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Note

Synthesis of *N*-(2-acetamido-2,3-dideoxy-D-glucopyranos-3-yl)glycyl-L-alanyl-D-isoglutamine analogues of muramyl dipeptide

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N-Acetylmuramyl-L-alanyl-D-isoglutamine **13** (muramyl dipeptide, MDP), a constituent of most bacterial cell-wall peptidoglycans, is the minimal adjuvant active structure capable of replacing whole mycobacterial cells in Freund's complete adjuvant for increasing the levels of humoral antibodies against a given antigen and for inducing delayed hypersensitivity[1,2]. It also stimulates non-specific resistance against bacterial, viral, and parasite infections[3–5]. However, MDP (**13**) has such untoward effects as pyrogenicity[6–8], transitory leukopenia[6], thrombocytolysis[8], and somnogenicity[9]. Consequently, the MDP structure has been extensively modified[4,10–12] and it has been found that the alkyl esters of the D-glutamic acid analogue are less pyrogenic than MDP[13,14], and that substitution of the D-lactic acid residue of MDP by hydroxyacetic acid (nor-MDP) gives a compound that is less active and less toxic than MDP[11,12].

We now describe the synthesis of *N*-(2-acetamido-2,3-dideoxy-D-glucopyranos-3-yl) derivatives of glycyl-L-alanyl-D-isoglutamine methyl ester (**11**) and glycyl-L-alanyl-D-glutamine methyl ester (**12**) in which the lactic acid residue of MDP (**13**) has been replaced by glycine.

Benzyl 2-acetamido-3-azido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-glucopyranoside[15–17] (**2**) was prepared (75%) by an improved procedure involving the treatment of the 3-*O*-mesyl-α-D-allopyranoside **1** with sodium azide and tetrabutylammonium hydrogen sulfate in *N*,*N*-dimethylformamide, which avoided[16] competing elimination of MsO-3. The high chemoselectivity towards substitution of this phase-transfer reaction is favoured by the greater dissociation of the ionic pair azide anion–quaternary ammonium cation as compared to azide anion–alkaline cation[18,19] and by the higher polarity of the solution produced by the tetrabutylammonium salt[19]. Catalytic (Pd/C) hydrogenation of **2** at room temperature gave 74% of the 3-amino-3-deoxyglucopyranoside **3**[20].

Reaction of the benzyl (**3**) and methyl 3-amino-3-deoxyglucopyranosides (**4**)[17] with ethyl bromoacetate–pyridine gave benzyl (**5**, 67%) and methyl 3-deoxy-3-[(ethoxycarbonylmethyl)amino]-α-D-glucopyranosides (**6**, 64%), respectively. The ^{1}H-n.m.r. spectra of **5** and **6** showed signals corresponding to the N–CH_2–CO

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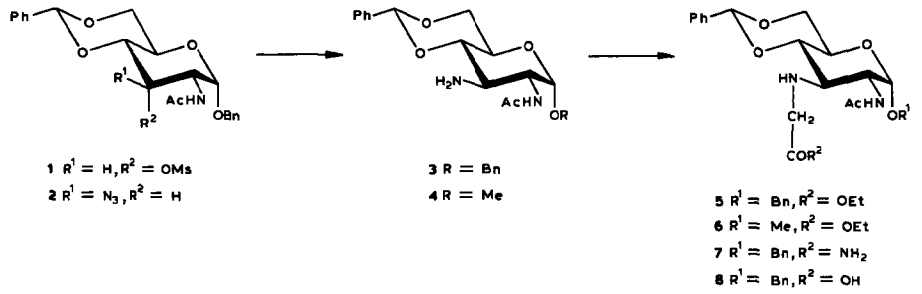
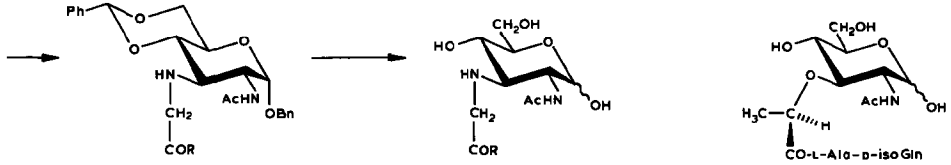


methylene group (δ 3.45) and to the ethyl ester residue, which confirmed the proposed structures. The *gluco* configuration of **5** and **6** was confirmed by the high values (8–11 Hz) of $J_{2,3}$ and $J_{3,4}$.

Treatment of **5** with ammonia–methanol afforded the amide **7**, the ^{1}H-n.m.r. spectrum of which contained two one-proton singlets (δ 7.02 and 7.28) for a $CONH_2$ group. Saponification of the ethyl ester group of **5** with ethanolic potassium hydroxide at room temperature afforded 82% of the free acid **8**.

Coupling of **8** with L-alanyl-D-isoglutamine methyl ester, using dicyclohexylcarbodi-imide and *N*-hydroxysuccinimide as the activating agents, yielded, respectively, *N*-(benzyl 2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-glucopyranosid-3-yl)glycyl-L-alanyl-D-isoglutamine methyl ester (**9**, 73%) and *N*-(benzyl 2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-glucopyranosid-3-yl)glycyl-L-alanyl-D-glutamine methyl ester (**10**, 78%). The ^{1}H-n.m.r. spectra of **9** and **10** showed all the expected bands and confirmed that no racemisation had taken place during the condensation step.

Hydrogenolysis (Pd/C) of the benzyl and benzylidene groups of **9** and **10** gave the desired *N*-(2-acetamido-2,3-dideoxy-D-glucopyranos-3-yl)glycyl-L-alanyl-D-isoglutamine methyl ester (**11**, 68.5%) and *N*-(2-acetamido-2,3-dideoxy-D-glucopyranos-3-yl)glycyl-L-alanyl-D-glutamine methyl ester (**12**, 68%).



EXPERIMENTAL

General. — Melting points are uncorrected. ^{1}H-N.m.r. spectra (internal Me_4Si) were recorded with Varian XL-300 (300 MHz) and EM-390 (90 MHz) spectrometers, i.r. spectra with a Perkin–Elmer 257 spectrophotometer, and optical

rotations at 23 ±2° with a Perkin–Elmer 141 polarimeter. T.l.c. was performed on silica gel 60 F_{254} (Merck), p.l.c. on silica gel PF_{254} (Merck), and column chromatography on silica gel 60 (Merck) with detection, as appropriate, by u.v. light (254 nm) or by charring with sulfuric acid.

Benzyl 2-acetamido-3-azido-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranoside (**2**). — A mixture of **1**[15] (10 g, 0.02 mol), sodium azide (13 g, 0.2 mol), tetrabutylammonium hydrogen sulfate (6.8 g, 0.02 mol), and *N,N*-dimethylformamide (125 mL) was heated to 100° for 20 h, and then poured into water and ice (500 mL). The solid was collected, dried, and recrystallised from ethanol to give **2** (6.36 g, 75%), m.p. 248–249°, $[\alpha]_D$ +106° (*c* 1, methyl sulfoxide); lit.[15] m.p. 244–245°, $[\alpha]_D$ +97° (*c* 1, methyl sulfoxide).

Benzyl 2-acetamido-3-amino-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranoside (**3**). — A mixture of **2** (2 g, 4.7 mmol), methanol (100 mL), and 10% Pd/C (0.6 g) was hydrogenated at 15 p.s.i. and 25° for 6 h, then filtered, and concentrated. Column chromatography (ethyl acetate–methanol, 10:1) of the residue gave **3** (1.4 g, 74%), m.p. 241–244° (dec.) (from methanol–water), $[\alpha]_D$ +120° (*c* 1, chloroform); lit.[20] m.p. 247–250°, $[\alpha]_D^{20}$ +92° (*c* 1, chloroform). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 1.90 (s, 3 H, NAc), 3.23–4.43 (m, 6 H, H-2,3,4,5,6,6), 4.55 and 4.74 (AB system, 2 H, J_{gem} 12 Hz, OCH_2Ph), 4.90 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.66 (s, 1 H, C*H*Ph), 8.34 (d, 1 H, $J_{NH,2}$ 8 Hz, N*H*Ac), 8.56 (bs, 2 H, NH_2).

Benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-3-[(ethoxycarbonylmethyl)amino]-α-D-glucopyranoside (**5**). — A mixture of **3** (1 g, 2.4 mmol), ethyl bromoacetate (0.6 mL, 5 mmol), pyridine (0.64 mL, 8 mmol), and acetonitrile (20 mL) was heated to reflux for 20 h and then concentrated. A solution of the residue in chloroform was washed with water, dried (Na_2SO_4), filtered, and concentrated. P.l.c. (ethyl acetate) of the residue gave **5** (0.77 g, 67%), m.p. 206–208° (from ethyl acetate–hexane), $[\alpha]_D$ +89° (*c* 1, chloroform). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 1.08 (t, 3 H, OCH_2CH_3), 1.86 (s, 3 H, NAc), 3.01 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8 Hz, H-3), 3.33–4.23 (m, 5 H, H-2,4,5,6,6), 3.45 (s, 2 H, $NHCH_2CO$), 3.97 (q, 2 H, OCH_2CH_3), 4.51 and 4.70 (AB system, 2 H, J_{gem} 12 Hz, OCH_2Ph), 4.78 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.60 (s, 1 H, C*H*Ph), 7.90 (d, 1 H, $J_{NH,2}$ 9 Hz, N*H*Ac).

Anal. Calc. for $C_{26}H_{32}N_2O_7$: C, 64.46; H, 6.61; N, 5.78. Found: C, 64.36; H, 6.89; N, 6.18.

Methyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-3-[(ethoxycarbonylmethyl)amino]-α-D-glucopyranoside (**6**). — A mixture of **4**[17] (2 g, 6.2 mmol), ethyl bromoacetate (1.49 mL, 14 mmol), pyridine (1.28 mL, 16 mmol), and acetonitrile (25 mL) was heated to reflux for 24 h, and then worked-up as indicated for **5**, to afford **6** (1.5 g, 64%), m.p. 190°, $[\alpha]_D$ +71° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.20 (t, 3 H, OCH_2CH_3), 2.06 (s, 3 H, NAc), 2.97 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 9 Hz, H-3), 3.33 (s, 3 H, OMe), 3.50–4.36 (m, 7 H, H-2,4,5,6,6, $NHCH_2CO$), 4.09 (q, 2 H, OCH_2CH_3), 4.79 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.53 (s, 1 H, C*H*Ph), 6.45 (d, 1 H, $J_{NH,2}$ 9 Hz, N*H*Ac).

Anal. Calc. for $C_{20}H_{28}N_2O_7$: C, 59.70; H, 8.45; N, 6.16. Found: C, 59.31; H, 8.18; N, 6.11.

Benzyl 2-acetamido-4,6-O-benzylidene-3-[(carbamoylmethyl)amino]-2,3-dideoxy-α-D-glucopyranoside (**7**). — A mixture of **5** (0.1 g, 0.2 mmol) in saturated $MeOH/NH_3$ (20 mL) was stirred for 20 h at room temperature, then concentrated at reduced pressure to 4–5 mL, and cooled to 0° for 4 h. The precipitate was collected to give **7** (0.075 g, 82%), m.p. 257–259° (dec.) (from methanol–ethyl ether), $[\alpha]_D$ +95° (*c* 0.5, methyl sulfoxide). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 1.86 (s, 3 H, NAc), 2.88 (dd, 1 H, $J_{2,3} \simeq J_{3,4}$ = 9 Hz, H-3), 3.21 (s, 2 H, NHCH_2CO), 3.26–4.23 (m, 5 H, H-2,4,5,6,6), 4.49 and 4.69 (AB system, 2 H, J_{gem} 12.5 Hz, OCH_2Ph), 4.80 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 5.61 (s, 1 H, C*H*Ph), 7.02 and 7.28 (2 bs, 2 H, $CONH_2$), 8.08 (d, 1 H, $J_{NH,2}$ 7 Hz, N*H*Ac).

Anal. Calc. for $C_{24}H_{29}N_3O_6$: C, 63.30; H, 6.37; N, 9.23. Found: C, 63.24; H, 6.59; N, 8.95.

Benzyl 2-acetamido-4,6-O-benzylidene-3-[(carboxylmethyl)amino]-2,3-dideoxy-α-D-glucopyranoside (**8**). — A solution of **5** (0.3 g, 0.6 mmol) in ethanolic 1% KOH (30 mL) was stirred for 20 h at room temperature. Water (5 mL) was added and the mixture was slowly passed through a column of Amberlite IRC-50 (H^+) resin (2 g). The eluate was concentrated under reduced pressure to give **8** (0.23 g, 82%), m.p. 228–232° (dec.) (from methanol–ethyl ether),$[\alpha]_D$ +47° (*c* 0.5, methyl sulfoxide); ν_{max}^{Nujol} 1640 (NAc), 1655 (COOH), and 2540–3700 cm^{-1} (OH, NH). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 1.89 (s, 3 H, NAc), 2.98 (m, 1 H, H-3), 3.39 (s, 2 H, NHCH_2CO), 3.48–4.38 (m, 5 H, H-2,4,5,6,6), 4.50 and 4.70 (AB system, 2 H, J_{gem} 12 Hz, OCH_2Ph), 4.81 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 5.63 (s, 1 H, C*H*Ph), 7.96 (d, 1 H, $J_{NH,2}$ 9 Hz, N*H*Ac).

Anal. Calc. for $C_{24}H_{28}N_2O_7$: C, 63.16; H, 6.14; N, 6.14. Found: C, 62.94; H, 6.04; N, 6.19.

N-*(Benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranosid-3-yl)glycyl-L-alanyl-D-isoglutamine methyl ester* (**9**). — To an ice-cooled solution of **8** (0.46 g, 1.0 mmol) in dry tetrahydrofuran (15 mL) were added *N*-hydroxysuccinimide (0.115 g, 1.0 mmol) and dicyclohexylcarbodi-imide (0.206 g, 1.0 mmol). The mixture was stirred in an ice bath for 3 h and then at room temperature for 1 h. The 1,3-dicyclohexylurea was collected and washed with tetrahydrofuran, the combined filtrate and washings were cooled (ice-bath), and L-alanyl-D-isoglutamine methyl ester trifluoroacetate (0.34 g, 1.0 mmol) and triethylamine (0.14 mL, 1.0 mmol) were added. The mixture was stirred overnight at room temperature and then concentrated. P.l.c. (chloroform–methanol, 20:1) of the residue gave **9** (0.49 g, 73%), m.p. 234–236° (from ethyl acetate–methanol), $[\alpha]_D$ +64.5° (*c* 0.5, methyl sulfoxide). ^{1}H-N.m.r. data [$(CD_3)_2SO$, 300 MHz]: δ 1.07 (d, 3 H, CHCH_3), 1.57–1.98 (m, 2 H, CHCH_2CH_2), 1.87 (s, 3 H, NAc), 2.28 (t, 2 H, CH_2CH_2CO), 2.93 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.1 Hz, H-3), 3.10 and 3.28 (AB system, 2 H, J_{gem} 16.6 Hz, NHCH_2CO), 3.45–3.71 (m, 3 H, H-4,5,6′), 3.50 (s, 3 H, OMe), 3.80 (ddd, 1 H, $J_{1,2}$ 3.5, $J_{NH,2}$ 8.5 Hz, H-2), 4.0–4.19 (m, 3 H, CH_3C*H*NH, CH_2C*H*NH, H-6″), 4.51

and 4.71 (AB system, 2 H, J_{gem} 12.5 Hz, OCH_2Ph), 4.77 (d, 1 H, H-1), 5.58 (s, 1 H, C*H*Ph), 7.10 (s, 1 H, CONH), 7.20–7.40 (m, 11 H, CONH, Ph), 7.93, 8.00, and 8.06 (3 d, 3 H, *J* 7–8.5 Hz, 3 CHN*H*CO).

Anal. Calc. for $C_{33}H_{43}N_5O_{10}$: C, 59.19; H, 6.43; N, 10.46. Found: C, 59.22; H, 6.50; N, 10.35.

N-*(Benzyl 2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-*D*-glucopyranosid-3-yl)glycyl-*L*-alanyl-*D*-glutamine methyl ester* (**10**). — To an ice-cooled solution of **8** (0.3 g, 0.66 mmol) in dry tetrahydrofuran (15 mL) were added *N*-hydroxysuccinimide (0.76 g, 0.66 mmol) and dicyclohexylcarbodi-imide (0.136 g, 0.66 mmol). The mixture was stirred at 0° for 3 h and then at room temperature for 1 h. The 1,3-dicyclohexylurea was collected and washed with tetrahydrofuran, the combined filtrate and washings were cooled (ice-bath), and L-alanyl-D-glutamine methyl ester hydrochloride (0.176 g, 0.66 mmol) and triethylamine (0.1 mL, 0.6 mmol) were added. The mixture was stirred overnight at room temperature and then concentrated. P.l.c. (chloroform–methanol, 20:1) of the residue gave **10** (0.344 g, 78%), m.p. 257–259° (dec.) (from ethyl acetate–methanol), $[\alpha]_D$ +68.5° (*c* 0.5, methyl sulfoxide). ^{1}H-N.m.r. data [$(CD_3)_2SO$, 300 MHz]: δ 1.14 (d, 3 H, CHCH_3), 1.68–2.01 (m, 2 H, CHCH_2CH_2), 1.86 (s, 3 H, NAc), 2.09 (t, 2 H, CH_2CH_2CO), 2.95 (dd, 1 H, $J_{2,3} \simeq J_{3,4}$ = 10.1 Hz, H-3), 3.23 (s, 2 H, NHCH_2CO), 3.58 (s, 3 H, OMe), 3.53–3.80 (m, 3 H, H-4,5,6′), 3.89 (m, 1 H, H-2), 4.08–4.40 (m, 3 H, CH_3C*H*NH, CH_2C*H*NH, H-6″), 4.49 and 4.70 (AB system, 2 H, J_{gem} 12 Hz, OCH_2Ph), 4.61 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.53 (s, 1 H, C*H*Ph), 6.78 and 7.32 (2 s, 2 H, $CONH_2$), 8.01, 8.05, and 8.42 (3 d, 3 H, *J* 7.5–9 Hz, 3 CHN*H*CO).

Anal. Calc. for $C_{33}H_{45}N_5O_{10}$: C, 59.19; H, 6.43; N, 10.46; Found: C, 59.15; H, 6.57; N, 10.28.

N-*(2-Acetamido-2,3-dideoxy-*D*-glucopyranos-3-yl)glycyl-*L*-alanyl-*D*-isoglutamine methyl ester* (**11**). — A mixture of **9** (0.27 g, 0.41 mmol), acetic acid (20 mL), and 10% Pd/C (100 mg) was hydrogenated at 15 p.s.i. for 40 h at room temperature, then filtered, and concentrated. Column chromatography (chloroform–methanol–acetic acid, 60:10:3) of the residue gave **11** as a foam (0.14 g, 68%), $[\alpha]_D$ +4.5° (*c* 0.5, methanol; equil.). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 1.2 (d, 3 H, CHCH_3), 1.82 (s, 3 H, NHAc), 2.28 (t, 2 H, CH_2CH_2CO), 3.57 (s, 3 H, OMe), 4.88 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1).

Anal. Calc. for $C_{19}H_{33}N_5O_{10}$: C, 46.43; H, 6.72; N, 14.26. Found: C, 46.32; H, 6.93; N, 13.95.

N-*(2-Acetamido-2,3-dideoxy-*D*-glucopyranos-3-yl)glycyl-*L*-alanyl-*D*-glutamine methyl ester* (**12**). — A mixture of **10** (0.27 g, 0.41 mmol), acetic acid (20 mL), and 10% Pd/C (100 mg) was hydrogenated at 15 p.s.i. for 40 h at room temperature, and then worked-up, as indicated for **11**, to give **12** as a foam (0.14 g, 68%), $[\alpha]_D$ +6.5° (*c* 0.5, methanol; equil.). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 1.26 (d, 3 H, CHCH_3), 1.86 (s, 3 H, NHAc), 1.72–2.26 (m, 4 H, CH_2CH_2CO), 3.65 (s, 3 H, OMe), 4.92 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1).

Anal. Calc. for $C_{19}H_{33}N_5O_{10}$: C, 46.44; H, 6.72; N, 14.26. Found: C, 46.21; H, 6.97; N, 13.89.

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