SYNTHESES OF SOME STERICALLY PURE *N*-PROTECTED PEPTIDES BY STEPWISE ADDITION OF AMINO ACIDS TO THE CARBOXYL END OF THE PEPTIDE CHAIN*

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Since only the azide procedure for forming the peptide bond proceeds without racemization,[1] the synthesis of peptides by the stepwise addition of amino acids to the carboxyl end of the growing peptide chain has only rarely been used.

Table 1
SYNTHESIS OF Dim-L-Val-L-Ala-L-Leu-OMe[a] FROM OPTICALLY PURE Dim-L-Val-L-Ala-OH[b]

Condensing Agent	Solvent	Temp.	Isolation Procedure	Steric Purity (%)		
				Val	Ala	Leu
DCC	$CHCl_3$	0–4°	not isolated	98	96	99
DCC	$CHCl_3$	0–4	recrystallized	100	100	99
CDI	THF	0–4	not isolated	98	97	99
CDI	THF	0–4	recrystallized	100	100	99
Woodward's reagent K	CH_3CN	25	recrystallized	100	99	99

[a] The following abbreviations are used: Ala, alanine; Cbz, carbobenzoxy; CDI, carbonyldiimidazole; DDC, dicyclohexylcarbodiimide; Dim (dimedonyl), 5,5-dimethylcyclohex-2-en-1-one-3-yl; Leu, leucine; Phe, phenylalanine; Orn, ornithine; Val, valine; TFA, trifluoroacetic acid.

[b] A sensitive gas chromatography procedure was used to determine the optical purity of the products (Halpern, B., and Westley, J. W., *Biochem. biophys. Res. Commun.*, 1965, **19**, 361). Results have been corrected for 1·5% D-proline in the resolving agent.

In the present work, we have prepared several sterically pure *N*-protected peptides, by stepwise synthesis from the carboxylic end of the peptide chain, using carbonylbisimidazole as the condensing agent. By an appropriate choice of the coupling conditions the degree of racemization, as determined by gas chromatography, was kept down to 3–4%. Use of dicyclohexylcarbodiimide and Woodward's reagent K gave similar results. The crude, slightly racemized, products were crystalline and a single recrystallization effected complete removal of the unwanted diastereoisomer as indicated by gas chromatography (Table 1).

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[1] Schröder, E., and Lübke, K., "The Peptides." Vol. I, p. 236. (Academic Press: New York 1965.)

By analogous procedures a peptide chain was then extended from sterically pure Dim-L-Leu-L-Val-OH and the following peptides, Dim-L-Leu-L-Val-L-Ala-OMe, Dim-L-Leu-L-Val-L-Ala-D-Phe-OMe, and Dim-L-Leu-L-Val-L-Ala-D-Phe-L-ε-Cbz-Orn-OMe, were obtained sterically pure in 40–60% yield. This simple procedure has also been applied successfully to prepare some sterically pure Cbz-protected peptide esters such as Cbz-L-Leu-L-Val-L-Ala-OMe and Cbz-L-Leu-L-Val-L-Ala-L-Phen-OMe.

While the lack of a suitable gas chromatographic optical analysis for polyfunctional amino acids has restricted our present studies to peptides derived from the neutral amino acids, this limitation has now been overcome by the development of a suitable analytical technique for all but four of the protein amino acids.[2]

Experimental

All optical rotation measurements were carried out in methanol solution (*c*, 0·1) on an O. C. Rudolph 80 Polarimeter. We wish to thank Dr L. Throop and his associates for carrying out these determinations.

Dim-L-Val-L-Ala-OMe

Dim-L-Val-OH[3] (1·8 g) was dissolved in dry chloroform (15 ml) and the solution cooled to −5°. L-Ala-OMe (derived from 2·09 g of the hydrochloride) and DCC (1·53 g) in cold chloroform (10 ml) was added and the reaction mixture stirred at 0° overnight. The formed urea was then filtered off and the chloroform solution washed with 1N HCl, H_2O, $NaHCO_3$ (10%), and H_2O. After drying and evaporating, the product was recrystallized from $CHCl_3$/ether: yield 1·1 g; m.p. 190–191°; $[\alpha]_D^{25}$ −105° (Found: C, 63·0; H, 8·9; N, 8·8. Calc. for $C_{17}H_{28}N_2O_4$: C, 62·92; H, 8·69; N, 8·63%).

Dim-L-Val-L-Ala-OH

Dim-L-Val-L-Ala-OMe (1 g) was refluxed with saturated $NaHCO_3$ solution (25 ml) until everything dissolved. The solution was then acidified to a pH 4 and the precipitate extracted into ethyl acetate. After washing with water and drying and concentrating, the product crystallized: yield 0·8 g; m.p. 246°; $[\alpha]_D^{25}$ −112° (Found: C, 62·1; H, 8·6; N, 9·1. Calc. for $C_{16}H_{26}N_2O_4$: C, 61·9; H, 8·4; N, 9·0%).

Dim-L-Val-L-Ala-L-Leu-OMe

Dim-L-Val-L-Ala-OH (200 mg) and carbonyldiimidazole (113 mg) in dry tetrahydrofuran (15 ml) were stirred at 0° for 1½ hr. L-Leu-OMe (93·4 mg) (prepared from the HCl salt) dissolved in tetrahydrofuran (1 ml) was then added and the reaction mixture left at room temperature overnight. The solvent was then removed, the residue redissolved in ethyl acetate, and after washing with 1N HCl, H_2O, $NaHCO_3$, and water, the residual solution was evaporated to dryness for gas chromatographic analysis. Recrystallization of the residue from chloroform/light petroleum yielded 120 mg: m.p. 199°; $[\alpha]_D^{25}$ −93° (Found: C, 62·3; H, 9·4; N, 9·5. Calc. for $C_{23}H_{39}N_3O_5$: C, 63·1; H, 9·0; N, 9·6%). Similarly, Dim-L-Val-L-Ala-OH (200 mg), Woodward's reagent K (162 mg), and triethylamine (64·6 mg) was stirred for 1 hr. L-Leu-OMe (93·4 mg) in acetonitrile (2 ml) was then added. After 12 hr at room temperature the reaction was worked up as above: yield 150 mg; m.p. 199°.

Dim-L-Leu-L-Val-OMe[3]

Dim-L-Leu-OH (17·2 g), L-Val-OMe (9·8 g) (prepared from HCl salt), and DCC (14 g) in chloroform (300 ml) were condensed at 0° as described above: yield 18·2 g; m.p. 186–187° ($CHCl_3$/light petroleum); $[\alpha]_D^{25}$ −87°.

[2] Halpern, B., and Westley, J. W., *Tetrahedron Lett.*, 1966, **21**, 2283.
[3] Halpern, B., *Aust. J. Chem.*, 1965, **18**, 417.

Dim-L-Leu-L-Val-OH

Dim-L-Leu-L-Val-OMe (18 g) was saponified with refluxing saturated sodium bicarbonate (150 ml) as described above: yield 17 g; m.p. 263–266° (methanol/H_2O); $[\alpha]_D$ −77° (Found: C, 64·5; H, 9·4; N, 8·1. Calc. for $C_{19}H_{32}N_2O_4$: C, 64·6; H, 9·1; N, 7·9%).

Dim-L-Leu-L-Val-L-Ala-OMe

Dim-L-Leu-L-Val-OH (13 g) in dry DMF (220 ml) was cooled to 0° and *NN'*-carbonyldiimidazole (5·98 g) added. After 1 hr L-Ala-OCH_3 (3·8 g) (prepared from the HCl salt) was added. After addition of ethyl acetate (700 ml) the product was worked up as described: yield 12·3 g; m.p. 240–242° (methanol/light petroleum); $[\alpha]_D$ −125° (Found: C, 62·6; H, 9·0; N, 9·7. $C_{23}H_{38}N_3O_5$: C, 63·1; H, 9·0; N, 9·6%).

Dim-L-Leu-L-Val-L-Ala-OH

Dim-L-Leu-L-Val-L-Ala-OMe (6 g) was refluxed in saturated $NaHCO_3$ (50 ml) and the reaction worked up as described: yield 5 g; m.p. 270–271° (methanol/H_2O); $[\alpha]_D$ −38° (Found: C, 62·1; H, 9·0; N, 9·95. Calc. for $C_{22}H_{37}N_3O_5$: C, 62·4; H, 8·8; N, 9·9%).

Dim-L-Leu-L-Val-L-Ala-D-Phe-OMe

Dim-L-Leu-L-Val-L-Ala-OH (1·33 g) in abs. DMF (10 ml) and carbonyldiimidazole (0·52 g) were stirred at 0° for 1 hr. After addition of D-Phe-OMe (0·58 g) (prepared from the HCl salt) and stirring overnight at room temperature, the reaction was worked up: yield 1·1 g; m.p. 214–216° (methanol/ether); $[\alpha]_{600}$ −77° (Found: N, 9·5. Calc. for $C_{32}H_{48}N_4O_6$: N, 9·6%).

Dim-L-Leu-L-Val-L-Ala-D-Phen-OH

The methyl ester (1·0 g) from above was hydrolysed with saturated $NaHCO_3$ (15 ml): yield 0·65 g; m.p. 238–240° (ethyl acetate/ether) (Found: N, 9·4. Calc for $C_{31}H_{46}N_4O_6$: N, 9·8%).

Dim-L-Leu-L-Val-L-Ala-D-Phe-L-γ-Cbz-Orn-OCH_3

The Dim-tetrapeptide-OH (0·36 g) in abs. DMF (8 ml) was condensed with carbonyldiimidazole (0·11 g) followed by the addition of γ-Cbz-L-Orn-OCH_3 (0·17 g): yield 0·34 g; m.p. 145–147° (methanol/ether) (Found: N, 9·7. Calc. for $C_{45}H_{64}N_6O_9$: N, 10·1%).

By essentially the same procedures the following Cbz peptides were prepared:

N-*Cbz-L-Leu-L-Valine*

M.p. 110–111° (ether/light petroleum); $[\alpha]_D$ −27° (Found: C, 62·8; H, 7·8; N, 7·8. Calc. for $C_{19}H_{28}N_2O_5$: C, 62·6; H, 7·7; N, 7·7%).

N-*Cbz-L-Leu-L-Val-L-Ala-OMe*

M.p. 175–176° (MeOH/ether/light petroleum); $[\alpha]_D$ −70° (Found: C, 61·55; H, 7·7; N, 9·35. Calc. for $C_{23}H_{35}N_3O_6$: C, 61·45; H, 7·85; N, 9·35%).

N-*Cbz-L-Leu-L-Val-L-Ala-OH*

M.p. 203–205° (MeOH/H_2O); $[\alpha]_D$ −55° (Found: C, 60·2; H, 7·6; N, 9·2. Calc. for $C_{22}H_{33}N_3O_6$: C, 60·7; H, 7·6; N, 9·65%).

N-*Cbz-L-Leu-L-Val-L-Ala-L-Phe-OMe*

M.p. 198–200° (ethyl acetate); $[\alpha]_D$ −48° (Found: C, 64·8; H, 7·0; N, 9·5. Calc. for $C_{32}H_{44}N_4O_7$: C, 64·4; H, 7·4; N, 9·4%).

Steric Analyses of Peptides by Gas Chromatography

Dimedonyl peptides (25 mg) were hydrolysed directly with 6N HCl at 135° (sealed tube) overnight, but Cbz derivatives were first treated with HBr/CH_3COOH (2 ml) before hydrolyses. The reaction mixtures were then lyophylized and the residue esterified with thionyl chloride/

methanol reagent (5 ml)[4] by refluxing for 30 min. The solution was then evaporated to dryness and TFA-L-prolyl chloride (0·1 mg in 1 ml CH_2Cl_2) was added to the residue. After neutralizing the reaction mixture with anhydrous triethylamine (approx. 0·02 ml), the solution was stirred for 30 min. After washing with 1N HCl, H_2O, $NaHCO_3$, and H_2O, a portion (*c.* 2μl) was injected into the gas chromatograph (see Table 2).

TABLE 2

STERIC PURITY OF *N*-PROTECTED PEPTIDE ESTERS

Gas chromatographic analyses were carried out on a Wilkens Aerograph chromatograph using the same column and conditions previously described.[2] All starting materials were analysed for steric purity before use in peptide syntheses and the condition of hydrolyses were checked on samples of sterically pure L-Leu-L-Val-L-Ala and L-Val-L-Ala-L-Leu. In these cases <1% of the D-amino acids were formed

Peptide Derivative	Steric Purity after Hydrolysis				
	Leu	Val	Ala	Phe	Orn
Dim-L-Leu-L-Val-L-Ala-OMe	99	98	99		
Dim-L-Leu-L-Val-L-Ala-D-Phe-OMe	98	98	98	100	
Dim-L-Leu-L-Val-L-Ala-D-Phe-ε-Cbz-L-Orn-OMe	99	98	99	100	not detd.
Cbz-L-Leu-L-Val-L-Ala-OMe	100	99	99		
Cbz-L-Leu-L-Val-L-Ala-L-Phe-OMe	99	99	99	100	

[4] Brenner, M., and Huber, W., *Helv. chim. Acta*, 1953, **36**, 1109.