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Artificial Receptors That Provides a Preorganized Hydrophobic Environment: A Biomimetic Approach to Dopamine Recognition in Water

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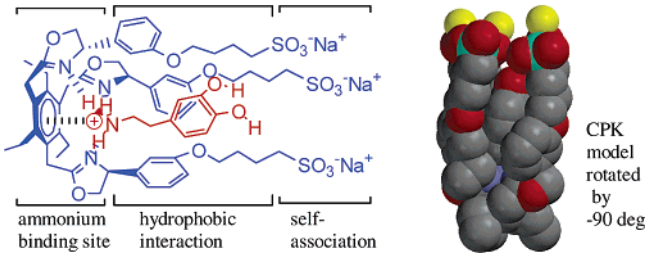

The recognition of dopamine in water has been achieved with tripodal oxazoline-based artificial receptors, capable of providing a preorganized hydrophobic environment by rational design, which mimics a hydrophobic pocket predicted for a human D2 receptor. The receptors show an amphiphilic nature owing to the presence of hydrophilic sulfonate groups at the periphery of the tripodal oxazoline ligands, which seems to contribute in forming the preorganized hydrophobic environment. The artificial receptors recognized dopamine hydrochloride in water with reasonable selectivity among various organoammonium guests examined. The observed binding behavior of the receptors was explained by evoking guest inclusion in the preorganized hydrophobic pocket-like environment and not by simple ion-pairing interactions. The rationally predicted 1:1 inclusion binding mode was supported by binding studies such as with a reference receptor that cannot provide a similar binding pocket, Job and VT-NMR experiments, electrospray ionization mass analysis, and guest selectivity data. This study implies that an effective hydrophobic environment can be generated even from an acyclic, small molecular artificial receptor. Such a preorganized hydrophobic environment, as being utilized in biological systems, can be effectively used as a complementary binding force for the recognition of organoammonium guests such as dopamine hydrochloride in water.

Introduction

Dopamine receptors (DRs), a member of the super family of G-Protein coupled receptors, are known to play an important role in cellular signaling processes in the nervous system.[1] These DRs are also ideal targets for treating schizophrenia and Parkinson's disease. Despite the biological importance, their exact binding mechanisms in the biological systems are yet to be understood completely. Meanwhile, considerable efforts have been focused on the development of artificial DRs in order to unravel dopamine binding mechanisms at the molecular level in biological systems.[2] Consequently, a sizable number of artificial receptors have been developed to date. The structural features of most of these artificial DRs, however, are far from those in biosystems. Most of these artificial receptors provide a single binding site either for the ammonium ion or the catechol hydroxyl groups of dopamine, and in most cases their binding ability is limited to organic solvents only.[3] Thus, the recognition of dopamine in water or at biological pH still remains a challenging task.[4] A recently predicted binding domain of a D2

(1) (a) Jackson, D. M.; Westlind-Danielsson, A. *Pharmacol. Ther*. **1994**, *64*, 291−369. (b) Strange, P. G. *Adv. Drug Res*. **1996**, *28*, 313−351.

(2) (a) Schmidtchen, F. P. *Z. Naturforsch., C: J. Biosci*. **1987**, *42*, 476−485. (b) Kimura, E.; Fujioka, H.; Kodama, M. *J. Chem. Soc., Chem. Commun*. **1986**, 1158−1159. (c) Hayakawa, K.; Kido, K.; Kanematsu, K. *J. Chem. Soc., Perkin Trans. 1* **1988**, 511−519. (d) Park, C. E.; Jung, Y. G.; Hong, J. I. *Tetrahedron Lett*. **1998**, *39*, 2353−2356. (e) Paugam, M.-F.; Valencia, L. S.; Bogess, B.; Smith, B. D. *J. Am. Chem. Soc*. **1994**, *116*, 11203−11204. (f) Paugam, M.-F.; Biens, J. T.; Smith, B. D.; Christoffels, A. J.; de Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc*. **1996**, *118*, 9820−9825.

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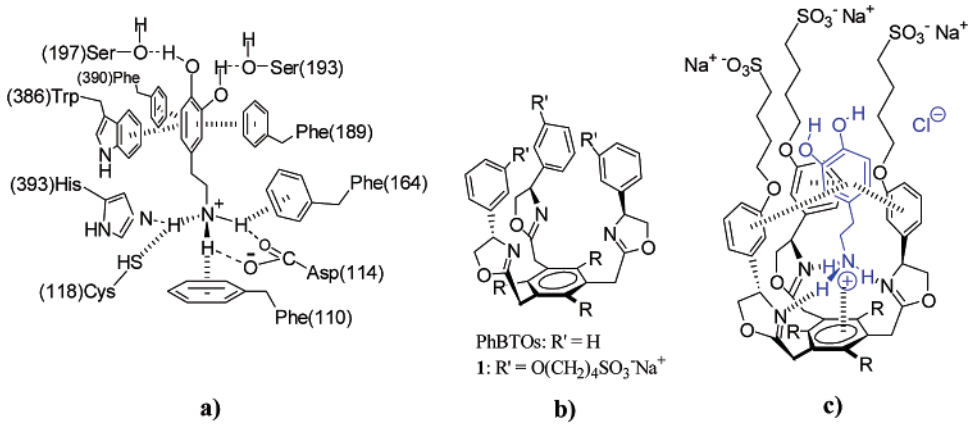

FIGURE 1. (a) Simplified schematic representation of dopamine binding interactions predicted in a biosystem. (b) PhBTOs and designed biomimetic receptors **1**. (c) Proposed interactions of designed hosts **1** with dopamine·HCl in water.

human dopamine receptor[5] (Figure 1a) indicates that dopamine is buried in a hydrophobic pocket provided by three aromatic nuclei of Phe-189, Phe-390, and Trp-386. Additionally, hydrogen bonding interactions between the dopamine ammonium ion and carboxylate group of Asp-114 and between the dopamine catechol hydroxyl groups and serine-197 and serine-193 are found to exist as major forces of binding interactions. In general, it is believed that a balanced combination of electrostatic interactions, hydrogen bonding, a hydrophobic environment, $\pi-\pi$ stackingm and van der Waals forces are crucial for the design of artificial receptors in water.[6] Biomimetic and shape-selective artificial adrenergic receptors that can provide ditopic binding sites, i.e., two different binding sites for ammonium and catechol, were employed with reasonable success to achieve shape-selective recognition of noradrenaline in aqueous medium by Schrader and workers.[7] Recently, the same group reported excellent adrenergic artificial receptors that can provide strong hydrophobic interactions along with other binding interactions.[7c,d] In these receptors the hydrophobic environment is two-dimensional and arises from either a rigid or cyclic structure of the host. We reasoned that artificial receptors that can provide a preorganized, three-dimensional hydrophobic environment similar to the recently predicted D2 human dopamine receptors might also be useful for binding catechol amines, particularly dopamine.[5] As far as we know, a preorganized hydrophobic environment,[8] particularly built from an acyclic host system, has been rarely realized for the molecular recognition in aqueous medium. Herein, we wish to present a novel class of artificial dopamine receptors, partial biomimics of a recently predicted D2 human dopamine receptor, which can provide a preorganized hydrophobic environment for dopamine binding in sole water.

On the basis of our experience in the field of molecular recognition and sensing with benzene-based tripodal receptors, particularly, the phenylglycinol-derived tripodal oxazolines (PhBTOs),[9] we reasoned that a tripodal receptor unit should be an ideal candidate for the recognition of dopamine as its ammonium salt, because C_3 symmetric tripodal oxazoline receptors can provide both a complementary binding geometry for the dopamine ammonium ion and a preorganized hydrophobic environment, albeit very flexible, for the dopamine phenylethyl moiety. To this end, we were particularly intrigued with the preorganized hydrophobic environment, whether it can be generated from acyclic precursors as those in biomolecules and, once generated, if it can be effective or not in water for the recognition of organoammonium salts such as dopamine and related amines. The structures of the designed receptors **1** are shown in Figure 1b. The butanesulfonate groups at the periphery of the receptors were introduced for dual purposes: the one obvious reason is to confer water solubility to the receptor and the other is to provide enhanced preorganization as reasoned in the following. We reasoned that there could be an additional driving force for the preorganization of the designed host molecules, that is, an amphiphilic nature of the receptor structure, in addition to the 2,4,6-trialkyl substituents on the core benzene ring in **1** (**1a**, R = Me; **1b**, R = Et), which drive the three oxazoline ligands to organize on one side of the core benzene ring.[10] The amphiphilic nature of the host could result from the self-association of all the sulfonate groups in water. Through the sulfonate self-association, the oxazolinyl phenyl substituents of the receptors may provide a highly preorganized and reasonably "rigid" hydrophobic environment, suitable for binding the hydrophobic moiety of guest organoammonium ions. Thus, from Figure 1, it can be seen that the receptor design is

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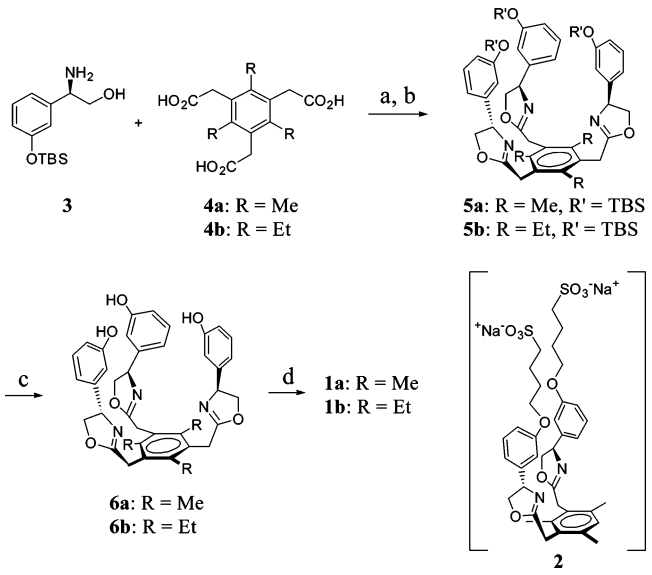
SCHEME 1. Synthesis of Receptors 1 and Reference Compound 2[a]



[a] Reaction conditions: (a) $(COCl)_2$, Et_3N, CH_2Cl_2; (b) MsCl, Et_3N, DMAP, CH_2Cl_2, 45% (**5a**), 29% (**5b**); (c) 1 N NaOH, MeOH, rt, 6 h, 99%; (d) NaH, DMF, 1,4-butanesultone, rt, 36 h, 63% (**1a**), 65% (**1b**).

intended to provide a preorganized hydrophobic pocket-like environment to the dopamine's lipophilic moiety. The receptor is expected to bind dopamine in a 1:1 fashion via tripodal hydrogen bonding between the oxazolines of the receptor and the ammonium ion of the dopamine.[9] The ether linkage allows a synthetically simple manipulation for the introduction of the butanesulfonate groups to our PhBTOs. Also, these ethereal or the sulfonate oxygens may provide hydrogen bonding sites for the catechol hydroxyl groups of dopamine. In such a case, the designed receptors **1** would show higher selectivity toward dopamine compared to simple 2-phenethylamine. Furthermore, a higher selectivity for dopamine over the other adrenergic amines with α- or β-branches could be expected for the simple reason that PhBTOs accommodate linear ammonium ions better than α- or β-branched ammonium ions owing to their shape complemetarity.[9a,b] Also, cation-π interactions between the ammonium and the core benzene ring of the host are expected to have a stabilizing influence on the host−guest complexation process.

Results and Discussion

The synthesis of the receptors **1** is depicted in Scheme 1. The tricarboxylic acid **4a** and **4b**[9b] were converted to the corresponding oxazolines by treating with amino alcohol **3**.[11] Deprotection of the *tert*-butyldimethylsilyl (TBS) group with aqueous hydroxide[12] and subsequent introduction of the butanesulfonate group yielded receptors **1** in high yields. The bis(oxazoline) analogue **2** as a reference compound was synthesized similarly, with which valuable information on the binding mode was obtained.

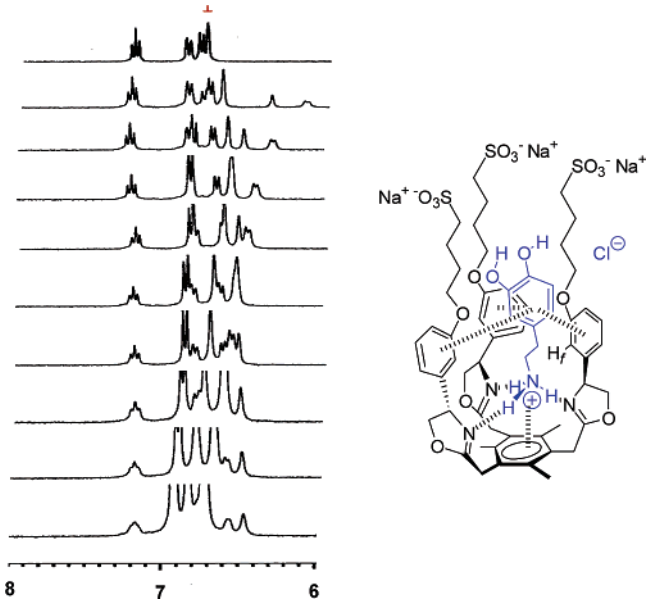


FIGURE 2. ^1H NMR spectral changes of **1a** (4.0 mM) upon addition of dopamine hydrochloride (0, 0.8, 1.6, 2.5, 3.3, 4.1, 4.9, 6.6, 9.8, 16.5, 50 equiv) in water. Only an enlarged region (6−8 ppm) is shown, in which a phenyl proton H_f of the receptor is indicated with arrows (guest equivalents from 0.0 to 16.5).

Our initial experimentation to evaluate the potential of our receptors **1** as DRs and to validate our reasoning began with the ^1H NMR titration of receptor **1a** with increasing concentrations of dopamine hydrochloride in pure D_2O (Figure 2).[13] The protons of the oxazoline phenyl rings (peaks at δ 6.78, 6.89, 6.92, 7.26 ppm) and the oxazoline ring protons (peaks at δ 4.11, 4.67, 5.12 ppm) showed significant complexation-induced chemical shifts (CICS). However, the CICS of the oxazoline ring protons could not be followed during NMR titration because they merged with the solvent peak; therefore, the phenyl ring protons were followed. We observed upfield CICS for the oxazoline phenyl protons (for example, peaks at δ 6.78, 6.89 ppm), which is ascribed to the aromatic $\pi-\pi$ stacking between the aromatic ring of the dopamine and that of the receptor **1a**. Also, the CH_2 protons of the dopamine (peaks at δ 2.82, 3.12 ppm) displayed significant upfield shifts upon complexation ($\Delta\delta$ = 0.45, 0.75 ppm, respectively, at the saturation point), which can be explained by assuming that the dopamine binds inside the hydrophobic pocket composed of the three phenyl rings as predicted. Such upfield shifts of the α- and β-methylene units of organoammonium ions were ascribed to diamagnetic shielding by the surrounding phenyl rings, which was previously identified by an X-ray crystal structure of an inclusion complex of PhBTOs.[9a] A Job plot[14] was obtained by the continuous variations method (Figure 3), which also suggested the proposed 1:1 binding fashion. An association constant of K_{assoc} = 161 M^{-1} was obtained by nonlinear least-squares fitting for the ^1H

(10) For selected examples for the use of other benzene-based tripodal ligands in molecular recognition and self-assembly, see: (a) Metzger, A.; Lynch, V. M.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 862−865. (b) Niikura, K.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533−8534. (c) Szabo, T.; O'Leary, B. M.; Rebek, J., Jr. *Angew. Chem., Int. Ed.* **1998**, *37*, 3410−3413 (d) Sato, K.; Arai, S.; Yamagishi, T. *Tetrahedron Lett.* **1999**, *40*, 5219−5222. (e) Chin, J.; Walsdorff, C.; Stranix, B.; Oh, J.; Chung, H. J.; Park, S.-M.; Kim, K. *Angew. Chem., Int Ed.* **1999**, *38*, 2756−2759. (f) Lavigne, J. L.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666−3669. (g) Rekharsky, M.; Inoue, Y.; Tobey, S.; Metzger, A.; Anslyn, E. *J. Am. Chem. Soc.* **2002**, *124*, 14959−14967.

(11) Ahn, K. H. and co-workers, manuscript in preparation.

(12) Attempts to deprotect the silyl group under various other known conditions that use a fluoride or hydrofluoric acid source failed.

(13) See Supporting Information for complete spectral changes.

(14) (a) Job, P. *Compt. Rend.* **1925**, *180*, 928. (b) Blanda, M. T.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626−4636.

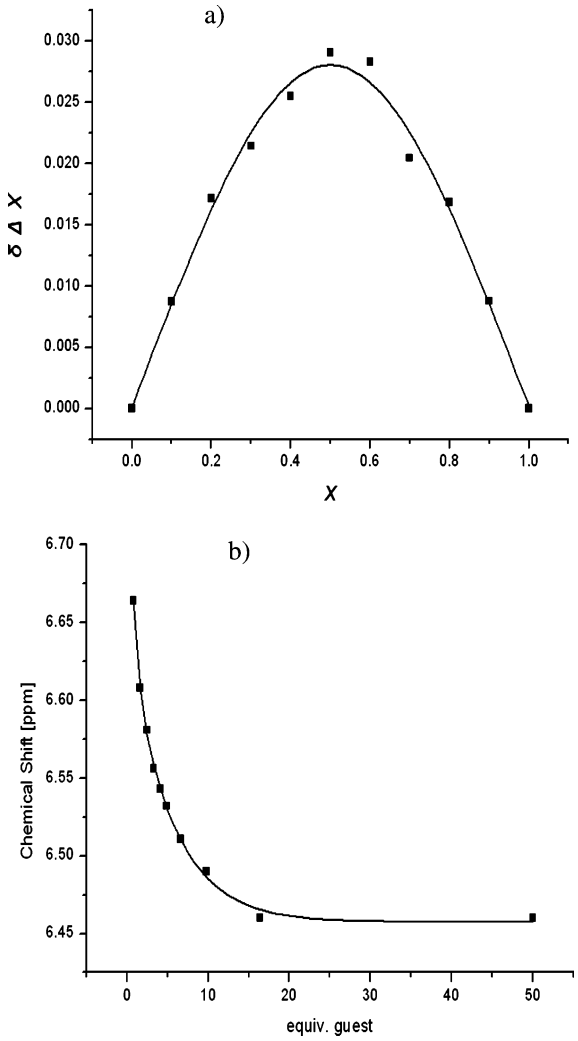


FIGURE 3. (a) A Job plot for the same process. (b) ^{1}H NMR titration curve for complexation of receptor **1a** with dopamine hydrochloride in D_2O: complexation-inducedchemical shift of the H_f vs guest equivalent.

NMR titration curve using WinEQNMR program.[15] This K_{assoc} value is better than or comparable to literature values determined in aqueous medium.[16] In water, the desired hydrogen bonding and hydrophobic interactions are likely to compete with "nonspecific" interactions such as ion-pairing, which may account for the observed weak association constants.

Further support for the predicted 1:1 host−guest complexation process was obtained by electrospray ionization (ESI) mass spectrometry.

The ESI MS spectrum of an equimolar solution of the receptor **1a** and dopamine hydrochloride (Figure 4) exhibited peaks corresponding to the 1:1 receptor−guest complex: $[R + G + H^+ - Cl^-]^{2+}$, $m/z = 637.18$; $[R + G + Na^+ - Cl^-]^{2+}$, 648.19; $[R + G - Cl^-]^+$, 1273.34; where R represents the receptor **1a** and G represents dopamine·HCl. In addition to these, a 1R:2G complex, $[R + 2G + H^+ - Cl^-]^{2+}$ at $m/z = 713.73$, was also observed as minor peak. The observation of such a 1R:2G complex peak may be explained by further existence of ionic interactions between the 1R:1G inclusion complex and the guest.

To get an insight on the host−guest complexation process, we evaluated the thermodynamic parameters for the binding process using the variable temperature NMR technique (VT NMR). Thus, the binding process was followed by ^{1}H NMR at temperatures in the range of 5−65 °C, and the van't Hoff equation was employed to extract the thermodynamic data. The thermodynamic data so obtained for the binding process between receptor **1a** and dopamine hydrochloride showed that the host−guest complexation involves negative enthalpy and positive entropy changes ($\Delta H° = -3.8$ kJ·mol^{-1} and $T\Delta S° = 8.9$ kJ·mol^{-1} ($T = 303$ K); from which $K_{assoc} = 148$ M^{-1} can be extracted, similar to that obtained by the NMR titration). Thus, the host−guest complexation process is entropy-driven rather than enthalpy-driven. The entropy gain would have resulted from the release of a number of water molecules during the binding process of the ionic guest in going from the solvated state into the preorganized hydrophobic pocket. This entropy-driven binding process implies that simple ion-pairing interactions between the guest ammonium ions with the host sulfonate groups are not the main interactions responsible for the observed association constants, since there is no reason to expect a positive entropy change for such a recombination process of ion pairs between the sodium butanesulfonate and the ammonium chloride in water. If such a recombination process of ion pairs were the dominant source of the binding affinity observed, we should have obtained a different stoichiometry, not the 1:1 binding mode, from the Job plot and ESI MS analysis, because 3 molar equiv of ionic guests were required to match the three sulfonate groups of the hosts. Also, a large difference in the basicity of oxazoline nitrogen ($pK_a \approx 5$)[9a] and the dopamine amine ($pK_a = 10.6$) excludes a possible proton-transfer complex formation between these two groups.[17] To exclude a possibility of such simple ionic interactions as the major binding force, we also performed an NMR titration of **1a** with dopamine hydrochloride in a phosphate buffer solution (pH 7.0). The significant binding affinity ($K_{assoc} = 122 \pm 18$ M^{-1}) so obtained, which is almost comparable with those in pure water, rules out such an ion-pairing process for the observed binding affinity. However, the ion-pairing process should not be excluded but rather be incorporated with "overall" binding processes, of which only the inclusion binding mode as shown in Figure 1c manifests itself in the binding behavior observed by NMR experiments.

The ability of our receptors **1** to provide the predicted, highly preorganized hydrophobic pocket-like environment for the dopamine binding could be further supported by comparing its dopamine binding ability with the reference receptor **2**, which, as can be easily reasoned from its open structure (Scheme 1), cannot provide a preorganized hydrophobic pocket-like environment for the guest binding. When the complexation of the reference receptor with dopamine hydrochloride was followed by ^{1}H NMR, otherwise under identical conditions as that of receptor **1a**, we could not observe any CICS. This observation supports that the binding affinity observed for our receptor **1a** is originated mainly from its 3D hydrophobic environment and

(15) (a) Schneider, H. J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442−6448. (b) Hynes, M. J. *J. Chem. Soc., Dalton Trans.* **1993**, 311−312.

(16) Association constants reported for dopamine in aqueous media. In ref 7a, $K_{assoc} = 246 \pm 38\%$ M^{-1} in MeOH/D_2O (1: 1). In ref 7b, $K_{assoc} = 142 \pm 14\%$ M^{-1} in D_2O/MeOH (1:1). In ref 4a log $K_{assoc} = 1.2$ M^{-1} in D_2O by ^{1}H NMR. In ref 4b, maximum of $K_{assoc} = 63.4 \pm 2$ M^{-1} in aqueous NaCl by pH-metric titration.

(17) Sánchez-Revera, A. E.; Corona-Avendaño, S.; Alarcón-Angeles, G.; Rojas-Hernández, A.; Ramírez-Silva, M. T.; Romero-Romo, M. A. *Spectrochim. Acta, Part A* **2003**, *59*, 3193−3203.

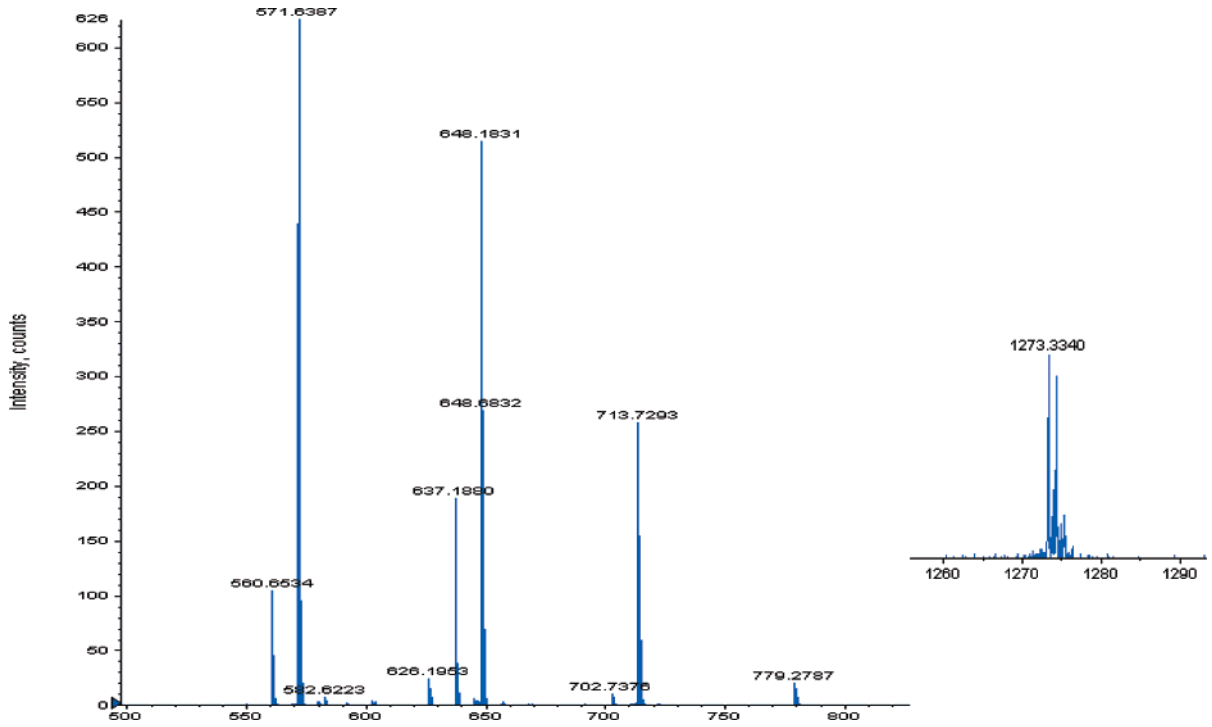


FIGURE 4. ESI mass spectrum of a 1:1 mixture of receptor **1a** and dopamine·HCl.

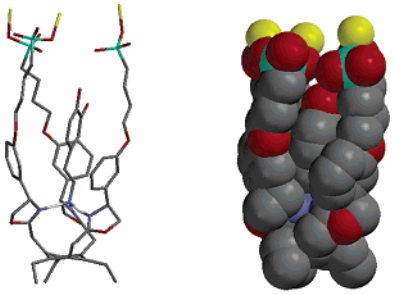


FIGURE 5. Model structure and its CPK view of an inclusion complex between **1b** and dopamine ammonium ion.

not from the simple ion-pairing; were the latter situation operating, there is no reason to expect such a difference in the binding affinity between the two receptors **1a** and **2**. Our efforts to get additional evidence for the guest binding in the hydrophobic pocket by NOESY experiments did not yield fruitful results, possibly owing to a highly flexible nature of the inclusion complex.

A molecular modeling study[18] indicated that the distance between the aromatic protons of the hosts and the guest protons were above the NOE scale (Figure 5).

To validate our reasoning that the butanesulfonate arms may associate themselves, which in turn assist the preorganization process, we have studied the self-associating ability of our receptors **1**. The receptors **1** may undergo self-association through a shuttlecock shape, which can be obtained as the sulfonate groups associate themselves. Thus, the ^{1}H NMR spectra of the receptors were recorded at various concentrations (0.1−20 mM in D_2O) and the chemical shifts of the phenyl ring protons were followed (Figure 6). A plot of chemical shift as the function of host concentration yielded a curve, and analysis of the curve yielded the corresponding self-association constants for the monomer−dimer equilibrium. Although the self-association constants (K_{sa}) obtained were quite low, being $K_{sa} = 40 \pm 3$ and 73 ± 9 [M^{-1}] for the receptors **1a** and **1b**, respectively, this result shows an amphiphilic nature of our receptors in water.[19]

The slightly higher self-association constant for the receptor **1b** compared to **1a** could have resulted from its higher degree of preorganization, as the 2,4,6-triethyl substituents are known to show higher preorganizing ability than the 2,4,6-trimethyl substituents. Moreover, this difference in the self-association constants indicates that the receptors' self-association results from the preorganized shuttlecock structures in which the sulfonate groups are on one side of the core benzene ring. In other words, the butanesulfonate groups associate themselves in water, and this process assists the preorganization of the receptors as we intended in their design. We observed frothing upon shaking the test solutions containing either free receptors or a mixture of receptor and guest. This observation also suggests the existence of the amphiphilic average nature of our receptors. Thus, our reasoning that the introduction of alkyl sulfonate groups at appropriate position to our PhBTO system would lead to water-soluble receptors that can provide highly preorganized structures is validated.

A close look at Tables 1 and 2 reveals an interesting and nonnegligible observation that the association constants of the receptors **1** toward a particular ammonium guest studied do not show a considerable increase upon changing from the 2,4,6-trimethyl based receptor **1a** to the 2,4,6-triethyl based **1b**. We were intrigued by this little change because it is well-known

(18) Molecular mechanics computation was performed using Spartan '04 Windows from Wavefunction, Inc.

(19) The amphiphilic nature of our receptors open up their possible application to the molecular recognition at the air/water interface by embedding them in a monolayer of stearic acid, as beautifully demonstrated by Schrader and co-workers; see: Molt, O.; Schrader, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 5509−5513.

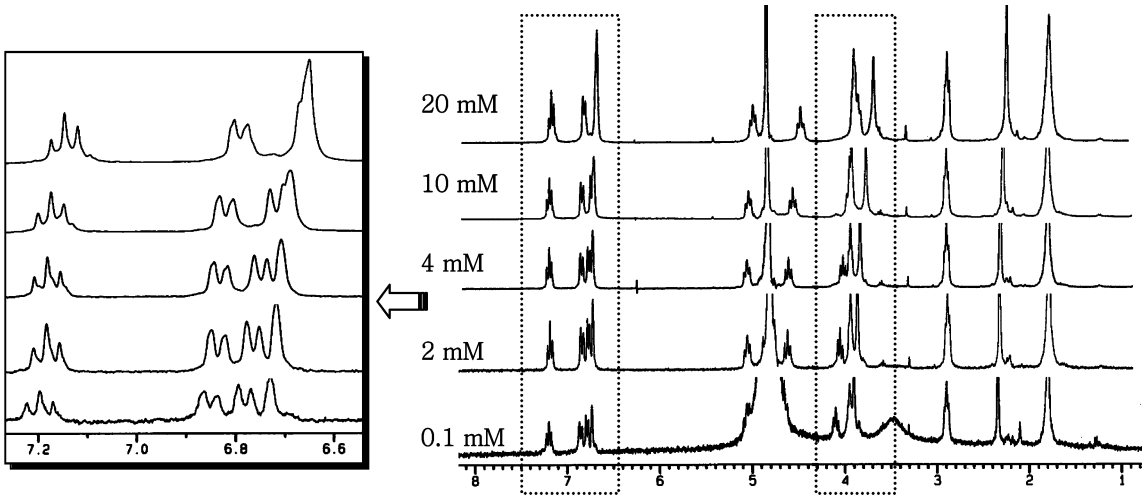


FIGURE 6. Stack plots of ^{1}H NMR spectra of receptor **1a** showing its self-aggregation behavior upon increasing concentration (A region around 7 ppm is enlarged and shown at left).

TABLE 1. Association Constants Obtained for the Complexation of Receptor 1a toward Various Organoammonium Ions in D_2O by ^{1}H NMR Titrations

guest[a]	$K_{assoc(1:1)}$ [M^{-1}][b]	$-\Delta G$ [kJ mol^{-1}]	$\Delta\delta_{sat}$ [ppm][c]	stoichiometry[d]
2-phenethylamine	82 ± 12%	10.99	0.29	1:1
tyramine	101 ± 7%	11.51	0.32	1:1
dopamine	161 ± 16%	12.67	0.32	1:1
catechol	<1			
ethanolamine	<1			
acetylcholine	<1			
adrenaline	<1			
noradrenaline	67 ± 6%	10.48	0.13	1:1
DL-tyrosine methyl ester	65 ± 11%	10.41	0.17	1:1
GABA	<1			

[a] As the hydrochloride salt. [b] Errors are calculated as standard deviations from the nonlinear regression. [c] Largest shifts from selected CH protons. [d] Determined by Job plots and curve fitting of the titration curves.

TABLE 2. Association Constants Obtained for Complexes of Receptor 1b and Various Organoammonium Ions in D_2O by ^{1}H NMR Titrations

guest[a]	$K_{assoc(1:1)}$ [M^{-1}][b]	$-\Delta G$ [kJ mol^{-1}]	$\Delta\delta_{sat}$ [ppm][c]	stoichiometry[d]
2-phenethylamine	86 ± 14%	11.11	0.32	1:1
tyramine	92 ± 16%	11.27	0.33	1:1
dopamine	178 ± 15%	12.92	0.36	1:1
catechol	<1			
ethanolamine	<1			
acetylcholine	<1			
adrenaline	<1			
noradrenaline	74 ± 14%	10.73	0.21	1:1
DL-tyrosine methyl ester	72 ± 17%	10.66	0.22	1:1
GABA	<1			

[a] As the hydrochloride salt. [b] Errors are calculated as standard deviations from the nonlinear regression. [c] Largest shifts from selected CH protons. [d] Determined by Job plots and curve fitting of the titration curves.

that a benzene based tripodal system with 2,4,6-triethyl substituents has better tendency toward preorganization of the 1,3,5-tripodal ligands than does the 2,4,6-triemethyl analogue.[10] We have observed, in the case of a simple PhBTO receptor, an increase up to 2 orders of magnitude in the association constants toward linear organoammonium ions, in going from the 2,4,6-trimethyl based receptor to the 2,4,6-triethyl based ones in organic medium.[9a] This increase in the association constants was attributed to the tendency of the 2,4,6-triethyl groups to force a higher degree of preorganization than that of the 2,4,6-trimethyl groups. The absence of such substantial increase in the association constants in the cases of receptors **1a** and **1b** may be understood as follows. Even with the 2,4,6-trimethyl substituents, the receptor **1a** might have gained a fair degree of preorganization due to the amphiphilic nature of the receptors in water by the presence of the ionic sulfonate groups; hence, the effect of the 2,4,6-triethyl groups in the preorganization is less felt. Thus, the above observation further augments the predicted participation of the sulfonate groups in the preorganization of the receptors.

The complex forming ability of receptors **1** was examined for other biogenic organoammonium ions (Figure 7), which are structurally related to dopamine, and the obtained association constants (K_{assoc}) are collected in Tables 1 and 2.

The trend of the binding constants observed reveals again that the aromatic $\pi-\pi$ interactions and hydrophobic nature of the guest are essential for the binding process; i.e., ethanolamine, acetylcholine, and GABA which do not have an aromatic nucleus did not show any binding. This result clearly indicates that a simple ion-pairing process between the host and guest, apparent in these cases if any, does not lead to any change in the NMR titration spectra; hence, the observed association constants in other cases are owing to the predicted hydrogen bonding and hydrophobic interactions. If only the hydrophobic interactions alone were responsible for binding, then catechol should have shown association. However, absence of such association indicates that the hydrogen bonding of ammonium ions to the tripodal oxazoline nitrogens, which can be only provided in the hydrophobic pocket-like environment. Thus, how well the ammonium guests fit in the binding pocket of receptors **1** should affect the binding process. Consequently, the unbranched 2-phenylethylammonium analogues (2-phenylethylamine, tyramine, and dopamine salts) that fit better in the binding pocket showed stronger binding affinities compared to the branched ones (noradrenaline and tyrosine methyl ester salts).

As predicted, the receptors **1** showed higher selectivity toward the linear 2-phenethylammonium analogues such as dopamine over the branched ones such as noradrenaline salt. As mentioned previously, the tripodal oxazolines offer only three complement-

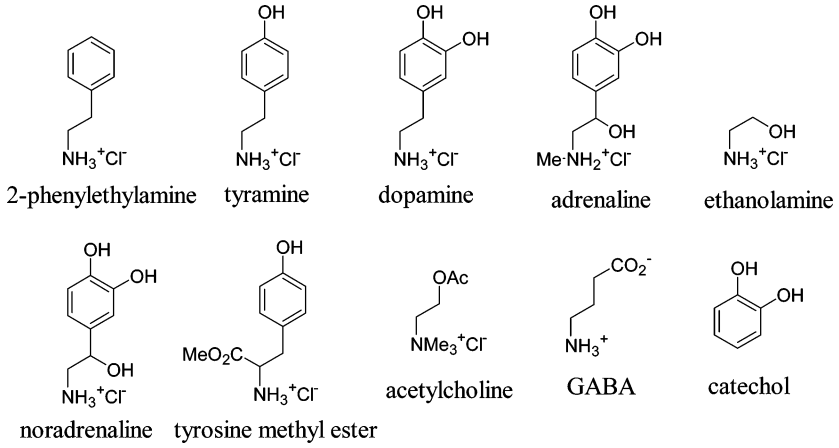


FIGURE 7. Structures of the guest ammonium ions studied.

ing hydrogen bonding sites that can accommodate linear ammonium ions much better than α- or *β*-branched ammonium ions.[9] In the hydrophobic pocket generated by the tripodal oxazoline ligands, we can expect that guests such as catechol that has no ammonium group, acetylcholine that is quaternary ammonium salt, and adrenaline that is a secondary amine salt cannot or weakly bind, as demonstrated. This relationship between the substrate structures and binding affinity is correlated very well with the previously observed results with PhBTOs.[9] This structure−affinity correlation is the most obvious evidence for the proposed inclusion binding mode.

A notable feature is that the receptors **1** show higher affinity toward dopamine compared to other structurally related ammonium ions, 2-phenylethylamine and tyramine. This selectivity suggests the participation of the catechol hydroxyl groups in the host−guest interactions. The binding of the dopamine in the hydrophobic pocket results in such a fashion that the catechol hydroxyl groups extend out of the hydrophobic pocket (Figure 1b),[20] where they may enter into hydrogen bonding with the sulfonate or the phenyl ether oxygens of the receptor. The CPK model in Figure 5 suggests that the catechol hydroxyl groups can have hydrogen bonding with sulfonate groups. The trend of the K_{assoc} values in the Table 1 and Table 2, dopamine (two OH groups) > tyramine (one OH group) > 2-phenethylamine (no OH group), is in accordance with this explanation. Our attempts to get supporting evidence for the supposed hydrogen bonding participation by the sulfonate or phenyl ether oxygens in aqueous media, which is an obviously challenging task, through IR and NMR studies were not successful.

Conclusion

The molecular recognition of dopamine hydrochloride in sole water has been achieved by a rationally designed, new class of artificial receptors that provides a highly preorganized hydrophobic environment, mimicking a hydrophobic pocket predicted for a human D2 receptor. The new receptors were derived from our benzene-based tripodal oxazoline system by introducing butanesulfonate groups at the periphery of the oxazoline ligands, which endowed the receptors with not only water solubility but also enhanced preorganization proposed. Our artificial receptors recognized dopamine hydrochloride with reasonable selectivity among various organoammonium salts evaluated, which could be explained by complementary molecular interactions involving a hydrophobic pocket-like environment and not by simple ion-pairing interactions. Binding studies such as with a reference compound that lacks a similar hydrophobic environment as well as by Job and VT-NMR experiments, electrospray ionization mass analysis, and guest selectivity data gave supporting results for the inclusion binding mode suggested. An amphiphilic nature of the receptors, evidenced by their self-association constants and their froth-forming tendency in water, is supposed to contribute to the receptors' preorganization, thereby enabling the acyclic receptors with an effective pocket-like hydrophobic environment. We took an advantage of this preorganization to recognize dopamine hydrochloride and related ions in water. An acyclic receptor system providing such a highly preorganized hydrophobic pocket for guest binding in water is indeed unique. This idea seems to be general and could be extended to design a range of receptors for recognition and sensing of biogenic molecules in water. A synergistic effect of stronger binding interactions such as ionic salt bridges and preorganized hydrophobic interactions should result in new receptors with more improved affinity, which is a subject of our next study.

Experimental Section

All commercial reagents are of ACS reagent grade and used as supplied. All solvents were dried over 4 Å molecular sieves when necessary. Column chromatography was carried out on silica gel having 230−400 mesh. Melting points were obtained with an electrothermal capillary apparatus and are uncorrected. Optical rotations were measured using a sodium lamp (D line, 589 nm) and are reported in degrees with concentration in unit of 10 mg mL^{-1}. ^1H and ^{13}C spectra were recorded at ambient temperature and all chemical shifts are reported as δ in parts per million (ppm) downfield from tetramethylsilane ($\delta = 0.0$) using the residual solvent signal as an internal standard. Mass spectral data are reported in the unit of mass to charge (*m*/*z*).

(*S*,*S*,*S*)-2-{[3,5-Bis({4-[3-(*tert*-butyldimethylsilanyloxy)phenyl]-4,5-dihydrooxazol-2-yl}methyl)-2,4,6-trimethyl]phenyl}methyl-4-[3-(*tert*-butyldimethylsilanyloxy)phenyl]-4,5-dihydrooxazole (5a). To a suspension of triacid **4** (1.30 g, 4.42 mmol) in dichloromethane (63 mL) were added oxalyl chloride (1.93 mL, 22.1 mmol) and *N*,*N*-dimethylformamide (0.15 mL, 2.21 mmol). After being stirred for 24 h at room temperature, solvent and excess oxalyl chloride were evaporated under reduced pressure. The crude acyl chloride was immediately used for the next reaction without further

(20) See ref 9b for the X-ray crystal structure of the inclusion complex of PhBTO with 2-phenethylammonium ion. This reveals that the phenyl ring of a 2-phenyethylammonium ion is not completely buried in the hydrophobic pocket provided by the tripodal host.

purification. To a solution of amino alcohol **3** (3.31 mg, 12.4 mmol) and triethylamine (3.1 mL, 22.1 mmol) in dichloromethane at 0 °C was added the above acyl chloride in dichloromethane (43 mL) dropwise via cannula. After being stirred for 12 h at room temperature, methanesulfonyl chloride (1.13 mL, 14.6 mmol), triethylamine (4.9 mL, 35.4 mmol), and 4-(dimethylamino)pyridine (162 mg, 1.33 mmol) were added to the reaction mixture. After being stirred for additional 24 h at room temperature, the mixture was poured into an Erlenmeyer flask containing a mixture of water/dichloromethane. The combined organic layer was extracted, washed with brine, dried over anhydrous $MgSO_4$, and concentrated to dryness. The crude residue was purified by column chromatography (hexane/EtOAc, 6:4) to afford the desired tris(oxazoline) **5a** (1.84 g, 45%) as a white solid: mp 93−94 °C; $[\alpha]^{18}_D = -40.2$ (c = 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CHCl_3$) δ 7.13 (dd, J = 8.4, 7.8 Hz, 3H), 6.78 (d, J = 7.8 Hz, 3H), 6.70 (d, J = 8.4 Hz, 3H), 6.69 (s, 3H), 5.07 (dd, J = 10.2, 9.0 Hz, 3H), 4.53 (dd, J = 10.2, 8.4 Hz, 3H), 3.40 (dd, J = 9.0, 8.4 Hz, 3H), 3.83 (s, 6H), 2.48 (s, 9H), 0.96 (s, 27H), 0.16 (s, 18H); ^{13}C NMR (75 MHz, $CHCl_3$) δ 167.5, 156.2, 144.6, 136.3, 131.1, 130.0, 119.9, 119.6, 118.8, 75.2, 69.8, 30.5, 26.1, 18.6, 17.7, −4.0; MS (FAB) m/z (rel intensity) 988 (M + 1, 100), 738 (12), 250 (31). Anal. Calcd for $C_{57}H_{81}N_3O_6Si_3 \cdot 1/2H_2O$: C, 68.63; H, 8.29; N, 4.21. Found: C, 68.60; H, 8.44; N 4.32.

(*S*,*S*,*S*)-2-[(3,5-Bis{[4-(3-hydroxyphenyl)-4,5-dihyrooxazol-2-yl]methyl}-2,4,6-trimethyl)phenyl]methyl-4-(3-hydroxyphenyl)-4,5-dihydrooxazole (6a). Compound **5a** (50 mg, 0.05 mmol) was dissolved in methanol (1.7 mL) and 1 N NaOH solution (0.5 mL, 0.5 mmol) was added dropwise during 30 min under stirring at room temperature. After being stirred for 6 h, the crude product was cooled to 0 °C and neutralized with 0.5 N HCl, during which the product was precipitated. The precipitate was filtered and washed with distilled water to afford a desired product **6a** (33 mg, 99%) as white solids: mp > 195 °C, decomposed; $[\alpha]^{19}_D = -42.0$ (c = 1.20, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.63 (s, 3H), 7.04 (dd, J = 8.4, 7.8 Hz, 3H), 6.60−6.54 (m, 9H), 4.98 (dd, J = 9.6, 8.1 Hz, 3H), 4.52 (dd, J = 9.6, 8.7 Hz, 3H), 3.84 (dd, J = 8.7, 8.1 Hz, 3H), 3.71 (s, 6H), 2.34 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.7, 158.3, 145.3, 135.9, 131.4, 130.3, 117.8, 114.9, 114.2, 75.0, 69.4, 30.3, 17.6; MS (FAB) m/z (rel intensity) 646 (M + 1, 5), 460 (12), 307 (64), 154 (100); HRMS (FAB) calcd for $C_{39}H_{39}N_3O_6$ 646.2917, found 646.2916.

(*S*,*S*,*S*)-4-{3-[2-(2,4,6-Trimethyl-3,5-bis-{4-[3-(4-sulfobutoxy)-phenyl]-4,5-dihydrooxazol-2-ylmethyl}benzyl)-4,5-dihydrooxazol-4-yl]phenoxy}butane-1-sulfonic Acid Trisodium Salt (1a). Solid NaH (30 mg, 1.24 mmol) was added in small portions into a solution of phenol-BTO **6a** (200 mg, 0.31 mmol) in dry DMF (5 mL). After the generation of hydrogen gas subsided, the reaction mixture was stirred at room temperature for 30 min, and then 1,4-butanesultone (0.13 mL, 1.24 mmol) was introduced into the reaction through the septum via a syringe. The resulting mixture was subsequently stirred at room temperature for 36 h, diluted with CH_2Cl_2 (5 mL) ,and filtered to give a pale brown powder. The crude product was dissolved in a minimum amount of methanol and precipitated out with EtOAc. The precipitate was filtered and washed with EtOAc to give the desired product **1a** (220 mg, 63%): mp >270 °C, decomposed; $[\alpha]^{23}_D = -39.2$ (c = 0.5, CH_3OH); ^{1}H NMR (300 MHz, D_2O) δ 7.25 (t, J = 7.9 Hz, 3H), 6.91 (dd, J = 1.9, 8.2 Hz, 3H), 6.82 (d, J = 7.7 Hz, 3H), 6.78 (s, 3H), 5.12 (t, J = 8.1 Hz, 3H), 4.66 (t, J = 9.2 Hz, 3H), 4.08 (t, J = 8.2 Hz, 3H), 4.01 (t, J = 5.5 Hz, 6H), 3.89 (s, 6H), 2.96 (t, J = 7.3 Hz, 6H), 2.37 (s, 9H), 1.86 (m, 12H); ^{13}C NMR (75 MHz, D_2O) δ 168.1, 157.9, 143.2, 135.2, 129.9, 129.6, 118.4, 113.3, 112.2, 74.3, 67.2, 67.2, 50.2, 28.6, 26.9, 20.4, 16.0; MS (FAB) m/z (rel intensity) 1120 (M + 1, 90), 1098 (100), 1076 (49), 1053 (53); HRMS (FAB) calcd for $C_{51}H_{61}N_3O_{15}S_3Na_3$ 1120.2958, found 1120.2946.

Evaluation of the Complex Stoichiometry. The stoichiometry of the complexes was determined according to the Job's method of continuous variations. Equimolar amounts of host and guest were dissolved in D_2O. These solutions were distributed among 10 NMR tubes in such a way that the molar fraction X_H ($X_H = [H]_0/([H]_0 + [G]_0)$) in the resulting solutions decreased from 0.0 to 1.0. The complexation-induced chemical shifts (CICS) were multiplied by X_H and plotted against X_H itself (Job plot).

ESI Mass Analysis. A sample solution (20 μL) of an 1:1 mixture of host **1a** and dopamine hydrochloride (each 1.4 μL in distilled water) was introduced at flow rates of 5 μL min^{-1} and ion spray potential of 4.0 kV (positive ESI). About 60 scans were averaged to improve the signal-to-noise ratio. The region of m/z 1260−1290 is magnified and shown separately after m/z 800 (Figure 4).

Evaluation of Association Constant K_{assoc}. The host compound was dissolved in 6.6 mL of D_2O, and the resulting solution was evenly distributed among 11 NMR tubes. The first NMR tube was sealed without any guest. The guest (100 equiv corresponding to the host) was also dissolved in 1.22 mL of D_2O and added in increasing amounts to the NMR tubes, so that finally solutions with the following relative amounts (equiv) of the guest versus host compound were obtained: 0, 0.8, 1.6, 2.4, 3.2, 4.1, 4.9, 6.5, 9.8, 16.4, 50.0. All $\Delta\delta$ values refer to the standard of the pure host compound. Volume and concentrations changes were taken into account during analysis. The association constants were calculated by nonlinear regression methods.

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Supporting Information Available: Synthesis and characterization of compounds **5b**, **6b**, **1b**, and **2**; NMR titration data, and ^{1}H/^{13}C NMR spectra of compounds **1**, **2**, **5**, and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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