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SEGMENT COUPLING IN PEPTIDE SYNTHESIS—II

A SIMPLE PREDICTIVE EQUATION CORRELATING RACEMIZATION AND PRIMARY STRUCTURE

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Abstract—A predictive equation based on extrathermodynamic assumptions is proposed, that allows the prediction of the degree of epimerization in tripeptide model reactions of condensation of peptide segments as a function of the primary structure. The experimental validation of this equation is presented in the field of the 125 tripeptides containing alanine, leucine, phenylalanine, valine and isoleucine, the prediction requires 13 epimerization measurements on reference reactions. Sixteen statistically chosen predictions are checked experimentally showing a good fit in spite of the drastic basic assumptions needed to set up the equation.

The condensation of peptide segments is a good strategy for peptide synthesis. The separation of the product from truncated or deleted sequences is considerably facilitated. The main drawback of this method lies in the easy racemization of the carboxylic segment of the C-terminal residue, when this residue is neither glycine, nor proline. This racemization goes mostly through an oxazolone by reaction of the penultimate residue on the terminal activated carboxylate. Considerable efforts have been made to forecast this reaction and its dependence upon the reagents and the coupling conditions. However, very little has been done concerning the influence of the primary sequence in the vicinity of the coupling point on the extent of the racemization.

Concluding his recent review on the racemization in peptide synthesis,[1] Kemp pointed out five "unresolved problems". The last is the "development of a procedure for predicting the chiral purity expected for a coupling reaction carried out with a given pair of peptide fragments".

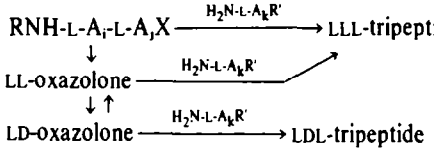
Two steps can be considered for this development: "A first step in developing such a procedure involves determining whether the racemization level can be analyzed in terms of separate contributions from substituents, coupling agent, and conditions. A second step involves determining the degree to which the entire substituent contribution is controlled by the penultimate and COOH-terminal acyl substituents, together with the amine substituent. Implicit in a prediction procedure is an understanding of the scope of 5(4H)-oxazolone induced epimerization at the penultimate site".

The aim of the present paper is to give a general basis for an answer to Kemp's problem, and a partial experimental justification. A preliminary note gave some verifications of a general predictive equation.[2]

The predictive equation

The general reaction scheme of a coupling reaction is often complex; several active species are generally involved, especially when a nucleophilic additive, such as hydroxybenzotriazole is added in order to minimize racemization and other side reactions.

The racemization[3] can be expressed by the ratio of the epimerized product; it is actually the result of the competition between activated species aminolysis and cyclization to a steady quantity of oxazolone; under normal reaction conditions, the oxazolone undergoes epimerization more readily than ring opening, either directly by aminolysis, or indirectly to an epimerized active intermediate.[4] This last possibility is very probable in the presence of a nucleophilic additive. It is necessary to consider a simplified scheme. It has been shown by Goodman that the simplest scheme to which the competition aminolysis-racemization could be reduced, is the following:



Even this scheme does not allow the expression of the epimerization ratio $\rho = \%\ (LDL)/\%(LLL)$ as a simple factorizable function of the various rate constants. Hence we expressed this ratio as the simple ratio of an epimerization to a coupling rate constant. The epimerization ratio may be assumed to depend only on the nature of the amino acids A_i, the participating aminoacid, A_j, the activated amino acid and A_k the amino component.

$$\rho_{ijk} = \frac{k^r_{ijk}}{k^c_{ijk}}$$

Furthermore, we shall assume that k^r_{ijk} is independent of the nature of A_k and that k^c_{ijk} is independent of the nature of A_i. This procedure presents a drastic simplification but leads to conclusions that, with experience, we can compare.

It has been shown by Kemp[5] that a factorization of the coupling rate constant for the coupling of the A_j and A_k residues can be expressed as

$$k^c_{jk} = \frac{k^c_{jo} \cdot k^c_{ok}}{k^c_{oo}} \qquad (1)$$

where the subscript o refers to a standard amino acid. As we assume that the coupling rate depends only on the

nature of the A_j and A_k residues, this expression can be written for a triplet ijk:

$$k^c_{ijk} = k^c_{ojk} = \frac{k^c_{ojo} \cdot k^c_{ook}}{k^c_{ooo}} \quad (2)$$

We shall then assume that this expression can be applied to racemization rate constants, though no supporting experimental evidence is available:

$$k^r_{ij} = \frac{k^r_{io} \cdot k^r_{oj}}{k^r_{oo}} \quad (3)$$

as the epimerization rate is independent of A_k

$$k^r_{ijk} = \frac{k^r_{ioo} \cdot k^r_{ojo}}{k^r_{ooo}} \quad (4)$$

It is possible to derive an expression for ijk, from this. The details of this development are described in Appendix I and the simple predictive equation (5) is given.

$$\rho_{ijk} = \frac{\rho_{ioo} \cdot \rho_{ojo} \cdot \rho_{ook}}{\rho^2_{ooo}} \quad (5)$$

This approach possesses the advantage of *not requiring kinetic experiments but only the measurement of the ratio of the epimer yields.* The equation, if experimentally valid, can be applied to the field of the 18 racemizable coded amino-acids. The knowledge of ρ_{ooo}, ρ_{ioo}, ρ_{ojo} and ρ_{ook} requires $(3 \times 17) + 1 = 52$ reference measurements and provides the prediction of $18^3 - 52 = 5780$ cases, for any case of segment coupling. The experimental validation of this law would justify our simplifying assumptions and would provide synthetic chemists with a very useful tool for segment planning. Indeed, an epimerization index will be available for any peptide link in the chain and in the absence of glycine or proline will allow the selection of the low epimerization bonds rather than the high epimerization bonds. We chose to check the validity of the prediction law in the field of five non-functional amino-acids, alanine, leucine, phenylalanine, valine and isoleucine. Thirteen reference measurements are needed to forecast 112 cases.

RESULTS AND DISCUSSION

Obviously, eqn (5) is applicable to a set of data obtained under the same experimental conditions. It is necessary to fix these conditions: nature of the coupling reagent, solvent, base, ions, concentration of the reagent, and temperature. Our model reaction consists of the coupling of Boc dipeptides by our BOP reagent, which involves the formation of active esters of hydroxy benzotriazole.[6]

$$\text{Boc-LA}_i\text{-LA}_j\text{-OH} + \text{H}_2\text{-N-LA}_k\text{-OMe} \xrightarrow[\text{Base}]{\text{BOP}} \text{Boc-LA}_i\text{-LA}_j\text{-LA}_k\text{OMe}$$

Alanine was chosen as a standard amino acid in each position i, j and k. Reference tripeptides for the application of eqn (6) are reported in the first row of Table 2. Proline and glycine were excluded from these models as their presence in position i or k was unrealistic from the synthesis chemist's point of view; racemization studies on large sets of tripeptides have been recently undertaken by other authors, but the structural variations were often limited by the performances of the diastereoisomeric mixture analysis. Ion exchange chromatography generally requires the presence of a polar residue such as lysine;[7] hplc left many diastereoisomeric pairs unresolved.[8]

^{1}H NMR at 250 MHz was chosen to carry out this analysis. Indeed the estimation of the diastereoisomeric contents on the basis of the integration of methyl ester signals was achieved in 28 out of the 29

Table 1. Chemical shifts (Hz at 250 MHz) of methylester protons of diastereoisomeric tripeptides

$BocA_iA_jA_kOMe$	ν_{LLL}	ν_{LDL}	$\Delta\nu$	$BocA_iA_jA_kOMe$	ν_{LLL}	ν_{LDL}	$\Delta\nu$
A A A	936	930	6	F A V	935	926	9
L A A	935.5	930	5.5	F A I	933	924	9
F A A	933.5	924.5	9	F A F	917	912	5
V A A	938	930	8	A L L	929.5	922	7.5
I A A	936	931	5	A F I	918.2	910.5	7.7
A L A	933.5	928.5	5	A I V	933.5	926.5	7
A F A	925	920	5	V F L	918.5	912.5	6
A V A	934.5	930	4.5	V V L	928	920	8
A I A	933	930	3	V I V	932.5	925	7.5
A A L	932	926	6	I L A	930.8	926.3	4.5
A A F	923.5	922	1.5	I V A	932.5	928	4.5
A A V	937.5	930	7.5	L V I	932	926	6
A A I	934.5	928	6.5	L I A	933	930	3
A L I	932.5	923.5	9	L V V	933.5	924.5	9
F A L	930.5	921.5	9				

Table 2. Epimerization data for reference reactions

Boc-$A_iA_jA_k$OMe	ρ_{ijk} (%)
A A A	6.7
L A A	6.1
F A A	5.4
V A A	8.7
I A A	8.1
A L A	6.8
A F A	8.9
A V A	17.2
A I A	19.0
A A L	5.7
A A F	6.9
A A V	9.3
A A I	6.5

diastereoisomeric pairs observed. The separation between the two signals was generally greater than 3 Hz at 250 MHz. The signals for LLL isomers were always located at lower field than the signals for LDL isomers. Figure 1 shows a typical pattern of this situation. Table 1 summarizes the chemical shifts of the methyl ester signal for 29 diastereoisomeric pairs.

The presence of an aromatic ring was not necessary for diastereoisomeric discrimination.[9] Furthermore, if an aromatic ring in position i or j gave a positive contribution to the chemical shift difference, a phenylalanine in position k, acting as a magnetic screen on the methyl ester, gave a negative contribution to this difference. Hence, the signals of Boc-Ala-Ala-Phe-OMe diastereoisomers differed by only 1.5 Hz, but could be fairly easily separated by adding 10% of EuFod shift reagent[10] and recording the spectrum at 45°. This method is then universal; however it was limited to the measurement of epimerization ratios higher than 3%. The coupling conditions for the "2 + 1" model reactions were chosen in order to give epimerization ratios in the range of 3 to 25%. For this purpose, reagents were used in equimolar proportions at 0.1M, in order to slow down the coupling rate.[11] Dimethylformamide was chosen as solvent, triethylamine as base; amino acid methyl esters were introduced as chlorhydrates. Pure LLL and LDL stereoisomers of each tripeptide were synthetized stepwise by non racemizing procedures, in order to provide reference compounds. Epimerization ratios for the 13 reference reactions are reported in Table 2. From these values it was possible to get predicting values for the 112 other possible reactions involving the five non functional amino acids.

The experimental validation of eqn (5) must be supported by a statistically fair sample of this population. Sixteen tripeptide forming reactions were chosen for which epimerization ratios were measured under the same conditions. This data is reported in Table 3 and compared with the corresponding data obtained from equation (5) (Fig. 2). The choice of the items belonging to

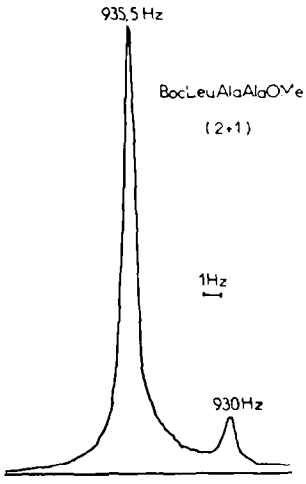


Fig. 1. ^{1}H NMR spectrum of the methylester part of a typical diastereoisomeric mixture.

the test sample is important. The breakdown of the sample population into epimerization ratio groups must reflect the breakdown of the total population.

This breakdown was made as reported in Table 4. Furthermore, the test reactions involved every five amino acids in every three positions i, j and k. The sample size was determined from statistical considerations.

The (pessimistic) hypothesis of a normal distribution law of the measurement errors allows the correlation between the certainty of the proportion of satisfied forecasts from eqn (5), the size of the sample and the precision required for the forecast (Appendix 2). For the sample of 16 items, this correlation results in the data reported in Table 5. For instance, it appears from this data that there is a 95% chance for equation (5) to give 80% correct forecasts with a precision of 20%. If a precision of 30% is achieved, every forecast will be found to be true. Hence we are confident that Kemp's question is, at least partly, answered. A prediction law exists that correlates highly significantly the epimerization in fragment coupling with primary structure; we are now working on the extension of its application to the wider field of functional amino acids. Some major problems remain for which we have not yet any experimental answer: what happens when we change experimental conditions (coupling reagent, base, or salt). Obviously ρ_{ijk} can vary by a large amount; however we feel that the epimerization indexes defined by eqn (6)

$$r_{ijk} = \frac{\rho_{ijk}}{\rho_{ooo}} = r_{ioo} \times r_{ojo} \times r_{ook} \qquad (6)$$

could be more independent of experimental conditions.

In this case these epimerization indexes would be helpful tools for synthetic chemists; regardless of the relatively low precision, the possibility of simply evaluating an epimerization index for each peptide bond in a chain will provide supplementary information for strategic choices.

Furthermore, we feel that our method for setting a provision law depending on more than two parameters is rather general and could be used in other field of organic chemistry.

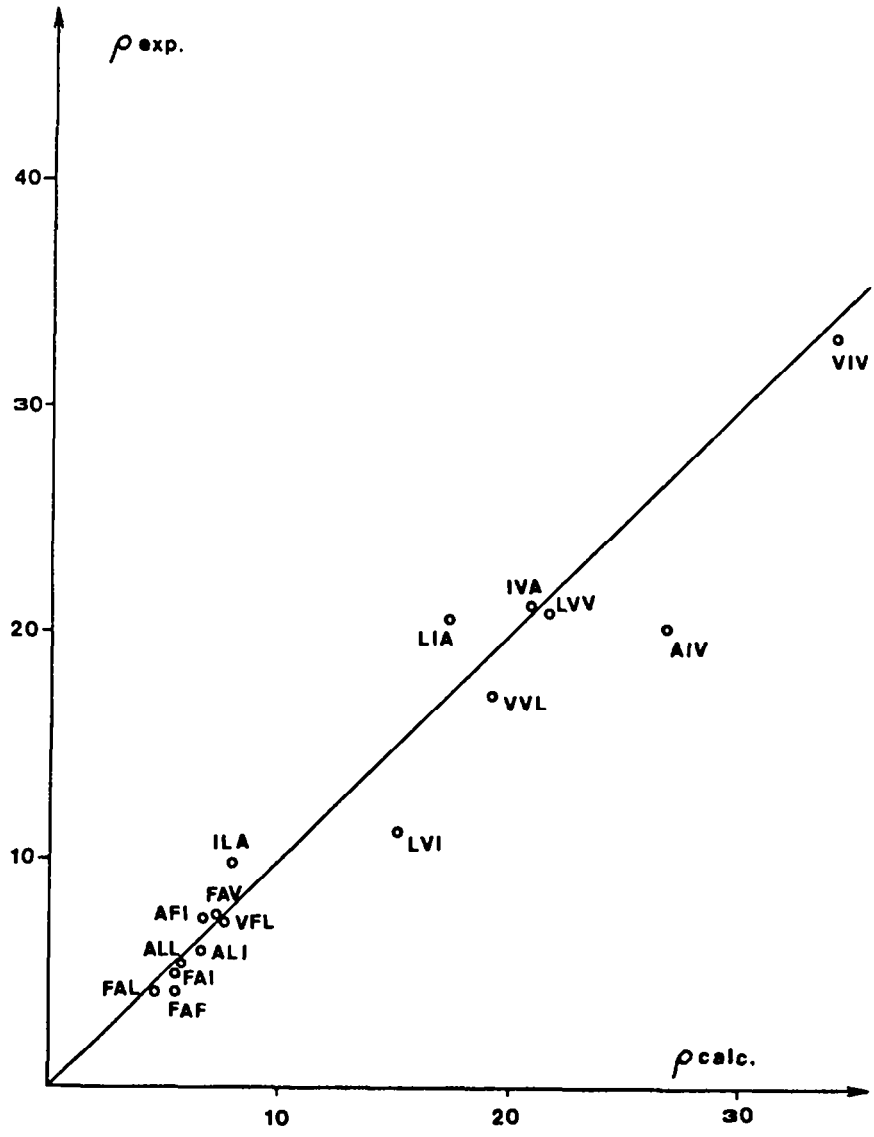


Fig. 2. Experimental *vs* calculated epimerization for test sample. (The straight line is the first diagonal, not the very near regression line).

Appendix 1

From eqns (2) and (3) one can write the epimerization ratio

$$\rho_{ijk} = \frac{k^r_{ijk}}{k^c_{ijk}} = \frac{k^r_{ioo} \times k^r_{ojo}}{k^r_{ooo}} \times \frac{k^c_{ooo}}{k^c_{ojo} \times k^c_{ook}} \quad (6)$$

that is

$$\rho_{ijk} = \frac{k^r_{ioo}}{k^c_{ook}} \times \rho_{ojo} \times \frac{1}{\rho_{ooo}}. \quad (7)$$

An expression for k^r_{ioo}/k^c_{ook} is easily found:

$$\frac{k^r_{ioo}}{k^c_{ook}} = k^r_{ioo} \times \frac{k^c_{ioo}}{k^c_{ioo}} \times \frac{k^r_{ook}}{k^r_{ook}} \times \frac{1}{k^c_{ook}} \quad (8)$$

$$= \rho_{ioo} \times \rho_{ook} \times \frac{k^c_{ioo}}{k^r_{ook}}. \quad (9)$$

As from initial assumption $k^c_{ioo} = k^c_{ooo}$ and $k^r_{ook} = k^r_{ooo}$, the predictive equation follows:

$$\rho_{ijk} = \frac{\rho_{ioo} \times \rho_{ojo} \times \rho_{ook}}{\rho^2_{ooo}} \quad (5)$$

Appendix 2

Assuming that the proportions ρ_ϵ of satisfied predictions with a precision better than ϵ are normally distributed, the probability P that the proportion of the whole population of prediction, ρ_ϵ, differ from the measured proportion on the sample, $\hat{\rho}_\epsilon$, can be compared to a given risk α, through the classical expression of the statistical Normal Law:

$$P(|\rho_\epsilon - \hat{\rho}_\epsilon| > \eta(\epsilon, \alpha)) < \alpha \quad (10)$$

where $\eta(\epsilon, \alpha)$, the uncertainty on the proportion of

Table 3. Epimerization data for test tripeptides

Boc-$A_1A_jA_K$OMe	Calc. %	Found %
A L I	8.4	8.3
F A L	4.8	4.5
A L L	5.8	5.5
F A V	7.4	7.5
I L A	7.9	10
A F I	6.6	7.5
F A F	5.5	4.5
V F L	7.6	7.3
F A I	5.2	5.3
L V I	15	11.4
V V L	18.9	17.2
L I A	17.2	20.6
L V V	21.6	20.6
I V A	20.7	20.8
A I V	26.3	19.9
V I V	34	32.5

Table 4. Compared breakdowns of predicted epimerization in the total population and in the test population

Racemization range	Total population breakdown % (number of items)	Test population breakdown % (number of items)
0-5	2.6 (3)	6.2 (1)
5-10	49.1 (55)	50 (8)
10-15	12.5 (14)	6.2 (1)
15-20	15.1 (17)	12.5 (2)
20-25	15.1 (17)	12.5 (2)
25-30	2.6 (3)	6.2 (1)
30-35	2.6 (3)	6.2 (1)

satisfied predictions is:

$$\eta(\epsilon, \alpha) = t(\alpha)\sqrt{\left(\frac{\hat{\rho}_\epsilon(1-\hat{\rho}_\epsilon)}{n}\right)} \quad (11)$$

where n is the sample size and $t(\alpha)$ is defined as:

$$P(0 \leq T < t(\alpha)) = \frac{1-\alpha}{2} \quad (12)$$

where T is the normal, centered, reduced variable.

One can write the estimation of ρ_ϵ under the more popular form:

$$\rho_\epsilon = \hat{\rho}_\epsilon \pm \eta(\epsilon, \alpha) \quad (13)$$

From eqn (13) it can be deduced that the uncertainty $\eta(\epsilon, \alpha)$ could be smaller than a given value η_0 if

$$n > \frac{t^2(\alpha)}{\eta_0^2}\hat{\rho}_\epsilon(1-\hat{\rho}_\epsilon) \quad (14)$$

We had no *a priori* estimation of $\hat{\rho}_\epsilon$ before any measurement; however, the maximal value of $\hat{\rho}_\epsilon(1-\hat{\rho}_\epsilon)$

Table 5. Statistical amount of satisfied predictions as a function of the required precision

ϵ (a)	$\hat{\rho}_\epsilon$ (b)	$\eta_{(\epsilon,\alpha)}$ (c)
5	55	12
10	60	12
15	70	10
20	80	8
25	90	4
30	100	-

(a) Precision of the prediction %

(b) Proportion of fit in the test sample %

(c) Uncertainty on the proportion of satisfied prediction %. α = 5 % (see eq. (13) Appendix II).

is 0.25 for $\hat{\rho}_\epsilon = 50\%$. At the risk $\alpha = 5\%$, $t(\alpha) = 1.96$ and the sample size can be safely chosen as

$$n > \frac{1}{\eta_o^2} \qquad (15)$$

An uncertainty below 10% would need more than 100 experiments; this evaluation is obviously pessimistic and even meaningless on a population of 112 individuals where an other distribution law, such as binomial law, would be considered.

The value n = 16 was chosen, for which uncertainty below 25% could be secured. Above this value, the rentability of experimental work, expressed by $d\eta/dn$ drops below 0.1%.

In Table 5 are reported the proportions of fit $\hat{\rho}_\epsilon$ as a function of the relative precision ϵ. The range of ρ_ϵ values for the total population at the risk of 5% can be determined from eqn (13).

EXPERIMENTAL

Boc-amino-acids and amino-acid methyl esters were purchased commercially. BOP reagent was synthetized according to a published method.[6c] Acetonitrile was distilled over phosphorus pentoxide and stored over molecular sieves 3 Å; dimethylformamide was distilled over calcium hydride; triethylamine was first distilled over potassium hydroxide then over ninhydrin. tlc analysis were performed on Kieselgel G precoated plates. Preparative chromatographic column were filled with Kieselgel 0.05–0.2 mm; the eluent was hexane–ethyl acetate (40–60). Optical rotations were measured with a Perkin–Elmer 451 apparatus in a 10 cm cell in ethanol (c = 1). Melting points were determined on a Koffler plate and were not corrected. All products were recrystallized in diethylether.

High resolution ^{1}H NMR spectra were plotted on a CAMECA 250 spectrometer in the C.W. mode and $CDCl_3$ was used as solvent with TMS as internal standard. Elemental analyses were obtained from the "Service Central de Microanalyse du CNRS".

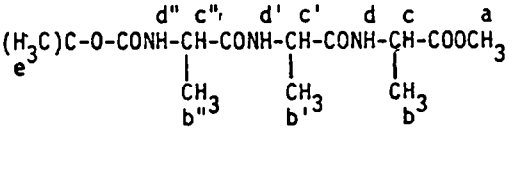
Stepwise synthesis of the reference peptides

The following standard procedure was followed for the synthesis of the 26 reference tripeptides.

Boc-L-alanyl-L-alanyl-L-alanine methyl ester

An acetonitrile solution (5 ml) of 189 mg of Boc-L-alanine (1 mmol), 139.5 mg of methylalaninate methyl ester hydrochloride (1 mmol) and 422 mg of BOP (1 mmol) were treated with 202 mg (2 mmol) of triethylamine under stirring for 2 hr. The reaction mixture was then diluted with water and extracted with ethyl acetate. The organic phase was washed with 3N hydrochloric acid solution, saturated sodium hydrogen carbonate and saturated sodium chloride. It was then dried over magnesium sulfate, filtered and concentrated by rotatory evaporation. 265 mg of Boc-L-Ala-L-Ala-OMe were then obtained whose purity was checked by tlc before deblocking.

The intermediate product was treated by a 1-1 mixture of trifluoroacetic acid and methylene chloride (3.4 ml). The reaction was monitored by tlc. After 0.5 hr the solvents were evaporated under vacuum. The remaining acid was thoroughly removed by 5 coevaporations with hexane. $CF_3CO_2^-$, H_2N^+-L-Ala-L-Ala-OMe was obtained as a yellow oil. That oil was dissolved in 5 ml of acetonitrile and coupled with 189 mg of Boc-L-Alanine (1 mmol) as previously described. The same workup gave the crude product as a pale yellow solid that was chromatographed. 310 mg of pure product were obtained (90%). m.p. 190°; $[\alpha]_D^{20} = -69.3$; NMR: δ 1.36, 1.39, 1.40 (3d, $J_{bc} = J_{b'c'} = J_{b''c''} = 7.5$ Hz, 9 H, 3 H_b + 3 $H_{b'}$ + 3 $H_{b''}$); 1.44 (s, 9 H, H_e); 3.74 (s, 3 H, H_a); 4.19 (br.m., 1 H, $H_{c''}$); 4.54 (m, 2 H, $H_c + H_{c'}$); 5.13 (d, $J_{d'c''} = 7.5$ Hz, 1 H, $H_{d''}$); 6.87, 6.93 (2 d, $J_{dc} = J_{d'c'} = 7.5$ Hz, 2 H, $H_d + H_{d'}$). Calc. for $C_{15}H_{27}O_6N_3$: C, 52.16; H, 7.87; N, 12.16; Found: C, 51.84; H, 7.76; N, 12.14%.

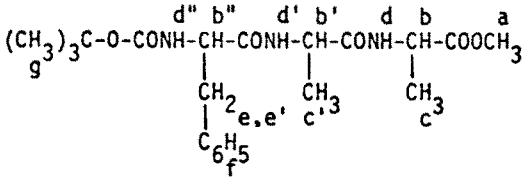


Boc-L-alanyl-D-alanyl-L-alanine methyl ester

M.p. 102°; $[\alpha]_D^{20} = -2.6$; NMR: δ 1.36, 1.38, 1.40 (3 d, $J_{bc} = J_{b'c'} = J_{b''c''} = 7.5$ Hz, 9 H, 3 H_b + 3 $H_{b'}$ + 3 $H_{b''}$); 1.44 (s, 9 H, H_e); 3.72 (s, 3 H, H_a); 4.18 (br.m., 1 H, $H_{c''}$); 4.57 (m, 2 H, $H_b + H_b$); 5.44 (m, 1 H, $H_{d''}$); 7.19, 7.34 (2 d, $J_{dc} = J_{d'c'} = 7.5$ Hz, 2 H, $H_d + H_{d'}$). Calc. for $C_{15}H_{27}O_6H_3$: C, 52.16; H, 7.87; N, 12.16; Found: C, 52.00; H, 7.77; N, 11.98%.

Boc-L-*phenylalanyl*-L-*alanyl*-L-*alanine methyl ester*

M.p. 163°; $[\alpha]_D^{20} = -30.8$; NMR: δ1.34, 1.39 (2 d, J = 7 Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.38 (s, 9 H_g); 3.08 (dd, $J_{eb'} = 7.5$ Hz, $J_{ee'} = 14$ Hz, 1 H, H_e); 3.10 (dd, $J_{e'b'} = 6$ Hz, $J_{ee'} = 14$ Hz, 1 H, $H_{e'}$); 3.73 (s, 3 H, H_a); 4.34–4.64 (br.m., 3 H, H_b + $H_{b'}$ + $H_{b''}$); 5.27 (d, $J_{d''b''} = 8$ Hz, 1 H, $H_{d''}$); 6.78 (m, 2 H, H_d + $H_{d'}$); 7.14–7.32 (m, 5 H, H_f). Calc. for $C_{21}H_{31}O_6N_3$: C, 59.84; H, 7.41; N, 9.96; Found: C, 59.49; H, 7.22; N, 9.72%.

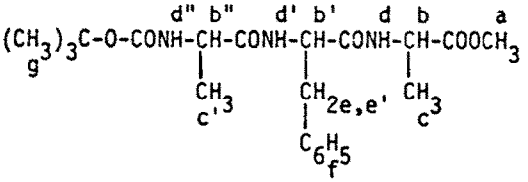


Boc-L-*phenylalanyl*-D-*alanyl*-L-*alanine methyl ester*

M.p. 152°; $[\alpha]_D^{20} = -20.9$; NMR: δ 1.18 (d, $J_{c'b'} = 7$ Hz, 3 H, $H_{c'}$); 1.40 (d, $J_{cb} = 7$ Hz, 3 H, H_c); 1.38 (s, 9 H, H_g); 3.00 (dd, $J_{eb'} = 7.5$ Hz, $J_{ee'} = 14$ Hz, 1 H, H_e); 3.06 (dd, $J_{e'b'} = 7.5$ Hz, $J_{ee'} = 14$ Hz, 1 H, $H_{e'}$); 3.69 (s, 3 H, H_a); 4.36 (m, 1 H, $H_{b'}$); 4.53 (m, 2 H, H_b + $H_{b'}$); 5.54 (d, $J_{d'b'} = 7$ Hz, 1 H, $H_{d'}$); 6.89 (d, J = 8 Hz, 1 H); 7.45 (d, J = 7 Hz, 1 H), $H_{d'}$ + H_d; 7.14–7.32 (m, 5 H_f). Calc. for $C_{21}H_{31}O_6N_3$: C, 59.84; H, 7.41; N, 9.96; Found: C, 59.69; H, 7.06; N, 10.17%.

Boc-L-*ananyl*-L-*phenylalanyl*-L-*alanine methyl ester*

M.p. 168°; $[\alpha]_D^{20} = -29.9$; NMR: δ 1.29, 1.33 (2 d, $J_{cb} = J_{c'b'} = 7$ Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.40 (s, 9 H, H_g); 3.08 (dd, $J_{eb'} = 6.5$ Hz, $J_{ee'} = 13.5$ Hz, 1 H, H_e); 3.11 (dd, $J_{e'b'} = 6$ Hz, $J_{ee'} = 13.5$ Hz, 1 H, $H_{e'}$); 3.70 (s, 3 H, H_a); 4.12 (br.m., $H_{b'}$); 4.49 (dq, $J_{bc} = J_{bd} = 7$ Hz, 1 H, H_b); 4.71 (ddd, $J_{b'e} = 6.5$ Hz, $J_{b'e'} = 6$ Hz, $J_{b'd'} = 8$ Hz, 1 H, $H_{b'}$); 5.02 (d, $J_{d''b''} = 6.5$ Hz, 1 H, $H_{d''}$); 6.74 (m, 1 H, H_d); 6.80 (d, $J_{d'b'} = 8$ Hz, 1 H, $H_{d'}$); 7.14–7.36 (m, 5 H, H_g). Calc. for $C_{21}H_{31}O_6N_3$: C, 59.84; H, 7.41; N, 9.96; Found: C, 59.62; H, 7.37; N, 9.68%.

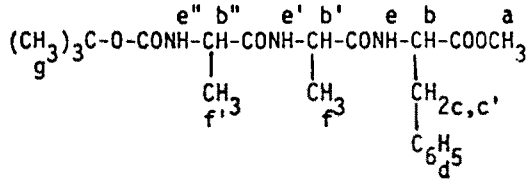


Boc-L-*alanyl*-D-*phenylalanyl*-L-*alanine methyl ester*

M.p. 131°; $[\alpha]_D^{20} = -14.0$; NMR: δ 1.24, 1.26 (2 d, $J_{cb} = J_{c'b'} = 7$ Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.52 (s, 9 H, H_g); 3.03 (dd, $J_{eb'} = 6.5$ Hz, $J_{ee'} = 13.5$ Hz, 1 H, H_e); 3.16 (dd, $J_{e'b'} = 6$ Hz, $J_{ee'} = 13.5$ Hz, 1 H, $H_{e'}$); 3.68 (s, 3 H, H_a); 4.08 (dq, $J_{b'd'} = J_{b'c'} = 7$ Hz, 1 H, $H_{b'}$); 4.49 (dq, $J_{bc} = J_{bd} = 7$ Hz, 1 H, H_b); 4.74 (m, 1 H, $H_{b'}$); 5.14 (m, 1 H, $H_{d''}$); 6.74 (m, 1 H, H_d); 6.88 (d, $J_{d'b'} = 7.5$ Hz, 1 H, $H_{d'}$); 7.16–7.36 (m, 5 H, H_f). Calc. for $C_{21}H_{31}O_6N_3$: C, 59.84; H, 7.41; N, 9.96; Found: C, 59.87; H, 7.26; N, 9.97%.

Boc-L-*alanyl*-L-*alanyl*-L-*phenylalanine methyl ester*

M.p. 157°; $[\alpha]_D^{20} = -36.3$; NMR: δ 1.29, 1.32 (2 d, $J_{fb'} = J_{f'b'} = 7$ Hz, 6 H, 3 H_f + 3 $H_{f'}$); 1.44 (s, 9 H, H_g); 3.08 (dd, $J_{cb} = 6.5$ Hz, $J_{cc'} = 14$ Hz, 1 H, H_c); 3.11 (dd, $J_{c'b} = 6$ Hz, $J_{cc'} = 14$ Hz, 1 H, $H_{c'}$); 3.69 (s, 3 H, H_a); 4.16 (br.m., 1 H, $H_{b'}$); 4.49 (dq, $J_{b'c'} = J_{b'f} = 7$ Hz, 1 H, $H_{b'}$); 4.82 (ddd, $J_{bc} = 6.5$ Hz, $J_{bc'} = 6$ Hz, $J_{be} = 8$ Hz, 1 H, H_b); 5.24 (d, $J_{e'b'} = 7.5$ Hz, 1 H, $H_{e'}$); 6.91, 6.95 (2 d, $J_{eb} = 8$ Hz, $J_{e'b'} = 7.5$ Hz, 2 H, H_e + $H_{e'}$); 7.12–7.45 (m, 5 H, H_d). Calc. for $C_{21}H_{31}O_6N_3$: C, 59.84; H, 7.41; N, 9.96; Found: C, 59.81; H, 7.45; N, 9.76%.

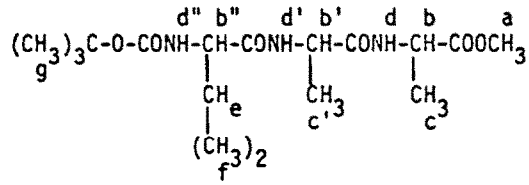


Boc-L-*alanyl*-D-*alanyl*-L-*phenylalanine methyl ester*

M.p. 152°; $[\alpha]_D^{20} = +8.7$; NMR: δ 1.25, 1.31 (2 d, $J_{fb'} = J_{f'b'} = 7$ Hz, 6 H, 3 H_f + 3 $H_{f'}$); 1.43 (s, 9 H, H_g); 3.02 (dd, $J_{cb} = 7.5$ Hz, $J_{cc'} = 13.5$ Hz, 1 H, H_c); 3.16 (dd, $J_{c'b} = 6$ Hz, $J_{cc'} = 13.5$ Hz, 1 H, $H_{c'}$); 3.68 (s, 3 H, H_a); 4.21 (br.m., 1 H, $H_{b'}$); 4.51 (m, H_b); 4.82 (ddd, $J_{bc} = 7.5$ Hz, $J_{bc'} = 6$ Hz, $J_{be} = 7.5$ Hz, 1 H, H_b); 5.24 (d, $J_{e'b'} = 7.5$ Hz, 1 H, $H_{e'}$); 6.97 (d, $J_{eb} = 7.5$ Hz, 1 H, H_e); 7.08 (d, $J_{e'b'} = 8$ Hz, 1 H, $H_{e'}$); 7.12–7.32 (m, 5 H, H_d). Calc. for $C_{21}H_{31}O_6N_3$: C, 59.84; H, 7.41; N, 9.96; Found: C, 59.97; H, 7.60; N, 10.00%.

Boc-L-*valyl*-L-*alanyl*-L-*alanine methyl ester*

M.p. 150°; $[\alpha]_D^{20} = -6.17$; NMR: δ 0.91, 0.95 (2 d, $J_{fe} = 7$ Hz, 6 H, H_f); 1.38, 1.39 (2 d, $J_{cb} = J_{c'b'} = 7$ Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.44 (s, 9 H, H_g); 2.10 (m, 1 H, H_e); 3.74 (s, 3 H, H_a); 4.05 (dd, $J_{b'd'} = 7.5$ Hz, $J_{b'e} = 7$ Hz, 1 H, $H_{b'}$); 4.55, 4.65 (2 dq, $J_{bc} = J_{b'c'} = J_{b'd'} = 7$ Hz, 2 H, H_b + $H_{b'}$); 5.54 (d, $J_{d'b'} = 7.5$ Hz, 1 H, $H_{d'}$); 7.24, 7.39 (2 d, $J_{d'b'} = J_{db} = 7$ Hz, 2 H, H_d + $H_{d'}$). Calc. for $C_{17}H_{31}O_6N_3$: C, 54.69; H, 8.57; N, 11.26; Found: C, 54.77; H, 8.26; N, 11.18%.

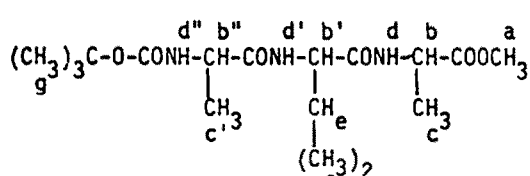


Boc-L-*valyl*-D-*alanyl*-L-*alanine methyl ester*

M.p. 140°; $[\alpha]_D^{20} = -0.1$; NMR: δ 0.93, 0.96 (2 d, $J_{fe} = 7$ Hz, 6 H, H_f); 1.39, 1.42 (2 d, $J_{cb} = J_{c'b'} = 7$ Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.43 (s, 9 H, H_g); 2.11 (m, 1 H, H_e); 3.72 (s, 3 H, H_a); 3.98 (m, 1 H, $H_{b'}$); 4.16, 4.27 (2 dq, $J_{bc} = J_{bd} = J_{b'c'} = J_{b'd'} = 7$ Hz, 2 H, H_b + $H_{b'}$); 5.51 (d, $J_{d'b'} = 8$ Hz, 1 H, $H_{d'}$); 7.23, 7.50 (2 d, $J_{d'b'} = J_{db} = 7$ Hz, 2 H, H_d + $H_{d'}$). Calc. for $C_{17}H_{31}O_6N_3$: C, 54.69; H, 8.57; N, 11.26; Found: C, 54.60; H, 8.39; N, 11.13%.

Boc-L-*alanyl*-L-*valyl*-L-*alanine methyl ester*

M.p. 173°; $[\alpha]_D^{20} = -56.8$; NMR: δ 0.93, 0.97 (2 d, $J_{fe} = 7$ Hz, 6 H, H_f); 1.38, 1.41 (2 d, $J_{cb} = J_{c'b'} = 7$ Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.44 (s, 9 H, H_g); 2.17 (br.m., 1 H, H_e); 3.74 (s, 3 H, H_a); 4.22 (br.m., 1 H, $H_{b'}$); 4.32 (dd, $J_{b'd'} = 8.5$ Hz, $J_{b'e} = 7$ Hz, 1 H, $H_{b'}$); 4.56 (dq, $J_{bd} = J_{bc} = 7$ Hz, 1 H, H_b); 5.23 (d, $J_{d'b'} = 7$ Hz, 1 H, $H_{d'}$); 6.94 (m, 2 H, H_d + $H_{d'}$). Calc. for $C_{17}H_{31}O_6N_3$: C, 54.69; H, 8.57; N, 11.26; Found: C, 54.20; H, 8.53; N, 9.97%.



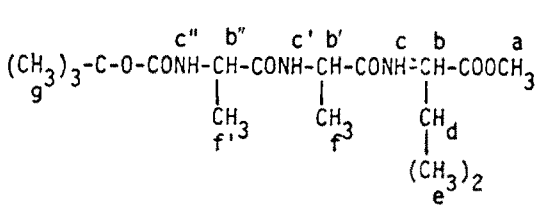
Boc-L-*alanyl*-D-*valyl*-L-*alanine methyl ester*

M.p. 127°; $[\alpha]_D^{20} = -12.5$; NMR: δ 0.93, 0.96 (2 d, $J_{fe} = 6.5$ Hz, 6 H, H_f); 1.38, 1.41 (2 d, $J_{cb} = J_{c'b'} = 7.5$ Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.43

(s, 9 H, H_g); 2.20 (br.m., 1 H, H_e); 3.72 (s, 3 H, H_a); 4.25 (m, 1 H, $H_{b'}$); 4.39 (dd, $J_{b'd'} = 8.5$ Hz, $J_{b'e} = 7$ Hz, 1 H, $H_{b'}$); 4.56 (dq, $J_{bd} = J_{bc} = 7$ Hz, 1 H, H_b); 5.51 (m, 1 H, $H_{d'}$); 7.16 (d, $J_{d'b'} = 8.5$ Hz, 1 H, $H_{d'}$); 7.30 (d, $J_{db} = 7$ Hz, 1 H, H_d). Calc. for $C_{17}H_{31}O_6N_3$: C, 54.69; H, 8.57; N, 11.26; Found: C, 54.89; H, 8.62; N, 10.50%.

Boc-L-alanyl-L-alanyl-L-valine methyl ester

M.p. 148°; $[\alpha]_D^{20} = -58.3$; NMR: δ 0.90, 0.93 (2 d, $J_{ed} = 5$ Hz, 6 H, H_e); 1.35, 1.38 (2 d, $J_{b'f} = J_{b'f'} = 7$ Hz, 6 H, 3 H_f + 3 $H_{f'}$); 1.43 (s, 9 H, H_g); 2.16 (br.m., 1 H, H_d); 3.75 (s, 3 H, H_a); 4.23 (br.m., 1 H, $H_{b'}$); 4.50 (dd, $J_{bd} = 5$ Hz, $J_{bc} = 9$ Hz, 1 H, H_b); 4.60 (dq, $J_{b'c'} = J_{b'f} = 7$ Hz, 1 H, $H_{b'}$); 5.36 (d, $J_{c'b'} = 7.5$ Hz, 1 H, $H_{c'}$); 7.11 (m, 2H, $H_c + H_{c'}$). Calc. for $C_{17}H_{31}O_6H_3$: C, 54.69; H, 8.57; N, 11.26; Found: C, 54.80; H, 8.61; N, 11.35%.

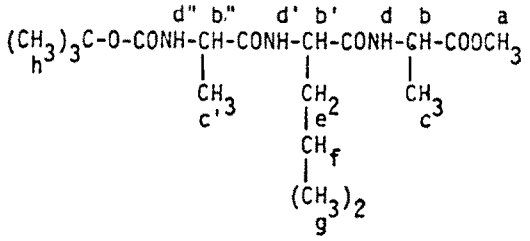


Boc-L-alanyl-D-alanyl-L-valine methyl ester

M.p. 147°; $[\alpha]_D^{20} = -1.7$; NMR: δ 0.92, 0.95 (2 d, $J_{ed} = 7$ Hz, 6 H, H_e); 1.36, 1.40 (2 d, $J_{b'f} = J_{b'f'} = 7$ Hz, 6 H, 3 H_f + 3 $H_{f'}$); 1.43 (s, 9 H, H_g); 2.17 (m, 1 H, H_d); 3.72 (s, 3 H, H_a); 4.20 (br.m., 1 H, $H_{b'}$); 4.49 (dd, $J_{bd} = 5.5$ Hz, $J_{bc} = 9$ Hz, 1 H, H_b); 4.64 (dq, $J_{b'c'} = J_{b'f} = 7$ Hz, 1 H, $H_{b'}$); 5.40 (d, $J_{c'b'} = 7$ Hz, 1 H, $H_{c'}$); 7.13 (d, $J_{c'b'} = 7$ Hz, 1 H, $H_{c'}$); 7.27 (d, $J_{cb} = 9$ Hz, 1 H, H_c). Calc. for $C_{17}H_{31}O_6N_3$: C, 54.69; H, 8.57; N, 11.26; Found: C, 54.75; H, 8.75; N, 11.41%.

Boc-L-leucyl-L-alanyl-L-alanine methyl ester

M.p. 143°; $[\alpha]_D^{20} = -59.9$; NMR: δ 0.92, 0.94 (2 d, $J_{gf} = 6$ Hz, 6 H, H_g); 1.38, 1.40 (2 d, $J_{bc} = J_{b'c'} = 7$ Hz, 6 H, $H_c + H_{c'}$); 1.44 (s, 9 H, H_h); 1.50–1.78 (br.m., 3 H, 2 H_e + H_f); 3.74 (s, 3 H, H_a); 4.17 (br.m., 1 H, $H_{b'}$); 4.53, 4.58 (2 dq, $J_{bc} = J_{bd} = J_{b'c'} = J_{b'd'} = 7$ Hz, 2 H, $H_b + H_{b'}$); 5.37 (d, $J_{d'b'} = 7$ Hz, 1 H, $H_{d'}$); 7.18, 7.34 (2 d, $J_{db} = J_{d'b'} = 7$ Hz, 2 H, $H_d + H_{d'}$). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.51; N, 10.85; Found: C, 55.98; H, 83.7; N, 11.03%.

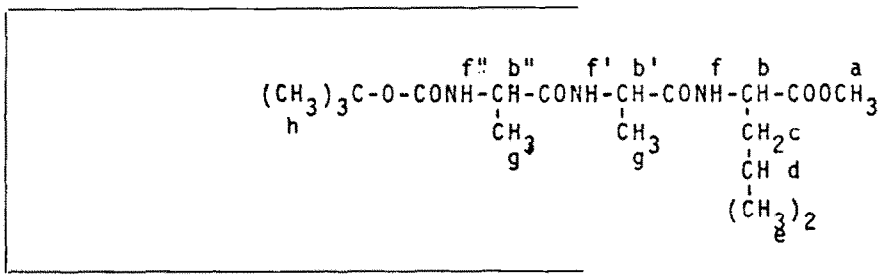


Boc-L-alanyl-D-leucyl-L-alanine methyl ester

M.p. 142°; $[\alpha]_D^{20} = +9.4$; NMR: δ 0.91, 0.95 (2 d, $J_{gf} = 5.5$ Hz, 6 H, H_g); 1.36 (d, $J_{b'c'} = 7$ Hz, 3 H, $H_{c'}$); 1.40 (d, $J_{b'c'} = 7$ Hz, 3 H, H_c); 1.43 (s, 9 H, H_h); 1.50–1.75 (br.m., 3 H, 2 H_e + H_f); 3.71 (s, 3 H, H_a); 4.16 (m, 1 H, $H_{b'}$); 4.51 (m, 1 H, H_b); 4.53 (dq, $J_{bc} = J_{bd} = 7.5$ Hz, 1 H, H_b); 5.33 (m, 1 H, $H_{d'}$); 6.99 (d, $J_{d'b'} = 8.5$ Hz, 1 H, $H_{d'}$); 7.18 (d, $J_{db} = 7.5$ Hz, 1 H, H_d). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.52; N, 10.85; Found: C, 55.97; H, 8.80; N, 10.95%.

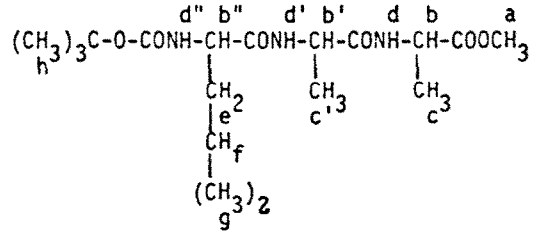
Boc-L-alanyl-L-alanyl-L-leucine methyl ester

M.p. 160°; $[\alpha]_D^{20} = -58.7$; NMR: δ 0.91, 0.92 (2d, $J_{ed} = 6$ Hz, 6 H, H_e); 1.35, 1.38 (2d, $J_{b'g} = J_{b'g'} = 7$ Hz, 6 H, 3 H_g + 3 $H_{g'}$); 1.44 (s, 9 H, H_h); 1.48–1.73 (br.m., 3 H, 2H_c + H_d); 3.72 (s, 3H, H_a); 4.23 (m, 1H, $H_{b'}$); 4.53–4.65 (br.m., 2H, $H_b + H_{b'}$); 5.34 (d, $J_{fb'} = 7$ Hz, 1H, H_f); 7.10 (d, $J_{fb} = J_{fb'} = 8$Hz, 2H, $H_f + H_f$). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.52; N, 10.85; Found: C, 55.90; H, 8.62; N, 10.82%.



Boc-L-leucyl-D-alanyl-L-alanine methyl ester

M.p. 120°; $[\alpha]_D^{20} = -4.7$; NMR: δ 0.93, 0.95 (2 d, $J_{gf} = 6$ Hz, 6 H, H_g); 1.39, 1.41 ($J_{bc} = J_{b'c'} = 7.5$ Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.43 (s, 9 H, H_h); 1.48–1.73 (br.m., 3 H, 2 H_e + H_f); 3.72 (s, 3 H, H_a); 4.13 (br.m., 1 H, $H_{b'}$); 4.56, 4.63 (2 dq, $J_{bc} = J_{bd} = J_{b'c'} = J_{b'd'} = 7.5$ Hz, 2 H, $H_b + H_{b'}$); 5.36 (d, $J_{d'b'} = 9.5$ Hz, 1 H, $H_{d'}$); 7.15, 7.44 (2 d, $J_{db} = J_{d'b'} = 7.5$ Hz, 2 H, $H_d + H_{d'}$). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.52; N, 10.85; Found: C, 56.11; H, 8.61; N, 11.05%.



Boc-L-alanyl-L-leucyl-L-alanine methyl ester

M.p. 180°; $[\alpha]_D^{20} = -64.6$; NMR: δ 0.91, 0.93 (2 d, $J_{gf} = 6$ Hz, 6 H, H_g); 1.35, 1.40 (2 d, $J_{bc} = J_{b'c'} = 7$ Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.44 (s, 9 H, H_h); 1.48–1.74 (br.m., 3 H, 2 H_e + H_f); 3.73 (s, 3 H, H_a); 4.19 (br.m., 1 H, $H_{b'}$); 4.48 (m, 1 H, $H_{b'}$); 4.54 (dq, $J_{bc} = J_{bd} = 7$ Hz, 1 H, H_b); 5.16 (m, 1 H, $H_{d'}$); 6.82 (d, $J_{d'b'} = 8$ Hz, 1 H, $H_{d'}$); 6.91 (d, $J_{db} = 7$ Hz, 1 H, H_d). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.52; N, 10.85; Found: C, 55.57; H, 8.40; N, 10.59%.

Boc-L-alanyl-D-alanyl-L-leucine methyl ester

M.p. 142°; $[\alpha]_D^{20} = +5.7$; NMR: δ 0.92, 0.94 (2d, $J_{ed} = 6$Hz, 6H, H_e); 1.36, 1.38 (2d, $J_{b'g} = J_{b'g'} = 7$ Hz, 6 H, 3 H_g + 3 $H_{g'}$); 1.44 (s, 9 H, H_h); 1.55–1.73 (br.m., 3H, 2H_c + H_d); 3.70 (s, 3H, H_a); 4.16 (m, 1H, $H_{b'}$); 4.48–4.63 (br.m., 2H, $H_b + H_{b'}$); 5.32 (d, $J_{fb'} = 7$Hz, 1H, H_f); 7.07 (d, $J_{fb'} = 7$ Hz, 1H, H_f); 7.16 (d, $J_{fb} = 8$ Hz, 1H, H_f). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.51; N, 10.85; Found: C, 55.19; H, 8.29; N, 10.88%.

Boc-L-isoleucyl-L-alanyl-L-alanine methyl ester

M.p. 160°; $[\alpha]_D^{20} = -64.3$; NMR: δ 0.88 (t, $J_{hc} = 7.5$ Hz, 3 H, H_h); 0.92 (d, $J_{fe} = 6.5$ Hz, 3 H, H_f); 1.38, 1.40 (2d, $J_{bc} = J_{b'c'} = 7$ Hz, 6 H, 3 H_c + 3 $H_{c'}$); 1.44 (s, 9 H, H_i); 3.74 (s, 3 H, H_a); 4.04 (m, 1H, $H_{b'}$); 4.54 (dq, $J_{b'd'} = J_{b'c'} = 7$ Hz, 1H, $H_{b'}$); 4.61 (dq, $J_{bd} = J_{bc} = 7.5$ Hz, 1H, H_b); 5.00 (d, $J_{d'b'} = 8.5$ Hz, 1H, $H_{d'}$); 7.23 ($J_{d'b'} =$

7 Hz, 1H, $H_{d'}$); 7.38 (d, J_{db} = 7.5 Hz, 1H, H_d). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.52; N, 10.85; Found: 55.85; H, 8.71; N, 10.75%.

Boc-L-isoleucyl-D-alanyl-L-alanine methyl ester

M.p. 147°; $[\alpha]_D^{20}$ = +0.2; NMR: δ 0.89 (t, J_h = 7.5 Hz, 3H, H_h); 0.93 (d, J_{fe} = 6.5 Hz, 3H, H_f); 1.39, 1.41 (2d, J_{cb} = $J_{c'b'}$ = 7 Hz, 6H, $3H_c$ + $3H_{c'}$); 1.43, (s, 9H, H_i); 3.72 (s, 3H, H_a); 3.94, dd, $J_{b'e}$ = $J_{b'd''}$ = 7 Hz, 1H, $H_{b'}$); 4.53 (dq, $J_{b'd'}$ = $J_{b'c'}$ = 7 Hz, 1H, $H_{b'}$); 4.57 (dq, J_{bd} = J_{bc} = 7 Hz, 1H, H_b); 5.42 (d, $J_{d''b''}$ = 7 Hz, 1H, $H_{d''}$); 7.12 (d, $J_{d'b'}$ = 7 Hz, 1H, $H_{d'}$); 7.33 (d, J_{db} = 7 Hz, 1H, H_d). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.52; N, 10.85; Found: C, 55.66; H, 8.47; N, 10.82%.

Boc-L-alanyl-L-isoleucyl-L-alanine methyl ester

M.p. 181°; $[\alpha]_D^{20}$ = −67.3; NMR: δ 0.88 (t, J_{hg} = 7.5 Hz, 3H, Hh); 0.94 (d, J_{fe} = 6.5 Hz, 3H, H_f); 1.34, 1.40 (2d, J_{cb} = $J_{c'b'}$ = 7 Hz, 6H, $3H_c$ + $3H_{c'}$); 1.44 (s, 9H, H_i); 3.73 (s, 3H, H_a); 4.25 (br.m., 1H, $H_{b'}$); 4.37 (dd, $J_{b'd'}$ = 9 Hz, $J_{b'e}$ = 7 Hz, 1H, $H_{b'}$); 4.56 (dq, J_{bd} = J_{bc} = 7.5 Hz, 1H, H_b); 5.35 (d, $J_{d''b''}$ = 7 Hz, 1H, $H_{d''}$); 7.07 (d, $J_{d'b'}$ = 9 Hz, 1H, $H_{d'}$); 7.10 (d, J_{db} = 7.5 Hz, 1H, H_d). Calc. for $C_{18}H_{33}O_6N_3$; C, 55.81; H, 8.52; N, 10.85; Found: C, 55.73; H, 8.57; N, 10.75%.

Boc-L-alanyl-D-isoleucyl-L-alanine methyl ester

M.p. 153°; $[\alpha]_D^{20}$ = −13.5; NMR: δ 0.90 (t, J_{hg} = 7.5 Hz, 3H, H_h); 0.93 (d, J_{fe} = 7 Hz, 3H, H_f); 1.37, 1.41 (2d, J_{cb} = $J_{c'b''}$ = 7 Hz, 6H, $3H_c$ + $3H_{c'}$); 1.43 (s, 9H, H_i); 3.72 (s, 3H, H_a); 4.15 (dq, $J_{b''d''}$ = $J_{b''c'}$ = 7 Hz, 1H, $H_{b''}$); 4.35 (dd, $J_{b'd'}$ = 9 Hz, $J_{b'e}$ = 6.5 Hz, 1H, $H_{b'}$); 4.55 (dq, J_{bd} = J_{bc} = 7.5 Hz, 1H, H_b); 5.09 (d, $J_{d''b''}$ = 7 Hz, 1H, $H_{d''}$); 6.73 (d, $J_{d'b'}$ = 9 Hz, 1H, $H_{d'}$); 6.81 (m, 1H, H_d). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.52; N, 10.85; Found: C, 55.84; H, 8.45; N, 10.62%.

Boc-L-alanyl-L-alanyl-L-isoleucine methyl ester

M.p. gum >40°; $[\alpha]_D^{20}$ = −48.0; NMR: δ 0.89 (t, J_{fe} = 7 Hz, 3H, H_f); 0.90 (d, J_{dc} = 7 Hz, 3H, H_d); 1.35, 1.38 (2d, $J_{hb'}$ = $J_{h'b''}$ = 6.5 Hz, 6H, $3H_h$ + $3H_{h'}$); 1.44 (s, 9H, H_i); 1.90 (br.m., 1H, H_c); 3.73 (s, 3H, H_a); 4.27 (br.m., 1H, $H_{b'}$); 4.55 (dd, J_{bg} = 8.5 Hz, J_{bc} = 5 Hz, 1H, H_b); 4.69 (dq, $J_{b'h}$ = 6.5 Hz, $J_{b'g'}$ = 7.5 Hz, 1H, $H_{b'}$); 5.59 (d, $J_{g'b''}$ = 7 Hz, 1H, $H_{g''}$); 7.32 (2d, J_{gb} = 8.5 Hz, $J_{g'b'}$ = 7.5 Hz, 2H, H_g + $H_{g'}$). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.52; N, 10.85: Found: C, 55.80; H, 8.79; N, 10.94%.

Boc-L-alanyl-D-alanyl-L-isoleucine methyl ester

M.p. gum >40°; $[\alpha]_D^{20}$ = +6.0; NMR: δ 0.90 (t, J_{fe} = 6.5 Hz, 3H, H_f); 0.92 (d, J_{dc} = 6.5 Hz, 3H, H_d); 1.36, 1.39 (2d, $J_{bb'}$ = $J_{h'b''}$ = 7 Hz, 6H, $3H_h$ + $3H_{h'}$); 1.44 (s, 9H, H_i); 1.91 (br.m., 1H, H_c); 3.68 (s, 3H, H_a); 4.53 (dd, J_{bg} = 8.5 Hz, J_{bc} = 5.5 Hz, 1H, H_b); 4.64 (dq, $J_{b'g'}$ = 7 Hz, $J_{b'h}$ = 7 Hz, 1H, $H_{b'}$); 5.48 (d, $J_{g''b''}$ = 7 Hz, 1H, $H_{g''}$); 7.21 (d, $J_{g'b'}$ = 7 Hz, 1H, Hg'); 7.35 (J_{gb} = 8.5 Hz, 1H, H_g). Calc. for $C_{18}H_{33}O_6N_3$: C, 55.81; H, 8.52; N, 10.85; Found: C, 55.65; H, 8.43; N, 10.67%.

Products for epimerization measurements

We describe here one example out of the 26 series studied.

Boc-L-valyl-L-valine. 310 mg of Boc-L-valyl-L-valine methyl ester prepared according to our standard coupling procedure (m.p. 165–166;[12] $[\alpha]_D^{20}$ = −32.4°) were dissolved in 7 ml of dioxan. Sodium hydroxide solution (0.3 N, 3 ml) was added. After one hour, the solution was adjusted to pH 3 with 3 N HCl solution. The organic phase was washed thrice with saturated sodium chloride solution, dried over magnesium sulphate and evaporated: 280 mg of the title product were obtained (95%). In order to check if any racemization had occurred at this stage[13] a fraction was reesterified by a diazomethane solution and gave a sample of the starting material identical to the original one (m.p. 165–166°; $[\alpha]_D^{20}$ = −32.6°).

Boc-L-valyl-L, D-valyl-L-leucine methyl ester. 280 mg of the preceding material (0.9 mmol) was dissolved in 9 ml of dimethylformamide with 170 mg (0.9 mmol) of leucine methyl ester hydrochloride and 400 mg of BOP (0.9 mmol). 185 mg of triethylamine (1.8 mmol) were then added. After 2.5 hr the standard workup gave a crude product that was filtered on a short column of silicagel. The non-separation of the diastereoisomers was checked by tlc, 370 mg of title product were obtained (95%). Indeed in the cases of AFA and ALA, the chromatographic behavior of the diastereoisomers were different enough to cause their separation. This artefact was the cause of mistaken data in our preliminary communications.[2,14] The high resolution ^1H NMR spectra were performed on a solution of 50 mg of the compound in 0.5 ml of deuteriochloroform.

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