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Electronic Supporting Information for

A Highly Versatile Fluorenone-Based Macrocycle for the Sensitive Detection of Polycyclic Aromatic Hydrocarbons and Anions

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**These authors contributed equally to this work*

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MATERIALS AND METHODS

All the starting materials, reagents, and solvents were purchased from Sigma Aldrich, Acros Organics, TCI chemicals, Alfa Aesar, or Fisher Scientific and were used as received. Reactions were all monitored via analytical thin layer chromatography (TLC) using polyester backed TLC plates. Visualization was accomplished with UV light at 254 nm. Flash column chromatography was performed with SiliaFlash F60 (230-400 mesh) or using automated flash chromatography (Yamazen Smart Flash AI-580S & AKROS). UV-VIS spectra were recorded on a Shimadzu UV-3600 Plus spectrophotometer. Fluorescence spectra were recorded on a Shimazdu RF-6000 fluorophotometer with 3.0 nm excitation and 3.0 nm emission slit widths.

^{1}H and ^{13}C NMR spectra were taken on a Bruker 300 MHz spectrometer and were recorded in $CDCl_3$ and DMSO-*d6* at room temperature. Chemical shifts (δ) are reported in parts per million relative to chloroform at 7.26 ppm, dimethyl sulfoxide at 2.59 ppm, or to tetramethylsilane (TMS) at 0.00 ppm for ^{1}H NMR and relative to $CDCl_3$ at 77.16 ppm or DMSO at 40.76 ppm for ^{13}C NMR spectra.

METHODS FOR MASS SPECTROMETRY DETECTION

Compounds **1** and **2** were dissolved in a mixture of water/acetonitrile (50/50) or chloroform to make 1 mg/mL or 0.285 mg/mL solutions respectively, and further diluted to 5 µg/mL in methanol/water (50/50) to produce an analytical standard. The latter was infused into a Thermo Scientific LTQ Orbitrap XL™ mass spectrometer at a rate of 15 µL/min using an electrospray ionization source in a positive mode. The rest of the ionization source and ion optics parameters were as follows: sheath gas 25, auxiliary gas 6, spray voltage 5 kV, capillary temperature 275 °C, capillary voltage 47 V, tube lens 165 V, multipole 00 offset -5.5 V, lens 0 -6.0 V, multipole 0 offset -5.75 V, lens 1 -10.0 V, gate lens -46.0 V, multiple 1 offset -19.5 V, multipole RF amplitude 400.0 V, front lens -6.75 V. The mass spectra were collected using full scan mode with a resolution of 30000 in the range between 60 and 600 amu. The spectra were averaged over 2 microscans with 10.0 ms maximum injection time and 2.0×10^5 ions for AGC target settings.

METHODS FOR COMPUTATIONAL EXPERIMENTS

Computational work was performed with Spartan software (Spartan 10, version 1.1.0), obtained from Wavefunction, Inc. CA. All calculations were performed using equilibrium geometry at the ground state, HF-DFT (B3LYP, 6-31G*) level. All the conformations shown were energy-minimized.

METHODS FOR FLUORESCENCE EXPERIMENTS

12 μL of a 5 mM solution of the analyte was added to a cuvette containing 2.0 mL of chloroform. In a separate cuvette, 12 μL of a 5 mM of the analyte was added to 2.0 mL (30 μM) of **1** in chloroform. Both samples were excited at the analyte's excitation wavelength and the fluorescence emission spectra were recorded. Both the excitation slit width and the emission slit width were 3.0 nm. All fluorescence spectra were integrated vs. wavenumber on the X-axis using OriginPro Version 9.1.

The fluorescence change was determined using the following equation:

$$Fluorescence\ change\ (\%) = \frac{Fl_m - Fl_a}{Fl_a}$$

Where Fl_a is the integrated fluorescence emission of the analyte and Fl_m is the integrated fluorescence emission of the analyte in the presence of macrocycle **1.**

Analyte	Excitation Wavelength (nm)
4	275
5	343
6	295
7	321

METHODS FOR LIMIT OF DETECTION EXPERIMENTS

Reference: Cheng, D.; Zhao, W.; Yang, H.; Huang, Z.; Liu, X.; Han, A. "Detection of Hg^{2+} by a FRET Ratiometric Fluorescent Probe Based on a Novel BODIPY-RhB System." *Tetrahedron Lett.* **2016**, *57*, 2655-2659.

The limit of detection (LOD) is defined as the lowest concentration of analyte at which a signal can be detected. To determine this value, the following steps were performed for each macrocycle-analyte combination. In a quartz cuvette, 2.5 mL of a 3 μM solution of **1** in $CHCl_3$ was added. The fluorescence emission spectra were recorded. Six repeat measurements were taken.

Next, 3 μL of analyte (0.5 mM) was added, and again the solution was excited at the analyte's excitation wavelength, and the fluorescence emission spectra were recorded. Six repeat measurements were taken. This step was repeated for 6 μL of analyte, 12 μL of analyte, 18 μL of analyte, 24 μL of analyte, 30 μL of analyte, 36 μL of analyte, and 42 μL of analyte. All of the fluorescence emission spectra were integrated vs. wavenumber on the X-axis, and calibration curves were generated. The curves plotted the analyte concentration in μM on the X-axis, and the fluorescence change on the Y-axis. The curve was fitted to a straight line and the equation of the line was determined.

The limit of detection is defined according to the following equation:

$$LOD = 3(SD_{blank})/m$$

Where SD_{blank} is the standard deviation of the blank sample and m is the slope of the calibration curve.

METHODS FOR UV/VIS ABSORPTION SPECTROSCOPY EXPERIMENTS

For polycyclic aromatic hydrocarbons (PAHs): The absorption spectra of a 30 μM solution of both 1 and each guest (PAH) were collected separately. For the 1:1 absorption spectra, 12 μL of a guest solution of 5.10^{-3} M was added to 2 mL of a 30 μM solution of 1, the solution was shaken and data was collected.

For anion binding experiments: In a quartz cuvette, 2.5 mL of a 10 μM solution of 1 was added. During titration, aliquots of a 4 mM solution of the anion (as its tetrabutylammonium salt) were added to the cuvette. The solution was shaken and data was collected following each addition.

METHODS FOR ^{1}H NMR EXPERIMENTS

Titration experiments: Solutions of receptor 1 (1 mM, DMSO-*d6*) were titrated by adding known quantities of a stock 20mM solution of tetrabutylammonium fluoride. The chemical shifts of the triazole protons were monitored and plotted. Nonlinear curve fitting method was employed to compare against a standard 1:2 host-guest interaction model.

Calculation of other complexed species: Interferences from the *in situ* generation of HF and HF_2^- were quantified based on stoichiometric analyses of their integrated peak ratios against that of the predominant complexed species. With the overall concentration of the receptor 1 held constant throughout the titration, the percentage of each complexed species is calculated according to the equation shown below:

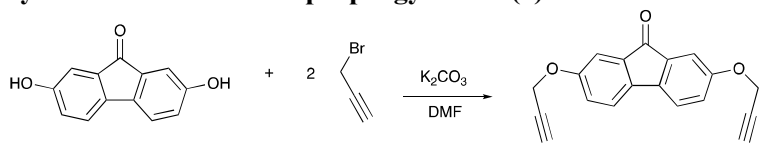
% complexed species *n* = (Integrated area of peak for species *n*) / (Sum of the integrated peak areas of all the complexed species)

METHODS FOR JOB PLOT ANALYSIS

Job's plot experiment: Stock solutions of the macrocycle **1** and TBAF (3.2 mM each) were prepared separately in deuterated DMSO. The ^{1}H NMR spectra was taken for each of 11 different solutions (total volume 0.5 mL) containing the macrocycle **1** and the tetrabutylammonium salt in the following molar fraction ratio (of the macrocycle): 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0. δ is measured with respect of the triazole proton of **1**.

SYNTHETIC PROCEDURES

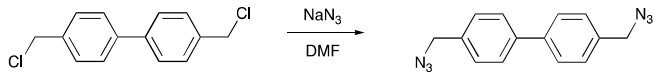
Synthesis of fluorenone-propargyl ether (2)



2

In a 25 mL round-bottomed flask containing 15 mL of *N,N*-dimethylformamide (DMF), 2,7-dihydroxy-9-fluorenone (compound **8**) (212 mg, 1.0 mmol, 1.0 eq.) and potassium carbonate (414 mg, 3.0 mmol, 3.0 eq.) were added. The reaction mixture was stirred at room temperature for 30 min, then propargyl bromide (0.379 mL, 5.0 mmol, 5 eq.) was added and the reaction mixture was stirred for 24 hours at room temperature. After 24 hours, distilled water (100 mL) was added and the product was extracted with ethyl acetate (3x10 mL). The organic layer was washed with water (3 x 10 mL) and dried over Na_2SO_4. The pure compound was isolated as an orange solid after recrystallization from chloroform (244 mg, 85% yield). ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 7.61 (d, 2 H, J = 8.1 Hz), 7.19 (d, 2 H, J = 2.4 Hz), 7.13 (dd, 2 H, J = 8.1, 2.5 Hz), 4.90 (d, 4 H, J = 2.4 Hz), 3.64 (t, 2 H, J = 2.3 Hz). ^{13}C NMR (100 MHz, DMSO-*d6*): δ (ppm) = 193.2, 158.0, 138.1, 136.0, 121.3, 120.7, 110.9, 78.0, 76.1, 56.2. ESI-TOF-MS: ESI MS calcd for $C_{19}H_{12}O_3$ m/z 288.0786, found $[M+Na]^+$ m/z 311.0679.

Synthesis of 4,4'-bis(azidomethyl)-1,1'-biphenyl (3)

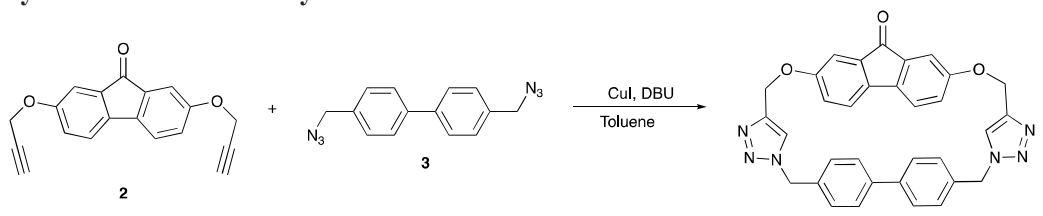


3

In a 50 mL round-bottom flask containing 25 mL of DMF, 4,4′-bis(chloromethyl)-1,1′-biphenyl (251 mg, 2.0 mmol, 1.0 eq.) and NaN_3 (390 mg, 6.0 mmol, 6.0 eq) were added. The reaction mixture was stirred at 60 °C for 16 hours, at which point water (100 mL) was added and the product was extracted with Et_2O (3x10 mL). The combined organic phases were washed with water and brine, and then dried over Mg_2SO_4. The pure compound was isolated as a white solid after evaporation of Et_2O in 90% yield (475 mg). ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 7.72 (d, 4 H, J = 7.6 Hz), 7.47 (d, 4 H, J = 7.6 HZ), 4.50 (s, 4 H).

The spectroscopic characteristics were in good agreement with those found in the literature (*J. Polym. Sci., Part A: Polym. Chem.* **2014**, *52*, 223–231).

Synthesis of the macrocycle 1



2 3 1

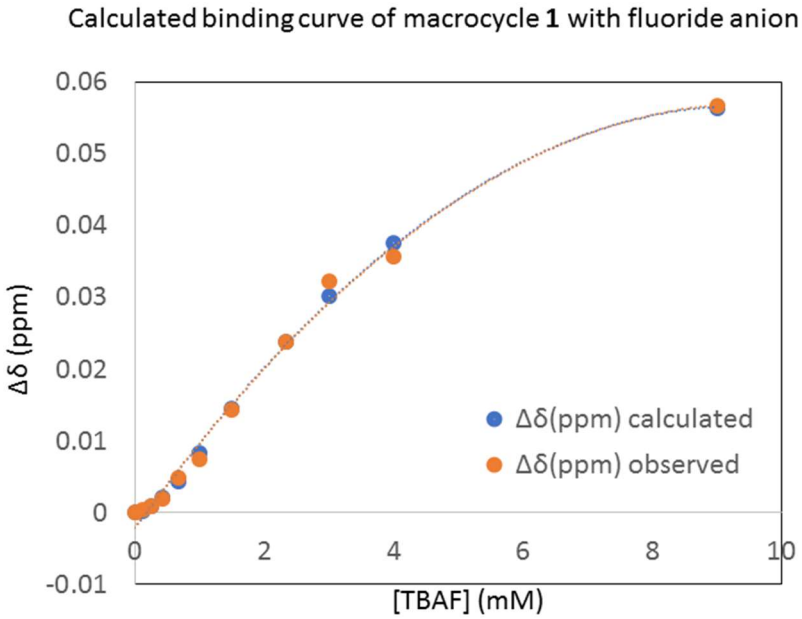
Under nitrogen, 1,8-diaza[5.4.0] bicycloundec-7-ene (DBU) (0.4 mL, 2.25 mmol) and CuI (5 mg, 0.026 mmol) were added to dry toluene (200 mL), degassed for 30 min and heated to 70 °C. Then **2** (58 mg, 0.20 mmol) and **3** (54 mg, 0.20 mmol) in dry toluene (100 mL) were added to the solution dropwise over 10 h and stirred for another 12 h. The mixture was then cooled to room temperature. The filtrate was concentrated in vacuum, and the product was purified by column chromatography (SiO_2, $CHCl_3$/MeOH 99:1) to afford **1** (79 mg, 0.14 mmol, 71% yield) as a light orange solid. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 8.15 (s, 2 H), 7.52 (d, 2 H, J = 8.1 Hz), 7.23 (d, 4 H, J = 8.1 Hz), 7.00 (dd, 2 H, J = 8.3, 2.5 Hz) 6.90 (d, 2 H, J = 2.4 Hz) 6.86 (d, 4 H, J = 8.1 Hz) 5.62 (s, 4 H), 5.40 (s, 4 H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ (ppm) = 192.7, 157.4, 143.9, 139.5, 137.2, 136.7, 135.4, 127.6, 127.1, 125.8, 121.5, 113.1, 79.7, 61.8, 52.8. ESI-TOF-MS: MS calcd for $C_{19}H_{12}O_3$ m/z 552.1909, found $[M+H]^+$ m/z 553.1958.

SUMMARY TABLES FOR ^{1}H NMR EXPERIMENTS

Chemical shift changes of the triazole proton of macrocycle **1** in the presence of 10 equivalents of each anion (as its tetrabutylammonium salt). The changes are calculated relative to the peak position for free macrocycle **1**.

Anion	Δδ (ppm)
F^-	0.1002
CN^-	0.0021
SCN^-	0.0025
N_3^-	0.0024

^{1}H SUMMARY DATA FOR FITTING OF THE NMR TITRATION DATA TO A NONLINEAR BINDING ISOTHERM



A nonlinear curve fitting method was employed to compare against a standard 1:2 host-guest interaction model, using the following equation[i]:

$$\Delta\delta = (\Delta\delta_{HG}K_1[G_0] + \Delta\delta_{HG2}K_1K_2[G_0]^2) / (1 + K_1[G_0] + K_1K_2[G_0]^2)$$

where, $\Delta\delta$ is the observed change in the chemical shift of the host H; $\Delta\delta_{HG}$ is the change in the chemical shift of host H at the first binding event; $\Delta\delta_{HG2}$ is the overall change in the chemical shift of host H, at the second binding event; K_1 is the association constant value for the first binding event to the host H; K_2 is the association constant value for the second binding event to HG; and $[G_0]$ is the concentration of guest.

SUMMARY TABLES FOR LIMIT OF DETECTION EXPERIMENTS

With macrocycle 1:

Analyte	Equation	R^2	LOD (nM)
4	y = 0.6473x + 2.7969	0.995	28.8 ± 0.1
5	y = 15.766x + 113.52	0.964	2.2 ± 0.8
6	y = 1.1217x + 9.5484	0.973	37.2 ± 0.1
7	y = 11.148x + 152.42	0.952	4.2 ± 0.0

Without macrocycle:

Analyte	Equation	R^2	LOD (nM)
4	y = 0.3163x + 1.0405	0.997	166.5 ± 1.4
5	y = 1612.9x + 16780	0.9836	30.1 ± 0.9
6	y = 0.7606x+ 2.577	0.992	59.5 ± 0.7
7	y = 0.3848x + 2.2844	0.9898	204.8 ± 1.1

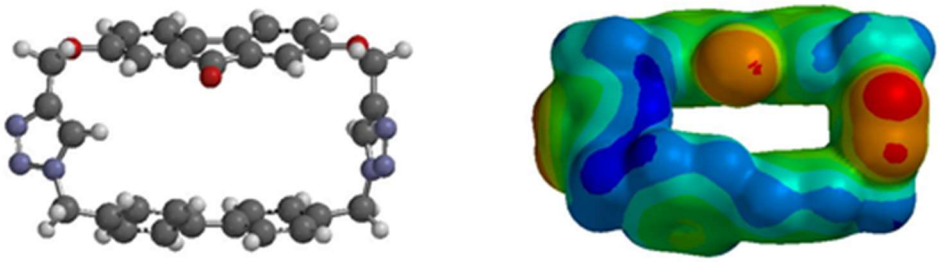
SUMMARY TABLES FOR JOB PLOT ANALYSIS

[**1**] (mM)	[F^-](mM)	γ_1	δ (ppm)	Δδ (ppm)	γ_1*Δδ
3.2	0	1	8.128	0	0
2.88	0.32	0.9	8.1295	0.0015	0.00135
2.56	0.64	0.8	8.1304	0.0024	0.00192
2.24	0.96	0.7	8.1315	0.0035	0.00245
1.92	1.28	0.6	8.132	0.004	0.0024
1.6	1.6	0.5	8.1321	0.0041	0.00205
1.28	1.92	0.4	8.1325	0.0045	0.0018
0.96	2.24	0.3	8.133	0.005	0.0015
0.64	2.56	0.2	8.1329	0.0049	0.00098
0.32	2.88	0.1	8.133	0.005	0.0005
0	3.2	0			

SUMMARY FIGURES FOR COMPUTATIONAL EXPERIMENTS

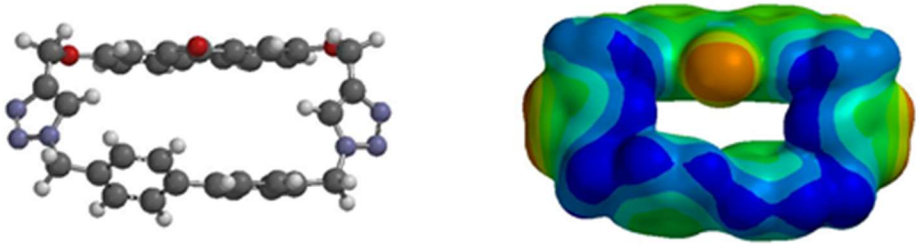
Representative energy-minimized conformations and potentials of macrocycle **1** as deduced by ab initio HF-DFT (B3LYP, 6-31G*) level measurements. The energy of the conformation is shown beneath each structure. The structure shown in the paper is structure **1** with E = 727.6230 KJ/mol. Cavity dimensions of structure 1: 10.6 Å x 5.043 Å.

Structure 1:



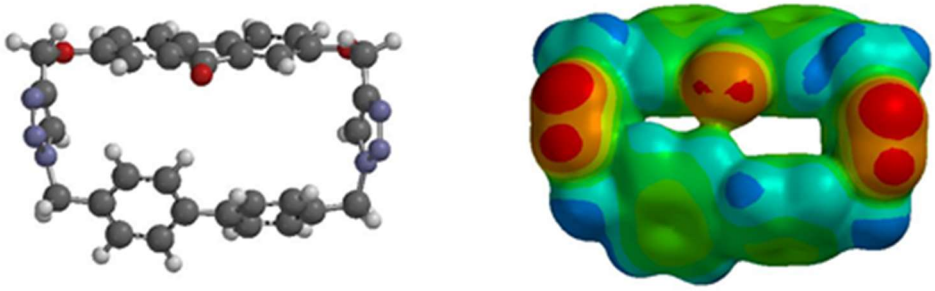
E = 727.6230 KJ/mol

Structure 2:



E = 736.5206 KJ/mol

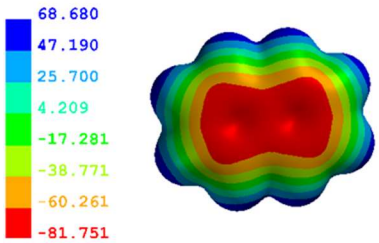
Structure 3:



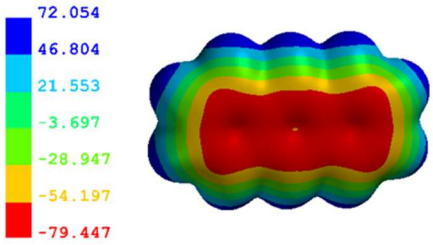
E = 741.7002 KJ/mol

Electrostatic potential maps of analytes **4-7**:

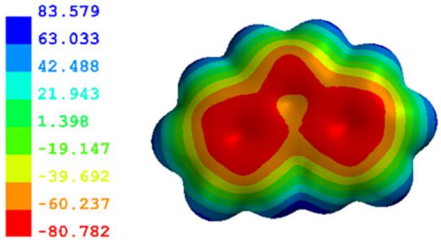
Analyte **4**:



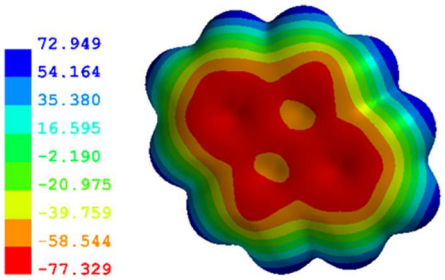
Analyte **5**:



Analyte **6**:



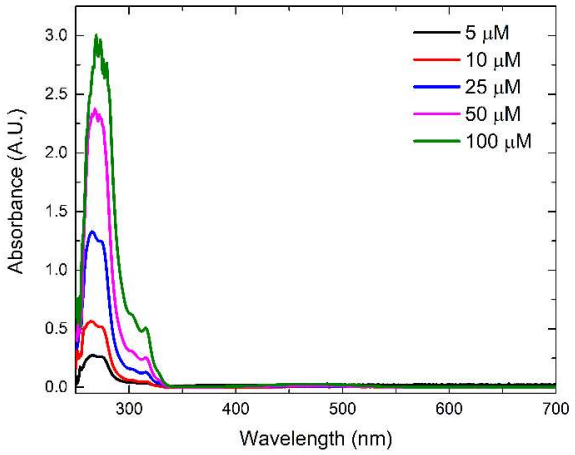
Analyte **7**:



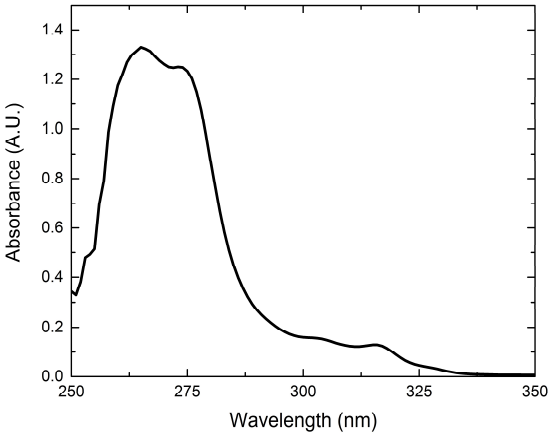
SUMMARY FIGURES FOR ABSORBANCE EXPERIMENTS

The concentration of the analyte and **1** taken separately was 30 μM. The final concentrations of the analyte and **1** in the 1:1 mixture were 30 μM.

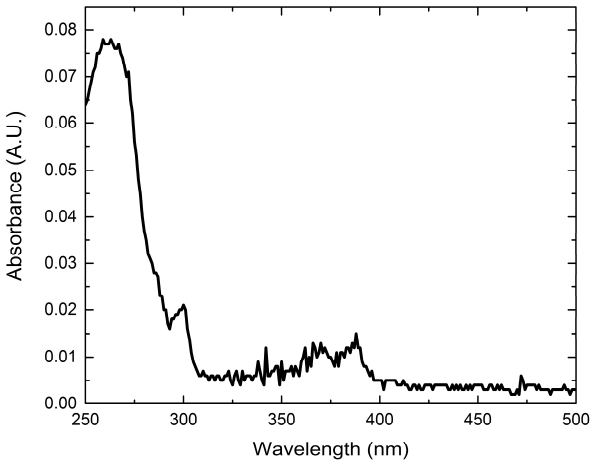
Absorbance Spectra of the Macrocycle without Analyte



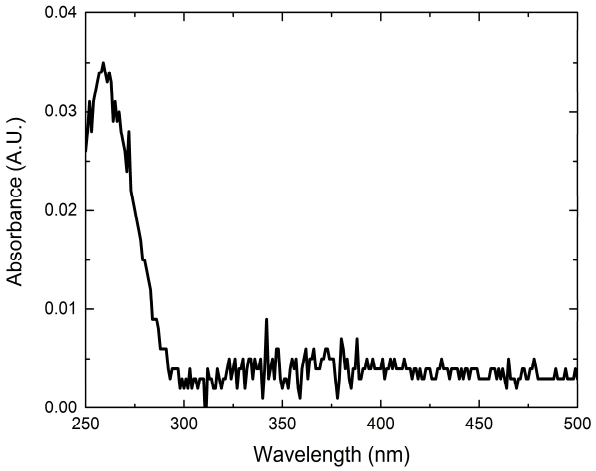
Zoomed in on the shorter wavelength spectral region:



Absorbance spectrum of compound **8**:

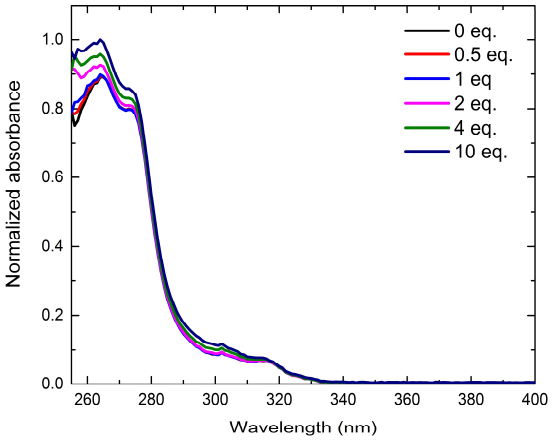


Absorbance spectrum of compound **9**:

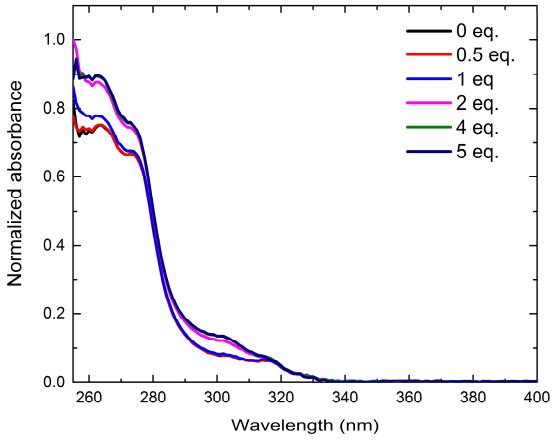


Anion experiments: The concentration of **1** was kept constant throughout the titration at 10 μM.

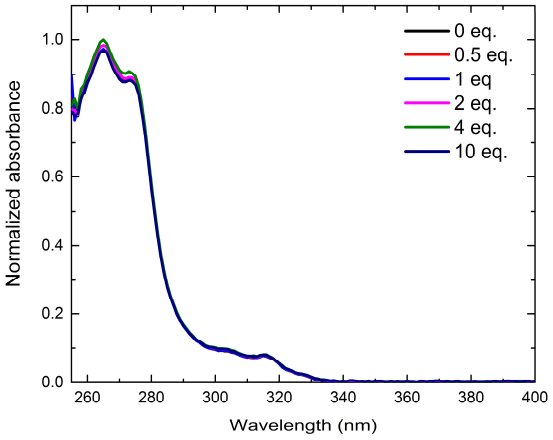
Thiocyanate:



Azide:

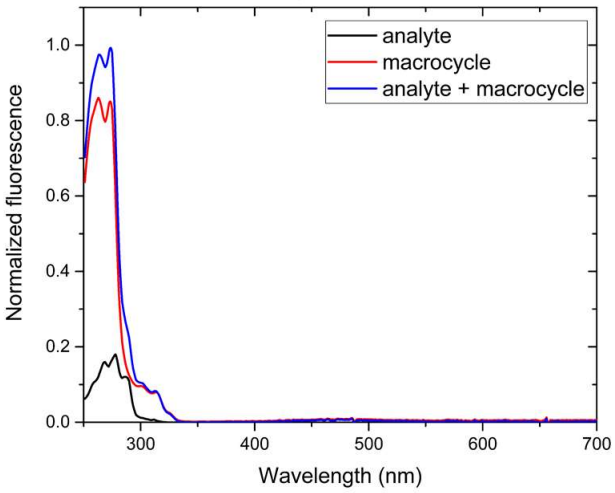


Cyanide:

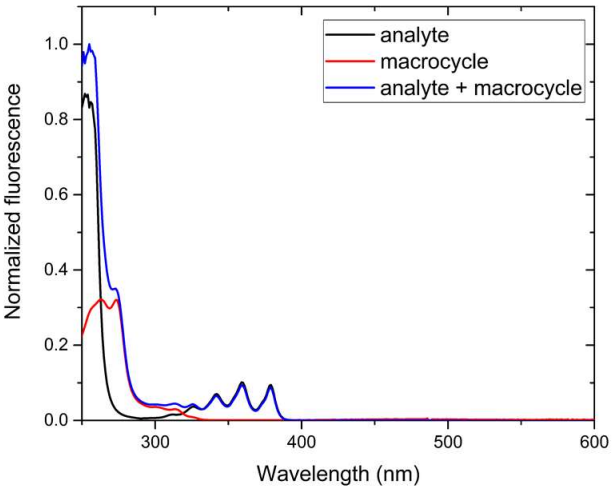


SUMMARY FIGURES FOR FLUORESCENCE EXPERIMENTS

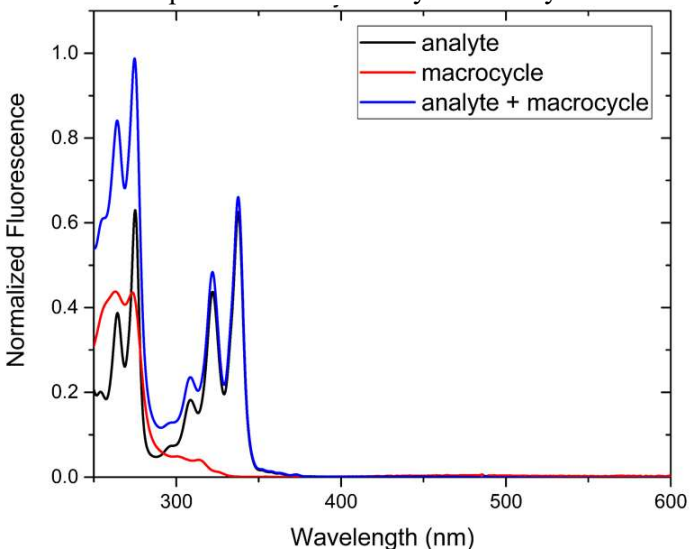
Fluorescence Spectra of the Macrocycle with Naphthalene



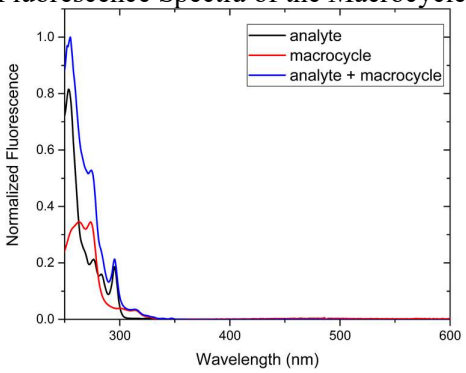
Fluorescence Spectra of the Macrocycle with Anthracene



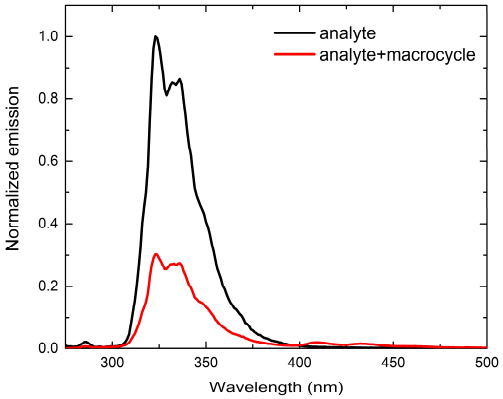
Fluorescence Spectra of the Macrocycle with Pyrene



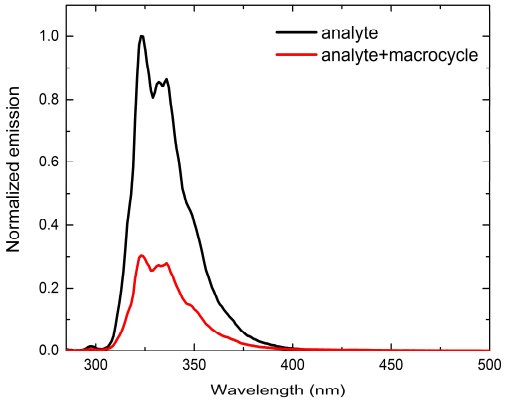
Fluorescence Spectra of the Macrocycle with Phenanthrene



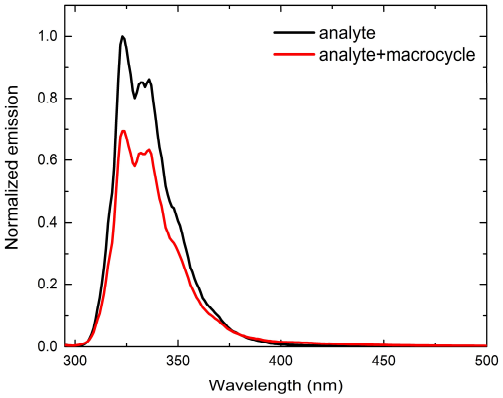
Macrocycle with naphthalene
265 nm excitation:



275 nm excitation:



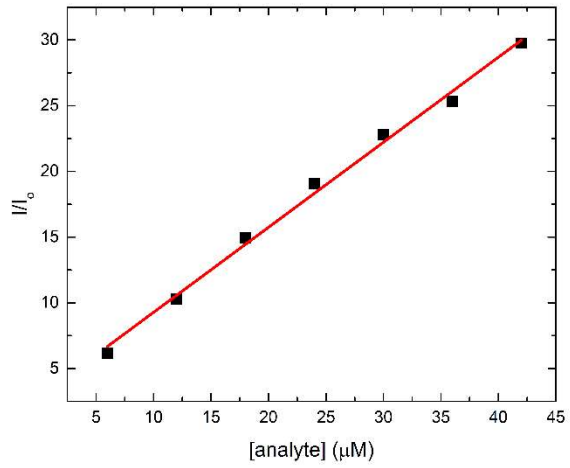
285 nm excitation:



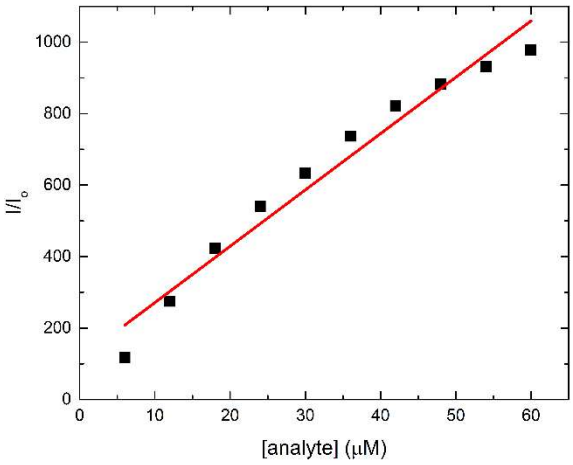
SUMMARY FIGURES FOR LIMIT OF DETECTION EXPERIMENTS

With macrocycle 1:

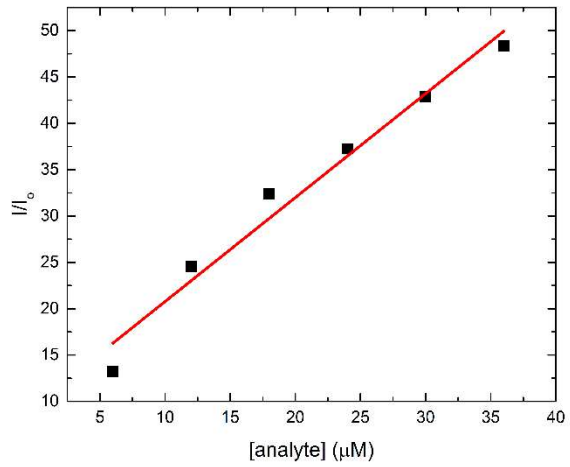
Naphthalene



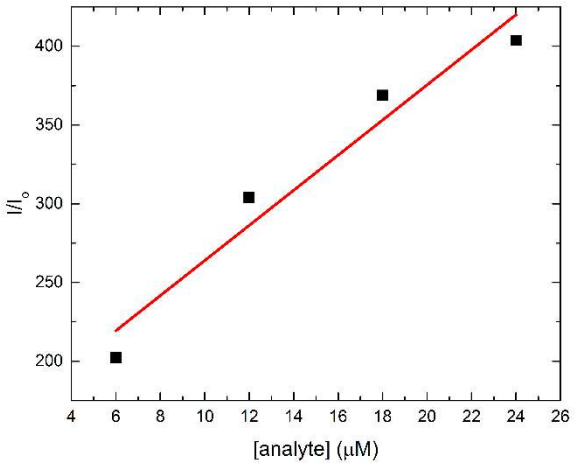
Anthracene



Phenanthrene

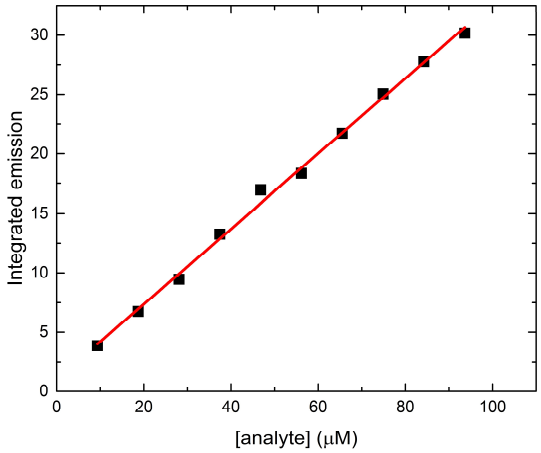


Pyrene

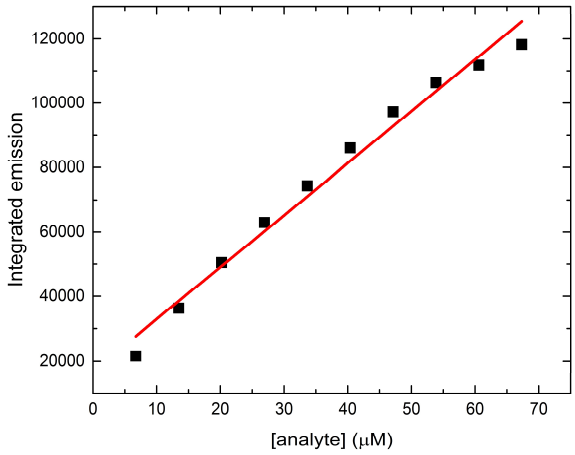


LOD experiments without macrocycle 1:

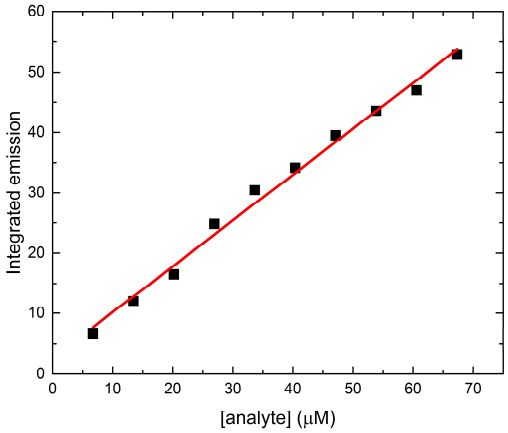
Naphthalene



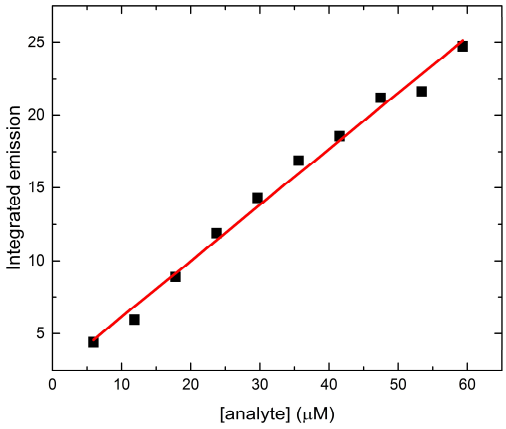
Anthracene



Phenanthrene

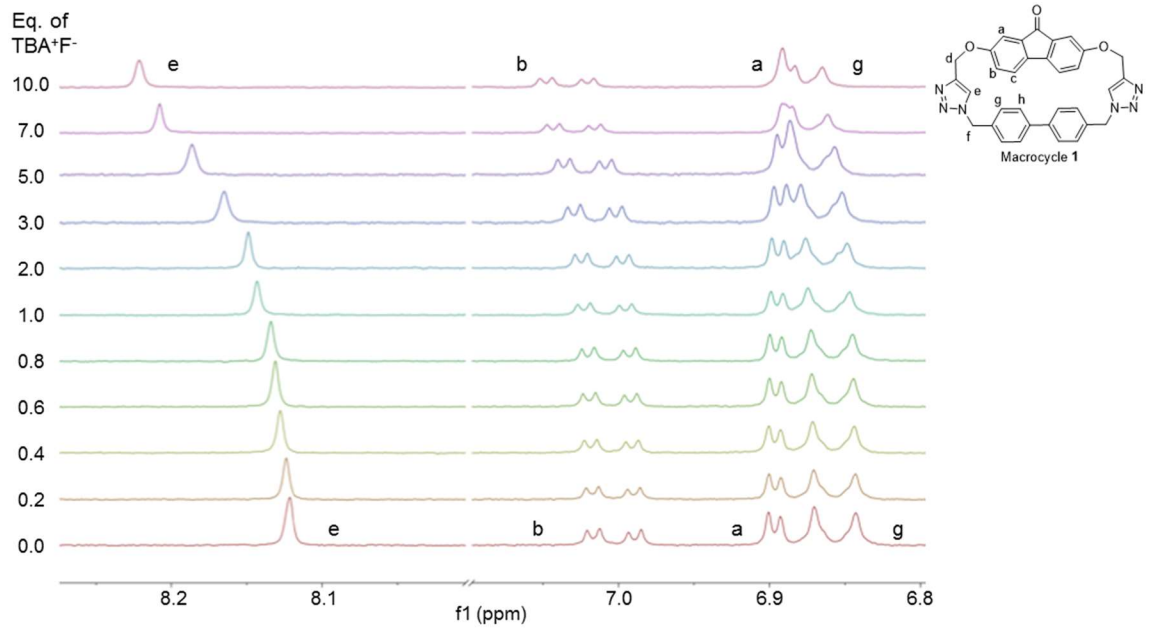


Pyrene

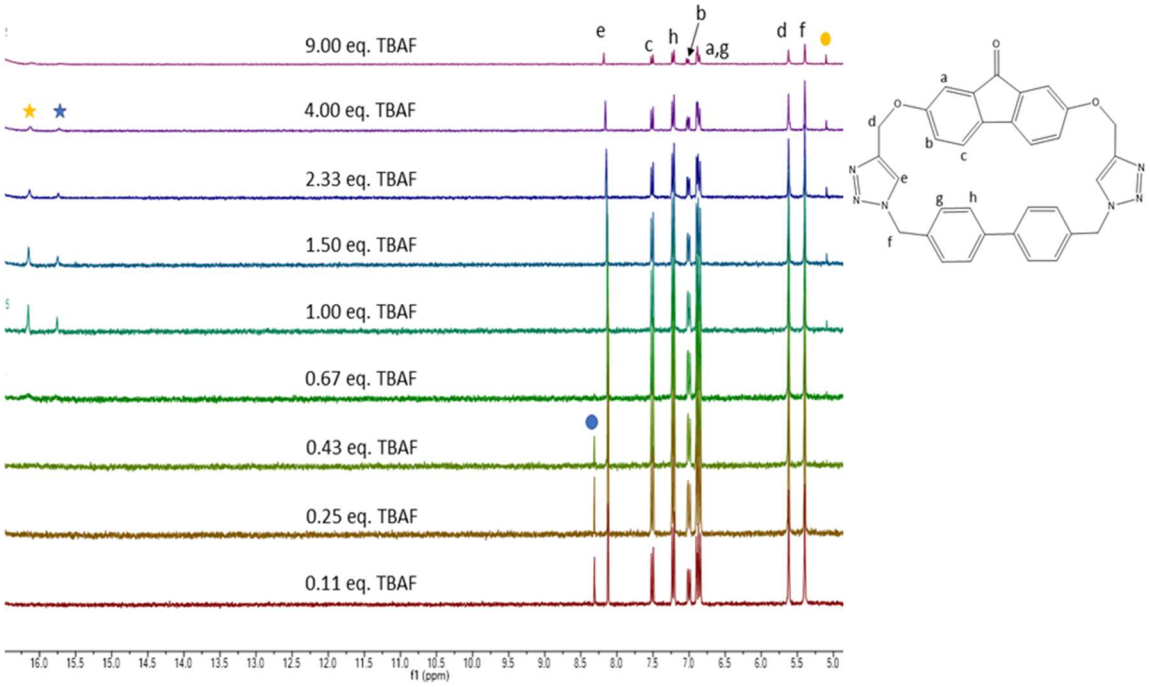


SUMMARY FIGURES FOR NMR TITRATION EXPERIMENTS

a) ^{1}H NMR titration of **1** with TBAF indicating chemical shifts in the triazole, biphenyl, and fluorenone protons.



b) ^{1}H NMR titration of **1** with TBAF indicating the formation of other complexes from interfering fluorine species

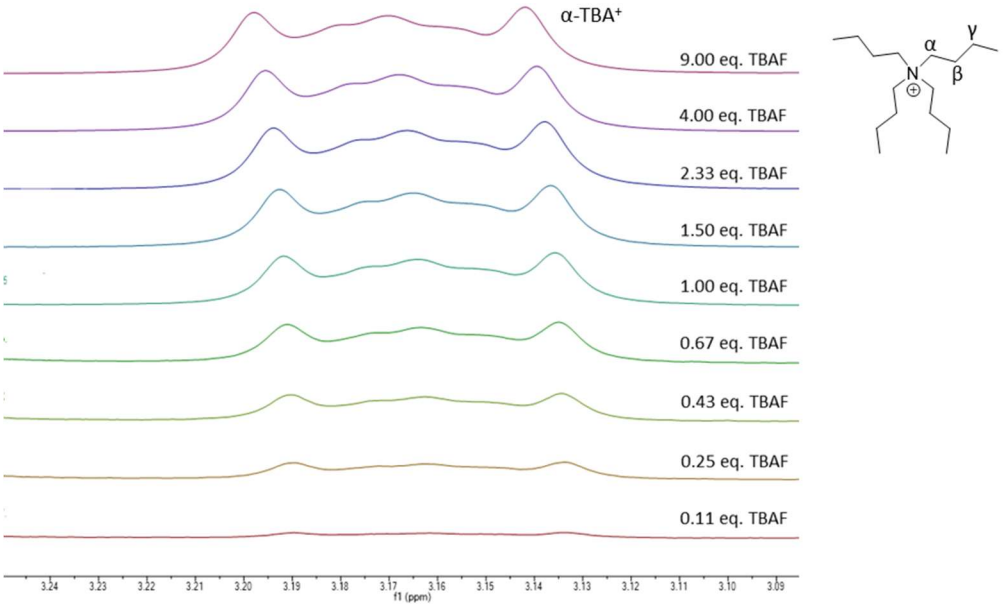


c) Calculated relative percentages of interfering fluorine species in the complexation

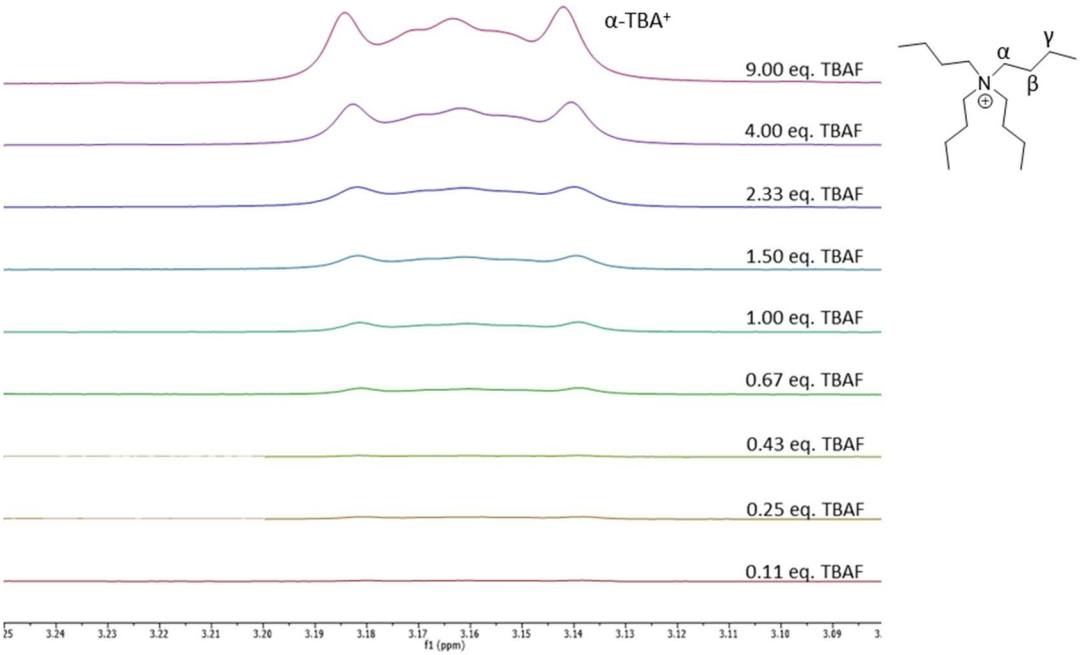
Eq. of TBAF	NH-bonded **1**[F^-]$_2$ (blue circle) (%)	**1**[F^-]$_2$ (%)	**1**[HF_2]$^-$ (yellow circle) (%)
0.11	21.26	78.74	0.00

0.25	16.66	83.34	0.00
0.43	10.71	89.29	0.00
0.67	0.00	100.0	0.00
1.00	0.00	95.24	4.76
1.50	0.00	94.34	5.66
2.33	0.00	94.34	5.66
4.00	0.00	92.60	7.40
9.00	0.00	90.91	9.09

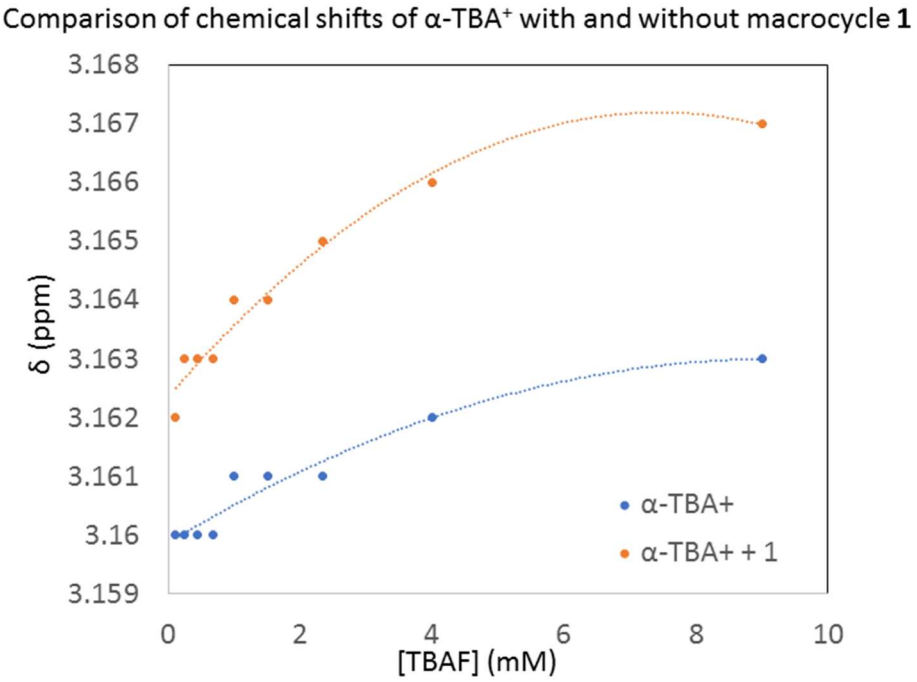
c) Chemical shift changes of α-TBA^+ protons at 1 mM concentration of **1**



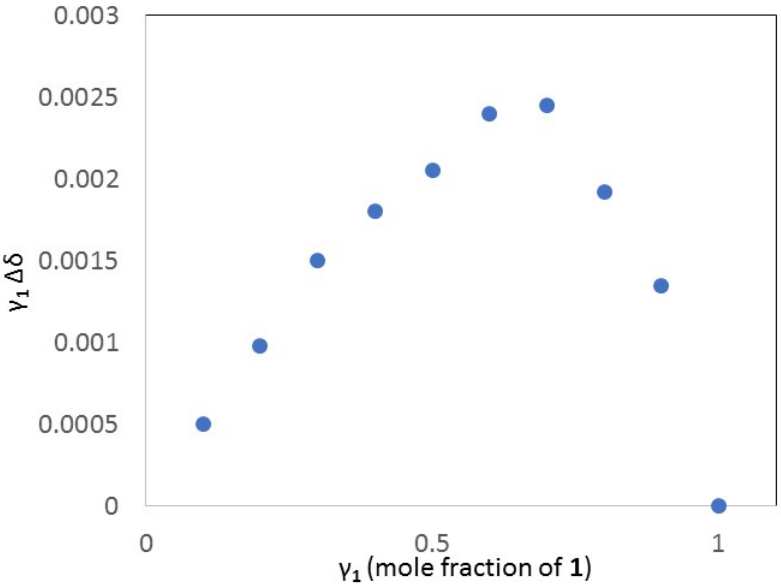
d) Chemical shift changes of α-TBA^+ protons at 0mM concentration of **1**



e) Plot of chemical shift changes of α-TBA^+ protons (c) vs. (d)

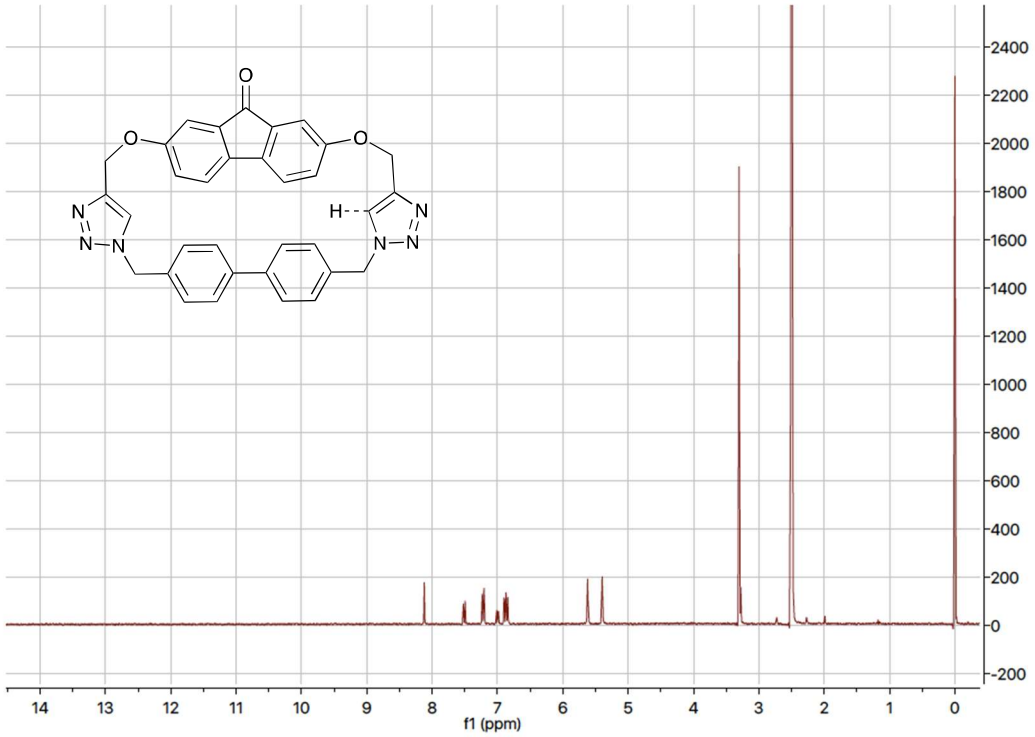


SUMMARY FIGURE FOR JOB PLOT ANALYSIS

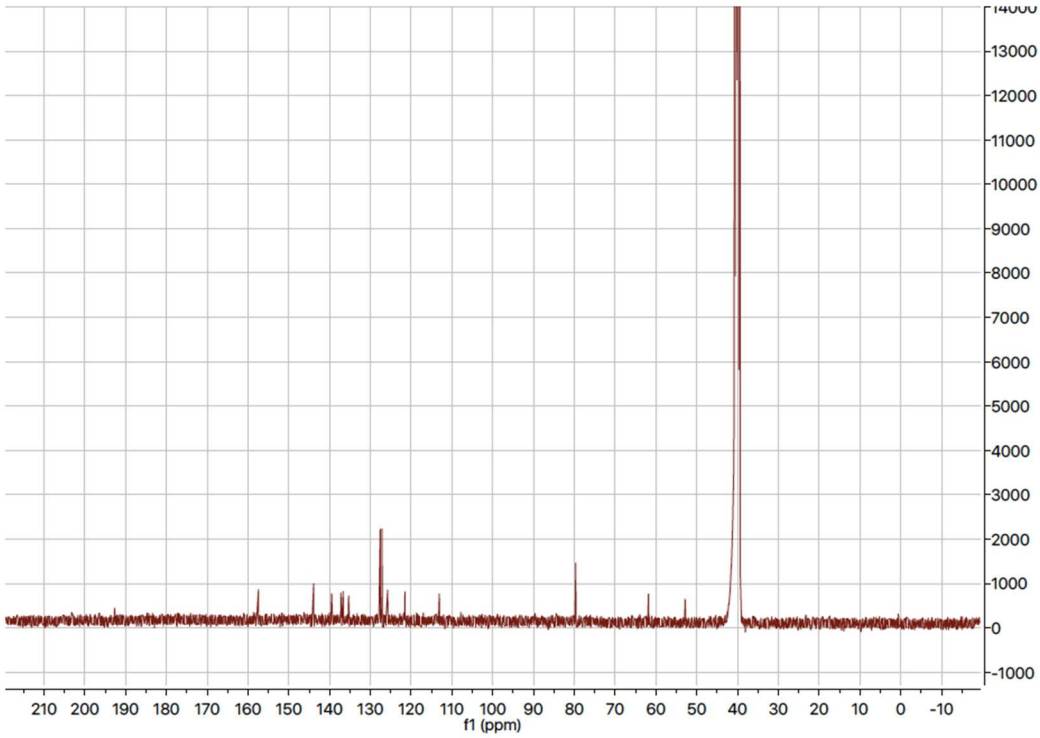


COPIES OF ALL SPECTRA

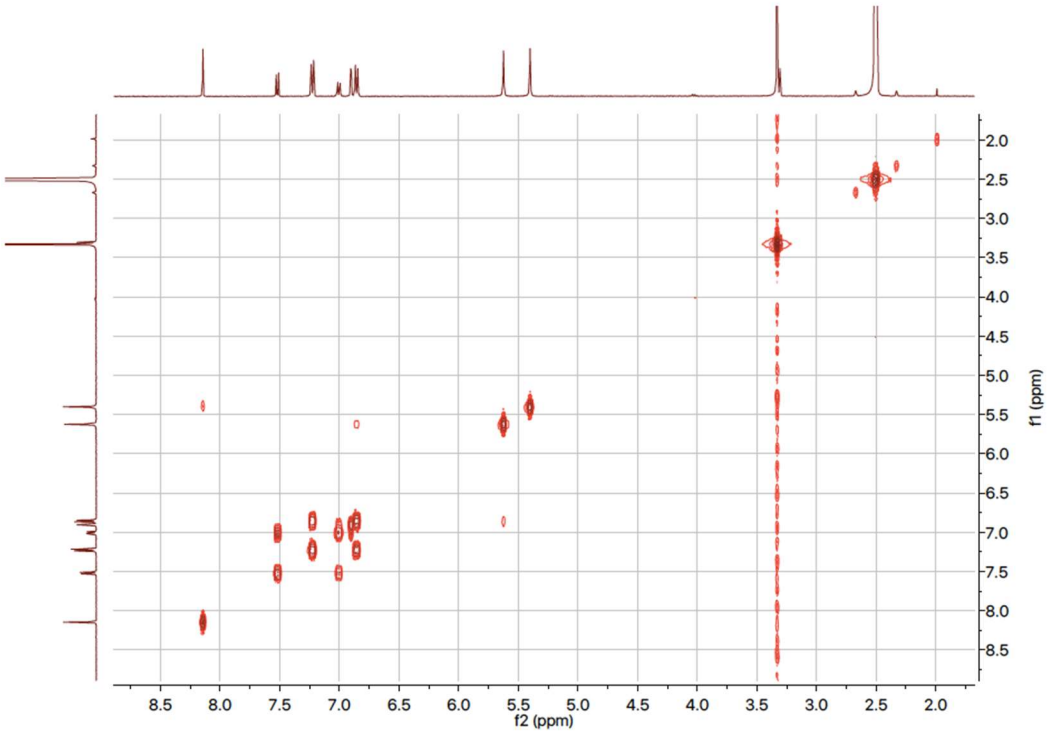
^{1}H NMR spectrum of compound **1** in DMSO-*d6*



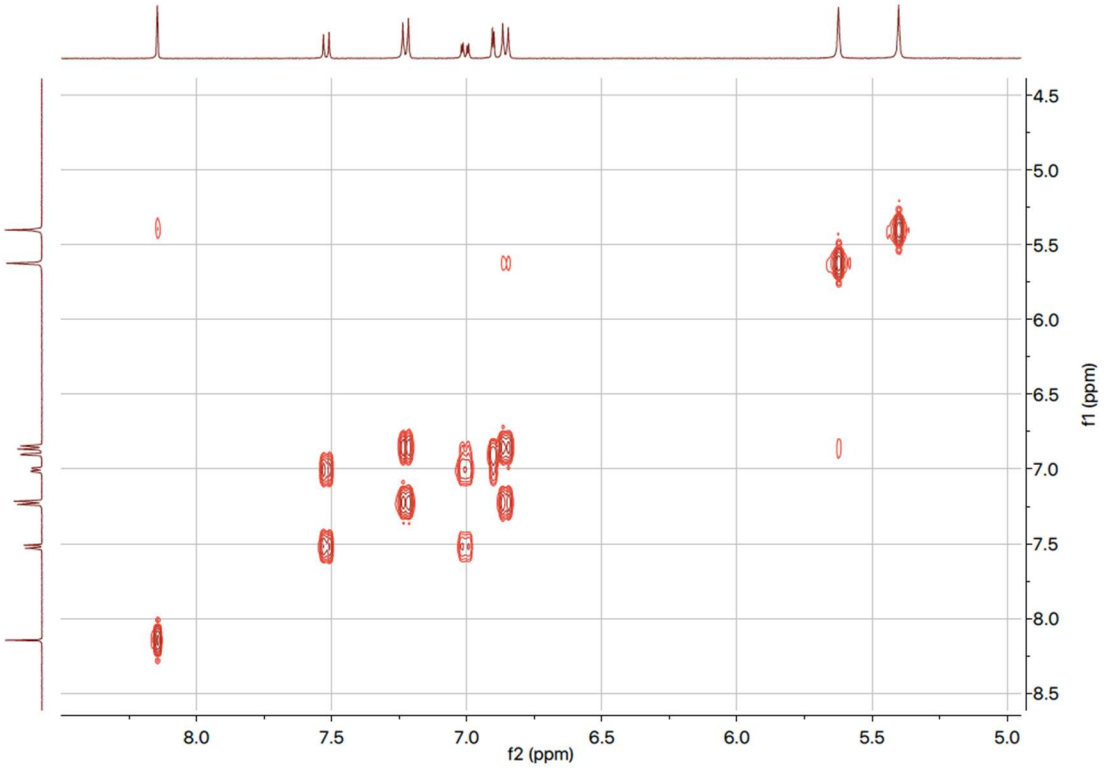
^{13}C NMR of **1** in DMSO-*d6*



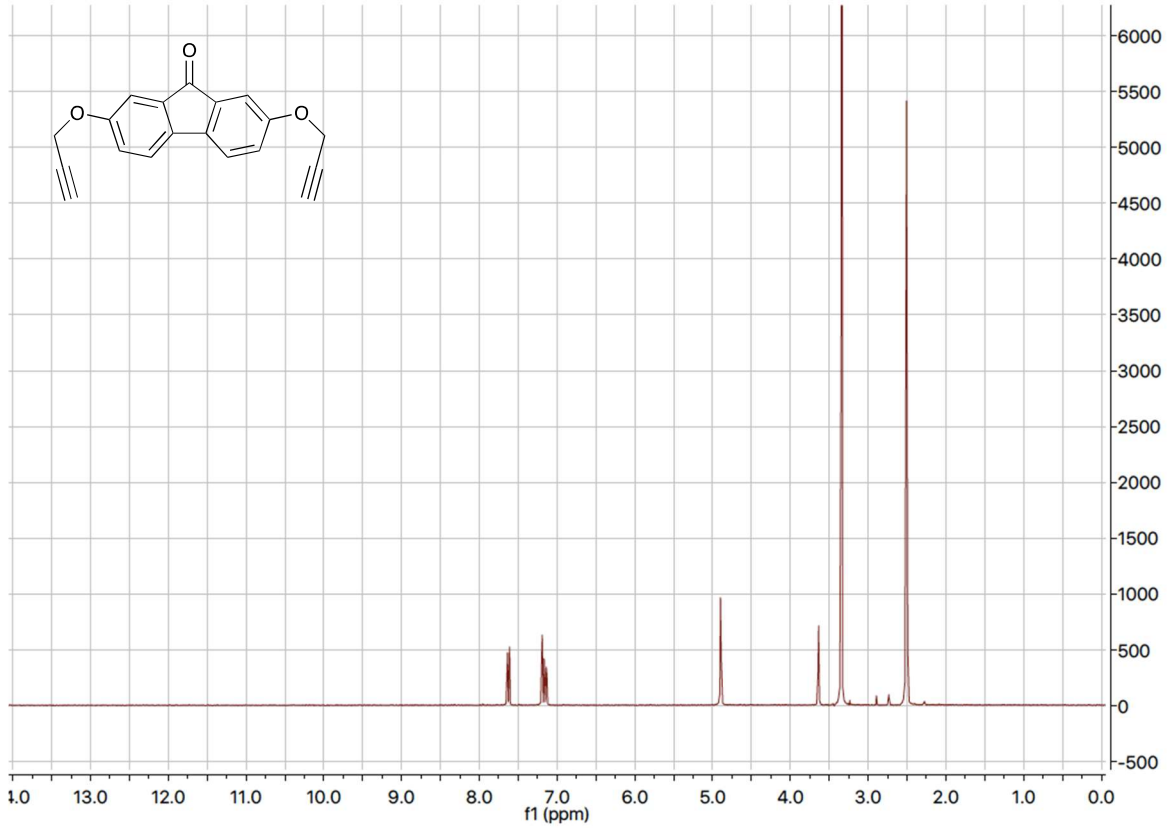
COSY NMR spectrum of compound **1**



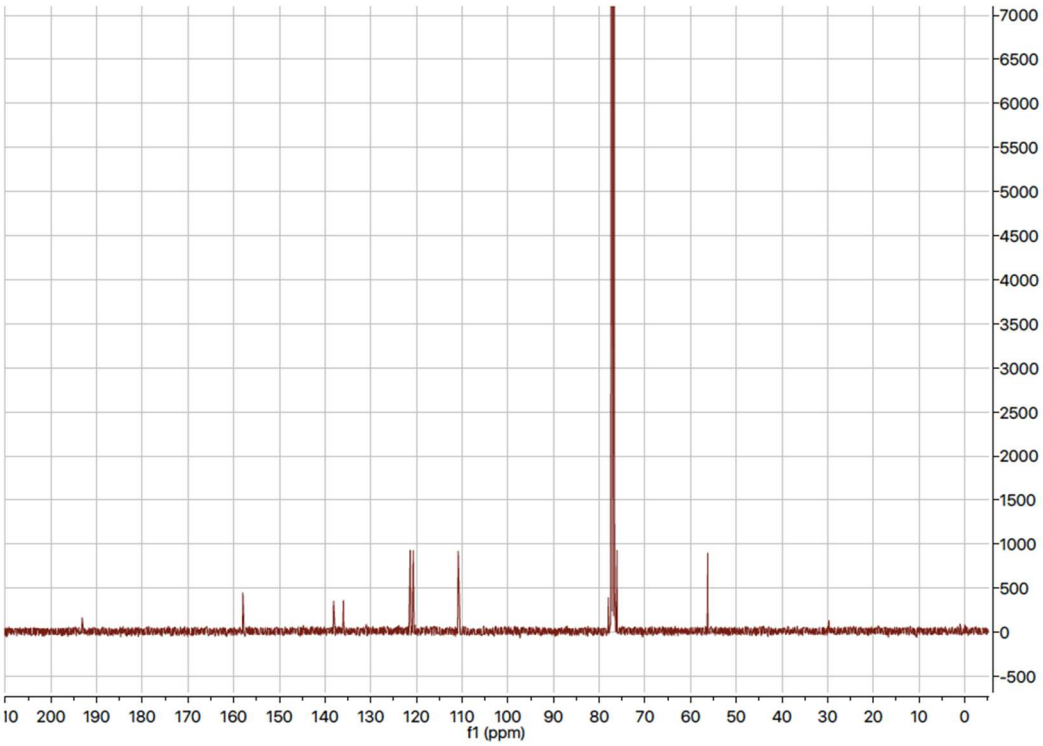
Zoomed-in close-up on the COSY NMR



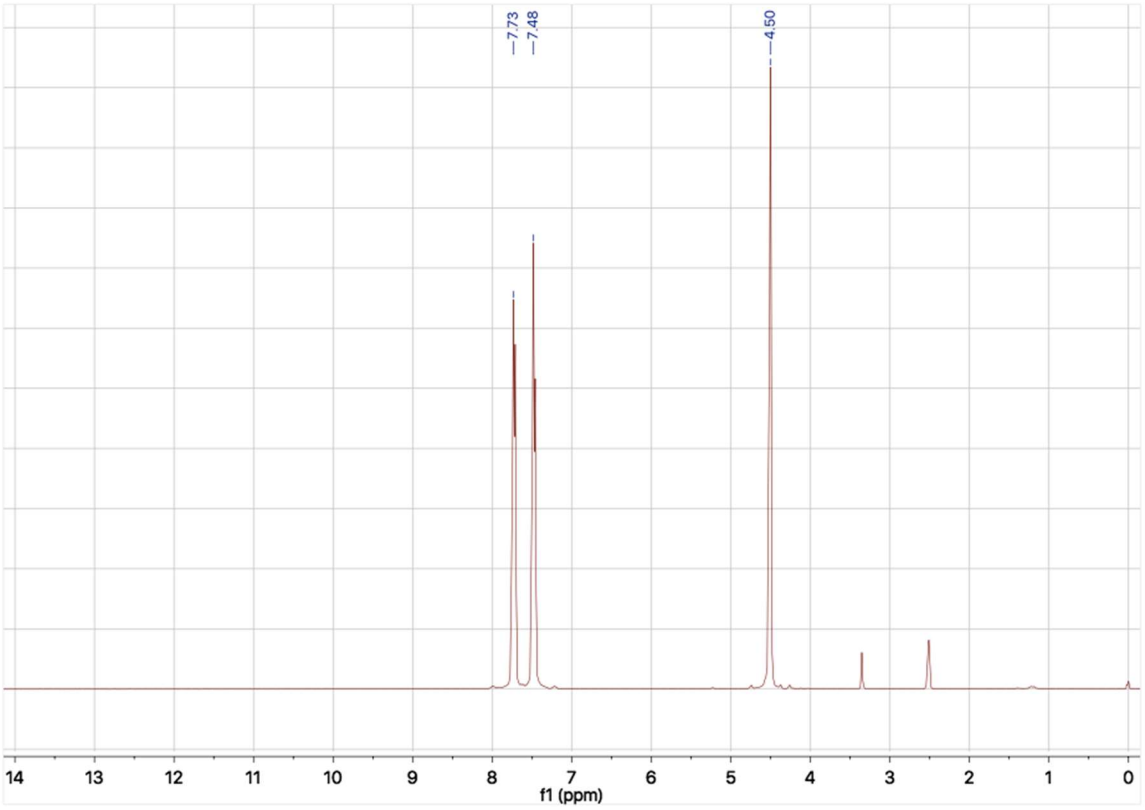
^{1}H NMR of **2** in DMSO-d6



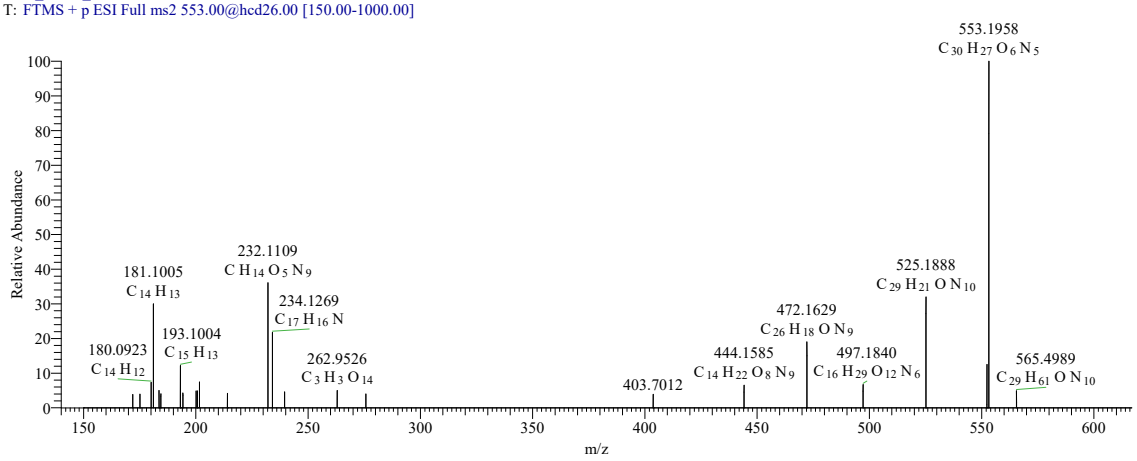
^{13}C NMR of **2** in CDCl3



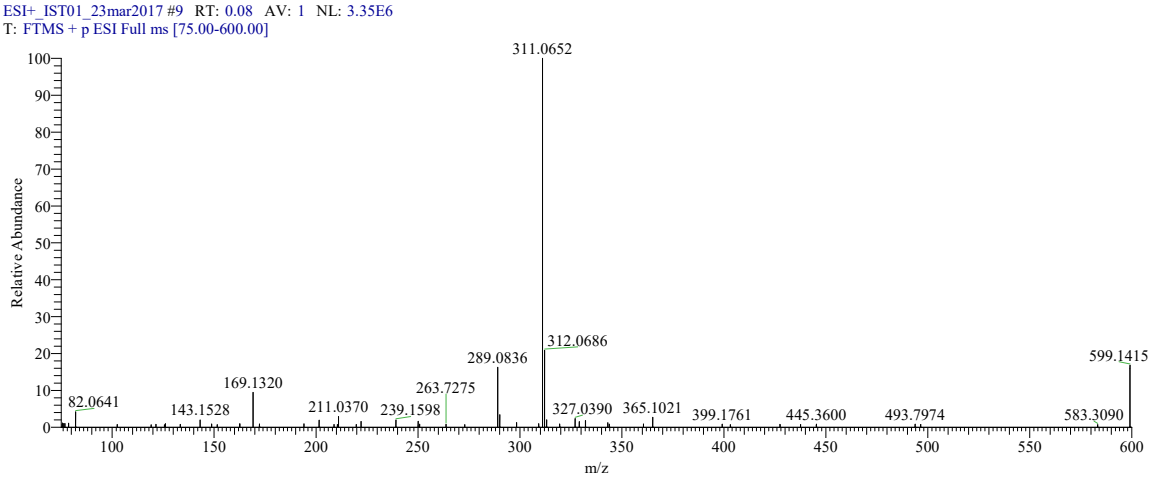
^{1}H NMR of **3** in DMSO-d6



High resolution mass spectrometry of compound **1**



High resolution mass spectrometry of compound **2**



[i] P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305-1323.