Supporting Information for

An Original Class of Small Sized Molecules as Versatile Fluorescent Probes for Cellular Imaging

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Abstract: An unusual class, compact in sizes, of fluorescent probes based on pyridazino-1,3a,6a-triazapentalene scaffolds exhibits highly fluorescent properties (quantum yield values up to 73%, large Stokes shifts, emission wavelengths located in the green-yellow range, excellent solubility) with very good photostability suitable for optical imaging applications.

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1. General remarks

Materials and methods

Nuclear Magnetic Resonance (NMR) spectra were recorded on a 250 or 400 MHz Bruker NMR spectrometers in $CDCl_3$ or DMSO. All chemical shift values are reported in parts per million (ppm) with coupling constant (J) values reported in Hz. All spectra were referenced to the $CDCl_3$ residual solvent peak $CHCl_3$ (δ = 7.26 ppm) for ^1H NMR and the $CDCl_3$ solvent peak (δ = 77.16 ppm) for ^{13}C NMR. The notation of signals is: Proton: δ chemical shift in ppm (multiplicity, J value(s), number of protons). Carbon: δ chemical shift in ppm. Fluorine: δ chemical shift in ppm. Splitting patterns are assigned s = singlet, b = broad, d = doublet, td = triplet of doublet, dt = doublet of triplet, t = triplet, q = quartet, app= apparent.

All reaction were monitored by thin-layer chromatography (TLC) analysis using silica gel (60 F254). THF and ACN were purified with a dry station GT S100, dichloromethane were freshly distilled over calcium hydride and stored under N_2. Other solvents, unless otherwise stated, were purchased in reagent grade or anhydrous quality and used as received. Reagents were either purchased directly from commercial suppliers or prepared according to literature procedures. Yields of all the compounds refer to isolated compounds. Chromatography: Separations were carried out on Silica gel 60, (40-63 µ, 60 Å) purchased from Sigma Aldrich. High Resolution Mass Spectrometry (HRMS) were recorded on Maxis Bruker 4G. Melting points (mp [C°]) were taken on open capillary tubes using a Electrothermal IA 9100 apparatus.

Compound **2** was synthesized according to literature known procedure [1] developed previously in our laboratory. The starting 3-methoxy and 4-methoxypyrazoles were obtained according to the procedures described by Janin *et al.* and Oslob *et al.* [2][3] The other azoles were purchased from commercial suppliers and were used without further purification.

ATTENTION: Azides derivatives must be carefully handles due to their potential instability.

Optical spectroscopy

All optical measurements were carried out at room temperature using degassed (with argon during 30 - 60 minutes) spectroscopic grade solvents purchased from Thermofisher. UV-Vis spectra were obtained on a Varian Cary 50 scan spectrophotometer by using a rectangular quartz micro cell (Hellma, 104-QS, light path : 10mm,1.0 mL). Fluorescence spectroscopic studies (emission/excitation spactra) were performed with a Varian Cary Eclipse spectrophotometer using a fluorescence quartz ultra-micro cell (Hellma, 105.250 QS, light path: 10x 2mm, 50 µL). Emission spectra were recorded under the same conditions after excitation at the corresponding maximum absorption wavelength.

Quantum Yield Measurement

The photoluminescence quantum yield of a dye or material is defined as follows:

$$\Phi = P_E/P_A$$

Where $P_{E,A}$ are the number of photons absorbed and emitted respectively. Quantum yields of our tricyclic compounds were determined using a well-known relative method with Coumarine 153 (λ_{max} = 421 nm, λ_{em} = 531 nm in ethanol, Φ_{em}= 0.38 in Ethanol) [4] as standard fluorophore.

The quantum yield of the compounds in organic or aqueous solvents was calculated according to the following equation:

$$\Phi_{sample} = \Phi_{standard} \times (I_{sample} / I_{standard}) \times (A_{standard} / A_{sample}) \times (n_{sample} / n_{standard})^2$$

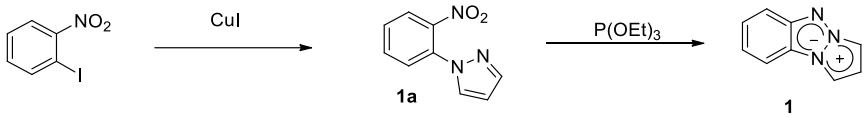
Φ denotes the quantum yield; **I** denotes the area under the fluorescence band; **A** denotes the absorbance (in the range 0.01 – 0.1 AU); **n** denotes the refractive index of the solvent (at 25 °C). All compound solutions (in DMSO, $CHCl_3$ or water) were freshly prepared before each spectroscopic analysis, degassed with argon (during 30-40 minutes) and protected from direct light throughout the analysis.

Solvatochromism Measurements

Solvatochromism measurements were performed by monitoring absorption and emission intensities with tricyclic triazapentalenes **4** and tetrazapentalene **4b**. Tests were done using a large range of solvents, all purchased from Thermofisher having a spectrophotometric grades, kept under dry conditions and protected from light. All solutions were prepared using polar aprotic and polar protic solvents as : DMSO, MeOH, acetone, CH_2Cl_2, $CHCl_3$, CH_3CN, H_2O at identical concentrations (~ 6.2 .10-5 M)

2. Synthesis of fused tricyclic (hetero)aryl-1,3a,6a-triazapentalene scaffolds and tetrazapentalene analogs

2.1. Synthesis of benzo-1,3a,6a-triazapentalene 1 by desoxygenated cyclisation



1-(2-nitrophenyl)-1H-pyrazole (1a) : Under a nitrogen atmosphere, in a 100 mL simple bottle equipped with a magnetic stir bar was placed pyrazole (3 g, 12 mmol, 1 equiv.), 2-iodo-1nitro-benzene (1.2 g, 18mmol, 1.5 equiv.), copper oxide (I) (0.17 g, 1 mmol, 10 mol%), cesium carbonate (7.9 g, 24 mmol, 2 equiv.) and anhydrous dimethylformamide (25 mL). The mixture solution was stirred at 100 ˚C for 20 hours, after cooling down to room temperature, the solvent was removed *in vacuum*. To the resulting crude product was added 50 mL of diethyl acetate and 50 mL of water. The extracted organic phases were then washed with sat. aq. NaCl (3 x 50 mL), then dried on magnesium sulfate ($MgSO_4$). The volatiles were removed *under vacuum* and the crude residue was purified by flash column chromatography (PE/EA = 7/3) to yield 1-(2-nitrophenyl)-1H-pyrazole (1.7 g, 75 %) as a yellow solid. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.75 (d, *J* = 1.9 Hz, 1H), 7.72 (d, *J* = 2.5 Hz, 1H), 7.67 (dd, *J* = 7.4, 1.4 Hz, 1H), 7.62 – 7.57 (m, 1H), 7.56 – 7.47 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.74, 133.43, 130.19, 128.80, 126.73, 125.48, 108.63. MS (IC+) calcd. for 190, found 190. Analysis data are in agreement with literature. [5]

Benzo[d][1,2,3]triazolo[1,2-a][1,2,3]triazol-10-ium-9-ide (1) : A suspension of bicycle (**1a**) (760 mg, 4.02 mmol, 1 equiv.) in $(EtO)_3P$ (15 mL, 87 mmol, 22 equiv.) was subjected to a microwave irradiation cycle : 5 min to attain 176 °C and then 90 min at 176 °C. After cooling down to room temperature, the resulting mixture was concentrated under vacuo and the crude residue was purified by flash column chromatography (PE/EA = 8/2, then 5/5) to give the expected tricycle (**2a**) (151 mg, 24 %) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.67 - 7.62 (m, 1H), 7.60 (d, *J* = 2.6 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.36 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 6.96 (ddd, *J* = 8.1, 7.0, 1.0 Hz, 1H), 6.79 (t, *J* = 2.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 126.26, 116.17, 112.93, 109.74, 108.91, 104.57, 103.77. HRMS (ESI) calcd. for $C_6H_5N_6$: 161.0570, found 161.0570. Analysis data are in agreement with literature. [6]

2.2. Synthesis of pyrimidyl-1,3a,6a-triazapentalene 3 by thermolysis induced cyclisation

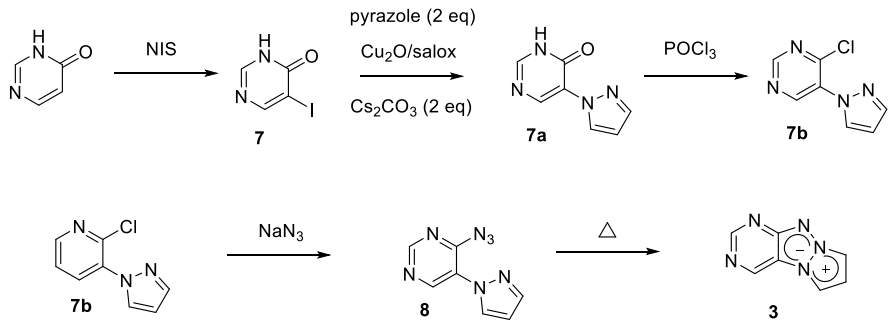


Figure S1. Synthesis of pyrimidyl-1,3a,6a-triazapentalene **3**.

5-iodopyrimidin-4(3H)-one (7): Under an argon atmosphere, in a 100 mL simple bottle equipped with a magnetic stir bar was placed 4-(3H)-pyrimidinone (750 mg, 7.8 mg, 1 equiv.) and glacial acetic acid (27 mL). Then N-iodosuccinimide (2.04 g, 8.61 mmol, 1.1 equiv.)

was added by small portions and the mixture solution was stirred at 50 ˚C for 4 hours. After cooling down to 10 °C using a cold water bath, the resulting precipitate was filtered and washed with cold water. The obtained residue was washed with cold ethanol and dried under vacuum to afford 5-iodopyrimidin-4(3H)-one (1.74 g, 89%) as white powder. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.94 (s, 1H), 8.44 (s, 1H), 8.18 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.76, 160.00, 159.02, 150.23, 90.99. MS (IC+) calcd. for 223, found 223.

5-Pyrazol-1-yl-3H-pyrimidin-4-one (7a) : Under a nitrogen atmosphere, in a microwave tube equipped with a magnetic stir bar was placed the Cu_2O (11.2 mg, 0.078 mmol, 0.05 equiv.), 2-Hydroxybenzaldehyde oxime (42.8 mg, 0.31 mmol, 0.2 equiv), pyrazole (163.5 mg, 2.35 mmol, 1.5 equiv.), cesium carbonate (1.02, 3.12 mmol, 2 equiv.), 5-iodopyrimidin-4(3H)-one **7** (350 mg, 1.56 mmol, 1 equiv.) and anhydrous dimethylformamide (1.75 mL). The tube was evacuated, back-field with argon and capped with a top. The tube was then subjected to a microwave irradiation cycle: 5 min to attain 170 °C and then 70 min at 170 °C. After cooling down to room temperature, the resulting mixture was concentrated under vacuo and the crude residue was purified by flash column chromatography (DCM/MeOH = 10/0, 97/3, 95/5 and 90/10) to give the expected bicycle (239 mg, 95 %) as a yellow solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 2.4 Hz, 1H), 8.48 (s, 1H), 8.22 (s, 1H), 7.74 (d, J = 1.2 Hz, 1H), 6.61 – 6.38 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.53, 140.65, 131.16, 106.89. HRMS (ESI) calcd. for $C_7H_7N_4O$: 163.0614, found 163.0612.

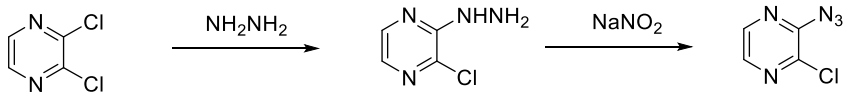
4-Chloro-5-pyrazol-1-yl-pyrimidine (7b): A solution of 5-Pyrazol-1-yl-3H-pyrimidin-4-one **(7a)** in phosphorus oxychloride (0.61 mL, 6.54 mmol, 20 equiv.) stirred at 110 ˚C for 1 hour. After cooling down to room temperature and quenching with cold water, the organic materials were extracted with 3 x 20 mL ethyl acetate. The combined organic phases were washed with sat. aq. NaCl (1 x 50 mL), then dried on magnesium sulfate ($MgSO_4$) and concentrated *in vacuo*. The desired compound 4-chloro-5-pyrazol-1-yl-pyrimidine **7** was obtained as brown solid and engaged in the next step without further purification (unstable compound). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.98 (s, 1H), 8.08 (d, J = 2.6 Hz, 1H), 7.84 (d, J = 1.7 Hz, 1H), 6.59 (dd, J = 2.5, 1.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.31, 154.42, 142.69, 131.13, 108.52. HRMS (ESI) calcd. for $C_7H_6ClN_4$: 181.0276, found 181.0276.

4-azido-5-(1H-pyrazol-1-yl)pyrimidine (8): Under an argon atmosphere, in a microwave tube equipped with a magnetic stir bar was placed 4-chloro-5-pyrazol-1-yl-pyrimidine **7b** (100 mg, 0.55 mmol, 1 equiv.) and anhydrous dimethylformamide (1.7 mL). After sodium azide (72 mg, 1.11mL, 2 equiv.) is added slowly and the mixture solution was stirred at 50 ˚C for 15 hours, then at 70 ˚C for 2 hours. After cooling down to room temperature and quenching with cold water, the organic materials were extracted with 3 x 20 mL ethyl acetate. The combined organic phases were washed with sat. aq. NaCl (1 x 20mL), then dried on magnesium sulfate ($MgSO_4$). The volatiles were removed under *vacuum* and the crude residue was purified by flash column chromatography (Cyclohexane/EA = 8 /2) to yield 4-azido-5-(1H-pyrazol-1-yl)pyrimidine **8** (23 mg, 22%) as a white powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.58 (s, 1H), 9.21 (d, J = 2.7 Hz, 1H), 9.08 (s, 1H), 7.90 (d, J = 1.7 Hz, 1H), 6.66 (dd, J = 2.7, 1.8 Hz, 1H). HRMS (ESI) calcd. $C_7H_6N_7$: 188.0679, found 188.0680.

Pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-d]pyrimidin-9-ium-10-ide (3): Compound (**3**) was synthesized using general method of thermolysis for triazapentalens preparation described below. Compound was obtained as a beige powder, yield = 66% (15mg). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.88 (s, 1H), 7.96 (d, J = 3.2 Hz, 1H), 7.89 (d, J = 2.4 Hz, 1H), 6.93 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.88, 156.43, 136.24, 113.86, 111.40, 109.73, 109.40. HRMS (ESI) calcd. for $C_7H_6N_5$: 160.0618, found 160.0619.

2.3. Synthesis of pyrazinyl-1,3a,6a-triazapentalenes 4, 4e, 4d, 4f, 4i, 4h, 4g, 4j and tetrazapentalenes analogs 4a, 4b and 4c by thermolysis induced cyclisation

2.3.1. Synthesis of the key intermediate 2-azido-3-chloro-pyrazine 5



5a **5**

(3-chloropyrazin-2-yl)hydrazine (5a): Hydrazine monohydrate (6.84 mL, 138.2 mmol, 2.1 equiv.) is added dropwise in a round bottomed flask containing a solution of 2,3-dichloropyrazine (10.0 g, 65.8 mmol, 1 equiv.) in ethanol (400 mL). The reaction mixture is

stirred under reflux for 6h. A second portion of hydrazine monohydrate (1.71 mL, 34.6 mmol, 0.25 equiv.) is then added. After 1 hour at reflux, the mixture was then concentrated under reduced pressure. The crystalline solid was filtrated on Millie-Pore, washed with cold ethanol to afford the hydrochloric salt of (3-chloropyrazin-2-yl)hydrazine as a yellowish solid (14.9 g, quant.) The compound was synthesized following procedure from literature. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.04 (d, *J* = 2.8 Hz, 1H), 7.58 (dd, *J* = 2.8 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 154.3, 141.9, 134.8, 131.9. Analysis data are in agreement with literature. [7]

2-azido-3-chloro-pyrazine (5): 3-chloropyrazin-2-yl)hydrazine **5a** (5 g, 27.6 mmol, 1 equiv.) is solubilized in a 10% aqueous solution of acetic acid (100 mL). The reaction media is cooled at 0°C, followed by a dropwise addition of a solution of sodium nitrite (2.1 g., 30 mmol, 1.1 equiv.) in distilled water (7 mL). After 30 min. stirring at 0°C, the aqueous layer is extracted twice with EtOAc. The organic layer is then washed with brine, dried over $MgSO_4$ and concentrated *in vacuum,* and the crude residue was purified by flash column chromatography (pure DCM) to yield 2-azido-3-chloro-pyrazine as a yellowish powder (3.12 g., 73%) ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.75 (d, *J* = 4.6 Hz, 1H), 8.13 (d, *J* = 4.6 Hz, 1H) δ ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.42, 134.18, 118.35. MS calcd. for $C_4H_2N_5Cl$: 156, found 156.

2.3.2. General methods for tricyclic triazapentalenes and tetrazapentalenes synthesis (One pot reaction)

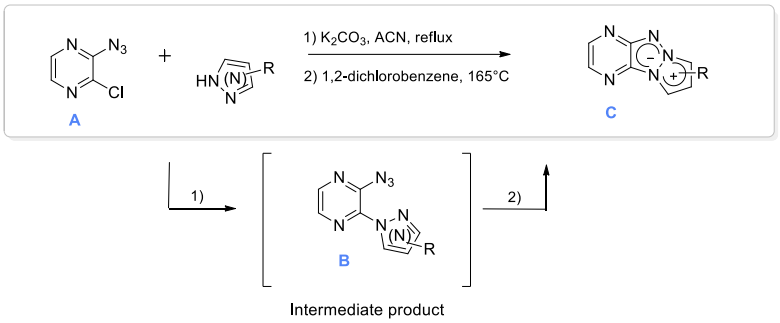
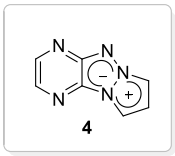


Figure S2. General method for tricyclic triazapentalenes and tetrazapentalenes synthesis.

Under an argon atmosphere, the nucleophile (1 equiv.) is solubilized in dry acetonitrile (0.14 - 0.16 M). The potassium carbonate (2 equiv.) is then introduced. The flask is then purged with argon, and allowed to stir at room temperature during 5 minutes. To this solution was then added portion wise the 2-azido-3-chloro-pyrazine **5** (1 equiv.), and the solution was stirred under reflux until the completion of the reaction. The mixture was then concentrated under reduced pressure, and to the crude material was added 1.2-dichlorobenzene (0.14 - 0.16 M). The atmosphere was then flushed and filled with argon, the reaction media was degassed, and the corresponding suspension was heated at 165°C until the total consumption of the bicyclic intermediate. After cooling down to room temperature, the solvent was eliminated by filtration on a silica pad (eluting petroleum ether). C was then eluted with more polar solvent (such as DCM / EtOAc) to afford desired tricyclic triazapentalene as fluorescent powders.

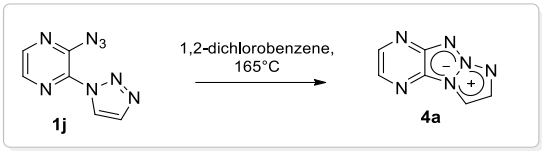
Pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4)


4

S_NAr: 3 hours under reflux. Thermolysis: 1h30 at 165°C. Purification: DCM 100% the DCM/MeOH 98/2. Yellow powder, 64%, 67 mg. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.44 (d, *J* = 2.6 Hz, 1H), 8.08 (d, *J* = 3.3 Hz, 1H), 7.90 (d, *J* = 2.6 Hz, 1H), 7.84 (d, *J* = 2.5 Hz, 1H), 6.93 (t, *J* = 2.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.64, 142.43, 129.02, 109.32, 108.80, 108.13. HRMS (ESI) calcd. for $C_7H_6N_5$: 160.0618, found 160.0615.

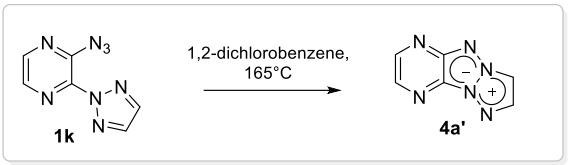
[1,2,3]triazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4a) and [1,2,3]-triazolo[2',1':1,2][1,2,3]triazolo[4,5-b]pyrazin-9-ium-10-ide (4a')

Structure discrimination between isomers **4a** and **4a'** was based on the ^{1}H NMR analysis of the isolated azides precursors **1j** and **1k**. The two triazole protons in **1j** are different in ^{1}H NMR while the two triazole protons in **1k** are identical.



Compound **1j**: ^{1}H RMN (400 MHz, $CDCl_3$) δ 9.18 (d, *J* = 1.3 Hz, 1H), 8.87 (d, *J* = 4.5 Hz, 1H), 8.33 (d, 1H, *J* = 4.5 Hz, 1H), 8.02 (d, *J* = 1.3 Hz, 1H). MS (IC+)**:** *m/z* 161 ($M+H^+-N_2$), 189 ($M+H^+$), 211 ($M+Na^+$).

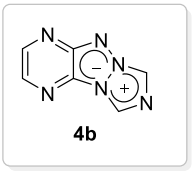
Compound **4a**: Yellowish powder, 47%, 41 mg. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.72 (d, *J* = 2.4 Hz, 1H), 8.35 (d, *J* = 2.4 Hz, 1H), 8.22 (d, *J* = 1.1 Hz, 1H), 8.02 (d, *J* = 1.1 Hz, 1H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.92, 144.68, 138.32, 135.51, 127.36, 104.83. HRMS (ESI) calcd. for $C_6H_5N_6$: 161.0570, found 161.0565.



Compound **1k**: ^{1}H RMN (400 MHz, $CDCl_3$) δ 8.83 (d, *J* = 4.5 Hz, 1H), 8.31 (d, *J* = 4.5 Hz, 1H), 8.23 (s, 2H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.08 (2C), 131.96, 118.47. MS (IC+): *m/z* 161 ($M+H^+-N_2$), 189 ($M+H^+$), 211 ($M+Na^+$). mp: 226°C.

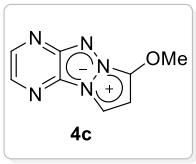
Compound **4a' :** Yellowish powder, 1%, 1 mg. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.75 (d, *J* = 2.5 Hz, 1H), 8.31 (d, *J* = 2.5 Hz, 1H), 8.26 (d, *J* = 2.0 Hz, 1H), 7.94 (d, *J* = 2.0 Hz, 1H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.29, 135.15, 132.91, 106.67. HRMS (ESI) calcd. for $C_6H_5N_6$: 161.0570, found 161.0565.

[1,2,4]triazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4b)



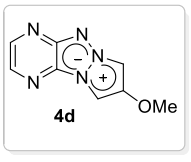
S_NAr: 3 hours under reflux. Thermolysis: 3h at 165°C. Purification: EP 100% then DCM 100% then DCM/MeOH 97/3. Yellow powder, 49%, 238 mg. m.p.: 185°C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.58 (d, *J* = 2.4 Hz, 1H), 8.46 (s, 1H), 8.03 (d, *J* = 2.4 Hz, 1H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.79, 145.45, 131.68, 123.44, 119.38. HRMS (ESI) calcd. for $C_6H_4N_6$: 161.0570, found 161.0568.

7-methoxypyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4c)



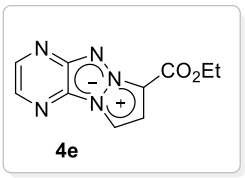
S_NAr: 2 hours under reflux. Thermolysis: 2h at 165°C. Purification: DCM/MeOH 97/3. Brown powder, 42%, 142 mg. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, *J* = 3.5 Hz, 1H), 8.19 (d, *J* = 2.6 Hz, 1H), 7.65 (d, *J* = 2.7 Hz, 1H), 6.97 (d, *J* = 3.5 Hz, 1H), 4.16 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.6, 141.7, 138.3, 129.8, 126.8, 112.5, 96.0, 60.0. IR (v, cm^{-1}): 3115, 3097, 1505, 1429, 1351, 1342, 1228, 1004, 959, 741. mp: 168°C (decomp.). HRMS (ESI) m/z: calculated for $C_8H_8N_5O$ $[M+H]^+$ 190.0723; found 190.0720.

8-methoxypyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4d)



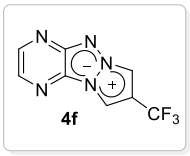
S_NAr: 18 hours under reflux. Thermolysis: 2h at 165°C. Purification: PE 100% then DCM/AcOEt 8/2. Yellow powder, 40%, 39 mg. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.40 (d, *J* = 2.7 Hz, 1H), 7.89 (d, *J* = 2.7 Hz, 1H), 7.70 (s, 1H), 7.66 (s, 1H), 3.95 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.07, 148.42, 142.45, 130.42, 129.89, 99.79, 95.08, 59.13 mp.: 220°C. HRMS (ESI)**:** $[M+H]^+$ calcd. 190.0723 for $C_8H_8N_5O$, found 190.0722.

7-(ethoxycarbonyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4e)



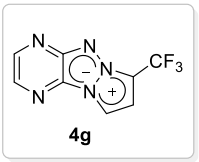
S_NAr: 3 hours under reflux. Thermolysis: 20min at 165°C. Purification: PE 100% then DCM/AcOEt 8/2. Yellow powder, 57%, 224 mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 2.6 Hz, 1H), 8.16 (d, *J* = 2.5 Hz, 1H), 8.03 (d, *J* = 3.6 Hz, 1H), 7.40 (d, *J* = 3.6 Hz, 1H), 4.54 (q, *J* = 7.2 Hz, 2H), 1.48 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.85, 151.52, 144.53, 133.75, 128.47, 112.35, 109.17, 61.86, 14.44. mp: 162°C. HRMS (ESI+) m/z calculated for $C_{10}H_{10}N_5O_2$: 232.0756, found 232.0827.

8-(trifluoromethyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4f)



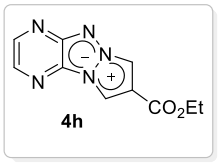
S_NAr: 2 hours under reflux. Thermolysis: 20min at 165°C. Purification: PE 100% then DCM 100% then DCM/AcOEt 8/1. Yellow-green powder, 73 %, 160 mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 2.6 Hz, 1H), 8.37 (s, 1H), 8.07 (s, 1H), 8.04 (d, *J* = 2.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.08, 144.62, 131.85, 128.61, 120.96 ($^1J_{C\text{-}F}$ = 166 Hz), 117.01 ($^2J_{C\text{-}F}$ = 25 Hz), 107.89 ($^3J_{C\text{-}F}$ = 3 Hz), 105.67 ($^3J_{C\text{-}F}$ = 3 Hz). mp: 202°C. HRMS (ESI+) m/z calculated for $C_8H_5F_3N_5$: 228,0419, found 228.0492.

7-(trifluoromethyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-die (4g)



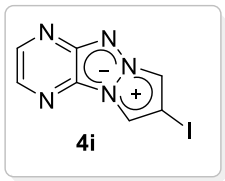
S_NAr: 7 hours under reflux. Thermolysis: 10min at 165°C. Purification: DCM 100%. Yellow-green powder, 77 %, 363 mg. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.64 (d, *J* = 2.4 Hz, 1H), 8.12 (d, *J* = 2.4 Hz, 1H), 8.11 (d, *J* = 3.6 Hz, 1H), 7.20 (d, *J* = 3.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.75, 144.73, 133.10, 128.63, 119.7 (q, $^1J_{C\text{-}F}$ = 166 Hz), 119.37, 110.5 (q, $^2J_{C\text{-}F}$ = 27 Hz), 109.28, 108.84 ($^3J_{C\text{-}F}$ = 2 Hz). mp: 152°C. HRMS (ESI+) m/z calculated for $C_8H_5F_3N_5$: 228,0419, found 228.0492.

8-(ethoxycarbonyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4h)



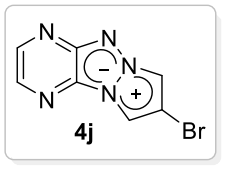
S_NAr: 7 hours under reflux. Thermolysis: 2h at 165°C. Purification: DCM 100% the DCM/AcOEt 9/1. Yellow powder, quant., 550 mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.51 (d, *J* = 1.9 Hz, 1H), 8.20 (s, 1H), 7.98 (d, *J* = 1.9 Hz, 1H) 4.45 (q, *J* = 7.5, 2H), 1.42 (t, *J* = 7.5, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.21, 152.82, 144.44, 131.25, 129.00, 118.49, 112.01, 109.25, 61.90, 14.43. mp.: 221°C. HRMS (ESI+) m/z calculated for $C_{10}H_{10}N_5O_2$: 232,0756, found 232.0827.

8-iodopyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4i)



S_NAr: 3 hours under reflux. Thermolysis: 40min at 165°C. Purification: PE 100% then DCM/AcOEt 8/2. Yellow-orange powder, 86%, 2.35g. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 2.6 Hz, 1H), 8.14 (s, 1H), 7.96 (d, *J* = 2.6 Hz, 1H), 7.87 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.91, 142.91, 130.07, 127.73, 113.37, 112.02. mp.: 255°C (decomp.). HRMS (ESI+) m/z calculated for $C_7H_5IN_5$ 285.9584, found 285.9582.

8-bromopyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4j)



S_NAr: 9 hours under reflux. Thermolysis: 20min at 165°C. Purification: PE 100% then DCM/AcOEt 10/0 to 9/1. Yellow powder, 97%, 1.34g. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 2.8 Hz, 1H, H), 8.11 (s,1H), 7.97 (d, *J* = 2.4 Hz, 1H, H), 7.87 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.48, 143.86, 131.27, 128.87, 110.12, 109.50, 98.49. mp.: >260°C. HRMS (ESI+) m/z calculated for $C_7H_5BrN_5$ 237.9723, found 237.9722.

2.3.2. General methods for diversification of triazapentalenes thanks to Suzuki coupling process.

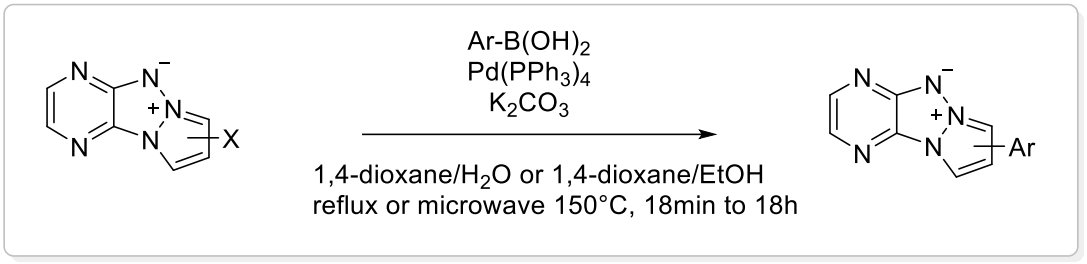
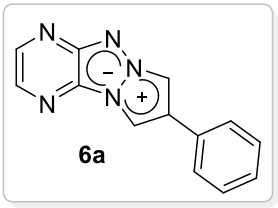


Figure S3. General method for diversification of triazapentalenes via Suzuki coupling process.

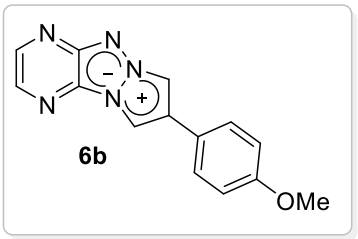
In a round bottom flask *(or sealed tube)* under an inert argon atmosphere, the halogenated triazapentalene **4j** (1eq.) was solubilized in 1,4-dioxane/H_2O(or 1,4-dioxane/EtOH) (2.5/1 : v/v ; 30mM). After introduction of the boronic acid (1.5eq.) and potassium carbonate (6eq.), the mixture was dégassed with argon for 5 to 10min. $Pd(PPh_3)_4$ (0.05eq.) was added and the suspension stirred under reflux *(or under microwaves irradiation at 150°C)* until total consumption of the starting halogenated compound. The mixture was cooling down to room temperature and solvents removed under vacuum. The crude compound was then purified by silica gel column chromatography.

8-phenylpyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6a)



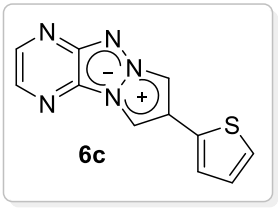
In a round bottom flask, starting from brominated **4j**, in 1,4-dioxane/H_2O under reflux for 45min. Purification: DCM/AcOEt 8/2. Yellow powder, 88%, 29mg. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.45 (d, *J* = 2.7 Hz, 1H), 8.28 (s, 1H), 8.09 (s, 1H), 7.93 (d, *J* = 2.7 Hz, 1H), 7.62 (dd, *J* = 8.2, 1.4 Hz, 2H), 7.55 – 7.36 (m, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 143.41, 130.46, 130.13, 129.55, 128.91, 127.05, 126.59, 106.72, 106.21. mp.: 240°C. HRMS (ESI+) m/z calculated for $C_{13}H_{10}N_5$ 236.0931, found 236.0931.

8-(4-methoxyphenyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6b)



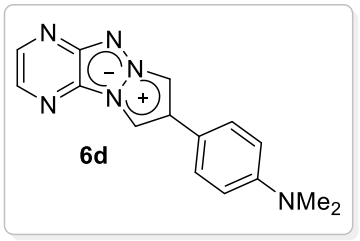
In a round bottom flask, starting from brominated **4j**, in 1,4-dioxane/H_2O under reflux for 5h. Purification: DCM/AcOEt 8/2 then DCM/MeOH 97/3. Yellow powder, 70%, 97mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 2.7 Hz, 1H), 8.19 (s, 1H), 8.03 (s, 1H), 7.91 (d, *J* = 2.7 Hz, 1H), 7.54 (d, *J* = 8.7 Hz, 2H), 7.02 (d, *J* = 8.7 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.42, 143.34, 130.50, 127.84, 126.93, 122.76, 114.97, 106.58, 105.66, 55.60. mp.: 270°C. HRMS (ESI+) m/z calculated for $C_{14}H_{12}N_5O$ 266.1036, found 266.1037.

8-(thiophen-2-yl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6c)



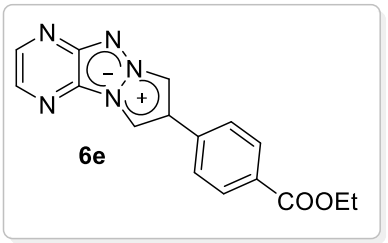
In a sealed tube, starting from brominated **4j**, in 1,4-dioxane/H_2O under microwaves irradiation at 150°C for 1h. Purification: DCM/AcOEt 10/0 to 9/1. Yellow powder, 86%, 67mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 2.0 Hz, 1H), 8.20 (s, 1H), 8.01 (s, 1H), 7.93 (d, *J* = 2.4 Hz, 1H), 7.37 (d, *J* = 5.1 Hz, 1H), 7.31 (d, *J* = 3.5 Hz, 1H), 7.13 (t, *J* = 3.65 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.31, 143.48, 132.06, 130.72, 129.35, 128.31, 126.07, 125.56, 120.91, 106.49, 105.95. mp.: 250°C. HRMS (ESI+) m/z calculated for $C_{11}H_8N_5S$ 242.0494, found 242.0497.

8-(4-(dimethylamino)phenyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6d)



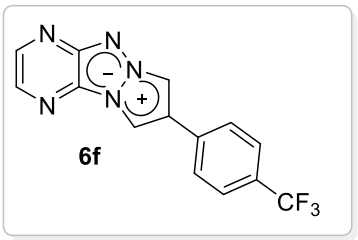
In a round bottom flask, starting from brominated **4j**, in 1,4-dioxane/H_2O under reflux for 18h. Purification: DCM/AcOEt 9/1 to 7/3. Yellow powder, 82%, 73mg. ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.86 (s, 1H), 8.34 (d, *J* = 2.8 Hz, 1H), 7.89 (d, *J* = 2.7 Hz, 2H), 7.70 (d, *J* = 9.0 Hz, 2H), 6.80 (d, *J* = 8.9 Hz, 2H), 2.95 (s, 6H). Due to the very low solubility of compound **11d** in DMSO and usual solvents, ^{13}C NMR (101 MHz, DMSO-d_6) was not recorded. mp.: >260°C. HRMS (ESI+) m/z calculated for $C_{15}H_{15}N_6$ 279.1353, found 279.1353.

8-(4-(ethoxycarbonyl)phenyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6e)



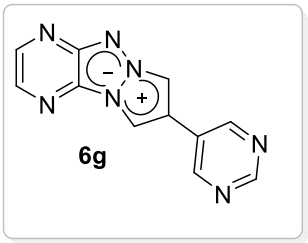
In a sealed tube, starting from brominated **4j**, in 1,4-dioxane/EtOH under microwaves irradiation at 150°C for 20min. Purification: DCM/MeOH 10/0 to 97/3. Yellow powder, 85%, 84mg. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (s, 1H), 9.08 (s, 1H), 8.40 (d, *J* = 2.7 Hz, 1H), 8.08 – 7.98 (m, 4H), 7.94 (d, *J* = 2.7 Hz, 1H), 4.34 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.26, 151.97, 142.66, 135.03, 129.79, 129.55, 129.19, 129.14, 126.07, 124.51, 108.93, 107.68, 60.81, 14.15. mp.: 268°C. HRMS (ESI+) m/z calculated for $C_{16}H_{14}N_5O_2$ 308.1142, found 308.1142.

8-(4-(trifluoromethyl)phenyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6f)



In a sealed tube, starting from brominated **4j**, in 1,4-dioxane/EtOH under microwaves irradiation at 150°C for 30min. Purification: DCM/MeOH 100/0 to 95/5. Yellow powder, 45%, 23mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, *J* = 2.6 Hz, 1H), 8.34 (s, 1H), 8.13 (s, 1H), 7.97 (d, *J* = 2.7 Hz, 1H), 7.75 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.99, 149.18, 142.70, 134.61, 129.60, 129.23, 128.43, 126.62, 126.01 (d, *J* = 3.7 Hz), 124.17, 109.05, 107.73. mp.: >260°C. HRMS (ESI+) m/z calculated for $C_{14}H_9F_3N_5$ 304.0805, found 304.0803.

8-(pyrimidin-5-yl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6g)



In a sealed tube, starting from brominated **4j**, in 1,4-dioxane/H_2O under microwaves irradiation at 150°C for 20min. Purification: DCM/MeOH 97/3 to 95/5. Yellow powder, 74%, 56mg. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.35 (s, 1H), 9.31 (s, 1H), 9.15 (s, 1H), 9.08 (s, 1H), 8.40 (d, *J* = 2.7 Hz, 1H), 7.94 (d, *J* = 2.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.09, 154.54, 152.44, 143.37, 130.34, 119.63, 109.28, 107.67. mp.: >260°C. HRMS (ESI+) m/z calculated for $C_{11}H_8N_7$ 238.0836, found 238.0837.

3. Theoretical approach: methodological details, HOMO and LUMO electronic densities, atomic contributions

TD-DFT calculations have been carried out with Gaussian09 [8] software, to understand the effect of the photophysical properties of nitrogen position on the aryl group of hetero aryl-1,3a,6a-triazapentalenes. CAM-B3LYP was considered as exchange-correlation functional, regarding the charged transfer involved during the first excitation from S0 to S1 and the 6-31+G* basis set was used. Solvent effects of DMSO were considered using polarizable continuum model (PCM). After geometry optimization of S0 and S1 states, absorption and emission wavelength were estimated from their equilibrium conformation in solvent.

Calculations show a good correlation between the prediction of absorption and emission wavelengths and the experimental data, in a qualitative way (Table S1). There is a systematic bias of around 58 nm between experimental and calculated values. Furthermore, the Stokes shift between absorption and emission is remarkably well predicted by TD-DFT calculations. In each case, first excited state is the lowest energy transition ππ*, corresponding to the HOMO - LUMO electronic transition.

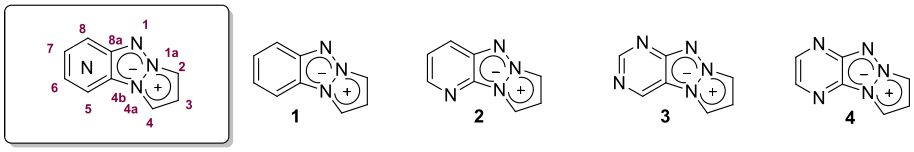
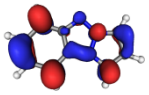
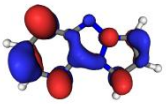
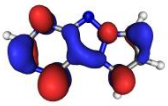
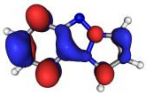


Figure S4. General representation of tricyclic fused triazapentalene with corresponding notation (this notation was attributed for ease of use)

Table S1. Calculated spectroscopic characterization and LUMO electronic density.

Compound	Solvent	$\lambda_{abs\ calc}$ [nm] [a]	Oscillator strength (u.a.)	$\lambda_{em\ calc}$ [nm] [b]	Δ [nm] [c]	Gap Energy (eV)	LUMO
1	DMSO	315	0.553	349	34	3.938	
2	DMSO	338	0.478	396	58	3.668	
3	DMSO	312	0.392	387	75	3.977	
4	DMSO	363	0.377	455	92	3.414	

[a] Calculated maximal absorption wavelength of the dye. [b] Calculated maximal emission wavelength of the dye. [c] Calculated Stokes Shift calculated as the difference between the maxima absorption and maxima emission.

HOMO and LUMO contribution of tricyclic triazapentalenes

All atomic contributions were calculated by Multiwfn software. [9] Results revealed that the LUMO is more impacted by the nitrogen substitution on the fused ring (Table S2) compared to the HOMO (Table S3). The HOMO atomic contributions of triazapentalene scaffold (positions 1 to 4b, Table S3) are not related to the chemical substituents of the fused ring. However, only the neighbourhood atoms of the atomic substitution are impacted by the HOMO such as position 6. Regarding the LUMO, the whole electronic density is displaced from the triazapentalene toward the fused ring (Table S1 and Figure S5). Those charge effects enhance the intramolecular charge transfer (ICT) process, and the fluorescence properties.

Table S2. Details of atomic contribution to the LUMO for each heavy atom, associated to the molecule considered.

Compound	Atomic contribution of each position											
	1	1a	2	3	4	4a	4b	5	6	7	8	8a
1	0.035	0.092	0.060	0.038	0.096	0.061	0.047	0.144	0.072	0.079	0.129	0.030
2	0.023	0.078	0.056	0.034	0.083	0.046	0.061	0.156	0.095	0.079	0.167	0.039
3	0.014	0.083	0.073	0.035	0.097	0.056	0.064	0.202	0.068	0.070	0.114	0.042
4	0.013	0.068	0.060	0.032	0.079	0.038	0.081	0.186	0.084	0.097	0.161	0.048

Table S3. Detail of atomic contribution to the HOMO for each heavy atom, associated to the molecule considered.

Compound	Atomic contribution of each position											
	1	1a	2	3	4	4a	4b	5	6	7	8	8a
1	0.246	0.039	0.127	0.031	0.128	0.022	0.084	0.030	0.102	0.021	0.069	0.053
2	0.254	0.041	0.133	0.031	0.130	0.041	0.093	0.024	0.092	0.020	0.061	0.053
3	0.261	0.052	0.128	0.031	0.132	0.027	0.098	0.028	0.093	0.021	0.058	0.043
4	0.249	0.043	0.126	0.029	0.122	0.024	0.091	0.021	0.114	0.037	0.061	0.045

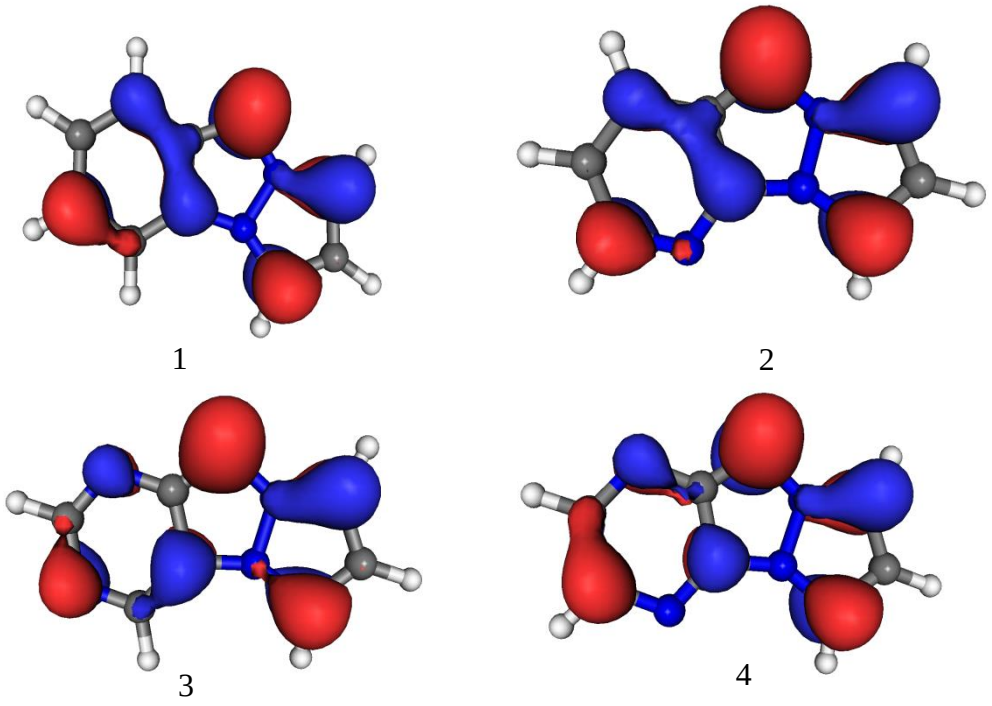


Figure S5. HOMO representation for compound 1, 2 3 and 4, respectively.

4. Photophysical measurements: absorption, emission, molar extinction coefficient, quantum yields

4.1 Optical properties of the unsubstituted triazapentalenes in DMSO

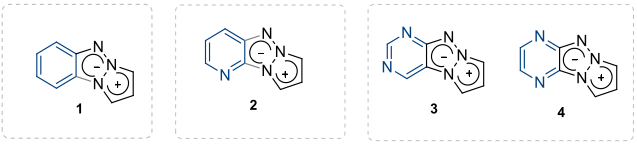
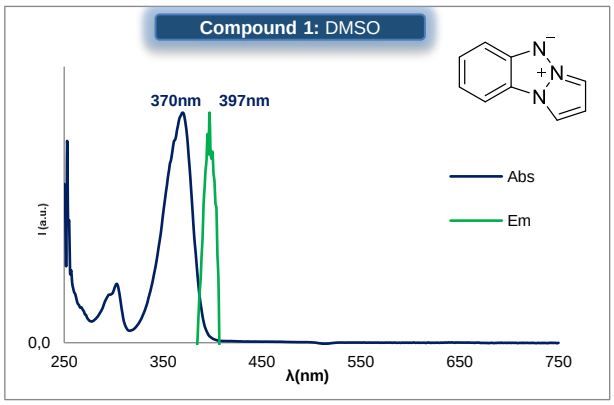
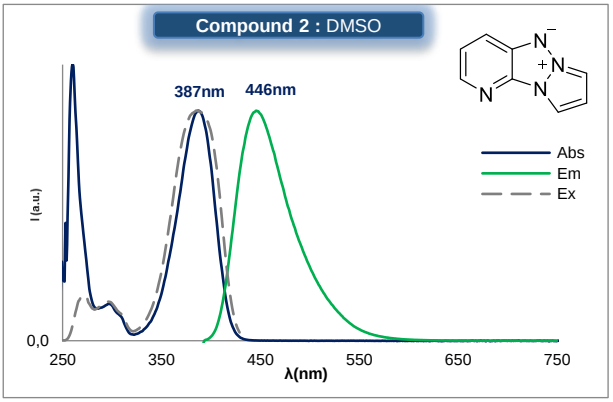


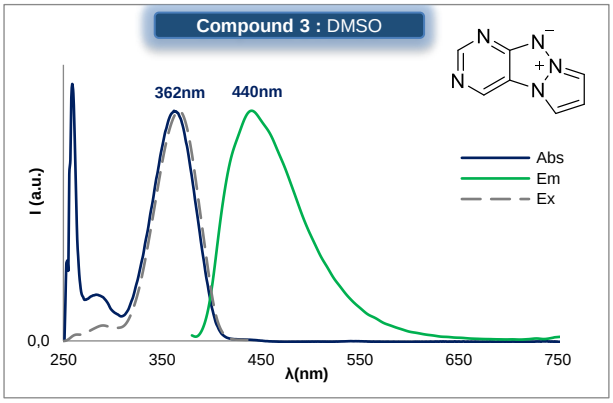
Table S4. Experimental excitation and emission values of corresponding tricyclic unsubstituted triazapentalenes.

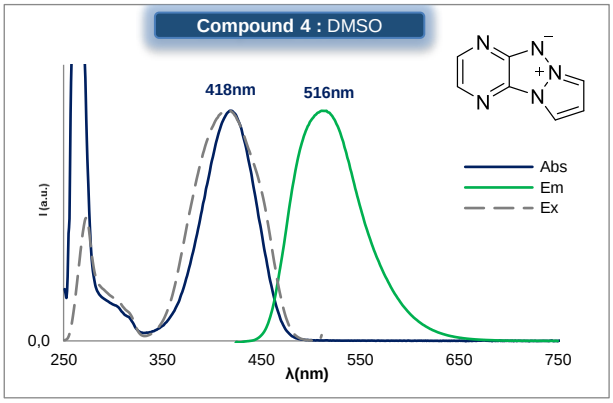
Compound	Solvent[a]	λ_{abs} (nm)	ε ($m^{-1}.cm^{-1}$)	λ_{em} (nm)	Stokes Shift (nm)	QY[b]	Brightness
1	DMSO	370	12700	397	27	< 0.001	< 13
2	DMSO	387	18700	446	59	0.018	340
3	DMSO	362	1200	440	78	0.15	180
4	DMSO	418	15500	516	98	0.15	2300

[a] Dry and degassed solvent with argon. [b] Determined by using Coumarin 153 (QY=0.38 in Ethanol) as standard (Excited at λ_{abs} of each product)

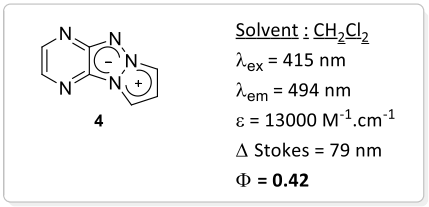








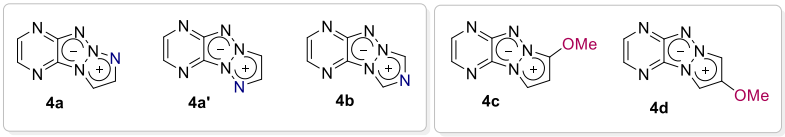
«Lead» probe :



Strong green fluorescence
Under UV irradiation (360 nm)
in CH_2Cl_2

4.2 Effect of the substitution on optical properties tricyclic pyrazinyltriazapentalenes and pyrazinyltetrazapentalenes.

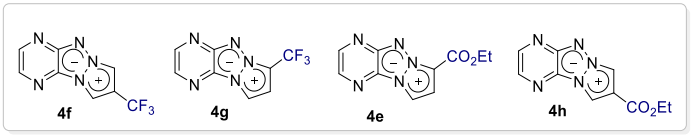
Table S5. Experimental excitation and emission values of triazapentalenes with an electron donating group and tetrazapentalenes.



Compound	Solvent[b]	λ_{abs} (nm)	ε ($m^{-1}.cm^{-1}$)	λ_{em} (nm)	Stokes Shift (nm)	QY[a]	Brightness
4a	CH_2Cl_2	391	12700	427	36	0.51	6418
4a'	DMSO	394	12000	444	50	0.07	840
4b	CH_2Cl_2	410	12133	470	63	0.37	4435
	DMSO	414	5748	499	85	0.50	2580
4d	CH_2Cl_2	410	7105	512	102	0.08	569
	DMSO	415	13700	521	106	0.03	411
4c	DMSO	436	13200	529	89	0.03	396

[a] Determined by using Coumarin 153 (QY=0.38 in Ethanol) as standard (Excited at λ_{abs} of each product)

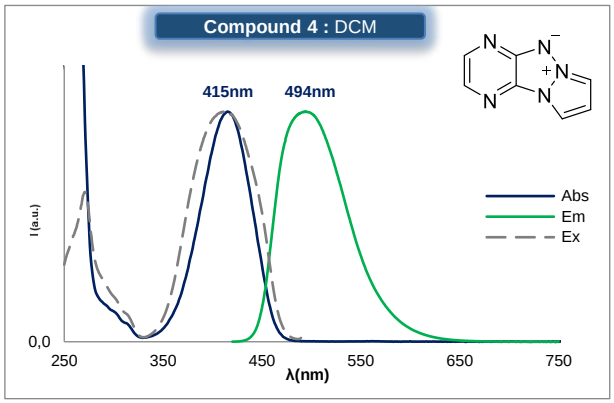
Table S6. Experimental excitation and emission values of triazapentalene derivatives with additional electron withdrawing groups.

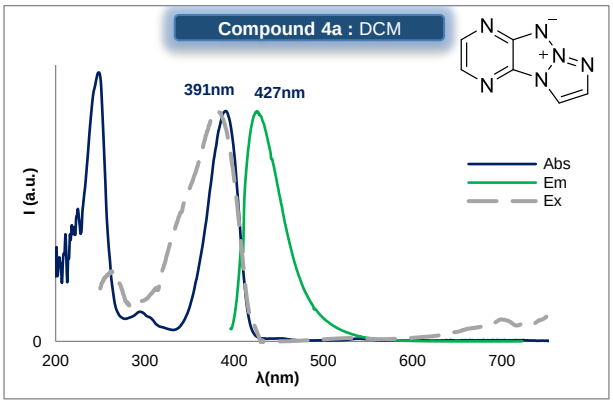


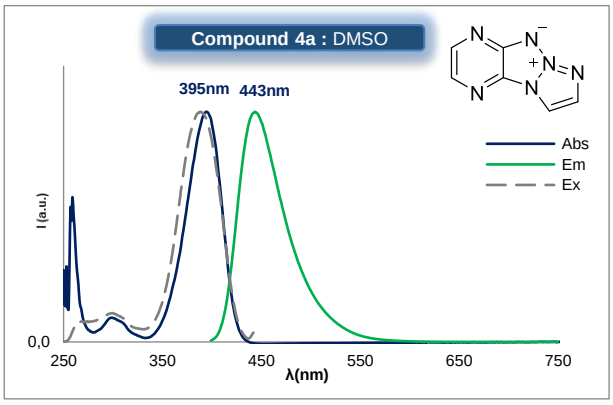
Compound	Solvent[b]	λ_{abs} (nm)	ε ($m^{-1}.cm^{-1}$)	λ_{em} (nm)	Stokes Shift (nm)	QY[a]	Brightness
4f	CH_2Cl_2	409	14911	467	58	0.52	7753
4g	CH_2Cl_2	403	14706	464	61	0.034	501
	DMSO	407	12225	489	81	0.23	2787
4e	CH_2Cl_2	416	24157	473	57	0.023	5556
	DMSO	421	23646	493	72	0.076	1798
4h	CH_2Cl_2	418	11470	473	55	0.73	8373
	DMSO	422	12444	496	74	0.54	6720

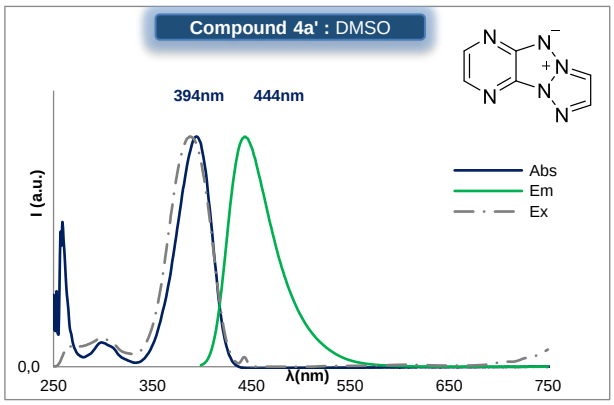
[a] Determined by using Coumarin 153 (QY=0.38 in Ethanol) as standard (Excited at λ_{abs} of each product)

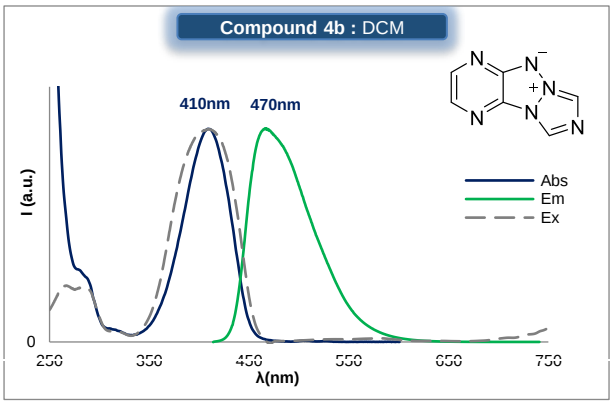
4.3 Photophysical profile.

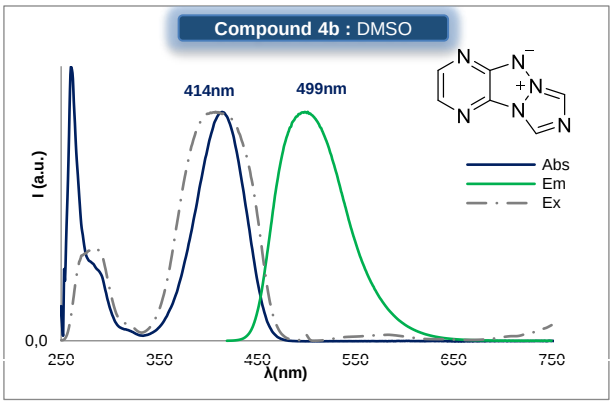


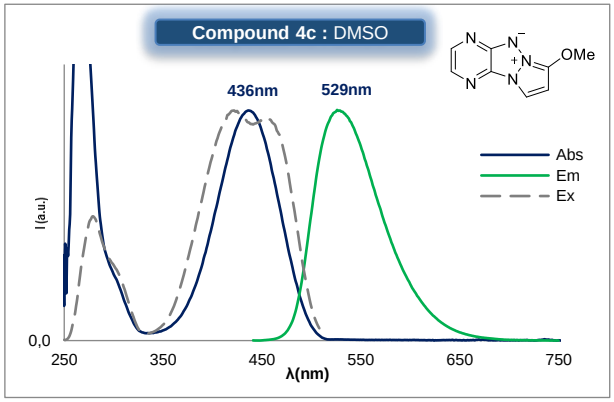


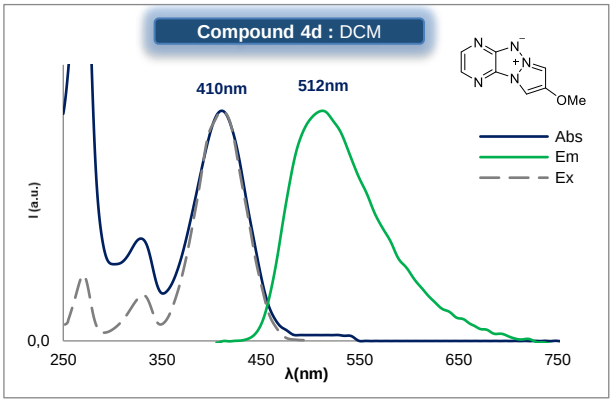


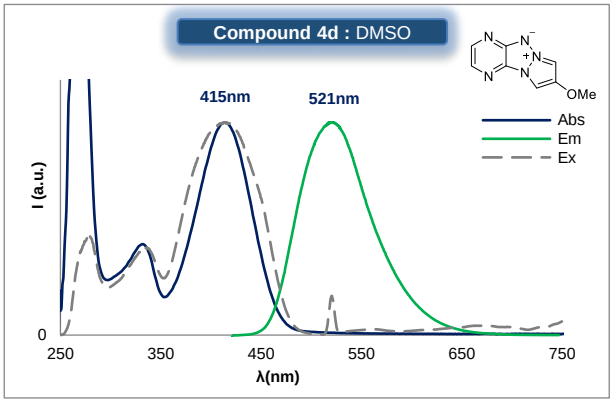


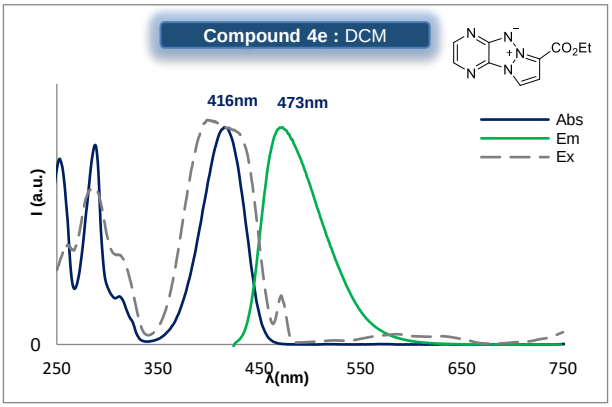


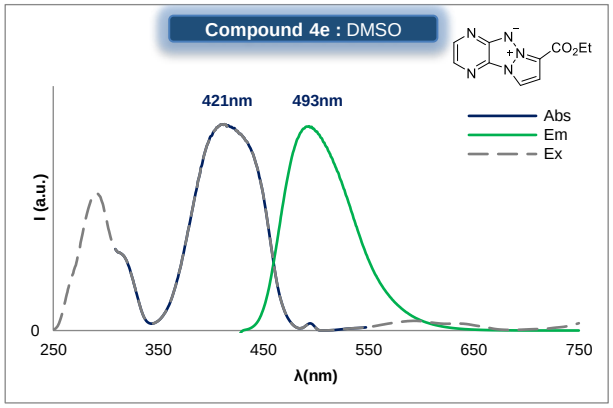


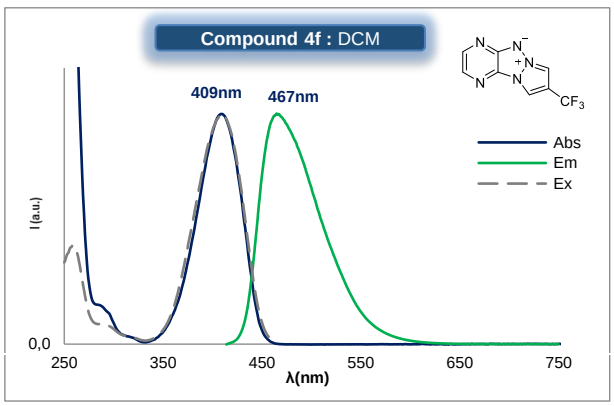

Compound 4c : DMSO
436nm
529nm
OMe
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)

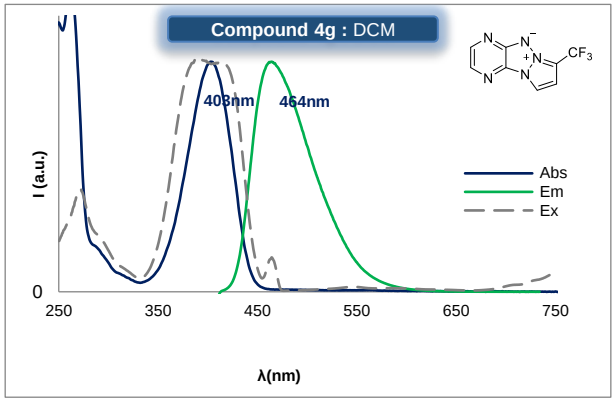

Compound 4d : DCM
410nm
512nm
OMe
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)

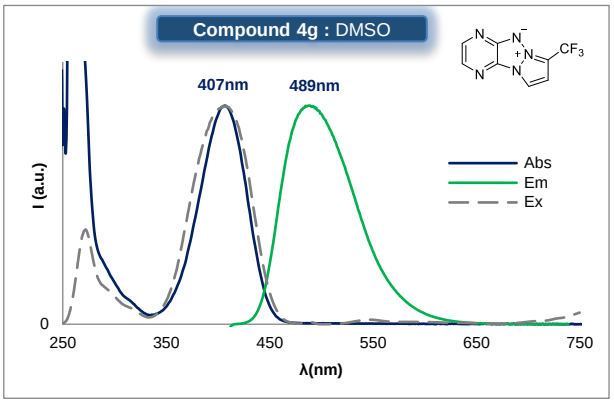

Compound 4d : DMSO
415nm
521nm
OMe
Abs
Em
Ex
I (a.u.)
0
250
350
450
550
650
750
λ(nm)

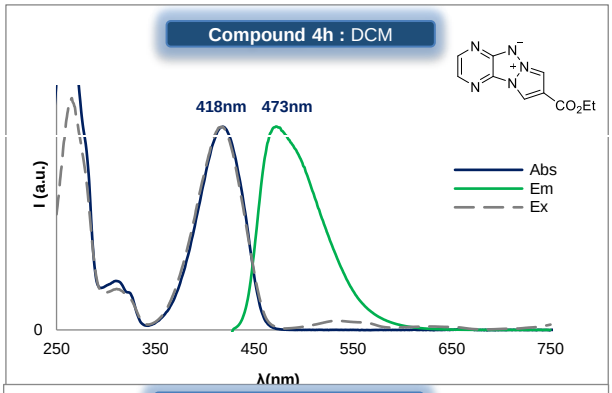

Compound 4e : DCM
416nm
473nm
CO2Et
Abs
Em
Ex
I (a.u.)
0
250
350
450
550
650
750
λ(nm)

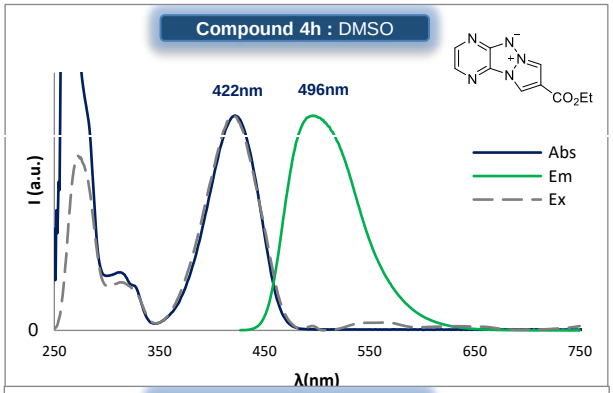

Compound 4e : DMSO
421nm
493nm
CO2Et
Abs
Em
Ex
I (a.u.)
0
250
350
450
550
650
750
λ(nm)

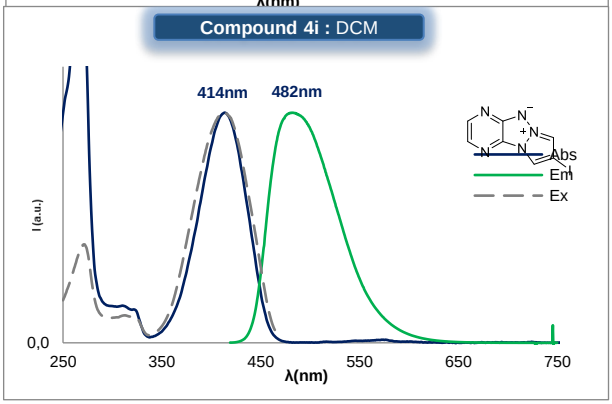

Compound 4f : DCM
409nm
467nm
CF3
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)

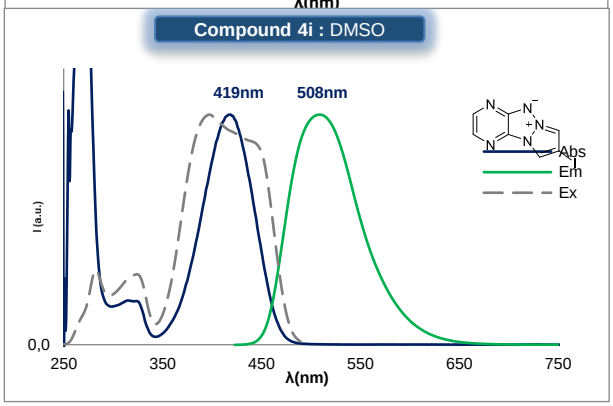

Compound 4g : DCM
408nm
464nm
Abs
Em
Ex
I (a.u.)
0
250
350
450
550
650
750
λ(nm)

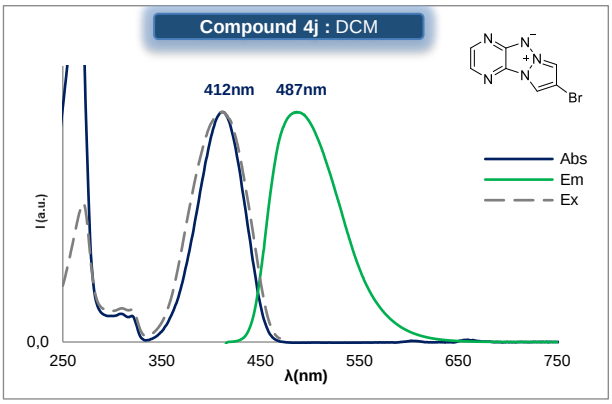

Compound 4g : DMSO
407nm
489nm
Abs
Em
Ex
I (a.u.)
0
250
350
450
550
650
750
λ(nm)

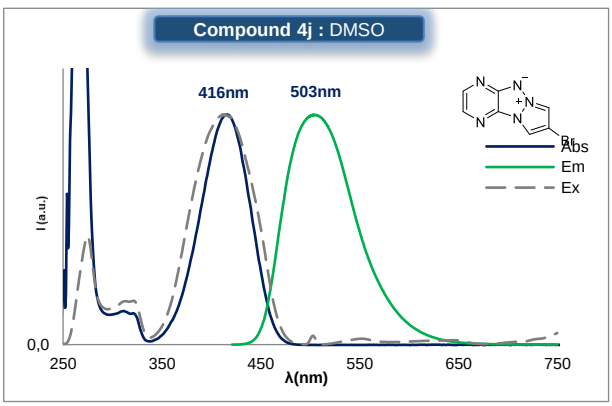

Compound 4h : DCM
418nm
473nm
Abs
Em
Ex
I (a.u.)
0
250
350
450
550
650
750

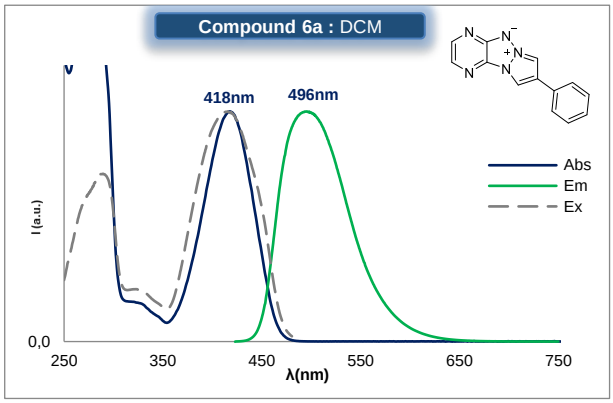

Compound 4h : DMSO
422nm
496nm
Abs
Em
Ex
I (a.u.)
0
250
350
450
550
650
750
λ(nm)

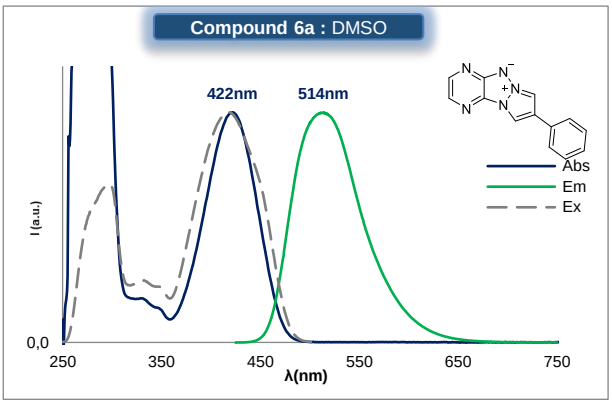

Compound 4i : DCM
414nm
482nm
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)

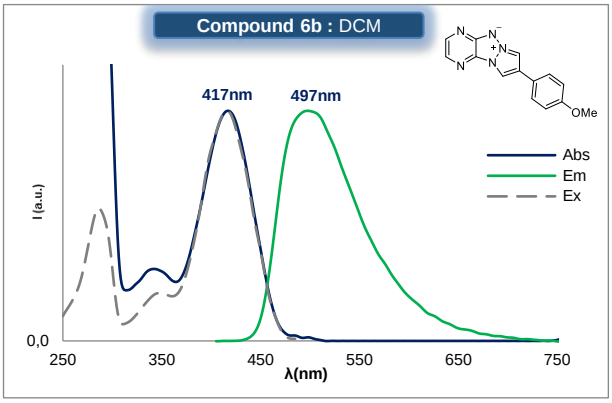

Compound 4i : DMSO
419nm
508nm
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)

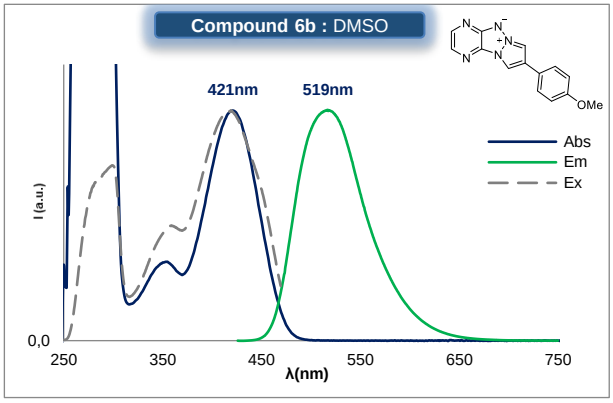

Compound 4j : DCM
412nm
487nm
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)

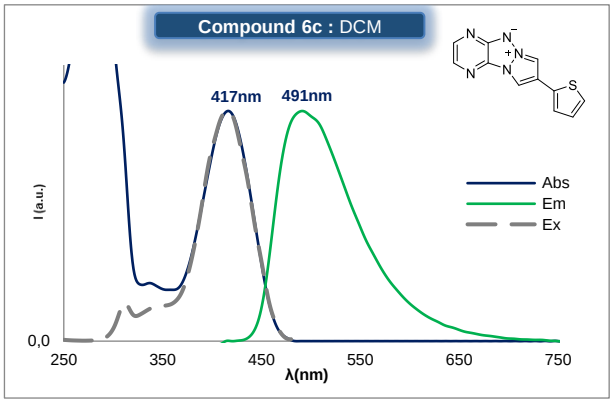

Compound 4j : DMSO
416nm
503nm
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)

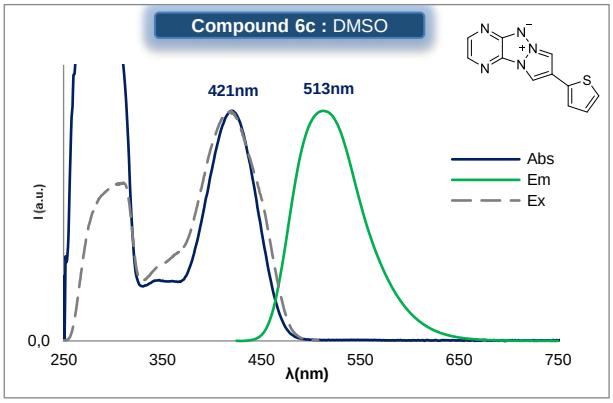

Compound 6a : DCM
418nm
496nm
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)

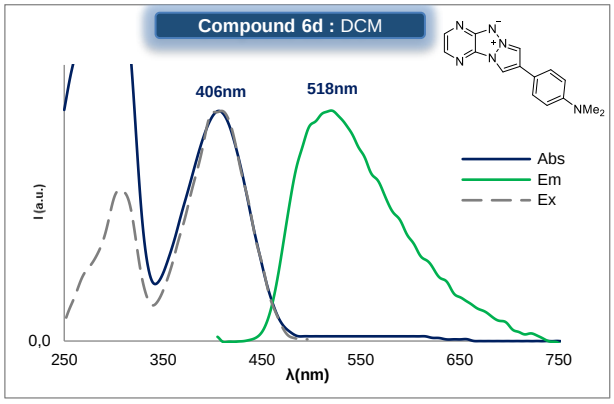

Compound 6a : DMSO
422nm
514nm
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)


Compound 6b : DCM
417nm
497nm
OMe
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)


Compound 6b : DMSO
421nm
519nm
OMe
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)


Compound 6c : DCM
417nm
491nm
S
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)


Compound 6c : DMSO
421nm
513nm
S
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)


Compound 6d : DCM
406nm
518nm
NMe2
Abs
Em
Ex
I (a.u.)
0,0
250
350
450
550
650
750
λ(nm)

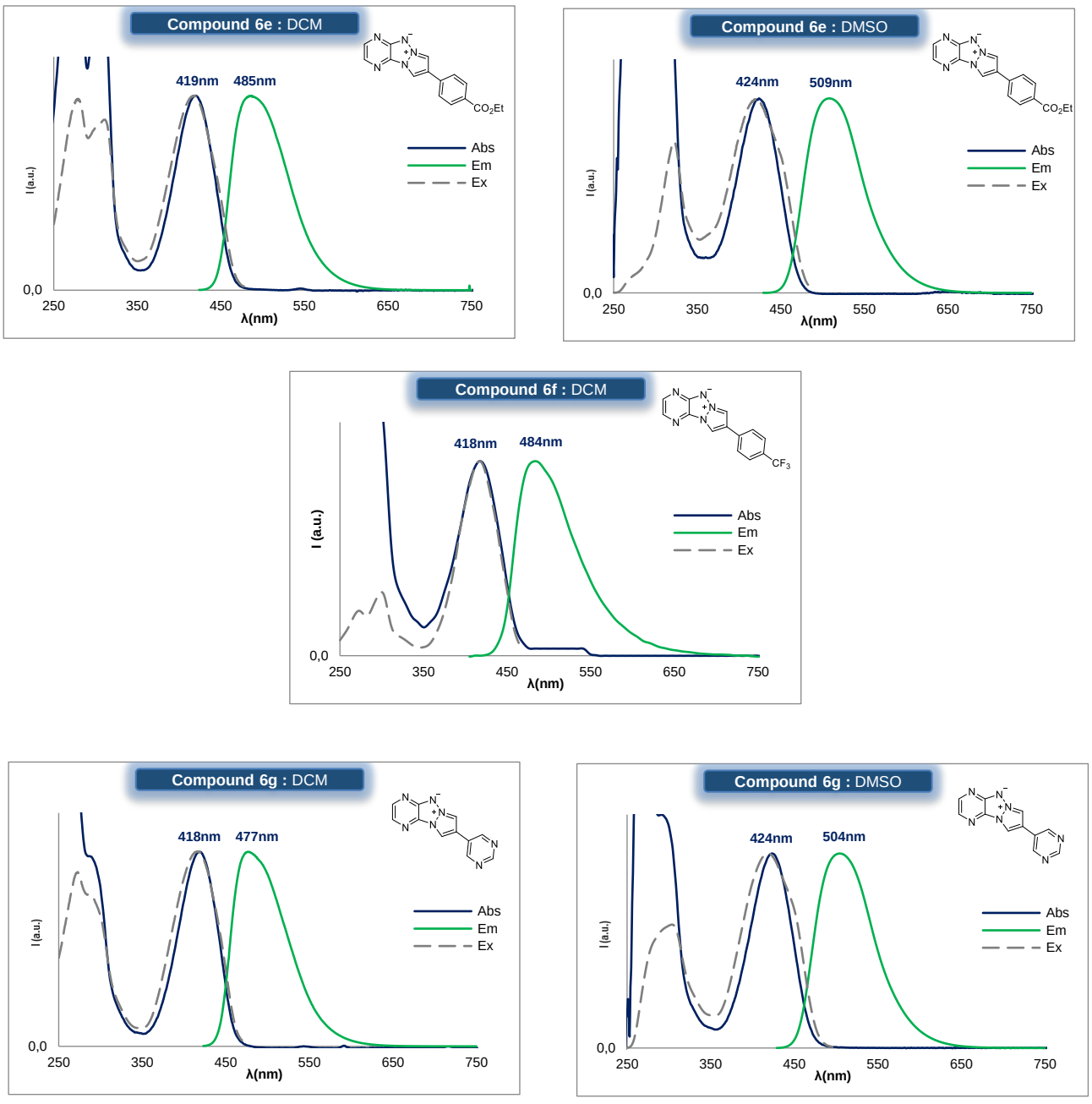


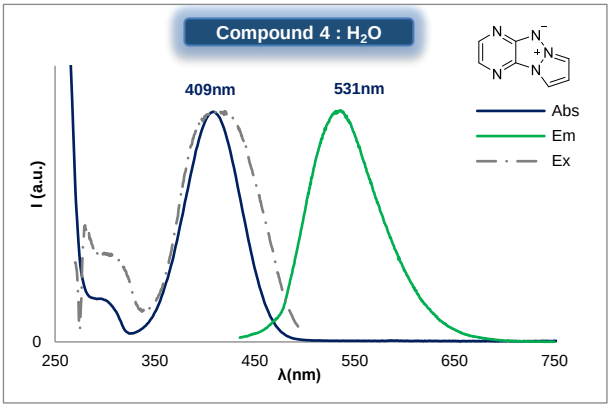
Figure S6. Photophysical profile

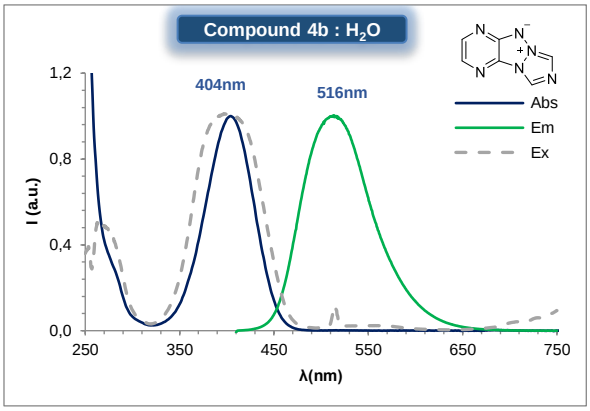
4.4 Water solubility of tricyclic triazapntalenes and optical properties in aqueous media.

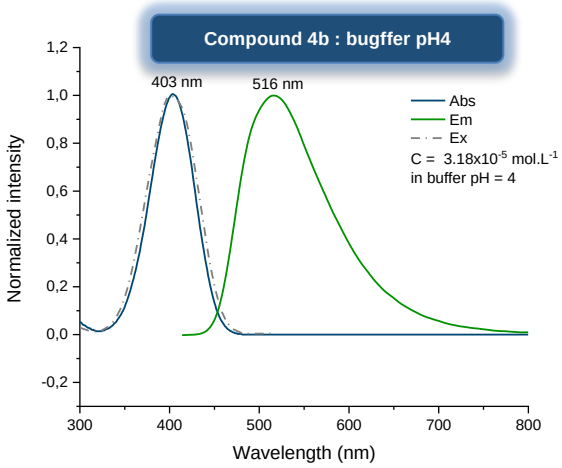
Table S7. Experimental optical properties of fluorescent pyrazinyl-triazapentalenes scaffolds **4** and **4b**

Compound	Solvent	λ_{abs} (nm)	ε ($m^{-1}.cm^{-1}$)	λ_{em} (nm)	Stokes Shift (nm)	QY[a]	Brightness
4	H_2O [b]	409	12200	531	122	0.02	244
4b	H_2O [c]	404	6500	516	112	0.02	130
4b	H_2O (pH4) [d]	403		516	113		

[a] Determined by using Coumarin 153 (QY=0.38 in Ethanol) as standard (Excited at λ_{abs} of each product). [b] Using ultrapure water, degassed with argon. [c] Using PBS aqueous solution, degassed with argon [d] Using citrate/phosphate buffer solution at pH 4.

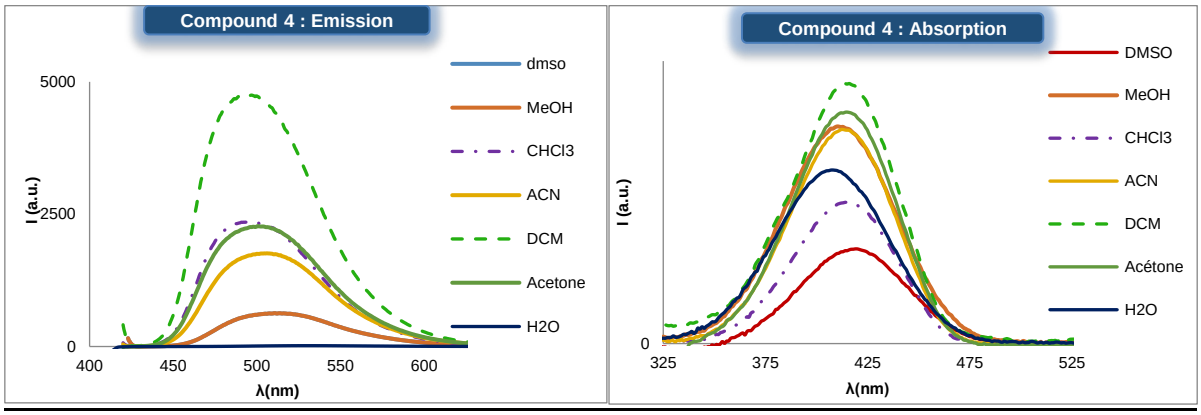






4.5 Solvatochromisme studies with compounds 4 and 4b.

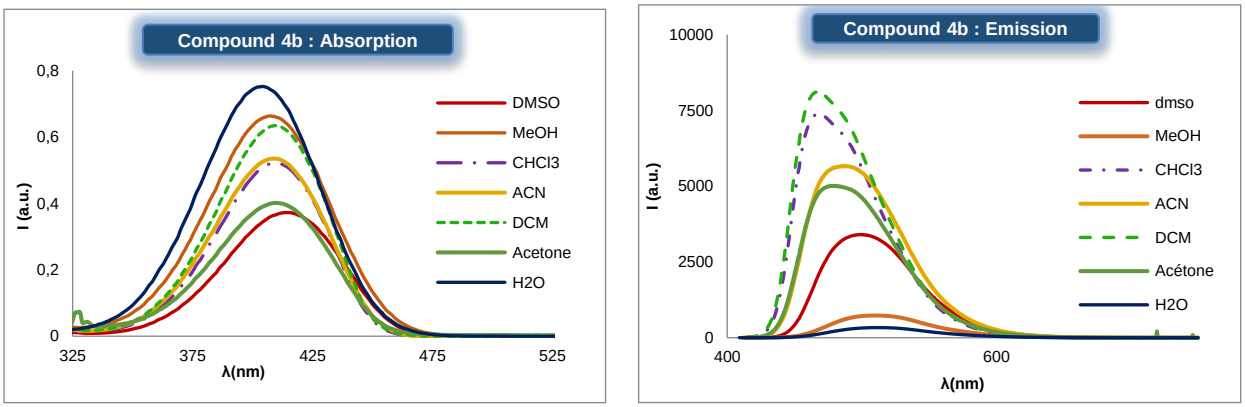
Compound 4:



Solvent	*H_2O*	*MeOH*	*DMSO*	*CH_3CN*	*Acetone*	*CH_2Cl_2*	*$CHCl_3$*
λ_{abs}(nm)	409	410	418	413	415	416	415
λ_{em}(nm)	531	511	516	506	500	507	500
Δ Stokes	**122**	**101**	**98**	**93**	**85**	**91**	**85**
QY	**0.2**		**0.15**			**0.42**	

Solvent polarity

Compound 4b:



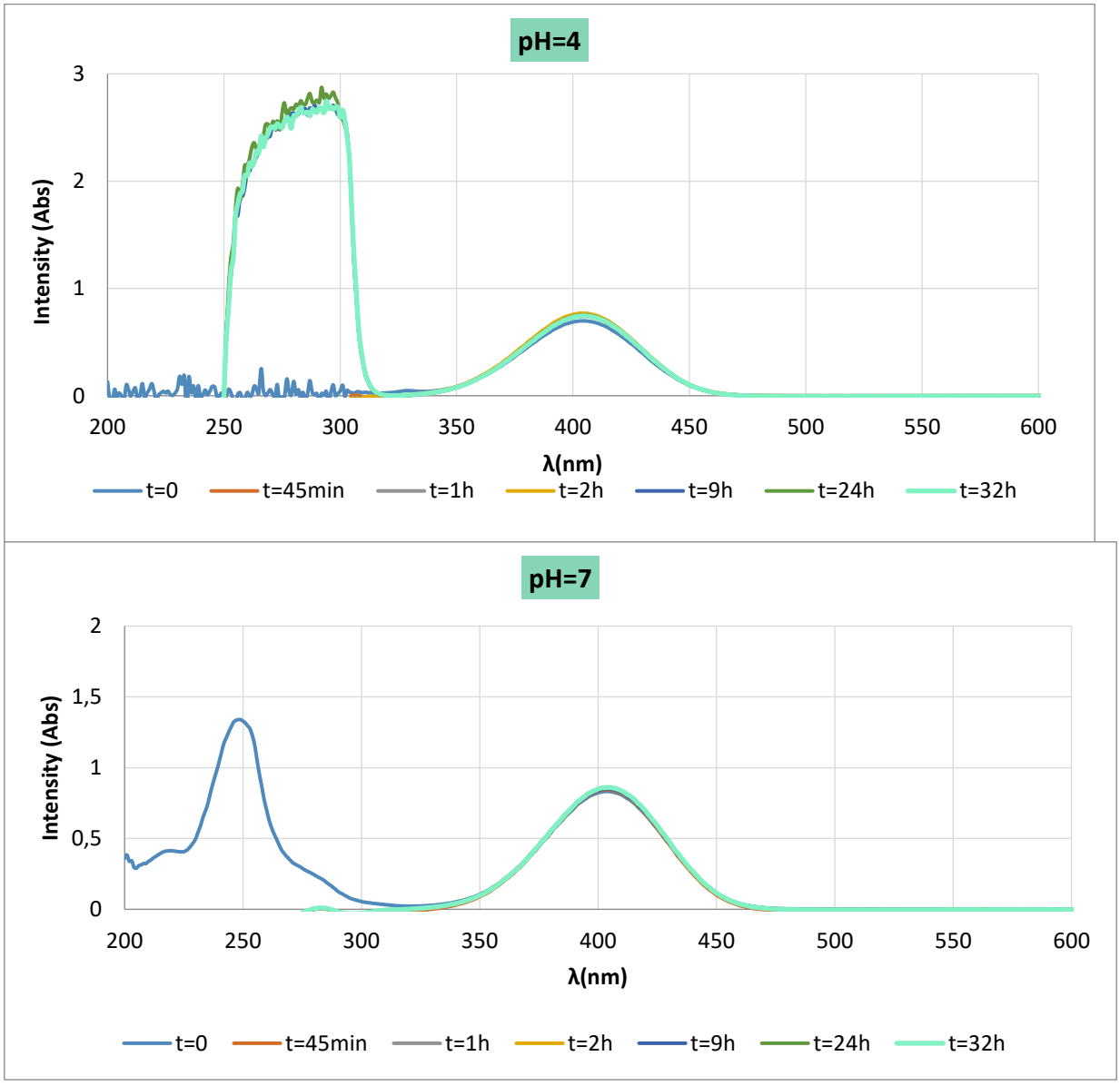
Solvent	*H_2O*	*MeOH*	*DMSO*	*CH_3CN*	*Acetone*	*CH_2Cl_2*	*$CHCl_3$*
λ_{abs}(nm)	404	407	414	409	410	410	409
λ_{em}(nm)	516	510	501	488	480	470	468
Δ Stokes	**112**	**103**	**87**	**79**	**70**	**60**	**59**
QY	**0.2**		**0.45**			**0.37**	

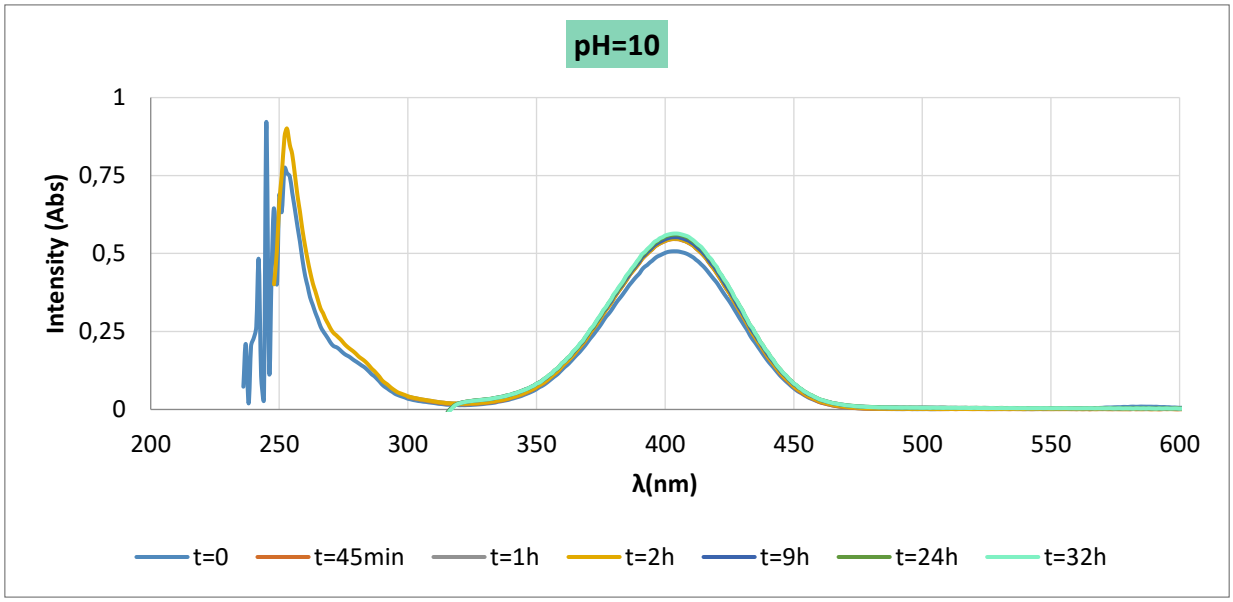
Solvent polarity

Figure S7. Solvatochromisme studies with compounds 4 and 4b

4.6 pH stability study for compound 4b.

Compound **4b** (6.10^{-5}mol.L^{-1} in buffer) stability was evaluated at pH= 4 (citrates buffer), pH = 7 (phosphates buffer) and pH = 10 (carbonate buffer) over a period of 32 hours. The perfect superposition of the UV spectra, recorded over a period of 32 hours, indicates that no structural change occurred for compound 4b at those pH.





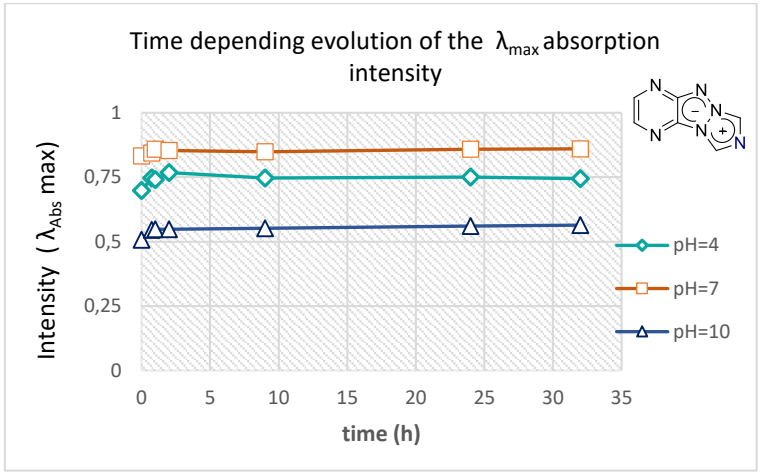


Figure S8. pH stability study for compound 4b

5. Cell Imaging

5.1 Epifluorescence Microscopy: Cellular Distribution

The HeLa (Human Cervical Carcinoma) cell line obtained from ATCC (Molsheim, France) was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% of heat-inactivated fetal bovine serum (FBS), 1% of 100× non-essential aminoacid solution, 1% of L-glutamine (GlutaMAX) and 1% of streptomycin/penycilin antibiotics. Cells were seeded in a 8-well Lab Tek Chamber coverglass (Nunc, Dutsher S.A., Brumath, France) at a density of $6 \cdot 10^4$ cells/well and cultured at 37°C in 5% humidified CO_2 atmosphere. After 24h the cell culture medium was removed, cells were washed twice with Opti-MEM medium (room temperature) and incubated with TTAZAP fluorescent probes during 1h and 30 min at 340 µM concentration. Prior to epifluorescence imaging, cells were washed twice with Opti-MEM (room temperature) in order to remove any non-specifically bound fluorescent probes. It should be noted that solutions of fluorescent probes were prepared in a DMSO and percentage of DMSO during epifluorescence microscopy was kept at 3%. Cells were observed with a Zeiss Axio Observer Z1 fluorescence inverted microscope (Zeiss, Le Pecq, France) equipped with an CCD camera (Orca-R2 Hamamatsu) and acquisition software Axiovision (Zeiss). The light source, Zeiss HXP 120 or Colibri, was combined

with the following filter cubes: (i) 417 nm band pass 60 nm filter for the excitation and 536 nm band pass 40 nm filter for emission in the visible range.

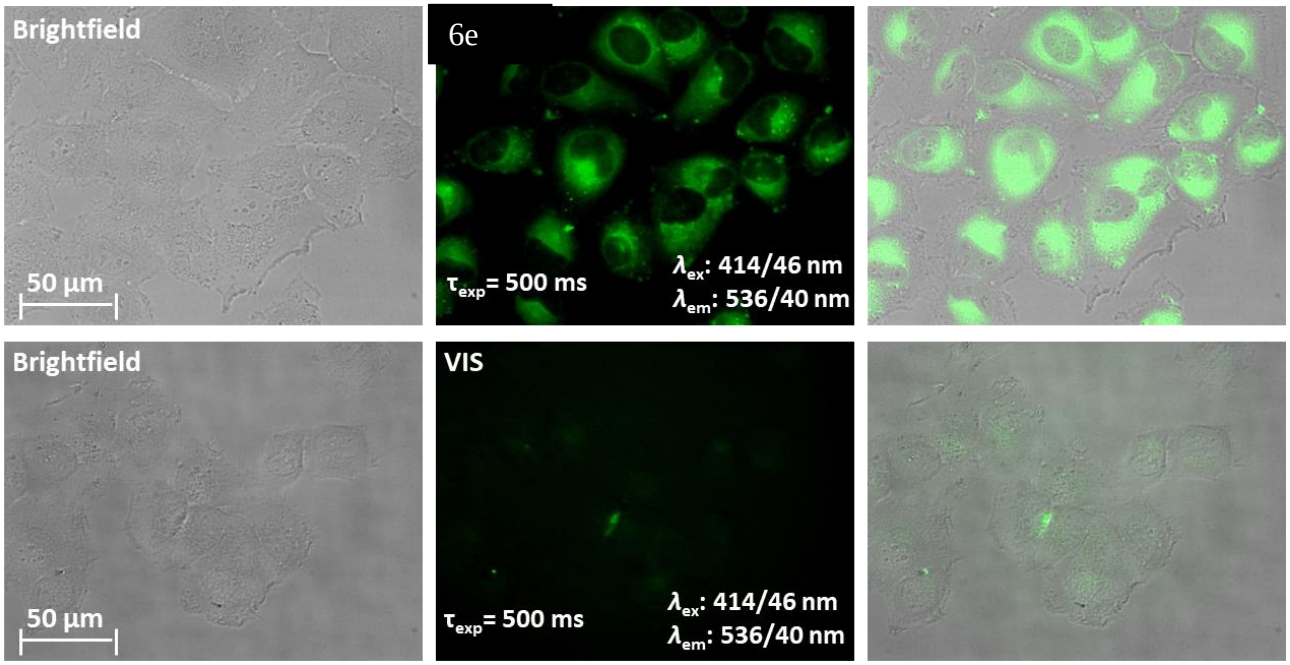
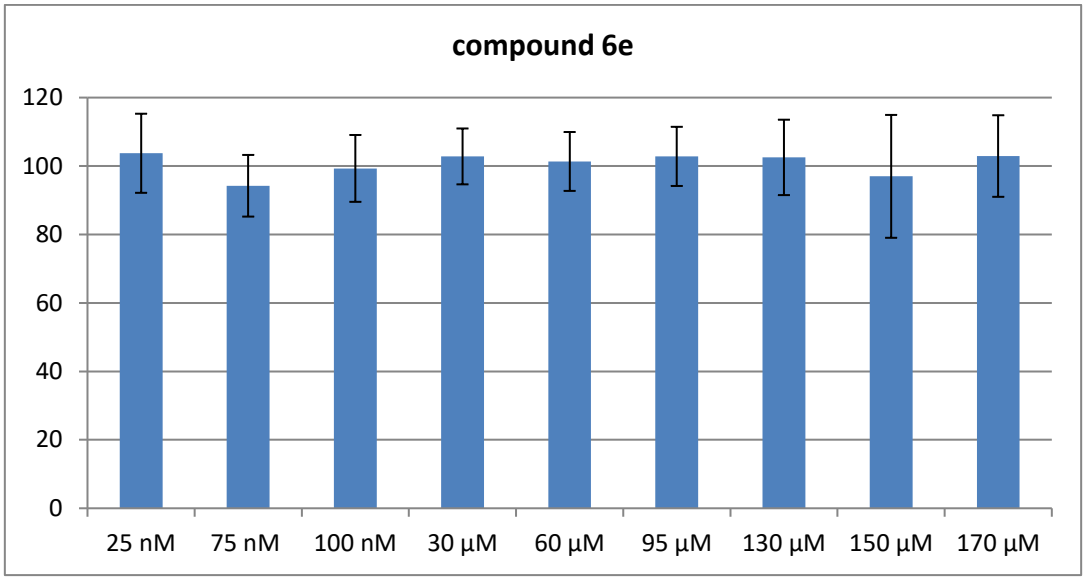


Figure S9. Epifluorescence Microscopy: Cellular Distribution

5.2 Cytotoxicity tests

Cytotoxicity tests were performed with the alamarBlue® assay (Invitrogen, France). Cells were seeded in 96-wale plates at the density of $1 \cdot 10^4$ cells per well and cultured at 37°C in a 5% humidified CO_2 atmosphere. After 24h of attachment, cells were incubated with different concentrations of TTAZAP fluorescent probes (i.e. 25 nM, 75 nM, 100 nM, 30 µM, 60 µM, 95 µM, 130 µM, 130 µM, 150 µM and 170 µM) during 24h followed by the incubation with the alamarBlue® (10% v/v) during 3-4 h at 37°C in a 5% humidified CO_2 atmosphere. Solutions of fluorescent probes were prepared in a DMSO and percentage of DMSO during cytotoxicity tests was kept at 1%. The fluorescence of alamarBlue® was measured with a plate reader (Victor 3V, Perkin Elmer, France) under an excitation at 530 nm and collecting the emission at 590 nm. Control cells were prepared under the same experimental conditions but without addition of fluorescent probes (with 3% of DMSO). For each fluorescent probe three individual experiments were performed in triplicates. An average value out of three experiments was calculated as a mean fluorescence value and corrected for the control cells. Data were presented as the mean ± RSD.



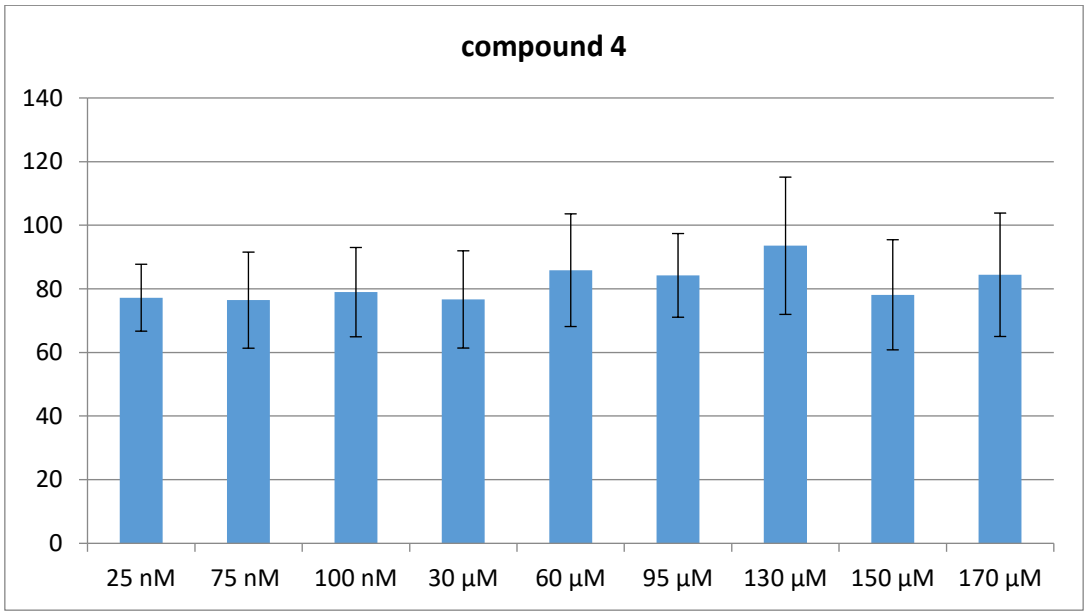


Figure S10. Cytotoxicity tests

5.3 Photostability

Photobleaching experiments were done on HeLa cells incubated during 1h and 30 min with a 30 µg/mL solution of the compound **6e** (top) or with 50 nM LysoTracker Green DND-26 (bottom) after exposure to the continuous excitation light at 414 nm, band pass 46 nm filter during different times: (A) 0 s, (B) 30 s, (C) 100 s, (D) 155 s, (E) 480 s. 63× objective.

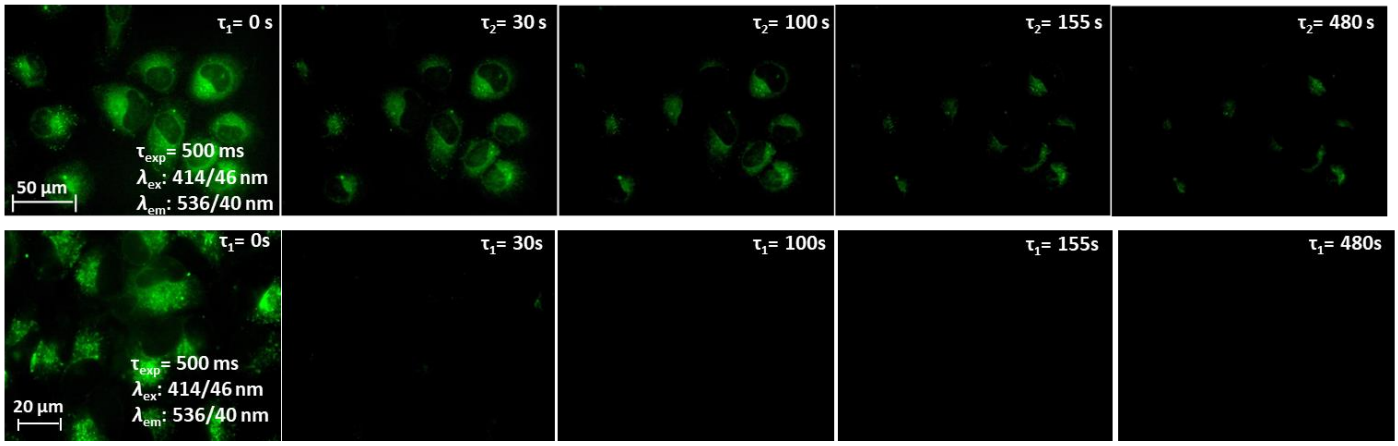
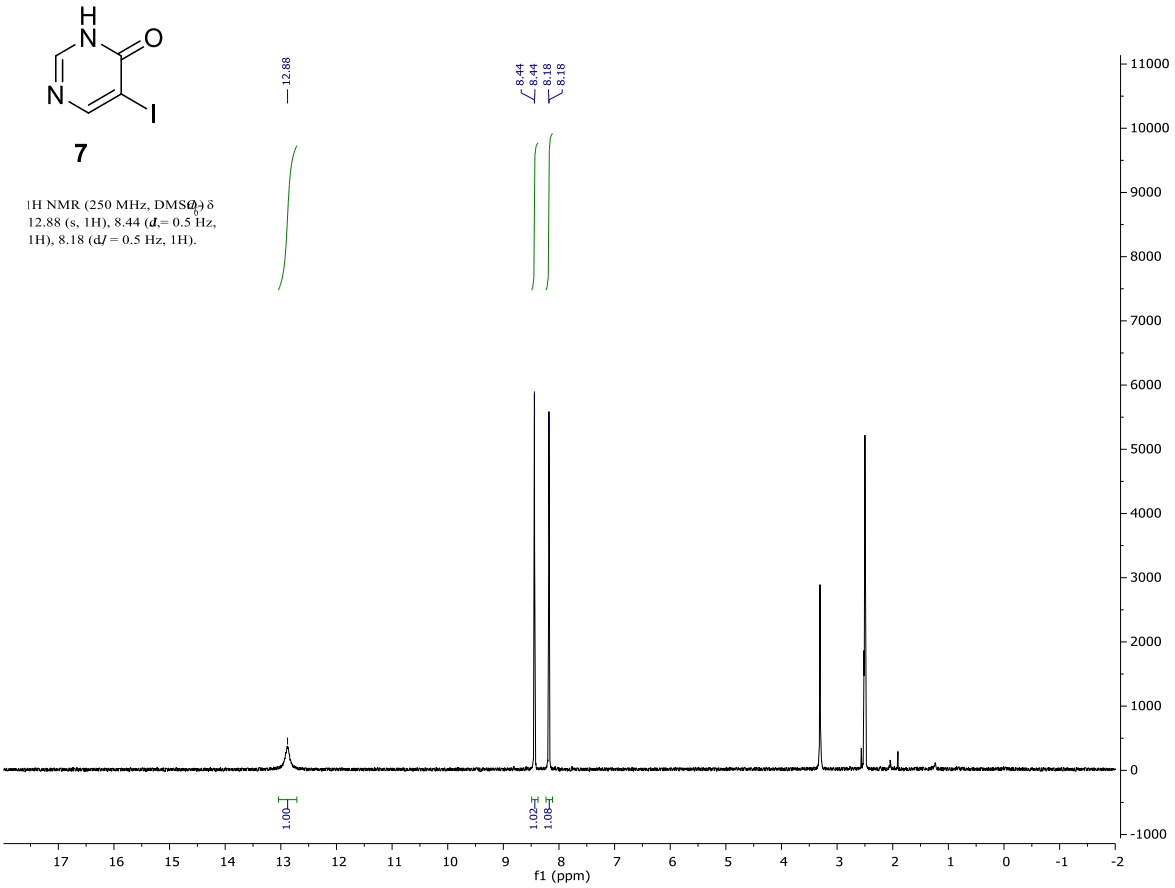


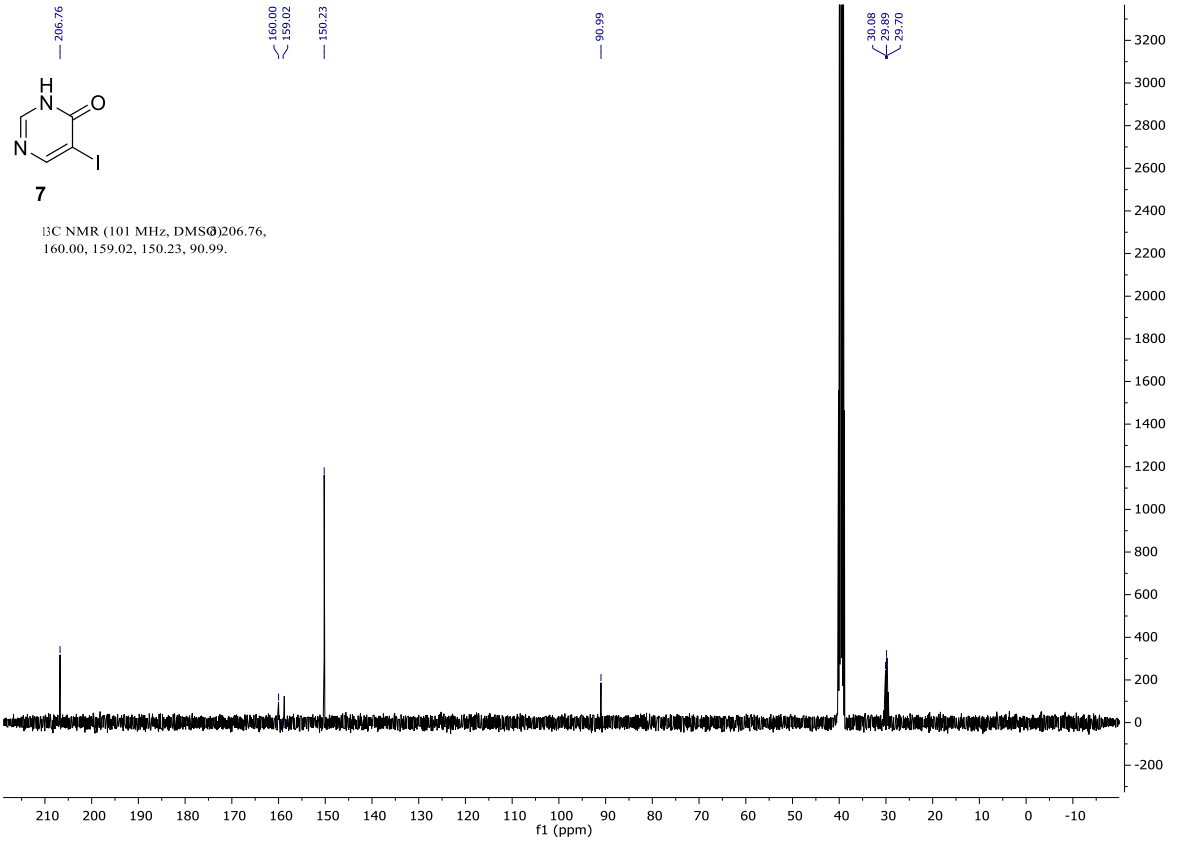
Figure S11. Photostability

6. NMR spectra

5-iodopyrimidin-4(3H)-one (7)

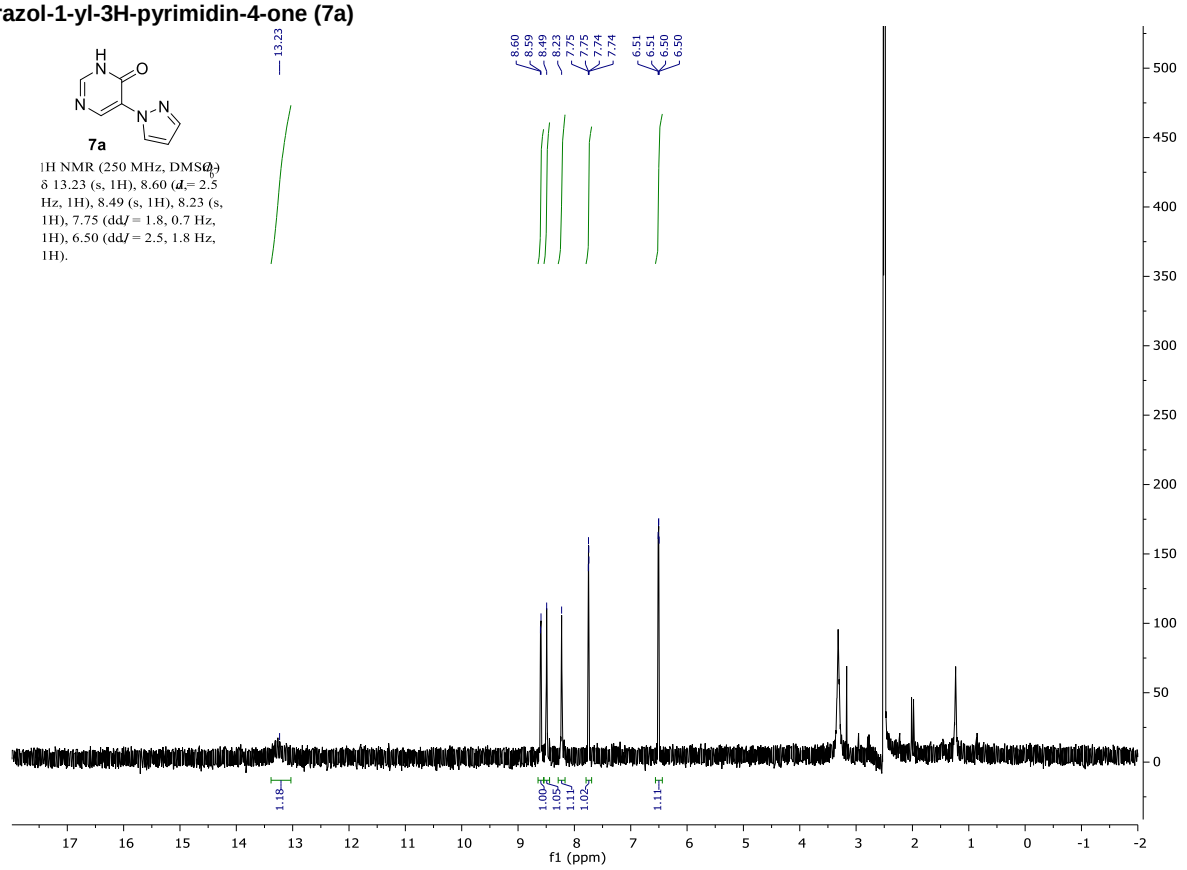


^{1}H NMR (250 MHz, DMSO-d_6) δ 12.88 (s, 1H), 8.44 (d, J = 0.5 Hz, 1H), 8.18 (d, J = 0.5 Hz, 1H).

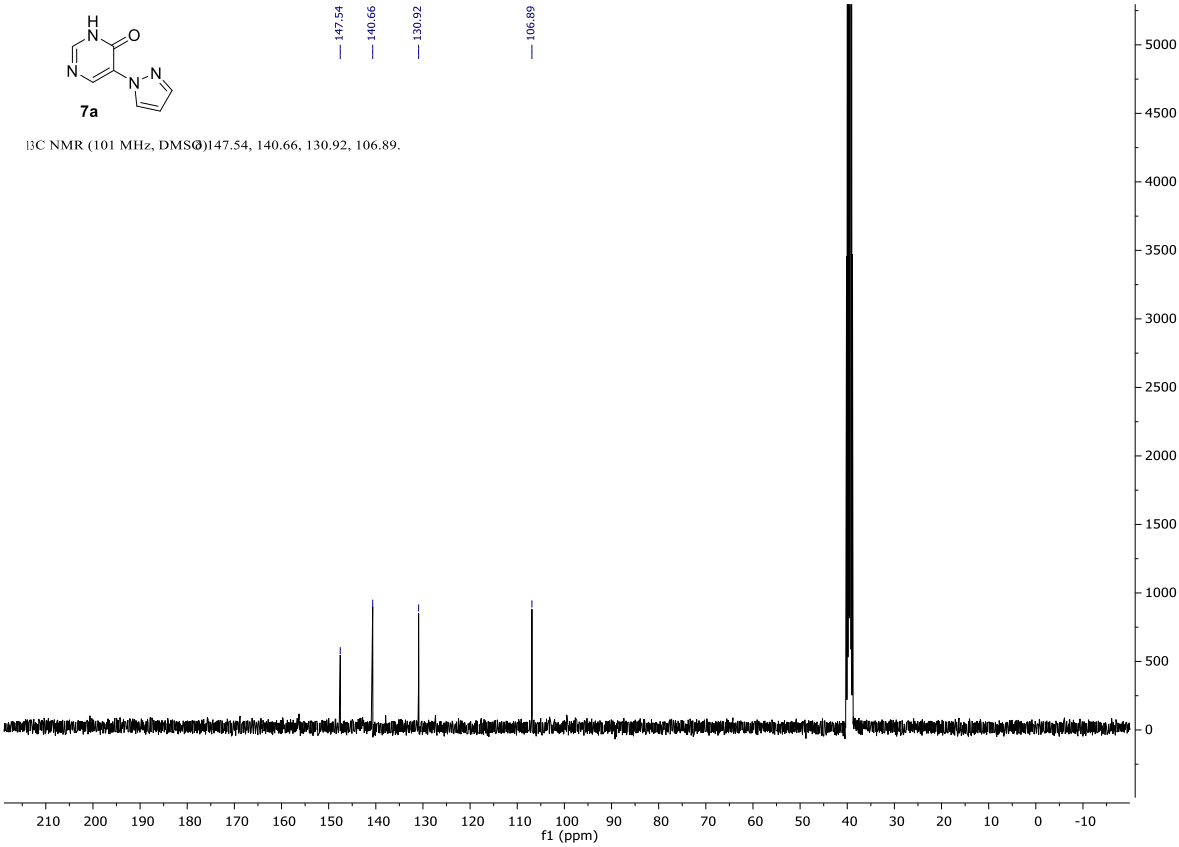


^{13}C NMR (101 MHz, DMSO-d_6) 206.76, 160.00, 159.02, 150.23, 90.99.

5-Pyrazol-1-yl-3H-pyrimidin-4-one (7a)

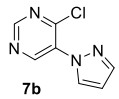


^{1}H NMR (250 MHz, DMSO-d_6) δ 13.23 (s, 1H), 8.60 (d, = 2.5 Hz, 1H), 8.49 (s, 1H), 8.23 (s, 1H), 7.75 (dd, J = 1.8, 0.7 Hz, 1H), 6.50 (dd, J = 2.5, 1.8 Hz, 1H).



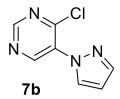
^{13}C NMR (101 MHz, DMSO-d_6) δ 147.54, 140.66, 130.92, 106.89.

4-Chloro-5-pyrazol-1-yl-pyrimidine (7b)



7b

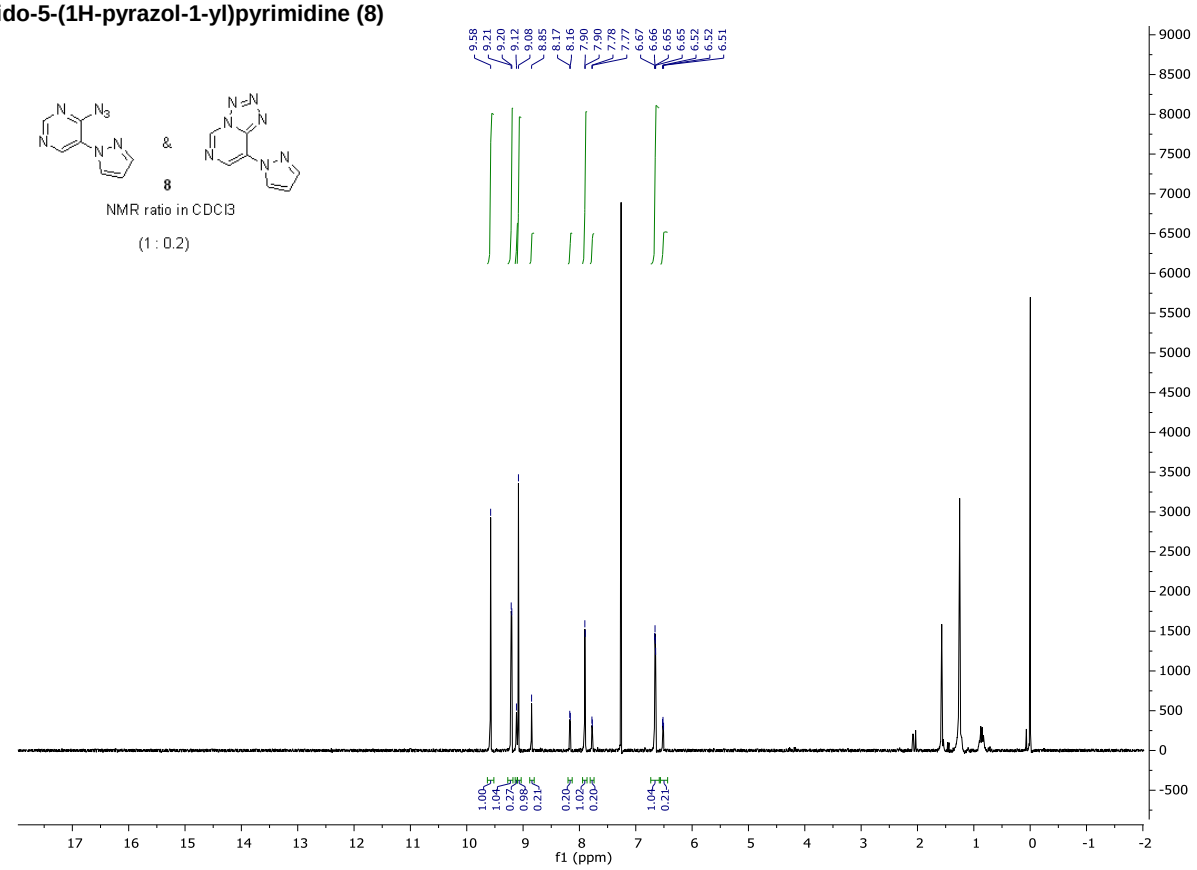
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.04 (d, *J* = 1.6 Hz, 1H), 8.98 (d, *J* = 1.6 Hz, 1H), 8.07 (d, *J* = 2.2 Hz, 1H), 7.84 (d, *J* = 2.0 Hz, 1H), 6.58 (dd, *J* = 2.2, 2.0 Hz, 1H).



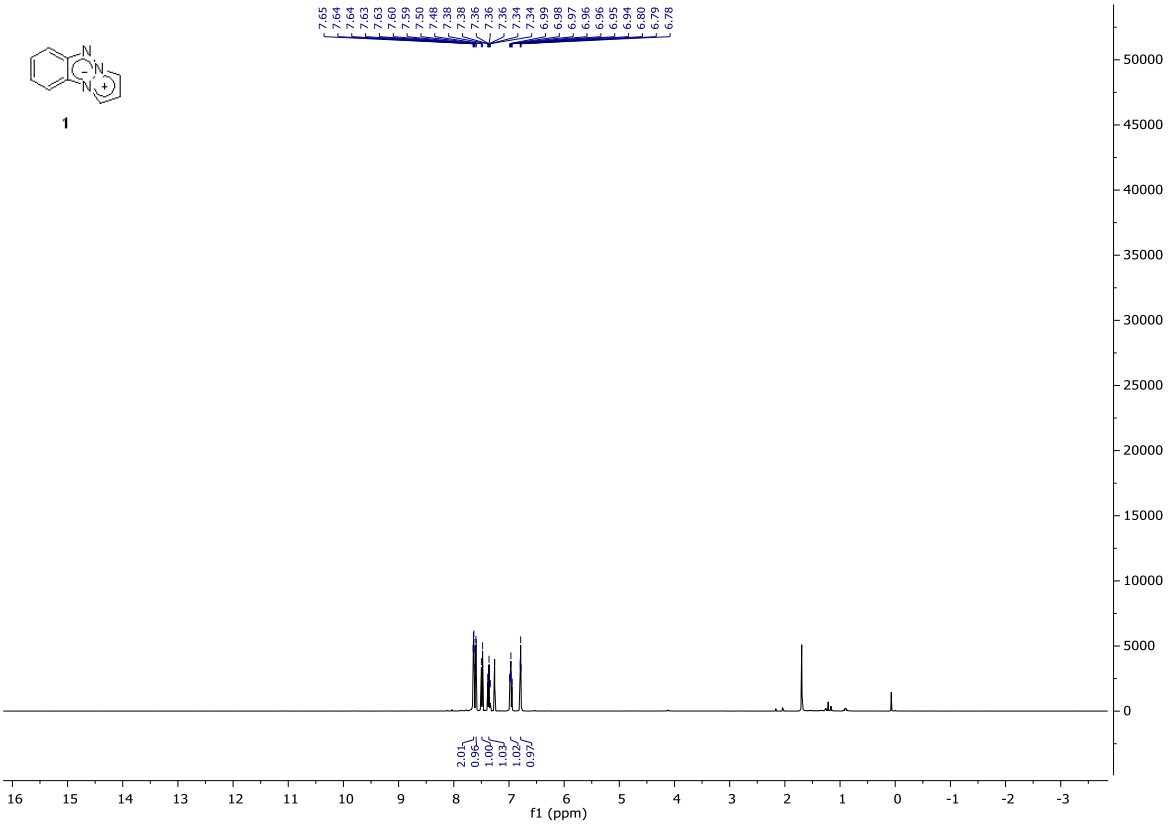
7b

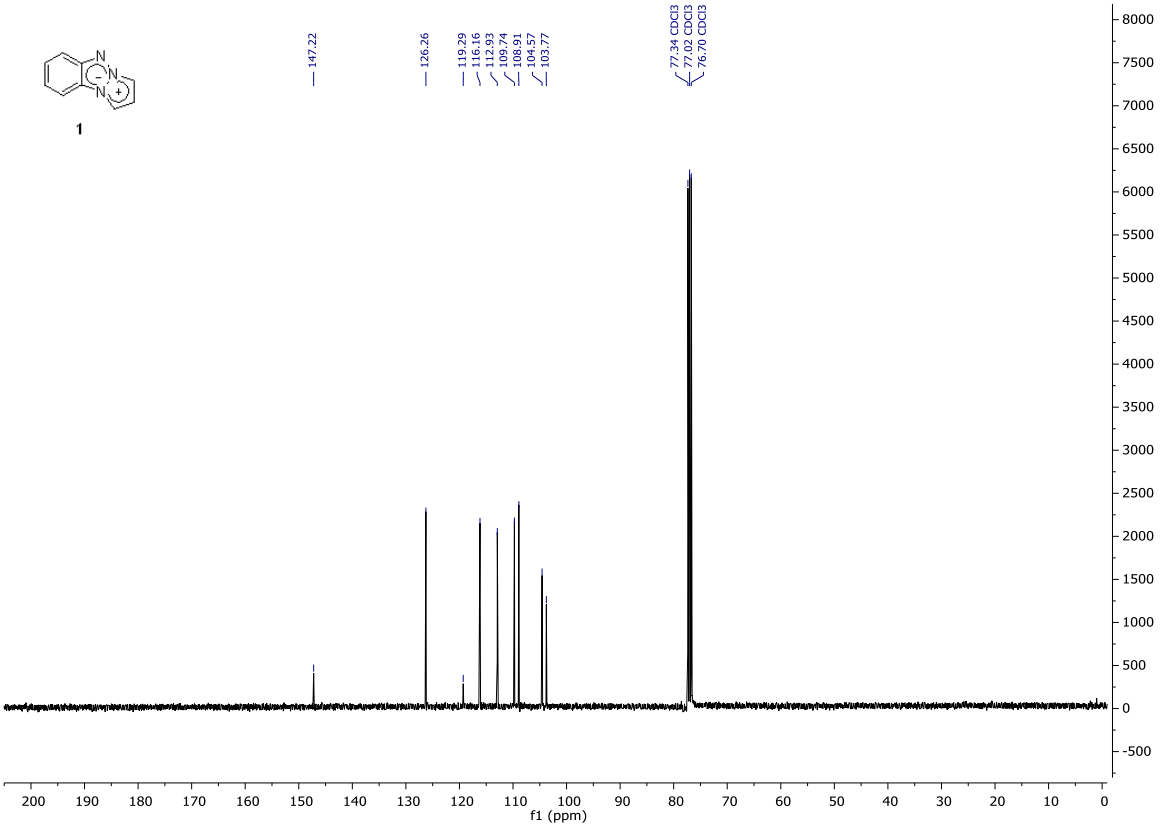
^{13}C NMR (101 MHz, CDCl$_3$) δ 156.57, 154.71, 153.13, 142.85, 131.25, 108.64.

4-azido-5-(1H-pyrazol-1-yl)pyrimidine (8)

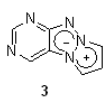


Benzo[d][1,2,3]triazolo[1,2-a][1,2,3]triazol-10-ium-9-ide (1)

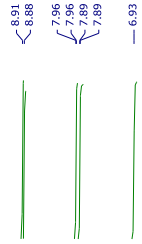




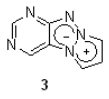
Pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-d]pyrimidin-9-ium-10-ide (3)



3



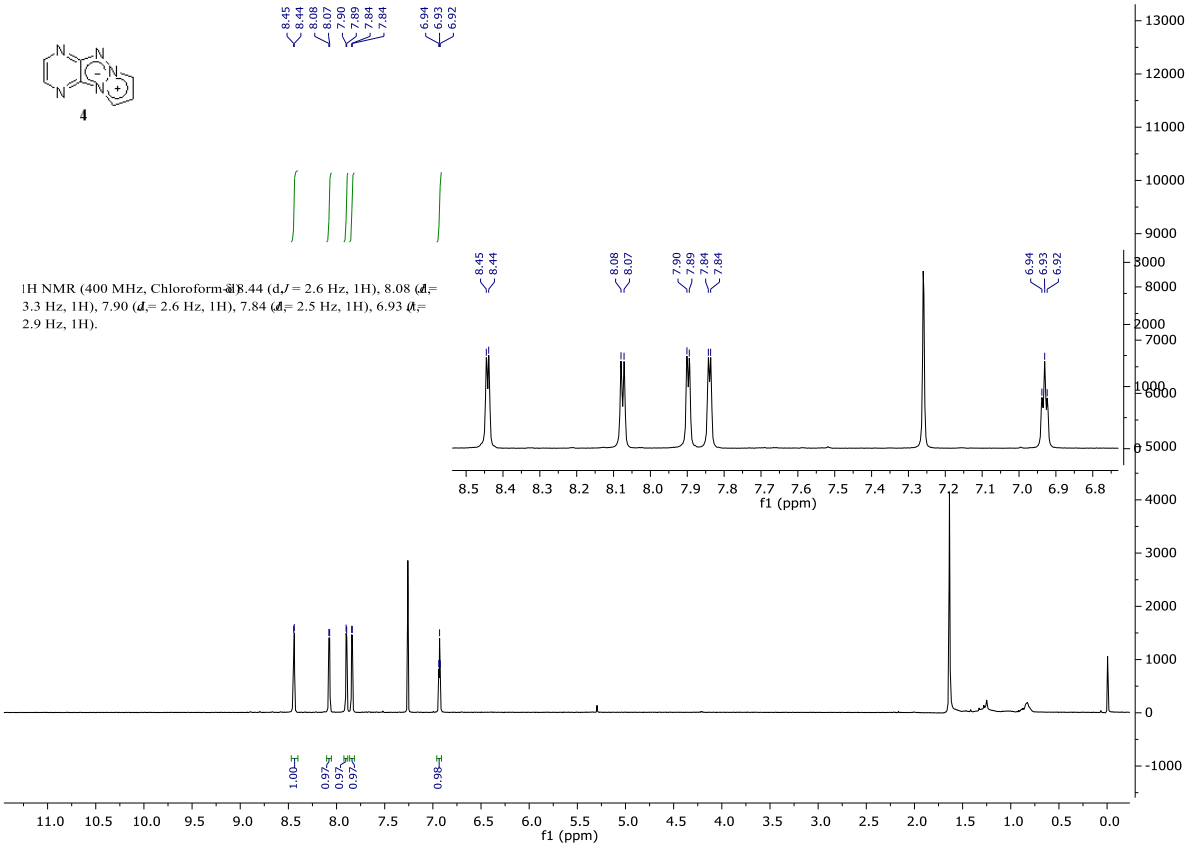
^{1}H NMR (400 MHz, Chloroform-d) δ 8.91 (s, 1H), 8.88 (s, 1H), 7.96 (d, J = 3.2 Hz, 1H), 7.89 (d, J = 2.4 Hz, 1H), 6.93 (s, 1H).



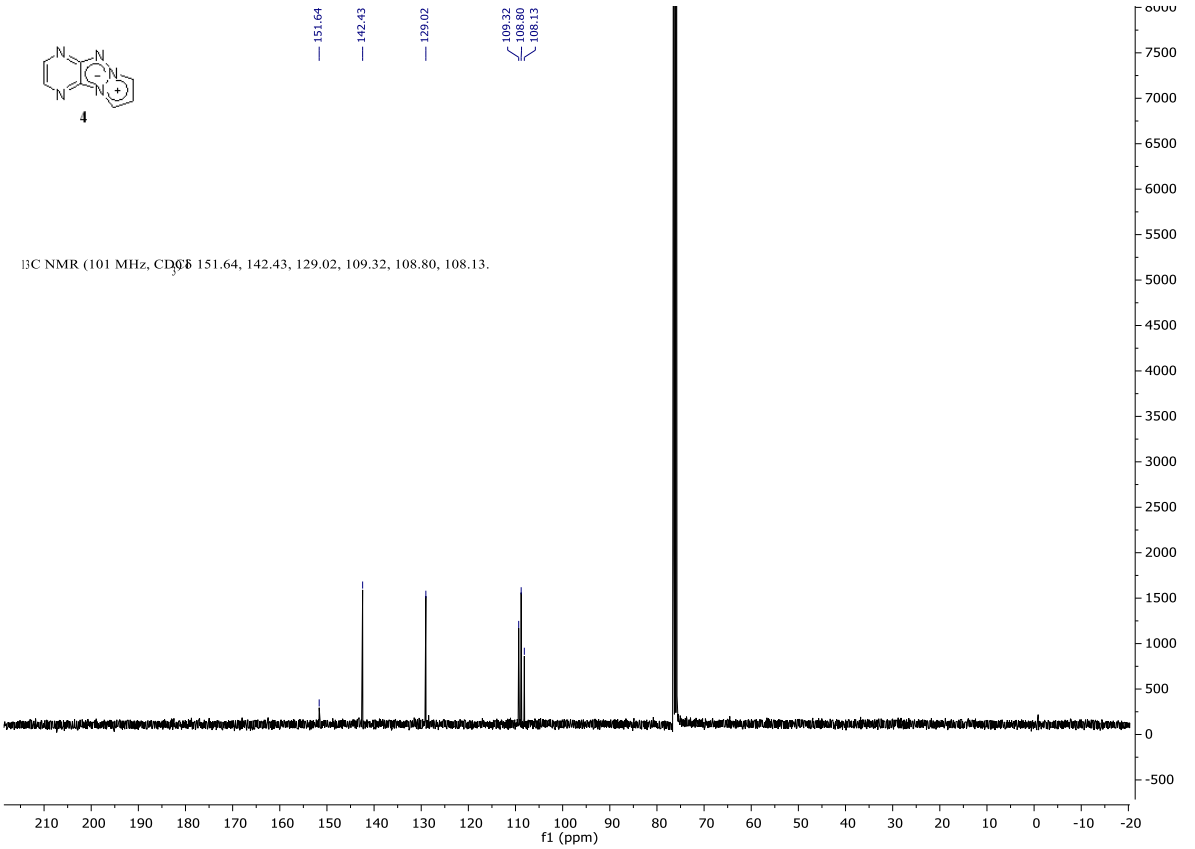
3

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.88, 156.43, 136.24, 113.86, 111.40, 109.73, 109.40.

Pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4)

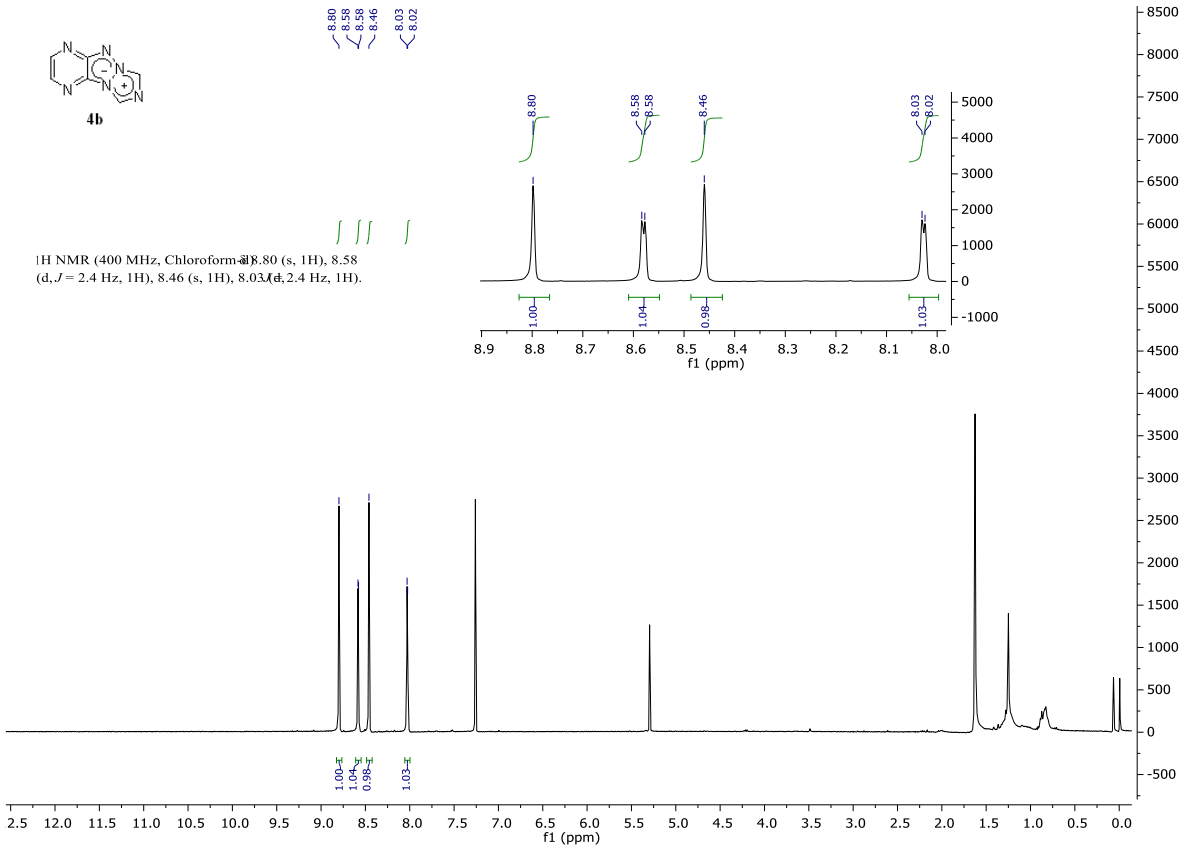


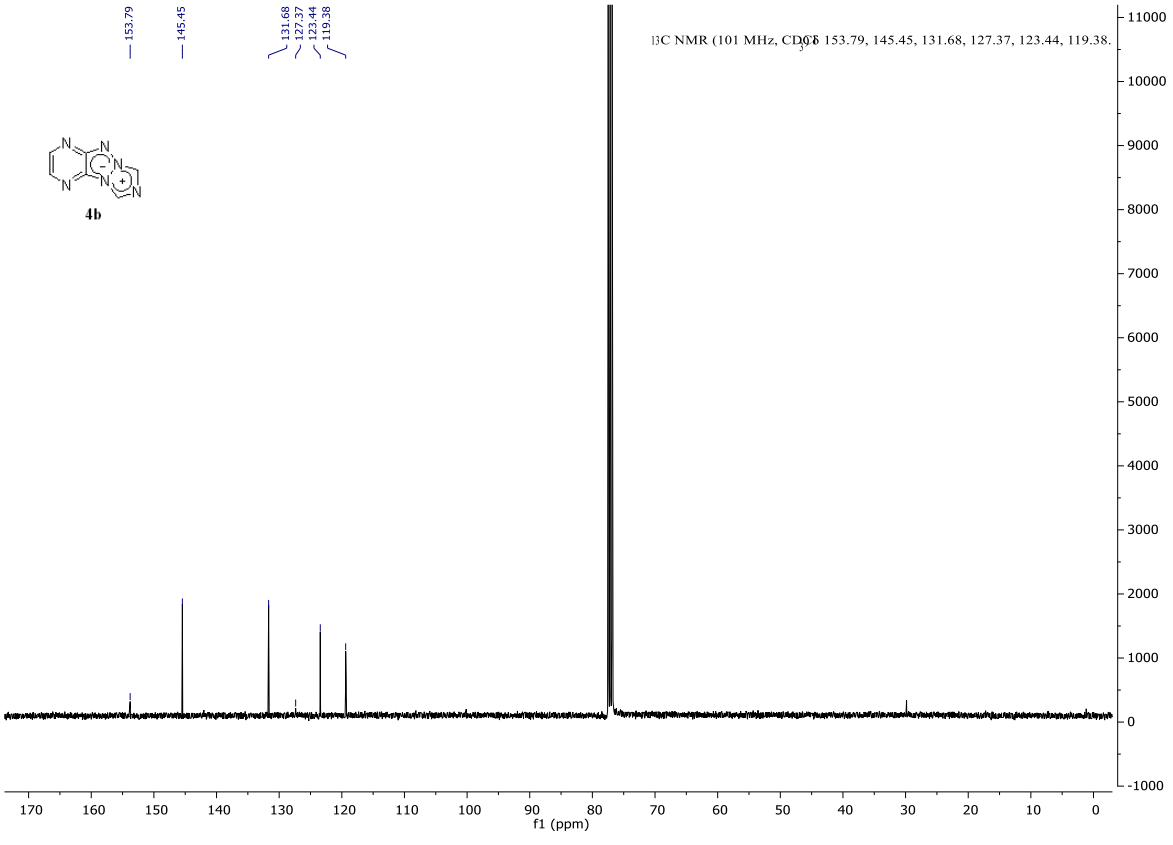
^{1}H NMR (400 MHz, Chloroform-d) δ 8.44 (d, J = 2.6 Hz, 1H), 8.08 (d, J = 3.3 Hz, 1H), 7.90 (d, J = 2.6 Hz, 1H), 7.84 (d, J = 2.5 Hz, 1H), 6.93 (d, J = 2.9 Hz, 1H).

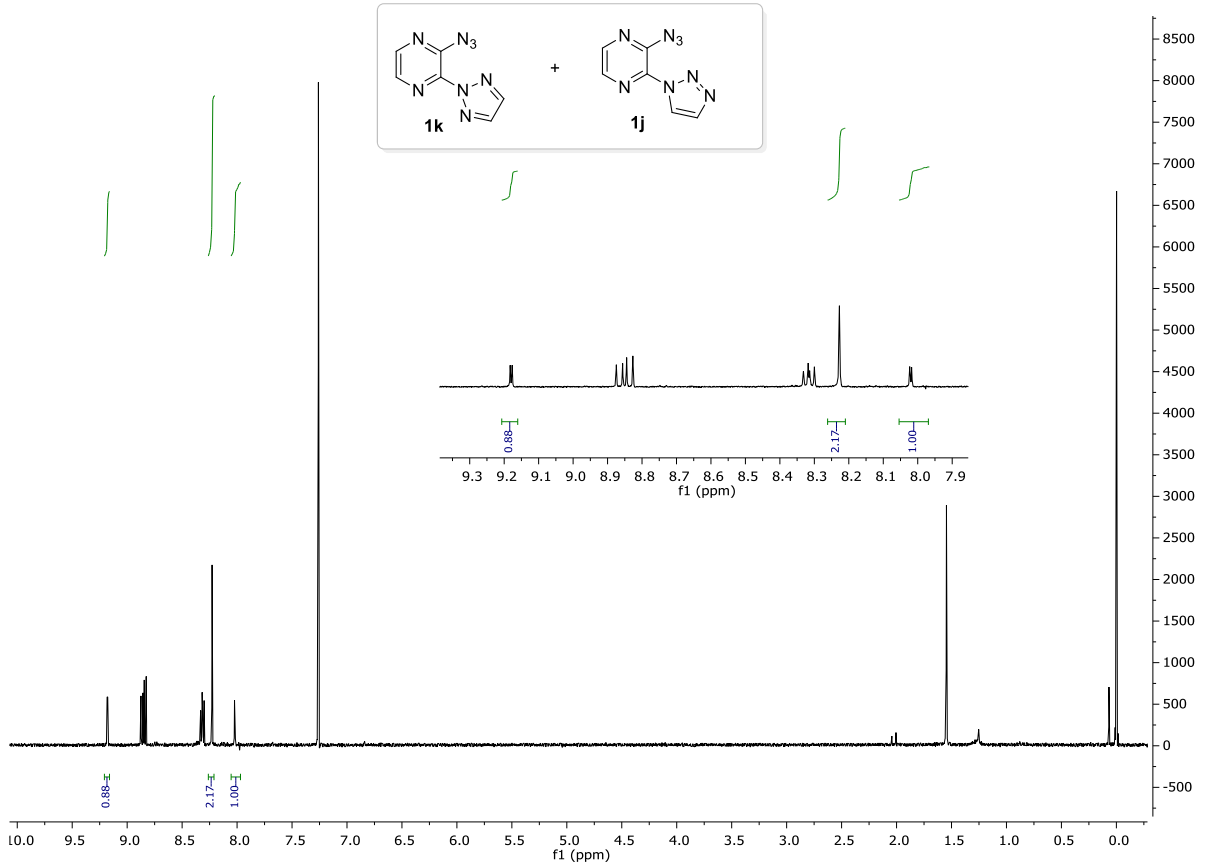


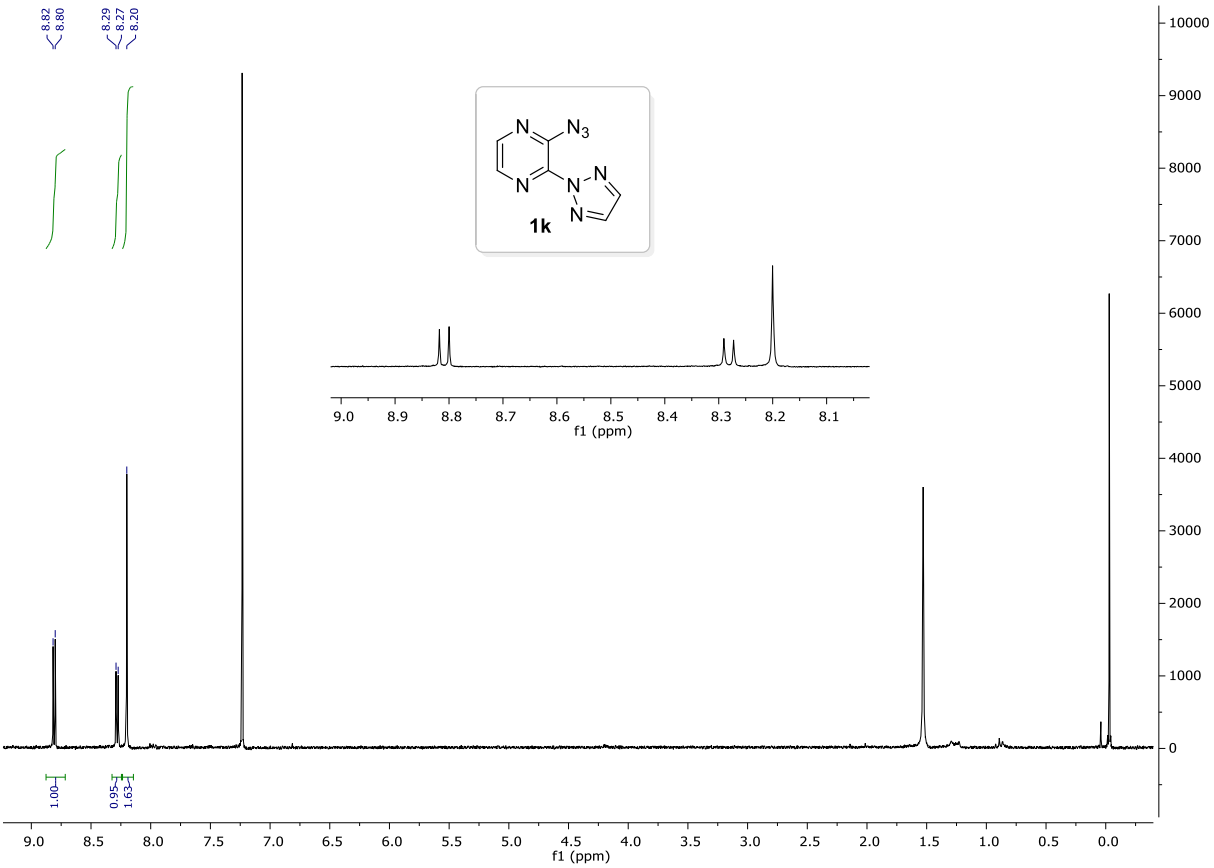
^{13}C NMR (101 MHz, CD_3CN) δ 151.64, 142.43, 129.02, 109.32, 108.80, 108.13.

[1,2,4]triazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4b)

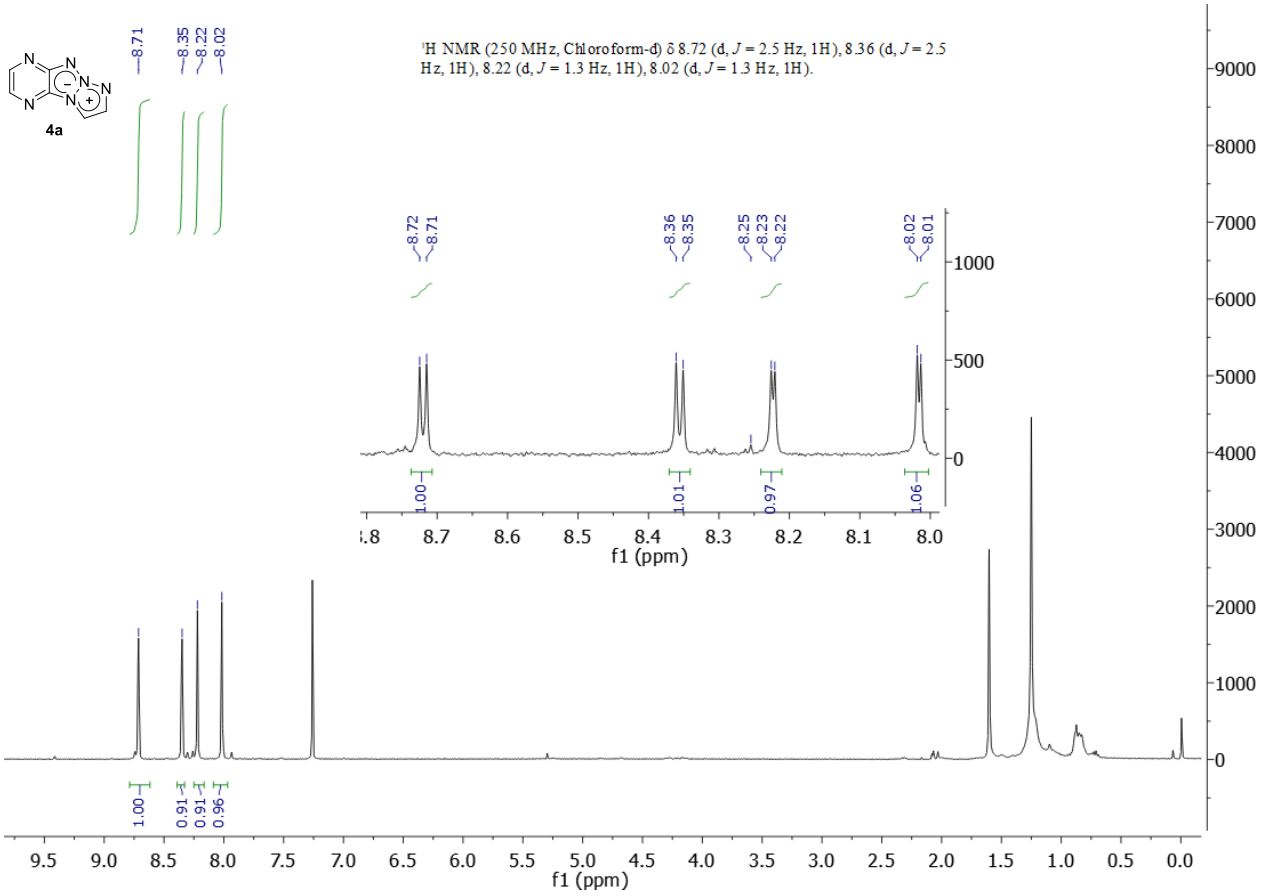


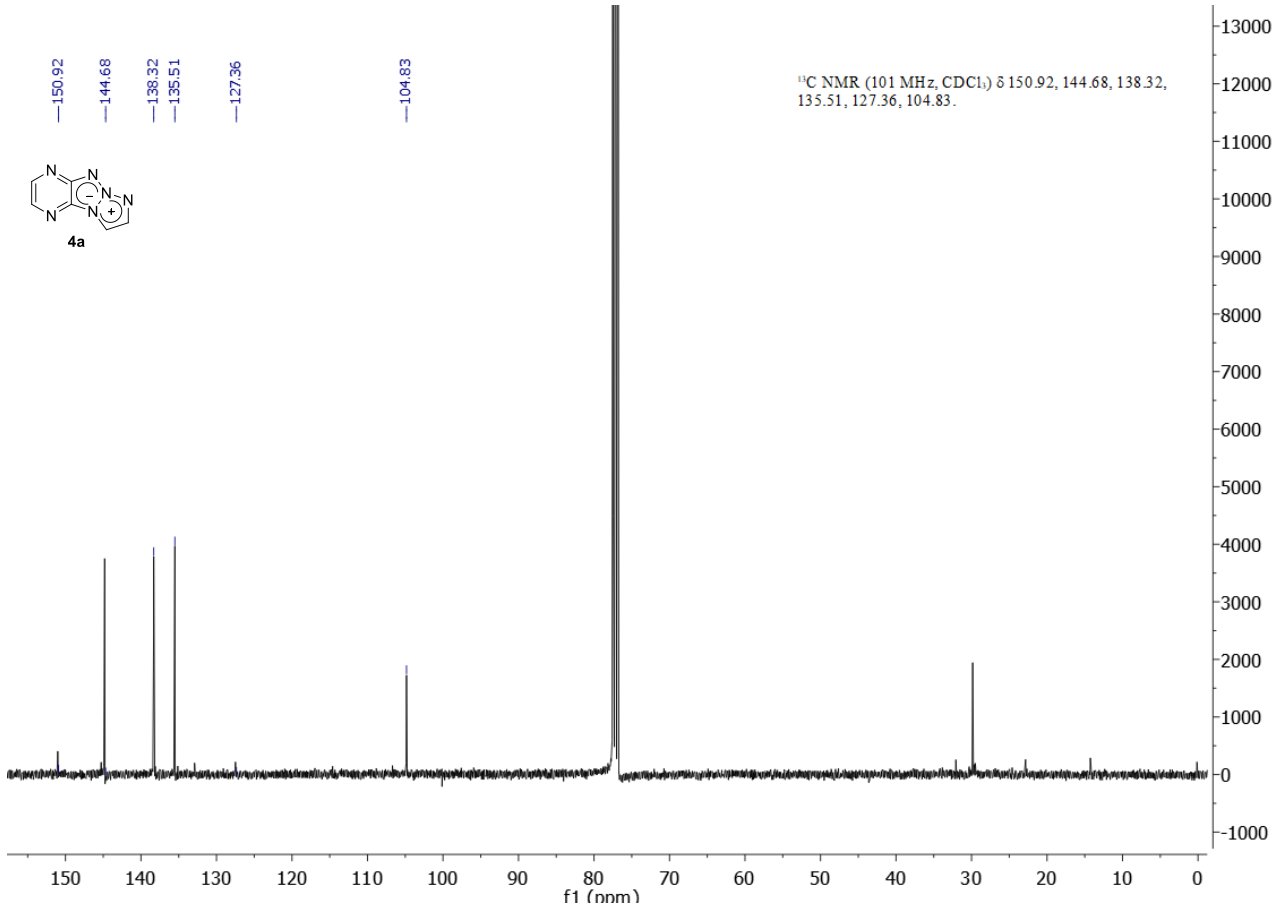



1k
+
1j
0.88
2.17
1.00
f1 (ppm)

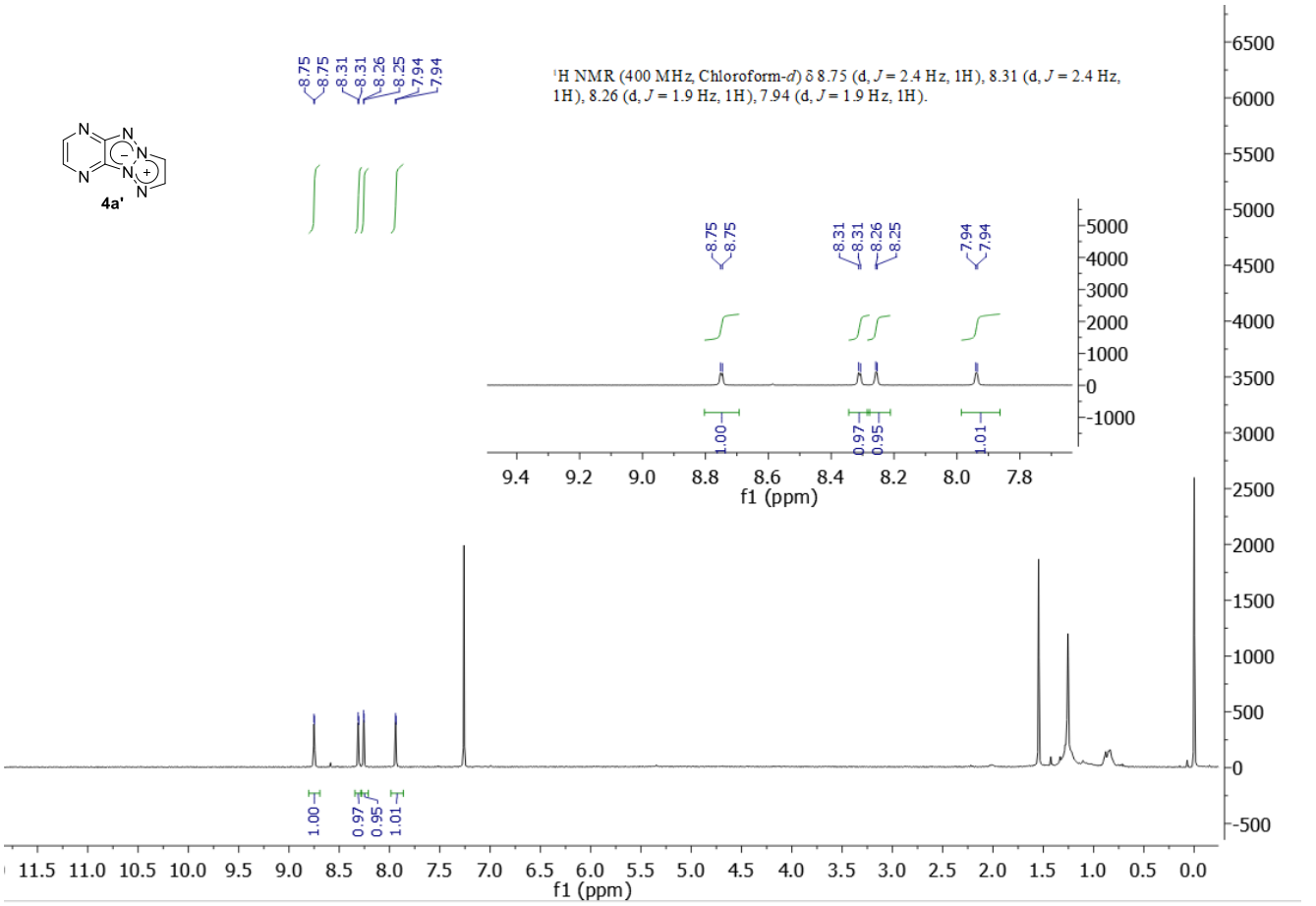

8.82
8.80
8.29
8.27
8.20
1k
1.00
0.95
1.63
f1 (ppm)

[1,2,3]-triazolo[2',1':1,2][1,2,3]triazolo[4,5-b]pyrazin-9-ium-10-ide (4a)

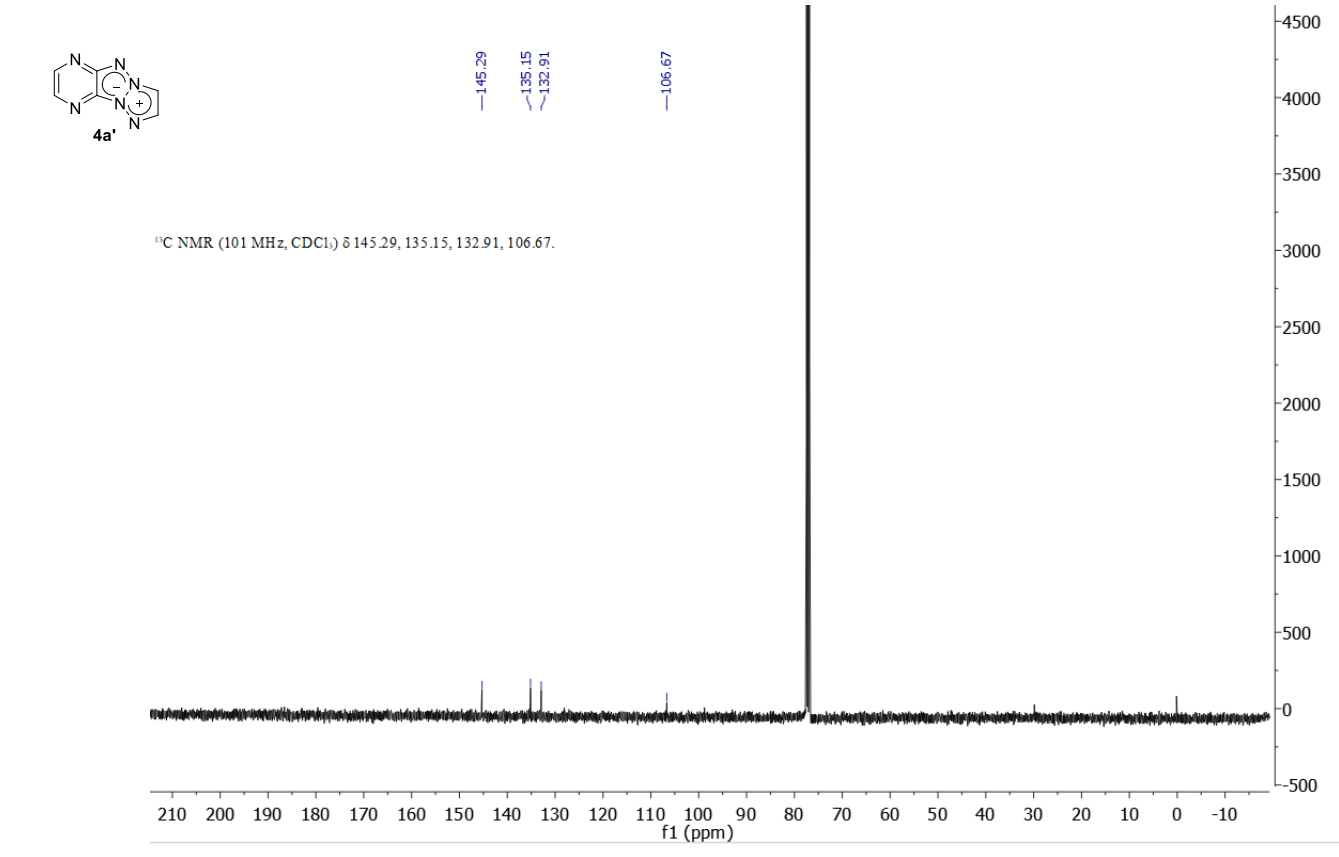




[1,2,3]triazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4a')

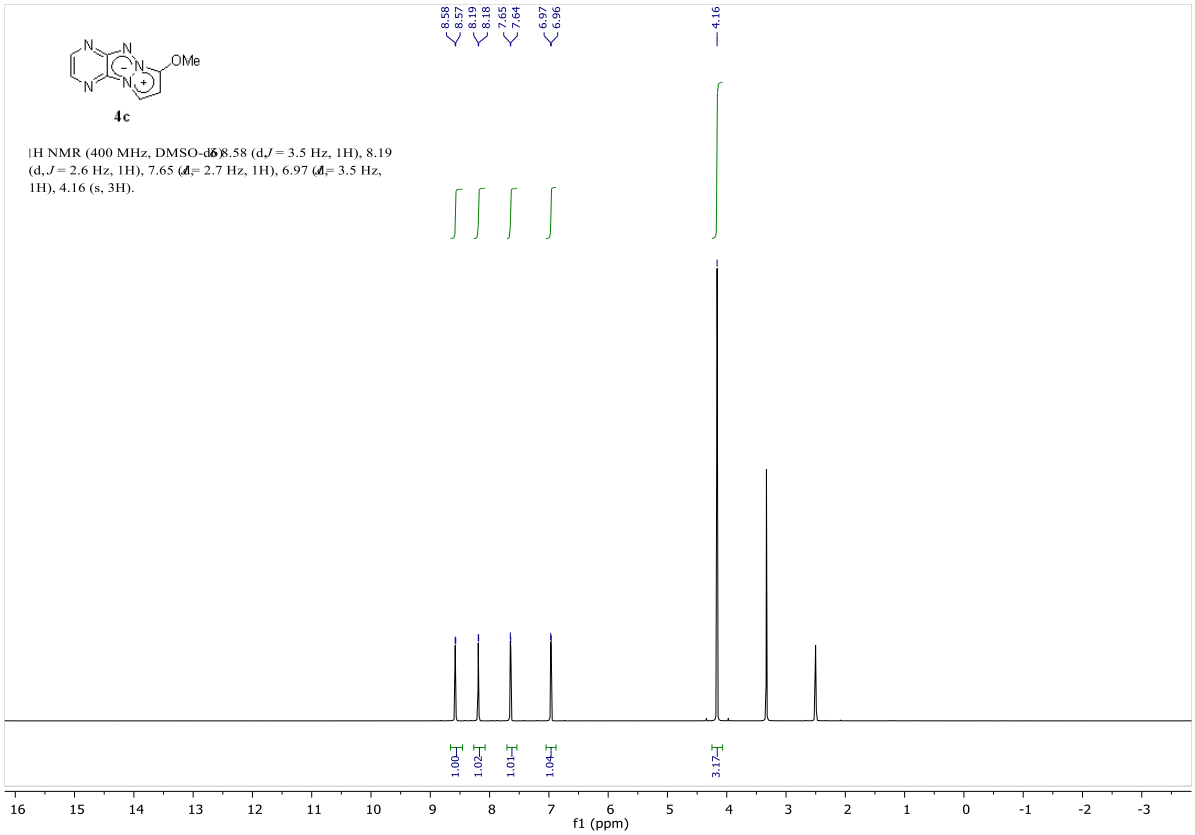


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.75 (d, *J* = 2.4 Hz, 1H), 8.31 (d, *J* = 2.4 Hz, 1H), 8.26 (d, *J* = 1.9 Hz, 1H), 7.94 (d, *J* = 1.9 Hz, 1H).

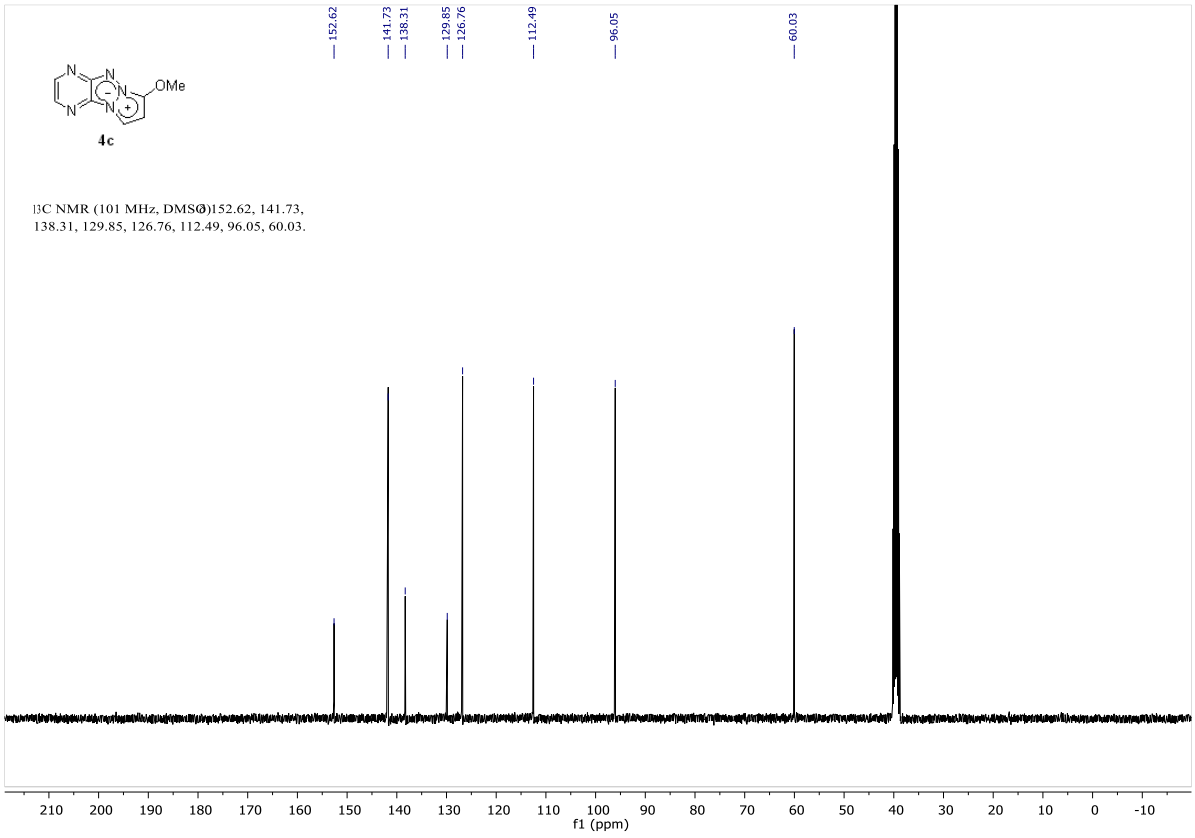


^{13}C NMR (101 MHz, $CDCl_3$) δ 145.29, 135.15, 132.91, 106.67.

7-methoxypyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4c)

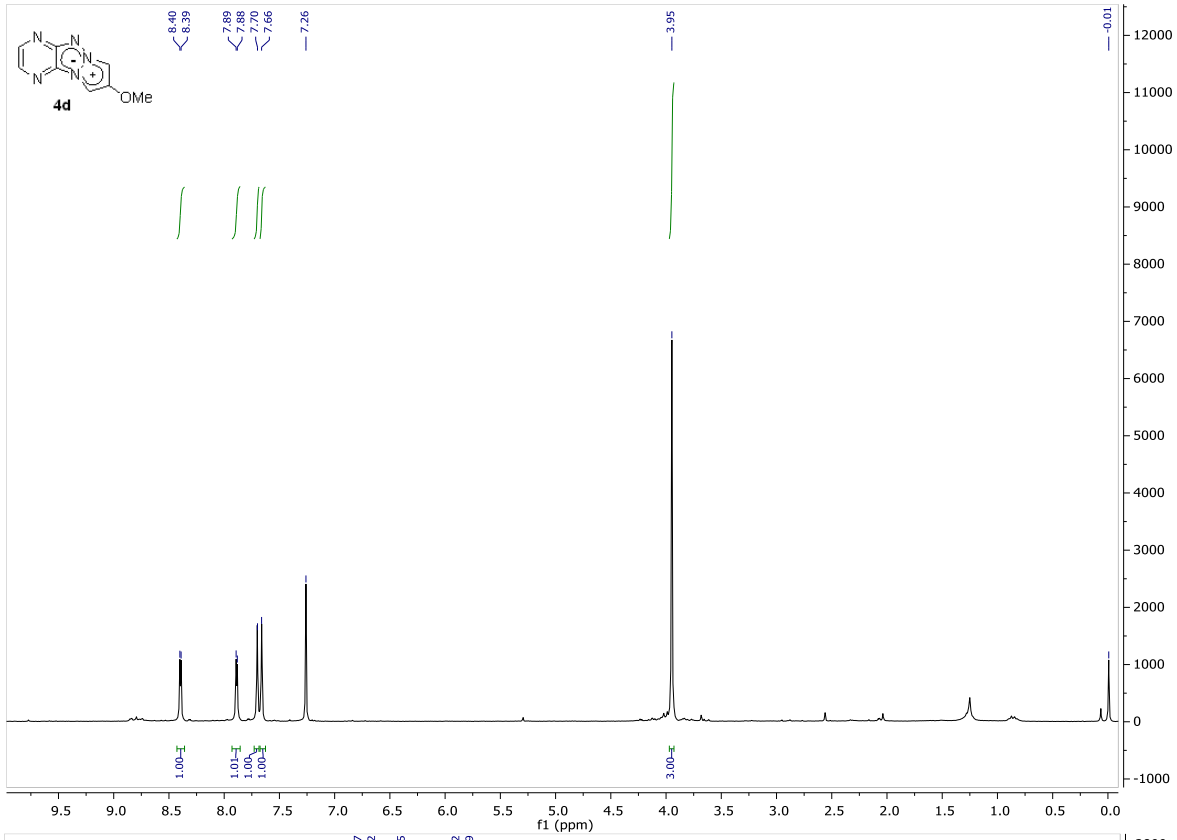


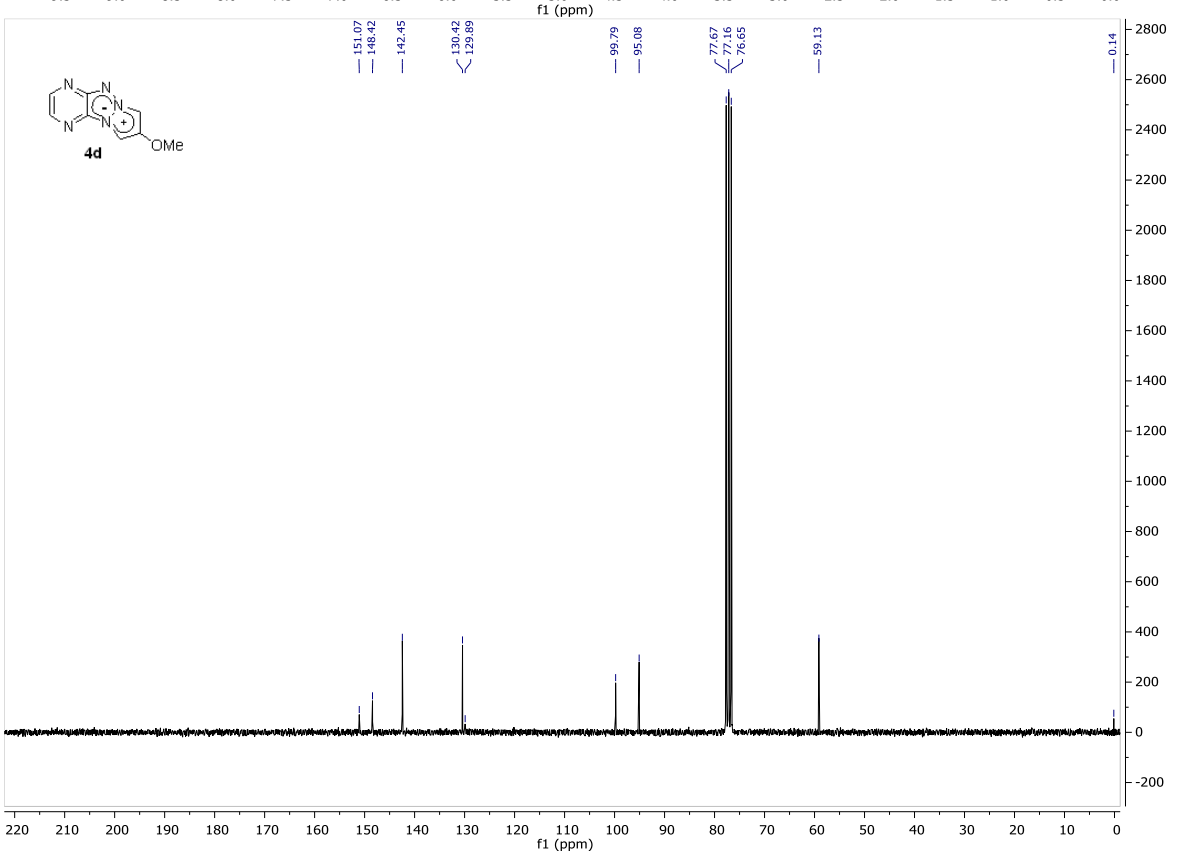
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 3.5 Hz, 1H), 8.19 (d, J = 2.6 Hz, 1H), 7.65 (d, J = 2.7 Hz, 1H), 6.97 (d, J = 3.5 Hz, 1H), 4.16 (s, 3H).



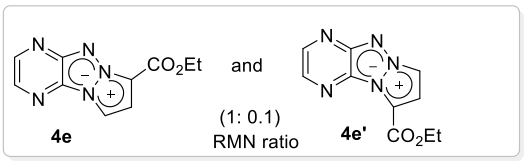
^{13}C NMR (101 MHz, DMSO-d_6) δ 152.62, 141.73, 138.31, 129.85, 126.76, 112.49, 96.05, 60.03.

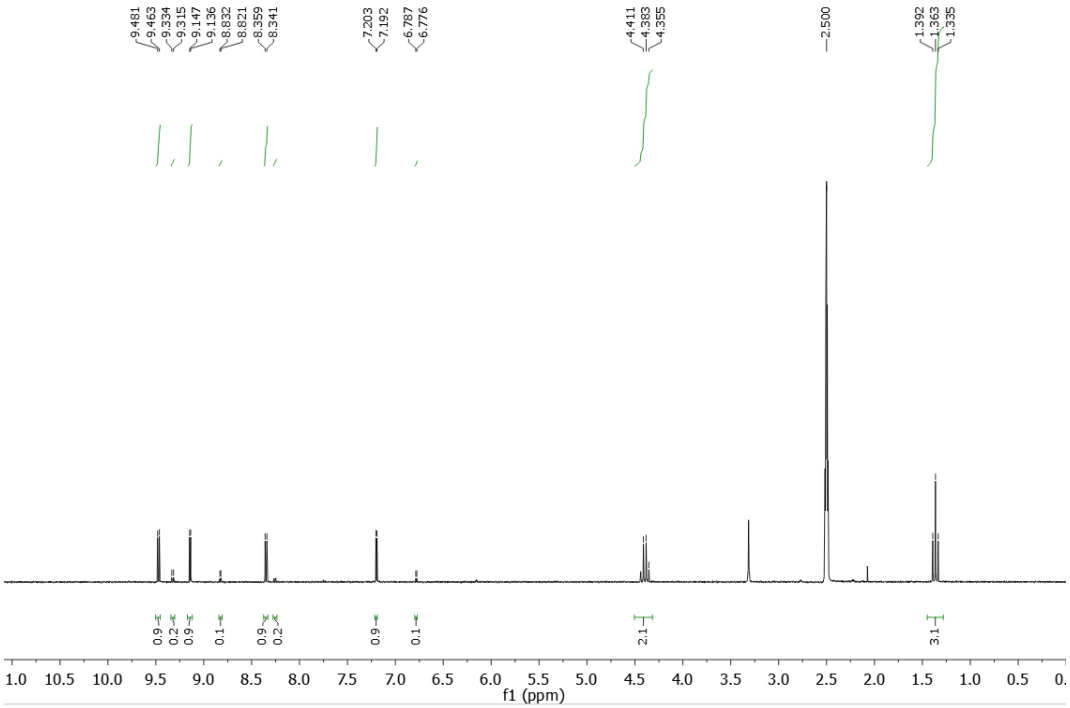
8-methoxypyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4d)



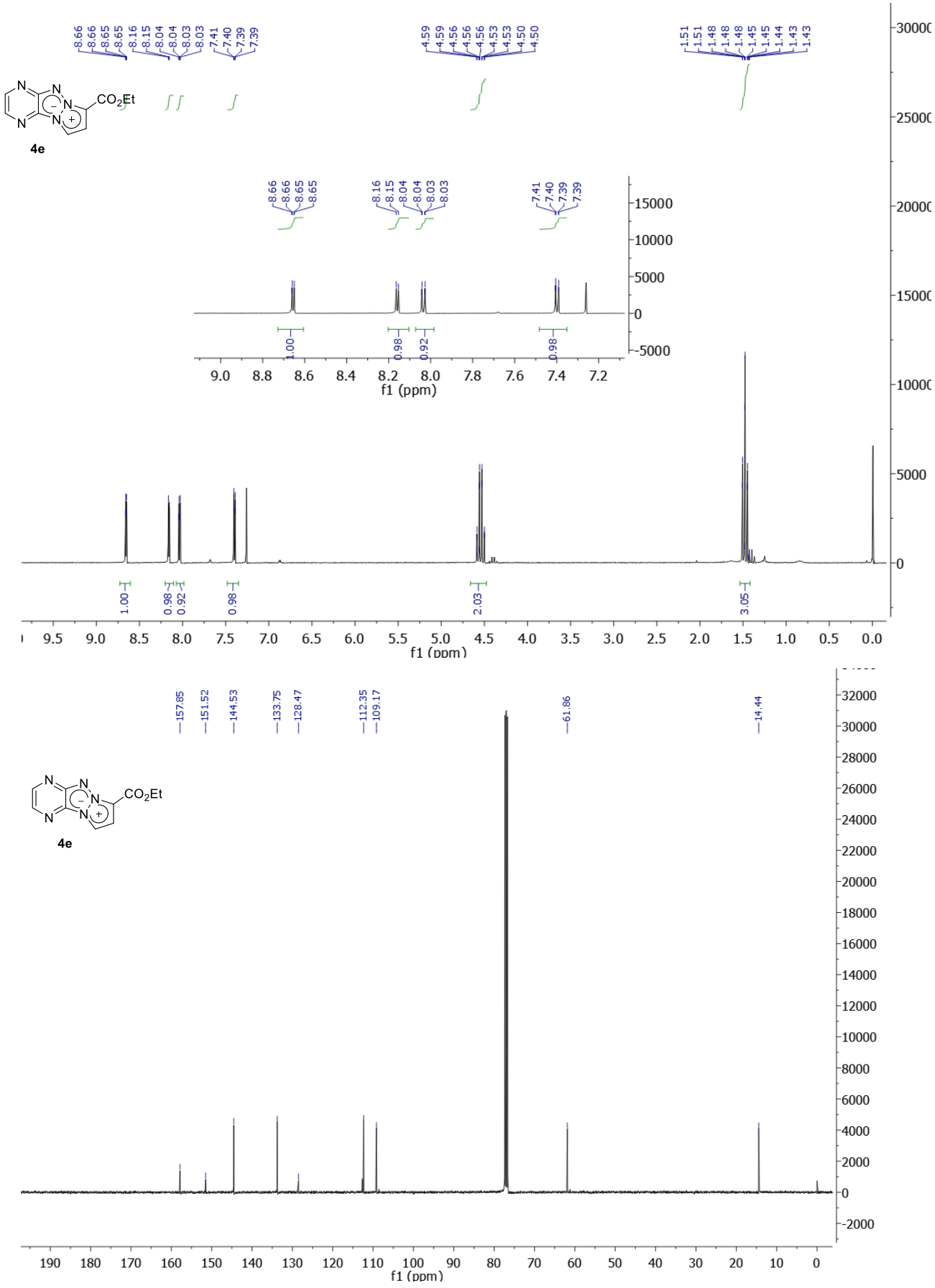


7-(ethoxycarbonyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4e) - crude product

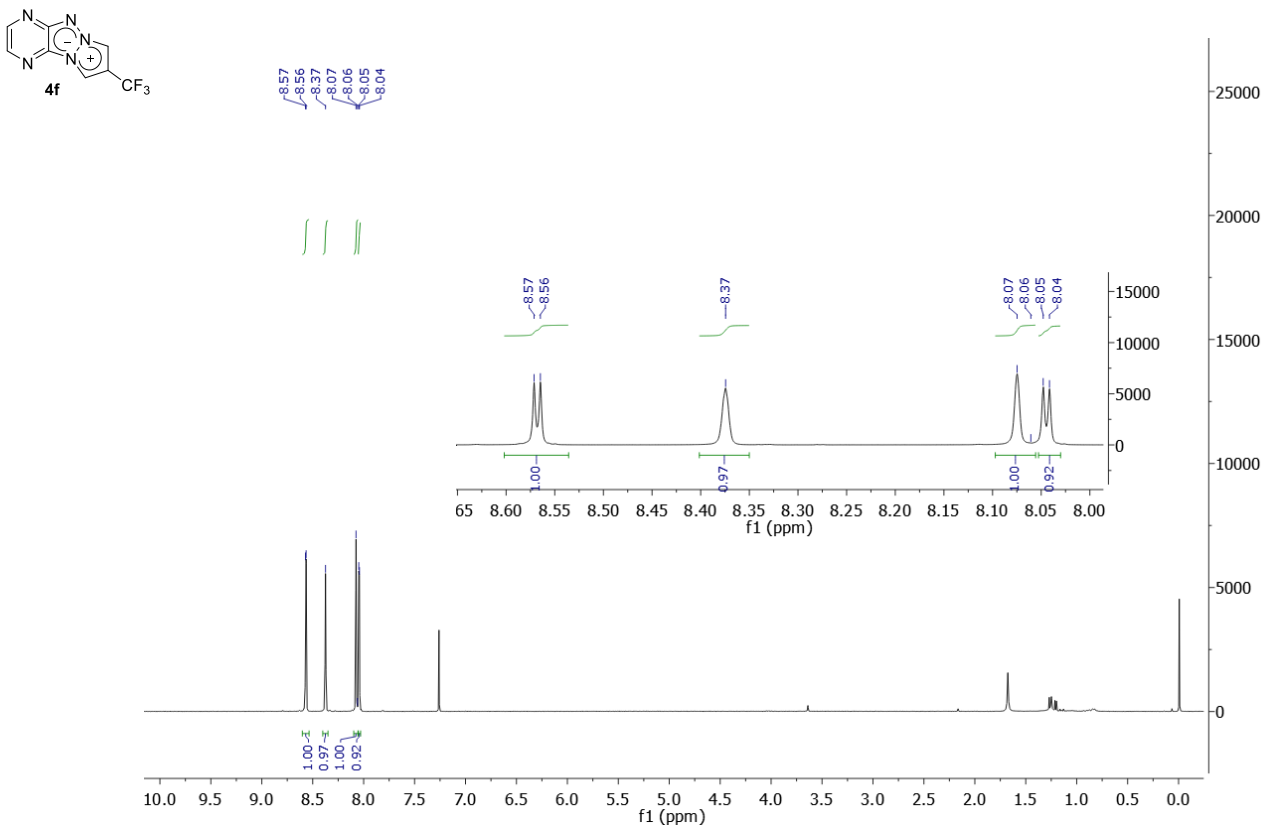


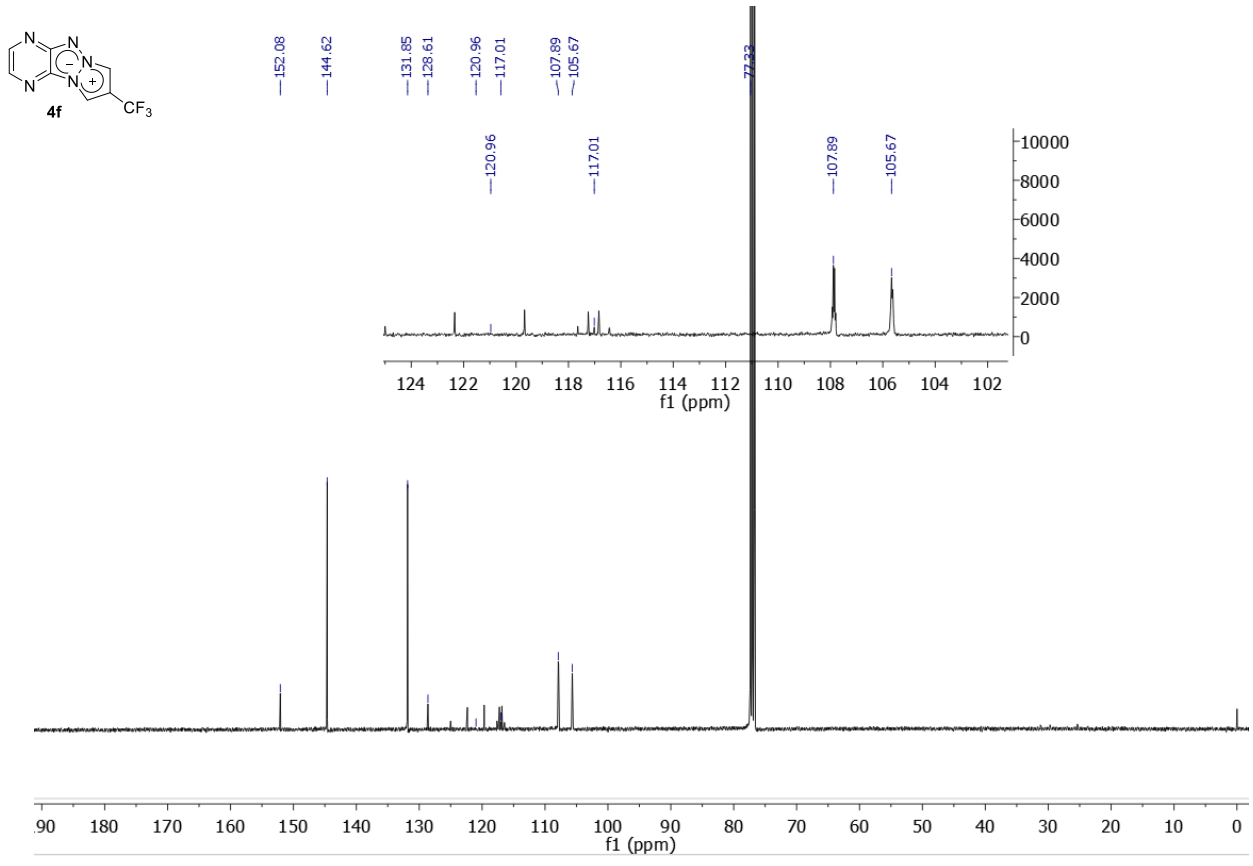


7-(ethoxycarbonyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4e)

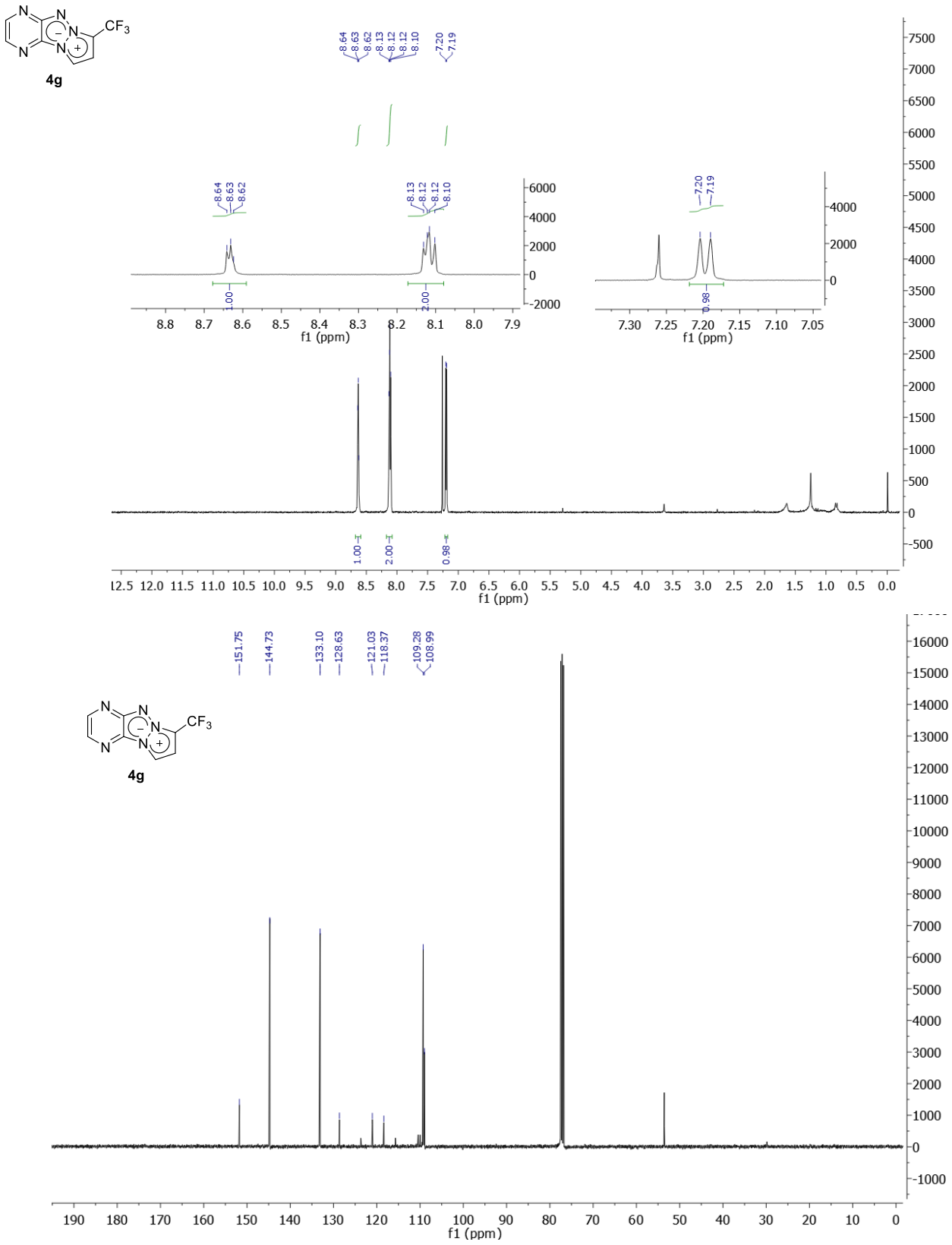


8-(trifluoromethyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4f)

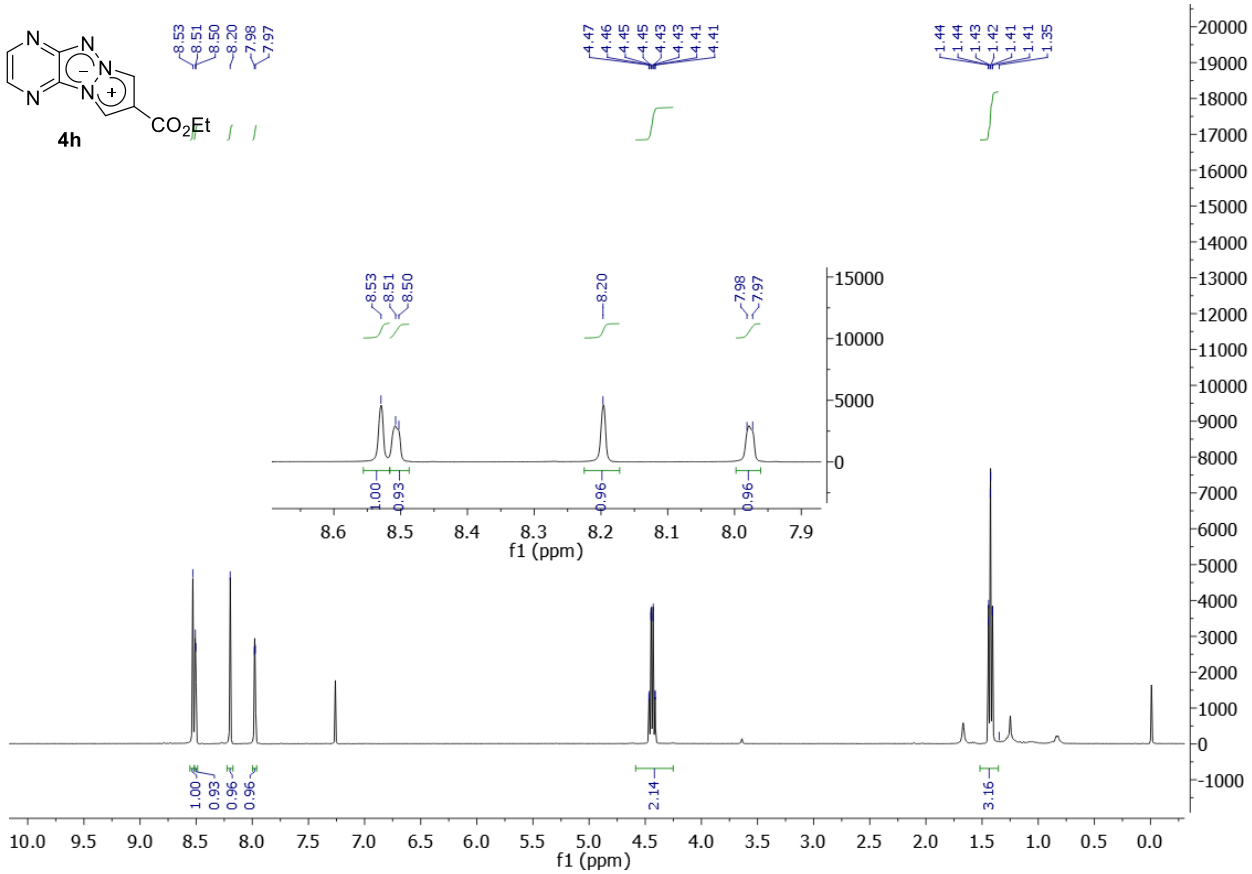


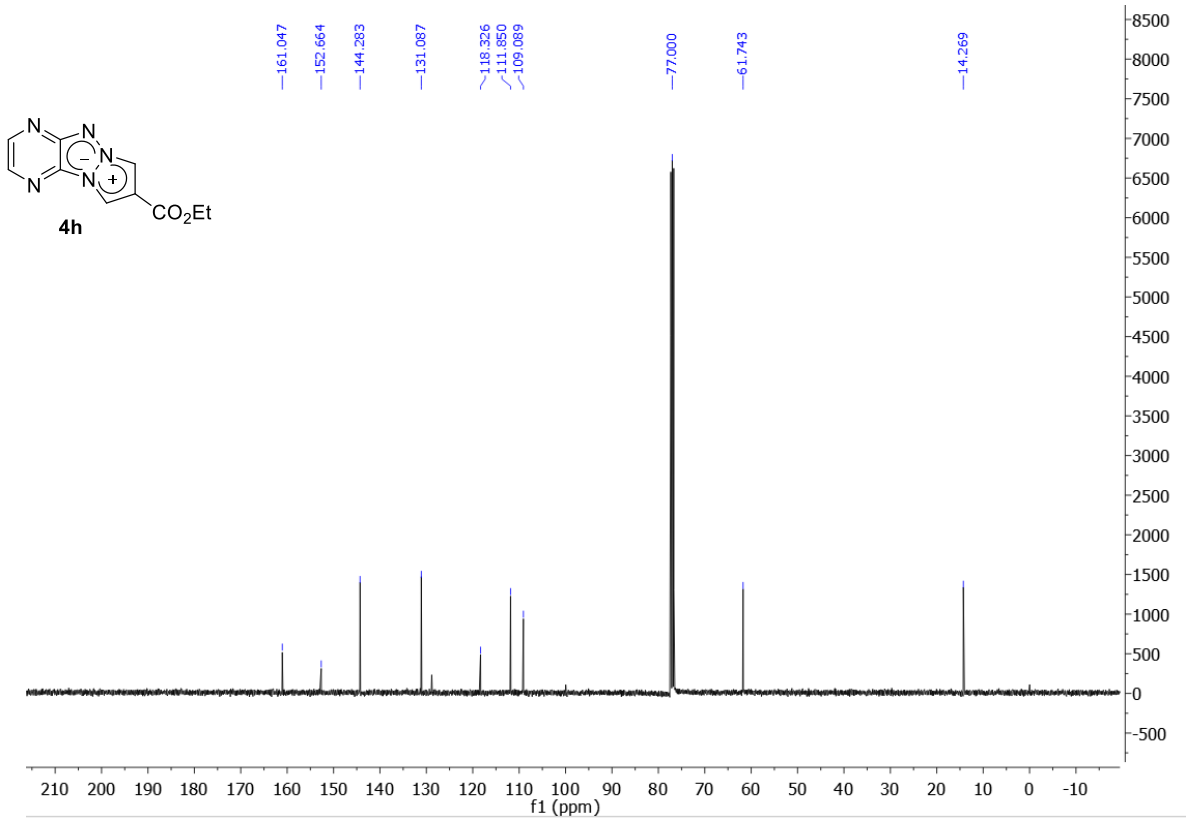


7-(trifluoromethyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-die (4g)

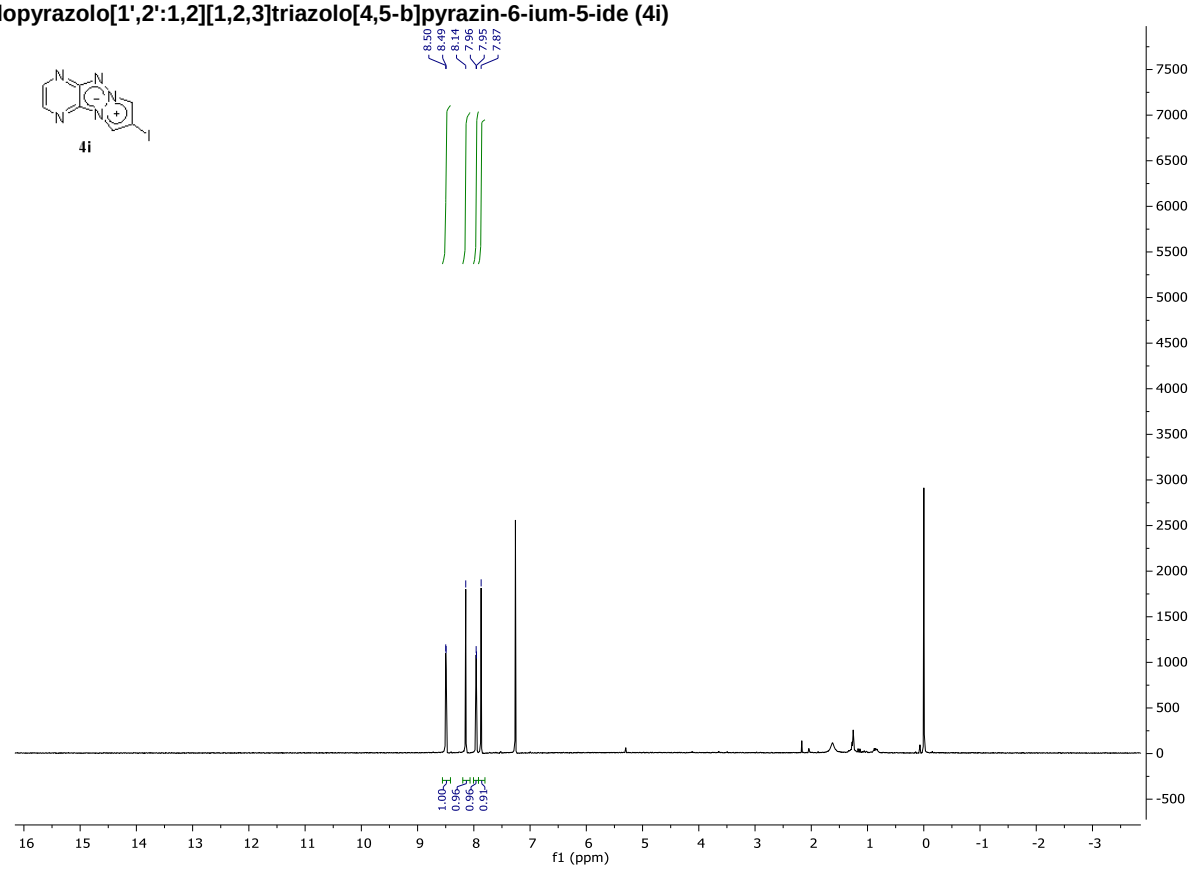


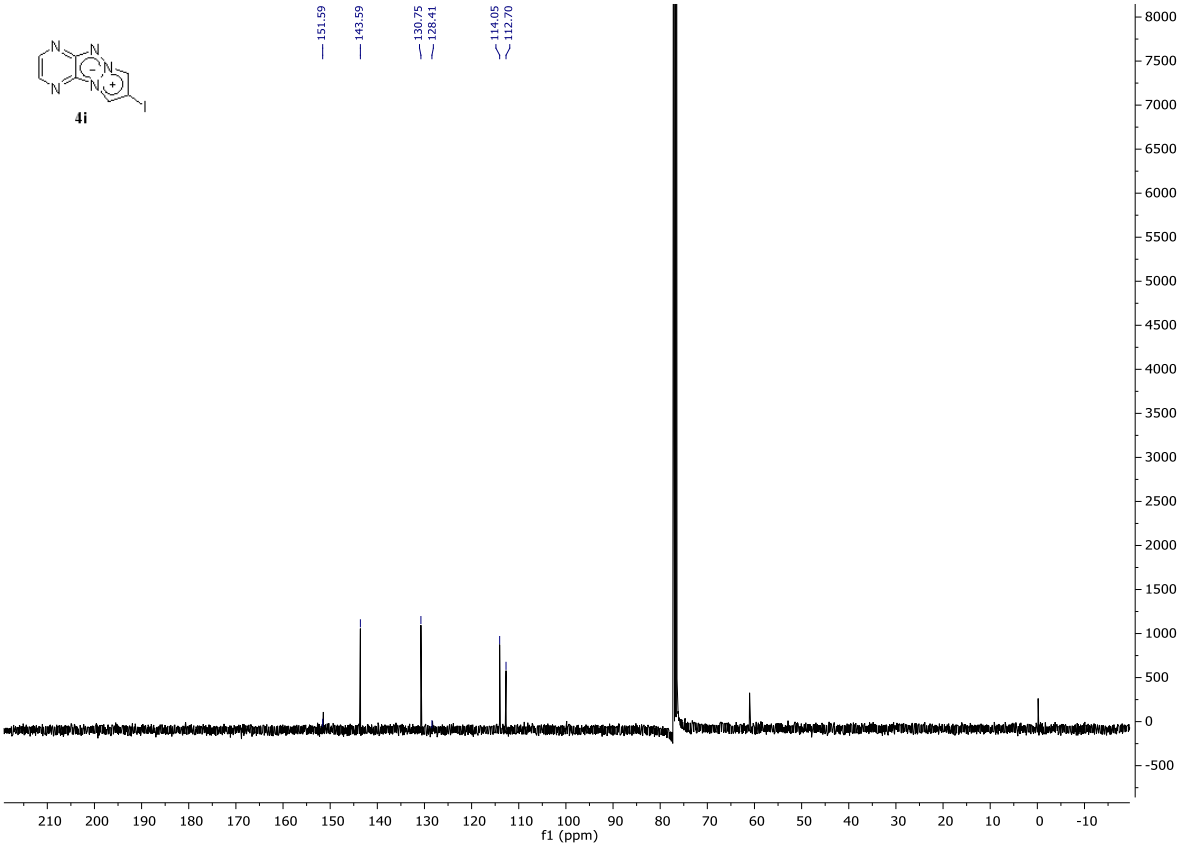
8-(ethoxycarbonyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4h)



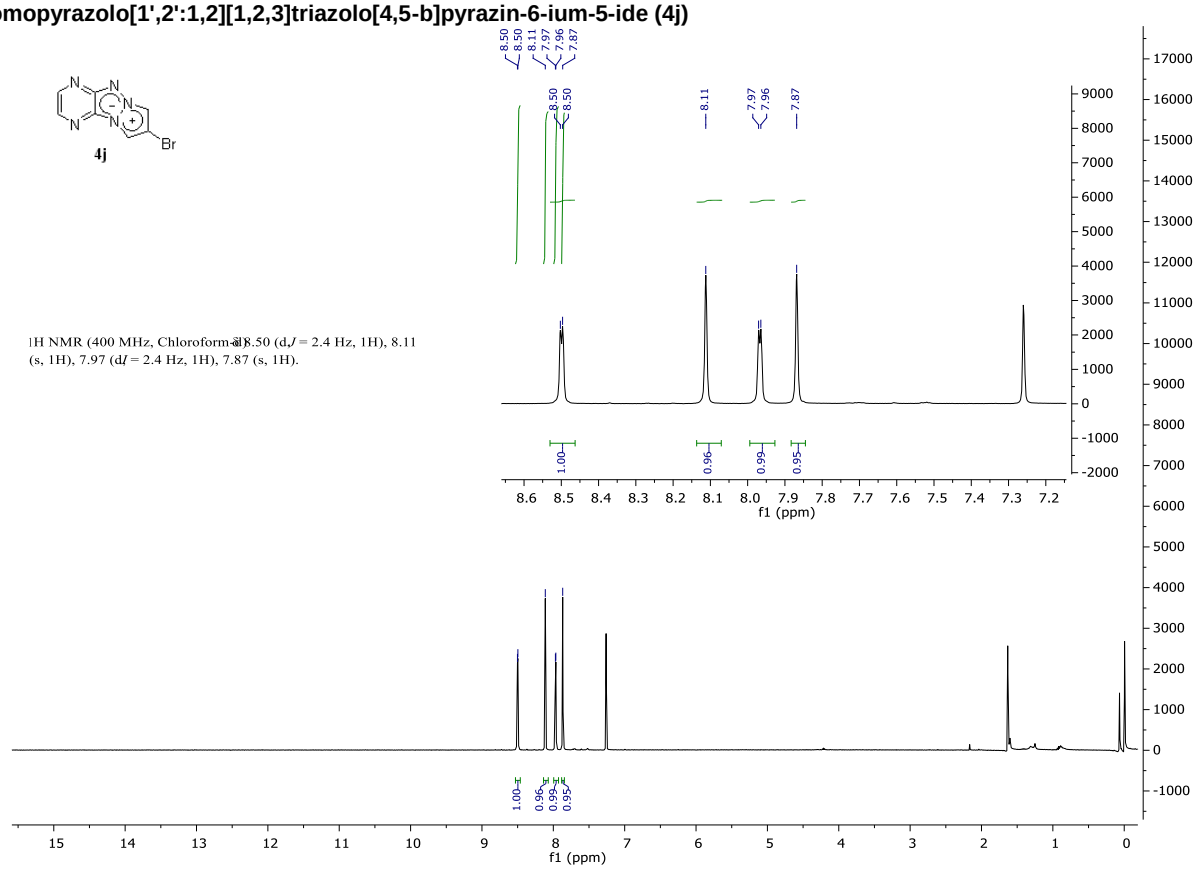


8-iodopyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4i)

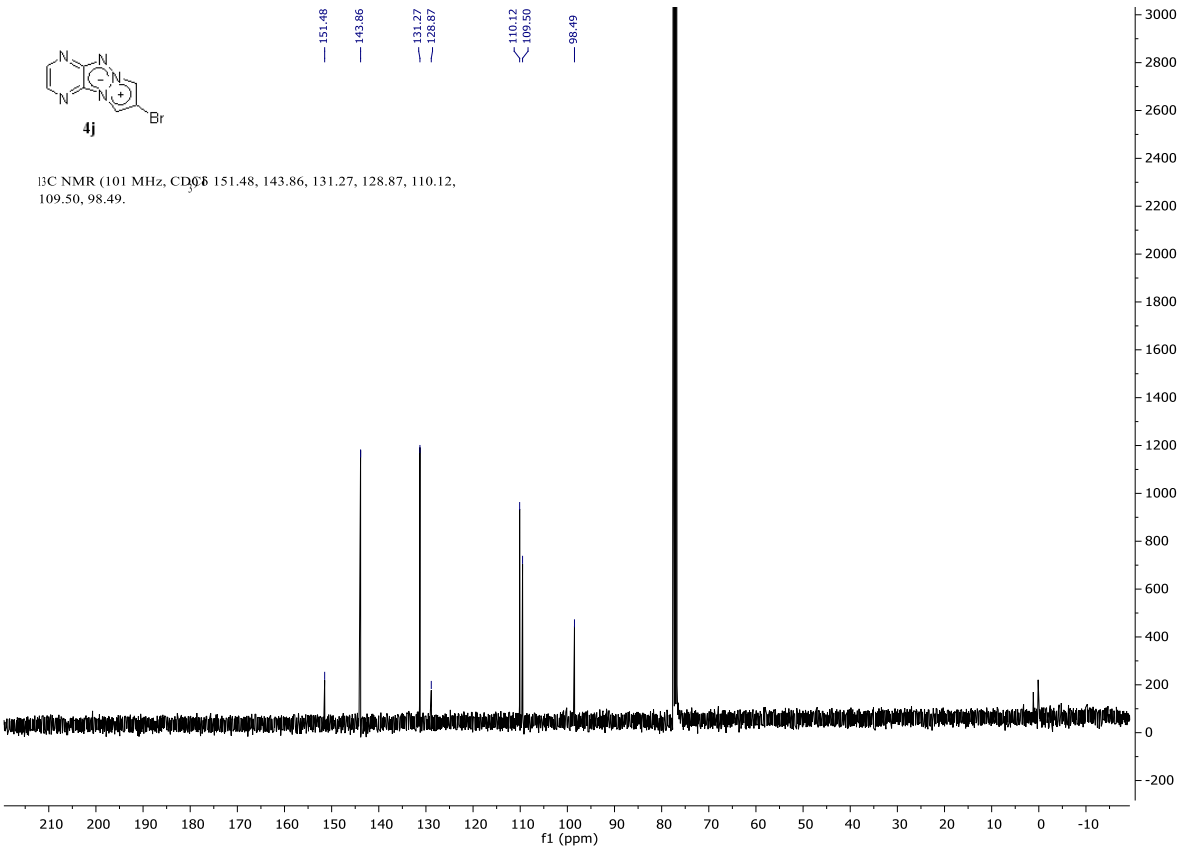




8-bromopyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (4j)

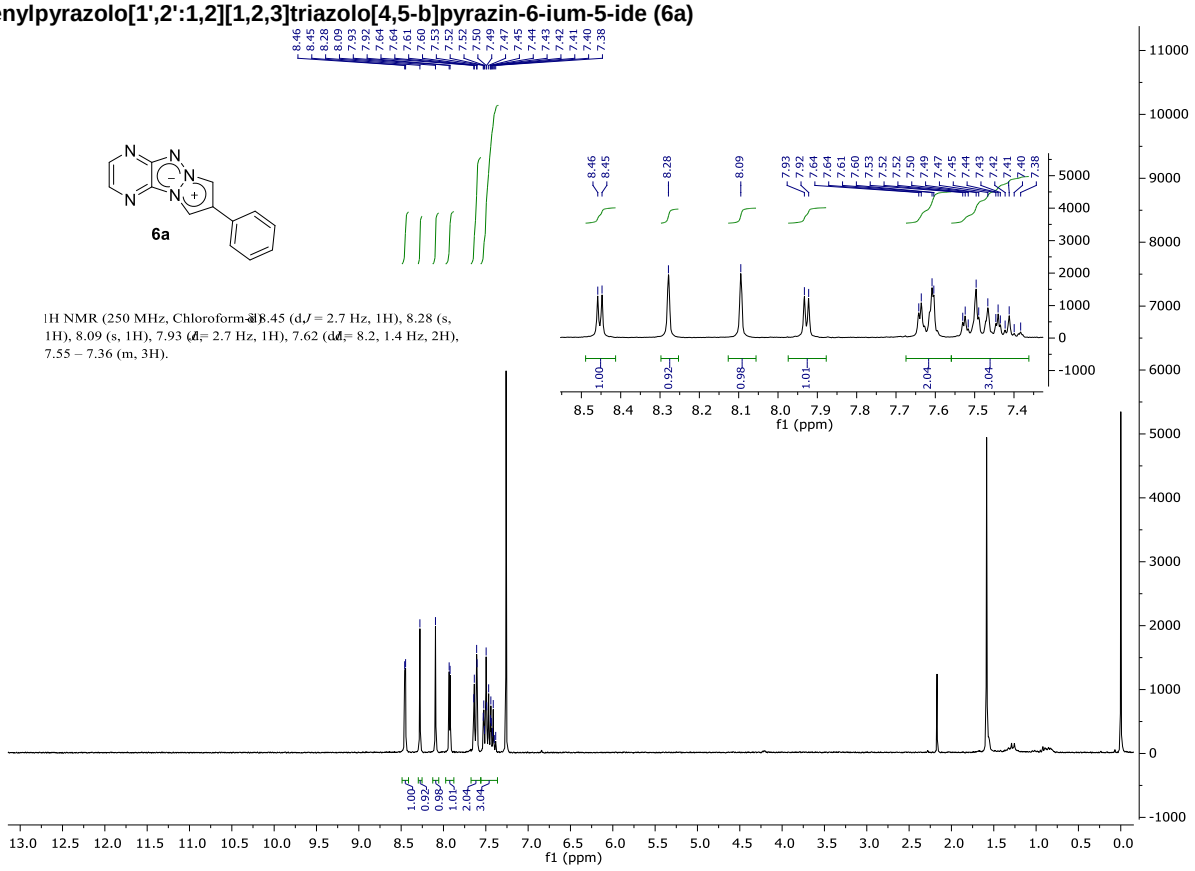


^{1}H NMR (400 MHz, Chloroform-d) δ 8.50 (d, J = 2.4 Hz, 1H), 8.11 (s, 1H), 7.97 (d, J = 2.4 Hz, 1H), 7.87 (s, 1H).

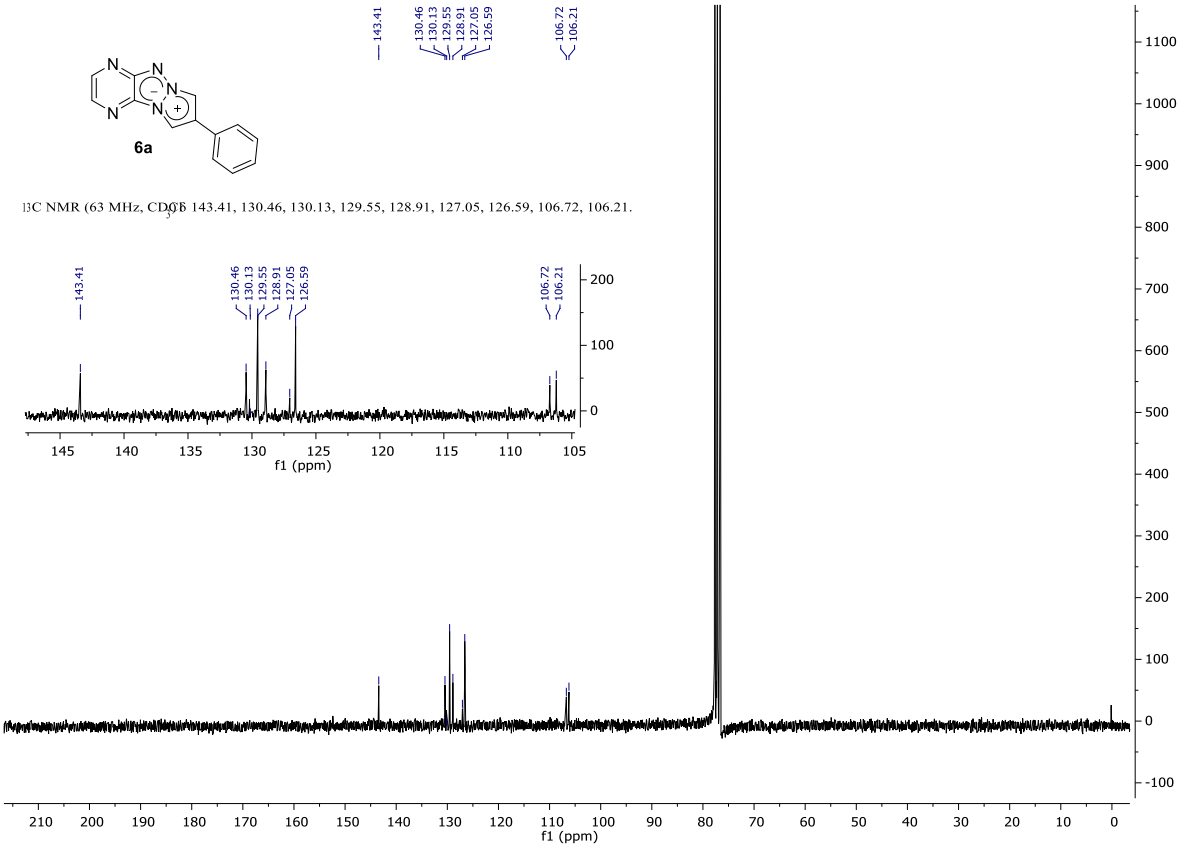


^{13}C NMR (101 MHz, $CDCl_3$) δ 151.48, 143.86, 131.27, 128.87, 110.12, 109.50, 98.49.

8-phenylpyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6a)

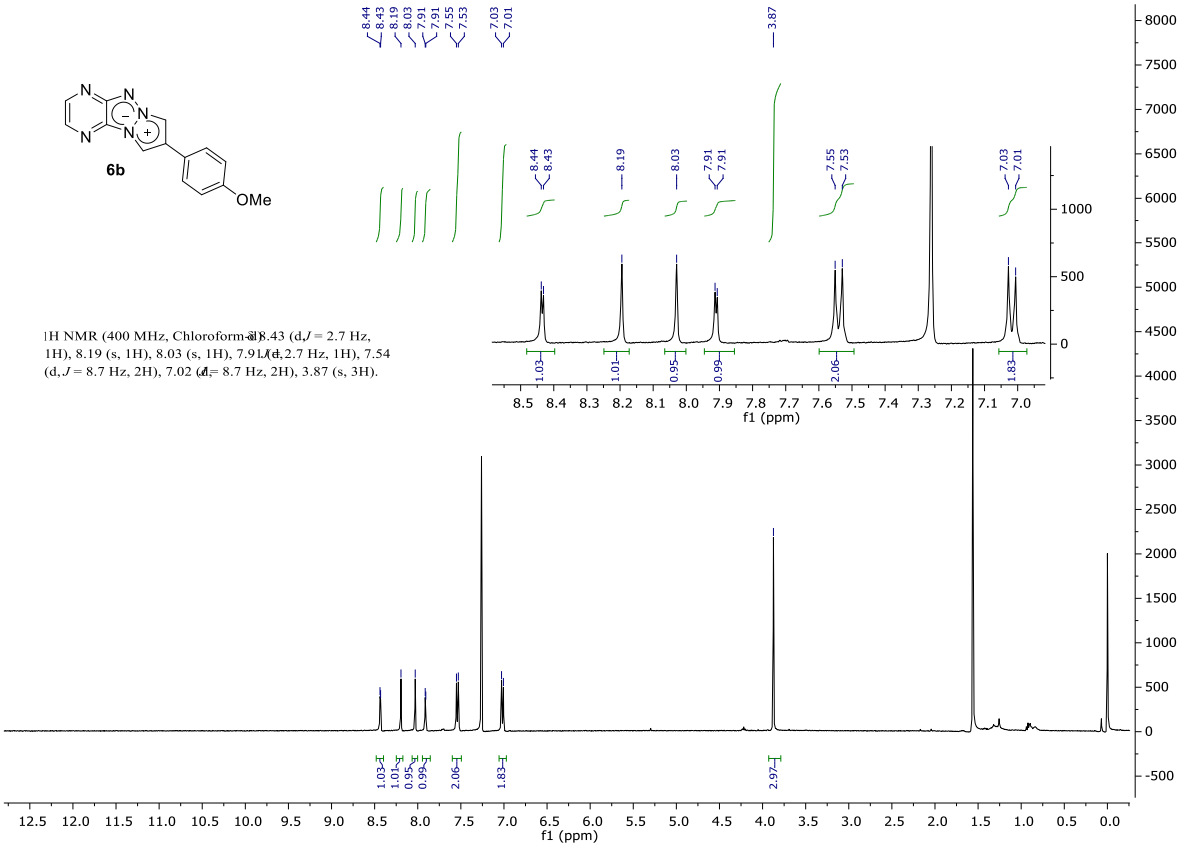


^{1}H NMR (250 MHz, Chloroform-d) δ 8.45 (d, J = 2.7 Hz, 1H), 8.28 (s, 1H), 8.09 (s, 1H), 7.93 (d, J = 2.7 Hz, 1H), 7.62 (dd, J = 8.2, 1.4 Hz, 2H), 7.55 – 7.36 (m, 3H).

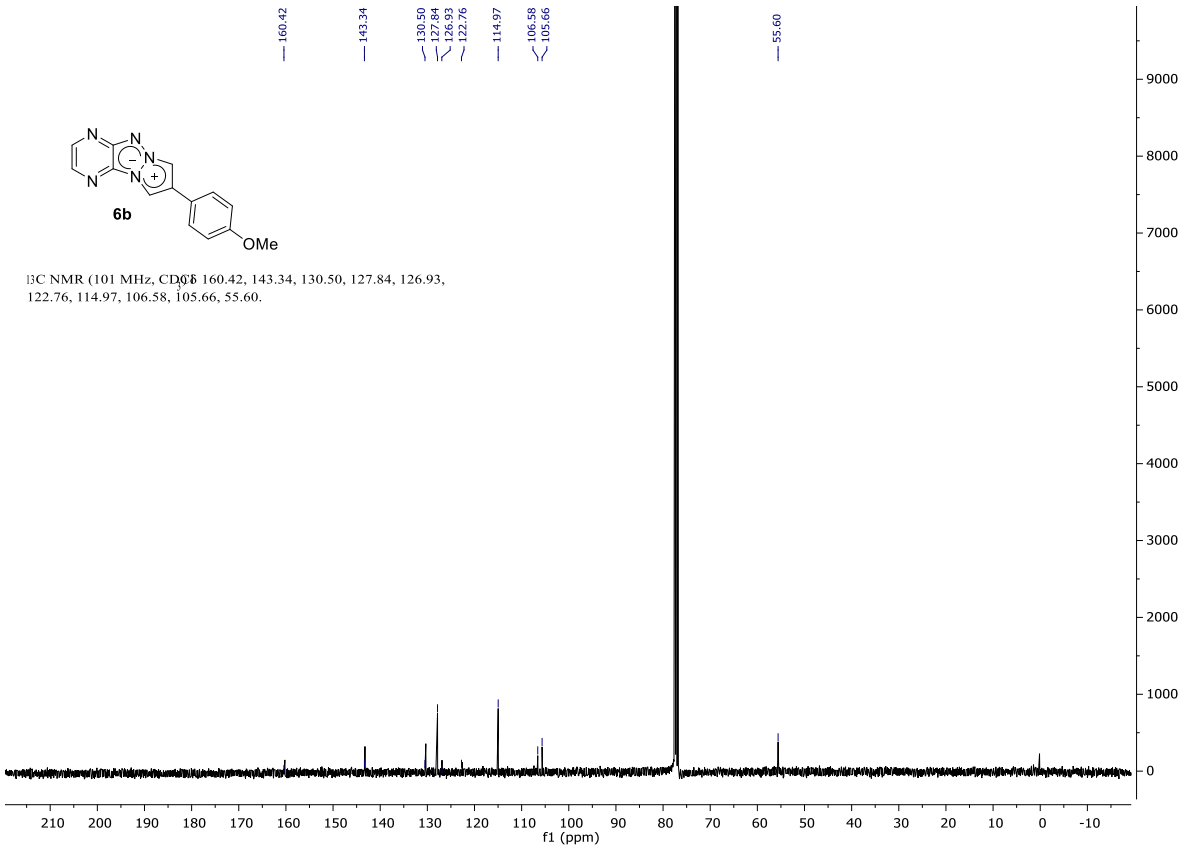


^{13}C NMR (63 MHz, $CDCl_3$) δ 143.41, 130.46, 130.13, 129.55, 128.91, 127.05, 126.59, 106.72, 106.21.

8-(4-methoxyphenyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6b)

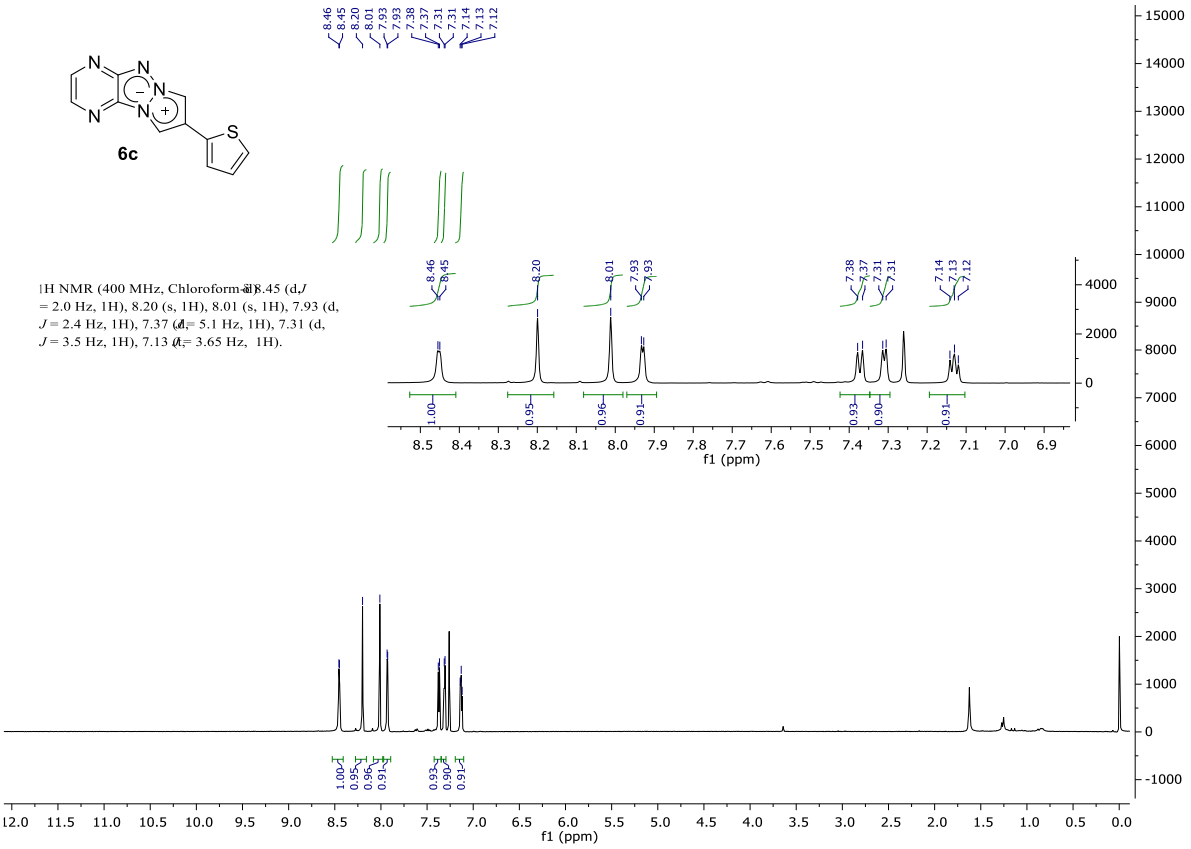


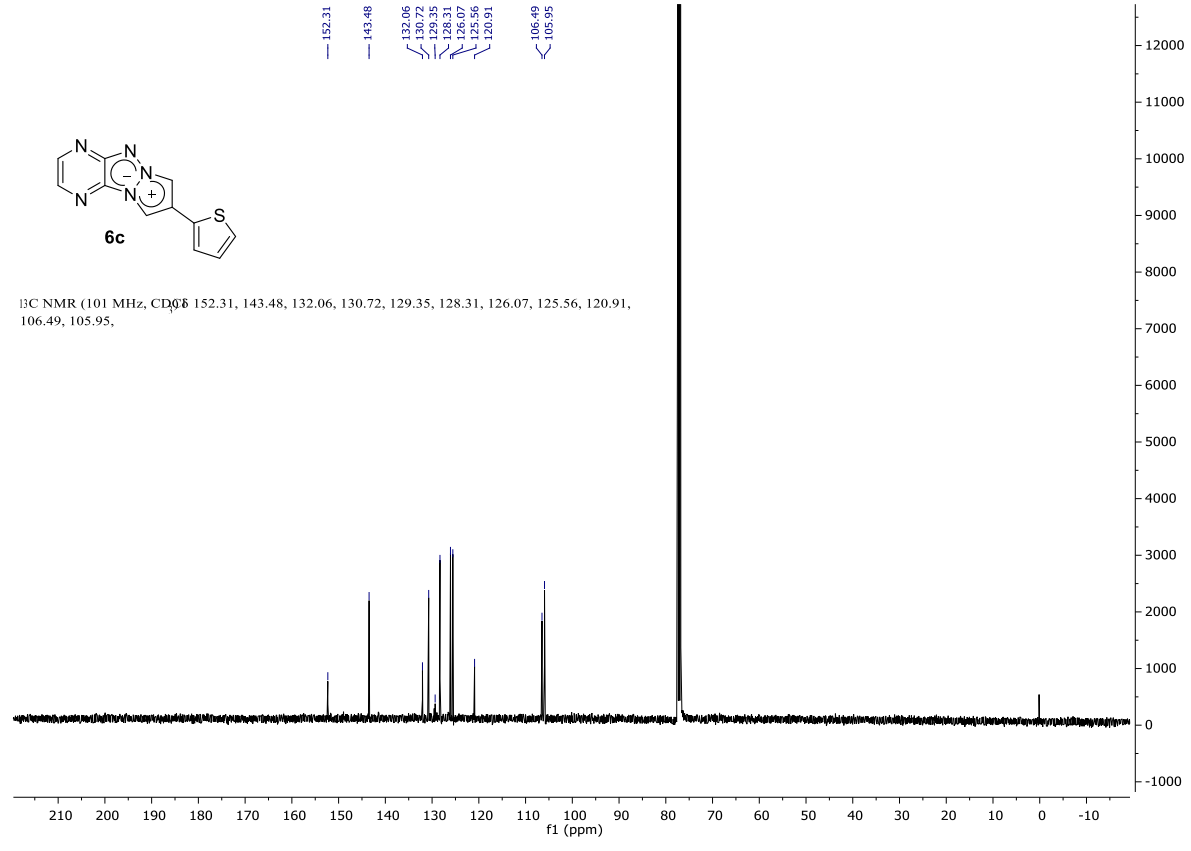
^{1}H NMR (400 MHz, Chloroform-d) δ 8.43 (d, J = 2.7 Hz, 1H), 8.19 (s, 1H), 8.03 (s, 1H), 7.91 (d, J = 2.7 Hz, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.02 (d, J = 8.7 Hz, 2H), 3.87 (s, 3H).



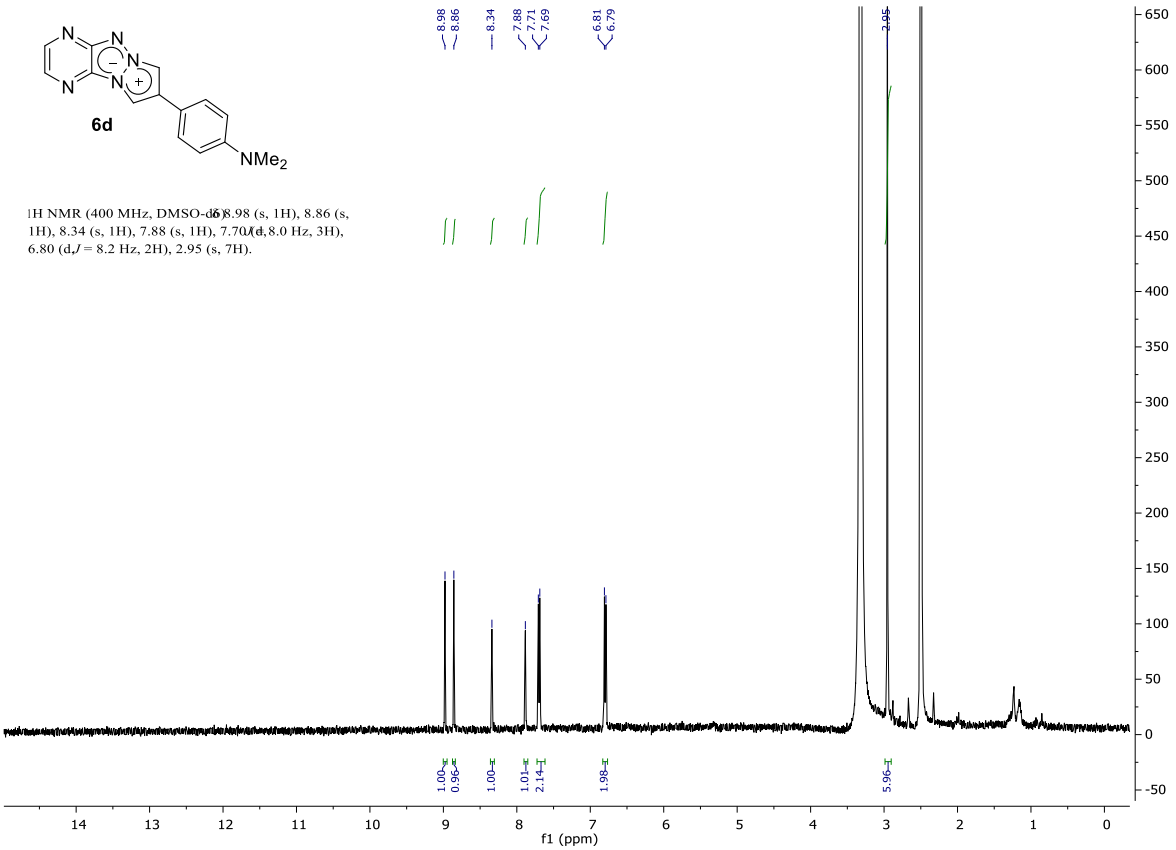
^{13}C NMR (101 MHz, CDCl$_3$) δ 160.42, 143.34, 130.50, 127.84, 126.93, 122.76, 114.97, 106.58, 105.66, 55.60.

8-(thiophen-2-yl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6c)

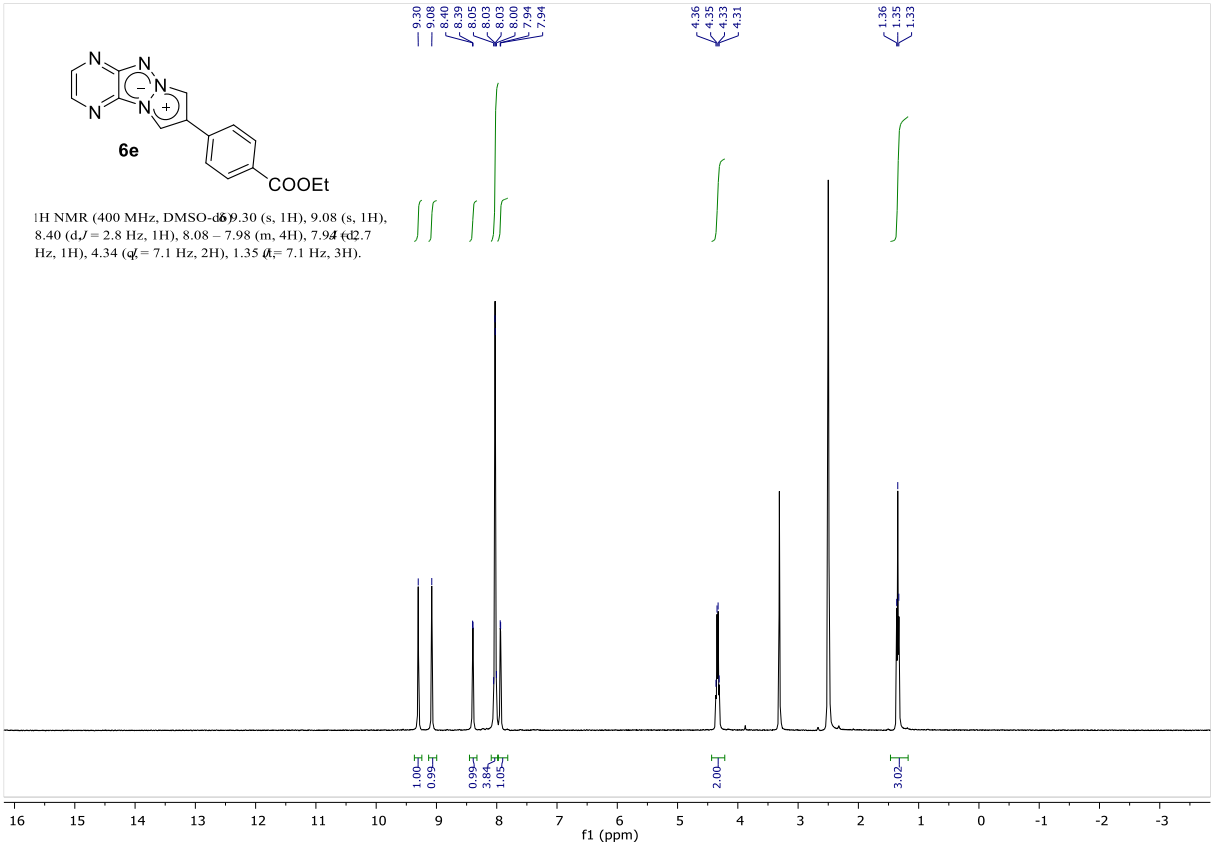


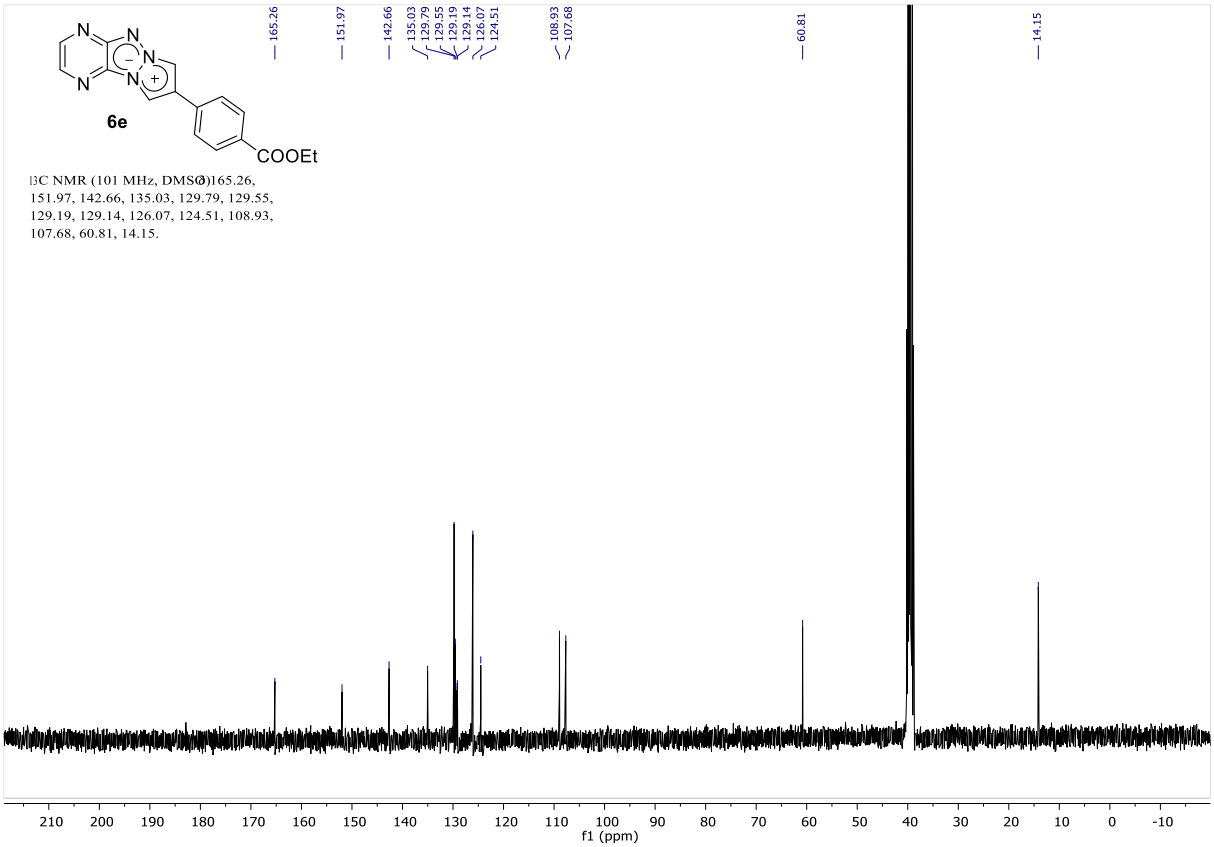


8-(4-(dimethylamino)phenyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6d)



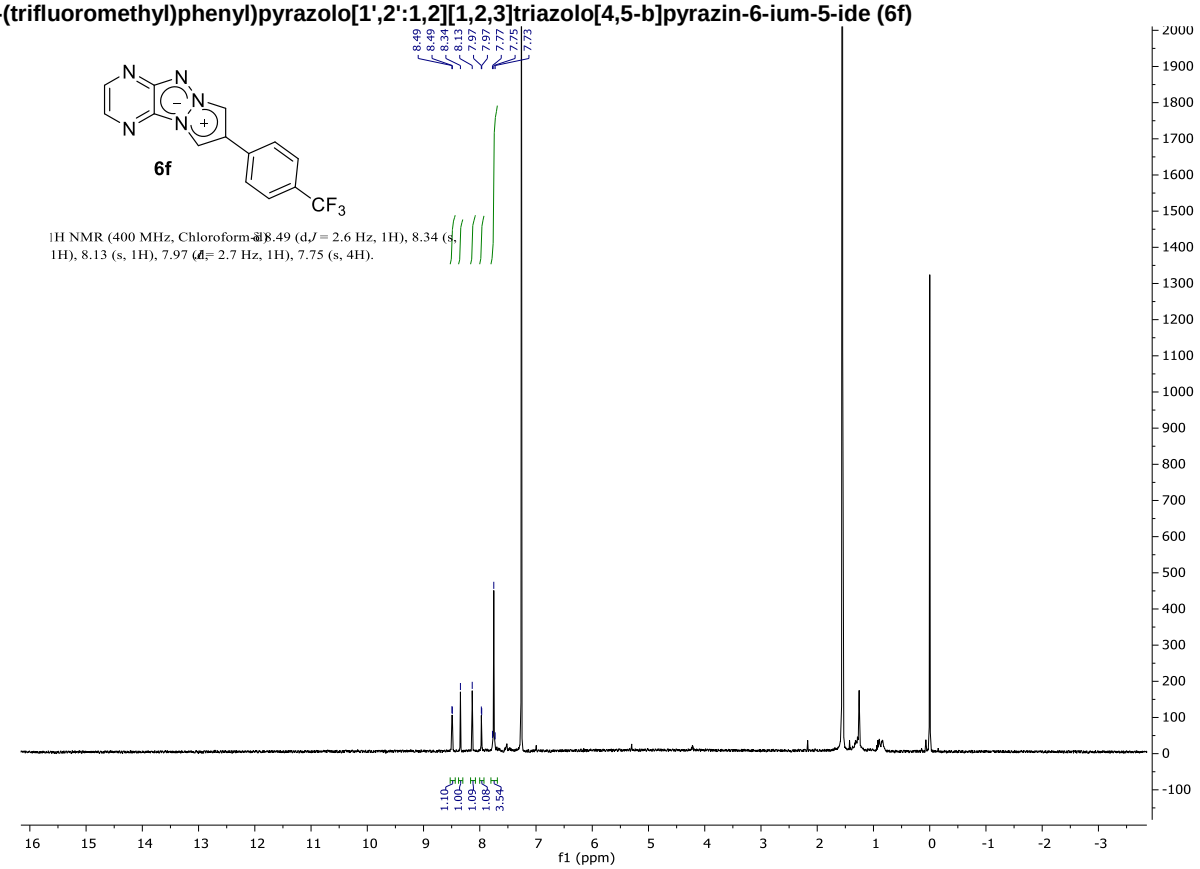
8-(4-(ethoxycarbonyl)phenyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6e)



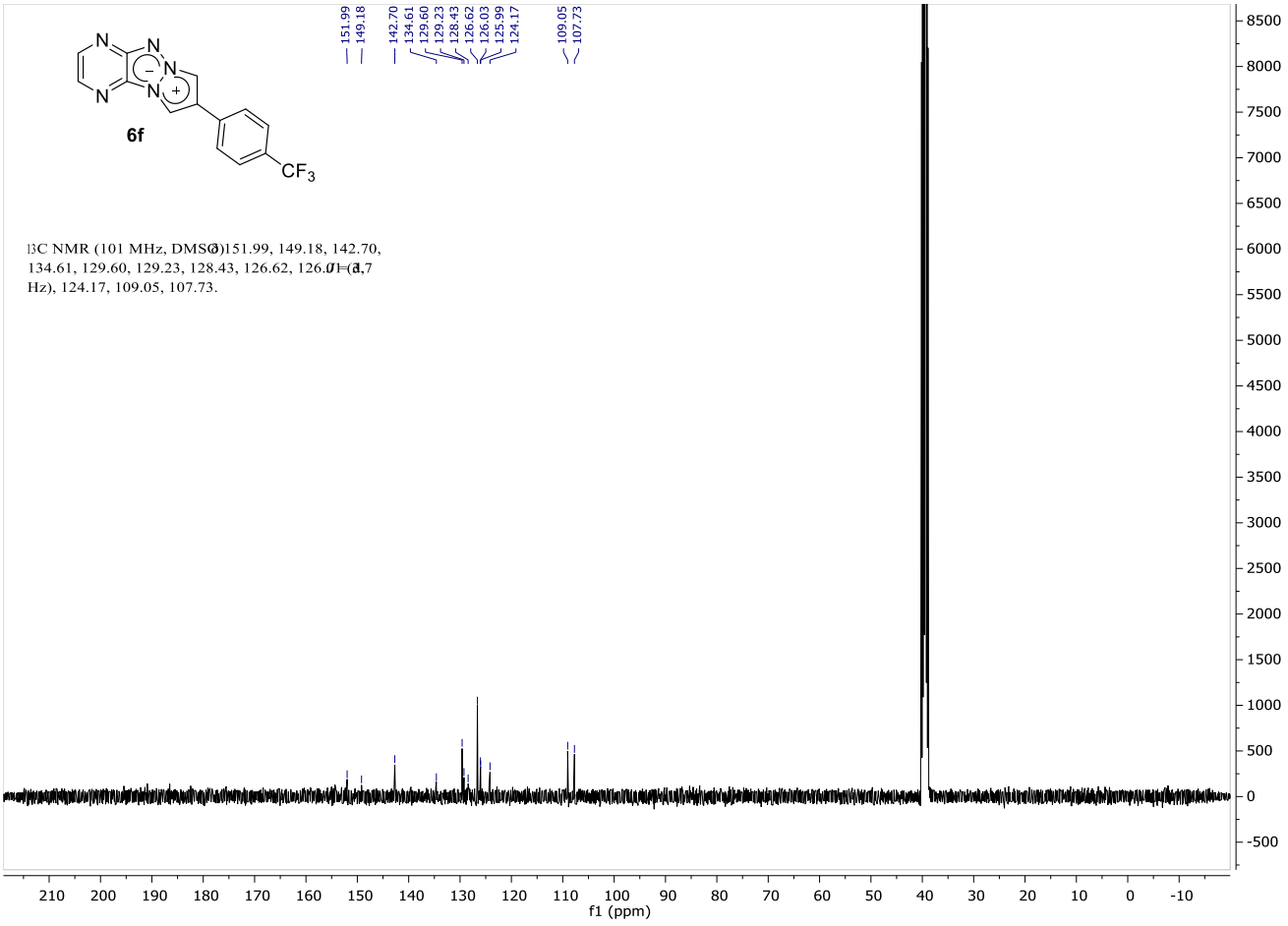


^{13}C NMR (101 MHz, DMSO) δ 165.26, 151.97, 142.66, 135.03, 129.79, 129.55, 129.19, 129.14, 126.07, 124.51, 108.93, 107.68, 60.81, 14.15.

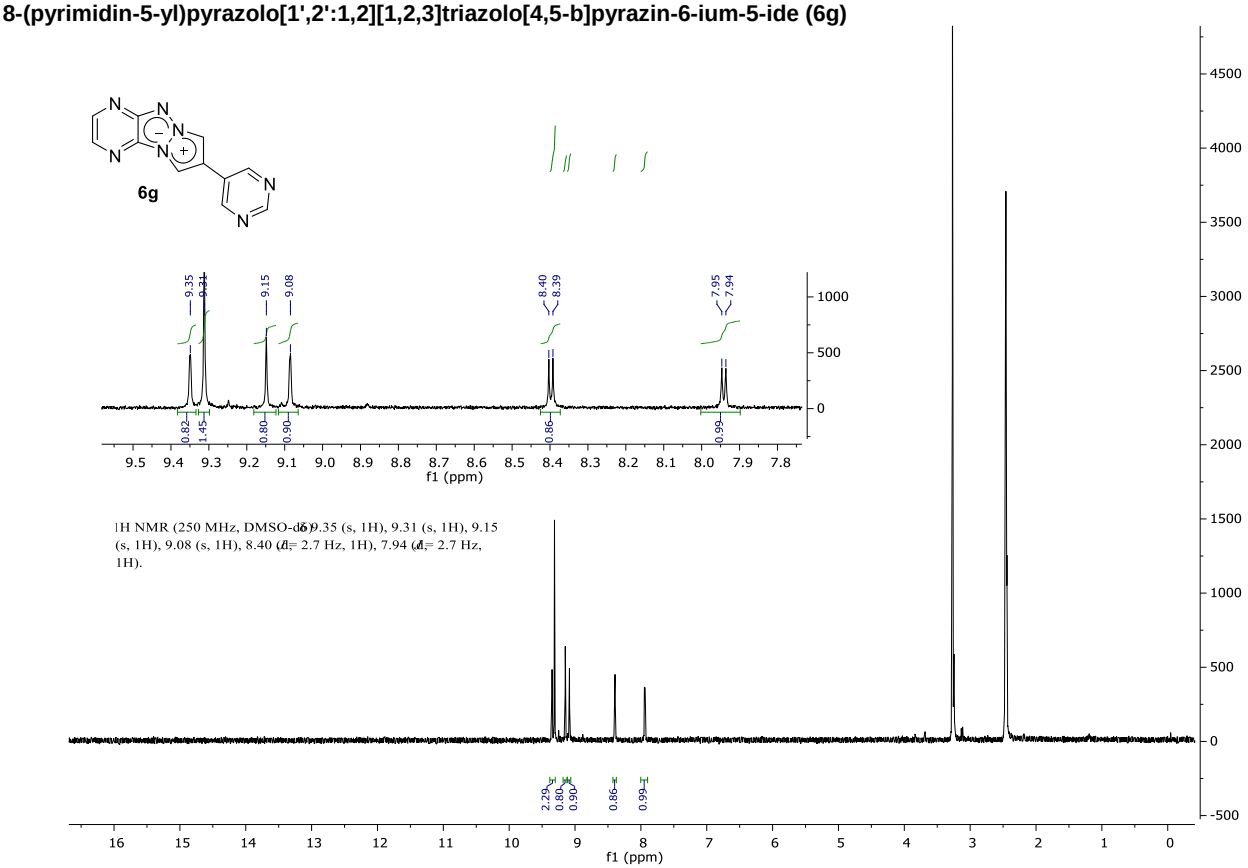
8-(4-(trifluoromethyl)phenyl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6f)

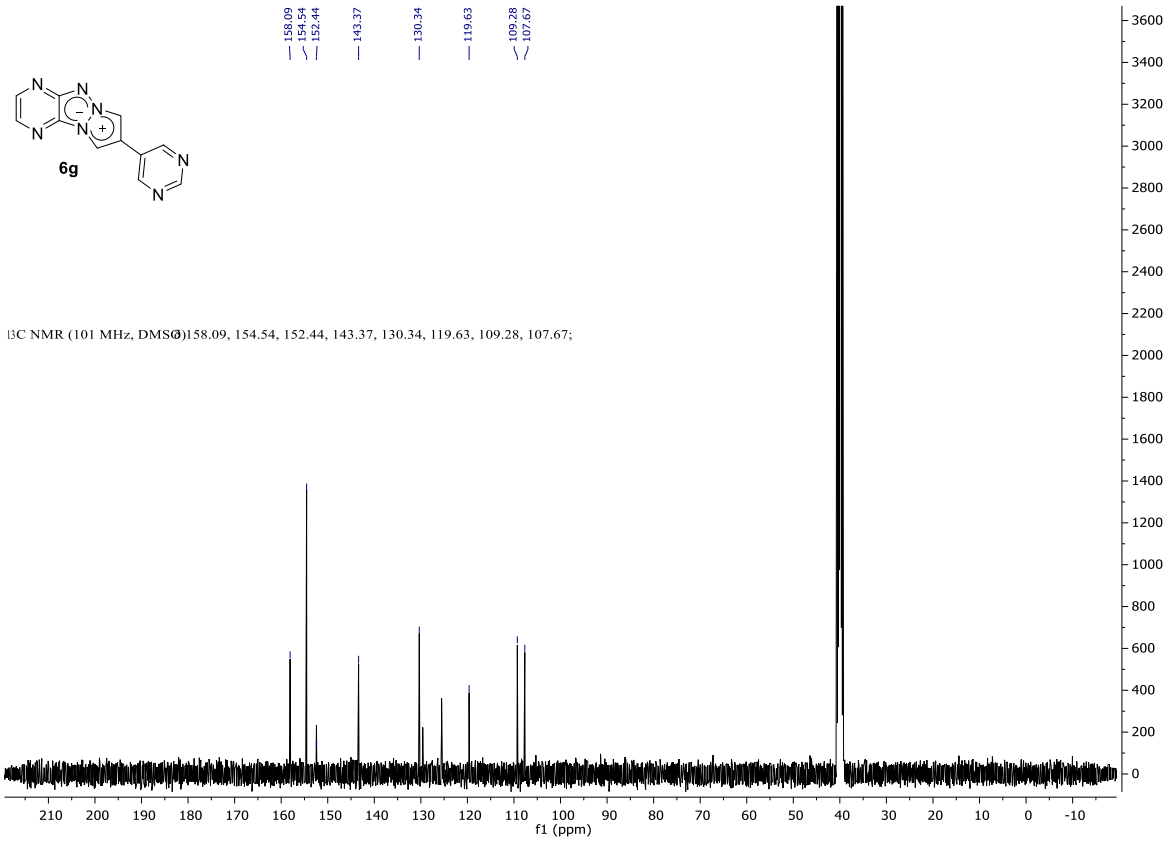


^{1}H NMR (400 MHz, Chloroform-d) δ 8.49 (d, J = 2.6 Hz, 1H), 8.34 (s, 1H), 8.13 (s, 1H), 7.97 (d, J = 2.7 Hz, 1H), 7.75 (s, 4H).



8-(pyrimidin-5-yl)pyrazolo[1',2':1,2][1,2,3]triazolo[4,5-b]pyrazin-6-ium-5-ide (6g)





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Author Contributions

Doina Sirbu and Nicolas Chopin performed the compound synthesis and spectroscopic characterisations. Julien Diharce and Pascal Bonnet worked on the theoretical calculations. Ivana Martinic, Svetlana Eliseeva and Stéphane Petoud were in charge of the cellular imaging. Gérald Guillaumet and Franck Suzenet designed research and were involved in the synthesis of the compounds. All contributors wrote the paper.