

Rational design of a fluorescent receptor for the recognition of anthrax biomarker dipicolinate.

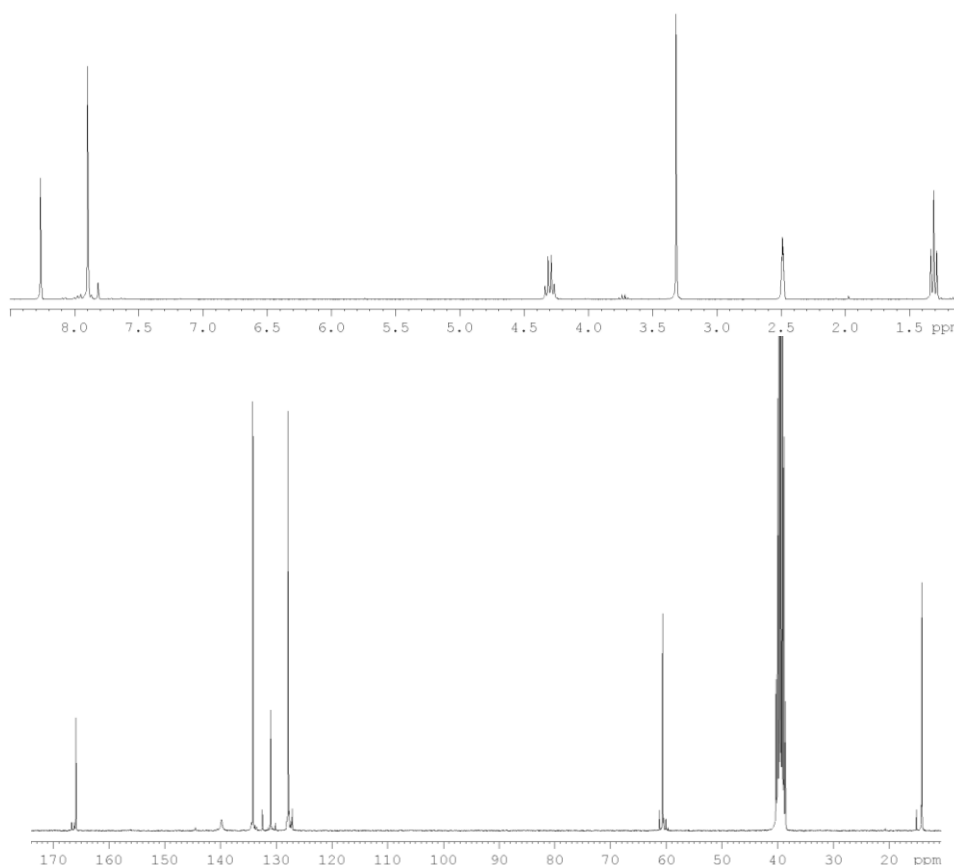
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ELECTRONIC SUPPLEMENTARY INFORMATION

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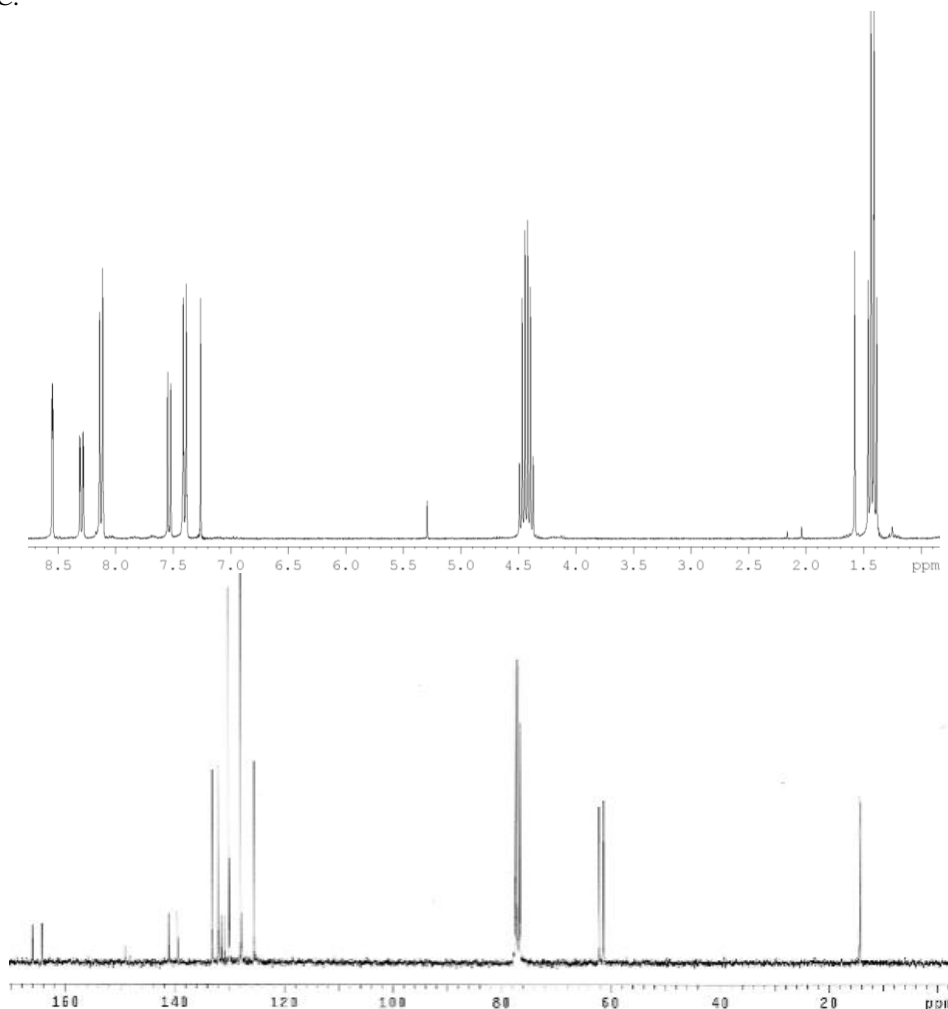
Reagents used as starting materials were commercially available and were used without further purification. Solvents were dried following the usual protocols (THF, Et₂O and Toluene were distilled from sodium wire with benzophenone indicator; CH₃CN and CH₂Cl₂ were distilled from CaCl₂; EtOH and MeOH were distilled from magnesium and stored with molecular sieves). Unless stated otherwise, all reactions were carried out under nitrogen atmosphere. Column chromatography was run with silica gel 60 A CC 70-200 μm as stationary phase and using HPLC grade solvents. Melting points were measured in a Reichert instrument and are not corrected. ¹H-NMR, ¹³C-NMR and NOESY experiments were recorded on either a Bruker AV300 instrument, or a Bruker AV400 instrument. Chemical shifts are referred to the residual peak of the solvent. In the experimental data “bp” stands for broad peak and “Cq” for quaternary carbon atom. Mass spectrometry was recorded on HPLC-MS TOF 6220 instrument. Absorption spectra were recorded on a Cary 5000 UV-vis-NIR spectrophotometer. Emission spectra were recorded on a Cary Eclipse spectrophotometer.

***p*-ethoxycarbonylphenylboronic acid (1).** Over a solution of *p*-carboxyphenylboronic acid (1 g, 6.03 mmol) in ethanol (40 mL) sulphuric acid was added (1 mL). The mixture was stirred under reflux for 8 h; the reaction was then cooled to room temperature and neutralised by adding NaHCO₃. The solution was concentrated under reduced pressure and extracted with AcOEt (3x20 mL). The organic extracts were combined, dried over anhydrous Na₂SO₄, filtered and evaporated to yield the desired product as a white solid (0.93 g, 80%). ¹H-NMR (200 MHz, DMSO-*d*₆); δ (ppm): 1.31 (t, *J* = 7 Hz, 3H), 4.30 (q, *J* = 7 Hz, 2H), 7.90 (s, 4H), 8.27 (s, 2H, OH). ¹³C-NMR (75 MHz, DMSO-*d*₆); δ (ppm): 14.2(CH₃), 60.7(CH₂), 127.9(2xCH), 131.0(C_q), 134.2(2xCH), 139.8(C_q), 165.9(C=O). HR-MS (*m/z*): (C₉H₁₁BO₄), found: 194.0861 (M⁺+1), calculated: 194.0865. Melting point: 175-176 °C.

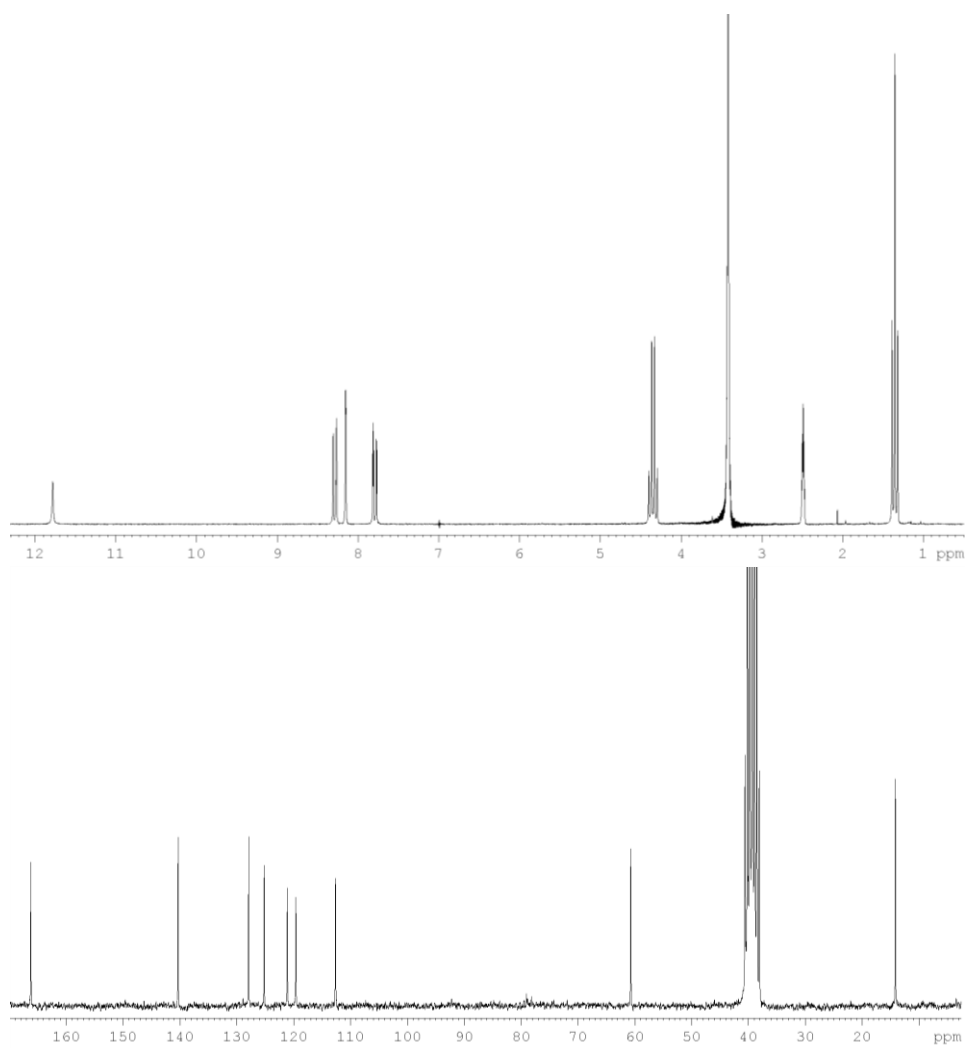


4,4'-bis(ethoxycarbonyl)-2-nitrobiphenyl (3). A solution of ethyl 4-iodo-3-nitrobenzoate (1.5 g, 4.67 mmol) in toluene (15 mL) was added over a degassed solution of tetrakis(triphenylphosphine)palladium(0) (0.16 g, 0.14 mmol) in toluene (10 mL). The mixture was stirred under nitrogen atmosphere for 15 min. Then, a suspension of *p*-ethoxycarbonylphenylboronic acid (1.36 g, 7 mmol) in toluene (15 mL) was added and the mixture was stirred for an additional 15 min.

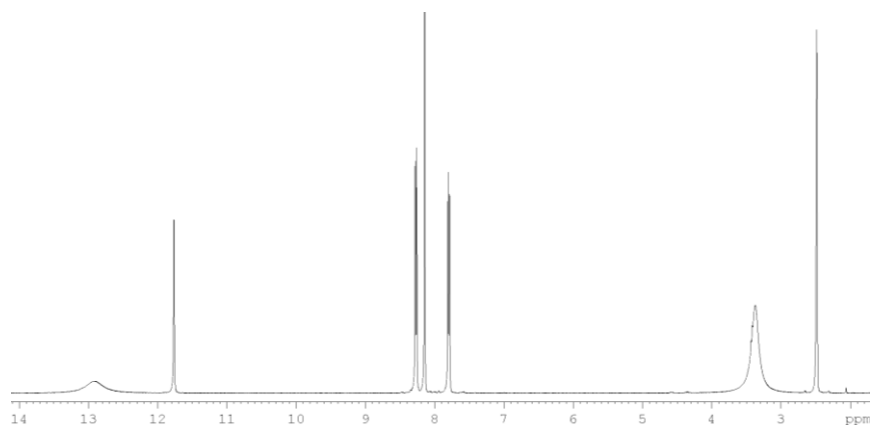
After that time, a deoxygenated Na_2CO_3 2 M (4.21 mL, 8.41 mmol) solution was added and the resulting suspension was stirred under reflux overnight. Passed this time, the crude was cooled to room temperature and concentrated under reduced pressure. The product was further purified by column chromatography (AcOEt, hexane 1:3) to yield a yellow solid (0.96 gr, 60 %). $^1\text{H-NMR}$ (300 MHz, CDCl_3); δ (ppm): 1.41-1.46 (m, 6H), 4.40-4.70 (m, 4H), 7.40 (d, $J = 6$ Hz, 2H), 7.54 (d, $J = 9$ Hz, 1H), 8.12 (d, $J = 6$ Hz, 2H), 8.30 (dd, $J_1 = 9$ Hz, $J_2 = 1.5$ Hz, 1H), 8.54 (d, $J = 1.5$ Hz, 1H). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3); δ (ppm): 14.3(CH_3), 14.3(CH_3), 61.2(CH_2), 62.0(CH_2), 125.4(CH), 127.8 (2x CH), 130.0 (2x CH), 130.9(C_q), 131.4(C_q), 132.0(CH), 133.0(CH), 139.4(C_q), 141.0(C_q), 149.0(C_q), 164.2($\text{C}=\text{O}$), 165.9($\text{C}=\text{O}$). HR-MS (m/z): ($\text{C}_{18}\text{H}_{17}\text{NO}_6$), found: 344.1123 ($\text{M}^+ + 1$), calculated: 344.1129. Melting point: 64-66 °C.

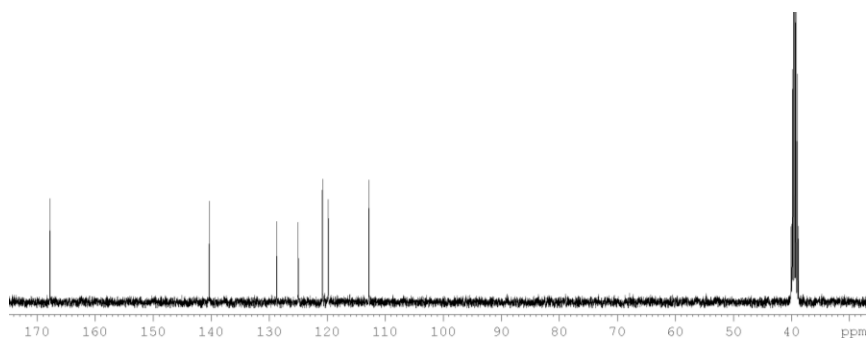


Diethyl carbazole-2,7-dicarboxylate (4). 4,4'-bis(ethoxycarbonyl)-2-nitrobiphenyl (0.5 g, 1.46 mmol) was dissolved in triethyl phosphite (20 mL) and refluxed for 8 h. After that, the remaining triethyl phosphite was evaporated under reduced pressure and dichloromethane was added in order to precipitate the desired product as a pale yellow solid (0.27 g, 60 %). $^1\text{H-NMR}$ (200 MHz, $\text{DMSO-}d_6$); δ (ppm): 1.35 (t, $J = 6$ Hz, 6H), 4.35 (q, $J = 6$ Hz, 4H), 7.80 (dd, $J_1 = 8$ Hz, $J_2 = 2$ Hz, 2H), 8.15 (d, $J = 2$ Hz, 2H), 8.29 (d, $J = 8$ Hz, 2H), 11.78 (s, 1H, NH). $^{13}\text{C-NMR}$ (50 MHz, $\text{DMSO-}d_6$); δ (ppm): 14.2 (2x CH_3), 60.7 (2x CH_2), 112.7 (2x CH), 119.6 (2x CH), 121.1(2x CH), 125.1 (2x C_q), 127.9 (2x C_q), 140.3 (2x C_q), 166.2 (2x $\text{C}=\text{O}$). HR-MS (m/z): ($\text{C}_{18}\text{H}_{17}\text{NO}_4$), found: 312.1234 ($\text{M}^+ + 1$), calculated: 312.1230. Melting point: 268-270 °C.



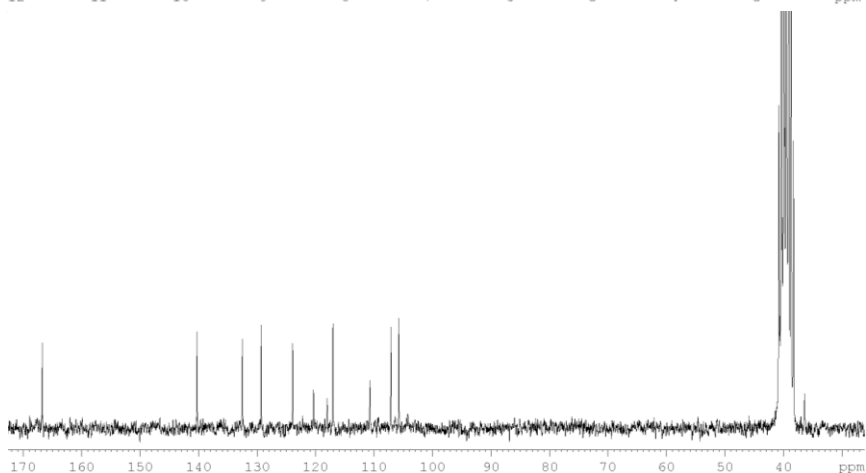
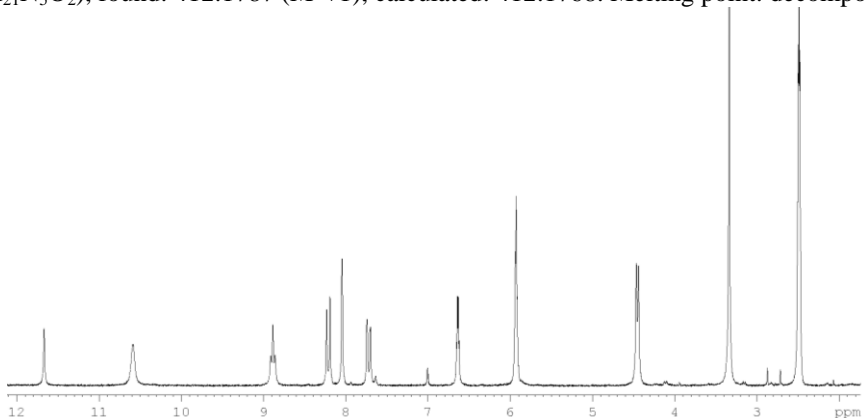
Carbazole-2,7-dicarboxylic acid (5). A suspension of diethyl carbazole-2,6-dicarboxylate (0.5 g, 1.16 mmol) in EtOH (70 mL) was refluxed till the complete solution of the solid. KOH (0.72 g, 12.86 mmol) in water (10 mL) was added and the mixture was refluxed for an additional 8 h. Then, the mixture was acidified by the addition of HCl 15% and the solvent was removed by rotary evaporation. The resulting solid was washed with distilled water (50 mL) and diethylether (50 mL) to give the desired product as a yellow solid (0.41 g, 100 %). ¹H-NMR: (400 MHz, DMSO-*d*₆); δ (ppm): 7.79 (d, *J* = 8.4 Hz, 2H), 8.14 (s, 2H), 8.27 (d, *J* = 8.4 Hz, 2H), 11.76 (s, 1H, NH), 12.91 (bp, 2H, COOH). ¹³C-NMR (100 MHz, DMSO-*d*₆); δ (ppm): 112.8(2xCH), 119.8(2xCH), 120.9(2xCH), 125.0(2xC_q), 128.8(2xC_q), 140.3(2xC_q), 167.8(2xC=O). HR-MS (*m/z*): (C₁₄H₉NO₄), found: 256.0613 (M⁺+1), calculated: 256.0604. Melting point: >300 °C.





bis(*N*-pyrrole-2-ylmethyl)carbazole-2,7-dicarboxamide (6). *N, N'*-carbonyldiimidazole (1.02 g, 6.27 mmol) was added over a stirred suspension of carbazole-2,7-dicarboxylic acid **5** (0.4 g, 1.57 mmol) in anhydrous DMF (30 mL), at room temperature. Once the mixture was completely dissolved, 2-aminomethylpyrrole (0.75 g, 7.84 mmol) in anhydrous DMF (5 mL) was added and the mixture was then stirred for 8 hours. After that, the solvent was removed under reduced pressure and the resulting crude was triturated with methanol to yield a white compound which corresponds to the pure product (0.42 g, 65%).

¹H-NMR (200 MHz, DMSO-*d*₆); δ (ppm): 4.46 (d, *J* = 6 Hz, 4H), 5.92-5.94 (m, 4H), 6.62-6.66 (m, 2H), 7.73 (dd, *J*₁ = 8.4 Hz, *J*₂ = 1.2 Hz, 2H), 8.05 (d, *J*₂ = 1.2 Hz, 2H), 8.22 (d, *J*₁ = 8.4 Hz, 2H), 8.89 (t, *J* = 6 Hz, 2H, NH amide), 10.59 (s, 2H, NH pyrrole), 11.67 (s, 1H, NH carbazole). ¹³C-NMR (50 MHz, DMSO-*d*₆); δ (ppm): 36.4 (2xCH₂), 105.8 (2xCH), 107.2 (2xCH), 110.8 (2xCH), 117.1 (2xCH), 118.1 (2xCH), 120.4 (2xCH), 124.0 (2xC_q), 129.3 (2xC_q), 132.5 (2xC_q), 140.3 (2xC_q), 166.7 (2xC=O). HR-MS (*m/z*): (C₂₄H₂₁N₅O₂), found: 412.1787 (M⁺+1), calculated: 412.1768. Melting point: decomposes >280 °C.



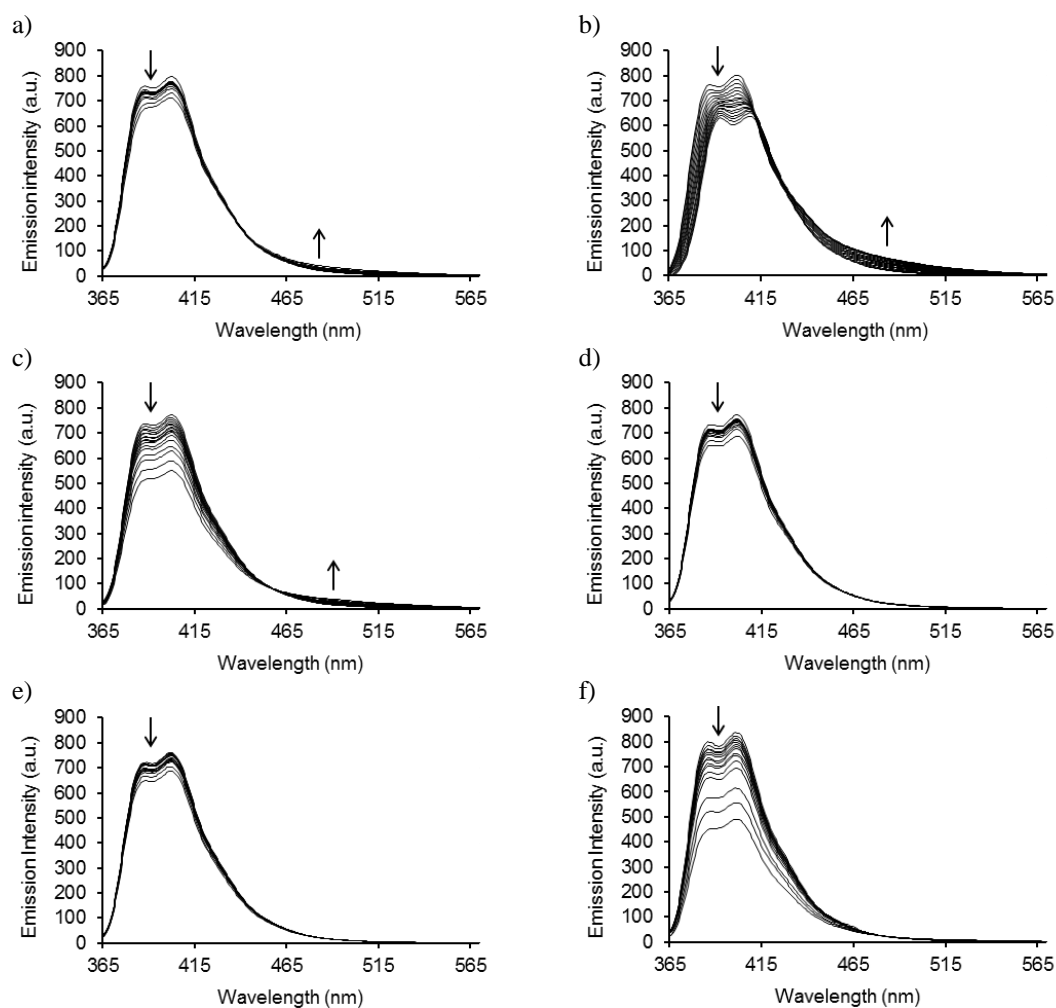


Figure 1. Evolution of the emission spectra of **6** ($2 \cdot 10^{-5}$ M in acetone-water, 2% v/v) upon titration with: a) phthalate, b) isophthalate, c) terephthalate, d) nicotinate, e) isonicotinate and f) picolinate.

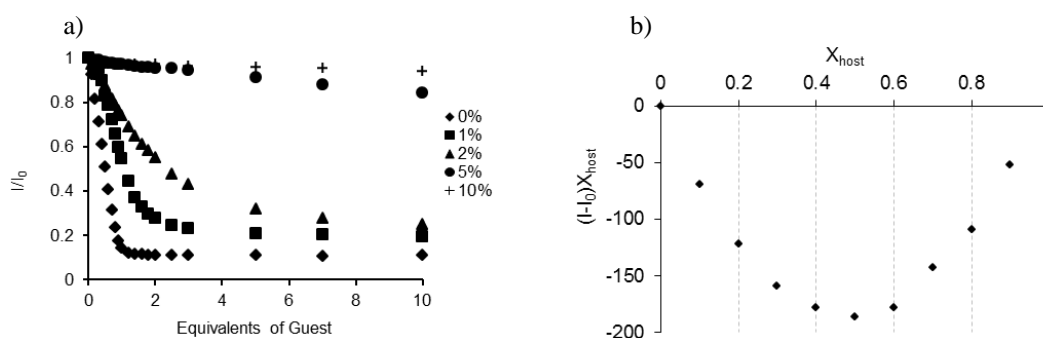


Figure 2. a) Titration curves of **6** ($2 \cdot 10^{-5}$ M) with dipicolinate anion in acetone-water solution at different water content (% v/v). b) Job plot (**6** + dipicolinate) in acetone-water, 2% v/v.

Table 1. Binding constants^a determined from fluorescence titrations in acetone water (2% v/v) solutions.

	Dipicolinate	Picolinate	Phthalate	Isophthalate	Terephthalate	Nicotinate	Isonicotinate
$K_{\text{assoc.}}$	$3.46 \cdot 10^4$	- ^b	- ^b	$1.12 \cdot 10^4$	$5.01 \cdot 10^3$	- ^b	- ^b

^a Errors for the calculated constants were below 5%. ^b Interaction was too weak to calculate a binding constant.

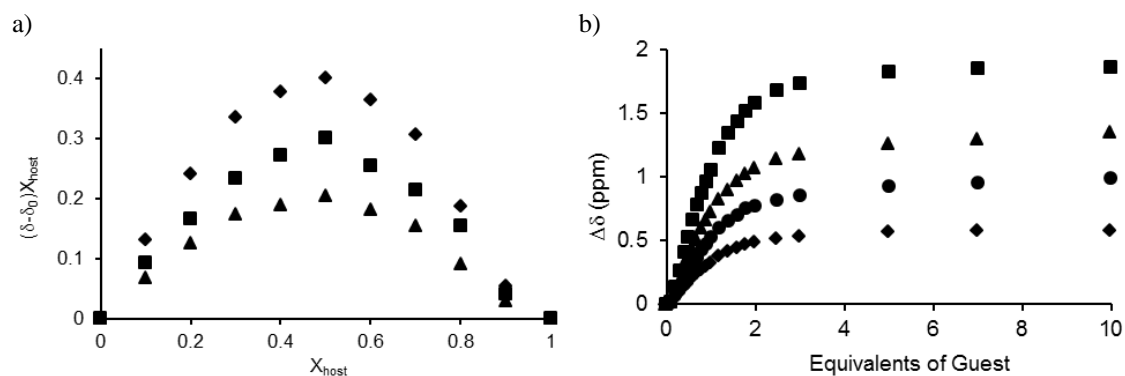


Figure 3. a) Job plots (**6** + dipicolinate) and b) titration curves from ^1H -NMR experiments ($[\mathbf{6}] = 2 \cdot 10^{-3}$ M in $\text{DMSO-}d_6$) following several nuclei: \blacksquare $\text{NH}_{\text{carbazole}}$ \blacktriangle $\text{NH}_{\text{pyrrole}}$, \bullet NH_{amide} and \blacklozenge CH (1-8).

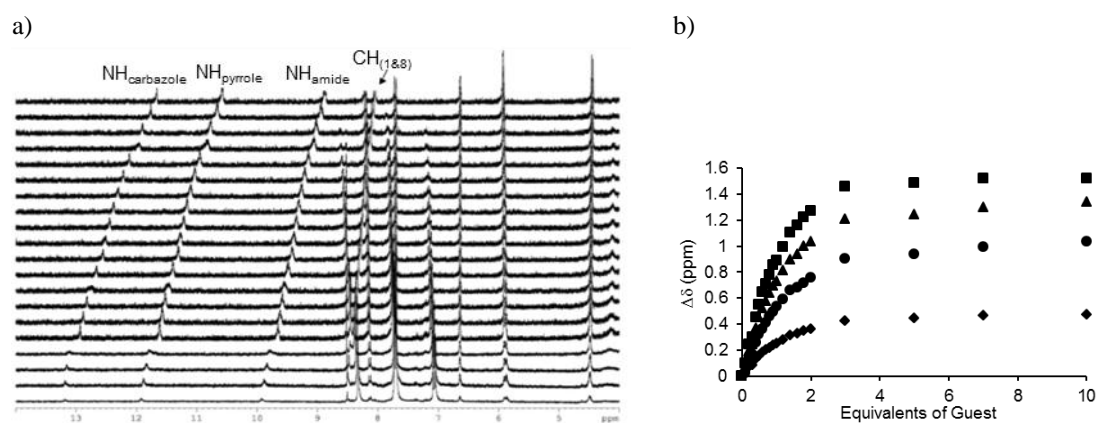


Figure 4. a) Evolution of the ^1H -NMR spectra and b) titration curves (**6** + isophthalate). $[\mathbf{6}] = 2 \cdot 10^{-3}$ M in $\text{DMSO-}d_6$; \blacksquare $\text{NH}_{\text{carbazole}}$ \blacktriangle $\text{NH}_{\text{pyrrole}}$, \bullet NH_{amide} and \blacklozenge CH (1-8).

Table 2. Binding constants^a determined from ^1H -NMR titrations in $\text{DMSO-}d_6$ solutions.

	dipicolinate	phthalate	isophthalate	terephthalate
$\text{NH}_{\text{carbazole}}$	521	20	487	110
NH_{amide}	473	28	424	101
$\text{NH}_{\text{pyrrole}}$	474	25	372	107
CH (1-8)	474	-	311	-

^a Errors for the calculated constants were below 10%.

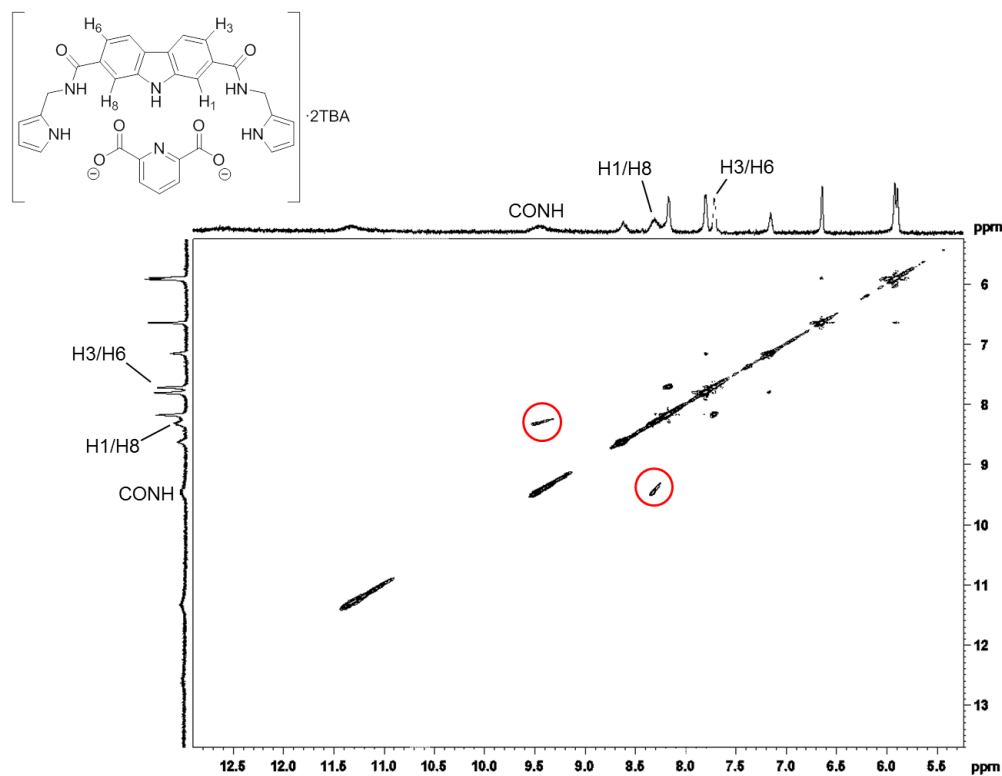
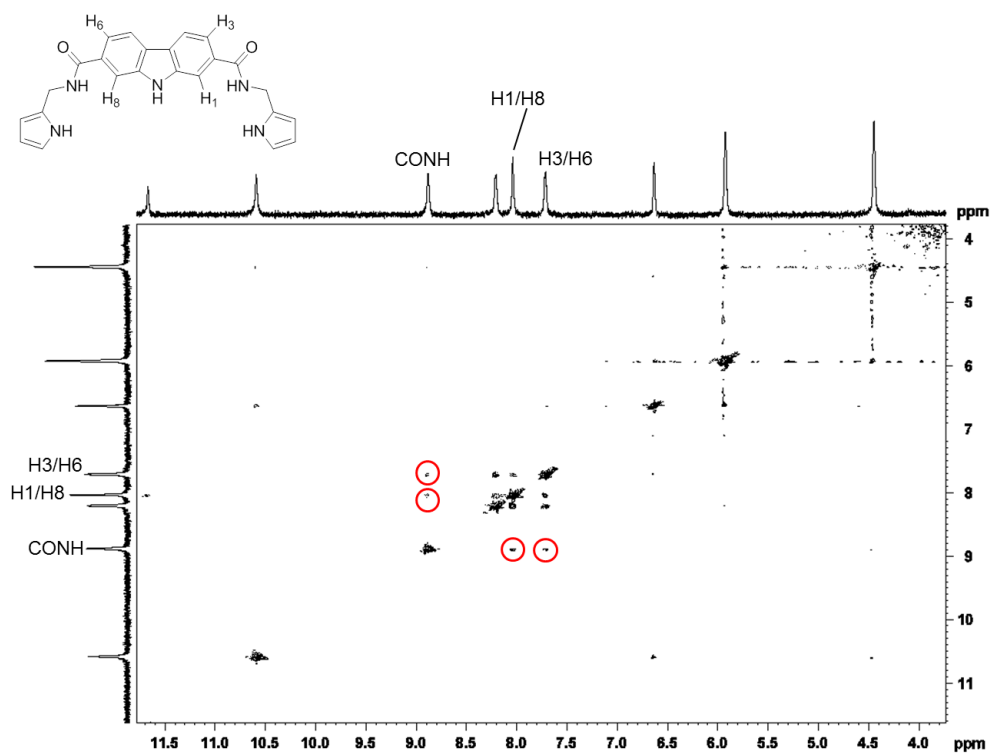


Figure 5. NOESY spectra in DMSO-*d*₆ solution.

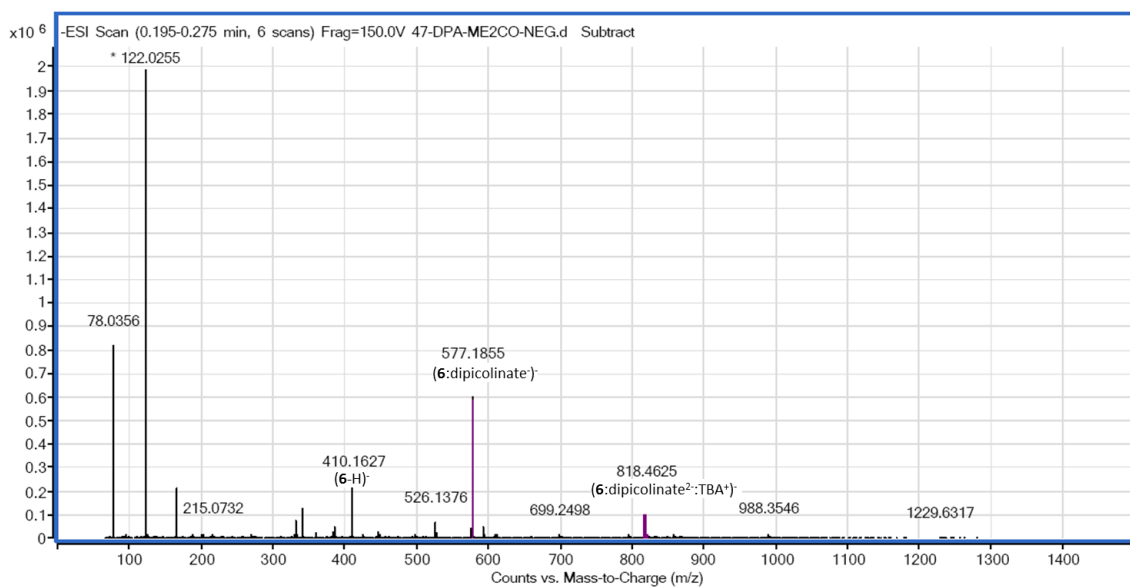


Figure 6. Mass spectrum for the complex **6**:dipicolinate.