H, 6''-H_{endo}), 3.18 (dd, $J = 17.5$ Hz, $J' = 5.5$ Hz, 1H, 6''-H_{exo}), 3.42–3.52 (complex signal, 3H, 11''-H and 1'-H_2), 4.00 (dt, $J = J' = 7.5$ Hz, 2H, 5'-H_2), 4.87 (s, NH, $^{+}$NH and OH), 5.58 (broad d, $J = 4.5$ Hz, 1H, 8''-H), 7.35 (dd, $J = 7.5$ Hz, $J' = 2.0$ Hz, 1H, 6-H), 7.47 (broad d, $J = 8.5$ Hz, 1H, 2''-H), 7.62 (broad s, 2H, 4''-H, 3-H), 7.76 (d, $J = 7.5$ Hz, 1H, 8-H), 7.78 (dd, $J = J' = 7.5$ Hz, 1H, 7-H), 8.04 (s, 1H, 1-H), 8.34 (d, $J = 8.5$ Hz, 1H, 1''-H).

^{13}C NMR (125.7 MHz, CD_3OD) δ: 23.5 (CH_3, 9''-CH_3), 25.1 (CH_2, C3'), 27.3 (CH, C11''), 27.9 (CH, C7''), 29.3 (CH_2, C13''), 29.9 (CH_2, C2'), 31.0 (CH_2, C4'), 36.1 (CH_2, C6''), 36.2 (CH_2, C10''), 40.7 (CH_2, C1'), 49.5 (CH_2, C5'), 115.7 (C, C12a''), 117.0 (C, C10a), 117.6 (C, C11a''), 118.6 (C, C4a), 118.8 (CH, C1), 119.1 (CH, C4''), 120.9 (CH, C8), 123.7 (CH, C3), 125.1 (CH, C8''), 125.8 (CH, C6), 126.7 (CH, C2''), 129.3 (CH, C1''), 134.6 (C, C9''), 134.8 (C, C8a), 135.2 (C, C9a), 138.8 (CH, C7), 140.2 (C, C3''), 140.9 (C, C4a''), 143.4 (C, C2), 151.4 (C, C5a''), 156.7 (C, C12''), 163.4 (C, C4), 163.7 (C, C5), 167.3 (C, CONH), 182.2 (C, C9), 193.7 (C, C10).

HRMS, ESI:
Calculated for ($C_{37}H_{34}{}^{35}ClN_3O_5 + H^{+}$): 636.2260.
Observed: 636.2255.

Elemental analysis: Calculated for $C_{37}H_{34}Cl\,N_3O_5 \cdot HCl \cdot 1.5H_2O$:
C: 63.52%; H: 5.47%; N: 6.01%; Cl: 10.13%.
Observed: C: 63.39%; H: 5.72%; N: 5.64%; Cl: 10.91%.

Preparation of (±)-*N*-{6-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]hexyl}-9,10-dihydro-4,5-dihydroxy-9,10-dioxoanthracene-2-carboxamide, (±)-93b.



In a triple neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, **40** (1.19 g, 4.18 mmol) was suspended in anhydrous CH_2Cl_2 (10 mL) and cooled to 0 ºC with an ice bath, then treated dropwise with freshly distilled Et_3N (1.16 mL, 842 mg, 8.34 mmol) and $ClCO_2Et$ (0.40 mL, 456 mg, 4.18 mmol). The resulting solution was stirred at 0 ºC for 30 min and treated with a solution of (±)-**92b** (1.60 g, 4.18 mmol) in anhydrous CH_2Cl_2 (12 mL). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (70 mL), the phases were separated and the aqueous phase was again extracted with CH_2Cl_2 (3 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a red solid (2.00 g), which was purified by column chromatography (silica gel, 120 g, 40–60 µm, Ø = 5 cm; #1–18, 1450 mL, hexane / EtOAc / Et_3N 50:50:0.2; #19–23, 500 mL, hexane / EtOAc / Et_3N 40:60:0.2; #24–28, 500 mL, hexane / EtOAc / Et_3N 30:70:0.2; #29–38, 500 mL, hexane / EtOAc / Et_3N 20:80:0.2; #39–63, 2000 mL, hexane / EtOAc / Et_3N 10:90:0.2; #64–83, 1500 mL, EtOAc / Et_3N 100:0.2; #84–93, 500 mL, EtOAc / MeOH / Et_3N 99.5:0.5:0; #89–93, 500 mL, EtOAc / MeOH / Et_3N 99:1:0.2; #94–98, 500 mL, EtOAc / MeOH / Et_3N 98:2:0.2; #99–109, 1000 mL, EtOAc / MeOH / Et_3N 95:5:0.2; #110–124, 1500 mL, EtOAc / MeOH / Et_3N 90:10:0.2; #125–139, 1000 mL, EtOAc / MeOH / Et_3N 80:20:0.2), to provide the hybrid (±)-**93b** (#41–128, 647 mg, 24% yield) as a red solid.

R_f = 0.54 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-93b·HCl.

In a 25 mL round-bottomed flask, (±)-**93b** (142 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (4 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (1.4 N, 0.50 mL) and evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**93b**·HCl (141 mg) as a yellow solid.

Melting point: 203–205 ℃ (CH_2Cl_2 / MeOH 88:12).

IR (KBr) v: 3500–2500 (max. at 3228, 3042, 2927, 2859, 2722, OH, NH, N^+H and CH st), 1627, 1586, 1570 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

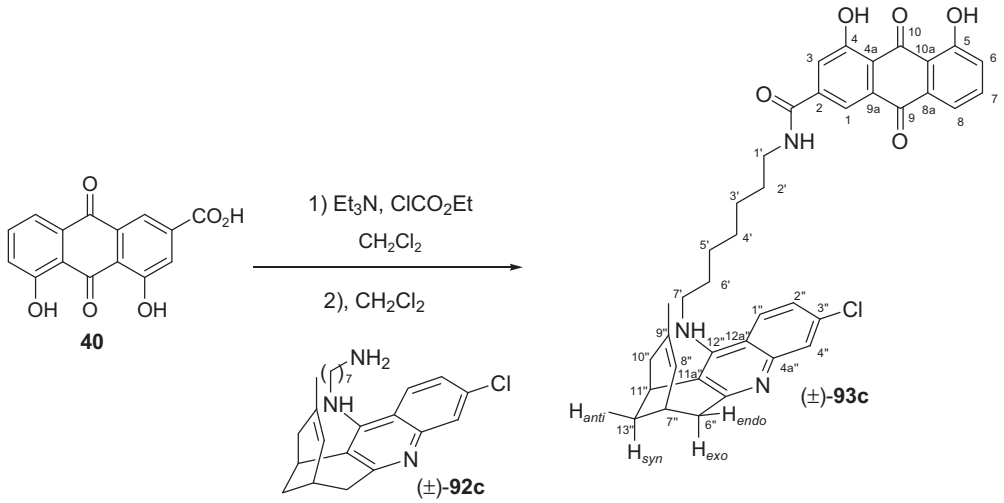
^1H NMR (500 MHz, CD_3OD) δ: 1.49–1.56 (complex signal, 4H, 3'-H_2, 4'-H_2), 1.59 (s, 3H, 9''-CH_3), 1.70 (tt, $J \approx J' \approx 6.7$ Hz, 2H, 2'-H_2), partially overlapped 1.87–1.94 (complex signal, 3H, 13''-H_{syn} and 5'-H_2), partially overlapped 1.94 (broad d, $J = 17.5$ Hz, 1H, 10''-H_{endo}), 2.07 (dm, $J = 12.0$ Hz, 1H, 13''-H_{anti}), 2.54 (broad dd, $J \approx 17.5$ Hz, $J' \approx 3.5$ Hz, 1H, 10''-H_{exo}), 2.77 (m, 1H, 7''-H), 2.82 (broad d, $J = 18.0$ Hz, 1H, 6''-H_{endo}), 3.17 (dd, $J = 18.0$ Hz, $J' = 5.0$ Hz, 1H, 6''-H_{exo}), 3.39–3.49 (complex signal, 3H, 11''-H, 1'-H_2), 3.96 (dt, $J = J' = 6.7$ Hz, 2H, 6'-H_2), 4.84 (s, NH, $^+$NH and OH), 5.58 (broad d, $J = 5.0$ Hz, 1H, 8''-H), 7.35 (dd, $J = 7.5$ Hz, $J' = 1.5$ Hz, 1H, 6-H), 7.46 (d, $J \approx 9.0$ Hz, 1H, 2''-H), 7.60 (s, 1H, 4''-H), 7.64 (s, 1H, 3-H), partially overlapped 7.75 (d, $J = 8.0$ Hz, 1H, 8-H), partially overlapped 7.77 (dd, $J \approx J' \approx 8.0$ Hz, 1H, 7-H), 8.07 (s, 1H, 1-H), 8.32 (d, $J \approx 9.0$ Hz, 1H, 1''-H).

^{13}C NMR (125.7 MHz, CD_3OD) δ: 23.4 (CH_3, 9''-CH_3), 27.2 (CH, C11''), 27.3 ($2CH_2$, C3', C4'), 27.9 (CH, C7''), 29.3 (CH_2, C13''), 30.0 (CH_2, C2'), 31.1 (CH_2, C5'), 36.1 ($2CH_2$, C6'', C10''), 40.7 (CH_2, C1'), 49.5 (CH_2, C6'), 115.7 (C, C12a''), 117.0 (C, C10a), 117.7 (C, C11a''), 118.6 (C, C4a), 118.8 (CH, C1), 119.1 (CH, C4''), 120.9 (CH, C8), 123.7 (CH, C3), 125.1 (CH, C8''), 125.8 (CH, C6), 126.6 (CH, C2''), 129.4 (CH, C1''), 134.6 (C, C9''), 134.8 (C, C8a), 135.3 (C, C9a), 138.8 (CH, C7), 140.2 (C, C3''), 141.0 (C, C4a''), 143.6 (C, C2), 151.3 (C, C5a''), 156.8 (C, C12''), 163.4 (C, C4), 163.8 (C, C5), 167.3 (C, CONH), 182.2 (C, C9), 193.8 (C, C10).

HRMS, ESI:
Calculated for ($C_{38}H_{36}{}^{35}ClN_3O_5 + H^+$): 650.2416.
Observed: 650.2414.

Elemental analysis: Calculated for $C_{38}H_{36}ClN_3O_5 \cdot HCl \cdot 1.25H_2O$:
C: 64.36%; H: 5.61%; N: 5.93%; Cl: 10.00%.
Observed: C: 64.52%; H: 5.55%; N: 5.83%; Cl: 10.50%.

Preparation of (±)-*N*-{7-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]heptyl}-9,10-dihydro-4,5-dihydroxy-9,10-dioxoanthracene-2-carboxamide, (±)-93c.



In a triple neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, **40** (1.43 g, 5.04 mmol) was suspended in anhydrous CH_2Cl_2 (15 mL) and cooled to 0 ºC with an ice bath, then treated dropwise with freshly distilled Et_3N (1.40 mL, 1.02 g, 10.1 mmol) and $ClCO_2Et$ (0.48 mL, 547 mg, 5.04 mmol). The resulting solution was stirred at 0 ºC for 30 min and treated with a solution of (±)-**92c** (2.00 g, 5.04 mmol) in anhydrous CH_2Cl_2 (9 mL). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (70 mL), the phases were separated and the aqueous phase was again extracted with CH_2Cl_2 (3 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a red solid (2.20 g), which was purified by column chromatography (silica gel, 110 g, 40–60 µm, Ø = 5 cm; #1–5, 500 mL, hexane / EtOAc / Et_3N 50:50:0.2; #6–29, 2000 mL, hexane / EtOAc / Et_3N 40:60:0.2; #30–34, 500 mL, hexane / EtOAc / Et_3N 30:70:0.2; #35–39, 500 mL, hexane / EtOAc / Et_3N 20:80:0.2; #40–44, 500 mL, hexane / EtOAc / Et_3N 10:90:0.2; #45–49, 500 mL, EtOAc / Et_3N 100:0.2; #50–54, 500 mL, EtOAc / MeOH / Et_3N 98:2:0.2; #55–59, 500 mL, EtOAc / MeOH / Et_3N 95:5:0.2; #60–69, 1000 mL, EtOAc / MeOH / Et_3N 90:10:0.2; #70–79, 1000 mL, EtOAc / MeOH / Et_3N 80:20:0.2), to provide the hybrid (±)-**93c** (#41–128, 864 mg, 26% yield) as a red solid.

R_f = 0.48 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-93c·HCl.

In a 25 mL round-bottomed flask, (±)-**93c** (328 mg, 0.49 mmol) was dissolved in CH_2Cl_2 (7 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (1.4 N, 0.70 mL) and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 / MeOH 3:1 (4 mL) and precipitated with EtOAc (12 mL), the solvent was decanted and the residue was dried and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**93c**·HCl (260 mg) as a yellow solid.

Melting point: 202–203 ℃ (EtOAc / CH_2Cl_2 / MeOH 75:19:6).

IR (KBr) v: 3500–2500 (max. at 3048, 2926, 2855, 2742, OH, NH, N^+H and CH st), 1654, 1628, 1593, 1583, 1569, 1546 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

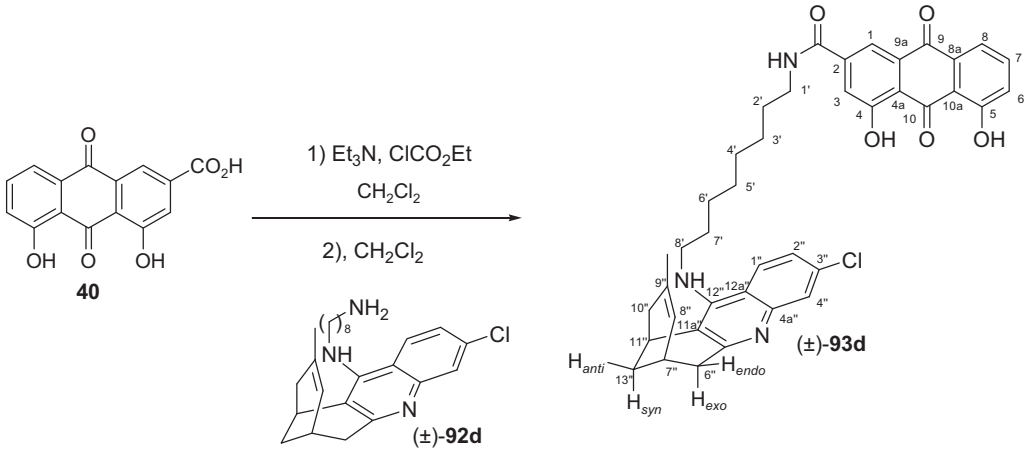
^1H NMR (500 MHz, CD_3OD) δ: 1.49 (complex signal, 6H, 3'-H_2, 4'-H_2, 5'-H_2), 1.58 (s, 3H, 9''-CH_3), 1.69 (tt, $J \approx J' \approx 6.5$ Hz, 2H, 2'-H_2), partially overlapped 1.87–1.94 (complex signal, 3H, 13''-H_{syn}, 6'-H_2), partially overlapped 1.94 (broad d, $J \approx 18.0$ Hz, 1H, 10''-H_{endo}), 2.07 (dm, $J = 12.0$ Hz, 1H, 13-H_{anti}), 2.53 (broad dd, $J = 18.0$ Hz, $J' = 5.0$ Hz, 1H, 10-H_{exo}), 2.75 (m, 1H, 7''-H), 2.79 (broad d, $J \approx 18.0$ Hz, 1H, 6''-H_{endo}), 3.14 (dd, $J \approx 18.0$ Hz, $J' = 5.5$ Hz, 1H, 6''-H_{exo}), 3.37–3.48 (complex signal, 3H, 11''-H and 1'-H_2), 3.92 (dt, $J = 7.5$ Hz, $J' = 4.0$ Hz, 2H, 7'-H_2), 4.84 (s, NH, $^+$NH and OH), 5.58 (broad d, $J = 5.0$ Hz, 1H, 8''-H), 7.36 (dd, $J = 8.5$ Hz, $J' = 1.0$ Hz, 1H, 6-H), 7.44 (dd, $J \approx 9.0$ Hz, $J' = 2.0$ Hz, 1H, 2''-H), 7.59 (d, $J = 2.0$ Hz, 1H, 4''-H), 7.66 (d, $J \approx 2.0$ Hz, 1H, 3-H), 7.73 (dd, $J = 7.5$ Hz, $J' = 1.5$ Hz, 1H, 8-H), 7.77 (dd, $J \approx J' \approx 7.5$ Hz, 1H, 7-H), 8.09 (d, $J = 2.0$ Hz, 1H, 1-H), 8.26 (d, $J = 9.0$ Hz, 1H, 1''-H).

^{13}C NMR (125.7 MHz, CD_3OD) δ: 23.4 (CH_3, 9''-CH_3), 27.3 (CH, C11''), 27.6 (CH_2), 27.7 (CH_2) (C4', C5'), 27.9 (CH, C7''), 29.3 (CH_2, C13''), 29.7 (CH_2), 29.9 (CH_2) (C2', C3'), 31.1 (CH_2, C6'), 36.0 (CH_2), 36.1 (CH_2) (C6'', C10''), 41.0 (CH_2, C1'), 49.7 (CH_2, C7'), 115.6 (C, C12a''), 117.1 (C, C10a), 117.6 (C, C11a''), 118.6 (C, C4a), 118.7 (CH, C1), 119.2 (CH, C4''), 120.9 (CH, C8), 123.7 (CH, C3), 125.1 (CH, C8''), 125.8 (CH, C6), 126.6 (CH, C2''), 129.3 (CH, C1''), 134.6 (C, C9''), 134.8 (C, C8a), 135.3 (C, C9a), 138.8 (CH, C7), 140.2 (C, C3''), 141.0 (C, C4a''), 143.7 (C, C2), 151.3 (C, C5a''), 156.6 (C, C12''), 163.5 (C, C4), 163.8 (C, C5), 167.4 (C, CONH), 182.2 (C, C9), 193.6 (C, C10).

HRMS, ESI:
Calculated for ($C_{39}H_{38}{}^{35}ClN_3O_5 + H^+$): 664.2573.
Observed: 664.2569.

Elemental analysis: Calculated for $C_{39}H_{38}ClN_3O_5 \cdot HCl \cdot 0.5H_2O$:
C: 66.01%; H: 5.68%; N: 5.92%; Cl: 9.99%.
Observed: C: 66.26%; H: 5.64%; N: 5.90%; Cl: 9.63%.

Preparation of (±)-*N*-{8-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]octyl}-9,10-dihydro-4,5-dihydroxy-9,10-dioxoanthracene-2-carboxamide, (±)-93d.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, **40** (213 mg, 0.76 mmol) was suspended in anhydrous CH_2Cl_2 (6.8 mL) and cooled to 0 ºC with an ice bath, then treated with anhydrous Et_3N (0.21 mL, 152 mg, 1.50 mmol) and $ClCO_2Et$ (72 µL, 82 mg, 0.76 mmol) dropwise. The resulting solution was stirred at 0 ºC for 30 min and treated with a solution of (±)-**92d** (307 mg, 0.75 mmol) in anhydrous CH_2Cl_2 (5.8 mL). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (40 mL), the phases were separated and the aqueous phase was again extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a red solid (740 mg), which was purified through column chromatography (silica gel, 44 g, 40–60 µm, Ø = 3 cm; #1–4, 300 mL, hexane / EtOAc / Et_3N 50:50:0.2; #5–8, 300 mL, hexane / EtOAc / Et_3N 40:60:0.2; #9–11, 300 mL, hexane / EtOAc / Et_3N 30:70:0.2; #12–14, 300 mL, hexane / EtOAc / Et_3N 20:80:0.2; #15–17, 300 mL, hexane / EtOAc / Et_3N 10:90:0.2; #18–20, 300 mL, EtOAc / Et_3N 100:0.2; #21–23, 300 mL, EtOAc / MeOH / Et_3N 98:2:0.2; #24–34, 600 mL, EtOAc / MeOH / Et_3N 95:5:0.2; #35–37, 300 mL, EtOAc / MeOH / Et_3N 90:10:0.2; #38–46, 600 mL, EtOAc / MeOH / Et_3N 80:20:0.2) to provide impure (±)-**93d** (#22–46, 165 g). This impure product was purified through column chromatography (silica gel, 13 g, 40–60 µm, Ø = 1.5 cm; #1–17, 100 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #18–26, 100 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #27–49, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2; #50–56, 100 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 97:3:0.2; #57–63, 100 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 90:10:0.2; #64–67, 50 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 80:20:0.2), to provide the hybrid (±)-**93d** (#32–64, 80 mg, 16% yield) as a red solid.

R_f = 0.30 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-93d·HCl.

In a 25 mL round-bottomed flask, (±)-**93d** (39 mg, 0.06 mmol) was dissolved in CH_2Cl_2 (4 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 2.30 mL) and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 / MeOH 1:1 (0.4 mL) and precipitated with EtOAc (0.8 mL), the solvent was decanted and the residue was dried and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**93d**·HCl (32 mg) as a yellow solid.

Melting point: 180–182 ℃ (EtOAc / CH_2Cl_2 / MeOH 66:17:17).

IR (KBr) v: 3500–2500 (max. at 3222, 3047, 3007, 2924, 2852, 2640, OH, NH, N^+H and CH st), 1766, 1674, 1628, 1607, 1582, 1566, 1522 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR (500 MHz, CD_3OD) δ: 1.44 (complex signal, 8H, 3'-H_2, 4'-H_2, 5'-H_2, 6'H_2), 1.59 (s, 3H, 9''-CH_3), 1.67 (tt, $J \approx J' \approx 7.0$ Hz, 2H, 2'-H_2), partially overlapped 1.86 (tt, $J \approx J' \approx 7.0$ Hz 2H, 7'-H_2), overlapped 1.85−1.94 (m, 1H, 13''-H_{syn}), partially overlapped 1.93 (broad d, $J \approx 18.0$ Hz, 1H, 10''-H_{endo}), 2.06 (dm, $J = 12.0$ Hz, 1H, 13''-H_{anti}), 2.53 (broad dd, $J \approx 18.0$ Hz, $J' = 4.5$ Hz, 1H, 10''-H_{exo}), 2.75 (m, 1H, 7''-H), 2.82 (broad d, $J = 18.0$ Hz, 1H, 6''-H_{endo}), 3.13 (dd, $J = 18.0$ Hz, $J' = 5.0$ Hz, 1H, 6''-H_{exo}), 3.39–3.47 (complex signal, 3H, 11''-H and 1'-H_2), 3.87 (dt, $J \approx J' \approx 6.0$ Hz, 2H, 8'-H_2), 4.86 (s, NH, $^+$NH and OH), 5.58 (broad d, $J = 5.0$ Hz, 1H, 8''-H), 7.26 (d, $J = 8.5$ Hz, 1H, 6-H), 7.35 (d, $J \approx 9.0$ Hz, 1H, 2''-H), 7.54 (s, 1H, 4''-H), 7.58 (s, 1H, 3-H), partially overlapped 7.57−7.61 (m, 1H, 8-H), 7.69 (dd, $J \approx J' \approx 8.0$ Hz, 1H, 7-H), 7.97 (s, 1H, 1-H), 8.19 (d, $J = 9.0$ Hz, 1H, 1''-H).

^{13}C NMR (125.7 MHz, CD_3OD) δ: 23.5 (CH_3, 9''-CH_3), 27.2 (CH, C11''), 27.5 (CH_2), 27.6 (CH_2), (C5', C6'), 27.8 (CH, C7''), 29.3 (CH_2, C13''), 29.86 (CH_2), 29.92 (CH_2), 30.0 (CH_2) (C2', C3', C4'), 31.1 (CH_2, C7'), 36.0 ($2CH_2$, C6'', C10''), 41.0 (CH_2, C1'), 49.7 (CH_2, C8'), 115.4 (C, C12a''), 116.7 (C, C10a), 117.5 (C, C11a''), 118.3 (C, C4a), 118.8 (CH, C1), 119.0 (CH, C4''), 120.8 (CH, C8), 123.7 (CH, C3), 125.1 (CH, C8''), 125.8 (CH, C6), 126.6 (CH, C2''), 129.2 (CH, C1''), 134.4 (C, C9''), 134.6 (C, C8a), 134.9 (C, C9a), 138.8 (CH, C7), 140.1 (C, C3''), 140.8 (C, C4a''), 143.6 (C, C2), 151.1 (C, C5a''), 156.5 (C, C12''), 163.3 (C, C4), 163.5 (C, C5), 167.1 (C, CONH), 181.8 (C, C9), 193.4 (C, C10).

HRMS, ESI:

Calculated for ($C_{40}H_{40}{}^{35}ClN_3O_5 + H^+$): 678.2729.

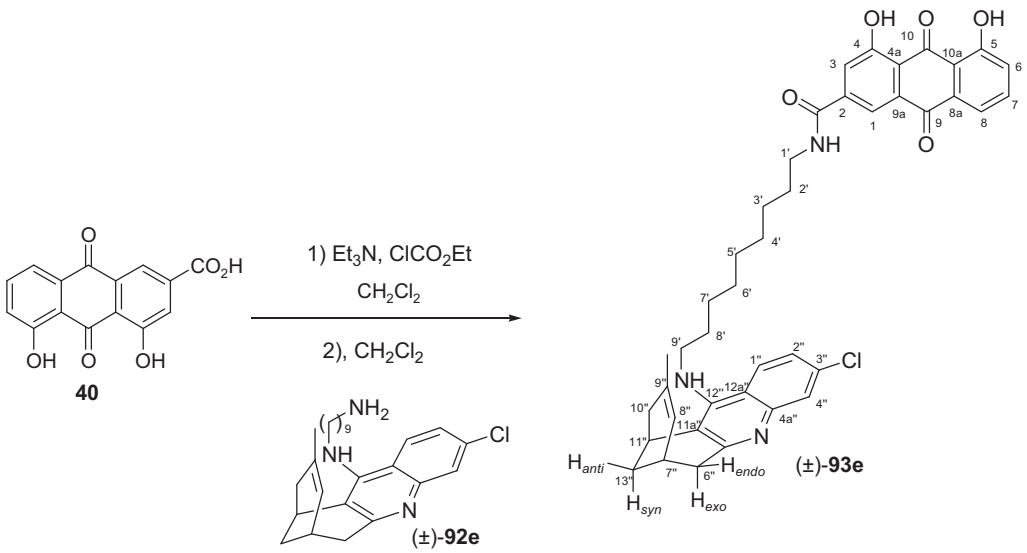
Observed: 678.2734.

Elemental analysis: Calculated for $C_{40}H_{40}ClN_3O_5 \cdot HCl \cdot H_2O$:

C: 65.57%; H: 5.92%; N: 5.73%; Cl: 9.68%.

Observed: C: 65.52%; H: 5.86%; N: 5.83%; Cl: 9.35%.

Preparation of (±)-*N*-{9-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]nonyl}-9,10-dihydro-4,5-dihydroxy-9,10-dioxoanthracene-2-carboxamide, (±)-93e.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, **40** (213 mg, 0.76 mmol) was suspended in anhydrous CH_2Cl_2 (7.0 mL) and cooled to 0 ºC with an ice bath, then treated with anhydrous Et_3N (0.21 mL, 152 mg, 1.51 mmol) and $ClCO_2Et$ (72 µL, 82 mg, 0.76 mmol) dropwise. The resulting solution was stirred at 0 ºC for 30 min, and treated with a solution of (±)-**92e** (321 mg, 0.76 mmol) in anhydrous CH_2Cl_2 (6.0 mL). The reaction mixture was stirred at r. t. for 3 days, diluted with % aq. Na_2CO_3 (50 mL), the phases were separated and the aqueous phase was again extracted with CH_2Cl_2 (3 x 35 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give a red solid (550 mg), which was purified by column chromatography (silica gel, 33 g, 40–60 µm, Ø = 3 cm; #1–5, 400 mL, hexane / EtOAc / Et_3N 50:50:0.2; #6–8, 300 mL, hexane / EtOAc / Et_3N 40:60:0.2; #9–11, 300 mL, hexane / EtOAc / Et_3N 30:70:0.2; #12–14, 300 mL, hexane / EtOAc / Et_3N 20:80:0.2; #15–17, 300 mL, hexane / EtOAc / Et_3N 10:90:0.2; #18–23, 600 mL, EtOAc / Et_3N 100:0.2; #24–29, 600 mL, EtOAc / MeOH / Et_3N 95:5:0.2; #30–32, 300 mL, EtOAc / MeOH / Et_3N 90:10:0.2; #33–35, 300 mL, EtOAc / MeOH / Et_3N 80:20:0.2), to provide the hybrid (±)-**93e** (#12–35, 118 mg, 23% yield) as a red solid.

R_f = 0.33 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-93e·HCl.

In a 25 mL round-bottomed flask, (±)-**93e** (118 mg, 0.17 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 1.0 mL) and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 / MeOH 1:1 (2 mL) and precipitated with EtOAc (5 mL), the solvent was decanted and the residue was dried and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**93e**·HCl (78 mg) as a yellow solid.

Melting point: 192–193 °C (EtOAc / CH_2Cl_2 / MeOH 70:15:15).

IR (KBr) v: 3500–2500 (max. at 3401, 2925, 2853, OH, NH, N^+H and CH st), 1628, 1584, 1566, 1524 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

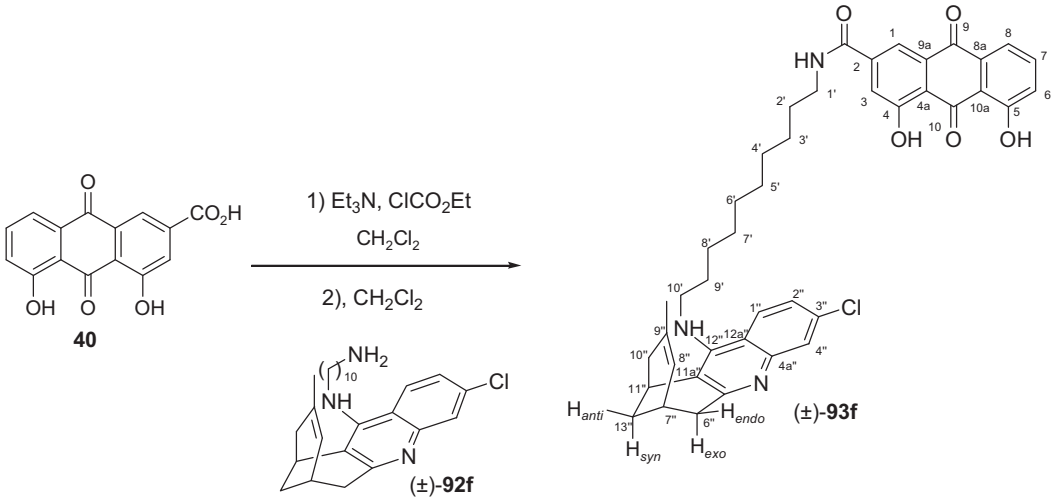
^1H NMR (500 MHz, CD_3OD) δ: 1.41 (complex signal, 10H, 3'-H_2, 4'-H_2, 5'-H_2, 6'H_2, 7'-H_2), 1.59 (s, 3H, 9''-CH_3), partially overlapped 1.64 (m, 2H, 2'-H_2), partially overlapped 1.66 (tt, $J \approx J' \approx 6.0$ Hz 2H, 8'-H_2), overlapped 1.84−1.94 (m, 1H, 13''-H_{syn}), partially overlapped 1.92 (broad d, $J = 17.0$ Hz, 1H, 10''-H_{endo}), 2.06 (dm, $J \approx 10.5$ Hz, 1H, 13''-H_{anti}), 2.53 (dm, $J \approx 17.0$ Hz, 1H, 10''H_{exo}), 2.76 (m, 1H, 7''-H), partially overlapped 2.79 (broad d, $J = 18.5$ Hz, 1H, 6''-H_{endo}), 3.14 (dd, $J = 18.0$ Hz, $J' = 3.5$ Hz, 1H, 6''-H_{exo}), 3.38–3.42 (complex signal, 3H, 11''-H, 1'-H_2), 3.86−3.96 (m, 2H, 9'-H_2), 4.85 (s, NH, $^+$NH and OH), 5.58 (broad d, $J = 4.0$ Hz, 1H, 8''-H), 7.31 (d, $J = 8.5$ Hz, 1H, 6-H), 7.38 (d, $J = 8.5$ Hz, 1H, 2''-H), 7.57 (s, 1H, 4''-H), 7.63 (s, 1H, 3-H), partially overlapped 7.64 (d, $J = 7.0$ Hz, 1H, 8-H), 7.72 (dd, $J \approx J' \approx 8.0$ Hz, 1H, 7-H), 8.04 (s, 1H, 1-H), 8.20 (d, $J = 8.5$ Hz, 1H, 1''-H), 8.82 (broad s, 1H, CONH).

^{13}C NMR (125.7 MHz, CD_3OD) δ: 23.5 (CH_3, 9''-CH_3), 27.2 (CH, C11''), 27.5 (CH_2), 27.6 (CH_2), (C6', C7'), 27.8 (CH, C7''), 29.3 (CH_2, C13''), 29.7 ($2CH_2$), 30.0 (CH_2), 30.1 (CH_2) (C2', C3', C4', C5'), 31.2 (CH_2, C8'), 36.0 ($2CH_2$, C6'', C10''), 41.0 (CH_2, C1'), 49.6 (CH_2, C9'), 115.4 (C, C12a''), 116.8 (C, C10a), 117.5 (C, C11a''), 118.4 (C, C4a), 118.8 (CH, C1), 119.1 (CH, C4''), 120.8 (CH, C8), 123.7 (CH, C3), 125.1 (CH, C8''), 125.8 (CH, C6), 126.6 (CH, C2''), 129.2 (CH, C1''), 134.6 (2C, C9'', C8a), 135.1 (C, C9a), 138.7 (CH, C7), 140.2 (C, C3''), 140.8 (C, C4a''), 143.7 (C, C2), 151.0 (C, C5a''), 156.6 (C, C12''), 163.4 (C, C4), 163.6 (C, C5), 167.3 (C, CONH), 182.0 (C, C9), 193.5 (C, C10).

HRMS, ESI:
Calculated for ($C_{41}H_{42}{}^{35}ClN_3O_5 + H^+$): 692.2886.
Observed: 692.2873.

Elemental analysis: Calculated for $C_{41}H_{42}ClN_3O_5 \cdot 1.3HCl \cdot 1.25H_2O$:
C: 64.61%; H: 6.06%; N: 5.51%; Cl: 10.70%.
Observed: C: 64.51%; H: 6.00%; N: 6.10%; Cl: 10.97%.

Preparation of (±)-*N*-{10-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]decyl}-9,10-dihydro-4,5-dihydroxy-9,10-dioxoanthracene-2-carboxamide, (±)-93f.



In a double neck 25 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, **40** (344 mg, 1.21 mmol) was suspended in anhydrous CH_2Cl_2 (12 mL) and cooled to 0 ºC with an ice bath, then treated with anhydrous Et_3N (0.34 mL, 247 mg, 2.44 mmol) and $ClCO_2Et$ (0.12 mL, 137 mg, 1.27 mmol) dropwise. The resulting solution was stirred at 0 ºC for 30 min and treated with a solution of (±)-**92f** (530 mg, 1.21 mmol) in anhydrous CH_2Cl_2 (9.0 mL). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (45 mL), the phases were separated and the aqueous phase was again extracted with CH_2Cl_2 (3 x 35 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a red solid (900 mg), which was purified by column chromatography (silica gel, 50 g, 40–60 µm, Ø = 3 cm; #1–10, 300 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #11–20, 300 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2; #21–48, 900 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2), to provide the hybrid (±)-**93f** (#35–41, 115 mg, 13% yield) as a red solid.

R_f = 0.53 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-93f·HCl.

In a 25 mL round-bottomed flask, (±)-**93f** (115 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 0.65 mL) and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 / MeOH 1:1 (0.8 mL) and precipitated with EtOAc (2.1 mL), the solvent was decanted and the residue was dried and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**93f**·HCl (95 mg) as a yellow solid.

Melting point: 171–173 ℃ (AcOEt / CH_2Cl_2 / MeOH 72:14:14).

IR (KBr) v: 3500–2500 (max. at 3229, 3049, 3002, 2925, 2852, OH, NH, N^+H and CH st), 1767, 1739, 1715, 1675, 1629, 1604, 1583, 1567, 1524 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

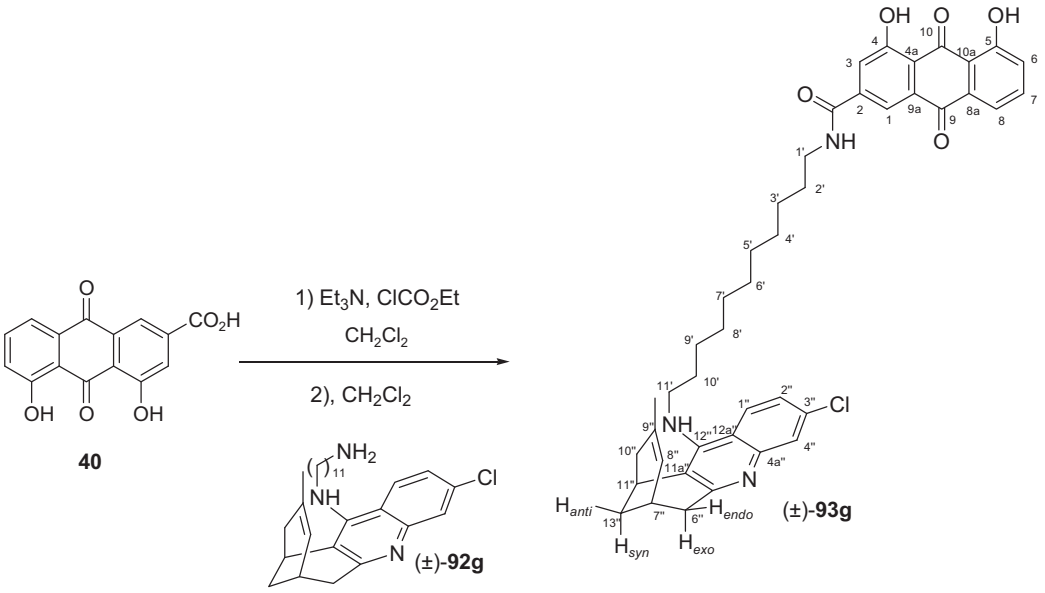
^1H NMR (500 MHz, CD_3OD) δ: 1.34−1.44 (complex signal, 12H, 3'-H_2, 4'-H_2, 5'-H_2, 6'H_2, 7'-H_2, 8'-H_2), 1.58 (s, 3H, 9''-CH_3), 1.66 (tt, $J \approx J' \approx 7.0$ Hz , 2H, 2'-H_2), 1.83 (tt, $J \approx J' \approx 7.0$ Hz, 2H, 9'-H_2), overlapped 1.90−1.93 (m, 1H, 13''-H_{syn}), partially overlapped 1.92 (broad d, $J = 17.0$ Hz, 1H, 10''-H_{endo}), 2.06 (dm, $J \approx 12.0$ Hz, 1H, 13''-H_{anti}), 2.52 (broad dd, $J \approx 17.0$ Hz, $J' \approx$ 4.3 Hz, 1H, 10''-H_{exo}), 2.75 (m, 1H, 7''-H), 2.82 (broad d, $J = 18.0$ Hz, 1H, 6''-H_{endo}), 3.15 (dd, $J \approx 18.0$ Hz, $J' = 5.3$ Hz, 1H, 6''-H_{exo}), 3.38–3.45 (complex signal, 3H, 11''-H and 1'-H_2), 3.87 (dt, $J \approx J' \approx 6.5$ Hz, 2H, 10'-H_2), 4.85 (s, NH, $^+$NH and OH), 5.57 (broad d, $J = 5.5$ Hz, 1H, 8''-H), 7.27 (dd, $J = 8.5$ Hz, $J' = 1.0$ Hz, 1H, 6-H), 7.39 (dd, $J = 9.5$ Hz, $J' = 1.5$ Hz, 1H, 2''-H), partially overlapped 7.59 (s, 1H, 4''-H), 7.60 (d, $J = 1.5$ Hz, 1H, 3-H), 7.63 (dd, $J = 7.5$ Hz, $J' = 1.0$ Hz, 1H, 8-H), 7.70 (dd, $J \approx J' \approx 8.0$ Hz, 1H, 7-H), 8.01 (d, $J = 1.5$ Hz, 1H, 1-H), 8.21 (d, $J = 9.5$ Hz, 1H, 1''-H).

^{13}C NMR (125.7 MHz, CD_3OD) δ: 23.5 (CH_3, 9''-CH_3), 27.2 (CH, C11''), 27.7 ($2CH_2$), 27.8 (CH + CH_2) (C6', C7', C8', C7''), 29.3 (CH_2, C13''), 30.03 (CH_2), 30.05 (CH_2), 30.22 (CH_2), 30.25 (CH_2) (C2', C3', C4', C5'), 31.2 (CH_2, C9'), 36.0 ($2CH_2$, C6'', C10''), 41.1 (CH_2, C1'), 49.6 (CH_2, C10'), 115.5 (C, C12a''), 116.8 (C, C10a), 117.5 (C, C11a''), 118.4 (C, C4a), 118.9 (CH, C1), 119.1 (CH, C4''), 120.9 (CH, C8), 123.7 (CH, C3), 125.1 (CH, C8''), 125.8 (CH, C6), 126.7 (CH, C2''), 129.3 (CH, C1''), 134.6 (2C, C9'', C8a), 135.0 (C, C9a), 138.7 (CH, C7), 140.2 (C, C3''), 140.8 (C, C4a''), 143.7 (C, C2), 151.0 (C, C5a''), 156.6 (C, C12''), 163.3 (C, C4), 163.6 (C, C5), 167.2 (C, CONH), 181.9 (C, C9), 193.5 (C, C10).

HRMS, ESI:
Calculated for ($C_{42}H_{44}{}^{35}ClN_3O_5 + H^+$): 706.3042.
Observed: 706.3038.

Elemental analysis: Calculated for $C_{42}H_{44}ClN_3O_5 \cdot HCl \cdot 0.5H_2O$:
C: 67.11%; H: 6.17%; N: 5.59%; Cl: 9.43%.
Observed: C: 66.76%; H: 6.26%; N: 5.27%; Cl: 9.91%.

Preparation of (±)-*N*-{11-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]undecyl}-9,10-dihydro-4,5-dihydroxy-9,10-dioxoanthracene-2-carboxamide, (±)-93g.



In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, **40** (491 mg, 1.73 mmol) was suspended in anhydrous CH_2Cl_2 (17 mL) and cooled to 0 ºC with an ice bath, then treated with anhydrous Et_3N (0.48 mL, 349 mg, 3.45 mmol) and $ClCO_2Et$ (0.16 mL, 187 mg, 1.73 mmol) dropwise. The resulting solution was stirred at 0 ºC for 30 min and treated with a solution of (±)-**92g** (785 mg, 1.73 mmol) in anhydrous CH_2Cl_2 (13 mL). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (100 mL), the phases were separated and the aqueous phase was again extracetd with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a red solid (1.6 g), which was purified by column chromatography (silica gel, 50 g, 40–60 μm, Ø = 3 cm; #1–15, 500 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #16–21, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2; #22–76, 1800 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2), to provide the hybrid (±)-**93g** (#36–47, 338 mg, 27% yield) as a red solid.

R_f = 0.50 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-93g·HCl.

In a 25 mL round-bottomed flask, (±)-**93g** (150 mg, 0.21 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.75 N, 0.83 mL) and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 / MeOH 1:1 (2.0 mL) and precipitated with EtOAc (3.0 mL), the solvent was decanted and the residue was dried and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**93g**·HCl (119 mg) as a yellow solid.

Melting point: 171–172 ℃ (AcOEt / CH_2Cl_2 / MeOH 1:1:3).

IR (KBr) v: 3500–2500 (max. at 3226, 3048, 3007, 2924, 2852, 2645, OH, NH, N^+H and CH st), 1765, 1675, 1628, 1607, 1583, 1567, 1522 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

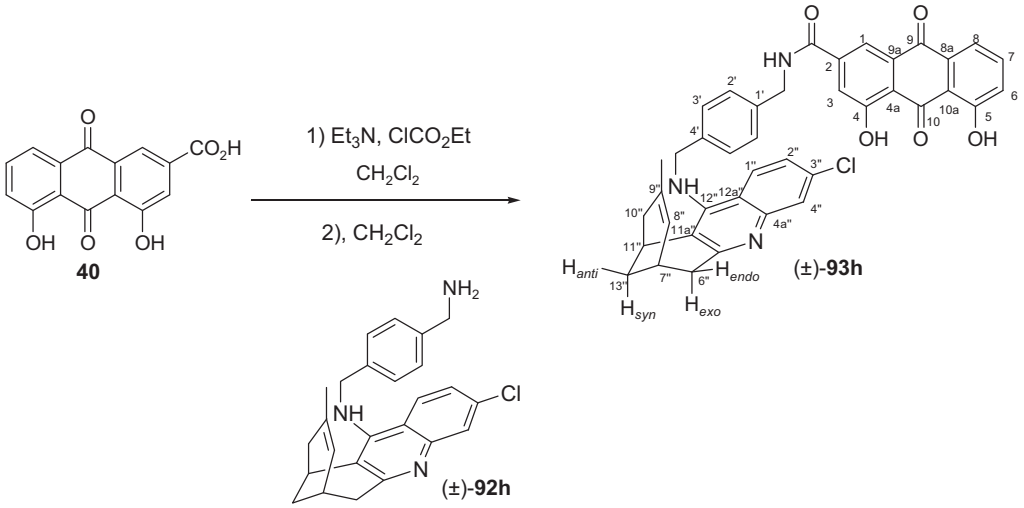
^1H NMR (500 MHz, CD_3OD) δ: 1.33−1.43 (complex signal, 14H, 3'-H_2, 4'-H_2, 5'-H_2, 6'H_2, 7'-H_2, 8'-H_2, 9'-H_2), 1.58 (s, 3H, 9''-CH_3), 1.66 (tt, $J \approx J' \approx 7.0$ Hz , 2H, 2'-H_2), 1.82 (tt, $J \approx J' \approx 17.0$ Hz, 2H, 10'-H_2), overlapped 1.89−1.93 (m, 1H, 13''-H_{syn}), partially overlapped 1.91 (broad d, $J = 17.0$ Hz, 1H, 10''-H_{endo}), 2.05 (dm, $J \approx 10.5$ Hz, Hz, 1H, 13''-H_{anti}), 2.52 (broad dd, $J \approx 17.0$ Hz, $J' \approx 4.5$ Hz, 1H, 10''-H_{exo}), 2.75 (m, 1H, 7''-H), 2.82 (broad d, $J = 18.0$ Hz, 1H, 6''-H_{endo}), 3.14 (dd, $J \approx 18.0$ Hz, $J' = 5.5$ Hz, 1H, 6''-H_{exo}), partially overlapped 3.37 (m, 1H, 11''-H), partially overlapped 3.40 (tt, $J \approx J' \approx 7.0$ Hz , 2H, 1'-H_2), 3.86 (dt, $J \approx J' \approx 7.0$ Hz, 2H, 11'-H_2), 4.85 (s, NH, $^+$NH and OH), 5.57 (broad d, $J = 5.5$ Hz, 1H, 8''-H), 7.23 (dd, $J = 8.5$ Hz, $J' = 1.0$ Hz, 1H, 6-H), 7.37 (dd, $J = 9.0$ Hz, $J' \approx 1.5$ Hz, 1H, 2''-H), partially overlapped 7.57 (d $J = 1.5$ Hz, 1H, 4''-H), partially overlapped 7.58 (d, $J = 2.0$ Hz, 1H, 3-H), partially overlapped 7.58 (dd, $J = 8.0$ Hz, $J' = 1.0$ Hz, 1H, 8-H), 7.67 (dd, $J \approx J' \approx 8.0$ Hz, 1H, 7-H), 7.97 (d, $J = 1.5$ Hz, 1H, 1-H), 8.20 (d, $J = 9.0$ Hz, 1H, 1''-H).

^{13}C NMR (125.7 MHz, CD_3OD) δ: 23.5 (CH_3, 9''-CH_3), 27.2 (CH, C11''), 27.7 (CH_2), 27.82 (CH, C7''), 27.83 ($2CH_2$) (C7', C8', C9', C7''), 29.3 (CH_2, C13''), 30.1 ($2CH_2$), 30.2 (CH_2), 30.36 (CH_2), 30.4 (CH_2) (C2', C3', C4', C5', C6'), 31.2 (CH_2, C10'), 36.0 ($2CH_2$, C6'', C10''), 41.2 (CH_2, C1'), 49.6 (CH_2, C11'), 115.4 (C, C12a''), 116.7 (C, C10a), 117.5 (C, C11a''), 118.3 (C, C4a), 118.9 (CH, C1), 119.1 (CH, C4''), 120.8 (CH, C8), 123.7 (CH, C3), 125.1 (CH, C8''), 125.8 (CH, C6), 126.6 (CH, C2''), 129.3 (CH, C1''), 134.5 (C, C9''), 134.6 (C, C8a), 134.9 (C, C9a), 138.7 (CH, C7), 140.2 (C, C3''), 140.8 (C, C4a''), 143.7 (C, C2), 151.0 (C, C5a''), 156.6 (C, C12''), 163.3 (C, C4), 163.5 (C, C5), 167.1 (C, CONH), 181.8 (C, C9), 193.4 (C, C10).

HRMS, ESI:
Calculated for ($C_{43}H_{46}{}^{35}ClN_3O_5 + H^+$): 720.3199.
Observed: 720.3195.

Elemental analysis: Calculated for $C_{43}H_{46}ClN_3O_5 \cdot HCl \cdot 0.5H_2O$:
C: 67.44%; H: 6.32%; N: 5.49%; Cl: 9.26%.
Observed: C: 67.52%; H: 6.46%; N: 5.30%; Cl: 9.51%.

Preparation of (±)-*N*-{4-{[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]methyl}benzyl}-9,10-dihydro-4,5-dihydroxy-9,10-dioxoanthracene-2-carboxamide, (±)-93h.



In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, **40** (548 mg, 1.93 mmol) was suspended in anhydrous CH_2Cl_2 (7 mL) and cooled to 0 ºC with an ice bath, then treated with anhydrous Et_3N (0.81 mL, 592 mg, 5.86 mmol) and $ClCO_2Et$ (0.18 mL, 208 mg, 1.93 mmol) dropwise. The resulting solution was stirred at 0 ºC for 30 min and treated with a solution of (±)-**92h** (780 mg, 1.93 mmol) in anhydrous CH_2Cl_2 (6 mL). The reaction mixture was stirred at r. t. for 3 days, diluted with 10 % aq. Na_2CO_3 (100 mL), the phases were separated and the aqueous phase was again extracted with CH_2Cl_2 (2 x 100 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a red solid (1.07 g), which was purified by column chromatography (silica gel, 40 g, 40–60 µm, Ø = 3 cm; #1–12, 400 mL, hexane / EtOAc / Et_3N 50:50:0.2; #13–16, 200 mL, hexane / EtOAc / Et_3N 45:55:0.2; #17–27, 400 mL, hexane / EtOAc / Et_3N 40:60:0.2; #28–33, 200 mL, hexane / EtOAc / Et_3N 30:70:0.2; #34–36, 100 mL, hexane / EtOAc / Et_3N 20:80:0.2; #37–39, 100 mL, EtOAc / Et_3N 100:0.2; #40–42, 100 mL, EtOAc / MeOH / Et_3N 95:5:0.2; #43–45, 100 mL, EtOAc / MeOH / Et_3N 90:10:0.2; #46–50, 200 mL, EtOAc / MeOH / Et_3N 80:20:0.2), to provide the hybrid (±)-**93h** (#17–23, 113 mg, 9% yield) as a red solid.

R_f = 0.57 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-93h·HCl.

In a 25 mL round-bottomed flask, (±)-**93h** (90 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (8 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.45 N, 0.96 mL) and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 / MeOH 1:1 (2 mL) and precipitated with EtOAc (4 mL), the solvent was decanted and the residue was dried and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**93h**·HCl (35 mg) as a yellow solid.

Melting point: 129–130 ℃ (EtOAc / CH_2Cl_2 / MeOH 4:1:1).

IR (KBr) v: 3500–2500 (max. at 3245, 3055, 2925, 2852, 2790, OH, NH, N^+H and CH st), 1705, 1673, 1629, 1607, 1583, 1560, 1516 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

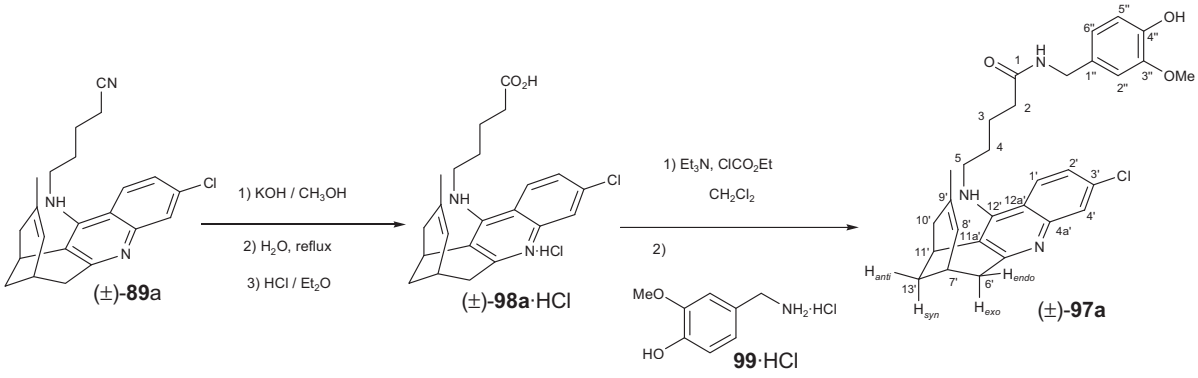
^1H NMR (500 MHz, CD_3OD) δ: 1.58 (s, 3H, 9''-CH_3), 1.89 (dm, *J* = 12.5 Hz, 1H, 13''-H_{syn}), 1.97 (broad d, *J* = 18.0 Hz, 1H, 10''-H_{endo}), 2.06 (dm, *J* = 12.5 Hz, 1H, 13''-H_{anti}), 2.56 (broad dd, *J* = 18.0 Hz, *J'* = 4.3 Hz, 1H, 10''-H_{exo}), 2.75 (m, 1H, 7''-H), 2.85 (broad d, *J* = 18.0 Hz, 1H, 6''-H_{endo}), 3.14 (dd, *J* = 18.0 Hz, *J'* = 5.5 Hz, 1H, 6''-H_{exo}), 3.46 (m, 1H, 11''-H), 4.58 (s, 2H, CH_2NH), 4.85 (s, NH, $^+$NH and OH), 5.17 (s, 2H, CH_2NHCO), 5.57 (broad d, *J* = 5.0 Hz, 1H, 8''-H), 7.07 (dd, *J* = 9.0 Hz, *J'* = 2.5 Hz, 1H, 2''-H), 7.25 (dd, *J* = 8.0 Hz, *J'* = 1.5 Hz, 1H, 6-H), 7.42 (d, *J* = 8.0 Hz, 1H) and 7.48 (d, *J* = 8.0 Hz, 1H) [2'(6')-H_2, 3'(5')-H_2], 7.57−7.58 (complex signal, 2H, 3-H and 4-H), partially overlapped 7.64 (dd, *J* = 7.5 Hz, *J'* = 1.5 Hz, 1H, 8-H), partially overlapped 7.66 (dd, *J* ≈ *J'* ≈ 8.0 Hz, 1H, 7-H), 7.99 (d, *J* = 2.0 Hz, 1H, 1-H), 8.12 (d, *J* = 9.0 Hz, 1H, 1''-H).

^{13}C NMR (125.7 MHz, CD_3OD) δ: 23.5 (CH_3, 9''-CH_3), 27.5 (CH, C11''), 27.8 (CH, C7''), 29.3 (CH_2, C13''), 36.0 (CH_2, C6''), 36.2 (CH_2, C10''), 44.3 (CH_2, CH_2NH), 51.9 (CH_2, *C*H_2NHCO), 115.4 (C, C12a''), 116.9 (C, C10a), 118.2 (C, C11a''), 118.5 (C, C4a), 118.8 (CH, C1), 119.0 (CH, C4''), 120.9 (CH, C8), 123.7 (CH, C3), 125.0 (CH, C8''), 125.8 (CH, C6), 126.6 (CH, C2''), 128.1 (CH) and 129.8 (CH) [C2'(6'), C3'(5')], 129.3 (CH, C1''), 134.6 (C, C9''), 134.7 (C, C8a), 135.0 (C, C9a), 137.8 (C) and 139.2 (C) (C1', C4'), 138.8 (CH, C7), 140.2 (C, C3''), 140.7 (C, C4a''), 143.1 (C, C2), 151.6 (C, C5a''), 157.1 (C, C12''), 163.3 (C, C4), 163.6 (C, C5), 167.1 (C, CONH), 182.0 (C, C9), 193.5 (C, C10).

HRMS, ESI:
Calculated for ($C_{40}H_{32}{}^{35}ClN_3O_5$ + H^+): 670.2103.
Observed: 670.2104.

Elemental analysis: Calculated for $C_{40}H_{32}ClN_3O_5 \cdot 1.1HCl \cdot 1.4H_2O$:
C: 65.32%; H: 4.92%; N: 5.71%; Cl: 10.12%
Observed: C: 65.32%; H: 5.06%; N: 6.09%; Cl: 10.15%

Preparation of (±)-5-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]-*N*-(4-hydroxy-3-methoxybenzyl)pentanamide, (±)-97a.



In a 50 mL round-bottomed flask equipped with magnetic stirrer and a condenser, (±)-**89a** (1.43 g, 3.91 mmol) was dissolved in MeOH (5 mL) and treated with a 40% sol. of KOH in MeOH (10 mL). The resulting suspension was stirred under reflux for 3 h, treated with H_2O (16 mL) and again stirred under reflux overnight. The resulting solution was cooled down to r. t., evaporated under reduced pressure, and treated with HCl / Et_2O (0.5 N, 156 mL) and again concentrated under reduced pressure to give a white solid (6.00 g), whose ^{1}H NMR spectrum was consistent with that expected for the desired acid, (±)-**98a**·HCl, and was used as a crude in the next step.

In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**98a**·HCl (3.00 g of a crude that could contain a maximum of 1.90 mmol of the desired acid) was suspended in anhydrous CH_2Cl_2 (20 mL), cooled to 0 ℃ with an ice bath and then treated dropwise with freshly distilled Et_3N (1.32 mL, 959 mg, 9.50 mmol) and $ClCO_2Et$ (0.18 mL, 206 mg, 1.90 mmol). The resulting suspension was stirred at 0 ℃ for 30 min and then treated with amine **99**·HCl (360 mg, 1.90 mmol). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (60 mL) and extracted with CH_2Cl_2 (2 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow solid (740 mg), which was purified through column chromatography (silica gel, 40–60 μm, 40 g, Ø = 3 cm; #1–17, 400 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #18–28, 400 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2; #29–54, 800 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #55–68, 800 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98.5:1.5:0.2), to provide starting nitrile (±)-**89a** (#31–36, 240 mg) and the hybrid (±)-**97a** (#48–52, 180 mg, 18% overall yield).

R_f = 0.19 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-97a·HCl.

In a 25 mL round-bottomed flask, (±)-**97a** (180 mg, 0.35 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.43 N, 2.42 mL), evaporated under

reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**97a**·HCl as a beige solid (176 mg).

Melting point: 132–133 ℃ (CH_2Cl_2 / MeOH 71:29).

IR (KBr) v: 3500–2500 (max. at 3250, 3061, 3012, 2925, 2857, 2801, OH, NH, N^+H and CH st), 1728, 1635, 1584, 1514 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

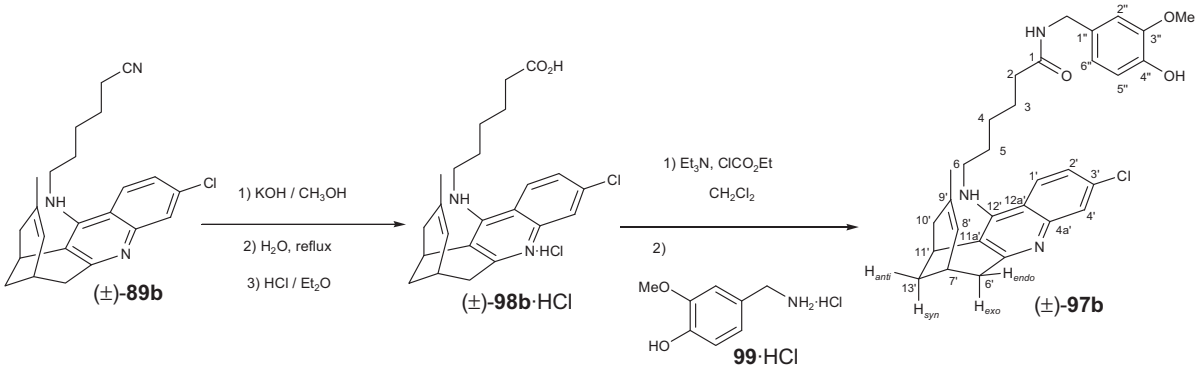
^1H NMR (400 MHz, CD_3OD) δ: 1.56 (s, 3H, 9'-CH_3), 1.77 (tt, $J \approx J' \approx$ 7.6 Hz, 2H, 3-H_2), partially overlapped 1.85–1.91 (tt, $J \approx J' \approx$ 7.2 Hz, 2H, 4-H_2), partially overlapped 1.90 (broad d, J = 18.0 Hz, 1H, 10'-H_{endo}), overlapped 1.88–1.92 (m, 1H, 13'-H_{syn}), 2.06 (dm, J = 11.2 Hz, 1H, 13'-H_{anti}), 2.34 (t, J = 6.8 Hz, 2H, 2-H_2), 2.53 (dd, J = 18.0 Hz, J' = 5.2 Hz 1H, 10'–H_{exo}), 2.75 (m, 1H, 7'-H), 2.87 (broad d, J = 18.0 Hz, 1H, 6'-H_{endo}), 3.19 (dd, J = 18.0 Hz, J' = 5.4 Hz, 1H, 6'-H_{exo}), 3.44 (m, 1H, 11'-H), 3.76 (s, 3H, 3''-OCH_3), 3.95 (t, $J \approx$ 7.0 Hz, 2H, 5-H_2), 4.23 (d, J = 4.0 Hz, 2H, CONHCH_2), 4.85 (s, NH, $^+$NH and OH), 5.56 (broad d, J = 4.8 Hz, 1H, 8'-H), 6.61 (d, J = 8.0 Hz, 1H), 6.66 (d, $J \approx$ 8.0 Hz, 1H) (5''-H, 6''-H), 6.80 (s, 1H, 2''-H), 7.48 (d, J = 9.2 Hz, 1H, 2'-H), 7.74 (s, 1H, 4'-H), 8.40 (d, J = 9.2 Hz, 1H, 1'-H), 8.39 (t, $J \approx$ 4.0 Hz, 1H, C1).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 23.8 (CH_2, C3), 27.3 (CH, C11'), 27.8 (CH, C7'), 29.3 (CH_2, C13'), 31.0 (CH_2, C4), 36.0 (CH_2, C6'), 36.1 ($2CH_2$, C10', C2), 43.9 (CH_2, CONHCH_2), 49.1 (CH_2, C5), 56.3 (CH_3, 3''-OCH_3), 112.4 (CH, C2''), 115.5 (C, C12a'), 116.0 (CH, C5''), 117.6 (C, C11a'), 119.1 (CH, C4'), 121.3 (CH, C6''), 125.1 (CH, C8'), 126.7 (CH, C2'), 129.3 (CH, C1'), 131.5 (C, C1''), 134.6 (C, C9'), 140.1 (C, C3'), 140.8 (C, C4a'), 146.7 (C, C4''), 148.8 (C, C3''), 151.2 (C, C5a'), 156.7 (C, C12'), 157.3 (C, CONH).

HRMS, ESI:
Calculated for ($C_{30}H_{34}{}^{35}ClN_3O_3 + H^+$): 520.2361.
Observed: 520.2357.

Elemental analysis: Calculated for $C_{30}H_{34}ClN_3O_3 \cdot HCl \cdot H_2O$:
C: 62.72%; H: 6.49%; N: 7.31%; Cl: 12.14%.
Observed: C: 63.28%; H: 6.67%; N: 7.17%; Cl: 11.80%.

Preparation of (±)-6-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]-*N*-(4-hydroxy-3-methoxybenzyl)hexanamide, (±)-97b.



In a 50 mL round-bottomed flask equipped with magnetic stirrer and a condenser, (±)-**89b** (1.60 g, 4.22 mmol) was dissolved in MeOH (5 mL) and treated with a 40% sol. of KOH in MeOH (11 mL). The resulting suspension was stirred under reflux for 3 h, treated with H_2O (15 mL) and again stirred under reflux overnight. The resulting solution was cooled down to r. t., evaporated under reduced pressure, treated with HCl / Et_2O (0.5 N, 170 mL) and again concentrated under reduced pressure to give a white solid (6.61 g), whose ^{1}H NMR spectrum was consistent with that expected for the desired acid, (±)-**98b**·HCl, and was used as a crude in the next step.

In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**98b**·HCl (6.26 g of a crude that could contain a maximum of 3.99 mmol of the desired acid) was suspended in anhydrous CH_2Cl_2 (25 mL), cooled to 0 ℃ with an ice bath and then treated dropwise with freshly distilled Et_3N (2.77 mL, 2.01 g, 19.9 mmol) and $ClCO_2Et$ (0.39 mL, 455 mg, 4.21 mmol). The resulting suspension was stirred at 0 ℃ for 30 min and treated with amine **99**·HCl (757 mg, 3.99 mmol). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (60 mL) and extracted with CH_2Cl_2 (2 x 35 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow solid (2.00 g), which was purified through column chromatography (silica gel, 40–60 μm, 90 g, Ø = 5 cm; #1–4, 500 mL, CH_2Cl_2 / 50% aq. NH_4OH 99:0.2; #5–9, 500 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2; #10–24, 1500 mL CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #25–52, 2500 mL CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98.5:1.5:0.2), to provide the hybrid (±)-**97b** (#33–40, 800 mg, 37% overall yield).

R_f = 0.20 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-97b·HCl.

In a 25 mL round-bottomed flask, (±)-**97b** (800 mg, 1.50 mmol) was dissolved in CH_2Cl_2 (15 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (1.45 N, 3.0 mL), then evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**97b**·HCl as a yellow solid (807 mg).

Melting point: 134–135 ℃ (CH_2Cl_2 / MeOH 83:17).

IR (KBr) v: 3500–2500 (max. at 3233, 3054, 3012, 2924, 2855, 2791, 2645, OH, NH, N^+H and CH st), 1632, 1583, 1517 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

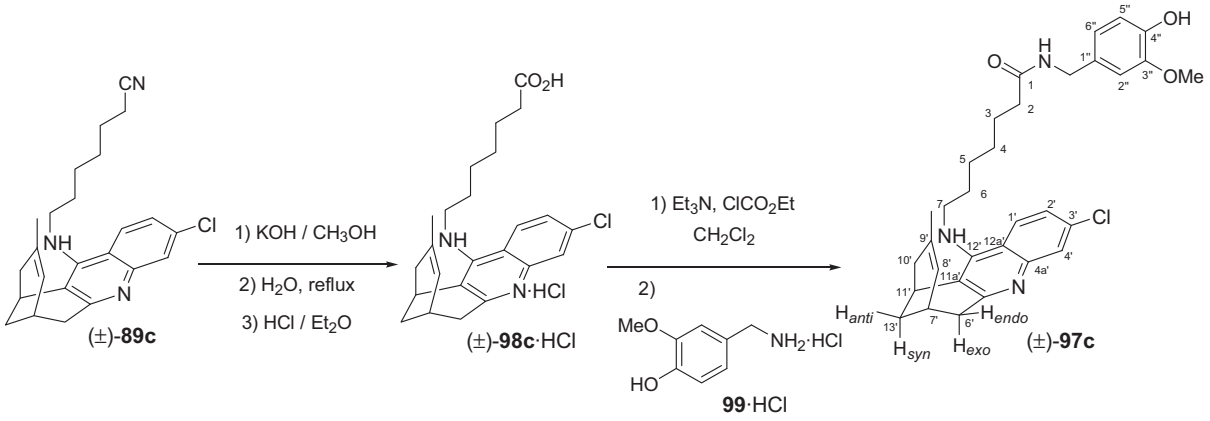
^1H NMR (400 MHz, CD_3OD) δ: 1.46 (tt, *J* ≈ *J'* ≈ 7.6 Hz, 2H, 4-H_2), 1.57 (s, 3H, 9'-CH_3), 1.71 (tt, *J* ≈ *J'* ≈ 7.6 Hz, 2H, 3-H_2), partially overlapped 1.87 (tt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, 5-H_2), partially overlapped 1.91 (broad d, *J* = 17.2 Hz, 1H, 10'-H_{endo}), overlapped 1.84–1.94 (m, 1H, 13'-H_{syn}), 2.06 (dm, *J* = 12.4 Hz, 1H, 13'-H_{anti}), 2.27 (t, *J* ≈ 7.6 Hz, 2H, 2-H_2), 2.54 (dd, *J* ≈ 17.2 Hz, *J'* ≈ 4.6 Hz, 1H, 10'-H_{exo}), 2.75 (m, 1H, 7'-H), 2.86 (broad d, *J* = 18.0 Hz, 1H, 6'-H_{endo}), 3.19 (dd, *J* = 18.0 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.43 (m, 1H, 11'-H), 3.80 (s, 3H, 3''-OCH_3), 3.94 (t, *J* = 7.2 Hz, 2H, 6-H_2), 4.24 (d, *J* = 5.2 Hz, 2H, CONHC*H*$_2$), 4.85 (s, NH, $^+$NH and OH), 5.57 (broad d, *J* = 4.8 Hz, 1H, 8'-H), 6.65–6.72 (complex signal, 2H, 5''-H and 6''-H), 6.84 (s, 1H, 2''-H), 7.53 (dd, *J* = 9.2 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.76 (d, *J* = 2.0 Hz, 1H, 4'-H), partially overlapped 8.33 (t, *J* ≈ 5.2 Hz, 1H, CONH), 8.35 (d, *J* = 9.2 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 26.4 (CH_2, C3), 27.3 (CH + CH_2, C4, C11'), 27.8 (CH, C7'), 29.3 (CH_2, C13'), 30.9 (CH_2, C5), 36.0 (CH_2, C6'), 36.1 (CH_2, C10'), 36.7 (CH_2, C2), 43.9 (CH_2, CONH*C*H_2), 49.5 (CH_2, C6), 56.4 (CH_3, 3''-OCH_3), 112.5 (CH, C2''), 115.6 (C, C12a'), 116.1 (CH, C5''), 117.6 (C, C11a'), 119.1 (CH, C4'), 121.3 (CH, C6''), 125.1 (CH, C8'), 126.7 (CH, C2'), 129.4 (CH, C1'), 131.5 (C, C1''), 134.6 (C, C9'), 140.1 (C, C3'), 140.9 (C, C4a'), 146.8 (C, C4''), 148.9 (C, C3''), 151.2 (C, C5a'), 156.8 (C, C12'), 157.6 (C, C1).

HRMS, ESI:
Calculated for ($C_{31}H_{36}{}^{35}ClN_3O_3 + H^+$): 534.2518.
Observed: 534.2527.

Elemental analysis: Calculated for $C_{31}H_{36}ClN_3O_3 \cdot HCl \cdot 0.75H_2O$:
C: 63.75%; H: 6.64%; N: 7.19%; Cl: 12.14%.
Observed: C: 64.25%; H: 6.88%; N: 7.01%; Cl: 11.66%.

Preparation of (±)-7-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]-*N*-(4-hydroxy-3-methoxybenzyl)heptanamide, (±)-97c.



In a 50 mL round-bottomed flask equipped with magnetic stirrer and a condenser, (±)-**89c** (800 mg, 2.03 mmol) was dissolved in MeOH (3 mL) and treated with a 40% sol. of KOH in MeOH (5.5 mL). The resulting suspension was stirred under reflux for 3 h, treated with H_2O (7 mL) and again stirred under reflux overnight. The reaction mixture was cooled down to r. t., evaporated under reduced pressure, treated with HCl / Et_2O (0.5 N, 72.5 mL) and again concentrated under reduced pressure to give a white solid (3.43 g) whose ^{1}H NMR spectrum was consistent with that expected for the desired acid, (±)-**98c**·HCl, and was used as a crude in the next step.

In a triple neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**98c**·HCl (3.43 g of a crude that could contain a maximum of 2.03 mmol of the desired acid) was suspended in anhydrous CH_2Cl_2 (24 mL), cooled to 0 ℃ with an ice bath and then treated dropwise with freshly distilled Et_3N (1.41 mL, 1.02 g, 10.1 mmol) and $ClCO_2Et$ (0.19 mL, 220 mg, 2.03 mmol). The resulting suspension was stirred at 0 ℃ for 30 min and treated with amine **99**·HCl (385 mg, 2.03 mmol). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (60 mL) and extracted with CH_2Cl_2 (2 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow solid (1.09 g), which was purified through column chromatography (silica gel, 40–60 μm, 40 g, Ø = 3 cm; #1–8, 300 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #9–18, 300 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2; #19–58, 1500 mL CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2), to provide the hybrid (±)-**97c** (#32–44, 490 mg, 44% overall yield).

R_f = 0.24 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-97c.HCl.

In a 25 mL round-bottomed flask, (±)-**97c** (490 mg, 0.89 mmol) was dissolved in CH_2Cl_2 (12 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (1.45 N, 1.89 mL), evaporated under

reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**97c**·HCl as a yellow solid (492 mg).

Melting point: 133–135 °C (CH_2Cl_2 / MeOH 86:14).

IR (KBr) v: 3500–2500 (max. at 3233, 3057, 3007, 2925, 2854, 2785, OH, NH, N^+H and CH st), 1630, 1599, 1583, 1566, 1513 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

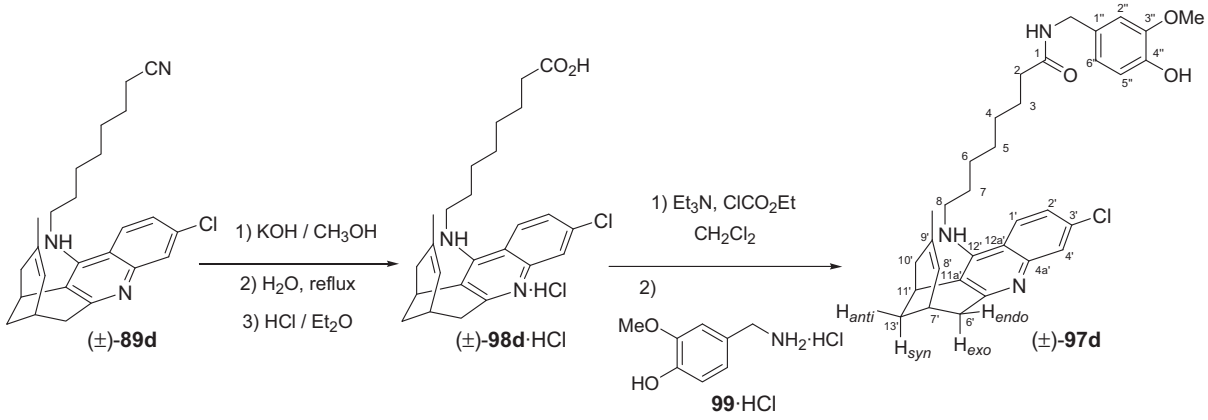
^1H NMR (400 MHz, CD_3OD) δ: 1.36–1.47 (complex signal, 4H, 4-H_2, 5-H_2), 1.58 (s, 3H, 9'-CH_3), 1.65 (tt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, 3-H_2), partially overlapped 1.81–1.90 (tt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, 6-H_2), partially overlapped 1.92 (broad d, *J* = 18.0 Hz, 1H, 10'-H_{endo}), partially overlapped 1.95 (dm, *J* = 12.8 Hz, 1H, 13'-H_{syn}), 2.08 (dm, *J* = 12.8 Hz, 1H, 13'-H_{anti}), 2.23 (t, *J* = 7.2 Hz, 2H, 2-H_2), 2.55 (dd, *J* ≈ 18.0 Hz, *J'* = 4.4 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.86 (broad d, *J* = 18.0 Hz, 1H, 6'-H_{endo}), 3.20 (dd, *J* = 18.0 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.44 (m, 1H, 11'-H), 3.81 (s, 3H, 3''-OCH_3), 3.93 (t, *J* = 6.6 Hz, 2H, 7-H_2), 4.24 (d, *J* = 5.6 Hz, 2H, CONHCH_2), 4.85 (s, NH, N^+H and OH), 5.58 (broad d, *J* = 4.4 Hz, 1H, 8'-H), 6.66–6.72 (complex signal, 2H, 5''-H and 6''-H), 6.84 (s, 1H, 2''-H), 7.54 (dd, *J* ≈ 9.6 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.75 (d, *J* ≈ 2.0 Hz, 1H, 4'-H), 8.30 (t, *J* ≈ 5.6 Hz, 1H, CONH), 8.35 (d, *J* = 9.6 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 26.8 (CH_2, C3), 27.3 (CH, C11'), 27.5 (CH_2, C4), 27.9 (CH, C7'), 29.3 (CH_2, C13'), 29.6 (CH_2, C5), 31.0 (CH_2, C6), 36.0 (CH_2, C6'), 36.1 (CH_2, C10'), 36.8 (CH_2, C2), 43.9 (CH_2, CONHCH_2), 49.6 (CH_2, C7), 56.4 (CH_3, 3''-OCH_3), 112.6 (CH, C2''), 115.7 (C, C12a'), 116.1 (CH, C5''), 117.6 (C, C11a'), 119.2 (CH, C4'), 121.4 (CH, C6''), 125.1 (CH, C8'), 126.7 (CH, C2'), 129.4 (CH, C1'), 131.6 (C, C1''), 134.6 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 146.8 (C, C4''), 148.9 (C, C3''), 151.2 (C, C5a'), 156.9 (C, C12'), 175.8 (C, C1).

HRMS, ESI:
Calculated for ($C_{32}H_{38}{}^{35}ClN_3O_3$ + H^+): 548.2674.
Observed: 548.2677.

Elemental analysis: Calculated for $C_{32}H_{38}ClN_3O_3$·HCl·0.75H_2O:
C: 64.26%; H: 6.83%; N: 7.03%; Cl: 11.86%.
Observed: C: 64.47%; H: 7.04%; N: 6.86%; Cl: 11.47%.

Preparation of (±)-8-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]-*N*-(4-hydroxy-3-methoxybenzyl)octanamide, (±)-97d.



In a 25 mL round-bottomed flask equipped with magnetic stirrer and a condenser, (±)-**89d** (540 g, 1.33 mmol) was dissolved in MeOH (1.76 mL) and treated with a 40% sol. of KOH in MeOH (3.5 mL) and EtOH (1 mL). The resulting suspension was stirred under reflux for 3 h, treated with H_2O (5 mL) and again stirred under reflux overnight. The reaction mixture was cooled down to r. t., evaporated under reduced pressure, treated with HCl / Et_2O (0.5 N, 47.2 mL) and again concentrated under reduced pressure to give a white solid (2.32 g), whose ^{1}H NMR spectrum was consistent with that expected for the desired acid, (±)-**98d**·HCl, and was used as a crude in the next step.

In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**98d**·HCl (2.00 g of a crude that could contain a maximum of 1.14 mmol of the desired acid) was suspended in anhydrous CH_2Cl_2 (6.5 mL), cooled to 0 ℃ with an ice bath and trea ted dropwise with freshly distilled Et_3N (0.79 mL, 576 mg, 5.70 mmol) and $ClCO_2Et$ (0.11 mL, 124 mg, 1.14 mmol). The resulting suspension was stirred at 0 ℃ for 30 min and treated with amine **99**·HCl (216 mg, 1.14 mmol). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (60 mL) and extracted with CH_2Cl_2 (2 x 35 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow solid (0.75 g), which was purified through column chromatography (silica gel, 40–60 µm, 30 g, Ø = 3 cm; #1–39, 1200 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #40–67, 1200 mL,CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2), to provide the hybrid (±)-**97d** (#56–60, 110 mg, 17% overall yield).

R_f = 0.29 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-97d·HCl:

In a 25 mL round-bottomed flask, (±)-**97d** (110 mg, 0.19 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 0.78 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**97d**·HCl as a yellow solid (104 mg).

Melting point: 126–127 °(CH_2Cl_2 / MeOH 88:12).

IR (KBr) ν: 3500–2500 (max. at 3253, 3056, 3012, 2927, 2854, 2790, OH, NH, N^+H and CH st), 1630, 1583, 1514 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

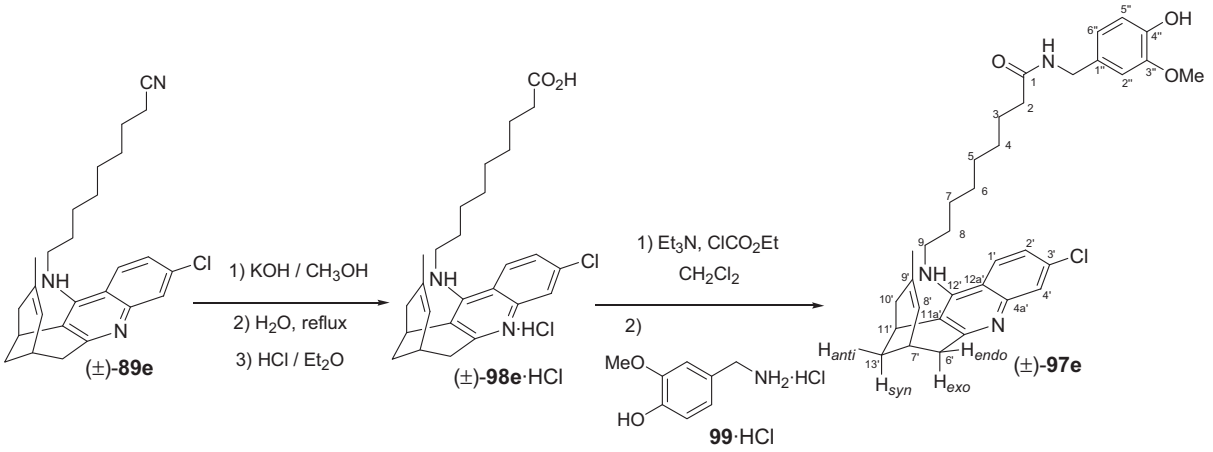
^1H NMR (400 MHz, CD_3OD) δ: 1.29–1.44 (m, 6H, 4-H_2, 5-H_2 and 6-H_2), 1.58 (s, 3H, 9'-CH_3), 1.62 (tt, $J \approx J' \approx 7.2$ Hz, 2H, 3-H_2), partially overlapped 1.79–1.88 (tt, $J \approx J' \approx 7.2$ Hz, 2H, 7-H_2), partially overlapped 1.92 (broad d, $J = 18.0$ Hz, 1H, 10'-H_{endo}), overlapped 1.90–1.97 (dm, $J \approx 10.2$ Hz, 1H, 13'-H_{syn}), 2.08 (dm, $J = 10.2$ Hz, 1H, 13'-H_{anti}), 2.21 (t, $J = 7.2$ Hz, 2H, 2-H_2), 2.54 (dd, $J = 18.0$ Hz, $J' = 4.0$ Hz, 1H, 10'-H_{exo}), 2.76 (m, 1H, 7'-H), 2.84 (dm, $J = 18.0$ Hz, 1H, 6'-H_{endo}), 3.20 (dd, $J = 18.0$ Hz, $J' = 5.8$ Hz, 1H, 6'-H_{exo}), 3.44 (m, 1H, 11'-H), 3.82 (s, 3H, 3''-OCH_3), 3.95 (t, $J = 7.4$ Hz, 2H, 8-H_2), 4.25 (d, $J = 4.4$ Hz, 2H, CONHCH_2), 4.85 (s, NH, N^+H and OH), 5.58 (broad d, $J = 4.4$ Hz, 1H, 8'-H), 6.70 (complex signal, 2H, 5''-H, 6''-H), 6.85 (s, 1H, 2''-H), 7.55 (dd, $J \approx 9.2$ Hz, $J' \approx 2.0$ Hz, 1H, 2'-H), 7.75 (d, $J = 2.0$ Hz, 1H, 4'-H), 8.29 (broad t, $J \approx 4.4$ Hz, 1H, CONH), 8.37 (d, $J = 9.2$ Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.4 (CH_3, 9'-CH_3), 26.8 (CH_2, C3), 27.3 (CH, C11'), 27.6 (CH_2, C4), 27.9 (CH, C7'), 29.3 (CH_2, C13'), 29.9 (CH_2), 30.0 (CH_2), 31.1 (CH_2) (C5, C6, C7), 36.0 (CH_2, C6'), 36.1 (CH_2, C10'), 36.9 (CH_2, C2), 43.9 (CH_2, CONHCH_2), 49.6 (CH_2, C8), 56.4 (CH_3, 3''-OCH_3), 112.5 (CH, C2''), 115.7 (C, C12a'), 116.1 (CH, C5''), 117.6 (C, C11a'), 119.2 (CH, C4'), 121.4 (CH, C6''), 125.1 (CH, C8'), 126.7 (CH, C2'), 129.4 (CH, C1'), 131.6 (C, C1''), 134.6 (C, C9'), 140.2 (C, C3'), 141.0 (C, C4a'), 146.8 (C, C4''), 149.0 (C, C3''), 151.3 (C, C5a'), 156.9 (C, C12'), 175.9 (C, C1).

HRMS, ESI:
Calculated for ($C_{33}H_{40}{}^{35}ClN_3O_3 + H^+$): 562.2831.
Observed: 562.2828.

Elemental analysis: Calculated for $C_{33}H_{40}ClN_3O_3 \cdot 1.1HCl$:
C: 65.81%; H: 6.88%; N: 6.98%; Cl: 12.36%.
Observed: C: 65.33%; H: 7.17%; N: 6.69%; Cl: 12.39%.

Preparation of (±)-9-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]-*N*-(4-hydroxy-3-methoxybenzyl)nonanamide, (±)-97e.



In a 25 mL round-bottomed flask equipped with magnetic stirrer and a condenser, (±)-**89e** (400 mg, 0.95 mmol) was dissolved in MeOH (1.5 mL) and treated with a 40% sol. of KOH in MeOH (2.5 mL). The resulting suspension was stirred under reflux for 3 h, treated with H_2O (3.5 mL) and again stirred under reflux overnight. The reaction mixture was cooled down to r. t., evaporated under reduced pressure, treated with HCl / Et_2O (0.5 N, 34.0 mL) and again concentrated under reduced pressure to give a white solid (1.71 g) whose ^{1}H NMR spectrum was consistent with that expected for the desired acid, (±)-**98e**·HCl, and was used as a crude in the next step.

In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**98e**·HCl (1.55 g of a crude that could contain a maximum of 0.86 mmol of the desired acid) was suspended in anhydrous CH_2Cl_2 (5 mL), cooled to 0 ℃ with an ice bath and treate d dropwise with freshly distilled Et_3N (0.58 mL, 419 mg, 4.15 mmol) and $ClCO_2Et$ (0.08 mL, 93 mg, 0.86 mmol). The resulting suspension was stirred at 0 ℃ for 30 min , and treated with amine **99**·HCl (163 mg, 0.86 mmol). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (60 mL) and extracted with CH_2Cl_2 (2 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow solid (450 mg), which was purified through column chromatography (silica gel, 40–60 µm, 20 g, Ø = 2,5 cm; #1–5, 200 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; # 6–40, 1300 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2), to provide starting nitrile (±)-**89e** (#13–16, 49 mg) and the hybrid (±)-**97e** (#19–23, 45 mg, 8% overall yield).

R_f = 0.33 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-97e·HCl.

In a 25 mL round-bottomed flask, (±)-**97e** (107 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (8 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (1.70 N, 0.33 mL), evaporated under

reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**97e**·HCl as a yellow solid (104 mg).

Melting point: 122 –123 ℃ (CH_2Cl_2 / MeOH 96:4).

IR (KBr) v: 3500–2500 (max. at 3250, 3059, 3002, 2926, 2854, 2795, OH, NH, N^+H and CH st), 1763, 1726, 1705, 1630, 1583, 1514 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

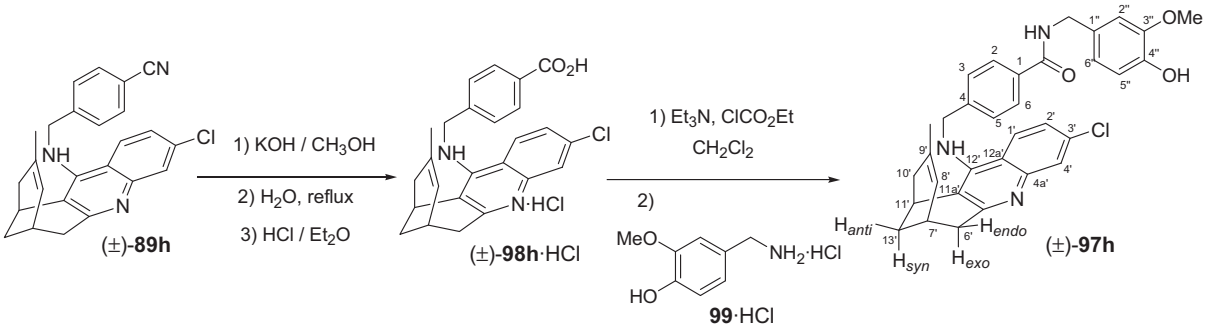
^1H NMR (400 MHz, CD_3OD) δ: 1.28–1.42 (m, 8H, 4-H_2, 5-H_2, 6-H_2 and 7-H_2), 1.58 (s, 3H, 9'-CH_3), partially overlapped 1.58−1.65 (tt, $J \approx J' \approx$ 7.4 Hz, 2H, 3-H_2), partially overlapped 1.79–1.91 (tt, $J \approx J' \approx$ 6.8 Hz, 2H, 8-H_2), partially overlapped 1.94 (broad d, J = 17.2 Hz, 1H, 10'-H_{endo}), overlapped 1.91–1.97 (m, 1H, 13'-H_{syn}), 2.08 (dm, $J \approx$ 10.6 Hz, 1H, 13'-H_{anti}), 2.21 (t, J = 7.4 Hz, 2H, 2-H_2), 2.55 (dd, J = 17.2 Hz, J' = 4.2 Hz, 1H, 10'-H_{exo}), 2.77 (m, 1H, 7'-H), 2.86 (dm, J = 17.6 Hz, 1H, 6'-H_{endo}), 3.20 (dd, J = 17.6 Hz, J' = 5.8 Hz, 1H, 6'-H_{exo}), 3.44 (m, 1H, 11'-H), 3.82 (s, 3H, 3''-OCH_3), 3.96 (t, J = 6.8 Hz, 2H, 9-H_2), 4.24 (s, 2H, CONHCH_2), 4.85 (s, NH, N^+H and OH), 5.58 (broad d, J = 4.8 Hz, 1H, 8'-H), 6.70 (complex signal, 2H, 5''-H, 6''-H), 6.85 (s, 1H, 2''-H), 7.55 (dd, $J \approx$ 9.2 Hz, J' = 2.0 Hz, 1H, 2'-H), 7.75 (d, J = 2.0 Hz, 1H, 4'-H), 8.28 (broad s, 1H, CONH), 8.38 (d, J = 9.2 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 26.9 (CH_2, C3), 27.3 (CH, C11'), 27.7 (CH_2, C4), 27.9 (CH, C7'), 29.3 (CH_2, C13'), 30.0 (CH_2), 30.1 (CH_2), 30.2 (CH_2), 31.2 (CH_2) (C5, C6, C7, C8), 36.0 (CH_2, C6'), 36.1 (CH_2, C10'), 37.0 (CH_2, C2), 43.9 (CH_2, CONH*C*H_2), 49.7 (CH_2, C9), 56.4 (CH_3, 3''-OCH_3), 112.5 (CH, C2''), 115.6 (C, C12a'), 116.1 (CH, C5''), 117.6 (C, C11a'), 119.1 (CH, C4'), 121.4 (CH, C6''), 125.1 (CH, C8'), 126.7 (CH, C2'), 129.5 (CH, C1'), 131.6 (C, C1''), 134.6 (C, C9'), 140.3 (C, C3'), 141.0 (C, C4a'), 146.8 (C, C4''), 149.0 (C, C3''), 151.2 (C, C5a'), 156.9 (C, C12'), 175.9 (C, C1).

HRMS, ESI:
Calculated for ($C_{34}H_{42}{}^{35}ClN_3O_3$ + H^+): 576.2987.
Observed: 576.2995.

Elemental analysis: Calculated for $C_{34}H_{42}ClN_3O_3 \cdot 1.1HCl \cdot 0.5H_2O$:
C: 65.31%; H: 7.11%; N: 6.72%; Cl: 11.91%.
Observed: C: 65.16%; H: 7.32%; N: 6.38%; Cl: 12.31%.

Preparation of (±)-4-{[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]methyl}-*N*-(4-hydroxy-3-methoxybenzyl)-benzamide, (±)-97h.



In a 50 mL round-bottomed flask equipped with magnetic stirrer and a condenser, (±)-**89h** (870 g, 2.18 mmol) was dissolved in MeOH (3 mL) and treated with a 40% sol. of KOH in MeOH (6 mL). The resulting suspension was stirred under reflux for 3 h, treated with H_2O (8 mL) and again stirred under reflux overnight. The reaction mixture was cooled down to r. t., evaporated under reduced pressure, treated with HCl / Et_2O (0.5 N, 81.6 mL) and again concentrated under reduced pressure to give a white solid (3.50 g), whose ^{1}H NMR spectrum was consistent with that expected for the desired acid, (±)-**98h**·HCl, and was used as a crude in the next step.

In a triple neck 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**98h**·HCl (3.50 g of a crude that could contain a maximum of 2.08 mmol of the desired acid) was suspended in anhydrous CH_2Cl_2 (27 mL), cooled to 0 ℃ with an ice bath and treated dropwise with freshly distilled Et_3N (1.41 mL, 1.03 g, 10.2 mmol) and $ClCO_2Et$ (0.20 mL, 225 mg, 2.08 mmol). The resulting suspension was stirred at 0 ℃ for 30 min and treated with amine **99**·HCl (394 mg, 2.08 mmol). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (60 mL) and extracted with CH_2Cl_2 (2 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow solid (1.12 g), which was purified through column chromatography (silica gel, 40–60 μm, 40 g, Ø = 3 cm; #1–9, 300 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #10–50, 1500 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2), to provide the hybrid (±)-**97h**·HCl (#31–44, 380 mg, 33% overall yield).

R_f = 0.24 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-97h·HCl.

In a 25 mL round-bottomed flask, (±)-**97h** (380 mg, 0.69 mmol) was dissolved in CH_2Cl_2 (12 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (1.45 N, 1.42 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**97h**·HCl as a yellow solid (382 mg).

Melting point: 209–210 ºC (CH_2Cl_2 / MeOH 89:11).

IR (KBr) v: 3500–2500 (max. at 3229, 3105, 3052, 3002, 2925, 2852, 2790, OH, NH, N^+H and CH st), 1635, 1602, 1583, 1560, 1508, 1501, 1458 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

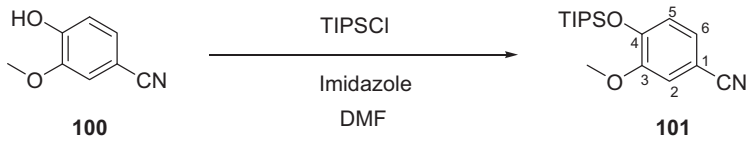
^1H NMR (400 MHz, CD_3OD) δ: 1.60 (s, 3H, 9'-CH_3), 1.98 (broad d, *J* = 17.6 Hz, 1H, 10'-H_{endo}), partially overlapped 2.00 (dm, *J* = 12.8 Hz, 1H, 13'-H_{syn}), 2.09 (dm, *J* = 12.8 Hz, 1H, 13'-H_{anti}), 2.55 (broad dd, *J* ≈ 17.6 Hz, *J'* = 4.4 Hz, 1H, 10'-H_{exo}), 2.78 (m, 1H, 7'-H), 2.91 (broad d, *J* = 17.6 Hz, 1H, 6'-H_{endo}), 3.24 (dd, *J* = 17.6 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.51 (m, 1H, 11'-H), 3.82 (s, 3H, 3''-OCH_3), 4.47 (d, *J* = 4.0 Hz, 2H, CONHCH_2), 4.85 (s, NH and $^+$NH), 5.24 (s, 2H, 4-CH_2NH), 5.59 (broad d, *J* = 4.8 Hz, 1H, 8'-H), 6.72 (d, *J* = 8.4 Hz, 1H, 5''-H), 6.78 (dd, *J* = 8.4 Hz, *J'* = 2.0 Hz, 1H, 6''-H), 6.93 (d, *J* = 2.0 Hz, 1H, 2''-H), 7.35 (dd, *J* = 8.8 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.52 [d, *J* ≈ 8.4 Hz, 2H, 3(5)-H], 7.76 (d, *J* = 2.0 Hz, 1H, 4'-H), 7.90 [d, *J* ≈ 8.4 Hz, 2H, 2(6)-H], 8.16 (d, *J* = 8.8 Hz, 1H, 1'-H), 8.89 (t, *J* ≈ 4.0 Hz, 1H, CONH).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 27.5 (CH, C11'), 27.9 (CH, C7'), 29.2 (CH_2, C13'), 36.1 (CH_2, C6'), 36.3 (CH_2, C10'), 44.4 (CH_2, CONH*C*H_2), 52.0 (CH_2, C4-CH_2NH), 56.3 (CH_3, 3''-OCH_3), 112.6 (CH, C2''), 115.6 (C, C12a'), 116.1 (CH, C5''), 118.4 (C, C11a'), 119.3 (CH, C4'), 121.4 (CH, C6''), 125.1 (CH, C8'), 126.8 (CH, C2'), 127.8 (CH), 129.1 (CH) [C2(6), C3(5)], 129.2 (CH, C1'), 131.6 (C, C1''), 134.6 (C, C9'), 135.4 (C, C1), 140.3 (C, C3'), 140.8 (C, C4a'), 142.5 (C, C4), 146.8 (C, C4''), 149.0 (C, C3''), 151.9 (C, C5a'), 157.4 (C, C12'), 169.4 (C, CONH).

HRMS, ESI:
Calculated for ($C_{33}H_{32}{}^{35}ClN_3O_3 + H^+$): 554.2205.
Observed: 554.2205.

Elemental analysis: Calculated for $C_{33}H_{32}ClN_3O_3 \cdot 1.2HCl \cdot 1.25H_2O$:
C: 63.89%; H: 5.80%; N: 6.77%; Cl: 12.57%.
Observed: C: 63.87%; H: 5.76%; N: 6.64%; Cl: 12.67%.

Preparation of 3-methoxy-4-(triisopropylsilyloxy)benzonitrile, 101.



100 **101**

In a triple neck 100 mL round-bottomed flask equipped an inert atmosphere and magnetic stirrer, 4-hydroxy-3-methoxybenzonitrile, **100** (3.00 g, 20.1 mmol) and imidazole (4.10 g, 60.2 mmol) were dissolved in anhydrous DMF (36 mL). TIPSCl (5.56 mL, 5.05 g, 26.2 mmol) was then added dropwise during 10 min. The reaction mixture was stirred at r. t. overnight, then concentrated under reduced pressure and the residue purified through column chromatography (silica gel, 40–60 μm, 105 g, ∅ = 3 cm; #1–23, 3.5 L, hexane), to provide **101** (#2–23, 6.25 g, quantitative yield) as a colorless oil.

R_f = 0.49 (silica gel, 10 cm, hexane / EtOAc 1:1).

Analytical sample of 101.

In a 25 mL round-bottomed flask, **101** (100 mg, 0.33 mmol) was dissolved in CH_2Cl_2 (12 mL), filtered with a PTFE filter (0.2 μm) and evaporated under reduced pressure to give, after drying under standard conditions, **101** as a colorless oil (98 mg).

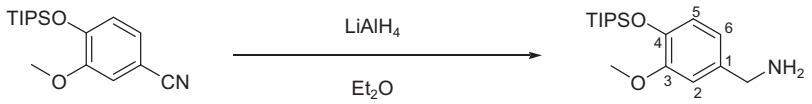
IR (neat) v: 2224 (CN st), 1595, 1571, 1545, 1513 (Ar–C–C st) cm^{-1}.

^{1}H NMR (400 MHz, CDCl) δ: 1.08 [d, *J* = 7.2 Hz, 18H, Si[CH(C*H*$_3$)$_2$]$_3$], 1.22−1.29 [m, 3H, Si[C*H*(CH$_3$)$_2$]$_3$], 3.82 (s, 3H, 3-OCH$_3$), 6.89 (d, *J* = 8.0 Hz, 1H, 5-H), 7.06 (d, *J* = 2.0 Hz, 1H, 2-H), 7.16 (dd, *J* = 8.0 Hz, *J'* = 2.0 Hz, 1H, 6-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 12.9 [CH, Si[*C*H(CH$_3$)$_2$]$_3$], 17.8 [CH$_3$, Si[CH(*C*H$_3$)$_2$]$_3$], 55.6 (CH$_3$, 3-OCH$_3$), 104.3 (C, C1), 115.0 (CH, C2), 119.4 (C, CN), 120.8 (CH, C5), 126.1 (CH, C6), 150.1 (C, C4), 151.2 (C, C3).

HRMS, ESI:
Calculated for ($C_{17}H_{27}NO_2Si$ + H^+): 306.1884.
Observed: 306.1883.

Preparation of 3-methoxy-4-(triisopropylsilyloxy)benzylamine, 102.



101 **102**

In a triple neck 250 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, $LiAlH_4$ (373 mg, 9.84 mmol) was added in portions to anhydrous Et_2O (576 mL), then a solution of **101** (2.00 g, 6.56 mmol) in anhydrous Et_2O (144 mL) was added. The reaction mixture was stirred at r. t. for 2 h, treated with wet Et_2O (800 mL) and evaporated under reduced pressure. The residue was suspended in CH_2Cl_2, filtered through Celite® and evaporated under reduced pressure to give the desired amine **102** (1.90 g, 94% yield).

R_f = 0.26 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 102.

In a 25 mL round-bottomed flask, **102** (100 mg, 0.32 mmol) was dissolved in CH_2Cl_2 (13 mL), filtered with a PTFE filter (0.2 µm), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **102** as a colorless oil (97 mg).

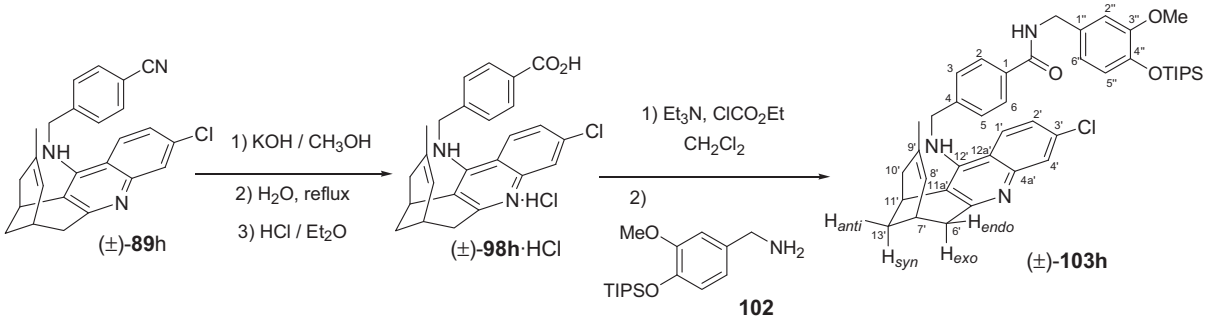
IR (neat) v: 3429, 3241 (NH st), 1585, 1514 (Ar–C–C st) cm^{-1}.

^1H NMR (400 MHz, CDCl) δ: 1.08 [d, *J* = 7.2 Hz, 18H, $Si[CH(CH_3)_2]_3$], 1.24 [m, 3H, $Si[CH(CH_3)_2]_3$], 1.64 (broad s, 2H, NH_2) partially overlapped 3.79 (d, *J* = 9.2 Hz, 2H, CH_2NH_2), partially overlapped 3.80 (s, 3H, 3-OCH_3), 6.71 (dd, *J* = 8.0 Hz, *J'* ≈ 1.6 Hz, 1H, 6-H), partially overlapped 6.80 (d, *J* = 1.6 Hz, 1H, 2-H), 6.82 (d, *J* = 8.0 Hz, 1H, 5-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 12.0 [CH, $Si[CH(CH_3)_2]_3$], 17.9 [CH_3, $Si[CH(CH_3)_2]_3$], 46.3 (CH_2, CH_2NH_2), 55.4 (CH_3, 3-OCH_3), 111.2 (CH, C2), 119.0 (CH, C6), 120.2 (CH, C5), 136.4 (C, C1), 144.3 (C, C4), 150.8 (C, C3).

HRMS, ESI:
Calculated for $M^+ - NH_2$ ($C_{17}H_{29}O_2Si + H^+$): 293.1930.
Observed: 293.1942.

Preparation of (±)-4-{[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-12-yl)amino]methyl}-*N*-[3-methoxy-4-(triisopropylsilyloxy)benzyl]benzamide, (±)-103h.



In a 50 mL round-bottomed flask equipped with magnetic stirrer and a condenser, (±)-**89h** (658 mg, 1.65 mmol) was dissolved in MeOH (2.5 mL) and then treated with a 40% sol. of KOH in MeOH (6.1 mL). The resulting suspension was stirred under reflux for 3 h, treated with H_2O (8 mL) and again stirred under reflux overnight. The reaction mixture was cooled down to r. t., evaporated under reduced pressure, treated with HCl / Et_2O (0.5 N, 50 mL) and again concentrated under reduced pressure to give a white solid (3.4 g), whose ^{1}H NMR spectrum was consistent with that expected for (±)-**98h**·HCl, and was used as a crude in the next step.

In a double neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**98h**·HCl (3.3 g of a crude that could contain a maximum of 1.60 mmol of the desired acid) was suspended in anhydrous CH_2Cl_2 (20 mL) cooled to 0 ℃ with an ice bath and treate d dropwise with freshly distilled Et_3N (1.11 mL, 808 mg, 8.00 mmol) and $ClCO_2Et$ (0.15 mL, 174 mg, 1.60 mmol) dropwise. The resulting mixture was stirred at 0 ℃ for 30 min and treated with a solution of **102** (494 mg, 1.60 mmol) in anhydrous CH_2Cl_2 (20 mL). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (70 mL) and extracted with CH_2Cl_2 (2 x 50 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow solid (1.39 g), which was purified through column chromatography (silica gel, 40–60 μm, 40 g, Ø = 3 cm; #1–24, 800 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #25–56, 800 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2), to provide the hybrid (±)-**103h** (#38–44, 919 mg, 81% overall yield).

R_f = 0.36 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-103h.

In a 25 mL round-bottomed flask, (±)-**103h** (80 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (2 mL), filtered with a PTFE filter (0.2 μm), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**103h** as a colorless oil (78 mg).

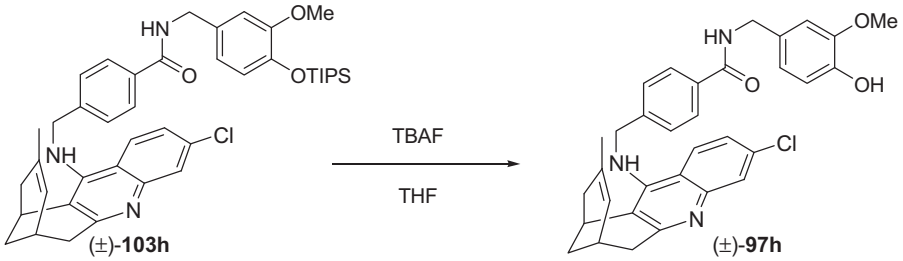
IR (KBr) ν: 3049 (NH st), 1726, 1710, 1640, 1607, 1583, 1553, 1514 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.08 [d, *J* = 7.2 Hz, 18H, Si[CH(C*H*$_3$)$_2$]$_3$], 1.24 [m, 3H, Si[C*H*(CH$_3$)$_2$]$_3$], 1.49 (s, 3H, 9'-CH_3), 1.73 (broad d, *J* = 16.8 Hz, 1H 10'-H$_{endo}$) 1.82 (dm, *J* = 12.0 Hz, 1H, 13'-H$_{syn}$), 1.97 (dm, *J* ≈ 12.0 Hz, 1H, 13'-H$_{anti}$), 2.45 (dd, *J* = 16.8 Hz, *J'* = 4.0 Hz, 1H, 10'-H$_{exo}$), 2.71 (m, 1H, 7'-H), 3.06−3.20 (complex signal, 3H, 6'-H$_{endo}$, 6'-H$_{exo}$ and 11'-H), 3.79 (s, 3H, 3''-OCH_3), 4.55 (d, *J* = 9.2 Hz, 2H, CONHC*H*$_2$), 4.76 (broad s, 2H, 4-C*H*$_2$NH), 5.51 (broad d, *J* = 5.6 Hz, 1H, 8'-H), 6.45 (s, 1H, CONH), 6.78 (dd, *J* = 8.0 Hz, *J'* = 2.0 Hz, 1H, 6''-H), partially overlapped 6.83 (d, *J* = 8.0 Hz, 1H, 5''-H), partially overlapped 6.85 (d, *J* = 2.0 Hz, 1H, 5''-H), 7.24 (dd, *J* = 8.8 Hz, *J'* = 2.0 Hz, 1H, 2'-H), 7.42 [d, *J* = 8.0 Hz, 2H, 3(5)-H], 7.80 [d, J = 8.0 Hz, 2H, 2(6)-H], 7.91 (d, *J* = 8.8 Hz, 1H, 1'-H), 8.04 (broad s, 1H, 4'-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 13.0 [CH, Si[*C*H(CH$_3$)$_2$]$_3$], 18.0 [CH_3, Si[CH(*C*H$_3$)$_2$]$_3$], 23.5 (CH_3, 9'-CH_3), 27.7 (CH, C11'), 28.3 (CH, C7'), 29.1 (CH_2, C13'), 37.4 (CH_2, C10'), 40.1 (CH_2, C6'), 44.3 (CH_2, CONH*C*H$_2$), 54.0 (CH_2, 4-CH_2NH), 56.7 (CH_3, 3''-OCH_3), 112.3 (CH, C2''), 119.3 (C, C12a'), 120.4 (CH, C6''), 120.5 (CH, C5''), 122.7 (C, C11a'), 124.9 (CH, C2'), 125.1 (CH, C1'), 125.6 (CH, C8'), 127.7 (CH), 127.8 (CH) [C2(6), C3(5)], 128.0 (CH, C4'), 131.1 (C, C1''), 131.7 (C, C9'), 134.2 (C, C1), 134.3 (C, C3), 143.0 (C, C4), 145.3 (C, C4''), 148.7 (C, C4a'), 149.7 (C, C12'), 151.2 (C, C3''), 159.1 (C, C5a'), 166.8 (C, CONH).

HRMS, ESI:
Calculated for ($C_{42}H_{52}{}^{35}ClN_3O_3Si + H^+$): 710.3539.
Observed: 710.3549.

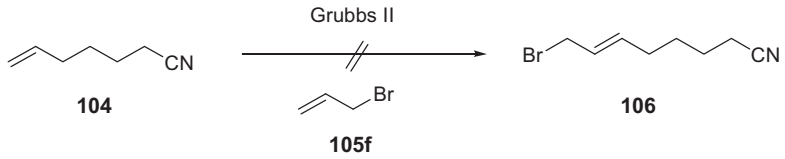
Preparation of hybrid huprine-capsaicin (±)-98h by deprotection of (±)-103h.



(±)-**103h** (±)-**97h**

In a 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, (±)-**103h** (520 mg, 0.73 mmol) was dissolved in anhydrous THF (15 mL) and treated dropwise with TBAF (1 M solution in THF, 1.10 mL, 1.10 mmol). The reaction mixture was stirred at r. t. for 4 h, diluted with 10% aq. Na_2CO_3 (70 mL) and extracted with CH_2Cl_2 (2 x 50 mL). The combined organic extracts were washed with H_2O (3 x 70 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the hybrid (±)-**97h** (380 mg, 94% yield).

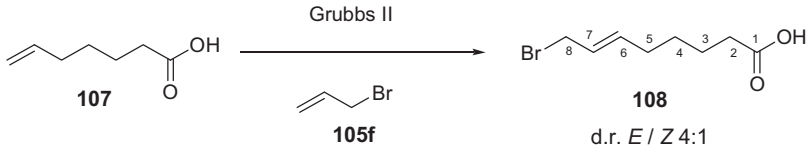
Attempted preparation of 8-bromo-6-octenenitrile, 106.



104 **105f** **106**

In a 5 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a condenser, **104** (172 mg, 1.58 mmol), allyl bromide (2.1 mL, 2.94 g, 24.3 mmol) and Grubbs II generation catalyst (136 mg, 0.16 mmol) were placed. The reaction mixture was stirred at 70 ºC overnight and purified through column chromatography (silica gel, 40–60 µm, 30 g, Ø = 3 cm; #1−6, 200 mL, hexane; #7–12, 200 mL, hexane / EtOAc 95:5), to provide only byproduct 1,4-dibromo-2-butene (#3–5, 122 mg).

Preparation of 8-bromo-6-octenoic acid, 108.



In a 5 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a condenser, 6-heptenoic acid, **107** (0.16 mL, 150 mg, 1.17 mmol), allyl bromide (1.5 mL, 2.10 g, 17.3 mmol) and Grubbs II generation catalyst (102 mg, 0.12 mmol) were placed. The reaction mixture was stirred at 70 ºC overnight and purified through column chromatography (silica gel, 40–60 µm, 30 g, Ø = 3 cm; #1−4, 150 mL, hexane; #5–14, 400 mL, hexane / EtOAc 90:10; #15–24, 400 mL, hexane / EtOAc 80:20), to provide 1,4-dibromo-2-butene (#7–9, 419 mg) and bromo acid **108** in a ratio *E* / *Z* 4:1 (^{1}H NMR) (#12–21, 248 mg, 96% yield).

R_f = 0.21 (silica gel, 10 cm, hexane / EtOAc 1:1).

Analytical sample of 108.

In a 25 mL round-bottomed flask, **108** (40 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (8 mL), filtered with a PTFE filter (0.2 µm) and evaporated under reduced pressure to give, after drying under standard conditions, **108** (38 mg) as a colorless oil.

.

IR (KBr) v: 1702 (C=O st) cm^{-1}.

^{1}H and ^{13}C NMR of the major diastereoisomer (*E*)-108.

^{1}H NMR (400 MHz, $CDCl_3$) δ: overlapped 1.43 (tt, *J* ≈ *J'* ≈ 7.6 Hz, 2H, 4-H_2), overlapped 1.63 (tt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, 3-H_2), 2.08 (dt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, 5-H_2), overlapped 2.35 (t, *J* = 7.4 Hz, 2H, 2-H_2), 3.92 (d, *J* = 6.8 Hz, 2H, 8-H_2), partially overlapped 5.68 (dt, *J* = 15.2 Hz, *J'* = 6.0 Hz, 1H), 5.75 (dt, *J* = 15.2 Hz, *J'* = 6.0 Hz, 1H) (6-H, 7-H), 11.0 (broad s, 1H, CO_2H).

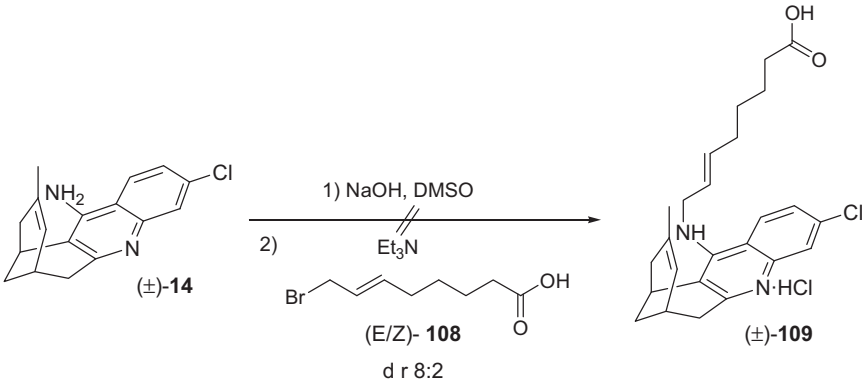
^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 24.0 (CH_2, C4), 28.1 (CH_2, C3), 31.5 (CH_2), 33.2 (CH_2), 33.8 (CH_2) (C2, C5, C8), 126.8 (CH, C6), 135.7 (CH, C7), 180.2 (C, C1).

Significant signals of ^{1}H and ^{13}C NMR of the minor diastereoisomer (*Z*)-108.

^{1}H NMR (400 MHz, $CDCl_3$) δ: overlapped 1.44 (tt, *J* ≈ *J'* ≈ 7.6 Hz, 2H, 4-H_2), 2.15 (ddt, *J* ≈ *J'* ≈ 7.2 Hz, *J''* = 1.6 Hz, 2H, 5-H_2), 3.97 (d, *J* = 8.4 Hz, 2H, 8-H_2), 5.57 (dt, *J* = 10.8 Hz, *J'* = 7.6 Hz, 1H), 5.97 (dt, *J* ≈ 10.8 Hz, = *J'* = 7.6 Hz, 1H) (6-H, 7-H)

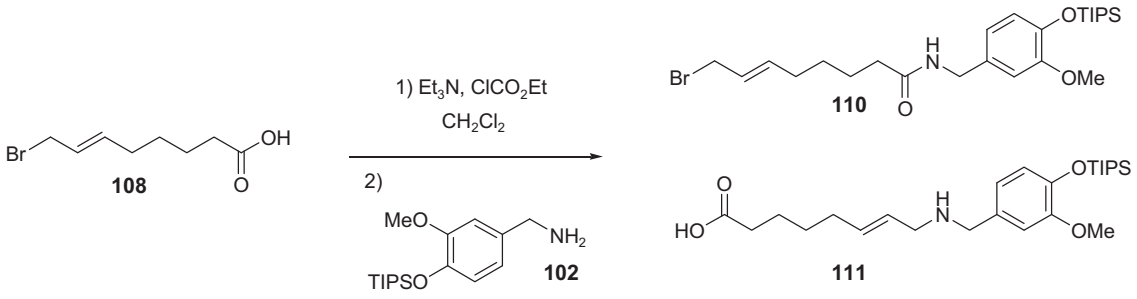
^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 24.2 (CH_2, C4), 26.6 (CH_2), 27.1 (CH_2) (C5, C8), 125.9 (CH, C6), 135.2 (CH, C7).

Attempted preparation of (±)-8-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta [*b*] quinolin-12-yl)amino]-6-octenoic acid, (±)-109.



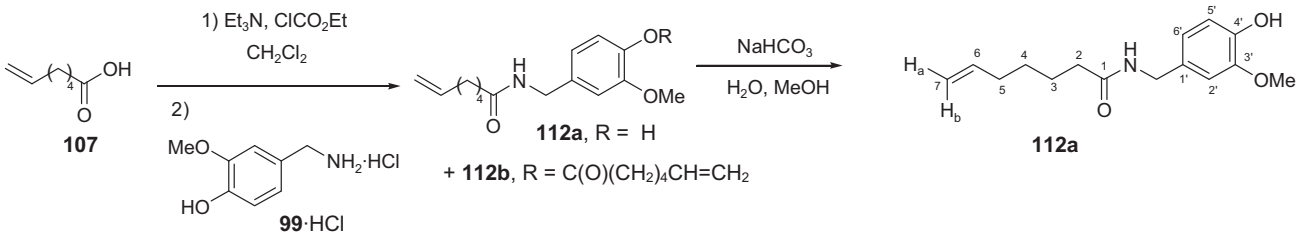
In a double neck 10 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (138 mg, 0.48 mmol) and NaOH (68 mg, 1.70 mmol) were suspended in anhydrous DMSO (4 mL). The suspension was stirred heating every 10 min approximately with a heat gun for 1 h and at r. t. for 1 h more, then treated dropwise with a solution of **108** (93 mg, 0.42 mmol) and freshly distilled Et_3N (0.06 mL, 44 mg, 0.43 mmol) in anhydrous DMSO (2 mL). TLC revealed the presence of starting material only.

Attempted preparation of 8-bromo-*N*-[3-methoxy-4-(triisopropylsilyloxy)benzyl]-6-octenamide, 110.



In a double neck 525 mL round-bottomed flask equipped with magnetic stirrer and an inert atmosphere, 8-bromo-6-heptenoic acid, **108** (773 mg, 3.51 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) and cooled to 0 ℃ with an ice bath, then t reated dropwise with freshly distilled Et_3N (1.10 mL, 798 mg, 7.90 mmol) and $ClCO_2Et$ (0.33 mL, 379 mg, 3.51 mmol). The resulting solution was stirred at 0 ℃ for 30 min and treated with amine **102** (1.08 g, 3.49 mmol). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (60 mL) and extracted with CH_2Cl_2 (3 x 35 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil that was purified through column chromatography (silica gel, 40–60 μm, 40 g, Ø = 3 cm; #1–17, 600 mL, CH_2Cl_2; #18–46, 1000 mL, CH_2Cl_2 / MeOH 99.5:0.5; #47–59, 400 mL CH_2Cl_2 / MeOH 95:5; #60–66, 200 mL CH_2Cl_2 / MeOH 90:10; #67–79, 400 mL CH_2Cl_2 / MeOH 80:20), to provide only traces of **110** and mainly impure carboxylic acid **111** (#47–59, 684 mg).

Preparation of *N*-(4-hydroxy-3-methoxybenzyl)-6-heptenamide, 112a.



In a double neck 50 mL round-bottomed flask equipped with magnetic stirrer and an inert atmosphere, 6-heptenoic acid, **107** (0.71 mL, 675 mg, 5.25 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) and cooled to 0 ℃ with an ice bath, then t reated dropwise with freshly distilled Et_3N (1.61 mL, 1.17 g, 11.6 mmol) and $ClCO_2Et$ (0.50 mL, 567 mg, 5.25 mmol). The resulting solution was stirred at 0 ℃ for 30 min, and treated with amine **99**·HCl (1.00 g, 5.25 mmol). The reaction mixture was stirred at r. t. for 3 days, then diluted with 10% aq. Na_2CO_3 (60 mL) and extracted with CH_2Cl_2 (3 x 40 mL). The combined organic extracts were washed with H_2O (3 x 60 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a colorless oil that consisted of a mixture of **112a** / **112b** in an approximate ratio of 9:1 (^{1}H NMR) (1.53 g). This mixture was transferred to a 500 mL round-bottomed flask equipped with magnetic stirrer and dissolved in MeOH (108 mL). The resulting solution was treated with H_2O (59 mL) and saturated aq. $NaHCO_3$ (80 mL), stirred at r. t. overnight, concentrated under reduced pressure and extracted with CH_2Cl_2 (3 x 90 mL). The combined organic extracts were washed with H_2O (3 x 150 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to provide **112a** (1.08 g, 79% yield) as a white solid.

R_f = 0.65 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 112a.

In a 25 mL round-bottomed flask, **112a** (100 mg, 0.38 mmol) was dissolved in CH_2Cl_2 (13 mL), filtered with a PTFE filter (0.2 µm), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying at 2 Torr. and r. t., **112a** as a white solid (97 mg).

Melting point: 57−59 ºC (CH_2Cl_2).

IR (KBr) v: 3379, 3344, 3076 (OH and NH st), 1640, 1601, 1549, 1522 (C=O and Ar–C–C st) cm^{-1}.

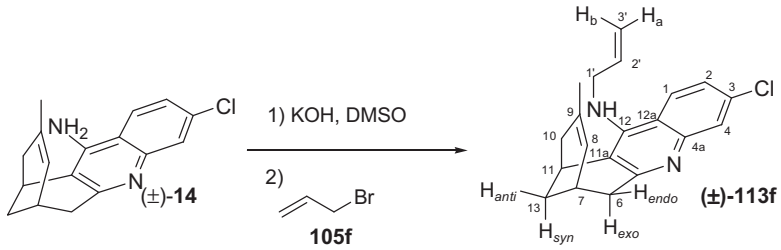
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (tt, $J \approx J' \approx 7.2$ Hz, 2H, 4-H_2), 1.66 (tt, $J \approx J' \approx 7.2$ Hz, 2H, 3-H_2), 2.05 (dtt, $J = J' = 7.2$ Hz, $J'' = 1.4$ Hz, 2H, 5-H_2), 2.19 (t, $J = 7.6$ Hz, 2H, 2-H_2), 3.85 (s, 3H, 3'-OCH_3), 4.33 (d, $J = 6.0$ Hz, 2H, CONHCH_2), 4.93 (ddt, J = 10.0 Hz, $J' = 2.0$ Hz, $J'' = 1.2$ Hz, 1H, 7-H_a), 4.98 (ddt, $J = 17.2$ Hz, $J' = 2.0$ Hz, $J'' = 1.6$ Hz, 1H, 7-H_b), 5.77 (ddt, $J = 17.2$ Hz, $J' = 10.0$ Hz, $J'' = 6.8$ Hz,

1H, 6-H), 5.79 (broad s, 1H, CONH), 6.74 (dd, *J* = 8.0 Hz, *J'* = 2.0 Hz, 1H, 6'-H), 6.79 (d, *J* = 1.6 Hz, 1H, 2'-H), 6.84 (d, *J* = 8.0 Hz, 1H, 5'-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 25.2 (CH_2, C3), 28.5 (CH_2, C4), 33.4 (CH_2, C5), 36.6 (CH_2, C2), 43.5 (CH_2, CONH*C*H_2), 55.9 (CH_3, 3'-OCH_3), 110.6 (CH, C2'), 114.3 (CH, C5'), 114.6 (CH_2, C7), 120.7 (CH, C6'), 130.3 (C, C1'), 138.4 (CH, C6), 145.1 (C, C4'), 146.7 (C, C3'), 172.7 (C, C1).

HRMS, ESI:
Calculated for ($C_{15}H_{21}NO_3 + H^+$): 264.1594.
Observed: 264.1592.

Preparation of (±)-3-chloro-6,7,10,11-tetrahydro-9-methyl-12-(2-propenylamino)-7,11-methanocycloocta[*b*]quinoline, (±)-113f.



In a double neck 10 mL round-bottomed flask equipped an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (500 mg, 1.76 mmol) and KOH (85% purity, 186 mg, 2.82 mmol) were suspended in anhydrous DMSO (6 mL). The suspension was stirred heating every 10 min approximately with a heat gun for 1 h and at r. t. for 1 h more, then treated dropwise with allyl bromide (0.15 mL, 213 mg, 1.76 mmol). The reaction mixture was stirred at r. t. overnight, then diluted with 2 N NaOH (15 mL) and extracted with EtOAc (3 x 35 mL). The combined organic extracts were washed with H_2O (3 x 25 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (654 mg), which was purified through column chromatography (silica gel, 40–60 μm, 33 g, Ø = 3 cm; #1–47, 1620 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #48–63, 500 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2; #64–79, 500 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2), to provide (±)-**113f** (#54–66, 314 mg, 55% yield).

R_f = 0.60 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-113f·HCl.

In a 25 mL round-bottomed flask, (±)-**113f** (52 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (1.4 N, 0.34 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**113f**·HCl as a yellow solid (53 mg).

Melting point: 228–230 ℃ (CH_2Cl_2 / MeOH 95:5).

IR (KBr) ν: 3500-2500 (max. at 3401, 3215, 3113, 3078, 3051, 3015, 2925, 2903, 2882, 2852, 2703, 2685, NH, N^+H and CH st), 1723, 1634, 1604, 1583, 1562, 1519 (Ar–C–C and Ar–C–N st) cm^{-1}.

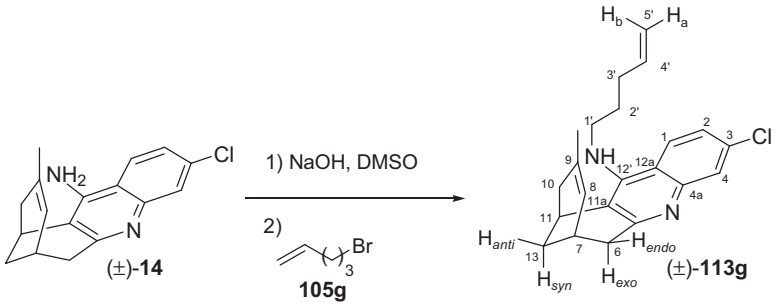
^1H NMR (400 MHz, CD_3OD) δ: 1.59 (s, 3H, 9-CH_3), partially overlapped 1.94 (broad d, J = 18.0 Hz, 1H, 10-H_{endo}), partially overlapped 1.96 (dm, J = 12.8 Hz, 1H, 13-H_{syn}), 2.09 (dm, J = 12.8 Hz, 1H, 13-H_{anti}), 2.55 (dd, J = 18.0 Hz, J' = 4.8 Hz, 1H, 10-H_{exo}), 2.79 (m, 1H, 7-H), 2.88 (dm, J = 17.6 Hz, 1H, 6-H_{endo}), 3.22 (dd, $J \approx$ 17.6 Hz, $J' \approx$ 5.2 Hz, 1H, 6-H_{exo}), 3.45 (m, 1H, 11-H), 4.58 (m, 2H, 1'-H_2), 4.85 (s, NH and $^+$NH), 5.39 (dtd, J = 10.4 Hz, J' = 2.0 Hz, J'' = 1.2 Hz, 1H, 3'-H_a), 5.41 (dtd, J = 17.2 Hz, J' =

2.0 Hz, *J''* = 1.2 Hz, 1H, 3'-H_b), 5.59 (broad d, *J* = 4.4 Hz, 1H, 8-H), 6.19 (ddt, *J* = 17.2 Hz, *J'* = 10.4 Hz, *J''* ≈ 4.4 Hz, 1H, 2'-H), 7.51 (dd, *J* = 9.6 Hz, *J'* ≈ 2.0 Hz, 1H, 2-H), 7.76 (d, *J* = 2.0 Hz, 1H, 4-H), 8.36 (d, *J* = 9.6 Hz, 1H, 1-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.4 (CH_3, 9-CH_3), 27.4 (CH, C11), 27.9 (CH, C7), 29.3 (CH_2, C13), 36.1 (CH_2, C6), 36.2 (CH_2, C10), 50.9 (CH_2, C1'), 115.5 (C, C12a), 117.8 (CH_2, C3'), 117.9 (C, C11a), 119.1 (CH, C4), 125.1 (CH, C8), 126.7 (CH, C2), 129.5 (CH, C1), 134.7 (C, C9), 135.1 (CH, C2'), 140.3 (C, C3), 140.9 (C, C4a), 151.5 (C, C5a), 157.4 (C, C12).

HRMS, ESI:
Calculated for ($C_{20}H_{21}N_2 + H^+$): 325.1466.
Observed: 325.1466.

Preparation of (±)-3-chloro-6,7,10,11-tetrahydro-9-methyl-12-(4-pentenylamino)-7,11-methanocycloocta[*b*]quinoline, (±)-113g.



In a double neck 50 mL round-bottomed flask equipped an inert atmosphere, magnetic stirrer and 4 Å molecular sieves, (±)-huprine Y, (±)-**14** (2.00 g, 7.04 mmol) and NaOH (563 mg, 14.1 mmol) were suspended in anhydrous DMSO (20 mL). The suspension was stirred heating every 10 min approximately with a heat gun for 1 h and at r. t. for 1 h more, then treated dropwise with 5-bromo-1-pentene (0.92 mL, 1.15 g, 7.74 mmol). The reaction mixture was stirred at r. t. overnight, then diluted with 2 N NaOH (30 mL) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with H_2O (3 x 50 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (1.97 g), which was purified through column chromatography (silica gel, 40–60 μm, 90 g, Ø = 5 cm; #1–64, 6800 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2), to provide (±)-**113g** (#22–51, 680 mg, 27% yield).

R_f = 0.63 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-113g·HCl.

In a 25 mL round-bottomed flask, (±)-**113g** (106 mg, 0.30 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.75 N, 1.2 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**113g**·HCl as a yellow solid (110 mg).

Melting point: 128–129 ℃ (CH_2Cl_2 / MeOH 89:11).

IR (KBr) ν: 3500-2500 (max. at 3226, 3111, 3049, 3004, 2925, 2854, 2717, NH, N^+H and CH st), 1717, 1699, 1684, 1669, 1629, 1582, 1569, 1560 (Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR (400 MHz, CD_3OD) δ: 1.57 (s, 3H, 9-CH_3), 1.91–2.02 (complex signal, 4H, 13-H_{syn}, 10-H_{endo} and 2'-H_2), 2.08 (dm, J = 12.8 Hz, 1H, 13-H_{anti}), 2.10 (dt, $J \approx J' \approx$ 7.2 Hz, 3'-H_2), 2.56 (dd, J = 17.6 Hz, J' = 4.8 Hz, 1H, 10-H_{exo}), 2.76 (m, 1H, 7-H), 2.89 (d, J = 17.6 Hz, 1H, 6-H_{endo}), 3.21 (dd, J = 17.6 Hz, J' = 5.2 Hz, 1H, 6-H_{exo}), 3.48 (m, 1H, 11-H), 3.99 (dt, $J \approx J' \approx$ 6.8 Hz, 2H, 1'-H_2), 4.86 (s, NH and $^+$NH), 5.00 (ddt, J = 10.4 Hz, $J' \approx J'' \approx$ 1.6 Hz, 1H, 5'-H_a), 5.04 (ddt, J = 17.2 Hz, $J' \approx J'' \approx$ 1.6 Hz, 1H, 5'-H_b),

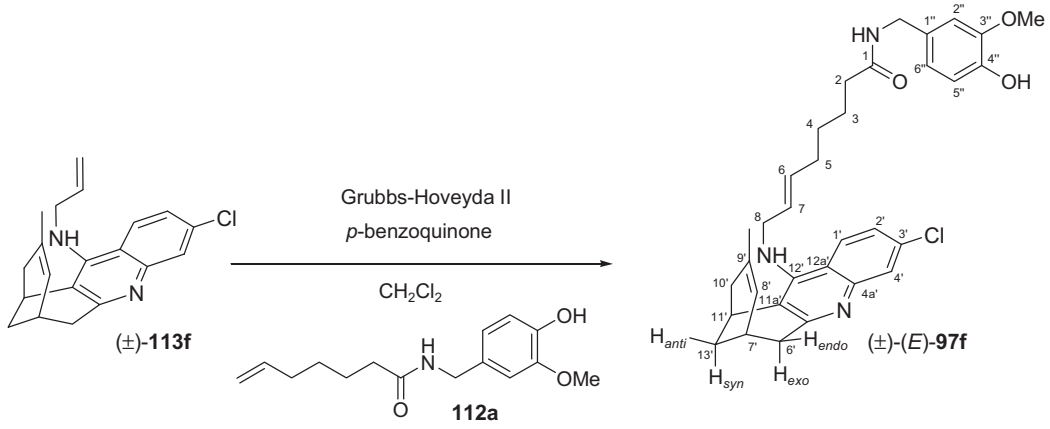
5.57 (broad d, *J* = 4.8 Hz, 1H, 8-H), 5.86 (ddt, *J* = 17.2 Hz, *J'* = 10.4 *J''* = 6.8 Hz, 1H, 4'-H), 7.52 (d, *J* ≈ 9.6 Hz, 1H, 2-H), 7.80 (s, 1H, 4-H), 8.36 (d, *J* = 9.6 Hz, 1H, 1-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9-CH_3), 27.2 (CH, C11), 27.8 (CH, C7), 29.3 (CH_2, C13), 30.5 (CH_2), 31.9 (CH_2) (C2', C3'), 36.0 (CH_2, C6), 36.2 (CH_2, C10), 50.9 (CH_2, C1'), 115.6 (C, C12a), 116.3 (CH_2, C5'), 117.9 (C, C11a), 119.1 (CH, C4), 125.1 (CH, C8), 126.6 (CH, C2), 129.4 (CH, C1), 134.5 (C, C9), 138.4 (CH, C4'), 140.1 (C, C3), 140.9 (C, C4a), 151.3 (C, C5a), 156.9 (C, C12).

HRMS, ESI:
Calculated for ($C_{22}H_{25}N_2 + H^+$): 353.1779.
Observed: 353.1777.

Preparation of (±)-(*E*)-8-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]-*N*-(4-hydroxy-3-methoxybenzyl)-6-octenamide, (±)-(*E*)-97f.

a) With Grubbs-Hoveyda II generation catalyst in anhydrous CH_2Cl_2



In a 5 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a condenser, (±)-**113f** (80 mg, 0.25 mmol) was dissolved in anhydrous CH_2Cl_2 (1.5 mL), treated with olefin **112a** (118 mg, 0.45 mmol), *p*-benzoquinone (3.00 mg, 0.03 mmol) and Grubbs-Hoveyda II generation catalyst (8 mg, 0.01 mmol). The reaction mixture was stirred under reflux for 3 days and purified through column chromatography (silica gel, 40–60 µm, 8 g, Ø = 1.5 cm; #1−9, 300 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #10–26, 600 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2), to provide the hybrid (±)-(*E*)-**97f** (#14–18, 12 mg, 9% yield).

R_f = 0.42 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-(*E*)-97f·HCl.

In a 25 mL round-bottomed flask, (±)-(*E*)-**97f** (64 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 0.46 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-(*E*)-**97f**·HCl (65 mg) as a yellow solid.

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Melting point: 135–136 ℃ (CH_2Cl_2 / MeOH 93:7).

IR (KBr) ν: 3500-2500 (max. at 3234, 3105, 3056, 3007, 2927, 2863, 2790, OH, NH, N^+H and CH st), 1633, 1599, 1583, 1566, 1514 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR (400 MHz, CD_3OD) δ: 1.41 (tt, $J \approx J' \approx 7.4$ Hz, 2H, 4-H_2), 1.58 (s, 3H, 9'-CH_3), partially overlapped 1.58−1.67 (tt, $J \approx J' \approx 7.2$ Hz, 2H, 3-H_2), 1.92 (broad d, J = 18.4 Hz, 1H, 10'-H_{endo}), overlapped 1.90–1.95 (m, 1H, 13'-H_{syn}), 2.07 (dm, J = 12.4 Hz, 1H, 13'-H_{anti}), partially overlapped 2.14 (dt, $J \approx J' \approx 6.6$ Hz, 2H, 5-H_2), 2.20 (t, J = 7.4 Hz, 2H, 2-H_2), 2.45 (dd, $J \approx 18.4$ Hz, J' = 6.0 Hz, 1H, 10'-

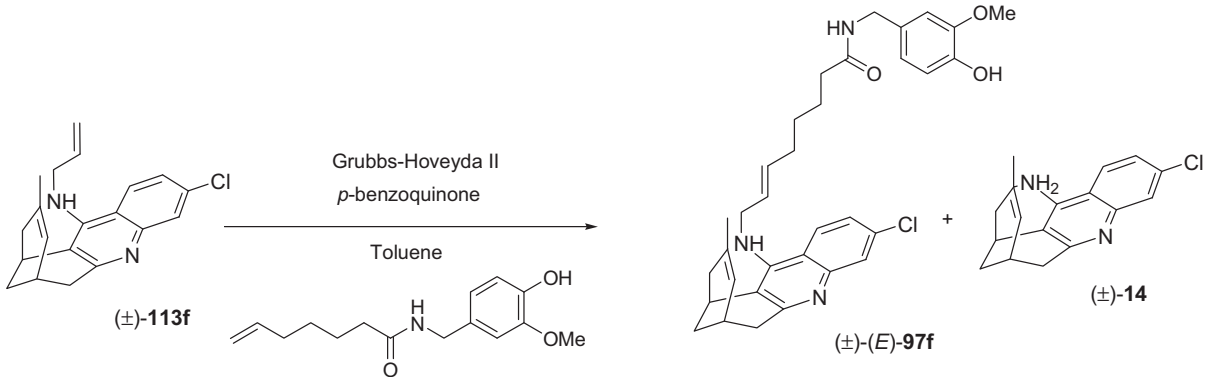
H_{exo}), 2.77 (m, 1H, 7'-H), 2.87 (broad d, *J* = 17.6 Hz, 1H, 6'-H_{endo}), 3.21 (dd, *J* = 17.6 Hz, *J'* = 5.6 Hz, 1H, 6'-H_{exo}), 3.44 (m, 1H, 11'-H), 3.80 (s, 3H, 3''-OCH_3), 4.24 (s, 2H, CONHCH_2), 4.51 (broad d, *J* = 3.2 Hz, 2H, 8-H_2), 4.85 (s, NH, N^+H and OH), 5.58 (broad d, *J* = 4.8 Hz, 1H, 8'-H), 5.73 (dt, *J* = 15.6 Hz, *J'* ≈ 4.0 Hz, 1H, 7-H), 5.80 (dt, *J* = 15.6 Hz, *J'* ≈ 6.4 Hz, 1H, 6-H), 6.68 (complex signal, 2H, 5''-H, 6''-H), 6.84 (s, 1H, 2''-H), 7.50 (dd, *J* = 9.2 Hz, *J'* = 2.4 Hz, 1H, 2'-H), 7.77 (d, *J* = 2.4 Hz, 1H, 4'-H), 8.38 (d, *J* = 9.2 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 26.3 (CH_2, C3), 27.3 (CH, C11'), 27.9 (CH, C7'), 29.3 (CH_2, C13'), 29.6 (CH_2, C4), 32.9 (CH_2, C5), 36.0 (CH_2, C6'), 36.2 (CH_2, C10'), 36.7 (CH_2, C2), 43.9 (CH_2, CONH*C*H_2), 50.4 (CH_2, C8), 56.3 (CH_3, 3''-OCH_3), 112.5 (CH, C2''), 115.5 (C, C12a'), 116.1 (CH, C5''), 117.8 (C, C11a'), 119.1 (CH, C4'), 121.3 (CH, C6''), 125.1 (CH, C8'), 126.6 (CH, C2'), 126.8 (CH, C6), 129.7 (CH, C1'), 131.5 (C, C1''), 134.6 (C, C9'), 135.1 (CH, C7), 140.3 (C, C3'), 140.9 (C, C4a'), 146.8 (C, C4''), 148.9 (C, C3''), 151.4 (C, C5a'), 157.2 (C, C12'), 175.7 (C, C1).

HRMS, ESI:
Calculated for ($C_{33}H_{38}{}^{35}ClN_3O_3$ + H^+): 560.2674.
Observed: 560.2671.

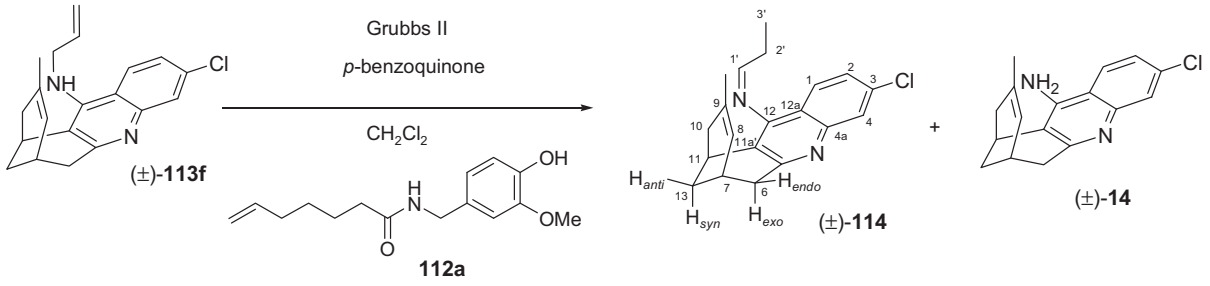
Elemental analysis: Calculated for $C_{33}H_{38}ClN_3O_3 \cdot HCl \cdot 0.75H_2O$:
C: 62.65%; H: 6.85%; N: 6.64%.
Observed: C: 62.75%; H: 6.35%; N: 6.81%.

b) In anhydrous toluene



In a 5 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a condenser, (±)-**113f** (73 mg, 0.22 mmol) was dissolved in anhydrous toluene (2 mL), treated with olefin **112a** (106 mg, 0.40 mmol), *p*-benzoquinone (2.04 mg, 0.02 mmol) and Grubbs-Hoveyda II generation catalyst (6 mg, 0.01 mmol). The reaction mixture was stirred at 70 ºC for 3 days and purified through column chromatography (silica gel, 40–60 µm, 10 g, Ø = 1.5 cm; #1−9, 290 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #10–15, 600 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2), to provide an inseparable mixture of (±)-(*E*)-**97f** / (±)-**14** in an approximate ratio 72:28 (^{1}HNMR) [#14–18, 18 mg, 10% yield of (±)-(*E*)-**97f**].

c) With Grubbs II generation catalyst in anhydrous CH_2Cl_2.



In a 5 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a condenser, (±)-**113f** (100 mg, 0.31 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL), treated with olefin **112a** (147 mg, 0.55 mmol), *p*-benzoquinone (3.30 mg, 0.03 mmol) and Grubbs II generation catalyst (13 mg, 0.01 mmol). The reaction mixture was stirred under reflux overnight and purified through column chromatography (silica gel, 40–60 µm, 10 g, Ø = 1.5 cm; #1−3, 50 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #4–18, 600 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2), to provide imine (±)-**114** (#3–5, 51 mg, 51% yield), starting **112a** (#7–8, 130 mg) and (±)-huprine Y, (±)-**14** (#9–14, 21 mg, 23% yield).

R_f = 0.83 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-114.

In a 25 mL round-bottomed flask, (±)-**114** (51 mg, 0.15 mmol) was dissolved in CH_2Cl_2 (8 mL), filtered with a PTFE filter (0.2 µm), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-**114** (48 mg) as a colorless oil.

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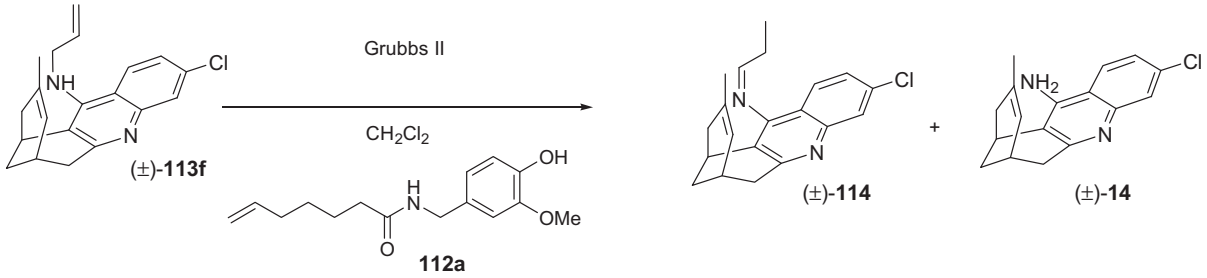
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (m, 3H, 3'-H_3), 1.50 (s, 3H, 9-CH_3), 1.79 (d, *J* = 16.8 Hz, 1H, 10-H_{endo}), 1.90 (d, *J* = 13.6 Hz, 1H, 13-H_{syn}), 1.95 (dm, *J* = 12.4 Hz, 1H, 13-H_{anti}), 2.37 (d, *J* = 16.0 Hz, 1H, 10-H_{exo}), partially overlapped 2.68 (m, 2H, 2'-H_2), partially overlapped 2.74 (m, 1H, 7-H), 3.05 (ddd, *J* = 17.6 Hz, *J'* ≈ *J''* ≈ 2.0 Hz, 1H, 6-H_{endo}), 3.16 (dd, *J* = 17.6 Hz, *J'* = 5.2 Hz, 1H, 6-H_{exo}), 3.29 (m, 1H, 11-H), 5.53 (broad d, *J* = 4.8 Hz, 1H, 8'-H), 7.29 (dd, *J* = 8.8 Hz, *J'* = 2.0 Hz, 1H, 2-H), 7.47 (d, *J* = 8.8 Hz, 1H, 1-H), 7.84 (s, 1H, 1'-H), 7.92 (d, *J* = 2.0 Hz, 1H, 4-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 9.70 (CH_3, C3'), 23.3 (CH_3, 9-CH_3), 27.8 (CH, C7), 28.4 (CH_2, C13), 28.7 (CH, C11), 29.6 (CH_2, C2'), 37.2 (CH_2, C6), 40.1 (CH_2, C10), 118.5 (C, C12a), 122.6 (C, C11a), 124.0 (CH, C1), 125.2 (CH, C8), 125.8 (CH, C2), 127.3 (CH, C4), 132.3 (C, C9), 134.4 (C, C3), 147.5 (C, C4a), 153.8 (C, C12), 159.7 (C, C5a), 170.3 (C, C1').

LRMS, ESI: 325.1490 ($C_{20}H_{21}{}^{35}ClN_2 + H^+$).

d) With Grubbs II generation catalyst, without p-benzoquinone in anhydrous CH_2Cl_2.

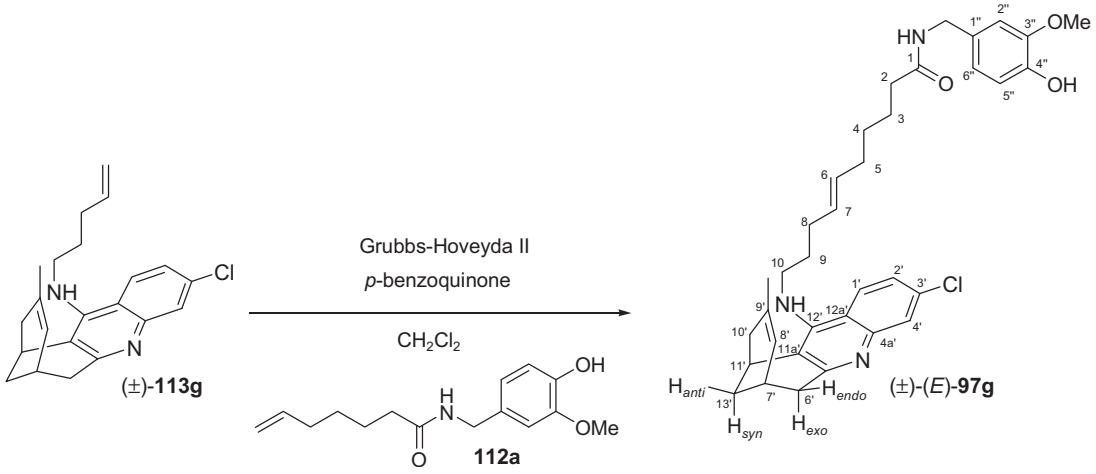


(±)-**113f** **112a** (±)-**114** (±)-**14**

In a 5 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a condenser, (±)-**113f** (100 mg, 0.31 mmol) was dissolved in anhydrous CH_2Cl_2 (1.5 mL) treated with olefin **112a** (147 mg, 0.55 mmol) and Grubbs II generation catalyst (13 mg, 0.01 mmol). The reaction mixture was stirred under reflux overnight and purified through column chromatography (silica gel, 40–60 µm, 25 g, Ø = 2 cm; #1−40, 50 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2) to provide imine (±)-**114** (#3–5, 42 mg, 42% yield), starting **112a** (#7–29, 127 mg) and (±)-huprine Y, (±)-**14** (#9–14, 50 mg, 56% yield).

Note: when the reaction was carried out with (±)-**113f** (400 mg, 1.23 mmol), **112a** (323 mg, 1.23 mmol) and Grubbs II generation catalyst (101 mg, 0.12 mmol), imine (±)-**114** and huprine (±)-**14** were obtained in 6% and 27% yield respectively.

Preparation of (±)-(*E*)-10-[(3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinolin-12-yl)amino]-*N*-(4-hydroxy-3-methoxybenzyl)--6-decenamide, (±)-(*E*)-97g.



In a 50 mL round-bottomed flask equipped with an inert atmosphere, magnetic stirrer and a condenser, (±)-**113g** (613 mg, 1.74 mmol) was dissolved in anhydrous CH_2Cl_2 (24 mL) treated with olefin **112a** (547 mg, 2.07 mmol), *p*-benzoquinone (17.3 mg, 0.17 mmol) and Grubbs-Hoveyda II generation catalyst (54 mg, 0.09 mmol). The reaction mixture was stirred under reflux for 3 days and purified through column chromatography (silica gel, 40–60 µm, 30 g, Ø = 2.5 cm; #1−40, 1300 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #41–77, 1000 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2), to provide the hybrid (±)-(*E*)-**97g** (#58–77, 73 mg, 7% yield).

R_f = 0.87 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of (±)-(*E*)-97g·HCl.

In a 25 mL round-bottomed flask, (±)-(*E*)-**97g** (73 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (8 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 0.49 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, (±)-(*E*)-**97g**·HCl (74 mg) as a yellow solid.

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Melting point: 129–130 ℃ (CH_2Cl_2 / MeOH 94:6).

IR (KBr) v: 3500-2500 (max. at 3253, 3061, 2997, 2926, 2857, 2795, 2651, OH, NH, N^+H and CH st), 1635, 1583, 1515 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

^1H NMR (400 MHz, CD_3OD) δ: 1.31 (tt, $J \approx J' \approx 7.6$ Hz, 2H, 4-H_2), 1.58 (s, 3H, 9'-CH_3), overlapped 1.53−1.65 (m, 2H, 3-H_2), 1.91–1.98 (complex signal, 6H, 13'-H_{syn}, 10'-H_{endo}, 8-H_2, 9-H_2), 2.05–2.15 (complex signal, 3H, 13'-H_{anti}, 5-H_2), 2.19 (t, J = 7.2 Hz, 2H, 2-H_2), 2.54 (d, J = 14.8 Hz, 1H, 10'-H_{exo}), 2.76 (m, 1H, 7'-H), 2.86 (d, J = 18.0 Hz, 1H, 6'-H_{endo}), 3.18 (d, $J \approx$ 18.0 Hz, 1H, 6'-H_{exo}), 3.44 (m, 1H,

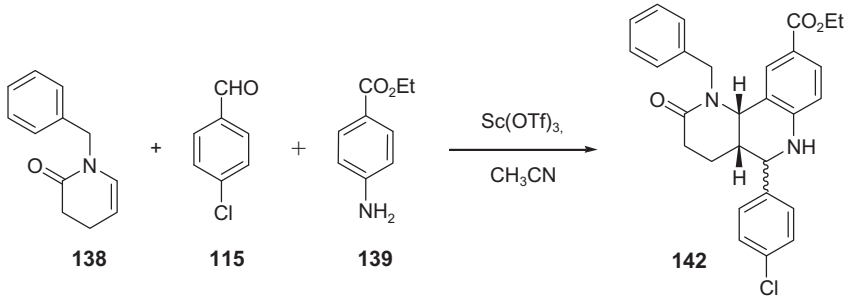
11'-H), 3.81 (s, 3H, 3''-OCH_3), 3.96 (m, 2H, 10-H_2), 4.23 (s, 2H, CONHCH_2), 4.85 (s, NH, N^+H and OH), 5.36 (dt, *J* = 15.2 Hz, *J'* = 6.0 Hz, 1H) and 5.42 (dt, *J* = 15.2 Hz, *J'* = 5.6 Hz, 1H) (6-H, 7-H), 5.57 (broad signal, 1H, 8'-H), 6.68 (complex signal, 2H, 5''-H, 6''-H), 6.83 (s, 1H, 2''-H), 7.52 (d, *J* = 8.8 Hz, 1H, 2'-H), 7.76 (s, 1H, 4'-H), 8.29 (broad s, 1H, CONH), 8.34 (d, *J* = 8.8 Hz, 1H, 1'-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 23.5 (CH_3, 9'-CH_3), 26.4 (CH_2, C3), 27.2 (CH, C11'), 27.8 (CH, C7'), 29.3 (CH_2, C13'), 30.0 (CH_2, C4), 30.6 (CH_2), 30.9 (CH_2), 33.1 (CH_2) (C5, C8, C9), 36.0 (CH_2, C6'), 36.2 (CH_2, C10'), 36.9 (CH_2, C2), 43.9 (CH_2, CONHCH_2), 49.1 (CH_2, C10), 56.3 (CH_3, 3''-OCH_3), 112.4 (CH, C2''), 115.6 (C, C12a'), 116.0 (CH, C5''), 117.6 (C, C11a'), 119.1 (CH, C4'), 121.3 (CH, C6''), 125.1 (CH, C8'), 126.6 (CH, C2'), 129.4 (CH, C1'), 129.9 (CH, C6), 131.6 (C, C1''), 132.8 (CH, C7), 134.5 (C, C9'), 140.2 (C, C3'), 140.9 (C, C4a'), 146.8 (C, C4''), 148.9 (C, C3''), 151.2 (C, C5a'), 157.2 (C, C12'), 175.7 (C, C1).

HRMS, ESI:
Calculated for ($C_{35}H_{42}{}^{35}ClN_3O_3 + H^+$): 588.2987.
Observed: 588.2969.

Elemental analysis: Calculated for $C_{35}H_{42}ClN_3O_3 \cdot HCl \cdot 0.5H_2O$:
C: 66.34%; H: 7.00%; N: 6.63%.
Observed: C: 66.18%; H: 7.06%; N: 6.33%.

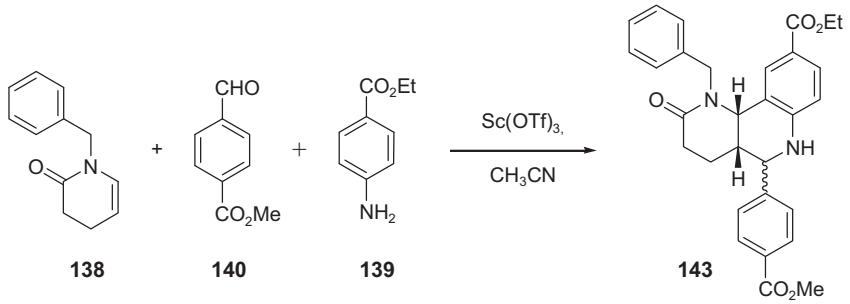
Preparation of ethyl 1-benzyl-5-(4-chlorophenyl)-1,2,3,4,4a,5,6,10b-octahydro-2-oxobenzo[*h*] [1,6]naphthyridine-9-carboxylate, 142 as a diastereomeric mixture.



138 **115** **139** **142**

In a triple neck 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of *p*-chlorobenzaldehyde, **115** (1.50 g, 10.7 mmol) and ethyl 4-aminobenzoate, **139** (1.76 g,10.7 mmol) in anhydrous CH_3CN (40 mL) was prepared, 4 Å molecular sieves and $Sc(OTf)_3$ (1.05 g, 2.13 mmol) were added and the mixture was stirred at r. t. under argon atmosphere for 5 min and then treated with a solution of the unsaturated lactam **138** (2.00 g, 10.7 mmol) in anhydrous CH_3CN (20 mL). The resulting suspension was stirred at r. t. under argon atmosphere for 3 days. Then, $Sc(OTf)_3$ (0.53 g, 1.07 mmol) was added and the reaction mixture was stirred at r. t. under argon atmosphere one more day, diluted with sat. aq. $NaHCO_3$ (35 mL) and extracted with EtOAc (3 x 45 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil (4.89 g) which was purified through column chromatography (silica gel, 35–70 μm, 140 g, Ø = 5 cm; #1–3, 300 mL, hexane; #4–7, 300 mL, hexane / EtOAc 90:10; #8–11, 300 mL, hexane / EtOAc 80:20; #12–15, 300 mL, hexane / EtOAc 70:30; #16–19, 300 mL, hexane / EtOAc 60:40; #20–23, 300 mL, hexane / EtOAc 50:50; #24-26, 300 mL, hexane / EtOAc 40:60; #27–29, 300 mL, hexane / EtOAc 30:70; #30-33, 300 mL, hexane / EtOAc 20:80; #34–36, 300 mL, hexane / EtOAc 10:90; #37-43, 600 mL, EtOAc), to provide **142** as a diastereomeric mixture in an approximate d.r. 10:7 (^{1}H NMR) (#27–41, 3.36 g, 66% yield) as a white solid.

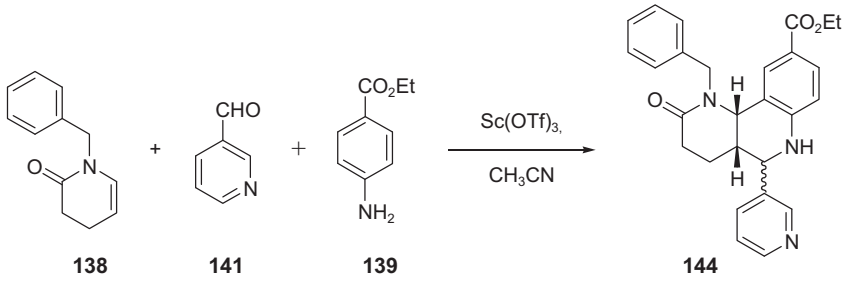
Preparation of ethyl 1-benzyl-5-(4-methoxycarbonylphenyl)-1,2,3,4,4a,5,6,10b- octahydro-2-oxobenzo[*h*][1,6]naphthyridine-9-carboxylate, 143 as a diastereomeric mixture.



138 **140** **139** **143**

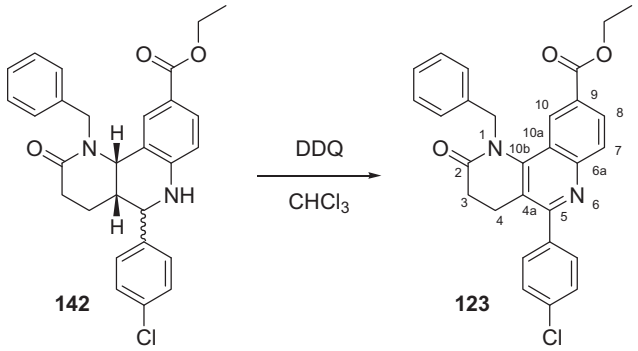
In a triple neck 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of methyl 4-formylbenzoate, **140** (1.75 g, 10.7 mmol) and ethyl 4-aminobenzoate, **139** (1.76 g, 10.7 mmol) in anhydrous CH_3CN (30 mL) was prepared, 4 Å molecular sieves, and $Sc(OTf)_3$ (1.05 g, 2.14 mmol) were added, and the mixture was stirred at r. t. under argon atmosphere for 5 min and then treated with a solution of the unsatured lactam **138** (2.00 g,10.7 mmol) in anhydrous CH_3CN (10 mL). The resulting suspension was stirred at r. t. under argon atmosphere for 3 days. Then, $Sc(OTf)_3$ (0.53 g, 1.07 mmol) was added and the reaction mixture was stirred at r. t. under argon atmosphere one more day, diluted with sat. aq. $NaHCO_3$ (35 mL) and extracted with EtOAc (3 x 75 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a solid (6.40 g), which was purified through column chromatography (silica gel, 35–70 µm, 130 g, Ø = 5 cm; #1–3, 400 mL, hexane; #4–6, 300 mL, hexane / EtOAc 90:10; #7–9, 300 mL, hexane / EtOAc 80:20; #10–12, 300 mL, hexane / EtOAc 70:30; #13–15, 300 mL, hexane / EtOAc 60:40; #16–18, 300 mL, hexane / EtOAc 50:50; #19–21, 300 mL, hexane / EtOAc 40:60; #22–24, 300 mL, hexane / EtOAc 30:70; #25–27, 300 mL, hexane / EtOAc 20:80; #28–30, 300 mL, hexane / EtOAc 10:90; #31–36, 630 mL, EtOAc), to provide **143** as a diastereomeric mixture in an approximate d.r. 3:5 (^{1}H NMR) (#20–34, 3.91 g, 73% yield) as a white solid.

Preparation of ethyl 1-benzyl-1,2,3,4,4a,5,6,10b-octahydro-2-oxo-5-(3-pyridyl)benzo[*h*] [1,6]naphthyridine-9-carboxylate, 144 as a diastereomeric mixture.



In a triple neck 100 mL round-bottomed flask equipped with magnetic stirrer and an inert atmosphere, a solution of 3-pyridinecarboxaldehyde, **141** (1.00 mL, 1.14 g, 10.7 mmol) and ethyl 4-aminobenzoate, **139** (1.76 g, 10.7 mmol) in anhydrous CH_3CN (40 mL) was prepared, 4 Å molecular sieves, and $Sc(OTf)_3$ (1.05 g, 2.14 mmol) were added, and the mixture was stirred at r. t. under argon atmosphere for 5 min and then treated with a solution of the unsatured lactam **138** (2.00 g, 10.7 mmol) in anhydrous CH_3CN (10 mL). The resulting suspension was stirred at r. t. under argon atmosphere for 3 days. Then, $Sc(OTf)_3$ (0.53 g,1.07 mmol) was added and the reaction mixture was stirred at r. t. under argon atmosphere one more day, diluted with sat. aq. $NaHCO_3$ (35 mL) and extracted with EtOAc (3 x 45 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a brown solid (5.20 g), which was purified through column chromatography (silica gel, 35–70 μm, 120 g, Ø = 5 cm; #1–3, 400 mL, hexane; #4–8, 500 mL, hexane / EtOAc 90:10; #9–13, 500 mL, hexane / EtOAc 80:20; #14–18, 500 mL, hexane / EtOAc 70:30; #19–23, 500 mL, hexane / EtOAc 60:40; #24–28, 500 mL, hexane / EtOAc 50:50; #29–33, 500 mL, hexane / EtOAc 40:60; #34–38, 500 mL, hexane / EtOAc 30:70; #39–43, 500 mL, hexane / EtOAc 20:80; #44–48, 500 mL, hexane / EtOAc 10:90; #49–53, 500 mL, EtOAc; #54–58, 500 mL, EtOAc / MeOH, 95:5; #59–64, 500 mL, EtOAc / MeOH, 90:10; #65–69, 500 mL, EtOAc / MeOH, 80:20), to provide **144** as a diastereomeric mixture in an approximate d.r. 1:1 (^{1}H NMR) (#57–67, 2.53 g, 54% yield) as a white solid.

Preparation of ethyl 1-benzyl-5-(4-chlorophenyl)-1,2,3,4-tetrahydro-2-oxobenzo[*h*][1,6] naphthyridine-9-carboxylate, 123.



In a 250 mL round-bottomed flask equipped with magnetic stirrer and an inert atmosphere, a solution of **142** (2.69 g, 5.66 mmol) in anhydrous $CHCl_3$ (88 mL) was prepared and DDQ (2.57 g, 11.3 mmol) was added. The reaction mixture was stirred at r. t. under argon atmosphere overnight, diluted with sat. aq. $NaHCO_3$ (150 mL) and extracted with CH_2Cl_2 (2 x 100 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give an orange solid (3.50 g), which was purified through column chromatography (silica gel, 35–70 μm, 120 g, Ø = 5 cm; #1–4, 500 mL, hexane; #5–17, 1300 mL hexane / EtOAc 90:10; #18–28, 1000 mL, hexane / EtOAc 85:15; #29–55, 300 mL, hexane / EtOAc 80:20; #56–60, 500 mL, hexane / EtOAc 70:30; #61–66, 500 mL, hexane / EtOAc 60:40; #67–71, 500 mL, hexane / EtOAc 50:50; #72–76, 500 mL, hexane / EtOAc 40:60; #77–78 300 mL, hexane / EtOAc 30:70; #79–80, 300 mL, hexane / EtOAc 20:80), to provide **123** (#20–80, 1.94 g, 73% yield) as a beige solid.

R_f = 0.93 (silica gel, 10 cm, hexane / EtOAc 1:1).

Analytical sample of 123.

In a 10 mL round-bottomed flask, **123** (91 mg, 0.19 mmol) was dissolved in CH_2Cl_2 (3 mL), filtered with a PTFE filter (0.2 μm) and washed with pentane (3 x 4 mL) to give, after drying under standard conditions, **123** (89 mg) as a white solid.

Melting point: 192–193 ºC (CH_2Cl_2).

IR (KBr) v: 1706, 1689, 1566 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (t, *J* = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 2.65 (broad t, *J* = 6.8 Hz, 2H, 4-H_2), 2.96 (broad t, *J* = 6.8 Hz, 2H, 3-H_2), 4.35 (q, *J* ≈ 7.0 Hz, 2H, $CO_2CH_2CH_3$), 5.41 (s, 2H, CH_2–Ph), 7.14 [dd, *J* = 6.4 Hz, *J'* = 1.6 Hz, 2H, N–CH_2–Ar–C2(6)-H], 7.22–7.29 [complex signal, 3H, N–CH_2–Ar–C4-H and N–CH_2–Ar–C3(5)-H], 7.49 [dt, *J* ≈ 8.4 Hz, *J'* ≈ 2.0 Hz, 2H, 5–Ar–C3(5)-H_2], 7.56

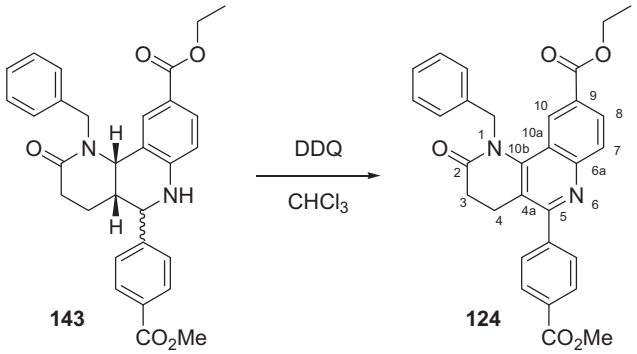
[d, *J* = 8.4 Hz, *J'* = 2.0 Hz, 2H, 5–Ar–C2(6)-H_2], 8.14 (d, *J* = 8.8 Hz, 1H, 7-H), 8.27 (dd, *J* = 8.8 Hz, *J'* = 1.6 Hz, 1H, 8-H), 8.74 (d, *J* = 1.6 Hz, 1H, 10-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 14.5 (CH_3, $CO_2CH_2\mathit{C}H_3$), 23.8 (CH_2, C3), 32.8 (CH_2, C4), 52.7 (CH_2, $\mathit{C}H_2$–Ph), 61.6 (CH_2, $CO_2\mathit{C}H_2CH_3$), 119.4 (C, C10a), 122.1 (C, C4a), 126.1 (CH, C7), 127.3 [2CH, N–CH_2–Ar–*C*2(6)], 127.7 (CH, C10), 128.0 (C, C9), 128.7 (CH, N–CH_2–Ar–*C*4), 128.9 (2CH), 129.0 (2CH) and 130.6 (2CH) [N–CH_2–Ar–*C*3(5), 5–Ar–*C*3(5) and 5–Ar–*C*2(6)], 130.9 (CH, C8), 135.5 (C, N–CH_2–Ar–*C*1), 137.2 (C) and 137.8 (C) (5–Ar–*C*1 and 5–Ar–*C*4), 148.1 (C, C6a), 150.4 (C, C5), 159.1 (C, C10b), 166.0 (C, $\mathit{C}O_2CH_2CH_3$), 172.6 (C, C2).

HRMS, ESI:
Calculated for ($C_{28}H_{23}{}^{35}ClN_2O_3$ + H^+): 471.1470.
Observed: 471.1469.

Elemental analysis: Calculated for $C_{28}H_{23}ClN_2O_3$:
C: 71.41%; H: 4.92%; N: 5.95%; Cl: 7.53%.
Observed: C: 71.44%; H: 4.94%; N: 5.68%; Cl: 7.36%.

Preparation of ethyl 1-benzyl-5-(4-methoxycarbonylphenyl)-1,2,3,4-tetrahydro-2-oxobenzo[*h*] [1,6]naphthyridine-9-carboxylate, 124.



In a 250 mL round-bottomed flask equipped with magnetic stirrer and an inert atmosphere, a solution of **143** (3.40 g, 6.80 mmol) in dry $CHCl_3$ (110 mL) was prepared and DDQ (3.06 g, 13.6 mmol) was added. The reaction mixture was stirred at r. t. under argon atmosphere overnight, diluted with sat. aq. $NaHCO_3$ (150 mL) and extracetd with CH_2Cl_2 (2 x 100 mL). The combined organic extracts were washed with H_2O (2 x 200 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give an orange solid (3.53 g), which was purified through column chromatography (silica gel, 35–70 μm, 124 g, Ø = 5 cm; #1–8, 800 mL, hexane; #9–23, 1500 mL, hexane / EtOAc 90:10; #24–43, 2000 mL, hexane / EtOAc 80:20; #44–63, 2500 mL, hexane / EtOAc 70:30; #64–68, 500 mL, hexane / EtOAc 60:40; #69–73, 500 mL, hexane / EtOAc 50:50; #74–78, 500 mL, hexane / EtOAc 40:60; #79–83, 500 mL, hexane / EtOAc 30:70; #84–88, 500 mL, hexane / EtOAc 20:80), to provide **124** (#46–66, 1.20 g, 36% yield) as a white solid.

R_f = 0.89 (silica gel, 10 cm, hexane / EtOAc 1:1).

Analytical sample of 124·HCl.

In a 25 mL round-bottomed flask, **124** (145 mg, 0.29 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.53 N, 1.70 mL) and washed with pentane (3 x 4 mL) to give, after drying under standard conditions, **124**·HCl (150 mg) as a white solid.

Melting point: 223–224 ºC (CH_2Cl_2 / MeOH 85:15).

IR (KBr) v: 3500–2500 (max. at 3409, 3126, 3101, 3033, 2981, 2954, 2917, 2847, $^+$NH and CH st), 1721, 1633, 1611, 1571, 1554, 1512 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24 (t, *J* = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 2.75 (broad t, *J* = 7.0 Hz, 2H, 4-H_2), 3.00 (broad t, *J* = 6.8 Hz, 2H, 3-H_2), 3.92 (s, 3H, CO_2CH_3), 4.26 (q, *J* = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 5.43 (s, 2H, CH_2–Ph), 7.12 [d, *J* = 6.8 Hz, 2H, N–CH_2–Ar–C2(6)-H], 7.23–7.31 [complex signal, 3H, N–

CH_2–Ar–C4-H and N–CH_2–Ar–C3(5)-H], 7.75 [d, *J* = 8.4 Hz, 2H, 5–Ar–C2(6)-H], 8.21 [d, *J* = 8.4 Hz, 2H, 5–Ar–C3(5)-H], 8.42 (d, *J* = 8.8 Hz, 1H, 8-H), 8.81–8.83 (complex signal, 2H, 7-H and 10-H).

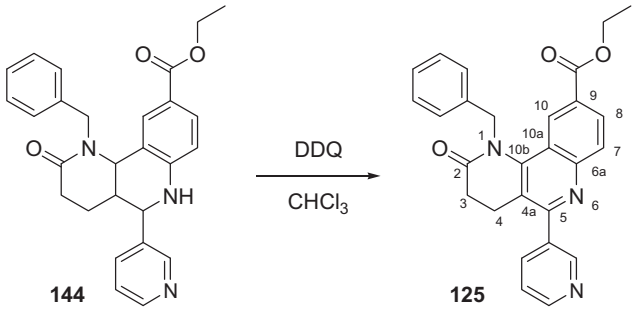
^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 14.2 (CH_3, $CO_2CH_2CH_3$), 22.6 (CH_2, C3), 31.2 (CH_2, C4), 52.6 (CH_3, CO_2CH_3), 53.8 (CH_2, CH_2–Ph), 62.2 (CH_2, $CO_2CH_2CH_3$), 119.1 (C, C10a), 122.2 (C, C4a), 124.0 (CH, C7), 126.6 (CH, C10), 126.7 [2CH, N–CH_2–Ar–*C*2(6)], 128.1 (CH, N–CH_2–Ar–*C*4), 129.0 [2CH, N–CH_2–Ar–*C*3(5)], 130.1 [2CH, 5–Ar–*C*(2)6], 130.1 (C, C9), 130.2 [2CH, 5–Ar–*C*3(5)], 133.0 (C, 5–Ar–*C*4), 133.2 (CH, C8), 134.7 (C, 5–Ar–*C*1), 135.7 (C, N–CH_2–Ar–*C*1), 142.6 (C, C6a), 154.4 (C, C5), 154.9 (C, C10b) 164.6 (C, $CO_2CH_2CH_3$), 166.1 (CO_2CH_3), 171.1 (C, C2).

HRMS, ESI:
Calculated for ($C_{30}H_{26}N_2O_5$ + H^+): 495.1914.
Observed: 495.1910.

Elemental analysis: Calculated for $C_{30}H_{26}N_2O_5$·HCl:
C: 67.86%; H: 5.13%; N: 5.28%; Cl: 6.68%.
Observed: C: 67.82%; H: 5.21%; N: 5.09%; Cl: 6.14%.

Preparation of ethyl 1-benzyl-1,2,3,4-tetrahydro-2-oxo-5-(3-pyridyl)benzo[*h*][1,6]naphthyridine-9-carboxylate, 125.

a) By oxidation of 144 with DDQ.



In a 250 mL round-bottomed flask, equipped with magnetic stirrer and an inert atmosphere, a solution of **144** (2.53 g, 5.73 mmol) in anhydrous $CHCl_3$ (83 mL) was prepared and DDQ (2.42 g, 10.7 mmol) was added. The reaction mixture was stirred at r. t. under argon atmosphere overnight, diluted with sat. aq. $NaHCO_3$ (200 mL) and extracted with CH_2Cl_2 (2 x 150 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give an orange solid (1.22 g), which was purified through column chromatography (silica gel, 35–70 μm, 110 g, Ø = 5 cm; #1–2, 300 mL, hexane / EtOAc / Et_3N 75:25:0.2; #3–10, 900 mL, hexane / EtOAc / Et_3N 60:40:0.2; #11–13, 300 mL, hexane / EtOAc / Et_3N 50:50:0.2; #14–16, 300 mL, hexane / EtOAc / Et_3N 40:60:0.2; #17–19, 300 mL, hexane / EtOAc / Et_3N 30:70:0.2; #20–29, 900 mL, hexane / EtOAc / Et_3N 20:80:0.2; #30–50, 2100 mL, hexane / EtOAc / Et_3N 10:90:0.2), to provide **125** (#31–41, 110 mg, 5% yield) as a beige solid.

Note: When the oxidation reaction was carried out starting from the crude of the multicomponent reaction, the two steps overall yield was 2%.

R_f = 0.60 (silica gel, 10 cm, hexane / EtOAc 1:1).

Analytical sample of 125·HCl.

In a 10 mL round-bottomed flask, **125** (51 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (4 mL), filtered with a PTFE filter (0.2 μm), treated with HCl / MeOH (0.53 N, 2.00 mL) and washed with pentane (3 x 4 mL) to give, after drying under standard conditions, **125**·HCl (53 mg) as a white solid.

Melting point: 185–186 ºC (CH_2Cl_2 / MeOH 67:33).

IR (KBr) v: 3500–2500 (max. at 3404, 3051, 2982, 3008, 2384, $^+$NH and CH st), 2095, 1974, 1691, 1587, 1596, 1553 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

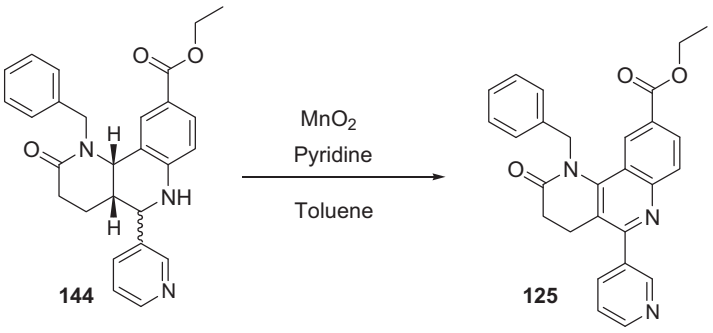
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.31 (t, *J* = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 2.76 (broad s, 2H, 4-H_2), 3.09 (broad s, 2H, 3-H_2), 4.32 (q, *J* = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 5.42 (s, 2H, CH_2–Ph), 7.13 [d, *J* = 7.2 Hz, 2H, N–CH_2–Ar–C2(6)-H], 7.21–7.29 [complex signal, 3H, N–CH_2–Ar–C3(5)-H and N–CH_2–Ar–C4-H], 8.09 (broad s, 1H 5–Ar–C5-H), 8.27–8.33 (complex signal, 2H, 7-H and 8-H), 8.66–8.78 (complex signal, 2H, 5–Ar–C4-H and 10-H), 8.97 (broad s, 1H, 5–Ar–C6-H), 9.24 (broad s, 1H, 5–Ar–C2-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 14.4 (CH_3, $CO_2CH_2CH_3$), 23.3 (CH_2, C3), 32.1 (CH_2, C4), 53.2 (CH_2, CH_2–Ph), 61.9 (CH_2, $CO_2CH_2CH_3$), 119.8 (C, C10a), 121.8 (C, C4a), 126.4 (CH, C7), 126.7 (CH, C10), 127.0 [2CH, N–CH_2–Ar–*C*2(6)], 127.9 (CH, N–CH_2–Ar–*C*4), 128.9 [2CH, N–CH_2–Ar–*C*3(5)], 129.3 (C, C9), 129.5 (CH, 5–Ar–*C*5) 130.7 (CH, C8), 136.5 (2C, N–CH_2–Ar–*C*1 and 5–Ar–*C*3), 142.6 (CH), 143.1 (CH) and 145.2 (CH) (5–Ar–*C*2, 5–Ar–*C*4 and 5–Ar–*C*6), 148.7 (C, C6a), 150.8 (C, C5), 151.4 (C, C10b), 165.3 (C, $CO_2CH_2CH_3$), 171.6 (C, C2).

HRMS, ESI:
Calculated for ($C_{27}H_{23}N_3O_3$ + H^+): 438.1812.
Observed: 438.1804.

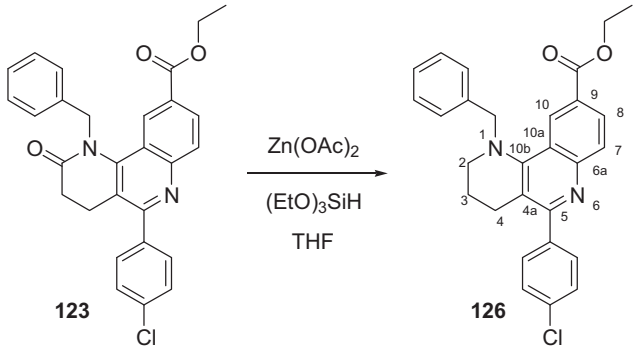
Elemental analysis: Calculated for $C_{27}H_{23}N_3O_3$·HCl·2.5H_2O:
C: 62.49%; H: 5.63%; N: 8.10%; Cl: 6.83%.
Observed: C: 62.53%; H: 5.12%; N: 8.02%; Cl: 7.00%.

b) By oxidation with MnO_2



In a 250 mL round-bottomed flask, equipped with magnetic stirrer, an inert atmosphere and a condenser, a solution of **144** (1.48 g, 3.36 mmol) in anhydrous toluene (160 mL) was prepared and treated with freshly distilled pyridine (1.26 mL, 2.31 g, 29.2 mmol) and MnO_2 (27.1 g, 311 mmol). The reaction mixture was stirred at 55 ºC for 4 h), when TLC showed total consumption of the starting material. The reaction mixture was then cooled to r. t., filtered through Celite® and evaporated under reduced pressure to give a brown oil (1.10 g), which consisted of a complex mixture that did not contain the desired quinoline **125**.

Preparation of ethyl 1-benzyl-5-(4-chlorophenyl)-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine-9-carboxylate, 126.



To a 25 mL oven dried sealed vessel equipped with magnetic stirrer, $Zn(OAc)_2$ (35 mg, 0.19 mmol) was added and the vessel was purged with argon, then, anhydrous THF (5 mL) and $(EtO)_3SiH$ (0.77 mL, 685 mg, 4.17 mmol) were added. The resulting suspension was stirred at r. t. for 30 min and then treated with a solution of lactam **123** (0.90 g, 1.91 mmol) in anhydrous THF (15 mL). The reaction mixture was stirred at 65 ºC for 48 h and monitored by TLC. The resulting mixture was transferred to a conical flask containing 1 N NaOH (15 mL), cooled to 0 ºC with an ice bath and vigorously stirred for 30 min. Then, EtOAc was added (15 mL), the phases were separated and the aqueous phase was extracted with EtOAc (2 x 15 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow solid (1.80 g), which was purified through column chromatography (silica gel, 35–70 µm, 110 g, Ø = 5 cm; #1–4, 100 mL, hexane; #5–10, 200 mL hexane / EtOAc 90:10; #11–22, 400 mL, hexane / EtOAc 85:15; #23–34, 400 mL, hexane / EtOAc 80:20; #35–39, 200 mL, hexane / EtOAc 75:25; #40–45, 200 mL, hexane / EtOAc 70:30; #46–50, 200 mL, hexane / EtOAc 60:40), to provide **126** (#15–21, 0.45 g, 52% yield) as a yellow solid and unreacted lactam **123** (#22–45, 0.29 g).

R_f = 0.76 (silica gel, 10 cm, hexane / EtOAc 1:1).

Analytical sample of 126·HCl.

In a 10 mL round-bottomed flask, **126** (90 mg, 0.20 mmol) was dissolved in CH_2Cl_2 (3 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 1.1 mL), evaporated under reduced pressure, and washed with pentane (3 x 4 mL) to give, after drying under standard conditions, **126**·HCl (92 mg) as a white solid.

Melting point: 227–228 ºC (CH_2Cl_2 / MeOH 73:27).

IR (KBr) v: 3500–2500 (max. at 3402, 3029, 2930, 2909, 2860, 2600, $^+$NH and CH st), 1716, 1653, 1626, 1595, 1576, 1560, 1521 (C=O, Ar–C–C and Ar–C–N st)cm^{-1}.

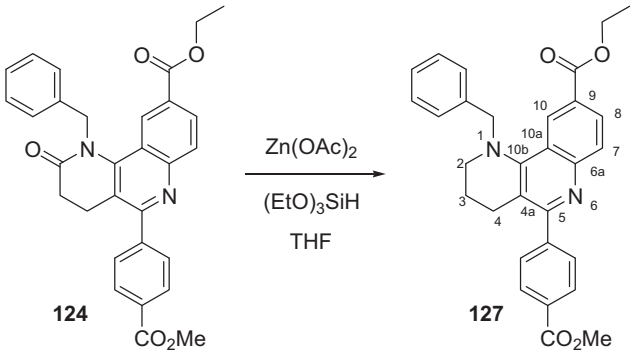
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.03 (t, *J* = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 2.00 (tt, *J* ≈ *J'* ≈ 4.0 Hz, 2H, 3-H_2), 2.87 (broad t, *J* = 6.0 Hz, 2H, 4-H_2), 3.70 (broad t, *J* = 5.4 Hz, 2H, 2-H_2), 4.10 (q, *J* ≈ 7.0 Hz, 2H, $CO_2CH_2CH_3$), 5.18 (s, 2H, CH_2–Ph), 7.34 [d, *J* = 8.4 Hz, 2H, 5–Ar–C3(5)-H], 7.39–7.45 [complex signal, 3H, N–CH_2–Ar–C4-H and N–CH_2–Ar–C2(6)-H], 7.52 [dd, *J* ≈ *J'* ≈ 7.4 Hz, 2H, N–CH_2–Ar–C3(5)-H], 7.65 [d, *J* = 8.4 Hz, 2H, 5–Ar–C2(6)-H], 8.18 (dd, *J* = 8.8 Hz, *J'* = 1.6 Hz 1H, 8-H), 8.63 (d, *J* = 1.6 Hz, 1H, 10-H), 8.82 (d, *J* = 8.8 Hz, 1H, 7-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 14.0 (CH_3, $CO_2CH_2CH_3$), 20.7 (CH_2, C3), 26.0 (CH_2, C4), 51.8 (CH_2, C2), 61.0 (CH_2, CH_2–Ph), 61.5 (CH_2, $CO_2CH_2CH_3$), 114.1 (C, C4a), 116.0 (C, C10a), 121.1 (CH, C7), 126.5 [2CH, N−CH_2−Ar−C2(6)], 127.1 (CH, C10), 128.5 (CH, N–CH_2–Ar–C4), 129.0 [2CH, N–CH_2–Ar–C3(5)], 129.2 (C, C9), 129.7 [2CH, 5–Ar–C3(5)], 131.6 [2CH, 5–Ar–C2(6)] 132.2 (CH, C8), 134.8 (2C) and 137.4 (C) (5–Ar–C1, 5–Ar–C4 and N–CH_2–Ar–C1), 141.8 (C, C6a), 150.0 (C, C5), 158.5 (C, C10b), 164.9 (C, $CO_2CH_2CH_3$).

HRMS, ESI:
Calculated for ($C_{28}H_{25}{}^{35}ClN_2O_2$ + H^+): 457.1677.
Observed: 457.1673.

Elemental analysis: Calculated for $C_{28}H_{25}ClN_2O_2 \cdot HCl \cdot 1.25H_2O$:
C: 65.18%; H: 5.57%; N: 5.43%; Cl: 13.74%.
Observed: C: 65.31%; H: 5.52%; N: 5.20%; Cl: 10.50%.

Preparation of ethyl 1-benzyl-1,2,3,4-tetrahydro-5-(4-methoxycarbonylphenyl)benzo[*h*][1,6] naphthyridine-9-carboxylate, 127.



To a 50 mL oven dried sealed vessel equipped with magnetic stirrer and an inert atmosphere, $Zn(OAc)_2$ (39 mg, 0.21 mmol) was added and the vessel was purged with argon, then, anhydrous THF (5 mL) and $(EtO)_3SiH$ (0.87 mL, 774 mg, 4.71 mmol) were added. The resulting supension was stirred at r. t. for 30 min and then treated with a solution of lactam **124** (1.06 g, 2.14 mmol) in anydrous THF (16 mL). The reaction mixture was stirred at 65 ºC for 48 h and monitored by TLC. The resulting mixture was transferred to a conical flask containing 1 N NaOH (15 mL), cooled to 0 ºC with an ice bath and vigorously stirred for 30 min. Then, EtOAc was added (40 mL), the phases were separated and the aqueous phase was extracted with EtOAc (2 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow solid (1.20 g), which was purified through column chromatography (silica gel, 35–70 µm, 110 g, Ø = 5 cm; #1–6, 550 mL, hexane; #7–16, 1000 mL, hexane / EtOAc 90:10; #17–21, 500 mL, hexane / EtOAc 80:20; #22–31, 1000 mL, hexane / EtOAc 70:30; #32–36, 500 mL, hexane / EtOAc 60:40), to provide **127** (#25–27, 0.40 g, 39% yield) as a yellow solid and unreacted **124** (#28–34, 0.22 g).

R_f = 0.89 (silica gel, 10 cm, hexane / EtOAc 1:1).

Analytical sample of 127·HCl.

In a 25 mL round-bottomed flask, **127** (190 mg, 0.39 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 2.20 mL) and washed with pentane (3 x 4 mL) to give, after drying under standard conditions, **127**·HCl (150 mg) as a white solid.

Melting point: 156–158 ºC (CH_2Cl_2 / MeOH 82:18).

IR (KBr) v: 3500–2500 (max. at 3406, 3219, 3102, 3043, 2985, 2950, 2844, 2656, $^+$NH and CH st), 1716, 1628, 1608, 1578, 1564 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.03 (t, *J* = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 2.00 (broad s, 2H, 3-H_2), 2.92 (broad s, 2H, 4-H_2), 3.76–3.82 (complex signal, 5H, 2-H_2 and CO_2CH_3), 4.11 (q, *J* = 7.2 Hz, 2H,

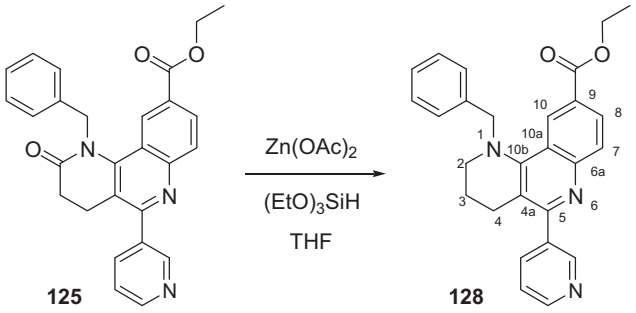
$CO_2CH_2CH_3$), 5.30 (s, 2H, CH_2–Ph), 7.40–7.46 [complex signal, 3H, N–CH_2–Ar–C2(6)-H and N–CH_2–Ar–C4-H], 7.53 [dd, $J \approx J' \approx 7.6$ Hz, 2H, N–CH_2–Ar–C3(5)-H] 7.82 [d, $J = 7.2$ Hz, 2H 5–Ar–C2(6)-H], 7.91 [d, $J = 7.2$ Hz, 2H 5–Ar–C3(5)-H], 8.15 (dd, $J = 9.2$ Hz, $J' = 1.6$ Hz, 1H, 8-H), 8.61 (d, $J = 9.2$ Hz, 1H, 7-H), 8.65 (d, $J = 1.6$ Hz, 1H, 10-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 13.9 (CH_3, $CO_2CH_2CH_3$), 20.6 (CH_2, C3), 25.7 (CH_2, C4), 51.8 (CH_2, C2), 52.1 [CH_3, CO_2CH_3), 60.8 (CH_2, CH_2–Ph), 61.3 (CH_2, $CO_2CH_2CH_3$), 114.0 (C, C4a), 115.8 (C, C10a), 121.8 (CH, C7), 126.2 [2CH, N–CH_2–Ar–C2(6)], 126.9 (C, C9), 127.2 (CH, C10), 128.3 (CH, N–CH_2–Ar–C4), 129.5 [4CH, 5–Ar–C3(5) and N–CH_2–Ar–C3(5)], 130.2 [2CH, 5–Ar–C(2)6], 131.7 (C, 5–Ar–C4), 132.0 (CH, C8), 134.8 (2C, 5–Ar–C1 and N–CH_2–Ar–C1), 141.5 (C, C6a), 149.4 (C, C5), 158.4 (C, C10b), 164.8 (C, $CO_2CH_2CH_3$), 165.6 (C, CO_2CH_3).

HRMS, ESI:
Calculated for ($C_{30}H_{28}N_2O_4 + H^+$): 481.2122.
Observed: 481.2111.

Elemental analysis: Calculated for $C_{30}H_{28}N_2O_4 \cdot HCl \cdot 1.75H_2O$:
C: 65.43%; H: 5.52%; N: 4.99%; Cl: 6.69%.
Observed: C: 65.69%; H: 5.97%; N: 5.11%; Cl: 6.46%.

Preparation of ethyl 1-benzyl-1,2,3,4-tetrahydro-5-(3-pyridyl)benzo[*h*][1,6]naphthyridine-9-carboxylate, 128.



To a 10 mL oven dried sealed vessel equipped with magnetic stirrer and an inert atmosphere, $Zn(OAc)_2$ (4.4 mg, 0.02 mmol) was added and the vessel was purged with argon, then, anhydrous THF (1 mL) and $(EtO)_3SiH$ (0.1 mL, 8.9 mg, 0.54 mmol) were added. The mixture was stirred at r. t. for 30 min and then treated with a solution of lactam **125** (115 mg, 0.26 mmol) in anhydrous THF (2 mL). The reaction mixture was stirred at 65 ºC for 48 h and monitored by TLC. The resulting mixture was transferred to a conical flask containing 1 N NaOH (4 mL), cooled to 0 ºC with an ice bath, and vigorously stirred for 30 min. Then, EtOAc (8 mL) was added, the phases were separated and the aqueous phase was extracted with EtOAc (2 x 8 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a brown solid (160 mg), which was purified through column chromatography (silica gel, 35-70 µm, 140 g, Ø = 1.5 cm; #1–6, 200 mL, hexane / EtOAc / Et_3N 50:50:0.2; #7–19, 400 mL, hexane / EtOAc / Et_3N 40:60:0.2; #20–22, 100 mL, hexane / EtOAc / Et_3N 30:70:0.2), to provide impure **128** (#9–21, 35 mg). This product was again purified through column chromatography (silica gel, 35–70 µm, 3.5 g, Ø = 1 cm; #1–7, 60 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #8–18, 150 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2), to provide still impure **128** (# 13-16, 21 mg). The impure product was dissolved in EtOAc (0.2 mL) and precipitaded with hexane (0.8 mL). The solvent was decanted and the residue was dried under vacuum to give pure **128** (16 mg, 15% yield) as a white solid.

R_f = 0.55 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 128·2HCl.

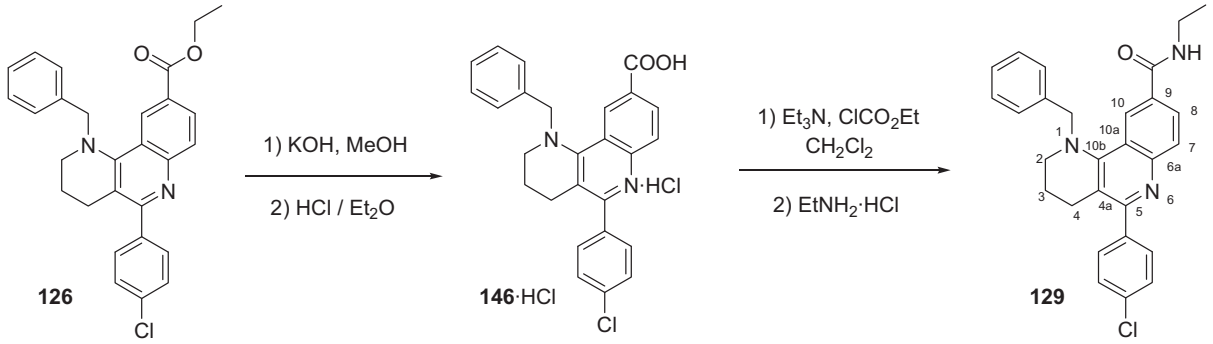
In a 5 mL round-bottomed flask, **128** (16 mg, 0.04 mmol) was dissolved in CH_2Cl_2 (2 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 0.60 mL) and washed with pentane (3 x 4 mL) to give, after drying under standard conditions, **128**·2HCl (17 mg) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.06 (t, *J* = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 2.13 (broad s, 2H, 3-H_2), 2.92 (broad s, 2H, 4-H_2), 3.74 (broad s, 2H, 2-H_2), 4.13 (q, *J* = 7.0 Hz, 2H, $CO_2CH_2CH_3$), 5.23 (broad s, 2H, CH_2–Ph), 7.38 [tt, *J* ≈ *J'* ≈ 7.2 Hz, 2H, N–CH_2–Ar–C3(5)-H], 7.38–7.48 [complex signal, 4H, 5–Ar–C5-H, N–CH_2–Ar–C2(6)-H and N–CH_2–Ar–C4-H], partially overlapped 8.12 (broad s, 1H, 5–Ar–C5-H), 8.26 (d, *J* = 9.2 Hz, 1H, 7-H), 8.67 (broad d, 1H, 8-H), 8.71 (broad signal, 2H, 10-H and 5–Ar–C4-H), 8.94 (broad s, 1H, 5–Ar–C6-H), 9.38 (broad s, 5–Ar–C2-H).

HRMS, ESI:
Calculated for ($C_{27}H_{25}N_3O_2 + H^+$): 424.2020.
Observed: 424.2013.

Elemental analysis: Calculated for $C_{27}H_{25}N_3O_2 \cdot 2HCl \cdot 3.5H_2O$:
C: 57.96%; H: 6.13%; N: 7.51%.
Observed: C: 58.13%; H: 5.80%; N: 7.70%.

Preparation of 1-benzyl-5-(4-chlorophenyl)-*N*-ethyl-1,2,3,4-tetrahydrobenzo[*h*][1,6] naphthyridine-9-carboxamide, 129.



In a 25 mL round-bottomed flask equipped with magnetic stirrer and a condenser, a suspension of ester **126** (0.20 g, 0.42 mmol) and KOH (85% purity, 0.09 g, 1.36 mmol) in MeOH (11 mL) was prepared. The reaction mixture was stirred under reflux for 24 h. The resulting solution was cooled down at room temperature and concentrated under reduced pressure. The solid residue (0.38 g) was treated with HCl / Et_2O (0.5 N, 18 mL) and the resulting suspension was concentrated under reduced pressure to give a white solid (0.34 g), whose ^{1}H NMR spectrum was consistent with that expected for carboxylic **146**·HCl and was used in the next step as a crude, without further purification.

In a 25 mL round-bottomed flask equipped with magnetic stirrer and an inert atmosphere, a solution of **146**·HCl (0.34 g of a crude that could contain a maximum of 0.44 mmol) in anhydrous CH_2Cl_2 (5 mL) was prepared, cooled to 0º C with an ice bath and treated dropwise with freshly distilled Et_3N (0.25 mL, 0.18 g, 1.78 mmol) and $ClCO_2Et$ (0.04 ml, 45.6 mg, 0.42 mmol). The resulting suspension was vigorously stirred at 0º C for 30 min and treated with $EtNH_2$·HCl (0.04 g, 0.49 mmol). The reaction mixture was stirred at r. t. for 3 days, diluted with 10% aq. Na_2CO_3 (15 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts were washed with H_2O (2 x 50 mL), dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a white solid (220 mg), which was purified through column chromatography (silica gel, 35–70 µm, 15 g, Ø = 1.5 cm; #1–5, 150 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #6–10, 150 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #11–15, 150 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2; #16–19, 150 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 97:3:0.2), to provide amide **129** (#11–15, 100 mg, 44% overall yield) as a white solid.

R_f = 0.54 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 129·HCl.

In a 10 mL round-bottomed flask, **129** (40 mg, 0.09 mmol) was dissolved in CH_2Cl_2 (3 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.53 N, 0.50 mL), evaporated under reduced pressure, and washed with pentane (3 x 4 mL) to give, after drying under standard conditions, **129**·HCl (43 mg) as a white solid.

Melting point: 149–151 ºC (CH_2Cl_2 / MeOH 73:27).

IR (KBr) v: 3500–2500 (max. at 3415, 3229, 3144, 3058, 3034, 2953, 2922, 2868, 2709, 2636, $^+$NH, NH and CH st), 1659, 1625, 1607, 1595, 1577, 1563, 1531 (C=O, Ar–C–C and Ar–C–N st) cm^{-1}.

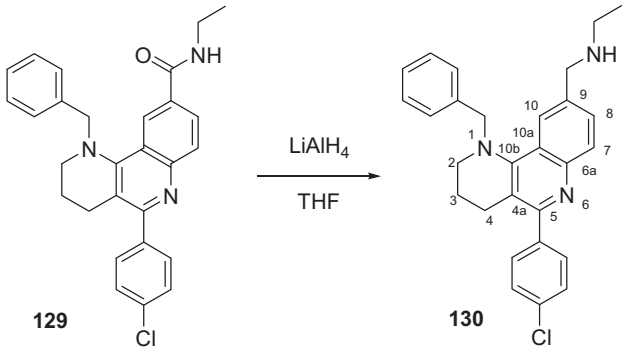
^{1}H NMR (400 MHz, CD_3OD) δ: 1.06 (t, *J* ≈ 7.2 Hz, 3H, $CONHCH_2CH_3$), 2.08 (tt, *J* ≈ *J'* ≈ 4.8 Hz, 2H, 3-H_2), 2.77 (t, *J* = 5.8 Hz, 2H, 4-H_2), 3.25 (q, *J* = 7.2 Hz, 2H, $CONHCH_2CH_3$), 3.68 (t, *J* = 5.2 Hz, 2H, 2-H_2), 4.80 (s, NH and $^+$NH), 5.28 (s, 2H, CH_2–Ph), partially overlapped 7.42–7.47 [complex signal, 3H, N–CH_2–Ar–C2(6)-H and N–CH_2– Ar–C4-H], partially overlapped 7.47–7.53 [dd, *J* ≈ *J'* ≈ 7.2 Hz, 2H, N–CH_2–ArC3(5)-H], 7.63–7.68 [complex signal, 4H, 5–Ar–C2(6)-H and 5–Ar–C3(5)-H], 7.91 (d, *J* = 8.8 Hz, 1H, 7-H), 8.15 (dd, *J* = 8.8 Hz, *J'* = 1.6 Hz, 1H, 8-H), 8.51 (d, *J* ≈ 1.6 Hz, 1H, 10-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 14.6 (CH_3, $CONHCH_2CH_3$), 21.1 (CH_2, C3), 26.4 (CH_2, C4), 35.8 (CH_2, C2), 53.1 (CH_2, $CONHCH_2CH_3$), 61.6 (CH_2, CH_2–Ph), 115.0 (C, C4a), 117.3 (C, C10a), 121.1 (CH, C7), 126.9 (CH, C10), 127.7 [2CH, N–CH_2–Ar–C2(6)], 129.2 (CH, N–CH_2–Ar–C4), 130.37 (CH, C8), 130.42 [2CH, N–CH_2–C3(5)], 131.6 [2CH, 5–Ar–C3(5)], 132.0 (C, C9), 132.2 [2CH, 5–Ar–C2(6)], 132.8 (C, 5–Ar–C4), 136.2 (C, 5–Ar–C1), 138.2 (C, N–CH_2–Ar–C1], 141.6 (C, C6a), 150.3 (C, C5), 159.8 (C, C10b), 168.1 (C, $CONHCH_2CH_3$).

HRMS, MALDI:
Calculated for ($C_{28}H_{26}{}^{35}ClN_3O + H^+$): 456.1837
Observed: 456.3648.

Elemental analysis: Calculated for $C_{28}H_{26}ClN_3O \cdot 1.1HCl \cdot 0.25H_2O$:
C: 66.58%; H: 5.62%; N: 8.32%; Cl: 14.74%.
Observed: C: 66.77%; H: 5.74%; N: 7.74%; Cl: 15.04%.

Preparation of *N*-{{1-benzyl-5-(4-chlorophenyl)-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridin-9-yl}methyl}ethanamine, 130.



In a 50 mL round-bottomed flask equipped with magnetic stirrer, condenser, and an inert atmosphere, a solution of amide **129** (100 mg, 0.22 mmol) in anhydrous THF (5 mL) was prepared, cooled to 0 ºC with an ice bath, and treated portionwise with solid $LiAlH_4$ (30 mg, 0.77 mmol). The resulting suspension was stirred under reflux overnight, cooled to 0 ºC with an ice bath and treated dropwise with 1 N NaOH (3 mL), then diluted with H_2O (8 mL) and extracted with EtOAc (3 x 8 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow solid (120 mg), which was purified through column chromatography (silica gel, 35–70 µm, 20 g, Ø = 2.5 cm; #1–9, 300 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #10–40, 1200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99.5:0.5:0.2; #41–65, 800 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #66–69, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2; #70–77, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 95:5:0.2), to provide amine **130** (#50-75, 43 mg, 44% yield) as a white solid and unreacted amide **129** (#19-26, 14 mg).

R_f = 0.44 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 130·2HCl.

In a 10 mL round-bottomed flask, **130** (35 mg, 0.08 mmol) was dissolved in CH_2Cl_2 (3 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.43 N, 1.7 mL), evaporated under reduced pressure, and washed with pentane (3 x 4 mL) to give, after drying under standard conditions, **130**·2HCl (38 mg) as a white solid.

Melting point: 149–151 ºC (CH_2Cl_2 / MeOH 73:27).

IR (KBr) ν: 3500–2500 (max. at 3380, 2924, 2853, 2662, 2522, $^+$NH, NH and CH st), 1631, 1596, 1578, 1563, 1518 (Ar–C–C and Ar–C–N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.29 (t, *J* = 7.2 Hz, 3H, 9-$CH_2NHCH_2CH_3$), 2.05 (tt, *J* ≈ *J'* ≈ 5.0 Hz, 2H, 3-H_2), 2.75 (t, *J* = 5.8 Hz, 2H, 4-H_2), 3.00 (q, *J* = 7.2 Hz, 2H, 9-$CH_2NHCH_2CH_3$), 3.65 (t, *J* = 5.2 Hz, 2H,

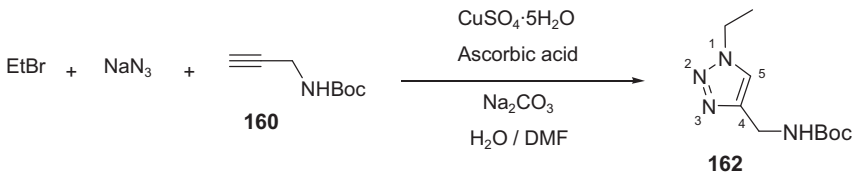
2-H_2), 4.14 (s, 2H, 9-C*H*$_2$NHCH$_2$CH$_3$), 4.87 (s, NH and $^+$NH), 5.35 (s, 2H, C*H*$_2$–Ph), 7.42 [dd, *J* ≈ *J'* ≈ 6.8 Hz, 2H, N–CH$_2$–C3(5)-H], 7.47–7.53 [complex signal, 3H, N–Ar–C4-H and N–CH$_2$–Ar–C2(6)-H], 7.66–7.71 [complex signal, 4H, 5–Ar–C2(6)-H and 5–Ar–C3(5)-H], 7.98 (d, *J* = 8.4 Hz, 1H, 7-H), 8.04 (d, *J* = 8.8 Hz, 1H, 8-H), 8.24 (s, 1H, 10-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 11.4 (CH_3, 9-$CH_2NHCH_2CH_3$), 21.3 (CH_2, C3), 26.5 (CH_2, C4), 43.8 (CH_2, C2), 51.4 (CH_2, 9-$CH_2NHCH_2CH_3$), 53.0 (CH_2, 9-$CH_2NHCH_2CH_3$), 61.7 (CH_2, CH_2–Ph), 115.5 (C, C4a), 118.1 (C, C10a), 121.9 (CH, C7), 128.4 [2CH, N–CH_2–Ar–*C*2(6)], 129.4 (CH, C10), 129.5 (C, C9), 129.6 (CH, N–CH_2–Ar–*C*4), 130.4 [2CH, N–CH_2–Ar–*C*3(5)], 130.5 [2CH, 5–Ar–*C*3(5)], 131.9 [2CH, 5–Ar–*C*2(6)], 132.5 (C, 5–Ar–*C*4), 135.2 (CH, C8), 137.1 (C, N–CH_2–Ar–*C*1), 138.2 (C, 5–Ar–*C*1), 140.9 (C, C6a), 150.4 (C, C5), 159.8 (C, C10b).

HRMS, ESI:
Calculated for ($C_{28}H_{28}{}^{35}ClN_3$ + H^+): 442.2045.
Observed: 442.2048.

Elemental analysis: Calculated for $C_{28}H_{28}ClN_3 \cdot 1.6HCl \cdot 2.6H_2O$:
C: 61.46%; H: 6.41%; N: 7.68%; Cl: 16.85%.
Observed: C: 61.81%; H: 6.28%; N: 7.06%; Cl: 16.90%.

Preparation of *tert*-butyl (1-ethyl-1*H*-1,2,3-triazol-4-yl)methylcarbamate, 162.



In a 100 mL round-bottomed flask equipped with magnetic stirrer, a solution of EtBr (0.53 mL, 774 mg, 7.10 mmol) in H_2O / DMF 1:4 (50 mL) was prepared and treated with NaN_3 (502 mg, 7.72 mmol), Na_2CO_3 (2.05 g, 19.3 mmol), ascorbic acid (907 mg, 5.15 mmol), $CuSO_4·5H_2O$ (641 mmg, 2.57 mmol) and *N*-Boc-propargylamine, **160** (1.00 g, 6.44 mmol). The reaction mixture was stirred at r. t. overnight, diluted with 20% aq. NH_4OH (200 mL), treated with solid EDTA (5 g approx.) and extracted with EtOAc (2 x 150 mL). The combined organic extracts were washed with H_2O (3 x 100 mL), dried with anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, washed with pentane (3 x 10 mL) and dried under vacuum, to give the triazole **162** (1.14 g, 71% yield) as a white solid.

R_f = 0.57 (silica gel, 10 cm, CH_2Cl_2 / MeOH 9:1).

Melting point: 77–78 ºC (EtOAc).

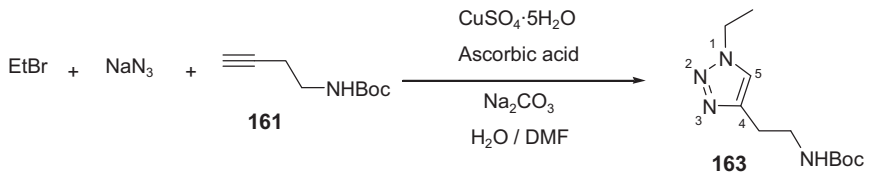
IR (neat) v: 3318 (NH st), 1685, 1675, 1528 (C=O, Ar–C–C and Ar–C–N st), 1249 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.40 [s, 9H, $C(CH_3)_3$], 1.51 (t, *J* = 7.2 Hz, 3H, N–CH_2–CH_3), 4.33–4.38 (complex signal, 4H, N–CH_2–CH_3 and 4–CH_2–NH), 5.21 (broad s, 1H, NH), 7.50 (s, 1H, 5-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 15.5 (CH_3, N–CH_2–*C*H_3), 28.4 [CH_3, C(*C*H_3)$_3$], 36.2 (CH_2, 4–*C*H_2–NH), 45.3 (CH_2, N–*C*H_2–CH_3), 79.7 [C, *C*$(CH_3)_3$], 121.3 (CH, C5), 145.6 (C, C4), 156.0 (C, C=O).

HRMS, ESI:
Calculated for ($C_{10}H_{18}N_4O_2$ + H^+): 227.1503.
Observed: 227.1493.

Preparation of *tert*-butyl (1-ethyl-1*H*-1,2,3-triazol-4-yl)ethylcarbamate, 163.



In a 100 mL round-bottomed flask equipped with magnetic stirrer, a solution of EtBr (0.65 mL, 949 mg, 8.71 mmol) in H_2O / DMF 1:4 (55 mL) was prepared and treated with NaN_3 (618 mg, 9.51 mmol), Na_2CO_3 (2.52 g, 23.8 mmol), ascorbic acid (1.12 g, 6.34 mmol), $CuSO_4{\cdot}5H_2O$ (790 mg, 3.17 mmol) and *N*-Boc-butinylamine, **161** (1.34 g, 7.93 mmol). The reaction mixture was stirred at r. t. overnight, diluted with 20% aq. NH_4OH (200 mL), treated with solid EDTA (5 g approx.) and extracted with EtOAc (2 x 150 mL). The combined organic extracts were washed with H_2O (3 x 100 mL), dried with anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, washed with pentane (3 x 10 mL) and dried under vacuum, to give the triazole **163** (1.48 g, 71% yield) as a white solid.

R_f = 0.50 (silica gel, 10 cm, CH_2Cl_2 / MeOH 9:1).

Melting point: 78–80 ºC (EtOAc).

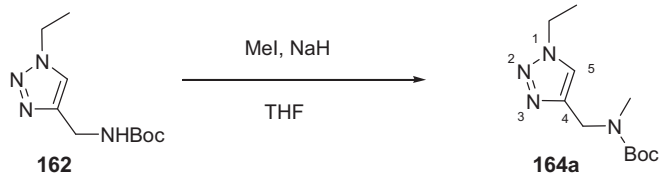
IR (neat) v: 3341 (NH st), 1692, 1512, 1504 (C=O, Ar–C–C and Ar–C–N st), 1249 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.40 [s, 9H, $C(CH_3)_3$], 1.51 (t, *J* = 7.2 Hz, 3H, N–CH_2–CH_3), 2.87 (t, *J* ≈ 6.8 Hz, 2H, 4–CH_2–CH_2–NH), 3.44 (dt, *J* ≈ *J'* ≈ 6.4 Hz, 2H, 4–CH_2–CH_2–NH), 4.35 (q, *J* = 7.2 Hz, 2H, N–CH_2–CH_3), 5.04 (broad s, 1H, NH), 7.35 (s, 1H, 5-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 15.6 (CH_3, N–CH_2–CH_3), 26.3 (CH_2, 4–CH_2–CH_2–NH), 28.5 [CH_3, $C(CH_3)_3$], 39.9 (CH_2, 4–CH_2–CH_2–NH), 45.2 (CH_2, N–CH_2–CH_3), 79.2 [C, $C(CH_3)_3$], 120.8 (CH, C5), 145.5 (C, C4), 156.1 (C, C=O).

HRMS, ESI:
Calculated for ($C_{11}H_{20}N_4O_2$ + H^+): 241.1659.
Observed: 241.1654.

Preparation of *tert*-butyl (1-ethyl-1*H*-1,2,3-triazol-4-yl)methyl(methyl)carbamate, 164a.



162 **164a**

In a 25 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of **162** (634 mg, 2.80 mmol) in anhydrous THF (6.5 mL) was prepared and cooled to 0 ºC with an ice bath, and treated with NaH (60% in mineral oil, 246 mg, 6.16 mmol) and MeI (0.19 mL, 433 mg, 3.05 mmol). The reaction mixture was stirred at r. t. for 3 h, when TLC revealed total consumption of the starting material, it was then cooled to 0 ºC with an ice bath, diluted dropwise with sat. aq. NH_4Cl (40 mL) and extracted with EtOAc (3 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, evaporated under reduced pressure, washed with pentane (3 x 10 mL) and dried under vacuum, to give **164a** (654 mg, 97% yield) as a colorless oil.

R_f = 0.64 (silica gel, 10 cm, CH_2Cl_2 / MeOH 9:1).

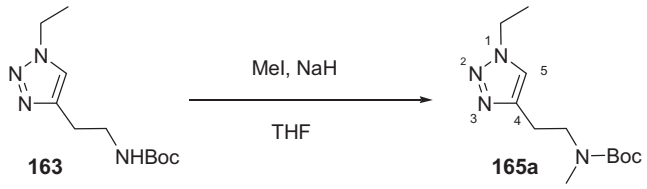
IR (neat) v: 1688 (C=O), 1245 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42 [s, 9H, $C(CH_3)_3$], 1.51 (t, J = 7.2 Hz, 3H, N–CH_2–CH_3), 2.87 (s, 3H, N–CH_3), 4.32–4.38 (m, 2H, N–CH_2–CH_3), 4.44 (s, 2H, 4–CH_2–N), 7.37–7.49 (m, 1H, 5-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 17.5 (CH_3, N–CH_2–CH_3), 30.5 [CH_3, $C(CH_3)_3$], 36.5 (CH_3, N–CH_3), 38.2 (CH_2, 4–CH_2–N), 47.3 (CH_2, N–CH_2–CH_3), 81.8 [C, $C(CH_3)_3$], 123.7 (CH, C5), 147.0 (C, C4), 157.9 (C, C=O).

HRMS, ESI:
Calculated for ($C_{11}H_{20}N_4O_2$ + H^+): 241.1659.
Observed: 241.1654.

Preparation of tert-butyl (1-ethyl-1*H*-1,2,3-triazol-4-yl)ethyl(methyl)carbamate, 165a.



163 **165a**

In a 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of **163** (1.70 g, 7.08 mmol) in anhydrous THF (17 mL) was prepared and cooled to 0 ºC with an ice bath, and treated with NaH (60% in mineral oil, 424 mg, 10.6 mmol) and MeI (0.48 mL, 1.10 g, 7.79 mmol). The reaction mixture was stirred at r. t. for 2 h. As TLC revealed the presence of starting material, the reaction mixture was treated with more NaH (60% in mineral oil, 424 mg, 10.6 mmol) and MeI (0.48 mL, 1.10 g, 7.71 mmol), and again stirred at r. t. for 1 h, cooled to 0 ºC with an ice bath, diluted dropwise with sat. aq. NH_4Cl (40 mL) and extracted with EtOAc (3 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, evaporated under reduced pressure, washed with pentane (3 x 10 mL) and dried under vacuum, to give **165a** (1.71 mg, 95% yield) as a colorless oil.

R_f = 0.58 (silica gel, 10 cm, CH_2Cl_2 / MeOH 9:1).

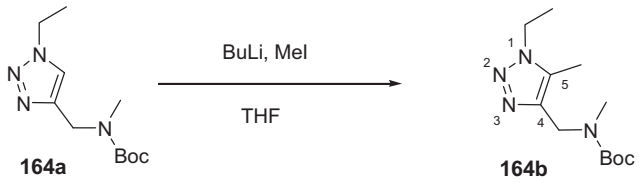
IR (neat) v: 1690 (C=O, st), 1249 (N−N=N st) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$) δ: 1.41 [s, 9H, $C(CH_3)_3$], 1.51 (t, J = 7.2 Hz, 3H, N–CH_2–CH_3), 2.82 (s, 3H, N–CH_3), 2.93 (broad s, 2H, 4–CH_2–CH_2–N), 3.52 (broad t, $J \approx$ 6.8 Hz, 2H, 4–CH_2–CH_2–N), 4.35 (q, J = 7.2 Hz, 2H, N–CH_2–CH_3), 7.28–7.41 (m, 1H, 5-H).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 15.6 (CH_3, N–CH_2–*C*H_3), 24.5 (CH_2, 4–*C*H_2–CH_2–N), 28.5 [CH_3, $C(CH_3)_3$], 34.4 (CH_3, N–CH_3), 45.2 (CH_2, 4–CH_2–*C*H_2–N), 48.9 (CH_2, N–*C*H_2–CH_3), 79.5 [C, *C*$(CH_3)_3$], 120.8 (CH, C5), 145.2 (C, C4), 155.8 (C, C=O).

HRMS, ESI:
Calculated for ($C_{12}H_{22}N_4O_2$ + H^+): 255.1816.
Observed: 255.1815.

Preparation of *tert*-butyl (1-ethyl-5-methyl-1*H*-1,2,3-triazol-4-yl)methyl(methyl)carbamate 164b.



164a **164b**

In a triple neck 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer a solution of **164a** (420 mg, 1.75 mmol) in anhydrous THF (30 mL) was prepared and cooled to -78 ºC with an acetone / dry ice bath, then treated with *n*-BuLi (2.5 M in hexanes, 1.05 mL, 2.62 mmol) and MeI (0.43 mL, 980 mg, 6.91 mmol). The reaction mixture was stirred at -78 ºC for 1 h, then warmed to r. t. and stirred 2 h more. The resulting solution was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (2 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, evaporated under reduced pressure, washed with pentane (3 x 10 mL) and dried under vacuum, to give **164b** (427 mg, 96% yield) as a colorless oil.

R_f = 0.57 (silica gel, 10 cm, CH_2Cl_2 / MeOH 9:1).

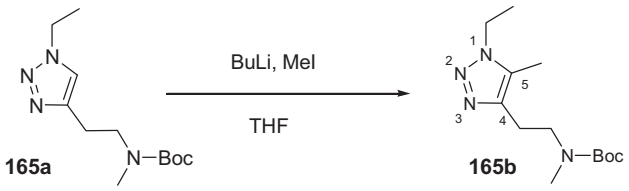
IR (neat) v: 1686 (C=O, st), 1240 (N−N=N st) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$) δ: partially overlapped 1.46 [s, 9H, $C(CH_3)_3$], overlapped 1.44–1.49 (t, J ≈ 7.2 Hz, 3H, N–CH_2–CH_3), 2.31 (s, 3H, 5–CH_3), 2.85 (s, 3H, N–CH_3), 4.26 (q, *J* = 7.2 Hz, 2H, N–CH_2–CH_3), 4.48 (s, 2H, 4–CH_2–N).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 7.8 (CH_3, 5–CH_3), 15.2 (CH_3, N–CH_2–CH_3), 28.5 [CH_3, $C(CH_3)_3$], 34.0 (CH_3, N–CH_3), 42.7 (CH_2, 4–CH_2–N), 43.1 (CH_2, N–CH_2–CH_3), 78.9 [C, $C(CH_3)_3$], 130.5 (C, C5), 142.0 (C, C4), 156.0 (C, C=O).

HRMS, ESI:
Calculated for ($C_{12}H_{22}N_4O_2$ + H^+): 255.1816.
Observed: 255.1811.

Preparation of *tert*-butyl (1-ethyl-5-methyl-1*H*-1,2,3-triazol-4-yl)ethyl(methyl)carbamate, 165b.



165a **165b**

In a 100 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of **165a** (942 mg, 3.71 mmol) in anhydrous THF (70 mL) was prepared and cooled to -78 ºC with an acetone / dry ice bath, then treated with *n*-BuLi (2.5 M in hexanes, 2.97 mL, 7.42 mmol) and MeI (0.92 mL, 2.10 g, 14.8 mmol). The reaction mixture was stirred at -78 ºC for 1 h, then warmed to r. t. and stirred 2 h more. The resulting solution was diluted with H_2O (45 mL) and extracted with CH_2Cl_2 (2 x 55 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, evaporated under reduced pressure, washed with pentane (3 x 10 mL) and dried under vacuum, to give **165b** (895 mg, 90% yield) as a colorless oil.

R_f = 0.57 (silica gel, 10 cm, CH_2Cl_2 / MeOH 9:1).

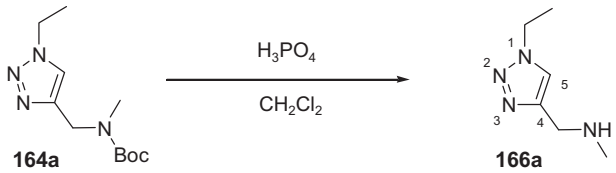
IR (neat) v: 1685 (C=O st), 1249 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.40 [s, 9H, $C(CH_3)_3$], 1.44 (t, *J* = 7.2 Hz, 3H, N–CH_2–CH_3), 2.20 (broad s, 3H, 5–CH_3) , 2.77 (s, 2H, 4–CH_2–CH_2–N), partially overlapped 2.77–2.82 (broad s, 3H, N–CH_3), 3.43 (t, *J* ≈ 7.2 Hz, 4–CH_2–CH_2–N), 4.23 (q, *J* = 7.2 Hz, 2H, N–CH_2–CH_3).

^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 7.7 (CH_3, 5–CH_3), 15.3 (CH_3, N–CH_2–CH_3), 23.9 (CH_2, 4–CH_2–CH_2–N), 28.5 [CH_3, $C(CH_3)_3$], 34.9 (CH_3, N–CH_3), 43.0 (CH_2, 4–CH_2–CH_2–N), 48.9 (CH_2, N–CH_2–CH_3), 79.4 [C, *C*$(CH_3)_3$], 129.2 (C, C5), 142.2 (C, C4), 155.7 (C, C=O).

HRMS, ESI:
Calculated for ($C_{13}H_{24}N_4O_2$ + H^+): 269.1972.
Observed: 269.1974.

Preparation of *N*-(1-ethyl-1*H*-1,2,3-triazol-4-yl)methyl-*N*-methylamine, 166a.



164a **166a**

In a 50 mL round-bottomed flask equipped with magnetic stirrer, a solution of **164a** (411 mg, 1.71 mmol) was dissolved in CH_2Cl_2 (6 mL) and treated with H_3PO_4 (85% purity, 2.96 mL, 25.7 mmol). The reaction mixture was stirred at r. t. for 2 h, cooled to 0 ºC with an ice bath, diluted with H_2O (15 mL), alkalinized dropwise to pH = 14 with 5 N NaOH and extracted with CH_2Cl_2 (2 x 30 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the amine **166a** (205 mg, 86% yield) as a colorless oil.

R_f = 0.07 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 166a·HCl.

In a 10 mL round-bottomed flask, **166a** (25 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (4 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 0.71 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **166a**·HCl (28 mg) as a beige sticky solid.

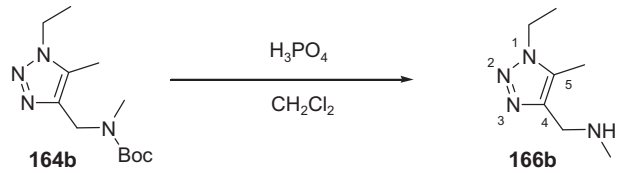
IR (neat) v: 3500–2500 (max. at 3386, 3126, 3080, 3028, 2981, 2935, 2756, 2695, $^+$NH, NH and CH st), 1234, (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.55 (t, $J \approx 7.6$ Hz, 3H, N–CH_2–CH_3), 2.76 (s, 3H, NH–CH_3), 4.35 (s, 2H, 4–CH_2–NH), 4.50 (q, J = 7.6 Hz, 2H, N–CH_2–CH_3), 4.96 (s, NH and $^+$NH), 8.22 (s, 1H, 5-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 15.8 (CH_3, N–CH_2–*C*H_3), 33.1 (CH_3, NH–CH_3), 44.2 (CH_2, 4–*C*H_2–NH), 46.8 (CH_2, N–*C*H_2–CH_3), 126.2 (CH, C5), 139.3 (C, C4).

HRMS, ESI:
Calculated for ($C_6H_{12}N_4$ + H^+): 141.1135.
Observed: 141.1133.

Preparation of *N*-[1-ethyl-5-methyl-1*H*-1,2,3-triazol-4-yl)methyl]-*N*-methylamine, 166b.



164b **166b**

In a 50 mL round-bottomed flask equipped with magnetic stirrer, a solution of **164b** (370 mg, 1.46 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with H_3PO_4 (85% purity, 2.52 mL, 21.9 mmol). The reaction mixture was stirred at r. t. for 4 h, cooled to 0 ºC with an ice bath, diluted with H_2O (15 mL), alkalinized dropwise to pH = 14 with 5 N NaOH and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the amine **166b** (205 mg, 92% yield) as a colorless oil.

R_f = 0.11 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 166b·HCl.

In a 10 mL round-bottomed flask, **166b** (24 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (4 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 0.62 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **166b**·HCl (26 mg) as a beige sticky solid.

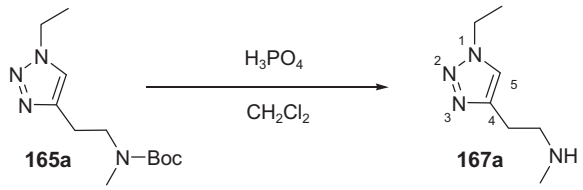
IR (neat) ν: 3500–2500 (max. at 3390, 2925, 2868, 2837, 2757, 2697, $^+$NH, NH and CH st), 1250 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.48 (t, *J* = 7.2 Hz, 3H, N–CH_2–CH_3), 2.41 (s, 3H, 5–CH_3), 2.76 (s, 3H, NH–CH_3), 4.27 (s, 2H, 4–CH_2–NH), 4.38 (q, *J* = 7.2 Hz, 2H, N–CH_2–CH_3), 4.87 (s, NH and $^+$NH).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 7.7 (CH_3, 5–CH_3), 15.2 (CH_3, N–CH_2–CH_3), 33.1 (CH_3, NH–CH_3), 43.6 (CH_2, 4–CH_2–NH), 44.4 (CH_2, N–CH_2–CH_3), 134.7 (C, C5), 136.8 (C, C4).

HRMS, ESI:
Calculated for ($C_7H_{14}N_4$ + H^+): 155.1291.
Observed: 155.1288.

Preparation of *N*-[2-(1-ethyl-1*H*-1,2,3-triazol-4-yl)ethyl]-*N*-methylamine, 165a.



In a 50 mL round-bottomed flask equipped with magnetic stirrer, a solution of **165a** (263 mg, 1.03 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with H_3PO_4 (85% purity, 1.80 mL, 15.6 mmol). The reaction mixture was stirred at r. t. for 4 h, cooled to 0 ºC with an ice bath, diluted with H_2O (10 mL), alkalinized dropwise to pH = 14 with 5 N NaOH and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the amine **167a** (143 mg, 90% yield) as a colorless oil.

R_f = 0.07 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 167a·HCl.

In a 10 mL round-bottomed flask, **167a** (40 mg, 0.26 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 1.04 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **167a**·HCl (41 mg) as a beige sticky solid.

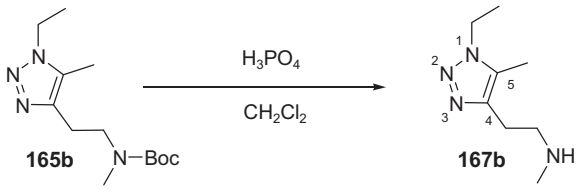
IR (neat) ν: 3500–2500 (max. at 3395, 3116, 3064, 2976, 2936, 2752, 2691, 2446, $^+$NH, NH and CH st), 1252 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.54 (t, *J* = 7.2 Hz, 3H, N–CH_2–CH_3), 2.76 (s, 3H, NH–CH_3), 3.17 (t, *J* = 7.2 Hz, 2H, 4–CH_2–CH_2–NH), 3.37 (t, *J* = 7.2 Hz, 2H, 4–CH_2–CH_2–NH), 4.47 (q, *J* = 7.2 Hz, 2H, N–CH_2–CH_3), 4.87 (s, NH and $^+$NH), 8.07 (s, 1H, 5-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 15.6 (CH_3, N–CH_2–*C*H_3), 22.9 (CH_2, 4–*C*H_2–CH_2–NH), 33.7 (CH_3, NH–CH_3), 47.1 (CH_2, 4–CH_2–*C*H_2–NH), 47.9 (CH_2, N–*C*H_2–CH_3), 124.6 (CH, C5), 143.3 (C, C4).

HRMS, ESI:
Calculated for ($C_7H_{14}N_4$ + H^+): 155.1291.
Observed: 155.1297.

Preparation of *N*-[2-(1-ethyl-5-methyl-1*H*-1,2,3-triazol-4-yl)ethyl]-*N*-methylamine, 165b.



In a 50 mL round-bottomed flask equipped with magnetic stirrer, a solution of **165b** (890 mg, 3.32 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with H_3PO_4 (85% purity, 5.70 mL, 49.4 mmol). The reaction mixture was stirred at r. t. for 1.5 h, cooled to 0 ºC with an ice bath, diluted with H_2O (15 mL), alkalinized dropwise to pH = 14 with 5 N NaOH aq. sol. and extracted with CH_2Cl_2 (3 x 35 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, to give the amine **167b** (495 mg, 89% yield) as a colorless oil.

R_f = 0.10 (Silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 167b·HCl.

In a 10 mL round-bottomed flask, **167b** (40 mg, 0.26 mmol) was dissolved in CH_2Cl_2 (6 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 1.04 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **167b**·HCl (41 mg) as a beige sticky solid.

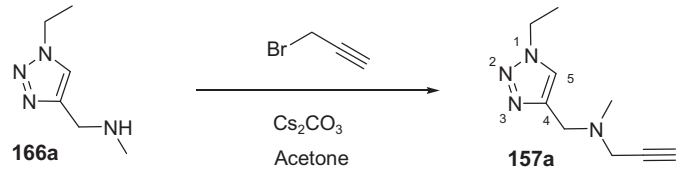
IR (neat) v: 3500–2500 (max. at 3374, 2981, 2937, 2746, 2692, 2446, $^+$NH, NH and CH st), 1236 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.49 (t, *J* = 7.2 Hz, 3H, N–CH_2–CH_3), 2.38 (s, 3H, 5–CH_3), 2.76 (s, 3H, NH–CH_3), 3.09 (t, *J* = 7.2 Hz, 2H, 4–CH_2–CH_2–NH), 3.34 (t, *J* = 7.2 Hz, 2H, 4–CH_2–CH_2–NH), 4.39 (q, *J* = 7.2 Hz, 2H, N–CH_2–CH_3), 4.86 (s, NH and $^+$NH).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 7.6 (CH_3, 5–CH_3), 15.0 (CH_3, N–CH_2–CH_3), 22.1 (CH_2, 4–CH_2–CH_2–NH), 33.7 (CH_3, NH–CH_3), 45.0 (CH_2, 4–CH_2–CH_2–NH), 49.0 (CH_2, N–CH_2–CH_3), 133.4 (C, C5), 140.1 (C, C4).

HRMS, ESI:
Calculated for ($C_8H_{16}N_4$ + H^+): 169.1448.
Observed: 169.1452.

Preparation of *N*-[(1-ethyl-1*H*-1,2,3-triazol-4-yl)methyl]-*N*-methyl-*N*-propargylamine, 157a.



In a 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of **166a** (85 mg, 0.61 mmol) was dissolved in anhydrous acetone (20 mL), cooled to 0 ºC with an ice bath and treated with Cs_2CO_3 (199 mg, 0.61 mmol) and propargyl bromide (80% in toluene, 0.09 mL, 0.61 mmol). The reaction mixture was stirred at r. t. overnight, then filtered and evaporated under reduced pressure to give an oil (161 mg), which was purified through column chromatography (silica gel, 40–60 µm, 20 g, Ø = 2.5 cm; #1–3, 100 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #4–8, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #9–14, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2; #15–20, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 97:3:0.2; #21–26, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 96:4:0.2; #27–32, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 90:10:0.2; #33–38, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 80:20:0.2), to provide the propargylamine **157a** (#11–13, 97 mg, 89%) as a colorless oil.

R_f = 0.33 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 157a·HCl.

In a 10 mL round-bottomed flask, **157a** (97 mg, 0.54 mmol) was dissolved in CH_2Cl_2 (4 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 2.18 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **157a**·HCl (99 mg) as a beige sticky solid.

IR (neat) ν: 3500–2300 (max. at 3412, 3205, 3126, 2987, 2941, 2568, 2477, 2381, $^+$NH and CH st), 2125 (C≡C st), 1213 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.56 (t, $J \approx 7.2$ Hz, 3H, N–CH_2–CH_3), 2.94 (s, 3H, N–CH_3), 3.39 (t, $J \approx 2.0$ Hz, 1H, propargyl CH), 4.11 (d, $J = 2.0$ Hz, 2H, propargyl CH_2), partially overlapped 4.50 (q, $J = 7.2$ Hz, 2H, N–CH_2–CH_3), partially overlapped 4.54 (s, 2H, 4–CH_2–N), 4.87 (s, $^+$NH), 8.26 (s, 1H, 5-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 15.7 (CH_3, N–CH_2–CH_3), 40.3 (CH_3, N–CH_3), 45.6 (CH_2, propargyl CH_2), 46.7 (CH_2, N–CH_2–CH_3), 50.3 (CH_2, 4–CH_2–N), 73.3 (C, propargyl C), 81.2 (CH, propargyl CH), 127.4 (CH, C5), 137.8 (C, C4).

HRMS, ESI:

Calculated for ($C_9H_{14}N_4 + H^+$): 179.1291.

Observed: 179.1289.

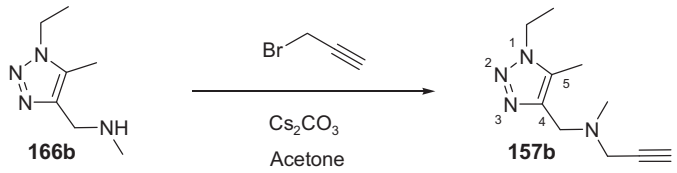
Elemental analysis: Calculated for $C_9H_{14}N_4 \cdot HCl \cdot 0.5 \cdot H_2O$:

C: 48.32%; H: 7.21%; N: 25.04%.

Observed: C: 48.82%; H: 7.41%; N: 23.33%.

Preparation of *N*-[(1-ethyl-5-methyl-1*H*-1,2,3-triazol-4-yl)methyl]-*N*-methyl-*N*-propargylamine, 157b.

a) At 0 ºC for 2 h.



In a 25 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of **166b** (128 mg, 0.83 mmol) was dissolved in anhydrous acetone (20 mL), cooled to 0 ºC with an ice bath and treated with Cs_2CO_3 (270 mg, 0.83 mmol) and propargyl bromide (80% in toluene, 0.12 mL, 0.83 mmol). The reaction mixture was stirred at 0 ºC for 2 h, then filtered and evaporated under reduced pressure to give a residue that was suspended in 5 N NaOH (10 mL) and extracted with EtOAc (2 x 15 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, to give the propargylamine **157b** (96 mg, 60% yield) as a yellow oil.

R_f = 0.55 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 157b·HCl.

In a 10 mL round-bottomed flask, **157b** (95 mg, 0.49 mmol) was dissolved in CH_2Cl_2 (5 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 1.98 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **157b**·HCl (96 mg) as a yellow sticky solid.

IR (neat) ν: 3500–2300 (max. at 3386, 3197, 2938, 2497, 2352, $^+$NH and CH st), 2123, (C≡C st), 1254 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.50 (t, *J* = 7.2 Hz, 3H, N–CH_2–CH_3), 2.45 (s, 3H, 5–CH_3), 3.01 (s, 3H, N–CH_3), 3.44 (t, *J* = 2.4 Hz, 1H, propargyl CH), 4.16 (d, *J* = 2.4 Hz, 2H, propargyl CH_2), 4.39 (q, *J* = 7.2 Hz, 2H, N–CH_2–CH_3), 4.52 (s, 2H, 4–CH_2–N), 4.84 (s, $^+$NH).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 8.0 (CH_3, 5–CH_3), 15.1 (CH_3, N–CH_2–CH_3), 40.3 (CH_3, N–CH_3), 44.5 (CH_2, propargyl CH_2), 45.6 (CH_2, N–CH_2–CH_3), 49.8 (CH_2, 4–CH_2–N), 73.1 (C, propargyl C), 81.5 (CH, propargyl CH), 135.1 (C, C5), 136.3 (C, C4).

HRMS, ESI:

Calculated for ($C_{10}H_{16}N_4 + H^+$): 193.1448.

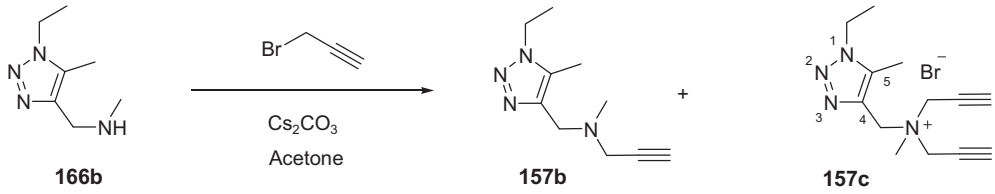
Observed: 193.1450.

Elemental analysis: Calculated for $C_{10}H_{16}N_4 \cdot HCl \cdot 0.5H_2O$:

C: 47.72%; H: 7.33%; N: 21.26%.

Observed: C: 47.30%; H: 7.86%; N: 22.06%.

b) At r. t. overnight: obtention of *N*-[(1-ethyl-5-methyl-1*H*-1,2,3-triazol-4-yl)methyl]-*N*-methyl-*N*,*N*-dipropargylammonium bromide, 157c.



In a 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of **166b** (117 mg, 0.76 mmol) was dissolved in anhydrous acetone (30 mL), cooled to 0 ºC with an ice bath and treated with Cs_2CO_3 (362 mg, 1.11 mmol) and propargyl bromide (80% in toluene, 0.16 mL, 1.11 mmol). The reaction mixture was stirred at r. t. overnight, then filtered and evaporated under reduced pressure to give an oil (358 mg), which was purified through column chromatography (silica gel, 40–60 µm, 15 g, Ø = 2.5 cm; #1–3, 100 mL, CH_2Cl_2 / 50% aq. NH_4OH 100:0.2; #4–9, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 99:1:0.2; #10–15, 200 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 98:2:0.2; #16–32, 700 mL, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 80:20:0.2), to provide the propargylamine **157b** (#8–10, 15 mg, 10% yield) as a colorless oil and dipropargylated **157c** (223 mg, 94% yield) as a brown oil.

$R_{f(\mathbf{157c})}$ = 0.04 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 157c.

In a 50 mL round-bottomed flask, **157c** (223 mg, 0.96 mmol) was dissolved in CH_2Cl_2 (20 mL), filtered with a PTFE filter (0.2 µm), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **157c** (213 mg) as a brownish oil.

IR (neat) v: 2124 (C≡C st), 1258 (N−N=N st) cm^{-1}.

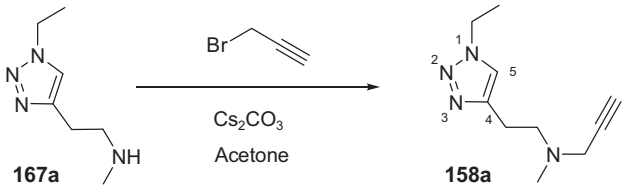
^{1}H NMR (400 MHz, CD_3OD) δ: 1.53 (t, *J* = 7.6 Hz, 3H, N–CH_2–CH_3), 2.73 (s, 3H, 5–CH_3), 2.85 (t, *J* = 2.4 Hz, 2H, propargyl CH), 3.42 (s, 3H, $^+$N–CH_3), 4.34 (q, *J* ≈ 7.6 Hz, 2H, N–CH_2–CH_3), 4.83 (dd, *J* = 16.0 Hz, *J'* = 2.4 Hz, 2H) and 4.91 (dd, *J* = 16.0 Hz, *J'* = 2.4 Hz, 2H) (propargyl CH_2), 5.34 (s, 2H, 4–CH_2–N^+)

^{13}C NMR (100.6 MHz, CD_3OD) δ: 8.8 (CH_3, 5–CH_3), 14.9 (CH_3, N–CH_2–CH_3), 43.6 (CH_2, N–CH_2–CH_3), 47.3 (CH_3, $^+$N–CH_3), 51.8 (2CH_2, propargyl CH_2), 55.7 (CH_2, 4–CH_2–N^+), 70.9 (2C, propargyl C), 80.3 (2CH, propargyl CH), 132.5 (C, C5), 136.5 (C, C4).

HRMS, ESI:
Calculated for ($C_{13}H_{19}N_4$ + H^+): 231.1604.

Observed: 231.1613.

Preparation of *N*-[2-(1-ethyl-1*H*-1,2,3-triazol-4-yl)ethyl]-*N*-methyl-*N*-propargylamine, 158a.



In a 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of **167a** (299 mg, 1.94 mmol) was dissolved in anhydrous acetone (30 mL), cooled to 0 ºC with an ice bath and treated with Cs_2CO_3 (630 mg, 1.94 mmol) and propargyl bromide (80% in toluene, 0.29 mL, 1.94 mmol). The reaction mixture was stirred at 0 ºC for 2.5 h, then filtered and evaporated under reduced pressure to give a residue that was suspended in 5 N NaOH (30 mL) and extracted with EtOAc (2 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, to give the propargylamine **158a** (220 mg, 59% yield) as a yellow oil.

R_f = 0.55 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 158a·HCl.

In a 25 mL round-bottomed flask, **158a** (184 mg, 0.95 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 3.84 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **158a**·HCl (189 mg) as a yellow sticky solid.

IR (neat) ν: 3500–2300 (max. at 3417, 3191, 2937, 2521, 2434, 2359, $^+$NH and CH st), 2121 (C≡C st), 1214 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.53 (t, *J* = 7.2 Hz, 3H, N–CH_2–CH_3), 3.05 (s, 3H, N–CH_3), 3.24 (t, *J* = 7.6 Hz, 2H, 4–CH_2–CH_2–N), 3.41 (t, *J* ≈ 2.8 Hz, 1H, propargyl CH), 3.62 (broad t, *J* ≈ 7.6 Hz, 2H, 4–CH_2–CH_2–N), 4.24 (d, *J* = 2.8 Hz, 2H, propargyl CH_2), 4.46 (q, *J* = 7.2 Hz, 2H, N–CH_2–CH_3), 4.87 (s, $^+$NH), 8.01 (s, 1H, 5-H).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 15.7 (CH_3, N–CH_2–*C*H_3), 21.6 (CH_2, 4–*C*H_2–CH_2–N), 40.7 (CH_3, N–CH_3), 46.3 (CH_2, propargyl CH_2), 46.8 (CH_2, N–*C*H_2–CH_3), 55.4 (CH_2, 4–CH_2–*C*H_2–N), 72.6 (C, propargyl C), 81.6 (CH, propargyl CH), 124.2 (CH, C5), 143.1 (C, C4).

HRMS, ESI:

Calculated for ($C_{10}H_{16}N_4$ + H^+): 193.1448.

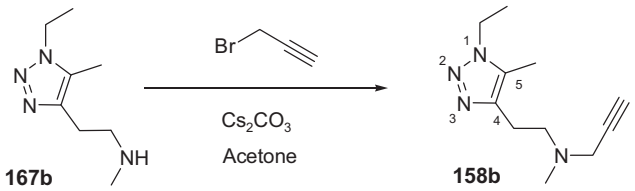
Observed: 193.1440.

Elemental analysis: Calculated for $C_{10}H_{16}N_4 \cdot HCl \cdot 1.5H_2O$:

C: 46.96%; H: 7.88%; N: 21.91%.

Observed: C: 47.44%; H: 7.68%; N: 21.85%.

Preparation of *N*-[2-(1-ethyl-5-methyl-1*H*-1,2,3-triazol-4-yl)ethyl]-*N*-methyl-*N*-propargylamine, 158b.



In a 50 mL round-bottomed flask equipped with an inert atmosphere and magnetic stirrer, a solution of **167b** (490 mg, 2.92 mmol) was dissolved in anhydrous acetone (40 mL), cooled to 0 ºC with an ice bath and treated with Cs_2CO_3 (952 mg, 2.92 mmol) and propargyl bromide (80% in toluene, 0.43 mL, 2.93 mmol). The reaction mixture was stirred at 0 ºC for 2.5 h, then filtered and evaporated under reduced pressure to give a residue that was suspended in 5 N NaOH (30 mL) and extracted with EtOAc (2 x 40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, to give the propargylamine **158b** (319 mg, 53% yield) as a yellow oil.

R_f = 0.59 (silica gel, 10 cm, CH_2Cl_2 / MeOH / 50% aq. NH_4OH 9:1:0.05).

Analytical sample of 158b·HCl.

In a 25 mL round-bottomed flask, **158b** (222 mg, 0.95 mmol) was dissolved in CH_2Cl_2 (12 mL), filtered with a PTFE filter (0.2 µm), treated with HCl / MeOH (0.75 N, 4.31 mL), evaporated under reduced pressure and washed with pentane (3 x 2 mL) to give, after drying under standard conditions, **158b**·HCl (230 mg) as a yellow sticky solid.

IR (neat) ν: 3500–2300 (max. at 3406, 3181, 2981, 2932, 2583, 2518, 2461, 2408, 2366, $^+$NH and CH st), 2123 (C≡C st), 1215 (N−N=N st) cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.56 (t, *J* = 7.2 Hz, 3H, N–CH_2–C*H*$_3$), 2.50 (s, 3H, 5–CH_3), 3.08 (s, 3H, N–C*H*$_3$), 3.35 (broad t, *J* = 7.8 Hz, 2H, 4–C*H*$_2$–CH_2–N), 3.45 (t, *J* ≈ 2.8 Hz, 1H, propargyl CH), 3.63 (t, *J* = 7.8 Hz, 2H, 4–CH_2–C*H*$_2$–N), 4.28 (d, *J* = 2.8 Hz, 2H, propargyl CH_2), 4.51 (q, *J* = 7.2 Hz, 2H, N–C*H*$_2$–CH_3), 4.94 (s, $^+$NH).

^{13}C NMR (100.6 MHz, CD_3OD) δ: 8.0 (CH_3, 5-CH_3), 14.4 (CH_3, N–CH_2–*C*H$_3$), 20.0 (CH_2, 4–*C*H$_2$–CH_2–N), 40.7 (CH_3, N–CH_3), 46.3 (CH_2, propargyl CH_2), 46.4 (CH_2, N–*C*H$_2$–CH_3), 54.0 (CH_2, 4–CH_2–*C*H$_2$–N), 72.5 (C, propargyl C), 81.8 (CH, propargyl CH), 136.3 (C, C5), 137.8 (C, C4).

HRMS, ESI:

Calculated for ($C_{11}H_{18}N_4 + H^+$): 207.1604.

Observed: 207.1609.

Elemental analysis: Calculated for $C_{11}H_{18}N_4 \cdot HCl \cdot 1.75H_2O$:

C: 48.17%; H: 8.27%; N: 20.43%.

Observed: C: 48.14%; H: 7.96%; N: 19.81%.

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7. COMMUNICATION OF RESULTS

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