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Design and synthesis of new KRN7000 analogues

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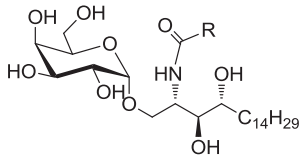
ABSTRACT

Presented by CD1d protein, KRN7000, a potent synthetic α-galactosylceramide, is known to stimulate the iNKT cells to produce different bioactive cytokines. Six new KRN7000 analogues, in which the amide bond in KRN7000 is replaced with O, NH, or ester groups incorporating variation of the acyl chain, or possessing an additional four-atom linker between the galactose and phytosphingosine moiety, were designed and synthesized. The synthetic compounds were evaluated for their ability to stimulate cytokine release and the preliminary structure–activity relationships were discussed. The synthetic strategy will benefit the construction of more KRN7000 derivatives, which may contribute to cytokine profile bias.

1. Introduction

Invariant natural killer T (iNKT) cells are regulatory T cells that display characteristics of both T cells and NK cells.[1] Mediated by semi-invariant T cell receptor (TCR), iNKT cells recognize bacterial or endogenous glycolipid antigens presented by CD1d, an antigen presenting protein related to major histocompatibility complex proteins.[2] Once stimulated, iNKT cells can elicit a number of cytokines, including pro-inflammatory T helper 1 (Th1) cytokines (e.g., IFN-γ, IL-2) and anti-inflammatory Th2 cytokines (e.g., IL-4).[3] Th1 cytokines are thought to be related to the antitumor, antiviral, and antibacterial activities, whereas Th2 cytokines may help to alleviate autoimmune diseases.[4] KRN7000 (Fig. 1), a synthetic glycolipid α-galactosylceramide (α-GalCer) generated from the structural modification of the marine natural product agelasphin-9b, is the prototypical ligand to stimulate iNKT cells when presented by CD1d expressed on the surface of antigen-presenting cells (APC).[5] However, the controversial effects resulted from both high level inductions of Th1 and Th2 cytokines and the anergic state of iNKT cells induced by KRN7000 limited its value in therapeutic applications.[6] Therefore, more effective antigens, which can selectively control the cytokine release profile by NKT cells toward either Th1 or Th2 were extensively explored.[7]



1 KRN7000 R = -$(CH_2)_{24}CH_3$
2 PBS-25 R = -$(CH_2)_6CH_3$
3 R = -$(CH_2)_7Ph$

Fig. 1. Chemical structures of **1**, **2**, **3**.

So far there have been a number of KRN7000 derivatives including modifications of the sugar moiety, the polar portion of the ceramide, the two lipid chains, and the glycosidic linkage.[7a,8] Some of them succeeded in leading to changes in the cytokine release profile, presumably through alteration of glycolipid/CD1d complex stability. For example, α-GalCer derivative **2** (PBS-25) with a shorter fatty acid chain have shown to induce a predominant production of Th2 cytokine over Th1 compared with that of KRN7000.[9] Compound **3** with introduction of an aromatic group to the fatty acid chain enhances the Th1 cytokine profile (Fig. 1).[10] The replacement of an amide function with an isosteric group, such as a triazole increases the IL-4 versus IFN-γ bias of released cytokines.[11] Replacement of the anomeric oxygen of α-GalCer by CH_2, the C-glycoside variant of KRN7000 stimulates strong Th1 responses in vivo from NKT cells.[12] Although advances on syntheses of KRN7000 analogues with improved activities have been achieved, the discovery of new KRN7000 derivatives with more potent properties is still in great demand. Herein we report the preparation of several new KRN7000 analogues with the modifications at the amide bond.

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2. Results and discussion

The structure–activity relationship (SAR) studies of α-GalCer were aided by two crystal structures of both mouse (m) and human (h) CD1d complex with **1** and by a ternary structure of hCD1d/KRN7000/hTCR complex.[13] The crystallographic studies identified that the lipid chains of α-GalCer were accommodated in the antigen-binding groove, consisting of two channels, lined with hydrophobic residues. Besides hydrophobic interactions, there are several hydrogen-bonding interactions between the surface residues of CD1d and the hydroxyl groups of KRN7000, which are considered to contribute to maintain KRN7000 in the correct position and orientation for recognition by the TCR. One of the hydrogen-bonding interactions is identified as the Thr156 of mCD1d with NH of the amide group of KRN7000 acting as an H-bond donor. In view of substitution of the amide group including triazole,[11] sulfonamide,[14] azetidine or pyrrolidine ring[15] and recently reported ester group,[16] it is seemed that the functional groups substituting amide of KRN7000 are mostly able to form H-bond.

In an effort to understand whether this H-bond ability is crucial for the activity and how this replacement influences cytokine profile, we designed three KRN7000 analogues **4**, **5**, and **6** (Fig. 2) in which the amide group was replaced with an ether or amino group, combining variations of the acyl chain from compounds **2** and **3**. To evaluate whether the two lipid chains of analogues linked at different hydroxyl positions can fit into the CD1d binding groove like KRN7000 or not, we decided to synthesize both ether and ester derivatives (**8**, **9**) as target molecules (Fig. 2). It was shown that an isoglobotrihexosylceramide (iGb3), which is a β-linked trihexosyl ceramide and may be an endogenous ligand for iNKT cells, stimulating both human and mouse NKT cells.[17] It was suggested that the variability of the NKT TCR β-chain enabled the accommodation of iGb3 via a different conformation of TCR or 'squashed' iGb3 headgroup.[13c,18] Comparing the larger trihexosyl headgroup of iGb3 with the simple galactose sugar of KRN7000, we designed compound **7** (Fig. 2), which has a four-atom linker between the sugar and ceramide part of KRN7000 via amide group to investigate the influence on cytokine profile. As far as we know, the linkage between the sugar and ceramide of KRN7000 has been less studied in the literature.

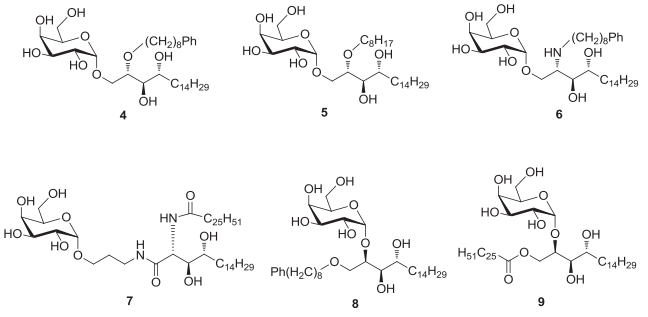
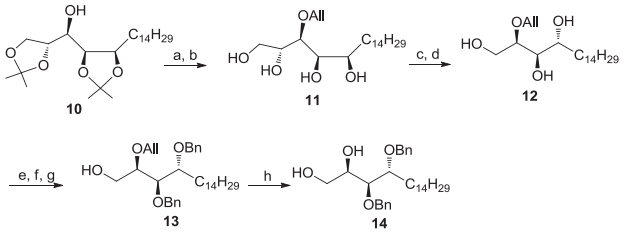


Fig. 2. Designed target KRN7000 analogues.

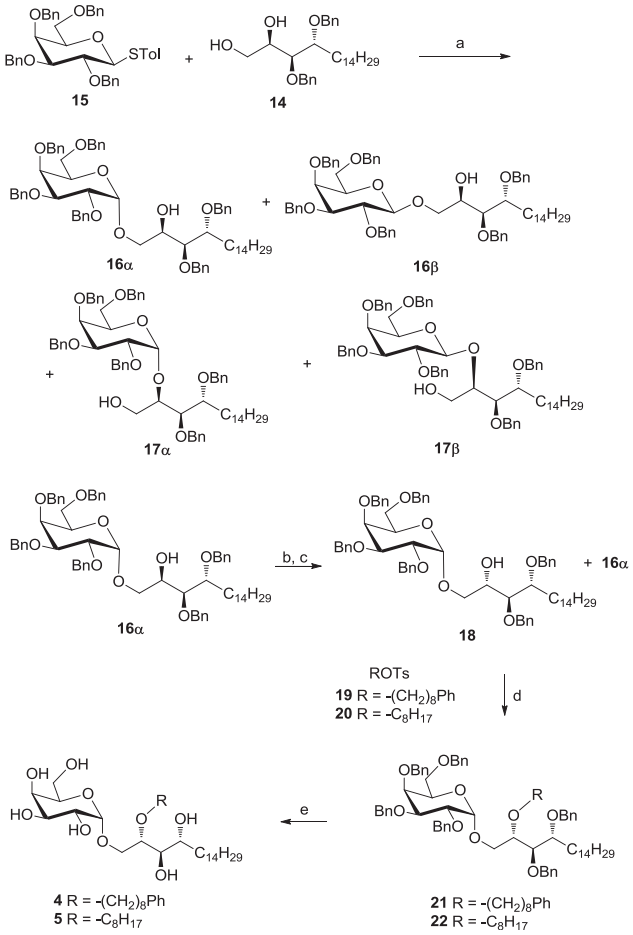
We therefore turned our attention to the synthesis of the target molecules. First of all, we decided to synthesize diol **14** as a glycosyl acceptor and invert the hydroxyl configuration after glycosylation (Scheme 1). Treatment of alcohol **10**[19] with allyl bromide and NaH, followed by removal of the propylidene group with acid, afforded compound **11**. Cleavage of the hydroxylmethylene arm in **11** by sodium periodate and further treatment with sodium borohydride, gave triol **12** in good yield. Regioselective protection of the primary hydroxyl group in **12** with trityl chloride and subsequent protection of the remaining free secondary hydroxyl groups as benzyl ether, followed by removal of the trityl group, provided intermediate **13** in 80% isolated yield. It is possible that the subsequent glycosylation



Scheme 1. Reagents and conditions: (a) AllBr, NaH, DMF, 0 °C, 95%; (b) HCl, MeOH, quant.; (c) $NaIO_4/H_2O$, THF, 96%; (d) $NaBH_4$, MeOH, 91%; (e) TrCl, Py, 50 °C; (f) BnBr, NaH, DMF, 0 °C; (g) $HOAc/H_2O$, 60 °C, 80%; (h) $PdCl_2$, MeOH, 84%.

with *N*-iodosuccinimide (NIS) will lead to byproducts resulting from the addition of electrophiles to the double bond of allyl group.[20] To avoid this, the allyl group in **13** was removed to afford glycosyl acceptor **14**.

With acceptor **14** in hand, the glycosylation reaction was carried out. As shown in Scheme 2, the coupling reaction of acceptor **14** and donor **15**[21] with NIS/TfOH as promoter provided the desired α-anomer **16α** as well as separable products **16β**, **17α**, **17β**. Other donors, such as *p*-methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidine-1-thio-β-D-galactopyranoside[21] were also attempted for the glycosylation of acceptor **14**, but they resulted in a small or no amount of the desired α configuration products with either NIS/TfOH or BSM/Tf_2O[22] as promoter. Subsequently, an attempt to convert **16α** to its triflate under the conditions of Tf_2O/KNO_2/DMF,

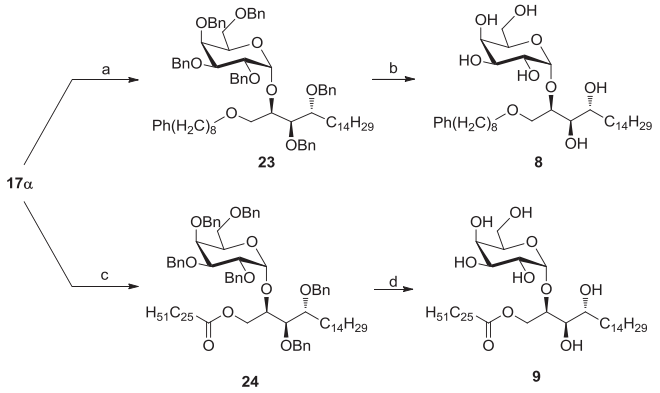


Scheme 2. Reagents and conditions: (a) NIS, TfOH, 4 Å MS, CH_2Cl_2, 43% for **16α**, 11% for **17α**, 18% for **16β**, 9% for **17β**; (b) PCC, 4 Å MS, CH_2Cl_2; (c) $NaBH_4$, MeOH, 98% over two steps (**18**:**16α**=1.6:1.0); (d) **19** and **20**, KOH, toluene, reflux, 85% for **21**, 65% for **22**; (e) H_2, Pd/C, CH_2Cl_2/MeOH, 98% for **4**, 96% for **5**.

which was followed by an S_N2 displacement with 8-phenyloctan-1-ol using NaH as base to directly afford product **21** with desired stereochemistry, was proved to fail. Fortunately, oxidation of **16α** with PCC followed by reduction with $NaBH_4$ eventually resulted in compound **18** with the inversion of C-2 configuration as the major product as well as the recovery of starting material **16α** in a ratio of 1.6:1. The substitution reaction of **18** with 8-phenyl-1-octanolyl *p*-toluenesulfonate **19**[23] and 1-octlyl *p*-toluenesulfonate **20**[24] yielded compounds **21** and **22**, respectively. Then, the catalytic hydrogenolysis of **21** and **22** gave the target compounds **4** and **5** very smoothly.

We were inclined to invert the C3–OH configuration of the reported intermediate **10** in the beginning for the synthesis of target compounds **4** and **5**. However, the S_N2 displacement of the mesylate or triflate of **10** with KOH gave no desired configuration inversion product but elimination product. It gave the same results when the mesylate or triflate of **10** was treated with allyl alcohol/NaH. Both the Mitsunobu reaction (allyl alcohol,[25] acetic acid or *p*-nitrobenzoic acid as nucleophile) and oxidation (TEMPO,[26] PCC, PDC, the Swern reagent, and the Dess–Martin reagent) followed by reduction failed to provide the desired inversion product, which may result from the steric hindrance of the two propylidene protecting groups in the proximity of hydroxyl group. Moreover, treatment of the triflate of **10** with KNO_2 in DMF proceeded,[27] but it only produced a trace amount of configuration inversion product.

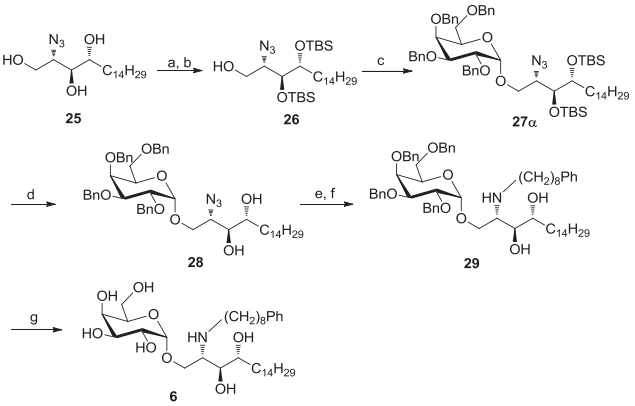
Coupling product **17α** was transformed into the final compound **8** by the same strategy as described in the preparation of **4**. On the other hand, esterification of **17α** with hexacosanoic acid, which was followed by hydrogenolysis, afforded another target molecule **9** (Scheme 3).



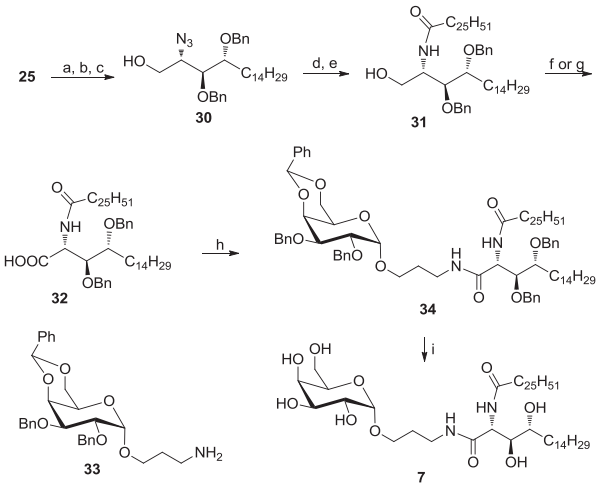
Scheme 3. Reagents and conditions: (a) **19**, KOH, toluene, reflux, 85%; (b) H_2, Pd/C, CH_2Cl_2/MeOH, 97%; (c) $C_{25}H_{51}COOH$, EDC, DMAP, CH_2Cl_2, 55%; (d) H_2, $Pd(OH)_2$/C, CH_2Cl_2/MeOH, 97%.

With respect to analogue **6**, we chose alcohol **26** as the glycosyl acceptor, which was obtained from the known triol **25**[19] (Scheme 4). Glycosylation of **26** with the galactosyl donor **15** was then performed, affording the desired α-galactoside **27α** as the major anomer. Cleavage of the TBS protecting group in **27α** with TBAF gave compound **28**. Reduction of the azido group in **28** followed by reductive amination provided compound **29**. In the final step, all benzyl protecting groups in **29** were removed with H_2 over $Pd(OH)_2$/C to yield target compound **6**.

The synthesis of analogue **7** started from the transformation of triol **25** to compound **30** according to the reported procedure[28] (Scheme 5). Reduction of azide **30** under the Staudinger conditions followed by acylation with hexacosanoic acid furnished alcohol **31** in excellent yield. Oxidation of **31** with DIAB/TEMPO produced acid **32**, it took relatively long time (3–4 days) and an excess amount of reagent (3 equiv TEMPO). Thus an alternative oxidation by using DIAB/TEMPO (2 equiv/1 equiv, overnight) and



Scheme 4. Reagents and conditions: (a) TBSOTf, 2,6-lutidine, CH_2Cl_2; (b) CF_3COOH, THF/H_2O, 85% over two steps; (c) **15**, NIS, TfOH, 4 Å MS, CH_2Cl_2, 53% for **27α**, 27% for its β-anomer; (d) TBAF, THF, 80%; (e) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, MeOH; (f) $Ph(CH_2)_7CHO$, $NaBH_3CN$, AcOH, MeOH, 51%; (g) H_2, $Pd(OH)_2$/C, CH_2Cl_2/MeOH, 78%.



Scheme 5. Reagents and conditions: (a) TrCl, Py, 50 °C; (b) BnBr/NaH/DMF; (c) HOAc/H_2O, 50 °C, 80% over three steps; (d) PPh_3/H_2O, THF, 60 °C; (e) $C_{25}H_{51}COOH$, HBTU, DIPEA, CH_2Cl_2, 98% over two steps; (f) DIAB/TEMPO, CH_2Cl_2, 81%; (g) (i) DIAB/TEMPO, CH_2Cl_2; (ii) $NaClO_2$, NaH_2PO_4, Bu_4NCl, CH_2Cl_2/H_2O, 90%; (h) **33**, HBTU, DIPEA, CH_2Cl_2, 71%; (i) H_2, $Pd(OH)_2$/C, $CHCl_3$/MeOH/HOAc, 74%.

further by $NaClO_2/NaH_2PO_4$ (30 min), was performed to give acid **32** in 90% yield. Coupling reaction of **32** and amine **33** afforded compound **34**, which was followed by catalytic hydrogenolysis over $Pd(OH)_2$/C to result in target molecule **7**.

The synthetic compounds **4**–**9** were evaluated for their ability to induce IL-4 and IFN-γ in vitro and in vivo, using KRN7000 as a positive control. It turned out that the analogues induced no detectable cytokine or proliferative response.

The ether analogues **4** and **5** were completely inactive on murine cells, which was consistent with the previous results reported previously.[16] The ether analogues **4** and **5** as well as the amine analogue **6**, which could be protonated at physiological pH, may be not able to form a H-bond with Thr156 of mCD1d. Taking all amide modifications together, we speculate that the ability to form H-bond involving H-bond donor and H-bond acceptor play a significant role in the activity and cytokine profile of NKT cells. Moreover, the modifications also increase the flexibility of the lipid chain, which may cause a different orientation of the acyl lipid in the compound, resulting in the analogues not to fit into the cavity of mCD1d. This result gave further evidence on the conjecture that the amide moiety of KRN7000 is crucial for maintaining the correct position and orientation to fit into the binding cavity and to be recognized by the TCR. The fact analogue **7** lost the activity may be attributed to the

distance between the galactose and ceramide moiety, which was also observed in C-KRN7000 analogue (increasing by one carbon).[29] The suitable tolerant distance between the galactose and ceramide part of KRN7000 needs to be investigated.

In conclusion, six new KRN7000 analogues with the modifications at the amide bond or the linkage between the sugar and ceramide were designed and synthesized. Although the synthetic route for some target molecules was uneven, the desired compounds were eventually prepared by the strategy that inverted the hydroxyl configuration after the glycosidic bond construction. These synthetic compounds were evaluated for their ability to stimulate cytokine release and the preliminary structure–activity relationships were discussed. Although the compounds are inactive on inducing the cytokine release, the results may be useful to guide to design the next generation of KRN7000 analogues. On the other hand, due to the potential applications of KRN7000 analogues in drug discovery, the disclosed approach may facilitate preparation of more KRN7000 derivatives with biological importance.

3. Experimental

3.1. General

Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon using flame-dried glassware and standard syringe/septa techniques. All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Dichloromethane (CH_2Cl_2) and pyridine were distilled over calcium hydride (CaH_2). Methanol was distilled from magnesium. DMF was stirred with CaH_2 and distilled under reduced pressure. Tetrahydrofuran (THF) was distilled over sodium/benzophenone. All reactions were carried out under anhydrous conditions with freshly distilled solvents, unless otherwise noted. Analytical TLC was performed on silica gel 60-F_{254} precoated on aluminum plates (E. Merck), with detection by UV (254 nm) and/or by staining with acidic ceric ammonium molybdate. Solvents were evaporated under reduced pressure and below 35 °C (bath). Organic solutions of crude products were dried over anhydrous Na_2SO_4. Column chromatography was performed employing silica gel (200–300 mesh). ^{1}H NMR spectra were recorded on a JEOL AL-300, Varian INOVA-500 or Advance DRX Bruker-400 spectrometers at 25 °C. Chemical shifts (in parts per million) were referenced to tetramethylsilane (δ=0 ppm) in deuterated chloroform. ^{13}C NMR spectra were obtained by using the same NMR spectrometers and were calibrated with $CDCl_3$ (δ=77.00 ppm) or CD_3OD (δ=49.00 ppm). Mass spectra were recorded using a PE SCLEX QSTAR spectrometer. High-resolution mass spectrometry was performed on a Bruker APEX IV. Elemental analysis data were recorded on a Vario EL-III element analyzer.

3.2. (2*R*,3*R*,4*R*,5*R*)-3-*O*-Allyl-1,2,3,4,5-nonadecanepentol (11)

Sodium hydride (420 mg, 0.01 mol) was added to a solution of (2*R*,3*R*,4*R*,5*R*)-3-hydroxy-1,2; 4,5-di-*O*-isopropylidenenonadecane **10**[19] (2.14 g, 0.005 mol) in dry DMF (15 mL) at 0 °C. The reaction mixture was stirred at the same temperature for 10 min before allyl bromide (0.66 mL, 0.9 g, 0.0075 mol) was added. The mixture was allowed to warm to room temperature and stirred for an additional 6 h. The mixture was poured into ice-water mixture and then extracted with EtOAc. The combined organic extract was dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 40:1) to give the intermediate as a colorless oil (2.23 g, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.94–5.86 (m, 1H), 5.31 (dq, *J*=17.3, 1.5 Hz, 1H), 5.13 (dq, *J*=10.5, 1.5 Hz, 1H), 4.31 (ddt, *J*=12.8, 6.0, 1.5 Hz, 1H), 4.18 (ddt, *J*=12.5, 5.0, 1.5 Hz, 1H), 4.12–4.06 (m, 3H), 4.03–3.98 (m, 2H), 3.60 (t, *J*=4.5 Hz, 1H), 1.69–1.59 (m, 2H), 1.58–1.48 (m, 2H), 1.46 (s, 3H), 1.41 (s, 3H), 1.40–1.22 (m, 28H), 0.88 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.0, 116.2, 108.4, 108.2, 79.0, 77.7, 77.66, 77.5, 72.9, 66.2, 31.9, 29.7, 29.65, 29.6, 29.55, 29.4, 27.2, 26.5, 26.3, 26.0, 25.2, 22.7, 14.1; ESI-HRMS $[M+Na]^+$ calcd for $C_{28}H_{52}NaO_5^+$ 491.3707, found 491.3703.

To a solution of above intermediate (2.23 g, 4.76 mmol) in MeOH (15 mL) was added HCl (0.8 mL, 9.28 mmol) dropwise at 0 °C and the mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by column chromatography on silica gel (petroleum ether/EtOAc/MeOH 10:10:1) to give compound **11** as white solids (1.84 g, 100%). ^{1}H NMR (500 MHz, CD_3OD) δ 5.99–5.92 (m, 1H), 5.39 (dq, *J*=17.5, 2.0 Hz, 1H), 5.11 (dq, *J*=10.3, 1.5 Hz, 1H), 4.20–4.16 (m, 2H), 3.82–3.76 (m, 1H), 3.76–3.70 (m, 2H), 3.62–3.51 (m, 3H), 1.86–1.81 (m, 1H), 1.62–1.53 (m, 1H), 1.42–1.18 (m, 24H), 0.88 (t, *J*=6.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 136.6, 116.7, 79.0, 75.0, 74.4, 73.1, 72.0, 64.5, 35.2, 33.1, 31.0, 30.8, 30.5, 26.6, 23.7, 14.4; ESI-HRMS $[M+H]^+$ calcd for $C_{22}H_{45}O_5^+$ 389.3262, found 389.3253.

3.3. (2*R*,3*R*,4*R*)-2-*O*-Allyl-1,2,3,4-octadecanetetrol (12)

To a solution of compound **11** (1.1 g, 2.83 mmol) in THF (15 mL) was added a solution of $NaIO_4$ (0.6 g, 2.83 mmol) in H_2O (15 mL) at 0 °C and the mixture was stirred at 0 °C for 1 day. The solvent was evaporated and the resulting residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 2:1) to give the intermediate as white solids (0.96 g, 96%). ^{1}H NMR (500 MHz, CD_3OD) δ 5.99–5.88 (m, 1H), 5.34–5.27 (m, 1H), 5.22–5.13 (m, 2H), 4.18–4.08 (m, 2H), 3.94–3.89 (m, 1H), 3.74–3.71 (m, 1H), 3.68–3.55 (m, 1H), 1.69–1.58 (m, 1H), 1.52–1.43 (m, 1H), 1.42–1.24 (m, 24H), 0.89 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 136.1, 135.8, 117.5, 117.2, 101.5, 95.7, 92.2, 85.6, 83.0, 82.3, 81.1, 78.9, 72.3, 71.8, 36.4, 34.3, 33.1, 30.8, 30.77, 30.74, 30.5, 26.9, 26.8, 23.7, 14.5; ESI-TOF-MS $[M+Na]^+$ calcd for $C_{21}H_{40}NaO_4^+$ 379, found 379; Anal. Calcd for $C_{21}H_{40}O_4$: C, 70.74; H, 11.31. Found: C, 70.79; H, 10.91.

To a solution of above intermediate (506 mg, 1.42 mmol) in dried MeOH (10 mL) was added $NaBH_4$ (55 mg, 1.42 mmol) at 0 °C and the mixture was stirred at room temperature for 4 h. The solvent was evaporated and the resulting residue was purified by column chromatography on silica gel (petroleum ether/EtOAc/MeOH 15:15:1) to give compound **12** as white solids (511 mg, 91%). ^{1}H NMR (500 MHz, CD_3OD) δ 6.00–5.93 (m, 1H), 5.28 (ddd, *J*=17.5, 3.5, 1.5 Hz, 1H), 5.12 (d, *J*=10.3 Hz, 1H), 4.22 (ddt, *J*=12.8, 5.5, 1.5 Hz, 1H), 4.13 (ddt, *J*=12.5, 5.5, 1.5 Hz, 1H), 3.71–3.68 (m, 3H), 3.58 (dt, *J*=2.5, 8.5 Hz, 1H), 3.37 (dd, *J*=8.3, 1.5 Hz, 1H), 1.81–1.75 (m, 1H), 1.59–1.52 (m, 1H), 1.40–1.24 (m, 24H), 0.89 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 136.85, 116.9, 79.8, 75.4, 73.4, 72.1, 62.8, 34.8, 33.1, 31.0, 30.8, 30.5, 26.6, 23.7, 14.4; ESI-TOF-MS $[M+Na]^+$ calcd for $C_{21}H_{42}NaO_4^+$ 381, found 381; Anal. Calcd for $C_{21}H_{42}O_4$: C, 70.34; H, 11.81. Found: C, 70.09; H, 11.53.

3.4. (2*R*,3*R*,4*R*)-2-*O*-Allyl-3,4-di-*O*-benzyl-1,2,3,4-octadecanetetrol (13)

A solution of **12** (175 mg, 0.448 mmol) and trityl chloride (180 mg, 0.634 mmol) in dry pyridine (10 mL) was stirred at 50 °C for 5 h. Pyridine was evaporated under reduced pressure and the residue was diluted with EtOAc, and washed with H_2O, saturated aqueous $NaHCO_3$ solution and brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was then dissolved in dry DMF (8 mL) and then treated with 60% NaH (84 mg, 2.0 mmol) for 10 min at 0 °C. BnBr (0.16 mL, 256 mg, 1.5 mmol) was added and the mixture was allowed to warm to room temperature and stirred for an additional 4.5 h. After the solvent was removed in vacuo, the residue was dissolved in water and extracted with EtOAc. The

organic extraction was concentrated and then dissolved in a mixture of HOAc and H_2O (5:1, 6 mL). The mixture was stirred at 60 °C overnight. Then the mixture was diluted with EtOAc, and washed with H_2O, saturated aqueous $NaHCO_3$ solution and brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 10:1) to give compound **13** as white waxy solids (211 mg, 80% for three steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.25 (m, 10H), 5.95–5.87 (m, 1H), 5.24 (dd, *J*=17.0, 1.5 Hz, 1H), 5.15 (dd, *J*=10.3, 1.0 Hz, 1H), 4.74 (d, *J*=11.5 Hz, 1H), 4.68 (d, *J*=11.0 Hz, 1H), 4.60 (d, *J*=11.5 Hz, 1H), 4.52 (d, *J*=11.5 Hz, 1H), 4.17–4.08 (m, 2H), 3.75–3.69 (m, 2H), 3.64–3.55 (m, 3H), 2.27 (t, *J*=6.0 Hz, 1H), 1.78–1.71 (m, 1H), 1.61–1.54 (m, 1H), 1.49–1.42 (m, 1H), 1.36–1.18 (m, 23H), 0.88 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.4, 134.9, 128.3, 128.0, 127.9, 127.6, 127.58, 117.0, 80.3, 80.1, 79.7, 74.0, 72.1, 72.0, 61.9, 31.9, 30.4, 29.7, 29.67, 29.63, 29.3, 25.8, 22.7, 14.1; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{35}H_{58}NO_4^+$ 556.4360, found 556.4358.

3.5. (2*R*,3*S*,4*R*)-3,4-Di-*O*-benzyl-1,2,3,4-octadecanetetrol (14)

A mixture of compound **13** (560 mg, 1.04 mmol) and $PdCl_2$ (36.9 mg, 0.208 mmol) in dry MeOH (10 mL) was stirred overnight. The reaction was then diluted with MeOH (10 mL), filtered through Celite and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 1:1) to give compound **14** as white waxy solids (436 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.25 (m, 10H), 4.74 (d, *J*=11.5 Hz, 1H), 4.66 (d, *J*=11.5 Hz, 1H), 4.59 (d, *J*=11.5 Hz, 1H), 4.55 (d, *J*=11.5 Hz, 1H), 3.91–3.87 (m, 1H), 3.70–3.64 (m, 3H), 3.54 (t, *J*=4.0 Hz, 1H), 3.26 (d, *J*=4.0 Hz, 1H), 2.16 (br s, 1H), 1.73–1.65 (m, 2H), 1.60–1.54 (m, 1H), 1.45–1.38 (m, 1H), 1.36–1.20 (m, 22H), 0.88 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.0, 137.8, 128.5, 128.4, 128.1, 128.0, 127.99, 127.8, 79.7, 79.1, 73.3, 72.7, 71.2, 64.0, 31.9, 30.8, 29.7, 29.65, 29.59, 29.56, 29.3, 25.5, 22.7, 14.1; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{32}H_{54}NO_4^+$ 883.5579, found 883.5540. The spectroscopic data coincide with those reported in the literature.[30]

3.6. 1-*O*-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-D-*arabino*-1,2,3,4-octadecanetetrol (16α) and 2-*O*-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-D-*arabino*-1,2,3,4-octadecanetetrol (17α)

To a mixture of donor **15**[21] (58.0 mg, 0.09 mmol), acceptor **14** (50.0 mg, 0.1 mmol), and 4 Å molecular sieves (400 mg) in CH_2Cl_2 (4 mL) was added NIS (25.6 mg, 0.108 mmol) at room temperature under Ar. The reaction mixture was stirred and TfOH (11 μL, 0.5 M in Et_2O, 0.0054 mmol) was added. After 2 h, the reaction was quenched with Et_3N (0.3 mL). The residue was diluted with CH_2Cl_2 and then filtered through Celite. The filtrate was concentrated, washed with H_2O, saturated aqueous $Na_2S_2O_3$ solution and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 4:1 to 2:1) to give compound **16α** (39.0 mg, 43%), **17α** (10.0 mg, 11%), **16β** (16.0 mg, 18%), and **17β** (8.0 mg, 9%) as syrup. For **16α**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.23 (m, 30H), 4.92 (d, *J*=11.5 Hz, 1H), 4.87 (d, *J*=4.0 Hz, 1H), 4.78 (t, *J*=11.5 Hz, 2H), 4.71–4.60 (m, 4H), 4.56–4.48 (m, 3H), 4.45 (d, *J*=12.0 Hz, 1H), 4.37 (d, *J*=11.5 Hz, 1H), 4.06–4.00 (m, 3H), 3.96–3.92 (m, 2H), 3.78–3.73 (m, 1H), 3.66–3.63 (m, 1H), 3.60–3.57 (m, 2H), 3,52–3.49 (m, 2H), 3.23 (d, *J*=5.0 Hz, 1H), 1.64–1.58 (m, 1H), 1.46–1.38 (m, 1H), 1.36–1.19 (m, 24H), 0.88 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.74, 138.67, 138.6, 138.4, 138.3, 138.0, 128.35, 128.27, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.36, 98.3, 79.8, 79.5, 79.1, 76.5, 75.0, 74.8, 73.6, 73.44, 73.40, 72.9, 72.7, 69.8, 69.5, 68.9, 31.9, 31.1, 29.8, 29.7, 29.4, 25.7, 22.7, 14.1; ESI-HRMS $[M+H]^+$ calcd for $C_{66}H_{85}O_9^+$ 1021.6194, found 1021.6172. For **17α**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33–7.21 (m, 30H), 5.02 (d, *J*=4.0 Hz, 1H), 4.91 (d, *J*=11.5 Hz, 1H), 4.81–4.68 (m, 5H), 4.60 (d, *J*=12.0 Hz, 1H), 4.55–4.48 (m, 3H), 4.38 (dd, *J*=16.0, 11.5 Hz, 2H), 4.16 (dd, *J*=6.8, 5.0 Hz, 1H), 4.05 (dd, *J*=10.0, 3.5 Hz, 1H), 3.94 (dd, *J*=10.0, 3.0 Hz, 1H), 3.85 (d, *J*=1.5 Hz, 1H), 3.81–3.67 (m, 5H), 3.52 (dd, *J*=9.8, 7.5 Hz, 2H), 3.31 (dd, *J*=9.5, 5.0 Hz, 1H), 1.72–1.65 (m, 2H), 1.48–1.40 (m, 2H), 1.38–1.18 (m, 22H), 0.88 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.8, 138.75, 138.6, 138.5, 138.4, 137.6, 128.39, 128.36, 128.2, 127.8, 127.7, 127.65, 127.5, 127.4, 99.4, 84.4, 80.3, 79.6, 79.0, 76.6, 75.1, 74.6, 73.5, 73.4, 73.2, 71.7, 70.4, 69.6, 63.2, 31.9, 30.6, 29.9, 29.7, 29.4, 25.9, 22.7, 14.1; ESI-HRMS $[M+H]^+$ calcd for $C_{66}H_{85}O_9^+$ 1021.6194, found 1021.6191. For **16β**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.23 (m, 30H), 4.94 (d, *J*=12.0 Hz, 1H), 4.87 (d, *J*=11.0 Hz, 1H), 4.74 (t, *J*=11.5 Hz, 2H), 4.72 (d, *J*=8.0 Hz, 1H), 4.70–4.58 (m, 4H), 4.53 (d, *J*=11.0 Hz, 1H), 4.43–4.35 (m, 3H), 4.09 (ddd, *J*=11.0, 6.5, 2.5 Hz, 1H), 3.96 (dd, *J*=6.3, 9.5 Hz, 1H), 3.90 (d, *J*=2.5 Hz, 1H), 3.81 (dd, *J*=7.8, 9.5 Hz, 1H), 3.69–3.65 (m, 2H), 3.59–3.50 (m, 5H), 3.23 (d, *J*=5.0 Hz, 1H), 1.63–1.38 (m, 2H), 1.42–1.16 (m, 24H), 0.88 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.7, 138.6, 138.5, 138.3, 138.2, 137.8, 128.4, 128.3, 128.2, 128.16, 128.1, 128.07, 127.9, 127.87, 127.8, 127.7, 127.6, 127.5, 104.1, 82.2, 79.9, 79.5, 78.9, 75.2, 74.6, 73.6, 73.5, 73.3, 73.0, 72.9, 70.9, 70.0, 68.6, 31.9, 31.2, 29.7, 29.67, 29.63, 29.4, 25.7, 22.7, 14.1; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{66}H_{88}NO_9^+$ 1038.6454, found 1038.6459. For **17β**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.20 (m, 30H), 4.94 (d, *J*=11.5 Hz, 1H), 4.88 (d, *J*=11.0 Hz, 1H), 4.80 (d, *J*=11.0 Hz, 1H), 4.74–4.67 (m, 3H), 4.62–4.56 (m, 4H), 4.46 (d, *J*=11.5 Hz, 1H), 4.31 (dd, *J*=18.0, 11.5 Hz, 2H), 4.00–3.96 (m, 1H), 3.92–3.88 (m, 2H), 3.71–3.52 (m, 6H), 3.49 (dd, *J*=7.0, 6.0 Hz, 1H), 3.38 (dd, *J*=8.8, 5.5 Hz, 1H), 3.03 (dd, *J*=9.0, 4.0 Hz, 1H), 1.68–1.60 (m, 2H), 1.48–1.40 (m, 2H), 1.34–1.18 (m, 22H), 0.88 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.84, 138.8, 138.6, 138.2, 138.0, 137.8, 128.5, 128.4, 128.37, 128.24, 128.22, 128.2, 127.8, 127.78, 127.6, 127.54, 127.49, 127.4, 104.0, 82.8, 82.1, 80.0, 79.7, 78.8, 76.5, 75.7, 74.6, 73.8, 73.5, 73.4, 73.2, 72.6, 71.6, 68.4, 63.3, 34.1, 31.9, 30.4, 29.9, 29.7, 29.6, 29.4, 25.6, 22.7, 14.1; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{66}H_{88}NO_9^+$ 1038.6454, found 1038.6446.

3.7. 1-*O*-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-D-*ribo*-1,2,3,4-octadecanetetrol (18)

To a mixture of **16α** (80.0 mg, 0.078 mmol) and 4 Å molecular sieves (300 mg) in CH_2Cl_2 (6 mL) was added PCC (52.0 mg, 0.24 mmol) at 0 °C. After stirred at room temperature for 3 h, the reaction mixture was diluted with CH_2Cl_2 and then filtered through Celite. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 5:1) to give the crude corresponding aldehyde, which was dissolved in MeOH (5 mL). $NaBH_4$ (9.0 mg, 0.24 mmol) was added to the solution at 0 °C. The mixture was allowed to warm to room temperature and stirred for 2 h. The solvent was evaporated and the resulting residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 5:1) to give **18** (48.0 mg) and **16α** (30.0 mg) as yellow oil (98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.23 (m, 30H), 4.92 (d, *J*=11.5 Hz, 1H), 4.85 (d, *J*=3.5 Hz, 1H), 4.82 (d, *J*=12.0 Hz, 1H), 4.81 (d, *J*=12.0 Hz, 1H), 4.74 (d, *J*=11.5 Hz, 1H), 4.73 (d, *J*=12.0 Hz, 1H), 4.68–4.54 (m, 4H), 4.47 (d, *J*=11.5 Hz, 1H), 4.44 (d, *J*=12.0 Hz, 1H), 4.37 (d, *J*=12.0 Hz, 1H), 4.05–3.91 (m, 6H), 3.68–3.65 (m, 1H), 3.62 (dd, *J*=6.8, 3.0 Hz, 1H), 3.57 (dd, *J*=10.8, 8.0 Hz, 1H), 3.50–3.43 (m, 2H), 3.40 (br s, 1H), 1.68–1.63 (m, 2H), 1.45–1.42 (m, 2H), 1.31–1.18 (m, 22H), 0.88 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.8, 138.63, 138.58, 138.3, 137.8, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 99.7, 80.4, 80.2, 79.2, 76.6, 74.9, 74.7, 73.7, 73.6, 73.5, 73.0, 72.6, 72.0, 70.6, 69.7, 69.1, 31.9, 29.9, 29.86, 29.7, 29.4, 25.8, 22.7, 14.1; ESI-HRMS $[M+Na]^+$ calcd for $C_{66}H_{84}NaO_9^+$ 1043.6008, found 1043.6018.

3.8. 1-*O*-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-2-*O*-(8″-phenyloctyl)-D-*ribo*-1,2,3,4-octadecanetetrol (21)

KOH (9.4 mg, 0.132 mmol) was added to a solution of compound **18** (45.0 mg, 0.044 mmol) in dry toluene (2 mL). The mixture was stirred for 10 min after which a solution of **19**[23] (22.2 mg, 0.062 mmol) in toluene (2 mL) was added to it. The resulting mixture was refluxed at 110 °C overnight and then concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 10:1) to give compound **21** as a yellow oil (45.0 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.14 (m, 35H), 4.94 (d, J=3.5 Hz, 1H), 4.93 (d, J=11.5 Hz, 1H), 4.81–4.55 (m, 8H), 4.43 (d, J=12.0 Hz, 1H), 4.42 (d, J=11.5 Hz, 1H), 4.33 (d, J=12.0 Hz, 1H), 4.05 (dd, J=9.5, 3.5 Hz, 1H), 3.99–3.95 (m, 4H), 3.75 (dd, J=5.5, 4.0 Hz, 1H), 3.70–3.56 (m, 4H), 3.53 (dd, J=9.3, 7.0 Hz, 1H), 3.45 (q, J=5.0 Hz, 1H), 3.35 (dt, J=11.3, 6.5 Hz, 1H), 2.57 (t, J=7.5 Hz, 2H), 1.68–1.61 (m, 1H), 1.56–1.19 (m, 37H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.8, 138.9, 138.84, 138.78, 138.1, 128.4, 128.31, 128.28, 128.2, 128.17, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 125.5, 98.1, 79.7, 79.2, 79.0, 78.9, 76.6, 75.0, 74.8, 73.7, 73.5, 72.9, 72.8, 71.8, 70.7, 69.2, 69.0, 68.0, 36.0, 31.9, 31.5, 30.2, 30.0, 29.9, 29.7, 29.6, 29.4, 26.3, 25.7, 22.7, 14.1; ESI-HRMS $[M+Na]^+$ calcd for $C_{80}H_{104}NaO_9^+$ 1231.7573, found 1231.7569.

3.9. 1-*O*-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-2-*O*-(8″-octyl)-D-*ribo*-1,2,3,4-octadecanetetrol (22)

The synthetic procedure was the same as that described in the synthesis of compound **21** using KOH (6.4 mg, 0.094 mmol), **18** (32.0 mg, 0.031 mmol), and **20**[24] (13.3 mg, 0.047 mmol) to afford **22** as a colorless oil (23.3 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.21 (m, 30H), 4.94 (d, J=3.2 Hz, 1H), 4.93 (d, J=12.0 Hz, 1H), 4.80 (d, J=12.0 Hz, 1H), 4.76 (d, J=12.0 Hz, 1H), 4.73 (d, J=11.2 Hz, 1H), 4.68–4.63 (m, 3H), 4.60 (d, J=11.6 Hz, 1H), 4.56 (d, J=11.2 Hz, 1H), 4.43 (d, J=11.6 Hz, 1H), 4.42 (d, J=12.0 Hz, 1H), 4.33 (d, J=11.6 Hz, 1H), 4.05 (dd, J=9.4, 3.4 Hz, 1H), 4.00–3.95 (m, 4H), 3.75 (dd, J=5.8, 3.8 Hz, 1H), 3.69–3.57 (m, 4H), 3.53 (dd, J=11.0, 7.4 Hz, 1H), 3.45 (dd, J=9.2, 6.0 Hz, 1H), 3.34 (dt, J=8.8, 6.8 Hz, 1H), 1.69–1.61 (m, 1H), 1.53–1.42 (m, 3H), 1.33–1.23 (m, 34H), 0.88 (t, J=6.8 Hz, 3H), 0.87 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.92, 138.83, 138.80, 138.76, 138.05, 128.31, 128.27, 128.20, 128.16, 127.85, 127.81, 127.75, 127.61, 127.51, 127.47, 127.37, 127.32, 98.04, 79.71, 79.08, 78.98, 78.88, 76.63, 75.03, 74.75, 73.68, 73.45, 72.95, 72.80, 71.81, 70.70, 69.24, 68.95, 67.93, 31.91, 31.89, 30.19, 29.99, 29.87, 29.72, 29.66, 29.56, 29.35, 26.34, 25.70, 22.68, 14.10; ESI-HRMS $[M+H]^+$ calcd for $C_{74}H_{101}O_9^+$ 1133.7440, found 1133.7487.

3.10. 1-*O*-(α-D-Galactopyranosyl)-2-*O*-(8′-phenyloctyl)-D-*ribo*-1,2,3,4-octadecanetetrol (4)

Pd/C (10% on carbon, 15 mg) was added to a solution of compound **21** (29.0 mg, 0.024 mmol) in MeOH/CH_2Cl_2 (5 mL, 2:3) at room temperature. The mixture was stirred under H_2 (3 atm) for 12 h and then filtered through Celite. The filter cake was rinsed with MeOH and CH_2Cl_2. The filtrate was concentrated, and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to provide **4** as a colorless oil (15.6 mg, 98%). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD=1:1) δ 7.27 (dd, J=8.3, 7.0 Hz, 2H), 7.20–7.16 (m, 3H), 4.89 (d, J=3.5 Hz, 1H), 4.03 (dd, J=11.0, 4.0 Hz, 1H), 3.98 (d, J=2.0 Hz, 1H), 3.87 (t, J=5.5 Hz, 1H), 3.83–3.73 (m, 4H), 3.72–3.61 (m, 4H), 3.52–3.47 (m, 1H), 3.42–3.37 (m, 1H), 2.60 (t, J=7.5 Hz, 2H), 1.72–1.66 (m, 1H), 1.64–1.50 (m, 6H), 1.48–1.39 (m, 2H), 1.38–1.15 (m, 29H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD=1:1) δ 143.0, 128.5, 128.4, 125.7, 99.4, 79.6, 72.9, 72.4, 70.5, 70.46, 70.2, 70.19, 69.3, 65.7, 62.2, 36.1, 33.7, 32.2, 32.1, 31.7, 30.1, 30.0, 29.9, 29.6, 29.5, 29.46, 26.9, 26.6, 26.3, 25.9, 22.8, 14.1; ESI-HRMS $[M+H]^+$ calcd for $C_{38}H_{69}O_9^+$ 669.4942, found 669.4943.

3.11. 1-*O*-(α-D-Galactopyranosyl)-2-*O*-(8′-octyl)-D-*ribo*-1,2,3,4-octadecanetetrol (5)

The synthetic procedure was the same as that described in the synthesis of compound **4** using Pd/C (10% on carbon, 20.0 mg) and **22** (29.9 mg, 0.0264 mmol) to afford **5** as a colorless oil (15.0 mg, 96%). ^{1}H NMR (500 MHz, CD_3OD) δ 4.84 (d, J=4.5 Hz, 1H), 4.05 (dd, J=11.0, 3.5 Hz, 1H), 3.89–3.86 (m, 2H), 3.77–3.67 (m, 7H), 3.60 (dd, J=11.0, 4.0 Hz, 1H), 3.52 (m, 1H), 3.41 (dt, J=9.5, 7.0 Hz, 1H), 1.64–1.52 (m, 4H), 1.37–1.28 (m, 34H), 0.90 (t, J=7.0 Hz, 3H), 0.89 (t, J=7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 100.8, 80.5, 74.6, 72.9, 72.4, 71.5, 71.2, 71.0, 70.3, 67.1, 62.8, 33.1, 32.8, 31.2, 30.9, 30.81, 30.76, 30.67, 30.51, 30.47, 27.43, 27.0, 23.8, 23.7, 14.5, 14.4; ESI-HRMS $[M+Na]^+$ calcd for $C_{32}H_{64}NaO_9^+$ 615.4443, found 615.4458.

3.12. 2-*O*-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-1-*O*-(8″-phenyloctyl)-D-*arabino*-1,2,3,4-octadecanetetrol (23)

The synthetic procedure was the same as that described in the synthesis of compound **21** using KOH (8 mg, 0.115 mmol), **17**α (39.0 mg, 0.038 mmol), and **19** (18.0 mg, 0.05 mmol) to afford **23** as a yellow oil (39.0 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.14 (m, 35H), 5.13 (d, J=3.0 Hz, 1H), 4.91 (d, J=11.5 Hz, 1H), 4.79 (d, J=12.0 Hz, 1H), 4.74–4.68 (m, 4H), 4.60 (d, J=12.5 Hz, 1H), 4.55 (d, J=11.5 Hz, 1H), 4.50–4.43 (m, 2H), 4.39 (d, J=12.0 Hz, 1H), 4.33 (d, J=11.5 Hz, 1H), 4.17 (t, J=6.5 Hz, 1H), 4.05–3.98 (m, 3H), 3.93 (dd, J=10.5, 5.5 Hz, 1H), 3.85 (t, J=4.5 Hz, 1H), 3.72–3.68 (m, 1H), 3.64–3.48 (m, 4H), 3.33–3.25 (m, 2H), 2.57 (t, J=7.5 Hz, 2H), 1.72–1.62 (m, 1H), 1.52–1.18 (m, 37H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.8, 139.3, 139.0, 138.8, 138.7, 138.1, 128.4, 128.31, 128.28, 128.2, 128.1, 128.09, 127.9, 127.74, 127.7, 127.6, 127.4, 127.3, 127.2, 127.1, 125.5, 99.2, 79.6, 79.2, 79.0, 78.4, 76.3, 75.1, 74.7, 73.6, 73.5, 73.2, 72.8, 71.4, 71.0, 70.4, 69.4, 69.0, 36.0, 31.9, 31.5, 30.4, 30.3, 30.0, 29.7, 29.54, 29.49, 29.4, 29.3, 26.2, 25.7, 22.7, 14.1; ESI-HRMS $[M+H]^+$ calcd for $C_{80}H_{105}O_9^+$ 1209.7759, found 1209.7750.

3.13. 2-*O*-(α-D-Galactopyranosyl)-1-*O*-(8′-phenyloctyl)-D-*arabino*-1,2,3,4-octadecanetetrol (8)

The synthetic procedure was the same as that described in the synthesis of compound **4** using Pd/C (10% on carbon, 10 mg) and **23** (16.0 mg, 0.013 mmol) to afford **8** as a colorless oil (8.6 mg, 97%). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD=1:1) δ 7.29–7.25 (m, 2H), 7.20–7.16 (m, 3H), 5.07 (d, J=4.0 Hz, 1H), 4.00–3.94 (m, 3H), 3.81–3.64 (m, 7H), 3.55 (dd, J=7.0, 2.0 Hz, 1H), 3.47–3.44 (m, 2H), 2.60 (t, J=7.5 Hz, 2H), 1.72–1.50 (m, 7H), 1.50–1.40 (m, 2H), 1.40–1.14 (m, 29H), 0.89 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD=1:1) δ 143.0, 128.6, 128.4, 125.7, 101.1, 78.0, 74.0, 72.1, 71.9, 71.0, 70.7, 70.3, 70.2, 69.4, 62.2, 36.1, 33.8, 33.7, 32.1, 31.7, 30.0, 29.9, 29.8, 29.64, 29.59, 29.4, 26.6, 26.3, 25.9, 22.8, 14.1; ESI-HRMS $[M+Na]^+$ calcd for $C_{38}H_{68}NaO_9^+$ 691.4761, found 691.4758.

3.14. 2-*O*-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-1-*O*-(8″-hexacosanoyloxy)-D-*arabino*-1,2,3,4-octadecanetetrol (24)

A mixture of compound **17α** (26.6 mg, 0.026 mmol), *n*-hexacosanoic acid (46.5 mg, 0.12 mmol), EDAC (100 mg, 0.52 mmol), and DMAP (64.3 mg, 0.52 mmol) in dry CH_2Cl_2 (8 mL) was stirred for 3 days at room temperature and then diluted with $CHCl_3$. The solution was washed with H_2O and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo. The residue was

purified by column chromatography on silica gel (petroleum ether/EtOAc 15:1) to give **24** as a colorless oil (20.0 mg, 55%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.19 (m, 30H), 5.12 (d, J=3.5 Hz, 1H), 4.90 (d, J=11.0 Hz, 1H), 4.78–4.65 (m, 5H), 4.59 (d, J=12.0 Hz, 1H), 4.53 (d, J=11.5 Hz, 1H), 4.49 (d, J=11.5 Hz, 1H), 4.48 (d, J=11.5 Hz, 1H), 4.39 (d, J=11.5 Hz, 1H), 4.35 (d, J=11.5 Hz, 1H), 4.33 (dd, J=11.5, 6.0 Hz, 1H), 4.24 (dd, J=12.0, 6.0 Hz, 1H), 4.13 (t, J=7.0 Hz, 1H), 4.05–3.99 (m, 3H), 3.98 (dd, J=10.5, 2.5 Hz, 1H), 3.78 (t, J=5.0 Hz, 1H), 3.69 (ddd, J=5.0, 4.5, 4.0 Hz, 1H), 3.58 (t, J=8.0 Hz, 1H), 3.49 (dd, J=9.0, 5.5 Hz, 1H), 2.25 (t, J=8.0 Hz, 2H), 1.71–1.62 (m, 2H), 1.54–1.50 (m, 2H), 1.42–1.21 (m, 68H), 0.88 (t, J=7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 138.7, 138.6, 138.0, 128.33, 128.30, 128.2, 128.14, 128.11, 127.8, 127.7, 127.6, 127.5, 127.3, 99.1, 79.5, 79.2, 79.1, 76.7, 76.1, 74.9, 74.8, 73.5, 73.4, 73.3, 72.7, 71.5, 69.6, 68.6, 64.1, 34.1, 31.9, 30.5, 29.9, 29.7, 29.5, 29.4, 29.2, 25.8, 24.9, 22.7, 14.1; ESI-HRMS $[M+H]^+$ calcd for $C_{92}H_{138}NO_{10}^+$ 1417.0315, found 1417.0295.

3.15. 2-*O*-(α-D-Galactopyranosyl)-1-*O*-(8″-hexacosanoyloxy)-D-*arabino*-1,2,3,4-octadecanetetrol (9)

A solution of compound **24** (38.8 mg, 0.028 mmol) in MeOH/CH_2Cl_2 (6 mL, 1:1) containing $Pd(OH)_2$/C (20%, 35.0 mg) was stirred for 1 h at room temperature under hydrogen atmosphere (balloon). The suspension was then filtered through a bed of Celite, and the filter cake was washed with CH_2Cl_2/MeOH (5:1). The combined filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to give **9** as white solids (23.0 mg, 97%). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD) δ 5.06 (d, J=4.0 Hz, 1H), 4.35 (dd, J=11.0, 5.5 Hz, 1H), 4.22 (dd, J=11.0, 7.0 Hz, 1H), 4.05 (m, 1H), 3.98 (d, J=2.5 Hz, 1H), 3.91 (t, J=6.0 Hz, 1H), 3.89–3.72 (m, 4H), 3.68 (m, 1H), 3.44 (dd, J=6.5, 2.0 Hz, 1H), 2.33 (t, J=7.5 Hz, 2H), 1.67–1.43 (m, 4H), 1.42–1.22 (m, 70H), 0.88 (t, J=7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 174.4, 101.2, 77.5, 73.0, 72.0, 70.9, 70.1, 69.9, 69.1, 63.5, 61.9, 34.2, 33.8, 31.9, 29.8, 29.6, 29.4, 29.2, 25.9, 24.9, 22.7, 14.1; ESI-HRMS $[M+Na]^+$ calcd for $C_{50}H_{98}NaO_{10}^+$ 881.7043, found 881.7052.

3.16. (2*R*,3*R*,4*R*)-2-Azido-3,4-di-*tert*-butyldimethylsilyloxy-1-octadecaneol (26)

To a stirred solution of compound **25**[19] (167.8 mg, 0.49 mmol) in 2,6-lutidine (5.5 mL) and CH_2Cl_2 (5.5 mL), TBSOTf (0.69 mL, 2.93 mmol) was added at 0 °C. After stirring at room temperature for 2.5 h, the reaction was quenched with MeOH (3 mL). The resulting mixture was concentrated in vacuo, and the remaining 2,6-lutidine was removed azeotropically with toluene. The residue was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether) to give the intermediate as a yellow oil (325.5 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (dd, J=10.4, 2.4 Hz, 1H), 3.75–3.66 (m, 2H), 3.61 (dd, J=6.0, 2.8 Hz, 1H), 3.59–3.57 (m, 1H), 1.56–1.30 (m, 2H), 1.30–1.20 (m, 24H), 0.92 (s, 9H), 0.91 (s, 9H), 0.89 (s, 9H), 0.88 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 75.8, 74.6, 65.4, 64.2, 32.9, 31.9, 29.9, 29.69, 29.67, 29.60, 29.4, 26.1, 26.0, 25.8, 25.3, 22.7, 18.3, 18.21, 18.20, 14.1, −4.0, −4.1, −4.5, −4.7, −5.47, −5.51; ESI-HRMS $[M+Na]^+$ calcd for $C_{36}H_{79}N_3NaO_3Si_3^+$ 708.5330, found 708.5321.

To a stirred solution of above intermediate (325.5, 0.48 mmol) in THF (15 mL), 10% aqueous trifluoroacetic acid (v/v, 3 mL) was added dropwise at 0 °C and the mixture was stirred overnight. The reaction was then quenched with 15% aqueous NaOH solution, diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 30:1 to 20:1) to give **26** as a colorless oil (238.1 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92–3.88 (m, 1H), 3.75 (dd, J=6.0, 2.4 Hz, 1H), 3.73–3.70 (m, 3H), 2.39 (t, J=6.0 Hz, 1H, OH), 1.54–1.42 (m, 1H), 1.42–1.26 (m, 25H), 0.91 (s, 18H), 0.88 (t, J=7.2 Hz, 3H), 0.13 (s, 3H), 0.11 (s, 6H), 0.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 76.0, 75.5, 64.7, 62.1, 33.8, 31.9, 29.8, 29.7, 29.65, 29.62, 29.56, 29.55, 29.4, 25.99, 25.97, 25.5, 22.7, 18.21, 18.18, 14.1, −4.0, −4.2, −4.5, −4.8; ESI-HRMS $[M+Na]^+$ calcd for $C_{30}H_{65}N_3NaO_3Si_2^+$ 594.4457, found 594.4445.

3.17. (2*R*,3*R*,4*R*)-1-*O*-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2-azido-3,4-di-*tert*-butyldimethylsilyloxy-octadecane (27α)

The synthetic procedure was the same as that described in the synthesis of compound **16α** using **15** (50.0 mg, 0.077 mmol), **26** (40.2 mg, 0.070 mmol), NIS (19.5 mg, 0.084 mmol), and TfOH (8.4 μL, 0.5 M in Et_2O, 0.0042 mmol) to afford **27α** (81.0 mg, 53%) and its β-anomer (41.2 mg, 27%) as colorless oil. For **27α**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.24 (m, 20H), 4.94 (d, J=11.2 Hz, 1H), 4.90 (d, J=3.6 Hz, 1H), 4.83 (d, J=12.0 Hz, 1H), 4.78 (d, J=12.0 Hz, 1H), 4.72 (m, 2H), 4.56 (d, J=11.6 Hz, 1H), 4.45 (d, J=12.0 Hz, 1H), 4.38 (d, J=11.6 Hz, 1H), 4.07–3.95 (m, 5H), 3.80–3.74 (m, 1H), 3.74–3.70 (m, 1H), 3.62 (dd, J=5.2, 3.6 Hz, 1H), 3.57–3.53 (m, 2H), 3.46 (dd, J=8.8, 5.6 Hz, 1H), 1.54–1.33 (m, 2H), 1.26 (m, 24H), 0.89–0.87 (m, 21H), 0.09 (s, 3H), 0.08 (s, 3H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9, 138.82, 138.79, 138.0, 128.34, 128.27, 128.18, 128.15, 127.8, 127.6, 127.5, 127.44, 127.35, 99.0, 78.7, 76.4, 76.1, 75.2, 74.7, 74.6, 73.4, 73.2, 73.0, 69.6, 69.3, 68.7, 63.8, 33.0, 31.9, 29.8, 29.67, 29.65, 29.60, 29.4, 26.0, 23.2, 22.7, 18.24, 18.16, 14.1, −4.1, −4.5, −4.7; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{64}H_{103}N_4O_8Si_2^+$ 1111.7309, found 1111.7271. For its β-anomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.23 (m, 20H), 4.96 (d, J=11.6 Hz, 1H), 4.94 (d, J=11.2 Hz, 1H), 4.77–4.69 (m, 3H), 4.44 (d, J=12.0 Hz, 1H), 4.40 (d, J=11.2 Hz, 1H), 4.38 (d, J=7.6 Hz, 1H), 4.00 (dd, J=10.8, 8.4 Hz, 1H), 3.91 (d, J=2.4 Hz, 1H), 3.87–3.81 (m, 2H), 3.76–3.72 (m, 2H), 3.64–3.59 (m, 2H), 3.57–3.50 (m, 3H), 1.53–1.35 (m, 2H), 1.26 (m, 24H), 0.87–0.86 (m, 21H), 0.08 (s, 3H), 0.07 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8, 138.5, 137.9, 128.4, 128.3, 128.2, 128.11, 128.05, 127.96, 127.9, 127.8, 127.5, 127.40, 127.36, 103.9, 82.2, 79.5, 75.9, 75.2, 74.8, 74.5, 73.6, 73.5, 73.4, 73.0, 69.4, 68.6, 63.5, 33.1, 31.9, 29.8, 29.68, 29.65, 29.59, 29.4, 26.04, 26.03, 25.5, 22.7, 18.22, 18.15, 14.1, −4.00, −4.03, −4.5, −4.8; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{64}H_{103}N_4O_8Si_2^+$ 1111.7309, found 1111.7278.

3.18. (2*R*,3*R*,4*R*)-1-*O*-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2-azido-1,3,4-octadecanetriol (28)

To a stirred solution of compound **27α** (83.1 mg, 0.076 mmol) in THF (4 mL), a solution of TBAF (1 M in THF, 460 μL, 0.46 mmol) was added at 0 °C. After stirring at room temperature overnight, the reaction was quenched with water, and the resulting mixture was extracted with EtOAc. The combined organic extract was successively washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 3:1) to give **28** as a colorless oil (52.7 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.25 (m, 20H), 4.91 (d, J=11.0 Hz, 1H), 4.89 (d, J=12.0 Hz, 1H), 4.78 (d, J=3.5 Hz, 1H), 4.76 (d, J=11.5 Hz, 1H), 4.72 (d, J=12.0 Hz, 1H), 4.66 (d, J=12.0 Hz, 1H), 4.55 (d, J=11.0 Hz, 1H), 4.49 (d, J=12.0 Hz, 1H), 4.42 (d, J=12.0 Hz, 1H), 4.16 (dd, J=10.5, 3.5 Hz, 1H), 4.05 (dd, J=10.0, 3.5 Hz, 1H), 3.99 (t, J=7.0 Hz, 1H), 3.95–3.93 (m, 1H), 3.92 (dd, J=10.0, 2.5 Hz, 1H), 3.81 (dd, J=10.5, 3.5 Hz, 1H), 3.74 (dd, J=6.5, 5.5 Hz, 1H), 3.62–3.58 (m, 1H), 3.55–3.48 (m, 2H), 3.46 (dt, J=5.0, 4.0 Hz, 1H), 3.34 (d, J=7.0 Hz, 1H), 2.24 (d, J=5.0 Hz, 1H), 1.54–1.46 (m, 2H), 1.40–1.26 (m, 24H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.3, 137.92, 137.87, 128.5, 128.4, 128.24,

128.18, 128.1, 127.96, 127.80, 127.7, 127.62, 127.59, 99.2, 79.2, 75.9, 75.2, 74.8, 74.7, 74.2, 73.4, 72.9, 72.8, 70.1, 69.1, 68.9, 60.0, 32.6, 31.9, 29.7, 29.4, 25.8, 22.7, 14.1; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{52}H_{75}O_8N_4^+$ 883.5579, found 883.5540.

3.19. (2*R*,3*R*,4*R*)-1-*O*-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2-(8″-phenyloctylamino)-1,3,4-octadecanetriol (29)

To a mixture of compound **28** (38.0 mg, 0.044 mmol) and $NiCl_2\cdot 6H_2O$ (10.7 mg, 0.044 mmol) in MeOH (5 mL), $NaBH_4$ (5.0 mg, 0.132 mmol) was added at 0 °C. The mixture was allowed to warm to room temperature and stirred for 3 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2 with several drops of ammonia. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was dissolved in MeOH (3 mL), after which the aldehyde derived from the treatment of PCC with 8-phenyloctan-1-ol (9.0 mg, 0.044 mmol) and acetic acid (10.1 μL, 0.176 mmol) were added. The reaction mixture was stirred for 30 min at room temperature. Sodium cyanoborohydride (8.7 mg, 0.132 mmol) was added to the resulting solution, and the mixture was stirred overnight at room temperature. Saturated aqueous $NaHCO_3$ was added to the resulting mixture and the aqueous layer was extracted twice with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (petroleum ether/EtOAc/Et_3N 1:1:0.02 to 1:1.5:0.02) to afford **29** as an oil (23.0 mg, 51% for two steps). ^1H NMR (500 MHz, $CDCl_3$) δ 7.38–7.15 (m, 25H), 4.93 (d, J=11.5 Hz, 1H), 4.86 (d, J=12.0 Hz, 1H), 4.78 (d, J=11.5 Hz, 1H), 4.77 (d, J=3.5 Hz, 1H), 4.74 (d, J=11.5 Hz, 1H), 4.65 (d, J=12.0 Hz, 1H), 4.56 (d, J=11.0 Hz, 1H), 4.47 (d, J=11.5 Hz, 1H), 4.39 (d, J=12.0 Hz, 1H), 4.04 (dd, J=10.0, 3.5 Hz, 1H), 3.98 (dd, J=10.0, 3.5 Hz, 1H), 3.95 (m, 1H), 3.91 (t, J=6.5 Hz, 1H), 3.86 (dd, J=10.0, 2.5 Hz, 1H), 3.58–3.56 (m, 2H), 3.53–3.49 (m, 2H), 3.35 (t, J=7.5 Hz, 1H), 2.81 (m, 1H), 2.70–2.66 (m, 1H), 2.58 (t, J=7.5 Hz, 2H), 2.52–2.46 (m, 1H), 1.69–1.51 (m, 4H), 1.43–1.21 (m, 36H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.8, 138.5, 138.4, 138.0, 137.8, 128.5, 128.42, 128.39, 128.36, 128.24, 128.21, 128.16, 128.0, 127.8, 127.6, 127.4, 125.6, 125.5, 99.1, 79.1, 76.8, 76.3, 74.8, 74.7, 74.1, 73.5, 72.8, 71.5, 70.2, 69.0, 66.5, 60.8, 46.7, 35.9, 34.4, 31.9, 31.4, 29.9, 29.70, 29.65, 29.4, 29.2, 27.0, 25.5, 22.7, 14.1; ESI-HRMS $[M+H]^+$ calcd for $C_{66}H_{94}N_1O_8^+$ 1028.6974, found 1028.6961.

3.20. (2*R*,3*R*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(8″-phenyloctylamino)-1,3,4-octadecanetriol (6)

The synthetic procedure was the same as that described in the synthesis of compound **9** using **29** (12.0 mg, 0.012 mmol) and $Pd(OH)_2/C$ (20%, 10.0 mg) to afford **6** as a colorless oil (6.1 mg, 78%). ^1H NMR (500 MHz, $CDCl_3/CD_3OD$) δ 7.27 (t, J=8.0 Hz, 2H), 7.18–1.16 (m, 3H), 4.91 (d, J=4.0 Hz, 1H), 4.10 (dd, J=11.5, 3.0 Hz, 1H), 3.93 (d, J=3.0 Hz, 1H), 3.86–3.77 (m, 8H), 3.71 (dd, J=10.5, 7.5 Hz, 2H), 3.64 (dd, J=9.0, 4.5 Hz, 1H), 3.47 (t, J=8.5 Hz, 1H), 3.41 (br, 1H), 2.96 (t, J=7.5 Hz, 2H), 2.60 (t, J=8.0 Hz, 2H), 1.84–1.80 (m, 2H), 1.74–1.65 (m, 2H), 1.64–1.50 (m, 2H), 1.44–1.20 (m, 32H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$) δ 142.6, 128.2, 128.0, 125.4, 99.3, 72.4, 71.0, 69.7, 69.5, 68.9, 63.1, 61.8, 60.0, 45.5, 37.2, 35.7, 34.2, 32.6, 31.7, 31.2, 29.5, 29.2, 29.0, 28.9, 27.8, 26.5, 25.2, 22.5, 19.5, 13.8; ESI-HRMS $[M+H]^+$ calcd for $C_{38}H_{70}NO_8^+$ 668.5096, found 668.5074.

3.21. (2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (30)

The synthetic procedure was the same as that described in the synthesis of compound **13** using **25** (130.0 mg, 0.38 mmol) to afford **30** as a yellow oil (159.0 mg, 80%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.25 (m, 10H), 4.73–4.54 (m, 4H), 3.92–3.86 (m, 1H), 3.83–3.75 (m, 1H), 3.72–3.61 (m, 3H), 2.53 (t, J=6.0 Hz, 1H), 1.70–1.50 (m, 2H), 1.45–1.18 (m, 24H), 0.88 (t, J=7.0 Hz, 3H). The spectroscopic data coincide with those reported in the literature.[31]

3.22. (2*S*,3*S*,4*R*)-2-Hexacosylamino-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (31)

Triphenylphosphine (237 mg, 0.905 mmol) was added to a stirred solution of azide **30** (315.5 mg, 0.603 mmol) in dry THF (8 mL) and the mixture was refluxed for 30 min before H_2O (2 mL) was added. After further stirring for 4 h, the solvent was removed. The residue was dissolved in dry CH_2Cl_2 (10 mL) and treated with cerotic acid (222 mg, 0.548 mmol), HBTU (250 mg, 0.658 mmol), and DIPEA (110.5 μL, 81.8 mg, 0.658 mmol) under Ar. After the mixture was stirred overnight at room temperature, the solvent was evaporated. The resulting residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 2:1) to afford **31** as white solids (470.0 mg, 98%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.39–7.26 (m, 10H), 6.03 (d, J=8.5 Hz, 1H), 4.72 (d, J=11.5 Hz, 1H), 4.64 (dd, J=25.3, 11.5 Hz, 2H), 4.45 (d, J=11.5 Hz, 1H), 4.16–4.12 (m, 1H), 4.00 (d, J=6.5 Hz, 1H), 3.72–3.67 (m, 2H), 3.63–3.58 (m, 1H), 3.06 (br s, 1H), 2.05–1.94 (m, 2H), 1.73–1.67 (m, 1H), 1.64–1.56 (m, 1H), 1.54–1.49 (m, 2H), 1.48–1.18 (m, 68H), 0.88 (t, J=7.0 Hz, 6H). The spectroscopic data coincide with those reported in the literature.[32]

3.23. (2*R*,3*S*,4*R*)-2-Hexacosylamino-3,4-di-*O*-benzyl-octadecanoic acid (32)

Method A. To a stirred solution of alcohol **31** (81.0 mg, 0.093 mmol) in CH_2Cl_2 (8 mL) was added sequentially TEMPO (44.3 mg, 0.278 mmol) and DIAB (304 mg, 0.93 mmol). After 72 h, the solvent was removed and the residue was purified by column chromatography on silica gel (petroleum ether/EtOAc/HOAc 2:1:0.03) to give compound **32** as white solids (66.0 mg, 81%).

Method B. To a stirred solution of alcohol **31** (65.0 mg, 0.074 mmol) in CH_2Cl_2 (2 mL) was added TEMPO (5 mg, 0.03 mmol) followed by iodobenzene diacetate (97 mg, 0.30 mmol). After 12 h, the reaction mixture was then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was dissolved in CH_2Cl_2 (5 mL) and treated with a solution of $NaClO_2$ (211 mg, 1.86 mmol) and $NaH_2PO_4\cdot 2H_2O$ (20 mg, 0.027 mmol) in H_2O (2 mL) with several drops of Bu_4NCl. After 0.5 h, the reaction mixture was acidified with 1 N HCl and then diluted with CH_2Cl_2. The organic phase was washed with H_2O, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc/HOAc 2:1:0.03) to give **32** as white solids (59.0 mg, 90%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.36–7.25 (m, 10H), 6.47 (d, J=6.5 Hz, 1H), 4.80 (d, J=6.0 Hz, 1H), 4.70 (dd, J=19.8, 11.5 Hz, 2H), 4.52 (dd, J=18.3, 12.0 Hz, 2H), 3.82–3.80 (m, 1H), 3.78–3.72 (m, 1H), 2.08–1.97 (m, 2H), 1.89–1.84 (m, 1H), 1.75–1.69 (m, 2H), 1.54–1.46 (m, 2H), 1.40–1.18 (m, 67H), 0.88 (t, J=7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 170.0, 137.4, 135.8, 128.9, 128.71, 128.66, 128.5, 128.1, 81.2, 80.0, 73.5, 72.4, 53.8, 36.4, 31.9, 30.7, 29.7, 29.6, 29.5, 29.4, 29.2, 25.8, 25.5, 22.7, 14.1; ESI-HRMS $[M+Na]^+$ calcd for $C_{58}H_{99}NNaO_5^+$ 912.7415, found 912.7416.

3.24. *N*-[3′-*O*-(2″,3″-Di-*O*-benzyl-4″,6″-*O*-benzylidene-α-D-galactopyranosyl)propyl] (2*R*,3*S*,4*R*)-2-hexacosylamino-3,4-di-*O*-benzyl-octadecanamide (34)

The glycosylation procedure was the same as that described in the synthesis of compound **16α**, using *p*-methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidine-1-thio-β-D-galactopyranoside[20] (219.0 mg,

0.395 mmol), 3-phthalimino-propanol[33] (81.0 mg, 0.395 mmol), NIS (119.0 mg, 0.474 mmol), and TfOH (47.4 μL, 0.5 M in Et_2O, 0.0237 mmol) to afford the α-anomeric coupling product (126.0 mg, 50%) as white solids. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (dd, *J*=5.8, 3.0 Hz, 2H), 7.70 (dd, *J*=5.5, 3.0 Hz, 2H), 7.51 (dd, *J*=7.8, 1.5 Hz, 2H), 7.41–7.23 (m, 13H), 5.48 (s, 1H), 4.91 (d, *J*=4.0 Hz, 1H), 4.82 (d, *J*=12.0 Hz, 1H), 4.77 (d, *J*=12.5 Hz, 1H), 4.69 (d, *J*=12.0 Hz, 1H), 4.67 (d, *J*=12.0 Hz, 1H), 4.23–4.18 (m, 2H), 4.06–4.01 (m, 2H), 3.96 (dd, *J*=10.0, 3.5 Hz, 1H), 3.85–3.67 (m, 4H), 3.52 (dt, *J*=10.0, 6.0 Hz, 1H), 2.05–1.99 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4, 138.9, 138.8, 137.9, 133.9, 132.1, 128.8, 128.3, 128.1, 127.9, 127.6, 127.56, 127.5, 126.4, 123.2, 101.1, 98.7, 76.1, 75.6, 74.8, 73.4, 72.2, 69.5, 66.2, 62.8, 35.5, 28.7; ESI-TOF-MS $[M+H]^+$ calcd for $C_{38}H_{38}NO_8^+$ 636, found 636; $[M+NH_4]^+$ calcd for $C_{38}H_{41}N_2O_8^+$ 653, found 653; $[M+Na]^+$ calcd for $C_{38}H_{37}NNaO_8^+$ 658, found 658; $[M+K]^+$ calcd for $C_{38}H_{37}KNO_8^+$ 674, found 674; Anal. Calcd for $C_{38}H_{37}NO_8$: C, 71.80; H, 5.87; N, 2.20. Found: C, 71.71; H, 5.89; N, 2.17.

A solution of above glycosylation product (112.0 mg, 0.039 mol) in aminomethane/MeOH (2 mL/2 mL) was refluxed for 0.5 h. The solvent was evaporated and the resulting residue was purified by column chromatography on silica gel (petroleum ether/EtOAc/ammonia 5:1:0.06) to give compound **33** as a yellow syrup (79.0 mg, 89%), which was directly used for the next reaction.

The coupling procedure was the same as that described in the synthesis of compound **31**, using amine **33** (70.0 mg, 0.142 mmol), acid **32** (101.0 mg, 0.114 mmol), HBTU (51.8 mg, 0.137 mmol), and DIPEA (23 μL, 17.0 mg, 0.137 mmol) to afford **34** as white waxy solids (110.0 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (dd, *J*=7.5, 2.0 Hz, 2H), 7.40–7.23 (m, 23H), 6.95 (t, *J*=5.5 Hz, 1H), 6.63 (d, *J*=7.5 Hz, 1H), 5.42 (s, 1H), 4.91 (d, *J*=12.0 Hz, 1H), 4.79 (d, *J*=12.0 Hz, 1H), 4.75 (d, *J*=3.5 Hz, 1H), 4.70–4.56 (m, 7H), 4.15–4.06 (m, 2H), 4.00 (dd, *J*=10.0, 3.5 Hz, 1H), 3.96–3.88 (m, 3H), 3.63–3.56 (m, 2H), 3.49 (s, 1H), 3.34–3.17 (m, 3H), 2.10–1.98 (m, 2H), 1.76–1.60 (m, 4H), 1.56–1.48 (m, 2H), 1.46–1.37 (m, 2H), 1.34–1.18 (m, 66H), 0.88 (t, *J*=7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 169.5, 138.9, 138.8, 138.3, 137.9, 128.8, 128.5, 128.3, 128.2, 128.1, 128.0, 127.95, 127.89, 127.6, 127.5, 127.4, 126.3, 101.0, 98.5, 81.1, 81.0, 76.3, 75.4, 74.5, 73.8, 73.3, 72.3, 72.1, 71.8, 69.4, 66.5, 62.6, 53.8, 37.4, 36.7, 31.9, 30.3, 29.7, 29.5, 29.4, 28.9, 27.7, 26.1, 25.6, 22.7, 14.1; ESI-HRMS $[M+H]^+$ calcd for $C_{88}H_{133}N_2O_{10}^+$ 1377.9955, found 1377.9962.

3.25. *N*-[3′-*O*-(α-D-Galactopyranosyl)propyl] (2*R*,3*S*,4*R*)-2-hexacosylamino-3,4-di-hydroxy-octadecanamide (7)

The synthetic procedure was the same as that described in the synthesis of compound **9**, using **34** (18.0 mg, 0.013 mmol) and $Pd(OH)_2$/C (10%, 15 mg) to afford **7** as white solids (9.0 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD=1:1) δ 4.98–4.92 (br m, 1H), 4.89 (d, *J*=4.0 Hz, 1H), 3.98 (d, *J*=2.5 Hz, 1H), 3.97–3.94 (br m, 1H), 3.90–3.73 (m, 6H), 3.70–3.65 (m, 1H), 3.57–3.48 (m, 2H), 3.44–3.38 (m, 1H), 2.35 (t, *J*=7.5 Hz, 2H), 1.90–1.81 (m, 2H), 1.80–1.60 (m, 5H), 1.44–1.20 (m, 67H), 0.89 (t, *J*=7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD=1:1) δ 99.6, 71.3, 70.9, 70.6, 69.7, 66.8, 66.0, 62.4, 35.1, 32.5, 31.4, 30.2, 29.9, 29.8, 29.6, 29.4, 28.8, 26.8, 26.3, 25.5, 25.4, 25.1, 23.2, 14.3; ESI-HRMS $[M+H]^+$ calcd for $C_{53}H_{105}N_2O_{10}^+$ 929.7769, found 929.7733.

3.26. Spleen cell proliferation assay

The splenocytes (8×10^5 cells/well) were plated in 96-well flat-bottom tissue culture plates with synthetic compound (100 ng/mL, 100 μL/well) diluted in 200 μL of medium. After 48 h at 37 °C, CCK-8 (20 μL) was added to the cultured cell and the colorimetric values were measured by a microplate reader with 600 nm as reference.

3.27. In vivo stimulation with synthetic compounds

Stock solutions of KRN7000 and synthetic compounds were prepared in 100% DMSO at a concentration of 1 mg/mL. Before use, the solutions were diluted with phosphate buffered saline (pH 7.4) to obtain a final concentration of 10 μg/mL. Mice were injected intraperitoneally with 5 μg of compound or with diluted DMSO alone. Sera were collected at two time points, and the levels of IFN-γ (at 16 h) and IL-4 (at 2 h) were measured by a standard sandwich ELISA using purified capture and biotin-conjugated detection monoclonal antibodies and standards. ELISAs were developed with TMB substrate, followed by evaluation using a microplate reader.

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Supplementary data

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References and notes

1. Bendelac, A.; Savage, P. B.; Teyton, L. *Annu. Rev. Immunol.* **2007**, *25*, 297–336.
2. (a) Brigl, M.; Brenner, M. B. *Annu. Rev. Immunol.* **2004**, *22*, 817–890; (b) Matsuda, J. L.; Mallevaey, T.; Scott-Browne, J.; Gapin, L. *Curr. Opin. Immunol.* **2008**, *20*, 358–368.
3. (a) Kronenberg, M. *Annu. Rev. Immunol.* **2005**, *23*, 877–900; (b) Crowe, N. Y.; Uldrich, A. P.; Kyparissoudis, K.; Hammond, K. J. L.; Hayakawa, Y.; Sidobre, S.; Keating, R.; Kronenberg, M.; Smyth, M. J.; Godfrey, D. I. *J. Immunol.* **2003**, *171*, 4020–4027; (c) Carnaud, C.; Lee, D.; Donnars, O.; Park, S.-H.; Beavis, A.; Koezuka, Y.; Bendelac, A. *J. Immunol.* **1999**, *163*, 4647–4650.
4. (a) Yu, K. O. A.; Porcelli, S. A. *Immunol. Lett.* **2005**, *100*, 42–55; (b) Taniguchi, M.; Harada, M.; Kojo, S.; Nakayama, T.; Wakao, H. *Annu. Rev. Immunol.* **2003**, *21*, 483–513; (c) Godfrey, D. I.; MacDonald, H. R.; Kronenberg, M.; Smyth, M. J.; Van Kaer, L. *Nat. Rev. Immunol.* **2004**, *4*, 231–237; (d) Gonzalez-Aseguinolaza, G.; Van Kaer, L.; Bergmann, C. C.; Wilson, J. M.; Schmieg, J.; Kronenberg, M.; Nakayama, T.; Taniguchi, M.; Koezuka, Y.; Tsuji, M. *J. Exp. Med.* **2002**, *195*, 617–624; (e) Berkers, C. R.; Ovaa, H. *Trends Pharmacol. Sci.* **2005**, *26*, 252–257.
5. (a) 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; (b) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176–2187; (c) Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591–5592; (d) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771–2784.
6. Parekh, V. V.; Wilson, M. T.; Olivares-Villagomez, D.; Singh, A. K.; Wu, L.; Wang, C.-R.; Joyce, S.; Van Kaer, L. *J. Clin. Invest.* **2005**, *115*, 2572–2583.
7. (a) Savage, P. B.; Teyton, L.; Bendelac, A. *Chem. Soc. Rev.* **2006**, *35*, 771–779; (b) Wu, D.; Fujio, M.; Wong, C.-H. *Bioorg. Med. Chem.* **2008**, *16*, 1073–1083; (c) Hsieh, M.-H.; Hung, J.-T.; Liw, Y.-W.; Lu, Y.-J.; Wong, C.-H.; Yu, A. L.; Liang, P.-H. *ChemBioChem* **2012**, *13*, 1689–1697; (d) Tashiro, T.; Shigeura, T.; Watarai, H.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem.* **2012**, *20*, 4540–4548.
8. (a) Banchet-Cadeddu, A.; Henon, E.; Dauchez, M.; Renault, J.-H.; Monneaux, F.; Haudrechy, A. *Org. Biomol. Chem.* **2011**, *9*, 3080–3104; (b) Venkataswamy, M. M.; Porcelli, S. A. *Semin. Immunol.* **2010**, *22*, 68–78; (c) Araki, M.; Miyake, S.; Yamamura, T. *Curr. Med. Chem.* **2008**, *15*, 2337–2345.
9. (a) Goff, R. D.; Gao, Y.; Mattner, J.; Zhou, D.; Yin, N.; Cantu, C., III; Teyton, L.; Bendelac, A.; Savage, P. B. *J. Am. Chem. Soc.* **2004**, *126*, 13602–13603; (b) Miyamoto, K.; Miyake, S.; Yamamura, T. *Nature* **2001**, *413*, 531–534.
10. (a) Fujio, M.; Wu, D.; Garcia-Navarro, R.; Ho, D. D.; Tsuji, M.; Wong, C.-H. *J. Am. Chem. Soc.* **2006**, *128*, 9022–9023; (b) Chang, Y.-J.; Huang, J.-R.; Tsai, Y.-C.; Hung, J.-T.; Wu, D.; Fujio, M.; Wong, C.-H.; Yu, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 10299–10304.
11. Lee, T.; Cho, M.; Ko, S.-Y.; Youn, H.-J.; Baek, D. J.; Cho, W.-J.; Kang, C.-Y.; Kim, S. *J. Med. Chem.* **2007**, *50*, 585–589.
12. Franck, R. W.; Tsuji, M. *Acc. Chem. Res.* **2006**, *39*, 692–701.
13. (a) Zajonc, D. M.; Cantu, C.; Mattner, J.; Zhou, D.; Savage, P. B.; Bendelac, A.; Wilson, I. A.; Teyton, L. *Nat. Immunol.* **2005**, *6*, 810–818; (b) Koch, M.; Stronge, V. S.; Shepherd, D.; Gadola, S. D.; Mathew, B.; Ritter, G.; Fersht, A. R.; Besra, G. S.; Schmidt, R. R.; Jones, E. Y.; Cerundolo, V. *Nat. Immunol.* **2005**, *6*, 819–826; (c) Borg, N. A.; Wun, K. S.; Kjer-Nielsen, L.; Wilce, M. C. J.; Pellicci, D. G.; Koh, R.; Besra, G. S.; Bharadwaj, M.; Godfrey, D. I.; McCluskey, J.; Rossjohn, J. *Nature* **2007**, *448*, 44–49.

14. Tashiro, T.; Hongo, N.; Nakagawa, R.; Seino, K.-I.; Watarai, H.; Ishii, Y.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem.* **2008**, *16*, 8896–8906.
15. Fuhshuku, K.-I.; Hongo, N.; Tashiro, T.; Masuda, Y.; Nakagawa, R.; Seino, K.-I.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem.* **2008**, *16*, 950–964.
16. Shiozaki, M.; Tashiro, T.; Koshino, H.; Nakagawa, R.; Inoue, S.; Shigeura, T.; Watarai, H.; Taniguchi, M.; Mori, K. *Carbohydr. Res.* **2010**, *345*, 1663–1684.
17. (a) Zhou, D.; Mattner, J.; Cantu, C., III; Schrantz, N.; Yin, N.; Gao, Y.; Sagiv, Y.; Hudspeth, K.; Wu, Y.-P.; Yamashita, T.; Teneberg, S.; Wang, D.; Proia, R. L.; Levery, S. B.; Savage, P. B.; Teyton, L.; Bendelac, A. *Science* **2004**, *306*, 1786–1789; (b) Mattner, J.; DeBord, K. L.; Ismail, N.; Goff, R. D.; Cantu, C.; Zhou, D.; Saint-Mezard, P.; Wang, V.; Gao, Y.; Yin, N.; Hoebe, K.; Schneewind, O.; Walker, D.; Beutler, B.; Teyton, L.; Savage, P. B.; Bendelac, A. *Nature* **2005**, *434*, 525–529.
18. (a) Scott-Browne, J. P.; Matsuda, J. L.; Mallevaey, T.; White, J.; Borg, N. A.; McCluskey, J.; Rossjohn, J.; Kappler, J.; Marrack, P.; Gapin, L. *Nat. Immunol.* **2007**, *8*, 1105–1113; (b) Zajonc, D. M.; Savage, P. B.; Bendelac, A.; Wilson, I. A.; Teyton, L. *J. Mol. Biol.* **2008**, *377*, 1104–1116.
19. Chiu, H.-Y.; Tzou, D.-L. M.; Patkar, L. N.; Lin, C.-C. *J. Org. Chem.* **2003**, *68*, 5788–5791.
20. Luo, S.-Y.; Kulkarni, S. S.; Chou, C.-H.; Liao, W.-M.; Hung, S.-C. *J. Org. Chem.* **2006**, *71*, 1226–1229.
21. Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734–753.
22. Wang, C.; Wang, H.; Huang, X.; Zhang, L.-H.; Ye, X.-S. *Synlett* **2006**, 2846–2850.
23. Pang, Y.-P.; Hong, F.; Quiram, P.; Jelacic, T.; Brimijoin, S. *J. Chem. Soc., Perkin Trans. 1* **1997**, 171–176.
24. Yoshida, Y.; Sakakura, Y.; Aso, N.; Okada, S.; Tanabe, Y. *Tetrahedron* **1999**, *55*, 2183–2192.
25. (a) Falck, J. R.; Yu, J.; Cho, H.-S. *Tetrahedron Lett.* **1994**, *35*, 5997–6000; (b) Aesa, M. C.; Baan, G.; Novak, L.; Szantay, C. *Synth. Commun.* **1995**, *25*, 1545–1550.
26. Ye, X.-S.; Huang, X.; Wong, C.-H. *Chem. Commun.* **2001**, 974–975.
27. Moriarty, R. M.; Zhuang, H.; Penmasta, R.; Liu, K.; Awasthi, A. K.; Tuladhar, S. M.; Rao, M. S. C.; Singh, V. K. *Tetrahedron Lett.* **1993**, *34*, 8029–8032.
28. Fan, G.-T.; 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.
29. Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Org. Lett.* **2004**, *6*, 4077–4080.
30. Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005**, *46*, 3165–3168.
31. Xia, C.; Schuemann, J.; Emmanuel, R.; Zhang, Y.; Chen, W.; Zhang, W.; De Libero, G.; Wang, P. G. *J. Med. Chem.* **2007**, *50*, 3489–3496.
32. Xia, C.; Yao, Q.; Schuemann, J.; Rossy, E.; Chen, W.; Zhu, L.; Zhang, W.; De Libero, G.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2195–2199.
33. Pascale, R.; Carocci, A.; Catalano, A.; Lentini, G.; Spagnoletta, A.; Cavalluzzi, M. M.; De Santis, F.; De Palma, A.; Scalera, V.; Franchini, C. *Bioorg. Med. Chem.* **2010**, *18*, 5903–5914.