Molecular Diagnostics: Synthesis of New Chromogenic Calix[8]arenes as Potential Reagents for Detection of Amines

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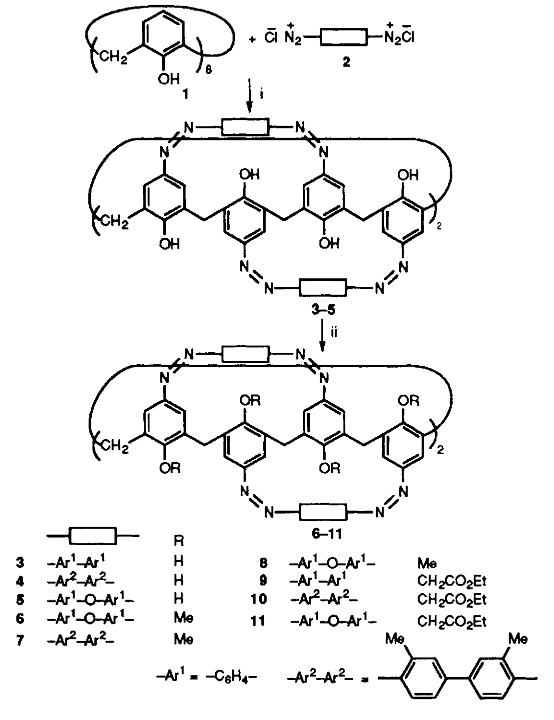
New chromogenic calixarenes **3–11**, potential diagnostic reagents for amines, are synthesized.

Amines are recognised best by their solubility, basic nature and their derivatisation or secondary reactions.[1] These reported procedures run into difficulties when several amines are present and do not afford a convenient procedure to distinguish mono-, di- and poly-amines.[2] Here we report the synthesis of new chromogenic calix[8]arenes **3–11**, which have a potential of providing new molecular diagnostics for the visual detection of amines and their classification. The colour change can also be followed spectrometrically (Table 1).

After several unsuccessful attempts, the diazo calixes **3–5** were prepared by coupling the debutylated calix[8]arenes[3,4] **1** with diazotized 4,4′-diaminobiphenyls **2** ($NaNO_2$–HCl, 0 °C) in DMF : MeOH (5 : 8, *v*/*v*) (Scheme 1). The compounds were purified by dissolving in pyridine followed by precipitation with dilute HCl and washing with $NaHCO_3$. The structures and possible conformations of the synthesized compounds were confirmed spectroscopically [^1H NMR, IR, UV, molecular mass determination (vapour pressure osmometry)] and by transformation to compounds **6–11** (reaction with MeI/NaH, $BrCH_2CO_2Et$/NaH).

The possibility of regioisomerism in **3–5** was examined by a careful analysis of the ^1H NMR spectra. For example, though the synthesized compounds **3–5** could theoretically have the bisazobridge across the 1,2-; 1,3-; 1,4-, 1,5-phenyl rings and also across two calixarene units to yield biscalixarene analogues, one could easily discern that biphenyl capping of phenyl rings of calix[8]arenes cannot be on adjacent phenyls due to geometric considerations. Out of the other possible 1,3-; 1,4- and 1,5-bisazo capping, the possibility of bridging between 1 and 5 phenyl rings was ruled out with the help of CPK models while interlinkage between two calix[8]arene units was ruled out by molecular mass determination and elemental analysis. This left the possibility of 1,4- and 1,3-bisazo bridging. We favour the 1,3- bisazo capping as the likely structure for **3–5** because only this situation would tally with the observed ^1H NMR spectra which show the methylene protons as a clear singlet at δ 3.86. This observation is in agreement with recent findings of Neri *et al.*[5,6] who synthesized different partially alkylated calix[*n*]arene (*n* = 6, 8) derivatives with C_3 and C_4 symmetry.

In a representative study it has been observed that the diazocalixes **3–5** exhibited a λ_{max} 382–383 nm while the studied amines [$R–NH_2$; R = Et, C_4H_9, Me_3C; Et_2NH; Et_3N;



Scheme 1 *Reagents and conditions*: i, DMF, MeOH (8 : 5 *v*/*v*, 0 °C, 3 h; ii, For **6–8**, MeI, NaH, DMF, 60 °C, 24 h; for **9–11**, $BrCH_2COOEt$, DMF, NaH, 80 °C, 24 h

Table 1 Colour change and shift in the λ_{max} of **3–5** on addition of amines

Amine added	Colour change	λ_{max} of calixarenes **3–5** in the presence of amines (shift/nm) **3**	**4**	**5**
—	Pale yellow	382	382	383
Ethylamine	Yellow to orange	420(38)	420(38)	420(37)
n-Butylamine	Yellow to orange	420(38)	420(38)	420(37)
tert-Butylamine	Yellow to orange	420(38)	420(38)	420(37)
Diethylamine	Yellow to orange	420(38)	420(38)	420(37)
Triethylamine	Yellow to orange	422(40)	422(40)	422(39)
Methylaziridine	Yellow to pale pink	424(42)	423(41)	420(39)
Ethylene-1,2-diamine[b]	Yellow to red	480(98)	482(100)	480(97)
Diethylene triamine[b]	Yellow to red	480(98)	480(98)	480(97)
Triethylene tetramine[b]	Yellow to red	480(98)	480(98)	480(97)
Aniline	No change	385(3)	385(3)	385(2)
p-Nitroaniline	No change	384(2)	384(2)	385(2)

[a] Total concentration of [amine] + [**3** or **4** or **5**] = 5 × 10^{-4} mol dm^{-3} in Me_2SO at 25 °C. [b] An additional peak was observed at 433 nm on addition of these amines.

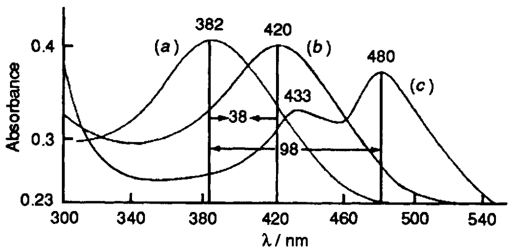


Fig. 1 Optical spectrum of (*a*) **3**; (*b*) **3** + *tert*-butylamine (1 : 1), (*c*) **3** + ethylene-1,2-diamine (1 : 1) in Me_2SO at 25 °C. (Solvent effects were eliminated by using blanks, [**3**] + [amine] = 5×10^{-4} mol dm^{-3}).

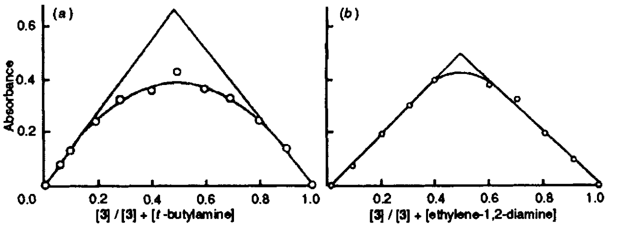


Fig. 2 Continuous variation plots for the formation of (*a*) **3**-*tert*-butylamine complex; (*b*) **3**-ethylene-1,2-diamine complex (25° C, Me_2SO), [**3**] + [amine] = 5×10^{-4} mol dm^{-3}

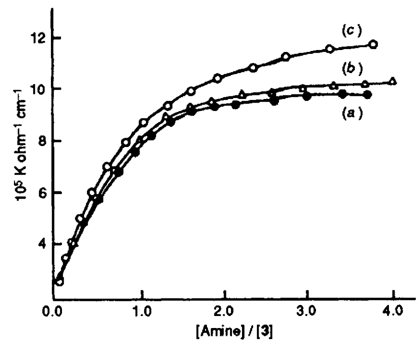


Fig. 3 Conductometric titration curves of **3** with (*a*) *tert*-butylamine; (*b*) diethylamine; (*c*) ethylene-1,2-diamine in Me_2SO at 25 °C

p-$XC_6H_4NH_2$ (X = H, NO_2); Me-$CHNHCH_2$; $H_2NCH_2CH_2NH_2$; diethylenetriamine, triethylenetetramine] did not absorb at this wavelength in their optical spectra. When 2.5×10^{-4} mol dm^{-3} solution of amines listed in Table 1 in Me_2SO was added to the Me_2SO solution (2.5×10^{-4} mol dm^{-3}) of diazocalix[8]arene **3**, a distinct colour change (Fig. 1) from yellow to red was observed which was confirmed by a bathochromic shift of 37 to 100 nm (Table 1). For instance, addition of *tert*-butylamine to **3** (2.5×10^{-4} mol dm^{-3}, Me_2SO) shifted its λ_{max} from 382 to 420 nm with a red shift of 38 nm. Similar addition of diamines however, shifted the λ_{max} of **3–5** to 480 nm with a bathochromic shift of 98–100 nm along with an additional major absorption at 433 nm. Addition of diethylenetriamine and triethylenetetramine did not reveal any further shift in the λ_{max} (>480 nm). Aromatic amines did not reveal any colour change or shift in the λ_{max} (Table 1).

The results can be explained if it is assumed that the calix[8]arenes [p-*tert*-butylcalix[n]arenes (n = 4, 6, 8) in earlier work[7–9]] can transfer a maximum of two protons out of the available eight protons. Conductometric titrations[8,9] (Fig. 3) show a continuous increase in conductivity until a plateau is reached on addition of **3** to the listed amines (Table 1) as well as the potentiometric titrations[6,7] of **3** (2.5×10^{-4} mol dm^{-3} in Me_2SO) with *tert*-butyl amine and ethylene-1,2-diamine which show one and two inflection points respectively. For instance, with ethylene-1,2-diamine, the first inflection point was observed when [**3**]/[ethylene-1,2-diamine] = 0.5 and the second inflection point was observed when [**3**]/[ethylene-1,2-diamine] = 1.0. An increase in the number of amino groups by using diethylenetriamine and triethylenetetramine (Table 1) did not show any increase in the number of inflection points in their potentiometric titration curves revealing thereby that **3** could transfer only two protons as analogously observed in the photometric titration of **3** with sodium hydroxide solution.

The utility of the synthesized chromogenic calixarenes as molecular diagnostics was examined by detecting the diamine (*e.g.*, 1,2-ethylenediamine) in the presence of a much higher background concentration of the monoamine (*e.g. tert*-butylamine) and *vice versa*. This indicated that the method offers a qualitative selectivity in polyamine analysis.

A preliminary study on the host–guest chemistry of diazo calixes **3–5** reveal that all amines except aniline and p-nitroaniline listed in Table 1 form 1 : 1 complexes with **3–5** as revealed by the Job's continuous plots for [**3**]/[**3**] + [amine] *vs* absorbance at 420 and 480 nm for *tert*-butylamine and ethylene-1,2-diamine respectively. ^{1}H NMR titration of **3** with *tert*-butylamine in $(CD_3)_2SO$ partially reveals the nature of the complex. The methyl singlet at δ 1.40 due to *tert*-butylamine was observed to shift to δ 1.56 ($\Delta\delta$ = +0.16 ppm) while the methylene protons of **3** at δ 3.86 did not show any significant shift in their ^{1}H NMR spectra. This observation suggests that the **3**-amine complex might be '*exo*' and could not be transformed to the '*endo*' complex as observed earlier by Gutsche *et al* with p-allylcalix[4]arene and *tert*-butylamine,[10–12] probably due to loss of flexibility in the calixarene owing to bridging of the opposite phenyl groups by the bisazobiphenyl linkage. It appears that the transfer of a second proton from the new chromogenic calix[8]arene derivatives to amines seems to be easier than the *exo*-to-*endo* transformation.[12]

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