Carbohydrate Research, 97 (1981) 337–345
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

The chemical modification of the C-2 substituent in the sugar moiety of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, and the immunoadjuvant activities*

AKIRA HASEGAWA, HIROYUKI OKUMURA, KAORU NISHIBORI, YOSHIMI KANEDA, MAKOTO KISO,
Department of Agricultural Chemistry, Gifu University, Kakamigahara, Gifu 504 (Japan)

AND ICHORO AZUMA

Institute of Immunological Science, Hokkaido University, Sapporo 060 (Japan)

(Received May 12th, 1981; accepted for publication, June 5th, 1981)

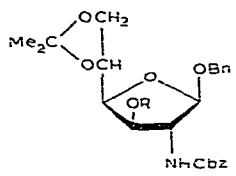
In our continuing effort[1] to clarify the structural requirements of the carbohydrate moiety in *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP), which is the minimal structure[3] required for the immunoadjuvant activity of the bacterial cell-wall peptidoglycans, it has been found that not only the position and the configuration of the linkage between the lactoyl-dipeptide and the sugar moiety, but also the configuration, and the chemical modification, of the functional groups in the carbohydrate moiety give various, important effects on the manifestation of the activity. The present report describes the chemical modification of the C-2 substituent in the sugar moiety, and the immunoadjuvant activities of the products.

Benzyl 2-(benzyloxycarbonylamino)-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside[4] (**1**) served as a convenient starting-material for the synthesis of the muramoyl dipeptide analogs described herein.

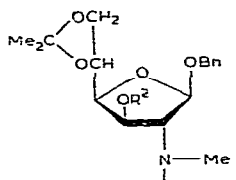
Treatment of **1** with lithium aluminum hydride in boiling oxolane gave benzyl 2-deoxy-5,6-*O*-isopropylidene-2-(methylamino)-β-D-glucofuranoside (**4**) in 77% yield. *N*-(Benzyloxycarbonyl)ation, or *N*-acetylation, of **4** respectively afforded benzyl 2-(*N*-benzyloxycarbonyl-*N*-methylamino)-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside (**5**) or benzyl 2-(*N*-acetyl-*N*-methylamino)-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside (**6**). 2-Deoxy-D-*arabino*-hexopyranose ("2-deoxy-D-glucose") was treated with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide in the presence of *p*-toluenesulfonic acid, giving methyl 2-deoxy-5,6-*O*-isopropylidene-D-*arabino*-hexofuranoside (**14**).

Compounds **1**, **5**, **6**, and **14** were condensed with L-2-chloropropanoic acid in

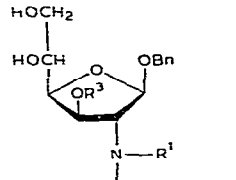
*Studies on Immunoadjuvant Active Compounds, Part XIV. For Part XIII, see ref. 1a. For a preliminary report on part of this work, see ref. 2.



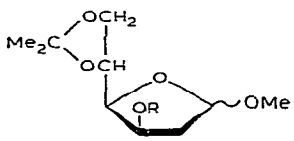
1 R = H
2 R = a
3 R = b
Bn = $PhCH_2$
Cbz = $PhCH_2OCO$
a = $-CH(Me)CO_2H$
b = $-CH(Me)CO$-L-Ala-D-isoGln—OBn



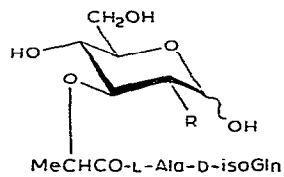
4 $R^1 = R^2 = H$
5 R^1 = Cbz, R^2 = H
6 R^1 = Ac, R^2 = H
7 R^1 = Cbz, R^2 = a
8 R^1 = Ac, R^2 = a
9 R^1 = Cbz, R^2 = b
10 R^1 = Ac, R^2 = b



11 R^1 = Cbz, R^2 = H, R^3 = b
12 R^1 = Cbz, R^2 = Me, R^3 = b
13 R^1 = Ac, R^2 = Me, R^3 = b



14 R = H
15 R = a
16 R = b



17 R = NHAc (MDP)
18 R = $NH_2 \cdot HCl$
19 R = NHMe
20 R = N(Me)Ac
21 R = H

the presence of sodium hydride, affording the corresponding 3-*O*-(D-1-carboxyethyl) derivatives, **2**, **7**, **8**, and **15**, respectively, in good yields. Coupling of the acids **2**, **7**, **8**, and **15** with L-alanyl-D-isoglutamine benzyl ester[5] was conducted with dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu) as the activating agents, to afford the corresponding dipeptides, **3**, **9**, **10**, and **16**, in good yields.

Removal of the isopropylidene group from compounds **3**, **9**, **10**, and **16** with 60% aqueous acetic acid at 40–45° respectively gave, in high yields, the diols **11–13**. Hydrogenolysis of the benzyl and benzyloxycarbonyl groups in compound **11** with hydrogen in the presence of 10% Pd–C catalyst, in methanol–acetic acid–water, followed by treatment with 0.1M hydrochloric acid, gave muramoyl-L-alanyl-D-isoglutamine hydrochloride (**18**) in good yield. By essentially the same procedure, compounds **12** and **13** yielded the corresponding carbohydrate analogs (**19** and **20**) of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, in almost quantitative yields.

Compound **16** was first treated with 80% aqueous trifluoroacetic acid, to convert it into the pyranoid structure, and then hydrogenolyzed with 10% Pd–C catalyst in ethanol, to give the desired 2-deoxy-3-*O*-(D-2-propanoyl-L-alanyl-D-isoglutamine)-D-*arabino*-hexopyranose (**21**).

TABLE I

ADJUVANT ACTIVITY OF SOME CARBOHYDRATE ANALOGS OF *N*-ACETYLMURAMOYL-L-ALANYL-D-ISOGLUTAMINE (MDP) 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] *at*	
	24 h	*48 h*
18	24.2	22.6
19	22.5	19.0
20	22.5	17.8
21	(10.6)	(3.3)
MDP (**17**)	21.0	19.0
Control[d]	0	0

[a]At a dose of 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 faint erythema. [d]ABA–*N*-acetyl-L-tyrosine in Freund's incomplete adjuvant.

The immunoadjuvant activities of compounds **18–21** on the induction of the delayed-type of hypersensitivity to *N*-acetyltyrosine–3-azobenzene-4′-arsonic acid (ABA-*N*-acetyltyrosine) were examined[6] in guinea-pigs (see Table I).

Compounds **18–20** exhibited strong activity that was even stronger than that of MDP, whereas the 2-deoxy-D-*arabino*-hexose analog (**21**) abolished the activity almost completely. In previous reports[1a,2,7] in this series, we showed that the D-glucopyranose and 2-acetamido-2-deoxy-D-mannopyranose analogs of MDP have potent activity, comparable to that of MDP. These results suggest that, for activity, the substituent on C-2 is not restricted to the acetamido group, and also that the configuration of C-2 is not important, but that the presence of a substituent such as an amine, an amine derivative, or a hydroxyl group at C-2 is essential for manifestation of the activity.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union PM-201 polarimeter, and i.r. spectra were recorded with a Jasco IRA-1 spectrophotometer. N.m.r. spectra were recorded at 60 MHz and 90 MHz with Hitachi R-24BS and R-22 spectrometers for solutions in chloroform-*d*, unless otherwise noted. N.m.r. data were confirmed by use of decoupling techniques. Preparative chromatography was performed on silica gel (Waco Co.; 300 mesh) with the solvent systems specified. Evaporations were conducted *in vacuo*.

Benzyl 2-(benzyloxycarbonylamino)-3-O-(D-1-carboxyethyl)-2-deoxy-5,6-O-iso-

propylidene-β-D-glucofuranoside (**2**). — To a stirred solution of **1** (2.0 g) in dry 1,4-dioxane (10 mL) was added sodium hydride in oil suspension (870 mg) (50% of sodium hydride by weight) at room temperature, and the mixture was stirred for 1.5 h at 90°. L-2-Chloropropanoic acid (640 mg) was added at 65°, and the mixture was stirred for 2 h at 60 to 65°, and cooled. 2M Hydrochloric acid was carefully added to the cooled mixture until pH 8 was reached, and the solution was evaporated. Chloroform (100 mL) and water (100 mL) were added to the mixture, and 2M hydrochloric acid was added, with stirring at 0°, to pH 4. The chloroform layer was washed with water, dried (sodium sulfate), and evaporated to a syrup which was chromatographed on a column of silica gel (30 g) with chloroform, and then with 100:1 chloroform–methanol. The latter eluate gave compound **2** (1.64 g, 71%) as a syrup; $[\alpha]_D^{25}$ −28° (*c* 0.5, chloroform); ν_{max}^{film} 3280 (NH), 2800–2700 (COOH), 1710 (C=O), 1690 and 1520 (amide), 840 (Me_2C), and 730 and 690 cm^{-1} (phenyl); n.m.r. data at 60 MHz: δ 1.22, 1.30 (9 H, Me_2C, MeCH), 4.40, 4.67 (2 d, 2 H, J_{gem} 11.5 Hz, benzyl methylene), 4.98 (s, 1 H, H-1), 5.02 (s, 2 H, CO_2CH_2Ph), 7.22 (s, 10 H, 2 Ph), and 8.95 (s, 1 H, COOH).

Anal. Calc. for $C_{27}H_{33}NO_9$: C, 62.90; H, 6.45; N, 2.72. Found: C, 62.97; H, 6.51; N, 2.64.

Benzyl 2-(benzyloxycarbonylamino)-2-deoxy-5,6-O-isopropylidene-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-β-D-glucofuranoside (**3**). — To a solution of **2** (600 mg) in dry 1,4-dioxane (15 mL) were added, with stirring, *N*-hydroxysuccinimide (HOSu) (160 mg) and dicyclohexylcarbodiimide (DCC) (300 mg), and the mixture was stirred for 1 h at room temperature; at that time, the starting material had been converted into the activated ester. The *N,N'*-dicyclohexylurea formed was filtered off, L-alanyl-D-isoglutamine benzyl ester trifluoroacetate[5] (540 mg) and triethylamine (0.2 mL) were added to the filtrate, and it was stirred for 3 h at room temperature, and then evaporated. The residue was chromatographed on a column of silica gel (30 g) with chloroform, and then with 70:1 chloroform–methanol. The latter eluate afforded 650 mg (69%) of **3** as crystals, m.p. 149°, $[\alpha]_D^{25}$ −39° (*c* 0.7, chloroform); ν_{max}^{KBr} 3280 (NH), 1720 and 1220 (ester), 1690, 1620, and 1520 (amide), 840 (Me_2C), and 720 and 690 cm^{-1} (phenyl); n.m.r. data at 60 MHz: δ 1.00–1.35 (12 H, Me_2C, 2 MeCH), 2.30–2.50 (m, 4 H, 2 CH_2), 5.02 (s, 1 H, H-1), 5.05 (s, 2 H, CO_2CH_2Ph), and 7.33 (s, 15 H, 3 Ph).

Anal. Calc. for $C_{42}H_{52}N_4O_{12}$: C, 62.67; H, 6.51; N, 6.97. Found: C, 62.78; H, 6.45; N, 7.09.

Benzyl 2-deoxy-5,6-O-isopropylidene-2-methylamino-β-D-glucofuranoside (**4**). — To a stirred solution of **1** (500 mg) in dry oxolane (20 mL) was gradually added lithium aluminum hydride (450 mg) at 0°, and the mixture was boiled under reflux for 19 h; at that time, the starting material was no longer detectable by t.l.c., and the excess of the reagent was decomposed by adding water at 0°. After filtration, the filtrate was evaporated, the residue extracted with chloroform, and the extract washed with water, dried (sodium sulfate), and evaporated to a syrup which was chromatographed on a column of silica gel (15 g) with chloroform and 100:1 chloroform–

methanol. From the latter eluate, compound **4** was obtained as a syrup, wt. 280 mg (77%); $[\alpha]_D^{25}$ −65° (*c* 0.29, chloroform); ν_{max}^{film} 3380 (OH), 3240 and 1640 (NH), 835 (Me_2C), and 730 and 690 cm^{-1} (phenyl); n.m.r. data at 90 MHz: δ 1.38, 1.45 (2 s, 6 H, Me_2C), 1.95 (broad s, 1 H, NH), 2.47 (s, 3 H, MeN), 4.47, 4.73 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 4.96 (s, 1 H, H-1), and 7.30 (s, 5 H, Ph).

Anal. Calc. for $C_{17}H_{25}NO_5$: C, 63.14; H, 7.79; N, 4.33. Found: C, 63.42; H, 2.93; N, 4.19.

Benzyl 2-(N-benzyloxycarbonyl-N-methylamino)-2-deoxy-5,6-O-isopropylidene-β-D-glucofuranoside (**5**). — A solution of **4** (250 mg) in 1,4-dioxane (10 mL) and 0.5M sodium carbonate (3.5 mL) at 0° was stirred whilst benzyloxycarbonyl chloride (200 mg) was added. The mixture was stirred for 1.5 h at 0°, evaporated, and the residue extracted with chloroform. The extract was successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried (sodium sulfate), and evaporated. The product, purified by chromatography on a column of silica gel (15 g) with 100:1 chloroform–methanol, was obtained as needles after recrystallization from ether, wt. 310 mg (88%); m.p. 84° (dec.), $[\alpha]_D^{25}$ −54.5° (*c* 0.5, chloroform); ν_{max}^{Nujol} 3425 (OH), 1680 (amide), 870 (Me_2C), and 740 and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{25}H_{31}NO_7$: C, 65.62; H, 6.83; N, 3.06. Found: C, 65.59; H, 6.85; N, 3.15.

Benzyl 2-(N-acetyl-N-methylamino)-2-deoxy-5,6-O-isopropylidene-β-D-glucofuranoside (**6**). — To a solution of **4** (230 mg) in methanol (3 mL) was added acetic anhydride (0.2 mL), and after 30 min, the mixture was evaporated. The product was chromatographed on a column of silica gel (15 g) with 100:1 chloroform–methanol, to give compound **6** (230 mg, 88%) as a syrup; $[\alpha]_D^{25}$ −64.5° (*c* 0.4, chloroform); ν_{max}^{film} 3370 (OH), 1630 (amide), 850 (Me_2C), and 745 and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.19; H, 7.65; N, 3.70.

Benzyl 2-(N-benzyloxycarbonyl-N-methylamino)-3-O-(D-1-carboxyethyl)-2-deoxy-5,6-O-isopropylidene-β-D-glucofuranoside (**7**). — To a stirred solution of **5** (100 mg) in dry 1,4-dioxane (1.0 mL) was added the sodium hydride reagent (20 mg), and the mixture was kept, with stirring, for 30 min at 60°. L-2-Chloropropanoic acid (35 mg) was added to the mixture, and it was stirred for 2 h at 60°; the mixture was then processed as described in the preparation of **2**, to afford compound **7** (69 mg, 60%) as a syrup; $[\alpha]_D^{25}$ −72° (*c* 0.39, chloroform); ν_{max}^{film} 2650–2600 (COOH), 1740 (C=O), 1690 (amide), 840 (Me_2C), and 730 and 685 cm^{-1} (phenyl); n.m.r. data at 90 MHz: δ 1.33, 1.42 (2 s, 6 H, Me_2C), 1.36 (d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeCH), 2.92 (s, 3 H, MeN), 4.51, 4.72 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.10 (s, 2 H, CO_2CH_2Ph), 5.15 (d, 1 H, $J_{1,2}$ 2.2 Hz, H-1), and 7.29, 7.31 (2 s, 10 H, 2 Ph).

Anal. Calc. for $C_{28}H_{35}NO_9$: C, 63.50; H, 6.66; N, 2.64. Found: C, 63.21; H, 6.93; N, 2.55.

Benzyl 2-(N-acetyl-N-methylamino)-3-O-(D-1-carboxyethyl)-2-deoxy-5,6-O-isopropylidene-β-D-glucofuranoside (**8**). — To a stirred solution of **6** (84 mg) in dry 1,4-dioxane (1.0 mL) was added the sodium hydride reagent (40 mg), and the mixture

was stirred for 30 min at 90°, and cooled. L-2-Chloropropanoic acid (30 mg) was added to the stirred mixture, and it was stirred for 1.5 h at 65°; t.l.c. then showed the reaction to be complete. The mixture was processed as already described, to give compound **8** (79 mg, 79%) as a syrup; $[\alpha]_D^{25}$ −53° (*c* 0.79, chloroform); ν_{max}^{film} 2600–2500 (COOH), 1740 (C=O), 1610 (amide), 850 (Me_2C), and 750 and 700 cm^{-1} (phenyl); n.m.r. data at 60 MHz: δ 1.25–1.46 (9 H, Me_2C, MeCH), 2.10, 2.26 (2 s, 3 H, AcN), 2.80, 3.05 (2 s, 3 H, MeN), 4.97, 5.27 (2 d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 7.33 (s, 5 H, Ph), and 7.95 (broad s, 1 H, COOH).

Anal. Calc. for $C_{22}H_{31}NO_8$: C, 60.40; H, 7.14; N, 3.20. Found: C, 60.21; H, 7.36; N, 3.05.

Benzyl 2-(N-*benzyloxycarbonyl*-N-*methylamino*)-*2-deoxy-5,6*-O-*isopropylidene*-(D-*2-propanoyl*-L-*alanyl*-D-*isoglutamine benzyl ester*)-β-D-*glucofuranoside* (**9**). — Compound **7** (39 mg) with the L-alanyl-D-isoglutamine derivative (34 mg) in dry 1,4-dioxane (1.0 mL) in the presence of HOSu (13 mg), DCC (23 mg), and triethylamine (9 mg), as described in the preparation of **3**, gave the product. It was purified by chromatography on a column of silica gel (10 g) with chloroform, and then with 50:1 chloroform–methanol. From the latter eluate, compound **9** was obtained as a syrup, wt. 55 mg (91%); $[\alpha]_D^{25}$ −42° (*c* 0.55, chloroform); ν_{max}^{film} 3280 (NH), 1720 and 1240 (ester), 1690, 1650, and 1500 (amide), 840 (Me_2C), and 740 and 680 cm^{-1} (phenyl); n.m.r. data at 60 MHz: δ 1.20–1.45 (12 H, Me_2C, 2 MeCH), 2.89 (s, 3 H, MeN), 4.48, 4.77 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.17–5.19 (5 H, 2 CO_2CH_2Ph, H-1), and 7.36 (s, 15 H, 3 Ph).

Anal. Calc. for $C_{43}H_{54}N_4O_{12}$: C, 63.06; H, 6.40; N, 6.84. Found: C, 63.21; H, 6.59; N, 6.58.

Benzyl 2-(N-*acetyl*-N-*methylamino*)-*2-deoxy-5,6*-O-*isopropylidene-3*-O-(D-*2-propanoyl*-L-*alanyl*-D-*isoglutamine benzyl ester*)-β-D-*glucofuranoside* (**10**). — Coupling of **8** (110 mg) with the L-alanyl-D-isoglutamine derivative (117 mg) in dry 1,4-dioxane (1.0 mL), by using HOSu (44 mg), DCC (78 mg), and triethylamine (31 mg) as described for **3**, gave **10** (134 mg, 73%) as a syrup, $[\alpha]_D^{25}$ −40° (*c* 0.67, chloroform); ν_{max}^{film} 3280 (NH), 1720 and 1220 (ester), 1660 and 1520 (amide), 850 (Me_2C), and 750 and 700 cm^{-1} (phenyl); n.m.r. data at 60 MHz: δ 1.27–1.44 (12 H, Me_2C, 2 MeCH), 2.06, 2.15 (2 s, 3 H, AcN), 2.80, 2.93 (2 s, 3 H, MeN), 5.08–5.16 (3 H, CO_2CH_2Ph, H-1), and 7.30 (s, 10 H, 2 Ph).

Anal. Calc. for $C_{37}H_{50}N_4O_{11}$: C, 61.14; H, 6.93; N, 7.71. Found: C, 61.00; H, 7.25; N, 7.53.

Benzyl 2-(N-*benzyloxycarbonylamino*)-*2-deoxy-3*-O-(D-*2-propanoyl*-L-*alanyl*-D-*isoglutamine benzyl ester*)-β-D-*glucofuranoside* (**11**). — A solution of **3** (170 mg) in 60% aqueous acetic acid (10 mL) was heated for 2 h at 40–45°; it was then evaporated at 40° to a syrup which was chromatographed on a column of silica gel (15 g) with chloroform and 30:1 chloroform–methanol. The latter eluant gave **11** (154 mg, 95%) as a syrup, $[\alpha]_D^{25}$ −57° (*c* 0.7, chloroform); ν_{max}^{film} 3360 (OH), 3240 (NH), 1720 and 1240 (ester), 1690, 1650, and 1510 (amide), and 730 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{39}H_{48}N_4O_{12}$: C, 61.24; H, 6.33; N, 7.33. Found: C, 61.08; H, 6.49; N, 7.15.

Benzyl 2-(N-*benzyloxycarbonyl*-N-*methylamino*)-*2-deoxy-3*-O-(D-*2-propanoyl*-L-*alanyl*-D-*isoglutamine benzyl ester*)-*β*-D-*glucofuranoside* (**12**). — Hydrolysis of **9** (55 mg) with 60% aqueous acetic acid (2 mL), as described for **11**, gave **12** (49 mg, 94%) as a syrup, $[\alpha]_D^{25}$ −26° (*c* 0.5, methanol); ν_{max}^{film} 3340–3250 (OH, NH), 1730 and 1240 (ester), 1690, 1600, and 1520 (amide), and 750 and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{40}H_{50}N_4O_{12}$: C, 61.68; H, 6.47; N, 7.19. Found: C, 61.42; H, 6.76; N, 6.95.

Benzyl 2-(N-*acetyl*-N-*methylamino*)-*2-deoxy-3*-O-(D-*2-propanoyl*-L-*alanyl*-D-*isoglutamine benzyl ester*)-*β*-D-*glucofuranoside* (**13**). — Hydrolysis of **10** (134 mg) with 60% aqueous acetic acid (5 mL), as described for **11**, gave **13** (98 mg, 77%) as a syrup, $[\alpha]_D^{25}$ −44° (*c* 0.44, chloroform); ν_{max}^{film} 3300–3250 (OH, NH), 1720 and 1230 (ester), 1650 and 1520 (amide), and 740 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{34}H_{46}N_4O_{11}$: C, 59.46; H, 6.75; N, 8.16. Found: C, 59.33; H, 6.89; N, 7.88.

Methyl 2-deoxy-5,6-O-*isopropylidene*-D-arabino-*hexofuranoside* (**14**). — A solution of 2-deoxy-D-*arabino*-hexopyranose (2.0 g) in dry *N,N*-dimethylformamide (10 mL) was stirred at 70–75°, while 2,2-dimethoxypropane (3.0 mL) and *p*-toluenesulfonic acid monohydrate (30 mg) were added; stirring was continued for 3 h at 75°. The mixture was cooled, and treated with Amberlite IRA-410 (OH^-) ion-exchange resin to remove the acid: the resin was filtered off, and washed with methanol. The filtrate and washings were combined, and evaporated to a syrup that was chromatographed on a column of silica gel (20 g) with (*a*) chloroform and (*b*) 200:1 chloroform-methanol. Eluant *b* gave compound **14** as a syrup, wt. 1.1 g (39%); $[\alpha]_D^{25}$ +33.5° (*c* 2.6, chloroform); ν_{max}^{film} 3420 (OH) and 850 cm^{-1} (Me_2C); n.m.r. data at 60 MHz: δ 1.30, 1.38 (2 s, 6 H, Me_2C), 3.30 (s, 3 H, MeO), and 5.13 (t, 1 H, $J_{1,2} = J_{1,2'} = 5.0$ Hz, H-1).

Anal. Calc. for $C_{10}H_{18}O_5$: C, 57.38; H, 7.88. Found: C, 57.55; H, 8.07.

Methyl 3-O-(D-*1-carboxyethyl*)-*2-deoxy-5,6*-O-*isopropylidene*-D-arabino-*hexofuranoside* (**15**). — To a stirred solution of **14** (750 mg) in dry 1,4-dioxane (4 mL) was added the sodium hydride reagent (660 mg), and the mixture was kept, with stirring, for 1.5 h at 95°. L-2-Chloropropanoic acid (750 mg) was added to the mixture, and it was stirred for 3 h at 65°; the mixture was then processed as described for **2**, affording compound **15** (780 mg, 83%) as a syrup, $[\alpha]_D^{25}$ +64° (*c* 1.25, chloroform); ν_{max}^{film} 2700–2600 (COOH), 1740 (C=O), and 850 cm^{-1} (Me_2C); n.m.r. data at 60 MHz: δ 1.28–1.42 (9 H, Me_2C, MeCH), 3.31 (s, 3 H, MeO), 5.12 (near t, 1 H, $J_{1,2} = J_{1,2'} = 4.8$ Hz, H-1), and 7.74 (s, 1 H, COOH).

Anal. Calc. for $C_{13}H_{22}O_7$: C, 53.78; H, 7.64. Found: C, 53.51; H, 7.86.

Methyl 2-deoxy-5,6-O-*isopropylidene-3*-O-(D-*2-propanoyl*-L-*alanyl*-D-*isoglutamine benzyl ester*)-D-arabino-*hexofuranoside* (**16**). — Coupling of **15** (125 mg) with the L-alanyl-D-isoglutamine derivative (260 mg), in dry 1,4-dioxane (1.5 mL) by using HOSu (98 mg), DCC (177 mg), and triethylamine (52 mg), as described for

the preparation of **3**, afforded compound **16** (154 mg, 62%) as a syrup, $[\alpha]_D^{25}$ +34° (*c* 0.96, chloroform); ν_{max}^{film} 3300 (NH), 1730 and 1250 (ester), 1650 and 1510 (amide), 840 (Me_2C), and 750 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{28}H_{41}N_3O_{10}$: C, 58.02; H, 7.13; N, 7.25. Found: C, 57.86; H, 7.33; N, 7.12.

*Muramoyl-*L*-alanyl-*D*-isoglutamine hydrochloride* (**18**). — Compound **11** (130 mg) was dissolved in methanol (20 mL) and water (2 mL), 10% Pd–C catalyst (150 mg) was added, and hydrogen was bubbled through while the mixture was stirred for 30 min at 30°. Acetic acid (0.2 mL) and water (10 mL) were added to the mixture, and hydrogen was bubbled through the mixture, with stirring, for 2 h at 30°, the course of the reaction being monitored by t.l.c. The catalyst was removed by filtration, 0.1M hydrochloric acid (1.70 mL) was added to the filtrate, and it was evaporated below 20°, to give a hygroscopic, amorphous mass (83 mg, quantitative yield), which showed a single spot in t.l.c.; m.p. 159–163° (dec.), $[\alpha]_D^{25}$ +55° (*c* 0.6, water; equil.); ν_{max}^{KBr} 3400–3200 (OH, NH), 1710 (C=O), and 1670–1620 and 1530 cm^{-1} (amide); n.m.r. data at 60 MHz (in D_2O): δ 1.35 (2 d, 6 H, $J_{CH_3,CH}$ 7.0 Hz, 2 MeCH), 4.88 (d, $J_{1,2}$ 8.5 Hz, H-1β), and 5.38 (d, $J_{1,2}$ 3.5 Hz, H-1α).

Anal. Calc. for $C_{17}H_{30}N_4O_{10} \cdot HCl$: C, 41.93; H, 6.42; N, 11.51. Found: C, 41.56; H, 6.84; N, 11.35.

*2-Deoxy-2-(methylamino)-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-*D*-glucopyranose* (**19**). — Compound **12** (49 mg) in methanol (10 mL)–acetic acid (1 mL)–water (10 mL) was hydrogenolyzed in the presence of 10% Pd–C catalyst (75 mg) as described in the preparation of **18**, to give **19** (29 mg, quantitative yield) as an amorphous mass, $[\alpha]_D^{25}$ +34.5° (*c* 0.35, water; equil.); ν_{max}^{KBr} 3360–3250 (OH, NH), 1720 (C=O), and 1660 and 1550 cm^{-1} (amide); n.m.r. data at 60 MHz (in D_2O): δ 1.26–1.40 (m, 6 H, 2 MeCH), 2.75 (s, 3 H, MeN), and 5.45 (d, $J_{1,2}$ 3.5 Hz, H-1α).

Anal. Calc. for $C_{18}H_{32}N_4O_{10}$: C, 46.54; H, 6.94; N, 12.06. Found: C, 46.21; H, 7.25; N, 11.88.

*2-(*N*-Acetyl-*N*-methylamino)-2-deoxy-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-*D*-glucopyranose* (**20**). — Hydrogenolysis of compound **13** (44 mg) with hydrogen in the presence of 10% Pd–C catalyst (80 mg) in methanol (5 mL)–acetic acid (0.5 mL)–water (5 mL), according to the procedure already described, gave **20** (31 mg, 96%) as an amorphous material; $[\alpha]_D^{25}$ +7° (*c* 0.42, water; equil.); ν_{max}^{KBr} 3360–3250 (OH, NH), 1710 (C=O), and 1650 and 1520 cm^{-1} (amide); n.m.r. data at 60 MHz (D_2O): δ 1.20–1.36 (m, 6 H, 2 MeCH), 2.00, 2.09 (2 s, 3 H, AcN), 2.70, 2.87 (2 s, 3 H, MeN), and 5.25 (d, $J_{1,2}$ 3.4 Hz, H-1α).

Anal. Calc. for $C_{20}H_{34}N_4O_{11}$: C, 47.42; H, 6.77; N, 11.06. Found: C, 47.05; H, 7.08; N, 10.91.

*2-Deoxy-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-*D*-arabino-hexopyranose* (**21**). — A solution of **16** (96 mg) in 80% aqueous trifluoroacetic acid (1.0 mL) was stirred for 7 h at room temperature, and then evaporated to a syrup which was chromatographed on a column of silica gel (5 g) with chloroform, and then with 20:1 chloroform–methanol. The latter eluate gave 2-deoxy-3-*O*-(D-2-propanoyl-L-

alanyl-D-isoglutamine benzyl ester)-D-*arabino*-hexopyranose (45 mg). The product was dissolved in ethanol (3 mL); 10% Pd–C catalyst (20 mg) was added, and hydrogen was bubbled through for 30 min while the solution was stirred at room temperature. After removal of the catalyst by filtration, the filtrate was evaporated, to give an amorphous mass of **21** (29 mg, 40%) which showed a single spot in t.l.c.; $[\alpha]_D^{25}$ +28° (*c* 0.28, water; equil.); ν_{max}^{KBr} 3400–3250 (OH, NH), 2750–2700 (COOH), 1720 (C=O), and 1650 and 1520 cm^{-1} (amide).

Anal. Calc. for $C_{17}H_{29}N_3O_{10}$: C, 46.89; H, 7.87; N, 9.65. Found: C, 46.55; H, 8.03; N, 9.51.

REFERENCES

1 (a) A. Hasegawa, Y. Kaneda, Y. Goh, K. Nishibori, M. Kiso, and I. Azuma, *Carbohydr. Res.*, 94 (1981) 143–163; (b) A. Hasegawa, E. Tanahashi, Y. Goh, M. Kiso, and I. Azuma, *ibid.*, 92 (1981) 75–84, and earlier papers.

2 M. Kiso, Y. Kaneda, H. Okumura, A. Hasegawa, I. Azuma, and Y. Yamamura, *Carbohydr. Res.*, 79 (1980) c17–c19.

3 (a) F. Ellouz, A. Adam, R. Ciorbaru, and E. Lederer, *Biochem. Biophys. Res. Commun.*, 59 (1974) 1317–1325; C. Merser, P. Sinaÿ, and A. Adam, *ibid.*, 66 (1975) 1316–1322; (b) S. Kotani, I. Morisaki, Y. Watanabe, F. Kinoshita, T. Shimono, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikehara, *Biken J.*, 18 (1975) 105–111.

4 A. Hasegawa, N. Aritake, and M. Kiso, *Carbohydr. Res.*, 52 (1976) 137–149.

5 S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 49 (1976) 533–539.

6 I. Azuma, E. Kamisango, I. Saiki, Y. Tanio, S. Kobayashi, and Y. Yamamura, *Infect. Immun.*, 29 (1980) 1193–1196.

7 A. Hasegawa, Y. Kaneda, M. Amano, M. Kiso, and I. Azuma, *Agric. Biol. Chem.*, 42 (1978) 2187–2189.