and reveals the sequence specificity of binding when the end-labeled products are subjected to high resolution denaturing gel electrophoresis. Reactions were performed in the presence of 12 mM Mg^{2+} and 135 mM KCl,[13] and enzymatic conditions were chosen to ensure that the extent of RNA:DNA hybridization was rate limiting.[14]

All probes bind and induce RNase H cleavage at their targeted site(s) (Figure 2A). Comparison of the site-specific cleavage induced by TOP **1** with that induced by probes **4** and **6** (which contain only one oligodeoxynucleotide) indicates a significant increase in yield at *both* sites when the two oligodeoxynucleotides are united in a single molecule. Comparison of TOP **2** with probes **5** and **7** shows the identical trend. Neither **10** nor **11** induces RNA cleavage at either site, demonstrating that the 5'-site cleavage enhancement depends on *sequence-specific* hybridization at the 3'-site. None of the TOPs induce cleavage at several partially complementary sites (Figure 1), providing evidence that secondary structure has been maintained.[15] Thus, TOPs **1** and **2** hybridize cooperatively and sequence-specifically to the SL RNA, and the hybridization efficiency of TOP **1** is higher.

Selective competition experiments demonstrate cooperative formation of a 1:1 complex. RNA was incubated with RNase H, TOP, and an excess of either UCCAAAAUUU or TCCAAAATTT. If binding of the TOP to the 5'-site depends explicitly on simultaneous binding to the 3'-site, and the concentration of the competing probe is high enough to displace the TOP 5'-end, then the TOP 3'-end should be unbound at equilibrium with a concomitant loss of RNase H sensitivity at bases 13-19.[16] If binding is noncooperative or multimeric, a significant fraction of TOP 3'-ends will be bound at the 5'-site and detected by RNase H. As shown in Figure 2B, competition with excess UCCAAAAUUU or TCCAAAATTT *causes the 5'-site cleavage yield to decrease for all three TOPs*. In contrast, cleavage at the 5'-site is unaffected when the experiment is performed in the presence of untethered oligonucleotides **8** (TCCAAAATTT) and **9** (GTTCTTC). Addition of noncomplementary AAAUUUUGGA has no (**1** or **2**) or little (**3**) effect on RNase H sensitivity at either site. Moreover, an oligoribonucleotide complementary to the 5'-site causes a reduction in cleavage yield at both the 5'- and 3'-sites when TOPs **1-3** are tested but not when the experiment is performed with **8** and **9**.[17] This data demonstrates that the two oligonucleotide segments within each TOP interact cooperatively, and both ends bind *simultaneously* to a single molecule of the SL RNA. Because they combine the increased sequence selectivity provided by two oligonucleotides with the structural specificity of a synthetic tether, *TOPs offer the potential to characterize and differentiate tertiary structures in globular RNAs and RNPs.*[18,19] Experiments to address this question are underway.

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Supplementary Material Available: Experimental procedures for the synthesis and characterization of **1-11** (2 pages). Ordering information is given on any current masthead page.

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(14) Increasing the amount of enzyme in the reaction mixture by 300% increased the fraction of RNA cleaved by less than 15%.
(15) Sites of partial complementarity are indicated in Figure 1 in boldface type. Our experiments do not exclude the possibility that the TOPs themselves influence RNA structure.
(16) The lifetime of the SL RNA:**1** complex is less than 5 min at 25 °C, assuring that equilibrium is established during a 2-h incubation with RNase H.
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Highly Selective Binding of Simple Peptides by a C_3 Macrotricyclic Receptor

Jong-In Hong, Sung Keon Namgoong, Anna Bernardi, and W. Clark Still*

Department of Chemistry, Columbia University
New York, New York 10027

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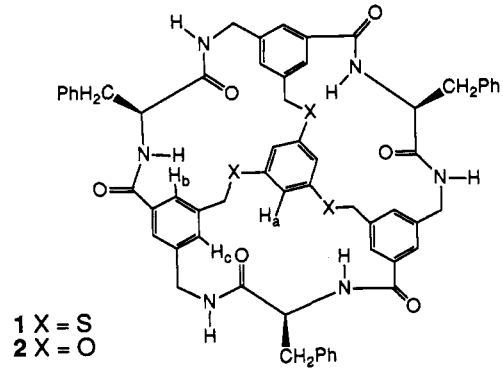
High selectivity in the binding of various substrates to a host molecule is often dependent upon conformational homogeneity and substantial host/guest contact. In this communication, we describe two chiral, C_3-symmetric receptors (**1** and **2**) having only limited conformational flexibility and deep basket-like binding sites.[1] These molecules bind diamides of certain amino acids with high selectivity which is dependent upon the nature of the amino acid side chain (~2 kcal/mol for serine vs alanine) and the identity of the *N*-alkyl substituent (>3 kcal/mol for methyl vs *tert*-butyl). They are also among the most enantioselective synthetic receptors yet prepared[2] and bind certain derivatives of L-amino acids with selectivities as high as 3 kcal/mol.



The syntheses (see supplementary material) of **1** and **2** utilized their C_3 symmetry and began with trialkylation of 1,3,5-trimercaptobenzene[3] or phloroglucinol with N-protected methyl 3-(aminomethyl)-5-(bromomethyl)benzoate. After coupling with Boc-L-phenylalanine (Phe), a triple macrolactamization via a tris(pentafluorophenyl ester) provided **1** and **2** in 30% and 7% yields, respectively.

Receptors **1** and **2** are exceptional in that Monte Carlo conformational searching[4] using the MacroModel/AMBER[5] force

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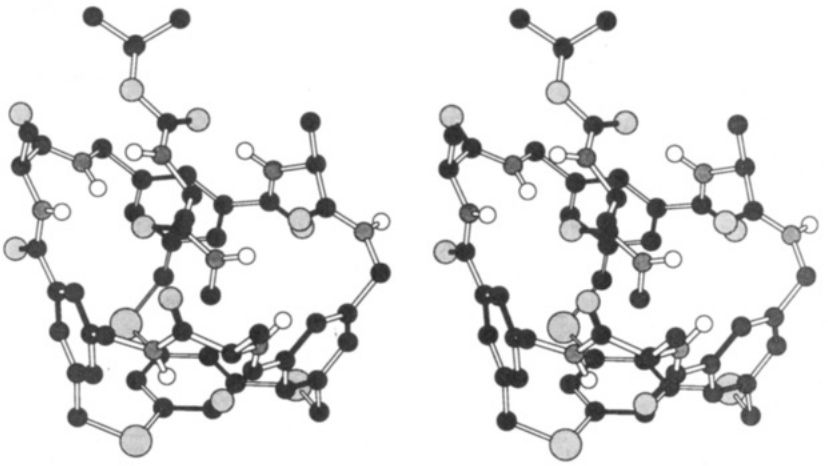

Figure 1.

Table I. ΔG's of Association (kcal/mol) of **1** and **2** with Simple Peptides

		$-\Delta G$,[a] kcal/mol		saturation,[b] %		$\Delta\Delta G$,[c] kcal/mol	
entry	peptide substrate	**1**	**2**	**1**	**2**	**1**	**2**
1	*N*-Boc-D-Ala-NHMe	1.7	2.1	53	70		
2	*N*-Boc-L-Ala-NHMe	3.9	3.8	93	90	2.2	1.7
3	*N*-Boc-L-Ala-NHBn	1.4		51			
4	*N*-Boc-L-Ala-NHtBu	nc[d]					
5	*N*-Boc-D-Val-NHMe	1.5	1.5	51	54		
6	*N*-Boc-L-Val-NHMe	4.4	4.0	79	74	2.9	2.5
7	*N*-Boc-D-Leu-NHMe	1.5	1.6	64	60		
8	*N*-Boc-L-Leu-NHMe	4.1	3.8	88	78	2.6	2.2
9	*N*-Boc-D-Ser-NHMe	3.8	4.4	86	94		
10	*N*-Boc-L-Ser-NHMe	>6.1	>6.2	95	96	>2.3	>1.8
11	*N*-Boc-L-Ser(OBn)-NHMe	3.1		83			
12	*N*-Boc-D-Thr-NHMe	3.2	3.6	84	90		
13	*N*-Boc-L-Thr-NHMe	>6.2	lg[e]	>95		>3.0	
14	*N*-Ac-D-Ala-NHMe	2.7		90			
15	*N*-Ac-L-Ala-NHMe	3.9		94		1.2	
16	*N*-Ac-D-Ala-NHtBu	2.0		59			
17	*N*-Ac-L-Ala-NHtBu	3.0		85		1.0	

[a] Measured by NMR titration at 25 °C with **1** or **2** at 0.5 mM concentration in $CDCl_3$. [b] Extent of extrapolated saturation at end of titration. [c] Enantioselectivity, $\Delta G(D) - \Delta G(L)$. [d] No complexation detected. [e] Too large to measure accurately.

field predicts them (Phe modeled by Ala) to exist largely in a single family of closely related conformations having near or perfect C_3 symmetry (see supplementary material). All low-energy conformations have Phe's folded into γ-turns around the periphery of a large binding cavity with dimensions (~6 Å diameter) similar to those of α-cyclodextrin. They differ primarily in the central ring Ar–X–CH_2–Ar′ torsion angles, differences that make only insignificant changes in the shape and nature of the binding cavity. These structures are compatible with available experimental evidence including NH–CH_α coupling constants ($J(\mathbf{1})$ = 8.1 Hz; $J(\mathbf{2})$ = 8.0 Hz)[6] and the presence of both free and hydrogen-bonded N–H infrared bands (3434, 3321 cm^{-1}) in dilute $CDCl_3$ solution. Simulated annealing suggests the conformation to change little upon binding: the lowest energy complex with Boc-L-alanine-NHMe found is shown in stereo in Figure 1. The molecular mechanics model of the complex is held together by three N—H/O═C hydrogen bonds.

As summarized in Table I, receptors **1** and **2** show high binding selectivity among simple peptides. With Boc-protected, *N*-methylamide amino acid derivatives, enantioselectivity ranges from 1.7 to 3.0 kcal/mol with the L isomer always being bound preferentially (entries 1/2, 5/6, 7/8, 9/10, 12/13). Side-chain functionality can also be distinguished by our receptors as shown in entries 1–8 vs 9,10 and 12,13. Here the side-chain hydroxyls of serine and threonine contribute ~2 kcal/mol to association energies and effectively distinguish these amino acids from Ala, Val, and Leu. Such hydroxylated L-amino acids bind better than *O*-benzyl-L-serine (entry 11) by ~3 kcal/mol.

Only Boc-protected peptides with small *N*-methyl C-termini bind tightly (entry 2 vs 3,4). The sensitivity of binding to C-terminal steric effects is compatible with a complex in which an *N*-methylamide is buried deep within the binding cavity as shown in Figure 1. This structure is supported by the NMR spectra of the complexes of **1** and **2** with Boc-L-threonine *N*-methylamide: in accord with the proposed structure which locates the *N*-methyl group near the shielding faces of all four macrocyclic aromatics, the *N*-methyl resonance shifts from 2.8 ppm to −0.8 ppm upon complexation. Similar shifts were found with other complexes of **1** and **2**. Additional support comes from intermolecular NOE experiments which indicate contacts between the threonine *N*-methyl and protons H_a, H_b, and H_c of **1**. Entries 14–17 suggest that other binding modes are available to amino acid derivatives having small N-terminal functionalities such as acetyl.

The high selectivity and generality of these simple receptors for L-amino acid derivatives make them resemble the binding sites of naturally occurring enzymes. Work directed toward extending their selectivity is in progress.[7]

Supplementary Material Available: Synthetic schemes for **1** and **2** and C_3 global minimum of **1** found by conformational search (2 pages). Ordering information is given on any current masthead page.

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(7) Supported by NIH GM44525 and NSF CHE89-11008.