Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Calix[2]benzimidazole: A New Luminescence Turn-On Host for Anions

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1. Experimental

Materials: All the materials described in the manuscript, including HPLC grade solvents, were purchased from Sigma-Aldrich and Fluka. Solvents and starting materials were used as received unless noted. 2,3-Diamino-benzoic acid methyl ester, **2**, was prepared according to a slightly modified literature procedure.**Error! Bookmark not defined.**

Methyl 2-(2-nitrophenyl)-1*H*-benzimidazole-4-carboxylate (4): A solution of methyl 2,3-diaminobenzoate **(2)** (1.6 gr, 8.2 mmole) and 2-nitrobenzaldehyde **(3)** (1.23 gr, 8.2 mmole) in freshly distilled nitrobenzene (250 ml) was refluxed for four days under nitrogen in the dark. Nitrobenzene was removed under reduced pressure to obtain the brown crude product. Subsequent purification by flash column chromatography (silica, 30% EtOAc in Hexane) afforded the product (1.7gr, 70% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 10.82 (br s, 1H, NH), 7.95-8.01 (m, 4H, CH-phenyl), 7.75 (t, 1H, J = 9.0 Hz, CH-phenyl), 7.66 (t, 1H, J= 9.0 Hz, CH-phenyl), 7.36 (t, 1H, J = 9.0 Hz, CH-phenyl), 4.01 (s, 3H, CH_3) ppm; mp= 208-210 °C; MS (CI): m/z= 298 (MH^+).

2-(2-nitrophenyl)-1*H*-benzimidazole-4-carboxylic Acid (5): Methyl 2-(2-nitrophenyl)-1*H*-benzimidazole-4-carboxylate **(4)** (2 gr, 6.7 mmole) was added to a solution of NaOH (1.6 gr, 0.04 mole) in methanol and water (125 ml) and the mixture was refluxed for 2h. The solvent was evaporated under reduced pressure to give a yellow solid. The solid was dissolved in water (100 ml) and neutralized at 0°C using conc. HCl. The precipitate formed was removed by filtration, washed with water, and dried under reduced pressure to give the product (1.8 gr, 95% yield) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6-0.1% D_2O): δ= 12.89 (s, 1H, COOH) , 7.922 (t, J=7.2 Hz, 2H, CH-phenyl), 7.833-7.882 (m, 2H, CH-phenyl), 7.78 (t, J=7.5 Hz, 1H, CH-phenyl), 7.329 (t, J=7.8 Hz ,1H, CH-phenyl) ppm; ^{13}C- NMR (300 MHz, DMSO-d_6): δ= 61.0, 100.76, 122.6, 125.5, 126.2, 132.3, 134.0, 134.2, 150.0, 167.4, 168.0, 173.3 ppm; mp>200°C; MS (TOP LD^-): m/z= 282 (M-H), 266 (M-OH).

2-(2-Aminophenyl)-1*H*-benzimidazole-4-carboxylate (6): A suspension of methyl 2-(2-Nitrophenyl)-1*H*-benzimidazole-4-carboxylate **(4)** (2 gr, 6.7 mmole) in MeOH (50 ml) was hydrogenated at normal pressure in the presence of Pd/C (10% w/w, 0.2 g) for 5 h. After filtration through Celite and removal of the solvent under reduced pressure, the resulting yellow-brown solid (1.8 gr, 98% yield) was used as is. ^{1}H NMR (300 MHz, DMSO-d_6-0.1% D_2O): δ= 7.89 (br s, 1H, NH_2) , 7.782 (d, 1H, J=7.5 Hz, CH-phenyl), 7.305 (t, 2H, J=7.8 Hz, Ch-phenyl), 7.173 (t, 2H, J=7.2 Hz, CH-phenyl), 6.846 (d, 1H, J=8.1 Hz, CH-phenyl), 6.645 (t, 1H, J=7.5 Hz, CH-phenyl), 3.95 (s, 3H, CH_3) ppm; mp>200°C; MS (CI): m/z= 266 [M^+].

2-(2-nitrophenyl)-N-propyl-3H-benzimidazole-4-carboxamide (10): NMM (0.35 ml, 3.18 mmole), and BOP (0.46 gr, 1.06 mmole) were added to a suspension of 2-(2-nitrophenyl)-1*H*-benzimidazole-4-carboxylic Acid **(5)** (0.3g, 1.06 mmole) and n-propylamine (0.09 ml, 1.06 mmole) in DCM (125 ml). The reaction mixture was refluxed for six days under nitrogen and washed with saturated $NaHCO_3$ aqueous solution. The organic layer was dried with sodium sulfate and evaporated under reduced pressure to give a brown solid. Subsequent purification by flash column chromatography (silica gel, 2% MeOH in DCM) gave the product as a yellow solid (0.18 gr, 53% yield). ^{1}H NMR (300 MHz, DMSO-d_6-0.1% D_2O): δ= 9.462 (s, 1H, CONH) , 8.216 (d, J=8.4 Hz, 1H, CH-phenyl), 8.089 (d, J=8.1 Hz ,1H, CH-phenyl), 8.022 (d, J=7.8 Hz, 1H, CH-phenyl), 7.892 (t, J=4.5 Hz ,1H, CH-phenyl), 7.792 (t, J=7.8 Hz, 2H, CH-phenyl), 7.405 (t, J=8.1 Hz, 1H, CH-phenyl), 3.367 (m, 2H, NCH_2), 1.61 (m, 2H, CH_2), 0.937 (t, J=7.5 Hz, 2H, CH_3) ppm; mp>200°C; MS (TOP LD$^+$): m/z= 325 (MH^+).

2-(2-aminophenyl)-N-propyl-3H-benzimidazole-4-carboxamide (11): A suspension of 2-(2-nitrophenyl)-N-propyl-3H-benzimidazole-4-carboxamide **(10)** (1 gr, 3.1 mmole) in MeOH (50 ml) was hydrogenated at atmospheric pressure in the presence of Pd/C (10% w/w, 0.1 g) for 12 h. After filtration through Celite and removal of the solvent under reduced pressure, the resulting yellow- brown solid (0.89 gr, 98%) was used as is, without further purification . ^{1}H NMR (300 MHz, DMSO-d_6-0.1% D_2O): δ= 9.52 (s, 1H, CONH), 7.95 (br s, 1H, NH_2), 7.82 (d, 1H, J=7.5 Hz, CH-phenyl), 7.35 (t, 2H, J=7.8 Hz, Ch-phenyl), 7.12 (t, 2H, J=7.2 Hz, CH-phenyl), 6.92 (d, 1H, J=8.1 Hz, CH-phenyl), 6.7 (t, 1H, J=7.5 Hz, CH-phenyl), 3.42 (m, 2H, NCH_2), 1.65 (m, 2H, CH_2), 1.02 (t, J=7.5 Hz, 2H, CH_3) ppm; mp>200°C; MS (TOF LD$^+$): m/z= 295 (MH^+), 335 ($M+K^+$).

2-(2-acetamidophenyl)-N-propyl-3H-benzimidazole-4-carboxamide (12): 2-(2-aminophenyl)-N-propyl-3H-benzimidazole-4-carboxamide **(11)** (200 mg, 0.7 mmole), was treated with Ac_2O (5 ml) at room temprature under magnetic stirring in the presence of $InCl_3$ (0.155mg, $0.7x10^{-3}$ mmole, 0.1 mol%) For 2 days. The white precipitate that developed was filtered and washed with Et_2O and dried under reduced to afford the product as a white solid (0.22 gr, 95% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 10.18 (s, 1H, CONH) , 8.622 (d, J=6.9 Hz, 1H, CH-phenyl), 8.492 (d, 1H, J=7.2 Hz, CH-phenyl), 8.179 (d, 1H, J=7.5 Hz, CH-phenyl), 7.919 (d, 1H, J=8.4 Hz, CH-phenyl), 7.821 (t, 1H, J=6.9 Hz, CH-phenyl), 7.693 (t, 1H, J=7.2 Hz, CH-phenyl), 7.565 (t, 1H, J=7.8 Hz, CH-phenyl), 3.64 (m, 2H, NCH_2), 1.82 (m, 2H, CH_2), 1.16 (t, J=6 Hz, 2H, CH_3) ppm; mp>200°C; MS (TOF LD$^+$): m/z= 337 (MH^+).

Methyl 2-(2-(2-(2-nitrophenyl)-1H-benzimidazole-4-carboxamido)phenyl)-1H-benzimidazole-4-carboxylate (7): NMM (0.32 ml, 2.96 mmole) and BOP (0.64 gr, 1.48 mmole) were added to a suspension of 2-(2-nitrophenyl)-1*H*-benzimidazole-4-carboxylic Acid **(5)** (0.4 gr, 1.48 mmole) and 2-(2-Aminophenyl)-1*H*-benzimidazole-4-carboxylate **(6)** (0.4 gr, 1.48 mmole) in DCM (125 ml). The reaction mixture was refluxed for six days under nitrogen and washed with saturated $NaHCO_3$ aqueous solution. The organic layer was dried with sodium sulfate and evaporated under reduced pressure to give a brown solid. Subsequent purification by flash column chromatography (Al_2O_3, 30% EtOAc in hexane) afforded the product as a yellow solid (0.32 gr, 41% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 13.78 (br s, 1H, CONH), 7.297-8.454 (m, 14H, CH-phenyl), 3.95 (s, 3H, CH_3) ppm; mp>200°C; MS (TOF LD+): m/z= 533.1 (45%, MH^+), 555.1 ($M+Na^+$). See supplementary material for the crystal structure of **7**.

2-(2-(2-(2-nitrophenyl)-1H-benzimidazole-4-carboxamido)phenyl)-1H-benzimidazole-4-carboxylic acid (8): Methyl 2-(2-(2-(2-nitrophenyl)-1H-benzimidazole-4-carboxamido)phenyl)-1H-benzimidazole-4-carboxylate **(7)** (0.1gr, 0.18mmole) was added to a solution of NaOH (0.04 gr, 1 mmole) in MeOH and water (125 ml) and the mixture was refluxed for 5h. The solvent was evaporated under reduced pressure to give a yellow solid. The solid was dissolved in water (300 ml) and neutralized at 0 °C with conc. HCl. The precipitate formed was removed by filtration, washed with water, and dried under reduced pressure to give the product as a gummy brownish solid (0.078 gr, 80% yield). ^{1}H NMR (300 MHz, DMSO-d_6-0.1% D_2O): δ= 13.3 (br s, 1H, COOH), 12.71 (br s, 1H, CONH), 7.302-8.091 (m, 14H, CH-phenyl), 3.96 (s, 3H, CH_3) ppm; mp>200°C; MS (TOF AP+): m/z= 519 (MH^+), 541.13 ($M+Na^+$).

2-(2-(2-(2-aminophenyl)-1H-benzo[d]imidazole-4-carboxamido)phenyl)-1H-benzo[d]imidazole-4-carboxylic acid (9): A suspension of 2-(2-(2-(2-nitrophenyl)-1H-benzimidazole-4-carboxamido)phenyl)-1H-benzimidazole-4-carboxylic acid (**8)** (0.68 gr, 1.3 mmole) in MeOH (50 ml) was hydrogenated at normal pressure in the presence of Pd/C (10% w/w,0.06 g) for 5 h. After filtration through Celite and removal of the solvent under reduced pressure, the resulting gummy yellow solid (640 mg, 100%) was used directly. ^{1}H NMR (300 MHz, DMSO-d_6-0.1% D_2O): δ= 13.35 (br s, 1H,

COOH), 6.664-8.11 (m, 14H, CH-phenyl); mp>200°C; MS (TOF LD+): *m*/*z*= 489 [MH^+], 511 [$M+Na^+$], 523 [$M+Cl^-$], 529 [$M+K^+$].

Calix[2]benzimidazole (1): NMM (0.3 ml, 2.66 mmole), followed by BOP (0.58 gr, 1.33 mmole) were added to a suspension of 2-(2-(2-(2-aminophenyl)-1H-benzo[d]imidazole-4-carboxamido)phenyl)-1H-benzo[d]imidazole-4-carboxylic acid **(12)** (0.64 gr, 1.33 mmole) in DCM (250 ml). The reaction mixture was refluxed for six days under nitrogen and washed with saturated $NaHCO_3$ aqueous solution. The organic layer was dried with sodium sulfate and evaporated under reduced pressure to give a brown solid. Subsequent purification by flash column chromatography (silica, 0.5% MeOH in DCM) afforded the product as a yellow solid (0.19 gr, 30% yield). ^{1}H NMR (500 MHz, DMSO-d_6-0.1% D_2O): δ= 12.26(s, 2H) , 7.957 (d, *J*=8 Hz, 2H, CH-phenyl), 7.863 (d, *J*=8.0 Hz, 2H, CH-phenyl), 7.787 (d, *J*=8 Hz, 4H, CH-phenyl), 7.594 (t, *J*=7 Hz, 2H, CH-phenyl), 7.428 (t, *J*=7 Hz, 4H, Ch-phenyl), 7.374 (t, *J*=8.0 Hz, 2H, CH-phenyl) ppm; mp>200°C; MS (TOF MS ES-): *m*/*z*=469 $(M-H)^+$.

2. Apparatus

NMR spectra were recorded on a Bruker AVANCE 500 spectrometer at 298±1 K. Mass spectra were recorded by using MALDI micro MX (MICROMASS). UV-Vis absorption spectra were recorded on a Shimadzu UV-1601 spectrometer. Fluorescence spectra were recorded on a Perkin–Elmer LS 50 luminescence spectrometer. All the optical measurements were performed in analytical grade solvents.

3. NMR

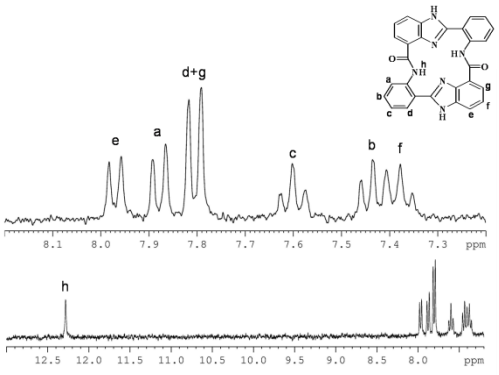

Figure S1 The ^{1}H NMR spectrum of **1** in 0.1% D_2O:DMSO-d_6.

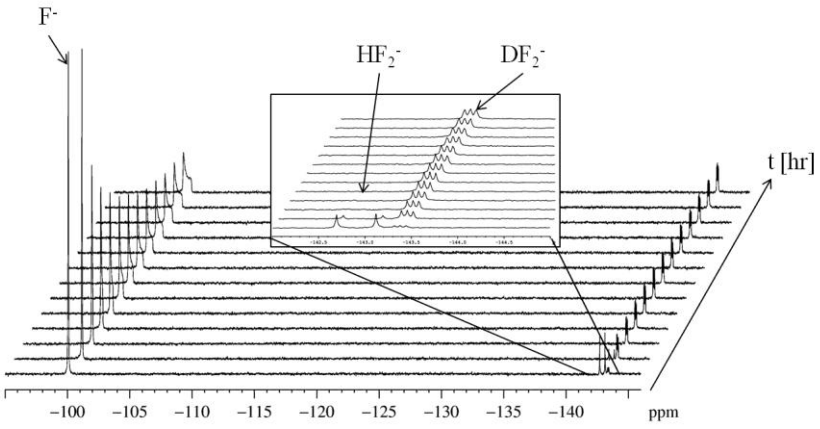

Figure S2 The change with time of the ^{19}FNMR spectrum of 0.02 M tetrabutyl ammonium fluoride (TBAF**)** in 0.1% D_2O:DMSO-d_6 showing the disappearance and shifting of the fluoride anion with the formation of DF_2.

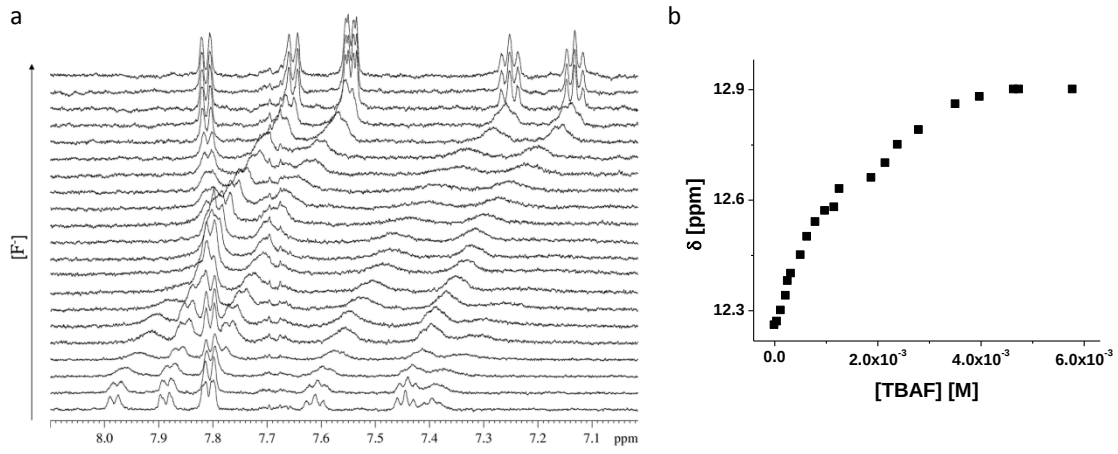

Figure S3 (a) ^{1}H NMR spectra of **1** (0.5 mM) in 0.1% D_2O:DMSO-d_6 in the presence of increasing concentration of TBAF; (b) The titration profile for the amide hydrogen's peak.

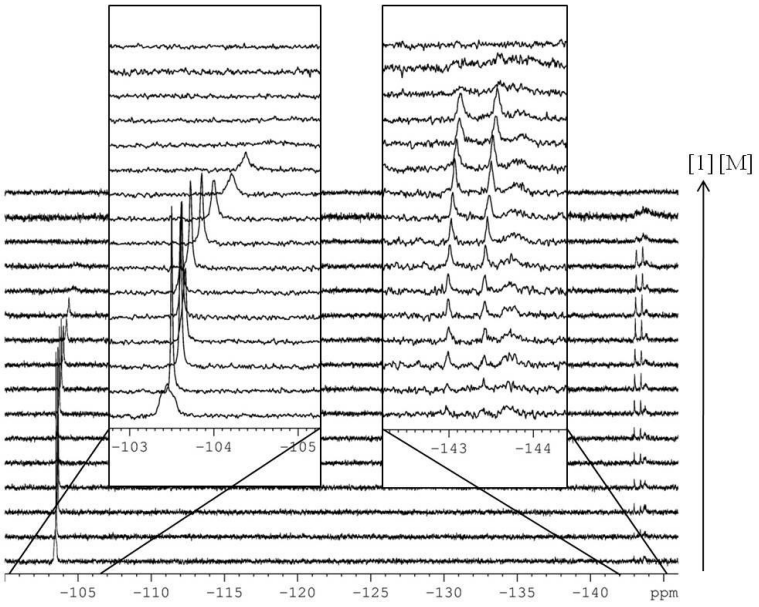

Figure S4 Titration ^{19}FNMR spectra of TBAF (8 mM) in 0.1% D_2O:DMSO-d_6 in the presence of increasing concentration of **1.**

4. Spectrophotoscopy

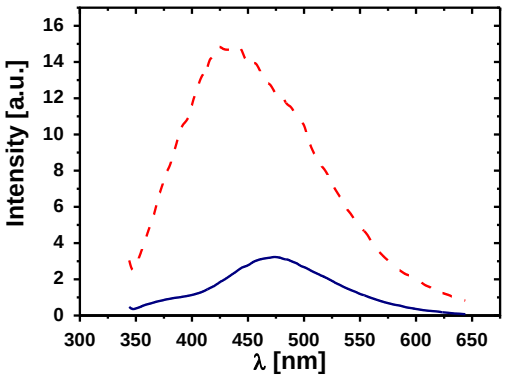

Figure S5 Fluorescence spectra of calix[2]benzimidazole, **1**, 1.5x10^{-3} M in 0.1% H_2O:DMSO excited at λ_{ex}=351.1nm at room temperature (▬) and at 82K (▪▪▪).

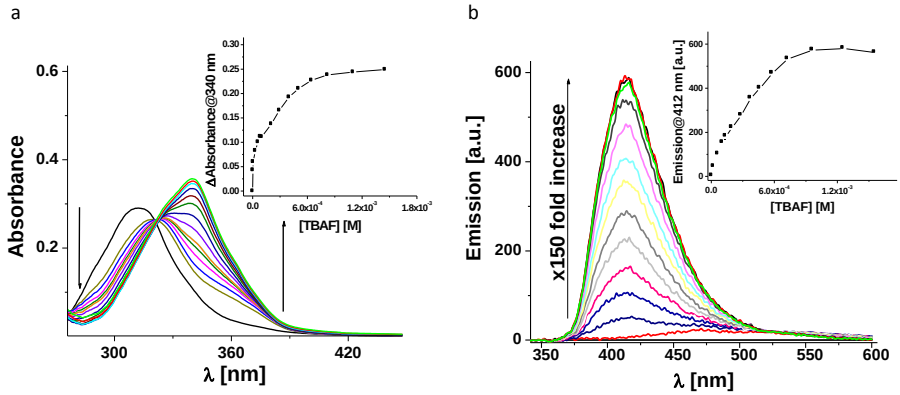

Figure S6 Absorption (a) and emission (b, λ_{ex}=322nm) spectra of a solution of 1.5x10^{-5} M calix[2]benzimidazole, **1**, in the presence of different concentrations of TBAF in 0.1% H_2O:DMSO.

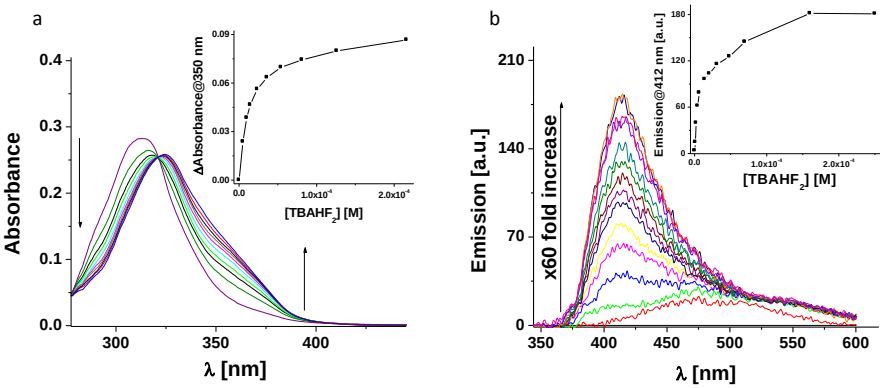

Fig. S7 Absorption (a) and emission (b, λ_{ex}=322nm) spectra of a solution of 1.5x10^{-5} M calix[2]benzimidazole, **1**, in the presence of different concentrations of tetrabutyl ammonium bifluoride ($TBAHF_2$) in 0.1% H_2O:DMSO.

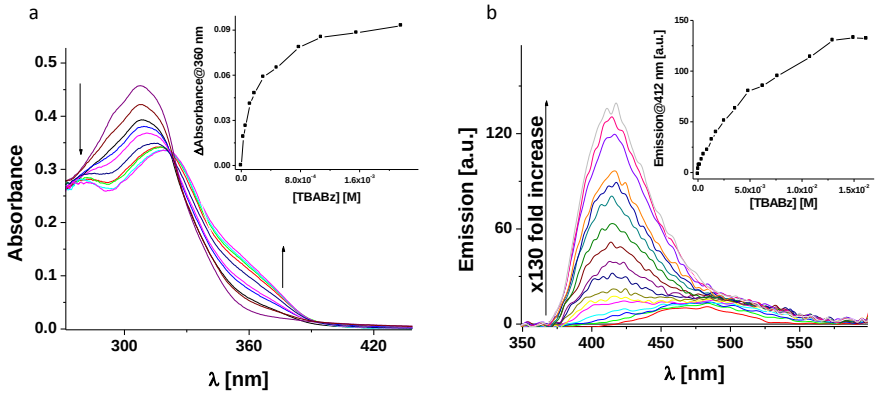

Fig. S8 Absorption (a) and emission (b, λ_{ex}=322nm) spectra of a solution of $1.5x10^{-5}$ M calix[2]benzimidazole, **1**, in the presence of different concentrations of tetrabutyl ammonium benzoate (TBABz) in 0.1% H_2O:DMSO.

5. Binding constant calculation

Binding constants were found by calculating the δ_{obs} and comparing the calculated values to the measured ones. The concentrations of the complex species of 1:1 and 2:1 host:guest stoichiometries were calculated by solving the following equations using Matlab for varying sets of values of K_1 and K_2:

$$\text{Host-H} + F^- \overset{K_1}{\rightleftharpoons} \text{Host-H}\cdots F^-$$

$$\text{Host-H}\cdots F^- + F^- \overset{K_2}{\rightleftharpoons} \text{Host}^- + HF_2^-$$

$$K_1 = \frac{[HG]}{[Host]_f [Guest]_f}, K_2 = \frac{[H_2G]}{[HG][Host]_f}$$

$$[Host]_f = [Host]_0 - [HG] - 2[H_2G]$$

$$[Guest]_f = [Guest]_0 - [HG] - [H_2G]$$

$$K_1 = \frac{[HG]}{([Host]_0 - [HG] - 2[H_2G])([Guest]_0 - [HG] - [H_2G])}, K_2 = \frac{[H_2G]}{[HG]([Host]_0 - [HG] - 2[H_2G])}$$

Using the calculated values of *HG* and H_2G concentrations, the value of δ_{obs} were calculated for varying sets of values of δ_{free}, δ_{HG} and δ_{H2G} by solving the following equation:

$$\delta_{obs} = \chi_f \cdot \delta_f + \chi_{HG} \cdot \delta_{HG} + \chi_{H_2G} \cdot \delta_{H_2G}$$

Where, $\chi_f = \frac{[Host]_f}{[Host]_0}, \chi_{HG} = \frac{[HG]}{[Host]_0}, \chi_{H_2G} = \frac{[H_2G]}{[Host]_0}$