Stannyl ceramides as efficient acceptors for synthesising β-galactosyl ceramides†

José Antonio Morales-Serna, Yolanda Díaz, M. Isabel Matheu and Sergio Castillón*

Received 5th June 2008, Accepted 15th July 2008
First published as an Advance Article on the web 13th August 2008
DOI: 10.1039/b809570a

β-Galactosyl ceramides have been obtained in excellent yields and stereoselectivities by reacting disarmed glycosyl donors with stannyl ethers. The broad compatibility of stannyl ethers with various leaving group–promoter pairs is demonstrated.

Introduction

Glycosphingolipids (GSLs) are used to bind cells by a large variety of pathogens such as virus, bacteria, fungus and parasites.[1] The oligosaccharide moieties present in GSLs are the adhesion point for these pathogens. Thus, it has been shown that several GSLs binds the protein gp120 of HIV virus.[1d]

The V3 loop of HIV-1 gp120 is known to interact with several GSLs (GalCer **1**, GM_3 **2**, iGB_3 **3**; see Fig. 1) and proteins (CD4, CCR5, CXCR4, GPR15/Bob) expressed by various cell types. In T lymphocytes, the V3 loop binds to Gb3 and GM3 with a low/moderate affinity. Accordingly, these GSLs should not be considered as true gp120 receptors but rather as auxiliary, albeit indispensable, fusion cofactors.[2] In contrast, the V3 loop interacts with GalCer with a high affinity, so that this major intestinal GSL has long been recognized as a real receptor for HIV-1.[3]

This and other findings have stimulated the research in the synthesis of glycosphingolipids,[1a,b,4] ceramides[4a,b] and sphingosines[4a,5] as an alternative to the natural sources.[6] The current retrosynthetic analysis reveal three important steps in the syntheses of β-GalCer **4**: (*i*) glycosylation of the sphingosine moiety, (*ii*) *N*-acylation with the fatty acid and finally (*iii*) elimination of the protecting groups (Fig. 2).

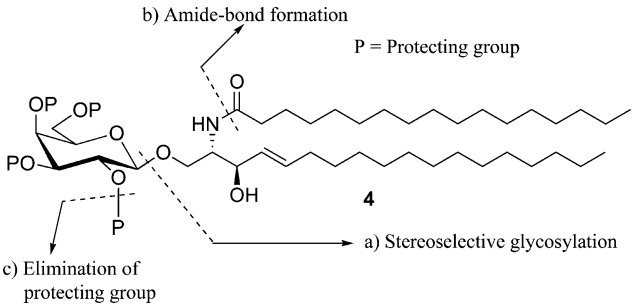


Fig. 2 Retrosynthetic analysis of β-galactosyl ceramide.

One of the main synthetic problems in the synthesis of glycosphingolipids is the glycosylation reaction,[7] and sphingosines are commonly used for glycosylation because low yields were obtained when ceramides like **5** are directly used in the reaction. That has been attributed to the low nucleophilicity of ceramides,[1a,8] which are extremely ordered as a result of the headgroup hydrogen bonding and the formation of micelles. This driving force for molecular self-assembly in ceramides allows them to have hexagonal and orthorhombic phases, structural organizations that are similar in the crystalline and hydrated states, and high stability.[9] The problem of glycosylating ceramides is usually circumvented by using azidosphingosine **6**. However, further reduction of the azido group and acylation is therefore required (Fig. 3).[10] In the glycosylation of azidosphingosine, glycosyl fluorides and trichloroacetimidates

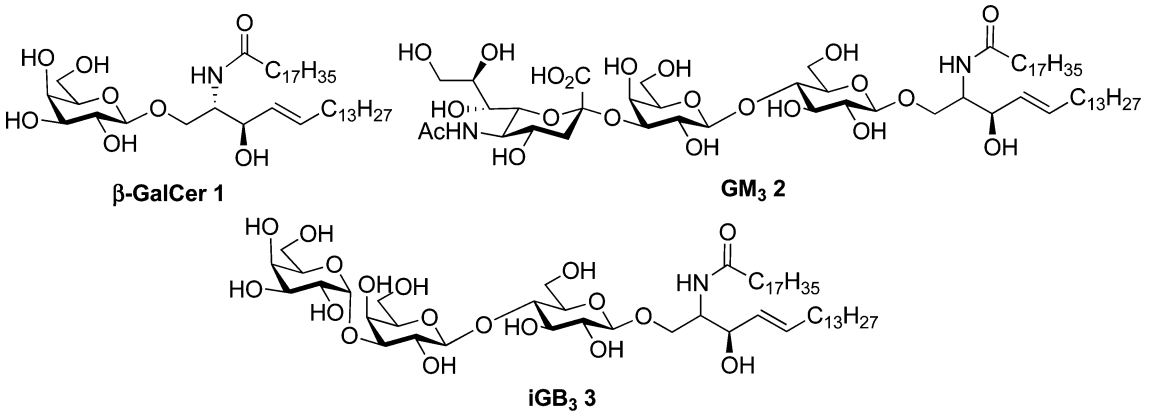


Fig. 1 Structures of β-GalCer, GM_3 and iGB_3.

Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, C/Marceli.li Domingo s/n, 43007, Tarragona, Spain. E-mail: sergio.castillon@urv.cat; Fax: +34 977 558446; Tel: + 34 977 559556

† Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR spectra for the compounds **10**, **16**, **17** and **18**. See DOI: 10.1039/b809570a

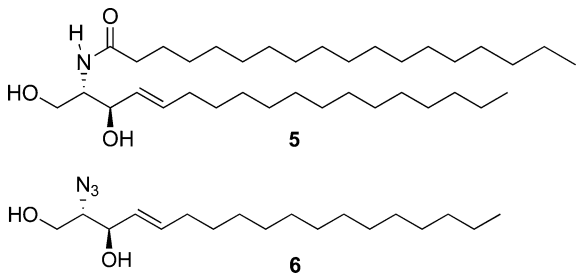


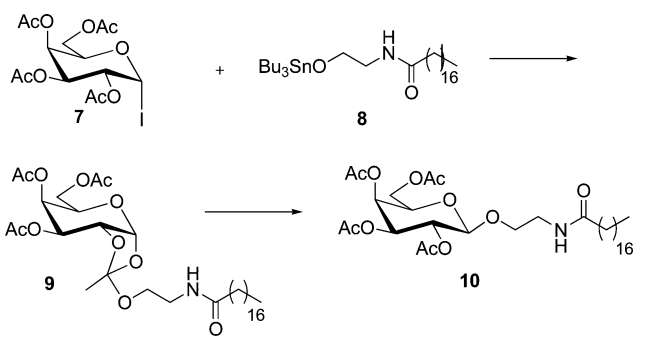
Fig. 3 Ceramide **5** and azidosphingosine **6**.

are the glycosyl donors that afforded the best yields.[7,11] α-Iodo-glycosides are employed as glycosyl donors in both armed[12,13] and disarmed[14] series, and the utility of α-iodo-glycosides in the synthesis of α-galactosyl-azidosphingolipids,[15] α-GalCer[16] and KRN7000[17] was recently demonstrated. The reaction proceeds by *in situ* anomerization of the α-iodo- to the more reactive β-iodo-derivative, from which the α-glycoside is obtained in excellent yield.

Stannyl ethers have been used in the synthesis of β-glycosides from 1,2-anhydro-glucose (1,2-oxiranes)[18] and β-glycosides obtained from seleno glycosides.[19] With this background and with the aim of preparing β-glycosphingolipids, we proposed a strategy involving coupling of α-galactosyl iodide and a stannyl ether.[20] The latter increases the nucleophilicity of the oxygen without significant changes in the basicity. In this work, we show that stannyl ethers can be used with different leaving group–promoter pairs, affording in all the cases excellent yields and stereoselectivities.

Results and discucion

Initially, we explored the reaction of tetra-*O*-acetyl-α-iodogalactose **7** with the amide **8**, which was expected to be a simple ceramide model (Table 1, Scheme 1). The reaction is essentially driven under neutral conditions and affords first the orthoester **9**, which is further isomerized to the β-anomer in high yields. The reaction was carried out in dichloromethane or toluene at different temperatures and in the presence or absence of TBAI. When the reaction was carried out at room temperature in dichloromethane, the orthoester **9** was obtained, and further treatment[21,22] with $BF_3{\cdot}OEt_2$ afforded **10** in 62% yield for the two steps (Table 1, entry 1). The yield increased to 88% (entry 2) when the reaction was heated to reflux. However, 93% yield was obtained performing the reaction in toluene at 80°C (entry 3). In this case the presence of TBAI was also necessary for the reaction to proceed



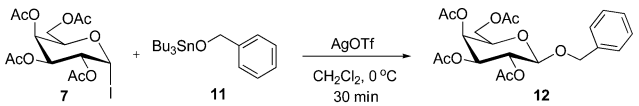
Scheme 1

Table 1 Synthesis of compound **10** by glycosylation or stannyl amide **8** with iodide **7** in presence of TBAI followed by isomerization[a]

Entry	Solvent	Temp/°C	Yield (%)[b]	Ratio α:β
1	CH_2Cl_2	rt	62	0 : 1
2	CH_2Cl_2	reflux	88	0 : 1
3	Toluene	80	93	0 : 1
4[c]	Toluene	80	—	—

[a] *Reagents and conditions*: **7** (1.2 mmol), **8** (1 mmol), Bu_4NI (0.10 mmol), 18 h and then $BF_3{\cdot}Et_2O$ (3 mmol), CH_2Cl_2 (20 mL), rt, 3 h. [b] Yields of isolated product (for the two steps) after chromatographic purification. [c] In the absence of TBAI.

Table 2 Glycosylation of **11** with the iodide **7** in presence of AgOTf to give **12**[a]



Entry	Equiv. AgOTf	Time/h	Yield (%)[b]	Ratio α:β
1	1	0.5	63	0 : 1
2	1	2	63	0 : 1
3	2	0.5	68	0 : 1
4	3	0.5	99	0 : 1
5	4	0.5	99	0 : 1

[a] *Reagents and conditions*: **7** (1.2 mmol), **11** (1 mmol), CH_2Cl_2 (15 mL), 0 °C, 30 min. [b] Yields of isolated product alter chromatographic purification.

(entry 4). These results demonstrated the efficiency of stannyl ethers in the glycosylation of ceramides with α-iodogalactose in presence of TBAI as promoter.[20]

At this point, we considered the possibility of using other promoters. Therefore, a screening of alternative promoters for the glycosylation reaction was conducted, focusing on the replacement of TBAI by AgOTf. Thus, when the α-iodogalactose **7** was reacted with benzyl tributylstannyl ether **11** at 0 °C in the presence of 1 equivalent of AgOTf, the β-glycoside **12** was obtained in 63% yield after 0.5 hours (Table 2, entry 1).

Longer reaction times did not affect the yield (entry 2). The stoichiometry of AgOTf has a strong influence on the yield, and when 3 or 4 equivalents were used, **12** was obtained in quantitative yield in a short time (entries 3–5). In the ^1H NMR spectrum of the crude reaction the β-anomer was the only product detected, and the orthoester or elimination products were not observed. When the reaction was carried out with benzyl alcohol as glycosyl acceptor under the optimized conditions, only 42% yield was obtained. These results indicated that the presence of stannyl ether is required to facilitate the reaction.

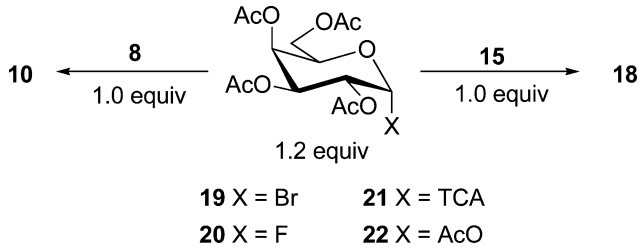
Then, we tried to glycosylate the stannyl ether **8** (Scheme 1), using AgOTf as promotor. When the galactosyl iodide **7** was reacted with **8** in the presence of only AgOTf, a mixture of the orthoester **9** and the corresponding β-*O*-glycoside **10** (ratio 1 : 2) was obtained. The ^1H NMR spectrum of **9** showed characteristic signals at δ 5.79 (1H, d, J= 3.6 Hz, H1) and 1.6 (3H, s, methyl group). The mixture of **9** and **10** was treated with $BF_3{\cdot}OEt_2$ to convert **9** into **10**, giving the β-*O*-glycoside in 90% overall yield. Adding $SnCl_2$ did not seem to affect the ratio between **9** and **10**, and the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) exclusively afforded the orthoester as a consequence

of the decrease of the acidity. It should be noted that pivaloyl-protected glycosyl donors do not totally prevent the formation of the orthoester.[23]

To test the scope of these glycosylation protocols in the synthesis of biologically relevant glycolipids, glycosylation of azidosphingosine, ceramide and analogues was tested (Table 3). When the stannyl ether **13**, a ceramide analogue, was reacted with **7** following the general protocol of glycosylation employing TBAI as promoter, the orthoester was exclusively obtained, and treatment with $BF_3{\cdot}OEt_2$ afforded the glycolipid **16** in 93% yield. Similar yields were obtained when AgOTf was used as promoter (Table 3, entry 1).

The classical protocol for the selective glycosylation of diols requires a series of programmed protection–deprotection steps to ensure that only one of the hydroxyls reacts. Azido-sphingosine has two hydroxyl functions in positions 1,3 that can be simultanously protected as stannyl acetal. When stannyl acetal **14**, obtained from azidosphingosine by reaction with Bu_2SnO with water exclusion, was reacted with **7** under standard conditions, the β-*O*-glycoside **17** was obtained in 94% yield after orthoester isomerization. In this case, the use of AgOTf as promoter afforded similar yields (entry 2). The reaction was fully chemo- and stereoselective, involving exclusively the primary hydroxyl group. Similarly, the stannyl acetal **15**, obtained by reaction of ceramide and Bu_2SnO, was also reacted with the glycosyl iodide **7** in the presence of TBAI or AgOTf, and the corresponding reaction products were treated with $BF_3{\cdot}OEt_2$ to obtain the galactosyl ceramide **18** in 90% yield. The reaction was again fully chemo- and stereoselective and the yields obtained are close to those obtained by enzymatic procedures.[24] Pleasingly, the yields obtained with AgOTf were similar to those afforded when TBAI was the promoter.

We also investigated the reactivity of stannyl ethers with various leaving groups such as bromide, fluoride, trichloroacetimidate and acetate under the appropriate reaction conditions. This study focused on the use of stannyl ethers **8** and **15**, which were reacted with glycosyl donors **19–22** (Scheme 2). It was expected that the bromo derivative **19** would behave similarly to iodo derivative

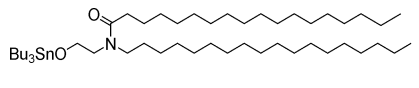
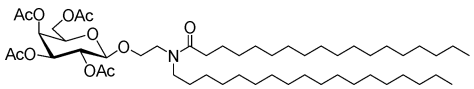
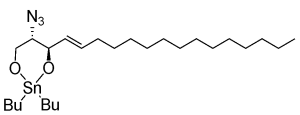
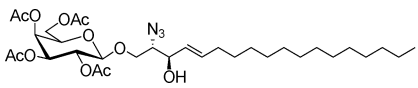
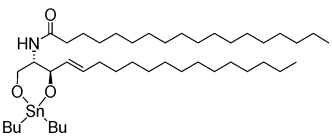
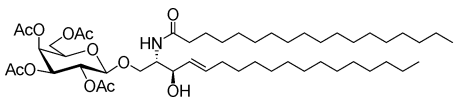


Scheme 2 Examination of various leaving groups in the glycosylation of ceramides.

7 when AgOTf is used as promoter. Effectively, when **19** was reacted with the stannyl ether **8**, a mixture of galactoside **10** and the orthoester **9** was obtained. Futher isomerization by treatment with $BF_3{\cdot}OEt_2$ exclusively afforded the β-galactoside **10** in similar yield to the obtained from α-iodogalactose (Table 4, entry 1). Using the glycosyl fluoride **20** as donor, the stannyl ethers **8** and **15** as acceptors and the couple $SnCl_2$/AgOTf as promoter, β-galactosides **10** and **18** together with the respective orthoesters, were also obtained. In the presence of $BF_3{\cdot}OEt_2$, these mixtures evolved to compounds **10** and **18**, which were obtained in high yields and exclusively as β-anomers (entries 3 and 4). When the trichloroacemidate **21** was used as donor, the glycosylation of **8** and **15** in the presence of $BF_3{\cdot}OEt_2$ afforded the orthoesters as major products, despite the presence of substoichiometric amounts of $BF_3{\cdot}OEt_2$. The orthoesters were, however, then isomerized in good yields to give the β-anomers by adding additional amounts of $BF_3{\cdot}OEt_2$ (entries 5 and 6).

The reaction of penta-*O*-acetyl-β-D-galactopyranose (**22**), a readily accessible glycosyl donor, was also probed with the stannylated acceptors **8** and **15** in the presence of a large excess of $BF_3{\cdot}OEt_2$, which directly afforded the β-glycoside **10** (76%) and **18** (70%). In this case, the *O*-glycosides were obtained in moderate yields since the acetate groups were hydrolyzed to give the partially deprotected compound (entries 7 and 8). These results reveal that stannyl ethers enhanced the nucleophilicity of hydroxyl groups,

Table 3 Glycosylation of **13–15** using **7** as glycosyl donor and TBAI or AgOTf as promoters to afford the orthoester followed by isomerization to give the glycolipids **16–18**

Entry	Stannylated glycosyl acceptor		Glycolipid		Promoter	Yield (%)[c]
1		**13**		**16**	TBAI[a] AgOTf[b]	93 91
2		**14**		**17**	TBAI[a] AgOTf[b]	94 93
3		**15**		**18**	TBAI[a] AgOTf[b]	90 90

[a] *Reagents and conditions*: **7** (1.2 mmol), **13**, **14** and **15** (1 mmol), Bu_4NI (0.10 mmol), 18 h, and then $BF_3{\cdot}Et_2O$ (3 mmol), CH_2Cl_2 (20 mL), rt, 3 h. [b] *Reagents and conditions*: **7** (1.2 mmol), **13**, **14** or **15** (1mmol), AgOTf (3.0 mmol), DTBMP (1.2 mmol), 4 Å MS, CH_2Cl_2 (20 mL), 0 °C, 3 h, and then $BF_3{\cdot}Et_2O$ (3 mmol), CH_2Cl_2 (15 mL), rt, 3 h. [c] Yields of isolated product (for the two steps, glycosylation and isomerization) after chromatographic purification.

Table 4 Galactosylation of stannylceramides **8** and **15** with different donor–promoter pairs[a]

Entry	Acceptor	Donor (X)	Promoter	Time/h	Product	Yield (%)[b]
1	**8**	**19** (Br)	AgOTf (3 mmol)	5	**10**	88
2	**15**	**19** (Br)	AgOTf (3 mmol)	5	**18**	85
3	**8**	**20** (F)	$SnCl_2$/AgOTf (3 mmol)	3	**10**	91
4	**15**	**20** (F)	$SnCl_2$/AgOTf (3 mmol)	3	**18**	90
5	**8**	**21** (TCA)	$BF_3{\cdot}OEt_2$ (0.5 mmol)	3	**10**	85
6	**15**	**21** (TCA)	$BF_3{\cdot}OEt_2$ (0.5 mmol)	3	**18**	82
7	**8**	**22** (OAc)	$BF_3{\cdot}OEt_2$ (6 mmol)	4	**10**	73
8	**15**	**22** (OAc)	$BF_3{\cdot}OEt_2$ (6 mmol)	4	**18**	70

[a] *Reagents and conditions for glycosylation*: see ref. 25 (entries 1 and 2); ref. 26 (entries 3 and 4); ref. 27 (entries 5 and 6), and ref. 28 (entries 7 and 8). *Reagents and conditions for isomerization*: glycosylation product (1 mmol), $BF_3{\cdot}Et_2O$ (3 mmol), CH_2Cl_2 (15 mL), rt, 3 h. [b] Yields of isolated product (for the two steps, glycosylation and isomerization) after chromatographic purification.

which make them react with unique selectivity with a variety of leaving groups. Despite the basicity of stannyl ethers, although less basic than alkoxides, no elimination products (glycals) were observed.

Conclusions

β-Galactosyl ceramides were obtained in high yield and stereoselectivity by reacting disarmed galactosyl donors with stannyl ceramides. The stannyl ethers are compatible with a variety of glycosyl donor–promoter pairs, making this procedure very general and predictable for accesing β-galactosyl ceramides in high yield.

Experimental section

General experimental

All reactions were conducted under a dried argon stream. Solvents (CH_2Cl_2 99.9%, benzene 99.9%) were purchased in capped Pure Solv System-4® bottles, used without further purification and stored under argon. Yields refer to the chromatographically and spectroscopically (^1H and ^{13}C) homogeneous materials, unless otherwise stated. TMSI was stored at –15 °C under a dry atmosphere. All other solvents and reagents were used without further purification. The sphingosine was purchased from Avanti Polar Lipids Inc (Alabaster, AL) and azidosphingosine was synthesized using a literature procedures.[4a] All glassware was flame-dried before use. Reactions were monitored by TLC carried out on 0.25 mm E. Merck silica gel plates. 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 (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 ^1H, 77.23 ppm ^{13}C) or Me_4Si as an internal reference (0.00 ppm) Chemical shifts are relative to the deuterated solvent peak and are in parts per million (ppm).

Preparation of stannyl ether 8

A mixture of *N*-(2-hydroxyethyl)stearamide (100 mg, 0.305 mmol) and bis(tributyltin) oxide (91 mg, 0.152 mmol) in 20 ml of dry toluene was heated to reflux overnight and was subjected to azeotropic dehydration using a Dean–Stark apparatus or 4 Å molecular sieves. Removal of solvent under reduced pressure afforded the stannyl ether **8**, which was used for the glycosyl-coupling reaction without further purification.

Preparation of stannyl acetal 15

A mixture of *N*-((2*S*,3*R*,*E*)-1,3-dihydroxyoctadec-4-en-2-yl)-stearamide (100 mg, 0.176 mmol) and dibutyltin oxide (44 mg, 0.176 mmol) in dry toluene (20 mL) was heated to reflux overnight and was subjected to azeotropic dehydration using a Dean–Stark apparatus or 4 Å molecular sieves. Removal of solvent under reduced pressure afforded the stannyl ether **15**, which was used for the glycosyl-coupling reaction without further purification.

Synthesis of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-octadecenoyl-*N*-octadecyl-2-aminoethanol (10)

General procedure of glycosylation employing TBAI as promoter, and isomerization. The following protocol was followed prior to the glycosylation reaction: 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose and the alcohol **8** were separately dried by co-distillation with toluene (3 × 5 mL) in dried flasks and then were placed under vacuum for 1 h. TBAI was added to a dried flask with a magnetic stirring bar and was co-distilled with dry toluene (2 × 5 mL) in the dark. Activated 4 Å molecular sieves were added to the flask, and the mixture was co-distilled with toluene (5 mL) once more before being placed under vacuum for 1 h. Complete water exclusion is crucial to achieve good yields.

A solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose previously dried (142 mg, 0.366 mmol) in CH_2Cl_2 (3 mL) was cooled to 0°C under argon in the dark, and TMSI (88 mg, 0.439 mmol) was added to the stirred mixture. The reaction was stirred for 20 min at 0 °C. The reaction was stopped by adding 3 mL of dry toluene and co-distilled three times with dry toluene to obtain compound **7** as a slightly yellow oil, which was then dissolved in anhydrous toluene (5 mL) and kept under argon.

To a stirred mixture of TBAI (11 mg, 0.030 mmol) and 4 Å molecular sieves (300 mg) in anhydrous toluene (5 mL) under argon at room temperature was added *via* syringe a solution of stannyl derivative **8** (188 mg, 0.305 mmol) in dry toluene (5 mL), and a solution of **7** (0.366 mmol) in dry toluene (5 mL). The reaction mixture was stirred at 80 °C in the dark for 18 h and then diluted with AcOEt (15 mL) and cooled to 0 °C. The white

precipitate was removed by filtration through a pad of Celite. The organic layer was concentrated *in vacuo* to get the orthoester **9**, which was co-distilled with dry toluene (3 × 5 mL) and placed under vacuum for 1 h before the next reaction.

A solution containing the orthoester **9** in anhydrous CH_2Cl_2 (5 mL) was cooled to 0 °C under argon atmosphere, and freshly distilled $BF_3{\cdot}EtO_2$ (129 mg, 0.915 mmol) was added to the stirred mixture. The resulting reaction mixture was stirred for 3 h at room temperature, quenched with saturated aqueous $NaHCO_3$ solution and 25 mL of AcOEt added. The aqueous phase was extracted with AcOEt (2 × 15 mL), and the combined organic extracts were washed with saturated aqueous $Na_2S_2O_3$ solution (2 × 10 mL) and brine (3 × 10 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel using hexane–AcOEt–MeOH (85 : 10 : 5) as eluent to give 186 mg (93%) of **10** as the only anomer. TLC (hexane–AcOEt–MeOH 60 : 30 : 10) R_f 0.40; m.p. 136–138 °C; $[\alpha]_D^{25}$ −5 (c = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.85 (1H, t, J = 5.2 Hz), 5.39 (1H, d, J = 3.2 Hz), 5.19 (1H, dd, J = 10.8, 8.4 Hz), 5.01 (1H, dd, J = 10.8, 3.6 Hz), 4.46 (1H, d, J = 8.4 Hz), 4.16–4.13 (2H, m), 3.91 (1H, t, J = 6.4 Hz), 3.87 (1H, ddd, J = 10.4, 6.4, 4.0 Hz), 3.67 (1H, ddd, J = 10.4, 6.8, 3.6 Hz), 3.51 (2H, q, J = 5.2 Hz), 2.17 (2H, t, J = 7.2 Hz), 2.07 (3H, s), 2.06 (3H, s), 2.04 (3H, s), 1.98 (3H, s), 1.61 (2H, quint, J = 7.2, Hz), 1.33 (2H, sext, J = 7.6, Hz); 1.25 (26H, m), 0.87 (3H, t, J = 7.2 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 172.0, 170.2, 170.0, 169.9, 168.8, 100.0, 71.8, 70.9, 68.2, 67.0, 61.2, 61.0, 41.6, 36.5, 31.8, 29.5, 28.6, 25.6, 22.7, 20.7, 20.5 14.1. Anal. Calcd. for $C_{34}H_{59}NO_{11}$: C, 62.08; H, 9.04; N, 2.11. Found: C, 62.02; H, 9.08; N, 2.21.

General procedure for glycosylation employing AgOTf as promoter, and isomerization. The following protocol was followed prior to the glycosylation reaction. The acceptor 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose and acceptor **8** were azeotropically distilled with dry toluene (3 × 5 mL) in a dried flask and placed under vacuum for 1 h before the reaction. The AgOTf was added to a dried flask with a magnetic stirring bar. The flask was wrapped in Al foil and azeotropically distilled with dry toluene (2 × 5 mL) in the dark. Activated 4 Å molecular sieves was added to the flask, and the mixture was azeotropically distilled with benzene (5 mL) once more before being placed under vacuum for 1 h.

A solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (286 mg, 0.733 mmol) in CH_2Cl_2 (3 mL) was cooled to 0 °C 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 °C. The reaction was stopped by adding 3 mL of dry toluene and azeotropically distilled three times with dry toluene. The slightly yellow oil **7** was dissolved in CH_2Cl_2 (5 mL) and kept under argon.

To a stirred mixture of AgOTf (471 mg, 1.83 mmol) and 4 Å molecular sieves (600 mg) in dry CH_2Cl_2 (5 mL) under argon and at room temperature a solution of **8** (377 mg, 0.611 mmol) in dry CH_2Cl_2 (5 mL) and a solution of **7** (335 mg, 0.733 mmol) in dry CH_2Cl_2 (5 mL) were added *via* syringe. The reaction mixture was stirred at 0 °C in the dark for 3 h before being allowed to warm to 25 °C and further stirred for 3 h. The mixture was diluted with AcOEt (15 mL) and cooled to 0 °C. The white precipitate formed was removed by filtration through a pad of Celite. The organic layer was concentrated *in vaccuo* to give a crude residue, which mainly consisted of orthoester **9**. This crude product was azeotropically dried with dry toluene (3 × 5 mL) and placed under vacuum for 1 h before the next reaction.

A solution containing the orthoester **9** in CH_2Cl_2 (10 mL) was cooled to 0 °C under argon atmosphere, and freshly distilled $BF_3{\cdot}EtO_2$ (260 mg, 1.833 mmol) was added to the stirred mixture. The resulting reaction mixture was stirred for 3 h at room temperature, then quenched with saturated aqueous $NaHCO_3$ solution, and AcOEt (25 mL) added. The aqueous phase was extracted with AcOEt (2 × 20 mL), and the combined organic extract was washed with saturated aqueous $Na_2S_2O_3$ solution (2 × 20 mL) and brine (3 × 20 mL), dried with anhydrous Na_2SO_4, and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel using hexane–AcOEt–MeOH (85 : 10 : 5) as eluent to give 373 mg of pure **10** (93%).

Synthesis of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-octadecenoyl-*N*-octadecyl-2-aminoethanol (16)

TLC (hexane–AcOEt–MeOH 60 : 30 : 10) R_f 0.50; m.p. 136–138 °C; $[\alpha]_D^{25}$ −11 (c = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz $CDCl_3$): δ 5.37 (1H, d, J = 3.2 Hz), 5.17 (1H, dd, J = 10.4, 8.0 Hz), 5.0 (1H, dd, J = 10.4, 3.2 Hz), 4.47 (1H, d, J = 8.0 Hz), 4.17–4.01 (2H, m), 3.96 (1H, t, J = 6.4 Hz), 3.9 (1H, ddd, J = 10.0, 6.0, 4.0 Hz), 3.77 (1H, ddd, J = 10.0, 6.4, 3.6 Hz), 3.52 (2H, q, J = 5.0 Hz), 3.27 (2H, t, J = 7.2 Hz), 2.32 (2H, t, J = 7.2 Hz), 2.09 (3H, s), 2.04 (3H, s), 2.02 (3H, s), 1.01 (3H, s), 1.62–1.54 (4H, m), 1.25 (58H, m), 0.87 (6H, t, J = 7.2 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 173.0, 172.9, 170.8, 170.5, 170.0, 169.0, 100.1, 72, 71, 68.8, 67.1, 61.5, 58.9, 50.2, 47.3, 34.3, 31.8, 30.4, 29.6, 29.3, 28.9, 28.6, 27.7, 22.7,20.8, 20.58, 14.1. Anal. Calcd. for $C_{52}H_{95}NO_{11}$: C, 68.61; H, 10.52; N, 1.54. Found: C, 68.59; H, 10.55, N, 1.49.

Synthesis of (2*S*,3*S*)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-azido-octadec-4-ene-1,3-diol (17)

TLC (hexane–AcOEt–MeOH 60 : 30 : 10) R_f 0.60; m.p. 50–52 °C; $[\alpha]_D^{25}$ −21 (c = 1.0, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$): δ 5.8 (1H, dt, J = 15.6, 6.4 Hz), 5.48 (1H, dd, J = 15.6, 7.2 Hz), 5.37 (1H, d, J = 3.2 Hz), 5.19 (1H, dd, J = 10.5, 7.5 Hz), 5.0 (1H, dd, J = 10.5, 3.2 Hz), 4.5 (1H, d, J = 8.0 Hz), 4.25 (1H, dd, J = 6.8, 6.0 Hz), 4.2–4.06 (2H, m), 3.96 (1H, t, J = 6.4 Hz), 3.93 (1H, dd, J = 12.8, 6.0 Hz), 3.69 (1H, dd, J = 10.4, 4.4 Hz), 3.45 (1H, m), 2.06 (2H, q, J = 7.2 Hz), 2.05 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 1.96 (3H, s), 1.36 (2H, m), 1.25 (20H, m), 0.87 (3H, t, J = 7.2 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.2, 169.3, 169.2, 165.0, 139.0, 122.7, 100.5, 72.8, 72.5, 72.0, 71.0, 68.3, 68.1, 63.5, 61.8, 32.4, 31.9, 29.6–28.7, 22.7, 20.6, 20.5, 14.0. Anal. Calcd. for $C_{32}H_{53}N_3O_{11}$: C, 58.61; H, 8.15; N, 6.41. Found: C, 58.80; H, 8.17; N, 6.28.

Synthesis of (2*S*,3*S*)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-2-hexacosanoylamino-octadec-4-ene-1,3-diol (18)

TLC (hexane–AcOEt–MeOH 60 : 30 : 10) R_f 0.60; m.p. 50–52 °C; $[\alpha]_D^{25}$ −13.5 (c = 1.0, $CHCl_3$). ^{1}H NMR (400, MHz $CDCl_3$): δ 5.79 (1H, t, J = 7.0 Hz), 5.75 (1H, dt, J = 15.3, 6.7 Hz), 5.49 (1H, dd, J = 15.3, 7.5 Hz), 5.38 (1H, d, J = 3.2 Hz), 5.20 (1H, dd, J = 10.6, 8 Hz), 5.0 (1H, dd, J = 10.6, 3.6 Hz), 4.47 (1H, d, J = 8 Hz), 4.25 (1H, dd, J = 7.5, 3.4 Hz), 4.16 (1H, dd, J = 10.0, 4.6 Hz),

4.15–3.99 (3H, m), 3.92 (1H, t, $J = 6.4$ Hz), 3.65 (1H, dd, $J = 10.0$, 3.2 Hz), 2.20 (2H, t, $J = 7.5$ Hz), 2.05 (3H, s), 2.04 (3H, s), 2.03 (2H, dt, $J = 7.2$, 6.7, Hz), 2.03 (3H, s), 1.99 (3H, s), 1.61 (2H, quint, $J = 7.5$ Hz), 1.35 (2H, m), 1.34–1.20 (48H, m), 0.87 (6H, t, $J = 6.9$, Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 174.3, 170.2, 170.1, 169.9, 168.8, 133.9, 128.8, 101.0, 71.9, 71.7, 71.0, 68.2, 68.1, 67.1, 61.3, 53.0, 36.0, 31.9, 31.5, 29.0, 29–28.8, 25.5, 21.2, 20.6, 20.5, 13.4. Anal. Calcd. for $C_{49}H_{87}NO_{12}$: C, 66.7; H, 9.94; N, 1.59. Found: C, 66.97; H, 9.63, N, 1.38.

General procedure for glycosylation of 8 and 15, using the glycosyl donors 19–22

Glycosyl donors **19**, **20** and **22**, and the precursor of **21**, and the acceptors **8** and **15**, were carefully azetropically dried as described in the general procedures for synthesising the compound **10**. The glycosylation reaction was carried out following reported procedures (see Table 4), affording the orthoester, which was isomerized to the β-galactosyl ceramide following the isomerization procedure described in the preparation of **10**.

Acknowledgements

Financial support from DGESIC CTQ-2005–03124/BQU (Ministerio de Educación y Ciencia, Spain) is acknowledged. We are also grateful to the Servei de Recursos Cientifics (URV) for its technical assistance. Fellowship from DURSI (Generalitat de Catalunya) and Fons Social Europeu to JAMS is gratefully acknowledged.

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