Host-Guest Complexation. 50. Potassium and Sodium Ion-Selective Chromogenic Ionophores

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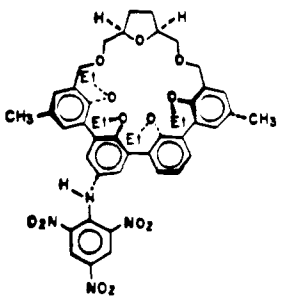
Abstract: Making use of the principles of complementarity and preorganization, we have designed two potassium and one sodium ion-selective chromogenic ionophores useful for colorimetric assays of body and other fluids. Practical syntheses of **1-4** are described, and the UV-visible spectral changes in the presence and absence of Na^+ and K^+ ions are reported at pH's that optimize the spectral differences. Compounds **3** and **4** exhibit high sensitivity (detection limits of $\sim 4 \times 10^{-7}$ M for K^+ and 2×10^{-5} M for Na^+) and selectivities estimated to be greater than 1000 for both $[K^+]/[Na^+]$ and $[Na^+]/[K^+]$, respectively, in essentially aqueous solution. Compounds **1** and **2** show highly sensitivity (10^{-5} M for K^+) and high selectivity for K^+ over Na^+ in 80% dioxane-20% water.

Takagi et al.[1] were the first to bond a corand (benzo-15-crown-5) to a chromogenic indicator system (picrylamine) to compose an analytical reagent (**5**) useful for measuring colorimetrically the amount of K^+ in the presence of Na^+.[1] The method



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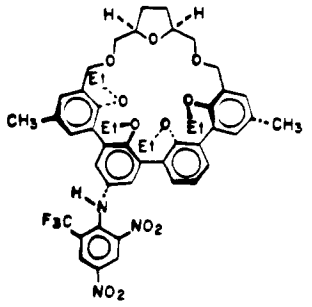

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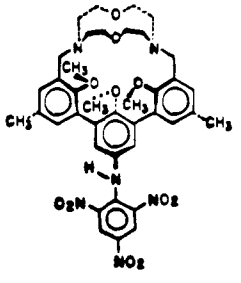

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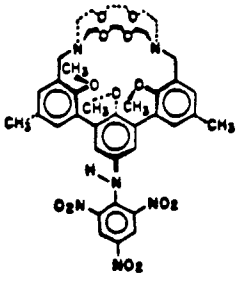

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involved a K^+-selective extraction (factor, $K^+/Na^+ > 100$) from water into chloroform of $K^+\mathbf{5}^-\cdot\mathbf{5}$ vs ($Na^+\mathbf{5}^- + Na^+\mathbf{5}^-\cdot\mathbf{5}$). The importance of Na^+ and K^+ assays coupled with the growing sophistication in host design for alkali-metal ion selectivity has led to many studies of potentially useful chromogenic ligand systems, most of which are based on easily synthesized corands or cryptands. The extensive literature has been thoroughly reviewed.[2,3]

Spherand **6** binds Na^+ and K^+ picrates at 25 °C in $CDCl_3$ saturated with D_2O with $-\Delta G°$ values of 19.3 and <6 kcal mol^{-1}, respectively.[4] This system provides an extreme example of the application of the principles of preorganization and complementarity to structural recognition in complexation.[5] The high binding and selectivity shown by **6** was largely retained by the chromogenic indicator system **7**.[6] Thus in 80% dioxane-20% water, the pK_a values of **7**·Li^+, **7**·Na^+, **7**·K^+, and **7** were, respectively, 5.9, 6.9, 12.7, and 13. The blue color of **7**·Na^+ could be detected at concentrations as low as 10^{-8} M in the presence of other common ions.[6]

In this paper we report the development of chromogenic indicator systems **1-4** for commercial use as Na^+ and K^+ assays in serum and other solutions. Their design was inspired by the observations that parent compounds **8**,[7] **9**,[8] and **10**[8] combined very strong binding with very high selectivity. At 25 °C in $CDCl_3$ saturated with water, the picrate salt-ligand association constant (K_a) ratios were as follows: **8**, $K_a^{K^+}/K_a^{Na^+}$, 2000;[7] **9**, $K_a^{Na^+}/K_a^{K^+}$, 10 000;[8] **10**, $K_a^{K^+}/K_a^{Na^+}$, 10 000.[8] The values of the association constants involving the preferred ions were as follows: **8**, $K_a^{K^+}$, 3×10^8; **9**, $K_a^{Na^+}$, 1×10^{15}; **10**, $K_a^{K^+}$, 9×10^{13}.[7,8] We hoped to make systems workable at pH's as close to neutral as possible and useful in a single phase rich in water.

Modified cryptahemispherands **9** and **10** were particularly attractive as ion-selective systems since crystal structures of the three complexes **9**·Na^+, **10**·Na^+, and **10**·K^+·H_2O had been determined.[9] That of **9**·Na^+ is capsular, with its Na^+···N and Na^+···O average distances of 2.63 and 2.40 Å being close to the standard distances[10] of 2.66 and 2.35 Å, respectively. Thus the Na^+ ion simultaneously contacts all of the heteroatoms of the ligand system. In contrast, although the crystal structure of **10**·Na^+ shows it to be capsular, the Na^+ appears to be too small to simultaneously contact more than five of the nine ligands. The two Na^+···N distances in **10**·Na^+ are 3.18 and 3.64 Å, well above the standard value of 2.45 Å.[10] Five of the Na^+···O distances are between 2.53 and 2.69 Å, while the other two are 2.78 and 2.85 Å,[9] compared to the standard distance of 2.35 Å.[10] Moreover, the dimensions of the cavity in the crystal structures of **10**·Na^+ and **10**·Cs^+ are almost the same. The N···N distance in **10**·Na^+ is 6.68 Å and in **10**·Cs^+ is 6.67 Å, while the average Ar-Ar dihedral angles are nearly equal (57° for **10**·Na^+ and 56° for **10**·Cs^+).[9] Thus in solution, the Na^+ in **9**·Na^+ is undoubtedly closely held, while the Na^+ in **10**·Na^+ is probably "rattling

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(1) Takagi, M.; Nakamura, H.; Ueno, K. *Anal. Lett.* **1977**, *10*(13), 1115-1122.
(2) Takagi, M.; Ueno, K. *Top. Curr. Chem.* **1984**, *121*, 39-65.
(3) Lohr, H.-G.; Vögtle, F. *Acc. Chem. Res.* **1985**, *18*, 65-72.
(4) Cram, D. J.; Lein, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 3657-3668.
(5) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1039-1067.
(6) Cram, D. J.; Carmack, R. A.; Helgeson, R. C. *J. Am. Chem. Soc.* **1988**, *110*, 571-577.
(7) Artz, S. P.; Cram, D. J. *J. Am. Chem. Soc.* **1984**, *106*, 2160-2171.
(8) Cram, D. J.; Ho, S. P. *J. Am. Chem. Soc.* **1986**, *108*, 2998-3005.
(9) Cram, D. J.; Ho, S. P.; Knobler, C. B.; Maverick, E.; Trueblood, K. N. *J. Am. Chem. Soc.* **1986**, *108*, 2989-2998.
(10) These values were obtained by adding the ionic radii of the metal ions to the van der Waals radii of the ligating atoms. Radii were taken from: Pauling, L. C. *Nature of the Chemical Bond*, 2nd ed.; Cornell University: Ithaca, New York, 1940; pp 189, 350.

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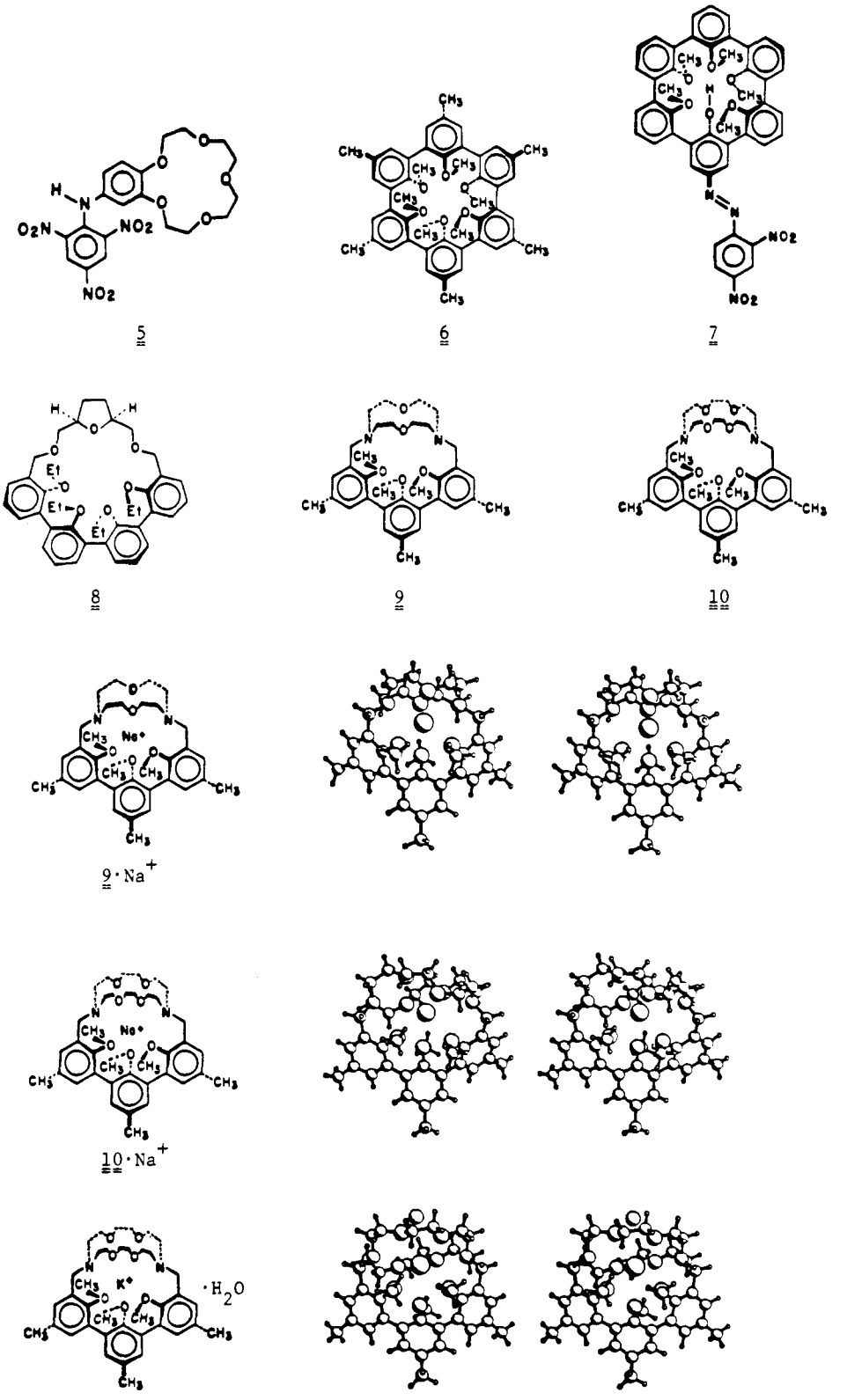
Chart I

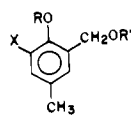


around". In **10**·K^+, the complex is capsular, and the K^+ ion contacts all nine heteroatoms simultaneously.

We presumed at the outset of this research that the presence of a picrylamine group substituted in a position remote from the binding site should not affect the selectivity of these highly preorganized hosts. We also considered it likely that the positive charge of an ionic guest contacting all heteroatoms of the binding site should acidify the picrylamine hydrogen more than no guest at all or a guest which contacted only 56% of the binding sites at a time (as in **10**·Na^+). The experiments reported here were designed to test this hypothesis.

Results and Discussion

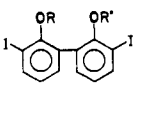
Syntheses. The syntheses of hemispherands **1** and **2** were highly convergent and involved substituted aryl compounds **11–24** as intermediates. Substituted phenol **11**[11] was treated with NaH–



11, R = R' = H, X = Br

12, R = R' = Et, X = Br

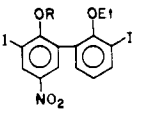
13, R = R' = Et, X = $B(OH)_2$



14, R = R' = CH_3

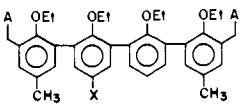
15, R = R' = H

16, R = H, R' = Et



17, R = H

18, R = Et



19, A = OEt, X = NO_2

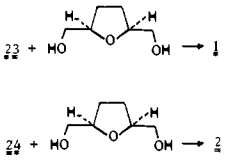
20, A = OEt, X = NH_2

21, A = OEt, X = 2,4,6-$(O_2N)_3C_6H_2NH$

22, A = OEt, X = 2,4-$(O_2N)_2$-6-$CF_3C_6H_2NH$

23, A = Br, X = 2,4,6-$(O_2N)_3C_6H_2NH$

24, A = Br, X = 2,4-$(O_2N)_2$-6-$CF_3C_6H_2NH$



23 + HO OH → **1**

24 + HO OH → **2**

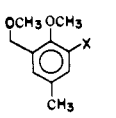
Et_2SO_4 in $(CH_2)_4O$ to give diether **12** (66% yield). This compound was metalated with BuLi–THF at −78 °C, and the organometallic that formed was added to $(CH_3O)_3B$ at −78 °C to minimize the displacement of two alkoxy groups by ArLi. The arylboronate ester was hydrolyzed with hydrochloric acid at 25 °C, and the arylboronic acid that formed (**13**, 80%) was stored at 5 °C as an uncharacterized oil.

Substituted biphenyl compound **14**[12] was demethylated with BBr_3 to provide biphenol **15** (93%), which was fully characterized. This material was alkylated with EtI–K_2CO_3 in acetone to give a 70% yield of the desired half-phenol–half-ether **16**, which was characterized, and 26% of the unwanted 3,3′-diiodo-2,2′-diethoxy-1,1′-biphenyl as byproduct. Nitration of **16** (CH_3CO_2H–HNO_3) gave selective substitution of the phenol ring to yield **17** (56%), which was characterized. This monophenol (**17**) was O-alkylated with Et_2SO_4–K_2CO_3–acetone to produce diiodo diether **18** (92%).

One of the two key reactions in the syntheses of **1** and **2** was the 2-fold Suzuki[13] aryl–aryl coupling between diiodide **18** and boronic acid **13** to provide **19** (79%, fully characterized). The reaction was conducted under argon in a refluxing mixture of reactants, ethanol, water, benzene, Na_2CO_3, and $[(C_6H_5)_3P]_4Pd$.[13] The nitro group of **19** was reduced to the amino group in **20** (78%) with $Fe(CO)_5$.[14] Amine **20** was fully characterized. This amine when treated with picryl chloride in CH_3OH–$NaHCO_3$ gave **21** (92%). Similarly, when **20** was treated with 2,4-dinitro-6-[(trifluoromethyl)chloro]benzene, **22** (86%) was obtained. Both **21** and **22** were fully characterized. Treatment of benzyl ethers **21** and **22** with HBr in $CHCl_3$ gave, respectively, benzyl bromides **23** (95%) and **24** (93%), both of which were fully characterized.

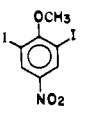
The second key reactions in the syntheses were the macrocyclizations leading to **1** and **2**. These 2-fold alkylations were carried out at high dilution. A mixture of *cis*-2,5-bis(hydroxymethyl)tetrahydrofuran[15] and either dibromide **23** or **24** dissolved in THF was added to a suspension of NaH in refluxing THF. Cycles **1** and **2** were produced in 37% and 19% yields, respectively, and were fully characterized.

Cryptahemispheraplex **4**·NaBr was prepared by two methods. In the first method, hydroxyphenol **11**[11] was dimethylated with Me_2SO_4–NaH–THF to give diether **25** (90%, characterized). This material was metalated with BuLi–THF at −78 °C, and the organometallic formed was added to a solution of $(CH_3O)_3B$ in



25, X = Br

26, X = $B(OH)_2$



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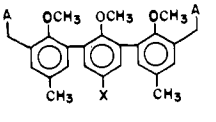
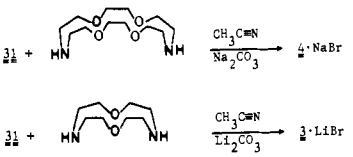


28, A = OCH_3, X = NO_2

29, A = OCH_3, X = NH_2

30, A = OCH_3, X = 2,4,6-$(O_2N)_3C_6H_2NH$

31, A = Br, X = 2,4,6-$(O_2N)_3C_6H_2NH$



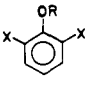
31 + HN NH $\xrightarrow[Na_2CO_3]{CH_3C\equiv N}$ **4**·NaBr

31 + HN NH $\xrightarrow[Li_2CO_3]{CH_3C\equiv N}$ **3**·LiBr

THF at −80 °C to produce the $ArB(OCH_3)_2$ intermediate. This material was hydrolyzed at 25 °C with hydrochloric acid to give arylboronic acid **26** (63%), which was characterized only by its ^1H NMR spectrum and mp. It was stable to storage at 5 °C. Iodination of 4-nitrophenol gave 2,5-diiodo-4-nitrophenol,[16] which was methylated with CH_3I–K_2CO_3 to give ether **27**[16] (42%). The diiodo compound **27** was subjected to a 2-fold Suzuki coupling reaction[13] with 2 mol of arylboronic acid **26** to provide the terphenyl pentaether **28** (97%), which was fully characterized. The nitro group of **28** was reduced with $Fe(CO)_5$[14] to the amino group of **29** (89%). This amine was fully characterized. Treatment of **29** with picryl chloride in $NaHCO_3$–CH_3OH gave **30** (95%, fully characterized). When mixed with HBr in $CHCl_3$, **30** afforded the dibromide **31** (93%, fully characterized).

The crucial cyclizations were carried out in $CH_3C\equiv N$. Thus alkylation of 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane by dibromide **31** in the presence of Na_2CO_3 gave **4**·NaBr (84%, fully characterized), as well as a small amount of **4**·KBr, which was also characterized. Apparently **4**·NaBr scavenged a small amount of K^+ ion from the silica gel column used in purification to give **4**·KBr. Similarly, when dibromide **31** was used to alkylate 1,7-diaza-4,10-dioxadodecane[9] in CH_3CN–Li_2CO_3, **3**·LiBr (56%, fully characterized) was produced, as well as a small amount of **3**·NaBr. The conversion of **3**·LiBr to **3**·NaBr probably occurred on the silica gel used in the purification.

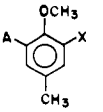
The second method of preparing a complex of **4** involved a different reaction for macroring formation. Dibromophenol **32**[17]



32, R = H, X = Br

33, R = $CH_2C_6H_5$, X = Br

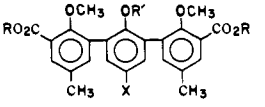
34, R = $CH_2C_6H_5$, X = $B(OH)_2$



35, A = X = I

36, A = I, X = CO_2H

37, A = I, X = CO_2CH_3



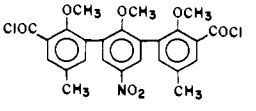
38, R = CH_3, R' = $CH_2C_6H_5$, X = H

39, R = CH_3, R' = H, X = H

40, R = CH_3, R' = H, X = NO_2

41, R = CH_3, R' = CH_3, X = NO_2

42, R = H, R' = CH_3, X = NO_2



43

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(13) Modeled after: Miyoura, N.; Yanagi, T.; Suzuki, A. *Synth. Commun.* **1981**, *11*(7), 513–519.

(14) Abbayes, H.; Alper, H. *J. Am. Chem. Soc.* **1977**, *99*, 98–101.

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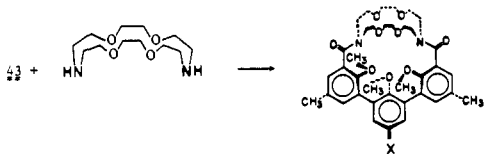
was benzylated with $C_6H_5CH_2Br$–K_2CO_3–acetone to give **33** (80%, characterized). This dibromo ether was dimetalated with *sec*-BuLi–THF at −78 °C, and the organometallic was added to $(CH_3O)_3B$ in THF at −78 °C. The resulting aryldiboronic ester was hydrolyzed with hydrochloric acid to give the aryldiboronic acid **34** (91%), which was characterized only by its ^1H NMR spectrum. This intermediate was stored at 5 °C. Diiodoaryl ether **35**[18] was monometalated with BuLi–Et_2O at −78 °C and carbonated to give acid **36** (64%), which when treated with CH_2N_2 gave iodo ester **37** (90%). Both **36** and **37** were fully characterized. These two compounds were subjected to a 2-fold Suzuki aryl-to-aryl coupling reaction[13] to give trisether diester **38** (67%), characterized only by its ^1H NMR spectrum. The benzyl ether protecting group of **38** was removed with Pd–H_2 to provide phenol **39** (77%, fully characterized). This substance was nitrated (HNO_3–CH_3CO_2H–$CHCl_3$) to produce **40** (91%, only ^1H NMR spectral characterization), whose hydroxyl group was methylated with Me_2SO_4–K_2CO_3–acetone to provide ester ether **41** (93%, characterized only by its ^1H NMR spectrum). The two ester groups of **41** were hydrolyzed with LiOH, and the salt produced was acidified to give **42** (74%), which was fully characterized. Treatment of diacid **42** with $SOCl_2$ gave the diacid chloride **43** (~100%), characterized only by its ^1H NMR spectrum. This inaterial was used to diacylate 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane in C_6H_6–Et_3N under high-dilution conditions to produce diamide **44** (60%, fully characterized). The nitro group



44, X = NO_2

45, X = NH_2

of **44** was reduced (Pd–H_2–DMF) to give amine **45** (97%, characterized only by its ^1H NMR spectrum). The two amide groups of **45** were reduced with $H_3B{\cdot}S(CH_3)_2$ to afford triamine **46**, which without isolation was mixed with picryl chloride to give **4** complexed with adventitious salts. A solution of this material in CH_2Cl_2 was shaken with aqueous NaCl to give complexed product. Chromatographic purification (silica gel) gave **47** (same as **4**·NaCl, 38%) and **4**·KCl (6%). The former complex was characterized by its FABMS and ^1H NMR spectra, which were essentially the same as those obtained from **4**·NaBr synthesized by the first method. The first method is obviously shorter and affords the desired cryptahemispheraplex in better yield.

Free hosts **3** and **4** were never isolated uncontaminated with complexed salt. They are such strong ligands for the alkali-metal salts that when in solution, they scavenge them from materials with which they come in contact. In contrast, **3**·LiX and **4**·NaX can be easily handled and stored as solids and are less subject to both oxidation and guest alteration than the free hosts. Dilute solutions of **3**·LiX in 1% $Et(OCH_2CH_2)_2OH$–99% H_2O (v/v) when mixed with dilute solutions of buffered (pH 7) aqueous NaX undergo guest exchange to produce **3**·NaX over a period of 3 h at 37 °C. Dilute solutions of **4**·NaX in 1% $Et(OCH_2CH_2)_2OH$–99% H_2O (v/v) undergo instantaneous guest exchange to give **4**·KX when mixed with dilute solutions of buffered (pH 7–8) aqueous KX. The differences in binding of **3** for Na^+ over Li^+ and of **4** for K^+ over Na^+ are so great, and the binding of each host for its favored ion is so strong, that, under practical working conditions, the cationic guest exchanges go essentially to completion. The use of forms of **3** and **4** as analytical reagents for blood analysis has been reported.[19]

Determination of pK_a Values of 1–4 and Their Complexes. The substituted picric acid dissociation constants were determined

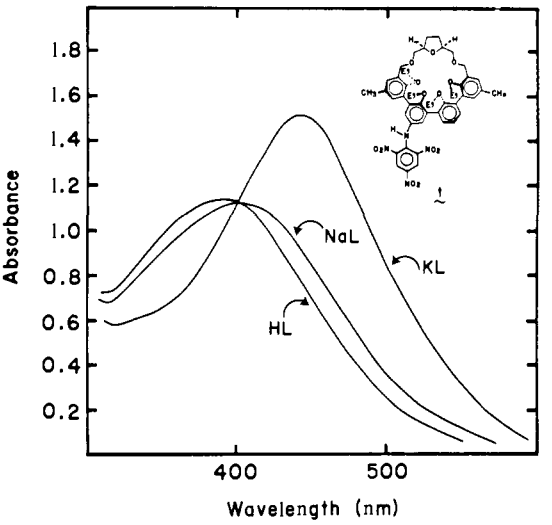


Figure 1. Differences in electronic spectra of **1** (**1** = HL) at pH 10.0 in 80% dioxane–20% water (v/v), 1.07×10^{-4} M in **1**, 0.1 M in CHES buffer: no added salt (curve HL); 0.010 M in NaCl (curve NaL); 0.010 M in KCl (curve KL).

spectrophotometrically from the molar absorptivities (ϵ) exemplified in Table I (also see the Experimental Section). Absorbances (A) were determined at λ_{max} in media 0.020 M in HCl to suppress ionization of the NH bond; media 0.020 M in $(CH_3)_4NOH$ to ensure complete ionization of the NH bond; and in media buffered (0.020 M concentration) to known pH's. These spectra were also taken in the presence of 0.010 M NaCl, in the presence of 0.010 M KCl, and in the absence of added salt. Figures 1–6 provide examples of the spectra taken in various media. The resulting data coupled with eq 1–4 were used to calculate the pK_a values for **1**–**4** in their various forms in the media indicated (Table II).

$$pK_a = pH + \log (HL/L^-) \quad (1)$$

$$\frac{HL}{L^-} = \frac{A_{a\lambda}\epsilon_{L^-,b\lambda} - A_{b\lambda}\epsilon_{L^-,a\lambda}}{A_{b\lambda}\epsilon_{HL,a\lambda} - A_{a\lambda}\epsilon_{HL,b\lambda}} \quad (2)$$

$$\frac{A_{a\lambda}}{l} = \epsilon_{HL,a\lambda}[HL] + \epsilon_{L^-,a\lambda}[L^-] \quad (3)$$

$$\frac{A_{b\lambda}}{l} = \epsilon_{HL,b\lambda}[HL] + \epsilon_{L^-,b\lambda}[L^-] \quad (4)$$

In eq 1–4, HL is the acid and L^- is the basic form of the picryl system, A is the observed absorbance, ϵ is the molar absorptivity, aλ is the wavelength maxima of HL, bλ is the wavelength maxima of L^-, and l is the light path length in centimeters.

The validity of this procedure depends on the assumption that the metal-ion-complexed forms of both the protonated and unprotonated forms of the chromophore have the same spectral characteristics. Spectra of the various forms of **3** and **4** demonstrated this assumption to apply. However, the complexed forms of **1** and **2** are slightly different from the corresponding noncomplexed forms (see Table I). Accordingly, we substituted the molar absorptivities of the complexed forms for the uncomplexed (substitute $\epsilon_{HL\cdot M}$ for ϵ_{HL}, and ϵ_{ML} for ϵ_{L^-}, Table I) in order to obtain more realistic estimates of the pK_a's of **1** and **2**.

Factors that influence the pK_a's include the ionic strength of the media and the amount of water-miscible organic solvent present. Determination of pK_a's for **1** and **2** were made in both 40% (v/v) and 80% (v/v) 1,4-dioxane in water. The influence of the amount of dioxane on the pK_a of the buffer was used, and hence the ionization reaction pH was minimal with the zwitterionic buffers, $(CH_2)_5CHNH(CH_2)_3SO_3H$ (CAPS), and $(CH_2)_5CHNH(CH_2)_2SO_3H$ (CHES). However, the higher the dioxane concentration, the stronger became the cation binding of **1** or **2**.

The ionic strength of the media used for determinations made with indicators **1** and **2** was relatively low (0.020 M buffer), and no corrections for ionic strength effects were made. Compounds

(18) Wilkinson, J. H. *J. Chem. Soc.* **1951**, 626–627.
(19) Kumar, A.; Chapoteau, E.; Czech, B. P.; Gebauer, C. R.; Chimenti, M. Z.; Raimondo, O. *Clin. Chem.* **1988**, *34*, 1709–1712.

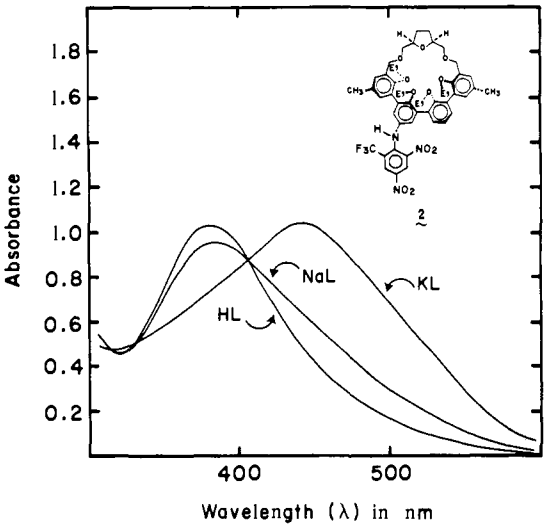


Figure 2. Differences in electronic spectra for **2** (**2** = HL) at pH 10.0 in 80% dioxane–20% water (v/v), 7.65×10^{-5} M in **2**, 0.020 M in CAPS buffer: without added salt (curve HL); 0.010 M in NaCl (curve NaL); 0.010 M in KCl (curve KL).

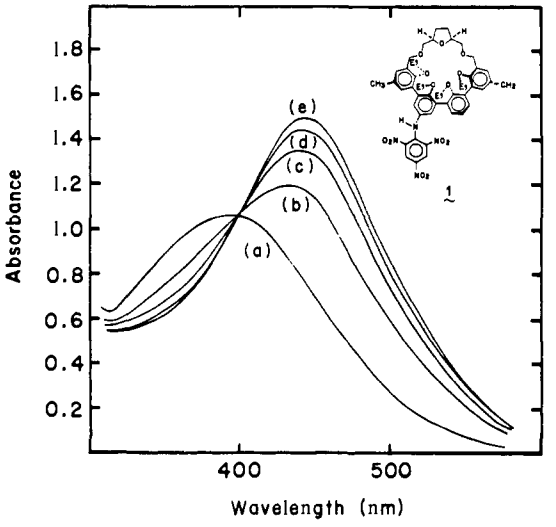


Figure 3. Potassium ion response curves in 80% dioxane–20% water (v/v), 0.020 M in CHES buffer at pH 10.0: (a) 0.0 equiv of KCl/equiv of **1** at 1.07×10^{-4} M; (b) 0.5; (c) 1.0; (d) 2.0; (e) 4.0.

3 and **4** showed a stronger dependence of pK_a on ionic strength, particularly when complexed to the more strongly binding cations. Therefore, the final values of pK_a for **3** and **4** are those obtained by extrapolating experimentally determined pK_a's at different ionic strengths to zero ionic strength. The errors estimated for the pK_a's were taken from errors in the intercepts observed in plots of pK_a data vs ionic strength. The 1% (v/v) of $Et(OCH_2CH_2)_2OH$ in water used as medium for **3** and **4** kept these compounds in solution and had a negligible effect on the pK_a values. Table II records the pK_a values for **1**–**4** and their complexes.

Interesting and useful conclusions are drawn from the pK_a data of Table II. In 80% dioxane–20% water (v/v), HL of **1** is ~0.34 pK_a unit higher valued than NaL, which is ~1.5 pK_a unit higher than KL. In 40% dioxane–60% water (v/v), HL of **1** is ~0.1 pK_a unit higher valued than NaL, which is ~0.8 pK_a unit higher than KL. Thus the *medium richer in dioxane provides the greater selectivity of* **1** *for* K^+ *over* Na^+. Figure 1 shows visually how much more sensitive **1** is to the presence of K^+ than to Na^+ in pH 10 buffer in 80% dioxane–20% water (v/v). Similarly, Figure 2 shows the same kind but somewhat smaller effects for **2**. Thus **1** appears to be superior to **2** as a chromogenic indicator system. Figure 3 provides a graphic measure of how **1** at pH 10 in 80% dioxane–20% water (v/v) responds to incremental additions of

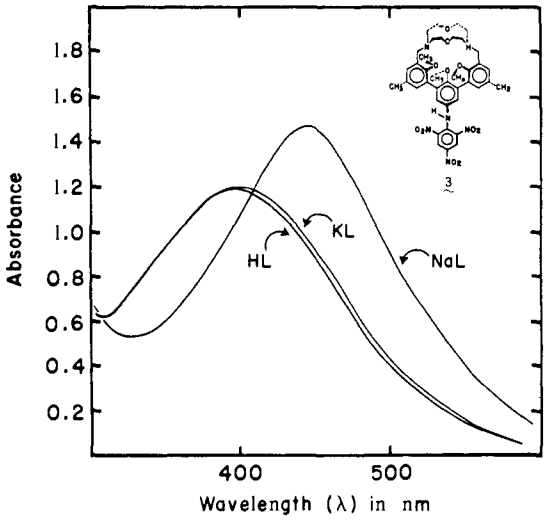


Figure 4. Differences in electronic spectra for **3**·LiCl (**3**·LiCl = HL) at pH 8.0 in 1% $Et(OCH_2CH_2)_2OH$–99% water (v/v), 1.00×10^{-4} M in **3**·LiCl, 0.29 M in imidazolium acetate: without added salt (curve HL); 0.010 M in NaCl (curve NaL); 0.010 M in KCl (curve KL). Samples left 16 h at 37 °C before scanning at 25 °C.

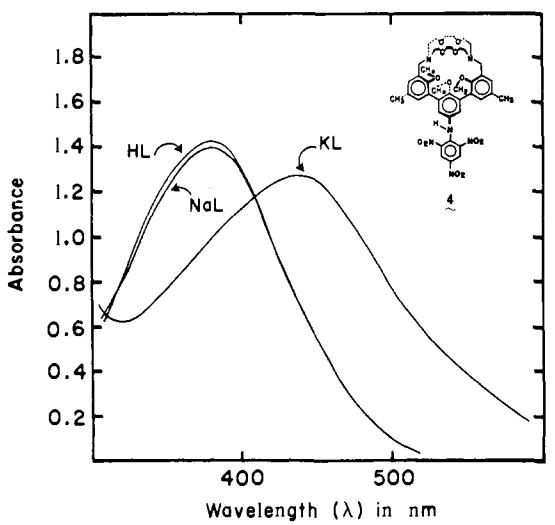


Figure 5. Differences in electronic spectra for **4**·NaCl (**4**·NaCl = HL) at pH 7.0 in 1% $Et(OCH_2CH_2)_2OH$–99% water (v/v), 1.0×10^{-4} M in **4**·NaCl, 0.29 M in imidazolium acetate: without added salt (curve HL); 0.010 M in NaCl (curve NaL; 0.010 M in KCl (curve KL).

KCl, while runs 16–20 of Table I record the λ_{max} and ϵ values for each curve. The fact that the ϵ values increase in passing from a ratio of 2:1 to 4:1 $[K^+]/[\mathbf{1}]$ shows that, even at the latter ratio, **1** is not fully complexed at its concentration of 1.07×10^{-4} M. We conclude that **1** is a potentially useful colorimetric indicator system for measuring K^+ ion concentrations in the 10^{-4}–10^{-5} M range in the presence of Na^+ at pH 10 in 80% dioxane–20% water (v/v). Unreported results in other comparable organic–water media show that a variety of media could be used.

System **3**·LiBr was designed to quantitatively distinguish between Na^+ and K^+. Table II shows that the pK_a of **3**·LiCl and **3**·KCl are both 7.85 in what is essentially water. This identity of values suggests that K^+, even when present at concentrations 100 times as high as Li^+, does not form capsular **3**·K^+. This result is compatible with the inability of ions with the diameter of potassium to be encapsulated by **3** in CPK molecular models. Moreover, the UV–visible spectrum of **3**·Li^+ at pH 8 is nearly identical with that of **3**·Li^+ in the presence of K^+ (Figure 4). Table II also shows that the pK_a of **3**·Na^+ is 6.95, 0.9 units lower than that of **3**·Li^+. Moreover, Figure 4 and runs 40–42 of Table I show that **3**·Li^+ and **3**·Na^+ possess widely different spectra. For example, at pH 8, **3**·Li^+ gives λ_{max} = 395 nm (ϵ = 11 800 M^{-1} cm^{-1}),

Table I. Absorptivities at Maximum and Useful Wavelengths of Chromogenic Species of **1**–**4**

run	no. compd	concn, $\times 10^5$ M	solvent, % v/v	additive final concn	species[a]	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	λ_{nm}[b]	ϵ^c, M^{-1} cm^{-1}
1	**1**	7.56	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M HCl	HL	388	10300	459	4600
2	**1**	7.56	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M HCl, 0.010 M NaCl	HL·Na^+	388	10300	454	4630
3	**1**	7.56	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M HCl, 0.010 M KCl	HL·K^+	380	10600	449	3770
4	**1**	7.56	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M $(CH_3)_4NOH$	L^-	459	15400	388	7500
5	**1**	7.56	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M $(CH_3)_4NOH$, 0.010 M NaCl	NaL	454	15400	388	7360
6	**1**	7.56	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M $(CH_3)_4NOH$, 0.010 M KCl	KL	449	16350	380	7320
7	**1**	7.56	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M HCl	HL	380	12900	455	8500
8	**1**	7.56	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M HCl, 0.010 M NaCl	HL·Na^+	380	13500	455	8700
9	**1**	7.56	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M HCl, 0.010 M KCl	HL·K^+	380	12900	455	6000
10	**1**	7.56	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M $(CH_3)_4NOH$	L^-	455	16500	380	7000
11	**1**	7.56	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M $(CH_3)_4NOH$, 0.010 M NaCl	NaL	455	16700	380	7100
12	**1**	7.56	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M $(CH_3)_4NOH$, 0.010 M KCl	KL	455	16300	380	6900
13	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES[d] (pH 10)	HL	392	11500		
14	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 10), 0.010 M NaCl	NaL	403	11600		
15	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 10), 0.010 M KCl	KL	443	14300		
16	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 10), 0 equiv[e] KCl	HL	395	10040		
17	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 10), 0.5 equiv[e] KCl	KL	433	11230		
18	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 10), 1.0 equiv[e] KCl	KL	440	12785		
19	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 10), 2.0 equiv[e] KCl	KL	442	13625		
20	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 10), 4.0 equiv[e] KCl	KL	442	14113		
21	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 9)	HL	389	10300		
22	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 9), 0.010 M NaCl	HL·Na^+	389	10300		
23	**1**	10.7	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M CHES (pH 9), 0.010 M KCl	KL	427	10800		
24	**2**	7.64	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M HCl	HL	378	13600	457	3700
25	**2**	7.64	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M HCl, 0.010 M NaCl	HL·Na^+	380	13600	448	4300
26	**2**	7.64	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M HCl, 0.010 M KCl	HL·K^+	372	14400	448	3200
27	**2**	7.64	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M $(CH_3)_4NOH$	L^-	457	15700	378	8700
28	**2**	7.64	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M $(CH_3)_4NOH$, 0.010 M NaCl	NaL	448	17500	380	8100
29	**2**	7.64	80% $O(CH_2CH_2)_2O$–20% H_2O	0.020 M $(CH_3)_4NOH$, 0.010 M KCl	KL	448	19600	378	5930
30	**2**	7.64	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M HCl	HL	372	12200	458	4000
31	**2**	7.64	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M HCl, 0.010 M NaCl	HL·Na^+	372	13300	448	6400
32	**2**	7.64	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M HCl, 0.010 M KCl	HL·K^+	372	12200	448	3350
33	**2**	7.64	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M $(CH_3)_4NOH$	L^-	458	13200	372	6200
34	**2**	7.64	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M $(CH_3)_4NOH$, 0.010 M NaCl	NaL	448	12700	372	5800
35	**2**	7.64	40% $O(CH_2CH_2)_2O$–60% H_2O	0.020 M $(CH_3)_4NOH$, 0.010 M KCl	KL	448	14000	372	6000
36	**3**·LiBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.10 M HCl	HL	380	14700	451	5810
37	**3**·LiBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.10 M $(CH_3)_4NOH$	L^-	451	18000	380	9600
38	**4**·NaBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.10 M HCl	HL	379	11700	448	4390
39	**4**·NaBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.10 M $(CH_3)_4NOH$	L^-	448	16700	379	7452
40[f]	**3**·LiBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.30 M IMA[g] (pH 8.0)	LiL	395	11800		
41[f]	**3**·LiBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.30 M IMA[g] (pH 8.0), 0.010 M KCl	KL	398	11930		
42[f]	**3**·LiBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.30 M IMA[g] (pH 8.0), 0.010 M NaCl	NaL	445	14600		
43	**4**·NaBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.30 M IMA[g] (pH 7.0)	NaL	380	14130		
44	**4**·NaBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.30 M IMA[g] (pH 7.0), 0.010 M NaCl	NaL	380	13860		
45	**4**·NaBr	10.0	1% $CH_3(OCH_2CH_2)_2OH$–99% H_2O	0.30 M IMA[g] (pH 7.0), 0.010 M KCl	KL	433	12530		
46	**4**·NaBr	27	3% $CH_3(OCH_2CH_2)_2OH$–97% H_2O	0.29 M TEA (pH 7.3), 0.000 mM KCl	NaL	390	10100	500[h]	2150
47	**4**·NaBr	27	3% $CH_3(OCH_2CH_2)_2OH$–97% H_2O	0.29 M TEA (pH 7.3), 0.025 mM KCl	NaL + KL	393	9919	500[h]	2590
48	**4**·NaBr	27	3% $CH_3(OCH_2CH_2)_2OH$–97% H_2O	0.29 M TEA (pH 7.3), 0.050 mM KCl	NaL + KL	397	9852	500[h]	3110
49	**4**·NaBr	27	3% $CH_3(OCH_2CH_2)_2OH$–97% H_2O	0.29 M TEA (pH 7.3), 0.075 mM KCl	NaL + KL	402	9693	500[h]	3480
50	**4**·NaBr	27	3% $CH_3(OCH_2CH_2)_2OH$–97% H_2O	0.29 M TEA (pH 7.3), 0.125 mM KCl	NaL + KL	408	9600	500[h]	4370

[a] HL is uncomplexed, substituted picrylamine; HL·M^+ is complexed picrylamine; L^- is uncomplexed picrylamine anion; ML is complexed picrylamine anion, a zwitterion. [b] Wavelength of λ_{max} of conjugate base or conjugate acid of species. [c] Molar absorptivity of conjugate base at λ_{max} of acid species or molar absorptivity of conjugate acid at λ_{max} of basic species. [d] CHES is $CH_2(CH_2CH_2)_2NCH_2CH_2SO_3H$. [e] Equivalents based on **1** present equals 1 equiv. [f] Incubated 26 h at 37 °C before spectra taken. [g] IMA is imidazolium acetate. [h] Wavelength chosen to maximize sensitivity to K^+ ion concentrations.

3·K^+ provides λ_{max} at 398 nm (ϵ = 11930 M^{-1} cm^{-1}), whereas for **3**·Na^+, λ_{max} appears at 445 nm (ϵ = 14600 M^{-1} cm^{-1}). Thus **3**·Li^+ appears to be a good colorimetric indicator system for measuring Na^+ ion concentrations in the presence of K^+ in water. The system suffers somewhat from the limitation that, at 25 °C, Na^+ completely displaces Li^+ from **3**·Li^+ to give **3**·Na^+ only over a period of several hours. However, measurements of Na^+ ion concentrations can readily be made by measuring the reaction rate of Na^+ + **3**·Li → **3**·Na^+ + Li^+ at 30 °C or higher temperatures. Attempts to isolate free **3** in a pure state free of Na^+ failed. Even **3**·LiBr scavenges Na^+ from soft glass, water, and chromatographic materials.

System **4**·NaBr was designed to distinguish quantitatively between K^+ and Na^+. Table II provides the following pK_a values in essentially aqueous solution: **4**·NaBr, 7.75; **4**·NaBr in the presence of 100 equiv of NaBr, 7.75; **4**·NaBr in the presence of 100 equiv of KCl, 7.05. Thus **4**·K^+ is about 0.7pK_a units more acidic than **4**·Na^+. Figure 5 shows graphically the substantial difference between the UV–visible spectrum of **4**·Na^+ and **4**·K^+ in water buffered at pH 7.0. Runs 43–45 of Table I show that the λ_{max} of **4**·NaCl in pH 7.0 buffer is at 380 nm ($\epsilon \sim$ 13800 M^{-1} cm^{-1}), whereas **4**·KCl has its λ_{max} at 433 nm ($\epsilon \sim$ 12500 M^{-1} cm^{-1}). Runs 46–50 of Table I and Figure 6 made in 3% $CH_3(OCH_2CH_2)_2OH$–97% H_2O (v/v) in pH 7.3 buffer take advantage of the large spectroscopic window available for measuring K^+ concentration at wavelengths where **4**·Na^+ hardly absorbs (e.g. at λ = 500 nm, see Figure 5). A plot of the concentration of K^+ against absorbance at λ = 500 nm is nicely linear, as are other plots in the presence of much larger excesses of Na^+. The displacement of Na^+ by K^+ in **4**·Na^+ occurs essentially instantaneously on the human time scale (unlike the displacement of Li^+ by Na^+ in **3**·Li^+).

Effect of pH and of the Presence of Dioxane on the Selectivity of 4 for Potassium over Sodium Ion in Aqueous Systems. Ex-

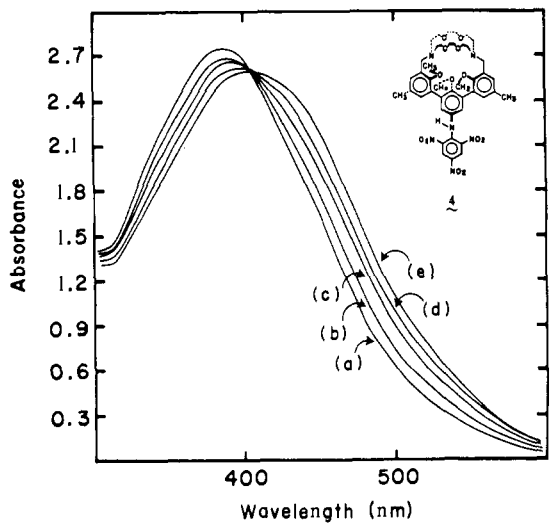


Figure 6. Potassium ion response curves in 3% $Et(OCH_2CH_2)_2OH$–97% water (v/v), 2.7×10^{-4} M in **4**·NaBr, 0.1 M in $N(CH_2CH_2OH)_3$·HCl buffer at pH 7.3: (a) no added salt; (b) 0.025 mM in KCl; (c) 0.05; (d) 0.075; (e) 0.125.

Table II. Values of pK_a of **1**–**4** and Their Complexes

		pK_a in water diluted with organic solvents (v/v)		
compd	form	80% O-$(CH_2CH_2)_2O$[a]	40% O-$(CH_2CH_2)_2O$[a]	1% EtO-$(CH_2CH_2O)_2H$[b]
1	HL	11.10 ± 0.04	10.23 ± 0.08	
1	NaL	10.76 ± 0.02	10.12 ± 0.06	
1	KL	9.35 ± 0.10	9.27 ± 0.09	
2	HL	10.77 ± 0.04	10.15 ± 0.04	
2	NaL	10.40 ± 0.15	10.05 ± 0.02	
2	KL	9.42 ± 0.20	9.20 ± 0.15	
3·LiBr	LiL			7.85 ± 0.10
3·NaBr	NaL			6.95 ± 0.05
3·KBr	KL			7.85 ± 0.10
4·NaBr	NaL			7.75 ± 0.10
4·NaBr[c]	NaL			7.75 ± 0.10
4·NaBr[d]	KL			7.05 ± 0.05

[a] Solutions 0.020 M in $(CH_2)_5CHNH(CH_2)_3SO_3H$ (CAPS) buffer, 0.010 M in NaCl, or KCl where indicated as present; pK_a values are average of three determinations ± 1 standard deviation, uncorrected for ionic strength due to salt or activity coefficient changes due to organic solvent. [b] Solutions range from 0.01 to 0.4 M in $N(CH_2CH_2OH)_3$·HCl (TEA) buffer concentration; 0.010 M in NaCl or KCl where indicated as present; ± estimated error in extrapolated pK_a values to zero ionic strength. [c] 100 equiv of NaBr were also present (**4**·NaBr = 1 equiv). [d] 100 equiv of KBr were also present (**4**·NaBr = 1 equiv).

periments were conducted to determine the effect on the absorbance of various forms of **4** at λ = 450 nm when the percentages of dioxane in water were varied and when the pH's of the solutions were changed by the presence of various tertiary amine–sulfonic acid buffers at 0.10 M concentration. Table III presents the results in the form of ΔA values, where ΔA = absorbance with added salt − absorbance without added salt. Note that the concentration of added NaCl or KCl present was 500 times the concentration of the **4**·NaBr indicator system, thus rendering inconsequential the amount of NaBr originally present in the complexed reagent. However, at high dioxane concentration the amount of NaBr originally present in **4**·NaBr is sufficient to influence the pK_a of the chromophore. In run 1 made in 1% dioxane in water (v/v) with added NaCl, the response was very low at all pH's except 8.0, which gave a ΔA value of 0.026. Run 2 in the same media except that in which KCl was added gave responses with ΔA values of 0.414 at pH 6.6, 0.512 at pH 7.0, and 0.098 at pH 8.0. Thus K^+ complexed to **4** in essentially water solution greatly acidifies the N–H bond of the indicator system so that at pH 7.0, it is highly ionized. Runs 3 and 4 made in 25% dioxane in water provided smaller ΔA values. The maximum ΔA value for added Na^+ was 0.086 (reached at pH 9), whereas the maximum ΔA value for added K^+ was 0.352 (reached at pH 8). Run 5 made in 50% dioxane in water provides a maximum response to added Na^+ of ΔA = 0.072 at pH 9.0, whereas run 6 in the same medium shows a maximum response to K^+ of ΔA = 0.061 at pH 8.

These results provide the striking conclusion that *the greatest NH acidifying power of K^+ over that of Na^+ is observed in 99% water–1% dioxane (v/v) at pH 7.0. The acidifying power of K^+ over that of Na^+ decreases in 75% water–25% dioxane and disappears in 50% water–50% dioxane.* Control experiments demonstrated that this dramatic solvent effect is *not due to slow cation exchange between* **4**·Na^+ and **4**·K^+ but is associated with equilibria. This solvent effect runs contrary to the behavior of **1**, which shows somewhat higher acidifying power of NH by K^+ over that of Na^+ as the concentration of dioxane in water *increases* in passing from 40% dioxane to 80% dioxane in water (v/v). Thus the difference in pK_a between **1**·K^+ and **1**·Na^+ in 40% dioxane–60% water is 0.85 units, whereas the respective difference in 80% dioxane–20% water is 1.40 units, in each media **1**·K^+ being more acidic than **1**·Na^+ (see Table II). Compound **4**, present as its NaBr complex rather than as a free host, also increases its binding strength toward Na^+ and K^+ as the solvent becomes less polar. However, at very high K_a values, the chromophore cannot distinguish between K^+ complex and the original **4**·NaBr complex.

These results show that **4**·Na^+ has close to ideal characteristics for use as a chromogenic indicator system for measuring K^+ concentrations in serum: it is stable, and soluble in media over 97% by volume in water; it is operable at close to physiological pH; it is sensitive at low concentrations of K^+; it is not affected by the presence of physiological concentrations of Na^+, Ca^{2+}, and Mg^{2+} or other ions present in serum.[19]

Selectivity of Cryptahemispherand Systems 3 and 4. Selectivities were estimated using Technicon ChromoLyte reagents for potassium and sodium ions based on **3** and **4**. The ChromoLyte Potassium Reagent is an aqueous solution 0.36 mM in **4**·NaBr, pH 7.3 buffer, stabilizers, and surfactant, whereas the ChromoLyte Sodium Reagent is an aqueous solution 0.50 mM in **3**·LiBr, pH 7.5 buffer, stabilizers, and surfactant. The Technicon RA-1000 Clinical Analyzer was employed to obtain sensitivity values for each ion. The following instrumental parameters were used: sample volume for K^+, 3.5 μL, for Na^+, 4 μL; reagent volume for K^+, 350 μL, and for Na^+, 390 μL; temperature, 37 °C; path length 7 mm; wavelength, 500 nm; delay for K^+, 2 min, and for Na^+, 30 s; incubation time for K^+, none; for Na^+, 3.5 min.

Table III. Effect of pH and Dioxane Concentration in Water on Responses of the Absorbance of **4**·NaBr at 0.10 mM Concentration to the Presence of NaCl or KCl at 50 mM Concentration at 25 °C

run no.	dioxane in water, % (v/v)	kind of added salt	ΔA[a] values at λ = 450 nm at various pH's MES:[b] 6.0	MES:[b] 6.6	HEPES:[c] 7.0	HEPES:[c] 8.0	CHES:[d] 9.0
1	1	NaCl	0.005	0.000	0.002	0.026	0.000
2	1	KCl	0.003	0.414	0.512	0.098	0.006
3	25	NaCl	0.002	0.001	0.000	0.016	0.086
4	25	KCl	0.014	0.035	0.018	0.352	0.175
5	50	NaCl	0.002	0.000	0.013	0.039	0.072
6	50	KCl	0.032	0.012	0.007	0.061	0.005

[a] ΔA = A of added salt − A of no added salt. [b] MES is $O(CH_2CH_2)_2NCH_2CH_2SO_3H$ buffer at 0.10 M concentration. [c] HEPES is $HOCH_2CH_2N(CH_2CH_2)_2NCH_2CH_2SO_3H$ at 0.10 M concentration. [d] CHES is $CH_2(CH_2CH_2)_2NCH_2CH_2SO_3H$ at 0.10 M concentration.

Selectivity for the potassium reagent (based on **4**) was estimated from the ratio of potassium sensitivity to sodium sensitivity (in water) in which sensitivity equals the slope of the response line for each ion. Eight aqueous solutions, each 140 mM in NaCl but varying in KCl concentrations between 1.0 and 10.0 mM, gave ΔA values ($A_{sample} - A_{blank}$) that varied from 0.0035 to 0.4575 absorbance units to provide a linear plot of $[K^+]$ vs ΔA whose least-squares slope was 47.2 mA/mM. Twelve aqueous solutions, each 4 mM in KCl but varying in NaCl concentrations between 0 and 200 mM, gave ΔA values that varied from 0.1907 to 0.1969 absorbance units to provide a linear plot of $[Na^+]$ vs ΔA whose least-squares slope was 0.0306 mA/mM. Thus potassium ion selectivity = 47.2/0.0306 ~ 1500.

Similar attempts were also made to determine sodium ion selectivity by comparing the sensitivity toward each ion obtained in a rate reaction. Nine aqueous solutions, each 4 mM in KCl but varying from 0 to 200 mM in NaCl, gave ΔA values ($A_{3.5min} - A_{0.5min}$) that varied from 0.0029 to 0.5014 absorbance units to provide a linear plot of $[Na^+]$ vs ΔA whose least-squares slope was 2.54 mA/mM. Five aqueous solutions (no NaCl present) varying in KCl concentrations from 0 to 200 mM gave ΔA values ($A_{3.5min} - A_{0.5min}$) that varied from 0.0026 to 0.0048 (absorbance precision: one standard deviation = 0.002 absorbance units). Since these latter data are within the noise level of the instrument, we conclude that an accurate determination of the Na^+ over K^+ selectivity is not possible. However, an estimate can be made. The selectivity relationship of **4** for K^+ over Na^+ of 1500 in water compares with the value of $K_a^{K^+}/K_a^{Na^+} \sim 10000$ for parent compound **10** in $CHCl_3$. The value of $K_a^{Na^+}/K_a^{K^+} \sim 10000$ for parent compound **9** in $CHCl_3$ suggests that, by analogy, the selectivity of **3** for Na^+ over K^+ in water should be over 1000 as well.

Experimental Section

General Procedures. Tetrahydrofuran (THF) and Et_2O were distilled under N_2 from sodium benzophenone ketyl; CH_2Cl_2 was distilled and redistilled from CaH_2 when dryness was required; and benzene was distilled from $LiAlH_4$. Dimethylformamide (DMF) and dimethylacetamide (DMA) were allowed to stand over 3-Å molecular sieves activated by heating to 360 °C for 5 h and cooling in a dessicator. Dimethyl sulfoxide (DMSO) was stirred with activated alumina for 2 h, refluxed over CaO for 4 h under argon, cooling and stirred (12 h) with CaH_2, and finally distilled at 43–45 °C (0.1 mm). All reactions were conducted under an argon atmosphere. Column chromatography was performed using silica gel 60 (E. M. Merck, particle size, 0.063–0.200 mm, 70–230 mesh ASTM). Preparative thin-layer chromatography employed 2-mm silica gel plates (E. M. Merck, 60 F_{254}). Thin-layer chromatography was conducted on plastic-backed, precoated silica gel plates (E. M. Merck F_{254}, 0.2-mm thickness). Dry columns were packed with silica gel (E. M. Merck 60 F_{254}). Melting points were recorded on a Mel-Temp or Thomas-Hoover melting point apparatus and are uncorrected. Mass spectra were recorded on a Kratos AE-1 Model MS-9 double-focusing spectrometer at 16 or 70 eV at inlet temperatures indicated, and FABMS were determined on a ZABSE instrument. ^1H NMR spectra were recorded at 200 MHz on a Bruker WP-200 spectrometer. Chemical shifts refer to Me_4Si as an internal standard. The UV–visible spectra were recorded on a Beckman DU-8 spectrophotometer. An Orion 601-A digital ion analyzer was used in the pH measurements.

2-Bromo-6-(ethoxymethyl)-4-methylethoxybenzene (12). To a solution of 40 g (0.18 mol) of 2-bromo-6-(hydroxymethyl)-4-methylphenol (**11**)[11] in 1 L of THF under argon at 0 °C was added 30 g (0.63 mol) of NaH (50% in mineral oil). After the mixture was warmed to 25 °C, 94 g (0.61 mol) of diethyl sulfate was added, the mixture was refluxed 18 h and cooled to 0 °C, and CH_3OH was added to decompose the excess NaH. Concentration of the mixture of 200 mL and dilution with $CHCl_3$ (0.5 L) and saturated aqueous NaCl (0.6 L) gave an organic layer that was dried and evaporated. The residue was dissolved in 100 mL of cyclohexane and chromatographed on silica gel (500 g). Elution of the column with benzene–cyclohexane (1:4) gave 33 g (66%) of **12** as a colorless oil. The mass spectrum (70 eV) gave a molecular ion at m/e 272 ($-Br^{79}$). ^1H NMR (200 MHz, $CDCl_3$): δ 1.25 (t, CH_2CH_3, 3 H), 1.43 (t, CH_2CH_3, 3 H), 2.29 (s, $ArCH_3$, 3 H), 3.98 (q, OCH_2, 2 H), 4.5 (q, OCH_2, 2 H), 7.16 (d, Ar*H*, 1 H), 7.29 (d, Ar*H*, 1 H). Anal. Calcd for $C_{12}H_{17}BrO_2$: C, 52.76; H, 6.27. Found: C, 52.66; H, 6.30.

3,3′-Dilodo-2,2′-dihydroxy-1,1′-biphenyl (15). To a solution of 20 g (43 mmol) of 3,3′-diiodo-2,2′-dimethoxy-1,1′-biphenyl[12] in 1 L of CH_2Cl_2 at −10 °C was added 37 g (0.15 mol) of BBr_3. The mixture was warmed to 25 °C, stirred 6 h, and cooled to 0 °C, and the excess BBr_3 was decomposed by dropwise addition of H_2O. Addition of 400 mL of H_2O and extractive workup gave the crude product, which was recrystallized from CH_2Cl_2–cyclohexane (300 mL of 1:2) to give 17.5 g (93%) of **15** as a white solid, mp 157–158 °C. The mass spectrum (70 eV) showed a molecular ion at m/e 438. ^1H NMR (200 MHz, $CDCl_3$): δ 5.87 (s, O*H*, 2 H), 6.80 (t, Ar*H*, 2 H), 7.22 (m, Ar*H*, 2 H), 7.75 (m, Ar*H*, 2 H). Anal. Calcd for $C_{12}H_8I_2O_2$: C, 32.91; H, 1.84. Found: C, 32.90; H, 1.89.

3,3′-Diiodo-2-ethoxy-2′-hydroxy-1,1′-biphenyl (16). To a mixture of 16.5 g (37.7 mmol) of **15** and 21.5 g (0.14 mol) of ethyl iodide in 110 mL of acetone under argon at 25 °C was added 5.5 g (39.8 mmol) of K_2CO_3, and the suspension was stirred for 72 h. The acetone and excess ethyl iodide were evaporated, and the residue was partitioned between CH_2Cl_2 (400 mL) and 10% aqueous NaCl (500 mL). Extractive workup and concentration of the organic solution to 50 mL was followed by chromatography of the solution on 300 g of alumina (MCB, activated) made up in benzene. Elution of the column with benzene gave 48 g (26%) of byproduct 3,3′-diiodo-2,2′-diethoxy-1,1′-biphenyl as a white foam. Further elution of the column with ethyl ether gave 12.3 g (70%) of **16** as a white foam. The mass spectrum gave a molecular ion (70 eV) at m/e 466. ^1H NMR (200 MHz, $CDCl_3$): δ 1.23 (t, CH_2CH_3, 3 H), 3.73 (q, OCH_2, 2 H), 6.77 (t, Ar*H*, 1 H), 6.97 (t, Ar*H*, 1 H), 7.30 (m, Ar*H*, 2 H), 7.82 (m, Ar*H*, 2 H). Anal. Calcd for $C_{14}H_{12}I_2O_2$: C, 36.08; H, 2.60. Found: C, 35.89; H, 2.56.

3,3′-Diiodo-5-nitro-2′-ethoxy-2-hydroxy-1,1′-biphenyl (17). To a solution of 1.05 g of **16** in 40 mL of CH_3CO_2H was added 0.5 mL of 70% HNO_3. The mixture was stirred 30 min and diluted with 20 mL of H_2O, and the resulting suspension was stirred 2 h at 25 °C, filtered, and dried at 25 °C under vacuum. This material was recrystallized from CH_2Cl_2–C_2H_5OH to give 640 mg (56%) of **17** as pale yellow crystals, mp 141–142 °C. The mass spectrum (70 eV) gave the expected molecular ion at m/e 511. ^1H NMR (200 MHz, $CDCl_3$): δ 1.27 (t, CH_3, 3 H), 3.82 (q, CH_2, 2 H), 7.05 (t, Ar*H*, 1 H), 7.36 (m, Ar*H*, 1 H), 7.93 (m, Ar*H*, 1 H), 8.26 (d, Ar*H*, 1 H), 8.71 (d, Ar*H*, 1 H). Anal. Calcd for $C_{14}H_{11}I_2NO_4$: C, 32.90; H, 2.17. Found: C, 32.78; H, 2.25.

3,3′-Dliodo-2,2′-diethoxy-5-nitro-1,1′-biphenyl (18). A suspension of 0.61 g (1.2 mmol) of **17**, 2.0 g (13 mmol) of Et_2SO_4, and 2.5 g of K_2CO_3 in 75 mL of acetone under N_2 was refluxed 8 h and evaporated under reduced pressure, the residue was dissolved in 10% NH_4OH and $CHCl_3$ (300 mL of each) and stirred 1 h, and the layers were separated. The organic extract was dried, concentrated to 10 mL, and added to an Al_2O_3 column (50 g) made up in benzene. Elution of the column with benzene (1 L) gave 594 mg (92%) of **18** as a colorless glass. The mass spectrum (70 eV) gave the expected molecular ion at m/e 539. ^1H NMR (200 MHz, $CDCl_3$): δ 1.13–1.20 (m, CH_3, 6 H), 3.61–3.74 (m, CH_2, 4 H), 6.93 (t, Ar*H*, 1 H), 7.37 (m, Ar*H*, 1 H), 7.87 (m, Ar*H*, 1 H), 8.30 (d, Ar*H*, 1 H), 8.67 (d, Ar*H*, 1 H). Anal. Calcd for $C_{12}H_{15}I_2NO_2$: C, 35.65; H, 2.80. Found: C, 35.77; H, 2.79.

3,3‴-Bis(ethoxymethyl)-5,5‴-dimethyl-5′-nitro-2,2′,2″,2‴-tetraethoxy-1,1′:3′,1″:3″,1‴-quaterphenyl (19). The compound 2-ethoxy-3-(ethoxymethyl)-5-methylphenylboronic acid (**13**) was prepared as follows. To a solution of 29 g (106 mmol) of **12** in 400 mL of THF under argon at −78 °C was added 45 mL of 2.4 M BuLi (hexane). After the solution was stirred 8 min, the lithiation solution was cannulated over 8 min into 96 g (0.92 mol) of trimethyl borate in 250 mL of THF at −78 °C. The mixture was stirred 30 min at −78 °C, warmed to 0 °C over 45 min, diluted with 400 mL of 2 N hydrochloric acid, and stirred 1 h at 25 °C. Ether (0.5 L) was added, the mixture was stirred 6 h at 25 °C, and the layers were separated. The aqueous layer was extracted with fresh ether (2 × 200 mL). The combined ether extracts were extracted with 3 N aqueous NaOH (4 × 200 mL). The base extracts were cooled to 5 °C and acidified to pH 1 with concentrated hydrochloric acid. Extraction of the aqueous solution with ether (3 × 200 mL), and evaporation of the ether extracts (no drying) at 25 °C (30 mm) gave ~20 g (80%) of **13** as a moist oil, which was stored at 5 °C and used without further purification.

A mixture of 539 mg (1.0 mmol) of diiodide **18**, 1.2 g (5.5 mmol) of boronic acid **13**, 50 mg (0.04 mmol) of tetrakis(triphenylphosphine) palladium, 5 mL of EtOH, 10 mL of 2 M aqueous Na_2CO_3, and 20 mL of benzene under argon was refluxed for 8 h, cooled to 25 °C, and diluted with benzene (100 mL) and 10% NaCl (300 mL).[13] The organic layer was dried, concentrated to 15 mL, and added to an alumina column (75 g) made up in 1:1 benzene–hexane. Elution of the column with benzene (2 L) gave 528 mg (79%) of **19**, mp 93–95 °C. The mass spectrum gave the expected molecular ion at m/e 671. ^1H NMR (200 MHz, $CDCl_3$): δ 0.77–1.32 (m, CH_2CH_3, 18 H), 2.34 (s, $ArCH_3$, 3 H), 2.35 (s, $ArCH_3$, 3 H), 3.47–3.68 (m, OCH_2CH_3, 12 H), 4.58 (s, $ArCH_2$, 4 H), 7.24–7.44 (m, Ar*H*, 7 H), 8.27 (m, ArH, 2 H). Anal. Calcd for $C_{40}H_{49}NO_8$: C,

71.51; H, 7.35. Found: C, 71.51; H, 7.21.

5′-Amino-3,3‴-bis(ethoxymethyl)-5,5‴-dimethyl-2,2′,2″,2‴-tetraethoxy-1,1′:3′,1″:3″,1‴-quarterphenyl (20). To a mixture of 740 mg (1.1 mmol) of **19** in 20 mL of C_6H_6 and 20 mL of aqueous 1 N NaOH under argon was added 0.53 g (2.7 mmol) of $Fe(CO)_5$.[14] The mixture was stirred for 8 h at 25 °C, 100 mL of C_6H_6 was added, and the suspension was filtered through Celite. The benzene layer was dried (K_2CO_3), concentrated to 20 mL, and added to an alumina column (100 g made up in CH_2Cl_2). Elution of the column with 9:1 and 4:1 CH_2Cl_2–Et_2O mixtures (2 L each) gave 550 mg (78%) of **20** as a light brown foam. The mass spectrum (70 eV) gave the expected molecular ion at *m/e* 641. ^1H NMR (200 MHz, $CDCl_3$): δ 0.70–1.31 (m, CH_2CH_3, 18 H), 2.32 (s, $ArCH_3$, 3 H), 2.33 (s, $ArCH_3$, 3 H), 3.34–3.71 (m, OCH_2CH_3, 12 H), 4.59 (s, $ArCH_2$, 4 H), 6.70–6.80 (m, Ar*H*, 2 H) and 7.07–7.43 (m, Ar*H*, 7 H). Anal. Calcd for $C_{40}H_{51}NO_6$: C, 74.85; H, 8.01. Found: C, 74.65; H, 7.92.

3,3‴-Bis(ethoxymethyl)-5,5‴-dimethyl-2,2′,2″,2‴-tetraethoxy-5′-(2,4,6-trinitroanilino)-1,1′:3′,1″:3″,1‴-quaterphenyl (21). A mixture of 550 mg (0.86 mmol) of **20**, 250 mg (1.14 mmol) of picryl chloride (CTC Organics), and 72 mg (0.86 mmol) of $NaHCO_3$ in 45 mL of CH_3OH under argon at 25 °C was stirred for 4 h and evaporated at 30 °C (20 mm), and the residue was dissolved in $CHCl_3$–H_2O (100 mL of each). The $CHCl_3$ layer was dried, concentrated to 10 mL, and added to a silica gel column (75 g) made up in CH_2Cl_2. Elution of the column with 500 mL of CH_2Cl_2 gave unreacted picryl chloride. Further elution with 19:1 CH_2Cl_2–Et_2O (1 L) gave 700 mg (92%) of **21** as an orange foam. The mass spectrum gave the expected molecular ion at *m/e* 850. ^1H NMR (200 MHz, $CDCl_3$): δ 0.76–1.31 (m, CH_2CH_3, 18 H), 2.33 (s, $ArCH_3$, 3 H), 2.34 (s, $ArCH_3$, 3 H), 3.51–3.71 (m, OCH_2CH_3, 12 H), 4.58 (s, $ArCH_2$, 4 H), 7.08–7.38 (m, ArH, 9 H), 9.06 (s, Ar*H* (picryl), 2 H), 10.32 (s, NH, 1 H). Anal. Calcd for $C_{46}H_{52}N_4O_{12}$: C, 64.78; H, 6.14. Found: C, 64.66; H, 6.12.

3,3‴-Bis(ethoxymethyl)-5,5‴-dimethyl-5′-[2,4-dinitro-6-(trifluoromethyl)anilino]-2,2′,2″,2‴-tetraethoxy-1,1′:3′,1″:3″,1‴-quaterphenyl (22). The procedure used to conert **20** to **21** was used to convert **20** to **22** except that 2,4-dinitro-6-[(trifluoromethyl)chloro]benzene was substituted for picryl chloride. The product, **22** was purified by column chromatography on silica gel with 30:60 pentane–ethyl acetate (v/v) as the mobile phase. The product, **22**, was isolated as a yellow foam in 86% yield. MS (70 eV): *m/e* 876 (M^+). ^1H NMR (200 MHz, $CDCl_3$): δ 0.60–1.40 (m, CH_2CH_2, 18 H), 2.32 (s, $ArCH_3$, 6 H), 3.25–3.80 (m, CH_2O, 12 H), 4.57 (s, $ArCH_2$, 4 H), 6.74 (s, N*H*, 1 H), 6.96–7.50 (m, ArH, 9 H), 8.65 (d, ArH, 1 H), 8.89 (d, ArH, 1 H). Anal. Calcd for $C_{47}H_{52}F_3N_3O_{10}$: C, 64.45; H, 5.98. Found: C, 64.41; H, 6.12.

3,3‴-Bis(bromomethyl)-5,5‴-dimethyl-2,2′,2″,2‴-tetraethoxy-5′-(2,4,6-trinitroanilino)-1,1′:3′,1″:3″,1‴-quaterphenyl (23). Anhydrous HBr gas was bubbled into a solution of 700 mg (0.82 mmol) of **21** in 250 mL of $CHCl_3$ for 10 min. After the solution was stirred an additional 10 min, the solution was poured into 800 mL of H_2O and stirred an additional 30 min. The organic layer was dried, concentrated to 10 mL, and flash chromatographed on 60 g of silica gel made up in CH_2Cl_2. Elution of the column with CH_2Cl_2 gave 720 mg (95%) of **23** as an orange foam. The mass spectrum (70 eV) showed a molecular ion at *m/e* 920 (^{79}Br). ^1H NMR (200 MHz, $CDCl_3$): δ 0.77–1.27 (m, CH_2CH_3, 12 H), 2.33 (s, $ArCH_3$, 6 H), 3.48–3.76 (m, OCH_2CH_3, 8 H), 4.62 (s, $ArCH_2$, 4 H), 7.08–7.40 (m, Ar*H*, 9 H), 9.07 (s, Ar*H* (picryl), 2 H), 10.32 (s, NH, 1 H). Anal. Calcd for $C_{42}H_{42}Br_2N_4O_{10}$: C, 54.68; H, 4.59. Found: C, 54.73; H, 4.59.

3,3‴-Bis(bromomethyl)-5,5‴-dimethyl-5′-[2,4-dinitro-6-(trifluoromethyl)anilino]-2,2′,2″,2‴-tetraethoxy-1,1′:3′,1″:3″,1‴-quaterphenyl (24). The procedure used to convert **21** to **23** was used to transform **22** to **24** in 93% yield. ^1H NMR (200 MHz, $CDCl_3$): δ 0.6–1.40 (m, CH_2CH_3, 12 H), 2.31 (s, $ArCH_3$, 6 H), 3.27–3.95 (m, OCH_2, 8 H), 4.60 (s, CH_2Br, 4 H), 6.93–7.50 (m, ArH, 9 H), 7.68 (s, N*H*, 1 H), 8.67 (d, ArH, 1 H), 8.90 (d, Ar*H*, 1 H). Anal. Calcd for $C_{43}H_{42}Br_2F_3N_3O_8$: C, 54.62; H, 4.48. Found: C, 54.64; H, 4.45.

(20*R*,23*S*)-14,29-Dimethyl-31,33,34,35-tetraethoxy-4-(2,4,6-trinitroanilino)-18,25,32-trioxahexacyclo[25.3.1.1^{2,6}.1^{7,11}.1^{12,16}.1^{20,23}]pentatriaconta-1(31),2,4,6(35),7,9,11(34),12,14,16(33),27,29-dodecaene (Racemate) (1). To a refluxing suspension of 1.8 g (37.5 mmol) of NaH (50% mineral oil) in 150 mL of THF under argon was added a solution of 0.75 g (0.81 mmol) of **23** and 115 mg (0.87 mmol) of *cis*-2,5-bis(hydroxymethyl)tetrahydrofuran[15] in 400 mL of THF over 8 h. The mixture was refluxed an additional 10 h and cooled to 25 °C, excess NaH was decomposed with CH_3OH, and the solvent was evaporated at 30 °C (30 mm). The residue was dissolved in 500-mL portions of $CHCl_3$ and 10% aqueous NaCl. The aqueous layer was acidified to pH 1 with 6 N HCl (aqueous), and the organic layer was dried, concentrated to 15 mL, and added to a silica gel column (100 g) made up in CH_2Cl_2. Elution of the column with CH_2Cl_2 (1.5 L) gave 278 mg (37%) of **23**. Further elution of the column with 9:1 and 3:1 CH_2Cl_2–$(CH_3)_2CO$ mixtures (2 L of each) gave after evaporation under vacuum a red oil. Pentane was added to this oil to precipitate product, which was washed with pentane to give 110 mg of **1** as an orange-red foam. The mass spectrum (70 eV) gave the expected molecular ion at *m/e* 892. A FAB mass spectrum (*m*-nitrobenzyl alcohol dispersion) gave a molecular ion as well as M + 18 (M + H_2O), M + 23 (M + Na), and M + 39 (M + K). ^1H NMR (200 MHz, $CDCl_3$): δ 0.48–1.13 (m, CH_2CH_3, 12 H), 1.80–2.33 (m, $ArCH_3$, CH_2CH_2, 10 H), 3.30–4.82 (m, OCH_2, CH_2CHO, 18 H), 7.03–7.28 (m, Ar*H*, 9 H), 9.09 (s, Ar*H* (picryl), 2 H), 10.36 (s, NH, 1 H). Anal. Calcd for $C_{48}H_{52}N_4O_{13}\cdot C_5H_{12}$ (pentane): C, 65.96; H, 6.68. Found: C, 65.79, 65.71; H, 6.75, 6.82.

(20*R*,23*S*)-14,29-Dimethyl-4-[2,4-dinitro-6-(trifluoromethyl)anilino]-31,33,34,35-tetraethoxy-18,25,32-trioxahexacyclo[25.3.1.1^{2,6}.1^{7,11}.1^{12,16}.1^{20,23}]pentatriaconta-1(31),2,4,6(35),7,9,11(34),12,14,16(33),27,29-dodecaene (Racemate) (2). The procedure used to convert **23** to **1** was applied to the transformation of **24** to **2** in 19% yield (orange-red foam). The FABMS (thioglycerol dispersion) gave M^+ at *m/e* 915, as well as $(M + 18)^+(2\cdot H_2O)$, $(M + 23)^+(2\cdot Na)^+$, $(M + 39)^+(M + K)^+$, and $(M + 55)^+(M + Fe)$. ^1H NMR (200 MHz, $CDCl_3$): δ 0.45–1.10 (m, CH_2CH_3, 12 H), 1.80–2.33 (m, $ArCH_3$, CH_2CH_2, 10 H), 3.30–4.78 (m, CH_3CH_2, CH_2O, CHO, 18 H), 6.97–7.28 (m, Ar*H*, 9 H), 7.72 (s, N*H*, 1 H), 8.68 (d, Ar*H*, 1 H), 8.91 (d, Ar*H*, 1 H). Anal. Calcd for $C_{49}H_{52}F_3N_3O_{11}\cdot 0.5C_5H_{12}$: C, 64.97; H, 6.14. Found: C, 65.13, 65.18, 65.12; H, 6.30, 6.41, 6.13.

2-Bromo-6-(methyloxymethyl)-4-methylanisole (25). To a solution of 2-bromo-6-(hydroxymethyl)-4-methylphenol (**11**)[11] (23.4 g, 0.11 mol) in THF (600 mL) under argon at 0 °C was added NaH (60% in mineral oil, 15.2 g, 0.38 mol). The mixture was warmed to room temperature, Me_2SO_4 (45.7 g, 0.36 mol) was added, and the mixture was refluxed over 18 h. After cooling the mixture to 0 °C, methanol was added to decompose excess NaH. The solvent was removed in vacuo to give a residue, which was column chromatographed (flash) on silica gel with benzene–cyclohexane (1:4 to 1:1 by volume) to afford 23.7 g (90%) of **25** as a colorless liquid, bp 104–106 °C (1 mm). Anal. Calcd for $C_{10}H_{13}BrO_2$: C, 49.00; H, 5.35. Found: C, 49.11; H, 5.34.

2,6-Bis[3-(methoxymethyl)-2-methoxy-5-methylphenyl]-4-nitroanisole (28). Starting material 2,6-diiodo-4-nitroanisole[16] (**27**) was prepared as follows. To a solution of 39 g (0.1 mol) of 2,6-diiodo-4-nitrophenol (iodination of commerically available *p*-nitrophenol via literature preparation)[16] were added 28 g (0.2 mol) of K_2CO_3 and 40 g (0.28 mol) of iodomethane in 500 mL of dimethylformamide under argon, and the solution was heated at 70 °C for 60 h. Portions (15 g) of K_2CO_3 and CH_3I were added to the mixture four times after 12, 24, 36, and 48 h of reaction times. The mixture was cooled to 25 °C, $CHCl_3$ (600 mL) and H_2O (1.2 L) were added, the layers were separated, and the organic layer was extracted with H_2O (2 × 1 L). The organic layer was dried, concentrated to 60 mL, and chromatographed on 500 g of Al_2O_3 made up in CH_2Cl_2. Elution of the column with CH_2Cl_2 (2 L) gave 17 g (42%) of **27**,[16] mp 133–135 °C (EtOH). ^1H NMR (200 MHz, $CDCl_3$): δ 3.94 (s, OCH_3, 3 H), 8.64 (s, Ar*H*, 2 H). This material was used without further purification.

The other reaction component, 2-methoxy-3-(methoxymethyl)-5-methylphenylboronic acid (**26**) was prepared as follows. To a solution of 52 g (0.2 mol) of **25** in 500 mL of THF under argon at −78 °C was added 83 mL of 2.6 M BuLi (hexane). After stirring 6 min, the lithiation solution was cannulated over 20 min into 137 g (1.3 mol) of trimethyl borate in 500 mL of THF at −78 °C. The mixture was stirred 30 min at −78 °C, warmed to 0 °C over 45 min, diluted with 300 mL of 2 N hydrochloric acid, and stirred 10 h at 25 °C. Ether (0.6 L) was added, the mixture was stirred 6 h at 25 °C, and the layers were separated. The aqueous layer was extracted with fresh ether (2 × 200 mL). The combined ether extracts were extracted with 3 N aqueous NaOH (4 × 200 mL). The basic extracts were cooled to 5 °C, acidified to pH 1 with concentrated hydrochloric acid, and extracted with ether (4 × 200 mL). Evaporation of the ether extracts at 25 °C (30 mm) gave 28 g (63%) of **26** as a colorless solid, mp 52–54 °C, which was stored at 5 °C and used without further purification. ^1H NMR (200 MHz, $(CD_3)_2CO$): δ 2.29 (s, $ArCH_3$, 3 H), 3.38 (s, OCH_3, 3 H), 3.80 (s, OCH_3, 3 H), 4.45 (s, $ArCH_2$, 2 H), 7.29 (d, ArH, 1 H), 7.52 (d, Ar*H*, 1 H).

Compounds **26** and **27** were submitted to the Suzuki coupling reaction[13] to give **28** as follows. To a mixture of 9.0 g (43 mmol) of **26** and 6.8 g (16.8 mmol) of **27** in 130 mL of benzene and 33 mL of ethanol under argon was added 66 mL of 2 M aqueous Na_2CO_3. To this vigorously stirred two-phase mixture was added 0.6 g (0.52 mmol) of tetrakis(triphenylphosphine)palladium(0). The mixture was refluxed 48 h (100 mg of fresh catalyst was added after 24 h). The layers were separated, and the organic layer was dried, concentrated to 40 mL, and added to an Al_2O_3 column (400 g) made up in benzene. Elution of the column with benzene and CH_2Cl_2 gave 7.87 g (97%) of **28** as a colorless

foam. The mass spectrum (70 eV) gave the expected molecular ion at m/e 481. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.36 (s, ArCH_3, 6 H), 3.30 (s, OCH_3, 3 H), 3.47 (s, OCH_3, 6 H), 3.49 (s, OCH_3, 6 H), 4.54 (s, ArCH_2, 4 H), 7.12 (d, ArH, 2 H), 7.28 (d, ArH, 2 H), 8.25 (s, ArH, 2 H). Anal. Calcd for $C_{27}H_{31}NO_7$: C, 67.35; H, 6.49. Found: C, 67.27; H, 6.38.

4-Amino-2,6-bis[3-(methoxymethyl)-2-methoxy-5-methylphenyl]anisole (29). To a mixture of 4.57 g (9.5 mmol) of **28** in 150 mL of benzene and 150 mL of 1 N aqueous NaOH under argon was added 4.5 g (23 mmol) of $Fe(CO)_5$.[14] The mixture was stirred 20 h at 25 °C, 300 mL of benzene was added, and the suspension was filtered through Celite. The benzene layer was dried (K_2CO_3), concentrated to 40 mL, and added to a silica gel column (150 g) made up in CH_2Cl_2. Elution of the column with 10% ether–90% CH_2Cl_2 gave traces of unidentified material. Further elution with CH_3OH–CH_2Cl_2 mixtures (1–2% CH_3OH) gave 3.8 g (89%) of **29** as a yellow foam. The mass spectrum (70 eV) gave the expected molecular ion at m/e 451. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.33 (s, ArCH_3, 6 H), 3.14 (s, OCH_3, 3 H), 3.45 (s, OCH_3, 6 H), 3.51 (s, OCH_3, 6 H), 4.54 (s, ArCH_2, 4 H), 6.70 (s, ArH, 2 H), 7.13 (s, ArH, 2 H), 7.19 (s, ArH, 2 H). Anal. Calcd for $C_{27}H_{33}NO_5$: C, 71.82; H, 7.37. Found: C, 71.75; H, 7.56.

2,6-Bis[3-(methoxymethyl)-2-methoxy-5-methylphenyl]-4-(2,4,6-trinitroanilino)anisole (30). A mixture of **29** (2.75 g, 6.1 mmol), picryl chloride (2.00 g, 8.1 mmol) and $NaHCO_3$ (0.51 g, 6.1 mmol) in methanol (325 mL) under argon, at room temperature, was stirred overnight. The solvent was removed in vacuo, at room temperature, and the residue was dissolved in a $CHCl_3$–H_2O mixture (100 mL each). The chloroform layer was dried ($MgSO_4$), concentrated to 10 mL, and column chromatographed (flash) on silica gel with petroleum ether–ethyl acetate (2:1 by volume) to give 3.82 g (95%) of **30** as an orange-red foam.

The mass spectrum (70 eV) gave the expected molecular ion at m/e 662. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35 (s, ArCH_3, 6 H), 3.23 (s, OCH_3, 3 H), 3.46 (s, OCH_3, 6 H), 3.53 (s, OCH_3, 6 H), 4.53 (s, ArCH_2, 4 H), 7.09–7.25 (m, ArH, 6 H), 9.08 (s, ArH, 2 H), 10.29 (s, NH, 1 H). Anal. Calcd for $C_{33}H_{34}N_4O_{11}$: C, 59.81; N, 5.17. Found: C, 59.86; H, 5.36%.

2,6-Bis[3-(bromomethyl)-2-methoxy-5-methylphenyl]-4-(2,4,6-trinitroanilino)anisole (31). Anhydrous HBr gas was bubbled into a solution of 3.0 g (4.5 mmol) of **30** in 300 mL of $CHCl_3$ for 10 min. After stirring an additional 10 min, the solution was poured into 800 mL of H_2O and stirred an additional 30 min. The organic layer was dried, concentrated to 20 mL, and flash chromatographed on 100 g of silica gel made up in CH_2Cl_2. Elution of the column with CH_2Cl_2 gave 3.2 g (93%) of **31** as an orange foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.34 (s, ArCH_3, 6 H), 3.23 (s, OCH_3, 3 H), 3.62 (s, OCH_3, 6 H), 4.61 (s, ArCH_2, 4 H), 7.09–7.24 (m, ArH, 6 H), 9.09 (s, ArH, 2 H), 10.30 (s, NH, 1 H). Anal. Calcd for $C_{31}H_{28}Br_2N_4O_9$: C, 48.97; H, 3.71. Found: C, 48.70; H, 3.78%.

5,15-Dimethyl-36,37,38-trimethoxy-10-(2,4,6-trinitroanilino)-22,25,30,33-tetraoxa-1,19-diazapentacyclo[17.8.8.1^{3,7}.1^{8,12}.1^{13,17}]octatriaconta-3,5,7(38),8,10,12(37),13,15,17(36)-nonaene (4) as 4·NaBr. To a vigorously stirred acetonitrile (120-mL) mixture containing anhydrous sodium carbonate (0.85 g, 8 mmol) was added at reflux over a period of 20 h 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane ([1.2]kryptafix, E. Merck) (0.42 g, 1.6 mmol) in CH_3CN (35 mL) and dibromide **31** (1.22 g, 1.6 mmol) in CH_3CN (35 mL). After the addition was completed, reflux was continued for an additional 15 h, and then the solvent was removed in vacuo at room temperature. The residue was column chromatographed (flash) on silica gel with CH_2Cl_2–CH_3OH (95:5 to 90:10 by volume) to give 1.30 g (84%) of **4**·NaBr as a dark red glass. A small amount of **4**·KBr was also isolated as a deep red glass. A FAB mass spectrum of **4**·NaBr (*m*-nitrobenzyl alcohol dispersion) gave no peak for the free ligand, but a base peak at m/e 883 (M + 23) corresponding to the M + Na^+ ion and a lower intensity ion at 899 (M + 39) corresponding to M + K^+. ^{1}H NMR (200 MHz, $CDCl_3$) of **4**·NaBr: δ 2.36 (s, ArCH_3, 6 H), 2.84 (s, OCH_3, 3 H), 3.48 (s, OCH_3, 6 H), 2.18–4.10 (m, NCH_2, OCH_2, 24 H), 2.67 (d, ArCH_2N, 2 H), 4.20 (d, ArCH_2N, 2 H), 7.03 (d, ArH, 2 H), 7.12 (d, ArH, 2 H), 7.17 (s, ArH, 2 H), 9.09 (s, ArH, 2 H). Anal. Calcd for $C_{43}H_{52}N_6O_{13}$·NaBr: C, 53.59; H, 5.44. Found: C, 53.38; H, 5.70. Anal. Calcd for $C_{43}H_{52}N_6O_{13}$·KBr: C, 52.71; H, 5.35. Found: C, 52.62; H, 5.51.

5,15-Dimethyl-30,31,32-trimethoxy-10-(2,4,6-trinitroanilino)-22,27-dioxa-1,19-diazapentacyclo[17.5.5.1^{3,7}.1^{8,12}.1^{13,17}]dotriaconta-3,5,7-(32),8,10,12(31),13,15,17(30)-nonaene (3) as 3·LiBr. To a vigorously stirred acetonitrile mixture (100 mL) containing anhydrous Li_2CO_3 (0.49 g, 6.6 mmol) was added at reflux over a period of 20 h 1,7-diaza-4,10-dioxadodecane[8] (0.22 g, 1.25 mmol) in CH_3CN (27 mL) and dibromide **31** (0.95 g, 1.25 mmol) in CH_3CN (27 mL). After the addition was completed, reflux was continued for an additional 15 h, the solvent was removed in vacuo at room temperature, and the residue was column chromatographed (flash) on silica gel with CH_2Cl_2–CH_3OH (95:5 by volume) to afford 0.54 g (56%) of **3**·LiBr as an orange glass, which was slightly contaminated by **3**·NaBr (10%) on average, by atomic absorption). A middle-cut fraction from the column provided an analytically pure sample of **3**·LiBr. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.36 (s, ArCH_3, 6 H), 2.53 (s, OCH_3, 3 H), 3.49 (s, OCH_3, 6 H), 2.51–4.08 (m, NCH_2, OCH_2 16 H), 3.15 (d, ArCH_2N, 2 H), 4.12 (d, ArCH_2N, 2 H), 7.05 (d, ArH, 2 H), 7.14 (d, ArH, 2 H), 7.28 (s, ArH, 2 H), 9.09 (s, ArH, 2 H). Anal. Calcd for $C_{39}H_{44}N_6O_{11}$·LiBr·2H_2O: C, 52.30; H, 5.40. Found: C, 52.57; H, 5.10.

2-(Benzyloxy)-1,3-dibromobenzene (33). A suspension of 30 g (0.12 mol) of **32**,[17] 34 g (0.2 mol) of benzyl bromide, and 30 g (0.22 mol) of anhydrous K_2CO_3 in 600 mL of acetone was refluxed for 48 h, the mixture was evaporated under reduced pressure, the residue was dissolved in $CHCl_3$ and H_2O (600 mL of each), and the layers were separated. The organic extract was dried, concentrated to 50 mL, and added to an Al_2O_3 column (400 g) made up in 1:1 cyclohexane–benzene. Elution of the column with 3 L of 1:1 (by volume) cyclohexane–benzene gave 32.6 g (80%) of **33**, mp 45–47 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.04 (s, OCH_2, 2 H), 6.86–7.66 (m, ArH, 8 H). Anal. Calcd for $C_{13}H_{10}Br_2O$: C, 45.65; H, 2.95. Found: C, 45.95; H, 3.09.

3-Iodo-2-methoxy-5-methylbenzoic Acid (36). A solution of 100 g (0.27 mol) of 2,6-diiodo-4-methylanisole (**35**)[18] in 1 L of ether under argon was cooled to −78 °C. A 110-mL portion of 2.5 M BuLi was added over 5 min and the resulting mixture stirred 10 min at −78 °C. Carbon dioxide gas was vigorously bubbled through the suspension for 20 min, and the cold bath was allowed to warm to 25 °C over 10 h. The suspension was diluted with 600 mL of 1 N aqueous NaOH, and the layers were separated. The aqueous layer was acidified with 6 N HCl, and the white solid was collected and dried at 25 °C under vacuum to give 50 g (64%) of crude **36**. ^{1}H NMR (200 MHz, $(CD_3)_2CO$): δ 2.33 (s, ArCH_3, 3 H), 3.85 (s, OCH_3, 3 H), 7.64 (d, ArH, 1 H), 7.86 (d, ArH, 1 H). Anal. Calcd for $C_9H_9IO_3$: C, 37.01; H, 3.11. Found: C, 37.26; H, 3.22.

Methyl 3-Iodo-2-methoxy-5-methylbenzoate (37). To a solution of 50 g (0.17 mol) of **36** in 400 mL of ether at 10 °C was added excess CH_2N_2 (in ether). After stirring 10 min at 25 °C, the excess CH_2N_2 was decomposed with acetic acid and the ether evaporated. The residue was dissolved in 40 mL of CH_2Cl_2 and flash chromatographed on 300 g of silica gel made up in CH_2Cl_2. Elution of the column with CH_2Cl_2 gave 47 g (90%) of **37** as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.30 (s, ArCH_3, 3 H), 3.85 (s, OCH_3, 3 H), 3.92 (s, OCH_3, 3 H), 7.59 (d, ArH, 1 H), 7.78 (d, ArH, 1 H). Anal. Calcd for $C_{10}H_{11}IO_3$: C, 39.24; H, 3.62. Found: C, 39.31; H, 3.62.

2,6-Bis(3-carbomethoxy-2-methoxy-5-methyl)phenol (39). This compound was prepared through a Suzuki coupling[13] between ester **37** and 2-(benzyloxy)-1,3-phenyldiboronic acid (**34**), whose preparation is first described. To a solution of 13.3 g (38.9 mmol) of **33** in 350 mL of THF under argon at −78 °C was added 85 mL of 1.4 M *sec*-butyllithium (cyclohexane). After stirring 8 min, the lithiation solution was cannulated over 8 min into 150 g (1.4 mol) of trimethyl borate in 350 mL of THF at −78 °C. The mixture was stirred 30 min at −78 °C, warmed to 0 °C over 1 h, diluted with 500 mL of 2 N hydrochloric acid, and stirred 1 h at 25 °C. Ether (0.8 L) was added, the mixture was stirred 8 h at 25 °C, and the layers were separated. The aqueous layer was extracted with fresh ether (2 × 200 mL). Evaporation of the ether extracts (no drying) at 25 °C (30 mm) gave ~7.8 g (91%) of **34** as a moist oil, which was stored at 5 °C and used without further purification. ^{1}H NMR (200 MHz, $(CD_3)_2CO$): δ 5.04 (s, ArCH_2, 2 H) 7.14–7.86 (m, ArH, 8 H).

To a mixture of 7.8 g (35 mmol) of **34** and 27 g (88 mmol) of **37** in 200 mL of benzene and 50 mL of ethanol under argon was added 100 mL of 2 M aqueous Na_2CO_3. To this vigorously stirred two-phase mixture was added 1.2 g (1 mmol) of tetrakis(triphenylphosphine)palladium(0), and the mixture was refluxed 48 h (note: 100 mg of fresh catalyst was added after 24 h of reflux).[13] The layers were separated, and the organic layer was dried, evaporated, and dissolved in 40 mL of CH_2Cl_2. The mixture was separated by flash chromatography on silica gel (250 g) made up in CH_2Cl_2. Elution of the column with ether–CH_2Cl_2 mixtures (1 and 2% ether, 2 L of each) gave 12.8 g (67%) of **38** as a colorless foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.32 (s, ArCH_3, 6 H), 3.57 (s, OCH_3, 6 H), 3.93 (s, OCH_3, 6 H), 4.33 (s, OCH_2, 2 H), 6.60–7.61 (m, ArH, 12 H). This compound, **38**, without further characterization was reduced to **39** as follows. A suspension of 2 g (2 mmol) of 10% palladium on carbon and 11.1 g (20.6 mmol) of **38** in 250 mL of ethyl acetate was hydrogenated (3 atm of H_2) in a Parr shaker for 2 h. The mixture was filtered, the solvent of the filtrate was evaporated, and the residue was dissolved in 30 mL of CH_2Cl_2. The product was purified by flash chromatography on silica gel (150 g) made up in CH_2Cl_2. Elution of the column with 2% ether–98% CH_2Cl_2 gave 7.1 g (77%) of **39** as a colorless foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.38

(s, ArCH$_3$, 6 H), 3.60 (s, OCH$_3$, 6 H), 3.92 (s, OCH$_3$, 6 H), 6.97–7.63 (m, ArH, 7 H). Anal. Calcd for $C_{26}H_{26}O_7$: C, 69.32; H, 5.82. Found: C, 69.44; H, 5.75.

2,2″-Dimethoxy-5,5″-dimethyl-2′-methoxy-5′-nitro[1,1′:3′,1″-terphenyl]-3,3″-dicarboxylic Acid (42). To a stirred solution of 7.1 g (15.8 mmol) of **39** in 500 mL of 1:1 $CHCl_3$–CH_3CO_2H was added 20 mL of 70% HNO_3 over 2 min. After stirring 15 min, the solution was diluted with H_2O (1.2 L) and $CHCl_3$ (200 mL), and the organic layer was extracted with H_2O (3 × 1.2 L), dried, concentrated to 25 mL, and flash chromatographed on silica gel (200 g) made up in CH_2Cl_2. Elution of the column with CH_2Cl_2 (1 L) and 49:1 CH_2Cl_2–Et_2O (3 L) gave 7.1 g (91%) of **40** as a yellow foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.42 (s, ArCH$_3$, 6 H), 3.65 (s, OCH$_3$, 6 H), 3.94 (s, OCH$_3$, 6 H), 7.36 (d, ArH, 2 H), 7.72 (d, ArH, 2 H), 8.30 (s, ArH, 2 H). Without further characterization, **40** was methylated to give **41** as follows.

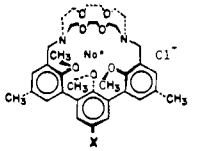
A mixture of 7.1 g (14.3 mmol) of **40**, 20 g (0.16 mol) of Me_2SO_4, and 22 g (0.16 mol) of K_2CO_3 in 500 mL of acetone under argon was refluxed 24 h and evaporated, and the residue was dissolved in 1 L of 1:1 $CHCl_3$–H_2O (v). The organic layer was dried, concentrated to 25 mL, and flash chromatographed on 200 g of silica gel made up in CH_2Cl_2. Elution of the column with CH_2Cl_2 (1 L) and 49:1 CH_2Cl_2–ether (v) (2 L) gave 6.8 g (93%) of diester **41** as a colorless foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.39 (s, ArCH$_3$, 6 H), 3.30 (s, OCH$_3$, 3 H), 3.60 (s, OCH$_3$, 6 H) 3.94 (s, OCH$_3$, 6 H), 7.34 (d, ArH, 2 H), 7.68 (d, ArH, 2 H), 8.25 (s, ArH, 2 H). Without further characterization, **41** was hydrolyzed to diacid **42** as follows.

To a solution of 8 g (15.7 mmol) of **41** in 325 mL of CH_3OH was added 100 mL of H_2O and then 12 g (0.29 mol) of LiOH·H_2O. After stirring 14 h at 25 °C, the mixture was diluted with 400 mL of H_2O and extracted with CH_2Cl_2 (2 × 50 mL), and the aqueous layer was acidified to pH 1 with concentrated aqueous HCl. The aqueous suspension was extracted with ether (3 × 300 mL), the ether was evaporated, and the residue was dried for 16 h at 95 °C (0.01 mm) to give 5.6 g (74%) of **42** as an amorphous yellow powder, mp 247–253 °C (dec). ^{1}H NMR (200 MHz, $(CD_3)_2CO$): δ 2.42 (s, ArCH$_3$, 6 H), 3.37 (s, OCH$_3$, 3 H), 3.65 (s, OCH$_3$, 6 H), 7.45 (d, ArH, 2 H), 7.75 d, ArH, 2 H), 8.25 (s, ArH, 2 H). Anal. Calcd for $C_{25}H_{23}NO_9$: C, 62.37; H, 4.81. Found: C, 62.03; H, 4.72.

5,15-Dimethyl-10-nitro-36,37,38-trimethoxy-22,25,30,33-tetraoxa-1,19-diazapentacyclo[17.8.8.1^{3,7}.1^{13,17}]octatriaconta-3,5,7(38),8,10,12-(37),13,15,17(36)-nonaene-2,18-dione (44). A suspension of 2.44 g (5 mmol) of **42** in 8 mL (110 mmol) of purified $SOCl_2$ was stirred 2 h at 25 °C under argon (**42** dissolved after ~30 min). Dry benzene (30 mL) was added and the solution evaporated at 40 °C (30 mm) to remove the excess $SOCl_2$. This procedure was repeated three times. The crude product was dried at 25 °C (0.01 mm) to give 2.6 g (~100%) of **43** as a yellow foam, which was used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.44 (s, ArCH$_3$, 6 H), 3.33 (s, OCH$_3$, 3 H), 3.66 (s, OCH$_3$, 6 H), 7.44 (d, ArH, 2 H), 8.00 (d, ArH, 2 H, 8.32 (s, ArH, 2 H).

Diacid chloride **43** (2.6 g, 5 mmol) was dissolved in 150 mL of anhydrous benzene and transferred in 50-mL portions to a 50-mL gas-tight syringe. Similarly, 1.3 g (5 mmol) of [2.2]kryptofix (E. Merck Chemicals) together with 1.5 g (15 mmol) of pure triethylamine was dissolved in 150 mL of anhydrous benzene and transferred to a 50-mL gas-tight syringe. These solutions were added via a syringe pump to an oven-dried 2-L Morton flask containing 1200 mL of anhydrous benzene over 2 h with vigorous mechanical stirring under argon at 12 °C. After stirring for 8 h at 12 °C, the suspension was warmed to 25 °C and filtered to remove triethylamine hydrochloride, and the filtrate was evaporated. The residue was dissolved in 40 mL of CH_2Cl_2 and flash chromatographed on silica gel (150 g) made up in CH_2Cl_2. Elution of the column with acetone–dichloromethane mixtures (10–30% of acetone) gave 2.1 g (60%) of **44** as a white solid, which darkens above 320 °C and melts/decomposes at ~345 °C. The mass spectrum (70 eV) showed a molecular ion at *m/e* 707. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.37 (s, ArCH$_3$, 6 H), 2.85 (s, OCH$_3$, 3 H), 3.41 (s, OCH$_3$, 6 H), 3.05–3.88 (m, NCH$_2$, OCH$_2$, 22 H), 4.30 (d, NCH$_2$, 2 H), 7.17–7.23 (m, ArH, 4 H), 8.35 (s, ArH, 2 H). Anal. Calcd for $C_{37}H_{45}N_3O_{11}$·0.5H_2O: C, 62.00; H, 6.47. Found: C, 61.89; H, 6.33.

5,15-Dimethyl-36,37,38-trimethoxy-10(2,4,6-trinitroanilino)-22,25,30,33-tetraoxa-1,19-diazapentacyclo[17.8.8.1^{3,7}.1^{8,12}.1^{13,17}]octatriaconta-3,5,7(38),8,10,12(37),13,15,17(36)-nonaene (47). A suspension of 560 mg (0.79 mmol) of **44** and 1 g of 10% palladium on charcoal in 200 mL of dimethylformamide was hydrogenated (3 atm of H_2) in a Parr shaker for 2 h. The catalyst was removed by filtration, the filtrate was diluted with $CHCl_3$ (500 mL) and H_2O (1.2 L), and the layers were separated. The organic layer was extracted with fresh H_2O (3 × 1.2 L), dried (K_2CO_3) and evaporated to give 520 mg (97%) of amine **45** as a colorless foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.32 (s, ArCH$_3$, 6 H), 2.66 (s, OCH$_3$, 3 H), 3.41 (s, OCH$_3$, 6 H), 3.06–3.96 (m, NCH$_2$, OCH$_2$, 22 H), 4.28 (d, NCH$_2$, 2 H), 6.80 (s, ArH, 2 H), 7.08 (s, ArH, 2 H), 7.13 (s, ArH, 2 H). This material was converted to **46** as follows. A



46, X = NH_2

47, X = 2,4,6-$(O_2N)_3C_6H_2NH$

solution of 490 mg (0.72 mmol) of **45** in 100 mL of THF was heated to reflux under argon, and 2.0 mL (20 mmol) of borane–methyl sulfide was added. The methyl sulfide–THF was slowly distilled from the mixture over 70 min. The remaining solution (30 mL) was cooled to 5 °C, 5 N aqueous NaCl was cautiously added to decompose excess borane, and THF (30 mL) and 5 N aqueous NaCl (50 mL) were added. The mixture was stirred for 10 days at 25 °C, the THF was evaporated, and the residue was extracted with CH_2Cl_2 (2 × 50 mL). The organic extracts were filtered through phase-separator paper, concentrated to 5 mL, and diluted with 150 mL of CH_3OH. To the triamine **46** solution was added 0.4 g (4.8 mmol) of $NaHCO_3$ and 0.2 g (0.81 mmol) of picryl chloride. The mixture was stirred for 25 min at 25 °C, and shaken with 40 mL of CH_2Cl_2 and 100 mL of 1 N aqueous NaCl. The layers were separated, and the organic layer (no drying) was added to a silica gel column (100 g) made up in 2% CH_3OH–98% CH_2Cl_2. Elution of the column with CH_3OH–CH_2Cl_2 mixtures (2–5% CH_3OH) gave 40 mg (6%) of the **4**·KCl complex. Further elution of the column with CH_3OH–CH_2Cl_2 mixtures (10–20% CH_3OH) gave 250 mg (38%) of **47** (same as **4**·NaCl) as an orange foam. A FAB mass spectrum (*m*-nitrobenzyl alcohol dispersion) gave a base peak at *m/e* 883 $(M + 23)^+$ corresponding to the $(M + Na)^+$ ion and a lower intensity ion at 889 $(M + 39)^+$ (25% the intensity of 883) corresponding to the $(M + K)^+$ ion. ^{1}H NMR for (200 MHz, CD_2Cl_2) for **4**·NaCl: δ 2.33 (s, ArCH$_3$, 6 H), 2.12–4.00 (m, NCH$_2$, OCH$_2$, 24 H) 2.95 (d, ArCH$_2$N, 2 H), 4.06 (d, ArCH$_2$N, 2 H), 7.02–7.13 (m, ArH, 6 H), 8.85 (s, ArH, 2 H). These spectra are essentially the same as those of **4**·NaBr prepared by the first method.

Solutions for Ultraviolet–Visible Spectra of Various Forms of 1–4. Compound **1** was dissolved in pure 1,4-dioxane to provide a 1.91 × 10^{-4} M stock solution. In a 1-cm optical cuvette, 0.80 mL of this stock solution, 0.80 mL of dioxane, 0.40 mL of 0.10 M aqueous HCl, and 0.020 mL of either H_2O, 1.0 M aqueous NaCl, or 1.0 M aqueous KCl were added, mixed, and scanned (vs H_2O) on a Beckman DU-8 UV-visible spectrometer from 300 to 700 nm. Solutions 80% in dioxane (by volume) but substituting 0.10 M aqueous $(CH_3)_4NOH$, 0.10 M aqueous $CH_2(CH_2CHNH(CH_2)_3SO_3H$ (CAPS, pH 10, 25 °C) buffer, or 0.10 M aqueous $CH_2(CH_2CH_2)CHNHCH_2CH_2SO_3H$ (CHES, pH 9 or pH 10, 25 °C) buffer for the 0.10 M aqueous HCl solution, were also prepared, and the spectra were scanned. Solutions in 40% dioxane–60% water (v/v) were similarly prepared using 800 μL of **1** stock solution and 800 μL of distilled water in place of the dioxane. All solutions were finally 7.56 × 10^{-5} M in **1**. Table I reports the results.

With procedures modeled after the above, a stock solution in 1,4-dioxane, 1.91 × 10^{-4} M in **2** was prepared, which was used to obtain final solutions for spectroscopic scanning that were 7.64 × 10^{-5} M in **2**. Wavelength maxima (λ_{max}) and molar absorptivities (ε) for the various forms of **1** and **2** are shown in Table I for both 40% and 80% dioxane solutions.

The spectra of the various forms of **3** and **4** were determined in 0.10 mM solutions of the substances in 1% (v/v) $Et(OCH_2CH_2)_2OH$ in water. Stock solutions of **3**·LiBr and **4**·NaBr in $Et(OCH_2CH_2)_2OH$ were prepared, which were 10 mM. In 1-cm optical cuvettes, 20 μL of stock solution was pipetted followed by 2 mL of 0.10 M aqueous HCl or 0.10 M $(CH_3)_4NOH$ solution. The resulting mixtures were scanned from 300 to 700 nm. Table I records the wavelength maxima and molar absorptivities (ε) for the various forms of **3** and **4**. In the determinations of pK_a values for **3** and 4, TEA $(N(CH_2CH_2OH)_3$·HCl) buffers were employed at final concentrations ranging from 0.01 to 0.40 M to allow extrapolation to zero ionic strength.

Responses to sodium or potassium ions at pH 7 and 8 were determined in a similar manner for both **3** and 4. In these runs, 0.30 M solutions of imidazolium acetate buffers of pH 7 or 8 were substituted for the aqueous HCl or $(CH_3)_4NOH$ solutions used above, and 20 μL of either distilled water, 1.0 M in NaCl, or 1.0 M in KCl were added to their respective cuvettes. Thus the final concentrations of buffer in these solutions were 0.29 M. For determinations involving the conversion of **3**·LiBr to **3**·NaBr, or **3**·KBr, the solutions were incubated at 37 °C for 16 h before the spectra were recorded (the reaction is over within 1 h at

37 °C). The conversion of 4·NaBr to 4·KBr is essentially instantaneous on the human time scale.

Figures 1-6 illustrate the different kinds of plots obtained (wavelength vs absorbance) involving various forms of **1-4**. Figure 1 corresponds to Table I run numbers 13-15, Figure 3, to run numbers 16-20, Figure 4, to run numbers 40-42, Figure 5, to run numbers 43-45, and Figure 6, to run numbers 46-50.

The solutions used for determining the ΔA values of Table III were prepared as follows. Aqueous 0.10 M buffer solutions were prepared to provide pH values of 6.0 (MES), 6.6 (MES), 7.0 (HEPES), 8.0 (CHES), and 9.0 (CHES) at 25 °C. To each of these was added an amount of 4·NaBr dissolved in dioxane to provide a final concentration of 0.10 mM of 4·NaBr. The volumes of dioxane were varied to achieve concentrations of 1%, 25%, and 50% by volume of dioxane. Thus three sets of reagent solutions were prepared, all being 0.10 mM in 4·NaBr. Each set comprised the five pH levels with buffer at 0.10 M concentration, but each set varied from one another only in dioxane concentrations. To 2 mL of each sample of reagent was added 0.10 mL of 1.0 M NaCl or 1.0 M KCl in water in an optical cuvette to give a final salt concentration of 50 mM. After mixing, the absorbance of each solution was measured on a Beckman DU-8 spectrophotometer at 300-700 nm. The absorbances at λ = 450 nm were read and are recorded in Table III.

Scavenging of Radicals by Vitamin E in the Membranes As Studied by Spin Labeling

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Abstract: The scavenging of radicals by α-tocopherol in the liposomal membranes was studied by using a spin label technique. It has been shown experimentally that α-tocopherol can scavenge lipophilic radicals that are closer to the membrane surface more efficiently than those that reside deep in the lipid region of the bilayer membrane. It was also found that α-tocopherol effectively scavenges aqueous oxygen radicals attacking from oustide of the membrane.

It is now generally accepted that the autoxidation of lipids in biological membranes is associated with a variety of important pathological events and aging, and the generation, reactions, and scavenging of radicals in the membranes have received great interest.[1] Vitamin E (tocopherols) is known to function as a lipophilic, chain-breaking antioxidant.[2] The chemistry of the mode of action of tocopherols in the homogeneous solution is now fairly well understood,[3,4] but the detailed mechanism for the inhibition of oxidation by tocopherols in the membrane has not been clearly elucidated. It has been shown that α-tocopherol, the most potent tocopherol, is retained in the membrane[5] and suppresses the oxidations of phospholipid liposomal membranes[6-9] and erythrocyte membranes,[10-12] although the antioxidant activity of α-tocopherol is considerably smaller in the membranes than in the homogeneous solution.[13] One of the important questions is how the phenolic hydrogen of α-tocopherol, which is accepted to be located at the surface of the membrane,[14,15] can interact with lipid peroxyl radical, which must reside deep in the bilayer membranes. The objective of the present study is to study the scavenging of radicals by α-tocoperhol in the phosphatidylcholine liposomal membranes by using a spin label technique.[16,17]

Experimental Section

Materials. Commercial soybean phosphatidylcholine (PC) purchased from Daigo Chemical Co. (Osaka) was purified with alumina and silica gel columns as previously.[18] Dimyristoylphosphatidylcholine (14:0 PC) obtained from Sigma Chemical Co. (St. Louis, MO) and dilinoleoylphosphatidylcholine (18:2 PC) obtained from Nihon Seika Co. (Osaka) were used without further purification. (*R,R,R*)-α-tocopherol (α-Toc), 2,2,5,7,8-pentamethyl-6-chromanol (PMC), (*R,R,R*)-δ-tocopherol (δ-Toc), and tocol (Toc) were kindly supplied from Eisai Co. (Tokyo) and used as received. A water-soluble radical initiator, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), and oil-soluble radical initiator, 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN), were obtained from Wako Pure Chemical Co. (Osaka).

Five kinds of *N*-oxyl-4,4′-dimethyloxazolidine derivatives of stearic acid, 5-NS, 7-NS, 10-NS, 12-NS, and 16-NS were used as spin probes, where the nitroxide group is attached at various positions along the fatty acid chain to situate the nitroxide groups at different depths in the hydrophobic interior of the phospholipid bilayer. The spin probes were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Methods. The liposomal membranes were prepared as follows as reported previously.[18] PC and lipid-soluble additives such as antioxidants, spin probes, or AMVN, when necessary, were dissolved in chloroform, and the solution was transferred to a round-bottom flask. Chloroform was removed on a water aspirator by using a rotary vacuum evaporator to obtain a thin film on the flask wall. An appropriate amount of 0.1 M NaCl aqueous solution was added, and the film was slowly peeled off by shaking to obtain white, milky, multilamellar liposome suspensions. They were sonicated to obtain unilamellar liposomal membranes and subjected to oxidation when AAPH was used as a radical initiator. The

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