Boradiazaindacene-Appended Calix[4]arene: Fluorescence Sensing of pH Near Neutrality

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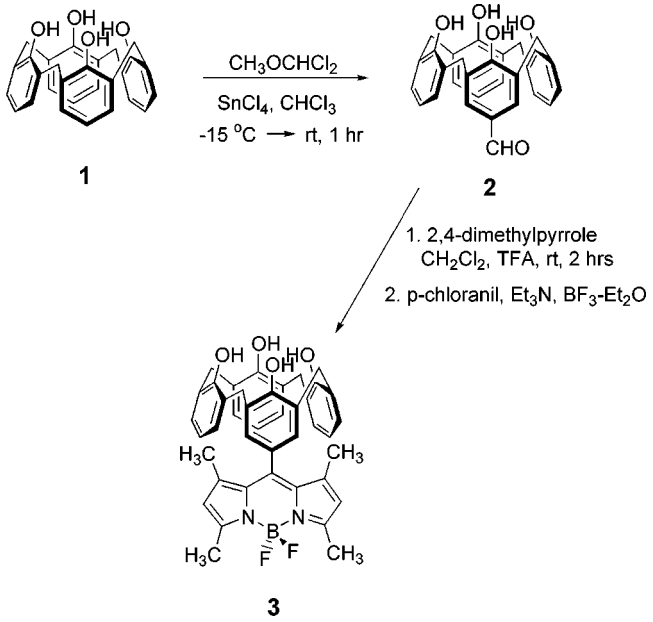
Fluorescent molecular sensors of near neutral pH are an attractive target of supramolecular design.[1] We now report the synthesis of a novel boradiazaindacene appended calix[4]arene which signals pH changes by larger than 10-fold change in the emission intensity at 509 nm. Two-thirds of the total fluorescence enhancement takes place within the pH range of 8.2−5.6.

4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) derivatives are highly fluorescent dyes[2] that have applications in many different areas.[3] In 8-phenylboradiazaindacene derivatives, the phenyl π-system is virtually decoupled from the rest of the molecule especially when the 1 and 7 positions are substituted. Strong PET[4] (photoinduced electron transfer) activity is possible from this orthogonal substituent, creating yet to be explored opportunities for molecular sensing/signaling. 4′-Hydroxyphenyl-,[5] 4′-dialkylaminophenyl-,[6] and 4′-azathiacrownphenyl-substituted[7] boradiazaindacenes all showed interesting PET-mediated fluorescence signals, thus acting as sensors for pH in the first two cases and for soft transition metal cations in the last case. Remarkable photophysical properties of these dyes, like high quantum yield, high extinction coefficient, and narrow emission peak leading to higher peak emission intensity, would be best exploited if the sensing of the targeted analyte can be carried out in a physiologically relevant range. While the 4-hydroxyphenyl[5] derivative senses the alkaline region, the dialkylaminophenyl[6] derivative senses pH changes in the acidic region of the pH scale. Thus, BODIPY-based pH sensing near neutrality remains an important target.

Scheme 1. Synthesis of the Calix[4]arene−BODIPY Conjugate



Calixarenes, on the other hand, proved themselves to be very useful scaffolds[8] in a number of supramolecular designs. Calixarene-based sensors and light harvesting systems were reported in recent years.[9] In one of the earlier examples of calixarene-based ion sensing,[9d] an MLCT luminophore was attached to a calix[4]arene, and at slightly alkaline pH the Ru(II) luminescence was quenched as a result of PET from the calixarene-phenolate ions. In our own effort toward fluorescent ion sensors[1d,10] and molecular logic elements,[11] we are particularly interested in strong fluorescence signals at biologically relevant analyte concentrations. Thus, the boradiazaindacene-appended calix[4]arene (**3**) looked like a promising candidate for the realization of at least some of these goals. We expected that the internal hydrogen bonding leading to altered pK_a values for the phenol−phenolate equilibrium in calix[4]arene would bring the dynamic range of the chemosensor near pH 7.

The compound **3** was synthesized in two steps from the parent calix[4]arene (Scheme 1). Standard formylation

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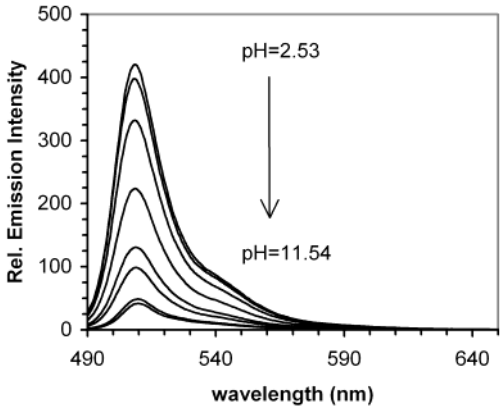

Figure 1. Fluorescence emission spectrum of compound **3** in dilute (2.0 μM) aqueous (50:50 water/ethanol) solutions with different apparent pH: 2.53, 3.34, 5.56, 6.04, 6.44, 7.11, 8.17, 11.54. Excitation was at 480 nm.

procedure of calixarenes with some modifications yielded the monoaldehyde **2**. The BODIPY unit was then assembled[12] using dimethylpyrrole and BF_3-Et_2O. The desired product with intense green fluorescence was purified by silica gel chromatography and preparative TLC (5:1 chloroform−hexane). ^{1}H NMR spectrum of the compound revealed that the AB coupled methylene protons of the calixarene ($ArCH_2Ar$) were very broad due to reduced conformational mobility, in part a result of the intrusion of boradiazaindacene unit into the calixarene cavity.

Interestingly, all four phenolic protons appeared as a singlet suggesting a fast proton exchange at the lower rim. Absorption spectrum showed a sharp peak at 490 nm in 50% ethanol−water, which is invariant at different pH values. The extinction coefficient was determined to be 55 000 M^{-1} cm^{-1}. The emission peak at 509 nm again was unaltered in different pH solutions, but the emission intensity is very much pH dependent. There is a more than 10-fold change in the emission intensity, and two-thirds of this change is within the pH range of 5.6−8.2. The fluorescence signal on pH changes is fully reversible. The analysis of intensity changes as a function of pH by using the Henderson−Hasselbach-type mass action equation[13] yielded one apparent pK_a of 6.5. The first deprotonation is expected to be at the distal phenol unit with a pK_a of 4.5,[9d] but high-efficiency PET takes place only when adjacent phenol groups deprotonate at slightly higher pH. The quantum yield of emission for compound **3** was determined to be 0.67 in ethanol; this yields a remarkable intrinsic brightness[14] value of 37 000. Thus, orthogonal attachment of a BODIPY unit to calix[4]arene affords a fluorescent chemosensor with many desirable qualities. Considering the fact that calixarane solubilities can be modulated to a great extent, we believe compound **3** is likely to serve as a prototype of a novel family of calixarene-based chemosensors for pH. Alternatively, immobilization of **3** on different solid matrixes may lead to LED compatible sensors for pH. Further work along these lines is in progress.

Experimental Section

General Methods. All reagents were purchased from commercial suppliers and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution with TMS as an internal reference at 400 MHz (^{1}H) and 100 MHz (^{13}C). Mass spectra were recorded by electron impact (70 eV). Elemental analyses were performed by TUBITAK Instrumental Analysis Laboratory, Ankara, Turkey. The fluorescence quantum yield of compound **3** was determined in reference to fluorescein, which has a reported[15] quantum yield of 0.925 in 1 M aqueous NaOH.

5-Formylcalix[4]arene-25,26,27,28-tetrol (2). Calix[4]arene (1.2 g, 2.8 mmol) was dissolved in chloroform (30 mL), and the solution was cooled to −10 °C. 1,1-Dichlorodimethyl ether (0.42 g, 3.4 mmol) and $SnCl_4$(3.78 g, 14.5 mmol) were rapidly added. The reaction mixture was then stirred at room temperature for 40 min and quenched with water. The organic layer was separated, washed three times with water, and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The oily residue was purified by silica gel column chromatography (petroleum ether/$CHCl_3$ 8:7). The yield was 0.32 g (26%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.52−3.57 (br m, 4H), 4.19−4.24 (br m, 4H), 6.63−6.71 (m, 3H), 7.01 (m, 7H), 7.52 (s, 2H), 9.71 (s, 1H), 9.91−10.33 (br s, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) 30.4, 30.5, 122.1, 127.3, 127.6, 128.5, 130.3, 130.7, 131.2, 157.1, 158.9, 190.1; MS (EI) *m/e* 452 (M^+). Anal. Calcd for $C_{29}H_{24}O_5$: C, 76.98; H, 5.35. Found: C, 76.83; H, 5.52.

BODIPY−Calix[4]arene (3). Into a solution of 2,4-dimethylpyrrole (0.102 g, 0.69 mmol) and 5-formylcalix[4]arene (0.150 g, 0.335 mmol) in N_2-saturated dichloromethane (100 mL) was added trifluoroacetic acid (6.6 μL, 0.086 mmol). The clear yellow solution was stirred for 3 h at room temperature under N_2. A solution of *p*-chloranil (0.170 g, 0.69 mmol) in dichloromethane (44 mL) was then added. The reaction mixture turned dark red, and the stirring was continued for another 30 min. Then, 100 mL portions of Et_3N and $BF_3{\cdot}Et_2O$ were added until a bright green fluorescence was observed. The solution was then washed with water (4 × 100 mL), and the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The desired product was purified by silica gel column chromatography ($CHCl_3$), and further purification was achieved by preparative TLC ($CHCl_3$/hexane 5:1) The yield was 0.056 g (25%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (s, 6H), 2.42 (s, 6H), 3.2−4.4 (br m, 8H), 5.76 (br s, 2H), 6.61−6.70 (m, 3H), 6.84 (s, 2H), 6.89 (d, 2H), 6.96−7.04 (m, 4H), 9.65 (s, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) 13.5, 30.3, 30.5, 120.0, 120.9, 121.2, 121.8, 126.7, 127.0, 127.1, 127.3, 127.5, 127.6, 127.8, 128.1, 128.5, 130.5, 140.1, 147.7, 147.9, 148.3, 154.1; MS (EI) *m/e* 670 (M+). Anal. Calcd for $C_{41}H_{37}N_2O_4BF_2$: C, 73.44; H, 5.56; N, 4.18. Found: C, 73.13; H, 5.62; N, 4.09.

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