Síntesi i aplicació de ψ-dipèptids amb estructura de 3 aminopiperidona. Síntesi de ψ-melanotans

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SÍNTESI I APLICACIÓ DE ψ-DIPÈPTIDS AMB ESTRUCTURA DE 3-AMINOPIPERIDONA. SÍNTESI DE ψ-MELANOTANS

JORDI MAS PONS

2010

EXPERIMENTAL SECTION

CHAPTER 7. EXPERIMENTAL SECTION

7.1. Main solvents and reagents

Solvents: • DCM, THF, Hexane, EtOAc • MeOH, Et_2O • Toluene, $CHCl_3$ • Benzene • N,N-dimetilformamide • Piperidine	 SDS (analytical grade) SDS (synthetical grade) Panreac Merck (puris.) SDS (Peptide Synthesis grade) SDS (Peptide Synthesis grade)
HPLC solvents: • Acetonitril • H_2O • Methanol • TFA	 J. T. Baker, Ultra Gradient HPLC grade Mili-Q (Millipore filtration system) Chromanorm for HPLC Isocratic Grade Fluorochem
Silica	SDS (Silice 60, 35-70 μm)
TLC	Merck (aluminium sheets, silica gel 60 F_{254}
Celite®	Fluka
Amino acids	Novabiochem, IRIS Biotech
Coupling agents: • DIPCDI • PyBOP • HOBt • HOAt • DIEA • CDI	 Fluka Novabiochem Novabiochem Novabiochem Sigma Sigma-Aldrich
Resins: • Rink Amide • Sieber Amide	 IRIS Biotech Novabiochem

Table I. Main reagents and solvents used in experimental procedures.Reagents used in this

PhD Thesis has been purchased to the main providers as Sigma-Aldrich, Acros…Purification of solvents and reagents has been done if necessary using general protocols described in literature.[195]

7.2. Materials and Instrumentation

RMN	Varian Mercury-400 MHz Bruker Avance 300 MHz Bruker Avance 400 MHz
IR	Thermo Nicolet FT-R Nexus
Mass spectroscopy	• MALDI Voyager DE RP time-of-flight (TOF) spectrometer (Applied Biosystems, Foster City, USA) using ACH matrix. • ESI , CI or EI recorded on HP 5989 A (Agilent Technologies)
Analitical HPLC	Waters Alliance 2695 (Waters, MA, USA) with PDA 995 detector. Column: Symmetry C_{18} (4.6 x 150 mm, 5 μm). Solvents: H_2O with 0.045% TFA and ACN with 0.036% TFA; flow: 1 mL/min
Semipreparative HPLC	Waters chromatography system with dual absorbance detector (Waters 2487), using automatic injection (Waters 2700). Column: Symmetry C_{18} (19 x 100 mm, 5 μm). Solvents: H_2O with 0.1% TFA and ACN with 0.05% TFA. Flow: 15 mL/min
HPLC-MS	Waters Alliance 2796 with a dual absorbance detector (Waters 2487) and ESI-MS Micromass ZQ (Waters) chromatography system. Column: Symmetry 300 C_{18} (3.9 x 150 mm, 5 μm). Solvents: H_2O and ACN with 0.1% formic acid. Flow: 0.3 mL/min
Polarimeter	Perkin Elmer 241 Polarimeter, Na lamp (D).
Lyophilizer	Virtis Freezamobile 12 EL

Table II. Main instruments used in experimental procedures.

[195] Armarego, W. L. F.; Perrin, D. D., Purification of Laboratory Chemicals, Butterworth Heinemann.

Nuclear Magnetic Ressonance

1H NMR spectra were recorded in $CDCl_3$ unless otherwise stated and were referenced either to TMS or to the residual solvent peak, and peaks are reported in ppm downfield of TMS. Multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), double doublet (dd), triple doublet (td), doble doublet doublet (ddd), multiplet (m), broad signal (bs). Coupling constants (*J*) were reported in hertz.

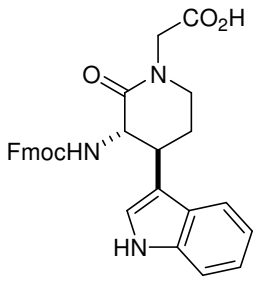
13C NMR spectra were referenced to the solvent peak and are reported in ppm downfield of TMS.

Infrared spectra

Only characteristic absorptions are reported in cm-1.

7.3. Experimental procedures of chapter 3

Àcid *trans*- *i cis*-3-[9-Fluorenilmetoxicarbonilamino)-4-(3-indolil)-2-oxopiperidin acètic (13)



***trans*-13**

A solution of compound **trans-27** (155 mg, 0.27 mmol, 1 eq.) in 10 mL of a ^{i}PrOH/H_2O/AcOH (1:1:2) mixture was warmed to 100 ºC and stirred for 24 h. Then, solvent was evaporated obtaining ***trans*-13** (121 mg, 87%).

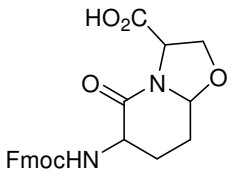
IR (KBr): ν 3400, 1712, 1640, 1246 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 2.15-2.36 (m, 2H, H-5), 3.33-3.45 (m, 1H, H-6), 3.62-3.77 (m, 2H, H-4 i H-6), 4.02 and 4.31 (2d, J_{AB}=17.0 Hz, 1H each one, CH_2-CO_2H), 4.06-4.25 (m, 3H, Fmoc-9, CH_2-Fmoc), 4.43-4.46 (m, 1H, H-3), 5.85 (bs, 1H, NH), 7.04-7.12 (m, 1H, H-5'), 7.16 (t, *J* = 7.2 Hz, 2H, H-6' and H-2'), 7.33 (t, *J* = 7.6 Hz, 3H, H-7' and Fmoc-2 and -7), 7.40 (d, *J* = 7.2 Hz, 2H, Fmoc-3 and -6), 7.43 (d, *J* = 7.0 Hz, 2H, Fmoc-1 and -8), 7.59 (d, *J* = 7.2 Hz, 1H, H-4'), 7.70 (d, *J* = 7.5 Hz, 2H, Fmoc-4 and -5), 8,22 (bs, 1H, NH).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 29.9 (C-5), 35.8 (C-4), 47.1 (Fmoc-9), 48.6 (C-6), 50.0 (CH_2-CO_2H), 57.3 (C-3), 67.2 (CH_2-Fmoc), 111.8 (CH-7'), 116.3 (C-3'), 118.7 (CH-4'), 119.5 (CH5'), 120.0 (Fmoc-4 and -5), 121.6 (CH-2'), 122.1 (CH-6'), 125.5 (C-3'a), 127.2 (Fmoc-1 and -8), 127.7 (Fmoc-3 and -6), 136.4 (C-7'a), 141.3 (Fmoc-4a and -4b) and 144.0 (Fmoc-8a and -9a), 157.2 (CO-carbamate), 171.1 and 172.0 (CO-acid and lactam).

MALDI-TOF (ACH): 548,17 $[M+K]^+$; 532,19 $[M+Na]^+$; 510,20 $[M+H]^+$.

(3S, 6S, 9S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxo-7,1-oxazabicyclo[4.3.0]-nonan-9-carboxylic acid (15)



15

Methyl esther **44** (550 mg; 1,26 mmol) was taken up with isopropanol (20 mL) and THF (7 mL) and $CaCl_2$ was added. Separately $LiOH{\cdot}H_2O$ (212 mg; 5,04 mmol) was dissolved in water (8 mL). The aqueous solution was then added to the reaction mixture and stirred as a cloudy white solution for 2h. The organic solvents were removed under vacuum and the resulting residue was taken up with HCl 1N (15 mL) and extracted with CH_2Cl_2 (5 x 15 mL). The combined organic layers were then washed with Brine, dried over $MgSO_4$ and concentrated to white solid **15** (492 mg, 92%).

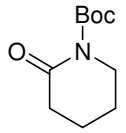
IR (NaCl): ν 3330, 3063, 1722, 1665 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.68 (bs, 1H, H-8), 1.89 (bs, 1H, H-7), 2.38 (bs, 2H, H-7 and H-8), 4.11 (m, 2H, H-2 and H-6), 4.22 (t, *J* = 6.3 Hz, 1H, Fmoc-9), 4.42 (m, 3H, H-2 and Fmoc-CH_2), 4.65 (bs, 1H, H-3), 4.86 (bs, 1H, H-9), 5.65 (bs, 1H, NH), 7.31 (td, *J* = 7.4 and 0.9 Hz, 2H, Fmoc-3 and -6), 7.40 (t, *J* = 7.5 Hz, 2H, Fmoc-2 and -7), 7.59 (d, *J* = 7.4 Hz, 2H, Fmoc-4 and -5), 7.75 (d, *J* = 7.5 Hz, 2H, Fmoc-1 and -8).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 24.9 (C-7), 26.9 (C-8), 47.3 (Fmoc-9), 52.1 (C-6), 56.9 (C-3), 67.4 (Fmoc-CH_2), 67.8 (C-2), 88.2 (C-9), 120.2 (Fmoc-1 and -8), 125.3 (Fmoc-4 and -5), 127.3 (Fmoc-3 and -6), 128.0 (Fmoc-2 and -7), 141.5 (Fmoc-4a and -4b), 143.9 (Fmoc-8a and -9a), 156.6 (N-CO), 163.2 (C-5), 170.7 (CO_2H).

ESI-MS: m/z 445.3 $(M+Na)^+$, 423.1$(M+H)^+$, 201.2 $(M\text{-Fmoc})^+$.

1-(*tert*-butoxyicarbonyl)piperidin-2-one (17)



17

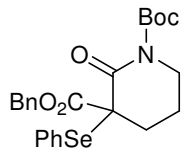
To a solution of δ-valerolactam (20 g, 201.7 mmol) in anydrous CH_2Cl_2 (100 mL), Et_3N (56.2 mL, 403.5 mmol), Boc_2O (88 g, 403.5 mmol) and 4-DMAP (24.6 g, 201.7 mmol) were added and the mixture was stirred under argon atmosphere for 20 h at rt. Product **17** (32.2 g, 81%) was obtained after column chromatography (hexane/ethyl acetate 1:1) and lyophilization.

^{1}H RMN (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, CH_3-^{t}Bu); 1.83 (m, 4H, H-4, H-5); 2.52 (td, *J* = 4.8 and 1.6 Hz, 2H, H-3); 3.67 (td, *J* = 5.6 and 1.0 Hz, 2H, H-6).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 20.7 (C-5), 22.9 (C-4), 28.2 (CH_3-^{t}Bu), 35.0 (C-3), 46.5 (C-6), 82.9 (C-^{t}Bu), 152.9 (CO-lactam) and 171.5 (CO_2^{t}Bu).

ESI-MS (+) m/z: 144, 200 $[M+H]^+$, 222 $[M+Na]^+$.

3-(Benziloxicarbonil)-1-(*tert*-butoxicarbonil)-3-(fenilselenil)piperidin-2-ona (18)



18

To a solution of N-(tert-butoxycarbonyl)-2-piperidone **17** (8.8 g, 44.1 mmol, 1 eq) in freshly distilled THF (200 mL) under argon atmosphere at -78 ºC, a solution of 1M LHMDS in THF (48.6 mL, 48.6 mmol, 1.1 eq) was added and the mixture was stirred for 30 min at -78 ºC. Benzyl chloroformate (8.2 mL, 61.8 mmol, 1.4 eq) was then added dropwise and stirring was continued for 30 min. Next, a solution of 1M LHMDS in THF was added (48.6 mL, 48.6 mmol, 1.1 eq) and the mixture was allowed to stir 30 min at -78 ºC. Then, a solution of phenylselanyl chloride (11.8 g, 61.8 mmol, 1.4 eq) in dry THF (80 mL) was added and the mixture was allowed to rt for 2 h. The reaction was quenched with 1N solution of HCl (200 mL), extracted with ethyl acetate and washed with saturated solution of $NaHCO_3$ and Brine. Organic phase was dried with Na_2SO_4, filtered and evaporated under reduced pressure. Crude product was purified by column chromatography (hexane/ethyl acetate 8:2) obtaining piperidone **18** (19.64 g, 91 %) as yellow oil.

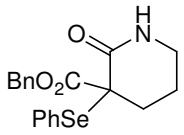
IR (NaCl): ν 1719 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, CH_3-^{t}Bu), 1.58 (m, 1H, H-5 ax), 1.72 (dq, *J* = 14.3 and 5.8 Hz, 1H, H-5 eq), 1.92 (ddd, *J* = 13.9, 10.1 and 5.7 Hz, 1H, H-4 ax), 2.19 (dt, *J* = 13.6 Hz and 5.6 Hz, 1H, H-4 eq), 3.30 (dt, *J* = 13.5 and 6.4 Hz, 1H, H-6 eq), 3.53 (ddd, *J* = 13.2, 8.0 and 5.2 Hz, 1H, H-6 ax), 5.20 and 5.09 (2 d, *J* = 12.4 Hz, 1H each one, CH_2-Ph), 7.16-7.49 (m, 10H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 21.1 (C-5), 28.2 (CH_3-^{t}Bu), 32.0 (C-4), 45.5 (C-6), 57.1 (C-3), 67.9 (CH_2-Ph), 83.7 (C-*t*-butyl), 126.7 and 135.5 (C_6H_5-*ipso*-Bn i C_6H_5-*ipso*-Ph), 128.3, 128.5, 128.7, 129.0, 129.9 and 138.7 (C_6H_5), 153.0 (CO-lactam), 168.0 and 169.6 (CO-esters).

EI-MS m/z (%): 489 (8) $[M+H]^+$, 389 (6), 157 (10), 91 (100), 57 (84).

3-(Benziloxicarbonil)-1-(tert-butioxicarbonil)-5,6-dihidropiridin-2-(1H)-ona (19)



19

2,6-lutidine (9.2 mL, 79.2 mmol, 2 eq.) was added to a solution of **18** (19.32 g, 39.6 mmol, 1 eq.) in anhydrous CH_2Cl_2 (100 mL). To this mixture TMSOTf (10.75 mL, 59.4 mmol, 1.5 eq.) was added slowly and the mixture was stirred 1h at rt. The reaction was quenched with saturated solution of NH_4Cl and extracted with Et_2O. Organic phase was washed with H_2O and Brine, dried over Na_2SO_4 and solvent was evaporated to dryness. Product **19** (11.97 g, 80%) was obtained by precipitation in Et_2O as a white solid.

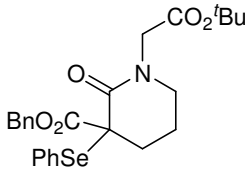
IR (NaCl): ν 1736, 1669 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.56 (m, 1H, H-5 ax), 1.79 (m, 1H, H-5 eq), 1.94 (ddd, *J* = 13.7, 11.9 and 3.4 Hz, 1H, H-4 ax), 2.16 (ddd, *J* = 13.7, 4.8 and 3.7 Hz, 1H, H-4 eq), 3.24 (m, 2H, H-6), 5.80 (bs, 1H, NH), 7.22- 7.56 (m, 10H, C_6H_5)

^{13}C-RMN (100 MHz, $CDCl_3$): δ 20.6 (C-5), 32.4 (C-4), 42.4 (C-6), 53.7 (C-3), 67.8 (CH_2-Ph), 126.9 and 135.7 (C_6H_5-*ipso*-Bn i C_6H_5-*ipso*-Ph), 128.3, 128.4, 128.7, 128.9 and 138.6 (C_6H_5), 168.7 i 170.7 (CO-ester and lactam).

EI-MS m/z (%): 389 (16), $[M+H]^+$, 157 (16), 91 (100), 77 (16), 65 (19).

3-(Benziloxicarbonil)-1-[(tert-butoxicarbonil)metil]-3-(fenilselanil)piperidin-2-ona (20)



20

To a solution of 19 (10 g, 25.7 mmol, 1 eq.) in anhydrous CH_3CN (100 mL) under inert atmosphere, TBAB (33.2 g, 103.0 mmol, 4 eq.) and K_2CO_3 (35.6 g, 257 mmol, 10 eq.) were added. Tert-butyl bromoacetate (5.7 mL, 38.6 mmol, 1.5 eq.) was then added and the mixture was allowed to stir vigorously for 36 h at rt. After that time the reaction was filtered and solvent was evaporated under reduced pressure. The resulting crude was purified by column chromatography (hexane/ethyl acetate 6:4) to obtain product 20 (12.19 g, 91%).

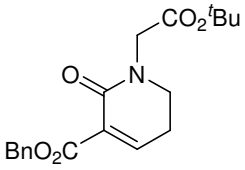
IR (NaCl): ν 1739, 1649 cm^{-1}.

^{1}H-RMN (**400 MHz, $CDCl_3$):** δ 1.47 (s, 9H, CH_3-^{t}Bu), 1.63 (m, 1H, H-5 ax), 1.85 (d, *J* = 14.0 Hz, 1H, H-5 eq), 1.99 (ddd, *J* = 13.4, 11.9 and 3.6 Hz, 1H, H-4 ax), 2.15 (dt, *J* = 13.8 Hz and 4.4 Hz, 1H, H-4 eq), 3.27 (ddd, *J* = 11.6; 6.2 and 4.0 Hz, 1H, H-6 eq), 3.33 (ddd; *J* = 11.7; 9.9 and 5.3 Hz, 1H, H-6 ax), 3.87 and 4.13 (2 d, *J* = 17.0 Hz, 1H each one, CH_2-CO_2*t*Bu), 5.22 (s, 2H, CH_2-Ph), 7.21-7.53 (m, 10 H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 21.1 (C-5), 28.3 (CH_3-^{t}Bu), 32.5 (C-4), 49.2 (C-6), 49.2 (CH_2-CO_2^{t}Bu), 53.9 (C-3), 67.7 (CH_2-Ph), 82.2 (C-^{t}Bu), 127.3 and 135.8 (C_6H_5-*ipso*-Bn and C_6H_5-*ipso*-Ph), 128.2, 128.3, 128.6, 128.8 and 138.6 (C_6H_5), 167.0, 167.8 i 170.6 (CO-esters and lactam).

CI-MS m/z: 520 $[M+NH_4]^+$, 503 $[M+H]^+$, 502 $[M]^+$.

3-(benziloxicarbonil)-1-[(tert-butoxicarbonil)metil]-5,6-dihidropiridin-2(1*H*)-ona (21)



21

A solution of *m*CPBA (6.3 g, 36.5 mmol, 1.5 eq.) in CH_2Cl_2 (50 mL) was slowly added to a solution of piperidone **20** (12.19 g, 24.3 mmol, 1 eq) in CH_2Cl_2 (70 mL) at 0 ºC and the mixture was stirred for 15 min at this temperature and 1h at rt. Then saturated solution of $NaHCO_3$ (40 mL) was added and the reaction is allowed to stir 10 min. Aqueous phase was extracted with CH_2Cl_2 and the collected organic phases were washed with saturated solution of NaCl, dried over anh. Na_2SO_4 and evaporated to dryness. Product **21** (8.6 g) was obtained as a colorless oil and was used without further purification.

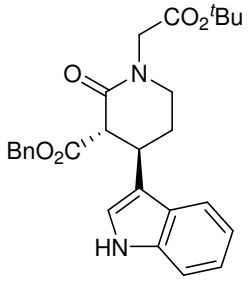
IR (NaCl): ν 1739, 1661 cm^{-1}.

1H-RMN (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, CH_3), 2.56 (td, *J* = 6.8 and 4.5 Hz, 2H, H-5), 3.50 (t, *J* = 6.9 Hz, 2H, H-6), 4.13 (s, 2H, CH_2-$CO_2{}^t$Bu), 5.26 (s, 2H, CH_2-Ph), 7.25-7.45 (m, 6H, C_6H_5 and H-4).

13C-RMN (100 MHz, $CDCl_3$): δ 24.6 (C-5), 28.3 (CH_3-^{t}Bu), 46.1 (C-6), 49.1 (CH_2-$CO_2{}^t$Bu). 67,0 (CH_2-Ph), 82.2 (C-^{t}Bu), 128.3, 128.4 and 128.7 (C_6H_5), 129.2 and 136.1 (C_6H_5-*ipso* and C-3), 147.5 (C-4), 161.2, 164.2 and 168.4 (CO-esters and lactam).

CI-MS m/z: 345 [M+H]+, 244, 91.

***trans*/*cis*-(3-benziloxicarbonil)-1-[(tert-butoxicarbonil)metil]-4-(3-indolil)piperidin-2-ona (22)**



22

Dihydropiperidone **21** (6 g, 17.4 mmol, 1 eq.) was placed in a 100 mL round bottom flask and was solved in anh. CH_2Cl_2 under Ar atmosphere. Montmorillonite KSF® (Aldrich, 3.5 g) and indole (2.05 g, 17.4 mmol, 1 eq.) were then added and the mixture was stirred for 24 h at rt. Then, the mixture is filtered over Celite and solvent was evaporated. The resulting crude was purified by column chromatography (hexane/ethyl acetate 6:4) and product **22** (4.77 g, 76%) was obtained as a mixture of diastereomers with a 9:1 (*trans*:*cis*) ratio.

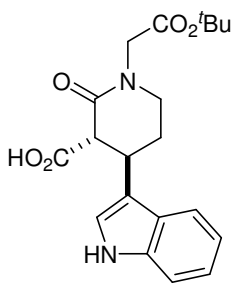
IR (NaCl): ν 1739, 1733, 1650, 1640, 1634 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.46 (s, 9H, $CH_3{}^tBu$), 2.12 (m, 1H, H-5 ax), 2.30 (dq, *J* = 4.0 and 13.5 Hz, 1H, H-5 eq), 3.38 (ddd, *J* = 4.0, 5.5 and 11.7 Hz, 1H, H-6eq), 3.57 (td, *J* = 4.5 i 11.5 Hz, 1H, H6ax), 3.85 (m, 2H, H-3 and H-4), 4.02 (m, 2H, H-3 and H-4), 3.86 and 4.26 (2d, *J* = 17.0 Hz, 2H each one, $CH_2CO_2{}^tBu$), 5.02 and 5.08 (2d, *J* = 12.4 Hz, 2H each one, CH_2Ph), 6.97 (d, *J* = 8.5 Hz, 1H, H-2), 6.98 (d, *J* = 2.0 Hz, 1H, H-2'), 7.09 (t, *J* = 8.0 Hz, 1H, H5'), 7.18 (t, *J* = 8.0 Hz, 1H, H-6'), 7.35 (d, *J* = 8.0 Hz; 1H, H-7'), 7.57 (d, *J* = 8.0 Hz, 1H, H-4'), 8.12 (bs, 1H, NH).

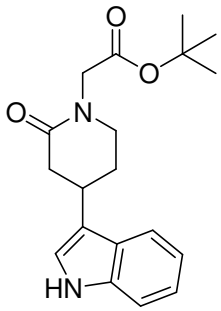
^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.3 (CH_3-^{t}Bu), 29.4 (C-5), 34.5 (C-4), 48.3 (C-6), 49.7 (CH_2-$CO_2{}^t$Bu), 55.9 (C-3), 67.0 (CH_2-Ph), 82.3 (C-^{t}Bu), 111.7 (CH-7'), 116.4 (C-3'), 118.9 (CH-4'), 119.8 (CH-5'), 121.5 (CH-2'), 122.5 (C-6'), 126.2 (C-3'a), 127.8, 127.9, 128.0, 128.3 and 128.5 (C_6H_5), 135.8 (C_6H_5-*ipso*), 136.6 (C-7'a), 166.8, 168.0 and 170.4 (CO-esters and lactam).

MALDI-TOF (ACH) m/z: 501 $[M+K]^+$, 485 $[M+Na]^+$.

Acids *trans- and cis*-1-[(tert-butoxicarbonil)metil]-4-(3-indolil)-2-oxopiperidina 3- carboxílics (23)



23



23a

To a solution of piperidone ***trans*/*cis*-22** (1.5 g, 3.24 mmol, 1 eq.) in CH_2Cl_2 (40 mL), 10% Pd/C (0.6 g) was added and the mixture was hydrogenated for 3h at atmospheric pressure. The reaction was filtered over Celite and solvent was evaporated under reduced pressure to give product ***trans*/*cis*-23** (1.18 g, 98%) as colorless oil. Piperidone **23a** was obtained as byproduct when the evaporation of the solvent was done while heating the water bath of the rotaevaporator.

***Compound trans*/*cis*-23:**

IR (NaCl): ν 3357, 1737, 1636, 1631 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.47 (s, 18H, CH_3-^{t}Bu), 2.10-2.38 (m, 4H, H-5), 3.22-3.43 (m, 4H, H-6), 3.67 (d, *J* = 5.2 Hz, 1H, H-3), 3.77 (d, *J* = 6Hz, 1H, H-3), 3.85 and 4.28 (2d, J_{AB} = 16.8 Hz, 1H each one, CH_2-CO_2^{t}Bu), 3.91 and 4.10 (2d, J_{AB} = 17.0 Hz, 1H each one, CH_2-CO_2^{t}Bu), 4.12-4.18 (m, 1H, H-4), 4.40-4.46 (m, 1H, H-4), 7.04-7.15 (m, 4H, H-2' i H-5'), 7.16-7.22 (m, 2H, H-6'), 7.32-7.39 (m, 2H, H-7'), 7.57-7.64 (m, 2H, H-4'), 8.19 (bs, 1H, NH), 8.28 (bs, 1H, NH).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 27.7 and 27.8 (C-5), 28.3 (CH_3-^{t}Bu), 31.3 and 31.7 (C-4), 47.3 i 47.6 (C-6), 48.1 and 53.0 (C-3), 50.3 i 50.6 (CH_2-CO_2^{t}Bu), 82.8 i 82.9 (C-^{t}Bu), 111.5 and 111.7 (CH-7'), 116.3 (C-3'), 118.8 i 118.9 (CH-4'), 119.7 i 119.8, 121.3 and 121.9 (C-5' i C-6'), 122.5 i 122.6 (C-2'), 126.1 and 126.8 (C-3'a), 136.5 i 136.6 (C-7'a), 167.0, 167.3, 168.9, 169.0, 169.5 and 172.1 (CO-ester, lactam and acid).

EI-MS m/z: 182 (11), 165 (1), 137 (34), 109 (21), 57 (100)

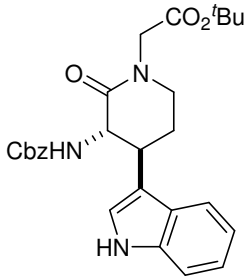
Compound 23a:

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.48 (s, 9H, CH_3-^{t}Bu), 2.02-2.11 (m, 1H, H-5), 2.26-2.35 (m, 1H, H-5), 2.65 (dd, *J* = 17.4 and 9.6 Hz, 1H, H-3ax), 2.92 (ddd, *J* = 17.4, 5.4 and 1.5 Hz, 1H, H-3eq), 3.35 (dt, *J* = 11.6 and 5.0 Hz, 1H, H-4), 3.46-3.55 (m, 2H, H-6); 4.03 and 4.11 (2d, J_{AB} = 17.0 Hz; 1H each one, CH_2-CO_2^{t}Bu), 7.02 (d, *J* = 2.4 Hz, 1H, H-2'), 7.11 (td, *J* = 7.6 and 0.8 Hz, 1H, H-5'), 7.20 (td, *J* = 7.2 and 0.8 Hz, 1H, H-6'), 7.38 (d, *J* = 8.0 Hz, 1H, H-7'), 7.59 (d, *J*=7.8 Hz, 1H, H-4'), 8,34 (bs, 1H, NH-indole).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.3 (CH_3-^{t}Bu), 29.8 (C-5), 30.4 (C-4), 38.7 (C-3), 48.4 (C-6), 49.6 (CH_2-CO_2^{t}Bu), 82.3 (C^{t}Bu), 111.9 (CH-7'), 118.0 (C-3'), 118.8 (CH-4'), 119.3 (CH-5'), 120.9 (CH-2'), 122.4 (CH-6'), 126.4 (C-3'a), 136.9 (C-7'a), 168.6 and 170.9 (CO-ester and lactam).

ESI-MS (+) m/z: 329.3 $(M+H)^+$, 351.2 $(M+Na)^+$, 657.5 $(2M+H)^+$.

***trans*- i *cis*-(3-Benziloxicarbonilamino)-1-[(*tert*-butoxicarbonil)metil]-4-(3-indolil)piperidin-2-ona (25)**



***trans*-25**

To a solution of piperidone **23** (1.71 g, 4.6 mmol, 1 eq.) in anhydrous benzene (30 mL), DPPA (2.47 mL, 11.5 mmol, 2.5 eq.) and Et_3N (1.6 mL, 11.5 mmol, 2.5 eq.) were added and the mixture was stirred at 50 ºC for 2 h. After that time, benzyl alcohol (1.2 mL, 11.5 mmol, 2.5 eq.) and dibutyltin dilaurate (0.28 mL, 0.46 mmol, 0.1 eq.) were added and the mixture was allowed to stir at 80 ºC for 4 h. After cooling to rt, Et_2O was added and the organic phase was washed with H_2O and Brine, dried with anh. Na_2SO_4 and evaporated under reduced pressure. Product ***trans*-25** (1.25 g, 56%) was obtained after column chromatography of the crude (hexanes/ethyl acetate 6:4).

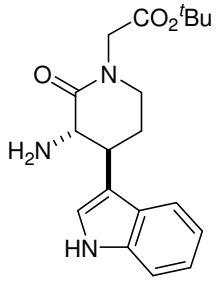
IR (NaCl): ν 3401, 1735, 1725, 1648 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.48 (s, 9H, $CH_3{}^tBu$), 2.13-2.33 (m, 2H, H-5), 3.30-3.40 (m, 1H, H-6), 3.45-3.53 (m, 2H, H-4 and H-6), 4.06 (s, 2H, $CH_2CO_2{}^tBu$), 4.40 (t, J = 10.0 Hz, 1H, H-3), 4.90 and 4.97 (2d, J_{AB} = 12.4 Hz, 1H each one, CH_2Ph), 5.25 (bs, 1H, NH-carbamate), 6.99-7.09 (m, 2H, H-2' i H-5'), 7.10-7.16 (m, 2H, C_6H_5), 7.17 (t, J = 8.0 Hz, 1H, H-6'), 7.22-7.27 (m, 3H, C_6H_5), 7.35 (d, J = 8.2 Hz, 1H, H-7'), 7.52 (d, J = 7.9 Hz, 1H, H-4'), 8.44 (bs, 1H, NH-indole).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.4 ($CH_3{}^tBu$), 30.6 (C-5), 38.6 (C-4), 48.2 (C-6), 49.8 ($CH_2CO_2{}^tBu$), 57.3 (C-3), 66.9 (CH_2Ph), 82.3 ($C{}^tBu$), 111.6 (CH-7'), 117.0 (C-3'), 119.0 (CH-4'), 119.8 (CH-5'), 120.7 (CH-2'), 122.4 (CH-6'), 126.2 (C-3'a), 128.0 and 128.6 (C_6H_5), 136.7 (C-7'a), 156.2 (CO-carbamate), 168.3 and 169.8 (CO-ester and lactam).

CI-MS m/z : 478 $[M+H]^+$, 495 $[M+NH_4]^+$.

***trans*- i *cis*-3-Amino-1-[(tert-butoxicarbonil)metil]-4-(3-indolil)piperidin-2-ona (11)**



26

Lactam **25** (491 mg, 1.02 mmol, 1eq.) was dissolved in CH3OH (30 mL) and hydrogenated at atmospheric pressure for 3h using 10% Pd/C (160 mg) as catalyst. Then, the mixture was filtered and solvent was evaporated to dryness obtaining the aminopiperidone **26** (330 mg, 95%) as colorless oil.

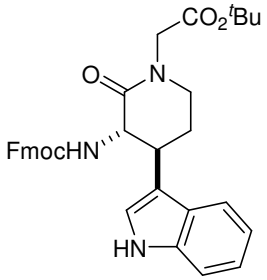
IR (NaCl): ν 3303, 1739, 1650 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.49 (s, 9H, CH_3-^{t}Bu), 2.14-2.21 (m, 1H, H-5), 2.28-2.38 (m, 3H, H-5 and NH_2), 3.28-3.43 (m, 2H, H-4 and H-6), 3.58 (dd, *J* = 11.4 and 4.0 Hz, 1H, H-6ax), 3.77 (d, *J* = 10.4 Hz, 1H, H-3), 3.94 and 4.17 (2d, J_{AB} = 17.0 Hz, 1H each one, CH_2-CO_2^{t}Bu), 7.10 (m, 2H, H-2' and H-5'), 7.19 (t, *J* = 7.6 Hz, 1H, H-6'), 7.39 (d, J = 8.0 Hz; 1H, H-7'), 7.66 (d, *J* = 8.0 Hz; 1H, H-4'), 8.56 (bs, 1H, NH-indole).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.3 (CH_3-^{t}Bu), 29.0 (C-5), 38.6 (C-4), 48.5 (C-6), 49.6 (CH_2-CO_2^{t}Bu), 56.8 (C-3), 82.3 (C-^{t}Bu), 111.8 (CH-7'), 116.2 (C-3'), 119.3 (CH-4'), 119.7 (CH-5'), 122.3 (CH-2'), 122.4 (CH-6'), 126.4 (C-3'a), 136.8 (C-7'a), 168.3 (CO-lactam), 174.2 (CO-ester).

EM-ESI m/z (%): 344 $[M+H]^+$, 288 $[(M-^{t}Bu)+H]^+$.

***Trans*- i *cis*-3-[9-Fluorenilmetoxicarbonilamino-1-[(*tert*-butoxicarbonil)metil]-4-(3-indolil)piperidin-2-ona (12)**



***trans*-27**

To a 100 mL round bottom flask, aminopiperidone **26** (330 mg, 0.96 mmol, 1eq.) is dissolved in acetone (15 mL). $NaHCO_3$ (120 mg, 1.44 mmol, 1.5 eq.) and Fmoc-OSu (482 mg, 1.44 mmol, 1.5 eq.) were then added and the mixture is stirred overnight at rt. Solvent was evaporated and resulting residue was redissolved in CH_2Cl_2 and washed with 0.1N HCl and H_2O. Organic phase was dried with anh. Na_2SO_4, filtered and evaporated. Product ***trans*-27** is obtained (390 mg, 72%) after silica gel column chromatography (hexane/ethyl acetate 7:3) as white foam.

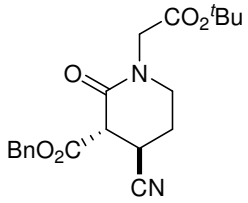
IR (NaCl): ν 3415, 1747, 1725, 1618 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.49 (s, 9H, CH_3-^{t}Bu), 2.15-2.23 (m, 1H, H-5), 2.24-2.35 (m, 1H, H-5), 3.29-3.41 (m, 1H, H-6), 3.55-3.71 (m, 2H, H-4 and H-6), 4.02 and 4.19 (2d, J_{AB} = 17.0 Hz, 1H each one, CH_2-CO_2^{t}Bu), 4.06-4.20 (m, 3H, Fmoc-CH_2 and Fmoc-9), 4.45 (t, J = 8.8 Hz, 1H, H-3), 5.38 (bs, 1H, NH-carbamate), 7.03-7.11 (m, 2H, H-2' and H-5'), 7.12-7.23 (m, 3H, H-6', Fmoc-3 and -6), 7.29-7.35 (m, 3H, H-7', Fmoc-2 and -7), 7.37 i 7.40 (2d, J = 8.0 Hz, 1H each one, Fmoc-4 and -5), 7.57 (d, J = 7.5 Hz, 1H, H-4'), 7.69 (d, J = 7.5, 2H, Fmoc-1 and -8), 8.40 (bs, 1H, NH-indole).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.4 (CH_3-^{t}Bu), 30.2 (C-5), 36.5 (C-4), 47.2 (Fmoc-9), 48.3 (C-6), 49.9 (CH_2-CO_2^{t}Bu), 57.4 (C-3), 67.2 (CH_2-Fmoc), 82.4 (C-^{t}Bu), 111.7 (CH-7'), 116.7 (C-3'), 118.7 (CH-4'), 119.7 (CH-5'), 120.0 (Fmoc-4 and -5), 121.4 (CH-2'), 122.3 (CH-6'), 125.5 (Fmoc-1 and -8), 127.2 (Fmoc-2 and -7), 127.7 (Fmoc-3 and -6), 136.5 (C-7'a), 141.3, 141.4, 144.1 and 144.2 (C_6H_5-*ipso*), 157.0 (CO-carbamate), 168.4 and 169.9 (CO-ester and lactam).

MALDI-TOF (ACH): 604 $[M+K]^+$, 588 $[M+Na]^+$.

***Trans- i cis*-(3-Benziloxicarbonil)-1-[(*tert*-butoxicarbonil)metil]-4-cianopiperidin-2-ona (28)**



***trans/cis*-28**

To a solution of **21** (3.0 g, 8.7 mmol, 1 eq.) in DMF (20 mL), NH_4Cl (699 mg, 13.08 mmol, 1.5 eq.) and a solution of KCN (1.13 g, 17.4 mmol, 2 eq.) in H_2O (7.5 mL) were added and the mixture was warmed to 90 ºC for 20 min. Then, the solution was diluted with H_2O and was extratcted with ethyl acetate. The organic phase was washed with sat. solution of NaCl, dried over anh. Na_2SO_4, filtered and evaporated at reduced pressure. The resulting crude was purified by silica flash chromatography (hexane/ethyl acetate 2:1) to obtain a mixture of diastereomers ***trans*/*cis*-28** (2.8 g, 87%) in a 20:1 ratio.

IR (NaCl): ν 2245, 1744, 1666, 1650, 1644 cm^{-1}.

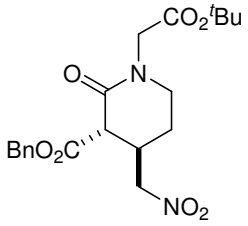
^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.46 (s, 9H, CH_3-^{t}Bu), 2.12-2.22 (m, 1H, H-5), 2.33-2.41 (m, 1H, H-5), 3.31-3.36 (m, 3H, H-4 and H-6)*, 3.44-3.55 (m, 3H, H-4 and H-6), 3.63 and 4.40 (2d, J_{AB} = 17.2 Hz, 1H each one, CH_2-CO_2^{t}Bu)*, 3.72 (d, J = 8.5 Hz; 1H, H-3), 3.78 (d, J = 5.8 Hz, 1H, H-3)*, 3.97 and 4.05 (d, J_{AB} = 17.2 Hz, 1H each one, CH_2CO_2^{t}Bu), 5.27 (s, 2H, CH_2Ph), 7.30-7.41 (m, 5H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 24.9 (C-5), 28.0 (C-4), 28.3 (CH_3-^{t}Bu), 46.7(C-6), 49.7 (CH_2-CO_2^{t}Bu), 51.3 (C-3), 68.3 (CH_2-Ph), 82.8 (C^{t}Bu), 118.7 (CN), 128.4 (C_6H_5-*p*), 128.7 (C_6H_5-*o*), 128.8 (C_6H_5-*m*), 135.1 (C_6H_5-i*pso*), 162.8, 167.3 and 167.6 (CO-esters i lactam).

EI-MS m/z (%): 316 (4), [M-*t*Bu]$^+$, 299(7), 271 (12), 225 (16), 181 (17), 104(27), 91(100), 57(92).

* Signals corresponding to racemate *cis*.

***trans*-(3-Benziloxicarbonil)-1-[(*tert*-butoxicarbonil)metil]-4-nitrometilpiperidin-2-ona (trans-29)**



trans-29

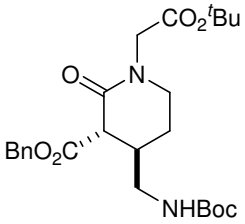
To a solution of dihydropiridone **21** (1.0 g, 2.9 mmol, 1 eq.) in CH_3NO_2 (9.4 mL, 173.9 mmol, 60 eq.), DBU (0.045 mL, 0.29 mmol, 0.1 eq.) was added and the mixture was stirred for 1 h. Solvent was then evaporated and the resulting crude was purified by column chromatography. Product **trans-29** (884 mg, 75%) was obtained as colorless oil.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.46 (s, 9H, CH_3-^{t}Bu); 1.74-1.85 (m, 1H, H-5); 2.09-2.16 (m, 1H, H-5); 2.97-3.16 (m, 1H, H-4); 3.36 (d, J = 9.1 Hz; 1H, H-3); 3.38-3.51 (m, 2H, H-6); 3.76 i 4.26 (2d, J_{AB} = 17.2 Hz; 1H each one, CH_2-$CO_2{}^t$Bu); 4.36-4.39 (m, 2H, CH_2-NO_2); 5.23 (s, 2H, CH_2Ph) 7.30-7.39 (m, 5H, C_6H_5).

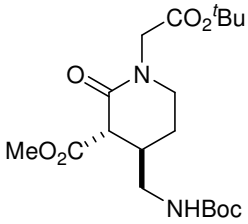
^{13}C-RMN (100 MHz, $CDCl_3$): δ 26.4 (C-5), 28.3 (CH_3-^{t}Bu), 35.3 (C-4), 46.8 (C-6), 49.6 (CH_2-$CO_2{}^t$Bu), 51.9 (C-3), 67.9 (CH_2Ph), 77.5 (CH_2-NO_2), 82.6 (C-^{t}Bu), 128.5, 128.6, 128.7, 128.8 and 128.9 (C_6H_5), 135.4 (C_6H_5-*ipso*), 164.5, 167.6 and 168.8 (CO esters and lactam).

CI-MS m/z: 424 $[M+NH_4]^+$; 407 $[M+H]^+$.

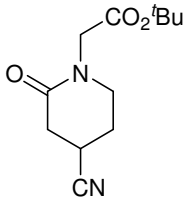
***trans*-[(3-Benziloxicarbonil)-1-[(*tert*-butoxicarbonil)metil]-4-(tert-butoxicarbonilamino)metil-piperidin-2-ona**



***trans*-31**



***trans*-32**



33

Procedure A

To a solution of nitromethyl piperidone ***trans*-29** (113 mg, 0.37 mmol, 1 eq.) in CH_3OH (10 mL), $NiCl_2$ (9.5 mg, 0.04 mmol, 0.1 eq.) and Boc_2O (161.5 mg, 0.74 mmol, 2 eq.) were added and the mixture was cooled to 0 ºC. $NaBH_4$ (41.6 mg, 1.1 mmol, 3 eq.) was then addedand the reaction was allowed to stir for 3 h at rt. Diethylenetriamine was added (0.040 mL, 0.37 mmol, 1 eq.) and stirred 30 minuts. After that time solvent was evaporated to dryness, residue was redissolved in ethyl acetate and washed with saturated solution of $NaHCO_3$. Organic phase was dried with Na_2SO_4, filtered and rotaevaporated to give the protectec amine ***trans*-31** (85 mg, 61%).

Procedure B

When conditions described above were applied over compound ***trans*-28** (147 mg; 0.54 mmol, 1 eq.) in CH_3OH (10 mL) using $NiCl_2$ (12.8 mg, 0.05 mmol, 0.1 eq.), Boc_2O (235 mg, 1.08 mmol, 2 eq.) and $NaBH_4$ (143 mg, 3.78 mmol, 7 eq.) and stirring 24 h at rt, compounds ***trans*-31** (64 mg, 32%), ***trans*-32** (5 mg, 3%) and decarboxylation product **33** (5 mg, 4%) were obtained.

Compound *trans*-31:

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.39 and 1.41 (2s, 18H, CH_3-^{t}Bu), 1.52-1.73 (m, 1H, H-5), 1.92-2.02 (m, 1H, H-5), 2.37-2.50 (m, 1H, H-4), 3.03-3.17 (m, 2H, CH_2-NHBoc), 3,23 (d, J = 9.7 Hz, 1H, H-3), 3.28-3.47 (m, 2H, H-6), 3.77 and 4.15 (2d, J_{AB} = 17.2 Hz, CH_2-$CO_2{}^t$Bu), 4.73 (bs, 1H, NH), 5.15 and 5.20 (2d, J_{AB} = 12.4 Hz, 1H each one, CH_2Ph), 7.26-7.37 (m, 5H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 25.2 (C-5); 28.2 and 28.6 (CH_3-^{t}Bu); 37.5 (C-4); 43.9 (CH_2-NH-Boc); 47.6 (C-6); 49.6 (CH_2-$CO_2{}^t$Bu); 53.3 (C-3), 67.4 (CH_2Ph), 82.2 (C^{t}Bu), 128.3, 128.4, 128.5, 128.7 and 128.8 (C_6H_5), 135.8 (C_6H_5-*ipso*), 156.2, 166.0, 167.9 and 170.4 (CO-esters, lactam and carbamate).

MALDI-TOF (ACH): 499.3 $(M+Na)^+$, 515.2 $(M+K)^+$;

Compound *trans*-32:

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.43 and 1.46 (s, 9H, CH_3-^{t}Bu), 1.62-1.72 (m, 1H, H-5), 1.97-2.05 (m, 1H, H-5), 2.43-2.53 (m, 1H, H-4), 3.10-3.25 (m, 2H, CH_2-NHBoc), 3.21 (d, *J* = 10.0 Hz, 1H, H-3), 3.36 (ddd, *J* = 11.8, 5.0 and 4.2 Hz, 1H, H-6eq), 3.45 (td, *J* = 11.0 and 4.8 Hz, 1H, H-6ax), 3.79 and 4.20 (2d, J_{AB} = 16.0 Hz, 1H each one, CH_2-$CO_2{}^t$Bu), 4,66 (bs, 1H, NH).

MS-EI m/z (%): 344 (2), 288 (8), 271 (13), 214 (45), 167 (27), 110 (13), 57 (100).

Compound 33:

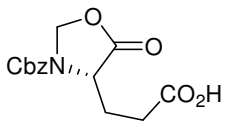
IR (NaCl): ν 2241, 1738, 1652 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, CH3-*t*Bu); 2.11-2.30 (m, 2H, H-5); 2.69 (dd, *J* = 17.6 and 8.0 Hz; 1H, H-3ax); 2.80 (dd, *J* = 17.6 and 5.4 Hz; 1H, H-3eq); 3.05-3.16 (m, 1H, H-4); 3.42-3.56 (m, 2H, H-6); 3.83 and 4.21 (2d; *JAB* = 17.2 Hz; 1H each one, CH2CO2*t*Bu).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 24.3 (C-4), 25.9 (C-5), 28.0 (CH_3-^{t}Bu), 34.1 (C-3), 46.2 (C-6), 48.9 (CH_2-$CO_2{}^t$Bu), 82.1 (C^{t}Bu), 119.7 (CN), 165.7 and 167.4 (CO-ester and lactam).

EM-EI m/z (%): 182 (11), 165 (1), 137 (34), 109 (21), 57 (100).

(S)-N-benzyloxicarbonyl-4-(carboxyethyl)-1,3-oxazolidin-5-one (37)



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(S)-benziloxicarbonilglutamic acid (25 g, 186.6 mmol, 1 eq.), paraformaldehyde (5.60, 186.6 mmol, 1 eq.) and p-toluenesulfonic acid·H_2O (1.01, 5.33 mmol, 0.06 eq) were solve ethyl acetate and washed with H_2O (3 x 300 mL). Organic phase was dried with Na_2SO_4 and evaporated at reduced pressure to obtain product **37** (26 g, 100%) as a yellow oil used without purification for the next reaction.

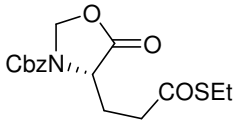
IR (NaCl): ν 3332, 1803, 1713 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 2.16-2.21 (m, 1H, H-1'a), 2.29-2.37 (m, 1H, H-1'b), 2.50 (bs, 2H, H-2'), 4.40 (t, J = 5.8 Hz, 1H, H-4), 5.18 (s, 2H, CH_2-Ph), 5.22 (d, J = 4.4 Hz, 1H, H-2a), 5.53 (bs, 1H, H-2b), 7.34-7.37 (m, 5H, C_6H_5), 9.55 (bs, 1H, CO_2H).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 26.0 (C-1'), 29.4 (C-2'), 54.1 (C-4), 68.5 (CH_2-Ph), 78.1 (C-2), 128.6 (C_6H_5–*p*), 128.9 (C_6H_5-*o*), 129.0 (C_6H_5-*m*), 135.4 (C_6H_5-*ipso*), 153.3 (N-CO), 171.9 (C-5), 178.0 (CO_2H).

ESI-MS m/z: 294 $(M+H)^+$.

(S)-N-benziloxycarbonyl-4-(ethylthiocarbonilethyl)-1,3-oxazolidin-5-one (38)



38

To a solution of the acid **37** (18.34 g, 62.5 mmol, 1 eq) in CH_2Cl_2 (75 mL), DPPA (33.7 mL, 156.4 mmol, 2.5 eq), EtSH (13.9 mL, 187.6 mmol, 3 eq.) and Et_3N (17.4 mL, 125.1 mmol, 2 eq.) were added and the mixture was stirred for 24 h at rt. The product was washed with H_2O (3 x 40 mL) and organic phase was dried over Na_2SO_4 and solvent was evaporated to dryness. Product **38** (19.29 g, 96%) was obtained as a dark yellow oil after column chromatography (hexane/ethyl acetate 9:1).

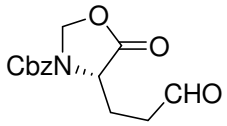
IR (NaCl): ν 2967, 1715, 1412 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.23 (t, *J* = 7.4 Hz, 3H, CH_3), 2.23-2.26 (m, 1H, H-1'a), 2.33-2.43 (m, 1H, H-1'b), 2.67 (bs, 2H, H-2'), 2.86 (q, *J* = 7.5 Hz, S-CH_2), 4.36 (t, *J* = 5.2 Hz, 1H, H-4), 5.19 (s, 2H, CH_2-Ph), 5.23 (d, *J* = 4.7 Hz, 1H, H-2a), 5.53 (bs, 1H, H-2b), 7.37 (bs, 5H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 14.9 (CH3), 23.6 (C-1'), 26.4 (C-2'), 39.0 (S-CH_2), 54.2 (C-4), 68.4 (CH_2-Ph), 77.6 (C-2), 128.6 (C_6H_5–*p*), 128.9 (C_6H_5-*o*), 129.0 (C_6H_5-*m*), 135.6 (C_6H_5-*ipso*), 153.1 (N-CO), 171.8 (C-5), 198.0 (COS).

ESI-MS m/z: 338 $(M+H)^+$.

(S)-N-benzyloxicarbonyl-4-(formiethyl)-1,3-oxazolidin-5-one (39)



39

To a solution of tioester **38** (16.61 g, 49.24 mmol, 1 eq) in freshly distilled acetone (90 mL) in a round bottom flask containing 4A molecular sieves, 10% Pd/C (1.45 g) was added and stirred 5 min at rt. Et_3SiH (23.47 mL, 147.72 mmol, 3 eq) was then added slowly and the mixture was allowed to stir for 2 h at rt. Pd/C was removed by filtration and solvent was evaporated at reduced pressure. Compound **39** (10.32 g, 76%) was obtained as a dark yellow oil without further purification.

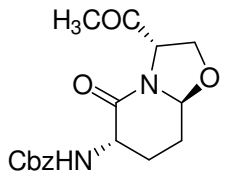
IR (NaCl): ν 2955, 1711 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 2.17-2.25 (m, 1H, H-1'a), 2.26-2.37 (bs, 1H, H-1'b), 2.60 (bs, 2H, H-2'), 4.37 (t, *J* = 6.0 Hz, 1H, H-4), 5.18 (s, 2H, CH_2-Ph), 5.20 (d, *J* = 3.6 Hz, 1H, H-2a), 5.52 (bs, 1H, H-2b), 7.38-7.39 (m, 5H, C_6H_5), 9.69 (bs, 1H, CHO).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 23.6 (C-1'), 39.0 (C-2'), 54.1 (C-4), 68.4 (CH_2-Ph), 78.0 (C-2), 128.6 (C_6H_5–*p*), 128.8 (C_6H_5-*o*), 129.0 (C_6H_5-*m*), 135.4 (C_6H_5-*ipso*), 153.2 (N-CO), 171.9 (C-5), 200.1 (CHO).

ESI-MS: m/z 301 $(M+Na)^+$, 279 $(M+H)^+$.

methyl (3S, 6S, 9S)-3-benzyloxicarbonylamino-2-oxo-7,1-oxazabicyclo[4.3.0]nonan-9- carboxylate (40)



40

A solution of aldehyde **39** (11.27 g, 40.65 mmol, 1 eq.) and serine methyl ester hydrochloride (6.96 g, 44.7 mmol, 1.1 eq.) in anhydrous pyridine (400 mL) was stirred for 6 days at r.t. in presence of 4Å molecular sieves. Then the mixture was filtered and solvent was evaporated at reduced pressure. The redidue was redissolved in anhydrous CH_3OH (335 mL), K_2CO_3 (3.93 g, 28.45 mmol, 0.7 eq) was added and the mixture was allowed to stir at rt until product disappeared (5 h). Finally, the reaction crude was filtered and solvent was evaporated. Bicyclic product **40** (6.35 g, 45%) was obtained after column chromatography (CH_2Cl_2/CH_3OH 99:1) as only isomer.

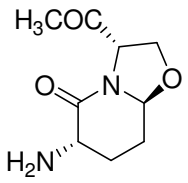
IR (NaCl): ν 3339, 1722, 1666 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.63-1.86 (m, 2H, H-7a and H-8a); 2.32-2.37 (m, 1H, H-8b), 2.51 (bs, 1H, H-7b), 3.77 (s, 3H, CO_2CH_3), 3.84 (dd, *J* = 9.2 and 7.2 Hz, 1H, H-2a), 4.12-4.22 (m, 1H, H-6), 4.44 (t, *J* = 8.8 Hz, 1H, H-2b), 4.68 (dd, J = 8.4 and 7.2 Hz, 1H, H-3), 4.91 (dd, *J* = 9.6 Hz and 4.4 Hz, 1H, H-9), 5.12 (s, 2H, CH_2-Ph), 5.47 (bs, 1H, NH), 7.34-7.36 (m, 5H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 25.2 (C-7), 26.9 (C-8), 52.2 (C-6), 52.9 (CH_3), 56.1 (C-3), 67.1 (CH_2-Ph), 68.7(C-2), 88.3 (C-9), 128.3 (C_6H_5-*m* and –*p*), 128.7 (C_6H_5-*o*), 136.6 (C_6H_5-*ipso*), 156.7 (NH-CO), 167.1 (C-5), 170.4 (CO_2Me).

ESI-MS: m/z 371 $(M+Na)^+$, 349 $(M+H)^+$.

(3S, 6S, 9S)-3-amino-2-oxo-7,1-oxazabicyclo[4.3.0]nonan-9-methyl carboxylate (43)



43

To a solution of the bicyclic compound **40** (6.48 g, 18.61 mmol, 1 eq.) in CH3OH (185 mL), Pd/C 10% (0,65 g) was added and the mixture was hydrogenated during 6 h at atmospheric pressure. The mixture was filtered and evaporated at reduced pressure to give directly the amine **43** (3.68 g, 92%) as a brown solid.

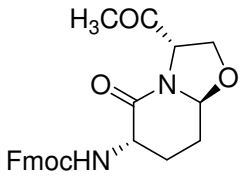
IR (NaCl): ν 3411, 1739, 1658 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1,55-1,73 (m, 2H, H-7a and H-8a); 2,21 (bs, 2H, NH_2), 2,25-2,48 (m, 2H, H-7b and H-8b); 3.33 (dd, *J* = 10.8 and 5.6 Hz, 1H, H-6); 3.78 (s, 3H, CO_2CH_3); 3.85 (dd, *J* = 8.8 and 7.2 Hz, 1H, H-2a); 4.44 (t, *J* = 8.8 Hz, 1H, H-2b), 4.70 (t, *J* = 8.0 Hz, 1H, H-3), 4.92 (dd, *J* = 8.8 and 4.4 Hz, 1H, H-9).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 26.6 (C-7), 27.4 (C-8), 52.3 (C-6), 52.9 (CH_3), 56.0 (C-3), 68.7 (C-2), 88.6 (C-9), 170.6 (C-8), 171.1 (CO_2Me).

ESI-MS: m/z 215 $(M+H)^+$.

(3S, 6S, 9S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxo-7,1-oxazabicyclo[4.3.0] nonan-9-methylcarboxylate (44)



44

Compound **43** (3.4 g, 15.9 mmol, 1 eq.) was dissolved in a mixture of H_2O/dioxane (1:1) (30 mL) and the mixture was acidified to pH = 8-9 with a 5% solution of Na_2CO_3. Then, Fmoc-OSu (5.89, 1.47 mmol, 1.1 eq) was added and the mixture was stirred for 2 days at rt. During the extraction with MTBE a white precipitate was observed in the aqueous phase which was filtered giving the desired product **44** (6.10 g, 88%).

IR (NaCl): ν 3336, 1723, 1665 cm^{-1}.

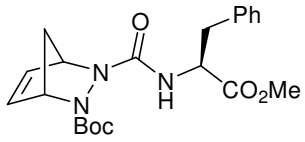
^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.58-1.74 (m, 2H, H-7a and H-8a); 2.36 (bs, 1H, H-8b), 2.57 (bs, 1H, H-7b), 3.79 (s, 3H, CO_2CH_3), 3.85 (t, *J* = 8.2, 1H, H-2a), 4.18 (bs, 1H, Fmoc-9) 4.23 (t, *J* = 7.0 Hz, 1H, H-6), 4.40 (bs, 1H, Fmoc-CH_2), 4.46 (t, *J* = 8.8 Hz, 1H, H-2b), 4.70 (t, *J* = 7.6 Hz, 1H, H-3), 4.92 (bs, 1H, H-9), 5.50 (bs, 1H, NH), 7.31 (td, *J* = 7.2 and 1.2 Hz, 2H, Fmoc-3 and Fmoc-6), 7.40 (t, *J* = 7.4 Hz, 2H, Fmoc-2 and Fmoc-7), 7.60 (dd, *J* = 7.2 and 4 Hz, 2H, Fmoc-4 and Fmoc-5), 7.76 (d, *J* = 7.2 Hz, 2H, Fmoc-1 and Fmoc-8).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 25.3 (C-7), 26.8 (C-8), 47.4 (Fmoc-9), 52.4 (C-6), 53.0 (CH_3), 56.1 (C-3), 67.3 (Fmoc-CH_2), 68.7(C-2), 88.3 (C-9), 120.0 (Fmoc-4 and -5) 125.4 (Fmoc-1 and -8), 127.3 (Fmoc-2 and -7) 127.9 (Fmoc-3 and -6), 141.5 (Fmoc-4a and -4b), 144.0 (Fmoc-8a and 9a), 156.8 (N-CO), 166.9 (C-5), 170.4 (CO_2Me).

ESI-MS: m/z 437 $(M+H)^+$, 215 $(M\text{-Fmoc})^+$.

7.4. Experimental procedures of chapter 4

Tert-butyl 3-[(S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-2,3-diaza-bicyclo[2.2.1]hept-5-ene-2-carboxylate (60)



60

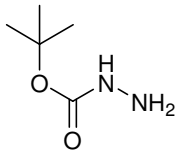
A solution of **64** (0.10 g, 0.30 mmol, 1 eq.) in CH_2Cl_2 (2 mL) was cooled to 0 ºC and cyclopentadiene was then added. Lead (IV) acetate (133 mg, 0.30 mmol, 1 eq.) was added carefully and the mixture was allowed to stir for 2 h at rt. Lead was removed by filtration and solvent was evaporated to dryness. Bicyclic hydrazine **60** (88 mg, 73%) was obtained after column chromatography (2% MeOH in DCM) as white solid.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.44 (s, 18H, $CH_3{}^tBu$), 1.52-1.67 (m, 4H, H-7), 3.05-3.11 (m, 4H, CH_2-Ph), 3.67 and 3.68 (2s, 6H, CH_3-O), 4.69-4.81 (m, 2H, Hα), 4.95 and 4.98 (2s, 1H each one, H-1 and H-4), 5.22 (s, 2H, H-1 and H-4), 6.21 (d, *J* = 7.7 Hz, 1H, NH urea), 6.28 (d, *J* = 7.5 Hz, 1H, NH urea), 6.45-6.50 (m, 4H, H-5 and H-6), 7.08-7.16 (m, 4H, C_6H_5-*o*), 7.21-7.28 (m, C_6H_5-*m* and –*p*).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.0 ($CH_3{}^tBu$), 38,3 and 38.6 (CH_2-Ph), 47.6 and 47.8 (C-7), 52.1 (CH_3-O), 54.2 and 54.4 (Cα), 64.7, 65.0 and 65.2(C-1 and C-4), 82.6 and 82.7 (C^tBu), 126.9 (C_6H_5-*p*) 128.5 (C_6H_5-*o*), 129.3 and 129.4 (C_6H_5-*m*), 135.9 and 136.2 (C_6H_5-*ipso*),159.0 (CO carbamate), 161.6 (CO urea), 172.0 and 172.1 (CO ester).

ESI-MS: m/z 302 $(M\text{-}Boc)^+$, 402 $(M+H)^+$, 424 $(M+Na)^+$.

Tert-butyl carbazate (63)



63

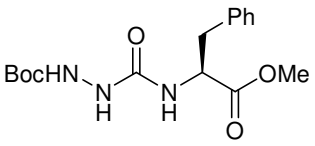
Hydrazine monohydrate (5.05 mL, 100 mmol, 1 eq.) was stirred in 20 mL of isopropanol at 0 ºC for 15 min., and treated dropwise with a solution of Boc_2O (10.0 g, 45.8 mmol, 0.46 eq.) in 10 mL of isopropanol. The reaction turned cloudy upon addition and stirring was continued at rt for 20 min. The solvent was removed by rotary evaporation and the residue was dissolved in CH_2Cl_2 and dried over $MgSO_4$. The CH_2Cl_2 was removed by evaporation at reduced pressure to obtain t-butyl carbazate **63** (5.61 g, 92%) as white solid.

IR (NaCl): ν 3330, 2978, 2933, 1704, 1632, 1494 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, CH_3-^{t}Bu), 3.69 (bs, 2H, NH_2), 5.83 (bs, 1H, NHCO).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.2 (CH_3^{t}Bu), 81.7 (C^{t}Bu), 158.1 (CO carbamate).

Tert-butyl-2-([(S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]carbamoyl)-1-carboxylate (64)



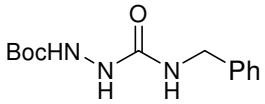
64

Methyl (*S*)-(-)-2-isocyanato-3-phenylpropanoate (0.69 mL, 3.78 mmol, 1 eq.) was dissolved in CH_2Cl_2 (2 mL) under argon atmosphere. Boc-hydrazine **63** (0.5 g, 0.76 mmol, 1 eq.) dissolved in CH_2Cl_2 (4 mL) was then added and the mixture was stirred for 2 h at rt. The solvent was removed by rotary evaporation and compound **64** (1.17 g, 92%) was obtained without further purification.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, CH_3-^{t}Bu), 3.09 (dd, *J* = 5.8 and 1.4 Hz, 2H, CH_2-Ph), 3.69 (s, 3H, CO_2CH_3), 4.75 (dt, *J* = 8.0 and 5.9 Hz, 1H, Hα), 5.99 (d, *J* = 7.9 Hz, 1H, NH urea), 6.60 (bs, 1H, NH), 6.82 (bs, 1H, NH), 7.11-7.15 (m, 2H, C_6H_5), 7.21-7.30 (m, 3H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.2 (CH_3^{t}Bu), 38.3 (CH_2-Ph), 52.2 (CH_3-O), 53.9 (Cα), 81.7 (C^{t}Bu), 126.9 (C_6H_5-*p*), 128.5 (C_6H_5-*o*), 129.4 (C_6H_5-*m*), 136.2 (C_6H_5-*ipso*), 156.2 (CO carbamate), 158.1 (CO urea), 172.8 (CO ester).

Tert-butyl 2-(benzylcarbamoyl)-1-carboxylate (79)



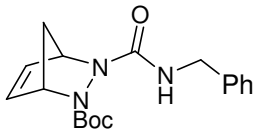
79

To a solution of benzyl isocyanate (0.47 mL, 3.78 mmol, 1 eq.) in CH_2Cl_2 (5 mL), tert-butyl carbazate **63** (0.5 g, 3.78 mmol, 1 eq.) dissolved in CH_2Cl_2 (5 mL) was added and the mixture was stirred for 2 h at rt. After solvent evaporation compound **79** (964 mg, 93%) was obtained without further purification.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.43 (s, 9H, CH_3-*t*Bu), 4.36 (dd, *J* = 5.8 and 1.7 Hz, 2H, CH_2-Ph), 5.99 (bs, 1H, NH urea), 6.75-7.00 (m, 2H, NH-NH), 6.82 (bs, 1H, NH), 7.20-7.38 (m, 5H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.2 ($CH_3{}^t$Bu), 43.7 (CH_2-Ph), 82.0 ($C{}^t$Bu), 127.1 (C_6H_5-*p*), 127.4 (C_6H_5-*o*), 128.5 (C_6H_5-*m*), 139.0 (C_6H_5-*ipso*), 156.5 (CO carbamate), 159.2 (CO urea).

Tert-butyl 3-(benzylcarbamoyl)-2,3-diaza-bicyclo[2.2.1]hept-5-ene-2-carboxylate (80)



80

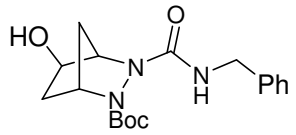
A solution of **79** (964 mg, 3.63 mmol, 1 eq.) in CH_2Cl_2 (10 mL) was cooled to 0 ºC and cyclopentadiene (0.9 mL, 10.91 mmol, 3.5 eq.) was then added. Lead (IV) acetate (1.6 g, 3.63 mmol, 1 eq.) was added carefully and the mixture was allowed to stir for 2 h at rt. Lead was removed by filtration and solvent was evaporated to dryness. Bicyclic hydrazine **80** (1.12 g, 94%) was obtained after column chromatography (2% MeOH in DCM).

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.39 (s, 9H, CH_3tBu), 1.62-1.70 (m, 2H, H-7), 4.39 (d, J = 5.8 Hz, 2H, CH_2-Ph), 4.96 (bs, 1H, H-1 or H-4), 5.26 (bs, 1H, H-1 or H-4), 6.15 (bs, 1H, NH urea), 6.46 (d, 1H, H-5 or H-6), 6.53 (d, 1H, H-5 or H-6), 7.17-7.31 (m, 5H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.0 (CH_3*t*Bu), 47.3 (CH_2-Ph), 47.7 (C-7), 64.8 and 65.2 (C-1 and C-4), 82.6 (C*t*Bu), 127.2, 127.3 and 128.5 (C_6H_5), 138.9 (C_6H_5-*ipso*), 159.1 (CO carbamate), 162.0 (CO urea).

ESI-MS: m/z 230 $(M\text{-}Boc)^+$, 252 $[M\text{-}(Boc)+H]^+$, 330 $(M+H)^+$, 452 $(M+Na)^+$.

Tert-butyl 3-(benzylcarbamoyl)-5-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (81)



81

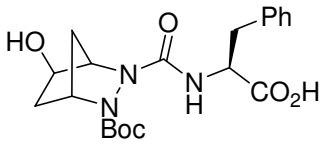
A mixture of $[Rh(cod)Cl]_2$ (2.9 mg, 0.006 mmol, 0.01 eq.), (*S*,*S*)-bdpp (5.3 mg, 0.012 mmol, 0.02 eq.) and hydrazine **80** (190 mg, 0.58 mmol, 1 eq.) was dried under vacuum for 1 h and placed under argon. Freshly distilled DME (2.5 mL) was then added at -50 ºC and the mixture was warmed to 0 ºC and stirred at this temperature for 1 h. Catecholborane (0.123 mL, 1.16 mmol, 2 eq.) was then added and the temperature is maintained at 0 ºC for 7 h. EtOH (0.58 mL) was then added to quench the reaction and the cooling bath was removed. When the reaction became orange, 30% H_2O_2 (0.58 mL) and NaOH (3M in H_2O, 0.98 mL) were added turning the solution to black. After stirring 15 h at rt, NaOH (1M in H_2O, 5 mL) was added and the mixture was extracted with EtOAc (3 x 10 mL) and the organic phase was washed with 1M NaOH (2 x 10 mL), H_2O (10 mL) and saturated solution of NaCl (10 mL). After solvent evaporation at reduced pressure, product the crude was purified by column chromatography (hexane / ethyl acetate 1:1) to give alcohol **81** (86 mg, 41%) as a mixture of regioisomers.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.43 (s, 18H, $CH_3{}^tBu$), 1.52-1.64 (m, 4H, H-6), 2.04 (d, *J* = 10.7 Hz, 4H, H-7), 4.26 (d, *J* = 6.5 Hz, 2H, H-5), 4.42 (dd, *J* = 5.8 and 2.8Hz, 2H, CH_2-Ph), 4.51 (bs, 4H, H-1 and H-4), 5.98 (t, *J* = 5.6 Hz, 2H, NH), 7.26-7.33 (m, 5H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.0 ($CH_3{}^tBu$), 33.7 (C-7), 38.3 (C-6), 44.3 (CH_2-Ph), 47.7 (C-5), 59.5 (C-1), 64.2 (C-1 or C-4), 82.9 (C^tBu), 127.3 and 128.6 (C_6H_5), 138.6 (C_6H_5-*ipso*).

ESI-MS: m/z 270 $[M-(Boc)+H]^+$, 370 $(M+Na)^+$.

(2S)-2-(2-(tert-butoxycarbonyl)-5-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-3-carboxamido)-3-phenylpropanoic acid (83)



83

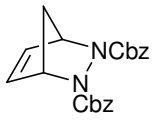
A mixture of $[Rh(cod)Cl]_2$ (2.5 mg, 0.005 mmol, 0.01 eq.), (*S*,*S*)-bdpp (4.4 mg, 0.010 mmol, 0.02 eq.) and hydrazine **60** (190 mg, 0.47 mmol, 1 eq.) was dried under vacuum for 1 h and placed under argon. Freshly distilled DME (2 mL) was then added at -50 ºC and the mixture was warmed to -20 ºC and stirred at this temperature for 1 h. Catecholborane (0.101 mL, 0.95 mmol, 2 eq.) was then added and the mixture was stirred for 12 h at 0 ºC. EtOH (0.47 mL) was then added to quench the reaction and the cooling bath was removed. When the reaction became orange, 30% H_2O_2 (0.47 mL) and NaOH (3M in H_2O, 0.79 mL) were added turning the solution to black. After stirring 1 h 30 min at rt, NaOH (1M in H_2O, 5 mL) was added and the mixture was extracted with EtOAc (3 x 10 mL) and the organic phase was washed with 1M NaOH (2 x 10 mL), H_2O (10 mL) and saturated solution of NaCl (10 mL). Aqueous phase was then acidified and reextracted with EtOAc (3 x 10 mL). After solvent evaporation at reduced pressure, product the crude was purified by column chromatography (3% MeOH in DCM) to give alcohol **83** (114 mg, 60%) as a mixture of regioisomers.

1H-RMN (400 MHz, CD_3OD): δ 1.45 and 1.46 (2s, 22H, $CH_3{}^tBu$ and H-6), 1.88-2.11 (m, 4H, H-7), 3.02-3.23 (m, 4H, CH_2-Ph), 4.07 (bs, 2H, H-1 or H-4), 4.17-4.25 (m, 2H, H-5), 4.48 (bs, 1H, H-1 or H-4), 4.57-4.69 (m, 2H, Hα), 7.17-7.32 (m, C_6H_5).

^{13}C-RMN (100 MHz, CD_3OD): δ 27.0 ($CH_3{}^tBu$), 32.8 and 33.1 (C-7), 37.2 and 37.5 (C-6), 38,3 and 38.6 (CH_2-Ph), 54.2 (Cα), 59.2 and 59.3 (C-1 or C-4), 64.2 and 64.3 (C-1 or C-4), 82.5 (C^tBu), 126.5 (C_6H_5-*p*), 128.0 (C_6H_5-*o*), 129.1 (C_6H_5-*m*), 136.7 (C_6H_5-*ipso*), 161.5 (CO urea), 173.2 (CO acid).

ESI-MS: m/z 306 $[M-(Boc)+H]^+$, 328 $[M-(Boc)+Na]^+$, 406 $(M+H)^+$, 428 $(M+Na)^+$.

Dibenzyl-2,3-diazabicyclo[2.2.1]hept-5-ene 2,3-dicarboxylate



84

Dibenzyl azodicarboxylate (25.7 g, 86.15 mmol, 1 eq.) was dissolved in CH_2Cl_2 (50 mL) and was cooled to 0 ºC under argon atmosphere. Cyclopentadiene (17.7 mL, 215.4 mmol, 2.5 eq.) was then added and the temperature was allowed to warm at rt. After 3h solvent was removed under reduced pressure and the resulting residue was stirred overnight in cyclohexane (100 mL). Product **84** (27.94 g, 89%) was obtained as a white solid which was filtered and dried in vacuum.

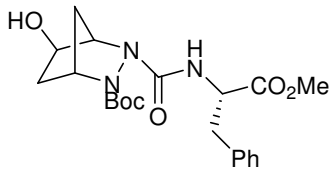
IR (KBr): ν 3342, 3044, 1747 (NCO), 1730 (NCO), 1510, 1455 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): 1.73 (m, 2H, H-7), 5.18 (s, 6H, H-1, H-4 and CH_2-Ph), 6.46 (bs, 2H, H-5 and H-6), 7.34 (m, 10H, C_6H_5).

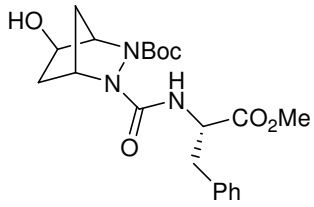
^{13}C-RMN (100 MHz, $CDCl_3$): 48.4 (C-7), 65.7 (C-1 and C-4), 68.3 (CH_2-Ph), 128.1 (CH-Ar-*p*), 128.4 (C_6H_5-*o*), 128.7 (C_6H_5-*m*), 136.8 (C_6H_5-*ipso*), 159.0 (COcarbamate).

EM-ESI m/z: 365 $(M+H)^+$, 387 $(M+Na)^+$, 403 $(M+K)^+$, 751 $(2M+Na)^+$.

tert-butyl 3-([(*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]carbamoyl)-5-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate(85a) and tert-butyl 3-([(*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]carbamoyl)-6-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (85b)



Boc-AzaSer 85a



Boc-AzaSer 85b

Methyl (*S*)-(-)-2-isocyanato-3-phenylpropionate (0.076 mL, 0.42 mmol, 1 eq.) was added to a solution of **100a** (90 mg, 0.42 mmol, 1 eq.) in CH_2Cl_2 (5 mL) and the mixture was stirred for 2h at rt. Solvent was removed under vacuum and the crude reaction was then purified by silica gel flash chromatography (Hexane / ethyl acetate 4:6) to give **AzaSer 85a** as a white solid (170 mg, 96%).

AzaSer 85b (298 mg, 94 %) is obtained following the same procedure starting from compound **100b** (161 mg, 0.75 mmol, 1 eq.) and methyl (S)-(-)-2-isocyanato-3.phenylpropionate (0.136 mL, 0.75 mmol, 1 eq.) in CH_2Cl_2 (6 mL).

AzaSer 85a:

IR (KBr): ν 3415, 2978, 2953, 1741, 1655, 1521, 1456, 1438, 1369, 1203, 1162 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.44 (bs, 10H, ^{t}Bu and H-6), 1.62 (d, *J* = 10.7 Hz, 1H, H-7), 1.93 (d, *J* = 10.4 Hz, 1H, H-7), 2.07 (m, 1H, H-6), 3.09 (m, 2H, CH_2-Ph), 3.68 (s, 3H, OCH_3), 4.07 (bs, 1H, H-5), 4.25 (s, 1H, H-1), 4.62 (s, 1H, H-4), 4.75 (dd, *J* = 13.4 and 6.9 Hz, 1H, H-α), 6.22 (d, *J* = 7.6 Hz, 1H, NH), 7.10-7.29 (m, 5H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.3 (CH_3-tBu), 33.8 (C-7), 38.5 (C-6), 38.6 (CH_2-Ph), 52.4 (OCH_3), 54.6 (C-α), 59.2 (C-4), 64.7 (C-1), 83.0 (C-tBu), 127.2 (C_6H_5-*p*), 128.8 (C_6H_5-*o*), 129.4 (C_6H_5-*m*), 136.2 (C_6H_5-*ipso*), 157.6 (NH-CO-NH), 161.1 (OCO-NH), 172.6 (CO_2Me).

ESI-HRMS (+): m/z 320.17 $[(M\text{-}Boc)+H]^+$, 420.17 $(M+H)^+$, 442.20 $(M+Na)^+$, 861.42 $(2M+Na)^+$.

AzaSer 85b:

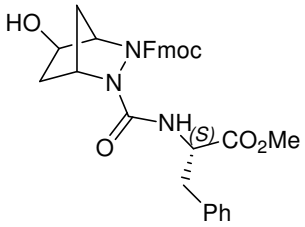
IR (KBr): ν 3413, 2978, 2952, 1743, 1659, 1518, 1455, 1437, 1369, 1158, 1119 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.37 (s, 9H, tBu), 1.45 (m, 2H, H-6 and H-7), 1.93 (m, 2H, H-6 and H-7), 3.01 (d, *J* = 6.0 Hz, 2H, CH_2-Ph), 3.61 (s, 3H, OCH_3), 4.08 (d, *J* = 5.2 Hz, 1H, H-5), 4.35 (s, 1H, H-1), 4.38 (s, 1H, H-4), 4.64 (m, 1H, H-α), 5.97 (d, *J* = 8.2 Hz, 1H, NH), 7.06 (dd, *J* = 6.5 Hz and 1.3 Hz, 2H, C_6H_5), 7.17 (m, 3H, H-Ar).

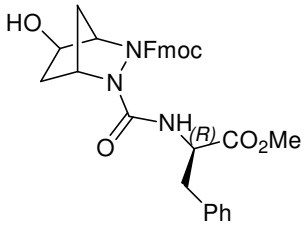
^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.2 (CH_3-tBu), 33.9 (C-7), 38.2 (C-6), 38.3 (CH_2-Ph), 52.4 (OCH_3), 54.4 (C-α), 59.7 (C-4), 64.4 (C-1), 69.4 (C-5), 82.9 (CtBu), 127.2 (C_6H_5-*p*), 128.8 (C_6H_5-*o*), 129.4 (C_6H_5-*m*), 136.2 (C_6H_5-*ipso*), 158.4 (NH-CO-NH), 160.2 (OCO-NH), 172.4 (CO_2Me).

ESI-HRMS (+): m/z 320.17 $[(M\text{-}Boc)+H]^+$, 420.22 $(M+H)^+$, 442.20 $(M+Na)^+$, 839.44 $(2M+H)^+$.

(9H-fluoren-9-yl)methyl 3-([(S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]carbamoyl]-6-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (86b)



Fmoc-azaSer-Phe 86b



Fmoc-azaSer-Phe 86c

To a solution of **101b** (130 mg, 0.39 mmol, 1 eq.) in CH_2Cl_2 (3 mL), methyl (*R*)-(-)-2-isocyanato-3-phenylpropionate (0.070 mL, 0.39 mmol, 1 eq.) was added and the mixture was stirred for 2h. After solvent evaporation, the crude was purified in column chromatography (2% MeOH in CH_2Cl_2) giving **Fmoc-AzaSer-Phe 86b** (174 mg, 82%).

Azadipeptide **Fmoc-AzaSer-D-Phe 86c** (343 mg, 93%) was synthesized following the same procedure, starting from **101b** (230 mg, 0.68 mmol, 1 eq.) in CH_2Cl_2 (5 mL) and using the enantiomer methyl (*S*)-(–)-2-isocyanato-3-phenylpropionate (0.125 mL, 0.68 mmol, 1 eq.) as acylating agent.

Compound 86b:

IR (KBr): ν 3412, 3028, 2950, 1739, 1658, 1521 1478, 1450, 1322, 1282, 1201, 1137 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.38-1.49 (m, 2H, H-6 and H-7), 1.76 (bs, 1H, OH), 1.92-2.02 (m, 2H, H-6 and H-7), 3.03 (t, *J* = 6.3 Hz, 2H, CH_2-Ph), 3.66 (s, 3H, OCH_3), 4.10 (t, *J* = 6.7 Hz, 1H, Fmoc-9), 4.16 (d, *J* = 6.4 Hz, H-5), 4.32-4.52 (m, 4H, H-1, H-4 and CH2-Fmoc), 4.68 (dd, *J* = 14.0 and 6.4 Hz, 1H, Hα), 5.98 (bs, 1H, NH urea), 7.01-7.05 (m, 2H, C_6H_5-*o*), 7.08-7.22 (m, 3H, C_6H_5-*m* and –*p*), 7.28-7.35 (m, 2H, Fmoc-2 and -7), 7.41 (t, *J* = 6.4 Hz, 2H, Fmoc-3 and -6), 7.54 (dd, *J* = 7.1 and 5.1 Hz, 2H, Fmoc-1 and -8), 7.77 (d, *J* = 7.4 Hz, 2H, Fmoc-4 and -5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 34.0 (C-7), 38.1 (C-6), 38.6 (CH_2-Ph), 47.1 (Fmoc-9), 52.5 (OCH_3), 54.2 (Cα), 59.9 (C-1), 64.5 (C-4), 68.5 (CH_2-Fmoc), 69.5 (C-5), 120.3

(Fmoc-4 and -5), 125.3 (Fmoc-1 and -8), 127.3 and 127.4 (Fmoc-2 and -7), 128.1 (Fmoc-3 and -6), 128.7, 129.3 (C_6H_5), 136.0 (C_6H_5-*ipso*), 141.6 (Fmoc-4a and -4b), 143.5 (Fmoc-8a and 9a), 159.1 (CO carbamate), 160.0 (CO urea), 172.4 (CO ester).

ESI-MS (+): m/z 542.1 $(M+H)^+$, 564.2 $(M+Na)^+$, 1105.5 $(2M+Na)^+$.

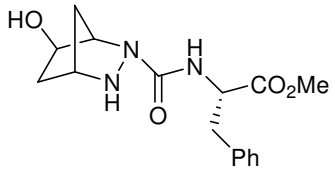
Compound 86c:

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.40-1.49 (m, 2H, H-6 and H-7), 1.81 (bs, 1H, H-6), 1.91-1.98 (m, 1H, H-7), 2.99 (dd, *J* = 13.9 and 6.9 Hz, 1H, CH_2-Ph), 3.11 (dd, *J* = 13.8 and 5.4 Hz, 1H, CH_2-Ph), 3.58 (s, 3H, OCH_3), 3.97 (d, *J* = 5.7 Hz, 1H, H-5), 4.15 (bs, 1H, Fmoc-9), 4.38-4.50 (m, 4H, H-1, H-4 and CH_2-Fmoc), 4.72 (dd, *J* = 13.4 and 6.9 Hz, 1H, Hα), 6.05 (d, *J* = 7.9 Hz, 1H, NH urea), 7.05-7.29 (m, 5H, C_6H_5), 7.27-7.35 (m, 2H, Fmoc-2 and -7), 7.39 (t, *J* = 7.4 Hz, 2H, Fmoc-3 and -6), 7.53 (d, *J* = 7.5 Hz, 2H, Fmoc-1 and -8), 7.75 (d, *J* = 7.5 Hz, 2H, Fmoc-4 and -5).

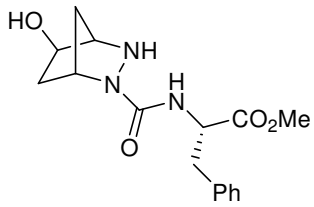
^{13}C-RMN (100 MHz, $CDCl_3$): δ 33.9 (C-7), 38.4 (C-6), 38.6 (CH_2-Ph), 47.1 (Fmoc-9), 52.5 (OCH_3), 54.3 (Ca), 59.8 (C-1), 64.6 (C-4), 68.6 (CH_2-Fmoc), 69.3 (C-5), 120.3 (Fmoc-4 and -5), 125.2 and 125.3 (Fmoc-1 and -8), 127.3 and 127.4 (Fmoc-2 and -7), 128.1 (Fmoc-3 and -6), 128.7, 128.9, 129.4 and 129.5 (C_6H_5), 136.1 (C_6H_5-ipso), 141.5 and 141.6 (Fmoc-4a and -4b), 143.5 and 143.7 (Fmoc-8a and 9a), 159.1 (CO carbamate), 160.9 (CO urea), 172.1 (CO ester).

ESI-MS (+): m/z 542.1 $(M+H)^+$, 564.2 $(M+Na)^+$, 1105.5 $(2M+Na)^+$.

(2S)-methyl 2-(5-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-3-carboxamido)-3-phenylpropanoate (87a) and (2S)-methyl 2-(5-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxamido)-3-phenylpropanoate (87b)



87a



87b

85a (196 mg, 0.47 mmol, 1 eq.) was dissolved in a mixture of 50%TFA in CH_2Cl_2 (4 mL) and the mixture was stirred 1h at rt. Solvent and excess of TFA was removed under vacuum and crude reaction mixture was washed with ammonia and extracted with CH_2Cl_2. The combined organic layers were dried with $MgSO_4$, filtered and concentrated and the crude was then purified by silica gel chromatography. Compound was obtained **87a** (111 mg, 74%). Compound **87b** (81 mg, 69%) is obtained with the same procedure starting from compound **85b** (154 mg, 0.37 mmol, 1 eq.) and 50% TFA in CH_2Cl_2 (3 mL).

Compound 87a:

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.30-1.48 (m, 1H, H-6), 1.57 (bs, 1H, H-7), 1.83-2.00 (m, 2H, H-6 and H-7), 3.02 (dd, *J* = 13.9 and 7.2 Hz, 1H, CH_2-Ph), 3.14 (dd, *J* = 13.9 and 5.5 Hz, 1H, CH_2-Ph), 3.45 (s, 1H, H-1), 3.70 (s, 3H, CH_3-O), 3.79 (bs, 1H, H-5), 4.43 (bs, 1H, H-4), 4.47 (dd, *J* = 12.8 and 6.8 Hz, 1H, Hα), 6.64 (bs, 1H, NH urea), 7.10-7.14 (m, 2H, C_6H_5-*o*), 7.20-7.31 (C_6H_5-*m* and –*p*).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 38.2 (C-7), 38.5 (C-6), 40.4 (CH_2-Ph), 52.5 (CH3-O), 54.0 (Cα), 56.9 (C-4), 62.5 (C-1), 127.1, 128.7and 129.4 (C_6H_5), 136.5 (C_6H_5-ipso), 172.9 (CO ester).

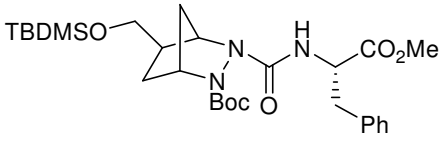
MS-ESI (+): 320 $(M+H)^+$, 260 $[(M-CO_2CH_3)+H]^+$, 639 $(2M+H)^+$.

Compound 87b:

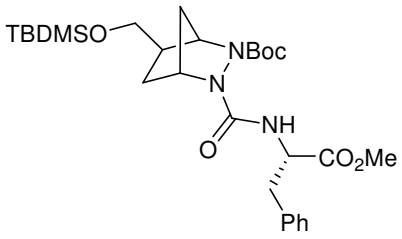
^{1}H-RMN (400 MHz, CD_3OD): δ 1.36-1.42 (m, 1H, H-6), 1,67 (bs, 1H, H-7), 1.87 (bs, 1H, H-6), 2.04 (bs, 1H, H-7), 3.06 (dd, *J* = 13.8 and 7.8 Hz, 1H, CH_2-Ph), 3.15 (dd, *J* = 13.8 and 5.3 Hz, 1H, CH_2-Ph), 3.63 (d, *J* = 3.3 Hz, 1H, H-1), 3.72 (s, 3H, CH_3-O), 3.93 (d, *J* = 6.2 Hz, 1H, H-5), 4.11 (bs, 1H, H-4), 4.56 (dd, *J* = 7.7 and 5.4 Hz, 1H, Hα), 7.17-7.31 (m, 5H, C_6H_5).

MS-ESI (+): 320 $(M+H)^+$, 260 $[(M-CO_2CH_3)+H]^+$, 639 $(2M+H)^+$.

tert-butyl 3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-5-((tert-butyldimethylsilyloxy)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (88a) and tert-butyl 3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-6-((tert-butyldimethylsilyloxy)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (88b)



88a



88b

To a solution of **103a** (165 mg, 0.48 mmol) in CH_2Cl_2 (5 mL), methyl (*S*)-(-)-2-isocyanato-3-phenylpropionate (0.088 mL, 0.48 mmol) was added and the mixture was stirred for 2 h at RT. After evaporation of the solvent the crude was purified by silica flash chromatography (Hexane / ethylacetate 6:4) to obtain **88a** (166 mg, 62 %).

Compound **88b** (200 mg, 74%) was obtained following the same procedure, starting from **103b** (170 mg, 0.49 mmol, 1 eq.) in CH_2Cl_2 (5 mL) and adding methyl (*S*)-(-)-2-isocyanato-3-phenylpropionate (0.091 mL, 0.49 mmol, 1 eq.).

Compoun 88a:

Yellow oil

IR (NaCl): 3429, 3029, 2953, 2930, 2856, 1743, 1720, 1676, 1512, 1368, 1256 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.01 (2s, 3H each one, CH_3-Si), 0.86 (s, 9H, ^{t}Bu-Si), 1.23-1.33 (m, 1H, H-6), 1.41-1.43 (m, 1H, H-7), 1.44 (s, 9H, $CH_3{}^t$Bu), 1.64 (d, *J* = 9.2 Hz; H-7), 1.85-1.99 (m, 1H, H-6), 2.05-2.,15 (m, 1H, H-5), 3.02 (dd, *J* = 13.8 and 6.6 Hz; 1H CH_2-Ph), 3.10 (dd, *J* = 13.9 and 5.8 Hz; 1H, CH_2-Ph), 3.40 (dd, *J* = 10.4 and 6.6 Hz; 1H, CH_2-O), 3.54 (dd, *J* = 10.4 and 5.0 Hz; 1H, CH_2-O), 3.67 (s, 3H, CH_3-O), 4.29 (s, 1H, H-1), 4.54 (s, 1H, H-4), 4.73 (dd, *J* = 13.9 and 6.6 Hz; 1H, H-α), 6.10-6.25 (m, 1H, NH), 7.07-7.13 (m, 2H, C_6H_5-*o*), 7.17-7.27 (m, 3H, C_6H_5-*m* and -*p*).

^{13}C-RMN (100 MHz, $CDCl_3$): δ -5.3 (CH_3-Si), -5.2 (CH_3-Si), 18.5 (C-^{t}Bu-Si), 26.1 (CH_3-^{t}Bu-Si), 28.3 (CH_3-^{t}Bu), 31.3 (C-6), 35.4 (C-7), 38.8 (CH_2-Ph), 41.5 (C-5), 52.3 (CH_3-O),

54.5 (Cα), 60.7 (C-1), 62.2 (C-4), 64.6 (CH_2-O), 82.5 (C-^{t}Bu), 127.1 (C_6H_5-*p*), 128.6(C_6H_5-*o*), 129.5(C_6H_5-*m*), 136.4 (C_6H_5-*ipso*), 157.8 (CO carbamate), 160.9 (CO urea), 172.3 (CO ester).

ESI-MS (+): m/z 448 $[(M\text{-Boc})+H]^+$, 492 $[(M\text{-}^t\text{Bu})+H]^+$, 548 $(M+H)^+$.

Compound 88b:

Yellow oil

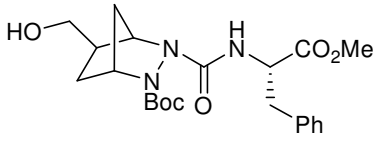
IR (NaCl): 3427, 2954, 2930, 2857, 1746, 1713, 1681,1510, 1367 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.00 (s, 6H, CH_3-Si), 0.85 (s, 9H, ^{t}Bu-Si), 1.07 (m, 1H, H_6), 1.41 (s, 9H, ^{t}Bu), 1.46 (m, 1H, H-6), 1.64 (d, *J* = 10.5 Hz, H-7), 1.94 (m, 1H, H-7), 2.07 (m, 1H, H-6), 3.06 (d, *J* = 6.1 Hz, 2H, CH_2-Ph), 3.25 (t, *J* = 9.2 Hz; 1H, CH_2-O), 3.50 (dd, *J* = 10.4 and 4.9 Hz; 1H, CH_2-O), 3.64 (s, 3H, OCH_3), 4.39 (s, 1H, H-4), 4.50 (s, 1H, H-1), 4.72 (dt, *J* = 8.0 and 6.2Hz; 1H, Hα), 6.10 (bs, 1H, NH), 7.16 (m, 5H, C_6H_5).

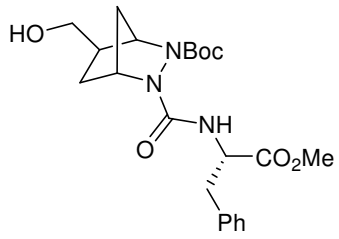
^{13}C-RMN (100 MHz, $CDCl_3$): δ -5.3 (CH_3-Si), -5.2 (CH_3-Si), 18.5 (C-^{t}Bu-Si), 26.1 (CH_3-^{t}Bu-Si), 28.3 (CH_3-^{t}Bu), 30.7 (C-6), 35.0 (C-7), 38.5 (CH_2-Ph), 42.1 (C-5), 52.3 (CH_3-O), 54.4 (Cα), 60.1 (C-1 o C-4), 62.6 (C-1 o C-4), 64.7 (CH_2-O), 82.5 (C-^{t}Bu), 127.1 (C_6H_5-*p*), 128.7(C_6H_5-*o*), 129.4(C_6H_5-*m*), 136.4 (C_6H_5-*ipso*), 158.1 (CO carbamate), 160.7 (CO urea), 172.5 (CO ester).

ESI-MS (+): m/z 448 $[(M\text{-Boc})+H]^+$, 548 $(M+H)^+$.

tert-butyl 3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-5-(hydroxymethyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (89a) and tert-butyl 3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-6-(hydroxymethyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (89b)



89a



89b

To a solution of **88a** (123 mg, 0.22 mmol, 1 eq.) in anhidrous THF (3 mL), tetrabutylammonium fluoride (TBAF) (118 mg, 0.44 mmol, 2 eq.), was added and the mixture was stirred for 2 h at RT. The mixture was purified by silica flash chromatography (Ethylacetate 100%) to obtain **89a** (78 mg, 80 %) as yellow oil.

Compound **89b** (51 mg, 65%) was obtained as yellow oil following the procedure described above starting from **88b** (100 mg, 0.18 mmol, 1eq.) in THF (3 mL) with TBAF (95 mg, 0.36 mmol, 2 eq.) at rt for 2h.

Compound 89a:

IR (NaCl): 3428, 2973, 2931, 2868, 1736, 1668, 1512, 1368, 1159 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.19-1.22 (m, 1H, H-6), 1.46 (s, 10H, CH_3-^{t}Bu and H-7), 1.58-1.67 (m, 1H, H-7), 1.90-2.14 (2H, H-6 and OH), 2.16-2.26 (m, 1H, H-5), 3.05 (dd, *J* = 13.9 and 6.7 Hz, 1H, CH_2-Ph), 3.12 (dd, *J* = 13.8 and 5.8 Hz, 1H, CH_2-Ph), 3.40 (dd, *J* = 10.9 and 8.3 Hz, 1H, CH_2-O), 3.49 (dd, *J* = 11.1 and 5.9 Hz, 1H, CH_2-O), 3.69 (s, 3H, OCH_3), 4.35-4.43 (m, 1H, H-1), 4.61 (s, 1H, H-4), 4.71-4.78 (m, 1H, Hα), 6.15-6.26 (m, 1H, NHurea), 7.10-7.17 (m, 2H, C_6H_5-*o*), 7.19-7.30 (m, 3H, C_6H_5-*m* and -*p*).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.3 (CH_3^{t}Bu), 31.5 (C-6), 35.0 (C-7), 38.7 (CH_2-Ph), 42.1 (C-5), 52.4 (CH_3-O), 54.5 (Cα), 60.5 (C-1), 61.5 (C-4), 64.7 (CH_2-O), 82.7 (C^{t}Bu), 127.2 (C_6H_5-*p*), 128.7 (C_6H_5-*o*), 129.5 (C_6H_5-*m*), 136.4 (C_6H_5-ipso), 158.0 (CO carbamate), 160,8 (CO urea), 172,4 (CO ester).

ESI-MS (+): m/z 334 $[(M\text{-}Boc)+H]^+$, 434 $(M+H)^+$.

Compound 89b:

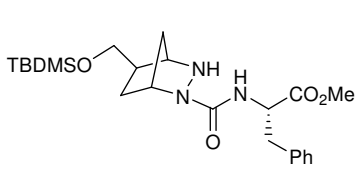
IR (NaCl): 3419, 2978, 2934, 2874, 1740, 1664, 1514, 1368, 1161 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.00-1.14 (m, 1H, H-6), 1.45 (s, 9H, CH_3-^{t}Bu), 1.56-1.66 (m, 2H, H-7), 1.77-1.95 (2H, H-5 and H-6), 3.03 (dd, *J* = 13.9 and 7.1 Hz, 1H, CH_2-Ph), 3.18 (dd, *J* = 13.9 and 5.5 Hz, 1H, CH_2-Ph), 3.35 (dd, *J* = 10.8 and 8.7 Hz, 1H, CH_2-O), 3.49 (dd, *J* = 10.9 and 5.4 Hz, 1H, CH_2-O), 3.70 (s, 3H, OCH_3), 4.46 (s, 1H, H-4), 4.51 (s, 1H, H-1), 4.74-4.85 (m, 1H, Hα), 6.13 (d, *J* = 7.8 Hz, 1H, NH-urea), 7.13-7.17 (m, 2H, C_6H_5-*o*), 7.20-7.29 (m, 3H, C_6H_5-*m* and -*p*).

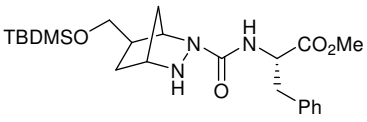
^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.2 (CH_3^{t}Bu), 31.1 (C-6), 35.2 (C-7), 38.9 (CH_2-Ph), 42.4 (C-5), 52.4 (OCH_3), 54.2 (Cα), 60.2 (C-1), 61.8 (C-4), 64.6 (CH_2-O), 82.9 (C^{t}Bu), 127.1 (C_6H_5-*p*), 128.7 (C_6H_5-*o*), 129.5 (C_6H_5-*m*), 136.4 (C_6H_5-*ipso*), 158.2 (CO-carbamate), 172.4 (CO-ester).

ESI-MS (+): m/z 334 $[(M\text{-Boc})+H]^+$, 434 $(M+H)^+$, 888 $(2M+H)^+$.

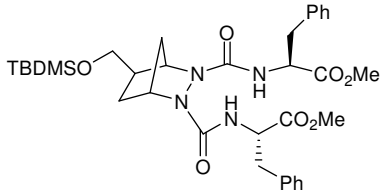
(2S)-methyl 2-(5-((tert-butyldimethylsilyloxy)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxamido)-3-phenylpropanoate (90a) and (2S)-methyl 2-(5-((tert-butyldimethylsilyloxy)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-3-carboxamido)-3-phenylpropanoate (90b)



90a



90b



90c

Compound **102** (330 mg, 0.64 mmol, 1 eq.) was placed in a 50 mL round bottom flask and was dissolved in CH_2Cl_2 (8 mL). Methyl (*S*)-(-)-2-isocyanato-3-phenylpropionate (129 μL, 0.71 mmol, 1.1 eq.) was added and the mixture was hydrogenated for 2h using $Pd(OH)_2$ (60 mg) as catalyst. The mixture was then filtered and solvent was evaporated under reduced pressure. **90b** (100 mg, 35%) and a mixture of **90a** and **90c** were obtained after silica gel flash chromatography (DCM/Ethyl acetate 9:1). A second purification by column chromatography (Hexane/diethyl ether 4:1) afforded **90a** (96 mg, 34%) and **90c** (60 mg, 14%).

Compound 90a:

IR (NaCl): 3301, 2952, 2856, 1744, 1664, 1497, 1256 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.02 and 0.03 (2s, 6H, CH_3-Si), 0.87 (s, 9H, CH_3^{t}Bu-Si), 1.36 (ddd, *J* = 13.0, 5.0 and 2.9 Hz, 1H, H-6), 1.44 (d, *J* = 10.8 Hz, 1H, H-7), 1.57-1.65 (m, 1H, H-6), 1.78 (d, *J* = 10.3 Hz, 1H, H-7), 2.12-2.10 (m, 1H, H-5), 2.59 (bs, 1H, NH), 3.05 (dd, *J* = 13.8 and 6.8 Hz, 1H, CH_2-Ph), 3.13 (dd, *J* = 13.8 and 5.7 Hz, 1H, CH_2-Ph), 3.40 (dd, *J* = 10.3 and 6.8 Hz, 1H, CH_2-O), 3.48 (dd, *J* = 10.4 and 5.7 Hz, 1H, CH_2-O), 3.63 (bs, 1H, H-1), 3.69 (s, 3H, OCH_3), 4.38 (s, 1H, H-4), 4.71-4.77 (m, 1H, Ha), 6.66 (d, *J* = 8.5 Hz, 1H, NH urea), 7.11-7.15 (m, 2H, C_6H_5-*o*), 7.19-7.30 (m, 3H, C_6H_5-*m* and –*p*).

^{13}C-RMN (100 MHz, $CDCl_3$): δ -5.3 and -5.2 (CH_3-Si), 18.5 (C^{t}Bu-Si), 26.1 (CH_3-^{t}Bu-Si), 33.4 (C-6), 36.3 (C-7), 38.6 (CH_2-Ph), 42.8 (C-5), 52.3 (OCH_3), 54.1 (Cα), 57.8 (C-1 o C-4), 60.3 (C-1 o C-4), 64.8 (CH_2-O), 127.0 (C_6H_5-*p*), 128.6 (C_6H_5-*o*), 129.4 (C_6H_5-*m*), 136.7 (C_6H_5-*ipso*), 161.0 (CO urea), 172.9 (CO ester).

ESI-MS (+): m/z 448 $(M+H)^+$, 895 $(2M+H)^+$.

Compound 90b:

IR (NaCl): 3374, 2926, 2854, 1741, 1664, 1512 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.00 and 0.09 (2s, 6H, CH_3-Si), 0.85 (s, 10H, CH_3*t*Bu-Si and H-6), 1.57 (m, 4H, H-5, H-6 and H-7), 2,96 (dd, *J* = 13.8 and 7.5 Hz, 1H, CH_2-Ph), 3.11 (m, 1H, CH_2-Ph), 3.18 (dd, *J* = 10.2 and 8.7 Hz, 1H, CH_2-O), 3.39 (dd, *J* = 10.3 and 4.9 Hz, 1H, CH_2-O), 3.52 (s, 1H, H-1 o H-4), 3.66 (s, 3H, CH_3-O), 3.90 (bs, 1H, NH), 4.24 (s, 1H, H-1 o H-4), 4.76 (m, 1H, H-α), 6.52 (s, 1H, NH urea), 7.09-7.24 (m, 5H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ -5.2 and -5.1 (CH_3-Si), 18.5 (C*t*Bu-Si), 26.1 (CH_3-*t*Bu-Si), 31.8 (C-6), 38.0 (C-7), 38,9 (CH_2-Ph), 42.7 (C-5), 52.4 (CH_3-O), 53.9 (C-α), 57.6 (C-1 o C-4), 59.7 (C-1 o C-4), 65.1 (CH_2-O), 127.0 (CH-Ar-*p*), 128.6 (CH-Ar-*o*), 129.5 (CH-Ar-*m*), 136.7 (C-Ar-*ipso*), 160.6 (CO urea), 173.0 (CO ester).

MS-ESI (+): 448 $(M+H)^+$, 470 $(M+Na)^+$, 895 $(2M+H)^+$.

Compound 90c:

IR (KBr): 3285, 2926, 1746, 1661, 1532, 1360, 1255, 1213 cm^{-1}.

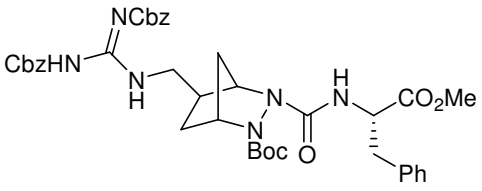
^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.00 and 0.01 (2s, 6H, CH_3-Si), 0.86 (s, 9H, CH_3*t*Bu-Si), 0.99 (dd, *J* = 12.8 and 5.1 Hz, 1H, H-6), 1.11 (d, *J* = 10.2 Hz, 1H, H-7), 1.49-1.66 (m, 3H, H-5, H-6 and H-7), 2.87-3.01 (m, 2H, CH_2-Ph), 3.18-3.28 (m, 3H, CH_2-Ph and CH_2-O), 3.44 (dd, *J* = 10.2 and 4.5 Hz; 1H, CH_2-O), 3.68 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.24 (s, 1H, H-1), 4.49 (s, 1H, H-4), 4.65 (td, *J* = 8.9 and 5.6 Hz, 1H, Hα), 4.74 (td, *J* = 9.5 and 5.1 Hz, 1H, Hα), 6.20 (d, *J* = 8.3 Hz, 1H, NH urea), 6.31 (d, *J* = 8.1 Hz, 1H, NH urea), 7.13-7.29 (m, 10H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ -5.3 and -5.2 (CH_3-Si), 18.5 (C*t*Bu-Si), 26.1 (CH_3-*t*Bu-Si), 31.1 (C-6), 35.3 (C-7), 37.7 and 37.8 (CH_2-Ph), 41.2 (C-5), 52.5 and 52.6 (OCH_3), 54.5 and 55.0 (Cα), 60.5 (C-1), 62.5 (C-4), 64.3 (CH_2-O), 127.1 (C_6H_5-*p*), 128.7 (C_6H_5-*o*);

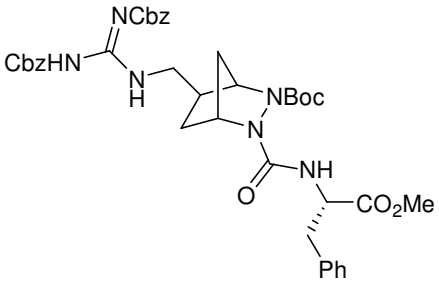
129.3 (C_6H_5-*m*), 136.6 and 136.7 (C_6H_5-*ipso*), 159.9 and 161,1 (CO urea), 172.3 and 172.5 (CO ester).

MS-ESI (+): 448 $(M\text{-}C_{11}H_{12}NO_3)^+$, 653 (M+H), 1306 $(2M+H)^+$.

tert-butyl 3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-5-((2,3-bis(benzyloxycarbonyl)guanidino)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (91a)



91a



91b

To a solution of **89a** (30 mg, 0.07 mmol) in anhidrous THF (4 mL), N,N'-Di-Cbz-guanidine **104** (69 mg, 0.21 mmol) and PPh_3 (183 mg, 0.7 mmol) were added under inert atmosphere. The mixture was cooled to 0 ºC and DEAD (0.320 mL, 0.7 mmol) was added dropwise. After the addition was complete the reaction mixture was allowed warm to rt and stir overnight. The reaction was quenched with water and the solvent was evaporated under reduced pressure. **91a** (42 mg, 82%) was isolated by flash column chromatography on silica gel (CH_2Cl_2 / diethylether 8:2).

Compound **91b** (96 mg, 75%) is obtained following the same procedure starting from **89b** (75 mg, 0.17 mmol, 1 eq.), N,N'-Di-Cbz-guanidine (0.17 g, 0.52 mmol, 3 eq.), PPh3 (68 mg, 0.26 mmol, 1.5 eq.) and 40% DEAD in toluene (0.120 mL, 0.26 mmol, 1.5 eq.) in THF (6 mL). Purification was achieved by column chromatography of the crude (CH_2Cl_2 / diethylether 8:2).

Compound 91a:

Colorless oil

IR (NaCl): ν 3392, 3030, 2976, 1720, 1677, 1612, 1510, 1370 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.10-1.20 (m, 1H, H-6), 1.43 (s, 9H, $CH_3{}^tBu$), 1.50 (d, *J* = 11.0 Hz, 1H, H-7), 1.69 (d, *J* = 10.4 Hz, 1H, H-7), 1.82-1.99 (m, 1H, H-6), 2.40-2.51 (m, 1H, H-5), 3.00-3.13 (m, 2H, CH_2-Ph), 3.67 (s, 3H, CH_3-O), 3.76-3.85 (m, 2H, CH_2-N), 3.92-4.01 (m, 1H, CH_2-N), 4.29 (s, 1H, H-4), 4.48 (s, 1H, H-1), 4.72 (m, 1H, H-α), 5.11 (s, 2H, CH_2-Ph), 5.22 and 5.26 (2d, *J* = 12.0 Hz, 1H each one, CH_2-Ph), 6.17 (d, *J* = 7.4 Hz, 1H, NH urea), 7.11 (d, *J* = 7.6 Hz, 2H, H-Ar), 7.18-7.37 (m, 13H, H-Ar), 9.25 (s, 1H, NH-Cbz), 9.44 (s, 1H, NH-Cbz).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.3 (CH_3-^{t}Bu), 32.8 (C-6), 34.9 (C-7), 38.6 (CH_2-Ph), 39.2 (C-5), 46.8 (CH_2-N), 52.3 (CH_3-O), 54.6 (C-α), 60.4 (C-1), 61.8 (C-4), 67.0 (CH_2-Ph), 69.5 (CH_2-Ph), 82.6 (C^{t}Bu), 127.2, 127.9, 128.0, 128.6, 128.7, 128.8, 129.0, 129.1, 129.5 (C-Ar), 134.6, 136.2, 137.1 (C-Ar-*ipso*), 155.9, 157.9 and 160.8 (CO carbamate), 160. 9 (C=N), 163.9 (CO urea), 172.4 (CO ester).

MS-ESI (+): 743 $(M+H)^+$.

Compound 91b:

Colorless oil

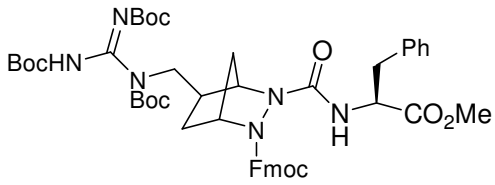
IR (NaCl): ν 3390, 3286, 3031, 2979, 1721, 1678, 1612, 1513, 1369, 1236, 1215 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.16 (m, 1H, H-6), 1.43 (s, 9H, CH_3^{t}Bu), 1.50 (d, *J* = 11.0 Hz, 1H, H-7), 1.69 (d, *J* = 10.8 Hz, 1H, H-7), 1.88 (m, 1H, H-6), 2.44 (m, 1H, H-5), 3.07 (t, *J* = 5.9 Hz, 2H, CH_2-Ph), 3.67 (s, 3H, CH_3-O), 3.81 (dd, *J* = 13.4 and 5.2 Hz, 1H, CH_2-N), 3.97 (m, 1H, CH_2-N), 4.29 (s, 1H, H-1), 4.48 (s, 1H, H-4), 4.67-4.75 (m, 1H, H-α), 5.10 (d, *J* = 8.2 Hz, 2H, CH_2-Ph), 5.21 and 5.26 (2d, *J* = 12.0 Hz, 1H each one; CH_2-Ph), 6.17 (d, *J* = 7.2 Hz, 1H, NH urea), 7.11 (d, *J* = 6.9 Hz, 2H, C_6H_5), 7.18-7.40 (m, 13H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.3 (CH_3-^{t}Bu), 32.8 (C-6), 34.9 (C-7), 38.6 (CH_2-Ph), 39.2 (C-5), 46.8 (CH_2-O), 52.3 (CH_3-O), 54.6 (C-α), 60.4 (C-1), 61.8 (C-4), 67.0 (CH_2-Ph), 69.5 (CH_2-Ph), 82.6 (C^{t}Bu), 127.1, 128.0, 128.6, 128.7, 128.8, 129.0, 129.1, 129.4 (C-Ar), 134.6, 136.2, 137.1 (C-Ar-*ipso*), 155.9, 157.9 and 160.8 (CO carbamate), 163.9 (CO urea), 172.4 (CO ester).

MS-ESI (+): 743 $(M+H)^+$, 643 $(M\text{-}Boc)^+$.

(9H-fluoren-9-yl)methyl 3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-5-((1,2,3-tris(tert-butoxycarbonyl)guanidino)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (92)



92

To a solution of **105** (679 mg, 0.98 mmol, 1 eq.) in CH_2Cl_2 (30 mL), methyl (*S*)-(-)-2-isocyanato-3-phenylpropionate (0.178 mL, 0.98 mmol, 1 eq.) and the mixture was allowed to stir for 2 h at rt. Solvent was then removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane / ethyl acetate 2:1) to yield compound **92** (597 mg; 68 %) as yellow oil.

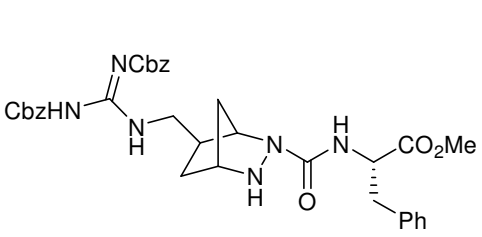
IR (KBr): ν 3421, 2978, 1743, 1685, 1655, 1609, 1508, 1405, 1369 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.20-1.37 (m, 2H, H-6), 1.46-1.49 (m, 28H, $CH_3{}^tBu$ and H-7), 1.74 (d, *J* = 10.7 Hz, 1H, H-7), 2.33-2.42 (m, 1H, H-5), 2.93-3.10 (m, 2H, CH_2-Ph), 3.54-3.78 (m, 5H, OCH_3 and CH_2-N), 4.10-4.14 (m, 1H, Fmoc-9), 4.32 (s, 1H, H-1), 4.36-4.42 (m, 2H, CH_2Fmoc), 4.61 (s, 1H, H-4), 4.64-4.76 (m, 1H, H-α), 6.20 (bs, 1H, NH urea), 7.05 (d, *J* = 7.4 Hz, 2H, C_6H_5), 7.11-7.22 (m, 3H, C_6H_5), 7.29-7.35 (m, 2H, Fmoc-2 and -7), 7.40 (t, *J* = 7.4 Hz, 2H, Fmoc-3 and -6), 7.57 (dd, *J* = 11.8 and 7.4 Hz, 2H, Fmoc-1 and -8), 7.76 (d, *J* = 7.5 Hz, 2H, Fmoc-4 and -5), 10.68 (s, 1H, NH carbamate).

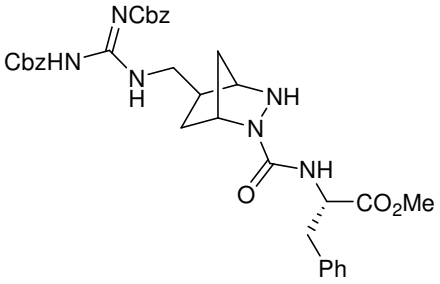
^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.1 and 28.3 ($CH_3{}^tBu$), 32.9 (C-6), 34.9 (C-7), 38.5 (CH_2-Ph), 39.2 (C-5), 47.1 (Fmoc-9), 49.8 (CH_2-N), 52.3 (OCH_3), 54.4 (C-α), 60.7 (C-1), 62.0 (C-4), 68.5 (CH_2Fmoc), 83.8 ($C{}^tBu$), 120.2 (Fmoc-4 and -5), 125.3 (Fmoc-1 and -8), 127.1 (Fmoc-2 and -7), 127.4 (C_6H_5-*p*), 128.0 (Fmoc-3 and -6), 128.6 (C_6H_5-*o*), 129.5 (C_6H_5-*m*), 136.4 (C_6H_5-*ipso*), 141.6 (Fmoc-4a and -4b), 143.6 (Fmoc-8a and -9a), 153.5 and 158.4 (CO carbamates), 160.4 (CO urea), 172.3 (CO ester).

ESI-MS (+): m/z 597 $[M-(Boc)_3+H]^+$, 697 $[M-(Boc)_2+H]^+$, 797 $[M-(Boc)+H]^+$, 897 $(M+H)^+$.

(2S)-methyl 2-(5-((2,3-bis(benzyloxycarbonyl)guanidino)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-3-carboxamido)-3-phenylpropanoate (93a)



93a



93b

A solution of **91a** (87 mg, 0.12 mmol, 1eq.) in ethyl acetate (3 mL) was cooled at 0 ºC Then, HCl was bubbled for 5 min. and the mixture was stirred for 40 min at 0 ºC. Solvent and excess of reagent was removed by rotary evaporation and **93a** (54 mg, 72 %) was obtained.

Compound **93b** (63 mg, 94%) was obtained following the procedure described above starting from compound **91b** (78 mg, 0.10 mmol, 1 eq.) solved in saturated solution of HCl in ethyl acetate (3 mL).

Compound 93a:

IR (NaCl): ν 3252, 2987, 1787, 1693, 1519, 1245, 1202, 1183 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.18 (ddd, *J* = 12.5, 4.5 and 2.5 Hz, 1H, H-6), 1.40-1.55 (m, 2H, H-7), 1.56-1.65 (m, 2H, H-6), 1.65-1.76 (m, 1H, NH-2), 2.32-2.51 (m, 1H, H-5), 3.04-3.15 (m, 2H, CH_2-Ph), 3.55 (s, 1H, H-1), 3.70 (s, 3H, OCH_3), 3.77 (dd, *J* = 14.2 and 4.7 Hz, 1H, CH_2-N), 3.85-3,93 (m, 1H, CH_2-N), 4.26 (bs, 1H, H-4), 4.66-4.74 (m, 1H, Hα), 5.06-5,16 (m, 2H, CH_2-Ph), 5.19-5.28 (m, 2H, CH_2-Ph), 6.57 (bs, 1H, NH urea), 7.05-7.39 (m, 15H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 30.9 (C-6), 33.4 (C-7), 38.5 (CH_2-Ph), 40.3 (C-5), 47.0 (CH_2-N), 52.4 (CH_3-O), 54.0 (C-α), 59.7 (C-1 o C-4), 62.5 (C-1 o C-4), 67.0 (CH_2-Ph), 69.4 (CH_2-Ph), 127.1, 127.9, 128.0, 128.6, 128.7, 128.8, 129.0, 129.1 and 129.4 (C_6H_5), 134.7, 136.6 and 137.2 (C_6H_5-*ipso*), 155.9 and 160.9 (CO carbamates), 163.9 (CO urea), 172.8 (CO ester).

MS-ESI (+): 643 $(M+H)^+$, 509 $[(M-CO_2Bn)+H]^+$.

Compound 93b:

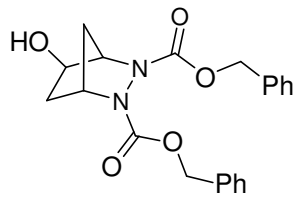
IR (NaCl): ν 3378, 3063, 2945, 1698, 1450, 1342, 1285, 1202, 1089 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.98-1.15 (m, 1H, H-6), 1.43-1.51 (m, 1H, H-7), 1.55-1.80 (m, 3H, H-6, H-7 and NH-2), 2.10 (bs, 1H, H-5), 2.96 (dd, *J* = 14.4 and 6.0 Hz, 1H, CH_2-Ph), 3.08 (dd, *J* = 13.6 and 5.4 Hz, 1H, CH_2-Ph), 3.36 (s, 1H, H-1), 3.68 (s, 3H, OCH_3), 3.71-3.77 (m, 1H, CH_2-N), 3.92 (dd, *J* = 13.8 and 10.5 Hz, 1H, CH_2-N), 4.31 (bs, 1H, H-4), 4.69-4.76 (m, 1H, Hα), 5.06-5,25 (m, 4H, CH_2-Ph), 6.53 (bs, 1H, NH urea), 7.07-7.40 (m, 15H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 29.9 (C-6), 33.5 (C-7), 38.1 (CH_2-Ph), 39.0 (C-5), 47.3 (CH_2-N), 52.3 (OCH_3), 54.0 (C-α), 57.6 (C-4), 59.8 (C-1), 67.2 (CH_2-Ph), 69.5 (CH_2-Ph), 127.1; 128.0, 128.1, 128.6, 128.9, 129.1and 129.5 (C_6H_5), 134.5, 136.6 and 137.0 (C_6H_5-*ipso*), 156.0 and 160.8 (CO carbamates), 164.0 (CO urea), 172.8 (CO ester).

MS-ESI (+): 643 $(M+H)^+$, 1285 $(2M+H)^+$.

5-Hydroxy-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Dibenzyl Ester



95

A mixture of $[Rh(cod)Cl]_2$ (39 mg, 0.08 mmol, 0.01 eq.), (S,S)-bdpp (70 mg, 0.16 mmol, 0.02 eq.) and bicyclic hydrazine **84** (3.0 g, 8.24 mmol, 1 eq.) was dried under vacuum for 1 h and placed under argon. Freshly distilled DME (33 mL) was then added at -50 ºC and the mixture was stirred at this temperature for 30 min. Catecholborane (1.75 mL, 16.48 mmol, 2 eq.) was then added and the reaction became orange but remained heterogeneous. Temperature was maintained at -50 ºC for 30 min and was quenched with EtOH (9 mL). The cooling bath was then removed and 30% H_2O_2 (9 mL) and NaOH (3M in H_2O, 15.3 mL) were added turning the solution to black. After stirring 15 h at rt, NaOH (1M in H2O, 80 mL) was added and the mixture was extracted with EtOAc (3 x 150 mL). The organic phase was washed with 1M NaOH (2 x 150 mL), H_2O (150 mL) and saturated solution of NaCl (150 mL). After solvent evaporation at reduced pressure, product the crude was purified by column chromatography (hexane / ethyl acetate 1:1) to give alcohol **95** (2.41 g, 76%) as colorless oil.

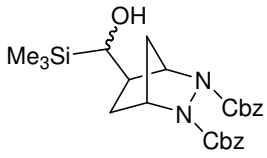
IR(NaCl): ν 3480, 3063, 3033, 2944, 1737, 1713, 1498, 1455, 1391, 1325 cm^{-1}.

^{1}H-RMN (400 MHz, DMSO-d_6, 70 ºC): 1.46 (dt, *J* = 13.7 and 2.5 Hz, 1H, H-6), 1.54 (d, *J* = 10.4 Hz, 1H, H-7), 1.98 (d, *J* = 10.4 Hz, 1H, H-7), 1.98-2.04 (m, H-6), 4.28 (d, *J* = 7.0 Hz, 1H, H-5), 4,52 (bs, 1H, H-4), 4,68 (bs, 1H, H-1), 5.16 (m, 4H, CH_2-Ph), 7.35 (m, 10H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): 34.0 (C-7), 38.0 (C-6), 59.6 (C-1), 64.3 (C-4); 68.1 and 68.2 (CH_2-Ph), 70.4 (C-5); 128.0, 128.3, 135.8 and 135.9 (C_6H_5), 155.0 (CO carbamate).

HRMS-ESI(+): 383.17 $(M+H)^+$, 405.15 $(M+Na)^+$, 787.31 $(2M+Na)^+$.

dibenzyl 5-(hydroxy(trimethylsilyl)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2,3-dicarboxylate (93)



96

$[Rh(COD)Cl]_2$ (40 mg, 0.082 mmol), (R,R)-BDPP (70 mg, 0.16 mmol) and **84** (3 g, 8,24 mmol) were placed in a round bottom flask, dried under vacuum (0.1 mmHg) for 1 h, and then placed under argon. DME (33mL) was degassed at -50ºC an added to the mixture at this temperature. The yellow-green slurry was stirred at -50ºC for 30 min. Catecholborane (1.76 mL, 16,5 mmol) was then added dropwise and the mixture became orange but remained heterogeneous. The reaction was kept at -50ºC for an additional 30 min. Solvent and excess reagent were then carefully removed under vacuum (0.1 mmHg, 3h) to give the intermediate borane as a dark yellow foam. A solution of the intermediate borane in THF (49 mL) under argon was then added over 2 M solution of trimethylsilyldiazomethane in Et_2O (20.5 mL, 41.2 mmol). After refluxing overnight, 80 mL of freshly prepared 1:1 mixture of 2N aqueous sodium hydroxide and 30% hydrogen peroxide were then added dropwise at 0ºC, turning the solution to black, and the mixture was stirred for an additional 4 h at RT. After extraction with EtOAc (3x100 mL), the combined organic layers were washed with 1M HCl (100mL), dried over $MgSO_4$, filtered and concentrated. The crude reaction mixture was then purified by silica gel flash chromatography (cyclohexane / ethylacetate 2:1) to give **96** (2.6g, 68%).

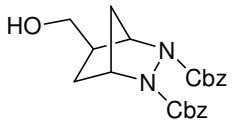
IR (NaCl): ν 3491, 3033, 2954, 1713, 1391, 1325, 1267, 1120 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.02 (s, 9H, CH_3-Si), 1.58 (t, *J* = 10.5 Hz, 1H, H-6), 1.77 (bs, 2H, H-7), 1.95 (d, *J* = 9.7 Hz, 1H, H-6), 2.23 (bs, 2H, H-5 and OH), 2.86 (d, *J* = 9.8 Hz, 1H, CH-OH), 4.46 (bs, 1H, H-1 or H-4), 4.84 (bs, 1H, H-1 or H-4), 5.11-5.26 (m, 4H, CH_2-Ph), 7.28-7.37 (m, 10H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 2.7 (CH_3-Si), 35.1 (C-7), 37.0 (C-6), 44.0 (C-5), 61.2 (C-1), 62.2 (C-4), 67.0 (CH-OH), 68.2 (CH_2-Ph), 128.3, 128.4 and 128.7 (C_6H_5), 136.3 (C_6H_5-*ipso*), 157.8 (CO carbamate).

ESI-MS: 397 $[(M\text{-}TMS)+H]^+$, 469 $(M+H)^+$, 937 $(2M+H)^+$.

Dibenzyl 5-(hydroxymethyl)-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (101)



97

A solution of **96** (1g, 2,13 mmol) in THF (10 mL) was added with tetrabutylammonium fluoride (1.12g, 4,26 mmol) and stirred at rt for 24 h. More reagent was then added (1.12 g, 4,26 mmol) and the mixture was kept at rt until complete consumption of the starting material. After concentration under vacuum, the crude reaction mixture was purified by silica gel flash chromatography (cyclohexane / ethylacetate 6:4) to give **97** (716 mg, 85%).

White solid

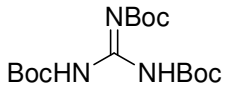
IR (NaCl): ν 3471, 1710, 1391, 1399, 1329, 1294 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): 1.06-1.25 (m, 1H, H-6), 1.56-1.71 (m, 2H, H-7), 1.77-1.95 (m, 1H, H-6), 2.15 (bs, 1H, H-5), 2.64 (bs, 1H, OH), 3.28 (t, *J* = 9.1 Hz, 1H, CH_2-OH), 3.43 (bs, 1H, CH_2-OH), 4.36-4.72 (m, 2H, H-1 and H-4), 5.16 (s, 4H, CH_2-Ph), 7.30 (m, 10H, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): 31.9 (C-6), 35.5 (C-7), 42.7 (C-5), 60.6 and 62.3 (C-1 and C-4), 64.2 (CH_2-O), 68.2 (CH_2-Ph), 128.2 (C_6H_5-*p*), 128.4 (C_6H_5-*o*), 128.7(C_6H_5-*m*), 136.2 (C_6H_5-*ipso*), 157.7 (CO carbamate).

ESI-MS (+): m/z 397 $(M+H)^+$.

***N,N´,N´´*-Tri-Boc-guanidine (98)**



98

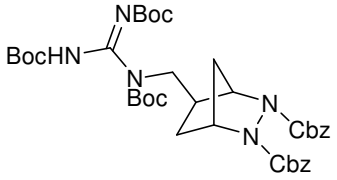
A mixture of KOH (2,81g; 50 mmol, 1 eq) and Na_2CO_3 (5,30 g; 50 mmol, 1 eq.) was finely ground in a mortar and transfered to a round bottom flask. DMSO (50 mL) was then added and the resulting suspension was allowed to stir for 5 min at room temperature. Guanidine hydrochloride (4.78 g, 50 mmol, 1 eq.) was added and the mixture was stirred for 5 min. After the addition of Boc_2O (51,7 g; 225 mmol, 4.5 eq.) the mixture was stirred for 60 h at 40ºC. Cold water was added and the white precipitate obtained was filtered, dried under vaccum and recristalized from acetonitrile to obtain **98** as colorless needles (14.06 g, 78%).

IR (KBr): ν 3292, 2983, 2953, 1728, 1717, 1642, 1541, 1430, 1370, 1312, 1236, 1124.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.51 (s, 27H, $CH_3{}^tBu$).

MS-ESI m/z (%): 360 $(M+H)^+$, 304 $(M-{}^tBu)^+$, 260 $(M-Boc)^+$.

dibenzyl 5-((1,2,3-tris(tert-butoxycarbonyl)guanidino)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2,3-dicarboxylate (99)



99

A solution of the alcohol **97** (2g; 5 mmol), PPh_3 (2,6 g; 10 mmol); and previously prepared triprotected guanidine **98** (3,6 g; 10 mmol) in anhydrous THF (80 mL) was cooled to -5 ºC under Ar atmosphere. DEAD 40% in toluene (4,6 mL; 10 mmol) was added dropwise and the reaction mixture was heated at 40ºC for 4h. The solution was then cooled to rt and the precipitate of excess **98** that formed was collected by filtration and was washed with a mixture of THF/hexanes 1:1. The filtrate was concentrated in vacuum and the **99** (3,0 g; 81%) was isolated by flash column chromatography on silica gel (hexane/ethyl acetate 7:3).

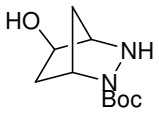
IR (KBr): ν 2979, 1758, 1735, 1654, 1609, 1394, 1369, 1243, 1140 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.22-1.31 (m, 1H, H-6), 1.42-1.53 (m, 27H, $CH_3{}^tBu$), 1.57-1.61 (m, 1H, H-6), 1.67 (d, *J* = 10.6 Hz, 1H, H-7), 1.77-1.85 (m, 1H, H-7), 1.87-2.02 (m, 1H, H-5), 2.44 (bs, 1H, OH), 3.62-3.75 (m, 2H, CH_2-N), 4.41-4.77 (m, 2H, H-1 and H-4), 5.02-5.28 (m, 4H, CH_2-Ph), 7.27-7.35 (m, 10H, C_6H_5), 10.71 (NH).

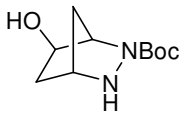
^{13}C-RMN (100 MHz, $CDCl_3$): 28.1 and 28.3 ($CH_3{}^tBu$), 31.1 (C-6), 35.3 (C-7), 42.7 (C-5), 49.8 (CH_2-N), 60.5 and 62.6 (C-1 and C-4), 68.2 (CH_2-Ph), 81.0, 82.8 and 83.8 ($C{}^tBu$), 127.9 (C_6H_5-*p*), 128.1 (C_6H_5-*o*), 128.7(C_6H_5-*m*), 136.3 (C_6H_5-*ipso*), 149.5 and 153.4 (CO carbamates).

ESI-MS (+): 738 $(M+H)^+$, 760 $(M+Na)^+$, 1475 $(2M+H)^+$.

tert-butyl 5-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (100a) and tert-butyl 6-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (100b)



100a



100b

To a solution of alcohol **95** (600 mg, 1.56 mmol, 1 eq.) in CH_3OH (6 mL), Boc_2O (342 mg, 1.56 mmol, 1 eq.) in methanol (6 mL) and $Pd(OH)_2$ (100 mg) were added and the mixture was hydrogenated for 2h. After that time reaction mixture was filtered and evaporated to dryness. The resulting yellow oil was precipited with diethyl ether, obtaining a white solid as the minor regioisomer **100a** (128 mg, 38%). The major regioisomer **100b** (151 mg, 45%) was obtained by purification by column chromatography (5% MeOH in CH_2Cl_2).

Compound 100a:

IR (KBr): ν 3421, 2978, 1718, 1701 1697, 1676, 1396, 1367 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.48 (bs, 10H, CH_3-^{t}Bu and H-6), 1.69 (d, *J* = 10.4 Hz, 1H, H-7), 2.04 (d, *J* = 10.4 Hz, 1H, H-7), 2.11 (ddd, *J* = 13.7, 6.9 and 2.6 Hz, 1H, H-6), 3.76 (bs, 1H, H1), 4.14 (d, *J* = 6.9 Hz, 1H, H5), 4.20 (bs, 1H, H4).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.7 (CH_3-^{t}Bu), 35.5 (C-7), 40.8 (C-6), 57.0 (C-4), 61.6 (C-1), 71.0 (C-5), 80.9 (C-^{t}Bu), 155.2 (OCO-NH).

HRMS-ESI (-): 212.08, 227.17, 250.08.

Compound 100b:

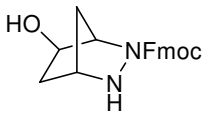
IR (KBr): ν 3398, 2978, 2933, 1701, 1560, 1393, 1367, 1252 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): 1.43-1.47 (m, 10H, CH_3-^{t}Bu and H-6), 1.70 (dt, *J* = 10.0 and 2.0 Hz, 1H, H-7), 1.97 (d, *J* = 10.3 Hz, 1H, H-7), 2.19 (ddd, *J* = 13.5, 6.8 and 2.3 Hz, 1H, H-6), 3.60 (bs, 1H, H-1), 4.13 (d, *J* = 5.7 Hz, 1H, H-5), 4.37 (bs, 1H, H-4).

^{13}C-RMN (100 MHz, $CDCl_3$): 28.6 (CH_3-^{t}Bu), 35.1 (C-7), 41.3 (C-6), 56.3 (C-4), 62.8 (C-1), 72.7 (C-5), 80.8 (C-^{t}Bu), 155.9 (OCO-NH).

HRMS-ESI (-): 212.08, 227.08.

(9H-fluoren-9-yl)methyl 6-hydroxy-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (100)



101b

To a solution of alcohol **95** (1.00 g, 2.6 mmol, 1eq.) in a mixture of THF/water in a 4:1 ratio (10 mL), Fmoc-Osu (1.05 g, 3.12 mmol, 1.2 eq.), $NaHCO_3$ (0.524 g, 6.24 mmol, 2.4 eq.) and $Pd(OH)_2$ (50 mg) were added and the mixture was hydrogenated for 2h. The mixture was filtered and solvent was removed under reduced pressure. The crude was redissolved in ethyl acetate and was extracted with ethyl acetate (3 x 10 mL), washed with Brine (15 mL) and dried with Na_2SO_4. The crude was purified in column chromatography (5% MeOH in CH_2Cl_2) and **101b** is obtained (580 mg; 66%) as white foam.

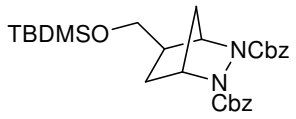
IR (KBr): ν 3422, 3066, 2941, 1718, 1704, 1655, 1457, 1420 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): 1.40 (d, *J* = 13.6 Hz, 1H, H-6), 1.66 (d, *J* = 10.4 Hz, 1H, H-7), 1.98 (d, *J* = 10.3 Hz, 1H, H-7), 2.05 (bs, 1H, H-6); 3.57 (s, 1H, H-1), 4.05 (d, *J* = 6.6 Hz, 1H, H-5), 4,23 (t, *J* = 6.9 Hz, 1H, Fmoc-9), 4.32 (bs, 1H, H-4), 4.42 (dq, *J* = 10.5 and 7.0 Hz, 2H, CH_2-Fmoc), 7.30 (t, *J* = 7.5 Hz, 2H, Fmoc-2 and -7), 7.39 (t, *J* = 7.5 Hz, 2H, Fmoc-3 and -6), 7.59 (d, *J* = 7.4 Hz, 2H, Fmoc-1 and -8), 7.75 (d, *J* = 7.5 Hz, 2H, Fmoc-4 and -5).

^{13}C-RMN (100 MHz, $CDCl_3$): 35.4 (C-7), 41.2 (C-6), 47.4 (Fmoc-9), 56.5 (C-4), 62.8 (C-1), 67.6 (CH_2-Fmoc), 72.4 (C-5), 120.2 (Fmoc-4 and -5), 125.3 (Fmoc-1 and -8), 127.3 (Fmoc-2 and -7), 128.0 (Fmoc-3 and -6), 141.5 (Fmoc-4a and -4b), 144.1 (Fmoc-8a and 9a), 156. 3 (CO carbamate).

ESI-MS (+): 159 $[M-(C_{14}H_{10})]^+$, 179 $[(C_{14}H_{10})+H]^+$, 337 $(M+H)^+$, 378 $(M+ACN)^+$, 673 $(2M+H)^+$.

Dibenzyl 5-[(*tert*-butyldimethylsilyloxy)methyl]-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (102)



102

To an ice-cooled stirred solution of **97** (1.10 g, 2.77 mmol, 1 eq.) and EtN_3 (0.54 mL, 3.88 mmol, 1.4 eq.) in CH_2Cl_2 (15 mL), TBDMSOTf (0.76 mL, 3.32 mmol, 1.2 eq.) was added dropwise. The solution was stirred for 2 h at 0ºC under Ar atmosphere. After evaporation of the solvent the crude mixture was purified by silica gel flash chromatography (cyclohexane / ethylacetate 8:2) to give **102** (1.36 g, 96%) as yellow oil.

Yellow oil

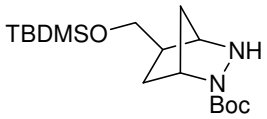
IR (KBr): ν 3033, 2951, 2885, 2856, 1741, 1705, 1498, 1455, 1389, 1322, 1258, 1112 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.03 (2s, 3H each one, CH_3-Si), 0.85 (s, 9H, ^{t}Bu-Si), 1.22 (s, 1H, H-6), 1.59 (d, *J* = 10.4 Hz, H-7), 1.71 (d, *J* = 9.8 Hz, 1H, H-7), 1.84 (s, 1H, H-6), 2.16 (s, 1H, H-5), 3.29 (m, 1H, CH_2-O), 3.51 (s, 1H, CH2-O), 4.52 (m, 2H, H-1 and H-4), 5.15 (m, 4H, CH_2-Ph), 7.29 (m, 10H, C_6H_5).

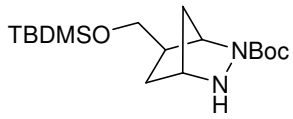
^{13}C-RMN (100 MHz, $CDCl_3$): δ -5.1 (CH_3-Si), 18.4 (C-^{t}Bu-Si), 26.1 (CH_3-^{t}Bu-Si), 31.5 (C-6), 35.5 (C-7), 42.2 (C-5), 60.7 and 62.8 (C-1 and C-4), 64.4 (CH_2-O), 68.2 (CH_2-Ph), 128.1 (C_6H_5-*p*), 128.3(C_6H_5-*o*), 128.7(C_6H_5-*m*), 136.2 (C_6H_5-*ipso*), 157.5 (CO carbamate).

ESI-MS (+): m/z 511 $(M+H)^+$, 1043 $(2M+Na)^+$.

tert-butyl 5-((tert-butyldimethylsilyloxy)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (103a) and tert-butyl 6-((tert-butyldimethylsilyloxy)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (103b)



103a



103b

A solution of **102** (350 mg, 0.68 mmol, 1eq.), Boc_2O (149 mg, 0.68 mmol, 1 eq.) and $Pd(OH)_2$ (45 mg) in MeOH (12 mL) was hydrogenated at P_{atm} for 45 min at RT. The reaction mixture was filtered through Celite and the resulting oil was purified by silica flash chromatography (Hexane / ethylacetate 7:3) to give **103a** (90 mg, 38 %) and **103b** (106 mg, 45 %).

Compound 103a

Yellow oil

IR (NaCl): ν 3349, 2956, 2930, 2857, 1712, 1693, 1519, 1391, 1366, 1254 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.01 (s, 6H, CH_3-Si), 0.85 (s, 9H, ^{t}Bu-Si), 1.13 (ddd, *J* = 12.7, 5.4 and 2.2 Hz, 1H, H-6), 1.45 (s, 9H, ^{t}Bu), 1.61 (d, *J* = 9.6 Hz, 1H, H-7), 1.72 (d, *J* = 10.1 Hz, 1H, H-7), 1.86 (m, 1H, H-6), 2.08 (m, 1H, H-5), 3.27 (dd, *J* = 10.4 and 8.4 Hz, 1H, CH_2-O), 3.47 (dd, *J* = 10.4 and 5.2Hz, 1H, CH_2O), 3.63 (s, 1H, H-4), 4.26 (s, 1H, H-1).

^{13}C-RMN (100 MHz, $CDCl_3$): δ -5.3 (CH_3-Si), -5.2 (CH_3-Si), 18.4 (C-^{t}Bu-Si), 26.1 (CH_3-^{t}Bu-Si), 28.7 (CH_3-^{t}Bu), 32.7 (C-6), 36.6 (C-7), 44.3 (C-5), 57.3 (C-1), 59.9 (C-4), 65.1 (CH_2-O), 80.3 (C-^{t}Bu), 155.7 (CO carbamate).

ESI-MS (+): m/z 243 $[(M-Boc)+H]^+$, 343 $(M+H)^+$, 686 $(2M+H)^+$.

Compound 103b

Yellow oil

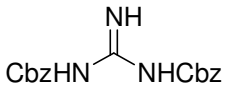
IR (NaCl): ν 3348, 2955, 2929, 2857, 1764, 1721, 1525, 1390, 1365 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.01 (2s, 6H, CH_3-Si), 0.85 (s, 9H, ^{t}Bu-Si), 1.19 (m, 1H, H_6), 1.43 (s, 9H, ^{t}Bu), 1.58 (m, 1H, H-7), 1.72 (m, 2H, H-6 and H-7), 2.14 (m, 1H, H-5), 3.25 (t, *J* = 9.5 Hz, 1H, CH_2-O), 3.42 (dd, *J* = 10.5 and 5.4 Hz, 1H, CH_2-O), 3.66 (s, 1H, H-1), 4.27 (s, 1H, H-4).

^{13}C-RMN (100 MHz, $CDCl_3$): δ -5.2 (CH_3-Si), -5.1 (CH_3-Si), 18.5 (C-^{t}Bu-Si), 26.1 (CH_3-^{t}Bu-Si), 28.7 (CH_3-^{t}Bu), 32.9 (C-6), 36.0 (C-7), 44.1 (C-5), 57.7 (C-1), 59.3 (C-4), 65.0 (CH_2-O), 80.3 (C-^{t}Bu), 155.4 (CO carbamate).

ESI-MS (+): m/z 243 $[(M\text{-Boc})+H]^+$, 343 $(M+H)^+$, 685 $(2M+H)^+$.

***N,N*-Di-Cbz-guanidine (104)**



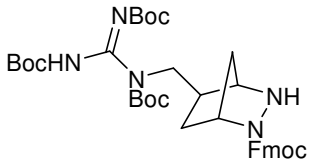
104

CH_2Cl_2 (64 mL) was added to a solution of guanidine hydrochloride (3 g, 31.4 mmol) and sodium hydroxide (6.3 g, 157 mmol) in H_2O (32 mL), and the resulting mixture was cooled to 0 ºC. Benzyloxycarbonyl chloride (13.4 mL, 94.2 mmol) was added dropwise with vigorous stirring over a period of 30 min. After the addition was complete, stirring was continued for 20 h at 0 ºC. The mixture was diluted with CH_2Cl_2 (80 mL), the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (80 mL). The extracts were combined, washed with H_2O, and dried with $MgSO_4$. After filtration and removal of the solvent under reduced pressure, the crude product was recrystallized from methanol to obtain **104** (8.6 g, 83 %).

IR (KBr): ν 3400, 3238, 1732, 1681, 1652, 1621, 1567, 1557, 1311, 1295, 1226 cm^{-1}.

^{1}H-RMN (400 MHz, DMSO-*d*$_6$): 5.12 (s, 4H, CH_2-Ph), 7.31-7.39 (m, 10H, C_6H_5), 8.68 (bs, 2H, NHCO), 10.88 (bs, 1H, NH).

(9H-fluoren-9-yl)methyl 5-((1,2,3-tris(tert-butoxycarbonyl)guanidino)methyl)-2,3-diaza-bicyclo[2.2.1]heptane-2-carboxylate (105)



105

To a solution of **99** (2.4 g, 3.25 mmol, 1 eq.) in ethyl acetate (70 mL), Fmoc-Osu (1.10 g, 3.25 mmol, 1 eq.), 2,2-bipyridyl (254 mg, 1.62 mmol) and 10 % Pd-C (93 mg) were added and the mixture was hydrogenated until complete consumption of starting material. After filtration, solvent was eliminated under reduced pressure and the crude was purified by silica gel column chromatography (hexane / ethyl acetate 8:2) giving **105** (863 mg; 33%) as yellow foam.

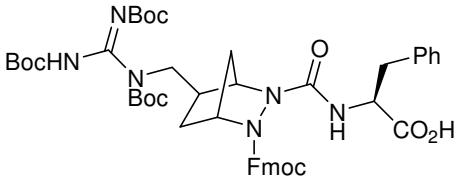
IR (KBr): ν 3374, 2978, 2931, 1758, 1718, 1610, 1517, 1452, 1369, 1245, 1139 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.15 (dd, *J* = 12.8 and 5.0 Hz, 1H, H-6); 1,48 and 1,50 (2s, 27H, $CH_3{}^tBu$); 1.74 (d, *J* = 10.4 Hz, 1H, H-7); 1.83-1.90 (m, 2H, H-6 and H-7), 2.24-2.35 (m, 1H, H-5), 3.64 (dd, *J* = 14.0 and 10.9 Hz, 1H, CH_2-N), 3.74 (dd, *J* = 14.2 and 5.7 Hz, 1H, CH_2-N), 3.89 (s, 1H, H-1), 4.26 (t, *J* = 7.1 Hz, 1H, Fmoc-9), 4.34 (s, 1H, H-4), 4.43 (d, *J* = 6.8 Hz, 2H, CH_2-Fmoc), 7.30 (t, *J* = 7.4 Hz, 2H, Fmoc-2 and -7), 7.39 (t, *J* = 7.4 Hz, 2H, Fmoc-3 and -6), 7.61 (d, *J* = 7.4 Hz, 2H, Fmoc-1 and -8), 7.76 (d, *J* = 7.5 Hz, 2H, Fmoc-4 and -5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.1 and 28.3 ($CH_3{}^tBu$), 34.5 (C-6), 36.7 (C-7), 41.3 (C-5), 47.5 (Fmoc-9), 50.2 (CH_2-N), 57.5 (C-4), 59.9 (C-1), 67.4 (CH_2-Fmoc), 83.7 ($C{}^tBu$), 120.2 (Fmoc-4 and -5), 125.4 (Fmoc-1 and -8), 127.3 (Fmoc-2 and -7), 127.9 (Fmoc-3 and -6), 141.5 (Fmoc-4a and -4b), 144.2 (Fmoc-8a and -9a), 153.6, 153.8 and 156.0 (CO carbamate).

MS-ESI (+): 392 $[M-(Boc)_3+H]^+$, 492 $[M-(Boc)_2+H]^+$, 592 $[M-(Boc)+H]^+$, 692 $(M+H)^+$, 1383 $(2M+H)^+$.

(2S)-2-(5-((1,2,3-tris(tert-butoxycarbonyl)guanidino)methyl)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)-2,3-diaza-bicyclo[2.2.1]heptane-3-carboxamido)-3-phenylpropanoic acid (108)



108

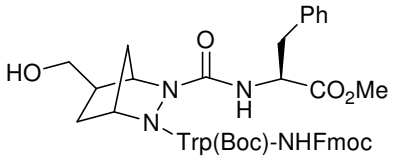
92 (800 mg; 0,89 mmol) was taken up in *i*PrOH (20 mL) and $CaCl_2$ (1,58 g; 14,26 mmol) was added. Separately, $LiOH \cdot H_2O$ (149 mg; 3,56 mmol) was dissolved in H_2O (5 mL) and was added to the reaction mixture which was stirred as a cloudy white solution for 2 h. Organic solvent was removed by evaporation and the resulting residue was then acidified to pH 2 with HCl 1N and was extracted with CH_2Cl_2 (5 x 30 mL). The combined organic layers were then washed with HCl 1N (1 x 50 mL) and Brine (1 x 50 mL), dried over $MgSO_4$ and concentrated to dryness. Compound **108** (584 mg, 74%) was used without further purification.

^{1}H-RMN (400 MHz, CD_3OD): δ 1.01-1.11 (m, 1H, H-6), 1.45-1.52 (m, 28H, $CH_3{}^tBu$ and H-7), 1.61-1.68 (m, 2H, H-6 and H-7), 2.17-2.30 (m, 1H, H-5), 3.02 (dd, *J* = 13.8 and 7.7 Hz, 1H, CH_2-Ph), 3.11-3.20 (m, 1H, CH_2-Ph), 3.43-3.55 (m, 2H, CH_2-N), 4.08-4.25 (m, 2H, H-1 and Fmoc-9), 4.30-4.51 (m, 3H, CH_2-Fmoc and H-4), 4.57 (dd, *J* = 7.9 and 4.5 Hz, 1H, Hα), 7.08-7.17 (m, 5H, C_6H_5), 7.27-7.33 (m, 2H, Fmoc-2 and -7), 7.38 (t, *J* = 7.4 Hz, 2H, Fmoc-3 and -6), 7.58 (t, *J* = 7.3 Hz, 2H, Fmoc-1 and -8), 7.79 (d, *J* = 7.7 Hz, 2H, Fmoc-4 and -5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.2 and 28.4 ($CH_3{}^tBu$), 32.9 (C-6), 35.0 (C-7), 38.4 (CH_2-Ph), 39.0 (C-5), 47.1 (Fmoc-9), 49.8 (CH_2-N), 54.5 (C-α), 60.6 (C-1), 62.1 (C-4), 68.4 (CH_2Fmoc), 83.9 ($C{}^tBu$), 120.1 (Fmoc-4 and -5), 125.4 (Fmoc-1 and -8), 127.1 (Fmoc-2 and -7), 127.4 (C_6H_5-*p*), 128.0 (Fmoc-3 and -6), 128.5 (C_6H_5-*o*), 129.5 (C_6H_5-*m*), 136.5 (C_6H_5-*ipso*), 141.5 (Fmoc-4a and -4b), 143.7 (Fmoc-8a and -9a), 153.5 (CO carbamate), 160.4 (CO urea), 174.5 (CO acid).

MALDI-TOF (ACH): m/z 883.4 $(M+H)^+$, 905.4 $(M+Na)^+$, 921.4 $(M+K)^+$.

tert-butyl 3-(3-(3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-5-(hydroxymethyl)-2,3-diaza-bicyclo[2.2.1]heptan-2-yl)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-oxopropyl)-1H-indole-1-carboxylate (110)



110

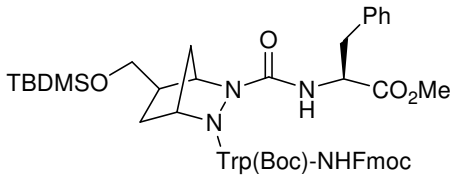
111 (250 mg; 0,26 mmol) was dissolved in a mixture of AcOH:H_2O:THF in a ratio 3:1:1 (10 mL) and the solution was stirred overnight at rt.Then organic solvent was evaporated and the residue was extracted with ethyl acetate and washed with 5% aquous solution of $NaHCO_3$. After purification by silica gel flash chromatography (hexane / ethyl acetate 1:1) alcohol **110** (126 mg; 58 %) was obtained as colorless oil.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.94 (dd, *J* = 12.6 and 6.1 Hz, 1H, H-6), 1.32-1.43 (m, 2H, H-5 and H-7), 1.60-1.68 (m, 11H, $CH_3{}^tBu$, H-6 and H-7), 2.78-2.92 (m, 2H, CH_2-ind and CH_2-Ph), 3.11 (s, 4H, CH_3-O and CH_2-ind), 3.24-3.41 (m, 3H, CH_2-O and CH_2-Ph), 4.17 (t, *J* = 6.8 Hz, 1H, Fmoc-9), 4.22-4.34 (m, 2H, CH_2-Fmoc), 4.37 (s, 1H, H-1), 4.74-4.82 (m, 1H, H-α Phe), 4.92 (s, 1H, H-4), 5.00-5.06 (m, 1H, H-α Trp), 5.25 (bs, 1H, NH Trp), 7.13-7.17 (m, 1H, H-Ar), 7.20-7.29 (m, 7H, H-Ar, Fmoc-2 and -7), 7.33 (d, *J* = 7,8 Hz, 1H, H-Ar), 7.38 (t, *J* = 7,5 Hz, 2H, Fmoc-3 and -6), 7.52 (dd, *J* = 10.9 and 7.75 Hz, 3H, H-Ar, Fmoc-1 and -8), 7.59 (bs, 2H, H-Ar and NH urea), 7.74 (d, *J* = 7.5 Hz, 2H, Fmoc-4 and -5), 8,11 (d, *J* = 7.8 Hz, H-Ar).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 25.8 (CH_2-ind), 28.4 ($CH_3{}^tBu$), 31.5 (C-6), 36.4 (C-7), 37.0 (CH_2-Ph), 40.4 (C-5), 47.2 (Fmoc-9), 51.8 (C-α Trp), 51.9 (CH_3-O), 54.4 (C-α Phe), 59.9 (C-4), 61.8 (C-1), 64.0 (CH_2-O), 67.8 (CH_2-Fmoc), 84.2 ($C{}^tBu$), 114.8, 115.4, 119.6, 120.2, 123.3, 125.3, 127.3, 128.0, 128.6, 129.4, 135.7, 137.8, 141.4, 143.8, 143.9 (C_6H_5), 149.9 and 157.2 (CO carbamates), 160.2 (CO urea), 172.2 (CO ester), 179.3 (CO amide).

MALDI-TOF (ACH): m/z 842.4 $(M+H)^+$, 864 $(M+Na)^+$, 880 $(M+K)^+$.

tert-butyl 3-(3-(3-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-5-((tert-butyldimethylsilyloxy)methyl)-2,3-diaza-bicyclo[2.2.1]heptan-2-yl)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-oxopropyl)-1H-indole-1-carboxylate (111)



111

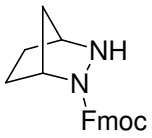
To a mixture of PyBOP (468 mg; 0,9 mmol), HOAt (122 mg; 0,9 mmol) and Fmoc-Trp(Boc)-OH (710 mg; 1,35 mmol) in CH_2Cl_2 (15 mL), DIPEA (313 μL; 1,8 mmol) was added and the mixture was stirred for 20 min at 0ºC. Then, **110** was added and the mixture was allowed to stir at rt for 4h. After that time more PyBOP (468 mg; 0,9 mmol), HOAt (122 mg; 0,9 mmol), Fmoc-Trp(Boc)-OH (710 mg; 1,35 mmol) and DIPEA (313 μL; 1,8 mmol) were added and reaction was stirred overnight. The crude was washed with a 5% aq solution of $NaHCO_3$ (3 X 10 mL) and saturated solution of NH_4Cl (2 X 10 mL). The organic phase was dried over $MgSO_4$, filtered and solvent was removed under vacuum. **111** (310 mg; 72 %) was obtained after purification by column chromatography as a colorless oil.

IR (NaCl): ν 3335, 3062, 2953, 2929, 1731, 1704, 1673, 1530, 1452, 1369, 1256 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 0.04 (s, 6H, CH_3-Si), 0.89 (s, 9H, CH_3-^{t}Bu-Si), 1.10 (dd, *J* = 12.9 and 5.9 Hz, 1H, H-6), 1.34 (d, *J* = 10.2 Hz, 1H, H-7), 1.40 (m, 1H, H-5), 1.68 (m, 11H, $CH_3{}^t$Bu, H-6 and H-7), 2.80 (dd, *J* = 13.9 and 11.5 Hz, 1H, CH_2-ind), 2.89 (t, *J* = 13.0 Hz, 1H, CH_2-Ph), 3.12 (m, 4H, CH_3-O and CH_2-ind), 3.24 (dd, *J* = 9.9 and 6.1 Hz, 1H, CH_2-O), 3.30 (dd, *J* = 14.2 and 3.1 Hz, 1H, CH_2-Ph), 3.49 (dd, *J* = 9.3 and 2.8 Hz, 1H, CH_2-O), 4.18 (t, *J* = 7.0 Hz, 1H, Fmoc-9), 4.25 (m, 1H, CH_2-Fmoc), 4.32 (dd, *J* = 10.1 and 7.6 Hz, 1H, CH_2-Fmoc), 4.39 (s, 1H, H-1), 4.74 (m, 1H, H-α Phe), 4.85 (s, 1H, H-4), 5,.05 (m, 1H, H-α Trp), 5.21 (s, 1H, NH Trp), 7.15 (dd, *J* = 5.2 and 3.3 Hz, 1H, H-Ar), 7.20 (m, 4H, C_6H_5), 7.27 (m, 3H, Fmoc-2; Fmoc-7 and C_6H_5), 7.33 (d, *J* = 7.7 Hz, 1H, C_6H_5), 7.38 (t, *J* = 7.5 Hz, 2H, Fmoc-3 and -6), 7.52 (m, 3H, C_6H_5 and NH urea), 7.59 (d, *J* = 6.9 Hz, Fmoc-1 and -8), 7.74 (d, *J* = 7.6 Hz, Fmoc-4 and -5), 8.11 (d, *J* = 7.8 Hz, C_6H_5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ -5.3 and -5.2 (CH_3-Si), 18.5 (C^{t}Bu-Si), 26.1 (CH_3^{t}Bu-Si), 28.4 (CH_3-^{t}Bu), 31.3 (C-6), 36.9 (C-7), 37.0 (CH_2-Ph), 39.8 (C-5), 47.2 (Fmoc-9), 51.7 (C-α Trp), 52.0 (CH_3-O), 54.6 (C-α Phe), 60.9 (C-4), 61.9 (C-1), 64.1 (CH_2-O), 67.8 (CH_2-Fmoc), 84.1 (C^{t}Bu), 114.8, 115.4, 119.6, 120.2, 124.6, 125.1, 125.3, 126.8, 127.3, 127.4, 128.0, 128.6, 129.3, 135.8, 137.7, 141.4, 143.9 (C_6H_5), 149.8 and 157.0 (CO carbamates), 160.2 (CO urea), 172.4 (CO ester), 179.1 (CO amide).

ESI-MS (+): m/z 857 $[(M\text{-Boc})+H]^+$, 957 $(M+H)^+$.



114

To a solution of **116** (0.70 g, 1.54 mmol, 1 eq.) in ethyl acetate (8 mL), $Pd(OH)_2$ (65 mg) was added and the mixture was allowed to stirr overnight. The catalyst was then filtered through celite and the filtrate was concentrated. The crude product was purified by column chromatography (Hexane/ethyl acetate 7:3) to yield **114** (362 mg, 73%) as colorless oil.

^{1}H-RMN (400 MHz, $CDCl_3$): 1.24-1.77 (m, 4H, H-5 and H-6), 2.01 (d, *J* = 10.3 Hz, 2H, H-7), 3.64 (s, 1H, H-1), 4,25 (t, *J* = 7.0 Hz, 1H, Fmoc-9), 4.35 (bs, 1H, H-4), 4.45 (t, *J* = 6.8 Hz, 2H, CH_2-Fmoc), 7.26-7.33 (m, 2H, Fmoc-2 and -7), 7.40 (t, *J* = 7.6 Hz, 2H, Fmoc-3 and -6), 7.61 (d, *J* = 7.6 Hz, 2H, Fmoc-1 and -8), 7.76 (d, *J* = 7.6 Hz, 2H, Fmoc-4 and -5).

^{13}C-RMN (100 MHz, $CDCl_3$): 29.9 (C-5), 35.3 (C-6), 41.2 (C-7), 47.5 (Fmoc-9), 56.2 (C-4), 60.6 (C-1), 67.6 (CH_2-Fmoc), 120.2 (Fmoc-4 and -5), 125.3 (Fmoc-1 and -8), 127.3 (Fmoc-2 and -7), 128.0 (Fmoc-3 and -6), 141.7 (Fmoc-4a and -4b), 144.2 (Fmoc-8a and 9a), 156.4 (CO carbamate).

MS-ESI (+): m/z 321 $(M+H)^+$, 362 $(M+MeCN)^+$, 641 $(2M+H)^+$.

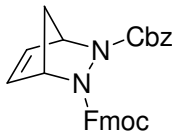
115

To a solution of **117** (2.00 g, 4.78 mmol, 1 eq.) in CH_2Cl_2 (7 mL), TFA (7 mL) was then added and the mixture was stirred for 2 h at rt. Excess of the reagent and solvent were evaporated under N_2 stream and then under vacuum. The residue was dissolved in CH_2Cl_2 and was washed with saturated solution of $NaHCO_3$. Organic phase was then evaporated to dryness and the crude product was purified by column chromatography (hexane/ethyl acetate 7:3) to yield compoun **115** (1.24 g, 81%) as yellow solid.

IR (KBr): ν 3414, 3255, 3066, 2953, 1719, 1541, 1248, 1202, 1168 cm^{-1}.

^{1}H-RMN (400 MHz, $CDCl_3$): 1.26-2.00 (m, 2H, H-7), 4.22 (t, *J* = 6.2 Hz, Fmoc-9), 4.32-4.71 (m, 2H, CH_2-Fmoc), 5.92-6.31 (m, 2H, H-5 and H-6), 7.25-7.34 (m, 2H, Fmoc-2 and -7), 7.41 (t, *J* = 7.4 Hz, 2H, Fmoc-3 and -6), 7.57 (d, *J* = 7.6 Hz, 2H, Fmoc-1 and -8), 7.77 (d, J = 7.6 Hz, 2H, Fmoc-4 and -5).

MS-ESI (+): m/z 140 $(M-C_{14}H_{11})^+$, 179 $(C_{14}H_{11})^+$, 319 $(M+H)^+$, 637 $(2M+H)^+$.

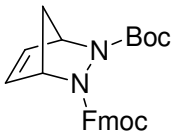


116

Cyclopentadiene (4.5 mL, 54.04 mmol, 3.5 eq.) was added to a solution of **118** (6.00 g, 15.44 mmol, 1 eq.) in CH_2Cl_2 (40 mL) at 0 ºC. Lead tetraacetate (IV) (6.84 g, 15.44 mmol, 1 eq.) was then added slowly and the mixture was stirred for 2 h at 0 ºC. The mixture was filtered and concentrated under reduced pressure. Compound **116** (6.23 g, 89%) was obtained after purification by column chromatography hexane/ethyl acetate 7:3).

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.60-1.72 (m, 2H, H-7), 4.22 (bs, 1H, Fmoc-9), 4.40-4.66 (m, 2H, CH_2-Fmoc), 4.91-5.28 (m, 4H, CH_2-Ph, H-1 and H-4), 6.44 (bs, 2H, H-5 and H-6), 7.29-7.39 (m, 7H, Fmoc-2 and -7 and C_6H_5), 7.42 (t, J = 7.3 Hz, Fmoc-3 and -6), 7.63 (d, *J* = 7.1 Hz, 2H, Fmoc-1 and -8), 7.79 (d, *J* = 7.4 Hz, 2H, Fmoc-4 and -5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 47.1 (Fmoc-9), 48.1 (C-7), 65.3 and 65.4 (C-1 and C-4), 68.1 (CH_2Fmoc), 120.0 (Fmoc-4 and -5), 125.1(Fmoc-1 and -8), 127.1 (Fmoc-2 and -7), 127.8 (Fmoc-3 and -6), 128.0, 128.2 and 128.5 (C_6H_5), 135.9 (C_6H_5-*ipso*), 141.4 (Fmoc-4a and -4b), 143.7 (Fmoc-8a and -9a), 158.8 (CO carbamate).



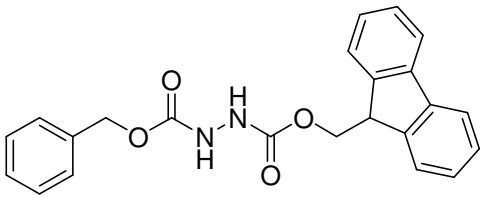
117

Cyclopentadiene (3.3 mL, 39.5 mmol, 3.5 eq.) was added to a solution of **119** (4.00 g, 11.28 mmol, 1 eq.) in CH_2Cl_2 (100 mL) at 0 ºC. Lead tetraacetate (IV) (5.00 g, 11.28 mmol, 1 eq.) was then added slowly and the mixture was stirred for 3 h at 0 ºC. The mixture was filtered to removed the lead and filtrate was concentrated under reduced pressure. Compound **117** (4.12 g, 87%) was obtained after purification by column chromatography hexane/ethyl acetate 7:3).

^{1}H-RMN (400 MHz, $CDCl_3$): δ 1.49 (s, 9H, CH_3-^{t}Bu), 1.66-1.72 (m, 2H, H-7), 4.24 (t, J = 6.2 Hz, 1H, Fmoc-9), 4.40-4.62 (m, 2H, CH_2-Fmoc), 5.07 (bs, 2H, H-1 and H-4), 6.46 (bs, 2H, H-5 and H-6), 7.31 (t, J = 7.2 Hz, 2H, Fmoc-2 and -7), 7.40 (t, J = 7.4 Hz, Fmoc-3 and -6), 7.66 (d, *J* = 7.2 Hz, 2H, Fmoc-1 and -8), 7.76 (d, *J* = 7.4 Hz, 2H, Fmoc-4 and -5).

^{13}C-RMN (100 MHz, $CDCl_3$): δ 28.3 (CH_3^{t}Bu), 47.1 (Fmoc-9), 48.1 (C-7), 65.3 and 65.4 (C-1 and C-4), 68.1 (CH_2Fmoc), 120.0 (Fmoc-4 and -5), 125.1(Fmoc-1 and -8), 127.1 (Fmoc-2 and -7), 127.8 (Fmoc-3 and -6), 141.4 (Fmoc-4a and -4b), 143.7 (Fmoc-8a and -9a), 158.8 (CO carbamate).

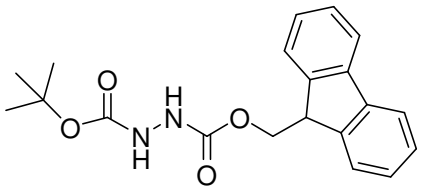
ESI-MS (+): m/z 179 $(C_{14}H_{11})^+$, 140 $[(M\text{-}Boc)\text{-}(C_{14}H_{11})]^+$, 319 $[(M\text{-}Boc)]^+$, 419 $(M+H)^+$.



118

A solution of 10% $NaHCO_3$ in H_2O (80 mL, 95.2 mmol, 5.3 eq.) and Fmoc-Cl (5.10 g, 19.2 mmol, 1.1 eq.) were added to a solution of benzyl carbazate (3 g, 18 mmol, 1 eq.) in THF (70 mL) and the mixture was stirred overnight. The crude product was purified by chromatography (hexane/ethyl acetate 7:3) to yield compound **118** (6.54g, 93%) as white foam.

^{1}H-RMN (400 MHz, $CDCl_3$): δ 4.22 (bs, 1H, Fmoc-9), 4.44 (d, J = 6.8 Hz, 2H, CH_2-Fmoc), 5.16 (s, 2H, CH_2Ph), 6.66 (bs, 2H, NH), 7.25-7.35 (m, 7H, Fmoc-2 and -7 and C_6H_5), 7.39 (t, *J* = 7.4 Hz, 2H, Fmoc-3 and -6), 7.56 (d, *J* = 6.9 Hz, 2H, Fmoc-1 and -8), 7.75 (d, *J* = 7.6 Hz, 2H, Fmoc-4 and -5).



119

To a solution of tert-butyl carbazate (2.10 g, 15.9 mmol, 1 eq.) in dioxane (30 mL), a solution of 10% $NaHCO_3$ in H_2O (80 mL, 95.4 mmol, 6 eq.) was added and stirred for 5 min. Fmoc chloride (4.93 g, 19.1 mmol, 1.2 eq.) was then added and the mixture was stirred overnight at rt. After solvent evaporation, the crude product was purified by column chromatography (hexane/ethyl acetate 8:2) to yield **119** (4.21 g, 74%) as a white solid.

^{1}H-RMN (400 MHz, CD_3OD): δ 1.46 (s, 9H, $CH_3{}^tBu$), 4.23 (t, *J* = 6.8 Hz, 1H, Fmoc-9), 4.30-4.39 (m, 2H, CH_2-Fmoc), 7.30 (td, *J* = 7.4 and 0.8 Hz, 2H, Fmoc-2 and -7), 7.38 (t, *J* = 7.4 Hz, 2H, Fmoc-3 and -6), 7.66 (d, *J* = 7.4 Hz, 2H, Fmoc-1 and -8), 7.79 (d, *J* = 7.5 Hz, 2H, Fmoc-4 and -5).

^{13}C-RMN (100 MHz, CD_3OD): δ 27.4 ($CH_3{}^tBu$), 47.1 (Fmoc-9), 67.4 (CH_2Fmoc), 80.6(C-^{t}Bu), 119.7 (Fmoc-4 and -5), 125.1(Fmoc-1 and -8), 127.0 (Fmoc-2 and -7), 127.6 (Fmoc-3 and -6), 141.4 (Fmoc-4a and -4b), 143.9 (Fmoc-8a and -9a).

MS-ESI (+): m/z 179 $(C_{14}H_{11})^+$, 255 $(M\text{-Boc})^+$, 299 $(M\text{-}^tBu)^+$, 355 $(M+H)^+$, 609 ([2M-Boc]+H)$^+$, 709 $(2M+H)^+$.

7.5. Experimental procedures of chapter 5

7.5.1. General procedures for solid phase peptide synthesis

Manual solid-phase peptide synthesis was performed in polypropylene syringes, each fitted with a polyethylene porous disk, using the Fmoc/^{t}Bu strategy. Solvents and soluble reagents were removed by suction. Washings between deprotection, coupling ans subsequent deprotection steps were carried out with DMF and DCM using 10 mL of solvent/g of resin each time. The Fmoc group was removed by treatment with 20% piperidine in DMF and acetylation steps were performed with Ac_2O-DIEA-DMF (1:2:7). Couplings and washes were performed at 25 ºC. Couplings were monitored by Kaiser method. Manual parallel solid-phase synthesis was carried out in a VacMan® vacuum manifold for fast removal of excess of reagents an solvents. Resins were swollen in DCM for 30 min and DMF (5 x 0.5 min).

7.5.1.1. Protocol for the incorporatation of the first amino acid

The first amino acid is incorporated to Rink Amide MBHA resin by forming an amide bond. This resin allows the obtention of peptidil carboxamide.

Step	Reagents	Operation	Treatments	Time
1	DMF	Wash	5	0.5 min
2	20% piperidine/DMF	Deprotection	2	10 min
3	DMF	Wash	5	0.5 min
4	DCM	Wash	5	0.5 min
5	DMF	Wash	5	0.5 min
	Fmoc-aa-OH (3 eq.)			
6	DIPCDI (3eq.)	Coupling	1	2 h
	HOBt (3 eq.)			
7	DMF	Wash	5	0.5 min
8	DCM	Wash	5	0.5 min

Table III. General protocol for the incorporation of first amino acid.

Previosly swollen Rink Amide MBHA resin was treated with 20% piperidine in DMF (2 x 10 min) to remove the Fmoc group and washed with DMF (5 x 0.5 min) and DCM (5 x 0.5 min) and DMF (5 x 0.5 min). For pre-activation of the first amino acid, 3 eq. of

DIPCDI and 3 eq. of HOBt were added to a solution of 3 eq. of protected amino acid (0.1 M) in DMF. After 3 min of pre-activation, the mixture was added to the resin and it was allowed to react for 2 h with occasional manual stirring. The resin was then washed with DMF (5 x 0.5 min) and DCM (5 x 0.5 min) and coupling was controlled by Kaiser test.

With a reduction of the loading of the resin was required only 0.4 eq. of DIPCDI, 0.4 eq. of HOBt and 0.4 eq. of Fmoc protected amino acid were used. Once firts amino acid was coupled, the loading of the resin was calculated by measuring the Fmoc groups remaining on the resin. For this, a small amount of resin (1 mg) was treated with 20% piperidine in DMF (10 mL) for 30 min. Nα-Fmoc deprotection with piperidine gives the fulvene-piperidine adduct, which can be quantitatively determined by spectrophotometric measurements at 290 nm using the following equation:

$$\text{Loading (mmol/g)} = Abs_{sample} \times 0.4^{a}$$

a based on $\varepsilon_{290nm} = 5800\ mol^{-1} \cdot cm^{-1}$

(ε depends also on the specifications of the spectrometer)

Step	Reagents	Operation	Treatments	Time
1	DMF	Wash	5	0.5 min
2	20% piperidine/DMF	deprotection	2	10 min
3	DMF	Wash	5	0.5 min
4	DCM	Wash	5	0.5 min
5	Fmoc-aa-OH (0.4 eq.) DIPCDI (0.4 eq.) HOBt (0.4 eq.)	Coupling	1	2 h
6	DMF	Wash	5	0.5 min
7	Ac_2O (10 eq.) DIEA (20 eq.)	Capping	2	20 min
8	DMF	Wash	5	0.5 min
9	DCM	Wash	5	0.5 min

Table IV. Incorporation of the first amino acid to reduce the loading of the resin to 0.2 mmol/g

7.5.1.2. Iterative peptide assembly

Deprotection

The resin was treated with 20% piperidine in DMF (2 x 10 min) and subsequently washed with DMF (5 x 0.5 min), DCM (5 x 0.5 min) and DMF (5 x 0.5 min).

Step	Reagents	Operation	Treatments	Time
1	DMF	Solvatation	5	0.5 min
2	20% piperidine/DMF	Deprotection	2	10 min
3	DMF	Wash	5	0.5 min
4	DCM	Wash	5	0.5 min
5	DMF	Wash	5	0.5 min

Table V. Protocol for Fmoc removal.

Amino acid coupling

Two methods have been used for the elongation of the peptide.

DIPCDI/HOBt

This method is based on the formation of benzotriazole ester of the N-Fmoc protected amino acid. We used the following reagents: DIPCDI (3 eq), HOBt (3 eq.) and N-Fmoc-aa-OH (3 eq.). This method is used for the comercial available amino acids.

Step	Reagents	Operation	Treatments	Time
1	DMF	Solvatation	5	0.5 min
2	20% piperidine/DMF	Deprotection	2	10 min
3	DMF	Wash	5	0.5 min
4	DCM	Wash	5	0.5 min
5	DMF	Wash	5	0.5 min
6	Fmoc-aa-OH Coupling reagents	Coupling	1	1 h
7	DMF	Wash	5	0.5 min
8	DCM	Wash	5	0.5 min
9	DMF	Wash	5	0.5 min

Table VI. General protocol for peptide elongation.

A preactivated solution of 3 eq. of N-Fmoc protected amino acid in DMF (3 mL) using 3 eq. of DIPCDI and 3 eq. of HOBt was added to the resin. After 1 h, the resin was washed with DMF (5 x 0.5 min) and DCM (5 x 0.5 min).

PyBOP/HOBt/DIEA

This method is based on the formation of the benzotriazole ester of N-Fmoc protected amino acid and it has been used fort he coupling of pseudodipeptide **13**, and amino acids following diazanorbornane pseudodipeptides **108**, **101b**, **114** and **115**.

In this method, a preactivated solution of 3 eq. of N-Fmoc protected amino acid in DMF (3 mL) using 3 eq. of PyBOP, 3 eq. of HOBt or HOAt and 6 eq. of DIEA was added and was allowed to stand for 1 h. After this time the resin was washed with DMF (5 x 0.5 min) and DCM (5 x 0.5 min).

Capping or N-terminal acetylation

With the aim of avoiding the formation of byproducts of difficult separation, capping of uncoupled amines in the polymeric support is required. For this, a solution of acetic anhydride and DIEA in DMF (1:2:7) is added to the resin (2 x 20 min). The resin is, then washed with DMF (5 x 0.5 min) and DMF (5 x 0.5 min).

Step	Reagents	Operation	Treatment	Time
1	DMF	Solvatation	5	0.5 min
2	Ac2O (10 eq.) DIEA (20 eq.)	Acetylation	2	20 min
3	DMF	Wash	5	0.5 min
4	DCM	Wash	5	0.5 min
5	DMF	Wash	5	0.5 min

Table VII. General method for amine acetylation.

7.5.1.3. Macrocyclic lactam ring formation

Typical procedure for Alloc/Allyl removal and macrocyclization

To the pre-swollen resin, under Ar atmosphere, dried DCM (1 mL) and phenylsilane (24 eq.) were added. After 5 min, a solution of $Pd[PPh_3]_4$ (0.1 eq.) in dried DCM (1 mL) was added and the mixture was allowed to stand for 20 min. After this time the resin was drained off, washed with DCM (5 x 1 min) and the deprotection procedure was

repeated twice more, allowing the mixture to stand for 20 min. Finally, the resin was successively washed with DCM (5 x 1 min), DMF (5 x 1 min) and DCM (5 x 1 min).

Step	Reagents	Operation	Treatments	Time
1	DCM	Solvatation	5	0.5 min
2	$PhSiH_3$ (24 eq.) $Pd[PPh_3]_4$ (0.1 eq.)	Deprotection	3	20 min
3	DCM	Wash	5	0.5 min
4	DMF	Wash	5	0.5 min
5	DCM	Wash	5	0.5 min

Table VIII. General method fort he Alloc/allyl removal.

Solid phase side chain cyclization (Rink Amide MBHA)

The macrocyclic lactam ring formation was then mediated by addition of PyBOP (3 eq.), HOAt (3 eq.) and DIEA (6 eq.) for 2 h. The process was repeated if necessary (Kaiser test was used to monitor completion).

Step	Reagents	Operation	Treatment	Time
1	DMF PyBOP (3 eq.)	Solvatation	5	0.5 min
2	HOAt (3 eq.) DIEA (6 eq.)	Cyclization	1	2 h
3	DMF	Wash	5	0.5 min
4	DCM	Wash	5	0.5 min
5	DMF	Wsh	5	0.5 min

Table IX. General procedure for lactam ring formation.

Cleavage of Rink Amide peptidil resin

A mixture of TFA/TIS/H_2O (95:2.5:2.5 v:v:v) was added. After 2 h, the resin was removed from solution by filtration. Then, the resin was washed with TFA (4 x 5 mL) and the crude peptide was recovered by precipitation with cold MTBE giving a white powder. Precipitate was then resuspended in H_2O/MeCN and liophylized.

Step	Reagents	Operation	Treatment	Time
1	DMF	Wash	5	0.5 min
2	DCM	Wash	5	0.5 min
3	TFA/TIS/H_2O (95:2.5:2.5)	Cleavage	2	1.5 h
4	TFA	Wash	4	2 min

Table X. General method for peptide cleavage from Rink Amide resin and side chain deprotection.

Crude peptides were analyzed by analytical HPLC and MALDI-TOF and purified by semi-preparative HPLC if necessary.

Cleavage of Sieber Amide peptidil resin

Cleavage of peptide-resin bond without side chain deprotection could be achieved using 1% TFA in DCM, following the protocol described in table XI.

Step	Reagents	Operation	Treatment	Time
1	DCM	Solvatation	5	0.5 min
2	1% TFA/DCM	Cleavage	3	15 h
3	DCM	Wash	5	0.5 min

Table XI. General protocol for peptide cleavage from the Sieber Amide resin.

Filtrates are collected in a round bottom flask containing a solution of 10% pyridine in MeOH. Solvents and excess of reagents were evaporated at reduced pressure and the resulting crude was precipitated in H_2O.

Solution phase cyclization (Sieber Amide)

When Sieber Amide resin was used, cyclization was carried out in solution. For this, PyBOP (1.5 eq.), HOBt (1.5 eq.) and DIEA (3 eq.) were added to a solution of protected linear peptide (10^{-4} M) in a mixture of DMF/DCM (97:3 v:v).

Solution side chain deprotection (Sieber Amide)

Side chains of cyclic peptide were deprotected using a mixture of TFA/TIS/H_2O (95:2.5:2.5 v:v:v) for 2 h. Excess of reagents were evaporated under Ar stream and peptide was precipitated with cold MTBE.

Crude peptide was analyzed by analytical HPLC and MALDI-TOF and was purified by semipreparative HPLC.

7.5.2. Analytical methods

7.5.2.1 Kaiser Test[196]

The Kaiser test is a colorimetric test to detect the presence of free terminal amino groups in solid phase peptide synthesis. We used it to make sure that each coupling step in peptide synthesis goes to completion. It is based on the reaction of ninhydrin with amino groups to form a blue adduct. Therefore, an incomplete coupling cycle will lead to a positive Kaiser test, demonstrated by the development of a blue color, while coupling to completion will yield a negative (yellow) test.

To perform this test it is necessary to prepare two different solutions:

Kaiser A solution: 40 g of phenol were dissolved in 10 mL of absolute EtOH. In a separate flask a soltion of 65 mg of KCN in 100 mL of H_2O was prepared, and 2 ml of this solution were diluted in 100 mL of pyridine which was distilled over ninhydrin. These two solutions were stirred separately for 45 min with 4 g of Amberlite MB-3 resin, filtered and combined. This is the Kaiser A solution.

Kaiser B solution: 2.5 g of ninhydrin were dissolved in 50 mL of absolute EtOH and solutions were stored in amber dripper bottles.

The recommended standard procedure consist in the addition of 3 drops of Kaiser A solution and 1 drop of Kaiser B solution over a few resin beads, and heat the mixture to 110 ºC for 3 min. If the test is positive the resin and solution turned to blue. However, when resin and solution are colourless to light yellow the test is negative.

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[196] Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I., *Anal. Biochem.*, **1970**, *34*, 595-598.

7.5.2.2. High-performance liquid chromatography

Peptides were analized by analytical HPLC with a PDA detector, using a reverse-phase Symmetry C_{18} column (4.6 x 150 mm, 5 μm) and linear gradients of MeCN with 0.036% TFA into H_2O with 0.045% TFA. The system was run at a flow rate of 1.0 mL/min over 15 min.

7.5.2.3. Mass spectrometry (MALDI-TOF)

Molecular mass of all peptides were determined by MALDI-TOF mas spectrometry. For the preparation of the samples, 1 μL of a peptide solution (1 mg/ml) and 1 μL of the matrix were mixed over the MALDI plate and it was allowed to evaporate.

To record our mass spectra, CHCA matrix (α-cyano-4-hydroxycinnamic acid)[197] was used. A concentration of 10 mg/mL of this matrix was prepared using a mixture of MeCN/H_2O (1:1) with 0,1% TFA as solvent.

7.6. Synthesis of peptides

7.6.1. Synthesis of MT2

Rink amide MBHA resin (300 mg, 0.56 mmol/g) was placed in a 10 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was swollen with DCM for 30 min and firsts amino acid N^{α}-Fmoc-Lys(Alloc)-OH (228 mg, 0.50 mmol, 3 eq.), was coupled after Fmoc removal as described in section 7.5.1.1. The following amino acids were then added to the growing peptide chain by stepwise addition of N^{α}-Fmoc-Trp(Boc)-OH (265 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-Arg(Pbf)-OH (327 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-D-Phe-OH (195 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-His(Trt)-OH (312 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-Asp(Allyl)-OH (199 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-Nle-OH (178 mg, 0.50 mmol, 3 eq.) as describe in table VI, using DIPCDI (78 μL, 0.50 mmol, 3 eq.) and HOBt (68 mg, 0.50 mmol, 3 eq.) in DMF (1 mL). In all cases, after 60 min of coupling, the ninhydrin test was negative. The N^{α}-Fmoc protecting groups were removed as described in table V. The peptide resin was then washed with DMF and DCM as described above and next coupling step was then initiated in a stepwise

[197] Beavis, R. C.; Chaudhary, T.; Chait, B. T., *Org. Mass Spectrom.*, **1992**, *27*, 156-158.

manner. The terminal N^{α}-Fmoc group was removed in the usual manner and the amino group was acetylated following the procedure described in table VII. Peptide resin was then washed with DMF (5 x 0.5 min) and DCM (5 x 0.5 min) and was incubated with $Pd[PPh_3]_4$ an $PhSiH_3$ as described in section 7.5.1.3. The resin was washed with DCM (5 x 0.5 min), DMF (5 x 0.5 mi) and again with DCM (5 x 0.5 min) and then was cyclized with PyBOP (175 mg, 0.33 mmol, 2 eq.), HOAt (46 mg, 0.33 mmol, 2 eq.) and DIEA (115 μL, 0.67 mmol, 4 eq.) for 2 h. Cleavage from the resin and side chain deprotection was achieved following the general procedure for Rink Amide resin described above. TFA was then removed by evaporation under nitrogen, and the peptide was precipitated with cold anhydrous MTBE, dissolved in H_2O-MeCN (1:1) and then lyophilized. The cyclic crude peptide was purified by semi-preparative HPLC (see conditions below) giving **MT2** with a purity of 95%.

Melanotan 2:

Resin: Rink Amide MBHA
Loading: 0.56 mmol/g
Scale: 0.168 mmol
Yield: 66 mg (38%)
Purification: 0 to 23% MeCN for 1 min and 23 to 25 % MeCN over 20 min.
Purity: 95%
Characterization:

- HPLC (from 24 to 26% MeCN over 15 min, t_R = 8.0 min, 95%; column: Symmetry C_{18}, 5 mm, 7.8 x 100 mm, detection at 220 nm).
- MALDI-TOF (m/z calcd. for $C_{50}H_{69}N_{15}O_9$ 1023.54; found 1024.4 $[M+H]^+$, 1046.4 $[M+Na]^+$, 1062.4 $[M+K]^+$.
- ^{1}H-RMN, 600 MHz, solvent H_2O:D_2O, 25 ºC (ppm).

Residue	NH	H-α	H-β	H-γ	H-δ	H-ε	HZ
Nle	8.08	3.93	1.36	0.96			
Asp	8.38	4.34	2.63/2.42				
His	8.33	4.00	2.93/2.78				
D-phe	8.26	4.30	2.90/2.61				
Arg	7.68	4.03	1.36/1.29	1.04	2.82	6.87	
Trp	8.31	4.37	3.39				
Lys	7.87	3.94	1.46/1.42	1.047/0.96	1.25/1.13	2.95/2.89	7.77

	Aromatic H									
Residue	HD1	HD2	HE1	HE3	HZ1	HZ2	HH2	HZ3	QD	QE
His	6.81	6.81	8.21							
D-Phe					7.03				6.96	7.08
Trp	7.01		9.95	7.42		7.23	6.91	6.99		

7.6.2 Synthesis of {Trp} melanotans 121a and 121b

General procedures describe above were followed for the synthesis of peptides 121a and 121b. Rink amide resin (300 mg, 0.56 mmol/g) was placed in a 10 mL polypropylene syringe fitted with a polyethylene filter disc. Coupling of the first residue was carried out as describe above. After Fmoc deprotection, lactam **13** (128 mg, 0.25 mmol, 1.5 eq.), PyBOP (131 mg, 0.25 mmol, 1.5 eq.), HOAt (34 mg, 0.25 mmol, 1.5 eq.) and DIEA (86.3 mL, 0.50 mmol, 3 eq.) were added and the mixture was allowed to stand for 1 h. After coupling of pseudodipeptide **13**, N^{α}-Fmoc-Arg(Pbf)-OH (327 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-D-Phe-OH (195 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-His(Trt)-OH (312 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-Asp(Allyl)-OH (199 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-Nle-OH (178 mg, 0.50 mmol, 3 eq.) were added sequentially to the resin as described for the synthesis of **MT2**. Acetylation of terminal amino group, removal of Alloc/allyl, cyclization and cleavage was performed as described above to obtain peptides **121a** and **121b** which were separated by semi-preparative HPLC.

Peptide 121a:

Resin: Rink Amide MBHA
Loading: 0.56 mmol/g
Scale: 0.168 mmol
Yield: 33 mg (19%)
Purification: 0 to 23% MeCN for 1 min and 23 to 26 % MeCN over 20 min.
Purity: 99%
Characterization:

- HPLC (from 10 to 35% MeCN in H_2O over 8 min, t_R = 7.5 min, 99%; column: SunFire 3.5 μm, 4.6 x 100 mm, detection at 220 nm).
- MALDI-TOF (m/z calcd. for $C_{54}H_{74}N_{16}O_{10}$ 1106.58; found 1107.6 $[M+H]^+$, 1129.6 $[M+Na]^+$, 1145.6 $[M+K]^+$.
- ^{1}H-RMN, 600 MHz, solvent H_2O:D_2O (9:1), 25 ºC (ppm).

Residue	NH	H-α	H-β	H-γ	H-δ	H-ε	HZ
Nle	8.09	3.90	1.37				
Asp	8.26	4.31	2.52/2.33				
His	8.12	4.21	2.60/2.50				
D-Phe	8.28	4.22	2.86/2.76				
Arg	7.73	3.85	0.91/0.81	0.39/0.32	2.35/2.23	6.56	
{Trp}-Gly	8.04	4.51/3.69(Gly)	3.49				
Lys	8.26	4.09	1.19/1.11	1.63/1.49	1.39/1.26	2.98/2.88	7.87

	Aromatic H									
Residue	HD1	HD2	HE1	HE3	HZ1	HZ2	HH2	HZ3	QD	QE
His	6.83									
D-Phe									6.82	7.03
{Trp}-Gly	6.93		9.98	7.41		7.20	6.87	6.94		

Peptide 121b:

Resin: Rink Amide MBHA
Loading: 0.56 mmol/g
Scale: 0.168 mmol
Yield: 37 mg (20%)
Purification: 0 to 25% MeCN for 1 min and 25 to 27 % MeCN over 20 min.
Purity: 88%
Characterization:

- HPLC (from 10 to 35% MeCN in H_2O over 8 min, t_R = 7.7 min, 88%; column: SunFire 3.5 μm, 4.6 x 100 mm, detection at 220 nm).
- MALDI-TOF (m/z calcd. for $C_{54}H_{74}N_{16}O_{10}$ 1106.58; found 1107.5 $[M+H]^+$, 1129.5 $[M+Na]^+$, 1145.6 $[M+K]^+$.

7.6.3. Synthesis of {Arg} melanotan 120

For the synthesis of ψ-melanotan **120**, Rink Amide MBHA resin (300 mg, 0.56 mmol/g) was used. Coupling of first amino acid was performed as described above. Then, N^{α}-Fmoc-Trp(Boc)-OH (265 mg, 0.50 mmol, 3 eq.) and diazanorbornane **108** (296 mg, 0.33 mmol, 2 eq) were added as described in table VI using DIPCDI (78 μL, for 0.50

mmol and 3 eq.; 52 μL for 0.33 mmol and 2 eq.) and HOBt (68 mg for 0.50 mmol and 3 eq.; 45 mg for 0.33 mmol and 2 eq.) as coupling agents. Next, N^{α}-Fmoc-D-Phe-OH (651 mg, 1.68 mmol, 10 eq.) was coupled using PyBOP (874 mg, 1.68 mmol, 10 eq.), HOAt (229 mg, 1.68 mmol, 10 eq.) and DIEA (575 mL, 3.36 mmol, 20 eq.). The process was repeated once more as coupling was still not complete as monitored by analytical HPLC. Subsequent peptide elongations were carried out as described in table VI using DIPCDI (78 μL, 0.50 mmol, 3 eq.) and HOBt (68 mg, 0.50 mmol, 3 eq.). Capping and removal of Alloc/allyl was performed as described for the synthesis of **MT2** and peptide was cyclized on-resin using PyBOP (349 mg, 0.67 mmol, 4 eq.), HOAt (91 mg, 0.67 mmol, 4 eq.) and DIEA (230 mL, 1.34 mmol, 8 eq.) in DMF (3 mL) for 24 h. Cleavage, side chains deprotection, and work-up were carried out as described above.

Peptide 120:

Resin: Rink Amide MBHA
Loading: 0.56 mmol/g
Scale: 0.168 mmol
Yield: 34 mg (19%)
Purification: 0 to 23% MeCN for 1 min and 22 to 25 % MeCN over 20 min.
Purity: 94%
Characterization:

- HPLC (from 15 to 40% MeCN in H_2O over 8 min, t_R = 4.4 min, 94%; column: SunFire 3.5 μm, 4.6 x 100 mm, detection at 220 nm).
- MALDI-TOF (m/z calcd. for $C_{50}H_{69}N_{15}O_9$ 1023.54; found 1024.4 $[M+H]^+$, 1046.4 $[M+Na]^+$, 1062.4 $[M+K]^+$.

7.6.4. Synthesis of ψ-melanotan 122

Peptide **122** was synthesized following the procedure described for the sinthesis of **MT2**. Starting from Rink amide resin (300 mg, 0.56 mmol/g), first amino acid, N^{α}-Fmoc-Lys(Alloc)-OH (228 mg, 0.50 mmol, 3 eq.), was coupled after Fmoc removal as described in section 7.5.1.1. N^{α}-Fmoc-Trp(Boc)-OH (265 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-Arg(Pbf)-OH (327 mg, 0.50 mmol, 3 eq.), oxazolopiperidone **15** (142 mg, 0.33

mmol, 2 eq.), N^{α}-Fmoc-Asp(Allyl)-OH (199 mg, 0.50 mmol, 3 eq.) and N^{α}-Fmoc-Nle-OH (178 mg, 0.50 mmol, 3 eq.) were added sequentially to the H-Lys(Alloc)-rink amide resin as described for the synthesis of **MT2**, using DIPCDI (78 μL for 0.50 mmol and 3 eq; 52 μL for 0.33 mmol and 2 eq.) and HOBt (68 mg, 0.50 mmol, 3 eq.; 45 mg for 0.33 mmol and 2 eq.). Capping, Alloc/allyl removal, cyclization and cleavage were carried out as described for the synthesis of **MT2**.

Peptide 122:

Resin: Rink Amide MBHA
Loading: 0.56 mmol/g
Scale: 0.168 mmol
Yield: 68 mg (44%)
Purification: 0 to 22% MeCN for 1 min and 22 to 24 % MeCN over 20 min.
Purity: 99%
Characterization:

- HPLC (from 10 to 40% MeCN in H_2O over 8 min, t_R = 6.5 min, 99%; column: SunFire 3.5 μm, 4.6 x 100 mm, detection at 220 nm).
- MALDI-TOF (m/z calcd. for $C_{43}H_{63}N_{13}O_{10}$ 921.48; found 922.5 $[M+H]^+$, 944.5 $[M+Na]^+$, 960.5 $[M+K]^+$.
- ^{1}H-RMN, 600 MHz, solvent H_2O:D_2O (9:1), 25 ºC (ppm).

Residue		NH	Ha	Hb	Hg	Hd	He	Hz
Nle		8.10	4.06	1.43	1.30	0.81/0.48	1.81	
Asp		8.28	4.55	2.60/2.44				
Lactam **15**	Aa 3	8.54	3.66	2.15/1.97	1.76/1.39	4.73		
	Aa 4		4.33	3.66				
Arg		7.79	4.23	1.68/1.60	1.42/1.32	2.96/3.05	7.12	
Trp		8.43	4.37	3.06/3.00				
Lys		8.02	3.87	1.45/1.32	1.07/0.96	1.19	3.03/2.87	7.86

Aromatic H						
Residue	HD1	HE1	HE3	HZ2	HH2	HZ3
Trp	7.03	9.94	7.43	7.26	7.00	6.91

7.6.5. Synthesis of ψ-melanotan 123

N^{α}-Fmoc-Asp(Allyl)-OH (199 mg, 0.50 mmol, 3 eq.) was coupled to Rink Amide MBHA resin (300 mg, 0.56 mmol/g) as described in table III. Oxazolopiperidone **15** (142 mg, 0.33 mmol, 2 eq.), N^{α}-Fmoc-Trp(Boc)-OH (265 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-Arg(Pbf)-OH (327 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-D-Phe-OH (195 mg, 0.50 mmol, 3 eq.) and Fmoc-His(Trt)-OH (312 mg, 0.50 mmol, 3 eq.) were added successively to H-Asp(Allyl)-rink amide resin using DIPCDI (78 μL for 0.50 mmol and 3 eq; 52 μL for 0.33 mmol and 2 eq.) and HOBt (68 mg, 0.50 mmol, 3 eq.; 45 mg for 0.33 mmol and 2 eq.) in DMF (2 mL). Alloc removal was performed following the protocol described in section 7.5.1.3. Then, the terminal N^{α}-Fmoc group was removed in the usual manner (see table V) and peptide was cyclized as described above using PyBOP (175 mg, 0.33 mmol, 2 eq.), HOAt (46 mg, 0.33 mmol, 2 eq.) and DIEA (115 mL, 0.67 mmol, 4 eq.) for 2 h. Cleavage, deprotection and precipitation were carried out as described above.

Peptide 123:

Resin: Rink Amide MBHA
Loading: 0.56 mmol/g
Scale: 0.168 mmol
Yield: 72 mg (46%)
Purification: 0 to 22% MeCN for 1 min and 22 to 23 % MeCN over 20 min.
Purity: 98%
Characterization:

- HPLC (from 10 to 40% MeCN in H_2O over 8 min, t_R = 5.7 min, 98%; column: SunFire 3.5 μm, 4.6 x 100 mm, detection at 220 nm).
- MALDI-TOF (m/z calcd. for $C_{44}H_{54}N_{14}O_9$ 922.42; found 923.4 $[M+H]^+$, 945.5 $[M+Na]^+$, 961.5 $[M+K]^+$.
- ^{1}H-RMN, 600 MHz, solvent H_2O:D_2O (9:1), 25 ºC (ppm).

Residue		NH	Ha	Hb	Hg	Hd	He
Asp		8.32	4.35	2.69/2.51			
His		8.09	4.35	2.83/2.75			
D-Phe		8.54	4.31	2.81/2.72			
Arg		7.88	3.95	1.21/1.04	0.82/0.67	2.65	6.78
Trp		7.80	4.37	3.11/3.02			
Lactam **15**	Aa 6	7.94	3.64	2.05/1.58	1.51/1.30	4.71	
	Aa 7		4.40	4.20/3.80			

Aromatic H									
Residue	HD1	HD2	HE1	HE3	HZ2	HH2	HZ3	QD	QE
His		6.79							
D-Phe								6.96	7.10
Trp	7.04		9.92	7.40	7.24	6.99	6.92		

7.6.6. Synthesis of ψ-melanotans 124 and 125

Synthesis of ψ-melanotan **124** started with the coupling of N^{α}-Fmoc-Asp(Allyl)-OH (199 mg, 0.50 mmol, 3 eq.) to Rink Amide MBHA resin (300 mg, 0.56 mmol/g) as described above. Then, the H-Asp(Allyl)-O-rink amide resin is incubated with CDI (272 mg, 1.68 mmol, 10 eq.) for 20 min and the process was repeated twice. After washing the resin with DMF (5 x 0.5 min), DCM (5 x 0.5 min) and again DMF (5 x 0.5 min) diazanorbornane **101a** (169 mg, 0.50 mmol, 3 eq.) in a mixture of DCM/DMF (1:1) was added and the mixture was allowed to stand for 2 h. Capping of the resin with Ac_2O (159 μL, 1.68 mmol, 10 eq.) and DIEA (575 μL, 3.36 mmol, 20 eq.) in DMF (3 mL) was carried out in order to minimize purification difficulties. Resin was then washed with DMF (5 x 0.5 min) and DCM (5 x 0.5 min) and N^{α}-Fmoc-Trp(Boc)-OH (885 mg, 1.68 mmol, 10 eq.) was coupled using PyBOP (874 mg, 1.68, 10 eq.), HOAt (229 mg, 1.68 mmol, 10 eq.) and DIEA (575 mL, 3.36 mmol, 20 eq.). The process had to be repeated as coupling was not complete (monitored by HPLC). Amino acids N^{α}-Fmoc-Arg(Pbf)-OH (327 mg, 0.50 mmol, 3 eq.), N^{α}-Fmoc-D-Phe-OH (195 mg, 0.50 mmol, 3 eq.) and Fmoc-His(Trt)-OH (312 mg, 0.50 mmol, 3 eq.) were added using DIPCDI (78 μL, 0.50 mmol, 3 eq.) and HOBt (68 mg, 0.50 mmol, 3 eq.) but in the case of N^{α}-Fmoc-Arg(Pbf)-OH and N^{α}-Fmoc-D-Phe-OH it was necessary another coupling reaction. Removal of Alloc group in the usual manner followed by terminal N^{α}-Fmoc removal gives the

protected linear peptide which was cyclized as described above, allowing the mixture to stand for 24 h.

Peptide **125** was obtained following the same protocol, starting from Rink amide resin (300 mg, 0.56 mmol/g) and adding diazanorbornane **114** (162 mg, 0.50 mmol, 3 eq.). N^{α}-Fmoc-Trp(Boc)-OH (885 mg, 1.68 mmol, 10 eq.) was coupled using PyBOP (874 mg, 1.68, 10 eq.), HOAt (229 mg, 1.68 mmol, 10 eq.) and DIEA (575 mL, 3.36 mmol, 20 eq.) and a second coupling with N^{α}-Fmoc-Trp(Boc)-OH (885 mg, 1.68 mmol, 10 eq.) was coupled using PyBOP (874 mg, 1.68, 10 eq.), oxyma pure (239 mg, 1.68 mmol, 10 eq.) and DIEA (575 mL, 3.36 mmol, 20 eq.).

Peptide 124:

Resin: Rink Amide MBHA
Loading: 0.56 mmol/g
Scale: 0.168 mmol
Yield: 6 mg (3%)
Purification: 0 to 21% MeCN for 1 min and 21 to 23 % MeCN over 20 min.
Purity: 92%
Characterization:

- HPLC (from 10 to 40% MeCN in H_2O over 8 min, t_R = 5.6 min, 92%; column: SunFire 3.5 μm, 4.6 x 100 mm, detection at 220 nm).
- MALDI-TOF (m/z calcd. for $C_{42}H_{52}N_{14}O_8$ 880.41; found 881.4 $[M+H]^+$, 903.5, $[M+Na]^+$, 919.4 $[M+K]^+$.

Peptide 125:

Resin: Rink Amide MBHA
Loading: 0.56 mmol/g
Scale: 0.168 mmol
Yield: 2 mg (1%)
Purification: 0 to 21% MeCN for 1 min and 21 to 23 % MeCN over 20 min.
Purity: 95%
Characterization:

- HPLC (from 10 to 40% MeCN in H_2O over 8 min, t_R = 5.9 min, 95%; column: SunFire 3.5 μm, 4.6 x 100 mm, detection at 220 nm).
- MALDI-TOF (m/z calcd. for $C_{42}H_{52}N_{14}O_7$ 864.41; found 865.4 $[M+H]^+$, 887.4 $[M+Na]^+$, 903.4 $[M+K]^+$.
- ^{1}H-RMN, 600 MHz, solvent H_2O:D_2O (9:1), 25 ºC (ppm).

Residue	NH	H-α	H-β	H-γ	H-δ	H-ε
Asp	8.105	4.93	3.09	2.98		
His		4.03	2.54			
D-Phe	8.35	4.36	2.79/2.70			
Arg	8.05	4.31	0.51	0.36	2.55	6.75
Trp		4.32	2.75/2.60			
Aza-**114**			n.d	n.d	n.d	