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A Color Version of the Hinsberg Test: 1°–3° Amine Indicator

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Abstract: The Hinsberg test to recognize the type of amine (1°–3° amines), which has been established for more than 100 years and well documented in textbooks, is not possible without conducting complicated organic reactions. We report for the first time unique chemosensors that are capable of showing selective color changes toward 1°–3° amines as a color version of the Hinsberg test. This simple and straightforward qualitative analysis, using the synthesized novel compounds herein, can be considered a new innovative tool for discriminating 1°–3° amines as an alternative to the historical Hinsberg test.

Keywords: calixarenes · Hinsberg test · receptors · sensors · supramolecular chemistry

Introduction

One of the most pressing challenges in the design of chemosensors is to achieve a visual discrimination for different types of biomolecules. A simple monitoring system distinguishing among amines, amino acids, or proteins would be extremely useful in environmental technology and biological technology.[1] The Hinsberg test[2] was developed more than 100 years ago for the determination of amine types (1°–3°) and has been well documented in the literature.[3] This test, however, is conducted on the basis of organic reactions. Recently, a series of macrocyclic chromophores that can visually change upon the addition of amines have gained additional attention.[4–8]

A number of macrocyclic compounds' ability to selectively recognize cations, anions, and neutral molecules, have been investigated.[9] For amine recognition, it has also been reported that macrocyclic polyethers bind primary ammonium ions by anchoring the $R\text{-}NH_3^+$ group into their circular cavity by means of three hydrogen bonds (N–H···O).[10] However, the selective recognition of specific alkylamines has not been clarified due to the lack of selectivity. To overcome the low selectivity and low sensitivity associated with the discrimination of amines with regard to color changes, we report unique chemosensors **1** and **2**, which are capable of exhibiting selective color changes toward 1°-3° amines, as a color version of the Hinsberg test. Compounds **1**/**2b** and **2a** (see Scheme 1) show high selectivity for 1° amines, and for both 1° and 2° amines respectively, in contrast to more highly substituted ammonium ions. The selectivity would be applicable for the binding of biologically active ions, such as noradrenaline and norephedrine, with respect to their *N*-methylated derivatives, adrenaline and ephedrine.[11]

Results and Discussion

Calix[4]arene was reacted with pentaethyleneglycol ditosylate in the presence of Cs_2CO_3, followed by a diazo-coupling reaction to yield **1** in the cone conformation, confirmed by ^1H and ^{13}C NMR spectroscopy.[12] Single crystals of **1** were obtained as orange-red plates in CH_3CN/CH_2Cl_2 at room

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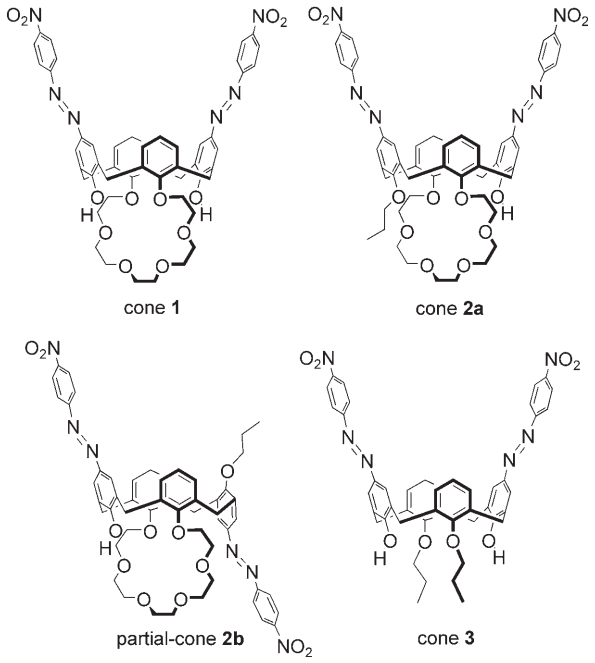

Scheme 1. Azo-coupled calix[4]arene compounds. **2** has two conformers: (*cis*) cone conformer (**2a**) and (*trans*) partial cone conformer (**2b**).

temperature. The crystal structure of **1** is monoclinic and has a centrosymmetric space group *C*2/*c*, in which the calix[4]arene group is in the cone conformer.[13] The oxygen atoms of the crown-ether loop in Figure 1 (top) are disordered due to intramolecular interactions with CH_3CN molecules. The intermolecular interaction arises from displaced π–π stacking[14] between phenylazo groups, at the plane-to-plane distance of 3.46 Å (center to center distance is 4.00 Å), with an offset angle of 58.8° (Figure 1, bottom).

Compound **2** was prepared in a moderate yield by the reaction of **1** with 1-iodopropane in the presence of NaH. As the oxygen atom of the propyloxy unit is not hydrogen bonded, **2** is expected to show two possible conformers: (*cis*) cone conformer (**2a**) and (*trans*) partial cone conformer (**2b**). Compound **2a** shows a pair of doublets at 3.74 and 4.51 ppm in the ^{1}H NMR spectra, as well as a single peak at 30.8 ppm in the ^{13}C NMR spectra. In contrast, the ^{13}C NMR spectra of **2b** shows two peaks at 32.0 and 38.0 ppm for the $ArCH_2Ar$ bridge carbons. The NMR spectra of **2b**, together with the X-ray crystallographic analysis of a single crystal formed as a red plate in CH_3CN, clearly indicate a partial cone conformation. The crystal structure is triclinic with a centrosymmetric space group $P\bar{1}$ (Figure 2).[13] As the two conformers have slightly different values for the retention factor (R_f), they were separated by column chromatography.

The binding characteristics of **1**–**3** with various amines were investigated by UV-visible spectroscopy. On the basis of UV band shifts of **1** and **2a** (Figures 3 and 4) upon the addition of 1°, 2°, or 3° amines in a $CHCl_3$ solution, we obtained association constants ($\log K_a$) for various amine molecules (Table 1).[15,16] For 1° amines, the $\log K_a$ values are

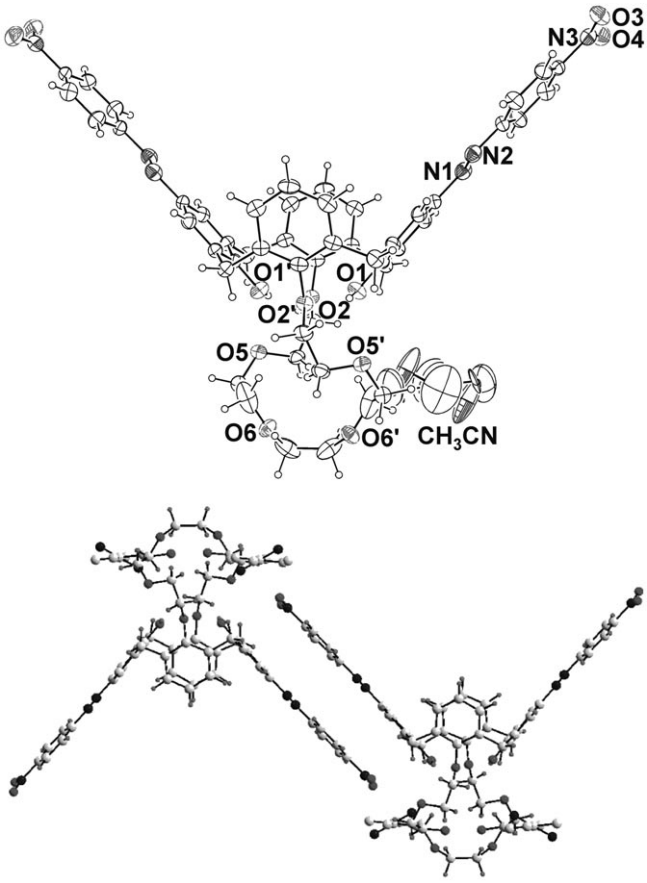

Figure 1. Top: Crystal structure of **1**·(CH_3CN) with 30% probability of the displacement ellipsoids. Bottom: Ball and stick representation showing the π–π stacking between phenylazo groups.

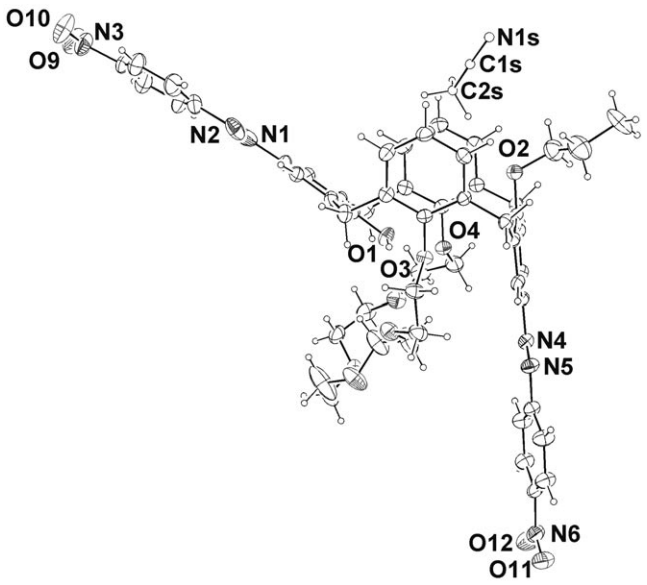

Figure 2. Crystal structure of partial cone **2b**·(CH_3CN) with 30% probability of the displacement ellipsoids. The atoms of CH_3CN are shown as small spheres for visual distinction.

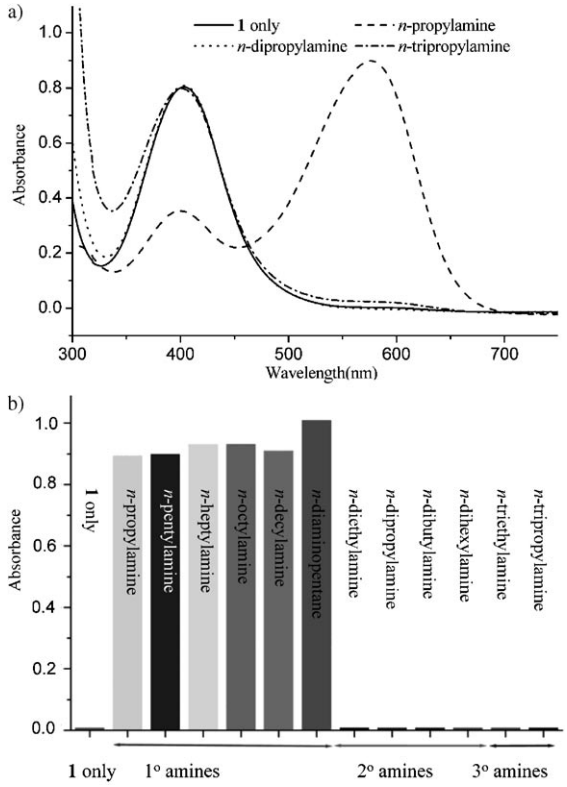

Figure 3. a) Spectral changes of **1** (0.01 mM) upon the addition of mono-, di-, and tripropylamine (10000 equiv) in $CHCl_3$. b) Absorbance of **1** (0.01 mM) at 576 nm upon the addition of various amines (10000 equiv) in $CHCl_3$.

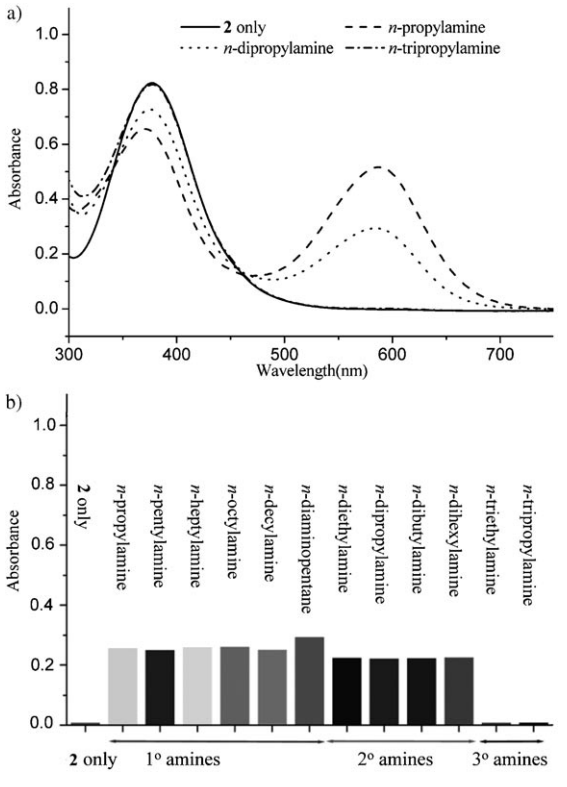

Figure 4. a) Spectral changes of **2a** (0.01 mM) upon the addition of mono-, di-, and tripropylamine (10000 equiv) in $CHCl_3$. b) Absorbance of **2a** (0.01 mM) at 587 nm upon the addition of various amines (10000 equiv) in $CHCl_3$.

≈4.2, larger than those for 2° amines. The $\log K_a$ values of **2a** for 2° amines are ≈3.6, similar to that for 1° amines. Among four receptors, **1** remarkably responded to the 1° amines, exhibiting a distinct color change from orange (complementary to the absorption of 401 nm) to blue (complementary to the absorption of 576 nm) with an isosbestic point at 450 nm. Furthermore, it should be noted that only one isosbestic point appears in the spectra of **1** with an amine in $CHCl_3$, supporting that the complex stoichiometry for **1** with an amine is 1:1. Further evidence of 1:1 complex formation in the **1**–*n*-propylamine complex was obtained by FAB mass spectroscopy, as shown in Figure 5.

Interestingly, we observed that **1** responded to 1° amines only, not to either 2° or 3° amines, thereby no color change was noted on addition of 2° or 3° amines to the solution of **1** (Figure 3). The bathochromic shifts of **1** upon the complexation of 1° amine are believed to arise from the phenolic oxygen atoms of the calix[4]arene, which become highly polarized. This polarization results in higher stabilization of the excited states relative to the ground states, to give the red-shift of the band in UV spectra.[17] For the selective color changes of **1** for 1° amines, it is conceivable that transfer of the phenolic proton to the nitrogen atom of the 1° amine, provides hydrogen bonds between three hydrogen atoms in the ammonium salt and the oxygen atoms of crown-6. Evidence for the hydrogen bonds can be seen in the solid-state structure of **2b**–*n*-propylamine (see Figure 9 below). The hydrogen bonding was also proven by changes in chemical shift of the ^{1}H NMR spectra (Supporting Information, Figure S4).

Table 1. Association constants of the ligands for various amines in $CHCl_3$.[a]

Amine	$\log K_a$		
	1	**2a**	**3**
n-diaminopentane	4.42 ± 0.03	4.00 ± 0.04	–
n-propylamine	4.12 ± 0.06	3.68 ± 0.06	–
n-pentylamine	4.17 ± 0.04	3.65 ± 0.07	–
n-heptylamine	4.22 ± 0.03	3.73 ± 0.05	–
n-octylamine	4.22 ± 0.02	3.74 ± 0.06	–
n-decylamine	4.20 ± 0.03	3.72 ± 0.06	–
n-diethylamine	– [b]	3.64 ± 0.04	–
n-dipropylamine	–	3.63 ± 0.05	–
n-dibutyllamine	–	3.66 ± 0.07	–
n-dihexylamine	–	3.52 ± 0.06	–
n-triethylamine	–	–	–
n-tripropylamine	–	–	–

[a] The K_a [M^{-1}] values were obtained by using the ENZFITTER program based on the 1:1 complexation phenomena between receptor and respective amines. [b] Not determined due to absence of color change.

By utilizing a competition experiment, we measured the changes of the absorption intensity upon the addition of the 1° alkylamine (*n*-nonylamine) to the chloroform solution of

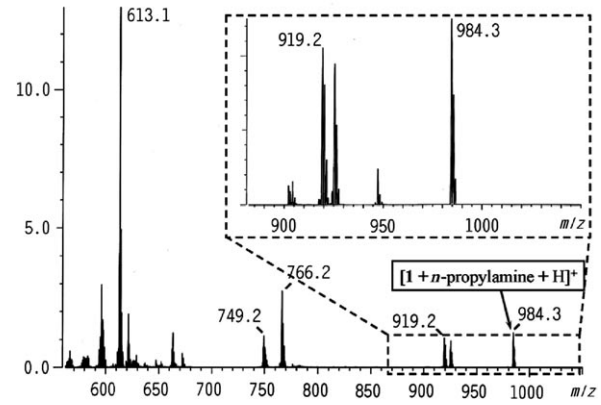

Figure 5. FAB-mass spectrum of **1**+*n*-propylamine in $CHCl_3$.

1 containing 1250 equivalents of the 2° amine (diethylamine). Addition of even a small amount of the *n*-nonylamine under these conditions results in a marked increase in absorption intensity at 574 nm (Figures 6 and S6). In the reverse case, addition of 2° amine to the solution (**1**+1° alkylamine), no spectral change was observed (Figure S7). These findings predict that **1** could selectively recognize a 1° amine, even in the presence of a larger amount of 2° amine.

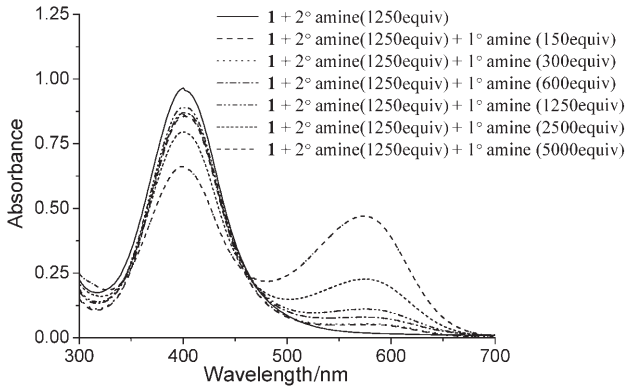

Figure 6. Competitive titration profiles of **1** (0.01 mM) containing 1250 equivalents of 2° amine (diethylamine) upon the addition of the 1° amine (*n*-nonylamine) in $CHCl_3$.

To gain an insight into the role of the crown ring in amine selectivity, **3**, without the crown loop, was prepared as a reference and tested for the color change toward various amines. No color change in the UV spectra was observed upon the addition of excess ($>10^4$ equiv) of all amines (Figures S8 and S9), from which it can be concluded that the crown-ether loop of **1** plays a critical role in the selective discrimination of the 1°–3° amines as previously mentioned.

The pK_a values of **1**, **2a**, **2b**, and **3** in the solution of H_2O and 1,4-dioxane (v/v = 1:2) were 7.64, 8.14, 9.25, and 9.65, respectively.[18] Compounds **1**, **2a**, and **2b** have a phenolic hydroxy group that is susceptible to deprotonation in the presence of 1°/2° amines, and is able to form hydrogen bonds between the 1°/2° ammonium ions and the crown-6-ring oxygen atoms. In particular **2a**, having a monopropyloxy group on the lower rim of the calix[4]arene, responded to both 1°and 2° amines rather than 3° amines. The color of **2a** changed from orange (378 nm) to blue (587 nm) with an isosbestic point at 462 nm (Figures 4, S13, and S14). Upon testing, the sensitivity of **2a** was lower than that of **1** due to the lower acidity of **2a**. In contrast, the **2b** responds to 1° amines only, though with lower sensitivity than **1** (Figures 7 and S15).

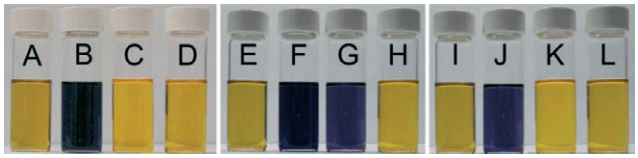

Figure 7. Color changes of **1**, **2a**, and **2b** (0.005 mM) upon the addition of 10000 equiv of 1°, 2°, and 3° amines in $CHCl_3$: A) **1** only; B) **1**+1° amine; C) **1**+2° amine; D) **1**+3° amine; E) **2a** only; F) **2a**+1° amine; G) **2a**+2° amine; H) **2a**+3° amine; I) **2b** only; J) **2b**+1° amine; K) **2b**+2° amine; L) **2b**+3° amine.

We further investigated the stability of the **1**–amine and **2a**–amine complexes using the density functional theory (Supporting Information). The calculations predict that in the cases of **1** with 1° amines and of **2a** with both 1° and 2° amines, the ionic complex is favored by transferring the phenolic hydrogen to the amine nitrogen. In contrast the case of **1** with 2° amines, the neutral complex is favored without deprotonation (Table 2, Figure 8). The most surprising and unexpected result from our calculations, in support of the experimental observation, is that the more acidic host **1** does not form an ionic complex with more basic 2° amines. Notably in both the crystal structure and the theoretically predicted structure of **1**, the two phenoxyl hydrogen atoms are well buried inside the lower rim of the calixarene, due to the two hydrogen bonds. Abstraction of one of these phenoxyl hydrogen atoms is most likely to depend on the proximity of the NH_2 group of the approaching amine. In this case, a 1° amines' less bulky alkyl group is then at a dihedral angle of $\approx 180^o$ with respect to the phenoxyl oxygen and can easily approach near to the phenoxyl hydrogen, leading to the formation of an ionic complex by deprotonation of the phenoxyl hydrogen. In contrast, the approaching 2° amine is

Table 2. Relative change in total energies [kcal mol^{-1}] of neutral forms of **1** and **2** complexed with the primary amine (CH_3NH_2) and secondary amine ($(CH_3)_2NH$) relative to their deprotonated anionic forms **1′**$^-$ and **2′**$^-$ complexed with the cationic primary and secondary aminines ($CH_3NH_3^+$ and $(CH_3)_2NH_2^+$) in $CHCl_3$ solution.[a]

	1′$^-$**/1**	**2a′**$^-$**/2**
$CH_3NH_3^+$ vs. CH_3NH_2	−1.3	−5.2
$(CH_3)_2NH_2^+$ vs. $(CH_3)_2NH$	+4.9	−5.8

[a] Calculations were carried out at the B3LYP/6–31G* level. Calculations in the solvent phase were performed by using the Self-Consistent Reaction Field-Isodensity Polarizable Continuum Model (SCRF-IPCM).

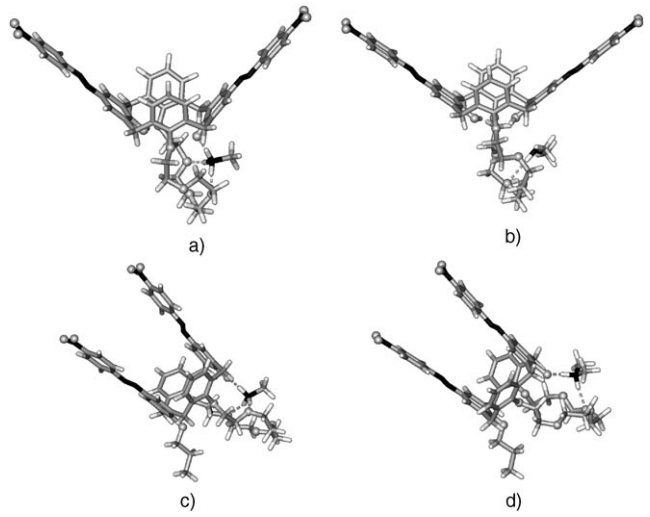

Figure 8. B3LYP/6–31G* optimized geometry of the stable complexes a) $\mathbf{1'}^{-}$–$CH_3NH_3^+$, b) **1**-$(CH_3)_2NH$, c) $\mathbf{2a'}^{-}$–$CH_3NH_3^+$, and d) $\mathbf{2a'}^{-}$–$(CH_3)_2NH_2^+$. Oxygen atoms are represented by grey balls; carbon, hydrogen, and nitrogen by grey, white, and black color, respectively. Hydrogen bonds are shown as dotted lines.

increasingly affected by the steric hindrance of the aromatic ring of the phenoxyl group. As the two alkyl groups are at ≈90° dihedral angle with respect to the phenoxy oxygen, the deprotonation of the phenoxy hydrogen is not warranted.

The spectral-peak shift, from ≈390 to ≈580 nm (by ≈190 nm), for **1** (mixing with 1° amines) and for **2a** (mixing with 1° and 2° amines) is theoretically due to the absorption involving the phenylazo-phenol anion moiety in the receptor amine ionic complexes. The large blue-shifts originate from charge transfer from the electron-rich phenol anion center to the azo moiety and the nitrophenyl center, in conjunction with the transformation of −N=N− to =(N−N$^-$)− and =N−N= (Figure S16). This is consistent with the blue-shifts[19] of other compounds with a diazo moiety. This kind of color change, for organic molecules as a result of deprotonation followed by a charge-transfer mechanism, is well known in the field of chromogenic anion receptors.[20] The absorption peaks for the nitrophenylazo-phenol (Azo−OH) moiety and its deprotonated anionic form $(Azo{=}O)^-$ are predicted to be 391 and 595 nm (red-shifted by ≈200 nm), respectively, at the level of Zindo//B3LYP/6–311+G* theory.[21] Consistent results were also obtained for the free receptors (**1** and **2a**) and their deprotonated anionic receptors at the Zindo//B3LYP/6–31G* level (S19 and S20). The absorption peaks corresponding to ≈580 nm are due to the transition from the HOMO of the phenylazo-phenol anion to the third LUMO. Therefore, the color changes of **1** with 1° amines and of **2a** with both 1° and 2° amines are due to the formation of ionic complexes (Figures 8 and S18).

With regard to the critical role of the crown loop in amine selectivity, we were able to obtain a crystal structure of the ionic salt of **2b**–*n*-propylamine (black-red colored crystal). The crystal structure displays the *n*-propylammonium cation encapsulated within the crown-6 and is bound to the quinone group through hydrogen bonding (Figure 9).[13] Here, the *n*-propylamine is bound between the crown ether

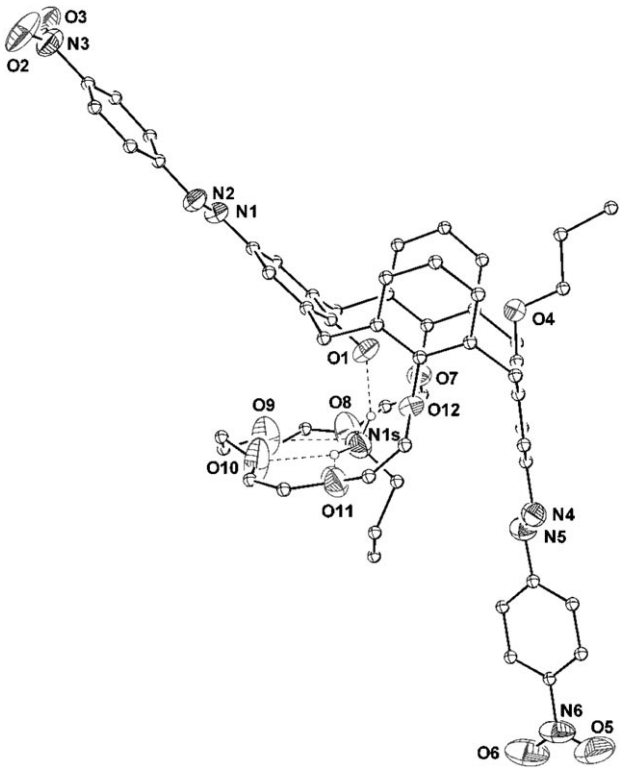

Figure 9. Crystal structure of $(propylamonium)^+(\mathbf{2b})^-$ complex with 30% probability of the displacement ellipsoids. The carbon atoms are shown in spherical form for the clarity of structure, while hydrogen atoms are omitted.

and the azo group, unlike the predicted structure of the **1**/**2a**–amine ionic complexes. The binding of *n*-propylamine to the crown-ether from the opposite side of the azo group, would lead to minimal interaction between the already buried phenyloxy group (Figure 2) and the approaching 1° amine. However, in the case of 2° amines, the binding to the crown loop is most likely to occur opposite to the azo moiety. The insertion of a bulky 2° amine between the crown group and the azo moiety would be highly unfavorable, due to the steric hindrance. In such a situation, in analogy with the binding pattern of the 2° amine with **1**, the buried phenoxyl group of **2b** will not donate an hydrogen atom to the approaching 2° amine, therefore, avoiding the formation of ionic complex. A more careful structural analysis, particularly for the phenylazo-phenol and phenyl(propoxyphenyl)diazine, of the X-ray structure of the **2b**–propylamine complex (Figure 9) and the hydrogen bonding interactions (Table 3) shows the deprotonated phenolic group complexing with the 1° propylamine. This is inferred by the single-bond-like N1−N2 bond length (1.281 Å) in comparison with the length of the N1=N2 double bond (1.218 Å). The structural changes correlate well with deprotonation,

Table 3. Structure data of hydrogen-bonding interactions in $(C_3H_7NH_3)^+$ $(\mathbf{2b})^-\cdot 3(CH_3CN)$.

Interaction	N–H [Å][a]	N···O [Å]	H···A [Å]	∢(N–H···O) [°]
N1s–H1s···O1	0.890	2.639	1.815	152.79
N1s–H2s···O8	0.890	3.117	2.280	156.69
N1s–H2s···O9	0.890	3.023	2.357	131.66
N1s–H3s···O10	0.890	2.998	2.170	154.49
N1s–H4s···O10	0.890	3.108	2.443	131.82

[a] Fixed distance and calculated position.

followed by charge-transfer-induced color changes in the phenyloxy-azo moiety (Figures S16 and 23). Additionally, we also analyzed the IR spectra of **1** and **2a** (in $CHCl_3$) along with **1**+1° amine, **2a**+1° amine, in the same way as the nitrophenylazo-phenol (Azo–OH) moiety, and its deprotonated anionic form $(Azo{=}O)^-$ (Figures S21, S22 and Table S1).

Based on the unique visual color changes of **1**, **2a**, and **2b** mentioned above and shown in Figure 7, we now propose a qualitative analysis of unknown amines by following the simple two-step procedure (flow chart is given in Figure 10). 1) Add an excess amount of an unknown amine into a

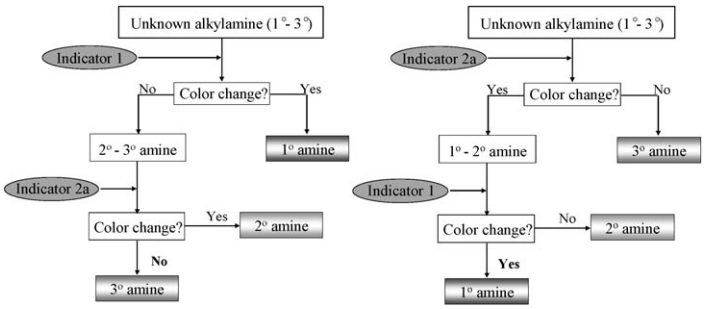

Figure 10. Flow chart for determination of the amines.

$CHCl_3$ solution of **1** (or **2b**, though less sensitive). If the color changes from orange to deep-blue, the unknown amine must be a 1° amine; therefore, 2° and 3° amines are excluded. 2) Subsequently, add the unknown amine (2° or 3°) into another $CHCl_3$ solution of **2a**. If the color changes from orange to deep-blue, it must be a 2° amine; otherwise, it is a 3° amine. An alternative test is also given in Figure 10.

Conclusion

Amine receptors based on phenyldiazo-calix[4]crown-6 (cone **1**, cone **2a**, and partial cone **2b**) were synthesized and their conformations characterized by NMR spectroscopy and X-Ray crystallography. Cone **1** can discriminate only 1° amines from 1°, 2° and 3° amines, whereas cone **2a** responds to both 1° and 2° amines. The crystal structure of *n*-propylamine-complexed partial-cone **2b** and the calculation data of the complexes based on the density functional theory show that there are intermolecular hydrogen bonding interactions between ammonium hydrogen atoms and crown-6 oxygen atoms, which mainly control the amine selectivity. We accordingly introduce for the first time an effective and informative method for determining the types of amines, with combinational use of indicators, cone **1** and cone **2a**. This simple and straightforward qualitative analysis, with diazocalix[4]crowns **1** and **2a**, parallels the historical Hinsberg test and can be considered as an innovative tool in discriminating 1°–3° amines.

Experimental Section

Synthesis: Compounds **2** and **3** were prepared by using the following procedures reported in literature.[9]

5,17-Bis[(4-nitrophenyl)(azo)phenyl]-26-mono(1-propyloxy)-25,27-calix[4]monocrown-6, (2a and 2b): A mixture of 1-iodopropane (0.18 g, 1.08 mmol) in 5 ml of, 1,4-dioxane (5 mL) was added dropwise to a mixture of **1** (0.20 g, 0.22 mmol) and NaH (0.0432 g, 1.08 mmol, 60% dispersion) in 1,4-dioxane (25 mL) under N_2. The reaction mixture was refluxed for 24 h. After the mixture was cooled to 0 °C, 10 mL of aqueous MeOH was added and the solvent was removed in vacuo. CH_2Cl_2 (100 mL) and a 10% HCl solution (100 mL) were added to the reaction mixture. The organic layer was separated, dried over anhydrous $MgSO_4$ and then concentrated in vacuo to provide a reddish powder. Column chromatography using a mixture of ethyl acetate and hexane (1:4) as an eluent on silica gel gave 0.11 g of **2a** in 52% yield. Purification by column chromatography with a mixture of ethyl acetate and hexane (2:1) as an eluent on silica gel gave 0.07 g of **2b** in 32% yield.

Compound 2a: M.p. 114–116 °C; ^{1}H NMR (400 MHz; $CDCl_3$): $\delta = 8.41$–8.36 (dd, $J = 8.87$ Hz, 4H; NO_2ArH_o), 8.05–7.98 (dd, $J = 8.74$ Hz, 4H; NO_2ArH_m), 7.88 (s, 2H; ArH_m), 7.83 (s, 2H; ArH_m), 6.55–6.53 (d, $J = 7.77$ Hz, 4H; ArH_m), 6.49–6.45 (t, $J = 7.46$ Hz, 4H; ArH_p), 4.76–4.72 (d, $J = 13.8$ Hz, 2H; $ArCH_2Ar$), 4.52–4.50 (d, $J = 13.7$ Hz, 2H; $ArCH_2Ar$), 4.09–3.39 (m, 26H; OCH_2CH_2O, $ArCH_2Ar$, and $CH_2CH_2CH_3$), 2.52–2.49 (m, 2H; $CH_2CH_2CH_3$), 0.99–0.94 ppm (t, $J = 6.48$ Hz, 3H; $CH_2CH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.0$, 159.2, 157.1, 156.7, 149.1, 148.4, 148.6, 146.0 138.7, 133.2, 132.8, 130.7, 130.2, 129.1, 125.4, 124.5, 123.7, 76.0, 73.6, 72.4, 71.2, 70.9, 30.8, 23.4, 10.8 ppm; IR (KBr pellet): $\tilde{\nu} = 3405$, 1521, 1343 cm^{-1} (NO_2); MS (FAB): m/z: calcd $[M^+]$: 967.0; found: 967.0; elemental analysis calcd (%) for $C_{53}H_{54}N_6O_{12}$: C 65.83, H 5.63; found: C 65.82, H 5.65.

Compound 2b: M.p. 149–151 °C; ^{1}H NMR (400 MHz; $CDCl_3$): $\delta = 8.44$–8.36 (d, $J = 8.8$ Hz, 2H; NO_2ArH_o), 8.36 (d, $J = 8.9$ Hz, 2H; NO_2ArH_m), 8.08 (d, $J = 8.8$ Hz, 2H; NO_2ArH_m), 8.01 (s, 2H; N_2Ar-H_o), 7.97 (d, $J = 8.9$ Hz, 2H; NO_2Ar-H_m), 7.80 (s, 2H; N_2Ar-H_o), 7.58 (s, 1H; ArO*H*), 7.04 (d, $J = 7.3$ Hz, 2H; ArH_m), 6.92 (d, $J = 7.6$ Hz, 2H; ArH_m), 6.78 (t, $J = 7.5$ Hz, 2H; ArH_p), 4.34 (d, $J = 13.4$ Hz, 2H; $ArCH_2Ar$), 4.12 (d, $J = 14.5$ Hz, 2H; OCH_2CH_2O), 3.95 (t, $J = 14.6$ Hz, 2H; $CH_2CH_2CH_3$), 3.89 (s, 4H; $ArCH_2Ar$), 3.88–3.42 (m, 18H; OCH_2CH_2O), 3.38 (d, $J = 13.9$ Hz, 2H; $ArCH_2Ar$), 1.26 (m, 2H; $CH_2CH_2CH_3$), 0.73 ppm (t, $J = 7.4$ Hz, 3H; $CH_2CH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 161.7$, 159.0, 157.1, 156.8, 154.8, 149.1, 148.5, 147.9, 146.0 135.7, 133.4, 133.2, 130.4, 129.6, 127.1, 125.6, 125.5, 125.4, 125.2, 123.8, 123.5, 74.3, 74.2, 72.1, 71.3, 70.1, 38.2, 31.2, 24.1, 10.7 ppm; IR (KBr pellet): $\tilde{\nu} = 1521$, 1343 cm^{-1} (NO_2); MS (FAB): m/z: calcd $[M^+]$: 967.20; found: 968.10; elemental analysis calcd (%) for $C_{53}H_{54}N_6O_{12}$: C 65.83, H 5.59; found: C 65.85, H 5.55.

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