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Function-Oriented Synthesis of a Didesmethyl Triazacryptand Analogue for Fluorescent Potassium Ion Sensing

Richard D. Carpenter^{[a],[b]} and Alan S. Verkman^{[a].*}

^[a]Departments of Medicine and Physiology, University of California, San Francisco, Health Sciences East, Room 1246, Box 0521, 513 Parnassus Ave., San Francisco, CA 94143-0521, USA

^[b]Department of Biomedical Engineering, University of California, Davis, Genome and Biomedical Sciences Facility, 451 Health Sciences Dr., Davis, CA 95616, USA

Abstract

Triazacryptand (TAC)-based fluorescent K⁺ sensors have broad biomedical utility, yet their advancement has been hindered because of their challenging synthesis. Herein, an efficient synthesis is reported that delivers a didesmethyl tri-azacryptand (ddTAC) K⁺ sensor in twofold fewer steps and ninefold higher overall yield than the original TAC synthesis. Our synthesis utilizes a C–O dianionic oxidative macrocyclization and reports new examples of aminoarylations and a microwave route to xanthylum chromophores. The K⁺ sensitivity and selectivity of the ddTAC-based sensor are comparable to the TAC-based sensor.

Keywords

Biosensors; Potassium; Ionophores; Cryptands; Dianionic oxidation

Introduction

Fluorescent potassium (K⁺) ion sensors have great potential utility in the fields of cell biology, neuroscience, drug discovery, and clinical medicine. Figure 1a shows the generic structure of one type of ion sensor, which consists of ionophore, linker, and chromophore domains. With suitable linker and chromophore domains, ion binding to the ionophore region can increase fluorescence of the chromophore by a charge-transfer mechanism.^[1–6] The K⁺ sensor shown in Figure 1b contains two macrocyclic rings and multiple heteroatoms in its triazacryptand (TAC) ionophore and a chromophore with its π system in conjugation with the linker and ionophore regions.^[7–9]

He et al. reported the first TAC K⁺ sensor, yet their sensor contained ionophore and chromophore moieties that were not in π conjugation, and thus it was of limited use.^[10] The key precursor of He et al., TAC-CHO (**2a** in Figure 2), was synthesized in 10 steps (longest linear sequence being 9 steps) and 2% overall yield. In addition to the low overall yield of **2a**, its synthesis was difficult to reproduce, as it required two challenging 21-membered macrocyclization reactions. Nonetheless, on the basis of the route to **2a** reported by He et al.,

we synthesized a series of water-soluble K^+ sensors with different optical and physical properties^[11] and demonstrated their application to measurement of extracellular space K^+ concentration in brain^[12] and airway/ocular surface liquids^[13] and to K^+ transporter drug discovery.^[14] Recently, we reported new ionophore **1** with excellent K^+ sensitivity and selectivity,^[15] yet its synthesis utilizes an ionophore bromide precursor that, compared to TAC-CHO precursor **2a**, limits further chromophore and bioconjugate derivatization.

Our goal here was to develop an alternative, practical synthetic route to an ionophore aldehyde precursor with reduced number of steps and improved overall yield over the original synthetic route to **2a**. After studying the scheme reported by He et al.^[10] we speculated that the bistolyl moieties likely served as blocking groups, thereby enabling regioselective Vilsmeier–Haack formylation to afford **2a**.^[16,17] Therefore, we recognized that removing these remote methyl groups on the TAC ionophore would result in a simplified structure without a predicted effect on K^+ binding. Herein, we report a streamlined synthesis of the functionally comparable didesmethyl triazacryptand (ddTAC) K^+ sensors **3b** and **3c** by using a key template-directed dianionic oxidation reaction to close the alkyl phenyl ether macrocycle. Our efficient and streamlined route to ddTAC should enable the advancement of TAC-based K^+ sensors by using the ddTAC ionophore in place of the TAC ionophore.

Results and Discussion

Our streamlined route maximizes step-economy based on function-oriented synthesis, convergence, and new reaction methodology.^[18,19] The synthesis of ddTAC-CHO **3a**, the key ionophore aldehyde precursor that is subsequently conjugated with various chromophores, began with a metal-mediated bisaminoarylation as outlined in Scheme 1. While metal-mediated aminoarylations, so-called Buchwald–Hartwig reactions,^[20] have been key transformations in the synthesis of anilines, there are no examples, to the best of our knowledge, of the use of β -amino ethers, iodophenols, diamines, or any type of cyclic secondary amine. Given this, the sophistication of both reactants, and the expense of the diazacryptand, optimization reactions were carried out by using morpholine and piperazine as proof-of-principle substrates. During these optimization reactions, it was found that the use of DMA as a solvent, a predissolved 1 N solution of NaOH, 2-iodophenol (2.8 equiv.), catalytic cop-per(I) iodide, and microwave heating to 105 °C for 12 min afforded the desired *N*-arylated products with an average conversion of 79% determined by LC–MS and with complete chemoselectivity. Using these conditions with the desired diazacryptand starting material rapidly afforded bisarylated amine **4** (76% yield; Scheme 1), which represents the only example of a β -amino ether coupled with an iodo-phenol. Of note, this transformation uses commercially available 2-iodophenol in slight excess (2.8 equiv.). Preparation of TAC-CHO **2a** by this route would require 2-iodo-5-methylphenol, which, despite its structural simplicity, adds one or two synthetic transformations from either an electrophilic aromatic iodination of *m*-cresol^[21] or an *o*-lithiation^[22] and subsequent demethylation of 3-methyl-anisole.^[23]

This bisaminoarylation was then followed by a Finkelstein-modified Williamson ether synthesis of bisphenol **4** with *tert*-butyl bis(2-chloroethyl)carbamate to afford mono-*O*-alkylated intermediate **5** with an adorning alkyl chloride motif. Finkelstein-modified Williamson ether synthesis under dilute conditions afforded Boc-protected amine **6**; however, the yields were poor and variable (26±19%; *n* = 3) for this two-step sequence.

This disappointing intramolecular displacement macrocyclization employing a phenoxide anion is, nonetheless, an improvement over a similar transformation that was attempted but ultimately unsuccessful with the imidazole system.^[15] To overcome this unsuccessful displacement reaction with the imidazole system, we recently developed a new template-

directed organodipotassium oxidative macrocyclization to install a C–N bond^[15] that was inspired by Sarpong's organodilithium oxidation.^[24,25] During the course of this work, we discovered that our organodipotassium oxidation conditions could be used to close a variety of rings and make C–N or N–N bonds from either metal–halogen exchange or deprotonation of acidic C–H or N–H.^[15] Although unprecedented, we speculated that organodipotassium oxidation of **5** (structure shown in Scheme 1) would similarly make macrocycle **6**; the phenol would be deprotonated and the alkyl chloride would undergo metal–halogen exchange to produce dianions that are held in close proximity through neighboring chelating groups prior to oxidation to install a C–O bond. Gratifyingly, alkyl chloride **5** was treated with Li⁰ sticks and KO^tBu (Schlosser's conditions) at cryogenic temperature, followed by treatment with diphenyl diselenide and TFA to afford amine **7** (74% over three steps) containing the bismacrocyclic entity of **3a** (Scheme 2).

A series of control experiments were then performed to ensure that this transformation likely goes through a dianionic intermediate and not a nucleophilic displacement. Notably, combining any two of the three reagents (Li⁰, KO^tBu, and diphenyl diselenide) with **5** did not result in any appreciable macrocyclization (i.e., **6**). Therefore, a likely pathway in this reaction involves elemental lithium reducing the phenolic hydrogen and undergoing lithium–halogen exchange, the potassium alkoxide solution for transmetalation and substrate templation, and diphenyl diselenide as an appropriate oxidant in the presence of an organometallic species.

With the construction of bismacrocycle **7** complete, benzaldehyde fragment **8** was synthesized (Scheme 3). The synthesis of the aldehyde fragment began with *O*-alkylation of electron-deficient 3-hydroxy-4-nitrobenzaldehyde under Finkelstein conditions to obtain **9** in 68% yield. Tin(II) chloride reduction of the nitro group^[26] afforded aniline **10** (88%) followed by diazotization to presumably furnish diazonium salt **8**, which was not isolated.

Crude diazonium aldehyde fragment **8** was then cannulated into a chilled DMSO solution (0 °C) of bismacrocyclic fragment **7**. This solution was then warmed to room temperature and, following purification by column chromatography, afforded didesmethylated TAC-CHO **3a** in 83% yield over two steps. This metal-free aminoarylation represents a rare example of a secondary amine reacting with an aryldiazonium salt, as thiols, water, or iodide have been the nucleophiles of choice.^[27] In eight total steps, with the longest linear sequence being five steps, ddTAC-CHO **3a** was synthesized in 28% overall yield from commercially available starting materials.

Having devised an efficient and high-yielding route to ddTAC-CHO **3a**, our attention turned towards the ionophore–chromophore union (Scheme 4). To investigate whether the remote 4-methyl groups on the TAC ionophore affect probe K⁺ sensitivity and selectivity, as well as pH sensitivity, ddTAC-CHO **3a** was conjugated to both xanthylium and BODIPY chromophores to generate sensors **3b** and **3c** (Figure 2). Briefly, aldehyde **3a** was treated with 3-dimethylaminophenol and a catalytic amount of acid followed by oxidation with tetrachloro-1,4-benzoquinone to afford ddTAC-Red (**3b**) in 62% yield from ddTAC-CHO **3a**. Notably, key to the xanthylium chromophore synthesis is the use of microwave heating, which rapidly afforded the desired chromophores >100-fold faster and, remarkably, in 12-fold higher yield than the previously described route.^[10] Similarly, **3a** was treated with 2,4-dimethylpyrrole and a catalytic amount of TFA in a Friedel–Crafts reaction, followed by oxidation with DDQ and treatment with BF₃·OEt₂ to afford K⁺ ddTAC-BODIPY (**3c**) in 58% yield over three steps.

The cation sensitivity and selectivity and the pH sensitivity of ddTAC-Red **3b** and ddTAC-Lime **3c** were determined and compared with those of TAC-based K⁺ sensors **2b/2c**. Sensor

selectivity was assessed via titration with several alkali and alkali earth cations (Figure 3b). ddTAC-Derived sensors **3b/3c** and TAC-derived sensors **2b/2c** showed comparably low sensitivity to Na⁺, Li⁺, Mg²⁺, and Ca²⁺ (data shown only for Na⁺) and comparable modest sensitivity to Rb⁺ and Cs⁺. Finally, pH sensitivity was determined by titration with HCl at different concentrations of K⁺ (Figure 3c). Sensors **2b/2c** and **3b/3c** were comparably insensitive to pH values greater than 6 and sensitive to pH values lower than 6. This is likely due to the presence of multiple Lewis basic heteroatoms that are present in either ionophore.

Conclusions

In conclusion, the didesmethyl modification with two 4-methyl groups removed results in a simplified sensor that retains the fluorescence properties of TAC-based K⁺ sensors, regardless of the chromophore. Functionally simplified precursor ddTAC-CHO **3a** is synthesized in twofold fewer total steps and with a ninefold increase in overall yield compared to the original synthesis of **2a**. Additionally, this report introduces a new C–O bond-forming dianionic oxidative macrocyclization, a much improved ionophore–chromophore union, and new examples of metal-mediated and metal-free aminoarylations. Future efforts will be focused on creating conjugates of **3** for various molecular, cellular, translational, and clinical applications.

Experimental Section

Experiment Setup and Equipment Details

Oxygen- and moisture-sensitive reactions were carried out in flame- or oven-dried glassware sealed with a rubber septum under positive pressure of argon from a balloon. Sensitive liquids and solutions were transferred by syringe or cannula through septa under positive pressure of argon. The term “concentration” refers to rotary evaporation (Büchi) with a vacuum pump at a pressure of 3 Torr. Residual solvents were removed from nonvolatile products on a vacuum line with a pressure of 0.1–0.3 Torr. The temperature of microwave reactions was maintained by using a Biotage microwave reactor, which heated the sealed samples to the indicated temperature in 100–120 s and maintained that temperature for the duration of the reactions. Proof-of-principle reactions, carried out by using morpholine and piperazine to optimize the synthesis of **4**, were analyzed by TLC and LC–MS and were neither purified nor characterized by additional analytical methods.

Reagents, Solvents, and Buffers

Reagents and solvents were purchased from commercial vendors and used without further purification with the exception of the following: Ether and THF were distilled from sodium–benzophenone ketyl under an atmosphere of argon (**CAUTION**: do not distill to dryness). Dichloromethane was distilled from calcium hydride under an atmosphere of argon. Elemental lithium was weighed out in an oven-dried (120 °C) screw-cap vial that was cooled in a desiccator by employing Drierite as the desiccant. TAC-Red and TAC-Lime are described elsewhere.^[12,14] HEPES solution contained (in mM): 145 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 10 D-glucose, and 10 HEPES (pH 7.4). The K⁺-free solution was prepared by replacing 5 mM KCl with NaCl. The 200 mosm/L K⁺-free solution was prepared by removing 50 mM NaCl and 5 mM KCl. The high-K⁺ solution was prepared by replacing 100 mM NaCl with KCl. Other solvents and reagents were used without purification unless otherwise noted.

Chromatography and Purification

Thin-layer chromatography (TLC) used glass plates coated with silica gel 60 F254, developed in a chamber, and visualized under ultraviolet light ($\lambda_{\text{ex}} = 254 \text{ nm}$) as well as by

treatment with iodine or potassium permanganate solution. Column chromatography used silica gel 60 packed in glass columns with indicated eluents. Precipitation was carried out by bubbling in hydrogen chloride (generated from the dropwise addition of concentrated sulfuric acid to solid sodium chloride) into a dropping funnel containing anhydrous ether (ca. 3 h) followed by the dropwise addition of this solution to a concentrated anhydrous ether solution of the amine-containing compound. Following vacuum filtration, the amine-HCl salt was dissolved in sodium hydrogen carbonate and ether and extracted as indicated. Recrystallization was carried out by dissolving the compound with the indicated boiling solvent followed by gradual cooling to ambient temperature or treatment with the indicated nonpolar cosolvent at ambient temperature.

Physical Properties and Spectroscopic Measurements

^1H and ^{13}C NMR spectra were obtained by using Bruker Instruments (300 and 500 MHz for ^1H ; 75 and 125 MHz for ^{13}C). ^1H and ^{13}C NMR spectra were recorded in CDCl_3 and $[\text{D}_6]\text{DMSO}$ with the solvent signals used as a reference (CDCl_3 : 7.26 ppm for ^1H , NMR 77.16 for ^{13}C NMR; $[\text{D}_6]\text{DMSO}$: 2.50 ppm for ^1H , 39.52 ppm for ^{13}C NMR). ^{13}C NMR spectra were recorded with complete hetero-decoupling and with nOe. LC-MS was accomplished by using a Waters Alliance instrument equipped with a Nova-Pak C_{18} column (3.9×150 mm). All yields reported refer to isolated material judged to be homogeneous by TLC and NMR spectroscopy. HRMS was performed at the University of California, Riverside. Fluorescence data shown in Figure 3 was obtained by using a Spex fluorimeter (λ_{ex} = 480 nm for xanthylium chromophores; λ_{ex} = 500 nm for BODIPY chromophores) with all solutions balanced with either sodium chloride or choline chloride in order to keep ionic strength constant at 200 mM.

Sample Handling and Storage

Because derivatives of **2** and **3** bind to K^+ with high affinity, all prepared compounds described herein that contain a cryptand ring should be handled with care. No animal toxicity data exist for these compounds. All sample vials were wrapped in foil, sealed in a plastic bag, and stored at $-20\text{ }^\circ\text{C}$.

2,2'-(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)di-phenol (4)—1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane (700 mg, 2.68 mmol), 2-iodophenol (847 μL , 7.50 mmol), copper(I) iodide (51 mg, 0.268 mmol), and a predissolved solution of 1 N NaOH (2 mL) were dissolved in DMA (8 mL) in a sealable microwave vial. This mixture was flushed with argon, sealed, and microwave irradiated at $105\text{ }^\circ\text{C}$ for 12 min. The reaction mixture was then cooled to ambient temperature, diluted with water (20 mL), and extracted with diethyl ether (3×15 mL). The combined organics were washed with water (50 mL), brine (50 mL), dried (MgSO_4), and concentrated. The concentrate was precipitated by the dropwise addition of concentrated hydrogen chloride in anhydrous ether and filtered. To remove the unwanted HCl salt, the precipitate was taken up in diethyl ether (15 mL) and saturated NaHCO_3 (15 mL). Following separation, the organic layer was washed with brine (15 mL), dried (MgSO_4), and concentrated to afford **4** (1.81 g, 76%) as a light brown solid. ^1H NMR (300 MHz, CDCl_3 , $25\text{ }^\circ\text{C}$): δ = 6.74–6.58 (m, 8 H), 3.62–3.57 (m, 1 H), 2.81, (t, J = 4.8 Hz, 8 H) ppm. ^{13}C NMR (75 MHz, CDCl_3 , $25\text{ }^\circ\text{C}$): δ = 145.3, 135.8, 119.7, 118.9, 115.6, 115.3, 70.2, 49.2 ppm. MS (ESI): m/z = 47.3 [$\text{M} + \text{H}$] $^+$. HRMS (ESI): calcd. for $\text{C}_{24}\text{H}_{35}\text{N}_2\text{O}_6$ [$\text{M} + \text{H}$] $^+$ 447.2490; found 447.2495. Purity was found to be 99% on the basis of HPLC analysis.

tert-Butyl 2-Chloroethyl(2-{2-[16-(2-hydroxyphenyl)-1,4,10,13-tetra-oxa-7,16-diazacycloocta-decan-7-yl]phenoxy}ethyl)carbamate (5)—Diphenol **4** (500 mg, 1.12 mmol), *tert*-butyl bis(2-chloroethyl) carbamate (271 mg, 1.12 mmol), sodium iodide

(16 mg, 0.112 mmol), and sodium carbonate (59 mg, 0.56 mmol) were dissolved in acetone (50 mL). The reaction mixture was stirred for 16 h before dilution with water (40 mL) and extraction with diethyl ether (2 × 30 mL). The combined organics were washed with brine (50 mL), dried (MgSO₄), and concentrated. This residue was crystallized with CHCl₃/petroleum ether and filtered to presumably afford crude **5**, which was quickly used without further purification or characterization.

***tert*-Butyl-2,2'-[2,2'-(1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane-7,16-diyl)-bis(2,1-phenylene)]bis(ethoxy)carbamate (**6**)**

Method A: Lithium sticks (10 mm diameter; 7 mg, 1.00 mmol) were quickly added to a flame-dried flask that was back-filled with argon. THF (5 mL) was added, and the suspension was chilled to −78 °C. After cooling this solution for 10 min, a −78 °C chilled solution of crude alkyl chloride **5** (65 mg, 0.100 mmol) in THF (2 mL) was added via cannula over 5 min. After cooling for 20 min, potassium *tert*-butoxide (1.0 M in THF, 1.00 mL, 1.00 mmol) was added, and the reaction mixture was stirred for an additional 20 min. To this solution was added diphenyl diselenide (312 mg, 0.500 mmol) and the temperature was kept at −78 °C for 2 h, followed by warming the solution to 0 °C (ca. 2.5 h). The reaction was quenched with saturated aqueous ammonium chloride (10 mL) and saturated aqueous sodium bisulfite (10 mL). The resulting solution was extracted with diethyl ether (3×20 mL), and the combined organics were washed with brine (35 mL), dried (MgSO₄), vacuum filtered, and concentrated. This residue was crystallized with CHCl₃/petroleum ether and filtered to afford **6** as a light-brown solid (46 mg, 74% from **4**).

Method B: Crude alkyl chloride **5** (65 mg, 0.100 mmol) was dissolved in acetone (10 mL) and treated with a mixture of sodium iodide (75 mg, 0.500 mmol) and sodium carbonate (53 mg, 0.500 mmol) portionwise over 1 h. Following this, the temperature of the reaction was gradually raised to 50 °C over 2 h and kept at this temperature for 8 h before dilution with water (15 mL) and extraction with diethyl ether (2×15 mL). The combined organics were washed with brine (25 mL), dried (MgSO₄), and concentrated. The crude residue was crystallized with CHCl₃/petroleum ether and filtered to afford **6** as a light brown solid (16±12 mg, 26±19%; *n* = 3). ¹H NMR (300 MHz, CDCl₃, 25 °C): δ = 6.74–6.58 (m, 8 H), 3.61–3.56 (m, 24 H), 2.80 (t, *J* = 4.8 Hz, 8 H), 1.46, (s, 9 H) ppm. ¹³C NMR (75 MHz, CDCl₃, 25 °C): δ = 156.2, 145.1, 135.6, 119.9, 118.9, 115.8, 115.3, 80.0, 70.1, 70.0, 61.6, 52.5, 49.1, 28.4 ppm. MS (ESI): *m/z* = 60.8 [M + H − *t*Bu]⁺. HRMS (ESI): calcd. for C₃₃H₅₀N₃O₈⁺ 616.3592; found 560.2963 (−*t*Bu). Purity was found to be 97% and 99% for methods A and B, respectively, on the basis of HPLC analysis.

2,2'-[2,2'-(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)-bis(2,1-phenylene)]bis(ethoxy)amine (7**)**—Boc-Protected amine **6** was dissolved in TFA/CH₂Cl₂ (1:1, 50 mL), and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated and treated with saturated aqueous sodium hydrogen carbonate. The solid was filtered to afford **7** (428 mg, 74% from **4**) as a light brown solid. ¹H NMR (300 MHz, CDCl₃, 25 °C): δ = 6.73–6.58 (m, 8 H), 3.96 (br. s, 1 H), 3.68 (t, *J* = 5.0 Hz, 4 H), 3.61–3.57 (m, 20 H), 2.84 (t, *J* = 4.9 Hz, 8 H) ppm. ¹³C NMR (75 MHz, CDCl₃, 25 °C): δ = 145.2, 135.7, 119.9, 118.9, 115.8, 115.3, 70.3, 70.1, 61.3, 50.3, 49.7 ppm. MS (ESI): *m/z* = 516.8 [M + H]⁺. HRMS (ESI): calcd. for C₂₈H₄₂N₃O₆⁺ 516.3068; found 516.3061. Purity was found to be 100% on the basis of HPLC analysis.

3-(2-Methoxyethoxy)-4-nitrobenzaldehyde (9**)**—3-Hydroxy-4-nitrobenzaldehyde (1 g, 5.99 mmol), 1-chloro-2-ethoxyethane (684 μL, 7.19 mmol), sodium iodide (1.07 g, 7.19 mmol), and sodium carbonate (733 mg, 7.19 mmol) were dissolved in DMF (70 mL). The reaction mixture was kept at room temperature for 1 h and then heated to 75 °C for 16 h

before dilution with water (40 mL) and extraction with diethyl ether (2×30 mL). The combined organics were washed with brine (50 mL), dried (MgSO₄), and concentrated. This residue was purified by column chromatography (hexane/EtOAc, 95:5) to afford **9** (795 mg, 68 %) as a light yellow solid. ¹H NMR (300 MHz, [D₆]DMSO, 25 °C): δ = 10.0 (s, 1 H), 8.02 (d, *J* = 8.3 Hz, 1 H), 7.58 (d, *J* = 1.6 Hz, 1 H), 7.48 (dd, *J* = 1.5, 8.3 Hz, 1 H), 3.50 (t, *J* = 6.5 Hz, 2 H), 3.34 (t, *J* = 5.3 Hz, 2 H), 3.24 (s, 3 H) ppm. ¹³C NMR (75 MHz, [D₆]DMSO, 25 °C): δ = 192.2, 151.6, 140.9, 139.8, 125.8, 124.2, 119.7, 118.9, 73.8, 60.0, 57.9 ppm. MS (ESI): *m/z* = 226.2 [M + H]⁺. HRMS (ESI): calcd. for C₁₀H₁₂NO₅⁺ 226.0710; found 226.0706. Purity was found to be 98% on the basis of HPLC analysis.

4-Amino-3-(2-methoxyethoxy)benzaldehyde (10)—Nitrobenzene **9** (500 mg, 2.22 mmol) was dissolved in methanol (300 mL) followed by the addition of tin(II) chloride dihydrate (15.01 g, 66.7 mmol). This reaction mixture was heated at reflux for 6 h and then cooled to ambient temperature. The solution was concentrated and then dissolved in ethyl acetate (200 mL). The solution was washed with saturated aq. NaHCO₃ (3 × 100 mL), dried (MgSO₄), and concentrated to afford **10** (795 mg, 68%) as a light yellow solid. ¹H NMR (300 MHz, [D₆]DMSO, 25 °C): δ = 9.70 (s, 1 H), 7.29 (d, *J* = 2 Hz, 1 H), 7.27–7.23 (m, 1 H), 6.92 (d, *J* = 4 Hz, 1 H), 3.49 (t, *J* = 6.5 Hz, 2 H), 3.35–3.32 (m, 2 H), 3.24 (s, 3 H) ppm. ¹³C NMR (75 MHz, [D₆]DMSO, 25 °C): δ = 191.0, 152.1, 145.8, 128.8, 124.4, 115.5, 114.3, 73.8, 60.0, 58.0 ppm. MS (ESI): *m/z* = 196.1 [M + H]⁺. HRMS (ESI): calcd. for C₁₀H₁₄NO₃⁺ 196.0968; found 196.0970. Purity was found to be 97% on the basis of HPLC analysis.

ddTAC-CHO (3a)—A solution of aniline **10** (50 mg, 0.256 mmol) in concentrated HCl (2 mL) was cooled to 0 °C. A solution of NaNO₂ (17 mg, 0.256 mmol) in H₂O (3 mL) was added, and the mixture was stirred at 0 °C for 1 h. This mixture, presumably containing diazonium salt **8**, was added dropwise over 30 min to a solution of **7** (131 mg, 0.256 mmol) in CH₃CN (10 mL) at 0 °C. The reaction mixture was warmed to 25 °C, and the solution was stirred at this temperature for 12 h. The mixture was slowly diluted with saturated aqueous sodium hydrogen carbonate (75 mL) and extracted with diethyl ether (3×100 mL). The combined extracts were washed with brine (100 mL), dried (MgSO₄), and concentrated. This residue was purified by column chromatography (hexane/EtOAc, 90:10) to afford **3a** (147 mg, 83%) as a light-brown solid: ¹H NMR (300 MHz, CDCl₃, 25 °C): δ = 9.69 (s, 1 H), 7.48–7.08 (m, 3 H), 6.79–6.58 (m, 8 H), 3.73–3.48 (m, 24 H), 3.39 (s, 3 H), 2.82–2.74 (m, 12 H) ppm. ¹³C NMR (75 MHz, CDCl₃, 25 °C): δ = 191.0, 155.5, 148.0, 147.5, 145.0, 136.9, 135.5, 128.2, 125.6, 120.1, 119.0, 116.0, 115.8, 115.3, 113.7, 69.4, 68.9, 60.8, 50.8, 49.2 ppm. MS (ESI): *m/z* = 694.5 [M + H]⁺. HRMS (ESI): calcd. for C₃₈H₅₂N₃O₉⁺ 694.3698; found 694.3693. Purity was found to be 98% on the basis of HPLC analysis.

ddTAC-Red (3b)—In a sealable microwave vial, ddTAC-CHO **3a** (15 mg, 21.6 μmol) and 3-dimethylaminophenol (6.5 mg, 47.6 μmol) were dissolved in acetic acid (1 mL). A catalytic amount of *p*-toluenesulfonic acid (PTSA) was then added, and the vial was sealed and microwave irradiated at 130 °C for 5 min. After cooling, the reaction mixture was treated with 3 M sodium acetate in water and the precipitated solid was collected by centrifugation, washed with water and dried, giving a reddish-brown solid. This solid was then dissolved in methanol/chloroform (3:1, 1 mL) and transferred to a sealable microwave vial. Tetrachloro-1,4-benzoquinone (11 mg, 43.2 μmol) was then added, and the vial was sealed and microwave irradiated at 115 °C for 18 min. The reaction mixture was then filtered, and the filtrate was concentrated. The residue was dissolved in a minimal volume of ether, precipitated with a saturated solution of hydrogen chloride in ether, and filtered to afford ddTAC-Red (**3b**) as a dark solid (12 mg, 62% from ddTAC-CHO **3a**). ¹H NMR (500 MHz, [D₆]DMSO/D₂O = 1:1, 25 °C): δ = 6.65–6.52 (m, 9 H), 6.43–6.40 (m, 8 H), 3.48–

3.23 (m, 24 H), 3.21 (s, 3 H), 2.82–2.59 (m, 24 H) ppm. ^{13}C NMR (125 MHz, $[\text{D}_6]\text{DMSO}/\text{D}_2\text{O} = 1:1$, 25 °C): $\delta = 145.8, 144.6, 136.6, 136.1, 120.3, 119.5, 117.8, 115.5, 115.0, 114.7, 74.2, 70.3, 70.0, 60.6, 58.6, 51.4, 49.0, 40.7$ ppm. MS (ESI): $m/z = 465.8$ $[\text{M} + \text{H} - \text{Cl}]^{2+}$. HRMS (ESI): calcd. for $\text{C}_{54}\text{H}_{69}\text{ClN}_5\text{O}_9^+$ 966.4778; found 465.7542 $[\text{M} + \text{H} - \text{Cl}]^{2+}$. Purity was found to be 99% on the basis of HPLC analysis.

ddTAC-Lime (3c)—ddTAC-Lime **3c** was synthesized in 58% yield from ddTAC-CHO **3a** by using the TAC-Lime protocol that is described elsewhere.^[8] ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}/\text{D}_2\text{O} = 1:1$, 25 °C): $\delta = 6.66\text{--}6.52$ (m, 9 H), 6.42–6.39 (m, 8 H), 6.27 (s, 1 H), 5.52 (s, 2 H), 3.51–3.34 (m, 24 H), 3.23 (s, 3 H), 2.62–2.58 (m, 24 H), 2.10 (s, 6 H), 1.94 (s, 6 H) ppm. ^{13}C NMR (125 MHz, $[\text{D}_6]\text{DMSO}/\text{D}_2\text{O} = 1:1$, 25 °C): $\delta = 145.6, 144.4, 136.6, 136.1, 126.9, 126.7, 119.8, 119.6, 118.9, 117.01, 116.96, 115.1, 114.9, 114.7, 113.8, 113.6, 107.21, 107.18, 74.1, 70.10, 70.07, 60.4, 60.3, 58.3, 51.6, 49.1, 13.0, 12.9, 12.2$ ppm. MS (ESI): $m/z = 912.7$ $[\text{M} + \text{H}]^+$. HRMS (ESI): calcd. for $\text{C}_{50}\text{H}_{65}\text{BF}_2\text{N}_5\text{O}_8^+$ 912.4889; found 912.4894. Purity was found to be 100 % on the basis of HPLC analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Hiratani K, Albrecht M. Chem Soc Rev. 2008; 37:2413–2421. [PubMed: 18949114]
2. Metrangola P, Meyer F, Pilati T, Resnati G, Terraneo G. Angew Chem. 2008; 120:6206–6220. Angew Chem Int Ed. 2008; 47:6114–6127.
3. Maeda H. Eur J Org Chem. 2007:5313–5325.
4. Enoki T, Miyazaki A. Chem Rev. 2004; 104:5449–5477. [PubMed: 15535656]
5. McCusker JK. Acc Chem Res. 2003; 36:876–887. [PubMed: 14674779]
6. Rosokha SV, Kochi JK. J Org Chem. 2002; 67:1727–1737. [PubMed: 11895385]
7. Duan X, Liu L, Feng F, Wang S. Acc Chem Res. 2010; 43:260–270. [PubMed: 19954139]
8. Nolan EM, Lippard SJ. Chem Rev. 2008; 108:3443–3480. [PubMed: 18652512]
9. Valeur B, Leray I. Coord Chem Rev. 2000; 205:3–40.
10. He H, Mortellaro MA, Leiner MJP, Fratz RJ, Tusa JK. J Am Chem Soc. 2003; 125:1468–1469. [PubMed: 12568593]
11. Magzoub M, Padmawar P, Dix JA, Verkman AS. J Phys Chem B. 2006; 110:21216–21221. [PubMed: 17048948]
12. Padmawar P, Yao X, Bloch O, Manley GT, Verkman AS. Nat Methods. 2005; 2:825–827. [PubMed: 16278651]
13. Namkung W, Song Y, Mills AD, Padmawar P, Finkbeiner WE, Verkman AS. J Biol Chem. 2009; 284:15916–15926. [PubMed: 19364771]
14. Namkung W, Padmawar P, Mills AD, Verkman AS. J Am Chem Soc. 2008; 130:7794–7795. [PubMed: 18512924]
15. Carpenter RD, Verkman AS. Org Lett. 2010; 12:1160–1163. [PubMed: 20148571]
16. Lellouche JP, Kotlyar V. Synlett. 2004:564–571.
17. Tasneem. Synlett. 2003:138–139.
18. Wender PA, Verma VA, Paxton TJ, Pillow TH. Acc Chem Res. 2008; 41:40–49. [PubMed: 18159936]

19. Wender PA, Miller BL. *Nature*. 2009; 460:197–201. [PubMed: 19587760]
20. Maes BUW, Kosmrlj J, Lemiere GLF. *J Heterocycl Chem*. 2002; 39:535–543.
21. Narender N, Srinivasu P, Kulkarni SJ. *Synth Commun*. 2002; 32:2319–2324.
22. Townsend CA, Bloom LM. *Tetrahedron Lett*. 2001; 22:3923–3924.
23. Coleman RS, Guernon JM, Roland JT. *Org Lett*. 2000; 2:277–280. [PubMed: 10814301]
24. Bisai A, West SP, Sarpong R. *J Am Chem Soc*. 2008; 130:7222–7223. [PubMed: 18479095]
25. West SP, Bisai A, Lim AD, Narayan RR, Sarpong R. *J Am Chem Soc*. 2009; 131:11187–11194. [PubMed: 19591469]
26. Okuyama M, Laman H, Kingsbury SR, Visintin C, Leo E, Eward KL, Stoeber K, Boshoff C, Williams GH, Selwood DL. *Nat Methods*. 2007; 4:153–159. [PubMed: 17220893]
27. Filimonov VD, Trusova M, Postnikov P, Krasnokutskaya EA, Lee YM, Hwang H, Kim H, Chi KW. *Org Lett*. 2008; 10:3961–3964. [PubMed: 18722457]

Figure 1.

(a) Schematic of a generic ion sensor, where ion binding produces an increase in chromophore fluorescence via a charge-transfer mechanism; (b) chemical structure of a K⁺ sensor in a K⁺-bound conformation, showing triazacryptand ionophore (blue), linker (green), and xanthylium chromophore (red) domains.

Figure 2.

Structures of sensors **1**, **2b–e**, and **3b–c**, with **2a** and **3a** serving as key sensor precursors. In this report, sensors **2b/3b** and **2c/3c** are compared in their sensitivity and selectivity for K^+ and their insensitivity to pH.

Figure 3.

(a) Fluorescence emission at 570 nm for xanthylum-derived TAC-Red and ddTAC-Red (2b/3c) and at 533 nm for BODIPY-derived TAC-Lime and ddTAC-Lime (2c/3c) at indicated $[K^+]$. (b) Fluorescence of 2b/3b and 2c/3c in the presence of indicated cations. (c) Fluorescence as a function of pH at indicated $[K^+]$. Samples in (a–c) were excited at 480 nm for xanthylum-derived sensors and 500 nm for BODIPY-derived sensors. Solutions in (ac) were balanced with NaCl or choline chloride to maintain a constant ionic strength of 200 mM.

Scheme 1.
Initial attempts towards bismacrocylic fragment 7.

Scheme 2.

Improved route to bismacrocycle **7** featuring a dianionic oxidation macrocyclization presumably via templating of one or more K⁺ ions. The structures of **5–7** are shown in Scheme 1.

Scheme 3.
Synthesis of ddTAC-CHO **3a**.

Scheme 4.

Synthesis of ddTAC-Red (**3b**) and ddTAC-Lime (**3c**). Reagents and conditions: (a) 3-(dimethylamino)phenol, PTSA, propionic acid, MW, 130 °C, 5 min; (b) tetrachloro-1,4-benzoquinone, MeOH/CHCl₃, MW, 115 °C, 18 min; (c) 2,5-dimethylpyrrole, TFA, CH₂Cl₂; (d) BF₃·OEt₂, DIEA, CH₂Cl₂; (e) DDQ, CH₂Cl₂.