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N-(GLUCOPYRANOSID-3-YL)-D-ALANYL-L-ALANYL-D-ISOGLUTAMINE AND RELATED TRIPEPTIDE ANALOGUES OF MURAMYL DIPEPTIDE.

Ana Calvo-Mateo, María-José Camarasa and Federico G. De las Heras*.

Instituto de Química Médica C.S.I.C., Juan de la Cierva 3, 28006-Madrid, Spain.

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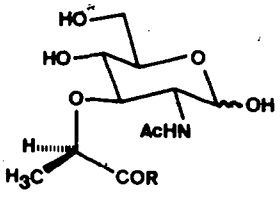
Abstract - Four analogues of the immunoadjuvant Muramyl dipeptide in which the D-lactic acid have been replaced by D-alanine and L-alanine have been prepared. Condensation of methyl and benzyl 2-acetamido-3-amino-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranosides 3 and 4 with ethyl D,L-2-bromopropionate afforded the corresponding methyl 3-[[D- and L-1-(ethoxycarbonyl)ethyl]amino]-gluco-pyranosides 5 and 9 and the corresponding benzyl glycosides 6 and 10. The absolute configurations of 5, 6, 9 and 10 were unequivocally determined by condensation of 3 and 4 with ethyl L-2-bromopropionate. Hydrolysis of 6 and 10 with KOH/MeOH, gave the benzyl 3-[[D- and L-1-(carboxy)ethyl]amino]-gluco-pyranosides 8 and 12, which by condensation with L-Ala-D-isoGln-OMe and L-Ala-D-Gln-OMe followed by hydrogenolysis afforded the desired N-(2-acetamido-2,3-dideoxy-D-glucopyranosid-3-yl) derivatives of D-Ala-L-Ala-D-isoGln-OMe, D-Ala-L-Ala-D-Gln-OMe, L-Ala-L-Ala-D-isoGln-OMe, and L-Ala-L-Ala-D-Gln-OMe,

N-[2-O-(2-Acetamido-2,3-dideoxy-D-glucopyranosid-3-yl)-D-lactoyl]-L-alanine-D-isoglutamine (1, Muramyl dipeptide, MDP) has been shown to be the minimal adjuvant active structure capable of replacing whole mycobacterial cells in Freund's complete adjuvant for increasing levels of humoral antibodies against a given antigen and for inducing delayed hypersensitivity[1,2]. MDP stimulates non specific resistance against bacterial, viral and parasite infections[3-5] and also shows anti-tumor activity[6]. However, it has undesired side effects such as transitory leukopenia[7], pyro-genicity[7-9], thrombocytolysis[9] and somnogenicity[10], which prompted the chemical modification of



1, (MDP), R = L-Ala-D-isoGln

2, (Murabutide), R = L-Ala-D-Gln-$OCH_2(CH_2)_2CH_3$

MDP structure[4,11-14]. The main conclusions derived so far from the structure-activity relationship studies are [4,11,12]: a) only slight structural changes can be introduced in the muramyl residue, b) the L-Ala residue can be replaced by other aliphatic L-aminoacids, and c) the D-Glu residue is essential, but the functionality of D-Glu is important for modulating biological acitivty. For example, Murabutide, 2, a MDP analog which is under clinical trials, and other alkyl ester

derivatives of the D-Gln and D-isoGln residues are less pyrogenic than MDP[15,16].

On the other hand, in the series of the naturally occurring, immunostimulant peptide FK-156[17], D-lactoyl-L-Ala-D-isoGlu-(L)-meso-A_2mp(L)-Gly, it has been demonstrated that the absolute configuration of the D-lactic acid is of little importance for the immunoadjuvant activity[18].

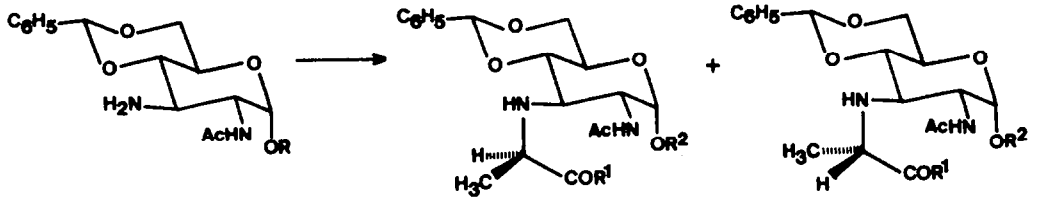
Taking into account all these facts we have prepared analogues of MDP in which the D-lactic acid residue has been replaced by D-alanine and L-alanine to give N-(2-acetamido-2,3-dideoxy-D-glucopyranosid-3-yl) tripeptide analogues of MDP, in which the tripeptide side chain is D-Ala-L-Ala-D-isoGln-OMe (15), D-Ala-L-Ala-D-Gln-OMe (16), L-Ala-L-Ala-D-isoGln-OMe (19), and L-Ala-L-Ala-D-Gln-OMe (20).

RESULTS AND DISCUSSION

Condensation of methyl 3-amino-3-deoxy-glucopyranoside 3[19] with ethyl D,L-2-bromopropionate in the presence of pyridine afforded methyl 3-deoxy-3-[[R and S-1(ethoxycarbonyl)ethyl]amino]-gluco-pyranosides 5 and 9 in 33 and 31% yield, respectively. A similar condensation of benzyl 3-amino-3-deoxy-glucopyranoside 4[20] showed some stereoselectivity and afforded a mixture of benzyl 3-deoxy-3-[[R and S-1-(ethoxycarbonyl)ethyl]amino]-glucopyranosides 6 and 10 in 24.4 and 36.6% yield, respectively. The two compounds of each diastereoisomeric pair were very similar to each other, and were isolated by a tedious preparative TLC chromatography.

The gluco configuration of the four muramic acid analogues 5, 6, 9 and 10 was confirmed by the high values of the coupling constants $J_{2,3}$ and $J_{3,4}$, which were in the range of 8.2-11.3 Hz.

The absolute configuration of the alanyl residue was determined chemically, by condensation of 3 and 4 with optically active ethyl L-2-bromopropionate[21]. These reactions afforded a (85 : 15) mixture of the methyl glucosides 5 and 9, and a (80 : 20) mixture of the benzyl derivatives 6 and 10, respectively, which indicated that partial racemization at C-2 of the propionic acid residue took place during the condensation. In its reaction with amines, the 2-bromopropionate undergoes a Walden inversion[22]. Therefore, the two major products of each reaction, i.e. 5 and 6, will be muramic acid analogues, that is, will have a D-alanine residue with a R absolute configuration, and the two minor compounds 9 and 10, will be analogues of isomuramic acid, having a L-alanine residue, with a S absolute configuration.



3 , R = Me

4 , R = Bn

5 , R^1 = OEt ; R^2 = Me

6 , R^1 = OEt ; R^2 = Bn

7 , R^1 = NH_2 ; R^2 = Bn

8 , R^1 = OH ; R^2 = Bn

9 , R^1 = OEt ; R^2 = Me

10 , R^1 = OEt ; R^2 = Bn

11 , R^1 = NH_2 ; R^2 = Bn

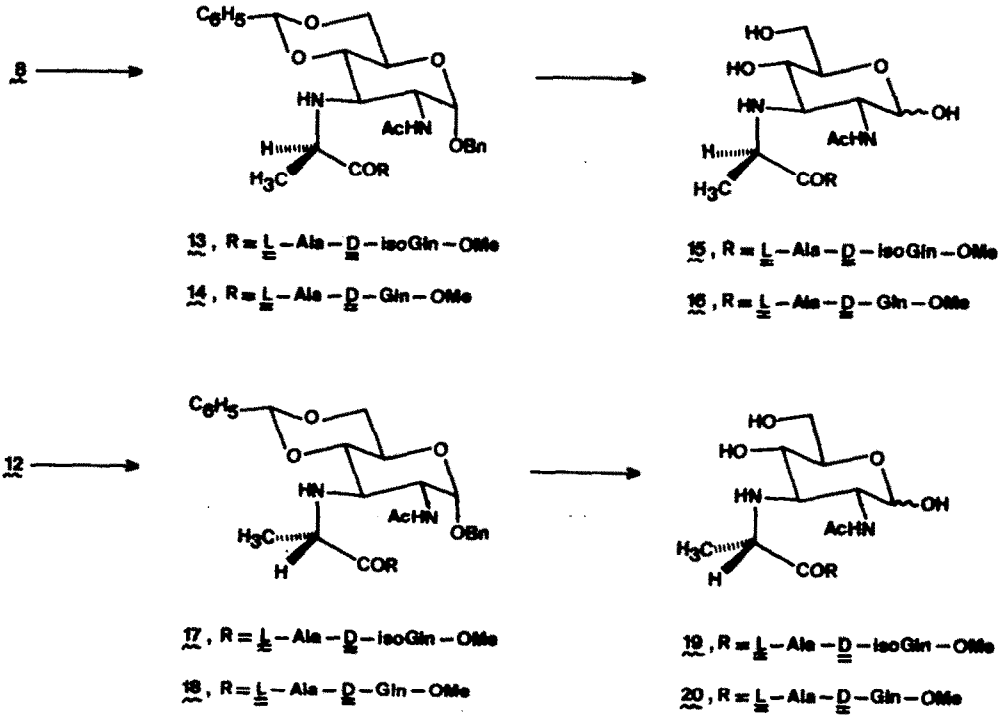
12 , R^1 = OH ; R^2 = Bn

This conclusion is supported by comparison of the optical rotation values of 5, 6 and 9, 10, with those of several pairs of muramic and isomuramic acid derivatives and several pairs of N-substituted D- and L-alanine ethyl esters. As long as the optical rotations are measured in methanol or chloroform, the D-1-carboxyethyl derivatives, analogues of muramic acid, are more dextrorrotatory than the corresponding L-1-carboxyethyl isomers, analogues of isomuramic acid[23-25]. Similarly, the N-substituted-D-alanine ethyl esters are more dextrorrotatory than the corresponding L-enantiomers[26,27]. The optical rotation values of 5 and 6, higher than those of 9 and 10, were in

agreement with this rule and the above structural assignment.

Treatment of 6 and 10 with methanolic ammonia, afforded the corresponding amides 7 and 11 in 75 and 73% yield, respectively. No racemization at the alanine quiral C-2 atom was observed. Saponification of the ethyl ester group of 6 and 10 with a 1% solution of potassium hydroxyde in ethanol, followed by treatment of the resulting carboxylic acid derivatives with IRC-50 cation exchange resin, afforded, without racemization at the alanine C-2 position, the free acids 8 and 12 in 79 and 77% yield respectively.

Reaction of the muramic acid derivative 8 with L-alanyl-D-isoglutamine methyl ester and L-alanyl-D-glutamine methyl ester, using dicyclohexylcarbodiimide and N-hydroxysuccinimide as the activating agents afforded, without racemization, the corresponding N-(glucopyranosid-3-yl)-D-alanyl-L-alanyl-D-isoglutamine methyl ester 13 and N-(glucopyranosid-3-yl)-D-alanyl-L-alanyl-D-glutamine methyl ester 14 in 71 and 72% yield, respectively. Similarly, condensation of the iso-muramic acid analogue 12 with the same dipeptides by the same coupling procedure, afforded the corresponding N-(glucopyranosid-3-yl) derivatives of L-alanyl-L-alanyl-D-isoglutamine methyl ester 17, and L-alanyl-L-alanyl-D-glutamine methyl ester 18 in 70 and 73% yield, respectively.



Condensation of a mixture of diasteroisomers 8 and 12 with L-alanyl-D-isoglutamine methyl ester and L-alanyl-D-glutamine methyl ester, afforded in each case a mixture of two MDP analogues 13 and 17, and 14 and 18, respectively, which were much easier to isolate and purify than the mixture of the N-(glucopyranosid-3-yl)-D- and L-alanine derivatives 8 and 12. Thus, once the stereochemistry of 13, 14, 17 and 18 was unequivocally determined, the preparation of these MDP analogues was conveniently carried out starting from the mixture of diastereoisomeric ethyl esters 6 and 10. These compounds were readily prepared by condensation of 4 with the commercially available ethyl D,L-2-bromopropionate, and the only chromatographic separation was performed only after their transformation into the corresponding protected glycopeptides.

Hydrogenolysis of the benzyl and benzylidene groups of 13, 14, 17 and 18 in acetic acid using 10% Pd/C as catalyst gave the desired N-(2-acetamido-2,3-dideoxy-D-glucopyranosid-3-yl) derivatives

of D-alanyl-L-alanyl-D-isoglutamine methyl ester 15, D-alanyl-L-alanyl-D-glutamine methyl ester, 16, L-alanyl-L-alanyl-D-isoglutamine methyl ester 19, and L-alanyl-L-alanyl-D-glutamine methyl ester 20, in 74, 69, 70 and 68% yield, respectively.

EXPERIMENTAL

M.ps were measured with a Kofler hot-stage apparatus. ^{1}H NMR spectra were recorded with a Varian EM-390 or a Varian XL-300 spectrometer operating at 90 or 300 MHz, respectively, with Me_4Si as internal standard. IR spectra were recorded with a Perkin-Elmer 257 spectrophotometer. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Analytical TLC was performed on aluminium sheets coated with a 0.2 mm layer of silica gel 60 F_{254} (Merck), and preparative layer chromatography was performed on 20 x 20 cm glass plates coated with a 2 mm layer of silica gel PF_{254} (Merck).

Methyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-3-[[-D-1-(ethoxycarbonyl)ethyl]amino]-α-D-glucopyranoside (5) and methyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-3-[[L-1-(ethoxycarbonyl)ethyl]amino]-α-D-glucopyranoside (9). A mixture of 3[19] (2 g, 6.2 mmol), ethyl D,L-2-bromopropionate (2,6 mL, 14 mmol), pyridine (1.6 mL, 20 mmol) and acetonitrile (25 mL) was heated to reflux for 24 h. The solvent was removed and a chloroform solution of the residue was washed with water, dried (Na_2SO_4), filtered and concentrated. Preparative TLC of the residue (hexane-chloroform-ethyl acetate, 4:4:1) showed two major bands. The fastest moving band gave 5 (0.87 g, 33%); m.p. 167-168° (from ethyl acetate-hexane); $[\alpha]_D$ + 62.2° (c 1, chloroform); IR (nujol) 1750 cm^{-1} (CO_2Et). ^{1}H NMR ($CDCl_3$, 90 MHz): δ 1.26(t, 3H, CH_3-CH_2-), 1.28(d, 3H, CH_3-CH), 2.10(s, 3H, NAc), 2.94(dd, 1H, H-3, $J_{2,3}$= 11 Hz, $J_{3,4}$= 9 Hz), 3.37(s, 3H, OCH_3), 3.37-4.32(m, 6H, H-2, H-4, H-5, H-6, CH-CH_3), 4.12 (q, 2H, O-CH_2-CH_3), 4.70(d, 1H, H-1, $J_{1,2}$= 4 Hz), 5.56(s, 1H, CH-C_6H_5), 6.46(d, 1H, NHAc, $J_{NH,2}$= 9 Hz). Anal. Calcd. for $C_{21}H_{30}N_2O_7$: C, 59.71; H, 7.01; N, 6.63. Found: C, 59.35; H, 6.98; N, 6.78.

The slowest running band gave 9 (0.83 g, 31%); m.p. 190-191° (from ethyl acetate-hexane); $[\alpha]_D$ + 17° (c 1, chloroform); IR (nujol) 1750 cm^{-1} (CO_2Et); ^{1}H NMR[$(CD_3)_2SO$, 90 MHz]: δ 1.01 (t, 3H, CH_3-CH_2), 1.21(d, 3H, CH_3-CH), 2.04(s, 3H, NAc), 2.99(dd, 1H, H-3, $J_{2,3}$= 10 Hz, $J_{3,4}$= 9 Hz), 3.40 (s, 3H, OCH_3), 3.40-4.30(m, 6H, H-2, H-4, H-5, H-6, CH-CH_3), 4.71(d, 1H, H-1, $J_{1,2}$= 4 Hz), 5.43 (s, 1H, CH-C_6H_5). Anal. Calcd. for $C_{21}H_{30}N_2O_7$: C, 59.71; H, 7.01; N, 6.63. Found: C, 59.30; H, 7.02; N, 6.81.

Reaction of methyl 2-acetamido-3-amino-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranoside (3) with ethyl L-2-bromopropionate. A mixture of 3 (0.5 g, 1.55 mmol), ethyl L-2-bromopropionate freshly prepared[21] (0.65 mL, 3.5 mmol), pyridine (0.4 mL, 5 mmol) and acetonitrile (15 mL) was heated to reflux for 20 h. The solvent was removed and a chloroform solution of the residue was washed with water, dried (Na_2SO_4), filtered and concentrated. Preparative TLC of the residue (hexane-chloroform-ethyl acetate, 2:2:1) gave a (7:3) mixture of 5 and 9 as a solid (0.40 g, 62%), which was separated and identified as indicated before.

Benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-3-[[D-1-(ethoxycarbonyl)ethyl]amino]-α-D-glucopyranoside (6) and benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-3-[[L-1-(ethoxycarbonyl)ethyl]amino]-α-D-glucopyranoside (10). A mixture of 4[20] (1 g, 2.4 mmol), ethyl D,L-2-bromopropionate (1.9 mL, 10 mmol), pyridine (1.2 mL, 15 mmol) and acetonitrile (20 mL) was heated to reflux for 20 h. The solvent was removed and a chloroform solution of the residue was washed with water, dried (Na_2SO_4), filtered and concentrated. Preparative TLC of the residue (hexane-n-butanol, 20 : 1) showed two major bands. The fastest moving band gave 6 (0.29 g, 24.4%); m.p. 184-185° (from ethyl acetate-hexane); $[\alpha]_D$ + 93° (c 1, chloroform); IR (nujol) 1750 cm^{-1} (CO_2Et); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.12(d, 3H, CH-CH_3), 1.13 (t, 3H, CH_2-CH_3), 1.90(s, 3H, NAc), 2.90(dd, 1H, H-3, $J_{2,3}$= 11.3 Hz, $J_{3,4}$= 9.9 Hz), 3.44-3.80(m, 4H, H-4, H-5, H-6a, CH-CH_3), 3.86(ddd, 1H, H-2, $J_{1,2}$= 3.7 Hz, $J_{NH,2}$= 8.8 Hz), 3.96-4.15 (m, 3H, H-6e, O-CH_2-CH_3), 4.53, 4.70(AB system, 2H, O-CH_2-C_6H_5, J_{gem}= 12.4 Hz), 4.77(d, 1H, H-1), 5.63(s, 1H, CH-C_6H_5), 7.77(d, 1H, CONH). Anal. Calcd. for $C_{27}H_{34}N_2O_7$: C, 65.06; H, 6.82; N, 5.62. Found: C, 64.70; H, 6.94; N, 5.25.

The slowest running band gave 10 (0.44 g, 36.6 %); m.p. 201-202° (from ethyl acetate-hexane);

$[\alpha]_D$ + 52° (c 1, chloroform); IR (nujol) 1750 cm^{-1} (CO_2Et). ^1H NMR [$(CD_3)_2SO$, 300 MHz]: δ 0.99 (t, 3H, CH_2-CH_3), 1.12(d, 3H, CH_3-CH), 1.86(s, 3H, NAc), 2.98(dd, 1H, H-3, $J_{2,3}$= 10.5 Hz, $J_{3,4}$= 8.2 Hz), 3.4-4.2(m, 8H, H-2, H-4, H-5, H-6, $CH-CH_3$, $O-CH_2-CH_3$), 4.51, 4.70 (AB system, 2H, $O-CH_2-C_6H_5$, J_{gem}= 11.8 Hz), 4.73(d, 1H, H-1, $J_{1,2}$= 3.2 Hz), 5.56(s, 1H, $CH-C_6H_5$), 8.0(d, 1H, NHAc, $J_{NH,2}$= 8.9 Hz). Anal. Calcd. for $C_{27}H_{34}N_2O_7$: C, 65.06; H, 6.82; N, 5.62. Found: C, 64.82; H, 6.98; N, 5.43.

Reaction of benzyl 2-acetamido-3-amino-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranoside (4) with ethyl L-2-bromopropionate. A mixture of 4 (0.5 g, 1.2 mmol), ethyl L-2-bromopropionate freshly prepared[21] (0.65 mL, 3.5 mmol), pyridine (0.6 mL, 7.5 mmol) and acetonitrile (15 mL) was heated to reflux for 20 h. The solvent was removed and a chloroform solution of the residue was washed with water, dried (Na_2SO_4), filtered and concentrated. Preparative TLC of the residue (hexane-chloroform-acetonitrile, 3: 2: 1) gave a (7 : 3) mixture of 6 and 10 as a solid (0.35 g, 58%), wich was separated and identified as indicated before.

Benzyl 2-acetamido-4,6-O-benzylidene-3-[[D-1-(carboxamide)ethyl]amino]-2,3-dideoxy-α-D-glucopyranoside (7). A mixture of 6 (0.1 g, 0.2 mmol) in $MeOH/NH_3$ (100 mL) was stirred for 20 h at room temperature. The solution was concetrated at reduced pressure to 4-5 mL and cooled to 0° for 4 h to complete crystallization. The precipitate was filtered to give 7 (0.07 g, 75%); m.p. 250-255° (from methanol-ethyl ether); $[\alpha]_D$ + 83° (c 0.5, dimethyl sulfoxide); IR (nujol) 1640, 1655 cm^{-1} (CONH, $CONH_2$); ^1H NMR [$(CD_3)_2$ SO, 300 MHz]: δ 1.11(d, 3H, $CHCH_3$), 1.88(s, 3H, NAc), 2.80(dd, 1H, H-3, $J_{2,3} \simeq J_{3,4}$= 10.3 Hz), 3.28-3.88(m, 5H, H-2, H-4, H-5, H-6a, $CHCH_3$), 4.12(m, 1H, H-6e), 4.50, 4.69(AB system, 2H, $O-CH_2-C_6H_5$, J_{gem}= 13.4 Hz), 4.86(d, 1H, H-1, $J_{1,2}$= 3.6 Hz), 5.62(s, 1H, $CH-C_6H_5$), 7.08, 7.46(2s, 2H, $CONH_2$), 8.09(d, 1H, NHAc, $J_{NH,2}$= 8 Hz). Anal. Calcd. for $C_{25}H_{31}N_3O_6$: C, 63.96; H, 6.61; N, 8.95. Found: C, 63.66; H, 6.88; N, 8.47.

Benzyl 2-acetamido-4,6-O-benzylidene-3-[[L-1-(carboxamide)ethyl]amino]-2,3-dideoxy-α-D-glucopyranoside (11). A mixture of 10 (0.1 g, 0.2 mmol) in $MeOH/NH_3$ (100 mL) was stirred for 20 h at room temperature. The reaction mixture was worked up,as indicated before for 7,to give 11 (0.07 g, 73%); m.p. 269-274° (dec.) (from methanol-ethyl ether); $[\alpha]_D$ + 99° (c 0.5, dimethyl sulfoxide); IR (nujol) 1640, 1655 cm^{-1} (CONH, $CONH_2$); ^1H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.10(d, 3H, $CH-CH_3$), 1.90 (s, 3H, NAc), 2.89(dd, 1H, H-3, $J_{2,3} \simeq J_{3,4}$= 11.5 Hz), 3.30-3.62(m, 2H, H-4, $CH-CH_3$), 3.68-3.78(m, 2H, H-5, H-6a), 3.99(ddd, 1H, H-2, $J_{1,2}$= 3.5 Hz, $J_{NH,2}$= 9.4 Hz), 4.13(dd, 1H, H-6e, $J_{6a,6e}$=7.9, $J_{5,6e}$= 2.8 Hz), 4.51, 4.71(AB system, 2H, $O-CH_2-C_6H_5$, J_{gem}= 12.4 Hz), 4.71(d, 1H, H-1), 5.62(s, 1H, $CH-C_6H_5$), 7.06, 7.46 (2s, 2H, $CONH_2$), 8.10(d, 1H, NHAc). Anal. Calcd. for $C_{25}H_{31}N_3O_6$: C, 63.96; H, 6.61; N, 8.95. Found: C, 63.58; H, 6.76; N, 8.74.

Benzyl 2-acetamido-4,6-O-benzylidene-3-[[(D-1-carboxy)ethyl]amino]-2,3-dideoxy-α-D-glucopyranoside (8). A solution of 6(0.1 g, 0.2 mmol) in 1% KOH/EtOH (15 mL), was stirred for 20 h at room temperature. Water (5 mL) was added and the mixture was slowly passed through an Amberlite IRC-50 (H^+) (2 g) column. The eluate was concentrated under reduced pressure to give 8 (0.074 g, 79%); m.p. 228-230° (from methanol-ethyl ether); $[\alpha]_D$ + 80° (c 0.5 dimethyl sulfoxide); IR (nujol) 1640 cm^{-1} (NAc), 1655 cm^{-1} (COOH), 2800-3500 cm^{-1} (OH, NH); ^1H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.15(d, 3H, $CH-CH_3$), 1.88(s, 3H, NAc), 2.89(dd, 1H, H-3, $J_{2,3}$= 10.8 Hz, $J_{3,4}$= 9.3 Hz), 3.49(q, 1H, $CH-CH_3$), 3.58(t, 1H, H-4, $J_{4,5}$= 9.4 Hz), 3.68-3.80(m, 2H, H-5, H-6a), 3.66(ddd, 1H, H-2, $J_{1,2}$= 3.5 Hz, $J_{NH,2}$= 8.2 Hz), 4.12(dd, 1H, H-6e, $J_{6a,6e}$= 9.4 Hz, $J_{5,6e}$= 3 Hz), 4.52, 4.70(AB system, 2H, $O-CH_2-C_6H_5$, J_{gem}= 12.4 Hz), 4.83(d, 1H, H-1), 5.63(s, 1H, $CH-C_6H_5$), 7.92(d, 1H, NHAc, $J_{NH,2}$= 8.2 Hz). Anal. Calcd. for $C_{25}H_{30}N_2O_7$: C, 63.83; H, 6.38; N, 5.96. Found: C, 63.78; H, 6.47; N, 5.56.

Benzyl 2-acetamido-4,6-O-benzylidene-3-[[(L-1-carboxy)ethyl]amino]-2,3-dideoxy-α-D-glucopyranoside (12). A solution of 10 (0.1 g, 0.2 mmol) in 1% KOH/EtOH (15 mL), was stirred for 20 h at room temperature. The reaction mixture was worked up as indicated before for 8 to give 12 (0.72 g, 77%); m.p. 242-245° (from methanol-ethyl ether); $[\alpha]_D$ + 102° (c 0.5, dimethyl sulfoxide); IR (nujol) 1640 cm^{-1} (NAc), 1655 cm^{-1} (COOH), 2600-3600 cm^{-1} (OH, NH); ^1H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.18(d, 3H, $CHCH_3$), 1.88(s, 3H, NAc), 3.02(dd, 1H, H-3, $J_{2,3} \simeq J_{3,4}$= 10.2 Hz), 3.53(q, 1H, $CH-CH_3$), 3.61(dd, 1H, H-4, $J_{4,5}$= 8.8 Hz), 3.66-3.80(m, 2H, H-5, H-6a), 3.95(m, 1H, H-2), 4.14(dd, 1H, H-6e, $J_{6a,6e}$= 8.4 Hz, $J_{5,6e}$= 2.9 Hz), 4.52, 4.72(AB system, 2H, $O-CH_2-C_6H_5$, J_{gem}= 12.5 Hz), 4.73 (d, 1H, H-1, $J_{1,2}$= 3.3 Hz), 5.63(s, 1H, $CH-C_6H_5$), 8.7(d, 1H, NHAc, $J_{NH,2}$= 8 Hz). Anal. Calcd. for

$C_{25}H_{30}N_2O_7$: C, 63.83; H, 6.38; N, 5.96. Found: C, 63.56; H, 6.52; N, 5.63.

N-(benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-alanyl-L-alanyl-D-isoglutamine methyl ester (13). To an ice-cooled solution of 8 (0.047 g, 0.1 mmol) in dry tetrahydrofuran (4 mL), N-hydroxysuccinimide (0.011 g, 0.1 mmol) and dicyclohexylcarbodiimide (0.021 g, 0.1 mmol) were added. The mixture was stirred in an ice bath for 3 h and then at room temperature for 1 h. The 1,3-dicyclohexylurea formed was removed by filtration and washed with tetrahydrofuran. The combined filtrate and washings were cooled again in an ice bath and L-alanyl-D-isoglutamine methyl ester trifluoroacetate (0.034 g, 0.1 mmol), triethylamine (0.14 mL, 0.1 mmol) and tetrahydrofuran (3 mL) were added. The mixture was stirred overnight at room temperature. After evaporation of the solvent, the residue was chromatographed (preparative TLC) with chloroform-methanol (20:1) to give 13 (0.048 g, 71%); m.p. 258-262° (dec.) (from ethyl acetate-methanol); $[\alpha]_D$ + 59° (c 0.5, dimethyl sulfoxide); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.09(d, 3H, $CHCH_3$), 1.18(d, 3H, $CHCH_3$), 1.84(s, 3H, NAc), 1.66-2.10(m, 2H, $CH-CH_2-CH_2$), 2.28(t, 2H, CH_2-CH_2-CO), 2.92(dd, $J_{2,3} \simeq J_{3,4}$= 10.8 Hz), 3.35(q, 1H, H-3, Glc-NH-CH-CH_3), 3.56(m, 4H, H-5, OCH_3), 3.68-3.79(m, 2H, H-4, H-6a), 3.90(ddd, 1H, H-2, $J_{NH,2}$= 9.1, $J_{1,2}$= 3.7 Hz), 4.13-4.20(m, 3H, H-6e, $CONHCHCH_3$, NH-$CH-CH_2$), 4.52, 4.71(AB system, 2H, $O-CH_2-C_6H_5$, J_{gem}= 12.7 Hz), 4.77(d, 1H, H-1), 5.62(s, 1H, $CH-C_6H_5$), 7.10, 7.46 (2s, 2H, $CONH_2$), 7.84, 8.06, 8.12(3d, 3H, NHCO, J= 6.4-9.1 Hz). Anal. Calcd. for $C_{34}H_{45}N_5O_{10}$: C, 59.74; H, 6.59; N, 10.25. Found: C, 59.57; H, 6.98; N, 10.52.

N-(benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranosid-3-yl)-L-alanyl-L-alanyl-D-isoglutamine methyl ester (17). Coupling of the acid 12 (0.047 g, 0.1 mmol) with L-alanyl-D-isoglutamine methyl ester trifluoroacetate (0.034 g, 0.1 mmol) by using N-hydroxysuccinimide (0.011 g, 0.1 mmol), dicyclohexylcarbodiimide (0.021 g, 0.1 mmol) and triethylamine (0.015 mL, 0.1 mmol), according to the procedure described for 13, afforded 17 (0.048 g, 70%); m.p. 278-280° (dec.) (from ethyl acetate-methanol); $[\alpha]_D$ + 62.5° (c 0.5, dimethyl sulfoxide); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz]: δ 0.80(d, 3H, $CHCH_3$), 1.09(d, 3H, $CH-CH_3$), 1.87(s, 3H, NAc), 1.64-2.08(m, 2H, NH-CH-CH_2) , 2.27(t, 2H, CH_2-CH_2-CO), 2.97(dd, 1H, H-3, $J_{2,3} \simeq J_{3,4}$= 10.6 Hz), 3.36(q, 1H, Glc-NH-CH-CH_3), 3.53(dd, 1H, H-6a, $J_{6a,6e} \simeq J_{5,6a}$= 9.4 Hz), 3.58(s, 3H, OCH_3), 3.68(dd, 1H, H-4, $J_{4,5}$= 9.5 Hz), 3.78(dt, 1H, H-5, $J_{5,6e}$= 3.8 Hz), 3.92-4.60(m, 2H, H-2, H-6e), 4.11-4.27(m, 2H, CONH-CH-CH_3, NH-$CH-CH_2$), 4.51, 4.73 (AB system, 2H, $O-CH_2-C_6H_5$, J_{gem}= 12.4 Hz), 4.71(d, 1H, H-1, $J_{1,2}$= 3.1 Hz), 5.52(s, 1H, $CH-C_6H_5$), 7.17, 7.46(2s, 2H, $CONH_2$), 7.96, 8.09, 8.13(3d, 3H, 3 CONH, J= 7-9.1 Hz). Anal. Calc. for $C_{34}H_{45}N_5O_{10}$: C, 59.74; H, 6.59; N, 10.25. Found: C, 59.43; H, 6.36; N, 10.21.

Reaction of a mixture of 8 and 12 with L-alanyl-D-isoglutamine methyl ester. Coupling of a (2:3) mixture of 8 and 12 (0.4 g, 0.85 mmol) with L-alanyl-D-isoglutamine methyl ester trifluoroacetate (0.293 g, 0.85 mmol) by using N-hydroxysuccinimide (0.098 g, 0.85 mmol), dicyclohexylcarbodiimide (0.176 g, 0.85 mmol) and triethylamine (0.13 mL, 0.85 mmol) according to the procedure already described , afforded a mixture of two compounds which were separated by preparative TLC (chloroform-methanol, 20:1). The fastest moving band gave 17 (0.244 g, 42%). The slowest running band gave 13 (0.157 g, 27%).

N-(benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-alanyl-L-alanyl-D-glutamine methyl ester (14). Coupling of the acid 8 (0.047g, 0.1 mmol) with L-alanyl-D-glutamine methyl ester hydrochloride (0.027 g, 0.1 mmol) by using N-hydroxysuccinimide (0.011 g, 0.1 mmol), dicyclohexylcarbodiimide (0.021 g, 0.1 mmol) and triethylamine (0.015 mL, 0.1 mmol) according to the procedure described before for 13, afforded 14 (0.049 g, 72%); m.p. 216-217° (from ethyl acetate-methanol); $[\alpha]_D$ + 56° (c 0.5, dimethyl sulfoxide); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.10(d, 3H, $CHCH_3$), 1.20(d, 3H, $CHCH_3$), 1.82(s, 3H, NAc), 1.73-1.92(m, 2H, $CH-CH_2-CH_2$), 2.08(t, 2H, CH_2-CH_2-CO), 2.91(dd, 1H, H-3, $J_{2,3}$= 11.4 Hz, $J_{3,4}$= 8.8 Hz), 3.34(q, 1H, Glc-NH-CH-CH_3), 3.57-3.75(m, 3H, H-4, H-5, H-6a), 3.59(s, 3H, OCH_3), 3.88(ddd, 1H, H-2, $J_{NH,2}$= 8.6, $J_{1,2}$= 3.4 Hz), 4.14(dd, 1H, H-6e, $J_{6a,6e}$= 8.3, $J_{5,6e}$= 2.9 Hz), 4.21-4.33(m, 2H, CONH-CH-CH_3, NH-$CH-CH_2$), 4.51, 4.71(AB system, 2H, $CH_2-C_6H_5$, J_{gem}= 12.6 Hz), 4.77(d, 1H, H-1), 5.61(s, 1H, $CH-C_6H_5$), 6.79, 7.46(2s, 2H, $CONH_2$), 7.82, 8.06, 8.37(3d, 3H, 3 CONH, J= 7.7-8.6 Hz). Anal Calcd. for $C_{34}H_{45}N_5O_{10}$: C, 59.74; H, 6.59; N, 10.25. Found: C, 59.60; H, 6.77; N, 10.12.

N-(benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranosid-3-yl)-L-alanyl-L-alanyl-D-glutamine methyl ester (18). Coupling of the acid 12 (0.047 g, 0.1 mmol) with L-alanyl-D-glutamine

methyl ester hydrochloride (0.027 g, 0.1 mmol) by using N-hydroxysuccimide (0.011 g, 0.1 mmol), dicyclohexylcarbodiimide (0.021 g, 0.1 mmol) and triethylamine (0.015 mL, 0.1 mmol), according to the procedure already described, afforded compound 18 (0.05 g, 73%); m.p. 249-251° (from ethyl acetate-methanol); $[\alpha]_D$ + 62° (c 0.5 dimethyl sulfoxide); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.01(d, 3H, $CHCH_3$); 1.12(d, 3H, $CHCH_3$), 1.87(s, 3H, NAc), 1.70-2.02(m, 2H, $CH-CH_2$), 2.10(t, 2H, CH_2-CH_2-CO), 3.02(dd, 1H, H-3, $J_{2,3} \simeq J_{3,4}$= 10 Hz), 3.23(q, 1H, Glc-NH-CH-CH_3), 3.57(s, 3H, OCH_3), 3.51-3.80(m, 3H, H-4, H-5, H-6a), 3.95(ddd, 1H, H-2, $J_{1,2}$= 3.5, $J_{NH,2}$= 8.9 Hz), 4.14(dd, 1H, H-6e, $J_{6a,6e}$= 8.7, $J_{5,6e}$= 3.5 Hz), 4.17-4.30(m, 2H, NH-CH-CH_3, NH-CH-CH_2), 4.51, 4.72(AB system, 2H, $CH_2-C_6H_5$, J_{gem}= 12.5 Hz), 4.73(d, 1H, H-1), 4.54(s, 1H, $CH-C_6H_5$), 6.81, 7.43(2s, 2H, $CONH_2$), 8.12, 8.17, 8.35 (3d, 3H, 3 NHCO, J= 7.3-8.9 Hz). Anal. Calcd. for $C_{34}H_{45}N_5O_{10}$: C, 59.74; H, 6.59; N, 10.25. Found: C, 59.36; H, 6.79; N, 10.02.

Reaction of a mixture of 8 and 12 with L-alanyl-D-glutamine methyl ester. Coupling of a (2:3) mixture of the acids 8 and 12 (0.4 g, 0.85 mmol) with L-alanyl-D-glutamine methyl ester hydrochloride (0.228 g, 0.85 mmol) by using N-hydroxysuccinimide (0.098 g, 0.85 mmol) and dicyclohexylcarbodiimide (0.176 g, 0.85 mmol) and triethylamine (0.13 mL, 0.85 mmol) according to the procedure described, afforded a mixture of compounds which were separated by preparative TLC (chloroform-methanol, 20:1). The fastest moving band gave 14 (0.232 g, 40%) and the slowest gave 18 (0.162 g, 28%).

N-(2-acetamido-2,3-dideoxy-D-glucopyranosid-3-yl)-D-alanyl-L-alanyl-D-isoglutamine methyl ester (15). A mixture of 13 (0.2 g, 0.42 mmol), acetic acid (20 mL) and 10% Pd/C (0.07 g) was hydrogenated at 15 psi for 40 h at room temperature. The catalyst was filtered and the filtrate concentrated. The residue was chromatographed (silica gel column) with chloroform-methanol-acetic acid (60:10:3) to give 15 as a foam (0.157 g, 74%); $[\alpha]_D$ + 7° (c 0.5, methanol; equil.); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.09(d, 3H, $CHCH_3$), 1.21(d, 3H, $CHCH_3$), 1.80(s, 3H, NAc), 1.66-2.04(m, 2H, $CH-CH_2$), 2.28(t, 2H, CH_2CH_2CO), 3.02(dd, 1H, H-3, $J_{2,3} \simeq J_{3,4}$= 10.5 Hz), 3.58(s, 3H, OCH_3), 4.85 (d, 1H, H-1, $J_{1,2}$= 4 Hz), 7.11 (m. 2H, $CONH_2$), 7.40, 7.66, 8.08(3d, 3H, 3 CONH, J= 7-12 Hz). Anal. Calcd. for $C_{20}H_{35}N_5O_{10}$: C, 47.52; H, 6.93; N, 13.86. Found: C, 47.15; H, 7.26; N, 13.73.

N-(2-acetamido-2,3-dideoxy-D-glucopyranosid-3-yl)-D-alanyl-L-alanyl-D-glutamine methyl ester (16). Hydrogenolysis of compound 14 (0.2 g, 0.42 mmol) with hydrogen in the presence of 10% Pd/C (0.07 g), in acetic acid, according to the procedure described before for 15 gave 16 (0.146 g, 69%) as a foam; $[\alpha]_D$ + 6° (c 0.5 methanol; equil.); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.10(d, 3H, $CHCH_3$), 1.20(d, 3H, $CHCH_3$), 1.78(s, 3H, NAc), 2.10(t, 2H, CH_2CO), 3.07(dd, 1H, H-3, $J_{2,3} \simeq J_{3,4}$= 10 Hz), 3.62 (s, 3H, OCH_3), 4.85(d, 1H, H-1, $J_{1,2}$ = 4 Hz), 6.80(broad s, 2H, $CONH_2$), 8.13, 8.38, 8.44 (3d, 3H, 3 NHCO, J= 7-8 Hz). Anal. Calcd. for $C_{20}H_{35}N_5O_{10}$: C, 47.52; H, 6.93; N, 13.86. Found: C, 47.48; H, 7.15; N, 13.88.

N-(2-acetamido-2,3-dideoxy-D-glucopyranosid-3-yl)-L-alanyl-L-alanyl-D-isoglutamine methyl ester (19). A mixture of 17 (0.2 g, 0.42 mmol), acetic acid (20 mL) and 10% Pd/C (0.07 g) was hydrogenated at 15 psi for 40 h at room temperature. The reaction mixture was worked up as indicated before for 15 to give 19 as a foam (0.148 g, 70%); $[\alpha]_D$ + 8° (c 0.5, methanol; equil.); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.15(d, 3H, $CHCH_3$), 1.21(d, 3H, $CHCH_3$), 1.86(s, 3H, NAc), 2.28(t, 3H, CH_2-CH_2CO), 3.59(s, 3H, OCH_3), 7.13(s, 2H, $CONH_2$), 7.38, 7.48, 8.15(3d, 3H, 3 NHCO, J= 8-9 Hz). Anal. Calcd. for $C_{20}H_{35}N_5O_{10}$: C, 47.52; H, 6.93; N, 13.86. Found: C, 47.32; H, 7.30; N, 13.58.

N-(2-acetamido-2,3-dideoxy-D-glucopyranosid-3-yl)-L-alanyl-L-alanyl-D-glutamine ester (20). Compound 18 (0.2 g, 0.42 mmol), in acetic acid (20 mL) was hydrogenolyzed in the presence of 10% Pd/C catalyst (0.07 g) as described in the preparation of 15, to give 20 (0.144 g, 68%) as a foam; $[\alpha]_D$ + 7.5° (c 0.5, methanol; equil.); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.12(d, 3H, $CHCH_3$), 1.21(d, 3H, $CHCH_3$), 1.86(s, 3H, NAc), 2.10(t, 2H, CH_2CH_2CO), 3.61(s, 3H, OCH_3), 4.85(d, 1H, H-1, $J_{1,2}$= 4 Hz), 6.77(m, 2H, $CONH_2$), 7.32-8.30(m, 3H, 3 NHCO). Anal. Calcd. for $C_{20}H_{35}N_5O_{10}$: C, 47.52; H, 6.93; N, 13.86. Found: C, 47.21; H, 7.03; N, 13.76.

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