723. *Bis-o-phenylene Pyrophosphite: A New Reagent for Peptide Synthesis. Part II.* Some Peptide Syntheses with the New Reagent.*

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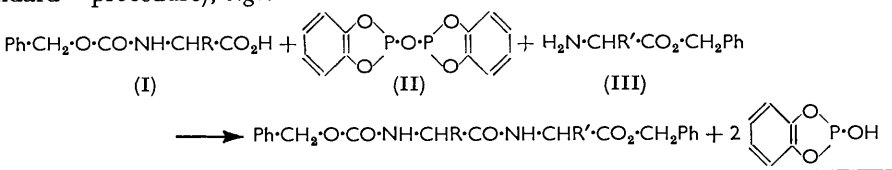
The use of bis-*o*-phenylene pyrophosphite as a reagent for the synthesis of peptides is described. The experimental procedure is simple and the yields are good, and, although racemisation occurs in certain circumstances, the new method is recommended for general use.

Ester-interchange during the catalytic hydrogenolysis of peptide benzyl esters can be avoided by using t-butyl alcohol as solvent.

In Part I * we described the preparation of bis-*o*-phenylene pyrophosphite (II), in good yield and by a simple procedure, from catechol and phosphorus trichloride. We now describe the use of this reagent in peptide syntheses, as a substitute for the widely used, but less easily prepared, tetraethyl pyrophosphite.[1,2]

As with tetraethyl pyrophosphite,[1] so with the new reagent, peptide syntheses can be carried out either by direct interaction of the reagent with the two reactants (the " standard " procedure), *e.g.*:



* Part I, Crofts, Markes, and Rydon, *J.*, 1958, 4250.

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

[2] For some important synthetical applications of tetraethyl pyrophosphite see, *inter alia*, Anderson, *ibid.*, 1953, **75**, 6081; du Vigneaud, Ressler, Swan, Roberts, and Katsoyannis, *ibid.*, 1954, **76**, 3115; Gish and du Vigneaud, *ibid.*, 1957, **79**, 3579; Ressler and du Vigneaud, *ibid.*, p. 4511; Katsoyannis, Gish, and du Vigneaud, *ibid.*, p. 4516.

or by so-called " anhydride " or " amide " procedure, involving preliminary reaction of the pyrophosphite with, respectively, the carboxyl (I), or amino-component (III), followed by reaction of the resulting mixed anhydride or phosphoramidite with the second reactant, (III) or (I).

The " standard " procedure is by far the most convenient of the three, and we have not found either of the alternatives to possess any marked advantages in the cases we have investigated. Tetraethyl pyrophosphite is generally used in diethyl hydrogen phosphite as solvent; with bis-*o*-phenylene pyrophosphite, the more readily available pyridine has been satisfactory † and has the advantage that amino-acid or peptide esters can be used as their hydrohalides or toluene-*p*-sulphonates. The experimental procedure is very simple, an *N*-protected (*N*-benzyloxycarbonyl- or *N*-phthaloyl-) amino-acid or peptide, *e.g.*, (I), and an equimolecular amount of an amino-acid or peptide ester, *e.g.*, (III), conveniently, although not necessarily, as its hydrochloride or toluene-*p*-sulphonate, being heated in pyridine on the steam-bath for half an hour with a 10% excess of bis-*o*-phenylene pyrophosphite; the cooled mixture is then poured into water, and the product isolated by an appropriate procedure.

In the following Table are recorded the yields, of purified product, obtained in seventeen peptide syntheses carried out by this " standard " procedure (the arrangement and abbreviations are those used by Goodman and Kenner,[4] *e.g.*, Z = Ph·CH_2·O·CO–). It will be seen that the yields are, in general, very satisfactory; excluding the tyrosine peptides and the experiments in which extensive racemisation occurred, the average yield of purified material is 67%; in most cases the crude product, obtained in substantially higher yield, is sufficiently pure for preparative purposes. The method is unsuitable for the synthesis of tyrosine peptides without prior protection of the phenolic hydroxyl group; this is not wholly unexpected in view of the ease with which this hydroxyl group undergoes *O*-phosphorylation.[5]

Product	Reactants	Yield (%)
Z·Gly·Leu·OMe	Z·Gly·OH; H·Leu·OMe	88
Z·Gly·Leu·OBz	Z·Gly·OH; H·Leu·OBz	76
Phth·Gly·Tyr·OBz	Phth·Gly·OH; H·Tyr·OBz	21
Z·Gly·Gly·Cys(SBz)·OBz	Z·Gly·Gly·OH; H.Cys(SBz)·OBz	74
Z·Cys(SBz)·Gly·Gly·OEt	Z·Cys(SBz)·OH; H·Gly·Gly·OEt	52
Z·Cys(SBz)·Gly·Gly·OBz	Z·Cys·(SBz)·OH; H·Gly·Gly·OBz	56
Z·Gly·Gly·Gly·OBz	Z·Gly·Gly·OH; H·Gly·OBz	79
Z·Leu·Gly·Gly·OBz	Z·Leu·OH; H·Gly·Gly·OBz	69
Z·Met·Gly·Gly·OBz	Z·Met·OH; H·Gly·Gly·OBz	59
Z·Tyr·Gly·Gly·OBz	Z·Tyr·OH; H·Gly·Gly·OBz	25
Z·Gly·Leu·Gly·OBz	Z·Gly·Leu·OH; H·Gly·OBz	38 *
Phth·Gly·Tyr·Gly·OBz	Phth·Gly·Tyr·OH; H·Gly·OBz	14 *
Z·Gly·Gly·Leu·OBz	Z·Gly·Gly·OH; H·Leu·OBz	70
Z·Gly·Gly·Met·OBz	Z·Gly·Gly·OH; H·Met·OBz	40
Z·Gly·Gly·Phe·OBz	Z·Gly·Gly·OH; H·Phe·OBz	70
Z·Gly·Gly·Tyr·OBz	Z·Gly·Gly·OH; H·Tyr·OBz	45
Z·Gly·Gly·Gly·Gly·OBz	Z·Gly·Gly·OH; H·Gly·Gly·OBz	70

* Product extensively racemised.

It is of considerable importance to know in what circumstances a peptide synthesis may be accompanied by racemisation and we have accordingly examined this aspect. As with other methods, no racemisation occurred when the amino-component, *e.g.*, (III), alone contained an asymmetric centre or in coupling *N*-benzyloxycarbonyl-L-amino-acids

† Since our work was completed, Maclaren [3] has reported the use of pyridine as a solvent in syntheses with tetraethyl pyrophosphite.

[3] Maclaren, *Australian J. Chem.*, 1958, **11**, 360.

[4] Goodman and Kenner, *Adv. Protein Chem.*, 1957, **12**, 465; cf. Erlanger and Brand, *J. Amer. Chem. Soc.*, 1951, **73**, 3508.

[5] Ashbolt and Rydon, *Biochem. J.*, 1957, **66**, 237.

with amino-acid or peptide esters; this point was established in the coupling of *N*-benzyloxycarbonylglycylglycine with L-leucine benzyl ester, and of *N*-benzyloxycarbonyl-L-leucine with glycylglycine benzyl ester, by the rigorous method of hydrogenolysis of the product, followed by complete hydrolysis and examination of the optical rotatory power of the hydrolysate, rather than the usual, but less conclusive, method of comparing the optical rotatory power of the product with that of a specimen made by a method known, or presumed, not to cause racemisation. As in syntheses by other reagents,[6] however, extensive racemisation occurred in coupling carboxyl components of the type X·NH·CHR·CO·NH·CHR'·CO_2H, possibly owing to oxazolone formation;[6,7] racemisation occurs similarly with tetraethyl pyrophosphite.[1] With our reagent, racemisation was observed when the " amide " and the " anhydride " procedure were used, as well as in the " standard " conditions; racemisation was not suppressed when pyridine was replaced by diethyl phosphite as solvent.

During this work, we synthesised diglycyl-L-leucine by way of L-leucine benzyl ester toluene-*p*-sulphonate and *N*-benzyloxycarbonyldiglycyl-L-leucine benzyl ester; by a coincidence, both intermediates exhibited zero rotation, although the final product was shown, by the hydrolysis procedure already mentioned, to have full optical activity.

The new method of peptide synthesis described in this paper has a number of advantages; the experimental procedure is very simple and the reagent used is easily prepared and may be purified by vacuum-distillation and stored indefinitely in a stoppered bottle. Since the method was first developed, in 1955, it has been used extensively by colleagues for many peptide syntheses, in addition to those described in the present paper; at present it is one of the three methods which we generally prefer for peptide synthesis, the others being the azide [8] and the carbodi-imide [9] method.

Benzyl esters are frequently used, particularly in conjunction with *N*-benzyloxycarbonyl groups, in peptide synthesis, both protecting groups being eventually removed by catalytic hydrogenolysis, generally in ethanol or aqueous ethanol, often with the addition of acetic acid. In this work, we observed that catalytic hydrogenolysis of *N*-benzyloxycarbonyldiglycyl-leucine benzyl ester and of *N*-benzyloxycarbonyl-leucylglycylglycine benzyl ester was not entirely satisfactory in ethanolic solvents, the products being contaminated with some tripeptide ethyl ester; similar difficulties have been encountered in other cases. This unwanted ester-interchange can be entirely avoided by using t-butyl alcohol in place of ethanol and we recommend this as a general procedure.

Experimental

Pyridine was dried for some weeks over potassium hydroxide, shaken overnight with freshly ignited barium oxide, and then distilled over barium oxide. Light petroleum denotes the fraction of b. p. 60—80°. Rotations were measured in 2 dm. tubes. The purity of all products was checked by chromatography on Whatman No. 1 paper, with butan-1-ol–pyridine–water (39 : 21 : 39) or butan-1-ol–acetic acid–water (100 : 17 : 38) as developing solvent.

Benzyl Ester Toluene-p-*sulphonates*.—The following modified procedure [10,11] was used: The amino-acid or peptide (0·1 mole) and toluene-*p*-sulphonic acid monohydrate (0·1 mole) were refluxed with benzyl alcohol (100—250 ml.) in 1—4 volumes of benzene in an apparatus fitted with a Dean and Stark tube to remove entrained water. Refluxing was continued for some hours after water ceased to be produced; benzene and some excess of benzyl alcohol were removed under reduced pressure (max. temperature 125°), and the residue was ground and slurried with ether. The insoluble product was then recrystallised.

[6] Kenner, *Chem. Soc. Special Publ.*, 1955, **2**, 103; cf. Goodman and Kenner, ref. 4.
[7] Young, Wood, Joyce, and Anderson, *J. Amer. Chem. Soc.*, 1956, **78**, 2126.
[8] Curtius, *Ber.*, 1902, **35**, 3226.
[9] Sheehan and Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067; Khorana, *Chem. and Ind.*, 1955, 1087; Sheehan, Goodman, and Hess, *J. Amer. Chem. Soc.*, 1956, **78**, 1367.
[10] Miller and Waelsch, *J. Amer. Chem. Soc.*, 1952, **74**, 1092; Cipera and Nicholls, *Chem. and Ind.*, 1955, 16.
[11] Hooper, Rydon, Schofield, and Heaton, *J.*, 1956, 3148.

The following were prepared in this way:

Glycine benzyl ester toluene-p-*sulphonate* (83%), m. p. 132°, from methanol–di-isopropyl ether (Found: C, 57·0; H, 5·5; N, 4·0. $C_{16}H_{19}O_5NS$ requires C, 57·0; H, 5·7; N, 4·2%).

Glycylglycine benzyl ester toluene-*p*-sulphonate (this compound was first prepared in these laboratories by Dr. D. Morris [12]), m. p. 153° (from ethanol) (76% yield).

L-*Leucine benzyl ester toluene*-p-*sulphonate* (74%), m. p. 157° (from benzene–light petroleum), $[\alpha]_D^{23\cdot5}$ 0·0° (*c* 2·00 in ethanol) (Found: C, 60·8; H, 7·1; N, 3·9. $C_{20}H_{27}O_5NS$ requires C, 61·0; H, 6·9; N, 3·6%); this salt was precipitated from the reaction mixture with light petroleum.

DL-*Methionine benzyl ester toluene*-p-*sulphonate* (60%), m. p. 129—131° (from ethyl acetate) (Found: C, 55·3; H, 6·2; N, 3·5. $C_{19}H_{25}O_5NS_2$ requires C, 55·4; H, 6·1; N, 3·4%).

DL-*Phenylalanine benzyl ester toluene*-p-*sulphonate* (59%), m. p. 149° (from water) (Found: C, 65·2; H, 5·7; N, 3·4. $C_{23}H_{25}O_5NS$ requires C, 64·6; H, 5·9; N, 3·3%).

L-Tyrosine benzyl ester toluene-*p*-sulphonate (this compound was first prepared in these laboratories by Dr. D. Morris [12]), m. p. 178° (from water), $[\alpha]_D^{23}$ −10·3° (*c* 1·00 in 60% acetone) (85% yield) (Found: C, 62·3; H, 5·4; N, 3·4. Calc. for $C_{23}H_{25}O_6NS$: C, 62·3; H, 5·7; N, 3·2%).

Peptide Syntheses.—Unless otherwise stated, the following, " standard," procedure was employed:

Equimolecular amounts of the carboxylic acid and amino-reactants, in anhydrous pyridine, were treated with bis-*o*-phenylene pyrophosphite (10% excess) (calcium chloride guard-tube). The mixture was then heated on a steam-bath for 30 min., cooled, and poured into ice and water. Next morning, the product was separated. If solid, it was filtered off, washed with N-sodium hydrogen carbonate, N-hydrochloric acid, and water, dried, and recrystallised; if an oil, it was dissolved in ethyl acetate, washed similarly, dried, and recovered.

In the " amide " procedure, the amino-reactant was heated, in pyridine, on the steam-bath for 2 min. with bis-*o*-phenylene pyrophosphite before addition of the carboxylic acid reactant. In the " anhydride " procedure, the carboxylic acid reactant and the pyrophosphite were similarly heated together for 2 min. before addition of the amine reactant.

N-*Benzyloxycarbonylglycyl*-L-*leucine Benzyl and Methyl Ester.*—*N*-Benzyloxycarbonylglycine [13] (8·37 g.) and L-leucine benzyl ester toluene-*p*-sulphonate (15·74 g.) were coupled in pyridine (25 ml.) by using bis-*o*-phenylene pyrophosphite (12·94 g.). The product (16·1 g., 98%) was an oil. Reprecipitation from ethyl acetate with light petroleum gave the *peptide benzyl ester* as an oil (12·6 g., 76%), $[\alpha]_D^{19}$ −11·1° (*c* 2·39 in ethyl acetate) (Found: C, 67·5; H, 7·1; N, 6·1. $C_{23}H_{28}O_5N_2$ requires C, 67·0; H, 6·8; N, 6·8%).

The *methyl ester*, prepared similarly in 88% yield from L-leucine methyl ester hydrochloride,[14] was an oil, $[\alpha]_D^{20\cdot5}$ −9·0° (*c* 2·05 in dioxan) (Found: C, 60·8; H, 7·0; N, 7·7. $C_{17}H_{24}O_5N_2$ requires C, 60·7; H, 7·2; N, 8·3%). Saponification (shaking for 45 min. with N-sodium hydroxide, followed by acidification) afforded *N*-benzyloxycarbonylglycyl-L-leucine (31%, after recrystallisation from aqueous ethanol), m. p. 102°, $[\alpha]_D^{20}$ −18·3° (*c* 1·39 in N-sodium hydroxide) (Vaughan [15] gives m. p. 99—100°, and Goldschmidt and Lautenschlager [16] give m. p. 104°, $[\alpha]_D^{14\cdot5}$ −17·9°).

N-*Phthaloyglycyl*-L-*tyrosine Benzyl Ester.*—N-Phthaloylglycine [17] (6·15 g.) and L-tyrosine benzyl ester toluene-*p*-sulphonate (13·30 g.) were coupled in pyridine (20 ml.), by using bis-*o*-phenylene pyrophosphite (9·70 g.). Several recrystallisations of the crude product (8·20 g., 60%), m. p. 172—176°, from ethyl acetate–light petroleum gave the pure *peptide benzyl ester* (2·88 g., 21%), m. p. 189°, $[\alpha]_D^{19}$ +54·4° (*c* 1·43 in ethyl acetate) (Found: N, 6·5. $C_{26}H_{22}O_6N_2$ requires N, 6·1%). Shaking this ester (4·50 g.), in ethanol (150 ml.) and water (30 ml.), in hydrogen, with 5% palladised charcoal, gave *N*-phthaloylglycyl-L-tyrosine (2·42 g., 67%), m. p. 257—258° (from methanol–di-isopropyl ether), $[\alpha]_D^{21}$ +75·0° (*c* 1·00 in 80% acetone (Turner [18] gives m. p. 241—244° and Hanson and Illhardt [19] give m. p. 235—237°).

N-*Benzyloxycarbonyldiglycyl*-S-*benzyl*-L-*cysteine Benzyl Ester.*—*N*-Benzyloxycarbonylglycylglycine [13] (2·66 g.) and *S*-benzyl-L-cysteine benzyl ester toluene-*p*-sulphonate [11] (4·73 g.) were

[12] Morris, Ph.D. Thesis, Manchester, 1956.
[13] Bergmann and Zervas, *Ber.*, 1932, **65**, 1192.
[14] Schott, Larkin, Rockland, and Dunn, *J. Org. Chem.*, 1947, **12**, 490.
[15] Vaughan, *J. Amer. Chem. Soc.*, 1952, **74**, 6137.
[16] Goldschmidt and Lautenschlager, *Annalen*, 1953, **580**, 68.
[17] Drechsel, *J. prakt. Chem.*, 1883, **27**, 418.
[18] Turner, *J. Amer. Chem. Soc.*, 1953, **75**, 2388.
[19] Hanson and Illhardt, *Z. physiol. Chem.*, 1954, **298**, 210.

coupled in pyridine (10 ml.), by using bis-*o*-phenylene pyrophosphite (3·23 g.). Recrystallisation of the solid product (4·84 g., 88%), m. p. 109—111°, from ethyl acetate–light petroleum gave the pure peptide ester (4·05 g.; 74%), m. p. 115·5—116·5°, $[\alpha]_D^{22\cdot5}$ −35·0° (*c* 0·54 in ethanol) (Lautsch and Kraege [20] give m. p. 115·5—116·5°, $[\alpha]_D^{21}$ −32·3°).

N-*Benzyloxycarbonyl*-S-*benzyl*-L-*cysteinylglycylglycine Benzyl and Ethyl Esters.*—*N*-Benzyloxycarbonyl-*S*-benzyl-L-cysteine [21] (1·725 g.) and glycylglycine benzyl ester toluene-*p*-sulphonate (1·975 g.) were coupled in pyridine (3·5 ml.), by using bis-*o*-phenylene pyrophosphite (1·615 g.). The crude product (2·22 g., 78%), m. p. 120—123°, recrystallised from aqueous ethanol, affording the *peptide benzyl ester monohydrate* (1·58 g., 56%), m. p. 135°, $[\alpha]_D^{18}$ −14·7° (*c* 1·01 in ethyl acetate) (Found: C, 61·6, 62·1, 61·7; H, 5·7, 6·0, 5·8; N, 7·5; S, 5·5. $C_{29}H_{31}O_6N_3S,H_2O$ requires C, 61·4; H, 5·9; N, 7·4; S, 5·6%).

The ethyl ester, prepared similarly from glycylglycine ethyl ester hydrochloride, in 52% yield, and recrystallised from ethyl acetate–light petroleum, had m. p. 111—113°, $[\alpha]_D^{18}$ −12·0° (*c* 3·21 in ethanol) (Hooper *et al.*[11] give m. p. 114°, $[\alpha]_D^{20}$ −12·9°).

N-*Benzyloxycarbonyldiglycylglycine Benzyl Ester.*—*N*-Benzyloxycarbonylglycylglycine [13] (2·66 g.) and glycine benzyl ester toluene-*p*-sulphonate (3·37 g.) were coupled in pyridine (10 ml.) by using bis-*o*-phenylene pyrophosphite (3·23 g.). The crude product (3·84 g., 93%), m. p. 156—158°, recrystallised from aqueous ethanol, affording the peptide ester (3·28 g., 79%), m. p. 161° (Found: C, 61·2; H, 5·7; N, 10·2. Calc. for $C_{21}H_{23}O_6N_3$: C, 61·1; H, 5·6; N, 10·2%) (Hooper *et al.*[11] give m. p. 148° for material prepared by the mixed anhydride procedure).

N-*Benzyloxycarbonyl*-L-*leucylglycylglycine Benzyl Ester.*—*N*-Benzyloxycarbonyl-L-leucine [22] (7·95 g.) and glycylglycine benzyl ester toluene-*p*-sulphonate (11·82 g.) were coupled in pyridine (21 ml.) by using bis-*o*-phenylene pyrophosphite (9·70 g.). The crude product (10·4 g., 74%), m. p. 115—117°, $[\alpha]_D^{21}$ −11·0° (*c* 2·00 in dioxan), recrystallised from ethyl acetate–light petroleum, yielding the *peptide ester* (9·7 g., 69%), m. p. 117—118°, $[\alpha]_D^{21}$ −11·7° (*c* 2·00 in dioxan) (Found: C, 63·3, 63·4; H, 6·8, 6·9; N, 8·9. $C_{25}H_{31}O_6N_3$ requires C, 63·9; H, 6·7; N, 9·0%); two further recrystallisations, thorough washing with N-sodium hydrogen carbonate and a further recrystallisation removed a little yellow impurity, raising the m. p. to 122—123°, but leaving $[\alpha]_D$ unchanged. A similar yield was obtained by the " amide " procedure.

This ester (5·30 g.), in t-butyl alcohol (150 ml.) and water (30 ml.), was shaken in hydrogen over 5% palladised charcoal, water (10 ml. portions) being added from time to time to keep the peptide in solution. When absorption was complete, the mixture was heated on the steam-bath for a few minutes and filtered hot. Evaporation of the filtrate gave 2·47 g. (89%) of chromatographically pure L-leucylglycylglycine, $[\alpha]_D^{20}$ +57·3° (*c* 5·01 in water); recrystallisation from aqueous propan-2-ol gave 1·47 g. (53%), $[\alpha]_D^{21}$ +57·2° (Schott *et al.*[14] give $[\alpha]_D^{25}$ +57·7°). The crude tripeptide (1·0000 g.) was heated on a steam-bath for 24 hr. with constant-boiling hydrochloric acid (9 ml.) in a previously heat-treated 10 ml. graduated flask fitted with a ground-in double-surface condenser, then cooled to room temperature. The volume was made up to 10 ml. with constant-boiling hydrochloric acid; the resulting solution had $[\alpha]_D^{19}$ +1·76°; a similarly treated mixture of L-leucine (0·5348 g.) and glycine (0·6121 g.) had $[\alpha]_D^{17}$ +1·73°. Hydrolysis of the peptide to L-leucine and glycine was shown by paper chromatography to be complete.

N-*Benzyloxycarbonyl*-DL-*methionylglycylglycine Benzyl Ester.*—*N*-Benzyloxycarbonyl-DL-methionine [23] (2·83 g.) and glycylglycine benzyl ester toluene-*p*-sulphonate (3·94 g.) were coupled in pyridine (7 ml.) by using bis-*o*-phenylene pyrophosphite (3·23 g.). Recrystallisation of the crude product (3·47 g., 71%) from aqueous ethanol gave the *peptide ester* (2·85 g., 59%), m. p. 115·5—117° (Found: C, 59·4; H, 5·7; N, 8·6. $C_{24}H_{29}O_6N_3S$ requires C, 59·1; H, 6·0; N, 8·6%).

N-*Benzyloxycarbonyl*-L-*tyrosylglycylglycine Benzyl Ester.*—*N*-Benzyloxycarbonyl-L-tyrosine [13] (0·75 g.) and glycylglycine benzyl ester toluene-*p*-sulphonate (0·95 g.) were coupled in pyridine (2 ml.) by using bis-*o*-phenylene pyrophosphite (0·81 g.). The crude product (0·98 g., 79%), m. p. 80—88°, recrystallised from ethyl acetate–light petroleum and from aqueous ethanol, yielding the peptide ester (0·31 g., 25%), m. p. 136°, $[\alpha]_D^{18}$ −8·5° (*c* 1·40 in acetone) (Morris [12] gives m. p. 132—133°, $[\alpha]_D^{16}$ −8·5°).

[20] Lautsch and Kraege, *Chem. Ber.*, 1956, **89**, 737.
[21] Harington and Mead, *Biochem. J.*, 1936, **30**, 1598.
[22] Bergmann, Zervas, and Fruton, *J. Biol. Chem.*, 1936, **115**, 593.
[23] Dekker and Fruton, *ibid.*, 1948, **173**, 471.

N-Benzyloxycarbonylglycyl-L-leucylglycine Benzyl Ester.—(i) *N*-Benzyloxycarbonylglycyl-L-leucine (3·23 g.) and glycine benzyl ester toluene-*p*-sulphonate (3·37 g.) were coupled, in pyridine (7 ml.), by the " standard " procedure, with bis-*o*-phenylene pyrophosphite (3·23 g.). The crude product, isolated with ethyl acetate, solidified (3·26 g., 70%) on treatment with ethyl acetate–light petroleum and had m. p. 122—125°, $[\alpha]_D^{15·5}$ −14·6° (*c* 2·00 in ethanol). Recrystallisation from aqueous ethanol, followed by washing with N-sodium hydrogen carbonate, N-hydrochloric acid and water and two further recrystallisations, gave 1·76 g. (38%), m. p. 127—128°, $[\alpha]_D^{16·5}$ −15·4° (*c* 2·00 in ethanol). This material (1·52 g.) was hydrogenolysed as usual in aqueous t-butyl alcohol over palladised charcoal; the product (0·63 g., 80%) had $[\alpha]_D^{17·5}$ −19·3° (*c* 2·50 in water), indicating it to be about 55% racemised (Bergmann *et al.*[24] give $[\alpha]_D^{24}$ −41·2° for pure glycyl-L-leucylglycine).

(ii) A similar coupling, carried out by the " amide " procedure, gave a crude product (3·85 g., 82%) with m. p. 92—94°, $[\alpha]_D^{18·5}$ −8·8° (*c* 2·01 in ethanol). This was recrystallised once from aqueous ethanol, washed with N-sodium hydrogen carbonate, N-hydrochloric acid, and water, and then fractionally crystallised from aqueous ethanol, affording much extensively racemised material (1·51 g., 32%), m. p. 128—130°, $[\alpha]_D^{17·5}$ + 2·4° (*c* 2·00 in ethanol), and a little optically pure *peptide ester* (0·36 g., 8%), m. p. 102—104°, $[\alpha]_D^{17·5}$ −31·8° (*c* 2·00 in ethanol) (Found: C, 63·9; H, 6·6; N, 8·9. $C_{25}H_{31}O_6N_3$ requires C, 63·9; H, 6·7; N, 9·0%).

N-Phthaloylglycyl-L-tyrosylglycine Benzyl Ester.—*N*-Phthaloylglycyl-L-tyrosine (0·368 g.) and glycine benzyl ester toluene-*p*-sulphonate (0·377 g.) were coupled in pyridine (1·5 ml.) with bis-*o*-phenylene pyrophosphite (0·300 g.). The crude product (0·39 g., 76%), m. p. 113—118°, was thrice recrystallised from aqueous ethanol, once from aqueous 2-ethoxyethanol, and once from ethyl acetate–light petroleum, giving the partially racemised *peptide ester* (0·07 g., 14%), m. p. 167—169°, $[\alpha]_D^{20}$ +14·6° (*c* 0·65 in 2-ethoxyethanol) (Found: C, 64·4; H, 4·9; N, 8·2. $C_{28}H_{25}O_7N_3$ requires C, 65·2; H, 4·9; N, 8·2%); acid hydrolysis gave a product from which 10% of DL-tyrosine was isolated. Neither the yield nor the quality of the product was improved by using the " amide " coupling procedure.

N-Benzyloxycarbonyldiglycyl-L-leucine Benzyl Ester.—*N*-Benzyloxycarbonylglycylglycine [13] (7·98 g.) and L-leucine benzyl ester toluene-*p*-sulphonate (11·80 g.) were coupled in pyridine (21 ml.) with bis-*o*-phenylene pyrophosphite (9·70 g.). The product, isolated with ethyl acetate, was a yellow oil which crystallised on trituration with ethyl acetate–light petroleum; this was suspended in boiling ether (250 ml.) and precipitated with light petroleum. The solid (11·07 g., 79%), m. p. 91—92°, recrystallised from ethyl acetate–light petroleum, giving the pure *peptide ester* (9·86 g., 70%), m. p. 93—94°, $[\alpha]_D^{23·5}$ 0·0° (*c* 2·00 in chloroform) (Found: C, 63·9; H, 6·6; N, 8·9. $C_{25}H_{31}O_6N_3$ requires C, 63·9; H, 6·7; N, 9·0%).

This ester (9·10 g.) in t-butyl alcohol (170 ml.) and water (30 ml.), was shaken in hydrogen over 5% palladised charcoal for 34 hr. Working up as usual gave crude diglycyl-L-leucine (4·15 g., 81%), $[\alpha]_D^{18}$ −29·9° (*c* 2·01 in water), which crystallised from water as the *monohydrate*, m. p. 217° (decomp.), $[\alpha]_D^{19}$ −29·9° (*c* 2·00 in water) (Found: C, 46·3, 46·5; H, 7·9, 7·9; N, 16·0, 16·2. $C_{10}H_{19}O_4N_3,H_2O$ requires C, 45·6; H, 8·0; N, 16·0%) (Fruton *et al.*[25] give $[\alpha]_D^{26}$ −28·0° for the anhydrous tripeptide). The crude tripeptide monohydrate (1·0735 g.) was hydrolysed with constant-boiling hydrochloric acid as described for leucylglycylglycine; the hydrolysate, made up to 10 ml., had α_D^{17} +1·76°.

N-Benzyloxycarbonyldiglycyl-DL-methionine Benzyl Ester.—*N*-Benzyloxycarbonylglycylglycine [13] (5·32 g.) and DL-methionine benzyl ester toluene-*p*-sulphonate (8·23 g.) were coupled in pyridine (10 ml.) with bis-*o*-phenylene pyrophosphite (7·35 g.). The gummy product, isolated with ethyl acetate, crystallised on addition of light petroleum to a concentrated solution in ethyl acetate. Recrystallisation of this crude product (6·29 g., 65%) from ethyl acetate–light petroleum gave the pure *peptide ester* (3·90 g., 40%), m. p. 92—94° (Found: C, 58·8; H, 5·8; N, 8·5; S, 6·4. $C_{24}H_{29}O_6N_3S$ requires C, 59·1; H, 6·0; N, 8·6; S, 6·6%).

N-Benzyloxycarbonyldiglycyl-DL-phenylalanine Benzyl Ester.—*N*-Benzyloxycarbonylglycylglycine [13] (2·66 g.) and DL-phenylalanine benzyl ester toluene-*p*-sulphonate (4·27 g.) were coupled in pyridine (10 ml.) with bis-*o*-phenylene pyrophosphite (3·23 g.). The crude product, isolated with ethyl acetate, crystallised when rubbed with light petroleum. The solid (4·12 g., 82%), m. p. 101—103°, recrystallised from aqueous ethanol, giving the *peptide ester* (3·52 g., 70%), m. p. 113—113·5° (Found: C, 67·0; H, 5·7; N, 8·3. $C_{28}H_{29}O_6N_3$ requires C, 66·8;

[24] Bergmann, Zervas, and Fruton, *J. Biol. Chem.*, 1935, **111**, 225.
[25] Fruton, Smith, and Driscoll, *ibid.*, 1948, **173**, 457.

H, 5·8; N, 8·3%). Use of the benzyl ester hydrochloride instead of the toluene-*p*-sulphonate in this preparation gave a poorer yield (56%).

N-*Benzyloxycarbonyldiglycyl*-L-*tyrosine Benzyl Ester*.—*N*-Benzyloxycarbonylglycylglycine [13] (5·32 g.) and L-tyrosine benzyl ester toluene-*p*-sulphonate (8·87 g.) were coupled in pyridine (13 ml.) with bis-*o*-phenylene pyrophosphite (7·06 g.). The crude product (7·88 g., 76%), m. p. 139—141°, recrystallised several times from aqueous methanol, gave the pure tripeptide ester (4·71 g., 45%), m. p. 161°, $[\alpha]_D^{19}$ +12·8° (*c* 2·00 in dioxan) (Morris [12] gives m. p. 161—162°, $[\alpha]_D^{18}$ +13·7°).

N-*Benzyloxycarbonyltriglycylglycine Benzyl Ester*.—*N*-Benzyloxycarbonylglycylglycine [13] (2·66 g.) and glycylglycine benzyl ester toluene-*p*-sulphonate (3·95 g.) were coupled in pyridine (10 ml.) with bis-*o*-phenylene pyrophosphite (3·23 g.). Recrystallisation of the crude product (3·90 g., 83%), m. p. 193—195°, from aqueous pyridine gave the pure *peptide ester* (3·29 g., 70%), m. p. 199—200° (Found: C, 58·8; H, 5·7; N, 11·8. $C_{23}H_{26}O_7N_4$ requires C, 58·7; H, 5·6; N, 11·9%).

N-*Benzyloxycarbonylglycyl*-L-*phenylalanylglycine Ethyl Ester*.—*N*-Benzyloxycarbonylglycyl-L-phenylalanine (1·42 g.), redistilled glycine ethyl ester (0·41 g.), and bis-*o*-phenylene pyrophosphite (1·29 g.), in diethyl phosphite (5 ml.), were heated on a steam-bath for 30 min. The mixture was cooled and treated with water (20 ml.). The precipitated oil was dissolved in ethyl acetate and washed with N-hydrochloric acid, N-sodium hydrogen carbonate, and water, dried, and recovered. Addition of water to a concentrated solution in ethanol gave the extensively racemised peptide ester (0·77 g., 44%), m. p. 113—118°, $[\alpha]_D^{18\cdot5}$ −1·6° (*c* 2·00 in ethanol); recrystallisation from aqueous ethanol raised the m. p. to 123—124° (Found: N, 9·8. Calc. for $C_{23}H_{27}O_6N_3$: N, 9·5%) (Sheehan and Hess [9] give m. p. 118—119°, $[\alpha]_D^{27}$ −13·5°).

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