Influence of Secondary Structure (Helical Conformation) on Stereoselectivity in Peptide Couplings

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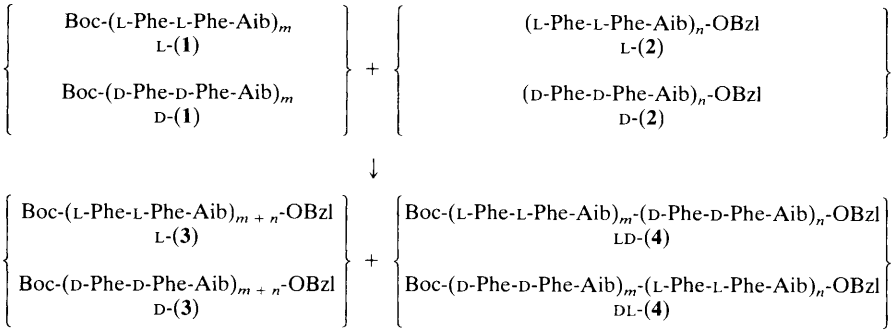
The stereoselectivity in the peptide bond formation was investigated and it was demonstrated that secondary structure–secondary structure interaction is significant in affecting the stereochemical course of peptide bond formation.

The influence of primary structure on the behaviour of organic substances has been extensively, often systematically, investigated, resulting in a vast amount of information from which it is possible to predict with confidence the chemical properties and reactivities of many classes of compounds. In contrast, very little is known about the effect of secondary structure–secondary structure interactions on chemical reactivities. We even feel that there has been no rigorous demonstration of the existence and degree, if it exists, of such an interaction in non-biological systems. Here we present a dramatic example observed in the oligopeptide area.[1]

We chose to study the stereoselectivity in the peptide bond



Scheme 1

formation outlined in Scheme 1 for several reasons. First and most important, as we have discussed previously,[2] oligopeptides composed of the tripeptide unit -(Phe-Phe-Aib)-,† form stable right- or left-handed helices,‡ which are very soluble in a wide range of organic solvents. Second, the α-aminoisobutyric acid (Aib) residue at the C-terminal avoids complications due to racemization during peptide bond formation. Third, the Aib residue at the C-terminal eliminates the influence of a chiral centre adjacent to an activated form of the carboxylic acid on the stereochemical course of the bond formation. Experimentally, we investigated the stereoselectivity of peptide bond formation by using the racemic form of both substrates, keeping the molar ratio of L-(**1**), D-(**1**), L-(**2**), and D-(**2**) constant throughout the coupling reaction.§

Racemic oligopeptides used for this study were synthesized as follows: the tripeptide units Boc-L-Phe-L-Phe-Aib-OBzl and Boc-D-Phe-D-Phe-Aib-OBzl were prepared and purified as described elsewhere.[2] Oligopeptides Boc-(Phe-Phe-Aib)$_m$-OBzl belonging to the L- as well as the D-phenylalanine series were obtained from the L- and D-tripeptide units, respectively, by standard synthetic methods. A 1:1 mixture of these optically active oligopeptides, followed by deprotection of the amino or carboxylic acid group, gave racemic oligopeptides Boc-(Phe-Phe-Aib)$_m$-OBzl, *i.e.* L-(**1**) + D-(**1**), or -(Phe-Phe-Aib)$_n$-OBzl, *i.e.* L-(**2**) + D-(**2**).

Using the case of $m = n = 2$, preliminary studies were made to identify a suitable coupling reagent and solvent system; under DCC/THF–H_2O (4:1) (DCC = dicyclohexylcarbodiimide; THF = tetrahydrofuran) conditions, Boc-(Phe-Phe-Aib)$_2$ [racemic, *i.e.* L-(**1**) + D-(**1**)] and (Phe-Phe-Aib)$_2$-OBzl [racemic, *i.e.* L-(**2**) + D-(**2**)] yielded a 5.5:1.0 mixture of Boc-(Phe-Phe-Aib)$_4$-OBzl [racemic, *i.e.* L-(**3**) + D-(**3**)] and Boc-(L-Phe-L-Phe-Aib)$_2$(D-Phe-D-Phe-Aib)$_2$-OBzl [racemic, *i.e.* LD-(**4**) + DL-(**4**)], while DCC–CH_2Cl_2 gave a ratio of 3:2, DCC–DMF 3:2 (DMF = dimethylformamide), DCC–THF 3:2, DCC–HOBT[3]/CH_2Cl_2 1:1, DCC–HOBT/THF 1:1, and DCC–HOBT/THF–H_2O (4:1) 1:1 (HOBt = hydroxybenzotriazole). Thus, the DCC/THF–H_2O (4:1) conditions were chosen for the coupling of other peptides, giving the results summarized in Table 1.

Table 1. Product ratios, [L-(**3**) + D-(**3**)]: [LD-(**4**) + DL-(**4**)] for the peptide coupling reaction:[a]
[L-(**1**) + D-(**1**)] + [L-(**2**) + D-(**2**)] → [L-(**3**) + D-(**3**)] + [LD-(**4**)] + DL (**4**)]

		(2)			
	[c]	$n = 1$	$n = 2$	$n = 3$	$n = 4$
[b]	1.1:1.0[d]	0.9:1.0[d]	1.6:1.0[d]	2.6:1.0[d]	[f]
(1) $m = 1$	[f]	0.8:1.0[d]	1.8:1.0[d]	3.2:1.0[d]	5.0:1.0[e]
(1) $m = 2$	1.4:1.0[d,e]	1.2:1.0[d,e]	5.5:1.0[d,e]	8.6:1.0[d,e]	6.6:1.0[d,e]
(1) $m = 3$	[f]	1.4:1.0[d]	4.8:1.0[d,e]	5.5:1.0[d,e]	[f]
(1) $m = 4$	0.9:1.0[d]	1.1:1.0[d]	5.0:1.0[d,e]	8.2:1.0[e]	[f]

[a] The coupling reaction was conducted under the following conditions. A mixture of racemic carboxylic acid L-(**1**) + D-(**1**) (5.0 μmol) and racemic amine hydrochloride salt L-(**2**) + D-(**2**) (5.0 μmol) was dissolved in 1.5 ml of aq. THF (THF:H_2O = 4:1). To this solution were added *N*-methylmorpholine (5.0 μmol) and DCC (10.0 μmol) at room temperature. The reaction mixture was stirred for 36 h at room temperature, and the solvent was removed *in vacuo*. By silica gel t.l.c, the crude products (typically 30—40% yield) containing all coupled products were collected and analysed by n.m.r. spectroscopy and/or h.p.l.c. [b] Boc-Phe-Aib (racemic). [c] -Phe-OBzl (racemic). [d] Ratio determined from a 500 MHz ^{1}H n.m.r. spectrum. [e] Ratio determined by analytical h.p.l.c. (Waters; eluant EtOAc–CH_2Cl_2). [f] Data not yet available.

Several significant conclusions can be drawn from these results. First, the effect of the α-chiral centre of the terminal amino acid residue in the amine component is insignificant in the present cases.[4] Second, the diastereoselectivity for the coupling increases with the size of peptide substrates. Third and most important, a sharp increase in the diastereoselectivity is observed from tripeptides to hexapeptides; it is particularly significant for (Phe-Phe-Aib)$_n$-OBzl. The coupling diastereoselectivity is best in the cases where both substrates are hexa- or higher-peptides. In connection with this, it is important to point out that helix formation is observed for hexa- or higher-peptides composed of Boc-(Phe-Phe-Aib)$_x$-OBzl ($x > 1$) but not for the tripeptide.[2]

On the basis of these experiments, we can conclude that the origin of the significant diastereoselectivity observed is the

† Nomenclature according to IUPAC–IUB Joint Commission on Biochemical Nomenclature, 'Nomenclature and Symbolism for Amino Acids and Peptides, Recommendations 1983,' *Eur. J. Biochem.*, 1984, **138**, 9.

‡ It is known that oligopeptides composed of L-amino acids form right-handed helices while D-amino acids form left-handed helices.

§ Using one of the two components in an optically active form, a kinetic resolution of racemates of the other components is feasible: for example, racemic hexapeptide carboxylic acid L-(**1**) + D-(**1**) ($m = 2$, 2.0 equiv.) and L-nonapeptide amine L-(**2**) ($n = 3$, 1.0 equiv.), when treated with DCC (2.0 equiv.) in aqueous THF (1:4) at room temperature for 36 hours, gave the coupled product (30% isolated yield for the major product) with a 6.0:1.0 ratio favouring L-(**3**) ($m + n = 5$) over DL-(**4**) ($m = 2$, $n = 3$). For the case of L-(**1**) ($m = 4$, 1.0 equiv.) + L-(**2**) + D-(**2**) ($n = 3$, 2.0 equiv.), a ratio 5.9:1.0 of L-(**3**) ($m + n = 7$) and LD-(**4**) ($m = 4$, $n = 3$) was observed (room temperature, 36 h; *ca.* 30% isolated yield).

secondary structure–secondary structure interaction. However, no experimental evidence is available at this time to suggest a specific mode of the interaction of substrates.

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