Chromogenic Diaza-Crown Ether Dicarboxylic Acids for Determination of Calcium Ions

Richard A. Bartsch,*,† Eddy Chapoteau,‡ Bronislaw P. Czech,*,‡ Jan Krzykawski,† Anand Kumar,‡ and Thomas W. Robison†

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas 79409, and Diagnostics Division, Miles Inc., Tarrytown, New York 10591

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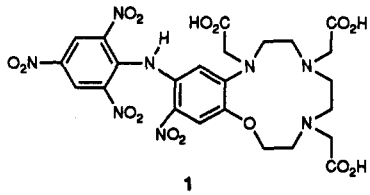
The synthesis of two novel calcium chromoionophores **2** and **3**, which are based on a benzodiaza-crown ether with an inward-facing methoxyl group and bear two acetic acid groups and a 2,4,6-trinitroanilino chromophore, is described. Calcium and magnesium responses at pH optima are determined. Compound **2** exhibits a 31-nm bathochromic shift of the absorption maximum, an increase in the absorptivity upon complexation with Ca^{2+}, and good selecitivity for Ca^{2+} over Mg^{2+} at pH 10.3. The response of chromoionophore **2** is linear from 0 to 8×10^{-3} M Ca^{2+}.

Introduction

Current colorimetric methods for the determination of Ca^{2+} in blood serum, which are based on acyclic chromogenic compounds, such as *o*-cresolphthalein complexone or Arsenazo III, suffer from deficiencies related to short reagent stability, sensitivity to carbon dioxide, and various interferences. In the past Ca^{2+} selective, chromogenic compounds derived from crown ether phenols[1-3] and calixarenes,[4] for use in extraction photometry and optical fiber sensors, were reported. Due to our interest in the application of macrocylic chromoionophores in clinical diagnostics, we have examined the possibility of designing a macrocyclic chromogenic compound which would provide a new reagent with improved characteristics for Ca^{2+} determination. Recently,[5] we have reported the 12-membered triazaoxamacrocycle-*N,N',N''*-triacetic acid **1** which exhibited very high selectivity of Ca^{2+} over Mg^{2+}. In this paper we present a different approach which produced two chromogenic compounds **2** and **3**. An



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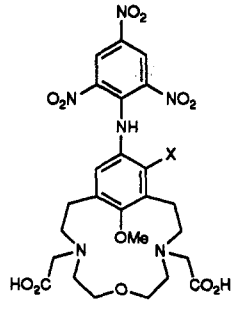
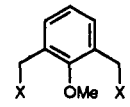


	X
2	H
3	NO_2

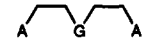
inward-facing methoxyl group is incorporated into a benzodiaza-14-crown-4 macrocycle which bears two acetic acid groups and a 2,4,6-trinitroanilino chromophore. The presence of an intraannular methoxyl group should facilitate interaction between the chromophore and the bound cation, thus providing good sensitivity. The additional nitro group in compound **3** is intended to increase the N–H group acidity of the 2,4,6-trinitroanilino substituent located ortho to it.

Results and Discussion

Synthesis. The initial route to cyclic diamine **16** involved cyclization of diacid chloride **4**[6] with commercially available bis(2-aminoethyl) ether (**8**) to form cyclic diamide **15** (50%), followed by reduction with diborane in THF to provide diazacrown ether **16** in 66% yield. The second route to **16** utilized reduction of dinitrile **5**[6] to diamine **6** with $BH_3 \cdot Me_2S$ in THF.[7] The relatively unstable diamine



	X
4	C(O)Cl
5	CN
6	CH_2NH_2
7	CH_2NHTs



	A	G
8	NH_2	O
9	OMs	O
10	OH	NTs
11	OMs	NTs
12	N_3	NTs
13	NH_2	NTs
14	OTs	NTs

6 was reacted directly with tosyl chloride to give disulfonamide **7** in an overall 85% yield. Cyclization of **7** by reaction with the dimesylate of diethylene glycol (**9**)[8,7] and Cs_2CO_3 in DMF produced cyclic sulfonamide **17** in 63%

† Texas Tech University.
‡ Miles, Inc.
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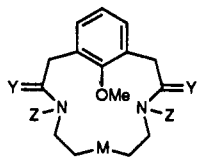
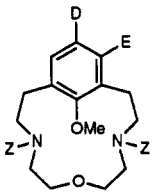
yield. Attempts to detosylate 17 with concentrated H_2SO_4 at 100 °C resulted in total decomposition. Detosylation of 17 was accomplished with 6% sodium amalgam and Na_2HPO_4 in dioxane–MeOH solvent to afford cyclic diamine 16 in 98% yield. Presumably, the relief of steric strain in the rigid cyclic structure of 17 rendered the detosylation surprisingly facile.



	M	Y	Z
15	O	O	H
16	O	H_2	H
17	O	H_2	Ts
18	NTs	O	H
19	NTs	H_2	Ts
20	NTs	H_2	H

The ease with which disulfonamide 17 could be detosylated was an early indication of the difficulty that would be encountered in alkylating the amine nitrogen atoms. Reaction of cyclic diamine 16 with ethyl bromoacetate and K_2CO_3 in DMF gave only moderate yields (38–66%) of diethyl ester 21. In an alternative approach, ester 21 was obtained in 91% yield by reaction of the diamine with NaH in DMF, followed by the addition of ethyl bromoacetate.



	D	E	Z
21	H	H	CH_2CO_2Et
22	NO_2	H	CH_2CO_2Et
23	NH_2	H	CH_2CO_2Et
24	NH-picryl	H	CH_2CO_2Et
25	NH-picryl	NO_2	CH_2CO_2Et
26	H	H	CH_2CO_2Me
27	NO_2	H	CH_2CO_2Me
28	NH_2	H	CH_2CO_2Me
29	NH-picryl	H	CH_2CO_2Me
30	NH-picryl	NO_2	CH_2CO_2Me
31	H	H	CH_2CO_2H

Nitration of 21 required more rigorous conditions than one would expect for an anisole derivative. Examination of CPK space-filling models suggests strong steric interaction between the macrocyclic ring and the methyl group of the intraannular methoxyl oxygen in 21. This reduces the effective overlap between the nonbonding electron pairs on the oxygen and the π system of the aromatic ring which in turn reduces reactivity of the compound for electrophilic aromatic nitration. Such reduced reactivity in substituted anisoles has been observed before.[9] Attempts to nitrate diester 21 with fuming HNO_3 in $CHCl_3$ at 0 °C and at room temperature yielded only the recovered substrate. Similar results were obtained with NO_2BF_4. However, at 70 °C reaction with NO_2BF_4 in nitromethane produced the nitrated compound 22 in 58% yield.

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Reduction of the nitro group in 22 with H_2 and 10% Pd/C in EtOH gave the substituted aniline 23 which was reacted with picryl chloride to afford chromogenic diester 24 (64% overall after two steps) as the potassium salt. Hydrolysis of 24 with KOH in 95% EtOH–dioxane gave chromogenic dicarboxylic acid 2 in 85% yield as the dipotassium salt.

There was an interest in lowering the working pH of chromogen 2. This could be accomplished by the introduction of a nitro group ortho to the chromogenic function. The acidity of the amine should be increased significantly due to resonance interactions with the *o*-nitro group. Examination of a CPK model, however, indicated that that the *o*-nitro group might lack the ability to freely rotate about the C–N bond due to steric congestion. Therefore, the feasibility of nitrating 24 and the effectiveness of lowering the pK_a of the amine proton were uncertain.

Diester 24 was treated with fuming HNO_3 in $CHCl_3$ at 50 °C to afford nitrated diester 25 in 70% yield. Attempted hydrolysis of the nitrated dimethyl ester 24 provided some unexpected results. Mild conditions (5 equiv of KOH, 3–4 h, room temperature) led to partial hydrolysis, whereas extended reaction times resulted in decomposition. Therefore, it was decided to prepare the nitrated dimethyl ester 30 which should undergo hydrolysis more readily. Alkylation of diamine 16 with methyl bromoacetate which gave dimethyl ester 26 was followed by nitration to produce 27 in 81% yield. Reduction of 27 to the corresponding aniline 28 and coupling with picryl chloride afforded chromogenic diester 29 in 53% yield for the two-step process. Nitration to form 30 was accomplished in 58% yield with fuming HNO_3. Hydrolysis of diester 30 under mild conditions using KOH in 95% EtOH at room temperature produced chromogenic nitrated diacid 3 in 86% yield as a hydrochloride salt. The nonchromogenic diaza crown ether dicarboxylic acid 31 was obtained by basic hydrolysis of diethyl ester 21 in 72% % yield. An attempt was made to prepare cyclic triamine 20 which would have three ring nitrogens for attachment of ionizable side arms. Treatment of N-tosyl diethanolamine (10)[10] with mesyl chloride gave dimesylate 11[11] in quantitative yield. The reaction of 11 with NaN_3 under phase-transfer catalysis conditions produced diazide 12 in 53% yield. Reduction of 12 with $LiAlH_4$ afforded the mono-protected triamine 13.[12] High dilution cyclization of 13 with diacid chloride 4 gave only a 21% yield of diamide 18. In view of the observed instability of triamine 13 and the low cyclization yield, an alternative route was explored. Cyclization of disulfonamide 7 by reaction with ditosyl sulfonamide 14[10] and Cs_2CO_3 in DMF gave a 47% yield of cyclic trisulfonamide 19. The only attempt to obtain cyclic triamine 20 by detosylation of 19 involved the use of concentrated H_2SO_4 at 100 °C and led to total decomposition.

New compounds were characterized by IR and ^1H NMR spectra and by elemental analysis. Structural rigidity in macrocyclic diamides 15 and 18 was evident from their ^1H NMR spectra. For both compounds the diastereotopic benzylic protons appeared as AB patterns in the 300-MHz ^1H NMR spectra. For ether diamide 15, the four absorptions were at 3.246, 3.295, 3.731, and 3.780 ppm with a calculated[13] chemical shift difference of 144 Hz and a

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Table 1. UV-vis Spectral Characteristics of Chromogenic Compounds 2 and 3

compd	form[a]	λ_{max}, nm	$\epsilon(\lambda_{max})$
2	HL	375	10 500
	L^-	446	17 700
3	HL	358	9600
	L^-	436	19 600

[a] HL is the nonionized ligand in 0.10 M HCl, and L^- is fully ionized ligand in 1.0 M (TMA)OH.

geminal coupling constant of 14.8 Hz. For tosylamide diamide 18, the four peaks were at 3.309, 3.357, 3.898, and 3.916 ppm with a calculated chemical shift difference of 167 Hz and a geminal coupling constant of 14.2 Hz. If one assumes that a larger chemical shift difference results from slower interconversion of the benzylic hydrogens on the NMR time scale, then the ring tosylamide diamide 18 has greater rigidity than that of ether diamide 15.

Spectral Characteristics and Cation Responses of Chromogenic Compounds 2 and 3. Wavelength maxima (λ_{max}) and molar absorptivities (ϵ) of the acid and base forms of chromogenic compounds 2 and 3 are recorded in Table 1. To suppress ionization of the N–H bond and thereby obtain the spectrum for HL, the nonionized form of the ligands, the absorbance was determined in 0.1 M HCl. Likewise, full ionization of the N–H bond was ensured by use of 0.1 M tetramethylammonium hydroxide, (TMA)OH, to obtain the L^- form. The chromophore system of compound 2 exhibited an absorption maximum for the acid form at 375 nm which upon full ionization shifted bathochromically to 446 nm with an increase in the molar absorptivity. The acid form of compound 3 had its maximum at 358 nm, which upon ionization shifted to 436 nm with more than a 2-fold increase in the molar absorptivity.

At pH 10.3, chromoionophore 2 exhibits very high selectivity for Ca^{2+} over Mg^{2+} (Table 2). Complexation of Ca^{2+} is accompanied by a 31-nm bathochromic shift and an increase in absorptivity (Figure 1). Furthermore, ligand 2 becomes more acidic by nearly 0.5 pK_a units in the presence of Ca^{2+} (Table 2). As shown in Figure 2, the response of chromoionophore 2 to Ca^{2+} is linear over the entire clinical range from 0 to 4×10^{-3} M Ca^{2+}.

For compound 3, which contains an additional nitro group located ortho to the picrylamino function, the pK_a value is 2.9 units lower than that for 2. However, the presence of Ca^{2+} affects neither the absorption maximum nor pK_a and has only a very slight influence on the absorptivity (Table 2). One of two possible explanations for the contrasting behavior of 3 and 2 is that the incorporation of a fourth nitro group makes the chromophore system of the former so electron deficient that the oxygen of the methoxyl group loses its metal-sensing ability. Alternatively, the examination of CPK space-filling molecular models suggests that the additional nitro group may exert a steric effect and prevent a planar arrangement of the anion which is formed by ionization of the chromophore, reducing the resonance interaction.

From the linear relationship shown in Figure 2, the sensitivity of chromoionophore 2 to Ca^{2+} is calculated to be 15 milliabsorbance units per mmol of Ca^{2+}. This is considerably lower than would be anticipated from the pK_a of 0.5 and the 31-nm spectral shift noted for chromoionophore 2 in the presence of Ca^{2+}. To probe the reason for this low sensitivity, binding of Ca^{2+} by the model nonchromogenic diaza-crown ether dicarboxylic acid 31 was measured in four buffered alkaline aqueous solutions at 25 °C. The stability constants, which were determined by potentiometry with a Ca^{2+}-selective electrode, are presented in Table 3. Stability constants for complexation of Ca^{2+} are found to increase as the pH is enhanced from 8.4 to 11.0. However, even at the most alkaline pH, the binding of Ca^{2+} by model compound 31 is weak. Therefore, it seems reasonable to ascribe the low sensitivity of chromoionophore 2 to weak binding of Ca^{2+}.

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Experimental Section

General Methods. Melting points were determined on a Fisher Johns melting point apparatus and are uncorrected. IR spectra were obtained with Nicolet MX-S FT-IR spectrophotometer. ^1H NMR spectra were recorded with Bruker 200- and 300-MHz spectrometers, and chemical shifts are reported in parts per million (δ) downfield from TMS. The UV–vis spectra were recorded on a Beckman DU-8 spectrophotometer. An Orion 601-A digital ion analyzer was used in the pH measurements. Combustion analysis was performed by Galbraith Laboratories (Knoxville, TN) and Desert Analytics (Tuscon, AZ).

Materials. Unless specified otherwise, reagent-grade reactants and solvents were used as received from chemical suppliers. Compounds 4 and 5 were available from an earlier work.[6] Compounds 9,[8] 10,[10] and 14[10] were prepared according to literature procedures.

Cyclic Diamide 15. Solution A (30 mL) containing diacid chloride 4[6] (0.56 g, 2.10 mmol) in THF and solution B (30 mL) containing bis(2-aminoethyl) ether (8, 0.22 g, 2.10 mmol) and triethylamine (0.87 g, 8.56 mmol) were added simultaneously to rapidly stirred THF (200 mL). After being stirred for 12 h, the reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was chromatographed on alumina with CH_2Cl_2 followed by MeOH as eluents to give diamide 15 (0.31 g, 50%) as a white, fluffy solid with mp 158–161 °C. IR (deposit from $CDCl_3$ solution on a NaCl plate): 3375 (NH), 1666 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.10–3.40 (m, 10H), 3.76 (d, 2H), 3.80 (s, 3H), 6.31 (br s, 2H), 7.00–7.20 (m, 3H). Anal. Calcd for $C_{15}H_{18}N_2O_4$: C, 62.06; H, 6.25. Found: C, 62.48; H, 6.56.

Diaza-Crown Ether 16. Method A. BH_3·Me_2S complex (2 M) in THF (8.0 mL, 16 mmol) was added dropwise to a solution of cyclic diamide 15 (0.59 g, 2.00 mmol) in THF (10 mL), and the solution was refluxed for 9 h. Water (15 mL) was added slowly, the solvent was removed in vacuo to give a white solid to which was added 6 N HCl (10 mL) and water (10 mL), and the mixture was refluxed for 12 h. Aqueous NH_4OH was added to pH 10. The aqueous solution was extracted with CH_2Cl_2 and dried (Na_2CO_3). Evaporation of the solvent in vacuo gave cyclic diamine 16 (0.35 g, 66%) as a light yellow oil. IR (neat): 3343 (NH) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.58 (br s, 2H), 2.49–2.76 (m, 8H), 3.11–3.25 (m, 4H), 3.45–3.49 (m, 4H), 3.71 (s, 3H), 7.00–7.13 (m, 3H). Anal. Calcd for $C_{15}H_{24}N_2O_2$: C, 68.15; H, 9.15. Found: C, 67.84; H, 9.40.

Method B. Cyclic disulfonamide 17 (1.00 g, 1.75 mmol), Na_2HPO_4 (1.04 g, 7.33 mmol), and 6% sodium amalgam (10.48 g, 27.34 mmol) were combined and brought to reflux in a 4:3 MeOH–dioxane solvent mixture (35 mL) for 12 h. The solvent was removed in vacuo, and CH_2Cl_2 was added to the residue. The insoluble solids were filtered, and the filtrate was passed through a short bed of alumina with CH_2Cl_2–MeOH (19:1) as eluent to afford 16 (0.45 g, 98%) as a colorless oil.

2,6-Bis[2′-(*p*-toluenesulfonamido)ethyl]anisole (7). To a refluxing solution of dinitrile 5[6] (0.93 g, 5.00 mmol) in THF (10 mL) was added 10 M BH_3·Me_2S complex (3.3 mL, 33 mmol), and the solution was refluxed for 1 h. After 6 N HCl (6 mL) was added slowly, the mixture was stirred at reflux for 14 h. After the mixture was cooled in an ice bath, 25% aqueous NaOH (20 mL) was added while the temperature was maintained below 20 °C. Tosyl chloride (2.38 g, 12.5 mmol) was added, and the reaction mixture was stirred at reflux for 4 h. The mixture was cooled,

Table 2. Spectral Responses of Chromogenic Compounds 2 and 3 to Ca^{2+} and Mg^{2+} at the pH Optimum[a] and Their pK_a Values at 25 °C

compd	optimum pH	form[b]	λ_{max}, nm	$\epsilon(\lambda_{max})$	pK_a[c]
2	10.3[d]	L	401	10 000	10.58 ● 0.05
		CaL	432	11 700	10.11 ± 0.10
		MgL	402	10 100	10.53 ● 0.08
3	10.0[d]	L	436	15 500	7.65 ● 0.03
		CaL	436	16 500	7.64 ± 0.03
		MgL	436	15 500	7.64 ● 0.01

[a] The pH at which the difference in response of the ligand to a large excess of Ca^{2+} versus Mg^{2+} is maximized. [b] L is the uncomplexed ligand; CaL and MgL are the compound in the presence of a large excess of Ca^{2+} and Mg^{2+}, respectively. [c] pK_a values are averaged of three determinations ± standard deviation. [d] The buffer was 0.10 M CAPS (3-(cyclohexylamino)-1-propanesulfonic acid) adjusted with (TMA)OH.

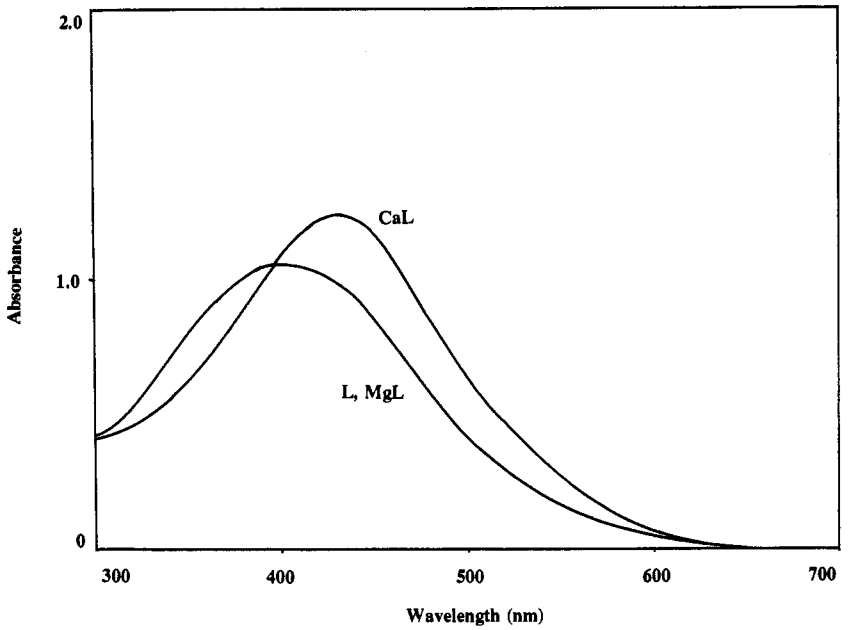

Figure 1. UV-vis spectra for chromogenic compound 2 (L) and for calcium (CaL) and magnesium (MgL) responses in water at pH 10.3.

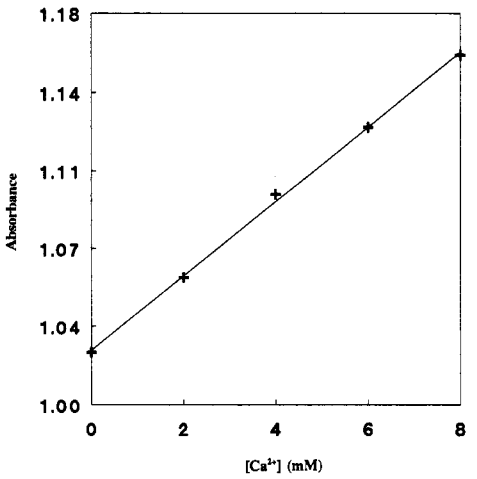

Figure 2. Linear relationship between the absorbance of the calcium complex of 2 and the concentration of Ca^{2+} at pH 10.3.

and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4). The solvent was removed in vacuo, and the residue was chromatographed on silica gel with CH_2Cl_2–EtOAc (19:1) followed by CH_2Cl_2–MeOH (99:1) as eluents to give diamide 7 (2.15 g, 85%) as a viscous, light yellow oil. IR (deposit from $CDCl_3$ solution on a NaCl plate): 3278 (NH), 1325, 1158 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.40 (s, 6H), 2.77 (t, 4H), 3.19 (q, 4H), 3.62 (s, 3H), 4.96 (t, 2H), 6.93 (m, 3H), 7.25 (d, 4H), 7.88 (d, 4H). Anal. Calcd for $C_{25}H_{30}N_2O_5S_2$: C, 59.65; H, 6.01. Found: C, 59.44; H, 5.93.

Table 3. Effect of pH on Complexation[a] of Ca^{2+} by Diaza-Crown Ether Diacid 31

pH	8.4	9.9	10.6	11.0
buffer[b]	CHES	CHES	CAPS	CAPS
K_s (M^{-1})	22	1.4×10^2	3.9×10^2	7.1×10^2

[a] Stability constants (K_s) were determined by potentiometry with a Ca^{2+}-selective electrode. [b] CHES is 0.10 M 2-(cyclohexylamino)-ethanesulfonic acid adjusted with (TMA)OH; CAPS is 0.10 M 3-(cyclohexylamino)-1-propanesulfonic acid adjusted with (TMA)OH.

Cyclic Disulfonamide 17. A solution of ditosylamide 7 (1.22 g, 2.43 mmol) and dimesylate 9 (0.64 g, 2.43 mmol) in DMF (20 mL) was added at a rate of 1.1 mL/h with a syringe pump to a rapidly stirred suspension of Cs_2CO_3 (1.74 g, 5.35 mmol) in DMF (100 mL) at 75 °C. After the addition was completed, the reaction mixture was stirred for an additional hour at 75 °C. The solvent was removed in vacuo, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was separated and dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was chromatographed on silica gel with CH_2Cl_2–EtOAc (49:1) as eluent to afford 17 (0.88 g, 63%) as a fluffy, white solid with mp 72–73 °C. IR (deposit from $CDCl_3$ solution on a NaCl plate): 1339, 1159 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.41–2.49 (m, 8H), 2.62–2.72 (m, 2H), 2.86–2.95 (m, 2H), 3.02 (m, 2H), 3.14–3.21 (m, 4H), 3.43–3.63 (m, 4H), 3.65 (s, 3H), 7.01–7.06 (t, 1H), 7.18 (d, 4H), 7.69 (d, 4H). Anal. Calcd for $C_{29}H_{36}N_2O_6S_2$: C, 60.81; H, 6.34. Found: C, 61.06; 6.29.

Diaza-Crown Ether Diethyl Ester 21. Method A. A mixture of cyclic diamine 16 (0.32 g, 1.21 mmol), ethyl bromoacetate (0.81 g, 4.84 mmol), and K_2CO_3 (0.67 g, 4.84 mmol) in acetonitrile (20 mL) was stirred at 45 °C for 12 h. The solvent was removed in vacuo, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was separated, dried (Na_2-

SO_4), and evaporated in vacuo. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (93:7) as eluent to give **21** (0.35 g, 66%) as a light yellow, viscous oil. IR (neat): 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.27 (t, 6H), 2.40–2.53 (m, 4H), 2.70–3.02 (m, 10H), 3.12–3.17 (m, 2H), 3.46 (dd, 4H), 3.69 (s, 3H), 4.15 (q, 4H) 6.96–7.05 (m, 3H). Anal. Calcd for $C_{23}H_{36}N_2O_6$: C, 63.28; H, 8.31. Found: C, 63.51; H, 8.26.

Method B. Sodium hydride (0.18 g of 60% dispersion in mineral oil, 4.54 mmol) was added to a solution of cyclic diamine **16** (0.40 g, 1.51 mmol) in DMF (20 mL), and the mixture was stirred for 30 min. Ethyl bromoacetate (0.55 g, 3.30 g) was added in one portion, and the mixture was stirred at room temperature for 24 h. The reaction was quenched by addition of several drops of water, and the solvent was removed in vacuo. The residue was partitioned between CH_2Cl_2 and water. The organic layer was separated, dried (Na_2SO_4), and passed through a bed of alumina with CH_2Cl_2–MeOH (98:2) as eluent to afford **21** (0.60 g, 91%) as a light yellow oil.

Nitrated Diaza-Crown Ether Diethyl Ester 22. A solution of diester **21** (0.28 g, 0.64 mmol) in nitromethane (10 mL) was heated to 70 °C, and nitronium tetrafluoroborate (0.42 g, 3.20 mmol) was added in one portion. The solution was stirred for 2 h and cooled in an ice bath, and a small amount of K_2CO_3 was added followed by several drops of water. The solvent was removed in vacuo, and CH_2Cl_2 was added to the residue. The mixture was filtered, and the filtrate was evaporated in vacuo. The residue was chromatographed on alumina with CH_2Cl_2–MeOH (96:4) as eluent to give diester **22** (0.18 g, 58%) as a dark orange oil. IR (neat): 1732 (C=O); 1519, 1341 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.29 (t, 6H), 2.45–3.20 (m, 16H), 3.46 (dd, 4H), 3.76 (s, 3H), 4.14 (q, 4H), 7.95 (s, 2H). Anal. Calcd for $C_{23}H_{35}N_3O_8$: C, 57.37; H, 7.33. Found: C, 57.70; H, 7.38.

2,4,6-Trinitroanilino Diaza-Crown Ether Diethyl Ester 24. A catalytic amount of *p*-toluenesulfonic acid and 10% Pd/C (0.04 g) were added to a solution of the nitrated diethyl ester **22** (0.39 g, 0.81 mmol) in 95% EtOH, and the mixture was shaken under 50 psi of hydrogen for 24 h. The catalyst was removed by filtration, and the solvent was removed in vacuo to give an oil which was filtered through a bed of alumina with CH_2Cl_2–MeOH (98:2) as eluent to produce slightly impure amine **23** (0.40 g) as an oil which was used in the next step without further purification. ^1H NMR ($CDCl_3$): δ 1.28 (t, 6H), 2.31–3.69 (m, 23H), 4.15 (q, 4H), 6.37 (s, 2H).

Amine **23** (0.40 g) was dissolved in MeOH (10 mL), and picryl chloride (0.28 g, 1.13 mmol) and anhydrous K_2CO_3 (0.16 g, 1.13 mmol) were added. The mixture was stirred at room temperature for 18 h. The solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2. The mixture was filtered, and the filtrate was chromatographed on silica gel with CH_2Cl_2–MeOH (19:1) as eluent to give **24** (0.35 g, 64%) as a glass. IR (deposit from $CDCl_3$ solution on a NaCl plate): 1725 (C=O), 1510, 1334 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.26 (t, 6H), 2.31–3.22 (m, 16H), 3.44 (dd, 4H), 3.70 (s, 3H), 4.14 (q, 4H), 6.76 (s, 2H), 9.07 (s, 2H). Anal. Calcd for $C_{29}H_{37}KN_6O_{12}$: C, 49.71; H, 5.32. Found: C, 50.11; H, 5.35.

Chromogenic Diaza-Crown Ether Dicarboxylic Acid 2. Potassium hydroxide (0.06 g, 0.98 mmol) was added to a solution of diester **24** (0.13 g, 0.20 mmol) in a mixture of 95% EtOH–dioxane (15:1, 16 mL), and the solution was stirred at room temperature for 4 h. The solution was acidified with 6 N HCl to pH 1, and the solvent was removed in vacuo. Absolute EtOH was added, and the solvent was evaporated in vacuo. This operation was repeated several times. Finally, the residue was dissolved in a minimum amount of EtOH and filtered. Addition of anhydrous ethyl ether to the filtrate formed a precipitate which was filtered and washed with large amounts of ethyl ether. Drying under vacuum afforded diacid **2** (0.12 g, 85%) as an orange solid with mp > 160 °C dec. IR (KBr): 3395 (OH), 3312 (NH), 1736 (C=O), 1535, 1337 (NO_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.86–4.29 (m, 20H), 7.18 (s, 2H), 8.97 (s, 2H), 10.28 (br s, 1H). Anal. Calcd for $C_{25}H_{28}K_2N_6O_{12}$·2HCl: C, 39.74; H, 4.00. Found: C, 39.42; H, 3.99.

Nitrated Chromogenic Diaza-Crown Diethyl Ester 25. A mixture of 0.5 mL each of $CHCl_3$, acetic acid, and fuming HNO_3 was added to a solution of chromogenic diethyl ester **24** (0.10 g, 0.15 mmol) in $CHCl_3$ (15 mL) at 50 °C and the mixture was stirred for 10 min. The reaction mixture was neutralized with K_2CO_3, and the organic layer was dried (Na_2SO_4). The solvent was removed in vacuo to give an orange oil which was chromatographed on silica gel with CH_2Cl_2–MeOH as eluent to afford **25** (0.075 g, 70%) as a red-brown oil. IR (neat): 3350 (NH), 1735 (C=O), 1516, 1336 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.29 (t, 6H), 2.18-3.75 (m, 23H), 4.20 (m, 4H), 7.94 (s, 1H), 9.05 (s, 2H). Anal. Calcd for $C_{29}H_{37}N_7O_{14}$: C, 49.22; H, 5.27. Found: C, 49.03; H, 5.16.

Diaza-Crown Ether Dimethyl Ester 26. Following method B for the preparation of diethyl ester **21**, reaction of cyclic diamine **16** (0.40 g, 1.51 mmol), NaH (0.18 g, 4.54 mmol), and methyl bromoacetate (0.69 g, 4.54 mmol) in DMF (15 mL) for 22 h gave **26** (0.53 g, 85%) as a yellow oil. IR (neat): 1735 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.40–2.52 (m, 4H), 2.70–3.15 (m, 12H), 3.48 (dd, 4H), 6.98–7.06 (m, 3H). Anal. Calcd for $C_{21}H_{32}N_2O_6$: C, 61.75; H, 7.90. Found: C, 61.56; H, 7.98.

Chromogenic Diaza-Crown Ether Dimethyl Ester 29. Following the procedure given for the preparation of diethyl ester **22**, reaction of dimethyl ester **26** (0.39 g, 0.95 mmol) gave **27** (0.35 g, 81%) as an oil. IR (neat): 1734 (C=O), 1515, 1330 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.45–3.18 (m, 16H), 3.36 (dd, 4H), 3.60 (m, 9H), 7.79 (s, 2H).

Following the procedure given for the preparation of chromogenic diethyl ester **24**, nitro compound **27** (0.29 g, 0.53 mmol) was reduced to amino compound **28** and coupled with picryl chloride to give chromogenic dimethyl ester **29** (0.19 g, 58%) as an orange-red glass. IR (neat): 3350 (NH), 1736 (C=O), 1512, 1339 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.68–3.18 (m, 16H), 3.53 (br s, 4H), 3.69–3.71 (m, 9H), 6.84 (s, 2H), 9.06 (s, 2H), 9.77 (br s, 1H). Anal. Calcd for $C_{27}H_{34}N_6O_{12}$: C, 51.10; H, 5.40. Found: C, 50.99; H, 5.26.

Nitrated Chromogenic Diaza-Crown Ether Dicarboxylic Acid 3. Following the procedure given for the preparation of nitrated chromogenic diethyl ester **22**, chromogenic dimethyl ester **29** (0.12 g, 0.19 mmol) was nitrated to produce nitrated chromogenic dimethyl ester **30** (0.07 g, 58%) as a dark-orange oil. IR (neat): 3330 (NH), 1736 (C=O), 1512, 1339 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.23–4.47 (m, 29H), 6.83 (s, 1H), 9.10 (br s, 2H), 10.71 (br s, 1H).

Potassium hydroxide (0.07 g, 1.00 mmol) was added to a solution of chromogenic dimethyl ester **29** (0.070 g, 0.10 mmol) in a 15:1 mixture of 95% EtOH and dioxane (16 mL), and the solution was stirred at room temperature for 7 h. The solution was acidified with 6 N HCl to pH 1, and the solvent was removed in vacuo. Absolute EtOH was added followed by evaporation in vacuo. The addition and evaporation of EtOH was repeated several times. Finally, the solid residue was dissolved in a minimum amount of EtOH, and the mixture was filtered. Anhydrous ethyl ether was added to the filtrate. The resulting precipitate was collected by filtration and dried under vacuum to give acid **3** (0.064 g, 86%) as an orange solid which decomposed upon heating. IR (KBr): 3386 (OH), 1736 (C=O), 1534, 1500, 1343 (NO_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.59–4.20 (m, 25H), 7.41 (s, 1H), 9.04 (s, 2H). Anal. Calcd for $C_{25}H_{26}N_7O_{14}$·4HCl: C, 37.66; H, 4.17. Found: C, 37.46; H, 4.27.

Dimesylate of *N*-Tosyldiethanolamine (11).[11] A solution of mesyl chloride (54.4 g, 0.47 mol) in CH_2Cl_2 (100 mL) was added dropwise to a suspension of *N*-tosyldiethanolamine (**10**,[10] 50.0 g, 0.19 mol) in a solution of triethylamine (57.7 g, 0.57 mol) and CH_2Cl_2 (400 mL), and the mixture was cooled to below −10 °C in an ice–salt bath. After being stirred for an additional 15 min, the reaction mixture was allowed to warm to room temperature and was washed with 100 mL each of cold water, 10% HCl, saturated aqueous Na_2CO_3, and brine. The organic layer was dried ($MgSO_4$), and the solvent was removed in vacuo to give dimesylate tosylamide **11** (78.9 g, 100%) as a white solid with mp 44–45 °C. IR (neat): 1332, 1255 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H), 3.08 (s, 6H), 3.41 (t, 4H), 4.32 (t, 4H), 7.28 (d, 2H), 7.62 (d, 2H).

Diazide 12. A mixture of dimesylate **11** (78.9 g, 0.19 mol), sodium azide (37.55 g, 0.58 mol), and Aliquat 336 in water (400 mL) was vigorously stirred at reflux for 2 h. The reaction mixture was extracted with ethyl ether, the organic layer was dried ($MgSO_4$), and the solution was concentrated to 75 mL. After column chromatography on alumina with petroleum ether–ethyl

ether (gradient elution), petroleum ether was added to the pure azide fraction to induce crystallization which gave diazide **12** (31.2 g, 53%) after drying at room temperature in vacuo (**heating to dryness was avoided due to potential explosiveness of azides**). IR (deposit from $CDCl_3$ solution on a NaCl plate): 2939, 2924, 2125 (N_3) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.44 (s, 3H), 3.29 (t, 4H), 3.54 (t, 4H), 7.34 (d, 2H), 7.71 (d, 2H).

Diamine 13.[12] A solution of diazide **12** (31.2 g, 0.10 mol) in THF (200 mL) was added to a suspension of $LiAlH_4$ (10.1 g, 0.27 mol) in THF (250 mL) at −10 °C over a 1-h period. After the addition was completed, the reaction mixture was warmed to 10 °C for 30 min and then cooled to −15 °C before adding 5% aqueous NaOH (20 mL). The mixture was stirred overnight and filtered. The filtrate was evaporated in vacuo, and the residue was chromatographed on alumina with CH_2Cl_2–MeOH (gradient elution) to afford diamine **13** (16.5 g, 66%) as a light yellow oil. IR (neat): 3371, 3306 (NH) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H), 2.94–3.79 (m, 12H), 3.86 (s, 3H), 6.80 (t, 2H), 7.06–7.30 (m, 6H), 7.53 (d, 2H).

Cyclic Diamide 18. Solution A (20 mL) containing diacid chloride **4** (2.30 g, 8.85 mmol) in toluene and solution B (19 mL) containing diamine sulfonamide **13** (2.28 g, 8.85 mmol) and triethylamine (2.69 g, 26.35 mmol) in CH_2Cl_2 were added simultaneously to rapidly stirred toluene (500 mL) at room temperature during a 6-h period. After being stirred for an additional 18 h, the reaction mixture was filtered and the solvent was removed in vacuo. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (19:1) as eluent to give **18** (0.83 g, 21%) as a white solid with mp 75–77 °C. IR (deposit from $CDCl_3$ solution on a NaCl plate): 3380 (NH), 1660 (C=O), 1338, 1163 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H), 2.94–3.79 (m, 12H), 3.86 (s, 3H), 6.80 (t, 2H), 7.06–7.30 (m, 5H), 7.53 (d, 2H). Anal. Calcd for $C_{22}H_{27}N_3O_6S\cdot H_2O$: C, 57.00; H, 6.31. Found: C, 57.27; H, 5.94.

Cyclic Trisulfonamide 19. A solution of disulfonamide **7** (0.75 g, 1.49 mmol) and ditosyl sulfonamide **14** (0.75 g, 1.32 mmol) in DMF (15 mL) was added dropwise over a 6-h period to a suspension of Cs_2CO_3 (0.97 g, 2.98 mmol) in DMF (20 mL) at 90 °C. After the solution was stirred for an additional 14 h, the solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2. The solution was washed with water, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (49:1) as eluent to give **19** (0.45 g, 47%) as a white solid with mp 104–106 °C. IR (deposit from $CDCl_3$ solution on a NaCl plate): 1339, 1159 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.04–3.82 (m, 28H), 6.95–7.01 (m, 6H). Anal. Calcd for $C_{36}H_{43}N_3O_7S_3$: C, 59.47; H, 5.96. Found: C, 59.43; H, 5.90.

Diaza-Crown Ether Dicarboxylic Acid 31. Potassium hydroxide (0.06 g, 1.07 mmol) was added to a solution of diester **21** (0.12 g, 0.30 mmol) in a 5:1 dioxane–water mixture (10 mL), and the mixture was stirred at room temperature for 12 h. The solution was acidified to pH 4 with concentrated HCl and evaporated in vacuo. The solid residue was treated with MeOH (5 mL) and filtered to remove KCl. The filtrate was evaporated in vacuo and treated again with MeOH (2.5 mL) and filtered. Evaporation of the solvent followed by drying under vaccum gave diacid **31** (0.085 g, 72%) as a white solid with mp 79–81 °C. IR (KBr): 3398 (OH), 1740 (C=O) cm^{-1}. ^1H NMR (CD_3OD): δ 2.76–3.92 (m, 23 H), 7.09–7.22 (m, 3H), 10.07 (br s, 2H). Anal. Calcd for $C_{19}H_{28}N_2O_6$: C, 59.99; H, 7.42. Found: 60.05; H, 7.21.

UV–vis Spectroscopic Properties of Chromogenic Compounds 2 and 3 and Their Responses to Calcium and Magnesium. Chromoionophore **2** was dissolved in 0.10 M (TMA)OH to make a stock solution of 1×10^{-2} M. For the nonionized form, a solution was made from 0.020 mL of the stock solution and 2.0 mL of 0.10 M HCl and scanned in a 1-cm cuvette from 700 to 300 nm with a Beckman DU-8 spectrophotometer. Molar absorptivities (ϵ) at wavelength maxima (λ_{max}) were calculated according to Beer's law. A similar procedure was used for the ionized form where 0.10 M (TMA)OH was substituted for the 0.10 M HCl.

The reagents for obtaining responses to Ca^{2+} and Mg^{2+} consisted of 1×10^{-4} M **2** and an appropriate buffer. The final concentrations of Ca^{2+} and Mg^{2+} in each 1-cm cuvette was 1.0×10^{-2} M.

For demonstration of the linear response of **2** to Ca^{2+} (Figure 2) a stock solution of 1.25×10^{-4} M **2** in 0.05 M CHES (pH 10.3) was prepared. To 0.96 mL of this solution 0.04 mL of aqueous calcium chloride solutions (0–8 mM) were added, and the change in absorbance at 446 nm was measured in a 1-cm cuvette.

Determination of Stability Constants. The stability constants were determined for complexation of Ca^{2+} by compound **31** in buffered aqueous solutions at 25 ± 0.5 °C by the Frensdorff method[14] using a Ca^{2+}-selective electrode prepared according to the literature method.[15]

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(15) Moody, G. J.; Nassory, N. S.; Thomas, J. D. R. *Analyst* **1978**, *103*, 68.