Carbohydrate Research, 103 (1982) 273–280
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Synthesis of 4-acetamido-*N*-acetyl-4-deoxy- and 4,6-di(acetamido)-*N*-acetyl-4,6-dideoxy-muramoyl-L-alanyl-D-isoglutamine derivatives*

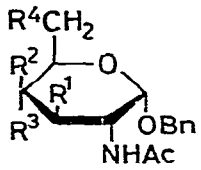
AKIRA HASEGAWA, EIJI TANAHASHI, YASUHIKO GOH, AND MAKOTO KISO
Department of Agricultural Chemistry, Gifu University, Kakamigahara, Gifu 504 (Japan)
(Received November 9th, 1981; accepted for publication, December 9th, 1981)

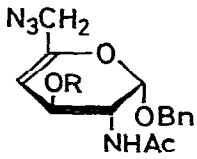
In a preceding paper[1], we described the synthesis of some analogs of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) having modified substituents at C-4, and C-4 and C-6, of the carbohydrate moiety in MDP, which is the minimal immunoadjuvant-active structure[2] of bacterial cell-wall peptidoglycans, in order to clarify the structural requirements of the sugar moiety for the activity. We now describe the synthesis of 4-acetamido-*N*-acetyl-4-deoxy- and 4,6-di(acetamido)-*N*-acetyl-4,6-dideoxy-muramoyl-L-alanyl-D-isoglutamine derivatives.

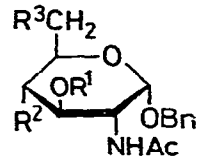
Benzyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside[3] (**1**) and the corresponding 3,6-di-*O*-benzoyl derivative (**2**), prepared from **1** by selective benzoylation, served as convenient starting-materials for the synthesis of the title compounds. Treatment[4,5] of **1** or **2** with triphenylphosphine in carbon tetrachloride respectively gave benzyl 2-acetamido-3-*O*-benzoyl-4,6-dichloro-2,4,6-trideoxy-α-D-galactopyranoside (**9**) or benzyl 2-acetamido-3,6-di-*O*-benzoyl-4-chloro-2,4-dideoxy-α-D-galactopyranoside (**3**), with inversion of the configuration of C-4, in good yield. The D-*galacto* configuration of compounds **3** and **9** was based on n.m.r. spectroscopy; the n.m.r. spectra of **3** and **9** showed the H-3 signals as a doublet of doublets ($J_{2,3}$ 10.0, $J_{3,4}$ 4.0 Hz) at δ 5.46 for **3**, and at δ 5.41 for **9**, indicating the structures shown for the galactopyranoside derivatives **3** and **9**. Treatment of **4**, derived from **3** by debenzoylation, with 2,3-dihydro-4*H*-pyran in the presence of *p*-toluenesulfonic acid afforded **5**, which was converted into benzyl 2-acetamido-4-azido-2,4-dideoxy-3,6-di-*O*-(tetrahydropyran-2-yl)-α-D-glucopyranoside (**6**) by heating with sodium azide in dry *N,N*-dimethylformamide for 24 h at 140°. Hydrolytic removal of the tetrahydropyranyl groups of **6** under mildly acidic conditions gave **7**, which was treated with chlorotriphenylmethane in pyridine to afford the 6-*O*-trityl derivative **8**. Condensation of **8** with L-2-chloropropanoic acid in the presence of sodium hydride, and hydrolysis of the product, gave the 3-*O*-(D-1-carboxyethyl) derivative (**15**) in good yield.

*Studies on Immunoadjuvant Active Compounds, Part XIX. For Part XVIII, see ref. 1.

0008-6215/82/0000–0000/S 02.75,







1 R^1 = OBz, R^2 = H, R^3 = R^4 = OH
2 R^1 = R^4 = OBz, R^2 = H, R^3 = OH
3 R^1 = R^4 = OBz, R^2 = Cl, R^3 = H
4 R^1 = R^4 = OH, R^2 = Cl, R^3 = H
5 R^1 = R^4 = OThp, R^2 = Cl, R^3 = H
6 R^1 = R^4 = OThp, R^2 = H, R^3 = N_3
7 R^1 = R^4 = OH, R^2 = H, R^3 = N_3
8 R^1 = OH, R^2 = H, R^3 = N_3, R^4 = OTr
9 R^1 = OBz, R^2 = R^4 = Cl, R^3 = H
10 R^1 = OH, R^2 = R^4 = Cl, R^3 = H
11 R^1 = OH, R^2 = H, R^3 = R^4 = N_3
12 R^1 = OAc, R^2 = H, R^3 = R^4 = N_3

13 R = H
14 R = Ac

Ac = MeCO
Bn = $PhCH_2$
Bz = PhCO
Thp = tetrahydropyran-2-yl
Tr = Ph_3C

15 R^1 = a, R^2 = N_3, R^3 = OH
16 R^1 = a, R^2 = R^3 = N_3
17 R^1 = b, R^2 = N_3, R^3 = OH
18 R^1 = b, R^2 = R^3 = N_3
19 R^1 = c, R^2 = NHAc, R^3 = OH
20 R^1 = c, R^2 = R^3 = NHAc

a = -CH(Me)CO_2H
b = -CH(Me)CO-L-Ala-D-isoGln-OBn
c = -CH(Me)CO-L-Ala-D-isoGln

On the other hand, benzyl 2-acetamido-4,6-dichloro-2,4,6-trideoxy-α-D-galactopyranoside (**10**), derived from **9** by debenzoylation, was treated with an excess of sodium azide in dry *N*,*N*-dimethylformamide for 20 h at 140°, to afford benzyl 2-acetamido-4,6-diazido-2,4,6-trideoxy-α-D-glucopyranoside (**11**) in 54% yield, together with benzyl 2-acetamido-6-azido-2,4,6-trideoxy-β-L-*threo*-hex-4-enopyranoside (**13**) as a minor product. Compounds **11** and **13** were respectively acetylated with acetic anhydride in pyridine, to produce the 3-acetates (**12** and **14**) in quantitative yield. The structures of **11** and **13** were determined from the n.m.r. data of the corresponding 3-*O*-acetyl derivatives (**12** and **14**); a significant signal in the n.m.r. spectrum of **12** was a one-proton triplet at δ 5.21 ($J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), and the n.m.r. data for **14** showed the H-3 signal as a doublet of doublets, at δ 5.45 ($J_{2,3}$ 8.6, $J_{3,4}$ 3.0 Hz), and the H-4 signal as a doublet at δ 4.91. Other n.m.r. data are given in the Experimental section, and are respectively consistent with structures **12** and **14**.

Condensation of **11** with L-2-chloropropanoic acid as already described gave benzyl 2-acetamido-4,6-diazido-2,4,6-trideoxy-3-*O*-(D-1-carboxyethyl)-α-D-glucopyranoside (**16**). Coupling of the acids **15** and **16** with L-alanyl-D-isoglutamine benzyl ester, using dicyclohexylcarbodiimide and *N*-hydroxysuccinimide (DCC–HOSu) as the activating agents in dry 1,4-dioxane, respectively afforded the corresponding lactoyl dipeptide derivatives (**17** and **18**) in almost quantitative yield.

Hydrogenolytic removal of the benzyl group and reduction of the azide group in compounds **17** and **18**, in methanol, with hydrogen in the presence of 10% Pd–C catalyst at room temperature, and *N*-acetylation of the respective product, afforded the title compounds (**19** and **20**) in high yields.

EXPERIMENTAL

General methods. — See ref. 1.

Benzyl 2-acetamido-3,6-di-O-benzoyl-2-deoxy-α-D-glucopyranoside (**2**). — To a solution of benzyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside[3] (**1**; 3.2 g) in pyridine (10 mL) was added benzoyl chloride (1.2 g) at −25°. The mixture was kept for 10 h at −10°; by that time, most of the starting material had been converted into the 3,6-dibenzoate. The mixture was extracted with chloroform, and 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 (50 g) with 200:1 chloroform–methanol, was obtained as a syrup: wt. 3.5 g (88%), $[\alpha]_D^{25}$ +106° (*c* 0.24, methanol); ν_{max}^{film} 3340–3250 (OH, NH), 1720 and 1270 (ester), 1660 and 1530 (amide), and 750, 710, and 690 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.70 (s, 3 H, AcN), 4.44, 4.72 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 4.87 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.39 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz, H-3), 6.12 (d, 1 H, $J_{2,NH}$ 10.0 Hz, NH), 7.23 (s, 5 H, Ph), and 7.20–8.03 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{29}H_{29}NO_8$: C, 67.04; H, 5.63; N, 2.70. Found: C, 67.21; H, 5.85; N, 2.67.

Benzyl 2-acetamido-3,6-di-O-benzoyl-4-chloro-2,4-dideoxy-α-D-galactopyranoside (**3**). — To a solution of **2** (3.3 g) in dry carbon tetrachloride (50 mL) was added triphenylphosphine (3.3 g), and the mixture was boiled under reflux, with stirring, for 15 h. It was then cooled, the precipitate was filtered off, and the filtrate was evaporated. The residue was chromatographed on a column of silica gel (60 g), with chloroform, to give a crystalline mass. Recrystallization from ether afforded the 4-chloro compound **3** (3.3 g, 97%) as needles, m.p. 141°, $[\alpha]_D^{25}$ +130° (*c* 1.0, methanol); ν_{max}^{Nujol} 3270 (NH), 1730, 1710, and 1260 (ester), 1640 and 1550 (amide), and 740 and 700 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.84 (s, 3 H, AcN), 4.51, 4.77 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 4.66 (dd, 1 H, $J_{3,4}$ 4.0, $J_{4,5}$ 1.0 Hz, H-4), 5.07 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.46 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 4.0 Hz, H-3), 5.72 (d, 1 H, $J_{2,NH}$ 9.5 Hz, NH), 7.29 (s, 5 H, Ph), and 7.22–8.10 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{29}H_{28}ClNO_7$: C, 64.74; H, 5.25; N, 2.60. Found: C, 64.59; H, 5.24; N, 2.58.

Benzyl 2-acetamido-4-chloro-2,4-dideoxy-α-D-galactopyranoside (**4**). — To an ice-cooled solution of **3** (1.78 g) in dry methanol (20 mL) was added sodium methoxide (30 mg), and the solution was kept for 30 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 a crystalline mass. Recrystallization from ether afforded **4** (900 mg, 83%) as needles, m.p. 199–201°, $[\alpha]_D^{25}$ +228° (*c* 0.2, methanol); ν_{max}^{Nujol} 3330, 3250, and 3180 (OH, NH), 1640 and 1540 (amide), and 740 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{15}H_{20}ClNO_5$: C, 54.63; H, 6.11; N, 4.25. Found: C, 54.49; H, 6.23; N, 4.21.

Benzyl 2-acetamido-4-chloro-2,4-dideoxy-3,6-di-O-(*tetrahydropyran-2-yl*)-α-D-*galactopyranoside* (**5**). — To a stirred solution of **4** (600 mg) in dry 1,4-dioxane (10 mL) were added 2,3-dihydro-4*H*-pyran (1 g) and *p*-toluenesulfonic acid monohydrate (10 mg). The mixture was stirred at room temperature; after 2 h, the starting material and mono-*O*-tetrahydropyran-2-yl derivative were no longer detectable by t.l.c. The mixture was treated with Amberlite IRA-410 (OH^-) resin to remove the acid, and evaporated to a syrup. The product was purified by chromatography on a column of silica gel (20 g) with chloroform, and then with 250:1 chloroform–methanol. The latter eluate gave **5** (850 mg, 94%) as an amorphous material; ν_{max}^{Nujol} 3240 (NH), 1650 and 1540 (amide), and 730 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{25}H_{36}ClNO_7$: C, 60.29; H, 7.29; N, 2.81. Found: C, 60.33; H, 7.25; N, 2.99.

Benzyl 2-acetamido-4-azido-2,4-dideoxy-3,6-di-O-(*tetrahydropyran-2-yl*)-α-D-*glucopyranoside* (**6**). — To a solution of **5** (600 mg) in dry *N*,*N*-dimethylformamide (10 mL) was added sodium azide (800 mg), and the mixture was heated, with stirring, for 24 h at 140°. 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 a syrup which was purified by chromatography on a column of silica gel (20 g) with chloroform. Compound **6** was obtained as a syrup, 295 mg (49%); ν_{max}^{film} 3300 (NH), 2080 (N_3), 1660 and 1530 (amide), and 730 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{25}H_{36}N_4O_7$: C, 59.51; H, 7.19; N, 11.10. Found: C, 59.80; H, 7.42; N, 10.95.

Benzyl 2-acetamido-4-azido-2,4-dideoxy-α-D-*glucopyranoside* (**7**). — A solution of **6** (200 mg) in 70% aqueous acetic acid (10 mL) was heated for 1 h at 40°, and then evaporated to a syrup. The residue was chromatographed on a column of silica gel (10 g) with chloroform and then 100:1 chloroform–methanol. The latter eluate gave **7** (120 mg, 90%) as needles, m.p. 174–176°, $[\alpha]_D^{25}$ +245° (*c* 0.7, chloroform) (lit.[6] m.p. 177°, $[\alpha]_D^{20}$ +217°); ν_{max}^{Nujol} 3330–3260 (OH, NH), 2100 (N_3), 1640 and 1540 (amide), and 730 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{15}H_{20}N_4O_5$: C, 53.56; H, 5.99; N, 16.66. Found: C, 53.52; H, 6.20; N, 16.58.

Benzyl 2-acetamido-4-azido-2,4-dideoxy-6-O-*trityl*-α-D-*glucopyranoside* (**8**). — To a solution of **7** (90 mg) in pyridine (2 mL) was added trityl chloride (85 mg), and the mixture was heated for 5 h at 90–95°, cooled, and evaporated. The product was purified by chromatography on a column of silica gel (10 g) with (*a*) 150:1 and (*b*) 100:1 chloroform–methanol. Eluant (*b*) gave **8** (130 mg, 84%) as needles, m.p. 174–175°, $[\alpha]_D^{25}$ +74° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3300–3250 (OH, NH), 2080 (N_3), 1650 and 1530 (amide), and 750, 730, 700, and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{34}H_{34}N_4O_5$: C, 70.57; H, 5.92; N, 9.68. Found: C, 70.54; H, 5.86; N, 9.71.

Benzyl 2-acetamido-3-O-benzyl-4,6-dichloro-2,4,6-trideoxy-α-D-galactopyranoside (**9**). — To a solution of[3] **1** (1.5 g) in dry carbon tetrachloride (50 mL) was added triphenylphosphine (3.0 g), and the mixture was boiled under reflux for 16 h. The procedure described for **3** gave **9** (1.37 g, 84%) as needles, m.p. 160–162°, $[\alpha]_D^{25}$ +232° (*c* 0.27, methanol); ν_{max}^{Nujol} 3240 (NH), 1720 and 1270 (ester), 1640 and 1560 (amide), and 730–690 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.83 (s, 3 H, AcN), 3.43–3.67 (m, 2 H, H-6,6'), 4.55, 4.78 (2 d, 2 H, J_{gem} 11.5 Hz, benzyl methylene), 4.67 (dd, 1 H, $J_{3,4}$ 4.0, $J_{4,5}$ 1.5 Hz, H-4), 5.01 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.41 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 4.0 Hz, H-3), 5.73 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 7.33 (s, 5 H, Ph), and 7.22–8.08 (m, 5 H, Ph).

Anal. Calc. for $C_{22}H_{23}Cl_2NO_5$: C, 58.41; H, 5.13; N, 3.10. Found: C, 58.39; H, 5.12; N, 3.03.

Benzyl 2-acetamido-4,6-dichloro-2,4,6-trideoxy-α-D-galactopyranoside (**10**). — To a solution of **9** (470 mg) in methanol (10 mL) was added sodium methoxide (20 mg), and the mixture was kept for 30 min at room temperature. After using the same procedure as for the preparation of **4**, compound **10** was obtained as needles (350 mg, 97%), m.p. 198–203° (dec.), $[\alpha]_D^{25}$ +243° (*c* 0.5, methanol); ν_{max}^{Nujol} 3340 (OH), 3250 (NH), 1650 and 1540 (amide), and 730 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{15}H_{19}Cl_2NO_4$: C, 51.73; H, 5.50; N, 4.02. Found: C, 51.51; H, 5.54; N, 3.96.

Benzyl 2-acetamido-4,6-diazido-2,4,6-trideoxy-α-D-glucopyranoside (**11**) *and benzyl 2-acetamido-6-azido-2,4,6-trideoxy-β-L-threo-hex-4-enopyranoside* (**13**). — To a solution of **10** (360 mg) in dry *N,N*-dimethylformamide (2 mL) was added sodium azide (670 mg), and the mixture was heated, with stirring, for 20 h at 140°. The procedure used for the preparation of **6** gave compound **11** (200 mg, 54%) as needles, m.p. 128–129°, $[\alpha]_D^{25}$ +145° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3270 (OH, NH), 2100 and 2070 (N_3), 1620 and 1540 (amide), and 730 and 690 cm^{-1} (phenyl); and compound **13** (80 mg, 24%) as an amorphous material which was characterized after acetylation.

Anal. Calc. for $C_{15}H_{19}N_7O_4$ (for **11**): C, 49.85; H, 5.30; N, 27.14. Found: C, 49.69; H, 5.21; N, 27.30.

Benzyl 2-acetamido-3-O-acetyl-4,6-diazido-2,4,6-trideoxy-α-D-glucopyranoside (**12**). — A sample of **11** (200 mg) was acetylated at room temperature with acetic anhydride in pyridine, and the product was purified by chromatography on a column of silica gel (20 g) with chloroform. The acetate **12** was obtained as needles (220 mg, 99%), m.p. 132°, $[\alpha]_D^{25}$ +215° (*c* 0.24, chloroform); ν_{max}^{Nujol} 3200 (NH), 2080 (N_3), 1750 and 1250 (ester), 1640 and 1550 (amide), and 730 and 690 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.88 (s, 3 H, AcN), 2.09 (s, 3 H, AcO), 3.43–3.72 (m, 4 H, H-4,5,6,6'), 4.29 (m, 1 H, H-2), 4.50, 4.72 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 4.93 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.21 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.82 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), and 7.32 (s, 5 H, Ph).

Anal. Calc. for $C_{17}H_{21}N_7O_5$: C, 50.61; H, 5.25; N, 24.31. Found: C, 50.55; H, 5.26; N, 24.56.

*Benzyl 2-acetamido-3-O-acetyl-6-azido-2,4,6-trideoxy-β-*L-threo-*hex-4-enopyranoside* (**14**). — A sample of **13** (100 mg) was acetylated at room temperature with acetic anhydride–pyridine, and the procedure just described gave **14** (105 mg, 93%) as needles, m.p. 103–104°, $[\alpha]_D^{25}$ +234° (*c* 0.23, chloroform); ν_{max}^{Nujol} 3230 (NH), 2070 (N_3), 1730 and 1260 (ester), 1690 (C=C), 1630 and 1520 (amide), and 740 and 690 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.90 (s, 3 H, AcN), 2.02 (s, 3 H, AcO), 3.59 (s, 2 H, H-6,6'), 4.36 (m, 1 H, H-2), 4.60, 4.82 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 4.91 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4), 5.45 (dd, 1 H, $J_{2,3}$ 8.6, $J_{3,4}$ 3.0 Hz, H-3), 6.02 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), and 7.30 (s, 5 H, Ph).

Anal. Calc. for $C_{17}H_{20}N_4O_5$: C, 56.66; H, 5.59; N, 15.55. Found: C, 56.51; H, 5.58; N, 15.49.

*Benzyl 2-acetamido-4-azido-3-*O-(D-*1-carboxyethyl*)-*2,4-dideoxy-α-*D-*glucopyranoside* (**15**). — To a stirred solution of **8** (130 mg) in dry 1,4-dioxane (3 mL) was added sodium hydride in oil suspension (30 mg; 50% of sodium hydride by weight). The mixture was kept for 30 min at 90°, and then L-2-chloropropanoic acid (25 mg) was added, with stirring, at 60°. The mixture was stirred for 1 h at 60°, cooled, and evaporated. Chloroform (30 mL) and water (10 mL) were added to the residue, and then 2M hydrochloric acid was added, with stirring, at 0°, to pH 3. The chloroform layer was separated, and the aqueous solution was thoroughly extracted with chloroform. The chloroform layer and extracts were combined, and evaporated. A solution of the residue in 70% aqueous acetic acid (10 mL) was heated for 30 min at 50°, cooled, and evaporated, and the residue was chromatographed on a column of silica gel (10 g) with (*a*) chloroform, (*b*) 100:1, and (*c*) 20:1 chloroform–methanol. Eluant (*c*) gave **15** (70 mg, 76%) as an amorphous material, $[\alpha]_D^{25}$ +149° (*c* 0.62, chloroform); ν_{max}^{KBr} 3350–3250 (OH, NH), 2070 (N_3), 2700–2450 (OH), 1730 (C=O), 1640 and 1530 (amide), and 730 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{18}H_{24}N_4O_7$: C, 52.93; H, 5.92; N, 13.72. Found: C, 52.65; H, 6.25; N, 13.49.

*Benzyl 2-acetamido-4,6-diazido-3-*O-(D-*1-carboxyethyl*)-*2,4,6-trideoxy-α-*D-*glucopyranoside* (**16**). — To a stirred solution of **11** (220 mg) in dry 1,4-dioxane (2 mL) was added the sodium hydride reagent (45 mg), and the mixture was kept, with stirring, for 30 min at 90°, and then cooled. L-2-Chloropropanoic acid (80 mg) and the sodium hydride reagent (40 mg) were added to the stirred mixture, which was then kept at 65°, 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 **15** gave compound **16** (180 mg, 68%) as needles, m.p. 169°, $[\alpha]_D^{25}$ +193° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3240 (NH), 2700–2500 (OH), 2100 and 2080 (N_3), 1730 (C=O), 1620 and 1540 (amide), and 730 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{18}H_{23}N_7O_6$: C, 49.88; H, 5.35; N, 22.62. Found: C, 49.63; H, 5.42; N, 22.80.

*Benzyl 2-acetamido-4-azido-2,4-dideoxy-3-*O-(D-*2-propanoyl*-L-*alanyl*-D-*isoglu-*

tamine benzyl ester)-α-D-*glucopyranoside* (**17**). — To a solution of **15** (60 mg) in dry 1,4-dioxane (1 mL) were added, with stirring, *N*-hydroxysuccinimide (HOSu) (30 mg) and dicyclohexylcarbodiimide (DCC) (40 mg), and the mixture was stirred for 30 min at room temperature; at that time, the starting acid had been converted into the activated ester. L-Alanyl-D-isoglutamine benzyl ester trifluoroacetate (70 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 (10 g) with (*a*) 100:1 and (*b*) 30:1 chloroform–methanol. Eluant (*b*) gave **17** (95 mg, 93%) as crystals, m.p. 216–217°, $[\alpha]_D^{25}$ +144° (*c* 0.2, methanol); ν_{max}^{KBr} 3350 (OH), 3240 (NH), 2090 (N_3), 1730 and 1250 (ester), 1670, 1620, and 1540–1520 (amide), and 730 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{33}H_{43}N_7O_{10}$: C, 56.80; H, 6.21; N, 14.05. Found: C, 56.69; H, 6.33; N, 14.12.

Benzyl 2-acetamido-4,6-diazido-2,4,6-trideoxy-3-O-(D-*2-propanoyl*-L-*alanyl*-D-*isoglutamine benzyl ester*)-α-D-*glucopyranoside* (**18**). — Coupling of **16** (80 mg) with the L-alanyl-D-isoglutamine derivative (100 mg) in dry 1,4-dioxane (2 mL) by using HOSu(40 mg), DCC (70 mg), and triethylamine (0.1 mL), as described in the preparation of **17**, afforded compound **18** (130 mg, 98%) as crystals, m.p. 217–218°, $[\alpha]_D^{25}$ +147° (*c* 0.3, methanol); ν_{max}^{Nujol} 3240 (NH), 2080 (N_3), 1720 and 1250 (ester), 1660, 1640, and 1530 (amide), and 730 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{33}H_{42}N_{10}O_9$: C, 54.84; H, 5.86; N, 19.38. Found: C, 54.69; H, 6.02; N, 19.29.

Benzyl 2,4-di(acetamido)-2,4-dideoxy-3-O-(D-*2-propanoyl*-L-*alanyl*-D-*isoglutamine*)-α-D-*glucopyranoside* (**19**). — Compound **17** (52 mg) was dissolved in methanol (10 mL), 10% Pd–C catalyst (100 mg) was added, and hydrogen was bubbled through while the mixture was stirred for 1 h at room temperature; at that time, the starting material was no longer detectable by t.l.c. Acetic anhydride (1 mL) was added to the mixture, and it was kept for 30 min at room temperature. The catalyst was removed by filtration, and the filtrate was lyophilized, to give **19** as a hygroscopic, amorphous mass (46 mg, 93%), which showed a single spot in t.l.c.; $[\alpha]_D^{25}$ +75° (*c* 0.2, methanol); ν_{max}^{KBr} 3350–3220 (OH, NH), 1720 (C=O), 1650 and 1530 (amide), and 730 and 690 cm^{-1} (phenyl); n.m.r. data (in D_2O): δ 1.20, 1.32 (2 d, 6 H, $J_{Me,CH}$ 7.0 Hz, 2 MeC), 1.82, 1.98 (2 s, 6 H, 2 AcN), 4.84 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), and 7.33 (s, 5 H, Ph).

Anal. Calc. for $C_{28}H_{41}N_5O_{11}$: C, 53.92; H, 6.63; N, 11.23. Found: C, 53.56; H, 6.95; N, 11.15.

Benzyl 2,4,6-tri(acetamido)-2,4,6-trideoxy-3-O-(D-*2-propanoyl*-L-*alanyl*-D-*isoglutamine*)-α-D-*glucopyranoside* (**20**). — Hydrogenation of compound **18** (61 mg) with hydrogen in the presence of 10% Pd–C catalyst (100 mg) in methanol (10 mL), and subsequent *N*-acetylation with acetic anhydride (1 mL) according to the procedure described in the preparation of **19**, yielded **20** (54 mg, 96%) as an amorphous material; $[\alpha]_D^{25}$ +96° (*c* 0.3, methanol); ν_{max}^{KBr} 3320–3220 (NH), 1720 (C=O), 1650 and 1530 (amide), and 730 and 690 cm^{-1} (phenyl); n.m.r. data (in D_2O): δ 1.19, 1.33 (2 d,

6 H, $J_{Me,CH}$ 7.0 Hz, 2 MeC), 1.83, 1.92, 1.97 (3 s, 9 H, 3 AcN), 4.84 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), and 7.33 (s, 5 H, Ph).

Anal. Calc. for $C_{30}H_{44}N_6O_{11}$: C, 54.20; H, 6.67; N, 12.64. Found: C, 54.05; H, 6.99; N, 12.51.

REFERENCES

1 A. HASEGAWA, E. TANAHASHI, Y. GOH, AND M. KISO, *Carbohydr. Res.*, 103 (1982) 263–272.
2 (a) F. ELLOUZ, A. ADAM, R. CIORBARU, AND E. LEDERER, *Biochem. Biophys. Res. Commun.*, 59 (1974) 1317–1325; (b) C. MERSER, P. SINAŸ, AND A. ADAM, *ibid.*, 66 (1975) 1316–1322; (c) S. KOTANI, Y. WATANABE, F. KINOSHITA, T. SHIMONO, I. MORISAKI, T. SHIBA, S. KUSUMOTO, Y. TARUMI, AND K. IKENAKA, *Biken J.*, 18 (1975) 105–111.
3 M. PARQUET AND P. SINAŸ, *Carbohydr. Res.*, 18 (1971) 195–202.
4 J. B. LEE AND T. J. NOLAN, *Can. J. Chem.*, 44 (1966) 1331–1334.
5 A. HASEGAWA, E. TANAHASHI, Y. GOH, M. KISO, AND I. AZUMA, *Carbohydr. Res.*, 92 (1981) 75–84.
6 W. MEYER ZU RECKENDORF AND N. WASSILIANDOU-MICHELI, *Chem. Ber.*, 105 (1972) 2998–3013.