611. *The Synthesis of Peptide Derivatives of Basic Amino-acids.*

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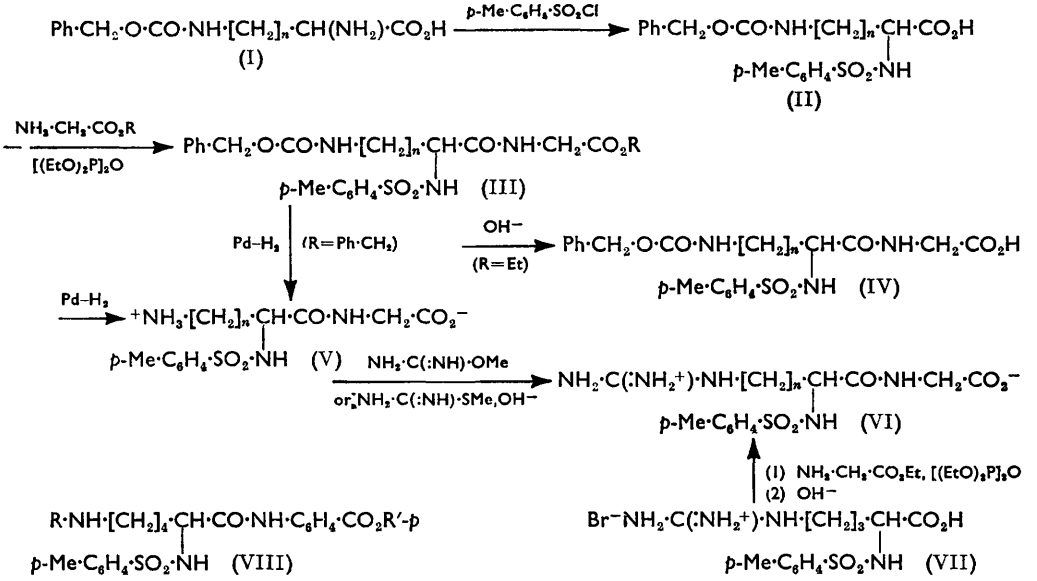
ω-*N*-Benzyloxycarbonyl-α-*N*-toluene-*p*-sulphonyldiamino-acids have been converted into peptide derivatives in high yield by means of tetraethyl pyrophosphite.[1] After removal of the *C*-terminal ester and the benzyloxycarbonyl groups, treatment with *O*-methyl*iso*urea or *S*-methyl*iso*thiourea afforded ω-guanidino-α-*N*-toluene-*p*-sulphonyl-peptides.

Peptide derivatives of basic amino-acids were required for a study of the specificities of trypsin and thrombin. Although lysine peptides have been known for a long time, derivatives of arginine were almost inaccessible until recently, owing to the difficulty of

[1] Anderson, Blodinger, and Welcher, *J. Amer. Chem. Soc.*, 1952, **74**, 5309.

protecting the guanidino-group. Glycyl-L-arginine was synthesised from nitro-L-arginine [2] but, since α-*N*-benzyloxycarbonylnitro-L-arginine could not be converted into an acid chloride or azide, no arginyl peptides were synthesised until the introduction of methods involving unsymmetrical acid anhydrides.[3]



We envisaged the annexed alternative approach, a suggestion independently outlined by Fruton.[4] It appeared attractive because (i) protection of the α-amino-group by a toluene-*p*-sulphonyl residue would be expected to give substrates with high sensitivity towards trypsin [5] and thrombin,[6] and (ii) introduction of the guanidino-group at a late stage would be expected to simplify the synthesis of peptides of homologues of arginine.

Conversion of diamino-acids into their ω-*N*-benzyloxycarbonyl derivatives by the general method of Synge,[7] Neuberger and Sanger,[8] or Harris and Work [9] gave satisfactory yields only in the case of L-lysine. In agreement with Harris and Work,[9] δ-*N*-benzyloxycarbonyl-DL-ornithine was obtained in 35—40% yield. Greatly improved yields were obtained by treatment of the copper derivative of the amino-acid with benzyl chloroformate in presence of excess of magnesium oxide. Reaction of ω-*N*-benzyloxycarbonyldiamino-acids with toluene-*p*-sulphonyl chloride in aqueous acetone or water–ether mixtures proceeded smoothly. Coupling of δ-*N*-benzyloxycarbonyl-α-*N*-toluene-*p*-sulphonyl-DL-ornithine with glycine ethyl ester by Anderson and Young's diethyl phosphorochloridite method [10] gave only a modest yield of peptide derivative (III; R = Et, $n = 3$). The use of tetraethyl pyrophosphite,[1] however, almost always afforded excellent yields of the desired compounds (III). Ethyl esters (III; R = Et) were hydrolysed to the acids (IV), and these were hydrogenolysed to α-*N*-toluene-*p*-sulphonylpeptides (V).

[2] Bergmann, Zervas, and Rinke, *Z. physiol. Chem.*, 1934, **224**, 40.
[3] (*a*) Anderson, *J. Amer. Chem. Soc.*, 1953, **75**, 6081; (*b*) Hofmann, Rheiner, and Peckham, *ibid.*, p. 6084; 1956, **78**, 238; Gish and Carpenter, *ibid.*, 1953, **75**, 5872; Van Orden and Smith, *J. Biol. Chem.*, 1954, **208**, 751.
[4] Fruton, *Adv. Protein Chem.*, 1949, **5**, 1.
[5] Schwert, Neurath, Kaufman, and Snoke, *J. Biol. Chem.*, 1948, **172**, 221.
[6] Sherry and Troll, *ibid.*, 1954, **208**, 95.
[7] Synge, *Biochem. J.*, 1948, **42**, 99.
[8] Neuberger and Sanger, *ibid.*, 1943, **37**, 515.
[9] Harris and Work, *ibid.*, 1950, **46**, 582.
[10] Anderson and Young, *J. Amer. Chem. Soc.*, 1952, **74**, 5307.

Alternatively, benzyl esters (III; R = Ph·CH_2) were hydrogenolysed directly to the latter compounds. The free ω-amino-group was converted into a guanidino-residue by reaction with *O*-methyl*iso*urea or *S*-methyl*iso*thiourea in alkaline solution at room temperature.[11]

It is, of course, vital that peptide syntheses should proceed without racemisation. As a check, we synthesised α-*N*-toluene-*p*-sulphonyl-L-arginylglycine by two methods. The first started from L-ornithine and followed the route outlined above. In the second procedure, based on Anderson's method,[3a] L-arginine was converted into α-*N*-toluene-*p*-sulphonyl-L-arginine hydrobromide (VII) and thence into the desired peptide derivative (VI; $n = 3$). The two products had optical rotations which did not differ by a statistically significant amount.

The successful use of chromogenic substrates [12] in enzyme studies prompted an attempt to synthesise α-*N*-toluene-*p*-sulphonyl-L-lysine *p*-carboxyanilide (VIII; R = R′ = H), since *p*-aminobenzoic acid is readily assayed spectrophotometrically. Unfortunately, ε-*N*-benzyloxycarbonyl-α-*N*-toluene-*p*-sulphonyl-L-lysine *p*-benzyloxycarbonylanilide (VIII; R = Ph·CH_2·O·CO, R′ = Ph·CH_2) was obtained in poor yields by the pyrophosphite procedure [1] and several variations of the method of Vaughan [13a] and Boissonnas.[13b] Moreover, attempts to remove protecting groups by hydrogenolysis or hydrogen bromide in acetic acid [14] were unsuccessful.

An early idea to employ phthaloyl groups was abandoned after the observation that phthaloyl peptides were too unstable for use as enzyme substrates in alkaline solution.[15]

Experimental

L-α*γ*-Diaminobutyric acid dihydrochloride, prepared by Adamson's method,[16] had m. p. 203—204° (decomp.), $[\alpha]_D^{20}$ +11·6° (*c* 3·67 in H_2O).

L-Ornithine monohydrochloride, prepared by Hunter's method,[17] had m. p. 235—237° (decomp.), $[\alpha]_D^{20}$ +11·0° (*c* 5·76 in H_2O) (Found: C, 35·7; H, 7·8. Calc. for $C_5H_{13}O_2N_2Cl$: C, 35·6; H, 7·8%).

ε-*N*-Benzyloxycarbonyl-L-lysine (I; $n = 4$), prepared by Neuberger and Sanger's method,[8] had m. p. 250—252° (decomp.).

δ-*N*-*Benzyloxycarbonyl*-DL-*ornithine* (I; $n = 3$).—A solution of the copper complex of DL-ornithine (from 6 g. of DL-ornithine monohydrobromide) was treated with benzyl chloroformate (6 c.c.) portionwise with stirring during 30 min. at 0° in presence of magnesium oxide (5·6 g.). Stirring was continued for a further 2 hr. at room temperature, and the precipitated copper complex was collected, washed, suspended in 0·9N-hydrochloric acid and decomposed with hydrogen sulphide. The combined filtrate and washings from copper sulphide were brought to pH 4—5 with aqueous ammonia, and the product (6·83 g., 91%) was collected; it had m. p. 255—257° (decomp.) after recrystallisation from 50% aqueous ethanol (Found: C, 58·7; H, 7·2. Calc. for $C_{13}H_{18}O_4N_2$: C, 58·6; H, 6·8%).

The L-derivative (89%), obtained by the same procedure, had m. p. 253—255° (decomp.), $[\alpha]_D^{21}$ +22·7° [*c* 2·9 in aqueous acetone (1 : 1) containing 2 mols. of hydrogen chloride]. In the same solvent, Synge [7] records $[\alpha]_D^{16}$ +17° (Found: C, 58·6; H, 6·8%).

α-*Amino*-γ-*benzyloxycarboxyamido*-L-*butyric acid* (I; $n = 2$).—This was obtained from L-α*γ*-diaminobutyric acid by the foregoing procedure. The product (62%), after recrystallisation from aqueous ethanol (1 : 1), had m. p. 235—236° (decomp.) in agreement with Zaoral,

[11] Wheeler and Jamieson, *J. Biol. Chem.*, 1907, **4**, 111; Greenstein, *ibid.*, 1935, **109**, 529, 541; Stevens and Bush, *ibid.*, 1950, **183**, 139; Hughes, Saroff, and Carney, *J. Amer. Chem. Soc.*, 1949, **71**, 2476.

[12] Ravin and Seligman, *J. Biol. Chem.*, 1951, **190**, 391; Gomori, *Proc. Soc. Exp. Biol. Med.*, 1954, **87**, 559.

[13] (*a*) Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547; (*b*) Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874.

[14] Ben-Ishai, *J. Org. Chem.*, 1954, **19**, 62.

[15] Hanson and Illhardt, *Z. physiol. Chem.*, 1954, **298**, 210.

[16] Adamson, *J.*, 1939, 1564.

[17] Hunter, *Biochem. J.*, 1939, **33**, 27.

Rudinger, and Šorm [18] (Found: C, 57·5; H, 6·5; N, 10·8. Calc. for $C_{12}H_{16}O_2N_2$: C, 57·1; H, 6·4; N, 11·1%).

ε-N-*Benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-L-*lysine* (II; $n = 4$).—ε-*N*-Benzyloxycarbonyl-L-lysine (1·94 g.) in 1·25N-sodium hydroxide (15 c.c.) was treated with toluene-*p*-sulphonyl chloride (2 g.) in acetone (15 c.c.). Next day, the solution was acidified and diluted with water. The resultant oil solidified at 0° overnight, and the *product* (2·31 g., 77%), recrystallised from benzene, had m. p. 123—124° (Found: C, 57·9; H, 5·9; N, 6·1; S, 7·3. $C_{21}H_{26}O_6N_2S$ requires C, 58·0; H, 6·0; N, 6·5; S, 7·4%).

δ-N-*Benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-DL-*ornithine* (II; $n = 3$).—Reaction of δ-*N*-benzyloxycarbonyl-DL-ornithine in aqueous sodium hydroxide with an ethereal solution of toluene-*p*-sulphonyl chloride during 6 hr., followed by acidification, afforded an oil which crystallised at 0°. The *product* (93%), recrystallised from benzene or ethyl acetate–light petroleum (b. p. 60—80°), had m. p. 134—135° (Found: C, 57·6; H, 5·6; N, 6·8; S, 7·4. $C_{20}H_{24}O_6N_2S$ requires C, 57·1; H, 5·8; N, 6·7; S, 7·6%). The L-*enantiomorph* (82%) was obtained in a similar manner. Recrystallised from benzene, it had m. p. 120·5—121·5° (Found: C, 57·1; H, 5·8; N, 6·7; S, 7·6%).

γ-*Benzyloxycarboxyamido*-α-*toluene*-p-*sulphonamido*-L-*butyric Acid* (II; $n = 2$).—This was prepared (73%) in the manner described for the corresponding lysine derivative. After recrystallisation from chloroform, it had m. p. 149·5—150·5°, in agreement with Rudinger [19] (Found: C, 56·2; H, 5·3; N, 7·3. Calc. for $C_{19}H_{22}O_6N_2S$: C, 56·1; H, 5·5; N, 6·9%). The compound was also prepared by Rudinger's [19] method in 52% yield.

ε-N-*Benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-L-*lysylglycine Ethyl Ester* (III; R = Et, $n = 4$).—ε-*N*-Benzyloxycarbonyl-α-*N*-toluene-*p*-sulphonyl-L-lysine (6·5 g.), glycine ethyl ester (1·55 g.), and tetraethyl pyrophosphite (4·25 g.) in diethyl hydrogen phosphite (15 c.c.) were heated at 100° for 2 hr. with exclusion of moisture, cooled, and poured into water. The oily product was extracted into ethyl acetate and washed successively with saturated sodium hydrogen carbonate, dilute hydrochloric acid, and water. The extract was dried and evaporated; the *product* (6·7 g., 86%), m. p. 103—104°, crystallised after addition of light petroleum (b. p. 60—80°) (Found: C, 58·0; H, 6·4; N, 8·0; S, 6·2. $C_{25}H_{33}O_7N_3S$ requires C, 57·8; H, 6·4; N, 8·1; S, 6·2%). When the reaction was carried out at 60° for 1 hr. the yield dropped to 65%. Reaction of this ester with ethanolic ammonia at room temperature afforded ε-N-*benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-L-*lysylglycineamide* (92%), m. p. 164·5—165·5° (from ethanol) (Found: C, 56·1; H, 6·2; N, 11·5; S, 6·3. $C_{23}H_{30}O_6N_4S$ requires C, 56·3; H, 6·2; N, 11·4; S, 6·5%).

ε-N-*Benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-L-*lysylglycine Benzyl Ester* (III; R = Ph·CH_2 $n = 4$).—This *peptide derivative* (92%) was synthesised by the same method from glycine benzyl ester hydrobromide and 1 equiv. of triethylamine. After recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°), it had m. p. 127·5—128·5° (Found: C, 61·9; H, 5·9; N, 7·2; S, 5·3. $C_{30}H_{35}O_7N_3S$ requires C, 61·9; H, 6·1; N, 7·2; S, 5·5%).

δ-N-*Benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-DL-*ornithylglycine Ethyl Ester* (III; R = Et, $n = 3$).—This *compound* (80%), synthesised by the method used for the lysine derivative, had m. p. 124·5—125·5° (Found: C, 57·3; H, 6·3; N, 8·5. $C_{24}H_{31}O_7N_3S$ requires C, 57·0; H, 6·2; N, 8·3%). The phosphorochloridite method [10] afforded an identical product in 32% yield.

The L-*enantiomorph* (82%), synthesised by the pyrophosphite procedure,[1] had m. p. 132·5—133·5° after recrystallisation from ethyl acetate–light petroleum (b. p. 40—60°) (Found: C, 57·0; H, 6·3; N, 8·4%). Reaction of the racemate with ethanolic ammonia afforded δ-N-*benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-DL-*ornithylglycineamide* (82%), m. p. 192—193° after recrystallisation from ethanol (Found: C, 55·2; H, 6·1; N, 12·0; S, 7·0. $C_{22}H_{28}O_6N_4S$ requires C, 55·4; H, 5·9; N, 11·8; S, 6·7%).

δ-N-*Benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-L-*ornithylglycine Benzyl Ester* (III; R = Ph·CH_2, $n = 3$).—Prepared in the same way as the L-lysine-analogue, this *compound* (79%) had m. p. 132—133° after recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) (Found: C, 61·1; H, 5·8; N, 8·0; S, 6·0. $C_{29}H_{33}O_7N_3S$ requires C, 61·4; H, 5·9; N, 7·4; S, 5·7%).

γ-*Benzyloxycarboxyamido*-α-*toluene*-p-*sulphonamido*-L-*butyrylglycine Benzyl Ester* (III; R = Ph·CH_2, $n = 2$).—Prepared by the method used for the L-lysine analogue, this *compound*

[18] Zaoral, Rudinger, and Šorm, *Chem. Listy*, 1953, **47**, 427; *Chem. Abs.*, 1955, **49**, 179.
[19] Rudinger, *Coll. Czech. Chem. Comm.*, 1954, **19**, 365.

(72%) had m. p. 156—157° after recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) (Found: C, 60·2; H, 5·6; N, 7·8. $C_{28}H_{31}O_7N_3S$ requires C, 60·7; H, 5·6; N, 7·6%).

ε-N-*Benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-L-*lysylglycine* (IV; $n = 4$).—A solution of the ethyl ester (5·17 g.) in acetone (20 c.c.) and N-sodium hydroxide (20 c.c.) was left overnight at room temperature. The oil, which separated after acidification, solidified at 0°. Crystallisation from ethyl acetate–light petroleum (b. p. 60—80°) afforded the *acid* (needles), m. p. 120—121°, although one batch (prisms) had m. p. 140—141°. The two specimens had identical elementary compositions and infrared spectra (Found: C, 56·5; H, 6·0; N, 8·2. $C_{23}H_{29}O_7N_3S$ requires C, 56·2; H, 5·9; N, 8·5%).

δ-N-*Benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-DL-*ornithylglycine* (IV; $n = 3$) was obtained in almost theoretical yield by the method described for the foregoing compound. After crystallisation from ethyl acetate or aqueous acetone, it had m. p. 155—156° (Found: C, 55·5; H, 5·7; N, 8·9; S, 6·6. $C_{22}H_{27}O_7N_3S$ requires C, 55·3; H, 5·7; N, 8·8; S, 6·7%). The L-*enantiomorph* (79%), prepared in the same manner, had m. p. 164·5—165·5° after recrystallisation from ethyl acetate–light petroleum (b. p. 40—60°) (Found: C, 55·3; H, 5·6; N, 9·3%).

α-N-*Toluene*-p-*sulphonyl*-L-*lysylglycine* (V; $n = 4$).—(*a*) Hydrogenolysis of ε-*N*-benzyloxycarbonyl-α-*N*-toluene-*p*-sulphonyl-L-lysylglycine (IV; $n = 4$) in methanol containing a few drops of acetic acid with palladous oxide as catalyst afforded a theoretical yield of *product*, which crystallised from water as a monohydrate, m. p. 242—244° (decomp.), $[\alpha]_D^{23}$ −29·8° (*c* 2·3 in N-HCl) (Found: C, 48·2; H, 6·7; N, 11·6; S, 8·7. $C_{15}H_{23}O_5N_3S,H_2O$ requires C, 48·0; H, 6·7; N, 11·2; S, 8·5%).

(*b*) Hydrogenolysis of ε-*N*-benzyloxycarbonyl-α-*N*-toluene-*p*-sulphonyl-L-lysylglycine benzyl ester (III; R = Ph·CH_2, $n = 4$) in methanol afforded the same compound (92%), m. p. and mixed m. p. 242—244° (decomp.), $[\alpha]_D^{21}$ −30·0° (*c* 3·32 in N-HCl).

α-N-*Toluene*-p-*sulphonyl*-DL-*ornithylglycine* (V; $n = 3$).—This *compound* (94%) was obtained by hydrogenolysis of the δ-*N*-benzyloxycarbonyl derivative (IV; $n = 3$) in methanol containing a few drops of acetic acid in the presence of palladous oxide. Recrystallised from aqueous acetone, it had m. p. 220—222° (decomp.) (Found: C, 48·8; H, 6·1; N, 12·2. $C_{14}H_{21}O_5N_3S$ requires C, 49·0; H, 6·2; N, 12·2%). The L-*enantiomorph* (88%) was obtained in the same manner, having m. p. approx. 205° (decomp.) after darkening above 190° (the behaviour depends on the rate of heating), $[\alpha]_D^{22}$ −31·4° (*c* 1·2 in N-HCl) (Found: C, 48·7; H, 6·5; N, 12·6%).

γ-*Amino*-α-*toluene*-p-*sulphonamido*-L-*butyrylglycine* (V; $n = 2$).—γ-Benzyloxycarboxyamido-α-toluene-*p*-sulphonamido-L-butyrylglycine benzyl ester was hydrogenolysed in the usual way. The *product* (85%), crystallised from water–ethanol–ether or water–acetone–ether, had m. p. 219—221° (decomp.), $[\alpha]_D^{21}$ −18·4° (*c* 3 in N-HCl) (Found: C, 47·1; H, 5·8; N, 12·7. $C_{13}H_{19}O_5N_3S$ requires C, 47·4; H, 5·8; N, 12·8%).

α-N-*Toluene*-p-*sulphonyl*-DL-*ornithine* was prepared by hydrogenolysis of the δ-*N*-benzyloxycarbonyl derivative (II; $n = 3$). It (75%) had m. p. 212·5—213·5° (decomp.) after recrystallisation from water–ethanol–ether (Found: C, 50·6; H, 6·6; N, 9·2. $C_{12}H_{18}O_4N_2S$ requires C, 50·3; N, 6·3; N, 9·8%).

α-N-*Toluene*-p-*sulphonyl*-DL-*arginine*.—α-*N*-Toluene-*p*-sulphonyl-DL-ornithine (206 mg.) and *S*-methyl*iso*thiuronium iodide (180 mg.) were kept in a mixture of aqueous ammonia (*d* 0·880; 2 c.c.) and methanol (0·5 c.c.) at room temperature during 4 days. α-N-*Toluene*-p-*sulphonyl*-DL-*arginine* (164 mg., 69%) separated and was crystallised from water. It decomposed gradually above 250°, but did not melt below 330° (Found: C, 47·6; H, 6·2; N, 16·9; S, 9·7. $C_{13}H_{20}O_4N_4S$ requires C, 47·5; H, 6·1; N, 17·1; S, 9·8%). α-*N*-Toluene-*p*-sulphonyl-L-arginine, m. p. 256—257° (decomp.), was prepared by the method of Bergmann, Fruton, and Pollock,[20] and was converted into the *hydrobromide* (VII), m. p. 197—199° (decomp.) (after recrystallisation from ethanol–ether) (Found: C, 38·2; H, 5·3; N, 13·3. $C_{13}H_{21}O_4N_4SBr$ requires C, 38·1; H, 5·2; N, 13·7%).

α-N-*Toluene*-p-*sulphonyl*-L-*homoarginylglycine* (VI; $n = 4$).—α-*N*-Toluene-*p*-sulphonyl-L-lysylglycine (335 mg.) and *O*-methyl*iso*uronium hydrogen sulphate (242 mg.) were kept in aqueous sodium hydroxide (1·65 c.c.) at pH 10—11 at room temperature overnight. Some *product* separated and a further quantity was obtained by adjusting the pH of the filtrate to 7 and keeping the solution overnight at 0°. The combined crops (50%), recrystallised from

[20] Bergmann, Fruton, and Pollock, *J. Biol. Chem.*, 1939, **127**, 643.

water, had m. p. 262—264° (decomp.), $[\alpha]_D^{21}$ −33·2° (*c* 1·69 in N-HCl) (Found: C, 48·0; H, 6·4; N, 18·0. $C_{16}H_{25}O_5N_5S$ requires C, 48·1; H, 6·3; N, 17·5%). The *picrate* of this compound crystallised from water, having m. p. 184—185° (Found: C, 41·8; H, 4·8; N, 17·7; S, 5·0. $C_{22}H_{28}O_{12}N_8S$ requires C, 42·0; H, 4·5; N, 17·9; S, 5·1%).

α-N-*Toluene*-p-*sulphonyl*-DL-*arginylglycine* (VI; $n = 3$).—Reaction of α-*N*-toluene-*p*-sulphonyl-DL-ornithylglycine with *O*-methyl*iso*uronium hydrogen sulphate or *S*-methyl*iso*-thiuronium iodide in aqueous sodium hydroxide at pH 10·5—11·0 during 4 days afforded 82% and 71% respectively of the *arginine derivative* which crystallised from water as a dihydrate, m. p. 265—266° (decomp.) (Found: C, 42·9; H, 6·4; N, 16·4. $C_{15}H_{23}O_5N_5S,2H_2O$ requires C, 42·7; H, 6·5; N, 16·6%).

α-N-*Toluene*-p-*sulphonyl*-L-*arginylglycine* (VI; $n = 3$).—(*a*) By using *S*-methyl*iso*-thiuronium iodide, this *compound* (56%) was obtained as the sesquihydrate by the method described above. After recrystallisation from water, it melted over a range with frothing and had $[\alpha]_D^{21}$ −36·8° (*c* 1·28 in 10% H_2SO_4) (Found: C, 44·0; H, 6·6; N, 17·2. $C_{15}H_{23}O_5N_5S,1\frac{1}{2}H_2O$ requires C, 43·7; H, 6·4; N, 17·0%).

(*b*) α-*N*-Toluene-*p*-sulphonyl-L-arginine hydrobromide (2·05 g.), glycine ethyl ester (0·52 g.), and tetraethyl pyrophosphite (2·85 g.) were heated in diethyl hydrogen phosphite (5 c.c.) at 100° for 1 hr. with exclusion of moisture. Methanol (10 c.c.) and ether (250 c.c.) were added to the cooled solution and the mixture was left at 0° overnight. The supernatant liquid was decanted and the residual gum was dissolved in methanol (10 c.c.) and N-sodium hydroxide (10 c.c.). After 4 hr. at room temperature, the solution was adjusted to pH 7 with dilute hydrochloric acid, and the product (1·05 g., 50%) was caused to crystallise by addition of acetone and ether to faint turbidity followed by storage at 0° overnight. After recrystallisation from water, it melted unsharply and had $[\alpha]_D^{21}$ −36·6° (*c* 1·3 in 10% H_2SO_4). The two samples were indistinguishable on a paper chromatogram irrigated with butan-1-ol–acetic acid–water (4 : 1 : 5).

ε-N-*Benzyloxycarbonyl*-α-N-*toluene*-p-*sulphonyl*-L-*lysine* p-*Benzyloxycarbonylanilide* (VIII; R = Ph·CH_2·O·CO, R′ = Ph·CH_2).—Several attempts to synthesise this *compound* from ε-*N*-benzyloxycarbonyl-α-*N*-toluene-*p*-sulphonyl-L-lysine and benzyl *p*-aminobenzoate by using tetraethyl pyrophosphite afforded yields of 15—38%. Several experiments using the chloroformate method of peptide synthesis [13] are summarised below:

Chloroformate	Et	Et.	Et	Bu^s
Solvent	Chloroform	Dioxan	Tetrahydrofuran	Tetrahydrofuran
Yield (%)	11	14	21	6

Crystallised from ethanol, it had m. p. 144—145° (Found: C, 65·3; H, 6·1; N, 6·6. $C_{35}H_{37}O_7N_3S$ requires C, 65·3; H, 5·8; N, 6·5%).

Attempted hydrogenolysis of this compound resulted in a 92% recovery of starting material. Reaction with hydrogen bromide in acetic acid gave no identifiable product.

δ-N-*Benzyloxycarbonyl*-α-N-*phthaloylglycyl*-DL-*ornithine Methyl Ester*.—*N*-Phthaloylglycine (410 mg.), δ-*N*-benzyloxycarbonyl-DL-ornithine methyl ester hydrochloride (633 mg.), triethylamine (205 mg.), and tetraethyl pyrophosphite (706 mg.) were heated in diethyl hydrogen phosphite (2 c.c.) at 100° for 105 min. Isolation in the usual way afforded the *peptide derivative* (481 mg.), m. p. 176·5—177·5° after recrystallisation from aqueous ethanol (Found: C, 61·7; H, 5·4. $C_{24}H_{25}O_7N_3$ requires C, 61·7; H, 5·3%).

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