# Sequential Polypeptides. Part I. Use of Mono-esters of Catechol in the Synthesis of Sequential Polypeptides [1]

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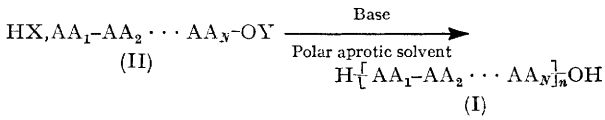
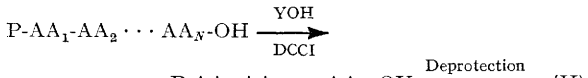
Benzyloxycarbonylpeptide 2-benzyloxyphenyl esters have been shown to be useful intermediates for the racemisation-free synthesis of sequential polypeptides. Simultaneous removal of the benzyloxycarbonyl and benzyl groups with hydrogen bromide in acetic acid (or, less satisfactorily, by hydrogenolysis) gives a peptide 2-hydroxyphenyl ester salt, which is not isolated but is polymerised immediately by triethylamine in dimethyl sulphoxide. Syntheses of poly(glycylglycyl-L-phenylalanine), poly-(β-alanyl-L-phenylalanine) and poly(glycyl-L-prolyl-L-alanine) are described as examples. In the last case the polymer (molecular weight by the Archibald method $1{\cdot}20 \pm 0{\cdot}1 \times 10^4$) had an optical rotation more than twice as great as a preparation of similar molecular weight obtained previously by use of a 4-nitrophenyl ester. Attempts to extend the versatility of the method by using alternative methods of blocking the phenolic group have so far proved fruitless.

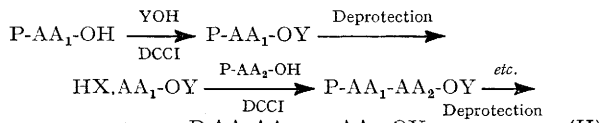
THE most successful route to sequential polypeptides (I) [2] has involved peptide active esters (II) [3] as generalised in Scheme 1. If the *C*-terminal residue in the monomer (II) is glycine or an imino-acid, this is a satisfactory and convenient approach: the required monomer can then be prepared simply by deprotection of an acylpeptide active ester after direct activation of the corresponding free acid (Scheme 2), and the risk of racemisation is not incurred during polymerisation. If, on the other hand, the *C*-terminal residue is an optically active α-amino-acid, then serious racemisation may result if the active ester group is introduced as in Scheme 2, so that circuitous and frequently troublesome 'backing-off' [3] manoeuvres must be used for the preparation of the monomers (Scheme 3). Furthermore, slight racemisation of the *C*-terminal residue may also occur during polymerisation. Judicious choice of conditions can reduce the risk of racemisation to very low levels, but as Rydon has pointed out [4] the requirements for an optically pure polymer are very stringent: even if only 1% of condensation steps occur with concomitant racemisation, then after a degree of polymerisation of 100 has been attained *ca.* 39% of the resulting polymer chains will have an inverted configuration at one residue at least. It seems likely that the presence even of a very small number of inverted residues could lead to disproportionate conformational consequences, and the problem is exacerbated by the lack of methods for detecting small amounts of racemate in polymers. The slightest doubt




SCHEME 1




SCHEME 2




SCHEME 3

AA = amino-acid residue; X = anion of a strong acid; Y = active ester group; P = *N*-protecting group; DCCI = dicyclohexylcarbodi-imide.

about the optical integrity of sequential polypeptides prepared in this way must necessarily undermine the

[1] Preliminary communication, J. H. Jones, *Chem. Comm.*, 1969, 1436.

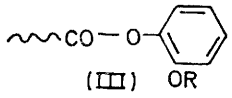
[2] For recent references to the synthesis and study of sequential polypeptides see 'Amino-acids, Peptides, and Proteins,' Chem. Soc. Specialist Periodical Report, 1969, **1**, 143, 200; 1970, **2**, p. 169.

[3] D. F. DeTar, in 'Peptides,' ed. H. C. Beyerman, A. van de Linde, and W. Maassen van den Brink, North Holland Publishing Co., Amsterdam, 1967, p. 125, and references cited therein.

[4] H. N. Rydon, Lecture delivered at the Chemical Society Anniversary Meeting, Exeter, 1967.

confidence with which physicochemical and biological studies of these materials are interpreted. Clearly procedures are required which can be shown in model systems to be entirely free from the danger of racemisation.

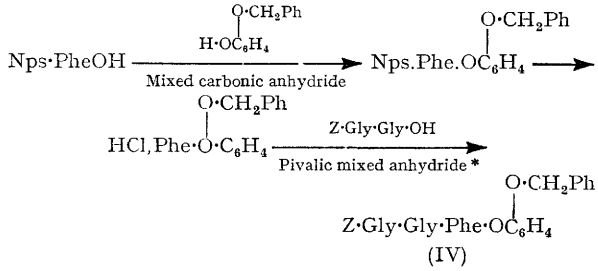
Previous work [5] in this laboratory has shown that 2-hydroxyphenyl esters (III; R = H) undergo rapid



(III)

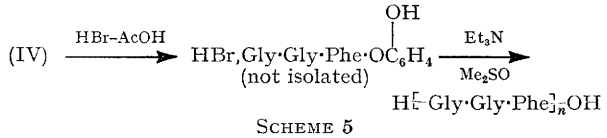
coupling but are highly resistant to racemisation—a finding which was attributed to intramolecular general base catalysis. Such intramolecular catalysis accelerates aminolysis but not oxazolone formation (the principal cause of racemisation in peptide synthesis), since the nucleophile in oxazolone formation is an oxygen atom which is indifferent towards base catalysis because it bears no hydrogen atom. 2-Hydroxyphenyl esters are best prepared by acidolysis or hydrogenolysis of the corresponding 2-benzyloxyphenyl esters (III; R = $PhCH_2$), which are unreactive towards nucleophilic attack. The 2-benzyloxyphenyl group can therefore be used in a 'safety-catch' [6] method of protecting carboxy-groups. These features of 2-hydroxyphenyl esters led to the suggestion [5] that they might prove to be of special value for synthesis of sequential polypeptides and the present work is an examination of this application.

For initial evaluation, we chose the synthesis of poly(glycylglycyl-L-phenylalanine), which had previously been synthesised by DeTar and his colleagues [7] and by Kovacs and his co-workers.[8] The fully protected intermediate (IV) required for this synthesis was prepared as shown in Scheme 4. Purification of (IV) caused us much trouble until we discovered by accident the curious fact that it forms a crystalline and easily purified 1 : 1 chloroform solvate. Treatment of this chloroform solvate with hydrogen bromide in acetic acid gave a deprotected and activated monomer which was polymerised without purification or delay by means of triethylamine in a small volume of dimethyl sulphoxide (Scheme 5). A rigid gel resulted, and crude poly(glycylglycyl-L-phenylalanine) was obtained in 45% yield after trituration with water followed by methanol and then ether. Mass spectrometry indicated the presence of some cyclo-glycylglycyl-L-phenylalanyl-glycylglycyl-L-phenylalanine, but this was no longer evident after exhaustive extraction with methanol in a Soxhlet apparatus followed by reprecipitation from trifluoroacetic acid with ether. Elemental analysis showed the presence of tenaciously adsorbed solvents, but no other impurities were shown by the n.m.r. spectrum. Complete hydrolysis and comparison of the optical rotation of the hydrolysate with that of a control mixture of glycine and phenylalanine showed, within the limits of this method (2—3%),[7,8] that the polymer was optically pure. A rough estimate of the molecular weight by end-group analysis with 2,4-dinitrofluoro-benzene gave a number average molecular weight of *ca.* $5 \times 10^3$. Hydrogenation of compound (IV) in glacial acetic acid–

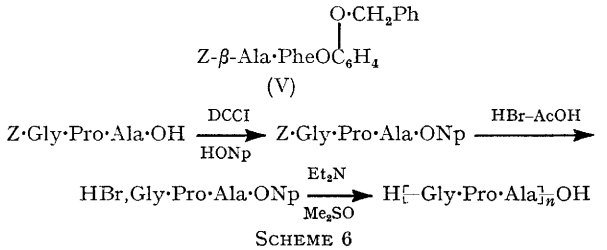



* Or *via* the mercaptopyridyl ester.

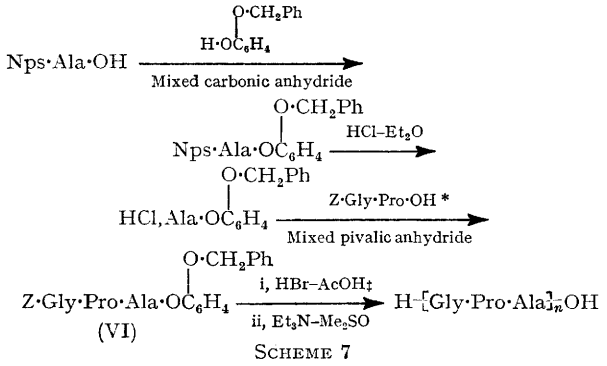
SCHEME 4




SCHEME 5




SCHEME 6




SCHEME 7

‡ Removal of protecting groups by hydrogenolysis gave a less satisfactory polymer in lower yield.

Abbreviated designations for amino-acid residues (all of which are L) used here and elsewhere in this paper are as recommended I.U.P.A.C. Information Bulletin No. 26, 1966. Nps = 2-Nitrophenylsulphenyl; Z = benzyloxycarbonyl;

$-C_6H_4$(O·$CH_2$Ph) = 2-benzyloxyphenyl; $-C_6H_4$(OH) = 2-hydroxyphenyl.

[5] J. H. Jones and G. T. Young, *J. Chem. Soc.* (*C*), 1968, 436.
[6] J. Rudinger, *Pure Appl. Chem.*, 1963, **7**, 335.
[7] D. F. DeTar and N. F. Estrin, *Tetrahedron Letters*, 1966, 5985.
[8] J. Kovacs, R. Gianotti, and A. Kapoor, *J. Amer. Chem. Soc.*, 1966, **88**, 2282.

trifluoroacetic acid over palladium–charcoal and subsequent steps and purification as just outlined gave a polymer of lower molecular weight in poorer yield. Detailed studies on these preparations of poly(glycylglycyl-L-phenylalanine) were, however, inhibited by the extreme insolubility and intractability of this polymer.

We therefore turned to alternative sequential polypeptides for further evaluation, and first attempted the synthesis of poly-(β-alanyl-L-phenylalanine) *via* the fully protected intermediate (V) by a route analogous to that used for poly(glycylglycyl-L-phenylalanine). The β-alanine polymer, however, proved to be a remarkably refractory material, being easily soluble only in trifluoroacetic acid, and we abandoned it after a cursory examination.

The sequential polytripeptide poly(glycyl-L-prolyl-L-alanine) seemed a more satisfactory objective for a thorough investigation of the 2-hydroxyphenyl ester method. This polymer has been prepared *via* a 4-nitrophenyl ester by Blout and his associates as shown in Scheme 6,[9] and extensively investigated as a collagen model in *X*-ray diffraction,[10] conformational,[11,12] and enzymic studies.[13]

Poly(glycyl-L-prolyl-L-alanine) was prepared in this work as indicated in Scheme 7. The fully protected intermediate (VI) was first obtained [1] as an amorphous material, but it was purified by reprecipitation and used in subsequent steps without difficulty. We have now, however, obtained this substance in crystalline form. The crude polymer (66% yield) was practically insoluble in water but dissolved easily in acetic acid and remained in solution when diluted with water. The solution thus obtained was dialysed, freeze-dried, and then dried to constant weight at 100°/0·1 mm., giving poly(glycyl-L-prolyl-L-alanine) as a white fluffy powder. Some of the properties of this polymer are summarised in the Table,

Properties of poly(glycyl-L-prolyl-L-alanine)

| | 2-Hydroxyphenyl ester route | 4-Nitrophenyl ester route (data from refs. 9 and 11) |
|---|---|---|
| % Hydration (w/w) | 12·5 [a] | <1 |
| Solubility in $H_2O$ | Very sparingly soluble [b] | Soluble |
| $[\alpha]_{546}$ | −426° (*c* 0·05 in $H_2O$) [c] | −208° (*c* 0·08 in $H_2O$) [d] |
| Mol. wt. (Archibald method) | 1·2 ± 0·1 × $10^4$ | 1·40 ± 0·05 × $10^4$ |

[a] Calc. from results of elemental analysis. Apart from firmly held water, the polymer was pure, as judged by the C:N ratio, the n.m.r. spectrum, and amino-acid analysis. [b] Solutions of concentration up to 0·1% in water could be obtained by heating at 50° for several hr. [c] Measurements were made at 20° after equilibration of the solutions at 20° for 24 hr. [d] At 25°: misquoted as being at 20° in the preliminary communication.[1]

together with published data [9] on polymer obtained by the 4-nitrophenyl ester route. Despite the closeness of their molecular weights these two preparations have strikingly different solubilities and optical rotations. These discrepancies must be ascribed to the presence of some racemised residues in the 4-nitrophenyl ester product, since the 2-hydroxyphenyl ester product is optically pure (see later). Racemisation could have intervened at either or both of two stages in the 4-nitrophenyl ester synthesis (Scheme 6), *i.e.* the activation and polymerisation stages. In spite of this the 4-nitrophenyl ester product was shown by enzymic means to contain few if any racemised residues,[9] although the possibility of slight racemisation could not be ruled out.[12] The data in the Table therefore serve as a cautionary indication of the serious extent to which racemisation can affect the properties of a sequential polypeptide. Furthermore, it can be inferred from the fact that racemisation of a small number of residues causes such a large decrease in rotation that the reduction in rotation is mainly due to the loss of a contribution from conformational asymmetry, *i.e.* that poly(glycyl-L-prolyl-L-alanine) does have an ordered conformation in aqueous solution at room temperature. This is precisely opposite to the conclusion drawn from studies [11] on poly(glycyl-L-prolyl-L-alanine) obtained by the 4-nitrophenyl ester route.

While this work was in progress, Professor E. R. Blout informed us that he and his co-workers had recently prepared poly-(L-prolyl-L-alanylglycine),[14] which has the same amino-acid sequence as poly(glycyl-L-prolyl-L-alanine) except at the termini. Their synthesis of poly(L-prolyl-L-alanylglycine) entirely precluded racemisation, since the *C*-terminal residue of the polymerising unit was glycine. Their preparation [14] has almost the same specific rotation as the poly(glycyl-L-prolyl-L-alanine) described here, and the c.d. spectra [14] of the two preparations are essentially identical, both qualitatively and quantitatively. This comparison, which we believe to be valid because the molecular weights of the two preparations are very close, constitutes unequivocal proof that racemisation did not occur in the present synthesis.

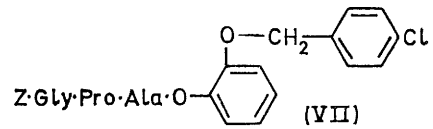
These preliminary experiments demonstrate that 2-hydroxyphenyl esters are suitable intermediates for the synthesis of simple sequential polypeptides, with the substantial advantage that racemisation does not occur. However the general route so far developed (Schemes 4, 5, and 7) lacks versatility, since the preferred conditions for activation by cleavage of the benzyl ether group (6N-hydrogen bromide in acetic acid) prohibit the use of most currently available methods of side-chain protection. In addition, most of the benzyloxycarbonylpeptide 2-benzyloxyphenyl esters we have so far prepared have been difficult to crystallize. In the interests of

[9] S. M. Bloom, S. K. Dasgupta, R. P. Patel, and E. R. Blout, *J. Amer. Chem. Soc.*, 1966, **88**, 2035.
[10] W. Traub and A. Yonath, *J. Mol. Biol.*, 1967, **25**, 351.
[11] P. J. Oriel and E. R. Blout, *J. Amer. Chem. Soc.*, 1966, **88**, 2041.
[12] F. R. Brown, tert., J. P. Carver, and E. R. Blout, *J. Mol. Biol.*, 1969, **39**, 307.
[13] K. I. Kivirikko and D. J. Prockop, *J. Biol. Chem.*, 1969, **244**, 2755; E. Adams, S. Antoine, and A. Goldstein, *Biochim. Biophys. Acta*, 1969, **185**, 251.
[14] F. R. Brown, tert., G. P. Lorenzi, and E. R. Blout, to be published.

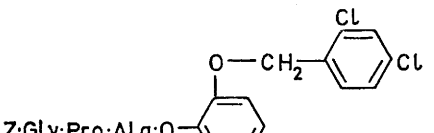
greater synthetic flexibility and improved crystallinity we have examined alternative means of blocking the phenolic group until its catalytic assistance is required. Other work [15] in this laboratory has shown that the t-butyl group cannot be used for this purpose, since 2-t-butoxyphenol is difficult to prepare in a pure condition and the preparation of esters from it is subject to severe steric hindrance. We therefore turned our attention to variously substituted benzyl groups for protection.

The introduction of electron-donating substituents into the aromatic ring of protective groups based on benzyl alcohol confers enhanced lability to acid: the 4-methoxybenzyloxycarbonyl,[16] 4-methoxybenzyl ester,[17] and 2,4,6-trimethylbenzyl ester [18] methods of protection are of this type. We have however been unable to prepare either 2-(4-methoxybenzyloxy)phenol or 2-(2,4,6-trimethylbenzyloxy)phenol from catechol: in both cases only the corresponding diethers could be isolated.

Conversely, the introduction of electron-withdrawing substituents into the aromatic rings of benzyl-based protecting groups gives increased stability towards acidolysis. This is the basis of the use of 4-nitrobenzyloxycarbonyl,[19] 4-nitrobenzyl ester,[20] and 4-chlorobenzyl ester [21] groups, which resist hydrogen bromide in acetic acid but are cleaved by hydrogenolysis. The 4-nitrobenzyl group seemed inappropriate for our purpose, because work-up after hydrogenation would probably be attended by difficulties arising from the formation of 4-aminotoluene. We did, however, investigate the use of two chlorinated benzyl groups. 2-(4-chlorobenzyloxy)phenol and 2-(2,4-dichlorobenzyloxy)phenol were prepared from catechol and the appropriate benzyl chloride without difficulty and used in syntheses of the fully protected monomers (VII) and (VIII) by routes




(VII)




(VIII)

similar to that shown in Schemes 4 and 7. All of the intermediates and both final products in these syntheses were crystalline. However, neither (VII) nor (VIII) was of any value for sequential polypeptide synthesis since, in both cases, n.m.r. spectroscopy showed that the scission of the substituted benzyl ether group was incomplete even after extended periods of catalytic hydrogenation.

## EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus, optical rotations with a Perkin-Elmer 141 automatic polarimeter (solutions in a 1 dm. cell), i.r. spectra with a Perkin-Elmer 257 spectrometer, u.v. spectra with a Carey 14M spectrometer, and n.m.r. spectra with a Perkin-Elmer R14 spectrometer operating at 100 MHz, with tetramethylsilane as internal standard (by Mrs. E. E. Richards). Mass spectra were determined (by Dr. R. T. Aplin) with an A.E.I. MS9 spectrometer operating at 70 ev: samples were introduced into the source by way of the direct insertion lock. Viscosities were determined with an Ubbelohde viscometer (British Standard number BS/IP/MSL/2) at 25·0 ± 0·1°. The amino-acid analysis was performed by Dr. S. S. Husain and the ultracentrifuge molecular weight determination was carried out by Professor E. R. Blout and his colleagues.

Evaporation was performed with a rotary evaporator, and solutions in organic solvents were dried over magnesium sulphate. Dimethyl sulphoxide was dried by distillation from calcium hydride. Triethylamine was dried by distillation from sodium; traces of primary and secondary amine contaminants were removed by refluxing with 4-nitrophenyl acetate followed by two distillations. T.l.c. was performed on unbaked Kieselgel G plates with ethyl acetate as eluant and iodine vapour for detection. Light petroleum was of b.p. 40—60°.

*2-Benzyloxyphenol.*—The modified [5] method of Druey [22] was used, except that the residue remaining after removal of ethanol was taken up in benzene instead of ether and washed with water instead of N-sodium hydroxide; excess of catechol was quantitatively removed in this way.

*2-Nitrophenylsulphenyl-L-phenylalanine 2-Benzyloxyphenyl Ester.*—Ethyl chloroformate (2·1 ml., 20 mmoles) was added to a solution of 2-nitrophenylsulphenyl-L-phenylalanine [23] (6·34 g., 20 mmoles) and triethylamine (2·8 ml., 20 mmoles) in chloroform (50 ml.) with stirring at −5°. After 5 min., a solution of 2-benzyloxyphenol (4·0 g., 20 mmoles) and triethylamine (2·8 ml., 20 mmoles) in chloroform (20 ml.) was added. The cooling bath was removed after 15 min., and the mixture was left at room temperature overnight. After removal of solvent, the residue was distributed between ethyl acetate and water, and the organic layer was washed with N-sulphuric acid, water, 10% sodium hydrogen carbonate, and water, and dried. Removal of solvent gave an oil which crystallised from ethyl acetate–light petroleum to give a yellow solid (8·50 g., 85%), m.p. 128—130°. Recrystallisation from ethyl acetate–light petroleum gave the *ester*, m.p. 129—131°, $[\alpha]_D^{20}$ −58·9° (*c* 1·0 in EtOAc), $\nu_{max.}$ ($CHCl_3$) 1750 cm.$^{-1}$; τ ($CDCl_3$) 1·7—2·0 (1H, complex, proton *ortho* to nitro-group), 2·4—3·2 (17H, complex, other aromatic protons), 5·0 (2H, s, O·C$H_2$·Ph), 5·8—7·5 (4H, complex, N*H*·C*H*·C$H_2$Ph) (Found: C, 67·25; H, 4·7; N, 5·9; S, 6·8. $C_{28}H_{24}N_2O_5S$ requires: C, 67·2; H, 4·8; N, 5·6; S, 6·4%).

[15] J. R. Smith and G. T. Young, unpublished work.
[16] F. C. McKay and N. F. Albertson, *J. Amer. Chem. Soc.*, 1957, **79**, 4686.
[17] F. Weygand and K. Hunger, *Chem. Ber.*, 1962, **95** 1.
[18] R. Ledger and F. H. C. Stewart, *Austral. J. Chem.*, 1968, **21**, 1101; F. H. C. Stewart, *ibid.*, p. 2831.
[19] F. H. Carpenter and D. T. Gish, *J. Amer. Chem. Soc.*, 1952, **74**, 3818.
[20] H. Schwarz and K. Arrakawa, *J. Amer. Chem. Soc.*, 1959, **81**, 5691; R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, 1959, **42**, 972.
[21] L. Kisfaludy and M. Löw, *Acta Chim. Acad. Sci. Hung.*, 1965, **44**, 33.
[22] J. Druey, *Bull. Soc. chim. France*, 1935, **52**, 1737.
[23] L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.*, 1963, **85**, 3660.

L-*Phenylalanine 2-Benzyloxyphenyl Ester Hydrochloride*.—A solution of hydrogen chloride in dioxan (7·8N; 3 ml.) was added to a solution of 2-nitrophenylsulphenyl-L-phenylalanine 2-benzyloxyphenyl ester (3·38 g.) in ethyl acetate (50 ml.) at room temperature. After 5 min., the solution was diluted with ether (150 ml.) and stored at 0° for 1 hr. The precipitate was filtered off, washed with ether, and recrystallised from ethanol–ether to give the *ester hydrochloride* (2·13 g., 82%), m.p. 200—201°, $[\alpha]_D^{20}$ −4·1° (*c* 1·0 in EtOH), $\nu_{max.}$ (Nujol) 1772 cm.$^{-1}$ (Found: C, 68·7; H, 5·7; Cl, 9·55; N, 3·8. $C_{22}H_{22}ClNO_3$ requires C, 69·0; H, 5·8; Cl, 9·3; N, 3·65%).

*Benzyloxycarbonylglycylglycyl-L-phenylalanine 2-Benzyloxyphenyl Ester*.—(a) *By the mercaptopyridyl ester method* (with K. Lloyd). Triethylamine (0·125 g., 1·25 mmole) was added to a suspension of benzyloxycarbonylglycylglycine 2-mercaptopyridyl ester [24] (0·359 g., 1 mmole) and L-phenylalanine 2-benzyloxyphenyl ester hydrochloride (0·384 g., 1 mmole) in chloroform (2 ml.). After 10 min., the mixture was diluted with ethyl acetate (25 ml.), washed with brine, 2N-sulphuric acid, saturated sodium hydrogen carbonate, and brine, and dried. Removal of solvents left a glass, which crystallised from chloroform–light petroleum to give (after drying at 20°/12 mm. overnight) chromatographically pure *protected tripeptide chloroform solvate*, m.p. 56—88°, $R_F$ 0·69, $[\alpha]_D^{20}$ −17·9° (*c* 1·0 in $CHCl_3$), $\nu_{max.}$ (Nujol) 1640, 1690, 1720, and 1760 cm.$^{-1}$; τ ($CDCl_3$) 2·5—3·4 (22H, complex, aromatic, chloroform, and peptide protons), 4·29 (1H, t, *J* 6 Hz, urethane NH), 4·8—5·1 (4H, complex, O·C*H*$_2$Ph groups), 5·7—6·5 (5H, complex, NH·C*H*$_2$·CO and C*H*·$CH_2$Ph) and 6·6—7·3 (2H, complex, CH·C*H*$_2$Ph) (Found: C, 58·8; H, 4·8; Cl, 15·1; N, 6·1. $C_{34}H_{33}N_3O_7$,$CHCl_3$ requires C, 58·8; H, 4·8; Cl, 14·9; N, 5·9%).

(b) *By the mixed pivalic anhydride method* (with A. W. Williams) Pivaloyl chloride (3·22 g., 26·7 mmoles), dissolved in tetrahydrofuran (20 ml.), was added during 5 min. with stirring at 0° to a solution of benzyloxycarbonylglycylglycine [25] (7·10 g., 26·7 mmoles) and *N*-methylmorpholine (5·40 g., 53·4 mmoles) in a mixture of tetrahydrofuran (125 ml.) and dioxan (125 ml.). The temperature was maintained at 0° for 25 min., and then a suspension of L-phenylalanine 2-benzyloxyphenyl ester hydrochloride (10·8 g., (28 mmoles) in a mixture of *N*-methylmorpholine (2·83 g., 28 mmoles), tetrahydrofuran (100 ml.), and dioxan (100 ml.) was added. The ice-bath was removed after a further 30 min., and the mixture was allowed to attain room temperature during 2 hr. After removal of the solvents, the residue was distributed between ethyl acetate and water, and the organic layer was washed with 2N-hydrochloric acid, water, 10% sodium hydrogen carbonate, and water, and dried. The ethyl acetate was removed, and the residual oil was dissolved in chloroform (40 ml.). Analytically pure protected tripeptide chloroform solvate (15·5 g., 80·5%), identical with the product from method (a), crystallised on addition of light petroleum (500 ml.).

*Crude Poly(glycylglycyl-L-phenylalanine)*.—*Method* (*a*). Benzyloxycarbonylglycylglycyl-L-phenylalanine 2-benzyloxyphenyl ester chloroform solvate (3·98 g., 5·5 mmoles) was dissolved, with the exclusion of moisture, in a solution of hydrogen bromide in acetic acid (25 ml.; 45% w/v). After 1 hr., the solution was concentrated at 50°/12 mm. to *ca.* 5 ml., and ether (100 ml.) was added. The oil which separated was allowed to settle, washed by decantation with three further 100 ml. portions of ether, and dried at 20°/0·1 mm. The resulting sticky orange foam was dissolved in dimethyl sulphoxide (5 ml.) by warming at 40°, and triethylamine (0·7 g., 7 mmoles) was added. After 48 hr., the solution had stiffened to a rigid gel, which was triturated with water (200 ml.). The precipitate was filtered off, washed with water (200 ml.), methanol (40 ml.), and ether (100 ml.), and dried overnight at 20°/12 mm. to give *crude polymer* (751 mg., 45%) as a buff powder, $\nu_{max.}$ (Nujol) 3300, 1645br, and 1520br, cm.$^{-1}$. When this material was heated at 300° in the ion source of the mass spectrometer, an intense peak was observed at *m/e* 522, indicating contamination with cyclo-glycylglycyl-L-phenylalanylglycylglycyl-L-phenylalanine (calc. molecular weight for the cyclohexapeptide 522). When the aqueous washings were set aside for 24 hr., filtration gave a brown powder (*ca.* 20 mg.), which was identified as cyclo-glycylglycyl-L-phenylalanylglycylglycyl-L-phenylalanine by mass spectrometry ($M^+$ 522) and by i.r. spectrometry [the i.r. spectrum (KBr) was identical to that published [26] for the DD-isomer].

*Method* (*b*). Benzyloxycarbonylglycylglycyl-L-phenylalanine 2-benzyloxyphenyl ester chloroform solvate (3·6 g., 5 mmoles), dissolved in a mixture of glacial acetic acid (50 ml.) and trifluoroacetic acid (1·0 ml.), was hydrogenated at 20°/760 mm. for 5 hr. over 10% palladium–charcoal (3·6 g.). Removal of solvents after filtration gave an oil which was triturated with three 100 ml. portions of ether and dried at 20°/0·1 mm. for 1 hr. The colourless residue was dissolved in dimethyl sulphoxide (5 ml.), and triethylamine (1·4 ml., 10 mmoles) was added. After 2 days, the resulting gel was triturated with water (250 ml.). Filtration and washing with water (250 ml.), methanol (40 ml.), and ether (100 ml.) followed by drying overnight at 20°/12 mm. gave *crude polymer* (505 mg., 38%) as a grey powder of i.r. spectrum identical with that of the product from method (*a*).

*Purification of Poly(glycylglycyl-L-phenylalanine)*.—The crude products from three preparations by method (*a*) were combined (2·136 g.) and subjected to exhaustive extraction with methanol in a Soxhlet apparatus for 12 hr. The residue was washed with ether, dried (weight 1·773 g.), and dissolved in trifluoroacetic acid (20 ml.). Addition of ether (500 ml.) after filtration gave a gel which was filtered off, washed with ether, and dried to constant weight at 80°/0·01 mm. (3 days) giving *polymer* (1·540 g.) as a cream powder, $[\alpha]_D^{20}$ +25·0° (*c* 2·09 in $CHCl_2$·$CO_2H$), $\nu_{max.}$ (Nujol) 3300, 1640, and 1520 cm.$^{-1}$, η sp/c 0·136 dl./g. (*c* 1·03 in $CHCl_2$·$CO_2H$); τ ($CF_3$·$CO_2H$) 1·8—2·4 (3H, NH), 2·4—2·9 (5H, Ph), 4·7—5·2 (1H, α-H of phenylalanine residue), 5·5—6·0 (4H, α-H of glycine residues), and 6·6—7·0 (2H, broadened d centred at 6·80, PhC*H*$_2$); no impurity absorptions were evident (Found: C, 57·2; H, 5·5; F, 3·7; N, 14·0. Calc. for $C_{13}H_{15}N_3O_3$: C, 59·8; H, 5·8; N, 16·1%). A nitrogen content of 14·0% corresponds to 87% purity. The polymer was insoluble in acetic acid, dimethylformamide, 8M-urea, saturated aqueous lithium bromide, and *m*-cresol, slightly soluble, in hexamethylphosphoramide, dimethyl sulphoxide, and 90% formic acid, but freely soluble in dichloroacetic and trifluoroacetic acids.

*Estimation of the Molecular Weight of Poly(glycylglycyl-L-phenylalanine) by End-group Analysis*.—2,4-Dinitrofluoro-

[24] K. Lloyd and G. T. Young, to be published.
[25] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
[26] R. Schwyzer and T.-K. Aung, *Helv. Chim. Acta*, 1962, **45**, 859.

benzene (0·2 g.) was added to a stirred suspension of polymer (15 mg.) in a mixture of sodium hydrogen carbonate solution (10%; 1 ml.) and ethanol (2 ml.). After 24 hr. at room temperature the mixture was acidified with concentrated hydrochloric acid and diluted with aqueous ethanol (1 : 1; 7 ml.). Isolation by centrifugation, washing with water (3 × 10 ml.), ethanol (6 × 10 ml.), and ether (3 × 10 ml.), and drying at 20°/0·1 mm. gave *dinitrophenylated polymer*, $\lambda_{max.}$ 360 nm., $E^{1\%}_{1\,cm.}$ 35 in dimethyl sulphoxide. Comparison with 2,4-dinitrophenylglycylglycine [$\lambda_{max.}$ 360 nm. ($\varepsilon$ 1·8 × $10^4$) in dimethyl sulphoxide] gives a number average molecular weight of *ca.* 5 × $10^3$.

*Verification of the Optical Purity of Poly(glycylglycyl-L-phenylalanine) by Examination of the Optical Rotation of the Hydrolysate.*—Hydrolysis of the polymer (83·9 mg., 0·280 mmole after correction for 87% purity) with concentrated hydrochloric acid–water (4 : 1) in a sealed tube for 21·5 hr. at 110° followed by evaporation and dissolution of the residue in 1·82 ml. of pH 7·03 phosphate buffer gave a solution of optical rotation −0·316° (D-line, 20°). Treatment of a control mixture of L-phenylalanine (46·6 mg., 0·280 mmole) and glycine (42 mg., 0·56 mmole) under precisely the same conditions gave a solution of optical rotation −0·308°. This corresponds to an optical purity of 103%: assessment of optical purity by this method is subject to errors of 2—3%.[7,8]

*Benzyloxycarbonyl-β-alanyl-L-phenylalanine 2-Benzyloxyphenyl Ester* (with K. Lloyd).—Triethylamine (0·125 g., 1·25 mmole) was added to a suspension of benzyloxycarbonyl-β-alanine 2-mercaptopyridyl ester[24] (0·316 g., 1·0 mmole) and L-phenylalanine 2-benzyloxyphenyl ester hydrochloride (0·384 g., 1·0 mmole) in chloroform (2 ml.). After 10 min. at room temperature, the mixture was diluted with ethyl acetate (25 ml.), washed with brine, 2N-sulphuric acid, saturated sodium hydrogen carbonate, and brine, and dried. Removal of solvent gave a white solid (0·512 g., 93%), m.p. 136—140°. Recrystallisation from chloroform–light petroleum gave *protected dipeptide*, m.p. 138·5—140·5°, $R_F$ 0·84, $[\alpha]_D^{20}$ −21·8° (*c* 0·8 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1645, 1695, and 1770 $cm.^{-1}$; $\tau$ ($CDCl_3$) 2·5—3·2 (20H, complex, aromatic and peptide protons), 3·94 (1H, d, *J* 7 Hz, urethane NH), 4·6—5·1 (5H, complex, O·C$H_2$Ph groups and C$H$·$CH_2$-Ph), 6·4—7·1 (4H, complex, CH·C$H_2$Ph and NH·C$H_2$·$CH_2$), and 7·77 (2H, t, *J* 6 Hz, NH·$CH_2$·C$H_2$·CO) (Found: C, 71·4; H, 5·7; N, 5·3. $C_{33}H_{32}N_2O_6$ requires C, 71·7; H, 5·8; N, 5·1%).

*Poly-(β-alanyl-L-phenylalanine).*—Benzyloxycarbonyl-β-alanyl-L-phenylalanine 2-benzyloxyphenyl ester (2·72 g., 5 mmoles) was dissolved, with exclusion of moisture, in a solution of hydrogen bromide in acetic acid (45% w/v; 15 ml.) and set aside at room temperature for 1 hr. Addition of anhydrous ether (200 ml.) caused separation of an oil, which was washed several times by decantation with fresh ether. Removal of last traces of ether *in vacuo* gave a sticky foam, which was dissolved in the minimum amount of dimethyl sulphoxide (8·5 ml.) by warming at 40°. Triethylamine (0·8 g., 8 mmoles) was added, and the mixture was set aside at room temperature. After 2·5 days, the resulting stiff paste was triturated with water (200 ml.), and the solid was filtered off, washed with more water (200 ml.), methanol (25 ml.), and ether (100 ml.), and dried at 40°/0·1 mm., giving crude polymer (39 mg., 39%) as a buff powder, $[\alpha]_D^{20}$ −9·0° (*c* 1·21 in $CHCl_2$·$CO_2H$); $\nu_{max.}$ (Nujol) 3305, 1645, and 1545 $cm.^{-1}$; $\eta$ sp/c 0·09 dl/g (*c* 0·89 in $CHCl_2$·$CO_2H$); $\tau$ ($CF_3$·$CO_2H$) 1·8—3·2 (7H, complex, NH and aromatic protons), 4·8—5·4 (1H, α-H of phenylalanine residue), and 5·9—7·8 (6H, complex, other protons); *no* impurity absorptions were evident (Found: C, 61·45; H, 6·2; N, 12·05. $C_{12}H_{14}N_2O_2$ requires C, 66·2; H, 6·5; N, 12·9%). When the polymer was heated at 300° in the ion source of the mass spectrometer, a strong peak at *m/e* 436 was observed (calc. *M* for cyclo-β-alanylphenylalanyl-β-alanylphenylalanine 436). Extraction in a Soxhlet apparatus with methanol for 3 days caused a 20% loss in weight, but a strong peak at *m/e* 436 was still observed in the mass spectrum. The polymer was insoluble in dimethylformamide and dimethyl sulphoxide, soluble with difficulty in dichloroacetic acid, but dissolved freely in trifluoroacetic acid. On attempted hydrolysis of the polymer (28 mg.) with 7N-hydrochloric acid (3 ml.) in a sealed evacuated tube at 110°, dissolution did not occur during 50 hr., and the solid darkened to a brown sludge. Dinitrophenylation of the polymer by the method described for poly(glycylglycyl-L-phenylalanine) gave material of $\lambda_{max.}$ 353 nm., $E^{1\%}_{1\,cm.}$ 51 in dichloroacetic acid. Comparison with 2,4-dinitrophenyl-β-alanine [$\lambda_{max.}$ 353 nm. ($\varepsilon$ 1·73 × $10^4$) in dichloroacetic acid] gives a number average molecular weight of *ca.* 3400.

*L-Alanine 2-Benzyloxyphenyl Ester Hydrochloride.*—2-Nitrophenylsulphenyl-L-alanine 2-benzyloxyphenyl ester (60 g.; obtained as an impure oil in 95% yield by the method described for the analogous L-phenylalanine derivative) was dissolved in anhydrous ether (400 ml.) and a solution of hydrogen chloride in anhydrous ether (200 ml.; 3N) was added. The oil which separated solidified overnight, giving a yellow powder (36·2 g.). Two recrystallisations from ethanol–ether gave *ester hydrochloride* (25·1 g., 58%) as needles of indefinite m.p. 160—170°, $[\alpha]_D^{20}$ −14·6° (*c* 1·0 in $CHCl_3$), $\nu_{max.}$ (Nujol) 1760 $cm.^{-1}$; $\tau$ ($CDCl_3$) 0·5—1·7 (3H, $NH_3^+$), 2·2—2·9 (9H, s, at 2·67 superimposed on a complex system, aromatic protons), 5·01 (2H, s, C$H_2$Ph), 5·65 (1H, q, *J* 8 Hz, C$H$·$CH_3$), and 8·42 (3H, d, *J* 8 Hz, CH·C$H_3$) (Found: C, 62·4; H, 5·95; Cl, 11·45; N, 4·8. $C_{16}H_{18}ClNO_3$ requires C, 62·5; H, 5·9; Cl, 11·5; N, 4·55%).

*Benzyloxycarbonylglycyl-L-prolyl-L-alanine 2-Benzyloxyphenyl Ester.*—Pivaloyl chloride (1·20 g., 10 mmoles), dissolved in methyl cyanide (5 ml.) was added with stirring at 0° to a solution of benzyloxycarbonylglycyl-L-proline[9,27] (3·06 g., 10 mmoles) and *N*-methylmorpholine (1·01 g., 10 mmoles) in methyl cyanide (15 ml.). After 10 min., a suspension of L-alanine 2-benzyloxyphenyl ester hydrochloride (3·08 g., 10 mmoles) in a mixture of *N*-methylmorpholine (1·01 g., 10 mmoles) and methyl cyanide (5 ml.) was added. The mixture was left at 0° for a further 15 min., and then allowed to attain room temperature during 2·5 hr. After removal of the methyl cyanide, the residue was distributed between ethyl acetate (50 ml.) and water, and the organic phase was washed with saturated sodium hydrogen carbonate, N-hydrochloric acid, and brine, and dried. The solution was concentrated to *ca.* 10 ml. and light petroleum (200 ml.) was added. The oil thus obtained was reprecipitated twice from ethyl acetate with light petroleum and finally dried at 35°/0·1 mm to give chromatographically pure *protected tripeptide* (4·25 g., 76%) as a crisp foam of indefinite m.p., $R_F$ 0·36, $[\alpha]_D^{20}$ −76·8° (*c* 1·0 in EtOAc), $\nu_{max.}$ ($CHCl_3$) 1765, 1720, 1680, and 1655 $cm.^{-1}$; $\tau$ ($CDCl_3$) 2·4—3·2 (15H, complex, aromatic protons and peptide NH), 4·14 (1H, broadened t, *J* 6 Hz, urethane NH), 4·91 (2H, s, O·C$H_2$Ph), 4·98 (2H, s, other benzylic protons), 5·0—5·4 (1H, complex,

[27] H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.*, 1956, 3642.

α-H of alanine residue), 5·45—5·60 (1H, complex, α-H of proline residue), 6·06 (2H, d, *J* 6 Hz, NH·C*H*$_2$), 6·3—6·9 (2H, complex, N·C*H*$_2$·CH$_2$), 7·6—8·4 (4H, complex, N·CH$_2$·CH$_2$·C*H*$_2$), and 8·65 (3H, d, *J* 8 Hz, CH·C*H*$_3$); traces of ethyl acetate (quartet centred at 5·9, singlet at 8·0, and triplet centred at 8·78) were evident (Found: C, 66·0; H, 6·05; N, 6·9. $C_{31}H_{33}N_3O_7$ requires C, 66·5; H, 5·9; N, 7·5%). This material was used as such in the polymerisations described later but when a small sample was set aside with light petroleum, crystals formed after many months. Recrystallisation of these from ethyl acetate–light petroleum gave *protected tripeptide*, m.p. 105—107°, $[\alpha]_D^{20}$ −78·0° (*c* 1 in EtOAc), spectroscopic properties as already detailed except that solvent bands were absent from the n.m.r. spectrum (Found: C, 66·5; H, 5·9; N, 7·5%).

*Crude Poly(glycyl-L-prolyl-L-alanine).—Method* (*a*). Benzyloxycarbonylglycyl-L-prolyl-L-alanine 2-benzyloxyphenyl ester (5·59 g., 10 mmoles) was dissolved in a solution of hydrogen bromide in acetic acid (35 ml.; 45% w/v). After 1 hr., addition of ether (200 ml.) gave a solid which was washed by decantation with three further portions (200 ml.) of ether and dried at 20°/0·1 mm. to give crude peptide active ester hydrobromide (4·1 g., 100%) as an extremely hygroscopic white powder. This product was dissolved, with warming at 50°, in dimethyl sulphoxide (4·0 ml.), and triethylamine (2·0 ml., 14 mmoles) was added to the stirred solution after the flask had been flushed with dry nitrogen. After 2 days the resulting stiff paste was triturated with ethanol (200 ml.), and the suspension was centrifuged. The solid thus obtained was washed with more ethanol (4 × 50 ml.) and ether (200 ml.), extracted with dichloromethane in a Soxhlet apparatus for 6 hr., and dried, giving crude polymer as a white powder (1·38 g., 66%), $\nu_{max.}$ (Nujol) 3300, 1640, and 1540 cm.$^{-1}$. When the crude polymer was heated to 360° in the ion source of the mass spectrometer, neither the cyclotripeptide (*M* 220) nor the cyclohexapeptide (*M* 440) was detected.

*Method* (*b*). Deprotection and activation of benzyloxycarbonylglycyl-L-prolyl-L-alanine 2-benzyloxyphenyl ester by catalytic hydrogenolysis in glacial acetic acid containing trifluoroacetic acid (1 equiv.) in the presence of palladium–charcoal followed by polymerisation and isolation as described under method (*a*) gave crude polymer as a buff powder of similar properties in 45% yield. On dialysis as described later much of this material proved to be of low molecular weight and it was not investigated further.

*Purification of Poly(glycyl-L-prolyl-L-alanine).*—Crude polymer (503 mg.) obtained by method (*a*) was dissolved in glacial acetic acid (5 ml.) and the solution was diluted with water (50 ml.). The clear solution thus obtained was dialysed against water (5 l.) for a total of 24 hr., the water being changed after 4 and 12 hr. The resulting cloudy solution was filtered through a no. 2 sinter, then freeze-dried, and the product was finally dried to constant weight at 100°/0·1 mm., giving *purified polymer* as a fluffy white-powder (380 mg.), $\nu_{max.}$ (Nujol) 3350, 1650, and 1550 cm.$^{-1}$; $\eta$ sp/c 0·306 dl/g (*c* 1·21 in CHCl$_2$·CO$_2$H); $\tau$ (CF$_3$·CO$_2$H) 1·8—2·4 (2H, NH), 4·8—6·6 (6H, complex, α-protons and N·C*H*$_2$·CH$_2$), 7·2—8·1 (4H, N·CH$_2$·C*H*$_2$·C*H*$_2$·CH), and 8·1—8·6 (3H, broadened d, centred at 8·40, Me); no impurity absorptions were detected; amino-acid analysis: Gly 1·00; Pro 0·95; Ala 1·05; weight average mol. wt. (Archibald method): 1·20 ± 0·1 × 10$^4$ (Found: C, 46·6; H, 6·4; N, 16·4. Calc. for $C_{10}H_{15}N_3O_3$: C, 53·3; H, 6·65; N, 18·65%). These figures indicate *ca.* 12·5% water in this preparation, which is otherwise pure as judged from the C : N ratio (Found: 2·85. Calc.: 2·85).

The polymer was freely soluble in acidic solvents, *m*-cresol, 2,2,2-trifluoroethanol, and dimethyl sulphoxide, but almost insoluble in water. Aqueous solutions of concentration less than 0·1% for optical rotation measurements were obtained by heating at *ca.* 50° for several hours followed by equilibration at 20° for 24 hr. Four separate determinations of $[\alpha]_{546}^{20}$ gave values between −418° and −440° (corrected for 12·5% hydration), with a mean value of −426° (*c* 0·05 in H$_2$O). The poly(glycyl-L-prolyl-L-alanine) [mol. wt. (Archibald method [11]) 1·40 ± 0·05 × 10$^4$] prepared by the 4-nitrophenyl ester route by Blout and his co-workers [9] had $[\alpha]_{546}^{25}$ −206° (*c* 0·08 in H$_2$O).

*Attempted Preparation of 2-(2,4,6-Trimethylbenzyloxy)-phenol.—Method* (*a*). The method used by Druey [22] for the preparation of 2-benzyloxyphenol, with modifications as given later for the preparation of 2-(4-chlorobenzyloxy)-phenol, was used, starting with 2,4,6-trimethylbenzyl chloride [28] (8·45 g., 0·05 mole). After removal of sodium chloride no solid separated out on cooling and after the benzene extraction procedure, evaporation gave a brown oil. Distillation at 0·4 mm. gave only 2,4,6-trimethylbenzyl ethyl ether as an oil in 35% yield, b.p. 68—70°/0·4 mm., $n_D^{20}$ 1·5028, $\nu_{max.}$ (film) 1100 cm.$^{-1}$; $\tau$ (CCl$_4$) 3·30 (2H, s, aromatic protons), 5·61 (2H, s, C*H*$_2$·OEt), 6·58 (2H, q, *J* 7 Hz, O·C*H*$_2$·CH$_3$), 7·7 (6H, s, symmetrical methyl groups), 7·79 (3H, s, other Me on benzene ring), and 8·85 (3H, t, *J* 7 Hz, O·CH$_2$·C*H*$_3$) (lit.,[29] b.p. 115—116°/14 mm., $n_D^{20}$ 1·5003).

*Method* (*b*). The method used by Henbest and his co-workers [30] for the preparation of 4-methoxybenzyloxybenzene was adapted as follows. A solution of 2,4,6-trimethylbenzyl chloride [28] (8·45 g., 0·05 mmole) in acetone (20 ml.) was added to a solution of catechol (6·05 g., 0·055 mole) in acetone (20 ml.). Potassium carbonate (24 g.) was added and the suspension was heated at its b.p. for 9 hr. with stirring. After filtration, removal of solvent gave a pale brown solid which was taken up in benzene (100 ml.). The organic phase was washed with water (4 × 100 ml.), dried, and evaporated leaving a brown semi-solid residue. The solid (1·05 g.) which separated on trituration with ethanol was filtered off. Recrystallisation from ethanol gave *1,2-bis-(2,4,6-trimethylbenzyloxy)benzene*, m.p. 143—144°, $\nu_{max.}$ (CS$_2$) 1250 cm.$^{-1}$, $\lambda_{max.}$ (Et$_2$O) 274 (ε 3933) and 221 nm. (29,500); $\tau$ (CCl$_4$) 3·15 (4H, s, aromatic protons of benzylic groups), 3·30 (4H, s, other aromatic protons), 5·11 (4H, s, O·C*H*$_2$·C$_6$H$_2$Me$_3$), and 7·77 (18H, s, methyl groups) (Found: C, 83·4; H, 7·8. $C_{26}H_{30}O_2$ requires C, 83·5; H, 7·8%). The mass spectrum showed a molecular ion at *m/e* 374 (Calc. *M* 374) and the fragmentation pattern was consistent with the expected structure. Evaporation of the liquors gave a chromatographically impure oil (5·80 g.) which on distillation at 0·3 mm. gave only unchanged chloride (1·1 g.).

*Attempted Preparation of 2-(4-Methoxybenzyloxy)phenol.*—The preparation of 2-(4-methoxybenzyloxy)phenol from 4-methoxybenzyl chloride [31] (31·3 g., 0·2 mole) was at-

[28] R. C. Fuson and N. Rabjohn, *Org. Synth.*, Coll. Vol. III, 1955, 557.

[29] C. R. Hauser and D. N. Van Eenam, *J. Amer. Chem. Soc.*, 1957, **79**, 6277.

[30] H. B. Henbest, J. A. W. Reid, and C. J. M. Stirling, *J. Chem. Soc.*, 1961, 5239.

[31] R. Quelet and J. Allard, *Bull. Soc. chim. France*, 1937, **4**, 1468.

tempted by method (*b*) used for the unsuccessful preparation of 2-(2,4,6-trimethylbenzyloxy)phenol, with the following modifications. After filtration of the mixture the acetone was removed and ethanol (200 ml.) was added. The resulting suspension was set aside at 5° overnight. Filtration gave a light brown powder (5·23 g.), which was recrystallised twice from ethanol to give 1,2-*bis*-(4-*methoxybenzyloxy*)*benzene* as needles, m.p. 124—125°, $\nu_{max.}$ ($CS_2$) 1250 cm.$^{-1}$, $\lambda_{max.}$ ($Et_2O$), 275 (ε 7240) and 230 nm. (34,700); τ ($CDCl_3$) 2·69 (4H, low-field d of ABq, *J* 9 Hz, aromatic protons of benzylic groups), 3·12 (4H, s, aromatic protons of ring derived from catechol), 3·15 (4H, high-field d of ABq, *J* 9 Hz, aromatic protons of benzylic groups), 4·99 (4H, s, O·C$H_2$·$C_6H_4$·$OCH_3$), and 6·28 (6H, s, methyl groups) (Found: C, 75·7; H, 6·2. $C_{22}H_{22}O_4$ requires C, 75·4; H, 6·3%). The ethanol was removed and the residue was distributed between benzene (250 ml.) and water (250 ml.). The organic phase was washed with water (4 × 200 ml.) and dried. Evaporation gave a chromatographically impure brown oil (21·8 g.). Attempted distillation at 0·1 mm. resulted in decomposition.

2-(4-*Chlorobenzyloxy*)*phenol*.—The method used by Druey [22] for the preparation of 2-benzyloxyphenol was modified as follows. Catechol (22 g., 0·2 mole) was dissolved in ethanol (100 ml.) under nitrogen, and a solution of sodium ethoxide [sodium (4·6 g., 0·2 mole) in ethanol (70 ml.)] was added. This was followed immediately by a solution of 4-chlorobenzyl chloride (32·2 g., 0·2 mole) in ethanol (75 ml.). A slow stream of nitrogen was passed as the mixture was heated at its b.p. for 3 hr. After acidification (litmus) of the hot mixture with concentrated hydrochloric acid, the sodium chloride was filtered off. The solid which separated on cooling gave 1,2-*bis*-(4-*chlorobenzyloxy*)*benzene* as needles, m.p. 88·5—89·5° (from ethanol), $\nu_{max.}$ ($CS_2$) 1255 cm.$^{-1}$, $\lambda_{max.}$ ($Et_2O$) 277 (ε 2461) and 223 nm. (29,880); τ ($CCl_4$) 2·72 (8H, s, aromatic protons of benzylic groups), 3·18 (4H, s, other aromatic protons), and 5·01 (4H, s, O·C$H_2$·$C_6H_6Cl$) (Found: C, 67·0; H, 4·6; Cl, 19·45. $C_{20}H_{16}Cl_2O_2$ requires C, 66·9; H, 4·6; Cl, 19·8%). The mass spectrum showed a molecular ion at *m/e* 358 with isotope peaks at *m/e* 359, 360, 361, and 362, the relative intensities of these peaks being consistent with the molecular formula $C_{20}H_{16}Cl_2O_2$. The ethanol was removed from the filtrate and the residue was distributed between benzene (200 ml.) and water (200 ml.). The organic phase was washed with water (5 × 200 ml.) and dried. Evaporation gave a light brown oil (29·3 g., 62%), which crystallised overnight at 5°. Recrystallisation from light petroleum gave the *monoether* as white needles (22·5 g., 47%), m.p. 45—53°, $\nu_{max.}$ ($CS_2$) 3550 and 1265 cm.$^{-1}$; $\lambda_{max.}$ (EtOH) 276 (ε 1919) and 221 nm. (12,200); τ ($CCl_4$) 2·68 (4H, s, aromatic protons of benzylic group), 3·1—3·4 (4H, complex, protons of phenolic ring), 4·5—4·7 (1H, OH), and 4·96 (2H, s, O·C$H_2$·$C_6H_4Cl$) (Found: C, 66·4; H, 5·0; Cl, 15·35. $C_{13}H_{11}ClO_2$ requires C, 66·5; H, 4·7; Cl, 15·2%). The mass spectrum showed a molecular ion at *m/e* 234 with isotope peaks at *m/e* 235, 236, and 237, the relative intensities being consistent with the molecular formula $C_{13}H_{11}ClO_2$.

2-(2,4-*Dichlorobenzyloxy*)*phenol*.— 2-(2,4-Dichlorobenzyloxy)phenol was prepared from 2,4-dichlorobenzyl chloride (19·5 g., 0·1 mole) by the method used for 2-(4-chlorobenzyloxy)phenol. A white powder (3·4 g.) separated from the ethanolic solution on cooling after removal of the sodium chloride. Recrystallisation from ethanol gave 1,2-*bis*-(2,4-*dichlorobenzyloxy*)*benzene* as white needles, m.p. 141—142°, $\nu_{max.}$ ($CS_2$) 1255 cm.$^{-1}$; $\lambda_{max.}$ ($Et_2O$) 276 (ε 1632) and 218 nm. (24,320); τ ($CDCl_3$) 2·3—2·9 (6H, complex, aromatic protons of benzylic groups), 3·05 (4H, s, other aromatic protons), and 4·82 (4H, s, O·C$H_2$·$C_6H_3Cl_2$) (Found: C, 56·0; H, 3·45; Cl, 32·9. $C_{20}H_{14}Cl_4O_2$ requires: C, 56·0; H, 3·3; Cl, 33·2%). The mass spectrum showed a molecular ion at *m/e* 426, with isotope peaks at *m/e* 427, 428, 429, 430, 431, and 432, the relative intensities being consistent with the molecular formula $C_{20}H_{14}Cl_4O_2$. Crude 2-(2,4-dichlorobenzyloxy)phenol was obtained as a yellow crystalline mass (11·7 g., 43%) after removal of the diether. Recrystallisation from light petroleum gave the *monoether* as white needles (9·3 g., 35%), m.p. 42—46°, $\nu_{max.}$ ($CS_2$) 3550 and 1625 cm.$^{-1}$; $\lambda_{max.}$ (EtOH) 276 (ε 3383) and 218 nm. (19,630); τ ($CDCl_3$) 2·3—3·2 (7H, complex, aromatic protons), 4·29 (1H, s, OH), and 4·83 (2H, s, O·C$H_2$·$C_6H_3Cl_2$) (Found: C, 57·9; H, 3·95; Cl, 26·15. $C_{13}H_{10}Cl_2O_2$ requires: C, 58·0; H, 3·7; Cl, 26·4%). The mass spectrum showed a molecular ion at *m/e* 268 with isotope peaks at *m/e* 269, 270, 271, and 272, the relative intensities being consistent with the molecular formula $C_{13}H_{10}Cl_2O_2$.

L-*Alanine* 2-(4-*Chlorobenzyloxy*)*phenyl Ester Hydrochloride*.—This compound was prepared from 2-(4-chlorobenzyloxy)-phenol on a 10 mmole scale by the method described for the corresponding benzyloxyphenyl ester. Recrystallisation from chloroform–ether gave the *ester hydrochloride* as white needles (87%), m.p. 114·5—116·5°, $[\alpha]_D^{20}$ −12·2° (*c* 1 in $CHCl_3$); $\nu_{max.}$ (Nujol) 1770 cm.$^{-1}$; τ ($CDCl_3$) 0·8—2·0 (3H, $NH_3^+$), 2·6—3·4 (8H, complex, aromatic protons), 5·05 (2H, s, O·C$H_2$·$C_6H_4Cl$), 5·3—5·8 (1H, NH·C*H*·CO), and 8·39 (3H, d, *J* 7 Hz, CH·C$H_3$) (Found: C, 55·7; H, 5·2; Cl, 21·15; N, 4·1. $C_{16}H_{17}Cl_2NO_3$ requires C, 56·0; H, 5·0; Cl, 20·8; N, 4·1%).

*Benzyloxycarbonylglycyl*-L-*prolyl*-L-*alanine* 2-(4-*Chlorobenzyloxy*)*phenyl Ester*.—This compound was prepared from alanine 2-(4-chlorobenzyloxy)phenyl ester hydrochloride as described for benzyloxycarbonylglycyl-L-prolyl-L-alanine 2-benzyloxyphenyl ester, on a 2 mmole scale. Evaporation gave an oil which solidified to a white solid when triturated with light petroleum. Recrystallisation from ethyl acetate–light petroleum gave chromatographically pure ($R_F$ 0·34) *protected tripeptide* as white needles (0·907 g., 75%), m.p. 129·5—130·5°, $[\alpha]_D^{20}$ −70·3° (*c* 1 in EtOAc); $\nu_{max.}$ ($CHCl_3$) 1765, 1720, 1680, and 1655 cm.$^{-1}$; τ ($CDCl_3$) 2·4—3·2 (14H, complex, aromatic protons and peptide NH), 4·05—4·35 (1H, urethane NH), 4·88 (2H, s, PhC$H_2$·O), 5·00 (2H, s, O·C$H_2$·$C_6H_4Cl$), 5·22 (1H, complex, α-H of alanine residue), 5·4—5·6 (1H, complex, α-H of proline residue); 6·02 (2H, d, *J* 5 Hz, NH·C$H_2$), 6·3—6·8 (2H, complex, N·C$H_2$·$CH_2$), 7·6—8·4 (4H, complex, N·$CH_2$·-C$H_2$·$CH_2$), and 8·60 (3H, d, *J* 7 Hz, CH·C$H_3$) (Found: C, 62·4; H, 5·6; Cl, 6·0; N, 7·0. $C_{31}H_{32}ClN_3O_7$ requires C, 62·7; H, 5·4; Cl, 6·0; N, 7·1%).

*Benzyloxycarbonyl*-L-*alanine* 2-(2,4-*Dichlorobenzyloxy*)-*phenyl Ester*.—This compound was prepared from benzyloxycarbonyl-L-alanine and 2-(2,4-dichlorobenzyloxy)phenol on a 5 mmole scale by the method used for 2-nitrophenylsulphenyl-L-phenylalanine 2-benzyloxyphenyl ester, except that the washing operations were performed with chloroform as solvent. Removal of chloroform gave a white crystalline solid (1·50 g., 63%). Recrystallisation from ethyl acetate–light petroleum gave chromatographically pure ($R_F$ 0·68) *ester* as white needles (1·20 g., 51%), m.p. 94—96°, $[\alpha]_D^{20}$ −22·1 (*c* 1 in $CHCl_3$), $\nu_{max.}$ (Nujol) 1770 and 1690 cm.$^{-1}$; τ ($CDCl_3$) 2·4—3·2 (12H, complex, aromatic protons),

4·5—5·0 (5H, complex, benzylic and urethane protons), 5·28 (1H, complex, NH·C*H*·CO), and 8·53 (3H, d, *J* 7 Hz, CH·C*H*$_3$) (Found: C, 60·5; H, 4·5; Cl, 15·0; N, 3·1. $C_{24}H_{21}Cl_2NO_6$ requires C, 60·8; H, 4·4; Cl, 14·7; N, 3·0%).

L-*Alanine 2-(2,4-Dichlorobenzyloxy)phenyl Ester Hydrobromide*.—A solution of hydrogen bromide in acetic acid (0·16 ml.; 5·6N) was added to a mixture of benzyloxycarbonyl-L-alanine 2-(2,4-dichlorobenzyloxy)phenyl ester (98·4 mg., 0·2 mmole) and glacial acetic acid (0·05 ml.). The mixture was swirled for 15 min. until a clear solution was obtained. After 30 min. the acetic acid was partially removed *in vacuo*, and ether (100 ml.) was added. The solid which separated was filtered off to give the *ester hydrobromide* as white needles (60·7 mg., 70%), m.p. 135—136°, $[\alpha]_D^{20}$ −13·2° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1770 cm.$^{-1}$; $\tau$ ($CDCl_3$) 1·0—1·8 (3H, $NH_3^+$), 2·5—3·4 (7H, complex, aromatic protons), 4·94 (2H, s, O·C*H*$_2$·C$_6$H$_3$Cl$_2$), 5·2—5·8 (1H, complex, C*H*·CH$_3$), and 8·35 (3H, d, *J* 7 Hz, CH·C*H*$_3$) (Found: C, 45·65; H, 3·75; Br, 19·25; Cl, 16·55; N, 3·5. $C_{16}H_{16}BrClNO_3$ requires C, 45·6; H, 3·8; Br, 19·0; Cl, 16·9; N, 3·3%).

*Benzyloxycarbonylglycyl-L-prolyl-L-alanine 2-(2,4-Dichlorobenzyloxy)phenyl Ester*.—This was prepared from alanine 2-(2,4-dichlorobenzyloxy)phenyl ester on a 2 mmole scale by the method used for benzyloxycarbonylglycyl-L-prolyl-L-alanine 2-benzyloxyphenyl ester. The crude product was obtained as a white solid, which on recrystallisation from ethyl acetate–light petroleum gave chromatographically pure ($R_F$ 0·34) *protected tripeptide* as white needles (0·968 g., 77%), m.p. 100—103°, $[\alpha]_D^{20}$ −67·3° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1765, 1720, 1680, and 1655 cm.$^{-1}$; $\tau$ ($CDCl_3$ 2·4—3·2 (13H, complex, aromatic protons and peptide NH), 4·05—4·35 (1H, urethane NH), 4·87 (4H, superimposed singlets, PhC*H*$_2$·O and O·C*H*$_2$·C$_6$H$_3$Cl$_2$), 5·26 (1H, complex, α-H of alanine residue), 5·35—5·60 (1H, complex, α-H of proline residue), 6·01 (2H, d, *J* 5 Hz, NH·C*H*$_2$), 6·2—6·8 (2H, complex, N·C*H*$_2$·CH$_2$), 7·6—8·3 (4H, complex, N·CH$_2$·C*H*$_2$·C*H*$_2$), and 8·55 (3H, d, *J* 7 Hz, CH·C*H*$_3$) (Found: C, 59·45; H, 5·2; Cl, 11·55; N, 6·7. $C_{31}H_{31}Cl_2N_3O_7$ requires C, 59·3; H, 4·9; Cl, 11·3; N, 6·7%).

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