SYNTHESIS OF O-(2-DEOXY-2-STEAROYLAMINO-β-D-GLUCOPYRANOSYL)-(1→4)-N-ACETYLNORMURAMOYL-L-α-AMINOBUTANOYL-D-ISOGLUTAMINE, A LIPOPHILIC DISACCHARIDE ANALOGUE OF MDP

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Partial N-deacetylation of compound *II* with barium hydroxide afforded benzyl 2-acetamido-3-O-allyl-4-O-(2-amino-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-α-D-glucopyranoside (*III*) in high yield. Compound *III* was N-acylated with stearic acid in the presence of DCC and the obtained product was converted into benzyl 2-acetamido-6-O-benzyl-3-O-carboxymethyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-α-D-glucopyranoside (*VII*). Coupling of compound *VII* with L-α-aminobutanoyl-D-isoglutamine benzyl ester followed by hydrogenolysis of the product *VIII* afforded compound *IX*.

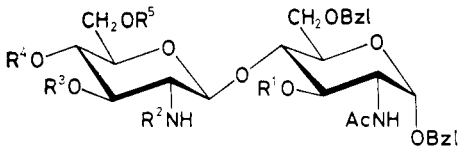
Some time ago we described[1,2] the preparation of O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-N-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine*. This compound exhibits a markedly higher immunoadjuvant activity than MDP (MurNAc-L-Ala-D-isoGln) with simultaneous suppression of undesired side-effects such as pyrogenicity and thrombocytolysis. The synthesis of O-(2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-(1→4)-N-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine** (*IX*) was motivated by communications[4-6] reporting enhanced antitumour and antiviral activity of some MDP derivatives with large lipophilic groups, particularly when these compounds are built into liposomes. MDP and its lipophilic analogue built into liposomes also significantly inhibit the in vitro HIV virus replication[7,8].

As starting material for the preparation of compound *IX* we utilized benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-O-allyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (*I*) the preparation of which we described already earlier[1]. On reaction with 2,2-dimethoxypropane and catalytic amount of trifluoromethanesulfonic acid in acetone, compound *I* was converted into benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-3-O-allyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (*II*). Partial N-deacetylation of the isopropylidene

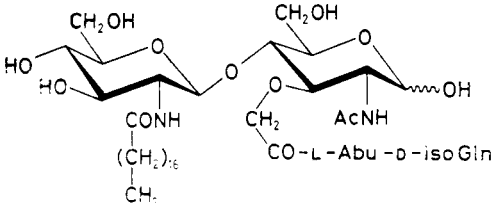
* Normuramic acid is the trivial name for 2-amino-3-O-carboxymethyl-2-deoxy-D-glucopyranose.

** For preliminary communication see ref.[3].

derivative *II* with barium hydroxide in aqueous methanol at 120°C afforded the key intermediate, benzyl 2-acetamido-3-O-allyl-4-O-(2-amino-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-α-D-glucopyranoside (*III*). Under the above-mentioned conditions the N'-acetyl group (neighbouring with the free OH-3' group) on the sugar ring is cleaved selectively. The regioselectivity of the reaction is probably due to the free vicinal OH group rather than the different configuration at the anomeric centers of the sugar moieties because N-deacetylation of benzyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-α- and -β-D-glucopyranoside with barium hydroxide proceeds in the same manner[9]. Compound *III* was N-acylated[10] with stearic acid and N,N'-dicyclohexylcarbodiimide in dichloromethane and the isopropylidene protecting group was then removed by heating with 90% acetic acid. The obtained benzyl 2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-4-O-(2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-α-D-glucopyranoside (*IV*) was O-benzylated with benzyl bromide and barium hydroxide in N,N-dimethylformamide to give benzyl 2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-2-



I, R^1 = All ; R^2 = Ac ; R^3 = R^4 = R^5 = H
II, R^1 = All ; R^2 = Ac ; R^3 = H ; R^4 + R^5 = $(CH_3)_2C$
III, R^1 = All ; R^2 = R^3 = H ; R^4 + R^5 = $(CH_3)_2C$
IV, R^1 = All ; R^2 = $CH_3(CH_2)_{16}CO$; R^3 = R^4 = R^5 = H
V, R^1 = All ; R^2 = $CH_3(CH_2)_{16}CO$; R^3 = R^4 = R^5 = Bzl
VI, R^1 = H ; R^2 = $CH_3(CH_2)_{16}CO$; R^3 = R^4 = R^5 = Bzl
VII, R^1 = CH_2COOH ; R^2 = $CH_3(CH_2)_{16}CO$; R^3 = R^4 = R^5 = Bzl
VIII, R^1 = CH_2CO-L-Abu-D-isoGln(OBzl) ; R^2 = $CH_3(CH_2)_{16}CO$; R^3 = R^4 = R^5 = Bzl



IX

Ac = acetyl ; All = allyl ; Bzl = benzyl ; Abu = α-aminobutanoic acid ; isoGln(OBzl) = isoglutamine, benzyl ester

-deoxy-2-stearoylamino-β-D-glucopyranosyl)-α-D-glucopyranoside (*V*). High conversion (75%) in the preparation of *V* from *IV* was achieved by combination of mechanical stirring[1] of the reaction mixture at room temperature with sonication. Deallylation of compound *V* by catalytic isomerization of the allyl to the propenyl group with Wilkinson catalyst, followed by acid hydrolysis of the product[11], led to benzyl 2-acetamido-6-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-α-D-glucopyranoside (*VI*).

TABLE I

^{1}H NMR parameters of sugar moieties in compounds *II*—*VIII*

Parameter	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
δ(H-1)	4·87 d	4·93 d	4·86 d	4·93 d	4·91 d	5·06 d	4·94 d
δ(H-2)	4·16 dd	4·24 ddd	4·16 dd	4·19 ddd	4·21 ddd	4·11 ddd	4·19 ddd
δ(H-3)	3·56 dd	3·54 dd	3·51 dd	3·51 dd	3·47 dd	3·54—3·64 m	3·58 dd
δ(H-4)	3·82 dd	4·01 dd	3·88 dd	3·92 dd	3·90 dd	3·99 t	3·48—3·75 m
δ(H-5)	3·73 ddd	3·77 ddd	3·71 ddd	3·36 ddd	3·37 ddd	3·39 ddd	3·37 ddd
δ(H-6a)	3·60 dd	3·63 dd	3·59 dd	3·48 dd	3·51 dd	3·42 dd	3·48—3·75 m
δ(H-6b)	3·70 dd	3·89 dd	3·66 dd	3·70 dd	3·58—3·78 m	3·66 dd	3·48—3·75 m
δ(H-1′)	4·52 d	4·27 d	4·40 d	4·52 d	4·59 d	4·52 d	4·47 d
δ(H-2′)	3·64 dd	2·57 dd	3·54—	3·60—	3·58—	3·54—	3·48—
δ(H-3′)	3·45 dd	3·26 t	—3·68 m	—3·72 m	—3·78 m	—3·54 m	—3·75 m
δ(H-4′)	3·51 dd	3·47 dd					
δ(H-5′)	3·12 ddd	3·06 dt					
δ(H-6a′)	3·71 t	3·67 t	3·61 dd			3·74 dd	
δ(H-6b′)	3·88 dd	3·86 dd	3·85 dd			3·54—3·64 m	
δ(NH)	[a]	5·58 d	[a]	5·53 d	5·55 d	[a]	6·28 d
J(1, 2)	3·7	3·8	3·7	3·8	3·6	3·7	3·9
J(2, 3)	10·6	10·6	10·6	10·7	10·6	10·7	10·3
J(2, NH)	[a]	9·3	[a]	9·3	9·3	8·9	9·0
J(3, 4)	8·6	9·0	8·8	8·8	8·8	8·7	[a]
J(4, 5)	9·5	10·1	10·6	9·7	9·6	8·2	9·5
J(5, 6a)	2·2	1·8	3·2	2·2	2·2	2·2	2·4
J(5, 6b)	3·7	3·3	3·4	3·8	3·8	4·6	4·1
J(6a, 6b)	−10·8	−11·0	−10·7	−10·6	−10·6	−10·6	[a]
J(1′, 2′)	8·2	8·0	7·3	7·5	8·5	8·5	8·3
J(2′, 3′)	9·8	9·6	[a]	[a]	[a]	[a]	[a]
J(3′, 4′)	9·1	9·3	[a]	[a]	[a]	[a]	[a]
J(4′, 5′)	9·5	9·5	[a]	[a]	[a]	[a]	[a]
J(5′, 6a′)	10·4	10·5	6·2	[a]	[a]	2·2	[a]
J(5′, 6b′)	5·4	5·5	3·4	[a]	[a]	[a]	[a]
J(6a′, 6b′)	−10·7	−10·6	−12·3	[a]	[a]	−10·6	[a]

[a] Value not determined.

TABLE II

^{1}H NMR parameters of nonsugar moieties in compounds *II*— *VIII*

Moiety	Chemical shifts, ppm (*J*)						
	II[a]	*III*[b]	*IV*	*V*	*VI*	*VII*[c]	*VIII*[d]
$NHCOCH_3$	1·94 s	1·93 s	1·94 s	1·93 s	1·94 s	1·95 s	1·92 s
CH_2=$CHCH_2$	5·81 dddd (5·1, 5·8, 10·5, 17·4)	5·82 dddd (5·0, 6·0, 10·4, 17·4)	5·82 dddd (5·6, 5·9, 10·5, 17·3)	5·77 dddd (4·9, 6·1, 10·5, 17·3)	—	—	—
	5·11 ddt (1·4, 1·4, 1·8, 10·5)	5·01 ddt (1·1, 1·1, 2·0, 10·4)	5·13 ddt (1·3, 1·3, 1·7, 10·5)	4·98 ddd (1·3, 1·3, 2·2, 10·5)	—	—	—
	5·21 dq (1·7, 1·7, 1·7, 17·4)	5·19 dq (1·8, 1·8 1·8, 17·4)	5·22 dq (1·7, 1·7, 1·7, 17·3)	5·15 ddt (1·7, 1·7 2·2, 17·3)	—	—	—
	4·00 ddt (1·5, 1·5, 5·8, 12·6)	3·98 ddt 1·4, 1·4, 6·0, 12·6)	3·99 ddt (1·3, 1·3, 5·9, 12·7)	3·97 ddt 1·2, 1·2, 6·1, 13·4)	—	—	—
	4·28 ddt (1·5, 1·5, 5·1, 12·6)	4·28 ddt (1·5, 1·5, 5·0, 12·6)	4·39 ddt (1·7, 1·7, 5·6, 12·7)	4·41 ddt (1·5, 1·5, 4·9, 13·4)	—	—	—
$OCH_2C_6H_5$[e]	4·46 d (11·8)	4·46 d (11·8)	4·46 d (11·7)	4·46 d (11·8)	4·45 d (11·9)	4·47 d (12·0)	4·47 d (12·0)
	4·68 d (11·8)	4·68 d (11·8)	4·67 d (11·7)	4·66 d (12·0)	4·67 d (11·9)	4·61 d (12·0)	4·65 d (12·0)
	4·51 d (11·8)	4·50 d (12·2)	4·49 d (12·0)	4·51 d (12·0)	4·53 d (11·9)	4·50 d (12·0)	4·46 d (12·0)
	4·74 d (11·8)	4·71 d (12·2)	4·75 d (12·0)	4·75 d (12·0)	4·73 d (11·9)	4·77 d (12·0)	4·71 d (12·0)
	—	—	—	4·58 d (11·0)	4·58 d (11·0)	4·56 d (10·8)	4·53 d (11·2)
	—	—	—	4·73 d (11·0)	4·74 d (11·0)	4·71 d (10·8)	4·71 d (11·2)
	—	—	—	4·59 d (11·5)	4·59 d (11·6)	4·57 d (11·6)	4·59 d (11·4)
	—	—	—	4·78 d (11·5)	4·78 d (11·6)	4·78 d (11·6)	4·78 d (11·4)
	—	—	—	4·43 d (12·0)	4·45 d (12·1)	4·41 d (12·0)	4·46 d (12·0)
	—	—	—	4·57 d (12·0)	4·59 d (12·1)	4·56 d (12·0)	4·56 d (12·0)

TABLE II
(*Continued*)

Moiety	Chemical shifts, ppm (*J*)						
	II[a]	*III*[b]	*IV*	*V*	*VI*	*VII*[c]	*VIII*[d]
OStear	—	—	2·02 m	1·87 m	1·89 m	1·85 m	1·89 m
	—	—	2·05 m	1·96 m	1·94 m	1·94 m	1·92 m
	—	—	1·20— —1·56 m[f]	1·16— —1·66 m[f]	1·06— —1·72 m[f]	1·16— —1·63 m[f]	1·16— —1·44 m[f]
	—	—	0·88 t[g] (7·1)	0·88 t[g] (7·1)	0·85 t[g] (7·1)	0·88 t[g] (7·1)	0·88 t[g] (7·1)

Other signals: [a] $NHCOCH_3$: 1·90 s; $(CH_3)_2$: 1·43 s, 1·50 s; [b] $(CH_3)_2$: 1·42 s, 1·47 s; [c] CH_2COOH: 4·27 d (17·2), 4·35 d (17·2); [d] CH_2COOR: 4·15 d (15·5), 4·42 d (15·5), L-Abu-D-isoGln(OBzl): 4·47 d (11·7), 4·53 d (11·7), 4·36 t (7·5), 4·35 t (7·5), 1·90 m, 1·82 m, 1·64 m, 0·85 t (7·1); [e] aromatic protons for all compounds in region 7·18—7·70 ppm; [f] intensity of 30 protons; [g] intensity of 3 protons.

TABLE III
^{13}C NMR chemical shifts (in ppm) of compounds *II*—*IX*

Carbon	*II*	*III*	*IV*	*V*	*VI*	*VII*[a]	*VIII*[b]	*IX*[c]
C-1	96·73 d	96·98 d	96·18 d	96·89 d	96·75 d	96·65 d	96·54 d	90·39 d
C-2	52·02 d	51·94 d	52·18 d	52·16 d	53·29 d	53·49 d	52·71 d	51·96 d
C-3	78·13 d	78·30 d	77·87 d	78·35 d	81·07 d	80·39 d	78·80 d	78·17 d
C-4	77·53 d	75·88 d	75·90 d	78·35 d	78·14 d	78·46 d	78·26 d	76·23 d
C-5	70·63 d	70·80 d	71·14 d	70·72 d	70·10 d	70·29 d	70·41 d	70·93 d
C-6	68·43 t	67·95 d	68·48 d	68·00 t	67·83 t	67·38 t	67·84 t	67·94 t
C-7	101·00 d	103·67 d	100·51 d	100·07 d	101·30 d	99·84 d	99·76 d	101·12 d
C-8	57·47 d	58·72 d	56·05 d	56·31 d	55·21 d	56·21 d	56·36 d	55·93 d
C-9	66·87 d	67·06 d	70·58 d	74·74 d	74·55 d	74·80 d	74·47 d	70·93 d
C-10	72·46 d	73·85 d	74·07 d	76·28 d	76·21 d	75·52 d	75·56 d	74·17 d
C-11	74·04 d	74·42 d	75·90 d	81·67 d	81·67 d	81·27 d	81·30 d	75·93 d
C-12	61·92 t	62·11 t	61·64 t	68·74 t	68·80 t	68·44 t	68·62 t	61·38 t

TABLE III
(*Continued*)

Carbon	*II*	*III*	*IV*	*V*	*VI*	*VII*[a]	*VIII*[b]	*IX*[c]
allyl	72·69 t	72·29 t	72·46 t	74·57 t	—	—	—	—
	134·99 t	135·26 t	135·93 t	135·76 t	—	—	—	—
	116·01 t	115·98 t	115·04 t	115·86 t	—	—	—	—
$NHCOCH_3$	170·42 s	169·57 s	169·72 s	169·71 s	170·03 s	170·73 s	171·00 s	169·69 s
	22·83 q	23·25 q	22·52 q	23·33 q	23·38 q	23·19 q	23·03 q	22·85 q
	172·17 s	—	—	—	—	—	—	—
	22·83 q	—	—	—	—	—	—	—
NHStear	—	—	172·92 s	172·91 s	172·84 s	172·74 s	173·21 s	173·32 s
	—	—	36·17 t	36·88 t	36·71 t	36·81 t	36·73 t	35·99 t
	—	—	31·43 t	31·90 t	31·92 t	31·90 t	31·87 t	31·48 t
	—	—	29·21 t	29·69 t	29·71 t	29·69 t	29·65 t	29·26 t
	—	—	29·21 t	29·57 t	29·53 t	29·53 t	29·50 t	29·20 t
	—	—	29·21 t	29·51 t	29·44 t	29·41 t	29·44 t	29·16 t
	—	—	29·00 t	29·43 t	29·35 t	29·34 t	29·38 t	28·97 t
	—	—	28·85 t	29·34 t	25·39 t	25·51 t	29·30 t	28·89 t
	—	—	25·32 t	25·50 t	22·68 t	22·67 t	25·51 t	25·36 t
	—	—	22·22 t	22·67 t	14·11 q	14·11 q	22·63 t	22·28 t
	—	—	13·90 t	14·10 q	—	—	14·04 q	14·16 q
OBzl	69·83 t	69·72 t	68·62 t	69·72 t	69·87 t	69·80 t	69·75 t	—
	73·90 t	73·36 t	72·60 t	73·04 t	73·36 t	73·33 t	73·18 t	—
	—	—	—	73·37 t	73·52 t	73·46 t	73·47 t	—
	—	—	—	73·50 t	74·51 t	74·43 t	74·40 t	—
	—	—	—	74·42 t	74·76 t	74·71 t	74·59 t	—
	136·84 s	137·02 s	137·17 s	137·16 s	137·25 s	137·01 s	135·57 s	—
	137·50 s	137·80 s	138·26 s	138·07 s	137·71 s	137·88 s	136·93 s	—
	—	—	—	138·12 s	138·15 s	137·92 s	137·92 s	—
	—	—	—	138·25 s	138·65 s	137·92 s	138·00 s	—
	128·02 d	127·81 d	127·15 d	127·41 d	127·66 d	127·46 d	127·49 d	—
	128·17 d	127·90 d	127·46 d	127·59 d	127·73 d	127·61 d	127·59 d	—
	128·33 d	127·96 d	127·71 d	127·69 d	127·81 d	127·69 d	127·61 d	—
	128·45 d	128·02 d	128·02 d	127·83 d	127·89 d	127·84 d	127·72 d	—
	128·58 d	128·42 d	—	127·99 d	127·95 d	127·97 d	127·85 d	—
	—	128·45 d	—	128·07 d	128·00 d	128·04 d	128·07 d	—
	—	—	—	128·28 d	128·26 d	128·39 d	128·13 d	—
	—	—	—	128·35 d	128·38 d	128·50 d	128·32 d	—
	—	—	—	128·48 d	128·42 d	128·68 d	128·34 d	—
	—	—	—	128·63 d	128·58 d	128·80 d	128·52 d	—

[a] $-CH_2COOH$: 70·00 t, 172·74 s; [b] ^{13}C NMR chemical shifts of peptide residue on C-3 in Table IV; [c] in hexadeuteriodimethyl sulfoxide ($(CD_3)_2SO$ = 39·7 ppm), ^{13}C NMR chemical shifts of peptide residue on C-3 in Table IV.

Benzyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-carboxymethyl-4-O-(3,4,6-tri-O-benzyl-2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-α-D-glucopyranoside (*VII*) was prepared by O-alkylation of compound *VI* with chloroacetic acid and sodium hydride in dioxane[12]. Condensation of acid *VII* with L-α-aminobutanoyl-D-isoglutamine benzyl ester trifluoroacetate[1] using N,N′-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole afforded N-{2-O-[benzyl 2-acetamido-6-O-benzyl-2,3-dideoxy-4-O-(3,4,6-tri-O-benzyl-2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-α-D-glucopyranosid-3-yl]-glycoloyl}-L-α-aminobutanoyl-D-isoglutamine benzyl ester (*VIII*). Hydrogenolysis of the benzyl groups in *VIII* on a palladium catalyst in acetic acid furnished the desired compound *IX*.

The obtained lipophilized disaccharide-dipeptide *IX* exhibits higher immunoadjuvant activity than MDP, displays an antitumour and radioprotective effect, is apyrogenic and does not induce thrombocytolysis[13]. The immunomodulatory activity of compound *IX* was determined in a delayed type hypersensitivity skin test with ovalbumin as antigen, and in a comitogenic test with T-mitogen-stimulated thymocytes. The antitumour activity was determined by following the inhibition of tumour growth of subcutaneous transplants of nonmetastazing 3-methylcholanthrene-induced MC11 fibrosarcoma in syngeneic male mice (inbred strain B10). The radioprotective activity was determined on the basis of the number of colony-forming units in spleen of mice irradiated with 4 Gy doses. The compound *IX* also adjusts the immunoregulatory index in vitro in lymfocytes from sclerosis multiplex and subacute sclerosing panencefalitis patients[14].

TABLE IV

^{13}C NMR chemical shifts of peptide residue in compound *VIII* in deuteriochloroform ($CDCl_3$ = 77·00 ppm) and *IX* in hexadeuteriodimethyl sulfoxide ($(CD_3)_2SO$ = 39·70 ppm)

Carbon	*VIII*	*IX*
C-1	69·87 t	70·26 t
C-2	172·03 s	171·37 s
C-3	55·23 d	53·74 d
C-4	26·29 t	27·14 t
C-5	10·21 q	10·09 q
C-6	171·00 s	170·69 s
C-7	52·43 d	53·21 d
C-8	172·10 s	170·69 s
C-9	30·56 t	30·69 t
C-10	33·80 t	31·48 t
C-11	173·76 s	173·32 s
$-O-CH_2-Ph$	66·56 t	—

The structure of the synthesized compounds *II*–*IX* was confirmed by detailed ^{1}H and ^{13}C NMR analyses. Confirmation of structure and assignment of the individual signals in the spectra of *II*, *III*, *V* and *VI* was also done using homo- and hetero-correlated 2D COSY NMR spectra. The results of these measurements are summarized in Tables I–IV.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter at 25°C. NMR spectra were taken on a Varian XL-200 spectrometer in FT mode at 200 MHz (^{1}H spectra) and at 50·3 MHz (^{13}C spectra) in deuteriochloroform, using tetramethylsilane as internal standard for the ^{1}H NMR and deuteriochloroform (δ 77·0) or hexadeuteriodimethyl sulfoxide (δ 39·7) signals (for compound *IX*) as standards for the ^{13}C NMR spectrum. Chemical shifts are given in ppm (δ-scale) and coupling constants (J) in Hz. Thin-layer chromatography was performed on Silufol UV_{254} sheets, column chromatography on silica gel Silpearl (both Kavalier, Votice, Czechoslovakia). High-performance liquid chromatography was carried out on 250 × 4 mm or 250 × 17 mm columns packed with Separon SGX C18 (5 μm and 10 μm, respectively; Laboratorní přístroje, Prague). Solutions were evaporated on a rotatory evaporator. Amino acid analyses were obtained with a Durrum amino acid analyser (samples were hydrolyzed with 4M-HCl at 110°C for 8 h). Analytical samples were dried at 6·5 Pa and 25°C for 8 h.

Benzyl 2-Acetamido-4-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-3-O-allyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (*II*)

Trifluoromethanesulfonic acid (500 μl) was added at room temperature to a stirred suspension of benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-O-allyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside[1] (*I*; 12·9 g, 20 mmol) in 2,2-dimethoxypropane (100 ml) and acetone (500 ml). After stirring for 2 h the resulting solution was neutralized with triethylamine (2 ml) and evaporated. The residue was dissolved in chloroform (500 ml), the solution was washed with water (2 × 150 ml), dried over anhydrous sodium sulfate and the solvent was evaporated. The remaining solid was extracted with light petroleum (2 × 100 ml) and the insoluble residue crystallized from methanol–ether–light petroleum, yielding 10·8 g (79%) of compound *II*, m.p. 256–259°C (decomp.); $[\alpha]_D$ +61° (*c* 1·0, methanol). For $C_{36}H_{48}N_2O_{11}$ (684·8) calculated: 63·14% C, 7·07% H, 4·09% N; found: 62·98% C, 7·03% H, 4·12% N.

Benzyl 2-Acetamido-3-O-allyl-4-O-(2-amino-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-α-D-glucopyranoside (*III*)

Compound *II* (10·0 g, 14·6 mmol), barium hydroxide octahydrate (80 g, 254 mmol), and methanol–water mixture (3 : 2, 500 ml) were stirred in a pressure vessel at 120°C for 10 h. After cooling, the solvents were evaporated and the residue was extracted with chloroform (3 × 300 ml). The chloroform extract was washed with water (2 × 200 ml), dried over anhydrous sodium sulfate and the chloroform was evaporated. Chromatography of the residue on a column of silica gel (700 g) in chloroform–methanol–triethylamine (200 : 10 : 1) afforded 7·4 g (79%) of solid compound *III*; $[\alpha]_D$ +69° (*c* 0·3, chloroform). For $C_{34}H_{46}N_2O_{10}$ (642·8) calculated: 63·54% C, 7·21% H, 4·36% N; found: 63·40% C, 7·12% H, 4·28% N.

Benzyl 2-Acetamido-3-O-allyl-6-O-benzyl-2-deoxy-4-O-
-(2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-α-D-glucopyranoside (*IV*)

A solution of N,N'-dicyclohexylcarbodiimide in dichloromethane (11 ml of 1M solution) was added at room temperature to a stirred solution of compound *III* (6·5 g, 10·1 mmol) and stearic acid (2·9 g, 10·2 mmol) in dichloromethane (100 ml). After stirring for 6 h, the separated N,N'-
-dicyclohexylurea was filtered off, washed with dichloromethane (100 ml) and the combined filtrates were taken down. The residue was heated to 90°C with 90% acetic acid (100 ml) for 2 h under stirring. After evaporation, the residue was chromatographed on a column of silica gel (400 g) in chloroform–methanol (10 : 1), affording 5·3 g (60%) of the solid compound *IV*; $[\alpha]_D$ −44° (*c* 0·3, acetic acid). For $C_{49}H_{76}N_2O_{11}$ (869·2) calculated: 67·71% C, 8·81% H, 3·22% N; found: 68·09% C, 8·53% H, 3·32% N.

Benzyl 2-Acetamido-3-O-allyl-6-O-benzyl-4-O-(3,4,6-tri-
-O-benzyl-2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside (*V*)

Anhydrous barium hydroxide powder was prepared by equilibration of barium oxide (24 g, 156·5 mmol) with barium hydroxide octahydrate (6·17 g, 19·56 mmol). A solution of compound *IV* (4·35 g, 5 mmol) in N,N-dimethylformamide (50 ml) followed by benzyl bromide (4·2 ml, 34·9 mmol) was added through a septum under argon and the mixture was sonicated in a ultrasonic cleaner (Bandelin — Model Sonorex TK52) at room temperature for 12 h. The mixture was diluted with chloroform (600 ml), washed with 10% acetic acid (2 × 100 ml) and water (100 ml), dried over anhydrous sodium sulfate, and the solvent was evaporated. The residue was dried at 80°C and 1·32 Pa for 2 h and subjected to chromatography on a column of silica gel (450 g) in chloroform–ethyl acetate (4 : 1) to give 4·3 g (75%) of solid compound *V*; $[\alpha]_D$ +43° (*c* 0·4, chloroform). For $C_{70}H_{94}N_2O_{11}$ (1 139·5) calculated: 73·78% C, 8·34% H, 2·46% N; found: 73·69% C, 8·15% H, 2·49% N.

Benzyl 2-Acetamido-6-O-benzyl-4-O-(3,4,6-tri-O-benzyl-2-
-deoxy-2-stearoylamino-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside (*VI*)

Compound *V* (3·2 g, 2·8 mmol) and tris(triphenylphosphine)rhodium(I) chloride (278 mg, 0·3 mmol) were refluxed with an ethanol–toluene–water mixture (7 : 3 : 1, 110 ml) for 8 h under stirring. After addition of formic acid (3 ml), the mixture was refluxed for an additional hour, cooled and the solvents were evaporated. Chromatography on a column of silica gel (150 g) in chloroform–ethyl acetate (4 : 1) afforded 2·7 g (88%) of solid compound *VI*; $[\alpha]_D$ +51° (*c* 0·3, chloroform). For $C_{67}H_{90}N_2O_{11}$ (1 099·5) calculated: 73·19% C, 8·25% H, 2·55% N; found: 73·34% C, 8·17% H, 2·56% N.

Benzyl 2-Acetamido-6-O-benzyl-4-O-(3,4,6-tri-O-benzyl-2-deoxy-2-
-stearoylamino-β-D-glucopyranosyl)-2-deoxy-3-O-carboxymethyl-α-D-glucopyranoside (*VII*)

A stirred mixture of compound *VI* (2·6 g, 2·36 mmol), sodium hydride (288 mg, 12 mmol) and dioxane (25 ml) was heated at 95°C for 2 h. After cooling to room temperature, chloroacetic acid (300 mg, 3·17 mmol) was added and the mixture was stirred at 65°C for 4 h. After cooling to room temperature, solid carbon dioxide was added, the excess hydride was decomposed with water and the solvent was evaporated. Acetic acid (10%, 35 ml) was added, the mixture was extracted with chloroform (3 × 100 ml) and the chloroform extract was dried over anhydrous sodium sulfate. After evaporation, the residue was chromatographed on a column of silica gel

in chloroform–ethyl acetate–formic acid (800 : 200 : 1) to give 1·93 g (71%) of solid compound *VII*; $[\alpha]_D$ +34° (*c* 0·4, chloroform). For $C_{69}H_{92}N_2O_{13}$ (1 157·5) calculated: 71·60% C, 8·01% H, 2·42% N; found: 71·33% C, 7·86% H, 2·47% N.

N-{2-O-[Benzyl 2-Acetamido-6-O-benzyl-4-O-(3,4,6-tri-O-benzyl-2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl]-glucoloyl}-L-α-aminobutanoyl-D-isoglutamine Benzyl Ester (*VIII*)

A solution of tert-butoxycarbonyl-L-α-aminobutanoyl-D-isoglutamine benzyl ester[1] (650 mg, 1·54 mmol) in a mixture of dichloromethane–trifluoroacetic acid (17 : 3, 20 ml) was allowed to stand at room temperature for 50 min. After evaporation, the sirupy residue was extracted with diethyl ether (2 × 40 ml). The insoluble material was dried at room temperature and 1·32 Pa for 2 h and then dissolved in dioxane (25 ml). The obtained solution of L-α-aminobutanoyl-D-isoglutamine benzyl ester trifluoroacetate (1·54 mmol) was used in the condensation with acid *VII*.

To a stirred solution of compound *VII* (1·5 g, 1·3 mmol) and 1-hydroxybenzotriazole monohydrate (185 g, 1·37 mmol) in dichloromethane (10 ml) was added 1M solution of N,N′-dicyclohexylcarbodiimide in dichloromethane (1·3 ml) at 0°C. After stirring for 1 h, the above-described solution of L-α-aminobutanoyl-D-isoglutamine benzyl ester trifluoroacetate (1·54 mmol) in dioxane (25 ml) and triethylamine (600 µl, 4·3 mmol) were added. The mixture was stirred at 0°C for 2 h and at room temperature for 12 h and the solvents were evaporated. The residue was dissolved in chloroform (200 ml), the solution was washed successively with 0·01M-HCl (2 × 50 ml), saturated solution of sodium hydrogen carbonate (2 × 50 ml) and water (50 ml), and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was chromatographed on a column of silica gel (200 g) in chloroform–methanol (20 : 1) to give 1·35 g (71%) of solid compound *VIII*; $[\alpha]_D$ +25° (*c* 0·3, chloroform). For $C_{85}H_{113}N_5O_{16}$ (1 460·9) calculated: 69·89% C, 7·80% H, 4·79% N; found: 69·53% C, 7·59% H, 5·03% N.

O-(2-Deoxy-2-stearoylamino-β-D-glucopyranosyl)-(1→4)-N-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine (*IX*)

Compound *VIII* (980 mg, 0·67 mmol) was hydrogenolyzed in acetic acid (60 ml) over 5% palladium catalyst on carbon (2 g) at room temperature for 15 h. After this time the vessel was flushed with nitrogen, the catalyst was filtered off and washed with acetic acid (100 ml). The filtrate was taken down and the residue chromatographed on silica gel C18 in methanol–water (4 : 1). The homogeneous fraction was evaporated, the residue taken up in acetic acid and freeze-dried; yield 434 mg (70%) of compound *IX*. Amino acid analysis: glutamic acid 1·03, α-aminobutyric acid 1·04, normuramic acid 0·97, glucosamine 0·91. For $C_{43}H_{77}N_5O_{16}$ (920·1) calculated: 56·13% C, 8·44% H, 7·61% N; found: 56·09% C, 8·67% H, 7·42% N.

REFERENCES

1. Farkaš J., Ledvina M., Brokeš J., Ježek J., Zajíček J., Zaoral M.: Carbohydr. Res. *163*, 63 (1987).
2. Ledvina M., Farkaš J., Zajíček J., Ježek J., Zaoral M.: Collect. Czech. Chem. Commun. *54*, 2784 (1989).
3. Ledvina M., Šaman D.: *Abstr. Pap. 5th Eur. Symp. Carbohydr., August 21—25, 1989, Prague* (M. Černý and P. Drašar, Eds), Commun. A 163. Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences Prague 1989.

4. Kotani S., Takada H., Tsujimoto M., Ogawa T., Mori Y., Koga T., Iribe H., Tanaka A., Nagao S., Mc Ghee J. R., Michalek S. M., Morisaki I., Nishimura C., Ikeda S., Kohashi O., Ogawa H., Ozawa S., Hamada S., Kawata S., Shiba T., Kusumoto S. in: *Progress in Immunology V.* (Y. Yamamura and T. Tada, Eds), p. 1359. Academic Press, Tokyo 1983.
5. Adam A., Lederer E.: Med. Res. Rev. *4*, 111 (1984).
6. Leclerc C., Vogel F. R.: CRC Crit. Rev. Terapeut. Drug Carrier Syst. *2*, 353 (1986).
7. Masihi K. N., Lange W., Rohde-Schulz B., Chedid L.: AIDS Res. Hum. Retroviruses *6*, 393 (1990).
8. Lazdins J. K., Wood-Cook K. A., Walker M. R., Alteri E.: AIDS Res. Hum. Retroviruses *6*, 1157 (1990).
9. Kiso M., Tanaka S., Tanahashi M., Fujishima Y., Ogawa Y., Hasegawa A.: Carbohydr. Res. *148*, 221 (1986).
10. Nakamoto S., Takahashi T., Ikeda K., Achiwa K.: Chem. Pharm. Bull. *35*, 4517 (1987).
11. Corey J. E., Suggs J. W.: J. Org. Chem. *38*, 3224 (1973).
12. Chaturvedi N. C., Khosla M. C., Anand N.: J. Med. Chem. *9*, 971 (1966).
13. Hříbalová V., Bubeník J., Kofránek V.: Private communication.
14. Mitrová E.: Private communication.

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