

# Immunomodulatory $\alpha$ -Galactoglycosphingolipids: Synthesis of 2'-Fluoro-2'-deoxy- $\alpha$ -galactosylceramide and an Evaluation of Its Immunostimulating Properties<sup>[‡]</sup>

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In the framework of a project to assess the immunomodulating properties of  $\alpha$ -galactosyl glycosphingolipids, the total synthesis of 1-O-(2-fluoro-2-deoxy- $\alpha$ -D-galactopyranosyl)-2-docosanoylamino-1,3,4-octadecanetriol (**1**), a 2'-fluoro analogue of the immunostimulating  $\alpha$ -galactoglycosphingolipids, was accomplished. The glycosidation reaction of the azido precursor of sphingosine was performed using the Mu-

kaiyama glycosidation reaction. The stimulation of mouse splenocyte proliferation by the 2'-fluoro analogue was highly reduced compared to that of the  $\alpha$ -galactoglycosphingolipids, thereby confirming that a free galactose 2-OH group is essential for the immunostimulatory activity.

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## Introduction

*Agelas* and *Axinella* sponges<sup>[1–4]</sup> have been shown to produce  $\alpha$ -galactoglycosphingolipids ( $\alpha$ -Gal-GSLs), which are glycosphingolipids with an  $\alpha$ -galactopyranose as the first sugar attached to the ceramide head-group (e.g. agelasphin **2**). They are immunostimulating agents that are capable of activating the NKT cells' response<sup>[5]</sup> when presented by Antigen Presenting Cells (APC).<sup>[6]</sup> The first event in this process is recognition of  $\alpha$ -Gal-GSL by the TCR receptor of the NKT cells, which can only happen when the glycolipid binds a protein called CD1d, located on the surface of the APCs.

Some years ago, in a survey of the immunostimulatory activity of several natural  $\alpha$ -Gal-GSLs, we found that glycosylation of the galactose 2-OH group results in complete loss of activity.<sup>[4]</sup> More recently, it has been demonstrated that  $\alpha$ -Gal-GSLs with a glycosylated 2'-OH retain in vitro activity following the intracellular hydrolysis of the interglycosidic linkage by  $\alpha$ -glycosidases identified in the lysosomes of APCs.<sup>[7]</sup> These findings pointed to a crucial role of the galactose 2-OH group for the immunostimulatory activity

of  $\alpha$ -Gal-GSLs, and raised the question of why the 2-O-substitution makes  $\alpha$ -Gal-GSLs inactive. Two hypotheses can be proposed in this respect: (a) it is the mere steric hindrance of the additional sugar that prevents the glycolipid from binding to the TCR receptor, or (b) the 2-OH of the first sugar is the very group involved in a specific interaction at the active site of the receptor.

Even though quite a large number of natural  $\alpha$ -Gal-GSLs are available, they are not the best compounds to be used for this type of experiments. Natural  $\alpha$ -Gal-GSLs differ from simple  $\alpha$ -galactopyranosylceramide mostly in the presence of additional sugars and, given the glycosidase capability of APC cells, this prevents any sound structure–activity relationship study. Therefore, we synthesized some  $\alpha$ -Gal-GSL analogues modified at the key position 2, i.e. 2'-deoxy- $\alpha$ -D-galactopyranosylceramide (**3**),<sup>[8]</sup> and 2'-O-methyl- $\alpha$ -D-galactopyranosylceramide<sup>[9]</sup> (**4**). This synthetic modification resulted in loss of the capacity to stimulate the proliferation of murine NKT cells for compound **3**, and in a strong reduction of the activity for compound **4**. These results support the speculation that the galactose 2-OH group is responsible for the binding to the TCR receptor, most likely through a hydrogen bond.

In this paper, we attempt to gain further insight into the TCR receptor binding nature by introducing a fluorine atom in place of the galactose 2-OH group. The capacity of organically bound fluorine to act as an OH mimic and enter into hydrogen bonding as an acceptor has been widely discussed, and it is now generally acknowledged that the fluorine atom can indeed act as hydrogen-bond acceptor, although the resulting F...H hydrogen bonds are clearly weaker (about half as much) than O...H hydrogen bonds.<sup>[10]</sup>

[‡] Synthesis of Glycolipids, 3. Part 2: Ref.<sup>[8]</sup>

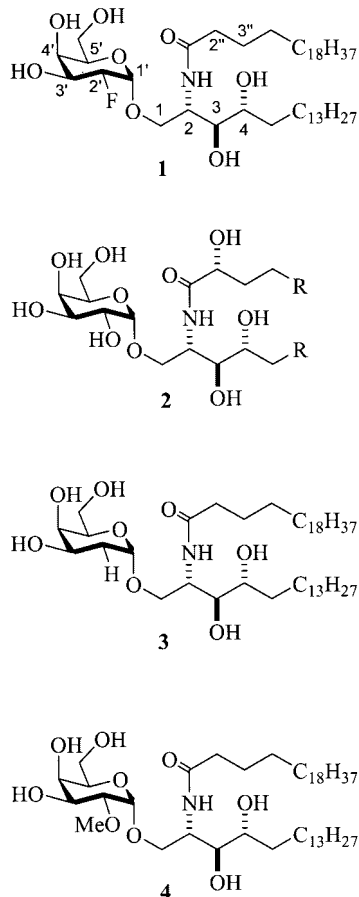
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We report below the first total synthesis of 2'-fluoro-2'-deoxy- $\alpha$ -D-galactopyranosylceramide (**1**), and a preliminary evaluation of its biological activity using lymphocyte proliferation tests (Scheme 1).



Scheme 1.

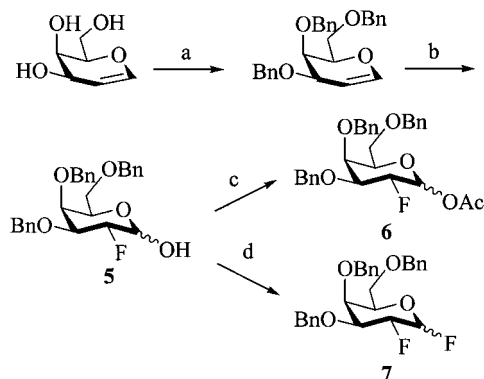
## Results and Discussion

A glycosphingolipid is made by assembling three building blocks: a saccharide chain, a sphingoid base, and a fatty acid. The crucial step of synthesis of an  $\alpha$ -galactoglycosphingolipid is the glycosidation of a suitably protected sphingoid base. To build up the target compound **1** we took advantage of our previous experience in synthesizing  $\alpha$ -Gal-GSLs.<sup>[8,9]</sup> We used the azido precursor of the sphingosine, rather than the sphingosine itself or the ceramide, as glycosyl acceptor because the former is a better nucleophile,<sup>[11]</sup> and the appropriate glycosyl fluoride as glycosyl donor.

The use of glycosyl fluorides in oligosaccharide synthesis was pioneered by the Mukaiyama group.<sup>[12]</sup> They demonstrated that glycosyl fluorides can be activated for glycosidation of alcohols in the presence of silver perchlorate and tin dichloride. If an ester protecting group is present at position 2, the mechanism involves the participation of this group, and this directs the reaction to the  $\beta$  product. Without a participating group at the 2-position,  $\alpha$ -glycosides are formed preferentially. The Mukaiyama glycosidation reac-

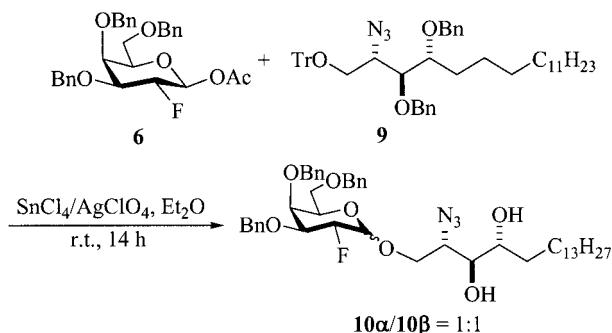
tion has been most often used to perform the stereoselective  $\alpha$ -glycosidation of the synthetic precursor of ceramide.<sup>[9,13]</sup> This reaction can provide a very high  $\alpha$ -selectivity, and offers the additional advantage that its stereoselectivity is not dependent on the configuration of the glycosyl fluoride used as substrate. However, the yields are in most cases only moderate.

Over the years, several improved versions of the original Mukaiyama glycosidation reaction have been proposed in an attempt to improve the yield of the reaction. One interesting modification,<sup>[14]</sup> involving a glycosyl acetate as glycosyl donor and an azidosphingosine activated with a trityl group, has been used recently by our group to prepare 2'-O-methyl- $\alpha$ -D-galactopyranosylceramide (**4**). In that case, the reaction provided considerably better yields than the original Mukaiyama reaction, while the stereoselectivity remained excellent. Therefore, it was natural for us to use this reaction as the key step for the synthesis of **1**. The required glycosyl donor **6** was prepared in only three steps from commercial galactal as follows (Scheme 2). After benzylation of the galactal (Sigma, 462233) by a standard procedure,<sup>[15]</sup> the obtained tri-O-benzylgalactal was converted into the corresponding 2-deoxy-2-fluorotri-O-benzyl-D-galactopyranose (**5**) by treatment with Selectfluor as the fluorinating agent.<sup>[16]</sup> Selectfluor reacts regioselectively with glycals in the presence of water to give specific fluorination at position 2. The reaction is remarkably stereoselective because of the directing effect of the axial protecting group at position 4, and only the equatorially fluorinated products **5** was obtained as an  $\alpha/\beta$  anomeric mixture. Finally, compound **5** was acetylated to give **6**, once again as an  $\alpha/\beta$  anomeric mixture.



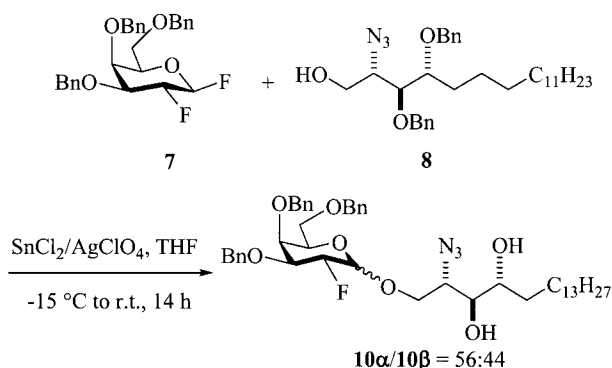
Scheme 2. Synthesis of the glycosyl donors **6** and **7**. Reagents: (a) NaH, DMF, 0 °C, then BnBr (90%); (b) F-TEDA-BF<sub>4</sub>, DMF/H<sub>2</sub>O (1:1), 12 h, room temp.; (c) Ac<sub>2</sub>O in py, room temp., quantitative; (d) DAST in THF, -30 °C, (71 %).

Compound **6** was used in the subsequent glycosylation reaction together with the tritylated azidosphingosine **9**, in the presence of AgClO<sub>4</sub> and SnCl<sub>4</sub> (Scheme 3). Unfortunately, the outcome of the reaction was different from what we expected. We observed complete loss of stereoselectivity, with a ratio between the  $\alpha$  isomer **10a** and the  $\beta$  isomer **10b** of about 1:1. In addition, the yield was low, only 32% overall, and it was not possible to recover either the tritylated azide **9** or the unprotected azide **8** as unreacted material.



Scheme 3. Modified Mukaiyama reaction applied to the synthesis of  $\alpha$ -Gal-GSL's.

At this point, we decided to go back to the original Mukaiyama glycosidation reaction, which involves a glycosyl fluoride as the glycosyl donor and  $\text{AgClO}_4$  and  $\text{SnCl}_2$  as a Lewis acid catalytic system. The bis-fluorinated glycosyl donor **7** was prepared by treatment of the 2-fluorosugar **5** with DAST ( $\text{Et}_2\text{NSF}_3$ ), a well known reagent for mild and direct transformation of glycosyl hemiacetals into glycosyl fluorides, in THF.<sup>[17]</sup> Compound **7** was obtained in 71% yield. The stereoselectivity of the glycosidation reaction (Scheme 4), although slightly better (the **10 $\alpha$ /10 $\beta$**  ratio was 56:44), was still disappointing. However, the yield of the reaction was much higher (49%,  $\alpha + \beta$ ), and, moreover, we could recover as much as 41% of the azidosphingosine **8**, so that the overall yield of **10 $\alpha$**  and **10 $\beta$**  based on the consumed **8** was 84%, and the yield of **10 $\alpha$**  alone was 47%.

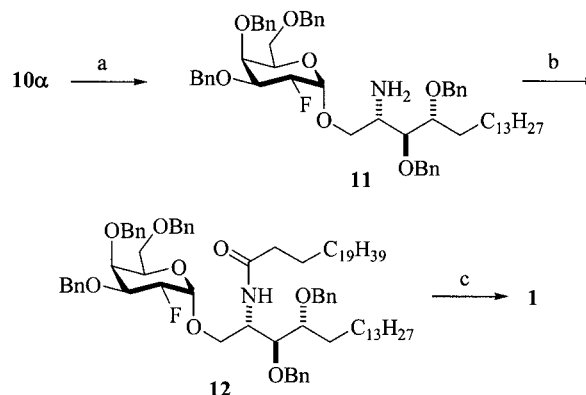


Scheme 4. Original Mukaiyama reaction applied to the synthesis of  $\alpha$ -Gal-GSL's.

The reason for the loss of selectivity of the Mukaiyama reaction when a 2-fluoroglycosyl donor is used can only be hypothesized at this stage. The mechanism postulated for this reaction is of the  $\text{S}_{\text{N}}1$  type, with the bulky perchlorate ion forming an ion pair with the oxocarbenium ion intermediate. The perchlorate ion prefers to stay on the less-hindered  $\beta$  face of the ion, and therefore the nucleophile attacks the  $\alpha$  face preferentially.<sup>[18]</sup> The presence of the fluorine atom could affect the stereochemical outcome of the reaction in two (nonmutually exclusive) ways – steric and electronic. On one hand, the limited steric hindrance of the fluorine atom (much smaller than any protected OH group, including  $\text{OCH}_3$ ) would allow the perchlorate ion to stay

on both faces of the cation, thus allowing the nucleophile to attack both sides. On the other hand it is well known that the strong electron-withdrawing effect of a fluoro substituent can significantly destabilize an oxocarbenium ion,<sup>[19]</sup> so the observed loss of selectivity could be explained by the higher reactivity of the oxocarbenium ion intermediate, which could prevent ion-pair formation.

Finally, the target compound **1** (29% overall yield based on the azidosphingosine **6** used as starting material) was obtained by reduction of the azide group with triphenyltin hydride, amidation with docosanoyl chloride, and removal of the benzyl protecting groups by hydrogenolysis over Pd/C, as previously reported (Scheme 5).<sup>[8]</sup>



Scheme 5. Final steps of the synthesis of compound **1**. Reagents: (a)  $\text{Ph}_3\text{SnH}$ , AIBN, benzene, reflux (81%); (b)  $\text{C}_{21}\text{H}_{43}\text{COCl}$ , pyridine/ $\text{CH}_2\text{Cl}_2$  (84%); (c)  $\text{H}_2$ ,  $\text{Pd}(\text{OH})_2/\text{C}$ ,  $\text{EtOH}/\text{AcOH}$ , 40 °C (73%).

Preliminary evaluation of the biological activity of compound **1** compared to  $\alpha$ -Gal-GSL agelasphin (**2**) was performed using the splenocyte proliferation test. As expected, compound **2** stimulated spleen cell proliferation from C57Bl/6 mice in a dose-dependent manner. The activity peaked after 72 h of incubation at 37 °C. On the contrary, when compound **1** was tested under the same experimental conditions, no significant proliferation was seen at any of the doses tested (Figure 1, panel A). Furthermore, different doses of compound **1** were added to compound **2** and proliferation evaluated after 72 h (Figure 1, panel B). No negative or positive interaction was observed between the two compounds, indicating that the stimulatory activity of **2** is not modified by the presence of **1**. These data also exclude any toxicity of compound **1** on normal splenocytes. These results indicate that compound **1** is unable to interact with the CD1d molecules and/or be appropriately presented by APC.<sup>[7]</sup>

Overall, these results strongly confirm that a free galactose 2-OH group is essential for the activity of  $\alpha$ -Gal-GSLs, and suggest that this hydroxyl group is the key group involved in the binding to the receptor. A more extensive examination of the biological activity of compound **2** and other modified  $\alpha$ -Gal-GSLs is in progress and will be reported elsewhere.

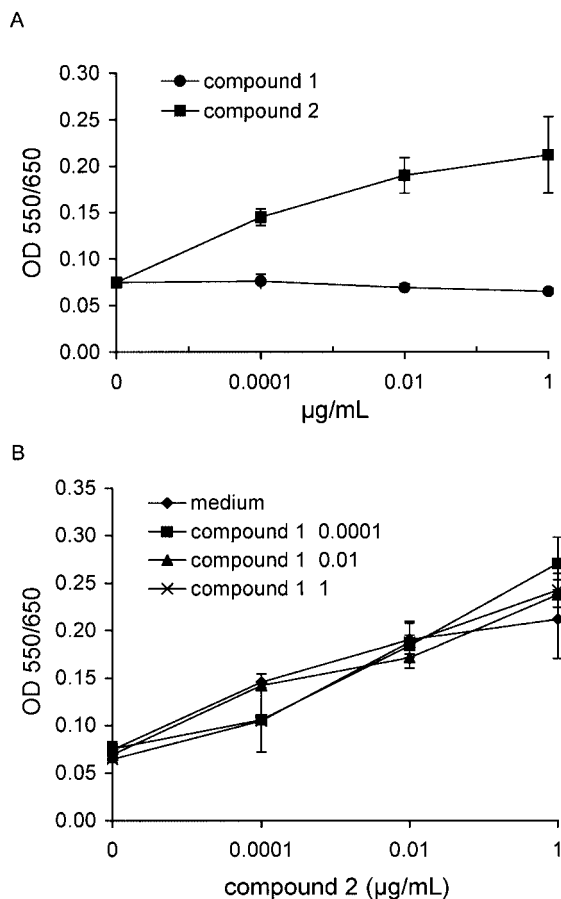


Figure 1. Proliferative response of C57Bl/6 spleen cells to 2'-fluoro-2'-deoxy- $\alpha$ -galactosylceramide (**1**) or  $\alpha$ -galactosylceramide (**2**). Panel A: Dose-dependent proliferation of C57Bl/6 spleen cells to compound **1** or compound **2** after 72 h of culture. The results are representative of three different experiments conducted in quadruplicate. Data are expressed as mean  $\pm$  SD. Panel B: Lack of interactions between compound **1** and compound **2** when tested in combination on C57Bl/6 spleen cells. The results are representative of two different experiments conducted in quadruplicate. Data are expressed as mean  $\pm$  SD.

## Experimental Section

**General Remarks:** High-resolution ESI mass spectra were recorded on a Micromass QTOF Micro mass spectrometer, dissolving the sample in MeCN/H<sub>2</sub>O (1:1) with 0.1% TFA. ESI MS/MS experiments were performed on a Finnigan LCQ ion-trap mass spectrometer. The spectra were recorded by infusion into the ESI source using MeOH/CHCl<sub>3</sub> (4:1) as the solvent. Optical rotations were measured at 589 nm on a Perkin–Elmer 192 polarimeter using a 10-cm microcell. <sup>1</sup>H and <sup>13</sup>C NMR spectra were determined on a Varian UnityInova 500 spectrometer at 500.13 and 125.77 MHz, respectively; chemical shifts were referenced to the residual solvent signal (CDCl<sub>3</sub>:  $\delta_{\text{H}}$  = 7.26,  $\delta_{\text{C}}$  = 77.0 ppm. CD<sub>3</sub>N:  $\delta_{\text{H}}$  = 8.71, 7.56, and 7.19,  $\delta_{\text{C}}$  = 149.8, 135.3, and 123.4 ppm). The spectra of new compounds were assigned thanks to COSY and HSQC two-dimensional NMR experiments.

**3,4,6-Tri-*O*-benzyl-2-deoxy-2-fluoro-D-galactopyranosyl Acetate (**6**):** H<sub>2</sub>O (6 mL) and 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (Selectfluor, 1.1 g, 3.08 mmol) were added to a solution of tri-*O*-benzylgalactal (640 mg, 1.54 mmol) in DMF (6 mL) and the reaction was allowed

to proceed for 12 h at room temperature. The reaction was quenched with water (200 mL) and then extracted with EtOAc (3  $\times$  300 mL). The combined organic extracts were dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude fluoride **5** was dissolved in pyridine (2.0 mL) and Ac<sub>2</sub>O (0.2 mL) was added. After 12 h, the reaction was quenched with MeOH, and after a further 30 min the mixture was dried under reduced pressure to give compound **6** (761 mg, 1.54 mmol, quantitative) as a mixture of anomers,  $\alpha/\beta$  = 4:3;  $[\alpha]_{\text{D}} = +68$  ( $c$  = 3.0, CHCl<sub>3</sub>), which was used in the next step without further purification.

**$\alpha$ -Anomer:** Colorless oil. ESI MS (positive ion):  $m/z$  = 517 [M + Na]<sup>+</sup>. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 2.09 (s, 3 H, acetyl protons), 3.51 (dd,  $J$  = 9.6 and 6.4 Hz, 1 H, 6b-H), 3.56 (dd,  $J$  = 9.6 and 8.7 Hz, 1 H, 6a-H), 4.09–4.02 (m, 2 H, 3-H and 5-H), 4.12 (br. t,  $J$  = 2.9 Hz, 1 H, 4-H), 4.48 and 4.41 (AB system,  $J$  = 11.7 Hz, 1 H each, geminal benzyl protons), 4.74 and 4.72 (AB system,  $J$  = 11.7 Hz, 1 H each, geminal benzyl protons), 4.84 and 4.51 (AB system,  $J$  = 11.7 Hz, 1 H each, geminal benzyl protons), 4.97 (ddd,  $J$  = 49.4, 9.8, 4.1 Hz, 1 H, 2-H), 6.34 (br. d,  $J$  = 4.1 Hz, 1 H, 1-H), 7.41–7.21 (15 H, aromatic protons) ppm. <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 69.6 (CH<sub>2</sub>, C-6), 73.1 (CH, C-5), 73.6 (d,  $J_{\text{C,F}}$  = 2 Hz, CH<sub>2</sub>, benzyl methylene group), 74.4 (CH<sub>2</sub>, benzyl methylene group), 76.2 (CH<sub>2</sub>, benzyl methylene group), 76.5 (d,  $J_{\text{C,F}}$  = 8 Hz, CH, C-4), 78.2 (d,  $J_{\text{C,F}}$  = 16 Hz, CH, C-3), 89.1 (d,  $J_{\text{C,F}}$  = 187 Hz, CH, C-2), 90.9 (d,  $J_{\text{C,F}}$  = 23 Hz, CH, C-1), 129.5–128.7 (CH, aromatic C atoms), 139.7–139.2 (C, aromatic C atoms), 170.9 (C, acetyl CO) ppm.

**$\beta$ -Anomer:** Colorless oil. ESI MS (positive ions):  $m/z$  = 517 [M + Na]<sup>+</sup>. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 2.09 (s, 3 H, acetyl protons), 3.57–3.55 (m, 2 H, 6-H<sub>2</sub>), 3.89–3.81 (m, 2 H, 3-H and 5-H), 4.02 (m, 1 H, 4-H), 4.48 and 4.40 (AB system,  $J$  = 11.7 Hz, 1 H each, geminal benzyl protons), 4.64 (ddd,  $J$  = 52.5, 9.6, 7.9 Hz, 1 H, 2-H), 4.72 and 4.70 (AB system,  $J$  = 11.7 Hz, 1 H each, geminal benzyl protons), 4.83 and 4.52 (AB system,  $J$  = 11.7 Hz, 1 H each, geminal benzyl protons), 5.71 (dd,  $J$  = 7.9 and 4.7 Hz, 1 H, 1-H), 7.41–7.21 (15 H, aromatic protons) ppm. <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 69.2 (CH<sub>2</sub>, C-6), 73.8 (d,  $J_{\text{C,F}}$  = 2 Hz, CH<sub>2</sub>, benzyl methylene group), 74.3 (CH<sub>2</sub>, benzyl methylene group), 75.4 (CH, C-5), 75.7 (d,  $J_{\text{C,F}}$  = 9 Hz, CH, C-4), 76.1 (CH<sub>2</sub>, benzyl methylene group), 81.3 (d,  $J_{\text{C,F}}$  = 16 Hz, CH, C-3), 91.7 (d,  $J_{\text{C,F}}$  = 184 Hz, CH, C-2), 93.3 (d,  $J_{\text{C,F}}$  = 25 Hz, CH, C-1), 129.5–128.7 (CH, aromatic C atoms), 139.7–139.2 (C, aromatic C atoms), 170.8 (C, acetyl CO) ppm.

**3,4,6-Tri-*O*-benzyl-2-deoxy-2-fluoro-D-galactopyranosyl Fluoride (**7**):** The crude fluoride **5**, prepared from tri-*O*-benzylgalactal (640 mg, 1.54 mmol) as described above, was dissolved in dry THF (17 mL) and cooled to –30 °C. DAST (0.24 mL, 1.8 mmol) was rapidly added and the cooling bath removed immediately. After stirring for 30 min at room temperature, the mixture was cooled again to –30 °C and quenched with MeOH (2 mL). After evaporation of solvent under reduced pressure, water (200 mL) was added to the residue, which was extracted with DCM (3  $\times$  300 mL). The combined organic layers were dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by column chromatography on SiO<sub>2</sub> (CH<sub>2</sub>Cl<sub>2</sub>) to give 495 mg (1.09 mmol, 71%) of **7** (mixture of anomers,  $\alpha/\beta$  = 2:1).

**$\alpha$ -Anomer:** Colorless oil,  $[\alpha]_{\text{D}} = +6$  ( $c$  = 0.2, CHCl<sub>3</sub>). ESI MS (positive ions):  $m/z$  = 477 [M + Na]<sup>+</sup>. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 3.63–3.59 (m, 2 H, 6-H<sub>2</sub>), 4.03 (ddd,  $J$  = 10.3, 10.3, 2.7 Hz, 1 H, 3-H), 4.09 (m, 1 H, 4-H), 4.16 (br. t,  $J$  = 6.6 Hz, 1 H, 5-H), 4.54 and 4.46 (AB system,  $J$  = 11.7 Hz, 1 H each, geminal benzyl protons), 4.85 and 4.73 (AB system,  $J$  = 11.7 Hz, 1 H each, geminal benzyl protons), 4.97 and 4.60 (AB system,  $J$  = 11.7 Hz, 1 H each,



geminal benzyl protons), 5.01 (dddd,  $J = 49.2, 24.1, 9.7, 2.7$  Hz, 1 H, 2-H), 5.81 (br. dd,  $J = 54.3$  and  $2.7$  Hz, 1 H, 1-H), 7.45–7.28 (15 H, aromatic protons) ppm.  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 67.8$  ( $\text{CH}_2$ , C-6), 71.9 (d,  $J_{\text{C,F}} = 3$  Hz, CH, C-5), 72.9 (d,  $J_{\text{C,F}} = 2$  Hz,  $\text{CH}_2$ , benzyl methylene group), 73.5 ( $\text{CH}_2$ , benzyl methylene group), 74.7 (d,  $J_{\text{C,F}} = 9$  Hz, CH, C-4), 75.0 ( $\text{CH}_2$ , benzyl methylene group), 76.2 (d,  $J_{\text{C,F}} = 16$  Hz, CH, C-3), 88.8 (dd,  $J_{\text{C,F}} = 188$  and  $25$  Hz, CH, C-2), 104.8 (dd,  $J_{\text{C,F}} = 227$  and  $24$  Hz, CH, C-1), 128.5–127.5 (CH, aromatic C atoms), 138.0–137.5 (C, aromatic C atoms) ppm.

**$\beta$ -Anomer:** Colorless oil,  $[\alpha]_{\text{D}} = +3$  ( $c = 0.2$ ,  $\text{CHCl}_3$ ). ESI MS (positive ions):  $m/z = 477$  [ $\text{M} + \text{Na}$ ] $^+$ .  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 3.71$ – $3.64$  (m, 3 H, 3-H and 6-H<sub>2</sub>), 3.74 (br. t,  $J = 6.7$  Hz, 1 H, 5-H), 3.99 (m, 1 H, 4-H), 4.52 and 4.46 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.79 and 4.72 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.83 (dddd,  $J = 52.2, 15.8, 9.3, 6.6$  Hz, 1 H, 2-H), 4.96 and 4.63 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 5.27 (ddd,  $J = 53.4, 6.6, 5.7$  Hz, 1 H, 1-H), 7.46–7.29 (15 H, aromatic protons) ppm.  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 67.9$  ( $\text{CH}_2$ , C-6), 72.8 (d,  $J_{\text{C,F}} = 2$  Hz,  $\text{CH}_2$ , benzyl methylene group), 73.5 (d,  $J_{\text{C,F}} = 9$  Hz, CH, C-4), 73.6 ( $\text{CH}_2$ , benzyl methylene group), 73.9 (d,  $J_{\text{C,F}} = 4$  Hz, CH, C-5), 74.8 ( $\text{CH}_2$ , benzyl methylene group), 78.9 (dd,  $J_{\text{C,F}} = 16$  and  $9$  Hz, CH, C-3), 91.5 (dd,  $J_{\text{C,F}} = 184$  and  $24$  Hz, CH, C-2), 107.1 (dd,  $J_{\text{C,F}} = 216$  and  $27$  Hz, CH, C-1), 128.5–127.6 (CH, aromatic C atoms), 138.0–137.5 (C, aromatic C atoms) ppm.

**Modified Mukaiyama Glycosidation Reaction:**  $\text{SnCl}_4$  (50  $\mu\text{L}$  of a 0.5 M toluene solution, 0.025 mmol) was added to a suspension of  $\text{AgClO}_4$  (7.0 mg, 0.034 mmol) in anhydrous  $\text{Et}_2\text{O}$  (2 mL). The mixture was stirred for 4 h, and compound **6** (170 mg, 0.34 mmol) and compound **9** (130 mg, 0.17 mmol)<sup>[8]</sup> were added simultaneously in an anhydrous  $\text{Et}_2\text{O}$  solution (2 mL). After 14 h, the reaction mixture was diluted with DCM (30 mL), washed with a saturated  $\text{NaHCO}_3$  solution (20 mL), dried with  $\text{Na}_2\text{SO}_4$ , and taken to dryness. The residue was chromatographed by HPLC ( $n$ -hexane/ $\text{EtOAc}$ , 9:1) to give 26 mg (0.027 mmol, 16%) of the  $\alpha$ -glycoside **10a** and 26 mg (0.027 mmol, 16%) of  $\beta$ -glycoside **10b**.

**Original Mukaiyama Glycosidation Reaction:** A solution of the azidosphingosine **8** (190 mg, 0.36 mmol)<sup>[9]</sup> and the fluoride **7** (495 mg, 1.09 mmol) in dry THF (3 mL) was added at  $-15^\circ\text{C}$  to a flask containing solid  $\text{AgClO}_4$  (225 mg, 1.09 mmol) and  $\text{SnCl}_2$  (205 mg, 1.09 mmol). After stirring for 12 h at room temperature, the mixture was filtered through Celite and the solvents evaporated. The reaction mixture was diluted with DCM (30 mL), washed with a saturated  $\text{NaHCO}_3$  solution (20 mL), dried with  $\text{Na}_2\text{SO}_4$ , and taken to dryness. The residue was chromatographed by HPLC ( $n$ -hexane/ $\text{EtOAc}$ , 9:1) to give 97 mg (0.101 mmol, 28%) of the  $\alpha$ -glycoside **10a** and 72 mg (0.076 mmol, 21%) of the  $\beta$ -glycoside **10b** ( $\alpha/\beta = 56:44$ ), along with 77 mg (0.147 mmol, 41%) of unreacted **8**.

**(2S,3S,4R)-2-Azido-3,4-di-*O*-benzyl-1-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro- $\alpha$ -D-galactopyranosyl)-1,3,4-octadecanetriol (10a):** Colorless oil,  $[\alpha]_{\text{D}} = +53$  ( $c = 1.1$ ,  $\text{CHCl}_3$ ). ESI MS (positive ions):  $m/z = 981$  [ $\text{M} + \text{Na}$ ] $^+$ .  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.88$  (t,  $J = 6.6$  Hz, 3 H, H<sub>3</sub>-18), 1.26 (alkyl chain  $\text{CH}_2$  protons), 1.35 (m, 1 H, 6b-H), 1.45 (m, 1 H, 6a-H), 1.59 (m, 1 H, 5b-H), 1.69 (m, 1 H, 5a-H), 3.55–3.46 (m, 2 H, 6'-H<sub>2</sub>), 3.65–3.59 (m, 2 H, 2-H and 4-H), 3.76–3.70 (m, 2 H, 1b-H and 3-H), 3.98–3.95 (m, 2 H, 5'-H and 4'-H), 4.08–3.99 (m, 2 H, 1a-H and 3'-H), 4.47 and 4.39 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.62 and 4.52 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.74 and 4.62 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl

protons), 4.82 and 4.69 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.92 (m,  $J_{\text{H,F}} = 49$  Hz, 0.5 H, the high field part of the 2'-H signal), 4.94 and 4.56 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 5.04–4.98 (m, 1.5 H, 1'-H and the low field part of the 2'-H signal), 7.42–7.24 (25 H, aromatic protons) ppm.  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.1$  ( $\text{CH}_3$ , C-18), 22.6 ( $\text{CH}_2$ , C-17), 25.5 ( $\text{CH}_2$ , C-6), 29.8–29.6 ( $\text{CH}_2$ , alkyl chain  $\text{CH}_2$  groups), 29.9 ( $\text{CH}_2$ , C-5), 31.9 ( $\text{CH}_2$ , C-16), 61.6 (CH, C-2), 68.5 ( $\text{CH}_2$ , C-6'), 68.8 ( $\text{CH}_2$ , C-1), 69.8 (CH, C-5'), 72.1 ( $\text{CH}_2$ , benzyl methylene group), 73.0 ( $\text{CH}_2$ , benzyl methylene group), 73.5 ( $\text{CH}_2$ , benzyl methylene group), 73.8 ( $\text{CH}_2$ , benzyl methylene group), 74.9 ( $\text{CH}_2$ , benzyl methylene group), 75.2 (d,  $J = 7$  Hz, CH, C-4'), 76.7 (d,  $J = 15$  Hz, CH, C-3'), 78.5 (CH, C-3), 79.5 (CH, C-4), 89.1 (d,  $J = 189$  Hz, CH, C-2'), 97.6 (d,  $J = 21$  Hz, CH, C-1'), 128.4–127.6 (CH, aromatic C atoms) ppm.

**(2S,3S,4R)-2-Azido-3,4-di-*O*-benzyl-1-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro- $\beta$ -D-galactopyranosyl)-1,3,4-octadecanetriol (10b):** Colorless oil,  $[\alpha]_{\text{D}} = +9$  ( $c = 1.1$ ,  $\text{CHCl}_3$ ). ESI MS (positive ions):  $m/z = 981$  [ $\text{M} + \text{Na}$ ] $^+$ .  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.88$  (t,  $J = 6.6$  Hz, 3 H, H<sub>3</sub>-18), 1.26 (alkyl chain  $\text{CH}_2$  protons), 1.32 (m, 1 H, 6b-H), 1.39 (m, 1 H, 6a-H), 1.56 (m, 1 H, 5b-H), 1.65 (m, 1 H, 5a-H), 3.49 (m, 1 H, 3-H) 3.53 (d,  $J = 5.3$  Hz, 1 H, 6'b-H), 3.61–3.54 (m, 2 H, 6'a-H and 5'-H), 3.63 (m, 1 H, 3'-H), 3.71 (t,  $J = 4.5$  Hz, 1 H, 4-H), 3.77 (m, 1 H, 2-H), 3.84 (dd,  $J = 10.7$  and  $3.6$  Hz, 1 H, 1b-H), 3.94 (br. t,  $J = 2.9$  Hz, 1 H, 4'-H), 4.09 (dd,  $J = 10.7$  and  $6.8$  Hz, 1 H, 1a-H), 4.43 and 4.38 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.44–4.38 (m, 3 H, 1'-H and two benzylic protons), 4.49 and 4.57 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.68 and 4.63 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.76 and 4.66 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.77–4.64 (2'-H submerged by another signal), 4.92 and 4.59 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 7.39–7.20 (25 H, aromatic protons) ppm.  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.1$  ( $\text{CH}_3$ , C-18), 20.8 ( $\text{CH}_2$ , C-17), 24.8 ( $\text{CH}_2$ , C-6), 29.3–29.1 ( $\text{CH}_2$ , alkyl chain  $\text{CH}_2$  groups), 29.7 ( $\text{CH}_2$ , C-5), 30.9 ( $\text{CH}_2$ , C-16), 61.6 (CH, C-2), 68.0 ( $\text{CH}_2$ , C-6'), 68.7 ( $\text{CH}_2$ , C-1), 71.6 ( $\text{CH}_2$ , benzyl methylene group), 72.4 ( $\text{CH}_2$ , benzyl methylene group), 72.6 ( $\text{CH}_2$ , benzyl methylene group), 73.2 ( $\text{CH}_2$ , benzyl methylene group), 73.4 (CH, C-3), 73.8 (CH, C-4'), 74.5 ( $\text{CH}_2$ , benzyl methylene group), 78.6 (CH, C-4), 79.2 (CH, C-3'), 80.0 (CH, C-5'), 91.4 (CH, d,  $J = 60$  Hz, C-2'), 100.5 (CH, C-1'), 127.8–127.2 (CH, aromatic C atoms) ppm.

**(2S,3S,4R)-2-Amino-3,4-di-*O*-benzyl-1-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro- $\alpha$ -D-galactopyranosyl)-1,3,4-octadecanetriol (11):**  $\text{Ph}_3\text{SnH}$  (434  $\mu\text{L}$ , 597 mg, 1.72 mmol) and a small amount of AIBN were added to a solution of azide **10a** (165 mg, 0.172 mmol) in dry benzene (10 mL), and the resulting solution was allowed to react for 2 h under reflux at  $80^\circ\text{C}$ . The solution was cooled to room temperature and concentrated under reduced pressure. Column chromatography on  $\text{SiO}_2$  ( $n$ -hexane/ $\text{EtOAc}$ , 6:4 with 0.1% pyridine) gave 142 mg (0.152 mmol, 88%) of **11** as a colorless oil.  $[\alpha]_{\text{D}} = +33$  ( $c = 1.1$ ,  $\text{CHCl}_3$ ). ESI MS (positive ions):  $m/z = 933$  [ $\text{M} + \text{H}$ ] $^+$ .  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.88$  (t,  $J = 6.6$  Hz, 3 H, H<sub>3</sub>-18), 1.26 (alkyl chain  $\text{CH}_2$  protons), 1.35 (m, 1 H, 6b-H), 1.45 (m, 1 H, 6a-H), 1.59 (m, 1 H, 5b-H), 1.69 (m, 1 H, 5a-H), 3.14 (m, 1 H, 2-H), 3.52–3.43 (m, 2 H, 6'b-H and 1b-H), 3.60–3.53 (m, 2 H, 6'a-H and 3-H), 3.73 (m, 1 H, 4-H), 4.03–3.94 (m, 4 H, 1a-H, 4'-H, 3'-H, and 5'-H), 4.46 and 4.39 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.64 and 4.54 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.76 and 4.57 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.81 and 4.70 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons),

4.92 (m,  $J_{\text{H,F}} = 49$  Hz, 0.5 H, the high field part of the 2'-H signal), 4.93 and 4.57 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 5.04–4.98 (m, 1.5 H, 1'-H and the low field part of the 2'-H signal), 7.42–7.23 (25 H, aromatic protons) ppm.  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.1$  ( $\text{CH}_3$ , C-18), 22.7 ( $\text{CH}_2$ , C-17), 25.8 ( $\text{CH}_2$ , C-6), 29.8–29.6 ( $\text{CH}_2$ , alkyl chain  $\text{CH}_2$  groups), 30.5 ( $\text{CH}_2$ , C-5), 31.9 ( $\text{CH}_2$ , C-16), 52.6 ( $\text{CH}$ , C-2), 68.5 ( $\text{CH}_2$ , C-6'), 69.5 ( $\text{CH}$ , C-5'), 71.8 ( $\text{CH}_2$ , C-1), 72.0 ( $\text{CH}_2$ , benzyl methylene group), 72.8 ( $\text{CH}_2$ , benzyl methylene group), 73.6 ( $\text{CH}_2$ , benzyl methylene group), 74.9 ( $\text{CH}_2$ , benzyl methylene group), 75.4 (d,  $J = 8$  Hz, CH, C-4'), 77.0 (CH, C-3'), 79.9 (CH, C-4), 81.6 (CH, C-3), 89.3 (d,  $J = 187$  Hz, CH, C-2'), 97.7 (d,  $J = 21$  Hz, CH, C-1'), 128.4–127.5 (CH, aromatic C atoms), 138.7–137.8 (C, aromatic C atoms) ppm.

**(2S,3S,4R)-3,4-Di-O-benzyl-2-docosanoylamino-1-O-(3,4,6-tri-O-benzyl-2-deoxy-2-fluoro- $\alpha$ -D-galactopyranosyl)-1,3,4-octadecanetriol (12):** A solution of docosanoic acid (156 mg, 0.46 mmol) in  $\text{SOCl}_2$  (1.0 mL, 1.64 g, 13.8 mmol) was refluxed for 90 min, and the excess of  $\text{SOCl}_2$  was then removed under reduced pressure. A solution of amine **11** (142 mg, 0.152 mmol) in dry pyridine (3 mL) and dry  $\text{CH}_2\text{Cl}_2$  (3 mL) was added to the obtained docosanoyl chloride. After 12 h, the solvents were removed under vacuum, and the residue was partitioned between  $\text{CH}_2\text{Cl}_2$  (100 mL) and a saturated  $\text{NaHCO}_3$  aqueous solution (50 mL). The organic phase was dried with  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. It was then subjected to column chromatography on  $\text{SiO}_2$  (*n*-hexane/EtOAc, 8:2) to give 183 mg (0.146 mmol, 96%) of **12** as a colorless oil.  $[\alpha]_{\text{D}}^{25} = +21$  ( $c = 1.1$ ,  $\text{CHCl}_3$ ). ESI MS (positive ions):  $m/z = 1255$  [ $\text{M} + \text{H}$ ] $^+$ .  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.88$  (t,  $J = 6.8$  Hz, 6 H, 18-H<sub>3</sub> and 22''-H<sub>3</sub>), 1.26 (alkyl chain  $\text{CH}_2$  protons), 1.58–1.49 (m, 3 H, 3''-H<sub>2</sub> and 5b-H), 1.69 (m, 1 H, 5a-H), 2.01 (m, 2 H, 2''-H<sub>2</sub>), 3.55–3.44 (m, 3 H, 6'b-H, 6'a-H, and 4-H), 3.84–3.76 (m, 2 H, 1b-H and 3-H), 3.97–3.89 (m, 4 H, 1a-H, 5'-H, 3'-H, and 4'-H), 4.24 (m, 1 H, 2-H), 4.48 and 4.39 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.61 and 4.51 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.79 and 4.69 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.80 and 4.53 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.91 and 4.56 (AB system,  $J = 11.7$  Hz, 1 H each, geminal benzyl protons), 4.95 (1 H, double multiplet,  $J = 51$  Hz, 2'-H), 4.96 (d,  $J = 3.6$  Hz, 1 H, 1'-H), 5.87 (d,  $J = 8.9$  Hz, 1 H, 2-NH), 7.42–7.20 (25 H, aromatic protons) ppm.  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.1$  ( $\text{CH}_3$ , C-18 and C-22''), 22.6 ( $\text{CH}_2$ , C-17 and C-21''), 24.7 ( $\text{CH}_2$ , C-3''), 29.8–29.1 ( $\text{CH}_2$ , alkyl chain  $\text{CH}_2$  groups), 30.3 ( $\text{CH}_2$ , C-5), 31.9 ( $\text{CH}_2$ , C-16 and C-20''), 34.1 ( $\text{CH}_2$ , C-2''), 50.1 (CH, C-2), 68.8 ( $\text{CH}_2$ , C-6'), 69.0 ( $\text{CH}_2$ , C-1), 69.9 (CH, C-5'), 71.9 ( $\text{CH}_2$ , benzyl methylene group), 72.8 ( $\text{CH}_2$ , benzyl methylene group), 73.4 ( $\text{CH}_2$ , benzyl methylene group), 73.6 ( $\text{CH}_2$ , benzyl methylene group), 74.9 ( $\text{CH}_2$ , benzyl methylene group), 75.3 (CH, d,  $J = 8$  Hz, C-4') 77.4 (CH, C-3'), 78.8 (CH, C-3), 80.1 (CH, C-4), 89.2 (CH, d,  $J = 188$  Hz, C-2'), 98.1 (CH, d,  $J = 21$  Hz, C-1'), 128.4–127.5 (CH, aromatic C atoms), 138.6–137.5 (C, aromatic C atoms), 172.9 (C, C-1'') ppm.

**(2S,3S,4R)-1-O-(2-Deoxy-2-fluoro- $\alpha$ -D-galactopyranosyl)-2-docosanoylamino-1,3,4-octadecanetriol (1):** Compound **12** (80 mg, 0.064 mmol) was subjected to hydrogenolysis over  $\text{Pd}(\text{OH})_2/\text{C}$  (50 mg, 20% w/w) as previously reported.<sup>[8]</sup> The reaction mixture was purified by reversed-phase HPLC chromatography on RP-18 silica gel (MeOH 100%) to give 38 mg (0.047 mmol, 73%) of compound **1** as an amorphous solid.  $[\alpha]_{\text{D}}^{25} = +4$  ( $c = 0.4$ ,  $\text{CHCl}_3$ ). HRESI MS (positive ions):  $m/z = 804.6745$  ([ $\text{M} + \text{H}$ ] $^+$ ,  $\text{C}_{46}\text{H}_{91}\text{FNO}_8$  gives 804.6729). ESI MS (positive ions):  $m/z = 804$  [ $\text{M} + \text{H}$ ] $^+$ .  $^1\text{H}$  NMR (500 MHz,  $[\text{D}_5]\text{pyridine}$ ):  $\delta = 0.86$  (t,  $J =$

7.1 Hz, 6 H, 18-H<sub>3</sub> and 22''-H<sub>3</sub>), 1.33–1.16 (alkyl chain  $\text{CH}_2$  protons), 1.50–1.34 (m, 3 H, 7a-H and 4''-H<sub>2</sub>), 1.69 (m, 1 H, 6b-H), 1.85 (m, 2 H, 3''-H<sub>2</sub>), 1.99–1.88 (m, 2 H, 5b-H and 6a-H), 2.22 (m, 1 H, 5a-H), 2.50 (m, 2 H, 2''-H<sub>2</sub>), 4.23 (br. t,  $J = 6.8$  Hz, 1 H, H-4), 4.35 (dd,  $J = 10.9$  and 5.3 Hz, 1 H, 6'b-H), 4.47–4.39 (m, 3 H, 1b-H, 6'a-H, and 3-H), 4.56–4.51 (m, 2 H, 5'-H and 4'-H), 4.61 (m, 1 H, 3'-H), 4.64 (dd,  $J = 10.9$  and 3.2 Hz, 1 H, 1a-H), 5.17 (m, 1 H, 2-H), 5.32 (ddd,  $J = 50.4$ , 9.6, 3.8 Hz, 1 H, 2'-H), 5.49 (d,  $J = 3.8$  Hz, 1 H, 1'-H), 8.78 (d,  $J = 8.6$  Hz, 1 H, 2-NH) ppm.  $^{13}\text{C}$  NMR (500 MHz,  $[\text{D}_5]\text{pyridine}$ ):  $\delta = 14.2$  ( $\text{CH}_3$ , C-18 and C-22''), 22.9 ( $\text{CH}_2$ , C-17 and C-21''), 26.4 ( $\text{CH}_2$ , C-3''), 26.5 ( $\text{CH}_2$ , C-6), 30.2–29.5 ( $\text{CH}_2$ , alkyl chain  $\text{CH}_2$  groups), 32.1 ( $\text{CH}_2$ , C-16 and C-20''), 33.7 ( $\text{CH}_2$ , C-5), 36.7 ( $\text{CH}_2$ , C-2''), 52.6 (CH, C-2), 62.2 ( $\text{CH}_2$ , C-6'), 69.1 ( $\text{CH}_2$ , C-1), 69.3 (CH, C-3'), 71.5 (CH, C-4'), 72.6 (CH, C-4), 72.8 (CH, C-5'), 76.1 (CH, C-3), 91.1 (d,  $J = 188$  Hz, CH, C-2'), 98.5 (d,  $J = 21$  Hz, CH, C-1'), 173.6 (C, C-1'') ppm.

**Lymphocyte Proliferation Test:** Spleens from C57Bl/6 mice (Charles River, Italia, Calco, Como) were aseptically removed, minced, and cell suspensions were incubated at  $5 \times 10^5$  per well in 96-well microtiter plates (3799 Costar, Italia; Milan, Italy) using RPMI 1640 medium supplemented with 100  $\text{U mL}^{-1}$  of penicillin, 2 mM glutamine, and 100  $\mu\text{g mL}^{-1}$  streptomycin, 20 mM Hepes buffer (Euroclone, Celbio, Milan, Italy), and 10% FCS (Euroclone, Celbio, Milan, Italy). All reagents were free of endotoxin contamination by the LAL assay. Various doses of the  $\alpha$ -Gal-GSL, either agelasphin (**2**) or the 2'-fluoro analog **1**, were added. Concanavaline A (1  $\mu\text{g mL}^{-1}$ ) was used as positive control. Cell proliferation was measured using the already described MTT assay.<sup>[20]</sup> Plates were incubated for 72 h at 37 °C in 5%  $\text{CO}_2$ , then 20  $\mu\text{L}$  of a 5  $\text{mg mL}^{-1}$  solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; M-2128 Sigma) in PBS were added for an additional 3 h at 37 °C. The plates were then centrifuged, the supernatants discarded, and the dark-blue formazan crystals dissolved in 100  $\mu\text{L}$  of lysing buffer consisting of a solution 20% (w/v) of SDS (Sigma), 40% of *N,N*-dimethylformamide (Merck) in  $\text{H}_2\text{O}$  at pH 4.7 adjusted with 80% acetic acid. The plates were then read on a microplate reader (Molecular Devices Co., Menlo Park, CA, USA) using a test wavelength of 550 nm and a reference wavelength of 650 nm. The results are expressed as OD 550/OD650 which mean that the values at OD 650 have been subtracted from the values at OD 550. All the tests were performed at least three times in quadruplicate and the statistical analysis was performed by one-way ANOVA with Scheffe F-test post hoc. *p* Values less than 0.05 were considered to be significant.

**Supporting Information:**  $^1\text{H}$  NMR of compound **1a**; 1D and 2D NMR spectra of compound **1b**;  $^1\text{H}$  NMR and CD spectra of the degradation products **2–5** (see also the footnote on the first page of this article).

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[1] V. Costantino, E. Fattorusso, A. Mangoni, M. Aknin, E. M. Gaydou, *Liebigs Ann. Chem.* **1994**, 1181–1185.

[2] V. Costantino, E. Fattorusso, A. Mangoni, *Liebigs Ann.* **1995**, 1471–1475.

- [3] V. Costantino, E. Fattorusso, A. Mangoni, *Liebigs Ann.* **1995**, 2133–2136.
- [4] V. Costantino, E. Fattorusso, A. Mangoni, M. Di Rosa, A. Ianaro, P. Maffia, *Tetrahedron* **1996**, *52*, 1573–1578.
- [5] T. Natori, N. Morita, K. Akimoto, Y. Koezuka, *Tetrahedron* **1994**, *50*, 2771–2784.
- [6] N. Dutronc, S. A. Porcelli, *Tissue Antigens* **2002**, *60*, 337–353.
- [7] T. I. Prigozy, O. Naidenko, P. Qasba, D. Elewaut, L. Brossay, A. Khurana, T. Natori, Y. Koezuka, A. Kulkarni, M. Kronenberg, *Science* **2001**, *291*, 664–667.
- [8] L. Barbieri, V. Costantino, E. Fattorusso, A. Mangoni, E. Aru, S. Parapini, D. Taramelli, *Eur. J. Org. Chem.* **2004**, 468–473.
- [9] V. Costantino, E. Fattorusso, C. Imperatore, A. Mangoni, *Tetrahedron* **2002**, *58*, 369–375.
- [10] H. Takemura, M. Kotoku, M. Yasutake, T. Ahinmyozu, *Eur. J. Org. Chem.* **2004**, 2019–2024.
- [11] R. Schmidt, in *Comprehensive Organic Synthesis* (Ed.: B. M. Trost), Pergamon Press, London, **1990**; Vol. 6, pp. 33–64.
- [12] T. Mukaiyama, Y. Murai, S. Shoda, *Chem. Lett.* **1981**, 431–432.
- [13] K. Akimoto, T. Natori, M. Morita, *Tetrahedron Lett.* **1993**, *34*, 5593–5596.
- [14] S. Houdier, P. J. A. Vottéro, *Tetrahedron Lett.* **1993**, *34*, 3283–3284.
- [15] M. Chmielewski, I. Fokt, J. Grodner, G. Gryniewicz, W. Szeja, *J. Carbohydr. Chem.* **1989**, *8*, 735–744.
- [16] M. D. Burkart, Z. Zhang, A. Hung, C. Wong, *J. Am. Chem. Soc.* **1997**, *119*, 11743–11746.
- [17] W. Rosebroox, D. A. Riley, P. A. Lartey, *Tetrahedron Lett.* **1985**, *26*, 3–4.
- [18] T. Mukaiyama, T. Takeshima, M. Katsuraba, H. Aizawa, *Chem. Lett.* **1991**, 533.
- [19] M. N. Namchuk, J. D. McCarter, A. Becalski, T. Andrews, S. G. Withers, *J. Am. Chem. Soc.* **2000**, *122*, 1270–1277.
- [20] T. J. Mosmann, *Immunol. Methods* **1983**, *65*, 55–63.

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