Carbohydrate Research 346 (2011) 914–926

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

An improved synthesis of dansylated α-galactosylceramide and its use as a fluorescent probe for the monitoring of glycolipid uptake by cells

Janice M. H. Cheng [a,b], Stephanie H. Chee [a], Deborah A. Knight [a], Hans Acha-Orbea [c], Ian F. Hermans [a,d], Mattie S. M. Timmer [b,*], Bridget L. Stocker [a,*]

[a] Malaghan Institute of Medical Research, PO Box 7060, Wellington, New Zealand
[b] School of Chemical and Physical Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand
[c] Department of Biochemistry CIIL, University of Lausanne, Chemin des Boveresses 155, 1066 Epalinges, Switzerland
[d] School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand

ARTICLE INFO

Article history:
Received 16 December 2010
Received in revised form 14 February 2011
Accepted 15 February 2011
Available online 19 February 2011

Keywords:
α-GalCer
Cancer
Fluorescent probe
Synthesis
Glycolipid

ABSTRACT

A highly efficient synthesis of the biologically important fluorescent probe dansyl α-GalCer is presented. Key in our strategy is the incorporation of the fluorescent dansyl group at an early stage in the synthesis to facilitate in the monitoring and purification of intermediates via TLC and flash column chromatography, respectively, and the use of a high yielding α-selective glycosylation reaction between the phytosphingosine lipid and a galactosyl iodide donor. The ability of dansyl α-GalCer to activate *i*NKT cells and to serve as a fluorescent marker for the uptake of glycolipid by dendritic cells is also presented.

1. Introduction

In 1993, the pharmaceutical division of Kirin Breweries isolated a series of novel α-galactosyl ceramides from the marine sponge *Agelas mauritianus*.[1,2] Of the series, agelasphin-9b (AGL-9b, **1**, Fig. 1), consisting of a galactosyl moiety α-linked to a ceramide containing an N-acylated phytosphingosine backbone, exhibited potent anti-tumour activity against in vivo models of several murine tumour cells and later served as the parent compound for subsequent analogue syntheses and structure–activity studies.[3] During the course of this structure–activity work, KRN7000 (**2**) was found to have similar anti-tumour activity to **1** and, due to its easier synthesis, was deemed the more suitable candidate for clinical use. Since then, KRN7000, now widely known as α-galactosyl ceramide (α-GalCer), has been reported to have potential in the treatment of several diseases including cancer, malaria, type I diabetes, and multiple sclerosis, and equally importantly, has been shown to exert its therapeutic activity via its ability to bind to CD1d [a member of the CD1 family of proteins found on the surface of antigen presenting cells (APCs)] and activate a subset of T cells known as invariant natural killer T (*i*NKT) cells.[4–6] This discovery was remarkable and provided the first evidence that glycolipids, like their protein counterparts, can be presented by APCs and recognised by T cells to invoke an immune response.

Given the therapeutic potential of α-GalCer, much effort has been spent in developing robust routes for its synthesis.[3,7–10] In addition, a number of α-GalCer-derivatives containing a fluorophore[11–13] or a biotinylated probe[11,14,15] have been prepared with the objective of using these substrates to better understand the mechanism of *i*NKT cell activation by α-GalCer.[13,16,17] Though the ability of α-GalCer to bind to CD1d and activate *i*NKT cells has been robustly studied, only little information is known about lipid trafficking and how this influences presentation by CD1d and activation of *i*NKT cells.[18] Accordingly, many groups have focussed on derivatising the lipid portion of α-GalCer with a reporter probe in order to address such issues.[12–14] However, as CD1d binds lipid chains within deep hydrophobic pockets,[19] it has been proposed that the addition of a label on the lipid may interfere with α-GalCer-CD1d association,[17] and moreover, may influence intracellular trafficking.[18,20–22] Conversely, modelling of the CD1d-α-GalCer complex suggests that the hydroxyl groups at C4″ and C6″ on galactose are not involved in complex formation.[23] This theory is also supported by the preparation of a α-GalCer analogue containing an additional α-linked galactose at the C6″ position which was shown to stimulate *i*NKT cells without the need for processing.[24] Taken as a whole, it is generally accepted that the CD1d-glycolipid-NKT cell

* Corresponding authors. Tel.: +64 4 499 6914x813; fax: +64 4 499 6915 (B.L.S.).
E-mail addresses: mattie.timmer@vuw.ac.nz (M.S.M. Timmer), bstocker@malaghan.org.nz (B.L. Stocker).

0008-6215/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2011.02.014

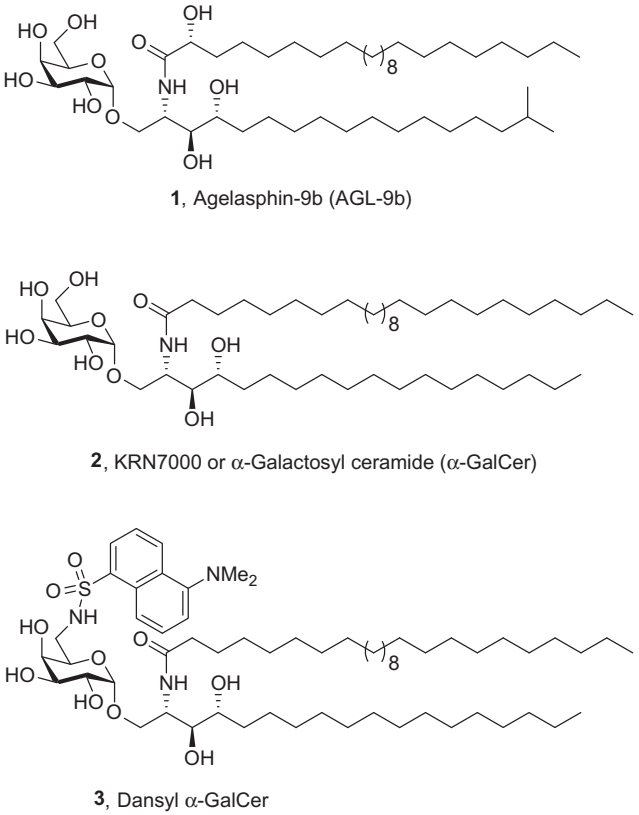


Figure 1. α-Galactosyl ceramide and derivatives.

receptor interaction tolerates the appendage of small molecules at C6″ and that this is an ideal position to attach a fluorescent reporter group. Indeed, this was the conclusion made by Zhou et al. who developed a strategy for the preparation of dansylated α-GalCer derivative **3**.[11]

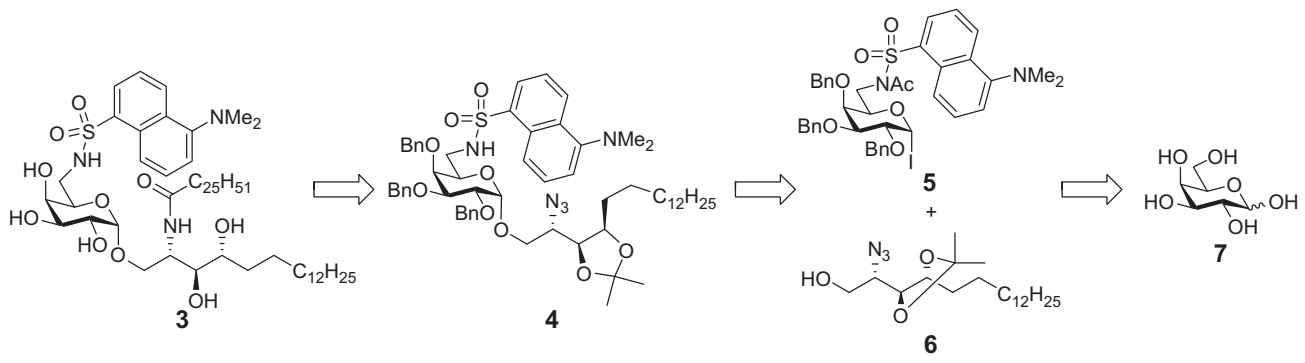
Despite there being much interest in α-GalCer, the need for a more efficient synthesis of an appropriately labelled fluorescent α-GalCer derivative remains. Indeed, our own desire to understand more about the mechanism by which α-GalCer is transferred to resident dendritic cells (DCs) during cancer immunotherapy,[25,26] drove us to devise an improved strategy for the synthesis of the dansylated α-GalCer derivative **3** (Fig. 1). Our objective was to achieve a robust synthesis of **3** with high reaction yields throughout, and to assess the ability of **3** to both activate *i*NKT cells and to serve as a fluorescent reporter group. The results of these studies are reported herein.

2. Results and discussion

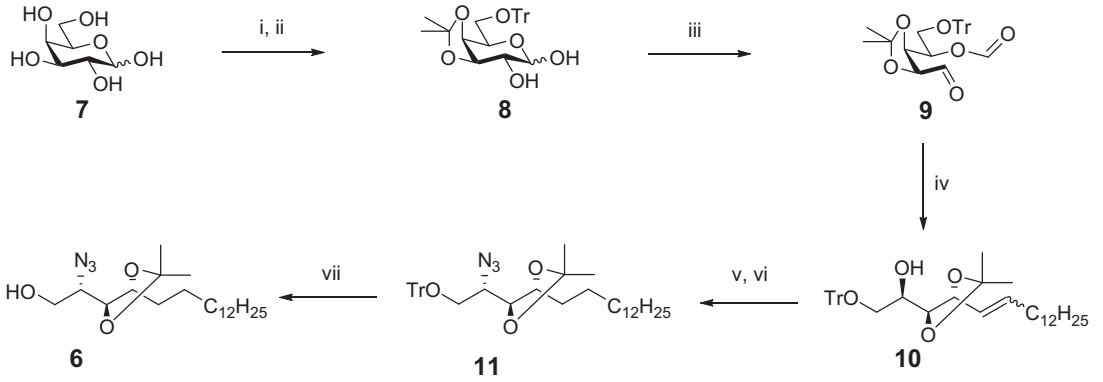
2.1. Synthesis of dansylated α-GalCer

Our retrosynthetic analysis for the preparation of dansylated α-GalCer **3** is presented in Scheme 1. We chose to prepare **3** from the mono-lipidated derivative **4**, itself formed via the coupling of dansylated galactosyl iodide donor **5** to the phytosphingosine backbone **6**. Donor **5** and acceptor **6** are in turn both readily prepared from D-galactose (**7**). The reasoning behind these key disconnects are twofold. First, though it is possible to couple the complete ceramide lipid backbone to a suitably protected galactose donor, the ceramide is a particularly poor acceptor and yields for these glycosylations are typically modest (ca. 25–55%). This is a general phenomenon largely independent of the type of galactose donor used.[8,15,27–31] There are also advantages to be gained by incorporating the fluorescent dansyl group at an early stage in the synthesis for it has been well documented that molecules containing a chromophore are more easily monitored by TLC and purified by flash column chromatography.[32] Accordingly, in contrast to other syntheses of fluorescent α-GalCer probes,[11–13] we envisioned a glycosylation reaction involving the use of a fluorescent galactose donor, such as **5**.

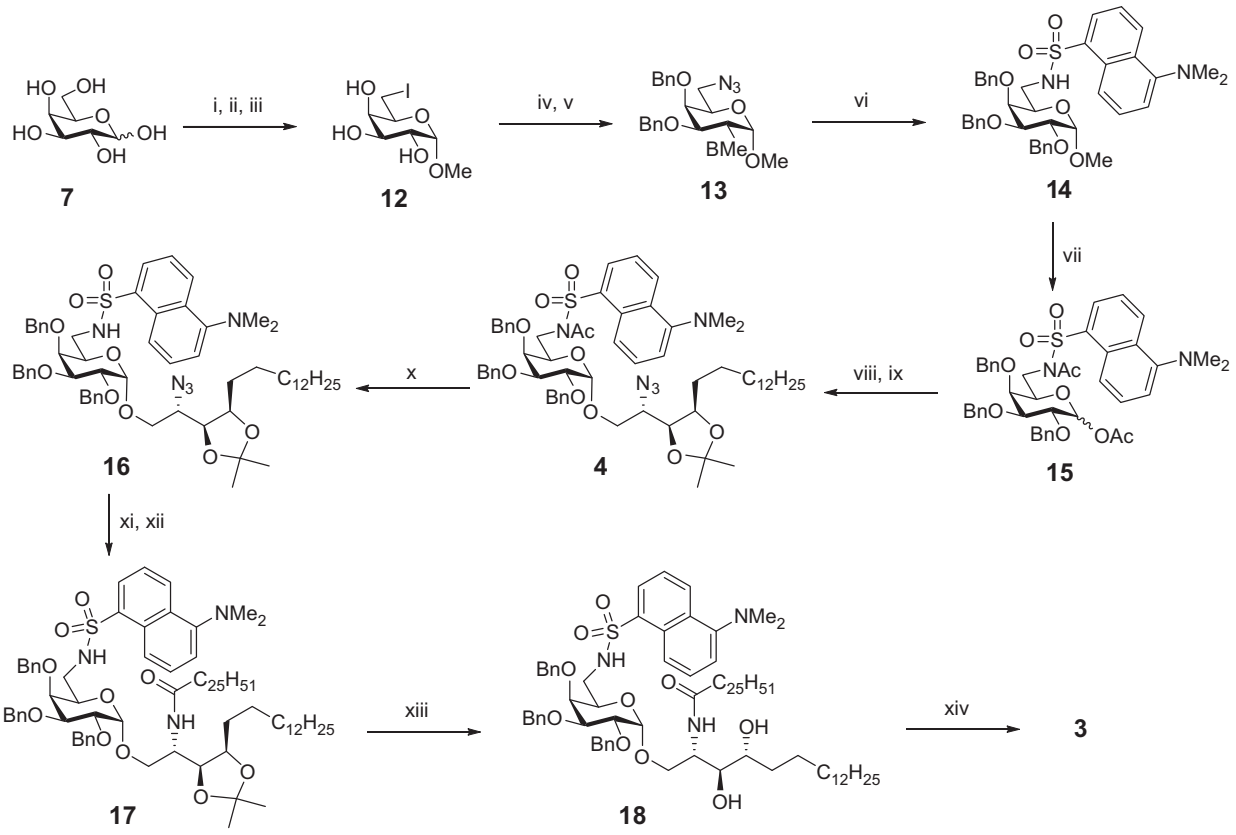
With our synthetic strategy in place, our first goal was the preparation of the phytosphingosine backbone **6** (Scheme 2). Here, our lipid synthesis commenced with the selective tritylation of D-galactose (**7**) at the primary position,[33] followed by installation of an isopropylidene at the 3- and 4-positions to give the diol **8** in 85% (over two steps).[34] Diol **8** was then treated with a solution of $NaIO_4$ in THF/H_2O, which resulted in cleavage of the diol and formation of the formate ester **9** in quantitative yield. As a temporary protecting group, the formate at the 4-position was a pivotal step in our strategy as it prevented cyclisation to the corresponding D-lyxofuranose derivative, which in our hands, gave only modest yields (50–60%) when subject to the subsequent Wittig reaction. Treatment of ester **9** with excess ylide (BuLi, 2.4 equiv, phosphonium bromide, 2.5 equiv), however, gave the corresponding alkene **10** in good (78%) yield and in an *E*:*Z* ratio of approximately 1:10 based on ^1H NMR analysis. Subsequent conversion of alkene **10** to the required phytosphingosine backbone then followed via quantitative hydrogenation, using 1.5 wt % Pd/C, and treatment with triflic anhydride to give the intermediate triflate, which was converted to azide **11** in situ. During the installation of the azide functionality, it was found that the best yields were obtained when the reaction was preformed at −15 °C as higher temperatures resulted in the elimination of the triflate and the formation of the corresponding olefinic by-product. Selective removal of the trityl ether in the presence of the isopropylidene group was then attempted. This transformation has proven problematic in the past,[7]



Scheme 1. Retrosynthetic analysis for the preparation of dansylated α-GalCer **3**.



Scheme 2. Reagents and conditions: (i) TrCl, pyridine, rt, 48 h; (ii) acetone, $CuSO_4$, rt, 3 d, 85% (two steps); (iii) $NaIO_4$, THF, H_2O, 0 °C to rt, overnight, quant.; (iv) BuLi (2.4 equiv), $BrPh_3PC_{13}H_{27}$ (2.5 equiv), THF, −78 °C, 15 min, then **9**, −78 °C to rt, 4.5 h, 78%; (v) H_2, Pd/C, THF, rt, overnight, quant.; (vi) Tf_2O, CH_2Cl_2, pyridine, −15 °C, 45 min, then NaN_3, DMF, −15 °C to rt, overnight, 81%; (vii) TFA, TES, $Me_2C(OMe)_2$, CH_2Cl_2, 0 °C, 2 min, 63%.



Scheme 3. Regents and conditions: (i) acetone, $ZnCl_2$, H_2SO_4, rt, 24 h, 92%; (ii) PPh_3, I_2, Imid., 90 °C, 5 h, 85%; (iii) AcCl, MeOH, 40 °C, 72 h, 92%; (iv) NaN_3, DMF, 80 °C, 5 h, 82%; (v) BnBr, NaH, DMF, 0 °C to rt, 2 h, 99%; (vi) PMe_3, THF, 0 °C to rt, 1 h, then dansyl chloride, 1 M NaOH, 0 °C to rt, 22 h, 93%; (vii) AcOH, Ac_2O, H_2SO_4, 0 °C, 3 h, quant.; (viii) TMSI, CH_2Cl_2, 0 °C, 1 h; (ix) TBAI, DiPEA, benzene, **6**, 70 °C, 20 h, 94% (two steps); (x) NaOMe, MeOH, CH_2Cl_2, rt, 3 d, quant.; (xi) PMe_3, 1 M NaOH, 0 °C to rt, 21 h; (xii) $HOC(O)C_{25}H_{51}$, EDCI, DMAP, CH_2Cl_2, rt, 43 h, 68% (two steps); (xiii) AcOH, H_2O, THF, 50 °C, 72 h, 74%; (xiv) H_2, $Pd(OH)_2$/C, $CHCl_3$, EtOH, rt, 17 h, 62%.

and resulted in the authors adopting a four-step deprotection/reprotection strategy to generate a modified 3-O, 4-O-benzylated phytosphingosine derivative. In view of the lability of the isopropylidene group, we treated azide **11** with TFA and TES, then added 2,2-dimethoxypropane to the reaction vessel to reinstall the isopropylidine, should it be cleaved under the acidic reaction conditions. Indeed, using this approach, phytosphingosine **6** was obtained in a respectable 63% yield. Numerous attempts were then made to further optimise the reaction including the addition of MeOH (to scavenge the liberated TES cation, which was sometimes found to be present on the 1-position of the lipid), however, this did not lead to any improvements in yield. The use of boron trichloride or formic acid to cleave the trityl group resulted in a lower yield of product. Despite this, in sum, the core phytosphingosine backbone **6** was prepared in seven steps and in a good overall yield of 34%. This represents one of the most efficient syntheses of the phytosphingosine backbone to date.[35,36]

Having completed the synthesis of the required phytosphingosine lipid, we then turned our attention to the preparation of the dansylated galactose donor. Our strategy commenced with the formation of the common azide (**13**)[11] using a modified sequence of reactions that first led to the formation of iodide **12** via the

selective installation of isopropylidenes at the 1,2 and 3,4 positions of D-galactose (**7**), subsequent installation of the iodide at the primary position, and then simultaneous cleavage of the isopropylidenes and Fisher glycosylation using a 5% solution of AcCl in MeOH (Scheme 3). This route provided a convenient means by which to prepare gram quantities of iodide **13** in excellent overall yield (72%, three steps) and without the need for column chromatography. The iodide in **12** was then displaced to give the intermediate azide, which was subsequently benzylated to give the fully protected azide **13**, again in excellent overall yield.

With the objective of installing the fluorescent group at an early stage in the synthesis, the dansyl group was then incorporated onto the galactose residue **13** in a two-step one-pot procedure involving the reduction of the azide to an amine using Staudinger conditions[10] and subsequent treatment with dansyl chloride in the presence of 1 M NaOH. Using this strategy, dansylated galactose **14** was prepared in excellent (93%) yield. Dansylated galactose **14** is a bright-green oil, and as such, is readily identified during flash column chromatography whereby the yellow-green band can be followed by eye during the purification process. Furthermore, incorporation of the dansyl flurophore also aids in the monitoring of the reaction by TLC analysis with **14** glowing bright white under UV light (λ = 254 nm). Treatment of **14** with a solution of AcOH, Ac_2O and H_2SO_4 at 0 °C for 3 h then resulted in the quantitative formation of acetate **15**. Here, it should be noted that in addition to the conversion of the anomeric methoxy to the acetate, the amine of the dansyl group was also acetylated. Though not optimal, the over-acetylation of dansyl groups has been previously reported[37] and we were confident that the superfluous acetyl could be readily removed during a subsequent step of the synthesis. A dramatic change in colour was also observed upon monitoring the reaction by TLC analysis (λ = 254 nm), with the methyl glycoside **14** being bright white, whilst the corresponding acetate **15** was mauve (see Supplementary data). As with all dansylated compounds prepared, acetate **15** was a fluorescent green colour, which allowed for its ready purification by flash column chromatography and illustrates the added value of the early incorporation of the dansyl-group into the synthetic plan.

Forging ahead with our synthesis, we thus converted glycosyl donor **15** to the corresponding glycosyl iodide via methodology based on seminal work by Gervay-Hague.[38–41] Though a number of galactosyl donors have been prepared and coupled to the phytosphingosine backbone en route to the preparation of α-GalCer,[7,9,10,27–29,42–45] few of these glycosylation reactions have been reported to be both high-yielding and highly α-selective.[38,46–48] To this end, we chose the glycosyl iodide route of Gervay-Hague and were delighted to report that glycolipid **4** could be prepared in 94% yield and as only the α-anomer, as determined by a ^1H $J_{1,2}$ coupling constant of 3.2 Hz and a ^{13}C–^1H ($^1J_{CH}$) coupling of 172.2 Hz.[49]

With glycolipid **4** in hand, we then set about installing the C26 acyl chain. First, the acetyl on the sulfonimide was removed using Zemplén conditions[50] to give the desired sulfonamide **16** in quantitative yield. Both glycolipid **4** and sulfonamide **16** had identical R_f values (0.40; PE/EA, 2/1, v/v), though were different colours when observed via TLC analysis. With glycolipid **4** being mauve in colour (UV light, λ = 254 nm) and sulfonamide **16** bright white, this greatly aided in the monitoring of the reaction. The isopropylidene group was then removed and the diol product subjected to hydrogenation conditions using $Pd(OH)_2/C$ in the presence of HCl in an attempt to simultaneously remove the benzyl groups and reduce the azide to an amine. Numerous attempts were made to achieve this transformation, yet despite the addition of HCl to protonate the resultant amine and prevent poisoning of the palladium catalyst, only partial debenzylation was observed. In view of this, we changed our strategy and first reduced the azide in **16** to the amine, using Staudinger methodology, then coupled the product to hexacosanoic acid via an EDCI/DMAP condensation protocol to give fully protected dansylated α-GalCer **17** in good (68%) overall yield. Removal of the isopropylidene group occurred smoothly to give **18** in 74% yield. Finally, global deprotection, again using $Pd(OH)_2/C$ as the catalyst, gave dansyl α-GalCer **3** in 62% yield, which glowed bright white under UV light (λ = 366 nm). Though glycolipids such as **3** have been reported to be notoriously difficult to solubilise and hence assign,[51,52] using a variety of 2D NMR spectroscopy techniques (COSY, HSQC, HMBC) with spectra recorded in pyridine-d_5, all ^{13}C and ^1H resonances were fully assigned. Furthermore, the dansyl group of **3** allowed for the ready detection of the compound by way of HPLC analysis (see Supplementary data). Thus, in summary, dansyl α-GalCer **3** was prepared in 14 linear steps from the commercially available and inexpensive D-galactose and in 16% overall yield. This represents a highly efficient synthesis of this important biological probe and is comparable to the work of Zhou et al. whereby the probe was prepared in <10% yield from 6-azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose.[11]

2.2. The effects of α-GalCer 3 on the stimulation of *i*NKT cells in vitro and in vivo

Having developed an efficient synthesis of dansyl α-GalCer **3**, we then set about to investigate whether **3** could activate *i*NKT cells in a manner similar to that of its parent compound, α-GalCer (**2**).[6,7,9,11,28,30,45] This was achieved by analysing levels of the cytokine IL-2 released by the Vα14$^+$ *i*NKT cell hybridoma[4] DN32.D3 following in vitro stimulation with the glycolipid compounds and by examining the expression of the co-stimulatory molecule, CD86, on splenic DC and B cells after injection of the compounds into mice.[9] The latter assay is based on the observation that CD86 is upregulated as a consequence of *i*NKT cells interacting with, and subsequently driving, DC and B cell maturation.[53,54]

To assess the ability of **3** to stimulate DN32.D3 *i*NKT cell hybridoma cells, titrated amounts of dansyl α-GalCer **3**, or α-GalCer **2**, were incubated for 24 h with the hybridoma cells in the presence of a constant number of murine DCs from the immortalised line, DC2114,[55] and supernatants were then collected and analysed for IL-2. Proliferation of the IL-2-dependent cell line HT-2 was used as a read-out of IL-2 levels. As illustrated (Fig. 2A), both α-GalCer **2** and dansyl α-GalCer **3** stimulated release of IL-2 in a dose-dependent manner, with α-GalCer **2** driving a higher level of this cytokine. This observation is consistent with literature precedent for weaker cytokine profiles generated by similar C6″-substituted α-GalCer derivatives, but is nonetheless indicative that dansyl α-GalCer **3** is indeed capable of *i*NKT cell activation.[11,15] The ability of dansyl α-GalCer **3** to stimulate *i*NKT cells in vivo was assessed by injecting mice with 200 ng of dansyl α-GalCer **3** or α-GalCer **2**, and then determining the expression of the maturation marker CD86 on the surface of splenic B cells and DCs by flow cytometry 20 h later. As shown, administration of fluorescent analogue **3** stimulated the maturation of B cells and DCs (Fig. 2B) with activities resembling that of the parent compound **2**. To confirm that this in vivo activity was mediated by *i*NKT cells, the experiment was repeated in CD1d deficient mice. Here, no up-regulation of CD86 was observed on DCs (Fig. 2C), confirming that dansyl α-GalCer **3**, like α-GalCer **2**, activates APCs by stimulating *i*NKT cells in a CD1d dependent manner.

2.3. Uptake of α-GalCer by dendritic cells

Having established that dansyl α-GalCer **3** binds to CD1d and activates *i*NKT cells in a manner similar to that of α-GalCer **2**, we then set out to investigate the ability of dansyl α-GalCer **3** to

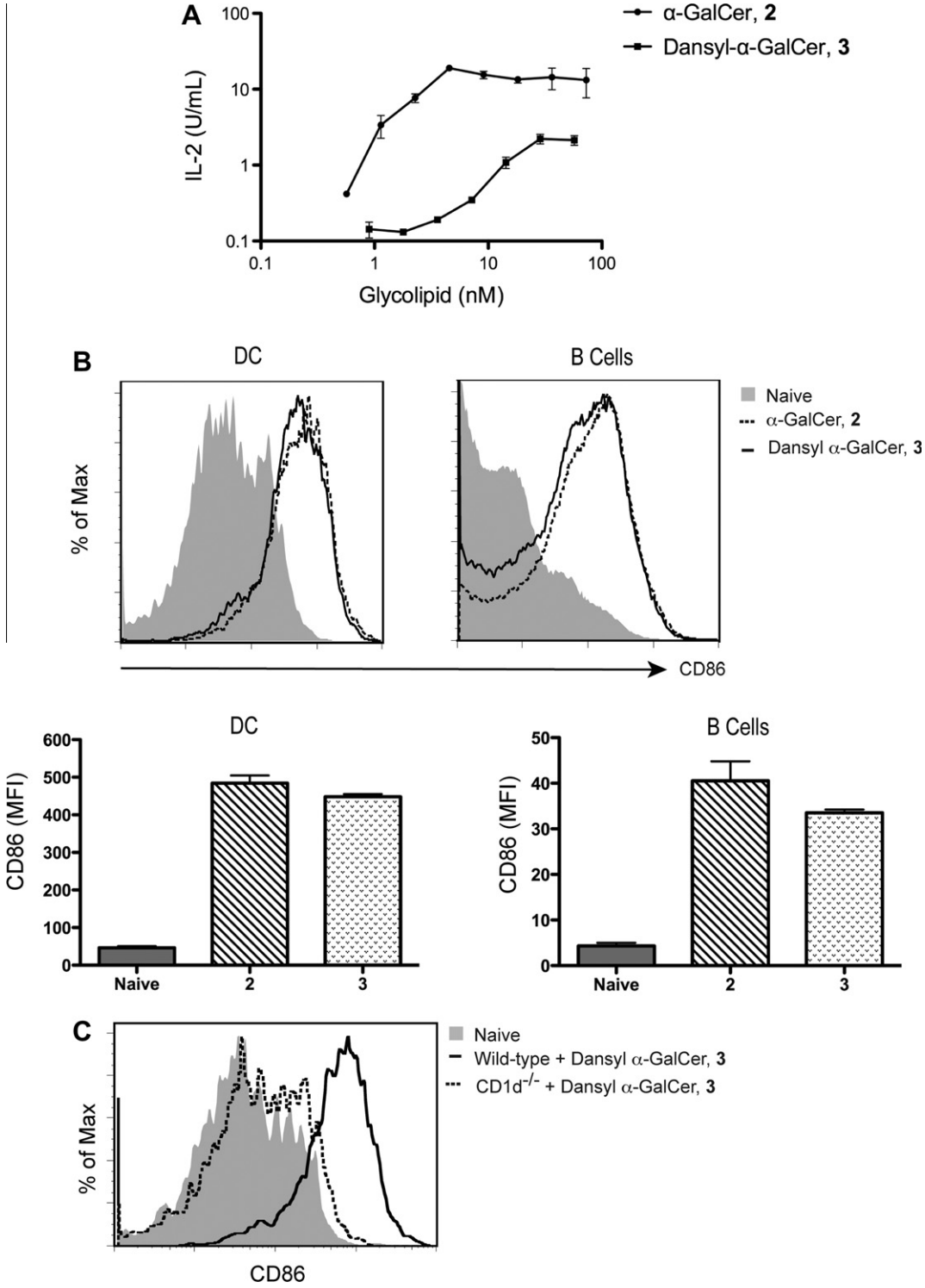


Figure 2. Dansyl α-GalCer **3** stimulates *i*NKT cells in vitro and in vivo: (A) production of IL-2 by *i*NKT cells (DN32.D3) in the presence of titrated doses of α-GalCer **2** or dansyl α-GalCer **3**; (B) dansyl α-GalCer **3** drives the maturation of splenic DCs and B cells in vivo. C57BL/6 mice were injected intravenously with 200 ng of α-GalCer **2** ($n = 3$) or dansyl α-GalCer **3** ($n = 3$), and spleens were removed 20 h after injection for antibody labelling and flow cytometry. The expression of CD86 on $CD11c^+$ dendritic cells and B220 B cells were assessed; (C) 200 ng of dansyl α-GalCer **3** was injected intravenously into wild type (C57BL/6) and $CD1d^{-/-}$ mice, and spleens were removed 20 h after injection for antibody labelling and flow cytometry. The spleen of an untreated wild type (C57BL/6) mouse was used as control. The expression of CD86 on $CD11c^+$ dendritic cells was assessed. Mean fluorescence intensities (MFI) ± SD are presented.

function as a fluorescent reporter group, which has previously not been reported. The wavelengths of maximum absorbance and emission of **3** were determined by spectrofluorimetry to be 343 nm and 510 nm, respectively, and these maxima were used in further analyses. The DC cell line, DC2114 was incubated with 4 μg of dansyl α-GalCer **3** for 24 h and uptake of the fluorescent glycolipid was analysed by flow cytometry (Fig. 3). A distinct shift in mean fluorescence intensity was observed for cells treated with dansyl α-GalCer **3**, but not for those treated with α-GalCer **2** or controls (untreated and vehicle), illustrating the ability of dansyl

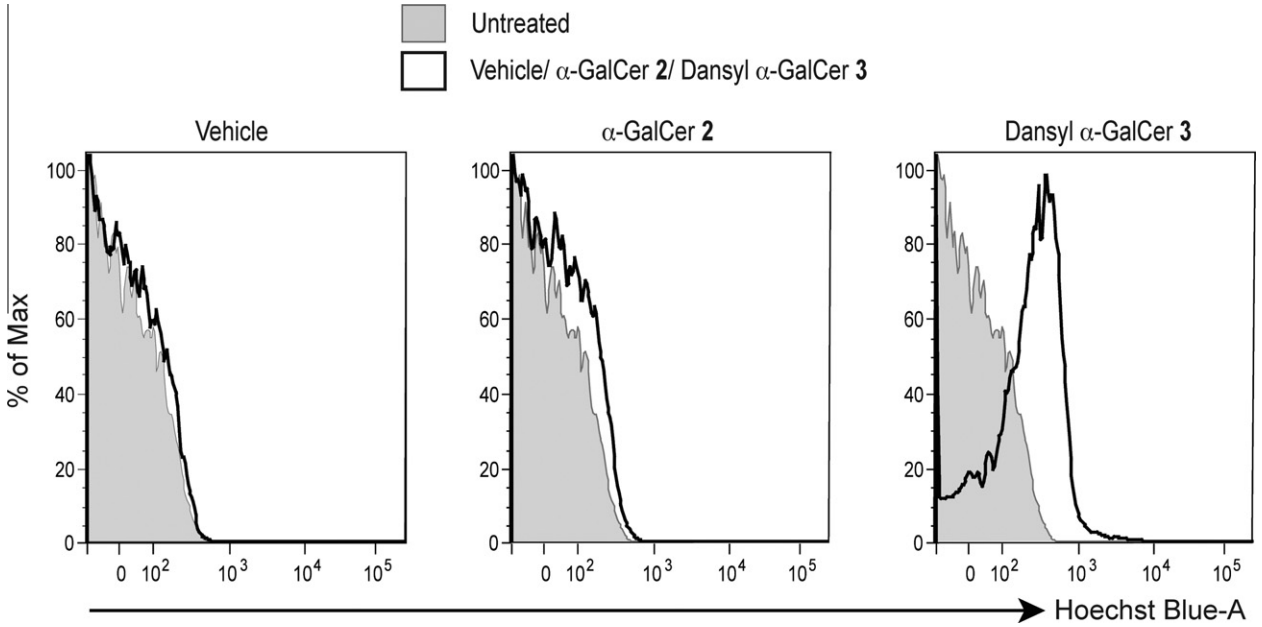


Figure 3. Cells that have taken up dansyl α-GalCer **3** can be detected by flow cytometry. 1×10^6 cells (DC2114 cell line) were incubated for 24 h with 4 μg of dansyl α-GalCer **3**, α-GalCer **2** or vehicle only. Cells were excited with the UV laser, and detected by the Hoechst Blue-A detector.

α-GalCer **3** to be used as a fluorescent probe to detect sub-populations of DCs that present the glycolipid.

3. Conclusion

In summary we have presented an efficient synthesis of the biologically important probe, dansyl α-GalCer **3**. En route to the synthesis of this glycolipid, we developed a highly efficient synthesis of the phytosphingosine lipid acceptor. In addition, we found that the early incorporation of the fluorescent group greatly aids in the monitoring and purification of reaction products. The ability of dansyl α-GalCer **3** to activate *i*NKT cells and to be used as a fluorescent probe for the analysis of glycolipid uptake by DCs has also been illustrated. Given the importance of α-GalCer **2**, it is anticipated that dansyl α-GalCer **3** will find wide application as a molecular tool for the better understanding of α-GalCer trafficking and presentation by CD1d.

4. Experimental

4.1. General

Unless otherwise stated all reactions were performed under N_2. Prior to use, THF (Pancreac) was distilled from $LiAlH_4$, pyridine was dried over 4 Å molecular sieves (4 Å MS), acetone (Pure Science) was dried over 3 Å MS, CH_2Cl_2 (Pancreac) was distilled from P_2O_5, TMSI (Aldrich) was distilled from antimony powder (M&B) and stored over Cu(s) powder (Hopkin & Williams), and H_2O and benzene (Fisher Scientific) were distilled. Trityl chloride (Acros), $CuSO_4$ (Fluka), $NaIO_4$ (M&B), anhydrous Et_2O (Pancreac), *n*-BuLi (Aldrich), 1-bromotridecane (Aldrich), PPh_3 (Merck), Pd/C (Aldrich, 10 wt %), Tf_2O (Aldrich), NaN_3 (BDH), anhydrous DMF (Acros), TFA (Aldrich), triethylsilane (Fluka), $Me_2C(OMe)_2$ (Aldrich), $ZnCl_2$ (Aldrich), H_2SO_4 (Lab-Scan), I_2 (BDH), imidazole (Aldrich), AcCl (B&M), BnBr (Fluka), NaH (Avocado Research Chemicals, 60% dispersion in mineral oil), PMe_3 (Aldrich, 1 M in THF), dansyl chloride (Acros), AcOH (Ajax Finechem), Ac_2O (Peking Reagent), TBAI (Riedel-de Haen), DiPEA (Aldrich), NaOMe (Janssen Chimica), $C_{25}H_{51}COOH$ (Acros), EDCI (Aldrich), DMAP (Merck), $Pd(OH)_2/C$ (Aldrich, 20 wt %), EtOAc (Pancreac), hexanes (Fisher Scientific), petroleum ether (Pure Science), MeOH (Pure Science), $CHCl_3$ (Pancreac), EtOH (absolute, Pure Science), NaOH (Pure Science), $NaHCO_3$ (Pure Science), NaCl (Pancreac) were used as received. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC-analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm, with fluorescent indicator UV_{254}) with detection by UV-absorption (short wave UV—254 nm; long wave UV—366 nm), by dipping in 10% H_2SO_4 in EtOH followed by charring at ~150 °C, by dipping in I_2 in silica, or by dipping into a solution of ninhydrin in EtOH followed by charring at ~150 °C. Column chromatography was performed on Pure Science silica gel (40–63 μm). AccuBOND II ODS-C18 (Agilent) was used for reverse phase chromatography. High-resolution mass spectra were recorded on a Waters Q-TOF Premier™ Tandem Mass Spectrometer using postive electro-spray ionisation. Optical rotations were recorded using a Perkin–Elmer 241 polarimeter or Autopol II (Rudolph Research Analytical) at 589 nm (sodium D-line). Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) sampling accessory and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were recorded at 20 °C in D_2O or $CDCl_3$ using either a Varian INOVA operating at 500 MHz or Varian VNMRS operating at 600 MHz. Chemical shifts are given in ppm (δ) relative to TMS. NMR peak assignments were made using COSY, HSQC and HMBC 2D experiments. Melting points were obtained using the Gallenkamp Melting Point Apparatus. Spectrofluorimetry measurements were carried out on the Horiba Jobin-Yvon Fluorolog-3 spectrofluorometer using the three dimensional (3D) scanning mode. High-pressure liquid chromatography was performed on Waters 600 liquid chromatograph with a photodiode array detector (Waters 2996) and a C-18 column (Waters Xbridge 5 μm).

4.2. 3,4-*O*-Isopropylidene-6-*O*-triphenylmethyl-D-galactopyranoside (8)

Trityl chloride (16.4 g, 58.8 mmol) was added to a solution of D-galactose (**7**) (10 g, 55.5 mmol) in pyridine (150 mL). The reaction mixture was stirred at 50 °C for 48 h and concentrated in vacuo.

The resulting residue was purified by chromatography through a short silica column and the tritylated galactose was eluted with acetone and crystallised from EtOH to afford the tritylated galactose (23.4 g, 55.4 mmol, quant.) [R_f: 0.17 (EtOAc)] as a white powder. To a solution of tritylated galactose (15 g, 35.5 mmol) in anhydrous acetone (750 mL) was added copper sulfate (60 g, 380 mmol). After stirring the reaction mixture for 3 d at room temperature, carbon was then added and the solution filtered through Celite. The filtrate was concentrated in vacuo and purified by flash column chromatography. Elution with 1:1 (v/v) hexanes/EtOAc afforded the title compound **8** (13.9 g, 30.1 mmol, 85%, two steps) as a clear oil. R_f: 0.65 (EtOAc); $[\alpha]_D^{20} = +18.0$ (*c* 1.0, $CHCl_3$); IR (film) 3406, 3059, 3034, 2986, 2936, 1597, 1491, 1449, 1380, 1219, 1162, 1132, 1079, 1032, 909, 872, 814, 776, 732, 705 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.48–7.46 (m, 6H, CPh_3, CH-*o*), 7.30 (t, $J_{o,m} = J_{m,p} = 7.4$ Hz, 6H, CPh_3, CH-*m*), 7.26–7.23 (m, 3H, CPh_3, CH-*p*), 5.23 (t, $J_{1,2} = J_{1,OH} = 3.7$ Hz, 1H, H-1α), 4.34–4.29 (m, 3H, H-3, H-4, H-5), 3.87–3.85 (m, 1H, H-2), 3.02 (dd, $J_{6a,6b} = 9.1$ Hz, $J_{6a,5} = 6.4$ Hz, 1H, H-6a), 3.33 (dd, $J_{6a,6b} = 9.1$ Hz, $J_{6b,5} = 6.4$ Hz, 1H, H-6a), 3.02 (d, $J_{OH,1} = 3.6$ Hz, 1H, 1-OH), 2.45–2.44 (m, 1H, 2-OH), 1.44 (s, 3H, CH_3 *i*Pr), 1.36 (s, 3H, CH_3 *i*Pr); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.9 (C-*i*, CPh_3), 128.8 (C-*o*, CPh_3), 127.8 (C-*m*, CPh_3), 127.0 (C-*p*, CPh_3), 109.5 (C_q *i*Pr), 91.2 (C-1), 86.8 (C_q CPh_3), 75.4 (C-3), 72.9 (C-4), 69.1 (C-2), 67.9 (C-5), 62.8 (C-6), 27.4, 25.7 (2 × CH_3 *i*Pr); HRMS(ESI) *m/z* calcd for $[C_{28}H_{30}O_6+Na]^+$: 485.1940, obsd.: 485.1949.

4.3. 4-*O*-Formyl-2,3-*O*-isopropylidene-5-*O*-triphenylmethyl-D-lyxose (9)

A solution of $NaIO_4$ (0.46 g, 2.14 mmol) in H_2O (4 mL) was added to **8** (0.66 g, 1.42 mmol) dissolved in 4 mL THF at 0 °C. After stirring at room temperature overnight, the reaction mixture was diluted with EtOAc (15 mL) and washed with water (20 mL), saturated $NaHCO_3$ solution (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated in vacuo to afford the title compound **9** (0.65 g, 1.42 mmol, quant.) as a clear oil, which was carried through without further purification. R_f: 0.48 (PE/EA, 1/1, v/v); IR (film) 3450, 3060, 3025, 2989, 2938, 2360, 2341, 2252, 1732, 1597, 1491, 1449, 1383, 1218, 1160, 1076, 987, 907, 764, 729, 701, 648 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.60 (d, $J_{1,2} = 2.4$ Hz, 1H, H-1), 8.00 (s, 1H, HCO_2), 7.44–7.41 (m, 6H, CPh_3, CH-*o*), 7.34–7.30 (m, 6H, CPh_3, CH-*m*), 7.27–7.24 (m, 3H, CPh_3, CH-*p*), 5.18 (q, $J_{3,4} = J_{4,5a} = J_{4,5b} = 4.9$ Hz, 1H, H-4), 4.74 (dd, $J_{2,3} = 7.7$ Hz, $J_{3,4} = 4.2$ Hz, 1H, H-3), 4.31 (dd, $J_{2,3} = 7.7$ Hz, $J_{1,2} = 2.4$ Hz, 1H, H-2), 3.40 (dd, $J_{5a,5b} = 9.8$ Hz, $J_{4,5a} = 5.6$ Hz, 1H, H-5a), 3.37 (dd, $J_{5a,5b} = 9.8$ Hz, $J_{4,5b} = 5.6$ Hz, 1H, H-5b), 1.56 (s, 3H, *i*Pr CH_3), 1.42 (s, 3H, *i*Pr CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.1 (C-1), 159.7 (C-6), 128.6 (CH-*o*, CPh_3), 127.9 (CH-*m*, CPh_3), 127.2 (CH-*p*, CPh_3), 111.1 (C_q *i*Pr), 87.2 (C_q CPh_3), 80.2 (C-2), 77.0 (C-3), 69.5 (C-4), 61.8 (C-5), 26.8, 25.1 (2 × CH_3 *i*Pr); HRMS(ESI) *m/z* calcd for $[C_{28}H_{28}O_6+Na]^+$: 483.1784, obsd.: 483.1793.

4.4. 3,4-*O*-Isopropylidene-1-*O*-triphenylmethyl-D-*arabino*-octadec-5-en-1,2,3,4-tetraol (10)

A mixture of 1-bromotridecane (7.0 mL, 27.4 mmol) and triphenylphosphine (7.18 g, 27.4 mmol) was heated at 140 °C under N_2 atmosphere for 5 h. The reaction mixture was allowed to cool to room temperature and formed a solid gel. The gel was dissolved in boiling dry acetone (36 mL) and dry diethyl ether (87 mL) was added and the compound left to crystallise, which greatly improved in subsequent batches via the use of seed crystals. The crystals were filtered under N_2 atmosphere to afford $BrPh_3PC_{13}H_{27}$ (11.1 g, 21.1 mol, 77%) as white needles. *n*-BuLi (6.6 mL, 13.2 mmol) was added to $BrPh_3PC_{13}H_{27}$ (7.0 g, 13.7 mmol) dissolved in distilled THF (130 mL) at −78 °C and stirred for 15 min. To the bright orange mixture, a solution of formate ester **9** (co-evaporated with dry toluene × 3) in THF (55 mL) was added. The reaction mixture was stirred at −78 °C for 1 h, then warmed to room temperature and stirred for a further 3.5 h before being quenched with NH_4Cl solution (150 mL), concentrated, and redissolved in ethyl acetate (100 mL). The organic layer was washed with saturated $NaHCO_3$ solution (100 mL), water (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography and the desired product eluted with 8:1 (v/v) petroleum ether/EtOAc to afford alkene **10** as a clear oil (2.57 g, 4.29 mmol, 78%) in an approximate *E*:*Z*, 1:10. R_f: 0.33 (PE/EA, 5/1, v/v); $[\alpha]_D^{20} = -22.2$ (*c* 1.0, $CHCl_3$); IR (film) 3559, 3058, 3023, 2924, 2854, 1598, 1491, 1449, 1380, 1214, 1162, 1073, 898, 880, 763, 746, 705 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.48–7.44 (m, 6H, CPh_3, CH-*o*), 7.33–7.29 (m, 6H, CPh_3, CH-*m*), 7.26–7.23 (m, 3H, CPh_3, CH-*p*), 5.59 (dd, $J_{5,6} = 11.3$ Hz, $J_{6,7} = 6.0$ Hz, 1H, H-6), 5.55 (dd, $J_{5,6} = 11.3$ Hz, $J_{4,5} = 7.7$ Hz, 1H, H-5), 4.92 (dd, $J_{4,5} = 7.7$ Hz, $J_{3,4} = 6.9$ Hz, 1H, H-4), 4.23 (dd, $J_{3,4} = 6.9$ Hz, $J_{2,3} = 4.3$ Hz, 1H, H-3), 3.77 (pent, $J_{1,2} = J_{2,3} = J_{2,OH} = 5.4$ Hz, 1H, H-2), 3.18 (dd, $J_{1a,1b} = 9.4$ Hz, $J_{1a,2} = 5.0$ Hz, 1H, H-1a), 3.13 (dd, $J_{1a,1b} = 9.4$ Hz, $J_{1b,2} = 6.4$ Hz, 1H, H-1b), 2.03–1.98 (m, 1H, H-7a), 1.82–1.77 (m, 1H, H-7a), 1.51 (s, 3H, CH_3 *i*Pr), 1.41 (s, 3H, CH_3 *i*Pr), 1.33–1.23 (m, 20H, H-8–H-17), 0.90 (t, $J_{17,18} = 7.0$ Hz, 3H, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.9 (C-*i*, CPh_3), 135.4 (C-6), 128.7 (CH-*o*, CPh_3), 127.8 (CH-*m*, CPh_3), 127.0 (CH-*p*, CPh_3), 125.0 (C-5), 108.4 (C_q *i*Pr), 86.7 (C_q CPh_3), 77.5 (C-3), 73.0 (C-4), 69.2 (C-2), 65.0 (C-1), 31.9, 29.70, 29.67, 29.62, 29.52, 29.50, 29.4, 29.3, 27.6, 22.7 (C-7–C-17), 27.4, 25.1 (2 × CH_3 *i*Pr), 14.2 (C-18); HRMS(ESI) *m/z* calcd for $[C_{40}H_{54}O_4+Na]^+$: 621.3920, obsd.: 621.3926.

4.5. 2-Azido-3,4-*O*-isopropylidene-1-*O*-triphenylmethyl-D-*arabino*-octadecane-1,3,4-triol (11)

Pd/C (1.5 wt %) was added to a solution of **10** (2.52 g, 4.21 mmol) in distilled THF (42 mL) and stirred under H_2 (g) overnight at room temperature. The reaction mixture was then filtered through Celite and concentrated to afford 3,4-*O*-isopropylidene-1-*O*-triphenylmethyl-D-*arabino*-octadecane-1,2,3,4-tetraol as a clear oil (2.53 g, 4.21 mmol, quant.), which was used without further purification. R_f: 0.44 (PE/EA, 5/1, v/v); $[\alpha]_D^{20} = -4.0$ (*c* 1.0, $CHCl_3$); IR (film) 3560, 3060, 3023, 2923, 2853, 1598, 1491, 1449, 1379, 1216, 1073, 899, 872, 762, 746, 705 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.47 (d, $J_{o,m} = 7.7$ Hz, 6H, CPh_3, CH-*o*), 7.31 (t, $J_{o,m} = J_{m,p} = 7.7$ Hz, 6H, CPh_3, CH-*m*), 7.27–7.23 (m, 3H, CPh_3, CH-*p*), 4.15 (dd, $J_{2,3} = 6.0$ Hz, $J_{3,4} = 3.8$ Hz, 1H, H-3), 4.11–4.07 (m, 1H, H-4), 3.74 (dt, $J_{1,2} = 9.5$ Hz, $J_{2,3} = 6.0$ Hz, 1H, H-2), 3.24–3.19 (m, 2H, H-1a, H-1b), 2.34 (d, $J_{OH,2} = 5.9$ Hz, 1H, OH), 1.71–1.64 (m, 2H, H-5), 1.51–1.28 (m, 24H, H-6–H-17), 1.46 (s, 3H, *i*Pr CH_3), 1.37 (s, 3H, *i*Pr CH_3), 0.89 (t, $J_{17,18} = 6.9$ Hz, 3H, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.9 (C-*i*, CPh_3), 128.7 (CH-*o*, CPh_3), 127.8 (CH-*m*, CPh_3), 127.1 (CH-*p*, CPh_3), 107.7 (C_q *i*Pr), 86.8 (C_q CPh_3), 77.5 (C-4), 77.0 (C-3), 69.0 (C-2), 65.2 (C-1), 31.9, 29.72, 29.68, 29.63, 29.62, 29.57, 29.39, 26.8, 22.7 (C-5–C-17), 27.4, 25.2 (2 × CH_3 *i*Pr), 14.2 (C-18); HRMS(ESI) *m/z* calcd for $[C_{40}H_{56}O_4+Na]^+$: 623.4076, obsd.: 623.4080. 3,4-*O*-Isopropylidene-1-*O*-triphenylmethyl-D-*arabino*-octadecane-1,2,3,4-tetraol (1.59 g, 2.65 mmol) was then co-evaporated with dry toluene (×3), dissolved in a mixture of dry CH_2Cl_2 (8 mL) and distilled pyridine (0.54 mL) and cooled to −15 °C. Triflic anhydride (0.67 mL, 3.97 mmol) was added dropwise over 5 min and stirred at −15 °C for 45 min to afford the triflate intermediate [R_f: 0.56 (PE/EA, 5/1, v/v)]. Dry DMF (27 mL) was cooled to −15 °C and added to the reaction mixture, followed by the addition of sodium azide (0.69 g, 10.61 mmol). The reaction mixture was then warmed to room temperature,

stirred overnight, and quenched by adding ice water (120 mL). The desired product was extracted with EtOAc (2 × 150 mL) and the combined organic layers washed with saturated $NaHCO_3$ solution (200 mL), water (200 mL) and brine (200 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by silica gel flash column chromatography and eluted with 1.5% EtOAc/hexanes to afford the title compound **11** (1.35 g, 2.16 mmol, 81%) as an amorphous white solid. R_f: 0.61 (PE/EA, 5/1, v/v); $[\alpha]_D^{25} = +7.0$ (*c* 1.0, $CHCl_3$), Lit:[56] $[\alpha]_D^{26} = +9.0$ (*c* 1.0, $CHCl_3$); IR (film) 3060, 2923, 2853, 2097, 1598, 1491, 1379, 1370, 1246, 1219, 1156, 1073, 1034, 900, 869, 762, 744, 703 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, $J_{o,m}$ = 7.3 Hz, 6H, CPh_3, CH-*o*), 7.32 (t, $J_{o,m} = J_{m,p}$ = 7.6 Hz, 6H, CPh_3, CH-*m*), 7.26 (d, $J_{o,m}$ = 7.1 Hz, 3H, CPh_3, CH-*p*), 4.13–4.09 (m, 1H, H-4), 3.89 (dd, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 5.5 Hz, 1H, H-3), 3.55 (dd, $J_{1a,1b}$ = 9.9 Hz, $J_{1a,2}$ = 2.5 Hz, 1H, H-1a), 3.50 (dt, $J_{2,3}$ = 9.5 Hz, $J_{1a,2}$ = 2.5 Hz, 1H, H-2), 3.37 (dd, $J_{1a,1b}$ = 9.9 Hz, $J_{1b,2}$ = 7.8 Hz, 1H, H-1b), 1.59–1.28 (m, 26H, H-5–H-17), 1.27 (s, 3H, *i*Pr CH_3), 1.24 (s, 3H, *i*Pr CH_3), 0.90 (t, $J_{17,18}$ = 6.9 Hz, 3H, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.8 (C-*i*, CPh_3), 128.7 (CH-*o*, CPh_3), 127.9 (CH-*m*, CPh_3), 127.1 (CH-*p*, CPh_3), 108.1 (C_q CPh_3), 87.3 (C_q *i*Pr), 77.9 (C-4), 75.9 (C-3), 64.6 (C-1), 60.8 (C-2), 32.0, 29.73, 29.69, 29.66, 29.64, 29.57, 29.4, 29.3, 26.5, 22.7 (C-5–C-17), 28.0, 25.7 (2 × CH_3 *i*Pr), 14.2 (C-18); HRMS(ESI) *m/z* calcd for $[C_{40}H_{55}N_3O_3+Na]^+$: 648.4141, obsd.: 648.4145.

4.6. 2-Azido-3,4-*O*-isopropylidene-D-*ribo*-octadecane-1,3,4-triol (6)

Triethylsilane (91.8 μL, 0.58 mmol) was added to a stirred solution of azide **11** (181 mg, 0.289 mmol) (co-evaporated with dry toluene × 3) in dry CH_2Cl_2 (5 mL) at 0 °C. Trifluoroacetic acid (TFA) (90 μL, 1.17 mmol) was added very slowly, allowing the reaction mixture to return to a colourless solution before adding the next drop. After stirring for 2 min, 2,2-dimethoxypropane (7 μL, 0.057 mmol) was added and the reaction mixture stirred for a further 1 min before being quenched with saturated $NaHCO_3$ solution (50 mL). The product was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers washed with water (80 mL) and brine (80 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by silica gel flash column chromatography and eluted with 5:1 (v/v) petroleum ether/EtOAc to afford the title compound **6** (69.3 mg, 0.18 mmol, 63%) as an amorphous white solid. R_f: 0.21 (PE/EA, 5/1, v/v); $[\alpha]_D^{20} = +24.0$ (*c* 1.0, $CHCl_3$); IR (film) 3426, 2923, 2853, 2098, 1465, 1370, 1247, 1220, 1170, 1063, 909, 869, 734 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.21–4.17 (m, $J_{3,4}$ = 5.7 Hz, H-4), 4.0 (ddd, $J_{1a,1b}$ = 11.8 Hz, $J_{1a,OH}$ = 5.1 Hz, $J_{1a,2}$ = 4.4 Hz, 1H, H-1a), 3.97 (dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 5.7 Hz, 1H, H-3), 3.87 (ddd, $J_{1a,1b}$ = 11.8 Hz, $J_{1b,OH}$ = 6.6 Hz, $J_{1b,2}$ = 5.6 Hz, 1H, H-1b), 3.48 (ddd, $J_{2,3}$ = 9.6 Hz, $J_{1b,2}$ = 5.6 Hz, $J_{1a,2}$ = 4.4 Hz, 1H, H-2), 2.20 (dd, $J_{1b,OH}$ = 6.6 Hz, $J_{1a,OH}$ = 5.1 Hz, 1H, OH), 1.63–1.55 (m, 3H, H-5a, H-5b, H-6a), 1.43 (s, 3H, *i*Pr CH_3), 1.34 (s, 3H, *i*Pr CH_3), 1.41–1.26 (m, 23H, H-6b, H-7–H-17), 0.88 (t, $J_{17,18}$ = 7.0 Hz, 3H, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 108.4 (C_q *i*Pr), 77.7 (C-4), 76.7 (C-3), 63.9 (C-1), 61.2 (C-2), 31.9, 29.70, 29.69, 29.68, 29.66, 29.65, 29.60, 29.58, 29.53, 29.39, 29.36, 26.52, 22.7 (C-5–C-17), 28.0, 25.5 (2 × CH_3 *i*Pr), 14.1 (C-18); HRMS(ESI) *m/z* calcd for $[C_{21}H_{41}N_3O_3+Na]^+$: 406.3046, obsd.: 406.3049.

4.7. Methyl 6-deoxy-6-iodo-α-D-galactopyranoside (12)

To a mixture of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[57,58] (2.6 g, 10 mmol), PPh_3 (3.93 g, 15 mmol) and imidazole (1.36 g, 20 mmol) in dry THF (100 mL), was added iodine (3.81 g, 15 mmol) in small portions. After refluxing for 1 h, the reaction mixture was cooled to room temperature, and quenched by the addition of 10% aq $Na_2S_2O_4$. The product was extracted with EtOAc, and the combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated. Distillation of the residue gave 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose as a yellow oil (3.14 g, 8.48 mmol, 85%). AcCl (1.8 mL) was added dropwise to a mixture of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3.14 g, 8.48 mmol) in MeOH (60 mL). After stirring for 2 d, the mixture was concentrated in vacuo and the residue crystallised from MeOH, to give the title compound **12** (2.37 g, 7.80 mmol, 92%) as white crystals. Mp 175.0–175.6 °C, Lit:[59] 162 °C; R_f = 0.43 (MeOH/EtOAc, 1/9, v/v); $[\alpha]_D^{21} = +129.0$ (*c* 1.0, H_2O), $[\alpha]_D^{21} = +122.1$ (*c* 1.0, MeOH), Lit:[59] $[\alpha]_D^{22} = +142.0$ (*c* 1.0, H_2O); IR (film) 3354, 3227, 3007, 2953, 2935, 2906, 2836, 1454, 1417, 1360, 1347, 1297, 1257, 1200, 1145, 1132, 1102, 1078, 1026, 999, 935, 886, 854, 790, 721, 691, 657 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.69 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 3.97 (dd, $J_{3,4}$ = 3.3, $J_{4,5}$ = 1.2 Hz, 1H, H-4), 3.90 (ddd, $J_{4,5}$ = 1.2 Hz, $J_{5,6a}$ = 5.3, $J_{5,6b}$ = 8.0 Hz 1H, H-5), 3.75 (dd, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.2 Hz, 1H, H-2), 3.69 (dd, $J_{2,3}$ = 10.2, $J_{3,4}$ = 3.3 Hz, 1H, H-3), 3.46 (s, 3H, OMe), 3.37 (dd, $J_{5,6a}$ = 5.3, $J_{6a,6b}$ = 10.2 Hz, 1H, H-6a), 3.33 (dd, $J_{5,6b}$ = 8.0, $J_{6a,6b}$ = 10.2 Hz, 1H, H-6b); ^{13}C NMR (125 MHz, D_2O) δ 100.2 (C-1), 71.4 (C-5), 70.4 (C-4), 69.9 (C-2), 68.4 (C-3), 54.6 (OMe), 2.3 (C-6). HRMS(ESI) *m/z* calcd for $[C_7H_{13}IO_5+Na]^+$: 326.9700, obsd.: 326.9709.

4.8. Methyl 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranoside (13)

Sodium azide (4.28 g, 0.066 mmol) was added to iodide **12** (5.01 g, 0.016 mmol) dissolved in dry DMF (300 mL). After stirring overnight at room temperature, MeOH (150 mL) was added and the reaction mixture was concentrated in vacuo. The residue was purified by silica gel flash column chromatography and the product eluted with 8% MeOH/CH_2Cl_2. The residue was crystallised from MeOH to afford methyl 6-azido-6-deoxy-α-D-galactopyranoside (2.96 g, 14 mmol, 82%) as white crystals. Mp 173.8–174.1 °C, Lit:[60] 172–173 °C; R_f = 0.43 (MeOH/EtOAc, 1/9, v/v); $[\alpha]_D^{23} = +144.0$ (*c* 1.0, H_2O), Lit:[61] $[\alpha]_D^{22} = +154.0$ (*c* 0.8, H_2O); IR (film) 3371, 3238, 2934, 2091, 1642, 1459, 1349, 1297, 1245, 1137, 1107, 1080, 1029, 961, 791, 730, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.73 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 3.90 (ddd, $J_{5,6a}$ = 8.8 Hz, $J_{5,6b}$ = 4.0 Hz, $J_{4,5}$ = 1.1 Hz, 1H, H-5), 3.79 (dd, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 1.1 Hz, 1H, H-4), 3.77 (dd, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 3.72 (dd, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.3 Hz, 1H, H-3), 3.58 (dd, $J_{6a,6b}$ = 12.8 Hz, $J_{5,6a}$ = 8.8 Hz, 1H, H-6a), 3.44 (s, 3H, OCH_3), 3.27 (dd, $J_{6a,6b}$ = 12.8 Hz, $J_{5,6b}$ = 4.0 Hz, 1H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 100.2 (C-1), 70.0, 69.83, 69.77 (C-3, C-4, C-5), 68.6 (C-2), 54.3 (OCH_3), 51.3 (C-6); HRMS(ESI) *m/z* calcd for $[C_7H_{13}N_3O_5+Na]^+$: 242.0753, obsd.: 242.0749. Methyl 6-azido-6-deoxy-α-D-galactopyranose (1.53 g, 6.98 mmol) was then co-evaporated from dry DMF (×1), dissolved in dry DMF (150 mL) and cooled to 0 °C. Benzyl bromide (3.3 mL, 27.9 mmol) was added followed by sodium hydride (60% in oil suspension) (1.40 g, 34.9 mmol) and the reaction mixture allowed to warm to rt. After stirring for 2 h, the reaction was quenched with MeOH (20 mL) and concentrated in vacuo. The residue was taken up in EtOAc (100 mL) and the mixture was washed with water (100 mL), saturated $NaHCO_3$ solution (100 mL), water (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by silia gel flash column chromatography and eluted with 20:1 (v/v) petroleum ether/EtOAc to afford the title compound **13** (3.37 g, 6.88 mmol, 99%) as a clear oil. R_f: 0.72 (PE/EA, 1/1, v/v); $[\alpha]_D^{20} = +4.6$ (*c* 1.0, $CHCl_3$); IR (film) 2937, 2098, 1453, 1351, 1124, 1094, 1045, 789, 735 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.44–7.31 (m, 15H, H_{arom}), 5.03 (d, $J_{a,b}$ = 11.4 Hz, 1H, CH-a, 4-*O*-Bn), 4.93 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-a, 3-*O*-Bn), 4.87 (d, $J_{a,b}$ = 12.1 Hz,

1H, CH-a, 2-*O*-Bn), 4.78 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-b, 3-*O*-Bn), 4.72 (d, $J_{a,b}$ = 12.1 Hz, 1H, CH-b, 2-*O*-Bn), 4.72 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 5.03 (d, $J_{a,b}$ = 11.4 Hz, 1H, CH-b, 4-*O*-Bn), 4.07 (dd, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 3.96 (dd, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 2.6 Hz, 1H, H-3), 3.83 (dd, $J_{5,6a}$ = 8.3 Hz, $J_{5,6b}$ = 4.7 Hz, 1H, H-5), 3.80 (d, $J_{3,4}$ = 2.6 Hz, 1H, H-4), 3.53 (dd, $J_{6a,6b}$ = 12.7 Hz, $J_{5,6a}$ = 8.3 Hz, 1H, H-6a), 3.43 (s, 3H, OCH_3), 2.94 (dd, $J_{6a,6b}$ = 12.7 Hz, $J_{5,6b}$ = 4.7 Hz, 1H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.7 (C-*i*, 3-*O*-Bn), 138.4 (C-*i*, 2-*O*-Bn), 138.1 (C-*i*, 4-*O*-Bn), 128.52, 128.50, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6 (8 × CH_{arom}), 98.8 (C-1), 79.0 (C-3), 76.3 (C-2), 75.3 (C-4), 74.6 (CH_2, 4-*O*-Bn), 73.70, 73.65 (2 × CH_2, 2-*O*-Bn, 3-*O*-Bn), 69.8 (C-5), 55.5 (OCH_3), 51.5 (C-6); HRMS(ESI) *m/z* calcd for $[C_{28}H_{31}N_3O_5+Na]^+$: 512.2161, obsd.: 512.2159.

4.9. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-[*N*-(5-[dimethylamino]napth-1-ylsulfonyl) 'amido]-α-D-galactopyranoside (14)

Trimethylphosphine (1 M in THF) (10.2 mL, 10.2 mmol) was added to a solution of **13** (1.0 g, 2.04 mmol) (co-evaporated with toluene × 3) in distilled THF (10 mL) at 0 °C. After stirring for 15 min at 0 °C and 45 min at rt, the reaction mixture was cooled to 0 °C and dansyl chloride (1.38 g, 5.11 mmol) was added followed by the addition of NaOH (1 M, 8.17 mL, 8.17 mmol) over 10 min. The reaction was then allowed to warm to rt and after 6 h, further dansyl chloride (0.83 g, 3.06 mmol) and NaOH (1 M, 2.04 mL, 2.04 mmol) were added. After stirring for a further 16 h, the solution was diluted with EtOAc (50 mL), and the organic layer washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by silica gel flash column chromatography and eluted with 1:1 (v/v) petroleum ether/EtOAc to afford the title compound **14** (1.32 g, 1.89 mmol, 93%) as a lime green foam. R_f: 0.61 (PE/EA, 1/1, v/v); Glows white on TLC (λ = 254 nm); $[\alpha]_D^{19} = +9.2$ (*c* 1.0, $CHCl_3$); IR (film) 3293, 3063, 3030, 2988, 2349, 1588, 1574, 1497, 1326, 1200, 1143, 791, 735, 698 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, $J_{CH\text{-}2,CH\text{-}3}$ = 8.8 Hz, 1H, CH-2 Dan), 8.13 (d, $J_{CH\text{-}3,CH\text{-}4}$ = 7.4 Hz, 1H, CH-4 Dan), 8.08 (d, $J_{CH\text{-}7,CH\text{-}8}$ = 8.7 Hz, 1H, CH-8 Dan), 7.54 (dd, $J_{CH\text{-}7,CH\text{-}8}$ = 8.7 Hz, $J_{CH\text{-}6,CH\text{-}7}$ = 7.7 Hz, 1H, CH-7 Dan), 7.50 (d, $J_{CH\text{-}2,CH\text{-}3}$ = 8.8 Hz, 1H, CH-8 Dan), 7.38–7.26 (m, 15H, H_{arom}), 7.20–7.17 (m, 3H, H_{arom}, CH-6 Dan), 4.84 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-a, 4-*O*-Bn), 4.77 (d, $J_{a,b}$ = 12.0 Hz, 1H, CH-a, 2-*O*-Bn), 4.76 (d, $J_{a,b}$ = 11.5 Hz, 1H, CH-a, 3-*O*-Bn), 4.61 (d, $J_{a,b}$ = 12.0 Hz, 2H, CH-b, 2-*O*-Bn, CH-b, 3-*O*-Bn), 4.47 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 4.29 (dd, $J_{NH,6a}$ = 8.4 Hz, $J_{NH,6b}$ = 4.6 Hz, 1H, NH Dan), 3.86 (dd, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.4 Hz,1H, H-2), 3.65 (dd, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 2.7 Hz, 1H, H-3), 3.64 (br s, 1H, H-4), 3.48 (t, $J_{5,6}$ = 6.6 Hz, 1H, H-5), 3.23 (s, 3H, OCH_3), 2.99–2.77 (m, 2H, H-6a, H-6b), 2.88 (s, 6H, NMe_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.3 (2 × C-*i*, 2-*O*-Bn, 3-*O*-Bn), 137.9 (C-*i*, 4-*O*-Bn), 134.9 (C-4a Dan), 130.4 (C-2 Dan), 129.5, 128.9, 128.6, 128.5, 128.4, 128.3, 128.1, 127.8, 127.7, 127.6 (10 × CH_{arom}), 123.4 (C-3 Dan), 118.9 (C-8 Dan), 115.4 (C-6 Dan), 98.7 (C-1), 79.0 (C-3), 76.1 (C-2), 74.1 (CH_2, 4-*O*-Bn), 73.7 (C-4), 73.63, 73.59 (2 × CH_2, 2-*O*-Bn, 3-*O*-Bn), 68.5 (C-5), 55.4 (OCH_3), 45.5 (NMe_2), 43.3 (C-6); HRMS(ESI) *m/z* calcd for $[C_{40}H_{44}N_2O_7S+Na]^+$: 719.2756, obsd.: 719.2760.

4.10. 1-*O*-Acetyl-2,3,4-tri-*O*-benzyl-6-deoxy-6-[*N*-(5-[dimethylamino]napth-1-ylsulfonyl)acetimido]-α-D-galactopyranoside (15)

Concentrated sulfuric acid (63.1 μL, 1.18 mmol) was added dropwise to a solution of **14** (550 mg, 0.79 mmol) in Ac_2O (2.4 mL, 25.3 mmol) and acetic acid (0.32 mL, 5.53 mmol) at 0 °C. After stirring at 0 °C for 1 h and at rt for 2 h, the solution was diluted with CH_2Cl_2 (50 mL) and washed with water (50 mL), saturated $NaHCO_3$ solution (50 mL) and brine (100 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by silica gel flash column chromatography and eluted with 3:1 (v/v) petroleum ether/EtOAc to afford the title compound **13** (605 mg, 0.79 mmol) as a lime green foam. R_f: 0.66 (PE/EA, 1/1, v/v); Glows mauve on TLC (λ = 254 nm); $[\alpha]_D^{23} = +65.8$ (*c* 1.0, $CHCl_3$); IR (film) 3064, 3030, 2940, 2872, 1750, 1705, 1454, 1342, 1229, 1135, 1102, 1051, 1009, 788, 739, 699 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (br s, 1H, CH-2 Dan), 8.30 (d, $J_{CH\text{-}3,CH\text{-}4}$ = 7.4 Hz, 1H, CH-4 Dan), 7.85 (br s, 1H, CH-8 Dan), 7.58–7.53 (m, 2H, CH-3, CH-7 Dan), 7.44–7.29 (m, 15H, H_{arom}), 7.23 (br s, 1H, CH-6 Dan), 6.35 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 5.11 (d, $J_{a,b}$ = 11.6 Hz, 1H, CH-a, 4-*O*-Bn), 4.91 (d, $J_{a,b}$ = 11.6 Hz, 1H, CH-a, 3-*O*-Bn), 4.82 (d, $J_{a,b}$ = 11.6 Hz, 1H, CH-b, 3-*O*-Bn), 4.72 (s, 2H, CH-a, CH-b, 2-*O*-Bn), 4.69 (d, $J_{a,b}$ = 11.6 Hz, 1H, CH-b, 4-*O*-Bn), 4.29 (dd, $J_{5,6a}$ = 8.6 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-5), 4.18 (dd, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 4.10 (dd, $J_{6a,6b}$ = 15.2 Hz, $J_{5,6}$ = 8.6 Hz, 1H, H-6a), 4.07 (br d, $J_{3,4}$ = 2.6 Hz, 1H, H-4), 3.96 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 2.6 Hz, 1H, H-3), 3.85 (dd, $J_{6a,6b}$ = 15.2 Hz, $J_{5,6b}$ = 2.5 Hz, H1, H-6b), 2.93 (s, 6H, NMe_2), 2.18 (s, 3H, NAc), 2.15 (s, 3H, OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9 (C=O NAc), 169.7 (C=O OAc), 138.4 (C-*i*, 3-*O*-Bn), 138.3 (C-*i*, 4-*O*-Bn), 137.9 (C-*i*, 2-*O*-Bn), 134.7 (C-4a Dan), 131.2 (C-2 Dan), 129.2 (C-1/C-8a Dan), 128.53, 128.50, 128.45, 128.42, 128.40, 128.38, 128.01, 127.94, 127.89, 127.86, 127.82, 127.74, 127.72, 127.61, 127.5 (15 × CH_{arom}), 123.5 (C-3 Dan), 115.5 (C-6 Dan), 90.2 (C-1), 78.9 (C-3), 75.2 (C-2), 74.9 (C-4), 74.6 (CH_2, 4-*O*-Bn), 73.5 (CH_2, 3-*O*-Bn), 73.4 (CH_2, 2-*O*-Bn), 72.1 (C-5), 47.7 (C-6), 45.5 (NMe_2), 24.7 (NAc), 21.2 (OAc), HRMS(ESI) *m/z* calcd for $[C_{43}H_{46}N_2O_9S+Na]^+$: 789.2822, obsd.: 789.2819.

4.11. 2-Azido-3,4-*O*-isopropylidene-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-[*N*-(5-[dimethylamino]napth-1-ylsulfonyl)acetimido]-α-D-galactopyranosyl)-D-*ribo*-octadecane-1,3,4-triol (4)

Iodotrimethylsilane (65.3 μL, 0.46 mmol) was added to a solution of **15** (237 mg, 0.31 mmol) (co-evaporated with toluene × 3) in dry CH_2Cl_2 (2 mL) at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was concentrated in vacuo, azeotroped with toluene (×3) and redissolved in dry benzene (3 mL). In a separate flask, molecular sieves (4 Å, 50 mg), TBAI (342 mg, 0.93 mmol) and Di-PEA (53.8 μL, 0.31 mmol) were added to a solution of the lipid acceptor **6** (39.1 mg, 0.10 mmol) (co-evaporated from toluene × 3) in dry benzene (1 mL) at room temperature. After stirring the reaction mixture at 70 °C for 15 min, the glycosyl iodide was cannulated into the flask containing the lipid acceptor and stirred at 70 °C for 20 h. The reaction mixture was then diluted with EtOAc (10 mL), cooled to 0 °C and filtered. The filtrate was concentrated and the resulting residue purified by silica gel flash column chromatography. Elution with 7:1 (v/v) hexanes/EtOAc afforded the desired glycolipid **4** (103.9 mg, 0.095 mmol, 94%) as a lime green oil. R_f: 0.40 (PE/EA, 2/1, v/v); Glows mauve on TLC (λ = 254 nm); $[\alpha]_D^{24} = +40.9$ (*c* 1.0, $CHCl_3$); IR (film) 3064, 3031, 2923, 2853, 2789, 2099, 1704, 1572, 1455, 1347, 1231, 1147, 1096, 1045, 787, 735, 697 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (d, $J_{CH\text{-}2,CH\text{-}3}$ = 8.6 Hz, 1H, CH-2 Dan), 8.29 (d, $J_{CH\text{-}3,CH\text{-}4}$ = 7.3 Hz, 1H, CH-4 Dan), 7.75 (d, $J_{CH\text{-}7,CH\text{-}8}$ = 8.6 Hz, 1H, CH-8 Dan), 7.53 (t, $J_{CH\text{-}2,CH\text{-}3}$ = $J_{CH\text{-}7,CH\text{-}8}$ = 8.6 Hz, 2H, CH-3, CH-7 Dan), 7.43–7.2.8 (m, 15H, H_{arom}), 7.19 (d, $J_{CH\text{-}6,CH\text{-}7}$ = 7.5 Hz, 1H, CH-6 Dan), 5.11 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-a, 4′-*O*-Bn), 5.00 (d, $J_{1,2}$ = 3.2 Hz, 1H, H-1), 4.91 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-a, 2′-*O*-Bn), 4.80 (d, $J_{a,b}$ = 12.0 Hz, 1H, CH-a, 3′-*O*-Bn), 4.79 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-b, 2′-*O*-Bn), 4.74 (d, $J_{a,b}$ = 12.0 Hz, 1H, CH-b, 3′-*O*-Bn), 4.68 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-b, 4′-*O*-Bn), 4.25–4.18 (m, 3H, H-1a, H-5′, H-3), 4.15–4.05 (m, 4H, H-6′a, H-4, H-3′, H-2′), 4.00 (br s, 1H, H-4′), 3.85 (dd, $J_{6a',6b'}$ = 14.8 Hz, $J_{5,6b'}$ = 2.9 Hz, 1H, H-6′b), 3.74 (dd, $J_{1a,1b}$ = 10.8 Hz, $J_{1b,2}$ = 5.2 Hz, 1H, H-1b), 3.41 (ddd, $J_{2,3}$ = 9.5 Hz, $J_{1a,2}$ = 5.2 Hz, $J_{1b,2}$ = 2.5 Hz, 1H, H-2), 2.89 (s,

6H, NMe_2), 2.18 (s, 3H, NAc), 1.67–1.54 (m, 4H, H-5, H-6), 1.43 (s, 3H, CH_3 *i*Pr), 1.30 (s, 3H, CH_3 *i*Pr), 1.41–1.27 (m, 22H, H-7–H-17), 0.89 (t, $J_{17,18}$ = 7.0 Hz, 3H, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8 (C=O NAc), 152.2 (C-5 Dan), 138.9 (C-*i*, 3′-*O*-Bn), 138.8 (C-*i*, 2′-*O*-Bn), 138.6 (C-*i*, 4′-*O*-Bn), 134.5 (C-4a Dan), 131.5 (C-2 Dan), 131.4 (C-4 Dan), 129.9, (C-1 Dan), 129.6 (C-8a Dan), 128.7 (C-7 Dan), 128.43, 128.40, 128.37, 128.2, 127.7, 127.6, 127.53, 127.52, 127.43 (9 × CH_{arom}), 123.1 (C-3 Dan), 117.8 (C-8 Dan), 115.2 (C-6 Dan), 108.1 (C_q *i*Pr), 98.5 (C-1′), 78.8 (C-2′), 77.8 (C-4), 76.5 (C-3′), 75.5 (C-4′), 75.0 (C-3), 74.5 (CH_2, 4′-*O*-Bn), 73.7 (CH_2, 2′-*O*-Bn), 72.7 (CH_2, 3′-*O*-Bn), 70.0 (C-5′), 69.2 (C-1), 59.3 (C-2), 47.8 (C-6′), 45.4 (NMe_2), 31.9, 29.71, 29.68, 29.67, 29.66, 29.64, 29.61, 29.4, 26.6, 22.7 (C-5–C-17), 28.3, 25.8 (2 × CH_3 *i*Pr), 24.8 (NAc), 14.1 (C-18); HRMS(ESI) *m/z* calcd for $[C_{62}H_{83}N_5O_{10}S+Na]^+$: 1112.5758, obsd.: 1112.5767.

4.12. 2-Azido-3,4-*O*-isopropylidene-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-[*N*-(5-[dimethylamino]napth-1-ylsulfonyl)amido]-α-D-galactopyranosyl)-D-*ribo*-octadecane-1,3,4-triol (16)

Glycolipid **4** (42.5 mg, 0.039 mmol) was dissolved in a mixture of MeOH/CH_2Cl_2 (3:1, v/v) and to this was added NaOMe until the solution reached pH 9.0. After stirring at room temperature for 3 d in the dark, while maintaining pH 9.0 via the addition of NaOMe. The solution was then diluted with EtOAc (15 mL), and washed with saturated NH_4Cl solution (20 mL), water (20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo to afford the title compound **16** (41 mg, quant.) as a lime green oil, which was used without further purification. R_f: 0.40 (PE/EA, 2/1, v/v); Glows white on TLC (λ = 254 nm); $[\alpha]_D^{21} = +10.5$ (*c* 1.0, $CHCl_3$); IR (film) 3309, 3031, 2923, 2853, 2360, 2099, 1574, 1454, 1328, 1220, 1145, 1094, 1047, 791, 734, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, $J_{CH\text{-}2,CH\text{-}3\ Dan}$ = 8.4 Hz, 1H, CH-2 Dan), 8.17 (d, $J_{CH\text{-}3,CH\text{-}4\ Dan}$ = 7.3 Hz, 1H, CH-4 Dan), 8.09 (d, $J_{CH\text{-}7,CH\text{-}8\ Dan}$ = 8.7 Hz, 1H, CH-8 Dan), 7.56 (dd, $J_{CH\text{-}7,CH\text{-}8\ Dan}$ = 8.7 Hz, $J_{CH\text{-}6,CH\text{-}7\ Dan}$ = 7.6 Hz, 1H, CH-7 Dan), 7.53 (dd, $J_{CH\text{-}2,CH\text{-}3\ Dan}$ = 8.4 Hz, $J_{CH\text{-}3,CH\text{-}4\ Dan}$ = 7.3 Hz, 1H, CH-3 Dan), 7.38–7.26 (m, 13H, H_{arom}), 7.20–7.18 (m, 3H, H_{arom}, CH-6 Dan), 4.86 (d, $J_{a,b}$ = 11.8 Hz, 1H, CH-a, 4′-*O*-Bn), 4.79 (d, $J_{a,b}$ = 12.0 Hz, 1H, CH-a, 3′-*O*-Bn), 4.77 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1′), 4.75 (d, $J_{a,b}$ = 11.9 Hz, 1H, CH-a, 2-*O*-Bn), 4.66 (d, $J_{a,b}$ = 11.9 Hz, 1H, CH-b, 2-*O*-Bn), 4.63 (d, $J_{a,b}$ = 12.0 Hz, 1H, CH-b, 3-*O*-Bn), 4.53 (d, $J_{a,b}$ = 11.8 Hz, 1H, CH-b, 4-*O*-Bn), 4.34 (dd, $J_{6'b,\ NH}$ = 8.3 Hz, $J_{6'a,\ NH}$ = 4.5 Hz, 1H, NH), 4.13 (p, $J_{4,5}$ = 4.2 Hz, 1H, H-4), 4.04 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 5.4 Hz, 1H, H-3), 3.95 (dd, $J_{1a,1b}$ = 10.6 Hz, $J_{1a,\ 2}$ = 2.5 Hz, 1H, H-1a), 3.91 (dd, $J_{2',3'}$ = 10.1 Hz, $J_{1,2}$ = 3.6 Hz, 1H, H-2′), 3.79 (dd, $J_{2',3'}$ = 10.1 Hz, $J_{3',4'}$ = 2.7 Hz, 1H, H-3′), 3.70 (br s, 1H, H-4′), 3.69–3.68 (m, 1H, H-5′), 3.61 (dd, $J_{1a,1b}$ = 10.6 Hz, $J_{1b,2}$ = 6.4 Hz, 1H, H-1b), 3.38 (ddd, $J_{2,3}$ = 9.8 Hz, $J_{1b,2}$ = 6.4 Hz, $J_{1a,2}$ = 2.5 Hz, 1H, H-2), 2.92–2.81 (dd, $J_{5,6'a}$ = 7.1 Hz, $J_{6'a,NH}$ = 4.5 Hz, 1H, H-6′a), 2.83 (ddd, $J_{6'a,6'b}$ = 13.6 Hz, $J_{6'b,NH}$ = 8.3 Hz, $J_{5,6'b}$ = 6.1 Hz, 1H, H-6′b), 2.89 (s, 6H, NMe_2), 1.64–1.27 (m, 26H, H-5–H-17), 1.45 (s, 3H, CH_3 *i*Pr), 1.31 (s, 3H, CH_3 *i*Pr), 0.89 (t, $J_{17,18}$ = 7.0 Hz, 3H, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.1 (C-5 Dan), 138.7, 138.6 (2 × C-*i*, 2′-*O*-Bn, 3′-*O*-Bn), 138.0 (C-*i*, 4′-*O*-Bn), 134.7 (C-4a Dan), 130.5 (C-2 Dan), 129.9 (C-1/C-8a Dan), 129.6 (C-4 Dan), 129.5 (C-1/C-8a Dan), 128.9, 128.6, 128.4 (3 × CH_{arom}) 128.30 (C-7 Dan), 128.25, 128.22, 127.64, 127.63, 127.54, 127.48 (6 × CH_{arom}), 123.1 (C-3 Dan), 118.8 (C-8 Dan), 115.2 (C-6 Dan), 108.3 (C_q *i*Pr), 98.5 (C-1′), 78.6 (C-3′), 77.8 (C-4), 76.3 (C-2′), 75.2 (C-3), 74.1 (CH_2, 4′-*O*-Bn), 73.8 (C-4′), 73.7 (CH_2, 3′-*O*-Bn), 72.9 (CH_2, 2′-*O*-Bn), 69.5 (C-1), 69.2 (C-5′), 59.5 (C-2), 45.4 (NMe_2), 43.3 (C-6′), 31.9, 29.72, 29.69, 29.68, 29.64, 29.61, 29.4, 29.3, 26.6, 22.7 (C-5–C-17), 28.3, 25.7 (2 × CH_3 *i*Pr), 14.1 (C-18); HRMS(ESI) *m/z* calcd for $[C_{60}H_{81}N_5O_9S+Na]^+$: 1070.5653, obsd.: 1070.5657.

4.13. 1-*O*-(2,3,4-Tri-*O*-benzyl-6-deoxy-6-[*N*-(5-[dimethylamino]napth-1-ylsulfonyl)amido]-α-D-galactopyranosyl)-2-hexacosanoylamido-3,4-*O*-isopropylidene-D-*ribo*-octadecane-1,3,4-triol (17)

Trimethylphosphine (1 M in THF) (0.17 mL, 0.17 mmol) was added to a solution of **16** (34.7 mg, 0.033 mmol) (co-evaporated with toluene × 3) in distilled THF (0.2 mL) at 0 °C. After stirring the reaction mixture for 15 min at 0 °C and 45 min at rt, the solution was cooled to 0 °C and 1 M NaOH (aq) solution (0.33 mL, 0.33 mmol) added drop wise. The reaction mixture was then stirred at rt for 21 h, then diluted with EtOAc (20 mL), washed with water (2 × 20 mL) and brine (20 mL), dried ($MgSO_4$) and filtered. Concentration in vacuo afforded the amine [R_f: 0.11 (toluene/EtOAc, 3/2, v/v)] as a green oil, which was used without further purification. A solution of hexacosanoic acid (33.5 mg, 0.084 mmol), EDCI (16.2 mg, 0.084 mmol), and DMAP (0.4 mg, 0.0034 mmol) in dry CH_2Cl_2 (1 mL) was added to the amine dissolved in dry CH_2Cl_2 (1 mL). After stirring at room temperature for 43 h, the reaction mixture was diluted with EtOAc (20 mL) and washed with saturated $NaHCO_3$ solution (20 mL), water (20 mL) and brine (20 mL), dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and the residue was purified by silica gel column chromatography. Elution with 5:1 (v/v) hexanes/EtOAc afforded the desired product **17** (31.9 mg, 0.023 mmol, 68%) as a lime green oil. R_f: 0.79 (toluene/EtOAc, 3/2, v/v); Glows white on TLC (λ = 254 nm); $[\alpha]_D^{22} = +4.3$ (*c* 1.0, $CHCl_3$); IR (film) 2923, 2853, 2361, 1650, 1541, 1455, 1330, 1218, 1145, 1092, 1052, 791, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, $J_{CH\text{-}2,CH\text{-}3}$ = 7.4 Hz, 1H, CH-2 Dan), 8.19 (br s, 1H, CH-4 Dan), 8.16 (d, $J_{CH\text{-}7,CH\text{-}8}$ = 7.3 Hz, 1H, CH-8 Dan), 7.56 (t, $J_{CH\text{-}6,CH\text{-}7}$ = $J_{CH\text{-}7,CH\text{-}8}$ = 8.1 Hz, 1H, CH-7 Dan), 7.52 (t, $J_{CH\text{-}2,CH\text{-}3}$ = $J_{CH\text{-}3,CH\text{-}4}$ = 8.0 Hz, 1H, CH-3 Dan), 7.41–7.23 (m, 15H, H_{arom}), 7.20 (d, $J_{CH\text{-}6,CH\text{-}7}$ = 7.1 Hz, 1H, CH-6 Dan), 5.89 (d, $J_{NH,2}$ = 9.5 Hz, 1H, NH C_{26}), 4.92 (br s, 1H, NH Dan), 4.88 (d, $J_{a,b}$ = 11.5 Hz, 1H, CH-a, 4″-*O*-Bn), 4.89 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1″), 4.79 (d, $J_{a,b}$ = 11.4 Hz, 1H, CH-a, 2″-*O*-Bn), 4.76 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-a, 3″-*O*-Bn), 4.71 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-b, 3″-*O*-Bn), 4.64 (d, $J_{a,b}$ = 11.4 Hz, 1H, CH-b, 2″-*O*-Bn), 4.53 (d, $J_{a,b}$ = 11.5 Hz, 1H, CH-b, 4″-*O*-Bn), 4.18–4.14 (m, 1H, H-2), 4.07 (t, $J_{2,3}$ = $J_{3,4}$ = 6.9 Hz, 1H, H-3), 4.04–4.01 (m, 1H, H-4), 3.96 (dd, $J_{2'',3''}$ = 10.0 Hz, $J_{1'',2''}$ = 3.6 Hz, 1H, H-2″), 3.85 (dd, $J_{1a,1b}$ = 11.6 Hz, $J_{1a,2}$ = 4.6 Hz, 1H, H-1a), 3.82 (br s, 1H, H-4″), 3.77 (dd, $J_{2'',3''}$ = 10.0 Hz, $J_{3'',4''}$ = 2.7 Hz, 1H, H-3″), 3.71 (t, $J_{5'',6''a}$ = 6.6 Hz, 1H, H-5″), 3.59 (dd, $J_{1a,1b}$ = 11.6 Hz, $J_{1b,2}$ = 2.6 Hz, 1H, H-1b), 2.90 (s, 6H, NMe_2), 2.87–2.85 (m, 2H, H-6″a, H-6″b), 2.14 (dt, $J_{2'a,2'b}$ = 14.7 Hz, $J_{2'a,3'}$ = 7.5 Hz, 1H, H-2′a), 2.06 (dt, $J_{2'a,2'b}$ = 14.7 Hz, $J_{2'a,3'}$ = 7.5 Hz, 1H, H-2′b), 1.71 (br s, 2H, CH_2, H-5), 1.59 (m, 2H, H-3′), 1.47–1.43 (m, 68H, CH_2, H-6–H-17, H-4′–H-25′), 1.43 (s, 3H, CH_3 *i*Pr), 1.32 (s, 3H, CH_3 *i*Pr), 0.89 (t, $J_{17,18}$ = $J_{25',26'}$ = 7.0 Hz, 6H, H-18, H-26′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6 (C=O), 151.8 (C-5 Dan), 138.4 (C-*i*, 3″-*O*-Bn), 138.2 (C-*i*, 2″-*O*-Bn), 138.1 (C-*i*, 4″-*O*-Bn), 134.7 (C-4a/C-8a Dan), 130.3 (C-2 Dan), 129.8 (C-4a/C-8a Dan), 129.6 (C-8 Dan), 128.8, 128.72, 128.66, 128.60, 128.56, 128.5, 128.4, 128.15, 128.1, 128.0, 127.94, 127.88, 127.7, 127.6, 127.5 (15 × CH_{arom}), 128.21 (C-7 Dan), 123.3 (C-3 Dan), 119.1 (C-1 Dan), 115.2 (C-6 Dan), 107.8 (C_q *i*Pr), 99.3 (C-1″), 79.1 (C-3″), 77.7 (C-4), 76.6 (C-3), 76.5 (C-2″), 74.2 (CH_2, 4″-*O*-Bn), 73.7 (CH_2, 2″-*O*-Bn), 73.4 (C-4″), 73.0 (CH_2, 3″-*O*-Bn), 69.4 (C-1), 69.1 (C-5″), 49.1 (C-2), 45.5 (NMe_2), 43.3 (C-6″), 36.9 (C-2′), 31.95, 31.94, 31.6, 29.8, 29.73, 29.71, 29.70, 29.67, 29.65, 29.50, 29.47, 29.42, 29.39, 29.37, 28.83, 26.8, 22.7 (C-5–C-17, C-3′–C-25′), 27.8, 25.73 (2 × CH_3 *i*Pr), 25.72 (CH_2, C-3″), 14.1 (2 × CH_3, C-18, C-26′); HRMS(ESI) *m/z* calcd for $[C_{86}H_{133}N_3O_{10}S+Na]^+$: 1422.9609, obsd.: 1422.9615.

4.14. 1-*O*-(2,3,4-Tri-*O*-benzyl-6-deoxy-6-[*N*-(5-[dimethylamino]napth-1-ylsulfonyl)amido]-α-D-galactopyranosyl)-2-hexacosanoylamido-D-*ribo*-octadecane-1,3,4-triol (18)

Glycolipid **17** (31.9 mg, 0.023 mmol) was dissolved in a mixture of 8:4:1 (v/v/v) AcOH/H_2O/toluene and stirred at 50 °C for 1 d after which analysis by TLC revealed the presence of the starting material. The reaction mixture was concentrated in vacuo (to remove the acetone by product), then redissolved in the same mixture of solvents and stirred for a further day. This procedure was repeated until all starting material was converted to product via TLC analysis. The reaction mixture was then concentrated in vacuo and the residue was purified by silica gel flash column chromatography. Elution with 1:2 (v/v) hexanes/EtOAc afforded the diol **18** (23.0 mg, 0.017 mmol, 74%) as a lime green oil. R_f: 0.22 (PE/EA, 3/2, v/v); Glows white on TLC (λ = 254 nm); $[\alpha]_D^{21} = +13.7$ (*c* 1.0, $CHCl_3$); IR (film) 3357, 3032, 2922, 2852, 2360, 1643, 1536, 1498, 1312, 1143, 1092, 1050, 909, 789, 733, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, $J_{CH\text{-}2,CH\text{-}3}$ = 8.5 Hz, 1H, CH-2 Dan), 8.10 (d, $J_{CH\text{-}3,CH\text{-}4}$ = 7.4 Hz, 1H, CH-4 Dan), 8.07 (d, $J_{CH\text{-}7,CH\text{-}8}$ = 8.6 Hz, 1H, CH-8 Dan), 7.56 (dd, $J_{CH\text{-}7,CH\text{-}8}$ = 8.6 Hz, $J_{CH\text{-}6,CH\text{-}7}$ = 7.8 Hz, 1H, CH-7 Dan), 7.51 (dd, $J_{CH\text{-}2,CH\text{-}3}$ = 8.5 Hz, $J_{CH\text{-}3,CH\text{-}4}$ = 7.4 Hz, 1H, CH-3 Dan), 7.41–7.27 (m, 13H, H_{arom}), 7.22–7.18 (m, 3H, H_{arom}, CH-6 Dan), 6.31 (d, $J_{NH,2}$ = 8.3 Hz, 1H, NH C_{26}), 4.88 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-a, 4″-*O*-Bn), 4.83 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-a, 2″-*O*-Bn), 4.81 (d, $J_{a,b}$ = 11.2 Hz, 1H, CH-a, 3″-*O*-Bn), 4.75 (d, $J_{a,b}$ = 11.2 Hz, 1H, CH-b, 3′-*O*-Bn), 4.74 (d, $J_{1'',2''}$ = 3.7 Hz, 1H, H-1″), 4.66 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-b, 2″-*O*-Bn), 4.53 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-b, 4″-*O*-Bn), 4.51–4.49 (m, 1H, NH Dan), 4.30 (dd, $J_{2,3}$ = 8.1 Hz, $J_{1a/b,2}$ = 3.7 Hz, 1H, H-2), 3.95 (dd, $J_{2'',3''}$ = 10.0 Hz, $J_{1'',2''}$ = 3.7 Hz, 1H, H-2″), 3.87–3.84 (m, 3H, H-1a, H-4″, H-5″), 3.80 (dd, $J_{2'',3''}$ = 10.0 Hz, $J_{3'',4''}$ = 2.4 Hz, 1H, H-3″), 3.76 (dd, $J_{1a,1b}$ = 10.5 Hz, $J_{1b,2}$ = 4.1 Hz, 1H, H-1b), 3.65–3.64 (m, 1H, 3-OH), 3.54–3.51 (m, 2H, H-3, H-4), 2.89 (s, 6H, NMe_2), 2.83 (ddd, $J_{6''a,6''b}$ = 14.1 Hz, $J_{5,6''a}$ = 7.1 Hz, $J_{6''a,NH}$ = 4.4 Hz, 1H, H-6″a), 2.77 (ddd, $J_{6''a,6''b}$ = 14.1 Hz, $J_{6''b,NH}$ = 8.1 Hz, $J_{5,6''b}$ = 6.1 Hz, 1H, H-6″b), 2.56–2.55 (m, 1H, 4-OH), 2.25 (dt, $J_{2'a,2'b}$ = 14.9 Hz, $J_{2'a,3'}$ = 7.5 Hz, 1H, H-2′a), 2.19 (dt, $J_{2'a,2'b}$ = 14.9 Hz, $J_{2'b,3'}$ = 7.5 Hz, 1H, H-2′b), 1.67–1.15 (m, 74H, CH_2, H-5–H-17, H-3′–H-25′), 0.89 (t, $J_{17,18} = J_{25',26'}$ = 7.0 Hz, 6H, H-18, H-26′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5 (C═O), 152.0 (C-5 Dan), 138.2 (C-*i*, 3″-*O*-Bn), 137.9 (C-*i*, 4″-*O*-Bn), 137.8 (C-*i*, 2″-*O*-Bn), 134.1 (C-4a Dan), 130.6 (C-2 Dan), 129.9 (C-1 Dan), 129.6 (C-4 Dan), 129.5 (C-8a Dan), 128.9, 128.6, 128.54, 128.52, 128.2, 128.05, 128.00, 127.8, 127.5 (9 × CH_{arom}), 128.4 (C-7 Dan), 123.1 (C-3 Dan), 118.8 (C-8 Dan), 115.2 (C-6 Dan), 98.8 (C-1″), 79.4 (C-3″), 76.3 (C-3), 75.9 (C-2″), 74.2 (CH_2, 4″-*O*-Bn), 74.0 (CH_2, 2″-*O*-Bn), 73.3 (CH_2, 3″-*O*-Bn), 73.2 (C-4), 73.1 (C-4″), 69.6 (C-5″), 69.2 (C-1), 49.9 (C-2), 45.4 (NMe_2), 43.2 (C-6″), 36.8 (C-2′), 33.5 (C-5), 31.9, 29.73, 29.71, 29.68, 29.67, 29.6, 29.44, 29.38, 25.9, 25.7, 22.7 (C-6–C-17, C-3′–C-25′), 14.1 (2 × CH_3, C-18, C-26′); HRMS(ESI) *m/z* calcd for $[C_{83}H_{129}N_3O_{10}S+Na]^+$: 1382.9296, obsd.: 1382.9296.

4.15. 1-*O*-[6-Deoxy-6-[*N*-(5-[dimethylamino]napth-1-ylsulfonyl)amido]-α-D-galactopyranosyl]-2-hexacosanoylamido-D-*ribo*-octadecane-1,3,4-triol (3)

Pd/$(OH)_2$ (2.5 mol %) was added to a solution of diol **18** (9.9 mg, 0.0073 mmol) in a mixture of $CHCl_3$/EtOH (1 mL, 3/2, v/v) and the reaction stirred under H_2 (g) for 17 h at rt. The reaction mixture was then filtered through Celite, the Celite was washed thoroughly with $CHCl_3$/EtOH (3/2, v/v), and the filtrate was concentrated in vacuo. The residue was purified by silica gel flash column chromatography (10% MeOH/CH_2Cl_2), followed by reverse phase chromatography (ODS-C18 resin, product eluted with MeOH), and finally silica gel column chromatography by elution with 5% MeOH/CH_2Cl_2. This afforded the target compound **3** (4.9 mg, 4.49 mmol, 62%) as a yellow oil. R_f: 0.22 (CH_2Cl_2/MeOH, 92/8, v/v); Glows white on TLC (λ = 366 nm); $[\alpha]_D^{22} = +40.0$ (*c* 0.1, pyridine); IR (film) 3320, 2918, 2851, 2359, 1653, 1634, 1560, 1457, 1310, 1144, 1030, 791, 684 cm^{-1}; UV λ_{max}Abs = 343 nm, λ_{max}-Em = 510 nm; ^1H NMR (600 MHz, pyridine-d_5) δ 9.04 (d, $J_{CH\text{-}2,CH\text{-}3}$ = 8.7 Hz, 1H, CH-2 Dan), 8.61–8.57 (m, 2H, NH C_{26}, CH-4 Dan), 8.54 (d, $J_{CH\text{-}7,CH\text{-}8}$ = 8.6 Hz, 1H, CH-8 Dan), 7.54 (dd, $J_{CH\text{-}7,CH\text{-}8}$ = 8.6 Hz, $J_{CH\text{-}6,CH\text{-}7}$ = 7.4 Hz, 1H, CH-7 Dan), 7.51 (dd, $J_{CH\text{-}2,CH\text{-}3}$ = 8.7 Hz, $J_{CH\text{-}3,CH\text{-}4}$ = 7.6 Hz, 1H, CH-3 Dan), 7.12 (d, $J_{CH\text{-}6,CH\text{-}7}$ = 7.4 Hz, 1H, CH-6 Dan), 5.45 (d, $J_{1'',2''}$ = 3.9 Hz, 1H, H-1″), 5.23 (m, 1H, H-2), 4.54 (dd, $J_{1a,1b}$ = 10.9 Hz, $J_{1a,2}$ = 5.6 Hz, 1H, H-1a), 4.52 (dd, $J_{2'',3''}$ = 9.9 Hz, $J_{1'',2''}$ = 3.9 Hz, 1H, H-2″), 4.51 (t, $J_{5,6a} = J_{5,6b}$ = 6.6 Hz, 1H, H-5″), 4.35–4.30 (m, 3H, H-3, H-4, H-4″), 4.26 (dd, $J_{2'',3''}$ = 9.9 Hz, $J_{3'',4''}$ = 3.3 Hz, 1H, H-3″), 4.19 (dd, $J_{1a,1b}$ = 10.9 Hz, $J_{1b,2}$ = 4.8 Hz, 1H, H-1b), 3.85 (br s, 2H, H-6″a, H-6″b), 2.50 (dt, $J_{2'a,2'b}$ = 14.8 Hz, $J_{2'a,3'}$ = 7.8 Hz, 1H, H-2′a), 2.46 (dt, $J_{2'a,2'b}$ = 14.8 Hz, $J_{2'b,3'}$ = 7.8 Hz, 1H, H-2′b), 2.34–1.24 (m, 72H, CH_2, H-5–H-17, H-3′–H-25′), 0.86 (t, $J_{17,18} = J_{25',26'}$ = 7.0 Hz, 6H, H-18, H-26′); ^{13}C NMR (150 MHz, pyridine-d_5) δ 173.7 (C═O), 152.4 (C-5 Dan), 137.8 (C-4a Dan), 130.8 (C-1 Dan), 130.6 (C-8a Dan), 130.3 (C-8 Dan), 129.3 (C-4 Dan), 128.5 (C-3 Dan), 123.5 (C-7 Dan), 120.7 (C-2 Dan), 115.9 (C-6 Dan), 101.4 (C-1″), 76.8 (C-3), 72.8 (C-4), 71.42 (C-5″), 71.37 (C-3″), 71.2 (C-4″), 70.2 (C-2″), 68.5 (C-1), 51.5 (C-2), 45.52 (NMe_2), 45.16 (C-6″), 37.1 (C-2′), 34.6 (C-5), 32.42, 32.41, 30.8, 30.7, 30.5, 30.4, 30.34, 30.33, 30.32, 30.31, 30.29, 303.27, 30.26, 30.23, 30.21, 30.19, 30.14, 30.10, 29.92, 29.90, 26.8, 26.7, 23.24, 23.23 (C-6–C-17, C-3′–C-25′), 14.6 (2 × CH_3, C-18, C-26′); HRMS(ESI) *m/z* calcd for $[C_{62}H_{111}N_3O_{10}S+Na]^+$: 1112.7888, obsd.: 1112.7878.

4.16. Materials and methods for cell proliferation (IL-2) assay, in vivo DC maturation assay and in vitro treatment of dendritic cells

4.16.1. Mice

Breeding pairs of the inbred strains C57BL/6 were obtained from The Jackson Laboratories and from the Animal Resource Centre. All mice were maintained in the Biomedical Research Unit of the Malaghan Institute of Medical Research. CD1d$^{-/-}$ mice, which are devoid of CD1d-restricted *i*NKT cells, were also used.[62] The experiments were approved by the NZ national animal ethics committee and performed according to established national guidelines.

4.16.2. Solubilisation of glycolipid

α-GalCer (Industrial Research Ltd, New Zealand) and synthesised glycolipids were tested to be endotoxin-free at the sensitivity of 0.125 EU/mL with an endotoxin kit (Pyrotell, Limulus Amebocyte Lysate). Each glycolipid (1 mg) was dissolved in 200 μL of $CHCl_3$/MeOH/H_2O (10:10:3), heated at 37 °C for 15 min followed by sonication for 10 min. The solution was then diluted to 200 μg/mL in 0.5% Tween/phosphate-buffered saline (PBS) and left to sit at −4 °C overnight. The glycolipid was then heated at 80 °C for 5 min followed by sonication for 5 min then cooled at 0 °C for 5 min (×2) and left to sit at −4 °C overnight.

4.16.3. Cell proliferation (IL-2) assay

Dendritic cells (DC2114;[55] 2.5×10^4 cells) and murine NKT hybridoma cells (DN32.D3;[4] 1×10^5 cells) were incubated with various concentration of glycolipid (α-GalCer **2**: 583–0.569 nM; Dansyl α-GalCer **3**: 458–0.447 nM in twofold dilution) in 200 μL of complete medium consisting of Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 2 mM glutamine (Invitrogen), 1% penicillin–streptomycin (Invitrogen), 5×10^{-5} M 2-mercaptoethanol (Invitrogen) and 5% foetal bovine serum

(Sigma-Aldrich) at 37 °C for 24 h. Supernatant (100 μL) was added to 5×10^3 HT-2 cells in a flat-bottom 96-well plate, incubated at 37 °C for 24 h and incorporation of [^{3}H]thymidine over the final 6 h was measured with a beta-counter (Wallac). A standard curve was constructed by incubating HT-2 cells with known concentrations of IL-2 (200–0 U/mL in fivefold dilution). Each sample was analysed in triplicate.

4.16.4. Antigen presenting cell maturation assay

Glycolipid diluted to 2 μg/mL and 200 ng (in 100 μL) was administered iv to groups of C57BL/6 mice (n = 3) and spleens were removed 20 h later. Spleens were teased through a cell strainer, red blood cells (RBC) were lysed with RBC lysis buffer followed by antibody labelling for CD11c (clone HL3; BD Pharmingen), B220 (clone RA3-6B2 conjugated to A488; BD Pharmingen), CD86 (clone GL1 conjugated to PE; eBioscience) and propidium iodide (BD Pharmingen) and analysis by flow cytometry (BD FACSort). The same protocol was used for assessing expression of CD86 on CD11c$^+$ dendritic cells by CD1d$^{-/-}$ mice.

4.16.5. Uptake of glycolipid by dendritic cells in vitro

1×10^6 DC2114 cells were incubated with 4 μg of glycolipid in 1 mL cIMDM in a flat-bottom 6-well plate at 37 °C for 24 h. The cells were resuspended in FACS buffer, stained with viability dye (PI; BD Pharmingen) and analysed by flow cytometry (BD LSRII SORP; UV laser, filter 525/50, Hoechst Blue detector).

Acknowledgement

The authors would like to thank the Cancer Society of New Zealand and the Health Research Council of New Zealand for financial assistance.

Supplementary data

Supplementary data (^{1}H NMR and ^{13}C NMR spectra for all new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.02.014.

References

1. Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591–5592.
2. Akimoto, K.; Natori, T.; Morita, M. *Tetrahedron Lett.* **1993**, *34*, 5593–5596.
3. Morita, M.; Motoki, K.; Akimoto, K.; Natori, T., et al *J. Med. Chem.* **1995**, *38*, 2176–2187.
4. Bendelac, A.; Lantz, O.; Quimby, M. E.; Yewdell, J. W.; Bennink, J. R.; Brutkiewicz, R. R. *Science* **1995**, *268*, 863–865.
5. Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626–1629.
6. For some recent reviews see: (a) Godfrey, D. I.; Stankovic, S.; Baxter, A. G. *Nat. Rev. Immunol.* **2010**, *11*, 197–206; (b) Wu, D.; Fujio, M.; Wong, C.-H. *Bioorg. Med. Chem.* **2008**, *16*, 1073–1083; (c) Speak, A. O.; Cerundolo, V.; Platt, F. M. *Immunol. Cell Biol.* **2008**, *86*, 588–597; (d) Godfrey, D. I.; Berzins, S. P. *Nat. Rev. Immunol.* **2007**, *7*, 505–518; (e) Van Kaer, L. *Nat. Rev. Immunol.* **2005**, *5*, 31–42; (f) Zajonc, D. M.; Kronenberg, M. *Curr. Opin. Struct. Biol.* **2007**, *17*, 521–529; (g) Godfrey, D. I.; Kronenbreg, M. *J. Clin. Invest.* **2004**, *114*, 1379–1388.
7. Fan, G.; Pan, Y. S.; Lu, K. C.; Cheng, Y. P.; Lin, W.-C.; Lin, S.; Lin, C.-H.; Wong, C.-H.; Fang, J.-M.; Lin, C.-C. *Tetrahedron* **2005**, *61*, 1855–1862.
8. Park, J. J.; Lee, J. H.; Ghosh, S. C.; Bricard, G.; Vebjataswamy, M. M.; Porcelli, S. A.; Chung, S.-K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3906–3909.
9. Lee, A.; Farrand, K. J.; Dickgreber, N.; Hayman, C. M.; Jürs, S.; Hermans, I. F.; Painter, G. F. *Carbohydr. Res.* **2006**, *341*, 2785–2798.
10. Plettenburg, O.; Bodmer-Narkevitch, V.; Wong, C.-H. *J. Org. Chem.* **2002**, *67*, 4559–4564.
11. Zhou, X.-T.; Forestier, C.; Goff, R. D.; Li, C.; Teyton, L.; Bendelac, A.; Savage, P. V. *Org. Lett.* **2002**, *4*, 1267–1270.
12. Sakai, T.; Ehara, H.; Koezuka, Y. *Org. Lett.* **1999**, *1*, 359–361.
13. Vo-Hoang, Y.; Micouin, L.; Ronet, C.; Gachelin, G.; Bonin, M. *ChemBioChem* **2003**, *4*, 27–33.
14. Sakai, T.; Naidenko, O. V.; Iijima, H.; Kronenberg, M.; Koezuka, Y. *J. Med. Chem.* **1999**, *42*, 1836–1841.
15. Xia, C.; Zhang, W.; Zhang, Y.; Woodward, R. L.; Wang, J.; Wang, P. G. *Tetrahedron* **2009**, *65*, 6390–6395.
16. Sagiv, Y.; Hudspeth, K.; Mattner, J.; Schrantz, N.; Stern, R. K.; Zhou, D.; Savage, P. B.; Teyton, L.; Bendelac, A. *J. Immunol.* **2006**, *177*, 26–30.
17. Naidenko, O. V.; Maher, J. K.; Ernst, W. A.; Sakai, T.; Modlin, R. L.; Kronenberg, M. *J. Exp. Med.* **1999**, *190*, 1069–1080.
18. Barral, D. C.; Brenner, M. B. *Nat. Rev. Immunol.* **2007**, *7*, 929–941.
19. Zheng, Z.-H.; Castaño, A. R.; Segelke, B. W.; Stura, E. A.; Peterson, P. A.; Wilson, I. A. *Science* **1997**, *277*, 339–345.
20. Mukherjee, S.; Soe, T. T.; Maxfield, F. R. *J. Cell Biol.* **1999**, *144*, 1271–1284.
21. Moody, D. B.; Briken, V.; Cheng, T.-Y.; Roura-Mir, C.; Guy, M. R.; Geho, D. H.; Tykocinski, M. L.; Besra, G. S.; Porcelli, S. A. *Nat. Immunol.* **2002**, *3*, 435–442.
22. Singh, R. D.; Liu, Y.; Wheatley, C. L.; Holicky, E. L.; Makino, A.; Marks, D. L.; Kobayashi, T.; Subramaniam, G.; Bittman, R.; Pagano, R. E. *J. Biol. Chem.* **2006**, *281*, 30660–30668.
23. Kamada, N.; Iijima, H.; Kimura, K.; Harada, M.; Shimizu, E.; Motohashi, S.; Kawano, T.; Shinkai, H.; Nakayama, T.; Sakai, T.; Brossay, L.; Kronenberg, M.; Taniguchi, M. *Int. Immunol.* **2001**, *13*, 853–861.
24. Prigozy, T. I.; Naidenko, O.; Qasba, P.; Elewaut, D.; Brossay, L.; Khurana, A.; Natori, T.; Koezuka, Y.; Kulkarni, A.; Kronenberg, M. *Science* **2001**, *291*, 664–667.
25. Farrand, K. J.; Dickgreber, N.; Stoitzner, P.; Ronchese, F.; Petersen, T. R.; Hermans, I. F. *J. Immunol.* **2009**, *183*, 7732–7742.
26. Shin-ichiro Fujii, S.-H.; Goto, A.; Shimizu, K. *Blood* **2009**, *113*, 4262–4272.
27. Velmourougane, G.; Raju, R.; Bricard, G.; Im, J. S.; Besra, G. S.; Porcelli, S. A.; Howell, A. R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3386–3388.
28. Raju, R.; Castillo, B. F.; Richardson, S. K.; Thakur, M.; Severins, R.; Kronenbrg, M.; Howell, A. R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4122–4125.
29. Takikawa, H.; Muto, S.-E.; Mori, K. *Tetrahedron* **1998**, *54*, 3141–3150.
30. Ndonye, R. M.; Izmirian, D. P.; Dunn, M. F.; Yu, K. O. A.; Porcelli, S. A.; Khurana, A.; Kronenberg, M.; Richardson, S. K.; Howell, A. R. *J. Org. Chem.* **2005**, *70*, 10260–10270.
31. For a recent review highlighting glycosylation reactions using sphingosines and ceramides see: Morales-Serna, J. A.; Boutureira, O.; Diaz, Y.; Matheu, M. I.; Castillon, S. *Carbohydr. Res.* **2007**, *342*, 1595–1612.
32. For examples see: (a) Timmer, M. S. M.; Stocker, B. L.; Northcote, P. T.; Burkett, B. A. *Tetrahedron Lett.* **2009**, *50*, 7199–7204; (b) Caton-Williams, J.; Huang, Z. *Angew. Chem., Int. Ed.* **2008**, *47*, 1723–1725; (c) Aumüller, I.; Lindhorst, T. K. *Eur. J. Org. Chem.* **2006**, 1103–1108; (d) Sekine, M.; Aoyagi, M.; Ushioda, M.; Ohkubo, A.; Seio, K. *J. Org. Chem.* **2005**, *70*, 8400–8408.
33. Bhattacharjee, A. K.; Zissis, E.; Glaudemans, C. P. J. *Carbohydr. Res.* **1981**, *89*, 249–254.
34. Morgenlie, S. *Carbohydr. Res.* **1975**, *41*, 77–83.
35. The preparation of a 3-*O*, 4-*O* benzyl protected phytosphingosine lipid by Wong and co-workers (Ref. 10) is comparably efficient having been prepared from 2-deoxy galactose in 9 steps and 36% overall yield.
36. For a highly efficient synthesis of the fully deprotected phytosphingosine lipid (6-steps, 29% overall yield), see: Lin, C.-C.; Fan, G.-T.; Fang, J.-M. A. *Tetrahedron Lett.* **2003**, *44*, 5281–5283.
37. Yeager, A. R.; Finney, N. S. *J. Org. Chem.* **2005**, *70*, 1269–1275.
38. Du, W.; Gervay-Hague, J. *Org. Lett.* **2005**, *7*, 2063–2065.
39. Gervay, J.; Hadd, M. J. *J. Org. Chem.* **1997**, *62*, 6961–6967.
40. Gervay, J.; Nguyen, T. N.; Hadd, M. J. *Carbohydr. Res.* **1997**, *300*, 119–125.
41. Hadd, M. J.; Gervay, J. *Carbohydr. Res.* **1999**, *320*, 61–69.
42. Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771–2784.
43. Graziani, A.; Passacantilli, P.; Piancatelli, G.; Tani, S. *Tetrahedron: Asymmetry* **2000**, *11*, 3921–3937.
44. Murata, K.; Toba, T.; Nakanishi, K.; Takahashi, B.; Yamamura, T.; Miyake, S.; Annoura, H. *J. Org. Chem.* **2005**, *70*, 2398–2401.
45. Goff, R. D.; Gao, Y.; Mattner, J.; Zhou, D.; Yin, N.; Cantu, C.; Teyton, L.; Bendelac, A.; Savage, P. B. *J. Am. Chem. Soc.* **2004**, *126*, 13602–13603.
46. Franchini, L.; Matto, P.; Ronchetti, F.; Panza, L.; Barbieri, L.; Costantine, V.; Mangoni, A.; Cavallari, M.; Mori, L.; De Libero, G. *Bioorg. Med. Chem.* **2007**, *15*, 55529–55536.
47. Wenjum, D.; Kulkarni, S. S.; Gervay-Hague, J. *Chem. Commun.* **2007**, 2336–2338.
48. Schombs, M.; Park, F. E.; Du, W.; Kulkarni, S. S.; Gervay-Hague, J. *J. Org. Chem.* **2010**, *75*, 4891–4898.
49. Gorin, P. A. J.; Mazuzek, M. *Can. J. Chem.* **1975**, *53*, 1212–1223.
50. Zemplén, G.; Pascu, E. *Ber. Dtsch. Chem. Ges.* **1929**, 1613–1614.
51. Liu, Y.; Goff, R. D.; Zhou, D.; Mattner, J.; Sullivan, B. A.; Khurana, A.; Cantu, C., III; Ravkov, E. V.; Ibegbu, C. C.; Altman, J. D.; Teyton, L.; Bendelac, A.; Savage, P. B. *J. Immunol. Methods* **2006**, *312*, 34–39.
52. Taki, T.; Kuroyanagi, M.; Yoshioka, H.; Handa, S. *J. Biochem.* **1992**, *111*, 614–619.
53. Fujii, S.-I.; Shimizu, K.; Smith, C.; Bonifaz, L.; Steinman, R. M. *J. Exp. Med.* **2003**, *198*, 267–279.
54. Hermans, I. F.; Silk, J. D.; Gileadi, U.; Salio, M.; Mathew, B.; Ritter, G.; Schmidt, R.; Harris, A. L.; Old, L.; Cerundolo, V. *J. Immunol.* **2003**, *171*, 5140–5147.
55. Steiner, Q. G.; Otten, L. A.; Hicks, M. J.; Kaya, G.; Grosjean, F.; Saeuberli, E.; Lavanchy, C.; Beermann, F.; McClain, K. L.; Acha-Orbea, H. *Blood* **2008**, *111*, 2073–2082.
56. Lin, C.-C.; Fan, G.-T.; Fang, J.-M. *Tetrahedron Lett.* **2003**, *44*, 5281–5283.
57. Banks, M. R.; Blake, A. J.; Cadogan, J. I. G.; Dawson, I. M.; Gaur, S.; Gosney, I.; Gould, R. O.; Grant, K. J.; Hodgson, P. K. G. *J. Chem. Soc., Chem. Commun.* **1993**, 1146–1148.

58. Ballou, C. E.; Fischer, H. O. L. *J. Am. Chem. Soc.* **1954**, *76*, 3188–3193.
59. Lehmann, J.; Weckerle, W. *Carbohydr. Res.* **1972**, *22*, 23–35.
60. Hanessian, S. *J. Org. Chem.* **1969**, *34*, 675–681.
61. Zaliz, C. L. R.; Varela, O. *J. Carbohydr. Chem.* **2001**, *20*, 689–701.
62. Chen, Y.-H.; Chiu, N. M.; Mandal, M.; Wang, N.; Wang, C.-R. *Immunity* **1997**, *6*, 459–467.