

N-Terminal Substituent and Side-chain Influences on the Chemical Shifts of Protons in Model Dipeptide Systems

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¹H Chemical shift values of ester methyls in model dipeptide esters have been shown to be sensitive to (a) the nature and configuration of the constituent amino acids and (b) the spatial distance between the *N*-terminal aryl group and the ester protons. Chemical shift differences have been rationalised in terms of shielding effects as calculated from space-filling models and tables of 'ring current' effects.

Recent applications of n.m.r. techniques¹⁻⁴ to assess the degree of racemisation during peptide coupling highlight the versatility of the techniques and the wealth of information derived from monitoring intermediates formed during peptide coupling. The underlying principle is that n.m.r. signals from diastereoisomers sometimes do not overlap and can be used as a quantitative assessment of the amount of a diastereoisomer in a mixture.

Methods developed so far rely on different aromatic shielding effects experienced by specific protons in dipeptides where the amino-acid residues are the same configuration (e.g. L-L), as compared with their (D-L) counterparts. The model dipeptide systems usually include *N*-acyl groups on the *N*-terminal amino-acid residues and in terms of model testing for racemisation, these groups are advantageous since they give little protection from racemisation. The amounts of diastereoisomers in a particular mixture therefore are at levels which make integration values in the n.m.r. spectra into respectable quantitative assessments.

Yet techniques used in peptide synthesis in recent years have relied on *N*-terminal protection by urethane (ROCONH) protecting groups to limit racemisation as far as possible. In order to design an n.m.r.-based racemisation test which would reflect more closely the current synthetic strategies the influence of aromatic shielding effects on *C*-terminal ester groups of diastereoisomeric peptides have been investigated.

A series of *N*-aroyl diastereoisomeric dipeptide esters have been synthesised under conditions (coupling with *NN'*-dicyclohexylcarbodi-imide, DCCI) which give rise to racemisation, and their n.m.r. spectra have been determined in CDCl₃ solution. The chemical shifts of the ester methyl proton were measured for the diastereoisomeric mixture in each case, and identification of the correct diastereoisomeric ester signal was made by synthesising selected peptides in the L-L form by coupling the appropriate starting materials (*N*-aroylamino acid and amino acid methyl ester hydrochloride) using DCCI and *N*-hydroxybenzotriazole (HOBt), conditions known to limit racemisation.⁵

Results

In Figure 1 the results obtained from the present study have been augmented with results from related studies,^{1,2,6} so that the trends emerging can be demonstrated and rationalised. Figure 1 represents a plot of chemical shift values of the ester methyl protons (at 100 MHz) against the various *N*-aroyl and other *N*-substituents in the dipeptide system (I).

Variations in R are listed under the key 1-10 on the horizontal axis of Figure 1. Results are grouped into the dipeptide derivatives Ala-Ala, Ala-Val, Val-Val, and Ala-Phe. The results for the L-L and D-L configurations have been given different codes as specified in Figure 1. For convenience *N*-aroyl substituents which are closely related have been linked by lines. The following trends can be deduced.

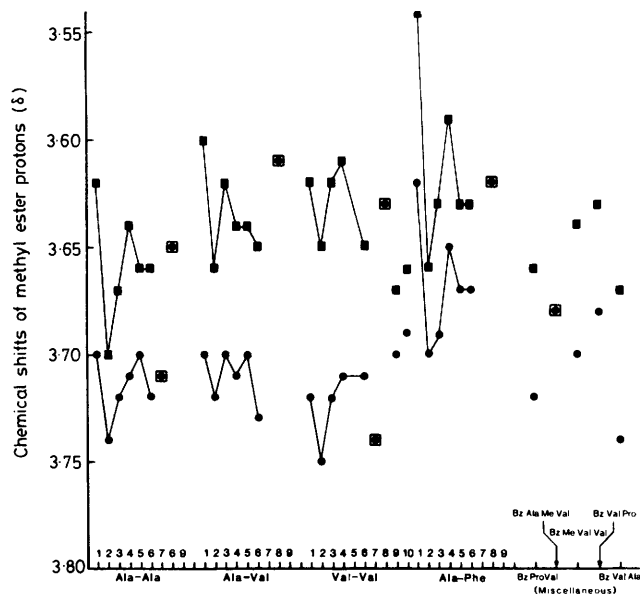
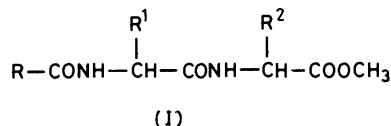


Figure 1. ■, D-L forms; ●, L-L forms; ■, D-L and L-L overlapping. *N*-protecting groups: 1, Bz; 2, *p*-NO₂Bz; 3, *p*-ClBz; 4, *p*-MeOBz; 5, 3,5-MeO₂Bz; 6, 3,4,5-MeO₃Bz; 7, benzyloxycarbonyl (Z); 8, fluorenylmethoxycarbonyl (Fmoc); 9, trifluoroacetyl; 10, cyclohexyl (Bz = PhCO)



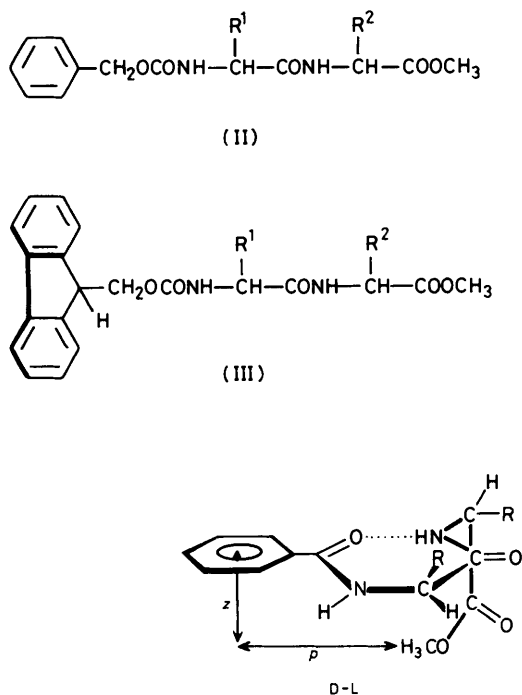
(a) *Influence of the N-Terminal Substituent.*—Taking the *N*-benzoyl substituent as standard it can be seen that the main deviation is in the result for the 4-nitrobenzoyl group in each set of results. The nitro group causes a stronger deshielding effect on the ester methyl protons than substituents such as methoxy and chlorine. The effect of the latter substituents does not seem to be so significant which is in line with recent ¹³C data⁷ on substituted acetophenones where the ¹³C chemical shift for the CO does not change significantly by varying the electron-donating substituents in the 4-position. The most plausible explanation for the *p*-nitrobenzoyl group effect is that the shielding cone of this aromatic system is vastly different from that of the more electron-rich systems. Whether this manifests itself as an across-space shielding effect or that it changes the hydrogen-bonding strength of the accepted seven-membered ring model for dipeptides would be very difficult to test.

However, some support for the former explanation can be derived from the observation that the ester methyls of dipeptides having an *N*-terminal *o*-nitrophenylsulphenyl group⁸ are at lower field positions (δ 3.73) and in this case hydrogen-bonding cannot take place.

In all cases studied where differences were seen between *D*-*L* and *L*-*L* forms, the shielding effect on the ester methyl of the *D*-*L* forms are greater than the *L*-*L* form. An explanation for this appears later in the discussion. The presence of an *N*-terminal aromatic system contributes *ca.* 0.03–0.04 p.p.m. to the shielding effects since the *N*-cyclohexyl and *N*-trifluoroacetyl derivatives¹ show methyl ester signal positions with a much smaller spread than their aromatic counterparts in the Val-Val system.

Changing the *N*-aroyl substituent to an aromatic urethane group causes a very distinctive change. In compound (II) with $R^1 = R^2 = \text{CH}(\text{CH}_3)_2$, *i.e.* the Val-Val system, no separation of the diastereoisomeric ester signals occurred and the ester signals appeared at relatively lower field values of δ 3.74 (δ 3.71 for *Z*-Ala-AlaOMe). In (III) the urethane system⁹ (Fmoc) is maintained but the aromatic character has been increased by the fluorenyl system. Again no separation of the diastereoisomeric ester signals occurred but in all the Fmoc-protected series studied (Ala-Ala), (Ala-Val), (Val-Val), (Ala-Phe), the signals were at higher field shifts (δ 3.65–3.61). An obvious explanation here is that the urethane group is probably less capable of forming a 'closely knit' hydrogen-bonded seven-membered ring system so that the selective influence of the aromatic system in the different diastereoisomeric forms does not materialise.

(b) *Influence of the Side-chain Groups.*—As can be seen in Figure 1 the changes in chemical shift of the ester signals are more dramatic if changes are made in the side-chain substituents. Increasing the bulkiness of the side-chain from methyl (Ala) to isopropyl (Val) causes a larger separation of ester chemical shift values between the *D*-*L* and *L*-*L* diastereoisomers. The valyl valine derivatives gives the greater separation in the whole series, and presumably is due to the



increased steric interaction provided by the bulky isopropyl side-chains.

Introduction of a benzyl group (Phe) as a side-chain for the *C*-terminal residue increases the shielding effects on the methyl ester protons but does this for both the *L*-*L* and *D*-*L* diastereoisomers. This therefore does not improve the model's potential as a means of assessing racemisation. However, the shielding effect on the *N*-terminal amino acid's side-chain caused by the *C*-terminal benzyl side chain, first recognised by Weinstein and Pritchard,³ is manifested in all the series we have studied. Differential diastereoisomeric signals for the side-chain methyl group of *N*-terminal alanine were even seen in the examples where urethane groups were used for *N*-protection and were a useful parameter to check on the composition of the diastereoisomeric mixture present.

Incorporation of *N*-methyl groups and proline residues as side-chains does not seem to affect^{1,2} the chemical shifts significantly, but complicates the interpretation due to the greater conformational flexibility of the molecules. The effect is more strongly manifested if these residues are in the *C*-terminal position.

Discussion

The seven-membered ring hydrogen-bonded cyclic model of a dipeptide system¹⁰ (Figure 2) appears to be the best model to explain the shielding effects. Earlier studies¹ showed that the separation of the methyl ester signals in diastereoisomeric *N*-aroyl dipeptide methyl esters were dependent on the polarity of the solvent. Increasing the proportion of [²H₆]DMSO in the CDCl₃ solutions used for the n.m.r. studies reduced the signal separation, so that in [²H₆]DMSO only, there was no separation between signals. Replacement of the central peptide *N*-H bond¹ by *N*-CH₃, thus removing the possibility of hydrogen-bonding, also removed diastereoisomeric ester signal separation. It is therefore reasonable to interpret the results in terms of the existence of the seven-membered hydrogen-bonded ring as the major conformation in non-polar solvents.

Model construction using CPK (Corey-Pauling-Koltun) space-filling kits showed quite definite differences between the relative 'through space' separation of the *N*-terminal aromatic residue and the *C*-terminal ester methyl protons, in the *D*-*L* and *L*-*L* forms. This is further support for the previous reports that the *D*-*L* form of dipeptides is more closely packed than the more extended *L*-*L* form.¹¹ By accurately measuring the distances between the relevant groups in the space-filled CPK models it was possible to correlate from tables of 'ring current' shielding effects¹² the expected chemical shift changes due to the aromatic ring. The *z* and *p* parameters used in the Tables¹² were measured as shown in Figure 2 from the molecular models and after averaging the distance for the dipeptide models (Ala-Ala, Ala-Val, and Val-Val) (Table 2) the iso-shielding position which the ester protons experience in the two forms (*D*-*L* and *L*-*L*) can be computed to be on average $\Delta\delta$ +0.04 and 0.007 p.p.m.,

Figure 2.

Table 1. Elemental analysis and physical data for the dipeptide derivatives

Dipeptide derivative ^b	Elemental analysis						¹ H N.m.r. signals at 100 MHz (δ; CDCl ₃)						Ester methyl			M.p. (°C) L-L ^a
	Found (%)			Required (%)			N-Protecting group	α-Protons	N-H	Side-chain protons			L-L	D-L	L-L ^a	
	C	H	N	C	H	N				L-L	D-L	L-L ^a				
Bz-Ala-L-AlaOMe	60.0	7.0	10.1	60.4	6.5	10.1	7.36 and 7.76 (ArH)	4.51, 4.81	7.32	1.64 (d), 1.4 (d) (2 × CH ₃)	3.70	3.62	3.70	147—148		
Bz-Ala-L-PheOMe	68.1	6.4	8.1	67.8	6.2	7.9	7.80 and 7.4 (ArH)	4.98	7.30	1.45 (2 d, CH ₃), 3.15 (t, CH ₂ Ph), 7.4 (ArH)	3.62	3.54				
Bz-Ala-L-ValOMe	62.6	7.45	9.2	62.7	7.2	9.15	7.9—7.3 (ArH)	5.00, 4.50	7.20	0.90 [m, (CH ₃) ₂ CH], 1.5 (dd, CH ₃), 2.1 (m, CH)	3.60	3.70				
Bz-Val-L-ValOMe	65.0	8.4	8.85	64.7	7.8	8.4	7.82 and 7.42 (ArH)	4.85, 4.52	7.30	0.90 [m, 2 (CH ₃) ₂ CH], 2.30 [m, 2 (CH ₃) ₂ CH]	3.72	3.62	3.71	172—174		
4-MeOBz-Ala-L-AlaOMe	58.6	6.8	9.1	58.4	6.5	9.1	6.90 (d, J 10 Hz), 8.0 (d, J 10 Hz), 3.8 (ArOCH ₃)	4.98, 4.65	7.50	1.45 (2 d, 2 CH ₃ CH)	3.71	3.64		140—141		
4-MeOBz-Ala-L-PheOMe	66.0	6.1	7.7	65.6	6.25	7.3	6.90—7.80 (ArH), 3.80 (ArOCH ₃)	4.98	7.50	1.45 (2 d, CH ₃), 3.15 (t, PhCH ₂)	3.65	3.59				
4-MeOBz-Ala-L-ValOMe	60.7	7.5	8.4	60.7	7.2	8.3	6.88 (d, J 10 Hz), 7.79 (d, J 10 Hz), 3.80 (ArOCH ₃)	4.98, 4.52	7.5—7.2	0.95 [m, (CH ₃) ₂ CH], 1.5 (2 d, CH ₃), 2.2 (m, CH)	3.71	3.64				
4-MeOBz-Val-L-ValOMe	62.8	8.1	7.9	62.6	7.7	7.7	7.79 (d, J 10 Hz), 6.88 (d, J 10 Hz), 3.80 (ArOCH ₃)	4.98, 4.60	7.50—7.15	1.10 [m, 2 (CH ₃) ₂ CH], 2.30 [m, 2CH(CH ₃) ₂]	3.71	3.61				
3,5-MeO ₂ Bz-Ala-L-AlaOMe	56.9	6.9	8.4	56.8	6.5	8.3	6.90 (ortho-ArH), 6.45 (meta-ArH), 3.78 (2 ArOCH ₃)	4.95, 4.65	7.65	1.45 (m, 2 CH ₃ CH)	3.70	3.66				
3,5-MeO ₂ Bz-Ala-L-PheOMe	63.5	6.5	7.0	63.8	6.3	6.8	6.4—7.2 (ArH), 3.77 (2 ArOCH ₃)	4.90	7.30	1.45 (2 d, CH ₃ CH), 3.15 (CH ₂ Ph)	3.67	3.63				
3,5-MeO ₂ Bz-Ala-L-ValOMe	59.1	7.4	7.8	59.0	7.1	7.6	6.90 (ortho-ArH), 6.45 (meta-ArH), 3.75 (2 ArOCH ₃)	5.00, 4.60	7.65	1.00 [m, (CH ₃) ₂ CH], 2.2 [m, (CH ₃) ₂ CH], 1.5 (m, CH ₃)	3.70	3.64				
3,5-MeO ₂ Bz-Val-L-ValOMe	60.8	7.85	7.1	60.9	7.6	7.1	6.95 (ortho-ArH), 6.45 (meta-ArH), 3.76 (2 ArOCH ₃)	4.85, 4.5	7.30	0.90 [m, 2 (CH ₃) ₂ CH], 2.20 [m, 2 (CH ₃) ₂ CH]	3.72	3.66				
4-NO ₂ Bz-Ala-L-AlaOMe	52.1	5.4	13.0	52.0	5.3	13.0	8.30 (d, J 10 Hz), 7.80 (d, J 10 Hz) (ArH)	4.98, 4.52	7.50	1.45 (2 d, 2 CHCH ₃)	3.74	3.70	3.75	180—182		
4-NO ₂ Bz-Ala-L-PheOMe	60.0	5.4	10.7	60.1	5.3	10.5	8.25 (d, J 10 Hz), 7.85 (d, J 10 Hz) (ArH)	4.98	7.65	1.45 (d, CHCH ₃), 7.10 (s, ArH), 3.15 (CH ₂ Ph)	3.70	3.66				
4-NO ₂ Bz-Ala-L-ValOMe	54.6	5.9	11.9	54.7	6.0	12.0	8.20 (d, J 10 Hz), 7.95 (d, J 10 Hz) (ArH)	4.90, 4.50	7.85, 7.20	0.95 [q, (CH ₃) ₂ CH], 2.2 [m, (CH ₃) ₂ CH], 1.5 (d, CH ₃ CH)	3.72	3.66	3.74	140—141		

Table I. (contd.)

Dipeptide derivatives ^b	Elemental analysis						¹ H N.m.r. signals at 100 MHz (δ : CDCl ₃)						Ester methyl			M.p. (°C)			
	Found (%)			Required (%)			N-Protecting group		α -Protons		N-H		Side-chain protons		L-L	D-L	L-L ^d		
	C	H	N	C	H	N	8.25 (d, J 10 Hz), 7.95 (d, J 10 Hz) (ArH)	7.18 (s, ArH), 3.72 (s, 3 ArOCH ₃) 7.0-7.3 (ArH, 2 + 5), 3.80 (br s, 3 ArOCH ₃) 7.18 (s, ArH), 3.82 (s, 3 ArOCH ₃)	4.70	4.95, 4.65	7.60	7.80	0.90 [m, 2 (CH ₃) ₂ CH], 2.30 [m, 2 (CH ₃) ₂ CH]	3.75	3.65	3.75	120-122		
4-NO ₂ Bz-Val-L-ValOMe	56.9	6.9	11.0	57.0	6.6	11.1													
3,4,5-MeO ₃ Bz-Ala-L-AlaOMe	55.1	6.6	7.7	55.4	6.5	7.6													
3,4,5-MeO ₃ Bz-Ala-L-PheOMe	62.3	6.6	6.6	62.2	6.3	6.3													
3,4,5-MeO ₃ Bz-Ala-L-ValOMe	57.8	7.3	7.5	57.6	7.1	7.1													
3,4,5-MeO ₃ Bz-Val-L-ValOMe	59.5	7.9	6.6	59.4	7.6	6.6													
4-CIBz-Ala-L-AlaOMe	53.9	5.7	9.1	53.8	5.4	9.0													
4-CIBz-Ala-L-PheOMe	62.0	6.0	7.6	61.7	5.5	7.2													
4-CIBz-Ala-L-ValOMe	56.4	6.3	8.0	56.4	6.2	8.2													
4-CIBz-Val-L-ValOMe	58.5	7.0	7.5	58.6	6.8	7.6													
Nps-Ala-L-AlaOMe ^b	47.7	5.2	12.5	47.7	5.2	12.8													
Nps-Ala-L-PheOMe	56.7	5.5	10.6	56.6	5.2	10.4													
Nps-Val-L-ValOMe	53.2	6.7	10.6	53.5	6.5	11.0													
FMOC-Ala-L-AlaOMe	66.4	6.2	7.4	66.7	6.1	7.1													
FMOC-Ala-L-PheOMe	77.1	5.65	6.3	71.2	5.9	5.9													
FMOC-Ala-L-ValOMe	68.3	7.1	7.0	67.9	6.6	6.6													
FMOC-Val-L-ValOMe	69.0	7.2	6.0	69.1	7.1	6.2													

^a Products from synthesis using DCCI-HOBT coupling method. ^b Trifluoroacetyl, benzyloxycarbonyl, and cyclohexyl protected peptide esters listed in Figure 1 were prepared in other projects.^{1,2,6}
^c Nps = *o*-nitrophenylsulphenyl.

Table 2.

Dipeptide	Form	Units of 0.139 nm		Calculated shielding values (p.p.m.)
		z	ρ	
Bz-Ala-AlaOMe	D-L	2.30	2.30	+0.068
	L-L	2.87	3.73	+0.007
Bz-Ala-ValOMe	D-L	2.71	2.99	+0.025
	L-L	2.87	4.02	+0.003
Bz-Val-ValOMe	D-L	2.87	2.87	+0.032
	L-L	2.87	3.45	+0.012
Average of above values	D-L	2.63	2.72	+0.042
	L-L	2.87	3.73	+0.007
Z-Ala-AlaOMe	D-L	3.45	5.17	-0.001
	L-L			
Z-Val-ValOMe	D-L	1.72	5.75	Outside range of Tables
	L-L			

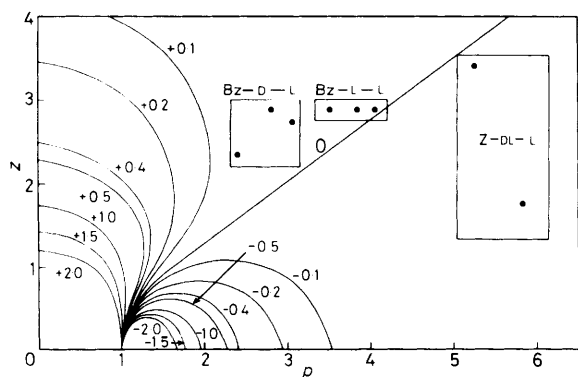


Figure 3. Positions of the ester methyls relative to the *N*-terminal aromatic rings (iso-shielding lines with values given in p.p.m.)

respectively. In practice the differences between the D-L and L-L ester chemical shifts have been between 0.04 and 0.1 p.p.m.

It can also be seen from the shielding effect contours (Figure 3, based on the diagram in ref. 13) why the urethane protecting groups do not selectively shield the L-L and D-L methyl protons. Probably the most important factor is not only the larger distance between the aromatic system and the ester group (estimated to be 0.2 nm from models) but also the models show an increased amount of flexibility in the movement of the aromatic system which could well average out any potential selective shielding effects. The 'closely knit' requirement for the aromatic shielding *N*-terminal system is lost in the urethane protected example, although the general increase in aromatic character of fluorenylmethoxycarbonyl does give an overall increase in the shielding of the *C*-terminal ester groups.

In summary, the development of new n.m.r. methods based on separate signals for D-L and L-L forms will have to depend more on emphasising conformational changes in the hydrogen-bonded cyclic model to maximise diastereoisomeric differences than to electrical influences on aromatic systems in the *N*-terminal position.

Experimental

¹H N.m.r. spectra were determined at 100 MHz on a Varian HA-100 instrument using CDCl₃ as solvent and tetramethylsilane as internal standard for all spectra. C, H, and N microanalyses were carried out using a Carlo Erba 1106 analyser. The purity of

products was routinely checked using Kieselgel G t.l.c. plates developed in chloroform or chloroform-methanol (9:1) with compounds either located using u.v. light (on GF₂₅₄ Kieselgel G) or using I₂ vapour. M.p.s were determined on a Kofler block and are uncorrected. Since most of the dipeptide derivatives were prepared as mixtures of diastereoisomers, m.p.s are not a meaningful assessment of purity and are therefore omitted in such cases.

Preparation of N-Aroyl Derivatives of Amino Acids.—*N*-Benzoyl and substituted *N*-benzoyl derivatives were synthesised from the corresponding amino acids by the conventional Schotten-Baumann method¹³ using the acid chlorides as the acylating agent.

The amino acid (0.01 mol) in 2*M*-sodium hydroxide (6 ml) was cooled in an ice-bath and stirred. Over 15–30 min the appropriate acid chloride (0.011 mol) was added in about ten equal portions, while 2*M*-NaOH was also added dropwise while the reaction mixture was vigorously stirred. Stirring was continued overnight at room temperature and the mixture then poured into water (20 ml). Acidification with concentrated hydrochloric acid yielded a precipitate, which was purified by recrystallisation. The following derivatives were prepared: *N*-benzoyl-L-alanine, m.p. 149–150 °C (lit.,¹³ 150–151 °C); *N*-benzoyl-L-valine, m.p. 130–132 °C (lit.,¹³ 131–132 °C); *N*-4-nitrobenzoyl-L-alanine, m.p. 165–166 °C (lit.,¹⁴ 191–193 °C for DL-isomer), δ ([²H₆]DMSO) 1.50 (3 H, d, CHCH₃), 4.50 (H, m, NHCHCO), 8.16 and 8.30 (4 H, 2 d, ArH), and 9.18 (H, d, NH); *N*-4-nitrobenzoyl-L-valine, m.p. 227–228 °C (lit.,¹⁵ 163 °C for DL-form) (Found: C, 54.2; H, 5.4; N, 10.3. Calc. for C₁₂H₁₄N₂O₅: C, 54.1; H, 5.3; N, 10.5%), δ (CD₃OD) 1.05 [6 H, d, (CH₃)₂CH], 2.25 [H, m, (CH₃)₂CH], 4.55 [H, d, NHCHCO], 7.95 (2 H, d, *J* 10 Hz, ArH), 8.25 (2 H, d, *J* 10 Hz, ArH), and 8.40 (H, d, CONH); *N*-4-chlorobenzoyl-L-alanine, m.p. 150 °C (lit.,¹⁶ 180–182 °C for DL-isomer) (Found: C, 52.6; H, 4.4; N, 6.0. Calc. for C₁₀H₁₀ClNO₃: C, 52.75; H, 4.4; N, 6.15%), δ ([²H₆]DMSO) 1.40 (3 H, d, CHCH₃), 4.45 (H, t, CHCH₃), 7.50 (2 H, d, *J* 9 Hz, ArH), 7.90 (2 H, d, *J* 9 Hz, ArH), and 8.75 (H, d, NH); *N*-4-chlorobenzoyl-L-valine, m.p. 110 °C (Found: C, 56.8; H, 5.6; N, 5.3. C₁₂H₁₄ClNO₃ requires C, 56.4; H, 5.5; N, 5.5%), δ ([²H₆]DMSO) 0.95 [6 H, 2 d, (CH₃)₂CH], 2.25 [H, m, (CH₃)₂CH], 4.35 (H, t, NHCHCO), 7.50 (2 H, d, *J* 9 Hz, ArH), 7.95 (2 H, d, *J* 9 Hz, ArH), and 8.5 (H, d, NH); *N*-4-methoxybenzoyl-L-alanine, m.p. 150–153 °C (lit.,¹⁶ 178–180 °C for DL-isomer) (Found: C, 59.0; H, 5.8; N, 6.3. Calc. for C₁₁H₁₃NO₄: C, 59.2; H, 5.8; N, 6.3%), δ ([²H₆]DMSO) 1.4 (3 H, d, CHCH₃), 3.79 (3 H, s, ArOCH₃), 4.45 (H, t, CHCH₃), 6.98 (2 H, d, *J* 10 Hz, ArH), 7.80 (2 H, d, *J* 10 Hz, ArH), and 8.5 (H, d, NH); *N*-4-methoxybenzoyl-L-valine, m.p. 165–169 °C (Found: C, 62.4; H, 6.5; N, 5.1. C₁₃H₁₇NO₄ requires C, 62.15; H, 6.8; N, 5.6%), δ ([²H₆]DMSO) 1.0 [6 H, d, (CH₃)₂CH], 2.25 [H, m, (CH₃)₂CH], 3.76 (3 H, s, ArOCH₃), 4.35 (H, t, NHCHCO), 6.95 (2 H, d, *J* 10 Hz, ArH), 7.90 (2 H, d, *J* 10 Hz, ArH), and 8.20 (H, d, CONH); *N*-3,5-dimethoxybenzoyl-L-alanine, m.p. 192 °C (Found: C, 56.9; H, 6.0; N, 5.4. C₁₂H₁₅NO₅ requires C, 56.9; H, 5.9; N, 5.5%), δ ([²H₆]DMSO) 1.45 (3 H, d, CHCH₃), 3.88 (6 H, s, 2 ArOCH₃), 4.45 (H, q, NHCHCO), 6.6 [H, m, Ar(4)H], 7.1 [2 H, m, 2 Ar(2)H], and 8.65 (H, d, NH); *N*-3,5-dimethoxybenzoyl-L-valine, m.p. 182–183 °C (Found: C, 59.6; H, 6.9; N, 4.8. C₁₄H₁₉NO₅ requires C, 59.8; H, 6.8; N, 5.0%), δ ([²H₆]DMSO) 1.0 [6 H, d, (CH₃)₂CH], 2.25 [H, m, (CH₃)₂CH], 3.76 (6 H, s, ArOCH₃), 4.45 (H, t, NH, CHCO), 6.65 [H, m, Ar(4)H], 7.1 [2 H, m, 2 Ar(2)H], and 9.35 (H, d, NH); *N*-3,4,5-trimethoxybenzoyl-L-alanine, m.p. 190 °C (Found: C, 55.6; H, 6.2; N, 5.2. C₁₃H₁₇NO₆ requires C, 55.1; N, 6.0; N, 4.9%), δ ([²H₆]DMSO) 1.45 (3 H, d, CHCH₃), 3.8 and 3.7 (9 H, 2 s, 3 × ArOCH₃), 4.48 (H, t, NHCH), 7.25 (2 H, s, ArH), and 8.6 (H, d, NH); *N*-3,4,5-trimethoxybenzoyl-L-valine, m.p. 150 °C

(Found: C, 57.7; H, 7.0; N, 4.2. $C_{15}H_{21}NO_6$ requires C, 57.9; H, 6.75; N, 4.5%), δ ($[^2H_6]$ DMSO) 1.00 [6 H, d, $(CH_3)_2CH$], 2.20 [H, m, $(CH_3)_2CH$], 3.72 and 3.84 (9 H, 2 s, $3 \times ArOCH_3$), 4.48 (H, t, $NHCHCO$), 7.44 (2 H, s, ArH), and 8.45 (H, d, NH).

o-Nitrophenylsulphenyl (Nps), derivatives of L- and D-alanine and L- and D-valine were prepared by standard methods.¹⁷ Similarly, Fmoc derivatives of the same amino acids were prepared from published data.⁹

General Method for Synthesis of Diastereoisomeric Mixtures of N-Protected Dipeptide Esters.—*NN'*-Dicyclohexylcarbodiimide (2 mmol) was added to an ice-cooled stirred solution of *N*-protected amino acid (2 mmol) and amino acid ester hydrochloride¹⁸ (2 mmol) in purified chloroform (30 ml) containing triethylamine (2 mmol). The mixture was stirred at room temperature overnight, then cooled in ice, and the precipitated dicyclohexylurea filtered off. The total filtrate was evaporated to dryness and the residue tritiated with chloroform to remove further amounts of the dicyclohexylurea. The chloroform-soluble fraction was washed in turn with portions (2×15 ml) of *m*-HCl, 0.5M-NaHCO₃, and water and dried (MgSO₄). The solvent was evaporated *in vacuo*, and the residue was purified by recrystallisation from chloroform–light petroleum mixtures. Physical data for the compounds synthesised are recorded in Table 1. Yields of purified material in the range 50–60% were generally obtained.

Synthesis of L–L-Forms of the Dipeptide Esters.—To prevent the racemisation of the *N*-terminal residue inherent in the general method above, optically pure forms were synthesised using *N*-hydroxybenzotriazole as an additive.⁵

NN'-Dicyclohexylcarbodiimide (2 mmol) was added to a cooled (-20°C) stirred solution of the *N*-protected amino acid (2 mmol), amino acid methyl ester hydrochloride (2 mmol), and *N*-hydroxybenzotriazole (2 mmol) in purified chloroform (30 ml) and triethylamine (2 mmol). The mixture was stirred at -20°C for 12 h when the products were worked up as described in the general method. Compound data have been summarised in Table 1.

Measurement of Parameters from Corey–Pauling–Koltun Models and Conversion into Shielding Effects.—Several measurements of the z and ρ distances (see Figure 2) were made and

converted into 'benzene ring units' of 0.139 nm and the average readings recorded as in Table 2. Corresponding shielding values could then be derived from tables published by Haigh and Mallion.¹²

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