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Stereochemical preference for heterochiral coupling controls selectivity in competitive peptide synthesis

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Competitive activated couplings of *N*-phthaloyl amino acids with amino acid dimethylamides show little selectivity among substrates in respect of their sidechains, but a consistent and significant preference for heterochiral outcomes.

Spontaneous abiotic formation of peptides from amino acid derivatives is a topic of considerable interest within prebiotic chemistry,[1] peptide synthesis[2] and combinatorial chemistry.[3] Whilst non-random thermal condensations in amino acid mixtures are seen as routes to incipient informational biopolymers,[4] evidence for selectivity at lower temperatures is equivocal.[2,5] Moreover, we are unaware of any study that addresses the question of stereoselectivity in competitive reactions where epimerizing equilibration is not a factor. We have found that, in DCC/HOBt-mediated coupling of *N*-phthaloyl amino acids and amino acid dimethylamides, stereoselectivity not only is the dominant controlling factor but also favours reaction between substrates of opposite configuration. An explanation of this phenomenon implies that such selectivity could be more widely applicable, with potentially significant consequences.

N-Phthaloyl (Pth) and *N*,*N*-dimethylamido (DMA) derivatives were selected for convenient chromophorically-based detection and as an inert C-terminus, respectively. Competitive couplings were carried out mostly pair-wise, where one type of substrate had the opportunity to react with two of the other type of substrate, the latter usually present in considerable molar excess. In an illustrative procedure, a solution of Pth-L-phenylalanine 1-hydroxybenzotriazole (HOBt) ester (0.1 mmol) in CH_2Cl_2 (10 cm^3), prepared *in situ* from Pth-L-phenylalanine, HOBt (0.12 mmol) and DCC (0.12 mmol), was added to racemic phenylalanine dimethylamide hydrobromide (1.0 mmol) dissolved in CH_2Cl_2 (25 cm^3) and Et_3N (1.2 mmol).[6] The mixture was stirred for two days, the CH_2Cl_2 removed by evaporation, the residue taken up in the initial HPLC eluent (30% aq. MeCN) and a homogeneous sample analysed by the area ratio of peaks identified by standards[7] as the L,L- and D,L-diastereoisomers of the corresponding derivatized dipeptides.

The outcomes of 28 such competition experiments are summarised in Tables 1 and 2, results being averaged in the cases where experiments were repeated. Table 1 shows the consistent and significant preference for heterochiral couplings, irrespective of whether the excess reagent is the Pth component or the DMA component. In order to confirm that products resulted from kinetic rather than thermodynamic control, we allowed one experiment to proceed with 1 equiv. of the racemic DMA and found the ratio of diastereomers to be close to 1 : 1 at completion. Table 2 demonstrates that when a choice of amino acids is presented, selectivity additional to that of stereochemistry is only significant in respect of the preferred coupling with glycyl residues.

The presence of one reactant in significant excess allows the approximation that product ratios in competing reactions correspond with the ratio 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. Further confirmation of a substantial preference for heterochiral coupling was found in the outcome of reactions between equimolar racemic reagents. Thus, the percentages of heterochiral diastereoisomer in reactions between racemic Pth-alanine and racemic dimethylamides of alanine, valine and phenylalanine were 75.6, 89.3 and 87.9, respectively. More complex mixtures also behaved consistently. For example, reaction between 1 equiv. of Pth-L-phenylalanine and a mixture of 5 equiv. each of the dimethylamides of L-alanine, L-phenyl-

Table 1 Competition outcomes in reactions between Pth-L-X and racemic Y-DMA using DCC/HOBt coupling

X	Y	Initial [Y-DMA]/[Pth-L-X]	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
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
Val	Val	10.0	96
Val	Ala	10.0	95
Val	Phe	8.3	83

[a] Determined by integration of HPLC traces, typically at 220 nm using a 250 × 4.6 mm (ID) Phenomenex Luna C18 (2) column at 35 °C and a linear mobile phase gradient from 30 to 95% MeCN in water over 20 min. Error < ± 5%. [b] Racemic Pth-X and L-Y-DMA.

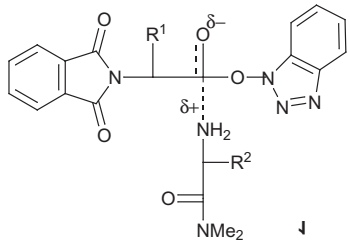
Table 2 Competition outcomes in reactions between Pth-X and equimolar mixtures of Y-DMA and Z-DMA using DCC/HOBt coupling.

X	Y	Z	Initial {[Y] + [Z]}/[X][b]	Final [XY]/[XZ][c]	% 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-Va	10.0	4.2	—
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

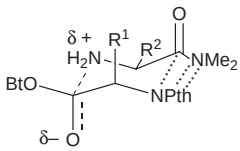
[a] See Table 1. [b] {[Y-DMA] + [Z-DMA]}/[Pth-X]. [c] [PthXYDMA]/[PthXZDMA].

alanine and *racemic* valine gave a product mixture with a ratio 15 : 6 : 4(LL):75(LD), respectively.

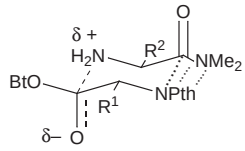
The dominance of chirality over sidechain as an influence in these competitive reactions may be explained by considering the probable structure of the activated complex **1**, where it can



be seen that the two α-carbon atoms are separated by only two other atoms, whereas β-carbon atoms are more remote from potentially interacting centres. The consistent preference for a heterocyclic outcome is less readily anticipated, but would follow if **1** gained secondary stabilization by interactions between the terminal groups in a six-membered chair conformation, where the more favoured diequatorial disposition of sidechains would lead to the observed results (**2b**).



L, L
2a



L, D
2b

If valid, this interpretation would suggest that the selectivity is largely independent of activating and terminal groups as long as the latter interact positively. (Similar independence has been noted in respect of amino acid selectivity in the reactions of different amino acids with the phosphoanhydrides of alanine and various nucleoside monophosphates; specificity was unaffected by alternative nucleosides.[8]) The results support Dose's contention that rate differences in condensations in amino acid mixtures would generally be too small to direct sidechain sequences,[5] but introduce the prospect that, in the absence of epimerization equilibria, such sequences in extendable systems would entail alternating configurations at C_α until interrupted by a glycyl residue. We are presently exploring the generality of this effect with other systems.

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Notes and references

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