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SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME LIPOPHILIC DERIVATIVES OF 1-THIO-MURAMOYL-L-ALANYL-D-ISOGLUTAMINE*

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ABSTRACT

2-*N*-Octadecanoyl derivatives of 1-*S*-acetyl-, 1-*S*-octadecanoyl-, and of 6-*O*-octadecanoyl-1-*S*-octadecanoyl-1-thiomuramoyl-L-alanyl-D-isoglutamine were synthesized from benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(octadecanoylamino)-β-D-glucopyranoside. Their immunoadjuvant activities were examined in guinea-pigs.

INTRODUCTION

In the course of recent studies on the structure–activity relationship of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine** (MDP), which is the minimal structure[2] required for the immunoadjuvant activity of the bacterial cell-wall peptidoglycans, it has been shown that introduction[3–5] of lipophilic character at the restricted position of the sugar skeleton in MDP and its carbohydrate analogs carrying strong, adjuvant activity causes potent antitumor and antiinfection activities, based on the immune reaction, that are not found for MDP itself, and abolishes the pyrogenicity, which is one of the side effects of MDP. However, the position of introduction of lipophilicity into the molecule is critical for the activity, as the lipophilic analogs[6] at both C-2 and C-6 in 6-amino-6-deoxymuramoyl dipeptide (having strong, adjuvant activity) completely lost the activity. In view of these facts, it seemed important to elucidate the relationship between the position of introduction of lipophilicity into the sugar moiety of MDP analogs, and the biological activities. We now describe the synthesis of lipophilic derivatives at C-2, at both C-1 and C-2, and at C-1, C-2, and C-6, in 1-thiomuramoyl-L-alanyl-D-isoglutamine[7], and their immunoadjuvant activities.

*Studies on Immunoadjuvant Active Compounds, Part 28. For Part 27, see ref. 1.
**N-[2-O-(2-Acetamido-2,3-dideoxy-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine.

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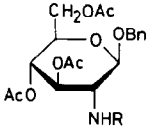
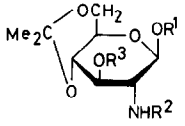
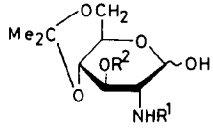
RESULTS AND DISCUSSION

O-Deacetylation of benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(octadecanoylamino)-β-D-glucopyranoside[8] (**1**) with methanolic sodium methoxide, and subsequent 4,6-*O*-isopropylidenation[9] with 2,2-dimethoxypropane in *N,N*-dimethylformamide and 1,4-dioxane in the presence of *p*-toluenesulfonic acid, gave benzyl 2-deoxy-4,6-*O*-isopropylidene-2-(octadecanoylamino)-β-D-glucopyranoside (**2**) in 95% yield. Condensation of **2** with L-2-chloropropanoic acid in the presence of sodium hydride, followed by methyl esterification with ethereal diazomethane, afforded benzyl 2-deoxy-4,6-*O*-isopropylidene-3-*O*-[D-1-(methoxycarbonyl)ethyl]-2-(octadecanoylamino)-β-D-glucopyranoside (**3**) in good yield. Treatment of **3** with chromium trioxide–pyridine complex[7,10] in the presence of acetic anhydride in dichloromethane for 3 h at 45° gave crystalline 1-*O*-benzoyl derivative **4** in 90% yield; significant signals in the n.m.r. spectrum were a three-proton singlet at δ 3.75 (MeO), a one-proton doublet at δ 5.71 ($J_{1,2}$ 8.0 Hz, H-1), and a five-proton multiplet at δ 7.32–8.15 (PhCO), indicating the structure shown for the 1-*O*-benzoyl-β-D-pyranose form **4**. *O*-Debenzoylation of **4** with methanolic sodium methoxide gave **5**.





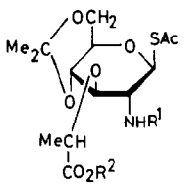


1 R = a
Bn = $PhCH_2$
a = octadecanoyl

2 R^1 = Bn, R^2 = a, R^3 = H
3 R^1 = Bn, R^2 = a, R^3 = b
4 R^1 = Bz, R^2 = a, R^3 = b
Bz = PhCO
b = $-CH(Me)CO_2Me$

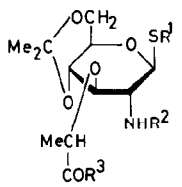
5 R^1 = a, R^2 = b

Treatment of **5** with carbon tetrachloride and tris(dimethylamino)phosphine[7,11] in dichloromethane for 15 min at −50° gave a quantitative yield of the alkoxytris(dimethylamino)phosphonium chloride, which was used for the next reaction without isolation; it was treated with potassium thioacetate[5b,7], to afford 1-*S*-acetyl-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[D-1-(methoxycarbonyl)ethyl]-2-(octadecanoylamino)-1-thio-β-D-glucopyranose (**6**) in 91% yield. The n.m.r. spectrum of **6** showed a three-proton singlet at δ 2.33 (*S*-Ac), and H-1 as a doublet at δ 5.08 ($J_{1,2}$ 10.6 Hz), indicating the structure of the 1-*S*-acetyl-β-D-glucopyranose derivative **6**. Hydrolysis of the *S*-acetyl and methyl ester groups in compound **6**, and subsequent, selective *S*-acetylation in methanol with acetic anhydride, in the presence of triethylamine, gave **7** in good yield. Coupling of **7** with L-alanyl-D-isoglutamine methyl ester, using dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu) as the activating agents, afforded *N*-{2-*O*-[1-*S*-acetyl-2,3-dideoxy-4,6-*O*-isopropylidene-2-(octadecanoylamino)-1-thio-β-D-glucopyranose-3-yl]-D-lactoyl}-L-



6 R^1 = a, R^2 = Me

7 R^1 = a, R^2 = H

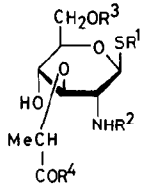


8 R^1 = Ac, R^2 = a, R^3 = c

9 R^1 = H, R^2 = a, R^3 = c

10 R^1 = R^2 = a, R^3 = c

c = L-Ala-D-isoGln-OMe



11 R^1 = Ac, R^2 = a, R^3 = H, R^4 = c

12 R^1 = R^3 = H, R^2 = a, R^4 = c

13 R^1 = R^3 = H, R^2 = a, R^4 = d

14 R^1 = R^2 = a, R^3 = H, R^4 = c

15 R^1 = R^2 = R^3 = a, R^4 = c

d = L-Ala-D-isoGln

alanyl-D-isoglutamine methyl ester (**8**); this was used as an intermediate for the synthesis of the 2-*N*-octadecanoyl-1-thiomuramoyl dipeptides described herein. Hydrolytic removal of the isopropylidene group in **8** under mild, acidic conditions gave **11** in quantitative yield; this was treated with sodium methoxide in methanol, to afford *N*-{2-*O*-[2,3-dideoxy-2-(octadecanoylamino)-1-thio-β-D-glucopyranose-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine methyl ester (**12**); saponification of the methyl ester group in **12** gave the 2-*N*-octadecanoyl-1-thiomuramoyl dipeptide **13**.

In order to prepare the more lipophilic analogs of **11**, bearing the lipid moiety at C-1, and at both C-1 and C-6, condensation of **9**, formed by selective hydrolysis of the *S*-acetyl group in **8**, with octadecanoyl chloride in pyridine–dichloromethane

TABLE I

ADJUVANT ACTIVITY OF SOME LIPOPHILIC DERIVATIVES OF 1-THIOMURAMOYL-L-ALANYL-D-ISOGLUTAMINE ON THE INDUCTION OF DELAYED-TYPE HYPERSENSITIVITY TO ABA-*N*-ACETYLTYROSINE IN GUINEA-PIGS

Compound[a]	*Skin reaction with ABA-BSA*[b] *(100 μg) (diam. in mm + SE)*[c]	
	24 h	*48 h*
11	17.5 ±0.5	12.5 ±1.1
12	13.0 ±0.9	7.5 ±0.2
13	17.6 ±1.3	14.8 ±2.0
14	12.0 ±0	(4.8 ±0.3)
15	13.9 ±0.9	(8.3 ±0.6)
1-Thio-MDP[d]	23.1 ±1.2	24.0 ±0.3
1-*S*-Octadecanoyl-1-thio-MDP[d]	22.7 ±0.8	25.3 ±0.3
MDP	20.9 ±0.5	22.4 ±1.0
Control[e]	0	0

[a]Dose: 100 μg. [b]Azobenzenearsonate–*N*-acetyl-L-tyrosine–bovine serum albumin. [c]The data indicate the average diameter ± the standard error (SE) of the skin reaction (induration) of four guinea-pigs; the values in parentheses indicate the size of the erythema. [d]See ref. 5b. [e]ABA-*N*-acetyltyrosine in Freund's incomplete adjuvant.

gave **10**, which was converted, by hydrolytic removal of the isopropylidene group, into the desired *N*-{2-*O*-[2,3-dideoxy-1-*S*-octadecanoyl-2-(octadecanoylamino)-β-D-glucopyranose-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine methyl ester (**14**); characteristic signals in the n.m.r. spectrum of **14** were a three-proton singlet at δ 3.75 (MeO) and a one-proton doublet at δ 5.12 ($J_{1,2}$ 10.0 Hz, H-1).

In a similar way, condensation of **14** with octadecanoyl chloride afforded the 1,2,6-trioctadecanoyl derivative **15** in good yield.

Immunoadjuvant activities of the compounds (**11–15**) thus obtained, on the induction of the delayed type of hypersensitivity to *N*-acetyl-L-tyrosine-3-azobenzene-4′-arsonic acid (ABA-*N*-acetyltyrosine) in guinea-pigs were examined[12] (see Table I). All of the compounds had a distinct, but weak, immunoadjuvant activity, as compared to those of MDP, 1-thio-MDP, and 1-*S*-octadecanoyl-1-thio-MDP. This clearly indicates that introduction of lipophilic character on C-2 in the 1-thiomuramoyl dipeptide is unfavorable to the activity, and that the position of introduction of lipophilicity into the molecule is important for manifestation of the activity.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Evaporations were conducted *in vacuo*. Preparative chromatography was performed on silica gel (Waco Co.; 200 mesh) with the solvent systems specified. Specific rotations were determined with a Union PM-201 polarimeter, and i.r. spectra were recorded with a Jasco A-100 spectrophotometer. N.m.r. data were recorded at 90 MHz with a Hitachi R-22 spectrometer.

Benzyl 2-deoxy-4,6-O-isopropylidene-2-(octadecanoylamino)-β-D-glucopyranoside (**2**). — To an ice-cooled solution of benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(octadecanoylamino)-β-D-glucopyranoside (**1**; 2.4 g) in methanol (150 mL) was added sodium metal (100 mg), and the mixture was kept for 30 min at room temperature, and then treated with Amberlite IR-120 (H^+) resin to remove the base; the resin was filtered off and washed with methanol. The filtrate and washings were combined, and evaporated. To a solution of the residue in *N,N*-dimethylformamide (15 mL) and 1,4-dioxane (70 mL) were added 2,2-dimethoxypropane (10 mL) and *p*-toluenesulfonic acid monohydrate (50 mg), and the mixture was stirred for 1 h at 40–45°. and then treated with Amberlite IR-410 (OH^-) resin. After removal of the resin, the solution was evaporated to a syrup which crystallized from ether–hexane. Recrystallization from ether gave **2** (2.0 g, 95%) as needles; m.p. 118°, $[\alpha]_D^{25}$ −65.5° (*c* 0.6, chloroform): ν_{max}^{Nujol} 3400–3250 (OH, NH), 2920 and 2840 (Me, methylene), 1640 and 1540 (amide), and 860 cm^{-1} (Me_2C).

Anal. Calc. for $C_{34}H_{57}NO_6$: C, 70.92; H, 9.98; N, 2.43. Found: C, 70.86; H, 9.85; N, 2.40.

Benzyl 2-deoxy-4,6-O-isopropylidene-3-O-[D-1-(methoxycarbonyl)ethyl]-2-

*(octadecanoylamino)-β-*D*-glucopyranoside* (**3**). — To a stirred solution of **2** (2.37 g) in dry 1,4-dioxane (40 mL) was added sodium hydride in oil suspension (500 mg; 50% of sodium hydride by weight) at room temperature. The mixture was stirred for 1 h at 95°, and cooled to 65°, and then L-2-chloropropanoic acid (670 mg) was added dropwise, with stirring, at 65–70°. The mixture was stirred for 30 min at 95°, and cooled; 2M hydrochloric acid was carefully added to the cooled mixture until pH 8 was reached, and the mixture was evaporated to a syrup which was extracted with chloroform. The extract was successively washed with 2M hydrochloric acid and water, dried (sodium sulfate), and evaporated. To a solution of the residue in ether (50 mL) was added an ethereal solution of diazomethane, the course of the reaction being monitored by t.l.c. The mixture was now evaporated to a syrup which was chromatographed on a column of silica gel (40 g) with chloroform, and then with 100:1 chloroform–methanol. The latter eluate gave compound **3** (2.15 g, 79%) as needles; m.p. 97°, $[\alpha]_D^{25}$ −31° (*c* 0.6, chloroform); ν_{max}^{KBr} 3260 (NH), 2920 and 2840 (Me, methylene), 1730 and 1260 (ester), 1640 and 1550 (amide), and 860 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 0.88 (near t, 3 H, $J_{Me,CH}$ 5.0 Hz, $MeCH_2$), 1.27 (m, 30 H, 15 CH_2), 1.34 (d, 3 H, $J_{Me,CH}$ 7.2 Hz, MeCH), 1.38, 1.49 (2 s, 6 H, Me_2C), 2.13–2.30 (m, 2 H, CH_2CO), 3.71 (s, 3 H, MeO), 4.44 (q, 1 H, $J_{CH,Me}$ 7.2 Hz, CHMe), 4.56, 4.90 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 4.68 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), and 7.29 (s, 5 H, Ph).

Anal. Calc. for $C_{38}H_{63}NO_8$: C, 68.95; H, 9.59; N, 2.12. Found: C, 68.87; H, 9.62; N, 2.08.

*1-*O*-Benzoyl-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[*D*-1-(methoxycarbonyl)-ethyl]-2-(octadecanoylamino)-β-*D*-glucopyranose* (**4**). — To a stirred solution of dry pyridine (1.1 mL) and dichloromethane (6 mL) was added chromium trioxide (700 mg), and the mixture was stirred for 15 min at room temperature. A solution of **3** (700 mg) in dichloromethane (6 mL) was added, with stirring, to the mixture, followed by acetic anhydride (0.8 mL), and it was stirred for 3 h at 45°, the course of the reaction being monitored by t.l.c. The mixture was passed through a column of silica gel (100 g) with ethyl acetate, to give the crude product, which was purified by chromatography on a column of silica gel (100 g) with chloroform and 200:1 chloroform–methanol. The latter eluate afforded **4** (640 mg, 89.5%) as needles; m.p. 65–66°, $[\alpha]_D^{25}$ −44° (*c* 0.6, chloroform); ν_{max}^{KBr} 3260 (NH), 2920 and 1840 (Me, methylene), 1730 and 1260 (ester), 1640 and 1550 (amide), 860 (Me_2C), and 700 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 0.87 (near t, 3 H, $J_{Me,CH}$ 5.6 Hz, $MeCH_2$), 1.11–1.30 (m, 30 H, 15 CH_2), 1.38 (d, 3 H, $J_{Me,CH}$ 7.2 Hz, MeCH), 1.31, 1.40 (2 s, 6 H, Me_2C), 2.18 (m, 2 H, CH_2CO), 3.75 (s, 3 H, MeO), 4.55 (q, 1 H, $J_{CH,Me}$ 7.2 Hz, CHMe), 5.71 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 6.71 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), and 7.32–8.15 (m, 5 H, Ph).

Anal. Calc. for $C_{38}H_{61}NO_9$: C, 67.52; H, 9.10; N, 2.07. Found: C, 67.41; H, 8.96; N, 2.10.

*2-Deoxy-4,6-*O*-isopropylidene-3-*O*-[*D*-1-(methoxycarbonyl)ethyl]-2-(octadecanoylamino)-*D*-glucopyranose* (**5**). — To an ice-cooled solution of **4** (290 mg) in

methanol (10 mL) was added sodium metal (20 mg), and the mixture was kept for 30 min at room temperature, and then treated with Amberlite IR-120 (H^+) resin. The product was purified by chromatography on a column of silica gel (20 g) with chloroform and 100:1 chloroform–methanol. The latter eluate afforded **5** (200 mg, 82%) as a syrup; $[\alpha]_D^{25}$ +23° (*c* 1.2, chloroform; equil.); ν_{max}^{film} 3300 (OH, NH), 1730 and 1220 (ester), 1640 and 1540 (amide), and 850 cm^{-1} (Me_2C).

Anal. Calc. for $C_{31}H_{57}NO_8$: C, 65.11; H, 10.05; N, 2.45. Found: C, 64.96; H, 10.13; N, 2.38.

1-S-Acetyl-2-deoxy-4,6-O-isopropylidene-3-O-[D-*1-(methoxycarbonyl)ethyl]-2-(octadecanoylamino)-1-thio-β-*D-*glucopyranose* (**6**). — To a solution of **5** (200 mg) in dry dichloromethane (4 mL) and carbon tetrachloride (200 mg), cooled to −60°, was added dropwise, with stirring, an ice-cooled solution of tris(dimethylamino)phosphine (150 mg) in dry dichloromethane (1 mL). The mixture was stirred for 15 min at −50°; at that time, all of the starting material had been converted into the salt. Potassium thioacetate (200 mg) and Drierite (500 mg; W. A. Hammond Co.) were added to the mixture, which was then stirred overnight at room temperature; the precipitates were filtered off, and washed with dichloromethane, and the filtrate and washings were combined, washed with water, dried (sodium sulfate), and evaporated. The residue was chromatographed on a column of silica gel (10 g) with chloroform and 200:1 chloroform–methanol. The latter eluate afforded **6** (200 mg, 91%) as needles, after recrystallization from ethanol–ether; m p. 94°, $[\alpha]_D^{25}$ +19.5° (*c* 0.5, chloroform); ν_{max}^{KBr} 3300 (NH), 2930 and 2850 (Me, methylene), 1740 and 1220 (ester), 1700 (*S*-acetyl), 1650 and 1540 (amide), and 860 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 0.88 (near t, 3 H, J_{Me,CH_2} 5.0 Hz, $MeCH_2$), 1.18–1.28 (m, 30 H, 15 CH_2), 1.33 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 1.35, 1.47 (2 s, 6 H, Me_2C), 2.33 (s, 3 H, AcS), 3.76 (s, 3 H, MeO), 4.52 (q, 1 H, $J_{CH,Me}$ 7.0 Hz, CHMe), 5.08 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), and 6.75 (d, 1 H, $J_{NH,2}$ 8.0 Hz, NH).

Anal. Calc. for $C_{33}H_{59}NO_8S$: C, 62.92; H, 9.44; N, 2.22. Found: C, 62.87; H, 9.43; N, 2.19.

*1-S-Acetyl-3-O-(*D-*1-carboxyethyl)-2-deoxy-4,6-O-isopropylidene-2-(octadecanoylamino)-1-thio-β-*D-*glucopyranose* (**7**). — To a solution of **6** (400 mg) in 1,4-dioxane (20 mL) and methanol (20 mL) was added 0.2M potassium hydroxide (20 mL), and the mixture was stirred for 30 min at room temperature, and then treated with Amberlite IRC-50 (H^+) resin to remove the base. After evaporation of the solvents, the residue was dissolved in methanol (10 mL); acetic anhydride (3 mL) and triethylamine (3 mL) were added, with stirring, to the solution at 0°, and it was stirred for 30 min at 0°, and evaporated. The residue was chromatographed on a column of silica gel (20 g) with (*a*) chloroform, (*b*) 150:1, and (*c*) 70:1 chloroform–methanol. Eluant (*c*) afforded compound **7** (280 mg, 72%) as a syrup; $[\alpha]_D^{25}$ +36° (*c* 0.8, chloroform); ν_{max}^{film} 3320 (NH), 2930 and 2840 (Me, methylene), 2750–2500 (COOH), 1730 (C=O), 1700 (*S*-acetyl), 1650 and 1530 (amide), and 855 cm^{-1} (Me_2C).

Anal. Calc. for $C_{32}H_{57}NO_8S$: C, 62.40; H, 9.33; N, 2.27. Found: C, 62.25; H, 9.41; N, 2.19.

N-{2-O-[1-S-*Acetyl*-2,3-*dideoxy*-4,6-O-*isopropylidene*-2-(*octadecanoylamino*)-1-*thio*-β-D-*glucopyranosy*-3-*yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**8**). — To a solution of **7** (450 mg) in dry 1,4-dioxane (10 mL) were added, with stirring, *N*-hydroxysuccinimide (126 mg) and dicyclohexylcarbodiimide (300 mg), and the mixture was stirred for 30 min at room temperature; at that time, compound **7** had been converted into the activated ester. A solution of L-alanyl-D-isoglutamine methyl ester trifluoroacetate (370 mg) in dry 1,4-dioxane (3 mL) and triethylamine (150 mg) was added to the mixture, and it was stirred for 1 h at room temperature, and then evaporated to a syrup which was extracted with chloroform. The extract was washed with water, dried (sodium sulfate), and evaporated. The residue was chromatographed on a column of silica gel (40 g) with (*a*) chloroform, (*b*) 100:1, (*c*) 50:1, and (*d*) 30:1 chloroform–methanol. Eluant (*d*) afforded **8** (360 mg, 59.5%) as an amorphous mass; m.p. 50–53°, $[\alpha]_D^{25}$ +12° (*c* 0.6, chloroform); ν_{max}^{KBr} 3370–3260 (NH), 2930 and 2840 (Me, methylene), 1740 and 1260 (ester), 1700 (*S*-acetyl), 1660 and 1530 (amide), and 860 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 0.87 (near t, 3 H, J_{Me,CH_2} 6.0 Hz, $MeCH_2$), 1.15–1.35 (m, 30 H, 15 CH_2), 1.30–1.49 (2 s, 2 d, 12 H, Me_2C, 2 MeCH), 2.31 (s, 3 H, AcS), 3.70 (s, 3 H, MeO), 5.23 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), and 6.05, 6.78, 6.95, and 7.50 (5 H, 3 NH, NH_2).

Anal. Calc. for $C_{41}H_{72}N_4O_{11}S$: C, 59.39; H, 8.75; N, 6.76. Found: C, 59.95; H, 8.88; N, 6.73.

N-{2-O-[2,3-*Dideoxy*-4,6-O-*isopropylidene*-2-(*octadecanoylamino*)-1-*thio*-β-D-*glucopyranose*-3-*yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**9**). — To a solution of **8** (150 mg) in methanol (10 mL) was added sodium metal (10 mg), and the mixture was kept for 30 min, treated with Amberlite IR-120 (H^+) resin, and then evaporated, to give **9** (130 mg, 92%) as crystals; m.p. 75–78°, $[\alpha]_D^{25}$ +12.5° (*c* 0.4, chloroform); ν_{max}^{KBr} 3360–3270 (NH), 2930 and 2840 (Me, methylene), 2550 (SH), 1730 and 1260 (ester), 1640 and 1530 (amide), and 860 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 0.87 (near t, 3 H, $J_{Me,CH}$ 6.0 Hz, $MeCH_2$), 1.15–1.25 (m, 30 H, 15 CH_2), 1.32–1.50 (2 s, 2 d, 12 H, Me_2C, 2 MeCH), and 3.69 (s, 3 H, MeO).

Anal. Calc. for $C_{39}H_{70}N_4O_{10}S$: C, 59.51; H, 8.97; N, 7.12. Found: C, 59.43; H, 9.08; N, 7.05.

N-{2-O-[2,3-*Dideoxy*-4,6-O-*isopropylidene*-1-S-*octadecanoyl*-2-(*octadecanoylamino*)-1-*thio*-β-D-*glucopyranose*-3-*yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**10**). — To a stirred solution of **9** (100 mg) in dichloromethane (4 mL) and pyridine (0.5 mL) was added dropwise a solution of octadecanoyl chloride (60 mg) in dichloromethane (2 mL) at −50°, and the mixture was stirred for 1.5 h at 0°; methanol (0.5 mL) was added, and the mixture evaporated, and the residue was extracted with chloroform. The extract was successively washed with 2M sodium carbonate, 2M hydrochloric acid, and water, dried (sodium sulfate), and evaporated to a syrup which was chromatographed on a column of silica gel (15 g) with

(*a*) chloroform, (*b*) 80:1, and (*c*) 30:1 chloroform–methanol. Eluant (*c*) gave compound **10** (120 mg, 90%) as crystals; m.p. 62–65°, $[\alpha]_D^{25}$ +19° (*c* 0.3, chloroform); ν_{max}^{KBr} 3350 (NH), 2930 and 2830 (Me, methylene), 1730 and 1260 (ester), 1700 (*S*-acyl), 1650 and 1530 (amide), and 855 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 0.88 (m, 6 H, 2 $MeCH_2$), 1.25–1.32 (m, 60 H, 30 CH_2), 1.33–1.50 (2 s, 2 d, 12 H, Me_2C, 2 MeCH), 3.72 (s, 3 H, MeO), and 5.21 (d, 1 H, $J_{1,2}$ 10.2 Hz, H-1).

Anal. Calc. for $C_{57}H_{104}N_4O_{11}S$: C, 64.98; H, 9.95; N, 5.32. Found: C, 64.79; H, 9.90; N, 5.38.

N-{2-O-[1-S-*Acetyl-2,3-dideoxy-2-(octadecanoylamino)-1-thio-β*-D-*glucopyranose-3-yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**11**). — A solution of **8** (130 mg) in 80% aqueous acetic acid (5 mL) was heated for 2 h at 45°, cooled, and evaporated, and the residue crystallized from ether, giving **11** (120 mg, 98%) as crystals; m.p. 163–166°, $[\alpha]_D^{25}$ +17.5° (*c* 0.3, chloroform); ν_{max}^{KBr} 3400 and 3270 (OH, NH), 2930 and 2840 (Me, methylene), 1740 and 1250 (ester), 1700 (*S*-acyl), and 1640 and 1540 cm^{-1} (amide); n.m.r. data (in 1:1 chloroform-*d*–methanol-d_4): δ 0.88 (near t, 3 H, J_{Me,CH_2} 5.6 Hz, $MeCH_2$), 1.18–1.30 (m, 30 H, 15 CH_2), 1.33–1.46 (m, 6 H, 2 MeCH), 2.32 (s, 3 H, AcS), 3.68 (s, 3 H, MeO), and 5.12 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1)

Anal. Calc. for $C_{38}H_{68}N_4O_{11}S$: C, 57.84; H, 8.69; N, 7.10. Found: C, 57.65; H, 8.76; N, 6.90.

N-{2-O-[2,3-*Dideoxy-2-(octadecanoylamino)-1-thio-β*-D-*glucopyranose-3-yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**12**). — *S*-Deacetylation of **11** (80 mg) with sodium metal (4 mg) in methanol (10 mL), as described in the preparation of **9**, afforded **12** (74 mg, 98%) as crystals; m.p. 105–107°, $[\alpha]_D^{25}$ +2.3° (*c* 0.5, chloroform); ν_{max}^{KBr} 3430–3220 (OH, NH), 2920 and 2840 (Me, methylene), 1725 and 1240 (ester), and 1650 and 1525 cm^{-1} (amide).

Anal. Calc. for $C_{36}H_{66}N_4O_{10}S$: C, 57.88; H, 8.91; N, 7.50. Found: C, 57.81; H, 9.02; N, 7.46.

N-{2-O-[2,3-*Dideoxy-2-(octadecanoylamino)-1-thio-β*-D-*glucopyranose-3-yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine* (**13**). — To a solution of **12** (40 mg) in methanol (5 mL) was added 0.2M potassium hydroxide (3 mL), and the solution was stirred for 20 min at room temperature, and then treated with Amberlite IR-120 (H^+) resin; the resin was filtered off, and washed with methanol. The filtrate and washings were combined, and evaporated, to give **13** (38 mg; quantitative) as crystals; m.p. 125–132° (dec.), $[\alpha]_D^{25}$ +4.0° (*c* 0.4, chloroform); ν_{max}^{KBr} 3350–3240 (OH, NH) 2920 and 2840 (Me, methylene), 1720 (C=O), and 1660 and 1530 cm^{-1} (amide).

Anal. Calc. for $C_{35}H_{64}N_4O_{10}S$: C, 57.35; H, 8.80; N, 7.64. Found: C, 57.09; H, 9.03; N, 7.59.

N-{2-O-[2,3-*Dideoxy-1*-S-*octadecanoyl-2-(octadecanoylamino)-1-thio-β*-D-*glucopyranose-3-yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**14**). — A solution of **10** (80 mg) in 80% aqueous acetic acid (5 mL) was heated for 2.5 h at 45°, and evaporated to a crystalline mass. Recrystallization from ether–hexane gave **14**

(70 mg, 91%); m.p. 140–146° (dec.), $[\alpha]_D^{25}$ +16.5° (*c* 0.38, chloroform); ν_{max}^{KBr} 3360–3230 (OH, NH), 2920 and 2840 (Me, methylene), 1740 and 1240 (ester), and 1650 and 1525 cm^{-1} (amide); n.m.r. data (in 1:1 chloroform-*d*–methanol-d_4): δ 0.86 (m, 6 H, 2 $MeCH_2$), 3.75 (s, 3 H, MeO), and 5.12 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1).

Anal. Calc. for $C_{54}H_{100}N_4O_{11}S$: C, 63.99; H, 9.95; N, 5.53. Found: C, 63.86; H, 9.82; N, 5.48.

N-{*2-O-[2,3-Dideoxy-6-O-octadecanoyl-1-S-octadecanoyl-2-(octadecanoylamino)-1-thio-β-*D-*glucopyranose-3-yl]*-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**15**). — To a solution of **14** (40 mg) in dry dichloromethane (2 mL) and dry pyridine (0.2 mL) was added, with stirring, a solution of octadecanoyl chloride (30 mg) in dichloromethane (2 mL) at −40°, and the mixture was stirred for 9.5 h at 0°, the course of the reaction being monitored by t.l.c. Methanol (1 mL) was added, and the mixture was evaporated to a syrup which was chromatographed on a column of silica gel (20 g) with (*a*) chloroform, (*b*) 80:1, (*c*) 40:1, and (*d*) 30:1 chloroform–methanol. Eluant (*d*) afforded **15** (40 mg, 79%) as crystals; m.p. 158–165° (dec.), $[\alpha]_D^{25}$ +1.7° (*c* 0.12, chloroform); ν_{max}^{KBr} 3350–3260 (OH, NH), 2920 and 2830 (Me, methylene), 1730 and 1250 (ester), and 1650 and 1520 cm^{-1} (amide); n.m.r. data (in 1:1 chloroform-*d*–methanol-d_4): δ 0.80–0.95 (m, 9 H, 3 $MeCH_2$), 3.70 (s, 3 H, MeO), and 5.12 (d, 1 H, $J_{1,2}$ 10.2 Hz, H-1).

Anal. Calc. for $C_{72}H_{134}N_4O_{12}S$: C, 67.87; H, 10.55; N, 4.38. Found: C, 67.80; H, 10.63; N, 4.29.

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