PREPARATION OF N-ACETYLNORMURAMYL-α-AMINOBUTYRYL--D-ISOGLUTAMINYL-LYSYL-LYSYL-LYSINE*

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The title compound (*I*) has been prepared by solid-phase synthesis. In aqueous solution the compound *I* forms a mixture of α- and β-anomers.

N-Acetylnormuramyl-α-aminobutyryl-D-isoglutamine[1,**] inhibits significantly the growth of cholanthrene-induced tumors. A change in acidobasic properties may influence its behaviour in the organism (transport, elimination, metabolism, persistence, accumulation in various tissues, *etc.*) and thus also its cancerostatic properties. In order to check this assumption, we synthesized N-acetylnormuramyl-α-aminobutyryl-D-isoglutaminyl-lysyl-lysyl-lysine (*I*). The compound was prepared by solid-phase synthesis[2,3]. Merrifield resin, esterified[4] with N^{ε}-*p*-toluenesulfonyllysine, was condensed successively with two lysine moieties, isoglutamine moiety, α-aminobutyric acid moiety and finally with 1-α-O-benzyl-4,6-O-benzylidene-N-acetylnormuramic acid. The α-amino functions were protected with tert-butyloxycarbonyl group and 1,5 equivalent of 1-hydroxybenzotriazole[5] was used in the coupling reaction. The condensation of protected N-acetylnormuramic acid[6] was carried out for 3 h without repeating. The protected glycopeptide was removed from the resin by ammonolysis[7] and the protecting groups were cleaved off by sodium in liquid ammonia[8]. The free glycopeptide was desalted on a column of Amberlite IRC-50 and purified by high performance liquid chromatography. The product was eluted from the column in two fractions, corresponding to the α- and β-anomers. Equilibration of both fractions in water for 24 h afforded identical mixtures of both anomeric forms. In a delayed type hypersensitivity test, using the 24 M type protein as antigene, the immunoadjuvant activity of compound *I* was comparable with that of N-acetylnormuramyl-α-aminobutyryl-D-isoglutamine.

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** Unless stated otherwise, the optically active amino acids are of the L-configuration and the sugar component of the D-configuration. Symbols usual in the amino acid and sugar chemistry are employed.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotation was measured on a Perkin–Elmer 141 polarimeter. Purity of the products was checked by thin-layer chromatography (Kavalier, Votice, Czechoslovakia; detection by chlorination method[9,10]) and by paper electrophoresis on a Whatman No 3 MM paper at 700 V (about 50 V/cm) in aqueous acetic acid (pH 2·5); detection with ninhydrin. Samples for the amino acid analysis were hydrolyzed for 8—20 h in 6M-HCl at 110°C. Samples for the elemental analysis were dried for 8 h at 10 Pa and 100°C over P_2O_5. The buffered hydrolyzates (pH 2·2) were analyzed on an amino acid analyser Beckman-Spinco, model 120B. The colour value for normuramic acid was 3·08; the values were corrected for 25% decomposition during the hydrolysis[11]. Preparative HPLC was performed on a 25 × 2 cm column of Separon R with methanol–water–trifluoroacetic acid (5 : 95 : 0·2) as the mobile phase, the compounds being detected at 210 nm using a UVM 4 spectrophotometer (Developmental Workshops of Czechoslovak Academy of Sciences, Prague).

1-α-O-Benzyl-4,6-O-benzylidene-N-acetylnormuramyl-α-aminobutyryl-D-isoglutaminyl-
-N^{ε}-*p*-toluenesulfonyllysyl-N^{ε}-*p*-toluenesulfonyllysyl-N^{ε}-*p*-toluenesulfonyllysine Amide (*II*)

The synthesis was carried out on a chloromethylated polystyrene resin, cross-linked by 2% of divinylbenzene (Calbiochem, Los Angeles, USA), containing 0·96 mmol Cl/g. After esterification[4] with Boc-Lys(Tos) the resin contained 0·50 mmol Lys/g. The synthesis was performed with 2 g of the resin (1 mmol of Lys) on a manually controlled semiautomatic synthetizer of our own construction. The α-amino groups were protected with Boc group, N^{ε}-amino group in Lys with *p*-toluenesulfonyl group. 1-α-O-Benzyl-4,6-O-benzylidene-N-acetylnormuramic acid[6] was used as protected sugar component. The couplings were carried out according to the scheme described preciously[3] (programme No 6). The protected normuramic acid was coupled for 3 h without repetition; in all the couplings 1·5 equivalent of 1-hydroxybenzotriazole hydrate was employed. Ammonolysis[7] afforded 1·2 g (79%) of the product, m.p. 201—204°C which upon two crystallizations melted at 207—209°C. The product was chromatographically homogeneous in 1-butanol–acetic acid–water (4 : 1 : 1) and 1-butanol–acetic acid–pyridine–water (15 : 3 : 12 : 10), $[\alpha]_D^{20}$ +25·4° (*c* 0·26, dimethylformamide). For $C_{72}H_{97}N_{11}O_{19}S_3.H_2O$ (1 535) calculated: 56·33% C, 6·50% H, 10·04% N; found: 56·72% C, 6·36% H, 9·97% N. Amino acid composition: NorMur 0·95, Abu 1·02, Glu 1·02, Lys 2·40 (8 h hydrolysis); Abu 1·03, Glu 1·01, Lys 2·96 (20 h hydrolysis).

N-Acetylnormuramyl-α-aminobutyryl-D-isoglutaminyl-lysyl-lysyl-lysine Amide (*I*)

The protected glycopentapeptide *II*, m.p. 201—204°C (200 mg; 0·13 mmol) was reduced with sodium in liquid ammonia and desalted on a column of Amberlite IRC 50 according to ref.[11], affording 174 mg (76%) of lyophilizate which on electrophoresis exhibited three minor impurities (about 10%). Purification by preparative HPCL yielded 87 mg (38%) of product, $[\alpha]_D^{20}$ +21·2° (after 24 h 19·3°) (*c* 0·2; water), nitrogen content 15·12% (the lyophilizate contained 86% of compound *I*). Amino acid composition: NorMur 0·86, Abu 1·10, Glu 1·06, Lys 2·97 (8 h hydrolysis).

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