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Synthesis of *N*-[2-*O*-(2-Acetamido-1,5-anhydro-2,3-dideoxy-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine (1-Deoxy-MDP) and Some of Its Lipophilic Analogs, and Their Immunoadjuvant Activities†

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N-[2-*O*-(2-Acetamido-1,5-anhydro-2,3-dideoxy-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine (1-deoxy-MDP) and its lipophilic analogs were synthesized from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride. The immunoadjuvant activity was examined in order to clarify the structural requirements of the carbohydrate moiety and the effects of the lipophilic character for this activity in *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP).

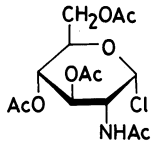
In the course of an investigation on the relationships between the immunoadjuvant activity of MDP and the structure of the carbohydrate moiety, it was proved that not only the restricted configuration of the sugar moiety[2～4] but also chemical modifications[1,5,6] of the functional group in the carbohydate moiety produced various important effects on the manifestation of activity. Moreover, it has been shown that the introduction[5,7～9] of lipophilic character at the restricted position of the sugar skeleton in MDP caused potent anti-infection activity, based on the immune reaction, as well as strong, immunoadjuvant activity. In view of these facts, we now describe the synthesis of 1-deoxy-MDP and some of its lipophilic analogs at the C-6 position in the sugar moiety, and their immunoadjuvant activities.

RESULTS AND DISCUSSION

Treatment[10] of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**1**) with tributyltin hydride in the presence of α,α′-azobis(isobutanonitrile) in dry toluene for 45 min at 90°C gave 2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-glucitol (**2**) in a 97.5% yield. *O*-Deacetylation of **2** and subsequent acetonation[11] using 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid monohydrate in *N*,*N*-dimethylformamide (DMF) afforded 2-acetamido-1,5-anhydro-2-deoxy-4,6-*O*-isopropylidene-D-glucitol (**4**) in a high yield. Compound **4** was condensed with L-2-chloropropanoic acid in dry 1,4-dioxane to give the corresponding 3-*O*-(D-1-carboxyethyl) derivative (**5**) in an 84% yield, which was converted into the methyl ester (**6**) by treatment with diazomethane in an ether solution.

Coupling of **5** with L-alanyl-D-isoglutamine methyl ester was conducted, with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as the activating agents, to afford *N*-[2-*O*-(2-acetamido-1,5-anhydro-2,3-dideoxy-4,6-*O*-isopropylidene-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (**7**) in a 91% yield. Hydrolysis of the isopropylidene

† Studies on Immunoadjuvant Active Compounds. Part XXIX. For Part XXVIII, see ref. 1.



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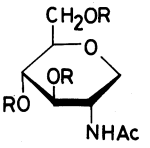
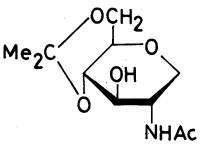


2 R = Ac
3 R = H



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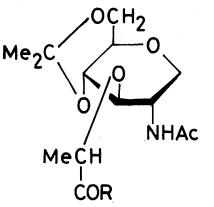
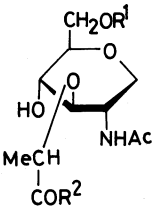
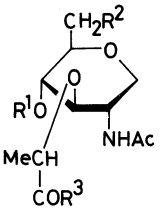


5 R = OH
6 R = OMe
7 R = a
a = L-Ala-D-isoGln
OMe



8 R^1 = H, R^2 = a
9 R^1 = H, R^2 = b
10 R^1=CO$(CH_2)_6$Me, R^2=a
11 R^1=CO$(CH_2)_{10}$Me, R^2=a
12 R^1=CO$(CH_2)_{14}$Me, R^2=a
13 R^1=CO$(CH_2)_{16}$Me, R^2=a
14 R^1=CO$[(CH_2)_{13}Me]_2$, R^2=a
b=L-Ala-D-isoGln



15 R^1=H, R^2=OH, R^3=OMe
16 R^1=H, R^2=Br, R^3=OMe
17 R^1=Thp, R^2=Br, R^3=OMe
18 R^1=Thp, R^2=Br, R^3=a
19 R^1=Thp, R^2=SAc, R^3=a
20 R^1=H, R^2=SAc, R^3=a
21 R^1=H, R^2=SCO$(CH_2)_{16}$Me, R^3=a
Thp=tetrahydropyran-2-yl

group in **7** under mild, acidic conditions gave the 1-dexoy-MDP methyl ester (**8**) in a quantitative yield, which was converted into 1-deoxy-MDP[4] (**9**) by saponification with 0.2 M potassium hydroxide.

In order to clarify the introduction effect of the acyl groups for the activity, we synthesized several, lipophilic analogs at the C-6 position in the glucitol moiety of 1-deoxy-MDP methyl ester (**8**). When treated with octanoyl, dodecanoyl, hexadecanoyl, octadecanoyl or 2-tetradecyl-hexadecanoyl chloride[9] in pyridine and dichloromethane at $-30°C$, compound **8** afforded the corresponding 6-*O*-(fatty acyl) derivatives (**10**$\sim$**14**).

For the synthesis of 6-*S*-acyl derivatives of **8**, *O*-deisopropylidenation of **6** by mild acid hydrolysis gave 2-acetamido-1,5-anhydro-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-D-glucitol (**15**). Treatment[12] of **15** with triphenylphosphine and carbon tetrabromide in pyridine for 2 hr at 40°C gave the 6-bromo derivative (**16**) in an 83% yield, which was converted into the crystalline 4-*O*-tetrahydropyran-2-yl derivative (**17**). Saponification of **17** with 0.2 M aqueous potassium hydroxide in 1,4-dioxane gave the acid, which was used for the next reaction without purification. Coupling of the acid with L-alanyl-D-isoglutamine methyl ester as described for compound **7** afforded *N*-{2-*O*-[2-acetamido-1,5-anhydro-6-bromo-4-*O*-(tetrahydropyran-2-yl)-2,3,6-trideoxy-D-glucitol-3-yl]-D-lactoxyl}-L-alanyl-D-isoglutamine methyl ester (**18**) in a quantitative yield. When treated with potassium thioacetate in acetone, compound **18** afforded the 6-*S*-acetyl derivative (**19**) in an 82% yield, which, on mild acid hydrolysis, was converted into *N*-[2-*O*-(2-acetamido-6-*S*-acetyl-1,5-anhydro-6-thio-2,3,6-trideoxy-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (**20**). *S*-Deacetylation of **19**, *S*-acylation with octadecanoyl chloride, and subsequent, hydrolytic removal of the isopropylidene group with 80% aqueous acetic acid yielded the 6-*S*-octadecanoyl derivative (**21**).

The immunoadjuvant activities of the compounds thus obtained on the induction of the delayed type of hypersensitivity to *N*-acetyl-L-tyrosine-3-azobenzene-4′-arsonate (ABA-*N*-acetyltyrosine) in guinea-pigs were examined[13] (see Table I). 1-Deoxy-MDP (**9**) and its methyl ester (**8**) showed strong activity, almost comparable to that of MDP, indicating that the presence of the sugar skeleton was not critical for the activity, and that a restricted configuration and conformation of the carbohydrate moiety in MDP was important. 6-*O*-

Table I. Adjuvant Activity of 1-Deoxy-MDP and Its Related Compounds on the Delayed-type Hypersensitivity to ABA-*N*-Acetyltyrosine in Guinea-pigs

Compounds[a]	Skin reaction with ABA-BSA[b] (100 μg) (diam. in mm ± SE)[c] at	
	24 hr	48 hr
8	20.3 ± 0.3	14.5 ± 0.5
9	20.4 ± 0.6	14.0 ± 1.0
10	(4.8 ± 1.7)	0
11	(4.8 ± 1.7)	0
12	9.5 ± 2.7	(4.5 ± 2.7)
13	18.5 ± 0.9	(12.3 ± 0.4)
14	20.6 ± 1.0	16.6 ± 1.0
20	(9.9 ± 0.9)	0
21	(8.6 ± 0.7)	0
MDP	21.5 ± 1.0	15.3 ± 0.3
Control[d]	0	0

[a] Dose: 10 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] ABA-*N*-acetyltyrosine in Freund's incomplete adjuvant.

Acyl and 6-*S*-acyl derivatives (**10**～**13**, **20** and **21**) exhibited weak, or no, adjuvant activity; interestingly, the 6-*O*-2-tetradecyl-hexadecanoyl derivative (**14**) showed even stronger activity than those of 1-deoxy-MDP and MDP. Moreover, all of the 1-deoxy-MDP analogs were found to be non-pyrogenic at a dose of 75 μg/kg in rabbits.[14]

EXPERIMENTAL

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 at 25°C, and IR spectra were recorded with a Jasco A-100 spectrophotometer. NMR spectra were recorded at 90 MHz with a Hitachi R-22 spectrometer.

2-Acetamido-3,4,6-tri-O-acetyl-1,5-anhydro-2-deoxy-D-*glucitol* (**2**). To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**1**, 39.35 g) and azobis(isobutanonitrile, 8.84 g) in dry toluene (200 ml) under a nitrogen atmosphere was added, with stirring, tributyltin hydride (34.8 ml), and the mixture was stirred for 45 min at 90°C. A solution of potassium fluoride dihydrate (20 g) in water (80 ml) was added to the mixture, which was then vigorously stirred for 15 min. The precipitates were filtered off and washed with chloroform. The filtrate and washings were combined, washed with water, dried (sodium sulfate), and then evaporated to a crystalline mass. Recrystallization from ethyl acetate–ether gave **2** (34.8 g, 97.5%) as needles, mp 156°C, $[\alpha]_D$ +7.1° (c=1.1, chloroform); IR ν_{max}^{Nujol} cm^{-1}: 3250 (NH), 1740 and 1230 (ester), and 1640 and 1560 (amide); NMR (1:1 $CDCl_3$–CD_3OD): δ 1.94 (s, 3H, AcN), 2.04, 2.06, 2.10 (3s, 9H, 3AcO), and 4.95～5.07 (m, 2H, H-3, 4).

Anal. Found: C, 50.68; H, 6.42; N, 4.31. Calcd. for $C_{14}H_{21}NO_8$: C, 50.75; H, 6.39; N, 4.23%.

2-Acetamido-1,5-anhydro-2-deoxy-D-*glucitol* (**3**). To a solution of **2** (6.0 g) in methanol (60 ml) was added sodium methoxide (100 mg) at 0°C, and after 10 min, the solution was treated with Amberlite IR-120 (H^+) resin to remove the base. The product was crystallized from ethanol–ether to give **3** (3.2 g, 86.5%) as needles, mp 204°C, $[\alpha]_D$ +20.3° (c=0.6, methanol); IR ν_{max}^{Nujol} cm^{-1}: 3450, 3380, 3300 and 3220 (OH, NH), and 1640 and 1560 (amide).

Anal. Found: C, 46.95; H, 7.59; N, 6.75. Calcd. for $C_8H_{15}NO_5$: C, 46.82; H, 7.37; N, 6.83%.

2-Acetamido-1,5-anhydro-2-deoxy-4,6-O-isopropylidene-D-*glucitol* (**4**). To a solution of **3** (3.1 g) in DMF (20 ml) were added 2,2-dimethoxypropane (8 ml) and *p*-toluenesulfonic acid monohydrate (100 mg), before the mixture was stirred for 2 hr at room temperature and then treated with Amberlite IR-410 (OH^-) resin. After filtration, the colorless solution was evaporated to a syrupy product which was crystallized from ether. The crystals were obtained as needles (3.6 g, 97%), mp 158.5°C, $[\alpha]_D$ −18.6° (c=1.2, 4:1 chloroform–methanol); IR ν_{max}^{Nujol} cm^{-1}: 3300, 3260 (OH, NH), 1670 and 1570 (amide), and 850 (Me_2C); NMR (1:1 $CDCl_3$–CD_3OD): δ 1.44, 1.53 (2s, 6H, Me_2C) and 2.00 (s, 3H, AcN).

Anal. Found: C, 53.88; H, 7.89; N, 5.76. Calcd for $C_{11}H_{19}NO_5$: C, 53.87; H, 7.81; N, 5.71%.

2-Acetamido-1,5-anhydro-3-O-(D-*1-carboxyethyl*)*-2-deoxy-4,6-O-isopropylidene*-D-*glucitol* (**5**). To a stirred solution of **4** (9.0 g) in dry 1,4-dioxane (150 ml) was added sodium hydride in an oil suspension (5.0 g; 50% of sodium hydride by weight) at 60°C. The mixture was kept for 1 hr at 95°C, and then a solution of L-2-chloropropanoic acid (2.0 g) in dry 1,4-dioxane (20 ml) was added, with stirring, at 65°C. The mixture was stirred for 5 hr at 95°C, and cooled; 2 M hydrochloric acid was then carefully added to the cooled mixture until pH 8 was reached, and the mixture was evaporated. Chloroform (100 ml) and water

(100 ml) were added to the residue, and 2 M hydrochloric acid was added to pH 3. The chloroform layer was separated, and the aqueous solution was throughly extracted with chloroform. The chloroform layer and the extracts were combined, washed with water, dried (sodium sulfate), and evaporated to a syrup, which was chromatographed on a column of silica gel (200 g) with chloroform, and then with 50 : 1 chloroform–methanol. The latter eluate gave compound **5** (9.8 g, 84.5%) as crystals, mp 75°C, $[\alpha]_D$ +28.5° (c=1.1, chloroform); IR ν_{max}^{Nujol} cm^{-1}: 3300 (NH), 2650~2500 (OH), 1720 (C=O), 1630 and 1560 (amide), and 850 (Me_2C); NMR ($CDCl_3$): δ 1.39, 1.49 (2s, 6H, Me_2C), 1.43 (d, 3H, $J_{Me,CH}$ 7.0 Hz, MeCH), 2.06 (s, 3H, AcN), 4.50 (q, 1H, $J_{CH,Me}$ 7.0 Hz, CHMe) and 8.29 (d, 1H, $J_{NH,2}$ 9.5 Hz, NH).

Anal. Found: C, 52.74; H, 7.23; N, 4.56. Calcd. for $C_{14}H_{23}NO_7$: C, 52.99; H, 7.31; N, 4.41%.

*2-Acetamido-1,5-anhydro-2-deoxy-4,6-O-isopropylidene-3-O-[*D*-1-(methoxycarbonyl)ethyl]-*D*-glucitol* (**6**). To a solution of **5** (200 mg) in ether (10 ml) was added a large excess of an ethereal solution of diazomethane, and after 10 min, the mixture was evaporated to give **6** in a quantitative yield, mp 104~105°C, $[\alpha]_D$ +16.5° (c=0.5, methanol); IR ν_{max}^{Nujol} cm^{-1}: 3320 (NH), 1735 and 1220 (ester), 1670 and 1545 (amide), and 850 (Me_2C).

Anal. Found: C, 54.25; H, 7.53; N, 4.33. Calcd. for $C_{16}H_{25}NO_7$: C, 54.37; H, 7.60; N, 4.30%.

*N-[2-O-(2-Acetamido-1,5-anhydro-2,3-dideoxy-4,6-O-isopropylidene-*D*-glucitol-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl ester* (**7**). To a solution of **5** (2.3 g) in dry 1,4-dioxane (20 ml) were added, with stirring, *N*-hydroxysuccinimide (HOSu, 1.25 g) and dicyclohexylcarbodiimide (DCC, 3.0 g), before the mixture was stirred for 30 min at room temperature. L-Alanyl-D-isoglutamine methyl ester trifluoroacetate (3.2 g) and triethylamine (0.5 ml) were added to the mixture, which was stirred for 1 hr at room temperature; the precipitates were filtered off, and then washed with 1,4-dioxane. The filtrate and washings were combined and evaporated to a syrup which was extracted with chloroform. The extract was washed with water, dried (sodium sulfate) and evaporated to a syrup, which was chromatographed on a column of silica gel (50 g) with (a) chloroform, (b) 100:1 chloroform–methanol, and (c) 30 : 1 chloroform–methanol. Eluant (c) afforded **7** (3.5 g, 91%) as crystals, mp 84~85°C, $[\alpha]_D$ +15° (c=3.2, chloroform); IR ν_{max}^{Nujol} cm^{-1}: 3300 (NH), 1730 and 1270 (ester), 1660 and 1540 (amide), and 850 (Me_2C); NMR ($CDCl_3$): δ 1.31~1.50 (m, 12H, Me_2C, 2MeCH), 1.98 (s, 3H, AcN) and 3.64 (s, 3H, MeO).

Anal. Found: C, 52.20; H, 7.42; N, 10.55. Calcd. for $C_{23}H_{38}N_4O_{10}$: C, 52.07; H, 7.22; N, 10.56%.

*N-[2-O-(2-Acetamido-1,5-anhydro-2,3-dideoxy-*D*-glucitol-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl ester* (**8**). A solution of **7** (100 mg) in 80% aqueous acetic acid (2 ml) was heated for 2 hr at 45°C, and then evaporated. The residue was crystallized from ether to give **8** in a quantitative yield, mp 236~237°C, $[\alpha]_D$ +14.5° (c=0.2, methanol); IR ν_{max}^{Nujol} cm^{-1}: 3400, 3370 and 3300 (OH, NH), 1740 and 1230 (ester), and 1680, 1660, 1640 and 1550 (amide); NMR (1 : 1 $CDCl_3$–CD_3OD): δ 1.31, 1.40 (2d, 6H, $J_{Me,CH}$ 7.0 Hz, 2MeCH), 2.02 (s, 3H, AcN) and 3.68 (s, 3H, MeO).

Anal. Found: C, 48.95; H, 6.83; N, 11.36. Calcd. for $C_{20}H_{34}N_4O_{10}$: C, 48.97; H, 6.99; N, 11.42%.

*N-[2-O-(2-Acetamido-1,5-anhydro-2,3-dideoxy-*D*-glucitol-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine* (**9**). To a solution of **8** (50 mg) in methanol (15 ml) was added 0.1 M potassium hydroxide (5 ml). The solution was stirred for 5 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 **9** (quantitative) as crystals that showed a single spot in TLC mp 182~183°C, $[\alpha]_D$ +10.5° (c=0.8, methanol; lit.[4] $[\alpha]_D$ +15.3° (c=0.72, methanol).

Anal. Found: C, 47.61; H, 6.95; N, 11.68. Calcd. for $C_{19}H_{32}N_4O_{10}$: C, 47.89; H, 6.77; N, 11.76%.

*N-[2-O-(2-Acetamido-1,5-anhydro-2,3-dideoxy-6-O-octanoyl-*D*-glucitol-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl ester* (**10**). To a solution of **8** (60 mg) in dry pyridine (2.4 ml) and dichloromethane (0.4 ml) was added, with stirring, a solution of octanoyl chloride (23 mg) in dichloromethane (1 ml) at −30°C, before the mixture was stirred overnight at −30°C; methanol (1 ml) was then added to the mixture, and the solution was evaporated. The residue was chromatographed on a silica gel plate (Kieselge 60 F-254; E. Merck, Darmstadt, West Germany) with 5 : 1 chloroform–methanol to afford **10** (51 mg, 68%), mp 119~120°C, $[\alpha]_D$ +13.5° (c=0.4, 1 : 1 chloroform–methanol); IR ν_{max}^{KBr} cm^{-1}: 3300 (OH, NH), 2940 and 2850 (Me, methylene), 1740 and 1250 (ester), and 1660 and 1540 (amide); NMR (1 : 1 $CDCl_3$–CD_3OD): δ 0.86 (near t, 3H, J_{Me,CH_2} 6.0 Hz, $MeCH_2$), 1.27~1.45 (m, 10H, $5CH_2$), 1.36, 1.40 (2d, 6H, $J_{Me,CH}$ 7.6 Hz, 2MeCH), 1.93 (s, 3H, AcN) and 3.67 (s, 3H, MeO).

Anal. Found: C, 54.53; H, 7.88; N, 9.16. Calcd. for $C_{28}H_{48}N_4O_{11}$: C, 54.53; H, 7.85; N, 9.09%.

*The other N-[2-O-(2-acetamido-1,5-anhydro-2,3-dideoxy-6-O-acyl-*D*-glucitol-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl esters* (**11**, **12**, **13** and **14**) were prepared from **8** by 6-*O*-acylation, according to the procedure described for compound **10**.

Compound **11** was obtained as crystals in a 62% yield, mp 234~236°C, $[\alpha]_D$ +29° (c=1.0, 1 : 1 chloroform–methanol); IR ν_{max}^{KBr} cm^{-1}: 3280 (NH), 2940 and 2850 (Me, methylene), 1735 and 1220 (ester), and 1680, 1660, 1640, 1550 and 1540 (amide); NMR (1 : 1 $CDCl_3$–CD_3OD): δ 0.88 (near t, 3H, J_{Me,CH_2} 6.2 Hz, $MeCH_2$), 1.26~1.45 (m,

16H, $8CH_2$), 1.35, 1.38 (2d, 6H, $J_{Me,CH}$ 7.4 Hz, 2MeCH), 1.92 (s, 3H, AcN) and 3.63 (s, 3H, MeO).

Anal. Found: C, 57.01; H, 8.55; N, 8.19. Calcd. for $C_{32}H_{56}N_4O_{11}$: C, 57.13; H, 8.39; N, 8.33%.

Compound **12** was obtained as crystals in a 56% yield, and gave similar spectral data to those of compound **11**; mp 196°C, $[\alpha]_D$ +7.8° (c=0.4, 1:1 chloroform–methanol).

Anal. Found: C, 59.11; H, 8.93; N, 7.65. Calcd. for $C_{36}H_{64}N_4O_{11}$: C, 59.32; H, 8.85; N, 7.69%.

Compound **13** was obtained as crystals in a 54% yield, mp 215~219°C, $[\alpha]_D$ +15.0° (c=0.2, 1:1 chloroform–methanol).

Anal. Found: C, 60.23; H, 9.25; N, 7.42. Calcd. for $C_{38}H_{68}N_4O_{11}$: C, 60.29; H, 9.05; N, 7.40%.

Compound **14** was obtained as crystals in a 54% yield, mp 164°C, $[\alpha]_D$ +4.0° (c=0.4, 1:1 chloroform–methanol); IR ν_{max}^{KBr} cm^{-1}: 3300 (OH, NH), 2940 and 2850 (Me, methylene), 1730 and 1260 (ester), and 1660, 1550 and 1540 (amide); NMR (1:1 $CDCl_3$–CD_3OD): δ 0.86 (near t, 6H, J_{Me,CH_2} 6.4 Hz, $2MeCH_2$), 1.15~1.50 (m, 52H, $26CH_2$), 1.36, 1.39 (2d, 6H, $J_{Me,CH}$ 6.5 Hz, 2MeCH), 1.93 (s, 3H, AcN) and 3.66 (s, 3H, MeO).

Anal. Found: C, 64.71; H, 10.20; N, 6.00. Calcd. for $C_{50}H_{92}N_4O_{11}$: C, 64.90; H, 10.02; N, 6.06%.

*2-Acetamido-1,5-anhydro-2-deoxy-3-O-[*D*-1-(methoxycarbonyl)ethyl]-*D*-glucitol* (**15**). A solution of **6** (500 mg) in 80% aqueous acetic acid (2 ml) was heated for 2 hr at 45°C and then evaporated. The residue was crystallized from ether to afford **15** as needles in a quantitative yield, mp 123°C, $[\alpha]_D$ +38° (c=0.4, methanol).

Anal. Found: C, 49.72; H, 7.18; N, 4.75. Calcd. for $C_{12}H_{21}NO_7$: C, 49.78; H, 7.27; N, 4.81%.

*2-Acetamido-1,5-anhydro-6-bromo-2,6-dideoxy-3-O-[*D*-1-(methoxycarbonyl)ethyl]-*D*-glucitol* (**16**). To a solution of **15** (220 mg) in pyridine (15 ml) were added triphenylphosphine (430 mg) and carbon tetrabromide (290 mg), before the mixture was stirred for 2 hr at 40°C. Methanol (5 ml) was added to the mixture, and it was then evaporated at 45°C to a syrup, which was chromatographed on a column of silica gel (20 g) with chloroform and 200:1 chloroform–methanol. The latter eluate gave **16** (200 mg, 83%) as crystals, mp 176°C, $[\alpha]_D$ +50.5° (c=1.4, 9:1 chloroform–methanol); IR ν_{max}^{Nujol} cm^{-1}: 3350, 3230 (OH, NH), 1730 and 1230 (ester), and 1640 and 1560 (amide); NMR (1:1 $CDCl_3$–CD_3OD): δ 1.45 (d, 3H, $J_{Me,CH}$ 7.0 Hz, MeCH), 2.03 (s, 3H, AcN), 3.03 (t, 1H, $J_{3,4}=J_{4,5}$= 10.0 Hz, H-4), 3.78 (s, 3H, MeO), 4.44 (dd, 1H, $J_{2,3}$ 5.0, $J_{3,4}$ 10.0 Hz, H-3) and 4.80 (q, 1H, $J_{CH,Me}$ 7.0 Hz, CHMe).

Anal. Found: C, 40.52; H, 5.66; N, 3.86. Calcd. for $C_{12}H_{20}NO_6Br$: C, 40.69; H, 5.69; N, 3.95%.

*2-Acetamido-1,5-anhydro-6-bromo-2,6-dideoxy-3-O-[*D*-1-(methoxycarbonyl)ethyl]-4-O-(tetrahydropyran-2-yl)-*D*-glucitol* (**17**). To a stirred solution of **16** (140 mg) in dry 1,4-dioxane (5 ml) were added 2,3-dihydro-4*H*-pyran (0.1 ml) and *p*-toluenesulfonic acid monohydrate (10 mg). The mixture was stirred for 2 hr at room temperature, before being treated with Amberlite IR-45 (OH^-) resin to remove the acid, and evaporated. The residue was purified by chromatography on a column of silica gel (10 g) with chloroform, and then with 200:1 chloroform–methanol. The latter eluate gave **17** (161 mg, 93%) as needles, mp 140°C, $[\alpha]_D$ +64.5° (c=1.6, chloroform); IR ν_{max}^{KBr} cm^{-1}: 3350 (NH), 1730 and 1230 (ester), and 1670, 1640 and 1540 (amide); NMR ($CDCl_3$): δ 1.42 (d, 3H, $J_{Me,CH}$ 6.6 Hz, MeCH), 1.45~2.05 (m, 8H, $4CH_2$), 2.00 (s, 3H, AcN), 2.98 (near t, $J_{3,4}=J_{4,5}$=11.0 Hz, H-4), 3.78 (s, 3H, MeO), 4.83 (q, 1H, $J_{CH,Me}$ 6.6 Hz, CHMe) and 7.93 (near d, 1H, $J_{NH,2}$ 5.5 Hz, NH).

Anal. Found: C, 46.71; H, 6.53; N, 3.20. Calcd. for $C_{17}H_{28}NO_7Br$: C, 46.58; H, 6.44; N, 3.20%.

*N-{2-O-[2-Acetamido-1,5-anhydro-6-bromo-4-O-(tetrahydropyran-2-yl)-2,3,6-trideoxy-*D*-glucitol-3-yl]-*D*-lactoyl}-*L*-alanyl-*D*-isoglutamine methyl ester* (**18**). To a solution of **17** (150 mg) in 1,4-dioxane (5 ml) was added 0.1 M potassium hydroxide (5 ml), before the solution was stirred for 5 min at room temperature and treated with Amberlite IRC-50 (H^+) resin; the resin was then filtered off, and washed with 1,4-dioxane. The filtrate and washings were combined and evaporated to give the free acid. Coupling of the acid with L-alanyl-D-isoglutamine methyl ester trifluoroacetate (130 mg) was performed as described for the preparation of **7**, using HOSu (60 mg) and DCC (90 mg). The product was purified by chromatography on a column of silica gel (10 g) with (a) 100:1, (b) 50:1 and (c) 30:1 chloroform–methanol. Eluant (c) afforded **18** (210 mg, 96%) as an amorphous mass, mp 195~198°C, $[\alpha]_D$ +35° (c=2.0, 4:1 chloroform–methanol); IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1740 and 1260 (ester), and 1680, 1640 and 1550 (amide).

Anal. Found: C, 46.98; H, 6.55; N, 8.61. Calcd. for $C_{25}H_{41}N_4O_{10}Br$: C, 47.10; H, 6.48; N, 8.79%.

*N-{2-O-[2-Acetamido-6-S-acetyl-1,5-anhydro-4-O-(tetrahydropyran-2-yl)-6-thio-2,3,6-trideoxy-*D*-glucitol-3-yl]-*D*-lactoyl}-*L*-alanyl-*D*-isoglutamine methyl ester* (**19**). To a solution of **18** (200 mg) in dry acetone (5 ml) was added potassium thioacetate (200 mg), before the mixture was stirred overnight at room temperature and evaporated. The residue was extracted with chloroform, and the extract was washed with water, dried (sodium sulfate) and evaporated to a syrup, which was chromatographed on a column of silica gel (10 g) with (a) 100:1, (b) 50:1 and (c) 30:1 chloroform–methanol. Eluant (c) gave **19** (160 mg, 82%) as a syrup, $[\alpha]_D$ +29° (c=0.9, 1:1 chloroform–methanol); IR ν_{max}^{film} cm^{-1}: 3280 (NH), 1730 and 1250 (ester), 1690 (*S*-acetyl), and 1660, 1545 and 1530 (amide); NMR (1:1 $CDCl_3$–CD_3OD): δ: 1.36 (2d, 6H, $J_{Me,CH}$ 6.8 Hz, 2MeCH), 1.95 (s, 3H, AcN), 2.35 (s, 3H, AcS) and 3.65 (s, 3H, MeO).

Anal. Found: C, 51.01; H, 7.09; N, 8.79. Calcd. for

$C_{27}H_{44}N_4O_{11}S$: C, 51.25; H, 7.01; N, 8.85%.

*N-[2-O-(2-Acetamido-6-S-acetyl-1,5-anhydro-6-thio-2,3,6-trideoxy-*D*-glucitol-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl ester* (**20**). A solution of **19** (50 mg) in 80% aqueous acetic acid (3 ml) was heated for 3 hr at 60°C, and then evaporated. The residue was crystallized from ether to afford **20** (38 mg, 80%), mp 100~103°C, $[\alpha]_D$ +24.5° ($c=1.1$, chloroform–methanol); IR ν_{max}^{KBr} cm^{-1}: 3400~3250 (OH, NH), 1720 and 1250 (ester), 1690 (*S*-acetyl), and 1650 and 1540 (amide).

Anal. Found: C, 46.75; H, 6.58; N, 9.80. Calcd. for $C_{22}H_{36}N_4O_{11}S$: C, 46.80; H, 6.43; N, 9.92%.

*N-[2-O-(2-Acetamido-1,5-anhydro-6-S-octadecanoyl-6-thio-2,3,6-trideoxy-*D*-glucitol-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl ester* (**21**). To a solution of **19** (50 mg) in methanol (3 ml) was added sodium methoxide (8 mg), before the mixture was stirred for 30 min at room temperature, and then treated with Amberlite IRC-50 (H^+) resin to remove the base. The product was dissolved in pyridine (1.8 ml) and dry dichloromethane (0.5 ml), and a solution of octadecanoyl chloride (30 mg) in dry dichloromethane (1 ml) was added at −20°C to the mixture; this was stirred for 3 hr at 0°C, and then evaporated. A solution of the residue in 80% aqueous acetic acid (5 ml) was heated for 3 hr at 60°C, and evaporated. The product was chromatographed on a silica gel plate (Kieselgel 60 F-254) with 5 : 1 chloroform–methanol, to afford **21** (38 mg), 61%), mp 168~170°C, $[\alpha]_D$ +4.5° ($c=0.4$, 1 : 1 chloroform–methanol); IR ν_{max}^{KBr} cm^{-1}: 3400~3260 (OH, NH), 2930 and 2850 (Me, methylene), 1730 and 1240 (ester), 1690 (*S*-acyl), and 1660, 1550 and 1540 (amide); NMR ($CDCl_3$): δ 0.88 (near t, 3H, J_{Me,CH_2} 6.0 Hz, $MeCH_2$), 1.20~1.45 (m, 36H, $15CH_2$, 2MeCH), 1.98 (s, 3H, AcN) and 3.68 (s, 3H, MeO).

Anal. Found: C, 57.61; H, 8.88; N, 6.95. Calcd. for $C_{38}H_{68}N_4O_{11}S$: C, 57.84; H, 8.69; N, 7.10%.

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