SYNTHESIS OF PEPTIDES, GLYCO DERIVATIVES AND GLYCOPEPTIDES FROM BACTERIAL CELL WALLS

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Synthesis in solution and in solid phase was used to prepare alanyl-D-isoglutamine (*VI*), alanyl-D-isoglutaminyl-N$^{\varepsilon}$-*p*-toluenesulfonyl-lysyl-D-alanine methyl ester (*XIII*), alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyl-lysyl-D-alanine methyl ester (*XIV*), alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyl-lysyl-D-alanyl-pentaglycine methyl ester and amide (*XXXI*, *XXXII*), alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyl-lysyl-D-alanyl-pentaglycyl—┐
alanyl-D-isoglutaminyl-lysyl-D-alanine methyl ester (*XXXV*), alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyl-lysyl-D-alanyl-pentaalanyl┐
alanyl-D-isoglutaminyl-lysyl-D-alanine methyl ester (*XXXVII*), N-acetylmuramyl-alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyl-lysyl-D-alanyl-pentaglycine amide (*XXXIX*), N-acetylmuramyl-alanyl-E-isoglutaminyl-N$^{\varepsilon}$-acetyl-lysyl-D-alanyl-pentaglycyl—┐
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alanyl-D-isoglutaminyl-lysyl-D-alanine methyl ester (*XLI*), and N-acetylmuramyl-alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyl-lysyl-pentaalanyl———┐
N-acetylmuramyl-alanyl-D-isoglutaminyl-lysyl-D-alanine methyl ester (*XLIII*). The tetrapeptides, nonapeptides, and tridecapeptides show a pronounced pyrogenic effect. Immunoadjuvant activity was observed not only with the glycopeptides but also with nonapeptide *XXXI*.

The supporting element of the bacterial cell wall forms a heteromerous, polydisperse biopolymer[1-6], peptidoglycan[7].* It consists of a polysaccharide and a polypeptide moiety. In the sugar component N-acetyl-D-glucosamine residues alternate with the residues of N-acetylmuramic acid.** The polypeptide consists of tetrapeptide units which are attached through their α-amino groups to the carboxyl function of N-ace-

* Synonyms: Basal structure[8], mucopeptide[9], murein[1], glycosaminopeptide[10], glycopeptide[11]. In this study the name peptidoglycan is attributed to naturally occurring components and the name glycopeptide to synthetic components of the bacterial wall.

** The symbols and abbreviations usual in the chemistry of peptides and saccharides were used. Unless stated otherwise the optically active amino are of L-configuration. To 2-acetamido-2-deoxy-3-O-[(*R*)-1-carboxyethyl]-D-glucose the name N-acetylmuramic acid (NAM-OH, residue NAM) is ascribed and benzyl-2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside is named 1-O-benzyl-4,6-O-benzylidene-N-acetylmuramic acid (BBM-OH, residue BBM). The protected muramic acid is in the derivatives and peptides present in the form of the α-anomer.

tylmuramic acid. The degree of substitution of the carboxyl groups varies with the individual microorganisms. The subunits can be loose or linked to one another (directly or by a peptide bridge). The polypeptide then cross-links the polysaccharide chains and has the character of a sequential polymer. A characteristic feature of the polypeptide is the alternation of L-amino acids with amino acids of D configuration in the peptide subunit and the presence of isopeptide bonds. There are numerous types of the polypeptide component of the peptidoglycan.

The peptidoglycan has an atractive macro- and microstructure and shows numerous biological effects. The confrontation of the various properties with chemical architecture poses the general question of the relation between function and effect in biological systems. The peptidoglycan is therefore an interesting object for studies on the relationship of chemical structure and biological effects. This problem has been attacked from two different points, *i.e.* by the isolation and investigation of peptidoglycan fragments and by the synthetic approach. A considerable number of partial data on the structure-activity relationship are available (*cf.*,*e.g.*[12-30]). In the synthetic field attention has been focused primarily on the most easily available fragments of the peptide moiety of the peptidoglycan (the subunit and higher peptides) containing a lysine residue[30,31] in position 3. The corresponding glycopeptides were prepared later[32,31]. Analogous studies on peptides[33,31] and glycopeptides[34] containing α,α′-diaminopimelic acid (L, D; L, L) in position 3 followed. The syntheses in the peptide field have arrived at the stage of a tridecapeptide[35] (lysine-containing) or a tetrapeptide[31] (containing α,α′-diaminopimelic acid); the largest glycopeptides synthetized were the lysine-containing glycohexapeptide[31,36] and the glycotetrapeptide[34] containing L, L-α, α′-diaminopimelic acid. Save for a few exceptions[37-39] the syntheses of fragments of the peptide moiety of the peptidoglycan and of all glycopeptides were effected in solution.

TABLE I
Program of Solid-Phase Syntheses

Step	Reagent	Time, min	Repetition
1	CF_3COOH/CH_2Cl_2 (30%)	5 + 15	—
2	CH_2Cl_2	3 × 1	—
3	$(C_2H_5)_3N/CH_2Cl_2$ (10%)	3 × 2	—
4	CH_2Cl_2	5 × 1	—
5	protected amino acid (3 mol) N,N′-dicyclohexylcarbodiimide (3 mol) N-hydroxybenztriazole (3 mol)	60	2 times
6	methanol	3 × 2	2 times
7	CH_2Cl_2	3 × 1	2 times

The aim of the present study has been to provide additional information on the relations between chemical structure and biological effects of glycopeptides from bacterial cell walls, above all on the role of the complexity of the glycopeptides (defined by the number and arrangement of monomer elements in the molecule) yet also on relations between the microstructure (local structure) of the monomer unit and its biological effects. The task of the synthetic part of the study was to prepare the fundamental structure unit of the peptidoglycan from *Staphylococcus aureus* (Copenhagen), *i.e.* the glycotridecapeptide containing two residues of N-acetylmuramic acid substituted by tetrapeptide subunits linked to one another by a pentaglycine bridge (Fig. 1) and its alanine analog (with pentaalanine as the connecting bridge). We planned our synthesis in a manner which would permit us to check the biological effects of the individual structural elements of the units synthetized, both in relation to their primary structure and to their increasing complexity. The scheme of the synthesis is shown in Fig. 2. From the very beginning of work we con-

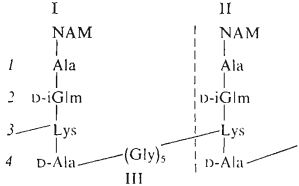

FIG. 1

Structural Unit of *Staphylococcus aureus* Copenhagen

I, II peptide subunits, III connecting bridge. Lysine in position 3 is bonded through its α-amino group to the γ-carboxyl group of D-isoglutamine in position 2. The amino group of pentaglycine is linked to the carboxyl group of D-alanine in position 4. The carboxyl group of the bridge is bonded to the ε-amino group of lysine in subunit II. The peptidoglycan monomer unit (glyconon apeptide) is marked by a dotted line.

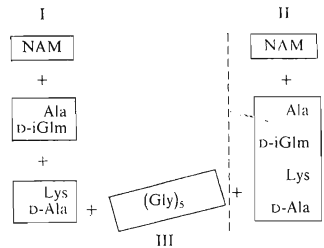

FIG. 2

Scheme of Synthesis of Peptidoglycan Fragments

The sugar component was introduced into the synthetized peptides. The highest peptide (nonapeptide) synthetized in solution is marked by a dotted line.

sidered both synthesis in solution and solid phase synthesis[40-42]. We assumed that the preparation of high molecular weight products will be effected by solid phase synthesis and that the transition from solid phase to solution will emerge from the synthetic work itself. The α-amino groups were protected mostly by benzyloxycarbonyl residues during syntheses in solution whereas tert-butyloxycarbonyl residues were employed as protecting groups in solid phase. The ε-amino group of lysine was protected by tosylation or acetylation in both cases. The removal of the protecting groups from the glycopeptides prepared was usually effected by sodium in liquid ammonia[43].

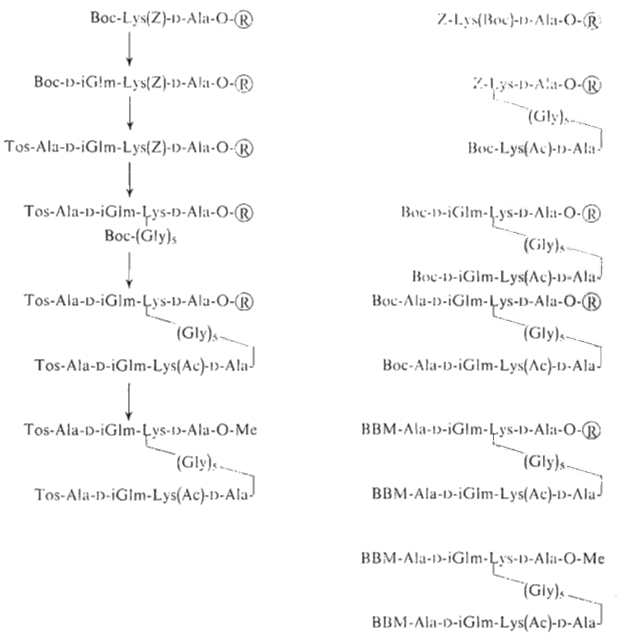

FIG. 3

Scheme of Solid-Phase Synthesis of *p*-Toluenesulfonyltridecapeptide Ester *XXXIV* and of Protected Glycotridecapeptide Ester *XL*

The tetrapeptide subunit, alanyl-D-isoglutaminyl-N$^{\varepsilon}$-*p*-toluenesulfonyllysyl-D-alanine methyl ester (*XIII*) and alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanine methyl ester (*XIV*) were obtained by fragment condensation of benzyloxycarbonyl-[44] and tert-butyloxycarbonylalanyl-D-isoglutamine[45,46], respectively, with N$^{\varepsilon}$-*p*-toluenesulfonyllysyl-D-alanine methyl ester and N$^{\varepsilon}$-acetyllysyl-D-alanine methyl ester, respectively, by the carbodiimide method or by the method of mixed anhydrides. Benzyloxycarbonylalanyl- and tert-butyloxycarbonylalanyl-D-isoglutamine were synthetized by condensation of the accordingly protected alanine[47,48] *p*-nitrophenyl ester with isoglutamine[49]. The benzyloxycarbonyl residue and the tert-butyloxycarbonyl residue were removed in a solution of hydrogen bromide in acetic acid[50] and in a solution of trifluoroacetic acid in dichloromethane[51] respectively. The obtained tetrapeptide ester salts afforded the corresponding free tetrapeptide esters[43] after deionization (on ion exchangers). A turning point on the way from synthesis in solution to solid phase synthesis became the preparation of nonapeptides *XXVIII*–*XXX*. We obtained information from orienting experiments showing that these peptides can be prepared faster, easier, and in a better purity by the Merrifield method rather than in the classical manner. Even the synthesis of intermediary pentaglycine in solid phase was far easier and faster and afforded a much higher yield of a considerably cleaner product than the synthetic procedures in solution (the method of mixed anhydrides, the carbodiimide method, the method of activated esters, *i.e.* *p*-nitrophenyl esters, stepwise synthesis form the C-terminus, fragment condensation). We did not examine this problem in detail yet it is likely that most of the complications observed with syntheses in solution are connected with the reactivity of the acylamino group of glycine (*e.g.*[52] and the references quoted in this paper) and with the troublesome removal of byproducts. The latter can easily be removed during solid-phase synthesis (they remain in solution). Nonapeptides, higher peptides, and the corresponding glycopeptides were prepared by solid phase synthesis. The synthesis of the tridecapeptides is shown in Fig. 3. We followed a scheme developed earlier[53] based on repeated condensation and repeated deblocking. We bonded benzyloxycarbonyl-N$^{\varepsilon}$-tert-butyloxycarbonyllysine to D-alanine esterified to the resin, split off the tert-butyloxycarbonyl residue in a solution of trifluoroacetic acid in dichloromethane, and condensed 5 glycine residues, D-alanine, and benzyloxycarbonyl-N$^{\varepsilon}$-acetyllysine to the ε-amino group of lysine. The benzyloxycarbonyl residues were cleaved off by treatment with hydrogen bromide in acetic acid and D-isoglutamine and alanine were condensed to the α-amino groups of both lysines. The protected tridecapeptide was removed from the resin by reesterification[54]. When the crude reaction mixture was subjected to thin-layer chromatography, it showed the presence of an intensive spot of the main product and of two minor impurity spots (about c. 10–20% of the main product). After a cursory purification the tert-butyloxycarbonyl residues were removed in trifluoroacetic acid, the product was deionized and purified by continuous free-flow electrophoresis[55] and gel filtration. The product

obtained behaved as a homogeneous substance when subjected to thin-layer chromatography and paper electrophoresis. Its amino acid analysis[56] and elemental analysis corresponded to the expected composition. Tridecapeptide *XXXVI* containing the pentaalanine bridge was prepared in an analogous manner.

The sugar component, N-acetylmuramic acid (NAM-OH) was introduced into the peptides in the form of 1-O-benzyl-4,6-O-benzylidene-N-acetylmuramic acid[57] (BBM-OH). The latter was prepared essentially according to paper[58] with a minor modification. The acylation of the peptides by the protected sugar component was effected by the method of mixed anhydrides; *XXVI* was prepared by the method of activated esters. An intermediary product of the preparation of *XXII* was BBM--alanine methyl ester (*XIX*) which we obtained by the condensation of BBM-OH with alanine methyl ester. The ammonolysis of *XIX* afforded the amide from which we removed the protecting groups and obtained *XXII*. Amide *XXIII* was prepared by the same procedure. Diamide *XXV* was synthetized in two different manners. In the first case we condensed BBM-OH with alanyl-D-glutamine methyl ester (which had been obtained by esterification of benzyloxycarbonylalanyl-D-isoglutamine[44] by diazomethane, removal of the protecting group in a solution of hydrogen bromide in acetic acid and deionization) to *XXIV*. Product *XXIV* was subjected to ammonolysis, the protecting groups were removed and *XXV* was obtained. In the second case benzyloxycarbonylalanine[59] was condensed with D-glutamic acid diamide, the protected dipeptide diamide was deblocked and deionized as in the preceding case and the free dipeptide diamide was condensed with BBM-OH. *XXV* was prepared from the condensation product by the procedure used in the first synthesis. We introduced the sugar component into alanyl-D-isoglutaminyl-N^{ε}-acetyllysyl--D-alanine (prepared from benzyloxycarbonylalanyl-D-isoglutaminyl-N^{ε}-acetyllysyl--D-alanine (*XII*) by decarbobenzoxylation in a solution of hydrogen bromide in acetic acid and deionization) *via* the N-hydroxysuccinimide ester[46]. The protected glyconopapeptide amide *XXXVIII* was synthetized by the same approach. The higher glycopeptides were prepared – like the higher peptides – also by solid phase synthesis. Before we started these operations we had compared the Merrifield method with the classical procedure since there is very little experience with solid phase synthesis of glycopeptides. We chose for this purpose BBM-alanyl-D-isoglutaminyl-N^{ε}-acetyllysyl-D-alanine amide (*XXVI*) whose synthesis in solution proceeds smoothly. The solid phase synthesis of *XXVI* proceeded readily and without any complications. The product obtained had the same properties as the compound synthetized in solution and the total yield was much higher. Before the solid phase synthesis of glycotridecapeptides *XLI* and *XLIII* we had prepared the corresponding protected tridecapeptides (with amino-terminal α-groups protected by a tert-butyloxycarbonyl residue), removed the tert-butyloxycarbonyl residues by treatment with trifluoroacetic acid in dichloromethane and after neutralization carried out a single step condensation with BBM-OH. The protected glycotridecapeptides were split off from the

resin by reesterification, the protecting groups were removed by sodium in liquid ammonia[43], and the products obtained were purified by gel filtration.

The prepared peptides and glycopeptides were tested for pyrogenic and immunoadjuvant effects.* We may summarize that pyrogenic effects were observed with all peptides higher than dipeptides and with all glycopeptides. The immunoadjuvant effects were observed as expected, with the glycopeptides. Contrary to our expectation, these effects were also observed with nonapeptide *XXXI* administered in a water-in-oil emulsion. According to this observation and contrary to the present views, the presence of the sugar component in the peptide seems not to be essential for the peptide to produce the immunoadjuvant effect.

EXPERIMENTAL

The melting points were determined on a Kofler block and are not corrected. The optical activity was measured in Perkin–Elmer type 141 polarimeter, the infrared spectra in Zeiss (Jena, GDR) model UR-20 spectrophotometer. The preparative electrophoreses were carried out in a modified apparatus of Hannig[55], manufactured in the workshops of this Institute. The purity of the intermediary products was checked by chromatography on Silufol layer sheets (Kavalier, Votice) and on DC-Fertigplatten Kieselgel 60 (Merck) in the following systems: 1-butanol–acetic acid–water (4 : 1 : 1) (S_1); 1-butanol–acetic acid–water (4 : 1 : 5) (S_2); 1-butanol–acetic acid–pyridine–water (15 : 3 : 12 : 10) (S_3), (60 : 4 : 45 : 30) (S_4); ethyl acetate–acetic acid–pyridine–water (5 : 1 : 5 : 3) (S_5), (5 : 5 : 5 : 3) (S_6); 1-butanol–ethyl acetate–acetic acid–water (5 : 5 : 1 : 3) (S_7); tert-butanol–dimethylformamide–acetic acid–water (2 : 1 : 3 : 2) (S_8); 1-butanol–tert-butanol–acetic acid–water (2 : 2 : 1 : 1) (S_9); methanol–chloroform (15 : 85) (S_{10}). The detection was effected by ninhydrin or chlorination[60,61]. The samples for amino acid analysis were hydrolyzed 8—48 h in 6M-HCl at 110°C. The hydrolysates were dissolved in the buffer at pH 2·2 and analyzed in Beckman-Spinco Model 120B Amino Acid Analyzer. When hydrolysates of glycopeptides containing muramic and glutamic acid were analyzed, the pH of the first buffer was decreased to pH 3 in order to obtain a good resolution of these two acids. A color value of 3·08 was used for muramic acid. The values obtained were corrected for 25% decomposition of muramic acid during the hydrolysis; this value was derived from the investigation of the kinetics of decomposition of free and protected muramyl peptides. Unless stated otherwise the samples for elemental analysis were dried 24 h at 10 Pa and 50—120°C over phosphorus pentoxide.

The solid phase syntheses were carried out on chloromethylated polystyrene resin cross-linked by 2% of divinylbenzene (Calbiochem, Los Angeles, U.S.A., chlorine content 5·9%), esterified with Boc-Gly-OH (glycine content 0·80 mmol/g resin) and Boc-D-Ala-OH (alanine content 0·602 mmol/g resin) in a semiautomatic synthetizer of own construction. The Boc residue was used as the mobile protecting group; in the case of differential protection it was used in combination with the Z residue. The N^{ε}-amino groups of lysine were protected by acetylation. The program of the syntheses is shown in Table I. After the first amino acid had been coupled, the unreacted amino groups were blocked by 30 min acetylation in the mixture dichloromethane–acetic anhydride–triethylamine (25 : 3 : 1). All condensations were carried out with a three-fold molar excess (with respect to the first amino acid bonded to the resin) of the protected amino acid,

* A detailed pharmacological study of the products synthetized will be presented in a separate paper.

dicyclohexylcarbodiimide or, alternatively, N-hydroxybenztriazole[62] (during condensation of Boc-D-iGlm and in the subsequent coupling steps). When protected muramic acid was being attached a single-step 3 h coupling was used. The benzyloxycarbonyl protecting group was removed by repeated treatment (5 and 15 min) with a 10% solution of hydrogen bromide in glacial acetic acid. The resin was moreover washed 5 times with methanol after the deblocking.

Benzyloxycarbonyl-N$^{\varepsilon}$-*p*-toluenesulfonyllysyl-D-alanine Methyl Ester (*I*)

This product was prepared by the condensation of benzyloxycarbonyl-N$^{\varepsilon}$-*p*-toluenesulfonyllysine with D-alanine methyl ester according to paper[63]. M.p. 142—143°C, $[\alpha]_D^{25}$ +7·2° (*c* 1·0, methanol), —1·9° (*c* 0·5, pyridine). Recorded data[63,64]: m.p. 143—145°C, $[\alpha]_D^{20}$ +7·0° (*c* 1·0, methanol) 141—142°C, $[\alpha]_D^{30}$ +0·6° (*c* 5·0, pyridine).

Benzyloxycarbonyl-N$^{\varepsilon}$-acetyllysyl-D-alanine Methyl Ester (*II*)

To a solution of 41·0 g (0·127 mol) of benzyloxycarbonyl-N$^{\varepsilon}$-acetyllysine, 16·8 ml (0·127 mol) of N-ethylpiperidine, and 10·2 ml (0·127 mol) of pyridine in 350 ml chloroform, 15·7 ml (0·127 mmol) of pivaloyl chloride was added at —10°C. The mixture was set aside for 5 min at 0°C, than cooled down to —10°C and a solution of 17·6 g (0·127 mol) of D-alanine methyl ester hydrochloride and 16·8 ml (0·127 mol) of N-ethylpiperidine in 230 ml of chloroform were added with stirring. The mixture was set aside overnight at 0°C. The solvents were distilled off, the dry residue was triturated with ethyl acetate and dried. Yield 45·6 g (88%), m.p. 167—169°C. Recrystallization from water afforded 38·0 g (73·5%) of a product melting at 168—169°C, $[\alpha]_D^{20}$ +10·8° (*c* 0·5, 50% acetic acid), homogeneous when subjected to TLC-M in systems S_2 and S_3. For $C_{20}H_{29}N_3O_6$ (407·5) calculated: 58·95% C, 7·17% H, 10·31% N; found: 58·65% C, 7·24% H, 10·38% N.

Benzyloxycarbonylalanyl-D-isoglutamine (*III*)

A mixture of benzyloxycarbonylalanine *p*-nitrophenyl ester[47] (20·65 g, 0·06 mol), 13·6 g (0·06 mol) of D-isoglutamine hydrobromide, 60 ml of 2M-NaOH (0·12 mol), and 60 ml of dimethylformamide was stirred overnight. The mixture was diluted with 160 ml of water and nitrophenol was extracted five times with ether. The aqueous solution was acidified by hydrochloric acid to pH 2—3, the product which had separated was extracted with ethyl acetate, the extracts were dried by sodium sulfate and evaporated under reduced pressure. Yield 20·3 g (96%), m.p. 154—155°C. Recrystallization from the mixture ethyl acetate–ethanol–diisopropyl ether afforded 15·8 g (75%) of a product of m.p. 155·5—156°C. The product can be also crystallized from the mixture ethanol–ethyl acetate–petroleum ether. The yield is lower in this case. $[\alpha]_D^{23}$ +1·7° (*c* 1·0, acetic acid), $[\alpha]_D^{25}$ +10·2° (*c* 2, dimethylformamide). For $C_{16}H_{21}N_3O_6$ (351·4) calculated 54·69% C, 6·02% H, 11·96% N; found: 54·72% C, 6·11% H, 12·02% N. The product shows a tendency to crystallize as a dimethylformamide solvate. M.p. 95—97°C $[\alpha]_D^{21}$ +9·1° (*c* 0·3, dimethylformamide). For $C_{16}H_{21}N_3O_6.(CH_3)_2N.CHO$ (424·5) calculated: 54·76% C, 6·65% H, 13·20% N; found: 53·89% C, 6·52% H, 13·33% N. The unsolvated product can be converted into the solvate by recrystallization from aqueous dimethylformamide. The composition of this product and its m.p. are the same as those of the solvate formed spontaneously. The melting points of both products are without depression. Both the unsolvated product and the dimethylformamide solvate are homogeneous when tested by thin-layer chromatography (TLC-S) in systems S_2 and S_3. Recorded data[44,36]: m.p. 128—129°C, $[\alpha]_D^{30}$ +8·7° (*c* 2·3, dimethylformamide); m.p. 146—148°C, $[\alpha]_D^{25}$ +1·4° (*c* 1·0, acetic acid).

Tert-butyloxycarbonylalanyl-D-isoglutamine (*IV*)

This compound was prepared by the above procedure except that dioxane was used as a solvent, the pH of the aqueous layer after the extraction of *p*-nitrophenol was adjusted to 4 (HCl), and the product was crystallized from wet ethyl acetate. From 36·1 g (0·113 mol) of tert-butyloxycarbonyl-alanine *p*-nitrophenyl ester[48] and 26·5 g (0·116 mol) of D-isoglutamine hydrobromide, using 115 ml of dioxane and 117 ml of 2M-NaOH, 27·4 g (58%) was obtained of a product of m.p. 88—93°C. This product, homogeneous when tested by thin-layer chromatography (TLC-S) in systems S_1 and S_8 was used for the subsequent synthesis. Additional crystallization from wet ethyl acetate afforded a product of m.p. 98—100°C, $[\alpha]_D^{25}$ —8·5° (*c* 1·0 methanol). The elemental analysis of the product corresponded to the solvate containing 1 mol of ethyl acetate. Recorded data[45,46]: m.p. 99·5—101°C, $[\alpha]_{28}^{D}$ —8·2° (*c* 1·0, methanol); 94—98°C, $[\alpha]_D^{25}$ —9·0° (*c* 1·0, methanol).

Tert-butyloxycarbonylalanyl-D-isoglutamine Benzyl Ester (*V*)

The procedure described in paper[65] was employed. The ethyl acetate solvate of tert-butyloxy-carbonyl-D-isoglutamine (17·1 g, 42 mmol) was refluxed 2 h with 5·55 ml (46·5 mmol) of benzyl bromide and 8·45 ml (42 mmol) of dicyclohexylamine in 105 ml of dimethylformamide. The dicyclohexylamine hydrobromide which had separated was filtered off and the filtrates were evaporated under reduced pressure. The dry residue was triturated with 100 ml of petroleum ether, the solvent was decanted off and its residues evaporated *in vacuo*. A solution (50 ml) of sodium bicarbonate was added to the dry residue. The product obtained after short trituration was filtered off, washed with two 20 ml portions of a solution of sodium bicarbonate on the filter, then with three 20 ml portions of water, and dried. Yield 14·6 g (85%), m.p. 132—138°C. Re-crystallization from ethyl acetate–petroleum ether afforded 13·85 g (78%) of a product of m. p. 138—139°C, $[\alpha]_D^{24}$ —8·8° (*c* 0·65, methanol). The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-S) in systems S_1 and S_3. Recorded data[45,46]: m.p. 137·5—138·5°C, $[\alpha]_D^{28}$ —8·1° (*c* 1·0, methanol); 140°C, $[\alpha]_D^{25}$ —9° (*c* 1·0, methanol).

Alanyl-D-isoglutamine (*VI*)

Benzyloxycarbonylalanyl-D-isoglutamine (1·5 g, 4·3 mmol) was hydrogenated in 250 ml of methanol in the presence of 0·5 g of Pd/C catalyst (20%). After the end of hydrogenation the catalyst was filtered off, the filtrates were taken to dryness under reduced pressure, the dry residue was freed of the organic solvent by repeated evaporation with water, dissolved in 20 ml of water, and lyophilized. The yield was 0·72 g (77%) of a product homogeneous when subjected to thin-layer chromatography (TLC-S) in system S_9. M.p. 128—130°C, $[\alpha]_D^{22}$ +23·3° (*c* 0·9, water). The elemental analysis after 24 h drying at 100 Pa and room temperature over phosphorus pentoxide and sodium hydroxide still corresponded to a partly solvated product. For $C_8H_{15}N_3O_4.1/2\ CH_3OH.1/4\ H_2O$ (237·7) calculated: 42·94% C, 7·42% H, 17·67% N; found: 43·05% C, 7·02% H, 17·98% N. Recorded data[46] for a hydrate containing 1/4 H_2O, m.p. 83°C and $[\alpha]_D^{28}$ +14·4° (*c* 0·96, water).

Benzyloxycarbonylglutamic Acid Diamide (*VII*)

Benzyloxycarbonylglutamic acid dimethyl ester (prepared from 5·6 g (0·02 mol) of benzyl-oxycarbonyl-D-glutamic acid by treatment with diazomethane) was dissolved in 200 ml of a 20% solution of ammonia in methanol. The mixture was allowed to stand in a pressure vessel 3 days at room temperature. The solvent was distilled off under reduced pressure. The dry residue (5·5 g,

i.e. 100%, m.p. 185—190°C) was recrystallized from methanol. Yield 5·0 g (90%), m.p. 198 to 192°C. This product was used in the subsequent experiments. Additional crystallization from methanol afforded a product of constant melting point 194—196°C, $[\alpha]_D^{20}$ —8·8° (*c* 0·4, dimethylformamide). Recorded data[66] for the L-isomer: m.p. 194—196°C.

Benzyloxycarbonylalanyl-D-glutamic Acid Diamide (*VIII*)

Benzyloxycarbonyl-D-glutamic acid diamide (3·5 g, 12·5 mmol) was decarbobenzoxylated by 5 min heating to 60°C in 30 ml of 15% hydrogen bromide solution in acetic acid. D-Glutamic acid diamide hydrobromide was precipitated from the reaction mixture by the addition of 500 ml of ether. The hydrobromide was filtered off and recrystallized from methanol–ether. Yield 2·75 g (97·5%), m.p. 178—181°C. The hydrobromide was suspended in 20 ml of chloroform, treated with an equivalent quantity (1·34 ml, 12·2 mmol) of N-methylmorpholine, and the suspension added at —10°C to the mixed anhydride prepared from 2·72 g (12·2 mmol) of benzyloxycarbonylalanine, 1·34 ml (12·2 mmol) of N-methylmorpholine, and 1·17 ml (12·2 mmol) of ethyl chloroformate in 10 ml of dimethylformamide at 0°C. The mixture was set aside for 30 min at 0°C and then overnight at room temperature. The solvents were evaporated under reduced pressure, the dry residue was repeatedly (always 3 times) triturated with water, dilute hydrochloric acid (3%), a saturated solution of sodium bicarbonate, water, and dried. Yield 2·4 g (57%), m.p. 204—208°C. Recrystallization from a mixture of ethanol, water and ether afforded 2·16 g (51%) of a product of m.p. 210—212°C, $[\alpha]_D^{20}$ 0° (*c* 0·3, dimethylformamide), $[\alpha]_{365}^{20}$ +13·14° (*c* 0·2, dimethylformamide). For $C_{16}H_{22}N_4O_5$ (348·4) calculated: 55·16% C, 6·36% H, 16·08% N; found: 55·26% C, 6·28% H, 15·92% N.

Tert-butyloxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-D-alanine Methyl Ester (*IX*)

A solution of 10·3 g (0·1 mol) of D-alanine methyl ester in 20 ml of chloroform was added at —10°C to a solution of the mixed anhydride prepared from 38·0 g (0·1 mol) of tert-butyloxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyllysine[67], 16·1 ml (0·2 mol) of pyridine, and 11·9 ml) (0·1 mol) of pivaloyl chloride in 120 ml of chloroform at —3°C. The mixture was allowed to stand 30 min at 0°C and overnight at room temperature. The solvent was distilled off under reduced pressure, the dry residue was triturated with water and extracted with ethyl acetate. The ethyl acetate extracts were repeatedly (always 3 times) extracted with dilute hydrochloric acid (*c.* 3%), a saturated solution of sodium bicarbonate, and water. The extracts were dried by solid sodium sulfate and evaporated under reduced pressure. The dry residue crystallized when triturated with petroleum ether. Recrystallization from ethyl acetate–light petroleum afforded 32·6 g; (70%) of a product of m.p. 81—83°C. Additional crystallization from the same solvents gave a product of m.p. 82—83°C, $[\alpha]_D^{25}$ +6·2° (*c* 1·5, methanol). The product was pure when subjected to thin-layer chromatography (TLC-S) in system S_{10}. For $C_{23}H_{35}N_3O_7$ (465.5) calculated: 59·34% C, 7·58% H, 9·03% N; found: 59·05% C, 7·74% H, 9·17% N.

Benzyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-*p*-toluenesulfonyllysyl-D-alanine Methyl Ester (X)

Methyl ester *I* (4·4 g, 8·5 mmol) was heated in 17 ml of a 15% solution of hydrogen bromide in acetic acid 5 min at 50°C. The dipeptide ester hydrobromide was precipitated from the reaction mixture by the addition of excess ether and subsequently powderized by repeated trituration with dry ether. The product was filtered off and dried *in vacuo* over phosphorus pentoxide and potassium hydroxide. It was then suspended in 20 ml of chloroform and 80 ml of a solution

of ammonia in chloroform and *c.* 1 g of active charcoal were added to the suspension. The mixture was filtered after several minutes and the filtrates were evaporated under reduced pressure. The dry residue was evaporated twice with chloroform (3·1 g) and dissolved in 20 ml of dimethylformamide. The solution was cooled down to 0°C and a solution of 2·46 g (7 mmol) of benzyloxycarbonylalanyl-D-isoglutamine, 1·06 g of N-hydroxybenztriazole in 25 ml of dimethylformamide, and a solution of 1·6 g (7·7 mmol) of dicyclohexylcarbodiimide in 5 ml of dimethylformamide were added. The mixture was maintained 1 h at 0°C and then set aside overnight at room temperature. Dicyclohexyl urea which had separated was filtered off, the filtrates were evaporated under reduced pressure, the dry residue triturated with 5 ml of dimethylformamide, and dicyclohexyl urea which had precipitated was again filtered off. The filtrates were treated with a saturated solution of sodium bicarbonate. The product which had separated crystallized during the trituration. It was filtered off after 2 h of standing in the refrigerator (0°C) and repeatedly (3 times) triturated with a solution of sodium bicarbonate, water, 3% hydrochloric acid and water. It was filtered off and dried. Yield 3·7 g (73%), m.p. 155—160°C. Double crystallization from aqueous ethanol afforded 2·9 g (58%) of a product of m.p. 163—165°C. For $C_{33}H_{46}N_6O_{10}S.1/2\ H_2O$ (727·8) calculated: 54·50% C, 6·44% H, 11·55% N, 4·41% S; found: 54·50% C, 6·57% H, 11·70% N, 4·47% S. Amino acid analysis: Ala 2·04, Glu 1·04, Lys 0·92.

Benzyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanine Methyl Ester (*XI*)

Methyl ester *II* (4·3 g, 10·5 mmol) was heated in 35 ml of a 15% solution of hydrogen bromide in acetic acid 5 min at 60°C. The dipeptide ester hydrobromide was precipitated from the mixture by an excess of ether and was washed by repeated trituration with ether. The solvent was decanted off and its residues removed *in vacuo*. The dry residue was dissolved in 20 ml of methanol. The solution was treated with stirring with a suspension of Ostion AT in methanol until the reaction for bromide ions became negative. The ion exchange resin was filtered off and the filtrates were evaporated under reduced pressure. The dry residue (2·73 g) was dissolved in 30 ml of dimethylformamide, cooled down to —10°C, and added to a solution of mixed anhydride prepared from 3·51 g (10 mmol) of benzyloxycarbonylalanyl-D-isoglutamine, 1·1 ml (10 mmol) of N-methylmorpholine, and 0·96 ml (10 mmol) of ethyl chloroformate in 35 ml of dimethylformamide at 0°C. The mixture was allowed to warm up to room temperature, then set aside for 12 h and the solvents evaporated under reduced pressure. The dry residue was repeatedly (3 times) triturated with 3% hydrochloric acid, water, a saturated solution of sodium bicarbonate, and water; it was then filtered off and dried. Yield 4·2 g (69%), m.p. 249—251°C. Recrystallization from ethanol–water afforded 3·65 g (60%) of a product of m.p. 253—255°C. For $C_{28}H_{42}N_6O_9$ (606·7) calculated: 55·43% C, 6·98% H, 13·85% N; found: 55·40% C, 6·82% H, 14·00% N. Amino acid composition: Ala 1·98; Glu 1·03; Lys 1·00.

Benzyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanine Amide (*XII*)

A suspension of benzyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanine methyl ester (*XI*, 1·4 g, 2·3 mmol) in 250 ml of a 20% solution of ammonia in methanol was allowed to stand in a pressurized vessel 7 days at room temperature. The solvent was evaporated off under reduced pressure. The residue was dried (yield 1·36 g, 100%), m.p. 240—245°C. Recrystallization from ethanol–water afforded 1·12 g (82%) of a product of m.p. 244—246°C, $[\alpha]_{280,365,436}^{20}$ 0° (*c* 0·8, dimethylformamide). For $C_{27}H_{41}N_7O_8$ (591·7) calculated: 54·81% C, 6·98% H, 16·57% N; found: 54·54% C, 6·74% H, 16·57% N. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-S) in systems S_1 and S_3.

Alanyl-D-isoglutaminyl-N$^{\varepsilon}$-*p*-toluenesulfonyllysyl-D-alanine Methyl Ester (*XIII*)

Methyl ester *X* (0·73 g, 1 mmol) was heated in 5 ml of a 15% solution of hydrogen bromide in acetic acid 5 min at 60°C. The tetrapeptide ester hydrobromide obtained was dissolved in 5 ml of methanol, 20 ml of a suspension of Ostion AT in methanol was added and the mixture was stirred until the reaction for bromide ions became negative. The ion exchange resin was filtered off and the filtrates were evaporated under reduced pressure. The residue crystallized when rubbed under ethyl acetate. The product was filtered off and dried. Recrystallization from ethanol–ethyl acetate afforded 0·33 g (57%) of a product of m.p. 174—176°C, $[\alpha]_D^{24}$ +1·5° (*c* 1·0, methanol). For $C_{25}H_{40}N_6O_8S$ (584·7) calculated: 51·36% C, 6·89% H, 14·37% N, 5·48% S; found: 51·11% C, 7·06% H, 14·19% N, 5·27% S. Amino acid analysis: Ala 2·10, Glu 1·06, Lys 0·88. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-S) in systems S_2 and S_3 and to paper electrophoresis (Whatman 3MM, 5% acetic acid; pyridine acetate buffer at pH 5·7).

Alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanine Methyl Ester (*XIV*)

Methyl ester *XI* (1·21 g, 2 mmol) was decarbobenzoxylated in 40 ml of *c.* 15% solution of hydrogen bromide in acetic acid and deionized (Ostion AT) by the procedure described in the preceding experiment. The yield was 0·74 g (78%) of a crude product of m.p. 205—210°C. Recrystallization from a mixture of methanol and ether increased the m.p. to 210—212°C, $[\alpha]_D^{20}$ +14·1° (*c* 0·4, water). For $C_{20}H_{36}N_6O_7.1/2\,H_2O$ (481·6) calculated: 49·88% C, 7·74% H, 17·45% N; found: 49·77% C, 7·57% H, 16·97% N. Amino acid analysis: Ala 1·99, Glu 1·00, Lys 1·02. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-M, S_1; TLC-S, S_3) and paper electrophoresis (Whatman 3MM, pyridine acetate buffer, pH 5·7).

Benzyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-*p*-toluenesulfonyllysyl-D-alanine (*XV*)

Methyl ester *X* (7·19 g, 10 mmol) was suspended in a mixture of 30 ml of 65% ethanol and 2·5 ml of 4M-NaOH. The mixture was stirred 1 h, the undissolved compound was filtered off, the filtrates were acidified to pH 4—4·5 by hydrochloric acid and concentrated under reduced pressure. The product which had separated was filtered off, dried, and recrystallized twice from a mixture of methanol and diisopropyl ether. Yield 5·8 g (82%) of a product of m.p. 196—198°C, $[\alpha]_D^{22}$ +0·8° (*c* 2·0, methanol). For $C_{32}H_{44}N_6O_{10}S$ (704·8) calculated: 54·53% C, 6·29% H, 11·92% N, 4·55% S; found: 54·59% C, 6·52% H, 11·72% N, 4·31% S. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-S, S_1, S_9).

Tert-butyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-D-alanine Methyl Ester (*XVI*)

Methyl ester *IX* (5·5 g, 11·8 mmol) was set aside for 20 min at room temperature with 12·5 ml of a 80% solution of trifluoroacetic acid in dichloromethane. The solution was evaporated under reduced pressure and dissolved in 30 ml of methanol. This solution was treated with stirring with a suspension of Ostion AT in methanol until the reaction for trifluoroacetate ions became negative (*c.* 130 ml). The ion exchange resin was filtered off and the filtrates were evaporated under reduced pressure. The dry residue was dissolved in 15 ml of dimethylformamide and added to a solution of 4·8 g (11·8 mmol) of the ethyl acetate solvate of tert-butyloxycarbonyl-D-isoglutamine and 1·6 g (11·8 mmol) of N-hydroxybenztriazol in 50 ml of dimethylformamide. The solution was cooled down to 0°C and treated with a solution of 2·7 g (13 mmol) of dicyclohexylcarbodiimide in 10 ml of dimethylformamide. The mixture was maintained 1 h at 0°C, then al-

lowed to warm up to room temperature, and set aside overnight. Dicyclohexylurea which had separated was filtered off, the filtrates were evaporated under reduced pressure, the residue dissolved in 15 ml of dimethylformamide, dicyclohexylurea filtered off, and the filtrates evaporated under reduced pressure. The residue crystallized when triturated with a saturated solution of sodium bicarbonate. It was filtered off and repeatedly (3 times) triturated with a solution of sodium bicarbonate, water, 3% hydrochloric acid, and water, then filtered off and dried. Yield 6·3 g (80%), m.p. 196—203°C (decomposition). Recrystallization from methanol–water afforded 5·6 g (72%) of a product of m.p. 202—204°C, $[\alpha]_D^{24}$ —10·3° (*c* 1·0, acetic acid). For $C_{31}H_{48}N_6O_{10}$ (664·8) calculated: 56·0% C, 7·28% H, 12·64% N; found: 55·79% C, 7·41% H, 12·53% N. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-S) in systems S_2 and S_{10}.

Tert-butyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-D-alanine (*XVII*)

Methyl ester *XVI* (3·3 g, 5 mmol) was stirred 2 h at room temperature with a mixture of 2·5 ml of 2M-NaOH and 5 ml of ethanol. A small portion of the undissolved product was filtered off, the filtrates were diluted with 10 ml of water, and acidified to pH 3·5 by 0·5M-HCl. The oily product which had separated crystallized after several hours of standing at 0°C. It was filtered off, washed on the filter with a small volume of water, dried, and dissolved in 10 ml of dimethylformamide; 2 ml of dicyclohexylamine were added to the solution. After several minutes of standing the dicyclohexylammonium salt was precipitated from the mixture by an excess of ether. The dicyclohexylammonium salt was crystallized from methanol–ether (with the admixture of a small amount of dicyclohexylamine) until a constant-melting product (164—166°C) was obtained. The dicyclohexylammonium salt was decomposed by adding an excess of 1M-NaOH, dicyclohexylamine was extracted with ethyl acetate, and the aqueous layer was acidified to pH 3·5 by 3% hydrochloric acid. The crystalline product which had separated was filtered off and recrystallized from methanol–water. The yield was 1·56 g (48%) of a product of m.p. 182—183°C (decomposition), $[\alpha]_D^{25}$ —7·6° (*c* 1·0, acetic acid). For $C_{30}H_{46}N_6O_{10}$ (650·7) calculated: 55·37% C, 7·12% H, 12·91% N; found: 55·41% C, 7·19% H, 13·03% N. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-S) in systems S_2 and S_{10}.

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramic Acid (*XVIII*)

To a solution of 12·0 g (30 mmol) of benzyl-2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside in 600 ml of tetrahydrofuran was added 6·0 g of sodium–potassium alloy (1 : 3) and the mixture was refluxed (under a drying tube) with stirring (by a vibrator) for 1 h. Afterwards it was cooled down to 10—15°C and treated dropwise 15 min with 27·2 ml (210 mmol) of D,L-2-bromopropionic acid ethyl ester in 100 ml of tetrahydrofuran keeping the temperature below 20°C. Finely ground sodium iodide (18 g, 120 mmol) was added and the mixture was refluxed 2 h with stirring. The solvent was distilled off under reduced pressure, a mixture of 600 ml of methanol and 600 ml of 0·5M-NaOH was added to the dry residue, and the mixture refluxed for 1 h. The solution was concentrated to *c.* 1/3 its volume under reduced pressure, and water (*c.* 4 l) was added. A small quantity of the compound which had not dissolved was filtered off and the filtrates were acidified to pH 3—4 by hydrochloric acid with cooling. The product which had separated was filtered off, washed with water on the filter (until the reaction of the filtrate for Cl^--ions was negative), and dried at room temperature over phosphorus pentoxide *in vacuo*. Yield 13·5 g (96%), m.p. 234—240°C. The dry product was pulverized, suspended in 300 ml of methanol, the suspension heated to boil and filtered hot. Water (1500 ml) was added to the filtrates and the mixture was allowed to stand 2 h in an ice bath. The product

which had separated was filtered off and dried. Yield 12·5 g (88·5%), m.p. 240—244°C, $[\alpha]_D^{22}$ +100·1° (*c* 0·5, methanol). This product was used in subsequent syntheses. Additional crystallization from methanol afforded a product of m.p. 242—245°C, $[\alpha]_D^{20}$ +113° (*c* 0·32, methanol). Recorded data[57,58]: m.p. 237—239°C, $[\alpha]_D^{18}$ +115° (*c* 1·3, methanol); m.p. 236—238°C, $[\alpha]_D^{20}$ +102° (*c* 0·5, methanol).

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramylanine Methyl Ester (*XIX*)

To a solution of 2·36 g (5 mmol) of acid *XVIII* and 0·55 ml (5 mmol) of N-ethylmorpholine in 20 ml of dimethylformamide, 0·475 ml (5 mmol) of ethyl chloroformate was added with stirring at 0°C. The mixture was maintained 5 min at 0°C, then cooled down to —10°C and 0·7 g (5 mmol) of alanine methyl ester hydrochloride and 0·55 ml of N-methylmorpholine in 15 ml of dimethyl formamide were added. The mixture was maintained 10 min at —10°C and then allowed to warm up to room temperature. Water (100 ml) was added after 1 h, the product which had separated was filtered off and triturated 3 times with 1% hydrochloric acid, water, a saturated solution of sodium bicarbonate, and water. Subsequently it was filtered off and dried. Yield 2·55 g (92%), m.p. 223—227°C. Recrystallization from dimethylformamide–water afforded 2·33 g (84%) of a product of m.p. 226—228°C, $[\alpha]_D^{20}$ +82·9° (*c* 0·52, dimethylformamide). For $C_{29}H_{36}N_2O_9$ (556·6) calculated: 62·58% C, 6·52% H, 5·03% N; found: 62·80% C, 6·75% H, 5·33% N. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-M) in systems S_2 and S_3.

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-alanine (*XX*)

Methyl ester *XIX* (0·556 g, 1 mmol) was dissolved in 20 ml of dimethylformamide, a solution of 0·04 g (1 mmol) of NaOH in 2 ml of water was added and the mixture was stirred 1 h at room temperature. It was subsequently diluted with 50 ml of water, a small quantity of undissolved product was filtered off, and the filtrates acidified by acetic acid to pH 4. The product which had separated was filtered off, washed with water on the filter, and dried. Yield 0·32 g (59%), m.p. 232—238°C (decomposition). The m.p. increased to 237—240°C after recrystallization from methanol–water. $[\alpha]_D^{20}$ +93·7° (*c* 0·5, methanol). For $C_{28}H_{34}N_2O_9$ (542·6) calculated: 61·98% C, 6·32% H, 5·16% N; found: 61·99% C, 6·22% H, 4·88% N. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-M, S_2, S_3). Recorded data[58]: m.p. 222—225°C (decomposition), $[\alpha]_D^{20}$ +61° (*c* 0·5, methanol).

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-D-alanine Methyl Ester (*XXI*)

It was prepared by the same procedure and from the same quantity of starting material as the L-isomer. The yield of the crude product was 2·67 g (96%), m.p. 175—185°C. Two crystallizations from ethanol–water afforded 1·98 g (74%) of a product of m.p. 194—196°C, $[\alpha]_D^{20}$ +111·7° (*c* 0·5, dimethylformamide). For $C_{29}H_{36}N_2O_9$ (556·6) calculated: 62·58% C, 6·52% H, 5·03% N; found: 62·38% C, 6·47% H, 5·13% N. Chromatographically homogeneous (TLC-M, S_2 and S_3).

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-alanine Amide (*XXII*)

Methyl ester *XIX* (1·0 g, 1·8 mmol) was allowed to stand (pressurized vessel) 3 days with 100 ml of a 20% ammonia solution in methanol. The solvent was evaporated under reduced pressure. Yield 0·97 g (100%) of a product of m.p. 247—263°C. Recrystallization from dimethylformamide–water afforded 0·83 g (86%) of a product of m.p. 263—264°C, $[\alpha]_D^{20}$ +75·5° (*c* 0·5, dimethyl-

formamide). For $C_{28}H_{35}N_3O_8$ (541·6) calculated: 62·09% C, 6·51% H, 7·76% N; found: 61·89% C 6·65% H, 8·01% N. Chromatographically homogeneous (TLC-M, S_2, S_3).

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-D-alanine Amide (*XXIII*)

It was prepared by the same procedure and in the same yield as the L-isomer, m.p. 280—281°C, $[\alpha]_D^{20}$ +107·5° (*c* 0·5, dimethylformamide). For $C_{28}H_{35}N_3O_8$ (541·6) calculated: 62·09% C, 6·51% H, 7·76% N; found: 62·08% C, 6·49% H, 7·85% N. Chromatographically homogeneous (TLC-M, S_2, S_3).

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-alanyl-D-isoglutamine Methyl Ester (*XXIV*)

Acid *XVIII* (1·93 g, 4·05 mmol) was dissolved in a mixture of 20 of dimethylformamide and 0·45 ml (4·05 mmol) of N-methylmorpholine. The solution was cooled down to —10°C and 0·39 ml (4·05 mmol) of ethyl chloroformate was added. The mixture was maintained 5 min at 0°C and cooled down to —10°C; 0·94 g (4·05 mmol) of alanyl-D-isoglutamine methyl ester (prepared from benzyloxycarbonylalanyl-D-isoglutamine methyl ester by the procedure described for the preparation of compound *XI*) was added. The mixture was allowed to stand 1 h at 0°C and overnight at room temperature. Subsequently 70 ml of water was added, the product which had separated was filtered off and freed of acidic and basic impurities by repeated (3 fold) trituration with dilute hydrochloric acid (1%), water, a solution of sodium bicarbonate, and water. The product was then filtered off and dried. Yield 2·24 g (80%), m.p. 225—247°C. Two crystallizations from dimethylformamide–water afforded 1·83 g (65%) of a product of m.p. 260—262°C, $[\alpha]_D^{20}$ +104·6° (*c* 0·5, dimethylformamide). For $C_{34}H_{44}N_4O_{11}$ (684·7) calculated: 59·64% C, 6·48% H, 8·18% N; found: 59·53% C, 6·41% H, 8·09% N. Amino acid analysis: Mur 1·17, Ala 0·92, Glu 0·91. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-M) in systems S_2 and S_3.

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-alanyl-D-glutamic Acid Diamide (*XXV*)

a) *Ammonolysis.* Methyl ester *XXIV* (0·3 g, 0·44 mmol) was allowed to stand in a pressurized vessel with 30 ml of a 20% ammonia solution in methanol 6 days at room temperature. After the solvent had been evaporated off under reduced pressure *c.* 0·295 g (100%) of a product of m.p. 245—263°C was obtained. The yield of the diamide after recrystallization from dimethylformamide–water was 0·23 g (78%), m.p. 262—264°C, $[\alpha]_D^{20}$ +87·4° (*c* 0·3, dimethylformamide). For $C_{33}H_{43}N_5O_{10}.H_2O$ (687·7) calculated: 57·63% C, 6·60% H, 10·18% N; found: 57·63% C, 6·55% H, 9·98% N. Chromatographically homogeneous (TLC-M, S_2, S_3).

b) Method of mixed anhydrides. The synthesis was effected by the procedure described for the preparation of compound *XXIV* from 2·74 g (5·8 mmol) of protected muramic acid, 0·635 ml (5·8 mmol) of N-methylmorpholine, 0·555 ml (5·8 mmol) of ethyl chloroformate, and 1·25 g (5·8 mmol) of alanyl-D-glutamic acid diamide (obtained from benzyloxycarbonyl-D-glutamic acid diamide by the procedure described for the preparation of compound *XI* (the hydrobromide and the free diamide were crystalline)). The diamide was added to the reaction mixture in a solution of 20 ml of dimethylformamide and 15 ml of dimethyl sulfoxide. The yield of the crude product was 3·8 g (98%), m.p. 245—260°C. Recrystallization from dimethylformamide–water afforded 3·3 g (81%) of the diamide of m.p. 262—264°C, showing no depression of the melting point when mixed with the product obtained by ammonolysis. $[\alpha]_D^{20}$ +89·5° (*c* 0·3, dimethylformamide). For $C_{33}H_{43}N_5O_{10}.H_2O$ (687·7) calculated: 57·63% C, 6·60% H, 10·18% N; found:

57·47% C, 6·37% H, 10·08% N. Amino acid composition: Mur 0·94, Ala 1·01, Glu 1·03. Chromatographically homogeneous (TLC-M, S_2, S_3).

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-alanyl-D-isoglutaminyl-N^{ε}-acetyllysyl--D-alanine Amide (*XXVI*)

A solution of 0·65 g (1·42 mmol) of alanyl-D-isoglutaminyl-N^{ε}-acetyllysyl-D-alanine amide in 10 ml dimethylformanide, 10 ml tetrahydrofurane and 7 ml water (the free tetrapeptide amide was prepared from amide *XII* by the procedure described for the preparation of compound *XIV*) was added with stirring to a solution of 0·89 g (1·56 mmol) of 1-O-benzyl-4,6-O-benzylidene--N-acetylmuramic acid N-hydroxysuccinimide ester[46] in a mixture of 10 ml dimethylformamide and 10 ml tetrahydrofurane. The reaction mixture was stirred about 2 h at 0°C and then set aside for 24 h at room temperature. Subsequently 50 ml of a solution of sodium bicarbonate was added. After 30 min at 0°C the product which had separated was filtered off and acidic and basic contaminants removed by its trituration (3-fold) with a solution of sodium bicarbonate, 1% hydrochloric acid, saturated solution of sodium bicarbonate, and water. The product was then filtered off and dried. Yield 1·25 g (97%), m.p. 294—300°C. The crude product was triturated and boiled with methanol (3 times with 30 ml). The insoluble moiety was recrystallized from 70% dimethylformamide. The yield was 0·95 g (74%) of a product of m.p. 300—303°C, $[\alpha]_D^{20}$ +30° (*c* 0·5, dimethylsulfoxide). For $C_{44}H_{62}N_8O_{13}$ (911·0) calculated: 58·01% C, 6·86% H, 12·30% N; found: 58·22% C, 7·03% H, 12·03% N. Amino acid analysis: Mur 1·04, Ala 2·06, Lys 1·01, Glu 1·03. Chromatographically homogeneous (TLC-S, S_1, S_3).

Tert-butyloxycarbonylalanyl-D-isoglutaminyl-N^{ε}-acetyllysyl-D-alanine Methyl Ester (*XXVII*)

The synthesis was effected with 4·84 g of the resin esterified with Boc-D-Ala-OH (3·0 mmol of D-Ala/g resin). The increase in resin weight after completion of the synthesis was 1·01 g (91%). The resin (2·9 g) was suspended in 40 ml of methanol containing 1 ml of triethylamine and the mixture was stirred 24 h at room temperature. The resin was filtered off and washed with methanol on the filter. The filtrates were evaporated under reduced pressure. The yield of the peptide, chromatographically homogeneous (TLC-S, S_1 and S_8) was 0·76 g (88%), m.p. 228—230°C (decomposition). This product was immediately used in subsequent work. A sample of the peptide was recrystallized twice from methanol–ether. The m.p. did not practically change (230°C, decomposition), $[\alpha]_D^{20}$ —4·6° (*c* 0·5, dimethylformamide). For $C_{25}H_{44}N_6O_9 \cdot 0{\cdot}5\ H_2O$ (581·7) calculated: 51·62% C, 7·80% H, 14·45% N; found: 51·74% C, 7·73% H, 14·23% N. Amino acid composition: Ala 1·97, Glu 0·97, Lys 1·05.

Tert-butyloxycarbonylalanyl-D-isoglutaminyl-N^{ε}-acetyllysyl-D-alanyl-pentaglycine Methyl Ester (*XXVIII*)

The synthesis was carried out with 7·0 g of esterified resin (5·6 mmol Gly). The weight increase was 3·0 g (80%). The resin (5·0 g) was suspended in 150 ml of methanol and 4 ml of triethylamine. The mixture was stirred 24 h at room temperature and evaporated under reduced pressure; the dry residue was extracted with a boiling mixture of methanol and water (4 : 1, 3 times). The extracts were pooled and the reaction product was precipitated by ether. Yield 1·72 g (72%), m.p. 210—213°C. Double reprecipitation from the mixture water–methanol–ether afforded 1·46 g (61%) of a product of m.p. 213—215°C, $[\alpha]_D^{20}$ 0° (*c* 0·2, dimethylformamide). For $C_{35}H_{59}$·$N_{11}O_{14} \cdot 1{\cdot}5\ H_2O$ (885·0) calculated: 47·51% C, 7·06% H, 17·41% N; found: 47·29% C, 6·85% H, 17·39% N. Amino acid analysis: Ala 1·82, Glu 0·96, Lys 1·0, Gly 5·2. The product was chromatographically homogeneous (TLC-S, S_1, S_8).

Tert-butyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanyl-pentaglycine Amide (*XXIX*)

The resin (5·0 g) from the preceding experiment was ammonolyzed 24 h at room temperature in 150 ml of methanol saturated with ammonia at 0°C. The solvent was evaporated under reduced pressure, the dry residue was extracted with two 25 ml portions of a boiling mixture of methanol and water (4 : 1). The extracts were pooled and the reaction product precipitated by ether. Yield 1·67 g (71%), m.p. 221—225°C (decomposition). Double reprecipitation from a mixture methanol–water–ether afforded 1·36 g (58%) of a product of m.p. 231—234°C (decomposition), $[\alpha]_D^{20}$ 0° (*c* 0·2, dimethylformamide). For $C_{34}H_{58}N_{12}O_{13}.1{\cdot}5\ H_2O$ (869·9) calculated: 46·94% C, 7·07% H, 19·32% N; found: 46·97% C, 6·81% H, 19·22% N. Amino acid composition: Ala 1·81, Glu 0·93, Lys 1·0, Gly 5·2. When subjected to thin-layer chromatography (TLC-S, S_1, S_8) the product behaved as a homogeneous compound.

Benzyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-D-alanyl-pentaglycine Methyl Ester (*XXX*)

The synthesis was carried out in the manner described for the preparation of compound *XXVIII*. A quantity of 6·1 g of the esterified resin (4·9 mmol of Gly) afforded 2·6 g (54%) of a product of m.p. 226—228°C. Double crystallization from 50% methanol yielded 1·8 g (37%) of a product of m.p. 229—230°C, $[\alpha]_D^{24}$ —1·0° (*c* 1·0, dimethylformamide). For $C_{44}H_{61}N_{11}O_{15}.H_2O$ (1 002) calculated: 52·74% C, 6·34% H, 15·38% N; found: 52·48% C, 6·09% H, 15·23% N. Amino acid composition: Ala 2·0, Glu 1·04, Lys 0·95, Gly 4·73. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC–S, S_1, S_3).

Alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanine-pentaglycine Methyl Ester (*XXXI*)

Methyl ester *XXVIII* (0·4 g, 0·45 mmol) was dissolved in a mixture of 3 ml of dichloromethane and 12 ml of trifluoroacetic acid. The mixture was allowed to stand 30 min at room temperature, evaporated under reduced pressure, and the dry residue triturated with ether. The product which had separated was filtered off, dried, dissolved in 3 ml of 90% methanol, and filtered through a column of Ostion AT (40 ml). The product-containing effluents (according to the reaction with ninhydrin) were evaporated under reduced pressure. The yield was 0·23 g (65%) of free nonapeptide ester of m.p. 208—211°C. Double reprecipitation from a mixture water–methanol–ether afforded a product of m.p. 215—217°C, $[\alpha]_D^{20}$ +10·5° (*c* 0·2, water). For $C_{30}H_{51}N_{11}O_{12}.2\ H_2O$ (793·9) calculated: 45·39% C, 6·98% H, 19·41% N; found: 45·76% C, 6·75% H, 19·12% N. Amino acid analysis: Ala 1·92, Glu 0·94, Lys 1·02, Gly 5·05. Chromatographically (TLC-S, S_9, S_{10}) and electrophoretically (on Whatman 3MM paper, 800 V, 1M-CH_3COOH) homogeneous.

Alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanyl-pentaglycine Amide (*XXXII*)

This compound was prepared by the procedure described in the preceding experiment except that the desalting was carried out in 75% methanol. Amide *XXIX* (0·3 g, 0·34 mmol) afforded 0·19 g (72%) of a crude product of m.p. 220°C (decomposition). The latter was reprecipitated twice from a mixture water–methanol–ether before analysis. M.p. 225°C (decomposition), $[\alpha]_D^{20}$ +9·1° (*c* 0·2, water). For $C_{29}H_{50}N_{12}O_{11}.2{\cdot}5\ H_2O$ (787·8) calculated: 44·21% C, 7·04% H, 21·33% N; found: 44·26% C, 6·95% H, 21·20% N. Amino acid analysis: Ala 1·93, Glu 0·96, Lys 1·03, Gly 5·07. Chromatographically and electrophoretically homogeneous under conditions described in the preceding experiment.

Alanyl-D-isoglutaminyl-lysyl-D-alanyl-pentaglycine Methyl Ester (*XXXIII*)

Methyl ester monohydrate *XXX* (0·33 g, 0·33 mmol) was heated with 6 ml of a 15% solution of hydrogen bromide in acetic acid 5 min at 60°C. The nonapeptide hydrobromide ester was precipitated from the mixture by an excess of ether. The precipitated was rubbed with ether, the latter was decanted off and its residues removed by evacuation. The dry residue was dissolved in 15 ml of water, 2·5 g of silver carbonate was added to the solution, and the reaction mixture was stirred 1 h at room temperature. The silver salts were removed by centrifugation, the supernatant was freed of silver ions by hydrogen sulfide, silver sulfide was filtered off, and the filtrates evaporated under reduced pressure. The dry residue (300 mg, smeary) showed the presence of two compounds when subjected to thin-layer chromatography (TLC-S) in system S_5 and to paper electrophoresis in pyridine acetate buffer at pH 5·7. The product was purified by continuous free-flow electrophoresis in pyridine acetate buffer (0·04M with respect to pyridine and 0·05M with respect to acetic acid, at 2700 V). The course of the electrophoresis was checked by thin-layer chromatography (TLC-S) in system S_5. The fractions containing pure products were pooled and evaporated under reduced pressure. The dry residues were desalted by filtration through a column of Amberlite CG 50 (filtration and washing with 0·25% acetic acid, elution by 50% acetic acid). The effluents were diluted with water (they were *c.* 20% with respect to acetic acid) and lyophilized. The yield was 26 mg (11%) of an electrophoretically slower fraction and 62·5 mg (26%) of the faster fraction. Both fractions showed the same amino acid composition. Slower fraction: Ala 2·0, Glu 1·0, Lys 0·99, Gly 5·40; faster fraction: Ala 2·13, Glu 1·05, Lys 1·00, Gly 4·72. The slower compound is an acid according to its infrared spectrum.

Tos-Ala-D-iGlm-Lys(Ac)-D-Ala-$(Gly)_5$—┐
Tos-Ala-D-iGlm-Lys-D-Ala-OMe (*XXXIV*)

The synthesis was carried out with 1·85 g of the esterified resin (1·15 mmol of D-Ala). The weight increase after the completion of the synthesis was 0·85 g (58%). The product was split off from the resin by reesterification by the procedure used for the preparation of methyl ester *XXVIII*. The resin was extracted with two 25 ml portions of hot dimethylformamide after the reesterification. The extracts were pooled, the solvent was distilled off under reduced pressure, and the product was triturated with ether. Subsequently it was filtered off and dried. In this manner 1·45 g of the resin yielded 0·45 g (53%) of a product of m.p. 200—205°C. The m.p. increased after double recrystallization from 5% acetic acid to 207—209°C. $[\alpha]_D^{20}$ —18·1° (*c* 0·2, dimethylformamide). For $C_{61}H_{93}N_{17}O_{21}S_2.CH_3COOH$ (1525) calculated: 49·63% C, 6·41% H, 15·62% N; found: 49·37% C, 6·57% H, 14·56% N. Amino acid analysis: Ala 4·0, Glu 2·02, Lys 1·85, Gly 5·13. The product was chromatographically homogeneous (TLC-S, S_1, S_8).

H-Ala-D-iGlm-Lys(Ac)-D-Ala-$(Gly)_5$—┐
H-Ala-D-iGlm-Lys-D-Ala-OMe (*XXXV*)

Protected tridecapeptide ester *XXXIV* (0·25 g, 0·165 mmol) was reduced by sodium in liquid ammonia and desalted in the usual manner[43]. Lyophilization of the desalted solution afforded 98 mg (62%) of crude peptide which was purified by continuous free-flow electrophoresis (1M-CH_3. .COOH as carrier electrolyte, at 2500 V). The fractions containing the pure product were pooled and lyophilized; the purity of the fractions was checked by chromatography (TLC-S, S_3 and S_{10}) and by paper electrophoresis (Whatman 3MM, 1M-CH_3COOH, 800 V. 1 h). The yield was 46 mg of a product of $[\alpha]_D^{20}$ +13·3° (*c* 0·2, water), chromatographically homogeneous (TLC-S, S_3, S_{10}). The product was dried 10 h at 100°C and 10 Pa over phosphorus pentoxide before

elemental analysis. For $C_{47}H_{81}N_{17}O_{17}.3\,CH_3COOH.3\,H_2O$ (1390) calculated: 45·78% C, 7·18% H, 17·12% N; found: 45·92% C, 7·30% H, 17·10% N. Amino acid analysis: Ala 3·9, Glu 2·0, Lys 2·01, Gly 5·1. The product behaved as a homogeneous compound when subjected to thin-layer chromatography (TLC-S, S_3, S_{10}) and paper electrophoresis (Whatman 3MM, 1M-CH_3.COOH, 800 V, 1 h).

Boc-Ala-D-iGlm-Lys(Ac)-D-Ala-(Ala)$_5$—┐
Boc-Ala-D-iGlm-Lys-D-Ala-OMe (*XXXVI*)

The synthesis of compound *XXXVI* was effected according to the same scheme as the preparation of compound *XXXIV*. The quantity of the resin was 2·42 g (1·5 mmol of D-Ala). The weight increase of the resin after completion of the synthesis was 1·0 g (54%). The protected peptide was split off from the resin by reesterification which was carried out by the same procedure as in the case of compound *XXVIII*. The yield was 0·32 g (51%), m.p. >360°C. The product was twice recrystallized from methanol–ether before analysis. M.p. >360°C. $[\alpha]_D^{20}$ —9·9 (*c* 0·2, dimethylformamide). For $C_{62}H_{107}N_{17}O_{21}.CH_3OH$ (1458) calculated: 51·88% C, 7·67% H, 16·32% N; found: 51·85% C, 7·59% H, 16·11% N.

H-Ala-D-iGlm-Lys(Ac)-D-Ala-(Ala)$_5$—┐
H-Ala-D-iGlm-Lys-D-Ala-OMe (*XXXVII*)

The preparation of compound *XXXVII* was carried out in the same manner as the preparation of free nonapeptide amide *XXXII*. Protected tridecapeptide ester *XXXVI* (0·1 g) afforded 78 mg of the crude peptide which was purified by continuous free-flow electrophoresis. The same procedure as that described for compound *XXXV* was used. The course of the purification was checked by paper electrophoresis (Whatman 3MM, 1M-CH_3COOH, 800 V, 1 h). The fractions containing the homogeneous product were pooled and lyophilized. The yield of the pure glycopeptide was 43 mg, $[\alpha]_D^{20}$ +52·7° (*c* 0·2, water). The sample for analysis was dried in the same manner as compound *XXXV*. For $C_{52}H_{91}N_{17}O_{17}.2\,CH_3COOH.2\,H_2O$ (1383) calculated: 48·65% C, 7·51% H, 17·24% N; found: 48·53% C, 7·30% H, 17·11% N. Amino acid analysis: Ala 8·7, Glu 1·88, Lys 2·13. Chromatographically pure (TLC-S, S_3, S_{10}).

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanine Amide (*XXVI*). Solid-Phase Synthesis

A part (2·9 g) of the resin with bonded tert-butyloxycarbonylalanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanine (prepared during the synthesis of compound *XXVII*) was subjected to deblocking, neutralization, and condensation with acid *XVIII* (2·55 g). The weight increase of the resin after completion of the synthesis was 0·44 g (85%). The protected glycotetrapeptide was split off from the resin by reesterification carried out in the manner used for the preparation of protected nonapeptide amide *XXIX*. After the ammonolysis had been completed and the solvent evaporated the product was extracted with hot dimethylformamide (two 25 ml portions) and the solvent was distilled off under reduced pressure. The dry residue solidified after trituration with ether. It was filtered off and dried. Yield 1·13 g (82%), m.p. 299—302°C which remained unaltered after double crystallization from methanol–water. $[\alpha]_D^{20}$ +60·0° (*c* 0·5, dimethylformamide). For $C_{44}H_{62}N_8O_{13}.H_2O$ (929·0) calculated: 56·88% C, 6·94% H, 12·06% N; found: 56·64% C, 6·80% H, 11·92% N. Amino acid analysis: Mur 0·92, Ala 2·01, Glu 1·02, Lys 0·97. Chromatographically homogeneous (TLC-S, S_1, S_3).

1-O-Benzyl-4,6-O-benzylidene-N-acetylmuramyl-alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanyl-pentaglycine Amide (*XXXVIII*)

A solution of 0·33 g (0·455 mmol) of amide *XXXII* in a mixture of 20 ml of dimethylformamide and 3 ml of water was added with stirring to a solution of 0·258 g (0·455 mmol) of 1-O-benzyl-4,6-benzylidene-N-acetylmuramic acid N-hydroxysuccinimide ester in 15 ml of dimethylformamide cooled down to 0°C. The mixture was allowed to warm up to room temperature and while stirred overnight it jellified. It was cooled down to 0°C and 80 ml of 1% hydrochloric acid was added. The product which had separated was filtered off and 3 times triturated with dilute hydrochloric acid, a solution of sodium bicarbonate, and water. It was then filtered off and dried. The yield was 0·361 g (66%) of a product of m.p. 248—265°C. Recrystallization from dimethylformamide–ether afforded 0·284 g of glycononapeptide amide of m.p. 268—270°C, $[\alpha]_D^{22}$ +46·75° (*c* 0·3, dimethylformamide). For $C_{54}H_{77}N_{13}O_{18}.H_2O$ (1214) calculated: 53·41% C, 6·56% H, 14·99% N; found: 53·36% C, 6·46% H, 14·83% N. Amino acid analysis: Mur 1·09, Ala 2·07, Glu 1·00, Lys 0·91, Gly 5·5. Chromatographically homogeneous (TLC-S, S_1, S_3).

N-Acetylmuramyl-alanyl-D-isoglutaminyl-N$^{\varepsilon}$-acetyllysyl-D-alanyl-pentaglycine Amide (*XXXIX*)

The removal of the benzyl and benzylidene group was carried out by sodium in liquid ammonia using the procedure described in paper[43]. The monohydrate of protected glycononapeptide amide *XXXVIII* (59 mg, 0·0485 mmol) afforded 37·8 mg of the lyophilisate dried 24 h over phosphorus pentoxide at 10 Pa. For $C_{40}H_{67}N_{13}O_{18}$ (1018) calculated: 17·89% N; found: 16·39% N. The peptide content of the lyophilisate was 92%, the yield of the lyophilisate (calculated in terms of the nitrogen content of the lyophilisate) was 70%. $[\alpha]_D^{22}$ +21·2° (after 24 h +19·3°) (*c* 0·5, water). The compound was dried 9 h at 60°C and 10 Pa over phosphorus pentoxide before analysis. For $C_{40}H_{67}N_{13}O_{18}.CH_3COOH.H_2O$ (1096) calculated: 46·02% C, 6·71% H, 16·61% N; found: 46·02% C, 6·43% H, 16·35% N. Amino acid analysis: Mur 1·04, Ala 1·82, Lys 1·04, Glu 0·91, Gly 5·18. Chromatographically homogeneous (TLC-S, S_3, S_8).

BBM-Ala-D-iGlm-Lys(Ac)-D-Ala-$(Gly)_5$—┐
BBM-Ala-D-iGlm-Lys-D-Ala-OMe (*XL*)

The synthesis was carried out with 2·42 g of the resin (containing 1·5 mmol of D-Ala). The weight increase of the resin after completion of the synthesis was 1·52 g (54%). The protected glycopeptide was split off from the resin by reesterification by the procedure used for compound *XXVIII*. The yield of the product was 1·58 g (51%), m.p. 295—300°C, which increased to 302—305°C (decomposition) after double crystallization from methanol–water. $[\alpha]_D^{20}$ +50·9° (*c* 0·2, dimethylformamide). For $C_{97}H_{135}N_{19}O_{31}.3\,H_2O$ (2117) calculated: 55·03% C, 6·71% H, 12·57% N; found: 54·74% C, 6·35% H, 12·39% N. Amino acid analysis: Mur 1·85, Ala 4·11, Glu 2·0, Lys 1·87, Gly 5·18. Chromatographically homogeneous (TLC-S, S_1, S_8).

NAM-Ala-D-iGlm-Lys(Ac)-D-Ala-$(Gly)_5$—┐
NAM-Ala-D-iGlm-Lys-D-Ala-OMe (*XLI*)

The protected glycopeptide from the preceding experiment (0·3 g) was reduced by sodium in liquid ammonia, desalted, and isolated according to the procedure described in paper[43]. The yield was 0·175 g (71%) of crude lyophilisate which was purified by gel filtration on Sephadex. The crude lyophilisate (30 mg) was dissolved in 1 ml of 1M-CH_3COOH and placed onto a column packed with Sephadex G-25 (100 × 1 cm). The purification was carried out in 1M-CH_3COOH,

the flow rate was 6 ml/h. The fractions containing the pure product (according to chromatographic analysis, TLC-S, S_{11}) were pooled and lyophilized. The yield of the pure lyophilisate was 27 mg, $[\alpha]_D^{20}$ +14·0 (*c* 0·2, water). The sample was dried 10 h at 100°C and 10 Pa before analysis. For $C_{69}H_{115}N_{19}O_{31}.CH_3COOH.2{\cdot}5\ H_2O$ (1812) calculated: 47·07% C, 6·90% H, 14·69% N; found: 46·82% C, 6·77% H, 14·54% N.

BBM-Ala-D-iGlm-Lys(Ac)-D-Ala-(Ala)$_5$—┐
BBM-Ala-D-iGlm-Lys-D-Ala-OMe (*XLII*)

A part of the resin (1·1 g) with the bonded tridecapeptide (synthesis of compound *XXXVI*) was subjected to deblocking and neutralization. Subsequently it was acylated with acid *XVIII* (1·0 g). The product was split off from the resin according to the procedure described for the synthesis of compound *XXVIII*. Yield 0·54 g (52%), m.p. 290—293°C. Double crystallization from methanol–water afforded a product of m.p. 295—297°C (decomposition), $[\alpha]_D^{20}$ +54° (*c* 0·2, dimethyl sulfoxide). For $C_{102}H_{145}N_{19}O_{31}.2\ H_2O$ (2169) calculated: 56·47% C, 6·92% H, 12·27% N; found: 56·56% C, 6·74% H, 11·98% N. Amino acid analysis: Mur 1·96, Ala 8·85, Glu 1·82, Lys 2·0. Chromatographically pure (TLC-S, S_1, S_8).

NAM-Ala-D-iGlm-Lys(Ac)-D-Ala-(Ala)$_5$—┐
NAM-Ala-D-iGlm-Lys-D-Ala-OMe (*XLIII*)

The removal of the protectings groups and the isolation of the product after the reduction were carried out according to a general scheme[43]. Protected glycotridecapeptide ester *XLII* (0·1 g) afforded 60 mg (83%) of a crude and 45 mg (62%) of a pure lyophilisate. (The purification was carried out as described for compound *XLI*). $[\alpha]_D^{20}$ +15·9° (*c* 0·2, water). The sample for analysis was dried in the same manner as product *XLI*. For $C_{74}H_{125}N_{19}O_{31}.CH_3COOH.1{\cdot}5\ H_2O$ (1862) calculated: 49·03% C, 7·04% H, 14·29% N; found: 48·85% C, 6·89% H, 14·35% N.

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