GLYCOLIPIDS: SYNTHESIS AND MULTIVALENT SYSTEMS.

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PhD THESIS

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Supervised by Prof. Sergio Castillón Miranda and
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Departament de Química Analítica i Química Orgànica

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FEM CONSTAR que el present treball, que porta per títol "Glycolipids: Synthesis and Multivalent Systems", que presenta Isidro Felipe Cobo Cardenete per a l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció i que compleix els requisits per a poder optar a la Menció Europea.

Tarragona, 25 de gener de 2012

Dr. Sergio Castillón Miranda   Dra. Maria Isabel Matheu Malpartida

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Isidro Cobo

Tell me your secrets and ask me your questions

Oh, let's go back to the start.

Running in circles, coming up tails

Heads on a science apart

Nobody said it was easy

It's such a shame for us to part

Nobody said it was easy

No one ever said it would be this hard

Oh, take me back to the start…

The Scientist. COLDPLAY

List of Abbreviations

Ac: acetyl

AcOH: acetic acid

appt: apparent triplet

Alk: alkylic

Ar: aromatic

ATR: Attenuated Total Reflectance

BSA: Bovine Serum Albumin

brs: broad singlet

Bn: benzyl

Bu: butyl

Bz: benzoyl

ºC: Celsius degree

cAMP: cyclic adenosine monophosphate

CAN: Cerium (IV) ammonium nitrate

Cer: Ceramide

CO(imid)$_2$: carbonyldiimidazole

COSY: Correlation Spectroscopy

(+)-CSO: (+)-camphorsulfonyloxaziridine

CTA: Cholera Toxin subunit A

CTB: Cholera Toxin subunit B

CuAAC: Copper(I)-catalyzed Azide-Alkyne Cycloaddition.

Cy: cyclohexyl

δ: chemical shifts

d: doublet

DBTO: Dibenzothiophene-5-oxide

1,2-DCE: 1,2-Dichloroethane

DCM: dichloromethane

dd: doublet of doublet

ddd: doublet of doublet of doublet

DFT: Density Functional Theory

DIPEA: N,N-Diisopropylethylamine

(-)-DIPT: (-)-diisopropyl tartrate

DMAP: 4-Dimethylaminopyridine

DMF: dimethylformamide

dr: diasteromeric ratio

DTBMP: Di-*tert*-butyl-4-methyl-pyridine

DYKAT: Dynamic kinetic asymmetric transformation

EDC: *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride

ee: enantiomeric excess

equiv: equivalent

Et: ethyl

FCC: Flash Column Chromatography

FT-IR: Fourier transform infrared spectroscopy

Fmoc: 9-Fluorenylmethoxycarbonyl

Gal: Galactose

Glyc: Glycal

GM1: monosialotetrahexosylganglioside

GPC: Gel Permeation Chromatography

GSL: Glycosphigolipid

HA: hemagglutinin antibody

HATU: O-(7-Azabenzotriazol-1-yl-N,N,N′,N′-tetramethyluronium hexafluorophosphate

HCCA: α-Cyano-4-hydroxycinnamic acid

HFIP: 1,1,1,3,3,3-Hexafluoro-2-propanol

HMBC: Hetereonuclear Multiple Bond Correlation

HMQC: Hetereonuclear Multiple Quantum Correlation

HSQC: Hetereonuclear Single-Quantum Correlation

HRMS: high resolution mass spectrometry

HRP: horseradish peroxidase

Hz: Hertz

IAA: trans-3-indoleacrylic acid

J: coupling constant

μwave: microwave

m: multiplet

Maj: major

MALDI: Matrix-Assisted Laser Desorption/Ionization

Mes: mesityl

Min: minor

M_n: molecular mass on numer

mp: melting point

M_{theo}: theoretical molecular mass

M_w: molecular mass on weight

Naph: naphthyl

NIS: *N*-iodosuccinimide

NMR: nuclear magnetic resonance

PBS: phosphate buffered saline

PDI: polydispersity index

Pfp: pentafluorophenyl

Ph: Phenyl

PMMA: poly(methyl methacrylate)

ppm: parts per million

^{i}Pr$_2$NEt: N,N-Diisopropylethylamine

p-TolSCl: p-tolylsulphenyl chloride

Py: Pyridyl

Rf: retardation factor

RI: refractive index

r.t.: room temperature

SEC: Size Exclusion Chromatography

t: triplet

TBAI: tetrabutylamonium iodide

TBDPS: *tert*-butyldiphenylsilyl

TBDPSCl: *tert*-butyldiphenylsilyl chloride

TBS: *tert*-butyldimethylsilyl

TBSOTf: *tert*-butyldimethylsilyl trifluoromethanesulfonate

TBTA: Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine

TEA: triethylamine

TFA: trifluoroacetic acid

thermodyn.: thermodynamic

THF: tetrahydrofuran

TIPS: triisopropylsilyl

TLC: Thin Layer Chromatography

TMS: trimethylsilyl

TOF: time-of-flight

Tol: para-tolyl

TsOH: phenylmethylsulfonyl acid

Tf: triflyl

Tr: Trityl

TTBP: 2,4,6-Tri-*tert*-butylpyrimidine

υ: frequency

UV: Ultraviolet

Table of Contents

Chapter 1

General Introduction and Objectives

1.1. Glycolipids: Structure and Classification

Oligosacharide moieties in glycoconjugates usually contain biological information codified in their structure. Such structures offer specific biological properties to the proteins or lipid attached to them. Moreover, their presence can be crucial for cellular functions when such glycoconjugates belong to a plasmatic membrane as it will be discussed in the following sections.

In particular, glycolipids are composed by a carbohydrate unit attached to a hydrophobic aglycone. A part of the cell glycocalix they are present on the surface of eukaryote organisms and they can be classified in three main families depending on how the carbohydrate and the lipid moieties are assembled: glycosphingolipids, glycoglycerolipids and lipopolysacarides (Figure 1.1.). As part of the cell glycocalix they are present on the surface of eukaryote organisms. In deep, glycosphigolipids are present mainly on animal cell while glycoglycerolipids and lipopolysacarides are abundant on bacterial and plant cells.[1]
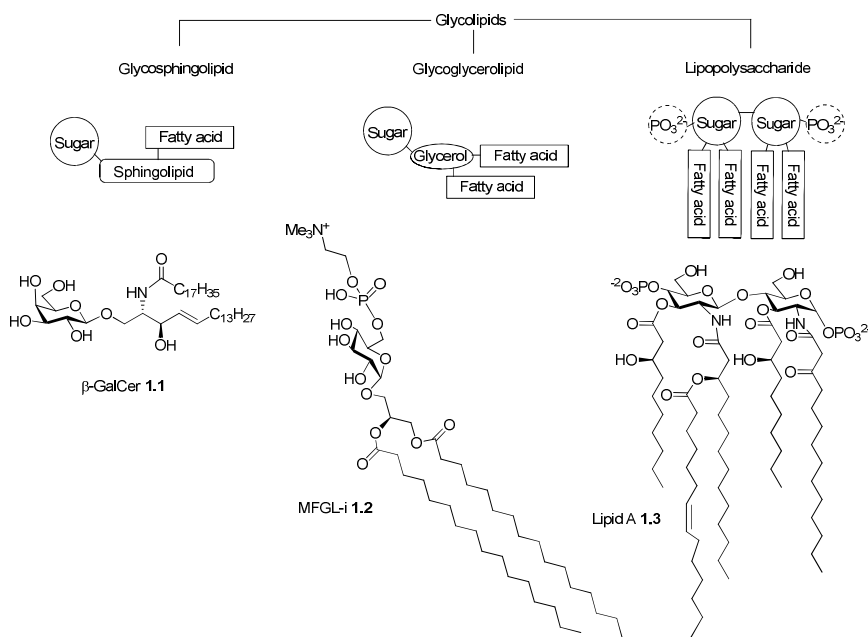
Figure 1.1. Exemples of families of glycolipids

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[1]   (a) Ernst, B.; Hart, G.; Sinaÿ, P. *Carbohydrates in Chemistry and Biology. Part II: Biology of Saccharides*, Vol. 3, 4; Wiley-VCH, Weinheim, 2000.

In the context of our research, glycosphingolipids (GSL's) deserve special interest because of their biological relevance. Each GSL carries a hydrophobic ceramide (Cer) moiety and a hydrophilic extracellular oligosaccharide chain or monosaccharide unit which emerges from the membrane surface (Figure 1.2.).
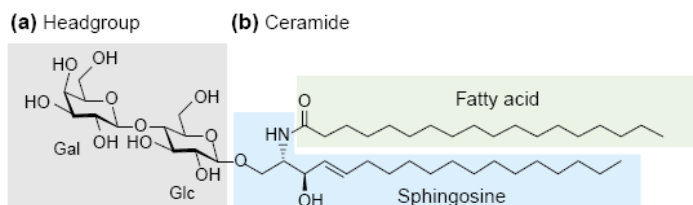
Figure 1.2. Tipical structure of a GSL

Ceramide in turn, is formed by a long chain amino alcohol (sphingoid base) commonly with 18-20 carbon atoms, N-linked to a fatty acid. The sphingoid base may be hydroxylated, and the most frequently occurring contains a C4-C5 double bound in the *trans*-D-*erythro* family. Less frequent are sphinganines, that lack the double bond or phytosphingosine that carries an hydroxyl group on C4 (Figure 1.3.).
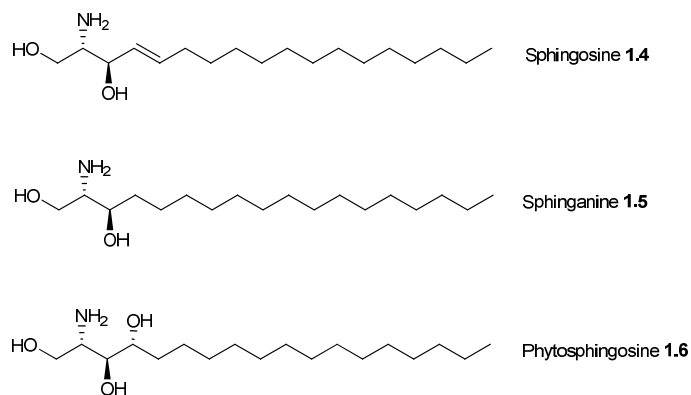
Figure 1.3. Sphingoid bases

The saccharide moiety is represented by a single saccharide unit, as in the case of cerebrosides (β-Galcer **1.1**, Figure 1.4.); sulphated mono- or di-saccharides, as in the case of sulphatides (Sulfatide β-Galcer **1.7**, Figure 1.4.); and as linear

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or branched oligosaccharide chain (iGB$_3$ **1.8** or GM$_3$ **1.9**, Figure 1.4.). The saccharide units present in glycosphingolipids can be galactose, glucose, *N*-acetylglucosamine, *N*-acetylgalactosamine, fucose, sialic acid and glucuronic acid. The mono- or multi-sialosylated glycosphingolipids are named gangliosides that, together with sulphatides, constitute the group of acidic glycosphingolipids. The remainder glycosphingolipids are neutral glycosphingolipids. Thus, glycosphingolipids are generally classified as follows:

- Cerebrosides, which contain one sugar residue (β-GalCer **1.1**)
- Sulfatides, whose structure contain one sugar residue with a sulphate group (Sulfatide β-GalCer **1.7**)
- Neutral Glycosphingolipids (iGB$_3$ **1.8**)
- Gangliosides (GM$_3$ **1.9**)
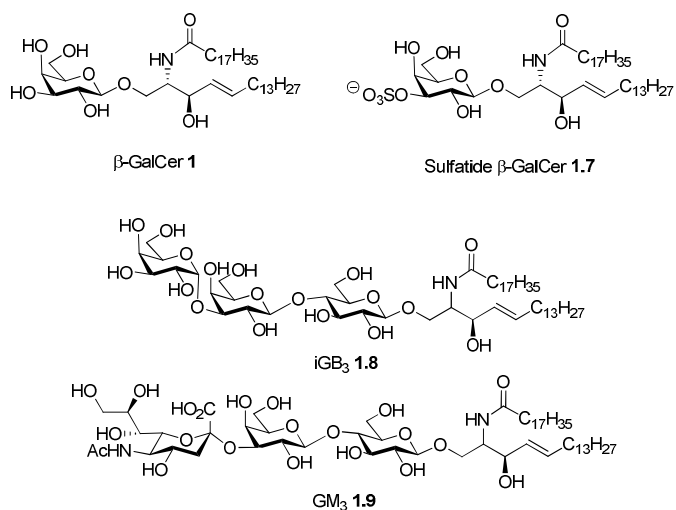
Figure 1.4. Examples of naturally occurring GSLs

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1.2. Glycosphingolipids as mamalian membrane receptors

1.2.1. Subcelular distribution and supramolecular organization

Organization of membrane glycosphingolipids and proteins into non-uniform domains has been an area of research interest leading to the concept of cell membrane 'lipid rafts'[2] (Figure 1.5.). These domains are defined as nano-assemblies of sphingolipid, cholesterol and glycosylphosphatidylinositol anchored proteins that fluctuate on a subsecond time scale.[3] However, this concept has suffered through a period of controversy,[4] but it is now considered as a valid paradigm of physiological membrane structure[5] of increasing relevance, particularly in signalling[6] and intracellular traffic.[7]
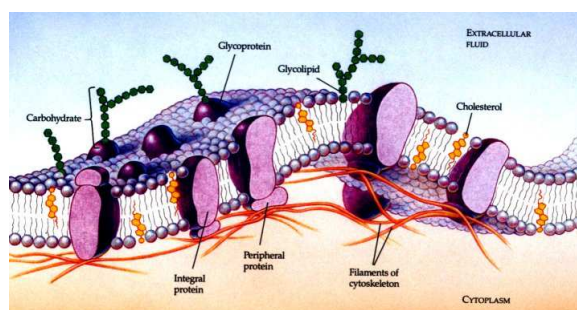
Figure 1.5. Schematic representation of a cell membrane

[2] (a) Simons, K.; Ikonen, E. *Nature* **1997**, *387*, 569-572. (b) Simons, K.; Ehehalt, R. *J. Clin. Invest.* **2002**, *110*, 597-603.

[3] Lingwood, D.; Kaiser, H. J.; Levental, I.; Simons, K. *Biochem. Soc. Trans.* **2009**, *37*, 955-960.

[4] (a) Munro, S. *Cell* **2003,** *115*, 377-388. (b) Hancock, J. F. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 456-462.

[5] (a) Lenne, P.F.; Wawrezinieck, L.; Conchonaud, F; Wurtz, O.; Boned, A,; Guo, X. J.; Rigneault, H; He, H.-T.; Marget, D. *EMBO J.* **2006**, *25*, 3245-3256. (b) Baumgart, T.; Hammond, A. T.; Sengupta, P.; Hess, S. T.; Holowka, D. A.; Baird, B. A.; Webb, W. W. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 3165-3170. (c) Lasserre, R.; Guo, X.-J.; Conchonaud, F.; Hamon, Y.; Hawchar, O.; Bernard, A.-M.; M'Homa Soudja, S.; Lenne, P.-F.; Rigneault, H.; Olive, D.; Bismuth, G.; Nunès, J. A.; Payrastre, B.; Marguet, D.; He, H-T. *Nat. Chem. Biol.* **2008**, *4*, 538-547.

[6] Zech, T.; Ejsing, C.S.; Gaus, K.; de Wet, B.; Shevchenko, A.; Simons, K.; Harder, T. *EMBO J.* **2009**, *28*, 466-476.

[7] Jackson, C.L. *J. Cell Sci.* **2009**, *122*, 443-452.

Membrane heterogeneity by rafts is believed to rely on the selective lipid–lipid interaction. For *in vitro* model membrane systems, this is illustrated by the sterol-dependent phase separation of sphingolipids from the more unsaturated glycerophospholipids[8] (Figure 1.6.). However, model membranes do not resemble to normal cell membrane physiology. Moreover, it has not been fully established that the cellular membranes correlate to a liquid ordered phase (raft, Lo) and a liquid-crystal fluid phase (Lc) completely. Very recent work suggested that lipid-based phase segregation principles cooperate with other lateral specificities, possibly chemical interactions involving proteins, to laterally organize function.[9]
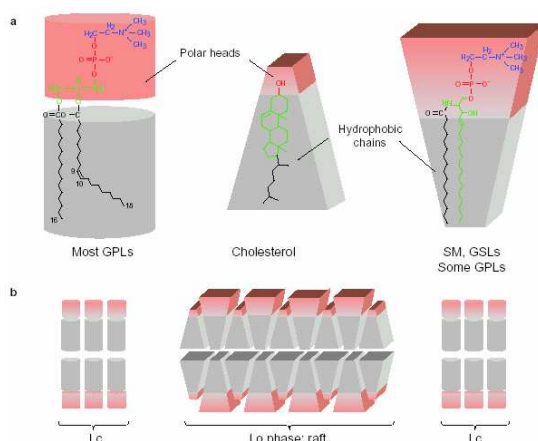
Figure 1.6. Simplified model of organization of GSL's in microdomains

In this context, primarily glycolipid receptors have been shown to function in bacterial/host interactions[10] and speculated to provide the basis and specificity for the initial attachment of the parasite to the host mucosal cell surface, for exemple. Such interactions have been described essentially limited to

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8 (a) Ahmed, S. N.; Brown, D. A.; London, E. *Biochemistry* **1997**, *36*, 10944-10953. (b) Tokumasu, F.; Jin, A. J.; Dvorak, J. A. *J. Electron Microsc.* **2002**, *51*, 1-9.

9 Kaiser, H. J.; Lingwood, D.; Levental, I.; Sampaio, J. L.; Kalvodova, L.; Rajendran, L.; Simons, K.; *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16645-16650.

10 (a) Karlsson, K. *Chem Phys Lipids* **1986**, *42*, 153-172. (b) Strömberg, N.; Deal, C.; Nyberg, G.; Normark, S.; So, M.; Karlsson, K.-A. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4902-4906. (c) Strömberg, N.; Ryd, N.; Lindberg, A. A.; Karlsson K.-A. *FEBS Lett.* **1988**, *232*, 193-198.

recognition of the carbohydrate moiety such as galacto,[11] sulfatide,[12] ganglio[13] series and globo[14] series core glycolipids. However, several studies have shown that the lipidic moiety of glycolipid also modulates the recognition process. Such phenomenon is commented below.

The term 'aglycone modulation' of GSL receptor function has been used to describe this effect, which relies not only on the composition of the ceramide moiety itself, but also on the composition of the surrounding lipid membrane microenvironment.[15] Thus membrane lipid organization in terms of lipid microdomains or lipid rafts may regulate the bioavailability of GSLs for interaction.

Further studies of microbial interaction with target cell glycolipids often showed that the binding specificity is dependent not only the carbohydrate sequence but also on the character of the lipid moiety itself[10c,16] (most often hydroxylation is required). These studies show that the regulation of lipid bounded to carbohydrate for recognition by exogenous ligands is more complex than for glycoconjugate carbohydrate sequences, for example, on proteins.

The question is not only about crypticity whereby cell membrane glycolipids can be masked by larger or other cell surface carbohydrates[17] or proteins, since

[11] (a) Schneiders, F. L.; Scheper, R. J.; von Blomberg, B. M. E.; Woltman, A. M.; Janssen, H. L. A.; van den Eertwegh, A. J. M.; Verheul, H. M. W.; de Gruijl, T. D.; van der Vliet, H. J. *Clin. Immunol.* **2011**, *140*, 130-141. (b) Hayakawa, Y.; Godfrey, D. I.; Smyth, M. J. *Curr. Med. Chem.* **2004**, *11*, 241-252.

[12] (a) Halder, R. C.; Jahng, A.; Maricic, I.; Kumar, V. *Neurochem. Res.* **2007**, *32*, 257-262. (b) Arrenberg, P.; Halder, R.; Kumar, V. *J. Cell. Physiol.* **2009**, *218*, 246-250.

[13] Houliston, R. S.; Yuki, N.; Hirama, T.; Khieu, N. H.; Brisson, J.-R.; Gilbert, M.; Jarrell, H. C. *Biochemistry* **2007**, *46*, 36-44.

[14] (a) Chatterjee, S.; Khutlar, M.; Shi, W. *Glycobiology* **1995**, *5*, 327-333. (b) Brown, K. E.; Anderson, S. M.; Young, N. S. *Science*, **1993**, *262*, 114-117.

[15] (a) Lingwood, C. A. *Can. J. Biochem.* **1979**, *57*, 1138-1143. (b) Kannagi, R.; Stroup, R.; Cochran, N.A.; Urdal, D.L.; Young, W.W. Jr.; Hakomori, S.-I. *Cancer Res.* **1983,** *43*, 4997-5005. (c) Stewart, R. J.; Boggs, J. *Biochemistry* **1990**, *29*, 3644-3653.

[16] (a) Strömberg, N.; Karlsson, K. A. *J. Biol. Chem*. **1990,** *265*, 11251-11258. (b) Ångström, J.; Tenenberg, S.; Milh, M. A.; Larsson, T.; Leonardsson, I.; Olsson, B.-M.; Ölwegård Halvarsson, M.; Danielsson, D.; Näslund, I.; Ljungh, Å.; Wadström, T.; Karlsson, K.-A. *Glycobiology* **1998,** *8*, 297-309.

[17] Wiels, J.; Holmes, E. H.; Cochran, N.; Tursz, T.; Hakomori, S.-I. *J. Biol. Chem.* **1984**, *259*, 14783-14787.

these binding effects can be demonstrated in simple GSL/cholesterol model membranes, but rather one of some '**allosteric**' mechanism where changes in the structure in a distal part of the molecule result in alteration of the conformation of the carbohydrate, to allow or restrict ligand binding.

1.2.2. Fisiological functions of GSL's on membranes and their on diseases

In the case of GSL's, the active mechanism of recognition can be classified in two main groups: interactions of GLS's with membrane receptors (*trans* recognition) and activity modulation of the proteins of the same membrane (*cis* recognition). Consequently, the fisiological functions of the GLS's on mammals are summarized next:[1a,18]

Cell adhesion and recognition: it takes place through two different kinds of mechanisms, recognition of equivalent proteins[19] or lectins,[18b] or carbohydrate-carbohydrate interaction with other GLS's.[20] Hence, GLS's play an important role in the initial stages of the embryogenesis, adhesion of neuronal cells and molecular adhesion to leukocytes.

Modulation of transmembrane signalization: GSL's function on the transduction of signals depens on the oligosaccharides embedded on the cellular external surface and the ceramide moiety.[21]

Immunomodulating propierties of glycolipids: modulation response of lymphocytes to antigens has suggested that GSL's can act as immunomodulating agents *in vivo.[22]* It is believed that some exogenous gangliosides can suppress or activate some immunologic responses depending on the structure and concentration of the gangliosides and the structure of the target cells.

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[18] (a) Fraser Reid, B.; Tatsuta, K.; Thiem, J.; Fraser-Reid, B. O. *Glycoscience: Chemistry and Chemical Biology*, vol. 3; Springer-Verlag, Berlin, 2001, pp: 2183-2249. (b) Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833-862.

[19] Fritsch, M.; Geilen, C. C.; Heidrich, C.; Reutter, W. *FEBS Lett.* **1995**, *376*, 159-163.

[20] Hakomori, S. *Pure & Appl Chem.* **1991**, *63*, 473-482.

[21] Testi, R. *Trends Biochem. Sci.* **1996**, *21*, 468-471.

[22] Bergelson, L. D. *Immunol. Today* **1995**, *16*, 483-486.

Modulators of growth factor receptor function: Several experimental evidences suggested that gangliosides are neuroregulatory factors *in vitro* and *in vivo*.[23] Disturbance of GSL expression and metabolism affects brain function, resulting in a variety of diseases.

As it has been commented previously, the interaction of pathogens to the membrane host is the first stage of an infectious process which is followed by the membrane penetration and the invasion of the tissues. For this reason, in the past few years, the field of GSLs research has been addressed as a strategy for preventing different diseases promoted by microbial infections such as the case of verotoxines,[24] cholera toxin,[25] HIV;[24c,26] as well to other processes in which they are involved, such as multi-system disorders of metabolism,[27] cancer,[28] lupus,[29] diabetes,[30] Alzheimer[31] and Parkinson.[32]

To increase the activity of glycosphingolipid compounds against diseases, two main strategies have been developed. The first approach consists of anchoring the oligosaccharide unit on a chemical matrix to obtain a multivalent neoglyconjugate.[33] The other approach consists of tuning the structure of the

[23] (a) Yu, R. K.; Nakatani, Y.; Yanagisawa, M. *J. Lipid Res.* **2009**, *50*, S440-S445. (b) Hakomori S. *J. Biol. Chem*. **1990**, *265*, 18713-18716.

[24] (a) Lingwood, C. A. *Biochim. Biophys. Acta* **1999,** *1455*, 375-386. (b) Abul-Milh, M.; Barnett Foster, D.; Lingwood, C. A. *Glycoconjugate J.* **2001,** *18*, 253-260. (c) Lingwood, C. A.; Binnington, B.; Manis, A.; Branch, D. R. *FEBS Letters* **2010,** *584*, 1879-1886.

[25] (a) Pacuszka, T.; Bradley, R. M.; Fishman, P. H. *Biochemistry* **1991**, *30*, 2565-2570. (b) McCann, J. A.; Mertz, J. A.; Czworkowski, J.; Picking, W. D. *Biochemistry* **1997**, *36*, 9169-9178. (c) Arosio, D.; Baretti, S.; Cattaldo, S.; Potenza, D.; Bernardi, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3831-3834.(d) Fujinaga, Y. *Toxin Rev.* **2006**, *25*, 47-59.

[26] (a) Viard, M.; Parolini, I.; Rawat, S. S.; Fecchi, K.; Sargiacomo, M.; Puri, A. *Glycoconjugate J.* **2004**, *20*, 213-222. (b) McReynolds, K. D.; Gervay-Hague, J. *Chem. Rev.* **2007**, *107*, 1533-1552.

[27] Xu, Y.-H.; Barnes, S.; Sun, Y.; Grabowski, G. A. *J. Lipid Res.* **2010**, *51*, 1643-1675.

[28] (a) Igarashi, Y.; Kannagi, R. *J. Biochem.* **2010**, *147*, 3-8. (b) Bieberich, E. *Glycoconjugate J.* **2004**, *21*, 315-327.

[29] Jury, E. C.; Kabouridis, P. S.; Flores-Borja, F.; Mageed, R. A.; Isenberg, D. A. *J. Clin. Invest.* **2004**, *113*, 1176-1187.

[30] Langeveld, M.; Aerts, J. M. F. G. *Prog. Lipid Res.* **2009**, *48*, 196-205.

[31] Mutoh, T.; Hirabayashi, Y.; Mihara, T.; Ueda, M.; Koga, H.; Ueda, A.; Kokura, T.; Yamamoto, H. *CNS Neurol. Disord. Drug Targets* **2006**, *5*, 375-380.

[32] Matsuoka, Y.; Saito, M.; LaFrancois, J.; Saito, M.; Gaynor, K.; Olm, V.; Wang, L.; Casey, E.; Lu, Y.; Shiratori, C.; Lemere, C.; Duff, K. *J. Neurosci.* **2003**, *23*, 29-33.

[33] Rojo, J.; Delgado, R. *Anti-Infect. Agents Med. Chem.* **2007**, *6*, 151-174.

natural GSL in order to find new mimetics with potencial biological activity[34] or to modify the hydrophobic part of GSLs, with the goal of obtaining water-soluble analogues[35] in which the conformation of the binding domain of the analogue is similar to GSLs. Several of these ideas will be discussed in the following sections.

1.3. Synthesis of glycosphingolipids

Since the isolation of a group of marine galactosylsphingolipids in the 1990's from *Agelas mauritianus*[36] to the posterior the synthesis of analogues,[37] a great interest has been aroused for this family of glycosphingolipids due to the potent antitumor activity found *in vivo*. Therefore, important contributions have been reported in last decades concerning the synthesis of such remarkable compounds.[38] Herein a brief description of the biosynthesis and the chemical synthesis of the glycosphingolipids is introduced.

1.3.1. Biosynthesis of glycosphingolipids

The formation of ceramide is carried out by membrane bound enzymes on the cytosolic leaflet of the endoplasmic reticulum (ER).[39] Starting from the amino acid L-serine **1.10** and two equivalents of the palmitoyl-coenzyme A **1.11**, dihydroceramide **1.14** is formed in three steps (Scheme 1.1.). This *N*-acyl-2-

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[34] Banchet-Cadeddu, A.; Hénon, E.; Dauchez, M.; Renault, J.-H.; Monneaux, F.; Haudrechya, A. *Org. Biomol. Chem.* **2011**, *9*, 3080-3104.

[35] (a) De Rosa, M.; Park, H.-J.; Mylvaganum, M.; Binnington, B.; Lund, N.; Branch, D.R.; Lingwood, C.A. *Biochim. Biophys. Acta* **2008**, *1780*, 347-352. (b) Fantini, J.; Hammache, D.; Delézay, O.; Yahi, N.; André-Barrès, C.; Rico-Lattes, I.; Lattes, A. *J. Biol. Chem.* **1997**, *272*, 7245-7252.

[36] (a) Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591-5592. (b) Akimoto, K.; Natori, T.; Morita, M. *Tetrahedron Lett.* **1993**, *34*, 5593-5596. (c) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771-2784.

[37] Franck, R.W.; Tsuji, M. *Acc. Chem. Res.* **2006**, *39*, 692-701.

[38] (a) Maccioni, H. J. F.; Quiroga, R.; Ferrari, M.L. *J. Neurochem.* **2011**, *117*, 589-602. (b) Morales-Serna, J.A.; Llaveria, J.; Díaz, Y.; Matheu, M.I.; Castillón, S. *Curr. Org. Chem.* **2010**, *14*, 2483-2521. (c) Morales-Serna, J.A.; Boutureira, O.; Díaz, Y.; Matheu, M.I.; Castillón, S. *Carbohydr Res.* **2007**, *342*, 1595-1612.

[39] Merrill Jr., A. H. *J. Biol. Chem*. **2002**, *277*, 25843-25846.

aminoalkyl-1,3-diol (*N*-acylsphinganine) **1.14** is dehydrogenated to ceramide **1.15** with a 4,5-trans-double bond by a dihydroceramide desaturase.
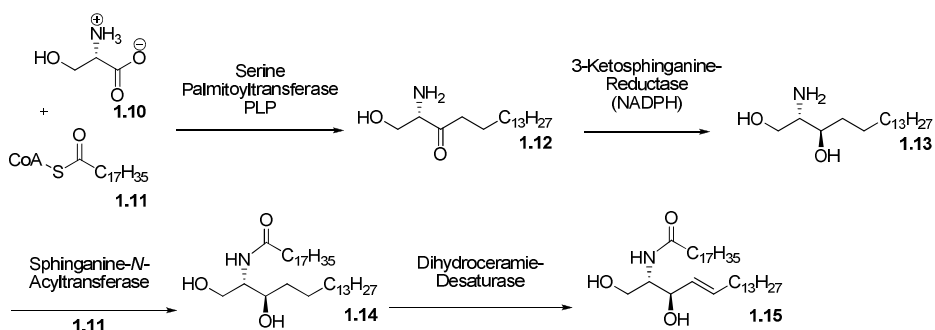
Scheme 1.1. Biochemical synthesis of ceramide **1.15**

Then, at the membranes of the Golgi apparatus, carbohydrate moieties are attached to ceramide leading to galactosylceramide, glucosylceramide, sphingomyelin and higher glycosphingolipids, which are synthesised by the stepwise addition of monosaccharides to glucosylceramide (Figure 1.7.).
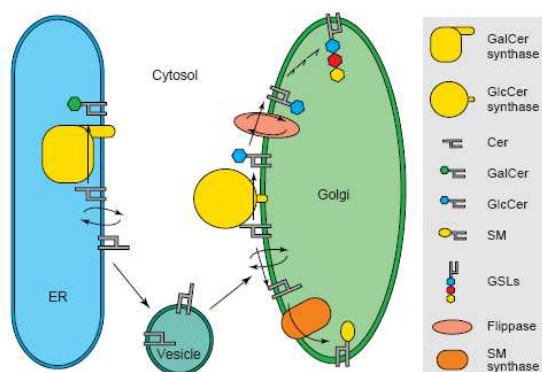
Figure 1.7. Biosynthesis of GSL's by metabolizing enzymes

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1.3.2. Design of glycosphingolipids

Due to the great interest of glycoconjugates for modern glycobiology, several chemical methodologies have provided suitable strategies to design glycosphigolipids[1c,38c,40] as depicted in Scheme 1.2.
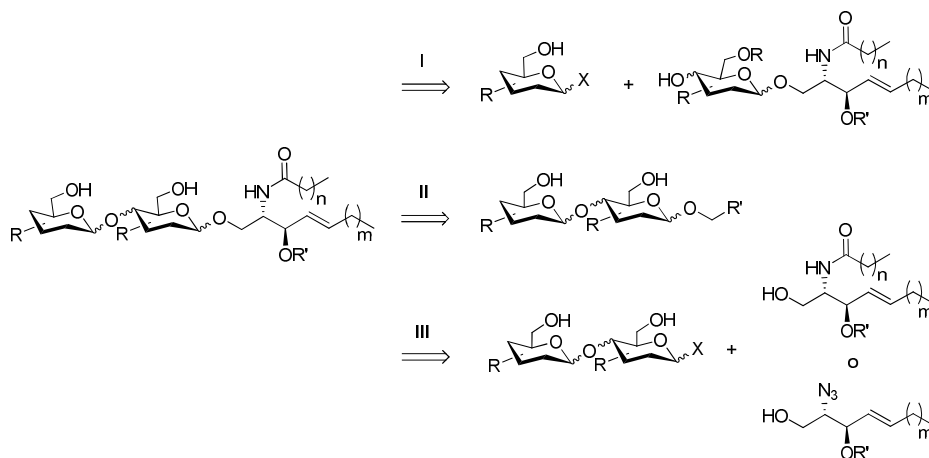
Scheme 1.2. General retrosynthetic plan for the preparation of GSLs

The first route (Scheme 1.2., I) consists of a sequential addition of conveniently protected monosaccharides which are covalent bonded to a growing fragment of the glycolipid. Generally, oligosaccharides are complex and the strategy of synthesis requires several sequences of protection-deprotection-activation reactions. It is necessary that the glycosyl acceptor was stable enough under glycosylation conditions but reactive enough at the same time to allow the oligosaccharide chain elongation. This synthetic route results too much lineal and for this reason is not employed very often.

The second approach (Scheme, 1.2., II) consists of growing the aglycone when it is already installed in the carbohydrate moiety. However, there are few

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[40] Khan, S. H.; O'Neill, R. A. *Modern Methods in Carbohydrate Synthesis,* Vol. 1; Harwood cademic Publishers, Amsterdam 1996.

examples of using this methodology in the literature[41] and they depend on the stability of the oligosaccharide residue on the condition reactions to construct the aglycone.

Finally, the third approach (Scheme 1.2., III) is a convergent synthesis, in which the oligosaccharide and the aglycone are synthesized independently and then they are joined by glycosylation reaction to obtain the desired GSL. This is the most employed strategy to obtain glycoconjugates and it will be discussed below.

As it is depicted in Scheme 1.3., the synthesis of the oligosaccharide residue may be prepared though two different ways. The first strategy (Scheme 1.3., I) consists of the classical glycosylation reaction between the convenient protected pyranoses.[1,40] Using this methodology, one of the carbohydrates participates as glycosyl donor whereas the other acts as glycosyl acceptor when it is activated by a promoter (Scheme 1.3., III). However, this strategy relies on the suitable choice of the different protecting groups to afford good yields and selectivities.[42]
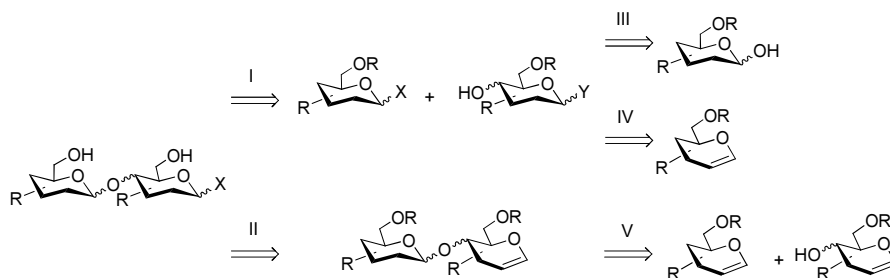
Scheme 1.3. Retrosynthetic analysis for the preparation of the oligosaccharide

Another variant of this classical strategy (Scheme 1.3., IV) employes glycals as precursors of glycosyl donors and glycosyl acceptors. Typical reactions to afford

[41] (a) Matto, P.; Modica, E.; Franchini, L.; Facciotti, F.; Mori, L.; De Libero, G.; Lombardi, G.; Fallarini, S.; Panza, L.i; Compostella, F.; Ronchetti, F. *J. Org. Chem.* **2007**, *72*, 7757-7760. (b) Rai, A. N.; Basu, A. *J. Org. Chem.* **2005**, *70*, 8228-8230. (c) Barrett, A. G. M.; Beall, J. C.; Braddock, D. C.; Flack, K.; Gibson, V. C.; Salter, M. M. *J. Org. Chem.* **2000**, *65*, 6508-6514.
[42] (a) Wong, C.-H.; Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A. *Chem Rev.* **1998**, *98*, 833-862. (b) Bernardi, A.; Cheshev, P. *Chem. Eur. J.* **2008**, *14*, 7434-7441.

these transformations are epoxydation, azidonitration and sulphonamidoglycosylation.[43]

The group of Danishefsky developed an alternative to the classic synthesis of oligosaccharides (Scheme 1.3., II) called 'glycal assembly method'.[1,40] This methodology allows a reiterative assembly of the carbohydrated residues because glycal can participate as glycosyl donor and/or glycosyl acceptor. This methodology is often more simply and avoids tedious manipulation of protecting groups.

Sphingolipids are formed by an aminoalcohol moiety (sphingoid base), a polar head and a fatty acid tail. The common structural unit for sphingolipids in eukaryotic cells is the sphingoid base D-*erythro*-sphingosine [(2*S*, 3*R*, 4*E*)-2-amino-3-hydroxyoctadeca-4-en-1-ol].[18a] Moreover, other similar structures such as phytosphingosine and sphingofungin are of wide interest[44] (Figure 1.8.).
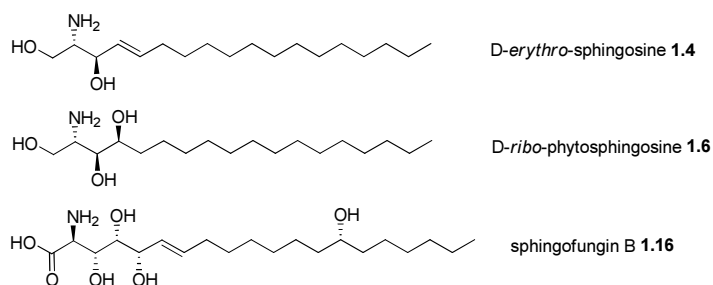
Figure 1.8. Examples of naturally occurring sphingoid bases

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[43] (a) Seeberger, P. H.; Bilodeau, M. T.; Danishefsky, S. J. *Aldrichimica Acta* **1997**, *30*, 75-92. (b) Guo, Z. *Carbohydrate Chemistry, Biology and Medical Applications,* Elsevier, Oxford, 2008.

[44] (a) Costantino, V.; Fattorusso, E.; Mangoni, A.; Di Rosa, M.; Ianaro, A.; Maffia, P. *Tetrahedron* **1996**, *52*, 1573-1578. (b) He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7618-7626. (c) Ndakala, A. J.; Hashemzadeh, M.; So, R. C.; Howell, A. R. *Org. Lett.* **2002**, *4*, 1719-1722. (d) Chiu, H.-Y.; Tzou, D.-L. M.; Patkar, L. N.; Lin, C.-C. *J. Org. Chem.* **2003**, *68*, 5788-5791.
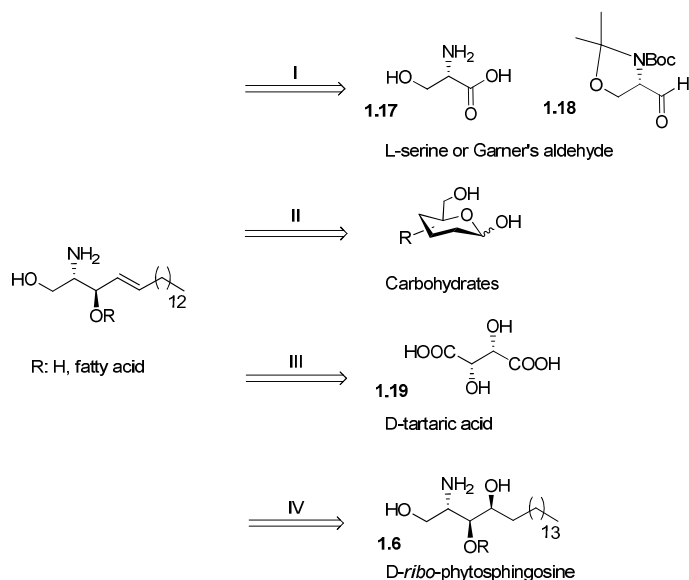
Scheme 1.4. General synthons for the preparation of sphingosine derivatives from quiral pool

Synthesis of sphingosine and its derivates has been revised in our group recently.[38b,45] The main important retrosynthetic strategies are depicted in Scheme 1.4. The quiral pool offers an attractive source of non racemic starting materials (Scheme 1.4., I, II, III and IV). Amino acids like L-Serine[46] and derivates like Garner's aldehyde[47] have been broadly employed for the synthesis of natural product that contain 1,2-aminoalcohol or a 1,3-diol moiety.[48] Carbohydrates have also been chosen as starting materials because of its high versatility. In this sense, sphingosine has been successfully obtained from D-xylose, D-arabinose, D-galactose, glycals, glucosamines derivatives among others.[49] Tartaric acid and its derivatives have been also employed as a chiral starting material in the synthesis of natural products such as D-*erythro*-sphingosine.[50] Moreover, commercially available D-*ribo*-phytosphingosine can

[45] Llaveria, J. *Doctoral Thesis*, Universitat Rovira i Virgili, Tarragona, **2011**.

[46] Herold, P. *Helv. Chim. Acta* **1988**, *71*, 354-362.

[47] Garner, P.; Park, J. M.; Malecki, E. *J. Org. Chem.* **1988**, *53*, 4395-4398.

[48] Koskinen, A. M. P.; Koskinen, P. M. *Synthesis* **1998**, 1075-1091.

[49] (a) Duclos Jr., R. I. *Chem. Phys. Lipids* **2001**, *111*, 111-138. (b) Milne, J. E.; Jarowicki, K.; Kocienski, P.J.; Alonso, J. *Chem. Commun.* **2002**, 426-427.(c) Reist, E. J.; Christie, P. H. *J. Org. Chem.* **1970**, *35*, 4127-4130. (b) Schmidt, R. R.; Zimmermann, P. *Tetrahedron Lett*. **1986**, *27*, 481-484. (d) Schmidt, R. R.; Bär, T.; Wild, R. *Synthesis* **1995**, 868-876. (e) Costantino, V.; Fattorusso, E.; Imperatore, C.; Mangoni, A. *Tetrahedron* **2002**, *58*, 369-376.

[50] Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005**, *46*, 1873-1875.

be transformed into D-*erythro*-sphingosine employing different methodologies.[38b,45]

On the other hand, several approaches that rely on asymmetric synthesis have afforded tools to create quiral compound from racemic starting materials in order to obtain the sphingosine scafold and their derivatives (Scheme 1.5., A-D). These strategies employ chiral reagents and auxiliaries or they are based on enantioselective catalytic procedures to generate the sphingoid amino alcohol moiety.

Among the approaches reported based on the use of chiral reagents and auxiliaries the must significative are summarized below:

a) The construction of the main functionalities in sphingosine from glycine-derived silicon enolate **1.20** using a zirconium-catalized asymmetric aldol reaction.[51]

b) The synthesis of the asymmetric vicinal amino alcohols for D-*erythro*-sphingosine has been also obtained from a highly diasteroselective *anti*-aminohydroxylation of an α,β-unsaturated ester **1.25**, *via* conjugate addition of lithium (*S*)-*N*-benzyl-*N*-(α-methylbenzyl)amide **1.26** and subsequent *in situ* enolate oxidation with (+)-(camphorsulfonyl)oxaziridine ((+)-CSO) as key steps.[52]

c) The addition reaction of chiral a guanidinium ylide **1.29** to α,β-insaturated aldehyde **1.28**. The corresponding aziridines 1.**30** and **1.31** formed can be derived into α-amino-β-hydroxy acid units *in route* D-*erythro*-sphingosine synthesis.[53]

d) The asymmetric synthesis of β-amino carbonyl derivatives **1.36** and **1.37** through stereoselective nucleophilic addition of enolates **1.35** to imines **1.34**. This Mannich-type reaction can be stereocontrolled to provide efficient routes to enantiomerically pure polyhydroxy-β-amino

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[51] Kobayashi, J.; Nakamura, M.; Mori, Y.; Yamashita, Y.; Kobayashi, S. *J. Am. Chem. Soc.* **2004**, *126*, 9192-9193.

[52] Abraham, E.; Davies, S. G.; Millican, N. L.; Nicholson, R. L.; Roberts, P. M.; Smith, A. D. *Org. Biomol. Chem.* **2008**, *6*, 1655-1664.

[53] (a) Disadee, W.; Ishikawa, T. *J. Org. Chem.* **2005**, *56*, 781-790. (b) Hu, X. E. *Tetrahedron* **2004**, *60*, 2701-2743.

acids, which have been applied in the enantiodivergent synthesis of L- and D-*erythro*-sphingosine.[54]
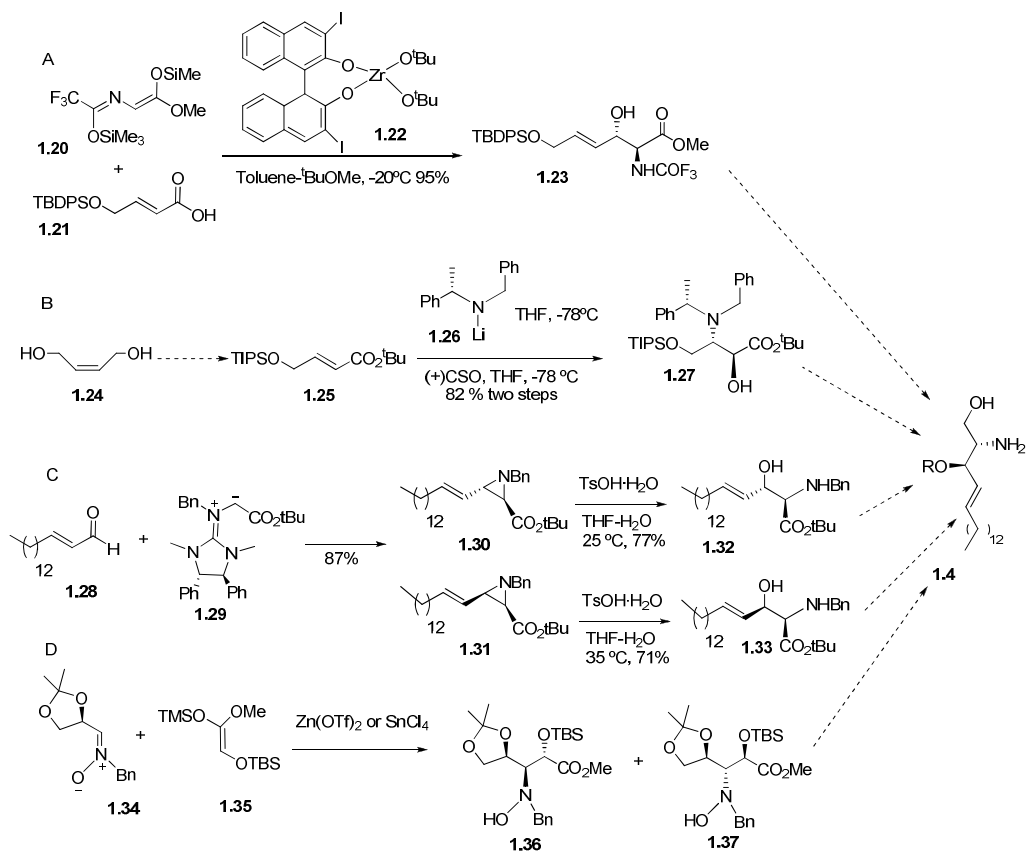
Scheme 1.5. Key steps in representative synthesis of 1.4 employing chiral reagents and auxiliaries.

Alternatively, the use of enantioselective catalytic procedures has been also studied as a key step to afford the sphingoid moiety with high selectivity in the D-erythro-sphingosine synthesis. The most representative approaches are summarized as follows (Scheme 1.6., A-E):

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[54] Merino, P.; Jimenez, P.; Tejero, T. *J. Org. Chem.* **2006**, *71*, 4685-4688.

a) The use a tin (II)-catalized asymmetric aldol reaction between trimethylsilylpropanal (**1.38**) with silylenol **1.39** in presence of chiral diamine **1.40**.[55]

b) The use of Sharpless asymmetric dihydroxylation of enyne ester **1.42** with AD-mix-β.[56]

c) An enantioselective epoxidation of diene **1.44** under Shi's asymmetric epoxidation conditions to afford a mixture of vinyl epoxides.[57]

d) The use of asymmetric Sharpless epoxydation to afford the desired sphingoid moiety and later a cross-metathesis reaction to elong the aliphatic chain in the presence of a phosphine-free Grubbs catalyst (Scheme 1.6., D).[58]

e) The use of palladium catalyzed dynamic asymmetric transformation (DYKAT) on a racemic butadiene monoepoxide **1.52** and later a cross-metathesis reaction to elong the aliphatic chain in presence of a Grubbs catalyst.[59]

[55] Kobayashi, S.; Furuta, T. *Tetrahedron* **1998**, *54*, 10275-10294.
[56] He, L.; Byun, H. S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7627-7633.
[57] Olofson, B.; Somfai, P. *J. Org. Chem.* **2002**, *67*, 8574-8583.
[58] Torsell, S.; Somfai, P. *Org. Biomol. Chem.* **2004**, *2*, 1643-1646.
[59] Llaveria, J.; Díaz, Y.; Matheu, M. I.; Castillón, S. *Org. Lett.* **2009**, *11*, 205-208.
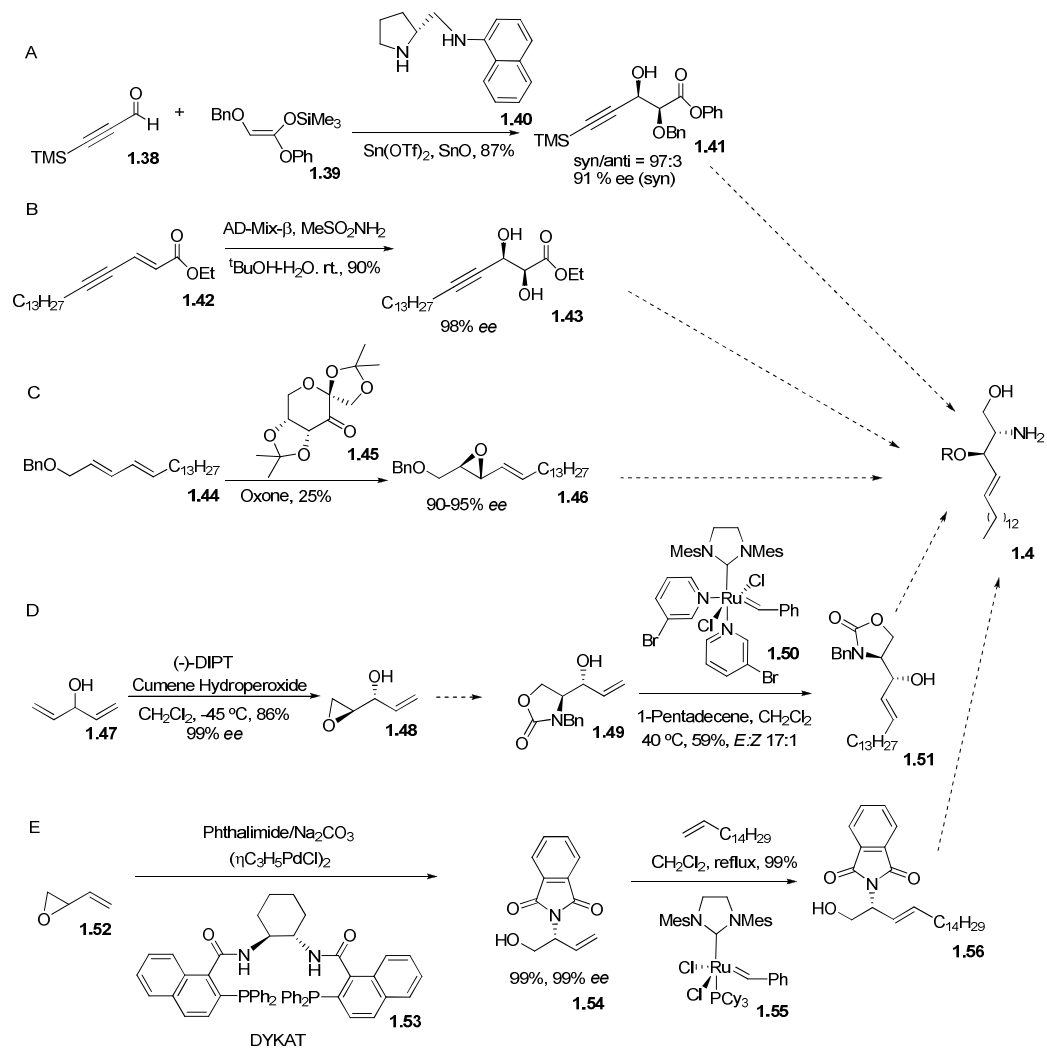
Scheme 1.6. Key steps in representative synthesis of 1.4 employing enantiomeric catalytic procedures.

1.3.2.3. Glycosylation reaction

In order to complete the synthesis of glycosphingolipids according to a covergent strategy (Scheme 1.2, III), it is necessary to attach the oligosaccharide moiety to the corresponding ceramide. Recently, our group has reviewed the recent contributions to the synthesis of GSL's, highlighting the improvements in glycosylation reactions leading to α and β glycosylsphingosines and ceramides as well as related compounds.[38c]

One of the main drawbacks of this convergent methodology is the direct glycosylation of *N*-acylsphingosine as a glycosyl acceptor. In general, rigorous conditions are required and yields and α/β selectivity are poor. This low reactivity is caused by the hindrance of the lipid chains and the existence of a disfavouring hydrogen bond that decreases the nucleophility of the glycosyl acceptor (Scheme 1.9.).[60] Therefore, the amino moiety of sphingosine derivatives is usually proctected as azide or imine groups to avoid the disfavouring hydrongen bond interaction in classical procedures. However, these routes require more synthetic steps and become more lineal.
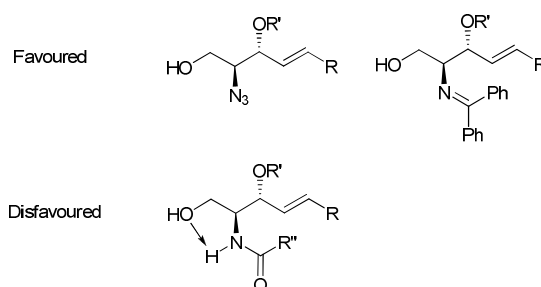
Figure 1.9. Proposed hydrogen bond models to explain the different reactivity of sphingoid bases

To solve this drawback, our group has proposed the activation of ceramides *via* the corresponding stannyl ether.[61] Such an idea permitted increasing the nucleophilicity of oxygen without significantly modifying the basicity of the glycosyl acceptor. Although the corresponding ortoester is obtained instead of the glycosylated product when the carbohydrate is protected with acetyl groups, ortoester may be rearranged to β-glycoside (90%) when it is treated with a Lewis acid (Scheme 1.7.).

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[60] (a) Schmidt, R. R.; Zimmermann, P. *Angew. Chem. Int. Ed. Engl*. **1986**, *25*, 725-726. (b) Polt, R.; Szabo, L.; Treiberg, J.; Li, Y.; Hruby, V. J. *J. Am. Chem. Soc*. **1992**, *114*, 10249-10258.

[61] Morales-Serna, J. A.; Boutureira, O.; Díaz, Y.; Matheu, M. I.; Castillón, S. *Org. Biomol. Chem.* **2008**, *6*, 443-446.
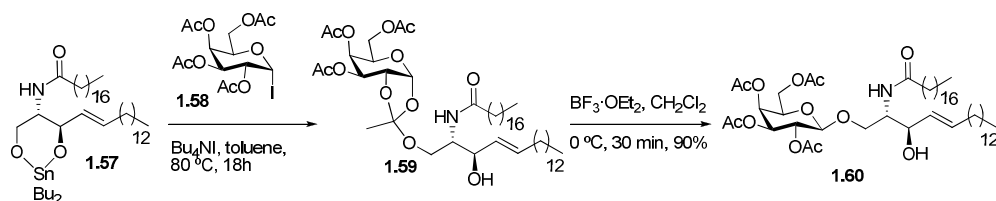
Scheme 1.7. Glycosylation procedure of stannylceramides.

Table 1.1. Examples of direct glycosylation of ceramides.

Entry	Donor	Acceptor	Conditions	Product (Yield)[a]
1[62]	**1.61**	**1.63**	$BF_3 \cdot OEt_2$, CH_2Cl_2, -20 to 0 ºC.	**1.65** (72%)
2[63]	**1.62**	**1.63**	$Sn(OTf)_2$, 1,1,3,3-tetramethylurea, 4 Å MS, r.t., 12 h.	**1.65** (47%)
3[64]	**1.62**	**1.63**	$Hg(CN)_2$, CH_3NO_2, 80 ºC, 2 h.	**1.65** (43%)
4[65]	**1.62**	**1.64**	$Hg(CN)_2$, CH_3NO_2/benzene, 35-40 ºC, 8-10 h.	**1.66** (50-70%)

[a] Yield over two steps. First the β-glycoside was obtained as a mixture of the α-glycoside and the corresponding orthoesters. Then the mixture was treated with TMSOTf to isomerize the orthoester to the β-glycoside which was isolated.

Although this strategy required two steps to afford the corresponding β-glycoside **1.60**, it made a remarkable improve in the direct glycosylation of ceramide (90% yield, Scheme 1.7.) compared to other reported procedures in

[62] Murakami, T.; Minamikawa, H.; Hato, M. *J. Chem. Soc. Perkin Trans. 1* **1992**, 1875-1876.

[63] Ohashi, K.; Kosai, S.; Arizuka, M.; Watanabe, T.; Fukunaga, M.; Monden, K.; Uchikoda, T.; Yamagiwa, Y.; Kamikawa, T. *Tetrahedron Lett.* **1988**, *29*, 1189-1192.

[64] Ohashi, K.; Kosai, S.; Arizuka, M.; Watanabe, T.; Yamagiwa, Y.; Kamikawa, T. *Tetrahedron* **1989**, *45*, 2557-2570.

[65] Shapiro, D.; Flowers, H. M. *J. Am. Chem. Soc.* **1961**, *83*, 3327-3332.

which the ceramide was not activated. In those cases, only modest to good yields (42-72%) were afforded.

In this context, contributions of the present work to the synthesis of glycosphingolipidic derivates employing new glycosylation methodologies will be introduced in the following chapters.

1.4. Strategies to increase binding properties: Multivalent Presentation.

Although protein-carbohydrate interactions are essential to many biological processes, individual interactions usually exhibit weak binding affinities as well as relatively low selectivities between similar carbohydrate ligands.[66] Nature's solution to this problem is to use multivalency.[67] Hence, multiple copies of the carbohydrate ligands are arranged on glycoprotein scaffolds or in patches of glycolipids on the surface of one cell, and multiple copies of lectins (or individial lectins with multiple binding sites) are displayed at the surface of another cell. When these two surfaces come together, the individual interactions reinforce one another to give overall a high avidity.

Importantly, multivalency should be differentiated from cooperativity. Cooperativity arises when the binding of one ligand influence the receptor's affinity towards subsequent ligands.[68] The interplay of individual interactions can lead to positive or negative cooperativity depending on whether one interaction favors or disfavors another.

1.4.1. Modes of multivalency

Multivalency is observed if the binding potency value recorded with a multivalent architecture having "x" epitopes is more than "x" times greater than that of the corresponding monovalent ligand. If this value (relative potency per

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[66] Lee, Y.C.; Lee, R.T. *Acc. Chem. Res.* **1995**, *28*, 321-327.

[67] Mammen, M.; Choi, S.-K.; Whitesides, G.M. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754-2794.

[68] Hunter, C. A.; Anderson, H. L. *Angew. Chem., Int. Ed.* **2009**, *48*, 7488-7499.

ligand) is identical to the monomeric reference, the effect occurring is purely statistical and no real affinity gain is observed.[69]

In multivalent interactions such as glycocluster effect, the receptor binding mechanisms depicted in Fig. 1.10. may happen independently or simultaneously, depending on the nature of the membrane receptor and structural features of the glycoconjugates.[70]

A) Statistical multivalent binding: Epitopes of a multivalent ligand may bind at a single site of its receptor, sliding and recapture of a second epitope increases the residence time and the binding affinity (Fig. 1.10., A). The proximity of additional epitopes promotes the recapture mechanism. This process is associated with a moderate gain of affinity with generally less than two orders of magnitude compared to the monovalent reference.[71] For polymeric glycoconjugates, however, the bind and slide process due to internal diffusion of the membrane receptor along the polymeric chain can lead to much higher affinity systems (Fig. 1.10., B).[72]

B) Chelate multivalent binding: This mode may operate if the distance between binding epitopes can span the distance between recognition sites of the membrane receptor. Thus, the multivalent ligand cross-links binding sites either in adjacent receptors or in a single multivalent receptor. (Fig 1.10, C and D).This process is associated with an over million-fold affinity enhancement in the case of pentameric toxins.[73]

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[69] Mulder, A.; Huskens, J.; Reinhoudt, D. N. *Org. Biomol. Chem.* **2004**, *2*, 3409-3424.

[70] Pohl, N.L.; Kiessling, L.L. *Synthesis*, **1999**, 1515-1519.

[71] (a) Quesenberry, M. S.; Lee, R. T.; Lee, Y. C. *Biochemistry*, **1997**, *36*, 2724-2732. (b) Benito, J. M.; Gómez-García, M.; Ortiz Mellet, C.; Baussanne, I.; Defaye, J.; García Fernandez, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 10355-10363.

[72] Dam, T. K.; Gerken, T. A.; Kavada, B. S.; Nascimento, K. S.; Moura, T.R.; Brewer, F. C. *J. Biol. Chem.* **2007**, *282*, 28256-28263.

[73] (a) Kitov, P.I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature*, **2000**, *403*, 669-672. (b) Fan, E.; Zhang, Z.; Minke, W. E.; Hou, Z.; Verlinde, C. L. M. J.; Hol, W. G. J. *J. Am. Chem. Soc.* **2000**, *122*, 2663-2664. (c) Zhang, Z.; Merritt, E. A.; Ahn, M.; Roach, C.; Hou, Z.; Verlinde, C. L. M. J.; Hol, W. G. J.; Fan, E. *J. Am. Chem. Soc.* **2002**, *124*, 12991-12998.
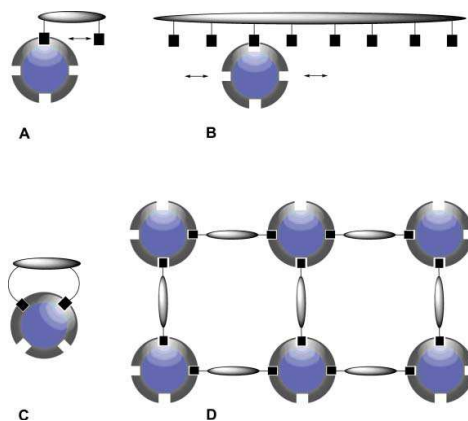
Figure 1.10. Modes of multivalency[74]

1.4.2. Structure of multivalent glycoconjugates

A large number of multivalent glycoconjugates with diverse scaffolds have been synthesized in the last decade. Such glycoconjugates can be classified in three distinct families according to disperty and core presentation.

A) Glycoclusters: A multivalent central scaffold (core) connected to the carbohydrate epitopes displayed directly at their periphery (Figure 1.11.).
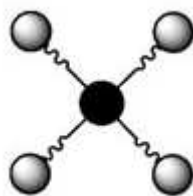
Figure 1.11. Schematic representation of a glycocluster

B) Glycodendrimers: This scaffolds are characterized by highly branched 'dendrons' or 'wedges' that emanate from a central multifunctional core unit. These well-defined polymers can be synthesized in a stepwise and controlled manner, providing increasing 'generations' of structures which are homogeneous or at least have very low polydispersities (Figure 1.12.).

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[74] Deniaud, D.; Julienne, K; Gouin, S. G. *Org. Biomol. Chem.* **2011,** *9,* 966-979.
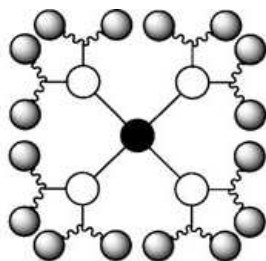
Figure 1.12. Schematic representation of a glycodendrimer

There are two main strategies for constructing dendrimers: divergent and convergent (Scheme 1.8.):[75]

In the divergent approach, the dendrimer is grown outwards from the core, with an increasing of the number of reactive functionalities being introduced with each new generation. Unfortunately, it carries the disadvantage that increasing numbers of reactions is necessary to be performed on each individual compound. Unavoidable side reactions lead consequently to mixtures of closely related compounds whose separation is quite difficult.[76]

In the convergent approach, many of the synthetic problems inherent at the divergent strategy are avoided. This methodology involves: the synthesis of carbohydrated dendrons as one of the structural components followed by the linking of these wedges to further branching components and then finally the attachment of these dendrons to the core.[77] This synthetic strategy also affords compounds with the highest possible structural homogeneities and monodispersities. However, this protocol typically requires larger quantities of the carbohydrate and these peripheral carbohydrates usually must be protected during the synthesis, resulting in further steric crowding that may block coupling efficiencies and necessitate subsequent deprotection steps.

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[75] Matthews, O. A.; Shipway, A. N.; Stoddart, J. F. *Prog. Polym. Sci.* **1998**, *23*, 1-56.
[76] Hummelen, J.C.; van Dongen, J. L. J.; Meier, E. W. *Chem. Eur. J.* **1997**, *3*, 1489-1493.
[77] Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1996**, *2*, 1115-1128.
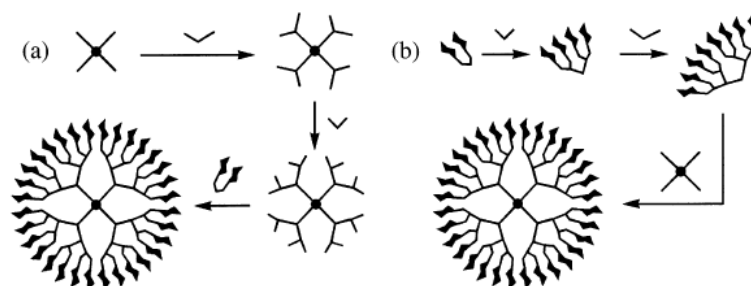
Scheme 1.8. a) Divergent synthesis of dendrimers, b) Convergent synthesis of dendrimers

C) Hyperbranched glycoconjugates: In this case the multivalent scaffold could be a polymer, a nanoparticle, a nanotube, etc. usually highly branched to emulate the dendritic disposition (Figure 1.13.). The main advantage of these macromolecules over dendrimers is the ease of their synthesis, although their structure is not as perfect as that of their dendritic counterparts. These structures are however receiving a growing interest, for instance, for their potential biomedical applications as drug carrier.[78]
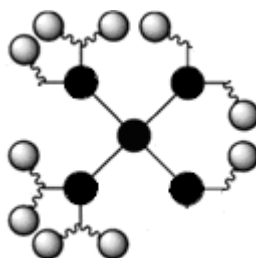
Figure 1.13. Schematic representation of a hyperbranched polymer

1.4.3. Remarks in the design of multivalent glycoconjugates

Direct evidence the importance of framework used to display the carbohydrates comes from early studies of Knowles, Wiley and coworkers,[79] who investigated three different classes of templates as inhibitors of influenza virus hemagglutinin. More recent works also explored the topology of

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[78] (a) Fox, M. E.; Szoka, F. C.; Fréchet, J. M. J. *Acc. Chem. Res.* **2009**, *42*, 1141-1151. (b) Carlmark, A.; Hawker, C.; Hult, A.; Malkoch, M. *Chem. Soc. Rev.* **2009**, *38*, 352-362. (c) Liu, M.; Fréchet, J. M. J. *Pharm. Sci. Technol. Today* **1999**, *2*, 393-401.

[79] Glick, G. D.; Toogood, P. L.; Wiley, D.C.; Skehel, J. J.; and Knowles, J. R. *J. Biol. Chem.* **1991**, *266*, 23660-23669.

glyconconjugates to increase the binding for lectins.[80] Generally, the conformational preferences of the linker residues profoundly affect the capacity of the derivatives to act as multivalent ligands. No single framework for presentation of multiple ligands can guarantee success.[81] Hence, some interesting features in the design of glycoconjugates are introduced bellow:

Shape of the multivalent glycoconjugate: Several works have exemplified how the topology of the multivalent glycoconjugates enhances their affinity for the receptor[80,82] (Figure 1.14.). Hence, to improve the complementary character between them, the multivant glyconjugate could be presented as a globular structure like those found in dendrimers,[83] liposomes,[84] nanoparticles,[85] fullerenes,[86]; and other structutures such as a linear structure as polymers,[87] peptoids,[80]; conical structure like calix[n]arenes,[80] cyclodextrins,[88]; square-planar structures like porphyrines;[80] among others.

[80] Cecioni, S.; Faure, S.; Darbost, U.; Bonnamour, I.; Parrot-Lopez, H.; Roy, O.; Taillefumier, C.; Wimmerová, M.; Praly, J.-P.; Imberty, A.; Vidal, S. *Chem. Eur. J.* **2011**, *17*, 2146-2159.

[81] Kiessling, L.L.; Pohl, N.L. *Chem. Biol.* **1996**, *3,* 71-77.

[82] (a)André, S.; Sansone, F.; Kaltner, H.; Casnati, A.; Kopitz, J.; Gabius, H. J.; Ungaro, R.; *ChemBioChem* **2008**, *9*, 1649-1661. (b) Cecioni, S.; Lalor, R.; Blanchard, B.; Praly, J.-P.; Imberty, A.; Matthews, S. E.; Vidal, S. *Chem. Eur. J.* **2009**, *15*, 13232 -13240.

[83] Clayton, R.; Hardman, J.; LaBranche, C.C.; McReynolds, K. D. *Bioconj. Chem.* **2011**, *22*, 2186-2197.

[84] (a) Kingery-Wood, I. E.; Williams, K.W.; Sigal, G.B.; Whitesides, G. M. *J. Am. Chem. Soc.* **1992**, *114*, 7303-7305. (b) Spevak, W.; Nagy, J.O.; Charych, D. H.; Schaefer, M. E.; Gilbert, J. H.; Bednarski, M. D. *J. Am. Chem. Soc.* **1993** *115*, 1146-l 147.

[85] Martínez-Ávila, O.; Hijazi, K.; Marradi, M.; Clavel, C.; Campion, C.; Kelly, C.; Penadés, S. *Chem. Eur. J.* **2009**, *15*, 9874-9888.

[86] Isobe, H.; Mashima, H.; Yorimitsu, H. Nakamura, E. *Org. Lett.* **2003**, *5*, 4461-4463.

[87] Disney, M. D.; Zheng, J.; Swager T. M.; Seeberger, P. H. *J. Am. Chem. Soc.* **2004**, *126*, 13343-13346.

[88] Mendez-Ardoy, A.; Guilloteau, N.; Di Giorgio, C.; Vierling, P.; Santoyo-Gonzalez, F.; Ortiz Mellet, C.; García Fernandez, J. M. *J. Org. Chem.* **2011**, *76*, 5882-5894.
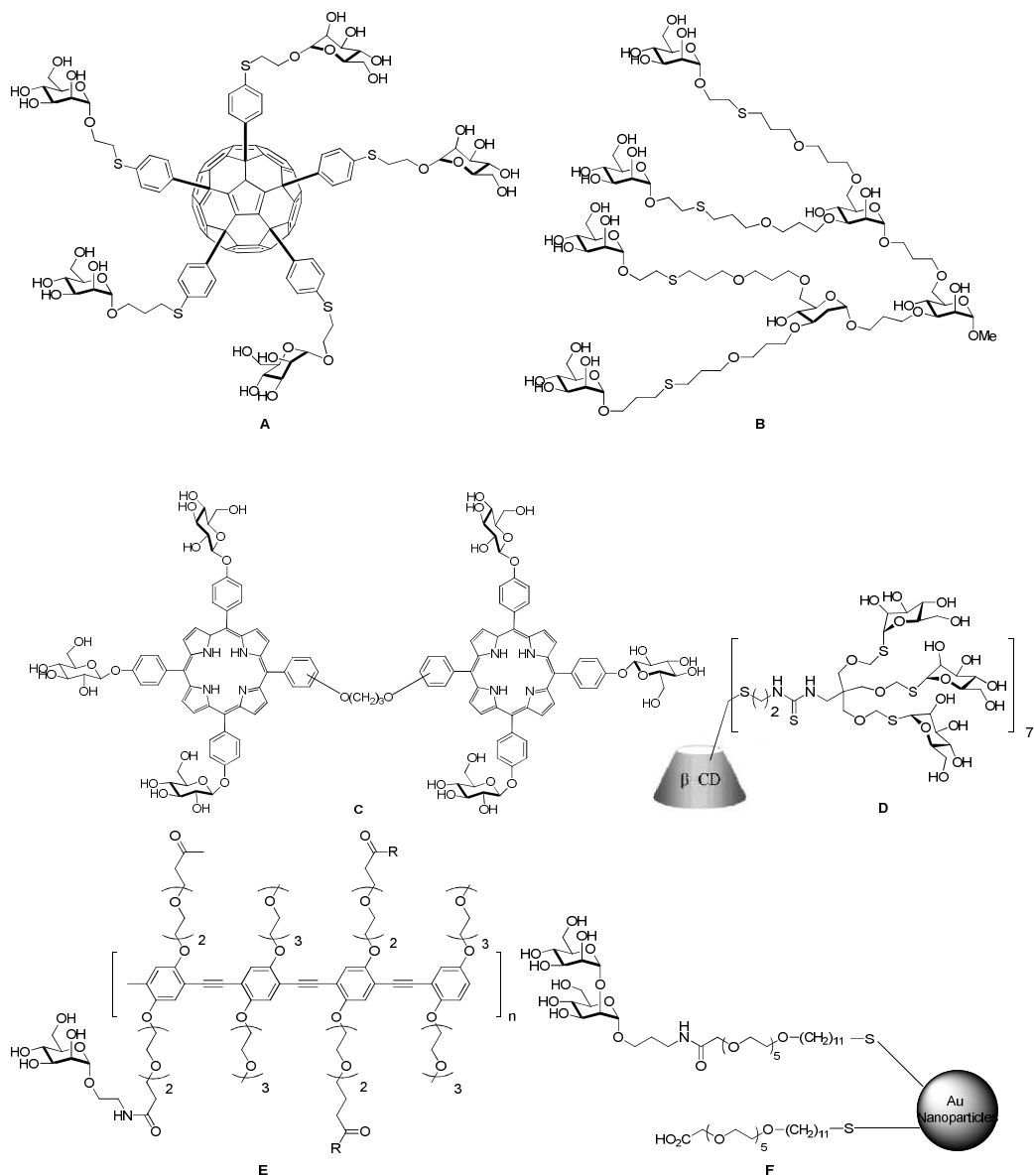
Fig. 1.14. Examples of multivalent structures and topologies: A) Fullerene pentavalent glycocluster.[86] B) Tetravalent mannoside dendrimer.[89] C) Glycosyl bis-porphyrin conjugates.[90] D) Cyclodextrin-centered glycocluster[91]. E) Carbohydrate-functionalized fluorescent polymer.[87] F) Gold manno-glyconanoparticles[85]

[89] Heidecke, C. D.; Lindhorst, T. K. *Chem. Eur. J.* **2007**, *13*, 9056-9067.

[90] Sol, V.; Chaleix, V.; Champavier, Y.; Granet, R.; Huang Y.-M.; Krausz, P. *Bioorg. Med. Chem.* **2006**, *14*, 7745-7760.

[91] Gómez-García, M.; Benito, J. M.; Rodríguez-Lucena, D.; Yu, J.-X.; Chmurski, K.; Ortiz Mellet, C.; Gutiérrez Gallego, R.; Maestre, A.; Defaye, J.; García Fernández, J. M. *J. Am. Chem. Soc.* **2005**, *127*, 7970-7971.

Rigidity of the core: In general, rigid architectures are disadvantageous for optimized lectin binding, due to the restricted spatial presentation adopted by ligands that are unable to match the specific topology of the receptors and spatial distances required. However, when the ligand geometry is appropriate, large benefits in term of affinity may happen.[92] Topological presentation of the sugar moieties can be fine-tuned when rigid scaffolds (such as calixarenes) and linkers are used.[80] Interestingly, several studies reported that glycoconjugates tethering conformationnaly constrained sugars can act as potencial selective inhibitors, able to discriminate between lectins with closely related sequences.[93]

The length of the spacer in the binding mode: Spatial distances between binding epitopes of a multivalent glycoconjugate can be fine-tuned with an appropriate selection of linker arm length.[94] For instance, this consideration may be critical for lectin affinity, specially if a chelate binding mode is participating. In general, rigid linkers should be much more effective than flexible ones because of the theoretical loss in conformational entropy upon binding. However, flexible linkers have been also used with success to design glycoconjugates able to chelate proteins binding sites, with some of the largest affinity enhancements described so far.[95] On the contrary, ligands attached to rigid spacers remain uncommon due to synthetic hurdles and the necessity to exactly match their size with the distance separating the receptor binding domains.[96]

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[92] (a) Vrasidas, I.; André, S.; Valentini, P.; Böck, C.; Lensch, M.; Kaltner, H.; Liskamp, R. M. J.; Gabius, H-J.; Pieters, R. J. *Org. Biomol. Chem*. **2003**, *1*, 803-810. (b) André, S.; Liu, B.; Gabius, H-J.; Roy, R. *Org. Biomol. Chem.* **2003**, *1*, 3909-3916.

[93] Sakai, S.; Shigemasa, Y.; Sasaki, T. *Tetrahedron Lett.* **1997**, *47*, 8145-8148.

[94] (a) Kitov, P. A.; Shimizu, H.; Homans, S. W.; Bundle, D. R. *J. Am.Chem. Soc.* **2003**, *125*, 3284-3294. (b) Yung, A.; Turnbull, W. B.; Kalverda, A. P.; Thompson, G. S.; Homans, S. W.; Kitov, P.; Bundle, D. R. *J. Am. Chem. Soc.* **2003**, *125*, 13058-13062.

[95] Krishnamurthy, V. M.; Semetey, V.; Bracher, P. J.; Shen, N.; Whitesides, G. M. *J. Am. Chem. Soc* **2007**, *129*, 1312-1320.

[96] Fan, E. K.; Zhang, Z. S.; Minke, W. E.; Hou, Z.; Verlinde, C. L. M. J.; Hol, W. G. J. *J. Am. Chem. Soc.* **2000**, *122*, 2663-2664.

The length of the spacer in intrinsic affinities and cross-linking events: The importance of the length of the linker is less intuitive when a multivalent ligand interacts at a single receptor binding site (Fig. 1.10, A) or when an aggregative process is occuring (Fig. 1.10, D). A careful selection of the binding assay can provide insights on particular binding events. For instance, binding affinities of multimeric lactosides based on carbohydrate scaffolds with different valencies and different linker lengths were measured toward biologically relevant galectins. Nearly identical binding affinities were recorded for derivatives differing in the length of the linkers when Enzyme Linked Lectin Assay (ELLA) was employed. In contrast, two-site "sandwich" ELLA revealed that multivalent derivatives bearing the longest spacers were more efficient for cross-linking lectins. Hence, intrinsic affinities, devoid of aggregation effects, and cross-linking capabilities are, therefore, not directly related phenomena that must be taking into consideration in neoglycoconjugate design for specific applications.[97]

Number of epitopes: Nowadays, it is well established that a higher number of epitopes doesn't necessary lead to a higher binding potency of the resulting glycoclusters. This is because a plateau of inhibition could be observed for glycoconjugates with valency in the middle of the series.[98] Moreover, several efforts have been made to predict enhancement expected from the multivalent presentation of binding epitopes taking into account if:

- A multivalent ligand is interacting intramolecularly with a multimeric receptor;[99]
- Chelating binding modes opperate as affinity enhancements;[100]

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[97] Gouin, S. G.; García Fernández, J. M.; Vanquelef, E.; Dupradeau, F-Y.; Salomonsson, E.; Leffler, H.; Ortega-Muñoz, F. M.; Nilsson, U. J.; Kovensky, J. *ChemBioChem*. **2010**, *11*, 1430-1442.

[98] (a) Ashton, P. R.; Hounsell, E. F.; Jayaraman, N.; Nilsen, T. M.; Spencer, N.; Stoddart, J. F.; Young, M. *J. Org. Chem.* **1998**, *63*, 3429-3437. (b) Pagé, D.; Roy, R. *Bioconjugate Chem.* **1997**, *8*, 714-723.

[99] Gargano, J. M.; Ngo, T.; Kim, J. Y.; Acheson, D. W. K.; Lees, W. J. *J. Am. Chem. Soc.* **2001**, *123*, 12909-12910.

[100] Kitov, P. L.; Bundle, D. R. *J. Am. Chem. Soc.* **2003**, *125*, 16271-16284.

- Diferent percentages of families of glycosides are grafted onto the same dendrimeric backbone.[101]

Such assumptions, however, may be still limited to ideal models, and that would be a misleading interpretation to consider these observations as general rules to predict the binding affinity of a neoglycoconjugate in a complex biological system.

1. 5. Objectives

With this background, the general objective of this work is to develop new methods for obtaining glycolipid analogues to β-GalCer of potential biological interest through chemical synthesis, focusing in the modification of the carbohydrate and lipid structure, and in the study of glycosylation reaction. This would afford new libraries of glycolipids which were not only tunned in the carbohydrate moiety but also in the ceramide part. Moreover, due to the fact that the availability of natural glycolipids in multigram scale is often limited and expensive for their studies against diseases, we hypothesize that anchoring synthetically more accessible β-GalCer analogues in a multivalent system could compensate its expected affinity loss due to its simplicity by increasing the number of copies of glycolipid moiety by the phenomenon so-called cluster effect. In this context, the specific objectives of the present work are the following:

1. To study the direct β-glycosylation reaction of long chain stannylated amido-alcohols in order to avoid the *in situ* formation of ortho-ester derivative. To achieve this purpose, the disarming protecting group 2,6-difluorobenzoyl will be tested because of the reported ability of such family of protecting groups to afford direct β-glycosides when they are placed on C-2 carbon at galactosyl donors. Moreover, monitoring of the glycosylation reaction and the identification of the glycosylation

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[101] Wolfenden, M. L.; Cloninger, M. J. *J. Am. Chem. Soc.*, **2005**, *127*, 12168-12169.

intermediates will be carried out through NRM spectroscopy (Scheme 1.9., Chapter 2).

Identification by NMR?
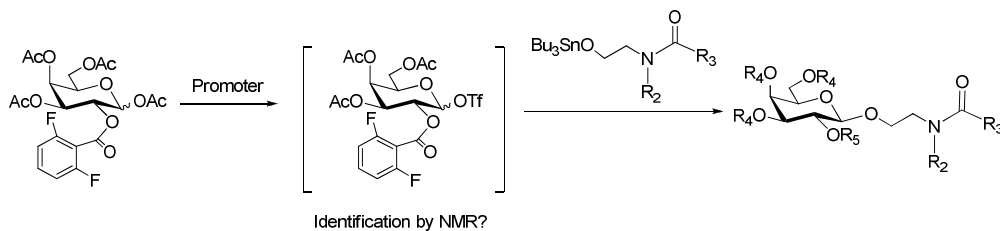
Scheme 1.9. Study of the direct β-glycosylation reaction of long chain stannylated amido-alcohols

2. To study the direct β- and α-glycosylation of stannylated ceramide derivatives of sphingosine and phytosphingosine with 2-deoxy-2-iodo-glycosyl donors. This study will check the generality of the glycosylation methodology developed by our group based on stannylated ceramides as effective glycosyl acceptors; and on the other hand, this methodology could afford new 2-deoxy-analogues of remarkable glycosphingolipids as β-GalCer and KRN7000 (Scheme 1.10., Chapter 3).
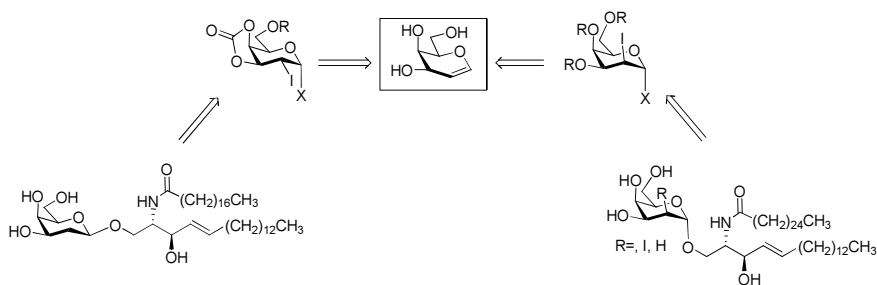
Scheme 1.10. Retrosynthetic analysis for the preparation of 2-deoxyglicolipids

3. To study modifications at C-2 on carbohydrate derivatives through cross coupling reactions. In this case, remarkable glycosyl precursors such as 2-iodoglycals of different configurations and with different protecting groups will be coupled with different boronic acids under phosphine free Suzuki-Miyaura reaction conditions in aqueous media in order to obtain 2-*C*-arylglycals. Once the generality of this reaction will be proved, the reactivity of 2-*C*-arylglycals will be studied in order to obtain 2-*C*-arylglycosides (Scheme 1.11., Chapter 4).

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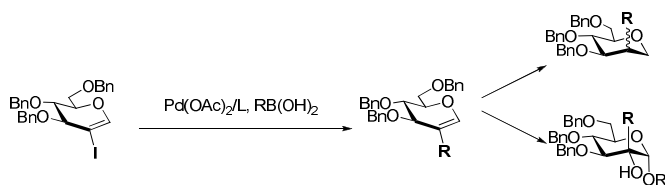
Scheme 1.11. Preparation of 2-C--arylglycosides

4. To synthetize multivalent systems based on β-GalCer derivates of different size, polarity and number of the aglycone chains. In order to achieve this objective, copper-catalized azide-alkyne cycloaddition (CuAAC) reaction will be studied on β-GalCer derivates modified with an azide moiety in the ω-carbon of the fatty acid chain, and scaffolds such as benzyl ethers and hyperbranched polymers (Boltorn H30) modified with acetylene groups. These materials will be employed in inhibition studies against the Cholera Toxin (Figure 1.15., Chaper 5).
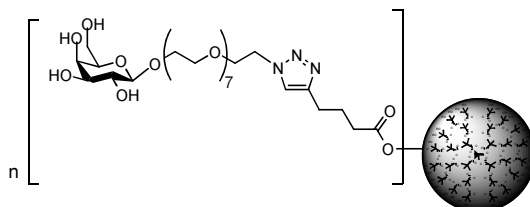
Figure 1.15. Representation of β-GalCer derivative supported on a hyperbranched polymer

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Chapter 2

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Synthesis of β-Galceramide analogues: Glycosylation of amidoalcohols using 2,6-diFBz-glycosyl donors

2.1. Introduction

As mentioned in chapter 1, glycosphingolipids (GSLs) are ubiquitous components of eukaryotic cell membranes.[1] Distributed mainly at the surface of the cell, they serve as distinguishing markers for cells and mediate cell-to-cell recognition and communication. Thus, GSLs have been known for many years to function in animal cells as antigens,[2] and receptors for microbial toxins, viruses and bacteria[3] as well as mediators of cell adhesion and modulators of signal transduction.[4] In recent years, these compounds have been studied as a strategy for pharmacological prevention of microbial infections (HIV),[5] cancer chemotherapy,[6] modifying the activity of receptors for insulin,[7] epidermal growth factor[8] and nerve growth factor[9] which may have potential effects in Alzheimer's[10] and Parkinson's[11] diseases.

GSLs consist of two structural elements (Figure 2.1.): a lipophilic membrane anchor, the ceramide portion, which is formed by a long chain aminoalcohol and a fatty acid, and a hydrophilic carbohydrate moiety, which protrudes from the cell surface.

[1] Hakomori, S. *Biochim. Biophys. Acta* **2008,** *1780*, 325-346.

[2] Hakomori, S. *Acta Anat.* **1998**, *161*, 79-90.

[3] Varki, A. *Glycobiology* **1993**, *3*, 97-130.

[4] (a) Hakomori, S. *Glycoconjugate J.* **2000**, *17*, 143-151. (b) Todeschini, A. R.; Hakomori, S. *Biochim. Biophys. Acta* **2008**, *1780*, 421-433.

[5] Svensson, M.; Frendeus, B.; Butters, T.; Platt, F.; Dwek, D.; Svanborg, C. *Mol. Microbiol.* **2003**, *47*, 453-461.

[6] Radin, N.S. *Eur. J. Biochem.* **2001**, *268*, 193-204.

[7] Allende, M.L.; Proia, R.L. *Curr. Opin. Struct. Biol.* **2002**, *12*, 587-592.

[8] Zhou, G.; Hakomori, S.; Kitamura, K.; Igarashi, Y. *J. Biol. Chem.* **1994**, *269*, 1959-1965.

[9] Mutoh, T.; Toluda, A.; Miyadai, T.; Hamaguchi, M.; Fujiki, N. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 5087-5091.

[10] Svennerholm, L.; Brane, G.; Karlsson, I.; Lekman, A.; Ramstorm, I.; Wikkelso, C. *Dementia Geriatr. Cognit. Disord.* **2002**, *14*, 128–136.

[11] Matsuoka, Y.; Saito, M.; LaFrancois, J.; Saito, M.; K. Gaynor, K.; Olm, V.; Wang, L.; Casey, E.; Lu, Y.; Shiratori, C.; Lemere, C.; Duff, K. *J. Neurosci* **2003**, *23*, 29-33.
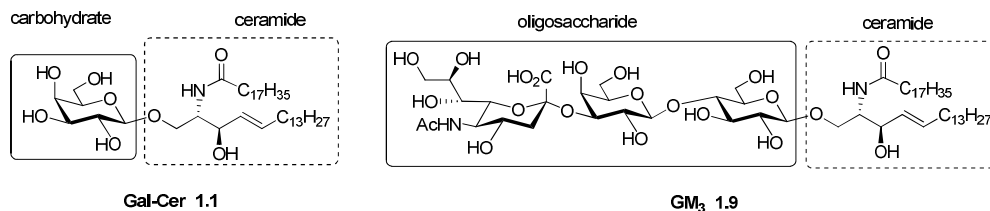
Figure 2.1. Some naturally occurring β-glycosphingolipids

Because of the biological importance of these compounds significant work has been devoted for preparing natural GSLs and analogues, with the goals of improving these properties and understanding the interactions responsible for biological activity.

A key step in the synthesis of GSLs is the formation of the glycosidic bond between a properly protected carbohydrate and ceramide or sphingosine.[12,13] To accomplish this key synthetic step, a variety of glycosyl donors have been utilized including glycosyl trichloroacetamidates, fluorides, phosphates and sulfides.[14] Regardless, the glycosylation reaction is still one of the main determining factors in the synthesis, because the yields in the direct glycosylation of ceramides are low. This problem has been attributed to the low nucleophilicity of ceramides,[12,15] as a result of head-group hydrogen bonding, and it is usually circumvented by using azidosphingosine derivate instead of the ceramide (Scheme 2.1.). However, further reduction of the azido group and acylation is therefore required.[15b,16]

[12] Vankar, Y. D.; Schmidt, R. R. *Chem. Soc. Rev.* **2000**, *29*, 201–216.

[13] Gigg, J.; Gigg, R. *Top. Curr. Chem.* **1990**, *154*, 77–139.

[14] For a review about the *O*-glycosylation of sphingosines and ceramides see: Morales-Serna, J. A.; Boutureira, O.; Díaz, Y.; Matheu, M. I.; Castillón, S. *Carbohydr. Res.* **2007**, *342*, 1595–1612.

[15] (a) Polt, R.; Szabo, L.; Treiberg, J.; Li, Y.; Hruby, V.J. *J. Am. Chem. Soc* **1992**, *114*, 10249-10258. (b) Schmidt, R.R.; Zimmermann, P. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 725-726.

[16] Schmidt, R.R.; Zimmermann, P. *Tetrahedron Lett.* **1986**, *27*, 481-484.
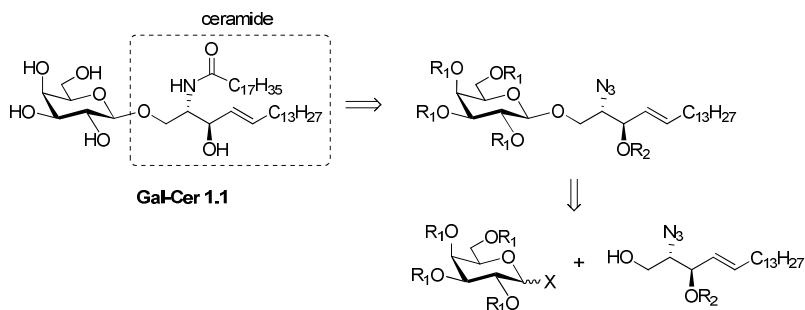
Scheme 2.1. Usual convergent retrosynthetic analysis of β-galactosylceramide

Our group has recently reported a new and highly efficient protocol for the direct glycosylation of ceramides which involves the reaction of stannyl ethers[17] with α-iodogalactose derivatives in the presence of TBAI as an activator. This approach allows increasing the nucleophilicity of ceramide oxygen without significantly modifying the basicity and provided a direct acces to both α-[18] and β-glycolipids[19] (Scheme 2.2.) starting from per-*O*-silylated (via a) or per-*O*-acetylated (via b) galactosyl iodides respectively.

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[17] (a) Kaji, E.; Shibayama, K.; In, K. *Tetrahedron Lett.* **2003**, *44*, 4881-4885. (b) Kartha, R. K. P.; Kiso, M.; Hasegawa, A.; Jennings, H. J. *J. Chem. Soc., Perkin Trans. 1*, **1995**, 3023-3026. (c) Garegg, P. J.; Malvisel, J.L.; Oscarson, S. *Synthesis*, **1995**, 409-414. (d) Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Griffith, D. A.; Oriyama, T.; Marsden, S.P. *J. Am. Chem. Soc.* **1995**, *117*, 1940-1953. (e) Vogel, K.; Sterling, J.; Herzig, Y.; Nudelman, A. *Tetrahedron*, **1996**, *52*, 3049–3056. (f) David, S.; Hanessian, S. *Tetrahedron*, **1985**, *41*, 643-663. (g) Yamago, S.; Yamada, T.; Hara, O.; Ito, H.; Mino, Y.;Yoshida, J.-I. *Org. Lett.* **2001**, *3*, 3867-3870. (h) Yamago, S.; Yamada, T.; Ito, H.; Hara, O.; Mino, Y.; Yoshida, J.-I. *Chem. Eur. J.* **2005**, *11*, 6159-6174.

[18] Boutureira, O.; Morales-Serna, J. A.; Díaz, Y.; Matheu, M.I.; Castillón, S. *Eur. J. Org. Chem.* **2008**, 1851-1854.

[19] (a) Morales-Serna, J.A.; Boutureira, O.; Díaz, Y.; Matheu, M.I.; Castillón, S. *Org. Biomol. Chem*. **2008**, *6*, 443-446. (b) Morales-Serna, J. A.; Díaz, Y.; Matheu, M. I.; Castillón, S. *Org. Biomol. Chem.* **2008**, *6*, 3831-3836.
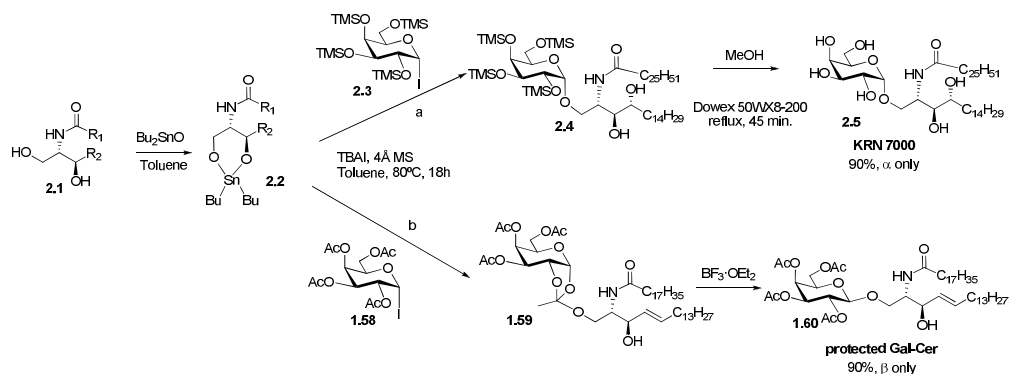
Scheme 2.2. Reported glycosylation of ceramides for the obtention of α- and β-glycolipids[18,19]

However, when the glycosylation is performed by using an acetylated glycosyl donor (Scheme 2.2., via b), the obtention of β-glycolipids is not direct and the corresponding orthoester is previously formed, a fact usually observed in the coupling of sterically hindered alcohols.[20] For this reason, a further treatment with BF₃·OEt₂ is necessary to rearrange the obtained orthoesters[21] to the corresponding β-anomers. The obtention of orthoester derivatives has been also decribed starting from 2-*O*-chloroacetylglycosyl donors.[22]

Often orthoester formation is obviated by using pivaloyl protecting group at O2.[23] However, in our hands, when starting from pivaloyl protected donor, a

[20] (a) Fürstner, A.; Jeanjean, F.; Razon, P.; Wirtz, C.; Mynott, R. *Chem. Eur. J.* **2003**, *9*, 320-326. (b) Gung, B. W.; Fox, R. M. *Tetrahedron* **2004**, *60*, 9405-9415. (c) Nicolaou, K. C.; Daines, R. A.; Ogawa, Y.; Chakraborty, T. K. *J. Am. Chem. Soc.* **1988**, *110*, 4696-4705. (d) Kuszmann, J.; Medgyes, G.; Boros, S. *Carbohydr. Res.* **2004**, *339*, 2407-1414. (e) Plante, O. J.; Palmacci, E. R.; Andrade, R. B.; Seeberger, P. H. *J. Am. Chem. Soc.* **2001**, *123*, 9545-9554.

[21] Some examples of isomerization of orthoesters to β-glycosides are: (a) Wang, W.; Kong, F. *J. Org. Chem.* **1998**, *63*, 5744-5745. (b) Sznaidman, M.L.; Johnson, S.C.; Crasto, C.; Hecht, S.M. *J. Org. Chem.* **1995**, *60*, 3942-3943. (c) Gass, J.; Strobl, M.; Kosma, P. *Carbohydr. Res.* **1993**, *244*, 69-84.

[22] (a) Wang, G.; Zhang, W.; Lu, Z.; Wang, P.; Zhang, X.; Li, Y. *J. Org. Chem.* **2009**, *74*, 2508-2515. (b) Hanashima, S.; Manabe, S.; Ito, S. *Angew. Chem. Int. Ed.* **2005**, *44*, 4218-4224. (c) Orgueira, H.A.; Bartolozzi, A.; Schell, P.; Litjens, R.E.J.N.; Palmacci, E.R.; Seeberger, P.H. *Chem. Eur. J.* **2003**, *9*, 140-169. (d) Fürstner, A.; Jeanjean, F.; Razon, P. *Angew. Chem. Int. Ed.* **2002**, *41*, 2097-2101; e) Ye, X.-S.; Wong, Ch.-H. *J. Org. Chem.* **2000**, *65,* 2410-2431. (e) Zegelaar-Jaarsveld, K.; Duynstee, H.I.; van der Marel, G.A.; van Boom, J.H. *Tetrahedron* **1996***, 52,* 3575-3592.

[23] (a) Harreus, A.; Kunz, H. *Liebigs Ann. Chem.* **1986**, 717-721. (b) Garegg, P. J.; Olsson, L.; Oscarson, S. *J. Org. Chem.* **1995**, *60*, 2200-2204. (c) Perrie, J.A.; Harding, J.R.; King, C.; Sinnott, D.; Stachulski, A.V. *Org. Lett.* **2003**, *5*, 4545-4548.

mixture of orthoester and β-glycoside was obtained, similar to that reported for related glycosyl donors.[24] Replacing a 2-*O*-acetyl by a 2-*O*-benzoyl is another common way to avoid orthoester formation, but more severe basic conditions are required for the removal of *O*-benzoates and in particular a 2-*O*-benzoate of galactose.[25]

Other recently reported bulky groups proposed in β-glycosylation reactions for avoiding orthoester formation are 2-chloro-2-methylpropanoic ester[26] or 4-acetoxy-2,2-dimethylbutanoic ester.[27] All these protecting groups have been proposed with the aim of affording a sterically demanding dioxolenium ion intermediate that prevents orthoester formation (Scheme 2.3., via a) to favour the obtention of the corresponding β-glycosides (Scheme 2.3., via b).
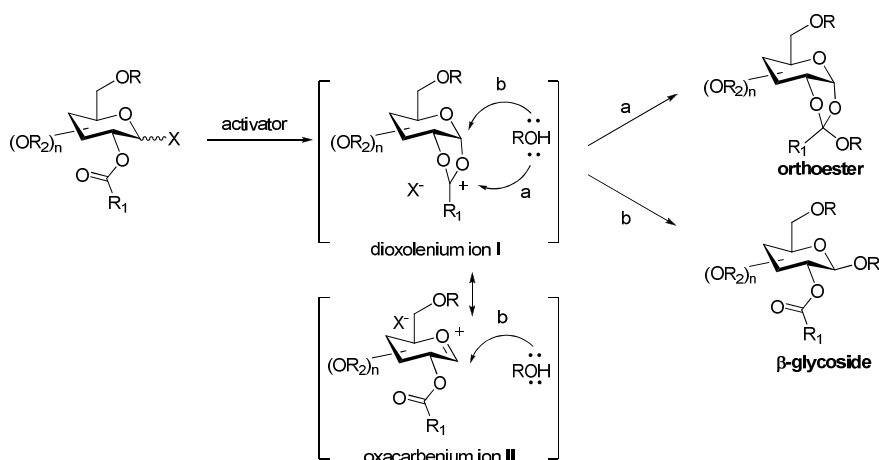
Scheme 2.3. Orthoester versus glycoside formation

In this context we envisage in a different approach to avoid orthoester formation during ceramide glycosylation: that is, to use a protecting group with a higher electron-withdrawing nature in order to destabilize the dioxolenium ion intermediate **I** (Scheme 2.3.) in the orthoester formation. In this sense,

[24] Vaughan, M. D.; Johnson, K.; DeFrees, S.; Tang, X.; Warren, R. A. J.; Withers, S. G. *J. Am. Chem. Soc.* **2006**, *128*, 6300-6301.
[25] Cato, D.; Buskas, T.; Boons, G.-J. *J. Carbohydr. Chem.* **2005**, *24*, 503-516.
[26] Szpilman, A.M.; Carreira, E.M. *Org. Lett.* **2009**, *11*, 1305-1307.
[27] (a) David, W.; Harry, E.; Yu, H. *PCT Int. Appl.* **2006** WO 2006/042200 A3. (b) Yu, H.; Williams, D.L.; Ensley, H.E. *Tetrahedron Lett.* **2005**, *46*, 3417-3421.

fluorobenzoyl groups have been described recently as alternatives to acetyl and benzoyl protective groups in glycopeptide synthesis.[28] Specifically, 2,6-difluorobenzoyl group combines the advantages of the benzoyl group in formation of glycosidic bonds with the ease of removal characteristic of the acetyl group.

In an effort to improve the direct glycosylation of ceramides applying our experience with stannyl ethers we show herein the use of highly disarmed glycosyl donors for direct accessing β-glycolipids without orthoester formation.

2.2. Results and Discussion

In general, easily accessible fully difluorobenzoylated galactosyl bromides have been found to be low reactive in the synthesis glycopeptides[28] (Scheme 2.4., A). Moreover, when other perfluorobenzoylated galactosyl sulfides were employed as glycosyl donors for the glycosylation of ceramides, good β-selectivities were obtained although yields were moderate to good 50-60%[29] (Scheme 2.4., B). However, no orthoester and better results in terms of yield to β-glycoside (63-74%) were obtained when only C-2 was functionalized with a difluorobenzoylated group on the glycosyl donor for the glycosylation of disaccharides[25] (Scheme 2.4., C).

With such precedents in mind, we decided to study the glycosylation reaction using less disarmed glycosyl donors, carrying only a difluorobenzoyl group at C-2 while acetyl esters were present at C-3, C-4 and C-6 position, and activating ceramide derivates as a stannyl ethers in order to achieve β-galactosides in just one glycosylation step.

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[28] Sjolin, P.; Kihlberg, J. *J. Org. Chem.* **2001**, *66*, 2957-2965.
[29] Wallner, F. K.; Norberg, H. A.; Johansson, A. I.; Mogemark, M.; Elofsson, M. *Org. Biomol. Chem.* **2005**, *3*, 309-315.
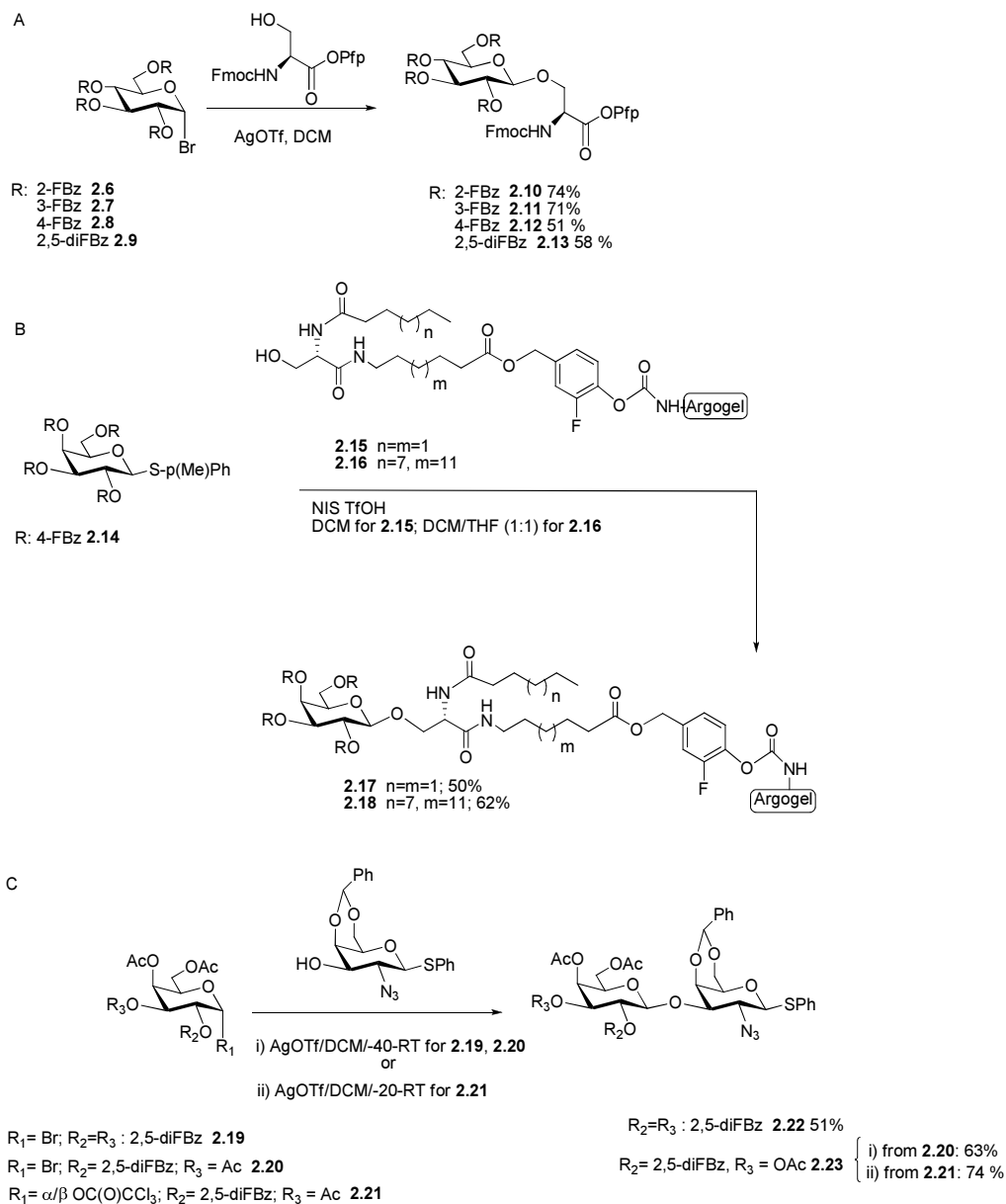
Scheme 2.4. Literature examples of glycosylation reactions employing fluorobenzoyl glycosyl donors[25,28,29]

43

2.2.1. Synthesis of the glycosylation starting materials

The preparation 2-difluorobenzoylated glycosyl donors at C-2 position can be easily afforded from commercial fully acetyl protected β-galactopyranose **2.24** (Scheme 2.5.). Selective deprotection of the acetyl group at C-2 of pentaacetyl-galactopyranoses in presence of aqueous trifluoroacetic acid (TFA/H$_2$O 10:1) was described by Chittenden.[30] This procedure afforded the alcohol **2.25** with a 83% yield after 5 h reaction. Then, the alcohol **2.25** was acylated with 2,6-difluorobenzoyl chloride in presence of DMAP and pyridine at 0 ºC for 5 h to afford **2.27** quantitatively.
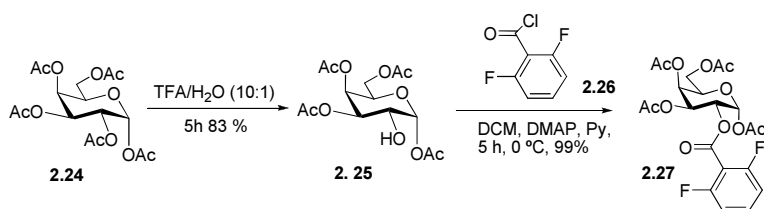
Scheme 2.5. Preparation of glycosyl donor **2.27**

On the other hand, the synthesis of amidoalcohols bering one (**2.32**) and two (**2.34**) long aliphatic chain was envisioned as a mimic of ceramide derivatives and to explore the direct β glycosylation of ceramides. Initial efforts were directed toward the synthesis of ceramide **2.34** (Scheme 2.6.) from 16-bromohexadecanoic acid **2.28**. Thus, 16-bromohexadecanoic acid **2.28** was transformed into the corresponding azide derivative **2.29** in 90% yield by treatment with NaN$_3$ in DMF at 90 °C.

2-Aminoethanol **2.30** was acylated with the stearoyl chloride **2.31** in CH$_2$Cl$_2$ to give the amide **2.32**[31] in 88% yield as is depicted in Scheme 2.6. Reduction of amide **2.32** with LiAlH$_4$ and acylation with **2.29** using coupling reagents[32] (HOBt and EDC) afforded the ceramide **2.34**.

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[30] Chittenden, G. J. F. *Carbohydr. Res.* **1988**, *183*, 140-143.
[31] Guan, L.-P.; Zhao, D.-H.; Xiu, J.-H.; Sui, X.; Piao, H.-R.; Quan, Z.-S. *Arch. Pharm. Chem. Life Sci.* **2009**, *342*, 34-40.
[32] Valeur, E.; Bradley, M. *Chem. Soc. Rev.* **2009**, *38*, 606-631.
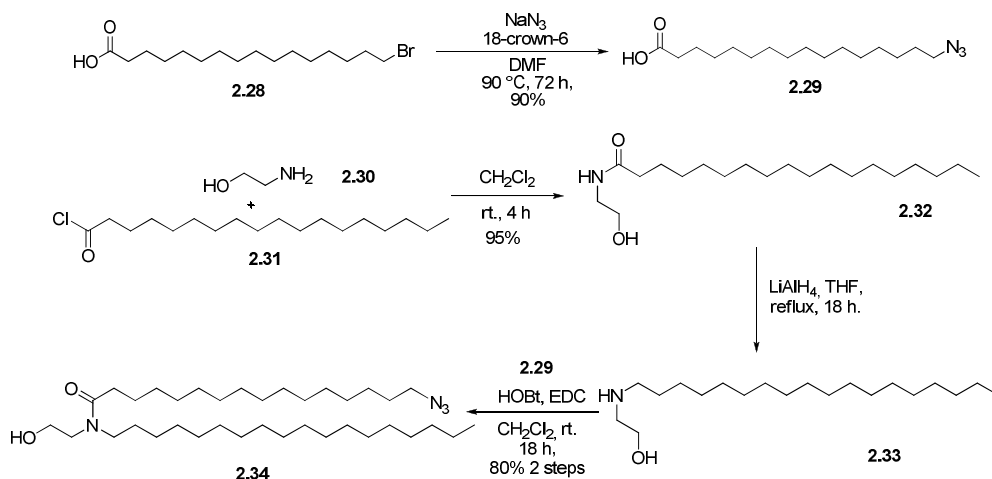
Scheme 2.6. Synthesis of amido alcohols **2.32** and **2.34**

2.2.2. Glycosylation of stannylamidoalchohols under strongly acidic conditions

Initially, we explored the reaction of 1-*O*-acetylgalactosyl donor **2.27** with a simple ceramide model, which was activated as the corresponding stannyl ether (**2.35**). The glycosylation reaction was studied by using different acidic conditions. In this sense, the use of Lewis acids in glycosylation of ceramides starting from penta-*O*-acetyl-β-D-galactose has been reported previously by Fantini et al.[33] Direct glycosylation of 1-*O*-acetylglycosyl donors is usually appreciated because the preparation of more activated glycosyl donors is avoided, thus decreasing the number of steps of the synthesis.

The first assay was performed using 6 equivalents of freshly distilled $BF_3·OEt_2$ added to an ice-cooled mixture of glycosyl donor and acceptor in dry CH_2Cl_2 under argon atmosphere. The reaction was warmed to room temperature but after 3 h of reaction the ^1H NMR spectrum from the crude only showed the presence of starting materials and the product that results of the hydrolysis of glycosyl donor (**2.40**). After crude purification, this product was obtained in a 80% yield and 1:4 β/α ratio (Table 2.1., entry 1).

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[33] Villard, R.; Hammache, D.; Delapierre, G.; Fotiadu, F.; Buono, G.; Fantini, J. *ChemBioChem* **2002**, *3*, 517-525.

In order to reduce the hydrolysis process, the reaction was performed using only 3 equivalents of $BF_3 \cdot OEt_2$ in the presence of activated 4Å MS but similar results were obtained after 4 h of reaction (Table 2.1., entry 2).

Other assays with Brönsted acids were also carried out. When a catalytic amount of TfOH (0.3 eq) was used, no evolution was observed during 3h of reaction (Table 2.1., entry 3). Nevertheless, the desired glycosylated product **2.37** was obtained by using an excess of TfOH (6 eq) (Table 2.1., entry 4). It is worth to note that only the β-glycoside was obtained and as we expected, no evidences of orthoester formation were found. In this case, the yield (48%) was not very high because products resulting from partial desprotection of galactose were also formed.

To improve the yield on glycosylation, promoter concentration was optimized. When 1 eq of TfOH was used no evolution was observed after 15 min. A second equivalent of acid was added without formation of glycosylated product either after 1 h of reaction (Table 2.1., entries 5 and 6). However, when 3 eq of TfOH were added, a complete conversion was achieved in 0.5 h. Regrettably, several purifications were needed to remove decomposition by-products and reactant **2.35** in excess, as a consequence, the desired β-glycoside was obtained in a 60% yield (Table 2.1., entry 7). Alternatively, dry toluene was tested as solvent but reaction became more complex so yield was lower (Table 2.1., entry 8).

In order to simplify the purification step, we considered not to use an excess of amide **2.35**. Moreover, the deprotection of glycosylated product **2.37** was carried out prior to purification in order to increase the difference of Rf between **2.38** and by-products. This methodology was applied under the optimized conditions of promoter concentration, obtaining the deprotected β-glycoside **2.38** in a good yield (85%) over the two steps (Table 2.1., entry 9).

Table 2.1. Glycosylation of lipids **2.35** and **2.36** with *O*-acetylglycosyl donors **2.24** and **2.27** promoted by Lewis and Brönsted acids

2.27 R_1=2,6-di-F-Bz   **2.35** R_2=H,
                           R_3 = $(CH_2)_{16}CH_3$

2.24 R_1=Ac

NaOMe/ MeOH

2.37 R_1 = 2,6-di-F-Bz, R_2=H, R_3 = $(CH_2)_{16}CH_3$, R_4= Ac
2.38 R_1= R_2= R_4= H, R_3 = $(CH_2)_{16}CH_3$
2.39 R_1= R_4= Ac, R_2=H, R_3 = $(CH_2)_{16}CH_3$

2.40 R_1=2,6-di-F-Bz R_4=Ac
2.41 R_1= R_4=Ac

2.36 R_2= $(CH_2)_{17}CH_3$,   NaOMe/
        R_3 = $(CH_2)_{14}CH_2N_3$ MeOH

2.42 R_1= 2,6-di-F-Bz, R_2=$(CH_2)_{17}CH_3$, R_3 = $(CH_2)_{14}CH_2N_3$, R_4= Ac
2.43 R_1= R_4= H, R_2=$(CH_2)_{17}CH_3$, R_3 = $(CH_2)_{14}CH_2N_3$
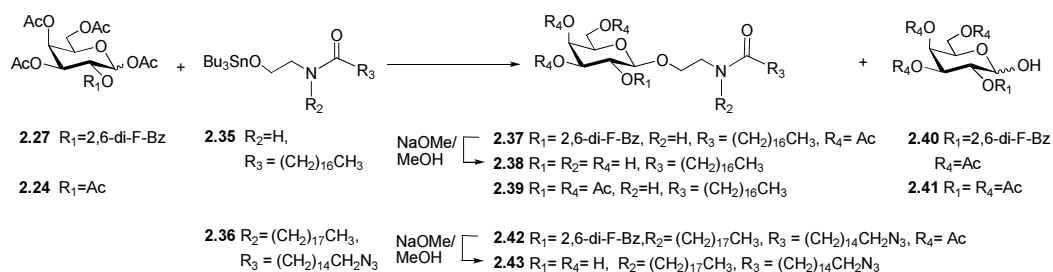
Entry[a]	Donor (eq)	Acceptor (eq)[b]	Promoter (equiv.)	t (h)	Reaction product	Yield[c] (%)
1	**2.27** (1)	**2.35** (1.1)	$BF_3 \cdot OEt_2$ (6)	3	**2.40**	80
2	**2.27** (1)	**2.35** (1.1)	$BF_3 \cdot OEt_2$ (3)	4	**2.40**	78
3	**2.27** (1)	**2.35** (1.1)	TfOH (0.3)	3	--	N.R.
4	**2.27** (1)	**2.35** (1.2)	TfOH (6)	0.25	**2.37**	48[d]
5	**2.27** (1)	**2.35** (1.1)	TfOH (1)	0.25	--	N.R.
6	**2.27** (1)	**2.35** (1.1)	TfOH (2)	1	--	N.R.
7	**2.27** (1)	**2.35** (1.2)	TfOH (3)	0.5	**2.37**	60[d]
8[f]	**2.27** (1)	**2.35** (1.2)	TfOH (3)	1	**2.37**	20
9	**2.27** (1.2)	**2.35** (1)	TfOH (3)	2	**2.38**	85[e]
10	**2.24** (1.2)	**2.35** (1)	TfOH (3)	2	**2.39**	18[e]
11	**2.27** (1.2)	**2.36** (1)	TfOH (3)	2	**2.43**	40[e]

[a] General conditions: Promoter was added to a mixture of donor, tributylstannyl acceptor, and 4 Å MS in the CH_2Cl_2 solvent unless otherwise indicated. [b] *In situ* tributylstannyl alkoxyde preparation prior to glycosylation reaction. [c] Isolated yield. [d] Yield of isolated product after two consecutive chromatographic purifications. [f] Toluene as a solvent. [e] Overall yield for the two steps. N.R.: no reaction.

To compare the advantages of using the 2,6-difluorobenzoyl group at position C-2 of glycosyl donor, the same methodology was applied to fully acetylated galactose **2.24** (Table 2.1, entry 10). In this case, the ^{1}H NMR spectrum of the crude reaction showed the presence of a mixture of products which results from the glycosyl donor hydrolysis with α configuration (**2.41α**), the glycosylated product **2.39** and the corresponding orthoester in a relation (0.5:1:3)

respectively. After acetyl deprotection and purification, glycosylated product **2.38** was obtained in very low yield (18%, Table 2.1, entry 10). This result demonstrates the importance of the presence of a difluorobenzoyl protecting group at O-2 in order to avoid the formation of the undesired orthoester.

In order to expand this methodology, glycosyl donor **2.37** was used in the glycosylation of more complex amidoalcohols such as ceramide **2.36**. Thus, under the optimized glycosylation conditions (Table 2.1., entry 9) and after subsequent acetyl deprotection, product **2.43** was isolated in a overall 40% yield (Table 2.1., entry 11).

Once we demonstrated that β-glycosylation of ceramide derivates was possible in presence of a strong protic acid and no traces of orthoester were observed, we decided to explore the glycosylation of lipids **2.35** and **2.36** in milder conditions.

2.2.3. Glycosylation of stannylamidoalchohols under milder conditions

The previous glycosylation study starting from 1-*O*-acetyl glycosyl derivatives demonstrated the convenience of using disarmed glycosyl donors but also showed the necessity of exploring other activation methods and different leaving groups for the glycosylation of low reactive ceramides. In this sense and, in an attempt to further broaden the scope of such disarmed glycosyl donors, we proceeded to study the glycosylation reaction using different leaving groups.

Glycosyl iodides are well known as excellent glycosyl donors[34] due to their superior reactivity compared to other glycosyl halides, and they have been used effectively in previous works in our group.[18,19] Thus, the conditions for the glycosylation of ceramides developed by us were tested with disarmed iodoglycoside **2.44** and stannyl amide **2.35**, using TBAI as a promoter and toluene as a solvent and heating to reflux (Table 2.2, entry 1). Unfortunately, a mixture of hydrolyzed glycoside **2.40** (89%) and the corresponding

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[34] (a) Gervay-Hague, J.; Hadd, M. J. *J. Org. Chem.* **1997**, *62*, 6961-6967. (b) Gervay-Hague, J.; Hadd, M. J. *Carbohydr. Res.* **1999**, *320*, 61-69. (c) Lam, S. N.; Gervay-Hague, J. *Org. Lett.* **2002**, *4*, 2039-2042.
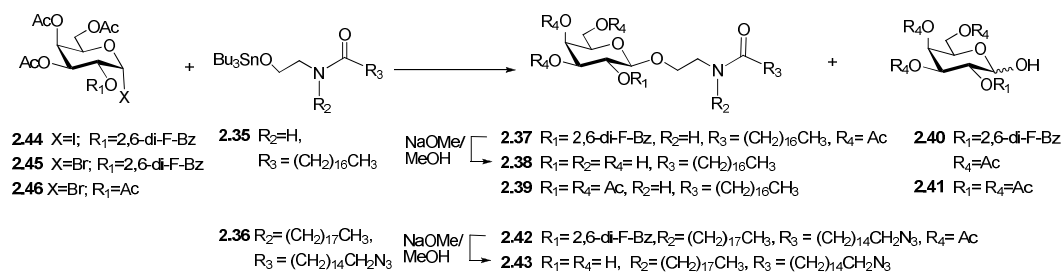
amidoalcohol **2.35** were recovered instead of the glycosylated product. By using AgOTf as a promoter and CH_2Cl_2 as a solvent at room temperature similar results were found (Table 2.2., entry 2). These results are consistent with the fact that iodoglycoside **2.44** is more reactive than the 1-*O*-acetyl donor **2.27** and therefore, product **2.44** might hydrolize before reacting with stannyl derivative **2.35**.

Consequently, the use of a less reactive glycosyl donor such as glycosyl bromide **2.45** was studied. Initially, the use of TBAI as a promoter was unsuccessful and a mixture of hydrolyzed donor **2.40α** and ceramide **2.35** was obtained again (Table 2.2., entry 3). Nevertheless, triflate salts such as AgOTf or $Hg(OTf)_2$ allowed to obtain glycosylated derivative **2.37** (Table 2.2., entries 4 and 5). However, conversions (30-35%) were not satisfactory enough because orthoester was detected as a by-product (characteristic signal:[19b] δ 5.71 ppm 1H, d, $J = 4.7$ Hz, 1H, H-1), and hydrolyzed glycosyl donor was also obtained. These results showed that under the less acidic conditions used for the activation of 1-haloglycosides the formation of orthoester products may be favoured after long reaction times. Nevertheless, the putative 2,6-difluorobenzoyl orthoester derivative should be easier to rearrange to the glycosylated product than the corresponding acetate orthoester in presence of acid promoter.[35] For this reason, the use of a as acid like $Sn(OTf)_2$ together with AgOTf was also studied. Thus, when a mixture of $AgOTf/Sn(OTf)_2$ was applied, quantitative formation of the glycosylated product **2.37** was observed in the reaction crude. Unfortunately, the yield obtained was not excellent because of the difficulties of purification (Table 2.2., entry 6). However, when the purification was performed after deprotection of the reaction crude, an excellent yield 90% for compound **2.38** was achieved over two steps (Table 2.2., entry 7).

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[35] Kong, F. *Carbohydr. Res.* **2007**, *342*, 345-373.

Table 2.2. Glycosylation of lipids **2.35** and **2.36** with glycosyl halydes **2.44-2.46**

2.44 X=I; R_1=2,6-di-F-Bz
2.45 X=Br; R_1=2,6-di-F-Bz
2.46 X=Br; R_1=Ac

2.35 R_2=H,
R_3 = $(CH_2)_{16}CH_3$

NaOMe/ MeOH

2.37 R_1= 2,6-di-F-Bz, R_2=H, R_3 = $(CH_2)_{16}CH_3$, R_4= Ac
2.38 R_1= R_2= R_4= H, R_3 = $(CH_2)_{16}CH_3$
2.39 R_1= R_4=Ac, R_2=H, R_3 = $(CH_2)_{16}CH_3$

2.40 R_1=2,6-di-F-Bz R_4=Ac
2.41 R_1= R_4=Ac

2.36 R_2= $(CH_2)_{17}CH_3$,
R_3 = $(CH_2)_{14}CH_2N_3$

NaOMe/ MeOH

2.42 R_1= 2,6-di-F-Bz, R_2= $(CH_2)_{17}CH_3$, R_3 = $(CH_2)_{14}CH_2N_3$, R_4= Ac
2.43 R_1= R_4= H, R_2= $(CH_2)_{17}CH_3$, R_3 = $(CH_2)_{14}CH_2N_3$

Entry[a]	Donor (eq)	Acceptor[b] (eq)	Promoter (eq)	T (°C)	t (h)	Reaction product	Yield[c] (%)
1[d]	**2.44** (1)	**2.35** (1.1)	TBAI (0.3)	reflux	15	**2.40**	89
2	**2.44** (1)	**2.35** (1.1)	AgOTf (3)	rt	24	**2.40**	87
3[d]	**2.45** (1)	**2.35** (1.2)	TBAI (0.3)	80	24	**2.40α**	80
4	**2.45** (1)	**2.35** (1.2)	AgOTf (3)	rt	24	**2.37**	35[e,f]
						2.40α	54
5	**2.45** (1)	**2.35** (1.2)	Hg(OTf)$_2$ (3)	rt	24	**2.37**	30[e,f]
						2.40α	57
6	**2.45** (1.2)	**2.35** (1)	AgOTf/Sn(OTf)$_2$ (3:3)	rt	24	**2.37**	53
7	**2.45** (1.2)	**2.35** (1)	AgOTf/Sn(OTf)$_2$ (3:3)	rt	24	**2.38**	90[g]
8	**2.46** (1.2)	**2.35** (1)	AgOTf/Sn(OTf)$_2$ (3:3)	rt	24	**2.38**	42[g]
9	**2.45** (1.2)	**2.36** (1)	AgOTf/Sn(OTf)$_2$ (3:3)	rt	40	**2.43**	8[g]
10	**2.45** (1.2)	**2.36** (1)	AgOTf/Sn(OTf)$_2$ (3:3)	40	24	**2.43**	63[g]

[a] General conditions: Promoter was added to a mixture of donor, tributylstannyl acceptor, and 4 Å MS in CH_2Cl_2 the solvent unless otherwise indicated. [b] *In situ* tributylstannyl alkoxide formation prior to glycosylation reaction. [c] Isolated yield. [d] Toluene as a solvent. [e] Total conversion. [f] Orthoester formation. [g] Overall yield for the two steps.

Alternatively, fully acetylated glycosyl bromide **2.46** was also used. In this case, the obtained yield 42% after deprotection of glycosylated product (Table 2.2., entry 8) was not as good as that by using 2,6-difluorobenzoate at position 2 (90%, Table 2.2., entry 7), showing again the effectiveness of using this disarmed glycosyl donor in the glycosylation reaction of stannylamide derivative **2.35**.

In order to test our methology to glycosylate more complex ceramides, the mixture Ag(OTf)/Sn(OTf)$_2$ was used starting from glycosyl bromide **2.45** and stannyl ether **2.36**. A low b 8% yield was obtained when the reaction was performed at room temperature during 40 h of reaction (Table 2.2., entry 9). However, the yield was improved by heating at 40 ºC during 24 h, although tedious purification of the deprotected product **2.43** hampered the achievement of a remarkable isolated yield (63% over two steps).

2.2.4. NMR studies on the glycosylation of stannylceramides promoted by TfOH

In the context of our study of stannylceramide glycosylation using 2,6-difluorobenzoyl glycosyl donors, one of our objectives was the possible detection of intermediates during the course of the reaction in such acidic conditions. In this sense, low-temperature NMR has been shown to be a powerful tool for monitor glycosylation process.[36] In particular, the logical use of ^{19}F NMR for the detection of anomeric triflates has been traditionally hampered by problems associated to the overlapping of such signals with those of the corresponding promoters (tipically AgOTf, TfOH, etc.) which avoids a reliable assignment of the triflate resonances and may lead to a wrong analysis.

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[36] (a) Kim, J.-H.; Yang, H.; Park, J.; Boons, G.-J. *J. Am. Chem. Soc.* **2005**, *127*, 12090-12097. (b) Callam, C. S.; Gadikota, R. R.; Krein, D. M.; Lowary, T. L. *J. Am. Chem. Soc.* **2003**, *125*, 13112-13119. (c) Nokami, T.; Shibuya, A.; Tsuyama, H.; Suga, S.; Bowers, A. A.; Crich, D.; Yoshida, J. *J. Am. Chem. Soc.* **2007**, *129*, 10922-10928. (d) Honda, E.; Gin, D. Y. *J. Am. Chem. Soc.* **2002**, *124*, 7343-7352. (e) Liu, J.; Gin, D. Y. *J. Am. Chem. Soc.* **2002**, *124*, 9789-9797. (f) Garcia, B.; Gin, D. Y. *J. Am. Chem. Soc.* **2000**, *122*, 4269-4279. (g) Gildersleeve, J.; Pascal, R. A., Jr.; Kahne, D. *J. Am. Chem. Soc.* **1998**, *120*, 5961-5969. (h) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217-11223. (i) Zeng, Y.; Wang, Z.; Whitfield, Huang, X. *J. Org. Chem.* **2008**, *73*, 7952-7962.

Therefore, ^{19}F NMR technique was chosen to perform this study because we anticipate that the presence of a fluorinated protecting group on glycosyl donor **2.27** may offer the possibility of exploiting the new ^{19}F NMR ressonaces in 2,6-di-F-Bz (-89 to -110 ppm for 2,6-di-F-Bz *versus* -74 to -78 ppm for triflates and triflic acid) of such intermediates and following its changes during the course of the reaction.

2.2.4.1. ^{19}F NMR monitoring of glycosylation reaction

In order to perform the study, the reaction was cooled immediately to -78º C upon addition of TfOH (3 eq.) to the mixture of products **2.27** and **2.35** in CDCl₃. ^{19}F NMR spectrum was recorded at that temperature but the signal associated to glycosyl donor **2.27** at δ -110.32 ppm did not suffer any change in chemical shift after 5 minutes of reaction. The sample was warmed to 20º C but neither change was detected after 5 minutes (Scheme 2.7.). This lack of reactivity at low temperature is reasonable taking into account that *O*-acetyl group is not usually a good leaving group in glycosylation reactions.
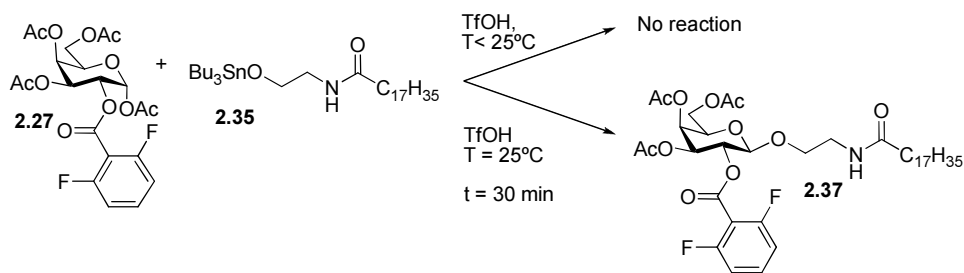

Scheme 2.7. Glycosylation of lipid **2.35** with glycosyl donor **2.27** with TfOH

^{19}F NMR experiment was repeated next at 25º C (Scheme 2.8.). After 5 minutes, the fluorine signal of the 2,6-difluorobenzoate group at δ -110.32 ppm disappeared, and two new peaks at δ -89.20 and -89.45 ppm started to appear. Those picks were tentatively attributed to the triflates **2.27a** and **2.27b,** respectively. However, the intensity of the signal at δ -89.20 was increasing, while the signal at δ -89.45 was reducing after 10 min of reaction. After 30 minutes of reaction, the signal at δ -89.45 disappeared completely and the signal

at δ -89.20 reached its maximum intensity. No signal at δ -112.0 corresponding to glycosylated compound **2.37** was observed during the experiment. Consequently, it was reasoned that the signal at δ -89.20 ppm could come from glycosylated compound protonated under the strong acid conditions of reaction (Scheme 2.8., product **2.37a**). To test this hypothesis, the reaction mixture was submitted to a basic work up. After that, the formation of the glycosylated compound was confirmed by means of the appearance of the known signal at δ -112.0 ppm in the ^{19}F NMR (Scheme 2.8., product **2.37**). Thus, on the basis of this NMR experiment, it is reasonable to think that the signal at δ -89.20 may be associated to glycosylated compound in acid conditions but the nature of the intermediate which fluorine signal appears at δ -89.45 ppm should be determined.

Regrettably, ^{1}H NMR and ^{13}C NMR experiments resulted not suitable to follow the course of the glycosylation reaction because TfOH environment not only decreased the sensibility of signals in the complex spectra obtained but it was also responsible for the decomposition during the same time frame (30 min).
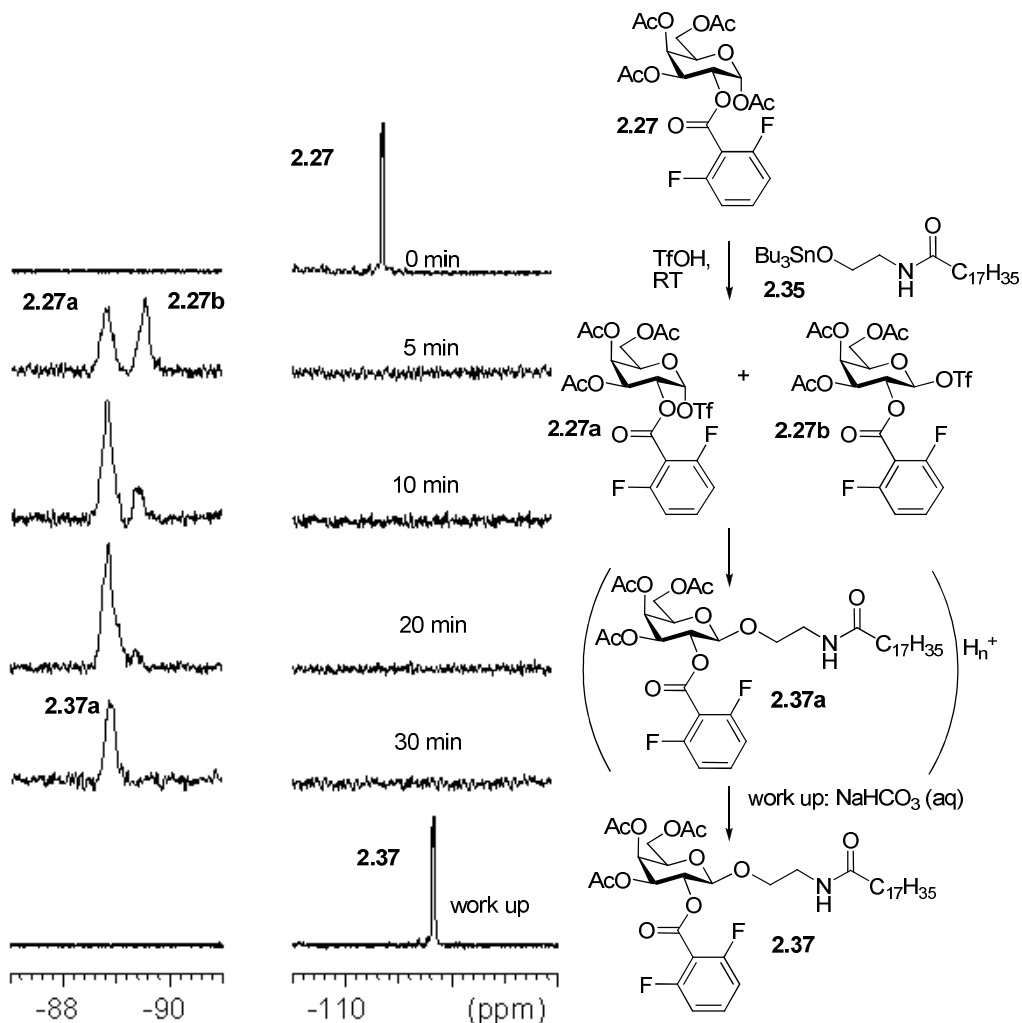
Scheme 2.8. Observation of anomeric triflates in acidic medium

Crich and Sun[36h] discovered that a mixture of an α/β glycosyl triflate (**2.48** and **2.49**), instead of dioxocarbenium ion, was formed during preactivation of peracetylglucosyl sulfoxide (**2.47**) in presence of Tf$_2$O and DTBMP in CD$_2$Cl$_2$ at low temperature (Scheme 2.9., A). In this case the two triflate species showed only one signal in the ^{19}F NMR spectrum but eight resonance signals corresponding to carbonyl carbons were detected at ^{13}C NMR. No evidence was found for dioxolenium carbons. On the other hand, the group of Huang[36i] observed the formation of α-glycosyl triflate (**2.48**) and dioxolenium ion (**2.50**) intermediates from glycosyl donors like peracetylglucosyl sulfides using the

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promoter system p-TolSCl/AgOTf but when the glycosyl donor contained multiple electro-withdrawing groups such perbenzoylgalactosyl sulphide (**2.51**), the triflate (**2.52**) was observed as a major product under the same conditions. Interestingly, the dioxolenium ion (**2.54**) was the major intermediate with a donor bearing electron-donating protective groups such as 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl derivatives under the same reaction conditions (Scheme 2.9., B)

Taking into account these precedents and according to the deactivating properties of our glycosyl donor, and the strong TfOH acid conditions[37] which may avoid the orthoester stabilization, the formation of glycosyl triflate intermediates may be reasonable in our case.
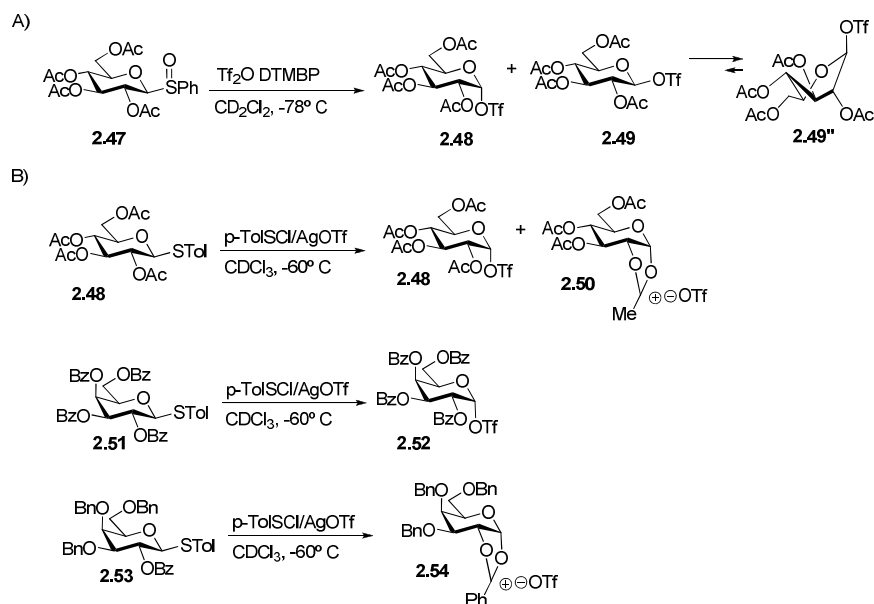

Scheme 2.9. Identification of triflate and dioxolenium intermediates by Crich (A)[36h] and Huang (B)[36i]

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[37] Banoub, J.; Bundle, D. R. *Can. J. Chem.* **1979**, *57*, 2091-2097.

2.2.4.2. Elucidation of glycosylation intermediates in presence of TfOH

To confirm the formation of the triflate species, the donor **2.27** in presence of TfOH in dry CD_2Cl_2 was studied by ^{19}F NMR spectroscopy. The ^{19}F NMR spectrum (Scheme 2.10.) showed two signals at the same chemical shift than those observed in the first experiment (compare Scheme 2.10. and Scheme 2.8. after 5 min) and a signal at -1.2 ppm corresponding to the fluorine atoms of the bonded triflate group (data not showed)
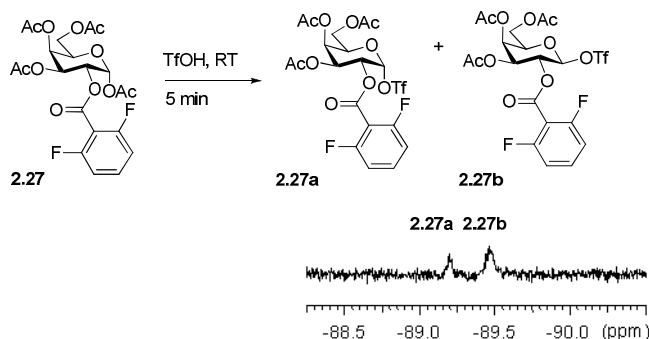

Scheme 2.10. Observation of anomeric triflates in acidic medium

This result suggested that during the glycosylation reaction (Scheme 2.8.) an α/β glycosyl triflate mixture is formed, showing two signals at δ -89.20 and -89.45 ppm in the ^{19}F NMR spectrum. This mixture rearranged to the more stable α-glycosyl triflate during the data experiment accumulation, with concomitant formation of protonated glycosylated product. The signals corresponding to fluorine atoms of difluorobenzoyl protecting group for the protonated product (**2.37a**) and the α-glycosyl triflate (**2.27a**) are overlapped (Scheme 2.9.).

It would be desirable to characterize the intermediates by ^1H and ^{13}C NMR to confirm their existence as triflates. Thus, we decided to carry out the addition of TfOH (3 eq.) to the solution of **2.27** in dry $CDCl_2$ at room temperature for 5 min to ensure the formation of the triflates and immediately freezing them at -78º C to start the acquisition. Moreover, the following NMR experiments were done in a 600 MHz NMR instrument in order to increase the sensitivity and to improve the resolution of the signals because the acid conditions make bands broader.

Initially, the sample was cooled to -78 ºC after TfOH activation. However, no ^{1}H NMR signal of the carbohydrate was observed because of the low solubility of the compounds in the cooled CD_2Cl_2. Several attempts to record a ^{1}H NMR spectra were carried out at higher temperatures (-40 ºC and -20 ºC) but it was only possible at 0 ºC. Fortunately, the mixture of triflates was stable enough to permit most of routine NMR experiments: ^{1}H, ^{13}C, COSY, HSQC, decoupled HMQC and HBMC.

Figure 2.2. shows the ^{1}H NMR spectra recorded at 0 ºC after 5 min to 4, 8, 12 and 20 h of addition of TfOH. It is appreciable the initial formation of triflates and a progressive decomposition through the pass of time.
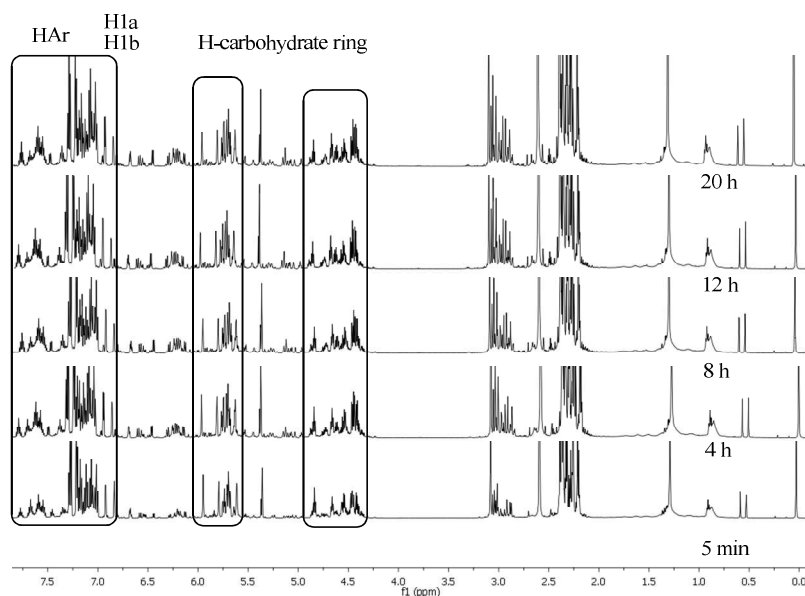

Figure 2.2. Monitorization of triflate mixture at 0º C for 20h by ^{1}H NRM. Diagnostic signals are highlighted to appreciate the relative stability of the intermediates

Acidic conditions dramatically influence the proton chemical shift and in the resolution of the signals because most of the signals are downfield around 1 ppm respect to the starting material **2.27** at neutral conditions and signals are quite broad. Thus, 2-D experiments are indispensable to establish a confident correlation of the signals. Figures 2.3. and 2.4. show ^{1}H and ^{13}C NMR spectra of diagnostic signals of triflates **2.27a** and **2.27b.** Figures 2.5 to 2.7 show COSY, HSQC, HMBC spectra for the intermediates. Signals in the ^{1}H and ^{13}C NMR

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spectra assigned taking into account the structural information from these bidimensional spectra.
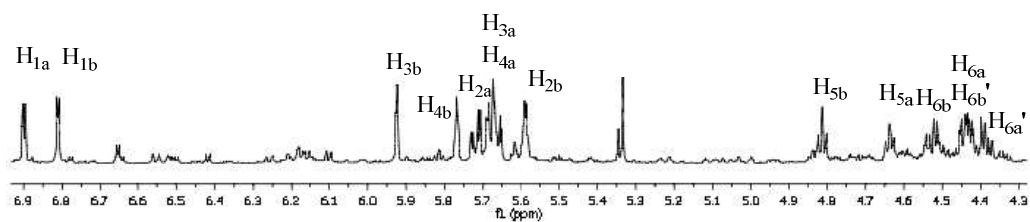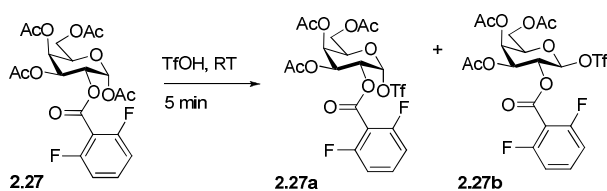
Figure 2.3. Selected signals from ^{1}H NMR experiment of intermediates **2.27a** and **2.27b**
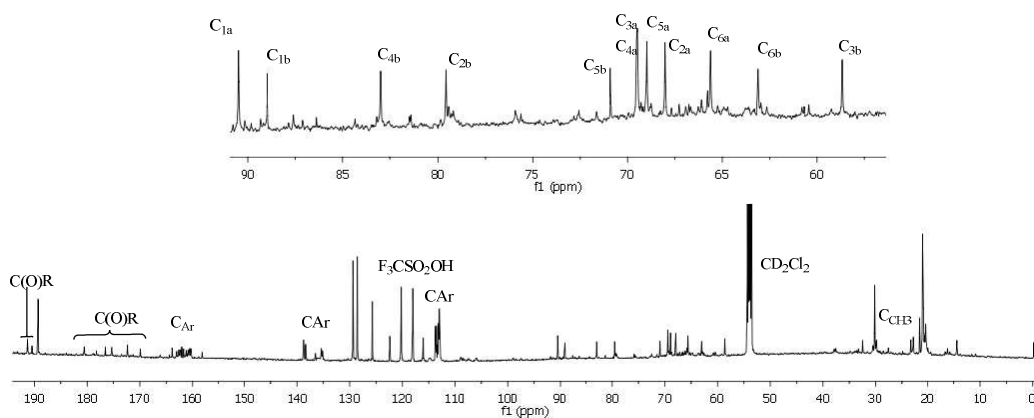
Figure 2.4. Selected signals from ^{13}C NMR experiment of intermediates **2.27a** and **2.27b**
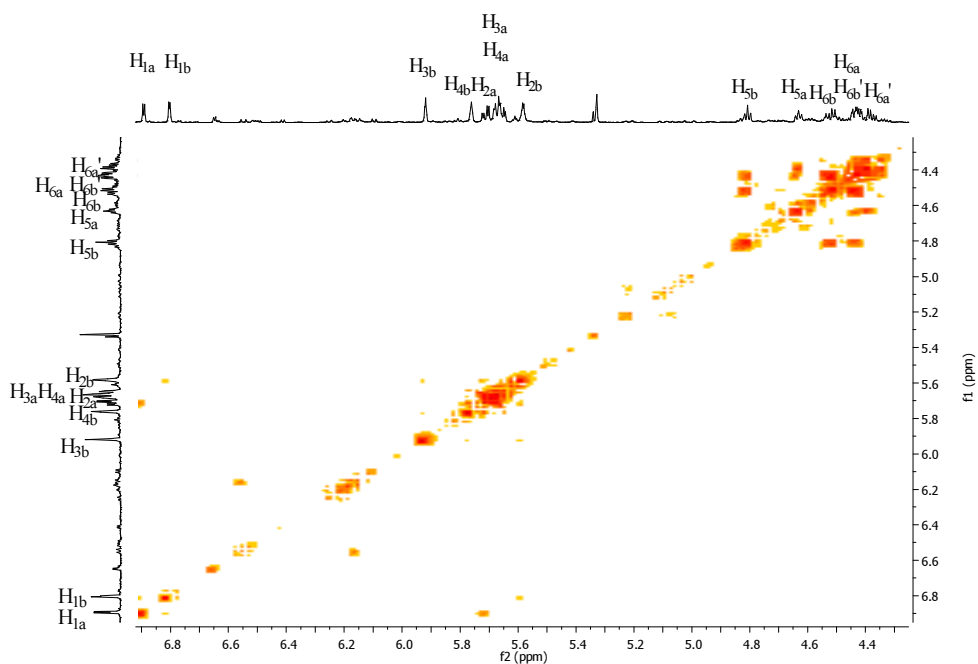
Figure 2.5. COSY NMR experiment of intermediates **2.27a** and **2.27b**

Figure 2.6. HSQC NMR experiment of intermediates **2.27a** and **2.27b**

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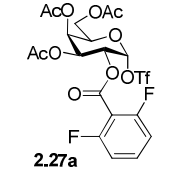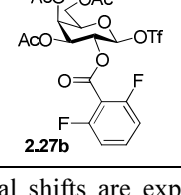
Figure 2.7. HMBC NMR experiment of intermediates **2.27a** and **2.27b**
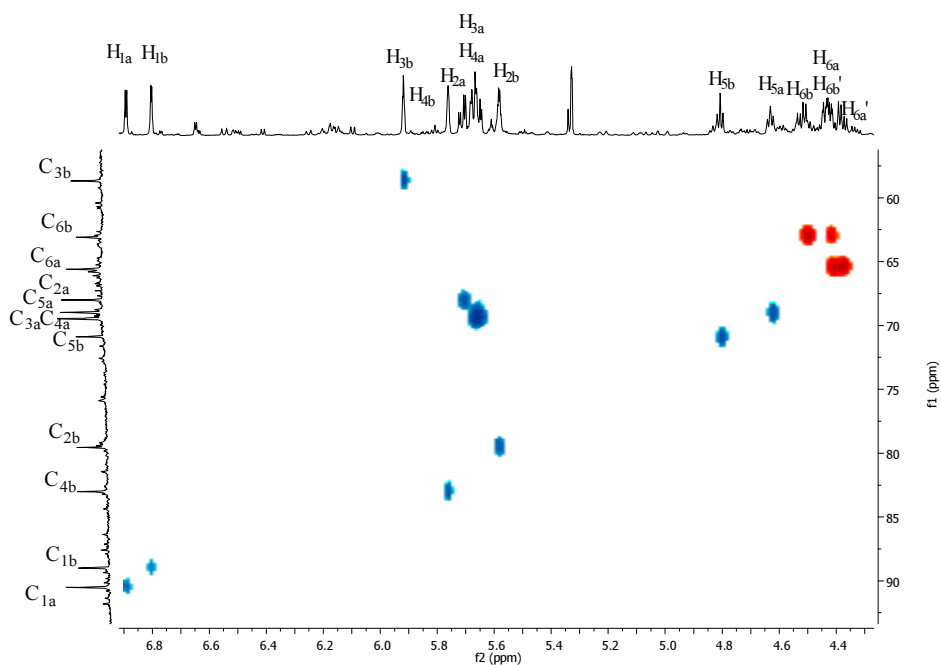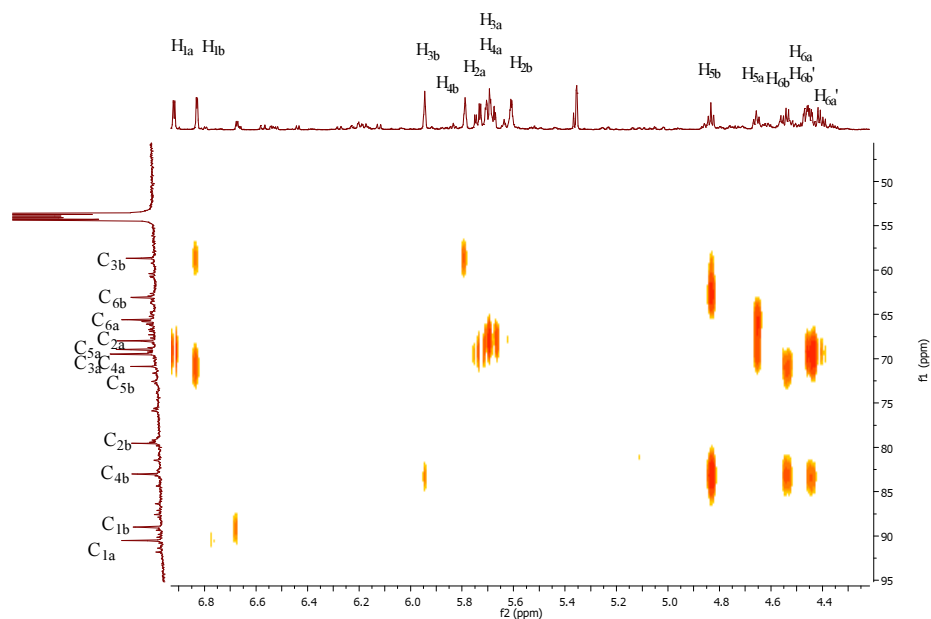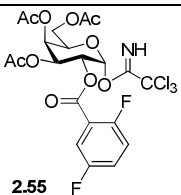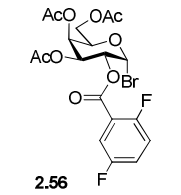
Although both anomeric protons (δ 6.89 ppm for isomer **2.27a** and 6.80 ppm for isomer **2.27b,** Figure 2.3.) appear at higher chemical shifts than described for some analogues in the literature (δ 6.40 ppm and 5.30 ppm of the anomeric protons for a α/β mixture tetraacetylglucopyranosyl triflate),[36h] The HSQC experiment (Figure 2.6.) showed that they correlate with carbons at 90.5 and 90.0 ppm, which clearly indicated that those signals correspond to anomeric protons. Their higher chemical shift must be caused by the low pH.

In general, values of ^{1}H chemical shift (δ) and coupling constants $^3J_{1,2}$ (3.6 Hz) and $^3J_{2,3}$ (10.8 Hz) for intermediate **2.27a** are in agreement with an α-glycosyl donor in $^4C_1$ chair conformation (Compare Table 2.3., Entries 1, 2 and 3). However, the small value of $^3J_{1,2}$ for **2.27b** did not fit with the expected for a β-glycosyl derivative which would be expected to be of 8-10 Hz instead of 2.0 Hz (Table 2.3., Entry 4).

Table 2.3. Comparison of selected data from ^{1}H NMR for glycosyl donors **2.27a, 2.27b, 2.55** and **2.56**

Entry[a]	Compound		H-1	H-2	H-3	H-4	H-5	H-6/H-6'
1[b]	**2.55**	δ	6.74		5.70-5.55		4.51	4.24-4.08
			(d)		(m)		(t)	(m)
		[3]J	2.2		n.d.		6.6	n.d.
2[b]	**2.56**	δ	6.80	5.30	5.60-5.55		4.54	4.25-4.09
			(d)	(dd)	(m)		(t)	(m)
		[3]J	3.8	2.2	n.d.		6.6	n.d.
				9.6				
3	**2.27a**	δ	6.89	5.71	5.68-5.66	5.68-5.66	4.64	4.46-4.38
			(d)	(dd)	(m)	(m)	(t)	(m)
		[3]J	3.6	3.6	n.d.	n.d.	6.6	n.d.
				10.8				
4	**2.27b**	δ	6.80	5.58	5.92	5.76	4.81	4.54-4.45
			(d)	(d)	(s)	(s)	(t)	(m)
		[3]J	2.4	1.8	-	-	6.0	n.d.
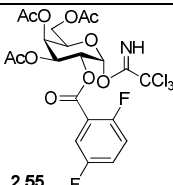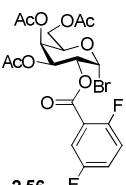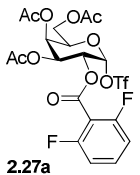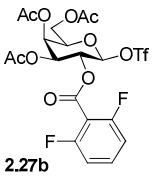
[a] Chemical shifts are expressed in ppm and coupling constants are expressed in Hz. [b] Data extracted from reference 25. n. d.: not determinable.

In the interpretation of the ^{13}C NMR spectra (Figure 2.4.), anomeric carbon signals were identified at δ 90.5 ppm for **2.27a** and 90.0 ppm for **2.27b** by HSQC NMR experiment (Figure 2.6.) as was commented previouly**.** Interestingly, The comparison of our intermediates with other galactosyl donors showed values of chemical shift at C-1 and C-2 by ^{13}C NMR, similar to those observed for intermediate **2.27a** (Table 2.4., Entry 3). In the case of **2.27b,** their δ values for C-2, C-3 and C-4 suffer a strong deviation compared to those of other glycosyl donors (Table 2.4., Entry 4).

Non-decoupled HMQC experiment was carried out to study the $^1J_{H1C1}$ for both isomers. In both cases $^1J_{H1C1}$ was higher than 180 Hz ($^1J_{H1C1}$ = 184 Hz for **2.27a**

and $^1J_{H1C1}$ = 189 Hz for **2.27b**) which was indicative that the H_1 was adopting an equatorial disposition towards the anomeric carbon. This observation was also in agreement with the $^3J_{1,2}$ on ^{1}H NMR for both compounds showed before in Table 2.3.

Table 2.4. Comparision of chemical shift of glycosyl donor by ^{13}C NMR

Entry[a]	Donor	C-1	C-2	C-3	C-4	C-5	C-6
1[b]	**2.55**	93.6	68.2	67.7	67.8	69.5	61.5
2[b]	**2.56**	87.8	68.2	71.5	69.0	67.3	61.0
3	**2.27a**	90.5	68.0	69.6	69.5	68.9	65.6
4	**2.27b**	90.0	79.5	58.7	83.0	70.8	63.1

[a] Chemical shifts are expressed in ppm. [b] Data extracted from reference 25

According to the observations by Crich and coworkers,[36h] we considered reasonable that a change of the chair conformation could explain why α and β mixture of anomers can be associated to the high values of both $^1J_{C1H1}$. According to the other spectroscopic data, structure **2.27a** could be attributed to the more stable α isomer in a $^4C_1$ chair conformation, whereas structure **2.27b** could be attributed to the less stable β isomer in which conformation has changed adopting the anomeric proton an equatorial disposition via a $^1C_4$ chair conformation or a skew or boat (**2.27b'**) in a similar way as observed by of Crich. However, it could be also attributed to the corresponding α-dioxolenium

intermediate in a conventional $^4C_1$ chair (**2.27b''**). In order to test this possibility, we analysed the coupling constant patern. The small $^3J_{2,3}$ of **2.27b** (2.4 Hz) supports better a β-triflate with a $^1C_4$ conformation (**2.27b'**) than a dioxocarbenium intermediate (**2.27b''**) (Scheme 2.11.).
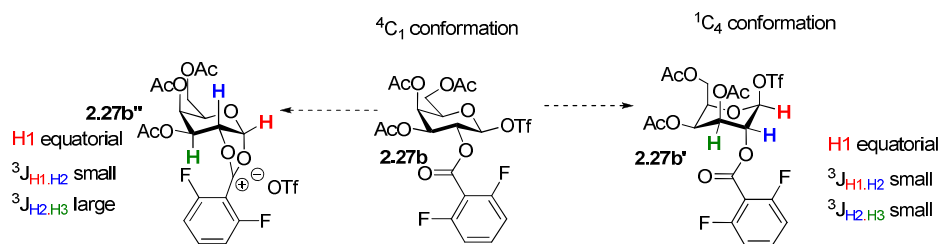
Scheme 2.11. Possible structures for a better description of **2.27b**

In order to support **2.27b'** structure, the coupling constants afforded by ^{1}H NMR were compared to the coupling constants simulated for a β triflate with $^1C_4$ configuration. Such structure was represented with the software ChemBio3D Ultra 11.0 and it was optimized employing molecular dynamics MM2 (Figure 2.8.).
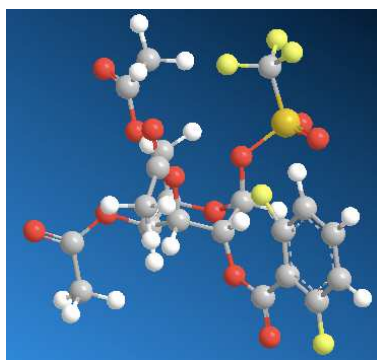
Figure 2.8. Optimized structure of **2.27b'** employing molecular dynamics MM2

These calculations provided the dihedral angles of all the hydrogen in the molecule which were exported to the software MestreJ.[38] This program displayed the teorical $^3J_{HH}$ according to the dihedral angle observed for the two hydrogen studied. The software offered different approximations like traditional

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[38] Navarro-Vazquez, A.; Cobas, J. C.; Sardina, F. J.; Casanueva, J.; Díez, E. *J. Chem. Inf. Comput. Sci.* **2004**, *44,* 1680-1685.

Karplus equation and newer approximations that consider the stereoelectronic effects of the closest substituents. Table 2.5. collects the $^3J_{H,H}$ according the Karplus equation and the Haasnoot-Altona-de Leeuw which considers the effect of electronegativity of the subtituents.

Table 2.5. Comparison of experimental and simulated $^3J_{H,H}$ for **2.27b'** in a $^1C_4$ chair conformation

Entry	J_{Hx-Hy}	Dihedral angle	^3J Karplus	^3J HLA	^3J experimental
1	J_{H1-H2}	67.83°	2.09	2.20	2.40
2	J_{H2-H3}	-76.29°	1.58	1.83	1.80
3	J_{H3-H4}	-49.50°	3.96	3.63	-
4	J_{H4-H5}	61.39°	2.65	2.77	-
5	J_{H5-H6}	-114.32°	3.17	1.16	6.0
6	$J_{H5-H6'}$	121.42°	4.08	3.47	n.d.

n.c.: not determinable.

Experimental $^3J_{1,2}$ and $^3J_{2,3}$ fitted well with the corresponding J calculated with both Karplus and HLA methods (Table 2.5., Entries 1 and 2). However, in the case of $^3J_{3,4}$ and $^3J_{4,5}$, the calculated J were slightly higher than the observed for **2.27b'** which were actually zero because the signals of H_3 and H_4 were actually singlets (Table 2.5., Entry 3 and 4). This observation could be explained if **2.27b'** behaved as a distorted $^1C_4$ chair. This idea would be in agreement with Crich's group observation for their β-tetraacetylglucosyl triflate with equatorial configuration of H-1.[36h] They proposed a $^1S_5$ twist boat conformation in which the triflate group assume a (pseudo)axial position as a consequence of the strongly electronegative nature of the triflate group and the anomeric effect[36h,39] (Scheme 2.12.). This conformation would also permit explaining other small coupling constants.
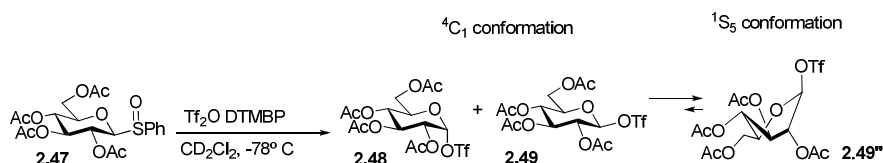

Scheme 2.12. Postulation of $^1S_5$ conformation for the β-triflate III' proposed by Crich

[39] Hall, L. D. *Can. J. Chem.* **1969**, *47*, 1-17.

In order to refine our proposal of a distorted $^1C_4$ chair conformation for **2.27b'**, NOESY experiment was also performed. Unfortunately, the long time acquisition of this experiment was not compatible with the inherent time-dependent decomposition of the glycosyl triflates along time, even though the experiment was performed a 0 ºC. Hence, the noise of the experiment interfered with a confident interpretation of the picks.

The obtention of the mixture of α-glycosyl triflate with a chair conformation $^4C_1$ (**2.27a**) and β-glycosyl triflate with a distort chair $^1C_4$ (**2.27b'**) explains the spectroscopic data commented previously for the activation of the disarmed galactosyl donor **2.27** (Scheme 2.13.).
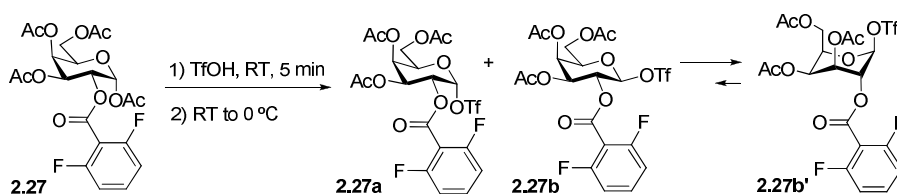
Scheme 2.13. Triflate intermediates proposed after activating **2.27**

To the best of our knowledge, this is the first evidence of the formation of glycosyl triflates intermediates under proton acid mediated glycosylations with disarmed glycosyl donors with a 2,6-diFBz group at C-2. On the other hand, the obtained results point out that the exclusive formation of β-glycosylated products using a highly disarmed glycosyl donor such as **2.27** when activated with TfOH may be in accord with a S_N2-like mechanism.

In conclusion, the use of highly disarmed glycosyl donors (**2.27** and **2.45**) allows for a direct glycosylation of stannylceramides (**2.35** and **2.36**) reducing the overall number of synthetic steps and providing access to β-glycolipids in a good yield and with complete chemo- and stereoselectivity.

The time-course of the reaction studied by ^{19}F NMR between highly disarmed glycosyl donor **2.27** and tributylstannyl acceptor **2.35** provides the first evidence

for the formation of glycosyl triflates as reaction intermediates in acidic medium. Moreover, ^{1}H, ^{13}C and bidimensional NMR spectroscopy at 0 ºC afforded more information about their configurations, which allowed to propose a ^{4}C$_1$ conformation for the α-triflate **2.27a** and a distorted chair for the β-triflate **2.27b'**.

2.3. Experimental Section

General Remarks: All reactions were conducted under a dried argon stream. CH$_2$Cl$_2$ (99.9%) was purchased in capped Pure Solv System-4$^®$ bottles and used without further purification and stored under argon. Toluene was purchased as a synthesis grade reagent from Scharlab$^®$. It was fleshly distilled over sodium and benzophenone under argon atmosphere every time it was required. Yields refer to the chromatographically and spectroscopically (^{1}H and ^{13}C) homogeneous materials. All other solvents and reagents were used without further purification. All glassware utilized was flame-dried before use. Reactions were monitored by TLC carried out on 0.25-mm E. Merck silica gel plates. Developed TLC plates were visualized under a short-wave UV lamp and by heating them after dipping in ethanol/H$_2$SO$_4$ (15:1). Flash column chromatography (FCC) was performed using flash silica gel (32-63 μm) and employed a solvent polarity correlated with TLC mobility. Optical rotations were measured at 598 nm on a Jasco DIP-370 digital polarimeter using a 100 mm cell. NMR experiments were conducted on a Varian 400 MHz instrument using CDCl$_3$ (99.9% D) as the solvent, with chemical shifts (δ) reference to internal standards CDCl$_3$ (7.26 ppm ^{1}H, 77.23 ppm ^{13}C) or Me$_4$Si as an internal reference (0.00 ppm). Chemical shifts are relative to the deuterated solvent peak and are in parts per million (ppm).

Activation of MSAW3000: Molecular sieves AW-300 (MSAW300) purchased from Aldrich (lot no. 04024CI) were ground and activated by heating over 200 °C under reduced pressure 12 h.

General glycosylation procedure for 1-*O*-acetylglycosides

The following protocol was followed prior to the glycosylation reaction. The glycosyl acceptor and the glycosyl donor were azeotroped out with dry toluene (3 x 5 mL) each one in independent dried flasks. After that, they were placed under vacuum for 1h.

To a stirred mixture of amidoalcohol (0.11 mmol), and 4-Å molecular sieves (36 mg) in dry CH_2Cl_2 (440 μL) under argon atmosphere at room temperature allyltributyltin (0.14 mmol) and TfOH (0.03 mmol) were added.

After stirring for 2 h, glycosyl donor (0.14 mmol) and more TfOH (0.30 mmol) were added to the mixture which was stirred for 0.5 h. The mixture was diluted with AcOEt (15 mL) and then washed with a sat. aq. $NaHCO_3$ (2 x 5 mL). The aqueous layer was extracted with AcOEt (3 x 15 mL), and the combined organic layers were washed with brine (7 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo* to yield a crude oil which was purified by column chromatography or submitted to subsequent hydrolysis.

General deprotection procedure

The hydrolysis of the glycosylation crude was carried out by addition of a 7% solution of MeONa in MeOH/CH_2Cl_2 (3:1, 5 mL/mmol glycosyl donor) at room temperature for 5 h. The solvent was removed under *vacuo*, and the resulting residue was purified by silica gel chromatography.

General glycosylation procedure for glycosyl halides

The following protocol was followed prior to the glycosylation reaction: glycosyl acceptor and glycosyl donor were separately dried by co-destillation with toluene (3 x 5 mL) with activated 4 Å molecular sieves in dried flasks. Then, they were placed under vacuum for 1 h, and after that, the flasks were placed in a dessicator under vacuum for 1 h. Complete water exclusion is crucial to achieve good yields.

A mixture of the corresponding amidoalcohol (0.11 mmol) and bis-(tri-n-butyltin) oxide (0.152 mmol) in 20 ml of dry toluene, was heated to reflux and was subjected to azeotropic dehydration using a Dean-Stark system or 4 Å molecular sieves overnight. Removal of solvent under reduced pressure afforded the stannyl ether, which was used for the glycosylation without further purification.

The promoter in a dried flask with a magnetic stirring bar and protected from the light was azeotroped with dry toluene (2 x 5 mL). Activated 4 Å molecular sieves (330 mg/mmol glycosyl donor o acceptor) were added to the flask, and the mixture was azeotroped with toluene once more (5mL) before placed under vacuum for 1 h.

A solution of stannyl ether (0.11 mmol) in dry CH_2Cl_2 (4 mL) and a solution of glycosyl halide (0.14 mmol) in dry CH_2Cl_2 (4 mL) were added to the promoter-molecular sieves mixture *via* syringe under argon atmosphere at room temperature. The mixture was stirred in the dark until no changes were observed by TLC. The mixture was diluted with AcOEt (12 mL), filtered through a pad of *Celite*, and rinsed with AcOEt (10 mL). Removal of the solvent under reduced pressure afforded the reaction crude wich was purified by column chromatography or submitted to subsequent hydrolysis.

1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,6-difluorobenzoyl)-α-D-galactopyranose (2.27)
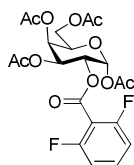
To a solution of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**2.25**)[30] (700 mg, 2.01 mmol) in dry pyridine (8 mL), 4-(dimethylamino)pyridine (49 mg, 0.402 mmol) was added and the solution was stirred at r.t for 30 min. 2,6-Difluorobenzoyl chloride (**2.26**) (0.5mL, 4.02 mmol) was added dropwise over a 10 min period and the stirring was continued for 18 h. The reaction was quenched by addition of methanol (4 mL) and after stirring for 1 h, the solution

was diluted with CH_2Cl_2 (120 mL) and washed with water (150 mL). The aqueous phase was extracted with CH_2Cl_2 (50 mL) and the combined organic phases were dried ($MgSO_4$) and evaporated to dryness. After purification by silica gel chromatography (AcOEt/hexanes 1:8), **2.27** was obtained as white solid (971 mg, 99%). Rf (AcOEt/hexane 1:1): 0.70; mp: 69-70 ºC; $[\alpha]_D^{25}$ = +106.9º, (c = 1.01, CH_2Cl_2); ^{1}H-$\{^{19}$F$\}$ NMR (400 MHz, $CDCl_3$) δ in ppm: 7.44 (at, $J_{Ar3,Ar4}$ = 8.4 Hz, 1H, H-Ar4), 6.90 (t, $J_{Ar3,Ar4}$ = 8.4 Hz, 2H, H-Ar3), 6.50 (d, $J_{1,2}$ 3.6 Hz, 1H, H-1), 5.52 (dd, $J_{3,2}$ = 10.4 Hz, $J_{3,4}$ = 2.4 Hz, 1H, H-3), 5.48 (d, $J_{4,3}$ = 2.4 Hz, 1H, H-4), 5.40 (dd, $J_{2,3}$ = 10.4 Hz, $J_{2,1}$ 3.6 Hz, 1H, H-2), 4.33 (t, $J_{5,6}$ = 6.8 Hz, 1H, H-5), 4.11-4.02 (m, 2H, H-6a, H-6b), 2.13, 2.07, 1.97, 1.94 (s, 12H, 4CH_3CO); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 170.3 (CO), 170.1 (CO), 170.1(2·CO), 168.9 (CO), 160.8 (dd, $J_{C,F}$ = 257.0 Hz, 2C, C-Ar2), 160.4 (C-Ar1), 133.7 (t, $J_{C,F}$ = 10.6 Hz, 1C, C-Ar4), 112.2 (dd, $J_{C,F}$ = 22.0 Hz, $J_{C,F}$ = 3.0 Hz, 2C, C-Ar3), 89.0 (C-1), 68.6 (C-2), 67.8 (C-4), 67.5 (C-3), 67.2 (C-5), 61.1 (C-6), 20.7, 20.6, 20.5, 20.4 (CH_3); FT-IR (ATR) ν in cm^{-1}: 2916, 2848, 1739, 1626, 1592, 1471, 1369, 1287, 1249, 1213, 1142, 1104, 1060, 1008, 933, 795; HR ESI-TOF MS for [M + Na]$^+$ calc for $C_{21}H_{22}F_2NaO_{11}$: 511.3788; found 511.3795 [M +Na]$^+$.

16-azidohexadecanoic acid (2.29)
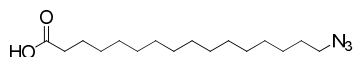
To a solution of 16-bromohexadecanoic acid **2.28** (1 g, 2.99 mmol) in freshly distilled DMF (50 mL) was added NaN_3 (1.94 g, 29.9 mmol) and a catalytic amount of 18-crown-6 (197 mg, 0.74 mmol), and the mixture was stirred at 90 °C for 72 h. The reaction mixture was poured into AcOEt-water (1:1, 80 mL) and the aqueous phase was extracted with AcOEt (3 x 20 mL). The organic phases were combined, washed with brine (3 x 20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using hexane/AcOEt (95:5) as the eluent to give **2.29** (799 mg, 90 %): Rf (Hexane/AcOEt 90:10): 0.70; mp: 45-46 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm 3.27 (t, $J_{16,15}$ = 7.2 Hz, 2H, H-16), 2.37 (t, $J_{2,3}$ =7.5 Hz, 2H, H-2), 1.62 (m, 4H, CH_2), 1.40-1.20 (m, 22H, CH_2); ^{13}C NMR (100.6

MHz, CDCl$_3$): δ 177.0 (CO), 51.4 (CH$_2$-N$_3$), 34.0 (C-2), 29.6 (CH$_2$), 29.5 (CH$_2$), 29.4 (CH$_2$), 29.2 (CH$_2$), 29.1 (CH$_2$), 29.0 (CH$_2$), 26.7 (CH$_2$), 24.6 (CH$_2$); FT-IR (ATR) ν in cm^{-1}: 3040, 2913, 2847, 2112, 1698, 1470, 1429, 1411, 1349, 1291, 1250, 943, 727; Anal. Calcd. for C$_{16}$H$_{31}$N$_3$O$_2$: C, 64.61; H, 10.51; N, 14.13. Found: C, 64.68; H, 10.52, N, 14.10.

Synthesis of *N*-(2-hydroxyethyl)stearamide (2.32)
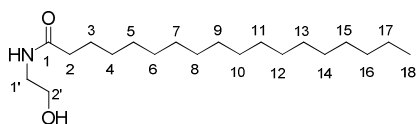
A solution of stearoyl chloride **2.31** (50 g 0.165 mol) in 250 mL of dry CH$_2$Cl$_2$ was cooled to 0°C. To this solution 2-amino ethanol **2.30** (100.78 g, 1.65 mol) was added in a slow drop-wise manner over a period of 30 min, resulting in the precipitation of a white solid. The reaction was stirred for 4 h at room temperature and then the mixture was filtered. The solid was washed with hexane and diethyl ether and dried *in vacuo*. The resulting crystalline material was recrystallized from CH$_2$Cl$_2$ to give 51.26 g of pure *N*-(2-hydroxyethyl)stearamide **2.32** (95%) as a white solid that was homogeneous: Rf (Hexane-AcOEt-MeOH 60:30:10) = 0.25; m.p. 53-55 $^\circ$C; ^{1}H NMR (400 MHz, CDCl$_3$): δ in ppm 5.94 (br s, 1H, OH), 3.72 (t, $J_{2',1'}$ = 5.2 Hz, 2H, H-2'), 3.42 (td, $J_{1',2'}$ = 5.2, 1.0 Hz, 2H, H-1'), 2.18 (t, $J_{2,3}$ = 7.2 Hz, 2H, H-2), 1.62 (quint, $J_{3,2}$ = 7.2 Hz, $J_{3,4}$ = 6.5 Hz, 2H, H-3), 1.28 (m, 28H, CH$_2$), 0.87 (t, $J_{18,17}$ = 6.8 Hz, 3H, H-18); ^{13}C NMR (100.6 MHz, CDCl$_3$): δ 172.6 (CO), 61.0 (C-2'), 41.6 (C-1'), 36.5 (C-2), 31.8 (CH$_2$), 29.5 (CH$_2$), 28.6 (CH$_2$), 25.6 (CH$_2$), 22.7 (CH$_2$), 14.1(C-18); FT-IR (ATR) ν in cm^{-1}: 3370, 3291, 3086, 2954, 2916, 2847, 1636, 1551, 1470, 1383, 1275, 1055, 720; Anal. Calcd. for C$_{20}$H$_{41}$NO$_2$: C, 73.34; H, 12.62; N, 4.28. Found: C, 73.30; H, 12.69; N, 4.20.

**Synthesis of *N*-(2-hydroxyethyl)-*N*-octadecylstearamide (2.34)**
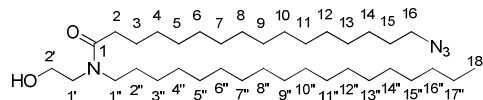
A 250 mL, three-necked, roundbottomed flask with a mechanical stirrer was fitted with a reflux condenser with drying tube, and a stoppered pressure-equalizing dropping funnel. The system was flushed with nitrogen or argon, and the flask was charged with 75 mL of dry THF and $LiAlH_4$ (1.45 g, 38.22 mmol). A mixture of 75 mL of THF and *N*-(2-hydroxyethyl)stearamide **2.32** (10 g, 30.58 mmol) was added, with stirring, at a rate sufficient to reach and maintain refluxing. After the addition was completed, the reaction mixture was kept boiling for 18 h. The flask was immersed in an ice bath, and 30 mL of water, 15 mL of 10% aqueous potassium hydroxide, and again 30 mL of water were added cautiously with very vigorous stirring. The reaction mixture was stirred for an additional 1h, filtered with suction, and the solid was washed with several 100-mL portions of ethyl acetate. The two layers were separated, and the aqueous phase was extracted with ethyl acetate (3 x 100 mL). The combined organic layers were dried over anhydrous sodium sulphate and concentrated *in vacuo* to get crude residue of 2-(ocatadecylamino) ethanol **2.33** as a white solid (6.7 g).

A solution of 2-(octadecylamino) ethanol **2.33** (1 g, 3.194 mmol), HOBt (586 mg, 3.832 mmol), EDC (734 mg, 3.832 mmol) and DIPEA (494 mg, 3.832 mmol) in 30 mL of dry CH_2Cl_2 was cooled to 0 $^{\circ}$C. Stearic acid (908 mg, 3.194 mmol) in 20 mL of dry CH_2Cl_2 was added drop wise over 6 h at 0 $^{\circ}$C and then the reaction was stirred under argon for 18 h at room temperature. The mixture was diluted with ethyl acetate (75 mL) and washed successively with HCl (10 % aqueous, 2 x 30 mL), $NaHCO_3$ (7 % aqueous, 2 x 30 mL), K_2CO_3 (7% aqueous, 2 x 30 mL) and brine (3 x 30 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using hexane/AcOEt/MeOH (85:10:5) as the eluent to give 1.67 g of pure *N*-(2-hydroxyethyl)-*N*-octadecylstearamide **2.34** as a waxy solid (80 % over two steps): Rf (Hexane/AcOEt/MeOH 60:30:10): 0.70; m.p. 50-52 $^{\circ}$C; ^{1}H NMR (400 MHz, CDCl$_3$): δ in ppm 3.75 (t, $J_{2',1'}$ = 5.2 Hz, 2H,

H-2'), 3.52 (t, $J_{1',2'}$ = 5.2 Hz, 2H, H-1'), 3.26 (t, $J_{1'',2''}$ =7.6 Hz, 2H, H-1''), 2.32 (t, $J_{16,15}$ =7.6 Hz, 2H, H-16), 1.62-1.54 (m, 4H, CH_2), 1.25 (m, 58H, CH_2), 0.87 (t, 6H, $J_{18'',17''}$ = 6.5 Hz, H-18''); ^{13}C NMR (100.6 MHz, CDCl$_3$): δ 173.0 (CO), 58.9 (C-2'), 50.2 (CH$_2$N$_3$), 50.2 (C-1''), 47.3 (C-1'), 34.3 (C-2), 31.8 (CH$_2$), 30.4 (CH$_2$), 29.6 (CH$_2$), 29.3 (CH$_2$), 28.9 (CH$_2$), 28.6 (CH$_2$), 27.7 (CH$_2$), 22.7 (CH$_2$), 14.1 (C-18''); FT-IR (ATR) ν in cm^{-1}: 3397, 2915, 2847, 2090, 1609, 1469, 1424, 1363, 1312, 1211, 1075, 718; Anal. Calcd. for C$_{38}$H$_{77}$NO$_2$: C, 78.69; H, 13.38; N, 2.41. Found: C, 78.73; H, 12.98, N, 2.45.

1-(β-D-galactopyranosyl)-*N*-octadecanoyl-2-amino-ethanol (2.38)
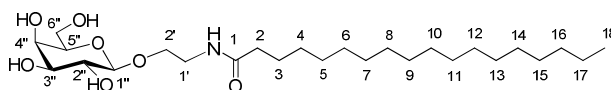
Departing from *N*-(2-hydroxyethyl)stearamide (**2.32**) (36 mg, 0.110 mmol), the glycoside **2.38** was obtained as a syrup according to the gereral procedure for glycosylation (46 mg, 0.094 mmol, 85% according to glycosylation procedure from *O*-acetylglycosides or 48 mg, 0.099 mmol, 90 % according to glycosylation procedure from *halo*-glycosides**):** Rf (CH$_2$Cl$_2$/MeOH 9:1): 0.16; $[\alpha]_D^{25}$ = +118.6º (*c* =0.0045, pyridine); ^{1}H NMR (CDCl$_3$:MeOD, 1:2, 400 MHz) δ in ppm: 4.21 (d, $J_{1'',2''}$ = 7.6 Hz, 1H, H1''), 3.96 (m, 1H, H-2a'), 3.84 (d, $J_{4'',3''}$ = 2.0 Hz, 1H, H-4''), 3.76-3.73 (m, 2H, H6a'', H6b''), 3.61 (m, 1H, H-2b'), 3.56-3.48 (m, 4H, H-2'', H-3'', H-5'', H-1a'), 3.35 (m, 1H, H-1b'), 2.17 (t, $J_{2,3}$ = 6.8 Hz, 2H, H-2), 1.57 (t, $J_{3,2}$ = 6.8 Hz, 2H, H-3), 1.40-1.25 (m, 26H, CH$_2$), 0.86 (t, $J_{18,17}$ = 6.8 Hz, 3H, H-18); ^{13}C NMR (CDCl$_3$:MeOD, 1:2, 100.6 MHz) δ in ppm:176.1 (CO), 104.5 (C-1''), 76.1 (C-5''), 74.3 (C-3''), 72.2 (C-2''), 69.9 (C-4''), 69.5 (C-2'), 62.5 (C-6''), 40.3 (C-1'), 37.0 (C-2), 32.8-26.8 (14 CH$_2$), 23.5 (C-17), 14.5 (C-18); FT-IR (ATR) ν in cm^{-1}: 3486, 2918, 2848, 1603, 1466, 1259, 1232, 1171, 1034, 630; ESI TOF for [M + Na]$^+$ calc for C$_{26}$H$_{51}$ NNaO$_7$ 512.3563, found 512.3551.

1-(β-D-galactopyranosyl)-N-(16-azidohexadecanoyl)-N-octadecanyl-2-amino-ethanol (2.43)
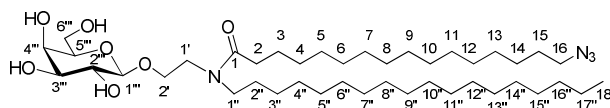
Departing from *N*-(2-hydroxyethyl)-*N*-octadecylstearamide (**2.34**) (65 mg, 0.110 mmmol), the glycoside **2.43** was obtained as a syrup according to the gereral procedure (33 mg, 0.043 mmol, 40 % according to glycosylation procedure from *O*-acetylglycosides or 52 mg, 0.069 mmol, 63 % according to glycosylation procedure from *halo*-glycosides): Rf (AcOEt/MeOH 9:1) 0.22; $[\alpha]_D^{25}$ = -5.8º, (c = 0.0065, pyridine); ^{1}H NMR (CDCl$_3$:MeOD, 1:2, 400 MHz) δ in ppm: 4.20 (t, $J_{1''',2'''}$ = 7.6 Hz, 1H, H-1'''), 3.96 (m, 1H, H-2a'), 3.84 (dd, $J_{4''',3'''}$ = 2.0 Hz, $J_{4''',5'''}$ = 1.8 Hz, 1H, H-4'''), 3.78-3.61 (m, 4H, H-6a''', H-6b''', H-2b', H-1a'), 3.56-3.33 (m, 6H, H-2''', H-5''', H-3''', H-1b', H-1''), 3.25 (t, $J_{16,15}$ = 6.8 Hz, 2H, H-16), 2.42 (t, $J_{2,3}$ = 8.0 Hz, 2H, H-2), 2.34 (dt, $J_{2,3}$= 8.0 Hz, 2H, H-2),[40] 1.61-1.50 (m, 8H, H-3, C*H*$_2$, H-2'', H-16), 1.40-1.25 (m, 46H, 23C*H*$_2$), 0.86 (t, $J_{18'',17''}$ = 6.8 Hz, 3H, H-18''), ppm; ^{13}C NMR (CDCl$_3$:MeOD, 1:2, 400 MHz) δ in ppm: 175.7 (*C*O), 105.2 (C-1'''), 76.7 (C-2'''), 75.0 (C-4'''), 72,4 (C-5'''), 70.2 (C-3'''), 68.5 (C-2'), 62.5 (C-6'''), 52.4 (C-16), 50.2 (C-1''), 47.0 (C-1'), 34.2 (C-2''), 33.9 (C-2), 33.1-30.0, 28.5, 28.0, 27.9, 27.8, 26.8, 26.2, 23.8 (CH$_2$), 14.5 (C-18), ppm; FT-IR (ATR) ν in cm^{-1}: 3330, 2917, 2848, 2812, 2776, 2682, 2099, 1614, 1466, 1350, 766; MALDI TOF for [M + Na]$^+$ calc for C$_{42}$H$_{82}$ N$_4$NaO$_7$ 777.6081, found 777.6901.

3,4,6-tri-*O*-acetyl-2-*O*-(2,6-difluorobenzoyl)-α-D-galactopyranosyl   iodide (2.44)

A solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**2.24**) (286 mg, 0.733 mmol) in CH$_2$Cl$_2$ (3 mL) was cooled to 0 ºC under argon in the dark, and TMSI (176 mg, 0.879 mmol) was added to the stirred mixture. The reaction was stirred for 20 min at 0 ºC. The reaction was stopped by adding 3 mL of dry

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[40] Signal associated to H-2 is doubled at room temperature due to the tertiary amide-imino alcoholate equilibrium. The signal coalesces once temperature is increased over 50 ºC.

toluene and azeotropically distilled three times with dry toluene. The slightly yellow oil **2.44** was dissolved in CH_2Cl_2 (5 mL) and was used immediately in the glycosylation reaction.

Low temperature NMR experiments

Identification of intermediates **2.27a** and **2.27b**
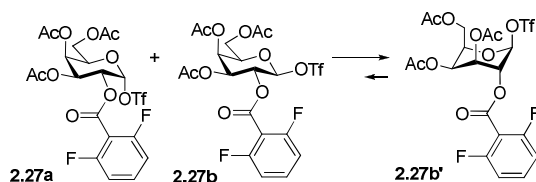
The donor **2.27** (69 mg, 0.141 mmol) was co-evaporated with dry toluene (2x), dissolved in CD_2Cl_2 (0.6 mL) fleshly distilled over P_2O_5 and transferred to an NMR tube under argon atmosphere. TfOH was also dried over 4 Å MS for 24 h. Then, the sample was treated with dried TfOH (50 μL, 0.565 mmol), shaken for 5 min and placed back in the NMR magnet at room temperature. The first ^{19}F NMR spectrum was immediately recorded to check if all the starting material was consumed. After that, the sample was ejected from the NMR magnet and cooled to 0º C in an ice bath meanwhile the NMR magnet was cooled at same temperature. Then, the sample was placed again in the NMR magnet to record ^{1}H, ^{13}C and bidimensional NMR experiments.

Selected signals for **2.27a**:
^{1}H NMR (CD_2Cl_2, 600 MHz) δ in ppm: 6.89 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.71 (dd, $J_{2,3}$ = 10.8 Hz, $J_{2,1}$ = 3.6 Hz, 1H, H-2), 5.68-5.66 (m, 2H, H-3, H-4), 4.46 (t, $J_{5,6}$ = 6.6 Hz, 1H, H-5), 4.46-4.38 (m, 2H, H-6, H-6'); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ in ppm: 179.9, 176.5, 175.3, 172.2, (CO), 163.7-160.3 (C-Ar), 138.8-135.1 (C-Ar), 113.7-112.7 (C-Ar), 90.5 (C-1), 69.6 (C-3), 69.4 (C-4), 68.9 (C-5), 68.0 (C-2), 65.6 (C-6), 20.8-20.5 (CH_3); ^{19}F NMR (CD_2Cl_2, 376 MHz) δ in ppm: -89.2.

Selected signals for **2.27b**:

^{1}H NMR (CD$_2$Cl$_2$, 600 MHz) δ in ppm: 6.80 (d, $J_{1,2}$ = 2.4 Hz, 1H, H-1), 5.92 (s, 1H, H-3), 5.76 (s, 1H, H-4), 5.58 (d, $J_{2,3}$ = 1.8 Hz, 1H, H-2), 4.81 (t, $J_{5,6}$ = 6.0 Hz, 1H, H-5), 4.54-4.45 (m, 2H, H-6, H-6'); ^{13}C NMR (CD$_2$Cl$_2$, 151 MHz) δ in ppm: 191.3, 190.4, 178.5, 169.9 (CO), 163.7-160.3 (C-Ar), 138.8-135.1 (C-Ar), 113.7-112.7 (C-Ar), 90.0 (C-1), 83.0 (C-4), 79.5 (C-2), 70.5 (C-5), 63.1 (C-6), 58.7 (C-3), 20.8-20.5 (CH$_3$); ^{19}F NMR (CD$_2$Cl$_2$, 376 MHz) δ in ppm: -89.4.

Chapter 3

Synthesis of 2-deoxygalactosyl ceramides

3. 1. Introduction

2-Deoxy-carbohydrates and 2,6-dideoxy-carbohydrates are structurally relevant components of numerous biologically important natural products[1] including antitumor drugs (anthracyclines, aureolic acids, calicheamicin, esperamicin), antibiotics active against Gram-positive bacteria (erythromycins, orthosomycins), antibiotics inhibiting platelet aggregation (angucyclines), cardiac glycosides (digitoxine) and antiparasitic agents (avermectins).[2] Due to their biological importance, efforts to develop chemical methods for the stereoselective synthesis of 2-deoxy-sugars relevant agents such as antibiotics and anti-cancer drugs have increased in the last years.[1, 2] However, if C-2 position of a glycosyl donor is deoxygenated, the construction of the glycosidic bonds with a complete stereocontrol of the reaction does not result trivial since the lack of a C-2 directing group usually leads to obtain 2-deoxyglycosides as mixture of anomers. Furthermore, the manipulation of 2-deoxyglycosides can be more difficult compared to their C-2 hydroxylated counterparts because of their susceptibility to hydrolysis.[3]

The formation of glycosidic bond is often controlled by the anomeric effect, which promotes axial (usually α- for $^4C_1$ chairs) glycoside formation.[4] The anomeric effect usually explains why the orientation of electronegative substituents, for example halides, *O*-aryl, *O*-alkyl, *S*-alkyl, *S*-aryl derivatives etc., bonded to the anomeric carbon of a pyranose ring tend to be axial.[5] It has been widely accepted that the electronwithdrawing character of the axial substituent (the α-anomer for D-sugars in the $^4C_1$ conformation) is stabilized by hyperconjugation between the non-bonding electron pair on O-5 and anti-bonding (σ^*) orbital of the exocyclic axial C-X bond (Figure 3.1.).

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[1] (a) Kirsching, A; Bechthold, A. F.-W.; Rohr, J. *Top. Curr. Chem.* **1997**, *188*, 1-84. (c) He, X. M.; Liu, H. W. *Curr. Opin. Chem. Biol.* **2002**, *6*, 590–597.

[2] (a) Kennedy, J. F.; White, C. A. *Bioactive Carbohydrates in Chemistry, Biochemistry, and Biology*, Chichester, Ellis Horwood, 1983. (b) Williams, N.; Wander, J. *The Carbohydrates: Chemistry and Biochemistry*, Vol. 1B; Pigman, W.; Horton, D. Eds., Academic Press, New York, 1980.

[3] Overend, W. G. ; Rees, C. W.; Sequeira, J. S. *J. Chem. Soc.* **1962**, 3429-3440.

[4] Carmona, A. T.; Moreno-Vargas, A. J.; Robina, I. *Curr. Org. Synth.* **2008**, *5*, 33-60.

[5] Juaristi, E.; Cuevas, G. *The Anomeric Effect*; CRC Press: Boca Raton, 1994.
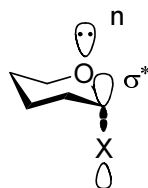
Figure 3.1. Anomeric effect stabilization of axial C–X bond at the anomeric center on a pyranose ring

However, a more detailed discussion of glycosidic bond formation in 2-deoxy-sugars can be contemplated when the protecting groups of the ring are considered. Scheme 3.1. depicts the reaction pathways available to an oxocarbenium ion (**3.3**) if the substituent at C-2 cannot stabilize the positive charge by participation. The oxocarbenium ion can be formed from a glycosyl donor like **3.1** if the departure of the leaving group is activated by a promoter or can be formed from a glycal like **3.2** if it is activated by the addition of a proton**.**

Then, the positive charge at C-1 in **3.3'** is stabilized by O-5. If the oxocarbenium ion adopts a half-chair conformation, the two faces of the ring can be attacked by the nucleophile. When the attack takes place axially, hyperconjugation between a non-bonding orbital of the ring oxygen and the anti-bonding orbital of the C-1 stabilizes the transition state that resemble a chair conformer.[5] On the other hand, when the attack takes place equatorially, the transition state must adopt a boat-like conformation to arise a proper orbital overlapping.
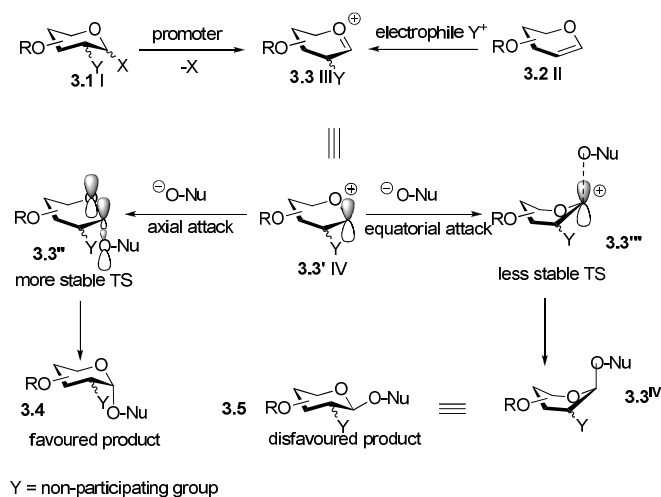
Scheme 3.1. Glycosylation with a non-particing group at C-2

However, this boat-like transition state is of higher energy than the chair-like transition state so the axial linkage is favoured (Scheme 3.1.) and therefore complete selectivity for these reactions is often difficult because factors such as protecting groups, promoter, solvent, temperature and the leaving group condition glycosylation yield and stereoselectivity.

With such observations in mind, the stereocontrolled formation of equatorial glycosides by this approach is complicated. However, there are indirect methodologies to achieve this target. It is possible to control the glycosylation if a participating neighboring group has been introduced previously at C-2 position. The neighboring group Y must have non-bonding electron pairs and it should be easy to remove in order to afford 2-deoxy glycosides. Hence, halogen, sulphur and selenium moieties are the most employed. (Scheme 3.2.).
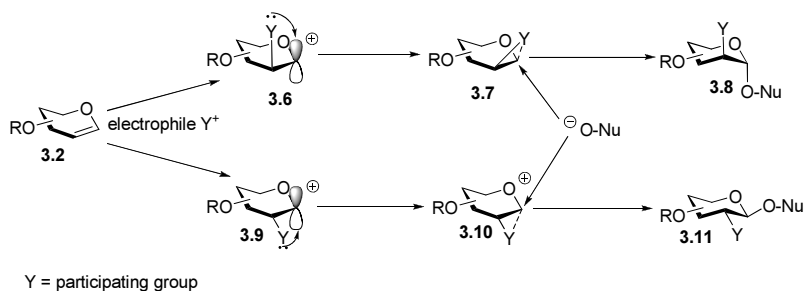
Scheme 3.2. Glycosylation with a particing group at C-2

81

Another strategy to enhance the preference for the glycosylated product with a equatorial configuration is forcing the oxocarbenium ion to adopt the boat-like conformation. In the case of the 2-deoxy-2-halo-galactosyl donors, Roush and co-workers observed that the β-selectivity of glycosylation reactions were considerably improved when the 3,4-hydroxyl moieties were protected with groups which can restrict the conformations of the chair such as 3,4-carbonate or 3,4-acetonide groups.[6] Therefore, such *cis*-fused cyclic 3,4-protecting group encourages the transition state to adopt the mentioned boatlike conformation with the C(2)-Y substituent in a pseudoaxial position, which should direct the glycosylation in a β-selective manner (Scheme 3.3.).[7]
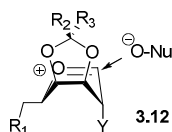
Scheme 3.3. Stereochemical consideration of the key boatlike oxocarbenium ion.

Glycolipids with a β-glycosidic linkage are also biologically active molecules. It is know that β-GalCer is able to induce apoptosis on human U937 leukemic cells and it is an agonist C6 glioma cells.[8] Although an extensive study in the ceramide moiety has been investigated,[8] to the best of our knowledge modifications on the polar part of glycolipids to include a 2-deoxy region have been less studied.

Due to the fact that the most efficient methodologies to obtain 2-deoxy-glycosides employ 2-deoxy-2-iodo glycosyl donor as a key intermediate for glycosylation, we envisioned the synthesis of a new precursor of 2-deoxy-α- and β-GalCer using this methodology. Moreover, the presence of a handle at C-2 with potential to be substituted (with azide group, thiol group, etc) will broaden the 2-substituted glycolipid analogues for biological evaluations. Indeed, this

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[6] Durham, T. B.; Roush, W. R. *Org. Lett.* **2003**, *5*, 1871-1874.
[7] Abdel-Rahman, A. A.-H.; Jonke, S.; El Ashry, E. S. H.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2002**, *41*, 2972-2974.
[8] Chang, Y.-T.; Choi, J.; Ding, S.; Prieschl, E. E.; Baumruker, T.; Lee, J.-M.; Chung, S.-K.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 1856-1857.

new anchoring point will allow the introduction of appropriate tags for different imaging modalities.
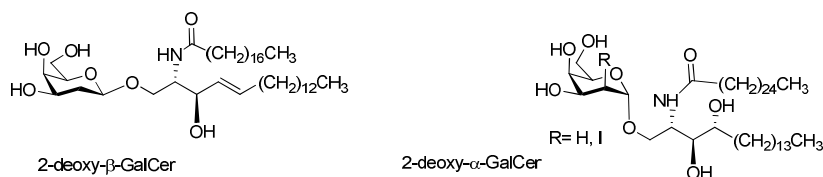
Figure 3.2. 2-deoxy derivatives of GalCer

The retrosynthetic scheme for the synthesis of 2-deoxy-β-GalCer is showed in Scheme 3.4. The stereoselectivity of the glycosylation reaction requires an iodine equatorial at position 2, which will be obtained from galactal protected at positions 3,4 as a carbonate, as mentioned before.
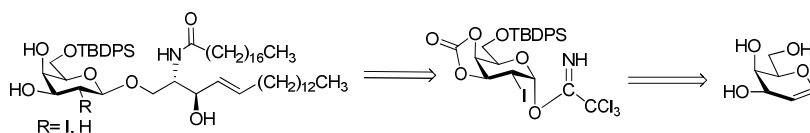
Scheme 3.4. Retrosynthetic analysis of 2-deoxy-β-GalCer

Scheme 3.5. shows the retrosynthetic analysis for 2-deoxy-α-GalCer. In this case, an iodine at position axial is needed in order to obtain the α-glycoside. For this purpose galactal must be protected as its peracetylated derivative. Glycosylation must give the 2-iodo-derivative, which after removal of protecting groups should afford the 2-deoxy-2-iodo-α-TalCer. Reduction of C-I bond should afford the 2-deoxy-α-GalCer.
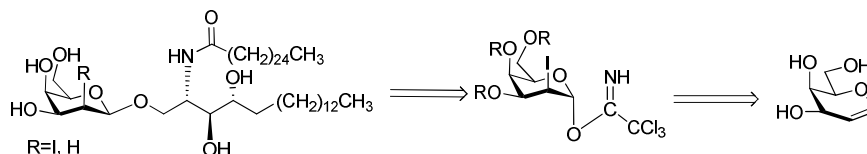
Scheme 3.5. Retrosynthetic analysis for 2-deoxy-α-GalCer and 2-deoxy-2-iodo-α-TalCer

3.2. Results and Discussion

3.2.1. Synthesis of 2-deoxy-β-GalCer analogue

As it has been mentioned before, the preparation of appropriate 2-deoxy-2-iodo-galactosyl donor for selective β-glycosylation bearing a 3,4-*cis*-fused cyclic protecting group[6] was carried out following the synthetic sequence depicted in Scheme 3.6.

Scheme 3.6. Synthesis of glycosyl donor **3.16**

Thus, using D-galactal (**3.13**) as starting material, primary alcohol was protected by reaction with *tert*-butyldiphenylsilyl chloride (TBDPSCl) to give **3.14** in 61% yield. Then, the positions 3 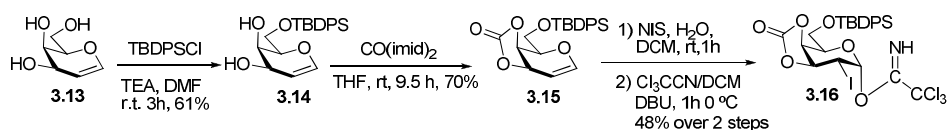and 4 of galactal **3.14** were protected by reaction with carbonyldiimidazole to provide the *cis*-fused cyclic carbonate **3.15** in 70% yield. The treatment of **3.15** with NIS/H_2O afforded an α/β mixture of 2-deoxy-2-iodoglactose derivatives as a major product, which was then treated with Cl_3CCN/CH_2Cl_2 and DBU to afford imidate **3.16** in 48% overall yieldafter 1 h at 0 ºC. Afterwards, the imidate **3.16** was employed in the glycosylation step without further purification to avoid a possible decomposition.[9]

Ceramides are poor nucleophiles because they are able to self assemble in hexagonal and orthorhombic phases as a result of their head group hydrogen bonding and van der Waals interactions.[10] To solve this drawback, stannyl ether

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[9] Boons, G-J.; Hale, K. *Organic Synthesis with Carbohydrates* Blackwell Science, Inc, Malden, Massachusetts, **2000**.

[10] (a) Schmidt, R. R.; Zimmermann, P. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 725–726. (b) Polt, R.; Szabo, L.; Treiberg, J.; Li, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **1992**, *114,* 10249-10258.

derived from ceramides is known to be a suitable strategy to increase the nucleophilicity of oxygen without significantly modifying the basicity.[11,12]

Table 3.1. Glycosylation assays of imidate **3.16** with stannyl ceramide **3.17**
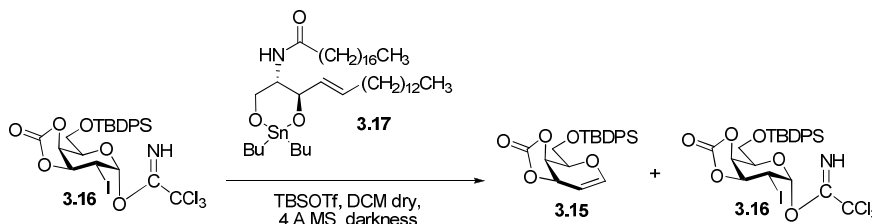
Entry	Promoter/Donor (mmol)/(mmol)	T (°C)	t (h)	Productᵃ
1	0.3	-78	24	(1:10) **3.15/3.16**
2	0.3	0	24	(2:1) **3.15/3.16**
3	0.6	r.t.	48	**3.15**
4ᵇ	0.3	-78	24	**3.15**

ᵃ Conversion of 95% observed by ¹H NMR. ᵇ Starting material 3.16 was purified by flash chromatography before use.

Stannyl ceramide **3.17** was prepared in situ by refluxing a solution of ceramide **1.15** in toluene with tributyltin oxide in a Dean-Stark system for 12 h.[12] Then, **3.17** was treated with a solution of trichloroacetimidate **3.16** in dry DCM prepared *in situ* in the presence of 4 Å MS (Table 3.1.). When the reaction was performed at -78 ºC, trichloroacetimidate **3.16** and trace amounts of glycal **3.15**

[11] (a) Kaji, E.; Shibayama, K.; In, K. *Tetrahedron Lett*. **2003**, *44*, 4881-4885. (b) Kartha, R. K. P.; Kiso, M.; Hasegawa, A.; Jennings, H. J. *J. Chem. Soc., Perkin Trans. 1* **1995**, 3023-3026. (c) Garegg, P. J.; Malvisel, J. L.; Oscarson, S. *Synthesis* **1995**, 409-414; (d) Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Griffith, D. A.; Oriyama, T.; Marsden, S. P.; *J. Am. Chem. Soc.* **1995**, *117*, 1940-1953; (e) Vogel, K.; Sterling, J.; Herzig, Y.; Nudelman, A. *Tetrahedron* **1996**, *52*, 3049-3056; (f) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643-663.
[12] (a) Morales-Serna, J. A.; Díaz, Y.; Matheu, M. I.; Castillón, S. *Eur. J. Org. Chem.* **2009**, 3849-3852. (b) Morales-Serna, J. A.; Díaz, Y.; Matheu, M. I.; Castillón, *Org. Biomol. Chem.* **2008,** *6*, 3831-3836. (c) Boutureira, O.; Morales-Serna, J. A.; Díaz, Y.; Matheu, M. I.; Castillón, *Eur. J. Org. Chem.* **2008**, 1851-1854. (d) Morales-Serna, J. A.; Boutureira, O.; Díaz, Y.; Matheu, M. I.; Castillón, S. *Org. Biomol. Chem.* **2008,** *6,* 443-446.

were obtained instead of the desired glycosylated product or the corresponding hydrolysed glycosyl donor (Table 3.1., Entry 1). Temperature was increased to force the glycosylation reaction but only the elimination product **3.15** was obtained even when ane excess of TBSOTf was added or longer reaction times were employed (Table 3.1., Entries 2 and 3).

The product distribution observed under the glycosylation conditions (Table 3.1.) and the formation of glycal **3.15** can be explained based in a previous work developed in our group.[13] It was found that 2-deoxy-2-iodo-galactosyl derivative **3.18** protected as 3,4-di-*O*-isopropilidene acetal underwent elimination at position 2 when a *N*-containing base **3.20** was present giving 2-iodo-galactals due to an elimination process as is described in Scheme 3.7.
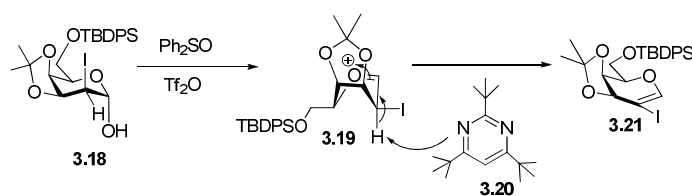
Scheme 3.7. Example of elimination pathway in 2-deoxy-2-iodo-glycosyl donors.

In our case, however, once the oxocarbenium ion is formed from substrate **3.16**, iodine adopts an axial configuration. This intermediate could be stabilised by hyperconjugative interactions between σ (C-I) and π^* (C-O) of the oxocarbenium.[14] Moreover, during the E1 elimination reaction, the new double bond can only be formed if the vacant p orbital of the carbocation and the breaking C-H or C-I bond are aligned in parallel. Therefore, the group to be eliminated must be in the axial position (Scheme 3.8.).

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[13] Rodríguez, M. A.; Boutureira, O.; Matheu, M. I.; Díaz, Y.; Castillón, S.; Seeberger, P. H. *J. Org. Chem.* **2007,** *72*, 8998-9001.
[14] Billings, S. B.; Woerpel, K. A. *J. Org. Chem.* **2006**, *71*, 5171-5178.
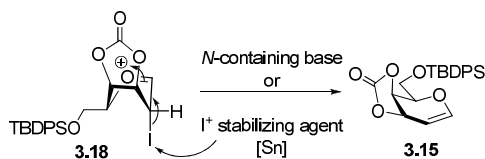
Scheme 3.8. Iodine elimination of the oxocarbeminum ion **3.18**

In the present study, it was reasonable to asume that the DBU present as a byproduct in the trichloroacetimidate **3.16** crude could be the base responsible of the elimination reaction. Hence, the formation of the more thermodynamically stable product **3.15** is favoured when the temperature was increased (Table 3.1., Entry 1 to 3).

To avoid the presence of base in the glycosylation reaction, the trichloroacetimidate **3.16** was purified by flash chromatography, taking into account their proven stability (Table 3.1., Entry 1). However, glycal **3.15** was recovered again when the reaction was carried out at -78 ºC (Table 3.1., Entry 4).

The lack of a base in the system and the formation of glycal at low temperatures indicated the stannyl ceramide was not a suitable glycosyl acceptor. The stannyl ceramide is not nucleophilic enough to avoid the inherent tendency of **3.16** to undergo elimination. Moreover, the presence of tin species may contribute to the stabilization of hypervalent iodine likely due to the formation of stable SnI_2 although the exact mechanism has not been stablished. However, some examples of stabilization of hypervalent iodine by tin (IV) had been identified in literature.[15]

At this point, another kind of glycosyl acceptor had to be chosen to avoid the low nucleophilicity of ceramides and the use of tin reagents to make the glycosylation successful. It has been broadly reported that convenient protected

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[15] (a) Davies, A. G.; Harrison, P. G. *J. Chem. Soc. C,* **1967**, 298-300. (b) Cox, P. J.; Doidge-Harrison, S. M. S. V.; Howie, R. A.; Nowell, I. W.; Taylor, O. J.; Wardell, James L. *J Chem. Soc., Perkin Trans 1* **1989**, *11*, 2017-2022.

azidosphingosines had been good glycosyl acceptors for glycosylation *en route* to the synthesis of glycolipids.[16]

For instance, (2*R*,3*S*)-2-azido-3-benzoylsphingosine (**3.22**), which had been prepared in our group according the procedure reported by Bittmann,[17] was chosen because its proctecting groups could be compatible with our glycosylation conditions. Thus, when **3.22** was treated with pure trichloroacetimidate **3.16** in presence of TBSOTf at -78 ºC under anhydrous conditions, glycoside **3.23** was obtained after 18 h with complete β selectivity in excellent yield (90%) (Scheme 3.9.). Moreover, the hypothesis that tin derivates assist the elimination of **3.16** was supported because byproducts of elimination such as glycal **3.15** were not isolated during the purification step.
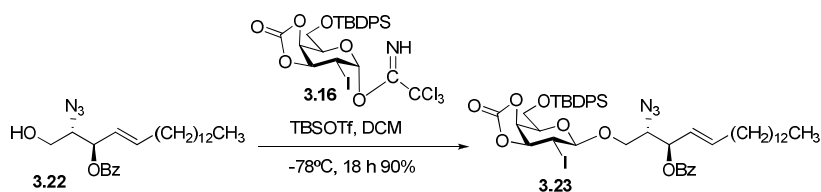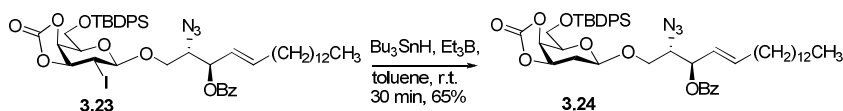

Scheme 3.9. Glycosylation of azidosphingosine **3.22**

To illustrate the potential of 2-iodo-glucosylazidosphingosine as a flexible precursor for the preparation of several 2-sunstituted and 2-deoxygenated analogues, **3.23** was treated with Bu_3SnH/Et_3B to remove the iodine moiety via a radical reaction (Scheme 3.10.). The reaction was monitorized by ^1H NMR and HSQC spectroscopy. Although identification of H-2 was difficult because of

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[16] (a) Xia, C.; Yao, Q.; Schuemann, J.; Rossy, E.; Chen, W.; Zhu, L.; Zhang, W.; De Libero, G.; Wang, P. G. *Bioorg. Medicinal Chem. Lett.* **2006**, *16*, 2195-2199. (b) Franchini, L.; Compostella, F.; Donda, A.; Mori, L.; Colombo, D.; De Libero, G.; M., P.; Ronchetti, F.; Panza, L. *Eur J. Org. Chem.* **2004**, *23*, 4755-4761. (c) Compostella, F.; Franchini, L.; De Libero, G.; Palmisano, G.; Ronchetti, F.; Panza, L. *Tetrahedron* **2002**, *58*, 8703-8708. (d) Castro-Palomino, J. C.; Simon, B.; Speer, O.; Leist, M.; Schmidt, R. R. *Chem. Eur. J.* **2001**, *7*, 2178-2184. (e) Hansen, H. C.; Magnusson, G. *Carbohydr. Res.* **1999**, *322*, 190-200. (f) Wilstermann, M.; Magnusson, G. *J. Org. Chem.* **1997**, *62*, 7961-7971. (g) Plewe, M.; Sandhoff, K.; Schmidt, R. R. *Carbohydr. Res.* **1992**, *235*, 151-161. (h) Zimmermann, P.; Greilich, U.; Schmidt, R. R. *Tetrahedron Lett.* **1990**, *31*, 1849-1852. (i) Singh, N. P.; Schmidt, R. R. *J. Carbohydr. Chem.* **1989**, *8*, 199-216. (k) Zimmermann, P.; Bommer, R.; Bare, T.; Schmidt, R. R. *J. Carbohydr. Chem.* **1988**, *7*, 435-452.
[17] Liu, Y.; Bittman, R. *Chem. Phys. Lipids* **2006**, *142*, 58-69.

its overlapping with other signals, the disappearance of the methyne signal at δ 19.1 ppm attributed to C-2 and the appearance of a new C-2 signal as a methylene at δ 32.1 ppm in the ^{13}C NMR spectrum indicated that the reduction was completed. After purification, the reduced glycoazidosphingolipid **3.24** was obtained with a 65% yield.

Scheme 3.10. Reduction of glycoside **3.23**

To reduce the azide moiety, the glycoazidosphingosine **3.24** was treated with triphenylphosfine[18] via Staudinger reaction for preserving other functionalities sensitive to a more severe reduction conditions. Without further purification, the corresponding amine was treated with stearic acid in presence of DMF, DIPEA and HATU at room temperature. However, the amine was not acylated after 18 h, and increasing the reactions neither forced the reaction to proceed (Table 3.2., Entries 1 and 2). The acylation reaction was also assayed with EDC, HOBt and DIPEA but no glycolipid was recovered (Table 3.2., Entry 3). We considered that the coupling reagents could have problems to access the amine group due to steric hindrance of the neighbouring protecting groups. Therefore, the stearic acid was activated as *N*-succinimidyl octadecanoate and the reaction was performed at 60 ºC in THF in a similar way as it was reported by Kim and co-workers[19] (Table 3.2., Entry 4). Under these conditions, the glycolipid **3.25** was recovered after in a 43% yield over two steps 18 h.

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[18] (a) Tian, W. Q.; Wang, Y. A. *J. Org. Chem*. **2004**, *69*, 4299-4308. (b) Lin, F. L.; Hoyt, H. M.; Halbeek, H. V.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc*. **2005**, *127*, 2686-2695.
[19] Kim, S.; Song, S.; Lee, T.; Jung, S.; Kim, D. *Synthesis* **2004**, *6*, 847-850.

Table 3.2. Study of the acylation reactions to obtaining glycolipid **3.25**

Entry	Fatty acid	Reagents	Solvent	Temperature (ºC)	Time (h)	Yield
1	Stearic acid	HATU DIPEA	DMF	r.t.	18h	-
2	Stearic acid	HATU DIPEA	DMF	r.t	48h	-
3	Stearic acid	EDC HOBt DIPEA	DMF	r.t.	18h	-
4	*N*-succinimidyl octadecanoate	TEA	THF	60	18h	43%

Although it remains to remove the protecting groups, to the best of our knowledge, this work represents the first example of synthesis of 2-deoxy-β-GalCer analogue (3.25) with complete β-stereoselectivity. The key step was the synthesis of 2-deoxy-2-iodo-glycosyl donor with *galacto* configuration which was achieved by the appropriate selection of the protecting groups.

3.2.2. Synthesis of 2-deoxy-2-iodo-α-TalCer analogue

As it was mentioned before, 2-deoxy-2-iodo-*talo*pyranoses may be excellent glycosyl donors to direct the glycosylation with α stereoselectivity. Hence, the synthesis of 2-iodopyranose **3.27** was necessary. Iodoacetoxylation of galactal **3.26** in presence of cerium (IV) ammonium nitrate (CAN), NaI and acetic acid (AcOH) in acetonitrile afforded **3.27** with the desired *talo* configuration in excellent yield (93%) (Scheme 3.11.).
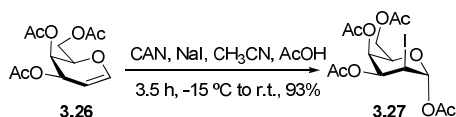
Scheme 3.11. Formation of 2-iodo-deoxy-glycosyl donor **3.27**

Although the mechanism is still unclear, the group of Roush[20] argued that the reaction could proceed by the addition of a iodine radical (I·) to the C-2 of the glycal like in the azidonitration reaction promoted by CAN[21]. In the case of the azidonitration reaction, it is known that ceric salts oxidize metallic azides to nitrogen quantitatively, and an azido radical has been suggested as intermediate which could be trapped by carrying out the reaction in the presence of olefins (Scheme 3.12., Equations 1 and 2). Then, if a radical intermediate is formed, it could be converted to the corresponding nitrate (**3.28**), by one of the pathways suggested for other radical nitrate conversions in CAN reactions such as an electron transfer reaction (Scheme 3.12., Equation 3), a ligand transfer reaction (Scheme 3.12., Equation 4), and reaction of the radical with nitrate to form a radical anion which is subsequently oxidized by a cerium(IV) to the neutral alkyl nitrate (Scheme 3.12., Equation 5).
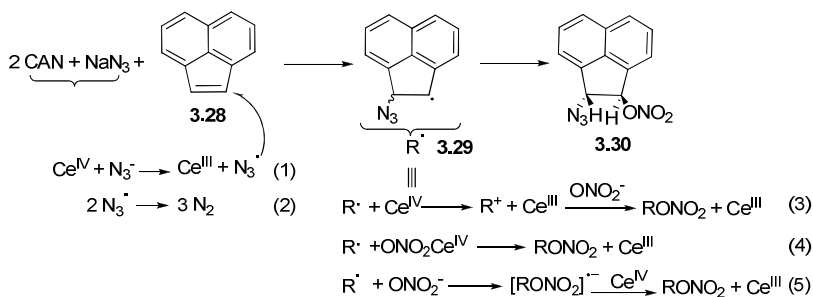
Scheme 3.12. Proposed mechanism for azidonitration of acenaphthene

To demonstrate that iodoacetoxylation of glycals proceed in a similar way as the azidonitration, the group of Roush treated cyclohexene under iodoacetoxylation conditions (CAN-NaI, AcOH). Under these conditions, 1-iodo-2-

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[20] Roush, R. W.; Narayan, S.; Bennett, C. E.; Briner, K. *Org. Lett.* **1999,** *6,* 895-887.
[21] (a) Trahanovsky, W. S.; Robbins, M. D. *J. Am. Chem. Soc.* **1971**, *93*, 5256-5258. (b) Trahanovsky, W. S.; Cramer, J. *J. Org. Chem.* **1971**, *36*, 1890-1893.

nitratocyclohexane (**3.32**) could be obtained (Scheme 3.13.). Therefore, they concluded that 2-iodo-1α-nitrates are intermediates in the CAN-NaI reactions of glycals and are converted to the iodo acetates after a final nitrate-acetate substitution reaction.
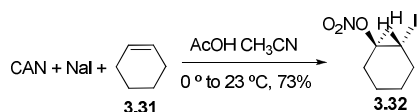
Scheme 3.13. Iodonitration of cyclohexene using CAN-NaI-AcOH

About the stereochemistry adopted by iodine group in a iodoacetylation of glycals, Roush and co-workers discussed that although it is known that the carbon based radicals generally add at the position C-2 of glycals with a *trans* configuration to the C-3 substituent,[22] they were unable to explain why the iodine radical adopts the configuration *cis* to C-3 subtituent for the examples that they studied on glycals.
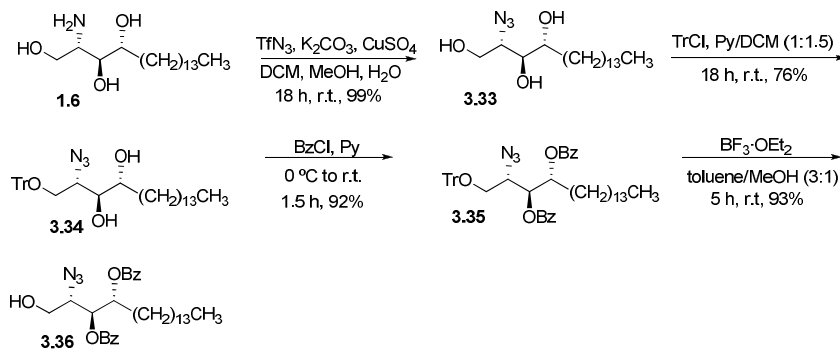
Scheme 3.14. Formation of azidophytosphingosine **3.36**

On the other hand, Scheme 3.14. depicts the synthetic route utilized for the preparation of azidophytosphingosine **3.26** from available phytosphingosine **1.6**. The amine moiety of phytosphingosine **1.6** was converted to azide employing the diazotransfer reaction to provide **3.33** in quantitative yield (99%).

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[22] (a) Linker, T.; Sommermann, T.; Kahlenberg, F. *J. Am. Chem. Soc.* **1997**, *119*, 9377-9384. (b) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244-1251. (c) Briner, K.; Vasella, A. *Helv. Chim. Acta* **1987**, *70*, 1341-1356.

Although the mechanism of diazotransfer reaction remains unclear, interesting contributions were reported by the group of Wong to explain this transformation.[23] They observed that divalent metal ions such as Cu (II) or Zn (II) were able to improve the reaction rate considerably. Hence, they proposed that the reaction could be promoted by the coordination of the amine to the metal catalyst under basic conditions to form **3.37** (Scheme 3.15.). Then a nucleophilic attack of **3.37** on the highly electrophilic triflyl azide followed by deprotonation, might form a metal-stabilized mixed tetrazene, **3.39**. The breakdown of **3.39**, via a reverse [3+2] dipolar cycloaddition, could afford the desired azide product and metal-triflyl imido complex **3.40**. Then, to be in agreement with the computational work by Brandt,[24] they assumed that complex **3.40** could be in equilibrium with **3.41**. At this point, they found reasonable that complexes **3.40** and **3.41** could derive from complex **3.42.** On the one hand, **3.42** may undergo amine complexation and then the proton transfer to provide **3.40** (with triflyl amide as one of the ligands). On the other hand, the transimination of **3.41** yield the transient metal-imido complex **3.42**. They found that this explanation was similar to the azide metathesis reaction reported by Bergman *et al.* with zirconium complexes.[25] Moreover, the imido-metal **3.42** complex could be engaged with triflyl azide in a [3+2] dipolar cycloaddition to alternatively provide **3.39**.

[23] Nyffeler, P. T.; Liang, C.-H.; Koeller, K. M.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 10773-10778.

[24] Brandt, P.; Söergren, M. J.; Andersson, P. G.; Norrby, P.-O. *J. Am. Chem.Soc.* **2000**, *122*, 8013-8020.

[25] Meyer, K. E.; Walsh, P. J.; Bergman, R. G. *J. Am. Chem. Soc.* **1995**, *117*, 974-985.
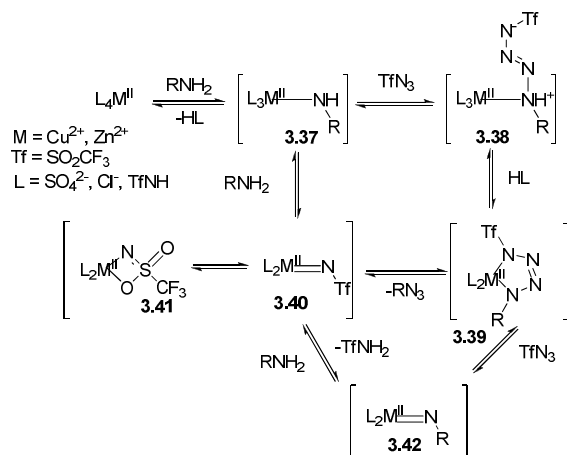
**Scheme 3.15.** Proposed mechanism for diazotransfer reaction.

Once azidophytosphingosine **3.33** was obtained, it was treated with trityl chloride in a mixture of pyridine/dichloromethane for 18 h to selectively protect the primary alcohol. The resulting azidophytosphingosine **3.34** was isolated in good 76% yield (Scheme 3.20.). Later, **3.34** was acylated by reaction with benzoyl chloride in pyridine from 0 ºC to room temperature for 1.5 h to yield the fully protected azidophytosphingosine **3.35**. Finally, **3.35** was treated with BF$_3$·OEt for 5 h to selectively cleave the trityl group. Alcohol **3.36** was obtained in excellent yield (93 %) and was ready to be used as a glycosyl acceptor for glycosylation.

It is well known that certain 1-*O*-acetylated-2-deoxy-2-iodo glycosides can undergo glycosylation when a strong Lewis acid such as TMSOTf is employed. Moreover, a similar Lewis acid promoter (TBSOTf) was successfully employed for the preparation of 2-deoxy-2-iodo-β-D-galactosyl ceramide, see previous section (Scheme 3.9.). Hence, the peracetylated 2-deoxy-2-iodogalactose **3.27** was treated with **3.36** in presence of TMSOTf at -78 ºC under anhydrous conditions for 18 h. To our delight, glycoside **3.43** was obtained in excellent 90% yield with complete α selectivity (Scheme 3.16.).
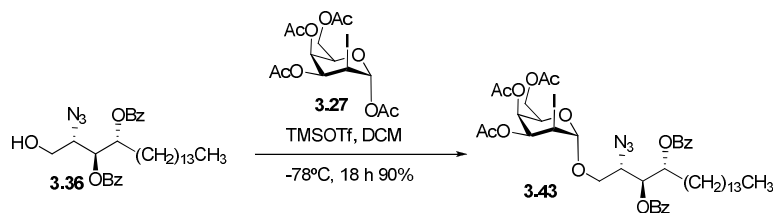
Scheme 3.16. Glycosylation of azidophytosphingosine **3.36**

Next, glycoazidophytosphingosine **3.43** was reduced with triphenylphosfine[26] (Scheme 3.17.) similarly to glycoazidosphigosine **3.24**, to afford the corresponding amine (Table 3.2.) which was treated without further purification, with hexacosanoic acid and HATU in a (55:15:30) mixture of DMF/CH$_2$Cl$_2$/Et$_2$O in presence of DIPEA as a base. After stirring at room temperature for 18 h the desired glycolipid **3.44** was isolated in 64% yield (Scheme 3.17.). The stereochemistry of iodine is appropriate for introduction of different substituents at C-2 by an S$_N$2 process, which will be tackled in the future. Moreover, reduction of C-I bond with HSnBu$_3$ under radical conditions and removal of protecting groups should afford the 2-deoxy-α-GalCer derivative.
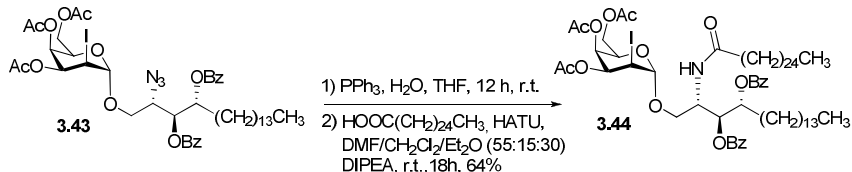
Scheme 3.17. Synthesis of glycolipid **3.45** from glycoside **3.44**

In summary, the use of the 2-deoxy-2-iodo-glycosyl donors (**3.16** and **3.27**) has been studied for the glycosylation reaction of stannyl ceramide **3.18** and azidosphingosine **3.22** and azidophitosphingosine **3.36** derivatives. The direct glycosylation of stannyl ceramide **3.18** was unsuccessful because of the side-reactions occurring between Sn (IV) species and iodine present in the glycosyl

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[26] (a) Tian, W. Q.; Wang, Y. A. *J. Org. Chem*. **2004**, *69*, 4299-4308. (b) Lin, F. L.; Hoyt, H. M.; Halbeek, H. V.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc*. **2005**, *127*, 2686-2695.

donor (**3.16**). However, direct glycosylation of azidosphingosine **3.22** and azidophytosphingosine **3.36** derivatives without tin activation afforded the corresponding glycosides **3.23** and **3.43** respectively in high yield. In both cases complete stereoselectivities were obtained due to the presence of both the 3,4-cyclic protecting group and the iodine configuration in the glycosyl donors. After several transformations, the corresponding derivatives of 2-deoxy-β-GalCer **3.25** and 2-deoxy-2-iodo-α-TalCer **3.44** were obtained. Further efforts to scale these products for their convenient deprotection in order to study its potencial biological activity are currently under investigation in our group.
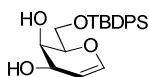
3. 3. Experimental section

N-stearoyl-D-*erythro*-sphingosine (1.15)
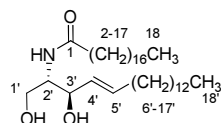
Stearic acid (**3.17**) (94 mg, 0.320 mmol), D-*erythro*-sphingosine (**1.4**) (115 mg, 0.384 mmol) and HATU (152 mg, 0.400 mmol) were dissolved in dry dimethylformamide (10.6 mL). DIPEA (195 μL, 1.120 mmol) was added to mixture that was stirred at room temperature for 13 h. The reaction was quenched by adding ethyl acetate and extracting with water. After concentration, the crude was purified by flash chromatography using a gradient 5:95 to 1:9 isopropanol/CHCl$_3$ as eluent to afford **1.15** as white solid (160 mg, 88% yield): Rf (1:9 isopropanol/CH$_3$Cl): 0.20; mp: 105-106 ºC; $[\alpha]_D^{20}$: -5.0º (c = 0.5, CHCl$_3$); ^{1}H NMR (400 MHz, 1:4 CDCl$_3$/CD$_3$OD) δ in ppm: 5.66 (dt, $J_{5',4'}$ = 15.2 Hz, $J_{5',6a'} = J_{5',6b'}$ = 6.4 Hz, 1H, H-5'), 5.40 (dd, $J_{4',5'}$ = 15.2 Hz, $J_{4',3'}$ = 6.8 Hz, 1H, H-4'), 4.09 (appt, $J_{3',2'} = J_{3',4'}$ = 6.8 Hz, 1H, H-3'), 3.76 (m, 2H, H-1a', H-2'), 3.55 (dd, $J_{1b',1a'}$ = 13.2 Hz, $J_{1b',2'}$ = 5.2 Hz, 1H, H-1b'), 2.13 (t, $J_{1,2}$ = 7.2 Hz, 2H, H-1), 1.98 (m, 2H, H-6'), 1.53 (m, 2H, H-2), 1.21 (m, 50H, H-alk), 0.80

(m. 6H, H-CH$_3$); ^{13}C NMR (100.6 MHz, 1:4 CDCl$_3$/CD$_3$OD) δ in ppm: 174.7 (CO), 134.0 (C-5'), 128.9 (C-4'), 73.4 (C-3'), 61.7 (C-1'), 54.8 (C-2'), 36.7 (C-1), 32.4 (C-6'), 31.9 (C-Alk), 29.7 (C-Alk), 29.6 (C-Alk), 29.5 (C-Alk), 29.4 (C-Alk), 29.4 (C-Alk), 29.3 (C-Alk), 29.2 (C-Alk), 25.8 (C-Alk), 22.7 (C-Alk), 14.1 (CH$_3$); FT-IR (neat) υ in cm^{-1}: 3309, 2917, 2849, 1642, 1548, 1467, 780, 628; HRMS (TOF ES+) for: C$_{36}$H$_{71}$NNaO$_3$$^+$ (*m/z*): calc. 588.5326; found: 588.5296.
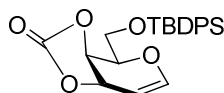
1,5-Anhydro-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-D-*lyxo*-hex-1-enopyranose (3.14)[27]

D-Galactal (**3.13**) (570 mg, 3.900 mmol) was diluted in DMF (1.7 mL). Triethylamine (1.6 µL, 11.70 mmol) and *t*-butyldiphenylsilyl chloride (1.2 µL, 4.290 mmol) were added. The reaction was stirred at room temperature for 3 h and then quenched by adding ethyl acetate and washing with water. After concentration of the organic layer, the crude was purified by flash chromatography using 3:7 ethyl acetate/hexanes as eluent to afford **3.14** as a colorless oil (910 mg, 61% yield): Rf (3:7 ethyl acetate/hexanes): 0.20; [α]$_D$^{20}: +1.60º (*c* = 2.2, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.75-7.69 (m, 4H, H$_{Ar}$), 7.48-7.40 (m, 6H, H$_{Ar}$), 6.37 (d, $J_{2,1}$ = 6.4 Hz, 1H, H-1), 4.72 (dd, $J_{1,2}$ = 6.4 Hz, $J_{2,3}$ = 1.6 Hz, 1H, H-2), 4.37 (brs, 1H, H-3), 4.15 (appt, $J_{3,4}$ = $J_{4,5}$ = 3.6 Hz, 1H, H-4), 4.01 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{6a,5}$ = 7.6 Hz, 1H, H-6a), 3.94-3.91 (m, 2H, H-5, H-6b), 3.15 (d, J = 5.2 Hz, 1H, H-OH), 2.89 (d, J = 10.0 Hz, 1H, H-OH), 1.10 (s, 9H, H-CH$_3$); ^{13}C NMR (100.6 MHz, CDCl$_3$) δ in ppm: 144.5 (C-1), 135.8 (C-Ar), 132.7 (C-Ar), 130.1 (C-Ar), 128.0 (C-Ar), 103.4 (C-2), 76.2 (C-5), 65.8 (C-3), 64.6 (C-4), 63.8 (C-6), 27.0 (CH$_3$), 19.3 (C(CH$_3$)$_3$); FT-IR (neat) υ in cm^{-1}: 3995, 3070, 2930, 2856, 1646, 1471, 1427, 1245, 1112, 745, 690; HRMS (TOF ES+) for C$_{22}$H$_{28}$NaO$_4$Si$^+$ (*m/z*): calc. 407.1649; found: 407.1636.
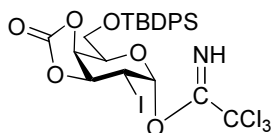
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[27] Gervay, J.; Peterson, J. M.; Oriyama, T.; Danishefsky, S. *J. Org. Chem.* **1993**, *58*, 5465-5468.

1,5-Anhydro-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-D-*lyxo*-hex-1-enopyranose 3,4-carbonate (3.15)[27]

6-*O*-(*tert*-butyldiphenylsilyl)galactal (**3.14**) (900 mg, 2.340 mmol) was treated with carbonyldiimidazole (552 mg, 3.065 mmol) in dry THF at room temperature for 9.5h. The crude was concentrated *in vacuo* and it was purified by flash chromatography using 1:3 ethyl acetate/hexanes as eluent. The protected product **3.15** was isolatated as syrup (675 mg, 70% yield): Rf (1:3 ethyl acetate/hexanes): 0.36; $[\alpha]_D^{20}$: -38.6º (c = 0.98, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.70-7.67 (m, 4H, H-Ar), 7.49-7.40 (m, 6H, HAr), 6.64 (d, $J_{1,2}$ = 6.4 Hz, 1H, H-1), 5.21 (dd, $J_{3,4}$ = 8.0 Hz, $J_{3,2}$ = 3.2 Hz, 1H, H-3), 5.05 (appdt, 1H, H-4), 4.95 (ddd, $J_{2,1}$ = 6.4 Hz, $J_{2,3}$ = 3.2 Hz, $J_{1,4}$ = 1.2 Hz, 1H, H-2), 4.02-3.95 (m, 3H, H-5, H-6a, H-6b), 1.10 (s, 9H, H-CH$_3$); ^{13}C NMR (100.6 MHz, CDCl$_3$) δ in ppm: 154.3 (CO), 149.3 (C-1), 135.7 (C-Ar), 132.9 (C-Ar), 130.2 (C-Ar), 128.1 (C-Ar), 98.2 (C-2), 74.0 (C-5), 73.1 (C-4), 69.1 (C-3), 62.0 (C-6), 27.0 (CH$_3$), 19.4 (C(CH$_3$)$_3$); FT-IR (neat) υ in cm^{-1}: 3067, 2936, 2857, 2361, 1801, 1647, 1428, 1369, 1244, 1110, 1012, 785, 703; HRMS (TOF ES+) for C$_{23}$H$_{26}$NaO$_5$Si$^+$ (*m/z*): calc. 433.1442; found: 433.1434.

6-*O*-(*tert*-butyldiphenylsilyl)-3,4-di-*O*-(carbonyl)-2-deoxy-2-iodo-α-D-*galacto*pyranosyl trichloroacetimidate (3.16)[6]
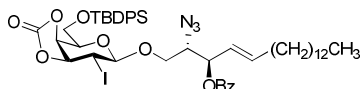

Galactal derivate **3.15** (675 mg, 1.640 mmol) was dissolved in tetrahydrofurane/water 1:1 (32 mL). To the resulting solution was added *N*-iodosuccinimide (443 mg, 1.968 mmol) at room temperature. After 5h of stirring, the starting galactal was consumed and the reaction was quenched by

the addition of 10 % $Na_2S_2O_3$ (20 mL) and the resulting mixture was stirred for 5 min. The mixture was extracted with three portions of ethyl acetate (20 mL each). The organic phase was washed with saturated NaCl solution, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography using 1:2 ethyl acetate/hexanes as eluent to afford the hemiacetal (816 mg) as syrup. This material was used directly in the next reaction.

The hemiacetal (816 mg, 1.476 mmol) was dissolved in dry CH_2Cl_2 (7.5 mL) under argon atmosphere and cooled to 0 ºC. Cl_3CCN (7.5 mL) was added and the solution was allowed to cool for 10 min. DBU (66 µL, 0.443 mmol) was added and the solution became yellow. The solution was stirred for 1.5h and then concentrated. The residue was quickly chromatographed on a short column of silica gel using 1:8 ethyl acetate/hexanes as eluent to obtain the imidate **3.16** (555 mg, 48% yield over two steps) as syrup: Rf (1:4 ethyl acetate/hexanes): 0.43; $[\alpha]_D^{20}$: + 26.6º (c = 4.4, CH_3Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 8.81 (s, 1H, H-CNH), 7.67-7.61 (m, 4H, H-Ar), 7.75-7.36 (m, 6H, H-Ar), 6.32 (d, $J_{1,2}$ = 4.0 Hz, 1H, H-1), 5.26 (dd, $J_{3,2}$ = 7.6 Hz, $J_{3,4}$ = 6.4 Hz, 1H, H-3), 4.87 (dd, $J_{4,3}$ = 6.4 Hz, $J_{4,5}$ = 2.4 Hz, 1H, H-4), 4.51 (ddt, $J_{5,6a}$ = 7.6 Hz, $J_{5,6b}$ = 6.0 Hz, $J_{5,4}$ = 2.4 Hz, 1H, H-5), 4.32 (dd, $J_{2,3}$ = 7.6 Hz, $J_{2,1}$ = 4.0 Hz, 1H, H-2) 3.95 (dd, $J_{6a,6b}$ = 10.8 Hz, $J_{6a,5}$ = 7.6 Hz, 1H, H-6a), 3.88 (dd, $J_{6a,6b}$ = 10.8 Hz, $J_{6b,5}$ = 6.0 Hz, 1H. H-6b), 1.06 (s, 9H, H-CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 160.1 (CNH), 153.1 (CO), 135.7 (C-Ar), 132.8 (C-Ar), 130.2 (C-Ar), 128.0 (C-Ar), 94.1 (C-1), 78.3 (C-3), 74.3 (C-4), 68.7 (C-5), 62.0 (C-6), 27.0 (CH_3), 19.4 ($C(CH_3)_3$), 19.1 (C-2); FT-IR (neat) υ in cm^{-1}: 3337, 3070, 2930, 2857, 1818, 1675, 1471, 1427, 1361, 1274, 1176, 1138, 1105, 1051, 997, 851, 739, 701, 643; HRMS (TOF ES+) for $C_{25}H_{27}Cl_3IKNO_6Si^+$ (m/z): calc. 735.9349; found: 735.9359.
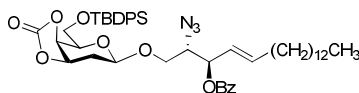
(2S,3R,4E)-2-azido-3-(benzoyloxy)-1-{[6-O-(tert-butyldiphenylsilyl)-3,4-di-O-(carbonyl)-2-deoxy-2-iodo-β-D-galactopyranosyl]oxy}-octadec-4-ene (3.23)[6]

(2R,3S)-2-azido-3-benzoylsphingosine (**3.22**)[17] (30 mg, 0.068 mmol) and imidate **3.16** (73 mg, 0.103 mmol) were codistilled three times with dry toluene in separated flasks. Activated 4 Å MS (50 mg) were added to the sphingosine derivate **3.22** containing flask under argon atmosphere. The mixture was dissolved in dry dichloromethane (1.5 mL) under argon atmosphere and cooled to -78 ºC. Imidate **3.16** was dissolved in dry dichloromethane (2 mL) and then was added via cannula to the sphingosine **3.22** solution. After the mixture was cooled for 10 min to -78 ºC, TBSOTf (8 μL, 0.034 mmol) was added. The reaction was stirred for 18 h at that temperature under argon atmosphere. Then, it was quenched with TEA and warmed to 23 °C. The resulting mixture was diluted with EtOAc and washed with saturated sodium hydrogencarbonate solution, saturated NaCl solution, dried over Na_2SO_4, filtered, and concentrated on a rotary evaporator. The crude was purified by flash chromatography using 1:8 ethyl acetate/hexane as eluent to afford **3.23** as syrup (60 mg, 90% yield): Rf (1:4 ethylacetate/hexane): 0.48; $[\alpha]_D^{20}$: -19.3º (c = 0.51, CH_3Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 8.04 (d, J = 8.4 Hz, 2H, H-Ar), 7.68-7.64 (m, 4H, H-Ar), 7.47-7.39 (m, 9H, H-Ar), 5.96 (dt, $J_{5,4}$ = 14.8 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, 1H, H-5), 5.62 (dd, $J_{3,4}$ = 8.4 Hz, $J_{3,2}$ = 4.0 Hz, 1H, H-3), 5.54 (dd, $J_{4,5}$ = 14.8 Hz, $J_{4,3}$ = 8.4 Hz, 1H, H-4), 5.13 (dd, $J_{3',4'}$ = 7.2 Hz, $J_{3',2'}$ = 6.4, 1H, H-3'), 4.74 (dd, $J_{4',3'}$ = 7.2 Hz, $J_{4',5'}$ = 2.0 Hz, 1H, H-4'), 4.69 (d, $J_{1',2'}$ = 6.8 Hz, 1H, H-1'), 4.09 (m, 1H, H-5'), 3.93 (m, 4H, H-2, H-2', H-6a', H-6b'), 3.80 (dd, $J_{1a,1b}$ = 10.0 Hz, $J_{1a,2}$ = 6.8 Hz, 1H, H-1a), 3.48 (dd, $J_{1b,1a}$ = 10.0 Hz, $J_{1b,2}$ = 6.0 Hz, 1H, H-1b), 2.06 (m, 2H, H-6), 1.30 (m, 22H, CH_2), 1.06 (s, 9H, tBu), 0.88 (t, $J_{18,17}$ = 6.4 Hz, 3H, H-18); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 165.4 (COPh), 153.1 (CO), 139.6 (C-5), 135.7 (C-Ar), 133.4 (C-Ar), 132.8 (C-Ar), 130.2 (C-Ar), 130.1 (C-Ar), 128.6 (C-Ar), 128.0 (C-Ar), 122.8 (C-4), 101.3 (C-1'), 80.3 (C-3'), 75.1 (C-3), 73.5 (C-4'), 72.3 (C-5'), 68.2 (C-1), 63.4 (C-2), 62.1 (C-6'),

32.4 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.43 (CH_2), 29.37 (CH_2), 29.2(CH_2), 28.7(CH_2), 27.0 (CH_3), 22.7 (CH_2), 19.4 ($C(CH_3)_3$), 19.1 (C-2'), 14.1 (C-18); FT-IR (neat) υ in cm^{-1}: 2926, 2854, 2102, 1818, 1723, 1265, 1112, 786, 710; HRMS (TOF ES+) for: $C_{48}H_{64}IN_3NaO_8Si^+$ (*m/z*): calc. 988.3400; found: 988.3363.

(2*S*,3*R*,4*E*)-2-azido-3-(benzoyloxy)-1-{[6-*O*-(*tert*-butyldiphenylsilyl)-3,4-di-*O*-(carbonyl)-2-deoxy-β-D-*galacto*pyranosyl]oxy}-octadec-4-ene (3.24)[28]
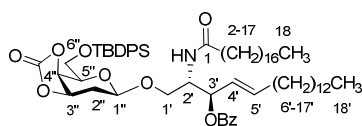

A solution of the glycoside **3.23** (33 mg, 0.034 mmol), Bu_3SnH (23 μL, 0.084 mmol) and Et_3B (8 μL, 1M in hexanes, 0.008 mmol) in 0.4 mL of toluene was stirred at room temperature. An aliquot of the reaction mixture was taken after 30 min and analyzed by 1H NMR and HSQC spectroscopy, showing that the reduction was complete. The reaction mixture was diluted with EtOAc (5 mL) and washed with an aqueous solution of $NaHCO_3$. The organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography using 1:4 ethyl acetate/hexanes as the eluent to afford the product **3.24** (18 mg, 65%) as a syrup: Rf (1:2 ethylacetate/hexane): 0.45; $[\alpha]_D^{20}$: -13.9º (*c* = 1.04, CH_3Cl); ^1H NMR (400 MHz, $CDCl_3$) δ in ppm: 8.04 (d, *J* = 8.4 Hz, 2H, H-Ar), 7.68-7.64 (m, 4H, H-Ar), 7.47-7.39 (m, 9H, H-Ar), 5.91 (dt, $J_{5,4}$ = 14.4 Hz, $J_{5,6a} = J_{5,6b}$ = 6.8 Hz, 1H, H-5), 5.59 (dd, $J_{3,4}$ = 8.4 Hz, $J_{3,2}$ = 4.0 Hz, 1H, H-3), 5.54 (dd, $J_{4,5}$ = 14.4 Hz, $J_{4,3}$ = 8.4 Hz, 1H, H-4), 4.97-4.92 (m, 2H, H-3',H-4'), 4.82 (t, $J_{1',2a'} = J_{1',2b'}$ = 3.6 Hz, 1H, H-1'), 3.96-3.76 (m, 5H, H-5', H-6a', H-6b', H-2, H-1a), 3.41 (dd, $J_{1b,1a}$ = 10.0 Hz, $J_{1b,2}$ = 6.0 Hz, 1H, H-1b), 2.13-2.02 (m, 4H, H-2a', H-2b', H-6a, H-6b), 1.30 (m, 22H, CH_2), 1.06 (s, 9H, *t*Bu), 0.88 (t, $J_{18,17}$ = 6.4 Hz, 3H, H-18); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 165.4 (COPh), 154.4 (CO), 139.0 (C-5), 135.7 (C-Ar), 133.4 (C-Ar), 132.8 (C-Ar), 130.2 (C-Ar), 130.1 (C-Ar), 128.6 (C-Ar), 128.0

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[28] Roush, W. R.; Narayan, S.; *Org. Lett.* **1999**, *1*, 899-902.

(C-Ar), 123.0 (C-4), 96.7 (C-1'), 75.5 (C-3), 72.4 (C-4'), 71.4 (C-5'), 70.8 (C-3'), 67.2 (C-1), 63.5 (C-2), 62.6 (C-6'), 32.4 (CH_2), 32.1 (C-2'), 31.9 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.43 (CH_2), 29.37 (CH_2), 29.2(CH_2), 28.9(CH_2), 27.0 (CH_3), 22.9 (CH_2), 19.4 ($C(CH_3)_3$), 14.1 (C-18); FT-IR (neat) υ in cm^{-1}: 3567, 2926, 2104, 1805, 1718, 1267, 1113, 766, 629; HRMS (TOF ES+) for: $C_{48}H_{65}N_3NaO_8Si^+$ (*m/z*): calc. 862.4433; found: 862.4441.

(2S,3R,4E)-2-N-stearoyl-3-(benzoyloxy)-1-{[6-*O*-(tert-butyldiphenylsilyl)-3,4-di-*O*-(carbonyl)-2-deoxy-β-D-galactopyranosyl]oxy}-4-octadecene (3.25)

On the one hand, to a solution of stearic acid (**3.17**) (71.7 mg, 0.252 mmol) in CH_2Cl_2 (3 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (53.4 mg, 0.272 mmol) and *N*-hydroxysuccinimide (34.8 mg, 0.302 mmol). The mixture was heated at 40 ºC for 3h, and then the mixture was poured into water (20 mL) and extracted with Et_2O (60 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the corresponding *N*-succinimidyl octadecanoate crude[29] (89 mg) was employed without further purification.

On the other hand, PPh_3 (12 mg, 0.045 mmol) and water (6 µL) were added to a stirred solution of azide derivative **3.24** (12.8 mg, 0.015 mmol) in THF (1 mL), and the mixture was stirred at 45ºC until TLC indicated the complete transformation of the starting azide into corresponding amine (about 12 h). After rotary evaporation, the amine residue, the *N*-succinimidyl octadecanoate (12 mg, 0.030 mmol) were redissolved in dry THF (2 mL). Then DIPEA (42 µL, 0.300 mmol) was added and the mixture was stirred at 60 ºC for 18 h. The solution was concentrated under vacuum, and the residue was purified by column chromatography on silica gel using 1:2:8 methanol/ethyl acetate/hexane as

[29] Howarth, N. M.; Lindsell, W. E.; Murray, E.; Preston, P. N. *Tetrahedron* **2005**, *61*, 8875-8887.

eluent to afford **3.25** as syrup (7 mg, 43 % yield over two steps): Rf (1:4 ethylacetate/hexane): 0.28; $[\alpha]_D^{20}$: -16.1° (c = 0.13, CH$_3$Cl); ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.94 (d, J = 7.5 Hz, 2H, H-Ar), 7.76 (t, J = 7.5 Hz, 4H, H-Ar), 7.51-7.38 (m, 9H, H-Ar), 5.81 (dt, $J_{5',4'}$ = 14.4 Hz, $J_{5',6a'} = J_{5,6b'}$ = 6.8 Hz, 1H, H-5'), 5.68 (dd, $J_{4',5'}$ = 14.4 Hz, $J_{4',3'}$ = 8.4 Hz, 1H, H-4'), 5.17 (t, $J_{3',4'} = J_{3',2'}$ = 8.4 Hz, 1H, H-3'), 4.94-4.84 (m, 2H, H-3'',H-4''), 4.80 (t, $J_{1'',2a''} = J_{1'',2b''}$ = 3.5 Hz, 1H, H-1'), 4.42-4.36 (m, 1H, H-2'), 4.03-3.60 (m, 3H, H-1a', H-6a'', H-6b'', H-5''), 3.53 (dd, $J_{1b,1a}$ = 10.0 Hz, $J_{1b,2}$ = 6.0 Hz, 1H, H-1b), 2.16-2.02 (m, 6H, H-2, H-2a'', H-2b'', H-6a', H-6b'), 1.65-1.10 (m, 52H, CH$_2$), 1.06 (s, 9H, tBu), 0.88 (m, 6H, H-18, H-18');^{13}C NMR (100.6 MHz, CDCl$_3$) δ in ppm: 173.8 (NCO), 164.5 (COPh), 154.6 (CO), 136.4 (C-5'), 135.7 (C-Ar), 135.7 (C-Ar), 135.4 (C-Ar), 133.3 (C-Ar), 133.0 (C-Ar), 131.6 (C-Ar), 130.2 (C-Ar), 130.1 (C-Ar), 128.5 (C-Ar), 128.4 (C-Ar), 128.1 (C-Ar), 128.1 (C-Ar), 127.1 (C-Ar), 125.2 (C-Ar), 124.4 (C-4'), 97.3 (C-1''), 83.4 (C-3'), 72.5 (C-4''), 71.3 (C-5''), 71.0 (C-3''), 68.9 (C-2'), 67.3 (C-1'), 62.6 (C-6''), 32.4 (CH$_2$), 32.1 (C-2'), 31.9 (CH$_2$), 29.7 (CH$_2$), 29.6 (CH$_2$), 29.4 (CH$_2$), 29.3 (CH$_2$), 29.2(CH$_2$), 28.9(CH$_2$), 27.0 (CH$_3$), 22.9 (CH$_2$), 19.4 (C(CH$_3$)$_3$), 14.1 (C-18, C-18'); FT-IR (neat) υ in cm^{-1}: 2923, 2853, 1807, 1738, 1461, 1114, 701, 631; HRMS (TOF ES+) for: C$_{66}$H$_{102}$NO$_9$Si $^+$ (m/z): calc. 1080.7318; found: 1080.7323.

1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-iodo-α-D-talose (3.27)[20]

3,4,6-Tri-*O*-acetyl-D-galactal (**3.26**, 690 mg, 2.458 mmol) was dissolved in CH$_3$CN (17 mL) and cooled to -15 °C. CAN (3503 mg, 6.391 mmol) and glacial acetic acid (1.4 mL) were added to the stirring mixture. Next NaI (479 mg, 3.115 mmol) in CH$_3$CN (8 mL) was added dropwise over approximately 30 min. The solution was then allowed to gradually warm to room temperature. After 3 h the reaction was complete, and the mixture was washed with 10% Na$_2$S$_2$O$_3$, saturated aqueous NaHCO$_3$, and brine. The organic layer was then dried over anhydrous Na$_2$SO$_4$, concentrated and purified by column chromatography using

1:2 ethyl acetate/hexanes as the eluent to afford the product **3.27** (539 mg, 93%) as a syrup: Rf (1:1 ethylacetate/hexane): 0.25; $[\alpha]_D^{20}$: +43.2º (c = 0.9, CH$_3$Cl); ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 6.44 (d, $J_{1,2}$ = 1.2 Hz, 1H, H-1), 5.37 (t, $J_{4,3} = J_{4,5}$ = 2.8 Hz, 1H, H-4), 4.83 (dd, $J_{3,2}$ = 4.8 Hz, $J_{3,4}$ = 2.8 Hz, 1H, H-3), 4.35 (ddd, $J_{5,6a}$ = 8.4 Hz, $J_{5,6b}$ = 6.4 Hz, $J_{5,4}$ = 2.8 Hz, 1H, H-5), 4.23 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,1}$ = 1.2 Hz, 1H, H-2), 4.13-4.11 (m, 2H, H-6a, H-6b), 2.13 (s, 3H, CH$_3$), 2.09 (s, 3H, CH$_3$), 2.03 (s, 3H, CH$_3$), 1.99 (s, 3H, CH$_3$); ^{13}C NMR (100.6 MHz, CDCl$_3$) δ in ppm: 170.5 (CO), 170.0 (CO), 169.5 (CO), 168.3 (CO), 96.0 (C-1), 69.1 (C-5), 64.9 (2C, C-3, C-4), 61.5 (C-6), 21.0 (CH$_3$), 20.9 (2C, CH$_3$), 20.7 (CH$_3$), 19.0 (C-2); FT-IR (neat) υ in cm^{-1}: 1749, 1434, 1372, 1218, 1133, 1073, 1051, 994, 939; HRMS (TOF ES+) for: C$_{14}$H$_{19}$INaO$_9$ $^+$ (m/z): calc. 480.9966; found: 480.9957.

2-azido-phytosphingosine (3.33)[30]

TfN$_3$ was freshly prepared prior to the reaction as follows: NaN$_3$ (4592 g, 15 mmol) was dissolved in H$_2$O (11 mL), and the obtained solution was cooled to 0 ºC. CH$_2$Cl$_2$ (11 mL) was added, followed by dropwise addition of Tf$_2$O over 15 min (795 µL, 4.710 mmol) with vigorous stirring of the solution at 0 ºC. After 2 h, an aqueous saturated solution of NaHCO$_3$ (10 mL) was carefully added, while stirring was continued until gas evolution had ceased. The reaction contents were then transferred to a separating funnel, and the phases were separated. The aqueous phase was washed with CH$_2$Cl$_2$ (2 x 15 mL). The combined organic phases were washed with NaHCO$_3$ solution (1 x 20 mL). The resulting solution of TfN$_3$ in CH$_2$Cl$_2$ was concentrated in *vacuo* until a volum of 5 mL and was used in the azidation step without further purification as follows: Amine **1.6** (500 mg, 1.570 mmol) and CuSO$_4$·5H$_2$O (4 mg, 0.015 mmol) were dissolved in H$_2$O (5 mL). The CH$_2$Cl$_2$ solution of TfN$_3$ was then added with vigorous stirring. CH$_3$OH (20 mL) was then added over 5 min. After 18 h, the reaction

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[30] (a) Garcia Diaz, Y. R.; Wojno, J.; Cox, L. R.; Besra, G. S. *Tetrahedron: Asymmetry* **2009**, *20*, 747-753. (b) Alper, P. B.; Hung, S-C.; Wong, C-H. *Tetrahedron Lett.* **1996**, *37*, 6029-6032.

mixture was diluted with H_2O (25 mL) and extracted with ethyl acetate (3 x 25 mL). The combined organic phases were dried over anhydrous Na_2SO_4, concentrated and purified by a silica plug and washed with ethyl acetate until complete elution of the product. Removal of the solvent under reduced pressure afforded azide **3.33** as a white solid (538 mg, 99%): Rf (5:20:75 $NH_4OH/CH_3OH/CH_2Cl_2$): 0.52; mp: 92-93 ºC; $[\alpha]_D^{20}$: +16.2º (c = 0.5, 1:1 $CH_3OH/CHCl_3$); ^{1}H NMR (400 MHz, 1:2 $CD_3OD/CDCl_3$) δ in ppm: 3.82 (dd, $J_{1a,1b}$ = 12.0 Hz, $J_{1a,2}$ = 4.8 Hz, 1H, H-1a), 3.69 (dd, $J_{1a,1b}$ = 12.0 Hz, $J_{1a,2}$ = 4.8 Hz, 1H, H-1b), 3.55-3.52 (m, 2H, H-3, H-4), 3.45-3.42 (m, 1H, H-2), 1.48-1.43 (m, 26H, H-Alk), 0.76 (t, $J_{18,17}$ = 6.0 Hz, H-18); ^{13}C NMR (100.6 MHz, 1:2 $CD_3OD/CDCl_3$) δ in ppm: 74.4 (C-3), 72.2 (C-4), 63.7 (C-2), 61.4 (C-1), 32.0 (C-Alk), 31.9 (C-Alk), 29.6 (C-Alk), 29.6 (C-Alk), 29.3 (C-Alk), 25.8 (C-Alk), 22.6 (C-Alk), 13.9 (C-18); FT-IR (neat) υ in cm^{-1}: 3319, 2915, 2847, 2116, 1463, 1247, 1152, 1070, 1008, 981, 880; HRMS (TOF ES+) for: $C_{18}H_{37}N_3NaO_3$ $^+$ (m/z): calc. 366.2727; found: 366.2723.

(2*S*, 3*S*, 4*R*)-2-Azido-1-*O*-trityl-3,4-octadecanetriol (3.34)[31]

To a solution of phytosphigosine **3.33** (538 mg, 1.570 mmol) in dry CH_2Cl_2 (5 mL) and dry pyridine (3.2 mL) was added chlorotriphenylmethane (525 mg, 1.884 mmol) and the mixture was stirred for 18h under argon atmosphere at room temperature. The resulting mixture was diluted in ethyl acetate (150 mL) and washed with a saturated aqueous $CuSO_4 \cdot 5H_2O$ solution (2 x 25 mL), saturated aqueous ammonium chloride solution (2 x 25 mL) and brine (25 mL). The organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography using 1:4 ethyl acetate/hexanes as the eluent to afford the product **3.34** (699 mg, 76%) as a syrup: Rf (1:4 ethyl acetate/hexanes): 0.31; $[\alpha]_D^{20}$: +9.0º (c = 1, $CHCl_3$); ^{1}H

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[31] Du, W.; Gervay-Hague, J. *Org. Lett.* **2005**, *7*, 2063-2065.

NMR (400 MHz, CDCl$_3$) δ in ppm: 7.49-7.46 (m, 6H, H-Ar3, H-Ar5), 7.33-7.29 (m, 6H, H-Ar2, H-Ar6), 7.26-7.22 (m, 3H, H-Ar4), 3.64 (dd, $J_{1a,1b}$ = 10.0 Hz, $J_{1a,2}$ = 3.6 Hz, H-1a), 3.60 (t, $J_{2,3}$ = $J_{3,4}$ = 4.4 Hz, 1H, H-3), 3.56-3.52 (m, 2H, H-2, H-4), 3.42 (dd, $J_{1b,1a}$ = 10.0 Hz, $J_{1b,2}$ = 3.6 Hz, H-1b), 2.54 (d, J = 5.2 Hz, 1H, OH), 2.00 (d, J = 4.8 Hz, 1H, H-OH), 1.53-1.24 (m, 26H, H-Alk), 0.89 (t, $J_{18,17}$ = 6.4 Hz, H-18); ^{13}C NMR (100.6 MHz, CDCl$_3$) δ in ppm: 143.6 (C-Ar1), 128.7 (C-Ar2, C-Ar6), 128.2 (C-Ar3, C-Ar5), 127.4 (C-Ar4), 87.9 (C(Ph)$_3$), 74.3 (C-3), 72.4 (C-4), 63.7 (C-1), 62.6 (C-2), 32.1 (C-Alk), 31.9 (C-Alk), 29.8 (C-Alk), 29.5 (C-Alk), 29.3 (C-Alk), 25.8 (C-Alk), 22.8 (C-Alk), 14.3 (C-18); FT-IR (neat) υ in cm^{-1}: 3434, 3058, 2922, 2852, 2096, 1490, 1449, 1268, 1071, 1031, 764, 744, 704, 633; HRMS (TOF ES+) for: C$_{37}$H$_{51}$N$_3$NaO$_3$$^+$ (*m/z*): calc. 608.3823; found: 608.3895.

(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzoyl-1-*O*-trityl-1,3,4-octadecanetriol (3.35)
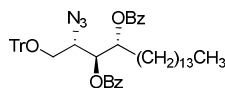
Phytosphigosine **3.34** (585 mg, 1.190 mmol) was dissolved in dry pyridine (4.6 mL) and cooled to 0 ºC. Benzoyl chloride (552 μg, 4.760 mmol) was added dropwise to the stirring mixture under argon atmosphere. After 30 min, the reaction was let warm to room temperature for 1 h. The resulting mixture was diluted in ethyl acetate (150 mL) and washed with a saturated aqueous CuSO$_4$·5H$_2$O solution (2 x 25 mL), saturated aqueous ammonium chloride solution (2 x 25 mL) and brine (25 mL). The organic extracts were dried over MgSO$_4$, filtered and concentrated. The crude product was purified by flash column chromatography using 1:9 ethyl acetate/hexanes as the eluent to afford the product **3.35** (866 mg, 92%) as an oil: Rf (1:4 ethyl acetate/hexanes): 0.45; [α]$_D^{20}$: -6.9º (*c* = 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.97-7.94 (m, 2H, H-Ar), 7.88 (m, 3H, H-Ar), 7.64-7.58 (m, 3H, H-Ar), 7.48-7.42 (m, 9H, H-Ar), 7.29-7.19 (m, 8H, H-Ar), 5.58-5.54 (m, 1H, H-4), 5.50 (dd, $J_{3,4}$ = 6.8 Hz, $J_{2,3}$ = 3.2 Hz, 1H, H-3), 3.98 (dt, $J_{2,1b}$ = 8.0 Hz, $J_{2,1a}$ = $J_{2,3}$ = 3.2 Hz, 1H, H-2), 3.58 (dd, $J_{1a,1b}$ = 10.0 Hz, $J_{1a,2}$ = 3.2 Hz, 1H, H-1a), 3.45 (dd, $J_{1b,1a}$ = 10.0 Hz, $J_{1b,2}$ = 8.0 Hz, 1H, H-1b), 1.86-1.77 (m, 2H, H-5), 1.46-1.21 (m, 24H, H-Alk),

0.90 (t, $J_{18,17}$ = 6.9 Hz, 3H, H-18); ^{13}C NMR (100.6 MHz, CDCl$_3$) δ in ppm: 165.7 (CO), 165.1 (CO), 143.5 (C-Ar), 133.4 (C-Ar), 133.2 (C-Ar), 130.1 (C-Ar), 129.7 (C-Ar), 129.5 (C-Ar), 128.8 (C-Ar), 128.4 (C-Ar), 128.0 (C-Ar), 127.27 (C-Ar), 87.7 (C(Ph)$_3$), 73.1 (C-4), 72.8 (C-3), 63.7 (C-1), 62.0 (C-2), 32.1 (C-Alk), 31.9 (C-Alk), 29.8 (C-Alk), 29.5 (C-Alk), 29.3 (C-Alk), 25.8 (C-Alk), 22.8 (C-Alk), 14.3 (C-18); FT-IR (neat) υ in cm^{-1}: 3060, 2923, 2852, 2097, 1723, 1601, 1490, 1449, 1260, 1092, 1025, 705, 632; HRMS (TOF ES+) for: C$_{37}$H$_{51}$N$_3$NaO$_5$$^+$ (*m/z*): calc. 816.4347; found: 816.4340.

(2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzoyloctadecan-1,3,4-triol (3.36)[31]

To a solution of phytosphigosine **3.35** (831 mg, 1.053 mmol) in dry toluene (4.5 mL) and dry CH$_3$OH (1.5 mL) was added BF$_3$·Et$_2$O (160 μg, 1.264 mmol) dropwise over 5 min under argon atmosphere. The mixture was stirred for 5 h at room temperature. The resulting mixture was quenched with TEA (0.5 mL), diluted in ethyl acetate (150 mL) and washed with a saturated aqueous NaHCO$_3$ solution (2 x 25 mL) and brine (25 mL). The organic extracts were dried over MgSO$_4$, filtered and concentrated. The crude product was purified by flash column chromatography using 1:4 ethyl acetate/hexanes as the eluent to afford the product **3.36** (539 mg, 93%) as a syrup: Rf (1:4 ethyl acetate/hexanes): 0.13; [α]$_D$^{20}: +21.2º (*c* = 0.8, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 8.11-7.99 (m, 4H, H-Ar), 7.64-7.51 (m, 2H, H-Ar), 7.43 (m, 4H, H-Ar), 5.60 (m, 2H, H-3, H-4), 4.09-3.91 (m, 1H, H-1a), 3.95-3.72 (m, 2H, H-1b, H-2), 3.06 (t, $J_{OH,1a}$ = $J_{OH,1b}$ = 5.8 Hz, 1H, OH), 2.09-1.74 (m, 2H, H-5), 1.61-1.09 (m, 24H, H-Alk), 0.91 (t, $J_{18,17}$ = 6.9 Hz, 3H, H-18); ^{13}C NMR (100.6 MHz, CDCl$_3$) δ in ppm: 166.17 (CO), 165.8 (CO), 133.7 (C-Ar), 133.3 (C-Ar), 130.0 (C-Ar), 129.8 (C-Ar), 129.2 (C-Ar), 128.7 (C-Ar), 128.5 (C-Ar), 73.40 (C-4), 72.97 (C-3), 63.3 (C-2), 62.3 (C-1), 32.0 (C-Alk), 29.8 (C-Alk), 29.7 (C-Alk), 29.7 (C-Alk), 29.6 (C-Alk), 29.5 (C-Alk), 29.4 (C-Alk), 25.6 (C-Alk), 22.8 (C-Alk), 14.21 (C-18). FT-IR (neat) υ in cm^{-1}: 3496, 2924, 2853, 2103, 1724, 1451, 1262, 1177,

1069, 1025, 710; HRMS (TOF ES+) for: $C_{32}H_{45}N_3NaO_5^+$ (m/z): calc. 574.3251; found: 574.3250.

### (2S,3S,4R)-2-azido-3,4-di-O-benzoyl-1-O-(3,4,6-tri-O-acetyl-2-deoxy-2-iodo-α-D-*talo*pyranosyl)-1,3,4-octadecantriol (3.43)

Phytosphingosine derivative (**3.36**) (250 mg, 0.452 mmol) and glycosyl donor **3.27** (270 mg, 0.591 mmol) were codistilled three times with dry toluene in separated flasks. Activated 4 Å MS (200 mg) were added to the phytosphingosine derivate **3.36** containing flask under argon atmosphere. The mixture was dissolved in dry dichloromethane (2.5 mL) under argon atmosphere and cooled to -78 ºC. Glycosyl donor **3.27** was dissolved in dry dichloromethane (2.5 mL) and then was added via cannula to the phytosphingosine **3.36** solution. After the mixture was cooled for 10 min to -78 ºC, TMSOTf (26 μL, 0.145 mmol) was added. The reaction was stirred for 18 h at that temperature under argon atmosphere. Then, it was quenched with TEA and warmed to 23 °C. The resulting mixture was diluted with EtOAc and washed with saturated sodium hydrogencarbonate solution, saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated on a rotary evaporator. The crude was purified by flash chromatography using 1:4 ethyl acetate/hexane as eluent to afford **3.43** as syrup (386 mg, 90% yield): Rf (1:4 ethyl acetate/hexanes): 0.13; $[\alpha]_D^{20}$: +21.1º (c = 0.7, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 8.06-7.97 (m, 4H, H-Ar), 7.64-7.55 (m, 2H, H-Ar), 7.51-7.41 (m, 4H, H-Ar), 5.56-5.47 (m, 2H, H-3, H4), 5.40-5.35 (m, 1H, H-4'), 5.32 (s, 1H, H-1'), 4.89 (appt, $J_{3',2'} = J_{3',4'}$ = 4.9 Hz, 1H, H-3'), 4.29 (d, $J_{2',3'}$ = 4.9 Hz, 1H, H-2'), 4.27 (td, $J_{5',6a'} = J_{5',6b'}$ = 6.5 Hz, $J_{5',4'}$ = 1.8 Hz, 1H, H-5'), 4.19-4.08 (m, 3H, H-6a', H-6b', H-1a), 3.99 (ddd, $J_{2,1b}$ = 8.0 Hz, $J_{2,1a}$ = 5.8 Hz, $J_{2,3}$ = 2.8 Hz, 1H, H-2), 3.70 (dd, $J_{1b,1a}$ = 10.6, $J_{1b,2}$ = 8.0 Hz, 1H, H-1b), 2.17 (s, 3H, CH$_3$), 2.06 (s, 3H, CH$_3$), 1.93 (s, 3H, CH$_3$), 1.85 (m, 2H, H-5), 1.50-1.17 (m, 24H, H-Alk), 0.87 (t, $J_{18,17}$ = 6.9 Hz, 1H, H-18); ^{13}C NMR (100.6 MHz, CDCl$_3$) δ in ppm: 170.54 ($\underline{C}$OCH$_3$), 170.0 ($\underline{C}$OCH$_3$), 169.5

($\underline{C}$OCH$_3$), 165.8 (COPh), 165.2 (COPh), 133.8 (C-Ar), 133.5 (C-Ar), 130.0 (C-Ar), 129.9 (C-Ar), 129.8 (C-Ar), 129.6 (C-Ar), 129.18 (C-Ar), 128.81 (C-Ar), 128.66 (C-Ar), 102.6 (C-1'), 72.9 (C-4), 72.7 (C-3), 68.5 (C-1), 67.4 (C-5'), 65.3 (C-4'), 65.1 (C-3'), 62.2 (C-6'), 61.0 (C-2), 32.0 (C-Alk), 30.2 (C-Alk), 29.8 (C-Alk), 29.6 (C-Alk), 29.4 (C-Alk), 25.4 (C-Alk), 22.8 (C-Alk), 21.1 (CH$_3$), 21.0 (CH$_3$), 20.7 (CH$_3$), 20.2 (C-2'), 14.2 (C-18); FT-IR (neat) υ in cm^{-1}: 2925, 2851, 2097, 1752, 1704, 1264, 984, 711; HRMS (TOF ES+) for: C$_{44}$H$_{60}$IN$_3$NaO$_{12}$$^+$ (m/z): calc. 972.3114; found: 972.3080.

(2S,3S,4R)-3,4-di-O-benzoyl-2-N-hexacosanoyl-1-O-(1,3,4,6-tetra-O-acetyl-2-deoxy-2-iodo-α-D-$talo$pyranosyl)-1,3,4-octadecanetriol (3.44)
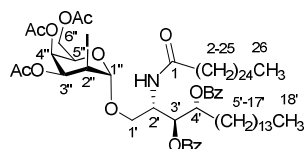
PPh$_3$ (23 mg, 0.088 mmol) and water (10 µL) were added to a stirred solution of azide derivative **3.43** (27.8 mg, 0.029 mmol) in THF (2 mL), and the mixture was stirred at room temperature until TLC indicated the complete transformation of the starting azide into corresponding amine (about 12 h). After rotary evaporation, the amine residue, the hexacosanoic acid (12.6 mg, 0.032 mmol) and HATU (13.7 mg, 0.036 mmol) were redissolved in dry mixture 55:15:30 DMF/CH$_2$Cl$_2$/Et$_2$O (2.75 mL). Then DIPEA (17 µL, 0.102 mmol) was added and the mixture was stirred for 18 h at room temperature. The solution was concentrated under vacuum, and the residue was purified by column chromatography on silica gel using 1:2:8 methanol/ethyl acetate/hexane as eluent to afford **3.44** as syrup (24 mg, 64 % yield over two steps): Rf (1:4:5 methanol/ethyl acetate/hexanes): 0.61; [α]$_D$^{20}: +38.8º (c = 0.47, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl3) δ in ppm: 8.13-8.00 (m, 2H, H-Ar), 8.02-7.90 (m, 2H, H-Ar), 7.64-7.35 (m, 6H, H-Ar), 5.62 (dd, $J_{2'-3'}$ = 8.7 Hz, $J_{3'-4'}$ = 3.0 Hz, 1H, H-3'), 5.47-5.42 (m, 1H, H-4'), 5.31 (d, J_{3-4} = 2.0 Hz, 1H, H-4''), 5.23 (d, $J_{NH,2'}$ = 9.1 Hz, 1H, NH), 5.08 (s, 1H, H-1''), 4.85 (dd, $J_{3'',2''}$ = 4.4 Hz, $J_{3'',4''}$ = 2.0 ,1H, H-3''), 4.54 (tt, $J_{2'-3'}$ = 8.7 Hz, $J_{2',1a'}$ = 3.4 Hz, $J_{2',1b'}$ = 2.9 Hz, 1H, H-2'), 4.22-4.03 (m, 4H, H-2'', H-5'', H-6a'', H-6b''), 3.85 (dd, $J_{1a'-1b'}$ = 10.5 Hz, $J_{1a'-2'}$ =

3.4 Hz, 1H, H-1a'), 3.58 (dd, $J_{1b',1a'}$ = 10.5 Hz, $J_{1b'-2'}$ = 2.9 Hz, 1H, H-1b'), 3.47-3.23 (m, 2H. H-2), 2.17-2.11 (m, 4H, CH_2), 2.11 (s, 3H, H-Ac), 2.03 (s, 3H, H-Ac), 1.96 (s, 3H, H-Ac), 1.68 (d, 8H, CH_2), 1.52-1.08 (m, 52H, CH_2), 0.94-0.83 (m, 6H, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 170.6 (MeCO), 170.3 (MeCO), 169.7 (MeCO), 166.7 (PhCO), 165.8 (PhCO), 156.3 (NCO), 133.8 (C-Ar1), 133.3 (C-Ar1), 130.2 (C-Ar), 130.0 (C-Ar), 129.9 (C-Ar), 129.6 (C-Ar), 128.9 (C-Ar), 128.6 (C-Ar), 103.3 (C-1''), 74.2 (C-4'), 73. 7 (C-3'), 68.1 (C-1'), 67.5 (C-5''), 65.5 (C-4''), 65.4 (C-3''), 62.1 (C-6''), 50.0 (C-2'), 41.7 (C-2), 32.1 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 28.7(CH_2), 25.8 (CH_2), 22.9 (CH_2), 21.1 (C-Me), 21.0 (C-Me), 20.8 (C-Me), 20.5 (C-2''), 14.3, 14.1 (C-18', C-26); FT-IR (neat) υ in cm^{-1}: 2924, 2850, 1751, 1719, 1653, 1522, 1456, 1276, 1233, 1120, 779, 632; HRMS (TOF ES+) for: $C_{70}H_{112}ILiNO_{13}^+$ (*m/z*): calc. 1308.7333; found: 1308.7354.

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Chapter 4

Phosphine-Free Suzuki-Miyaura Cross-Coupling in Aqueous Media Enables Acces to 2-C-Arylglycosides

4.1. Introduction

In the previous chapters, we focused our efforts to develop new glycosylation procedures to obtain glycolipids of potencial biological interest. In the way to achieve this target, we paid attention to some byproducts obtained during the glycosylation procedures studied in our group due to their high versatility.[1,2] Those compounds were glycals and 2-iodo-glycals. Glycals are not only employed as glycosyl donors[3] but they are also used as a precursors of other compounds of interest because of its polyvalent reactivity[4] (Scheme 4.1.).
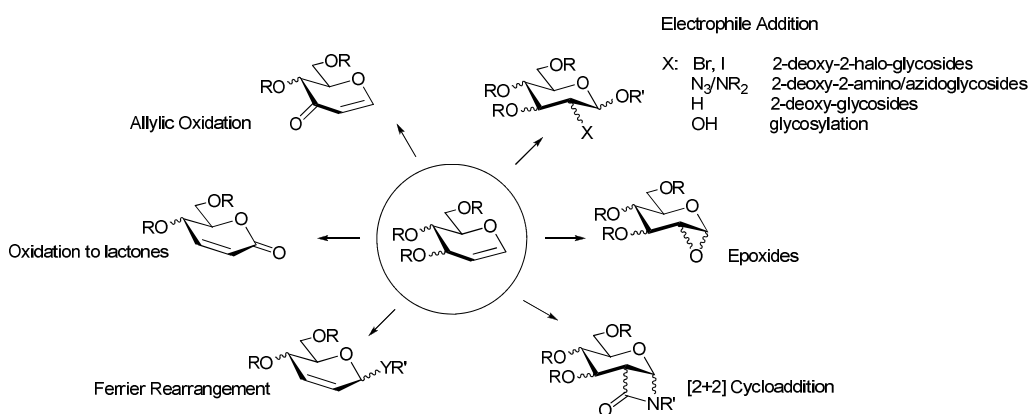
Scheme 4.1. Summary of reactivity of glycals

However, 2-iodo-glycals deserve a special attention because they has been scarcely studied and they could be a new family of versatile starting materials for obtaining branched carbohydrate derivatives through C-C cross-coupling reactions.

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[1] See glycosylation reaction of 2-deoxy-2-iodo-glycosyl donors with stannyl ceramides.

[2] Rodríguez, M. A.; Boutureira, O.; Matheu, M. I.; Díaz, Y.; Castillón, S.; Seeberger, P. H. *J. Org. Chem.* **2007**, *72*, 8998-9001.

[3] Davis, B. G.; Chambers, D.; Cumpstey, I.; France, R.; Gamblin, D. "Synthesis and Activation of Carbohydrate Donors: Acetates, Halides, Phenyl selenides and Glycals" *Carbohydrates*, Academic Press, New York, 2003.

[4] (a) McDonald, F. E.; Gleason, M. M. *J. Am. Chem. Soc*. **1996**, *118*, 6648-6659. (b) McDonald, F. E.; Bowman, J. L. *Tetrahedron Lett.* **1996**, *37*, 4675-4678. (c) McDonald, F. E.; Zhu, H. Y. H. *Tetrahedron* **1997**, *53*, 11061-11068. (d) McDonald, F. E. *Chem. Eur. J.* **1999**, 5, 3103-3106. (e) McDonald, F. E.; Reddy, K. S.; Diaz, Y. *J. Am. Chem. Soc.* **2000**, *122*, 4304-4309.

C-Arylglycosides are members of the *C*-glycosides[5] family of carbohydrate mimetics and their synthesis has attracted considerable interest due to the presence of such motifs in several naturally occurring bioactive products.[6] Many methods have been developed for the synthesis of 1-*C*-arylglycosides, where a carbon atom substitutes the anomeric glycosidic oxygen.[7] The most common method for 1-*C*-arylglycoside synthesis involves the use of catalytic transition metal-catalyzed reactions, in particular the addition of organometallic species to the sp^2-hybridized anomeric center of glycals.[8] Regioselectivity may be efficiently controlled using a directing halogen atom at the anomeric position[9] (*e.g.* 1-haloglycals). However, and when this regiocontrol element is missing, reactions often lead to the formation of Ferrier and other 2,3-unsaturated products due to β-elimination processes.[8]

Although the high demand for functionalized 1-*C*-arylglycosides stimulated extensive studies on metal-catalyzed C-C bond-forming reactions,[10] the development of more efficient methods that involve arylation at other positions is highly desirable.

[5] (a) Štambaský, J.; Hocek, M.; Kočovský, P. *Chem. Rev.* **2009**, *109,* 6729-6764. (b) Yuan, X.; Linhardt, R. J. *Curr. Top. Med. Chem.* **2005**, *5*, 1393-1430. (c) Du, Y.; Linhardt, R. J.; Vlahov, I. R. *Tetrahedron* **1998**, *54*, 9913-9959. (d) Beau, J.-M.; Gallagher, T.; *Top. Curr. Chem.* **1997**, *187*, 1-54. (e) Sinaÿ, P. *Pure Appl. Chem.* **1997,** *69*, 459-464. (f) Postema, M. H. D. *Tetrahedron* **1992**, *48*, 8545-8599.

[6] (a) Bililign, T.; Griffith, B. R.; Thorson, J. S. *Nat. Prod. Rep.* **2005**, *22,* 742-760. (b) Hultin, P. G. *Curr. Top. Med. Chem.* **2005**, *5*, 1299-1331. (c) Zou, W. *Curr. Top. Med. Chem.* **2005**, *5*, 1363-1391. (d) Moose, E. V.; Ben, R. N. *Curr. Top. Med. Chem.* **2005**, *5*, 1351-1361. (e) Compain, P.; Martin, O. R. *Bioorg. Med. Chem.* **2001**, *9*, 3077-3092. (f) Nicotra, F. *Top. Curr. Chem.* **1997**, *187*, 55-83.

[7] (a) Subrahmanyam, A. V.; Palanichamy, K.; Kaliappan, K. P. *Chem. Eur. J.* **2010**, *16*, 8545-8556 and references therein. (b) Lee, D. Y. W.; He, M. *Curr. Top. Med. Chem.* **2005**, *5*, 1333-1350. (c) Martin, S. F. *Pure Appl. Chem.* **2003,** *75*, 63-70.

[8] (a) Xiang, S.; Cai, S.; Zeng, J.; Liu, X.-W. *Org. Lett.* **2011**, *13*, 4608-4611. (b) Bai, Y.; Leow, M.; Zeng, J.; Liu, X.-W. *Org. Lett.* **2011**, *13*, 5648-5651. (c) Xiong, D.-C.; Zhang, L.-H.; Ye, X.-S. *Org. Lett.* **2009**, *11*, 1709-1712. (d) Li, H.-H.; Ye, X.-S. *Org. Biomol. Chem.* **2009**, *7*, 3855-3861. (e) de la Figuera, N.; Forns, P.; Fernández, J.-C.; Fiol, S.; Fernández-Forner, D.; Albericio, F. *Tetrahedron Lett.* **2005**, *46*, 7271-7274. (f) Ramnauth, J.; Poulin, O.; Rakhit, S.; Maddaford, S. P. *Org. Lett.* **2001**, *3*, 2013-2015.

[9] (a) Koester, D. C.; Liebeling, M.; Neufeld, R.; Werz, D. B. *Org. Lett.* **2010**, *12*, 3934-3937. (b) Potuzak, J. S.; Tan, D. S. *Tetrahedron Lett.* **2004**, *45*, 1797-1801. (c) Somsák, L. *Chem. Rev.* **2001**, *101*, 81-136. (d) Jeanneret, V.; Meerpoel, L.; Vogel, P. *Tetrahedron Lett.* **1997**, *38*, 543-546.

[10] Gong, H.; Gagné, M. R. *J. Am. Chem. Soc.* **2008,** *130,* 12177-12183.

C-Functionalizations at other positions of the sugar ring leading to C-branched sugars are by far less explored because they usually require many steps,[11] the use of strongly basic organolithium and Grignard reagents,[12] or the use of toxic reagents such as tin or mercury.[13] Particularly, the synthesis of 2-*C*-aryl-carbohydrate moieties[14] is rare even though they can be of potential interest for the development of new biologically active carbohydrate mimetics.

As part of our continuous interest in developing new catalytic tools for the efficient and fast access to carbohydrate derivatives,[15] we envisaged a general strategy for accessing 2-*C*-aryl-glycals (Scheme 4.2) as key intermediates for synthesizing 2-*C*-arylglycosides. We anticipated that this could be achieved through the use of 2-haloglycals as privileged starting materials for this

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[11] (a) Koester, D. C.; Holkenbrink, A.; Werz, D. B. *Synthesis* **2010**, 3217-3242. (b) Yin, J.; Linker, T.; *Org. Biomol. Chem.* **2009**, *7*, 4829-4831. (c) Sridhar, P. R.; Kumar, P. V.; Seshadri, K.; Satyavathi, R. *Chem. Eur. J.* **2009**, *15*, 7526-7529. (d) Elamparuthi, E.; Kim, B. G.; Yin, J.; Maurer, M.; Linker, T. *Tetrahedron* **2008**, *64*, 11925-11937. (e) Bouisset, T.; Gosselin, G.; Griffe, L.; Meillon, J.-C.; Storer, R. *Tetrahedron* **2008**, *64*, 6657-6661. (f) Xavier, N. M.; Rauter, A. P. *Carbohydr. Res.* **2008**, *343*, 1523-1539.

[12] Cleator, E.; McCusker, C. F.; Steltzer, F.; Ley, S. V. *Tetrahedron Lett.* **2004**, *45*, 3077-3080.

[13] Giese, B.; González-Gómez, J. A.; Witzel, T. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 69-70.

[14] (a) Robinson, T. V.; Pedersen, D. S.; Taylor, D. K.; Tiekink, E. R. T. *J. Org. Chem.* **2009**, *74*, 5093-5096. (b) Maurya, S. K.; Hotha, S. *Tetrahedron Lett.* **2006**, *47*, 3307-3310. (c) Willson, M.; Perie, J. *Spectrochim. Acta Part A* **1999**, *55*, 911-917. (d) Sugimura, H.; Osumi, K.; Koyama, T. *Chem. Lett.* **1991**, *20*, 1379-1382. (e) Augé, C.; Gautheron, C.; David, S.; Malleron, A.; Cavayé, B.; Bouxom, B. *Tetrahedron*, **1990**, *46*, 201-214. (f) Lee, J.B.; Scalon, B. *J. Chem. Soc. D, Chem. Comun.* **1969**, *17*, 955-956.

[15] (a) Boutureira, O.; Rodríguez, M. A.; Díaz, Y.; Matheu, M. I.; Castillón, S. *Carbohydr. Res.* **2010**, *345*, 1041-1045. (b) Boutureira, O.; Rodríguez, M. A.; Benito, D.; Matheu, M. I.; Díaz, Y.; Castillón, S. *Eur. J. Org. Chem.* **2007**, 3564-3572. (c) Rodríguez, M. A.; Boutureira, O.; Matheu, M. I.; Díaz, Y.; Castillón, S. *Eur. J. Org. Chem.* **2007**, 2470-2476. (d) Boutureira, O.; Matheu, M. I.; Díaz, Y.; Castillón, S. *Carbohydr. Res.* **2007**, *342*, 736-743. (e) Kövér, A.; Matheu, M. I.; Díaz, Y.; Castillón, S. *Arkivoc* **2007**, 364-379. (f) Boutureira, O.; Rodríguez, M. A.; Matheu, M. I.; Díaz, Y.; Castillón, S. *Org. Lett.* **2006**, *8*, 673-675. (g) Rodríguez, M. A.; Boutureira, O.; Arnés, X.; Díaz, Y.; Matheu, M. I.; Castillón, S. *J. Org. Chem.* **2005**, *70*, 10297-10310. (h) Arnés, X.; Díaz, Y.; Castillón, S. *Synlett* **2003**, 2143-2145.

transformation, featuring a regiocontrol element at the desired C-2 position[16] for posterior Suzuki-Miyaura cross coupling. Although iodo-derivates are preferable over chlorine or bromine for these reactions[17] they have not been used, probably due to the lack of a general method for their preparation.[2]
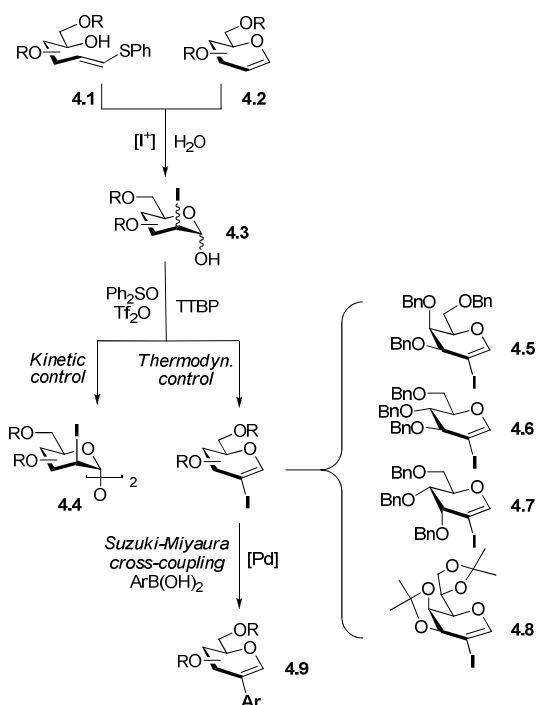
Scheme 4.2. General strategy towards the preparation of 2-aryl-glycals **4.9**

[16] (a) Hayashi, M.; Tsukada, K.; Kawabata, H.; Lamberth, C. *Tetrahedron,* **1999**, *55*, 12287-12294. (b) Hayashi, M.; Amano, K.; Tsukada, K.; Lamberth, C. *J. Chem. Soc., Perkin Trans. 1,* **1999**, 239-240. (c) Chemler, S. R.; Iserloh, U.; Danishefsky, S. J. *Org. Lett*. **2001**, *3*, 2949-2951. (d) Leibeling, M.; Milde, B.; Kratzert, D.; Stalke, D.; Werz, D. B. *Chem. Eur. J.* **2011**, *17*, 9888-9892. (e) Leibeling, M.; Koester, D. C.; Pawliczek, M.; Schild, S. C.; Werz, D. B. *Nat. Chem. Biol.* **2010**, *6*, 199-201. (e) Leibeling, M.; Koester, D. C.; Pawliczek, M.; Kratzert, D; Dittrich, B.; Werz, D.B. *Bioorg. Med. Chem.* **2010***, 18,* 3656-3667.
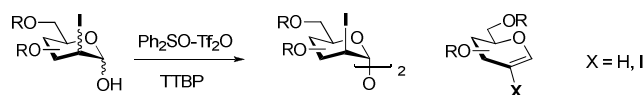
[17] (a) Gómez, A. M.; Pedregosa, A.; Valverde, S.; López, J. C. *Tetrahedron Lett.* **2003**, *44*, 6111-6116. (b) Gómez, A. M.; Danelón, G. O.; Pedregosa, A.; Valverde, S.; López, J. C. *Chem. Commun.* **2002**, 2024-2025.

4.2. Results and Discussion

4.2.1. Preparation of 2-I-glycals from 2-I-lactols

Starting 2-iodoglycals[2] were prepared by treating alkenyl sulfanyl derivatives **4.1** or commercial available glycals **4.2** with iodonium reagents in aqueous media to provide the corresponding 2-deoxy-2-iodo-pyranoses **4.3** (Scheme 4.2.) which were eliminated with Ph_2SO/Tf_2O and TTBP temperatures (-40 ºC or -60 ºC). Under these conditions, mixtures of 2-*I*-glycals (or glycals) or 1,1'-disacharides (trehaloses) were principally obtained (Scheme 4.2.).

Table 4.1. Selected experiments of dehydratation of 2-iodo-2-deoxy-pyranoses carried out in our group

Entry	2-iodo-lactol	Conditions	Product	Yield
1	**4.3a**	-50 ºC, 3h	**4.7**	22%
			4.7'	44%
2	**4.3b**	-60 ºC, 6h	**4.8**	73%
3	**4.3c**	-40 ºC, 5h	**4.4c**	54%
4	**4.3d**	-40 ºC, 5h	**4.4d**	84%

Thus, the examples **4.7** (22 % yield) and **4.8** (73 % yield) were obtained when dehydration of the corresponding 2-deoxy-2-iodo-pyranoses **4.3a** and **4.3b** was

performed respectively at low temperature (-50 ºC or -60 ºC)[2] (Table 4.1., Entries 1 and 2). However, it was observed that under this kinetic control[2,18] 2-deoxy-2-iodo-pyranoses **4.3c** and **4.3d** afforded principally the corresponding trehaloses instead of the desired 2-iodoglycals (Table 4.1., Entries 3 and 4).

Hence, an optimization of the original reaction conditions was necessary to promote the formation of the 2-iodoglycals **4.5** and **4.6**. We speculated that a termodynamical control of the reaction could drive the formation of the 2-iodo-glycals instead of the trehalose derivatives in the case of **4.3c** and **4.3d**. For this reason, once the corresponding 2-deoxy-2-iodo-pyranoses were treated under Ph_2SO/Tf_2O and TTBP promoters at low temperature, we warmed immediately the flask of the reaction to room temperature. For our delight, 2-iodo-glycal **4.5** and **4.6** were isolated in a 74 % and 64 % yield respectively after 5 h of reaction (Scheme 4.3.).
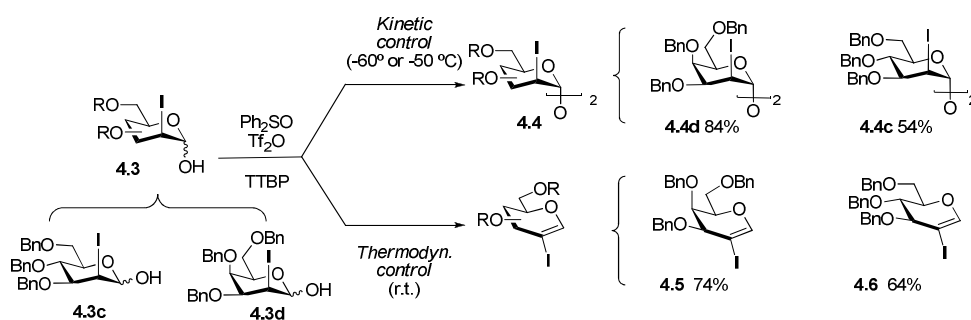
Scheme 4.3. Synthesis of 2-iodo-glycals **4.5** and **4.6**

With 2-iodo-glycals (**4.5**, **4.6**, **4.7** and **4.8**) in hand, the feasibility of the Suzuki-Miyaura coupling was examined below.

[18] Backus, K. M.; Boshoff, H. I.; Barry, C. S.; Boutureira, O.; Patel, M. K.; D'Hooge, F.; Lee, S. S.; Via, L. E.; Tahlan, K.; Barry, C. E.; Davis, B. G. *Nat. Chem. Biol.* **2011**, *7*, 228-235.

4.2.2. Generalities of Suzuki-Miyaura cross coupling and microwave assisted organic chemistry

It is well known that palladium can catalyze carbon-carbon bond formation between aryl or vinyl halides and sulfonates in presence of wide range of organometallic reagents in cross-coupling reactions.[19] The organometallic reagents used include organolithium, organomagnesium, and organozinc reagents, as well as cuprates, stannanes and organoboron compounds. This reaction is quite general for the formation of sp^2-sp^2 and sp^2-sp bonds in biaryls, dienes, polyenes and enynes.

In particular, the Suzuki-Miyaura reaction is the palladium-catalyzed cross coupling process in which a boron organometallic compound is employed.[20] The organoboron reagents that can undergo coupling include boronic acids,[21] boronate esters[22] and boranes.[23] One of the potencial advantages of the Suzuki-Miyaura reaction, especially when boronic acids are used, is that the resulting boronate derivatives are more innocuous by-products than the tin-derivated by-products generated in Stille-type couplings.

The mechanism for the Suzuki-Miyaura reaction is closely related to other cross-coupling methods (Scheme 4.4.). In general, the aryl halide or triflate reacts initially with the Pd(0) catalyst by oxidative addition. Then, the organoboron compound works as the source of the second organic group by transmetallation, and the disubstituted Pd(II) intermediate then undergoes reductive elimination. It appears that either the oxidative addition or the

[19] Diederich, F.; Stang, P. J. *Metal-Catalyzed Cross-Coupling Reactions*, Wiley-VCH, New York, 1998.

[20] Miyaura, N.; Yanagi, T.; Suzuki, A. *Synth. Commun.* **1981**, *11*, 513-519.

[21] (a) Roush, W.R.; Moriarty, K. J.; Brown, B. B. *Tetrahedron Lett.* **1990**, *31*, 6509-6512. (b) Roush, W. R.; Warmus, J. S.; Works, A. B. *Tetrahedron Lett.* **1993**, *34*, 4427-4430. (c) de Lera, A. R.; Torrado, A.; Iglesias, B.; Lopez, S. *Tetrahedron Lett.* **1992**, *33*, 6205-6208.

[22] (a) Ohe, T.; Miyaura, N.; Suzuki, A. *Synlett* **1990**, 221-223. (b) Fu, J.; Zhao, B.; Sharp, M. J.; Sniekus, V. *J. Org. Chem.* **1991**, *56*, 1683-1685.

[23] (a) Ohe, T.; Miyaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, *58*, 2201-2208. (b) Kobayashi, Y.; Shimazaki, H.; Taguchi, H.; Sato, F. *J. Org. Chem.* **1990**, *55*, 5324-5335.

transmetallation can be rate determining, depending on reaction conditions.[24] In the cases in which boronic acids are used as reactants, base catalysis is normally required and is believed that the mechanism involves the formation of the more reactive boronate anion in the transmetallation step.[25] In general, the choice of the base depens on the specific requirements of each reaction. For instance, specific bases as Cs_2CO_3[26] or TlOH[27] have been found preferable than NaOH.
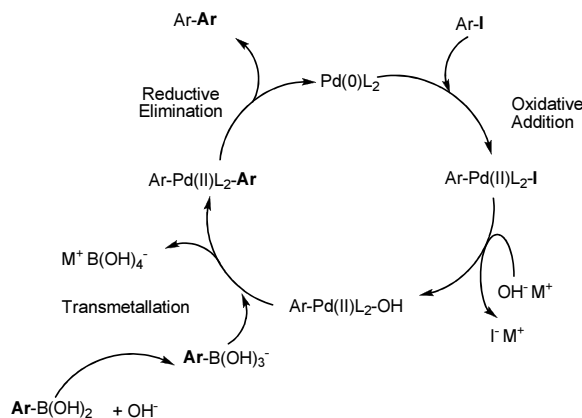
Scheme 4.4. General mechanism for the Suzuki-Miyaura cross-coupling using aryl iodides reagents

In the past decade, driving chemical reactions by microwave energy has become increasingly popular. The use of metal catalysts in conjunction with microwaves may have significant advantages over traditional heating methods since the inverted temperature gradient under microwave conditions may lead to an increased lifetime of catalyst through elimination of wall effects.[28] The first microwave-promoted Suzuki-Miyaura couplings were reported in 1996,[29] and since then a large number of investigations have been reviewed on this subject.[30]

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[24] Smith, G. B.; Dezeny, G. C.; Hughes, D. L.; King, A. D.; Verhoeven, T. R. *J. Org. Chem.* **1994**, *59*, 8151-8156.

[25] Matos, K.; Soderquist, J. B. *J. Org. Chem.* **1998**, *63*, 461-470.

[26] Littke, A. F.; Fu, G. C. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 3387-3388.

[27] Anderson, J. C.; Namli, H.; Roberts, C. A. *Tetrahedron* **1997**, *53*, 15123-15134.

[28] Kappe, C. O. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250.

[29] Larhed, M.; Hallberg, A. *J. Org. Chem.* **1996**, *61*, 9582-9584.

[30] Polshettiwar, V.; Decottignies, A.; Len, C.; Fihri, A. *Chem. Sus. Chem.* **2010**, *3*, 502-522.

Microwave irradiation is the electromagnetic irradiation in the frequency range of 0.3 to 300 GHz. The energy of the microwave photon in this frequency region (0.0016 eV) is too low to break chemical bonds and is also lower than the energy of Brownian motion. Therefore microwaves can not induce chemical reactions.[31]

Microwave-enhanced chemistry is based on the efficient heating of materials by "microwave dielectric heating" effects.[32] This phenomenon is dependent on the ability of a specific solvent or reagent to absorb microwave energy and convert it into heat. The electric component of an electromagnetic field causes heating by two main ways: dipolar polarization and ionic conduction.

According to dipolar polarization mechanism, when a sample is irradiated at microwave frequencies, the dipoles or ions start aligning in the applied electric field. As the applied field oscillates, the dipole or ion field attempts to realign itself with the alternating electric field (Figure 4.1.). In the process, energy is lost in the form of heat through molecular friction and dielectric loss. The generation of heat is related to the ability of the matrix to align itself with the frequency of the applied field. If the dipole does not have enough time to realign, or reorients too quickly with the applied field, no heating occurs.
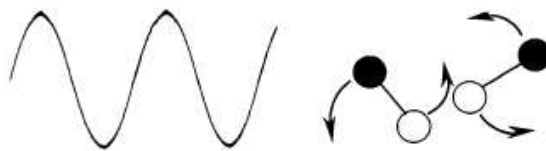
Figure 4.1. Dipolar molecules that try to align with an oscillating electric field

According to ionic conduction mechanism, if a sample contains ions, they will move through the solution under the influence of an electric field, resulting in expenditure of energy due to an increased collition rate, converting the kinetic

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[31] (a) Stuerga, D.; Delmotte, M. *Microwaves in Organic Synthesis*, Wiley-VCH, Weinheim, 2002. (b) M. D. P. Mingos Microwave-Assisted Organic Synthes, Blackwell, Oxford, 2004
[32] Lidström, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, 9225-9283.

energy to heat (Figure 4.2.). In general, this interaction results much stronger than the dipolar mechanism.
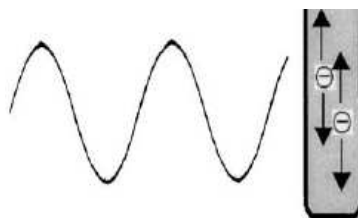
Figure 4.2. Charged compounds in a solution will follow the applied electric field

The ability of a substance to transform electromagnetic energy into heat at a given frequency and temperature is determined by the loss factor $\tan\delta$. This loss factor is expressed as the quotient $\tan\delta = \varepsilon''/\varepsilon'$, where ε'' is the dielectric loss, which is the efficiency with which electromagnetic radiation is converted into heat, and ε' is the dielectric constant describing the ability of molecules to be polarized by the electric field. A reaction medium with a high $\tan\delta$ value is more effitient for absorption and, consequently, for rapid heating. In general, solvents can be classified as high ($\tan\delta > 0.5$), medium ($\tan\delta$ 0.1-0.5), and low microwave absorbing ($\tan\delta < 0.1$). Values of $\tan\delta$ for solvent have been reported at literature.[33] Importantly, it has to be considered that a low $\tan\delta$ value does not discart a particular solvent from being used in a microwave-heated since the overall dielectric properties of the reaction medium will in most cases allow sufficient heating by microwaves.

The observed rate accelerations and sometimes altered product distributions compared to conventional oil-bath experiments have led to speculation on the existence of so-called "specific" or "nonthermal" microwave effects.[34]

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[33] Hayes, B. L. *Microwave Synthesis: Chemistry at the Speed of Light* CEM Publishing, Matthews NC, 2002.

[34] (a) Westaway, K. C.; Gedye, R. *J. Microwave Power* **1995**, *30*, 219-230. (b) Langa, F.; de la Cruz, P.; de la Hoz, A.; Díaz-Ortiz, A; Díez-Barra, E. *Contemp. Org. Synth.* **1997**, *4*, 373-386. (c) Perreux, L.; Loupy, A. *Tetrahedron* **2001**, *57*, 9199-9223. (d) Kuhnert, N. *Angew. Chem. Int. Ed.* **2002**, *41*, 1863-1866. (e) Strauss, C. R. *Angew. Chem. Int. Ed.* **2002**, *41*, 3589-3590.

However, most of scientists agree that in the majority of cases the reason for the observed rate enhancements is a thermal/kinetic effect as a consequence of the high reaction temperatures that can rapidly be attained when irradiating polar materials in a microwave field.[28]

4.2.3. Studing the scope of the microwave assisted phosphine-free Suzuki-Miyaura coupling on 2-iodo-glycals

A significant advance in Suzuki-Miyaura chemistry has been the observation that these couplings can be carried out using water as the solvent in conjunction with microwave heating.[35] Water is a nonexpensive, readily available, non-toxic, and non-flammable solvent so it has clear advantages in organic synthesis. With its comparatively medium loss factor (tanδ) of 0.123, water is also a potentially very useful solvent for microwave-mediated synthesis, especially in the high-temperature region accessible by using sealed vessel technology. Moreover, although palladium catalyst usually employes phosphine ligands to stabilize Pd(II) intermediates, efforts for effecting Suzuki-Miyaura coupling in absence of phosphine ligands have been alredy reported.[36] In particular, Davis and co-workers reported the sodium salt of 2-amino-4,6-dihydroxypyrimidine ligand forms a complex with Pd(OAc)$_2$ that is freely soluble in water (Figure 4.3.).

This phosphine free catalyst is active enough to mediate hindered, *ortho*-substituted biaryl couplings but mild enough for using on peptides and proteins under Suzuki-Miyaura conditions.[37] In a collaboration work with Prof. B.G. Davis group, we have acces to such Pd catalyst which let us to design a Suzuki-Miyaura methodology to afford 2-*C*-arylglycals from 2-iodoglycals.

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[35] (a) Leadbeater, N. E.; Marco, M. *Org. Lett.* **2002**, *4*, 2973-2976. (b) Leadbeater, N. E.; Marco, M. *J. Org. Chem.* **2003**, *68*, 888-892. (c) Bai, L.; Wang, J.-X.; Zhang, Y. *Green Chem.* **2003**, *5*, 615-617. (d) Leadbeater, N. E.; Marco, M. *Angew. Chem. Int. Ed.* **2003**, *42*, 1407-1409. (e) Leadbeater, N. E.; Marco, M. *J. Org. Chem.* **2003**, *68*, 5660-5667.

[36] (a) Wallow, T. L.; Novak, B. M. *J.Org. Chem.* **1994**, *59*, 5034-5037. (b) Badone, D.; Cardamone, M. B. R.; Ielmini, A.; Guzzi, U. *J. Org. Chem.* **1997**, *62*, 7170-7173.

[37] Chalker, J.M.; Wood, C.S.C.; Davis, B.G. *J. Am. Chem. Soc.* **2009**, *131,* 16346-16347.
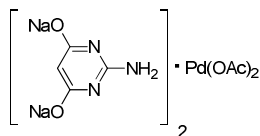
Figure 4.3. Phospine free Pd catalyst employed by Davis and co-workers in Suzuki-Miyaura cross-coupling

Table 4.2. Optimization of the reaction conditions of microwave-mediated palladium- catalyzed cross-coupling of iodogalactal **4.5** with PhB(OH)$_2$.[a]
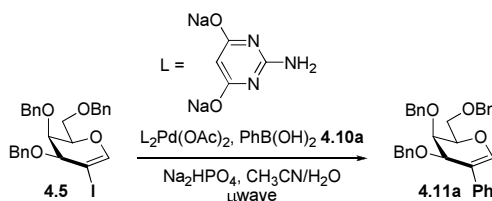

Entry	L$_2$Pd(OAc)$_2$ (mol %)	CH$_3$CN/H$_2$O (v/v)	T (ºC)	t (min)	Yield (%)[b]
1	2	1:3	100	300	82
2	0.1	1:3	100	30	NR[c]
3	2	1:1	100	30	90
4	5	1:1	80	190	84
5	2	1:1	40	720	NR[c]
6[d]	2	1:1	125	5	95

[a] Reactions were performed in a sealed vessel under single-mode microwave irradiation (65 W) with 2-iodo-glycal (1 equiv), PhB(OH)$_2$ (1.5 equiv), L$_2$Pd(OAc)$_2$ (up to 5 mol %), and Na$_2$HPO$_4$ (5 equiv) in solvent (0.02 M) unless otherwise indicated. [b] Isolated yield. [c] >98% starting material was recovered. [d] Microwave power (300 W). NR: No reaction

Initially, the Suzuki-Miyaura cross-coupling reaction was carried out starting from 2-iodo-tri-*O*-benzylgalactal (**4.5**) and phenylboronic acid (**4.10a**) in CH$_3$CN/H$_2$O 1:3 as the solvent, Na$_2$HPO$_4$ as base and 2% mol of L$_2$Pd(OAc)$_2$, were L= 2-amino-4,6-dihydroxypyrimidine (Table 4.2.). After 4 h at 100 ºC under microwave irradiation compound **4.11a** was isolated in 82% yield (Table

4.2., Entry 1). Attempts to decrease the catalyst loading were (Table 4.2., Entry 2). Changing the solvent ratio from 1:3 to 1:1 CH_3CN/H_2O increase the solubility of the starting material, improved the yield of **4.11a** to 90% after only 30 min at 100 ºC (Table 4.2., Entry 3). Decreasing the temperature had a negative effect in the yield. Thus, when the reaction was carried out at 80 ºC, 190 minutes were required to achieve 84% yield and at 40 ºC no reaction was observed after 12 hours (Table 4.2., Entries 4 and 5). However, an increase of the temperature to 125 °C allowed to obtain the coupling product **4.11a** with an excellent yield (95 %) after only 5 min of reaction (Table 4.2., Entry 6).

Globally, the use of this cheap and environmentally friendly catalyst provides several advantages. First, carrying out the reaction under aqueous conditions is key since the use of *nonpolar* solvents accelerates competing elimination reactions.[38] Secondly, the use of degassed solvents combined with expensive, easily oxidizable phosphines, that are particularly detrimental for the success of this reaction, is avoided. For example, we have observed 40% hydrodehalogenation of 2-iodogalactal **4.5** when reacted with PBu_3 under similar conditions to the optimized for the cross-coupling reaction (Scheme 4.5.).
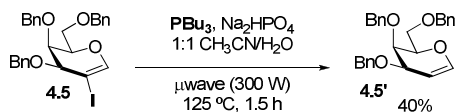

Scheme 4.5. Hydrodehalogenation of 2-iodogalactal **4.5** with PBu_3

The optimized reaction conditions (Table 4.2., Entry 6) were applied to benzyl protected 2-iodoglycals **4.6** and **4.7** and isopropylidene protected 2-iodoglycal (**4.8**) (Table 4.3.). In all cases, and independently of the sugar configuration and protecting groups present, the 2-phenyl-*C*-glycals **4.12**, **4.13** and **4.14** were obtained in excellent yields from the correspondent 2-iodoglycals. Only during the cross-coupling of **4.6** (Table 4.3., Entry 1), traces of tri-*O*-benzyl-D-glucal

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[38] Gong, H.; Sinisi, R.; Gagné, M. R. *J. Am. Chem. Soc.* **2007,** *129,* 1908-1909.

were formed as by-product. Such kind of dehalogenated products are common in typical Suzuki-Miyaura reactions.[39]

Table 4.3. Microwave-mediated palladium-catalyzed cross-coupling of iodoglycals **4.6-4.8 and** ArB(OH)$_2$.[a]
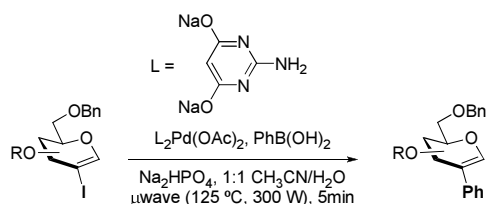
Entry	2-I-glycal	Product	Yield (%)[b]
1[c]	**4.6**	**4.12**	95
2	**4.7**	**4.13**	95
3	**4.8**	**4.14**	96

[a] Conditions: 2-I-glycal (1 equiv), PhB(OH)$_2$ (1.5 equiv), L$_2$Pd(OAc)$_2$ (2 mol %), and Na$_2$HPO$_4$ (5 equiv), in 1:1 CH$_3$CN/H$_2$O (0.02 M), single-mode microwave irradiation (125° C, 300 W). [b] Isolated yield. [c] Traces of tri-*O*-benzyl-D-glucal were also formed. [d] No acid work up was performed.

Encouraged by these results, a variety of arylboronic acids containing representative groups with potential in different imaging modalities (*e.g.* PET, MRI and fluorescence) were examined to expand the scope of the Suzuki-Miyaura cross-coupling with 2-iodoglycal **4.5** (Table 4.4.). Phenylboronic acids

[39] (a) Demchuk, O. M.; Yoruk, B.; Blackburn, T.; Snieckus, V. *Synlett* **2006**, 2908-2913. (b) Potuzak, J. S.; Tan, D. S. *Tetrahedron Lett.* **2004**, *45*, 1797-1801. (c) Navarro, O.; Marion, N.; Oonishi, Y.; Kelly III, R. A.; Nolan, S. P. *J. Org. Chem.* **2006**, *71*, 685-692. (d) Navarro, O.; Kaur, H.; Mahjoor, P.; Nolan, S. P. *J. Org. Chem.* **2004**, *69*, 3173-3180. (e) Urawa, Y.; Naka, H.; Miyazawa, M.; Souda, S.; Ogura, K. *J. Organomet. Chem.* **2002**, *653*, 269-278.

with electron-withdrawing groups (Entries 1, 3 and 7) or electron-donating groups (Entry 2) both afforded excellent results. Similarly, the use sterically hindered boronic acids (Entries 4, and 6), or hetereocyclic derivatives (Entry 7) also afforded excellent results although longer reaction times were required.

Table 4.4. Microwave-mediated palladium catalyzed cross-coupling of 2-iodogalactal **4.5** with arylboronicacids **4.10b-h.**[a]
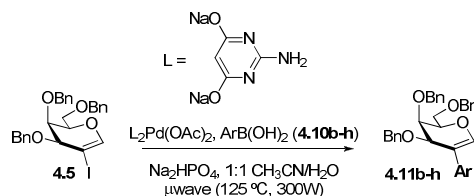
Entry	boronic acid	Ar	Product	t (min)	Yield (%)[b]
1	**4.10b**	4-CN-Ph	**4.11b**	5	95
2	**4.10c**	4-MeO-Ph	**4.11c**	5	95
3	**4.10d**	4-F-Ph	**4.11d**	5	90[c]
4	**4.10e**	2-Me-Ph	**4.11e**	40	90
5	**4.10f**	3-Py	**4.11f**	40	89
6	**4.10g**	1-Naph	**4.11g**	5	94
7	**4.10h**	3,5-(CF$_3$)$_2$-Ph	**4.11h**	5	95

[a] Conditions: 2-I-glycal (1 equiv), ArB(OH)$_2$ (1.5 equiv), L$_2$Pd(OAc)$_2$ (2 mol %), and Na$_2$HPO$_4$ (5 equiv.) in 1:1 CH$_3$CN/H$_2$O (0.02 M), single-mode microwave irradiation (125 °C, 300 W) with. [b] Isolated yield. [c] The arylglycal was recovered with traces of the corresponding glycal.

This effect could be explained because hindered boronic acids or boronic acids with coordinating hetereoatoms could make the transmetallation step slower.[24] Moreover, traces of tri-*O*-benzylgalactal were detected in the reactions with **4.10d** (Table 4.4., Entries 3). In order to better understand the origin of the dehalogenated glycal as a byproduct, some aspects of the Sukuki-Miyaura mechanism were revised for our catalytic system bellow.

### 4.2.4. Mechanistic considerations and phosphine-free hydrodehalogenation

The general Suzuki-Miyaura cross-coupling mechanism with 2-iodoglycals is depicted in Scheme 4.6. The main steps that operate in the transformation of the 2-iodoglycal into a 2-C-arylglycal are: a) the oxidative addition of the 2-iodoglycal to the Pd(0); b) participation of the base (Na_2HPO_4) to activate the arylboronic acid and to exchange an hydroxyl group by halogen in the Pd(II) system; c) transmetalation of the aryl group from the activated boronic acid to the Pd(II) system and d) reductive elimination of Pd(II) to Pd(0) which affords the 2-C-aryl glycal.

However, an hydrodehalogenation step may occur between the oxidative addition (Scheme 4.6., Step a) and the transmetallation step (Scheme 4.6., Step b) although several reports describe this hydrodehalogenation when using phosphine ligands[39a,b] in Suzuki-Miyaura cross-coupling reactions, there are also a few precedents in which reaction also occurs with other ligands different than phosphines.[39c-e] This side reaction is tentatively attributed to the formation of palladium hydride complexes likely by the reaction of the corresponding Pd-I species with the solvent system, typically water (or hydroxide) and several alcohols bearing α-protons (*e.g.* EtOH). In our case, this proton can potentially come either from water or CH_3CN.
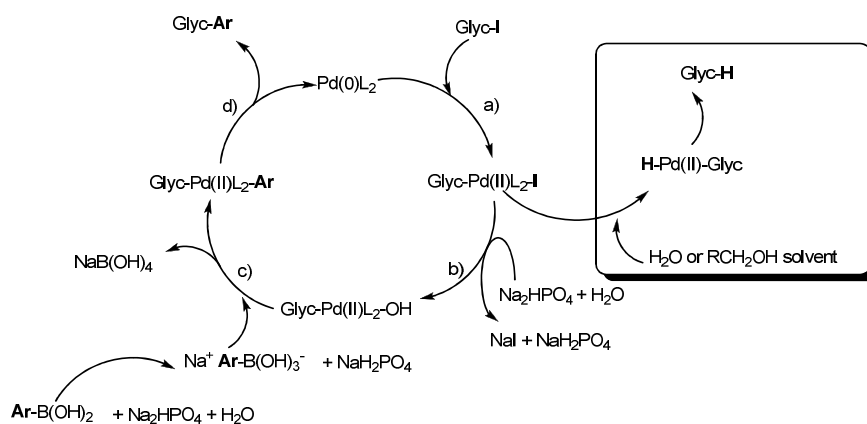

Scheme 4.6. Catalytic cycle for the Suzuki-Miyaura cross-coupling with 2-iodoglycals and putative origin of phosphine-free hydrodehalogenation

In order to confirm that H-2 from hydrodehalogenation comes from water and not from CH$_3$CN and thus gain insight into the mechanism for the formation of glycals, we treated 2-iodoglycal **4.5** under Suzuki-Miyaura cross-coupling conditions in the absence of phenylboronic acid and using mixtures of either D$_2$O or CD$_3$CN with their non-deuterated counterparts as a solvent system (Scheme 4.7.). These reactions were performed at 125 ºC under microwave irradiation for 3 h due to the hydrodehalogention of 2-iodoglycals is not a highly favoured process. Moreover, the initial catalyst load (2 mol%) was increased (gradually up to 15 mol%).

Scheme 4.7. Deuteration experiments with 2-iodoglycal **4.5**
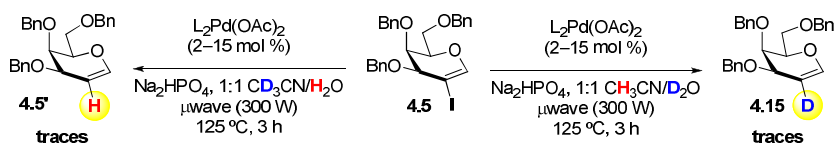
Analysis of the crude reaction mixtures by TLC and HRMS revealed the high stability of 2-iodoglycal **4.5** toward the Suzuki-Miyaura conditions in the absence of boronic acid, detecting only traces of both dehalogenated products. Interestingly, these experiments strongly suggest that H-2 comes from water as evidenced by the traces of 2-*d*-glycal detected only when D$_2$O was used as a deuterium source. Figure 4.4. depicts the high resolution mass spectra for 2-*d*-3,4,6-tri-*O*-benzyl-D-galactal (**4.15**) from the crude of the reaction performed in D$_2$O. The isotopic pick distribution for the molecular formula C$_{27}$H$_{27}$DNaO$_4^+$ (*m/z*) corresponding to the (M +Na)$^+$ ion was 440.1930 (I = 100 %), 441.1976 (I = 30%) and 442.8450 (I = 5%) which fits well with the stimation of theoretical isotope distribution. The pick corresponding to 439.1870 can be associated to the principal isotopic mass pick of the ion (M+Na)$^+$ of 3,4,6-tri-*O*-benzyl-D-galactal (**4.15**) (calc. 439.1880 for C$_{27}$H$_{28}$NaO$_4^+$). Hence, some non-deuterated galactal may be formed because of the exchange of D$_2$O with a labile H$^+$ in the system or by the inherent presence of amounts of H$_2$O in commercial D$_2$O. On the other hand, no 2-*d*-glycal **4.15** was detected when CD$_3$CN was employed instead; rather this reaction afforded traces of non-deuterated glycal.
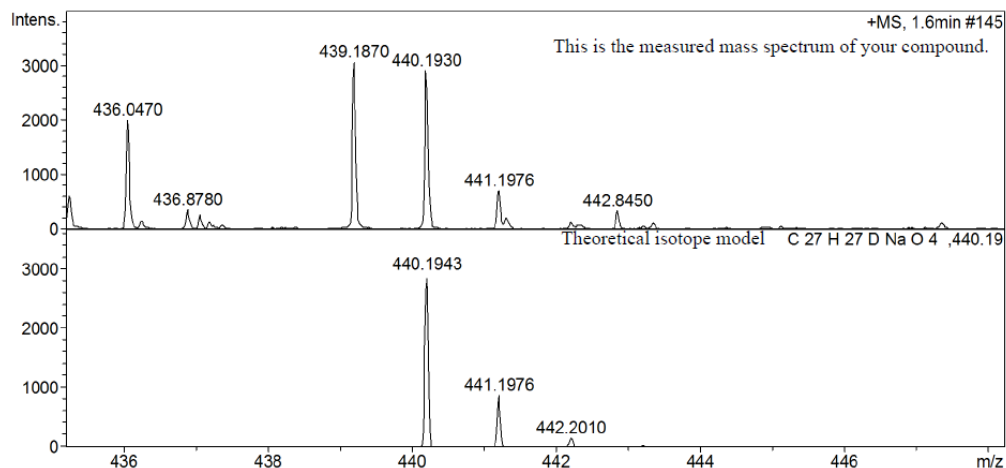
Figure 4.4. High resolution mass spectra for 2-*d*-3,4,6-tri-*O*-benzyl-D-galactal from the crude of the reaction performed in D_2O

4.2.5. Synthetic applications of 2-*C*-aryl-glycals: formation of alditols, epoxides and 2-*C*-aryl-glycosides

Having studied the Suzuki-Miyaura cross-coupling reaction with 2-iodoglycals, the potential of resulting 2-*C*-arylglycals as intermediates for the preparation of 2-*C*-arylglycoconjugates was investigated.

For instance, 1,5-anhydro-alditols are rarely occurring sugar derivative, being most of them biologically active compounds. For this reason, several methods for their syntheses are known.[40] However, to the best of our knowledge no examples for the synthesis of 2-deoxy-2-*C*-aryl-1,5-anhydro-alditol mimetics are reported. Hence, we decided to start the study of the chemical reactivity 2-*C*-aryl-glycals employing the hydrogenation reaction to obtain the first examples of 2-deoxy-2-*C*-aryl-1,5-anhydro-alditols.

Thus, 2-*C*-phenyl-2-deoxy-3,4,5-tri-*O*-benzyl-D-glucal (**4.12**) was efficiently hydrogenated with of Pd/C catalyst in methanol for 12 h to provide 2-*C*-phenyl-2-deoxy-1,5-anhydro-alditols in a 95 % yield with *manno* **4.16** and *gluco* **4.17** configuration and a diasteroisomeric ratio 10:1 (Scheme 4.8.). These

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[40] Nagarajan, M.; Murali, R. *Carbohydr. Res.* **1996**, *280*, 351-355.

configurations were evidencied by the analysis of diagnostic coupling constants between protons H-2 and H-3. In the case of **4.17**, the value of $^3J_{2,3}$ (6.0 Hz) indicates that the phenyl group has an axial configuration whereas the value of $^3J_{2,3}$ of **4.17** (12.0 Hz) indicates that the phenyl group is in a equatorial configuration. These observations were also confirmed by the key NOE correlations. For example, correlation between H-4 and protons of phenyl group and also between H-2 and H-3 was observed in the case of **4.16** whereas correlation between H-3 and protons group of phenyl and between H-2 and H-4 was observed in the case of **4.17** (Scheme 4.8.).
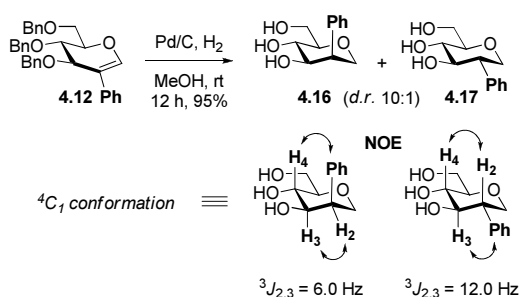

Scheme 4.8. Hydrogenation of 2-*C*-phenyl-2-deoxy-3,4,5-tri-*O*-benzyl-D-glucal (**4.12**)

In order to expand our studies on the synthetic applications of the 2-*C*-aryl glycals in organic chemistry, we attracted our attention in the preparation of challenging quaternary 2-*C*-aryl moieties. It is well known that glycals are useful synthons for the preparation of 2-hydroxy pyranosides via the corresponding 1,2-anhydropyranosides.[41] Several methods in direct oxidative glycosylation of simple glycals have been studied by Gin and co-workers.[41b-d] They have observed that the reaction pathway involved the enol ether moiety of the glycal which was activated with a triflyl sulfoxide, followed by oxygen transfer to the pyranose ring from the excess of sulfoxide reagent. Then, the presence of a nitrogenated base and MeOH promoted the formation of the oxirane moiety. It is worthy to note that these steps were carried out at low temperatures (-78 ºC) in order to preserve the stability of the intermediates that

[41] (a) Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1380-1419. (b) Di Bussolo, V.; Kim, Y.-J.; Gin, D.Y. *J. Am. Chem. Soc.* **1998**, *120*, 13515-13516. (c) Kim, J.-Y.; Di Bussolo, V.; Gin. D.Y. *Org. Lett.* **2001**, *3*, 303-306. (d) Honda, E.; Gin, D.Y. *J. Am. Chem. Soc.* **2002**, *124*, 7343-7352.

promote the formation of the oxirane ring. Once the ring is formed, the temperature can be increased slightly. Then, the epoxide is activated in presence of a Lewis acid in order to allow its ring-opening by a nucleophile. In this way, the glycoside is afforded in a *one-pot* manner. Moreover, depending on the nature of the sulphoxide specie employed (DBTO or Ph$_2$SO) is possible to control the stereochemistry of the epoxide ring to obtain the glycoside with 1,2-*trans* or 1,2-*cis* relative configuration (Scheme 4.9.). Thus, the reaction of tri-*O*-benzylglucal (**4.18**) with DBTO as sulphoxide agent, Tf$_2$O, ^{i}Pr$_2$NEt, MeOH, LiClO$_4$ and NaN$_3$ as glycosyl acceptor at afforded the α-*manno*-glycosyl azide **4.23** via oxirane **4.22** in a 73% yield. However, when the same substrate was treated under similar conditions but using Ph$_2$SO as the sulphoxide agent and TEA as a base, the β-*gluco*-glycosyl azide **4.25** was excusively obtained in a 79% yield via oxirane **4.24**.[41d]
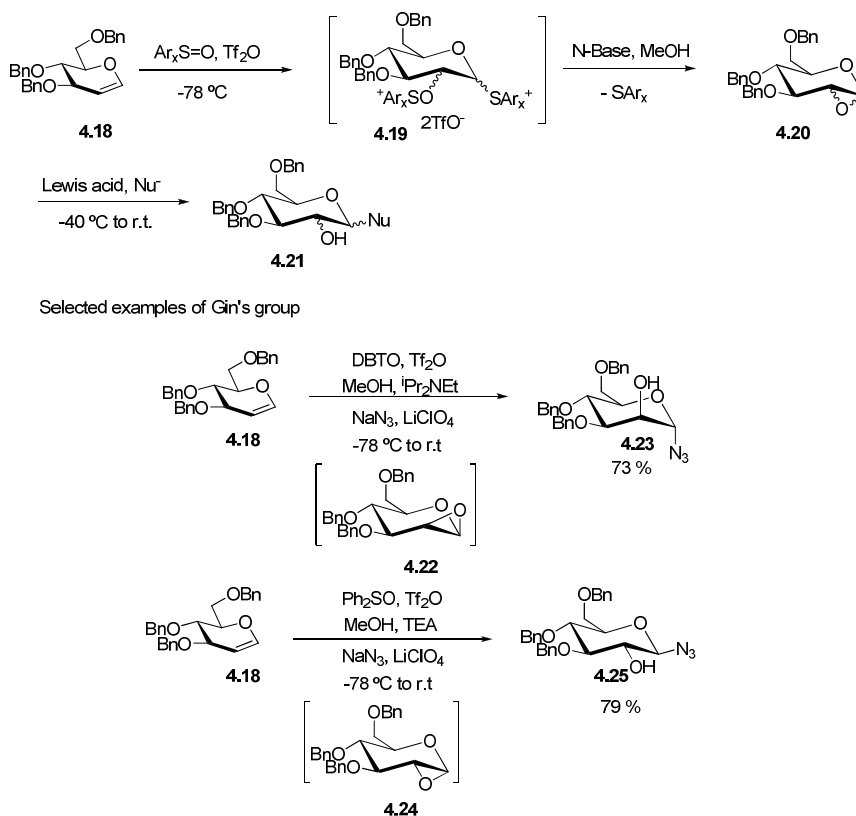

Scheme 4.9. Direct oxidative glycosylation pathway postulated by the group of Gin

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In our case, when direct oxidative glycosylation methods[41b-d] where applied to 2-*C*-phenyl-2-deoxy-3,4,5-tri-*O*-benzyl-D-glucal (**4.12**) using Ph_2SO or DBTO, Tf_2O and DTBMP, and glycosylation promoters (MeOH, TEA, H_2O, $ZnCl_2$) at low temperatures (-80 °C or -40 °C), only the starting material was recovered. Other attemps to carry out the reaction at higher temperatures (*e.g.* 0 ºC) only yielded complex sugar mixtures due to the decomposition of the epoxidation agents.[41d]

At this point, we considered that an epoxidation procedure which could be carried out at higher temperatures, such as Shi epoxidation, may be more suitable for our system. Using Shi's protocol,[42] the epoxidizing species are believed to be dioxiranes, which are powerful epoxidation reagents (Scheme 4.10.). These are not indefinitely stable, but can be generated *in situ* by oxidation of a ketone with potassium peroxymonosulfate (Oxone®). The intermediate sulphate formed, as a good leaving group, facilitates the ring closure to the dioxiranes. Taking into account that the ketone is regenerated, only catalytic amounts are needed. Reactions are conducted in buffered, often biphasic mixtures with phase transfer catalysts. Addition of K_2CO_3 to the reaction mixture increases the rate of formation of the dioxirane but also lowers the stability of Oxone®. However, at higher pH the Bayer-Villiger Oxidation as a side reaction is disfavoured, so the catalysts remain more active. Therefore the selfdecomposition of Oxone® at high pH can be avoided if the ketone is reactive enough. The enhancements in reaction rate can also be explained by a higher nucleophility of Oxone® under more basic conditions. In any case, a careful use of buffered media is often needed. Once the dioxirane is formed, the epoxidation of the alkene occurs by a concerted mechanism (Scheme 4.10.).[42]

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[42] Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry. Part B: Reactions and Synthesis*, Springer, New York, 2007.
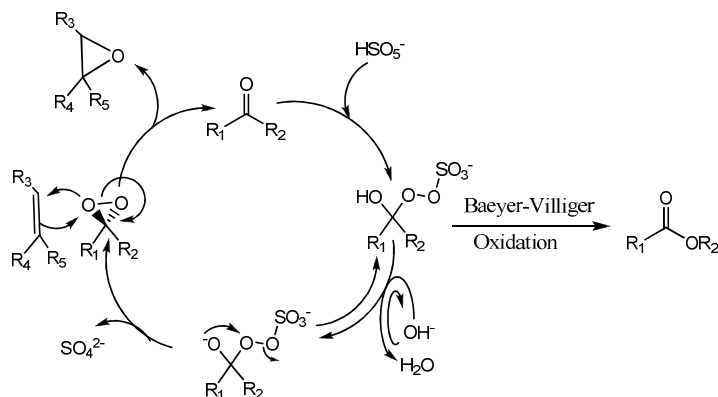
Scheme 4.10. Epoxidation of alkenes with a dioxirane agent

When 2-*C*-phenyl-2-deoxy-3,4,5-tri-*O*-benzyl-D-glucal (**4.12**) was treated with Oxone® and acetone[43] in a biphasic DCM-aqueous NaHCO$_3$ mixture from 0 °C to room temperature (Scheme 4.11.) compound **4.26** was quantitatively isolated after 9 h of reaction.
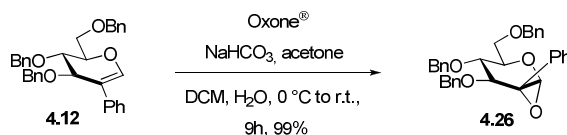
Scheme 4.11. Epoxidation of 2-*C*-arylglycal **4.12** under dioxyrane conditions
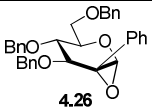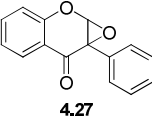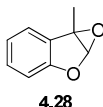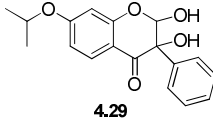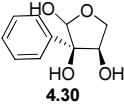
The formation of epoxide **4.26** under Shi's conditions instead was evidenced by comparison of δ ^{13}C-1 and δ ^{13}C-2 NMR data of those **4.26** with reported 2-aryl-*C*-oxiranes.[44] We noticed that **4.26** has chemical shifts for ^{13}C-1 (83.5 ppm) and ^{13}C-2 (62.4 ppm) (Table 4.6., Entry 1) more similar to 2-*C*-branched epoxides **4.27** and **4.28** (Table 4.6., Entries 2 and 3) rather than to corresponding 2-*C*-branched diols (Table. 4.6., Entries 4 and 5). Furthermore, although epoxides are sensitive to mass spectroscopy conditions and usually decomposes, the structure

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[43] Cheshev, P.; Marra, A.; Dondoni, A. *Carbohydr. Res.* **2006**, *341*, 2714-2716.

[44] See equivalent 2-*C*-aryl-oxiranic compounds: (a) Bernini, R.; Mincione, E.; Coratti, A.; Fabrizi, G.; Battistuzzi, G. *Tetrahedron*, **2004**, *60*, 967-971. (b) Adam, W.; Sauter, M. *Tetrahedron*, **1994**, *50,* 11441-11446. See equivalent 2-*C*-aryl-dihydroxylated compounds: (c)Varga, M.; Batori, S.; Kovari-Radkai, M.; Prohaszka-Nemet, I.; Vitanyi-Morvai, M.; Bocskey, Z.; Bokotey, S.; Simon, K.; Hermecz, I. *Eur. J. Org Chem.* **2001**, 3911-3920. (d) Robinson, T.V.; Pedersen, D.S.; Taylor, D.K.; Tiekink, E.R.T. *J. Org. Chem.* **2009**, *74*, 5093-5096.

of **4.26** could be also confirmed by high resolution mass spectroscopy in which the value $(M+Na)^+$ for the ion $C_{33}H_{32}NaO_5^+$ (*m/z*) was 531.2147 (calc 531.2142).

Table 4.5. Comparison of δ ^{13}C-1 and δ ^{13}C-2 NMR signals of 4.27 with reported 2-aryl-*C*-oxiranes.

| Entry	Compound	δ C-1 (ppm)	δ C-2 (ppm)	Reference
1	**4.26**	83.5	62.4	-
2	**4.27**	82.9	63.0	44a
3	**4.28**	88.7	63.7	44b
4	**4.29**	99.9 (Maj) 102.1(Min)	78.0 (Maj) 77.1 (Min)	44c
5	**4.30**	101.9 (Maj) 102.9 (Min)	78.6 (Maj) 81.2 (Min)	44d

However, the stereochemistry of the epoxide ring was not unequivocally stablished because NOE correlation did not offer any diagnostic evidence. Hence, we decided to open the epoxide ring under glycosylating conditions. It would be expected that if the phenyl group adquires an axial configuration, it would be a consequence of the opening of an equatorial epoxide ring in **4.26** whereas if the phenyl group acquires an equatorial configuration, it would be a consequence of the ring opening of an axially oriented epoxide ring in **4.26.**

Lewis acids had been reported as a suitable promotors for the glycosylation of 1,2- anhydrosugars.[45] In order to obtain 2-*C*-aryl-glycosides, compound **4.26** was dissolved in dichloromethane and treated with $H_2O/ZnCl_2$ or $NaN_3/LiClO_4$ at room temperature for 12 h, but only starting material was recovered.

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[45] Li, Y.; Tang, P.; Chen, Y., Yu, B. *J. Org. Chem.*, **2008**, *73*, 4323-4325.

However, in presence of catalytic of para toluene sulfonic acid (PTSA),[46] corresponding 2-*C*-phenyl-glycosides **4.31** and **4.32** were obtained in good yields (85-90 %) with exclusive α-selectivity after refluxing epoxide **4.26** in 1,2-dichloroethane for 12 h in the presence of EtOH or BnOH (Scheme 4.12.).
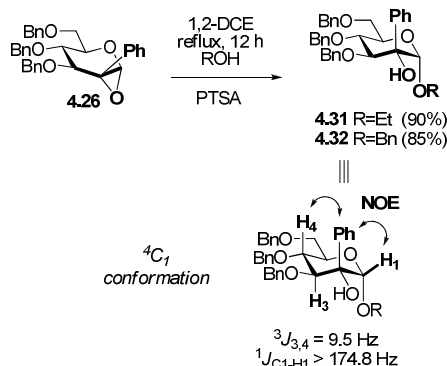

Scheme 4.12. Glycosidation of epoxide **4.26**

The stereochemistry of compounds **4.31** and **4.32** was initially deduced by analysis of diagnostic $^3J_{3,4} = 9.5$ Hz coupling constants that account for a $^4C_1$ conformation. Moreover, the anomeric $^1J_{C1-H1} = 174.8$ Hz coupling constant higher than 170 Hz is indicative of an α-configuration.[47] Finally, selective NOE irradiation of the aromatic protons at C-2 caused an enhancement of signals corresponding to H-1 and H-4, which confirmed the axial disposition of the Ph group at C-2 in both **4.31** and **4.32** (Scheme 4.12.). This evidende also permit deducing that the epoxide ring in **4.26** has α configuration.

More interestingly, when epoxide **4.26** was treated with the appropiately protected mannoside derivatives **4.33** or **4.34** with traces of PTSA in 1,2-dichloroethane for 12 h, disaccharides **4.35** and **4.36** were obtained (Scheme 4.13.). However, the yield obtained for these compounds was still low (35-42 %) so the optimization of reaction conditions would be further investigated in our group in order to afford new architectures for 1,2-manosides and 1,6-manosides.

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[46] Donnelly, J.A.; Keegan, J.R.; Quigley, K. *Tetrahedron*, **1980**, *36*, 1671-1680.
[47] Tvaroska, I.; Taravel, F. R. *Adv. Carbohydr. Chem. Biochem.* **1995**, *51*, 15-61.
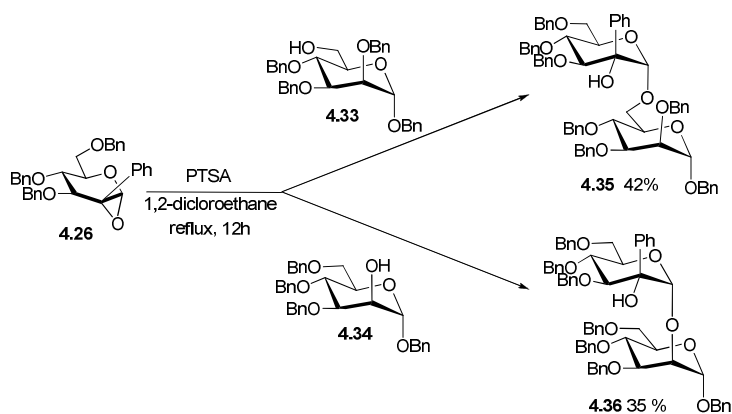
Scheme 4.13. Glycosylation of **4.26** to afford dissacarides **4.35** and **4.36**

In conclusion, we have developed a general catalytic strategy for the efficient synthesis of 2-*C*-arylglycals by phosphine-free Suzuki-Miyaura cross-coupling of 2-iodoglycals in aqueous media using an inexpensive Pd catalyst. To the best of our knowledge this transformation represents the first transition metal catalyzed 2-*C*-arylation of 2-haloglycals. The simplicity and relative mildness of this method allows the regioselective preparation of various 2-*C*-arylglycals with different configurations in excellent yields with no Ferrier or 2,3-unsaturated by-products detected. Notably the 2-iodoglycal substrates proved unstable in the presence of phosphine, necessiting systems that avoid their use. The elaboration of the 2-*C*-arylglycal moiety gives access to both 2-*C*-aryl-2-deoxy-1,5-anhydroalditols and challenging quaternary 2-*C*-aryl-α-glycosides which will broaden the plethora of *C*-arylglycosides at positions different than C-1. Further application of this methodology to the synthesis of more complex 2-*C*-aryl branched glycosides is currently under investigation

4.3. Experimental Section

Melting points (m.p.) were recorded on a Leica Galen III hot stage microscope equipped with a Testo 720 thermocouple probe and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AVII500 (500 MHz) or a Varian Mercury VX 400 (400 MHz) spectrometer, as indicated. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker AVII500 (125.8 MHz) or a Varian Mercury VX 400 (100.6 MHz) spectrometer, as indicated. Fluorine nuclear magnetic resonance (^{19}F NMR) spectra were recorded on a Bruker AVII500 (470.4 MHz) spectrometer. NMR Spectra were fully assigned using COSY, HSQC, HMBC and NOESY. All chemical shifts are quoted on the δ scale in ppm using residual solvent as the internal standard (^{1}H NMR: CDCl$_3$ = 7.26, CD$_3$OD = 4.87; DMSO-d_6 = 2.50 and ^{13}C NMR: CDCl$_3$ = 77.0; CD$_3$OD = 49.0; DMSO-d_6 = 39.5) and CFCl$_3$ as external standard for ^{19}F NMR. Coupling constants (J) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet and app = apparent.

Infrared (IR) spectra were recorded on a Bruker Tensor 27 Fourier Transform spectrophotometer using thin films on NaCl plates for liquids and oils and KBr discs for solids and crystals. Absorption maxima (v_{max}) are reported in wavenumbers (cm^{-1}).

Low resolution mass spectra (LRMS) were recorded on a Waters Micromass LCT Premier TOF spectrometer using electrospray ionization (ESI) and high resolution mass spectra (HRMS) were recorded on a Bruker MicroTOF ESI mass spectrometer. Nominal and exact m/z values are reported in Daltons. Other methods of ionization (EI, FI and FAB) are used where indicated and were recorded by the University of Oxford Mass Spectrometry Service in the Department of Chemistry.

Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1.0 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. Concentrations (c) are given in g/100 ml.

Thin layer chromatography (TLC) was carried out using Merck aluminium backed sheets coated with 60F254 silica gel. Visualization of the silica plates was achieved using a UV lamp (λ_{max} = 254 nm) and/or ammonium molybdate (5% in 2 M H_2SO_4) and/or potassium permanganate (5% $KMnO_4$ in 1 M NaOH with 5% potassium carbonate). Flash column chromatography was carried out using BDH 40–63 μm silica gel (VWR). Mobile phases are reported in relative composition (*e.g.* 1:1 EtOAc/petrol v/v).

Anhydrous solvents were purchased from Fluka or Acros. Triethylamine was stored over NaOH pellets. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Distilled water was used for chemical reactions and Milli–QR purified water for protein manipulations. Reagents were purchased from Aldrich and used as supplied, unless otherwise indicated. 'Petrol' refers to the fraction of light petroleum ether boiling in the range 40–60 ºC. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon or nitrogen. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulfate ($MgSO_4$) was used as drying agents after reaction workup, as indicated.

Preparation of Pd-pyrimidine catalyst
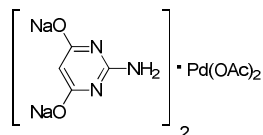
2-Amino-4,6-dihydroxypyrimidine (13 mg, 0.10 mmol) was added to a 0.1 M NaOH solution (2 mL). The pyrimidine ligand was dissolved by stirring for 2 minutes in a water bath preheated to 65 ºC. Pd(OAc)$_2$ (11.0 mg, 0.05 mmol) was added to the resulting solution. The mixture was stirred vigorously at 65 ºC for 30 minutes to give a homogenous yellow-orange solution. After cooling to room

temperature, the solution was diluted to 5 mL with distilled water to give a catalyst solution 0.01 M in Pd(II).

General procedure for the synthesis of 2-iodoglycals from glycals

NIS (1.5 mmol) was added to a solution of the corresponding 3,4,6-tri-*O*-benzyl-D-glycal (1 mmol) in 10:1 (v/v) CH_3CN/H_2O (20 mL) at room temperature. The reaction mixture was stirred at the same temperature for 3.5 h and the solvent evaporated. The crude was then diluted with EtOAc and washed with saturated aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was used in the next step without further purification. The crude was treated with a mixture of Ph_2SO (2 mmol), TTBP (3 mmol) and 4Å molecular sieves (0.4 g) in dry CH_2Cl_2 (25 mL) at –78 ºC for 30 min. Tf_2O (1 mmol) was then added and the reaction gradually warmed up to room temperature and stirred for 5 h. The reaction mixture was quenched with Et_3N and the solvent evaporated. The residue was purified by chromatographic techniques.

General procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals

An aliquot of 0.01 M Pd-pyrimidine catalyst solution (2 mol%) was added to a mixture of the corresponding 2-iodoglycal (1 mmol), boronic acid (1.5 mmol) and Na_2HPO_4 (5 mmol) in 1:1 (v/v) CH_3CN/H_2O (50 mL) at room temperature. The reaction mixture was microwave irradiated in a sealed tube at 125 ºC for 5 min using a CEM-Discover™ single-mode synthesizer (temperature control, fixed hold time off, normal absorption mode, 300 W). The crude was then diluted with EtOAc and washed with 5% aqueous HCl and brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by chromatographic techniques.

General procedure for the 1,2-anhydro-2-*C*-phenylglucopyranose ring-opening

A mixture of **4.26** (1 mmol), dry alcohol (3 mmol) and dry 4-toluenesulfonic acid (0.6–1 mol) in dry 1,2-dichloroethane (27 mL) was heated under reflux for 12 h. The crude was then concentrated under reduced pressure and the residue was purified by chromatographic techniques.

1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (4.5)
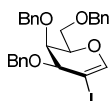

The title compound was prepared following the general procedure for the synthesis of 2-iodoglycals, starting from 3,4,6-tri-*O*-benzyl-D-galactal (737 mg, 1.8 mmol) and NIS (607 mg, 2.7 mmol) in 10:1 (v/v) CH_3CN/H_2O (36 mL). After standard workup the crude reaction was treated with Ph_2SO (746 mg, 3.5 mmol), TTBP (1.36 g, 5.3 mmol), 4Å molecular sieves (0.72 g) and Tf_2O (304 µL, 1.8 mmol) in dry CH_2Cl_2 (45 mL). After standard workup the crude was purified by column chromatography (1:8 EtOAc/petrol) to afford **4.5** (712 mg, 74%) as a yellowish solid. Rf (1:8 EtOAc/petrol): 0.24; m.p: 57–58 ºC; $[\alpha]_D^{20}$: +6.7 (*c* = 0.135, CH_2Cl_2); ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 7.33–7.25 (m, 15H, Ar), 6.61 (s, 1H, H-1), 4.80–4.37 (m, 6H, 3CH$_2$Ph), 4.33 (m, 1H, H-5), 4.08 (d, $J_{3,4}$ = 4.4 Hz, 1H, H-3), 4.03 (dd, $J_{4,3}$ = 4.4 Hz, $J_{4,5}$ = 3.6 Hz, 1H, H-4), 3.78 (dd, $J_{6a,6b}$ = 10.4 Hz, $J_{6a,5}$ = 8.0 Hz, 1H, H-6a), 3.68 (dd, $J_{6b,6a}$ = 10.4 Hz, $J_{6b,5}$ = 4.4 Hz, 1H, H-6b); ^{13}C NMR (CDCl$_3$, 100.6 MHz) δ in ppm: 147.7 (C-1), 138.1, 138.0, 137.9 (C, Ar), 128.6, 128.5, 128.2, 128.2, 128.2, 128.1, 128.0, 127.6 (CH, Ar), 76.0 (C-3), 75.9 (C-5), 74.1, 73.6 (2CH$_2$Ph), 73.6 (C-4), 73.4 (CH$_2$Ph), 73.1 (C-2), 68.0 (C-6); FT–IR (KBr) v in cm^{-1}: 3063, 3030, 2852, 1730, 1624, 1495, 1455, 1184, 1067, 734; HRMS (TOF ES+) for (M+Na)$^+$ $C_{27}H_{27}INaO_4^+$ (*m/z*): calc. 565.0846; found 565.0861.

1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*arabino*-hex-1-enitol (4.6)
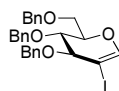
The title compound was prepared following the general procedure for the synthesis of 2-ioglycals, starting from 3,4,6-tri-*O*-benzyl-D-glucal (2.5 g, 6.0 mmol) and NIS (2.03 g, 9.0 mmol) in 10:1 (v/v) CH_3CN/H_2O (60 mL). After standard workup the crude reaction was treated with Ph_2SO (2.53 mg, 12.5 mmol), TTBP (4.61 g, 18.5 mmol), 4Å molecular sieves (2.4 g) and Tf_2O (1.0 mL, 6.1 mmol) in dry CH_2Cl_2 (150 mL). After standard workup the crude was purified by column chromatography (1:8 EtOAc/petrol) to afford **4.6** (2.08 g, 64%) as a yellowish solid. R_f (1:8 EtOAc/petrol): 0.23; m.p: 44-45 ºC; $[\alpha]_D^{20}$: +25.4 (*c* = 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ in ppm: 7.37-7.25 (m, 15H, Ar), 6.74 (s, 1H, H-1), 4.71-4.54 (m, 6H, 3CH_2Ph), 4.30 (m, 1H, H-5), 4.08 (d, $J_{3,4}$ = 5.0 Hz, 1H, H-3), 3.99 (dd, $J_{4,5}$ = 6.6 Hz, $J_{4,3}$ = 5.0 Hz, 1H, H-4), 3.80 (dd, $J_{6a,6b}$ = 10.7 Hz, $J_{6a,5}$ = 5.3 Hz, 1H, H-6a), 3.72 (dd, $J_{6b,6a}$ = 10.7 Hz, $J_{6b,5}$ = 3.8 Hz, 1H, H-6b); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ in ppm: 148.4 (C-1), 137.8, 137.7, 137.6 (C, Ar), 128.5, 128.4, 128.1, 128.0, 127.9, 127.7 (CH, Ar), 78.9 (C-3), 76.5 (C-5), 74.0 (C-4), 73.4, 73.1, 72.3 (3CH_2Ph), 70.3 (C-2), 67.8 (C-6); FT–IR (KBr) *v* in cm^{-1}: 3062, 3029, 2864, 1624, 1496, 1453, 1165, 1090, 733; HRMS (TOF ES+) for (M+Na)$^+$ $C_{27}H_{27}INaO_4^+$ (*m/z*): calc. 565.0846; found 565.0847.

Hydrodehalogenation of 2-iodoglycal 4.5 with PBu$_3$
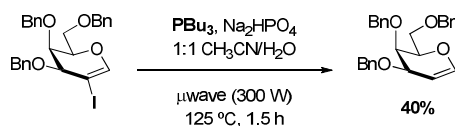
PBu$_3$ (14 µL, 0.056 mmol) was added to a solution of 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (**4.5**) (15 mg, 0.028 mmol) and Na_2HPO_4 (19.9 mg, 0.140 mmol) in 1:1 (v/v) CH_3CN/H_2O (1.4 mL) at room temperature. The reaction mixture was microwave irradiated in a sealed tube at 125 ºC for 1.5 h using a CEM-Discover™ single-mode synthesizer (temperature

control, fixed hold time off, normal absorption mode, 300 W). The crude was then diluted with EtOAc and washed with 5% aqueous HCl and brine. The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. ^{1}H and ^{13}C NMR analysis of the crude mixture revealed the formation of tri-*O*-benzyl-D-galactal in 40% conversion. HRMS (TOF ES+) for (M+Na)$^+$ C$_{27}$H$_{28}$NaO$_4^+$ (*m/z*): calc. 439.1880; found 439.1870; spectroscopic data was identical to that previously reported.[48]

1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-phenyl-D-*lyxo*-hex-1-enitol (4.11a)
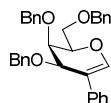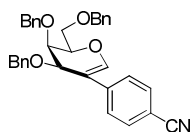

The title compound was prepared following the general procedure above, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (**4.5**) (20 mg, 0.037 mmol), phenylboronic acid **4.10a** (6.7 mg, 0.056 mmol), Na$_2$HPO$_4$ (26.3 mg, 0.185 mmol) and 0.01 M Pd-pyrimidine catalyst solution (74 µL, 0.74 µmol) in 1:1 (v/v) CH$_3$CN/H$_2$O (1.9 mL). After standard workup the crude was purified by column chromatography (1:8 EtOAc/petrol) to afford **4.11a** (17.3 mg, 95%) as a yellowish syrup. Rf (1:8 EtOAc/petrol): 0.23; [α]$_D^{20}$: -12.4 (*c* = 0.45, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) *δ* in ppm: 7.45-7.11 (m, 20H, Ar), 6.65 (s, 1H, H-1), 4.81-4.49 (m, 8H, 3CH$_2$Ph, H-3, H-5), 4.13 (appt, $J_{4,3} = J_{4,5} = 4.0$ Hz, 1H, H-4), 4.12-3.88 (m, 2H, H-6a, H-6b); ^{13}C NMR (CDCl$_3$, 125.8 MHz) *δ* in ppm: 141.9 (C-1), 138.4 , 138.2, 137.9, 137.4 (C, Ar), 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.4, 126.3, 125.9 (CH, Ar), 113.9 (C-2), 75.3 (C-5), 74.6 (C-4), 73.5, 73.4, 72.3 (3CH$_2$Ph), 71.1 (C-3), 68.1 (C-6); FT–IR (neat) *v* in cm^{-1}: 3456, 2063, 1642, 545; HRMS (TOF ES+) for (M+Na)$^+$ C$_{233}$H$_{32}$NaO$_4^+$ (*m/z*): calc. 515.2193; found 515.2195.

[48] Capozzi, G.; Falciani, C.; Menichetti, S.; Nativi, C.; Raffaelli, B. *Chem. Eur. J.* **1999**, *5*, 1748-1754.
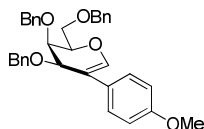
1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-(4-cyanophenyl)-D-*lyxo*-hex-1-enitol (4.11b)

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (**4.5**) (20 mg, 0.037 mmol), 4-cyanophenylboronic acid **4.10b** (8.2 mg, 0.056 mmol), Na_2HPO_4 (26.3 mg, 0.185 mmol) and 0.01 M Pd-pyrimidine catalyst solution (74 μL, 0.74 μmol) in 1:1 (v/v) CH_3CN/H_2O (1.9 mL). After standard workup the crude was purified by column chromatography (from 1:8 to 1:4 EtOAc/petrol) to afford **4.11b** (18.2 mg, 95%) as a yellowish syrup. Rf (1:4 EtOAc/petrol): 0.25; $[\alpha]_D^{20}$: +9.8 (*c* = 0.36, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) *δ* in ppm: 7.50-7.16 (m, 19H, Ar), 6.77 (s, 1H, H-1), 4.86-4.56 (m, 8H, $3CH_2Ph$, H-3, H-5), 4.13 (appt, $J_{4,3} = J_{4,5} = 4.0$ Hz, 1H, H-4), 4.02 (dd, $J_{6a,6b} = 11.0$ Hz, $J_{6a,5} = 8.0$ Hz, 1H, H-6a), 3.89 (dd, $J_{6b,6a} = 11.0$ Hz, $J_{6b,5} = 3.0$ Hz, 1H, H-6b); ^{13}C NMR ($CDCl_3$, 125.8 MHz) *δ* in ppm: 144.5 (C-1), 142.2 (C-1, ArCN), 138.1, 137.9, 137.4 (C, Ar), 132.3, 128.7, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8, 126.3 (CH, Ar), 119.5 (CN), 113.1 (C-2), 109.5 (C-4, ArCN), 75.9 (C-5), 74.0 (CH_2Ph), 73.6 (C-4), 73.6, 72.9 ($2CH_2Ph$), 70.7 (C-3), 68.1 (C-6); FT–IR (neat) *v* in cm^{-1}: 3064, 3031, 2924, 2870, 2226, 1726, 1632, 1602, 1496, 1454, 1270, 1027; HRMS (TOF ES+) for $(M+Na)^+$ $C_{33}H_{32}NaO_4^+$ (*m/z*): calc. 540.2145; found 540.2136.

1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-(4-methoxyphenyl)-D-*lyxo*-hex-1-enitol (4.11c)
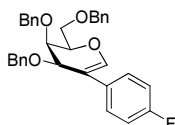

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (**4.5**) (20 mg, 0.037 mmol), 4-methoxyphenylboronic acid **4.10c** (8.4 mg, 0.056 mmol), Na_2HPO_4 (26.3 mg, 0.185 mmol) and 0.01 M Pd-pyrimidine catalyst solution (74 μL, 0.74 μmol) in 1:1 (v/v) CH_3CN/H_2O (1.9 mL). After standard workup the crude was purified by column chromatography (1:8 EtOAc/petrol) to afford **4.11c** (18.3 mg, 95%) as a yellowish syrup. Rf (1:8 EtOAc/petrol): 0.19; $[\alpha]_D^{20}$: –27.2 (*c* = 0.48, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* in ppm: 7.37-6.82 (m, 19H, Ar), 6.55 (s, 1H, H-1), 4.79-4.56 (m, 6H, $3CH_2Ph$), 4.51-4.49 (m, 2H, H-3, H-5), 4.12 (appt, $J_{4,3} = J_{4,5}$ = 4.0 Hz, 1H, H-4), 4.08 (dd, $J_{6a,6b}$ = 11.0 Hz, $J_{6a,5}$ = 8.0 Hz, 1H, H-6a), 3.88 (dd, $J_{6b,6a}$ = 11.0 Hz, $J_{6b,5}$ = 3.0 Hz, 1H, H-6b), 3.72 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 125.8 MHz) *δ* in ppm: 158.4 (C-4, ArOMe), 141.1 (C-1), 138.6, 138.4, 138.1 (C, Ar), 130.1 (C-1, ArOMe), 128.6, 128.5, 128.3, 128.0, 128.0, 127.9, 127.9, 127.7, 127.5, 127.4, 113.9 (CH, Ar), 113.9 (C-2), 75.3 (C-5), 74.8 (C-4), 73.7, 73.5, 72.7 ($3CH_2Ph$), 71.5 (C-3), 68.2 (C-6), 55.5 (OCH_3); FT–IR (neat) *v* in cm^{-1}: 3062, 3030, 3004, 2866, 1572, 1512, 1454, 1246, 1169, 1092, 1063, 866, 827, 800, 735, 697, 415; HRMS (TOF ES+) for $(M+Na)^+$ $C_{34}H_{34}NaO_5^+$ (*m/z*): calc. 545.2298; found 545.2292.

1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-(4-fluorophenyl)-D-*lyxo*-hex-1-enitol (4.11d)
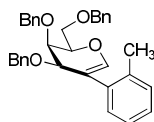

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (**4.5**) (20 mg, 0.037 mmol), 4-fluorophenylboronic acid **4.10d** (7.7 mg, 0.056 mmol), Na_2HPO_4 (26.3 mg, 0.185 mmol) and 0.01 M Pd-pyrimidine catalyst solution (74 μL, 0.74 μmol) in 1:1 (v/v) CH_3CN/H_2O (1.9 mL). After standard workup the crude was purified by column chromatography (1:9 EtOAc/petrol) to afford a yellowish syrup (18.8 mg) corresponding mainly to **4.11d** (ca 90 %) together with traces of the corresponding galactal which could not be separed of the mixture: Rf (1:9 EtOAc/petrol): 0.22; ^{1}H NMR (CDCl$_3$, 500 MHz) δ in ppm, selected signals: 7.38-6.92 (m, 19H, Ar), 6.57 (s, 1H, H-1), 4.81-4.49 (m, 8H, 3CH$_2$Ph, H-3, H-5), 4.13 (appt, $J_{4,3} = J_{4,5} = 4.0$ Hz, 1H, H-4), 4.06 (dd, $J_{6a,6b} = 11.0$ Hz, $J_{6a,5} = 8.0$ Hz, 1H, H-6a), 3.88 (dd, $J_{6b,6a} = 11.0$ Hz, $J_{6b,5} = 3.0$ Hz, 1H, H-6b); ^{13}C NMR (CDCl$_3$, 125.8 MHz) δ in ppm, selected signals: 161.8 (d, $J_{C,F} = 245.3$ Hz, C-4, ArF), 141.9 (C-1), 138.4, 138.3, 138.1 (C, Ar), 133.5 (C-1, ArF), 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.5 (CH, Ar), 115.2 (d, $J_{C,F} = 21.4$ Hz, C-2, C-3, ArF), 113.4 (C-2), 75.4 (C-5), 74.4 (C-4), 73.6, 73.5, 72.7 (3CH$_2$Ph), 71.5 (C-3), 68.1 (C-6); ^{19}F NMR (CDCl$_3$, 470.4 MHz) δ in ppm: -116.7 (m, 1F, ArF); HRMS (TOF ES+) for (M+Na)$^+$ C$_{33}$H$_{31}$FNaO$_4^+$ (*m/z*): calc. 533.2199; found 533.2096.

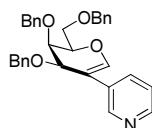**1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-(2-methylphenyl)-D-*lyxo*-hex-1-enitol (4.11e)**

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (**4.5**) (20 mg, 0.037 mmol), 2-methylphenylboronic acid **4.10e** (7.5 mg, 0.056 mmol), Na_2HPO_4 (26.3 mg, 0.185 mmol) and 0.01 M Pd-pyrimidine catalyst solution (74 µL, 0.74 µmol) in 1:1 (v/v) CH_3CN/H_2O (1.9 mL). The resulting mixture was microwave irradiated in a sealed tube at 125 ºC for 40 min. After standard workup the crude was purified by column chromatography (from petrol to 1:8 EtOAc/petrol) to afford **4.11e** (16.8 mg, 90%) as a yellowish syrup. Rf (1:4 EtOAc/petrol): 0.60; $[\alpha]_D^{20}$: -38.3 (c = 0.29, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ in ppm: 7.36-6.94 (m, 19H, Ar), 6.24 (s, 1H, H-1), 4.85-4.27 (m, 8H, 3CH_2Ph, H-3, H-5), 4.11 (appt, $J_{4,3} = J_{4,5} = 4.0$ Hz, 1H, H-4), 4.02 (dd, $J_{6a,6b} = 11.0$ Hz, $J_{6a,5} = 8.0$ Hz, 1H, H-6a), 3.80 (dd, $J_{6b,6a} = 11.0$ Hz, $J_{6b,5} = 3.0$ Hz, 1H, H-6b), 2.23 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ in ppm: 141.9 (C-1), 138.6, 138.4, 138.3, 137.9 (C, Ar), 136.9, 131.1, 130.0, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8, 127.5, 127.4, 127.3, 125.5 (CH, Ar), 114.2 (C-2), 75.6 (C-5), 73.6 (C-3), 73.1, 73.0, 73.0 (3CH_2Ph), 73.0 (C-4), 68.4 (C-6), 20.2 (CH_3); FT–IR (neat) v in cm^{-1}: 3062, 3029, 2961, 2919, 2864 2063, 1637, 1607, 1496, 1260, 1169, 1091, 1062, 1027, 867, 800, 734; HRMS (TOF ES+) for (M+Na)$^+$ $C_{34}H_{34}NaO_4^+$ (*m/z*): calc. 529.2369; found 529.2349.

1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-(3-pyridinyl)-D-*lyxo*-hex-1-enitol (4.11f)
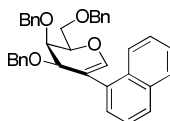

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (**4.5**) (20 mg, 0.037 mmol), 3-pyridinylphenylboronic acid **4.10f** (6.8 mg, 0.056 mmol), Na_2HPO_4 (26.3 mg, 0.185 mmol) and 0.01 M Pd-pyrimidine catalyst solution (74 μL, 0.74 μmol) in 1:1 (v/v) CH_3CN/H_2O (1.9 mL). The resulting mixture was microwave irradiated in a sealed tube at 125 ºC for 40 min. After standard workup the crude was purified by column chromatography (from 1:4 to 1:1 EtOAc/petrol) to afford **4.11f** (16.2 mg, 89%) as a yellowish syrup. Rf (1:1 EtOAc/petrol): 0.49; $[\alpha]_D^{20}$: -6.0 (*c* = 0.23, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) *δ* in ppm: 8.52 (brs, 1H, Py), 8.44 (brs, 1H, Py), 7.51-7.15 (m, 17H, Ar), 6.66 (s, 1H, H-1), 4.83-4.49 (m, 8H, 3CH₂Ph, H-3, H-5), 4.23 (appt, $J_{4,3} = J_{4,5} = 4.0$ Hz, 1H, H-4), 4.07 (dd, $J_{6a,6b} = 11.0$ Hz, $J_{6a,5} = 8.0$ Hz, 1H, H-6a), 3.87 (dd, $J_{6b,6a} = 11.0$ Hz, $J_{6b,5} = 3.0$ Hz, 1H, H-6b); ¹³C NMR (CDCl₃, 125.8 MHz) *δ* in ppm: 147.2 (CH, Py), 143.3 (C-1), 138.2, 138.0, 137.9 (C, Ar), 134.1 (C, Py), 128.7, 128.5, 128.5, 128.1, 128.1, 128.1, 127.9, 127.9, 127.8 (CH, Ar), 123.4 (CH, Py), 111.2 (C-2), 75.8 (C-5), 73.7 (C-4), 73.6, 73.6, 73.0 (3CH₂Ph), 71.3 (C-3), 68.1 (C-6); FT–IR (neat) *v* in cm⁻¹: 3030, 2960, 2924, 2854, 1635, 1541, 1511, 1496, 1455, 1259, 1169, 1092, 1027, 800, 735, 668; HRMS (TOF ES+) for (M+Na)⁺ $C_{32}H_{31}NNaO_4^+$ (*m/z*): calc. 516.2145; found 516.2145.

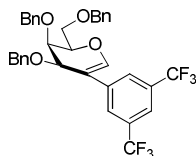**1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-(1-naphthyl)-D-*lyxo*-hex-1-enitol (4.11g)**

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (**4.5**) (20 mg, 0.037 mmol), 1-naphthylboronic acid **4.10g** (9.5 mg, 0.056 mmol), Na_2HPO_4 (26.3 mg, 0.185 mmol) and 0.01 M Pd-pyrimidine catalyst solution (74 µL, 0.74 µmol) in 1:1 (v/v) CH_3CN/H_2O (1.9 mL). After standard workup the crude was purified by column chromatography (from 1:20 to 1:9 EtOAc/petrol) to afford **4.11g** (18.8 mg, 94%) as a yellowish syrup: Rf (1:8 EtOAc/petrol): 0.22; $[\alpha]_D^{20}$: -3.4 (*c* = 0.44, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ in ppm: 8.04-6.75 (m, 22H, Ar), 6.40 (s, 1H, H-1), 4.81-4.55 (m, 4H, 2CH₂Ph), 4.52 (m, 1H, H-5), 4.49 (d, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 4.31 (d, J_{AB} = 11.5 Hz, 1H, CH₂Ph), 4.22 (appt, $J_{4,3} = J_{4,5}$ = 3.5 Hz, 1H, H-4), 4.10 (m, 2H, CH₂Ph, H-6a), 3.87 (dd, $J_{6b,6a}$ = 11.0 Hz, $J_{6b,5}$ = 4.0 Hz, 1H, H-6b); ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ in ppm: 142.8 (C-1), 138.4, 138.4, 138.3 (C, Ar), 135.1 (C-1, naph), 133.8, 133.7, 128.6, 128.5, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.2, 126.3, 125.9, 125.7, 125.4 (CH, Ar), 113.0 (C-2), 75.8 (C-5), 74.1 (C-3), 73.5, 73.1 (2CH₂Ph), 73.1 (C-4), 72.9 (CH₂Ph), 68.5 (C-6); FT–IR (neat) *v* in cm⁻¹: 3087, 3061, 2920, 1726, 1589, 1548, 1174, 1094, 861, 801, 778, 735, 697; HRMS (TOF ES+) for (M+Na)⁺ $C_{37}H_{34}NaO_4^+$ (*m/z*): calc. 565.2349; found 565.2336.

1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-(3,5-bis(trifluoromethyl)phenyl)-D-*lyxo*-hex-1-enitol (4.11h)
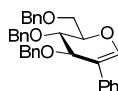

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*lyxo*-hex-1-enitol (**4.5**) (20 mg, 0.037 mmol), 3,5-bis(trifluoromethyl)phenyl boronic acid **4.10h** (14.2 mg, 0.056 mmol), Na_2HPO_4 (26.3 mg, 0.185 mmol) and 0.01 M Pd-pyrimidine catalyst solution (74 μL, 0.74 μmol) in 1:1 (v/v) CH_3CN/H_2O (1.9 mL). After standard workup the crude was purified by column chromatography (1:9 EtOAc/petrol) to afford **4.11h** (22.0 mg, 95%) as a yellowish syrup: R_f (1:9 EtOAc/petrol): 0.27; $[\alpha]_D^{20}$: -6.6 (*c* = 0.66, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ in ppm: 7.69-7.15 (m, 18H, Ar), 6.72 (s, 1H, H-1), 4.88-4.50 (m, 8H, $3CH_2Ph$, H-3, H-5), 4.18 (appt, $J_{4,3} = J_{4,5} = 3.5$ Hz, 1H, H-4), 4.00 (dd, $J_{6a,6b} = 11.0$ Hz, $J_{6a,5} = 8.0$ Hz, 1H, H-6a), 3.89 (dd, $J_{6b,6a} = 11.0$ Hz, $J_{6b,5} = 3.0$ Hz, 1H, H-6b); ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ in ppm: 144.4 (C-1), 139.7 (C-1, $Ar(CF_3)_2$), 138.1, 137.8, 137.5 (C, Ar), 131.6 (q, $J_{C,F} = 32.7$ Hz, C-3, C-5, $Ar(CF_3)_2$), 128.7, 128.6, 128.5, 128.2, 128.1, 127.8, 128.0, 127.4 (CH, Ar), 126.2 (C-2, C-6, $Ar(CF_3)_2$), 123.5 (q, $J_{C,F} = 273.0$ Hz, $2CF_3$), 120.0 (q, $J_{C,F} = 3.7$ Hz, C-4, $Ar(CF_3)_2$), 112.4 (C-2), 75.9 (C-3), 73.8 (CH_2Ph), 73.6 (C-4), 73.4, 72.3 ($2CH_2Ph$), 71.5 (C-5), 68.0 (C-6); ^{19}F NMR ($CDCl_3$, 470.4 MHz) δ in ppm: -68.8 (s, 6F, $Ar(CF_3)_2$); FT–IR (neat) *v* in cm^{-1}: 3089, 3064, 3032, 2922, 2868, 1636, 1614, 1496, 1454, 1389, 1364, 1343, 1278, 1179, 888, 844, 800, 736, 697, 682; HRMS (TOF ES+) for $(M+Na)^+$ $C_{35}H_{30}F_6NaO_4^+$ (*m/z*): calc. 651.1940; found 651.1945.

1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-phenyl-D-*arabino*-hex-1-enitol (4.12)

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*arabino*-hex-1-enitol (**4.6**) (20 mg, 0.037 mmol), phenylboronic acid **4.10a** (6.7 mg, 0.056 mmol), Na_2HPO_4 (26.3 mg, 0.185 mmol) and 0.01 M Pd-pyrimidine catalyst solution (74 μL, 0.74 μmol) in 1:1 (v/v) CH_3CN/H_2O (1.9 mL). After standard workup the crude was purified by column chromatography (1:8 EtOAc/petrol) to afford a yellowish syrup (17.3 mg) corresponding mainly to **4.12** (ca 95%) together with traces of the corresponding glucal which could not be separated of the mixture: Rf (1:8 EtOAc/petrol): 0.23; ^{1}H NMR (CDCl$_3$, 500 MHz) δ in ppm, selected signals: 7.34-7.02 (m, 20H, Ar), 6.84 (s, 1H, H-1), 4.73-4.40 (m, 8H, 3CH$_2$Ph, H-3, H-5), 4.08 (appt, $J_{4,3} = J_{4,5} = 4.0$ Hz, 1H, H-4), 3.88 (dd, $J_{6a,6b} = 10.5$ Hz, $J_{6a,5} = 8.0$ Hz, 1H, H-6a), 3.75 (dd, $J_{6b,6a} = 10.5$ Hz, $J_{6b,5} = 4.0$ Hz, 1H, H-6b); ^{13}C NMR (CDCl$_3$, 125.8 MHz) δ in ppm, selected signals: 143.1 (C-1), 138.1, 138.0, 137.9, 137.4 (C, Ar), 128.7, 128.5, 128.4, 128.1, 127.9, 127.9, 127.8, 127.8, 126.5, 126.1 (CH, Ar), 113.4 (C-2), 74.5 (C-5), 73.5 (CH$_2$Ph), 72.5 (C-3), 72.1, 70.1 (2CH$_2$Ph), 70.6 (C-4), 68.4 (C-6); HRMS (TOF ES+) for (M+Na)$^+$ C$_{33}$H$_{32}$NaO$_4$$^+$ (*m/z*): calc. 515.2193; found 515.2193.
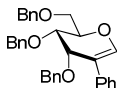
1,5-Anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-phenyl-D-*ribo*-hex-1-enitol (4.13)

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-iodo-D-*ribo*-hex-1-enitol (**4.7**)[2] (17 mg, 0.031 mmol), phenylboronic acid **4.10a** (5.7 mg, 0.047 mmol), Na_2HPO_4 (22.0 mg, 0.155 mmol) and 0.01 M Pd-pyrimidine catalyst solution (62 µL, 0.62 µmol) in 1:1 (v/v) CH_3CN/H_2O (1.6 mL). After standard workup the crude was purified by column chromatography (1:8 EtOAc/petrol) to afford **4.13** (14.5 mg, 95%) as a yellowish syrup: Rf (1:1 EtOAc/petrol): 0.40; $[\alpha]_D^{20}$: +153.4 (*c* = 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ in ppm: 7.38-7.15 (m, 20H, Ar), 6.78 (s, 1H, H-1), 4.81–4.45 (m, 7H, $3CH_2Ph$, H-3), 4.46 (dt, $J_{5,4}$ = 10.7 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 2.8 Hz, 1H, H-5), 4.13 (dd, $J_{4,5}$ = 10.7 Hz, $J_{4,3}$ = 3.5 Hz, 1H, H-4), 3.92 (d, $J_{6a,b}$ = $J_{6a,5}$ = 2.8 Hz, 2H, H-6a, H-6b); ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ in ppm: 144.2 (C-1), 138.6, 138.0, 137.9, 137.8 (C, Ar), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 126.2, 125.8, 125.6, 125.4 (CH, Ar), 113.9 (C-2), 75.2 (C-4), 73.6, 72.9 ($2CH_2Ph$), 72.6 (C-5), 72.1 (CH_2Ph), 69.6 (C-3), 68.7 (C-6); FT–IR (neat) v in cm^{-1}: 3443, 1637, 1495, 1453, 1261, 1195, 1026, 800, 733. 695, 412; HRMS (TOF ES+) for (M+Na)$^+$ $C_{33}H_{32}NaO_4^+$ (*m/z*): calc. 515.2193; found 515.2195.

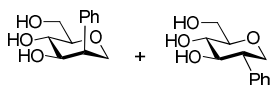**1,5-Anhydro-2-deoxy-2-*C*-phenyl-3,4:6,7-di-*O*-isopropylidene-D-*glycero*-D-*talo*-hept-1-enitol (4.14)**

The title compound was prepared following the general procedure for the Suzuki-Miyaura cross-coupling with 2-iodoglycals, starting from 1,5-anhydro-2-deoxy-3,4:6,7-di-*O*-isopropylidene-2-iodo-D-*glycero*-D-*talo*-hept-1-enitol (**4.8**)[2] (20 mg, 0.052 mmol), phenylboronic acid **4.10a** (9.5 mg, 0.078 mmol), Na_2HPO_4 (36.7 mg, 0.260 mmol) and 0.01 M Pd-pyrimidine catalyst solution (105 μL, 1.05 μmol) in 1:1 (v/v) CH_3CN/H_2O (2.6 mL). After standard workup the crude was purified by column chromatography (1:9 EtOAc/petrol) to afford **4.14** (16.5 mg, 96%) as a yellowish syrup: Rf (1:9 EtOAc/petrol): 0.33; $[\alpha]_D^{20}$: +128.6 (*c* = 0.72, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ in ppm: 7.45-7.23 (m, 5H, Ar), 6.82 (s, 1H, H-1), 5.10 (d, $J_{3,4}$ = 6.6 Hz, 1H, H-3), 4.61 (d, $J_{4,3}$ = 6.6 Hz, 1H, H-4), 4.46 (m, 1H, H-6), 4.16 (m, 2H, H-7a, H-7b), 3.84 (d, $J_{5,6}$ = 7.8 Hz, 1H, H-5), 1.37, 1.34, 1.32, 1.31 (s, 12H, $4CH_3$); ^13C NMR ($CDCl_3$, 125.8 MHz) δ in ppm: 142.8 (C-1), 136.8 (C, Ar), 128.7, 126.6, 125.6 (CH, Ar), 115.9 (C-2), 109.4, 103.0 (C_{ketal}), 75.8 (C-5), 74.1 (C-6), 72.8 (C-4), 70.7 (C-3), 66.7 (C-7), 26.8, 25.3, 22.7 ($4CH_3$); FT–IR (neat) *v* in cm^{-1}: 3427, 2986, 2934, 2093, 1637; HRMS (TOF ES+) for $(M+Na)^+$ $C_{33}H_{32}NaO_4^+$ (*m/z*): calc. 355.1516; found 355.1519.

1,5-Anhydro-2-deoxy-2-*C*-phenyl-D-mannitol (4.16) and 1,5-Anhydro-2-deoxy-2-*C*-phenyl-D-glucitol (4.17)

10% Pd/C (11.2 mg, 0.01 mmol Pd) was added to a solution of 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-phenyl-D-*arabino*-hex-1-enitol **4.12** (26 mg, 0.053 mmol) in dry and deoxygenated methanol (1mL) at room temperature. The mixture was stirred under H_2 (1 atm) at the same temperature for 12 h, filtered through a short path of Celite® 545 and concentrated under reduced pressure. The crude material was purified by column chromatography (17:2:1 EtOAc/MeOH/H_2O) to afford an inseparable 10:1 mixture of 1,5-anhydro-2-deoxy-2-*C*-phenyl-D-alditols **4.16** and **4.17** (11.2 mg, 95%) as a colorless syrup: Rf (17:2:1 EtOAc/MeOH/H_2O): 0.58; HRMS (TOF ES+) for (M+Na)$^+$ $C_{12}H_{16}NaO_4^+$ (*m/z*): calc. 247.0941; found 247.0939; Data for **4.16**: ^{1}H NMR (D_2O, 500 MHz) δ in ppm: 7.52-7.33 (m, 5H, Ar), 4.19-4.17 (m, 1H, H-1a), 4.02-3.94 (m, 3H, H-1b, H-3, H-6a), 3.84 (dd, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 6.0 Hz, 1H, H-6b), 3.63 (appt, $J_{4,5} = J_{4,3}$ = 9.5 Hz, 1H, H-4), 3.54-3.42 (m, 1H, H-5), 3.27 (dd, $J_{2,3}$ = 6.0 Hz, $J_{2,1b}$ = 1.5 Hz, 1H, H-2); ^{13}C NMR (D_2O, 125.8 MHz) δ in ppm: 139.7 (C, Ar), 130.1, 128.7, 127.1 (CH, Ar), 80.8 (C-4), 73.7 (C-3), 69.5 (C-1), 67.2 (C-5), 61.1 (C-6), 46.6 (C-2); Data for **4.17**: ^{1}H NMR (D_2O, 500 MHz) δ in ppm: 7.49-7.33 (m, 5H, Ar), 4.19-4.17 (m, 1H, H-6a), 4.02-3.94 (m, 1H, H-1a), 3.77 (dd, $J_{6b,6a}$ = 12.5 Hz, $J_{6b,5}$ = 6.0 Hz, 1H, H-6b), 3.63-3.60 (m, 2H, H-1b, H-3), 3.53-3.45 (m, 2H, H-4, H-5), 2.93 (ddd, $J_{2,3}$ = 16.0 Hz, $J_{2,1a}$ = 11.0 Hz, $J_{2,1b}$ = 4.5 Hz, 1H, H-2); ^{13}C NMR (D_2O, 125.8 MHz) δ in ppm: 137.1 (C, Ar), 128.9, 128.7, 127.6 (CH, Ar), 80.6 (C-4), 75.8 (C-3), 71.8 (C-1), 70.6 (C-5), 61.7 (C-6), 49.8 (C-2).

1,2-anhydro-2-*C*-phenyl-3,4,6-tri-*O*-benzyl-D-*gluco*pyranose (4.26)

A mixture of 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-2-*C*-phenyl-D-*arabino*-hex-1-enitol (**4.12**) (92 mg, 0.187 mmol), acetone (77 μL), saturated aqueous NaHCO$_3$ (1.25 mL) and CH$_2$Cl$_2$ (770 μL) was cooled to 0 ºC. A freshly 0.4 M aqueous solution of Oxone® (93 μL, 0.372 mmol) was added dropwise and the resulting mixture was vigorously stirred at the same temperature for 30 min. The reaction was allowed to warm to room temperature for 9 h. The crude was then diluted with CH$_2$Cl$_2$ and washed with brine. The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure to afford 1,2-anhydro-2-*C*-phenyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranose **4.26** (94 mg, 99%) as a yellowish syrup: Rf (1:8 EtOAc/petrol): 0.23; [α]$_D^{20}$: +19.9 (*c* = 0.50, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz) *δ* in ppm: 7.50-6.84 (m, 20H, Ar), 8.85 (m, 2H, CH$_2$Ph, H-1), 4.68-4.22 (m, 4H, 2CH$_2$Ph), 4.09 (d, $J_{3,4}$ = 8.2 Hz, 1H, H-3), 3.99-3.95 (m, 1H, H-5), 3.92 (d, J_{AB} = 11.5 Hz, 1H, CH$_2$Ph), 3.87-3.71 (m, 3H, H-4, H-6a, H-6b); ^{13}C NMR (CDCl$_3$, 100.6 MHz) *δ* in ppm: 138.2, 137.8, 137.0, 135.3 (C, Ar), 128.4, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.7, 127.4 (CH, Ar), 83.3 (C-1), 82.1 (C-3), 74.9 (CH$_2$Ph), 74.9 (C-4), 74.6, 73.6 (2CH$_2$Ph), 70.3 (C-5), 68.2 (C-6), 62.2 (C-2); FT-IR (neat) *v* in cm^{-1}: 3584, 3088, 3063, 3030, 2922, 2868, 1730, 1496, 1454, 1265, 1156, 1096, 1045, 666, 573, 550, 537; HRMS (TOF ES+) for (M+Na)$^+$ C$_{33}$H$_{32}$NaO$_5^+$ (*m/z*): calc. 531.2142; found 531.2147.

Ethyl 3,4,6-tri-*O*-benzyl-2-*C*-phenyl-α-D-glucopyranoside (4.31)

The title compound was prepared following the general procedure for oxirane ring opening, starting from 1,2-anhydro-2-*C*-phenyl-3,4,6-tri-*O*-benzyl-D-*gluco*pyranose (**4.26**) (6.0 mg, 0.011 mmol), EtOH (2 μL, 0.033 mmol) and 4-toluenesulfonic acid (2 mg, 0.011 mmol) in dry 1,2-dichloroethane (300 μL). After concentration under reduced pressure the crude was purified by column chromatography (1:9 EtOAc/petrol) to afford **4.31** (5.5 mg, 90%) as a yellowish syrup: Rf (1:4 EtOAc/petrol): 0.23; $[\alpha]_D^{20}$: +26.6 (c = 0.04, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ in ppm: 7.90-7.07 (m, 20H, Ar), 5.12 (d, J_{AB} = 11.5 Hz, 1H, CH$_2$Ph), 4.90 (s, 1H, H-1), 4.81 (d, J_{AB} = 11.5 Hz, 1H, CH$_2$Ph), 4.68 (d, J_{AB} = 11.5 Hz, 1H, CH$_2$Ph), 4.64 (d, J_{AB} = 11.5 Hz, 1H, CH$_2$Ph), 4.54 (d, J_{AB} = 11.5 Hz, 1H, CH$_2$Ph), 4.30 (d, J_{AB} = 11.5 Hz, 1H, CH$_2$Ph), 4.01 (d, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 3.94 (m, 1H, H-5), 3.86 (m, 2H, C$\underline{H}_2$CH$_3$, H-6a), 3.79 (dd, $J_{6b,6a}$ = 10.5 Hz, $J_{6b,5}$ = 2.0 Hz, 1H, H-6b), 3.70 (appt, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, 1H, H-4), 3.59 (dq, J = 9.5 Hz, J = 7.0 Hz, 1H, C$\underline{H}_2$CH$_3$), 3.20 (s, 1H, OH), 1.28 (t, J = 9.5 Hz, 3H, CH$_2$C$\underline{H}_3$); ^{13}C NMR (CDCl$_3$, 125.8 MHz) δ in ppm: 139.4, 138.8, 138.3 (C, Ar), 128.3, 128.2, 127.9, 127.6, 127.5, 127.4, 127.3 (CH, Ar), 101.5 (C-1), 86.0 (C-3), 77.5 (C-2), 75.8 (CH$_2$Ph), 75.5 (C-4), 75.0, 73.2 (2CH$_2$Ph), 71.5 (C-5), 68.8 (C-6), 63.8 ($\underline{C}$H$_2$CH$_3$), 15.2 (CH$_2$$\underline{C}H_3$); FT–IR (neat) v in cm^{-1}: 3356, 2920, 2850, 1608, 1453, 1166, 1051, 699; HRMS (TOF ES+) for (M+Na)$^+$ C$_{35}$H$_{38}$NaO$_6$$^+$ (*m/z*): calc. 577.2561; found 577.2558.
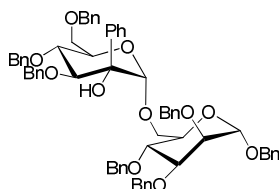
Benzyl 3,4,6-tri-*O*-benzyl-2-*C*-phenyl-α-D-glucopyranoside (4.32)

The title compound was prepared following the general procedure for ring oxirane opening, starting from 1,2-anhydro-2-C-phenyl-3,4,6-tri-*O*-benzyl-D-*gluco*pyranose (**4.26**) (10. mg, 0.020 mmol), BnOH (6 μL, 0.060 mmol) and 4-toluenesulfonic acid (2 mg, 0.011 mmol) in dry 1,2-dichloroethane (500 μL).

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After concentration under reduced pressure the crude was purified by column chromatography (1:9 EtOAc/petrol) to afford **4.32** (10.5 mg, 85%) as a yellowish syrup: Rf (1:4 EtOAc/petrol): 0.38; $[\alpha]_D^{20}$: +22.2 (c = 0.13, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ in ppm: 7.82-7.00 (m, 25H, Ar), 5.03 (d, J_{AB} = 11.0 Hz, 1H, CH$_2$Ph), 4.95 (s, 1H, H-1), 4.74 (d, J_{AB} = 11.0 Hz, 1H, CH$_2$Ph), 4.71 (d, J_{AB} = 11.0 Hz, 1H, CH$_2$Ph), 4.61 (d, J_{AB} = 11.0 Hz, 1H, CH$_2$Ph), 4.56 (d, J_{AB} = 11.0 Hz, 1H, CH$_2$Ph), 4.51 (d, J_{AB} = 11.0 Hz, 1H, CH$_2$Ph), 4.47 (d, J_{AB} = 11.0 Hz, 1H, CH$_2$Ph), 4.23 (d, J_{AB} = 11.0 Hz, 1H, CH$_2$Ph), 3.96 (d, $J_{3,4}$ = 10.0 Hz, 1H, H-3), 3.93 (m, 1H, H-5), 3.75 (dd, $J_{6a,6b}$ = 10.5 Hz, $J_{6a,5}$ = 3.5 Hz, 1H, H-6a), 3.70 (dd, $J_{6b,6a}$ = 10.5 Hz, $J_{6b,5}$ = 2.0 Hz, 1H, H-6b), 3.65 (appt, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, 1H, H-4), 3.09 (s, 1H, OH); ^{13}C NMR (CDCl$_3$, 125.8 MHz) δ in ppm: 139.4, 138.9, 138.3, 138.4, 136.8 (C, Ar), 133.43, 130.0, 129.1, 128.8, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.8 , 127.7, 127.6, 127.5 (CH, Ar), 101.4 (C-1), 86.2 (C-3), 77.8 (C-2), 76.0 (CH$_2$Ph), 75.8 (C-4), 75.2, 73.4 (2CH$_2$Ph), 72.0 (C-5), 70.1 (CH$_2$Ph), 68.9 (C-6); FT–IR (neat) v in cm^{-1}: 3584, 2921, 1607, 1453, 1166, 1050, 666; HRMS (TOF ES+) for (M+Na)$^+$ C$_{40}$H$_{40}$NaO$_6^+$ (m/z): calc. 639.2717; found 639.2708.

Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-2-*C*-phenyl-α-D-glucopyranosyl)-α-D-mannopyranose (4.35)

The title compound was prepared following the general procedure for ring opening, starting from 1,2-anhydro-2-*C*-phenyl-3,4,6-tri-*O*-benzyl-D-glucopyranose (**4.26**) (21.5 mg, 0.042 mmol), 1,2,3,4-tetra-*O*-benzyl-α-D-mannopyranoside (68.6 mg, 0.127 mmol) and 4-toluenesulfonic acid (2 mg, 0.011 mmol) in 1,2-dichloroethane (500 μL). After concentration under reduced pressure the crude was purified by column chromatography (1:8 EtOAc/petrol) to afford **4.35** (17.6 mg, 42 %) as a yellowish syrup: Rf (EtOAc/ petrol ether 1:4): 0.28; $[\alpha]_D^{20}$: +37.3 (c = 0.32, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ in ppm: 7.90 (dd, J = 10.0 Hz, J = 2.5 Hz, 2H, Ar), 7.38-7.15 (m, 36H, Ar), 7.06

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(dd, J = 9.0 Hz, J = 3.0 Hz, 2H, Ar), 5.05-4.93 (m, 3H, H-1a, CH$_2$Ph), 4.86 (d, J_{1b-2b} = 0.5 Hz, 1H, H-1b), 4.73-4.59 (m, 10H, CH$_2$Ph), 4.48 (d, J_{AB} = 15.0 Hz, 1H, CH$_2$Ph), 4.39 (d, J_{AB} = 15.0 Hz, 1H, CH$_2$Ph), 4.28 (d, J_{AB} = 15.0 Hz, 1H, CH$_2$Ph), 4.15-4.08 (m, 3H, H-4b, H-6b, H-6b'), 4.04 (d, J_{3a-4a} = 9.3 Hz, H-3a), 3.99-3.96 (m, 2H, H-3b, H-5b), 3.85-3.64 (m, 6H, H-5a, CH$_2$Ph, H-2b, H-6a, H-6a', H-4a); ^{13}C NMR (CDCl$_3$, 125.8 MHz) δ in ppm: 140.0, 139.0, 138.7, 138.5, 138.4, 138.1, 137.3, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4 (C-Ar), 103.2 (C-1a), 97.2 (C-1b), 85.6 (C-3a), 80.1 (C-3b), 78.1 (C-2a), 75.8 (C-4a), 75.5 (CH$_2$Ph), 75.5 (CH$_2$Ph), 75.1 (CH$_2$Ph), 74.8 (C-4b), 74.7 (C-5b), 73.3 (CH$_2$Ph), 73.0 (CH$_2$Ph), 72.3 (CH$_2$Ph), 71.9 (C-5a), 71.4 (C-2b), 69.2 (CH$_2$Ph), 68.9 (C-6a), 67.8 (C-6b); FT-IR (KBr) ν in cm^{-1}: 3583, 3062, 3030, 2923, 1496, 1454, 1055, 1028, 698, 666, 608; HRMS (TOF ES+) for (M+Na) C$_{67}$H$_{68}$NaO$_{11}^{+}$ (*m/z*): calc.1071.4654 ; found 1071.4632.
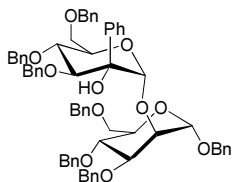
Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-*C*-phenyl-α-D-glucopyranosyl)-α-D-mannopyranose (4.36)

The title compound was prepared following the general procedure for ring opening, starting from 1,2-anhydro-2-*C*-phenyl-3,4,6-tri-*O*-benzyl-D-*gluco*pyranose (**4.26**) (20.5 mg, 0.040 mmol), 1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (64.9 mg, 0.120 mmol) and dry 4-toluenesulfonic acid (2 mg, 0.011 mmol) in dry 1,2-dichloroethane (1 mL). After concentration under reduced pressure the crude was purified by column chromatography (1:8 EtOAc/petrol) to afford **4.36** (14.7 mg, 35 %) as a yellowish syrup: Rf (EtOAc/petrol ether 1:4): 0.36; [α]$_D^{20}$: +25.6 (*c* = 0.08, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ in ppm: 7.90 (dd, J = 8.0 Hz, J = 1.5 Hz, 2H, Ar), 7.40-7.17 (m, 36H, Ar), 7.06 (dd, J = 6.0 Hz, J = 2.5 Hz, 2H, Ar), 5.10-5.08 (m, 2H, H-1b, CH$_2$Ph), 4.93 (s, 1H, H-1a), 4.81 (d, J_{AB} = 10.5, 1H, CH$_2$Ph), 4.67-4.53 (m, 8H, CH$_2$Ph),

158

4.46 (d, J_{AB} = 12.0 Hz, 1H, C$\underline{H}_2$Ph), 4.36 (d, J_{AB} = 12.0 Hz, 1H, C$\underline{H}_2$Ph), 4.30 (d, J_{AB} = 11.0 Hz, 1H, C$\underline{H}_2$Ph), 4.28 (d, J_{AB} = 15.0 Hz, 1H, C$\underline{H}_2$Ph), 4.17 (d, 1H, H-5b), 4.02 (d, $J_{3a\text{-}4a}$ = 9.5Hz, H-3a), 4.00 (m, 1H, H-5a), 3.88 (t, $J_{2b,1b}$ = $J_{2b\text{-}3b}$ = 3.8 Hz, 1H, H-2b), 3.87-3.78 (m, 4H, H-3b, H-4b, H-6a, H-6a'), 3.73-3.66 (m, 3H, H-6b, H-6b', H-4a); ^{13}C NMR (CDCl$_3$, 125.8 MHz) δ in ppm: 140.0, 138.6, 138.7, 138.5, 138.2, 137.7, 137.3, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.2, 128.2, 128.1, 128.0, 127.8, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4 (C-Ar), 105.5 (C-1a), 98.1 (C-1b), 85.9 (C-3a), 79.7 (C-5a), 79.0 (C-2b), 78.4 (C-2a), 75.9 (C-4a), 75.5 (C$\underline{H}_2$Ph), 75.5 (C$\underline{H}_2$Ph), 75.2 (C$\underline{H}_2$Ph), 75.0 (C-3b), 73.4 (C$\underline{H}_2$Ph), 73.3 (C$\underline{H}_2$Ph), 73.1 (C$\underline{H}_2$Ph), 72.1 (C-5b), 71.8 (C-4b), 69.4 (C$\underline{H}_2$Ph), 69.2 (C-6a), 69.1 (C-6b); FT-IR (KBr) ν in cm^{-1}:3583, 3062, 3030, 2962, 2921, 2853, 1454, 1208, 1052, 665, 608, 573, 537; HRMS (TOF ES+) for (M+Na) C$_{67}$H$_{68}$NaO$_{11}$$^+$ (*m/z*): calc.1071.4654 ; found 1071.4634.

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Chapter 5

Strong Inhibition of Cholera Toxin Binding by Hyperbranched Multivalent β-Galceramide Ligands

5.1. Introduction

Cholera is a disease caused by the gram-negative bacillus *Vibrio cholerae* and remains a major public health problem in Africa, Asia and Oceania, with 200,000-500,000 reported new cases each year.[1] In those regions, where sanitary provisions are poor, cholera is often endemic, and symptomless carriers usually induce epidemic outbreaks mostly among people with an impaired immune system such as young children, the elderly or travelers. Cholera is transmitted through contaminated food and water and, when untreated, leads to severe dehydration and shock. Without medical treatment, mortality associated with cholera infection is 20-50%.[2]

V. cholerae belongs to a family of infectious agents that enter cells *via* lipid rafts[3] and the endothelium reticulum (ER), it colonizes the small bowel using toxin-co-regulated pilus (TCP) and interacts with receptors on the intestinal epithelium.[4] Once attached, the bacterium secretes its toxin which is accompanied by the release of hemagglutinin/protease (HA/protease). This extracellular HA/protease is responsible for nicking the Cholera Toxin A subunit (CTA) at Arg192, yielding discrete CTA1 and CTA2 subunits which are solely connected by a single disulfide bond. This post-translational modification is critical for full activity of the toxin, leading to an increase in cyclic adenosine monophosphate (cAMP) production.[5] This causes massive secretion of electrolytes and water into the intestinal lumen, paralleled by excretion of the bacteria.[6]

[1] *WHO Weekly Epidemiological Record* **2010**, *85*, 293−308.

[2] Sack, D.A.; Sack, R.B.; Nair, G. B.; Siddique, A. K. *Lancet*, **2004**, *363*, 223−233.

[3] (a) DeMarco, M.L.; Woods, R. J. *Glycobiology*, **2009**, *19*, 344-355; (b) Sonnino, S.; Mauri, L.; Chigorno, V.; Prinetti, A. *Glycobiology*, **2007**, *17*, 1R-13R; c) Lingwood, C.A.; *Biochim. Biophys. Acta*, **1999**, *1455*, 375−386; (d) Lingwood, C. A. *Glycoconjugate J.* **1996**, *13*, 495-503; (e) Lingwood, C. A. *Curr. Opin. Struct. Biol.* **1992**, *2*, 693-700.

[4] Fujinaga, Y. *Toxin Rev.* **2006**, *25*, 47−59.

[5] Spangler, B. D. *Microbiol. Rev.* **1992**, *56*, 622-647.

[6] (a) Broeck, D. V.; Horvath, C.; De Wolf, M. J. S. *Int. J. Biochem. Cell Biol*. **2007**, *39*, 1771-1775; (b) Lencer, W. I.; Saslowsky, D. *Biochim. Biophys. Acta*, **2005**, *1746*, 314-321.

Current methods for prevention and control of cholera disease include vaccines based on either killed whole-cell bacteria or live attenuated bacteria consisting of the recombinant strain CVD 103 HgR, which lacks the gene encoding the A subunit.[1, 7]

Antibiotic treatment with tetracycline and furazolidane is also used and recommended to reduce severe diarrhea in Cholera patients. However, the use of antibiotics is not a viable solution because of the rapid increase in antibiotic resistance, particularly in endemic areas.[1,8] Alternatively, inhibition of toxin binding by targeting the Cholera Toxin B subunit-receptor (CTB-receptor) interaction is a particularly attractive approach with therapeutic potential for the treatment of cholera disease.

Cholera toxin consists of a single enzymatically active subunit A and five identical binding domains B which form a highly stable ringlike assembly. The infection mechanism is initiated by binding of each B subunit to up to five molecules of the soluble, monovalent oligosaccharide moiety (GM1os) of the cell surface glycolipid monosialoganglioside GM1 (**5.1**) (Figure 5.1.). This complex glycolipid is presented in the plasma membrane of vertebrate cells with the oligosaccharide chain exposed to the external environment.[9] The X-ray structure of CTB-GM1os shows a "two-fingered grip" of the sugar on the toxin comprising a sialic acid thumb and a Galβ(1→3)GalNAc forefinger. Interestingly, these two binding elements are connected through a 3,4-galactose residue which preorganize the motif to allow a highly efficient interaction with CTB.

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[7] (a) Silva, A. J.; Eko, F. O.; Benitez, J. A. *Biotechnol. Lett*. **2008**, *30*, 571−579; (b) Ryan, E. T.; Calderwood, S. B. *Clin. Infect. Dis*. **2000**, *31*, 561-565.

[8] Garg, P.; Chakraborty, S.; Basu, I.; Datta, S.; Rajendran, K.; Bhattacharya, T.; Yamasaki, S.; Bhattacharya, S. K.; Takeda, Y.; Balakrish Nair, G.; Ramamurthy, T. *Epidemiol. Infect*. **2000**, *124*, 393-399.

[9] Merritt, E. A.; Sarfaty, S.; van den Akker, F.; L'Hoir, C.; Martial, J. A.; Hol, W. G. *Protein Sci*. **1994**, *3*, 166-175.

Based on this analysis, most of the small[10] and multivalent[11] CTB-inhibitors that have been designed to date mimic the perfectly locked conformation of the two terminal key sugar elements, galactose and sialic acid, in GM1os. However, the specificity of the *lipid portion* of GM1 has received little attention in comparison with that of the carbohydrate moiety. As previously noted, GM1 is typically embedded in lipid membranes. Although the conformation of the carbohydrate head group of this ganglioside may be only weakly influenced by the membrane environment, the accessibility of its protein-binding epitope (GM1os) relies on head-group presentation (relative to the membrane surface). Early studies concluded that although the lipophilic moiety of the ganglioside is not essential for binding, it is important to preserve the structural integrity of lipid domains as key elements for the aforementioned carbohydrate head group presentation towards the CTB receptor.[12] Moreover, GM1 is also important for the internalization and activation of the toxin.[13] Consequently, and since the infection occurs *via* a lipid raft mediated process, it is reasonable to think that

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[10] (a) Cheshev, P.; Morelli, L.; Marchesi, M.; Podlipnik, Č.; Bergström, M.; Bernardi, A. *Chem. Eur. J.* **2010**, *16*, 1951-1967; b) Bernardi, A.; Cheshev, P. *Chem. Eur. J.* **2008**, *14*, 7434-7441 and references cited therein; (c) Podlipnik, Č.; Velter, I.; La Ferla, B.; Marcou, G.; Belvisi, L.; Nicotra, F.; Bernardi, A. *Carbohydr. Res.* **2007**, *342*, 1651−1660; (d) Pickens, J. C.; Merritt, E. A.; Ahn, M.; Verlinde, C. L. M. J.; Hol, W. G. J.; Fan, E. K. *Chem. Biol.* **2002**, *9*, 215-224.

[11] (a) Pieters, R. J. *Org. Biomol. Chem.* **2009**, *7*, 2013-2025; (b) Sisu, C.; Baron, A. J.; Branderhorst, H. M.; Connell, S. D.; Weijers, C. A. G. M.; de Vries, R.; Hayes, E. D.; Pukin, A. V.; Gilbert, M.; Pieters, R. J.; Zuilhof, H.; Visser, G. M.; Turnbull, W. B. *ChemBioChem*, **2009**, *10*, 329-337; (c) Liu, S.; Kiick, K. L. *Macromolecules*, **2008**, *41*, 764-772; d) Pukin, A. V.; Branderhorst, H. M.; Sisu, C.; Weijers, C. A. G. M.; Gilbert, M.; Liskamp, R. M. J.; Visser, G. M.; Zuilhof, H.; Pieters, R. J. *ChemBioChem*, **2007**, *13*, 1500-1503; (e) Branderhorst, H. M.; Liskamp, R. M. J.; Visser, G. M.; Pieters, R. J. *Chem. Commun.* **2007**, 5043-5045; (f) Rojo, J.; Delgado, R. *Anti-Infect. Agents Med. Chem.* **2007**, *6*, 151-174; (g) Arosio, D.; Fontanella, M.; Baldini, L.; Mauri, L.; Bernardi, A.; Casnati, A.; Sansone, F.; Ungaro, R. *J. Am. Chem. Soc.* **2005**, *127*, 3660-3661; (h) Pickens, J. C.; Mitchell, D. D.; Liu, J.; Tan, X.; Zhang, Z.; Verlinde, C. L. M. J.; Hol, W. G. J.; Fan, E. *Chem. Biol.* **2004**, *11*, 1205-1215; (i) Arosio, D.; Vrasidas, I.; Valentini, P.; Liskamp, R. M. J.; Pieters, R. J.; Bernardi, A. *Org. Biomol. Chem.* **2004**, *2*, 2113-2124; (j) Zhang, Z.; Pickens, J. C.; Hol, W. G. J.; Fan, E. *Org. Lett.* **2004**, *6*, 1377-1380; (k) Zhang, Z.; Merritt, E. A.; Ahn, M.; Roach, C.; Hou, Z.; Verlinde, C. L. M. J.; Hol, W. G. J.; Fan, E. *J. Am. Chem. Soc.* **2002**, *124*, 12991-12998; l) Vrasidas, I.; de Mol, N. J.; Liskamp, R. M. J.; Pieters, R. J. *Eur. J. Org. Chem.* **2001**, 4685-4692; (m) Thomson, J. P.; Schengrund, C. -L. *Biochem. Pharmacol.* **1998**, *56*, 591-597; (n) Thompson, J. P.; Schengrund, C. -L. *Glycoconjugate J.* **1997**, *14*, 837-845.

[12] Masserini, M.; Palestini, P.; Pitto, M.; Chigorno, V.; Tomasi, M.; Tettamanti, G. *Biochem. J.* **1990**, *271*, 107-111.

[13] (a) Miller, C. E.; Majewski, J.; Watkins, E. B.; Kuhl, T. L.; *Biophys. J.* **2008**, *95*, 629-640; (b) Wolf, Y. Fujinaga, A. A.; Lencer, W. I. *J. Biol. Chem.* **2002**, *18*, 16249-16256; (c) McCann, J. A.; Mertz, J. A.; Czworkowski, J.; Picking, W. D.; *Biochemistry*, **1997**, *36*, 9169-9178.

any change in the lipid composition or its molecular environment that might alter the lipid raft integrity will affect the oligosaccharide (GM1os) display toward the CTB receptor and hence, the overall infection process (although not the binding event).

Based on these findings, and considering the importance of both the sugar and lipid portion in the GM1 receptor, we envisioned an "artificial lipid raft" approach to designing cholera toxin inhibitors. In particular, we propose a systematic study aimed to develop multivalent (dendritic) water soluble mimics of GM1 receptors (with cross-linked lipid units) as new biomaterials which simulate the effect of the lipid moiety and membrane presentation of the carbohydrate head group (GM1os) in lipid rafts.

Although availability of gram quantities of GM1 is limited,[14] binding studies of GM1 have previously shown that its terminal galactose residue contributes a large portion of the binding energy.[15] Therefore, our approach will maintain this galactose residue together with the lipid portion in the design of a minimalist, synthetically more accessible version of GM1 glycolipid **5.1**. Thus, we postulated that clusters of naturally occurring *β*-galactosylceramide derivative **5.2** will behave similarly to GM1 (**5.1**) (Figure 5.1.). Hence the expected affinity loss due to the lack of the sialic acid unit in **5.1** would be compensated by increasing the number of copies of *β*-galceramide **5.2** by the phenomenon so-called glycoside cluster effect.[16] Additionally, the simplified model **2.43** that uses cheap, readily available building blocks has also been explored (Figure 5.1.).

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[14] Avanti Polar lipids, Inc. price list (updated June 2010): 93 US$/mg of Ganglioside GM1 from Ovine Brain.

[15] Turnbull, W. B.; Precious, B. L.; Homans, S. W. *J. Am. Chem. Soc.* **2004**, *126*, 1047-1054.

[16] Lundqist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555-578.
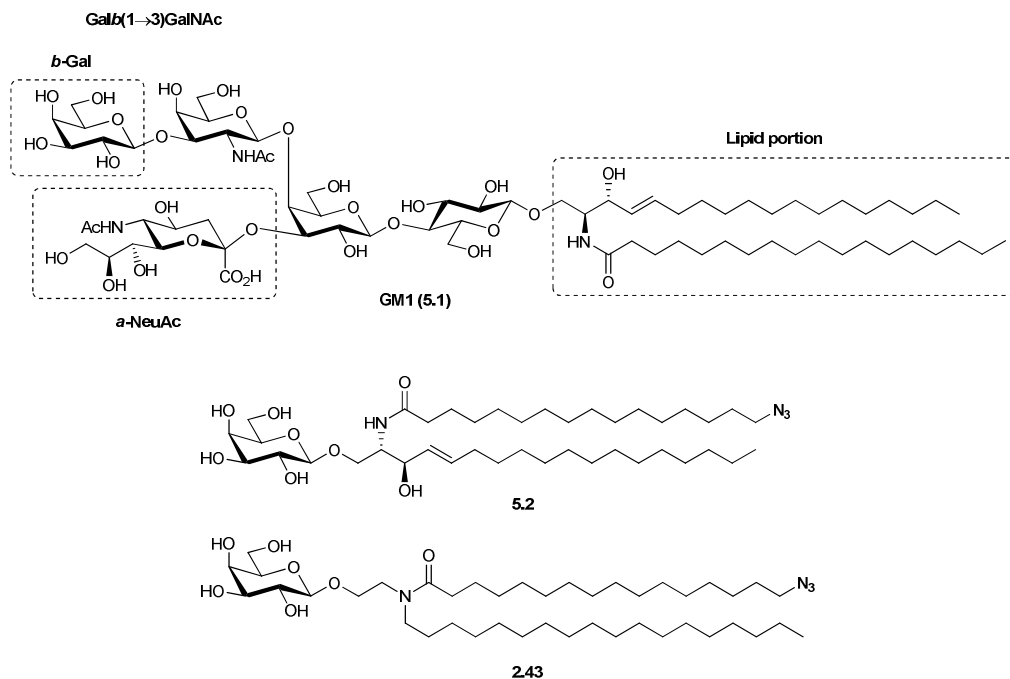
Figure 5.1. Structure of GM1 ligand **5.1** and *β*-Galcer analogues **5.2** and **2.43** mimicking key features (terminal *β*-Gal unit and lipid portion) in GM1 (**5.1**)

In this context, we designed multivalent clusters and hyperbranched dendritic polymers containing glycolipids analogues from **5.2** and/or **2.43** as a promising new class of biomimetic materials that fulfill the requirements mentioned above. Moreover, this study will let us compare if the glycolipid presentation of these multimeric materials is relevant for the interaction against the colera toxin.

5. 2. Results and Discussion

5.2.1. Anchoring glycolipids into multivalent clusters

As it was mentioned in the Chapter 1, glycoclusters are a multivalent central scaffold connected to carbohydrate moieties displayed directly at their periphery directly. Although they can not increase their generation as in the case of dendrimers, they constitute an easy way to obtain multivalent systems with a well-known number of epitopes. If the increase of the affinity promoted by the

multimeric system is widely superior than the associated to its valency, glycocluster effect could be operating.

Multivalent display of neoglycoconjugates, to mimic natural presentation of carbohydrate structures, attracts increasing applications of azide-alkyne cycloaddition chemistry. Due to the importance of this reaction in the context of this chapter, some of the more important features of its reactivity will be commented bellow:

5.2.1.1. Reactivity of catalyzed azide-alkyne cycloaddition

1,3-Dipolar cycloaddition reactions have been notably studied by Rolf Huisgen's group, whose work led to the formulation of the general concepts of these reactions.[17]

Although the reaction is highly exothermic its high activation barrier results in exceedingly low reaction rates for unactivated reactants even at elevated temperature.[18]

Moreover, since the differences in HOMO–LUMO energy levels for both azides and alkynes are of similar magnitude, both dipole-HOMO- and dipole-LUMO-controlled pathways operate in these cycloadditions. As a result, a mixture of regioisomeric 1,2,3-triazole products **5.5** and **5.6** is usually formed when an alkyne is unsymmetrically substituted (Scheme 5.1.).
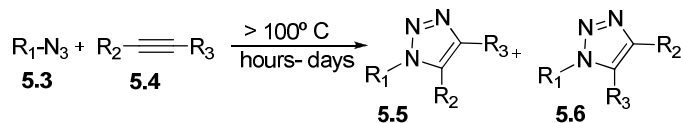
Scheme 5.1. The uncatalyzed termal cycloaddition of azide to alkynes results in mixtures of 1,4- and 1,5-disubstituted regioisomers

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[17] Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 565-598.
[18] Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210-216.

However, the reaction of azides with terminal alkynes (**5.7**) catalyzed by copper (I) catalysis, it is very fast accelerated and yield exclusively the corresponding 1,4-regioisomer (**5.8**) of the triazole products[19] (Scheme 5.2.). Moreover, Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) exhibits several features that makes this reaction unique among other block-ligation reactions as it was reported by the group of Fokin:[20]

- Generally, the reaction is not significantly affected by the electronic and steric properties of the groups attached to the alkyne and azide reactive centers.
- The CuAAC can be carried out in water and is not affected by most organic and inorganic functional groups; so protecting-group chemistry can be avoided for this kind of reactions.
- The rate of Cu(I)-catalyzed process is accelerated over 10^7 times that the uncatalyzed version.[18]
- The formation of the 1,2,3-triazole moiety offers: a high chemical stability, a strong dipole moment, an aromatic character and a good hydrogen-bond-accepting ability. These features make this unit a good replacement for the amide linkage in some cases.
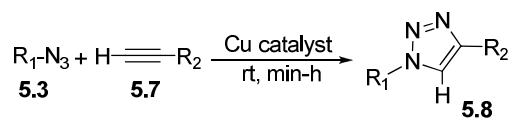
Scheme 5.2. Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC)

Catalytic cycle for CuAAC was reported by Fokin and co-workers based on DFT calculations[20] (Scheme 5.3.): The formation of copper acetylide **5.9** (Step a) probably occurs through the intermediacy of a π alkyne-copper complex. The π coordination of an alkyne to copper was calculated to move the pKa of the alkyne terminal proton down, bringing it into the proper range to be deprotonated in an aqueous medium. Then, the azide is activated by

[19] (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596-2599. (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057-3064.
[20] Hein, J. E.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1302-1315.

coordination to copper (step b), forming the intermediate **5.10**. The key bond-forming event takes place in the next step (step c), when **5.10** is converted to the unusual 6-membered copper metallacycle **5.11**. This step is endothermic by 12.6 kcal/mol with a calculated barrier of 18.7 kcal/mol, which is considerably lower than the barrier for the uncatalyzed reaction (approximately 26.0 kcal/mol), thus accounting for the enormous rate acceleration accomplished by Cu(I). Alternatively, the CuAAC mechanism was investigated by DFT calculations taking into account the possibility of the involvement of dinuclear copper(I) acetylides[21] (Scheme 5.3., B). In the transition state **5.14**, a second copper(I) atom, Cu^B, strongly interacts with the proximal acetylide carbon (C^1). A computational study reported by Straub compared dinuclear complexes with higher order aggregates and concluded that dinuclear intermediates were favored over the tetranuclear complexes.[21b]
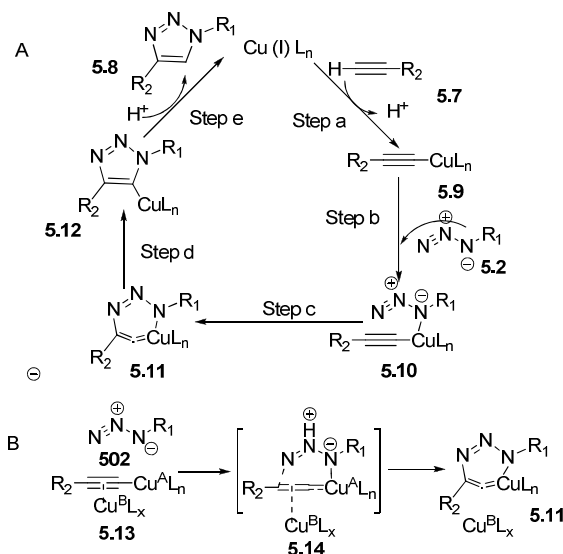
Scheme 5.3. Proposed catalytic cycles for CuAAC by Fokin and co-workers based on DFT calculations

The CuAAC reaction is not a true concerted cycloaddition, and its regiospecificity is explained by the binding of both azide and alkyne to copper prior to the formation of the C–C bond. Due to the energy barrier for the ring

[21] (a) Ahlquist, M.; Fokin, V. V. *Organometallics*, **2007**, *26*, 4389-4391. (b) Straub, B. F. *Chem. Commun.* **2007**, 3868-3870.

contraction of **5.11** is quite low, it evolves to the triazolyl–copper derivative **5.12**. Then, protolysis of **5.12** affords the triazole product **5.8**, thereby completing the catalytic cycle.

Due to thermodynamical unstabilitiy of Cu(I), it is common to generate it *in situ* through comproportionation of Cu(II) and Cu(0) salts in presence of a sacrifical reducing agent such as sodium ascorbate in aqueous alcohols as solvents. However, a wide number of different copper(I) sources can be utilized in the reaction such as copper(I) salts (CuI, CuBr) and coordination complexes ($[Cu(CH_3CN)_4]PF_6$,[19a] $(EtO)_3P•CuI$,[22] $[Cu(PPh_3)_3]Br$[23]) depending on the solubility of the system; although exclusion of oxygen may be required. Interestingly, addition of ligands such as tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) are able to accelerate the CuAAC reaction and stabilize the Cu(I) catalysts.[24] In addition, it appears to sequester copper(I) ions, so preventing damage to biological compounds.

Ideally, CuAAC work at room temperature, reaching full conversion within a few minutes, and providing quantitative isolated product yields but, in some cases, synthetically relevant click processes require high temperatures to proceed within a reasonable timeframe. Controlled microwave heating under sealed vessel conditions has been shown to reduce reaction times, increase product yields and to enhance product purities by reducing side reactions compared to conventional thermal methods.[25,26] As it was commented on the previous chapter, microwave chemistry generally relies on the ability of the reaction mixture to efficiently absorb microwave energy, taking advantage of ''microwave dielectric heating'' phenomena such as the dipolar polarization or

[22] Perez-Balderas, F.; Ortega-Muñoz, M.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Calvo-Flores, F. G.; Calvo-Asin, J. A.; Isac-García, J.; Santoyo-Gonzalez, F. *Org. Lett.* **2003**, *5*, 1951-1954.

[23] (a) Malkoch, M.; Schleicher, K.; Drockenmuller, E.; Hawker, C. J.; Russell, T. P.; Wu, P.; Fokin, V. V. *Macromolecules* **2005**, *38*, 3663-3678. (b) Wu, P.; Feldman, A. K.; Nugent, A. K.; Hawker, C. J.; Scheel, A.; Voit, B.; Pyun, J.; Fréchet, J. M. J.; Sharpless, K. B.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2004**, *43*, 3928-3932.

[24] Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853-2855.

[25] Kappe, C. O. *Chem. Soc. Rev.* **2008**, *37*, 1127-1139.

[26] (a) Caddick, S.; Fitzmaurice, R. *Tetrahedron*, **2009**, *65*, 3325-3355, and references therein; (b) P. Appukkuttan, P.; Van der Eycken, E. *Eur. J. Org. Chem.* **2008**, 1133-1155, and references therein.

ionic conduction mechanisms.[25] Thus, very efficient internal heat transfer decreases side effects which may lead to for example diminished catalyst deactivation.[25] For these reasons, many subsequent examples of 1,3-dipolar cycloaddition reactions applying this alternative energy transfer technique have been reported.[27]

To date, copper stands out as the only metal for the reliable, facile, and 1,4-regiospecific catalysis of the azide-alkyne system. However, ruthenium cyclopentadienyl complexes were found to catalyze the formation of the complementary 1,5-disubstituted triazole (**5.8**) from azides and terminal alkynes and also to engage internal alkynes in the cycloaddition[28] (Scheme 5.4.). While the scope and functional group compatibility of Ruthenium-catalized Azide-Alkyne Cycloaddtion (RuAAC) are excellent,[29] the reaction is more dependent on the solvents and the steric requirements of the azide substituents than CuAAC so applications of RuAAC are only beginning to appear.
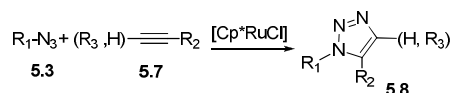
Scheme 5.4. Ruthenium-catalyzed Azide-Alkyne Cycloaddition (RuAAC)

Finally, new efforts in developing copper-free azide-alkyne cycloaddition under mild conditions have been reported recently.[30] They are based on the enhanced reactivity of cyclooctynes **5.15** with azides **5.3** to afford 1,2,3-triazoles **5.16** and **5.17** (Scheme 5.5.). This reaction has been employed to solve many problems associated with the toxicity of metallic catalyst when the coupling strategy is used in chemical biology and materials science, and it may have additional applications in these and other areas in the future.

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[27] Kappe, C.O.; Van der Eycken, E. *Chem. Soc. Rev.* **2009**, *39*, 1280-1290.

[28] Zhang, L.; Chen, X.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. *J. Am. Chem. Soc.* **2005**, *127*, 15998-15999.

[29] (a) Boren, B. C.; Narayan, S.; Rasmussen, L. K.; Zhang, L.; Zhao, H.; Lin, Z.; Jia, G.; Fokin, V. V. *J. Am. Chem. Soc.* **2008**, *130*, 8923-8930. (b) Rasmussen, L. K.; Boren, B. C.; Fokin, V. V. *Org. Lett.* **2007**, *9*, 5337-5339. (c) Majireck, M. M.; Weinreb, S. M. *J. Org. Chem.* **2006**, *71*, 8680-8683.

[30] Baskin, J. M; Bertozzi, C. R. *Aldrichimica Acta* **2010**, *43*, 15-23.
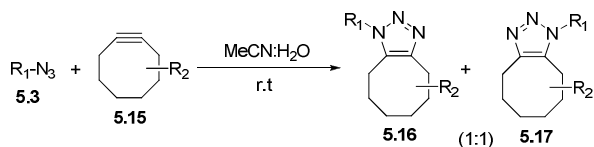
Scheme 5.5. Copper free cyclooctyne-azide cycloaddtion

With this background in mind, the synthesis of a family of new multimeric clusters of glycolipids will be discussed in the following section.

5.2.1.2. Synthesis of glycolipid-clusters

Initially a family of scaffolds based on benzyl bromide derivates was selected as starting materials for the synthesis of multimeric cores. Scheme 5.6. depicts the formation of the propargyl benzyl ethers **5.20**, **5.22** and **5.24** resulting from the nucleophilic substitution of propargyl alcohol over the corresponding benzyl bromides **5.19**, **5.21** and **5.23** respectively under Williamson conditions. In all cases the propargyl alcoholate was preformed by treatment of the corresponding alcohol with NaH. Then, the nucleophilic substitution of all the arms of the benzyl bromides with the propargyl alcoholate was performed in presence of TBAI at 70 ºC. The reactions were monitored by TLC and ^{1}H NMR to ensure complete substitution and yields 73-97% were good (Scheme 5.6.).
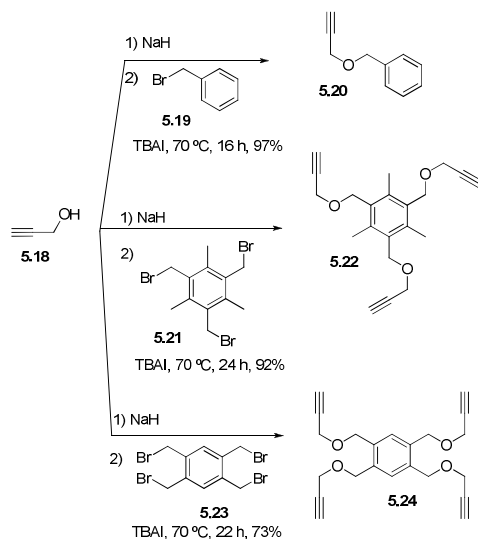
Scheme 5.6. Synthesis of the propargylic cores

Glycolipid **2.43** was prepared according the methodology developed in Chapter 2. The preparation of glycolipids with an azide group incorporated in the ceramide moiety was envisioned to be employed in the CuAAC reaction with the presented propargyl cores. Initially, the catalytic system employed consisted of generating Cu(I) *in situ* when $CuSO_4 \cdot 5H_2O$ was reduced in presence of sodium ascorbate. Moreover, to accelerate the process, glycolipid **2.43** and the monomeric core **5.20** dissolved in a mixture of *t*BuOH-H_2O (1:1) were heated at 100 ºC under microwave assistant irradiation. As a result, triazole **5.25** was obtained in 90% yield (Table 5.1., Entry 1). Consequently, the same conditions were tested for glycolipid **2.43** and the trimeric scaffold **5.22**, but in this case the reaction was not completed after 1 h. We considered reasonable that the steric hindrance exerted by the first glycolipid that underwent the cycloaddition may interference with the complete functionalization of all the arms on the core. Thus, the reaction under microwave irradiation was continued for further 2.5 h but the forced conditions only conducted to the partial decomposition of cluster **5.26** (Table 5.1., Entry 2). At this point, $[Cu(CH_3CN)_4]OTf$ was selected as a Cu(I) catalyst because of its solubility in aqueous alcohols. Since long reaction times were expected to complete the reaction, the ligand TBTA was employed due to its reported ability to avoid Cu(I) disproportionation.[24] With this precaution in mind, the CuAAC reaction between propargyl **5.20** and glycolipid **2.43** at the room temperature for 24 h afforded the triazole **5.25** with a 93% (Table 5.1., Entry 3). Then, the cyclooaddition between **2.43** and core **5.22** was assayed under similar conditions (Table 5.1., Entry 4). After 48 h of reaction, the cluster **5.26** was obtained in good yield (70%). Motivated for that succeed, cluster **5.24** and glycolipid **2.43** were treated under the same catalytic system (Table 5.1., Entry 5). Although the cluster **5.27** was also isolated, long reaction time (72 h) was needed. Moreover, the yield was not so good as in the other cases (only 25%) so we considered that high steric hindrance exerted by the lipid chains in a so small core may be responsible of this lowering yield.

Table 5.1. Synthesis of glycolipid clusters employing CuAAC reaction
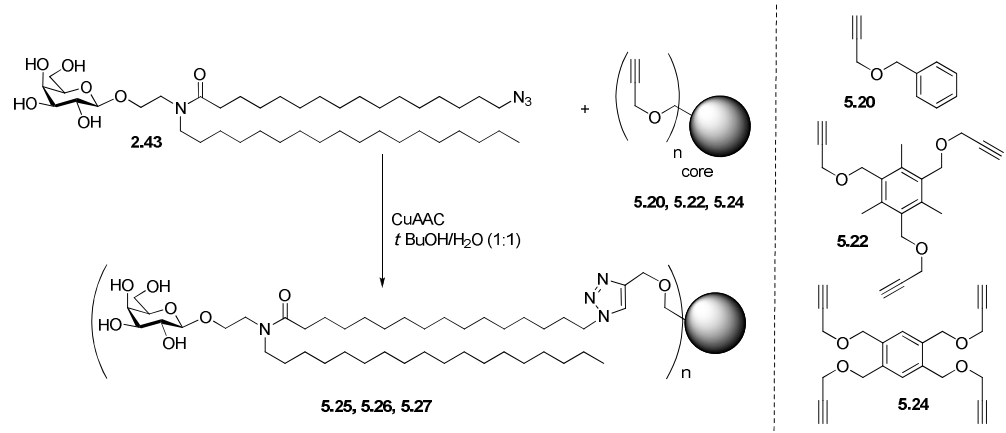
Entry[a]	Core	Glycolipid (eq)	Catalytic system	T	t	Product[b]
1	**5.20**	**2.43** (1.1)	CuSO₄·5H₂O Sodium ascorbate	100º C MW[c]	1 h	**5.25** (90%)
2	**5.22**	**2.43** (3.3)	CuSO₄·5H₂O Sodium ascorbate	100º C MW[c]	2.5 h	-[d]
3	**5.20**	**2.43** (1.1)	[Cu(CH₃CN)₄]OTf TBTA	r.t.	24 h	**5.25** (93%)
4	**5.22**	**2.43** (3.3)	[Cu(CH₃CN)₄]OTf TBTA	r.t.	48 h	**5.26** (70%)
5	**5.24**	**2.43** (4.4)	[Cu(CH₃CN)₄]OTf TBTA	r.t.	72 h	**5.27** (25%)

[a] General conditions: Glycolipid **2.43** and the corresponding core (1 eq.) were dissolved in a mixture of t-BuOH-H₂O (1:1). After that, the copper salt and the other promoters were added to the solution (1 mol% respect to the core). [b] Isolated yield. [c] Temperature achieved through microwave irradiation (Power = 300 W). [d] A complex mixture of decomposition products was recovered.

Apart from the glycolipid-cluster prepared to the date (Figure 5.2.), it would be convenient for our future studies of glycolipid presentation on multimeric compounds against the cholera toxin, the preparation of other compounds with different ceramide architectures. However, this synthetic approach was

abandoned because the glycolipid-clusters prepared were poorly solubles in water, even in the presence of 5% DMSO as co-solvent, and consequently they could not be employed for the cholera toxin inhibition studies.

For this reason, we planned to employ another platform such as hyperbranched polymers (Boltorn H30) because this scaffold was expected to increase the valency of our multimeric system and consequently, increasing the number of polar group heads without congesting too much the surface of the core. Thus, the final products may be water soluble enough to develop inhibition studies against cholera toxin. The discussion about anchoring glycolipids into hyperbranched polymers is presented below.
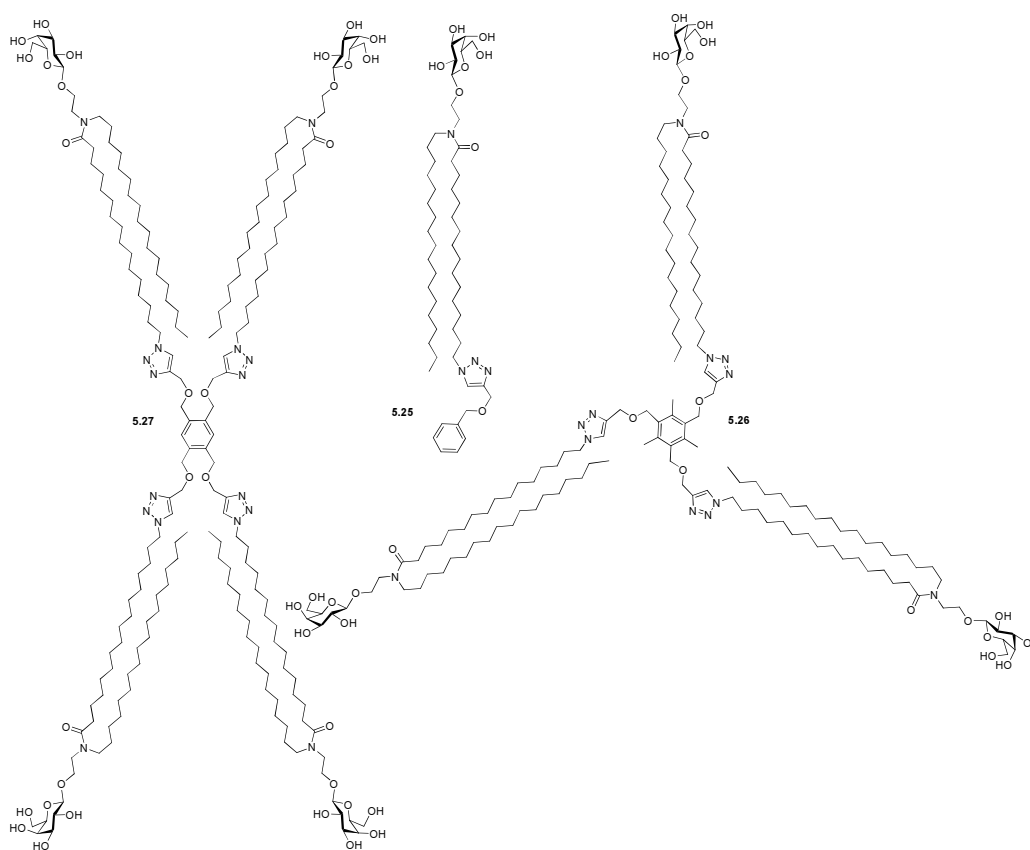
Figure 5.2. Glycolipid clusters **5.25**, **5.26** and **5.27**

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5.2.2. Anchoring glycolipids to hyperbranched polymers

Hyperbranched polymers represent an important part of the family of dendritic and multibranched polymers.[31] While dendrimers have perfect, monodisperse structures built around a core moiety with a branching point in every repeating unit, hyperbranched polymers are polydisperse and include some linear units in their molecular structure,[32] features that allow for mimicry the multivalent displaying of natural glycolipid receptors. Unlike dendrimers, randomly branched hyperbranched polymers with similar properties can be easily synthesized *via* one-step reactions providing access to valuable products for both small-scale and large-scale industrial applications. Their size and structure can be controlled by synthetic means and further modifications can ensure biocompatibility and biodegradability.[18] Thus, it seems certain that hyperbranched polymers will play a significant role in the future development of new biomedical devices for the treatment of human diseases.[33]

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[31] (a) Twyman, L. J.; King, A. S. H.; Burnett, J.; Martin, I. K. *Tetrahedron Lett*. **2004**, *45*, 433-435; (b) Haag, R. *Angew. Chem. Int. Ed*. **2004**, *43*, 278-282; (c) Gao, C.; Xu, Y.; Yan, D.; Chen, W. *Biomacromolecules* **2003**, *4*, 704-712; (d) Kolhe, P.; Misra, E.; Kannan, R. M.; Kannan, S.; Lieh-Lai, M. *Int. J. Pharm*. **2003**, *259*, 143-160; (e) Moorefield, C. N.; Newkome, G. R. *C. R. Chimie* **2003**, *6*, 715-724; (f) Ooya, T.; Lee, J.; Park, K. *J. Control. Release* **2003**, *93*, 121−127; (g) Frey, H.; Haag, R. *Rev. Mol. Biotechnol.* **2002**, *90*, 257-267; h) Crooks, R. M. *ChemPhyschem*, **2001**, *2*, 644-654; (i) Breitenbach, A. Li, Y. X.; Kissel, T. *J. Control Release*, **2000**, *64*, 167-178.

[32] (a) Voit, B. *J. Polym. Sc. Part A: Polym. Chem*. **2005**, *43*, 2679-2699; (b) Gao, C.; Yan, D. *Prog. Polym. Sci*. **2004**, *29*, 183-275; (c) Voit, B. I. *C. R. Chimie*, **2003**, *6*, 821-832; (d) Seiler, M. *Chem. Eng. Technol*. **2002**, *25*, 237-253; (e) Jikei, M.; Kakimoto, M. *Prog. Polym. Sci*. **2001**, *26*, 1233-1285; (f) Voit, B. *J. Pol. Sc. Part A: Polym. Chem*. **2000**, *38*, 2505-2525; (g) Kim, Y. M. *J. Pol. Sc. Part A: Polym. Chem*. **1998**, *36*, 1685-1698; (h) Zeng, F.; Zimmerman, S. C. *Chem. Rev*. **1997**, *97*, 1681-1712; (i) Tomalia, D.; Naylor, A. M.; Goddard III, W. A. *Angew. Chem. Int. Ed. Engl*. **1990**, *29*, 138-175.

[33] (a) Rojo, J.; Delgado, R. *Antimicrob. Agents Chemother*. **2004**, *54*, 579-581; (b) Lasala, F.; Arce, E.; Otero, J. R.; Rojo, J.; Delgado, R. *Antimicrob. Agents Chemother*. **2003**, *47*, 3970-3972; (c) Arce, E.; Nieto, P. M.; Díaz, V.; Castro, R. G.; Bernad, A.; Rojo, J. *Bioconjugate Chem*. **2003**, *14*, 817-823; (d) Voit, B.; Eigner, M.; Estel, K.; Wenzel, C.; Bartha, J. W. *Macromol. Symp*. **2002**, *177*, 147-154; (e) Klee, J. E.; Schneider, C.; Hölter, D.; Burgath, A.; Frey, H.; Mülhaupt, R. *Polym. Adv. Technol*. **2001**, *12*, 346−354; f) Haag, R.; *Chem. Eur. J*. **2001**, *7*, 327-335; (g) Pitois, C.; Wiesmann, D.; Lindgren, M.; Hult, A. *Adv. Mater*. **2001**, *13*, 1483-1487; (h) Duan, L.; Qiu, Y.; He, Q.; Bai, F.; Wang, L.; Hong, X. *Synthetic Met*. **2001**, *124*, 373-377; (i) van Benthem, R. A. T. M. *Prog. Org. Coat*. **2000**, *40*, 203-214; (j) Tao, X. T.; Zhang, Y. D.; Wada, T.; Sasabe, H.; Suzuki, H.; Watanabe, T.; Miyata, S. *Adv. Mater*. **1998**, *10*, 226-230.

As depicted in Figure 5.3., the goal of this work was to prepare different hyperbranched glycopolymers as a promising new class of biomimetic materials that fulfill the requirements mentioned above. Such compounds have been selected to study if a minimalist carbohydrate moiety replicated around the polymer is able to emulate the strong affinity of GM1 (**5.1**) and if the ceramide chain display is important for the inhibition of Cholera Toxin binding by hyperbranched multivalent *β*-Galceramide ligands.
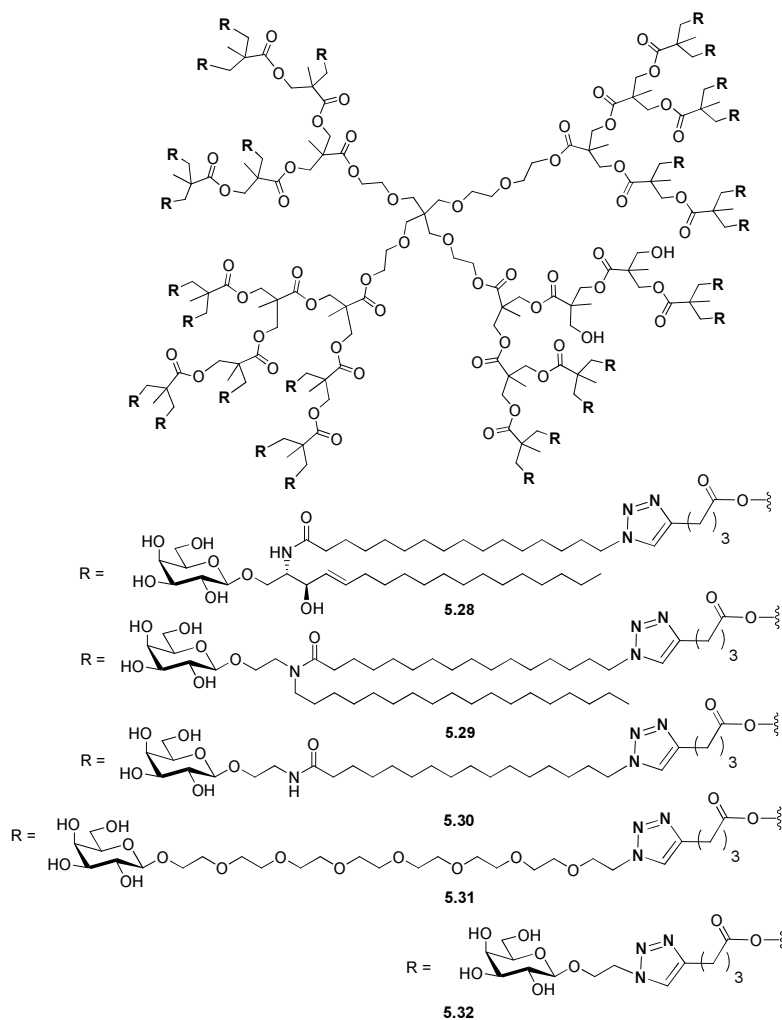
Figure 5.3. Hyperbranched polymers of *β*-Galcer **5.28** and *β*-Galcer analogues **5.29, 5.30, 5.31** and **5.32**

Hyperbranched polymers **5.28** and **5.29** (Figure 5.3.) were previously prepared in our group[34] from glycosides **5.28'** and **2.43** (Figure 5.4.). Such polymers were water soluble and they resulted promising materials when they were studied in the inhibition of HIV-1 rgp 120[35] and the recognition of lectin RCA120.[36] On the other hand, the synthesis of the other hyperbranched polymers **5.30**, **5.31** and **5.32** as well as their corresponding glycolipid starting material was carried out in the present work and it will be discussed in the following sections.

5.2.2.1. Preparation of β-GalCer analogues

Initially, it was necessary to prepare different glycolipid structures bearing an azide group to be employed in a CuAAC coupling. Moreover such glycolipidic structures (**5.33** and **5.34**) possessed an 2-azidoethanol spacer to study the effect of lack of long ceramide chain on the polymer, or 16-azidohexadecanoic acid to study the effect of a single chain ceramide on the polymer. 1-*O*-(2-azidoethyl)heptaethylene glycol was also considered to study the effect of a polar chain on the polymer (**5.35**), since PEG spacers confer a remarkable lower toxicity and immunogenicity, as well as a better biodistribution, to any molecule, polymer, or surface to which they are covalently bonded[37] (Figure 5.4.).

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[34] Morales-Serna, J. A., *Doctoral Thesis*, URV, **2010**.

[35] Morales-Serna, J. A.; Boutureira, O.; Serra, A.; Matheu, M. I.; Díaz, Y.; Castillón, S. *Eur. J. Org. Chem.* **2010**, 2657-2660.

[36] Manuscript under preparation.

[37] (a) Monfardini, C.; Veronese, F. M. *Bioconjugate Chem.* **1998**, *9*, 418-450. (b) Greenwald, R. B.; Choe, Y. H.; McGuire, J.; Conover, C. D. *Adv. Drug Delivery Rev.* **2003**, *55*, 217-250.
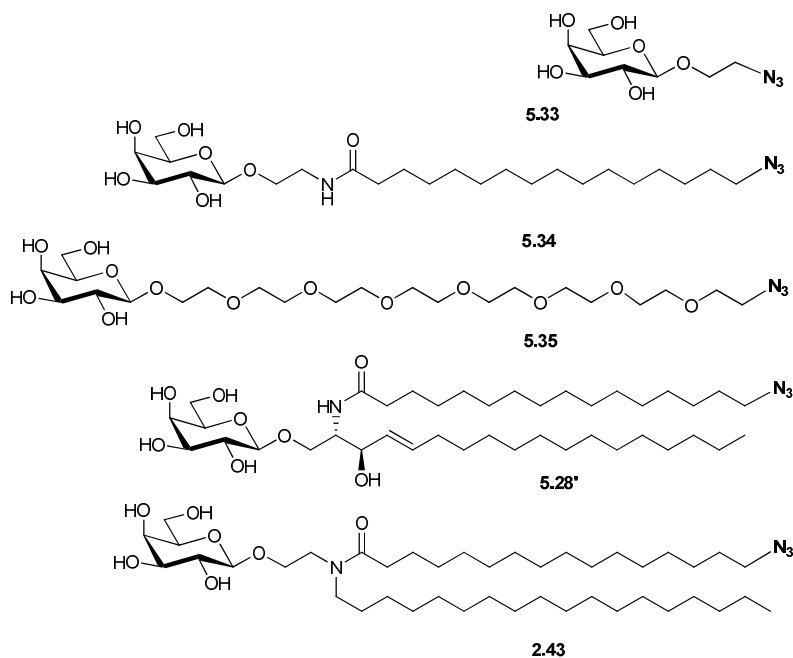
Figure 5.4. β-GalCel analogues with terminal azide group

In order to obtain the glycoside **5.33**, it was necessary to study previously the reaction conditions for glycosylation since mixtures of α and β anomers were inially obtainded (Table 5.2.). When the reaction was run at room temperature for long reaction time under strong Lewis acid conditions a mixture of α and β anomers were obtained. The β anomer could be isolated independently from the mixture by using 2-azidoethanol **5.36** or 2-chloroethanol **5.37** as a glycosyl acceptor (Table 5.2., Entries 1 and 2). The diasteroselectivity decreased when temperature was increased; even when a milder promoter such as AgOTf was employed (Table 5.2., Entry 3) and a complex mixture was obtained together with the desired product. However, better diasteroselectivities were obtained when lower temperatures and shorter reaction times were employed. Finally, glycoside **5.38** was obtained with complete β selectivity when toluene was employed as a solvent under similar reaction conditions (Table 5.1., Entry 4 and 5).

Table 5.2. Study of the β-glycosilation of ethanol derivates
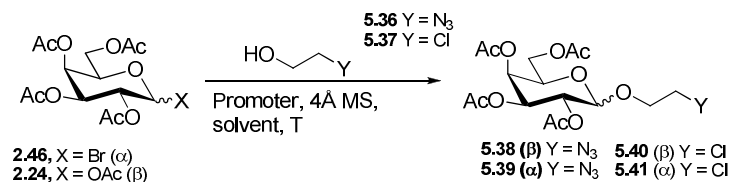
Entry	Donor (X)	Acceptor (Y)	Promoter	Solvent	T	t	Product
1	OAc (β)	N_3	$BF_3 \cdot OEt_2$	DCM	0 ºC to r.t	16 h	53% β/α = 8:1
2	OAc (β)	Cl	$BF_3 \cdot OEt_2$	DCM	0 ºC to r.t	16 h	65% β/α = 3.5:1
3	Br (α)	N_3	AgOTf	DCM	0 ºC to 35 ºC	17 h	22% complex mixture
4	Br (α)	N_3	AgOTf	DCM	-20 ºC to 0 ºC	5 h	44% β/α = 10:1
5	Br (α)	N_3	AgOTf	toluene	-20 ºC to 0 ºC	3 h	67% β

Once **5.38** was obtained, deprotection in basic conditions (MeONa 1M in MeOH) afforded glycoside **5.33** in good yields 95% (Scheme 5.7.).
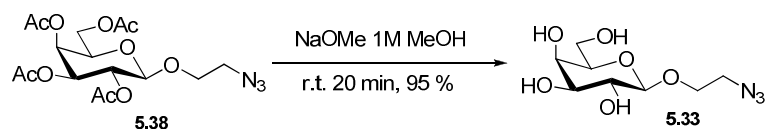
Scheme 5.7. Deprotection of glycoside **5.38**

In order to obtain **5.34**, glycoside **5.38** was employed as a precursor. Thus, **5.38** was submitted to hydrogenation conditions catalyzed by Pd/C in THF for 18 h (Scheme 5.8.). The formation of the corresponding amine was confirmed by ^{13}C NMR analysis after the disappearance of the signal of the carbon adjacent to azide (δ 50.7 ppm) and the appearance of a new signal at δ 41.6 ppm corresponding to the carbon adjacent to amine.
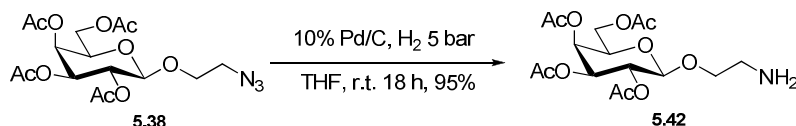
Scheme 5.8. Reduction of glycoside **5.38**

Amine **5.42** was acylated with lipid **2.29** employing coupling reagents (EDC, HOBt and DIPEA) to give the glycolipid **5.43** (56%) after 24 h at room temperature. Then, the deprotection of **5.43** under 1 M MeONa MeOH conditions for 20 min a room temperature (Scheme 5.9.) provided the desired glycolipid **5.34** in 95 % yield.
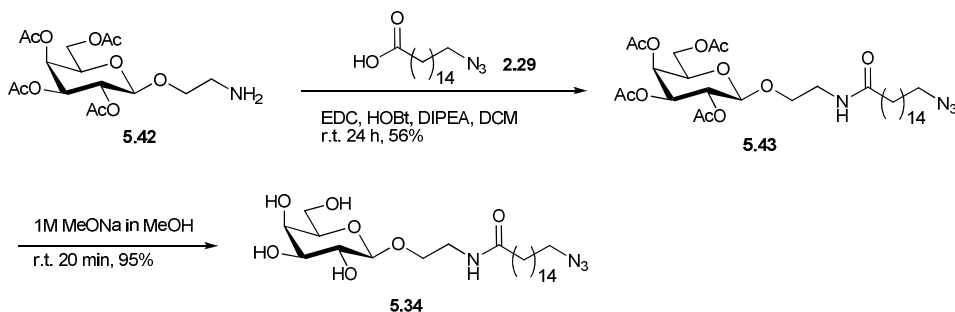
Scheme 5.9. Synthesis of glycolipid **5.34**

Verez-Bencomo[38] *et al.* reported the glycosylation of 5-azido-3-oxapentanol employing glycosyl donor **2.46** in good yield (83%) using Hg(CN)$_2$ as a promoter and acetonitrile as solvent. In our case, we respected their glycosylation conditions but we replaced the harmful Hg(CN)$_2$ by AgOTf. As a

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[38] González Nuñez, F.; Campos Valdés, M. T.; Aruca, E.; Schmidt, R. R.; Verez Bencomo, V. *J. Carbohydr. Chem*. **2003**, *22*, 395-406.

result, the ethylene glycol glycoside **5.45** was obtained in good yield (76%) (Scheme 5.10.). After that, **5.45** was deprotected under basic conditions (1M MeONa in MeOH, 20 min, r.t.) and the desired glycoside **5.35** was recovered in good yield (85%) after purification by a short C-18 column using a gradient of H_2O to H_2O/MeOH (1:1) as a eluent.
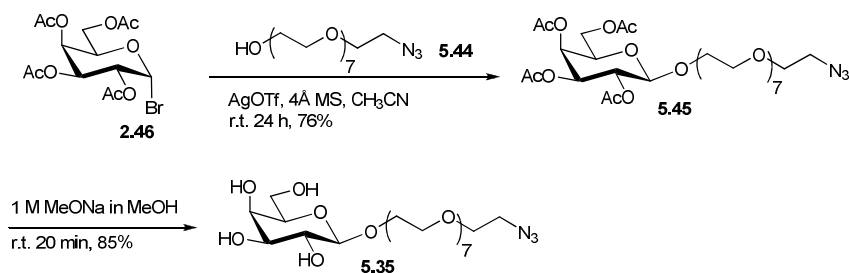
Scheme 5.10. Synthesis of glycolipid **5.35**

5.2.2.2. Synthesis of hyperbranched polymers based on β-GalCer analogues

Once the β-Galceramide ligand analogues were synthesized, we focused our efforts on the functionalization of comercially Boltorn H30 (**5.46**) to complete our library of glyco hyperbranched polymers. Boltorn H30 from Prestorp had already been characterized by Žagar and collaborators.[39] A good knowledge of the chemical structure of Boltorn H30 (**5.46**) was particularly important to understand how it could be influenced by its chemical modification. To illustrate the possible defects in the molecular architecture, a typical chemical structure of a third-generation is shown in Figure 5.5. To the commercial Boltorn H30 (**5.46**), Dendritic **D**, Linear **L**, and terminal **T** units can be distinguished both by ^{1}H NMR using the methyl signal at 1.4-1.0 ppm and ^{13}C NMR using either the carbonyl peaks at 175-171 ppm or that of the quaternary carbons at 50.2-46.2 ppm.

In order to introduce alkynyl moieties to the polymer, a partial modification of the hydroxylic groups of Boltorn H30 (**5.46**) was carried out with 5-hexynoic

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[39] Žagar, E.; Žigon, M.; Podzimek, S. *Polymer*, **2006**, *47*, 166-175.

acid (**5.47**) employing EDC, HOBt and DIPEA (Scheme 5.11.) according to the procedure previously reported in our group.[35] New pseudo-dendritic and pseudo-linear units are generated and ^{1}H NMR and ^{13}C NMR techniques are both useful in detecting these units in the case of the commercial Boltorn H30. All the units that can be found in a partially modified Boltorn H30 are depicted in Scheme 5.11. **D$_i$**, **L$_i$** and **T$_i$** are the initial dendritic, linear and terminal units, respectively; whereas **L$_u$** is a new linear unit created by the reaction of a **T$_i$** unit with one hexynoic acid; **D$_{1u}$** and **D$_{2u}$** arise from the reaction of one hexynoic acid with **L$_i$**, or of two hexynoic acid with **T$_i$**, respectively (Scheme 5.11.).
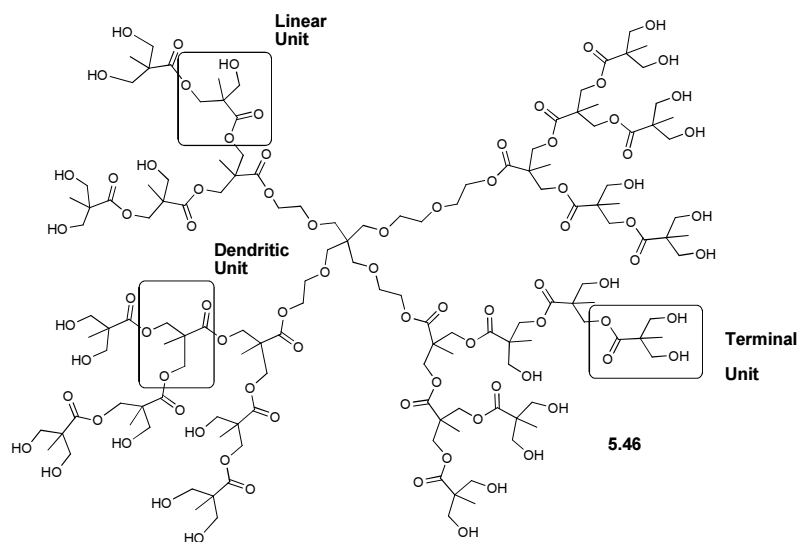
Figure 5.5. Typical structure of commercially Boltorn H30

The ^{1}H NMR spectrum of **5.48** indicated the presence of methyl groups at 1.26 ppm, acetylenic protons at 1.99 ppm, and methylene groups at 1.82, 2.24, and 2.48 ppm, which corresponds to protons β, α and γ to the acetylene moiety, respectively. The methylenic protons of Boltorn shell appeared at 3.68 ppm for alcohol and 4.24 ppm for the ester moieties. The ^{13}C NMR spectrum of the modified Boltorn displays C=O signals between 175-172 ppm than can be attributed to ester groups. Those signals corresponding to acetylenic carbons appear at 84.6 ppm and 71.3 ppm, whereas the methylene groups signals from Boltorn core can be found at 66-63 ppm and the methyl group can be found at

17 ppm. The signals at 34.9, 23.6, and 20.7 ppm correspond to methylene γ, β and α to triple bond, respectively. The chemical modification of Boltorn H30 induces a change in the peaks associated with quaternary carbons; this evolution is visible with a decrease in the intensity of T signals at 50.25 ppm, as well as the appearance of news peaks in the L and D areas between 46.2 ppm and 48.5 ppm.
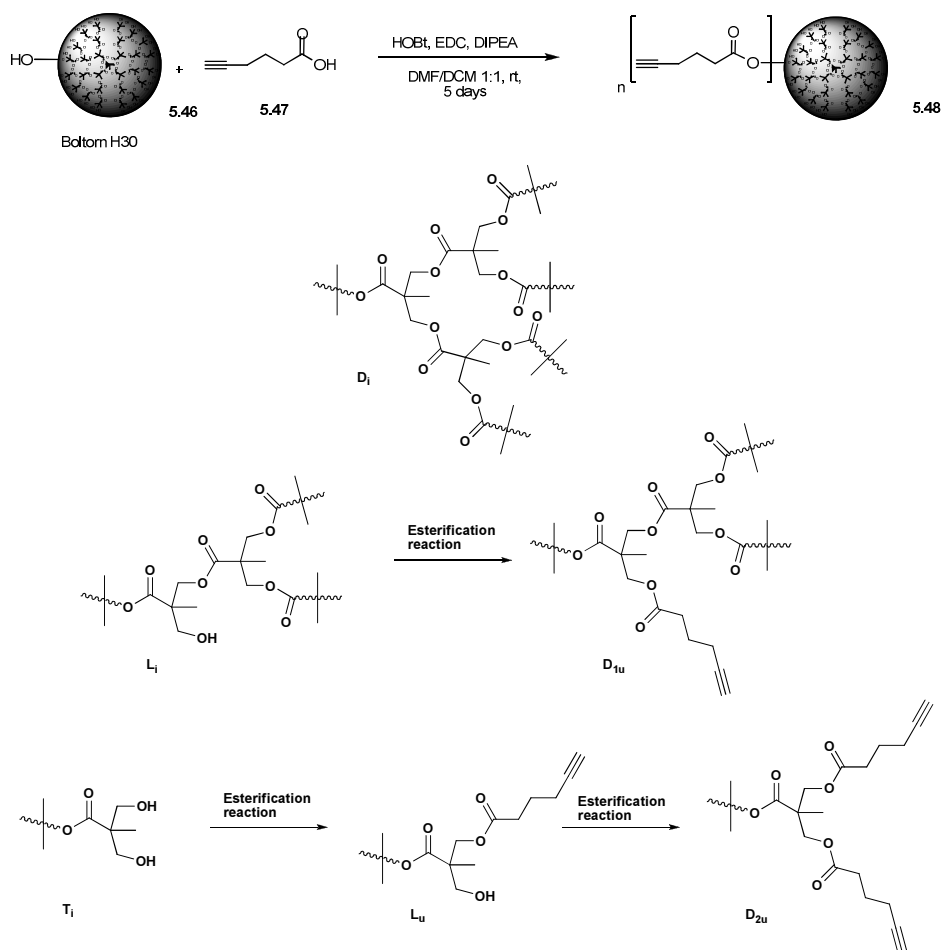
Scheme 5.11. Modification of Boltorn H30 with 5-hexynoyl ester units

Boltorn H30 is known to possess an average of 32 hydroxyl units.[39] It is possible to determinate the average number of hydroxyl modified with 5-hexynoic acid attending the modifications of the intensity of quaternary carbon areas.

However, this procedure requires of long time of accumulation at ^{13}C NMR experiment.[35]

Férnandez-Francos and co-workers reported recently a procedure to determinate the percentage of modification of Boltorn H30 through ^{1}H NMR spectroscopy.[40] According to their notation (Figure 5.6.), signals *A*, *B* and *C* correspond to the Boltorn H30 shell whereas the *A'* and *B'* correspond to the core (ethoxylated pentaerythriol). From the intensities of *C* and *A+A'* signals the intensity of signal *B* can be calculated according to the equations:

$$I_{Core} = I_{A+A'} + I_{B+B'} - \frac{4}{3}(I_C)$$

$$I_{B'} = \frac{10}{14} I_{Core}$$

$$I_B = I_{B+B'} - I_{B'}$$

When the modification takes place, *B* is transformed to *A* but *B'* remains constant. If the modification is total no *B* signals are left, so the ratio of methylene ester signals at 4.24 ppm and the signal of methylene bonded to ether groups (*B'*) that appear around 3.3-3.7 ppm can be calculated according to the following equation:

$$R_{100\%} = \frac{I_{A+A'} + I_B}{I_{B'}} \cdot 100$$

In order to calculate R for product **5.48** (R$_{5.48}$), the intensity of the signals *A+A'*, obtained from ^{1}H NMR spectrum of hyperbranched polymer **5.48**, was divided by *B+B'*. Finally, R$_{\mathbf{548}}$ was compared with the theorical R$_{100\%}$ to find out the degree of esterification obtained.

$$Modification(\%) = \frac{R_{548}}{R_{100\%}} \cdot 100$$

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[40] Fernández-Francos, X.; Foix, D.; Serra, A.; Salla, J. M.; Ramis, X. *Reac. Funct. Polym.* **2010**, *70*, 798-806.

According to this procedure, the degree of modification of hyperbranched polymer **5.48** was about 51% that means that an average of sixteen 5-hexynoate esters groups had been incorporated into the structure. With this information and the theoretical molecular mass (M_{theo}) of Boltorn H30 (3604 g/mol) reported by Žagar and co-workers, it is possible to calculate the M_{theo} for compoud **5.48** as 5110 g/mol. With this value, the yield estimated for the synthesis of hyperbranched polymer was 71%.

Entry	Compound	$I_{(A+A')}$	$I_{(B+B')}$	$I_{(C+C')}$
1[a]	Boltorn H30 **(5.46)**	33.44	60.89	52.95
2	**5.48**	2.34	1.00	1.69
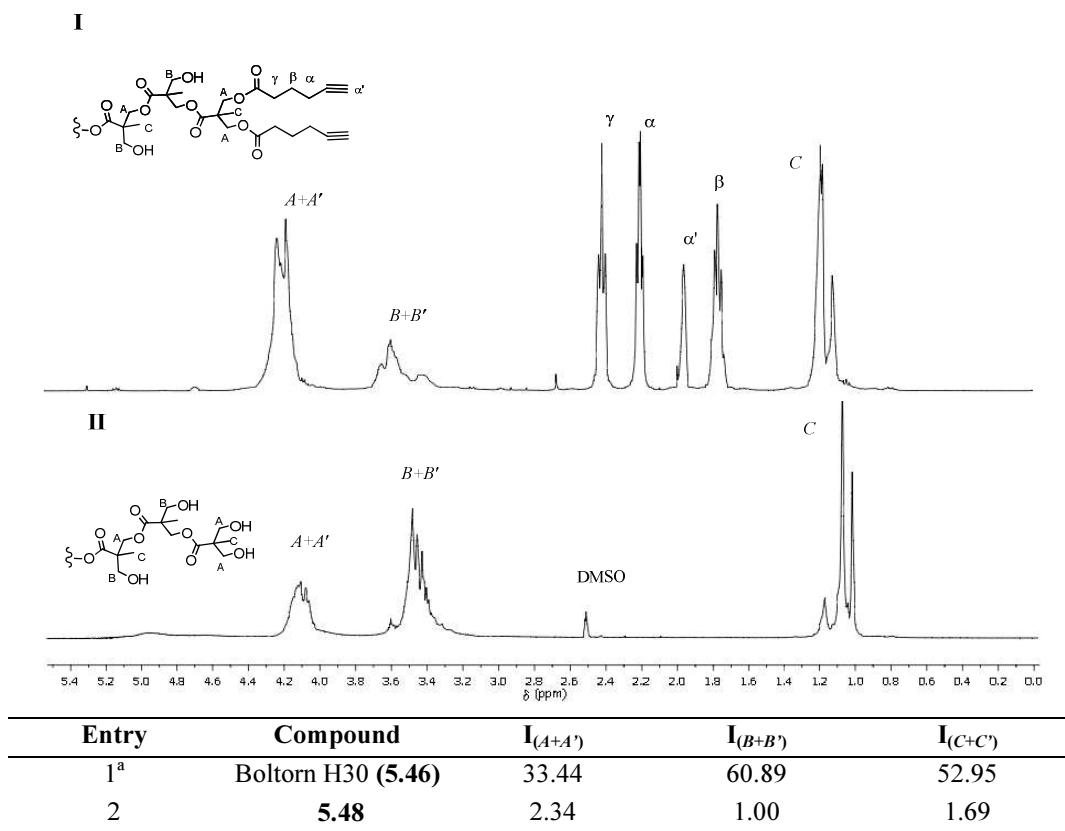
[a] Experimental value extracted from Žagar and co-workers[39]

Figure 5.3. ^{1}H NMR spectra of Boltorn H30 (**5.46**) (II) and the 5-hexynoic modified polymer **5.48** (I) with proton assignments and signal intensities.

Next, the hyperbranched polymer **5.48** was treated with glycoside **5.33** for 72 h in the presence of sodium ascorbate and $CuSO_4 \cdot 5H_2O$ in tBuOH/H_2O (Scheme 5.12.).[19a,35] After dialysis the corresponding glycohyperbranched polymer **5.32**

was obtained. The isolated product had a greenish color instead of being white. In case that **5.32** could be impurified by traces of Cu(II), the mixture was dialysed against in presence of 10 mM EDTA solution to complex any possible trace of free Cu(II). Although color intensity decreased, the color remained. Other methodologies for precipitate Cu(II) salts to CuO such as Benedict reaction[41] were tried but polyester structure of the Boltorn core did not stand the basic conditions of this reaction. Alternatively, metal scavenger QuadraSil MP® was employed to sequestrate Cu(II). This product consist of a mesoporus silica functionalised with a mercaptopropyl group compatible to aqueous and organic solvent and it is able to remove metals such as Cu(II), Cu(I), Pd(II), Pt(0), Rh(II), Ag(I), Ru(I) and Ni(II) in an 99-98% between minutes to hours according to the manufacturer's instructions. In our case, the hyperbranched polymer **5.32** was solved in water and stirred with the insoluble silica until the colour of the solution disappeared. Finally, the solution was filtered and freeze-dryed.
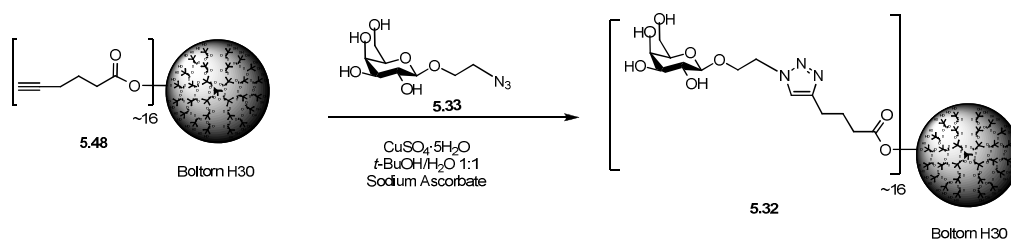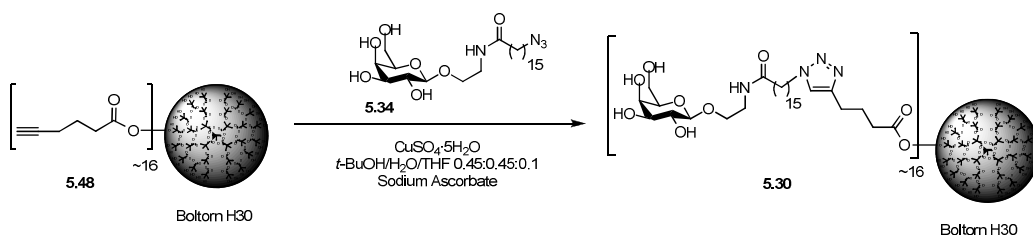

Scheme 5.12. CuAAC reaction between **5.48** and **5.33**

The analysis of the ^{1}H NMR spectra of glycohyperbranched polymer **5.32** revealed the presence of triazole protons at 8.90-8.60 ppm, and complete disappearance of the acetylenic signal at 1.95 ppm. That data demonstrated that the cycloaddition process worked quantitatively on the surface of the polymer. Moreover, ^{1}H NMR spectroscopic data also showed the protons of galactose and Boltorn core as broad and overlapped peaks between 4.57 ppm and 3.25 ppm. In ^{13}C NMR spectra, signals of carbonyl groups can be detected between 175 and 172 ppm, methylene groups between 66 and 63 ppm and quaternary carbons between 49 and 46 ppm. Unfortunately signals of the 1,2,3-triazol moiety did

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[41] Benedict, S. R. *J. Biol. Chem.* **1908,** *5*, 485-487.

not appeared because of relaxation problems, although it is possible to observe their projection on bidimesional experiments (HSQC, HMBC). Indeed, characteristic carbon of both the carbohydrate and the spacer were present.

A similar procedure was carried out to couple glycoside **5.34** to the hyperbranched polymer through CuAAC (Scheme 5.13.). In this case, the complete solubility was key to the success of the coupling. When the reaction was carried out with *t*-BuOH/H$_2$O/DCM (0.45:0.45:0.1) as solvent, it did not work. However, the product **5.30** was recovered satisfactory when the solvent was *t*-BuOH/H$_2$O/THF (0.45:0.45:0.1). Similarly, **5.30** was also dialysed agaisnt 10 mM EDTA and stirred in presence of QuadraSil MP to remove remaining traces of Cu(II).

Scheme 5.13. CuAAC reaction between **5.43** and **5.48**

Similarly, the analysis of the ^{1}H NMR spectra of glycohyperbranched polymer **5.30** revealed the presence of the triazole protons at 7.8 ppm, and complete disappearance of the acetylenic signal at 1.95 ppm. That data demonstrated that the cycloaddition process also worked quantitatively on the surface of this polymer. Moreover, ^{1}H NMR spectroscopic data also showed the protons of galactose and Boltorn core as broad and overlapped peaks between 4.57 ppm and 3.25 ppm and the protons corresponding to the aliphatic chain (1.40 ppm). In the ^{13}C NMR spectra, signals related to carbonyl groups can be observed between 175 and 172 ppm, methylene groups between 66 and 63 ppm and quaternary carbons between 49 and 46 ppm. Fortunately, the signals of the 1,2,3-triazol appeared in this case at 147.7 ppm (C-5) and 122.6 ppm (C-6). Moreover, characteristic carbons of carbohydrate moiety and spacer were also present.

Finally, the CuAAC reaction was carried out again to couple the glycoside **5.35** to hyperbranched polymer **5.31**. In this case, it was necessary to solubilise the system in *t*-BuOH/H$_2$O/THF (1:1:1) to obtain the glycohyperbranched polymer **5.31** satisfactory after a similar purification procedure (Scheme 5.14.).
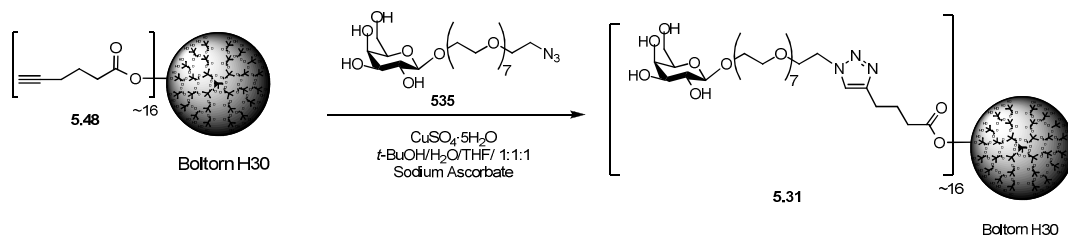
Scheme 5.14. CuAAC reaction between **5.35** and **5.48**

Similarly, the analysis of the ^{1}H NMR spectra of glycohyperbranched polymer **5.31** revealed the presence of the triazole protons at 7.80-7.68 ppm, and the complete disappearance of the acetylenic signal at 1.95 ppm was also observed. These data demonstrated that the cycloaddition process worked quantitatively on the surface of the polymer. Moreover, ^{1}H NMR spectroscopic data also showed the protons of galactose, ethylene glycol and Boltorn core as broad and overlapped peaks between 4.57 ppm and 3.25 ppm. In the ^{13}C NMR spectra signals of carbonyl groups between 175 and 172 ppm, of methylene groups between 66 and 63 ppm and of quaternary carbons between 49 and 46 ppm were also observed. Unfortunately, signals of the 1,2,3-triazol did not appeared again because of relaxation problems, although it is also possible to observe their projection on bidimesional experiments (HSQC, HMBC). Characteristic carbon of carbohydrate moiety and spacer were also present.

In order to determine the molecular mass for hyperbranched polymers, MALDI-TOF spectrometry was employed for the determination of the experimental molecular weigh of the hyperbranched polymer **5.48** and commercial Boltorn H30. Experimental conditions applied on previous works[35] which consist of using IAA as matrix were unsuccessful.

Other matrix conditions were studied to crystallize Boltorn H30 and **5.48.** Boltorn H30 was able to produce a MALDI spectrum when HCCA was employed as a matrix while **5.48** needed dithranol as a matrix and AgTFA as a doping agent to produce the spectra. Unfortunately, the mass registered was always lower than the expected. Although Boltorn H30 has M_{theo} = 3604 g/mol, only a molecular weigh of 1315 g/mol was found (Figure 5.7.). Similarly to the hyperbranched polymer **5.48** which was expected to have a molecular weight of 5110 g/mol, and only a molecular pick around 2250 g/mol was detected (Figure 5.8.).
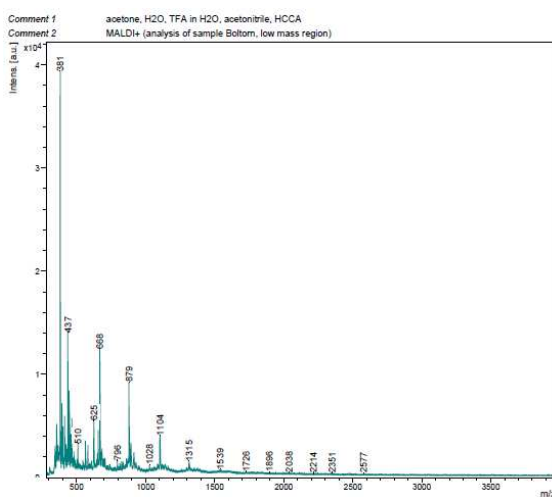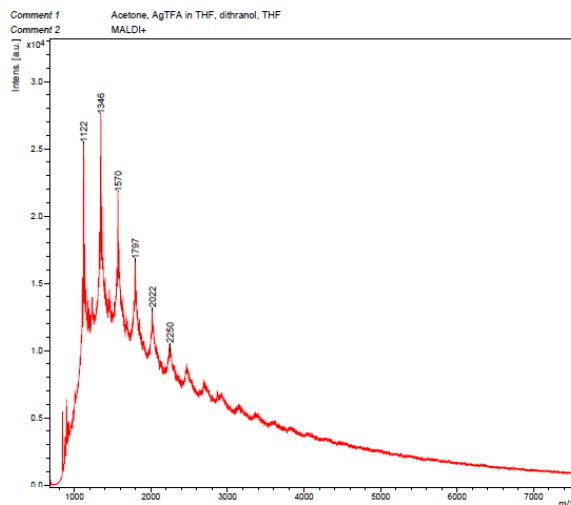

Figure 5.7. MALDI TOF of Boltorn H30

Figure 5.8. MALDI TOF of hyperbranched polymer **5.48**

The explanation for this unsastifactory results in MALDI-TOF spectrometry could be attributed to the inherent polydispersion associated to our hyperbranched polymers. It has been reported[42] that MALDI-TOF is suitable for hyperbranched polymers when molar masses are monodisperse.[42] The limitation is due to the fact that, in broadly distributed samples, the lower molar mass species are preferably activated for desorption and, hence, the higher molar masses are not detectable or their intensity is so low that they are ignored. Therefore information about the complete polydispersity of the hyperbranched sample cannot be achieved.

Other methods to determinate the molecular mass in polymers such as Size Exclusion Chromatography (SEC) were considered. However, this technique is limited because of the densely branched structure of the polymer. Their overall molecular density is increased compared to their linear analogues in a good solvent, and the well-known method for the determination of the full molar mass distribution of polymers, size-exclusion chromatography (SEC) with differential refractive index detection (DRI) or UV-detection and subsequent calibration with a linear polymer standard, can lead to strong deviations from the actual values of molar mass.

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[42] Voit, B. I.; Lederer, A. *Chem. Rev.* **2009**, *109*, 5924-5973.

In spite of this, molecular mass for our hyperbranched polymers **5.30**, **5.31**, **5.32** and **5.48** was measured by GPC/SEC analysis employing 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as a common solvent and poly(methyl methacrylate) PMMA as calibration patrons. These analyses were provided by the group of Prof. Muñoz-Guerra from Universitat Politècnica de Catalunya (UPC) (Table 5.3.). Although the restrictions of this technique for a hyperbranched structures, M_n and M_w obtained for our polymers were reasonable according to the calculated (M_{theo}) degree of functionalization observed by ^{1}H NMR (Table 5.3., Entries 2-5). Differences between M_n and M_w are in agreement with the high polydispersity index (PDI) observed (Table 5.4., Entries 2-5) because of the inherent polydispesity of starting Boltorn H30 (**546**) (Table 5.3., Entry 1). This also confirmed why a proper MALDI-TOF characterization was not obtained.[42]

**Table 5.3.** Comparison of the molecular mass measured by GPC with the calculed by ^{1}H NMR

Entry	Compound	M_n [a,b]	M_w [a,b]	PDI	M_{theo} [a,c]
1[d]	**5.46**	1410	3370	2.38	3604
2	**5.48**	2478	6801	2.78	5110
3	**5.32**	7302	15963	2.19	9097
4	**5.30**	8596	19446	2.26	13152
5	**5.31**	9937	24548	2.47	14031

[a] Molecular weight are expressed in g/mol. [b] Measured through GPC. [c] Calculated through ^{1}H NMR. [d] Data extracted from Žagar's group[39]

Finally, this family of hyperbranched polymers based on glycolipids was studied to determine its binding properties against cholera toxin. The results of its evaluation by ELISA-type assay are presented bellow.

5.2.3. Study of inhibition Cholera Toxin Binding by Hyperbranched Multivalent β-Galceramide Ligands

Enzime linked immunosorbent assay (ELISA) is based on the detection of binding of the antibody to an antigen by using an enzyme label. These antigen-antibody complexes are physically separated from free antigen and antibody using some type of solid phase system. Then, the enzyme acts on the colourless substrate to provide a coloured product which is detected (Scheme 5.15.).[43] ELISA has proven to be one of the most powerful tools available for probing recognition processes involving protein/protein, protein/glycoprotein, protein/glycolipid and glycoprotein/glycolipid interactions.[44]
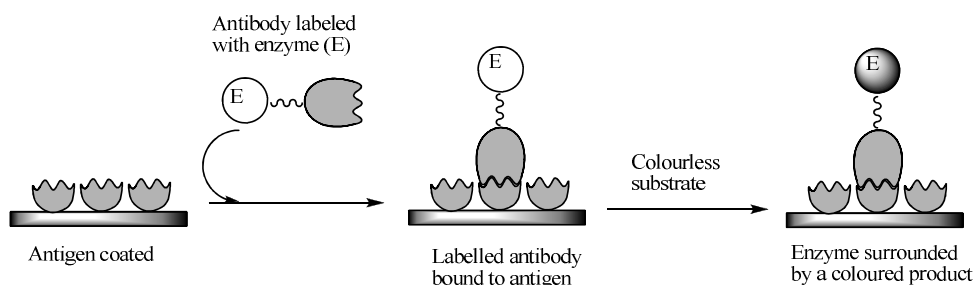
**Scheme 5.15.** Schematic basis of ELISA assay

In particular, studies of inhibition of antigen-antibody complexes by ELISA, the antigen immobilized first to the wells of ELISA plates, and then it is treated with samples containing the inhibitor and the enzyme-labeled antibody. Competition will occur between the immobilized antigen and the inhibitor with the enzyme-labeled antibody. The greater inhibition of the competitive agent, the greater the degree it will bind to the labeled antibody and, therefore, less labeled antibody will be available for binding to the immobilized antigen. After washing the plates only the enzyme-labeled antibody binding to the antigen coated on the wells will be available for detection. Consequently, the signal provided by the experiment can be correlated to the concentration of this competing agent.[43,45] In general, the strength of inhibition trend is expressed in terms of IC_{50} (M) of the

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[43] O'Kenedy, R.; Byrne, M.; O'Fagain, C.; Berns, G. *Biochem. Educ.* **1990**, *18*, 136-140.
[44] Gervay, J.; McReynolds, K. D. *Curr. Med. Chem.* **1999**, *6*, 129-153.
[45] Dawson, R. M. *J. Appl. Toxicol.* **2005**, *25*, 30-38.

competitive agent. Lower concentrations of the competing agent indicate greater inhibitor strength.
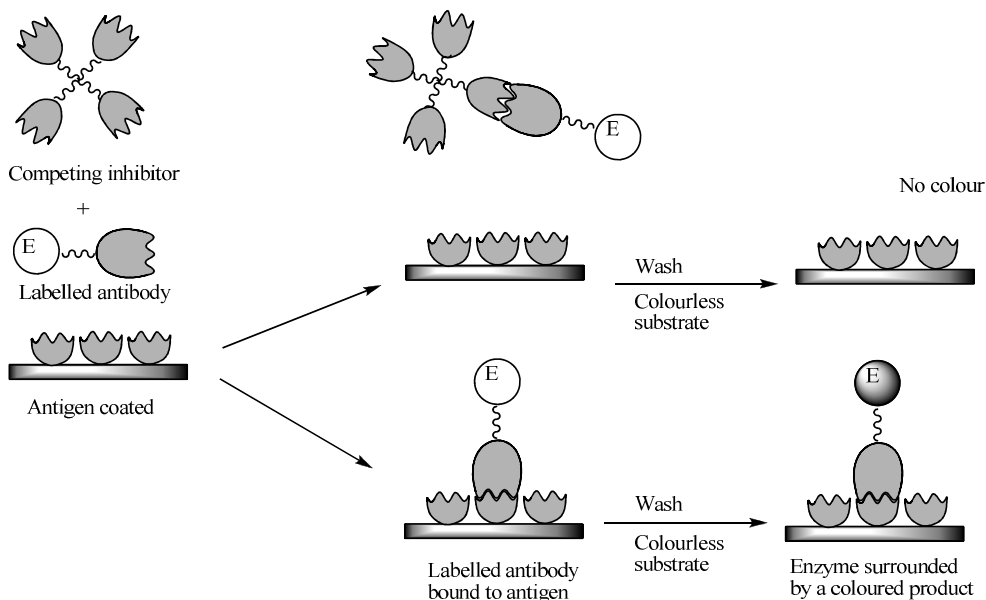
Scheme 5.16. Illustration of the competition between an immobilized antigen and a free multivalent antigen with an enzim labelled-antibody

Herein, it is shown the studies of Cholera toxin inhibition by hyperbranched multivalent β-Galceramide Ligands that was carried out in collaboration with Dr. Morales-Serna, who was responsible of the biological assay. In this assay, a GM1 ganglioside-coated 96 well plate was incubated with horseradish peroxidase labeled CTB_5 (CTB_5-HRP) and binding was inhibited with a range of different concentrations of hyperbranched polymers (Table 5.4.). In order to test if the hydroxylic surface of the Boltorn H30 platform affect to the inhibition, commercial Boltorn H30 (**5.46**) and alkyne functionalized Boltorn (**5.48**) were included in the assay but no significative inhibition was detected (Table 5.4., Entries 3 and 4). The monovalent β-Galcer analogues (Table 5.4., Entries 5, 6, 7, 8 and 9) exhibited a weak inhibitory potency with IC_{50} values between 20 and 95 mM. This slight decrease when compared with simple galactose (entry 1, IC_{50} = 240 mM) might be attributed to the oligovalency of these ligands, due to the formation of micellar or submicellar structures that increase the binding

affinities; a phenomenon previously described for GM1.[46] Next, hyperbranched structures with higher valency (16 epitopes: **5.33**, **5.34** and **5.35**) (20 epitopes: **5.28** and **5.29**), exhibited IC_{50} values (ca. 4.4 µM) in the same range as the GM1os derivative (Table 5.5., Entries 10, 11, 12, 13 and 15). The multivalency effect, as expressed by the relative potency per sugar, still increased in approximately 10^3 magnitud order when each hyperbranched polymer is compared with their respective monovalent ligand.

It is reasonable to assume that the strong binding was attributed to the combined use of β-Galcer ligands and their multivalent presentation on an hyperbranched polymer with long spacer arms. On the one hand, the more similar size of spacer arm to the natural GM1-Os, the better relative potencies were obtained (compare entries 10-12). This may be explained due to a better internalization of multivalent epitopes into the binding pocket of CTB_5-HRP. It is remarkable that hyperbranched polymer **5.31**, which contains an hydrophilic of the PEG spacer, gave the best result for one-chain based β-GalCer derivates. Possibly better solubility of PEG chain in polar solvents may facilitate a better accommodation of the epitopes on the solvent environment that make them more accessible to the CTB_5-HRP.

To explain the highest inhibition results obtained for the two-chain based β-GalCer hyperbranched polymers (**5.28** and **5.29**), it is not discarded the existence of an extra positive hydrophobic interaction between the known lipophilic patch[47] in the CTB binding region and the lipid moiety of β-GalCer residue on the polymers due to the relative proximity of the carbohydrate binding epitope and the lipid free moiety of these structures when compared to GM1.[48] Therefore, the complete β-GalCer architecture or its minimalist analogue on the Boltorn H30 is important to obtain the most competitive inhibiton results against cholera toxin.

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[46] Schwarzmann, G.; Mraz, W.; Sattler, J.; Schindler, R.; Wiegandt, H. *Hoppe Seylers Z Physiol. Chem.* **1978**, *359*, 1277-1286.

[47] (a) Bernardi, A.; Arosio, D.; Potenza, D.; Sánchez-Medina, I.; Mari, S.; Cañada, F. J.; Jiménez-Barbero, J. *Chem. Eur. J.* **2004**, *10*, 4395-4406; (b) Arosio, D.; Baretti, S.; Cattaldo, S.; Potenza, D.; Bernardi, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3831-3834.

[48] Hydrophobic spacers and/or thiourea linkers in lactose dendrimers contributed to the CTB-ligand binding affinity, see: ref. 11l.

Table 5.4. Inhibition studies using CTB_5 and multivalent ligands[a]

Entry	Compound	Valency	IC_{50} (M)	Rel. Potency (per sugar)
1[b]	Galactose	1	2.4 (±0.5) x 10^{-1}	1 (1)
2[b]	GM1os-$C_{11}H_{21}$	1	1.9 (±0.6) x 10^{-5}	12632 (12632)
3	Boltorn H30 **5.46**	-	No inhibition	-
4	Boltorn-alkyne **5.48**	-	No inhibition	-
5	β-Galcer **5.33**	1	2.0 (±0.5) x 10^{-1}	1.2 (1.2)
6	β-Galcer **5.34**	1	9.5 (±0.5) x 10^{-2}	2.5 (2.5)
7	β-Galcer **5.35**	1	9.0 (±0.5) x 10^{-2}	2.6 (2.6)
8	β-Galcer **5.28'**	1	9.0 (±0.4) x 10^{-2}	2.6 (2.6)
9	β-Galcer **2.43**	1	8.5 (±0.5) x 10^{-2}	2.8 (2.8)
10	Hyperbranched **5.32**	16	1.0 (±0.2) x 10^{-5}	24000 (1500)
11	Hyperbranched **5.30**	16	8.0 (±0.2) x 10^{-6}	30000 (1875)
12	Hyperbranched **5.31**	16	6.5 (±0.2) x 10^{-6}	36923 (2308)
13	Hyperbranched **5.28**	20	4.4 (±0.3) x 10^{-6}	54545 (2727)
14	Hyperbranched **5.29**	20	4.5 (±0.2) x 10^{-6}	53333 (2666)

[a] Determined in an ELISA experiment with 0.43 nM CTB_5-HRP and wells coated with 0.2 μg GM1. [b] See reference.[49]

[49] Pickens, J. C.; Mitchell, D. D.; Liu, J.; Tan, X.; Zhang, Z.; Verlinde, C. L. M. J.; Hol, W. G. J.; Fan, E. *Chem. Biol.* **2004**, *11*, 1205-1215.

In summary, data from this study indicate that hyperbranched polymers can be efficiently used as multivalent glyco(lipid) systems able to block toxin binding with potential applications to other surface receptor-mediated diseases. The best multivalent compounds here studied (**5.28** and **5.29**) exhibited >50,000-fold greater potency than monovalent galactose, providing a platform that mimic the natural GM1-enriched lipid domains. Therefore, advantages of the present method include:

- Operationally simple and robust method for accessing gram quantities of complex hyperbranched multivalent glycolipid ligands;
- The concentration-dependent micelle formation of simple glycolipid inhibitors is suppressed; hence avoiding different binding affinities due to different micelle sizes;
- And importantly, this method allows the use of whole glycolipids that normally are not employed as inhibitors since it has been shown that exogenous glycosphingolipids are integrated in cell membranes, therefore making nonsusceptible cells in susceptible to the toxic agent.[50]

5.3. Experimental Section

General Remarks: All reactions were conducted under a dried argon stream. Solvents (CH_2Cl_2 99.9%, toluene 99.9%, acetonitrile 99.9 %) were purchased in capped Pure Solv System-4® bottles and used without further purification and stored under argon. Yields refer to chromatographically and spectroscopically (^1H and ^{13}C) homogeneous materials, unless otherwise stated. All other solvents and reagents were used without further purification and purchased from Sigma-Aldrich. Boltorn H30 was supplied by Perstorp Company. All glassware utilized was flamedried before use. Reactions were monitored by TLC carried out on 0.25 mm E. Merck silica gel plates. Developed TLC plates were visualized under a short-wave UV lamp and by heating plates that were dipped in ethanol/H_2SO_4 (15:1). Flash column chromatography (FCC) was performed using flash silica gel or C-18 silica gel (32–63 μm) and employed a solvent

[50] Moss, J.; Fishman, P. H.; Manganiello, V.C.; Vaughan, M.; Brady, R. O. *Proc. Natl. Acad. Sci. U. S. A.* **1976**, *73*, 1034-1037.

polarity correlated with TLC mobility. Melting points, determined with Reichert apparatus, are uncorrected. Optical rotations were measured at 598 nm on a Jasco DIP-370 digital polarimeter using a 100 mm cell. FT IR spectroscopy was performed on Jasco FT/IR-600 Plus ATR Specac Golden Gate.

NMR experiments were conducted on a Varian 400 MHz instrument using CDCl$_3$ and D$_2$O (99.9% D) as the solvents. Chemical shifts are in ppm with respect to TMS (Tetramethylsilane).

Exact Mass was measured on Agilent G3250AA LC-MSTOF. Mass Spectrometry MALDI–TOF MS was performed on a Perseptive Biosystems Voyager DE-PRO spectrometer. Gel permeation chromatography (GPC) for determination of polymer molecular weights and their distributions was performed in a Waters equipment provided with RI and UV detectors using 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, 99% Apollo Scientific Lim.) containing sodium trifluoroacetate (98%, Sigma-Aldrich Co.; 6.8 g/L) as mobile phase. A total of 10 μL of 0.1% (w/v) sample solution was injected and chromatographed with a flow of 0.5 mL/min. HR5E Waters linear Styragel columns (7.8 × 300 mm, pore size 103-104 Å) packed with cross-linked polystyrene and protected with a precolumn (VanGuard, 1.8 μm, 2.1 × 5 mm) were used. Molecular weight averages and distributions were evaluated against poly(methyl methacrylate) standards.

**General procedure for CTB$_5$ inhibition assay (ELISA)**

A 96-well plate was coated with a solution of GM1 (100 μL, 2 μg mL^{-1}) in phosphate buffered saline (PBS buffer). Unattached ganglioside was removed by washing with PBS and the remaining binding sites of the surface were blocked with Bovine Serum Albumin (BSA) (1%); this was followed by washing with PBS. Samples of toxin–peroxidase conjugate (CTB–HRP; Sigma) and inhibitor in PBS with BSA (0.1%) and Tween-20 (0.05%) were incubated at room temperature for 2 h and were then transferred to the GM1-coated plate. After 30 min of incubation the solution was removed and the wells were washed with BSA (0.1 %)/Tween-20 (0.05%) in PBS. To identify toxin binding to surface-bound GM1 the wells were treated with a freshly prepared solution of *o*-

phenylenediamine/H_2O_2 in citrate buffer (100 μL) for 15 min. After being quenched with H_2SO_4 absorbance in each well was measured at 490 nm.

Benzylpropargylether (5.20)
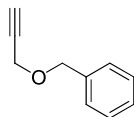
Propargylic alcohol (**5.18**) (3.4 mL, 58.5 mmol) was treated with NaH (2.34 g, 58.5 mmol) under argon atmosphere for 10 min to form the corresponding alcoholate. After that, benzyl bromide (**5.19**) (1.4 mL, 11.7 mmol) and TBAI (864 mg, 2.34 mmol) were added to the alcoholate suspension. Then, the mixture was stirred at 70 ºC for 16 h. The residue was purified by column chromatography (silica gel, ethyl acetate/ hexane 5: 95) to give **5.20** as syrup (1650 mg, 97%): Rf (EtOAc/Hexane 5:95): 0.29; ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 7.37 (m, 5H, H-Ar), 4.63 (s, 2H, H-1), 4.19 (s, 2H, H-1'), 2.49 (t, $J_{3'-1'}$ = 2.4 Hz, 1H, H-3'); ^{13}C NMR (CDCl$_3$, 100.3 MHz) δ in ppm: 137.4 (CAr-1), 128.8, 128.4, 128.2 (C-Ar), 79.6 (C-2'), 74.8 (C-3'), 71.8 (C-1), 57.3 (C-1'); FT-IR (ATR) ν in cm^{-1}: 2854, 1494, 1449, 1350, 1260, 1202, 1085, 1022, 937, 740; HRMS (TOF ES+) for (M+Na) $C_{10}H_{10}ONa$ (m/z): calc. 169.0624. Found: 169.0689.
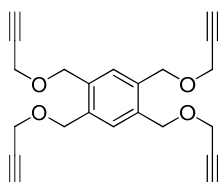
1,3,5-trimethyl-2,4,6-tris((prop-2-ynyloxy)methyl)benzene (5.22)
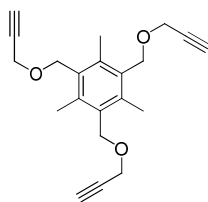
Propargylic alcohol (**5.18**) (4.9 mL, 84.0 mmol) was treated with NaH (3.36 g, 84.0 mmol) under argon atmosphere for 10 min to form the corresponding alcoholate. After that, commercial 1,3,5-tris (bromomethyl)-2,4,6-trimethylbenzene (**5.21**) (2000 mg, 5.60 mmol) and TBAI (414 mg, 1.12 mmol) were added to the alcoholate suspension. Then, the mixture was stirred at 70 ºC

for 24 h. The residue was purified by column chromatography (silica gel, ethyl acetate/ hexane 1:9) to give **5.22** as a white solid (1678 mg, 92%): Rf (EtOAc/Hexane 1:9): 0.28; mp: 107-108 ºC; ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 4.63 (s, 6H, H-1), 4.20 (d, $J_{1'-3'}$ = 2.4 Hz, 6H, H-1'), 2.49 (t, $J_{3'-1'}$ = 2.4 Hz, 3H, H-3'), 2.46 (s, 9H, H-Me); ^{13}C NMR (CDCl$_3$, 100.3 MHz) δ in ppm: 132.4 (CAr-1), 126.4 (C-Ar2), 78.6 (C-2'), 74.7 (C-3'), 66.7 (C-1), 57.3 (C-1'), 15.9 (C-Me); FT-IR (ATR) ν in cm^{-1}: 3287, 3249, 2927, 2898, 2850, 2107, 1575, 1496, 1458, 1433, 1345, 1264, 1240, 1089, 1050, 984, 925, 902, 827, 659; HRMS (TOF ES+) for (M+Na) C$_{21}$H$_{24}$NaO$_3$ (m/z): calc. 347.1618. Found: 347.1631.

1,2,4,5-tetrakis((prop-2-ynyloxy)methyl)benzene (5.24)

Propargylic alcohol (**5.18**) (5.2 mL, 88.9 mmol) was treated with NaH (3.56 g, 88.9 mmol) under argon atmosphere for 10 min to form the corresponding alcoholate. After that, 1,2,4,5-tetrakis((prop-2-ynyloxy)methyl)benzene (**5.23**) (2000 mg, 4.40 mmol) and TBAI (1310 mg, 3.55 mmol) were added to the alcoholate suspension. Then, the mixture was stirred at 70 ºC for 22 h. The residue was purified by column chromatography (silica gel, ethyl acetate/ hexane 15:85) to give **5.24** as a yellowish solid (1136 mg, 73%): Rf (EtOAc/Hexane 15:85): 0.32; mp: 53-55 ºC; ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 7.47 (s, 2H, H-Ar), 4.69 (s, 6H, H-1), 4.20 (d, $J_{1'-3'}$ = 2.4 Hz, 6H, H-1'), 2.53 (t, $J_{3'-1'}$ = 2.4 Hz, 3H, H-3'); ^{13}C NMR (CDCl$_3$, 100.3 MHz) δ in ppm: 135.7 (CAr-1), 130.3 (C-Ar2), 79.7 (C-2'), 75.0 (C-3'), 68.7 (C-1), 57.3 (C-1'); FT-IR (ATR) ν in cm^{-1}: 3272, 2916, 2876, 2855, 2792, 2124, 1720, 1457, 1390, 1340, 1310, 1258, 1242, 1193, 1075, 1011, 994, 894, 696, 652; HRMS (TOF ES+) for (M+Na) C$_{22}$H$_{22}$NaO$_4$ (m/z): calc. 347.1410. Found: 373.1430.

Cluster 5.25
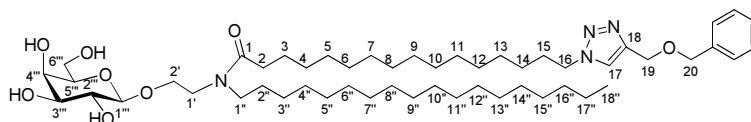
To a solution of propargilic core **5.20** (13 mg, 0.088 mmol) and glicolipid **2.43** (74 mg, 0.080 mmol) in a mixture of *t*-BuOH/H$_2$O (4 mL) was added TBTA (0.5 mg, 0.88 μmol) and [Cu(CH$_3$CN)$_4$]OTf (0.3 mg, 0.88 μmol). The reaction mixture was stirred at room temperature for 24 h. After evaporation, the crude product was azeotroped with toluene (3 x 5 mL). This residue was purified by silica gel chromatography (ethyl acetate/MeOH 9:1) to afford **5.25** as waxy solid (74 mg, 93%): Rf (EtOAc/Hexane 1:9): 0.28; [α]$_D^{25}$ = -6.1º, (*c* = 0.70, CHCl$_3$/MeOH 1:1); ^{1}H NMR (CDCl$_3$/MeOD 1:2, 400 MHz) δ in ppm: 7.97 (s, 1H, H-17), 7.34-7.29 (m, 5H, HAr), 4.63 (s, 2H, H-19), 4.57 (s, 2H, H-20), 4.38 (t, 2H, $J_{16\text{-}15}$ = 7.20 Hz, H-16), 4.22 (at, 1H, $J_{1'''\text{-}2'''}$ = 7.6 Hz, H-1'''), 3.97 (m, 1H, H-2a'), 3.83 (t, 1H, $J_{4'''\text{-}3'''} = J_{4'''\text{-}5'''}$ = 2.0 Hz, H-4'''), 3.78-3.61 (m, 4H, H-6a''', H-6b''', H-2b', H-1a'), 3.56 (m, 1H, H-2'''), 3.52-3.33 (m, 5H, H-5''', H-3''', H-1b', H-1''), 2.44 (t, 2H, $J_{2\text{-}3}$ = 7.2 Hz, H-2), 2.35 (t, 2H, $J_{2\text{-}3}$ = 7.2 Hz, H-2),[51] 1.89 (tt, 2H, J = 7.2 Hz, J = 6.8 Hz, H-15), 1.59-1.57 (m, 4H, H-2'', H-3), 1.39-1.20 (m, 52H, H3''-H17'', H-4-H-14), 0.89 (t, 3H, $J_{18''\text{-}17''}$ = 6.4 Hz, H-18''); ^{13}C NMR (CDCl$_3$/MeOD 1:2, 100.3 MHz) δ in ppm: 175.3 (CO), 138.0 (C-18) 137.6, 129.4, 129.0, 128.8, 125.0 (C-Ar), 105.2 (C-1'''), 76.7 (C-2'''), 74.4 (C-4'''), 72.4 (C-5'''), 70.3 (C-3'''), 68.5 (C-2'), 67.4 (C-20), 64.0 (C-6'''), 62.0 (C-19), 51.3 (C-1''), 49.6 (C-1'), 34.0 (C-2''), 33.9 (C-2), 33.1, 31.3, 30.8, 30.7, 30.6, 30.5, 30.1, 28.0, 27.8, 27.45, 26.8, 26.6, 23.8 (CH$_2$), 14.5 (C-18''); FT-IR (ATR) ν in cm^{-1}: 3364, 2921, 2852, 1730, 1625, 1462, 1368, 1091, 765; 630; MALDI-TOF for [M + Na]$^+$ C$_{52}$H$_{92}$ N$_4$NaO$_8$ (m/z) calc. 923.7; found 923.2.

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[51] Signal associated to H-2 is doubled at room temperature due to the tertiary amide-imino alcoholate equilibrium. The signal coalesces once temperature is increased over 50 ºC.

Cluster 5.26
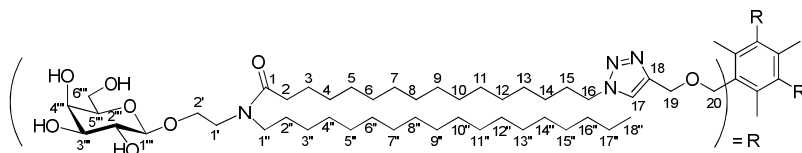
To a solution of propargilic core **5.22** (5 mg, 0.016 mmol) and glicolipid **2.43** (40 mg, 0.053 mmol) in a mixture of *t*-BuOH/H_2O (4 mL) was added TBTA (0.3 mg, 0.53 μmol) and $[Cu(CH_3CN)_4]OTf$ (0.2 mg, 0.53 μmol). The reaction mixture was stirred at room temperature for 2 days. After evaporation, the crude product azeotroped with toluene (3 x 5 mL). This residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 3:1) to afford **5.26** as waxy solid (28 mg, 70%): Rf (NH_4OH/MeOH/DCM 2:13:85) = 0.18; $[\alpha]_D^{25}$ = +0.35º (*c* = 2.0, $CHCl_3$/MeOH 1:1); ^1H NMR ($CDCl_3$-MeOD (1:2), 400 MHz): δ in ppm: 7.83 (s, 3H, H-17), 4.66 (s, 6H, H-19), 4.60 (s, 6H, H-20), 4.36 (t, 6H, J_{16-15} = 6.4 Hz, H-16), 4.21 (at, 3H, $J_{1'''-2'''}$ = 7.2 Hz, H-1'''), 3.96 (m, 3H, H-2a'), 3.84 (t, 3H, $J_{4'''-3'''} = J_{4'''-5'''}$ = 2.0 Hz, H-4'''), 3.78-3.61 (m, 12H, H-6a''', H-6b''', H-2b', H-1a'), 3.56 (m, 3H, H-2'''), 3.52-3.33 (m, 15H, H-5''', H-3''', H-1b', H-1''), 2.42 (t, 6H, J_{2-3} = 8.0 Hz, H-2), 2.35 (t, 6H, J_{2-3} = 8.0 Hz, H-2),[51] 2.29 (s, 9H, CH_3), 1.89 (tt, 6H, *J* = 7.2 Hz, *J* = 6.8 Hz, H-15), 1.59-1.57 (m, 12H, H-2'', H-3), 1.39-1.20 (m, 156H, H3''-H17'', H-4-H-14), 0.86 (t, 9H, $J_{18''-17''}$ = 6.4 Hz, H-18''); ^{13}C NMR ($CDCl_3$/MeOD 1:2, 100.3 MHz) δ in ppm: 175.7 (CO), 138.0 (C-18) 132.4 (CAr-1), 131.7 (C-17) 126.4 (C-Ar2), 103.3 (C-1'''), 76.7 (C-2'''), 75.0 (C-4'''), 72,4 (C-5'''), 70.2 (C-3'''), 68.2 (C-2'), 66.5 (C-20), 62.5 (C-6'''), 60.3 (C-19), 49.8 (C-1''), 45.2 (C-1'), 34.2 (C-2''), 32.6 (C-2), 32.4, 31.1-29.1, 28.9, 28.8, 28.4, 28.2, 26.8, 26.3, 26.1, 25.8, 24.8, 22.6 (CH_2), 14.6 (Ar-CH_3), 13.1 (C-18''); FT-IR (ATR) ν in cm^{-1}: 3370, 2922, 2853, 1737, 1623, 1459, 1375, 1284, 1092, 785, 628; MALDI-TOF for $[M + Na]^+$ $C_{147}H_{270}N_{12}NaO_{24}$ (m/z) calc. 2611.0; found 2613.2.

Cluster 5.27
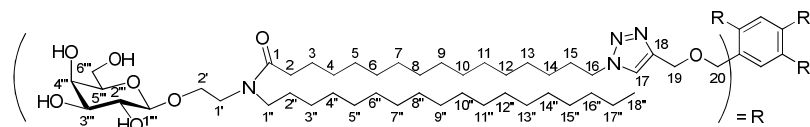
To a solution of propargilic core **5.24** (4 mg, 0.012 mmol) and glicolipid **2.43** (41 mg, 0.054 mmol) in a mixture of t-BuOH/H$_2$O (2 mL) was added TBTA (0.3 mg, 0.53 μmol) and [Cu(CH$_3$CN)$_4$]OTf (0.2 mg, 0.53 μmol). The reaction mixture was stirred at room temperature for 72 h. After evaporation, the crude product azeotroped with toluene (3 x 5 mL). This residue was purified by silica gel chromatography (from CH$_2$Cl$_2$/MeOH 3:1 to CH$_2$Cl$_2$/MeOH/H$_2$O 1:1:0.1) to afford **5.27** as waxy solid (10 mg, 25 %): Rf (NH$_4$OH/MeOH/DCM 2:23:75) = 0.15; $[\alpha]_D^{25}$ = +0.41° (c = 0.97, CHCl$_3$/MeOH 1:1); ^{1}H NMR (CDCl$_3$-MeOD (2:1), 400 MHz): δ, 7.72 (s, 4H, H-17), 7.47 (s, 2H, H-Ar), 4.65 (s, 8H, H-19), 4.64 (s, 8H, H-20), 4.36 (t, 8H, J_{16-15} = 7.2 Hz, H-16), 4.22 (at, 4H, $J_{1'''-2'''}$ = 7.6 Hz, H1'''), 3.99 (m, 4H, H2a'), 3.87 (t, 4H, $J_{4'''-3'''}$ = $J_{4'''-5'''}$ = 2.0 Hz, H4'''), 3.78-3.61 (m, 16H, H6a''', H6b''', H2b', H1a'), 3.56 (m, 4H, H2'''), 3.52-3.33 (m, 20H, H5''', H3''', H1b', H-1''), 2.39 (t, 8H, J_{2-3} = 8.0 Hz, H-2), 2.35 (t, 8H, J_{2-3} = 8.0 Hz, H-2),[51] 1.90 (bs, 8H, H-15), 1.59-1.57 (m, 16H, H-2'', H-3), 1.39-1.20 (m, 208H, H3''-H17'', H4-H14), 0.86 (bs, 12H, H-18''); ^{13}C NMR (CDCl$_3$:MeOD, 1:2, 100.3 MHz) δ in ppm: 175.7 (CO), 138.0 (C-18) 135.6 (CAr-1), 131.7 (C-17) 130.4 (C-Ar2), 104.6 (C-1'''), 75.9 (C-2'''), 74.4 (C-4'''), 71,9 (C-5'''), 70.3 (C-3'''), 69.5 (C-2'), 67.9 (C-20), 64.0 (C-6'''), 62.0 (C-19), 51.1 (C-1''), 46.6 (C-1'), 34.0 (C-2''), 33.8 (C-2), 32.6, 30.7-30.0, 29.9, 29.5, 29.5, 27.6, 27.3, 26.1, 25.8, 24.8, 22.6 (CH$_2$), 13.2 (C-18'') ppm; FT-IR (ATR) ν in cm^{-1}: 3365, 2924, 2853, 1737, 1623, 1461, 1282, 780, 630; MALDI-TOF for [M + Na]+ calc for C$_{190}$H$_{350}$N$_{16}$NaO$_{32}$ 3391.6; found 3394.3.

### Glycohyperbranched polymer (5.30)

Boltorn H30
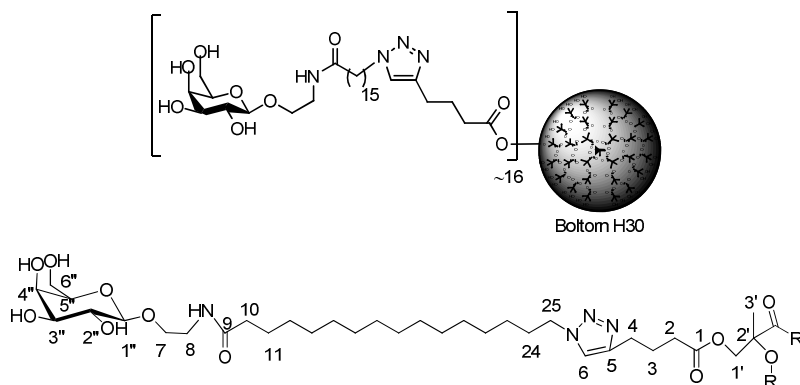
To a solution of dendritic polymer **5.48** (21 mg, 0.004 mmol) in THF (0.2 mL) was added a solution of glycoside **5.34** (100 mg, 0.200 mmol) in *t*-BuOH/H$_2$O 1:1 (2 mL) and a freshly prepared solution of CuSO$_4$·5H$_2$O (6 mg , 0.024 mmol) and sodium ascorbate (5 mg, 0.025 mmol) in *t*-BuOH/H$_2$O 1:1 (0.2 mL). The reaction mixture was stirred at room temperature for 72 h and then purified by dialysis (benzoylated tube flat width 32 mm (1.27''), from *t*-BuOH/H$_2$O/THF 0.45:0.45:0.1 to aquose EDTA [10 mM] to H$_2$O) for 3 days changing the solvent each 8h. After that, the sample was gently agitated with QuadraSil MP (150 mg) for 1 h. Then, the mixture was filtered and the solution was freeze dried to obtain **5.30** as lyophilised (50 mg, 96 %): ^{1}H NMR (CD$_2$Cl-MeOD (1:1), 400 MHz) δ in ppm: 7.80 (s, 16H, H-6), 4.57-4.37 (m, 126H, H-25, H-1', H-1''), 4.21-4.13 (m, 48H, H-7a, CH$_2$OH), 4.07 (bs, 16H, H-4''), 4.01-3.97 (bs, 32H, H-6''a, H-6''b), 3.86 (bs, 16H, H-7b), 3.77-3.53 (m, 80H, H-8, H-3'', H-2'', H-5''), 2.95 (bs, 32H, H-4), 2.63 (bs, 32H, H-2) 2.43 (bs, 32H, H-10), 2.18 (bs, 32H, H-3), 2.11 (bs, 32H, H-24), 1.83 (bs, 32H, H-11), 1.50 (bs, 55H, H-3') 1.40 (s, 352H, H-aliphatic);^{13}C NMR (D$_2$O, 100.6 MHz) δ in ppm: 175.9 (N-CO), 173.7-173.1 (CO, C-1), 147.7 (C-5), 122.6 (C-6), 104.4 (C-1''), 75.9 (C-2''), 74.3, 72.2 (C-3'' o C-5''), 69.7 (C-4''), 69.5 (C-7), 67.5-66.2 (C-1', CH$_2$OH), 62.1 (C-6''), 51.1 (C-25), 48.5-48.3 (C-2'), 40.2 (C-8), 37.0 (C-10), 33.9 (C-2), 31.0 (C-24), 30.4-30.1, 29.8 (CH$_2$), 26.7 (C-11), 25.4-25.3 (C-3, C-4), 18.2 (C-3'); FT-IR (ATR) ν in cm^{-1}: 3320, 2919, 2850, 1736, 1646, 1555, 1465, 1131; GPC MS: Mn = 8596 g/mol, Mw = 19446 g/mol, PDI = 2.26.

Glycohyperbranched polymer (5.31)

Boltorn H30
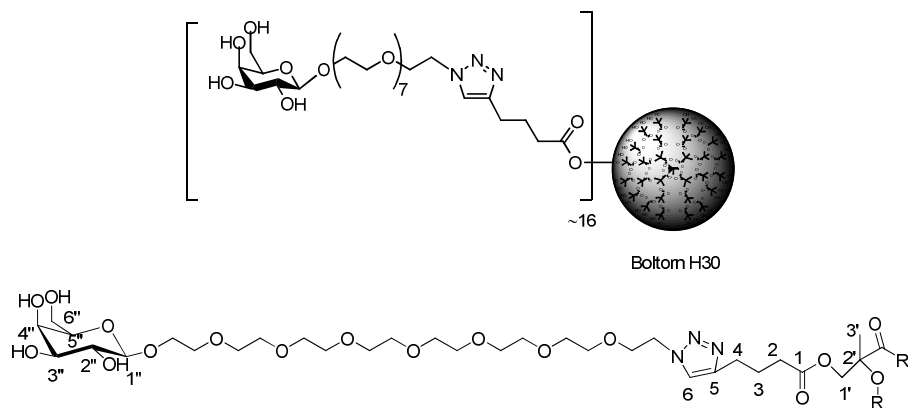
To a solution of dendritic polymer **5.48** (26 mg, 0.004mmol) in *t*-BuOH/H$_2$O/THF 1:1:1 (2 mL) was added a solution of glycoside **5.35** (111.4 mg, 0.200 mmol) in *t*-BuOH/H$_2$O/THF 1:1:1 (3 mL) and a freshly prepared solution of CuSO$_4$·5H$_2$O (6 mg , 0.024 mmol) and sodium ascorbate (5 mg, 0.025 mmol) in *t*-BuOH/H$_2$O 1:1 (0.2 mL). The reaction mixture was stirred at room temperature for 72 h and then purified by dialysis (benzoylated tube flat width 32 mm (1.27''), from *tert*-BuOH/H$_2$O/THF 1:1:1 to aquose EDTA [10 mM] to H$_2$O) for 3 days changing the solvent each 8h. After that, the sample was gently agitated with QuadraSil MP (150 mg) for 1 h. Then, the mixture was filtered and the solution was freeze dried to obtain **5.31** as lyophilised (52 mg, 93 %): ^{1}H NMR (D$_2$O, 400 MHz) δ in ppm: 7.80-7.68 (bs, 16H, H-6), 4.45-4.38 (bs, 75H, H-1'), 4.26 (d, J$_{1''-2''}$ = 8.0 Hz, 16H, H-1''), 4.19-4.00 (m, 54H, CH$_2$O), 3.92 (m, 16H, H-4''), 3.76 (bs, 72H, CH$_2$O), 3.68-3.36 (m, 466H, H-6''a, H-6''b, H-5'', CH$_2$O, H-3'', H-2''), 2.53 (bs, 32H, H-4), 2.08 (bs, 32H, H-2), 1.75 (bs, 32H, H-3), 1.08-1.02 (m, 55H, H-3'); ^{13}C NMR (D$_2$O, 100.6 MHz) δ in ppm: 174.6-170.2 (C(O)-O, C-1), 140.0 (C-5), 102.8 (C-1''), 75.1 (C-5''), 72.6 (C-3''), 70.7 (C-2''), 69.6-69.5 (CH$_2$O), 68.7-68.6 (CH$_2$OH), 68.5 (C-4'', C-1', CH$_2$O), 60.9 (C-6''), 57.7, 51.4 (C-25), 46.4 (C-2'), 32.9 (C-2), 24.0 (C-3, C-4), 17.0 (C-3'); FT-IR (ATR) ν in cm^{-1}: 3427, 2880, 1737, 1620, 1397, 1245, 1121, 785; GPC-MS: Mn = 9937 g/mol, Mw = 24548 g/mol, PDI = 2.47.

Glycohyperbranched polymer (5.32)
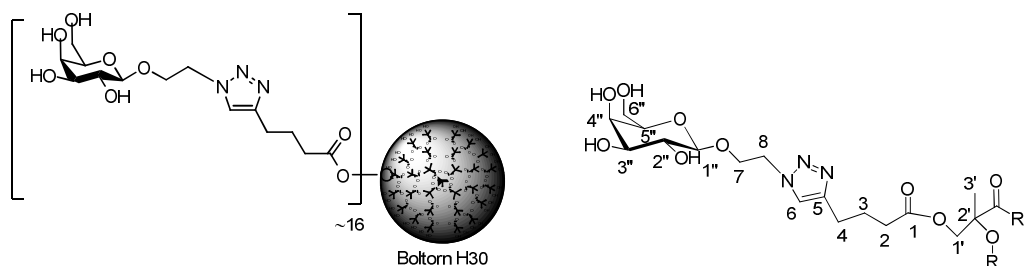
To a solution of dendritic polymer **5.48** (22 mg, 0.004mmol) in *t*-BuOH/H_2O 1:1 (1 mL) was added a solution of glycoside **5.33** (52 mg, 0.104 mmol) in *t*-BuOH/H_2O 1:1 (1 mL) and a freshly prepared solution of $CuSO_4 \cdot 5H_2O$ (7 mg, 0.028 mmol) and sodium ascorbate (6 mg, 0.031 mmol) in *t*-BuOH/H_2O 1:1 (0.1 mL). The reaction mixture was stirred at room temperature for 72 h and then purified by dialysis (benzoylated tube flat width 32 mm (1.27''), from *t*-BuOH/H_2O 1:1 to aquose EDTA [10 mM] to H_2O) for 3 days changing the solvent each 8h. After that, the sample was gently agitated with QuadraSil MP (150 mg) for 1 h. Then, the mixture was filtered and the solution was freeze dried to obtain **5.32** as lyophilised (34 mg, 94 %): ^{1}H NMR (D_2O, 400 MHz) δ in ppm: 8.90-8.60 (bs, 16H, H-6), 4.50 (bs, 32H, H-8), 4.34-4.15 (bs, 16H, H-1''), 4.22-4.05 (bs, 109H, H-1', H-7, CH_2OH), 3.88-3.81 (bs, 16H, H-4''), 3.75 (bs, 16H, H-6''a), 3.65-3.52 (bs, 32H, H-2'', H-6''b), 3.60-3.34 (bs, 32H, H-3'', H-5''), 2.70 (bs, 32H, H-4), 2.35-2.26 (bs, 32H, H-2), 2.05-1.80 (bs, 32H, H-3), 1.44 (bs, 55H, H-3');^{13}C NMR (D_2O, 100.6 MHz) δ in ppm: 174.6-173.2 (CO, C-1), 102.9 (C-1''), 75.0 (C-2''), 72.5, 70.4 (C-5''or C-3''), 68.4 (C-4''), 67.6 (C-7), 65.5-63.9 (C-1', CH_2OH), 60.8 (C-6''), 50.8 (C-8), 48.5-46.4 (C-2'), 32.9 (C-2), 23.4 (C-3, C-4), 17.0 (C-3'); FT-IR (ATR) ν in cm-1: 3385, 2941, 1734, 1608, 1390 1237, 1132, 1058; GPC-MS: M_n = 7302 g/mol, M_w = 15963 g/mol, PDI = 2.19.
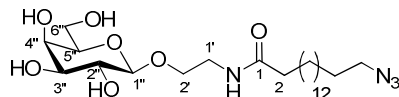
1-*O*-(2-Azidoethyl)-β-D-galactopyranoside (5.33)
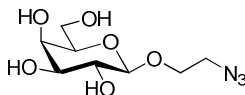
The glycoside **5.38** (306 mg, 0.73 mmol) was dried azeotropically with anhydrous toluene and kept at high vacuum for 2h. After that, it was dissolved in anhydrous MeOH (4 mL) and a freshly prepared solution of MeONa in anhydrous methanol was added (0.5 mL, 1 M). The mixture was stirred under argon pressure at room temperature for 20 min. The reaction was quenched by adding dry ice until pH 7 was reached. After removing the solvent, the crude was purified by column chromatography (silica gel, MeOH/DCM 1:9 v/v) to yield **5.33** as a syrup (173 mg, 95%): Rf (MeOH/DCM 1:9): 0.20; $[\alpha]_D^{20}$: +43.4º (0.38, DCM/MeOH 1:1); ^{1}H NMR (MeOD, 400 MHz) δ in ppm: 4.28 (d, $J_{1'-2'}$ = 7.2 Hz, 1H, H-1'), 4.05-4.00 (m, 2H, H-1a, H-1b), 4.09 (d, $J_{4'-3'}$ = 3.2 Hz, $J_{4'-5'}$ = 0.8 Hz, 1H, H-4'), 3.76-3.71 (m, 2H, H-6a', H-6b'), 3.57-3.47 (m, 5H, H-2', H-3', H-5', H-2a, H-2b); ^{13}C NMR (MeOD, 100.3 MHz) δ in ppm: 104.9 (C-1'), 76.6 (C-2'), 74.8, 72.4 (C-3', C-5'), 70.2 (C-4'), 69.2 (C-1), 62.4 (C-6'), 52.1 (C-2); FT-IR (ATR) ν in cm^{-1}: 3385, 2941, 1731, 1731, 1468, 1378, 1232, 1131, 1064, 998; HRMS (TOF ES+) for (M+Na) $C_8H_{15}N_3NaO_6$ (m/z): calc. 272.0853; found 272.0847.
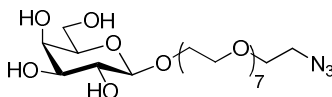
1-*O*-(2-(16-azidohexadecanamido)ethyl)-β-D-galactopyranoside (5.34)

The glycoside **5.43** (302 mg, 0.45 mmol) was dried azeotropically with anhydrous toluene and kept at high vacuum for 2h. After that, it was dissolved in anhydrous MeOH (4 mL) and a freshly prepared solution of MeONa in anhydrous methanol was added (0.2 mL, 1 M). The mixture was stirred under argon pressure at room temperature for 20 min. The reaction was quenched by adding dry ice until pH 7 was reached. After removing the solvent, the crude

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was purified by short column chromatography (silica gel, MeOH/DCM 1:9 v/v) to yield **5.34** as syrup (214 mg, 95%): Rf (MeOH/DCM 1:9): 0.17; $[\alpha]_D^{20}$: +43.4º (0.38, DCM/MeOH 1:1); ^{1}H NMR (CDCl$_3$/MeOD 3:1, 400 MHz) δ in ppm: 4.05 (d, $J_{1''-2''}$ = 7.2 Hz, 1H, H-1''), 3.78-3.74 (m, 1H, H-2a'), 3.72 (d, $J_{4''-3''}$ = 2.4 Hz, 1H, H-4''), 3.66-3.56 (m, 2H, H-6a'', H-6b''), 3.50-344 (m, 1H, H-2b'), 3.41-3.31 (m, 4H, H-2'', H-3'', H-5'', H-1a'), 3.21-3.18 (m, 1H, H-1b'), 3.10 (t, J_{16-15} = 6.8 Hz, 2H, H-16), 2.01 (t, J_{2-3} = 7.2 Hz, 2H, H-2), 1.43 (q, J = 7.2 Hz, 2H, H-3), 1.21-1.07 (m, 26H, CH$_2$); ^{13}C NMR (CDCl$_3$/MeOD 3:1, 100.3 MHz) δ in ppm: 103.4 (C-1''), 74.8 (C-2''), 73.3, 71.2 (C-3'', C-5''), 68.7 (C-4''), 68.6 (C-2'), 61.2 (C-6''), 51.4 (C-16), 39.2 (C-1'), 36.2 (C-2), 29.5-28.7, 26.7 (CH$_2$), 25.6 (C-3); FT-IR (ATR) ν in cm^{-1}: 3303, 2918, 2849, 2097, 1736, 1643, 1553, 1467, 1248, 1128, 1075, 722; HRMS (TOF ES+) for (M+Na) C$_{24}$H$_{46}$N$_4$NaO$_7$ (m/z): calc. 525.3259; found 525.3248.

1-*O*-(*O*-(2-azidoethyl)heptaethylene glycolyl)-β-D-galactopyranoside (5.35)

The glycoside **5.45** (438 mg, 0.603 mmol) was dried azeotropically with anhydrous toluene and kept at high vacuum for 2h. After that, it was dissolved in anhydrous MeOH (5 mL) and a freshly prepared solution of MeONa in anhydrous methanol was added (0.5 mL, 1 M). The mixture was stirred under argon pressure at room temperature for 20 min. The reaction was quenched by adding dry ice until pH 7 was reached. After removing the solvent, the crude was purified by column chromatography (C-18, H$_2$O to H$_2$O/MeOH 1:1 v/v) to yield **5.35** (287 mg, 85%): Rf (MeOH/EtOAc 3:7): 0.16; $[\alpha]_D^{20}$: +8.2º (*c* = 1.2, MeOH); ^{1}H NMR (D$_2$O, 400 MHz) δ in ppm: 4.37 (d, $J_{1'-2'}$ = 7.6 Hz, 1H, H-1'), 4.04 (dt, J = 8.4 Hz, J = 4.0 Hz, 1H, H-CH$_2$O), 3.87 (d, $J_{4'-3'}$ = 3.2 Hz, 1H, H-4'), 3.80-3.62 (m, 32H, CH$_2$O, H-6a'', H-6b'', H-5''), 3.60 (dd, $J_{3'-2'}$ = 9.6 Hz, $J_{3'-4'}$ = 3.2 Hz, 1H, H-3'), 3.51-3.45 (m, 3H, H-2', CH$_2$N$_3$); ^{13}C NMR (D$_2$O, 100.3 MHz) δ in ppm: 102.7 (C-1'), 75.0 (C-5'), 72.5 (C-3'), 70.6 (C-2'), 69.6-69.4 (CH$_2$O), 69.1 (C-4'), 68.5 (CH$_2$O), 60.8 (C-6'), 50.0 (CH$_2$N$_3$); FT-IR

(ATR) ν in cm^{-1}: 3420, 2855, 2104, 1299, 1119, 780; HRMS (TOF ES+) for (M+Na) $C_{22}H_{43}N_3NaO_{13}$ (m/z): calc. 580.2684; found 580.2650.

2-azidoethanol (5.36)
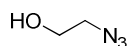
A mixture of 2-chloroethanol (4.1 mL, 60.5 mmol), NaN$_3$ (7.9 g, 121 mmol), and n-Bu$_4$NBr (487 mg, 1.5 mmol) was stirred vigorously for 18 h at 110 ºC. After cooling the mixture, the crude was purified by a short column chromatography (silica gel, Et$_2$O) to give **5.36** as a colorless liquid (4990 mg, 96%):[52] Rf (Et$_2$O): 0.77; ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 3.80 (t, J = 5.2 Hz, 2H, C$\underline{H}_2$OH), 3.47 (t, J = 5.2 Hz, 2H, CH_2N$_3$), 2.09 (bs, 1H, OH);^{13}C NMR (CDCl$_3$, 100.3 MHz) δ in ppm: 61.7 (CH$_2$OH), 53.7 (CH$_2$N$_3$); FT-IR (ATR) ν in cm^{-1}: 3356, 2934, 2877, 2092, 1441, 1347, 1286, 1061, 879, 629; HRMS (TOF ES+) for (M+H) $C_2H_6N_3O$ (m/z): calc. 88.0505; found 88.0530.

1-*O*-(2-Azidoethyl)-(2,3,4,6-tetra-*O*-acetyl)-β-ᴅ-galactopyranoside (5.38)
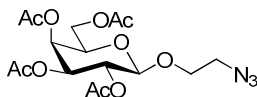

The 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2.46** (950 mg, 2.31 mmol) was prepared as it is reported by the literature[53] and then it was dried azeotropically with anhydrous toluene and kept at high vacuum for 2h. The promoter AgOTf (972 mg, 3.74 mmol) was dried azeotropically with anhydrous toluene and kept at high vacuum for 2h. After that, activated 4Å MS (950 mg) was added to the promoter and such mixture was stirred with a solution of ethanolazide (573 µL, 7.48 mmol) in anhydrous toluene (1 mL) under argon pressure at -20º C for 5 min. After that, a solution of **9** in anhydrous toluene (2.8 mL) was added to the mixture which was stirred under argon pressure at 0º C for

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[52] Xu, L.; Bittman R. *J. Org. Chem,* **2005**, *70*, 4746-4750.
[53] Bernardes, G. J. L.; Thompson, S.; Chalker, J. M.; Errey, J. C.; El Oualid, F.; Claridge, T. D. W.; Davis, B. G.; Grayson, E. J., *Angew. Chem. Int. Ed.* **2008**, *47*, 2244-2247.

3 h. The reaction was quenched by adding TEA (0.3 mL) and it was filtered through a pad of Celite. The residue was purified by column chromatography (silica gel, ethyl acetate/ hexane from 3:7 to 1:2 v/v) to give **5.38** (646 mg, 67%): Rf (EtOAc/Hexane 1:1): 0.39; $[\alpha]_D^{20}$: -12.0º (c = 1.7, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 5.39 (d, $J_{4'-3'}$ = 3.2 Hz, 1H, H-4'), 5.24 (dd, $J_{2'-3'}$ = 10.4 Hz, $J_{2'-1'}$ = 8.0 Hz, 1H, H-2'), 5.02 (dd, $J_{3'-2'}$ = 10.4 Hz, $J_{3'-4'}$ = 3.2 Hz, 1H, H-3'), 4.55 (d, $J_{1'-2'}$ = 8.0 Hz, 1H, H-1'), 4.20-4.10 (m, 2H, H-6a', H-6b'), 4.04 (ddd, J_{1a-1b} = 10.8 Hz, J_{1a-2b} = 4.4 Hz, J_{1a-2a} = 3.6 Hz, 1H, H-1a), 3.92 (dd, $J_{5'-6a'}$ = 7.2 Hz, $J_{5'-6b'}$ = 6.4 Hz, 1H, H-5'), 3.69 (ddd, J_{1b-1a} = 10.8 Hz, J_{1b-2a} = 8.8 Hz, J_{1b-2b} = 3.2 Hz, 1H, H-1b), 3.50 (ddd, J_{2a-2b} = 13.2 Hz, J_{2a-1b} = 8.8 Hz, J_{2a-1a} = 3.6 Hz, 1H, H-2a), 3.28 (ddd, J_{2b-2a} = 13.2 Hz, J_{2b-1a} = 4.4 Hz, J_{2b-1b} = 3.2 Hz, 1H, H-2b), 2.15 (s, 3H, CH$_3$), 2.06 (s, 3H, CH$_3$), 2.04 (s, 3H, CH$_3$), 1.98 (s, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$, 100.3 MHz) δ in ppm: 170.6 (C=O), 170.4 (C=O), 170.3 (C=O), 169.7 (C=O), 101.3 (C-1'), 71.1 (C-3'), 71.0 (C-5'), 68.7 (C-2'), 68.6 (C-1), 67.2 (C-4'), 61.4 (C-6'), 50.7 (C-2), 21.0 (CH$_3$), 20.9 (CH$_3$), 20.8 (CH$_3$), 20.7 (CH$_3$); FT-IR (ATR) ν in cm^{-1}: 2915, 1741, 1469, 1372, 1224, 1135, 1087, 1057, 630; HRMS (TOF ES+) for (M+Na) C$_{16}$H$_{23}$N$_3$NaO$_{10}$ (m/z): calc. 440.1276; found 440.1267.

1-*O*-(2-Aminoethyl)-(2,3,4,6-tetra-*O*-acetyl)-β-D-galactopyranoside (5.42)
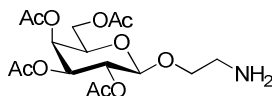
A suspension of Pd 10 % over C (85 mg) and the glycoside **5.38** (337 mg, 0.806 mmol) was prepared in anhydrous degassed THF (10 mL). The suspension was introduced to a low pressured reactor and the reaction was stirred under H$_2$ pressure (5 bar) at room temperature for 18 h. The crude was filtered though a pad of Celite to yield **5.42** as a syrup (315 mg, 99%) without further purification: Rf (MeOH/EtOAc/ 1:9): 0.11; $[\alpha]_D^{20}$: +13.0º (0.59, CH$_2$Cl$_2$); ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 5.31 (d, $J_{4'-3'}$ = 2.4 Hz, 1H, H-4'), 5.12 (dd, $J_{2'-3'}$ = 10.4 Hz, $J_{2'-1'}$ = 8.0 Hz, 1H, H-2'), 4.94 (dd, $J_{3'-2'}$ = 10.4 Hz, $J_{3'-4'}$ = 3.2 Hz, 1H, H-3'), 4.43 (d, $J_{1'-2'}$ = 8.0 Hz, 1H, H-1'), 4.10-4.00 (m, 2H, H-6a', H-6b'), 3.88 (m, 2H, H-5', H-1a), 3.54-3.49 (ddd, J_{1b-1a} = 10.4 Hz, J_{1b-2a} = 7.2 Hz,

$J_{1b,2b}$= 4.0 Hz, 1H, H-1b), 2.87-2.73 (m, 2H, H-2a, H-2b), 2.37 (bs, 2H, NH$_2$), 2.10 (s, 3H, CH$_3$), 2.01 (s, 3H, CH$_3$), 1.98 (s, 3H, CH$_3$), 1.92 (s, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$, 100.3 MHz) δ in ppm: 170.4 (C=O), 170.2 (C=O), 170.1 (C=O), 169.5 (C=O), 101.5 (C-1'), 70.8 (C-3'), 70.6 (C-5'), 68.7 (C-2'), 68.6 (C-1), 67.0 (C-4'), 61.3 (C-6'), 41.6 (C-2), 20.8 (CH$_3$), 20.7 (2CH$_3$), 20.6 (CH$_3$); FT-IR (ATR) ν in cm^{-1}: 3386, 1744, 1657, 1244, 1134, 1054, 760, 629; HRMS (TOF ES+) for (M+H) C$_{16}$H$_{26}$NO$_{10}$ (m/z): calc. 392.1551; found 392.1554.

1-*O*-(2-(16-azidohexadecanamido)ethyl)-(2,3,4,6-tetra-*O*-acetyl)-β-D-galactopyranoside (5.43)
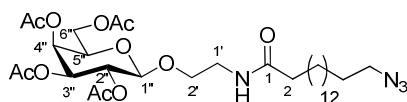
To a solution of 16-azidohexadecanoic acid[35] **2.29** (240 mg, 0.806 mmol) and glycoside **5.42** (315 mg, 0.806 mmol) in anhydrous DCM (5 mL) was added HOBt (131 mg, 0.967 mmol), EDC (185.4 mg, 0.967 mmol), and DIPEA (313 μL, 1.818 mmol). The reaction mixture was stirred at room temperature for 24h and then concentrated in vacuo. The residue was purified by column chromatography (silica gel, MeOH/EtOAc/hexane 1:2:7 v/v) to give **5.43** as syrup (305 mg, 56%): Rf (MeOH/EtOAc/Hexane 1:3:6): 0.20; [α]$_D^{20}$: +2.1º (0.24, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 5.37 (dd, $J_{4''-3''}$ = 3.6 Hz, $J_{4''-5''}$ = 1.2 Hz, 1H, H-4''), 5.16 (dd, $J_{2''-3''}$ = 10.4 Hz, $J_{2''-1''}$ = 8.0 Hz, 1H, H-2''), 4.99 (dd, $J_{3''-2''}$ = 10.4 Hz, $J_{3''-4''}$ = 3.6 Hz, 1H, H-3''), 4.44 (d, $J_{1''-2''}$ = 8.0, 1H, H-1''), 4.18-4.07 (m, 2H, H-6a'', H-6b''), 3.90 (ddd, $J_{5''-6a''}$ = 7.6 Hz, $J_{5''-6b''}$ = 6.4 Hz, $J_{5''-4''}$ = 1.2 Hz, 1H, H-5''), 3.86-3.82 (m, 1H, H-2a'), 3.68-3.63 (m, 1H, H-2b'), 3.50-3.38 (m, 2H, H-1a', H-1b'), 3.22 (t, J_{16-15} = 7.2 Hz, 2H, H-16), 2.15-2.11 (m, 5H, H-2, CH$_3$), 2.04 (s, 3H, CH$_3$), 2.02 (s, 3H, CH$_3$), 1.96 (s, 3H, CH$_3$), 1.60-1.54 (m, 4H, CH$_2$), 1.33-1.22 (m, 22H, CH$_2$); ^{13}C NMR (CDCl$_3$, 100.3 MHz) δ in ppm: 173.3 (HN-C=O), 170.5 (O-C=O), 170.3 (O-C=O), 170.2 (O-C=O), 169.7 (O-C=O), 101.5 (C-1''), 70.9 (C-5''), 70.8 (C-3''), 69.3 (C-2'), 69.0 (C-2''), 67.1 (C-4''), 61.4 (C-6''), 51.6 (C-16), 39.2 (C-1'), 36.8 (C-2), 29.7-28.9 (CH$_2$), 26.8 (CH$_2$), 25.8 (CH$_2$), 21.0 (CH$_3$), 20.8 (CH$_3$), 20.8 (CH$_3$), 20.7 (CH$_3$); FT-IR (ATR) ν in cm^{-1}: 2922, 2853, 2096, 1754, 1651, 1248, 1059,

784; HRMS (TOF ES+) for (M+Na) $C_{32}H_{54}N_4NaO_{11}$ (m/z): calc. 693.3681; found 693.3676.

1-*O*-(*O*-(2-azidoethyl)heptaethylene glycolyl)-(2,3,4,6-tetra-*O*-acetyl)-β-D-galactopyranoside (5.45)
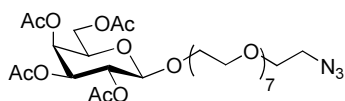
The 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2.46** (285.8 mg, 0.695 mmol) was prepared as it is reported at the literature[38] and then it was dried azeotropically with anhydrous toluene and kept at high vacuum for 2h. The promoter AgOTf (197 mg, 0.76 mmol) was dried azeotropically with anhydrous toluene and kept at high vacuum for 2h. After that, activated 4Å MS (285 mg) was added to the promoter and such mixture was stirred with a solution of *O*-(2-azidoethyl)heptaethylene glycol **5.44** (250 mg, 0.632 mmol) in anhydrous acetonitrile (1 mL) under argon pressure at room temperature for 5 min. After that, a solution of **2.46** in anhydrous acetonitrile (1.5 mL) was added to the mixture which was stirred under argon pressure at room temperature for 24 h. The reaction was filtered though a pad of Celite and concentred. The residue was purified by column chromatography (silica gel, MeOH/AcOEt/hexane from 1:3:6 to 1:6:3 v/v) to give **5.45** as syrup (348 mg, 76%): Rf (MeOH/EtOAc/Hexane 1:6:3): 0.17; $[\alpha]_D^{20}$: +31.0º (*c* = 0.9, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 5.36 (dd, $J_{4'-3'}$ = 3.6 Hz, $J_{4'-5'}$ = 1.2 Hz, 1H, H-4'), 5.15 (dd, $J_{2'-3'}$ = 10.4 Hz, $J_{2'-1'}$ = 8.0 Hz, 1H, H-2'), 4.99 (dd, $J_{3'-2'}$ = 10.4 Hz, $J_{3'-4'}$ = 3.6 Hz, 1H, H-3'), 4.53 (dd, $J_{1'-2'}$ = 8.0 Hz, 1H, H-1'), 4.13-4.06 (m, 2H, H-6a', H6b'), 3.93-3.88 (m, 2H, H-5', CH$_2$O), 3.73-3.56 (m, 29H, H-CH$_2$O), 3.34 (t, J = 5.2 Hz, 2H, CH$_2$N$_3$), 2.12 (s, 3H, CH$_3$), 2.04 (s, 3H, CH$_3$), 2.03 (s, 3H, CH$_3$), 1.96 (s, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$, 100.3 MHz) δ in ppm: 170.6 (C=O), 170.3 (C=O), 170.2 (C=O), 169.9 (C=O), 101.5 (C-1'), 70.9 (CH$_2$O), 70.7 (C-3'), 70.5 (C-5'), 70.4 (CH$_2$O), 70.2 (CH$_2$O), 70.1 (CH$_2$O), 70.0 (CH$_2$O), 69.9 (CH$_2$O), 69.2 (CH$_2$O), 68.9 (C-2'), 67.1 (C-4'), 61.3 (C-6'), 50.7 (CH$_2$N$_3$), 20.9 (CH$_3$), 20.8 (CH$_3$), 20.8 (CH$_3$), 20.7 (CH$_3$); FT-IR (ATR) ν in cm^{-1}: 2828,

2108, 1749, 1677, 1605, 1456, 1420, 1367, 1294, 1218, 1181, 915; HRMS (TOF ES+) for (M+Na) $C_{30}H_{51}N_3NaO_{17}$ (m/z): calc. 748.3111; found 748.3104.

Hyperbranched polymer (5.48)

To a solution of 5-hexynoic acid **5.47** (2276 µL, 20.01 mmol) and dialysed Boltorn H30 **5.46** (1060 mg, 0.294 mmol) in anhydrous DCM/DMF 1:1 (100 mL) was added HOBt (2703 mg, 20.01 mmol), EDC (3835 mg, 20.01 mmol), and DIPEA (3335 µL, 20.01 mmol). The reaction mixture was stirred at room temperature for 5 days and then concentrated in vacuo. The reaction was purified by dialysis (benzoylated tube flat width 32 mm (1.27''), DMF) for 3 days changing the solvent each 8h to give **5.48** as a syrup (1069 mg, 71 %). The amount of the 5-hexynoate groups added to Boltorn H30 surface corresponded to an average valency of 16, which represented a functionalization 51% of OH surface, estimated by integration of signals at ^{1}H NMR according to Fernández-Francos and co-workers methodology:[40] ^{1}H NMR (CDCl$_3$, 400 MHz) δ in ppm: 4.21-4.12 (m, 75H, H-1'), 3.65-3.35 (m, 32H, H-CH$_2$OH), 2.41 (t, J = 6.8Hz, 32H, H-2), 2.19 (td, J = 7.2Hz, J = 2.8 Hz, 32H, H-4), 1.95 (s, 16H, H-6), 1.77 (at, J = 7.2 Hz, 32H, H-3), 1.22-1.18 (m, 55 H, H-3');^{13}C NMR (CDCl$_3$, 100.6 MHz) δ in ppm: 173.1, 172.6, 172.4, 172.2, 171.9, 83.2 (C-5), 69.7 (C-6), 66.0-64.0 (C-1'), 48.7 (C-2'), 46.6-46.5 (C-2'), 32.7-32.6 (C-2), 23.6-23.5 (C-3), 17.9-17.5 (C-3', C-4); FT-IR (ATR) ν in cm^{-1}: 3529, 3285, 2942, 1730, 1468, 1380, 1237, 1130, 1053, 1011, 631; GPC-MS: M_n = 2478 g/mol, M_w = 6801 g/mol, PDI = 2.78.

Chapter 6

Conclusions

6.1. Conclusions

After the discussion of the results presented along this work, we may conclude that:

1. The use of highly disarmed glycosyl donors (**2.27** and **2.45**) permits a direct glycosylation of stannylceramides (**2.35** and **2.36**) reducing the overall number of synthetic steps by avoiding the orthoester formation and providing access to β-glycolipids in a good yield and with complete chemo- and stereoselectivity (Chaper 2).

2. The time-course ^{19}F NMR study for the reaction of highly disarmed glycosyl donor **2.27** with tributylstannyl acceptor **2.35** provided the first evidence for the formation of glycosyl triflates as reaction intermediates in protic acid medium (Chapter 2). ^{1}H, ^{13}C and bidimensional NMR spectroscopy at low temperature provided information about the configurations of glycosyl triflates identified by ^{19}F NMR. The obtained data indicate that α-glycosyl triflate has a chair conformation $^{4}C_1$ (**2.27a**) and β-glycosyl triflate adopts a distort chair $^{1}C_4$ (**2.27b'**) (Chapter 2)**.**

3. The direct glycosylation of stannyl ceramide **3.17** with the 2-deoxy-2-iodo glycosyl donor (**3.16**) was unsuccessful because of the side-reactions occurring between Sn (IV) species with iodine moiety present on the glycosyl donor (**3.16**). However, direct glycosylation of azidosphingosine **3.22** with 2-deoxy-2-iodo glycosyl donor (**3.16**) and azidophytosphingosine **3.36** with 2-deoxy-2-iodo glycosyl donor (**3.27**) afforded the corresponding glycosides **3.23** and **3.43** respectively in high yield. In both cases complete stereoselectivities were obtained. After several transformations, the corresponding derivatives of 2-deoxy-β-

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GalCer **3.25** and 2-deoxy-2-iodo-α-TalCer **3.44** were obtained. (Chapter 3).

4. 2-Iodoglycals were efficiently obtained by elimination of the corresponding 2-deoxy-2-iodopyranoses with Ph_2SO/Tf_2O and TTBP under thermodynamic conditions (Chapter 4).

5. A general catalytic strategy for the efficient synthesis of 2-*C*-arylglycals (**4.11a-h**, **4.12**, **4.13** and **4.14**) by phosphine-free Suzuki-Miyaura cross-coupling of 2-iodoglycals in aqueous media using an inexpensive Pd catalyst has been developed. To the best of our knowledge this transformation represents the first transition metal catalized 2-*C*-arylation of 2-haloglycals. The simplicity and relative mildness of this method allows the regioselective preparation of various 2-*C*-arylglycals with different configurations in excellent yields with no Ferrier or 2,3-unsaturated by-products detected (Chapter 4).

6. The elaboration of the 2-*C*-arylglycal moiety gives access to 2-*C*-aryl-2-deoxy-1,5-anhydroalditols (**4.16** and **4.17**) and challenging quaternary 2-*C*-aryl-α-glycosides (**4.31**, **4.32**, **4.35** and **4.36**) which will broaden the plethora of *C*-arylglycosides at positions different than C-1. (Chapter 4).

7. A family of glycolipidic clusters (**5.20**, **5.22** and **5.54**) was prepared by employing CuAAC reaction in order to test them as cholera toxin inhibitors. It was found that $[Cu(CH_3CN)_4]OTf$ and TBTA catalytic system was more suitable than $CuSO_4\cdot5H_2O$/Sodium ascorbate; the CuAAC reaction was enhanced by microwave heating due to relative stability of such compounds.

8. Hyperbranched polymers can be efficiently used as multivalent glyco(lipid) systems able to block cholera toxin binding with potential applications to other surface receptor-mediated diseases. The best multivalent compounds here studied (**5.28** and **5.29**) exhibited >50,000-fold greater potency than monovalent galactose, providing a platform that mimic the natural GM1-enriched lipid domains. Furthermore, a systematic study on the effect of the length, number of lipophilic chains and polarity was carried out, being those conjugates possessing two long spacers superior inhibitors of the cholera toxin binding.