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Minireview

Recent advances in the glycosylation of sphingosines and ceramides

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Abstract—Glycosphingolipids (GSLs) are ubiquitous components of eukaryotic cell membranes. They are highly bioactive and are involved in many aspects of cell signalling like cell–cell interaction, cell–substratum interaction and cell–pathogen interaction. GSLs also are involved in the modulation of signal transduction, resulting in regulation of cell proliferation and differentiation. The biological importance and complexity of these compounds afford many opportunities to prepare synthetic analogues for studies of their metabolism in intra- and intercellular processes. This review focuses on recent contributions in the synthesis of GSLs, highlighting improvements in glycosylation reactions leading to α and β glycosyl sphingosines and ceramides and related compounds. Literature from 2000 to the present is covered. The glycosylation reactions leading to the synthesis of GSLs are classified in function of the configuration of the created glycosidic bond (α or β) and of the acceptor used, either azido-sphingosine or ceramide.

Keywords: Carbohydrate; Glycosphingolipids; Glycosylation; Ceramides; Sphingosine; Phytosphintgosine

Contents

1. Introduction

Biological membranes[1] are described as a 'mosaic of lipid domains' where glycosphingolipids (GSLs) are building blocks of the plasma membrane and where their hydrophilic portions are exposed towards the cell

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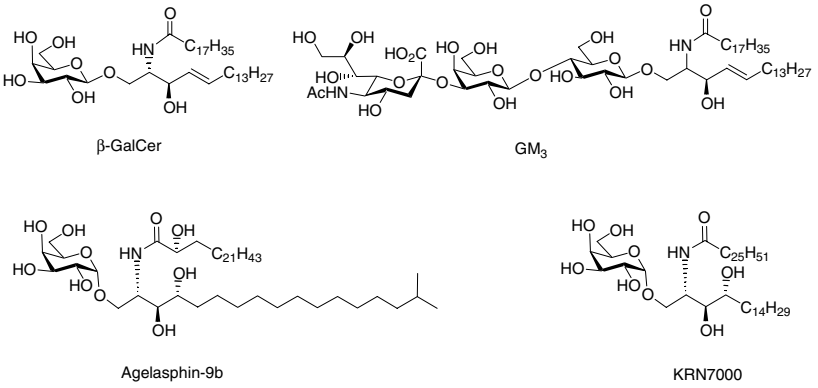


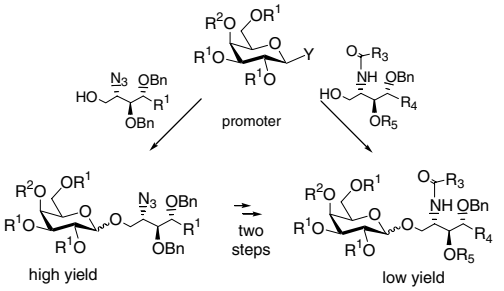
Figure 1.

surface and the hydrophobic moieties are inserted into the membrane layer. GSLs are involved in cellular trafficking, signalling functions,[2] interactions with external agents,[3] proliferation, differentiation, apoptosis and cellular embryogenesis.[4] The majority of GSLs are composed of a hydrophobic base, ceramide. Attached to this base is a hydrophilic group of core monosaccharides. Ceramide is composed of a long chain amino alcohol linked to a fatty acid, most commonly with a long chain, which is sometimes hydroxylated. The most frequently occurring long chains contain a C4–C5 double bond in the *trans*-D-*erythro* configuration (see β-GalCer and GM_3, Fig. 1). Less frequent are sphinganines, which lack the double bond, and phytosphingosine, which carries an additional hydroxyl group on C4, for example, as in Agelasphin-9b and α-galactosyl-ceramide (α-GalCer, KRN7000) (Fig. 1).[5] The carbohydrate moiety contains one or more monosacharides, and is linked to ceramide via a glycosidic bond. GSLs are classified as: (a) cerebrosides (containing a sugar residue), (b) sulfatides (containing a sugar residue with a sulfate group), (c) neutral glycosphingolipids (containing oligosaccharides with two or more sugar residues) and (d) gangliosides (containing neuraminic acid residues). The saccharide units present in GSLs are glucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, fucose, sialic acid and glucuronic acid.[6]

GSLs have been the subject of interesting studies as the molecular[7] basis of raft–pathogen interactions and the effect of such interactions.[8] However, in the past few years, the field of GSLs research has been addressed as a strategy for preventing different diseases: microbial infections (HIV),[9,10] cancer,[11] diabetes,[12,13] Alzheimer's[14,15] and Parkinson's.[16] To increase these activities, two alternatives have been developed. The first approach consists of anchoring the oligosaccharide units on a chemical matrix to obtain a multivalent neoglycoconjugate.[17,18] The other approach is to modify the structure of the hydrophobic part of GSLs, with the goal of obtaining water-soluble analogues[19] in which the conformation of the binding domain of the analogue is similar to GSLs.

There are three components of the immune systems in mammals. Two types of cells associated with these systems are necessary for the recognition of antigens. One family is B cells and the second is T cells. The third component involve CD1 (Cluster of differentiation 1) molecules.[20] At the molecular level, glycolipids have been shown to act as a connecting ligand presented by the CD1d molecule of antigen-presenting cells to the mouse Vα14 receptor and the human Vα24 receptor of natural killer T (NKT) cells. Upon recognition of the galactosylceramide in the context of CD1d, the NKT cell then is stimulated to produce interferon-γ (IFN-γ), interleukin-4 (IL-4) and interleukin-2 (IL-2).[21] The release of proinflamatory cytokines is believed to be responsible for the antitumour, antiviral, antibacterial effects of GSLs. Since the discovery of galactosyl-ceramides from marine sponges in 1993,[22] the potent immunostimulant activity of this family of molecules has been studied. Preliminary structure–activity studies suggested that structural variations in the lipid chains result in relatively small changes in IFN-γ and IL-4.[23] The immunostimulatory activities of GSLs analogues have led to the development of anticancer chemotherapeutics that are currently in clinical trials.[24]

In this context, significant work has been recently devoted to the preparation of 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 carbohydrate and ceramide or sphingosine.[5] To accomplish this key synthetic step, a variety of glycosyl donors have been utilized including glycosyl trichloroacetamidates, fluorides, phosphates and sulfides.[25] Regardless, the glycosylation reaction is still one of the main determining factors in the synthesis, because glycosylations of ceramides are generally plagued by low yields.[26] This problem has been attributed to the low nucleophilicity of



Scheme 1.

ceramides. One method for circumventing this challenge is to use azido-sphingosine instead of ceramide (Scheme 1).[27]

This mini-review will focus on recent contributions to the synthesis of GSLs, highlighting the improvements in glycosylation reactions leading to α and β glycosyl sphingosines and ceramides and related compounds. Literature from 2000 to the present is covered.[5,28,29] Due to the importance of α and β GSLs and their different biological properties, and to extend awareness and use of azido-sphingosine in synthesis, this review presents the synthesis of GSLs classified in function of the configuration of the created glycosidic bond (α or β) and of the acceptor used, either azido-sphingosine or ceramide.

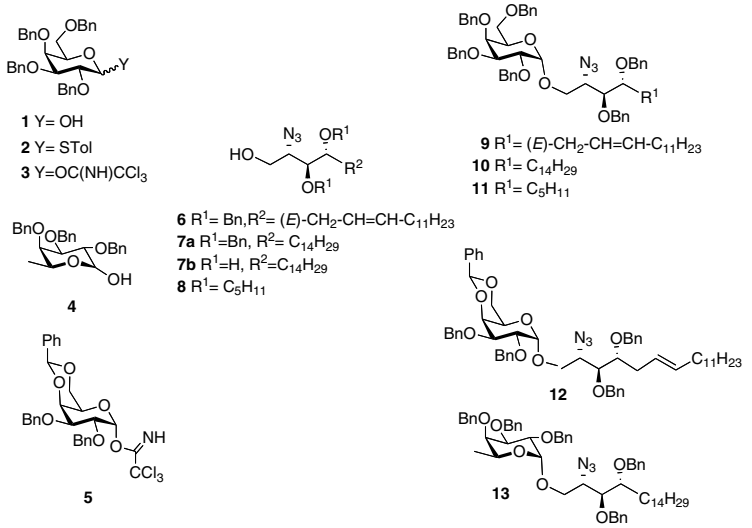
2. Synthesis of α-glycosyl sphingosines and ceramides

2.1. Glycosylation of azido-sphingosines

As mentioned previously, interest in α-glycosyl-ceramides derives from the relevant biological properties of these compounds, particularly those having phytosphingosine as a part of the ceramide moiety (α-GalCer). Consequently, most reports have focused on the synthesis of this compound and analogues. Although the formation of 1,2-*trans* glycosides can be easily achieved by taking advantage of neighbouring group assistance, such as *O*-acetyl or *O*-benzoyl at C-2, the stereospecific construction of 1,2-*cis* galactopyranosyl linkages has been one of the greatest challenges of glycoside synthesis. α-Gal-type linkages can be formed by working under thermodynamic conditions (anomeric effect), in appropriate solvents (ethereal solvent effect),[29] and by using non-participating protecting groups at the C2 hydroxyl, typically benzyl groups. Glycosyl trichloroacetimidates are the most popular glycosyl donors for glycosylation of azido-sphingosine, although SPh, OAc and other leaving groups have also been used. In Table 1 are collected several examples of the glycosylation of azido-sphingosines with galactose derivatives.

In the context of the synthesis of α-galactosyl-ceramide azido-sphingosine **6**, obtained in nine steps from 2-deoxy-D-*lyxo*-hexose in an overall yield of 36%, was

Table 1. Synthesis of α-galactosyl-azido-sphingosines **9**–**13**



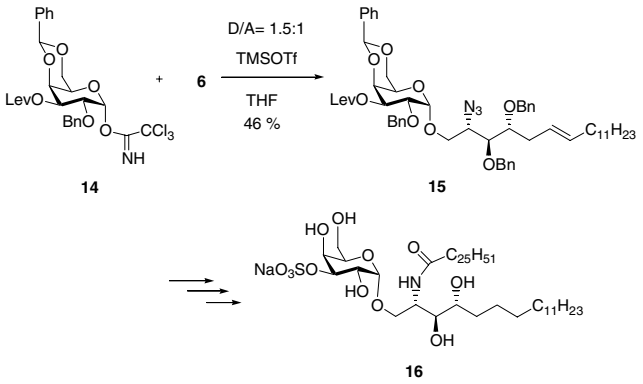
Entry	Acceptor	Donor	Promoter	Solvent	Product	Yield (%)	Ratio (α/β)	Ref.
1	**6**	**2**	DMTST	CH_2Cl_2	**9**	39	1:0	25
2	**6**	**3**	$BF_3{\cdot}OEt_2$	THF/Et_2O	**9**	50	2.9:1	25
3	**6**	**5**	$BF_3{\cdot}OEt_2$	THF/Et_2O	**12**	68	1:0	25 and 27
4	**7a**	**2**	NIS/TfOH	CH_2Cl_2	**10**	93	1:1	28
5	**7a**	**1**	Me_2S, 2-Cl–Py, Tf_2O	CH_2Cl_2	**10**	83	3:1	28
6	**8**	**1**	Me_2S, 2-Cl–Py, Tf_2O	CH_2Cl_2	**11**	nr	3:1	28
7	**7**	**4**	Me_2S, 2-Cl–Py, Tf_2O	CH_2Cl_2	**13**	nr	3:1	28

reacted with perbenzylated thiogalactoside **2** using dimethyl(methylthio)sulfonium triflate (DMTST) as the promoter. The reaction afforded the galactosyl-ceramide **9** in 39% yield as a pure α-isomer (Table 1, entry 1).[30] The use of NIS/TfOH as the promoter system gave complex mixtures as consequence of the addition of the electrophile to the double bond. The use of trichloroacetimidate **3** and $BF_3{\cdot}Et_2O$ as the promoter allowed the improvement of the yield to 50% but at the cost of decreasing the stereoselectivity ($\alpha/\beta = 2.9{:}1$) (Table 1, entry 2). Schmidt[31] had previously demonstrated that the 4,6-*O*-benzylidene-protected galactosyl trichloroacetimidate **5** reacts with unprotected azido-phytosphingosine **7b** to give the α-glycosylated product in 49% yield. Interestingly, when the 2-*O*-mesylate precursor of **7b** was used in the glycosylation, the yield improved to 73%, and no glycosylation of the secondary alcohols was observed. Thus, when the trichloroacetimidate **5** was reacted with the azido-phytosphingosine **6** using $BF_3{\cdot}OEt_2$ as a promoter, galactosyl-ceramide **12** was cleanly delivered in 68–70% yield.[30,32] The hydroxyl groups were deprotected and double bond reduced by treating **12** under hydrogenolytic conditions providing α-galactosyl-ceramide.

Coupling phytosphingosine **7** with the galactosyl donor **2** using NIS–TfOH as the promoter system afforded **10** in 93% yield, albeit as a 1:1 α/β mixture (Table 1, entry 4).[33] When a dehydrative glycosylation procedure[34] was used in the coupling of tetrabenzyl galactose **1** with the acceptor **7**, compound **10** was obtained in 83% yield and improved stereoselectivity ($\alpha/\beta = 3{:}1$) (Table 1, entry 5). α-Galactosyl-sphingosine **11** and α-fucosyl-sphingosine **13** were also synthesized in a 3:1 α/β ratio by dehydrative glycosylation of phytosphingosines **7** and **8**, with the glycosyl donors **1** and **4** (Table 1, entries 6 and 7). These compounds were transformed into the final galatosyl-ceramides by azide reduction, amide formation and debenzylation.

The 3-*O*-sulfo-α-galactosyl-ceramide **16**, which stimulates human NKT cells to secrete IL-4 and IFN-γ,[35] was prepared from 4,6-*O*-benzylidene protected-glycosyl trichloroacetimidate **14**, which has positions 2 and 3 differentially protected to facilitate selective deprotection and sulfation (Scheme 2). Donor **14** was coupled with azidosphingosine **6**, using a donor/acceptor ratio of 1.5:1, in the presence of TMSOTf to give **15** in 46% yield, exclusively as the α-anomer.

Several years ago, in the course of a study concerning the immunostimulatory activity of several natural α-Gal-GSLs, it was found that the glycosylation of 2-OH group of galactose resulted in a complete loss of activity,[36] and that the hydrolysis of the inter-glycosidic linkage by α-glycosidases can restore the activity. The crucial role of the galactose 2-OH group could be due to mere steric hindrance or to the fact that the 2-OH is involved in specific interactions at the binding site of

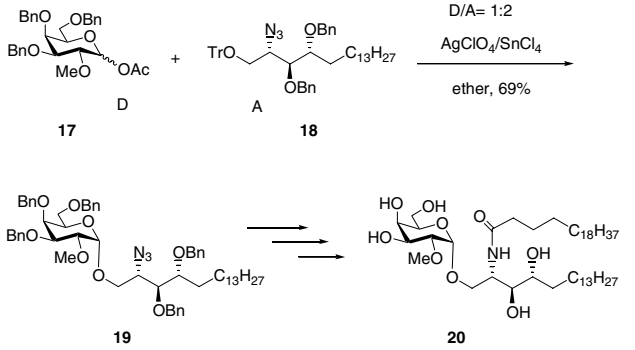


Scheme 2.

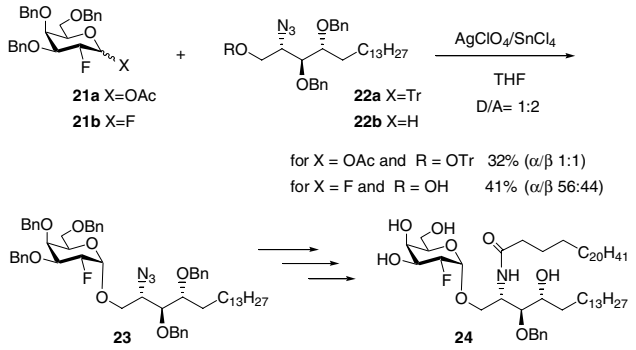
the receptor. In an attempt to clarify this effect, compounds **20**, **24** and **32**, in which the 2-OH of the galactose has been replaced by OMe, F or H were prepared. None showed significant immunostimulatory activity.

The synthesis of **20** is shown in Scheme 3. Intermediate **19** was synthesized using a modification of the Mukaiyama glycosylation procedure,[37] which involved the use of the glycosyl acetate **17** instead of the glycosyl fluoride, and $SnCl_4$–$AgClO_4$, instead of $SnCl_2$–$AgClO_4$. In addition, the alcohol of the sphingosine moiety was activated with a trityl group.[38] Coupling **17** and **18** under these conditions afforded **19** in 69% yield as an essentially pure α-anomer.

A similar procedure was used in the synthesis of the 2-deoxy-2-fluoro-galactosyl-sphingosine **23**, which was then converted into the 2-deoxy-2-fluoro-galactosyl-ceramide **24** (Scheme 4).[39] 2-Deoxy-2-fluoro-galactose **21a**, synthesized from 3,4,6-tri-*O*-benzyl-galactal, was reacted with the trityl derivative **22** (R = Tr) using $SnCl_4$–$AgClO_4$ as promoter affording **23** in 32% yield as a 1:1 anomeric mixture. When the reaction was carried out under standard Mukaiyama conditions from glycosyl fluoride **21b** and the non-tritrylated azidosphingosine **22** (R = H) using $SnCl_2$–$AgClO_4$, the yield increased slightly but the stereoselectivity was also essentially null. Comparing Schemes 2 and 3 it can be



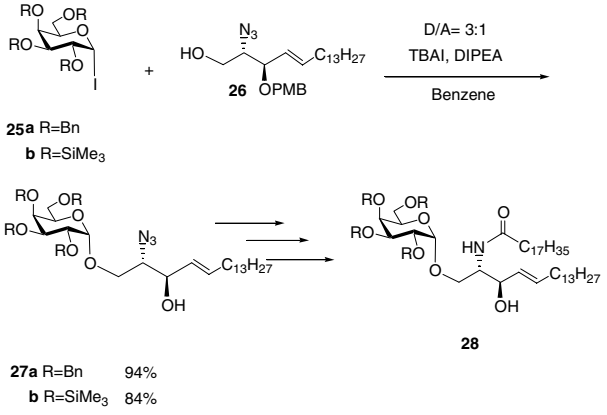
Scheme 3.



Scheme 4.

concluded that the presence of fluorine at position 2 has strong influence on the yield and stereoselectivity of glycosylation reaction.

Recently, it has been shown that glycosyl iodides[40] are excellent donors for glycosylation reactions. High α-stereoselectivity is achieved by in situ anomerization of the α-iodide to the more reactive β-iodide, from which the α-glycoside is exclusively formed as a consequence of an S_N2 displacement. Recently this procedure has been used in the synthesis of α-galactosyl-azido-sphingolipids **27** by reacting azido-sphingosine **26** with either the per-O-benzylated **25a** or per-O-silylated iodide **25b** donors in the presence of *n*-Bu_4NI (Scheme 5).[41] Compounds **27a**,**b** were obtained in remarkable yields of 94% and 84%, respectively, exclusively as the α-anomer.



Scheme 5.

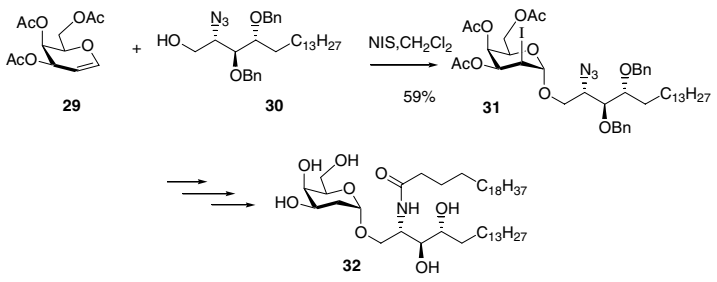
The 2-deoxy derivative **32** was synthesized by using 3,4,6-tri-*O*-acetyl-galactal as the chiral starting material for not only for the synthesis of phytosphingosine **30** but also as the starting material for glycosylation (Scheme 6).[42] Thus, stereoselective glycosylation was accomplished using **29** as the glycosyl donor in an iodonium-mediated coupling with the azido-sphingosine **30** to give **32**. The stereochemistry of this reaction was determined by the attack of iodine on the β-face of the double bond, which controls the selective formation of the α glycosidic bond.[43]

2.2. Glycosylation of ceramides

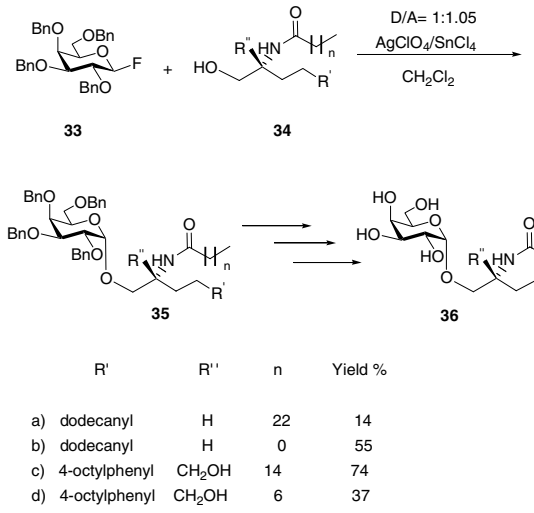
There are several examples of the use of perbenzylated-glycosyl fluorides in the glycosylation of structurally different ceramides to obtain α-glycosides (Schemes 7–10). Using $SnCl_2$–$AgClO_4$ as the promoter system, good stereoselectivity and moderate to poor yields are usually obtained. A series of α-mannosyl- and α-galactosyl-ceramide analogues **36**, incorporating sphinganines **34** in place of sphingosine, were prepared from the protected derivative **35**. These compounds were synthesized to examine their effects on immune responses by Vα19 NKT cells (Scheme 7). Compound **35** was synthesized by glycosylation of ceramide **34** with 2,3,4,6-tetra-*O*-benzyl-β-galactopyranosyl fluoride (**33**) in the presence of $SnCl_2$–$AgClO_4$. The α isomer was preferentially obtained (α/β = 9:1).[44]

The α-galactosyl-ceramide has proven to be an invaluable tool for enhancing understanding of the role of CD1d antigen presentation and NKT cell function. With the aim of elucidating this role, sphinganines **38a** and **38b**, analogues of α-GalCer, were prepared (Scheme 8). Glycosylation of ceramides **37a** and **38b** with glycosyl fluoride **33** in the presence of $SnCl_2$–$AgClO_4$ afforded **38a** and **38b** in low yields but with excellent α selectivity.[45]

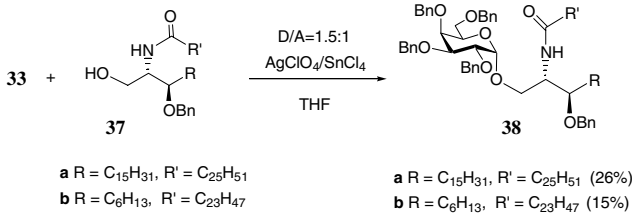
Modelling of the CD1d-α-GalCer complex suggested that the hydroxyl groups at C4 and C6 on galactose are not involved in complex formation. To quantify the association of glycolipids with CD1d and NKT cell



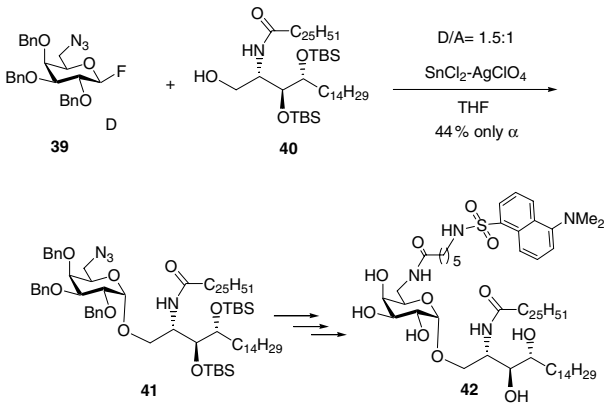
Scheme 6.



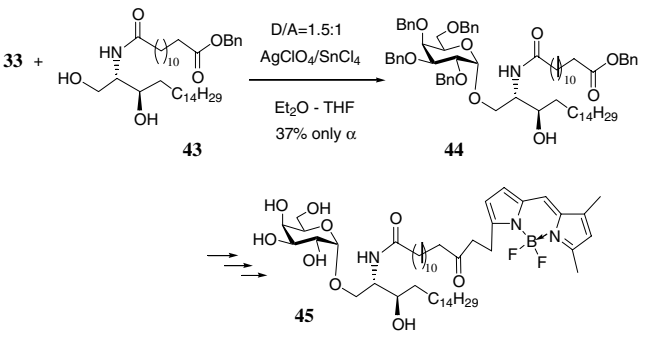
Scheme 7.



Scheme 8.



Scheme 9.

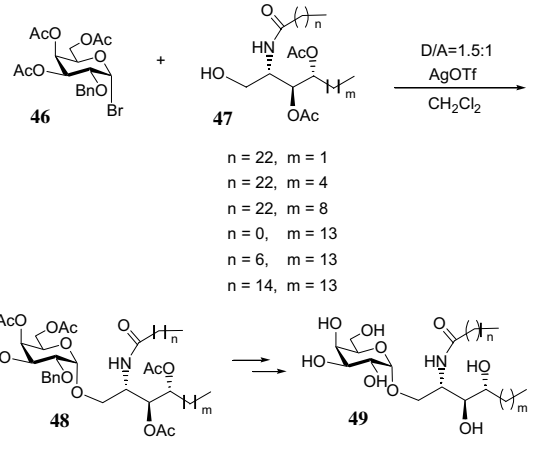


Scheme 10.

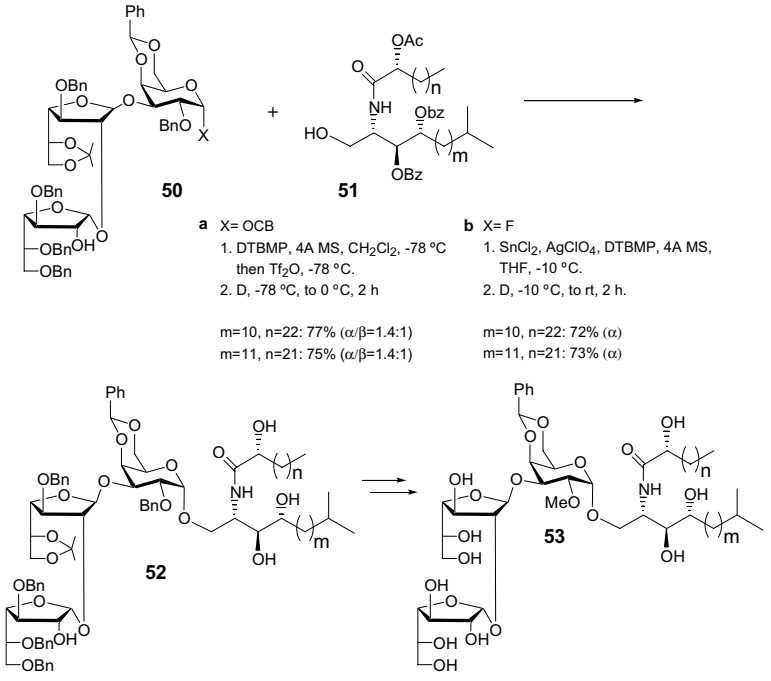
receptors, α-galactosyl-ceramides **42**, which have labels (fluorophores and biotin) appended at the C6 position of the sugar, were prepared. Glycosylation of ceramide **40** with 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-tribenzyl-galactopyranosyl fluoride **39** promoted by $SnCl_2$–$AgClO_4$ gave exclusively the α-anomer **41** in 44% yield (Scheme 9).[46] Compound **42** was prepared from **41** by removing the protecting groups and anchoring the label at C6.

α-Galactosyl-ceramide **45** containing BODIPY, a fluorescent group, was prepared with the purpose of visualizing the behaviour of this compound in vivo, and how the presence of this group affects biological activity (Scheme 9).[47] Ceramide **43** was glycosylated with the galactosyl fluoride **33** promoted by $SnCl_2$–$AgClO_4$ giving the ceramide **44** in 37% yield exclusively as the α-anomer. The lipidic chain in **44** was further elaborated and the protecting groups removed to give **45**.

A series of α-galactosyl-ceramides **49** in which the lipid chain lengths have been incrementally varied were prepared to study the effects of lipid chain lengths on cytokine release by natural killer T cells (NKT cells). They concluded that truncation of the phytosphingosine lipid chain increases the IL-4 versus INF-γ bias of released cytokines and that the length of the acyl chain in α-galactosyl-ceramides influences cytokine release profiles. With this purpose, ceramides **47** were glycosylated with α-galactosyl bromide **46** in the presence of silver triflate providing the α-galactosyl-ceramides **48** in good yield (62%) contaminated with small amounts of the β-anomers (Scheme 11).[48] Donor **46** was used because the selective deprotection of the benzyl group allows an easier separation of the β-anomer. It is remarkable that a relatively good yield is obtained in this case from the more classical glycosyl donors. This glycosyl donor differs from **33**, not only in the identity of the halogen at the anomeric position, but also by



Scheme 11.



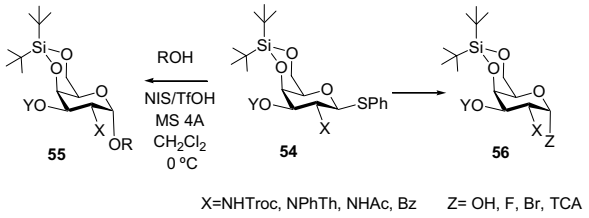
Scheme 12.

the presence of acetyl protecting groups on the hydroxyl groups at C3, C4 and C6.

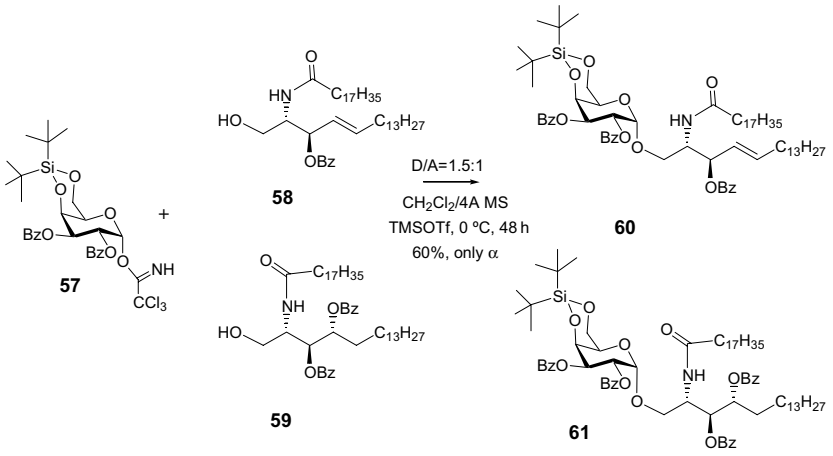
Agelagalastatin[49] **53** is a glycolipid isolated from the marine sponge *Agelas* sp., in addition to other relevant glycolipids such as longiside and α-GalCer,[50] which have shown inmunomodulating activity (Scheme 12). Agelagalastatin is composed of a trisaccharide containing two galactofuranose and one galactopyanose units, the latter of which is linked to phytosphingosine. In the context of the total synthesis of **53**, the stereoselective construction of the α-galactofuranoside and α-galactopyranoside bonds was performed by using the 2′-carboxybenzyl (CB) group as leaving group.[51] The synthesis of **52**, a precursor of the angelagalastatin **53**, was carried out by glycosylation of ceramide **51** with the trisaccharide **50a**, which has a 4,6-*O*-benzylidene protecting group. Using triflic anhydride as promoter, the yield of glycosylation was good (77%) but the stereoselectivity was very low (α/β = 1.4:1). When glycosylation was performed using the glycosyl fluoride **50b** and $SnCl_2$–$AgClO_4$ as promoter, the yield was also good (72%) and the α-anomer was exclusively obtained. In this case, the glycosylation reaction appears to be more dependent on the leaving group in the glycosyl donor and on the reaction conditions than on the presence of the 4,6-benzylidene group.

A particular problem in the synthesis of glycans is the synthesis of 2-deoxy-2-amino-α-glycosides, which usually require starting from 2-azido-derivatives to avoid participating groups. However, the anomeric selectivity and yield of glycosylation varies greatly depending on the structure of donors and acceptors. Alternatives to circumvent this problem include the use of 2-nitro-glycals[52] as glycosyl donors or the use of (1*S*)-phenyl-2-phenyl-2-(phenylsulfanyl)ethyl group at the C2-hydroxyl position.[53] Recently, it has been demonstrated that the presence of a 4,6-*O*-di-*tert*-butylsilylene group (DTBS) in galactosyl donors (e.g., **54**) allows the α-selective glycosylation of simple alcohols and carbohydrates to give **55** in excellent yields despite the presence of participating groups at the C2 position (Scheme 13).[54] Furthermore, the thio-glycoside **54** can be easily converted into other useful glycosyl donors **57** (Scheme 14), all of them with the α-configuration.

This discovery has been recently applied to the synthesis of α-GalCer,[55] and iGb3.[56] Remarkably, ceramides **58** and **59** were glycosylated with the glycosyl donor **57**, which have a participating group at C2 and trichloroacetimidate as a leaving group, to give the galactosylceramides **60** and **61**, respectively, in 60% yield exclusively as the α-anomers (Scheme 14). The presence of benzoate groups in the carbohydrate and in the lipidic



Scheme 13.



Scheme 14.

portion facilitates the deprotection process leading to α-GalCer.

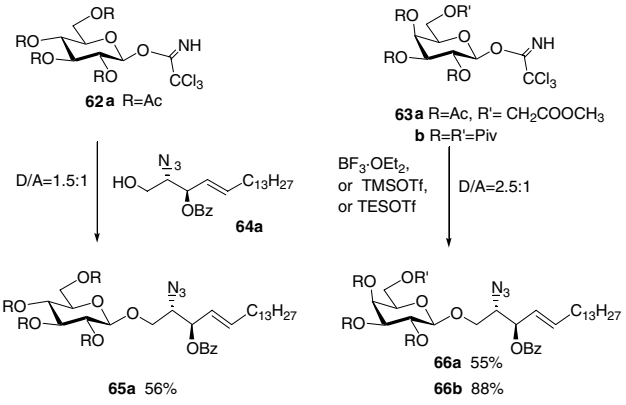
Similarly, the DTBS galactosyl donors **56** (Y = NHTroc, Z = SPh, TCA) have also been successfully used in the synthesis of α-galactosaminyl Ser/Thr sequences, obtaining in most cases, yields higher than 88% and almost exclusively the α-isomer.[55]

3. Synthesis of β-glycosyl sphingosines and ceramides

3.1. Glycosylation of azido-sphingosine

β-Glucosyl- and β-galactosyl-ceramides are frequently obtained by the azido-sphingosine method,[27] from glucose and galactose trichloroacetimidates protected with participating groups, mainly acetyl, benzoyl and pivaloyl esters (Scheme 15). The glycosyl-azido-sphingosines obtained are further converted into the amino derivatives, which are amidated to afford the final product.

Glucosyl-sphingosine **65** was prepared in 56% yield by reaction of **62a** with the azido-sphingosine **64a** in dichloromethane by using $BF_3{\cdot}OEt_2$ as promoter.[57] β-Galactosyl-azido-sphingosines **66** were prepared with the final objective of obtaining sulfated galactosyl-ceramides. Thus, **66a** was synthesized in 55% yield by reaction of



Scheme 15.

63a (the 6-OH was protected with CH_2COOMe to anchor the fluorescent dansyl group) with **64a** using TESOTf as promoter.[58] However, when a similar reaction was carried out from the pivaloyl derivative **63b** using $BF_3{\cdot}OEt_2$ as promoter, **66b** was obtained in an excellent 88% yield.[59] After deprotection of the sugar moiety and reduction of the azido group, the saturated fatty acid chains were introduced and the resulting compound was selectively sulfated[35] at O3 of the sugar moiety to afford **69a–c** (Fig. 2).

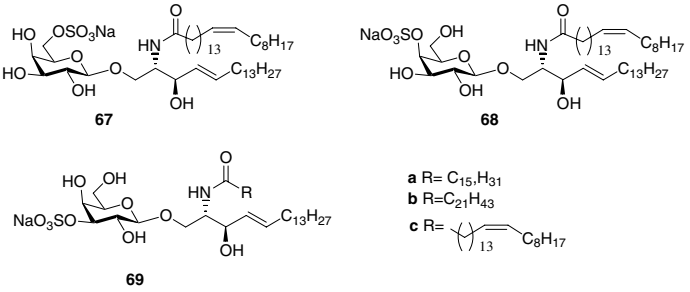
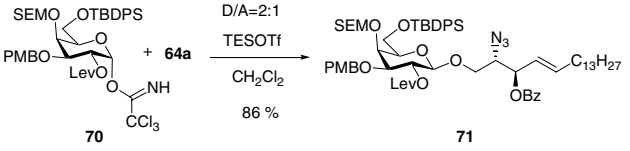


Figure 2.

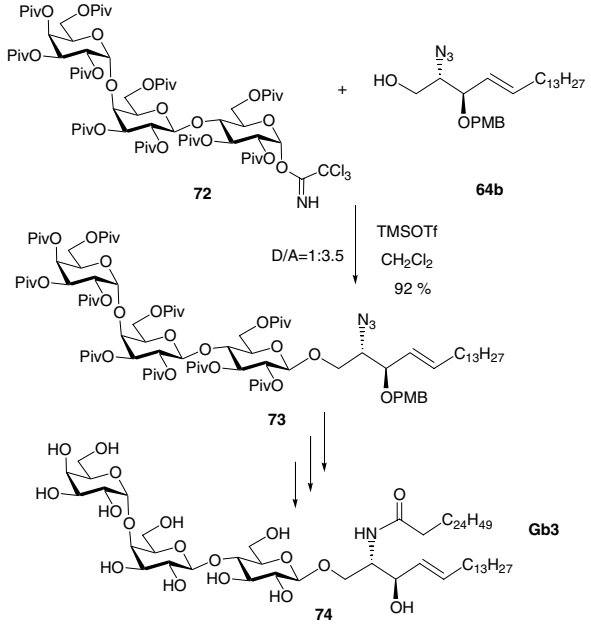


Scheme 16.

To assess the role of the position of the sulfate in CD1a-mediated T cell activation, the synthesis of three β-D-galactosyl-ceramides, bearing a sulfate ester at C2, C4 or C6 of galactose was carried out. Trichloroacetimidate **70**, with all the hydroxyl groups differentially protected, was reacted with **64a** using TESOTf as the activator to give the β-galactosyl-azido-sphingosine **71** in excellent yield (86%) (Scheme 16).[60] Compound **71** was further converted into ceramides **67**, **68** and **69c** by selective deprotection and sulfation.

The trisaccharides isoglobotrihexose, and globotrihexose, which are very difficult to obtain either from natural sources or by chemical synthesis, were synthesized by using enzymatic procedures. The suitably protected free trisaccharide was transformed into the corresponding trichloroacetimidate **72** and coupled with azido-sphingosine **64b** in the presence of TMSOTf to give the protected ceramides **73** in excellent yield (92%) and selectivity (Scheme 17).[61] When the trisaccharide was protected with benzoyl or acetyl groups, the yield decreased to 75% and 48%, respectively.[62] Removal of protecting groups under typical reaction conditions provided iGb3 and Gb3 (**74**).

Gangliosides are ubiquitously located in vertebrate cells and are particularly abundant in the nervous system. The synthesis of the glycosyl donor **75** started from glucosamine, which was further transformed into a galactosamine derivative by inverting the configuration at C4. Reaction of **64a** with the glycosyl donor **75** activated by $BF_3{\cdot}OEt_2$ afforded the β-linked glycosyl sphingosine derivative **76** in 36% yield (Scheme 18).[63]

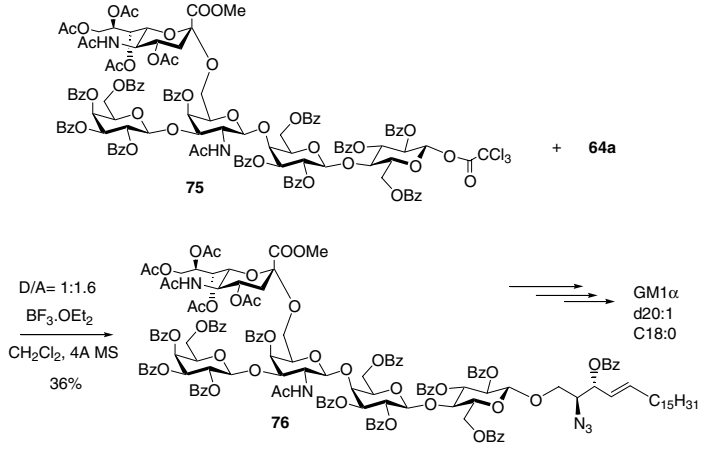


Scheme 17.

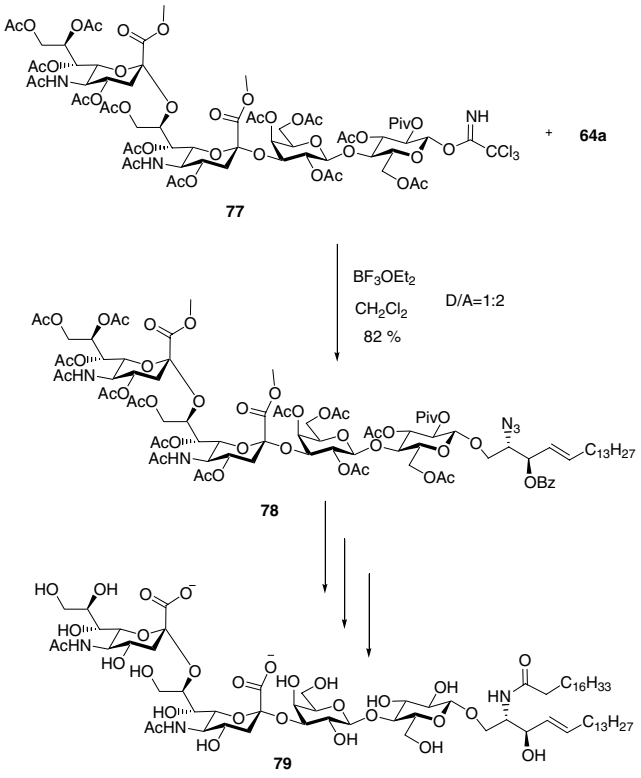
Compound **76** was then converted into GM1a by azide reduction, coupling with octadecanoic acid, methanolysis and saponification of the methyl ester.

In the synthesis of ganglioside GF3, the coupling between the oligosaccharide and azido-sphingosine **64a** was also carried out by activating trichloroacetimidate **77**, which is protected at O3 and O6 with acetate groups and at O2 with a pivaloyl group, with $BF_3{\cdot}OEt_2$. In this case, the yield of glycosyl-sphingosine **78** was 82% and only the isomer β was obtained (Scheme 19).[64] Compound **78** was then transformed into **79** following standard procedures.

The total synthesis of plakosides A and B, **83** and **86**, which are prenylated immunosuppressive marine galactosphingolipids, and their analogues was accomplished through an efficient and convergent strategy (Schemes



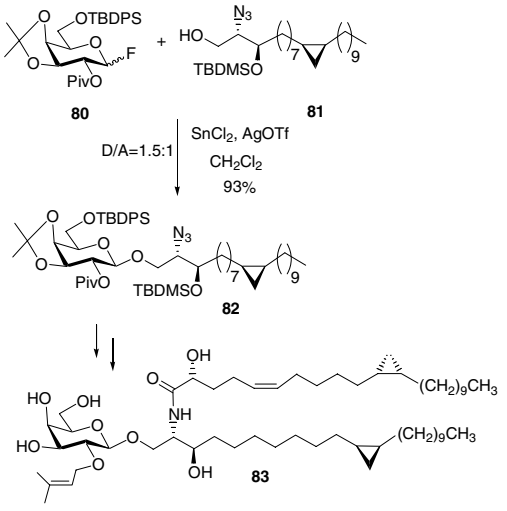
Scheme 18.



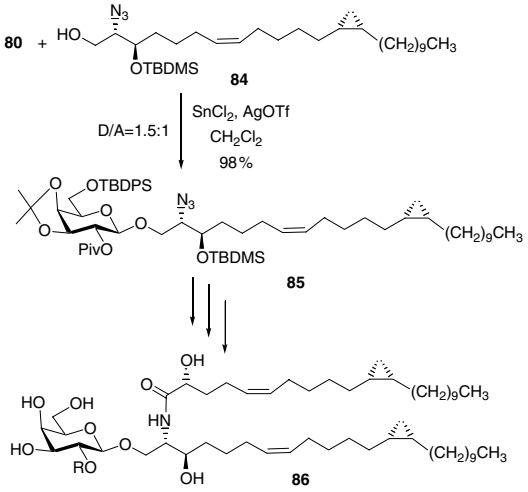
Scheme 19.

20 and 21). In this case, activation of galactosyl fluoride **80** in the presence of $SnCl_2$–$AgClO_4$ was used for the glycosylation of sphinganine analogues **81** and **84**.[65] Yields were practically quantitative, showing also the efficiency of galactosyl fluorides for the synthesis of β-galactosyl-ceramides.

It can be concluded that trichloroacetimidates and fluorides are efficient glycosyl donors for glycosylation of azido-sphingosine, and that better yields are obtained when there is a pivaloyl group at the 2-OH of the donor.



Scheme 20.

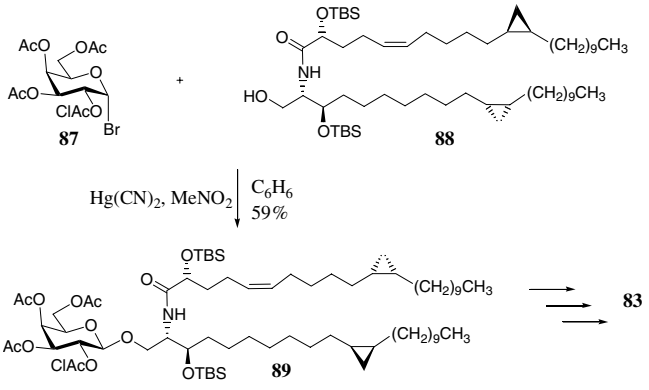


Scheme 21.

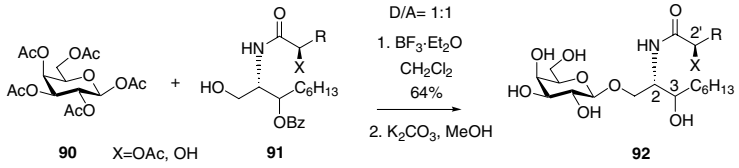
3.2. Glycosylation of ceramides

The total synthesis of plakosides A **83** was also carried out by direct glycosylation of ceramide **88** with the galactosyl bromide **87** in the presence of $Hg(CN)_2$ and nitromethane affording β-galactosyl-ceramide **89** in 59% yield.[66] A chloroacetate ester at O2 in the donor was used to avoid orthoester formation (Scheme 22).

GalCer **92** is a water-soluble analogue of natural GalCer that contains either a hexanoic or a decanoic acyl unit and a saturated nine-carbon sphingosine moiety, which was prepared from Garner's aldehyde.[67] These analogues were used to clearly establish the molecular basis for the selective recognition process between HIV-1 surface glycoprotein and GSL analogues within a GalCer monolayer. The authors provide evidence that this process involves conformations similar to the fundamental conformer of GalCer and demonstrate that the alkyl chains of the ceramide moiety are essential to the gp120 insertion into the monolayer. Glycosylation of ceramide **91** was carried out using 1,2,3,4,5-penta-*O*-acetyl-galactopyranose as donor and $BF_3{\cdot}OEt_2$ as the



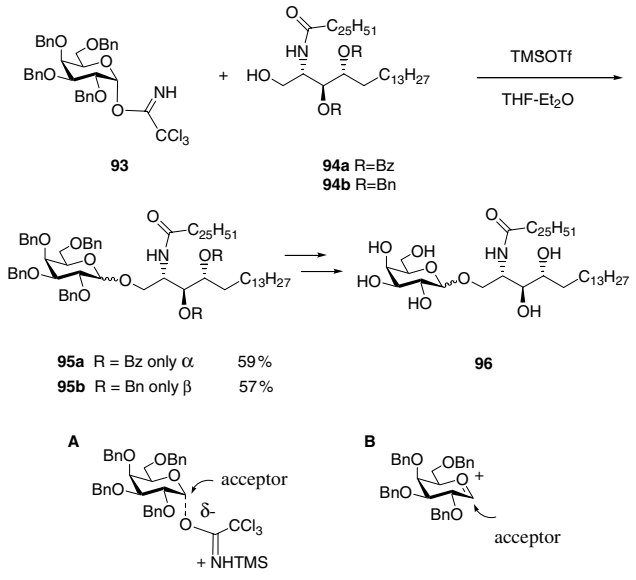
Scheme 22.



Scheme 23.

promoter affording the glycosylated product in 64% yield. Similar yields were obtained in other glycosylation procedures of ceramides (Scheme 23). The final GalCer **92** was obtained after removal of the protecting groups.

In the course of studies directed towards the synthesis of α-GalCer, the reaction of the glycosyl donor **93** with the differentially protected phytosphingosines **94a**,**b** was studied.[68,69] It is well known that benzyl groups are usually selected to protect the 2-OH position of glycosyl donors to give α-glycosides. However, unexpectedly, it was observed that the stereoselectivity of the reaction depended on the protecting group at the ceramide moiety. Thus, α-anomer **95a** was exclusively obtained from ceramide **94a** with a benzoyl protecting group, while the β-anomer **95b** was obtained when ceramide **94b** (R = Bn) was used as starting material (Scheme 24). It was suggested that **94a** reacts in a S_N1 manner (intermediate **B**), while when **94b** is used an S_N2 mechanism is followed (intermediate **A**). An armed–disarmed effect in the ceramide moiety is suggested for explaining this behaviour.

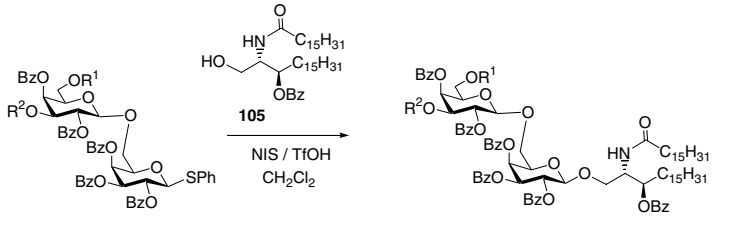


Scheme 24.

Unprotected glycosyl-ceramides **101**–**104** were isolated from the metacestodes of *Echinococcus multicularis*.[70] Coupling between benzoyl protected oligosaccharide thioglycosides **97**–**100** with ceramide **105** promoted by NIS–TfOH afforded the β-glycosides **101**–**104** in 44%, 33%, 54% and 27% yield, respectively. The fully protected glycosides were deprotected to give the four target glycosphingolipids (Scheme 25).

3.3. Enzymatic procedures

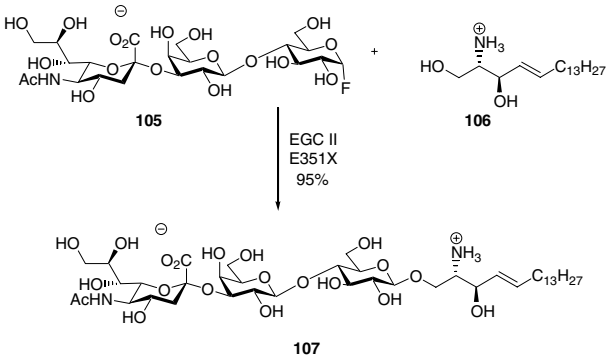
In recent years,[71] biocatalysis has become an established technology for synthetic production of fine chemicals. Enzymes are applied for transformations leading to complex target molecules, mainly because of their high selectivity and mild operational conditions. Novel enzymes[72] with improved properties are now accessible as a result of advances in high throughput screening methods, genomics and rational protein design. Recently, GSL syntheses through enzymatic catalysis by glycosynthases, generated from endoglycoceramidase II (EGC II), have been described.[73] By utilizing specific enzymatic catalysis, GSL **107** was obtained from the



97 R^1=R^2=Bz
98 R^1=*tetra*-Bz-β-Gal, R^2=ClAc
99 R^1=Lev, R^2=*tri*-Bn-α-Fuc
100 R^1=*tetra*-Bz-β-Gal, R^2=*tri*-Bn-α-Fuc

101 R^1=R^2=Bz (44%)
102 R^1=*tetra*-Bz-β-Gal, R^2=ClAc (33%)
103 R^1=Lev, R^2=*tri*-Bz-α-Fuc (54%)
104 R^1=*tetra*-Bz-β-Gal, R^2=*tri*-Bn-α-Fuc (27%)

Scheme 25.



Scheme 26.

fluoride **105** and the salt **106** in a highly regio- and stereoselective transformation (Scheme 26).

The ability of glycosidases[74] to use unactivated sugars and their broad specificity for aglycones, have limited synthetic applications. This is because the native enzymes perform poorly in organic media but the thermodynamic considerations require that water content to be minimized for maximal yields. In addition it is often difficult to satisfy the divergent solvent requirements of hydrophilic sugar donors and hydrophobic acceptors. However, when the reaction is carried out in plasticized glasses, high concentrations of both acceptor and sugar donor enable obtainment of glycosides **108** and **109** in good yields and high selectivity (Fig. 3).[75]

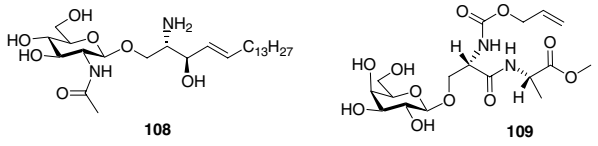


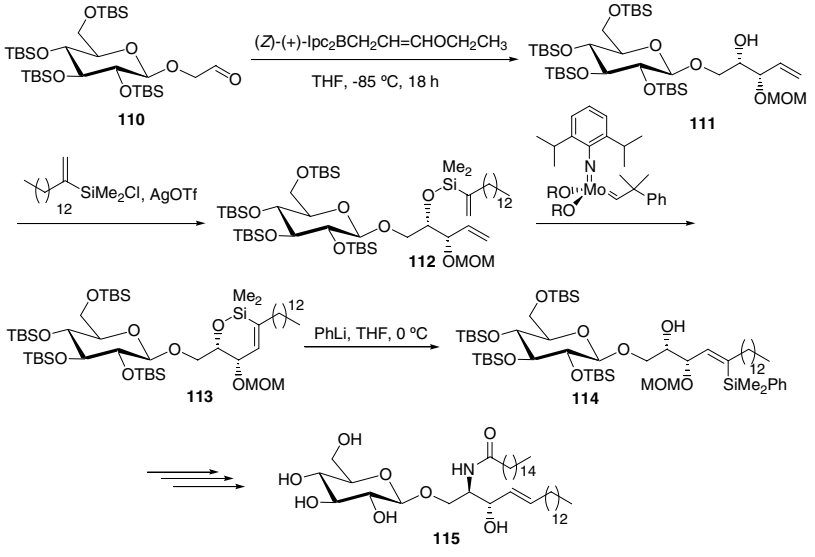
Figure 3.

3.4. Miscellaneous methods

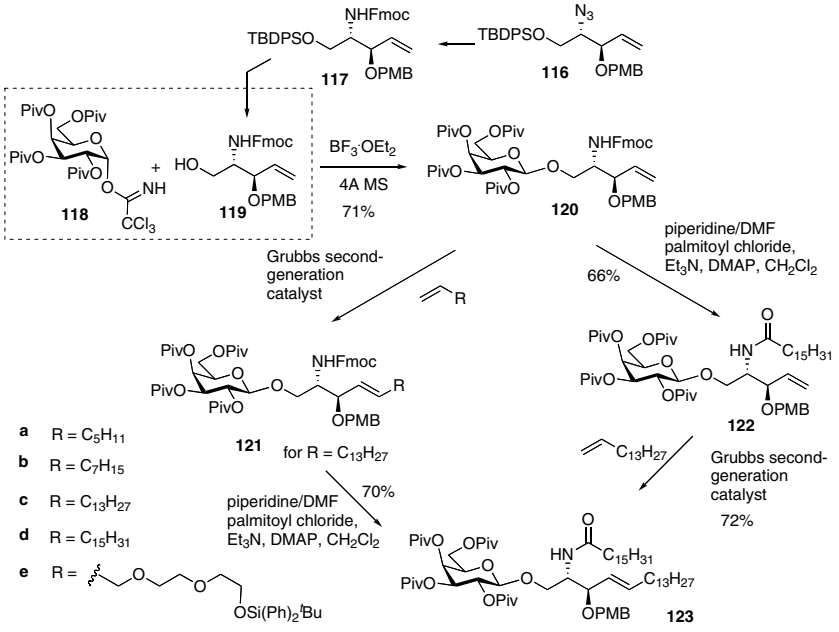
In the previous sections it has been shown that the different glycosylation procedures of azido-sphingosine and ceramides afford α- or β-glycosides. In this section alternative synthesis of glycosyl-ceramides, and some recent contributions in the synthesis of complex glycolipids will be discussed.

An alternative approach for the synthesis of glycosyl-ceramides consists of performing the glycosylation of small molecules, which are afterwards transformed into the corresponding glycosyl sphingosines and ceramides. Thus, starting from the previously prepared **110**, the construction of the adjacent stereocentres in the sphingolipid moiety was carried out by reagent-controlled asymmetric Brown allylboration to give MOM protected *syn*-diol **111** (Scheme 27).[76] The trans-configured double bond was obtained as a single geometric isomer by the use of a silicon-tethered ring closing olefin metathesis employing the Schrock carbene catalyst. This process allowed the transformation of **112** into **113**, and in situ PhLi-induced ring-opening of the intermediate siloxacycle gave **114**. This compound was subsequently transformed into the corresponding *anti*-azido alcohol by Mitsunobu reaction using diphenylphosphoryl azide followed by protodesilylation of the alkenyl-(dimethyl)phenyl silane and silyl deprotection with TBAF. Further group elaboration afforded ceramide **115**.

In a related procedure, starting from the building block **117**, a large diversity of GSLs can be accessed by sequentially introducing different sugars, acyl groups and main chains (Scheme 28). Additionally, the terminal alkene in **117** provides a useful chemical handle for alternative functionalization chemistries and bioconju-



Scheme 27.



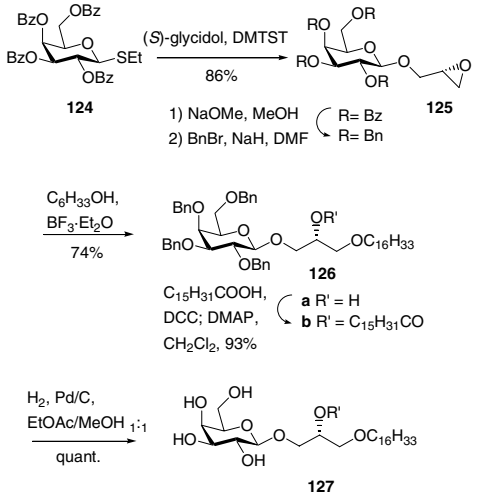
Scheme 28.

gation.[77] Because the olefin cross-metathesis reactions of the azide **116** suffered from poor yields and undesirable side reactions, the azide was reduced and the resulting amine was protected as the Fmoc carbamate. Alcohol **119** was efficiently glycosylated with the 2,3,4,6-tetra-*O*-pivaloyl trichloroacetimidate **118** using $BF_3{\cdot}Et_2O$ as the promoter to provide the desired β-linked glycoside **120** in 71% yield. It is worth noting that the glycosylation yield and anomeric selectivity obtained with the *N*-Fmoc-protected acceptor **116** is similar to that obtained with azido-sphingosines.

The *trans*-alkene **123** was formed directly from ceramide **122** with very high selectivity by cross-metathesis using Grubbs catalysts. Alternatively, it could be accessed from Fmoc-protected galactosyl-sphingosine **120**, followed by deprotection and amidation. While olefins **a**–**d** underwent facile cross-metathesis to afford the protected glycolipids in good yield, the presence of adjacent heteroatoms was problematic. The ethylene glycol derivative **120e** failed to provide any cross-coupled product.

Galactosyl-glycerolipids have been found in the HT29 human colon carcinoma cell line. The synthesis of galactosyl-glycerolipid **127** was carried out from (*S*)-glycidol, which was glycosylated with readily available galactosyl donor **124** and DMTST to produce galactosyl glycidol **125** in excellent yields (Scheme 29).[78] The epoxide was stable in these conditions. Lewis acid catalyzed opening of the epoxide with an alcohol afforded **126**. Subsequent palmitoylation followed by deprotection yielded the galactosyl and digalactosyl glycerolipids **127**.

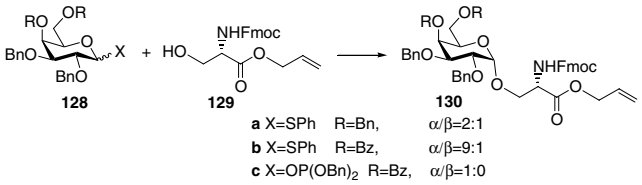
The tetrabenzyl thiogalactoside **128a** reacted with the serine derivative **129** in the presence of NIS–TfOH to



Scheme 29.

give a mixture of anomers **130a** ($\alpha/\beta = 2{:}1$) (Scheme 30).[33] The α/β selectivity was increased to 9:1 when the thiogalactoside **128b**, with benzoate protecting groups at O4 and O6, was used. Incorporation of the benzoyl group at the O4 and O6 positions of galactoside (or glucoside) donors, in comparison with benzyl groups, is known to enhance α-selectivity.[79] Interestingly, activation of the phosphite donor **128c** in the presence of TfOH gave only the α-glycosylation product in 95% yield.

An efficient route for synthesising sLe^x neoglycolipids **135a**–**c** has been developed.[80] Glycosylation of dialkylglycerols **131a**–**c** furnished the target molecules **135a**–**c** with one to three lactose units as spacers after deprotection (Scheme 31). The synthetic strategy was also ap-

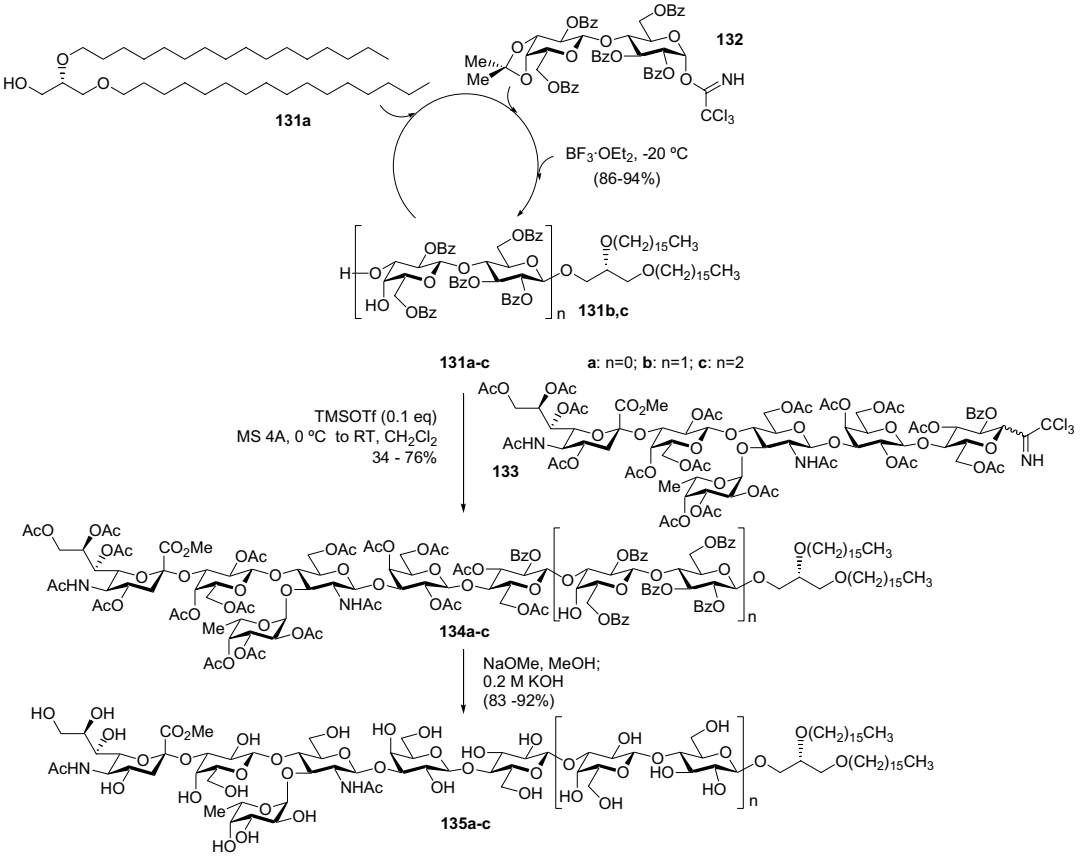


Scheme 30.

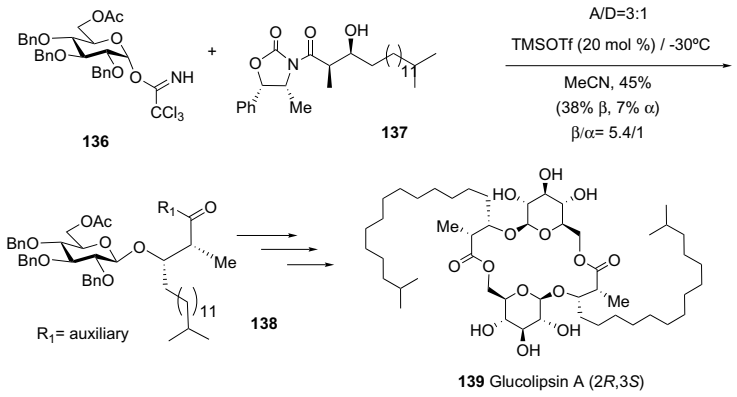
plied to the synthesis of the corresponding Lewis X (Le^x) derivatives. This paper studied the influence of the spacer structure and spacer length in regard to the mobility of the sLe^x epitope. The results obtained underline the dominating influence of the spacer in the presentation of the carbohydrate epitopes and thus on molecular recognition phenomena.

Glucolipsin A (**139**) is a complex macrocyclic glycoconjugate in which two glucosyl-lipid units are linked through ester groups formed between a carboxylic group of the lipidic chain and the 6-OH of the sugar ring (Scheme 32). Glycoconjugates of this type effectively inhibit the activity of the dual specific phosphatase Cdc25A with IC_{50} values in the micromolar range. The synthesis of the monomeric unit **138** was carried out by reaction of the donor trichloroacetimidate **136** with alcohol **137** always in moderate yields.[81] Glycosylation of the secondary alcohol in **137** proved to be difficult. Many promoters including TMSOTf, TBSOTf, TfOH, $BF_3{\cdot}Et_2O$, $SnCl_4$ and Cl_3CCHO[82] were tested. Although TBSOTf had previously been recommended as a superior promoter for glycosidation reactions of sensitive substrates, no improvement was observed in this case upon replacing TMSOTf by TBSOTf.[43c,83] The use of other donors, including the phenyl thioglycoside, the anomeric sulfoxide or the glycosyl fluoride under Mukaiyama conditions ($SnCl_2$, $AgClO_4$, MeCN) essentially met with failure.

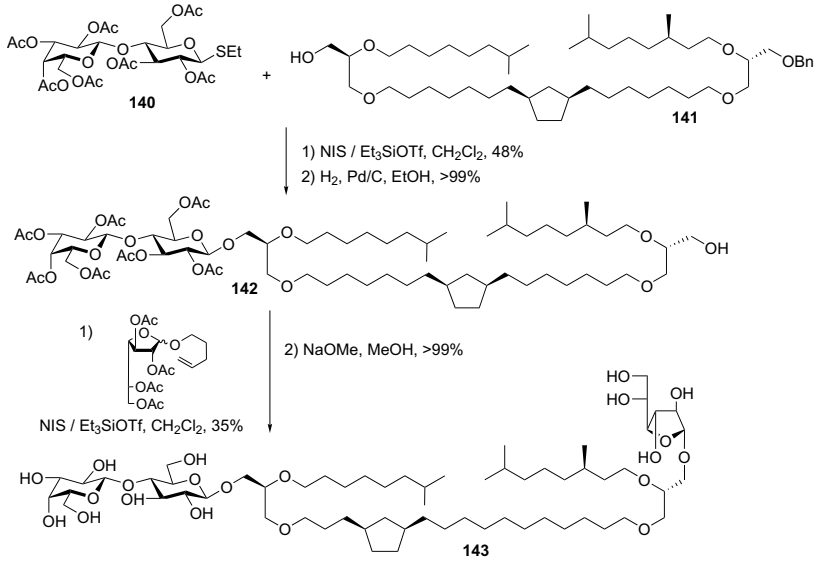
The unsymmetrical archaeal tetra ether glycolipid analogues **143** incorporate lactosyl and galactofuranosyl units as polar headgroups and an acyclic lipophilic backbone characterized by the presence of a bridging chain attached to two glycerols and containing a cis 1,3-disubstituted cyclopentane ring into the bridging chain.[84] The synthesis of this glycoside involved the construction of lipophilic monoprotected diols followed by the sequential introduction of the saccharidic moieties. The introduction of the lactosyl unit was performed from the corresponding lactosyl thioglycoside **144** by reaction with alcohol **145** and NIS–TESOTf (Scheme 33). Subsequent installation of the β-D-galactofuranosyl moiety in **142** was carried out using the pentenyl galactofuranoside as a glycosyl donor and NIS–TESOTf. The



Scheme 31.



Scheme 32.



Scheme 33.

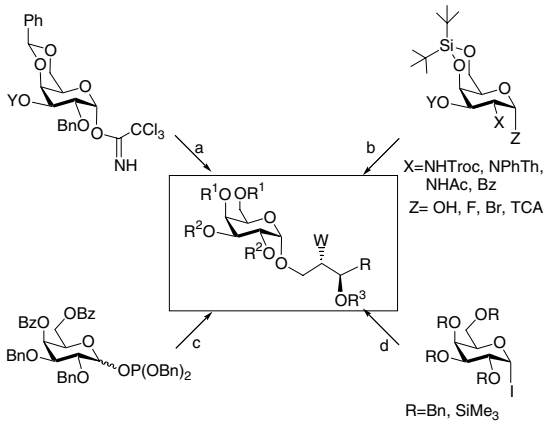
resulting product was treated afterwards with sodium methoxide to give product **143**. Both glycosylation procedures afforded moderate yields.

4. Conclusions

From the previous data, which is centred on the use of galactose derivatives as glycosyl donors, some conclusions can be drawn. First, glycosylation of azido-sphingosines provides better yields than glycosylation of ceramides, confirming an observation made by Schmidt several years ago. Yields higher than 80–90%, even quantitative yields, have been reported in the glycosylation of azido-sphingosines, while glycosylation of ceramides did not exceed 70% yield, and in many cases was below 50%.

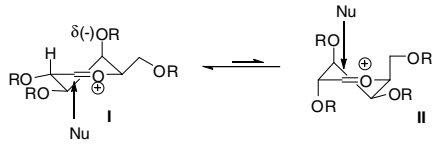
Second, with regard to the leaving groups present in the glycosyl donors many have been used with relative success, although glycosyl trichloroacetimidates and glycosyl fluorides have been more successfully and widely used. Recently, iodide has emerged as a promising leaving group in these glycosylation reactions, because the specific activation procedure and compatibility with different protecting groups, and because it provides high yields and selectivities in the synthesis of α-azido-glycosphingolipids (Scheme 34d).

Third, the stereoselectivity is influenced by the protecting groups present in the glycosyl donor. In general, obtaining the α or β glycoside depends on the presence of a non-participating or participating groups, respectively, on O2 of the glycosyl donor. However, this is not enough to obtain good yields and stereoselectivity, particularly in the synthesis of α-glycosides. Protection of hydroxyl groups at C4 and C6 of the galactosyl donor as benzoates, or more preferably as a benzylidene acetal, allows the formation of the α-anomer almost exclusively (Scheme 34a and c). Remarkably, protection with a



Scheme 34.

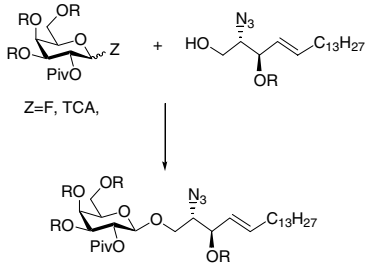
bis-*tert*-butylsilylene group allows production of the α-anomer independently of the protecting group on O2 (Scheme 34b). Oxocarbenium ions are postulated to be the common intermediates in most of these glycosylation procedures, and to rationalize the strong effects of substituents in the stereoselectivity, the recent contributions by Woerpel and co-workers[85] should be taken into account. According to this study, stereoselectivity in most glycosylations may be explained either by the conformational preference of the oxocarbenium ion and the reactivity of each conformer. The conformational preference in the galactopyrnosyl oxocarbenium ion is biased towards conformer **I** due to (a) the C–H bond at C-2 displaying an axial disposition, allowing hyperconjugative interactions between σ_{C-H} and π^*_{C-O}; and (b) if no other effects are operating (such as 1,3-diaxial interactions), the C4 hydroxyl group tends to occupy an axial position to maximize electrostatic attractive interactions between the partially negatively charged hydroxyl group and the positively charged carbon atom. The fact that using a benzoate as a protective group at C4 increases α-selectivity is in agreement with this previous observation, as the more electron withdrawing the C-4 substituent is, the more electrostatic stabilization results.



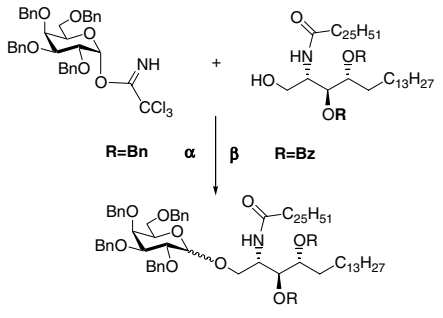
However, as stated before the stereoselectivity is also a product of the reactivity of each conformer towards nucleophilic attack. In this regard, conformer **I** is also the more reactive one as axial nucleophilic approach does not develop destabilizing interactions, whereas the reactivity of conformer **II** is seriously attenuated by negative steric interactions between the incoming nucleophile and the pseudo-axial substituent at C3. Furthermore, when either 4,6-*O*-benzylidene acetals or *bis-tert*-butylsilylene groups are present, glycosylation from conformer **II** would involve a nucleophilic approach from the *endo* face. Thus, both conformational and transition-state effects explain the excellent α-selectivity obtained in these galactosyl donors. A different case is for the glycosyl iodides, in which the stereoselectivy is the result of the faster reaction of β-anomer than the α-anomer, and the α selectivity observed depends on a S_N2 process from the β-iodide.

As reported before, the β-glycosphingolipids are easily obtained in all cases, but the yield of the process is very dependent on the protecting groups; the pivaloyl group affords the best results. This group must be used at least to protect the C2 hydroxyl group. Using glycosyl donors with trichloroacetimidate or fluoride as leaving groups, and azido-sphingosine as acceptor frequently affords yields higher than 90% with complete β-selectivity (Scheme 35).

Finally, a recent and relevant observation is the fact that α- or β-GSL's can be selectively obtained from a given glycosyl donor just by changing the protecting groups of hydroxyl of the ceramide moiety (Scheme 36). This has been rationalized suggesting a different reaction mechanism, S_N2 when R = *O*-benzyl, and S_N1 when R = *O*-benzoyl, and this has been related to an armed–disarmed effect in the acceptor.



Scheme 35.



Scheme 36.

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