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DIPEPTIDIC AMMONIUM ION BINDING BY A SYNTHETIC RECEPTOR

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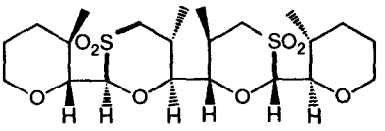
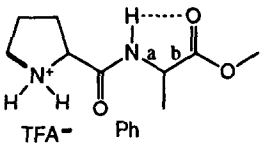
Abstract. A conformationally homogeneous synthetic host molecule binds proline-derived dipeptidic guests both enantioselectively and diastereoselectively.

Many naturally occurring receptors bind peptides with high sequence and stereoselectivity. Vancomycin, a clinically useful glycopeptide antibiotic, is one such receptor which associates both with bacterial cell wall intermediates and simple peptides terminating in D-Alanyl-D-Alanine.[1] While vancomycin binds Ac-D-Ala-D-Ala with an association free energy of -6.2 kcal/mol, substitution of either D-Ala residue by L-Ala decreases the binding energy by more than 3 kcal/mol. While we have yet to produce a synthetic receptor which is as sequence-selective as vancomycin, we recently described a sulfone-based podand ionophore, **1**, which bound certain monopeptidic ammonium ions with enantioselectivity as high as 1.5 kcal/mol.[2] Here we show that its stereoselective binding properties extend to dipeptide-like substrates. High enantioselectivity for the N-terminal substrate residue is always observed but selectivity for next residue is significant only with real dipeptides.

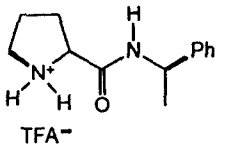
SCHEME 1



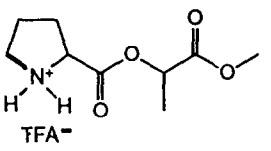
Podand Sulphone Receptor **1**



Pro-PheOMe Guests



Pro-PEA Guests



Pro-LacOMe Guests

In previous studies, we found that podand **1** selectively bound ammonium salts of L-amino acid derivatives. Among the amino acids we studied, derivatives of proline showed the most pronounced enantioselective binding ($\Delta\Delta G$ ~ 1.5 kcal/mol, 85% ee for L). Consequentially we studied the binding of **1** with dipeptide-like substrates derived from proline. When a D_2O solution of excess 1:1 L-Pro-L-AlaNHMe$^+$·PF_6^- and L-Pro-D-AlaNHMe$^+$·PF_6^- was shaken with ~10 mM **1** in $CDCl_3$, we found that the podand extracted primarily L-Pro-L-AlaNHMe into the organic phase.[3] Selectivity for the LL diastereomer was 50% de which corresponds to $\Delta\Delta G$ 0.65 kcal/mol. In this extraction, enough peptide was transfered to the organic phase to bind approximately 45% of the podand **1** in $CDCl_3$. Parenthetically, 18-crown-6 is a relatively poor host for our peptidic ammonium salts and extracts no observable guest from D_2O under the conditions of our experiments.

To study the origin of this selectivity, we measured the affinity of **1** for stereoisomeric proline derivatives[4] having two chiral centers. In these studies, we measured binding energies directly by ^1H NMR titrations in $CDCl_3$ to eliminate any effects due to differential hydration energies of diastereomeric substrates. To promote the solubility of our ammonium ions in $CDCl_3$, we chose hydrophobic substrates and trifluoroacetate (TFA^-) as the counterion. C-terminal substrate residues included an aminoacid ester (phenylalanine methyl ester) and analogs having no C-terminal functionality (α-phenethylamine) and no central amide bond (lactate methyl ester). Our results are summarized in Tables 1 and 2.

Table 1. Binding energies (kcal/mol) of stereoisomeric ammonium ions in $CDCl_3$[a]

Entry	Guest	$-\Delta G^c$ (sat)[b]	Entry	Guest	$-\Delta G^c$(sat)[b]	$\Delta\Delta G^d$
1	L-Pro-L-PheOMe$^+$TFA$^-$	3.21 (87%)	2	D-Pro-D-PheOMe$^+$TFA$^-$	2.20 (64%)	1.0
3	L-Pro-D-PheOMe$^+$TFA$^-$	2.67 (65%)	4	D-Pro-L-PheOMe$^+$TFA$^-$	1.85 (42%)	0.8
5	L-Pro-L-PEA$^+$TFA$^-$	3.64 (92%)	6	D-Pro-D-PEA$^+$TFA$^-$	2.24 (64%)	1.4
7	L-Pro-D-PEA$^+$TFA$^-$	3.72 (94%)	8	D-Pro-L-PEA$^+$TFA$^-$	2.24 (61%)	1.5
9	L-Pro-L-LacOMe$^+$TFA$^-$	3.57 (94%)	10	D-Pro-D-LacOMe$^+$TFA$^-$	2.58 (71%)	1.0
11	L-Pro-D-LacOMe$^+$TFA$^-$	3.48 (94%)	12	D-Pro-L-LacOMe$^+$TFA$^-$	2.59 (71%)	0.9

a. NMR titrations were carried out at 25°C by adding the guest to the podand sulfone host [1.5 mM] in $CDCl_3$. Binding energies were established by carrying out a nonlinear fit of the data to an ideal bimolecular binding equation of the form A + B <-> AB; b Percentage of total saturation achieved by the end of the titration; c. Free energy of association in kcal/mol, d Enantioselectivity in kcal/mol where $\Delta\Delta G=(\Delta G_{LL}-\Delta G_{DD})$ or $(\Delta G_{LD}-\Delta G_{DL})$.

As shown by the $\Delta\Delta G$ column in Table 1, podand **1** shows enantioselective binding corresponding to 0.8-1.5 kcal/mol for all substrates derived from L-proline. Similar enantioselectivity (~1.5 kcal/mol) was found for simple L-proline methyl esters and amides.[2]

This observation supports the notion that the geometrical mode of binding is similar for all substrates examined to that observed previously in x-ray structures of the complexes of **1**.

Comparing entries 1 and 3, we find diastereoselectivity corresponding to ~0.5 kcal/mol for the L-Pro-L-Phe methyl ester diastereomer which is similar to the above-mentioned diastereoselective extraction of the L-Pro-L-Ala methyl amide. Other comparisons are shown in Table 2 which summarizes all diastereoselectivities observed.

Table 2. Diastereomeric binding of ammonium ions in $CDCl_3$

Varying N-Terminal		Varying C-Terminal	
Entries	ΔΔG	Entries	ΔΔG
1-4	1.4	1-3	0.5
2-3	-0.5	2-4	0.4
5-8	1.4	5-7	-0.1
6-7	-1.5	6-8	0.0
9-12	1.0	9-11	0.1
10-11	-0.9	10-12	0.0

From Table 2, it may be seen that only in the case of the ProPhe substrates does the binding with **1** show any measurable sensitivity to the C-terminal stereochemistry. Selectivity observed with the ProPEA and ProLacOMe substrates derives almost totally from the chirality of proline. Thus the 0.4-0.6 kcal/mol diastereoselectivity observed with ProPheOMe and ProAlaNHMe substrates does not appear to originate solely from the properties of the C-terminal chiral center nor from its carboxylic substituent. Instead, a peptide link and a carboxylic chiral center substituent both seem necessary in order for **1** to sense the chirality of the C-terminal residue. It is interesting that the relatively subtle difference between ProAlaOMe and ProLacOMe is sufficient to give a measuable difference in diastereoselectivity.

While it is difficult to provide an convincing explanation of the ~0.5 kcal/mol diastereoselectivity with the dipeptidic substrates relative to the ProLac substrates, several differences between these structures may be important. First of all, the ProAla-derived substrates are composed of functional groups having the largest dipole moments and these may align favorably with the polar host functionality only in certain diastereomers. Another explanation for the different selectivity of ProPheOMe and ProLacOMe is the difference in the inherent conformational preferences for α-amido esters (e.g. preferred rotomers of bonds *a* and *b* in the Scheme) and α-ethero esters.[6] In any case, it is clear that subtle differences between guests can have significant effects on stereoselective binding properties and it may be difficult to quantify these without sophisticated computations.

During our binding measurements, we found that binding energies increased with the concentration of free trifluoroacetic acid (TFA) in the sample. With our ProPheOMe substrates and one equivalent of excess TFA, the binding energies with **1** were ~1 kcal/mol higher than those reported in Table 1. Since the binding energies of all substrates increased by approximately the same amount, we think it unlikely that the TFA is involved in a specific, stabilizing interaction with the complex. Instead, the TFA may be functioning by raising the energy of one of the unbound materials. Thus, TFA may associate with TFA^- and favor dissociation of the organoammonium trifluoroacetate ion pair leaving the substrate ammonium ion substrate more free for association with **1**.[7]

References and Notes:

(1) (a) Pekins, H. R. *Pharmac. Ther.* 1982, **16**, 181; (b) Barna, J. C. J.; Williams, D. H. *Ann. Rev. Microbiol.* 1984, **40**, 569; (c) Williamson, M. P.; Williams, D. H.; Hammond, S. J. *Tetrahedron* 1984, **40**, 569.
(2) Li, G.; Still, W. C. *J. Org. Chem.* 1991, **56**, 6964.
(3) Standard extraction method, see: (a) Cram, D. J. *Angew. Chem. Int. Ed. Engl.* 1988, **27**, 1009; (b) Iimori, T.; Erickson, S. D.; Rheingold, A. L.; Still, W. C. *Tetrahedron Lett.* 1989, **30**, 6947; (c) Wang, X.; Erickson, S. D.; Iimori, T.; Still, W. C. *J. Am. Chem. Soc.* 1992, **114**, 4128.
(4) All the guests are made by standard methods involving DCC coupling of Boc-Proline with phenylalanine methyl ester HCl salt, phenylethyl amine and methyl lactate followed by BOC deprotection using TFA in CH_2Cl_2.
(5) After deprotection of BOC group, the reaction mixture was concentrated and the resulting material (a) crystallized from hexane/$CHCl_3$ as thin needles. Anal. Calcd for $C_{19}H_{22}N_2O_7F_6$: C, 45.24; H, 4.36; N, 5.55. Found: C, 45.46; H, 4.20; N, 5.55, or (b) crystallized from toluene as thin plates. Anal. Found for LD isomer: C, 45.36; H, 4.29; N, 5.50. Anal Found for DL isomer: C, 45.29; H, 4.27; N,5.62.
(6) T. Head-Gordon, M. Head-Gordon, M.J. Risch, C.L. Brooks, J. A. Pople, *J. Am. Chem. Soc.*, **113**, 5989 (1991); H.-J. Bohm and S. Brode, *J. Am. Chem. Soc.*, **113**, 7129 (1991); J.A. Tucker, K.N. Houk and B.M. Trost, *J. Am. Chem. Soc.*, **112**, 5465 (1990).
(7) This work was supported by NIH Grant HL25364.