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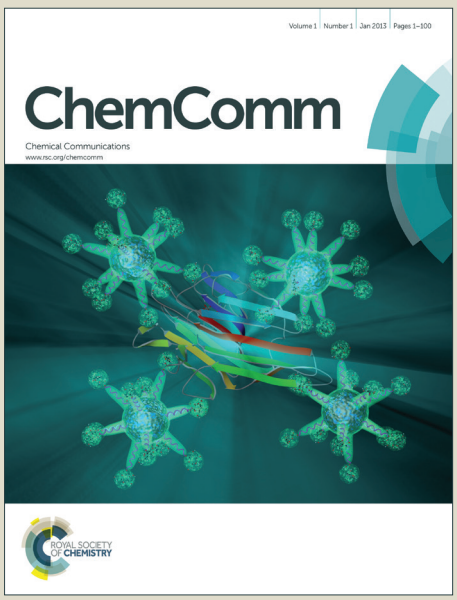

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Screw sense alone can govern enantioselective extension of a helical peptide by kinetic resolution of a racemic amino acid

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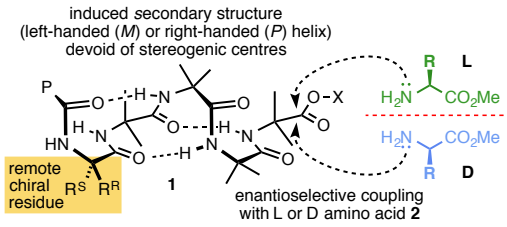
Liam Byrne,[a] Jordi Solà[a] and Jonathan Clayden[a]

Helical peptides built principally from the achiral quaternary amino acid Aib but with an induced preferred screw-sense exhibit enantioselectivity in their chain-extension reactions when presented with a racemic tertiary amino acid. This is the first demonstration that secondary structure alone, devoid of local chiral residues, can direct the enantioselectivity of peptide coupling.

Homochirality is a defining feature of life, and the driving force for the desymmetrisation of a presumed racemic, or almost racemic, mixture of prebiotic chiral building blocks has been the source of extensive study and speculation.[1-7] Mechanisms for chiral amplification by enantioselective fragment couplings have been demonstrated for nucleic acids[8] and for peptides.[7,9-12] For example, Kishi showed that pairs of helical peptides comprising largely Phe residues couple more rapidly when both partners contain the same enantiomer of phenylalanine, with mutual kinetic resolution of L-Phe and D-Phe containing peptides taking place.[9] Ghadiri showed that an enantioselective templated fragment coupling leads to a self-replicating system.[10] While coupling of amino acids to form dipeptides appears to favour heterochiral pairs under certain conditions,[13] chain extension of short peptide fragments by addition of single monomers generally favours the incorporation of residues of 'like' configuration.[14-16] An exception was identified by Toniolo *et al.*,[7] who found that chain extension of right handed 3_{10} helices formed from chiral, Cα-methylated quaternary residues preferentially incorporated D-Val residues on chain extension.

Specific stereocontrolling interactions with the terminal chiral residue of a growing peptide chain must play some role in these enantioselective couplings, but it is appealing to consider that the global chirality of the secondary structure of a peptide, especially a helical one, might also be an essential factor in the enantioselective chain extension reactions. Kishi[9] explored the role of matched secondary structure in the enantioselectivity of fragment coupling reactions by coupling helical peptides of defined screw sense in which the reactive terminus was an achiral residue Aib (2-aminoisobutyric acid), to minimise the influence of local configuration on the overall effect of global conformation. In Toniolo's work, the dependence of enantioselectivity on chain length strongly suggests that global conformation plays a role in determining enantioselectivity.[7]

In this paper we show that secondary structure *alone* is sufficient to select which of the two enantiomers of a chiral monomer are ligated to a peptide chain, even in chain extension reactions with a single monomer. Using a helical peptide structure devoid of stereogenic centres close to the reactive helix terminus, we find that a remotely induced screw-sense preference is sufficient to direct enantioselective chain extension by selection of the L or D enantiomer from a racemic pool of amino acid coupling partners.



Scheme 1: Induced secondary structure controlling enantioselective chain extension.

Oligomers of the achiral, non-proteinogenic amino acid Aib (2-aminoisobutyric acid) exist as a racemic mixture of rapidly interconverting left- and right-handed 3_{10} helical conformers.[17,18] However, this dynamic equilibrium can be perturbed by subtle chiral influences, such as the incorporation of a single chiral residue at the N[19-24] or C[25-30] terminus,[31,32] or reversible interaction between the helix terminus and a chiral ligand.[33,34] The induced screw-sense preference can be quantified using ^{1}H,[19] ^{13}C[22] or ^{19}F[35] NMR or circular dichroism (CD) techniques, and exploited in the remote induction of asymmetry at a new stereogenic centre up to 60 bonds from the source of screw-sense induction.[37] This ability to induce a quantifiable screw-sense preference in an Aib oligomer forms the foundation of the study we now describe.

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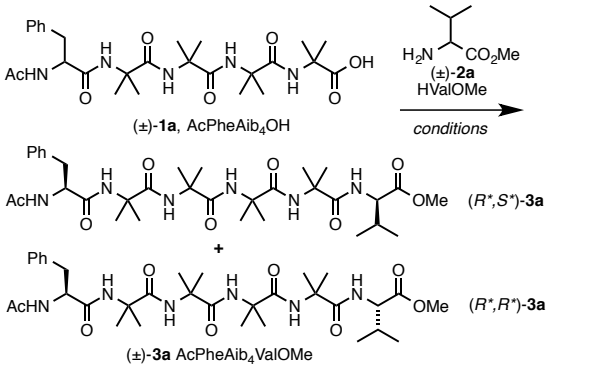
Our exploration of the inherent stereoselectivity in the chain extension of a helix made use of an oligomer of Aib **1**, induced to adopt either a left- or right-handed screw sense by a single N-terminal chiral residue, and allowed to undergo C-terminal chain extension by coupling with a racemic mixture of D- and L-amino esters **2** (Scheme 1).

Initial studies made use of the racemic pentapeptide (±)-AcPheAib$_4$OH **1a**, evaluating the diastereoselectivity of its coupling reactions by NMR (Table 1).[38] We had already calculated that an N-terminal L-Phe residue, protected as its acetamide, induces in an adjacent oligo-Aib domain a preference for a left-handed (*M*) helix[21] with a screw-sense ratio *M:P* of 80:20 (in methanol).[19] The coupling of (±)-**1a** with an excess (8 equiv) of (±)-ValOMe **2a** was initially attempted using conditions developed by Kishi[9] for the activation of peptides bearing a C-terminal Aib residue, namely DCC (*N,N'*-dicyclohexylcarbodiimide) as the coupling agent in a two-phase mixture of THF and water (Table 1, entry 1). After an acidic wash to remove the excess amino ester, ^{1}H NMR analysis of the crude reaction mixture indicated that the two diastereoisomers of **3a** were formed in a 1:1 ratio. This was confirmed after isolation of an analytically pure sample by column chromatography.[39]

Table 1: Diastereoselectivity in the coupling of **1a** and **2a**.



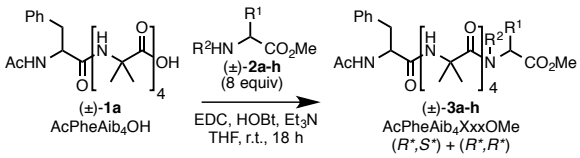
entry	reagents	base	**2a**, equiv	solvent	**3a**, yield (%)	**3a**, d.r. (*R*,S**): (*R*,R**)
1	DCC	NMM[a]	10	THF/H_2O (3:2)	96	50:50
2	DCC	NMM	10	1,4-dioxane	85	71:29
3	EDC,[b] HOBt[c]	Et_3N	10	CH_2Cl_2	84	63:37
4	EDC, HOBt	Et_3N	10	CH_3CN	86	65:35
5	EDC, HOBt	Et_3N	10	THF/TFE[d] (9:1)	63	73:27
6	EDC, HOBt	Et_3N	10	THF	95	75:25
7	EDC, HOBt	Et_3N	4	THF	82	72:28
8	EDC, HOBt	Et_3N	2	THF	78	68:32
9	EDC	–	10	THF	45	50:50
10	PyBOP[e]	Et_3N	10	THF	92	67:33

[a]*N*-methylmorpholine; [b]1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; [c]1-hydroxybenzotriaxole; [d]2,2,2-trifluoroethanol; [e]benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate.

Polar aprotic solvents such as THF enhance the ability of an Aib oligomer to maintain a uniform screw sense along its entire length relative to hydroxylic solvents such as methanol or water.[37,40] The reaction between (±)-**1a** and (±)-**2a** was therefore repeated in 1,4-dioxane (entry 2). After an identical work-up and purification procedure, the diastereoisomeric products (*R*,S**)- and (*R*,R**)-**3a** were isolated as a 71:29 mixture, albeit in slightly lower yield (the identification of the major diastereoisomer is described below). To facilitate purification, we switched to the water-soluble carbodiimide EDC, [41] with HOBt as a nucleophilic additive. A brief solvent screen (entries 3-6) identified THF as the optimal solvent, giving the highest diastereoisomeric ratio of 75:25. Further attempts to improve the diastereoselectivity using alternative coupling reagents were unsuccessful (Entries 9 and 10). Reducing the number of equivalents of amino ester **2** reduced both the d.r. and isolated yield of products (Entries 7 and 8).

Having identified optimal conditions for the reaction we screened the selectivity in the reaction of chain extension of (±)-**1a** with a range of racemic Cα-tertiary amino acid methyl esters, the results of which are summarised in Table 2. In most cases the products were isolated in good to excellent yields and with diastereoselectivities between 1.5:1 and 3:1. We attribute the poor yield obtained from the reaction with proline methyl ester (entry 8) to the difficulty of coupling a more hindered secondary amine with a Cα-quaternary amino acid. Of the amino acid esters tested, HValOMe **2a** reacted the most selectively, and in general the bulkier amino acid side chains gave higher diastereoselectivities.

Table 2: Diastereoselectivity with other amino esters **2**



entry	amino acid **2**	R^1	R^2	**3** yield (%)	**3**, d.r. (*R*,S**): (*R*,R**)
1	**2a**, Val	*i*-Pr	H	**3a**, 95	75:25
2	**2b**, Leu	*i*-Bu	H	**3b**, 76	68:32
3	**2c**, Phe	Bn	H	**3c**, 90	69:31
4	**2d**, Trp	CH_2-3-indole	H	**3d**, 84	66:34
5	**2e**, His	CH_2-4-imidazole	H	**3e**, 74	59:41
6	**2f**, Phg	Ph	H	**3f**, 80	59:41
7	**2g**, Ser	CH_2OH	H	**3g**, 64	58:42
8	**2h**, Pro	$-(CH_2)_3$	–	**3h**, 53	55:45

Varying the N-terminus of the peptide also had a significant effect on the outcome of the reaction linked closely to their ability to induce a screw-sense preference. Changing from an acetamide to a *p*-bromobenzamide to a Cbz carbamate led to a reduction in diastereoselectivity (Table 3, Entries 1-3). This is consistent with our earlier finding that Cα-tertiary amino acids capped with amides induce higher levels of screw-sense control than the corresponding carbamates.[19,42] Increasing the number of Aib residues between the chiral controller and the reactive site from 4 to 8 led to a small decrease in selectivity (Entries 1 and 4). This can be explained by the small but finite chance of a reversal of screw-sense preference at each Aib residue, leading to a decreasing probability that the two ends of the oligo-Aib domain will have the same screw sense and therefore an overall loss of local helicity excess as the C terminus moves further from the N terminal controlling residue. This decay in conformational preference is much more significant in methanol than in THF.[40] The modifications in selectivity observed on changing the N terminus and on lengthening the chain are consistent with the observed selectivity arising from propagation of chirality through the helix, rather than any

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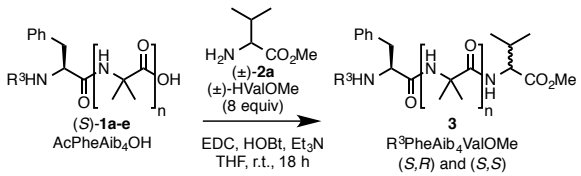
intermolecular interaction bringing the chiral centre into close proximity with the reactive site. [43]

Table 3: Effect on diastereoselectivity of protection and chain length of **1**



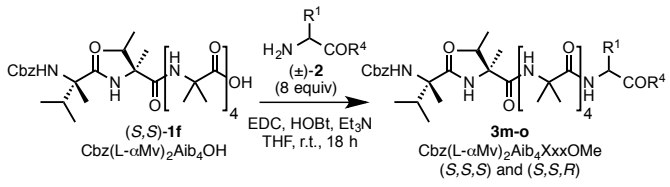
entry	**1**, R^3	*n*	**3** yield (%)	**3**, d.r. (*S,R*):(*S,S*)
1	**1a**,[a] Ac	4	**3a**, 95	75:25
2	**1b**,[b] *p*-BrBz[c]	4	**3i**, 94	71:29
3	**1c**,[b] Cbz[d]	4	**3j**, 92	67:33
4	**1d**,[b] Ac	8	**3k**, 72	67:33
5	**1e**,[b] Cbz	8	**3l**, 84	66:34

[a]Carried out with racemic **1a**; [b]Carried out with (*S*)-**1b-e**; [c]4-bromobenzoyl; [d]benzyloxycarbonyl.

Evidently the configuration of the Phe residue at the N terminus of the oligomer, through its conformational influence on the Aib chain, is able to dictate the diastereoselectivity with which remote couplings take place. The selectivities observed in the reactions of **1a** fall not far short of the 80:20 screw-sense control imparted by an N terminal Ac-Phe,[19] suggesting that each screw-sense conformer of the Aib_4 helix exerts a very high level of control over the diastereoselectivity of the coupling reaction.

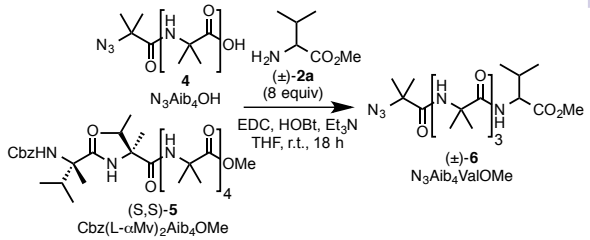
An N-terminal α-methylvaline dimer (αMv_2) induces an essentially quantitative screw-sense preference in Aib oligomers,[37] which, in an aprotic solvent, is maintained almost undiminished through the length of an Aib oligomer: in a Cbz(L-$\alpha Mv)_2Aib_n$ oligomer we deduce that the local screw-sense ratio, in THF, is 96:4 *P*:*M* at the sixth reside of the chain.[37] In order to exploit this ability to exert, remotely, a high level of control over the secondary structure of the peptide, enantiopure (*S,S*)-**1f** Cbz(L-$\alpha Mv)_2Aib_4OH$ was coupled with an excess of (±)-HValOMe **2a** under the optimised conditions from Table 1, entry 6. The product **3m** was formed with a d.r. of 84:16 (Table 4, entry 1). Similar selectivity was seen in the coupling with (±)-$HValNH_2$ **2b** (entry 2) and (±)-H-*tert*-LeuOMe **2h** (entry 3).

Table 4: Quantitative screw-sense control for maximal diastereoselectivity



entry	amino acid **2**	R^1	R^4	**3** yield (%)	**3**, d.r. (*S,S,S*):(*S,S,R*)
1	**2a**, Val	*i*-Pr	OMe	**3m**, 92	84:16
2	**2b**, Val	*i*-Pr	NH_2	**3n**, 95	79:21
3	**2h**, *tert*-Leu	*t*-Bu	OMe	**3o**, 93	85:15

The fact that these selectivities were determined by an intramolecularly and not an intermolecularly communicated conformational preference was established by carrying out a potentially enantioselective coupling reaction of (±)-**2a** with a mixture of an achiral helical peptide **4** in the presence of an equimolar amount of a chiral, helical, but unreactive peptide **5** (Scheme 2). The product **6** was formed as a racemic mixture.



Scheme 2: Diastereoselectivity results from intramolecular interactions.

In order to identify which enantiomer of **2** is selected by the peptide ... each case, authentic samples of (*S,S*)-**3j**, (*S,S,S*)-**3m** and (*S,S,R*)-**3** ... were synthesised using EDC/HOBt, under standard peptide coupling conditions.[44] Comparison of the HPLC retention times and ^1H N... spectra of **3j** with the mixtures formed by the coupling reactions indicated that Ac-L-Phe induces the Aib chain to couple selecti... with D amino esters (Figure 1). Conversely, the Cbz-(L-$\alpha Mv)_2$ controller induces the Aib chain to couple selectively with L a... esters. These outcomes are fully consistent with the helical screw sen... alone being the determining feature of the enantioselectivity, since N-terminal Ac-L-Phe induces an *M* screw sense[21] while N-terminal Cb... (L-$\alpha Mv)_2$ induces a *P* screw sense[19,37] (Scheme 3). [45]

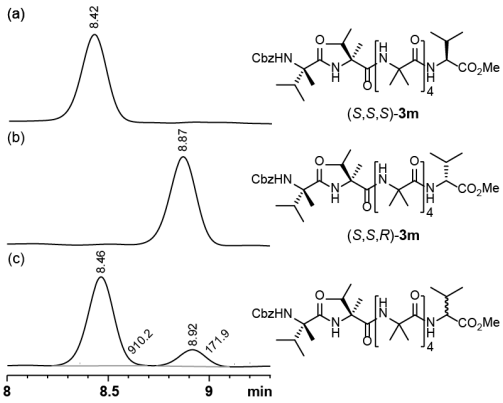
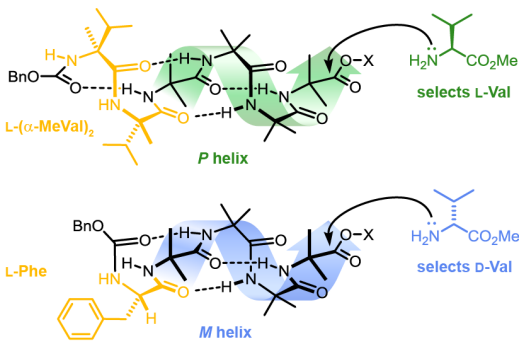


Figure 1: Analytical HPLC trace (see supplementary information for details) of (... an authentic sample of (*S,S,S*)-**3m**; (b) an authentic sample of (*S,S,R*)-**3m**; (c) t... reaction mixture (crude) of **3m** (relative peak areas shown)..



Scheme 3: Summary of the results

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We conclude that chain extension of a chiral peptide with a racemic pool of amino acids can lead to selective incorporation of one enantiomer purely as a result of the preferred screw sense of a peptide's helical secondary structure, even in the absence of local chiral residues. A right-handed helix selects preferentially the L enantiomer of the coupling partner, while a left handed helix selects the D enantiomer. Measured diastereoselectivies amount to >75% of the calculated magnitude of the local conformational control. While the conditions used here are evidently not prebiotically plausible, and the structures involved are 3_{10} helices[46] rather than the more widespread peptide α helices, these results illustrate the fact that kinetic, as well as thermodynamic factors, act in favour of the well-established[47] association of L-amino acids with right handed helices.

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[a] School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK. E-mail: clayden@man.ac.uk

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38. The use of racemic peptides avoids potential complications arising from mass action effects during the kinetic resolution.
39. It is noteworthy that, despite the distance (15 bonds) between the two chiral centres, the methyl ester signals of the two diastereomers are well resolved. This is most likely a result of a matched-mismatched effect of the screw-sense preferences induced by the N-terminal Phe and C-terminal Val residues (see ref 22.)
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42. A small change in selectivity may be ascribed to the change from (±)-Phe in **1a** to L-Phe in **1b-1e**, which means that **2** becomes enriched in the less reactive enantiomer as the reaction progresses. However, with an eight-fold excess of **2**, the effect of this change is expected to be negligible.
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