Stable Heterotopic Noncovalent Resorcin[4]arene Assemblies

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Keywords: Self-assembly / Vapor-pressure osmometry / Resorcin[4]arenes

Resorcin[4]arene tetracarboxylic acids **5**,**6** (A) and resorcin[4]arene tetrapyridines **2**,**3** (P) self-assemble in chloroform solution to form stable heterotopic AP dimers. Data from NMR titration and dilution experiments, as well as from vapor-pressure osmometry (VPO), indicate that the AP dimer is formed with an association constant greater than 10^7 M^{-1}. Solid–solution extraction experiments are indicative of the formation of a 2:1 trimer (A_2P), while self-associated homotopic species (A_2 and A_3) can be detected by NMR and VPO. Analysis of the heterotopic noncovalent assembly process over a range of compositions shows that these other species are much less stable than the AP heterodimer, which is the exclusive species at an A/P concentration ratio of 1:1 (> 99.7% of the total at 10 mM).

Introduction

Despite improvements in the modular synthesis of large functional molecules through the covalent combination of building blocks, for the construction of nanometer-scale structures this approach remains nontrivial. Our results in this area have illustrated both the strengths and the weaknesses of this synthetic strategy in relation to both well-defined receptor cavities[1] and unimolecular ion channels.[2] A promising alternative is the noncovalent combination of building blocks via self-assembly processes,[3,4] which can involve either self-complementary dimers (*homotopic* assembly)[3,5,6] or combinations of different building blocks (*heterotopic* assembly).[7]

Heterotopic self-assembly based on strong metal–bipyridine interactions is well-known.[4] Similarly, the interaction between carboxylic acids and pyridines has been successfully exploited in the construction of 1:1 associates of appropriately functionalized calix[4]arenes.[8,9]

Resorcin[4]arene cavitands are more rigidly preorganized than calix[4]arenes and offer a well-defined cavity for guest binding.[10] This preorganization can be expected to both direct the self-assembly process and stabilize the resulting noncovalent assembly. Examples of homotopic assembly have been reported by the groups of both Sherman[11] and Rebek.[12,13] In this paper, we describe the heterotopic self-assembly in solution of well-defined hydrogen-bonded dimers based on substituted resorcin[4]arenes. Assembly is driven by carboxylic acid–pyridine interactions aided by the complementary preorganization of the resorcin[4]arene building blocks.

Heterotopic noncovalent assembly poses additional challenges with regard to the characterization of the structures in solution compared to the species formed by homotopic assembly. Even the latter pose significant challenges in their NMR,[13] mass spectrometric,[14] and crystallographic analysis,[11] and provide new impetus for emerging techniques.[15] The additional components in a heterotopic assembly process increase both the number of possible species present and the complexity of the spectra. More importantly, the simplest heterotopic assembly involves at least two independent concentration terms, whereas the simplest homotopic dimerization (solvent as guest) involves only one. Heterotopic assembly also competes with potential homotopic assembly of one or both of the components. Thus, the stability of a heterotopic assembly can only be understood in terms of all the competing processes and the concentrations of all species in solution over a range of compositions. In this paper, we use a combination of NMR and vapor-pressure osmometry experiments in conjunction with solution modelling in order to provide a full description of a heterotopic noncovalent assembly process. We show that a heterotopic dimer species is both thermodynamically stable with respect to its components and is the predominant species at a 1:1 ratio of the building blocks.

Results and Discussion

Synthesis

Pyridyl-functionalized calix[4]arenes were obtained by alkylation of calix[4]arenes at the lower rim using the appropriate isomeric picolyl chlorides.[8] Alkylation of tetrahydroxycavitand **1** using an excess of 3-picolyl chloride in the presence of Cs_2CO_3 as a base in CH_3CN at room temperature gave tetra(3-pyridyl)resorcin[4]arene **2** (Scheme 1) in 37% yield. The moderate yield can probably be attributed to competing alkylation of the pyridyl ring, either in the starting material or in an alkylated resorcin[4]arene. Al-

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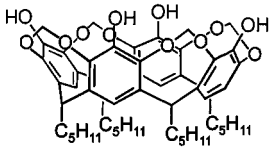

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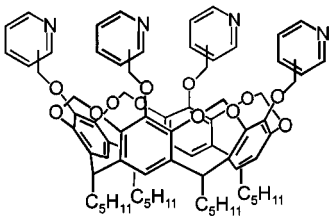
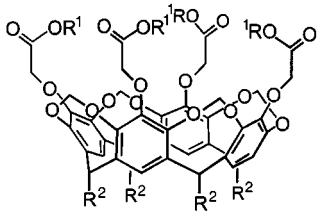


2 3-pyridyl
3 4-pyridyl



4 R^1 = -CH_3, R^2 = -C_5H_{11}
5 R^1 = -H, R^2 = -$C_{11}H_{23}$
6 R^1 = -H, R^2 = -C_5H_{11}

Scheme 1

kylation of tetrahydroxycavitand **1** with 4-picolyl chloride under the same reaction conditions afforded tetra(4-pyridyl)resorcin[4]arene **3** (Scheme 1) in 25% yield. That the symmetrical, fully substituted products had been formed was evident from a singlet in the ^{1}H-NMR spectra at around $\delta = 5$ attributable to the methylene protons adjacent to the pyridyl ring and two well-defined doublets due to the methylene protons of the cavitand (OCH_2O).

Previously, we described the synthesis of resorcin[4]arene tetracarboxylic acid **5** ($R^2 = -C_{11}H_{23}$, Scheme 1).[1b] Reaction of tetrahydroxycavitand **1** with methyl bromoacetate resulted in tetraester **4**, which was hydrolyzed with 2 N NaOH in THF to give resorcin[4]arene tetracarboxylic acid **6** ($R^2 = -C_5H_{11}$, Scheme 1) in 66% overall yield. Probably because of the aggregation discussed below, tetracarboxylic acid **6** gives rise to very broad signals in its ^{1}H-NMR spectrum in $CDCl_3$. In [D_6]DMSO, the spectrum is sharp and features a characteristic singlet at around $\delta = 4.5$ due to the methylene protons adjacent to the carboxylic acid group.

Noncovalent Assembly of Carboxylic Acid–Pyridine Combinations

Tetracarboxylic acid building blocks can, in principle, form a huge array of different complexes with tetrapyridyl building blocks. In a noncompeting solvent, such as chloroform, the important pairwise hydrogen-bond interactions occur between two acid groups or between an acid group and a pyridyl group. This limits the range of potential structures formed by intermolecular hydrogen bonds to the dimers AP and A_2, linear oligomers of the general formula $P(AP)_n$, and higher polycyclic oligomers of the general formulae A_n and $A(AP)_n$. If intramolecular carboxylic acid dimerizations are possible, then linear oligomers of the general formula $A(AP)_nA$ must also be considered. Although this is still a rich broth of potential species, the experiments discussed below only require consideration of A_2, A_3, AP, and the 2:1 adduct A_2P [$A(AP)_n$ where $n = 1$]. Following the convention for metal–ligand equilibria,[16] we define a cumulative formation constant (β_{ap}) for a species A_aP_p obtained from a mol of A and p mol of P (Equation 1):

$$aA + pP = A_aP_p \qquad \beta_{ap} = [A_aP_p]/([A]^a \times [P]^p) \qquad (1)$$

This definition covers all the cases listed above for an arbitrarily rich mixture of species. It also allows the derivation of stepwise association constants. For example, the association of A and AP to produce a species A_2P has a stepwise formation constant equal to β_{21}/β_{11}.

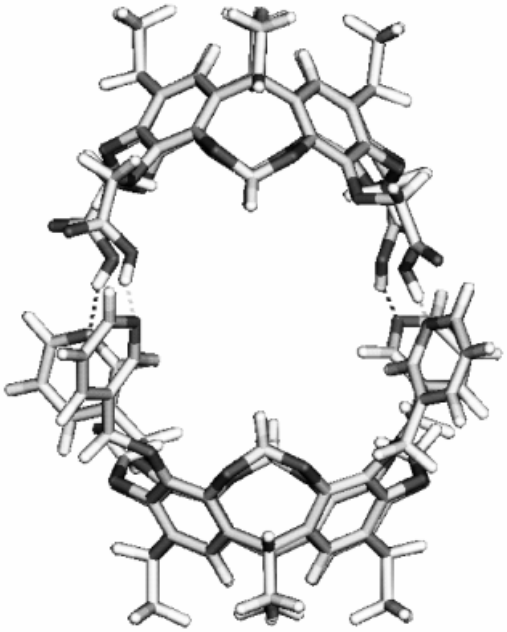


Figure 1. Representation of possible 1:1 complexes of resorcin[4]arene tetracarboxylic acid and tetra(3-pyridyl)resorcin[4]arene

Figure 1 depicts the type of structure expected for the 1:1 AP species formed from the core resorcinarenes **2** and **5** or **6**.

Homotopic Associations

Any hydrogen bonding between a carboxylic acid and a pyridine would have to overcome the self-association of the carboxylic acid.[17] Aggregation of the resorcinarene acids in chloroform is therefore expected and indeed the resorcin[4]arene tetracarboxylic acid **6** is quite insoluble in this solvent.[18] The ^{1}H-NMR spectrum of resorcin[4]arene tetracarboxylic acid **5** ($R^2 = -C_{11}H_{23}$) is concentration dependent over the experimentally accessible range (up to about 0.02 M) and the chemical shift changes on dilution

have been used in an attempt to determine a value for the dimerization constant β_{20} according to the method of Horman and Dreux.[19] However, the derived equilibrium constant proved to be a function of the probe signal used, indicating that such a simple model cannot be valid. A comparison experiment with phenyloxyacetic acid under the same experimental conditions yielded a value of 2.5×10^2 M^{-1} for each of the four signals (log $\beta_{20} = 2.4 \pm 0.1$). The corresponding value for **5** would have to be substantially greater (log $\beta_{20} > 4$) to account for the observed NMR changes.

NMR methods use observed shifts and assumed complex stoichiometries in order to derive association constants. An alternative technique, more sensitive to stoichiometry, is vapor-pressure osmometry (VPO).[20] Instrument response (R) is related to solute concentration for a mixture of solutes (c_i) by Equation 2:

$$R = K \times \Sigma\, c_i = K \times \Sigma\, (m_i/Mw_i)/m_s = K' \times \Sigma\, (m_i/Mw_i)/v_s \qquad (2)$$

Here, K and K' are calibration constants for the system determined using a high-purity solute (typically benzil), m_i and MW_i are the mass and molecular weight of the solute(s) of interest, and m_s and v_s denote the mass and volume of solvent. The technique is valid only for ideal solutes and so concentrations are extrapolated to infinite dilution in the standard state. The preferred concentration units of Equation (2) are mol L^{-1} (using K') rather than mol kg^{-1} in order to facilitate comparison with NMR experiments: for the dilute solutions considered here, the two may be interconverted through the solvent density. The conventional use of Equation 2 for a single solute of unknown molecular weight is first to determine K for the system and then to determine R for a series of solutions of known concentration (g/kg solution). Linear extrapolation of R/concentration *versus* concentration to infinite dilution gives the intercept $(R/K)_0$, i.e. the inverse molecular weight. However, solutions of interacting components are inherently nonideal and the dilution of media containing any noncovalently bound species will result in the dissociation of these into their components. The apparent molecular weight will therefore vary with concentration and the R/c *versus* c function will be nonlinear. More generally, the expected VPO response (R) for a noncovalently assembled species as a function of concentration can be expressed by some nonlinear function related to the formation constants of the species present.[21] VPO data obtained for noncovalent complexes in solution can be fitted to a model of the solution equilibria (Equation 1) using iterative values of the formation constants β_{ap}. The VPO experiment is particularly sensitive to dissociation and to the presence of minor species with significantly different molecular weights, such as higher oligomers in a solution composed principally of dimers.

The self-association of the tetracarboxylic acid **5** in ethanol-free chloroform, as studied by VPO, is illustrated in Figure 2, part A of which shows the direct R *versus* concentration relationship, while part B shows the same data in an R/concentration *versus* concentration representation. The data points in both plots are nonlinear, as expected for a

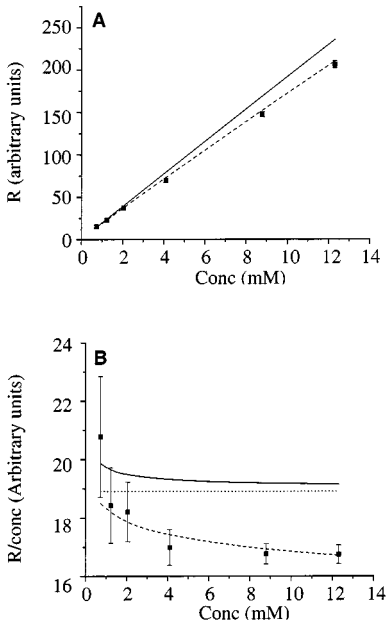


Figure 2. (A) Vapor-pressure osmometry response (R, arbitrary units) as a function of concentration of **5**; data are shown as solid squares with error bars; lines are calculated for log $\beta_{20} = 4.7$ (solid) and the fitted line for log $\beta_{20} = 5.4$ and log $\beta_{30} = 8.8$ (dashed); (B) Data corresponding to part A in the R/c *versus* c format, plus a dotted line calculated for a single solute with the molecular weight of the $(\mathbf{5})_2$ dimer

mixture of interacting compounds. A simple dimer model fails to fit the data and the poor fit of this model is illustrated for one of the values derived from the NMR experiments. The apparent molecular weight values appear to be significantly higher than would be expected for a simple A_2 dimer. As shown in Figure 2, the data fit can be substantially improved by assuming an additional trimeric species (A_3). A nonlinear fitting of the data (R *versus* c) gave values of log $\beta_{20} = 5.4 \pm 0.2$ and log $\beta_{30} = 8.8 \pm 0.2$. The stepwise formation of A_3 (from $A + A_2$) is weaker than the direct dimer formation by a factor of 100, hence A_3 is a minor species in solutions of pure A (<20%). It is "undetectable" by NMR due to rapid exchange and the small chemical shift differences between the dimer and trimer, although these have a sufficiently perturbing effect to prevent fitting of the NMR titration curves by a simple dimer model. Both the dimer and trimer are substantially more stable than the phenyloxyacetic acid dimer, indicating that the aggregates are stabilized by multiple hydrogen bonds.

Heterotopic Associations

The interaction between carboxylic acids and pyridine in chloroform solution has been shown to have comparable characteristics as those found in the more commonly used solvent water.[22,23] The magnitude of the expected interaction was assessed by an NMR titration experiment using the monofunctional analogues phenyloxyacetic acid and pyridine. Upon addition of phenyloxyacetic acid to a solution of pyridine in chloroform, the pyridine aromatic signals are shifted downfield while the signals of the acid are shifted upfield, as would be expected for hydrogen-bond

formation between the acid group and the nitrogen atom. In this case, the solution model consists of a dimerization and an association equilibrium. Using the values for β_{20} determined by dilution of the acid, the association constant β_{11} was evaluated as 2.5×10^2 M^{-1} (log $\beta_{11} = 2.5 \pm 0.06$).[23]

A 1:1 mixture of insoluble[24] resorcin[4]arene tetracarboxylic acid **6** ($R^2 = -C_5H_{11}$) and tetra(3-pyridyl)resorcin[4]arene **2** in $CDCl_3$ produces a clear solution on warming. This solubilization is accompanied by downfield shifts of several of the NMR signals of tetrapyridyl **2**. Furthermore, the sharp signals in the ^{1}H-NMR spectrum of the 1:1 mixture indicate that both resorcin[4]arenes retain a high average symmetry. The NMR spectrum remains virtually unaltered on dilution of the solution to a concentration of 0.15 mM, indicating a very strong interaction between the two components. Analogous extraction of tetra(4-pyridyl)resorcin[4]arene **3** by tetracarboxylic acid **6** and subsequent dilution of the solution of the 1:1 complex **6·3** gave comparable results. The low solubility of **6** compromises the interpretation that this represents a heterotopic self-assembly. Thus, we turned to the more soluble resorcin[4]arene tetracarboxylic acid **5** ($R^2 = -C_{11}H_{23}$).

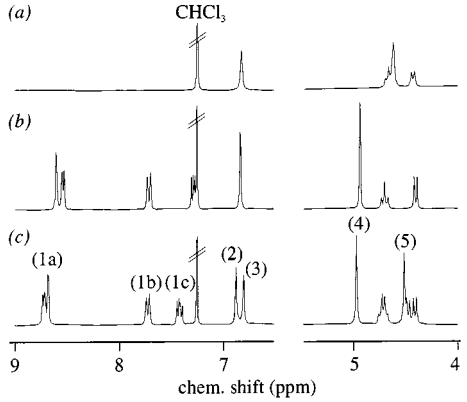


Figure 3. ^{1}H-NMR spectra of (a) resorcin[4]arene tetracarboxylic acid **5**, (b) tetra(3-pyridyl)resorcin[4]arene **2**, and (c) a 1:1 mixture of **5** and **2** in $CDCl_3$ at room temperature; 1. aromatic proton signals of the pyridyl fragment; 2. aromatic proton signal of **2**; 3. aromatic proton signal of **5**; 4. signal for OCH_2pyr protons, and 5. signal for $OCH_2C(O)$ protons

Titration of a solution of tetra(3-pyridyl)resorcin[4]arene **2** with a solution of resorcin[4]arene tetracarboxylic acid **5** in chloroform leads to downfield shifts of several ^{1}H-NMR signals of tetrapyridyl **2** and to upfield shifts of the singlet signals due to the aromatic protons and the $OCH_2C(O)$ protons of **5**. The ^{1}H-NMR spectra of tetracarboxylic acid **5** ($R^2 = -C_{11}H_{23}$), tetra(3-pyridyl) **2**, and a 1:1 mixture of **5** and **2** are given in Figure 3. Similar changes in chemical shifts were observed in the ^{1}H-NMR spectrum of a 1:1 mixture of tetra(4-pyridyl)resorcin[4]arene **3** and tetracarboxylic acid **5** in $CDCl_3$. Dilution of the 1:1 mixtures of **2** or **3** with **5** did not lead to a change in the chemical shift of any signal of either resorcin[4]arene component down to a concentration of 0.14 mM. The uncertainty in the largest shift (pyridyl 6-H of **2**) was less than 1% of the chemical shift difference between uncomplexed **2** and the 1:1 **2·5** complex at the lowest concentration (0.14 mM) and hence the association constant β_{11} must be $>>10^7$ M^{-1}. The 1:1 **3·5** complex possesses similar stability.

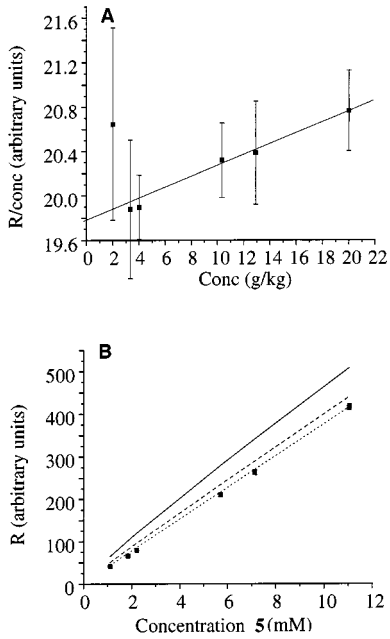


Figure 4. (A) *R*/*c versus c* (g/kg) for a 1:1 molar ratio mixture of **5** and **2** showing weighted linear regression. (B) Data corresponding to part A replotted as vapor-pressure osmometry response (*R*, arbitrary units) as a function of the molar concentration of **5** for a 1:1 mixture of **5** and **2**; lines are calculated for $\beta_{11} = 10^3$, 10^5, and 10^7 M^{-1} (solid, dashed, dotted, respectively)

The 1:1 complex is a sufficiently stable species to behave as a discrete solute in VPO experiments. Figure 4, part A shows the results obtained upon dilution of a 1:1 mixture of **5** and **2** in chloroform. The apparent molecular weight for this system, determined by extrapolation to infinite dilution, is 2740 ± 70 g/mol, in excellent agreement with the expected value of 2695 g/mol for **5·2**. Figure 4, part B shows the VPO response curves expected for different values of β_{11}. Like the NMR dilution experiment, VPO can give only a lower limit of $\beta_{11} >> 10^7$ M^{-1}.

The 1:1 heterodimer **5·2** was found to be stable to the addition of four equivalents of pyridine. No significant changes were observed in the signals of either of the resorcin[4]arenes, indicating that there must be a positive cooperative effect in the formation of this 1:1 complex. The complex can, however, be disrupted by the addition of four equivalents of the stronger base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and is apparently not formed in the competing solvent [D_6]DMSO. In both of these cases, the observed spectra for the 1:1 mixtures of **2** and **5** are the same as those of the separate components in the same solvent (base). This is what would be expected for a heterodimer maintained by hydrogen bonding between carboxylic acid and pyridine groups.[25]

Having established that the 1:1 heterodimer is a stable species, it was essential to demonstrate that the heterotopic self-assembly was not merely the result of mass action. Experiments with fixed 1:1 stoichiometries of soluble components will always favor a 1:1 complex at the expense of other

potential aggregates of other stoichiometries. Indeed, a Job plot experiment over a range of **2**:**5** ratios suggested that additional species enriched in **5** might also be important. The stoichiometry of these additional species was explored in an extraction experiment employing the insoluble tetraacid **6**. Mixtures of the insoluble tetracarboxylic acid **6** and varying amounts of tetra(3-pyridyl) **2** in chloroform were refluxed, and the resulting solutions were analyzed by ^{1}H-NMR. The results of the extraction experiments are presented in Figure 5. The amount of solubilized tetracarboxylic acid **6** was twice the amount of tetrapyridyl **2** added for compositions with up to half an equivalent of added tetrapyridyl **2**. Close scrutiny of the chemical shifts in the ^{1}H-NMR spectra of solutions to which more than half an equivalent of tetrapyridyl **2** had been added, revealed positional shifts for several signals, indicating that other associates were formed after the initial formation of the soluble 2:1 complex. At a 1:1 stoichiometry, the spectrum was comparable to that in Figure 3, part c, indicating the expected formation of the heterodimer. Dilution of a 2:1 mixture of **6** and **2** led to shifts in the positions of several signals due to both components, clearly indicating that the 2:1 complex is weaker than the 1:1 complex. The data suggest initial extraction of a 2:1 complex of tetracarboxylic acid **6** and tetrapyridyl **2**, followed by a shift to the 1:1 heterodimer as the concentration ratio becomes more favorable to the latter.

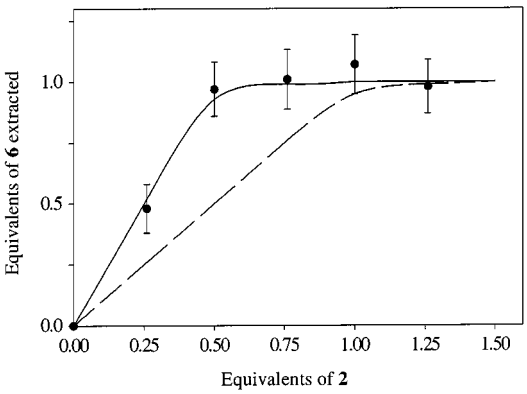


Figure 5. Extraction curve of resorcin[4]arene tetracarboxylic acid **6** ($R' = -C_5H_{11}$) in $CDCl_3$ by tetra(3-pyridyl)resorcin[4]arene **2**; solid line expected for 2:1 complex stoichiometry; dashed line expected for 1:1 complex stoichiometry

The cumulative association constant for the 2:1 complex (β_{21}) was determined by carrying out a dilution experiment using a 2:1 mixture of soluble tetracarboxylic acid **5** and tetrapyridyl **2** (monitoring the upfield shifts of 2-H and 5-H of the pyridyl moieties of **2** and of an aryl signal of **5**, and of the downfield shift of the signal due to the methylene unit adjacent to the carboxylic acid function in **5**). A simultaneous nonlinear fit to the four signals used the values for β_{20} and β_{30} determined as described above by VPO and an assumed value of 10^8 M^{-1} for β_{11}. The dilution data could be suitably fitted ($r^2 = 0.9999$) with a value of log β_{21} = 12.7 ± 0.2. The value of β_{21} is closely related with the value chosen for β_{11} (correlation 0.87). As a consequence, the *stepwise* constant for the association of **5** with the heterodimer **5**·**2** has a constant value of log K = 4.7 ± 0.2 from the ratio β_{21}/β_{11} irrespective of the value of β_{11} (in excess of 10^7 M^{-1}). Thus, at a stoichiometric ratio of 1:1, the heterodimer **5**·**2** is at least 500-fold more stable than any competing higher aggregate.

If the four species discussed thus far were to fully define the system, then it should be possible to choose a suitable ratio of components where the VPO experiment would be sensitive to shortcomings of the model. Figure 6, part A shows the calculated product distribution for the system based on the values derived above by NMR and VPO for a total concentration of 10 mM [A] + [P]. Near the maximum of the A_2P curve (A:P ratio 2:1), all species other than free P contribute 2% or more to the solution composition. The model at this stoichiometry also shows that the minor species will increase proportionally as the solution is diluted. The VPO response curve calculated at a 2:1 ratio of **5** to **2** is compared with the experiment in Figure 6, part B. Figure 6, part B is not a fit of a model to the data; it simply compares a prediction with the data. The *R*/*c versus c* function also fits the calculated curve (not shown). In both representations, the slope and curvature of the model line are found to be strongly dependent on the absolute values of the association constants chosen, with the exception that the ratio β_{21}/β_{11} must have a fixed value as discussed above. The excellent agreement indicates that the solution behavior over the range of compositions is correctly described by the four species discussed, and that the measured constants are essentially correct as well. The species distribution diagram

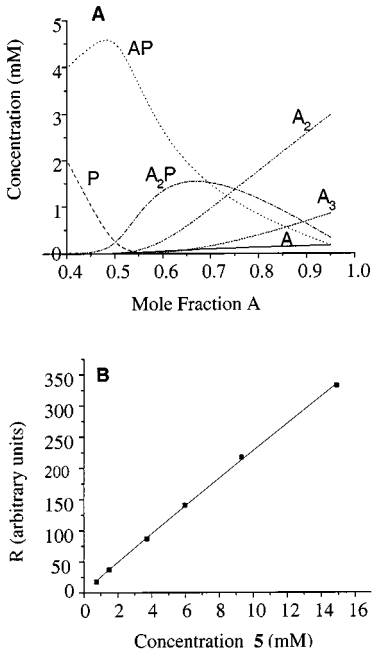


Figure 6. (A) Species distribution calculated for mixtures of tetracarboxylic acid (A) and tetrapyridine (P) as a function of mol fraction (total concentration = 10 mM, log β_{20} = 5.4; log β_{30} = 8.8; log β_{11} = 8.0; log β_{21} = 12.7); (B) Vapor-pressure osmometry response (*R*, arbitrary units) as a function of concentration for a 2:1 mixture of **5** and **2**; data shown as solid squares; lines are calculated from the species concentrations using the assumptions of part A

clearly illustrates that the heterodimer is formed virtually exclusively at a stoichiometric ratio of 1:1.

Conclusions

Noncovalent assembly of resorcinarene tetraacids and tetrapyridines leads to a very stable heterodimer. Other species can be observed or inferred from the experimental data at other stoichiometries, but at a 1:1 molar ratio virtually all the material is present as a single species (94.8% at 10 mM). Not only is the heterodimer very stable, it is substantially more stable than previously reported calixarene-based materials bearing the same functionalities.[8]

Homotopic self-assembly competes only with self-disassembly through dissociation. Heterotopic self-assembly has the same dissociative component, but has additional competitive associative processes to overcome. In this system, the AP heterodimer competes with additional species enriched in the acid component. Only two such processes have been detected:

$$AP + A <=> A_2P \quad (3)$$

$$AP + A <=> A_2 + P \quad (4)$$

Neither of these is significant at a 1:1 stoichiometry of the components, simply due to mass action. Even at other stoichiometric ratios these processes are roughly 500-fold less important than the main heterodimer formation. Only under extremes of concentration (neat A; insoluble A in the presence of excess P) can these species be observed directly.

Heterotopic self-assembly in this system is based on a very modest energetic difference between the acid–pyridine and acid–acid interactions (ca. 1 kJ/mol per interaction). Nonetheless, this difference is sufficient to drive the system towards a single heterodimer. Positive cooperativity must play a role, as indicated by the resistance of the **5**·**2** species to dissociation upon treatment with pyridine. It is also significant that species enriched in the pyridine component could not be observed in any experiment. Clearly, the preorganization and rigidity of the resorcinarene framework directs the noncovalent assembly process.

Experimental Section

General Information: Melting points are uncorrected. – ^{1}H NMR spectra were recorded in $CDCl_3$ solution using a Varian Unity 400 WB NMR spectrometer operating at 400 and 100 MHz for ^{1}H and ^{13}C, respectively. – Fast-atom bombardment (FAB) mass spectra were obtained on a Finnigan MAT 90 spectrometer with 3-nitrobenzyl alcohol as a matrix. Solvents were purified by standard procedures. All other chemicals were analytically pure and were used without further purification. All reactions were carried out under an argon atmosphere. The presence of solvent in the analytical samples was confirmed by ^{1}H-NMR spectroscopy. – Flash chromatography was performed on silica gel (SiO_2, Merck, 0.040–0.063 mm, 230–400 mesh). – Preparative thin-layer chromatography (PTLC) was performed using precoated silica plates (Merck, Kieselgel 60 F_{254}, 2 mm). Tetrahydroxycavitand **1**[26] and resorcin[4]arene tetracarboxylic acid **5** ($R^2 = -C_{11}H_{23}$)[1b] were obtained according to published procedures.

7,11,15,28-Tetrakis[(3-pyridyl)methoxy]-1,21–23,25-tetrapentyl Cavitand (2): A suspension of tetrahydroxycavitand **1** (0.50 g, 0.57 mmol), Cs_2CO_3 (3.70 g, 11.36 mmol), and 3-picolyl chloride (1.00 g, 6.10 mmol) in CH_3CN (60 mL) was stirred at room temperature for 2 weeks. The solvent was subsequently removed in vacuo and the residue was redissolved in CH_2Cl_2 (100 mL). The resulting solution was washed with H_2O (3 × 50 mL) and brine (3 × 50 mL), and dried over Na_2SO_4. The solvent was removed in vacuo and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH, 95:5) to afford **2**. Yield: 0.26 g (37%); m.p. 194–196 °C. – ^{1}H NMR: δ = 8.54 (s, 4 H, pyr-ArH^2), 8.46 (d, 4 H, J = 3.4 Hz, pyr-ArH^6), 7.65 (d, 4 H, J = 7.9 Hz, pyr-ArH^4), 7.3–7.2 (m, 4 H, pyr-ArH^5), 6.78 (s, 4 H, ArH), 5.73 (d, 4 H, J = 7.0 Hz, OCH_2O), 4.88 (s, 8 H, OCH_2Ar), 4.65 (t, 4 H, J = 7.9 Hz, ArCHRAr), 4.35 (d, 4 H, J = 7.3 Hz, OCH_2O), 2.2–2.1 [m, 8 H, $CH_2(CH_2)_3CH_3$], 1.4–1.2 [m, 24 H, $CH_2(CH_2)_3CH_3$], 0.85 [t, 12 H, J = 6.9 Hz, $CH_2(CH_2)_3CH_3$]. – ^{13}C NMR: δ = 149.2, 149.1, 148.2, 144.4, 139.0, 135.4, 133.2, 123.3, 114.6 (Ar), 72.7 (t, OCH_2pyr). – FAB-MS; *m/z*: 1245.5 $[M + H]^+$ (calcd. 1245.6). – $C_{76}H_{84}N_4O_{12}$ (1245.5): calcd. C 73.29, H 6.80, N 4.50; found C 73.32, H 6.84, N 4.49.

7,11,15,28-Tetrakis[(4-pyridyl)methoxy]-1,21–23,25-tetrapentyl Cavitand (3): This resorcinarene was prepared according to the procedure described for **2** using tetrahydroxycavitand **1** (0.25 g, 0.28 mmol), Cs_2CO_3 (1.85 g, 5.68 mmol), and 4-picolyl chloride (0.50 g, 3.05 mmol) in CH_3CN (30 mL). The crude product was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH, 95:5) to afford **3**. Yield: 0.09 g (25%); m.p. 103–104 °C. – ^{1}H NMR: δ = 8.59 (d, 8 H, $J \approx 5$ Hz, pyr-ArH^{3,5}), 7.29 (d, 8 H, $J \approx 5$ Hz, pyr-ArH^{2,6}), 6.76 (s, 4 H, ArH), 5.78 (d, 4 H, J = 7.0 Hz, OCH_2O), 4.98 (s, 8 H, OCH_2Ar), 4.72 (t, 4 H, J = 7.9 Hz, ArCHRAr), 4.47 (d, 4 H, J = 7.3 Hz, OCH_2O), 2.3–2.1 [m, 8 H, $CH_2(CH_2)_3CH_3$], 1.5–1.3 [m, 24 H, $CH_2(CH_2)_3CH_3$], 0.91 [t, 12 H, J = 6.9 Hz, $CH_2(CH_2)_3CH_3$]. – ^{13}C NMR: δ = 149.8, 148.0, 147.1, 139.1, 121.4, 114.7 (Ar), 73.4 (t, OCH_2pyr). – FAB-MS; *m/z*: 1245.5 $[M + H]^+$ (calcd. 1245.6). – $C_{76}H_{84}N_4O_{12}$ (1245.5): calcd. C 73.29, H 6.80, N 4.50; found C 73.26, H 6.80, N 4.39.

7,11,15,28-Tetrakis[(methoxycarbonyl)methoxy]-1,21–23,25-tetrapentyl Cavitand (4): A solution of tetrahydroxycavitand **1** (0.25 g, 0.28 mmol), K_2CO_3 (0.39 g, 2.84 mmol), and methyl bromoacetate (0.12 mL, 1.28 mmol) in CH_3CN (25 mL) was refluxed for 20 h. The mixture was then allowed to cool to room temperature and the solvent was removed in vacuo. The residue was redissolved in CH_2Cl_2 (50 mL) and the resulting solution was washed with 1 N HCl (10 mL), H_2O (3 × 10 mL), and brine (10 mL), and dried over $MgSO_4$. After filtration, the filtrate was concentrated to dryness to give **4**, which was used without further purification. Yield: 0.26 g (78%). – ^{1}H NMR: δ = 6.74 (s, 4 H, ArH), 5.66 (d, 4 H, J = 7.4 Hz, OCH_2O), 4.62 (t, 4 H, J = 7.9 Hz, ArCHRAr), 4.49 [s, 8 H, $OCH_2C(O)$], 4.37 (d, 4 H, J = 7.4 Hz, OCH_2O), 3.71 (s, 12 H, OCH_3), 2.2–2.0 [m, 8 H, $CH_2(CH_2)_3CH_3$], 1.4–1.2 [m, 24 H, $CH_2(CH_2)_3CH_3$], 0.86 [t, 12 H, J = 6.5 Hz, $CH_2(CH_2)_3CH_3$].

7,11,15,28-Tetrakis[(hydroxycarbonyl)methoxy]-1,21–23,25-tetrapentyl Cavitand (6): To a solution of **4** (0.26 g, 0.22 mmol) in THF (15 mL) was added 2 N NaOH (5 mL) and the mixture was stirred at room temperature for about 16 h. It was then acidified with 1 N HCl (15 mL) and the THF was removed under reduced pressure. The precipitate formed was collected by filtration through Celite and thoroughly washed with H_2O. After drying the solid at 80 °C in vacuo for 3 h, it was suspended in THF and filtered. The filtrate was concentrated to dryness to give pure **6**. Yield: 0.21 g (85%); m.p. 269–270 °C. – ^{1}H NMR ([D$_6$]DMSO): δ = 12.8 (br. s,

4 H, COOH), 7.28 (s, 4 H, ArH), 5.70 (d, 4 H, J = 7.1 Hz, OCH_2O), 4.51 (t, 4 H, J = 7.9 Hz, ArCHRAr), 4.45 [s, 8 H, $OCH_2C(O)$], 4.24 (d, 4 H, J = 7.0 Hz, OCH_2O), 2.3–2.1 [m, 8 H, $CH_2(CH_2)_3CH_3$], 1.5–1.2 [m, 24 H, $CH_2(CH_2)_3CH_3$], 0.88 [t, 12 H, J = 6.6 Hz, $CH_2(CH_2)_3CH_3$]. – ^{13}C NMR ([D$_6$]DMSO): δ = 170.2 (s, C=O). – FAB-MS; *m*/*z*: 1135.4 $[M + Na]^+$ (calcd. 1135.5). – $C_{60}H_{72}O_{20}\cdot 1.5H_2O$ (1140.3): calcd. C 63.20, H 6.63; found C 63.05, H 6.54.

Determination of Dimerization Constants for Resorcin[4]arene Tetracarboxylic Acid 5 and Phenyloxyacetic Acid: Dilution experiments were performed over the concentration ranges 0.29–10.0 mM for resorcin[4]arene **5** and 1.14–40.0 mM for phenyloxyacetic acid in $CDCl_3$ solution. Calculations were carried out according to the method of Horman and Dreux.[19]

Determination of Cumulative Formation Constants: The homotopic association of **5** was analysed from spectra recorded at eight concentrations in the range 0.14–20 mM by monitoring the signals as described in the text. Heterotopic association constants were determined by mixing solutions of the host and guest (10 mM each) in $CDCl_3$ at nine different ratios (1:9–9:1) and monitoring the chemical shifts. In each case, the known chemical shifts of the isolated components, the assumed chemical shifts of the major species, and the cumulative formation constants for the system were fitted to equations for the weighted average chemical shift and the overall mass balance using the program Scientist (MicroMath Inc.). For fits involving the 1:1 species, fixed values of β_{11} were used; the fitting of β_{21} used fixed values of β_{20} and β_{30} determined by VPO (see below).

Extraction of Resorcin[4]arene Tetracarboxylic Acid 6 by Tetrapyridyl Resorcin[4]arenes 2 and 3 in $CDCl_3$: To a series of vials containing 2.5 μmol of tetracarboxylic acid **6** each, increasing amounts (0.065–0.315 mL) of a 10 mM stock solution of tetrapyridylresorcin[4]arene in $CDCl_3$ were added, and then further $CDCl_3$ was added to make the total volume 0.5 mL. The mixtures were sonicated for at least 1 h and then heated under reflux for 5 min. in the closed vials. After allowing the mixtures to cool to room temperature, their ^{1}H-NMR spectra were recorded. In order to determine the fraction of tetracarboxylic acid that had dissolved, Equation 5 was applied.

$$F_A = \frac{I_A}{I_P} \times \frac{[P_0]}{[A_0]} \qquad (5)$$

In Equation 5, F_A is the fraction of carboxylic acid extracted, I_A and I_P are the integrals of the NMR signals of the acid and pyridyl protons, respectively, and $[P_0]/[A_0]$ gives the number of equivalents of the pyridyl component. The average of several integrals was used to calculate the fraction of acid extracted, giving the results depicted in Figure 5.

Vapor-Pressure Osmometry: The instrument (Osmomat 070) was operated at 35 °C using ethanol-free $CHCl_3$ as solvent and was calibrated daily using freshly prepared benzil standards prepared gravimetrically. Samples to be analysed were first placed in vacuo to remove volatile impurities, stock solutions were prepared gravimetrically, and aliquots for analysis were prepared by dilution. The solvent zero was periodically checked for instrument drift and a small time-dependent correction was applied to the values as required. Model calculations were performed numerically using the cumulative formation constants (molar units) by minimization of calculated differences in the mass balances.

Acknowledgments

The support of the Netherlands Organization for Scientific Research (NWO) through a visitors grant (T. M. Fyles) is gratefully acknowledged.

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Received July 19, 1999
[O99453]