Molecular recognition of xanthine alkaloids: First synthetic receptors for theobromine and a series of new receptors for caffeine

Shyamaprosad Goswami,* Ajit Kumar Mahapatra and Reshmi Mukherjee

Department of Chemistry, Bengal Engineering College (Deemed University), Howrah-711103, India

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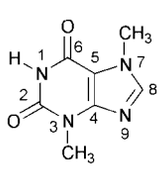
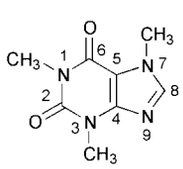
Synthetic receptors (**H3**, **H4**, **H5** and **H6**) are designed and synthesised for the first time for theobromine, a xanthine alkaloid used as a diuretic. The synthesis of the receptor **H6** is achieved by $Co(PPh_3)_3Cl$-mediated homocoupling of 3-(ethoxycarbonyl)benzyl bromide **12** under mild conditions. New caffeine receptors (**H7**, **H8** and **H9**) are designed and synthesised. The binding results of theobromine and caffeine (both by NMR and UV studies) are reported.

The design and synthesis of abiotic (*i.e.*, non-natural) receptors which undergo molecular recognition using weak non-covalent interactions is an emerging field[1,2] with many potential applications,[3,4] such as sensors,[5] carriers, and other molecular devices.[5] Such receptors can serve as models for the understanding of fundamental molecular recognition processes in biological systems.

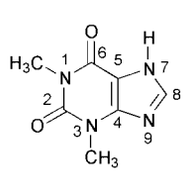
Xanthine derivatives (**G1**, **G2**, **G3**) have several pharmacological actions, such as antibronchospastic, CNS-stimulation, and tachycardia activity,[6] *etc*. We report here for the first time the design and synthesis of receptors **H3**, **H4**, **H5** and **H6** (the numbering of receptors starts from **H3**) to recognise theobromine (**G1**, a difficult recognition substrate because of its very poor solubility in both chloroform and methanol) and their binding efficacy to solubilise it in a less polar solvent like chloroform. It is one of our goals in molecular recognition research to make the insoluble bio-substrate (*e.g.*, urea[7]) soluble in chloroform (a common NMR solvent for binding studies) by hydrogen-bond complexation with the designed receptors quenching the individual self-polarity.



G1



G2



G3

The other xanthine derivative, caffeine (**G2**) (a trimethylxanthine), which is present in tea and coffee seeds, has long been known to be responsible for the stimulating effect of tea, and plays important roles in determining the liquor characters,[8] *i.e.* tea creaming (strength and briskness), for the desirable attributes of the beverage. So it has interested chemists for its complexation studies.[9]

Previously, complexation studies of caffeine in aqueous solution have been performed with a number of known polyphenols[10] and cyclodextrins.[10] We report here the recognition of caffeine in chloroform with our new synthetic amidic and non-amidic receptors **H7**, **H8** and **H9**.

Recognition of theobromine

Our designs are based on the structural features of theobromine which suggest that the two lactam carbonyl groups are good proton acceptors and that one imide N–H proton is a good proton donor like thymine[11] in the six-membered pyrimidine ring of xanthines and so it is an ADA (A, D = hydrogen-bond acceptor and donor, respectively)-type hydrogen-bonding system along with an AA system of the imidazole ring. We have designed different host molecules **H3**, **H4**, **H5** and **H6** for consideration of binding (structures of receptors and their complexes are shown in Fig. 1) on the basis of hydrogen-bond donor and acceptor properties of the guest theobromine and the corresponding receptors. The fact is that a binuclear guest such as theobromine needs more spacious receptors compared with **H4** and **H5**, therefore we designed the bigger receptor **H6**.

Synthesis of host molecules (H3, H4, H5 and H6)

The synthesis of the receptors **H3**, **H4**, **H5** and **H6** for theobromine is shown in Scheme 1. The synthesis of **H4** is achieved by the synthesis of carbamate **10** from *m*-aminobenzoic acid followed by formation of the amide bond by the mixed anhydride method. The synthesis of **H6**, having a bigger spacer, is made by the important Co^I coupling reaction[12] of **12** where the ester functionality survives. Compound **12** was prepared by the reaction between **11** and *N*-bromosuccinimide in the presence of benzoyl peroxide in carbon tetrachloride.

Result and discussion

Energy-minimised structures of theobromine complexes **A**, **B**, **C** and **D** with **H3**, **H4**, **H5** and **H6**, respectively, are shown in Fig. 2 using MMX (Serena Software 1993) calculations. Molecular modelling was done using standard constants, and the relative permittivity was maintained at 1.5. Alternative complex structures (Complexes **A′**, **B′**, **C′** and **D′** respectively) were obtained by flipping over the structure of theobromine, and the comparative energy values are shown in Table 1.

We therefore examined the simple pyridine diamide **H3** as the first candidate as a receptor for theobromine based on the triple hydrogen-bonding complementarity (DAD-ADA) between 2,6-diaminopyridines, but the six-membered ring containing the imide group of theobromine (Complex **A**) leaves the imidazole ring uncomplexed although **H3** makes **G1** soluble in chloroform. The comparative binding[13] results of the other designed receptors (**H4**, **H5** and **H6**) and the protons undergoing shifts

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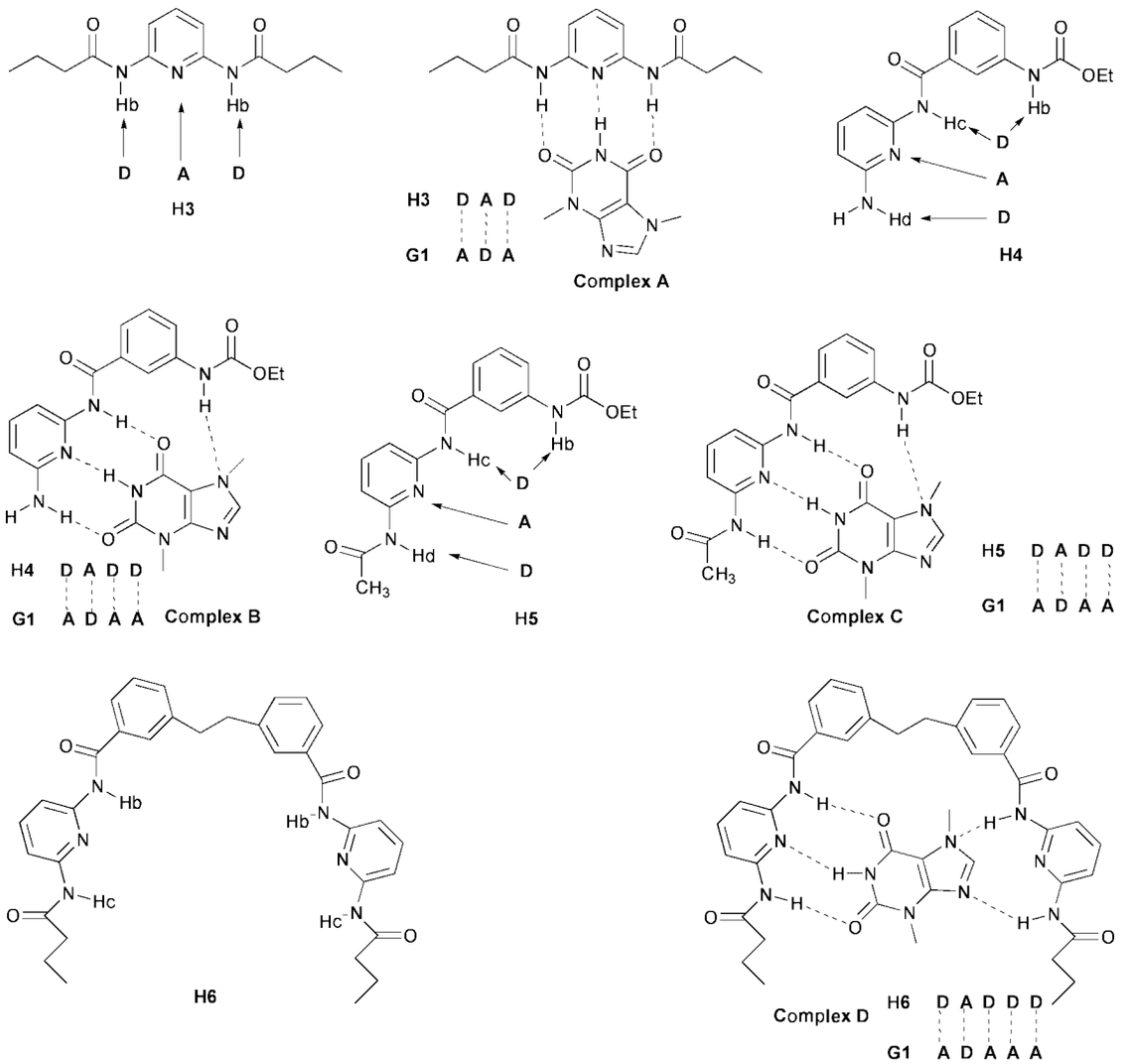


Fig. 1 Assignment of hydrogen-bond donors and acceptors in **H3**, **H4**, **H5** and **H6** and their probable hydrogen-bonding patterns in complexes **A**, **B**, **C** and **D** with **G1**.

Table 1 Energy-minimised values of different complexes in different mode of binding

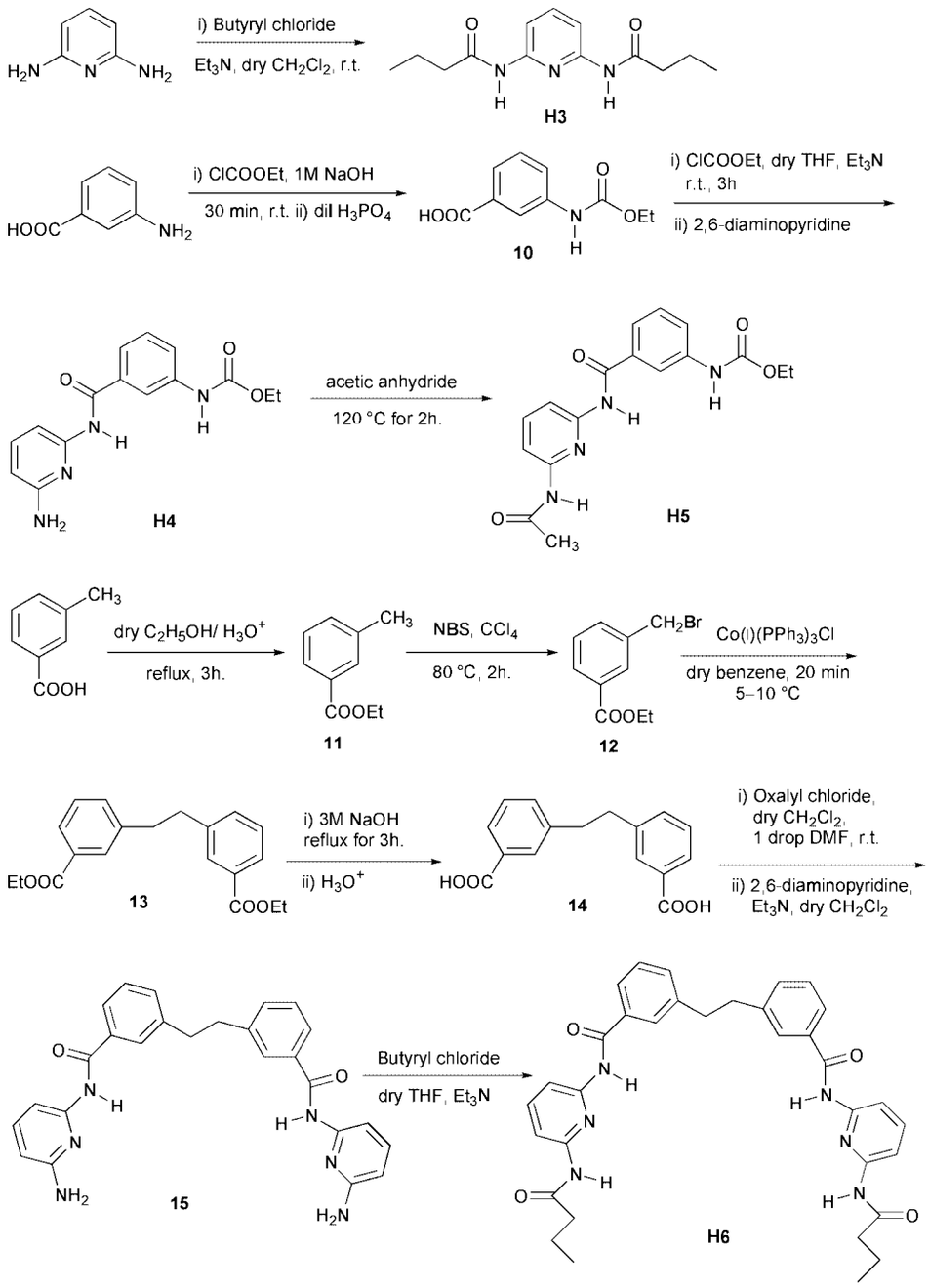
Complex	Energy[a]/kcal mol^{-1}	Complex	Energy[a]/kcal mol^{-1}
A	−20.620	**A′**	−20.709
B	−7.056	**B′**	−6.340
C	−9.749	**C′**	−10.522
D	−7.601	**D′**	−8.413

[a] 1 cal = 4.184 J.

Table 2 NH chemical shifts and association constants K_a for **G1** with different hosts (**H3**, **H4**, **H5** and **H6**)

Host	Guest	Different amide and amine proton shifts of host (Δδ/ppm)		Imide proton shift of guest (Δδ/ppm)		K_a/M^{-1}
H3	**G1**	NHb	0.37	NHa	0.61	Complex **A** 1.94×10^2
H4	**G1**	NHb	0.13	NHa	0.78	Complex **B**
		NHc	0.52			9.43×10^2
		NHd	0.15			
H5	**G1**	NHb	0.18	NHa	0.68	Complex **C**
		NHc	0.34			9.97×10^2
		NHd	0.39			
H6	**G1**	NHb	0.28	NHa	1.79	Complex **D**
		NHc	0.36			2.41×10^3

on complexation with theobromine are shown in Table 2. The assignments of amide and carbamate protons of **H4** are suggested from the ROESY experiment (500 MHz, Fig. 3). The assignment of protons of **H5** is connoted from a comparison of the downfield shifts of the corresponding protons due to acetylation of the pyridine amino group of **H4**. The different *syn* and *anti* forms of carbamates[14] **H4** and **H5** are shown in Fig. 4. With the incorporation of a carbamate moiety in the receptors **H4** and **H5**, the respective binding constant with **G1** is increased to some extent compared with that of **H3**. **H4** shows a small downfield chemical-shift change for the carbamate proton (Δδ 0.13 ppm) on complexation with theobromine (**G1**). Being frustrated with the isophthaloyl spacer for this type of binuclear guest, we then designed the bigger receptor **H6** (DADDD) having more space between the binding groups compared with the isophthaloyl spacer to accommodate freely the bicyclic theobromine to complex both the *N*-methylimidazole moiety as well as the pyrimidine ring. The receptor **H6** more efficiently solubilises **G1** by making one additional hydrogen bond with the most basic nitrogen [imidazole nitrogen (N-9) of purine ring] forming a total of four hydrogen bonds and leaving one amide NH in the host uncomplexed (NHa in Complex **D**). Interestingly these receptors do not bind theophylline, *i.e.* 1,3-dimethylxanthine **G3**, possibly due to the presence of the bulky N1–CH_3 group for which the adjacent two imide carbonyls cannot participate in hydrogen-bond formation with the amide protons of the receptors.



Scheme 1 Synthesis of the hosts **H3**, **H4**, **H5** and **H6**.

Recognition of caffeine

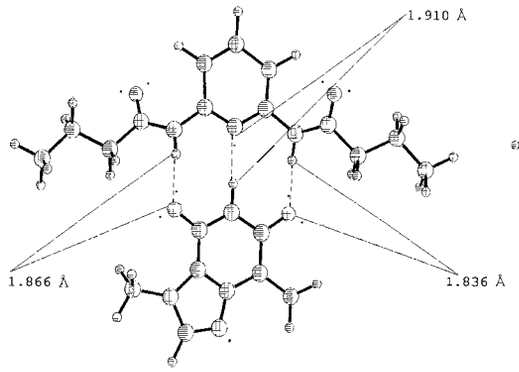
Previous receptors for caffeine's interaction with polyphenols[15] show the formation of 1 : 1 complexes between caffeine and known polyphenols in aqueous solution. In aqueous medium, caffeine forms a number of complexes of variable stoichiometry with polyphenols and aromatic hydroxy acids such as methyl gallate,[16] 3-nitrobenzoic acid,[16] 5-chlorosalicylic acid,[17] pyrogallol,[9] potassium chlorogenate[18] and cyclodextrins,[19] *etc*. For caffeine recognition, we designed first the tetraamide macrocyclic receptor **H7** containing the smaller isophthaloyl spacer which unfortunately failed to bind caffeine. We then designed polyphenolic receptors (**H8** and **H9**) which form 1 : 1 complexes with caffeine in chloroform.

Synthesis of caffeine receptors

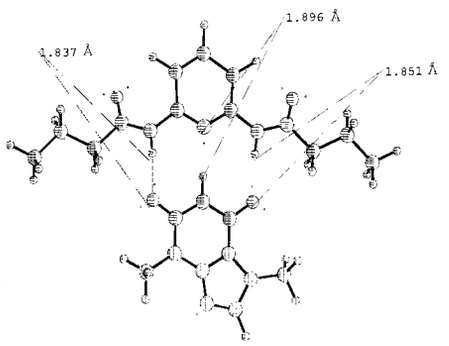
The synthesis of the macrocyle **H7** is achieved by high-dilution coupling of **17** with the bis(acid chloride) of **16** (Scheme 2). The syntheses of other compounds are straightforward and the procedures are mentioned in the Experimental section.

In the complexes with caffeine, the phenolic protons of **H8** and **H9** underwent upfield shifts (maximum $\Delta\delta$ of OH in **H8** = 0.015 ppm and that in **H9** = 0.84 ppm, respectively) as expected. Both **H8** and **H9** in organic solution may be intramolecularly hydrogen bonded, giving rise to two possible conformations in equilibrium as shown in Fig. 5. The intramolecularly hydrogen-bonded conformations have significant influence on the chemical shift of the imino C–H and also the complexation with caffeine.

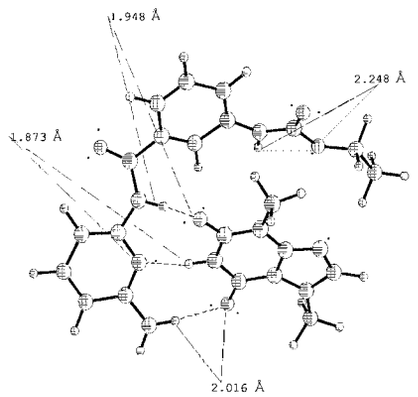
In **H9**, the imine hydrogen appears at δ 8.67 (intramolecular hydrogen bonds of OH with the imino nitrogen may cause imino C–H to appear at such a downfield position). Interestingly the two different phenolic OH protons of both **H8** and **H9** appear at different chemical shifts. The phenolic OH group which makes a stronger intramolecular hydrogen bond [probably the OH group *ortho* to the imino group is more acidic than the other OH group due to electron-withdrawing resonance of the imino nitrogen and the formation of a six-membered intramolecular hydrogen bond (Fig. 5)] appears more downfield. Surprisingly the most downfield OH proton of



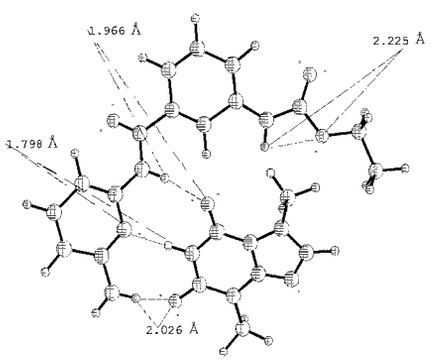
Complex **A**



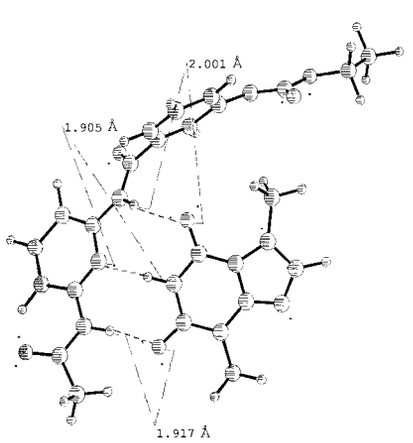
Complex **A′**



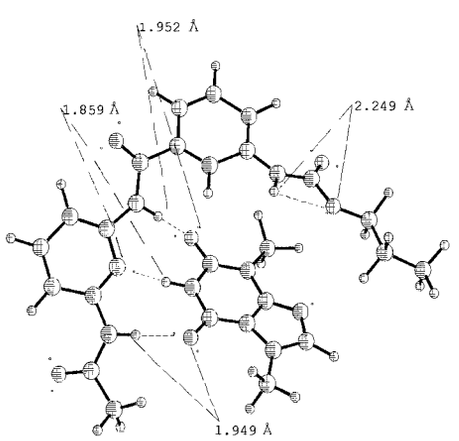
Complex **B**



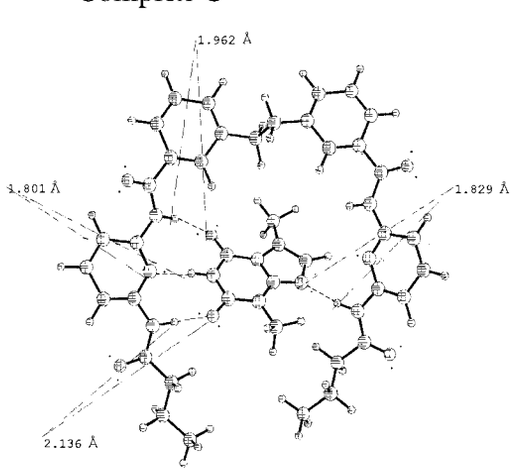
Complex **B′**



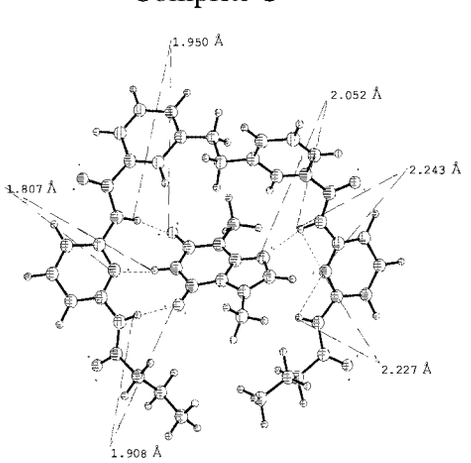
Complex **C**



Complex **C′**



Complex **D**



Complex **D′**

Fig. 2 Energy-minimised structures in different modes of complexes **A**, **B**, **C** and **D**.

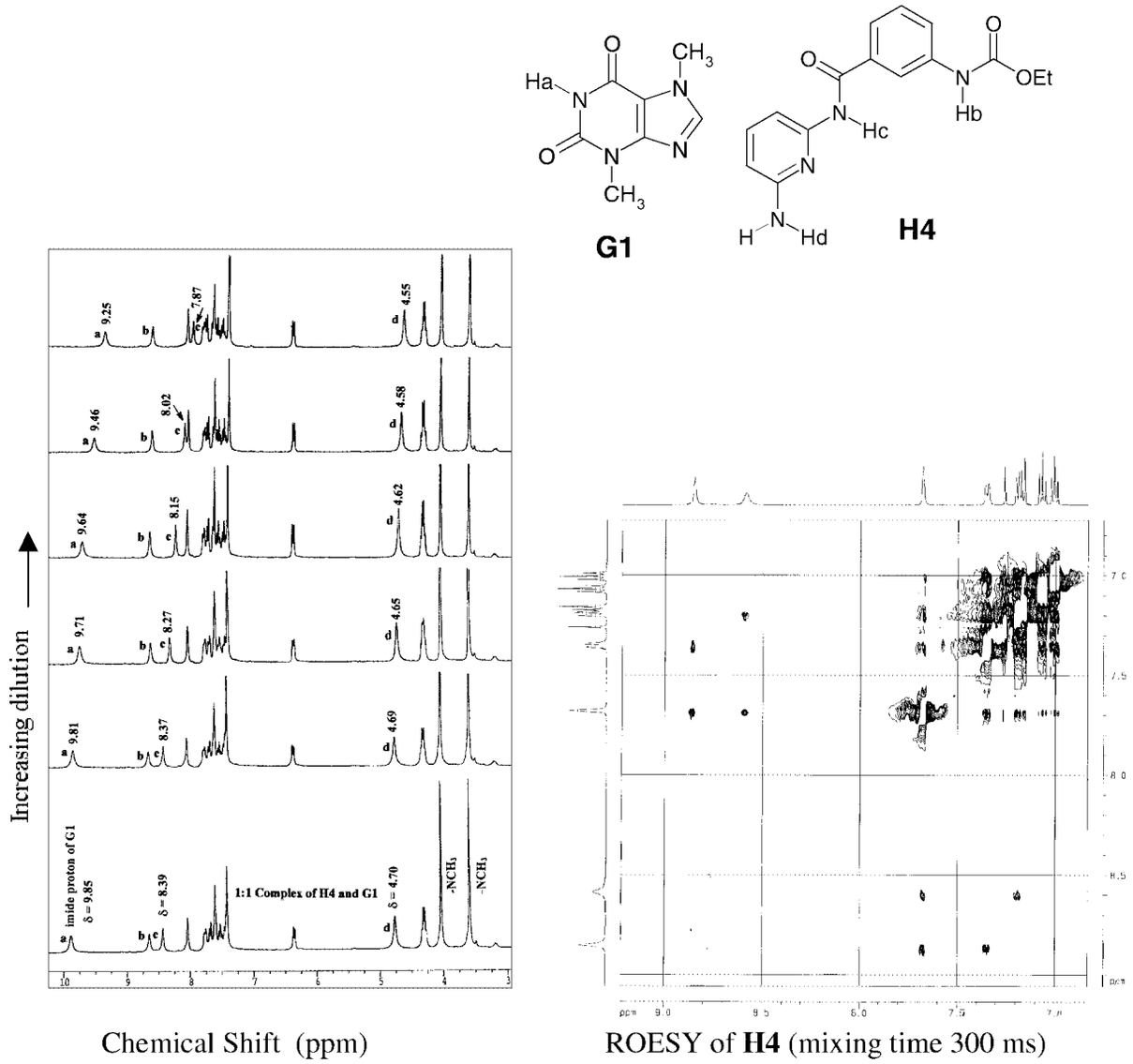


Fig. 3 300 MHz NMR spectra of 1 : 1 complex of **H4** and **G1** (0.02 mmol mL^{-1}) by gradual addition of $CDCl_3$ at 25 °C and the ROESY (500 MHz) spectrum of **H4**.

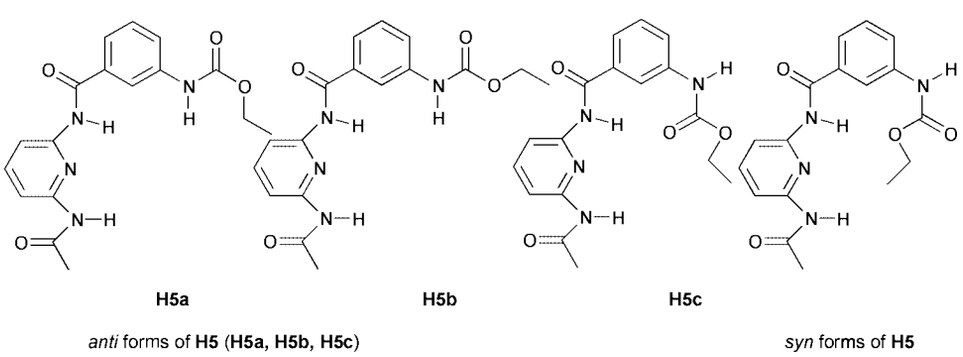
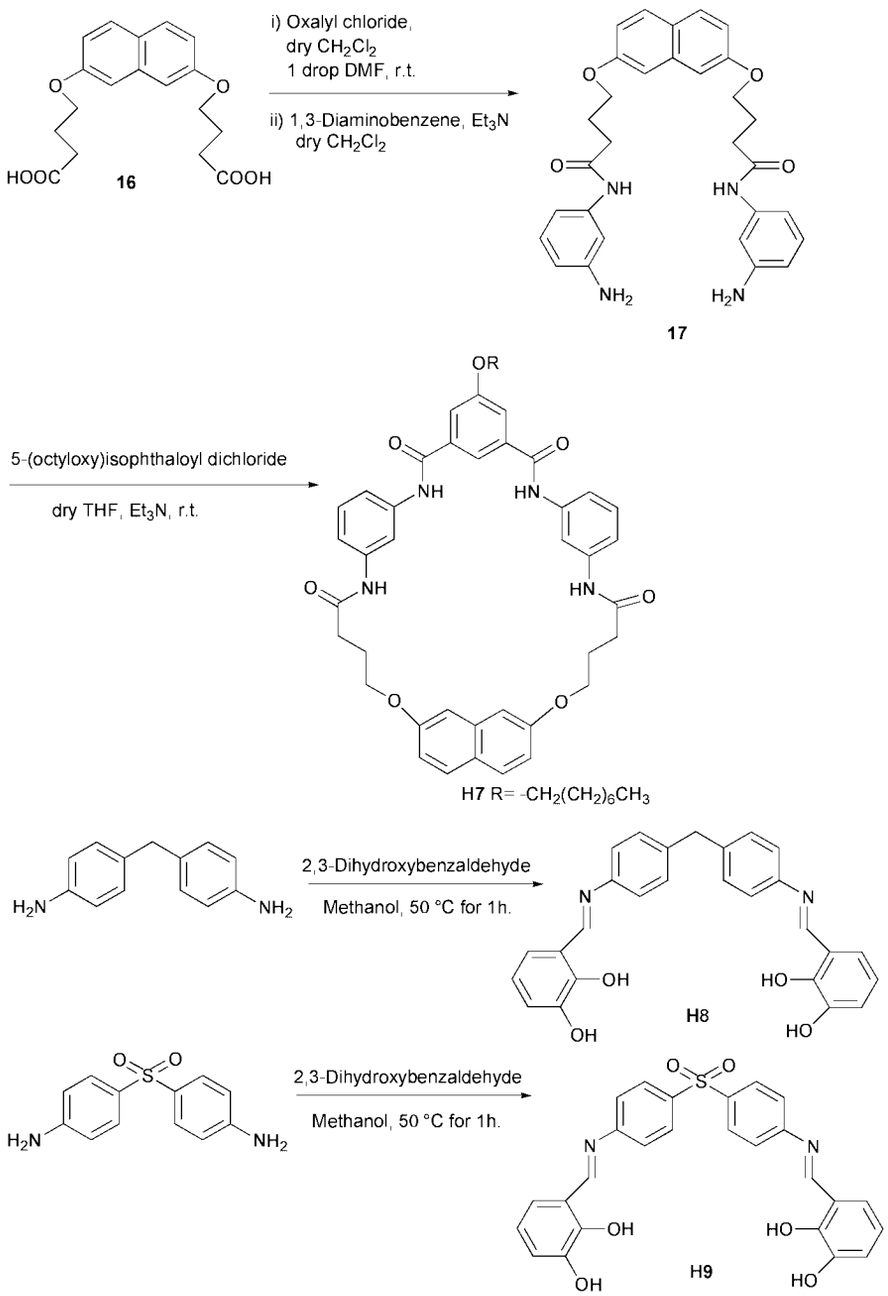


Fig. 4 *anti* and *syn* forms of **H5**.

H8, unlike **H9**, does not undergo any chemical-shift change on complexation with caffeine. The electron-withdrawing influence of a sulfonyl moiety, as well as it giving a wider spacing in **H9** compared with the bridging methylene group in **H8**, is more effective in increasing the binding affinity of **H9** with caffeine compared with **H8**. The values of association constants (K_a) for the formation of 1 : 1 complexes between caffeine and polyphenolic receptors have been calculated using the up field chemical-shift changes observed for phenolic OH during addition of increasing amounts of guest. The chemical-shift changes ($\Delta\delta$) of the protons of the polyphenol were monitored as a function of guest (caffeine) concentration. It is clear that the phenolic hydroxy group is a good proton donor but poor acceptor in hydrogen bonding, but the amide carbonyl [CO(NMe)] groups in caffeine may be good proton acceptors. Thus complexation reactions of caffeine with polyphenols, with hydrogen bonding between the polyphenol (proton donor) and caffeine (proton acceptor), may ultimately make specific



Scheme 2 Synthesis of the hosts **H7**, **H8** and **H9**.

contributions to the stability of the complexes **E** (1 : 1 complex of **H8** and **G2**, K_a = 0.56 × 10^2 M^{-1} at 25 °C) and **F** (1 : 1 complex of **H9** and **G2**, K_a = 1.36 × 10^2 M^{-1} at 25 °C) (Fig. 6).

Binding studies of H4, H5 and H6 with theobromine and H8 and H9 with caffeine

NMR method. The ^{1}H NMR binding studies were carried out in $CDCl_3$ and in a mixture of DMSO-d_6 (10%) in $CDCl_3$. The NMR spectrum of the host (**H8** or **H9**) at known concentration was recorded to obtain chemical shifts of unbound host and then small aliquots of the guest stock solution in $CDCl_3$ were added to the NMR tube *via* a microsyringe. The chemical shifts of selected protons of the host were monitored as a function of guest concentration. Addition of guest was continued until no further shift of the selected protons was observed. The binding constants were measured by using equation (1)[18] where Δ = chemical-shift change induced by substrate P at concentration $[P_0]$, $[P_0]$ = formal substrate concentration, K = equilibrium constant for the formation of 1 : 1 complex between caffeine and the substrate P, Δ_0^{AP} = chemical-shift difference between the caffeine resonance in the unbound state and the state in which it is totally in the form of a 1 : 1 complex.

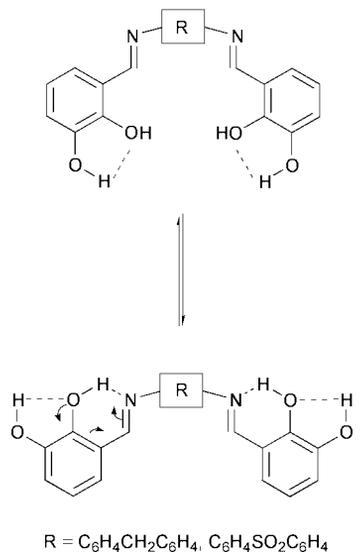
$$\frac{1}{\Delta} = \frac{1}{K \cdot \Delta_0^{AP}} \cdot \frac{1}{[P_0]} + \frac{1}{\Delta_0^{AP}} \quad (1)$$

In the case of theobromine, the titration with guests **H3**, **H4**, **H5** and **H6** was done by gradual dilution of the 1 : 1 complex of corresponding host and guest, and monitoring of the amide protons as a function of concentration. The binding constants were measured by using equation (2)[13a] where $\alpha = (\delta - \delta_0)/(\delta_{max} - \delta_0)$, δ_0 is the initial chemical shift (host only), δ is the chemical shift at each titration point, δ_{max} is the chemical shift when the receptor is entirely bound, and [c] = concentration of the host and guest.

$$K_{assoc} = \frac{\alpha}{(1 - \alpha)^2[c]} \quad (2)$$

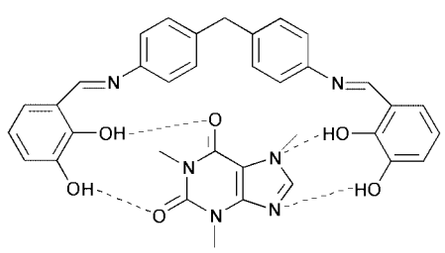
Table 3 Comparative binding study results for hosts **H4**, **H5**, **H6**, **H8**, **H9** by UV method

Host	Guest	Concentration of host	Concentration of guest	λ_{max}/nm monitored	K_a/M^{-1}
H4	**G1**	2.66×10^{-5} mol dm^{-3}	2.77×10^{-4} mol dm^{-3}	334	5.51×10^3
H5	**G1**	2.92×10^{-5} mol dm^{-3}	2.77×10^{-4} mol dm^{-3}	338	4.40×10^3
H6	**G1**	2.70×10^{-5} mol dm^{-3}	2.77×10^{-4} mol dm^{-3}	329	3.72×10^4
H8	**G2**	1.2×10^{-5} mol dm^{-3}	8.4×10^{-3} mol dm^{-3}	324	5.78×10^2
H9	**G2**	3.28×10^{-5} mol dm^{-3}	3.61×10^{-4} mol dm^{-3}	274	7.96×10^3

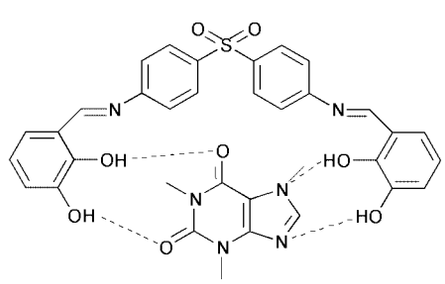


R = $C_6H_4CH_2C_6H_4$, $C_6H_4SO_2C_6H_4$

Fig. 5 Intramolecular hydrogen-bonded conformations of the imines **H8** and **H9**.



Complex **E**



Complex **F**

Fig. 6 Hypothetical complexation mode of caffeine with receptors **H8** and **H9**.

UV method. Stock solutions of different hosts were made in the order of 10^{-5} mol dm^{-3}. Aliquots of a solution of respective guest in the order of 10^{-4} mol dm^{-3} were added and the UV spectra were recorded for each addition for each host.

The concentrations of different hosts and the guest were calculated for Benesi–Hildebrand analyses.[13d,13e] The comparative binding results are shown in Table 3. The linear nature of the plot (Fig. 7) suggests a 1 : 1 stoichiometry for the different complexes.[13e]

Conclusions

In summary, we have developed a new class of biomimetic semirigid receptors for xanthine alkaloids that employs the recognition strategy of substrate-induced organisation of the binding site (induced fit) and we report the first receptors for theobromine which effectively solubilise it in nonpolar solvents such as chloroform. Systematic binding studies of caffeine with a series of newly designed diverse receptors show the importance of optimum space requirements especially in a macrocyclic receptor such as **H7** that fails to bind caffeine due to its smaller-than-optimum cavity. This follows the creation of a bigger synthetic cavity in the functionalised receptors for theobromine and caffeine. The synthesis of functionalised and more highly-spaced receptor **H6** for theobromine has been achieved by an important Co^{I} coupling reaction.[12]

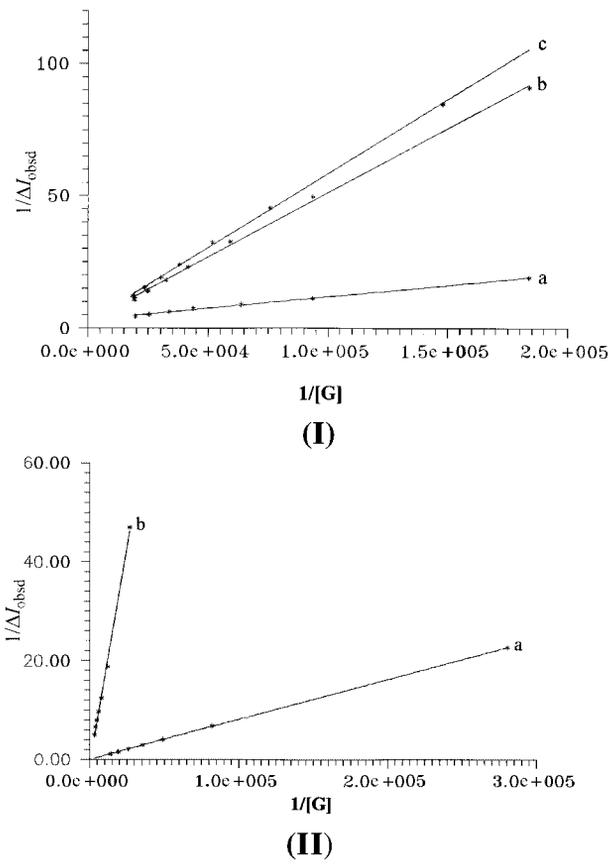


Fig. 7 (I) Benesi–Hildebrand plot of (a) **H6** with **G1**, (b) **H4** with **G1**, (c) **H5** with **G1**. (II) Benesi–Hildebrand plot of (a) **H9** with **G2**, (b) **H8** with **G2**.

Experimental

General

The following solvents were freshly distilled prior to use: tetrahydrofuran (THF) from sodium and benzophenone, ethanol from calcium oxide, magnesium turnings and I_2, methylene dichloride and triethylamine from calcium hydride. All other solvents and reagents were of reagent-grade quality and were used without further purification. Reactions were run under nitrogen unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on Bruker-AM-200 and AM-300 spectrometers. ROESY experiments were done on a Bruker-AM-500

spectrometer giving 300 ms mixing time. Chemical shifts are reported in δ ppm, and coupling constants J are in Hz. IR spectra were recorded on a Perkin-Elmer MODEL No-883 spectrometer; absorptions are reported in cm^{-1}. Melting points were recorded (uncorrected) in open capillaries with a hot-stage apparatus (Toshniwal). Elemental analyses were performed at IACS, Calcutta. NMR titrations were carried out in $CDCl_3$ except in the case of titration of **H9** with caffeine where d_6-DMSO was added (5%) to solubilise **H9** at 25 °C. Error limits of binding constants are within ±10%. The water content in the NMR solutions was checked by integration of the water peak at δ *ca.* 1.6 and was found to be 2–4 mM; in some cases where d_6-DMSO was used along with $CDCl_3$, water content was more likely to be 4–6 mM. Petroleum ether refers to the fraction with distillation range 60–80 °C.

N-[6-(Butyrylamino)pyridin-2-yl]butyramide H3

To an ice-cooled solution of 2,6-diaminopyridine (1 g, 9.17 mmol) and triethylamine (1.85 g, 18.34 mmol) in dry CH_2Cl_2 (40 mL) was added dropwise a solution of butyryl chloride (1.95 g, 18.34 mmol) in dry CH_2Cl_2 (20 mL). The mixture was stirred for 5 h at 0–10 °C and quenched with water (50 mL). The organic layer was separated, washed with 5% aq. sodium bicarbonate (20 mL), dried over sodium sulfate, and evaporated under reduced pressure. The crude solid was recrystallised from chloroform–hexane to afford **H3** (2.14 g, 94%) as a white solid, mp 101–102 °C; IR ν_{max} (KBr) 3320, 2950, 1669, 1531, 1455, 1304 cm^{-1}; ^{1}H NMR (200 MHz; $CDCl_3$) δ 8.52 (2H, br s), 7.92 (2H, d, *J* 8), 7.63 (1H, t, *J* 8), 2.40 (4H, t, *J* 7.6), 1.83–1.65 (4H, m), 1.00 (6H, t, *J* 6) (Calc. for $C_{13}H_{19}N_3O_2$: C, 62.63; H, 7.68; N, 16.85. Found: C, 62.54; H, 7.59; N, 16.82%).

3-(Ethoxycarbonylamino)benzoic acid 10

Ethyl chloroformate (1.98 g, 17.5 mmol) was added dropwise to a solution of 3-aminobenzoic acid (2 g, 14.6 mmol) and 1 M NaOH (50 mL) solution over a period of 30 min at room temperature. After stirring for an additional 3 h at room temperature, the mixture was neutralised by dil. phosphoric acid. The precipitate was filtered off, washed with cold water, and dried to afford **10** (2.6 g, 82%) as a white solid, mp 234–236 °C; IR ν_{max} (KBr) 3338, 2922, 1635, 1558,1238 cm^{-1}; ^{1}H NMR (200 MHz; $CDCl_3$) δ 8.87 (1H, br s), 8.02 (1H, s), 7.66–7.54 (2H, m), 7.20 (1H, d, *J* 8), 4.09 (2H, q, *J* 6), 1.22 (3H, t, *J* 6) (Calc. for $C_{10}H_{11}NO_4$: C, 57.74; H, 5.29; N, 6.69. Found: C, 57.65; H, 5.24; N, 6.61%).

{[3-(6-Aminopyridin-2-yl)carbamoyl]phenyl}carbamic acid ethyl ester H4

A mixture of **10** (0.5 g 2.39 mmol), ethyl chloroformate (0.26 g, 2.39 mmol), dry THF (50 mL), and triethylamine (0.4 mL) was stirred under argon at room temperature for 3 h. 2,6-Diaminopyridine (0.26 g, 2.39 mmol) was added to the mixture, which was then refluxed for an additional 3 h before being cooled to room temperature and the solvent was removed under reduced pressure. The residue was chromatographed (5% petroleum ether in chloroform) to afford **H4** (0.49 g, 64%) as grey solid, mp 199–200 °C (dec.); IR ν_{max} (KBr) 3401, 3279, 1732, 1617, 1454, 1309, 1234 cm^{-1}; ^{1}H NMR (200 MHz; 10% d_6-DMSO in $CDCl_3$) δ 8.75 (1H, br s, carbamate NH), 8.35 (1H, br s, pyr. amide), 7.78 (1H, s), 7.47 (1H, d, *J* 8), 7.30 (2H, t, *J* 8), 7.22–7.07 (2H, m), 6.02 (1H, d, *J* 8), 4.62 (2H, s), 3.97 (2H, q, *J* 6), 1.09 (3H, t, *J* 6); ^{1}H NMR (500 MHz; 10% d_6-DMSO in $CDCl_3$) δ 8.85 (1H, br s, carbamate NH), 8.58 (1H, br s, pyr. amide), 7.76 (1H, s), 7.35 (1H, d, *J* 7.75), 7.19 (1H, d, *J* 8), 7.16 (1H, d, *J* 8), 7.06 (1H, t, *J* 8), 7.00 (1H, t, *J* 8), 5.94 (1H, d, *J* 8), 4.74 (2H, br s), 3.85 (2H, q, *J* 7), 0.97 (3H, t, *J* 7); ^{13}C NMR (50 MHz; d_6-DMSO) δ_C 165.42, 158.25, 153.90, 149.99, 139.48, 139.19, 135.07, 128.82, 121.72, 121.35, 117.58, 104.32, 102.04, 60.47, 14.52; MS (EI) *m*/*z* (relative intensity) 300.1 (M^+, 56%), 254.1, 192.1, 145.6, 91.1 (Calc. for $C_{15}H_{16}N_4O_3$: C, 59.99; H, 5.37; N, 18.66. Found: C, 60.01; H, 5.20; N, 18.56%).

(3-{[6-(Acetylamino)pyridin-2-yl]carbamoyl}phenyl)carbamic acid ethyl ester H5

A mixture of **H4** (200 mg, 0.66 mmol) and distilled acetic anhydride (2 mL) was heated to 120 °C for 2 h. The reaction mixture was evaporated to dryness and the solid residue was dissolved in ethyl acetate. The organic layer was washed successively several times with 5% aq. sodium bicarbonate and water. The organic layer was separated, dried over Na_2SO_4, and evaporated to afford **H5** (0.21 g, 94%) as a grayish white solid, mp 220–223 °C (dec.); IR ν_{max} (KBr) 3265, 1729, 1620, 1445, 1312, 1250 cm^{-1}; ^{1}H NMR (200 MHz; $CDCl_3$) δ 8.34 (1H, br s, carbamate NH), 8.11 (1H, br s, pyr. aromatic amide), 8.04–8.01 (2H, m), 7.92 (1H, d, *J* 8), 7.75 (1H, d, *J* 8), 7.56 (2H, t, *J* 8), 7.42 (1H, d, *J* 6), 7.00 (1H, br s, pyr. N*H*COCH$_3$), 4.25 (2H, q, *J* 5), 2.23 (3H, s), 1.32 (3H, t, *J* 6); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 166.21, 160.34, 158.42, 154.45, 150.78, 139.32, 139.19, 135.98, 129.61, 121.36, 121.19, 117.62, 104.98, 103.07, 60.47, 28.56, 14.39; MS (EI) *m*/*z* 342.3 (M^+) (Calc. for $C_{17}H_{18}N_4O_4$: C, 59.65; H, 5.26; N, 16.37. Found: C, 59.42; H, 5.19; N, 16.13%).

1,2-Bis(3-carboxyphenyl)ethane 14

3-(Ethoxycarbonyl)benzyl bromide **12** (1.0 g, 4.1 mmol) was treated with $Co(PPh_3)_3Cl$ (4.34 g, 4.9 mmol) in degassed dry benzene (70 mL) at 5–10 °C for 20 min under argon atmosphere. The reaction mixture was filtered and the filtrate was washed with water and concentrated to dryness. The crude residue **13** was hydrolysed by addition 3 M NaOH (60 mL) and boiling for 3–4 h. The reaction mixture was cooled to room temperature and neutralised with dil. H_2SO_4. The precipitate was filtered off and the solid was washed several times with petroleum ether. Finally it was purified by chromatography on a silica gel column (3 : 1 ethyl acetate–methanol) to afford **14** (0.69 g, 78%) as a white solid, mp 225–228 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 8.19 (2H, s), 7.92 (2H, dd, *J* 2, *J* 8), 7.69 (2H, d, *J* 8), 7.43 (2H, t, *J* 8), 2.97 (4H, s) (Calc. for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 71.21; H, 5.19%).

1,2-Bis{3-[(6-amino-2-pyridyl)carbamoyl]phenyl}ethane 15

A slurry of **14** (0.2 g, 0.74 mmol) in dry CH_2Cl_2 (2 mL), oxalyl dichloride (0.8 mL) and a drop of DMF was stirred under an inert atmosphere for 3 h, resulting in a clear orange solution. The reaction mixture was evaporated to dryness under vacuum and the resulting light orange solid was re-dissolved in dry CH_2Cl_2 (50 mL). The dissolved acid chloride solution was added slowly *via* cannula to a vigorously stirred solution of 2,6-diaminopyridine (0.49 g, 4.44 mmol) in triethylamine (0.3 mL) and dry CH_2Cl_2 (50 mL) at 0 °C. The reaction mixture was allowed to attain room temperature and was then stirred for 24 h. The reaction mixture was concentrated, and washed with water to remove excess of 2,6-diaminopyridine and triethylamine hydrochloride. The crude product was purified by crystallisation from THF–hexane to afford **15** (0.24 g, 72%) as a light yellow powder; IR ν_{max} (KBr) 3398, 3282, 1617 cm^{-1}; ^{1}H NMR (200 MHz; $CDCl_3$) δ 8.58 (2H, br s), 8.20 (2H, s), 7.92 (2H, d, *J* 8), 7.69–7.65 (4H, m), 7.50 (2H, t, *J* 8), 7.43 (2H, t, *J* 8), 6.29 (2H, d, *J* 8), 4.40 (4H, br s), 2.97 (4H, s) (Calc. for $C_{26}H_{24}N_6O_2$: C, 69.01; H, 5.35; N, 18.57. Found: C, 69.11; H, 5.28; N, 18.42%).

1,2-Bis(3-{[6-(butyrylamino)-2-pyridyl]carbamoyl}phenyl)ethane H6

To a solution of **15** (0.2 g, 0.44 mmol) and triethylamine (0.13 mL, 0.88 mmol) in anhydrous THF (50 mL) was added

butyryl chloride (0.094 g, 0.88 mmol). After stirring at room temperature overnight, the reaction mixture was concentrated to give a sticky light yellow solid. Chromatography on silica gel using ethyl acetate–chloroform (1 : 3) as eluant yielded **H6** (0.16 g, 62%), mp 60–62 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 8.20 (2H, br s), 7.99 (2H, br s), 7.98–7.87 (4H, m), 7.83–7.64 (4H, m), 7.48–7.28 (6H, m), 3.03 (4H, s), 2.46 (4H, t, *J* 6), 1.53–151 (4H, m), 1.07 (6H, t, *J* 6); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 171.53, 165.72, 149.58, 140.85, 138.73, 134.07, 133.00, 128.64, 127.84, 124.08, 109.63, 109.56, 39.57, 21.27, 18.72, 13.63; MS (EI) *m*/*z* 592 (M^+, 15%), 549, 521, 479, 370, 344 (Calc. for $C_{34}H_{36}N_6O_4$: C, 68.90; H, 6.12; N, 14.18. Found: C, 68.47; H, 6.34; N, 13.99%).

Bis{4-[(2,3-dihydroxyphenyl)methylideneamino]phenyl}methane H8

Bis(4-aminophenyl)methane (0.2 g, 1.01 mmol) was added gradually to a methanolic (60 mL) solution of 2,3-dihydroxybenzaldehyde (0.28 g, 2.02 mmol) and the solution was stirred for 1 h at 50 °C. The orange solid precipitate was collected by filtration; mp 183 °C; IR ν_{max} (KBr) 3420, 1624, 1461, 1369, 1275, 1228 cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ 8.59 (2H, br s), 7.24–7.21 (6H, m), 7.03 (4H, d, *J* 7.5), 6.94 (4H, d, *J* 8), 6.82 (2H, t, *J* 8), 4.08 (2H, s); ^{1}H NMR (500 MHz; $CDCl_3$) δ 13.87 (2H, br s) (at 300 MHz it had broadened), 8.60 (2H, s), 7.28–7.24 (8H, m), 7.03 (2H, d, *J* 7.5), 6.94 (2H, d, *J* 8), 6.82 (2H, t, *J* 8), 5.79 (2H, br s), 4.05 (2H, s); MS (EI) *m*/*z* 438.3 (M^+, 81%) (Calc. for $C_{27}H_{22}N_2O_4$: C, 73.96; H, 5.06; N, 6.38. Found: C, 73.89; H, 5.26; N, 6.23%).

Bis{[(2,3-dihydroxyphenyl)methylideneamino]phenyl} sulfone H9

Bis(4-aminophenyl) sulfone (0.2 g, 0.81 mmol) was added gradually to a methanolic (60 mL) solution of 2,3-dihydroxybenzaldehyde (0.22 g, 1.61 mmol), the solution was stirred for 1 h at 50 °C and the orange solid precipitate was collected by filtration; mp 250 °C (dec.); IR ν_{max} (KBr) 3432, 1622, 1577, 1466, 1368, 1316, 1279, 1206; ^{1}H NMR (300 MHz; d_6-DMSO) δ 8.67 (4H, br s), 8.01 (4H, d, *J* 8), 7.64 (2H, s), 7.43 (4H, d, *J* 8), 7.05–6.98 (4H, m), 6.81 (2H, t, *J* 7.5); MS (EI) *m*/*z* 487.9 (M^+, 18%) (Calc. for $C_{26}H_{20}N_2O_6S$: C, 63.93; H, 4.13; N, 5.73. Found: C, 63.86; H, 4.25; N, 5.52%).

2,7-Bis{3-[(3-aminophenyl)carbamoyl]propoxy}naphthalene 17

2,7-Bis(3-carboxypropoxy)naphthalene **16** (0.36 g, 1 mmol) was suspended in dry CH_2Cl_2 and oxalyl dichloride (0.5 mL) and DMF (1 drop) were added; the reaction mixture was stirred at room temperature for 2 h. After some time all the starting material had dissolved. The solvent was removed on a rotary evaporator and the residue was kept under high vacuum for 3 h. The diiacid dichloride was used without purification.

To a solution of 1,3-diaminobenzene (0.702 g, 6.50 mmol) and triethylamine (0.25 mL) in dry THF (50 mL) was added dropwise a solution of the above diacid dichloride (0.300 g, 0.81 mmol) in dry THF (10 mL). After 3 h the reaction mixture was evaporated to dryness and the solid residue was washed several times with water. Purification by column chromatography (THF–CH_2Cl_2 eluant 20 : 80) gave diamine **17** (0.29 g, 52%) which was directly used for further reaction, mp 165 °C; IR ν_{max} (KBr) 3440, 3281, 1654, 1618, 1532 cm^{-1}; ^{1}H NMR (200 MHz; d_6-DMSO) δ 9.00 (2H, br s), 7.36 (2H, d, *J* 10), 7.04 (2H, s), 6.90–6.67 (8H, m), 6.26 (2H, d, *J* 8), 3.88 (4H, t, *J* 6), 3.12 (4H, br s), 2.33 (4H, t, *J* 6), 1.99–1.91 (4H, m); ^{13}C NMR (50 MHz; d_6-DMSO) δ_C 171.01, 157.19, 144.38, 139.72, 135.67, 129.18, 128.75, 123.91, 116.01, 115.30, 111.04, 107.69, 106.04, 66.96, 33.40, 24.92 (Calc. for $C_{30}H_{32}N_4O_4$: C, 70.29; H, 6.29; N, 10.93. Found: C, 70.21; H, 6.18; N, 10.79%).

Trisphenyl macrocycle H7

A solution of diamine **17** (0.1 g, 0.195 mmol) in THF (50 mL) and triethylamine (54 μL) was added simultaneously with a solution of 5-octyloxyisophthaloyl dichloride (0.065 g, 0.195 mmol) in THF (30 mL) to stirred THF (30 mL) at room temperature over a period of 3 h. The reaction mixture was evaporated to dryness and the residue was purified by column chromatography on silica gel (6% MeOH in $CHCl_3$ eluant). Crystallisation from $CHCl_3$–MeOH (1 : 1) afforded the product macrocycle **H7** (0.042 g, 28%); mp 310–311 °C (dec.); IR ν_{max} (KBr) 3309, 3055, 1978, 1820, 1721, 1649 cm^{-1}; ^{1}H NMR (200 MHz; $CDCl_3$) δ 9.42 (1H, br s), 9.10 (1H, br s), 8.00 (2H, br s), 7.87 (3H, m), 7.68 (2H, d, *J* 8), 7.55 (2H, s), 7.46 (2H, d, *J* 8), 7.13 (2H, t, *J* 8), 6.95 (2H, d, *J* 8), 6.80 (2H, d, *J* 8), 4.07 (4H, m), 3.81 (2H, t, *J* 6), 2.09–2.02 (4H, m), 1.61–1.63 (4H, m), 1.28–1.11 (12H, m), 0.78–0.73 (5H, m); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 179.71, 170.92, 164.72, 159.26, 156.86, 138.78, 138.41, 135.68, 135.29, 128.44, 124.54, 123.83, 117.22, 116.89, 116.12, 114.94, 111.88, 105.40, 71.04, 66.24, 38.23, 32.12, 29.01, 28.41, 24.21, 23.23, 22.39, 22.02, 13.51; HRMS (FAB) 771 (MH^+, 100%), 705, 453, 435, 307 (Calc. for $C_{46}H_{50}N_4O_7$: C, 71.66; H, 6.54; N, 7.27. Found: C, 71.53; H, 6.78; N, 7.32%).

Complex of H3–G1

^{1}H NMR (200 MHz; $CDCl_3$) δ 11.37 (1H_{G1}, br s), 8.95 (2H, br s), 7.96 (2H, d, *J* 8), 7.69 (1H, t, *J* 8), 7.59 (1H_{G1}, s), 4.00 (3H_{G1}, s, NCH_3), 3.59 (3H_{G1}, s, NCH_3), 2.43 (4H, t, *J* 7.6), 1.84–1.66 (4H, m), 1.00 (6H, t, *J* 6).

Complex of H4–G1

^{1}H NMR (300 MHz; 2% d_6-DMSO in $CDCl_3$) δ 9.85 (1H_{G1}, br s), 8.61 (1H, br s), 8.39 (1H, br s), 7.99 (1H, s), 7.72 (1H, d, *J* 8), 7.63 (1H, d, *J* 7.5), 7.55 (1H_{G1}, s), 7.50–7.41 (2H, m), 7.37 (1H, d, *J* 7.4), 6.34 (1H, d, *J* 7.8), 4.70 (2H, br s), 4.23 (2H, q, *J* 6.6), 3.96 (3H_{G1}, s, NCH_3), 3.52 (3H_{G1}, s, NCH_3), 0.86 (3H, t, *J* 6).

Complex of H5–G1

^{1}H NMR (200 MHz, $CDCl_3$) δ 9.84 (1H_{G1}, br s), 9.29 (1H, br s), 9.24 (1H, br s), 8.55 (1H, br s), 7.93 (1H, br s, *peri*), 7.90 (1H, d, *J* 8.6), 7.76 (1H, d, *J* 8), 7.62 (1H, d, *J* 8), 7.56–7.49 (2H, m), 7.45 (1H_{G1}, s), 7.29 (1H, t, *J* 7.9), 4.11 (2H, q, *J* 7.2), 3.84 (3H_{G1}, s), 3.39 (3H_{G1}, s), 2.16 (3H, s), 1.22 (3H, t, *J* 7.2).

Complex of H6–G1

^{1}H NMR (200 MHz; $CDCl_3$) δ 10.58 (1H_{G1}, br s), 8.38 (3H, br s), 8.24 (1H, br s), 7.99–7.86 (4H, m), 7.82–7.67 (4H, m), 7.52 (1H_{G1}, s), 7.49–7.23 (6H, m), 3.9 (3H_{G1}, s), 3.50 (3H_{G1}, s), 3.02 (s, 4H), 2.44 (4H, t, *J* 7.2), 1.87–1.69 (4H, m), 1.02 (6H, t, *J* 6).

Complex of H8–G2

^{1}H NMR (300 MHz; $CDCl_3$) δ 8.59 (4H, br s), 7.50 (1H_{G2}, s), 7.25 (6H, br s), 7.03 (4H, d, *J* 7.8), 6.95 (4H, d, *J* 7.2), 6.82 (2H, t, *J* 7.8), 4.04 (2H, s), 3.99 (3H_{G2}, s), 3.58 (3H_{G2}, s), 3.41 (3H_{G2}, s).

Complex of H9–G2

^{1}H NMR (300 MHz; 10% d_6-DMSO in $CDCl_3$) δ 8.66 (4H, br s), 8.0 (4H, d, *J* 8.4), 7.69 (1H_{G2}, s), 7.61 (2H, br s), 7.43 (4H, d, *J* 8.4), 7.05–6.98 (4H, m), 6.82 (2H, t, *J* 7.8), 3.99 (3H_{G2}, s), 3.56 (3H_{G2}, s), 3.31 (3H_{G2}, s).

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