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Synthesis of 4-*C*-methyl analogues of glucosylceramide

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ABSTRACT

Benzyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-*C*-methylene-α-D-*xylo*-hexopyranoside (**3**) was transformed with 3-chloroperoxybenzoic acid into the epoxides **4** and **5**. Reductive opening of the epoxide moiety in **4** furnished benzyl 2,3,6-tri-*O*-benzyl-4-*C*-methyl-α-D-glucopyranoside (**6**); subsequent hydrogenolytic *O*-debenzylation, per-*O*-acetylation, selective removal of the anomeric *O*-acetyl group, and then treatment with trichloroacetonitrile in the presence of base afforded 2,3,4,6-tetra-*O*-acetyl-4-*C*-methyl-D-glucopyranosyl trichloroacetimidate (**9**). Reaction of **9** with the 3-*O*-benzoyl-azidosphingosines **10a,b** in the presence of $BF_3 \cdot OEt_2$ gave (2*S*,3*R*,4*E*)-2-azido-3-benzoyloxy-1-(2,3,4,6-tetra-*O*-acetyl-4-*C*-methyl-β-D-glucopyranosyloxy)-4-octadecene (**14a**) and -4-dodecene (**14b**), respectively. Treatment of **14a,b** with triphenylphosphine and the carboxylic acid anhydride in the presence of water and then with methanolic sodium methoxide gave the title compounds **16a,b**.

INTRODUCTION

The biosynthesis of glycosphingolipids (GSLs) starts from ceramide as the substrate to which, with the help of activated sugars and glycosyl transferases as catalysts, the sugar residues are attached[1]. Thus, with UDP-glucose and glucosyl transferase, glucosylceramide is generated; then, with UDP-galactose and galactosyl transferase, the O-β-(1-4)-linked lactosylceramide is obtained which is the basic constituent of various types of GSLs. Modifications of the glucosyl moiety in the 4-position should provide interesting target molecules for biological testing[2,3]. Therefore, we planned to introduce at C-4 of the glucosyl moiety a *C*-methyl group, leading to glucosylceramide analogues **16** as target molecules that should not be galactosylated at O-4 due to steric hindrance. However, as a substrate analogue, compound **16a** could act as a competitive galactosyl transferase inhibitor. Synthesis of the truncated derivative **16b** has been envisaged[4–6] because

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advantageous properties for such sphingosines and ceramides, respectively, have been observed in biological testing[4,7].

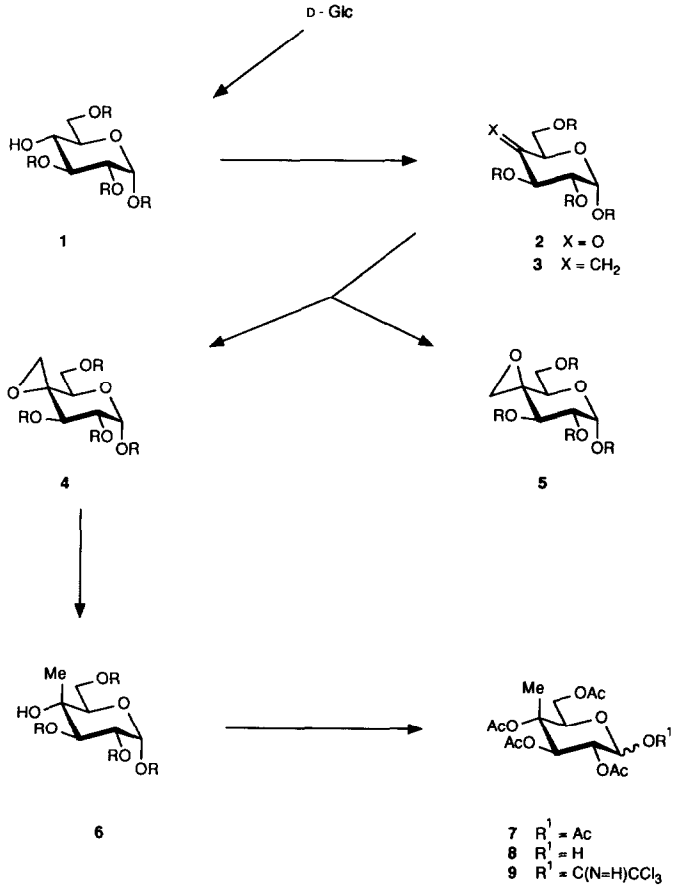
RESULTS AND DISCUSSION

The strategy selected for the synthesis of 4-*C*-methyl branched glucosylceramides **16** was based on the azidosphingosine glycosylation procedure for GSL synthesis introduced by us[8,9]. To this end, the 4-*C*-methylglucosyl donor **9** and the 3-*O*-protected azidosphingosines **10a,b** are required as essential building blocks.

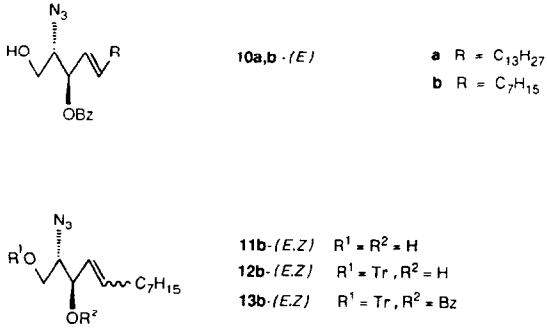
For the synthesis of **9**, the readily available *O*-benzyl-protected glucoside **1**[10,11] was transformed into the labile 4-ulose derivative **2**, and subsequently by Wittig reaction into the 4-deoxy-4-*C*-methylene derivative **3**, following known procedures[10]. Epoxidation of the alkene moiety in **3** with 3-chloroperoxybenzoic acid furnished the acid-sensitive epoxides **4** and **5** (overall yield from **1**, 36%; **4**:**5**-ratio, 1:2). Selective hydrogenolysis of the epoxide ring in **4** can be performed in ethyl acetate–methanol with palladium-on-$CaCO_3$ as catalyst, to give directly the desired 4-*C*-methylglucoside **6**, the structure of which could be assigned by comparison with authentic material obtained via a different route[12]. Reductive cleavage of the epoxide ring in **4** with $LiAlH_4$ as the reducing agent could also be successfully carried out, again providing **6** in high yield. Hydrogenolytic *O*-debenzylation of **6** with palladium-on-carbon as catalyst and subsequent *O*-acetylation with acetic anhydride in pyridine furnished the per-*O*-acetyl derivative **7** (1:1 anomeric mixture), which was selectively 1-*O*-deacetylated with hydrazinium acetate (method of Excoffier et al.[13]) to yield **8**. Treatment of **8** with trichloroacetonitrile in the presence of K_2CO_3 as base afforded the trichloroacetimidate **9** in high yield (83%, $\alpha:\beta \sim 1:1$)[14].

For the regioselective coupling of **9** with the azidosphingosine, 3-*O*-protected derivatives are required[8,9,15]. Successful reactions of various glycosyl donors with the 3-*O*-benzoyl derivative **10a**-(*E*)[8,15] encouraged us to synthesize also the truncated analogue **10b**-(*E*). For this purpose, the truncated azidosphingosine **11b** (obtained as a 7:1 *E*/*Z*-mixture)[5,16] was treated with trityl chloride in pyridine–dichloromethane (1:1), affording selectively the 1-*O*-trityl derivatives **12b**-(*E*,*Z*); 3-*O*-benzoylation with benzoyl chloride in pyridine–toluene (1:1) gave **13b**-(*E*,*Z*), which could be separated by MPLC; detritylation of the *E*/*Z*-mixture with $BF_3 \cdot OEt_2$ in methanol[17] afforded **10b** as an *E*,*Z*-mixture which could be readily fractionated by flash chromatography, thus providing pure **10b**-(*E*).

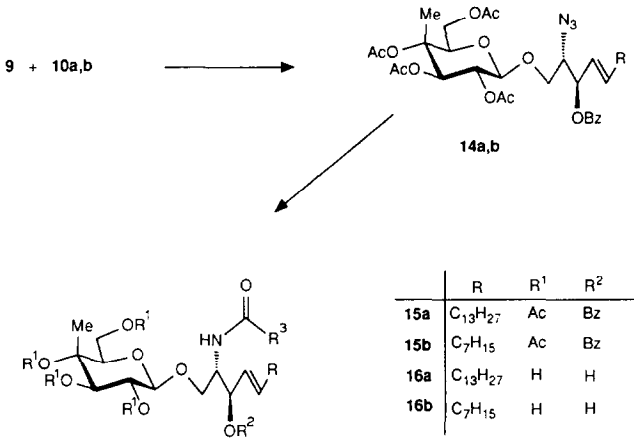
Glycosylation of acceptors **10a,b**-(*E*) with donor **9** in the presence of $BF_3 \cdot OEt_2$ as catalyst furnished, by neighbouring-group participation[14], the β-connected glycosides **14a** and **14b**, respectively. Azide group reduction in **14a** and subsequent *N*-palmitoylation was performed as a one-pot reaction with triphenylphosphine and palmitic anhydride in the presence of water[18,19], thus providing the *O*-acyl-protected target molecule **15a**; similarly, from **14b**, triphenylphosphine, and dodecanoic anhydride, compound **15b** was obtained. *O*-Deacylation of **15a,b** was carried



Scheme 1. R = Bzl.



Scheme 2.



	R	R^1	R^2	R^3
15a	$C_{13}H_{27}$	Ac	Bz	$C_{15}H_{31}$
15b	C_7H_{15}	Ac	Bz	$C_{11}H_{13}$
16a	$C_{13}H_{27}$	H	H	$C_{15}H_{31}$
16b	C_7H_{15}	H	H	$C_{11}H_{13}$

Scheme 3.

out with methanolic sodium methoxide (Zemplén conditions), thus affording the desired 4-*C*-methyl-glucosylceramides **16a,b**. The ^{1}H NMR data of the intermediates and of the final products are in accordance with the assigned structures.

EXPERIMENTAL

General methods.—Melting points are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 MC polarimeter. ^{1}H NMR spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) with a Bruker WM 250 (or AC 250) Cryospec instrument. R_F values refer to TLC performed on Silica Gel 60 F_{254} (Merck). Column chromatography was performed under normal pressure with silica gel (Merck, 70–230 mesh ASTM and 230–400 mesh ASTM for flash chromatography) and under elevated pressure with LiChroprep Si 60 (Merck, 15–25 μm). The bp of the light petroleum was 35–60°.

*Benzyl 2,3,6-tri-*O*-benzyl-α-*D*-*xylo*-hexopyranosid-4-ulose (2).*—This compound was prepared as described in ref. 10. From **1**[10,11] (90 g, 0.166 mol) was obtained crude **2** (98 g), which was used directly without purification for the synthesis of compound **3**.

*Benzyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-*C*-methylene-α-*D*-*xylo*-hexopyranoside (3).*—This compound was prepared as described in ref. 10. From crude **2** (66 g) was obtained crude **3** (53 g) which was used without purification for the synthesis of **4** and **5**.

*Benzyl 4,4^1-anhydro-2,3,6-tri-*O*-benzyl-4-*C*-hydroxymethyl-α-*D*-glucopyranoside (4) and benzyl 4,4^1-anhydro-2,3,6-tri-*O*-benzyl-4-*C*-hydroxymethyl-α-*D*-galacto-pyranoside (5).*—To a solution of crude **3** (25 g) in dry CH_2Cl_2 (250 mL) was added dry 3-chloroperoxybenzoic acid (90%; 8.8 g, 0.046 mol), with stirring at 0°, in

several portions. After 16 h at room temperature, the mixture was extracted with satd aq $NaHCO_3$ (50 mL), 1 M sodium bisulfite (50 mL), and satd aq $NaHCO_3$ (50 mL). The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure. Short-column chromatography (8:2 light petroleum–EtOAc) gave a syrup which was dissolved in EtOAc (50 mL). Light petroleum was added until crystallization of **5** began. The product was filtered off and the filtrate was concentrated under reduced pressure. The residual syrup, which consisted of a mixture of **4** and **5**, was purified by flash chromatography (8:2 light petroleum–EtOAc).

Compound **4** (3.36 g, 12% from **1**) was a colourless syrup; $[\alpha]_D^{22}$ +85° (*c* 1, $CHCl_3$); R_F 0.27 (9:1 light petroleum–EtOAc). ^1H NMR (250 MHz, $CDCl_3$): δ 2.81, 3.14 (2 d, 2 H, J_{gem} 5.2 Hz, H-4^{1}a,4^{1}b), 3.43 (m, 2 H, H-6a,6b), 3.58 (dd, 1 H, $J_{1,2}$ 3.6, $J_{2,3}$ 9.8 Hz, H-2), 4.23 (d, 1 H, $J_{2,3}$ 9.8 Hz, H-3), 4.35 (dd, 1 H, $J_{5,6a} = J_{5,6b} = 3.6$ Hz, H-5), 4.52–4.82 (m, 8 H, 4 CH_2Ph), 4.93 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 7.28–7.45 (m, 20 H, 4 Ph). *Anal.* Calcd for $C_{35}H_{36}O_6$: C, 76.06; H, 6.57. Found: C, 76.01; H, 6.65.

Compound **5** (6.86 g, 24% from **1**) formed colourless needles, mp 90° (from EtOH), $[\alpha]_D^{22}$ +51° (*c* 1, $CHCl_3$); R_F 0.21 (9:1 light petroleum–EtOAc). ^1H NMR (250 MHz, $CDCl_3$): δ 2.85, 2.98 (2 d, 2 H, J_{gem} 5.2 Hz, H-4^{1}a,4^{1}b), 3.31 (dd, 1 H, $J_{5,6a}$ 4.6, J_{gem} 9.8 Hz, H-6a), 3.43 (dd, 1 H, $J_{5,6b}$ 7.3, J_{gem} 9.8 Hz, H-6b), 3.78 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 9.8 Hz, H-2), 4.25 (d, 1 H, $J_{2,3}$ 9.8 Hz, H-3), 4.31 (dd, 1 H, $J_{5,6a}$ 4.6, $J_{5,6b}$ 7.3 Hz, H-5), 4.43–4.77 (m, 7 H, CH_2Ph), 4.91 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.92 (d, 1 H, J_{gem} 10.3, CH_2Ph), 7.25–7.44 (m, 20 H, 4 Ph).

Anal. Calcd for $C_{35}H_{36}O_6$: C, 76.06; H, 6.57. Found: C, 76.05; H, 6.56.

Benzyl 2,3,6-tri-O-benzyl-4-C-methyl-α-D-glucopyranoside (6).—Procedure (a). To a solution of **4** (190 mg, 0.34 mmol) in EtOAc (3 mL) and MeOH (3 mL) was added palladium-on-calcium carbonate (70 mg). The mixture was shaken under H_2 for 16 h. Removal of the catalyst, concentration under reduced pressure, and flash chromatography (8:2 light petroleum–EtOAc) of the residue yielded **6** (150 mg, 80%) as colourless crystals.

Procedure (b). To a suspension of lithium aluminium hydride (1 g) in dry tetrahydrofuran (150 mL) was added dropwise a solution of **4** (6.98 g, 12.6 mmol) in dry tetrahydrofuran (20 mL). After stirring for 4 h at room temperature, the mixture was cooled in an ice-bath, and MeOH was added dropwise until evolution of H_2 ceased. Then, 10% H_2SO_4 was added until the precipitate of aluminium hydroxide was fully dissolved. The organic solution was separated and the aqueous solution was extracted with diethyl ether (3 × 50 mL). The combined organic solutions were washed with brine (70 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Flash chromatography (9:1 light petroleum–EtOAc) yielded **6** (5.46 g, 78%) as colourless crystals, mp 69°, $[\alpha]_D^{22}$ +100° (*c* 1.9, EtOAc); R_F 0.60 (2:1 light petroleum–EtOAc).

Compound **6** was obtained independently as a by-product of the hydroboration of **3**[12]. The reported physical data are in accordance with those found here.

1,2,3,4,6-Penta-O-acetyl-4-C-methyl-D-glucopyranose (7).—To a solution of **6** (5.46 g, 9.82 mmol) in EtOAc (20 mL) and MeOH (20 mL) was added palladium-on-carbon (10%, 200 mg). After hydrogenolysis for 4 h, the mixture was filtered and concentrated under reduced pressure. A mixture of the residue, dry pyridine (15 mL), dry acetic anhydride (15 mL), and 4-(dimethylamino)pyridine (100 mg) was stirred for 16 h at room temperature and then concentrated at 10^{-2} Torr. Flash chromatography (6:4 light petroleum–EtOAc) of the residue yielded **7** (3.20 g, 81%; $\alpha:\beta = 1:1$) as a colourless syrup. ^{1}H NMR (250 MHz, $CDCl_3$) of the anomeric mixture: **7α**, δ 1.44 (s, 3 H, CH_3), 1.98, 2.01, 2.08, 2.09, 2.20 (5 s, 15 H, 5 OAc), 4.07 (dd, 1 H, $J_{5,6a}$ 8.0, J_{gem} 12.1 Hz, H-6a), 4.30 (dd, 1 H, $J_{5,6b}$ 2.3, J_{gem} 12.1 Hz, H-6b), 5.02 (dd, 1 H, $J_{1,2}$ 4.0, $J_{2,3}$ 10.4 Hz, H-2), 5.16 (dd, 1 H, $J_{5,6b}$ 2.3, $J_{5,6a}$ 8.0 Hz, H-5), 6.08 (d, 1 H, $J_{2,3}$ 10.4 Hz, H-3), 6.28 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1); **7β**, δ 1.44 (s, 3 H, CH_3), 1.97, 2.03, 2.07, 2.08, 2.11 (5 s, 15 H, 5 OAc), 4.09 (dd, 1 H, $J_{5,6a}$ 8.0, J_{gem} 12.1 Hz, H-6a), 4.30 (dd, 1 H, $J_{5,6b}$ 2.3, J_{gem} 12.1 Hz, H-6b), 4.90 (dd, 1 H, $J_{5,6b}$ 2.3, $J_{5,6a}$ 8.0 Hz, H-5), 5.09 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.8 Hz, H-2), 5.76 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.98 (d, 1 H, $J_{2,3}$ 9.8 Hz, H-3). *Anal.* Calcd for $C_{17}H_{24}O_{11}$: C, 50.50; H, 5.98. Found: C, 50.62; H, 6.30.

2,3,4,6-Tetra-O-acetyl-4-C-methyl-D-glucopyranose (8).—A solution of **7** (2.52 g, 6.21 mmol) and hydrazinium acetate (630 mg, 6.30 mmol) in dry $HCONMe_2$ (15 mL) was stirred for 4 h at 40°. After evaporation at 10^{-2} Torr, the residue was dissolved in CH_2Cl_2 (50 mL) and washed with brine (50 mL). The aqueous solution was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic solutions were dried ($MgSO_4$), and concentrated under reduced pressure. Flash chromatography (1:1 light petroleum–EtOAc) yielded **8** (1.92 g, 85%, $\alpha:\beta = 1:1$) as a colourless syrup, R_F 0.37 (1:1 light petroleum–EtOAc). ^{1}H NMR (250 MHz, $CDCl_3$) of the anomeric mixture: δ 1.43 (s, 3 H, CH_3), 1.97–2.09 (m, 12 H, 8 OAc), 4.06, 4.10 (2 dd, 1 H, $J_{5,6a}$ 8.1, $J_{6a,6b}$ 12.0 Hz, 2 H-6a), 4.29, 4.34 (2 dd, 1 H, $J_{5,6b}$ 2.4, $J_{6a,6b}$ 12.0 Hz, 2 H-6b), 4.81–4.87 (m, 2 H, H-1β, H-2α, H-2β, H-5α), 5.22 (dd, 0.5 H, $J_{5,6b}$ 2.4, $J_{5,6a}$ 8.1 Hz, H-5β), 5.44 (d, 0.5 H, $J_{1,2}$ 3.9 Hz, H-1α), 5.94 (d, 0.5 H, $J_{2,3}$ 9.4 Hz, H-3β), 6.09 (d, 0.5 H, $J_{2,3}$ 10.4 Hz, H-3α). *Anal.* Calcd for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12. Found: C, 49.73; H, 6.33.

2,3,4,6-Tetra-O-acetyl-4-C-methyl-D-glucopyranosyl trichloroacetimidate (9).—To a solution of **8** (2.11 g, 5.80 mmol) and trichloroacetonitrile (1.10 mL, 1.58 g, 10.9 mmol) in dry CH_2Cl_2 (15 mL) was added K_2CO_3 (500 mg). The mixture was stirred for 16 h at room temperature, then filtered through Celite and concentrated under reduced pressure. Flash chromatography (6:4 light petroleum–EtOAc) yielded **9** (2.43 g, 83%; $\alpha:\beta = 1:1$) as a colourless foam; R_F 0.30 (6:4 light petroleum–EtOAc). ^{1}H NMR (250 MHz, $CDCl_3$) of the anomeric mixture: **9α**: δ 1.45 (s, 3 H, CH_3), 2.01, 2.02, 2.10 (3 s, 9 H, 3 OAc), 4.08–4.17 (m, 1 H, H-6a), 4.29–4.37 (m, 1 H, H-6b), 5.04 (dd, 1 H, $J_{1,2}$ 4.1, $J_{2,3}$ 10.3 Hz, H-2), 5.25–5.28 (m, 1 H, H-5), 6.18 (d, 1 H, $J_{2,3}$ 10.3 Hz, H-3), 6.51 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1), 8.66 (s, 1 H, NH); **9β**: δ 1.47 (s, 3 H, CH_3), 2.00, 2.08, 2.09 (3 s, 9 H, 3 OAc), 4.08–4.17 (m, 1 H, H-6a), 4.29–4.37 (m, 1 H, H-6b), 4.98 (dd, 1 H, $J_{5,6b}$ 2.6, $J_{5,6a}$

7.8 Hz, H-5), 5.25 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 9.8 Hz, H-2), 5.88 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 6.00 (d, 1 H, $J_{2,3}$ 9.8 Hz, H-3), 8.73 (s, 1 H, NH). *Anal.* Calcd for $C_{17}H_{22}Cl_3NO_{10}$: C, 40.30; H, 4.38; N, 2.77. Found: C, 40.39; H, 4.47; N, 2.96.

(2S,3R)-2-Azido-1-triphenylmethyloxy-4-dodecen-3-ol (12b).—To a solution of **11b** (8.6 g, 35.6 mmol; $E:Z = 7:1$)[5,16] in dry pyridine (25 mL) and dry CH_2Cl_2 (25 mL) was added triphenylmethyl chloride (19.6 g, 70.3 mmol) with stirring. After 16 h at room temperature, the mixture was concentrated at reduced pressure and dissolved in diethyl ether (200 mL). The organic solution was extracted with satd aq $NaHCO_3$ (2×50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Flash chromatography (95:5 light petroleum–EtOAc) gave **12b** (17.1 g, quant; $E:Z = 7:1$) as a colourless oil which still contained triphenylmethanol; it was used without further purification in the next step. For analytical purposes, a small amount was purified by MPLC (95:5 light petroleum–EtOAc); separation of the *E* and *Z* isomers was not successful; R_F 0.62 (9:1 light petroleum–EtOAc). ^1H NMR (250 MHz, $CDCl_3$) of **12b**-*E*: δ 0.88 (t, 1 H, *J* 6.6 Hz, CH_3), 1.16–1.54 (m, 10 H, 5 CH_2), 1.90–2.06 (m, 3 H, 2 CH=CH–CH_2, OH), 3.30 (d, 2 H, *J* 5.5 Hz, CH_2O), 3.52 (dt, 1 H, $J = J' = 5.5$ Hz, CHN), 4.15–4.20 (m, 1 H, C*H*–OH), 5.31 (dd, 1 H, *J* 7.2, 15.4 Hz, C*H*=CH–CH_2), 6.15 (dt, 1 H, *J* 6.7, 15.4 Hz, CH=C*H*–CH_2), 7.19–7.45 (m, 15 H, 3 Ph). *Anal.* (for **12b**) Calcd for $C_{31}H_{37}N_3O_2$: C, 76.99; H, 7.71; N, 8.69. Found: C, 76.84; H, 7.75; N, 8.50.

(2S,3R,4E)-2-Azido-3-benzoyloxy-1-triphenylmethoxy-4-dodecene [13b-(E)] and (2S,3R,4Z)-2-azido-3-benzoyloxy-1-triphenylmethoxy-4-dodecene [13b-(Z)].—To a solution of **12b** (8.4 g, 17.4 mmol; $E:Z = 7:1$) in dry toluene (20 mL) and dry pyridine (20 mL) was added benzoyl chloride (4.0 mL, 4.8 g, 34.5 mmol). After stirring for 24 h at room temperature, the mixture was concentrated under reduced pressure and dissolved in diethyl ether (100 mL). The organic solution was extracted with satd aq $NaHCO_3$ (3×50 mL), and the aqueous solutions were combined and extracted with diethyl ether (3×50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Flash chromatography (95:5 light petroleum–EtOAc) yielded **13b** (6.8 g, 68% from **11b**; $E:Z = 7:1$) as a colourless oil, which was used as the *E/Z*-mixture in the next step. Separation of the *E/Z* isomers was possible by MPLC (30:1 light petroleum–EtOAc).

13b-(*E*): R_F 0.49 (95:5 light petroleum–EtOAc), $[\alpha]_D^{22}$ −20° (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* 6.8 Hz, CH_3), 1.22–1.25 (m, 10 H, 5 CH_2), 1.93–1.98 (m, 2 H, CH=CH–CH_2), 3.20 (dd, 1 H, *J* 5.2, 9.8 Hz, CH_2O), 3.29 (dd, 1 H, *J* 6.7, 9.8 Hz, CH_2O), 3.86 (ddd, 1 H, *J* 4.8, 5.2, 6.7 Hz, CHN), 5.42 (dd, 1 H, *J* 7.9, 15.4 Hz, C*H*=CH–CH_2), 5.63 (dd, 1 H, *J* 4.8, 7.9 Hz, C*H*=OBz), 5.81 (dt, 1 H, *J* 6.7, 15.4 Hz, CH=C*H*–CH_2), 7.18–7.48 (m, 17 H, Ph), 7.53–7.59 (m, 1 H, Ph), 7.93–7.97 (m, 2 H, Ph).

13b-(*Z*): R_F 0.52 (95:5 light petroleum–EtOAc); $[\alpha]_D^{22}$ +5° (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 3 H, *J* 6.8 Hz, CH_3), 1.24 (m, 10 H, 5 CH_2), 2.05–2.19 (m, 2 H, CH=CH–CH_2), 3.20 (dd, 1 H, *J* 4.7, 9.8 Hz, CH_2O), 3.29 (dd, 1

H, *J* 7.1, 9.8 Hz, CH_2O), 3.84 (ddd, 1 H, *J* 4.7, 4.8, 7.1 Hz, CHN), 5.39 (dd, 1 H, *J* 9.4, 10.9 Hz, C*H*=CH–CH_2), 5.67 (dt, 1 H, *J* 7.5, 10.9 Hz, CH=C*H*–CH_2), 5.91 (dd, 1 H, *J* 4.8, 9.4 Hz, C*H*–OBz), 7.17–7.46 (m, 17 H, Ph), 7.51–7.58 (m, 1 H, Ph), 7.91–7.95 (m, 2 H, Ph). *Anal.* [for **13b** (*E*/*Z*-mixture)] Calcd for $C_{38}H_{41}N_3O_3$: C, 77.65; H, 7.03; N, 7.15. Found: C, 77.62; H, 7.11; N, 7.00.

*(2S,3R,4E)-2-Azido-3-benzoyloxy-4-dodecen-1-ol [**10b**-(E)] and (2S,3R,4Z)-2-azido-3-benzoyloxy-4-dodecen-1-ol [**10b**-(Z)].*—To a solution of **13b** (8.16 g, 10.6 mmol; *E*:*Z* = 7:1) in dry toluene (30 mL) and dry MeOH (20 mL) was added dropwise diethyl ether–boron trifluoride (2 mL). After 2 h at room temperature, satd aq $NaHCO_3$ (50 mL) was added, the organic layer was separated, and the aqueous solution was extracted with diethyl ether (3 × 50 mL). The combined organic solutions were dried ($MgSO_4$) and concentrated under reduced pressure. Fractionation of the residue by flash chromatography (9:1 light petroleum–EtOAc) yielded **10b**-(*E*) (2.16 g, 59%) and **10b**-(*Z*) (0.30 g, 8%) as colourless oils.

10b-(*E*): R_F 0.10 (9:1 light petroleum–EtOAc); $[\alpha]_D^{22}$ −59° (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* 6.8 Hz, CH_3), 1.25–1.41 (m, 10 H, 5 CH_2), 2.04–2.12 (m, 3 H, CH=CH–CH_2, OH), 3.63 (dd, 1 H, *J* 7.0, 11.5 Hz, CH_2O), 3.75 (dd, 1 H, *J* 4.0, 11.5 Hz, CH_2O), 3.77–3.85 (m, 1 H, CHN), 5.55–5.65 (m, 2 H, C*H*=CH–CH_2, C*H*–OBz), 5.90–6.01 (m, 1 H, CH=C*H*–CH_2), 7.42–7.48 (m, 2 H, Ph), 7.55–7.61 (m, 1 H, Ph), 8.04–8.08 (m, 2 H, Ph).

10b-(*Z*): R_F 0.15 (9:1 light petroleum–EtOAc), $[\alpha]_D^{22}$ +7° (*c* 2, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* 6.6 Hz, CH_3), 1.24–1.39 (m, 10 H, 5 CH_2), 2.19–2.28 (m, 3 H, CH=CH–CH_2, OH), 3.63 (dd, 1 H, *J* 7.0, 11.6 Hz, CH_2O), 3.75 (dd, 1 H, *J* 3.9, 11.6 Hz, CH_2O), 3.83 (ddd, 1 H, *J* 3.9, 5.3, 7.0 Hz, CHN), 5.57 (ddd, *J* 1.3, 9.3, 10.9 Hz, C*H*=CH–CH_2), 5.81 (dt, 1 H, *J* 7.5, 10.9 Hz, CH=C*H*–CH_2), 5.97 (ddd, 1 H, *J* 0.7, 5.3, 9.3 Hz, C*H*–OBz), 7.42–7.48 (m, 2 H, Ph), 7.54–7.61 (m, 1 H, Ph), 8.04–8.07 (m, 2 H, Ph). *Anal.* Calcd for $C_{19}H_{27}N_3O_3$: C, 66.06; H, 7.88; N, 12.16. Found: C, 66.05; H, 7.69; N, 12.00.

*(2S,3R,4E)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-O-acetyl-4-C-methyl-β-D-glucopyranosyloxy)-4-octadecene (**14a**).*—To a solution of **10a**[8,15] (850 mg, 1.97 mmol) in dry *n*-hexane (6 mL) under an N_2 atmosphere was added dropwise diethyl ether–boron trifluoride (0.6 mL of a 0.2 M solution in CH_2Cl_2) and a solution of **9** (1.30 g, 2.56 mmol) in dry CH_2Cl_2 (3 mL). The mixture was stirred for 5 h at room temperature [TLC control with HPTLC Kieselgel 60 F_{254} (Merck), light petroleum–diethyl ether (1:1), R_F of orthoester = 0.50]. Then light petroleum was added and the mixture was filtered. The filtrate was washed with satd aq $NaHCO_3$ (20 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Flash chromatography (8:2 light petroleum–EtOAc) yielded **14a** (1.10 g, 72%) as a colourless syrup, $[\alpha]_D^{22}$ −33° (*c* 1, $CHCl_3$), which contained traces of orthoester; this material was used without further purification in the next step; R_F = 0.45 (HPTLC Kieselgel 60 F_{254} (Merck), 9:1 light petroleum–diethyl ether). ^1H NMR (250 MHz, $CDCl_3$): δ 0.88 (t, 3 H, *J* 6.8 Hz, CH_3), 1.24–1.33 (m, 22 H, 11 CH_2), 1.47 (s, 3 H, CH_3), 1.96–2.08 (m, 2 H, CH=CH–CH_2), 1.96, 2.00, 2.07, 2.08 (4 s, 12 H, 4 OAc),

3.62 (dd, 1 H, *J* 8.8, 12.0 Hz, CH_2O), 3.87–3.94 (m, 2 H, CH_2O, CHN), 4.00 (dd, 1 H, $J_{5,6a}$ 7.9, J_{gem} 12.0 Hz, H-6a), 4.25 (dd, 1 H, $J_{5,6b}$ 2.6, J_{gem} 12.0 Hz, H-6b), 4.57 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.79 (dd, 1 H, $J_{5,6b}$ 2.6, $J_{5,6a}$ 7.9 Hz, H-5), 5.00 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.8 Hz, H-2), 5.54 (ddt, 1 H, $J < 0.7$, 8.0, 14.7 Hz, C*H*=CH–CH_2), 5.63 (dd, 1 H, *J* 3.6, 8.0 Hz, C*H*–OBz), 5.90 (d, 1 H, $J_{2,3}$ 9.8 Hz, H-3), 5.93 (dt, 1 H, *J* 6.8, 14.7 Hz, CH=C*H*–CH_2), 7.42–7.48 (m, 2 H, Ph), 7.55–7.60 (m, 1 H, Ph), 8.03–8.07 (m, 2 H, Ph). *Anal.* Calcd for $C_{40}H_{59}N_3O_{12}$: C, 62.08; H, 7.68; N, 5.43. Found: C, 61.71; H, 7.76; N, 5.50.

*(2S,3R,4E)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-*O*-acetyl-4-*C*-methyl-β-*D*-glucopyranosyloxy)-4-dodecene (**14b**).*—To a solution of **10b** (510 mg, 1.48 mmol) in dry *n*-hexane (4 mL) under an N_2 atmosphere was added diethyl ether–boron trifluoride (1.9 mL of a 0.2 M solution in dry CH_2Cl_2) and a solution of **9** (970 mg, 1.91 mmol) in dry CH_2Cl_2 (2 mL). After stirring for 30 min at room temperature, the mixture was diluted with light petroleum (30 mL) and filtered. The filtrate was washed with satd aq $NaHCO_3$ (20 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Flash chromatography (8:2 light petroleum–EtOAc) of the residue yielded **14b** (440 mg, 44%) as a colourless syrup, $[\alpha]_D^{22}$ −32° (*c* 0.64, $CHCl_3$), $R_F = 0.25$ (8:2 light petroleum–ethyl acetate). ^1H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* 6.8 Hz, CH_3), 1.25–1.44 (m, 10 H, 5 CH_2), 1.40 (s, 3 H, CH_3), 1.80–2.13 (m, 2 H, CH=CH–CH_2), 1.96, 1.99, 2.07, 2.08 (4 s, 12 H, 4 OAc), 3.61 (dd, 1 H, *J* 8.5, 12.0 Hz, CH_2O), 3.88–3.93 (m, 2 H, CH_2O, CHN), 4.00 (dd, 1 H, $J_{5,6a}$ 7.9, J_{gem} 11.9 Hz, H-6a), 4.25 (dd, 1 H, $J_{5,6b}$ 2.5, J_{gem} 11.9 Hz, H-6b), 4.57 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.80 (dd, 1 H, $J_{5,6b}$ 2.5, $J_{5,6a}$ 7.9 Hz, H-5), 5.00 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.8 Hz, H-2), 5.50–5.65 (m, 2 H, C*H*=CH–CH_2, C*H*–OBz), 5.89 (d, 1 H, $J_{2,3}$ 9.8 Hz, H-3), 5.87–5.98 (m, 1 H, CH=C*H*–CH_2), 7.42–7.48 (m, 2 H, Ph), 7.54–7.61 (m, 1 H, Ph), 8.03–8.06 (m, 2 H, Ph). *Anal.* Calcd for $C_{34}H_{47}N_3O_{12} \cdot H_2O$: C, 57.70; H, 6.97; N, 5.94. Found: C, 57.70; H, 6.63; N, 6.00.

*(2S,3R,4E)-3-Benzoyloxy-2-hexadecanoylamino-1-(2,3,4,6-tetra-*O*-acetyl-4-*C*-methyl-β-*D*-glucopyranosyloxy)-4-octadecene (**15a**).*—A solution of **14a** (1.10 g), triphenylphosphine (560 mg, 2.13 mmol), and hexadecanoic anhydride (910 mg, 1.83 mmol) in dry tetrahydrofuran (20 mL) was stirred for 48 h at room temperature. Water (0.5 mL) was added and stirring continued for 48 h at room temperature. The mixture was concentrated under reduced pressure and coevaporated with pyridine (1 × 30 mL) and toluene (2 × 30 mL). MPLC (6:4 light petroleum–EtOAc) yielded **15a** (390 mg, 21% from **10a**), $[\alpha]_D^{22}$ −3° (*c* 1, $CHCl_3$), R_F 0.15 (7:3 light petroleum–EtOAc). ^1H NMR (250 MHz, $CDCl_3$): δ 0.85–0.90 (m, 6 H, 2 CH_3), 1.23–1.40 (m, 46 H, 23 CH_2), 1.35 (s, 3 H, CH_3), 1.40–1.63 (m, 2 H, CH_2), 1.84, 1.94, 2.03, 2.07 (4 s, 12 H, 4 OAc), 1.93–2.22 (m, 4 H, CH=CH–CH_2, NCO–CH_2), 3.63 (dd, 1 H, *J* 4.0, 9.8 Hz, CH_2O), 3.73 (dd, 1 H, $J_{5,6a}$ 7.9, J_{gem} 11.9 Hz, H-6a), 4.07 (dd, 1 H, *J* 3.4, 9.7 Hz, CH_2O), 4.17 (dd, 1 H, $J_{5,6b}$ 2.4, J_{gem} 11.9 Hz, H-6b), 4.46–4.51 (m, 1 H, CHN), 4.50 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.69 (dd, 1 H, $J_{5,6b}$ 2.4, $J_{5,6a}$ 7.9 Hz, H-5), 4.92 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.8 Hz, H-2), 5.42–5.58 (m, 2 H, C*H*=CH–CH_2, C*H*–OBz), 5.78–5.93 (m, 2 H, CH=C*H*–CH_2, NH), 5.87 (d, 1

H, $J_{2,3}$ 9.8 Hz, H-3), 7.40–7.46 (m, 2 H, Ph), 7.55–7.58 (m, 1 H, Ph), 8.00–8.04 (m, 2 H, Ph). *Anal.* Calcd for $C_{56}H_{91}NO_{13} \cdot H_2O$; C, 66.97; H, 9.33; N, 1.39. Found: C, 67.02; H, 9.39; N, 1.44.

*(2S,3R,4E)-3-Benzoyloxy-2-dodecanoylamino-1-(2,3,4,6-tetra-O-acetyl-4-C-methyl-β-D-glucopyranosyloxy)-4-dodecene (**15b**).*—A solution of **14b** (108 mg, 0.156 mmol), triphenylphosphine (60 mg, 0.228 mmol), and dodecanoic anhydride (78 mg, 0.80 mmol) in dry tetrahydrofuran (5 mL) was stirred for 36 h at room temperature. Water (0.2 mL) was added and stirring continued for 48 h at room temperature. The mixture was concentrated under reduced pressure, and coevaporated with pyridine (1 × 20 mL) and toluene (2 × 20 mL). Flash chromatography (7:3 light petroleum–EtOAc) of the residue and lyophilization from dioxane yielded **15b** (71 mg, 54%) as a colourless powder, $[\alpha]_D^{22}$ +5° (*c* 1, $CHCl_3$), R_F 0.17 (7:3 light petroleum–EtOAc). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.82–0.90 (m, 6 H, 2 CH_3), 1.24–1.35 (m, 26 H, 13 CH_2), 1.36 (3 H, CH_3), 1.59 (m, 2 H, CH_2), 1.84, 1.94, 2.03, 2.06 (4 s, 12 H, 4 OAc), 1.98–2.17 (m, 4 H, CH=CH–CH_2, NCO–CH_2), 3.63 (dd, 1 H, *J* 3.6, 9.8 Hz, CH_2O), 3.74 (dd, 1 H, $J_{5,6a}$ 7.9, J_{gem} 11.9 Hz, H-6a), 4.06 (dd, 1 H, *J* 3.6, 9.8 Hz, CH_2O), 4.17 (dd, 1 H, $J_{5,6b}$ 2.4, J_{gem} 11.9 Hz, H-6b), 4.45–4.51 (m, 1 H, CHN), 4.50 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.69 (dd, 1 H, $J_{5,6b}$ 2.4, $J_{5,6a}$ 7.9 Hz, H-5), 4.92 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.8 Hz, H-2), 5.43–5.58 (m, 2 H, C*H*=CH–CH_2, C*H*–OBz), 5.76–5.93 (m, 2 H, CH=C*H*–CH_2, NH), 5.87 (d, 1 H, $J_{2,3}$ 9.8 Hz, H-3), 7.40–7.46 (m, 2 H, Ph), 7.52–7.58 (m, 1 H, Ph), 8.00–8.04 (m, 2 H, Ph). *Anal.* Calcd for $C_{46}H_{71}NO_{13}$: C, 65.30; H, 8.46; N, 1.66. Found: C, 65.06; H, 8.43; N, 2.00.

*(2S,3R,4E)-2-Hexadecanoylamino-1-(4-C-methyl-β-D-glucopyranosyloxy)-4-octadecen-3-ol (**16a**).*—To a solution of **15a** (245 mg, 0.252 mmol) in dry CH_2Cl_2 (4 mL) was added sodium methoxide (0.3 mL of a 1 M solution in MeOH). The mixture was refluxed for 1 h, neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated under reduced pressure. Chromatography (9:1 $CHCl_3$–MeOH) of the residue and lyophilization from dioxane yielded **16a** (136 mg, 76%) as a white powder, $[\alpha]_D^{22}$ −3° (*c* 1, 9:1 $CHCl_3$–MeOH), R_F 0.49 (8:2 $CHCl_3$–MeOH). ^{1}H NMR [250 MHz, $CDCl_3/CD_3OD$ (9:1)]: δ 0.85–0.91 (m, 6 H, 2 CH_3), 1.12 (s, 3 H, CH_3), 1.23 (m, 46 H, 23 CH_2), 1.52–1.66 (m, 2 H, CH_2), 1.98–2.03 (m, 2 H, CH=CH–CH_2), 2.15–2.21 (m, 2 H, NCO–CH_2), 3.22 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.7 Hz, H-2), 3.33–3.40 (m, 1 H, CH_2O), 3.40 (d, 1 H, $J_{2,3}$ 9.7 Hz, H-3), 3.62–3.69 (m, 2 H, H-6a, CH_2O), 3.87 (dd, 1 H, $J_{5,6b}$ 3.2, J_{gem} 11.6 Hz, H-6b), 3.97–4.03 (m, 1 H, CHN), 4.09–4.15 (m, 2 H, H-5, CHO), 4.26 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 5.46 (dd, 1 H, *J* 7.2, 15.3 Hz, C*H*=CH–CH_2), 5.70 (dt, 1 H, *J* 6.5, 15.3 Hz, CH=C*H*–CH_2). *Anal.* Calcd for $C_{41}H_{79}NO_8 \cdot 2H_2O$: C, 65.65; H, 11.15; N, 1.86. Found: C, 65.78; H, 11.01; N, 1.97.

*(2S,3R,4E)-2-Dodecanoylamino-1-(4-C-methyl-β-D-glucopyranosyloxy)-4-dodecen-3-ol (**16b**).*—To a solution of **15b** (45 mg, 0.053 mmol) in dry CH_2Cl_2 (2 mL) was added sodium methoxide (0.15 mL of a 0.1 M solution in MeOH). After 1 h at room temperature, the mixture was neutralized with Amberlite IR-120 (H^+) resin,

filtered, and concentrated under reduced pressure. Flash chromatography (9:1 $CHCl_3$–MeOH) of the residue and lyophilization from dioxane yielded **16b** (29 mg, 96%) as a colourless powder, $[\alpha]_D^{22}$ −3° (*c* 1, 9:1 $CHCl_3$–MeOH), $R_F = 0.68$ (8:2 $CHCl_3$–MeOH). ^1H NMR [250 MHz, $CDCl_3/CD_3OD$ (9:1)]: δ 0.85–0.90 (m, 6 H, 2 CH_3), 1.13 (s, 3 H, CH_3), 1.26–1.37 (m, 26 H, 13 CH_2), 1.59 (m, 2 H, CH_2), 1.98–2.03 (m, 2 H, CH=CH–CH_2), 2.14–2.20 (m, 2 H, NCO–CH_2), 3.22 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.7 Hz, H-2), 3.33 (dd, 1 H, *J* 3.6, 7.8 Hz, CH_2O), 3.40 (d, 1 H, $J_{2,3}$ 9.7 Hz, H-3), 3.57–3.70 (m, 2 H, H-6a, CH_2O), 3.86 (dd, 1 H, $J_{5,6b}$ 3.6, J_{gem} 11.6 Hz, H-6b), 4.01–4.15, (m, 3 H, H-5, CHO, CHN), 4.26 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 5.46 (dd, 1 H, *J* 7.0, 15.3 Hz, C*H*=CH–CH_2), 5.71 (dt, 1 H, *J* 6.7, 15.3 Hz, CH=C*H*–CH_2). *Anal.* Calcd for $C_{31}H_{58}NO_8 \cdot 2H_2O$: C, 61.15; H, 10.26; N, 2.30. Found: C, 61.09; H, 10.17; N, 2.38.

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