

the overall intensities, which decrease in the order  $4 > 1 > 2 > 3$ . Table I lists fluorescence decay rate constants<sup>19a</sup> for **4** and electron-transfer rate constants<sup>19b</sup> and exothermicities<sup>19c</sup> for **1-3** in five solvents.

In solvents more polar than diethyl ether,<sup>25</sup> the electron-transfer rate constants in DSAs **2** and **3** are from three to seven and from four to 11 times larger, respectively, than those in DSA **1**. Hence, either  $|V|$  or the FCWDS must be larger in **2** and **3**. Since electron transfer in **1** is 0.3 eV more exothermic (Table I) than in **2** and the center to center distances ( $R_{cc}$ ) are comparable,  $|V|$  in DSA **2** must be larger than in DSA **1**.<sup>20</sup> This qualitative conclusion is supported by the  $|V|$ 's, listed in Table I, that were obtained<sup>21</sup> from the electron-transfer data using the semiclassical formulation of the transfer rate constant<sup>13</sup> in conjunction with Marcus' expression for the solvent reorganization energy<sup>22</sup> and the Born correction to the reaction exothermicity.<sup>4</sup> DSAs **1** and **2** are nearly linear and contain all trans arrays of spacer bonds; thus, the dominant contribution to  $|V|$  is through-bond coupling.<sup>4</sup>  $|V|$  in the symmetry-forbidden DSA, **1**, is only 4-7% as large as in the symmetry-allowed DSA, **2**. The reduction in  $|V|$  from **2** to **1** translates into a 200-800-fold reduction in the optimal transfer rate constant<sup>23</sup> for the symmetry-forbidden DSA, which represents the symmetry restriction to electron transfer in these molecules.

Qualitative comparison of the transfer rate constants in **3** to those of **1** and **2** is made difficult by the gauche link in the spacer of **3**. The shorter  $R_{cc}$  in **3** effects a decrease in the solvent reorganization energy and increases in the driving force and FCWDS in comparison to **2**. Furthermore, through-bond contributions to  $|V|$  are likely reduced by the gauche link<sup>4,12</sup> in **3**, whereas through-solvent coupling could be augmented.<sup>4e,24</sup> Neglecting the results from diethyl ether,<sup>25</sup> quantitative analysis (Table I) indicates that introduction of a single gauche link in the symmetry-allowed spacers (**2** → **3**) diminishes the optimal transfer rate constant<sup>23</sup> by a factor of 3-11. This concurs with results of Oliver et al., who have reported<sup>4b</sup> rate reductions by factors of 3-14 attending a single trans to gauche substitution in DSAs with nearly identical  $R_{cc}$ 's. We have not yet determined the contribution of through-solvent coupling in **3**. Furthermore, we have not identified the cause of the factor of 2 reduction in  $|V|$ (**2**) and  $|V|$ (**3**) observed in the more polar solvents, particularly since  $|V|$ (**1**) is effectively solvent independent. The larger  $|V|$  in THF, ethyl acetate, and ether (**3**) could originate from symmetry-dependent through-solvent coupling. Alternatively, inadequate values of the sol-

vent-dependent reaction and reorganization energies could be responsible.

Within the standard model,<sup>4,13,22</sup> these experiments demonstrate that electronic symmetry *can* modulate electron-transfer rate constants by at least 2 orders of magnitude. This observation is consistent with literature methods for evaluation of electronic coupling matrix elements, which reduce to 0 for appropriate DSA symmetries.<sup>3,8,9</sup> In order to experimentally achieve larger symmetry restrictions, the contributions of vibrations and vibronic coupling to the relaxation of the symmetry constraints must be better understood. If  $S_1-S_n$  vibronic coupling contributes significantly to the electronic mixing,  $|V|$  could be temperature dependent. Measurement of the temperature dependence of the electron-transfer rate constants and the dependence of the redox potential on solvent and temperature will provide more quantitative characterization of  $|V|$ . Moreover, elimination of the larger reaction exothermicity and conformational freedom associated with the acceptor in **1** will provide a more direct measure of symmetry effects in electron-transfer reactions. These experiments are currently in progress.

**Acknowledgment.** We gratefully acknowledge support of this work by the National Science Foundation. We thank ILC Technology for the donation of xenon laser lamps, Professor Dwight Sweigart for the electrochemical redox potential measurements and Professor Jan Verhoeven and Dr. Samir Farid for informative discussions.

## Tethered Oligonucleotide Probes. A Strategy for the Recognition of Structured RNA

Paul L. Richardson and Alanna Schepartz\*

Sterling Chemistry Laboratory, Yale University  
225 Prospect Street, New Haven, Connecticut 06511

Received February 7, 1991

The past decade has been marked by research directed toward the design of compounds capable of recognizing DNA sequences<sup>1,2</sup> and shapes.<sup>3</sup> Molecules capable of recognizing RNAs are virtually unknown,<sup>4</sup> largely because of the structural complexity of these macromolecules<sup>5</sup> and the scarcity of information describing their

(19) (a) Fluorescence waveforms obtained with a Hamamatsu R1645U microchannel plate connected to a Tektronix TD7912 digitizer. Lifetimes obtained by nonlinear least squares fitting waveforms to the system response convolved with a single exponential decay. (b) Electron-transfer rate constants determined as  $(1/\tau(X)) - (1/\tau(4))$ . (c) Redox potentials measured by using cyclic voltammetry at 22 °C in  $\text{CH}_3\text{CN}$  containing 0.1 M tetrabutylammonium hexafluorophosphate versus Ag/AgCl. The oxidation potential of the donor is 0.894 eV. The reduction potentials of the dicarbonyl ethylene and dicyanoethylene acceptors are -1.64 eV and -1.9 eV, respectively. The dicyanoethylene reduction waveform is irreversible at scan rates of 100 mV/s. The reduction potential is accurate to within 100 mV (see ref 4a). The reaction exothermicity was calculated as in ref 4a.

(20) The reaction exothermicities are insufficient to access the inverted region in these solvents.

(21) The matrix element calculation is sensitive to the Born radii assumed for the donor and acceptors. Changing the acceptor radii in the range from 3.0 to 5.0 Å generates  $|V(2)|/|V(1)|$  ratios of 3-40 in acetonitrile. The acceptor radii employed are 3.9 and 4.2 Å for the acceptors in **2** and **1**, respectively, and 5.0 Å for the donor. As experimental values for these radii are lacking, the radii were obtained from the equivalent excluded volume spheres. We are attempting to determine these radii from electrochemical measurements.

(22) (a) Marcus, R. A. *Can. J. Chem.* **1959**, *37*, 155. (b) Marcus, R. A. *J. Chem. Phys.* **1956**, *24*, 966.

(23) The optimal transfer rate constant is proportional to  $|V|^2$  and refers to the situation where FCWDS is 1.

(24) (a) Miller, J. R.; Beitz, J. V.; Huddleston, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 5057. (b) Miller, J. R.; Peoples, J. A.; Schmitt, M. J.; Closs, G. L. *J. Am. Chem. Soc.* **1982**, *104*, 6488. (c) Miller, J. R.; Beitz, J. V. *J. Chem. Phys.* **1981**, *74*, 6746. (d) Guarr, T.; McGuire, M. E.; McLendon, G. *J. Am. Chem. Soc.* **1985**, *107*, 5104.

(25) In diethyl ether, the nearly thermoneutral transfer in **2** and **3** could produce repopulation of  $S_1$  from the electron-transfer state, an increase in the observed lifetime, and a decreased  $|V|$ .

\* To whom correspondence should be addressed.

(1) Dreyer, G. B.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 968-972. Chu, C. F.; Orgel, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 963-967. Dervan, P. B. *Science* **1986**, *232*, 464-471. Chen, C. H. B.; Sigman, D. S. *Science* **1987**, *217*, 1197-1201. Helene, C.; Thuong, N. T. *Nucleic Acids Mol. Biol.* **1988**, *2*, 105-123. Corey, D. R.; Pei, D.; Schultz, P. G. *J. Am. Chem. Soc.* **1989**, *111*, 8523-8525. Pei, D.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 4579-4580. Corey, D. R.; Pei, D.; Schultz, P. G. *Biochemistry* **1990**, *28*, 8277-8286. Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645-650. Griffin, L. C.; Dervan, P. B. *Science* **1989**, *245*, 967-971. Strobel, S. A.; Dervan, P. B. *Science* **1990**, *249*, 73-75. Sun, J.-S., et al. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9198-9202. Francois, J. C.; Saison-Behmoaras, T.; Chassignol, M.; Thuong, N. T.; Helene, C. *J. Biol. Chem.* **1989**, *264*, 5891-5898.

(2) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543-584.

(3) Barton, J. K.; Basile, L. A.; Danishefsky, A.; Alexandrescu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1961-1965. Barton, J. K. *Science* **1986**, *233*, 727-734. Mei, H. Y.; Barton, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 7414-7416. Pyle, A. M.; Long, E. C.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 4520-4523.

(4) Zuckermann, R. N.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1766-1770. Chow, C. S.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 2839-2841. Carter, B. J.; de Vroom, E.; Long, E. C.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9373-9377.

(5) Wyatt, J. R.; Puglisi, J. D.; Tinoco, I., Jr. *BioEssays* **1989**, *11*, 100-106.

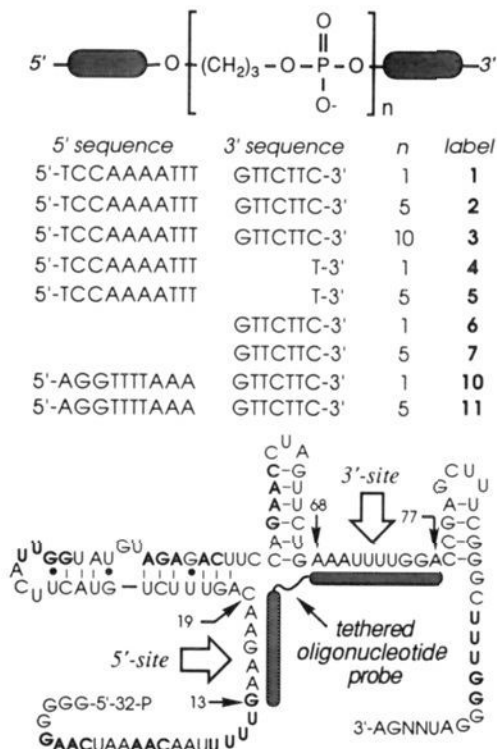
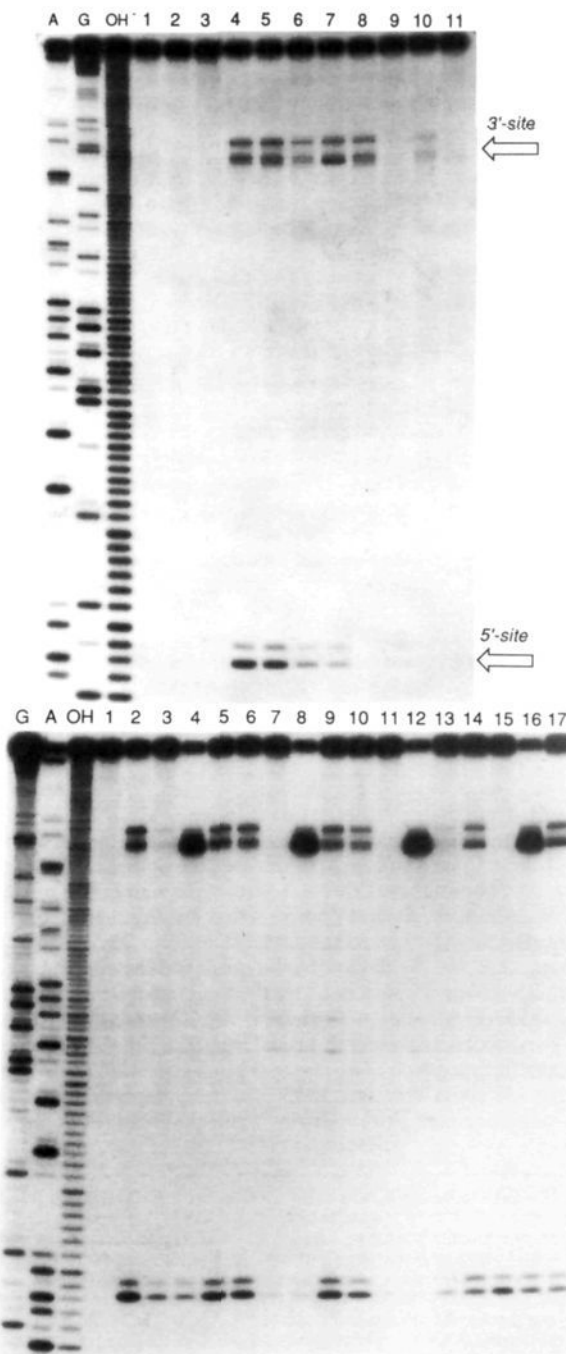


Figure 1.

tertiary structures.<sup>6</sup> In this communication we describe a strategy for the recognition of structured RNA.

Tethered oligonucleotide probes (TOPs) consist of two oligodeoxynucleotides linked by a tether. The oligodeoxynucleotides complement two *nonduplex and noncontiguous* sites on the RNA and are separated by a tether whose length spans the distance between them (Figure 1). We reasoned that if the two oligodeoxynucleotides in each TOP bound the RNA cooperatively,<sup>7</sup> a single molecule would result with greater sequence and structure specificity than the original two.<sup>8</sup> In this communication we report that TOPs bind cooperatively, monomerically, and with high affinity to two noncontiguous, nonhelical sites within the *Leptomonas collosoma* SL RNA.<sup>9</sup>

Each TOP contains the DNA complementary to RNA bases 13–19 (5'-site) and 68–77 (3'-site)<sup>10</sup> separated by a tether containing either one, five, or ten abasic phosphodiester units.<sup>11</sup> This tether mimics the chemical composition of DNA but permits considerable conformational flexibility. Binding was detected by using RNase H which selectively hydrolyzes RNA:DNA hybrids,<sup>12</sup>



**Figure 2.** Top (A): Autoradiogram of a 15% denaturing polyacrylamide gel illustrating RNase H cleavage of 5'-<sup>32</sup>P-end-labeled SL RNA in the presence of probes 1–9. Lanes labeled A, G, and OH<sup>-</sup> represent enzymatic (A,G) and alkaline (OH<sup>-</sup>) sequencing lanes. Lane 1: intact RNA control (no RNase H). Lane 2: RNase H control. Lane 3: 15 μM **1** (no RNase H). Lane 4: 150 nM **1**. Lane 5: 150 nM **2**. Lane 6: 150 nM **3**. Lane 7: 150 nM each **8** and **9**. Lane 8: 150 nM **4**. Lane 9: 150 nM **6**. Lane 10: 150 nM **5**. Lane 11: 150 nM **7**. Unless otherwise indicated, all reactions contained 1.5 μM SL RNA, 52 mM Tris-Cl 2 mM HEPES pH 8.0, 12 mM MgCl<sub>2</sub>, 1.5 mM DTT, 135 mM KCl, 0.7 mM EDTA, 0.5 μg of BSA, between 2 and 10 units of RNasin, 0.8 units of RNase H, and 5% glycerol. SL RNA was renatured (70 °C) and cooled slowly to 25 °C prior to addition of probe, RNasin, and RNase H. Reactions were incubated (2 h), quenched (100 mM EDTA), and electrophoresed. Bottom (B): Autoradiogram of a 15% denaturing polyacrylamide gel illustrating competition of 5'-<sup>32</sup>P-end-labeled SL RNA cleavage by UCCAAAAUUU, TCCAAAATT, and AAAUUUGGA. Lane 1: SL RNA control. Lanes 2–5: competition of **1** (150 nM). Lanes 6–9: competition of **2** (150 nM). Lanes 10–13: competition of **3** (150 nM). Lanes 14–17: competition of oligonucleotides **8** and **9** (150 nM each). Lanes 2, 6, 10, and 14: no inhibitor. Lanes 3, 7, 11, and 15: 15 μM UCCAAAAUUU. Lanes 4, 8, 12, and 16: 30 μM TCCAAAATT. Lanes 5, 9, 13 and 17: 15 μM AAAUUUGGA. Competitor and TOP were added simultaneously.

(6) Kim, S. H.; Sussman, J. L.; Suddath, F. L.; Quigley, G. J.; McPherson, A.; Wang, A. H. J.; Seeman, N. C.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4970–4974. Quigley, G. J.; Rich, A. *Science* **1976**, *194*, 796–806. Woo, N. H.; Roe, B. H.; Rice, A. *Nature* **1980**, *286*, 346–351. Westof, E.; Dumas, P.; Moras, D. *J. Mol. Biol.* **1985**, *184*, 119–145. Rould, M. A.; Perona, J. J.; Soll, D.; Steitz, T. A. *Science* **1989**, *246*, 1135–1142. tRNA NMR: Patel, D. J.; Shapiro, L.; Hare, D. Q. *Rev. Biophys.* **1987**, *20*, 78–90. Cheong, C.; Varrani, G.; Tinoco, I. *Nature* **1990**, *346*, 680–682. Puglisi, J. D.; Wyatt, J. R.; Tinoco, I., Jr. *Biochemistry* **1990**, *29*, 4215–4226. Varrani, G.; Wimberly, B.; Tinoco, I., Jr. *Biochemistry* **1989**, *28*, 7760–7772.

(7) Maher, L. J., III; Dolnick, B. J. *Arch. Biochem. Biophys.* **1987**, *253*, 214–220. Strobel, S. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 7286–7287. Horne, D. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 2435–2437.

(8) Becker, M. M.; Dervan, P. B. *J. Am. Chem. Soc.* **1979**, *101*, 3664–3666. Dervan, P. B.; Becker, M. M. *J. Am. Chem. Soc.* **1978**, *100*, 1968–1970. Chen, C.-W.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 4921–4922. Wakelin, L. P. G. *Med. Res. Rev.* **1986**, *6*, 275–340. See also: Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4046–4050.

(9) Bruzik, J. P.; Van Doren, K.; Hirsh, D.; Steitz, J. A. *Nature* **1988**, *335*, 559–562.

(10) Milhausen, M.; Nelson, R. G.; Sather, S.; Selkirk, M.; Agabian, N. *Cell* **1984**, *38*, 721–729.

(11) Seela, F.; Kaiser, K. *Nucleic Acids Res.* **1987**, *15*, 3113–3129.

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<sup>2+</sup> and 135 mM KCl,<sup>13</sup> and enzymatic conditions were chosen to ensure that the extent of RNA:DNA hybridization was rate limiting.<sup>14</sup>

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.<sup>15</sup> 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 UCCAAAUUU 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.<sup>16</sup> 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 UCCAAAUUU 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 AAAUUUUGA 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.<sup>17</sup> 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.<sup>18,19</sup> Experiments to address this question are underway.

**Acknowledgment.** We thank S. White, R. Gregorian, and D. Jeruzalmi for gifts of T7 RNA polymerase and T. Shrader, D. Crothers, J. Steitz, and members of the Schepartz group for helpful discussions. Paul Richardson is a Pfizer Predoctoral Fellow. This work was supported by the David and Lucile Packard Foundation,

Merck & Co., Inc., and the National Institutes of Health (GM43501).

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

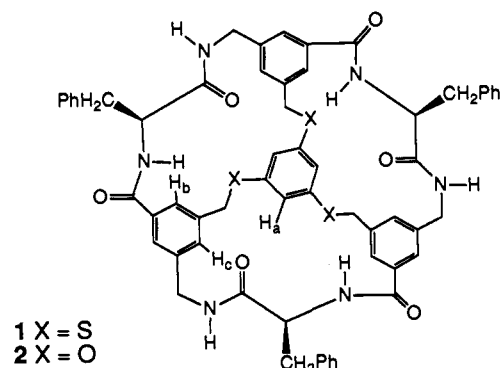
## Highly Selective Binding of Simple Peptides by a C<sub>3</sub> Macrotricyclic Receptor

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

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

Received April 5, 1991

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<sub>3</sub>-symmetric receptors (1 and 2) having only limited conformational flexibility and deep basket-like binding sites.<sup>1</sup> 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<sup>2</sup> 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<sub>3</sub> symmetry and began with trialkylation of 1,3,5-trimercaptobenzene<sup>3</sup> 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<sup>4</sup> using the MacroModel/AMBER<sup>5</sup> force

(12) Donis-Keller, H. *Nucleic Acids Res.* 1979, 7, 179-192.

(13) Knapp, G. *Methods Enzymol.* 1989, 180, 192-212.

(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.

(17) Richardson, P., unpublished results.

(18) Brimacombe, R. W. *Biochem. J.* 1985, 229, 1-17.

(19) Ehresmann, C.; Baudin, F.; Mougel, M.; Romby, P.; Ebel, J. P.; Ehresmann, B. *Nucleic Acids Res.* 1987, 15, 9109-9128. Wurst, R. M.; Vournakis, J. N.; Maxam, A. M. *Biochemistry* 1978, 17, 4493-4499. Lowman, H. B.; Draper, D. E. *J. Biol. Chem.* 1986, 261, 5396-5403. Brown, R. S.; Dewan, J. C.; Klug, A. *Biochemistry* 1985, 24, 4785-4801. Wang, X.; Padgett, R. A. *Proc. Natl. Acad. Sci. U.S.A.* 1989, 86, 7795-7799. Kean, J. M.; White, S.; Draper, D. E. *Biochemistry* 1985, 24, 5062-5070.

(1) Structurally related hosts: Kemp, D. S.; McNamara, P. E. *J. Org. Chem.* 1985, 50, 5834. Wambach, L.; Vogtle, F. *Tetrahedron Lett.* 1985, 26, 1483. Murakami, Y.; Kikuchi, J.; Tehma, H. *J. Chem. Soc., Chem. Commun.* 1985, 753. Fujita, T.; Lehn, J.-M. *Tetrahedron Lett.* 1988, 29, 1709. Ebmeyer, F.; Vogtle, F. *Angew. Chem., Int. Ed. Engl.* 1989, 28, 79. Askew, B. C. *Tetrahedron Lett.* 1990, 31, 4245. Garrett, T. M.; McMurray, T. J.; Hosseini, M. W.; Reys, Z. E.; Hahn, F. E.; Raymond, K. N. *J. Am. Chem. Soc.* 1991, 113, 2965. See also: Diederich, F. *Angew. Chem., Int. Ed. Engl.* 1988, 27, 362.

(2) Other enantioselective hosts for neutral molecules: Canceill, J.; Lacombe, L.; Collet, A. *J. Am. Chem. Soc.* 1985, 107, 6993. Pirkle, W. H.; Pochapsky, T. C. *J. Am. Chem. Soc.* 1987, 109, 5975. Sanderson, P. E. J.; Kilburn, J. D.; Still, W. C. *J. Am. Chem. Soc.* 1989, 111, 8314. Castro, P. P.; Georgiadis, T. M.; Diederich, F. *J. Org. Chem.* 1989, 54, 5834. Liu, R.; Sanderson, P. E. J.; Still, W. C. *J. Org. Chem.* 1990, 55, 5184. Jeong, K.-S.; Muehldorf, A. V.; Rebek, J. *J. Am. Chem. Soc.* 1990, 112, 6144.

(3) Bellavita, V. *Gazz. Chim. Ital.* 1932, 62, 655.