Carbohydrate Research, 92 (1981) 75–84
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

CHEMICAL MODIFICATION OF THE CARBOHYDRATE MOIETY IN *N*-ACETYLMURAMOYL-L-ALANYL-D-ISOGLUTAMINE, AND THE IMMUNOADJUVANT ACTIVITY*

AKIRA HASEGAWA, EIJI TANAHASHI, YASUHIKO GOH, MAKOTO KISO,
Department of Agricultural Chemistry, Gifu University, Kakamigahara, Gifu 504 (Japan)
AND ICHIRO AZUMA
Institute of Immunological Science, Hokkaido University, Sapporo 060 (Japan)

(Received December 3rd, 1980; accepted for publication, December 20th, 1980)

ABSTRACT

Five carbohydrate analogs of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine have been synthesized from benzyl 2-acetamido-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)-ethyl]-α-D-glucopyranoside (**1**) and the corresponding 6-*O*-benzoyl derivative (**2**). Chlorination of **1** and **2** with triphenylphosphine in carbon tetrachloride gave the 4,6-dichloro compound **3** and the 6-*O*-benzoyl-4-chloro compound (**4**), which were treated with tributyltin hydride, to yield benzyl 2-acetamido-2,4,6-trideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-*xylo*-hexopyranoside (**6**) and benzyl 2-acetamido-6-*O*-benzoyl-2,4-dideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-*xylo*-hexopyranoside (**7**), respectively. Methanesulfonylation of **8**, derived from **7** by debenzoylation, gave the 6-methanesulfonate, which underwent displacement with azide ion to afford benzyl 2-acetamido-6-azido-2,4,6-trideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-*xylo*-hexopyranoside (**10**). Hydrolysis of the methyl ester group in compounds **3**, **5** (debenzoylated **4**), **6**, **8**, and **10** gave the corresponding free acids, which were coupled with L-alanyl-D-isoglutamine benzyl ester, to yield the dipeptide derivatives in excellent yields. Hydrogenation of the dipeptide derivatives thus obtained gave the five carbohydrate analogs of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, respectively, in good yields. The immunoadjuvant activity of the *N*-acetylmuramoyl-dipeptide analogs was examined.

INTRODUCTION

The principal results of our recent studies on the relationship between the structure of the carbohydrate moiety and the activity in *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, which is the minimal structure[2,3] required for the immunoadjuvant

*Studies on Immunoadjuvant Active Compounds, Part XI. For Part X, see ref. 1.

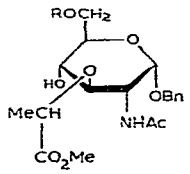
0008-6215/81/0000–0000/$ 02.50,

activity of bacterial cell-wall peptidoglycans, are the following. (*1*) The carbohydrate moiety is not restricted to 2-acetamido-2-deoxy-D-glucose, and can be replaced by 2-acetamido-2-deoxy-D-mannose[4], 2-acetamido-2-deoxy-D-galactose[5], 2,6-diamino-2,6-dideoxy-D-glucose[7], 2-acetamido-6-amino-2,6-dideoxy-D-glucose[8], 2,6-dideoxy-2,6-(diacylamino)-D-glucose[9a], and D-glucose[6] without decreasing the adjuvant activity. However, the substituents at C-2 and C-6 seem to be very critical for the manifestation of activity, because replacement[6,8,9b] of the hydroxyl group at C-2 or C-6 by a hydrogen atom was found to abolish the activity almost completely, and the D-*xylo* analog[8,9b] of the muramoyl dipeptide did not show any activity. (*2*) It has been found that not only is the sugar moiety essential for the activity[10,11], but also that the position and configuration of the linkage between the lactoyl-dipeptide and the sugar moiety are critically important[5].

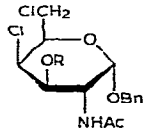
In view of these facts, our interest has been directed toward chemical modification of the substituent at C-4, or C-4 and C-6, of the carbohydrate moiety in *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, in order to clarify the structural requirements of the carbohydrate moiety for activity. We now describe the synthesis of three 4-deoxy analogs, and a 4-chloro-4-deoxy- and a 4,6-dichloro-4,6-dideoxy-D-*galacto* analog of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, and their immunoadjuvant activities.

RESULTS AND DISCUSSION

Benzyl 2-acetamido-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside[5,12,13] (**1**) and the corresponding 6-*O*-benzoyl derivative[13] (**2**) served as convenient starting-materials for the synthesis of all of the muramoyl dipeptide analogs described herein. Treatment[14] of **1** or **2** with triphenylphosphine in carbon tetrachloride respectively gave benzyl 2-acetamido-4,6-dichloro-2,4,6-trideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-galactopyranoside (**3**) or benzyl 2-acetamido-6-*O*-benzoyl-4-chloro-2,4-dideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-galactopyranoside (**4**), with inversion of the configuration of C-4, in good yields. The D-*galacto* configuration of compounds **3** and **4** was based on n.m.r. spectroscopy; the n.m.r. spectra of **3** and **4** showed the H-4 signal as a doublet of doublets, at δ 4.51 ($J_{3,4}$ 3.6, $J_{4,5}$ 2.0 Hz) for **3**, and at δ 4.45 ($J_{3,4}$ 3.6, $J_{4,5}$ 1.8 Hz) for **4**, indicating the structures

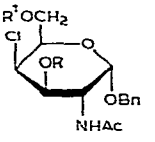


1 R = H
2 R = Bz
Bn = PhCH2
Bz = PhCO

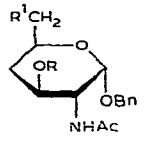


3 R = A

A = —CH(Me)—CO2Me



4 R = A, R1 = Bz
5 R = A, R1 = H



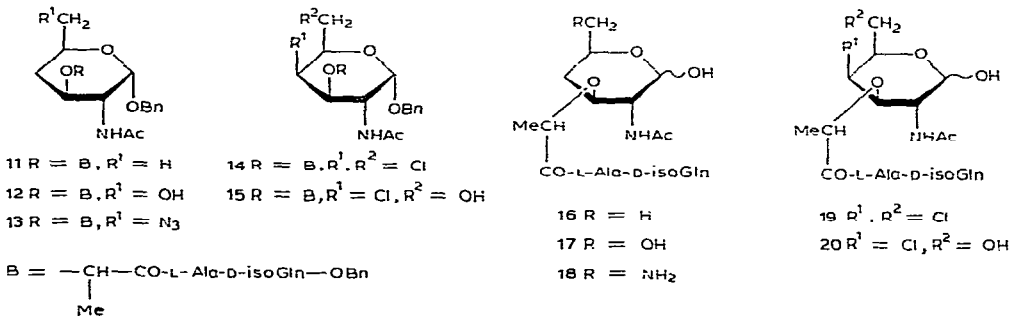
6 R = A, R1 = H
7 R = A, R1 = OBz
8 R = A, R1 = OH
9 R = A, R1 = OMs
10 R = A, R1 = N3
Ms = MeSO2

shown for the galactopyranoside derivatives, **3** and **4**. On radical dechlorination[15] with tributyltin hydride in the presence of α,α'-azobis(isobutanonitrile) in dry toluene, **3** and **4** respectively gave benzyl 2-acetamido-2,4,6-trideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-*xylo*-hexopyranoside (**6**) and benzyl 2-acetamido-6-*O*-benzoyl-2,4-dideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-*xylo*-hexopyranoside (**7**), in excellent yield.

Treatment of **8**, derived from **7** by debenzoylation, with methanesulfonyl chloride in pyridine afforded the 6-*O*-mesyl derivative **9**, which was converted, in 88% yield, into benzyl 2-acetamido-6-azido-2,4,6-trideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-*xylo*-hexopyranoside (**10**) by heating with sodium azide in *N*,*N*-dimethylformamide for 6 h at 70–80°.

Saponification of **6** with 0.1M aqueous potassium hydroxide in 1,4-dioxane gave the free acid, which was used for the next reaction without purification. Coupling of the acid with L-alanyl-D-isoglutamine benzyl ester[16] was conducted with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as the activating agents, to afford benzyl 2-acetamido-2,4,6-trideoxy-3-*O*-(D-2-propionyl-L-alanyl-D-isoglutamine benzyl ester)-α-D-*xylo*-hexopyranoside (**11**) in almost quantitative yield. In the same way, coupling of the free acids derived from compounds **3**, **5**, **8**, and **10** (by hydrolysis) with the L-alanyl-D-isoglutamine derivative yielded the corresponding dipeptides **12–15** in excellent yields.

Hydrogenolysis of the benzyl group in compound **11** with hydrogen in the presence of 10% Pd–C catalyst, in methanol–acetic acid–water, gave 2-acetamido-2,4,6-trideoxy-3-*O*-(D-2-propionyl-L-alanyl-D-isoglutamine)-D-*xylo*-hexopyranose (**16**) in good yield. By essentially the same procedure, compounds **12–15** yielded the corresponding carbohydrates analogs (**17–20**) of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, in good yields.



The immunoadjuvant activities of compounds **16–20** on the induction of the delayed type of hypersensitivity to *N*-acetyltyrosine-3-azobenzene-4′-arsonic acid (ABA-*N*-acetyltyrosine) were examined[17] in guinea-pigs (see Table I). All of the compounds exhibited weak, or no, adjuvant activity, indicating that the hydroxyl groups on C-4 and C-6 in the carbohydrate moiety of the minimal, adjuvant-active structure, namely, *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP), seem to be

TABLE I

ADJUVANT ACTIVITY OF SOME CARBOHYDRATE ANALOGS OF *N*-ACETYLMURAMOYL-L-ALANYL-D-ISOGLUTAMINE ON THE INDUCTION OF DELAYED-TYPE HYPERSENSITIVITY TO ABA-*N*-ACETYLTYROSINE IN GUINEA-PIGS

Compound[a]	*No. of animals*	*Skin reaction with* ABA–BSA[b] *(50 μg) at*	
		24 h (mm ±SE)	*48 h (mm ±SE)*[c]
16	4	(4.0 ±1.0)[d]	0
17	4	9.9 ±1.3	2.0 ±1.3
18	4	0	0
19	4	(3.3 ±0.7)[d]	0
20	4	9.3 ±0.3	3.0 ±0.7
N-Acetylmuramoyl-L-alanyl-D-isoglutamine (MDP)	5	19.7 ±0.5	16.7 ±1.4
Control	5	0	0

[a]At a dose of 100 μg. [b]Bovine serum albumin. [c]SE, Standard error. [d]Faint erythema.

important for manifestation of the activity. Replacement of the hydroxyl groups at C-4 and C-6 in MDP by a hydrogen atom (**16**) and a chlorine atom (**19**) led to almost complete loss of activity, showing that the hydroxyl group on C-6 in MDP is very critical for the activity, as compounds **17** and **20**, bearing a hydroxyl group on C-6, have a distinct, but weak, immunoadjuvant activity as compared to that of MDP.

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 90 MHz with a Hitachi R-22 spectrometer. 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-acetamido-4,6-dichloro-2,4,6-trideoxy-3-O-[D-*1*-(*methoxycarbonyl*)*ethyl*]-α-D-*galactopyranoside* (**3**). — To a solution of benzyl 2-acetamido-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**1**; 800 mg) in dry carbon tetrachloride (50 mL) was added triphenylphosphine (1.6 g), and the mixture was heated, with stirring, for 3 h at 80°. It was then cooled, the precipitate was filtered off, and the filtrate was evaporated. The residue was chromatographed on a column of silica gel (20 g), with chloroform, to give a crystalline mass. Recrystallization from ether–hexane afforded the 4,6-dichloride **3** (660 mg, 76%) as needles, m.p. 138°, $[\alpha]_D^{25}$ +209° (*c* 0.3, chloroform); ν_{max}^{Nujol} 3280 (NH), 1760, 1740, and 1200 (ester), 1650 and 1540 (amide), and 720 and 680 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*):

δ 1.47 (d, 3 H, $J_{CH_3,CH}$ 6.8 Hz, MeC), 2.00 (s, 3 H, AcN), 3.36–4.37 (m, 5 H, H-2,3,5,6,6'), 3.76 (s, 3 H, MeO), 4.12 (q, 1 H, J_{CH,CH_3} 6.8 Hz, CH), 4.51 (d of d, 1 H, $J_{3,4}$ 3.6, $J_{4,5}$ 2.0 Hz, H-4), 4.52, 4.68 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.39 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 7.07 (d, 1 H, $J_{2,NH}$ 5.2 Hz, NH), and 7.31 (s, 5 H, Ph).

Anal. Calc. for $C_{19}H_{25}Cl_2NO_6$: C, 52.54; H, 5.08; N, 3.23. Found: C, 52.51; H, 5.13; N, 3.28.

Benzyl 2-acetamido-6-O-benzoyl-4-chloro-2,4-dideoxy-3-O-[D-*1-(methoxycarbonyl)ethyl*]-α-D-*galactopyranoside* (**4**). — To a solution of **2** (1.5 g) in dry carbon tetrachloride (50 mL) was added triphenylphosphine (1.5 g), and the mixture was refluxed for 22 h. The same procedure as that described for **3** gave **4** (1.17 g, 75%) as needles, m.p. 169–170°, $[\alpha]_D^{25}$ +158° (*c* 0.3, chloroform); ν_{max}^{Nujol} 3300 (NH), 1760, 1725, and 1250 (ester), 1650 and 1555 (amide), and 730–680 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.48 (d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeC), 2.00 (s, 3 H, AcN), 3.75 (s, 3 H, MeO), 3.79–4.40 (m, 5 H, H-2,3,5,6,6'), 4.12 (q, 1 H, J_{CH,CH_3} 7.0 Hz, CH), 4.45 (d of d, 1 H, $J_{3,4}$ 3.6, $J_{4,5}$ 1.8 Hz, H-4), 4.45, 4.62 (2 d, 2 H, J_{gem} 11.5 Hz, benzyl methylene), 5.42 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 7.03 (d, 1 H, $J_{2,NH}$ 5.8 Hz, NH), 7.20 (s, 5 H, Ph), and 7.23–8.10 (m, 5 H, Ph).

Anal. Calc. for $C_{26}H_{30}ClNO_8$: C, 60.05; H, 5.82; N, 2.69. Found: C, 60.18; H, 5.88; N, 2.73.

Benzyl 2-acetamido-4-chloro-2,4-dideoxy-3-O-[D-*1-(methoxycarbonyl)ethyl*]-α-D-*galactopyranoside* (**5**). — To an ice-cooled solution of **4** (350 mg) in dry methanol (30 mL) was added M sodium methoxide (2 mL), and the solution was kept for 30 min at 10°, and then treated with Amberlite IR-120 (H^+) ion-exchange resin to remove the base; the resin was filtered off, and washed with methanol. The filtrate and washings were combined, and evaporated to a syrup which was purified by chromatography on a column of silica gel (20 g) with 100:1 chloroform–methanol. Compound **5** was obtained as needles, 270 mg (97%), m.p. 133–134°, $[\alpha]_D^{25}$ +205° (*c* 0.3, chloroform); ν_{max}^{Nujol} 3300 (NH), 1760, 1740, and 1230–1210 (ester), 1640 and 1550 (amide), and 730 and 680 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.45 (d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeC), 2.00 (s, 3 H, AcN), 2.35 (t, 1 H, $J_{6,OH}$ 6.0 Hz, OH-6), 3.76 (s, 3 H, MeO), 3.65–4.34 (m, 5 H, H-2,3,5,6,6'), 4.12 (q, 1 H, J_{CH,CH_3} 7.0 Hz, CH), 4.40 (d of d, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 1.2 Hz, H-4), 4.48, 4.65 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.38 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 7.07 (d, 1 H, $J_{2,NH}$ 4.6 Hz, NH), and 7.28 (s, 5 H, Ph).

Anal. Calc. for $C_{19}H_{26}ClNO_7$: C, 54.84; H, 6.30; N, 3.37. Found: C, 54.80; H, 6.25; N, 3.35.

Benzyl 2-acetamido-2,4,6-trideoxy-3-O-[D-*1-(methoxycarbonyl)ethyl*]-α-D-xylo-*hexopyranoside* (**6**). — To a solution of **3** (300 mg) and azobis(isobutanonitrile) (200 mg) in dry toluene (70 mL) under a nitrogen atmosphere was added, with stirring, tributyltin hydride (1.3 mL), and the mixture was stirred for 18 h at 120° (bath). The mixture was evaporated to a syrup which was chromatographed on a column of silica gel (30 g) with 200:1 chloroform–methanol, to afford compound **6** (230 mg, 91%) as a syrup, $[\alpha]_D^{25}$ +160° (*c* 0.35, chloroform); ν_{max}^{film} 3350 (NH),

1730 and 1220 (ester), 1660 and 1530 (amide), and 725 and 690 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.18 (d, 3 H, J_{5,CH_3} 6.0 Hz, Me), 1.35 (d, 3 H, $J_{CH_3,CH}$ 7.2 Hz, MeC), 1.86–2.15 (m, 2 H, H-4,4'), 2.00 (s, 3 H, AcN), 4.07 (q, 1 H, $J_{CH_3,CH}$ 7.2 Hz, CH), 4.44, 4.56 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.31 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 6.94 (d, 1 H, $J_{2,NH}$ 4.4 Hz, NH), and 7.29 (s, 5 H, Ph).

Anal. Calc. for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.38; H, 7.39; N, 3.80.

Benzyl 2-acetamido-6-O-benzoyl-2,4-dideoxy-3-O-[D-*1-(methoxycarbonyl)ethyl*]-α-D-xylo-*hexopyranoside* (**7**). — To a stirred solution of **4** (960 mg) and azobis(isobutanonitrile) (200 mg) in dry toluene (60 mL) under a nitrogen atmosphere was added tributyltin hydride (2.5 mL), and the mixture was stirred at 120° (bath), the progress of the reaction being monitored by t.l.c.; after 3 h, the starting material was no longer detectable. The same procedure used for the preparation of **6** gave compound **7** (820 mg, 92%) as needles, m.p. 151–152°, $[\alpha]_D^{25}$ +132° (*c* 0.5, chloroform); ν_{max}^{Nujol} 3300 (NH), 1740, 1720, 1705, and 1220 (ester), 1650 and 1550 (amide), and 740, 700, and 690 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.48 (d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeC), 1.47–2.22 (m, 2 H, H-4,4'), 2.02 (s, 3 H, AcN), 3.75 (s, 3 H, MeO), 3.52–4.33 (m, 5 H, H-2,3,5,6,6'), 4.11 (q, 1 H, $J_{CH_3,CH}$ 7.0 Hz, CH), 4.47, 4.67 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.40 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 7.02 (d, 1 H, $J_{2,NH}$ 5.0 Hz, NH), 7.25 (s, 5 H, Ph), and 7.40–8.10 (m, 5 H, Ph).

Anal. Calc. for $C_{26}H_{31}NO_8$: C, 64.31; H, 6.44; N, 2.89. Found: C, 64.25; H, 6.44; N, 2.81.

*Benzyl 2-acetamido-2,4-dideoxy-3-*O-[D-*1-(methoxycarbonyl)ethyl*]-α-D-xylo-*hexopyranoside* (**8**). — To a solution of **7** (720 mg) in methanol (30 mL) was added M sodium methoxide (3 mL), and the mixture was kept for 30 min at room temperature. After using the same procedure as for the preparation of **5**, compound **8** was obtained as a syrup (550 mg, 97%), $[\alpha]_D^{25}$ +88° (*c* 1.3, chloroform); ν_{max}^{film} 3320 (OH, NH), 1730 and 1220 (ester), 1650 and 1530 (amide), and 730 and 690 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.38 (d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeC), 1.52–2.05 (m, 2 H, H-4,4'), 2.02 (s, 3 H, AcN), 2.40 (near t, 1 H, OH-6), 3.52–3.68 (m, 2 H, H-6,6'), 3.70–4.10 (m, 3 H, H-2,3,5), 3.75 (s, 3 H, MeO), 4.12 (q, 1 H, J_{CH,CH_3} 7.0 Hz, CH), 4.42, 4.65 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.36 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 7.10 (d, 1 H, $J_{2,NH}$ 4.4 Hz, NH), and 7.29 (s, 5 H, Ph).

Anal. Calc. for $C_{19}H_{27}NO_7$: C, 59.83; H, 7.14; N, 3.67. Found: C, 59.58; H, 7.35; N, 3.61.

*Benzyl 2-acetamido-2,4-dideoxy-6-*O-*mesyl-3-*O-[D-*1-(methoxycarbonyl)ethyl*]-α-D-xylo-*hexopyranoside* (**9**). — To an ice-cooled solution of **8** (480 mg) in dry pyridine (5 mL) was added methanesulfonyl chloride (500 mg), and the mixture was kept overnight at 0°. The mixture was evaporated, the residue extracted with chloroform, and the extract successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried (sodium sulfate), and evaporated to give a crystalline product. Recrystallization from ether afforded **9** (470 mg, 83%) as needles, m.p. 127–128°, $[\alpha]_D^{25}$ +148° (*c* 0.3, methanol); ν_{max}^{Nujol} 3300 (NH), 1730 and 1250 (ester), 1640 and

550 (amide), 1180 (SO_2), and 720 and 690 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.38 (d, 3 H, $J_{CH_3,CH}$ 6.6 Hz, MeC), 1.40–2.18 (m, 2 H, H-4,4'), 2.01 (s, H, AcN), 3.05 (s, 3 H, MeS), 3.50–4.18 (m, 5 H, H-2,3,5,6,6'), 3.75 (s, 3 H, MeO), .11 (q, 1 H, J_{CH,CH_3} 6.6 Hz, CH), 4.47, 4.65 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), .40 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 7.12 (d, 1 H, $J_{2,NH}$ 4.0 Hz, NH), and 7.29 (s, 5 H, Ph).

Anal. Calc. $C_{20}H_{29}NO_9S$: C, 52.27; H, 6.36; N, 3.05. Found: C, 52.33, H, 6.35; N, 3.08.

Benzyl 2-acetamido-6-azido-2,4,6-trideoxy-3-O-[D-*1*-(*methoxycarbonyl*)*ethyl*]-α-D-xylo-*hexopyranoside* (**10**). — To a solution of **9** (400 mg) in dry *N*,*N*-dimethylformamide (5 mL) was added sodium azide (500 mg), and the mixture was heated, with stirring, for 6 h at 70–80°. It was then cooled, the salts were filtered off, and the filtrate was evaporated to a syrup which was extracted with chloroform. The extract was successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried (sodium sulfate), and evaporated, to give a crystalline mass. Recrystallization from ether–hexane afforded **10** (310 mg, 88%) as needles, m.p. 100°, $[\alpha]_D^{25}$ +155° (*c* 0.3, chloroform); ν_{max}^{Nujol} 3280 (NH), 2080 (azide), 1740 and 1230 (ester), 1640 and 1550 (amide), and 720 and 680 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.39 (d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeC), 1.22–2.11 (m, 2 H, H-4,4'), 2.00 (s, 3 H, AcN), 3.02–3.54 (m, 2 H, H-6,6'), 3.60–4.10 (m, 3 H, H-2,3,5), 3.74 (s, 3 H, MeO), 4.08 (q, 1 H, J_{CH,CH_3} 7.0 Hz, CH), 4.49, 4.69 (2 d, 2 H, J_{gem} 11.5 Hz, benzyl methylene), 5.38 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 7.02 (d, 1 H, $J_{2,NH}$ 4.6 Hz, NH), and 7.29 (s, 5 H, Ph).

Anal. Calc. for $C_{19}H_{26}N_4O_6$: C, 56.14; H, 6.45; N, 13.79. Found: C, 56.25; H, 6.41; N, 13.58.

Benzyl 2-acetamido-2,4,6-trideoxy-3-O-(D-*2-propionyl*-L-*alanyl*-D-*isoglutamine benzyl ester*)-α-D-xylo-*hexopyranoside* (**11**). — To a solution of **6** (100 mg) in 1,4-dioxane (5 mL) was added 0.1M potassium hydroxide (5.5 mL), and the solution was stirred for 5 min at room temperature, and then treated with Amberlite IR-120 (H^+) ion-exchange resin to remove the base; the resin was filtered off, and washed with methanol. The filtrate and washings were combined, and evaporated, to afford the crystalline, free acid which was used for the next reaction without purification.

To a solution of the acid in dry 1,4-dioxane (3 mL) were added, with stirring, *N*-hydroxysuccinimide (HOSu) (37 mg) and dicyclohexylcarbodiimide (DCC) (84 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. L-Alanyl-D-isoglutamine benzyl ester trifluoroacetate[16] (148 mg) and triethylamine (0.1 mL) were added to the mixture, and it was stirred for 1 h at room temperature, and then evaporated. The residue was chromatographed on a column of silica gel (15 g) with chloroform, and then with 50:1 chloroform–methanol. The latter eluate afforded 170 mg (97%) of **11** as crystals, m.p. 231°, $[\alpha]_D^{25}$ +93.5° (*c* 0.5, 1:1 acetic acid–methanol); ν_{max}^{Nujol} 3375, 3275 (NH), 1720 and 1260 (ester), 1675, 1640, and 1540 (amide), and 730–680 cm^{-1} (phenyl).

Anal. Calc. for $C_{33}H_{44}N_4O_9$: C, 61.86; H, 6.92; N, 12.57. Found: C, 61.85; H, 6.88; N, 12.56.

Benzyl 2-acetamido-2,4-dideoxy-3-O-(D-2-propionyl-L-alanyl-D-isoglutamine benzyl ester)-α-D-xylo-hexopyranoside (**12**). — To a solution of **8** (53 mg) in 1,4-dioxane (3 mL) was added 0.1M potassium hydroxide (2.8 mL), and the solution was stirred for 5 min at room temperature. The same procedure as that described for the preparation of **11** afforded the free acid. Coupling of the acid with the L-alanyl-D-isoglutamine derivative (77 mg) in dry 1,4-dioxane (3 mL) in the presence of HOSu (20 mg), DCC (43 mg), and triethylamine (0.1 mL), as in the preparation of **11**, 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. With the latter eluate, compound **12** was obtained as crystals, wt. 85 mg (93%), m.p. 207–208°, $[\alpha]_D^{25}$ +84° (*c* 0.5, 1:1 acetic acid–methanol); ν_{max}^{Nujol} 3360, 3275 (OH, NH), 1710 and 1260 (ester), 1660, 1640, and 1540 (amide), and 740–680 cm^{-1} (phenyl).

Anal. Calc. for $C_{33}H_{44}N_4O_{11}$: C, 60.35; H, 6.75; N, 8.53. Found: C, 60.28; H, 6.83; N, 8.49.

Benzyl 2-acetamido-6-azido-2,4,6-trideoxy-3-O-(D-2-propionyl-L-alanyl-D-isoglutamine benzyl ester)-α-D-xylo-hexopyranoside (**13**). — Hydrolysis of **10** (200 mg) with 0.1M potassium hydroxide (7.4 mL) in 1,4-dioxane (7 mL), with processing as already described, gave the free acid. Coupling of the acid with the L-alanyl-D-isoglutamine derivative (268 mg) in dry 1,4-dioxane (3 mL) by using HOSu (68 mg), DCC (150 mg), and triethylamine (0.1 mL) as described for **12**, gave **13** (330 mg, 98%) as crystals, m.p. 176°, $[\alpha]_D^{25}$ +64° (*c* 0.5, acetic acid); ν_{max}^{Nujol} 3275 and 3225 (NH), 2080 (azide), 1710 and 1210 (ester), 1670, 1630, and 1535 (amide), and 720 and 680 cm^{-1} (phenyl).

Anal. Calc. for $C_{33}H_{43}N_7O_9$: C, 58.14; H, 6.36; N, 14.35. Found: C, 58.06; H, 6.21; N, 14.25.

Benzyl 2-acetamido-4,6-dichloro-2,4,6-trideoxy-3-O-(D-2-propionyl-L-alanyl-D-isoglutamine benzyl ester)-α-D-galactopyranoside (**14**). — Hydrolysis of **3** (70 mg) with 0.1M potassium hydroxide (3.2 mL) in 1,4-dioxane (4 mL), as described for **13**, gave the free acid, which was condensed with the L-alanyl-D-isoglutamine derivative (88 mg) in dry 1,4-dioxane (2 mL) by using HOSu (22 mg), DCC (50 mg), and triethylamine (0.1 mL) as already described, to yield **14** (105 mg, 92%) as crystals, m.p. 246–247°, $[\alpha]_D^{25}$ +101.5° (*c* 0.54, acetic acid); ν_{max}^{Nujol} 3270 (NH), 1720 and 1210 (ester), 1670, 1640, 1540, and 1520 (amide), and 730, 710, and 680 cm^{-1} (phenyl).

Anal. Calc. for $C_{33}H_{42}Cl_2N_4O_9$: C, 55.85; H, 5.97; N, 7.90. Found: C, 55.83; H, 6.05; N, 7.92.

Benzyl 2-acetamido-4-chloro-2,4-dideoxy-3-O-(D-2-propionyl-L-alanyl-D-isoglutamine benzyl ester)-α-D-galactopyranoside (**15**). — Hydrolysis of **5** (70 mg) with 0.1M potassium hydroxide (3.2 mL) in 1,4-dioxane (4 mL) in the usual way gave the corresponding, free acid. Coupling of the acid with the peptide (90 mg) in dry 1,4-dioxane (2 mL) by using HOSu (23 mg), DCC (50 mg), and triethylamine (0.1 mL), as described in the preparation of **11**, afforded compound **15** (105 mg, 95%) as crystals, m.p. 236°, $[\alpha]_D^{25}$ +77° (*c* 0.5, acetic acid); ν_{max}^{Nujol} 3380 (OH), 3275 (NH),

1710 and 1260 (ester), 1660, 1640, and 1530 (amide), and 730, 710, and 680 cm^{-1} (phenyl).

Anal. Calc. for $C_{33}H_{43}ClN_4O_{10}$: C, 57.34; H, 6.27; N, 8.11. Found: C, 57.29; H, 6.41; N, 8.05.

*2-Acetamido-2,4,6-trideoxy-3-O-(*D*-2-propionyl-*L*-alanyl-*D*-isoglutamine)-*D*-xylo-hexopyranose* (**16**). — Compound **11** (50 mg) was dissolved in methanol (15 mL) and acetic acid (1 mL), 10% Pd–C catalyst (100 mg) was added, and hydrogen was bubbled through while the mixture was stirred for 30 min at room temperature. Water (10 mL) was added to the mixture; hydrogen was again bubbled through the mixture, with stirring, for 1 h at 30–40°, the course of the reaction being monitored by t.l.c. The catalyst was removed by filtration, and the filtrate was evaporated below 30°, to give a hygroscopic, amorphous mass (30 mg, quantitative yield), which showed a single spot in t.l.c.; $[\alpha]_D^{20}$ +41° (*c* 0.3, water; equil.); ν_{max}^{KBr} 3450–3250 (OH, NH), 1710 (C=O), and 1650 and 1520 cm^{-1} (amide); n.m.r. data (in D_2O): δ 1.26–1.44 (m, 6 H, 2 Me), 2.00 (s, 3 H, AcN), and 5.15 (d, $J_{1,2}$ 2.0 Hz, H-1α).

Anal. Calc. for $C_{19}H_{32}N_4O_9$: C, 49.55; H, 7.00; N, 12.17. Found: C, 49.23; H, 7.36; N, 11.89.

*2-Acetamido-2,4-dideoxy-3-O-(*D*-2-propionyl-*L*-alanyl-*D*-isoglutamine)-*D*-xylo-hexopyranose* (**17**), — Compound **12** (50 mg) was hydrogenated in the presence of 10% Pd–C catalyst (100 mg), in methanol (15 mL)–acetic acid (1 mL)–water (10 mL), as described in the preparation of **16**, to yield **17** (30 mg, 90%) as an amorphous material, $[\alpha]_D^{20}$ +51° (*c* 0.3, water; equil.); ν_{max}^{KBr} 3400–3250 (OH, NH), 1720 (C=O), and 1650 and 1520 cm^{-1} (amide); n.m.r. data (in D_2O): δ 1.32 and 1.39 (2 d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeC), 1.99 (s, 3 H, AcN), and 5.22 (d, $J_{1,2}$ 3.8 Hz, H-1α).

Anal. Calc. for $C_{19}H_{32}N_4O_{10}$: C, 47.89; H, 6.77; N, 11.76. Found: C, 47.55; H, 7.13; N, 11.49.

*2-Acetamido-6-amino-2,4,6-trideoxy-3-O-(*D*-2-propionyl-*L*-alanyl-*D*-isoglutamine)-*D*-xylo-hexopyranose* (**18**). — Compound **13** (50 mg) was hydrogenated in the presence of 10% Pd–C catalyst (100 mg), in methanol (15 mL)–acetic acid (5 mL)–water (10 mL), as already described, to give **18** (30 mg, 90%) as an amorphous mass; $[\alpha]_D^{20}$ +14° (*c* 0.3, water; equil.); ν_{max}^{KBr} 3400–3200 (OH, NH), 1720 (C=O), and 1670, 1640, 1550, 1530, and 1520 cm^{-1} (amide); n.m.r. data (in D_2O): δ 1.33 and 1.40 (2 d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeC), 2.00 (s, 3 H, AcN), and 5.26 (d, $J_{1,2}$ 3.0 Hz, H-1α).

Anal. Calc. for $C_{19}H_{33}N_5O_9$: C, 47.99; H, 7.00; N, 14.73. Found: C, 47.58; H, 7.35; N, 14.53.

*2-Acetamido-4,6-dichloro-2,4,6-trideoxy-3-O-(*D*-2-propionyl-*L*-alanyl-*D*-isoglutamine)-*D*-galactopyranose* (**19**). — Hydrogenation of compound **14** (50 mg) with hydrogen in the presence of 10% Pd–C catalyst (100 mg) in methanol (15 mL)–acetic acid (1 mL)–water (10 mL), according to the procedure already described, gave **19** (35 mg, 95%) as an amorphous material; $[\alpha]_D^{20}$ +55° (*c* 0.3, water; equil.); ν_{max}^{KBr} 3370–3250 (OH, NH), 1710 (C=O), and 1640, 1540, and 1520 cm^{-1} (amide);

n.m.r. data (in D_2O): δ 1.40 and 1.42 (2 d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeC), 2.00 and 2.01 (2 s, 3 H, AcN), and 5.26 (d, $J_{1,2}$ 4.0 Hz, H-1α).

Anal. Calc. for $C_{19}H_{30}Cl_2N_4O_9$: C, 43.11; H, 5.71; N, 10.58. Found: C, 42.92; H, 5.95; N, 10.33.

*2-Acetamido-4-chloro-2,4-dideoxy-3-*O-(D-*2-propionyl-*L-*alanyl-*D-*isoglutamine*)-D-*galactopyranose* (**20**). — Hydrogenation of compound **15** (50 mg) with hydrogen in the presence of 10% Pd–C catalyst (100 mg), in methanol (15 mL)–acetic acid (1 mL)–water (10 mL), according to the procedure described in the preparation of **16**, yielded **20** (32 mg, 90%) as an amorphous material; $[\alpha]_D^{20}$ +20° (*c* 0.3, water; equil.); ν_{max}^{KBr} 3500–3300 (OH, NH), 1720 (C=O), and 1660–1640 and 1550 cm^{-1} (amide): n.m.r. data (in D_2O); δ 1.38, 1.41 (2 d, 3 H, $J_{CH_3,CH}$ 7.0 Hz, MeC), 1.98 (s, 3 H, AcN), and 5.24 (d, $J_{1,2}$ 3.8 Hz, H-1α).

Anal. Calc. for $C_{19}H_{31}ClN_4O_{10}$: C, 44.66; H, 6.12; N, 10.97. Found: C, 44.28; H, 6.45; N, 10.70.

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