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Muramic acid derivatives as glycosyl donors for the synthesis of muramyl-containing glycosphingolipids and fatty acids

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ABSTRACT

2-Azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(1*R*)-(methoxycarbonyl)ethyl]-α-D-glucopyranosyl trichloroacetimidate (**3α**) has been used as the glycosyl donor in the synthesis of glycosphingolipids **14** and **27**. Reaction of **3α** with (2*S*, 3*R*, 4*E*)-2-azido-3-benzoyloxy-4-octadecen-1-ol (**6**) gave (2*S*, 3*R*, 4*E*)-2-azido-1-{2-azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(1*R*)-1-(methoxycarbonyl)ethyl]-β-D-glucopyranosyloxyl}-3-benzoyloxy-4-octadecene (**7**), which was converted into (2*S*, 3*R*, 4*E*)-1-{2-deoxy-2-hexadecanoylamino-3-O-[(2*R*)-propanoyl-(L-alanyl-D-isoglutamine benzyl ester)-2-yl]-β-D-glucopyranosyloxy}-2-hexadecanoylamino-4-octadecen-3-ol (**14**). Reaction of **3α** with *tert*-butyldimethylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**15**) gave *tert*-butyldimethylsilyl 2-azido-4-*O*-{2-azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(1*R*)-1-(methoxycarbonyl)ethyl]-β-D-glucopyranosyl}-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**16β**), which was converted into 1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-{4,6-di-*O*-acetyl-2-deoxy-2-hexadecanoylamino-3-*O*-[(2*R*)-propanoyl-(L-alanyl-D-isoglutamine methyl ester)-2-yl]-β-D-glucopyranosyl}-2-hexadecanoylamino-D-glucopyranose (**27**).

INTRODUCTION

The cell-wall petidoglycan of bacteria has a β-(1→4)-linked glycan chain, consisting of alternating 2-acetamido-2-deoxyglucose and *N*-acylmuramic acid residues that are cross-linked by a peptide chain (*e.g.*, L-Ala-D-iso-Gln-*meso*-DAP-D-Ala and glycine)[1]. The resulting peptidoglycan network (murein) and its fragments exhibit marked immunostimulatory and antitumour properties. The minimal structure for activity, the so-called Freund's complete adjuvant, is a "muramoyl dipeptide" (MDP, *N*-acetylmuramyl-L-alanyl-D-isoglutamine, **1**). Many investigations have been directed towards the synthesis of derivatives of MDP. It has been demonstrated that the carbohydrate moiety is responsible for the immunostimulatory properties and that the lipophilic groups and esterified isoglutamine residues can enhance the activity. Therefore, the synthesis of analogues of MDP, including glycosides and disaccharides that have lipophilic groups, is of special interest because of their potential[2,3] in combined chemotherapy and immunotherapy.

Glycosyl trichloroacetimidates, introduced in 1980 (ref. 4), are outstanding

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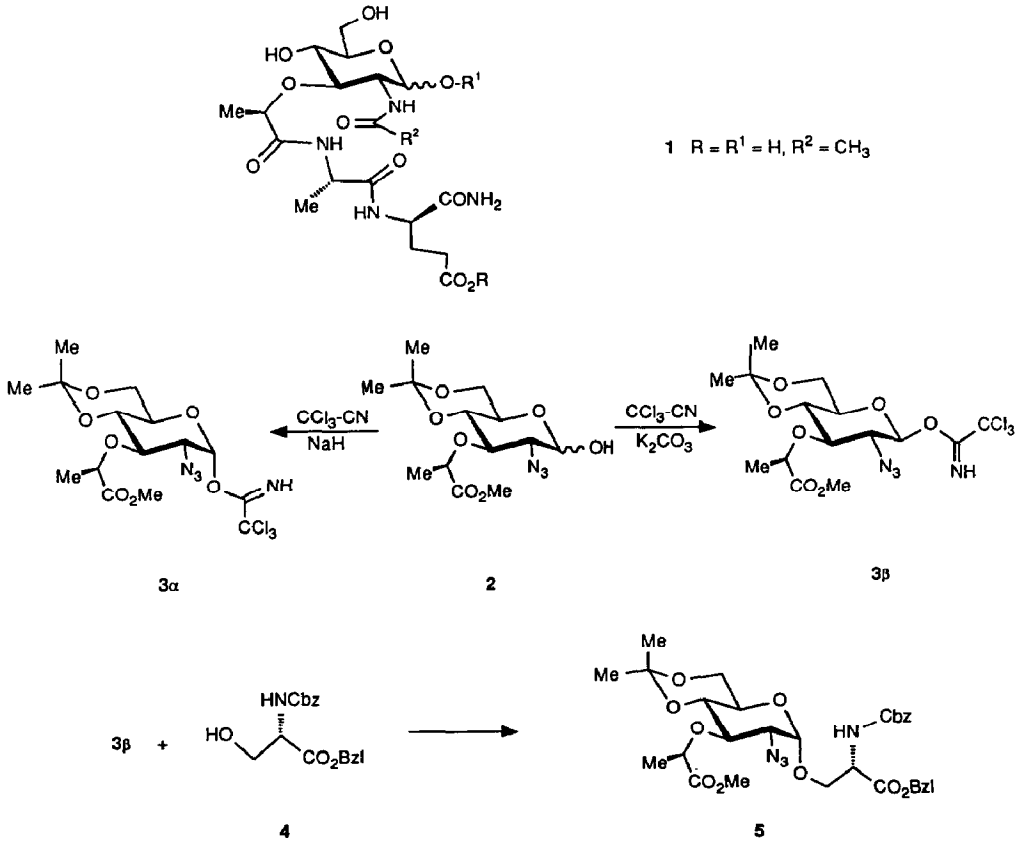
glycosyl donors[5-7] because of their stability, reactivity, diastereoselectivity, and general applicability. The application of the trichloroacetimidate method for muramylation is now reported.

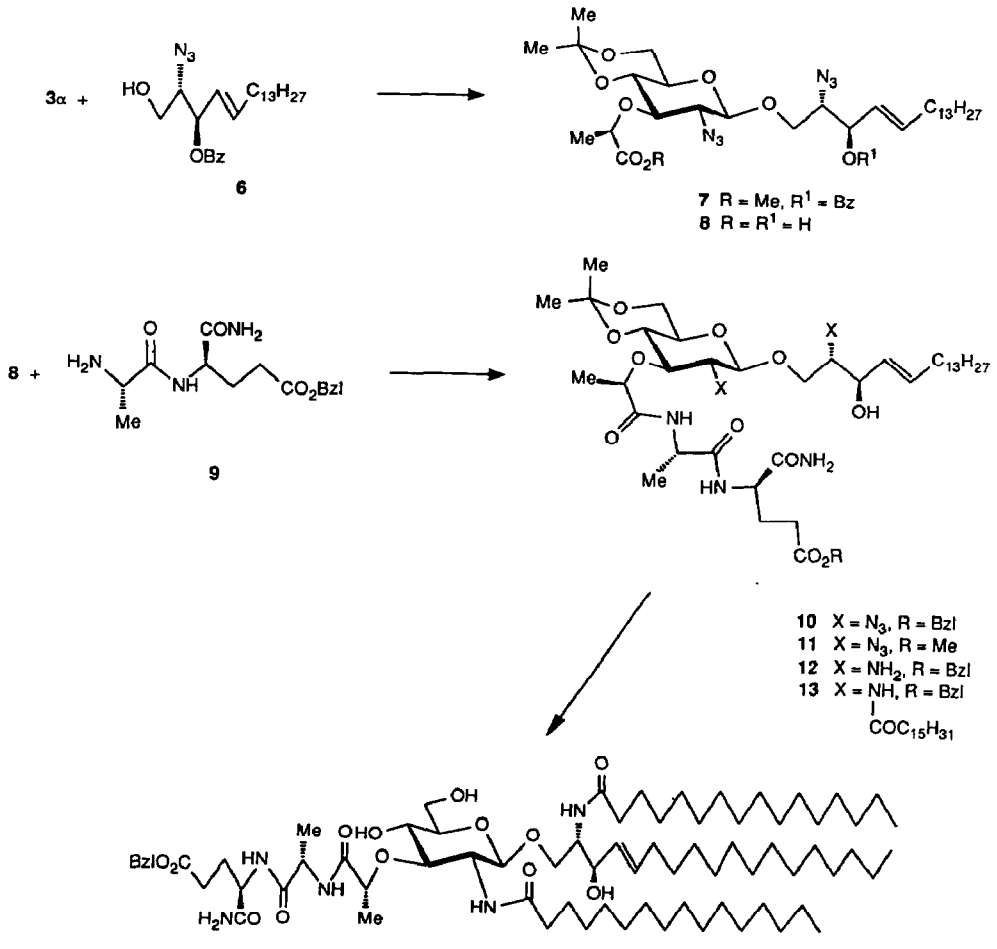
RESULTS AND DISCUSSION

The diastereocontrolled synthesis of the muramyl trichloroacetimidates **3** was based on the known[8-10] muramic acid derivative **2**. The previously used (2*S*)-2-chloropropionates afford mixtures of diastereomers[9,11]. Reaction of **2** with trichloroacetonitrile in the presence of a catalytic amount of sodium hydride afforded a mixture of the trichloroacetimidates **3α** and **3β**. However, the addition of excess of sodium hydride resulted in quantitative isomerisation of **3β** into the thermodynamically more stable anomer **3α**, which was isolated[8] crystalline (90% yield). The β-trichloroacetimidate **3β** was obtained crystalline (86%) as the kinetic product when potassium carbonate was used instead of sodium hydride.

Reaction of **3β** with the serine derivative **4**[12], catalysed by trimethylsilyl trifluoromethanesulfonate, gave exclusively the α-glycoside **5**, the structure of which was assigned on the basis of a comparison of the ^{1}H-n.m.r. data with published data[8].



In order to obtain an unusual glycosphingolipid from MDP and ceramide, the efficient azidosphingosine glycosylation procedure[13–15] was selected. The reaction of **3***α* and azidosphingosine **6**, catalysed by boron trifluoride etherate, furnished exclusively the *β*-glycoside **7** in high yield, and the *β* configuration was assigned on the basis of the ^{1}H-n.m.r. data (δ 4.24, d, $J_{1',2'}$ 7.6 Hz, H-1). Saponification of **7** with sodium hydroxide in aqueous 1,4-dioxane provided the carboxylic acid **8**, which was linked immediately to the L-Ala-D-iso-Gln derivative **9** (ref. 16), after treatment with *N*-hydroxysuccinimide–dicyclohexylcarbodi-imide[10,17] in tetrahydrofuran, to furnish the MDP derivative **10**. When this reaction was performed in dichloromethane–methanol, the methyl ester derivative **11** was formed. Reduction of the azide groups in **10** with hydrogen sulfide in aqueous pyridine[14,18] gave the diamine **12**, reaction of which with palmitoyl chloride in the presence of sodium acetate provided the *N*-palmitoyl derivative **13**. *O*-De-isopropy-

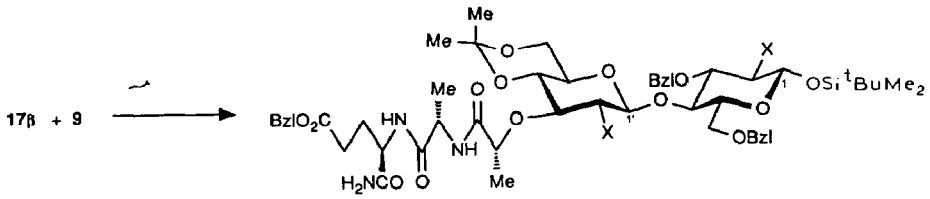


lidenation of **13** by treatment with trifluoroacetic acid then furnished the MDP glycosphingolipid **14** in good overall yield.

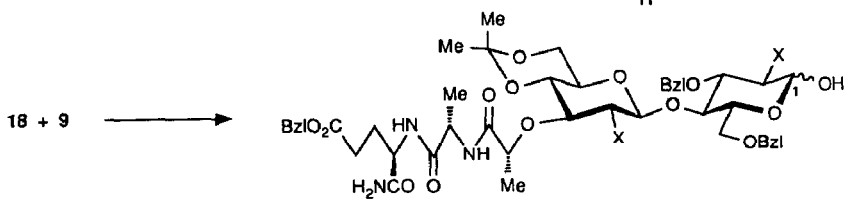
β-Glycosylation of the equatorial HO-4 of 2-amino-2-deoxyglucose derivatives frequently results in low yields and poor diastereoselectivity due to the low reactivity of the acceptor[8,19]. This phenomenon is particularly evident for glycosyl donors with participating protective groups[20] and acceptors[21] with an acyl group at position 6.

15

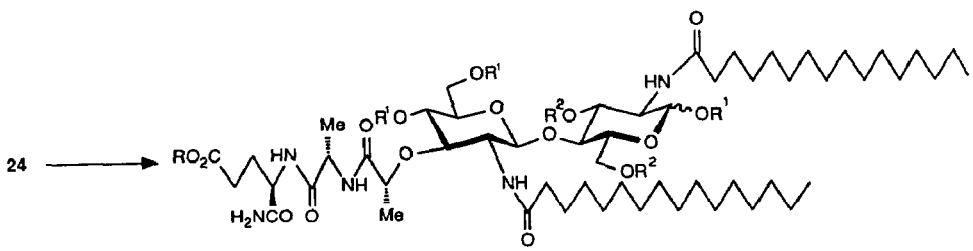
16β R = Me, X = H, Y = OSi^tBuMe_2
17β R = X = H, Y = OSi^tBuMe_2
18 R = H, X, Y = H, OH



19 X = N_3
20 X = NH_2
21 X = $NCOC_{15}H_{31}$
H



22 X = N_3
23 X = NH_2
24 X = $NCOC_{15}H_{31}$
H



25 R = R^2 = Bzl, R^1 = H
26 R = Me, R^1 = R^2 = H
27 R = Me, R^1 = R^2 = Ac

Reaction of **3***a* and the 2-amino-2-deoxyglucose derivative **15** (refs. 8, 21), catalysed by boron trifluoride etherate at ambient temperature, gave an acceptable yield of the *β*-(1→4)-linked disaccharide derivative **16***β*[21]. The structural assignment of **16***β* was based on the ^{1}H-n.m.r. data [δ 4.34 (d, $J_{1,2}$ 7.8 Hz, H-1) and 4.48 (d, $J_{1',2'}$ 7.6 Hz, H-1')]. Saponification of the methyl ester moiety in **16***β* with sodium hydroxide in aqueous 1,4-dioxane led to partial loss of the *tert*-butyldimethylsilyl group, so that **17***β* and **18** were obtained. Each compound was reacted immediately, either separately or in admixture, with the dipeptide derivative **9**, as described above, to give the disaccharide derivatives **19** and **22**, respectively. These compounds could be separated readily when a mixture of **17***β* and **18** was used in the reaction. Compounds **19** and **22** were transformed into the *N*-palmitoyl derivatives **21** and **24**, respectively, *via* the diamine intermediates **20** and **23**. Removal of ^{t}BuMe$_2$Si-1 from **21** with tetrabutylammonium fluoride in dichloromethane gave **24**, *O*-de-isopropylidenation of which with trifluoroacetic acid afforded **25**. Hydrogenolysis of **25** in methanol over Pd–C resulted in concomitant transesterification to give the MDP-containing glycolipid **26**. Acetylation of **26** furnished **27**, the ^{1}H-n.m.r. data of which indicated it to be mainly the *α* anomer.

EXPERIMENTAL

General methods. — Melting points are uncorrected. ^{1}H-N.m.r. spectra (internal Me$_4$Si) were recorded with Bruker WM 250 Cryospec and Jeol JNM-GX 400 instruments. R_F values refer to t.l.c. on silica gel (Merck). Column chromatography was carried out on silica gel (Merck 70–230 mesh ASTM and 230–400 mesh ASTM for flash chromatography under normal pressure, and Merck LiChroprep Si 60, 40–60 μm, for medium pressure operation). Light petroleum refers to the fraction b.p. 35–60°. Optical rotations were determined with a Perkin–Elmer 241 MC polarimeter.

*2-Azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(1*R*)-1-(methoxycarbonyl)ethyl]-β-*D*-glucopyranosyl trichloroacetimidate* (**3***β*). — To a solution of **2** (ref. 8) (5 g, 15.1 mmol) in dry dichloromethane (75 mL) was added potassium carbonate (10 g) and trichloroacetonitrile (6 mL). The mixture was stirred at room temperature for 5 h, then filtered through Celite, and concentrated under reduced pressure. Short-column chromatography (3:1 light petroleum–methyl acetate) of the residue on silica gel followed by recrystallisation from ether–light petroleum yielded **3***β* (6.2 g, 86%), m.p. 156°, $[\alpha]_D^{22}$ −19° (*c* 1, chloroform); R_F 0.49 (3:1 light petroleum–methyl acetate). ^{1}H-N.m.r. data ($CDCl_3$, 250 MHz). δ 8.83 (s, 1 H, NH), 5.63 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.46 (q, 1 H, *J* 7.0 Hz, MeC*H*), 3.97 (dd, 1 H, $J_{5,6e}$ 5.5, $J_{6a,6e}$ 10.7 Hz, H-6*e*), 3.80 (dd, 1 H, $J_{5,6a}$ 10.4 Hz, H-6*a*), 3.78 (s, 3 H, OMe), 3.75 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-4), 3.64 (dd, 1 H, $J_{2,3}$ 8.6 Hz, H-2), 3.62 (dd, 1 H, H-3), 3.36 (ddd, 1 H, $J_{4,5}$ 9.8 Hz, H-5), 1.51 (s, 3 H, CMe), 1.43 (d, 3 H, *Me*CH), 1.41 (s, 3 H, CMe).

The n.m.r. data agree with those reported[8].

*O-{2-Azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(1*R*)-1-(methoxycarbonyl)ethyl]-α-*D*-glucopyranosyl}-*N*-benzyloxycarbonyl-*L*-serine benzyl ester* (**5**). — A solution of **3***β* (2.2 g, 4.63 mmol) and **4** (3.63 g, 11 mmol) in dry ether containing powdered 4

Å molecular sieves and under nitrogen was stirred at room temperature for 2 h. The temperature was lowered to −20° and 0.01M trimethylsilyl trifluoromethanesulfonate in dry ether (40 mL, 0.4 mmol) was added dropwise. After 45 min, sodium hydrogen carbonate (2 g) was added, and the mixture was filtered and concentrated under reduced pressure. Short-column chromatography (2:1 light petroleum–methyl acetate) of the residue on silica gel and recrystallisation from ether–light petroleum afforded **5** (2.7 g, 91%), m.p. 81°, $[\alpha]_D^{22}$ +93° (*c* 1, chloroform); R_F 0.39. ^{1}H-N.m.r. data ($CDCl_3$, 250 MHz): δ 7.33–7.36 (m, 10 H, 2 Ph), 5.79 (d, 1 H, $J_{CH,NH}$ 8.24 Hz, NH), 5.22 (ABq, 2 H, CH_2Ph), 5.13 (ABq, 2 H, CH_2Ph), 4.74 (d, 1 H, $J_{1,2}$ 3.71 Hz, H-1), 4.55 (m, 1 H, Ser α-H), 4.50 (q, 1 H, $J_{6,7}$ Hz, MeC*H*), 4.00 (m, 2 H), 3.87–3.56 (m, 6 H), 3.76 (s, 3 H, OMe), 3.11 (dd, 1 H, $J_{2,3}$ 10.1, $J_{1,2}$ 3.97 Hz, H-2), 1.49 (s, 3 H, CMe), 1.41 (d, 3 H, *Me*CH), 1.40 (s, 3 H, CMe).

Anal. Calc. for $C_{31}H_{38}N_4O_{11}$ (642.7): C, 57.94; H, 5.96; N, 8.72. Found: C, 57.95; H, 5.99; N, 8.75.

(2S,3R,4E)-2-Azido-1-{2-azido-2-deoxy-4,6-O-isopropylidene-3-O-[(1R)-1-(methoxycarbonyl)ethyl]-β-D-glucopyranosyloxy}-3-benzoyloxy-4-octadecene (**7**). — A solution of **3***a* (1.28 g, 2.69 mmol) and **6**[14] (1 g, 2.33 mmol) in dry dichloromethane (50 mL) containing powdered 4 Å molecular sieves was stirred under nitrogen at room temperature for 3 h. 0.02M $BF_3{\cdot}Et_2O$ in dry dichloromethane (50 mL, 1 mmol) was added dropwise during 1 h. After 24 h, sodium hydrogen carbonate (5 g) was added, and the mixture was filtered and concentrated under reduced pressure. Column chromatography (6:1 light petroleum–methyl acetate) of the residue yielded **7** (1.47 g, 85%), isolated as a colourless oil, $[\alpha]_D^{20}$ −47° (*c* 2.9, dichloromethane); R_F 0.54 (3:1 light petroleum–methyl acetate). ^{1}H-N.m.r. data ($CDCl_3$, 250 MHz): δ 8.06 (m, 2 H, Ph), 7.57 (m, 1 H, Ph), 7.45 (m, 2 H, Ph), 5.96 (m, 1 H, BzOCHCH=C*H*), 5.58 (m, 2 H, BzOC*H*C*H*=CH), 4.43 (q, 1 H, *J* 6.8 Hz, MeC*H*), 4.24 (d, 1 H, *J* 7.6 Hz, H-1), 3.99–3.61 (m, 6 H, H-3,4,6*a*,6*e* and sphingosine CH_2O), 3.77 (s, 3 H, OMe), 3.42 (m, 2 H, H-2,2′), 3.14 (ddd, 1 H, $J_{4,5}$ 9.8 Hz, H-5), 2.07 (m, 2 H, C=CHCH_2), 1.48 (s, 3 H, CMe), 1.40 (d, 3 H, *Me*CH), 1.39 (s, 3 H, CMe), 1.24 [bs, 22 H, $(CH_2)_{11}$], 0.88 (t, 3 H, sphingosine CH_3).

Anal. Calc. for $C_{38}H_{58}N_6O_9$ (742.9): C, 61.44; H, 7.87; N, 11.31. Found: C, 61.49; H, 8.03; N, 11.0.

(2S,3R,4E)-2-Azido-1-{2-azido-3-O-[(1R)-1-carboxyethyl]-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyloxy}-4-octadecen-3-ol (**8**). — A solution of **7** (1.32 g, 1.78 mmol) in 1,4-dioxane (30 mL) was stirred with M potassium hydroxide (3 mL) at room temperature for 2 d, then neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated under reduced pressure. Short-column chromatography (9:1 chloroform–methanol) of the residue on silica gel yielded **8** (956 mg, 86%); R_F 0.56. ^{1}H-N.m.r. data ($CDCl_3$, 250 MHz): δ 5.75–5.84 (m, 1 H, H_2CC*H*=C), 5.50 (dd, 1 H, CH_2CH=C*H*), 4.41–4.48 (m, 2 H), 3.66–4.01 (m, 9 H), 3.42–3.51 (m, 1 H), 3.20–3.31 (m, 2 H), 2.07 (m, 2 H, C=CHCH_2), 1.51 (s, 3 H, CMe), 1.46 (d, 3 H, *J* 7.02 Hz, *Me*CH), 1.40 (s, 3 H, CMe), 1.26 [bs, 22 H, $(CH_2)_{11}$], 0.84 (t, 3 H, sphingosine CH_3). This product was used immediately in the next step.

(2S, 3R, 4E)-2-Azido-1-{2-azido-2-deoxy-4,6-O-isopropylidene-3-O-[(2R)-pro-

panoyl-(L-alanyl-D-isoglutamine benzyl ester)-2-yl]-β-D-glucopyranosyloxy}-4-octadecen-3-ol (**10**). — To a solution of **8** (174.5 mg, 0.28 mmol) in dry tetrahydrofuran (5 mL) was added *N*-hydroxysuccinimide (32.1 mg, 0.28 mmol) and dicyclohexylcarbodi-imide (57.6 mg, 0.28 mmol). The mixture was stirred 45 min at room temperature, cooled to 0°, and treated with a mixture of L-alanyl-D-isoglutamine benzyl ester hydrochloride[16] (104.1 mg, 0.3 mmol) and triethylamine (30.3 mg, 0.3 mmol) in tetrahydrofuran (7 mL) that had been sonicated for 10 min and cooled to 0°. The mixture was allowed to attain room temperature slowly, and, after 20 h, diluted with ether (50 mL), and washed with water (1 × 10 mL, 2 × 5 mL). The aqueous layer was re-extracted with ether, and the combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by m.p.l.c. (15:1 dichloromethane–methanol) yielded **10** (188.4 mg, 74%), isolated as a colourless oil, $[\alpha]_D^{20}$ −17° (*c* 0.55, dichloromethane); R_F 0.66 (9:1 chloroform–methanol). ^{1}H-N.m.r. data ($CDCl_3$, 400 MHz): δ 7.82 (d, 1 H, $J_{CH,NH}$ 5.86 Hz, Ala NH), 7.33–7.37 (m, 5 H, Ph), 7.13 (d, 1 H, $J_{CH,NH}$ 8.06, Gln NH), 6.79 (bs, 1 H, $CONH_2$), 5.8 (m, 1 H, $CH_2CH=C$), 5.5 (dd, 1 H, $CH_2CH=CH$), 5.4 (bs, 1 H, $CONH_2$), 5.13 (2 d, 2 H, CH_2Ph), 4.46 (m, 2 H, Gln α-H, H-1), 4.33 (q, 1 H, *J* 6.83 Hz, CH_3CH), 4.25 (dd, 1 H, $J_{5,6a}$ 0.9, $J_{6a,6e}$ 7.6 Hz, H-6*a*), 4.19 (dq, 1 H, Ala α-H), 3.97 (dd, 1 H, sphingosine CH_2O), 3.92 (dd, 1 H, sphingosine CH_2O), 3.80 (m, 2 H), 3.67 (m, 2 H), 3.44 (m, 2 H, H-2 and MeC*H*), 3.21 (m, 2 H, H-5), 2.58–2.64 (m, 1 H, Gln γ-CH_2), 2.52–2.44 (m, 1 H, Gln γ-CH_2), 2.24 (m, 1 H, Gln β-H), 2.05 (m, 3 H, $C=CHCH_2$ and Gln β-H), 1.5 (s, 3 H, CMe), 1.42 (d, 3 H, *J* 7.08 Hz, *Me*CH), 1.26 [bs, 22 H, $(CH_2)_{11}$], 0.88 (t, 3 H, *J* 6.59 Hz, sphingosine CH_3).

Anal. Calc. for $C_{45}H_{71}N_9O_{11}$ (914.1): C, 59.13; H, 7.83; N, 13.79. Found: C, 59.11; H, 7.70; N, 13.72.

(2S, 3R, 4E)-2-Azido-1-{2-azido-2-deoxy-4,6-O-isopropylidene-3-O-[(2R)-propanoyl-(L-alanyl-D-isoglutaminemethylester)-2-yl-β-D-glucopyranosyloxy}-4-octadecen-3-ol (**11**). — To a solution of **8** (624.78 mg, 1.0 mmol) in dry dichloromethane (30 mL) was added dicyclohexylcarbodi-imide (252 mg, 2.0 mmol) and *N*-hydroxysuccinimide (326 mg, 2.0 mmol). The mixture was stirred at room temperature for 1 h. The hydrochloride of **9** (380 mg, 1.1 mmol) in dry methanol was treated with Amberlite IRA-400 (HO^-) resin for 0.5 h. The mixture was filtered and concentrated under reduced pressure, and a solution of the free base in dichloromethane (40 mL) was added dropwise at −10° to the above solution of the activated ester. The mixture was allowed to attain room temperature slowly and, after 20 h, washed with water (3 × 15 mL). The aqueous layer was re-extracted with dichloromethane, and the combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (30:1 dichloromethane–methanol) of the residue yielded **11** (511 mg, 61%), isolated as a colourless oil, $[\alpha]_D^{20}$ −15.5° (*c* 2.85, methanol); R_F 0.65 (15:1 chloroform–methanol). ^{1}H-N.m.r. data ($CDCl_3$, 400 Hz): 7.87 (d, 1 H, $J_{CH,NH}$ 6.11 Hz, Ala NH), 7.31 (d, 1 H, $J_{CH,NH}$ 8.06 Hz, Gln NH), 6.87 (bs, 1 H, $CONH_2$), 5.8 (m, 1 H, $CH_2CH=C$), 5.67 (bs, 1 H, $CONH_2$), 5.51 (dd, 1 H, $CH_2CH=CH$), 4.48 (m, 2 H, Gln α-H and H-1), 4.32 (m, 2 H, MeC*H* and H-6*a*), 4.19 (dq, 1 H, Ala α-H), 3.97 (dd, 1 H, CH_2O), 3.92 (dd, 1 H, CH_2O), 3.78 (m, 2 H), 3.69 (s, 3 H, OMe), 3.66 (m, 2 H), 3.45 (m, 1 H, H-2), 3.22 (m, 2 H,

H-2,5), 2.51–2.59 (m, 1 H, Gln γ-H), 2.39–2.47 (m, 1 H, Gln γ-H), 2.18–2.27 (m, 1 H, Gln β-H), 1.96–2.09 (m, 3 H, Gln β-H, HC=CHCH_2), 1.51 (s, 3 H), 1.45 (d, 3 H, *J* 6.83 Hz, *Me*CH), 1.38 (s, 3 H, CMe), 1.37 (d, 3 H, Ala CH_3), 1.26 [bs, 22 H, $(CH_2)_{11}$], 0.88 (t, 3 H, *J* 6.84 Hz, sphingosine CH_3).

Anal. Calc. for $C_{39}H_{67}N_9O_{11}$ (838.0): C, 55.90; H, 8.06; N, 15.04. Found: C, 55.84; H, 8.05; N, 15.11.

(2S, 3R, 4E)-2-Amino-1-{2-amino-2-deoxy-4,6-O-isopropylidene-3-O-[(2R)-propanoyl-(L-alanyl-D-isoglutamine benzyl ester)-2-yl]-β-D-glucopyranosyloxy}-4-octadecen-3-ol (**12**). — Hydrogen sulfide was passed through a solution of **10** (172 mg, 0.19 mmol) in 1:1 pyridine–water (7 mL) for 15 min. The mixture was stirred for 24 h and concentrated under reduced pressure, and toluene was evaporated from the residue of crude **12**, R_F 0.1–0.54 (1:1 chloroform–methanol), which was used in the next step without further purification.

*(2S, 3R, 4E)-1-{2-deoxy-2-hexadecanoylamino-4,6-O-isopropylidene-3-O-[(2R)-propanoyl-(*L-alanyl-D-*isoglutamine benzyl ester)-2-yl]-β-D-glucopyranosyloxy}-2-hexadecanoylamino-4-octadecen-3-ol* (**13**). — To a solution of crude **12**, obtained from **10** (172 mg, 0.19 mmol), in tetrahydrofuran (15 mL) were added aqueous 50% sodium acetate (15 mL) and palmitoyl chloride (0.16 mL, 0.57 mmol) with vigorous shaking. After 1 h, the organic layer was separated, the aqueous layer was extracted with ether (3 × 50 mL), and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. M.p.l.c. (60:1 dichloromethane–methanol) of the residue yielded **13** (155.19 mg, 61% from **10**), m.p. 173°, $[\alpha]_D^{20}$ −9.5° (*c* 0.37, chloroform); R_F 0.45 (9:1 dichloromethane–methanol). ^{1}H-N.m.r. data ($CDCl_3$, 400 MHz): δ 7.29 (d, 1 H, $J_{CH,NH}$ 8.05 Hz, NH), 6.94 (d, 1 H, $J_{CH,NH}$ 6.59 Hz, NH), 6.78 (d, 1 H, $J_{CH,NH}$ 7.57 Hz, NH), 6.70 (bs, 1 H, $CONH_2$), 6.35 (d, 1 H, $J_{CH,NH}$ 7.81 Hz, NH), 5.77 (bs, 1 H, $CONH_2$), 5.70 (m, 1 H, CH_2=C*H*–C), 5.45 (dd, 1 H, CH_2CH=C*H*), 5.14 (ABq, 2 H, CH_2Ph), 4.55 (d, 1 H, $J_{1,2}$ 7.82 Hz, H-1), 4.49 (m, 1 H, Gln α-H), 4.28 (dq, 1 H, Ala α-H), 4.19 (q, 1 H, *J* 6.59 Hz, MeC*H*), 4.1 (bs, 1 H), 4.03 (bs, 1 H), 3.97 (dd, 1 H, sphingosine CH_2O), 3.89 (dd, 1 H, sphingosine CH_2O), 3.61–3.78 (m, 5 H), 3.24 (m, 2 H, H-5), 2.58–2.66 (m, 1 H, Gln γ-CH_2), 2.45–2.53 (m, 1 H, Gln γ-CH_2), 2.12–2.22 (m, 5 H, Gln β-H and 2 $COCH_2$), 1.99–2.04 (m, 3 H, Gln β-H and C=CH–CH_2), 1.69 (m, 2 H, $COCH_2$), 1.6 (m, 2 H, $COCH_2CH_2$) 1.5 (s, 3 H, CMe), 1.25–1.39 [m, 79 H, $(CH_2)_{11}$, 2 $(CH_2)_{12}$, *Me*CH, CMe, and Ala CH_3], 0.88 (t, 9 H, 2 palmitoyl CH_3 and sphingosine CH_3).

Anal. Calc. for $C_{77}H_{135}N_5O_{13}$ (1339.0): C, 69.07; H, 10.16; N, 5.23. Found: C, 68.85; H, 10.11; N, 4.88.

*(2S, 3R, 4E)-1-{2-Deoxy-2-hexadecanoylamino 3-O-[(2R)-propanoyl-(*L-*alanyl-*D-*isoglutamine benzyl ester)-2-yl]-β-*D-glucopyranosyloxy}-*2-hexadecanoylamino-4-octadecen-3-ol* (**14**). — To a stirred solution of **13** (97.2 mg, 72.6 μmol) in dichloromethane (100 mL) was added aqueous 60% trifluoroacetic acid (1.5 mL). After 2 h, the mixture was treated with an excess of sodium hydrogen carbonate, filtered, and concentrated under reduced pressure. Short-column chromatography (15:1 dichloromethane–methanol) of the residue on silica gel yielded **14** (90.8 mg, 95%), m.p. 177°, $[\alpha]_D^{20}$ −2.5° (*c* 0.18, methanol); R_F 0.25 (9:1 dichloromethane–methanol). ^{1}H-N.m.r. data [$(CD_3)_2SO$,

400 MHz]: δ 8.08 (d, 1 H, $J_{CH,NH}$ 8.06 Hz, NH), 7.7 (d, 1 H, $J_{CH,NH}$ 8.55 Hz, NH), 7.53 (d, 1 H, $J_{CH,NH}$ 6.59 Hz, NH), 7.34 (bs, 6 H, Ph, $CONH_2$), 7.09 (bs, 1 H, $CONH_2$), 6.98 (d, 1 H, $J_{CH,NH}$ 8.54 Hz, NH), 5.51 (m, 1 H, $CH_2CH=CH$), 5.36 (dd, 1 H, $CH_2CH=CH$), 5.36 (dd, 1 H, $CH_2CH=CH$), 5.25 (d, 1 H, J 6.35 Hz, HO-4), 5.08 (bs, 2 H, CH_2Ph), 4.81 (d, 1 H, $J_{1,2}$ 5.13 Hz), 4.56 (t, 1 H, HO-6), 4.34 (d, 1 H, J 8.06 Hz, H-1), 4.14–4.26 (m, 3 H, Gln α-H, Ala α-H, and MeCH), 3.16–3.85 (m, 10 H, sphingosine CH_2), H-2,2',3,4,5,6*a*,6*e*, and sphingosine OH), 2.37 (m, 2 H, Gln γ-CH_2), 2.04 (m, 5 H, Gln β-H and 2 $COCH_2$), 1.94 (m, 2 H, $C=CHCH_2$), 1.80 (m, 1 H, Gln β-H), 1.46 (bs, 4 H, 2 $COCH_2CH_2$), 1.24 [m, 76 H, 2 $(CH_2)_{12}$, $(CH_2)_{11}$, *Me*CH, and Ala CH_3], 0.86 (m, 9 H, 3 CH_3).

Anal. Calc. for $C_{74}H_{131}N_5O_{13}\cdot H_2O$ (1316.91): C, 67.49; H, 10.18; N, 5.32. Found: C, 67.52; H, 10.23; N, 5.20.

tert-*Butyldimethylsilyl 2-azido-4*-O-{*2-azido-2-deoxy-4,6*-O-*isopropylidene-3*-O-*[(1*R*)-1-(methoxycarbonyl)ethyl]-β*-D-glucopyranosyl}-*3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**16***β*). — To a solution of **3***α* (1.19 g, 2.5 mmol) and **15** (1 g, 2 mmol) in dry dichloromethane (50 mL) was added 0.025M $BF_3\cdot OEt_2$ in dichloromethane (20 mL, 0.5 mmol). After 14 h, sodium hydrogen carbonate (2.5 g) was added and the mixture was worked-up as described for **7**. M.p.l.c. (3:2 light petroleum–ether) of the product yielded **16***β* (617 mg, 38%), m.p. 98°, $[\alpha]_D^{20}$ −33° (*c* 1.4, methanol); R_F 0.6. ^{1}H-N.m.r. data ($CDCl_3$, 400 MHz): δ 7.28–7.39 (m, 10 H, 2 Ph), 4.78 (ABq, 2 H, CH_2Ph), 4.6 (ABq, 2 H, CH_2Ph), 4.48 (d, 1 H, $J_{1,2}$ 7.57 Hz, H-1), 4.4 (q, 1 H, J 6.83 Hz, MeCH), 4.34 (d, 1 H, J 7.81 Hz, H-1), 4.0 (m, 1 H, H-5'), 3.91 (dd, 1 H, $J_{6a,6e}$ 11.23, $J_{5,6e}$ 3.42 Hz, H-6*e*'), 3.72 (dd, 1 H, $J_{6a,6e}$ 11.23 Hz, H-6*e*), 3.66 (dd, 1 H, $J_{6a,6e}$ 10.99, $J_{5,6a}$ 5.37 Hz, H-6*a*'), 3.53 (dd, 1 H, $J_{6a,6e}$ 9.15, $J_{5,6a}$ 9.28 Hz, H-6*a*), 3.24–3.42 (m, 6 H, H-2,2',3,3',4,4'), 2.88 (m, 1 H, H-5), 1.44 (s, 3 H, CMe), 1.4 (d, 3 H, *Me*CH), 1.36 (s, 3 H, CMe), 0.93 (s, 9 H, ^{t}Bu), 0.16 (s, 3 H, SiMe), 0.15 (s, 3 H, SiMe).

Anal. Calc. for $C_{39}H_{56}N_6O_{11}Si$ (813.0): C, 57.62; H, 6.94; N, 10.34. Found: C, 57.94; H, 7.03; N, 10.42.

tert-*Butyldimethylsilyl 2-azido-4*-O-{*2-azido-3*-O*[(1*R*)-1-carboxyethyl]-2-deoxy-4,6*-O-*isopropylidene-β*-D-*glucopyranosyl}-*3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**17***β*) *and 2-azido-4*-O-{*2-azido*-3-O-*[(1*R*)-1-carboxyethyl-2-deoxy-4,6*-O-*isopropylidene-β*-D-*glucopyranosyl}-*3,6-di*-O-*benzyl-2-deoxy*-D-*glucopyranose* (**18**). — To a stirred solution of **16***β* (500 mg, 0.615 mmol) in 1,4-dioxane (15 mL) was added 0.1M potassium hydroxide (8 mL). T.l.c. (9:1 chloroform–methanol) after 15 min revealed ~90% of **17***β* (R_F 0.56) and a small proportion of **18** (R_F 0.46) and, after 1 h, a 1:1 mixture. The mixture was treated with Amberlite IR-120 (H^+) resin, filtered, and concentrated *in vacuo*. The components of the mixture could be isolated by m.p.l.c. (15:1 dichloromethane–methanol), but separation at the next stage (**19** and **22**) was easier. Therefore, only small quantities were purified. ^{1}H-N.m.r. data ($CDCl_3$, 400 MHz): **17***β*, δ 7.34 (m, 10 H, 2 Ph), 4.77 (d, ABq, 3 H, H-1 and CH_2Ph), 4.50–4.36 (m, 4 H, CH_2Ph, H-1, MeCH), 4.06 (m, 1 H, H-5'), 3.84 (dd, 1 H, $J_{6a,6e}$ 8.3 Hz, H-6*e*'), 3.70 (m, 3 H), 3.51 (dd, 1 H, H-6*a*), 3.42–3.26 (m, 4 H), 2.99 (m, 1 H), 2.88 (m, 1 H, H-5), 1.44 (s, 3 H, CMe), 1.44 (d, 3 H, *Me*CH), 1.37 (s, 3 H, CMe), 0.94 (s, 9 H, ^{t}Bu), 0.16 (s, 3 H, SiMe), 0.15 (s, 3 H, SiMe); **18**, δ 7.34 (m, 10 H, 2 Ph), 5.30 (d, 1 H, $J_{1,2}$ 3.17 Hz, H-1), 4.92–2.80 (m, 47 H), 1.44 (d, 3 H, *Me*CH), 1.42 (s, 3 H, CMe), 1.38 (s, 3 H, CMe).

tert-*Butyldimethylsilyl 2-azido-4-O-{2-azido-2-deoxy-4,6-O-isopropylidene-3-O-[(2R)-propanoyl-(L-alanyl-D-isoglutamine benzyl ester)-2-yl]-β-D-glucopyranosyl}-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside* (**19**) *and 2-azido-4-O-{2-azido-2-deoxy-4,6-O-isopropylidene-3-O-[(2R)-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-2-yl]-β-D-glucopyranosyl}-3,6-di-O-benzyl-2-deoxy-D-glucopyranose* (**22**). — To a solution of a 1:1 mixture of **17**β and **18** (386 mg, ~0.52 mmol) in dry tetrahydrofuran (12 mL) was added *N*-hydroxysuccinimide (115.1 mg, 1 mmol) and dicyclohexylcarbodi-imide (206.33 mg, 1 mmol). According to the procedure described for the preparation of **10**, L-alanyl-D-isoglutamine benzyl ester hydrochloride (208.2 mg, 0.6 mmol) and triethylamine (60.6 mg, 0.6 mmol) in tetrahydrofuran (20 mL) were added. M.p.l.c. (30:1 dichloromethane–methanol) yielded **19** (203.7 mg, 36%), m.p. 78°, $[\alpha]_D^{20}$ −6.7° (*c* 1.98, methanol); R_F 0.78 (9:1 chloroform–methanol); and **22** (197.5 mg, 39%), m.p. 87°, $[\alpha]_D^{20}$ +10° (after 24 h) (*c* 0.8, chloroform); R_F 0.59. ^{1}H-N.m.r. data ($CDCl_3$, 400 MHz): **19**, δ 7.72 (d, 1 H, $J_{CH,NH}$ 6.1 Hz, Ala NH), 7.25–7.71 (m, 16 H, 3 Ph, Gln NH), 6.76 (bs, 1 H, $CONH_2$), 5.6 (bs, 1 H, $CONH_2$), 5.13 (ABq, 2 H, CH_2Ph), 4.78 (ABq, 2 H, CH_2Ph), 4.6 (ABq, 2 H, CH_2Ph), 4.47 (m, 3 H, Gln α-H and H-1,1'), 4.34 (dd, 1 H, H-6*a*), 4.26 (q, 1 H, *J* 6.8 Hz, MeC*H*), 4.11 (m, 1 H, H-5'), 3.82 (dd, 1 H, $J_{6a,6e}$ 11.23, $J_{5,6e}$ 2.93 Hz, H-6*e*'), 3.71–3.77 (m, 2 H), 3.50 (dd, 1 H, H-6*a*), 3.42 (m, 2 H), 3.32 (m, 2 H), 3.25 (m, 1 H), 2.94 (m, 2 H, H-5), 2.57–2.63 (m, 1 H, Gln γ-CH_2), 2.46–2.51 (m, 1 H, Gln γ-CH_2), 2.25 (m, 1 H, Gln β-H), 2.03 (m, 1 H, Gln β-H), 1.45 (s, 3 H, CMe), 1.41 (d, 3 H, *J* 6.83 Hz, *Me*CH), 1.36 (s, 3 H, CMe), 1.35 (d, 3 H, Ala CH_3), 0.94 (s, 9 H, ^{t}Bu), 0.17 (s, 3 H, SiMe), 0.16 (s, 3 H, SiMe).

Anal. Calc. for $C_{53}H_{73}N_9O_{14}Si$ (1088.3): C, 58.49; H, 6.76; N, 11.58. Found: C, 58.50; H, 6.69; N, 15.54.

Compound **22**, ^{1}H-n.m.r. data: δ 7.68 (m, 1 H, Ala NH), 7.3–7.4 (m, 15 H, 3 Ph), 7.19 (d, 1 H, $J_{CH,NH}$ 8.06 Gln NH), 6.73 (bs, 1 H, $CONH_2$), 5.6 (bs, 1 H, $CONH_2$), 5.13 (ABq, 2 H, CH_2Ph), 4.95–3.21 (m, 28 H), 2.86 (m, 2 H, H-5), 2.61 (m, 1 H, Gln γ-CH_2), 2.48 (m, 1 H, Gln γ-CH_2), 2.23 (m, 1 H, Gln β-H), 2.03 (m, 1 H, Gln β-H), 1.43 (s, 3 H, CMe), 1.4 (d, 3 H, *Me*CH), 1.36 (s, 3 H, CMe), 1.34 (d, 3 H, Ala CH_3).

Anal. Calc. for $C_{47}H_{67}N_9O_{14}$ (974.1): C, 57.96; H, 6.11; N, 12.94. Found: C, 57.64; H, 6.06; N, 12.79.

tert-*Butyldimethylsilyl 2-amino-4-O-{2-amino-2-deoxy-4,6-O-isopropylidene-3-O-[(2R)-propanoyl-(L-alanyl-D-isoglutamine benzyl ester)-2-yl]-β-D-glucopyranosyl}-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside* (**20**). — Hydrogen sulfide was passed through a solution of **19** (186 mg, 0.17 mmol) in 1:1 pyridine–water (7 mL) for 15 min. The mixture was stirred for 24 h and worked-up, as described for **12**, to give crude **20**, R_F 0.54 (9:1 chloroform–methanol), which was used in the next step without further purification.

tert-*Butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-4-O-{2-deoxy-2-hexadecanoylamino-4,6-O-isopropylidene-3-O-[(2R)-propanoyl-(L-alanyl-D-isoglutamine benzyl ester)-2-yl]-β-D-glucopyranosyl}-2-hexadecanoylamino-β-D-glucopyranoside* (**21**). — Crude **20**, obtained from **19** (186 mg, 0.17 mmol), was shaken vigorously with aqueous 50% sodium acetate (15 mL) and palmitoyl chloride (0.15 mL, 0.51 mmol) as described

above for **13**. M.p.l.c. (60:1 dichloromethane–methanol) of the product yielded **21** (162 mg, 63% over both steps), m.p. 86°, $[\alpha]_D^{20}$ −7.4° (*c* 1.96, methanol); R_f 0.34 (15:1 dichloromethane–methanol). ^{1}H-N.m.r. data ($CDCl_3$, 400 MHz): δ 7.24–7.44 (m, 16 H, 3 Ph, Ala NH), 6.64 (d, 1 H, $J_{CH,NH}$ 6.84 Hz, Gln NH), 6.25 (d, 1 H, $J_{CH,NH}$ 8.55 Hz, palmitoyl NH), 6.2 (bs, 1 H, $CONH_2$), 6.15 (d, 1 H, $J_{CH,NH}$ 8.54 Hz, palmitoyl NH), 6.08 (bs, 1 H, $CONH_2$), 5.13 (ABq, 2 H, CH_2Ph), 4.9 (d, 1 H, $J_{1,2}$ 5.37 Hz, H-1), 4.62–4.71 (m, 3 H), 4.42–4.88 (m, 3 H), 4.3 (dd, 1 H, H-6*a*), 4.1 (q, 1 H, *J* 6.6 Hz, MeC*H*), 3.94 (m, 1 H), 3.72–3.86 (m, 8 H), 3.53–3.60 (m, 2 H), 3.17 (m, 1 H), 3.01 (m, 1 H, H-5), 2.53–2.60 (m, 1 H, Gln γ-CH_2), 2.42–2.46 (m, 1 H, Gln γ-CH_2), 2.08–2.35 (m, 5 H, Gln β-H and 2 $COCH_2$), 1.91–1.96 (m, 1 H, Gln β-H), 1.61 (m, 4 H, 2 $COCH_2CH_2$), 1.45 (s, 3 H, CMe), 1.25–1.39 [m, 57 H, 2 $(CH_2)_{12}$, *Me*CH, CMe, and Ala CH_3], 0.8–0.93 (m, 15 H, ^{t}Bu, 2 palmitoyl CH_3), 0.07 (2 s, 6 H, 2 SiMe).

Anal. Calc. for $C_{85}H_{137}N_5O_{16}Si$ (1513.2): C, 67.47; H, 9.13; N, 4.63. Found: C, 67.25; H, 9.40; N, 4.52.

*2-Amino-4-*O*-{2-amino-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(2*R*)-2-propanyloyl-(*L*-alanyl-*D*-isoglutamine benzyl ester)-2-yl]-β-*D*-glucopyranosyl}-3,6-di-*O*-benzyl-2-deoxy-*D*-glucopyranose* (**23**). — Hydrogen sulfide was passed through a solution of **22** (174 mg, 178.6 μmol) in 1:1 pyridine–water (7 mL) for 15 min. The mixture was stirred for 24 h and worked-up as described for **12**. Crude **23**, R_f 0.11–0.32 (9:1 chloroform–methanol), was used in the next step without further purification.

*3,6-Di-*O*-benzyl-2-deoxy-4-*O*-{2-deoxy-2-hexadecanoylamino-4,6-*O*-isopropylidene-3-*O*-[(2*R*)-propanoyl-(*L*-alanyl-*D*-isoglutamine benzyl ester)-2-yl]-β-*D*-glucopyranosyl}-2-hexadecanoylamino-*D*-glucopyranose* (**24**). — (*a*) A solution of crude **23**, obtained from **22** (174 mg, 178.6 μmol), in tetrahydrofuran (15 mL) was shaken vigorously with aqueous 50% sodium acetate (15 mL) and palmitoyl chloride (0.16 mL, 0.57 mmol) and then worked-up as described above for **13**. M.p.l.c. (35:1 dichloromethane–methanol) of the product yielded **24** (154.9 mg, 62% over both steps).

(*b*) To a stirred solution of **21** (114.8 mg, 75.87 μmol) in dichloromethane (5 mL) at −30° under nitrogen was added M tetrabutylammonium fluoride in tetrahydrofuran (0.5 mL, 0.5 mmol) dropwise. After 4 h, the mixture was allowed to attain room temperature, diluted with ether (200 mL), and washed with water (1 × 200 mL). The aqueous layer was re-extracted with ether (2 × 20 mL). The combined organic extracts were dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography (35:1 dichloromethane–methanol) of the residue yielded **24** (93.8 mg, 88.4%), m.p. 155°, $[\alpha]_D^{22}$ −3.9° (after 24 h) (*c* 1.8, chloroform); R_f 0.3 (15:1 dichloromethane–methanol). ^{1}H-N.m.r. data ($CDCl_3$, 400 MHz): δ 7.22–7.41 (m, 16 H, 3 Ph and Ala NH), 6.81 (d, 1 H, $J_{CH,NH}$ 6.6 Hz, Gln NH), 6.46 (bs, 1 H, $CONH_2$), 5.85 (d, 1 H, $J_{CH,NH}$ 8.79 Hz, palmitoyl NH), 5.24 (bs, 1 H, $CONH_2$), 5.12 (ABq, 2 H, CH_2Ph), 2.95–4.81 (22 H, 2 CH_2Ph, HO-1), MeC*H*, Ala α-H, and Gln α-H), 2.5–2.58 (m, 1 H, Gln γ-CH_2), 2.37–2.45 (m, 1 H, Gln γ-CH_2), 1.9–2.2 (m, 6 H, 2 Gln β-H and 2 $COCH_2R$), 1.53 (m, 4 H, 2 $COCH_2CH_2R$), 1.39 (s, 3 H, CMe), 1.37 (s, 3 H, CMe), 1.34 (d, 3 H, *J* 7.37 Hz, CH_3), 1.25 [m, 51 H, 2 $(CH_2)_{12}$, CH_3], 0.88 [t, 6 H, *J* 6.35 Hz, 2 palmitoyl CH_3).

Anal. Calc. for $C_{79}H_{123}N_5O_{16}$ (1398.9): C, 67.83; H, 8.86; N, 5.01. Found: C, 67.99; N, 8.87; N, 4.95.

3,6-Di-O-benzyl-2-deoxy-4-O-{2-deoxy-2-hexadecanoylamino-3-O-[(2R)-propanoyl-(L-alanyl-D-isoglutamine benzyl ester)-2-yl]-β-D-glucopyranosyl}-2-hexadecanoylamino-D-glucopyranose (**25**). — To a stirred solution of **24** (130 mg, 92.9 μmol) in dichloromethane (100 mL) was added aqueous 60% trifluoroacetic acid (1.5 mL). After 2 h, the mixture was worked-up as described above for **14**. Short-column chromatography (15:1 dichloromethane–methanol) of the product on silica gel yielded **25** (118.7 mg, 94%), m.p. 183°, $[\alpha]_D^{20}$ −2.9° →0° (24 h) (*c* 0.63, chloroform); R_F 0.48 (9:1 dichloromethane–methanol). ^{1}H-N.m.r. data [$(CD_3)_2SO$, 400 MHz]: δ 8.09 (d, 1 H, $J_{CH,NH}$ 8.06 Hz, NH), 7.79 (m, 2 H, NH, $CONH_2$), 6.66 (d, 1 H, $J_{CH,NH}$ 3.91 Hz, NH), 5.22 (d, 1 H, *J* 6.34 Hz, HO-4), 5.07 (bs, 2 H, CH_2Ph), 4.89 (m, 2 H), 4.52–4.6 (m, 4 H), 4.17–4.28 (m, 3 H), 4.08 (m, 1 H), 3.78–3.85 (m, 3 H), 3.67 (m, 5 H), 3.21–3.5 (m, 8 H), 3.05 (m, 1 H), 2.35 (m, 2 H, Gln γ-CH_2), 2.04 (m, 5 H, Gln β-H and 2 $COCH_2$), 1.77 (m, 1 H, Gln β-H), 1.24 [m, 54 H, 2 $(CH_2)_{12}$, *Me*CH, Ala CH_3), 0.86 (t, 6 H, *J* 6.59 Hz, 2 palmitoyl CH_3).

Anal. Calc. for $C_{76}H_{119}N_5O_{16}\cdot 0.5H_2O$ (1367.83): C, 66.74; H, 8.84; N, 5.12. Found: C, 66.74; H, 8.71; N, 4.94.

2-Deoxy-4-O-{2-deoxy-2-hexadecanoylamino-3-O-[(2R)-propanoyl-(L-alanyl-D-isoglutamine methyl ester)-2-yl]-β-D-glucopyranosyl}-2-hexadecanoylamino-D-glucopyranose (**26**). — A solution of **25** (37.7 mg, 27.7 μmol) and 10% Pd–C (20 mg) in dry methanol (2 mL) was stirred under hydrogen at room temperature. After 24 h, the mixture was diluted with methanol (20 mL), filtered, and concentrated *in vacuo* to yield **26** (25.6 mg) as a colourless oil, R_F 0.74 (5:1 chloroform–methanol).

1,3,6-Tri-O-acetyl-2-deoxy-4-O-{4,6-di-O-acetyl-2-deoxy-2-hexadecanoylamino-3-O-[(2R)-propanoyl-(L-alanyl-D-isoglutamine methyl ester)-2-yl]-β-D-glucopyranosyl}-2-hexadecanoylamino-D-glucopyranose (**27**). — A solution of **26** (12 mg, 11.0 μmol) in 1:1 pyridine–acetic anhydride (0.5 mL) was kept for 24 h, then concentrated *in vacuo*. Column chromatography (35:1 dichloromethane–methanol) of the residue yielded **27** (11.5 mg, 78%), m.p. 186°, $[\alpha]_D^{22}$ +7.1° (*c* 0.85, chloroform); R_F 0.5 (9:1 dichloromethane–methanol). ^{1}H-N.m.r. data ($CDCl_3$, 400 MHz): δ 7.19 (m, 1 H, NH), 7.02 (d, 1 H, $J_{CH,NH}$ 6.1 Hz, NH), 6.84 (m, 2 H, 2 NH), 6.1 (d, 1 H, *J* 3.66 Hz, H-1), 5.69 (m, 2 H, 2 NH), 5.22 (m, 1 H), 4.96 (m, 1 H), 4.80–3.50 (m, 14 H), 3.69 (s, 3 H, OMe), 2.53–2.59 (m, 1 H, Gln γ-CH_2), 2.39–2.46 (m, 1 H, Gln γ-CH_2), 2.2 (m, 1 H, Gln β-H), 2.06–2.17 (m, 20 H, 5 Ac, 2 $COCH_2$, and Gln β-H), 1.54 (m, 4 H, 2 $COCH_2CH_2$), 1.47 (d, 3 H, *J* 7.08 Hz, *Me*CH), 1.25 [m, 51 H, 2 $(CH_2)_{12}$, CH_3], 0.88 (t, *J* 6.6 Hz, 6 H, 2 palmitoyl CH_3).

Anal. Calc. for $C_{66}H_{113}N_5O_{21}\cdot 1.2H_2O$ (1334.28): C, 59.41; H, 8.72; N, 5.25. Found: C, 59.38; H, 8.41; N, 5.07.

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REFERENCES

1 D. E. S. Stewart-Tull and M. Daries (Eds.), *Immunochemistry of the Bacterial Cell Envelope*, Wiley, Chichester, 1985, and references therein.
2 P. Lefrancier and E. Lederer, *Fortschr. Chem. Org. Naturst.*, 40 (1981) 1–47.
3 P. Lefrancier and E. Lederer, *Pure Appl. Chem.*, 59 (1987) 449–454.
4 R. R. Schmidt and J. Michel, *Angew. Chem.*, 92 (1980) 763–764; *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 731–732.
5 R. R. Schmidt, *Angew. Chem.*, 98 (1986) 213–236; *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 212–235.
6 R. R. Schmidt, in W. Bartmann and K. B. Sharpless (Eds.), *Stereochemistry of Organic and Bioorganic Transformations*, Workshop Conferences Hoechst, Vol. 17, VCH Verlagsgesellschaft, Weinheim, 1987, pp. 169–189.
7 R. R. Schmidt, *Pure Appl. Chem.*, 61 (1989) 1257–1270.
8 W. Kinzy and R. R. Schmidt, *Justus Liebigs Ann. Chem.*, (1987) 407–415.
9 W. Kinzy, Diplomarbeit, University of Konstanz, 1983.
10 H. Paulsen, P. Himpkamp, and T. Peters, *Justus Liebigs Ann. Chem.*, (1986) 664–674.
11 P. H. Gross and M. Rimpler, *Justus Liebigs Ann. Chem.*, (1986) 37–45.
12 E. Baer, D. Buchnea, and H. C. Stancer, *J. Am. Chem. Soc.*, 81 (1959) 2166–2169.
13 R. R. Schmidt and P. Zimmermann, *Angew. Chem.*, 98 (1986) 722–723; *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 725–726.
14 R. R. Schmidt, T. Baer, and H.-J. Apell, *Angew. Chem.*, 99 (1987) 813–814; *Angew. Chem. Int. Ed. Engl.*, 26 (1987) 793–794; P. Zimmermann, R. Bommer, T. Baer, and R. R. Schmidt, *J. Carbohydr. Chem.*, 7 (1988) 435–452; T. Baer and R. R. Schmidt, *Justus Liebigs Ann. Chem.*, (1988) 669–674; N. P. Singh and R. R. Schmidt, *J. Carbohydr. Chem.*, 8 (1989) 199–216.
15 R. Bommer and R. R. Schmidt, *Justus Liebigs Ann. Chem.*, in press.
16 S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 49 (1976) 533–539.
17 E. Wünsch and F. Drees, *Chem. Ber.*, 99 (1966) 110–120; F. Weygand, D. Hoffmann, and E. Wünsch, *Z. Naturforsch., Teil B*, 21 (1966) 426–428.
18 T. Adachi, J. Yamada, I. Inove, and M. Saneyoshi, *Synthesis*, (1977) 45–46.
19 S. Kusumoto, M. Imoto, T. Ogiku, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 59 (1986) 1419–1423.
20 P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Carbohydr. Res.*, 77 (1979) c1–c4; P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Tetrahedron Lett.*, (1979) 4013–4016.
21 A. Termin and R. R. Schmidt, *Justus Liebigs Ann. Chem.*, in press.