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PERKIN TRANSACTIONS I
Organic and Bio-organic Chemistry

Polypeptides. Part XVI.[1] The Synthesis of Some Diastereoisomeric Poly-(γ-t-butyl glutamate)s

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The synthesis, by conventional methods, of six diastereoisomeric oligopeptides of γ-t-butyl glutamate and the polymerisation of five of them, by means of dicyclohexylcarbodi-imide in acetonitrile, are described. The resulting sequential diastereoisomeric poly-(γ-t-butyl glutamate)s had molecular weights in the range 6500—42,000.

It is widely held that the highly complex, and specific, conformation (secondary and tertiary structures) adopted by a protein molecule in solution is wholly determined by the sequence of amino-acid residues in the peptide chain of the protein (the primary structure). Investigations of protein denaturation [2] and *X*-ray crystallographic studies on protein crystals [3] have clearly demonstrated that the early view [4] that intra- and inter-chain hydrogen-bonding of peptide groups is the all-important factor governing the conformation of a protein molecule is inadequate, and that non-covalent interactions between amino-acid side-chains are at least equally as important. Although some success has attended attempts to predict protein and polypeptide conformations on the basis of amino-acid sequences by *ab initio* calculation,[5] it is clear that the fundamental problem requires further experiment for its satisfactory solution.

Although much valuable information on the effect of side-chains on conformation has been obtained by the study of poly-(α-amino-acid)s and co-poly-(α-amino-acid)s prepared by polymerising mixtures of *N*-carboxy-anhydrides,[6] these are oversimplified models. More useful and realistic models for the purpose in hand are the sequential polypeptides, with defined, repeating, amino-acid sequences, which can be obtained by the polymerisation of oligopeptides. The present paper is the first of a group which will be concerned with the synthesis of such polypeptides and the study of their conformations in solution by physicochemical, mainly spectroscopic, methods.

Earlier work on the synthesis of sequential polypeptides, summarised by Stewart [7] and De Tar,[8] includes few systematic studies of the effect of introducing varying proportions of a 'foreign' amino-acid into a poly(amino-acid) in a sequential manner. Three such studies are those of Fraser *et al.*[9] on the introduction of glycyl, L-valyl, and *S*-benzyl-L-cysteinyl residues into poly-(γ-methyl and -ethyl L-glutamate)s, which are essentially studies of the effect of introducing non-helicogenic residues into a moderately strong α-helix. Systematic studies of this kind, dealing with closely related series of sequential polypeptides, are clearly much more likely to lead to useful insight into the relationship between sequence and conformation, than studies of isolated and unrelated examples.

Our work is concerned with sequential polypeptides built up of amino-acid residues *A* and *B* which give rise to poly(amino-acid)s, H–A_n–OH and H–B_n–OH, with α-helical conformations of opposite sense in helicogenic solvents. In such a sequential polypeptide, of general formula H–(–A_m–B_n–)$_x$–OH, the two types of residue

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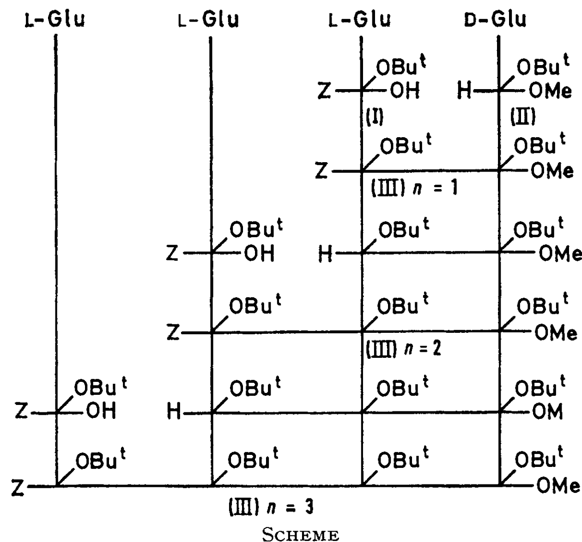
[2] Reviews: W. Kauzmann, *Adv. Protein Chem.*, 1959, **14**, 1; J. F. Brandts, in 'Structure and Stability of Biological Macromolecules,' ed. S. N. Timasheff and G. D. Fasman, Dekker, New York, 1967, ch. 3.
[3] Reviews: L. Stryer, *Ann. Rev. Biochem.*, 1968, **37**, 25; D. M. Blow and T. A. Steitz, *ibid.*, 1970, **39**, 63.
[4] L. Pauling, R. B. Corey, and H. R. Branson, *Proc. Nat. Acad. Sci. U.S.A.*, 1951, **37**, 205; L. Pauling and R. B. Corey, *ibid.*, p. 282.
[5] Reviews: H. A. Scheraga, *Adv. Phys. Org. Chem.*, 1968, **6**, 103; G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.*, 1968, **23**, 283; A. M. Liquori, *Quart. Rev. Biophys.*, 1969, **2**, 65.
[6] 'Poly-α-amino Acids: Protein Models for Conformational Studies,' ed. G. D. Fasman, Dekker, New York, 1967.
[7] F. H. C. Stewart, *Austral. J. Chem.*, 1965, **18**, 887.
[8] D. F. De Tar, in Peptides: Proc. Eighth European Peptide Symposium, 1967, p. 125.
[9] R. D. B. Fraser, B. S. Harrap, T. P. MacRae, F. H. C. Stewart, and E. Suzuki, *J. Mol. Biol.*, 1965, **12**, 482; 1965, **14**, 423; *Biopolymers*, 1967, **5**, 251.

exert opposing helix-forming tendencies and it seems possible that, at some defined value of m/n, the two will cancel, giving rise to a random structure; in the simplest case, as in the present paper, when A and B are the L- and D-enantiomers of the same amino-acid residue, the *meso*-polymer, H-(-A-B-)$_x$-OH, might be expected to be random. Alternatively, new types of conformation, more able than the α-helix to accommodate the two types of side-chain, may be encountered.

The present paper deals with the synthesis of diastereoisomeric sequential polypeptides containing L- and D-γ-t-butyl glutamyl residues. The cheapness and commercial availability of D-glutamic acid makes it more attractive than other D-amino-acids for this sort of work. The t-butyl ester group was chosen since it was hoped that its removal from poly-(γ-t-butyl glutamate)s to give the diastereoisomeric poly(glutamic acid)s, which we also wished to study, would be easier than that of the benzyl ester group from poly-(γ-benzyl glutamate).[10]

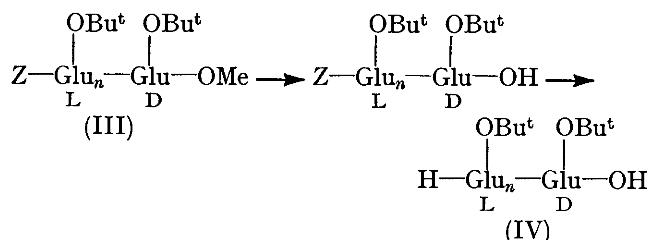
The synthesis of the required fully protected oligopeptides is outlined in the Scheme; * the benzyloxycarbonyl group was chosen for N-protection and the



SCHEME

methyl ester group for α-carboxy-protection because of the ease with which they can be removed selectively in the presence of t-butyl ester groups. α-Methyl N-benzyloxycarbonylglutamate was best prepared from N-benzyloxycarbonylglutamic acid by the action of dimethyl sulphate [11] and converted into the γ-t-butyl ester by the procedure of Klieger and Gibian; [12] the key intermediates (I) and (II) were prepared from this diester by saponification and hydrogenolysis, respectively.[12] The coupling reactions were brought about with dicyclohexylcarbodi-imide in dichloromethane at 0 °C; it was found convenient to mix together the dicyclohexylamine salt of the carboxy-component and the hydrochloride of the amino-component in dichloromethane and filter off the dicyclohexylammonium chloride before adding the di-imide. The yields were high (*ca.* 75%) and the products optically pure, as judged by the optical rotations of the acid hydrolysates. The hydrogenolyses were carried out in aqueous t-butyl alcohol, to avoid ester interchange.[13]

In this way the three oligopeptides (III; n = 1, 2, or 3), the LL- and DL-diastereoisomerides of (III; n = 1) and the LLL-diastereoisomer of (III; n = 2) were prepared. These were converted into the partially protected oligopeptides (IV), needed for the polymerisation experiments, by first removing the α-methyl ester group by saponification and then the benzyloxycarbonyl group by hydrogenolysis. Care was needed in the



saponifications, which were carried out at room temperature in aqueous acetone, with only a slight excess of alkali; the optimum reaction time was 30 min, longer times leading to the formation of cyclic imide (i.r. bands at 1655 and 1610 cm^{-1}); the use of purified (AnalaR) acetone gave better yields of purer products than those obtained with the ordinary reagent. The overall yields of the partially protected peptides (IV) from the fully protected peptides (III) were satisfactory (*ca.* 55%); all the products were crystalline and chromatographically homogeneous, although some of them gave gels on recrystallisation which were difficult to free from solvent.

Preliminary polymerisation studies were carried out on the LL-diastereoisomeride of (IV; n = 1); treatment of this compound in 5M-solution in dimethylformamide with dicyclohexylcarbodi-imide gave a low yield of dicyclohexylurea and the polymer produced was of low molecular weight (M_r by gel filtration, *ca.* 3000) and contaminated with a good deal of N-acylurea.[14,15] After several other trials, successful polymerisations were finally achieved by shaking a suspension of the oligopeptide (IV) in acetonitrile with dicyclohexylcarbodi-imide. The oligopeptides are notably less soluble in acetonitrile than the polypeptides derived from them and so pass progressively into solution as the polymerisation proceeds. The success of this procedure, unexpected in view of the low concentration of monomer during the polymerisation, is most probably due to the

* Here and elsewhere, the abbreviations for amino-acid residues and protecting groups are those recommended by the I.U.P.A.C.—I.U.B. Commission on Biochemical Nomenclature (*Biochem. J.*, 1967, **102**, 23).

[10] W. E. Hanby, S. G. Waley, and J. Watson, *J. Chem. Soc.*, 1950, 3239.

[11] G. H. L. Nefkens and R. J. F. Nivard, *Rec. Trav. chim.*, 1964, **83**, 199.

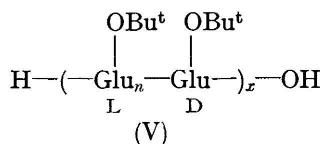
[12] E. Klieger and H. Gibian, *Annalen*, 1962, **655**, 195.

[13] P. C. Crofts, J. H. H. Markes, and H. N. Rydon, *J. Chem. Soc.*, 1959, 3610.

[14] V. Brückner, M. Kajtar, J. Kovacs, H. Nagy, and J. Wein, *Tetrahedron*, 1958, **2**, 211.

[15] J. H. Bradbury and D. C. Shaw, *Austral. J. Chem.*, 1959, **12**, 300.

known effect of acetonitrile in minimising *N*-acylurea formation in di-imide couplings.[16]



The sequential polypeptides prepared in this way comprise (V; $n = 1$) and its LL- and DL-diastereoisomerides (V; $n = 2$) and (V; $n = 3$); some of their properties are recorded in the Table. The molecular weights in the Table were determined by Sephadex gel-filtration, not of the t-butyl esters (V) themselves, but of the water-soluble poly(glutamic acid)s derived from them by treatment with cold 90% trifluoroacetic acid.[17] The extent of racemisation of the *C*-terminal residues during the polymerisations was determined by measuring the optical rotations of complete acid hydrolysates of the polymers. In the case of the *meso*-polypeptide (V; $n = 1$), and its DL-enantiomorph, attention may be drawn to the paradox that the rotation of the hydrolysate, and that of the polypeptide itself in helix-breaking solvents, is a direct measure of the degree of racemisation; the higher the rotation the greater the amount of racemisation. It will be seen that the degree of racemisation is considerable, the ratio of L- to D-residues diverging rather widely from the theoretical values. Although physicochemical measurements on these polypeptides have given useful results [18,19] the interpretation of these is hampered by the stereochemical inhomogeneity of the polypeptides.[20] Clearly, the dicyclohexylcarbodi-imide method of polymerisation, although simple and convenient, is not wholly satisfactory for our purpose; in later papers in this series it has been replaced by other methods causing less racemisation.

Poly-(γ-t-butyl glutamate)s (V)

n in (V)	Mol. wt.	x in (V)	L : D ratio Found	L : D ratio Theoretical
1 (LD)	11,500	31	56 : 44	50 : 50
1 (DL)	14,000	38	44 : 56	50 : 50
1 (LL)	42,000	114	87 : 13	100 : 0
2 (LLD)	6500	12		66 : 33
3 (LLLD)	14,000	19	80 : 20	75 : 25

EXPERIMENTAL

Whenever possible, the purity of important intermediates and end-products was confirmed by paper or thin-layer chromatography (two solvent systems); free amino-compounds were detected with ninhydrin in butanol and *N*-protected compounds by the chlorine–starch–iodide method.[21] Organic solutions were dried over magnesium sulphate and evaporated under reduced pressure. Light petroleum is the fraction of b.p. 60—80°. M.p.s are corrected. Unless otherwise indicated, all optical rotations were measured for solutions in methanol.

A. Synthesis of Oligopeptides

Dicyclohexylammonium α-methyl *N*-benzyloxycarbonyl-L-glutamate, m.p. 172—173°, $[\alpha]_D^{21}$ −10·7° (*c* 1·4) (lit.,[12] m.p. 172—173°, $[\alpha]_D^{25}$ −10·9°) and the D-isomer, m.p. 173—174°, $[\alpha]_D^{25}$ +11·0° (*c* 1·1), were prepared in 45% yield from the appropriate *N*-benzyloxycarbonylglutamic acid [22] by method (*c*) of Nefkens and Nivard [11] and converted in 90% yield into α-methyl *N*-benzyloxycarbonyl-L-glutamate, m.p. 68—69°, $[\alpha]_D^{25}$ −26·4° (*c* 1·1) (lit.,[12] m.p. 68—69°, $[\alpha]_D^{25}$ −25·9°), and the D-isomer, m.p. 68—69°, $[\alpha]_D^{19}$ +25·5° (*c* 1·8), by the procedure of Klieger and Gibian.[12] The latter compounds were converted into their t-butyl esters and these saponified, as described by Klieger and Gibian,[12] to give dicyclohexylammonium γ-t-butyl *N*-benzyloxycarbonyl-L-glutamate, m.p. 138—139°, $[\alpha]_D^{21}$ +7·0° (*c* 1·0) (lit.,[12] m.p. 139—140°, $[\alpha]^{25}$ +7·3°), and the D-isomer, m.p. 138—140°, $[\alpha]_D^{25}$ −6·2° (*c* 1·6), in 60% overall yield. The L-salt (1·69 g, 3 mmol), in 50% aqueous ethanol (33 ml), was shaken with Zeo-Karb 225 (acid form) for 30 min; filtration, evaporation, and extraction with ether gave *γ-t-butyl* N-*benzyloxycarbonyl-L-glutamate* (0·95 g, 90%) as an oil which crystallised when stored in a vacuum desiccator for several days; m.p. 79—80°, $[\alpha]_D^{21}$ −13·3° (*c* 2·3) (Found: C, 60·4; H, 7·1; N, 3·9. $C_{18}H_{32}N_2O_7$ requires C, 60·5; H, 6·9; N, 4·1%) (this compound was previously described [23] as an oil).

γ-t-Butyl α-methyl L-glutamate hydrochloride, prepared by hydrogenolysis [12] of the *N*-benzyloxycarbonyl derivative in 80% yield, had m.p. 125—126°, $[\alpha]_D^{21}$ +27·8° (*c* 1·0) (lit.,[12] m.p. 125—126°, $[\alpha]_D^{25}$ +23·9°); the D-isomer had m.p. 126—127°, $[\alpha]_D^{25}$ −26·6° (*c* 0·9).

Derivatives of t-Butyl Glutamylglutamate.—Dicyclohexylammonium γ-t-butyl *N*-benzyloxycarbonyl-L-glutamate (9·1 g, 17 mmol), in dichloromethane (43 ml), was added to a solution of γ-t-butyl α-methyl L-glutamate hydrochloride (4·4 g, 17 mmol) in the same solvent (43 ml) and the mixture was shaken mechanically for 1 h. The precipitated dicyclohexylammonium chloride was filtered off and dicyclohexylcarbodi-imide (3·6 g, 17·5 mmol) added to the filtrate, with ice-cooling. After 24 h at 0°, dicyclohexylurea was filtered off and the filtrate washed with 0·5M-hydrochloric acid and water, dried, and evaporated. The residue was dissolved in a little acetone and the solution kept at 0° for 5 h. A little more dicyclohexylurea was then filtered off and the filtrate was evaporated to dryness. Recrystallisation of the residue from ethyl acetate–light petroleum gave *di-γ-t-butyl α-methyl* N-*benzyloxycarbonyl-L-glutamyl-L-glutamate* (7·3 g, 83%), m.p. 46—47°, $[\alpha]_D^{23}$ −19·4° (*c* 1·0) (Found: C, 60·4; H, 7·4; N, 5·1. $C_{27}H_{40}N_2O_9$ requires C, 60·4; H, 7·5; N, 5·2%). *Di-γ-t-butyl α-methyl* N-*benzyloxycarbonyl-L-glutamyl-D-glutamate*, m.p. 64—65°, $[\alpha]_D^{25}$ +6·2° (*c* 2·8) (Found: C, 60·5; H, 7·7; N, 5·5%), and *di-γ-t-butyl α-methyl* N-*benzyloxycarbonyl-D-glutamyl-L-glutamate*, m.p. 60—61°, $[\alpha]_D^{25}$ −5·3° (*c* 1·1) (Found: C, 60·6; H, 7·1; N, 5·2%), were prepared similarly in 75 and 77% yield, respectively.

The LL-dipeptide triester (5 g, 9·3 mmol), in AnalaR

[16] S. Guttmann and R. A. Boissonnas, *Helv. Chim. Acta*, 1958, **41**, 1852; L. Benoiton and H. N. Rydon, *J. Chem. Soc.*, 1960, 3328.
[17] J. Kovacs and B. J. Johnson, *J. Chem. Soc.*, 1965, 6777.
[18] D. I. Marlborough and H. N. Rydon, in 'Some Newer Physical Methods in Structural Chemistry,' ed. R. Bonnett and J. G. Davis, United Trade Press, London, 1967, p. 211.
[19] P. M. Hardy, J. C. Haylock, D. I. Marlborough, H. N. Rydon, H. T. Storey, and R. C. Thompson, *Macromolecules*, in the press.
[20] *Cf.* P. M. Hardy, H. N. Rydon, and R. C. Thompson, following paper.
[21] H. N. Rydon and P. W. G. Smith, *Nature*, 1952, **169**, 122.
[22] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
[23] R. Schwyzer and H. Kapeller, *Helv. Chim. Acta*, 1961, **44**, 1991.

acetone (30 ml), was treated with M-sodium hydroxide (9·8 ml). After shaking the mixture for 30 min, 10% citric acid (10 ml) was added and the acetone was removed under reduced pressure at room temp. The residual aqueous solution was extracted several times with ethyl acetate; evaporation of the dried extract left the dipeptide dibutyl ester as a gum (4·6 g, 97%), which was chromatographically pure but which could not be induced to crystallise. *Di-γ-t-butyl* N-*benzyloxycarbonyl*-L-*glutamyl*-L-*glutamate* was characterised as its *dicyclohexylamine salt*, m.p. 143—144°, $[\alpha]_D^{21}$ −3·8° (*c* 1·6) (Found: C, 64·8; H, 8·6; N, 5·9. $C_{38}H_{60}N_3O_9$ requires C, 64·9; H, 8·6; N, 6·0%). The free dibutyl ester (4·3 g) was hydrogenated in aqueous 95% t-butyl alcohol (70 ml) over 5% palladised charcoal (200 mg) for 5 h at 4—5 atm. Filtration through kieselguhr, followed by evaporation and recrystallisation of the residue from methanol–ether, gave *di-γ-t-butyl* L-*glutamyl*-L-*glutamate* (1·8 g, 56%), m.p. 180—181°, $[\alpha]_D^{19}$ +25·3° (*c* 0·7) (Found: C, 54·7; * H, 8·4; N, 7·0. $C_{18}H_{32}N_2O_7$ requires C, 55·6; H, 8·3; N, 7·2%). *Di-γ-t-butyl* L-*glutamyl*-D-*glutamate* (70% yield), m.p. 132—133° (from ethyl acetate), $[\alpha]_D^{25}$ +21·7° (*c* 1·3) (Found: C, 54·4; H, 8·2; N, 7·5%), and *di-γ-t-butyl* D-*glutamyl*-L-*glutamate* (70% yield), m.p. 132—134°, $[\alpha]_D^{25}$ −21·7: (*c* 0·6) (Found: C, 55·2; H, 8·0; N, 6·7%), were prepared similarly.

Di-γ-t-butyl α-methyl *N*-benzyloxycarbonyl-L-glutamyl-L-glutamate (5·0 g) was hydrogenated in aqueous 95% t-butyl alcohol (85 ml) over 5% palladised charcoal (500 mg) for 5 h. After filtration, 0·5M-hydrochloric acid (15·7 ml) was added and the solution was evaporated to dryness. Recrystallisation of the residue from ethyl acetate–light petroleum gave *di-γ-t-butyl α-methyl* L-*glutamyl*-L-*glutamate hydrochloride* (3·2 g, 79%), m.p. 129—130°, $[\alpha]_D^{25}$ +6·3: (*c* 1·0) (Found: C, 52·2; H, 7·9; N, 6·2. $C_{19}H_{35}ClN_2O_7$ requires C, 52·0; H, 8·0; N, 6·4%). *Di-γ-t-butyl α-methyl* L-*glutamyl*-D-*glutamate hydrochloride*, m.p. 62—63° (from chloroform–light petroleum), $[\alpha]_D^{22}$ +35·5° (*c* 1·8) (Found: C, 51·5; H, 7·8; N, 6·9%), was prepared similarly in 70% yield.

Derivatives of t-Butyl Diglutamylglutamate.—Dicyclohexylammonium γ-t-butyl *N*-benzyloxycarbonyl-L-glutamate (2·25 g, 4·35 mmol) and di-γ-t-butyl α-methyl L-glutamyl-L-glutamate hydrochloride (1·9 g, 4·35 mmol) were coupled at 0° for 24 h with dicyclohexylcarbodi-imide (0·895 g, 4·35 mmol) in dichloromethane (20 ml). Work-up as usual and recrystallisation of the product from ethyl acetate–light petroleum gave *tri-γ-t-butyl α-methyl* N-*benzyloxycarbonyldi*-L-*glutamyl*-L-*glutamate* (2·2 g, 67%), m.p. 119—120°, $[\alpha]_D^{23}$ −26·7° (*c* 1·0) (Found: C, 59·7; H, 7·7; N, 5·7. $C_{36}H_{55}N_3O_{12}$ requires C, 59·9; H, 7·7; N, 5·8%). *Tri-γ-t-butyl α-methyl* N-*benzyloxycarbonyldi*-L-*glutamyl*-D-*glutamate*, prepared similarly in 73% yield, had m.p. 96—98°, $[\alpha]_D^{21}$ −10·6° (*c* 1·0) (Found: C, 59·9; H, 7·6; N, 6·0%).

Saponification of the above D-glutamate, followed by hydrogenation of the resulting non-crystalline product, by the methods previously described, gave *tri-γ-t-butyl di*-L-*glutamyl*-D-*glutamate* (50% yield), m.p. 162—164° (after recrystallisation first from methanol-ether and then from water), $[\alpha]_D^{25}$ +4·7° (*c* 1·2) (Found: C, 55·8; H, 8·7; N, 7·5. $C_{27}H_{47}N_3O_{10}$ requires C, 56·5; H, 8·3; N, 7·3%).

Derivatives of t-Butyl Triglutamylglutamate.—Hydrogenolysis of tri-γ-t-butyl α-methyl *N*-benzyloxycarbonyldi-L-glutamyl-D-glutamate (9·4 g), as described for the analogous dipeptide, gave tri-γ-t-butyl α-methyl di-L-glutamyl-D-glutamate hydrochloride (6·2 g, 77%) as a gum which, although chromatographically pure, resisted all attempts at crystallisation. This hydrochloride (6·05 g, 9·8 mmol) was coupled as usual with dicyclohexylammonium γ-t-butyl *N*-benzyloxycarbonyl-L-glutamate (5·3 g, 10 mmol) in dichloromethane (39 ml), by means of dicyclohexylcarbodi-imide (2·02 g, 9·8 mmol). Recrystallisation of the product from ethyl acetate–light petroleum gave *tetra-γ-t-butyl α-methyl* N-*benzyloxycarbonyltri*-L-*glutamyl*-D-*glutamate* (5·85 g, 67%), m.p. 109—112°, $[\alpha]_D^{21}$ −10·9° (*c* 1·0) (Found: C, 59·5; H, 7·8; N, 5·5. $C_{45}H_{70}N_4O_{15}$ requires C, 59·6; H, 7·8; N, 6·2%).

Saponification, followed by hydrogenolysis, as described for analogous lower peptides, gave *tetra-γ-t-butyl tri*-L-*glutamyl*-D-*glutamate* (58% yield), m.p. 146—149° (from water), $[\alpha]_D^{25}$ +1·4° (*c* 1·0) (Found: C, 56·0; H, 8·0; N, 7·3. $C_{36}H_{62}N_4O_{13}$ requires C, 57·0; H, 8·2; N, 7·4%).

B. Preparation and Properties of Polymers

The oligopeptide was suspended in acetonitrile (*ca.* 2 ml per mmol of peptide) and shaken at room temp. for 24 h with dicyclohexylcarbodi-imide (91 mmol per mmol of peptide). The insoluble dicyclohexylurea was filtered off and the filtrate was evaporated to dryness; in the case of the LL-dipeptide some polymer was recovered from the precipitated urea by extraction with chloroform at room temp. The residue was dissolved in dimethylformamide (2·5 ml per mmol of oligopeptide) and shaken for 10 min with G15-Sephadex (100 mg per ml). The gel was then filtered off; the polymer was precipitated from the filtrate with water and dried in a vacuum desiccator.

The following were prepared in this way: *poly-(γ-t-butyl*-L-*glutamyl-γ-t-butyl*-D-*glutamate)* (69% yield), $[\alpha]_D^{33.5}$ +1·5° (*c* 3·6 in $CHCl_3$) [Found: C, 58·6; H, 8·4; N, 7·8. $(C_9H_{15}NO_3)_n$ requires C, 58·4; H, 8·2; N, 7·6%]; *poly-(γ-t-butyl*-D-*glutamyl-γ-t-butyl*-L-*glutamate)* (74% yield), $[\alpha]_D^{33.5}$ −1·5° (*c* 2·5 in $CHCl_3$) (Found: C, 58·9; H, 8·3; N, 7·3%); *poly-(γ-t-butyl*-L-*glutamate)* (47% yield), $[\alpha]_D^{33.5}$ +20·5° (*c* 0·9 in $CHCl_3$); *poly[di-(γ-t-butyl*-L-*glutamyl)-γ-t-butyl*-D-*glutamate]* (44% yield), $[\alpha]_D^{33.5}$ −2·5° (*c* 2·3 in $CHCl_3$); *poly[tri-(γ-t-butyl*-L-*glutamyl)-γ-t-butyl*-D-*glutamate]* (74% yield), $[\alpha]_D^{33.5}$ +4·3° (*c* 2·7 in $CHCl_3$) (Found: C, 57·7; H, 8·0; N, 8·1%).

The extent of racemisation of the polymers was determined by hydrolysis at 90° with 5M-hydrochloric acid for 24 h, followed by determination of the optical rotation of the hydrolysate and comparison with a similarly treated mixture of L- and D-glutamic acids in the appropriate proportions. The results are given in the Table.

For the molecular weight determinations, the polypeptides were kept for 1 h at room temp. with aqueous 90% trifluoroacetic acid (5 ml). The product was precipitated with ether, collected by centrifugation, washed repeatedly with ether, and dried in a vacuum desiccator; the yields varied from 80 to 90%. The molecular weights of the poly(glutamic acid)s so obtained were determined by gel filtration on Sephadex (G75 or G100), with ribonuclease and α-chymotrypsin as standard. The molecular weights given in the Table are calculated from the results so obtained for the poly(glutamic acid)s.

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* Although chromatographically pure this and some other t-butyl esters prepared in this work gave consistently low carbon analyses.