MURAMYL-DIPEPTIDE ANALOGUES: SYNTHESIS AND BIOLOGICAL ACTIVITIES*,**

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Dedicated to the memory of Dr Karel Bláha.

In the search for immunoadjuvant active compounds without pyrogenic activity we prepared N-Ac-norMur-L-Abu-D-Gln-O-Bu (*V*), N-Ac-Mur-L-Abu-D-Gln-O-Bu (*VII*) and their respective α-benzylglycosides *VI* and *VIII*. All the prepared compounds are nonpyrogenic. In the delayed hypersensitivity test, compound *V* is inactive, *VI* is comparable to MDP, *VII* is more and *VIII* is less active than MDP.

Until now, about one thousand glycopeptides, bacterial wall fragments and their analogues have been prepared[1-4]. Many of them exhibit immunoadjuvant or antitumor activity. However, a great majority of these compounds have side-effects such as pyrogenicity, damage of rabbit blood plateles (either degranulation or complete lysis) etc. N-Ac-Mur-L-Ala-D-Gln-O-Bu (ref.[5-10]) or the corresponding methyl ester[5-9] (murabutide or muramethide) are potent immunoadjuvants but, unlike MDP, they are not pyrogenic even on intracerebroventricular application. N-Ac-norMur-L-Abu-D-iGln[11-20] exhibits an immunoadjuvant activity comparable with MDP. The compound has antitumor effect and in some vaccines is more effective than MDP. It is less pyrogenic and, contrary to MDP, evokes neither lysis of rabbit blood plateles nor degranulation. N-Ac-Glc-β-(1→4)-N-Ac-norMur-L-Abu-D--iGln[21-23] is stronger immunoadjuvant than MDP and is less pyrogenic, too. N-Ac-Glc-β-(1→4)-N-Ac-norMur(1-α-OBzl)-L-Abu-D-iGln(OBzl) (refs[18,21,22]) is also strong immunoadjuvant, however, it is not pyrogenic and does not cause thrombocytolysis or degranulation.

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** The symbols and abbreviations obey the published recommendations (Biochemical Nomenclature and Related Documents. International Union of Biochemistry, London 1978). In addition we use the following abbreviations: Mur, 2-amino-3-O-[(*R*)-1-carboxyethyl]-2--deoxy-D-glucose; norMur, 2-amino-3-O-carboxymethyl-2-deoxy-D-glucose; MDP, N-acetylmuramyl-L-alanyl-D-isoglutamine; Bu, butyl.

In the light of the above-mentioned facts it was of interest to prepare N-Ac-Mur-L-Abu-D-Gln-O-Bu (*VII*), N-Ac-norMur-L-Abu-D-Gln-O-Bu (*V*) and the respective 1-α-O-benzyl glycosides *VIII* and *VI*. The sugar component, in the form of benzyl 2-acetamido-4,6-O-benzylidene-3-O-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside[24] or benzyl 2-acetamido-4,6-O-benzylidene-3-O-carboxymethyl-2-deoxy-α-D-glucopyranoside[25], was condensed with H-L-Abu-D-Gln-O-Bu. The protecting groups in the fully protected muramyl (*IV*) and normuramyl (*III*) dipeptides were removed by catalytic hydrogenation. It is known[3] that hydrogenolysis of muramyl α-benzylglycosides is difficult. We made use of this fact in the simultaneous preparations of free glycopeptides and the corresponding α-benzylglycosides. The compounds *V*–*VIII* were isolated by preparative HPLC.

The purity and structure of compounds *I*, *II*, *V*–*VIII* have been confirmed by ^{1}H NMR spectra (Table I) and for compounds *VI* and *VIII* also by ^{13}C NMR spectra (Table II). Proton NMR spectra of compounds *V* and *VII*, taken immediately after dissolution of the sample in hexadeuterodimethyl sulfoxide, have shown the α : β anomer ratio to be about 2–2·5 : 1 whereas on prolonged standing the ratio was shifted in favour of the α-anomer to about 5 : 1. Table I lists ^{1}H NMR parameters of the α-anomer only. The presence of the β-anomer leads to splitting of some signals. The anomers can be identified by the H-1 signals: at about δ 5·0 (J(1, 2) = = 3·2 Hz) for the prevailing α-anomer and at about δ 4·5 (J(1, 2) = 8·0 Hz) for the β-anomer. The behaviour of the C(1) hydroxyl is interesting: the ^{1}H NMR spectra for the α-anomers *V* and *VII* exhibit, in addition to the vicinal interaction J(OH, H-1) = 4·5 Hz, a long-range coupling with the H-2 proton (1 Hz). Probably, the rotation about the C(1)—OH bond is restricted because of hydrogen bonding between C(1)—OH and the neighbouring carbonyl of the N-acetyl group, fixing geometry, advantageous for the long-range coupling.

The pyrogenicity was tested on rabbits. Each test was performed with groups of three animals, using MDP as a positive standard. Repeated experiments showed that compounds *V*–*VIII* are apyrogenic in doses of 40, 200 and 1 000 nmol. The delayed hypersensitivity tests were carried out on guinea pigs, using 400 nmol of the tested compound and 200 μg of ovalbumin in Freund's incomplete adjuvant. After two weeks, ovalbumin as sensitizing antigen was administered intradermally in doses 50 and 250 μg. After 24 h the skin induration was measured. As follows from repeated experiments, compound *V* is inactive, its α-benzyl glycoside *VI* is as active as, and compound *VII* more active than MDP. Compound *VIII* is less active than MDP.

EXPERIMENTAL

The melting points were determined on a Kofler block and are not corrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter, the obtained values are corrected according to nitrogen content in the lyophilizate. The IR spectra were recorded on a Zeiss (Jena, G.D.R.)

UR-20 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained in the FT mode with a Varian XL-200 spectrometer at 200 MHz and 50·3 MHz, respectively. Deuterochloroform or hexadeuterodimethyl sulfoxide were used as solvents with tetramethylsilane as internal standard. ^1H NMR spectra were analyzed on the basis of multiplicity of signals, decoupling experiments, exchange of labile protons for deuterium and comparison with literature data of similar compounds[26-28]. For the assignment of signals in ^{13}C NMR spectra the information from "attached proton test" spectra[30] and comparison with literature data for similar compounds[29] were used. The purity was checked by chromatography on Silufol sheets (Kavalier, Votice) in the following systems: 1-butanol–acetic acid–water (4 : 1 : 1) (*A*), chloroform–methanol (10 : 1) (*B*). The compounds were detected by ninhydrin or chlorination[31,32]. Samples for amino acid analysis were hydrolyzed with 6M-HCl at 110°C for 8 h. The hydrolyzates were analyzed on a Beckman–Spinco 120 B amino acid analyzer. When hydrolyzates containing muramic and glutamic acid were analyzed, the first buffer was adjusted to pH 3 in order to obtain good resolution of these two acids. A colour value of 3·08 was used for muramic and normuramic acid. The values obtained were corrected for 25% decomposition of muramic and normuramic acid during the 8 hour's hydrolysis[24]. Preparative HPLC was performed on a 16 × 250 mm column packed with Separon SGX C18 (10 μm), mobile phase water–methanol. Gradient for compound *V*: 0—10 min 25% methanol, 10—15 min gradient to 30% methanol, 15—30 min 30% methanol; compound *VII*: 0—5 min 0% methanol, 5—15 min gradient to 20% methanol, 15—20 min 20% methanol, 20—50 min gradient to 80% methanol. Flow rate 12 ml min^{-1}, two Knauer pumps type 64, detection with a Knauer variable wavelength monitor, 220 nm, 0·4 mm cell, recorder Shimadzu Chromatopac C-R3A. Injections 90—100 mg. Analytical HPLC was carried out on a 4 × 250 mm column packed with Separon SGX-RPS (10 μm). Mobile phase water–methanol or water–methanol–0·1% trifluoroacetic acid, gradient from 30 to 40% methanol, flow rate 1 ml min^{-1}, pump Spectra-Physics SP 8 700, detection with a Spectra-Physics SP 8 440 UV detector (220 or 254 nm), recorded by a Spectra-Physics SP 4 200 computing integrator.

All yields of the free glycopeptides are corrected for the content of the compounds in the lyophilizate, determined by nitrogen analysis. Analytical samples were dried over phosphorus pentoxide at 50—120°C/10 Pa for 8—24 h.

Boc-D-Gln-O-Bu (*I*)

A solution of caesium hydrogen carbonate (4·0 g; 20·6 mmol) in water (30 ml) was added to a solution of Boc-D-Gln (4·92 g; 20 mmol) in ethanol (120 ml) so as the final pH was 7. The solution was concentrated in vacuo, the residue was three times coevaporated with dioxane (100 ml) and dried in a desiccator over phosphorus pentoxide and sodium hydroxide to a constant weight. After addition of anhydrous dimethylformamide (100 ml) and butyl bromide (4·75 ml; 44 mmol), the reaction mixture was stirred for 48 h at room temperature, concentrated in vacuo to a thick slurry, the residue was mixed with ethyl acetate (150 ml) and the organic phase was washed with 1% hydrochloric acid (2 × 20 ml), water, saturated aqueous sodium hydrogen carbonate and water. The solution was dried over sodium sulfate, filtered, concentrated in vacuo and the product was precipitated with light petroleum. After standing at 0°C for 2 h, the product was collected and washed with light petroleum, m.p. 78—82°C, yield 5·3 g (88%), HPLC purity 94%. This compound, homogeneous on Silufol in systems A and B, was used in the next reaction step. A sample after crystallization from ethyl acetate–light petroleum melted at 81 to 82°C, $[\alpha]_D^{22}$ +22·8° (*c* 1·3, ethanol). For $C_{14}H_{26}N_2O_5$ (302·4) calculated: 55·61% C, 8·67% H, 9·26% N; found: 55·72% C, 8·56% H, 9·21% N. IR spectrum ($CHCl_3$), cm^{-1}: —$CONH_2$ 3 530, 3 415, 1 691, 1 607, 1 595; $(CH_3)_3$—C—O—CO—NH— 3 435, 1 705, 1 508, 1 172; ester 1 733, 1 172.

TABLE I

Proton NMR parameters of Boc-D-Gln-OBu (*I*) and Boc-L-Abu-D-Gln-OBu (*II*) in deuterochloroform and N-Ac-norMur-L-Abu-D-Gln-OBu (*V*), N-Ac-Mur-L-Abu-D-Gln-OBu (*VII*) and their 1-α-O-Bzl derivatives (*VI* and *VIII*) in hexadeuterodimethyl sulfoxide.

Proton	Chemical shifts (Coupling constants)					
	I	*II*	*VI*	*VIII*	*V*[a]	*VII*[a]
Sugar residue[b]						
H-1	—	—	4·74 (3·5)	4·81 (3·5)	4·94 (4·5; 3·5)	5·03 (4·3; 3·2)
H-2	—	—	3·84 (10·5; 8·2; 3·5)	3·74 (10·5; 7·5; 3·5)	3·74 (10·5; 8·5; 3·2)	3·62 (10·5; 7·2; 3·2)
C(1)—OH	—	—	—	—	6·60 (4·5; 1·2)	6·48 (4·3; 1·0)
C(4)—OH	—	—	5·71 (5·3)	5·29 (6·6)	5·59 (5·3)	5·18 (6·7)
C(6)—OH	—	—	4·60 (5·7)	4·55 (5·7)	4·44 (6·0)	4·53 (6·0)
N-Acetyl						
NH	—	—	8·12 (8·2)	8·16 (7·5)	8·00 (8·5)	8·08 (7·2)
CH_3	—	—	1·84	1·80	1·84	1·81
O-Benzyl						
CH_2	—	—	4·44 (12·3)	4·43 (12·5)	—	—
	—	—	4·68 (12·3)	4·66 (12·5)	—	—
C_6H_5	—	—	7·28—7·40	7·25—7·40	—	—

O—CHR—CO						
α-CHR	—	—	4·12 (15·8) 4·25 (15·8)	4·40 (6·8)	4·11 (15·5) 4·22 (15·5)	4·39 (6·7)
β-CH_3	—	—	—	1·24 (6·8)	—	1·26 (6·7)
L-Abu						
NH	—	5·10 (7·0)	7·79 (8·2)	7·59 (8·5)	7·80 (8·2)	7·62 (8·4)
α-CH	—	4·05 (7·0)	4·31	4·31	4·30	4·32
β-CH_2	—	1·89	1·48—2·02	1·47—2·03	1·49—2·04	1·49—2·04
γ-CH_3	—	0·97 (7·5)	0·88 (7·4)	0·86 (7·3)	0·88 (7·3)	0·88 (7·4)
D-Gln						
NH	5·51 (8·0)	7·09 (7·5)	8·37 (7·6)	8·42 (7·5)	8·37 (7·5)	8·41 (7·6)
α-CH	4·27	4·58	4·19	4·22	4·15	4·22
β-CH_2	1·95; 2·15	1·89; 2·20	1·48—2·02	1·47—2·03	1·49—2·04	1·49—2·04
γ-CH_2	2·33 (7·5)	2·29	2·11 (7·4)	2·11 (7·5)	2·12 (7·5)	2·12 (7·5)
$CONH_2$	6·13; 6·36	5·66; 6·34	6·73; 7·24	6·74; 7·23	6·73; 7·25	6·74; 7·24
O-Butyl						
α-CH_2	4·13 (6·8)	4·13 (6·8)	4·03	4·01	4·04	4·03
β-CH_2	1·63	1·63	1·48—2·02	1·47—2·03	1·49—2·04	1·49—2·04
γ-CH_2	1·37	1·39	1·31	1·30	1·32	1·32
δ-CH_3	0·93 (7·5)	0·93 (7·5)	0·84 (7·2)	0·82 (7·3)	0·85 (7·3)	0·83 (7·4)
tert-Butyl						
$C(CH_3)_3$	1·44	1·45	—	—	—	—

[a] The parameters for the major α-anomer are given only (see text); [b] the signals of the other sugar protons could not be analyzed.

Boc-L-Abu-D-Gln-O-Bu (*II*)

Boc-D-Gln-O-Bu (*I*; 4·53 g; 15 mmol) was dissolved in 55% solution of trifluoroacetic acid in dichloromethane (40 ml). After 30 min, the solution was concentrated in vacuo and the residue was coevaporated successively with dichloromethane, ether, benzene and ether (50 ml of each). The remaining oil was dried in vacuo (oil pump) at 50°C for 10 min. The obtained D-glutamine butyl ester trifluoroacetate was electrophoretically homogeneous at pH 2·5.

Boc-L-Abu (3·05 g; 15 mmol) was dissolved with stirring in anhydrous dimethylformamide (20 ml) and N-ethylpiperidine (2·08 ml; 15 mmol). The solution was cooled to −15°C and ethyl chloroformate (1·43 ml; 15 mmol) was added. After standing at 0°C for 10 min and cooling to −15°C, a cold solution of the amino component in dimethylformamide (20 ml) and N-ethylpiperidine (2·08 ml; 15 mmol) was added. The mixture was stirred at 0°C for 10 min and at 5°C for 2 h, concentrated in vacuo and dissolved in ethyl acetate (160 ml) and ethanol (10 ml). The reaction mixture was processed as described for compound *I*, yield 3·8 g (66%), m.p. 137–139°C, $[\alpha]_D^{22}$ −8·03° (*c* 0·5; ethanol), purity 99% (HPLC), homogeneous on Silufol in system *A*. Amino acid analysis: Abu 1·03, Glu 0·97, NH_3 1·07. For $C_{18}H_{33}N_3O_6$ (387·5) calculated: 55·80% C,

TABLE II

Carbon-13 NMR chemical shifts of 1-α-O-Bzl-N-Ac-norMur-L-Abu-D-Gln-OBu (*VI*) and 1-α-O--Bzl-N-Ac-Mur-L-Abu-D-Gln-OBu (*VIII*) in hexadeuterodimethyl sulfoxide

Carbon	*VI*	*VIII*	Carbon	*VI*	*VIII*
Sugar residue			L-Abu		
C-1	96·20	95·99	α-CH	51·91	51·82
C-2	53·33	53·33	β-CH_2	26·07	26·41
C-3	80·91	78·41	γ-CH_3	10·22	10·17
C-4	70·05	69·99	C=O	171·48[a]	171·99[a]
C-5	73·36	73·42	D-Gln		
C-6	60·69	60·79	α-CH	52·99	53·22
N-Acetyl			β-CH_2	26·80	26·91
C=O	173·49[a]	173·42[a]	γ-CH_2	30·31	30·29
CH_3	22·73	22·79	δ-$CONH_2$	170·44[a]	171·49[a]
O-Benzyl			C=O	169·68	169·70
CH_2	68·19	68·15	O-Butyl		
C_6H_5	137·87	137·96	α-CH_2	64·36	64·37
	128·42(2)	128·40(2)	β-CH_2	31·24	31·22
	127·90(2)	127·73(2)	γ-CH_2	18·73	18·72
	127·74	127·65	δ-CH_3	13·73	13·72
O—CH(R)—CO					
α-CH(R)	70·75	76·12			
β-CH_3	—	19·39			
C=O	171·95[a]	173·07[a]			

[a] The assignment of carbonyl signals can be interchanged.

8·58% H, 10·84% N; found: 56·08% C, 8·52% H, 10·96% N. IR spectrum ($CHCl_3$), cm^{-1}: —$CONH_2$ 3 530, 3 415, 1 693; $(CH_3)_3$—C—O—CO—NH— 3 440, 1 710, 1 501, 1 171; ester 1 731, 1 171; —CO—NH— 1 680, 1 520.

1-α-O-Benzyl-4,6-O-benzylidene-N-acetylnormuramyl-L-α-aminobutyryl-D-glutamine n-Butyl Ester (*III*)

Boc-L-Abu-D-Gln-O-Bu (*II*; 1·93 g; 5 mmol) was deprotected by treatment with 55% trifluoroacetic acid in dichloromethane (30 ml) for 30 min. The mixture was concentrated in vacuo and the remaining oil was repeatedly coevaporated with dichloromethane and then with ether (2 × 50 ml).

Ethyl chloroformate (0·48 ml; 5 mmol) was added at −10°C to sttirred solution of 1-α-O-benzyl-4,6-O-benzylidene-N-acetylnormuramic acid[25] (2·3 g; 5 mmol) and N-methylmorpholine (0·55 ml; 5 mmol) in dimethylformamide (30 ml). After standing at 0°C for 10 min, the mixture was cooled to −15°C and a cold solution of the amino component in dimethylformamide (20 ml), together with N-methylmorpholine (0·55 ml; 5 mmol), was added. The solution was stirred at 0°C for 15 min and at room temperature for 1 h. The product was precipitated with ice-cold water (100 ml), filtered and triturated on the filter successively with 1% hydrochloric acid (3 × 30 ml), water, saturated sodium hydrogen carbonate solution and water. Yield 2·48 g (68%) of material m.p. 193—198°C, uniform on Silufol in the systems *A* and *B*. Crystallization from hot dimethylformamide (20 ml) and precipitation with water (80 ml), standing in a refrigerator overnight, filtration, washing with water (3 × 30 ml) and drying in a desiccator afforded 2·0 g (55%) of the product, m.p. 240—245°C, $[\alpha]_D^{22}$ +82·7° (*c* 0·8, dimethylformamide); purity 100% (HPLC, 220 and 254 nm). Amino acid analysis: norMur 0·99, Abu 0·94, Glu 0·95, NH_3 1·10. For $C_{37}H_{50}N_4O_{11}$ (726·8) calculated: 61·14% C, 6·93% H, 7·71% N; found: 61·12% C, 6·82% H, 7·44% N. IR spectrum (KBr), cm^{-1}: —$CONH_2$ 3 405, 1 660; —CO—NH— 1 660, 1 559, 1 546; ester 1 740, 1 180; —O— 1 126, 1 094, 1 056, 1 030, 1 006; C_6H_5 700.

1-α-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-L-α-aminobutyryl-D-glutamine n-Butyl Ester (*IV*)

The compound was prepared in the same manner as the normuramyl derivative *III*, starting from 1-α-O-benzyl-4,6-O-benzylidene-N-acetylmuramic acid[24] (2·36 g; 5 mmol); yield 2·5 g (67·5%) of product, m.p. 200—205°C, uniform on Silufol in the system A. The compound was dissolved in hot dimethylformamide (20 ml) and precipitated with water. After standing for 2 h at 0°C, the product was filtered and washed with water, m.p. 206—207°C, yield 2·3 g (62%). Purity 80% (HPLC), the minor compound (20%, 220 and 254 nm) was the isomuramyl derivative. The product was not further purified. $[\alpha]_D^{22}$ +90·4° (*c* 0·9, dimethylformamide). Amino acid analysis: Mur 0·91, Abu 0·99, Glu 0·96, NH_3 1·09. For $C_{38}H_{52}N_4O_{11}$ (740·8) calculated: 61·61% C, 7·07% H, 7·56% N; found: 61·37% C, 6·78% H, 7·36% N. IR spectrum (KBr), cm^{-1}: —$CONH_2$ 1 685, 1 610; —CO—NH— 1 662, 1 559, 1 547, 1 534; ester 1 739, 1 184; —O— 1 129, 1 092, 1 059, 1 049, 1 034, 1 020, 1 006; C_6H_5 700.

N-Acetylnormuramyl-L-α-aminobutyryl-D-glutamine n-Butyl Ester (*V*)

A solution of palladium chloride in water (0·8 ml; corresponding to 0·19 g of palladium) was added to a suspension of purified charcoal (1 g) in water (50 ml) and the mixture was hydrogenated until the hydrogen consumption ceased (3 h). The catalyst was washed with water to neutrality, water was removed and the fully protected derivative *III* (1·0 g; 1·37 mmol) in acetic acid (50 ml;

distilled from chromium trioxide) was added. The compound was hydrogenated with stirring, for 24 h. The catalyst was filtered off and washed with 25% acetic acid (3 × 50 ml) and water (2 × 50 ml), the filtrate was concentrated in vacuo (bath temperature 40°C), mixed with water (50 ml) and freeze-dried; yield 871 mg. Preparative HPLC afforded two fractions which were repeatedly coevaporated with water and freeze-dried, affording 342 mg (44·2%) of *V* and 90 mg (10%) of 1-α-O-benzyl-N-acetylnormuramyl-L-α-aminobutyryl-D-glutamine n-butyl ester (*VI*). Both compounds were homogeneous on Silufol in systems *A* and *B*. The title product *V* had $[\alpha]_D^{22}$ +20·3° (*c* 0·3, water) (5 min), +17·2° (24 h). Amino acid analysis: norMur 1·07, Abu 1·0, Glu 1·09, NH_3 1·07. For $C_{23}H_{40}N_4O_{11}.H_2O$ (566·6) calculated: 48·76% C, 7·47% H, 9·89% N; found for undried sample: 48·90% C, 7·65% H, 9·93% N. For $C_{23}H_{40}N_4O_{11}$ (548·6) calculated: 10·21% N, glycopeptide content in the lyophilizate: 97·2%. IR spectrum (KBr), cm^{-1}: —CO— —NH— 1 666, 1 557; ester 1 734; —O— 1 150, 1 134, 1 086, 1 063, 1 044; —$CONH_2$ 1 696, 3 400. Purity 98% (HPLC, 220 nm, in 0·1% trifluoroacetic acid–methanol as well as in water–methanol).

Compound *VI*: $[\alpha]_D^{22}$ +74·4° (*c* 0·4, water). Amino acid analysis: norMur 1·04, Abu 1·0, Glu 1·07, NH_3 0·99. For $C_{30}H_{46}N_4O_{11}.1{\cdot}5\ H_2O$ (665·7) calculated: 54·12% C, 7·42% H, 8·42% N; for undried sample found: 53·86% C, 7·63% H, 8·54% N. For $C_{30}H_{46}N_4O_{11}$ (638·7) calculated: 8·77% N; glycopeptide content in lyophilizate: 97·4%. IR spectrum (KBr), cm^{-1}: —CO—NH— 1 666, 1 542; C_6H_5— 701; ester 1 736; —O— 1 157, 1 140, 1 054, 1 032; —$CONH_2$ 1 696,3 430. Purity 93% (HPLC, 220 and 254 nm, in 0·1% trifluoroacetic acid–methanol and in water–methanol).

N-Acetylmuramyl-L-α-aminobutyryl-D-glutamine n-Butyl Ester (*VII*)

The fully protected derivative *IV* (1·2 g; 1·62 mmol) and acetic acid (50 ml) were added to the catalyst, prepared from 1 g of charcoal (see the preceding experiment). After hydrogenation for 24 h, the catalyst was filtered off and washed with 25% acetic acid (3 × 50 ml) and water (2 × 50 ml). The filtrate was concentrated in vacuo and the residue was mixed with water (100 ml). The formed fine precipitate (80 mg) was filtered and washed with water (20 ml); according to TLC, m.p. and IR spectrum, this material was unreacted starting *IV*. The filtrate was concentrated in vacuo and freeze-dried; yield 892 mg. The compound was dissolved in water (5 ml) and acetic acid (2 ml) and was purified by preparative HPLC. The two obtained fractions were repeatedly coevaporated with water and freeze-dried, yield 177 mg (18%) of *VII* and 139 mg (12·5%) of 1-α-O-benzyl-N-acetylmuramyl-L-α-aminobutyryl-D-glutaminyl n-butyl ester (*VIII*). Both products were homogeneous on Silufol in systems *A* and *B*.

Product *VII*: $[\alpha]_D^{22}$ +31·1° (*c* 0·24, water), no change in rotation during 24 h. Amino acid analysis: Mur 1·04, Abu 0·92, Glu 1·03, NH_3 1·09. For $C_{24}H_{42}N_4O_{11}.H_2O$ (580·6) calculated: 49·65% C, 7·64% H, 9·65% N; for undried sample found: 49·70% C, 7·22% H, 9·37% N. For $C_{24}H_{42}N_4O_{11}$ (562·6) calculated: 9·69% N, glycopeptide content in lyophilizate: 94%; IR spectrum (KBr), cm^{-1}: —CO—NH— 1 663, 1 549, 3 300; ester 1 737; —O— 1 124, 1 060. —$CONH_2$ 1 696. Purity 99% (HPLC, 220 nm, in 0·1% trifluoroacetic acid–methanol as well as in water–methanol), two peaks (α and β anomers) in the ratio 76 : 24.

1-α-O-Benzyl-N-acetylmuramyl-L-α-aminobutyryl-D-glutaminyl n-butyl ester (*VIII*): $[\alpha]_D^{22}$ +82·9° (*c* 0·2, water). Amino acid analysis: Mur 0·91, Abu 1·0, Glu 1·1, NH_3 1·05. For $C_{31}H_{48}.N_4O_{11}.H_2O$ (670·7) calculated: 55·51% C, 7·51% H, 8·35% N; for undried sample found: 55·54% C, 7·26% H, 8·21% N. For $C_{31}H_{48}N_4O_{11}$ (652·7) calculated: 8·58% N, glycopeptide content in lyophilizate: 95·7%. IR spectrum (KBr), cm^{-1}: —CO—NH— 1 663, 1 556, 1 545, 1 534, 3 300; C_6H_5— 701; ester 1 738; —O— 1 125, 1 062, 1 048, 1 029; —$CONH_2$ 1 693, 1 631.

Purity 88% + 12% of a slower-eluted compound (HPLC, 220 and 254 nm, 0·1% trifluoroacetic acid–methanol or water–methanol).

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REFERENCES

1. Adam A., Lederer E.: Med. Res. Rev. *4*, 111 (1984).
2. Kotani S., Azuma I., Takada H., Tsujimoto M., Yamamura Y.: Adv. Exp. Med. Biol. *166*, 117 (1983).
3. Ježek J.: Chem. Listy *80*, 337 (1986).
4. Leclerc C., Vogel F. R.: CRC Crit. Rev. Ther. Drug Carrier Syst. *2*, 353 (1986).
5. Lefrancier P., Derrien M., Jamet X., Choay J., Lederer E., Audibert F., Parant M., Parant F., Chedid L.: J. Med. Chem. *25*, 87 (1982).
6. Krueger J. M., Walter J., Karnovsky M. L., Chedid L., Choay J. P., Lefrancier P., Lederer E.: J. Exp. Med. *159*, 68 (1984).
7. Parant M., Riveau G., Parant F., Chedid L.: Am. J. Physiol. *247*, C 169 (1984).
8. Riveau G., Parant M., Damais Ch., Parant F., Chedid L.: Am. J. Physiol. *250*, C 572 (1986).
9. Adam A., Petit J. F., Lefrancier P., Lederer E.: Mol. Cell. Biochem. *41*, 27 (1981).
10. Chedid L., Parant M., Audibert F., Riveau G., Parant F., Lederer E., Choay J., Lefrancier P.: Infect. Immun. *35*, 417 (1982).
11. McLaughlin Ch. A., Schwartzman S. M., Horner B. L., Jones G. H., Moffatt J. G., Nestor J. J., Tegg D.: Science *208*, 415 (1980).
12. Lopez-Berestein G., Mehta K., Mehta R., Juliano R. L., Hersh E. M.: J. Immunol. *130*, 1500 (1983).
13. Sharma S. D., Tsai V., Krahenbuhl J. L., Remington J. S.: Cell. Immunol. *62*, 101 (1981).
14. Fraser-Smith E. B., Matthews T. R.: Infect. Immun. *34*, 676 (1981).
15. Pekárek J., Rotta J., Zaoral M., Krchňák V., Straka R., Ježek J., Rýc M.: Exp. Cell Biol. *53*, 260 (1985).
16. Masihi K. N., Brehmer W., Lange W., Ribi E., Schwartzman S.: J. Biol. Response Modifiers *3*, 663 (1984).
17. Stevens V. C., Cinader B., Powell J. E., Lee A. C., Koh S. W.: Am. J. Reproductive Immunol. *1*, 315 (1981).
18. Rýc M., Rotta J., Straka R., Zaoral M., Farkaš J., Ledvina M., Ježek J., Pekárek J. in: *Biological Properties of Peptidoglycan* (P. H. Seidl and K. H. Schleifer, Eds), p. 383. Walter de Gruyter, Berlin 1986.
19. Rotta J., Zaoral M., Pekárek J., Ježek J., Rýc M., Straka R. in: *Recent Adv. Streptococci and Streptococcal Dis., Proc. 9th Lancefield Int. Symp. on Streptococci and Streptococcal Dis., 1984* (Y. Kimura, S. Kotani and Y. Shiokawa, Eds), p. 254. Reedbooks, Bracknell, U.K. 1985.
20. Rýc M., Rotta J., Straka R., Pokorný J., Hříbalová V., Zaoral M.: ibid. p. 196.
21. Farkaš J., Ledvina M., Brokeš J., Ježek J., Zajíček J., Zaoral M. in: *Synthetic immunomodulators and vaccines, Symposium Třeboň 1985* (M. Zaoral, Z. Havlas, O. Mikeš and Ž. Procházka, Eds), p. 123. Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 1986.
22. Zaoral M., Farkaš J., Ledvina M., Ježek J., Rotta J., Rýc M. in: *Biological Properties of Peptidoglycan* (P. H. Seidl and K. H. Schleifer, Eds), p. 379. Walter de Gruyter, Berlin 1986.

23. Farkaš J., Ledvina M., Brokeš J., Ježek J., Zajíček J., Zaoral M.: Carbohydr. Res. *163*, 63 (1987).
24. Zaoral M., Ježek J., Krchňák V., Straka R.: Collect. Czech. Chem. Commun. *45*, 1424 (1980).
25. Chaturvedi N. C., Khosla M. C., Anand N.: J. Med. Chem. *9*, 971 (1966).
26. Chapman B. E., Batley M., Redmond J. W.: Aust. J. Chem. *35*, 489 (1982).
27. McFarlane E. F., Martinic C.: Aust. J. Chem. *36*, 1087 (1983).
28. Okumura H., Azuma I., Kiso M., Hasegawa A.: Carbohydr. Res. *117*, 298 (1983).
29. Halls T. D. J., Raju M. S., Wenkert E., Zuber M., Lefrancier P., Lederer E.: Carbohydr. Res. *81*, 173 (1980).
30. LeCocq C., Lallemand J. Y.: J. Chem. Soc., Chem. Commun. *1981*, 150.
31. Reindel F., Hoppe W.: Chem. Ber. *87*, 1103 (1954).
32. Brenner M., Zimmermann J. P., Wehrmüller J., Quitt P., Hartmann A., Schneider W., Beglinger U.: Helv. Chim. Acta *40*, 1497 (1957).

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