JOC Article

Inclusion of Thiamine Diphosphate and *S*-Adenosylmethionine at Their Chemically Active Sites

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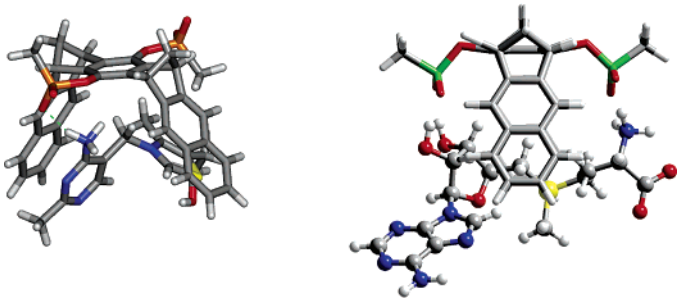

Molecular clips functionalized by phosphonate or phosphate groups bind thiamine diphosphate (TPP) and *S*-adenosylmethionine (SAM) with high affinity in water; both sulfur-based cofactors transfer organic groups to biomolecules. For TPP, various analytical tools point toward a simultaneous insertion of both heterocyclic rings into the electron-rich clip cavity. Similarly, SAM is also embedded with its sulfonium moiety inside the receptor cavity. This paves the way for enzyme models and direct interference with enzymatic processes.

Introduction

Thiamine diphosphate (TPP) is the cofactor of a large number of enzymes,[1] involved among others in decarboxylation[2] or oxidation reactions.[3] Most important, TPP catalyzes very interesting ligation reactions by virtue of its nucleophilic enamine intermediate, acting as an "umpoled" acyl carbanion equivalent.[4] The cofactor itself is bound in a typical V-shaped high-energy conformation, stabilized by hydrophobic interactions with large nonpolar amino acid side chains supporting both aromatic rings (Figure 1a).[5] However, if activated methyl groups are required, most biosyntheses rely on *S*-adenosylmethionine (SAM),[6] which catalyzes a wide range of essential methylation reactions.[7] For example, it converts ethanolamine into choline and noradrenaline into adrenaline, posttranscriptionally modifies t-RNA, and specifically methylates DNA bases, with far-reaching implications for mismatch repair and gene regulation.[8] Meanwhile, SAM is also used in the treatment of liver disorders and depressions.[9] Both cofactors have one common theme: they transfer organic groups to biological substrates (TPP, acyltransfer; SAM, alkyltransfer, Figure 1a and 1b, respectively). They are embedded in their respective enzymes mostly by means of hydrophobic interactions: aromatic and aliphatic side chains form a nonpolar binding pocket, which embraces the cofactor and locks it in its biologically active conformation, sometimes reinforced by an additional single salt bridge to an aspartate carboxylate.

Most recently we have presented a water-soluble molecular clip **1a′** featuring naphthalene sidewalls and

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(1) Review: Jordan, F. *Nat. Prod. Rep.* **2003**, *20*, 184−201.
(2) Schellenberger, A. *Biochim. Biophys. Acta* **1998**, *1385*, 177−186.
(3) Reed, L. *Acc. Chem. Res.* **1974**, *7*, 40−46.
(4) Lindquist, Y.; Schneider, G.; Ermler, U.; Sundstrom, M. *EMBO J.* **1992**, *11*, 2373−2379.
(5) Guo, F.; Zhang, D.; Kahyaoglou, A.; Farid, R. S.; Jordan, F. *Biochemistry* **1998**, *37*, 13379−13391.
(6) Review: Cheng, X. *Annu. Rev. Biophys. Biomol. Struct.* **1995**, *24*, 293−318.
(7) Mato, J. M.; Alvarez, L.; Ortiz, P.; Pajares, M. A. *Pharmacol. Ther.* **1997,** *73*, 265−280. Lu S. C. *Int. J. Biochem. Cell. Biol.* **2000**, *32*, 391−395.
(8) Modrich, P. *J. Biol. Chem.* **1989**, *264*, 6597−6600.
(9) Lieber, C. S. *Am. J. Clin. Nutr.* **2002**, *76* (suppl), 1183S−1187S. Mischoulon, D.; Fava, M. *Am. J. Clin. Nutr.* **2002**, *76* (suppl), 1158S−1161S.

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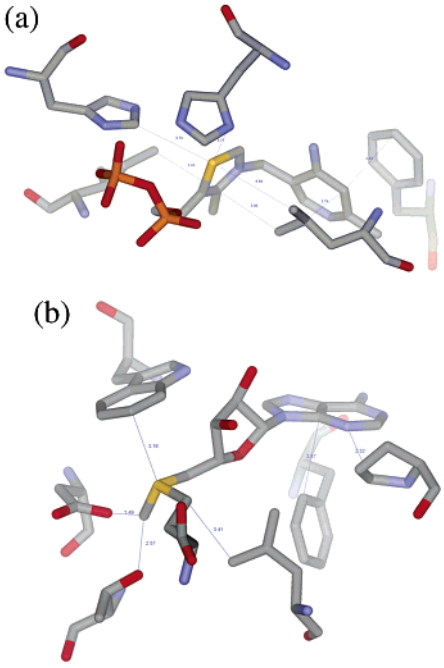
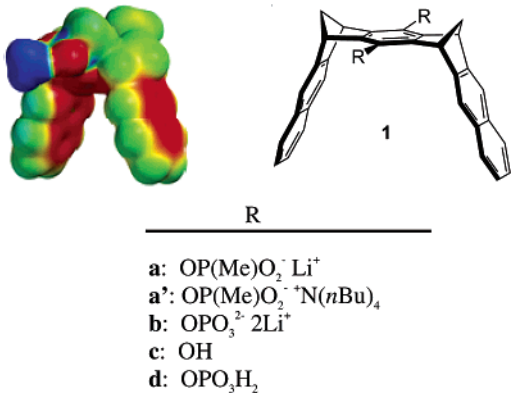


FIGURE 1. (a) V-Shaped conformation of a thiamine diphosphate derivative in its cavity in the yeast transketolase binding site.[10a] Note the stabilizing leucine residue supporting the convex aromatic face of the cofactor and forming a hydrophobic cleft together with Phe, His, and Ile side chains. (b) *S*-Adenosylmethionine in the DNA methyltransferase binding site.[10b] The sulfonium group is sandwiched between aromatic and aliphatic residues and simultaneously electrostatically stabilized by an aspartate counterion.

phosphonate anions. **1a′** is able to form stable inclusion complexes with *N*-alkylpyridinium ions and electron-poor aromatics in aqueous solution.[11,12]



We now report that *N*-alkylthiazolium cations such as thiamine **4** and TPP **5** (Figure 2) and *S*-dimethylarylsulfonium ions **6** and **7** and *S*-adenosylmethionine (SAM, **8**) (Figure 5) also represent excellent guest species for the electron-rich molecular clip **1a** or **1a′**. In addition, we describe the first phosphate-functionalized molecular

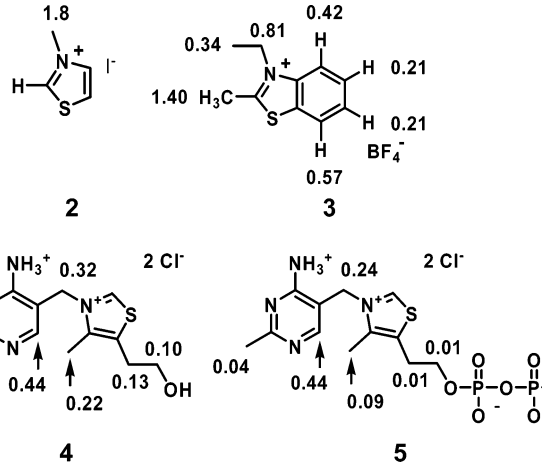


FIGURE 2. Guest molecules **2**–**5** (each with a thiazolium moiety), which form host–guest complexes with the bisphosphonate clip **1a′**, and the corresponding complexation-induced upfield ^{1}H NMR shifts of the guest protons (observed shifts: $\Delta\delta_{obs}$ except in the case of **2**: $\Delta\delta_{max}$).

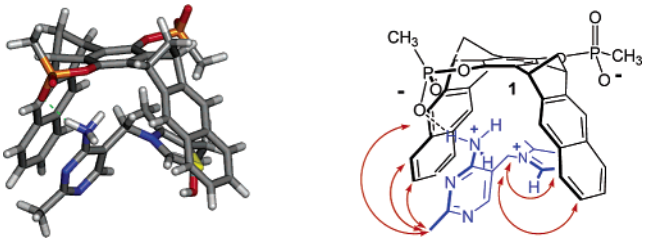
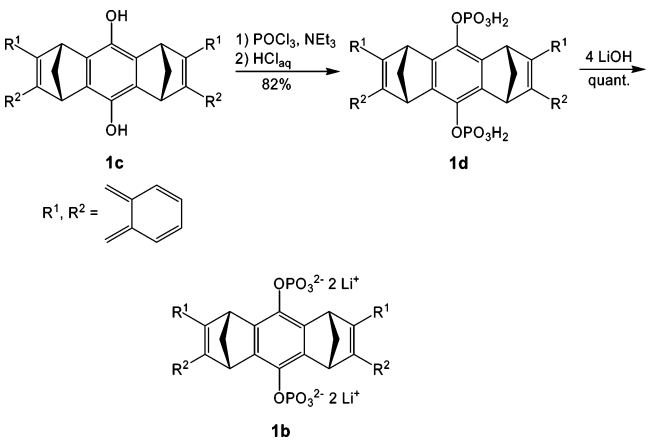


FIGURE 3. (Left) Calculated structure for the inclusion complex between thiamine **4** and clip **1a** (Monte Carlo simulation with MacroModel 7.0, OPLS-AA, 2000 steps, water). (Right) The intermolecular NOESY cross-peaks between host and guest are indicated by bent red double arrows.

clip **1b**, which even shows superior inclusion properties for electron-poor aromatic guests.

Results and Discussion

The phosphate-substituted clip **1b** was synthesized by reaction of the known hydroquinone clip **1c**[13] with $POCl_3$ and Et_3N in anhydrous THF followed by hydrolysis with dilute HCl and neutralization of the resulting bisdihydrogenphosphate-substituted clip **1d** with 4 equiv of LiOH.



(10) (a) König, S.; Schellenberger, A.; Neef, H.; Schneider, G. *J. Biol. Chem.* **1994**, *269*, 10879–10882. (b) Cheng, X.; Kumar, S.; Posfai, J.; Pflugrath, J. W.; Roberts, R. J. *Cell* **1993**, *74*, 299–307.

(11) Jasper, C.; Schrader, T.; Panitzky, J.; Klärner F.-G. *Angew. Chem.* **2002**, *114,* 1411–1415; *Angew. Chem., Int. Ed.* **2002**, *41*, 1355–1358.

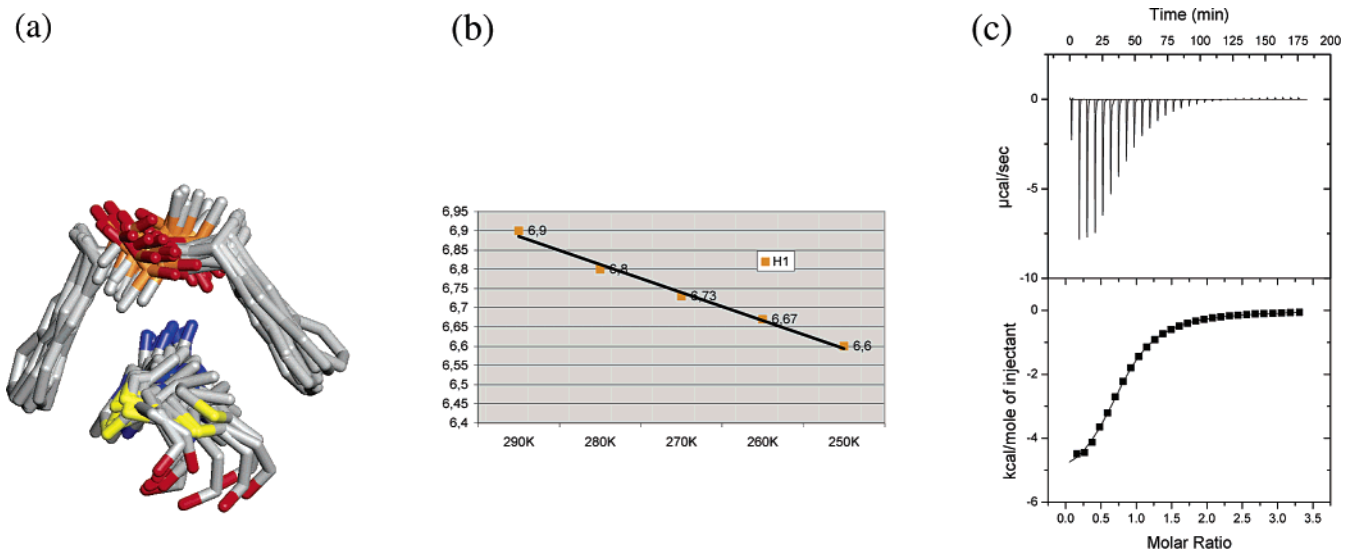


FIGURE 4. (a) Molecular dynamics calculation (MacroModel 7.0, 298 K, 10 ps with 1 ps snapshots). (b) Temperature dependence (290−190 K) of the chemical shift δ_{obs} observed for guest proton H1 (aromatic pyrimidine C−H) of thiamine **4** in the presence of **1a′** in CD_3OD ([**1a′**] = 1 mM, [**4**] = 1 mM, $K_a \geq 20\,000\ M^{-1}$). (c) Isothermal microcalorimetric titration curve between clip **1a′** (0.5 mM) and thiamine **4** (0.1−1.8 mM) in water.

TABLE 1. Binding Constants K_a and Free Energies ΔG Determined by NMR Dilution Titrations in D_2O for Complex Formation between Clip 1a′ and *N*-Alkylthiazolium Guest Molecules 2−5 at 20 °C

substrate	K_a [M^{-1}][a]	$-\Delta G$ [kcal/mol]
N-methylthiazolium **2**	1300 ± 400	4.2
thiamine **4**	19000 ± 6000	5.7
thiamine diphosphate **5**	14000 ± 5300	5.6

[a] Determined from the complexation-induced ^1H NMR shifts of the guest proton signals. The given errors are standard deviations between the K_a values from different NMR signals; the standard deviations from the nonlinear regressions were consistently lower.

Addition of the bisphosphonate host **1a′** to *N*-methylthiazolium iodide **2** furnished large upfield shifts of all guest protons, and a dilution titration of a 1:1 mixture, followed by curve-fitting with nonlinear regression[14] resulted in a moderate binding constant of $K_a = 1300\ M^{-1}$. Similar results were obtained for *N*-ethylbenzothiazolium salt **3**. Remarkably high binding constants, however, were produced by thiamine **4** (19 000 M^{-1}) and TPP **5** (14 000 M^{-1}), which strongly indicates that both rings participate in the interaction with the clip. The highest complexation-induced shifts are observed at protons from the central part of thiamine: the methylene bridge, the thiazolium methyl group, and especially the pyrimidine *ortho* proton in the 4-position are all considerably shifted upfield, while protons in the periphery of the cofactor are only slightly involved.

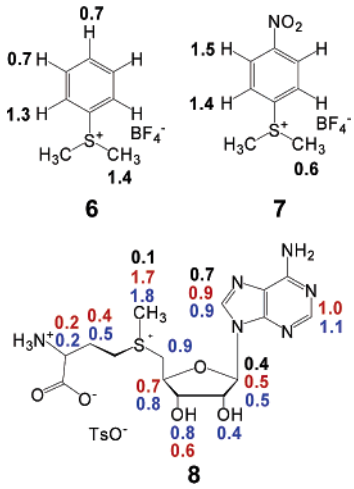


FIGURE 5. Guest molecules **6**−**8** with dimethylaryl- or trialkylsulfonium moieties, which form host−guest complexes with the bisphosphonate clip **1a** and the bisphosphate clip **1b**, respectively, and the complexation-induced upfield ^1H NMR shifts of the guest protons ($\Delta\delta_{max}$) determined for **6·1a**, **7·1a** in D_2O, and **8·1a** in CD_3OD ($\Delta\delta_{max}$ in black), in D_2O (phosphate buffer, pH = 7.2, $\Delta\delta_{max}$ in red) and **8·1b** in D_2O (phosphate buffer, pH = 7.1, $\Delta\delta_{max}$ in blue). The diastereotopic CH_2-$C\underline{H}_2$-S^+-$C\underline{H}_2$ protons of SAM show four or three complexation-induced upfield shifts ($\Delta\delta_{max}$ = 1.4, 4.7; 0.8, 1.1 and 0.6, 0.8; 0.9, respectively) in the ^1H NMR spectrum of complex **8·1a** and **8·1b**, respectively.

Interestingly enough, regardless of the starting geometries of the complex between clip **1a′** and thiamine **4**, Monte Carlo simulations in a continuous model imitating the water environment invariably converged at conformations with both heterocyclic rings inserted into the clip cavity, leading to a widened tip distance (Figure 3). Here all of those guest protons that experienced an upfield shift in the NMR titration are clearly in the shielding environment of the electron-rich naphthalene π-faces.[15] Additional confirmation of the calculated complex structure comes from a NOESY experiment in water: besides

(12) Fokkens, M.; Jasper, C.; Schrader, T.; Koziol, F.; Ochsenfeld, C.; Polkowska, J.; Lobert, M.; Kahlert, B.; Klärner, F.-G. *Chem. Eur. J.* **2005**, *11*, 477−494.

(13) Klärner, F.-G.; Panitzky, J.; Bläser, D.; Boese, R. *Tetrahedron* **2001**, *57*, 3673−3687.

(14) Wilcox, C. S. *Frontiers in Supramolecular Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds; VCH: New York, 1991; p 123. The dependence of the guest ^1H NMR shifts, $\Delta\delta$, on the host concentrations were analyzed by nonlinear regression using the program TableCurve 2D, version 5.01. In the case of the highly stable complexes the association constants, K_a, determined by ^1H NMR dilution experiments are only minimum values.

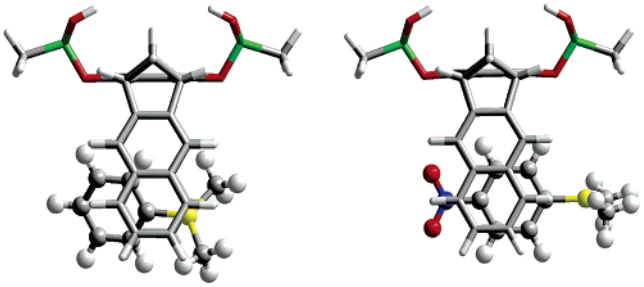


FIGURE 6. Structures of the complexes of phosphonate clip **1** (R = $OP(CH_3)O_2H$) with dimethylphenylsulfonium ion **6** (left) and dimethyl-*p*-nitrophenylsulfonium ion **7** (right) calculated by force field (MMFF 94 implemented in SPARTAN 04).[20] Note the shift of the aromatic moiety of **7** inside the clip cavity and the twist in the dimethylsulfonium group as it leaves the cavity.

TABLE 2. Binding Constants K_a and Free Energies ΔG Determined by ^{1}H NMR Titrations in Water for Complex Formation between Clip 1a or 1b and Di- and Trialkylsulfonium Guest Molecules 6−8 at 25 °C

complex	K_a [M^{-1}][a]	$-\Delta G$ [kcal/mol]
Ph-$S^+(CH_3)_2$ **6·1a**	2860 ± 420	4.7
p-NO_2-C_6H_4-$S^+(CH_3)_2$ **7·1a**	11500 ± 2700	5.5
SAM **8** ·**1a** (CD_3OD)	2960 ± 430	4.7
SAM **8** ·**1a** (buffer pH = 7.2)	1200 ± 300	4.2
SAM **8** ·**1b** (buffer pH = 7.1)	5390 ± 320	5.1

[a] The given errors are standard deviations between the K_a values from different NMR signals; the standard deviations from the nonlinear regressions were consistently lower.

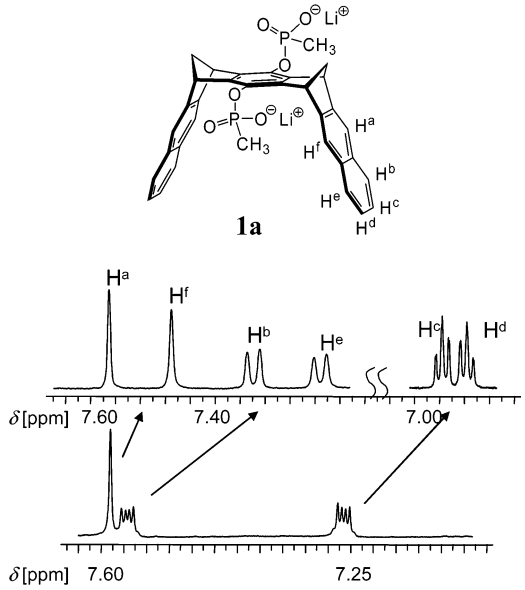


FIGURE 7. Chirality transfer from guest to host: SAM **8** inside the cavity of clip **1a** in CD_3OD. A similar splitting induced by guest **8** is observed for the corresponding protons H^a−H^f of bisphosphate host **1b**.

strong intramolecular NOEs a number of reciprocal intermolecular NOE cross-peaks are found with weak to medium intensity, which are only conceivable in a structure that brings both heterocyclic rings in close proximity to the clip walls. An overview of selected intermolecular NOE cross-peaks is given in the schematic of Figure 3. Since the acidic thiazolium CH-proton was

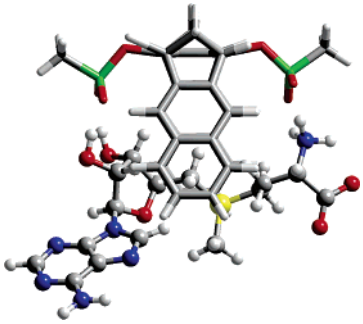
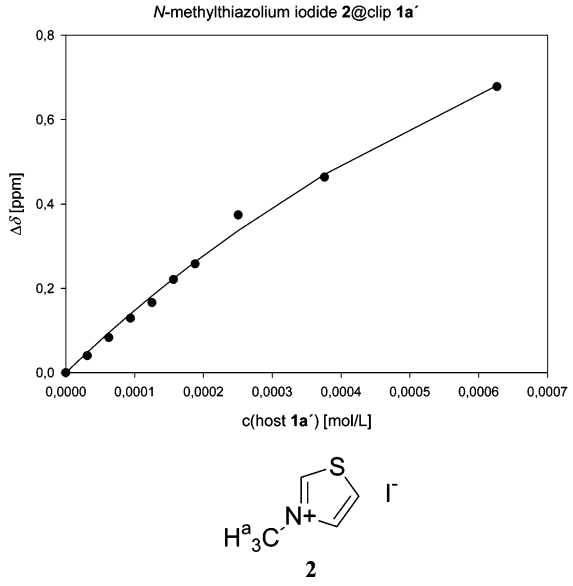


FIGURE 8. Structure of the complex between phosphonate clip **1a** and *S*-adenosylmethionine **8** calculated by force field (MMFF 94 implemented in SPARTAN 04) in the "gas phase".

TABLE 3. ^{1}H NMR Titration of Clip 1a′ with *N*-Methylthiazolium Iodide 2 in D_2O. Weighed Amounts: Host 1a′ 1.535 mg (1.425 μmol) in 650 μL D_2O; Guest 2 0.524 mg (2.307 μmol) in 250 μL D_2O

Nr.	V_{guest} (μL)	V_{host} (μL)	V_{total} (μL)	c_{guest} (mol/L)	c_{host} (mol/L)	δ_a (ppm)
0	25	0	700	$3.30\cdot10^{-4}$	0	4.2960
1	25	10	700	$3.30\cdot10^{-4}$	$3.13\cdot10^{-5}$	4.2556
2	25	20	700	$3.30\cdot10^{-4}$	$6.27\cdot10^{-5}$	4.2128
3	25	30	700	$3.30\cdot10^{-4}$	$9.40\cdot10^{-5}$	4.1667
4	25	40	700	$3.30\cdot10^{-4}$	$1.25\cdot10^{-4}$	4.1302
5	25	50	700	$3.30\cdot10^{-4}$	$1.57\cdot10^{-4}$	4.0752
6	25	60	700	$3.30\cdot10^{-4}$	$1.88\cdot10^{-4}$	4.0381
7	25	80	700	$3.30\cdot10^{-4}$	$2.51\cdot10^{-4}$	3.9222
8	25	120	700	$3.30\cdot10^{-4}$	$3.76\cdot10^{-4}$	3.8326
9	25	200	700	$3.30\cdot10^{-4}$	$6.27\cdot10^{-4}$	3.5176
					$\Delta\delta_{max}$ (ppm)	1.758
					K_a (mol^{-1})	1267 ± 367



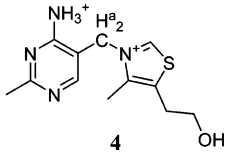
subject to fast proton exchange in aqueous solution, the complex was also diluted in H_2O applying the Watergate suppression.[16] Now the CH proton became visible at ~10 ppm and exhibited a large upfield shift of ~1 ppm to ~9 ppm. Clearly, not thiazolium's methyl group but the

(15) Numerous force-field calculations and crystal structures with included guest molecules have proven that the clip widening does not require high activation energy; to reach the above-discussed tip-widening, the penalty does not exceed 1 kcal/mol: Klärner, F.-G.; Kahlert, B. *Acc. Chem. Res.* **2003**, *36*, 919−932.

(16) Sklenar, V.; Piotto, M.; Leppik, R.; Saudek, V. *J. Magn. Reson. A* **1993**, *102*, 241−245.

TABLE 4. ^{1}H NMR Dilution Titration of Clip 1a′ with Thiamine·HCl in D_2O. Weighed Amounts: Host 1a′ 0.600 mg (0.818 μmol); Guest 4 0.078 mg (0.818 μmol) in 500 μL D_2O

Nr.	$V_{ref.\ soln.}$ (μL)	V_{total} (μL)	c_{guest} (mol/L)	c_{host} (mol/L)	δ_a (ppm)	δ_b (ppm)	δ_c (ppm)
0	reference	500	$2.83\cdot10^{-3}$	$1.38\cdot10^{-3}$	5.5294	7.6747	7.3326
1	25	500	$1.42\cdot10^{-4}$	$6.90\cdot10^{-5}$	5.2255	7.6161	7.2602
2	50	500	$2.83\cdot10^{-4}$	$1.38\cdot10^{-4}$	5.1768	7.5995	7.2536
3	75	500	$4.25\cdot10^{-4}$	$2.07\cdot10^{-4}$	5.1746	7.5973	7.2414
4	125	500	$7.08\cdot10^{-4}$	$3.45\cdot10^{-4}$	5.1326	7.5884	7.2381
5	250	500	$1.42\cdot10^{-3}$	$6.90\cdot10^{-4}$	5.1127	7.5796	7.2326
6	500	500	$2.83\cdot10^{-3}$	$1.38\cdot10^{-3}$	5.1039	7.5741	7.2292
				$\Delta\delta_{max}$ (ppm)	0.8954	0.1048	0.1063
				K_a (mol^{-1})	23100 ± 2772	12120 ± 1091	20470 ± 2661



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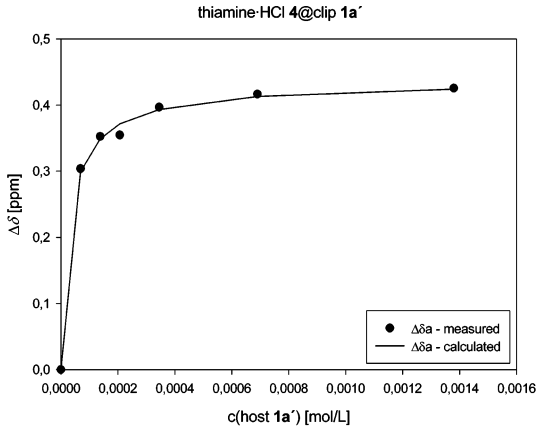
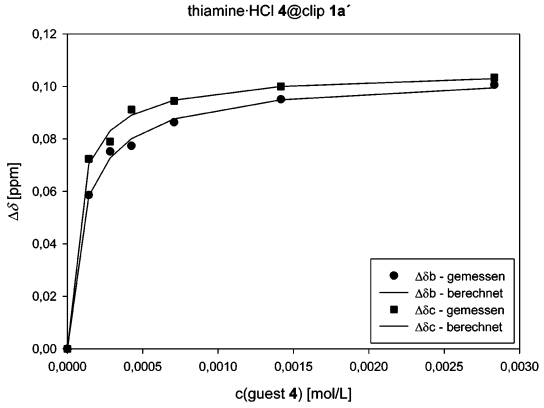
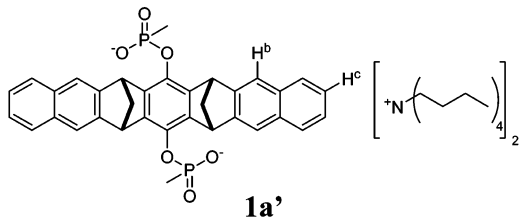




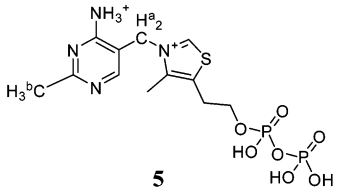


1a'

acidic CH proton resides in the shielding environment of the clip, a detail that is again in perfect agreement with the calculated structure. This arrangement of thiamine as totally embedded in a hydrophobic cavity is strongly reminiscent of its natural enzymatic environment. Although the naphthalene and bridgehead protons of clip **1a** are no longer equivalent in the calculated structure of complex **4·1a** (Figure 3) the ^{1}H NMR

TABLE 5. ^{1}H NMR Dilution Titration of Clip 1a′ with Thiamine Pyrophosphate in D_2O. Weighed Amounts: Host 1a′ 0.75 mg (0.69 μmol); Guest 5 0.38 mg (0.82 μmol) in 0.6 mL D_2O

Nr.	$V_{ref.\ soln.}$ (μL)	V_{total} (μL)	c_{guest} (mol/L)	c_{host} (mol/L)	δ_a (ppm)	δ_b (ppm)
0	Reference	600	$1.38\cdot10^{-3}$	$1.15\cdot10^{-3}$	5.5147	7.6747
1	30	600	$1.38\cdot10^{-3}$	$5.58\cdot10^{-5}$	5.3874	7.6293
2	60	600	$1.38\cdot10^{-3}$	$1.15\cdot10^{-4}$	5.3727	7.6101
3	90	600	$1.38\cdot10^{-3}$	$1.73\cdot10^{-4}$	5.3539	-
4	150	600	$1.38\cdot10^{-3}$	$2.29\cdot10^{-4}$	5.3329	7.6037
5	300	600	$1.38\cdot10^{-3}$	$6.58\cdot10^{-4}$	5.3031	7.5963
6	600	600	$1.38\cdot10^{-3}$	$1.15\cdot10^{-3}$	5.2765	7.5940
				$\Delta\delta_{max}$ (ppm)	0.3144	0.1308
				K_a (mol^{-1})	17850 ± 3927	10300 ± 2575



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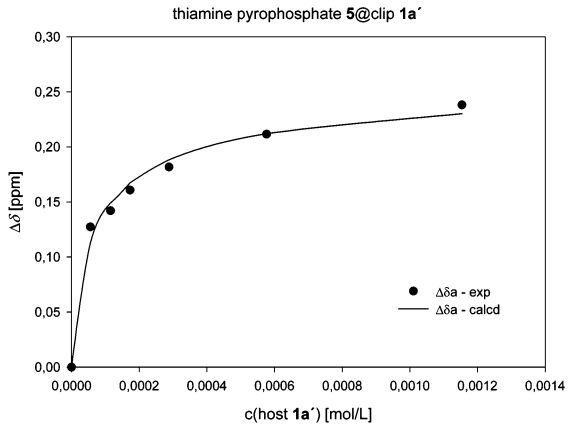
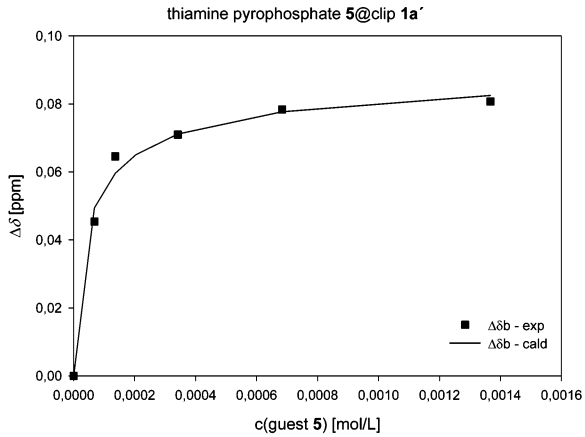
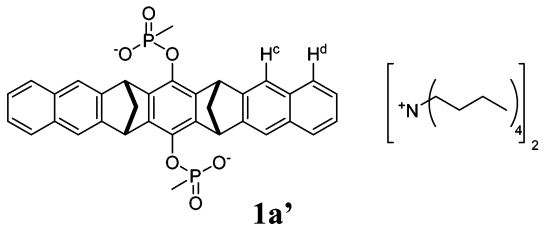




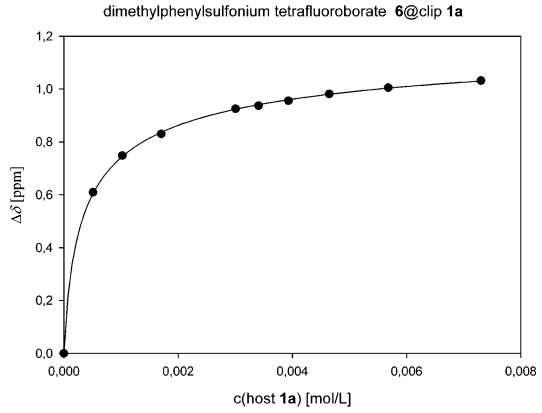


1a'

TABLE 6. ^{1}H NMR Dilution Titration of Clip 1a with Dimethylphenylsulfonium Tetrafluoroborate 6 in D_2O. Weighed Amounts: Host 1a 3.10 mg (5.11 μmol); Guest 6 1.20 mg (5.30 μmol) in 0.7 mL D_2O

sample	$V_{ref.\ soln.}$ [μL]	V_{total}[μL]	δ^a [ppm]	δ^b [ppm]	δ^c[ppm]	δ^d[ppm]
pure guest			3.2247	7.7125	7.7991	7.9380
9	500 Nr 8	1000	-	-	-	7.3288
8	3000	5000	2.4837	7.3108	7.4319	7.1904
7	1700	3000	2.3936	-	7.3834	7.1077
6	1500	1700	2.2829	7.2044	7.3279	7.0130
5	1300	1500	2.2704	7.1966	7.3178	7.0016
4	1100	1300	2.2447	7.1860	7.3073	6.9825
3	900	1100	2.2233	7.1718	7.2964	6.9571
2	700	900	2.1908	7.1571	-	6.9334
1	700	700	2.1579	7.1378	-	6.9060
[a] H-a (guest); [b] H-b (guest);						
[c] H-c (guest); [d] H-d (guest)			K [M^{-1}] = 2650	2850	2500	3450
			(± 265)	(± 313)	(± 250)	(± 414)

Average: K [M^{-1}] = 2860 ± 420



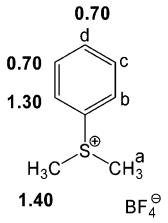


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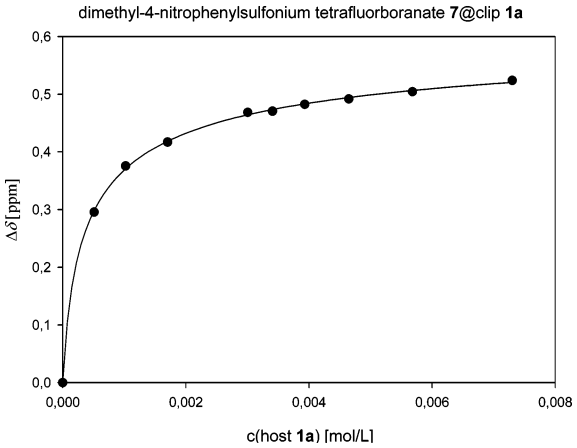
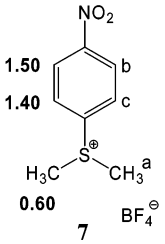
TABLE 7. ^{1}H NMR Dilution Titration of Clip 1a with Dimethyl-4-nitrophenylsulfonium Tetrafluoroborate 7 in D_2O. Weighed Amounts: Host 1a 3.10 mg (5.11 μmol); Guest 7 1.30 mg (4.80 μmol) in 0.7 mL D_2O

sample	$V_{ref.\ soln.}$ [μL]	V_{total}[μL]	δ^a [ppm]	δ^b [ppm]	δ^c[ppm]
pure guest			3.0984	7.9805	8.2898
9	500 Nr 8	1000	3.3665	-	-
8	3000	5000	2.9565	-	-
7	1700	3000	2.9153	7.0600	7.2275
6	1500	1700	2.8636	6.9898	7.1520
5	1300	1500	2.8616	6.9757	7.1485
4	1100	1300	2.8497	6.9641	7.1382
3	900	1100	2.8403	6.9569	7.1251
2	700	900	2.8278	6.9347	-
1	700	700	2.8083	6.9202	-
[a] H-a (guest); [b] H-b (guest);					
[c] H-c (guest);			K [M^{-1}] = 8500	12600	13500
			(± 1275)	(± 1380)	(± 1350)

Average: K [M^{-1}] = 11500 ± 2700





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spectrum of **4·1a** shows only four signals for these protons, indicating that a rotation of the guest inside the clip cavity and/or a complex dissociation/formation proceeds fast with respect to the NMR time scale, leading to an averaging of the NMR signals of the protons that are nonequivalent in the complex.

Subsequent molecular dynamics calculations predict that on the time scale of such a calculation (10 ps) the guest remains inside the widened gap of the clip but retains a high translational mobility, leading to a favorable entropy balance.[17] It seems that the two rings flip back and forth inside the cavity, so that always one can be engaged in π-stacking interactions with the host's naphthalene wall (Figure 4a). In a variable temperature ^{1}H NMR experiment a clear (1:1) solution of clip **1a′** and thiamine **4** in CD_3OD was obtained down to −100 °C. Substantial line-broadening occurred below 0 °C with concomitant upfield shifts of the ^{1}H NMR signals assigned to the guest protons (Figure 4b). These findings indicate that the host−guest equilibrium is shifted toward the complex and an exchange of guest protons (fast at room temperature) is decelerated. This exchange of guest protons may result from the fast dissociation/association process (exchange between the protons of free and complexed thiamine **4**) and/or from the dynamic equilibrium, which features complex structures with only one (either the thiazolium or the pyrimidinium) ring inserted into the cavity at the same time.

We also examined the complex formation between clip **1a′** and thiamine **4** with isothermal calorimetry (VP-ITC, MicroCal). Addition of **4** to an aqueous solution **1a′** was corrected by the corresponding dilution heats and produced a sigmoidal binding curve with a K_a value of 16 000 M^{-1} (±2%) and an approximate 1:1 stoichiometry (Figure 4b). The guest inclusion by clip **1a′** is almost entirely enthalpy-driven ($\Delta H = -5.5$ kcal/mol), with a negligible entropy contribution ($T\Delta S = +0.2$ kcal/mol). This points

(17) This is also confirmed by ITC measurements (vide infra), which show a large positive term for $T\Delta S$ with NAD^+ as opposed to TPP.

TABLE 8. ^{1}H NMR Titration of Clip 1a with *S*-Adenosylmethionine 8 in CD_3OD. Weighed Amounts: Host 1a 1.60 mg (2.64 μmol); Guest 8 3.32 mg (8.29 μmol) in 3.0 mL CD_3OD

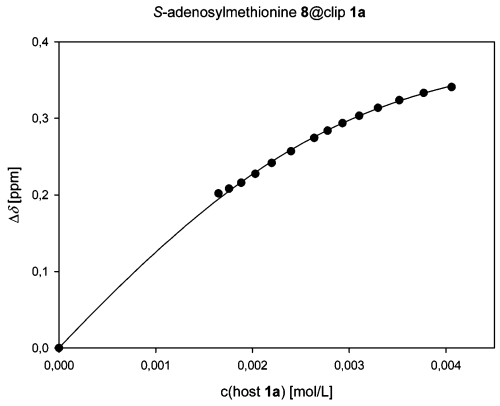
sample	V_{guest}[μL]	V_{total}[μL]	δ^a [ppm]	δ^b [ppm]	δ^c [ppm]
pure guest			6.1253	8.4702	8.5181
1	600	600	5.7868	-	-
2	50	650	-	-	-
3	50	700	5.7922	8.0047	7.9283
4	50	750	5.8017	8.0154	7.9397
5	50	800	5.8116	8.0277	7.9526
6	50	850	5.8220	8.0400	7.9668
7	50	900	5.8316	8.0535	7.9813
8	50	950	5.8415	8.0671	7.9968
9	100	1000	5.8509	8.0810	8.0116
10	100	1100	5.8683	8.1056	8.0397
11	100	1200	5.8837	8.1286	8.0652
12	100	1300	5.8979	8.1488	8.0898
13	100	1400	5.9095	8.1671	8.1100
14	100	1500	5.9172	8.1787	8.1242
15	100	1600	5.9234	8.1882	8.1349

[a] 1′-H (guest); [b] 2-H (guest);

[c] 8-H (guest); K [M^{-1}] = 3150 2500 3300

(± 315) (± 350) (± 360)

Average: K_a [M^{-1}] = 2960 ± 430



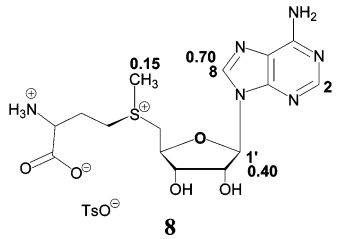


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TABLE 9. ^{1}H NMR Titration of Clip 1a with *S*-Adenosylmethionine 8 in Phosphate Buffer (pH = 7.2). Weighed Amounts: Host 1a 2.00 mg (3.30 μmol); Guest 8: 2.20 mg (5.51 μmol) in 3.0 mL Phosphate Buffer

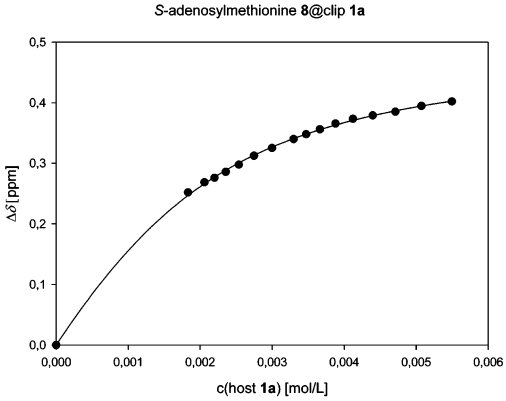
sample	V_{guest}[μL]	V_{total}[μL]	δ^a [ppm]	δ^b [ppm]	δ^c [ppm]	δ^d [ppm]
pure guest			6.1091	8.2806	8.2932	2.9667
1	600	600	5.7070	7.4033	7.5320	1.8083
2	50	650	5.7145	7.4169	7.5450	1.8398
3	50	700	5.7240	7.4311	7.5588	1.8704
4	50	750	5.7300	7.4424	7.5696	1.8963
5	50	800	5.7357	7.4506	7.5774	1.9139
6	50	850	5.7435	7.4667	7.5948	1.9490
7	50	900	5.7530	7.4822	7.6090	1.9789
8	50	950	5.7612	7.4986	-	2.0098
9	100	1000	5.7691	7.5134	-	2.0379
10	100	1100	5.7839	7.5421	-	2.0890
11	100	1200	5.7968	7.5661	7.6866	2.1303
12	100	1300	5.8114	7.5964	7.7150	2.1792
13	100	1400	5.8233	7.6200	7.7355	2.2158
14	100	1500	5.8331	-	7.7547	2.2483
15	100	1600	5.8404	-	7.7673	2.2682
16	200	1800	5.8571	7.6907	7.8001	-

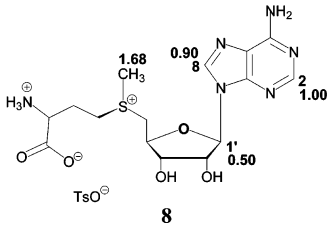
[a] 1′-H (guest); [b] 2-H (guest);

[c] 8-H (guest); [d] S^+-CH_3 (guest) K [M^{-1}] = 1086 1679 1357 540

(± 33) (± 84) (± 54) (± 27)

Average: K_a [M^{-1}] = 1200 ± 300





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to π-cation as well as π-stacking interactions as the main noncovalent attractive forces.[18]

Crystal structures demonstrate that a biomimetic recognition of SAM **8** must also feature a cavity for the inclusion of the trialkylsulfonium moiety (vide supra). To check the clip's complexation ability toward sulfonium ions, we chose dimethylphenyl- and dimethyl-*p*-nitrophenylsulfonium tetrafluoroborate **6** and **7** as valuable model compounds that undergo methyl transfer reactions comparable to those of SAM **8** in enzymes.[19] Both salts form stable host−guest complexes with the phosphonate clip **1a** in aqueous solution, which can be easily detected by substantial complexation-induced upfield shifts of the guest ^{1}H NMR signals. The association constants (K_a = 2860 and 11 500 M^{-1}, respectively) and the $\Delta\delta_{max}$ values (Figure 5) were determined by ^{1}H NMR dilution titration. Remarkably, the nitro group strongly influences the complex stability and structure. Comparison of the $\Delta\delta_{max}$ values of the guest signals between both complexes suggests that the two guest molecules are located at different positions inside the clip cavity. In the complex **6·1a** the *S*-methyl protons show a larger and the *m*-phenyl protons a smaller upfield shift than the corresponding protons of **7·1a**, indicating that in the first case the methyl protons and in the second case the phenyl protons are more strongly influenced by the magnetic anisotropy of the clip arene units.

The experimental NMR data are in good agreement with the complex structures obtained by force-field calculations (Figure 6). Accordingly, the nitro group causes a shift of the guest aryl moiety inside the clip

(18) Gallivan, J. P.; Dougherty, D. A. *J. Am. Chem. Soc.* **2000**, *122*, 870−874.

(19) Ngola, S. M.; Dougherty, D. A. *J. Org. Chem.* **1996**, *61*, 4355−4360. Kahn, K.; Bruice, T. C. *J. Am. Chem. Soc.* **2000**, *122*, 46−51.

TABLE 10. ^{1}H NMR Dilution Titration of Clip 1b with SAM 8 in Phosphate Buffer. Evaluation of the Guest Protons. Weighed Amounts: Host 1b 4.69 mg, 7.55 μmol; Guest 8 5.24 mg, 5.73 μmol. Dissolved in 3 mL of Phosphate buffer Containing 26.7 mg (222 μmol) of KH_2PO_4 and 6.2 mg (156 μmol) of NaOH. pH of the Phosphate buffer Containing the Weighed Amounts of Host 1b and Guest SAM 8 Was Determined to Be 7.06

sample	$V_{ref.soln.}$ [μL]	V_{total} [μL]	δ^a[ppm]	δ^b[ppm]	δ^c[ppm]	δ^d[ppm]	δ^e [ppm]	δ^f [ppm]	δ^g [ppm]
pure guest			3.7538	2.3205	6.1091	4.5784	8.2806	8.2932	2.9667
6	600	600	3.7156	2.2024	5.9836	-	8.0566	8.0207	2.5204
5	300	600	3.6973	2.1357	5.9188	4.2851	7.9409	7.8797	2.2933
4	150	600	3.6780	2.077	5.8544	4.1902	7.8299	7.7473	2.0770
3	75	600	3.6607	2.0271	5.7918	4.1025	7.7227	-	1.8620
2	38	600	3.6430	1.9704	5.7341	4.0208	7.6277	7.5060	1.6658
1	19	600	3.6260	1.9329	5.6832	3.9495	7.5514	7.4143	1.4955
[a] α-H (guest); [b] β-H (guest); [c] 1′-H (guest); [d] 4′-H (guest); [e] 2-H (guest); [f] 8-H (guest); [g] S^+CH_3-H (guest)									
	K_a [M^{-1}] =		5060	5770	5100	5660	5630	5770	5380
			(± 285)	(± 0)	(± 0)	(± 276)	(± 0)	(± 0)	(± 0)

Average: K_a [M^{-1}] = 5390 ± 320 (determined by averaging the K_a values obtained by nonlinear regression analysis for the ^{1}H NMR signals of the guest protons 1′-H, 2-H, α-H, β-H and S^+CH_3-H)

TABLE 11. ^{1}H NMR Dilution Titration of Clip 1b with SAM 8 in Phosphate Buffer. Evaluation of the Host Protons

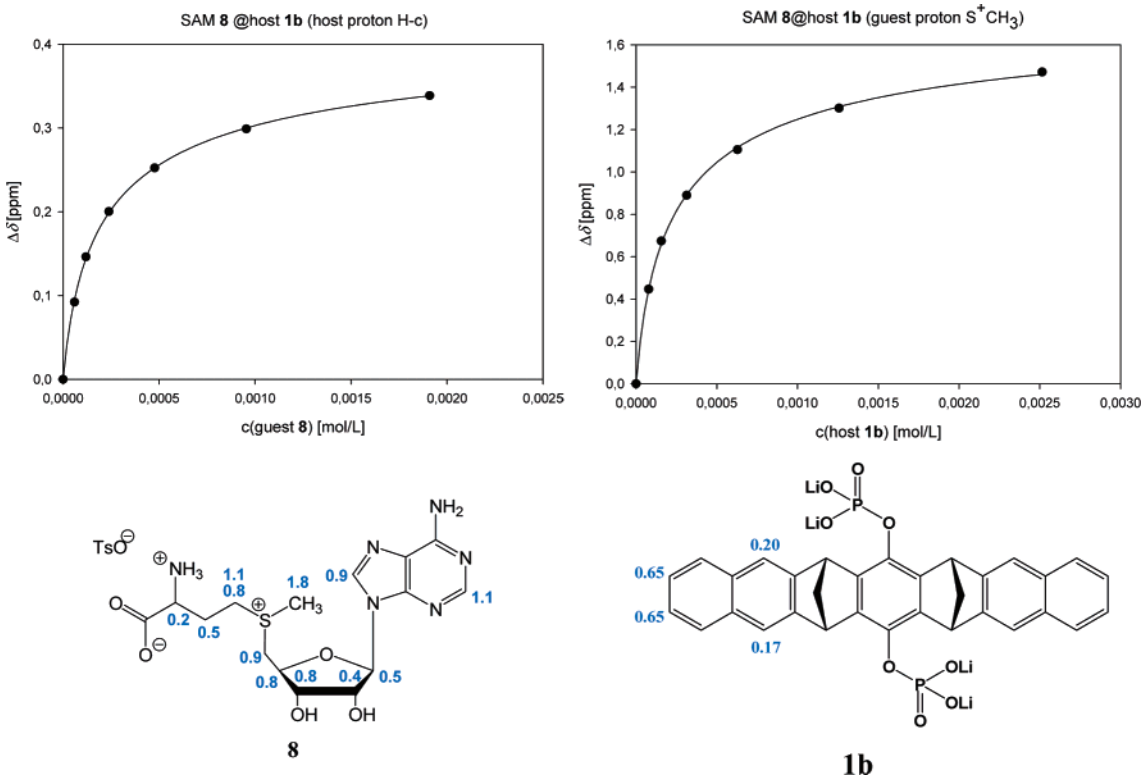
sample	$V_{ref.soln.}$ [μL]	V_{total} [μL]	δ^h[ppm]	δ^i[ppm]	δ^j[ppm]	δ^k[ppm]
pure guest			7.6699	7.6699	7.6336	7.2976
6	600	600	7.6533	7.6473	7.5659	7.2055
5	300	600	7.6391	7.6306	7.5206	7.1513
4	150	600	7.6252	7.6136	7.4765	7.0973
3	75	600	7.6117	7.5975	7.4342	7.0453
2	38	600	7.5997	7.5833	7.3958	6.9986
1	19	600	7.5874	7.5704	-	6.9589
[h] a-H (guest); [i] f-H (guest); [j] b-H, e-H (guest); [k] c-H, d-H (guest)						
	K_a [M^{-1}] =		4120	4800	5410	6040
			(± 295)	(± 0)	(± 333)	(± 72)

cavity. The finding that the complex of **1a** with the nitro-substituted guest **7** is more stable than the complex with the corresponding parent phenyl salt **6** is good evidence for the importance of the $\pi-\pi$ and CH$-\pi$ interactions between the guest arene moiety (whose positive polarization is enhanced by the nitro function) and the negatively polarized host arene units in these host−guest binding events.

Clip **1a** and **1b** also form stable complexes with *S*-adenosylmethionine (SAM, **8**). Their composition and geometries depend largely on the solvent. In methanol, **1a** selectively picks up the adenine moiety, leading to marked upfield shifts solely in this region (Figure 5). In pure water, however, the complex **8·1a** becomes insoluble and precipitates quantitatively when aqueous host and guest solutions are combined. The analysis by MS ESI-TOF indicated that the precipitate consists of a (1:1) adduct of **1a** and SAM **8**. In buffered aqueous solution (phosphate buffer, pH = 7.2) clip **1a** forms a soluble (1:1) complex with a binding constant of K_a = 1200 M^{-1} determined by ^{1}H NMR titration. The large complexation-induced ^{1}H NMR shifts of the S^+-CH_3 and the diastereotopic $C\underline{H}_2$-S^+-$C\underline{H}_2$ protons of SAM **8** (Figure 5) indicate that in this case the active trialkylsulfonium moiety is also included into the clip cavity. Similar structural results are obtained for bisphosphate clip **1b** in buffered solution. The complex **8·1b**, however, was determined to be substantially more stable (K_a = 5390 M^{-1}) than **8·1a** (Table 2). Clearly, in buffered aqueous solution, both complexes **8·1a** and **8·1b** contain the transferable methyl group, with 1.7 or 1.8 ppm maximum upfield shift, located inside the clip's cavity.

In the SAM complexes **8·1a** and **8·1b** a remarkable splitting is observed for the host proton signals assigned to the naphthalene sidewalls, which are equivalent in the free clips. In the complex the isolated naphthalene protons show two singlets and the protons at the terminal benzene ring an ABCD spectrum instead of one singlet and an A_2B_2 spectrum in the free clips (Figure 7). By contrast, both phosphonate or phosphate groups (^{31}P NMR signal at δ = 22.83 and 1.18 ppm, respectively) remain equivalent to each other in the complexes. Evidently, complex dissociation and association proceed rapidly with respect to the NMR time scale. These processes lead to an averaging of the signals and make the corresponding protons at the two naphthalene side-

SCHEME 1. Representative Curves of the Nonlinear Regression Analysis of the Guest Proton S^+-CH_3 and the Host Proton H-c



walls and the phosphonate or phosphate side chains equivalent to each other, whereas as a result of the chirality transfer of the guest to the host molecule, the front- and backside protons of each naphthalene sidewall remain nonequivalent. The question whether the (1:1) complex **8·1a** or **8·1b** consists of two rapidly equilibrating structures (one including the trialkylsulfonium group and the other the adenine unit inside the clip cavity) or of one structure (including both units into the clip cavity simultaneously comparable to the structure suggested for the thiamine complex **5·1a**) remains open.

Calculations provide only little information on the complex structures. Since force-field parameters of the sulfonium group are missing in MacroModel,[21] Monte Carlo simulations in a continuous model imitating the water environment are not feasible for complex **8·1a** or **8·1b**. In the structure of complex **8·1a** calculated by a conformational search performed by the force field MMFF94 in the gas phase implemented in SPARTAN 04[20] (Figure 8) the sulfonium function with its neighbor methyl and methylene groups are inserted into the clip cavity. However, the strength of the salt bridges and the hydrogen bonds are certainly overestimated in the gas-phase calculations, and hence the conformational lock resulting from the interaction between both phosphonate groups and the ribose and ammonium unit should not be overinterpreted.

Conclusion

In summary, we have demonstrated that clips **1a**,**b** functionalized with phosphonate or phosphate groups bind thiamine diphosphate **5** and *S*-adenosylmethionine **8** with high affinity in water. To the best of our knowledge, our new hosts are the first artificial receptors for both important cofactors.[22] Since various analytical tools unanimously point toward a simultaneous insertion of both heterocyclic rings of **5** into the cavity of **1a** with a distinct twist away from the biologically active V-conformation, it seems highly probable that "umpolung" reactions as well as other transformations using the thiazolium cofactor will be greatly affected if they take place in the altered microenvironment of the clip.[23] Similarly, an artifical methyltransferase is envisaged, which uses a metal ion chelate between the receptor's phosph(on)ate (**1a/b**) and the nucleophilic substrate to render the whole catalytic process intramolecular.[24] For an application in medicinal chemistry it would be of great interest whether the clip **1** is able to inhibit the enzymatic methyltransfer reaction, for example, of catechol-*O*-methyltransferase (COMT), by selective complexation of SAM **8**.[25] In a next step, we will also try to reach improved affinities and selectivities with a second gen-

(20) *SPARTAN 4.0*; Wavefunction Inc.: Irvine, California, 2003.

(21) Unfortunately, sulfonium ions are not parametrized in conventional modelling packages such as Macromodel, v. 6.5, Schrödinger, Inc., 1500 SW First Ave., Ste. 1180, Portland, OR 97201. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(22) Anion (e.g., TPP)-permeable metallopores: Das, G.; Onouchi, H.; Yashima, E.; Sakai, N.; Matile, S. *ChemBioChem* **2002**, *3*, 1089−1096.

(23) For attempts to alter the catalytic activity of thiazolium cations inside dendrophanes, see: Habicher, T.; Diederich, F.; Gramlich, V. *Helv. Chim. Acta* **1999**, *82*, 1066−1095.

(24) Initial investigations inside an acetate-modified tweezer show a marked effect on the methylation kinetics of NaI with dimethylphenylsulfonium tetrafluoroborate: Nellesen, A. Disertation, University of Duisburg-Essen, presumably 2005.

eration of our artificial hosts, which will be targeted to interfere with the natural biological processes mediated by TPP and SAM. Investigations in these directions will be carried out in our laboratories soon.

Experimental Section

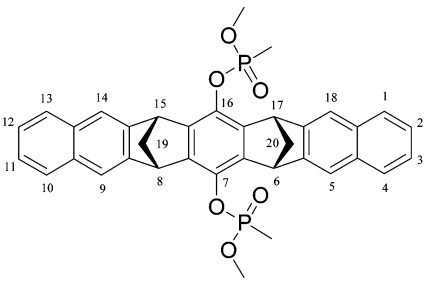
General Experimental Details. IR: Bio-Rad FTS 135. UV: J + M Tidas FG Cosytec RS 422. ^{1}H NMR, ^{13}C NMR, DEPT H,H−COSY, C,H−COSY, NOESY, HMQC, HMBC, ^{1}H NMR titration experiments: Bruker AMX 300 and DRX 500. The undeuterated amount of the solvent was used as an internal standard. The ^{1}H and ^{13}C NMR signals were assigned by the 2D experiments mentioned above. Positions of the protons of the methylene bridges are indicated by the letters *i* (*innen*, toward the center of the molecule) and *a* (*aussen*, away from the center of the molecule). MS: Fison Instruments VG ProSpec 3000 (70 eV). All melting points are uncorrected. Column chromatography: silica gel 0.063−0.2 mm. All solvents were distilled prior to use.

Syntheses. The clip **1a** was synthesized either directly by way of its phosphonic acid **1d** or by an indirect route via the methyl ester of **1d**, which can be purified by column chromatography.

(A) Direct Synthesis. The hydroquinone clip **1c** was synthesized according to ref 13. The 6,8,15,17-tetrahydro-6,17:8,15-dimethanoheptacene-7,16-bismethylphosphonic acid [R = OPO(CH$_3$)OH] **1d** was synthesized according to refs 11 and 12.

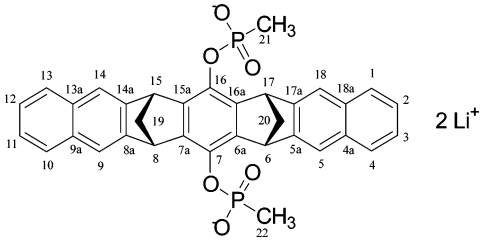
Bis(lithium)-6,8,15,17-tetrahydro-6,17:8,15-dimethanoheptacenyl-7,16-bismethylphosphonate, 1a. A solution of LiOH (3.69 mg, 0.154 mmol) in CH$_3$OH (0.3 mL) is added to the stirred suspension of phosphonic acid (R = OPO(CH$_3$)OH) **1d** (45.7 mg, 0.077 mmol) in dichloromethane (5 mL). The mixture is stirred at room temperature for 2 h, and then the solvent is evaporated to dryness. The residue is dissolved in methanol and subsequently membrane-filtered and again evaporated to dryness. The drying at 0.1 mbar furnishes 40.1 mg (66.2 μmoL, 86.7%) of **1a** as a white solid. Mp > 230 °C. ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.32 (d, ^2J(P-CH$_3$) = 16.45 Hz, 6H; P-CH$_3$), 2.34 (dt, ^2J(19-H^i, 19-H^a) = 7.9 Hz, 2H; 19-H^i, 20-H^i), 2.61 (dt, ^2J(19-H^a, 19-H^i) = 7.9 Hz, 2H; 19-H^a, 20-H^a), 4.78 (s, 4H; 6-H, 8-H, 15-H, 17-H), 7.18 (dd, ^3J(H-1, H-2) = 9.5 Hz, ^4J(H-4, H-2) = 3.0 Hz, 4H; 2-H, 3-H, 11-H, 12-H), 7.54 (dd, ^3J(H-2, H-1) = 9.5 Hz, ^4J(H-4, H-2) = 2.7 Hz, 4H; 1-H, 4-H, 10-H, 13-H), 7.61 (s, 4H; 5-H, 9-H, 14-H, 18-H). ^{13}C NMR (126 MHz, CD$_3$OD): δ 13.4 (d, ^1J(P,C) = 137.8 Hz; C-21, C-22), 65.7 (s, C-19, C-20), 120.8 (s, C-5, C-9, C-14, C-18), 125.9 (s, C-2, C-3, C-11, C-12), 128.6 (s, C-1, C-4, C-10, C-13), 133.5 (s, C-4a, C-9a, C-13a, C-18a), 142.1 (s, C-5a, C-8a, C-14a, C-17a), 148.9 (d, C-7, C-16), C-6, C-8, C-15, C-17: expected under the deuterium coupled septet of CD$_3$OD. ^{31}P NMR (202 MHz, CD$_3$OD): δ 22.93 (s). **MS** (ESI+, CH$_3$OH) *m/z*: 607 [M + H]$^+$, 1213 [2M + H]$^+$, 1819 [3M + H]$^+$.

(B) Indirect Synthesis. 7,16-*O*,*O*′-Bis(methylmethoxyphosphoryloxy)-6,8,15,17-tetrahydro-6,17:8,15-dimethanoheptacene. A 330 mg portion of the hydroquinone precursor **1c** (0.75 μmol) and 264 mg of methylphosphonic acid dichloride (1.98 mmol, 2.7 equiv) are dissolved in 33 mL of anhydrous THF and cooled to 0 °C. Then 264 μL of triethylamine (193 mg, 1.91 mmol, 3 equiv) is added dropwisely, followed by precipitation of a colorless solid after a few seconds. After 1 h, the solution is warmed to room temperature and stirred for another 1 h. The reaction mixture is quenched with 15 mL of anhydrous methanol and stirred for 16 h. Subsequently the solvent is evaporated to dryness. The remaining crude product is chromatographically purified over silica (300 mm × 10 mm, gradient elution with CH$_3$Cl/acetone = 10:1) and affords 260 mg of the methyl phosphonate clip (0.41 mmol, 55%) as a colorless solid. TLC: R_f = 0.41 (CH$_3$Cl/acetone =



3:1). Mp 110 °C. ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.65 (d, ^2J(P-CH$_3$) = 17.3 Hz, 6H; P-CH$_3$), 2.47 (d, ^2J(19-H^i, 19-H^a) = 8.3 Hz, 2H; 19-H^i, 20-H^i), 2.60−2.70 (m, 2H; 19-H^a, 20-H^a), 3.55, 3.56 (2 d, ^3J(P−OCH$_3$) = 11.23 Hz, 6H; P−OCH$_3$), 4.66, 4.70 (2 s, 4H; 6-H, 8-H, 15-H, 17-H), 7.20−7.35 (m, 4H; 2-H, 3-H, 11-H, 12-H), 7.59 (dd, ^3J(H-2, H-1) = 9.5 Hz, ^4J(H-2, H-4) = 2.4 Hz, 4H; 1-H, 4-H, 10-H, 13-H), 7.62, 7.66 (2 s, 4H; 5-H, 9-H, 14-H, 18-H) (diastereomers). ^{31}P NMR (81 MHz, CDCl$_3$): δ 29.48 (s). ^{13}C NMR (75.47 MHz, CDCl$_3$): δ 11.3 (d, ^1J(P,C) = 144.1 Hz; C-21, C-22), 48.5 (s, C-6, C-8, C-15, C-17), 53.1 (d, ^2J(P,C) = 6.8 Hz), 65.2 (s, C-19, C-20), 120.6 (d, C-5, C-9, C-14, C-18), 125.5 (s, C-2, C-3, C-11, C-12), 127.7, 127.8 (2 d, C-1, C-4, C-10, C-13), 132.2, 141.5, 146.3 (3 s, C-4a, C-5a, C-7, C-8a, C-9a, C-13a, C-14a, C-16, C-17a, C-18a). MS (ESI, CH$_3$OH) *m/z*: 623 [M + H$^+$], 645 [M + Na$^+$], 661 [M + K$^+$]. HR-MS: calcd for $C_{36}H_{32}O_6P_2$ 622.1674, found 622.1590.

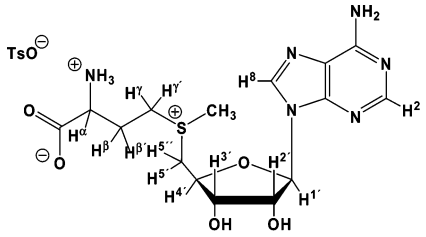
Bis(lithium)-6,8,15,17-tetrahydro-6,17:8,15-dimethanoheptacenyl-7,16-bismethylphos-phonate, 1a. A 20 mg (32 μmol) portion of the methyl phosphate clip (prepared in the previous step) is dissolved in 3 mL of 2-hexanone and after the addition of 6.1 mg (71 μmol) of dry LiBr the mixture is stirred under argon at 120 °C for 16 h. The resulting precipitate was centrifugated, washed three times with 2 mL of dry acetonitrile and dried in vacuo. This yielded 16 mg of brown product (26 μmol, 81%). Mp > 230 °C. ^{1}H NMR (300



MHz, CD$_3$OD): δ 1.34 (d, ^2J(P-CH$_3$) = 16.3 Hz, 6H; P-CH$_3$) 2.36 (d, ^2J(19-H^i, 19-H^a) = 7.6 Hz, 2H; 19-H^i, 20-H^i), 2.64 (d, ^2J(19-H^a, 19-H^i) = 7.9 Hz, 2H; 19-H^a, 20-H^a) 4.79 (s, 4H; 6-H, 8-H, 15-H, 17-H), 7.19 (dd, ^3J(H-1, H-2) = 9.3 Hz, ^4J(H-4, H-2) = 2.7 Hz, 4H; 2-H, 3-H, 11-H, 12-H), 7.55 (dd, ^3J(H-2, H-1) = 9.3 Hz, ^4J(H-4, H-2) = 3.0 Hz, 4H; 1-H, 4-H, 10-H, 13-H), 7.61 (s, 4H; 5-H, 9-H, 14-H, 18-H). ^{31}P NMR (81 MHz, CD$_3$OD): δ 26.04 (s). ^{13}C NMR (75.47 MHz, CD$_3$OD): δ 13.4 (d, ^1J(P,C) = 137.9 Hz; C-21, C-22), 65.7 (s, C-19, C-20), 120.8 (s, C-5, C-9, C-14, C-18), 125.9 (s, C-2, C-3, C-11, C-12), 128.6 (s, C-1, C-4, C-10, C-13), 133.5 (2 s, C-4a, C-9a, C-13a, C-18a), 142.1 (s, C-5a, C-8a, C-14a, C-17a), 148.8 (s, C-7, C-16), C-6, C-8, C-15, C17, expected under the deuterium coupled septett of CD$_3$OD. UV−vis (H$_2$O): λ = 218.6 nm (log ϵ = 9.93). MS (ESI, CH$_3$OH) *m/z*: 296 [M − 2Li$^+$]$^{2-}$, 593 [M − 2Li$^+$ + H$^+$]$^-$, 599 [M − Li$^+$]$^-$, 615 [M − 2Li$^+$ + Na$^+$]$^-$. HR-MS: calcd for $C_{34}H_{26}O_6LiP_2$ 599.1359, found 599.1330.

(6α,8α,15α,17α)-6,8,15,17-Tetrahydro-6:17,8:15-dimethanoheptacenyl-7,16-bisdihydrogenphosphate, 1d. POCl$_3$

(25) Lerner, Ch.; Ruf, A.; Gramlich, V.; Masjost, B.; Zürcher, G.; Jakob-Roetne, R.; Borroni, E.; Diederich, F. *Angew. Chem.* **2001**, *113*, 4164−4166; *Angew. Chem., Int. Ed.* **2001**, *21*, 4040−4042. Lerner, Ch.; Siegrist, R.; Schweizer, E.; Diederich, F.; Gramlich, V.; Jakob-Roetne, R.; Zürcher, G.; Borroni, E. *Helv. Chim. Acta* **2003**, *86*, 1045−1062.



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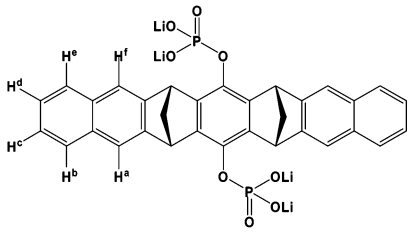

1b

(0.36 mL, 3.96 mmol) and triethylamine (0.34 mL, 2.45 mmol) are added to a stirred solution of hydroquinone clip **1c** (430 mg, 0.98 mmol) in anhydrous THF (45 mL) under argon atmosphere at 0 °C. After a few seconds a colorless solid precipitates. The mixture cooled to 0 °C is stirred for 2.5 h. Then the precipitate is filtered off and ca. 10 mL of aqueous HCl (2.5%) is added to the filtrate. After stirring the reaction mixture for 2 d at room temperature THF is evaporated. The white flaky precipitate is filtered off, washed several times with aqueous HCl (2.5%), and dried in vacuo. Yield: 480 mg (0.80 mmol, 82%) of phosphoric acid **1d** as bright ocher solid. Mp > 200 °C (decomposition). ^{1}H NMR (500 MHz, CD_3OD): δ 2.37 (dt, 2H, ^2J (19-H^a, 19-H^i) = 8.0 Hz, ^3J (19-H^a, 8-H) = 1.4 Hz, 19-H^a, 20-H^a), 2.61 (dt, 2H, 19-H^i, 20-H^i), 4.74 (s, 4H, 6-H, 8-H, 15-H, 17-H), 7.18 (m, 4H, 2-H, 3-H, 11-H, 12-H), 7.51 (m, 4H, 1-H, 4-H, 10-H, 13-H), 7.59 (s, 4H, 5-H, 9-H, 14-H, 18-H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 65.6 (s, CH_2, 19-C, 20-C), 121.3 (s, CH, C-5, C-9, C-14, C-18), 126.0 (s, CH, C-2, C-3, C-11, C-12), 128.7 (s, CH, C-1, C-4, C-10, C-13), 133.4 (s, C-4a, C-9a, C-13a, C-18a), 138.9 (dd, ^2J(C-7, P) = 8.5 Hz, ^5J(C-7, P) = 2.8 Hz, C-7, C-16), 142.4 (dd, ^3J(6a, P) = 2.8 Hz, ^4J(6a, P) = 4.2 Hz, C-6a, C-7a, C-15a, C-16a), 148.1 (s, C-5a, C-8a, C-14a, C-17a) (the signal of the bridgehead protons is overlapped by the signal of the CH_3OH). ^{31}P NMR (202 MHz, CD_3OD): δ 2.47 (s, $OP(O)(OH)_2$). MS (FAB): *m/z* 621 (M^+ + Na), 731 (M^+ + Cs).

Tetralithium-(6α,8α,15α,17α)-6,8,15,17-tetrahydro-6:17,8:15-dimethanoheptacenyl-7,16-bisphosphate, 1b. A solution of $LiOH\cdot H_2O$ (8.99 mg, 214.0 μmol) in 1 mL of CH_3OH is added to the stirred solution of phosphoric acid **1d** (32 mg, 53.5 μmol) in 5 mL of methanol at room temperature. After stirring the clear reaction solution for 30 min, methanol is evaporated in vacuo. The solid residue is dried several hours in vacuo to give the beige product in quantitative yield. Mp > 200 °C (decomposition). ^{1}H NMR (500 MHz, D_2O): δ 2.39 (d, 2H, ^2J (19-H^a, 19-H^i) = 8.2 Hz, 19-H^a, 20-H^a), 2.75 (d, 2H, 19-H^i, 20-H^i), 4.84 (s, 4H, 6-H, 8-H, 15-H, 17-H), 7.29 (m, 4H, 2-H, 3-H, 11-H, 12-H), 7.64 (m, 4H, 1-H, 4-H, 10-H 13-H), 7.68 (s, 4H, 5-H, 9-H, 14-H, 18-H). ^{1}H NMR (500 MHz, buffered D_2O, pH = 7.2): δ 2.40 (d, 2H, ^2J (19-H^a, 19-H^i) = 8.1 Hz, 19-H^a, 20-H^a), 2.71 (d, 2H, 19-H^i, 20-H^i), 7.30 (m, 4H, 2-H, 3-H, 11-H, 12-H), 7.63 (m, 4H, 1-H, 4-H, 10-H 13-H), 7.67 (s, 4H, 5-H, 9-H, 14-H, 18-H) (the signal of the bridgehead protons is overlapped by the HOD signal). ^{13}C NMR (125.7 MHz, D_2O): δ 47.8 (s, CH_2, C-19, C-20), 63.7 (s, CH, C-6, C-8, C-15, C-17), 119.3 (s, CH, C-5, C-9, C-14, C-18), 125.4 (s, CH, C-2, C-3, C-11, C-12), 127.6 (s, CH, C-1, C-4, C-10, C-13), 131.6 (s, C-4a, C-9a, C-13a, C-18a), 138.8 (dd, ^2J (C-7, P) = 7.0 Hz, ^5J (C-7, P) = 1.4 Hz, C-7, C-16), 141.2 (s, C-6a, C-7a, C-15a, C-16a), 148.7 (s, C-5a, C-8a, C-14a, C-17a). ^{31}P NMR (202 MHz, D_2O): δ 0.95 (s, 2P, OP(O)OLiOLi).

^{1}H NMR Titrations were carried out as described in ref 12. The association constants K_a and the maximum complexation-induced ^{1}H NMR shifts, $\Delta\delta_{max}$, were determined from the dependence of the guest ^{1}H NMR shifts, $\Delta\delta$, on the host concentrations by nonlinear regression analysis using the computer program TableCurve 2D, version 5.01.

Preparation of the Phosphate Buffer. A 88.9 mg (0.7 mmol) portion of KH_2PO_4 and 20.7 mg (0.5 mmol) of NaOH are dissolved in 10 mL of D_2O. The pH value of this solution was determined by electropotentiometrical measurements to be 7.11.

SAM 8·1b. ^{1}H NMR (500 MHz, buffered D_2O, pH = 7.06, 25 °C): δ_c 1.50 (s, 3 H, SAM **8**, *CH*$_3$), 1.93 (m, 2 H, SAM **8**, β-H), 2.55 (m, 2 H, SAM **8**, γ-H), 3.26 (t, 2 H, SAM **8**, 5′-H, 5″-H), 3.63 (t, 1 H, SAM **8**, α-H), 3.95 (m, 1 H, SAM **8**, 4′-H), 4.18 (t, 1 H, SAM **8**, 3′-H), 4.64 (t, 1 H, SAM **8**, 2′-H), 5.68 (d, 1 H, SAM **8**, 1′-H), 6.96 (m, 2 H, c-H, *c*′-H), 7.41 (s, 1 H, SAM **8**, 8-H), 7.55 (s, 1 H, SAM **8**, 2-H), 7.57 (s, 2 H, a′-H), 7.59 (s, 2 H, a-H).

Acknowledgment. The authors are indebted to Werner Karow for ESI-HRMS measurements and Heinz Bandmann for NMR investigations. This work was supported by the Deutsche Forschungsgemeinschaft.

Supporting Information Available: Molecular modeling and results of NOESY, VT, and ITC experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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