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Synthesis of N^{ϵ}-Glycyl-N^{α}-[2-(2-acetamido-2-deoxy-3-O-D-glucopyranosyl)-acetyl-L-alanyl-D-α-glutamyl]-L-lysyl-D-alanyl-D-alanine*

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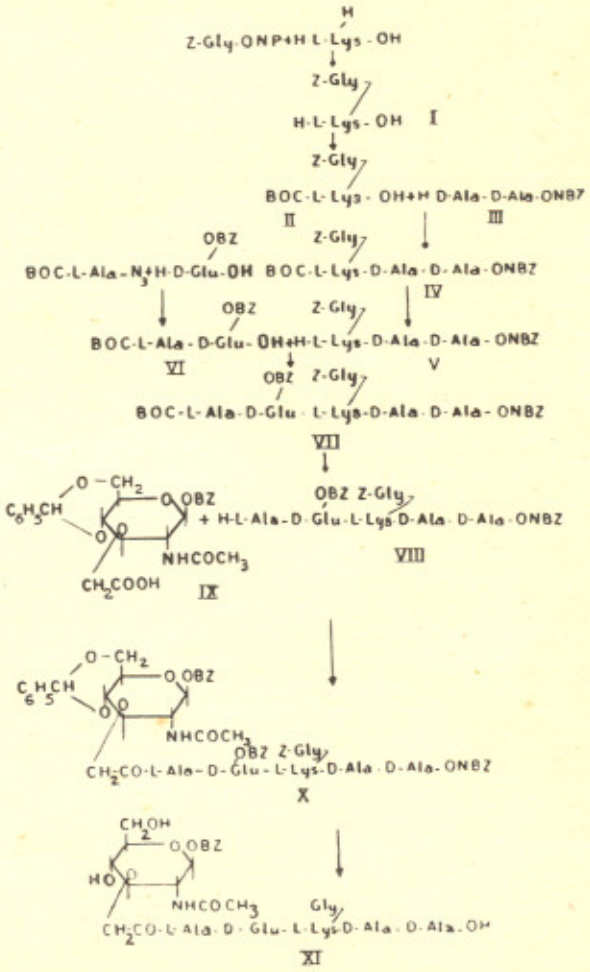
The synthesis of $^{\epsilon}$ N-glycyl-N^{α}-[2-(2-acetamido-2-deoxy-3-O-D-glucopyranosyl)-acetyl-L-alanyl-D-α-glutamyl]-L-lysyl-D-alanyl-D-alanine as an analogue of the N^{α},N^{ϵ}-disubstituted lysine glycopeptides present in bacterial cell walls is reported.

WE have been interested in the synthesis of the glycopeptides present in bacterial cell walls and certain related compounds[1]. An unusual feature of these glycopeptides is the presence of the N^{α},N^{ϵ}-disubstituted lysine residue[2,3] through which cross linkages may be possible[4] which would give strength and rigidity to the cell wall structure. With a view to making synthetic analogues of these glycopeptides which could possibly act as inhibitors of the biosynthesis of the cell wall glycopeptides, methods have been under investigation for the synthesis of lysine-containing glycopeptides substituted at both N^{α}- and N^{ϵ}-positions of lysine. The synthesis of N^{ϵ}-glycyl-N^{α}-[2-(2-acetamido-2-deoxy-3-O-D-glucopyranosyl)-acetyl-L-alanyl-D-α-glutamyl]-L-lysyl-D-alanyl-D-alanine is reported in this communication.

N^{ϵ}-(Benzyloxycarbonylglycyl)-L-lysine (I), obtained by the direct peptidation of lysine with benzyloxycarbonyl-glycine *p*-nitrophenyl ester[5], was converted into its N^{α}-*t*-butyloxycarbonyl derivative (II) and then condensed with D-alanyl-D-alanine *p*-nitrobenzyl ester (III)[6] to give N^{ϵ}-(benzyloxycarbonylglycyl)-N^{α}-*t*-butyloxycarbonyl-L-lysyl-D-alanyl-D-alanine nitrobenzyl ester (IV). Removal of the *t*-butyloxycarbonyl group with trifluoroacetic acid gave the tetrapeptide (V), which on condensation with *t*-butyloxycarbonyl-L-alanyl-γ-benzyl-D-glutamate (VI) using WRK gave the hexapeptide, N^{ϵ}-(benzyloxycarbonylglycyl)-N^{α}-(*t*-butyloxycarbonyl-L-alanyl-γ-benzyl-D-glutamyl)-L-lysyl-D-alanyl-D-alanine *p*-nitrobenzyl ester (VII). Compound (VI) was prepared by the condensation of *t*-butyloxycarbonyl-L-alanyl azide with glutamic acid γ-benzyl ester. The *t*-butyloxycarbonyl group was removed from (VII) with trifluoroacetic acid and the resulting hexapeptide (VIII) was condensed with benzyl 2-acetamido-4,6-O-benzylidene-3-O-carboxymethyl-2-deoxy-D-glucose (IX) using WRK to give the glycopeptide N^{ϵ}-[benzyloxycarbonylglycyl]-N^{α}-[2-(1-O-benzyl-2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-D-glucopyranosyl)-acetyl-L-alanyl-γ-benzyl-D-glutamyl]-L-lysyl-D-alanyl-D-alanine *p*-nitrobenzyl ester (X). The latter on hydrogenolysis using Pd/C



(10 per cent) in a vigorously stirred mixture of ethyl acetate and water gave the required glycopeptide (XI).

Experimental Procedure

Capillary melting points were determined on Totollis melting point apparatus (W. Buchi, Flawil/Switzerland) and are uncorrected. The homogeneity of the compounds was tested by thin layer chromatography on silica gel (200 mesh). Solvents used were: (A) *n*-butanol-acetic acid-water (50:10:40); (B) *n*-butanol-acetic acid-water-pyridine (30:6:24:20); (C) methanol-ethyl acetate (10:90); (D) chloroform-ethyl acetate (1:1); and (E) ethyl acetate. Protected

*The following abbreviations have been used: ONP=*p*-nitrophenyl ester; ONBZ=*p*-nitrobenzyl ester; Z=benzyloxycarbonyl; BOC=*t*-butyloxycarbonyl; OBZ=benzyl ester; WRK=N-ethyl-5-phenylisooxazolium-3′-sulphonate (Woodward's reagent K); DMF=dimethylformamide; TEA=triethylamine; Ala=alanine; Gly=glycine; Lys=lysine; and Glu=glutamic acid.

compounds were detected by spraying with water, or by exposure to iodine vapour, while the free peptides were detected by heating the plates at 150°C. for 30 min.

Nε-(Benzyloxycarbonylglycyl)-L-lysine (I) — It was prepared by a slight modification of the method described earlier[5]. A solution of benzyloxycarbonylglycine *p*-nitrophenyl ester (6·88 g.) in dioxane (20 ml.) was added dropwise under stirring to a cooled solution (0-5°) of lysine monohydrochloride (3·64 g.) and triethylamine (2·82 ml.) in water (10 ml.) and ethanol (60 ml.). The mixture was stirred for 2 hr below 10° and for 24 hr at 20°. Excess of ethyl acetate was added and the product which precipitated out was filtered, washed well with hot ethyl acetate and ether and crystallized from water; yield 65 per cent; m.p. 265°.

Nα-(t-Butyloxycarbonyl)-Nε-(benzyloxycarbonylglycyl)-L-lysine (II) — A suspension of Nε-benzyloxycarbonylglycyl-L-lysine (1·7 g.) in water (10 ml.) and *t*-butanol (30 ml.) was treated with *t*-butylazidoformate (1·5 ml.) and sodium bicarbonate (1·7 g.) and the mixture refluxed on the steam-bath for 6 hr. The mixture was concentrated *in vacuo* to remove *t*-butanol, the residue diluted with water, acidified with citric acid and extracted with ethyl acetate, the ethyl acetate extract washed with water, dried ($MgSO_4$) and evaporated to give (II) as an oil; yield 2·2 g.; R_f 0·5 (solvent D) and 0·8 (solvent E). (Found: C, 57·34; H, 7·56; N, 9·15. $C_{21}H_{31}N_3O_7$ requires C, 57·65; H, 7·14; N, 9·60%).

Nα-t-Butyloxycarbonyl-Nε-(benzyloxycarbonylglycyl)-L-lysyl-D-alanyl-D-alanine nitrobenzyl ester (IV) — To a stirred suspension of WRK (1·125 g.) in acetonitrile (15 ml.) kept at 0°, a solution of (II) (2·185 g.) and triethylamine (0·7 ml.) in acetonitrile (25 ml.) was added and the mixture stirred at 0° till a clear solution was obtained (1 hr). A solution of the hydrobromide of (III)[6] (2·1 g.) and triethylamine (0·8 ml.) in acetonitrile (25 ml.) was then added and the mixture stirred for an additional 1 hr at 0°, and kept overnight at room temperature. The precipitate was filtered, washed well with 0·5 per cent $NaHCO_3$, 5 per cent ice-cold citric acid and water, dried and crystallized from benzene to give (IV); m.p. 114-15°; yield 1·4 g.; $[\alpha]_D^{20}$ +20° (*c* 0·8, DMF); R_f 0·6 (solvent D) (Found: C, 57·64; H, 6·7; N, 11·43. $C_{34}H_{46}N_6O_{11}$ requires C, 57·12; H, 6·48; N, 11·76%).

t-Butyloxycarbonyl-L-alanyl-γ-benzyl-L-glutamate (VI) — A solution of *t*-butyloxycarbonyl-L-alanyl hydrazide (1·5 g.) in water (27 ml.) and acetic acid (9 ml.) was cooled to −10° and treated with $NaNO_2$ (0·6 g.). The mixture was shaken for 10 min. at −10° and the azide extracted with ether. The ethereal extract was washed with ice-cold $NaHCO_3$ (3 per cent) and water, and added to a cooled solution of γ-benzyl-D-glutamate (2·2 g.) and triethylamine (0·9 ml.) in DMF (45 ml.) and water (22·5 ml.). Ether was evaporated under vacuum, and the mixture kept stirring at 20°. After 2 days the solvent was removed under reduced pressure, the residue triturated with ice-cold citric acid solution and extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried ($MgSO_4$) and evaporated to dryness. The residue which solidified on trituration with petroleum ether was crystallized from ether-petroleum ether mixture to give (VI); m.p. 83-84°; yield 2 g.; $[\alpha]_D^{20}$ +18·34° (*c* 2, DMF) (Found: C, 59·23; H, 6·93; N, 7·18. $C_{20}H_{28}N_2O_7$ requires C, 58·80; H, 6·90; N, 6·85%).

Nε-(Benzyloxycarbonylglycyl)-Nα-(t-butyloxycarbonyl-L-alanyl-γ-benzyl-D-glutamyl)-L-lysyl-D-alanyl-D-alanine p-nitrobenzyl ester (VII) — The tetrapeptide (IV) was dissolved in ice-cold trifluoroacetic acid (12 ml.) and kept below 5° for 1 hr. Excess trifluoroacetic acid was removed under reduced pressure over sodium hydroxide pellets and the residue washed well with dry ether when 1·2 g. of trifluoroacetate of (V) was obtained.

To a stirred suspension of WRK (1·07 g.) in acetonitrile (15 ml.) kept at 0°, a solution of *t*-butyloxycarbonyl-L-alanyl-γ-benzyl-D-glutamate (0·7 g.) and triethylamine (0·22 ml.) in acetonitrile (15 ml.) was added, and the mixture stirred till a clear solution was obtained (1 hr). A solution of the trifluoroacetate of (V) and triethylamine (0·22 ml.) in acetonitrile (15 ml.) was added to the clear solution, and the mixture stirred for 1 hr at 0° and then kept overnight at room temperature. The solvent was removed under reduced pressure and the residue triturated with 0·5*N* warm sodium bicarbonate solution, filtered and washed with water, 5 per cent ice-cold citric acid solution and water and crystallized from DMF-ether mixture to give 0·9 g. of (VII); m.p. 181-2°; yield 0·9 g.; $[\alpha]_D^{20}$ −22° (*c* 0·5, DMF) R_f 0·8 (solvent A); R_f 0·8 (solvent B) (Found: C, 59·05; H, 6·62; N, 10·75. $C_{49}H_{64}N_8O_{15}$ requires C, 58·54; H, 6·41; N, 11·14%).

Nε-(Benzyloxycarbonylglycyl)-Nα-[2-(1-O-benzyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-acetyl-L-alanyl-γ-benzyl-D-glutamyl]-L-lysyl-D-alanyl-D-alanine p-nitrobenzyl ester (X) — The hexapeptide (VII, 600 mg.) was treated with ice-cold trifluoroacetic acid (6 ml.) and the mixture kept for 1 hr below 10°. Excess trifluoroacetic acid was removed under reduced pressure and the residue triturated with dry ether to give trifluoroacetate of (VIII); yield 0·6 g.

A solution of benzyl 2-acetamido-3-O-carboxymethyl-4,6-O-benzylidene-2-deoxyglucopyranose (IX, 230 mg.) and TEA (0·07 ml.) in acetonitrile (15 ml.) was added to a stirred suspension of WRK (115 mg.) in acetonitrile (10 ml.) at 0° and the mixture stirred when a clear solution was obtained (1 hr). A solution of trifluoroacetate of (VII) and triethylamine (0·07 ml.) in acetonitrile was then added and the mixture stirred for 1 hr at 0° and kept overnight at room temperature. The product which separated was filtered, washed with 0·5 per cent sodium bicarbonate solution (0·5 per cent), ice-cold citric acid solution (5 per cent) and water, and crystallized from ethanol; yield 0·3 g.; m.p. 220-1°; $[\alpha]_D^{20}$ +58° (*c* 0·5, DMF) R_f 0·5 (solvent C) (Found: C, 60·23; H, 6·47; N, 8·91. $C_{68}H_{81}N_9O_{20}$ requires C, 60·75; H, 6·07; N, 9·37%).

Nε-Glycyl-Nα-[2-(2-acetamido-2-deoxy-3-O-D-glucopyranosyl)-acetyl-L-alanyl-γ-D-glutamyl]-L-lysyl-D-alanyl-D-alanine (XI) — The compound (X) (200 mg.) was dissolved in excess ethyl acetate (200 ml.), glass distilled water (50 ml.) and Pd/C (10 per cent, 560 mg.) was added and the mixture vigorously stirred and hydrogenated at atmospheric temperature

and pressure till the absorption of hydrogen ceased. The catalyst was filtered off and the aqueous phase extracted twice with ethyl acetate and then stirred for 2 hr with 4 g. Dowex-50 (H^+ form). After filtering the resin the aqueous filtrate was lyophilized and the residue crystallized from methanol-ether to give (XI) as a colourless hygroscopic product; R_f 0·5 (solvent A); R_f 0·8 (solvent B); $[\alpha]_D^{30}$ +26·25° (*c* 1, MeOH) (Found: C, 47·45; H, 6·88; N, 13·20. $C_{32}H_{54}N_8O_{16}$ requires C, 47·63; H, 6·74; N, 13·89%).

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