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SYNTHESIS OF *N*-[2-*O*-(2-ACETAMIDO-2,3-DIDEOXY-5-THIO-D-GLUCOPYRANOSE-3-YL)-D-LACTOYL]-L-ALANYL-D-ISOGLUTAMINE*

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ABSTRACT

N-[2-*O*-(2-Acetamido-2,3-dideoxy-5-thio-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine, in which the ring-oxygen atom of the sugar moiety in *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) has been replaced by sulfur, was synthesized from 2-acetamido-2-deoxy-5-thio-α-D-glucopyranose (**1**). *O*-Deacetylation of the acetylated acetal, derived from the methyl α-glycoside of **1** by 4,6-*O*-isopropylidenation and subsequent acetylation, gave methyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-5-thio-α-D-glucopyranoside (**4**). Condensation of **4** with L-2-chloropropanoic acid, and subsequent esterification, afforded the corresponding ester, which was converted, *via* *O*-deisopropylidenation, acetylation, and acetolysis, into 2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-5-thio-α-D-glucopyranose (**12**). Coupling of the acid, formed from **12** by hydrolysis, with the methyl ester of L-alanyl-D-isoglutamine, and de-esterification, yielded the title compound.

INTRODUCTION

In the course of an investigation on the relationship between the immunoadjuvant activity of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) and the structure of the carbohydrate moiety, it was demonstrated that not only the restricted configuration of the sugar moiety[2] but also chemical modifications[3–6] of the functional group in the carbohydrate moiety produce various, important effects on the manifestation of activity. Moreover, it has been shown that introduction[3b,6–8] of lipophilic character at the restricted position of the sugar skeleton in MDP, and its carbohydrate analogs carrying adjuvant activity, causes potent antitumor and anti-infection activities, based on the immune reaction, that are not found for MDP itself, as well as strong, immunoadjuvant activities. In view of these facts, we now

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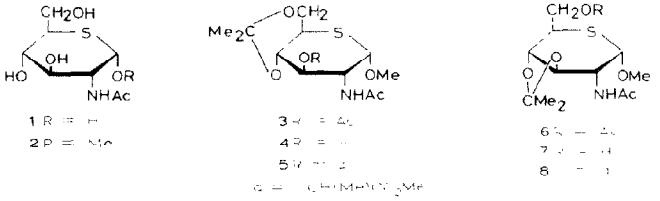
describe the synthesis of *N*-acetyl-5-thio-muramoyl-L-alanyl-D-isoglutamine, in which the ring-oxygen atom of the sugar moiety in MDP has been replaced by sulfur, the work being undertaken as part of a program aimed at finding a strong, immunoadjuvant-active compound.

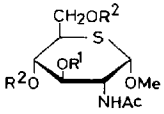
RESULTS AND DISCUSSION

Treatment of 2-acetamido-2-deoxy-5-thio-α-D-glucopyranose[9] (**1**) with methanol in the presence of Amberlite IR-120 (H^+) resin at 60° gave methyl 2-acetamido-2-deoxy-5-thio-α-D-glucopyranoside (**2**) in 86% yield. When treated with a large excess of 2,2-dimethoxypropane in dry 1,4-dioxane in the presence of *p*-toluenesulfonic acid for 40 min at room temperature, compound **2** gave a mixture showing a single spot in t.l.c., from which, after acetylation, methyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-isopropyridene-5-thio-α-D-glucopyranoside (**3**, 46%) and methyl 2-acetamido-6-*O*-acetyl-3,4-*O*-isopropylidene-5-thio-α-D-glucopyranoside (**6**; 52%) were isolated. On the other hand, when the reaction was performed in *N*,*N*-dimethylformamide (instead of 1,4-dioxane) as the solvent, compound **2** gave only the 4,6-*O*-isopropylidene derivative (**3**), in 83% yield. Hydrolysis of the *O*-acetyl group in **3** and **6** with sodium methoxide in methanol gave **4** and **7**, respectively, in good yield. When condensed with L-2-chloropropanoic acid in the presence of sodium hydride, followed by methylation with diazomethane, compounds **4** and **7** respectively gave methyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[D-1-(methoxycarbonyl)ethyl]-5-thio-α-D-glucopyranoside (**5**; 65%) and methyl 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-6-*O*-[D-1-(methoxycarbonyl)ethyl]-5-thio-α-D-glucopyranoside (**8**; 72%). All of the spectral features were consistent with structures **5** and **8**, respectively.

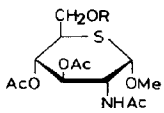


O-Deisopropylidenation of **5** or **8** by mild, acid hydrolysis, and subsequent acetylation with acetic anhydride in pyridine, respectively gave methyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-5-thio-α-D-glucopyranoside (**10**) and methyl 2-acetamido-3,4-di-*O*-acetyl-6-*O*-[D-1-(methoxycarbonyl)ethyl]-5-thio-α-D-glucopyranoside (**11**) in good yields. Treatment of **10** with acetic anhydride in acetic acid–sulfuric acid for 3 days at room tem-

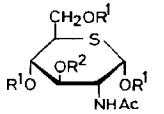
perature afforded crystalline 2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-5-thio-α-D-glucopyranose (**12**) in 94% yield; significant signals in its n.m.r. spectrum were a three-proton doublet at δ 1.39 ($J_{Me,CH}$ 6.4 Hz, MeCH), a one-proton doublet of doublets at δ 5.38 ($J_{3,4}$ 9.2, $J_{4,5}$ 10.5 Hz, H-4), and a one-proton doublet at δ 6.35 ($J_{1,2}$ 2.5 Hz, H-1). Other n.m.r. data are given in the Experimental section, and are consistent with structure **12**. Saponification of



9 R^1 = α, R^2 = H
10 R^1 = α, R^2 = Ac



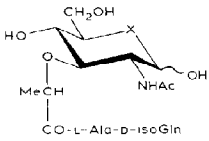
11 R = α



12 R^1 = Ac, R^2 = α
13 R^1 = H, R^2 = b

b = –CH(Me)-CO-L-Ala-D-isoGln-OMe

12, and coupling of the product with L-alanyl-D-isoglutamine methyl ester, using dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as the activating agents, yielded *N*-[2-*O*-(2-acetamido-2,3-dideoxy-5-thio-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (**13**) in 56% yield. Treatment of **13** with 0.1M potassium hydroxide gave **14** in quantitative yield.



MDP X = O
14 X = S

The immunoadjuvant activities of compounds **13** and **14** 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[10] (see Table I). Both of the compounds showed negligible activity, indicating that the ring-oxygen atom of the sugar skeleton in MDP is critical for 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.; 300 mesh) with the solvent systems specified. Specific rotations were determined with

TABLE I

ADJUVANT ACTIVITY OF *N*-ACETYL-5-THIOMURAMOYL-L-ALANYL-D-ISOGLUTAMINES ON THE INDUCTION OF 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 h	*48 h*
13	(9.9 ±0.2)	(4.5 ±0.6)
14	(13.4 ±0.9)	(8.4 ±0.7)
MDP	23.4 ±0.4	20.0 ±0.5
Control[d]	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]ABA-*N*-acetyltyrosine in Freund's incomplete adjuvant.

a Union PM-201 polarimeter, and i.r. spectra were recorded with a Jasco IRA-1 spectrophotometer. N.m.r. spectra were recorded at 90 MHz with a Hitachi R-22 spectrometer, and the n.m.r. data were confirmed by use of decoupling techniques.

*Methyl 2-acetamido-2-deoxy-5-thio-α-*D*-glucopyranoside* (**2**). — To a solution of 2-acetamido-2-deoxy-5-thio-α-D-glucopyranose[9] (**1**; 270 mg) in methanol (15 mL) was added Amberlite IR-120 (H^+) resin (3 g), and the mixture was stirred for 2 h at 65°. The resin was filtered off, and the filtrate evaporated to a syrup which crystallized from ether. Recrystallization from ethanol–ether afforded **2** (245 mg, 86%) as needles; m.p. 204°, $[\alpha]_D^{25}$ +243° (*c* 0.5, methanol); ν_{max}^{Nujol} 3350, 3270 (OH, NH), and 1630 and 1550 cm^{-1} (amide); n.m.r. data (in 1:1 methanol-d_4–D_2O): δ 2.02 (s, 3 H, AcN), 2.91–3.12 (m, 1 H, H-5), 3.40 (s, 3 H, MeO), and 4.47 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1).

Anal. Calc. for $C_9H_{17}NO_5S$: C, 43.01; H, 6.82; N, 5.57. Found: C, 43.22; H, 6.81; N, 5.53.

*Methyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-5-thio-α-*D*-glucopyranoside* (**3**) *and methyl 2-acetamido-6-*O*-acetyl-2-deoxy-3,4-*O*-isopropylidene-5-thio-α-*D*-glucopyranoside* (**6**). — A suspension of **2** (80 mg) in dry 1,4-dioxane (5 mL) and 2,2-dimethoxypropane (0.5 mL) was stirred at room temperature while *p*-toluenesulfonic acid monohydrate (5 mg) was added; stirring was continued for 40 min at room temperature. The mixture was treated with Amberlite IR-410 (OH^-) resin to remove the acid, and the resin was filtered off and washed with methanol. The filtrate and washings were combined, and evaporated to a syrup which was acetylated with acetic anhydride (1 mL)–pyridine (2 mL) at room temperature. The product was chromatographed on a column of silica gel (10 g) with chloroform and then 200:1 chloroform–methanol. With the latter eluant, compound **3** issued as the faster-moving component, and was obtained as a syrup (49 mg, 46%); $[\alpha]_D^{25}$ +138° (*c* 0.3, chloroform); ν_{max}^{Nujol} 3270 (NH), 1740 and 1240

(ester), 1660 and 1530 (amide), and 855 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 1.35, 1.47 (2 s, 6 H, Me_2C), 1.95 (s, 3 H, AcN), 2.04 (s, 3 H, AcO), 2.98–3.27 (m, 1 H, H-5), 3.40 (s, 3 H, MeO), 3.61–3.87 (m, 2 H, H-6,6'), 4.02 (dd, 1 H, $J_{3,4}$ 10.0, $J_{4,5}$ 9.0 Hz, H-4), 4.44 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 4.38–4.64 (m, 1 H, H-2), 5.10 (dd, 1 H, $J_{2,3}$ 9.0, $J_{3,4}$ 10.0 Hz, H-3), and 6.10 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH).

Compound **6** emerged as the slower-moving component (55 mg, 52%); $[\alpha]_D^{25}$ +188° (*c* 0.3, chloroform); ν_{max}^{Nujol} 3320 (NH), 1750 and 1240 (ester), 1660 and 1530 (amide), and 860 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 1.41, 1.42 (2 s, 6 H, Me_2C), 2.02 (s, 3 H, AcN), 2.09 (s, 3 H, AcO), 3.41 (s, 3 H, MeO), 4.46 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), and 6.68 (d, 1 H, $J_{NH,2}$ 8.0 Hz, NH).

Anal. Calc. for $C_{14}H_{23}NO_6S$: C, 50.43; H, 6.95; N, 4.20. Found: for compound **3**; C, 50.26; H, 6.83; N, 4.20; for compound **6**; C, 50.35; H, 6.99; N, 4.16.

*Methyl 2-acetamido-3-O-acetyl-2-deoxy-4,6-O-isopropylidene-5-thio-α-*D*-glucopyranoside* (**3**). — To a solution of **2** (150 mg) in *N,N*-dimethylformamide (10 mL) were added 2,2-dimethoxypropane (1 mL) and *p*-toluenesulfonic acid (10 mg). The mixture was stirred for 40 min at room temperature, and then treated with Amberlite IR-410 (OH^-) resin to remove the acid. The solution was evaporated to a syrup which was acetylated overnight at room temperature with acetic anhydride–pyridine. The product was purified by chromatography on a column of silica gel (20 g) with chloroform and then 200:1 chloroform–methanol. The latter eluate yielded **3** (165 mg, 83%) whose i.r. and n.m.r. spectra were identical with those of the sample prepared as in the previous section.

*Methyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-5-thio-α-*D*-glucopyranoside* (**4**). — To an ice-cooled solution of **3** (40 mg) in methanol (5 mL) was added sodium methoxide (5 mg); after 10 min, the solution was treated with Amberlite IR-120 (H^+) resin to remove the base. Compound **4** was obtained as a syrup (34 mg, 97%); $[\alpha]_D^{25}$ +160° (*c* 0.4, chloroform); ν_{max}^{film} 3260 (OH, NH), 1650 and 1540 (amide), and 850 cm^{-1} (Me_2C).

Anal. Calc. for $C_{12}H_{21}NO_5S$: C, 49.46; H, 7.27; N, 4.81. Found: C, 49.53; H, 7.46; N, 4.59.

*Methyl 2-acetamido-2-deoxy-3,4-O-isopropylidene-5-thio-α-*D*-glucopyranoside* (**7**). — *O*-Deacetylation of **6** (80 mg) with sodium methoxide (5 mg) in methanol (5 mL), as described in the preparation of **4**, afforded **7** (66 mg, 94%) as a syrup; $[\alpha]_D^{25}$ +171° (*c* 0.6, chloroform); ν_{max}^{film} 3300 (OH, NH), 1650 and 1540 (amide), and 855 cm^{-1} (Me_2C).

Anal. Calc. for $C_{12}H_{21}NO_5S$: C, 49.46; H, 7.27; N, 4.81. Found: C, 49.58; H, 7.33; N, 4.52.

*Methyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-[*D*-1-(methoxycarbonyl)ethyl]-5-thio-α-*D*-glucopyranoside* (**5**). — To a stirred solution of **4** (80 mg) in dry 1,4-dioxane (3 mL) was added sodium hydride in oil suspension (100 mg; 50% of sodium hydride by weight). The mixture was kept for 30 min at 90°, and then L-2-chloropropanoic acid (35 mg) was added, with stirring, at 60°. The mixture was stirred for 1.5 h at 90°, and cooled. Methanol (20 mL) was added to the solution.

The mixture was treated with Amberlite IRC-50 (H^+) resin while the pH of the mixture was adjusted to 8 by adding triethylamine. The resin was filtered off, and washed with methanol, and the filtrate and washings were combined, and evaporated. To a solution of the residue in methanol (5 mL) was added an ether solution of diazomethane; after 10 min, the excess of the reagent was decomposed by adding acetic acid, and the mixture was 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 (10 g) with chloroform, to afford compound **5** (75 mg, 65%) as needles; m.p. 118–120°, $[\alpha]_D^{25}$ +214° (*c* 0.3, chloroform); ν_{max}^{Nujol} 3330 (NH), 1740 and 1230 (ester), 1670 and 1530 (amide), and 860 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 1.37 (d, 3 H, $J_{Me,CH}$ 6.2 Hz, MeC), 1.38, 1.50 (2 s, 6 H, Me_2C), 2.03 (s, 3 H, AcN), 2.92–3.21 (m, 1 H, H-5), 3.34 (s, 3 H, MeO), 3.73 (s, 3 H, MeOCO), 4.58 (q, 1 H, $J_{CH,Me}$ 6.2 Hz, CH), 4.98 (d, 1 H, $J_{1,2}$ 2.6 Hz, H-1), and 8.00 (d, 1 H, $J_{NH,2}$ 5.5 Hz, NH).

Anal. Calc. for $C_{16}H_{27}NO_8S$: C, 48.84; H, 6.92; N, 3.56. Found: C, 48.80; H, 6.86; N, 3.49.

Methyl 2-acetamido-2-deoxy-3,4-O-isopropylidene-6-O-[D-*1-(methoxycarbonyl)ethyl*]*-5-thio-α-*D-*glucopyranoside* (**8**). — To a stirred solution of **7** (80 mg) in dry 1,4-dioxane (3 mL) was added the sodium hydride reagent (100 mg), and the mixture was kept, with stirring, for 1 h at 90–95°, and then cooled. L-2-Chloropropanoic acid (70 mg) and the sodium hydride reagent (40 mg) were added to the stirred mixture, which was then kept at 65–70°, the progress of the reaction being monitored by t.l.c.; after 1.5 h, the starting material was no longer detectable. The procedure used for the preparation of **5** gave compound **8** (83 mg, 72%) as a syrup; $[\alpha]_D^{25}$ +174° (*c* 0.25, chloroform); ν_{max}^{film} 3300 (NH), 1750 and 1230 (ester), 1650 and 1530 (amide), and 860 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 1.38 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeC), 1.40 (s, 6 H, Me_2C), 1.98 (s, 3 H, AcN), 3.38 (s, 3 H, MeO), 3.73 (s, 3 H, MeOCO), 4.00 (q, 1 H, $J_{CH,Me}$ 7.0 Hz, CH), 4.30–4.58 (m, 1 H, H-2), 4.59 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), and 6.26 (d, 1 H, $J_{NH,2}$ 8.0 Hz, NH).

Anal. Calc. for $C_{16}H_{27}NO_8S$: C, 48.84; H, 6.92; N, 3.56. Found: C, 48.76; H, 7.15; N, 3.48.

Methyl 2-acetamido-2-deoxy-3-O-[D-*1-(methoxycarbonyl)ethyl*]*-5-thio-α-*D-*glucopyranoside* (**9**). — A solution of **5** (200 mg) in 70% aqueous acetic acid (3 mL) was heated for 2.5 h at 45°, and evaporated, and the residue crystallized from ether to give **9** (165 mg, 96%) as needles; m.p. 233°, $[\alpha]_D^{25}$ +227° (*c* 0.3, chloroform); ν_{max}^{Nujol} 3430, 3360, and 3270 (OH, NH), 1730 and 1240 (ester), and 1640 and 1540 cm^{-1} (amide).

Anal. Calc. for $C_{13}H_{23}NO_7S$: C, 46.28; H, 6.87; N, 4.15. Found: C, 46.20; H, 6.84; N, 4.06.

Methyl 2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-[D-*1-(methoxycarbonyl)ethyl*]*-5-thio-α-*D-*glucopyranoside* (**10**). — Compound **9** (130 mg) was heated with a mixture of acetic anhydride (1 mL) and pyridine (2 mL) for 3 h at 50°. The mixture was evaporated, and the residue crystallized from ether–hexane to give **10** (155

mg, 95%) as needles; m.p. 115–116°, $[\alpha]_D^{25}$ +196° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3320 (NH), 1760, 1735, and 1250 (ester), and 1650 and 1530 cm^{-1} (amide); n.m.r. data (in chloroform-*d*): δ 1.35 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeC), 2.02, 2.03, 2.12 (3 s, 9 H, AcN, 2 AcO), 3.14–3.44 (m, 1 H, H-5), 3.38 (s, 3 H, MeO), 3.80 (s, 3 H, MeOCO), 4.94 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 5.31 (dd, 1 H, $J_{3,4}$ 10.5, $J_{4,5}$ 9.0 Hz, H-4), and 8.22 (d, 1 H, $J_{NH,2}$ 5.0 Hz, NH).

Anal. Calc. for $C_{17}H_{27}NO_9S$: C, 48.44; H, 6.46; N, 3.32. Found: C, 48.33; H, 6.29; N, 3.26.

*Methyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-[*D*-1-(methoxycarbonyl)-ethyl]-5-thio-α-*D*-glucopyranoside* (**11**). — A solution of **8** (25 mg) in 7:3 acetic acid–water (5 mL) was heated for 2 h at 45°; it was then evaporated to a syrup which was acetylated by heating with acetic anhydride (0.2 mL) and pyridine (0.5 mL) for 1 h at 50°. The reagents were removed by evaporation, and the residue was chromatographed on a column of silica gel (10 g) with 200:1 chloroform–methanol to give **11** (26 mg, 96%) as a syrup; $[\alpha]_D^{25}$ +150° (*c* 0.2, chloroform); ν_{max}^{film} 3280 (NH), 1750 and 1240 (ester), and 1660 and 1530 cm^{-1} (amide); n.m.r. data (in chloroform-*d*): δ 1.38 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeC), 1.96, 2.02, 2.04 (3 s, 9 H, AcN, 2 AcO), 3.22–3.56 (m, 1 H, H-5), 3.45 (s, 3 H, MeO), 3.73 (s, 3 H, MeOCO), 3.93 (q, 1 H, $J_{CH,Me}$ 7.0 Hz, CH), 4.44 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 4.57 (m, 1 H, H-2), 5.17 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 5.33 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.0 Hz, H-4), and 5.96 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH).

Anal. Calc. for $C_{17}H_{27}NO_9S$: C, 48.44; H, 6.46; N, 3.32. Found: C, 48.19; H, 6.58; N, 3.26.

*2-Acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-3-*O*-[*D*-1-(methoxycarbonyl)ethyl]-5-thio-α-*D*-glucopyranose* (**12**). — A sample of **10** (120 mg) was dissolved in a mixture of acetic anhydride (11 mL), acetic acid (5.5 mL), and sulfuric acid (0.23 mL), and the solution was kept for 3 days at room temperature, while the progress of the reaction was monitored by t.l.c. The mixture was poured into ice–water, the pH adjusted to 5 by addition of sodium hydrogencarbonate, and the solution thoroughly 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 (15 g) with (*a*) 150:1, and (*b*) 100:1 chloroform–methanol. Eluant (*b*) gave **12** (120 mg, 94%) as needles; m.p. 158–160°, $[\alpha]_D^{25}$ +191° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3340 (NH), 1750, 1250, and 1220 (ester), and 1670 and 1530 cm^{-1} (amide); n.m.r. data (in chloroform-*d*): δ 1.39 (d, 3 H, $J_{Me,CH}$ 6.4 Hz, MeC), 2.00, 2.09, 2.11, 2.15 (4 s, 12 H, AcN, 3 AcO), 3.31–3.55 (m, 1 H, H-5), 5.38 (dd, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 10.5 Hz, H-4), 6.35 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), and 8.23 (d, 1 H, $J_{NH,2}$ 4.0 Hz, NH).

Anal. Calc. for $C_{18}H_{27}NO_{10}S$: C, 48.10; H, 6.06; N, 3.12. Found: C, 48.23; H, 6.05; N, 3.12.

*N-[2-*O*-(2-Acetamido-2,3-dideoxy-5-thio-*D*-glucopyranose-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl ester* (**13**). — To an ice-cooled solution of **12** (100 mg) in methanol (10 mL) was added sodium methoxide (20 mg), and the mixture was kept for 10 min at room temperature, and then treated with Amberlite-IR-120

(H^+) resin to remove the base. After filtration, the solution was evaporated, and dried. A suspension of the residue in dry 1,4-dioxane (4 mL) was stirred at room temperature while *N*-hydroxysuccinimide (100 mg) and dicyclohexylcarbodiimide (210 mg) were added; stirring was continued for 30 min at room temperature. The 1,3-dicyclohexylurea formed was filtered off, and washed with dry 1,4-dioxane (2 mL). To a solution of the activated ester in dry 1,4-dioxane (6 mL) were added L-alanyl-D-isoglutamine methyl ester trifluoroacetate (100 mg) and triethylamine (0.05 mL), and the mixture was stirred for 1 h at room temperature, and then evaporated. The residue was chromatographed on a column of silica gel (20 g) with (*a*) 50:1, (*b*) 20:1, and (*c*) 10:1 chloroform–methanol. Eluant (*c*) afforded **13** (65 mg, 56%) as crystals; m.p. 90–95° (dec.), $[\alpha]_D^{25}$ +59° (*c* 0.2, methanol); ν_{max}^{KBr} 3350 (OH, NH), 1720 and 1250 (ester), and 1670, 1630, and 1520 cm^{-1} (amide).

Anal. Calc. for $C_{20}H_{34}N_4O_{10}S$: C, 45.97; H, 6.56; N, 10.72. Found: C, 45.68; H, 6.71; N, 10.55.

N-[*2-O-(2-Acetamido-2,3-dideoxy-5-thio*-D-*glucopyranose-3-yl*)-D-*lactoyl*]-L-*alanyl*-D-*isoglutamine* (**14**). — To a solution of **13** (22 mg) in methanol (2 mL) was added 0.1M potassium hydroxide (2 mL), and 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 below 30°, to give **14** (20 mg; quantitative) as crystals that showed a single spot in t.l.c.; m.p. 150–160° (dec.), $[\alpha]_D^{25}$ +55° (*c* 0.25, methanol); ν_{max}^{KBr} 3350 (OH, NH), 1720 (C=O), and 1620 and 1520 cm^{-1} (amide).

Anal. Calc. for $C_{19}H_{32}N_4O_{10}S$: C, 44.87; H, 6.34; N, 11.02. Found: C, 44.76; H, 6.53; N, 10.94.

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