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A Hydrosoluble Triphenylene That Preferentially Binds Acetylcholine, Epibatidine, and Nicotine

Cécile Givelet,[†] Thierry Buffeteau,[†] Françoise Arnaud-Neu,[‡] Véronique Hubscher-Bruder,[‡] and Brigitte Bibal*,[†]

Université de Bordeaux, Institut des Sciences Moléculaires, CNRS UMR 5255, 351 cours de la Libération, 33405 Talence, France, and Laboratoire de Chimie Physique, DSA, IPHC, UMR UDS-CNRS 7178, 25 rue Becquerel, 67087 Strasbourg, France

b.bibal@ism.u-bordeaux1.fr

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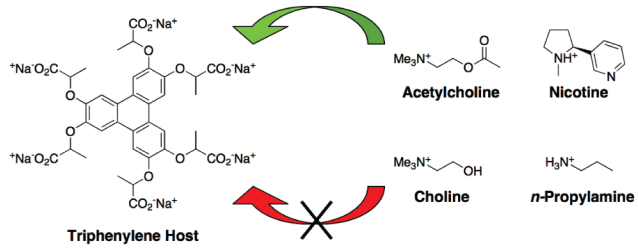
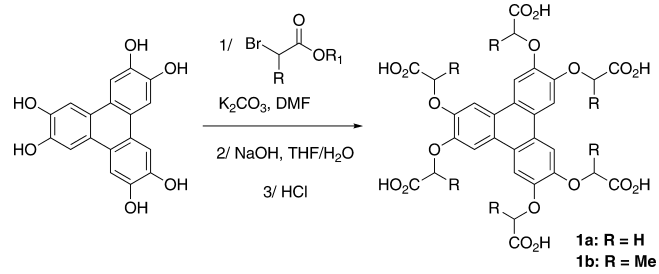


Synthesis and binding properties of a new hydrosoluble triphenylene **1b** are reported. Selective recognition of acetylcholine (ACh) against other aliphatic ammoniums is achieved by this flat receptor, which also forms complexes with epibatidine and nicotine. Ionic pairing and hydrophobic effects between host **1b** and ACh are studied by infrared spectroscopy.

Recognition of ammoniums in physiological medium by artificial receptors is an active field of research since the pioneer work of Cram, Lehn, and Pedersen.[1] Among biological ammoniums, the neurotransmitter acetylcholine (ACh) is an attractive target for natural and artificial receptors because of the implication of ACh in cerebral functions (memory, cognition, reward) and neuronal pathologies such as Alzheimer's disease.[2,3] In this context, recognition of ACh by artificial receptors in polar solvents and water became a major challenge.[4] Nevertheless, these studies are scarcely achieved in biomimetic conditions (neutral pH, in the presence of salts). Information about the molecular selectivity is also missing. For instance, the evaluation of other targets such as choline (Ch), a synaptic species structurally close to ACh,[5] as well as nicotine and epibatidine,[2c] two agonists of ACh, are poorly documented.

SCHEME 1. Molecular Structures and Synthesis of Hosts 1a,b



Recently, we reported that a triphenylene-based host can selectively bind catechols in chloroform.[6] Dynamic molecular mechanics and binding experiments showed that the new receptor interacts with a catechol molecule through a hydrogen bond assisted by a π-interaction between the triphenylene moiety and the guest. Thus a polyaromatic group was involved into combined interactions that enhance the strength of the host−guest association. In principle, a triphenylene core may participate in ammoniums binding in water through hydrophobic effects or/and a π-cation interaction. We thus decided to prepare a hydrosoluble triphenylene designed for ammonium recognition in buffered water. This polyaromatic moiety is expected to participate to the discrimination of ammoniums based upon their hydrophobicity.

In the literature, recognition of ammoniums in water is based upon the π-cation interaction[7] and hydrophobic effects[3] between aromatic cavitands and targets. Hence binding of ACh and similar ammoniums such as Ch was achieved with the same affinity. Weaker than a π-cation interaction, ion pair interactions may also be considered as a driving force for the complexation

[†] Université de Bordeaux.
[‡] DSA, IPHC.

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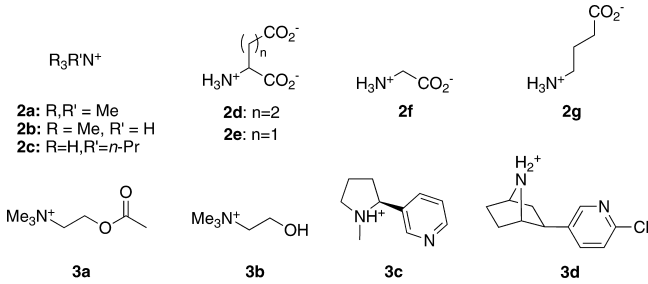

FIGURE 1. Molecular structures of model ammoniums **2a**−**c**, aliphatic neurotransmitters (**2d**−**g** and acetylcholine **3a**), and related ammoniums (choline **3b**, nicotine **3c**, and epibatidine **3d**).

of ammoniums.[7c] A few receptors did enhance ammonium recognition by combining a salt bridge to hydrophobic effects.[8] Our approach relies upon the amphiphilic receptors **1a,b** that associate anionic substituents (carboxylate groups at neutral pH) to favor a salt bridge, with a π-extended structure to promote hydrophobic effects and/or π-cation interactions (Scheme 1).

Herein we report the synthesis of the hosts **1a,b** and the binding properties of **1b** toward aliphatic ammoniums **2** and **3** in phosphate-buffered water (Figure 1). Guests **2** are hydrosoluble ammoniums: model compounds (**2a**−**c**), α-aminoacids (glutamate **2d**, aspartate **2e**, and glycine **2f**), and a γ-aminoacid (GABA **2g**), which are also neurotransmitters. Series **3** will allow comparison between ACh (**3a**), Ch (**3b**) and two agonists (nicotine **3c** and epibatidine **3d**).

The straightforward synthesis of **1a,b** was achieved in three steps (Scheme 1). 2,3,6,7,10,11-Hexahydroxytriphenylene[9] was allowed to react with commercial ethyl bromoacetate or (±)-methyl 2-bromopropionate in the presence of potassium carbonate. The resulting hexaesters were hydrolyzed with an excess of sodium hydroxide. After acidification and filtration, receptors **1a**[9] and**1b** were isolated as their hexa-acid form in 50% and 58% overall yield, respectively. Unfortunately, compound **1a** proved to be scarcely hydrosoluble. As known for amphiphilic hexa(*n*-alkyloxy)triphenylenes, **1a** self-aggregates in water.[10] Our effort thus concentrated on the new molecule **1b**, which was highly soluble in water and phosphate-buffered water (pH 7.1). This host was fully characterized and showed typical NMR spectra of hexasubstituted triphenylenes.[9] An acido-basic titration allowed determination of a pK_a for the six carboxylic functions around the mean value of 4.4. Compound **1b** was then considered as a hexacarboxylate sodium salt in phosphate buffer. In these conditions, its absorption spectrum showed a maximum at 277 nm, and its fluorescence emission spectrum (λ_{exc} 344 nm) had a maximum at 382 nm. This behavior is similar to those of hexa(*n*-alkyloxy)triphenylenes in polar solvents.[10]

Preliminary tests were run through proton NMR monitoring in phosphate-buffered D_2O (Na_2HPO_4 100 mM, pH 7.1). At first, dilution experiments proved that receptor **1b** does not self-aggregate in the 0.5−8 mM concentration range and can be used for recognition. Second, no salt effect was detected on host **1b** or model guest ACh (2 mM) in the presence of NaCl (0−100 mM). Therefore proton NMR and microcalorimetric titrations in buffered water between host **1b** (1 mM) and ammoniums **2** and **3** (0−25 mM) were undertaken (Table 1). As host signals were not shifted, NMR monitoring was done on guests protons. This observation also proved that a π-cation interaction does not take place between the triphenylene core and the ammoniums.

TABLE 1. Thermodynamic Data for Binding between Receptor 1b and Ammoniums 2a−g and 3a−d (Na_2HPO_4 buffer 100 mM, pH 7.1, 298 K)

	NMR titrations[b]	ITC titrations		
guest[a]	K_a (M^{-1})	K_a (M^{-1})	$-\Delta H$ (kJ mol^{-1})	$T\Delta S$ (J K^{-1} mol^{-1})
NMe_4^+ **2a**	<1	<1	*e*	*e*
2b−**g**	<1	n.d.	n.d.	
ACh **3a**	94	41 ± 4	28 ± 2	−19 ± 2
Ch **3b**	<1	<1[e]	*e*	
nicotine **3c**	700[c]; 124[d]	n.d.	n.d.	
epibatidine **3d**	1044[c]; 165[d]	n.d.	n.d.	

[a] Chloride as counterion. [b] Estimated errors ±10%. [c] K_1. [d] K_2. [e] Too little heat evolved to be interpreted. n.d. = not determined.

Interestingly, binding was only observed in the presence of ACh and its agonists. This observation simply reflects that **1b** cannot recognize α-aminoacid moieties (**2d**−**f**) and hydrophilic ammoniums (**2g** and **3b**). Model ammoniums **2a**−**c** also seem to be well-solvated and cannot interact with the host, even by ionic pairing. The stoichiometry of the complexes was determined by Job plots (see Supporting Information). Acetylcholine forms a 1:1 complex with **1b**, for which the association constant is 94 ± 9 M^{-1}. This binding is moderate, but to the best of our knowledge, this is the first example of a selective ACh binding against Ch in such an aqueous buffer. The microcalorimetric results corroborated the 1:1 stoichiometry of the complex involving **1b** and **3a** as well as the order of magnitude of the stability constant. They also show the predominance of the enthalpy contribution, the entropy change being negative and hence unfavorable. In comparaison, a tetracarboxylated cavitand that equally binds ACh and Ch (K_{assoc} are 1200 and 2600 M^{-1}, respectively) through classical hydrophobic effects had a strong entropic term with a small favorable enthalpic term.[5] Interactions between ACh and the flat receptor **1b** appeared to be different. Maximum binding-induced chemical shift variations were observed for the ammonium substituents (Table 2, + 0.18 for N^+Me), which may be due to ionic pairing with the host.

TABLE 2. Selected Binding-Induced Chemical Shift Variations Observed for Host 1b (1 mM) and Ammoniums 3a, 3c, and 3d (0.2−25 mM)[a,b]

	Δδ (ppm)			
	1b	**3a**	**3c**	**3d**
$H_{aromatic}$	<0.01		<0.01	+ 0.47; + 0.74
$H_{aliphatic}$	<0.01	+ 0.18 (N^+Me)	− 0.30 (N^+Me); − 0.50 ($\underline{CH}N^+R_3$)	+ 0.47 to + 1.21 ($\underline{CH}N^+HR_2$)

[a] D_2O, Na_2HPO_4 100 mM. [b] Full data are available in Supporting Information.

Nicotine and epibatidine are associated to receptor **1b** in 1:1 and 1:2 (host:guest) stoichiometries, with association constants stronger than that for ACh. Binding constants K_1 and K_2 are, respectively, in the 10^3 and 10^2 M^{-1} range (Table 1). Maximum variations of chemical shifts were also measured for these ammoniums (Table 2). Interestingly, no variation was observed on the aromatic protons of **3c**, meaning that classical hydro-

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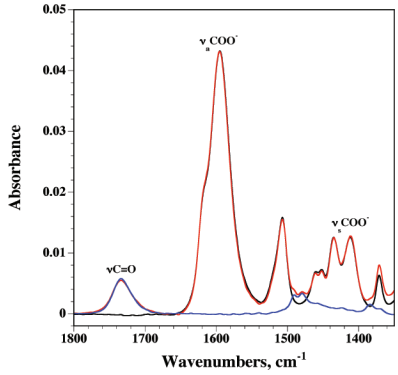

FIGURE 2. IR spectra (buffered D_2O, 1800−1350 cm^{-1}) of host **1b** (black), ACh **3a** (blue), and **1b**:**3a** mixture in a 1:1 ratio (red).

phobic effects between host and guest aromatic moieties did not take place in the presence of host **1b**. Several aliphatic protons signals were downshifted ($\Delta\delta$ −0.21 to −0.5 ppm). Nicotine thus interacts with **1b** through its tertiary ammonium, probably via ionic pairing. Concerning guest **3d**, each aromatic and aliphatic proton signals was upfield shifted in the presence of **1b** ($\Delta\delta$ +0.47 to +1.21 ppm). Association between **3d** and **1b** appeared to be a combination of several interactions, probably ionic pairing and hydrophobic effects between the aromatic moieties. Natural neuronal receptors also have different binding methods for ACh (π-cation), nicotine (H-bond), and epibatidine (π-cation and H-bond).[2c,7b] Artificial receptor **1b** has different binding modes with each of the three ammoniums.

Selectivity of host **1b** toward ACh against Ch was significant enough to undergo a detailed study. As ionic pairing seems to exist between host carboxylates and the ammonium group, infrared spectroscopy was chosen to monitor C═O environment within the **1b**:ACh complex.

Infrared spectra of ACh (blue) and receptor **1b** (black) were recorded in the 1800−1350 cm^{-1} region to assign carbonyl stretching bands (Figure 2). The band observed at 1734 cm^{-1} can be ascribed to the ester function of ACh and the two bands at 1595 and 1412 cm^{-1} were attributed, respectively, to the asymmetric and symmetric stretching vibrations (ν_a and ν_s) of the carboxylate groups in **1b**. The spectrum of a **1b**:**3a** mixture in a 1:1 ratio (red) was almost the addition of both spectra (Figure 2). Nevertheless, small displacements in wavenumbers were detected for C═O stretching bands, probably due to molecular interactions. In order to emphasize this phenomenon, mixtures with one partner in excess were prepared and recorded in each carbonyl region (Figure 3).

At first, the asymmetric (Figure 3a) and symmetric (Figure 3b) stretching bands of carboxylate functions were studied. Comparing infrared spectra of host **1b** (black) and a **1b**:**3a** mixture in a 1:1 ratio (red), 1:6 ratio (blue), and 1:18 ratio (green), we observed a displacement to higher wavenumbers for ν_a COO^- and to lower wavenumbers for ν_s COO^- when ACh was in excess to drive the complex formation. Such changes in the position of the carboxylate bands typically indicate that a lower interaction occurred between carboxylate groups and their counterions.[11] An ionic exchange between host sodium cations and ACh ammoniums took place. This assertion was also proved when receptor **1b** was provided with six tetramethyl-ammoniums (TMA) in place of sodium (orange, Figure 3a and b): ionic pairs were looser and carboxylate stretching bands (orange) were at higher ν_a COO^- and lower ν_s COO^- than those of COO^--Na^+ pairs (black). All these observations in the 1605−1585 and 1420−1405 cm^{-1} infrared regions are in accordance with host recognition of ACh through ionic exchange between a sodium (host) and ammonium (guest). Interestingly, FTIR spectra of a **1b**:Ch mixture showed ionic pairing between host carboxylates and guest ammonium, but to a lesser extent (see Supporting Information). This corroborates that the **1b**:Ch association was not significant.

Second, the ester carbonyl stretching band was also studied in the presence of a host excess (Figure 3c). The infrared spectra of **1b**:**3a** in a 1:0.33 ratio showed a displacement at higher wavenumbers (1736 cm^{-1}), which proved that the ester solvatation was modified in the presence of receptor **1b**. This was confirmed by the infrared spectrum of ACh in acetonitrile. In this solvent, solvation was achieved without hydrogen bond and C═O stretching band of ACh was present at 1750 cm^{-1}. A supplementary interaction thus occurs between host **1b** and the ester function of ACh, which is a hydrophobic effect. Therefore, the infrared study proved that **1b**:ACh involves a combination of salt bridge and hydrophobic effects, which is remarkable in strength (K_{assoc} 94 M^{-1}), according to ionic molecular receptors literature.[8a]

In summary, a hydrosoluble multivalent triphenylene **1b** was easily synthesized, and its binding properties were tested toward aliphatic ammoniums in phosphate-buffered water. Selective recognition was observed for ACh and its agonists nicotine and epibatidine. Investigation of the recognition of the two agonists is still in progress. An infrared study of **1b**:ACh association indicated that ionic pairing occurred between the guest ammonium and the host carboxylate, which is assisted by desolvatation of the guest ester. This might explain the fact that choline, a more hydrophilic molecule, does not interact with host **1b**. These combined weak associations are promising for fine-tuning of the selectivity. They should be reinforced using related multivalent triphenylene clips, under preparation.

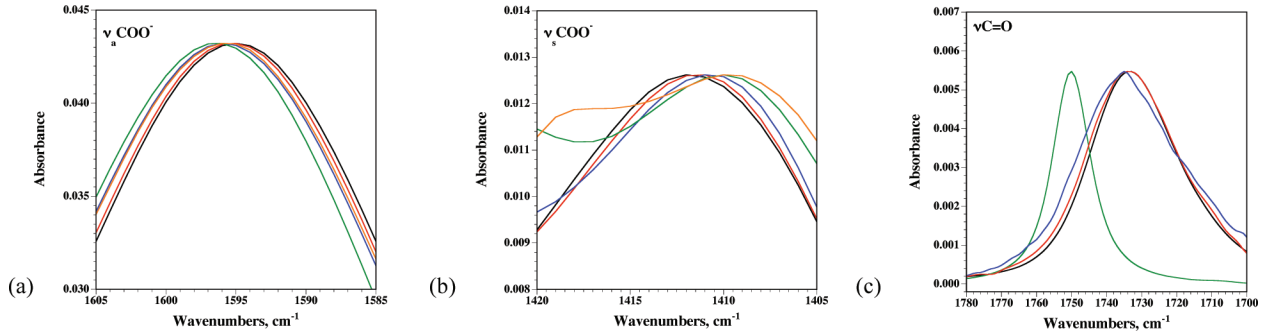

FIGURE 3. Normalized IR spectra (buffered D_2O) of host **1b** (black); **1b**:**3a** mixture in a 1:1 ratio (red), in 1:6 ratio (blue), and in 1:18 ratio (green); and **1b** with tetramethylammonium counterion (orange) recorded in (a) 1780−1700, (b) 1605−1585, and (c) 1420−1405 cm^{-1} regions.

Experimental Section

2,3,6,7,10,11-Hexahydroxytriphenylene and 2,3,6,7,10,11-hexa-(ethoxycarbonylmethoxy)triphenylene were prepared according to literature procedures.[9]

2,3,6,7,10,11-Hexa(ethoxycarbonyl(methyl)methoxy)triphenylene. To a solution of 2,3,6,7,10,11-hexahydroxytriphenylene (1.269 g, 4 mmol) and cesium carbonate (11.944 g, 36 mmol) in anhydrous DMF (40 mL) was added methyl 2-bromopropionate (4 mL, 36 mmol). The reaction mixture was stirred at room temperature overnight. Distilled water (50 mL) and ethyl acetate (50 mL) were added. After decantation, the aqueous phase was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with distilled water (2 × 200 mL) and brine (2 × 200 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The brown foam was dissolved with diethyl ether (10 mL), and hot pentane (200 mL) was added. After precipitation, the suspension was filtered, and the precipitate was dried under vacuum. The filtrate was concentrated in vacuo, suspended in diethylether/pentane, and filtrated, and the solid was dried. The experiment was repeated twice times. Compound was isolated as a yellow solid (2.151 g, 64%); R_f 0.25 (CH_2Cl_2/MeOH, 99:1); mp 73.5 °C (dec); IR (NaCl) ν 2956, 2859, 1755, 1618, 1505, 1429, 1377, 1214, 1164, 1098 cm^{-1}; ^1H NMR (250.13 MHz; $CDCl_3$; 295 K) δ 1.72 (18H, dd), 3.79 (18H, d), 5.07 (6H, m) and 7.83 (6H, m); ^{13}C NMR (75.5 MHz; $CDCl_3$; 295 K) δ 18.8, 52.4 (m), 74.9, 111.5, 125.0 (m), 148.2 and 172.7; LRMS (MALDI) *m/z* (%) 840.28 (100%, $[M]^+$); HRMS *m/z* (MALDI) calcd for $C_{42}H_{48}0_{18}$, 840.28317, found 840.28352.

General Procedure for the Saponification/Acidification Step. To a solution of a hexaester (0.63 mmol) in THF/water (3:1) was added NaOH (1 N, 7.56 mmol). The reaction mixture was stirred at room temperature overnight and concentrated in vacuo. A few milliliters of water and HCl (1 N) were successively added until pH was 3. After precipitation, the suspension was filtered, and the solid was dried under vacuum.

2,3,6,7,10,11-Hexa(carboxylic acidmethoxy)triphenylene 1a.[9] The general procedure applied to a solution of 2,3,6,7,10,11-hexakis (ethoxycarbonyl methoxy)triphenylene (300 mg, 0.355 mmol) in a THF/water solution (3:1, 60 mL) and a solution of NaOH (1 N, 4.26 mmol) gave **1a** as a white precipitate (220 mg, 92%): ^1H NMR (300 MHz, CD_3OD, 295 K) δ 5.03 (12H, s), 7.91 ppm (6H, s); LRMS (MALDI) *m/z* (%) 672 (25, $[M]^+$), 695 (60, $[M + Na]^+$).

2,3,6,7,10,11-Hexa(carboxylic acid(methyl)methoxy)triphenylene 1b. The general procedure applied to a solution of the corresponding hexaester (560 mg, 0.63 mmol) in THF/water (3:1, 40 mL) and NaOH (1N, 7.56 mL, 7.56 mmol) gave **1b** as a beige powder (429 mg, 90%); mp > 176 °C (dec.); IR (NaCl) ν 2975, 2889, 1731, 1620, 1508 (C═C), 1431, 1380, 1266, 1164, 1090 cm^{-1}; UV−vis (buffered D_2O, 2×10^{-5} M) λ_{max} (ε) 269 (39778), 277 (57629), 307 (13679), 344 nm (2422 mol^{-1} L cm^{-1}); ^1H NMR (250.13 MHz; DMSO; 295 K) δ 1.60 (18H, m), 5.21 (6H, m) and 7.91 (6H, m); ^{13}C NMR (50.2 MHz, DMSO, 295 K) δ 18.2, 73.3 (m), 110.4 (m), 123.6, 147.5 and 173.2; LRMS (MALDI) *m/z* (%) 756.66 (34, $[M]^+$), 779.67 (100, $[M + Na]^+$), 801.66 (42, $[M - H + 2Na]^+$); HRMS (MALDI) *m/z* calcd for $C_{36}H_{36}0_{18}Na$, 779.18179; found 779.17939.

FTIR Measurements. Infrared spectra of host **1b**, acetylcholine **3a**, and choline **3b**, as well as mixture of **1b** + **3a** and **1b** + **3b** were recorded with a FTIR spectrometer at a resolution of 4 cm^{-1}, by co-adding 50 scans. Samples were held in a CaF_2 cell with fixed path length of 45 μm. Concentration of host **1b** was fixed at 3 mM in D_2O solution (Na_2HPO_4 100 mM). Spectra of **1b** + **3a** were measured in 1:0.33, 1:1, 1:6, and 1:18 molar ratios, whereas the spectrum of **1b** + **3b** was measured in a 1:6 molar ratio. All infrared spectra were shown with solvent absorption subtracted out. Some infrared spectra were normalized to emphasize the frequency shift.

NMR Titrations. Proton NMR signals monitoring was conducted on guests **2** and **3**. All solutions were freshly prepared, and deuterated water was buffered with 100 mM Na_2HPO_4. A solution (250 μL) of host (2 mM) was introduced in each NMR tube (12−15 experiments per titration). Increasing aliquots of guest stock solution (∼50 mM) were added, and the total volume (500 μL) was adjusted with buffered D_2O. The titration data ($\Delta\delta$ ppm versus guest concentration) were fitted using the nonlinear curve-fitting procedure. The HypNMR2006 program was employed with (1:1) or (1:2) binding models.[12]

Microcalorimetric Titrations. Isothermal titration calorimetry experiments were performed at 25 °C on a solution of the host (2.5 mL, ca. 10^{-3} M) in buffered water (100 mM Na_2HPO_4) using a glass cell (4 mL). The heats of formation of the complexes were measured after addition of 17 × 15 μL aliquots of the guest (0.15−0.25 M) in the same medium. The enthalpy of complexation (ΔH) and the stability constants (K_{assoc}) were refined simultaneously from these data after correction for the heat of dilution of the guest determined in separate titrations without the host and using the ligand binding analysis program DIGITAM v. 4.1. Each titration was repeated twice at least.

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Supporting Information Available: Characterization of compounds, FTIR spectra, and titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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