

Enantioselection in peptide bond formation

Roger R. Hill,^{*,a} David Birch,^b Graham E. Jeffs^a and Michael North^{†b}

^a Department of Chemistry, The Open University, Milton Keynes, UK MK7 6AA.

E-mail: r.r.hill@open.ac.uk

^b Department of Chemistry, University of Wales, Bangor, Gwynedd, UK LL57 2UW

Received 5th December 2002, Accepted 29th January 2003

First published as an Advance Article on the web 25th February 2003

Selectivity in abiotic condensations of amino acids remains controversial and stereochemically little explored. We find that competitive activated couplings of *N*-acyl derivatives of glycine, alanine, valine, proline and phenylalanine with binary, ternary and quaternary mixtures of amides and esters of the same group of amino acids show little selectivity among the reactants, except with respect to configuration, where a consistent and significant preference for heterochiral outcomes, mostly >80%, is observed. One possible explanation of this selectivity predicts a predisposition to homochiral coupling under conditions that would require the two carboxyl functions to be co-facial in the activated complex.

Introduction

The formation of peptide bonds in biological protein synthesis is totally prescribed. Well-known but complex derivatives of α -amino acids, cofactors and templates ensure that, in each case, successive couplings involve a unique sequence of homochiral amino acids. Spontaneous abiotic formation of peptides from mixtures of amino acids have been widely investigated, however, and prompt much interest within the contexts of prebiotic chemistry,^{1,2} peptide synthesis³ and combinatorial chemistry.⁴ In principle, some selectivity among amino acids may be anticipated, leading to crudely 'informational' products. In practice, however, the significance of any selectivity remains controversial. Competitive condensations in aqueous solutions of proteinogenic amino acids mediated by a carbodiimide indicate a range of about three-fold in reactivity, too small to allow any meaningful sequence selection.⁵ On the other hand, coupling propensities in more recently reported condensations, in saturated salt solutions containing Cu(II) and on alumina surfaces, appear not only to have significant variation but also to align broadly with the dipeptide units occurring most frequently in the membrane proteins of archaeobacteria and prokaryotic cells.²

Stereochemical selectivity is fundamentally important as homochirality is considered a prerequisite for, rather than a consequence of life.⁶ Moreover, with no selectivity, only one molecule in 500 would be homochiral in a mixture of decapeptides, where secondary structure becomes significant.⁷ Studies of *N*-carboxyanhydride elongation indicate some bias towards a homochiral preference in extending a peptide chain,⁸ but the limited scope of both models and process prompt the examination of other systems before the wider significance of these results can be evaluated. Any such selectivity would be inconsequential if conditions allowed thermodynamically driven epimerization to occur, but this would, in any case, weaken the relevance of the experiments to protobiology.⁹ In spite of a great many theories and experimental studies, a plausible scenario for spontaneous abiotic assembly of a homochiral peptide remains a severe challenge.¹⁰

As a contrast to *N*-carboxyanhydride derivatives, we chose to investigate competitions between amino acid derivatives that involve other forms of activation popular in modern peptide synthesis, together with selective protection that is both convenient for analytical purposes and suitable for modelling

peptide elongation. For example, *N*-phthaloyl (Phth) amino acids are not only convenient for chromophorically-based detection in product analysis but also preclude oxazolone-mediated α -CH epimerization, and *N,N*-dimethylamido (DMA) derivatives of amino acids afford co-reactants with an inert *C*-terminus. In confining our studies to derivatives of glycine, alanine, phenylalanine, proline and valine, we also avoided potentially complicating factors with the more reactive side chains while retaining a stereochemically representative selection.

Competitive activated couplings involved presenting one type of substrate with the opportunity to react with two, three or four of the other type, the latter usually present in considerable molar excess. We found that stereochemical selectivity not only is the dominant factor controlling product distribution but, with *N*-terminal acyl protection, it also strongly favours reaction between substrates of opposite configuration.

The results we reported in a preliminary publication¹¹ have been extended to more complex mixtures of Phth- and DMA-derivatives and to show the effects of changes in activating group, solvent, *N*-protecting group and *C*-terminal group. The heterochiral bias remains the major influence on product distribution in the more complex mixtures and appears to be independent of activating and *C*-terminal groups. It is lost, however, when acyl *N*-protection is replaced by a urethane.

One possible explanation of these observations, supported by a solvent effect, implies that a predisposition to homochiral coupling would occur if the two reactants were linked through their carboxyl functions.

Results and discussion

The outcomes of 71 competitive coupling experiments are summarised in Tables 1–5. Table 1 shows, with one exception, a consistent and significant preference for heterochiral couplings, irrespective of whether the excess reagent is the Phth or the DMA component. Evidence for kinetic rather than thermodynamic control in these reactions can be seen from the experiment with equimolar reactants that, on completion, shows a product ratio close to 1 : 1. The heterochiral preference was further confirmed by the results from equimolar racemic reagents (not tabulated). Thus, the percentages of heterochiral diastereoisomer in reactions between racemic Phth-alanine and racemic dimethylamides of alanine, valine and phenylalanine were 75.6, 89.3 and 87.9, respectively. Results from more complex mixtures also emphasise a heterochiral preference when that option is available (Table 3). Tables 2 and 3 otherwise

[†] Present address: Department of Chemistry, Kings College, University of London, The Strand, London, UK WC2R 2LS.

Table 1 Competition outcomes in reactions between Phth-L-A and racemic B-DMA using DCC–HOBt coupling in CH₂Cl₂, except where indicated

A	B	Initial $\frac{[B-DMA]}{[Phth-L-A]}$	% LD-dipeptide derivative ^a
Ala	Ala	9.9	86
Ala	Ala	1.0	55
Ala	Ala	0.08 ^b	85
Ala	Phe	7.0	92
Ala	Phe	18.0	90
Ala	Phe	45.0	85
Ala	Val	8.0	94
Ala	Val	10.7	94 ^c
Ala	Val	9.5	83 ^d
Ala	Val	9.7	74 ^e
Phe	Phe	10.0	89
Phe	Phe	6.3	87
Phe	Phe	0.1 ^b	75
Phe	Ala	7.5	90
Phe	Val	10.0	94
Phe	Pro	10.0	54
Val	Val	10.0	96
Val	Ala	10.0	95 ^f
Val	Phe	8.3	83

^a Error $\leq \pm 5\%$. ^b Racemic Phth-A and L-B-DMA. ^c DCC–HOSu in CH₂Cl₂. ^d DCC–HOSu in DMF. ^e DCC–HOBt in DMF. ^f CZE analysis using a Beckman P/ACE instrument, fused silica capillary (40 cm \times 75 μ m id), 40 mmol SDS in 20 mmol borate/phosphate buffer, pH 8.6 adjusted with NaOH, benzyl alcohol as EOF marker, capillary temp. 25 °C, applied voltage 20 kV and UV detection at 214 nm.

demonstrate that when a choice of amino acid is present, selectivity additional to that of stereochemistry is only significant in respect of the preferred coupling with glycyl residues and of the slightly disfavoured coupling with phenylalanyl residues.

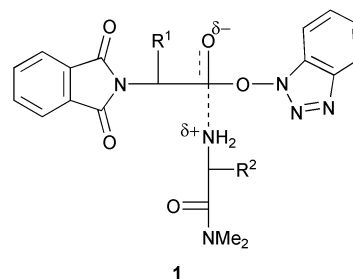
The presence of one reactant in excess allows the approximation that product ratios in competing reactions correspond with ratios of rate constants. Thus, the rate of peptide bond formation in these reactions is largely independent of β -substitution but significantly and uniformly faster between amino acid derivatives of opposite configuration. The probable activated complex for the hydroxybenzotriazole (HOBt)-mediated condensations, **1**, is generally consistent with this observation, for the α -carbon atoms are separated by two other atoms whereas any β -carbon atoms would be less sterically significant.

The consistent preference for heterochiral outcomes is less readily anticipated, but would follow if **1** gained secondary stabilization by interactions between the terminal groups in a six-membered chair conformation. If such were the case, the more favoured diequatorial disposition of side chains might be expected, and would lead to the observed results (**2**). (Similar

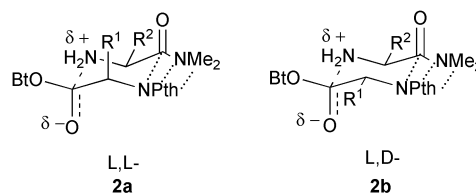
Table 3 Competition outcomes between Phth-A and equimolar mixtures of B-DMA, C-DMA and D-DMA using DCC–HOBt coupling^a

A	B	C	D	Product distribution (%) ^b		
				AB	AC	AD
Gly	Gly	L-Ala	L-Val	52	30	18
Gly	Gly	L-Val	L-Phe	54	33	12
Gly	Gly	L-Ala	L-Phe	54	39	7
L-Phe	L-Ala	L-Phe	L-Val	57	18	24
L-Phe	L-Ala	L-Phe	D-L-Val	15	6	4(L) 75(D)
L-Ala	L-Ala	L-Phe	D-L-Val	14	14	8(L) 64(D)

^a $\{[B-DMA] + [C-DMA] + [D-DMA]\} / [Phth-A] = 15.0$ except for the first experiment (10.0). ^b Error $\leq \pm 5\%$.



factors are considered to be stereochemically influential in the transition states of other reactions; for example, secondary orbital interactions in the Diels–Alder reaction¹² and ring-based conformational preferences in chelate-assisted aldol condensations.¹³) Some minor trends seen in Tables 1–3 would be consistent with this hypothesis; for example, the small but consistently progressive effect as the side chains become more bulky, the lesser selectivity seen when DMF, another amide, is used as solvent, the apparent insensitivity to a change in activating group (Table 1) and an additional influence that may be anticipated with benzyl side chains in close proximity with each other or with a six-membered ring.¹⁴ The exceptional result in Table 1 with the dimethylamide of proline may reflect some resistance to the *trans* ring fusion prescribed by a chair transition state.

**Table 2** Competition outcomes in reactions between Phth-X and equimolar mixtures of Y-DMA and Z-DMA using DCC–HOBt coupling

X	Y	Z	Initial $\frac{[Y-DMA] + [Z-DMA]}{[Phth-X]}$	Final $\frac{[PhthXYDMA]}{[PhthYDMA]}$	% LD dipeptide derivative ^a
L-Ala	L-Ala	L-Phe	6.0	1.0	—
L-Ala	L-Ala	L-Phe	0.17	0.8	—
L-Ala	L-Ala	D-Phe	13.0	0.3	78
L-Ala	D-Ala	L-Phe	10.2	3.5	78
L-Ala	L-Ala	L-Val	10.0	1.4	—
L-Ala	L-Ala	D-Val	11.0	0.2	85
L-Ala	D-Ala	L-Val	10.0	9.0	90
Gly	Gly	L-Ala	9.3	2.7	—
Gly	Gly	L-Phe	8.1	7.3	—
Gly	Gly	L-Val	10.0	4.2	—
L-Val	Gly	L-Ala	10.0	2.1	—
L-Val	Gly	L-Val	8.0	2.3	—
L-Val	Gly	L-Phe	8.8	9.0	—
L-Phe	L-Phe	D-Val	10.0	0.07	93
L-Phe	L-Phe	L-Val	10.0	1.06	—
L-Phe	D-Phe	L-Val	10.0	7.3	88

^a Error $\leq \pm 5\%$.

Table 4 Competition outcomes in reactions between *X*-*L*-*A*-OH and racemic *H*-*B*-*Y* using DCC–HOBt coupling in CH₂Cl₂, except where indicated

<i>X</i>	<i>A</i>	<i>B</i>	<i>Y</i>	Initial $\frac{[B-Y]}{[X-L-A]}$	% LD-dipeptide derivative ^a
Succ	Phe	Phe	NMe ₂	10.0	88
Ac	Pro	Phe	NMe ₂	10.0	52
Fmoc	Ala	Ala	NMe ₂	10.0	40
Fmoc	Phe	Phe	NMe ₂	10.0	49
Fmoc	Phe ^b	Phe	NMe ₂	10.0	39
Z	Ala	Ala	NMe ₂	9.2	37
Z	Phe	Phe	NMe ₂	10.8	53
Z	Val	Val	NMe ₂	10.0	44
Z	Ala	Ala	OMe	10.2	40 ^c
Z	Ala	Ala	OMe	11.0	38
Z	Phe	Phe	OMe	11.0	59
Z	Val	Val	OMe	10.5	54
Fmoc	Ala	Ala	OMe	11.0	37
Fmoc	Val ^b	Val	OMe	10.0	55
Fmoc	Ala	Ala	O-resin ^d	0.1	44
Fmoc	Ala	Ala	O-resin	1.0	49
Fmoc	Val	Val	O-resin	0.1	51
Fmoc	Val	Val	O-resin	1.0	50
Phth	Ala	Ala	O-resin	0.1	70
Phth	Ala	Ala	O-resin	1.0	52
Phth	Ala	Val	O-resin	0.1	69
Phth	Ala	Val	O-resin	1.0	56
Phth	Ala	Phe	O-resin	0.1	66
Phth	Ala	Phe	O-resin	1.0	56
Phth	Val	Val	NHMe	10.0	90
Succ	Phe	Val	NHMe	10.0	87
Phth	Ala	Ala	Ala-O-resin	0.1	96
Fmoc	Ala	Ala	Ala-O-resin	0.1	47

^a Error < ±5%. ^b Pfp (perfluorophenate) activated. ^c DCC–HOSu in CH₂Cl₂. ^d (CH₂C₆H₄OC₆H₄CHCH₂)_n; all experiments with resin based reactants used racemic *X*-*A*-OPfp and *L*-*B*-Oresin, with products converted to NMe₂ derivative for analysis.

Table 5 Enantioselectivity^a in the reactions between P¹NHCH(R¹)COX and H₂NCH(R²)COP²

$\frac{P^2 \rightarrow}{P^1}$	<i>X</i>	NMe ₂	NHMe	AlaOresin	Oresin	OMe
Phth	OBt–DCM	++	++			
Phth	OBt–DMF	+				
Phth	OSu–DCM	++				
Phth	OSu–DMF	+				
Phth	Pfp			++	+	(++)
Sucnmd	OBt–DCM	++	++			
Ac ^b	OBt–DCM	0				0
Fmoc	OBt–DCM	–(0)				–
Fmoc	Pfp			0	0 (–)	–
Z	OBt–DCM	0 (–)				0 (–)

^a Summarising outcomes of 74 reactions with derivatives of alanine, valine, phenylalanine and proline: ++ >80% LD; +60–80% LD; 0 <60% LD; –60–80% LL; minority outcome in parentheses. ^b *N*-acetylproline.

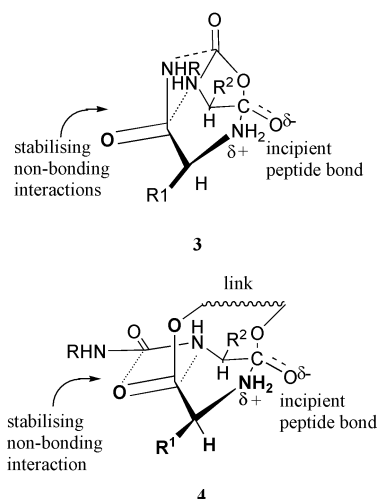
Table 4 shows outcomes with further structural variation in reactants. Thus, perfluorophenate activation, replacing the DMA group by a secondary amido function or the ester linkage of a Wang resin, and replacing phthalimido by succinimido all retain the heterochiral bias. Reactions with *N*-acetyl proline (another ‘oxazolone-safe’ reagent), however, show no selectivity but as before, one may anticipate a distinctive conformational influence on the outcome in this case.¹⁵ Urethane protection, even with DMA co-reactants, also leads to little or no selectivity, implying that the stabilising interaction proposed in **2** would need a significant electrophilic contribution from the *N*-protecting group. Table 5 summarises all the outcomes and suggests that selectivity requires an amide function at the *N*-terminus of the carboxy reactant but allows both amide and ester at the *C*-terminus of the amino reactant.

The results support Dose’s contention that rate differences in condensations in mixtures of activated amino acids would

generally be too small to direct side-chain sequences.⁵ At the same time, however, they demonstrate a clear possibility of stereochemical selectivity in abiotic condensations, which could be further enhanced with more closely interacting secondary features and lower temperatures.¹⁶ In the absence of epimerization equilibria, reactions analogous to those we have examined, for example, would convey a prospect of sequences in extendable systems having alternate configurations at C_α until interrupted by glycyl.

As mentioned earlier, a contrasting but weaker homochiral preference has been observed in chain extension mediated by *N*-carboxyanhydrides.⁸ A transition state that simultaneously minimises steric hindrance between side chains and allows secondary stabilizing interactions with the anhydride ring (**3**) predicts this outcome. We are thus prompted to consider what features might promote homochiral selection in the more general case, such as ours, of activated open-chain reactants. If the

stabilized activated complex was constrained to adopt a boat-like conformation similar to **3**, then the more favoured diequatorial disposition of side chains would correspond with homochiral configurations. Such conformations (**4**) would be prescribed if the two carboxyl derivatives were linked.



While the overall complexity of ribosomal protein synthesis¹⁷ obscures relevance of these ideas to the present day system, far less complex and more directly linked carboxyl derivatives, including mineral surface complexes,^{6,18} could apply in the prebiotic context, and a study of model compounds further exploring such concepts is in progress.

Conclusions

Significant enantioselection in abiotic peptide bond formation is demonstrable between *N*-acyl α -amino acids and α -amino acid amides or esters, in which heterochiral coupling predominates. This would be consistent with the tetrahedral intermediate being formed *via* an activated complex that can access a stabilising non-bonded interaction in the chair conformation of a quasi six-membered ring, thereby placing the side chains in the more favoured diequatorial positions. Such positions in the analogous boat-like conformation, expected when the two carboxyl functions are constrained to be on the same side of the ring, would lead to homochiral coupling. While sharing some similarities with those of the peptide transfer step of protein synthesis, these features are perhaps more indicative of one way homochiral peptides could have arisen prebiotically, given the probable availability of suitable carboxyl-binding templates.

Experimental section

General

Melting temperatures were measured using a Gallenkamp instrument. Optical rotations given in 10^{-1} deg cm² g⁻¹ were recorded on an Optical Activity Polar 2001 polarimeter, at 25 °C and a concentration of 0.01 g cm⁻³ in chloroform. Infrared spectra were recorded as films evaporated from CHCl₃ solutions on a Perkin-Elmer 1600 series FTIR spectrometer. ¹H NMR spectra were recorded at 250 MHz on a Bruker AM250 spectrometer fitted with a ¹H-¹³C dual probe, at 293 K. CDCl₃ was used as solvent and the spectra were internally referenced to the residual solvent peak. ¹³C NMR spectra were recorded at 62.9 MHz on the same spectrometer, at 293 K, using CDCl₃ and spectra were referenced to the solvent peak. Peak assignments were assisted by DEPT editing of the spectra. Elemental analyses were performed on a Carlo Erba Model 1106 or 1108 analyser. High resolution mass measurements were made on a VG ZAB-E spectrometer.

HPLC analyses were performed using a Waters Millennium System comprising a '616' quaternary gradient pump, a '996' photodiode array and a '717+' autosampler. Conditions were selected for optimum resolution of Phth-DMA dipeptide derivatives, including diastereoisomers, for each competition experiment using the synthesised standards, one of three columns (Phenomenex Luna C18 5 μ m 250 \times 4.6 mm id; Spherisorb S5 ODS1 250 \times 4.6 mm id; Hypercarb 5 μ m 100 \times 4.6 mm id) and aqueous acetonitrile gradients.¹⁹ Peak purities for Phth-DMA derivatives in competition mixtures were checked using protocols available from the system software, including visual scrutiny of superimposed UV spectra.

Wang resin and Fmoc- and ester derivatives of amino acids were obtained from Novabiochem or synthesized by standard procedures.²⁰ FmocOSu was kindly donated by Eastman Chemicals, Llangefni and the Sigma or Avocado Chemical Companies supplied all other chemicals.

General procedure for the synthesis of *N*-phthaloyl and *N*-succinimido amino acids²¹

Amino acid (0.02 mol) and the appropriate anhydride (0.02 mol) were refluxed in toluene (20 cm³) for 20 h.²² The protected amino acid crystallised on cooling and was isolated by filtration. Yields 62–83%.

General procedure for the synthesis of amino acid dimethylamides and methylamides

Z-protected amino acids were synthesised using *N*-(benzyloxy-carbonyloxy) succinimide.²³ Z-protected amino acid (0.017 mol) was dissolved in DCM (20 cm³) and HOBT²⁴ (0.018 mol) was added, followed by DCC (0.018 mol). To this solution, a mixture of dimethylamine or methylamine HCl (0.018 mol) and triethylamine (0.018 mol), suspended in DCM (10 cm³), was added and the mixture stirred at room temperature for 24 h. Precipitated DCHU was removed by filtration, DCM removed by rotary evaporation and subsequent trituration with cold EtOAc removed more DCHU. The EtOAc solution was washed with 0.5 M aq HCl (3 \times 15 cm³), 0.5 M aq KHCO₃ (3 \times 15 cm³), water (2 \times 15 cm³) and dried (anh. MgSO₄). Solvent removal left the Z-amino acid dimethylamide or methylamide as a crystalline solid in yields of 64–72%. These were treated with HBr–HOAc (45%, 5 cm³) for 2 h at room temperature. Addition of ice-cold Et₂O precipitated the amino acid dimethylamide or methylamide HBr salt which was recovered by filtration in yields of 42–53%.

General procedure for the synthesis of dipeptide derivatives

N-Protected amino acid (0.01 mol) and amino acid dimethylamide or methylamide–HBr (0.01 mol) were coupled using the above procedure to give crystalline solids which were purified by column chromatography on silica with Et₂O–MeOH as eluent; yields 10–24%. Methyl esters were prepared in a similar manner, coupling the *N*-protected amino acid with the amino acid methyl ester hydrochloride salt; yields 20–25%.

General procedure for competitive coupling experiments

N-Protected-L-amino acid (0.1 mmol) and racemic amino acid amide or ester halide salt (1.0 mmol) were allowed to react using the above coupling procedure for 48 h. The DCM was removed by rotary evaporation, the residue dissolved in the initial HPLC eluent (30% aq MeCN) and a homogenous sample analysed by the area ratio of peaks identified by standards as the L L and L D diastereoisomers of the corresponding derivatized peptides.

General procedure for resin coupling experiments

Experiments were conducted with freshly distilled solvents in a 25-cm³ vessel designed for solid-state synthesis.²⁵ In a typical

experiment, Fmoc-L-Ala-Wang resin (Novabiochem, 100 mg) was treated with piperidine (20% v/v in DMF) for 2 h, washed with DCM ($3 \times 10 \text{ cm}^3$) and DMF ($3 \times 10 \text{ cm}^3$), and the deprotected amine allowed to react with Fmoc-rac-Ala-Pfp (Novabiochem, 37 mg in 10 cm^3 DMF) for 24 h. The dipeptide derivative was released from the resin with TFA–DCM (1 : 1) and converted to the methyl ester with 9 : 1 MeOH–AcCl over 24 h in an overall yield of 57%.

Phth-Gly-Gly-DMA

Mp: 165 °C. (Found: C, 57.62; H, 5.23; N, 14.73. $\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}_4 \cdot 0.2 \text{ H}_2\text{O}$ requires: C, 57.41; H, 5.29; N, 14.35%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3314, 1721, 1650, 1418, 952, 714; δ_{H} 2.91 (s, 3H), 3.00 (s, 3H), 4.08 (d, 2H), 4.49 (s, 2H), 7.00 (br, s, 1H), 7.73 (m, 2H), 7.78 (m, 2H); δ_{C} 35.6, 35.9, 40.5, 41.4, 123.5, 123.6, 134.2, 166.0, 167.7.

Phth-Gly-L-Ala-DMA

Mp: 177 °C. $[a]_{\text{D}}$: –204.0. (Found: C, 60.08; H, 5.99; N, 13.96. $\text{C}_{15}\text{H}_{17}\text{N}_3\text{O}_4$ requires: C, 59.40; H, 5.68; N, 13.85%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3315, 1723, 1638, 1417, 953; δ_{H} 1.29 (d, 3H), 2.91 (s, 3H), 3.09 (s, 3H), 4.91 (q, 1H), 4.35 (s, 2H), 7.02 (br, s, 1H), 7.25 (m, 2H), 7.88 (m, 2H); δ_{C} 18.5, 35.8, 36.9, 40.5, 49.2, 123.5, 132.0, 134.1, 165.2, 167.8, 172.0; m/z (FAB+) 304.1294. ($\text{C}_{15}\text{H}_{18}\text{N}_3\text{O}_4$ requires 304.1297.)

Phth-Gly-L-Val-DMA

Mp: 154 °C. $[a]_{\text{D}}$: –145.5. (Found: C, 61.85; H, 6.67; N, 12.52. $\text{C}_{17}\text{H}_{21}\text{N}_3\text{O}_4$ requires: C, 61.62; H, 6.39; N, 12.68%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3296, 1719, 1630, 1420, 954, 714; δ_{H} 0.85 (d, 3H), 0.91 (d, 3H), 2.0 (q, 1H), 2.97 (s, 3H), 2.98 (s, 3H), 4.41 (d, 2H), 4.82 (q, 1H), 6.75 (br d, 1H), 7.69 (m, 2H), 7.88 (m, 2H); δ_{C} 17.4, 19.6, 24.9, 31.8, 33.9, 40.5, 53.8, 123.6, 132.11, 134.1, 165.9, 167.7, 171.4.

Phth-Gly-L-Phe-DMA

Mp: 96–98 °C. $[a]_{\text{D}}$: –125.0. (Found: C, 65.77; H, 5.63; N, 11.34. $\text{C}_{21}\text{H}_{21}\text{N}_3\text{O}_4$ requires: C, 65.85; H, 6.04; N, 10.97%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3407, 1718, 1635, 1419; δ_{H} 2.50 (s, 3H), 2.71 (s, 3H), 2.95 (m, 2H), 4.39 (s, 2H), 5.08 (q, 1H), 7.1 (m, 5H), 7.48 (br d, 1H), 7.68 (m, 2H), 7.84 (m, 2H); δ_{C} 24.9, 25.6, 39.1, 40.4, 50.5, 123.5, 127.0, 128.4, 129.4, 132.1, 134.1, 136.0, 165.6, 167.8, 171.0; m/z (FAB+) 380.1615. ($\text{C}_{15}\text{H}_{18}\text{N}_3\text{O}_4$ requires 380.1610.)

Phth-L-Val-Gly-DMA

Mp: 85–87 °C. $[a]_{\text{D}}$: –2.0. (Found: C, 61.43; H, 6.12; N, 12.60. $\text{C}_{17}\text{H}_{21}\text{N}_3\text{O}_4$ requires: C, 61.62; H, 6.39; N, 12.68%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3334, 1715, 1649, 1384, 1070, 718; δ_{H} 0.85 (d, 3H), 1.12 (d, 3H), 2.90 (m, 1H), 2.95 (s, 3H), 3.0 (s, 3H), 4.02 (d, 2H), 4.51 (d, 1H), 7.70 (m, 2H), 7.86 (m, 3H); δ_{C} 19.5, 20.1, 27.5, 35.5, 35.8, 41.7, 62.4, 123.7, 131.5, 134.3, 167.5, 168.2, 168.7.

Phth-L-Ala-L-Ala-DMA

Mp: 166–168 °C. $[a]_{\text{D}}$: +8.6. (Found: C, 61.11; H, 6.04; N, 13.28. $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4$ requires: C, 60.56; H, 6.03; N, 13.24%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3395, 2934, 1714, 1635, 1387, 1215, 754; δ_{H} 1.30 (d, 3H), 1.70 (d, 3H), 2.955 (s, 3H), 3.08 (s, 3H), 4.80–5.00 (m, 2H), 7.05 (br d, 1H), 7.70 (m, 2H), 7.84 (m, 2H); δ_{C} 14.8, 17.9, 35.2, 36.4, 45.4, 48.5, 123.1, 131.9, 133.7, 167.7, 168.0, 171.9; m/z (FAB+) 318.1450. ($\text{C}_{16}\text{H}_{20}\text{N}_3\text{O}_4$ requires 318.1454.)

Phth-L-Ala-D-Ala-DMA

Mp: 148–150 °C. $[a]_{\text{D}}$: –2.0. (Found: C, 60.14; H, 6.17; N, 13.29. $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4 \cdot 0.25 \text{ H}_2\text{O}$ requires C, 59.88; H, 6.09; N,

13.09%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3370, 2924, 1715, 1630, 1385, 1049, 881; δ_{H} 1.34 (d, 3H), 1.71 (d, 3H), 2.91 (s, 3H), 3.01 (s, 3H), 4.81–4.98 (m, 2H), 7.04 (br d, 1H), 7.68 (m, 2H), 7.82 (m, 2H); δ_{C} 15.2, 18.3, 35.7, 36.9, 45.8, 48.9, 123.5, 132.0, 134.1, 167.9, 168.2, 172.1.

Phth-L-Ala-L-Val-DMA

Mp: 152–154 °C. $[a]_{\text{D}}$: +34.3. (Found: C, 61.94; H, 6.63; N, 12.07. $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_4$ requires: C, 61.95; H, 6.76; N, 12.04%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3316, 2931, 1716, 1634, 1386, 1216, 756; δ_{H} 0.81 (d, 3H), 0.84 (d, 3H), 1.62 (d, 3H), 1.90–2.01 (m, 1H), 2.90 (s, 3H), 3.07 (s, 3H), 4.71 (m, 1H), 4.81 (m, 1H), 7.30 (br d, 1H), 7.69 (m, 2H), 7.82 (m, 2H); δ_{C} 15.3, 17.4, 19.6, 31.6, 35.7, 37.4, 49.11, 53.9, 123.5, 131.9, 134.2, 167.7, 169.0, 171.5.

Phth-L-Ala-D-Val-DMA

Mp: 118–120 °C. $[a]_{\text{D}}$: –3.1. (Found: C, 60.59; H, 6.58; N, 11.45. $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_4 \cdot 2\text{H}_2\text{O}$ requires: C, 60.49; H, 6.86; N, 11.76%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3314, 2932, 1716, 1634, 1388, 1216, 757; δ_{H} 0.90 (d, 3H), 0.93 (d, 3H), 1.71 (d, 3H), 1.94–2.13 (m, 1H), 2.91 (s, 3H), 3.10 (s, 3H), 4.82 (q, 1H), 4.91 (q, 1H), 6.75 (br d, 1H), 7.69 (d, 2H), 7.80 (d, 2H); δ_{C} 15.3, 17.4, 19.5, 31.6, 35.6, 37.3, 49.0, 53.9, 123.4, 131.9, 134.7, 167.7, 168.8, 171.4.

Phth-L-Ala-L-Phe-DMA

Mp: 108–112 °C. $[a]_{\text{D}}$: +18.1. (Found: C, 65.99; H, 5.86; N, 10.41. $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_4 \cdot 0.5 \text{ H}_2\text{O}$ requires: C, 66.15; H, 5.97; N, 10.52%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3317, 2928, 1712, 1631, 1135, 1175, 721; δ_{H} 1.78 (d, 3H), 2.57 (s, 3H), 2.86 (s, 3H), 2.86–3.09 (m, 2H), 4.99 (m, 1H), 5.04 (m, 1H), 6.95 (br d, 1H), 7.08–7.18 (m, 5H), 7.67 (m, 2H), 7.78 (m, 2H); δ_{C} 15.2, 35.6, 36.7, 39.4, 49.1, 50.7, 123.5, 127.0, 128.4, 129.5, 131.5, 131.9, 134.2, 136.0, 167.7, 168.3, 170.7.

Phth-L-Ala-D-Phe-DMA

Mp: 104–107 °C. $[a]_{\text{D}}$: –33.3. (Found: C, 64.91; H, 5.83; N, 10.16. $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_4 \cdot 3\text{H}_2\text{O}$ requires: C, 64.93; H, 6.07; N, 10.33%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3318, 2928, 1714, 1634, 1386, 1257, 723; δ_{H} 1.79 (d, 3H), 2.60 (s, 3H), 2.85 (s, 3H), 2.87–3.09 (m, 2H), 4.90 (m, 1H), 5.05 (m, 1H), 6.90 (br d, 1H), 7.09–7.19 (m, 5H), 7.68 (m, 2H), 7.79 (m, 2H); δ_{C} 15.2, 35.6, 36.8, 39.3, 49.1, 50.6, 123.4, 124.0, 127.0, 128.4, 129.5, 131.9, 134.1, 136.0, 167.7, 168.8, 171.4.

Phth-L-Val-L-Ala-DMA

Mp: 135–137 °C. $[a]_{\text{D}}$: +27.2. (Found: C, 62.42; H, 6.92; N, 12.02. $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_4$ requires: C, 62.59; H, 6.71; N, 12.16%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3392, 2965, 2932, 1716, 1636, 1384, 1070, 730; δ_{H} 0.85 (d, 3H), 1.11 (d, 3H), 1.32 (d, 3H), 2.85 (m, 1H), 2.91 (s, 3H), 3.02 (s, 3H), 4.41 (d, 1H), 4.88 (m, 1H), 7.70 (m, 3H), 7.88 (m, 3H); δ_{C} 18.3, 19.5, 20.0, 27.4, 35.7, 36.9, 45.7, 62.3, 123.6, 131.4, 134.2, 167.7, 168.1, 171.8.

Phth-L-Val-D-Ala-DMA

Mp: 123–125 °C. $[a]_{\text{D}}$: –12.0. (Found: C, 61.21; H, 6.57; N, 11.79. $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_4 \cdot \text{H}_2\text{O}$ requires: C, 61.00; H, 6.83; N, 11.86%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3419, 2932, 1716, 1636, 1384, 1070, 730; δ_{H} 0.87 (d, 3H), 1.12 (d, 3H), 1.34 (d, 3H), 2.90 (m, 1H), 2.98 (s, 3H), 3.08 (s, 3H), 4.41 (d, 1H), 4.87 (m, 1H), 7.72 (m, 2H), 7.75 (br d, 1H), 7.89 (m, 2H); δ_{C} 18.3, 19.5, 20.1, 27.5, 35.7, 36.9, 45.7, 62.3, 123.7, 131.5, 134.3, 167.7, 168.2, 172.0.

Phth-L-Val-L-Val-DMA

Mp: 170–171 °C. $[a]_{\text{D}}$: +0.7. (Found: C, 64.45; H, 7.10; N, 11.70. $\text{C}_{20}\text{H}_{27}\text{N}_3\text{O}_4$ requires: C, 64.32; H, 7.29; N, 11.25%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3344, 2967, 2932, 1716, 1637, 1385, 1216,

754; δ_{H} 0.86 (d, 3H), 0.92 (d, 3H), 0.98 (d, 3H), 1.12 (d, 3H), 2.02 (m, 1H), 2.89–2.91 (m, 1H), 2.91, 3.09 (s, 3H), 4.41 (d, 1H), 4.81 (m, 1H), 7.34 (br d, 1H), 7.72 (m, 2H), 7.89 (m, 2H); δ_{C} 17.5, 19.4, 19.6, 20.1, 27.4, 31.1, 35.7, 37.4, 53.9, 62.2, 123.6, 131.4, 134.3, 168.1, 168.5, 171.3.

Phth-L-Val-D-Val-DMA

Mp: 110–111 °C. $[\alpha]_{\text{D}}$: –0.8. (Found: C, 63.16; H, 7.04; N, 11.52. $\text{C}_{20}\text{H}_{27}\text{N}_3\text{O}_4 \cdot 0.5\text{H}_2\text{O}$ requires: C, 63.31; H, 7.35; N, 11.07%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands): 3351, 2967, 2930, 1716, 1638, 1384, 1216, 756; δ_{H} 0.81 (d, 3H), 0.90 (d, 3H), 0.98 (d, 3H), 1.12 (d, 3H), 2.00 (m, 1H), 2.84–2.88 (m, 1H), 2.89 (s, 3H), 3.17 (s, 3H), 4.38 (d, 1H), 4.81 (m, 1H), 7.34 (br d, 1H), 7.72 (m, 2H), 7.85 (m, 2H); δ_{C} 17.6, 19.4, 19.6, 20.1, 27.4, 31.1, 35.7, 37.4, 54.0, 62.1, 123.6, 131.4, 134.3, 168.2, 168.6, 171.4.

Phth-L-Val-L-Phe-DMA

Mp: 144–145 °C. $[\alpha]_{\text{D}}$: +5.2. (Found: C, 67.88; H, 6.78; N, 10.06. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4$ requires: C, 68.07; H, 6.88; N, 9.92%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3332, 2928, 1716, 1636, 1384, 1070, 730; δ_{H} 0.85 (d, 3H), 1.00 (d, 3H), 2.81–2.85 (m, 1H), 2.66 (s, 3H), 2.84 (s, 3H), 3.01 (m, 2H), 4.41 (d, 1H), 5.30 (m, 1H), 7.30 (br d, 1H), 7.70 (m, 2H), 7.81 (m, 2H); δ_{C} 19.5, 19.8, 27.4, 35.6, 36.8, 39.4, 50.3, 62.2, 123.7, 126.9, 128.3, 129.3, 131.5, 134.3, 136.2, 168.0, 168.2, 171.0.

Phth-L-Val-D-Phe-DMA

Mp: 103–105 °C. $[\alpha]_{\text{D}}$: –2.3. (Found: C, 67.46; H, 6.77; N, 10.08. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4 \cdot 0.5\text{H}_2\text{O}$ requires: C, 67.43; H, 6.52; N, 9.83%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3339, 2932, 1716, 1636, 1384, 1070, 730; δ_{H} 0.81 (d, 3H), 1.06 (d, 3H), 2.71 (s, 3H), 2.89 (s, 3H), 2.77–2.91 (m, 1H), 2.98 (m, 2H), 4.39 (d, 1H), 5.10 (q, 1H), 6.90 (m, 5H), 7.51 (br d, 1H), 7.70 (m, 2H), 7.79 (m, 2H); δ_{C} 19.5, 19.9, 27.3, 35.6, 36.8, 38.9, 50.4, 62.7, 123.6, 131.4, 134.3, 168.1, 168.5, 171.3.

Phth-L-Phe-L-Ala-DMA

Mp: 159–161 °C. $[\alpha]_{\text{D}}$: –9.7. (Found: C, 63.88; H, 6.17; N, 10.38. $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_4 \cdot \text{H}_2\text{O}$ requires: C, 64.22; H, 6.12; N, 10.21%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3422, 2922, 1715, 1635, 1384, 1104, 721; δ_{H} 1.25 (d, 3H), 2.87 (s, 3H), 3.02 (s, 3H), 3.51 (m, 2H), 4.85 (t, 1H), 5.15 (t, 1H), 7.05 (m, 5H), 7.12 (br d, 1H), 7.51 (m, 2H), 7.71 (m, 2H); δ_{C} 18.3, 35.7, 36.9, 45.9, 55.3, 123.5, 126.8, 128.5, 128.9, 131.5, 134.1, 134.7, 136.7, 167.3, 167.7, 171.8.

Phth-L-Phe-D-Ala-DMA

Mp: 116–117 °C. $[\alpha]_{\text{D}}$: –72.7. (Found: C, 66.59; H, 5.84; N, 10.45. $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_4 \cdot 0.25\text{H}_2\text{O}$ requires: C, 66.55; H, 5.94; N, 10.58%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3417, 1714, 1636, 1385, 1104, 720; δ_{H} 1.30 (d, 3H), 2.92 (s, 3H), 3.09 (s, 3H), 3.59 (m, 2H), 4.90 (t, 1H), 5.15 (t, 1H), 7.10–7.14 (m, 6H), 7.65 (m, 2H), 7.75 (m, 2H); δ_{C} 18.4, 35.6, 36.9, 45.9, 55.3, 123.5, 126.9, 128.5, 128.9, 131.5, 134.1, 134.7, 136.7, 167.3, 167.8, 171.8.

Phth-L-Phe-L-Val-DMA

Mp: 138–139 °C. $[\alpha]_{\text{D}}$: –40.6. (Found: C, 67.51; H, 6.54; N, 9.53. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4 \cdot 0.5\text{H}_2\text{O}$ requires: C, 67.43; H, 6.52; N, 9.83%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3308, 2929, 1714, 1635, 1384, 1215, 1103, 756; δ_{H} 0.85 (d, 3H), 1.00 (d, 3H), 1.90–1.98 (m, 1H), 2.94 (s, 3H), 3.01 (s, 3H), 3.55 (m, 2H), 4.86 (m, 1H), 5.15 (m, 1H), 7.01 (br d, 1H), 7.10 (m, 5H), 7.61 (m, 2H), 7.75 (m, 2H); δ_{C} 17.4, 19.5, 31.6, 34.7, 35.5, 37.2, 54.0, 55.3, 123.4, 126.9, 128.6, 128.9, 131.5, 134.1, 136.7, 167.8, 168.1, 171.3.

Phth-L-Phe-D-Val-DMA

Mp: 138–140 °C. $[\alpha]_{\text{D}}$: –108. (Found: C, 68.02; H, 6.74; N, 10.20. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4 \cdot \text{H}_2\text{O}$ requires: C, 68.07; H, 6.88; N, 9.91%);

$\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3318, 2932, 1716, 1634, 1387, 1216, 752; δ_{H} 0.87 (d, 3H), 1.02 (d, 3H), 1.95–2.12 (m, 1H), 2.94 (s, 3H), 3.00 (s, 3H), 3.55 (m, 2H), 4.86 (m, 1H), 5.18 (m, 1H), 6.98 (br d, 1H), 7.10 (m, 5H), 7.63 (m, 2H), 7.78 (m, 2H); δ_{C} 17.5, 19.6, 31.6, 34.7, 35.7, 37.4, 54.0, 55.4, 123.4, 126.9, 128.6, 128.9, 131.5, 134.1, 136.7, 167.8, 168.1, 171.3.

Phth-L-Phe-L-Phe-DMA

Mp: 128–130 °C. $[\alpha]_{\text{D}}$: –76.0. (Found: C, 71.71; H, 5.82; N, 9.17. $\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_4$ requires: C, 71.65; H, 5.76; N, 8.95%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3377, 2929, 1716, 1635, 1384, 1104, 753; δ_{H} 2.60 (s, 3H), 2.85 (s, 3H), 2.90–3.10 (m, 2H), 3.51 (m, 2H), 5.15 (m, 2H), 6.90 (br d, 1H), 7.10 (m, 10H), 7.61 (m, 2H), 7.75 (m, 2H); δ_{C} 34.6, 35.5, 36.8, 39.5, 55.3, 59.7, 123.5, 126.8, 127.0, 128.4, 128.5, 128.9, 129.4, 131.5, 134.1, 136.1, 136.7, 167.6, 167.7, 170.7.

Phth-L-Phe-D-Phe-DMA

Mp: 111–113 °C. $[\alpha]_{\text{D}}$: –46.9. (Found: C, 70.01; H, 5.88; N, 9.04. $\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_4 \cdot 0.6\text{H}_2\text{O}$ requires: C, 69.98; H, 5.88; N, 8.75%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3318, 2926, 1715, 1636, 1384, 1215, 756; δ_{H} 2.63 (d, 3H), 2.85 (d, 3H), 2.91–3.20 (m, 2H), 3.55 (m, 2H), 5.13 (m, 2H), 7.03 (br d, 1H), 7.12 (m, 10H), 7.65 (m, 2H), 7.78 (m, 2H); δ_{C} 34.6, 35.5, 36.8, 39.4, 50.6, 55.6, 123.5, 126.8, 127.0, 128.4, 128.6, 128.8, 129.4, 131.5, 134.1, 136.0, 136.6, 167.6, 167.8, 170.6.

Phth-L-Phe-L-Pro-DMA

Mp: 180–181 °C. $[\alpha]_{\text{D}}$: –148.8. (Found: C, 68.77; H, 6.53; N, 10.05. $\text{C}_{24}\text{H}_{25}\text{N}_3\text{O}_4$ requires: C, 68.72; H, 6.01; N, 10.02%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3331, 2929, 1716, 1648, 1382, 1101, 750, 721; δ_{H} 1.85–1.97 (m, 2H), 2.01–2.11 (m, 2H), 2.90 (s, 3H), 3.06 (s, 3H), 3.28–3.41 (m, 2H), 3.61–3.65 (m, 2H), 4.48–4.92 (m, 1H), 5.20–5.24 (m, 1H), 7.08–7.27 (m, 5H), 7.64 (m, 2H), 7.75 (m, 2H); δ_{C} 26.3, 26.4, 29.9, 35.5, 37.1, 46.8, 55.6, 58.5, 124.7, 124.9, 128.1, 129.8, 129.9, 130.0, 130.3, 130.5, 130.8, 135.4, 135.5, 166.9, 167.6, 171.1, 171.6.

Phth-L-Phe-LD-Pro-DMA (diastereomeric mixture)

Mp: 130–134 °C. $[\alpha]_{\text{D}}$: –103.0. (Found: C, 67.07; H, 6.29; N, 9.47. $\text{C}_{24}\text{H}_{25}\text{N}_3\text{O}_4 \cdot 0.5\text{H}_2\text{O}$ requires: C, 67.27; H, 6.12; N, 9.81%); distinctive ^1H NMR features, δ : 2.97 (s, 3H), 3.11 (s, 3H), 3.69–3.79 (m, 2H); distinctive ^{13}C NMR features, δ : 36.0, 38.5, 48.6, 56.9, 57.1, 166.3.

Z-L-Val-L-Val-OMe

Mp: 94–95 °C. $[\alpha]_{\text{D}}$: +1.5. (Found: C, 62.26; H, 7.64; N, 7.76. $\text{C}_{19}\text{H}_{28}\text{N}_2\text{O}_5 \cdot 0.2\text{H}_2\text{O}$ requires: C, 62.01; H, 7.78; N, 7.61%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3306, 2962, 1730, 1658, 1538, 1212, 1027; δ_{H} 0.89–1.00 (m, 12H), 2.15 (m, 2H), 3.72 (s, 3H), 4.05 (t, 1H), 4.52 (q, 1H), 5.10 (s, 2H), 5.45 (br d, 1H), 6.45 (br d, 1H), 7.38 (s, 5H); δ_{C} 17.7, 17.8, 18.9, 19.1, 31.2, 52.1, 57.1, 60.3, 66.9, 127.9, 128.1, 128.5, 171.5, 172.2.

Z-L-Val-D-Val-OMe

Mp: 142–143 °C. $[\alpha]_{\text{D}}$: –5.9. (Found: C, 62.01; H, 7.55; N, 7.83. $\text{C}_{19}\text{H}_{28}\text{N}_2\text{O}_5 \cdot 0.1\text{H}_2\text{O}$ requires: C, 62.31; H, 7.76; N, 7.65%); $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3386, 2958, 1731, 1688, 1646, 1540, 1250; δ_{H} 0.82–0.98 (m, 12H), 2.16 (m, 2H), 3.74 (s, 3H), 4.10 (t, 1H), 4.56 (q, 1H), 5.12 (s, 2H), 5.45 (br d, 1H), 6.44 (br d, 1H), 7.43 (s, 5H); δ_{C} 17.6, 17.8, 18.9, 19.3, 31.1, 52.2, 57.2, 60.4, 67.1, 128.0, 128.2, 171.5, 172.2.

Z-L-Phe-L-Phe-OMe

Mp: 134–136 °C. $[\alpha]_{\text{D}}$: –21.9. $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3306, 2928, 1652, 1538, 1454, 1216, 749, 699; δ_{H} 2.96–3.07 (m, 4H),

3.68 (s, 3H), 4.45 (m, 2H), 5.08 (s, 2H), 5.46 (br s, 1H), 6.45 (br s, 1H), 7.20–7.41 (m, 15H); δ_{C} 37.8, 52.4, 53.3, 127.1, 128.0, 128.2, 128.6, 128.7, 129.2, 129.3, 135.5, 136.1, 136.2, 170.5, 171.3; m/z (FAB+) 461.2070. ($\text{C}_{26}\text{H}_{29}\text{N}_2\text{O}_5$ requires 461.2076.)

Z-L-Phe-D-Phe-OMe

Mp: 132–134 °C. $[\alpha]_{\text{D}}^{25}$: –28.1. $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3378, 1658, 1539, 1216, 698; δ_{H} 2.96–3.02 (m, 4H), 3.66 (s, 3H), 4.49 (br d, 1H), 4.85 (q, 1H), 5.06 (s, 2H), 5.51 (br s, 1H), 6.50 (br s, 1H), 7.20–7.31 (m, 15H); δ_{C} 37.8, 38.5, 52.3, 53.0, 67.1, 127.1, 127.2, 128.0, 128.1, 128.2, 128.5, 128.6, 128.7, 129.2, 129.3, 135.5, 136.1, 136.2, 170.5, 171.5; m/z (FAB+) 461.2072. ($\text{C}_{26}\text{H}_{29}\text{N}_2\text{O}_5$ requires 461.2076.)

Z-L-Ala-L-Ala-OMe and Z-L-Ala-D-Ala-OMe

Were synthesised by the above general procedure and showed characterisation data consistent with that reported in the literature.²⁶

Z-L-Ala-L-Ala-DMA

Mp: 126–128 °C. $[\alpha]_{\text{D}}^{25}$: –1.9. (Found: C, 60.99; H, 7.34; N, 13.06. $\text{C}_{16}\text{H}_{23}\text{N}_3\text{O}_4 \cdot 0.2 \text{ H}_2\text{O}$ requires: C, 59.14; H, 7.26; N, 12.89%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3323, 2927, 1626, 1535, 1453, 1245, 1070; δ_{H} 1.24 (d, 3H), 1.48 (d, 3H), 2.91 (s, 3H), 3.01 (s, 3H), 4.23 (br q, 1H), 4.80 (q, 1H), 5.06 (s, 2H), 5.54 (br s, 1H), 7.30 (s, 5H), 7.40 (br s, 1H); δ_{C} 18.5, 19.2, 35.8, 36.9, 49.3, 50.6, 66.9, 128.0, 128.5, 171.5, 172.0.

Z-L-Ala-D-Ala-DMA

Mp: 133–135 °C. $[\alpha]_{\text{D}}^{25}$: –7.1. (Found: C, 60.99; H, 7.34; N, 13.06. $\text{C}_{16}\text{H}_{23}\text{N}_3\text{O}_4 \cdot 0.2 \text{ H}_2\text{O}$ requires: C, 59.14; H, 7.26; N, 12.89%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3280, 2926, 1631, 1538, 1450, 1252, 1043, 741, 700; δ_{H} 1.29 (d, 3H), 1.36 (d, 3H), 2.86 (s, 3H), 3.03 (s, 3H), 4.25 (br q, 1H), 4.80 (q, 1H), 5.02 (d, 2H), 5.54 (br s, 1H), 7.21 (s, 5H), 7.30 (br s, 1H); δ_{C} 18.0, 18.6, 35.9, 37.1, 49.3, 50.7, 66.7, 125.8, 126.3, 126.6, 127.9, 128.0, 172.3, 172.7; m/z (FAB+) 322.1767. ($\text{C}_{16}\text{H}_{24}\text{N}_3\text{O}_4$ requires 322.1767.)

Z-L-Phe-L-Phe-DMA

Mp: 115–117 °C. $[\alpha]_{\text{D}}^{25}$: –12.1. (Found: C, 69.68; H, 6.56; N, 9.03. $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_4 \cdot 0.5 \text{ H}_2\text{O}$ requires: C, 69.69; H, 6.68; N, 8.71%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3392, 1719, 1632, 1496, 1453, 1256, 1047, 743, 698; δ_{H} 2.57 (s, 3H), 3.00 (s, 3H), 3.01–3.16 (br q, 2H), 4.69 (q, 1H), 5.03 (s, 2H), 5.20 (q, 1H), 5.91 (br s, 1H), 7.05–7.34 (m, 15H), 8.11 (br s, 1H); δ_{C} 35.6, 35.8, 38.8, 39.6, 49.0, 55.9, 66.7, 126.8, 127.0, 127.9, 128.0, 128.4, 128.5, 129.4, 129.5, 129.8, 170.7, 170.9, 171.1.

Z-L-Phe-LD-Phe-DMA (diastereomeric mixture)

Mp: 115–118 °C. $[\alpha]_{\text{D}}^{25}$: +0.5. (Found: C, 70.81; H, 6.60; N, 8.95. $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_4$ requires: C, 71.02; H, 6.60; N, 8.87%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3279, 1049, 746. Distinctive ^1H NMR features, δ : 2.82 (s, 3H), 3.05 (d, 2H), 4.49 (q, 1H), 5.01–5.03 (m, 3H), 5.35 (br s, 1H), 6.96 (br s, 1H). Distinctive ^{13}C NMR features, δ : 38.8, 50.1, 50.3.

Fmoc-L-Ala-L-Ala-DMA

Mp: 198–199 °C. $[\alpha]_{\text{D}}^{25}$: –1.4. $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3294, 2928, 1718, 1636, 1508, 1449, 1248, 1117, 758; δ_{H} 1.29 (d, 3H), 1.35 (d, 3H), 2.92 (s, 3H), 3.05 (s, 3H), 4.20 (d, 2H), 4.82 (q, 1H), 5.45 (br d, 1H), 6.95 (br s, 1H), 7.20–7.40 (m, 4H), 7.57 (d, 2H), 7.74 (d, 2H); δ_{C} 18.6, 19.0, 35.8, 47.1, 47.2, 52.2, 67.1, 120.0, 125.2, 127.1, 127.2; m/z (FAB+) 410.2081. ($\text{C}_{23}\text{H}_{28}\text{N}_3\text{O}_4$ requires 410.2080.)

Fmoc-L-Ala-D-Ala-DMA

Mp: 145 °C. $[\alpha]_{\text{D}}^{25}$: +11.4. $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3410, 1718, 1636, 1521, 1449, 1248, 1118, 740; δ_{H} 1.29 (d, 3H), 1.35 (d, 3H),

2.91 (s, 3H), 3.00 (s, 3H), 4.15 (q, 1H), 4.18 (m, 2H), 4.32 (d, 2H), 5.40 (br d, 1H), 6.95 (br d, 1H), 7.20–7.39 (m, 4H), 7.37 (d, 2H), 7.57 (d, 2H); δ_{C} 18.5, 19.2, 35.8, 36.9, 45.4, 47.1, 52.1, 67.1, 120.0, 125.1, 127.0, 127.7; m/z (FAB+) 410.2075. ($\text{C}_{23}\text{H}_{28}\text{N}_3\text{O}_4$ requires 410.2080.)

Fmoc-L-Phe-L-Phe-DMA

Mp: 116–117 °C. $[\alpha]_{\text{D}}^{25}$: +1.2. (Found: C, 71.80; H, 6.45; N, 7.23. $\text{C}_{35}\text{H}_{35}\text{N}_3\text{O}_4 \cdot 1.25 \text{ H}_2\text{O}$ requires: C, 71.53; H, 6.43; N, 7.15%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3280, 3063, 2926, 1720, 1631, 1537, 1449, 1252, 1043, 740, 700; δ_{H} 2.59 (s, 3H), 2.86 (s, 3H), 2.99 (d, 2H), 3.09 (d, 2H), 4.09–4.30 (m, 3H), 4.45 (q, 1H), 4.62 (br d, 1H), 5.12 (q, 1H), 5.65 (br s, 1H), 7.18–7.44 (m, 14H), 7.55 (t, 2H), 7.77 (d, 2H); δ_{C} 35.5, 36.8, 38.6, 39.7, 47.1, 50.3, 55.9, 67.0, 112.0, 125.1, 127.0, 127.7, 128.4, 128.6, 136.1, 136.3, 141.3, 143.8, 170.1, 170.6.

Fmoc-L-Phe-D-Phe-DMA

Mp: 126–129 °C. $[\alpha]_{\text{D}}^{25}$: –18.2. (Found: C, 72.52; H, 6.52; N, 7.32. $\text{C}_{35}\text{H}_{35}\text{N}_3\text{O}_4$ requires: C, 72.77; H, 6.11; N, 7.27%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3279, 3062, 2922, 1720, 1633, 1496, 1450, 1250, 1046, 906, 737, 700; δ_{H} 2.57 (s, 3H), 2.82 (s, 3H), 3.08 (d, 4H), 4.17–4.43 (m, 3H), 4.53 (q, 1H), 5.07 (q, 1H), 5.52 (br d, 1H), 6.94 (br s, 1H), 7.15–7.43 (m, 14H), 7.53 (d, 2H), 7.65 (m, 2H); δ_{C} 35.6, 36.8, 39.0, 39.6, 47.1, 50.1, 56.0, 67.0, 112.0, 125.1, 127.1, 127.7, 128.5, 128.7, 129.4, 136.0, 136.3, 141.2, 143.8, 170.1, 170.7.

Fmoc-L-Val-LD-Val-OMe (diastereomeric mixture)

Mp: 138–140 °C. $[\alpha]_{\text{D}}^{25}$: +33.2. (Found: C, 68.19; H, 7.52; N, 8.82. $\text{C}_{26}\text{H}_{32}\text{N}_2\text{O}_5 \cdot 0.5 \text{ H}_2\text{O}$ requires: C, 68.33; H, 7.64; N, 8.85%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3373, 2964, 1717, 1542, 1451, 1218, 1100, 1027; δ_{H} 0.88 (d, 3H), 0.98 (d, 3H), 1.20 (d, 3H), 1.26 (d, 3H), 2.18 (m, 1H), 2.46 (m, 1H), 3.74 (s, 3H), 3.75 (s, 3H), 4.25 (m, 1H), 4.33 (m, 1H), 4.53 (d, 2H), 4.96 (t, 1H), 5.53 (d, 1H), 7.40 (m, 4H), 7.60 (d, 2H), 7.77 (d, 2H), 8.08 (d, 1H); δ_{C} 17.8, 18.9, 19.0, 19.3, 31.2, 47.1, 52.1, 52.2, 57.1, 68.1, 120.7, 120.9, 121.0, 121.2, 122.0, 126.1, 128.1, 129.0, 130.0, 142.3, 145.0, 172.2, 172.3, 173.2. HPLC (*cf* below): rt, 24.67 and 25.10 min; λ_{max} (eluent)/nm 220.0, 270.5, 292.2, 302.0.

Fmoc-L-Val-L-Val-OMe

Was isolated as a yellow oil without further purification. $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3372, 2931, 1713, 1537, 1450, 1216, 1101, 1025; δ_{H} 0.89 (d, 3H), 0.91 (d, 3H), 0.94 (d, 3H), 0.98 (d, 3H), 2.18 (m, 2H), 3.74 (s, 3H), 4.23 (m, 1H), 4.43 (m, 1H), 4.57 (d, 2H), 4.94 (t, 1H), 5.51 (br s, 1H), 7.40 (m, 4H), 7.62 (d, 2H), 7.78 (d, 2H), 8.05 (d, 1H). HPLC: rt, 24.67 min; λ_{max} (eluent)/nm 220.0, 270.5, 292.2, 302.0.

Fmoc-L-Ala-L-Ala-OMe and Fmoc-L-Ala-D-Ala-OMe

Were synthesised by the above general procedure and showed characterisation data consistent with that reported in the literature.²⁷

Suc-L-Phe-L-Phe-DMA

Mp: 145–148 °C. $[\alpha]_{\text{D}}^{25}$: –0.4. (Found: C, 67.13; H, 6.85; N, 10.36. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4$ requires: C, 67.46; H, 6.67; N, 10.26%; $\nu_{\text{max}}/\text{cm}^{-1}$ (selected bands) 3315, 2930, 2855, 1775, 1708, 1622, 1561, 1393, 1255, 1164, 751; δ_{H} 2.51 (d, 4H), 2.62 (s, 3H), 2.87 (s, 3H), 2.90–3.04 (m, 2H), 3.46–3.51 (m, 2H), 4.98 (q, 1H), 5.11 (q, 1H), 7.03 (br s, 1H), 7.12–7.29 (m, 10H); δ_{C} 27.9, 33.7, 35.6, 36.8, 39.4, 50.7, 55.4, 127.0, 127.1, 128.4, 128.6, 128.9, 129.4, 136.0, 136.4, 167.1, 176.7, 186.1.

Suc-L-Phe-LD-Phe-DMA (diastereomeric mixture)

Mp: 156–158 °C. $[\alpha]_{\text{D}}^{25}$: –76.8. (Found: C, 68.10; H, 6.90; N, 10.00. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4 \cdot 0.7 \text{ H}_2\text{O}$ requires: C, 68.17; H, 6.76; N,

9.94%); Distinctive IR bands (see above)/cm⁻¹: 1635, 1533, 1387, 751; distinctive ¹H NMR features, δ : 2.45 (d, 4H), 2.59 (d, 3H), 4.90 (q, 1H), 5.05 (q, 1H), 7.03 (br s, 1H); distinctive ¹³C NMR features, δ : 24.7, 25.4, 36.0, 38.7, 49.6, 53.5, 171.6, 182.4.

Suc-L-Phe-L-Val-DMA

Colourless oil. $[a]_D$: -37.2. ν_{\max} /cm⁻¹ (selected bands) 3315, 2932, 1708, 1648, 1534, 1388, 1153, 747; δ_H 0.85 (d, 3H), 0.93 (d, 3H), 2.09 (m, 1H), 2.58 (br s, 4H), 2.80 (d, 3H), 3.43 (m, 2H), 4.21 (t, 1H), 5.02 (q, 1H), 6.50 (br s, 1H), 6.82 (br s, 1H), 7.16–7.28 (m, 5H); δ_C 17.9, 19.2, 26.2, 27.8, 33.7, 34.8, 55.4, 59.2, 125.2, 127.2, 128.8, 129.2, 136.1, 168.1, 171.3, 176.8, 176.9; m/z (FAB+) 360.1921. (C₁₉H₂₆N₃O₄ requires 360.1923).

Suc-L-Phe-LD-Val-DMA (diastereomeric mixture)

Colourless oil. $[a]_D$: -13.4. Distinctive IR bands (see above)/cm⁻¹: 3326, 1654, 1216, 1163, 751; distinctive ¹H NMR features, δ : 2.73 (d, 4H), 2.80 (d, 3H); distinctive ¹³C NMR features, δ : 18.1, 18.3, 30.6, 34.0, 126.3, 126.7, 137.0, 182.4; m/z (FAB+) 360.1920. (C₁₉H₂₆N₃O₄ requires 360.1923).

N-Acetyl-L-Pro-L-Phe-DMA

Mp: 59–160 °C. $[a]_D$: -70.4. (Found: C, 64.21; H, 8.02; N, 12.64. C₁₈H₂₅N₃O₅·0.25 H₂O requires: C, 64.36; H, 7.95; N, 12.50%); ν_{\max} /cm⁻¹ (selected bands) 3448, 2926, 1627, 1531, 1443, 1420, 1249, 702; δ_H 1.16–1.22 (m, 2H), 1.86–1.92 (m, 2H), 2.06 (s, 3H), 2.67 (s, 3H), 2.80 (d, 1H), 2.85 (s, 3H), 2.89 (d, 1H), 3.36–3.49 (m, 2H), 4.47 (d, 1H), 5.12 (q, 1H), 7.15–7.27 (m, 5H), 7.71 (dd, 1H); δ_C 22.4, 22.6, 35.6, 36.8, 38.9, 39.2, 49.1, 50.0, 126.8, 128.2, 128.5, 129.2, 129.4, 136.0, 136.4, 170.5, 170.8.

N-Acetyl-L-Pro-LD-Phe-DMA (diastereomeric mixture)

Mp: 162 °C. $[a]_D$: -99.4. (Found: C, 64.23; H, 7.73; N, 12.20. C₁₈H₂₅N₃O₅·0.25 H₂O requires: C, 64.36; H, 7.95; N, 12.50%); Distinctive IR bands (see above)/cm⁻¹: 1633, 1542, 1451; distinctive ¹H NMR features, δ : 1.16–1.79 (m, 2H), 2.09 (s, 3H), 2.79 (s, 3H), 2.85 (s, 3H), 2.89 (d, 1H), 2.97 (dd, 1H), 5.01–5.06 (m, 1H), 7.37 (dd, 1H); distinctive ¹³C NMR features, δ : 39.4, 48.1, 50.0, 59.7.

Acknowledgements

We are grateful for facilities and helpful comments provided by Dr P J Murphy and Dr M Fletcher of the University of Wales, Bangor. We also thank the EPSRC Mass Spectrometry Service at the University of Wales, Swansea for low and high resolution mass spectra, and Jane Davies and Glyn O'Connell for other analytical services.

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