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Concise synthesis of clarhamnoside, a novel glycosphingolipid isolated from the marine sponge *Agela clathrodes*

Ning Ding, Chunxia Li, Yunpeng Liu, Zaihong Zhang and Yingxia Li*

Key Laboratory of Marine Drugs, The Ministration of Education of China, School of Pharmacy, Ocean University of China, Qingdao 266003, China

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Abstract—The first total synthesis of a novel α-galactoglycosphingolipid clarhamnoside has been achieved through a straightforward strategy. A thiogalactosyl donor with a benzylidene group at C-4 and C-6 and nonparticipating *p*-methoxybenzyl group at C-2 was successfully employed in the stereocontrolled syntheses of α-GalGSLs. The *N*-Phth-protected trifluoroacetimidate donor for terminal disaccharide was successfully applied in constructing the [GalNAcβ-(1→6)-Gal] glycosidic linkage.

Keywords: KRN7000; Clarhamnoside; Glycosphingolipids; Immunostimulatory; Sponge; Synthesis

1. Introduction

It is well established that sponges of the genus *Agelas* and *Axinella* produce α-galactoglycosphingolipids (α-GalGSLs), unique glycosphingolipids with an α-galactose as the first sugar of the carbohydrate chain, unlike the ubiquitous β-glycosidic bond from nearly all known higher animals and plants.[1] The scientific interests in α-GalGSLs have recently increased on account of the role they could play as therapeutic agents. α-GalGSLs are potent ligands of the MHC class I-like CD1d protein, which is present on the surface of the antigen-presenting cells (APCs) and is capable of activating in vitro and in vivo a specialized population of T cells, named natural killer T cells (NKT cells), which play an important role in regulating innate and adaptive immunity during infection, tumor growth, and autoimmune diseases.[2] α-Galactosyl ceramide (KRN7000), a potent analogue of the natural agelasphins isolated from the marine sponge *Agelas mauritianus*, is an important cerebroside exhibiting immunostimulatory activity and antitumor properties.[3] A truncated analogue of KRN7000, OCH, was found to selectively induce IL-4, as opposed to IFNγ, and to offer protection in mice against experimental autoimmune encephalomyelitis (EAE)[4] and more recently has been shown to offer protection against diabetes in NOD mice[5] and against collagen-induced arthritis.[6] Agelagalastatin, isolated from the Western Pacific marine sponge *Agelas* sp., displays significant in vitro inhibitory activities against human cancer cell growth.[7]

Besides its biological activities, spongal α-GalGSLs appear to be a quite peculiar class of molecules in terms of the structure of their carbohydrate moieties.[8] Clarhamnoside, a novel α-GalGSL, which was recently identified by the Mangoni group from new specimens of *A. clathrodes*,[9] bears quite a unique structure containing α-L-Rha*p*-(1→3)-β-D-Gal*p*NAc-(1→6)-α-D-Gal*p*-(1→2)-α-D-Gal*p*. It is one of the few natural α-GalGSLs glycosylated at the inner galactose 2-OH and the only α-GalGSL with an L-rhamnose unit in the sugar head.[9] In addition, the sequential two 1,2-*cis*-α-D-galactopyranosidic linkages [Galα-(1″→2′)-Galα-(1′→1)-Cer] are also an extraordinary and rare feature in nature (Fig. 1).

As part of our effort to understand the mechanism of CD1-mediated T-cell activation, we are interested in developing a facile approach to series of α-GalGSLs with a glycosylated 2′-OH or other substituting groups,

* Corresponding author. Tel.: +86 532 82032150; fax: +86 532 82033054; e-mail: liyx417@ouc.edu.cn

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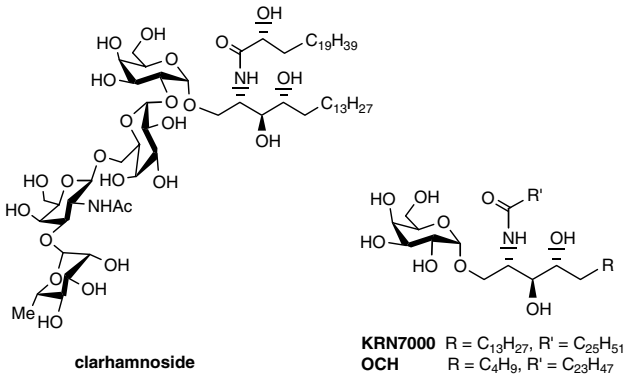


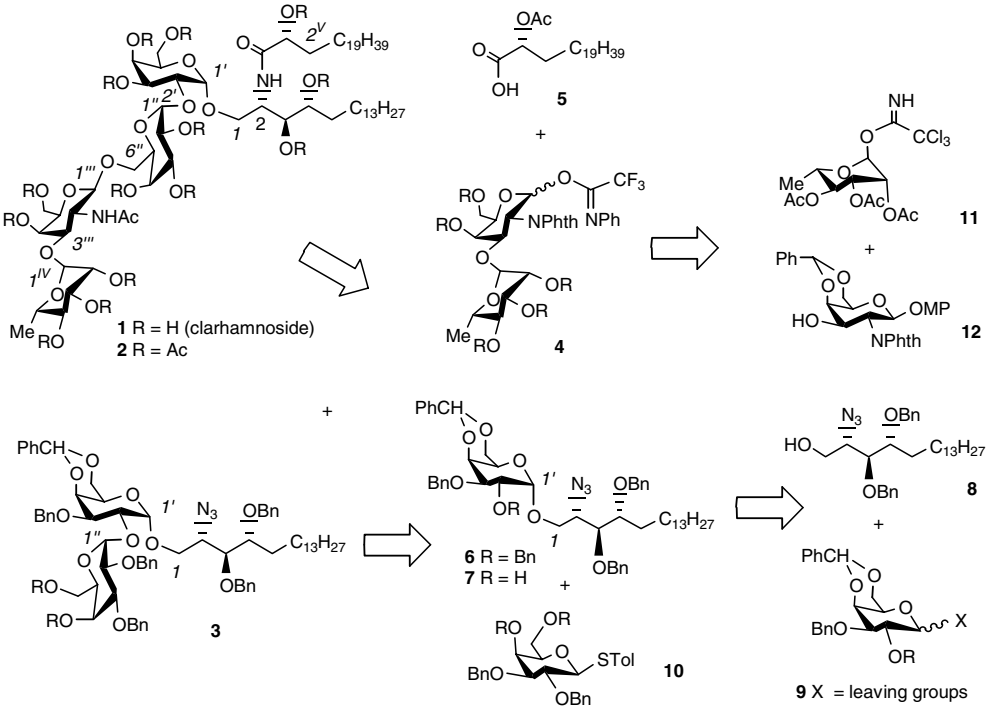
Figure 1. The structures of KRN7000, OCH, and clarhamnoside.

since structure–activity relationships showed that substitution on 2′-OH strongly affected the bioactivity of these compounds.[10] Although extensive syntheses of KRN7000 and its analogues[11] have been described, there are only a few works reported on the syntheses of C-2′ modified α-GalGSLs[10b,c,12] and spongal α-GalGSLs with a longer carbohydrate chain than KRN7000.[13] The unique structure and potent bioactivities make clarhamnoside a suitable goal for synthesis. Herein, we describe the concise synthesis of clarhamnoside using α-stereoselective glycosylations.

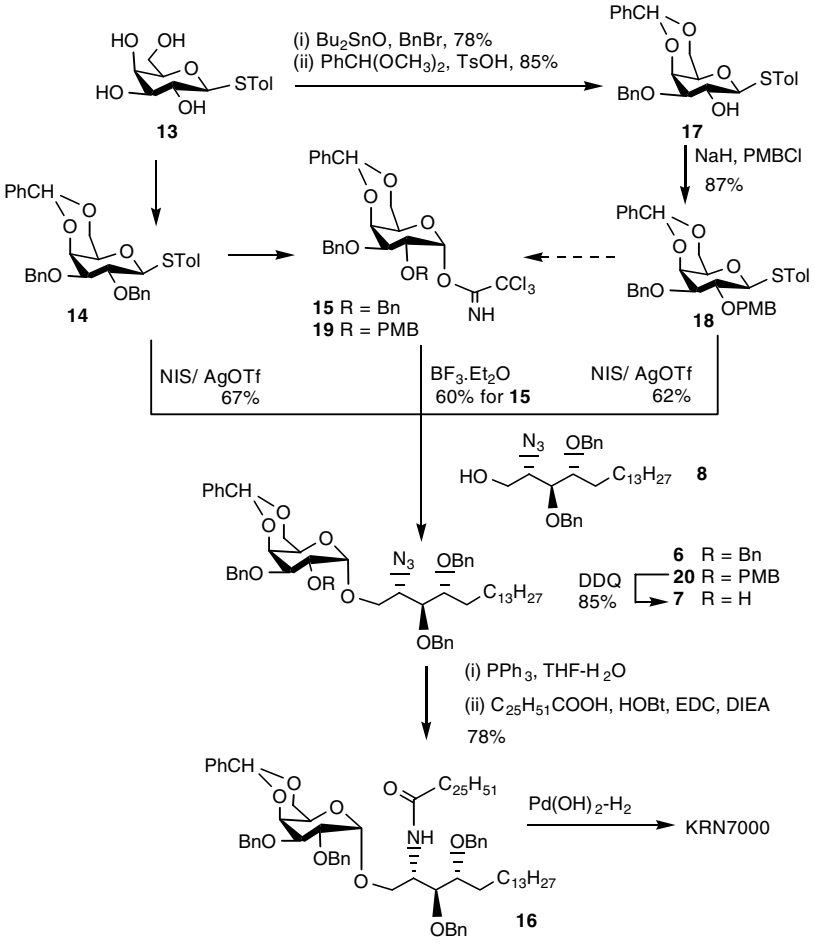
2. Results and discussion

Clarhamnoside consists of two synthetically distinct parts, the ceramide and the tetrasaccharide. A convergent synthesis thus calls for coupling of a tetrasaccharide donor with the aglycon. However, α-selective glycosylation of the ceramide with galactosyl donors has long been recognized as a difficult task,[13] especially for the inner galactose, which is (1→2) branched. The potent steric effect between the bulky ceramide residue and the tetrasaccharide donor requires attention in the convergent synthesis.[14,15] In addition, a general route for preparation of α-GalGSLs with a free 2′-OH group were needed in our later work. For this purpose, a straightforward strategy was described to complete the synthetic work. The retrosynthetic analysis of clarhamnoside is depicted in Scheme 1. α-Galactosyl lipid **7** with a free 2-OH group was anticipated as a key intermediate for elaboration of the tetrasaccharide lipid via sequential coupling with monosaccharide donor **10** and disaccharide donor **4**. The stereocontrolled construction of α-linked monosaccharide lipid **7** and disaccharide lipid **3** turned out to be the crucial steps in our synthesis.

For preparation of α-galactosyl ceramide (**6** or **7**), a major effort was to find a suitable galactosyl donor, which should be designed to obtain a high α:β ratio as well as a satisfactory yield. Recently, galactosyl trichloroacetimidate **15** with a benzylidene group at C-4 and C-6 and nonparticipating benzyl groups at C-2 and C-3 has been successfully used in the stereocontrolled syntheses of α-GalGSLs[16] (Scheme 2). This remarkable stereoselectivity could be attributed to the *cis*-decalin ring system with the equatorial phenyl group in **15** that hinders the attack from the β-face.[16] The result prompted



Scheme 1. Retrosynthetic analysis of clarhamnoside.

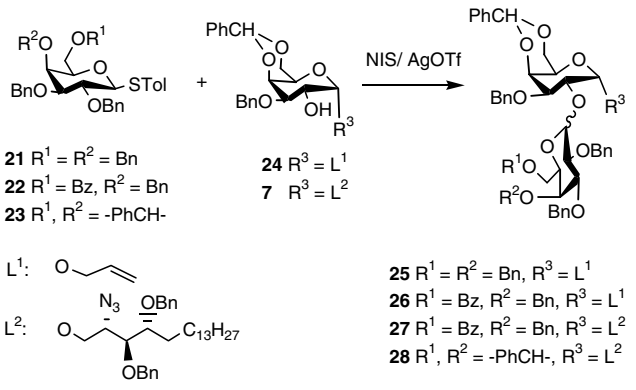


Scheme 2. Synthesis of KRN7000 and α-galactosyl ceramide **7**.

us to turn our attention to the corresponding thiogalactosyl donor **14** in forming α-GalGSLs. To the best of our knowledge, thiogalactoside **14** has not been successfully employed as a donor in the stereocontrolled syntheses of α-GalGSLs. It has been used, however, as an intermediate to trichloroacetimidate **15**.[16b,c] Fortunately, the glycosylation of azidosphingosine **8**[12] with **14** was carried out successfully in the presence of NIS/AgOTf to provide **6** exclusively and in a satisfactory 67% yield, while in our hands trichloroacetimidate **15** also delivered **6** exclusively in a clean reaction in 60% yield when BF_3·etherate was chosen as a mild promoter. Staudinger reduction[17] of the azido group in **6** led to an amine, which was then coupled with the hexacosanoic acid in the presence of 1-hydroxybenzotriazole (HOBt), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) to furnish **16**. After global deprotection, KRN7000 was obtained.[4a] Thus the research provides a more convenient donor than the most frequently used trichloroacetimidates for α-stereoselective preparation of KRN7000 analogues.

Since benzyl ethers have been excellent candidates as nonparticipating groups at C-2 to form α-galactosidic bonds, a PMB (*p*-methoxybenzyl) type ether should be a good choice and simultaneously serve as a protecting group at C-2 that can be selectively removed in the presence of benzyl groups. Therefore, thiolgalactosyl donor **18** with a PMB group at the 2-OH position was designed and rapidly prepared from the known thiolglycoside **13** in three steps. To our delight, the glycosylation of donor **18** with acceptor **8** was carried out smoothly to afford α-glycolipid **20** exclusively in a good yield (60%). Thus, after selective removal of the PMB group in **20**, the key 2′-OH free intermediate **7** for elaboration of disaccharide lipid **3** or other derivatives was readily obtained. It is worth noting that 3-*O*-benzyl-4,6-*O*-benzylidene-2-*p*-methoxybenzyl trichloroacetimidate **19** was difficult to prepare due to the lability of PMB ether during cleavage of various of anomeric protective groups, such as thioanisole, *p*-methoxyphenol, and allyl by using *N*-bromosuccinimide (NBS), ammonium cerium(IV) nitrate (CAN), and $PdCl_2$, respectively. Therefore, the application of thiolgalactosyl donor **18** instead of the corresponding trichloroacetimidate **19** provides a facile and effective strategy to achieve the syntheses of α-GalGSLs with a free 2′-OH.

Table 1. Introduce the second α-galactosidic unit to the 2′-OH of glycolipid **9**

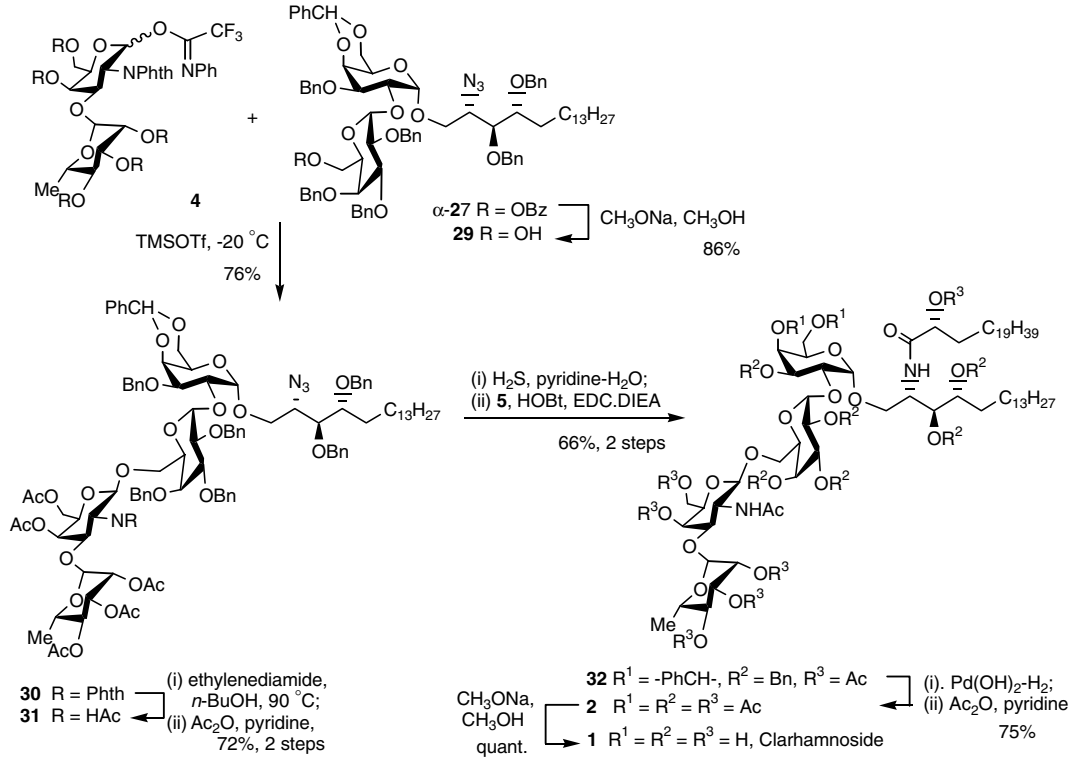


	Donor	Acceptor	Product	Yield (%)	α:β[a]
1	**21**	**24**	**25**	64	10:1
2	**22**	**24**	**26**	71	α
3	**22**	**7**	**27**	76	3:1
4	**23**	**7**	**28**	54	α

[a] The α:β ratio was determined by ^{1}H NMR spectroscopy.

Toward the target molecule **1**, the following effort was to efficiently introduce the second α-galactosidic unit to the 2′-OH of glycolipid **7** (Table 1). We have examined the glycosylation of two galactosyl donors (**21** and **22**) with easily prepared model acceptor **24** in our previous work.[18] Promoted with NIS/AgOTf in 4:1 Et_2O–CH_2Cl_2, the glycosylation of **21** with **24** gave disaccharide **25** as anomers (α:β 10:1, 64%), while donor **22** with a remote participating benzoyl group[19] showed an improvement in α-selectivity from α:β 10:1 to α only (71%). However, when the same conditions were applied to the glycosylation of bulky substituted glycosyl acceptor **7**, donor **22** afforded **27** in lower anomeric selectivity (α:β 3:1, 76%). The decreased α-selectivity with acceptor **7** declared the great influence of the bulky azidosphingosine residue. Fortunately, the α/β mixture of compound **27** was easily separated by column chromatography (1:200 acetone–toluene). The reaction was also accomplished to give α-**28** as the only identifiable anomer (54%) when donor **23** was employed; however, the manipulation of the benzylidene group to leave 6″-OH free was complex (in the presence of another benzylidene). Thus after removing the benzoyl group in 6″-OH of α-**27** (86%), the crucial building block **29** was obtained and used as a disaccharide acceptor for later glycosylation (Scheme 3).

Trifluoroacetimidate[20] **4** was employed as the donor for the glycosylation with **29**, which had been synthesized starting from L-rhamnosyl trichloroacetimidate donor **11** and 2-*N*-phthalimido protected **12** from a five-step procedure, and proved to be efficient in fragment coupling for a β-glycosidic bond in our previous work[18] (Scheme 3). The coupling was carried out in the presence of TMSOTf and 4 Å MS at −20 °C, affording the desired β-glycoside **30** in 76% good yield. Transformation of the *N*-phthalimido group into an



Scheme 3. Synthesis of clarhamnoside.

acetamido group was next accomplished using ethylenediamine, followed by acetylation to give **31** (72%). Staudinger reduction of the azide utilizing triphenylphosphine either in THF–water or pyridine–water caused decomposition of substance **31**. Another attempt to use zinc powder with AcOH[21] was shown to be innocuous toward **31**, but the reaction did not proceed efficiently. Finally, the reduction reaction was carried out smoothly with hydrogen sulfide (H_2S) in pyridine–water,[22] giving the desired amine intermediate, which was directly coupled with α-hydroxy acid **5** using HOBt and EDC·HCl as promoters to give the fully protected derivative **32** in 66% yield. α-Hydroxy acid **5** was prepared based on the method published by Chen et al.[23]

Removal of the benzylidene and benzyl ether groups in **32** by hydrogenolysis using palladium(II) hydroxide (75%) in 3:1 EtOAc–MeOH proved successful,[18] affording the desired tetrasaccharide intermediate, which was followed by acetylation to give clarhamnoside peracetate **2**. The diagnostic ^{1}H and ^{13}C NMR signals of **2** were shown to be identical to those provided in the literature.[9] Removal of the acyl groups in **2** using the reported procedure[9] eventually furnished the target glycolipid **1** (clarhamnoside). The ^{1}H NMR spectrum of clarhamnoside clearly shows the four anomeric protons (δ 5.78, 5.63, 5.56, 5.27) and two NH signals (δ 9.01, 8.59), which are in good agreement with those listed in the literature.[9] The ^{13}C NMR signals are also in good agreement with those listed in the literature, except for the presence of an additional carbonyl signal at 171.6 ppm (only one carbonyl signal δ 175.3 was listed in the literature).

3. Conclusions

In conclusion, we have achieved the first total synthesis of a novel α-GSL clarhamnoside in this paper by employing α-galactosyl lipid **7** as the key intermediate. Thiolgalactosyl donor **18**, which has a benzylidene group at C-4 and C-6 and a nonparticipating PMB group at C-2, has been successfully used to achieve the α-stereocontrolled synthesis of α-GalGSLs with 2′-OH groups free. The strategy described above provides a facile and efficient approach to syntheses of α-GalGSLs with glycosylated 2′-OH groups or other substituents. The syntheses of clarhamnoside analogues and their biological activities will be reported later.

4. Experimental

4.1. General

Solvents were purified in a conventional manner. Thin-layer chromatography (TLC) was performed on pre-coated E. Merck Silica Gel 60 F_{254} plates. Flash column chromatography was performed on silica gel (200–300 mesh, Qingdao, China). Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter. ^{1}H NMR and ^{13}C NMR spectra were taken on a Jeol JNM-ECP 600 spectrometer with tetramethylsilane (Me_4Si) as the internal standard, and chemical shifts are recorded as δ values. COSY, HMQC, and HMBC NMR spectra were routinely used to definitively assign the signals of ^{1}H and ^{13}C NMR spectra. Mass spectra were recorded on a Global Q-TOF mass spectrometer.

4.2. (2*S*,3*S*,4*R*)-1-*O*-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-azido-3,4-di-*O*-benzyl-octadecantriol (6)

Method A: To a solution of donor **14** (317.6 mg, 0.57 mmol), acceptor **8** (250.0 mg, 0.48 mmol), and 4 Å MS (80 mg) in 1:5:1 CH_2Cl_2–Et_2O–THF (21 mL) were added NIS (173.6 mg, 0.77 mmol) and AgOTf (12.3 mg, 0.05 mmol) at 0 °C under Ar. The reaction mixture was stirred at 0 °C for 12 h and then concentrated. The residue was diluted with CH_2Cl_2 (20 mL), washed with 10% $Na_2S_2O_3$ (20 mL × 1) and brine (20 mL × 1), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (7:1 petroleum ether–EtOAc) to give the desired product **6** (306.5 mg, 67.0%) as a colorless oil: R_f 0.35 (7:1 petroleum ether–EtOAc).

Method B: A solution of trichloroacetimidate **15** (50.0 mg, 0.085 mmol) and phytosphingosine derivative **8** (40.0 mg, 0.076 mmol) in dry Et_2O (3.5 mL) and dry THF (0.5 mL) was added to freshly dried powdered 4 Å MS (20 mg) and cooled to −20 °C. $BF_3{\cdot}Et_2O$ (47%, 22.4 mg, 0.074 mmol) was added to the solution, and the mixture was stirred at −20 °C for 1 h. The mixture was diluted with EtOAc (50 mL) and filtered through Celite. The organic layer was washed with satd aq $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography on silica gel (7:1 petroleum ether–EtOAc) to furnish **6** (48.4 mg, 60.0%) as a colorless oil: R_f 0.35 (7:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +50.1 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.52–7.31 (25H, Ph), 5.45 (s, 1H, PhC*H*), 4.97 (d, 1H, $J = 3.2$ Hz, H-1′), 4.87–4.48 (8H, 4PhCH_2), 4.15 (d, 1H, $J = 3.2$ Hz, H-4′), 4.09 (dd, 1H, $J = 10.1$, 3.7 Hz, H-2′), 4.08 (d, 1H, $J = 12.8$ Hz, H-6′a), 4.02 (dd, 1H, $J = 10.1$, 3.2 Hz, H-3′), 3.99 (d, 1H, $J = 9.6$ Hz, H-1a), 3.88 (d, 1H, $J = 12.4$ Hz, H-6′b), 3.74–3.67 (m, 3H, H-1b, H-3, H-4), 3.63 (m, 1H, H-2), 3.56 (s, 1H, H-5′), 1.67 (m, 1H), 1.53 (m, 1H), 1.41 (m, 1H), 1.31–1.20 (m, 16H), 0.88 (t, 3H, $J = 6.9$ Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 138.7, 138.3, 138.0, 137.8, 128.9, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7,

127.6, 127.5, 126.3, 101.0 (PhCH), 99.1 (C1′), 79.4 (C2), 78.9, 75.7 (C3′), 75.4 (C2′), 74.6 (C4′), 73.7, 73.5, 72.0, 69.3 (C6′), 68.4 (C1), 62.9 (C5′), 61.8, 31.9, 30.2, 30.0, 29.7, 29.3, 25.4, 22.7, 14.1; HRMS: (*m*/*z*) $[M+Na]^+$: calcd for $C_{59}H_{75}N_3O_8Na^+$ 976.5452, found 976.5428.

4.3. (2*S*,3*S*,4*R*)-1-*O*-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-hexacosanoylamino-3,4-di-*O*-benzyl-octadecantriol (16)

To a solution of compound **6** (130 mg, 0.136 mmol) in pyridine (5 mL) and water (0.5 mL) cosolvent system was added PPh_3 (71.5 mg, 0.27 mmol). The reaction mixture was heated at 50 °C for 5 h and concentrated. To a solution of the crude amino product and hexacosanoic acid (64.7 mg, 0.163 mmol) in dried CH_2Cl_2 (10 mL) were added HOBt (23.9 mg, 0.177 mmol), EDC·HCl (33.9 mg, 0.177 mmol), and DIPEA (26.4 mg, 0.204 mmol) at 0 °C under Ar. The reaction mixture was stirred at rt for 22 h, and then concentrated. The residue was partitioned between CH_2Cl_2 and water. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica gel (7:1 petroleum ether–EtOAc) to furnish **16** (138.7 mg, 78.1%) as a colorless oil: R_f 0.32 (7:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +48.2 (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.52–7.26 (25H, Ph), 5.80 (d, 1H, *J* = 8.3 Hz, NH), 5.45 (s, 1H, PhCH), 4.94 (d, 1H, *J* = 3.2 Hz, H-1′), 4.86–4.73 (8H, 4PhCH_2), 4.28 (m, 1H), 4.17 (d, 1H, *J* = 3.2 Hz, H-4′), 4.10 (d, 1H, *J* = 12.4 Hz), 4.06 (dd, 1H, *J* = 10.1, 3.2 Hz, H-2′), 3.94–3.90 (m, 3H), 3.80–3.74 (m, 2H), 3.57 (s, 1H, H-5′), 3.53 (m, 1H), 1.88 (m, 2H), 1.63 (m, 2H), 1.50–1.4 (m), 1.31–1.20 (m, 16H), 0.88 (t, 6H, *J* = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 172.5, 138.6, 138.5, 137.4, 128.3, 127.9, 109.6, 105.9, 87.3, 83.7, 79.9, 76.1, 73.4, 72.2, 71.9, 71.8, 66.3, 65.3, 49.3, 36.8, 31.9, 29.8–25.3, 22.7, 14.1; HRMS: (*m*/*z*) $[M+Na]^+$: calcd for $C_{85}H_{127}NO_9Na^+$ 1328.9409, found 1328.9412.

4.4. *p*-Methylphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-D-galactopyranoside (17)

Compound **13** (3.0 g, 10.5 mmol) and Bu_2SnO (3.9 g, 15.7 mmol) were stirred in MeOH (30 mL) and toluene (30 mL) at reflux under argon for 4 h. The solvent was evaporated under reduced pressure. The residue was dissolved in toluene (60 mL), then TBAB (0.68 g, 2.1 mmol) and BnBr (2.7g, 15.7 mmol) were added. The reaction mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure, and the residue was diluted with CH_2Cl_2 (100 mL) and washed with water (50 mL × 2). The organic extracts were combined, dried over $MgSO_4$, and concentrated. The resulting residue was purified by column chromatography (15:1 $CHCl_3$–MeOH) to furnish a white solid (3.1 g, 77.7%). The solid was dissolved in dry DMF (20 mL), then benzaldehyde dimethyl acetal (2.5 g, 16.3 mmol) and TsOH (125.4 mg, 0.7 mmol) were added. After rotating at 30 °C for 2 h and 50 °C for 2.5 h under reduced pressure, the mixture was neutralized by addition of Et_3N and then evaporated to dryness. Silica gel column chromatography of the crude product, using 3:1 petroleum ether–EtOAc as eluent, furnished **17** (3.22 g, 85.3%) as a foamy solid: R_f 0.33 (2:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +219.4 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.58–7.05 (14H, Ph), 5.41 (s, 1H, PhCH), 4.73 (d, 1H, *J* = 12.4 Hz, PhCHH), 4.70 (d, 1H, *J* = 12.4 Hz, PhCHH), 4.46 (d, 1H, *J* = 9.5 Hz, H-1), 4.35 (dd, 1H, *J* = 12.4, 1.4 Hz, H-6a), 4.12 (d, 1H, *J* = 2.9 Hz, H-4), 3.97 (dd, 1H, *J* = 12.4, 1.4 Hz, H-6b), 3.87 (dd, 1H, *J* = 9.54, 9.48 Hz, H-2), 3.50 (dd, 1H, *J* = 9.5, 2.9 Hz, H-3), 3.44 (s, 1H, H-5), 2.46 (br s, 1H, 2-OH), 2.33 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 138.4, 138.0, 137.8, 134.4, 129.7, 129.0, 128.4, 128.1, 127.9, 126.6, 126.5, 101.1 (PhCH), 87.1 (C1), 80.2 (C3), 73.3 (C4), 71.6 (PhCH_2), 70.0 (C5), 69.4 (C6), 67.1 (C2), 21.1 (CH_3); HRMS: (*m*/*z*) $[M+Na]^+$: calcd for $C_{27}H_{29}O_5S^+$, 465.1736, found 465.1738.

4.5. *p*-Methylphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside (18)

Compound **17** (2.5 g, 5.4 mmol) was dissolved in DMF (15 mL). The reaction mixture was cooled to 0 °C. Sodium hydride (60%, 318.0 mg, 8.0 mmol) was added, followed by *p*-methoxybenzyl chloride (1.52 g, 9.7 mmol). The reaction was monitored by TLC and shown to be complete after 2 h at 0 °C. The mixture was diluted with water (50 mL) and extracted with CH_2Cl_2 (50 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and concentrated. Silica gel column chromatography of the crude product, using 3:1 petroleum ether–EtOAc as eluent, furnished **18** (2.7 g, 86.9%) as a white solid; R_f 0.41 (2:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ −33.7 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.61–6.86 (18H, Ph), 5.45 (s, 1H, PhCH), 4.70 (d, 1H, *J* = 12.4 Hz, PhCHH), 4.68 (d, 1H, *J* = 12.4 Hz, PhCHH), 4.65 (d, 1H, *J* = 12.4 Hz, PhCHH), 4.61 (d, 1H, *J* = 12.4 Hz, PhCHH) 4.53 (d, 1H, *J* = 9.7 Hz, H-1), 4.32 (dd, 1H, *J* = 12.4, 1.4 Hz, H-6a), 4.10 (d, 1H, *J* = 2.9 Hz, H-4), 3.92 (dd, 1H, *J* = 12.4, 1.4 Hz, H-6b), 3.82 (dd, 1H, *J* = 9.7, 9.1 Hz, H-2), 3.78 (s, 3H, OCH_3), 3.58 (dd, 1H, *J* = 9.2, 3.2 Hz, H-3), 3.32 (s, 1H, H-5), 2.28 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 159.2, 138.1, 137.9, 137.5, 133.3, 129.8, 129.6, 128.3, 128.0, 127.7, 126.6, 113.7, 101.2 (PhCH), 86.6 (C1), 81.4, 75.1, 75.0, 73.6, 71.7, 69.7, 69.4, 55.2 (OCH_3), 21.1; HRMS: (*m*/*z*) $[M+Na]^+$: calcd for $C_{35}H_{37}O_6S^+$, 585.2311, found 585.2322.

4.6. (2*S*,3*S*,4*R*)-1-*O*-(3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl)-2-azido-3,4-di-*O*-benzyl-octadecantriol (20)

To a mixture of glycosyl donor **18** (1.37 g, 2.4 mmol), acceptor **8** (1.05 g, 2.0 mmol) and 4 Å MS (200 mg) in 1:5:1 CH_2Cl_2–Et_2O–THF (63 mL) were added NIS (723.2 mg, 3.2 mmol) and AgOTf (51.4 mg, 0.2 mmol) at −20 °C under Ar. The reaction mixture was stirred for 30 min at −20 °C and then warmed to 0 °C. After stirring for 12 h at 0 °C the reaction mixture was concentrated. The residue was diluted with CH_2Cl_2 (50 mL) and washed with 10% $Na_2S_2O_3$ (50 mL × 1) and brine (50 mL × 1), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (7:1 petroleum ether–EtOAc) to give the desired product **20** as a colorless oil (1.22 g, 61.9%): R_f 0.67 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +44.3 (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.52–6.76 (24H, Ph), 5.45 (s, 1H, PhC*H*), 4.93 (d, 1H, *J* = 3.7 Hz, H-1′), 4.82–4.48 (8H, 4PhCH_2), 4.15 (d, 1H, *J* = 3.2 Hz, H-4′), 4.07 (dd, 1H, *J* = 10.1, 3.7 Hz, H-2′), 4.06 (dd, 1H, *J* = 12.4, 1.4 Hz, H-6′a), 4.00 (m, 2H, H-3′, H-1a), 3.88 (dd, 1H, *J* = 12.4, 1.4 Hz, H-6′b), 3.74 (s, 3H, OCH_3), 3.74–3.67 (m, 3H), 3.62 (m, 1H), 3.56 (s, 1H, H-5′), 1.66 (m, 1H), 1.53 (m, 1H), 1.41 (m, 1H), 1.30–1.21 (m, 16H), 0.88 (t, 3H, *J* = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 159.1, 138.8, 138.4, 138.0, 137.8, 130.8, 129.4, 128.9, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 126.3, 113.6, 101.0 (Ph*C*H), 99.2 (C1′), 79.4, 78.9, 75.8 (C3′), 75.0 (C2′), 74.7 (C4′), 73.8, 73.2, 72.1, 69.3 (C6′), 68.4 (C1), 63.0 (C5′), 61.8, 55.2 (O*C*H_3), 31.9, 30.0, 29.7, 29.4, 25.4, 22.7, 14.1; HRMS: (*m*/*z*) $[M+Na]^+$: calcd for $C_{60}H_{77}N_3O_9Na^+$ 1006.5558, found 1006.5594.

4.7. (2*S*,3*S*,4*R*)-1-*O*-(3-*O*-Benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-azido-3,4-di-*O*-benzyl-octadecantriol (7)

A solution of **20** (550.0 mg, 0.56 mmol) in CH_2Cl_2 (15 mL) was treated with 1 mL of water and DDQ (190.4 mg, 0.84 mmol) and stirred at 0 °C for 3 h. The reaction mixture was poured into satd aq $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (50 mL × 2). The organic extract was washed with satd aq $NaHCO_3$ (50 mL × 2) and water, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (5:1 petroleum ether–EtOAc) to give the desired product **7** as a colorless oil (410.1 mg, 84.8%): R_f 0.58 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +67.5 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.52–7.25 (20H, Ph), 5.42 (s, 1H, PhC*H*), 5.03 (d, 1H, *J* = 3.7 Hz, H-1′), 4.76–4.53 (6H, 3PhCH_2), 4.21 (dd, 1H, *J* = 10.1, 3.7 Hz, H-2′), 4.15 (d, 1H, *J* = 3.2 Hz, H-4′), 4.11 (m, 1H, H-1a), 4.09 (dd, 1H, *J* = 12.4, 1.8 Hz, H-6′a), 3.89 (dd, 1H, *J* = 12.4, 1.4 Hz, H-6′b), 3.75 (dd, 1H, *J* = 10.1, 3.2 Hz, H-3′), 3.73 (m, 2H, H-1b, H-2), 3.67 (dd, 1H, *J* = 5.0, 4.1 Hz, H-3), 3.62 (m, 1H, H-4), 3.55 (s, 1H, H-5′), 1.67 (m, 1H), 1.55 (m, 1H), 1.41 (m, 1H), 1.31–1.22 (m, 16H), 0.88 (t, 3H, *J* = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 138.32, 138.26, 137.8, 137.7, 128.9, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 126.2, 100.9 (Ph*C*H), 100.1 (C1′), 79.3 (C4), 79.2 (C3), 76.3 (C3′), 73.9, 73.7 (C4′), 72.1, 71.6, 69.3 (C6′), 69.1 (C1), 68.0 (C2′), 63.2 (C5′), 62.0 (C2), 31.9, 30.0, 29.7, 29.6, 29.3, 25.3, 22.7, 14.1; HRMS: (*m*/*z*) $[M+Na]^+$: calcd for $C_{52}H_{69}N_3O_8Na^+$ 886.4982, found 886.4998.

4.8. (2*S*,3*S*,4*R*)-1-*O*-[6-*O*-Benzoyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzyl-idene-α-D-galactopyranosyl]-2-azido-3,4-di-*O*-benzyl-octadecantriol (27)

To a mixture of glycosyl donor **22** (52.8 mg, 0.08 mmol), acceptor **7** (63.4 mg, 0.096 mmol) and 4 Å MS (20 mg) in 1:5 CH_2Cl_2–Et_2O (3 mL) were added NIS (28.9 mg, 0.128 mmol) and AgOTf (2.0 mg, 0.008 mmol) at −20 °C under Ar. The reaction mixture was stirred for 30 min at −20 °C and then warmed to 0 °C. After stirring for 12 h at 0 °C, the reaction mixture was concentrated. The residue was diluted with CH_2Cl_2 (20 mL) and washed with 10% $Na_2S_2O_3$ (20 mL × 1) and brine (20 mL × 1), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (7:1 petroleum ether–EtOAc) to give the desired product **27** (84.9 mg, 75.8%) as a colorless oil: R_f 0.40 (7:1 petroleum ether–EtOAc); Representative ^{1}H NMR ($CDCl_3$) signals: δ 5.38 (s, 1H, PhC*H*-α), 5.35 (s, 0.33H, PhC*H*-β), 5.23 (d, 0.33H, *J* = 3.7 Hz, H-1′-β), 5.17 (s, 1H, H-1″-α), 5.12 (d, 0.33H, *J* = 11.5 Hz, H-1″-β) 5.10 (d, 1H, *J* = 3.7 Hz, H-1′-α); ^{13}C NMR ($CDCl_3$): δ 165.9, 138.8–137.7, 133.1, 132.6, 129.8–126.3, 101.1, 99.9, 98.5, 97.3, 79.4–79.0, 74.9–68.9, 63.6, 62.9, 62.2, 31.9, 29.9–29.4, 25.2, 22.7, 14.1. α/β Mixtures of compound **27** were easily separated by column chromatography on a silica gel column (250:1 toluene–acetone, 300–400 mesh) to give the desired α-**27**: R_f 0.10 (100:1 toluene–acetone,); $[\alpha]_D^{22}$ −2.0 (*c* 0.68, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.86–7.12 (35H, Ph), 5.38 (s, 1H, PhC*H*), 5.17 (s, 1H, H-1″), 5.11 (d, 1H, *J* = 3.6 Hz, H-1′), 4.95 (d, 1H, *J* = 11.0 Hz, C*H*HPh), 4.82–4.47 (m, 13H, C*H*HPh), 4.40 (t, 1H, *J* = 6.4 Hz, H-5″), 4.30 (dd, 1H, *J* = 10.1, 3.2 Hz, H-2′), 4.24 (m, 2H, H-6″a, H-6″b), 4.09 (s, 2H, H-2″, H-3″), 4.01 (dd, 1H, *J* = 10.1, 3.2 Hz, H-1a), 3.98 (d, 1H, *J* = 12.4 Hz, H-6′a), 3.93 (dd, 1H, *J* = 10.1, 3.2 Hz, H-3′), 3.87 (s, 1H, H-4″), 3.80 (m, 1H, H-2), 3.77 (d, 1H, *J* = 12.4 Hz, H-6′b), 3.59 (m, 1H, H-4), 3.54 (dd, 1H, *J* = 9.7, 9.6 Hz, H-1b), 3.48 (t, 1H, *J* = 5.0 Hz, H-3),

3.46 (s, 1H, H-5′), 1.61–1.53 (m, 4H), 1.30–1.20 (m, large band, alkyl chains), 0.88 (t, 3H, $J = 6.8$ Hz, CH_3); HRMS: (m/z) [M+Na]$^+$: calcd for $C_{86}H_{101}N_3O_{14}Na^+$ 1422.7184, found 1422.7176.

4.9. (2*S*,3*S*,4*R*)-1-*O*-[2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl]-2-azido-3,4-di-*O*-benzyl-octadecantriol (28)

To a mixture of glycosyl donor **23** (44.3 mg, 0.08 mmol), acceptor **7** (63.4 mg, 0.096 mmol) and 4 Å MS (20 mg) in 1:5 CH_2Cl_2–Et_2O (3 mL) were added NIS (28.9 mg, 0.128 mmol) and AgOTf (2.0 mg, 0.008 mmol) at −20 °C under Ar. The reaction mixture was stirred for 30 min at −20 °C and then warmed to 0 °C. After stirring for 12 h at 0 °C, the reaction mixture was concentrated. The residue was diluted with CH_2Cl_2 (20 mL) and washed with 10% $Na_2S_2O_3$ (20 mL × 1) and brine (20 mL × 1), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (7:1 petroleum ether–EtOAc) to give the desired product **28** (56.3 mg, 54.4%) as a colorless oil: R_f 0.68 (3:1 petroleum ether–EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.85–7.00 (35H, Ph), 5.46 (s, 1H, PhCH), 5.34 (s, 1H, PhCH), 5.12 (s, 1H, H-1″), 5.07 (d, 1H, $J = 3.3$ Hz, H-1′), 4.81-4.47 (5 PhCH_2), 4.29 (dd, 1H, $J = 10.1$, 3.2 Hz, H-2′), 4.18 (d, 1H, $J = 2.8$ Hz, H-4′), 4.05 (s, 2H, H-2″, H-3″), 4.01 (dd, 1H, $J = 10.1$, 3.7 Hz, H-1a), 3.99 (d, 1H, $J = 12.4$ Hz, H-6′a), 3.95 (dd, 1H, $J = 10.6$, 3.2 Hz, H-3′), 3.91 (d, 1H, $J = 12.8$ Hz, H-6″a), 3.90 (s, 2H, H-4″, H-5″), 3.82 (m, 2H, H-6′b, H-2), 3.58 (m, 1H, H-4, H-1b), 3.56 (s, 1H, H-5′), 3.51 (d, 1H, $J = 12.4$ Hz, H-6″b), 3.48 (dd, 1H, $J = 5.5$, 4.6 Hz, H-3), 1.68–1.20 (m, large band, alkyl chains), 0.88 (t, 3H, $J = 6.9$ Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 138.9, 138.5, 138.3, 138.0, 137.7, 132.0, 128.9, 128.7, 128.4–127.4, 126.4, 126.3, 114.3, 100.9 (2C), 98.3, 97.4, 79.3, 79.2, 76.0, 75.6, 74.7, 74.5, 73.9, 73.5, 73.3, 72.9, 72.1, 71.8, 71.1, 69.3, 69.2, 62.9, 62.7, 62.2, 55.6, 31.9, 29.9–29.4, 25.2, 22.7, 14.1; HRMS: (m/z) [M+Na]$^+$: calcd for $C_{79}H_{95}N_3O_{13}Na^+$ 1316.6765, found 1316.6767.

5. (2*S*,3*S*,4*R*)-1-*O*-[2,3,4-Tri-*O*-benzyl-α-D-galactopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl]-2-azido-3,4-di-*O*-benzyl-octadecantriol (α-29)

Compound α-**27** (272.4 mg, 0.20 mmol) was dissolved in dry CH_3OH (2 mL) and dry CH_2Cl_2 (2 mL). CH_3ONa (99%, 57 mg) was added, and the mixture was stirred for 20 h. After almost all starting material was consumed, Amberlyst 15 (H^+-form) was added to neutralize the CH_3ONa, the mixture was then diluted with CH_3OH, and the ion-exchange resin was filtered off. The resin was washed thoroughly, and the filtrate was concentrated. The residue was chromatographed on a silica gel column (3:1 petroleum ether–EtOAc) to give the desired product α-**29** (216.1 mg, 85.7%) as a foamy solid: R_f 0.35 (4:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +82.4 (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.57–7.26 (35H, Ph), 5.46 (s, 1H, PhCH), 5.12 (s, 2H, H-1′, H-1″), 4.97–4.53 (12H, 6PhCH_2), 4.34 (dd, 1H, $J = 10.1$, 3.2 Hz, H-2′), 4.17 (d, 1H, $J = 3.2$ Hz, H-4′), 4.12–4.01 (m, 6H), 3.87 (m, 1H), 3.83 (d, 1H, $J = 12.4$ Hz, H-6′b), 3.78 (s, 1H, H-4″), 3.66 (m, 2H), 3.59 (s, 1H, H-5′), 3.56 (m, 2H), 3.34 (dd, 1H, $J = 11.0$, 5.0 Hz, H-6″b), 1.68 (m, 1H), 1.59 (m, 1H), 1.35–1.28 (m, large band, alkyl chains), 0.94 (t, 3H, $J = 6.8$ Hz, –CH_3); ^{13}C NMR ($CDCl_3$): δ 138.8, 138.7, 138.4, 138.3, 138.2, 137.7, 128.8 128.3–127.4, 126.1, 100.7, 98.2, 96.8, 79.2, 78.9, 76.4, 75.3, 74.6, 74.3, 73.9, 73.3, 73.0, 72.9, 72.7, 72.0, 71.4, 70.6, 69.2, 69.1, 62.8, 62.4, 62.1, 31.8, 29.9, 29.7-29.6, 29.3, 25.1, 22.6, 17.9, 14.1; HRMS: (m/z) [M−N_2+Na]$^+$: calcd for $C_{79}H_{97}NO_{13}Na^+$ 1290.6858, found 1290.6856.

5.1. (2*S*,3*S*,4*R*)-1-*O*-[2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl]-2-azido-3,4-di-*O*-benzyl-octadecantriol (30)

To a mixture of α-**29** (216.1 mg, 0.17 mmol), **6** (167.4 mg, 0.20 mmol), and 4 Å molecular sieves (50 mg) in dried CH_2Cl_2 (15 mL) were added TMSOTf (3.7 mg, 0.017 mmol) at −20 °C under Ar protection. The mixture was stirred under these conditions for 1 h, then neutralized with Et_3N. The solid was then filtered off, and the filtrate was concentrated under vacuum to give a yellow syrup, which was purified by column chromatography (3:1 petroleum ether–EtOAc) to give compound **30** (240.0 mg, 75.9%) as a foamy solid: R_f 0.45 (3:2 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +45.8 (c 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.70–7.25 (39H, Phth, Ph), 5.34 (d, 1H, $J = 3.2$ Hz, H-4‴), 5.29 (s, 1H, PhCH), 5.03 (d, 1H, $J = 8.2$ Hz, H-1‴), 5.01 (dd, 1H, $J = 7.7$, 3.2 Hz), 4.99 (d, 1H, $J = 3.2$ Hz, Gal H-1), 4.96 (d, 1H, $J = 3.2$ Hz, Gal H-1), 4.92 (dd, 1H, $J = 10.1$, 9.6 Hz, H-4^{IV}), 4.92 (d, 1H, $J = 4.3$ Hz), 4.75 (d, 1H, $J = 11.0$ Hz, PhCHH), 4.70 (d, 1H, $J = 11.9$ Hz, PhCHH), 4.63 (m, 4H), 4.60 (br s, 1H, H-1^{IV}), 4.56 (m, 2H), 4.52–4.45 (m, 4H), 4.38 (m, 2H), 4.16–4.11 (m, 2H), 4.07 (dd, 1H, $J = 11.5$, 6.0 Hz), 4.04 (dd, 1H, $J = 9.7$, 9.6 Hz), 3.96–3.86 (m, 7H), 3.82 (br s, 1H), 3.77 (dd, 1H, $J = 10.1$, 3.2 Hz), 3.71 (m, 2H), 3.59 (t, 1H, $J = 6.4$ Hz), 3.54 (m, 2H), 3.47 (t, 1H, $J = 9.6$ Hz), 3.41 (m, 2H), 2.18

(s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.86 (s, 3H, CH_3CO), 1.83 (s, 3H, CH_3CO), 1.60 (m, 1H), 1.50 (m, 1H), 1.31–1.20 (broad band, alkyl chain protons), 1.17 (d, 3H, $J = 6.4$ Hz, CH_3-6^{IV}), 0.88 (t, 3H, $J = 6.8$ Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.5, 170.4, 170.1, 169.4, 169.3, 169.0, 167.3, 139.1, 138.9, 138.8, 138.7, 138.3, 137.8, 137.7, 134.0, 133.8, 131.4, 131.0, 128.9, 128.4-127.2, 126.3, 123.7, 123.1, 101.1 (Ph*C*H), 98.6, 98.5, 98.1, 97.7, 79.2, 79.1, 78.7, 76.1, 75.0, 74.65, 74.56, 74.3, 74.2, 73.5, 73.4, 73.2, 72.0, 71.9, 71.8, 71.0, 70.6, 69.9, 69.3, 69.1, 68.6, 68.2, 68.1, 67.3, 65.2, 62.8, 62.1, 61.8, 53.0, 31.9, 29.8-29.6, 29.3, 25.2, 22.7, 20.8, 20.7, 20.6, 20.5, 17.3, 14.1; HRMS: (m/z) [M+H]$^+$: calcd for $C_{109}H_{131}N_4O_{28}^+$ 1943.8950, found 1943.8872.

5.2. (2*S*,3*S*,4*R*)-1-*O*-[2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl]-2-azido-3,4-di-*O*-benzyl-octadecantriol (31)

A solution of **30** (271.0 mg, 0.14 mmol) in ethylenediamide (4 mL) and *n*-BuOH (8 mL) was heated at 90 °C for 6 h. The reaction mixture was cooled and concentrated under diminished pressure. The residue was then dissolved in pyridine (3 mL) and Ac_2O (3 mL). The mixture was stirred at rt for 12 h, MeOH (5 mL) was added, and the mixture was then concentrated to give a residue that was purified by column chromatography (3:2 petroleum ether–EtOAc) to give compound **31** (185.6 mg, 71.7%) as a foamy solid: R_f 0.80 (1:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +53.3 (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.51–7.26 (35H, Ph), 5.45 (d, 1H, $J = 6.8$ Hz, NH), 5.41 (s, 1H, PhCH), 5.22 (d, 1H, $J = 3.2$ Hz), 5.12–5.10 (m, 3H), 5.07 (m, 2H, sugar H-1, sugar H-1), 5.04 (d, 1H, $J = 9.6$ Hz, H-4^{IV}), 4.94 (d, 1H, $J = 2.3$ Hz), 4.92 (d, 1H, $J = 4.3$ Hz), 4.80–4.58 (m, 11H), 3.52–4.47 (m, 3H), 4.21–4.17 (m, 2H), 4.06–4.03 (m, 4H), 3.98–3.91 (m, 5H), 3.83 (dd, 1H, $J = 10.1$, 5.0 Hz), 3.82 (m, 1H), 3.79–3.76 (m, 2H), 3.71 (dd, 1H, $J = 6.9$, 6.4 Hz), 3.57 (m, 1H), 3.52–3.48 (m, 3H), 3.45 (t, 1H, $J = 5.0$ Hz), 2.85 (m, 1H), 2.11 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 1.28–1.23 (broad band, alkyl chain protons), 1.18 (d, 3H, $J = 6.4$ Hz, CH_3-6^{IV}), 0.88 (t, 3H, $J = 6.9$ Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.5, 170.2, 170.03, 170.00, 139.1, 138.8, 138.7, 138.6, 138.3, 137.8, 137.7, 129.1, 128.6–128.2, 127.9, 127.7, 127.6, 127.4, 127.2, 126.1, 100.7 (PhCH), 99.4, 98.3, 98.2, 97.5, 79.3, 79.0, 76.3, 75.1, 75.0, 74.5, 73.9, 73.5, 73.2, 73.0, 72.6, 72.5, 72.0, 70.7, 70.1, 69.9, 69.7, 69.2, 68.9, 68.8, 67.1, 63.0, 62.3, 62.0, 55.8, 31.9, 29.9-29.7, 29.4, 25.2, 23.3, 22.7, 20.9, 20.8, 20.7, 17.3, 14.1; HRMS: (m/z) [M+H]$^+$: calcd for $C_{109}H_{131}N_4O_{28}^+$ 1855.8923, found 1856.1011.

5.3. (2*S*,3*S*,4*R*)-1-*O*-[2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl]-2-[*N*-(*R*)-2-acetoxydocosanoylamino]-3,4-di-*O*-benzyl-octadecantriol (32)

Compound **31** (80.0 mg, 0.043 mmol) was dissolved in pyridine (3 mL) and H_2O (3 mL). H_2S, generated from FeS and 3 M H_2SO_4, was bubbled in over a period of 1 h. The reaction was kept sealed overnight. The solvent was removed in vacuo. The resulting residue was dissolved in CH_2Cl_2 (2 mL), and **5** (17.5 mg, 0.044 mmol), HOBt (6.5 mg, 0.048 mmol), EDC·HCl (9.2 mg, 0.048 mmol), DIPEA (7.6 mg, 0.060 mmol) were added. The reaction mixture was stirred under argon overnight. Solvent was removed in vacuo, and the resulting residue was purified by column chromatography (3:1 petroleum ether–EtOAc) to give compound **32** (62.9 mg, 66.0%) as a colorless oil: R_f 0.51 (1:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +0.3 (c 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.48–7.15 (35H, Ph), 6.64 (d, 1H, $J = 8.7$ Hz, NH), 5.41 (s, 1H, PhCH), 5.30 (d, 1H, $J = 6.8$ Hz, NH), 5.22 (d, 1H, $J = 3.2$ Hz), 5.10 (dd, 1H, $J = 10.1$, 3.7 Hz), 5.06 (dd, 1H, $J = 3.2$, 1.4 Hz), 5.03 (d, 1H, $J = 10.1$, 9.6 Hz), 5.00 (d, 1H, $J = 3.7$ Hz, sugar H-1), 4.97 (dd, 1H, $J = 7.8$, 4.6 Hz), 4.96 (d, 1H, $J = 4.1$ Hz, sugar H-1), 4.89 (d, 1H, $J = 11.0$ Hz), 4.79 (d, 1H, $J = 12.8$ Hz), 4.75 (d, 1H, $J = 8.2$ Hz, H-1‴), 4.73 (d, 1H, $J = 11.9$ Hz), 4.70–4.50 (m, 11H), 4.67 (m, 1H, sugar H-1), 4.40 (m, 1H, H-2^V), 4.21 (dd, 1H, $J = 6.8$, 6.4 Hz), 4.17 (dd, 1H, $J = 10.5$, 3.2 Hz), 4.14–4.10 (m, 4H), 4.08 (d, 1H, $J = 12.8$ Hz, H-6a′), 4.04 (dd, 1H, $J = 11.5$, 5.0 Hz), 4.02 (dd, 1H, $J = 10.1$, 3.2 Hz), 3.99–3.92 (m, 4H), 3.88–3.84 (m, 4H), 3.82 (d, 1H, $J = 11.9$ Hz, H-6b′), 3.69 (dd, 1H, $J = 6.9$, 6.4 Hz), 3.62 (dd, 1H, $J = 4.6$, 4.1 Hz), 3.54 (m, 1H), 3.51 (s, 1H, H-5′), 3.47 (dd, 1H, $J = 10.1$, 6.8 Hz), 2.92 (m, 1H), 2.15 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.76 (m, 1H), 1.57 (s, 3H, CH_3CONH), 1.40 (m, 1H), 1.30–1.21 (broad band, alkyl chain protons), 1.18 (d, 3H, $J = 6.4$ Hz, CH_3-6^{IV}), 0.88 (t, 6H, $J = 6.8$ Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 202.9, 172.03,171.21, 170.49, 170.46, 170.28, 170.18, 170.12, 170.04, 139.1, 138.61, 138.58, 138.46, 138.39, 138.20, 137.9, 129.1, 128.6, 128.41, 128.38, 128.32, 128.20, 127.99, 127.96, 127.77, 127.74, 127.70, 127,67, 127.56, 127.30, 127.21, 126.1, 100.7, 99.5, 99.3, 98.3, 95.9, 80.3, 79.5, 78.9, 75.8, 75.0, 74.6, 74.3, 73.3, 73.1, 72.9, 72.6, 72.1, 72.0, 70.8, 70.1, 69.9, 69.4, 69.3, 69.0, 68.8, 67.1, 63.1, 62.0, 60.4, 55.4, 50.6, 32.1, 31.9, 30.4, 29.8, 29.7, 29.6, 29.4, 25.8, 25.1, 23.2, 22.7, 21.1, 20.9,

20.8, 20.71, 20.68, 17.3, 14.21, 14.15; HRMS: (*m*/*z*) $[M+H]^+$: calcd for $C_{127}H_{176}N_2O_{30}^+$ 2232.2208, found 2232.2200.

5.4. Clarhamnoside peracetate 2

To a solution of **32** (50.0 mg, 0.036 mmol) in MeOH (1 mL) and EtOAc (3 mL) was added $Pd(OH)_2/C$ (75% wt% Pd dry basis on carbon, 25.0 mg), and the mixture was hydrogenated at rt for 12 h. The suspension was filtered through a Celite pad, and the filter cake was rinsed with 5:1 $CHCl_3$–MeOH (10 mL). The combined filtrate and washings were concentrated in vacuo. The residue was then dissolved in pyridine (1 mL) and Ac_2O (1 mL). After stirring at rt overnight, the excess Ac_2O was decomposed by adding MeOH. The mixture was concentrated in vacuo, and the resulting residue was purified by column chromatography (3:1 petroleum ether–EtOAc) to give clarhamnoside peracetate **2** (32.4 mg, 74.8%) as a colorless oil: R_f 0.75 (2:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +38 (*c* 0.43, $CHCl_3$); the diagnostic ^1H NMR and ^{13}C NMR signals of **2** were identical to those provided in the literature. HRMS: (*m*/*z*) $[M+H]^+$: calcd for $C_{94}H_{153}N_2O_{38}^+$ 1918.0132, found 1918.0101.

5.5. Clarhamnoside (1)

Clarhamnoside peracetate **2** (10.0 mg, 5.2 mmol) was dissolved in NaOMe in MeOH (3 mL of a 0.02 M solution). The reaction was allowed to proceed for 18 h at 25 °C, then the reaction mixture was dried under nitrogen, and the residue was partitioned between water and $CHCl_3$. After removal of the solvent, the organic layer gave a foamy solid (6.90 mg, quant): R_f 0.32 (4:1 $CHCl_3$–MeOH); $[\alpha]_D^{22}$ +22 (*c* 0.2, $CHCl_3$); ^1H NMR (pyridine-d_5): δ 9.04 (d, 1H, $J = 9.2$ Hz, N*H*), 8.59 (d, 1H, $J = 9.6$ Hz, N*H*), 5.78 (s, 1H, H-1^{IV}), 5.65 (dd, 1H, $J = 3.2$ Hz, H-1′), 5.56 (dd, 1H, $J = 2.8$ Hz, H-1″), 5.26 (d, 1H, $J = 7.8$ Hz, H-1‴), 5.25–4.25 (m, overlapped), 3.96 (m, 1H, H-5‴), 2.24–1.62 (m), 1.57 (d, 3H, $J = 5.9$ Hz, H-6^{IV}), 1.40–1.20 (m, large band, alkyl chains), 0.89 (m, 6H, C*H*$_3$); ^{13}C NMR (pyridine-d_5): δ 175.2 (*C*═O), 171.6 (*C*═O), 104.2, 102.4, 98.8, 98.0, 79.7, 76.6, 75.8, 75.4, 73.8, 72.9, 72.8, 72.7, 72.3, 71.1–70.1, 69.6, 69.2, 68.4, 62.3, 62.0, 57.3, 53.4, 50.9, 35.4, 33.6, 32.0, 30.3–29.5, 26.9, 26.6, 25.8, 23.2, 22.8, 19.1, 18.4, 14.2; HRMS (MALDI-TOF): (*m*/*z*) $[M+Na]^+$: calcd for $C_{66}H_{124}N_2O_{24}Na^+$ 1351.8444, found 1351.8436.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.05.018.

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