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Note

Synthesis and immunoadjuvant activity of *N*-[2-*O*-(2-acetamido-1,2,3,5-tetradeoxy-1,5-imino-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine*

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N-Acetylmuramoyl-L-alanyl-D-isoglutamine (MDP), a fragment of bacterial peptidoglycan, is capable of replacing whole mycobacteria in complete Freund's adjuvant[2]. In the course of our investigation of the relationship between the chemical structure and biological activities of MDP, it has been revealed that chemical modifications of the carbohydrate moiety produce various, important effects on the manifestation of the biological activities. MDP analogs in which the hydroxyl group at C-1 of the sugar skeleton is replaced by hydrogen[3] or an acylthio group[4] show the same potent immunoadjuvant activity as MDP. On the other hand, the replacement of the oxygen in the pyranose ring by a sulfur atom[5] reduces the activity, indicating the importance of closely retaining the conformation of the sugar skeleton. Very recently Barton *et al.*[6] reported the synthesis of carbocyclic analogs of MDP, but their biological activities were not described.

It is well known that 1,5-dideoxy-1,5-imino-D-glucitol (1-deoxynojirimycin), which is a glucose analog with an NH group substituting for the ring oxygen, has a potent inhibitory effect on α-glucosidase owing to a structural resemblance to glucose[7].

In view of these facts, we now describe as part of our continuing research on structure–activity relationships in the MDP series the synthesis and immunoadjuvant activity of *N*-acetyl-1,5-dideoxy-1,5-imino-muramoyl-L-alanyl-D-isoglutamine.

RESULTS AND DISCUSSION

We employed 2-azido-4,6-*O*-benzylidene-*N*-(*tert*-butoxycarbonyl)-1,2,5-trideoxy-1,5-imino-D-glucitol[8] (**1**), derived from 1,5-dideoxy-1,5-imino-D-glucitol (1-deoxy-

* Studies on immunoadjuvant active compounds, Part 42. For Part 41, see ref. 1.

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TABLE I

Adjuvant activity of MDP analogs on delayed-type hypersensitivity to ABA–*N*-acetyltyrosine in guinea pigs

Compound	*Dose (μg)*	*Skin reaction with ABA–BSA[a] (100 μg) (diam. in mm ± s.e.)[b]*	
		24h	*48h*
6	10	6.0 ± 0.7	0
9	10	6.5 ± 1.2	2.0 ± 4.0
MDP	10	20.3 ± 0.9	18.8 ± 1.0
Control[c]	–	6.3 ± 0.8	0

[a] Azobenzenearsonate–*N*-acetyl-L-tyrosine–bovine serum albumin. [b] The data indicate the average diameter ± the standard error (s.e.) of the skin reaction (induration) of four guinea pigs. [c] ABA–*N*-acetyltyrosine in Freund's incomplete adjuvant.

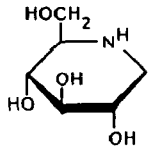
nojirimycin), as a starting material suitable for the synthesis of the title compound (**6**). The preparation of **1**, described in detail elsewhere[8], involved the successive 3-*O*-chloroacetylation, 2-O-methylsulfonylation, and sodium methoxide treatment of 4,6-*O*-benzylidene-*N*-(*tert*-butoxycarbonyl)-1,5-dideoxy-1,5-imino-D-glucitol to give 2, 3-anhydro-4,6-*O*-benzylidene-*N*-(*tert*-butoxycarbonyl)-1,5-dideoxy-1,5-imino-D-mannitol. The epoxide obtained was converted into **1** by treatment with sodium azide. It is noteworthy that diequatorial ring opening occurred preferentially to diaxial opening in this reaction, presumably because of a distortion of the pyranose ring characteristic of nojirimycin derivatives. Treatment of **1** with L-2-chloropropionic acid in the presence of sodium hydride gave the corresponding 3-*O*-(D-1-carboxyethyl) derivative (**2**) in a 72% yield. Coupling of **2** with L-alanyl-D-isoglutamine methyl ester, using dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as the activating agents, afforded *N*-[2-*O*-[2-azido-4,6-*O*-benzylidene-*N*- (*tert*-butoxycarbonyl) -1,2,3,5-tetradeoxy-1,5-imino-D-glucitol-3-yl]-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (**3**) in 91% yield.

The azido group in **3** was converted into acetamido by catalytic hydrogenation over palladium on carbon and subsequent acetylation with acetic anhydride, to give intermediate **4**. Treatment of **4** with 0.2M potassium hydroxide gave the free acid **5**. Finally, the *tert*-butoxycarbonyl and benzylidene groups were simultaneously removed by treatment with aqueous trifluoroacetic acid to yield the title compound **6** as its trifluoroacetate salt.

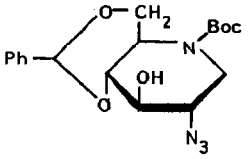
In order to prepare the 6-*O*-stearoyl derivative **9**, the benzylidene group in **4** was removed selectively by hydrogenolysis. Condensation of **7** with stearic acid using DCC and 4-dimethylaminopyridine in 1,4-dioxane–*N*,*N*-dimethylformamide gave **8**, which was converted, by hydrolytic removal of the *tert*-butoxycarbonyl group, into the desired *N*-[2-*O*-(2-acetamido-1,2,3,5-tetradeoxy-1,5-imino-6-*O*-octadecanoyl-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester trifluoroacetate **9**.

Compounds **6** and **9** showed negligible activity in the induction of delayed-type hypersensitivity in guinea pigs[9] (see Table I). As reported previously, 5-thio analogs of MDP also exhibit only negligible activity. Taking these facts into consideration, it may

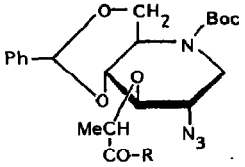
be concluded that the ring-oxygen atom in the sugar skeleton of MDP is essential for manifestation of the activity.



1-deoxynojirimycin

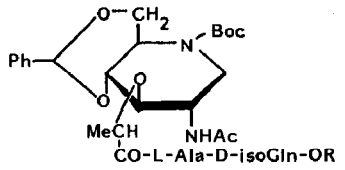


1



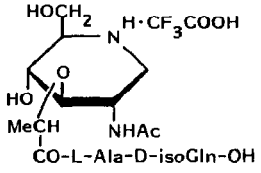
2 R = OH

3 R = L-Ala-D-isoGln-OMe

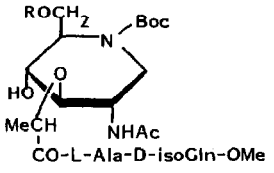


4 R = Me

5 R = H

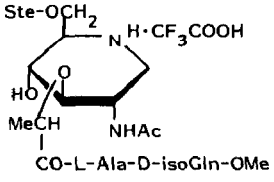


6



7 R = H

8 R = Ste



9

Ste: stearoyl

Boc: tert-butoxycarbonyl

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were measured with a Union PM-201 polarimeter, and i.r. spectra were recorded with a Jasco A-100 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 270 MHz with a JEOL JNM-GX-270 spectrometer, for solutions in chloroform-*d* unless otherwise noted. T.l.c. was performed on Silica Gel 60 (Merck, aluminum sheets), and column chromatography on silica gel

(Wako Co., 200 or 300 mesh) with the solvent systems (v/v) specified.

2-Azido-4,6-O-benzylidene-N-(tert-butoxycarbonyl)-3-O-(D-1-carboxyethyl)-1,2,5-trideoxy-1,5-imino-D-glucitol (**2**). — To a solution of 2-azido-4,6-*O*-benzylidene-*N*-(*tert*-butoxycarbonyl)-1,2,5-trideoxy-1,5-imino-D-glucitol[8] (**1**) (220 mg, 0.58 mmol) in 1,4-dioxane (3 mL) was added a 60% suspension of sodium hydride (42 mg of NaH, 1.75 mmol). The mixture was stirred for 1 h at 60°, then a solution of L-2-chloropropionic acid (100 mg, 0.92 mmol) in 1,4-dioxane (0.5 mL) was added with stirring. The mixture was stirred for 4 h at 60°, and concentrated. The residue was extracted with dichloromethane, and the extract was successively washed with 2M hydrochloric acid and water, dried (sodium sulfate), and concentrated to a syrup. The syrup was chromatographed on a column of silica gel with 40:1 dichloromethane–methanol to give compound **2** (188 mg, 72%) as a syrup; $[\alpha]_D$ −17.9° (*c* 0.7, CH_2Cl_2); i.r.: ν_{max} 3600–2400 (COOH), 3000, 2950 (CH_3, CH_2), 2120 (N_3), 1750, 1700 (C=O), 750, and 700 cm^{-1} (phenyl); ^{1}H-n.m.r.: δ 1.43–1.56 [m, 12 H, C*H*$_3$CH, (C*H*$_3$)$_3$C], 2.75 (dd, 1 H, J_{gem} 13.6, $J_{1ax,2}$ 9.2 Hz, H-1*ax*), 3.15 (m, 1 H, H-5), 3.76 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.8 Hz, H-3), 4.00 (dd, 1 H, $J_{1eq,2}$ 3.7 Hz, H-1*eq*), 4.29 (t, 1 H, $J_{5,6a} = J_{gem}$ = 11.4 Hz, H-6a), 4.54 (q, 1 H, *J* 7.0 Hz, CH$_3$C*H*), 4.98 (dd, 1 H, $J_{5,6b}$ 4.0 Hz, H-6b), 5.53 (s, 1 H, C*H*Ph), and 7.31–7.55 (m, 5 H, phenyl).

Anal. Calc. for $C_{21}H_{28}N_4O_7$ (504.47): C, 56.24; H, 6.29; N, 12.49. Found: C, 56.21; H, 6.13; N, 12.31.

N-*[2-O-[2-Azido-4,6-O-benzylidene-N-(tert-butoxycarbonyl)-1,2,3,5-tetradeoxy-1,5-imino-D-glucitol-3-yl]-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester* (**3**). — To a solution of **2** (30 mg, 0.054 mmol) in dry 1,4-dioxane (1 mL) was added *N*-hydroxysuccinimide (12 mg, 0.10 mmol) and dicyclohexylcarbodiimide (28 mg, 0.14 mmol), and the mixture was stirred for 1 h at room temperature. L-Alanyl-D-isoglutamine methyl ester trifluoroacetate (28 mg, 0.17 mmol) and triethylamine (1 drop) were added to the mixture, which was further stirred for 2 h at room temperature, and then the precipitate was filtered off and washed with 1,4-dioxane. The filtrate and washings were combined and concentrated to a syrup, which was chromatographed on a column of silica gel with (*A*) 150:1, (*B*) 40:1 dichloromethane–methanol. Elution with solvent *B* afforded compound **3** (40 mg, 91%) as a syrup; $[\alpha]_D$ −3.9° (*c* 0.9, CH_2Cl_2); i.r.: ν_{max} 3350 (NH), 3000, 2950, 2870 (CH_3, CH_2), 2120 (N_3), 1750 (ester), 1680, 1540 (amide), 740, and 700 cm^{-1} (phenyl); ^{1}H-n.m.r.: δ 1.33–1.40 (m, 6 H, 2 C*H*$_3$CH), 1.48 [s, 9 H, (C*H*$_3$)$_3$C], 1.80–2.47 (m, 4 H, C*H*$_2$C*H*$_2$CO of isoGln), 2.75 (dd, 1 H, J_{gem} 13.6, $J_{1ax,2}$ 11.0 Hz, H-1*ax*), 3.18 (m, 1 H, H-5), 3.36 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.8 Hz, H-3), 3.54 (m, 1 H, H-2), 3.63 (s, 3 H, C*H*$_3$OCO), 3.74 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4), 4.26–4.47 (m, 5 H, NHC*H* of isoGln, 2 CH$_3$C*H*, H-1*eq*,6a), 4.69 (dd, 1 H, J_{gem} 11.4, $J_{5,6b}$ 4.7 Hz, H-6b), 5.56 (s, 1 H, C*H*Ph), and 6.25–7.77 (m, 7 H, 2 N*H*, phenyl).

Anal. Calc. for $C_{30}H_{43}N_7O_{10}$ (661.71): C, 54.45; H, 6.54; N, 14.82. Found: C, 54.40; H, 6.39; N, 14.72.

N-*[2-O-[2-Acetamido-4,6-O-benzylidene-N-(tert-butoxycarbonyl)-1,2,3,5-tetradeoxy-1,5-imino-D-glucitol-3-yl]-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester* (**4**). — To a solution of **3** (320 mg) in methanol (2 mL), ethanol (3 mL), and acetic acid (0.5 mL) was added 10% palladium-on-carbon (400 mg), and the mixture was stirred for 2 h

in a hydrogen atmosphere. The catalyst was filtered off and washed with methanol. The filtrate and washings were then combined and concentrated. The residue was dissolved in methanol (2 mL) and acetylated with acetic anhydride (1 mL) for 1 h at room temperature. After concentration, the syrup obtained was chromatographed on a column of silica gel with 20:1 dichloromethane–methanol to give compound **4** (245 mg, 75%) as crystals, m.p. 208–210°; $[\alpha]_D$ +9.2° (*c* 0.2, 5:1 CH_2Cl_2–MeOH); i.r.: ν_{max} 3300 (NH), 2940, 2860 (CH_3, CH_2), 1750 (ester), 1650, 1540 (amide), 750, and 700 cm^{-1} (phenyl); ^{1}H-n.m.r. ($CDCl_3$ plus CD_3OD): δ 1.31–1.41 (m, 6 H, 2 C*H*$_3$CH), 1.48 [s, 9 H, (C*H*$_3$)$_3$C], 1.99 (s, 3 H, C*H*$_3$CO-N), 1.81–2.48 (m, 4 H, C*H*$_2$C*H*$_2$CO of isoGln), 2.76 (dd, 1 H, J_{gem} 13.2, $J_{1ax,2}$ 9.2 Hz, H-1*ax*), 3.27 (m, 1 H, H-5), 3.50 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3), 3.68 (s, 3 H, C*H*$_3$OCO), 3.74–3.88 (m, 2 H, H-2,4), 4.29–4.46 (m, 5 H, NHC*H* of isoGln, 2 CH_3C*H*, H-1*eq*,6a), 4.75 (dd, 1 H, J_{gem} 11.4, $J_{5,6eq}$ 4.4 Hz, H-6*eq*), 5.61 (s, 1 H, C*H*Ph), and 7.31–7.49 (m, 5 H, phenyl).

Anal. Calc. for $C_{32}H_{47}N_5O_{11}$ (677.75): C, 56.71; H, 6.98; N, 10.33. Found: C, 56.65; H, 6.82; N, 10.31.

N-*[2*-O-*[2-Acetamido-4,6*-O-*benzylidene*-N-*(*tert-*butoxycarbonyl)-1,2,3,5-tetradeoxy-1,5-imino*-D-*glucitol-3-yl]*-D-*lactoyl]*-L-*alanyl*-D-*isoglutamine* (**5**). — To a solution of **4** (24 mg) in a mixture of 1,4-dioxane (0.5 mL) and methanol (0.5 mL) was added 0.2M potassium hydroxide (1 mL), and the solution was stirred for 1 h at room temperature and then treated with Amberlite IR-120B (H^+) resin. The resin was filtered off and washed with methanol, and the filtrate and washings were combined and concentrated. This gave **5** (22 mg, quantitative) as a syrup, $[\alpha]_D$ +12.8° (*c* 0.4, MeOH); i.r.: ν_{max} 3300 (NH), 3000, 2930 (CH_3, CH_2), 1660 (C=O), 1640, 1540 (amide), 750, and 700 cm^{-1} (phenyl); ^{1}H-n.m.r. (CD_3OD): δ 1.20–1.38 [m, 15 H, 2 C*H*$_3$CH, (C*H*$_3$)$_3$C], 1.90 (s, 3 H, C*H*$_3$CO-N), 1.86–2.31 (m, 4 H, C*H*$_2$C*H*$_2$CO of isoGln), 2.68 (dd, 1 H, J_{gem} 13.6, $J_{1ax,2}$ 10.3 Hz, H-1*ax*), 3.21–3.98 (m, 4 H, H-2,3,4,5), 4.03–4.39 (m, 5 H, NHC*H* of isoGln, 2 CH_3C*H*, H-1*eq*,6a), 4.62 (dd, 1 H, J_{gem} 11.0, $J_{5,6b}$ 4.4 Hz, H-6b), 5.57 (s, 1 H, C*H*Ph), and 7.20–7.40 (m, 5 H, phenyl).

Anal. Calc. for $C_{31}H_{45}N_5O_{11}$ (663.72): C, 56.10; H, 6.83; N, 10.55. Found: C, 56.01; H, 6.90; N, 10.43.

N-*[2*-O-*(2-Acetamido-1,2,3,5-tetradeoxy-1,5-imino*-D-*glucitol-3-yl)*-D-*lactoyl]*-L-*alanyl*-D-*isoglutamine trifluoroacetate salt* (**6**). — A solution of **5** (20 mg) in a mixture of trifluoroacetic acid (2 mL) and water (0.4 mL) was stirred for 2.5 h at 0°, and then the solvent was evaporated. The residual syrup was dissolved in 1,4-dioxane, and the dioxane was removed by lyophilization to leave **6** (17 mg; quantitative), $[\alpha]_D$ +3.1° (*c* 0.1, MeOH); i.r.: ν_{max} 3350 (NH, OH), 2910, 2850 (CH_3, CH_2), 1660, 1530 (amide), 1110, and 1030 cm^{-1} (C-F).

Anal. Calc. for $C_{21}H_{34}F_3N_5O_{11}$ (589.52); C, 42.79; H, 5.81; N, 11.88. Found: C, 42.70; H, 5.92; N, 11.84.

N-*[2*-O-*[2-Acetamido*-N-*(*tert-*butoxycarbonyl)-1,2,3,5-tetradeoxy-1,5-imino*-D-*glucitol-3-yl]*-D-*lactoyl]*-L-*alanyl*-D-*isoglutamine methyl ester* (**7**). — To a solution of **4** (240 mg) in methanol (2 mL) and acetic acid (3 mL) was added 10% palladium-on-carbon (500 mg), and the mixture was stirred for 24 h in a hydrogen atmosphere. The

catalyst was filtered off and washed with methanol. The filtrate and washings were combined and concentrated. Compound **7** (198 mg, 95%) was isolated by recrystallization from ether, m.p. 110°, $[\alpha]_D$ +22.5° (*c* 1.2, 1:5 CH_2Cl_2–MeOH); i.r.: ν_{max} 3350 (NH, OH), 2980, 2940, 2850 (CH_3, CH_2), 1730 (C=O), 1660, and 1530 cm^{-1} (amide); ^{1}H-n.m.r. (CD_3OD): δ 1.33–1.42 (m, 6 H, 2 CH_3CH), 1.97 (s, 3 H, CH_3CO-N), and 3.69 (s, 3 H, CH_3OCO).

Anal. Calc. for $C_{25}H_{43}N_5O_{11}$ (589.64): C, 50.92; H, 7.35; N, 11.88. Found: C, 51.01; H, 7.31; N, 11.74.

N-*[2-O-[2-Acetamido*-N-*(*tert-*butoxycarbonyl)-1,2,3,5-tetradeoxy-1,5-imino-6-O-octadecanoyl-*D*-glucitol-3-yl]-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl ester* (**8**). — To a solution of **7** (48 mg, 0.081 mmol) in 1,4-dioxane (1 mL) and *N,N*-dimethylformamide (2 drops) were added octadecanoic acid (30 mg, 0.11 mmol), DCC (30 mg, 0.15 mmol), and a catalytic amount of 4-dimethylaminopyridine, and the mixture was stirred for 2.5 h at room temperature. Insoluble materials were filtered off, and the filtrate was concentrated. The residue obtained was subjected to preparative thin layer chromatography (Merck, Silica Gel/Kieselguhr F_{254}, 2.0 mm, 8:1 dichloromethane–methanol) to give compound **8** (56 mg, 81%), which was recrystallized from ether, m.p. 152.5–153.5°, $[\alpha]_D$ +3.2° (*c* 1.3, 2:1 CH_2Cl_2–MeOH); i.r.: ν_{max} 3400 (OH), 3350 (NH), 2940, 2870 (CH_3, CH_2), 1750 (ester), 1640, and 1580 cm^{-1} (amide); ^{1}H-n.m.r. ($CDCl_3$ plus CD_3OD): δ 0.88 (t, 3 H, CH_3CH$_2$), 1.26–1.48 [m, 45 H, 2 CH_3CH, 15 CH_2, (CH_3)$_3$C], 1.97 (s, 3 H, CH_3CO-N), and 3.69 (s, 3 H, CH_3OCO).

Anal. Calc. for $C_{43}H_{77}N_5O_{12}$ (856.11): C, 60.32; H, 9.06; N, 8.18. Found: C, 60.29; H, 9.10; N, 8.11.

N-*[2-O-[2-Acetamido-1,2,3,5-tetradeoxy-1,5-imino-6-O-octadecanoyl-D-glucitol-3-yl]-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester trifluoroacetate salt* (**9**). — Compound **9** was prepared from **8** by hydrolytic removal of the *tert*-butoxycarbonyl group, according to the procedure described for compound **6**, in a quantitative yield, $[\alpha]_D$ +13.9° (*c* 1.0, 1:1 CH_2Cl_2–MeOH); i.r.: ν_{max} 3400 (OH), 3320 (NH), 2920, 2850 (CH_3, CH_2), 1750 (ester), 1630 and 1570 (amide), 1210, 1160, and 1100 cm^{-1} (CF).

Anal. Calc. for $C_{40}H_{70}F_3N_5O_{11}$ (854.02): C, 60.32; H, 9.06; N, 8.18. Found: C, 60.30; H, 9.01; N, 8.09.

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