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Water-Soluble Calix[4]resorcinarenes with Hydroxyproline Groups as Chiral NMR Solvating Agents

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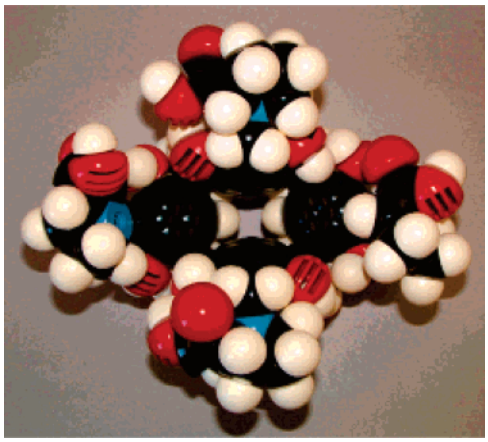

Water-soluble calix[4]resorcinarenes containing 3- and 4-hydroxyproline, D-nipecotic acid, (*S*)-2-(methoxymethyl)pyrrolidine, (*S*)-2-pyrrolidine methanol, and (*S*,*S*)-(+)-2,4-bis(methoxymethyl)pyrrolidine substituents are synthesized and evaluated as chiral NMR solvating agents. The derivatives with the hydroxyproline groups are especially effective at causing enantiomeric discrimination in the spectra of water-soluble cationic and anionic compounds with pyridyl, phenyl, and bicyclic aromatic rings. Binding studies show that mono- and ortho-substituted phenyl rings associate within the cavity of the calix[4]-resorcinarenes, as do naphthyl rings with mono-, 2,3-, and 1,8-substitution patterns. Anthracene derivatives with an amino or sulfonyl group at the 1-position bind within the cavity, as well. Aromatic resonances of the substrates exhibit substantial upfield shifts because of shielding from the aromatic rings of the calix[4]resorcinarene. The effectiveness of the reagents at producing chiral recognition in ^{1}H NMR spectra is demonstrated with sodium mandelate, the sodium salt of tryptophan, and doxylamine succinate. While no one reagent is consistently the most effective, the calix[4]resorcinarenes with *trans*-4-hydroxyproline and *trans*-3-hydroxyproline moieties generally produce the largest nonequivalence in the ^{1}H NMR spectra of the substrates.

Introduction

Optically pure chiral solvating agents are often used in NMR spectroscopy for the analysis of enantiomeric purity.[1] Association of the enantiomers with the chiral solvating agent can involve noncovalent interactions such as hydrogen bonding, other dipole−dipole interactions, and π-stacking between electron-rich and electron-deficient aromatic rings. Steric effects can also be important in the association process. Chiral solvating agents are simple to use since they can be added to the sample in the NMR tube and require no prior derivatization or purification. The potential for kinetic resolution or loss of configuration that can exist with chiral derivatizing agents is not a concern with chiral solvating agents. The associated complexes of a pair of enantiomers with the chiral solvating agent are diastereomers, which often accounts for the presence of nonequivalence in the NMR spectrum. Differences in

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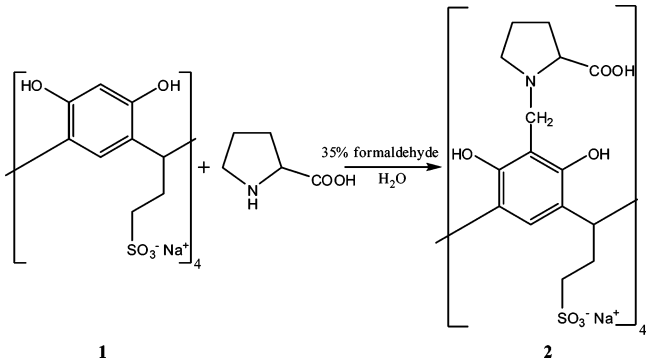
association constants of the enantiomers with the reagent may also account for the chiral recognition. These systems are usually under conditions of fast exchange such that the NMR spectrum of the substrate is a time average of the bound and unbound form. When the distinction is predominated by the diastereomeric nature of the associated complexes, high concentrations of the chiral solvating agent enhance the enantiomeric discrimination in the NMR spectrum.

Many compounds have been tested for their utility as chiral NMR solvating agents. The overwhelming majority of these are organic-soluble systems. In these cases, it is most desirable to use a relatively nonpolar solvent such as chloroform to promote association between the chiral solvating agent and the substrate. Polar solvents such as acetonitrile, methanol, acetone, and methyl sulfoxide effectively solvate the dipolar groups of the reagent and substrate and reduce formation of the complex. Host compounds such as crown ethers and cyclodextrins have been widely studied as chiral NMR solvating agents.[1] Crown ethers are effective for primary amines[2] and in the case of (18-crown-6)-2,3,11,12-tetracarboxylic acid for secondary amines.[3] Crown ethers are often used in polar solvents such as acetonitrile and methanol. Native cyclodextrins,[4] trimethyl-β-cyclodextrin,[5] and carboxymethylated cyclodextrins[6] are water-soluble reagents. Very few water-soluble chiral NMR shift reagents are known. Chiral, water-soluble micelles can cause chiral discrimination, although these systems have not been widely studied in NMR applications.[7] Since many pharmaceutical compounds are specifically designed to possess water solubility, and with growing emphasis on the use of water as a solvent in green chemistry, it is important to have water-soluble chiral NMR shift reagents.

Two other classes of host compounds that have been less studied as chiral NMR solvating agents are calixarenes and calixresorcinarenes. Calixarenes are prepared by the condensation of phenol and formaldehyde.[8,9] By varying the reaction conditions, it is possible to prepare calixarenes with different numbers of phenol rings in the cavity.[8] Calixresorcinarenes are prepared using resorcinol and virtually any aldehyde, preferentially forming a cavity compound with four resorcinol units.[8] The ability to vary the nature of the aldehyde provides a convenient means to alter the solubility properties of the resulting calix[4]resorcinarene. Derivatives suitable for use as chiral NMR solvating agents have been prepared by attaching optically pure substituent groups to the calixarene or calix[4]-resorcinarene.[6e,10] Studies of organic-soluble calix[4]arene and calix[4]resorcinarene derivatives as chiral NMR solvating agents are limited, primarily because favorable solvation of the enantiomers and host compound limits the complex formation needed for chiral discrimination.

SCHEME 1. Synthesis of 2



1 **2**

An exception occurs with water-soluble **2**, which contains optically pure prolinylmethyl groups that are attached to the resorcinol rings of a tetrasulfonated calix[4]resorcinarene (**1**).[11−13] The synthesis of **2** from a starting sulfonated calix[4]-resorcinarene is shown in Scheme 1. Compound **2** is an exceptional chiral NMR shift reagent for water-soluble substrates with a phenyl or bicyclic aromatic ring. Compound **2** adopts a cone configuration in solution or is in rapid exchange among a variety of possible configurations (Figure 1). Association occurs

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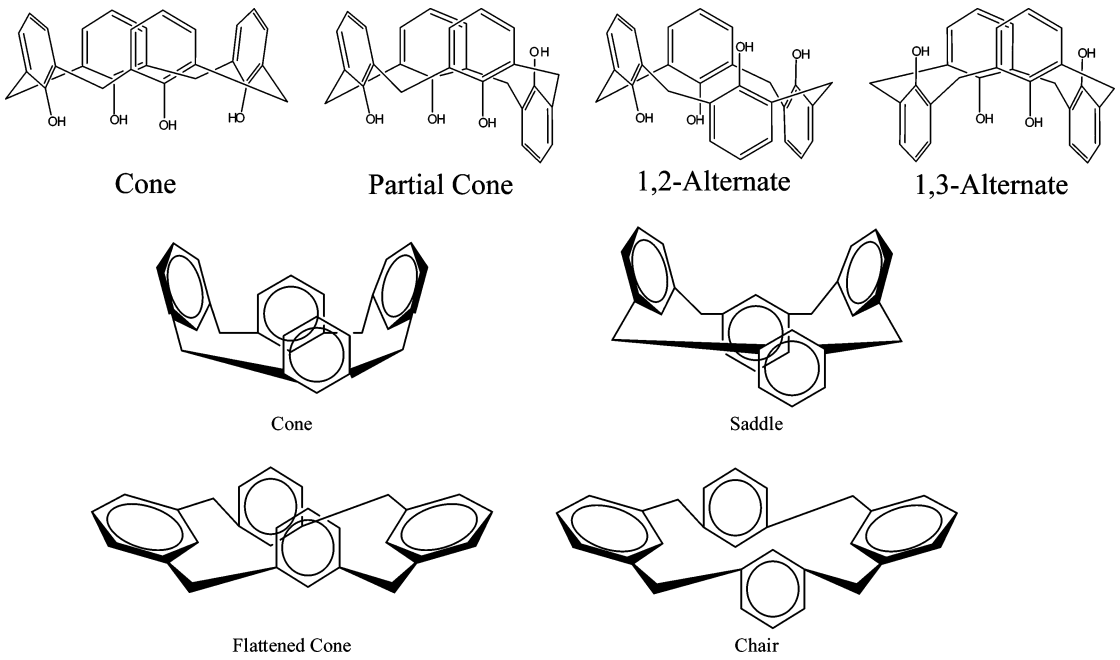

FIGURE 1. Possible configurations (top) and conformations (bottom) of calix[4]resorcinarenes.

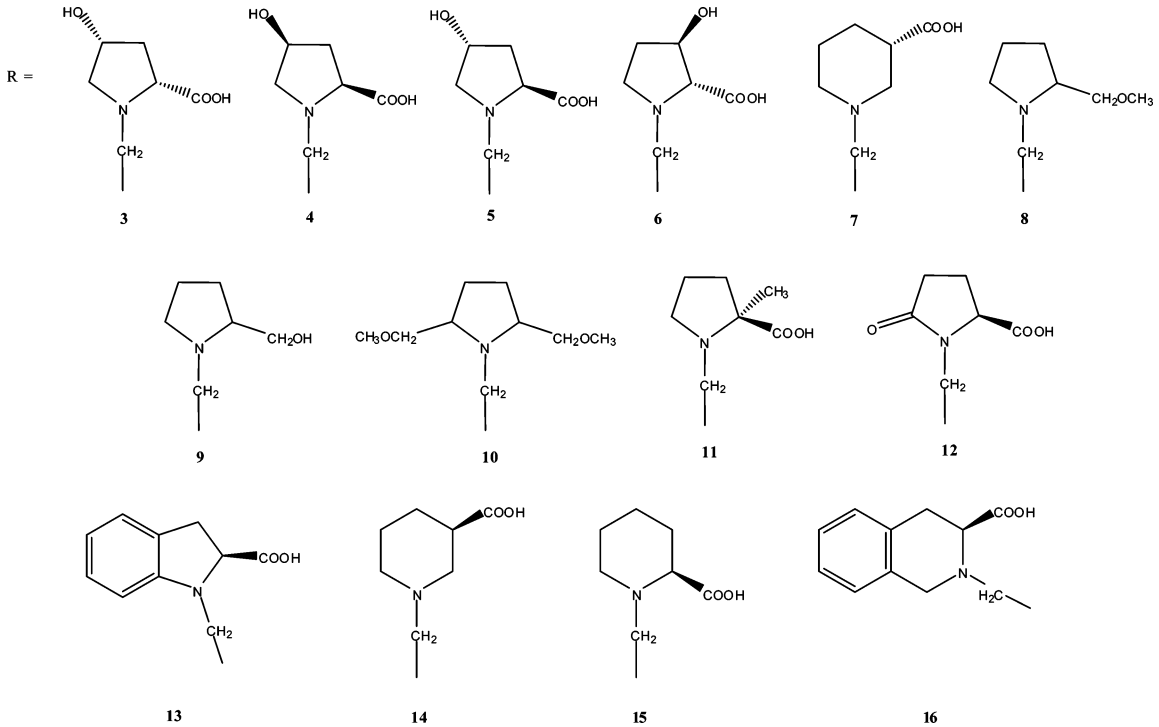

FIGURE 2. Substituent groups successfully (**3**−**10**) and unsuccessfully (**11**−**16**) attached to the sulfonated calix[4]resorcinarene in place of the prolinylmethyl group in Scheme 1.

through insertion of the hydrophobic aromatic ring of the substrate into the well-defined resorcinarene cavity, as evidenced by the large upfield shifts of the aromatic ring resonances of the substrate caused by shielding from the resorcinol rings of **2**. Attachment of optically pure *N*-methyl amino acids of alanine, valine, and leucine to the sulfonated calix[4]resorcinarene did not produce chiral NMR solvating agents as effective as **2**.[13] One reason was that the *N*-methyl amino acid derivatives were in several configurations and/or conformations in solution, which was indicated by broadening of the resonances of the calix[4]-resorcinarene.[13]

In this report, we describe the synthesis of sulfonated calix-[4]resorcinarenes similar to **2** that contain other pyrrolidine or piperidine groups (Figure 2, compounds **3**−**10**) and present preliminary data on their utility as chiral NMR solvating agents. Certain new derivatives are more effective in chiral recognition studies than **2**. We also describe further studies aimed at

elucidating the range of substrate compounds that effectively associate with **2**.

Results and Discussion

The aminomethylation of a tetrasulfonated calix[4]resorcinarene using L-proline under Mannich conditions has been reported (Scheme 1).[11−14] Calix[4]resorcinarene derivatives analogous to **2** that instead contain *cis*-4-hydroxy-D-proline (**3**), *cis*-4-hydroxy-L-proline (**4**), *trans*-4-hydroxy-L-proline (**5**), *trans*-3-hydroxy-L-proline (**6**), D-nipecotic acid (**7**), (*S*)-2-(methoxymethyl)pyrrolidine (**8**), (*S*)-2-pyrrolidine methanol (**9**), and (*S*,*S*)-(+)-2,4-bis(methoxymethyl)pyrrolidine (**10**) groups were successfully prepared using a modification of the same procedure (Figure 2). The reaction is conveniently monitored by the disappearance of the aromatic resonance at 6.35 ppm in the ^{1}H NMR spectrum. Attempts to prepare analogous derivatives with α-methyl-L-proline (**11**), (*S*)-(−)-2-pyrrolidone-5-carboxylic acid (**12**), (*S*)-(−)-indoline-2-carboxylic acid (**13**), L-(−)-nipecotic acid (**14**), L-(−)-pipecolinic acid (**15**), and (*S*)-(−)-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid (**16**) (Figure 2) were unsuccessful as the aromatic resonance at 6.35 ppm was never fully absent from the spectrum. Heating these reactions to reflux for 48 h also did not product the desired compounds.

Calix[4]resorcinarenes can exist as a series of isomers that differ by inversion of one or more rings, as well as a series of possible conformers (Figure 1). Previous work has noted that **2** either adopts a cone structure or is in fast exchange among interconverting configurations and conformations as evidenced by the presence of sharp resonances in the ^{1}H NMR spectrum.[11−13] Generally, the presence of sharp resonances in the NMR spectrum of a calix[4]resorcinarene is taken as an indication of only the cone structure.[8,9] Tetrasulfonated calix[4]resorcinarene derivatives with the *N*-methyl amino acids of alanine, valine, and leucine exhibited broadened ^{1}H NMR spectra, indicating that a slow exchange between several configurations/conformations occurred in solution.[13] The presence of several geometrical arrangements likely accounted for the general ineffectiveness of the *N*-methyl amino acid derivatives relative to **2** as chiral solvating agents for NMR spectroscopy.

For the new derivatives described herein, the ^{1}H and ^{13}C NMR spectra of **3**−**6** all had sharp resonances. Of particular interest is the behavior of the two hydroxy-substituted aromatic carbon resonances of **3**−**6**. Attachment of the optically pure chiral group renders these two carbon atoms diastereotopic, and there is a small resolution of the signals in the ^{13}C NMR spectrum. A similar behavior of the hydrogen resonances of a comparable calix[4]arene derivative with an optically pure amino acid has been noted, as well.[15] The spectrum of **7** was quite broadened, indicating the presence of several configurations or conformations in solution. The ^{1}H NMR spectra of **8**, **9**, and **10** initially exhibited sharp resonances over a broadened background, in which **9** seemed to have the largest proportion of the cone geometry and **10** the least. Over time, either in solution or on storage as a solid, **8**, **9**, and **10** isomerize such that the ^{1}H and ^{13}C NMR spectra are extremely broad. In contrast, the NMR spectra of compounds **2**−**6** do not broaden on standing in solution or after being stored in the solid state over a period of several months.

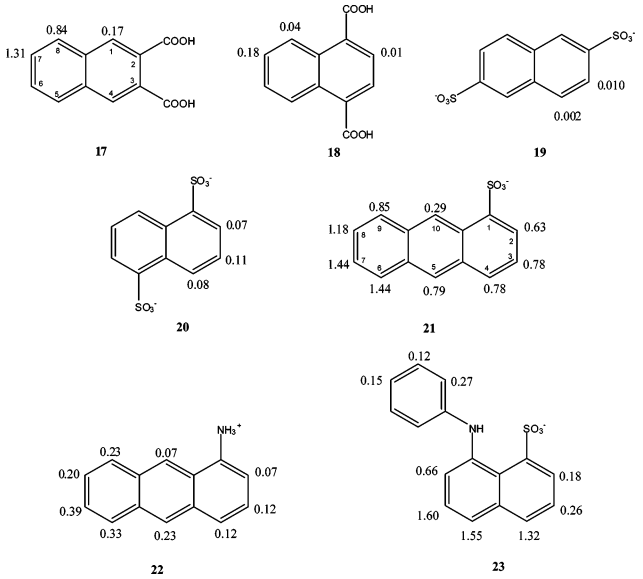

FIGURE 3. Structure of naphthyl- and anthryl-containing compounds used to examine binding with **2**. Numbers adjacent to positions represent chemical shifts of the hydrogen resonances of the substrate (10 mM) in the presence of **2** (35 mM). Conditions differed for **21** (2 mM substrate, 20 mM **2**) and **22** (1 mM substrate, 2 mM **2**).

Binding Studies of Aromatic Compounds with 2. Earlier studies have shown that mono-substituted and ortho-substituted phenyl rings[11−13] and mono-substituted naphthyl rings[12,13] form host−guest complexes with **2** in D_2O. Steric hindrance in substrates with meta- and para-substituted phenyl rings inhibits binding with **2**.[13] The association of variously substituted naphthyl- and anthryl-containing compounds with **2** was investigated using the series of achiral substrates shown in Figure 3. Carboxylic acid containing compounds were analyzed as their sodium salts. The shifts that were recorded in the ^{1}H NMR spectra of **17**−**23** with **2** are also included in Figure 3. The magnitude of the shifts in the spectra of substrates in the presence of **2** can be used to assess relative binding affinities as well as the geometry of association. Hydrogen atoms inserted more deeply into the calix[4]resorcinarene cavity experience greater shielding from the aromatic rings.

The resonances of the hydrogen atoms on the unsubstituted ring of **17** are highly shielded in the presence of **2**. The relative shift order $H_{6,7} > H_{5,8} > H_{1,4}$ indicates a geometry of association as shown in Figure 4a. A Job plot of **17** with **2** indicated that a 1:1 complex forms.[16] The association constant of **17** with **2** in D_2O is 169 M^{-1}, a value comparable to the association constants of other bicyclic substrates with **2**.[12,13,17,18] It is not surprising that a 2,3-disubstituted naphthalene ring would favorably associate with **2** since there is no steric hindrance to prevent binding.

The spectrum of compound **18** exhibits considerably smaller upfield shifts than **17** in the presence of **2**. The relative shift order $H_{6,7} > H_{5,8} > H_{2,3}$ indicates that the geometry of association of **18** with **2** is similar to that of **17** with **2**. However,

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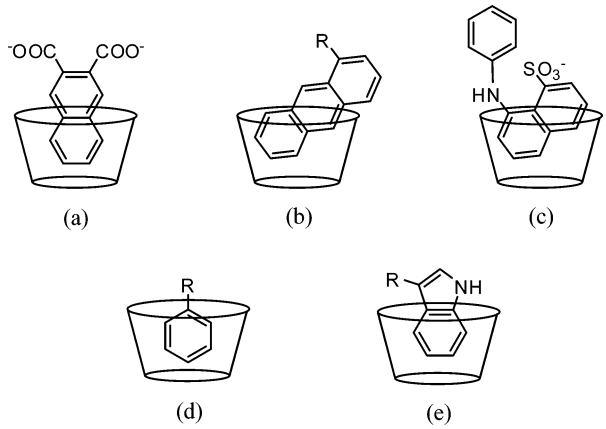


FIGURE 4. Proposed geometries of association of various substrates with the calix[4]resorcinarenes.

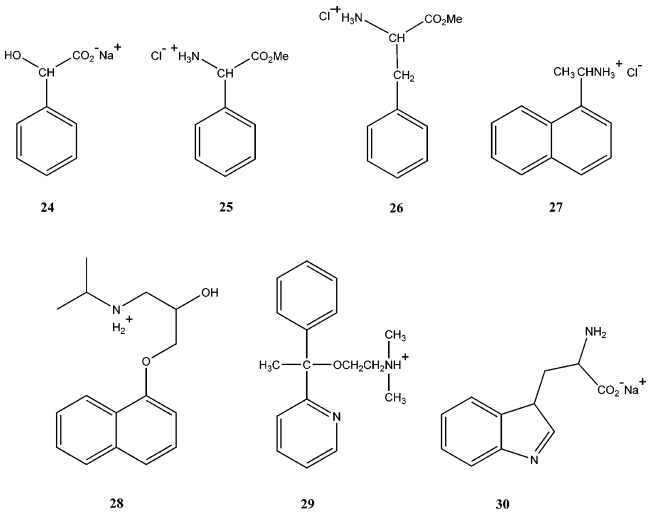
the carboxylate groups at the 1,4-positions significantly hinder association of **18** with **2**, which is likely from unfavorable steric effects. Similarly, the spectra of **19** and **20**, which have substituent groups at the 1,5- and 2,6-positions, exhibit only small shifts in the presence of **2**, again indicative of unfavorable steric hindrance caused by the substituents.

Two mono-substituted anthryl-containing compounds (**21** and **22**) are soluble in D_2O at low levels (1−2 mM) as their sodium and HBF_4 salts, respectively. Shifts in the ^1H NMR spectra of **21** and **22** indicate that both favorably associate with **2**. The relative shifts of the resonances of **21** and **22** indicate that the ring furthest from the substituent group inserts into the cavity of **2**. The consistent pattern in which H_6 and H_7 show the largest shifts for both substrates further implies that the ring is tipped in the cavity as shown in Figure 4b. The flattened cone conformation (Figure 1) of **2** produces a slot that is especially complementary to naphthyl rings and likely explains the ability of **21** and **22** to associate favorably, as well.[12,13]

Compound **23** possesses sterically unhindered phenyl and naphthyl rings. The contrast in shifts of the hydrogen resonances of the two rings indicates that the naphthyl ring preferentially binds in the cavity of **2**. The relative magnitude of the shifts of the naphthyl ring implies that the substrate is tipped in the cavity as shown in Figure 4c. The complexation geometry of compounds with **2** is likely influenced by unfavorable steric effects and favorable dipole−dipole interactions between the substrate substituent groups and proline residues. A Job plot of **23** with **2** confirms that a 1:1 complex forms. The association constant of **23** with **2** is 216 M^{-1}, which is consistent with previous measurements of association constants of naphthyl- and phenyl-containing substrates with **2**.[12,13]

Compounds **17** and **23** (10^{-4} M) exhibit appreciable fluorescence in solutions in D_2O. Addition of **2** (10^{-3} M) to solutions of **17** and **23** quenches the fluorescence, further indicating the favorable association of these substrates with **1**.

Evaluation of 7−10 as Chiral NMR Solvating Agents. Mandelic acid sodium salt (**24**), phenylglycine methyl ester hydrochloride (**25**), phenylalanine methyl ester hydrochloride (**26**), 1-(1-naphthyl)ethylamine hydrochloride (**27**), propranolol hydrochloride (**28**), and doxylamine succinate (**29**) provide a variety of aromatic ring functionalities that are useful for testing the effectiveness of **7**−**10** as chiral NMR solvating agents. **24**, **25**, and **26** contain phenyl rings, whereas **27** and **28** have bicyclic rings. The extra aliphatic carbon of **26** relative to **25** allows a comparison of the effect of the aliphatic group on chiral recognition. **27** and **28** have mono-substituted naphthyl rings and differ in the nature of the aliphatic group. **29** contains both a phenyl and a pyridyl ring. ^1H NMR spectra of **24**−**29** (10 mM) were recorded in the presence of **7**−**10** over the range of 2 to 40 mM.



The spectra of the substrates did shift in the presence of **7**−**10** in the general order **7** > **9** > **8** > **10**. The shifts with **8**, **9**, and **10** were much smaller than those with **2**. Furthermore, considerable broadening of the resonances of the substrates occurred at concentrations of the calix[4]resorcinarenes of 10 mM or higher. No enantiomeric discrimination is apparent in any of the spectra of **24**−**29** with **7**−**10**. The broadened peaks in the spectra of **7**−**10** showed no discernible sharpening in mixtures with substrates. Binding of the substrate in the cavity of the calix[4]resorcinarene might cause a redistribution in favor of the cone conformation, as observed to some extent in prior work on a calix[4]resorcinarene derivative with *N*-methyl alanine substituent groups.[13]

With **2**, the resonances of the diastereotopic hydrogen atoms of the methylene group between the aromatic and pyrrolidine rings exhibit an interesting behavior in NMR studies.[13] In the absence of substrate, the resonances of these diastereotopic hydrogen atoms exhibit identical chemical shifts in the NMR spectrum and appear as a deceptively simple singlet. With substrate bound in the cavity, these resonances shift upfield by approximately 0.3 and 0.5 ppm because of shielding from the aromatic ring of the substrate and are resolved into two doublets. Presumably, one hydrogen atom preferentially points in toward the cavity more than the other and experiences greater shielding. The resonances for the corresponding methylene group of **8** were readily discernible and consistently appear as two doublets with approximately the same chemical shifts in the presence or absence of substrates. For **9**, they tend to appear as a broadened set of resonances that do not shift or change appreciably in the presence of substrates. The indication is that binding of substrates with **8**, **9**, and **10** is not nearly as favorable as with **7** or **2**. Compounds **7**−**10** are not effective as chiral NMR solvating agents.

Evaluation of 3−6 as Chiral NMR Solvating Agents. Calix[4]resorcinarenes **3**−**6**, which contain different hydroxyproline substituent groups, exist in aqueous solution in the cone conformation or as a rapidly interconverting set of configurations and conformations as evidenced by their sharp NMR spectra. In contrast to **7**−**10**, they cause pronounced shifts and chiral

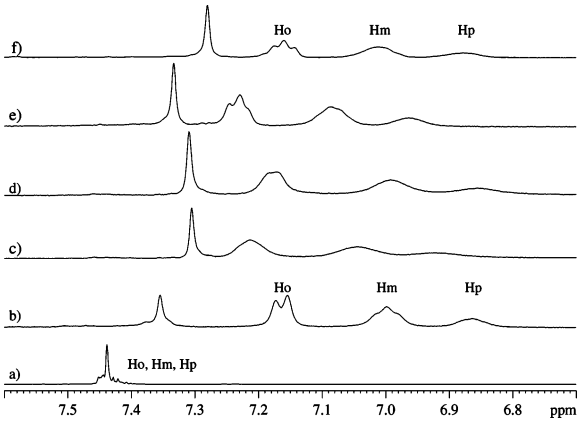


FIGURE 5. ^{1}H NMR spectrum (400 MHz, D_2O, 23 °C) of the aromatic resonances of (a) **24** (10 mM) enantiomerically enriched (2/3-(*R*), 1/3-(*S*)) with 10 mM (b) **2**, (c) **3**, (d) **4**, (e) **5**, (f) **6**.

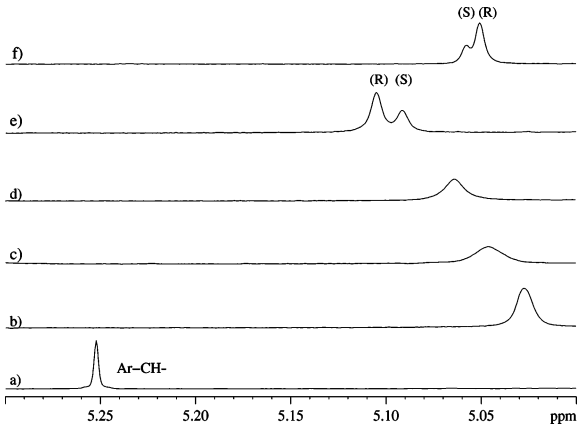


FIGURE 6. ^{1}H NMR spectrum (400 MHz, D_2O, 23 °C) of the methine resonance of (a) **24** (10 mM) enantiomerically enriched (2/3-(*R*), 1/3-(*S*)) with 10 mM (b) **2**, (c) **3**, (d) **4**, (e) **5**, (f) **6**.

TABLE 1. Comparative Shifts ($\Delta\delta$ in ppm) in the ^{1}H NMR Spectrum (400 MHz, D_2O, 23 °C) of 24 (10 mM) in the Presence of 2−6 (10 mM): Two Values Indicate Chiral Discrimination

	2	3	4	5	6
Ho	0.29	0.26	0.30	0.23/0.26	0.30/0.33
Hm	0.45	0.43	0.48	0.39	0.46
Hp	0.58	0.55	0.62	0.51	0.60
CH	0.23	0.24	0.22	0.18/0.19	0.23/0.24

recognition in the NMR spectra of substrates. Studies with **24**, **29**, and sodium tryptophan (**30**) are presented to demonstrate the effectiveness of **3**−**6** as chiral solvating agents for NMR spectroscopy.

The aromatic (Figure 5) and methine (Figure 6) resonances of **24** shift upfield in the presence of **3**−**6** (Table 1). The large upfield shifts indicate that the aromatic ring of **24** inserts into the cavity of the resorcinarenes. Job plots of **24** with **5** and **6** show that a 1:1 complex forms. The order of the shifts (Hp > Hm > Ho) suggests a geometry of association of **24** with **3**−**6** as shown in Figure 4d. Additionally, in the presence of **5** and **6**, the Ho (0.03 ppm) and methine (0.06 ppm) resonances (Figure 5e,f, Figure 6e,f, and Table 1) exhibit a small degree of enantiomeric discrimination. The larger discrimination of Ho relative to Hm and Hp likely occurs since Ho is closer to the chiral hydroxyproline substituents of **5** and **6**, whereas Hm and Hp are inserted deeper into the cavity. Enantiomeric discrimination is not observed in the presence of **2** at similar concentrations. An interesting observation is that the greatest enantiomeric discrimination does not always occur with the calix[4]-resorcinarene that causes the largest shifts, as **5** produces the smallest shifts of **3**−**6** in the spectrum of **24** but causes the largest NMR enantiodifferentiation. Presumably, the location of the hydroxyl group on the pyrrolidine ring of **5** and **6** is important in causing chiral recognition that is distinct from the other calix[4]resorcinarenes.

TABLE 2. Association Constants (M^{-1}) in D_2O at 23 °C of Compounds with Selected Calix[4]resorcinarenes

	2	5	6
(*R*)-**24**	33	22	48
(*S*)-**24**	36	32	37
D-**30**		127	115
L-**30**		135	102

TABLE 3. Comparative Shifts ($\Delta\delta$ in ppm) in the ^{1}H NMR Spectrum (400 MHz, D_2O, 23 °C) of 30 (10 mM) in the Presence of 2−6 (40 mM): Two Values Indicate Chiral Discrimination

	2	3	4	5	6
H_1	0.55	0.71/0.83	0.74/0.84	0.77/0.88	0.58/0.62
H_2	0.99	1.35	1.36	1.06	0.93/1.03
H_3	0.94	1.46	1.48	1.30	1.02
H_4	0.65	0.89	0.88	0.97/1.04	0.61/0.64
H_6[a]	0.09	0.01/0.02	0.01/0.02	0.01/0.02	0.06/0.09

[a] With 10 mM **30** and 10 mM calix[4]resorcinarene.

The reversal in shift order of the methine resonances of the two enantiomers with **5** and **6** is interesting. Association constants of (*R*)- and (*S*)-**24** with **5** and **6** are reported in Table 2. Association constants of **24** with **2**, which were previously reported, are included for comparison.[11] The relative values of the association constants of **24** with **2**, **5**, and **6** correlate with the relative magnitude of the shifts of the aromatic resonances. Furthermore, the association constant of the (*S*)-isomer is larger with **5**, whereas the value of the (*R*)-isomer is larger with **6**, consistent in each case with the relative shifts of the two enantiomers. However, the likely importance of the diastereomeric nature of the associated complexes cannot be dismissed as a contributing mechanism to the enantiomeric discrimination.

The shifts of the aromatic signals of **30** with **3**−**6** are reported in Table 3. Job plots of **30** with **2**,[12,13] **5**, and **6** show that 1:1 complexes form in each case. In the presence of **2**−**6**, the H_2 and H_3 resonances of **30** are shielded the most, H_1 and H_4 less so, and H_6 the least. The relative shielding supports a geometry of association as shown in Figure 4e. With **5**, the noticeably larger shielding of H_3 and H_4 compared to that of H_2 and H_1, respectively, implies that the ring is tipped in the cavity. **3**−**6** cause not only larger shifts in the spectrum of **30** than **2** but also substantially greater enantiomeric discrimination (Table 4). No one calix[4]resorcinarene is consistently the most effective. The largest discrimination for H_2 and H_6 of **30** occurs with **6**. Enantiodifferentiation in the H_1 resonance is larger with **3**−**5** than **6**. The best results for H_4 are obtained with **5**.

Association constants of **30** with **5** and **6** are reported in Table 2. In this case, the resonances of the D-isomer shift further with **5** and **6**, but the relative association constants only agree with this shift order for **6**. Clearly, the diastereomeric nature of the complexes is important in explaining the enantiomeric discrimination. Comparing results with **5** and **6**, the enantiomeric discrimination cannot be explained by differences in association

TABLE 4. Enantiomeric Discrimination ($\Delta\Delta\delta$ in ppm) in the ^{1}H NMR Spectrum (400 MHz, D_2O, 23 °C) of 30 (10 mM) in the Presence of 2−6 (40 mM)

	2	3	4	5	6
H_1	0	0.12	0.10	0.11	0.04
H_2	0	0	0	0	0.10
H_3	0	0	0	0	0
H_4	0	0	0	0.07	0.03
H_6[a]	0	0.01	0.01	0.01	0.03

[a] With 10 mM **30** and 10 mM calix[4]resorcinarene.

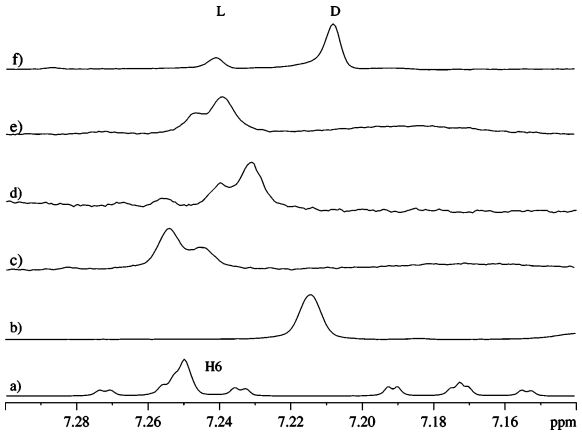


FIGURE 7. ^{1}H NMR spectrum (400 MHz, D_2O, 23 °C) of the H_6 resonance of (a) **30** (10 mM) enantiomerically enriched (2/3-L, 1/3-D) with 10 mM (b) **2**, (c) **3**, (d) **4**, (e) **5**, (f) **6**.

TABLE 5. Comparative Shifts ($\Delta\delta$ in ppm) in the ^{1}H NMR Spectrum (400 MHz, D_2O, 23 °C) of 29 (10 mM) in the Presence of 2−6 (10 mM): Two Values Indicate Chiral Discrimination

	2	3	4	5	6
H_2	0.20	0.17	0.21	0.19	0.22
H_3	0.32	0.20	0.25	0.22	0.27
H_4	0.44	0.40	0.51	0.44	0.41/0.44
H_3'	0.28/0.52	0.34/0.49	0.37/0.54	0.32/0.55	0.35/0.52
H_4'	0.33/0.96	0.42/0.85	0.48/0.91	0.43/0.85	0.46/0.85
H_5'	0.71	0.40	0.67	0.63	0.48/0.59
H_6'	0.23/0.28	0.25/0.28	0.31/0.34	0.24/0.29	0.23/0.27
CH_3	0.13	0.02	0.10/0.11	0.13/0.14	0.13/0.14

constants alone as H_1 and H_4 show larger enantiodifferentiation with **5**, whereas H_2 and H_6 show larger enantiomeric discrimination with **6**. The substantial improvement in effectiveness of **6** compared to that of **2** is illustrated by the series of spectra shown in Figure 7. An expansion of the H_6 resonance of an enantiomerically enriched mixture of **30** is shown, and the pronounced enantiomeric discrimination with **6** (Figure 7f) is apparent. Also apparent is the expected reversal in enantiomeric shift order between **3** and **4**. The small differences in shifts observed with **3** and **4** with the different substrates are the result of slight differences in concentration.

The shifts and enantiomeric discrimination in the spectrum of **29** with **3**−**6** are provided in Tables 5 and 6, respectively. Compound **29** has an aromatic and pyridyl ring, either of which can potentially bind within the cavity of the calix[4]resorcinarenes. Resonances of both rings are shielded. The aromatic resonances shift in the order Hp > Hm > Ho, which indicates that the aromatic ring inserts into the cavity. The magnitude of the shifts of the aromatic ring are smaller (0.20−0.44 ppm) than those of the pyridyl resonances (0.33−0.71 ppm), indicating a slight preference for insertion of the pyridyl ring of **29**.

TABLE 6. Enantiomeric Discrimination ($\Delta\Delta\delta$ in ppm) in the ^{1}H NMR Spectrum (400 MHz, D_2O, 23 °C) of 29 (10 mM) in the Presence of 2−6 (10 mM)

	2	3	4	5	6
H_2	0	0	0	0	0
H_3	0	0	0	0	0
H_4	0	0	0	0	0.03
H_3'	0.24	0.15	0.17	0.23	0.17
H_4'	0.63	0.43	0.43	0.42	0.39
H_5'	0	0	0	0	0.11
H_6'	0.05	0.03	0.03	0.05	0.04
CH_3	0	0	0.01	0.01	0.01

The magnitudes of shifts for the resonances of **29** are similar with all the calix[4]resorcinarenes (Table 5). When **29** complexes with **2**, certain resonances (H_3', H_4') show large enantiomeric discrimination; however, substantial broadening occurs in the ^{1}H NMR spectrum as the concentration of **2** is raised. The degree of broadening is not as pronounced with **3**−**6**. The degree of recognition shows that no one of the reagents is consistently the most effective. In the presence of **6**, H_4 and H_5' exhibit enantiomeric discrimination, which is not observed with any of the other calix[4]resorcinarenes. Presumably, dipole−dipole interactions of the hydroxyl group at the 3-position in the pyrrolidine ring of **6** is significant in causing enantiomeric discrimination in the spectrum of **29**. The largest discrimination for H_3' and H_4' occurs with **2**, whereas the greatest enantiodifferentiation for H_6' occurs with **2** and **5**.

Even though **3**−**6** do have a number of resonances in their NMR spectra that may overlap with the resonances of substrates, there are still certain regions of the spectra that are suitable for analysis. The aromatic region of **3**−**6** only contains a singlet. Addition of substrate does little to the chemical shift of this resonance in **3**−**6**, whereas the aromatic resonances of the substrate show sizable shielding that shifts them into a clear portion of the spectrum between 5 and 7 ppm. The spectra of **3**−**6** are free of resonances from 0 to 2 ppm so that methyl resonances of the substrate can be examined for enantiomeric discrimination. Finally, there are several gaps in the region from 2 to 5 ppm. When recording a series of spectra with increasing concentrations of **3**−**6**, it is often possible to find a set of conditions where substrate resonances in the 2−5 ppm region do not overlap with resonances of the calix[4]resorcinarene.

Aggregation Properties of 2−10. Dimerization of a calix[4]arene with alanine substituent groups has been observed in methanol.[19] This was the first report of such dimerization in a protic solvent. Addition of guest compounds caused the dimer to disassemble. Evidence for dimerization involved the observation that the chemical shifts of the calix[4]arene changed as a function of concentration. We observe changes in chemical shifts as a function of concentration for compounds **2**−**10** in D_2O at acidic pH values and without any pH adjustment. The spectra in Figure 8 provide an example of some of the changes in chemical shifts for two resonances of **9** over a concentration range of 0.1 to 10 mM. In one case, the changes are significant enough to show a slight resolution of the resonances of two diastereotopic hydrogen atoms. For each of **2**−**10**, certain resonances shifted over the range of 0.03−0.20 ppm. The changes in chemical shifts as a function of concentration imply that either dimerization or aggregation of **2**−**10** occurs in D_2O.

Attempts to fit the data to obtain dimerization constants provided inconsistent results, which were likely due to the

(19) Brewster, R. E.; Shuker, S. B. *J. Am. Chem. Soc.* **2002**, *124*, 7902−7902.

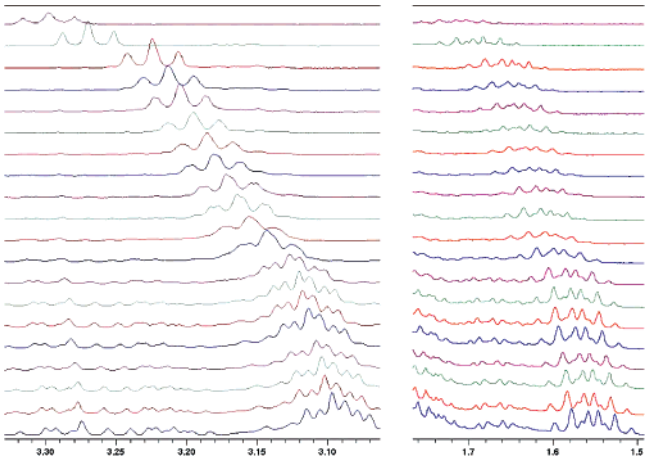


FIGURE 8. ^{1}H NMR spectrum (400 MHz, D_2O, 23 °C) of two resonances of **9** as the concentration is varied from 10 mM (bottom) to 0.1 mM (top). The pH of the sample adjusted to 2 by addition of DCl.

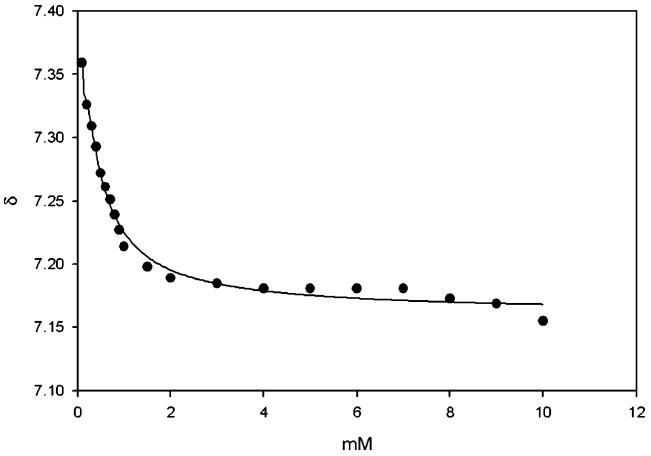


FIGURE 9. Plot of the chemical shift in the ^{1}H NMR spectrum (400 MHz, D_2O, 23 °C) of one resonance of **10** as the concentration is varied from 10 to 0.1 mM.

presence of higher order aggregates in the mixtures. The chemical shift data plotted in Figure 9 for one of the resonances of **10** are rather typical. Of particular significance is the additional change in chemical shift that begins to appear when the concentration is higher than 8 mM. The change in slope of the plot at the higher concentration regions indicates that larger aggregates are likely forming.

Job plots for several substrates all show 1:1 complexation with the calix[4]resorcinarenes, indicating that any dimerization or aggregation likely disassembles in the presence of substrate and does not inhibit association of the substrates with the cavity.

Conclusions

Water-soluble calix[4]resorcinarenes with hydroxyproline substituent groups are effective as chiral NMR solvating agents for substrates with pyridyl, phenyl, and bicyclic aromatic rings. Mono-substituted anthryl-containing compounds form host−guest complexes with the calix[4]resoncinarenes, as do naphthyl rings substituted at the 2,3- or 1,8-positions. Mono- or ortho-substituted phenyl rings bind within the cavity of the calix[4]-resorcinarenes, as well. While no one of these reagents is consistently the most effective at causing chiral discrimination in the ^{1}H NMR spectra of substrates, **5** and **6** generally produce larger enantiodifferentiation in more resonances than **2**, **3**, and **4**. Additional studies to expand the scope of substrates analyzed with **3**−**6** are currently underway.

Experimental Section

Reagents. The sulfonated calix[4]resorcinarene (**1**) and its prolinylmethyl derivative (**2**) were prepared and purified as its hydrated species as described in a published procedure.[11] Water-soluble derivatives of amines were obtained either by preparation and isolation of the corresponding hydrochloride salt (crystallization from a solution of the amine in methanol saturated with hydrogen chloride gas) or in solution by adding a stoichiometric equivalent of hydrochloric acid in deuterium oxide to the amine in solution. Similarly, water-soluble derivatives of carboxylic acids were obtained either by preparation and isolation of the corresponding sodium salt (crystallization by evaporation of a solution of the acid and a stoichiometric amount of sodium bicarbonate in water) or in solution by adding a stoichiometric amount of sodium hydroxide.

Synthesis of the Tetra-*cis*-4-hydroxy-D-prolinylmethyl Derivative (3). Tetrasulfonated calix[4]resorcinarene (**1**) (1.0 g, 0.9 mmol) and *cis*-4-hydroxy-D-proline (0.472 g, 3.6 mmol) were dissolved in distilled water (12 mL). Once fully dissolved, formaldehyde (37%, 0.440 mL) was added. The reaction mixture was purged with nitrogen gas and then allowed to stir under a nitrogen atmosphere. After 48 h, the reaction was dried by rotary evaporation and allowed to further dry by vacuum desiccation to yield **3** (1.397 g, 95% yield). To purify, solid **3** was dissolved in a minimal amount of distilled water (approximately 1 mL) and allowed to stir. The purified solid was triturated from solution with methanol, collected by vacuum filtration, and further dried by rotary evaporation: ^{1}H NMR (400 MHz, D_2O) δ = 7.18 (s, 4H), 4.67 (t, 4H), 4.47 (s, 12H), 4.14 (m, 4H), 3.32−3.19 (m, 8H), 2.90 (t, 8H), 2.58 (m, 8H), 2.66−2.14 (m, 8H); ^{13}C NMR (100 MHz, D_2O) δ = 173.6, 151.25, 126.29, 125.65, 125.56, 109.2, 81.6, 68.68, 67.2, 60.3, 48.7, 37.0, 33.8, 28.6. Anal. Calcd for $C_{60}H_{72}N_4O_{32}S_4Na_4 \cdot 6H_2O$: C, 38.54; H, 5.61; N, 2.99. Found: C, 38.50; H, 5.52; N, 2.85.

Synthesis of the Tetra-*cis*-4-hydroxy-L-prolinylmethyl Derivative (4). Using *N*-methyl-*cis*-4-hydroxy-L-proline, **4** was prepared by the same procedure as **3**: ^{1}H NMR (400 MHz, D_2O) δ = 7.18 (s, 4H), 4.68 (t, 4H), 4.49 (s, 12H), 4.15 (m, 4H), 3.34−3.19 (m, 8H), 2.91 (t, 8H), 2.59 (m, 8H), 2.69−2.19 (m, 8H); ^{13}C NMR (100 MHz, D_2O) δ = 173.6, 151.1, 126.28, 125.66, 125.56, 109.2, 81.6, 65.6, 67.2, 60.3, 48.7, 37.0, 33.8, 28.6. Anal. Calcd for $C_{60}H_{72}N_4O_{32}S_4Na_4 \cdot 7H_2O$: C, 38.17; H, 5.66; N, 2.97. Found: C, 38.05; H, 5.44; N, 3.07.

Synthesis of the Tetra-*trans*-4-hydroxy-L-prolinylmethyl Derivative (5). Using *N*-methyl-*trans*-4-hydroxy-L-proline, **5** was prepared by the same procedure as **3**: ^{1}H NMR (400 MHz, D_2O) δ = 7.19 (s, 4H), 4.67 (t, 4H), 4.54 (s, 12H), 4.31 (m, 4H), 3.68−3.24 (m, 8H), 2.89 (t, 8H), 2.59 (m, 8H), 2.43−2.14 (m, 8H); ^{13}C NMR (100 MHz, D_2O) δ = 172.8, 151.2, 126.2, 125.5, 125.4, 109.2, 81.6, 69.4, 67.9, 60.1, 48.7, 35.6, 33.7, 28.4. Anal. Calcd for $C_{60}H_{72}N_4O_{32}S_4Na_4 \cdot 15H_2O$: C, 38.92; H, 5.55; N, 3.02. Found: C, 38.89; H, 5.54; N, 2.92.

Synthesis of the Tetra-*trans*-3-hydroxy-L-prolinylmethyl Derivative (6). Using *N*-methyl-*trans*-3-hydroxy-L-proline, **6** was prepared by the same procedure as **3**: ^{1}H NMR (400 MHz, D_2O) δ = 7.10 (s, 4H), 4.63 (t, 4H), 4.54 (s, 12H), 4.47 (m, 4H), 3.63−3.32 (m, 8H), 2.87 (t, 8H), 2.53 (m, 8H), 2.07−1.95 (m, 8H); ^{13}C NMR (100 MHz, D_2O) δ = 170.7 151.0, 126.3, 125.52, 125.50, 109.4, 81.6, 76.0, 73.6, 52.6, 48.7, 33.8, 32.2, 28.5. Anal. Calcd for $C_{60}H_{72}N_4O_{32}S_4Na_4 \cdot 13H_2O$: C, 39.69; H, 5.44; N, 3.08. Found: C, 39.81; H, 5.45; N, 3.11.

Synthesis of the Tetra-D-(+)-nipecotic Acid Derivative (7). Using D-(+)-nipecotic acid, **7** was prepared by the same procedure

as **3** except that the reaction time was extended to 96 h. The ^{1}H NMR spectrum is highly broadened. Anal. Calcd for $C_{64}H_{80}N_4O_{28}S_4Na_4 \cdot 12H_2O$: C, 42.95; H, 5.86; N, 3.13. Found: C, 42.85; H, 5.72; N, 3.56.

Synthesis of the Tetra-(*S*)-(+)-2-methoxymethylpyrrolidinylmethyl Derivative (8). Using (*S*)-(+)-2-methoxymethylpyrrolidine, **8** was prepared by the same procedure as **3**: ^{1}H NMR (400 MHz, D_2O) δ = 7.16 ppm (s, 4H), 4.54 (t, 4H), 4.26 (dd, 8H), 3.64 (q, 4H), 3.56 (m, 4H), 3.35 (m, 4H), 3.23 (m, 12H), 2.89 (t, 8H), 2.60 (m, 8H), 2.16 (m, 4H), 2.02 (m, 4H), 1.86 (m, 4H), 1.75 (m, 4H). Anal. Calcd for $C_{64}H_{88}N_4O_{24}S_4Na_4 \cdot 17H_2O$: C, 42.14; H, 6.74; N, 3.38. Found: C, 41.81; H, 6.42; N, 3.07.

Synthesis of the Tetra-*N*-methyl-(*S*)-2-pyrrolidine Methanol Derivative (9). Using (*S*)-2-pyrrolidine methanol, **9** was prepared by the same procedure as **3**: ^{1}H NMR (400 MHz, D_2O) δ = 7.18 ppm (s, 4H), 4.53 (t, 4H), 4.18 (dd, 8H), 3.66 (s, 4H), 3.54 (m, 4H), 3.42 (m, 8H), 3.03 (d, 4H), 2.88 (t, 8H), 2.68 (m, 8H), 2.12 (m, 4H), 1.93 (m, 4H), 1.80 (m, 8H). Anal. Calcd for $C_{60}H_{80}N_4O_{24}S_4Na_4 \cdot 16H_2O$: C, 41.18, H, 6.45; N, 3.20. Found: C, 41.32; H, 6.29; N, 3.28.

Synthesis of the (*S*,*S*)-(+)-2,5-Bis(methoxymethyl)pyrrolidinylmethyl Derivative (10). Using (*S*,*S*)-(+)-2,5-bis(methoxymethyl)pyrrolidine, **10** was prepared by the same procedure as **3**: ^{1}H NMR (400 MHz, D_2O) δ = 7.17 ppm (s, 4H), 4.55 (t, 4H), 4.22 (dd, 8H), 3.76 (s, 4H), 3.60 (s, 4H), 3.36 (s, 8H), 2.91 (t, 8H), 2.70 (m, 8H), 2.11 (m, 8H), 1.80 (m, 8H). Anal. Calcd for $C_{72}H_{104}N_4O_{28}S_4Na_4 \cdot 16H_2O$: C, 43.63; H, 6.91; N, 2.83. Found: C, 43.77; H, 6.66; N, 3.02.

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Supporting Information Available: Procedures for obtaining NMR spectra, determining complex stoichiometries, and measuring association constants are provided. ^{1}H NMR spectra are provided for compounds **3**−**10**. Representative Job plots are provided. This material is available free of charge via the Internet at http// pubs.acs.org.

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