4.05 (1 H, d, J = 5.3), 4.19 (1 H, dd, J_1 = 2.8, J_2 = 8.7), 5.51-5.61 (1 H, m), 6.27 (1 H, d, J = 7.8), 6.52-6.60 (2 H, m), 6.76 (1 H, dd, J_1 = 7.8, J_2 = 10.7), 7.05-7.12 (2 H, m), 7.13-7.15 (1 H, m), 7.29-7.31 (1 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.1, 15.8, 23.4, 28.3, 31.7, 34.8, 45.7, 58.9, 63.6, 81.1, 84.1, 113.8, 123.1, 125.5, 130.2, 132.5, 155.2, 157.5, 167.0, 171.2; IR ($CHCl_3$) 3410 (m), 2990 (m), 2950 (m), 1730 (m), 1690 (s), 1625 (m), 1600 (w), 1500 (s), 1485 (s), 1400 (s), 1370 (m), 1315 (w), 1250 (s), 1160 (s), 1135 (m), 1095 (w), 1075 (w), 1030 (m), 980 (w), 905 (w), 860 (w), 830 (w) cm^{-1}; HRMS calcd for $C_{24}H_{34}N_3O_5$ (M + H) 444.251, found 444.247. Compound **22** can be obtained from **20b** by using same procedure, and all the physical and spectral data are identical.

Cyclo[*N*-[3-[4-(2-(*Z*)-aminovinyl)phenoxy]-(2*S*,3*S*)-prolyl]-(*S*)-isoleucyl] (23). To a solution of compound **22** (0.0580 g, 0.139 mmol) and thioanisole (0.173 g, 1.39 mmol) in methylene chloride (3 mL) at 5 °C was added trifluoroacetic acid (TFA) (0.316 g, 2.78 mmol). After 5 h, the reaction was diluted with methylene chloride (10 mL) and made basic with a saturated sodium bicarbonate solution (20 mL). The organic layer was separated and extracted with a 5% citric acid solution (2 mL, twice), and the aqueous layers were combined. The aqueous layer was made basic with a saturated $NaHCO_3$ solution, extracted with methylene chloride (20 mL, five times), dried over Na_2SO_4, and filtered, and the filtrate was concentrated under reduced pressure to provide pure **23** (0.0130 g, 28% yield): R_f 0.52 (methylene chloride:ethanol 90:10); $[\alpha]^{20}_D$ -145.9° (c 0.67, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80 (3 H, d, J = 6.9), 0.84-0.90 (3 H, m), 0.91-0.98 (1 H, m), 1.21-1.33 (1 H, m), 2.07-2.13 (1 H, m), 2.17-2.18 (2 H, m), 2.35-2.43 (1 H, m), 3.04-3.09 (1 H, m), 3.23-3.24 (2 H, m), 4.22-4.25 (1 H, m), 5.18-5.22 (1 H, m), 5.55 (1 H, d, J = 9.2), 6.42 (1 H, d, J = 7.6), 6.48 (1 H, d, J = 8.7), 6.59-6.63 (1 H, m), 7.09-7.10 (3 H, m), 7.23-7.25 (1 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.7, 15.7, 24.1, 33.8, 36.6, 46.7, 58.5, 69.6, 85.6, 118.2, 121.3, 122.2, 125.1, 130.0, 131.7, 132.1, 157.5, 167.0, 172.6; IR ($CHCl_3$) 3410 (s), 2970 (m), 2940 (m), 1690 (s), 1625 (m), 1600 (w), 1500 (m), 1475 (s), 1455 (w), 1260 (w), 1230 (w), 1165 (w), 1070 (w), 860 (w) cm^{-1}; HRMS calcd for $C_{19}H_{26}N_3O_3$ (M + H) 344.197, found 344.193.

(-)-Nummularine F (3). To a solution of compound **22** (0.013 g, 0.039 mmol) and *N*,*N*-dimethylglycine (0.082 g, 0.079 mmol) in methylene chloride (1 mL) at 5 °C was added 1,3-dicyclohexylcarbodiimide (DCC) (0.016 g, 0.079 mmol). After 1 h, the reaction was extracted with a 5% citric acid solution (1 mL). The aqueous layer was made basic to pH 8 with a 5% $NaHCO_3$ solution and extracted with EtOAc (1 mL, six times). The organic layers were combined, dried over Na_2SO_4, and filtered, and the filtrate was concentrated under reduced pressure to afford a crude residue. This material was purified by preparative thin-layer chromatography, eluting with methylene chloride:ethanol (90:10) to provide pure **3** (0.010 g, 59% yield): mp 152-154 °C (lit.[27] mp 120 °C); R_f 0.29 (methylene chloride:ethanol 90:10); $[\alpha]^{20}_D$ -197° (c 0.45, MeOH) (lit[27] $[\alpha]^{20}_D$ -204° (c 0.2, MeOH); ^{1}H NMR (500 MH, $CDCl_3$) δ 0.75 (3 H, d, J = 6.9), 0.85 (3 H, t, J = 7.3), 1.08-1.14 (1 H, m), 1.22-1.24 (1 H, m), 2.11-2.16 (1 H, m, J = 5.4, 7.1, 8.3, 10.6), 2.17-2.25 (1 H, m), 2.28 (6 H, s), 2.55-2.60 (1 H, m, J = 9.8, 10.6, 12.9), 3.04, 3.15 (2 H, AB q, δ_1 = 3.04, δ_2 = 3.15, J = 14.2), 3.42-3.48 (1 H, m, J = 5.4, 11.5, 12.9), 4.08 (1 H, dd, J_1 = 8.3, J_2 = 11.4), 4.18 (1 H, dd, J_1 = 3.1, J_2 = 8.7), 4.29 (1 H, d, J = 5.4), 5.56-5.61 (1 H, m, J = 5.4, 7.1, 9.8), 6.30 (1 H, d, J = 7.8), 6.52 (1 H, d, J = 10.6), 6.62 (1 H, d, J = 8.7), 6.76 (1 H, dd, J_1 = 7.8, J_2 = 10.6), 7.10 (1 H, d, J = 8.8), 7.14 (2 H, s), 7.30 (1 H, d, J = 8.8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.2 (q), 15.9 (q), 23.7 (t), 32.1 (t), 35.2 (d), 45.2 (q), 46.5 (t), 59.0 (d, C_5), 62.0 (t), 63.7 (d, C_8), 83.7 (d, C_9), 114.1 (d, C_1), 122.9 (d, C_{12}), 130.0 (d, C_{13}), 125.5 (d, C_2), 130.3 (d, C_{12}'), 132.6 (d, C_{13}'), 157.5 (s), 167.0 (s), 169.1 (s), 170.7 (s); IR ($CHCl_3$) 3410 (s), 2980 (m), 2950 (m), 2890 (m), 2870 (w), 2840 (w), 2790 (m), 1690 (s), 1625 (s), 1500 (s), 1480 (m), 1455 (w), 1360 (w), 1310 (w), 1255 (w), 1170 (w), 1115 (w), 1095 (w), 1080 (w), 1020 (w), 860 (w) cm^{-1}; HRMS calcd for $C_{23}H_{33}N_4O_4$ (M + H) 429.250, found 429.255.

Acknowledgment. Support from NSF (CHE 89-13869 A04) and a small instrumentation grant from the University of Pennsylvania Research Foundation are gratefully acknowledged. We thank Drs. G. Furst, J. Dykins, and P. Carroll of the University of Pennsylvania Analytical Facilities for their expert technical assistance.

Supplementary Material Available: 500-MHz 1D NMR (^{1}H, ^{13}C, DEPT), 2D NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C XHCORR) spectra and full-range EI mass spectra for **3**; ^{13}C NMR for all compounds; HRMS for **16** (30 pages). Ordering information is given on any current masthead page.

Approaches to Quantitative Supramolecular Chemistry. Hydrogen-Bond-Based Molecular Recognition Phenomena and Sigmoidal Behavior in Multicomponent Mixtures[1]

Craig S. Wilcox,*[,2] James C. Adrian, Jr., Thomas H. Webb, and Frank J. Zawacki

Contribution from the Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260. *Received July 13, 1992*

Abstract: In this paper we present NMR data from continuous titration competition experiments and describe a method for quantitative analysis of these data. Host **1** is evaluated and shown to have an extremely high affinity for adenine derivative **4** (K_a = 505 000 ± 100 000 M^{-1}). Two different experiments showing sigmoidal solute response are presented and analyzed. The methods discussed here promise greater accuracy than the formulae previously used for competition experiments because exact terms for all equilibria are included and the methods can be extended to analyses of more complex supramolecular ensembles. For example, a competition experiment here required adding host **1** to a 1.0 mM solution of 9-ethyladenine (**4**) in the presence of 20 mM dimethyleneurea (**3**). Before any host is added, 10% of the 9-ethyladenine is bound to dimethyleneurea and 12% of the dimethyleneurea is present as the dimer. These equilibria will obviously contribute to the observed chemical shifts for the solutes during the titration, and failure to consider these equilibria in calculations will lead to inaccurate results. These unnecessary inaccuracies can be avoided by using the methods detailed here.

By observation of natural phenomena and studies of artificial systems, it has been shown that orderly groups of hydrogen-bonding functional groups, arranged according to a reasoned plan, can effectively control and enhance solute-solute interactions.[3-7,11-15,21] This ability to control solute-solute interactions and to predict the stereochemical features of such interactions

(1) Eighteenth in a series on the Chemistry of Synthetic Receptors and Functional Group Arrays. Number 14: Smith, P. J.; Wilcox, C. S. *Tetrahedron* **1990**, *47*, 2617-2628.

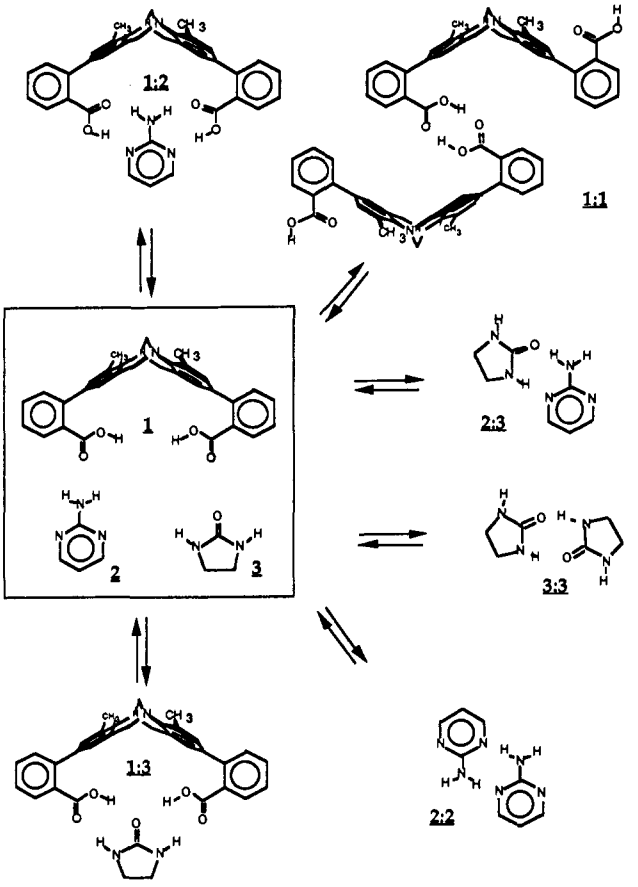
(2) Fellow of the Alfred P. Sloan Foundation, 1988-1991.

(3) (a) Mirsky, A. E.; Pauling, L. *Proc. Natl. Acad. Sci. U.S.A.* **1936**, *22*, 439. (b) Pauling, L. *J. Am. Chem. Soc.* **1940**, *62*, 2643. (c) Pauling, L.; Pressman, D. *J. Am. Chem. Soc.* **1945**, *67*, 1003. (d) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 964.

(4) (a) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89-112. (b) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1009-1020.

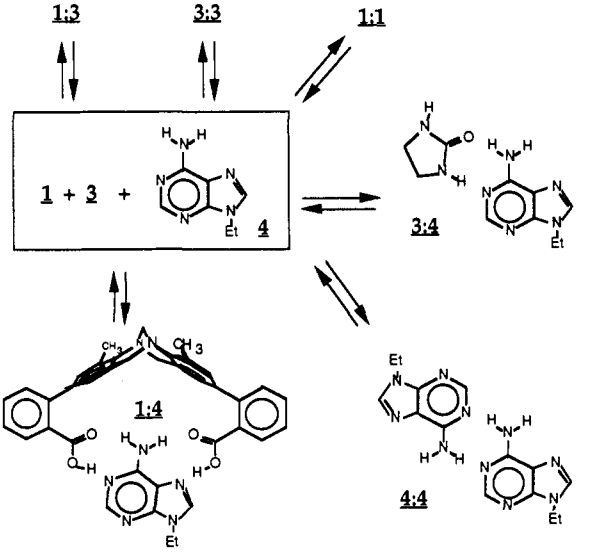
0002-7863/92/1514-10189$03.00/0

Scheme I



is required if chemists are to develop predictable, theoretically based methods to control and catalyze chemical reactions. The potential economic and environmental impact of such control is widely recognized.

Scheme II



By far the largest body of work in this field has dealt with the simplest molecular recognition process, one-to-one association of a single pair of solutes (eq 1). It is now apparent, however, that

$$A + B \rightleftarrows A{:}B \qquad (1)$$

applications of synthetic hydrogen-bonding systems for controlling solute interactions can go far beyond this simple model. Kelly has already demonstrated the use of a hydrogen-bond-based template for reaction control, and Rebek has shown that such templates can serve as catalysts for their own production.[8,9] The Kelly and Rebek systems rely on termolecular aggregates. Hydrogen-bonding interactions will play an important role as chemists continue to explore nature's methods for creating self-assembling efficacious molecular aggregates. Methods for quantitatively predicting the behavior of more complex hydrogen-bond-based phenomena will contribute to advances in this area.

With these thoughts in mind, an investigation of the behavior of three-component mixtures of hydrogen-bonding molecules was undertaken. The purpose of the study was to begin developing quantitative methods for dealing with multicomponent hydrogen-bond-forming mixtures of the type that may ultimately be used in self-assembling molecular systems. At the solute concentrations employed in this investigation, the molecules studied here (**1**–**4**) show no propensity to form termolecular assemblies. The system, however, is not simple and was chosen because it has some features that will be commonly encountered in more complex self-assembling molecular systems. For example, there are six possible one-to-one complexes that could arise from a mixture containing **1**, **2**, and **3** (Scheme I). This system, therefore, offers a challenging venue in which to test methods for dealing explicitly with complicated multiple equilibria such as those shown in

(5) (a) Rebek, J., Jr. *Acc. Chem. Res.* **1990**, *23*, 399. (b) Rebek, J., Jr. *Science* **1987**, *235*, 1478–1484.

(6) (a) Bell, T. W.; Liu, J. *J. Am. Chem. Soc.* **1988**, *110*, 3673. (b) Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8054. (c) Liu, R.; Sanderson, P. E.; Still, W. C. *J. Org. Chem.* **1990**, *55*, 5184. (d) Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 6409. (e) Kelly, T. R.; Maguire, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 6549. (f) Chapman, K. T.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 3075. (g) Neder, K. M.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 9412. (h) Aoyama, Y.; Tanaka, Y.; Toy, H.; Ogoshi, H. *J. Am. Chem. Soc.* **1988**, *110*, 634. (i) Hegde, V.; Madhukar, J. D.; Thummel, R. P. *J. Am. Chem. Soc.* **1990**, *112*, 4549. (j) Doig, A. J.; Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 338. (k) Hamilton, A. D.; Van Engen, D. *J. Am. Chem. Soc.* **1987**, *109*, 5035. (l) Chang, S. K.; Fan, E.; Van Engen, D.; Hamilton, A. D. *J. Am. Chem. Soc.* **1991**, *113*, 1318. (m) Hamilton, A. D. In *Advances in Supramolecular Chemistry*; Gokel, G., Ed.; JAI Press: Greenwich, 1990; Vol. 1. (n) Osterberg, C. E.; Arif, A. M.; Richmond, T. G. *J. Am. Chem. Soc.* **1988**, *110*, 6903. (o) Nowick, J. S.; Chen, J. S. *J. Am. Chem. Soc.* **1992**, *114*, 1107. (p) Huang, C. Y.; Cabell, L. A.; Lynch, V.; Anslyn, E. V. *J. Am. Chem. Soc.* **1992**, *114*, 1900. (q) Sheridan, R. E.; Whitlock, H. W. *J. Am. Chem. Soc.* **1986**, *108*, 7210–7211. (r) Gellman, S. H.; Adams, B. R. *Tetrahedron Lett.* **1989**, *30*, 3381. (s) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3138.

(7) Etter, M. *Acc. Chem. Res.* **1990**, *23*, 120–126.

(8) Kelly, T. R.; Zhao, C.; Bridger, G. J. *J. Am. Chem. Soc.* **1989**, *111*, 3744–3745.

(9) (a) Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 1249–1250. (b) Park, T. K.; Feng, Q.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1992**, *114*, 4529–4532.

(10) A study of hydrogen bonding between acids and bases and an analysis that was based on a model that included multiple equilibria have been reported: Davis, J. P.; Schuster, I. I. *J. Solution Chem.* **1984**, *13*, 167–178.

(11) Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem. Soc.* **1992**, *114*, 1398–1403.

(12) (a) de Boer, J. A. A.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1985**, *107*, 5347. (b) Wilcox, C. S.; Cowart, M. D. *Tetrahedron Lett.* **1986**, 5563. (c) Cowart, M. D.; Sucholeiki, I.; Bukownik, R. R.; Wilcox, C. S. *J. Am. Chem. Soc.* **1988**, *110*, 6204. (d) Petti, M. A.; Shepodd, T. J.; Barrans, R. E., Jr.; Dougherty, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 6825. (e) Schneider, H.-J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442–6448. (f) Horman, I.; Dreux, B. *Anal. Chem.* **1983**, *55*, 1219–1221. (g) Diederich, F.; Griebel, D. *J. Am. Chem. Soc.* **1984**, *106*, 8037–8046.

(13) Wilcox, C. S. In *Frontiers of Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: Weinheim, 1990.

(14) Cresswell, C. J.; Allred, A. L. *J. Am. Chem. Soc.* **1962**, *66*, 1469–1470.

(15) Horman, I.; Dreux, B. *Helv. Chim. Acta* **1984**, *67*, 754–764.

(16) (a) Draper, N. R.; Smith, H. *Applied Regression Analysis*; Wiley: New York, 1966; Chapters 1 and 2. (b) Lindley, D. V. *J. R. Stat. Soc., Suppl.* **1947**, *9*, 218–244. (c) Davies, O. L.; Goldsmith, P. L. *Statistical Methods in Research and Production*; Haffner: New York, 1972; p 209.

(17) Davis, W. H., Jr.; Pryor, W. A. *J. Chem. Ed.* **1976**, *53*, 285–287.

(18) Moore, J. W.; Pearson, R. G. *Kinetics and Mechanism*, 3rd ed.; Wiley: New York, 1981; pp 63–65.

(19) (a) Connors, K. A. *Binding Constants*; Wiley: New York, 1987; p 176. (b) Lin, S.-F.; Connors, K. A. *J. Pharm. Sci.* **1983**, *72*, 1333. (c) Lautsch, W.; Bandel, W.; Broser, W. *Z. Naturforsch.* **1956**, *11*, 282.

(20) For example, see: Diederich, F.; Dick, K. *J. Am. Chem. Soc.* **1984**, *106*, 8024–8036.

(21) Whitlock, B. J.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 3910–3915.

Scheme I or Scheme II.[10] The methods used here are presented in the context of nuclear magnetic resonance spectroscopy, but the general principles will be applicable to other spectroscopic methods.

Results and Discussion

The diacid **1** forms strongly hydrogen-bonded complexes with molecules such as 2-aminopyrimidine (**2**), dimethyleneurea (**3**), or 9-ethyladenine (**4**). Adrian and Wilcox used such host–guest interactions to explore how water can affect molecular recognition processes in chloroform and showed that small amounts of water can have a very large effect on the entropy and enthalpy changes associated with a binding event. Internal hydrogen bonds will inhibit guest binding, but they also have a beneficial effect: they can protect a host–guest system from the influence of small amounts of water or other inhibitory cosolutes.[11]

At the outset of this study, we knew that $K_{1:2}$ (Scheme I) had a value of about 10000 M^{-1}, that $K_{1:3}$ (Scheme I) was about 5-fold greater than $K_{1:2}$, and that $K_{1:4}$ (Scheme II) was 10–20 times greater than $K_{1:3}$. The principle objective was to use these guests in a series of inhibitor experiments to provide an accurate determination of the binding of 9-ethyladenine to host **1**. We wished to determine all the association constants shown in Schemes I and II and to test whether the weaker associations were of any importance under the conditions we and others have used for quantifying neutral molecule host–guest interactions. The first system to be examined was the combination of host **1** with guests **2** and **3**.

NMR spectroscopy is a powerful method for analyzing mixtures of molecules that form molecular complexes. Proton NMR chemical shifts are very sensitive to the environment surrounding the proton. Variations in observed NMR chemical shifts can be compared to the chemical shift changes that are predicted for various hypothetical models that may be proposed to describe solute behavior.[12,13] This approach to testing hypotheses concerning solute behavior requires the capability of predicting the chemical shift for a proton in a mixture of solutes. Such predictions are simplest in mixtures containing only two solutes. As more solutes and more possible molecular interactions are added, the predictions become more difficult.

Predicting the Chemical Shift for a Given Proton in These Termolecular Systems Requires Knowledge of Eight Quantities. In principle, it is simple to predict the chemical shift for a proton in the mixture of solutes **1**, **2**, and **3**. The various association equilibrium constants in this case are no greater than 10^6 M^{-1}. For any given proton only a single resonance is observed, and the position of the resonance represents an average value that depends on the probability of the proton being in any of several possible environments and on the chemical shift of the proton in those environments. For example, the observed chemical shift ($\delta_{NH,2}$-obs) of the N–H proton in solute **2** in the mixture of **1**, **2**, and **3** will depend on the relative amounts of the four species containing this proton (mole fractions of unbound **2** and the three complexes **1:2**, **2:2**, and **2:3** are symbolized as $X_{2,free}$, $X_{1:2}$, $X_{2:2}$, and $X_{2:3}$, respectively) and on the chemical shifts of the proton in each of these environments ($\delta_{2,free}$, $\delta_{NH,1:2}$, $\delta_{NH,2:2}$, and $\delta_{NH,2:3}$). If these eight quantities are known, then the observed shift can be expressed as in eq 2.

$$\delta_{NH,2}\text{-obs} = X_{2,free}\delta_{2,free} + X_{1:2}\delta_{NH,1:2} + X_{2:2}\delta_{NH,2:2} + X_{2:3}\delta_{NH,2:3} \quad (2)$$

Equation 2 provides a means of calculating the observed chemical shift for a proton in solute **2** in the presence of solutes **1** and **3**. This equation can be rewritten in a useful new form by eliminating $X_{2,free}$ ($X_{2,free} = 1 - X_{1:2} - X_{2:2} - X_{2:3}$) and collecting terms:

$$\delta_{NH,2}\text{-obs} - X_{2:2}(\delta_{NH,2:2} - \delta_{2,free}) - X_{2:3}(\delta_{NH,2:3} - \delta_{2,free}) = \delta_{2,free} + X_{1:2}(\delta_{NH,1:2} - \delta_{2,free}) \quad (3)$$

The left side of eq 3 represents the observed signal for a proton in **2** after correction for chemical shift effects due to dimerization [$X_{2:2}(\delta_{NH,2:2} - \delta_{2,free})$] and due to binding of **2** to the other guest, **3** [$X_{2:3}(\delta_{NH,2:3} - \delta_{2,free})$]. It is apparent that this "modified signal" varies linearly with the mole fraction of the host–guest complex **1:2**. The equation is useful because a plot of modified signal against $X_{1:2}$ should be linear with slope equal to the chemical shift difference between bound and unbound solute **2** proton and an intercept equaling the unbound chemical shift for the observed proton.

Table I. Association Constants ($K_{A:B}$) and ^{1}H-NMR Chemical Shift Data for Solutes in Schemes I and II[a]

entry	A:B	$K_{A:B}$ (M^{-1})	δ_B(unbound)[b]	δ_B(bound)[b]
1	**1:1**	13 ± 5	4.57 ± 0.01	4.14 ± 0.03
2	**2:2**	(1)[c]	4.97 ± 0.01	8.0 ± 0.7
3	**3:3**	4 ± 2	4.24 ± 0.02	6.76 ± 0.1
4	**4:4**	(1)[c]	5.46 ± 0.02	9.45 ± 1.4
5	**1:2**	9400 ± 400	4.94 ± 0.04[d]	6.63 ± 0.1
6	**2:3**	4 ± 2	4.24 ± 0.02	7.96 ± 0.05
7	**3:4**	3 ± 2	5.47 ± 0.02	7.67 ± 0.15
8	**1:3**	43800 ± 5000	4.17 ± 0.05	7.33 ± 0.08
9	**1:4**	505000 ± 100000	5.49 ± 0.03	8.25 ± 0.09

[a] Solvent, $CDCl_3$; T = 20 °C. [b] In ppm relative to TMS. [c] Effects on chemical shift were apparent, but binding is very weak. [d] Unbound shifts as determined by the regression analysis.

Dimerization Constants Can Be Directly Determined. Dimer formation constants $K_{1:1}$, $K_{2:2}$, $K_{3:3}$, and $K_{4:4}$ (Schemes I and II) are required if perfectly accurate host–guest association constants are to be determined. All dimerization constants were determined according to the method of Horman and Dreux after the technique of Creswell and Allred.[14,15] For example, dimerization of **2** was determined in the absence of the other three solutes by correlating changes in the chemical shift of the N–H proton with changes in the initial concentration of **2**. In this experiment, $X_{1:2} = X_{2:3} = 0.0$, so eq 3 can be rewritten as the simpler and more familiar NMR titration equation:

$$\delta_{NH,2}\text{-obs} = \delta_{2,free} + X_{2:2}(\delta_{NH,2:2} - \delta_{2,free}) \quad (4)$$

The dimerization constant $K_{2:2}$ is determined by successive approximation. An estimate of $K_{2:2}$ allows $X_{2:2}$ to be calculated for each of a series of initial solute concentrations. (There are only two states involved, and a simple quadratic equation provides a exact value for $X_{2:2}$.) A plot of observed chemical shift vs $X_{2:2}$ for this series will be linear if and only if the estimate of $K_{2:2}$ is correct. Deviations from linearity were quantified using the standard deviation of the residual errors as determined following linear regression.[16,17] (Residual errors were expressed as a fraction of mean response.) A quasi-Newtonian method was applied to find the value of $K_{2:2}$ that affords a plot that is most linear. The slope and intercept of this plot define the chemical shift of bound dimethyleneurea ($\delta_{NH,2:2}$) and the chemical shift of free dimethyleneurea at infinite dilution ($\delta_{2,free}$). In this way, the dimer formation constants $K_{1:1}$ = 13 ± 5 M^{-1}, $K_{2:2}$ = 1 M^{-1}, $K_{3:3}$ = 4 ± 2 M^{-1}, and $K_{4:4}$ = 1 M^{-1} (Table I, entries 1–4) were determined. Dimerization of the host **1** and of dimethyleneurea (**3**) are more important than dimerization of 2-aminopyrimidine (**2**) or 9-ethyladenine (**4**).

Host 1 Binds Moderately to 2-Aminopyrimidine. With the dimerization constants known, eq 3 can be rewritten for use in determining $K_{1:2}$, which is the association constant involving 2-aminopyrimidine (**2**) interactions with host **1**. In this case, $X_{2:3} = 0.0$ and eq 3 becomes

$$\delta_{NH,2}\text{-obs} - X_{2:2}(\delta_{NH,2:2} - \delta_{2,free}) = \delta_{2,free} + X_{1:2}(\delta_{NH,1:2} - \delta_{2,free}) \quad (5)$$

Although $X_{1:1}$ (the mole fraction of host in dimeric form) does not appear in this equation, $K_{1:1}$ is important because host dimerization is a mechanism that inhibits formation of host–guest complexes. The equilibria that are then important in predicting the chemical shift of the amino group of guest **2** in a mixture containing host **1** are as follows:

$$\mathbf{1} + \mathbf{1} \rightleftarrows \mathbf{1{:}1}\ (K_{1:1}) \quad (6)$$

$$\mathbf{2} + \mathbf{2} \rightleftarrows \mathbf{2{:}2}\ (K_{2:2}) \quad (7)$$

$$\mathbf{1} + \mathbf{2} \rightleftarrows \mathbf{1{:}2}\ (K_{1:2}) \quad (8)$$

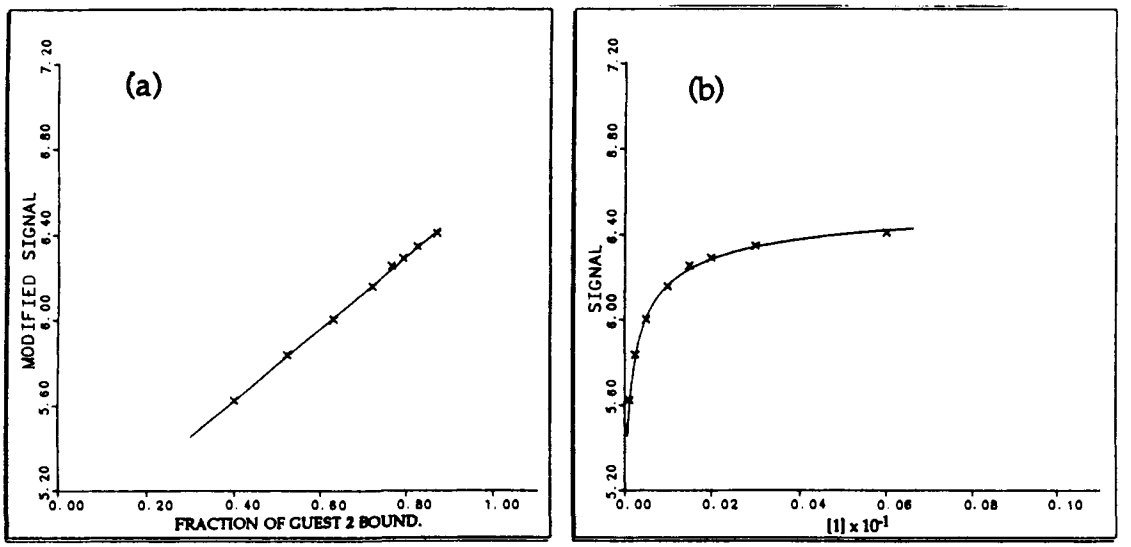


Figure 1. Observed chemical shift for guest **2** in a 1:1 mixture with host **1** in $CDCl_3$ at 20 °C. The concentration varied from 6 mM to 0.25 mM. (a) Modified shift data plotted according to eq 5 (see text). (b) Raw shift data plotted against host concentration. The solid lines represent the calculated responses based on eq 2 using the association constants and limiting chemical shifts summarized in Table I. All shifts measured in ppm relative to TMS.

These three equilibria involve five solutes. The canonical equations defining the equilibrium constants for these three reactions and solute mass balance constitute a set of simultaneous equations that must apply to any set of initial concentrations that lie within the bounds of the model. This set of equations can be solved to give the required mole fractions $X_{1:2}$ and $X_{2:2}$ in terms of five quantities (the initial concentrations of the two solutes, $K_{1:1}$, $K_{1:2}$, and $K_{2:2}$). An algebraic approach to this problem will not afford an analytical solution unless approximations are employed, because higher order polynomial equations arise.

A method of general utility that could be applied for determining the concentrations of all species in a complex equilibrating mixture of hosts, guests, and supramolecular structures was required. Numerical integration methods have long played an important role in analyses of chemical kinetics.[18] The quantitative modeling of supramolecular chemical systems will require more widespread use of these techniques. We used numerical integration to determine the concentrations (and mole fractions) of all species in this and other mixtures on the basis of the association constants and the initial concentration of the species.

In this way, given a partially hypothetical set of association constants (some association constants, e.g., dimerization constants, can be independently determined), mole fractions of all solute aggregates can be calculated. The method of Cresswell and Allred could again be applied to determine an unknown association constant ($K_{1:2}$). Because $K_{1:1}$, $K_{2:2}$, $\delta_{NH,2:2}$, $\delta_{2,free}$, and the initial concentrations of **1** and **2** are all known, there is only one unknown in Scheme I, the desideratum $K_{1:2}$. If an estimate of $K_{1:2}$ is made, then the system defined in eq 5 is complete. Given that estimate of $K_{1:2}$, numerical methods allow $X_{1:2}$ and $X_{2:2}$ to be calculated, and a plot of the left side of eq 5 against $X_{1:2}$ can be graphed. The estimate of $K_{1:2}$ is refined using quasi-Newtonian methods taking the best value of $K_{1:2}$ to be that value affording the most linear plot.

This approach was applied to analyze a data set obtained by successive dilutions of a deuteriochloroform solution containing host **1** (6 mM) and guest **2** (6 mM). NMR data were acquired for eight different concentrations of a 1:1 mixture of host **1** and guest **2**. These data were analyzed by the approach just described (Figure 1). It was found that $K_{1:2} = 9400 \pm 400\ M^{-1}$.

Including or not including the weaker associations has virtually no effect on this result. Assuming no host self-association and no guest self-association provides a calculated value for $K_{1:2}$ of $9390 \pm 400\ M^{-1}$, and changes in calculated unbound chemical shifts and guest chemical shifts are far smaller than the expected uncertainties. Therefore, in this instance, although there is some satisfaction to be derived from having dealt exactly with the additional equilibria, there is little benefit over the simpler curve fitting methods we and others have previously used.[12,13]

With the values of $K_{1:1}$, $K_{1:2}$, $K_{2:2}$, and $K_{3:3}$ available, the model system described in Scheme I is almost fully characterized. The plan was to observe the chemical shift of one guest in the presence of a second inhibitory guest and to use these data to determine the affinity of the host for the first guest.

Prior Inhibition Studies with Complex Systems. Competitive inhibition studies are common in the field of molecular association. Methods of graphical analysis for titration data from competitive spectrophotometry have been reviewed by Connors.[19] However, the method can be applied only if the analyte and the inhibitor do not bind to each other. Methods not limited to this condition would be more generally useful. Davis and Schuster analyzed a multiequilibria system, but their analysis required several simplifying assumptions and four equilibrium constants were fit simultaneously to the observed data by an iterative procedure.[10] By using a stepwise approach, we hoped in the present study to be able to determine one unknown at a time. Another method of analyzing data from competitive inhibition studies is based on algebraic conversion of the "apparent" association constant to the "real" association constant by use of a formula that incorporates the association constant for the inhibitor.[20] In a useful approach, Whitlock has devised a simple expression that relates the mole fractions bound for two competing guests to the relative association constants for the two guests.[21] Whitlock pointed out that these latter methods, too, can lead to errors if additional equilibria that may change the observed data for the analyte are not taken into account.

Guest–Guest Interactions Are Weak. A desirable model of the inhibitor experiment would include dimerization constants for all solutes and all solute–solute interaction constants. Missing guest–guest association constants were therefore determined using the same method that was described above and used to measure $K_{1:2}$ (Table I). Interaction between dimethyleneurea and 2-aminopyrimidine is weak ($K_{2:3} = 4 \pm 2\ M^{-1}$). Another weak guest–guest interaction was found between dimethyleneurea and 9-ethyladenine ($K_{3:4} = 3 \pm 2\ M^{-1}$). Although these association constants are very small in comparison with host–guest binding constants $K_{1:2}$, $K_{1:3}$, and $K_{1:4}$, because inhibitor concentrations are necessarily high, guest–guest interactions and guest dimerization events can introduce substantial errors in the determination of binding constants and limiting chemical shifts in these inhibition experiments. The magnitude of these errors depends on the methods used for analysis of the inhibition experiment.

Host 1 Binds Strongly to Dimethyleneurea. Five of the six equilibrium constants shown in Scheme I were determined by the

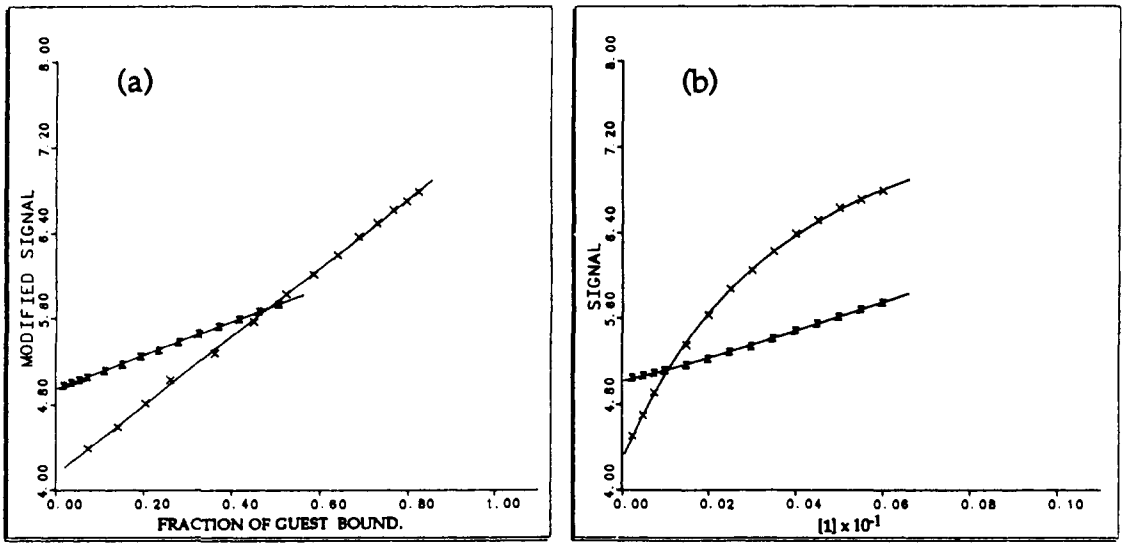


Figure 2. Chemical shifts observed for guest **3** (X, 0.001 M) and for guest **2** (X, 0.010 M) at 14 different concentrations of receptor **1**. (a) Linear plot of modified data according to eqs 3 and 9 (see text). (b) Observed NMR chemical shifts plotted as a function of receptor concentration. The solid lines represent the calculated responses based on the association constants and limiting chemical shifts summarized in Table I.

methods just described. The sixth and principle unknown association constant from Scheme I is the host:dimethyleneurea (**1**:**3**) association constant $K_{1:3}$. Data for this determination were acquired by taking 14 NMR spectra. For each observation, the concentration of the guest **3** was 0.001 M and the concentration of the inhibitor **2** was 0.01 M. Host concentration varied from 0.0002 M to 0.006 M. Figure 2 illustrates how the chemical shift of the N–H protons in both guests varied with changes in host concentration. To determine the value of $K_{1:3}$, an estimate of $K_{1:3}$ was combined with the other five known association constants in order to determine values for $X_{2:2}$, $X_{3:3}$, $X_{1:2}$, $X_{1:3}$, and $X_{2:3}$. Modified chemical shifts for solute **2** (corresponding to the left side of eq 3) were plotted against mole fraction $X_{1:2}$, and modified chemical shifts for solute **3** (corresponding to the left side of eq 9) were plotted against mole fraction $X_{1:3}$. The two plots were

$$\delta_{NH,3}\text{-obs} - X_{3:3}(\delta_{NH,3:3} - \delta_{3,free}) - X_{2:3}(\delta_{NH,2:3} - \delta_{3,free}) = \delta_{3,free} + X_{1:3}(\delta_{NH,1:3} - \delta_{3,free}) \quad (9)$$

made, and the estimate of $K_{1:3}$ was optimized on the basis of the fit of both plots to a straight line (Figure 2). Host **1** strongly binds to **3** ($K_{1:3}$ = 43 800 ± 5000 M^{-1}). The plots of modified signal were acceptably linear over the entire range of concentrations.

Host Dimerization and Dimethyleneurea:2-Aminopyrimidine Binding Compete with Dimethyleneurea:Host Binding. In this case, the weaker association events do have an effect on the measured association constant. If the weaker solute interactions are ignored, then analysis by this curve fitting method indicates that $K_{1:3}$ is about 41 300 M^{-1}—a result about 6% smaller than the result found when all equilibria are included. This diminishment in apparent affinity is expected. If the binding of dimethyleneurea to 2-aminopyrimidine is acknowledged, then it must be concluded that 2-aminopyrimidine is an inhibitor in two senses: it binds to the host strongly but it also binds to the guest. In the real mixture, this binding to the guest and the host–host dimerization processes both must be overcome in order for dimethyleneurea to bind to the host molecule. When these competitive processes are acknowledged and included in the model, the association of host with dimethyleneurea is recognized to be stronger (43 800 M^{-1}) than that which was calculated on the basis of a model in which the "weak" events were overlooked.

Other Analyses of the Data Can Be More Susceptible to Errors Caused Due to Guest–Guest Interactions. The difference in $K_{1:3}$ determined in these two models (with or without explicit analysis of the "weak" binding events) is small when the numerical equation fitting methods described here are used. If other calculation methods are applied to analyze these inhibition experiments, then ignoring the weak associations constants has a larger effect on the conclusions. For example, an analytical method described by Connors[19a] was applied to these data, and the result gave $K_{1:3}$ = 22 900 M^{-1}, a value only half the value found by our method. A plot of observed data against the best calculated data that could be generated using this alternative value of $K_{1:3}$ makes obvious the inappropriateness of this much weaker association constant (Figure 3).

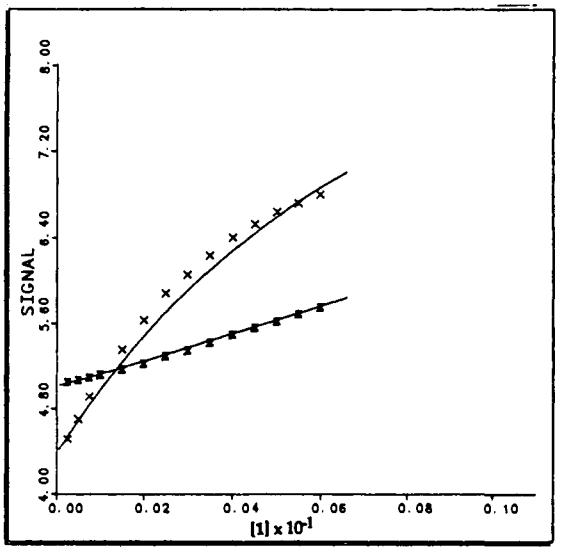


Figure 3. Same data as plotted in Figure 2b, but the solid line is calculated using $K_{1:3}$ = 22 900 M^{-1}. That this value is inappropriately low is obvious from the mismatch with the observed data.

The method that Whitlock developed for his "sliding scale" approach to measuring strong binding constants by NMR methods can be applied if the limiting chemical shifts for both types of complex (analyte with partner and inhibitor with partner) are known.[21] The method is very useful for strong binding host–guest systems in which bound chemical shifts can be determined directly. If inhibitor (**2** in this example) and analyte (**3** in this example) both bind to the host, then eq 10 will apply. Here F_2 and F_3

$$\frac{K_{1:3}}{K_{1:2}} = \frac{(1/F_2) - 1}{(1/F_3) - 1} \quad (10)$$

represent the fractions of inhibitor and analyte that are bound to host. If no confounding equilibria arise, then $F_2 = (\delta_{2,free} - \delta_{NH,2}\text{-obs})/(\delta_{2,free} - \delta_{NH,1:2})$ and $F_3 = (\delta_{3,free} - \delta_{NH,3}\text{-obs})/(\delta_{3,free} - \delta_{NH,1:3})$.

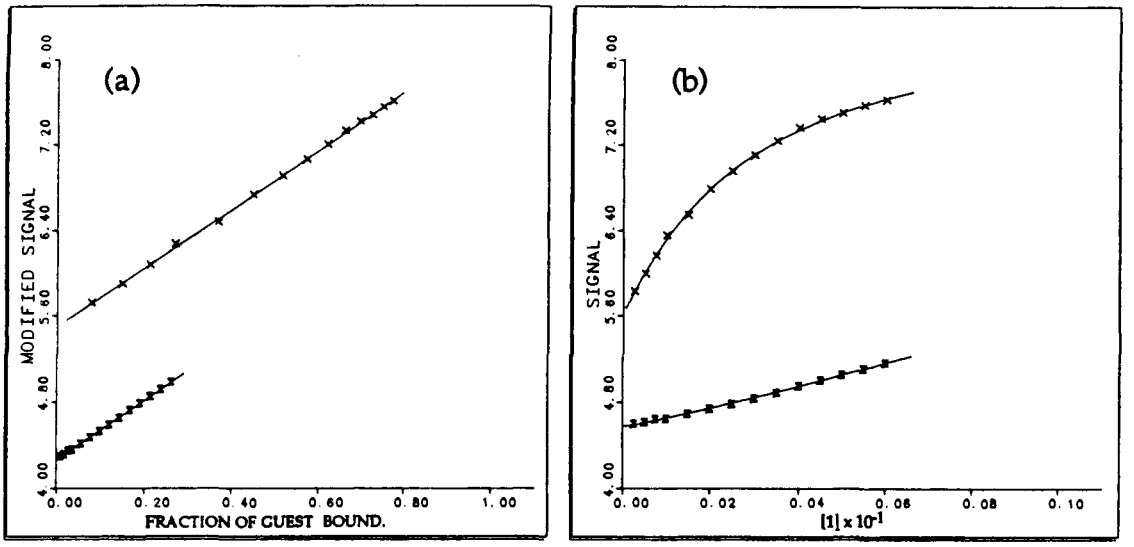


Figure 4. Chemical shifts observed for guest 4 (X, 0.001 M) and for guest 3 (X, 0.020 M) at 14 different concentrations of receptor 1. (a) Linear plot of modified data according to eqs 9 and 11 (see text). (b) Observed NMR chemical shifts plotted as a function of receptor concentration. The solid lines represent the calculated responses based on the association constants and limiting chemical shifts summarized in Table I.

Table II. Confounding Equilibria May Invalidate Eq 10

entry	[1] (M)[a]	δ_2(obs)[b]	δ_3(obs)[b]	$K_{1:3}$ (M^{-1})[c]
1	0.000 25	4.51	5.05	17 700
2	0.0005	4.70	5.07	25 700
3	0.000 75	4.91	5.09	33 400
4	0.001	5.12	5.12	37 700

[a] [2] = 0.010 M; [3] = 0.001 M. [b] In ppm relative to TMS. [c] Calculated using eq 10.

To evaluate this method, a mixture of the two guests 2 and 3 and the host 1 was prepared and chemical shifts for each guest were recorded for four different host concentrations. The observed chemical shifts for each guest ($\delta_{NH,2}$-obs and $\delta_{NH,3}$-obs) were used with the limiting chemical shifts shown in Table I to calculate four values of F_2 and F_3, and these values, in turn, were used with eq 10 to afford four determinations of $K_{1:3}$ (Table II).

In the present system, this approach to analyzing the data underestimates $K_{1:3}$. Under the conditions at which host is most limiting, the error is greatest. It is the weak association of dimethyleneurea with 2-aminopyrimidine that causes this large error. Because of this guest–inhibitor binding, the observed chemical shifts of the inhibitor at low host concentration are greater than they would be if only the host bound to the inhibitor. As a result, the calculated fraction of inhibitor bound to host is too large. This ultimately results in an underestimation of the association constant for the guest with the host. It is interesting that as the host approaches guest concentration, the error decreases. This is sensible because as more guest is bound to host, less is available to bind to the inhibitor, so the observed shifts for the inhibitor more accurately fit the mathematical model, which is based on only host–inhibitor interactions. The method of Whitlock and Whitlock is likely to be very useful in many cases, and when used correctly it can greatly simplify and expedite the determination of association constants. Our purpose in doing these calculations was not to discourage the reader from using the method. The point made here is simply that even small association constants, if not accounted for in a model system, can sometimes introduce large errors in conclusions.

Host 1 Binds Very Strongly to 9-Ethyladenine. The association of the host with 9-ethyladenine (4) was measured in the presence of dimethylurea (3) as a competitive inhibitor. The experiment and data analysis were similar to those described for determining $K_{1:3}$. Data for the determination of $K_{1:4}$ were acquired by taking 14 NMR spectra. For each spectrum, the concentration of the guest 4 was 0.001 M and the concentration of the inhibitor 3 was 0.02 M. Host concentration varied from 0.00025 M to 0.006 M. Chemical shift data for both solutes were fit to eqs 9 and 11. Host 1 binds very strongly to 9-ethyladenine (4) ($K_{1:4}$ = 505 000 ± 100 000 M^{-1}) (Figure 4).

$$\delta_{NH,4}\text{-obs} - X_{4:4}(\delta_{NH,4:4} - \delta_{4,free}) - X_{3:4}(\delta_{NH,3:4} - \delta_{4,free}) = \delta_{4,free} + X_{1:4}(\delta_{NH,1:4} - \delta_{4,free}) \quad (11)$$

The binding of host 1 to 9-ethyladenine is exceptionally strong. The free energy of binding exceeds binding energies reported previously for hydrogen-bond-based adenine receptors. Like Rebek's hosts and our previous hosts, host 1 can bind to adenine using both Watson–Crick and Hoogsteen binding motifs.[5,6b] Our other hosts related to 1 were designed to allow the carboxylic acids to converge at an angle complementary to the hydrogen-bonding surfaces of the adenine substrate.[6b] Host 1 is especially effective because the two methyl groups on the dibenzodiazocine nucleus open the "hinge angle" (the angle formed by the intersection of the planes defined the aromatic rings of the dibenzodiazocine). The result of opening this angle is that intramolecular association of the two carboxylic acids is inhibited. The host has an "open resting state".[11] Further increases in affinity can be expected if additional intermolecular interactions (for example, aromatic stacking interactions and dipole–dipole attractive forces) are combined with this efficient hydrogen-bonding structure.

Simulation of Sigmoidal Data—The Solute Chase Experiment. Any good quantitative supramolecular model must be able to predict the behavior of the system under new conditions. By the methods just described, all association constants and limiting chemical shifts for the two termolecular systems of Scheme I and Scheme II were determined (Table I). These conclusions can be further tested by modeling a more complicated, nonlinear titration experiment.

According to the association constants in Table I, a mixture of host 1 (3.6 mM) and a moderate affinity guest such as 3 (2.2 mM) should contain almost 2.2 mM of the complex and very little guest in the free state. The chemical shift observed for a proton in 3 will, therefore, be very near the shift predicted for 3 in the complex. Additions of small amounts of the more aggressive guest molecule 4 will initially have no effect on the chemical shift observed for guest 3. This is because there is excess host present, and as 4 is added it will be taken up by this excess host and not displace any of guest 3 from the binding site. When the free host is nearly depleted, however, the added 4 will have the effect of "chasing" the weaker binding guest from the host. Eventually, all of 3 will be removed from the receptor site and the observed chemical shift of 3 will again become nearly insensitive to added 4. A plot of observed chemical shift of 3 against titrant concentration would be sigmoid in form.

This experiment was carried out, and the observed changes in chemical shift were compared with changes expected on the basis

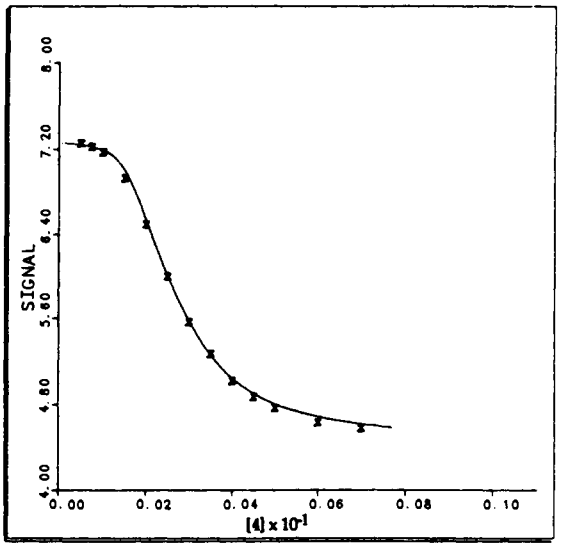


Figure 5. Chemical shifts observed for a solution containing host **1** (3.5 mM), guest **3** (2 mM), and varying amounts of guest **4** (0.25–7 mM) in $CDCl_3$ at 20 °C. The solid line represents calculated chemical shifts based on the association constants and limiting chemical shifts summarized in Table I.

of the quantitative model described in Scheme II and Table I (Figure 5).

Equation 12 was used to calculate the chemical shift expected for guest urea **3** under the conditions of this titration. The

$$\delta_{NH,3}\text{-obs} = \delta_{3,free} + X_{3:3}(\delta_{NH,3:3} - \delta_{3,free}) + X_{2:3}(\delta_{NH,2:3} - \delta_{3,free}) + X_{1:3}(\delta_{NH,1:3} - \delta_{3,free}) \quad (12)$$

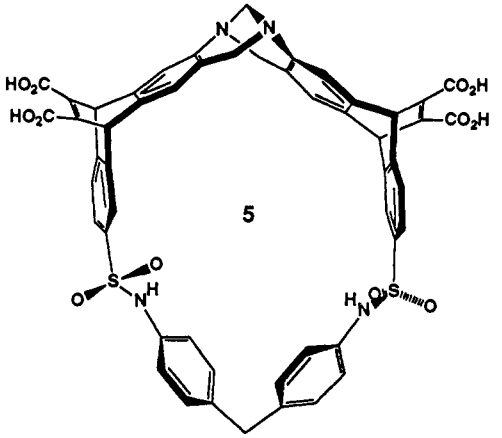
required mole fractions were calculated from the total solute concentrations and the required association constants. Some small modifications of limiting chemical shifts were required to get the calculated result shown in Figure 5, but in no case was it necessary to use a numerical value that was outside the bounds of uncertainty defined in Table I. Excellent agreement of the experimentally observed points and the calculated points is apparent.

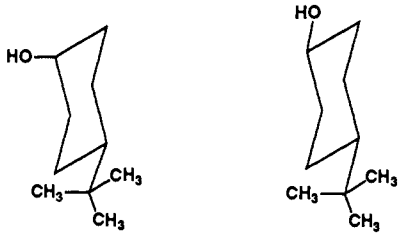
Some systematic differences between the data and the calculated result are apparent at higher concentration. Very small changes (0.01–0.03 ppm) in chemical shifts used in the calculation would provide a nearly perfect fit. Our purpose, however, was to test the predictive value of the methods and the data in Table I, so further "fine-tuning" of the calculated result was not pursued.

The prediction and confirmation of the rather complicated observed data illustrated in Figure 5 requires accuracy in each of the 27 parameters listed in Table I. Very small changes in association constants lead to quite obviously poor correlations with this sigmoidal data curve. The success of the model in simulating the behavior of this ensemble of three molecules is further evidence that the model is correct.

Sigmoidal Response Observed in Titrating a Mixture of Guests. There is another instance in which a titration-type experiment can lead to signals that vary sigmoidally with titrant concentration. Two guests that compete for a single receptor site will give rise to separately observable signals. If one guest binds to the receptor much more strongly than the other, then as the first increments of receptor are added to the guest mixture all the receptor will be occupied by the more strongly bound guest and the signal from the weakly bound guest will be unaffected. Only after all the strongly bound guest is consumed will receptor become available for the weak binding guest. At that point, the signal for the less agressive guest will undergo changes similar to that expected for a normal binding curve and reach a saturation level. Overall, a sigmoidal response will be observed for the more weakly bound guest. It should be possible for the two guest binding curves to be analyzed simultaneously using the methods described here and for both association constants to be determined from a single titration experiment.

This possibility was tested using host **5** in a one-pot titration with guests **6** and **7**. Host macrocycle **5** was reported by Webb et al. to bind diastereomeric forms of menthol with selectivity that indicated that axial groups on the cyclohexane nucleus may not fit in the host.[22] A mixture of *cis*- and *trans*-4-*tert*-butylcyclohexanol (**6** and **7**) was prepared in a phosphate buffer (D_2O) at pH 6.8. The concentration of **6** was 0.32 mM, and that of **7** was 0.68 mM. Throughout the titration (298 K, host varied from 0 to 1.7 mM), ionic strength was maintained (I = 0.34) by the use of added KCl. Observed chemical shift changes for the two *tert*-butyl groups are plotted in Figure 6a.





6 **7**

To determine the two association constants, the observed data were fit to eqs 13 and 14 and the association constants were varied

$$\delta_6\text{-obs} = \delta_{6,free} + X_{5:6}(\delta_{5:6} - \delta_{6,free}) \quad (13)$$

$$\delta_7\text{-obs} = \delta_{7,free} + X_{5:7}(\delta_{5:7} - \delta_{7,free}) \quad (14)$$

to optimize the linearity of both plots of observed solute chemical shift vs mole fraction solute bound (Figure 6b).

Host **5** binds to *trans-tert*-butylcyclohexanol more strongly than to *cis-tert*-butylcyclohexanol ($K_{5:6}$ = 6300 M^{-1}, $K_{5:7}$ = 43 000 M^{-1}). For the stronger binding host, chemical shift changes induced by added host follow an approximately hyperbolic function. For the weaker binding guest, sigmoidal changes are observed, just as expected on the basis of the above qualitative analysis.

The accuracy of this two-guest analysis was evaluated by doing separate titrations on the two pure diastereomeric guests. In the simple titration, with only one diastereomer present in each case, $K_{5:6}$ was found to be 8600 M^{-1} and $K_{5:7}$ was found to be 41 000 M^{-1}. As expected, no sigmoidal behavior was observed for either diastereomer when one-guest titrations were carried out. The differences between the two-guest and one-guest titration results are not large compared with expected uncertainties. Some of the observed difference is certainly due to the fact that accuracy is always better when one rather than two unknowns is fit to a metric plot. Also, complex solution behavior (especially guest aggregation and 2:1 complex formation) should be expected in studying the behavior of these very slightly soluble alicyclic substrates.

The diastereoselectivity exhibited in this example parallels our observations with this host and menthol diastereomers. Here,

(22) Webb, T. H.; Suh, H.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 8554–8555.

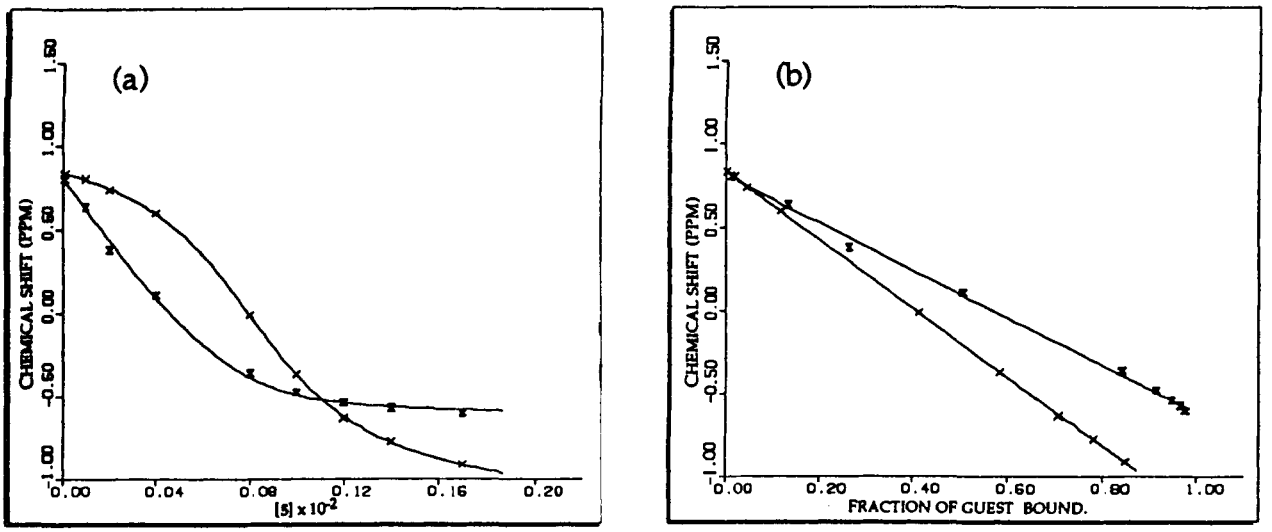


Figure 6. (a) Chemical shifts observed for a solution containing *cis*-4-*tert*-butylcyclohexanol (**6**, 0.32 mM) and *trans*-4-*tert*-butylcyclohexanol (**7**, 0.68 mM) and varying amounts of host **5** (0.01–1.7 mM) in D_2O at pH 6.8 (phosphate buffer) at 25 °C. Ionic strength (I = 0.34) was maintained by the use of KCl. Shifts are measured in ppm relative to an external standard (β-(trimethylsilyl)propionic acid in D_2O).

again, the cyclohexanoid guest bearing an axial substituent is less well accommodated in this shape-selective cavity. It is notable that the host-induced chemical shift for the *tert*-butyl group is much larger for the weakly bound guest than for the strongly bound guest. This result would be expected if the axial hydroxyl group causes the average position of the host to move closer to the *tert*-butyl group, and thus this observation further supports our hypothesis that this host will not readily accommodate axially substituted cyclohexanoid guests.

These data show that reasonably accurate association constants can be obtained from the sigmoidal response observed in such a two-guest/three-component experiment. While in this case the two guests could be easily separated and binding constants could be independently measured, this is not always the case. The analysis used here can also be applied to determining individual association constants for each enantiomer in a racemic mixture. The data also are important because they illustrate a second way in which sigmoidal signal response can arise in a three-solute system, and such behavior will certainly be observed more frequently when more complicated supramolecular ensembles are quantitatively evaluated.

Conclusions

As experiments in supramolecular chemistry become more sophisticated and multiple solutes are used to create new self-assembling systems, methods for quantitatively predicting the behavior of supramolecular systems must be evaluated. In this paper, a detailed analysis of three-component mixtures of hydrogen-bonding systems has been described. These experiments are similar to canonical competition experiments, but the data analysis demonstrates methods that handle explicitly many of the possible confounding equilibria that may arise in such systems, and quantitative evaluations of two different sigmoidally responding systems are detailed. The methods used are not limited to three-solute mixtures, and more complicated molecular ensembles may be analyzed by these methods. The results show that in the present case, neglect of minor binding phenomena does not lead to large errors when curve fitting methods are applied. The simplified formulae sometimes used to analyze inhibition experiments are more susceptible to errors induced by confounding equilibria and should be applied with due caution.

Experimental Section[23]

Methyl 4′-Amino-6-methylbiphenyl-2-carboxylate.[24] To a stirred solution of 20.2 g (63.8 mmol) of *N*,*N*-bis(trimethylsilyl)-4-bromoaniline[25] in 40.0 mL of ether at −78 °C under nitrogen was added dropwise over 45 min 83.0 mL (1.7 M, 141.1 mmol) of a solution of *tert*-butyllithium in pentane. The resulting mixture was stirred at −78 °C for 1 h and then allowed to warm to room temperature. The reaction mixture was concentrated under reduced pressure, affording a yellow solid which was subsequently redissolved in 40.0 mL of THF. The organolithium solution was then transferred via cannula to a stirred solution of 9.1 g (67.0 mmol) of fused $ZnCl_2$ in 40.0 mL of THF, and the mixture was stirred for 1 h at room temperature. The organozinc chloride solution was then transferred via cannula to a stirred solution of 19.3 g (70.0 mmol) of methyl 3-methyl-2-iodobenzoate[26] and $Ni(PPh_3)_4$ catalyst (prepared in situ by the reaction of 1.6 g (6.3 mmol) of $Ni(acac)_2$, 6.6 g (52.2 mmol) of PPh_3, and 6.3 mL (6.3 mmol) of diisobutylaluminum hydride) in 40.0 mL of THF and stirred overnight. The reaction mixture was poured into a separatory funnel containing 75 mL of 2 N HCl and 250 mL of ether and shaken, and the layers were separated. The ether layer was further extracted with two 50-mL portions of 2 N HCl. The combined aqueous portions were then allowed to stand. After 1 h, a red-brown oil separated, and the remaining aqueous portion was concentrated under reduced pressure to 50 mL. After the aqueous portion was allowed to stand for another 1 h, additional red-brown oil separated and was combined with the previously obtained material. The oil was then poured into a separatory funnel containing 100 mL of concentrated NH_4OH and 75 mL of CH_2Cl_2 and shaken. The organic layer was separated, and the remaining aqueous layer was further extracted with 2 × 75-mL portions of CH_2Cl_2. The combined organics were dried ($MgSO_4$), filtered, and concentrated in vacuo to afford 8.37 g (54%) of a red oil. ^1H NMR of the product oil indicated ≥95% purity; therefore no further purification was done. For characterization purposes, a small amount was distilled: bp 212–218 °C (1.5 mmHg); R_f = 0.13 (SiO_2, 5%, ethyl acetate/CH_2Cl_2); IR ($CHCl_3$) 3451, 3374, 3009, 2953, 2863, 1722, 1622, 1520, 1436, 1294, 1179, 1003, 828 cm^{-1}; 300 MHz ^1H NMR ($CDCl_3$) δ 7.58 (d, 1 H, J = 8 Hz), 7.36 (d, 1 H, J = 8 Hz), 7.25 (t, 1 H, J = 8 Hz), 6.95 (d, 2 H, J = 8 Hz), 6.72 (d, 2 H, J = 8 Hz), 3.78 (br s, 2 H), 3.57 (s, 3 H), 2.13 (s, 3 H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 169.3, 144.7, 141.5, 137.6, 132.8, 132.2, 130.4, 129.5, 126.6, 114.9, 51.8, 20.7; MS, m/e calcd for $C_{15}H_{15}NO_2$ (M^+) 241.1103, measured 241.1102. Anal. Calcd for $C_{15}H_{15}NO_2 \cdot 0.1H_2O$: C, 74.11; H, 6.30; N, 5.76. Found: C, 74.02; H, 6.36; N, 5.71.

6′,6″-Dimethyl-2,8-diphenyl-6*H*,12*H*-(5,11)-methanodibenzo[*b*,*f*]-[1,5]diazocine-2′,2″-dicarboxylic Acid. A solution of 5.0 g (20.7 mmol) of the above biphenylamine and 2.9 g (20.7 mmol) of hexamethyltetraamine in 40.0 mL of trifluoroacetic acid was stirred at room temperature. After 24 h, the trifluoroacetic acid was removed by distillation. The concentrated reaction mixture was taken up in 20.0 mL of water, poured into a separatory funnel, and basified by the addition of 50 mL of concentrated NH_4OH. The aqueous layer was then extracted with 3 × 100-mL portions of CH_2Cl_2. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo to yield 6.2 g of a yellow-brown glass foam. Preliminary purification by flash

(23) General experimental details and a description of the titration experiments have already been published.[11]

(24) The biphenyl synthesis described here is based on the methods of Negishi: Negishi, E.; King, A. O.; Okukado, N. *J. Org. Chem.* **1977**, *42*, 1821–1822.

(25) Pratt, J. R.; Massey, W. D.; Pinkerton, F. H.; Thames, S. F. *J. Org. Chem.* **1975**, *46*, 1090–1094.

(26) Mayer, F. *Chem. Ber.* **1911**, *44*, 2298–2305.

chromatography (75-mm × 40-mm column of SiO_2, eluted with 5/95% CH_3OH/CH_2Cl_2) afforded a yellow glass foam. Purification by flash chromatography (178-mm × 50-mm column of SiO_2, eluted with 2.5/97.5% CH_3OH/CH_2Cl_2) afforded 2.76 g (51%) of the dimethyl ester of the host dibenzodiazocine (purity ≈ 90%) as a yellow glass foam which was then crystallized from 95% ethanol (≈40 mL) to afford a first crop of 1.5 g as florets of fine yellow needles; a second crop (≈20 mL) of 430.0 mg was also obtained: mp 158.0–162.0 °C; R_f = 0.07 (SiO_2, 2.5/97.5%, CH_3OH/CH_2Cl_2); IR ($CHCl_3$) 3010, 2853, 1724, 1613, 1578, 1497, 1406, 1296, 1195, 1142, 1097, 1013, 840 cm^{-1}; 300 MHz ^1H NMR (DMF-d_7, +90 °C) δ 7.50 (*d*, 2 H, *J* = 8 Hz), 7.41 (d, 2 H, *J* = 8 Hz), 7.30 (t, 2 H, *J* = 8 Hz), 7.16 (d, 2 H, *J* = 8 Hz), 6.95 (d, 2 H, *J* = 8 Hz), 6.77 (s, 2 H), 4.73 (d, 2 H, *J* = 17 Hz), 4.36 (s, 2 H), 4.25 (d, 2 H, *J* = 17 Hz), 3.32 (br s, 6 H), 2.05 (s, 6 H); 300 MHz ^1H NMR ($CDCl_3$, −20 °C) δ 7.62 (m, 2 H), 7.36 (m, 4 H), 7.19 (m, 2 H), 7.04 (m, 2 H), 6.75 (m, 2 H), 4.79 (m, 2 H), 4.47 (m, 2 H), 4.26 (m, 2 H), 3.66 (s), 3.65 (s), 3.31 (s), 2.98 (s), 2.19 (s), 2.15 (s), 1.92 (s), 1.91 (s); 75 MHz ^{13}C NMR ($CDCl_3$, −20 °C) δ 169.5, 168.8, 168.7, 146.4, 141.1, 140.9, 140.8, 140.5, 137.2, 137.1, 136.8, 135.8, 135.5, 132.9, 132.1, 131.6, 131.4, 131.1, 127.8, 127.6, 127.2, 126.8, 126.5, 126.4, 124.4, 124.3, 66.7, 58.8, 58.6, 58.4, 51.9, 51.7, 51.0, 20.8, 20.7, 20.4; MS, *m/e* calcd for $C_{33}H_{30}N_2O_4$ (M^+) 518.2206, measured 518.2208. Anal. Calcd for $C_{33}H_{30}N_2O_4$: C, 76.43; H, 5.83; N, 5.40. Found: C, 76.17; H, 5.87; N, 5.42.

To a stirred heterogeneous mixture of 842.0 mg (35.1 mmol) of anhydrous lithium hydroxide in 6.0 mL of 4:1 (v:v) CH_3OH/water at room temperature was added a hot (50 °C) solution of 523.0 mg (1.0 mmol) of the above diester in 8.0 mL of 4:1 (v:v) CH_3OH/water and 2.0 mL of CH_2Cl_2. The flask was sealed with a wired septum, and the stirred mixture was heated at 50 °C. After 24 h, the excess solid LiOH was removed by filtration and the filtrate was concentrated in vacuo. The concentrate was diluted with 5 mL of water to afford a basic yellow homogeneous solution (pH ≥ 13.0). The basic solution was then acidified by the careful addition of 14.5 mL of 2 N HCl (final pH ≈ 2.0). The white precipitate was extracted with 3 × 20-mL portions of $CHCl_3$. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo to afford 560.0 mg of host as fine white needles. Recrystallization from ≈20 mL of $CHCl_3$ afforded 350.0 mg (first crop) followed by a second (≈10 mL) of 110.0 mg (total 92%) as fine colorless needles which become white and opaque on drying: mp 294–296 °C dec; IR ($CHCl_3$) 3250 (b), 2856, 1700, 1479, 1465, 1294, 1202, 750, 730, 662 cm^{-1}; 300 MHz ^1H NMR ($CDCl_3$, 23 °C) δ 7.44 (d, 2 H, *J* = 8 Hz), 7.38 (d, 2 H, *J* = 8 Hz), 7.27 (t, 2 H, *J* = 8 Hz), 7.11 (d, 2 H, *J* = 8 Hz), 6.98 (dd, 2 H, *J* = 8, 2 Hz), 6.63 (d, 2 H, *J* = 2 Hz), 4.68 (d, 2 H, *J* = 16 Hz), 4.59 (s, 2 H), 4.17 (d, 2 H, *J* = 16 Hz), 2.18 (s, 6 H); 125 MHz ^{13}C NMR ($CDCl_3$, 23 °C) δ 174.3, 146.3, 141.1, 137.0, 135.3, 132.7, 132.4, 128.8, 127.7, 127.4, 127.1, 125.5, 123.8, 67.9, 60.2, 20.5; MS, *m/e* calcd for $C_{31}H_{26}N_2O_4$ (M^+) 490.1893, measured 490.1894. Anal. Calcd for $C_{31}H_{26}N_2O_4 \cdot 1.0CHCl_3$: C, 63.08; H, 4.45; N, 4.58; Cl, 17.41. Found: C, 62.96; H, 4.59; N, 4.78; Cl, 17.04.

Acknowledgment. This work was funded by the National Institute of General Medical Sciences (GM-34846) and the Alfred P. Sloan Foundation. J.C.A. is pleased to acknowledge support from the Department of Education.

Supplementary Material Available: All NMR data required for the determination of the four dimerization constants and seven association constants discussed in this paper; NMR data for the solute chase experiment (Figure 5) (8 pages). Ordering information is given on any current masthead page.

Stereochemistry of Base-Catalyzed Ring Opening of 1,3,2-Oxathiaphospholanes. Absolute Configuration of 2-{*N*-[(R_c)-1-(α-Naphthyl)ethyl]amino}-2-thiono-1,3,2-oxathiaphospholanes and *O,S*-Dimethyl *N*-[(R_c)-1-(α-Naphthyl)ethyl]phosphoramidothioates†

B. Uznanski,‡ A. Grajkowski,‡ B. Krzyzanowska,‡ A. Kazmierkowska,‡ W. J. Stec,*,‡,§ M. W. Wieczorek,*,∥ and J. Blaszczyk∥

Contribution from the Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Department of Bioorganic Chemistry, 90-363 Lodz, Sienkiewicza 112, Poland, and Technical University of Lodz, Institute of Technical Biochemistry, 90-924 Lodz, Stefanowskiego 4/10, Poland. *Received May 8, 1992*

Abstract: Pure diastereoisomers of 2-{[(R_c)-1-(α-naphthyl)ethyl]amino}-2-thiono-1,3,2-oxathiaphospholane (**1**) and *O,S*-dimethyl *N*-[(R_c)-1-(α-naphthyl)ethyl]phosphoramidothioate (**2**) were obtained, and both "slow"-migrating isomers of **1** and **2** were studied by X-ray crystallography which demonstrated their (R_p,R_c) absolute configuration. Therefore, the absolute configuration of both "fast"-migrating isomers of **1** and **2** must be (S_p,R_c). In (R_p,R_c)-**1** oxathiaphospholane, the ring adopts the open-envelope conformation with the C2 atom in the flap position; the S1–P–O1 angle is 97°. DBU-assisted methanolysis of (R_p,R_c)-**1** ("slow") followed by S-methylation, gave (S_p,R_c)-**2** ("fast"). This result is interpreted in terms of an "adjacent" type mechanism of the regio- and stereoselective 1,3,2-oxathiaphospholane ring-opening process. Suggestions are presented regarding the absolute configuration at the phosphorus atom in diastereoisomers of 5′-O-protected nucleoside 3′-*O*-(2-thiono-1,3,2-oxathiaphospholanes), which are synthons for stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s.

In spite of numerous reports concerning the stereochemistry of P–S bond cleavage in acyclic alkyl thioloesters of phosphorus acids, the stereochemistry of the 1,3,2-oxathiaphospholane ring opening is rather obscure.[1] One can consider that the nucleophile may approach the phosphorus atom according to an "in-line" type mechanism via collinear attack from the side opposite to the endocyclic P–S bond and that the resulting intermediate collapses with the ring opening and the cleavage of the P–S bond (a, net inversion). Alternatively the ring opening may result from attack of the nucleophile from the side opposite to the most apicophilic endocyclic oxygen atom, resulting in a trigonal bipyramidal in-

† This paper is dedicated to Prof. F. H. Westheimer on the occasion of his 80th birthday.

‡ Polish Academy of Sciences.

§ Present address: J. E. Fogarty International Center, NIH, Building 16, Room 206, Bethesda, MD 20892.

∥ Technical University of Lodz.

(1) Hall, C. R.; Inch, T. D. *Tetrahedron* **1980**, *36*, 2059.

0002-7863/92/1514-10197$03.00/0