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Probing Polyvalency in Artificial Systems Exhibiting Molecular Recognition

David A. Fulton, Stuart J. Cantrill,‡ and J. Fraser Stoddart*

Department of Chemistry and Biochemistry, University of California—Los Angeles, 405 Hilgard Avenue, Los Angeles, California 90095-1569

stoddart@chem.ucla.edu

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An approach to the study of polyvalency—the interaction of polyvalent receptors with polyvalent ligands—in unnatural systems is outlined. In this study, the complexation of dibenzylammonium cations by dibenzo[24]crown-8 or benzometaphenylene[25]crown-8 is utilized as the component receptor—ligand interaction. Two analogous multivalent receptors—each containing either seven dibenzo[24]crown-8 (**DB24C8 CLUSTER**) or seven benzometaphenylene[25]crown-8 (**BMP25C8 CLUSTER**) moieties appended to a modified β-cyclodextrin core—were prepared in moderate yields. For each of these multivalent receptors, complementary mono- and divalent ligands containing one or two dialkylammonium centers, respectively, were prepared in good yields. These ligands contained fluorine atom substituents to allow their interactions with crown ether compounds to be probed by ^{19}F NMR spectroscopy. The complexation of these monovalent ligands with the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER** was studied by determining the average binding constant (K_{AVE}) between the receptors and ligands. The abilities of the crown ether clusters to complex with these monovalent ligands was compared with those of the monovalent crown ethers dibenzo[24]crown-8 and benzometaphenylene[25]crown-8. In both instances, it was found that clustering seven crown ethers together into one molecule is detrimental to the abilities of the crown ether moieties to complex with monovalent dialkylammonium ligands. The complexation of the divalent ligands by the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER** was then studied—again by determining K_{AVE}—and their abilities to complex with these ligands was compared with those of their respective component interactions. By determining K_{AVE} for the polyvalent interaction, it was possible to calculate an association constant, K_{POLY}, for the binding of the divalent ligands by the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER** compounds. In both instances K_{POLY} for the polyvalent interaction was found to be approximately 2 orders of magnitude higher than the association constants, K_A, for the component interaction.

Introduction

Natural phenomena provide a never-ending and fascinating source of inspiration for chemists seeking to transfer concepts from the biological world into the materials world. The so-called "glycoside cluster" effect[1] is a particularly interesting phenomenon in glycobiology. By exploiting the glycoside cluster effect, nature compensates for the inherently low binding affinities of carbohydrate ligands for lectins by clustering together carbohydrates and lectins on cell surfaces, leading to stronger, cooperative polyvalent interactions. The resulting gain in binding affinity can often be several orders of magnitude larger than that of a single component carbohydrate—lectin binding interaction. The interactions between carbohydrates and lectins involved in glycoside cluster effects are not the only examples of polyvalent interactions in nature. Indeed, polyvalent interactions involving multiple ligands and multiple receptors are almost certainly common in biology,[2] possibly as an evolutionary consequence of the relative ease of increasing binding affinities by multiplying the number of existing interactions, rather than addressing the more complicated task of evolving a new, stronger interaction.

There are several recent examples[3–5] that highlight the power of polyvalent ligands to act as extremely effective inhibitors of biological processes. One of these examples, the so-called STARFISH ligand, which is an oligovalent carbohydrate ligand that is able to inhibit Shiga-like toxins with subnanomolar activity, is a triumph of rational design.[3] Consisting of five "arms"—each termi-

* Corresponding author. Tel: (310) 206 7078. Fax: (310) 206 1843.
‡ Current Address: Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.

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(5) For a number of examples of multivalency in simple biological systems, such as antibodies, see ref 2.

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nated with a pair of Gb_3 trisaccharide analogues—emanating from a central core, the STARFISH ligand has been tailored to bind to the B-subunit pentamer of a Shiga-like toxin. Using crystallographic information to determine the spatial relationships between the binding sites of the Shiga-like toxin, the dimensions of the STARFISH ligand were designed so that it engages simultaneously, in an extremely effective fashion, to all 10 units of a pair of toxin B subunit pentamers.

Such rational design, however, is not necessarily required for the construction of an effective polyvalent inhibitor. It has been demonstrated recently that a polyacrylamide derivative bearing dodecapeptide ligands (approximately one ligand for every forty acrylamide units) is an efficacious inhibitor (IC_{50} = 20 nM in vitro) of the action of the anthrax toxin in an animal model.[4] The effectiveness of such "random" polyvalency, although poorly understood, does raise the issue of quantity versus quality in the design of polyvalent ligands. Given the amount of synthetic effort required to prepare effective, rationally designed, polyvalent ligands—as well as the realization that suitable crystallographic information is often not available—it may be that the most effective design of polyvalent receptors and ligands often will be one that emphasizes the quantity of interactions over the quality of design.

Systems that exhibit extremely strong association between "unnatural" ligands and receptors bearing multiple copies of their host and guest subunits also have been reported.[6,7] A structurally simple trivalent receptor−ligand pair, based upon the threading of dialkylammonium ions through the cavities of large-ring crown ethers, has been developed and displays very tight binding in relatively nonpolar organic solvents.[6,8] The association constant for this assembly in $CDCl_3/CD_3CN$ was determined by ^{1}H NMR spectroscopy to be greater than 10^6 M^{-1}, significantly larger than that[9] of the monomeric receptor and ligand pair (ca. 2×10^3 M^{-1}).

A receptor presenting three subunits of the antibiotic vancomycin about a trigonal hub binds extremely strongly (K_d $\sim 10^{-17}$ M) in water to a complementary ligand presenting three L-Lys-D-Ala-D-Ala tripeptide units.[7] Very careful analysis of the thermodynamics of the multivalent system led to the conclusion that although the enthalpy of binding was approximately 3 times that of the basic receptor−ligand pair (as is to be expected in an ideal trivalent host−guest system), the gains in the translational and rotational entropy of binding—brought about by linking the units together—were offset by a large loss of conformational entropy. This elegant study brings home the point that ideal multivalent receptors and ligands are those designed such that their subunits assemble together without freezing too many conformationally flexible bonds. Even when this feature is absent, this study demonstrates that extremely high binding constants can still be achieved in multivalent systems.

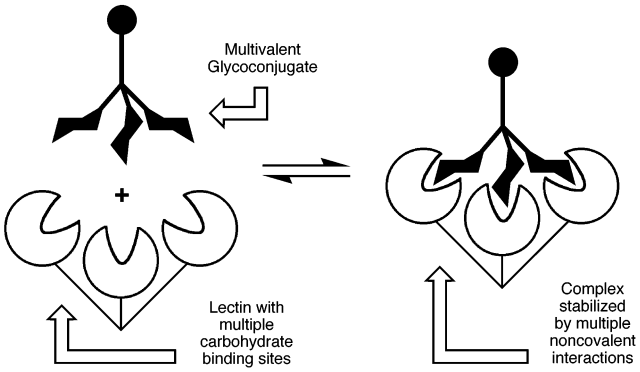

FIGURE 1. A graphical representation of a multivalent glycoconjugate binding with a multivalent lectin. In this instance, the complex is stabilized by multiple noncovalent interactions.

There are various contributing mechanisms[10] responsible for these gains in binding affinity that occur as a consequence of glycoside cluster effects, with chelate effects and statistical effects being two of the most important. In the chelate effect, a polyvalent carbohydrate ligand occupies several binding sites within a single lectin (Figure 1), leading to a stronger, cooperative binding interaction. In the case of the statistical effect, a polyvalent carbohydrate ligand presents a high local concentration of saccharide epitopes to a single binding site on the lectin (Figure 2), leading to slower dissociation rates and thus a larger binding affinity. Given the importance of the glycoside cluster effect in biological systems, it was decided to design and investigate an "unnatural" system that would allow an assessment of the contributions chelate and statistical effects make to polyvalent interactions. The ability to enhance noncovalent binding affinities within a given system, simply by expressing its component molecular recognition elements in a polyvalent fashion, remains a relatively unexplored[11] concept in supramolecular chemistry that could have important applications in materials science, such as in the preparation[8] of novel supramolecular polymers.[12]

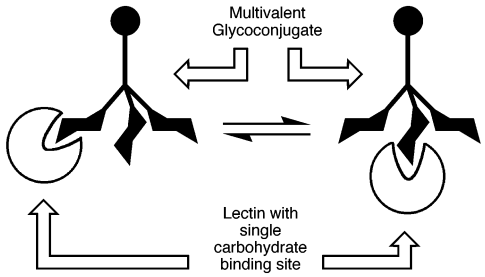

FIGURE 2. A graphical representation of the binding of a multivalent glycoconjugate with a monovalent lectin.

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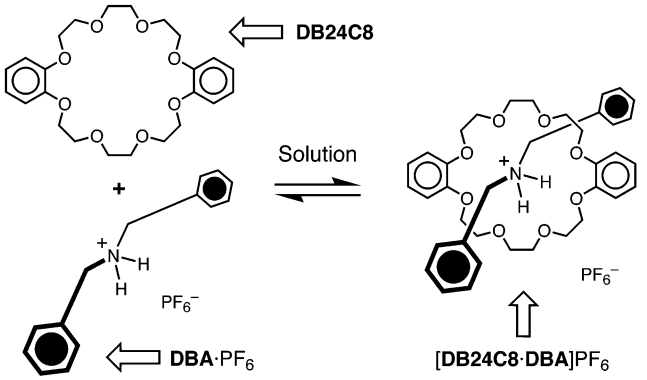


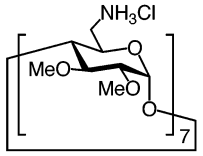
FIGURE 3. The complexation of dibenzylammonium hexafluorophosphate (**DBA**·PF_6) with the crown ether **DB24C8** to form a 1:1 complex with a pseudorotaxane geometry.

The complexation (Figure 3) of dibenzylammonium cations by dibenzo[24]crown-8 (**DB24C8**) is a well-studied binding motif in our laboratory[13] and others'[14] and therefore makes a good candidate for incorporation into multivalent conjugates. Additionally, cyclodextrins[15] (CDs) constitute interesting candidates as scaffolds for the construction of multivalent conjugates. It is possible to functionalize selectively the primary and/or secondary faces of CDs, allowing recognition elements to be attached to the CD scaffold spatially in a very precise manner, and with a large degree of directionality, i.e., all recognition elements are pointing in more or less one particular direction. The attachment of seven recognition elements to a β-CD scaffold also allows for the C_7 symmetry of the CD to be retained in the resulting conjugate, which could help to simplify the study of these compounds by NMR spectroscopic techniques. As all the experiments described in this paper have been performed in organic solvents—which essentially "switches off" the ability of the CD to complex with guest molecules—it can be safely assumed that the CD moieties of the cluster molecules will not have any significant effect on the outcome of these binding experiments.

This paper describes the synthesis of two CD-based crown ether cluster molecules, both of which can act as polyvalent receptors for mono- and divalent dialkylammonium ligands. The interaction of these polyvalent clusters with the mono- and divalent dialkylammonium ligands was then studied by NMR spectroscopic techniques in an effort to determine the contributions of chelate and statistical effects to the binding affinities of these systems.

Results and Discussion

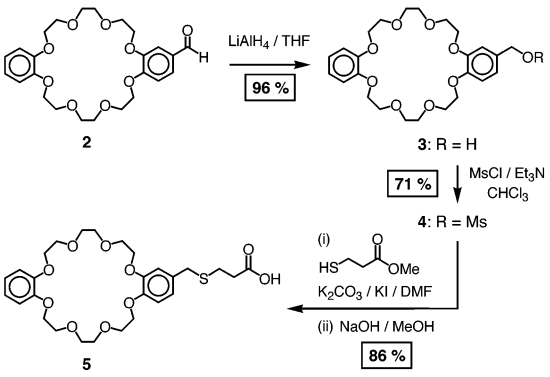
Synthetic Strategy. The grafting of appendages onto CD cores is the key step in the synthesis of any CD-based cluster compound. The perfunctionalization of a heptakisamino-β-CD with *N*-protected amino acids via amide bond formation to afford, after deprotection, water-soluble derivatives has already been successfully accomplished within our research group.[16] This amide bond-forming methodology has been employed also for the attachment of carbohydrate-containing appendages to heptakisamino-β-CD cores in the preparation of cyclodextrin-based carbohydrate cluster compounds.[17] Thus, extension of this methodology should permit the synthesis of CDs perfunctionalized with crown ether appendages. The heptakisamino-β-CD **1**, prepared[17] as its heptakishydrochloride salt from β-CD in four steps by a highly efficient methodology (89% overall yield), was chosen as a suitable CD core on account of the relative ease of its preparation and the potential for the perfunctionalization of its primary face via amide bond formation.



1

Synthesis of a Dibenzo[24]crown-8 Cluster. The **DB24C8** derivative **5** (Scheme 1), which contains an active carboxylic acid function at the end of a short spacer

SCHEME 1. Synthesis of a DB24C8 Derivative 5, Which Contains an Active Carboxyl Function at the End of a Short Spacer Arm



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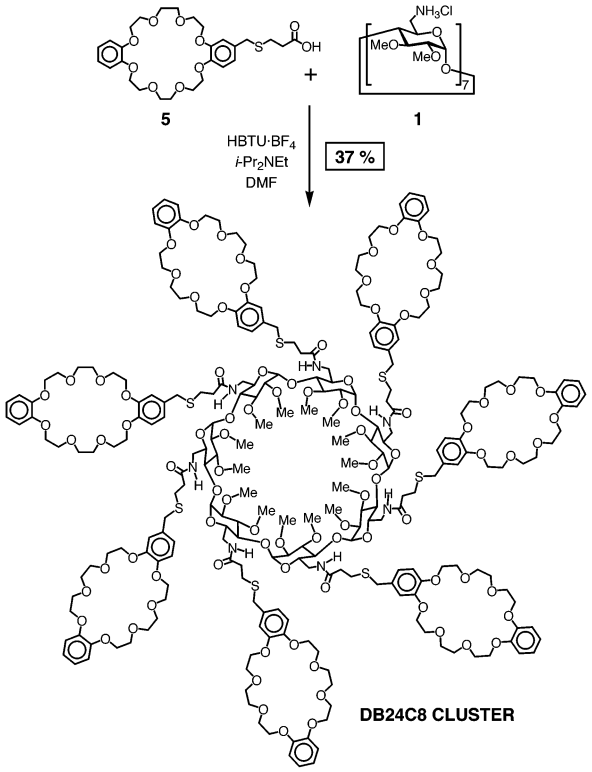
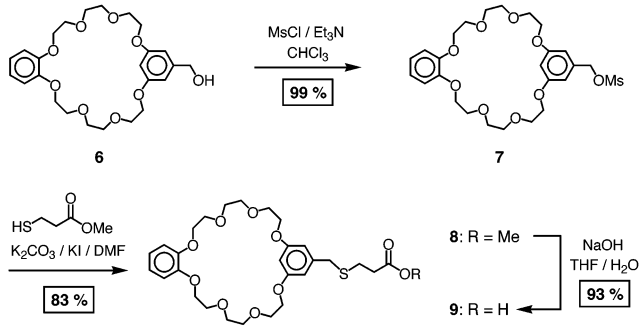
SCHEME 2. Reaction of the DB24C8 Derivative 5 with the Heptakisamino-β-CD Derivative 1 To Afford the DB24C8 Cluster



arm, was prepared in four steps from the known[18] formyl-substituted **DB24C8** macrocycle **2**. Reduction of **2** with $LiAlH_4$ in THF afforded smoothly the corresponding alcohol[19] **3** in 96% yield, which, following treatment with methanesulfonyl chloride and Et_3N in $CHCl_3$, yielded the mesylate **4**. Nucleophilic displacement with methyl 3-mercaptopropionate[20] afforded cleanly the methyl ester derivative that was then transformed readily to the carboxylic acid **5** upon saponification with NaOH in MeOH. This displacement/saponification approach to the synthesis of **5** was found to be far superior to the nucleophilic displacement reaction between the mesylate **4** and 3-mercaptopropionic acid. The amide coupling reagent *O*-benzotriazol-1-yl-*N*,*N*,*N*′*N*′-tetramethyluronium tetrafluoroborate (HBTU·BF_4) has been used effectively for the attachment of carbohydrate-containing ligands to cyclodextrin cores.[17,21] Thus, reaction (Scheme 2) of 14 equiv of the acid **5** with the heptakisamino-β-CD **1** in the presence of 14 equiv of HBTU·BF_4 and 16 equiv of *i*-Pr_2-NEt in DMF afforded the persubstituted **DB24C8 CLUSTER** in 37% yield after purification by gel filtration chromatography.

Synthesis of a Benzometaphenylene[25]crown-8 Cluster. A benzometaphenylene[25]crown-8 (**BMP25C8**) analogue of **5**, which also contains an active carboxylic acid function at the end of a short spacer arm, was prepared in three synthetic steps starting from the known[22] **BMP25C8** benzyl alcohol derivative **6** (Scheme 3). Treatment of **5** with methanesulfonyl chloride and Et_3N in $CHCl_3$ afforded quantitatively the mesylate **7**, which, upon reaction with methyl 3-mercaptopropionate, was transformed cleanly to the methyl ester derivative **8**. As observed previously, direct reaction of the mesylate **7** with 3-mercaptopropionic acid failed to produce the desired product either cleanly or in a reproducible fashion. Saponification of **8** with NaOH in THF/H_2O then afforded the desired carboxylic acid **9** in an overall yield of 76% from alcohol **6**. The crown ether derivative **9** was attached to the heptakisamino-β-CD **1** in the same manner as used for the formation of the **DB24C8 CLUSTER**. Reaction (Scheme 4) of 14 equiv of the acid **9** with the heptakisamino-β-CD **1** in the presence of 14 equiv of HBTU·BF_4 and 16 equiv of *i*-Pr_2NEt in DMF afforded the persubstituted **BMP25C8 CLUSTER** in 36% yield after purification by gel filtration chromatography.

SCHEME 3. The Synthesis of a BMP25C8 Derivative 9, Which Contains an Active Carboxyl Function at the End of a Short Spacer Arm



Characterization of Crown Ether Clusters. A detailed analysis of the ^1H NMR spectra of the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER** (the latter is depicted in Figure 4) was performed in order to ensure that complete perfunctionalization of the CD cores with crown ethers had occurred. The ^1H NMR spectra of the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER** were reasonably sharp and well-defined, features that are indicative of complete substitution of the CD core with crown ether appendages. A small degree of line broadening is evident in both of these ^1H NMR spectra, a phenomenon that has been observed[23] in several other instances of CDs persubstituted with carbohydrate appendages, which is thought to be a consequence of relatively slow dynamic processes occurring in these molecules. Nevertheless, it is observed (Figure 4) that

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(21) Attempts to utilize dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole as activating agents were unsuccessful, largely on account of difficulties encountered in removing the byproducts of the reactions. Although benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (Py-BOP) was found to act as a suitable activator, the removal of the tripyrrolidinophosphonium oxide byproduct was far from a straightforward exercise.
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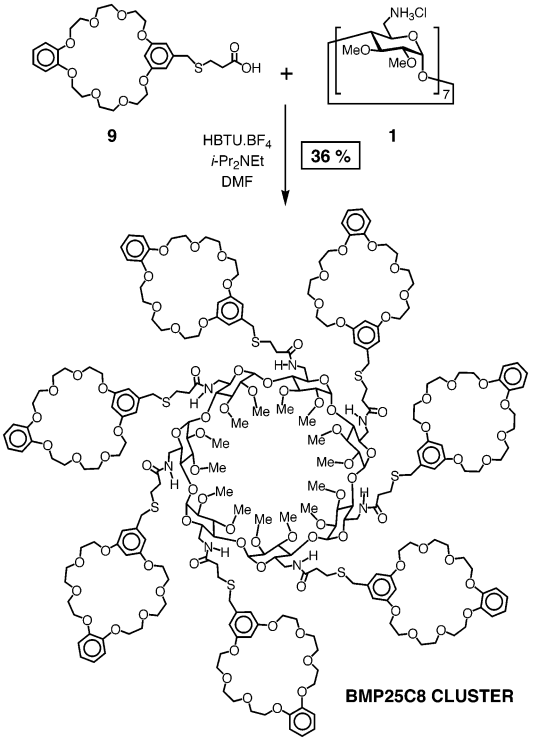
SCHEME 4. Reaction of the BMP25C8 Derivative 9 with the Heptakisamino-β-CD Derivative 1 To Afford the BMP25C8 Cluster



the signals corresponding to the CD protons H-1 and H-2 in the ^{1}H NMR spectrum of the **BMP25C8 CLUSTER** appear as sharp multiplets of correct integration, spectroscopic features that support very strongly the hypothesis that the CD core is persubstituted with seven crown ether appendages. The ^{1}H NMR spectrum of the **DB24C8 CLUSTER** is slightly less well defined than that of the **BMP25C8 CLUSTER**; in this instance the signals corresponding to the H-1 and H-2 protons in the CD torus appear as an almost-resolved doublet and a doublet of doublets, respectively. The quality of both these ^{1}H NMR spectra strongly supports the hypothesis that both the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER** are fully substituted with seven crown ether-containing appendages.

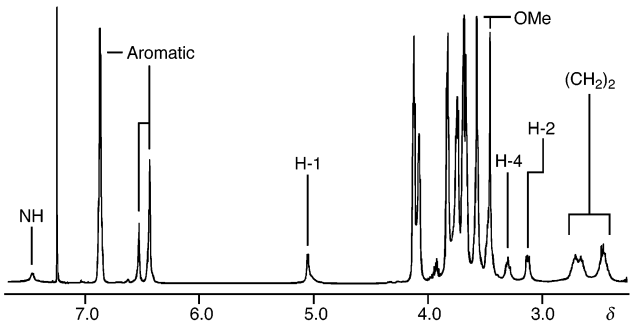


FIGURE 4. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of the **BMP25C8 CLUSTER**.

Further evidence that the CD cores in the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER** are each fully substituted with seven crown ether appendages is present in the ^{13}C NMR spectra of these compounds. The partially assigned ^{13}C NMR spectrum of the **DB24C8 CLUSTER** is shown in Figure 5. The signals are sharp and well-defined, a feature that is also evident in the spectrum of the **BMP25C8 CLUSTER**. The signals corresponding to carbon atoms in the CD torus have weaker intensities than those signals associated with carbon atoms in the crown ether appendages. Again, this phenomenon has been observed[23] previously in the ^{13}C NMR spectra of CD-based carbohydrate clusters—presumably as a result of some dynamic phenomena—and is thus not unexpected. Finally, further evidence to support the homogeneity of the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER** is found in the MALDI-TOF mass spectra of both of these compounds. In each instance, only a single peak corresponding to the molecular ion can be detected; there are no signals corresponding to ions derived from CD cores having less than seven crown ether appendages.

Synthesis of Mono- and Divalent Dialkylammonium Salts. Two pairs of mono- and bis(dialkylammonium) salts were prepared and were used as mono- and divalent ligands in the study of the binding properties of the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER**. Each ligand contains fluorine atom substituents, which allowed the binding with the crown ether clusters to be studied using ^{19}F NMR spectroscopy, a technique that has proven extremely useful[22] for studying the binding of complex dialkylammonium ion/crown ether systems. The mono- and dications **DBA-F$_2^+$** and **bis-DBA-F$_2^{2+}$**—each of which contains two para-substituted fluorine atoms—were used as ligands in investigations with the **DB24C8 CLUSTER**. The corresponding pair of mono- and dications **DBA-F$_4^+$** and **bis-DBA-F$_4^{2+}$** (Scheme 5) were used as ligands in binding studies with the **BMP25C8 CLUSTER**. It has been shown[22] that bis(3,5-difluorobenzyl)ammonium cations thread through the cavity of **BMP25C8** at a rate such that slow exchange of complexed and uncomplexed species is observed on the ^{19}F NMR time scale at 376 MHz. Therefore, binding constants can be determined[24] simply by integrating the signals of the bound and unbound species present in the ^{19}F NMR spectrum of a solution of the crown ether and the dialkylammonium cation, as is the case[9] with the binding of dibenzylammonium salts with **DB24C8** derivatives.

Thus, as reported previously,[22] condensation of the aldehyde **10** with the benzylamine **11** afforded (Scheme 5)—after reduction of the resulting imine, protonation with HCl and counterion exchange with NH_4PF_6—the *p*-fluoro-substituted monovalent cation **DBA-F$_2^+$** as its PF_6^- salt. Using an identical procedure,[22] the aldehyde **12** was reacted with the amine **13** to afford the PF_6^- salt of **DBA-F$_4^+$**. Condensation of **11** with the dialdehyde **14** afforded—after reduction of the resultant diimine with $NaBH_4$ followed by direct protonation with NH_4PF_6—the

(23) (a) García-López, J. J.; Santoyo-González, F.; Vargas-Berenguel, A.; Giménez-Martínez, J. J. *Chem. Eur. J.* **1999**, *5*, 1771−1784. (b) García-López, J. J.; Hernández-Mateo, F.; Isac-García, J.; Kim, J. M.; Roy, R.; Santoyo-González, F.; Vargas-Berenguel, A. *J. Org. Chem.* **1998**, *64*, 522−531. (c) Furuike, T.; Aiba, S.; Nishimura, S.-I. *Tetrahedron* **2000**, *56*, 9909−9915. (d) Fulton, D. A.; Stoddart, J. F. *Org. Lett.* **2000**, *2*, 1113−1116.

(24) Hill, Z. D.; MacCarthy, P. *J. Chem. Educ.* **1986**, *63*, 162−167. (b) Connors, K. A. *Binding Constants*; Wiley-Interscience: New York, 1987, pp 191−192. (c) Adrian, J. C.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 678−680. (d) Fielding, L. *Tetrahedron* **2000**, *56*, 6151−6170.

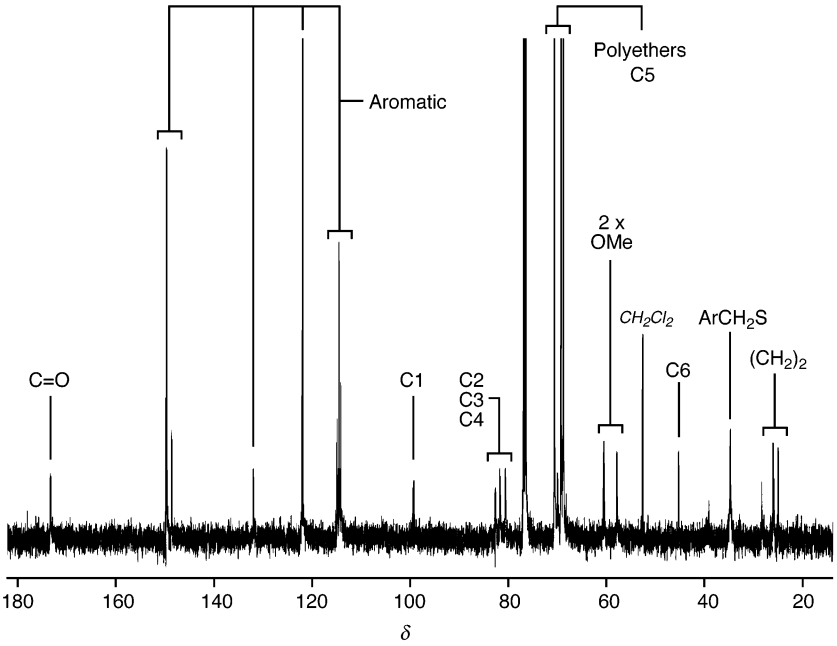
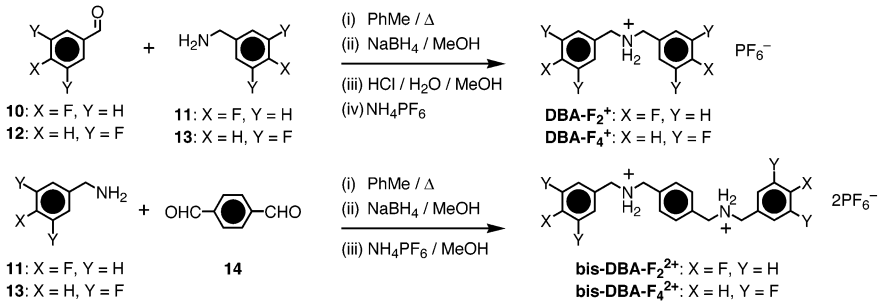


FIGURE 5. ^{13}C NMR Spectrum (125 MHz, $CDCl_3$) of the **DB24C8 CLUSTER**.

SCHEME 5. Synthesis of the Monovalent Ammonium Cations DBA-F_2^+ and DBA-F_4^+ and the Divalent Bisammonium Dications Bis-DBA-F_2^{2+} and Bis-DBA-F_4^{2+}



dication **bis-DBA-F_2^{2+}**. Condensation of **13** with **14** furnished, after similar treatment, the dication **bis-DBA-F_4^{2+}**. ^{19}F NMR spectroscopy was used to confirm that the compounds **DBA-F_2^+**, **DBA-F_4^+**, **bis-DBA-F_2^{2+}**, and **bis-DBA-F_4^{2+}** had been prepared as their PF_6^- salts. Integrations of the signals corresponding to the fluorine atom substituents and the PF_6^- anion gave the expected ratios in the case of all four compounds.

Statistical Effects. To probe statistical effects in polyvalency, a simple question was posed, namely, will a crown ether moiety bind a single dialkylammonium cation more strongly when the crown ethers are clustered together in a single molecule than when a single crown ether binds a single dialkylammonium cation? If the answer to this question is "yes", it can be concluded that clustering crown ethers together in a single molecule should lead to an increase in binding as a consequence of statistical effects.[25] Quantitatively, this question can be answered by determining the average binding constant (K_{AVE}) for the complexation of a dialkylammonium cation by a single crown ether moiety. The average binding constant, which is defined here as "a measure of the strength of the noncovalent binding interaction between a single crown ether moiety and a single dialkylammonium center," is defined mathematically as

$$K_{AVE} = \frac{[\text{complexed } NH_2^+ \text{ sites}]}{[\text{uncomplexed } NH_2^+ \text{ sites}][\text{uncomplexed crown ethers}]} \quad (1)$$

where an "NH_2^+ site" is a single dialkylammonium moiety within a compound containing one or more dialkylammonium sites.

In the first experiment performed, the binding of **DB24C8** with the dialkylammonium thread **DBA-F_2^+** was investigated by NMR spectroscopy. This experiment served as the model upon which the following NMR experiments were based. From the ^{19}F NMR spectrum (Figure 6) of a solution of **DB24C8** (7 mM) and the dialkylammonium thread **DBA-F_2^+** (14 mM) in CD_3CN, it is straightforward to calculate the concentrations of complexed and uncomplexed **DBA-F_2^+**. The concentrations of complexed and uncomplexed **DB24C8** can therefore be calculated, as it is known[22] that **DB24C8** binds **DBA-F_2^+** in a 1:1 complex with a pseudorotaxane geom-

(25) It is assumed that the electronic properties of the crown ethers are not significantly altered as a consequence of the presence of the tether that attaches the crown ether to the CD core.

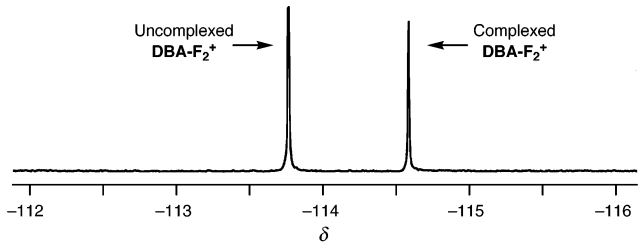


FIGURE 6. Partial ^{19}F NMR spectrum (376 MHz, CD_3CN) of a solution of **DB24C8** (7 mM) and **DBA-F_2^+** (14 mM), showing signals for both complexed and uncomplexed **DBA-F_2^+**.

etry. Thus, the average association constant (K_{AVE})[26] for the binding interaction between **DB24C8** and **DBA-F_2^+** can be calculated using eq 1. To obtain a better estimate for the value of K_{AVE} for this binding interaction, an NMR titration experiment was performed, in which the concentration of **DB24C8** was held constant at 7 mM throughout, while incrementally increasing the concentration of **DBA-F_2^+**. By performing this titration experiment, it is possible to plot a binding isotherm[27,28] (Figure 7) for the complexation of **DBA-F_2^+** with **DB24C8**. A "best-fit" isotherm has been drawn and serves as a useful comparison with other isotherms.[29] An accurate value of K_{AVE} was therefore determined by simply taking the mean of the K_{AVE} values, each of which corresponds to a data point on the binding isotherm. The standard deviation from this pool of data serves as an indication of the error in the calculated value of K_{AVE}. For the binding of **DB24C8** with the thread **DBA-F_2^+**, the mean value of K_{AVE} was calculated to be 790 ± 240 M^{-1}, a value that compares well[30] to the complexation of other dialkylammonium ions by **DB24C8**.

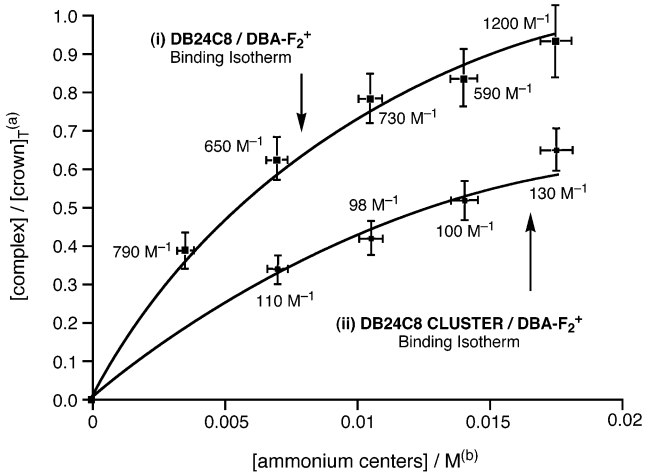


FIGURE 7. "Best fit" binding isotherms (solid lines) for (i) complexation of **DBA-F_2^+** by **DB24C8** and (ii) complexation of **DBA-F_2^+** by the **DB24C8 CLUSTER**. (a) The values represented on this axis represent the measured concentration of complexed dialkylammonium centers divided by the total concentration of crown ether in solution. Error bars on this axis are set at ±10% to reflect errors in the preparation of the sample and in determination of values of the integrals. (b) The values represented on this axis represent the total concentration of dialkylammonium cations in solution. Error bars on this axis are set at ±4% to reflect errors in the preparation of the sample.

A similar NMR titration experiment was performed to determine the mean value of K_{AVE} for the binding of thread **DBA-F_2^+** with the **DB24C8 CLUSTER**. In this NMR titration experiment, the concentration of the **DB24C8 CLUSTER** was held constant throughout at 1 mM, i.e., the concentration of crown ethers in solution remains constant at 7 mM, a concentration that is identical to that of **DB24C8** used in the previous experiment. A sample ^{19}F NMR spectrum from this titration experiment is shown in Figure 8. As expected, signals corresponding to complexed and uncomplexed **DBA-F_2^+** can be observed in the NMR spectrum. The signal corresponding to complexed **DBA-F_2^+** is broadened, presumably because (1) the fluorine atoms of complexed **DBA-F_2^+** are diastereotopic because of the chirality of the CD and (2) there are many different isomers of the complex formed when the cluster complexes with two or more dialkylammonium ion threads. Taken together, it is clear that the fluorine atoms of bound **DBA-F_2^+** can find themselves in many slightly differing magnetic environments upon complexation with the **DB24C8 CLUSTER**, leading to a complex, broadened signal.[31] Nevertheless, from the NMR spectrum, it is straightforward to determine the concentrations of uncomplexed and complexed **DBA-F_2^+** in solution—and therefore the concentrations of uncomplexed and complexed crown ether moieties—allowing a value of K_{AVE} to be obtained. This NMR experiment was repeated at different concentrations of **DBA-F_2^+** and the binding isotherm (Figure 7) plotted from the data points. From these data points, the mean K_{AVE} was determined to be 110 ± 33 M^{-1}.

The abilities of **BMP25C8** and the **BMP25C8 CLUSTER** to bind the dialkylammonium ion **DBA-F_4^+** were

(26) In this instance, $K_{AVE} = K_A$, where K_A is the binding constant between **DB24C8** and **DBA-F_2^+**.

(27) We have chosen to use binding isotherms, rather than Scatchard plots, to display our data. The main advantage of displaying data in Scatchard plots is that curvature of the data points suggests positive or negative cooperativity. (For a recent example of the use of Scatchard plots in determining the degree of cooperativity in a host–guest system similar to ours, see: Gibson, H. W.; Yamaguchi, N.; Hamilton, L.; Jones, J. W. *J. Am. Chem. Soc.* **2002**, *124*, 4653–4665.) Nonideal polyvalent systems are invariably uncooperative. It should be noted that Scatchard plots are mathematical approximations of the actual binding isotherm and thus are subject to the limitations of such an approximation. (There is some controversy about the advantages and limitations of Scatchard analysis; see ref 24b, as well as Klotz, I. M. *Acc. Chem. Res.* **1974**, *7*, 162–168). We decided, therefore, to model our systems on a 1:1 binding isotherm—arguably the purest way in which to represent receptor–ligand interactions—as polyvalent interactions can be simplified in their analysis to the interaction between one ligand and one receptor. Our experimental evidence reported in this paper suggests that this model is a valid one.

(28) To aid clarity, all binding isotherms presented in this work have been plotted using the approximation that [free ligand] = [total ligand].

(29) As the binding isotherms described here are only qualitative, only four to six data points were determined for every system discussed. Even this small number of data points allows a binding isotherm to be determined that is sufficiently accurate for the purposes of qualitative comparison. Bear in mind also that the association constant is calculated at each point in the isotherm using the single-point technique, and thus, we do not require multiple data points in order to utilize a curve-fitting approach to solve for K_{AVE}.

(30) (a) Ashton, P. R.; Fyfe, M. C. T.; Hickingbottom, S. K.; Stoddart, J. F.; White, A. J. P.; Williams, D. J. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2117–2128. (b) Ashton, P. R.; Bartsch, R. A.; Cantrill, S. J.; Hanes, R. E., Jr.; Hickingbottom, S. K.; Lowe, J. N.; Preece, J. A.; Stoddart, J. F.; Talanov, V. S.; Wang, Z.-H. *Tetrahedron Lett.* **1999**, *40*, 3661–3664.

(31) Although this signal is broad, we believe that it is still possible to obtain an accurate value for its area by integration.

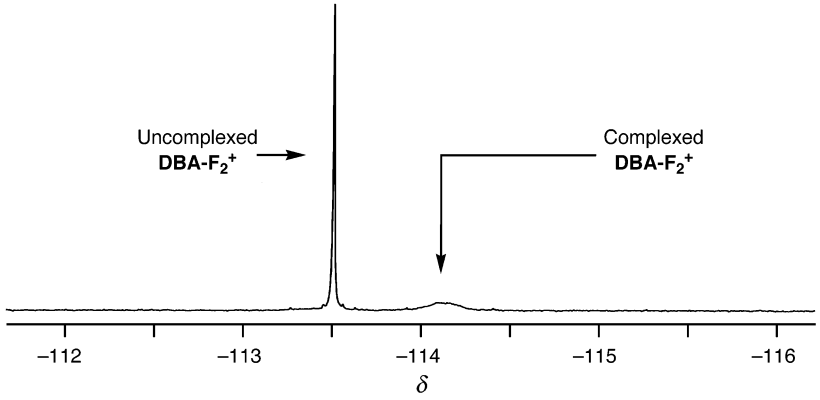


FIGURE 8. Partial ^{19}F NMR spectrum (376 MHz, CD_3CN) of a solution of the **DB24C8 CLUSTER** (1 mM) and **DBA-F$_2^+$** (14 mM), which shows signals for both complexed and uncomplexed **DBA-F$_2^+$**.

evaluated in a manner similar to that described for the binding of **DBA-F$_2^+$** by **DB24C8** and the **DB24C8 CLUSTER**. The binding isotherms[32] for the complexation of **DBA-F$_4^+$** by **BMP25C8** and the **BMP25C8 CLUSTER** are shown in Figure 9. The mean K_{AVE} for the binding[22] of **DBA-F$_4^+$** by **BMP25C8** was calculated to be 20 ± 6 M^{-1}, and the mean K_{AVE} for the binding of this ion by the **BMP25C8 CLUSTER** was calculated to be 11 ± 3 M^{-1}.

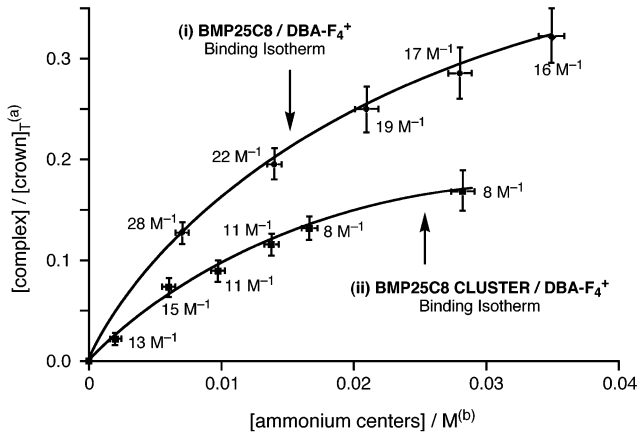


FIGURE 9. "Best fit" binding isotherms for (i) complexation of **DBA-F$_4^+$** by **BMP25C8** and (ii) complexation of **DBA-F$_4^+$** by the **BMP25C8 CLUSTER**. (a) The values represented on this axis represent the measured concentration of complexed dialkylammonium centers divided by the total concentration of crown ether in solution. (b) The values represented on this axis represent the total concentration of dialkylammonium cations in solution. Error bars are set as defined in Figure 7.

The results from all four experiments are summarized in Table 1. It appears that the clustering of seven **DB24C8** moieties into one cluster molecule is detrimental to the abilities of its crown ether moieties to bind the dialkylammonium cation **DBA-F$_2^+$**, relative to the ability of **DB24C8** to bind the same dialkylammonium cation. In terms of free energy, the costs of clustering **DB24C8** moieties together is ca. 1.1 kcal mol^{-1} in this instance. The same trend[33] is observed with the **BMP25C8 CLUSTER**, whose crown ether moieties bind the ammonium thread **DBA-F$_4^+$** less effectively than **BMP25C8** by ca. 0.4 kcal mol^{-1}. Therefore, the question of whether a crown ether moiety binds a single ammonium cation more strongly when the crown ethers are clustered together in a single molecule, compared to a single crown ether binding a single ammonium cation, can be answered with a very definite "no". This negative answer is probably a

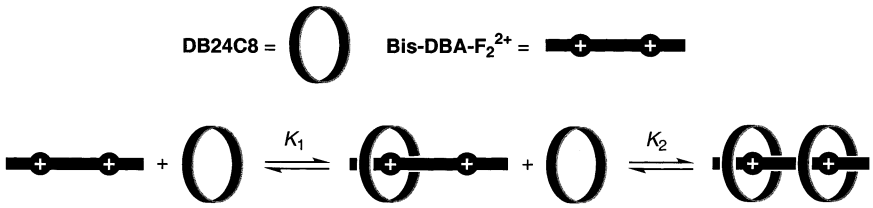
TABLE 1. Values of Mean K_{AVE} and $\Delta G°_{AVE}$ for the Interactions in CD_3CN at 300 K of DB24C8 and the DB24C8 CLUSTER with DBA-F$_2^+$ and of BMP25C8 and the BMP25C8 CLUSTER with DBA-F$_4^+$

host	guest	mean K_{AVE} (M^{-1})	$\Delta G°_{AVE}$ (kcal mol^{-1})
DB24C8	**DBA-F$_2^+$**	790 ± 240	-3.9 ± 0.2
DB24C8 CLUSTER	**DBA-F$_2^+$**	110 ± 33	-2.8 ± 0.2
BMP25C8	**DBA-F$_4^+$**	20 ± 6	-1.8 ± 0.2
BMP25C8 CLUSTER	**DBA-F$_4^+$**	11 ± 3	-1.4 ± 0.2

(32) The Weber rule of thumb (see, for instance: Tsukube, H.; Furuta, H.; Odani, A.; Takeda, Y.; Kudo, Y.; Inoue, Y.; Liu, Y.; Sakamoto, H.; Kimura, K. In *Comprehensive Supramolecular Chemistry*, Davies, J. E., Ed.; Pergamon Press: Oxford, 1996; Vol. 8, pp 425−482) states that accurate association constants can be obtained only at total concentrations of host and guest at which the minor component in the system is between 20 and 80% complexed. In the upper isotherm of Figure 9, all data are within this range, even the point labeled 28 M^{-1}, which corresponds to ca. 25% of the ammonium ions being complexed. Every point in the lower isotherm, however, corresponds to a situation in which there is less than 20% complexation of all of the species in solution. This situation arises because of the very weak association constant in this system (see discussion in the text), which results in only ca. 18% of the crown ether units being bound at 28 mM of **DBA-F$_4^+$**. Presumably, adding more of this salt would eventually result in greater than 20% of the crown ether units being complexed, but we did not wish to overload our solutions with salt in order to avoid affecting the dielectric constant of the medium. As such, the data points in this binding isotherm must be treated with caution, but, even so, the value of K_{AVE} in this system obviously is very low.

(33) It appears from the data presented in Table 1 that the magnitude of this detrimental binding effect, which occurs when crown ethers are clustered, is somewhat more pronounced in the **DB24C8** systems than in the **BMP25C8** systems. There are two principal reasons, however, why it is unwise to draw comparisons between the **DB24C8** and **BMP25C8** systems. First, these systems were studied at different concentrations of crown ether moieties; in the **DB24C8** system, the concentration of crown ethers was kept constant at 7 mM, while in the **BMP25C8** system, the concentration of crown ethers was kept constant at 14 mM. Recent research (Chang, T.; Heiss, A. M.; Cantrill, S. J.; Fyfe, M. C. T.; Pease, A. R.; Rowan, S. J.; Stoddart, J. F.; White, A. J. P.; Williams, D. J. *Org. Lett.* **2000**, *2*, 2947−2950) has indicated that the binding of dibenzylammonium cations by crown ethers is concentration dependent, that is, association constants can differ depending on the concentrations at which they are measured. Second, the **DB24C8** and **BMP25C8** systems are, almost certainly, not identical in terms of the enthalpy and entropy changes that occur when dibenzylammonium cations are bound. Again, it is concluded that it is unwise to draw comparisons between them.

SCHEME 6. A Graphical Representation of the Complexation of DB24C8 with the Divalent Bisammonium Dication Bis-DBA-F_2^{2+}



consequence of unfavorable electrostatic and steric effects, which occur when the crown ether clusters bind dialkylammonium ions. When a cluster binds a single dialkylammonium ion, the resulting complex carries a unit of positive charge. Even though solvent and anions will help to shield this bound positive charge from other unbound dialkylammonium ions, there will still be a certain degree of electrostatic repulsion between the positively charged complex and the positively charged unbound ions, thus reducing the ability of the cluster to bind any further dialkylammonium cations. Indeed, the more dialkylammonium cations complexed per cluster, the greater this electrostatic repulsion will become. Steric hindrance from bound threads and anions—which probably form loose ion pairs with bound ions—could also make it even more difficult for the crown ether clusters to bind any further dialkylammonium ions. Yamaguchi and Gibson reported[34] similar findings for the binding of dibenzylammonium cations by polymers containing appended **DB24C8** moieties. In that study, the authors suggested that electrostatic repulsion and steric hindrance were responsible for reducing the abilities of the appended crown ethers to complex with dibenzylammonium cations.

Probing Chelate Effects. The study of the contribution of chelate effects to polyvalent interactions is not straightforward. In this discussion, the system of nomenclature describing polyvalent interactions proposed by Whitesides and co-workers[2] is used. A polyvalent ligand can interact with a polyvalent receptor in numerous different ways to afford potentially a large number of species in solution. The value of K_{AVE} for a polyvalent system, relative to K_A for its component interaction, is by no means indicative of the strength of a polyvalent interaction. In other words, K_{AVE} for a polyvalent interaction can be of smaller value than K_A for the component interaction, but the binding constant for the polyvalent interaction (K_{POLY}) can still be extremely high. However, K_{AVE} for a polyvalent interaction is still a crucial quantity, since, with its careful interpretation, K_{AVE} can be used to determine K_{POLY} and, therefore, the free energy change for the polyvalent interaction, ΔG°_{POLY}.

In the first NMR experiment, the binding of **bis-DBA-F_2^{2+}** by **DB24C8** in CD_3CN, which follows the equilibria outlined in Scheme 6, was examined. The ^{19}F NMR spectrum of a solution of **bis-DBA-F_2^{2+}** (7 mM) and **DB24C8** (7 mM) is shown in Figure 10a. Four signals are observed in this spectrum: one signal corresponding to uncomplexed **bis-DBA-F_2^{2+}**, one signal for the 2:1 complex, and two signals corresponding to the 1:1 complex. Of the two signals arising from the 1:1 complex, one signal (the most downfield) corresponds to the fluorine atom adjacent to the uncomplexed dialkylammonium centers and the other signal (the most upfield) corresponds to the fluorine atoms adjacent to the complexed dialkylammonium center. From integrations of these signals, it is straightforward to calculate the concentrations of all species in solution, and thus, K_{AVE} can be calculated using eq 1. The microscopic binding constants K_1 (1500 ± 440 M^{-1}) and K_2 (730 ± 220 M^{-1}) were also determined.[35] As before, K_{AVE} was determined at various concentrations of **bis-DBA-F_2^{2+}** and a binding isotherm plotted (Figure 11).[36] A mean value of K_{AVE} was calculated from the data points, with the standard deviation taken to be a measure of the error in K_{AVE}. The mean value of K_{AVE} for the binding of **bis-DBA-F_2^{2+}** with **DB24C8** was calculated to be 1100 ± 340 M^{-1}.

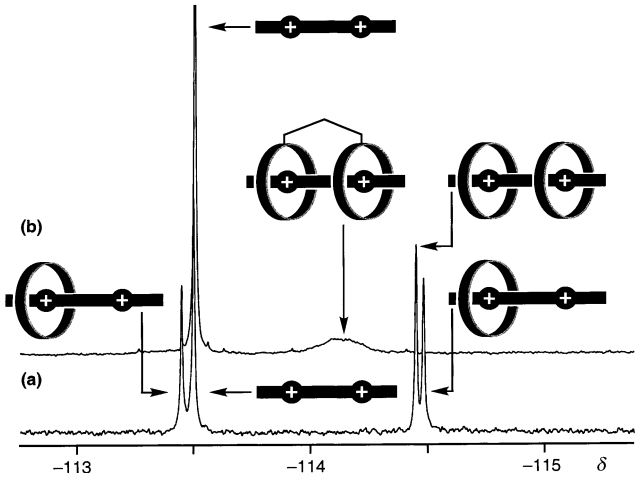


FIGURE 10. Partial ^{19}F NMR spectra (376 MHz, CD_3CN) of (a) a solution of **DB24C8** (7 mM) and **Bis-DBA-F_2^{2+}** (7 mM) and (b) a solution of the **DB24C8 CLUSTER** (1 mM) and **Bis-DBA-F_2^{2+}** (7 mM).

The binding of **bis-DBA-F_2^{2+}** with the **DB24C8 CLUSTER** was then investigated. The ^{19}F NMR spectrum of a solution containing the cluster (1 mM cluster, concentra-

(34) Yamaguchi, N.; Gibson, H. W. *Macromol. Chem. Phys.* **2000**, *201*, 815−824.

(35) The ratio of the microscopic binding constants (K_1/K_2) is approximately 2. For a ditopic system in which the recognition sites act independently, this ratio should be 4 (see ref 24b). The smaller observed ratio seems to suggest that this system behaves in a positively cooperative manner. This conclusion, however, might be erroneous. The assumption that the recognition sites act independently does not hold in such a system, because the crown ether unit in a 1:1 complex can shuttle between the two NH_2^+ centers of the dication. It is not intuitively obvious what effect such shuttling will have on the ratio K_1/K_2, but it seems possible that the rate at which a crown ether unit slips on to a dicationic thread (k_{on}) of a shuttling system will be double that exhibited in a system that cannot shuttle. If true, the effect would be to double K_2, and so K_1/K_2 would be expected statistically to be 2, rather than 4.

tion of crown ethers = 7 mM) and the **bis-DBA-F_2**$^{2+}$ (7 mM) is shown in Figure 10b. Two distinct signals are observed in this spectrum: a sharp signal (−113.5 ppm) corresponding to uncomplexed **bis-DBA-F_2**$^{2+}$ and a very broad signal (−114.1 ppm) corresponding to complexed **bis-DBA-F_2**$^{2+}$, where **bis-DBA-F_2**$^{2+}$ is complexed presumably by *two* crown ether moieties of the cluster. This assumption is made after comparison with the NMR spectrum in Figure 10a. In Figure 10b, there do not appear to be any signals at ca. −114.1 ppm—at least as can be detected by ^{19}F NMR spectroscopy—corresponding to the case in which a **bis-DBA-F_2**$^{2+}$ unit is complexed by only a *single* crown ether moiety of the cluster; i.e., in solution, **bis-DBA-F_2**$^{2+}$ appears to be either *completely uncomplexed* or *both* of its NH_2^+ centers are complexed by crown ether moieties. The signal corresponding to complexed **bis-DBA-F_2**$^{2+}$ has now moved downfield relative to the signal observed when **bis-DBA-F_2**$^{2+}$ is complexed by **DB24C8**, presumably as a consequence of the unique microenvironment that exists within the crown ether cluster.[37] By integration of the signals in the NMR spectrum, again it is straightforward to calculate K_{AVE}. By performing this NMR experiment at various concentrations of **bis-DBA-F_2**$^{2+}$, it is possible to construct a binding isotherm (Figure 11) and, by taking the average of these data points, calculate the mean K_{AVE} to be 160 ± 48 M^{-1}.

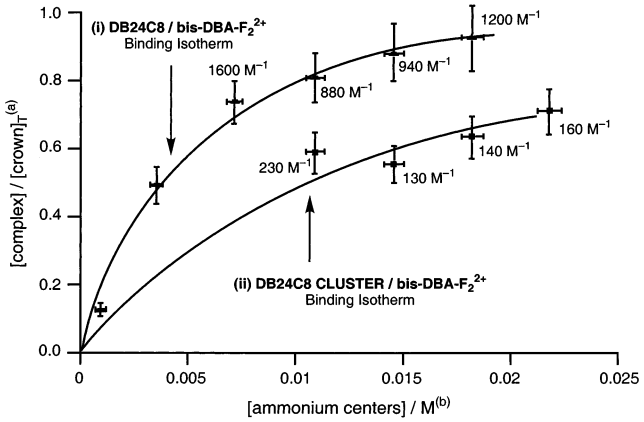


FIGURE 11. "Best fit" binding isotherms for (i) complexation of **Bis-DBA-F_2**$^+$ by **DB24C8** and (ii) complexation of **Bis-DBA-F_2**$^{2+}$ by the **DB24C8 CLUSTER**. (a) The values represented on this axis represent the measured concentration of complexed dialkylammonium centers divided by the total concentration of crown ether in solution. (b) The values represented on this axis represent the total concentration of dialkylammonium cations in solution. Error bars are set as defined in Figure 7.

The binding of **bis-DBA-F_4**$^{2+}$ with **BMP25C8** and the **BMP25C8 CLUSTER** was then investigated. The ^{19}F NMR spectrum of a solution of **BMP25C8** (14 mM) and **bis-DBA-F_4**$^{2+}$ (9 mM) in CD_3CN is shown in Figure 12a. Three signals can be observed in this NMR spectrum: a sharp singlet corresponding to unbound **bis-DBA-F_4**$^{2+}$, a sharp singlet corresponding to the 2:1 complex, and a broadened signal corresponding to the 1:1 complex. This signal for the 1:1 complex is broadened, since presumably the bound crown is shuttling between the two NH_2^+ centers of the threadlike dication at a rate approaching that of the ^{19}F NMR time scale at 376 MHz. Despite the fact that there is some partial overlap of the broadened signal (corresponding to the 1:1 complex) and the sharp singlet corresponding to uncomplexed **bis-DBA-F_4**$^{2+}$, it is still possible to integrate each of these signals and therefore calculate the concentrations of each of the species in solution and, hence, the values of the microscopic binding constants[38] K_1 and K_2, as well as K_{AVE}. By performing this NMR experiment at various concentrations of **bis-DBA-F_4**$^{2+}$, again it is possible to construct a binding isotherm (Figure 13). From the data points, the mean value of K_{AVE} was found to be 30 ± 9 M^{-1}.

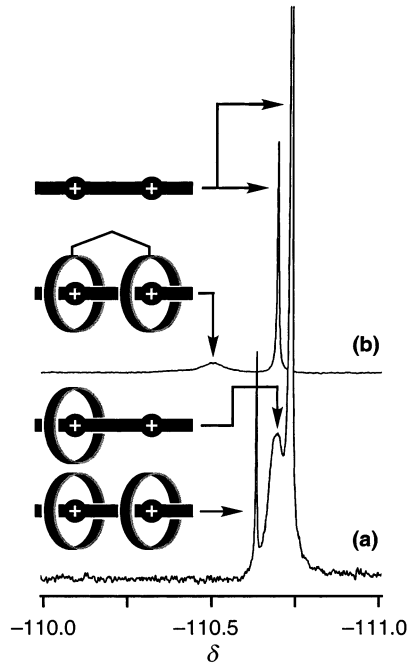


FIGURE 12. Partial ^{19}F NMR spectra (376 MHz, CD_3CN) of (a) a solution of **BMP25C8** (14 mM) and **Bis-DBA-F_4**$^{2+}$ (9 mM) and (b) a solution of the **BMP25C8 CLUSTER** (2 mM) and **Bis-DBA-F_4**$^{2+}$ (4 mM).

The ^{19}F NMR spectrum of a solution of the **BMP25C8 CLUSTER** (2 mM, concentration of crown ethers = 14 mM) and **bis-DBA-F_4**$^{2+}$ (4 mM) in CD_3CN is shown in Figure 12b. Two signals are observed in this spectrum: one signal corresponding to uncomplexed **bis-DBA-F_4**$^{2+}$ and another broad signal corresponding to aggregates in which **bis-DBA-F_4**$^{2+}$ units are each complexed by either one *or* two **BMP25C8** moieties. In the previous example of the binding of the **bis-DBA-F_2**$^{2+}$ by the **DB24C8 CLUSTER**, the NMR spectrum (Figure 10b) provided

(36) A couple of data points in the upper isotherm in Figure 11 exhibit greater than 80% complexation of the minor (crown ether) component in the equilibrium, and one point exhibits less than 20% complexation. As discussed in ref 32, such data may lead to unreliable values of the association constant. We believe, however, that the trend is clear in this series of experiments and that the accuracy of integration of ^{19}F NMR spectra makes these values significant enough to include them in the calculation of K_{AVE}.

(37) The possibility exists that dibenzylammonium cations can be bound by two crown ether moieties—contained within a single cluster—in a sandwich-like complex, as opposed to in a threaded pseudorotaxane-like geometry.

(38) The two values (K_1 = 55 ± 17 M^{-1}, K_2 = 25 ± 8 M^{-1}; K_1/K_2 = ca. 2.2) once again seem to suggest that positive cooperativity is in effect.

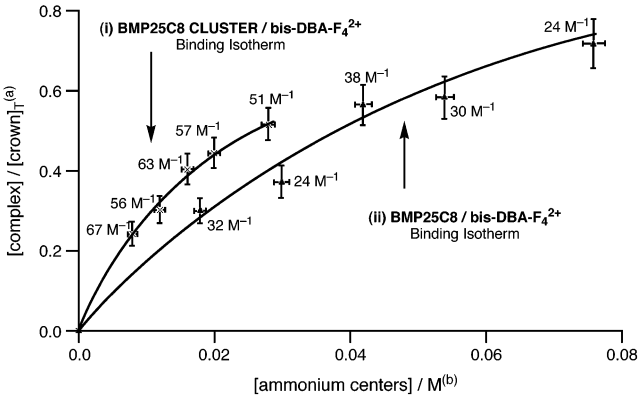


FIGURE 13. Binding isotherms for (i) complexation of **Bis-DBA-F$_4$**$^{2+}$ by the **BMP25C8 CLUSTER** and (ii) complexation of **Bis-DBA-F$_4$**$^{2+}$ by **BMP25C8**. (a) The values represented on this axis represent the measured concentration of complexed dialkylammonium centers divided by the total concentration of crown ether in solution. (b) The values represented on this axis represent the total concentration of dialkylammonium cations in solution. Error bars are set as defined in Figure 7.

good evidence that both NH_2^+ sites on the dication are complexed by crown ether moieties. Therefore, it follows logically that, in the present case of the binding of **bis-DBA-F$_4$**$^{2+}$ by the **BMP25C8 CLUSTER**, either both NH_2^+ centers of this dication are complexed simultaneously by two crown ether moieties or none are complexed at all. Making this assumption, it is possible to calculate K_{AVE} and, by repeating the experiment at various concentrations of **bis-DBA-F$_4$**$^{2+}$, a binding isotherm was constructed (Figure 13). Taking the mean of the data points yields a value of $K_{AVE} = 59 \pm 18$ M^{-1}.

The results from all four experiments conducted with the dicationic threads are summarized in Table 2. In the case of the **DB24C8**-based series of experiments, it appears that the mean K_{AVE} for the interaction of the **DB24C8 CLUSTER** with **bis-DBA-F$_2$**$^{2+}$ is lower than that for the interaction of **DB24C8** with **DBA-F$_2$**$^{2+}$ by almost an order of magnitude. Additionally, the value of the mean K_{AVE} for the interaction of the **DB24C8 CLUSTER** with **bis-DBA-F$_2$**$^{2+}$ is approximately 5 times smaller than the mean K_{AVE} for the component interaction of **DB24C8** with **DBA-F$_2$**$^+$. The reason for this reduction in the mean K_{AVE} is probably, at least in part, a consequence of the electrostatic and steric effects discussed previously for the complexation of monovalent

TABLE 2. Values of Mean K_{AVE} and ΔG°_{AVE} for the Interactions in CD_3CN at 300 K of DB24C8 and the DB24C8 CLUSTER with Bis-DBA-F$_2$ $^{2+}$ **and of BMP25C8 and the BMP25C8 CLUSTER with Bis-DBA-F$_4$**$^{2+}$

host	guest	mean K_{AVE} (M^{-1})	ΔG°_{AVE} (kcal mol^{-1})
DB24C8	**Bis-DBA-F$_2$**$^{2+}$	1100 ± 340	−4.1 ± 0.2
DB24C8 CLUSTER	**Bis-DBA-F$_2$**$^{2+}$	160 ± 48	−3.0 ± 0.2
BMP25C8	**Bis-DBA-F$_4$**$^{2+}$	30 ± 9	−1.8 ± 0.2
BMP25C8 CLUSTER	**Bis-DBA-F$_4$**$^{2+}$	59 ± 18	−2.4 ± 0.2

threads **DBA-F$_2$**$^+$ and **DBA-F$_4$**$^+$ by the clusters. In the case of the **BMP25C8**-based series of experiments, the value of the mean K_{AVE} for the interaction of the **BMP25C8 CLUSTER** with **bis-DBA-F$_4$**$^{2+}$ is about twice that of the mean K_{AVE} for the interaction of the dicationic thread with **BMP25C8** and approximately 3 times larger than that of the component interaction. Again, given the differences in concentrations at which the **DB24C8** and **BMP25C8** systems were investigated, and the intrinsic differences between both systems, it is probably unwise to try to draw comparisons.

With careful interpretation of the mean K_{AVE}, it is possible to calculate the binding constant (K_{POLY}) for the binding of 1 equiv of the **bis-DBA**$^{2+}$ threads by 1 equiv of the **CLUSTER** compounds to afford the 1:1 complex, i.e., where *one* **bis-DBA**$^{2+}$ molecule is complexed by *one* **CLUSTER** molecule, irrespective of the geometry of this complex. K_{AVE} has been defined in eq 1. Therefore, ΔG°_{AVE} can be defined as in eq 2.

$$\Delta G^\circ_{AVE} = -RT \ln K_{AVE} \quad (2)$$

For a polyvalent interaction, the total free energy change can be defined[2] by eq 3

$$\Delta G^\circ_{POLY} = N \Delta G^\circ_{AVE} \quad (3)$$

where N can be considered as the number of individual component interactions that constitute the multivalent interaction. In the case of the interaction where one bis-**DBA**$^{2+}$ dication is complexed by one **CLUSTER**, two NH_2^+ centers are simultaneously complexed by two crown ether moieties ($N = 2$). Thus, ΔG°_{POLY} can be calculated from eq 3, an equation that can also be expressed in terms of K_{POLY} and K_{AVE} (eq 4).

$$K_{POLY} = (K_{AVE})^N \quad (4)$$

Thus, for the binding of the **bis-DBA-F$_2$**$^{2+}$ and **bis-DBA-F$_4$**$^{2+}$ by the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER**, respectively, the quantities ΔG°_{POLY} and K_{POLY} can be calculated, and they are shown in Table 3. For the complexation of **bis-DBA-F$_2$**$^{2+}$ by the **DB24C8 CLUSTER**, K_{POLY} was calculated to be 2.6×10^4 M^{-1} ($\Delta G^\circ_{POLY} = -6.0$ kcal mol^{-1}), which is over 2 orders of magnitude larger than the component interaction, for which $K_{AVE} = 790$ M^{-1} ($\Delta G^\circ_{AVE} = -3.9$ kcal mol^{-1}). A similar trend is observed for the complexation of **bis-DBA-F$_4$**$^{2+}$ by the **BMP25C8 CLUSTER**, where K_{POLY} was calculated to be 3.5×10^3 M^{-1} ($\Delta G^\circ_{POLY} = -4.8$ kcal mol^{-1}), which is over 2 orders of magnitude larger than that of the component interaction, for which $K_{AVE} = 20$ M^{-1} ($\Delta G^\circ_{AVE} = -1.8$ kcal mol^{-1}). These results indicate that significant gains in binding affinity can be achieved within a supramolecular system by simply expressing its component interaction in a polyvalent fashion, even when each of the individual interactions within the polyvalent array are weaker than the component interaction. In both the **DB24C8** and **BMP25C8** systems, these gains in binding affinities are quite significant, especially when one considers that the multivalent receptor molecules are not particularly rigid, with their crown ether appendages having a large degree of conformational freedom, which in turn incurs a greater entropic penalty upon complexation.

Conclusions

The syntheses of two analogous multivalent receptors, the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER**—

TABLE 3. Calculated Values of K_{POLY} and ΔG°_{POLY} for the Interactions in CD_3CN at 300 K of DB24C8 and the DB24C8 CLUSTER with Bis-DBA-F_2^{2+} and of BMP25C8 and the BMP25C8 CLUSTER with Bis-DBA-F_4^{2+}

host	guest	mean K_{AVE} (M^{-1})	ΔG°_{AVE} (kcal mol^{-1})	K_{POLY} (M^{-1})	ΔG°_{POLY} (kcal mol^{-1})
DB24C8	**DBA-F_4^+**	790 ± 240	−3.9 ± 0.2		
DB24C8 CLUSTER	**Bis-DBA-F_2^{2+}**	160 ± 48	−3.0 ± 0.2	2.6(±0.8) × 10^4	−6.0 ± 0.2
BMP25C8	**DBA-F_4^+**	20 ± 6	−1.8 ± 0.2		
BMP25C8 CLUSTER	**Bis-DBA-F_4^{2+}**	59 ± 18	−2.4 ± 0.2	3.5(±1.0) × 10^3	−4.8 ± 0.2

each containing seven crown ether moieties attached to a β-CD core—have been accomplished. A series of complementary mono- and divalent dialkylammonium ligands—containing judiciously sited fluorine atom substituents[39]—was also prepared. These multivalent receptors and mono- and divalent ligands were used to study certain aspects of polyvalency, namely, statistical and chelate effects.

Studies of the interaction of both the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER** with their corresponding monovalent dialkylammonium ligands led to the finding that the crown ethers within the multivalent receptors were poorer hosts for dialkylammonium cations, when compared to the parent systems. The reason for this reduced binding by the multivalent receptors is probably a consequence of electrostatic repulsion and steric effects, both of which result from the build-up of ligands and their associated PF_6^- counterions within the complex.[40]

The abilities of the multivalent receptors, the **DB24C8 CLUSTER** and the **BMP25C8 CLUSTER**, to bind their corresponding divalent dialkylammonium ligands were investigated in an attempt to study the contributions of chelate effects to multivalency. In the case of the **DB24C8 CLUSTER**, we found that the K_{AVE} was lower than that of the component interaction, i.e., the crown ether moieties within this receptor bind a single dialkylammonium center—contained within the divalent ligand—less effectively than that of a single crown ether moiety binding a monovalent dialkylammonium cation. The opposite effect was found in the case of the **BMP25C8 CLUSTER** binding its corresponding divalent ligand. For both multivalent receptors, however, we found that K_{POLY} was approximately 2 orders of magnitude larger than their respective component interactions.

These results indicate that relatively high values of K_{POLY} can be obtained between two moieties simply by constructing receptors and ligands that express component recognition motifs in a polyvalent fashion.[41] It appears that expressing a recognition motif in a polyvalent fashion leads to high binding affinities, even when each interaction within the polyvalent assembly is not as strong as the component interaction. In other words, the quantity of binding interactions, as opposed to the quality, can be an effective strategy to adopt when seeking ways to construct supramolecular assemblies where each subunit is noncovalently bonded to another subunit with a strong affinity. The results of this initial study into polyvalency in a quite complicated unnatural system suggest that a more systematic study of polyvalency, whereby structurally simple di- and trivalent receptors and ligands are made by attaching crown ether and dialkylammonium moieties to a simple scaffold, should be undertaken before any detailed analysis of the factors at work in this system is done. Modifying the structures of the receptors and ligands by, for example, changing the rigidity of the scaffold onto which the receptor and ligand moieties are attached could yield valuable information about the subtle interplay between entropy and binding affinities, which is probably the most important and least understood aspect in the study of polyvalency.

Experimental Section

General. Compounds **1**,[17] **2**,[18] **6**,[22] **DBA-F_2·PF_6**,[22] and **DBA-F_4·PF_6**[22] were prepared according to published procedures. All solvents were used as purchased except for CH_2Cl_2, which was distilled from CaH_2, and THF, which was distilled from Na/benzophenone. Thin-layer chromatography (TLC) was carried out on aluminum sheets precoated with silica gel 60 F. The plates were examined under UV light or developed by charring with 5% H_2SO_4 in EtOH. Column chromatography was carried out using silica gel 60 F (230−400 mesh). Gel filtration chromatography was performed using a column packed with Sephadex LH-20 (3 cm × 90 cm) eluting with either MeOH or $CHCl_3$ at 1 mL min^{-1}. In both cases, the eluant was monitored with a refractive index detector. Fast atom bombardment mass spectra (FAB-MS) were obtained from a mass spectrometer. Matrix-assisted, laser-desorption−ionization, time-of-flight mass spectra (MALDI-TOF-MS) were recorded using a *trans*-indole acrylic acid matrix and an average of 50 laser shots per sample. ^{1}H NMR spectra were recorded at 200, 300, 400 or 500 MHz with either the residual solvent or external TMS (for ^{1}H NMR) as calibrants. ^{13}C NMR spectra were recorded at 75, 100 or 125 MHz. ^{19}F NMR spectra were recorded at 376 MHz using C_6F_6 as internal standard. The chemical shifts are expressed in ppm and the coupling constants from the ^{1}H NMR spectra in hertz (Hz) and are within a ca. ±0.5 Hz error range. The following abbreviations are used to explain the multiplicities: s, singlet; br s, broad singlet; br m, broad multiplet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets. Microanalyses were performed by Quantitative Technologies Inc., Whitehouse, NJ.

(2-Hydroxymethyl)dibenzo[24]crown-8 (3). To a solution of the aldehyde **2** (2.5 g, 5.25 mmol) in dry THF (100 mL) was added carefully $LiAlH_4$ (300 mg, 7.9 mmol) portionwise. The resulting suspension was left to stir under an atmosphere

(39) ^{19}F NMR spectroscopy was found to be an invaluable tool in the study of polyvalency in this unnatural system, allowing quantitative data to be obtained from binding experiments, which would not be possible by utilizing ^{1}H NMR spectroscopic techniques.

(40) It should be noted, however, that great care must be taken when making generalizations on the importance of statistical contributions to polyvalency based on the results reported here. The component interaction used in this study involves positively charged ligands and neutral receptors, which leads to complexes with resulting overall positive charge, a factor that is detrimental to the ability of the complex to further bind ligands. This phenomenon would not occur with a polyvalent system in which both the receptor and ligand, and their resulting complexes, have no overall charge.

(41) We do not wish to imply that it is easy to achieve significant enhancements in binding in multivalent systems over monomeric ones merely by force of numbers alone. These systems must still be designed such that recognition sites are ligated optimally, which means that, among other considerations, spacer units, linkers, and hubs must be complementary, relatively conformationally rigid, and not too sterically bulky so as to disturb the molecular recognition event.

of Ar at room temperature for 1 h. The reaction mixture was quenched by the dropwise addition of H_2O (ca. 1 mL) to the reaction, and $MgSO_4$ (ca. 1 g) then added to remove unreacted H_2O from the reaction mixture. The reaction mixture was filtered and evaporated to dryness to afford the alcohol **3** as a white solid (2.35 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.82 (8H, s), 3.89−3.92 (8H, m), 4.12−4.15 (8H, m), 4.75 (2H, s), 6.83−6.90 (7H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 65.2, 69.4, 69.9, 71.3, 112.9, 113.8, 114.0, 119.9, 121.4, 134.2, 148.9. FAB-MS: *m/z* 478 $[M]^+$. Anal. Calcd for $C_{25}H_{34}O_9$: C, 62.75; H, 7.16. Found: C, 62.93; H, 7.02. Spectral data are consistent with those reported previously.[19]

2-(Methanesulfonyloxymethyl)dibenzo[24]crown-8 (4). A stirred solution of the alcohol **3** (315 mg, 0.66 mmol) and Et_3N (552 μL, 3.96 mmol) in dry CH_2Cl_2 (30 mL) under an atmosphere of Ar was cooled to 0 °C on an ice bath. MsCl (204 μL, 2.64 mmol) was added dropwise and the solution left to stir at room temperature for 4 h. H_2O (30 mL) was added and the mixture stirred for 10 min, before being transferred to a separating funnel. The organic layer was dried ($MgSO_4$), filtered, and evaporated to dryness to afford a clear oil. Purification by column chromatography (SiO_2, EtOAc) afforded the pure mesylate **4** as an oil, which slowly solidified on standing (260 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.05 (3H, s), 3.77 (8H, s), 3.84−3.87 (8H, m), 4.08−4.11 (8H, m), 4.48 (2H, s), 6.75−6.85 (7H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 31.6, 46.5, 69.27, 69.35, 69.36, 69.74, 69.75, 69.85, 71.17, 71.20, 113.5, 114.0, 114.3, 121.4, 121.7, 139.0, 148.9, 149.0. FAB-MS: *m/z* 556 $[M]^+$. Anal. Calcd for $C_{26}H_{36}O_{11}S$: C, 56.11; H, 6.52. Found: C, 55.89; H, 6.62.

2-(2-(Hydroxycarbonyl)ethylthiomethyl)dibenzo[24]-crown-8 (5). To a stirred suspension of the mesylate **4** (698 mg, 1.25 mmol), K_2CO_3 (1.77 g, 12.8 mmol), and KI (10 mg) in DMF (20 mL) was added methyl 3-mercaptopropionate (0.8 g, 6 mmol). The suspension was stirred under an atmosphere of Ar at room temperature for 3 d. The reaction mixture was then evaporated to near dryness and the residue partitioned between H_2O (30 mL) and EtOAc (30 mL). HCl (2 M) was added dropwise and the mixture briefly shaken. This procedure was repeated until the pH of the water layer was ∼4. The organic layer was then dried ($MgSO_4$), filtered, and evaporated to dryness to afford the crude ester (990 mg), which was used without further purification in the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.72 (2H, t, *J* = 7.0 Hz), 2.90 (2H, t, *J* = 7.0 Hz), 3.65 (2H, s), 3.67 (3H, s), 3.80 (8H, s), 3.86−3.90 (8H, m), 4.11−4.13 (8H, m), 6.74−6.87 (7H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.2, 34.5, 36.1, 69.5, 69.6, 70.00, 70.02, 71.4, 113.8, 114.2, 114.5, 121.6, 131.1, 148.1, 149.0, 172.2. FAB-MS: *m/z* 603 $[M + Na]^+$. Anal. Calcd for $C_{29}H_{40}O_{10}S$: C, 59.98; H, 6.94. Found: C, 60.14; H, 7.01. A mixture of crude ester (990 mg), 1 M NaOH (13 mL), and tetrabutylammonium iodide (10 mg) in THF (20 mL) was vigorously stirred for 24 h. HCl (2 M) was then added dropwise until the pH of the mixture was ∼4, and the mixture evaporated to dryness. The resulting residue was then partitioned between H_2O (30 mL) and EtOAc (30 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated to dryness to afford the acid **5** as an oil that solidified upon standing (827 mg, 86% from **4**). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.52 (2H, t, *J* = 7.0 Hz), 2.64 (2H, t, *J* = 7.0 Hz), 3.65 (2H, s), 3.83 (8H, s), 3.89−3.91 (8H, m), 4.11−4.16 (8H, m), 6.76−6.89 (7H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.8, 34.2, 36.0, 69.3, 69.4, 69.80, 69.83, 71.1, 113.7, 114.0, 114.3, 121.4, 121.5, 130.9, 147.9, 148.81, 148.89, 176.3. FAB-MS: *m/z* 589 $[M + Na]^+$, 566 $[M]^+$. Anal. Calcd for $C_{28}H_{38}O_{10}S$: C, 59.35; H, 6.76. Found: C, 59.03; H, 6.62.

DB24C8 CLUSTER. To a stirred solution of the acid **5** (116 mg, 0.20 mmol) in DMF (10 mL) was added HBTU·BF_4 (86 mg, 0.23 mmol) and the reaction stirred at room temperature under an atmosphere of Ar for 2 h. *i*-Pr_2NEt (40 μL, 0.23 mmol) and the heptakisamino-β-CD derivative **1** (22 mg, 14 μmol) were added, and the reaction stirred for a further 3 d. The mixture was evaporated to dryness, and the residue was dissolved in EtOAc (30 mL) and washed with 1 M HCl (30 mL), saturated $NaHCO_3$ (30 mL), and brine (30 mL). The organic layer was then dried ($MgSO_4$), filtered, and evaporated to dryness to afford a clear oil. Purification by gel filtration chromatography (LH-20, 1:1 MeOH/$CHCl_3$) afforded the title compound (26 mg, 37%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.43−2.58 (14H, br m), 2.61−2.76 (14H, br m), 3.15 (7H, d, *J* = 7.0 Hz), 3.27 (7H, br m), 3.78−3.84 (56H, m), 3.85−3.93 (70H, m), 4.07−4.17 (56H, m), 5.07 (7H, br s), 6.71−6.81 (21H, m), 6.83−6.88 (28H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.3, 29.6, 35.9, 46.2, 58.5, 61.1, 69.3, 69.7, 70.5, 71.0, 80.9, 81.9, 82.7, 99.2, 113.7, 114.0, 114.2, 121.4, 121.6, 131.2, 147.7, 148.8, 172.0. MALDI-TOF: *m/z* 5188 $[M + Na]^+$. Anal. Calcd for $C_{252}H_{357}O_{91}N_7S_7 \cdot 2H_2O$: C, 58.19; H, 6.99. Found: C, 58.52; H, 7.13.

Benzo(5-methanesulfonyloxymethylmetaphenylene)-[25]crown-8 (7). A stirred solution of the alcohol **6** (1.013 g, 2.13 mmol) and Et_3N (880 μL, 6.32 mmol) in $CHCl_3$ (40 mL) was cooled to 0 °C. MsCl was then added dropwise (333 μL, 4.32 mmol) and the reaction was stirred for 10 min and then quenched with H_2O (40 mL). The mixture was transferred to a separating funnel, and the organic layer washed with H_2O (2 × 40 mL), dried ($MgSO_4$), and evaporated to dryness to afford the mesylate **7** as a clear oil which solidified upon standing (1.166 g, 99%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.84 (3H, s), 3.65 (8H, s), 3.73−3.80 (8H, m), 4.05−4.10 (8H, m), 5.05 (2H, s), 6.47 (2H, d, *J* = 2.2 Hz), 6.66 (1H, t, *J* = 2.2 Hz), 6.83 (4H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 38.3, 68.1, 68.8, 69.8, 70.88, 70.93, 71.3, 103.1, 108.1, 115.1, 121.6, 135.1, 148.9, 160.3. FAB-MS: *m/z* = 556 $[M]^+$, 461 $[M - SO_2CH_3]^+$. Anal. Calcd for $C_{26}H_{36}O_{11}S$: C, 56.11; H, 6.52. Found: C, 55.92; H, 6.36.

5-(2-(Methoxycarbonyl)ethylthiomethyl)-1,3-phenylenebenzo[25]crown-8 (8). To a stirred suspension of the mesylate **7** (960 mg, 1.73 mmol), K_2CO_3 (2.38 g, 17.2 mmol), and KI (10 mg) in DMF (30 mL) was added methyl 3-mercaptopropionate (1.02 g, 8.5 mmol). The suspension was stirred under an atmosphere of Ar at room temperature for 3 d. The reaction mixture was evaporated to near dryness and the residue partitioned between 1 M HCl (50 mL) and EtOAc (50 mL). The organic layer was washed with brine (50 mL), dried ($MgSO_4$), and evaporated to dryness. Purification by column chromatography (SiO_2; hexanes/EtOAc, 3:7) afforded the ester **8** as a clear oil which solidified on standing (839 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.50 (2H, t, *J* = 7.2 Hz), 2.63 (2H, t, *J* = 7.2 Hz), 3.56 (2H, s), 3.61 (3H, s), 3.65−3.69 (8H, m), 3.75−3.81 (8H, m), 4.08−4.10 (8H, m), 6.43 (2H, d, *J* = 2.0 Hz), 6.54 (1H, t, *J* = 2.0 Hz), 6.82−6.87 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.9, 33.9, 36.1, 51.4, 67.8, 68.7, 69.6, 70.66, 70.70, 101.0, 108.14, 114.9, 121.4, 139.8, 148.8, 159.8, 172.0. FAB-MS: *m/z* 580 $[M]^+$. Anal. Calcd for $C_{29}H_{40}O_{10}S$: C, 59.98; H, 6.94. Found: C, 60.21; H, 7.06.

5-(2-(Hydroxycarbonyl)ethylthiomethyl)-1,3-phenylenebenzo[25]crown-8 (9). A mixture of the ester **8** (830 mg, 1.43 mmol), NaOH (251 mg, 6.3 mmol), H_2O (2.8 mL), and THF (25 mL) was stirred vigorously for 22 h. EtOH (5 mL) was added to the mixture, and Amberlite resin IR-120 (H^+ form) was added until the pH reached ∼4. The mixture was filtered, and the filtrate evaporated to dryness. The resulting residue was redissolved in $CHCl_3$ and dried ($MgSO_4$). The organic layer was collected and evaporated to dryness to afford the acid **9** as an off-white solid (975 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.52 (2H, t, *J* = 7.0 Hz), 2.64 (2H, t, *J* = 7.0 Hz), 3.69−3.73 (8H, m), 3.79−3.81 (4H, m), 3.83−3.84 (4H, m), 4.10−4.14 (8H, m), 6.46 (2H, d, *J* = 2.2 Hz), 6.57 (1H, t, *J* = 2.2 Hz), 6.85−6.91 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.7, 34.0, 36.2, 67.8, 68.8, 69.6, 70.72, 70.74, 101.2, 108.2, 115.0, 121.5, 139.9, 148.8, 159.7, 159.8, 176.3. FAB-MS: *m/z* 566 $[M]^+$. Anal. Calcd for $C_{28}H_{38}O_{10}S$: C, 59.35; H, 6.76. Found: C, 59.53; H, 6.62.

BMP25C8 CLUSTER. To a stirred solution of the acid **9** (159 mg, 0.28 mmol) in DMF (10 mL) was added HBTU·BF_4

(130 mg, 0.34 mmol) and the reaction stirred at room temperature under an atmosphere of Ar for 2 h. *i*-Pr_2NEt (65 μL, 0.38 mmol) and the heptakisamino-β-CD derivative **1** (36 mg, 23 μmol) were then added, and the reaction stirred for a further 3 d. The reaction was evaporated to dryness, and the residue was dissolved in EtOAc (50 mL) and washed with 1 M HCl (50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was then dried ($MgSO_4$) and evaporated to dryness to afford a clear oil. Purification by gel filtration chromatography (LH-20, $CHCl_3$) afforded the title compound (43 mg, 36%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.44−2.53 (14H, m), 2.64−2.77 (14H, m), 3.14 (7H, dd, J = 2.9 Hz, 9.4 Hz), 3.31 (7H, t, J = 8.7 Hz), 3.47 (21H, br s), 3.46−3.51 (7H, m), 3.59 (35H, br s), 3.66−3.74 (63H, m), 3.73−3.77 (28H, m), 3.82−3.86 (42H, m), 4.08−4.15 (56H, m), 5.07 (7H, d, J = 2.9 Hz), 6.43−6.48 (14H, br m), 6.53−6.57 (7H, br m), 6.86−6.91 (28H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.1, 34.5, 36.2, 40.1, 58.5, 61.0, 67.9, 68.9, 69.7, 70.4, 70.8, 80.8, 81.9, 82.6, 99.0, 101.1, 108.4, 115.0, 121.5, 140.3, 148.9, 159.8, 171.9. MALDI-TOF: m/z 5185 $[M + Na]^+$. Anal. Calcd for $C_{252}H_{357}O_{91}N_7S_7 \cdot 2H_2O$: C, 58.19; H, 6.99. Found: C, 58.52; H, 7.30.

α,α′-Bis(4-fluorobenzyl)ammonium-*p*-xylene Bis(hexafluorophosphate) (Bis-DBA-F_2^{2+}). A solution of 4-fluorobenzylamine (**11**) (821 mg, 6.57 mmol) and terephthaldicarboxaldehyde (**14**) (436 mg, 3.25 mmol) in PhMe (50 mL) was heated under reflux for 20 h using a Dean−Stark apparatus. The resulting solution was evaporated to dryness, the residue was dissolved in dry MeOH (50 mL), $NaBH_4$ (1.24 g, 32.63 mmol) was added portionwise over a period of 10 min, and the reaction was left to stir at room temperature overnight. The reaction mixture was evaporated to dryness, and 2 M HCl (50 mL) was added to the residue. The resulting suspension was washed with $CHCl_3$ (50 mL), and 2 M NaOH solution was added to the aqueous layer until the pH reached ∼14. The aqueous layer was extracted with $CHCl_3$ (5 × 50 mL), and the organic extracts were combined, dried ($MgSO_4$), and evaporated to dryness. The resulting oil was dissolved in MeOH (25 mL), and saturated methanolic NH_4PF_6 solution was added dropwise until the precipitation of white solids ceased. The solids were filtered and dried to afford the title compound as a white powder (1.19 g, 62%). ^{1}H NMR (400 MHz, CD_3CN): δ = 4.34 (4H, s), 4.35 (4H, s), 7.10 (4H, br s), 7.28−7.33 (4H, m), 7.58−7.62 (4H, m), 7.61 (4H, s). ^{19}F NMR (376 MHz, CD_3CN): δ = −75.3 (6F, d, J = 707 Hz), −113.5 (2F, s). ^{13}C NMR (100 MHz, CD_3CN): δ = 51.77, 51.85, 116.8, 117.0, 131.8, 132.9, 133.7, 133.8. FAB-MS: m/z = 500 $[M - PF_6]^+$. Anal. Calcd for $C_{22}H_{24}F_{14}N_2P_2$: C, 41.01; H, 3.75. Found: C, 41.19; H, 3.98.

α,α′-Bis(3,5-difluorobenzyl)ammonium-*p*-xylene Bis(hexafluorophosphate) (Bis-DBA-F_4^{2+}). A solution of 3,5-difluorobenzylamine (**13**) (959 mg, 6.70 mmol) and terephthaldicarboxaldehyde (**14**) (447 mg, 3.33 mmol) in PhMe (50 mL) was heated under reflux for 20 h using a Dean−Stark apparatus. The resulting solution was evaporated to dryness, the residue was dissolved in dry MeOH (50 mL), $NaBH_4$ (2.53 g, 66.6 mmol) was added portionwise over a period of 10 min, and the reaction was stirred at room temperature overnight. The reaction mixture was worked up using a procedure similar to that described above for **bis-DBA-F_2^{2+}** to afford the title compound as a white powder (1.34 g, 61%). ^{1}H NMR (400 MHz, CD_3CN): δ = 4.24 (4H, s), 4.25 (4H, s), 7.04−7.12 (10H, m), 7.56 (4H, s). ^{19}F NMR (376 MHz, CD_3CN): δ = −72.3 (6F, d, J = 707 Hz), −110.4 (4F, s). ^{13}C NMR (100 MHz, CD_3CN): δ = 51.4, 52.0, 114.3, 114.5, 118.8, 131.9, 132.8, 134.9. FAB-MS: m/z = 535 $[M - PF_6]^+$. Anal. Calcd for $C_{22}H_{22}F_{16}N_2P_2$: C, 38.84; H, 3.26. Found: C, 38.73; H, 3.30.

Preparation of Solutions for NMR Studies. A stock solution of **DBA-F_2^+** was prepared by dissolving this salt in an appropriate volume of CD_3CN, as measured by a Gilson pipet, such that the concentration of the solution was 35 mM. A 14 mM stock solution of **DB24C8** in CD_3CN was prepared in the same manner. Solutions for NMR spectroscopic analysis were then prepared as follows. To a small vial were added, with an appropriate Gilson pipet, known volumes of the stock solutions of **DBA-F_2^+** and **DB24C8**, and the volume of CD_3CN was adjusted such that the final concentrations of the two components were as desired. After careful mixing, the resulting solution was transferred to an NMR tube and analyzed immediately. Solutions were discarded after use. This procedure was repeated to prepare samples corresponding to all other points on the binding isotherm, using the same stock solutions. For further experiments involving other dialkylammonium salts or crown ethers/crown ether clusters, fresh stock solutions of suitable concentrations were prepared, and the solutions were mixed as described using appropriate volumes of solutions and then diluted with CD_3CN so that the final solution contained both host and guest at the desired concentrations.

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