Polypeptides. Part XIX.[1] The Synthesis of Some Sequential Polypeptides of β-Methyl Aspartate and γ-Methyl Glutamate

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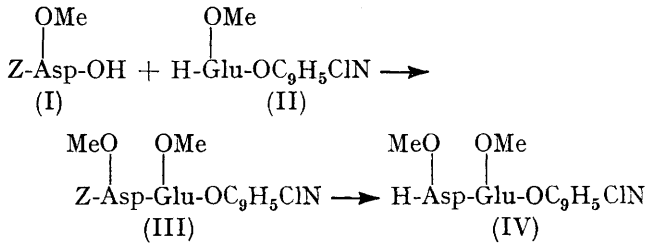
Four sequential polypeptides containing β-methyl aspartyl and γ-methyl glutamyl residues have been prepared by polymerisation of the corresponding oligopeptide *N*-succinimidyl esters, which were synthesised by conventional methods, using the benzyloxycarbonyl group for *N*-protection and the t-butyl ester group for carboxy-protection. The polypeptides had molecular weights (M_n) of about 10,000, and very little racemisation (*ca.* 2%) accompanied the polymerisations.

It is well established that, in helicogenic solvents, poly-(γ-methyl L-glutamate) adopts a right-handed α-helical conformation,[2] whereas poly-(β-methyl L-aspartate) forms a left-handed helix,[3] which is weak and probably a distorted α-helix.[4] We now describe the synthesis of two sequential polypeptides containing such residues, prepared for studies on their conformations in solution; also included, for comparative purposes, are two polypeptides containing β-methyl L-aspartyl and γ-methyl D-glutamyl residues. The methyl esters, rather than the benzyl esters, were chosen to simplify the interpretation of the n.m.r. spectra.

We originally envisaged the use of oligopeptide 5-chloro-8-quinolyl esters for the polymerisations, since such esters are highly crystalline and easily purified and are known to give optically pure products in peptide coupling reactions.[5] We proposed to use the 'backing off' strategy of Goodman and Stueben[6] and the dipeptide active ester (IV) was prepared in high yield by the dicyclohexylcarbodi-imide coupling of β-methyl *N*-benzyloxycarbonyl-L-aspartate (I) with α-5-chloro-8-quinolyl γ-methyl L-glutamate (II), followed by debenzyloxycarbonylation of the coupling product (III) with hydrogen bromide in acetic acid. Unfortunately the



hydrobromide of the active ester (IV) proved to be rather unstable, and polymerisations in dichloromethane and dimethylformamide gave mainly the dioxopiperazine and the acyclic tetrapeptide and very little polymer.

We turned, therefore, to the *N*-succinimidyl esters * for the polymerisations, since these had been found very satisfactory for the synthesis of other sequential polypeptides.[7] Details of the syntheses are given in Schemes 1—3. The benzyloxycarbonyl group was used throughout for *N*-protection and the t-butyl ester group for carboxy-protection; all the coupling reactions were

* *N*-Succinimidyl (Su) is used to denote the radical ⌜CO·[CH$_2$]$_2$·CO·N⌝– attached to oxygen; this name is widely used in peptide chemistry in place of the I.U.P.A.C. name 2,5-dioxopyrrolidin-1-yl.

[1] Part XVIII, P. M. Hardy, J. C. Haylock, and H. N. Rydon, *J.C.S. Perkin I*, 1972, 605.

[2] T. Yoshida, S. Sakurai, T. Okuda, and Y. Takagi, *J. Amer. Chem. Soc.*, 1962, **84**, 3590; B. S. Harrap, T. P. MacRae, F. H. C. Stewart, and E. Suzuki, *J. Mol. Biol.*, 1965, **12**, 482.

[3] M. Goodman, F. Boardman, and I. Listowsky, *J. Amer. Chem. Soc.*, 1963, **85**, 2491.

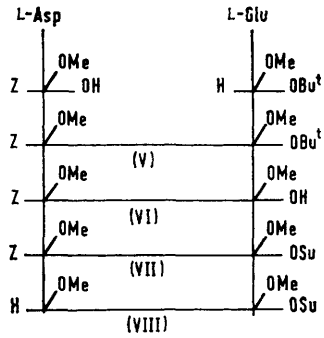
[4] P. M. Hardy, J. C. Haylock, D. I. Marlborough, H. N. Rydon, H. T. Storey, and R. C .Thompson, *Macromolecules*, 1971, **4**, 435.

[5] H.-D. Jakubke and A. Voigt, *Chem. Ber.*, 1966, **99**, 2944.

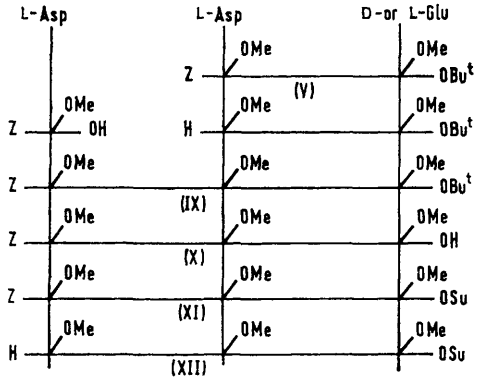
[6] M. Goodman and K. C. Stueben, *J. Amer. Chem. Soc.*, 1959, **81**, 3980.

[7] P. M. Hardy, H. N. Rydon, and R. C. Thompson, *J.C.S. Perkin I*, 1972, 5.

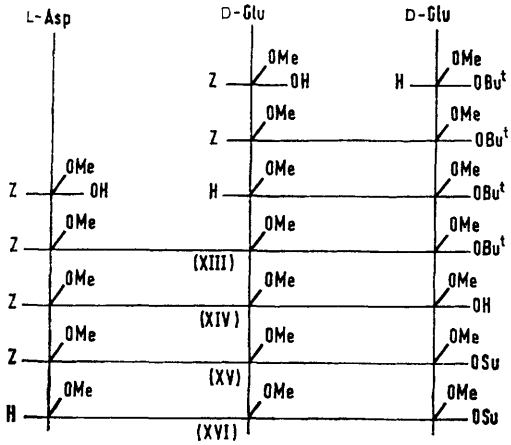
carried out with dicyclohexylcarbodi-imide. The t-butyl ester groups were removed from the fully protected



SCHEME 1 Synthesis of α-*N*-succinimidyl βγ-dimethyl L-aspartyl-L-glutamate



SCHEME 2 Synthesis of α-*N*-succinimidyl ββ′γ-trimethyl di-L-aspartyl-D- and -L-glutamates



SCHEME 3 Synthesis of α-*N*-succinimidyl βγγ′-trimethyl L-aspartyl-D-glutamyl-D-glutamate

oligopeptides (V), (IX), and (XIII) to give the corresponding acids (VI), (X), and (XIV); these were converted into the *N*-succinimidyl esters (VII), (XI), and (XV) with *N*-hydroxysuccinimide and dicyclohexylcarbodi-imide. Removal of the *N*-benzyloxycarbonyl group by the action of hydrogen bromide in acetic acid gave the required *N*-succinimidyl esters (VIII), (XII), and (XVI) as their hydrobromides. The yields were generally high and the fully protected succinimidyl esters were readily recrystallised; the hydrobromides of the debenzyloxycarbonylated compounds (VIII), (XII), and (XVI), however, were hygroscopic solids which could not be satisfactorily purified and were accordingly used at once for polymerisation.

The oligopeptide succinimidyl esters, released from the hydrobromides by addition of 1 equiv. of triethylamine, were allowed to polymerise at room temperature for 4 days in solvents chosen on the basis of small-scale trials. The crude polypeptides were freed from material of low molecular weight and partially fractionated by Soxhlet extraction with methanol and reprecipitation from suitable solvent pairs. The molecular weights of the purified polypeptides were determined by gel filtration, on Sephadex G150, of the derived poly-acids obtained by alkaline hydrolysis. These molecular weights, together with some details of the polymerisations, are given in Table 1. The molecular weights were sufficiently high (M_n *ca.* 10,000) for conformational studies, which will be reported elsewhere. The extent of racemisation during the polymerisations was determined by comparing the optical rotations of complete acid hydrolysates with those of control mixtures of the appropriate amino-acid methyl esters; as in previous work with succinimidyl esters [7] the amount of racemisation was very small (*ca.* 2%).

TABLE 1

Molecular weights of sequential polypeptides

Polypeptide	M_w	M_n	M_w/M_n
H-[-L-Asp(OMe)-L-Glu(OMe)-]$_n$OH	15,400	11,300	1·36
H-[-L-Asp(OMe)-L-Asp(OMe)-L-Glu(OMe)-]$_n$OH	15,500	12,200	1·27
H-[-L-Asp(OMe)-L-Asp(OMe)-D-Glu(OMe)-]$_n$OH	12,200	9900	1·23
H-[-L-Asp(OMe)-D-Glu(OMe)-D-Glu(OMe)-]$_n$OH	12,200	9900	1·23

EXPERIMENTAL

The purity of all intermediates and end-products was confirmed by t.l.c. (Kieselgel G) in at least two solvent systems. Compounds with free amino-groups were revealed with 0·3% ninhydrin in n-butanol at 100°; *N*-acylated compounds were revealed either by the chlorine–starch–iodide method [8] or by heating at 300° after spraying with aqueous 5% ammonium sulphate.[9]

Organic solutions were dried over magnesium sulphate; evaporations and concentrations were carried out under reduced pressure in a rotary evaporator. Light petroleum was the fraction of b.p. 60—80°. Optical rotations were measured with a Bendix-NPL polarimeter model 143C (path length 1 cm).

Synthesis of Oligopeptides

β-Methyl *N*-benzyloxycarbonyl-L-aspartate (I), prepared [10] from L-aspartic acid in 50% overall yield, had m.p. 92—93°, $[\alpha]_D^{22}$ −16·7° (*c* 2·4 in pyridine) (lit.,[10] m.p. 98°,

[8] H. N. Rydon and P. W. G. Smith, *Nature*, 1952, **169**, 922.
[9] T. Ziminski and E. Borowski, *J. Chromatog.*, 1966, **23**, 480.
[10] H. Schwarz, F. M. Bumpus, and I. H. Page, *J. Amer. Chem. Soc.*, 1957, **79**, 5697.

$[\alpha]_D^{25}$ −17·4°). This compound (5·6 g, 0·02 mol) and 5-chloro-8-hydroxyquinoline (5·4 g, 0·03 mol) were dissolved in ethyl acetate (60 ml). The solution was cooled to −10°, dicyclohexylcarbodi-imide (4·1 g, 0·02 mol) was added, and the mixture was shaken at 4° overnight. It was then filtered and the filtrate was washed with saturated aqueous sodium hydrogen carbonate (10 ml), M-hydrochloric acid (2 × 10 ml), and water (10 ml), and dried. Evaporation and recrystallisation from ethyl acetate–ether gave *α-5-chloro-8-quinolyl β-methyl N-benzyloxycarbonyl-L-aspartate* (7·2 g, 82%), m.p. 95—96°, $[\alpha]_D^{23}$ −12·9° (*c* 2·4 in $CHCl_3$) (Found: C, 59·9; H, 4·3; N, 6·5. $C_{22}H_{19}ClN_2O_6$ requires C, 59·7; H, 4·3; N, 6·3%).

γ-Methyl *N*-benzyloxycarbonyl-L- and -D-glutamates, prepared [11] from L- and D-glutamic acids in 40% overall yield, had m.p. 61°, $[\alpha]_D^{22}$ ±15·2° (*c* 7·0 in 1·4M-$KHCO_3$) (lit.,[11] m.p. 72—73°, $[\alpha]_D^{21}$ −15·3° for the L-isomer). This ester (45·7 g, 0·155 mol) in dichloromethane (110 ml) was kept in a pressure bottle at room temp. for 4 days with conc. sulphuric acid (1·6 ml) and liquid 2-methylpropene (160 ml), added at 0°. The product was freed from olefin by evaporation and the residual solution was washed with saturated aqueous sodium hydrogen carbonate (2 × 50 ml) and water (50 ml), dried, and evaporated. A portion (7·0 g, 0·02 mol) of the residual oil (52 g, 96%) was hydrogenated in acetic acid (60 ml) over 5% palladised charcoal (1 g) for 8 h. Filtration, evaporation, dissolution in anhydrous ether (60 ml), and addition of ethereal hydrogen chloride to the solution at −10° precipitated α-t-butyl *γ*-methyl L- and D-glutamates as the hydrochlorides, which were recrystallised from ethyl acetate; yield 3·9 g (77%), m.p. 134°, $[\alpha]_D^{22}$ ±22·0° (*c* 2·0 in EtOH) (lit.,[12] m.p. 135—135·5°, $[\alpha]_D^{20}$ +21·7° for the L-isomer).

α-5-Chloro-8-quinolyl γ-methyl N-benzyloxycarbonyl-L-glutamate, prepared in 86% yield from *γ*-methyl *N*-benzyloxycarbonyl-L-glutamate in the same manner as the analogous aspartic acid derivative and recrystallised from ethyl acetate, had m.p. 107—108°, $[\alpha]_D^{22}$ −12·5° (*c* 2·0 in AcOH) (Found: C, 60·6; H, 4·6; N, 6·3. $C_{23}H_{21}ClN_2O_6$ requires C, 60·5; H, 4·6; N, 6·1%). A sample (9·1 g, 0·02 mol) was kept for 1 h at room temp. with 20% hydrogen bromide in acetic acid (18 ml); the precipitated hydrobromide was filtered off and washed with anhydrous ether. Reprecipitation from trifluoroacetic acid with ether gave *α-5-chloro-8-quinolyl γ-methyl L-glutamate dihydrobromide* (9·5 g, 98%), decomp. 170°, $[\alpha]_D^{22}$ −10·0° (*c* 2·0 in H_2O) (Found: C, 37·9; H, 3·9; N, 5·6. $C_{15}H_{17}Br_2ClN_2O_4$ requires C, 37·2; H, 3·5; N, 5·8%).

Derivatives of Aspartylglutamic Acid.—Triethylamine (2·8 ml, 0·02 mol), followed by dicyclohexylcarbodi-imide (4·3 g, 0·021 mol), was added to a solution of α-t-butyl *γ*-methyl L-glutamate hydrochloride (5·1 g, 0·02 mol) and β-methyl *N*-benzyloxycarbonyl-L-aspartate (5·6 g, 0·02 mol) in anhydrous dichloromethane (60 ml) at 0°. The mixture was shaken overnight at 4° and then filtered. The filtrate was evaporated and the residue dissolved in ethyl acetate (80 ml). Acetic acid (0·2 ml) was added, and after a short time the solution was washed with M-hydrochloric acid (2 × 20 ml), saturated aqueous sodium hydrogen carbonate (2 × 20 ml), and water (20 ml), dried, and evaporated. The residue was kept for 1 h at −10° in acetone (20 ml). More dicyclohexylurea was filtered off and the filtrate was evaporated, giving α-t-butyl β*γ*-dimethyl *N*-benzyloxycarbonyl-L-aspartyl-L-glutamate (V) (9·4 g, 98%) as a viscous oil which could not be induced to crystallise; the LD-diastereoisomeride prepared similarly (97% yield) was also an uncrystallisable oil. Hydrogenation of both these compounds over 5% palladised charcoal in acetic acid by the procedure already described gave α-t-butyl β*γ*-dimethyl L-aspartyl-L- and -D-glutamate hydrochlorides (70%) as hygroscopic uncrystallisable gums. α-t-Butyl β*γ*-dimethyl *N*-benzyloxycarbonyl-L-aspartyl-L-glutamate (4·8 g, 0·01 mol) was kept for 4 h at room temp. in redistilled trifluoroacetic acid (10 ml); evaporation, trituration with anhydrous ether, and recrystallisation from ethyl acetate–light petroleum gave *βγ-dimethyl N-benzyloxycarbonyl-L-aspartyl-L-glutamate* (VI) (3·2 g, 75%), m.p. 98—99°, $[\alpha]_D^{22}$ +34·5° (*c* 2·0 in $CHCl_3$) (Found: C, 53·8; H, 5·6; N, 6·6. $C_{19}H_{24}N_2O_9$ requires C, 53·8; H, 5·7; N, 6·6%).

Dicyclohexylcarbodi-imide (2·1 g, 0·01 mol) was added at −10° to a solution of the ester (VI) (4·24 g, 0·01 mol) and *N*-hydroxysuccinimide (2·3 g, 0·02 mol) in dichloromethane (50 ml). The mixture was stirred at 4° for 12 h and then filtered, and the filtrate was evaporated. Acetic acid (0·1 ml) was added to the residue in ethyl acetate, and after a short time the solution was washed with water (5 × 20 ml), dried, and evaporated. The residue was kept for 12 h at −10° in acetone (15 ml). Filtration, evaporation, and trituration with anhydrous ether gave *βγ-dimethyl α-N-succinimidyl N-benzyloxycarbonyl-L-aspartyl-L-glutamate* (VII) (4·85 g, 93%), m.p. 78—81°, $[\alpha]_D^{22}$ −3·95° (*c* 1·7 in $CHCl_3$) (Found: C, 52·4; H, 5·2; N, 8·6. $C_{23}H_{27}N_3O_{11}$ requires C, 53·0; H, 5·2; N, 8·1%). This ester (4·17 g) was kept for 1 h at room temp. with 20% hydrogen bromide in acetic acid (12 ml). Precipitation with anhydrous ether (100 ml), followed by two further reprecipitations from trifluoroacetic acid (10 ml) with ether (100 ml) gave β*γ*-dimethyl α-*N*-succinimidyl L-aspartyl-L-glutamate hydrobromide (3·1 g, 83%) as a hygroscopic solid which could not be further purified.

Triethylamine (2·02 g, 0·02 mol), followed by dicyclohexylcarbodi-imide (2·1 g, 0·01 mol), was added to a solution at 0° of β-methyl *N*-benzyloxycarbonyl-L-aspartate (2·81 g, 0·01 mol) and α-5-chloro-8-quinolyl *γ*-methyl L-glutamate dihydrobromide (4·85 g, 0·01 mol) in dichloromethane (60 ml). Work-up in the usual manner, followed by recrystallisation from ethyl acetate, gave *α-5-chloro-8-quinolyl βγ-dimethyl N-benzyloxycarbonyl-L-aspartyl-L-glutamate* (III) (4·7 g, 80%), m.p. 139—140°, $[\alpha]_D^{22}$ −15·3° (*c* 2·0 in $CHCl_3$) (Found: C, 57·5; H, 5·3; N, 7·3. $C_{28}H_{28}ClN_3O_9$ requires C, 57·4; H, 4·8; N, 7·2%). This ester (5·9 g) was kept for 1 h at room temp. in 25% hydrogen bromide in acetic acid (12 ml). Precipitation with anhydrous ether (100 ml), followed by reprecipitation from trifluoroacetic acid (10 ml) with ether, gave *α-5-chloro-8-quinolyl βγ-dimethyl L-aspartyl-L-glutamate dihydrobromide* (5·2 g, 85%) as a hygroscopic solid, $[\alpha]_D^{22}$ −11·0° (*c* 1·8 in $Me_2N{\cdot}CHO$) (Found: C, 38·1; H, 3·8; N, 6·4. $C_{20}H_{24}ClBr_2N_3O_7,H_2O$ requires C, 38·2; H, 4·2; N, 6·7%).

Derivatives of Glutamylglutamic Acid.—*γ*-Methyl *N*-benzyloxycarbonyl-D-glutamate (8·85 g, 0·03 mol) and α-t-butyl *γ*-methyl D-glutamate hydrochloride (7·6 g, 0·03 mol) were coupled by the usual method in dichloromethane (90 ml) with the aid of triethylamine (4·2 ml, 0·03 mol)

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

[12] E. Taschner, A. Chimiak, B. Bator, and T. Sokolowska, *Annalen*, 1961, **646**, 134.

and dicyclohexylcarbodi-imide (6·2 g, 0·03 mol). Recrystallisation of the product from ethyl acetate–light petroleum gave *α-t-butyl γγ′-dimethyl* N-*benzyloxycarbonyl*-D-*glutamyl*-D-*glutamate* (13·7 g, 92%), m.p. 68—69°, $[\alpha]_D^{21}$ −1·5° (*c* 4·0 in $CHCl_3$), +16·5° (*c* 4·0 in AcOH) (Found: C, 58·4; H, 6·9; N, 5·3. $C_{24}H_{34}N_2O_9$ requires C, 58·3; H, 6·9; N, 5·7%). Hydrogenation in acetic acid gave α-t-butyl γγ′-dimethyl D-glutamyl-D-glutamate hydrochloride (6·8 g, 78%) as a hygroscopic uncrystallisable gum.

Derivatives of Diaspartylglutamic Acid.—β-Methyl *N*-benzyloxycarbonyl-L-aspartate (5·62 g, 0·02 mol) and α-t-butyl βγ-dimethyl L-aspartyl-L-glutamate hydrochloride (7·65 g, 0·02 mol) were coupled in dichloromethane (80 ml) with triethylamine (2·02 g, 0·02 mol) and dicyclohexylcarbodi-imide (4·3 g, 0·021 mol). Recrystallisation of the product from carbon tetrachloride gave *α-t-butyl ββ′γ-trimethyl* N-*benzyloxycarbonyldi*-L-*aspartyl*-L-*glutamate* (IX) (9·0 g, 74%), m.p. 116—117°, $[\alpha]_D^{22}$ +9·6° (*c* 2·0 in $CHCl_3$) (Found: C, 55·3; H, 6·7; N, 7·2. $C_{28}H_{39}N_3O_{12}$ requires C, 55·2; H, 6·5; N, 6·9%). The LLD-*diastereoisomeride*, prepared similarly in 76% yield, had m.p. 112—113°, $[\alpha]_D^{22}$ −9·8° (*c* 2·0 in $CHCl_3$) (Found: C, 55·1; H, 6·4; N, 7·1%).

These esters were kept for 1 h at room temp. in redistilled trifluoroacetic acid (2 ml per g). Evaporation, trituration with anhydrous ether, and recrystallisation from ethyl acetate gave *ββ′γ-trimethyl* N-*benzyloxycarbonyldi*-L-*aspartyl*-L-*glutamate* (X) (85%), m.p. 105—106°, $[\alpha]_D^{21}$ +10·5° (*c* 2·0 in $CHCl_3$) (Found: C, 52·3; H, 5·7; N, 7·7. $C_{24}H_{31}N_3O_{12}$ requires C, 52·1; H, 5·7; N, 7·6%) and its LLD-*diastereoisomeride* (88%), m.p. 142—143°, $[\alpha]_D^{22}$ −18·4° (*c* 2·0 in $CHCl_3$) (Found: C, 52·2; H, 5·7; N, 7·7%).

To the LLL-ester (X) (5·53 g, 0·01 mol) and *N*-hydroxysuccinimide (2·3 g, 0·02 mol) in dichloromethane (60 ml) at −10°, dicyclohexylcarbodi-imide (2·1 g, 0·01 mol) was added. After being shaken at 4° overnight, the mixture was worked up as usual. Recrystallisation from propan-2-ol gave *ββ′γ-trimethyl* α-N-*succinimidyl* N-*benzyloxycarbonyldi*-L-*aspartyl*-L-*glutamate* (XI) (5·9 g, 91%), m.p. 120—122°, $[\alpha]_D^{21}$ −1·5° (*c* 2·0 in $CHCl_3$) (Found: C, 51·8; H, 5·3; N, 9·1. $C_{28}H_{34}N_4O_{14}$ requires C, 51·7; H, 5·3; N, 8·6%). The LLD-*diastereoisomeride*, prepared similarly (87%), had m.p. 140—141°, $[\alpha]_D^{22}$ +4·1° (*c* 2·0 in $CHCl_3$) (Found: C, 51·3; H, 5·2; N, 9·0%). Treatment of these esters for 1 h at room temp. with 20% hydrogen bromide in acetic acid (3 ml per g), followed by working up as usual and reprecipitation from trifluoroacetic acid with ether gave *ββ′γ-trimethyl* α-N-*succinimidyl di*-L-*aspartyl*-L-*glutamate hydrobromide* (81%) (Found: C, 39·0; H, 4·8; N, 8·7. $C_{20}H_{29}BrN_4O_{12},H_2O$ requires C, 39·0; H, 5·1; N, 9·1%) and its LLD-diastereoisomeride (84%) as hygroscopic solids.

Derivatives of Aspartylglutamylglutamic Acid.—The following were prepared in similar manner to the diaspartylglutamic acid derivatives: *α-t-butyl βγγ′-trimethyl* N-*benzyloxycarbonyl*-L-*aspartyl*-D-*glutamyl*-D-*glutamate* (XIII) (73%) from β-methyl *N*-benzyloxycarbonyl-L-aspartate and α-t-butyl γγ′-dimethyl D-glutamyl-D-glutamate hydrochloride; m.p. 94—95° (from di-isopropyl ether), $[\alpha]_D^{21}$ +20·0° (*c* 2·0 in $CHCl_3$) (Found: C, 56·0; H, 6·5; N, 6·7. $C_{29}H_{41}N_3O_{12}$ requires C, 55·9; H, 6·6; N, 6·7%); *βγγ′-trimethyl* N-*benzyloxycarbonyl*-L-*aspartyl*-D-*glutamyl*-D-*glutamate* (XIV) (84%), m.p. 108—109° (from ethyl acetate–ether), $[\alpha]_D^{22}$ +7·5° (*c* 4·0 in $CHCl_3$) (Found: C, 52·6; H, 5·8; N, 7·5. $C_{25}H_{33}N_3O_{12}$ requires C, 52·9; H, 5·9; N, 7·4%); *βγγ′-trimethyl* α-N-*succinimidyl* N-*benzyloxycarbonyl*-L-*aspartyl*-D-*glutamyl*-D-*glutamate* (XV) (88%), m.p. 84—86° (from ethyl acetate–light petroleum), $[\alpha]_D^{23}$ +30·5° (*c* 4·0 in $CHCl_3$) (Found: C, 51·8; H, 5·4; N, 8·6%. $C_{29}H_{36}N_4O_{14}$ requires C, 52·4; H, 5·5; N, 8·4%); βγγ′-trimethyl α-N-succinimidyl L-aspartyl-D-glutamyl-D-glutamate hydrobromide (82%), too hygroscopic for analysis.

Preparation and Properties of Polymers

The α-*N*-succinimidyl ester hydrobromide (0·005 mol) was suspended in a suitable solvent (2—4 ml) and treated with purified triethylamine (0·005 mol). After 4 days at room temp., more solvent (2—4 ml) was added and the mixture was kept at room temp. for 2 more days. The product was triturated with water (10 ml) and then with methanol (10 ml). The solid was filtered off, dissolved in a little trifluoroacetic acid, and precipitated with water. The dried solid was extracted with hot methanol (Soxhlet) for 72 h and finally reprecipitated as indicated. The following polymers were prepared in this way.

Poly-(*β-methyl*-L-*aspartyl-γ-methyl*-L-*glutamate*), prepared in dioxan (2 × 2·3 ml), precipitated from *m*-cresol with ether, yield 23%, $[\alpha]_D^{33\cdot5}$ −54·2° (*c* 5·0 in $CF_3{\cdot}CO_2H$) [Found: C, 48·2; H, 6·0; N, 10·3. $(C_{11}H_{16}N_2O_6)_n$ requires C, 48·5; H, 5·9; N, 10·3%].

Poly[*di-*(*β-methyl*-L-*aspartyl*)-*γ-methyl*-L-*glutamate*], prepared in benzene (2 × 4 ml), precipitated from *m*-cresol with ether, yield 53%, $[\alpha]_D^{33\cdot5}$ −60·1° (*c* 5·0 in $CF_3{\cdot}CO_2H$) [Found: C, 47·9; H, 5·8; N, 10·6. $(C_{16}H_{23}N_3O_9)_n$ requires C, 47·9; H, 5·8; N, 10·5%].

Poly[*di-*(*β-methyl*-L-*aspartyl*)-*γ-methyl*-D-*glutamate*], prepared in tetrahydrofuran (2 × 4 ml), precipitated from trifluoroacetic acid with water, yield 28%, $[\alpha]_D^{33\cdot5}$ −11·0° (*c* 5·0 in $CF_3{\cdot}CO_2H$) [Found: C, 46·9; H, 5·7; N, 10·1. $(C_{16}H_{23}N_3O_9,0{\cdot}5H_2O)_n$ requires C, 46·9; H, 5·9; N, 10·3%].

Poly-(*β-methyl*-L-*aspartyl-γ-methyl*-D-*glutamyl-γ-methyl*-D-*glutamate*), prepared in dioxan (2 × 3 ml), precipitated from *m*-cresol with ether, yield 25%, $[\alpha]_D^{33\cdot5}$ +20·7° (*c* 5·0 in $CF_3{\cdot}CO_2H$) [Found: C, 48·8; H, 6·1; N, 10·2. $(C_{17}H_{25}N_3O_9)_n$ requires C, 49·2; H, 6·1; N, 10·1%].

Molecular Weight Determinations.—The polymers were shaken at room temp. overnight with aqueous 2M-sodium hydroxide; the demethylated products were precipitated with methanol, filtered off, washed with methanol, and dried *in vacuo*. Their molecular weights were determined by gel filtration on Sephadex G150, using the method, column, and poly-amino-acid calibration curve described previously.[7] The weight average molecular weights (M_w) were obtained from plots of optical density against molecular weight and the number average molecular weights (M_n) from plots of optical density divided by molecular weight against molecular weight. The values given in Table 1 for the polyesters are calculated from the molecular weights of the

TABLE 2

Polymer	$[\alpha]_{400}^{21}$ Polymer	$[\alpha]_{400}^{21}$ Control	% Racemisation
H-[-L-Asp(OMe)-L-Glu(OMe)-]$_n$OH	+65·8°	+67·2°	2·1
H-[-L-Asp(OMe)-L-Asp(OMe)-L-Glu(OMe)-]$_n$OH	+60·0	+61·6	2·6
H-[-L-Asp(OMe)-L-Asp(OMe)-D-Glu(OMe)-]$_n$OH	+10·3	+10·5	1·9
H-[-L-Asp(OMe)-D-Glu(OMe)-D-Glu(OMe)-]$_n$OH	−30·9	−31·7	2·5

demethylated products on the assumption that no degradation occurs during the alkaline hydrolysis; they are therefore minimum values.

Racemisation Tests.—The polymers (60—80 mg) were heated at 100° in sealed tubes for 13 h with 6M-hydrochloric acid. The resulting hydrolysates were made up to a convenient volume and their optical rotations were determined. Control experiments were carried out with appropriate mixtures of β-methyl L-aspartate and γ-methyl L- or D-glutamate. The results are given in Table 2.

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