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Supplementary Material Available: Experimental, spectroscopic, and analytical details and tables of atomic coordinates, bond angles and distances, anisotropic thermal parameters, and hydrogen-atom coordinates (10 pages); listing of observed and calculated structure factors (28 pages). Ordering information is given on any current masthead page.

Enantioselective Complexation of Simple Amides by a C_2 Host Molecule

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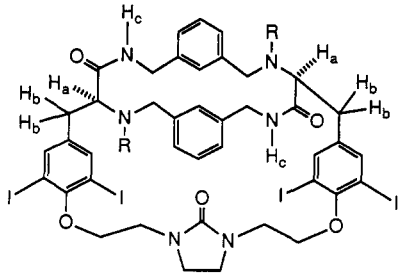
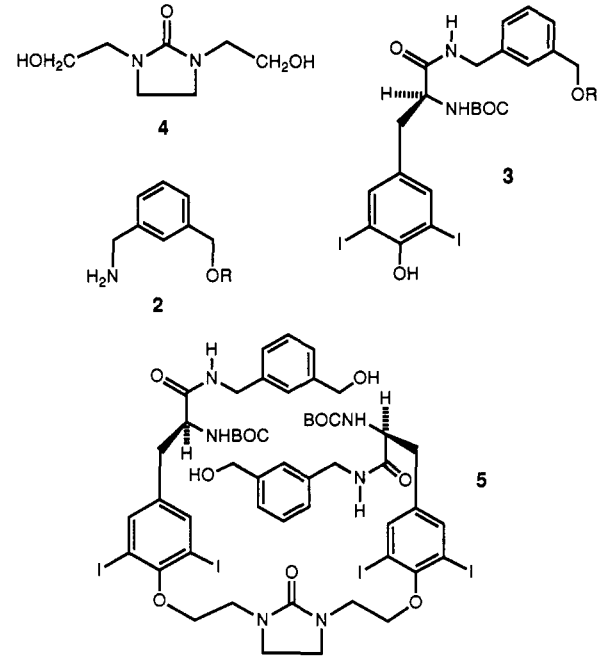
The creation of hydrogen bonds provides an effective driving force for forming molecular complexes in organic solvents.[1] When several hydrogen bonds can be made during complexation, substrates are often oriented within the binding site in geometries that maximize hydrogen bonding. When oriented binding in one geometry (or at most a few) can be achieved, there is potential for highly selective substrate binding. In this communication, we describe an enantiomerically pure, C_2 host molecule (**1**) that binds donor/acceptor guests by multiple hydrogen bonds. As we will show, **1** binds simple amides in benzene and distinguishes both energetically and spectrally between certain enantiomeric amides. This study describes one of the few synthetic hosts that show a measurable difference in its binding energies with enantiomeric neutral guests.[2]



1a R = H
1b R = CH_2Ph

Synthesis of **1** begins with L-BOC-diiodotyrosine. After condensation (DCC, HOBt, THF, 76%) with benzylic amine **2** (R = $SiPh_2tBu$) to give **3**, we used a double Mitsunobu reaction to join the phenolic peptide side chain to the diethanolurea **4**[3] and deprotected with Bu_4NF to provide **5** (39% yield). We then converted the benzylic alcohols to bromides (Ph_3P, CBr_4), removed the BOC protecting groups (TFA, CH_2Cl_2), and carried out an alkylative double macrocyclization (iPr_2NEt, CH_3CN, 2.5 mM, reflux) to give **1a** (23–47% yield from **5**). Treatment with excess BnBr gave **1b**.



X-ray structures of **1a** and **1b** were determined (see supplementary data).[4] As with a related meso host,[1h] **1** was found in two distinct conformations. These conformations differ most significantly by the orientation of their bridgehead hydrogens (H_a), which may point either away from (**1a**) or in toward (**1b**) the center of the host. Each conformation has an internal cavity which is occupied by CH_2Cl_2 in the crystal.

In addition to binding donor/acceptor heterocycles such as imidazole in organic solvents, **1b** (~2.0 mM) forms complexes with unhindered carboxylic amides in C_6D_6 (see Table I). Upon complexation, the NMR spectra of host and guest undergo major changes. For example, with *N*-methylacetamide the amide N–H's of both host and guest shift downfield by >1.0 ppm. The acetyl methyl undergoes a 0.5-ppm unfield shift, which is compatible with its location near a shielding face of an aromatic ring. We observed similar shifts in the other amide complexes examined. In the case of the *N*-methylacetamide complex, difference NOE studies further established proximity of the acetyl methyl with both the bridgehead hydrogens (H_a) and the amide N–H's (H_c) of the host. We also observed a strong intramolecular NOE between H_a and H_c. These NMR results are compatible with a structure for the complex that is related to the X-ray conformation of **1b** and found by molecular modeling to be as follows.

To locate low-energy structures of the **1b**/amide complex, we carried out local conformational searches using molecular dy-

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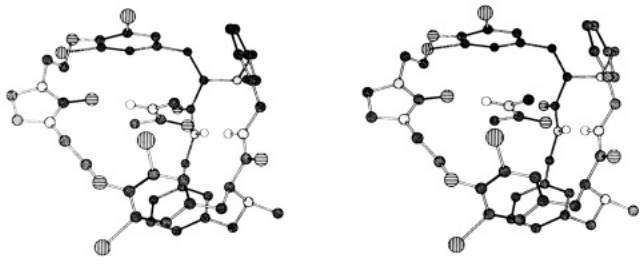
Table I. Free Energies of Association for **1b** and Amides in C_6D_6

substrate	binding energy, kcal/mol (enantiomer)	saturation achieved, %	enantioselection: $\Delta\Delta G$, kcal/mol
MeNHCOMe	−3.17	65	
MeNHCOBn	−2.18	62	
BnNHCOH	−3.24	64	
BnNHCOMe	−2.84	48	
$BnNHCOCF_3$	no complex observed		
BnNHCOEt	−2.33	67	
PhCHMeNHCOMe	−3.04 (*S*), −2.62 (*R*)	56 (*S*), 67 (*R*)	0.42
PhCHMeNHCOH	−3.18 (*S*), −2.85 (*R*)	57 (*S*), 48 (*R*)	0.33
PhCHMeNHCOEt	−1.80 (*S*), −1.55 (*R*)	56 (*S*), 45 (*R*)	0.25
1-NpCHMeNHCOMe	−2.56 (*S*), −2.31 (*R*)	57 (*S*), 51 (*R*)	0.25
BnOAlaNHCOMe	−2.29 (*S*), −1.81 (*R*)	64 (*S*), 50 (*R*)	0.48
MeOPGlyNHCOMe	−1.91 (*S*), −2.06 (*R*)	44 (*S*), 45 (*R*)	−0.15

namics[5] starting from the two conformations of **1** observed by X-ray crystallography. In these simulations, the benzyl groups of **1b** were replaced by methyls. After energy minimizing using the OPLS/AMBER force field[6] with *N*-methylacetamide in the binding cavity, we carried out 250 ps of molecular dynamics at 300 K. The average potential energy stabilized within the first 50 ps. Simulated annealing to ~50 K over 100 ps and energy minimizing gave the final conformers. The conformer of the complex derived from the **1b** crystal structure was found to be more stable by 2.5 kcal/mol in steric energy. When the rigid rotor/harmonic oscillator approximation is used, it is also higher in entropy by 8.8 cal deg^{-1} mol^{-1} than the **1a**-derived complex and thus is 5.1 kcal/mol more stable in free energy at 300 K. Its stereostructure is shown below:



As revealed in the structure above, the atoms bearing hydrogens that display the described NOE signals are indeed close in space. Furthermore, the observed coupling constants for hydrogens of the diiodotyrosine α and β carbons in the complex ($J_{a,b}$ = 2.8 and 9.2 Hz) are similar to those calculated by using Altona's equation[7] (1.4 and 9.8 Hz). If the **1**/amide complex has the geometry shown, then we would expect selective binding with the amides of primary amines having nitrogen attached to a chiral center of the *S* configuration.[8]

As summarized in the table, we do indeed find enantioselective binding of **1b** with certain chiral amides. Binding energies were measured by NMR titration, and error propagation analysis gives error limits of ±0.1 kcal/mol. While the chiral binding differences are not large, they lie well outside the error range of the measurements. Except for the acetamide of phenylglycine (PGly) methyl ester, which has substituents having similar steric demands,[9] it is the *S* enantiomer that binds more tightly. Distinctions between amide enantiomers were also observed by ^1H NMR. With PhCHMeNHCHO, for example, signals from the two enantiomers for the chiral methine hydrogen and the formamide C–H and N–H separated by >0.1 ppm upon treatment with **1b**.

It should be easy to design chiral hosts that bind enantiomeric guests with significantly different association energies because the thermodynamics of enantiomeric complexation are relatively simple. Enantiomeric guests have identical solvation energies, and differences in binding energies result exclusively from the relative stabilities of the complexes. In contrast, differences in the solvation energies of nonenantiomeric guests can have a major effect on selectivity.[10] Nevertheless, many previous reports of chiral hosts note little detectable difference in the energies of diastereomeric complexes. A likely explanation is that many different conformations of complexes are involved. In our host, cyclophane linkages, bridged macrocyclic structures, and C_2 symmetry all operate to reduce but not eliminate conformational heterogeneity. Further rigidification is clearly desirable and should provide enhanced enantioselection.[11]

Supplementary Material Available: Stereopair plots of the X-ray structures of **1a** and **1b** (1 page). Ordering information is given on any current masthead page.

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Proline Assignments and Identification of the Cis K116/P117 Peptide Bond in Liganded Staphylococcal Nuclease Using Isotope Edited 2D NMR Spectroscopy

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Proline is usually the most difficult type of amino acid residue to assign in a protein because the pyrrolidine ring lacks an amide proton, and therefore the essential sequential connectivities involving this proton are absent.[1,2] Although connectivities involving the proline δ-protons can substitute for the lacking amide proton connectivities,[1,3] the δ-protons are often difficult to identify because

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