Chromogenic Bis(urea) Spherands: Synthesis and Complexation of Lithium and Sodium Ions

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Syntheses of two novel 18-membered bis(urea) spherands bearing 2,4,6-trinitroanilino and 4-(diethylamino)phenylazo chromophores are described. The UV-vis spectral changes in the presence and absence of Na⁺ and Li⁺ ions are reported at pH optima. Chromogenic spherand 2 exhibits selectivity for Na⁺ over Li⁺ in chloroform-water extractions. In contrast, Li⁺ ions are bound better than Na⁺ by both spherands in homogeneous aqueous media. The pK_a values are determined for spherand 2 with Na⁺ and Li⁺ in 10% aqueous diethylene glycol monoethyl ether (DEGMEE). The extraction constants (K_{ex}) for K⁺, Na⁺, and Li⁺ are determined for 2 in chloroform saturated with water, and the extraction selectivity order is $Na^+ > Li^+ > K^+$.

Introduction

Chromogenic indicator systems based on macrocyclic hosts have been investigated widely for more than a decade.¹⁻³ More recently, several chromoionophores derived from powerful binders, such as spherands⁴ and cryptahemispherands,⁵ were reported. The high preorganization for binding present in cryptahemispherands made their complexation with alkali metal ions less solvent dependent⁶ and, for the first time, allowed practical application of such systems for determination of sodium and potassium in aqueous media.^{7–9}

It was demonstrated by Cram that appropriate incorporation of anisyl and cyclic urea units into macrocyclic structures provided hosts preorganized for strong complexation of alkali metal and ammonium cations.¹⁰⁻¹³ Due to our continuing interest in chromogenic ionophores for determination of physiologically important ions in blood and other biological fluids, we focused our attention on a recently reported bis(urea) sperand 1.13 The 18-membered, highly preorganized host 1 incorporates three p-methylanisyl and two cyclic urea units and binds Na⁺ almost as strongly $(-\Delta G(\text{CDCl}_3) = 19.1 \text{ kcal} \cdot \text{mol}^{-1})$ and selectively as some of the cryptahemispherands.¹⁴

Earlier, it had been shown that the strong binding and selectivity observed for nonchromogenic hosts was largely retained by closely related chromoionophores.^{4,5} Thus, we decided to synthesize chromoionophores derived from 1 to study their potential application for colorimetric de-

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termination of sodium and lithium in aqueous solutions. In the past, the ionizable picrylamine chromophore was used extensively in combination with various types of macrocyclic ionophores. The nonionizable azo-type chromophore was usually associated with chromogenic indicators containing phenol units.¹⁻³ To our knowledge, a combination of the azo-type chromophore with nonionizable cyclic ionophores has been studied rather sparsely.²

In this paper we wish to report synthetic strategies which were employed to incorporate 2,4,6-trinitroanilino and 4-(diethylamino)phenylazo chromogenic groups into the core spherand structure as well as to discuss chromogenic properties of the new indicators 2 and 3.

Results and Discussion

Syntheses. The known acid 4^{15} was treated with a $HNO_3-H_2SO_4$ mixture to give nitro compound 5 in 80% yield. A clean conversion of 5 to acid chloride 6 (84%) was accomplished at room temperature with oxalyl chloride in benzene. Refluxing 5 with SOCl₂ gave 6 contaminated with byproducts. A simplified literature procedure¹³ was applied to convert acid chloride 6 into bis(acylazide) 7. Due to potential explosiveness, azide 7 was directly rearranged to bis(isocyanate) 8, which again, without isolation, was treated with 2 equiv of known amine 9^{13} to produce bis-

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Table I. UV-vis Spectral Characteristics $(\lambda_{max}, nm and \epsilon(\lambda_{max}))$ of Chromogenic Compounds 2 and 3 in H.O-DEGMEE (90 + 10, v/v)

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compd	form ^a	λ _{max} , nm	$\epsilon(\lambda_{max})$
2	L-	446.6	16700
	HL	380.0	13700
3	L	474.0	27 680
	H+L	525.0, 340.0	4760, 19860

 $^{a}L^{-}$ is fully ionized ligand 2 and L is fully deprotonated ligand 3 in 0.1 M (TMA)OH; HL is nonionized ligand 2 and H⁺L is protonated ligand 3 in 0.1 M HCl.

(urea) compound 10 in a 69% overall yield from three steps.



A two-phase reaction of 10 with 1,3-propanediol ditosylate run for 7 days at room temperature, in a NaOH- H_2O -benzene system and with benzyltriethylammonium bromide as a catalyst, afforded bridged bis(urea) compound 11 in 48% yield. When the same reaction was run for 3 days at reflux the elimination reaction prevailed and only a very complicated mixture of products was obtained. The nitro group of 11 was reduced to the amino group of 12 (55%) with Fe(CO)₅. Amine 12 was treated with picryl chloride in NaHCO₃-CH₃OH to give 13 (82%). Bromination of 13 with HBr gas in CHCl₃ afforded dibromide 14 in 91% yield.

The macrocyclic ring formation which involved high dilution reaction of dibromide 14 with Na_2S-9H_2O in ethanol-benzene produced spheraplex 2-NaBr in 41% yield. Spherand 2 was fully characterized as its complex with LiBr.

A different strategy was applied to synthesize the second chromogenic spherand 3. Benzyl ether 11 was treated with HBr in CHCl₃ to provide dibromide 15 (95%). High dilution cyclization of 15 with Na₂S·9H₂O gave spheraplex 16-NaBr in 35% yield. Treatment of 16 with Fe(CO)₅¹⁶ afforded amine 17 (34%) which was then diazotized with NaNO₂-HCl and coupled with N,N-diethylaniline to produce the monohydrate of spherand 3 in 60% yield. Spherand 3 was fully characterized as its complex with NaBr.

Spectral Characteristics of Chromogenic Compounds 2 and 3. Wavelength maxima (λ_{max}) and molar absorptivities (ϵ) of the acid and base forms of the chro-

Table II. Spectral Responses of Chromogenic Spherands 2 and 3 to Sodium and Lithium at pH Optimum^a and pK_a Values of Spherand 2 and Its Complexes in H₂O-DEGMEE (90 + 10, v/v) at 25 °C

compd	optimum pH	form ^b	λ _{max} , nm	$\epsilon(\lambda_{max})$	pK_a^c
2	9.0	L_{f}	406	12000	8.95 ± 0.07
		NaL	424	12240	8.83 ± 0.03
		LiL	435	13070	8.63 ± 0.01
3	2.5	$\mathbf{L}_{\mathbf{f}}$	527; 343	4670; 19230	ND^d
		NaL	520; 337	5550; 19690	ND
		LiL	522; 338	5700; 18930	ND

^aBuffers: pH 9.0, 0.1 M CHES ((cyclohexylamino)ethanesulfonic acid); pH 2.5, 0.1 M MES (2-(N'-morpholino)ethanesulfonic acid. ^bL_f is the uncomplexed ligand; NaL and LiL is the compound in the presence of large excess of sodium and lithium ions, respectively. ^cpK_a values are averages of three determinations • standard deviation. ^dNot determined.

mogenic spherands 2 and 3 are recorded in Table I. To suppress ionization of the N-H bond in 2 and to ensure protonation of azo and/or amine nitrogens in 3 and thereby to obtain the HL and H⁺L spectra, respectively, absorbances were determined in 0.1 M HCl. Likewise, full ionization of the N-H bond in 2 and deprotonation of 3 was ensured by using 0.1 M TMA(OH) when obtaining L⁻ and L form spectra, respectively.

The chromophore system of spherand 2 exhibited the absorption maximum for the acid form at 380 nm which upon full ionization shifted bathochromically to ~447 nm with an increase in the molar absorptivity. It is interesting to note that two absorption maxima at 525 and 340 nm were observed for the acid form of spherand 3. According to the literature data¹⁷ concerning similar aminoazobenzene systems, the shorter wavelength maximum should be assigned to the amino nitrogen-protonated system while the azo nitrogen-protonated system gives rise to the absorption at the longer wavelength. It is apparent that for compound 3 in 0.1 M HCl the amino nitrogen-protonated form dominates over the azo nitrogen-protonated species.

The deprotonated form (L) of 3 showed only one absorption maximum at 474 nm and a substantial increase in the molar absorptivity.

Cation Responses. Due to our interest in the colorimetric determination of physiologically important sodium and lithium, responses of the two novel chromogenic spherands 2 and 3 to the presence of large excesses (100-200-fold) of sodium and lithium ions in 10% aqueous diethylene glycol monoethyl ether (DEGMEE) were determined. Absorption maxima and molar absorptivities at the pH optima are presented in Table II. (The pH optimum is defined as the pH at which the largest shift is observed between the absorption maxima for the sodium (NaL) and lithium (LiL) complexes.) At pH 9.0 for chromogenic spherand 2, there is an 11-nm shift in the absorption maximum to longer wavelength and a slightly higher molar absorptivity for LiL form versus the NaL form. A similar trend but with much smaller changes in the molar absorptivities at the wavelength maxima of both complexes (520 nm for Na⁺ and 522 nm for Li⁺) is observed at pH 2.5 for chromogenic spherand 3. The Li⁺ preference was also confirmed by the pK_a values determined in 10% aqueous DEGMEE for spherand 2 (see Table II). Thus, Li⁺ exhibits greater N-H acidifying power than Na⁺ shifting the pK_a from 8.95 for the free ligand (L) to 8.63 for the LiL complex.

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Table III. Cation Responses of Chromogenic Spherand 2 at pH 7.5 and 6.5 in CHCl₃-H₂O

pH	form	λ_{max} , nm	$\epsilon(\lambda_{max})$	
7.5ª	L_{f}	396.0	12100	
	KL	430.0	14920	
	NaL	446.0	20 1 50	
	LiL	445.0	19170	
6.5^{b}	$\mathbf{L}_{\mathbf{f}}$	385.0	13 500	
	KL	388.0	13 400	
	NaL	441.0	16000	
	LiL	429.0	13950	

^a0.1 M HEPES (N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid). ^b0.1 M MES (2-(N'-morpholino)ethanesulfonic acid). ^cL_f is the uncomplexed ligand; KL, NaL, and LiL are the compound in the presence of large excess of potassium, sodium, and lithium ions, respectively.

Table IV. Extraction Constants^{\circ} (K_{ex} and pK_{ex}) of Chromogenic Spherand 2 for Potassium, Sodium, and Lithium Chlorides at 25 °C in CHCl₃ Saturated with Water

	K+	Na ⁺	Li ⁺
K _{ex}	$1.03 \times 10^{-6} (\pm 0.2)$	$1.10 \times 10^{-4} (\pm 0.1)$	$1.08 \times 10^{-5} (\pm 0.2)$
pK _{ex}	5.99	3.96	4.97

 $^{\circ}$ CHCl₃-H₂O (pH 7.5; 0.1 M HEPES (N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid).

The data reported above obtained with aqueous systems seemingly contradict Cram's observations which reported sodium selectivity for the nonchromogenic spherand 1 in $\rm CHCl_3$ -water extractions.¹³ In fact, when our chromogenic spherand 2 was tested in $\rm CHCl_3$ -water extractions, it also exhibited preference for sodium over lithium confirming the earlier results. For comparison, UV-vis spectra of sodium and lithium responses in 10% aqueous DEGMEE and CHCl₃-H₂O systems are presented in Figure 1. Responses of chromogenic spherand 2 to potassium, sodium, and lithium at pH 7.5 and 6.5 in $CHCl_3$ are recorded in Table III. As the pH of the aqueous phase decreases the selectivity of Na⁺ over Li⁺ becomes more pronounced since sodium is bound more tightly than lithium. Extraction constants (K_{ex}) of spherand 2 for potassium, sodium and lithium chlorides were also determined (Table IV) and showed that the extraction selectivity order is $Na^+ > Li^+$ > K⁺. Thus, the dependance of selectivity on the solvent becomes apparent. It has been shown before that in the absence of other guests a molecule of water strongly complexes with spherands containing bis(urea) units spanning the two inward-facing carbonyl groups.¹³ Examination of CPK molecular models indicates that to complex sodium efficiently both the host spherand and the guest sodim cation must be dehydrated. It seems plausible that in largely aqueous systems the water molecule strongly bound to the host stays there and coparticipates with other binding sites in the complexation of lithium ion and that is why spherand 2 binds lithium preferentially over sodium.

In conclusion, chromogenic spherand 2 exhibits potential for the colorimetric determination of sodium using procedures allowing the extraction mode. Due to the lack of adequate selectivity for lithium over sodium in largely aqueous solutions the new chromogenic spherands 2 and 3 are unsuitable for direct colorimetric determination of lithium in clinical samples. Disappointingly, the chromogenic performance of spherand 3 containing the azotype chromophore was inferior to that usually observed for the picrylamine-functionalized binders.

Experimental Section

General Methods. All melting points are uncorrected. The ¹H and ¹³C NMR spectra were recorded at 200 and 50 MHz. Fast



Figure 1. UV-vis spectra of sodium (NaL) and lithium (LiL) responses in (a) 10% aqueous DEGMEE and (b) $CHCl_8-H_2O$.

atom bombardment (FAB) mass spectra were determined using *m*-nitrobenzyl alcohol (NOBA) as the matrix.

Materials. Unless specified otherwise, reagent-grade reactants and solvents were used as received from chemical suppliers. Benzene was dried over molecular sieves (4 Å). Pyridine was dried over KOH pellets. 2-Methoxy-1,3-benzenedicarboxylic acid (4)^{15b} and 2-methoxy-3-(methoxymethyl)-5-methylbenzeneamine (9)¹³ were prepared according to literature procedures.

2-Methoxy-5-nitro-1,3-ben zenedicar boxylic Acid (5). 2-Methoxy-1,3-benzenedicar boxylic acid (4) (7.00 g, 35.7 mmol) was cooled to 0 °C, and a cold nitrating mixture (15 mL) made of concentrated H_2SO_4 and fuming 90% HNO₃ (1.8:1, w/w) was added dropwise. After the addition was completed, the mixture was stirred at room temperature for 3 h. The yellow viscous liquid was poured over ice and the resulting aqueous solution was extracted with ether (3 × 20 mL), the combined extracts were dried (MgSO₄), and the solvent was removed in vacuo to afford a pale-yellow solid. The crude product was treated with CH_2Cl_2 , sonicated, and filtered to give diacid 5 (6.9 g, 80%) as a white solid with mp 185.5–187.5 °C: ¹H NMR (DMSO-d₆) δ 3.91 (s, CH_3O , 3 H), 8.55 (s, ArH, 2 H). Anal. Calcd for $C_9H_7NO_7$: C, 44.83; H, 2.93. Found: C, 44.88; H, 3.06.

2-Methoxy-5-nitro-1,3-benzenedicarboxylic Acid Chloride (6). Oxalyl chloride (13.5 g, 106 mmol) and two drops of dry pyridine were added to a suspension of diacid 5 (6.38 g, 26.5 mmol) in dry benzene (290 mL). The mixture was stirred for 24 h at room temperature, an additional 6 mL of oxalyl chloride and two drops of pyridine were added, and stirring was continued for another 4 days. The mixture was filtered, and the solvent and excess oxalyl chloride were removed in vacuo at room temperature to produce acid chloride 6 (6.17 g, 84%) as a pale-yellow liquid: ¹H NMR (CDCl₃) δ 4.05 (s, CH₃O, 3 H), 8.99 (s, ArH, 2 H).

N, N''-(2-Methoxy-5-nitro-1,3-phenylene)bis[N-[2-methoxy-3-(methoxymethyl)-5-methylphenyl]urea] (10). A modification of a literature procedure was used. A solution of NaN₃ (3.20 g, 49.2 mmol) in water (27 mL) was added dropwise to a solution of acid chloride 6 (5.34 g, 19.2 mmol) in acetone (180 mL) at 0 °C. The mixture was stirred at this temperature for 50 min, poured over ice, filtered, and washed with water. The wet diazide

7 was immediately redissolved in toluene, and the organic layer was separated from residual aqueous layer and dried $(MgSO_4)$ over 1 h. The drying material was filtered, and the drying was repeated. After filtration, the solution was concentrated to about 110-mL volume by removing excess toluene in vacuo at room temperature. The resulting solution was heated at 90 °C for 45 min, and a solution of aniline derivative 9 (7.66 g, 42.3 mmol) in toluene (40 mL) was added dropwise to the formed diisocyanate 8. The mixture was heated at 90 °C for 3 h and stirred overnight at room temperature. The precipitate was filtered and washed several times with toluene and petroleum ether to yield compound 10 (7.87 g, 69%) as an amorphous white solid with mp 240-242 °C dec: ¹H NMR (DMSO-d₆) δ 2.26 (s, ArCH₃, 6 H), 3.32 (s, ArCH₂OCH₃, 6 H), 3.70 (s, CH₃O, 6 H), 3.82 (s, CH₃O, 3 H), 4.41 (s, ArCH₂, 4 H), 6.83 (s, ArH, 2 H), 7.96 (s, ArH, 2 H), 8.82 (s, ArH, 2 H), 9.12 (s, NH, 2 H), 9.34 (s, NH, 2 H). Anal. Calcd for C₂₉H₃₅N₅O₉: C, 58.28; H, 5.90. Found: C, 57.94; H, 5.91.

1,1'-(2-Methoxy-5-nitro-1,3-phenylene)bis[tetrahydro-3-[2-methoxy-3-(methoxymethyl)-5-methylphenyl]-2(1*H*)-pyrimidinone] (11). A mixture of urea compound 10 (7.85 g, 13.1 mmol), 1,3-propaneditosylate (12.7 g, 33.0 mmol), and benzyltriethylammonium bromide (2.7 g, 10 mmol) in benzene (260 mL) was combined with a solution of NaOH (37.7 g, 0.94 mol) in water (77 mL) and vigorously stirred under argon, at room temperature, over a week. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were dried (MgSO₄), and the solvent was removed in vacuo. The dark residue was chromatographed twice on deactivated neutral alumina with petroleum ether-EtOAc (1:1) to give 11 (4.24 g, 48%) as a pale-yellow glass. Anal. Calcd for $C_{35}H_{43}N_5O_9$: C, 62.03; H, 6.39. Found: C, 62.05; H, 6.54.

1,1'-(2-Methoxy-5-amino-1,3-phenylene)bis[tetrahydro-3-[2-methoxy-3-(methoxymethyl)-5-methylphenyl]-2(1H)pyrimidinone] (12). A mixture of cyclic urea 11 (4.20 g, 6.20 mmol), Fe(CO)₅ (2.99 g, 15.2 mmol), 1 N NaOH (120 mL), and benzene (120 mL) was vigorously stirred under argon at room temperature for 18 h. Benzene (50 mL) was added, and the organic layer was separated. The solid residue and the aqueous phase were extracted with CH_2Cl_2 (2 × 20 mL), the combined extracts were filtered through Celite and dried (K_2CO_3) , and the solvent was removed in vacuo. The residue was flash chromatographed on silica gel with $CH_2Cl_2-CH_3OH$ (9:1) to afford amine 12 (2.22 g, 55%) as a white solid with mp 265 °C dec: ¹H NMR (CDCl₃) δ 2.10-2.25 (m, CH₂CH₂CH₂, 4 H), 2.26 (s, ArCH₃, 6 H), 3.39 (s, CH_2OCH_3 , 6 H), 3.55-3.75 (m, $CH_2 + NH$, 10 H), 3.78 (s, CH₃O, 3 H), 3.82 (s, CH₃O, 6 H), 4.45 (s, ArCH₂O, 4 H), 6.50 (s, ArH, 2 H), 7.05 (s, ArH, 4 H). Anal. Calcd for C₃₅H₄₅N₅O₇: C, 64.90; H, 7.00. Found: C, 64.97; H, 6.74.

1,1'-[2-Methoxy-5-(2,4,6-trinitroanilino)-1,3-phenylene]bis[tetrahydro-3-[2-methoxy-3-(methoxymethyl)-5-methylphenyl]-2(1H)-pyrimidinone] (13). A mixture of amine 12 (2.30 g, 3.55 mmol), NaHCO₃ (0.31 g, 3.69 mmol), and picryl chloride (0.88 g, 3.55 mmol) in methanol (200 mL) was stirred under argon for 3 h. The solvent was removed in vacuo, and the residue was flash chromatographed on silica gel with CH₂Cl₂-MeOH (95:5) to yield 13 (2.52 g, 82%) as a red glass: ¹H NMR (CDCl₃) δ 2.12-2.30 (m, ArCH₃ + CH₂CH₂CH₂, 10 H), 3.37 (s, CH₃OCH₂Ar, 6 H), 3.55-3.80 (m, CH₂N, 8 H), 3.74 (s, CH₃O, 6 H), 3.83 (s, CH₃O, 3 H), 4.41 (s, ArCH₂O, 4 H), 6.91 (s, ArH, 2 H), 6.95 (s, ArH, 2 H), 7.05 (s, ArH, 2 H), 8.91 (s, ArH, 2 H). Anal. Calcd for C₄₁H₄₆N₈O₁₃: C, 57.34; H, 5.40. Found: C, 57.08; H, 5.54.

1,1'-[2-Methoxy-5-(2,4,6-trinitroanilino)-1,3-phenylene]bis[tetrahydro-3-[2-methoxy-3-(bromomethyl)-5-methylphenyl]-2(1*H*)-pyrimidinone] (14). Hydrogen bromide gas was bubbled through a solution of 13 (2.52 g, 2.93 mmol) in CHCl₃ (250 mL) for 30 min. After being stirred for additional 45 min, the solution turned cloudy. More CHCl₃ (300 mL) was added, and the resulting clear solution was poured into 600 mL of water and stirred vigorously for 20 min. The organic layer was separated and dried (MgSO₄), and the solvent was removed in vacuo. The crude product was flash chromatographed on silica gel with CH₂Cl₂-MeOH (97:3) to afford dibromide 14 (2.56 g, 91%) as an orange-red foam: ¹H NMR (CDCl₃) δ 2.18-2.33 (m, ArCH₃ + CH₂CH₂CH₂, 10 H), 3.60-3.80 (m, CH₂N, 8 H), 3.85 (s, CH₃O, 3 H), 3.88 (s, CH₃O, 6 H), 4.49 (s, CH₂Br, 4 H), 6.97 (s, ArH, 2 H), 7.00 (s, NH, 1 H), 7.01 (s, ArH, 2 H), 7.06 (s, ArH, 2 H), 9.03 (s, ArH, 2 H). Anal. Calcd for $C_{39}H_{40}Br_2N_8O_{11}$: C, 48.97; H, 4.21. Found: C, 48.39; H, 4.25.

29,30,32-Trimethoxy-9-(2,4,6-trinitroanilino)-19,27-dimethyl-23-thia-2,6,12,16-tetraazahexacyclo[23.3.1.1^{2,6}.1^{7,11}.-1^{12,16}.1^{17,21}]tritriaconta-1(29),7,9,11(32),17,19,21(30),25,27-nonaene-31,33-dione (2). A solution of dibromide 14 (0.45 g, 0.47 mmol) in benzene (22 mL) was added via syringe pump to a vigorously stirred suspension of Na₂S·9H₂O (0.37 g, 1.56 mmol) in absolute EtOH (135 mL) over 13 h. After the addition was completed, the mixture was stirred an additional 12 h, the solvent was removed in vacuo, and the residue was flash chromatographed on silica gel with CH_2Cl_2 -MeOH (95:2 \rightarrow 80:20) containing 1% (wt/v) NaBr to get spherand 2-NaBr (0.18 g, 41%) as a dark red glass: ¹H NMR (CDCl₃) δ 2.21 (s, ArCH₃, 6 H), 2.25–2.55 (m, $CH_2CH_2CH_2$, 4 H), 3.78–4.06 (m, $CH_2N + CH_2S$, 12 H), 4.13 (s, CH₃O, 3 H), 4.15 (s, CH₃O, 6 H), 6.85 (s, ArH, 2 H), 6.92 (s, ArH, 2 H), 6.95 (s, ArH, 2 H), 8.96 (s, ArH, 2 H); MS (FAB, NOBA) m/e 851 (M + Na⁺, 27).

The free macrocycle 2 was obtained by extensive washing of a CH₂Cl₂ solution of 2·NaBr with deionized water: ¹NMR (CDCl₃) δ 2.10 (s, ArCH₃, 6 H), 2.15–2.40 (m, CH₂CH₂CH₂, 4 H), 3.60–4.05 (m, CH₂N + CH₂S + CH₃O, 21 H), 6.79 (brs, ArH, 6 H), 8.96 (s, ArH, 2 H).

A lithium spheraplex 2·LiBr was obtained by mixing 1 and LiBr in methanol: ¹H NMR (CDCl₃) δ 2.20 (s, ArCH₃, 6 H), 2.25–2.35 (m, CH₂CH₂CH₂, 4 H), 3.75–4.20 (m, CH₂N + CH₂S + CH₃O, 21 H), 6.84 (s, ArH, 2 H), 6.91 (s, ArH, 2 H), 6.97 (s, ArH, 2 H), 8.94 (s, ArH, 2 H); ¹³C NMR δ 21.03 (CH₃), 23.07 (CH₂CH₂CH₂CH₂), 36.18 (CH₂S), 48.38, 48.65 (CH₂N), 65.19, 65.67 (CH₃O), 118.97, 127.61, 128.41, 130.72, 131.29, 135.38, 136.34, 137.15, 138.36, 138.63, 139.22, 152.32, 152.63 (Ar), 155.48 [N-(C=O)N]. Anal. Calcd for C₃₉H₄₀N₈O₁₁S-LiBr: C, 51.16; H, 4.40. Found: C, 51.14; H, 4.52.

1,1'-(2-Methoxy-5-nitro-1,3-phenylene)bis[tetrahydro-3-[2-methoxy-3-(bromomethyl)-5-methylphenyl]-2(1H)-pyrimidinone] (15). Hydrogen bromide gas was bubbled for 45 min through a solution of 11 (3.80 g, 5.61 mmol) in CHCl₃ (500 mL) and stirred for 1 h at room temperature. Water (500 mL) was added, and the mixture was stirred vigorously for 30 min. The organic layer was separated and dried (Na₂SO₄), and the solvent was removed in vacuo. The crude product was recrystallized from petroleum ether-EtOAc (1:1) to afford dibromide 15 (4.2 g, 95%) as pale-yellow crystals with mp 148-150 °C: ¹H NMR (CDCl₃) δ 2.18-2.35 (m, ArCH₃ + CH₂CH₂CH₂, 10 H), 3.60-3.85 (m, CH₂Nr, 8 H), 3.92 (s, CH₃O, 6 H), 3.97 (s, CH₃O, 3 H), 4.50 (s, CH₂Br, 4 H), 7.05 (s, ArH, 2 H), 7.08 (s, ArH, 2 H), 8.10 (s, ArH, 2 H). Anal. Calcd for C₃₃H₃₇Br₂N₅O₇: C, 51.11; H, 4.81. Found: C, 51.17; H, 4.66.

29,30,32-Trimethoxy-9-nitro-19,27-dimethyl-23-thia-2,6,12,16-tetraazahexacyclo[23.3.1.1^{2,6},1^{7,11}.1^{12,16}.1^{17,21}]tritriaconta-1(29),7,9,11(32),17,19,21(30),25,27-nonaene-31,33-dione (16). A solution of dibromide 15 (0.77 g, 1.00 mmol) in ethanol (50 mL) was added to a suspension of Na₂S-9H₂O (0.80 g, 3.33 mmol) in ethanol (300 mL) during 20 h. The reaction mixture was stirred at room temperature for an additional 24 h. The solvent was removed in vacuo, and the residue was flash chromatographed on silica gel with CH₂Cl₂-MeOH (93:7) to produce sodium spheraplex 16 (0.23 g, 35%) as a yellow foam: ¹H NMR (CDCl₃) δ 2.24 (s, ArCH₃, 6 H), 2.30–2.60 (m, CH₂CH₂CH₂, 4 H), 3.80–4.18 (m, CH₂N + CH₂S, 12 H), 4.23 (s, CH₃O, 6 H), 4.30 (s, CH₃, 3 H), 6.90 (s, ArH, 2 H), 6.95 (s, ArH, 2 H), 8.03 (s, ArH, 2 H). Anal. Calcd for C₃₃H₃₇N₅O₇S-NaBr-0.5 H₂O: C, 52,18; H, 5.04. Found: C, 52.09; H, 5.42.

29,30,32-Trimethoxy-19,27-dimethyl-9-amino-23-thia-2,6,12,16-tetraazahexacyclo[23.3.1.1^{2,6},1^{7,11},1^{12,16},1^{17,21}]tritriaconta-1(29),7,9,11(32),17,19,21(30),25,27-nonaene-31,33-dione (17). 1 N NaOH (0.84 g, 21.0 mmol) and Fe(CO)₅ (0.36 mL, 2.73 mmol) were added to a solution of 16 (0.55 g, 0.86 mmol) in benzene (21 mL), and the mixture was stirred under argon at room temperature for 16 h. Benzene (20 mL) was added, and after stirring for 30 min the organic layer was separated and dried (Na₂SO₄) and the solvent was removed in vacuo. The crude reaction product was flash chromatographed on silica gel with CH₂Cl₂-MeOH (8:2) containing 1% (wt/vol) NaBr to give amine 17 (0.17 g, 34%) as a beige foam: ¹H NMR (CD₃OD) δ 2.17 (s, ArCH₃, 6 H), 2.20–2.30 (m, CH₂CH₂CH₂, 4 H), 3.65–4.05 (m, CH₂N + CH₂S, 12 H), 3.90 (s, CH₃O, 6 H), 4.03 (s, CH₃O, 3 H), 6.39 (s, ArH, 2 H), 6.84 (s, ArH, 2 H), 6.90 (s, ArH, 2 H). Anal. Calcd for $C_{33}H_{39}N_5O_5S$ -NaBr-0.5H₂O: C, 53.66; H, 5.59. Found: C, 53.74; H, 5.78.

29,30,32-Trimethoxy-9-[(4-nitrophenyl)azo]-19,27-dimethyl-23-thia-2,6,12,16-tetraazahexacyclo[23.3.1.1^{2,6},1^{7,11}.-1^{12,16},1^{17,21}]tritriaconta-1(29),7,9,11(32),17,19,21(30),25,27-nonaene-31,33-dione (3). A solution of amine 17 (0.20 g, 0.32 mmol) in a mixture of glacial acetic acid (6 mL) and 1 N HCl (1 mL) was cooled to 0 °C, and a solution of NaNO₂ (40 mg, 0.58 mmol) in a small amount of water was added. After the solution was stirred for 10 min a solution of N,N-diethylaniline (0.10 g, 0.67 mmol) in THF (4 mL) was added dropwise, and the mixture was stirred overnight at room temperature. The acid was neutralized with solid NaHCO₃, the solvent was removed in vacuo, and the residue was flash chromatographed on silica gel with CH₂Cl₂-MeOH (9:1) to give a fraction which was redissolved in CH_2Cl_2 and washed extensively with deionized water. The solvent was removed in vacuo to produce a monohydrate of spherand 3 (0.15 g, 60%) as a light brown glass: ¹H NMR (CDCl₃) δ 1.19 (t, CH₃CH₂, 6 H), 1.91 (brs, H₂O, 2 H), 2.12 (s, ArCH₃, 6 H), 2.20-2.50 $(m, CH_2CH_2CH_2, 4 H), 3.40 (q, CH_2CH_2N, 4 H), 3.65-4.10 (m, CH_2N, 4 H), 3.$ CH₂N + CH₂S + CH₃O, 21 H), 6.66 (d, ArH, 2 H), 6.83 (s, ArH, 4 H), 7.62 (s, ArH, 2 H), 7.73 (d, ArH, 2 H); ¹³C NMR δ 12.85, 20.76 (CH₃), 23.89 (CH₂CH₂), 33.87 (CH₂S), 44.92, 48.67, 48.75 (CH₂N), 61.19, 62.19 (CH₃O), 111.43, 120.53, 125.40, 128.94, 129.92, 132.83, 133.03, 143.68, 148.75, 150.27, 154.29, 154.83 (Ar), 157.83 [N(C=0)N].

The sodium spheraplex 3-NaBr was obtained by mixing 3 with NaBr in methanol, followed by short column chromatography (silica gel; CH_2Cl_2 -MeOH (9:1)): ¹H NMR ($CDCl_3$) δ 1.20 (t, CH_3CH_2 , 6 H), 2.19 (s, CH_3Ar , 6 H), 2.30–2.50 (m, $CH_2CH_2CH_2$, 4 H), 3.41 (q, CH_3CH_2 , 4 H), 3.75–4.25 (m, CH_2N + CH_2S + CH_3O , 21 H), 6.67 (d, ArH, 2 H), 6.82 (s, ArH, 2 H), 6.93 (s, ArH, 2 H), 7.61 (s, ArH, 2 H), 7.76 (d, ArH, 2 H); ¹³C NMR δ 12.82, 20.99 (CH₃), 23.16 ($CH_2CH_2CH_2$), 36.17 (CH_2S), 45.00, 48.67 (CH_2N), 65.37, 65.67 (CH_3O), 111.47, 119.47, 128.43, 130.52, 131.25, 136.13, 137.33, 137.76, 143.31, 151.03, 152.52, 154.30 (Ar), 155.65 [N-(C=O)N]; MS (FAB NOBA) m/e 800 (M + Na⁺, 63%). Anal. Calcd for $C_{43}H_{51}N_7O_5$ S-NaBr-0.5 H₂O: C, 58.03; H, 5.89. Found: C, 57.89; H, 5.63.

UV-vis Spectroscopic Properties of Chromogenic Compounds 2 and 3 and Determination of pK_a Values. Chromogenic compounds 2 and 3 were dissolved in diethylene glycol monoethyl ether (DEGMEE) to make a stock solution of $1.0 \times$ 10^{-2} M. For the nonionized form, solutions were made from 0.02 mL of the stock solution (2), 0.2 mL of DEGMEE, and 1.8 mL of 0.1 M HCl and 0.01 mL of the stock solution (3), 0.2 mL of DEGMEE, and 1.8 mL of 0.1 M HCl and scanned in a 1-cmpathlength cuvette from 700 to 300 nm with a Cary-3 spectrophotometer. Molar absorptivities (ϵ) at wavelength maxima (λ_{max}) were calculated according to Beer's law. A similar procedure was applied for the ionized form where 0.1 M HCl was substituted by 0.1 M TMA(OH).

For the pK_a determinations, absorbances were measured at the acid and base wavelength maxima of the chromogenic compounds in a zwitterionic buffer ((cyclohexylamino)ethanesulfonic acid (CHES)) at pH values equal to the pK_a and the $pK_a \pm 0.5$ units.

Responses to Sodium and Lithium in 10% Aqueous DEGMEE. The reagents for obtaining sodium and lithium responses consisted of 1.0×10^{-4} M (2) and 5.0×10^{-5} M (3) in 10% (v/v) DEGMEE/water and an appropriate buffer (Table II). Final concentrations of sodium and lithium ions in each cuvette was 1.0×10^{-2} M.

Responses to Potassium, Sodium, and Lithium in CHCl_s-Water. Equal volumes (3.0 mL) of a chloroform solution of 2 (1.0×10^{-4} M) and an aqueous solution made of an appropriate buffer (0.1 M MES (2-(N'-morpholino)ethanesulfonic acid) for pH 6.5 and 0.1 M HEPES (N-(2-hydroxyethyl)piperazine-N'-3propanesulfonic acid) for pH 7.5) containing from 0 to 0.04 M KCl, NaCl, and LiCl, respectively, were vortexed in a test tube for 2 min. The organic phase was separated and scanned from 700 to 300 nm on a Cary-3 spectrophotometer (Table III).

Determination of Extraction Constants. Chloroform solutions of chromogenic spherand 2 ($4.0 \times 10^{-5}-2.0 \times 10^{-4}$ M) were vortexed with solutions of 0.1 M HEPES (pH 7.5) containing fixed concentrations of metal ions ([K⁺] 3.3×10^{-3} , [Na⁺] 5.0×10^{-5} , [Li⁺] 4.8×10^{-4}). Absorbances were recorded at wavelength maxima of the complexes, i.e., at 445 nm for NaL and LiL and at 430 nm for KL. Based on these data the extraction constants were calculated for 2 according to the literature procedure.¹⁸

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