A Highly Selective Fluorescent Chemosensor for $H_2PO_4^-$ Based on a Calix[4]arene Tetraamide Derivative

Qi-Yin Chen[a,b] and Chuan-Feng Chen*[a]

Keywords: Calixarenes / Chemosensors / Dihydrogen phosphate / Fluorescence / Sulfonamides

A new neutral fluorescent chemosensor **5** based on a calix[4]arene tetraamide derivative has been synthesized. It exhibits high selectivity for $H_2PO_4^-$ over a wide range of anions, and the selectivity for $H_2PO_4^-$ is more than 2700-fold higher than for F^-. In acetonitrile, the fluorescence intensity of **5** is efficiently enhanced about 130% $[(I - I_0)/I_0]$ upon the addition of 5 equiv. of $H_2PO_4^-$. From the fluorescence titration experiments, the association constant for **5**/$H_2PO_4^-$ (1:2) was calculated to be 5.48×10^9 M^{-2}. Furthermore, the ^1H NMR spectral and X-ray crystal studies suggested that multiple hydrogen-bonding interactions occur between **5** and $H_2PO_4^-$.

Introduction

It is well known that anions play numerous fundamental roles in biological and chemical process,[1,2] therefore a great deal of attention has been paid to the design and synthesis of receptors that are efficient at detecting anions in solution.[3–5] Among all the detection methods, fluorescent chemosensors for anions have appeared to be particularly attractive due to their simplicity, high sensitivity, and high detection limits for trace chemicals detection.[3,6]

On account of their pivotal role in signal transduction, energy storage, and in the genes and hereditary elements in biological systems, phosphate anions are one of the most important constituents of living systems.[7,8] Considerable efforts have been undertaken in the last few decades to design new chemosensors, including chromogenic sensors[9–11] and fluorescent chemosensors,[12–17] for recognition of dihydrogen phosphate anions. However, most of these fluorescent receptors have limitations, including fluorescence quenching,[12–14] low signaling output,[16] the need for polar and unstable organic solvents,[17] and interference from other anions.[12,13b] In some systems, neutral receptors are necessary for the transport of phosphates through the cell membrane; this process is regulated by neutral binding proteins.

Calixarenes are an important class of macrocyclic compounds and are ideal platforms for the development of complexing agents for anions.[17–20] In addition, many effective receptors for anions based on amides have been successfully developed[16,17,19–24] due to their neutral and hydrogen-bond acceptor properties.[24] Compared with amides, sulfonamide-based receptors for anions are rare, even though they have a strong binding ability with anions and are readily available.[25–27] Herein, we report a novel fluorescent chemosensor (**5**) based on a disulfonamide derivative of calix[4]arene, which shows a highly selective and sensitive response towards $H_2PO_4^-$ over other anions under neutral conditions.

Results and Discussion

The synthesis of receptor **5** is depicted in Scheme 1. 1,3-Bis(chlorocarbonyl)-*p*-*tert*-butylcalix[4]arene (**1**)[28] was treated with mono-Boc-protected 1,2-diaminoethane[29] to produce compound **2**,[20] which was then treated with CF_3COOH to give **3**. In the presence of Et_3N under high dilution conditions, the reaction of **3** with anthracene-1,8-disulfonyl dichloride (**4**) afforded the product **5** in 71% yield. In the ^1H NMR spectrum of **5**, the methylene protons of the calix[4]arene appear as two double peaks, which indicates that the calix[4]arene unit adopts a cone conformation. The ^{13}C NMR and MALDI-TOF mass spectra are also consistent with this structure.

Single crystals suitable for X-ray crystallography were obtained from a solution of **5** in CH_2Cl_2/MeCN. The X-ray analysis of **5** (Figure 1) revealed that several intramolecular hydrogen bonds, with O(2)–H···O(1), O(4)–H···O(3), N(1)–H···O(4), N(2)–H···O(5), and N(4)–H···O(5) distances of 1.99, 1.95, 2.20, 2.11, and 2.20 Å, respectively, cause the calix[4]arene skeleton to be in a cone conformation and the cyclic chain to fold into an *S*-like conformation. Moreover, the amide N(3)H proton is positioned outward, which results in the formation of a dimer through two intermo-

[a] Laboratory of Chemical Biology, Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China
Fax: +86-10-6255-4449
E-mail: cchen@iccas.ac.cn
[b] Graduate School of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing 100080, China

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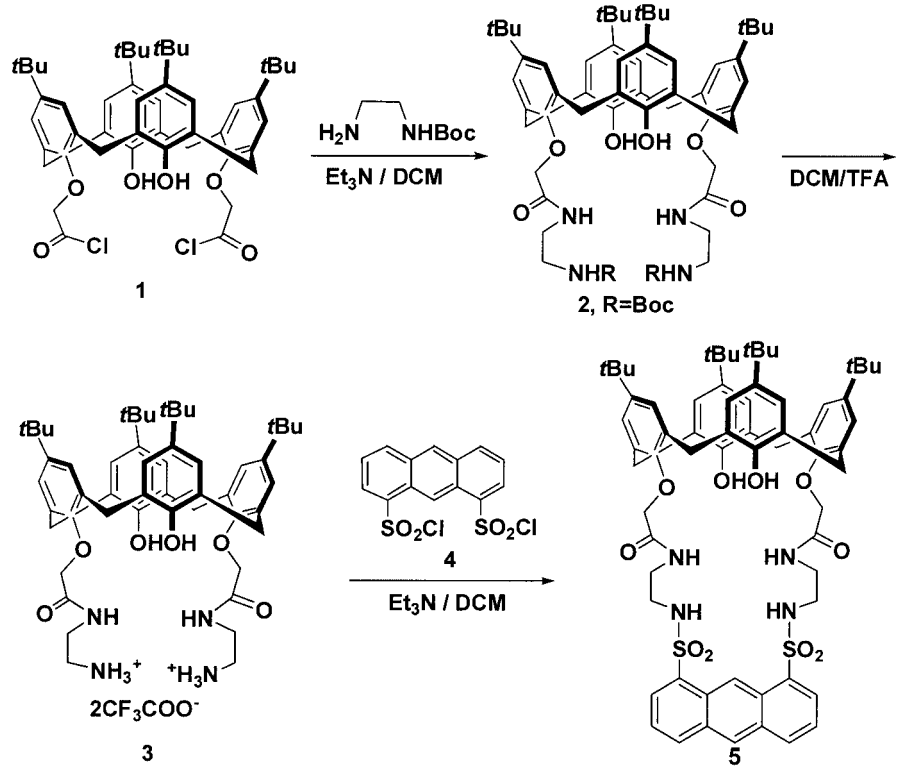


Scheme 1. Synthesis of compound **5**.

lecular hydrogen bonds involving N(3)–H···O′(7) and N′(3)–H···O(7). Thus, a preorganized structure is formed to accommodate incoming anions and binding anions effectively.

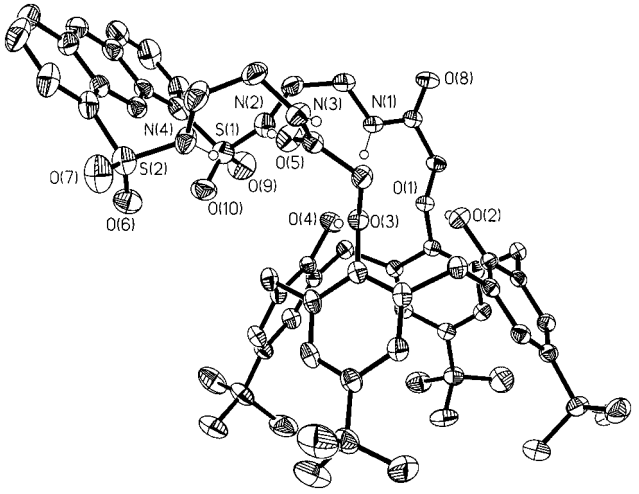


Figure 1. Crystal structure of compound **5**.

All the fluorescence titration experiments were performed in acetonitrile. We first of all investigated the binding properties of **5** towards different anions and found that diverse fluorescence behaviors occur. As shown in Figure 2, compound **5** shows characteristic emission bands, with λ_{max} (432 nm) attributed to the anthracene group in the absence of anions. When 5 equiv. of $H_2PO_4^-$ salts were added to a solution of **5** (1×10^{-5} M), a 134% fluorescence enhancement with a small concomitant blue shift (approx. 5 nm) was observed. This emission enhancement phenomenon is probably due to the inhibition of the PET quenching mechanism by hydrogen binding or an increase of the rigidity of the receptor.[3] Under the same conditions, 5 equiv. of AcO^- or Cl^- made the fluorescence intensity of **5** increase by 52% and 11%, respectively, and F^- and CN^- caused 34% and 4.4% fluorescence quenching of **5**, respectively. In the case of Br^-, I^-, HSO_4^-, and NO_3^- no obvious spectral changes were observed. Even in the presence of a large excess of the anions (up to 100 equiv.), only a very small degree of fluorescence quenching occurred.

The stoichiometry of the **5**/$H_2PO_4^-$ complex was determined by the method of continuous variations (Job's method)[30] (Figure 3). The result obtained from the Job plot unambiguously shows the formation of a 1:2 complex between **5** and $H_2PO_4^-$. Similarly, the stoichiometries of the **5**/F^- and **5**/AcO^- complexes were found to be 1:2 and 2:3, respectively (see Supporting Information). According to the good linear relationship of the titration plots, the stoichiometries of both the **5**/Cl^- and the **5**/CN^- complex were found to be 1:1.[31,32]

With the stoichiometry of the complex in hand, we could then determine the association constants (K_a) between **5** and anions from the fluorescence titration experiments (Figures 4 and 5).[31,33] As a result, K_a for the **5**/$H_2PO_4^-$ (1:2) complex was calculated to be 5.48×10^9 M^{-2} ($R > 0.9999$) from a nonlinear curve-fitting procedure of the fluorescence titration data. Based on the same method, K_a of the **5**/F^- (1:2) complex was calculated to be 2.02×10^6 M^{-2} ($R > 0.993$). The selectivity for dihydrogen phosphate ions is therefore more than 2700-fold higher than for fluoride

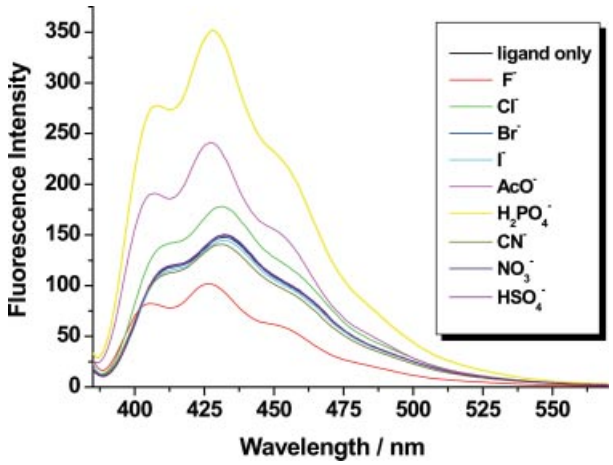


Figure 2. Fluorescent emission changes of **5** (10^{-5} M) upon the addition of various tetrabutylammonium salts (5 equiv.) in MeCN (λ_{ex} = 374 nm).

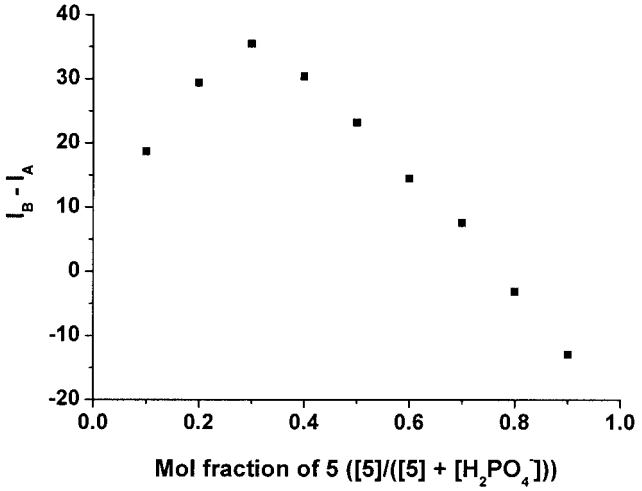


Figure 3. Job plot for the mixtures of **5** and $H_2PO_4^-$ ([**5**] + [G] = 2×10^{-5} M) in MeCN at 432 nm. I_A: fluorescence intensity of **5**; I_B: fluorescence intensity of **5** in the presence of the guest.

ions. Similarly, the K_a values for the **5**/AcO^- (2:3), **5**/Cl^- (1:1), and **5**/CN^- (1:1) complexes were estimated to be 1.26×10^7 $M^{-1.5}$ ($R > 0.9999$), 1.79×10^3 M^{-1} ($R > 0.9999$), and 9.68×10^3 M^{-1} ($R > 0.996$), respectively. From the results of the fluorescence titrations, we can see that **5** has a high sensitivity towards $H_2PO_4^-$ over other anions in neutral solution. Moreover, it can efficiently discriminate $H_2PO_4^-$ from not only F^- and AcO^-, which have similar basicities,[4,11] but also HSO_4^-, which has a similar tetrahedral structure.[12] Therefore, receptor **5** can be considered as a potentially powerful candidate for a practical fluorescent sensor for $H_2PO_4^-$.

In order to look further into the binding properties of receptor **5** with $H_2PO_4^-$, NMR titration experiments were carried out in a mixture of $CDCl_3$ and [D_6]DMSO (10:1, v/v). Partial ^1H NMR spectra of **5** in the absence and presence of anions are shown in Figure 6. Upon gradual addition of the $H_2PO_4^-$ salt to a solution of **5**, the ^1H NMR spectrum displayed dramatic changes (Figure 6). The signals for both two amide protons and two sulfonamide protons disappeared rapidly, even upon addition of only 0.5 equiv. of $H_2PO_4^-$. In the presence of 2 equiv. of $H_2PO_4^-$, the signal for the OH protons shows a significant downfield shift ($\Delta\delta$ = +1.64 ppm). Moreover, the signals for the Ar-CH_2Ar and OCH_2 protons and that at the 9-position of the anthracene moiety all shift downfield, as do the signals of the protons of $H_2PO_4^-$, upon the addition of anion. These facts indicate that the dihydrogen phosphate anion forms multiple hydrogen-bonding interactions not only with the amide and sulfonamide groups, but also with OH and wide-ranging CH protons, which results in the high selectivity and sensitivity of **5** for $H_2PO_4^-$ (Scheme 2). In the case of F^- (see Supporting Information), the chemical shift changes of the amide and hydroxy group protons in **5** are similar to, but obviously smaller than, those of $H_2PO_4^-$, which indicates that the interaction between **5** and F^- is weaker than

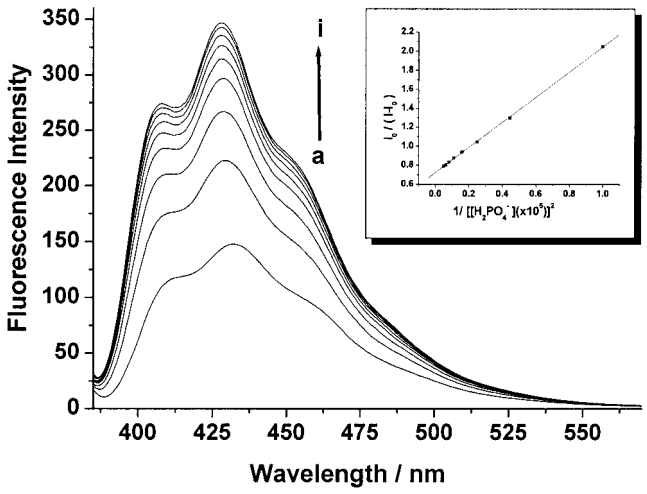


Figure 4. Fluorescent titrations of **5** (10^{-5} M) with $Bu_4N^+H_2PO_4^-$ in MeCN (λ_{ex} = 374 nm). From a → i [$H_2PO_4^-$]: 0, 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 equiv. Inset: plot of $I_0/(I - I_0)$ vs. $1/[H_2PO_4^-]^2$ at 432 nm.

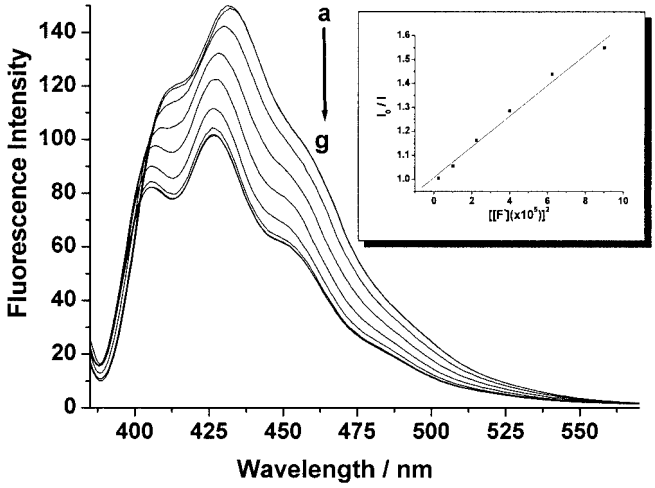


Figure 5. Fluorescent titrations of **5** (10^{-5} M) with $Bu_4N^+F^-$ in MeCN (λ_{ex} = 374 nm). From a →g [F^-]: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 equiv. Inset: plot of I_0/I vs. $[F^-]^2$ at 432 nm.

that between **5** and $H_2PO_4^-$. On the other hand, the signal for the proton at the 9-position of the anthracene moiety ($\Delta\delta$ = +0.25 ppm) shifts more downfield than that of $H_2PO_4^-$, which could be a reason for the different fluorescent changes between **5** and $H_2PO_4^-$ and F^-.

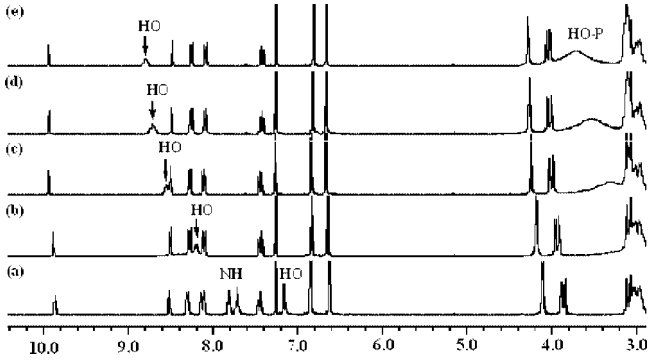
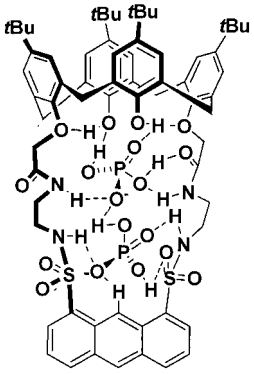


Figure 6. Partial ^1H NMR spectra (300 MHz) of compound **5** (6.32×10^{-3} M): (a) **5** only, (b) **5** + 0.5 equiv. $H_2PO_4^-$, (c) **5** + 1.0 equiv. $H_2PO_4^-$, (d) **5** + 1.5 equiv. $H_2PO_4^-$, (e) **5** + 2.0 equiv. $H_2PO_4^-$.



Scheme 2. Proposed binding mode of **5** with $H_2PO_4^-$.

Conclusions

In conclusion, we have presented a new fluorescence chemosensor, based on a calix[4]arene tetraamide derivative with a disulfonoanthracene group as the fluorophore, which displays not only a remarkable fluorescence-enhancement effect with $H_2PO_4^-$, but also high selectivity and sensitivity towards $H_2PO_4^-$ over other anions, including F^- and AcO^-, which have a similar basicity, and HSO_4^-, which has a similar structure, under neutral conditions. Therefore, **5** may be considered as a potentially practical $H_2PO_4^-$-selective fluorescent chemosensor.

Experimental Section

General Remarks: Solvents were dried and distilled before use according to standard procedures. Melting points were measured with a micromelting-point apparatus and are uncorrected. The fluorescence spectra were measured with a Hitachi F-4500 fluorescence spectrophotometer at 25 °C ($\lambda_{ex.}$ = 374 nm, the excitation and emission slit widths were 10 nm). The fluorescence titrations were performed with a series of 1×10^{-5} M acetonitrile solutions of compound **5**. ^1H and ^{13}C NMR spectra were recorded with a Bruker AM300 (300 MHz, chemical shifts in ppm, *J* in Hz). Mass spectra were obtained by the MALDI-TOF technique. Elemental analyses were performed with a Vario ELIII and a Carlo Erba 1106 analytical instrument.

Compound 2: A solution of **1** (2.28 g, 2.85 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise to a mixture of mono-Boc-protected 1,2-diaminoethane (2.74 g, 17.1 mmol) and triethylamine (8 mL) in dry CH_2Cl_2 (30 mL) in an ice bath. After stirring for 3 h, the solvent was evaporated under reduced pressure and the residue treated with MeOH to afford compound **2** (2.25 g, 75.3%). M.p. 184–186 °C. ^1H NMR ($CDCl_3$): δ = 1.07 (s, 18 H), 1.26 (s, 18 H), 1.34 (s, 18 H), 3.35 (m, 4 H), 3.44 (d, *J* = 13.32 Hz, 4 H), 3.52 (t, *J* = 5.78 Hz, 4 H), 4.17 (d, *J* = 13.32 Hz, 4 H), 4.61 (s, 4 H), 6.97 (s, 4 H), 7.07 (s, 4 H), 8.02 (s, 2 H), 8.97 (s, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 28.3, 31.00, 31.86, 32.17, 33.92, 34.20, 39.30, 40.80, 74.79, 79.27, 125.80, 126.35, 127.32, 132.54, 143.46, 143.66, 148.84, 149.07, 155.87, 168.69 ppm. MALDI-TOF: *m/z* = 1071.7 $[M + Na]^+$. $C_{62}H_{88}N_4O_{10}$ (1048.7): calcd. C 70.96, H 8.45, N 5.34; found C 70.94, H 8.24, N 5.40.

Compound 3: Trifluoroacetic acid (29 mL) was added, at 0 °C, to a solution of **2** (0.8 g, 0.765 mmol) in CH_2Cl_2 (12 mL). The mixture was stirred for 3 h and then concentrated in vacuo. The residue was treated with CH_2Cl_2 to afford compound **3** (0.8 g, 97.3%). M.p. 197–198 °C. ^1H NMR ($CDCl_3$): δ = 0.88 (s, 18 H), 1.19 (s, 18 H), 3.29 (m, 8 H), 3.66 (m, 4 H), 3.98 (m, 4 H), 4.47 (m, 4 H), 6.74 (s, 4 H), 6.99 (s, 4 H), 7.17 (m, 2 H), 8.06 (m, 6 H), 8.99 (m, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 28.68, 29.86, 29.97, 30.54, 32.88, 33.02, 36.21, 38.92, 72.99, 124.60, 125.11, 126.60, 131.07, 142.33, 147.31, 147.73, 148.10, 169.00 ppm. MALDI-TOF: *m/z* = 849.4 $[M - 2\ CF_3COO]^+$. $C_{56}H_{74}F_6N_4O_{10}\cdot 2H_2O$ (1112.6): calcd. C 60.42, H 7.06, N 5.03; found C 60.43, H 7.06, N 4.94.

Compound 5: A solution of **4**[34] (108 mg, 0.287 mmol) in dry CH_2Cl_2 (70 mL) was added dropwise to a mixture of compound **3** (308.5 mg, 0.287 mmol) and triethylamine (0.8 mL) in dry CH_2Cl_2 (800 mL) in an ice bath. After stirring at 0 °C for 2 h and then at room temperature for 5 h, the solvent was evaporated under reduced pressure. The residue was purified by column chromatography to give compound **5** (235 mg, 71.2%). M.p. 228–230 °C. ^1H NMR ($CDCl_3$): δ = 0.82 (s, 18 H), 1.25 (s, 18 H), 3.25 (d, *J* = 13.50 Hz, 4 H), 3.28 (m, 4 H), 3.45 (m, 4 H), 4.10 (d, *J* = 13.50 Hz, 4 H), 4.42 (s, 4 H), 6.50 (m, 2 H), 6.60 (m, 2 H), 6.63 (s, 4 H), 7.02 (s, 4 H), 7.52 (dd, *J* = 7.77, 8.34 Hz, 2 H), 8.14 (m, 2 H), 8.20 (d, *J* = 8.49 Hz, 2 H), 8.32 (d, *J* = 6.99 Hz, 2 H), 8.58 (s, 1 H), 10.00 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 30.88, 31.67, 33.89, 33.94, 39.91, 42.50, 74.99, 77.21, 121.34, 124.59, 125.45, 125.90, 126.38, 128.18, 129.12, 131.74, 131.98, 134.48, 135.11, 142.92, 147.66, 149.56, 150.09, 169.63 ppm. MALDI-TOF: *m/z* = 1151.3 $[M + H]^+$. $C_{66}H_{78}N_4O_{10}S_2$ (1150.5): calcd. C 68.84, H 6.83, N 4.87; found C 68.63, H 6.93, N 4.42.

X-ray Crystallographic Study: $C_{66}H_{78}N_4O_{10}S_2\cdot 3.5CH_3CN$, triclinic, space group $P\bar{1}$, *a* = 12.902(10), *b* = 16.305(13), *c* = 19.187(14)° Å, *α* = 104.224(13), *β* = 108.997(14)°, *γ* = 95.240(13)°, *V* = 3634(5) Å^3, $D_{calcd.}$ = 1.183 Mg m^{-3}, *Z* = 2, *T* = 293(2) K, 0.26 × 0.24 × 0.18 mm. Data collection was carried out with a Bruker–Nonius KappaCCD area detector and SHELXS-97 and SHELXL-97 were used for structure solution and refinement.[35] Of 12781 reflections collected, 6344 were found to be independent [*R*(int) = 0.0331], giving R_1 = 0.0741 for the observed unique reflections [$F^2 > 2\sigma(F^2)$] and wR_2 = 0.275 for all data. CCDC-256437 contains the supplementary crystallographic data for this paper. These data can be obtained

free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see also the footnote on the first page of this article): Fluorescence change ratios of **5** upon the addition of anions. Job plots for **5** and F^- and AcO^-. Fluorescent titrations of **5** with $Bu_4N^+AcO^-$, $Bu_4N^+CN^-$, and $Bu_4N^+Cl^-$. Partial ^1H NMR spectra of compound **5** upon the addition of F^-.

Acknowledgments

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