Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Strained Alkyne Substituted Near Infrared BF_2 Azadipyrromethene Fluorochrome

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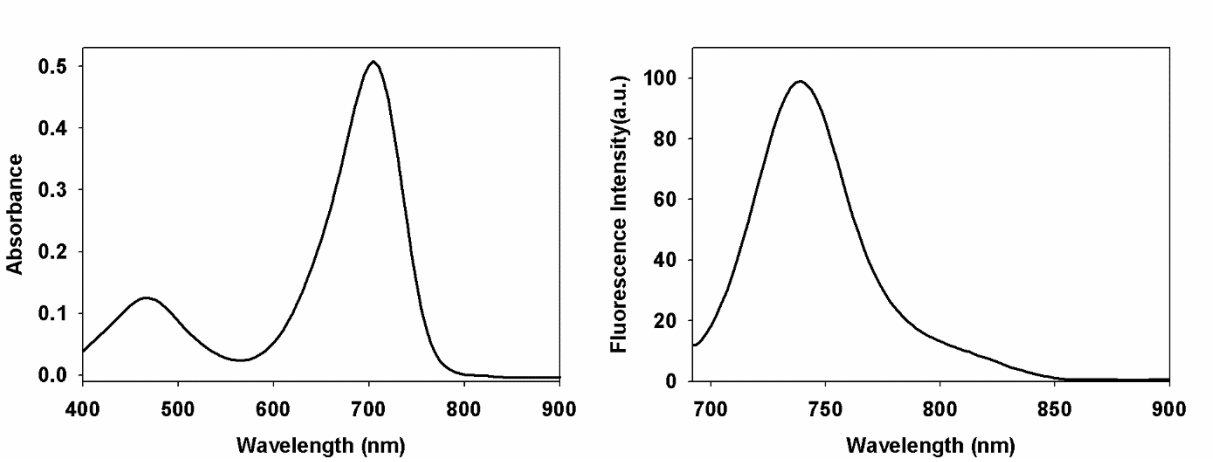
General Information and Materials

Synthetic reaction conditions and yields are not optimized. All reactions involving air-sensitive reagents were performed under nitrogen in oven-dried glassware using syringe-septum cap technique. All solvents were purified and degassed before use. Chromatographic separation was carried out under pressure on Apollo 60/40 silica gel and Merck alumina 90 using flash-column techniques. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel coated aluminum plates (60 Merck F_{254}) using UV light (254 nm) as visualizing agent. Unless it is specified, all reagents were used as received without further purifications. ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature at 400 MHz and 100 MHz respectively, and calibrated using residual non-deuterated solvent as an internal reference. Chemical shifts are reported in parts-per-million (ppm). On the basis of NMR and reverse phase HPLC all final compounds are >95% pure. MALDI-TOF MS analysis used a 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene] malononitrile (DCTB) matrix. The peptide *cyclo*[Arg-Gly-Asp-D-Phe-Lys (azide)] was purchased from Peptides International.

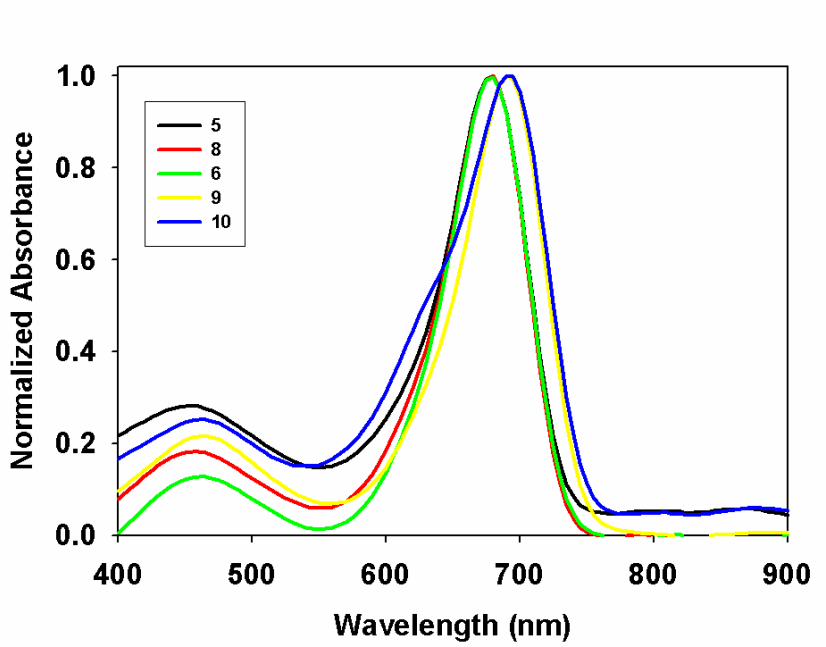
Fig. S1. Abs and emission properties of 3a in CrEL/water solution.



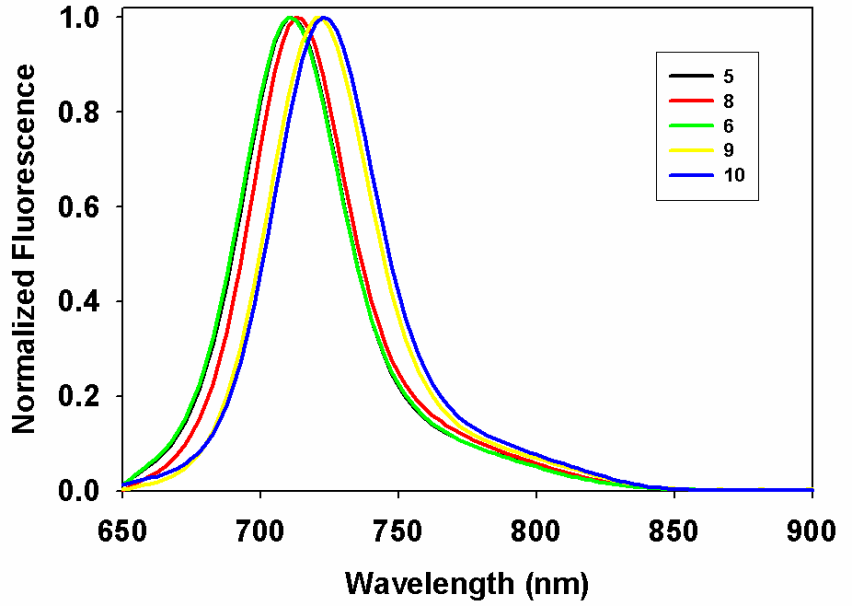
Solvent	Abs	Ex. Coeff.	Em
H_2O/CrEL[a]	709 nm	74,000	740 nm

[a]0.1% CrEL used.

Fig. S2. Normalized absorption and emission spectrum of 5, 6, 8, 9 and 10.[a]



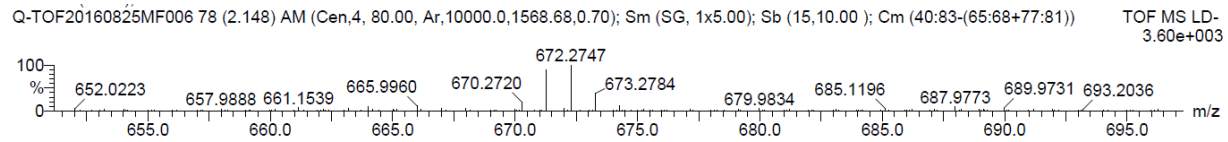
[a] Spectra for **5**, **6** and **8** were recorded in $CHCl_3$, **9** and **10** were recorded in water/1% SDS.



[a]Spectra for **5**, **6** and **8** were recorded in $CHCl_3$, **9** and **10** were recorded in water/1%SDS.

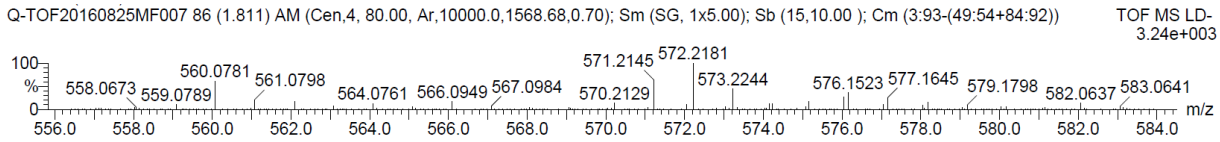
Fig. S3.

HRMS MALDI-QTOF of compound 5.



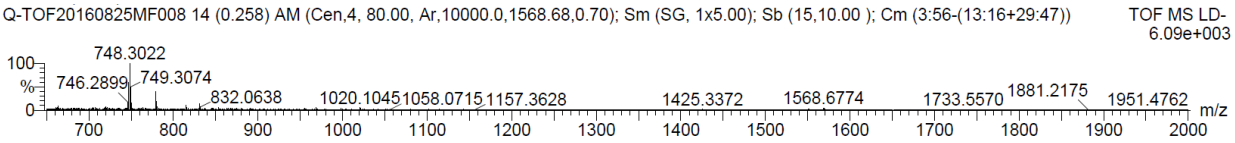
Minimum:				-1.5			
Maximum:		5.0	50.0	500.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
672.2747	672.2719	2.8	4.2	24.0	150.7	0.0	C39 H35 N4 O4 F2 B

HRMS MALDI-QTOF of compound 6.



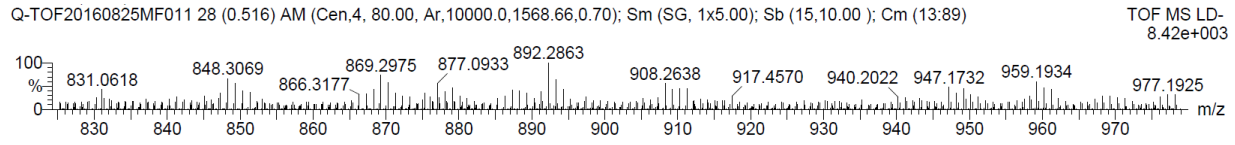
Minimum:				-1.5			
Maximum:		5.0	50.0	500.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
572.2181	572.2195	-1.4	-2.4	23.0	171.8	0.0	C34 H27 B N4 O2 F2

HRMS MALDI-QTOF of compound 8.



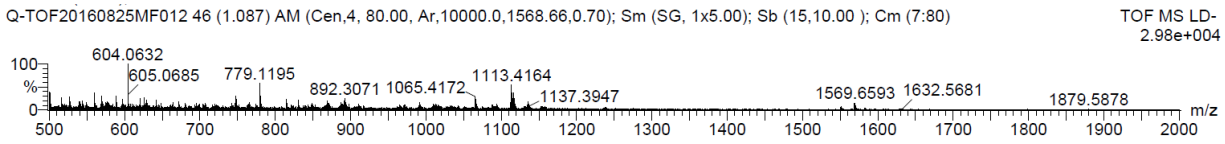
Minimum:				-1.5			
Maximum:		5.0	50.0	500.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
748.3022	748.3032	-1.0	-1.3	28.0	123.4	0.0	C45 H39 B N4 O4 F2

HRMS MALDI-QTOF of compound 4.



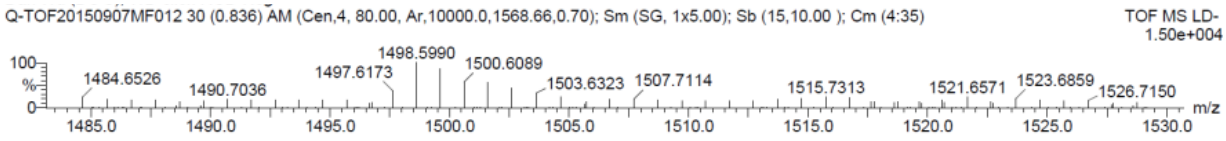
Minimum:				-1.5			
Maximum:		5.0	10.0	500.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
869.2975	869.2992	-1.7	-2.0	28.5	115.8	0.0	C48 H44 B N4 O7 F2 S

HRMS MALDI-QTOF of compound 9.



Minimum:				-1.5									
Maximum:		5.0	10.0	500.0									
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula						
1113.4164	1113.4163	0.1	0.1	31.5	81.9	0.0	C57	H60	B	N8	O11	F2	S

HRMS MALDI-QTOF of compound 10.



Minimum:				-1.5									
Maximum:		5.0	5.0	200.0									
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula						
1498.5990	1498.6026	-3.6	-2.4	41.5	61.5	0.0	C75	H83	B	N15	O14	F2	S

Fig. S4. Cell imaging with compound 10

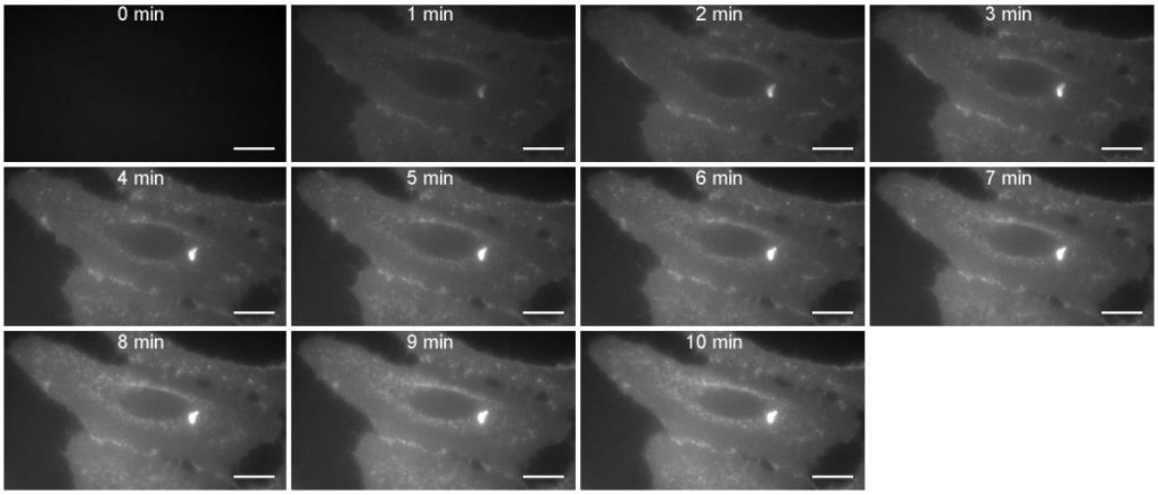


Fig. S4.1. HeLa-Kyoto cells after addition of **10** and the acquisition of a 4D video made up of z-scans acquired every sixty seconds over the course of 10 min. Increasing fluorescence on the cell surface is demonstrated in this montage summary of the video shown in movie S1. Scale bar = 10µm.

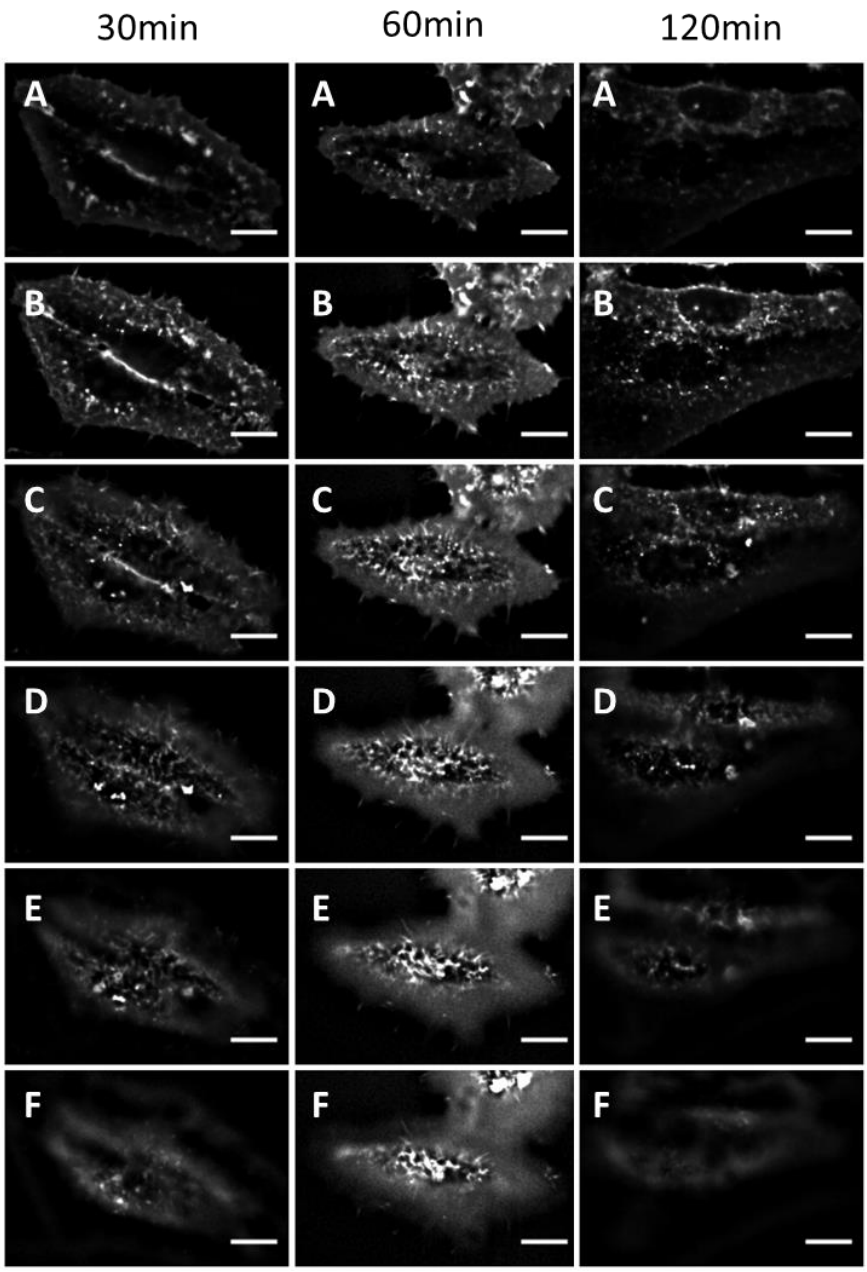


Fig. S4.2. HeLa-Kyoto cells were pre-incubated with **10** for 30, 60 and 120 min and z-scans were acquired. Six slices from the bottom (A) to top (F) show the localisation of **10** after the allotted time (see movie S2-4). Scale bar = 10 μm.

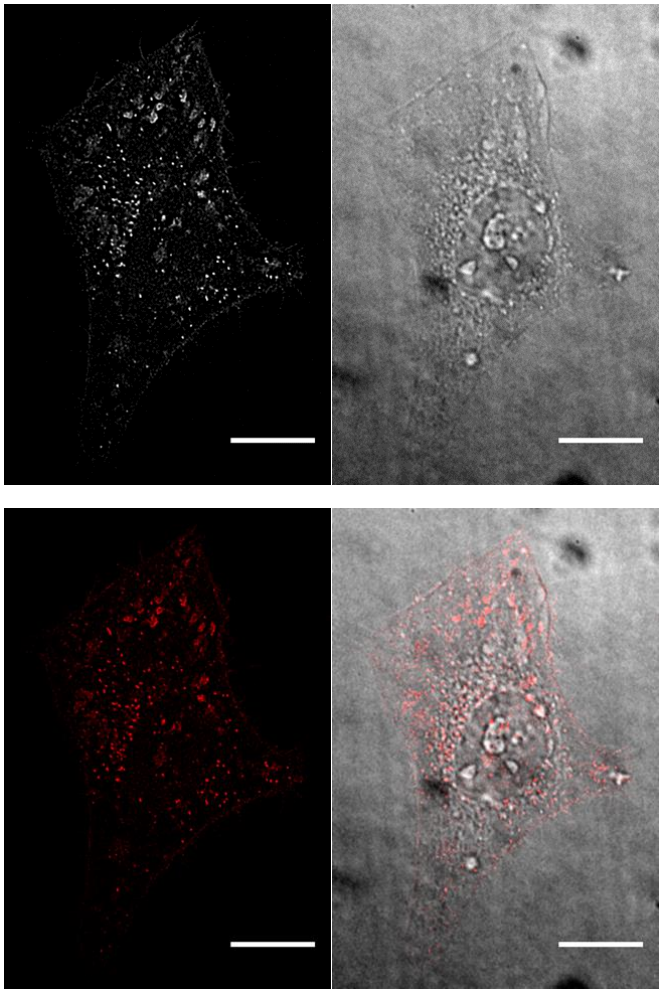


Fig. S4.3. After 120 min pre-incubation the fluorophore has been internalised by the cell, and the fluorescence has a more punctuated distribution. Top: Grey channel, bottom: red channel. Scale bar = 10 µm.

Fig. S5. *In vivo* imaging time course with compound 10.

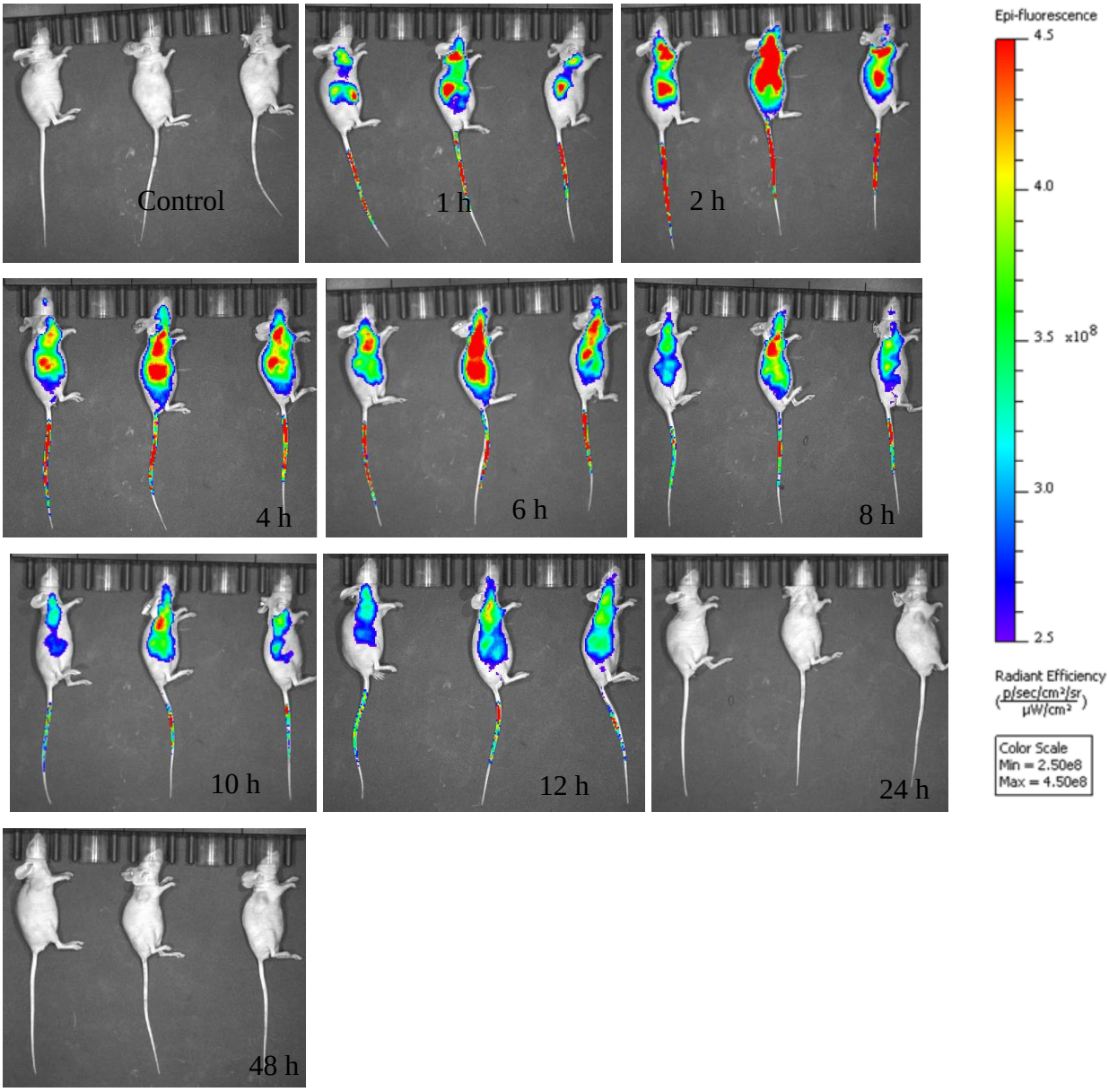
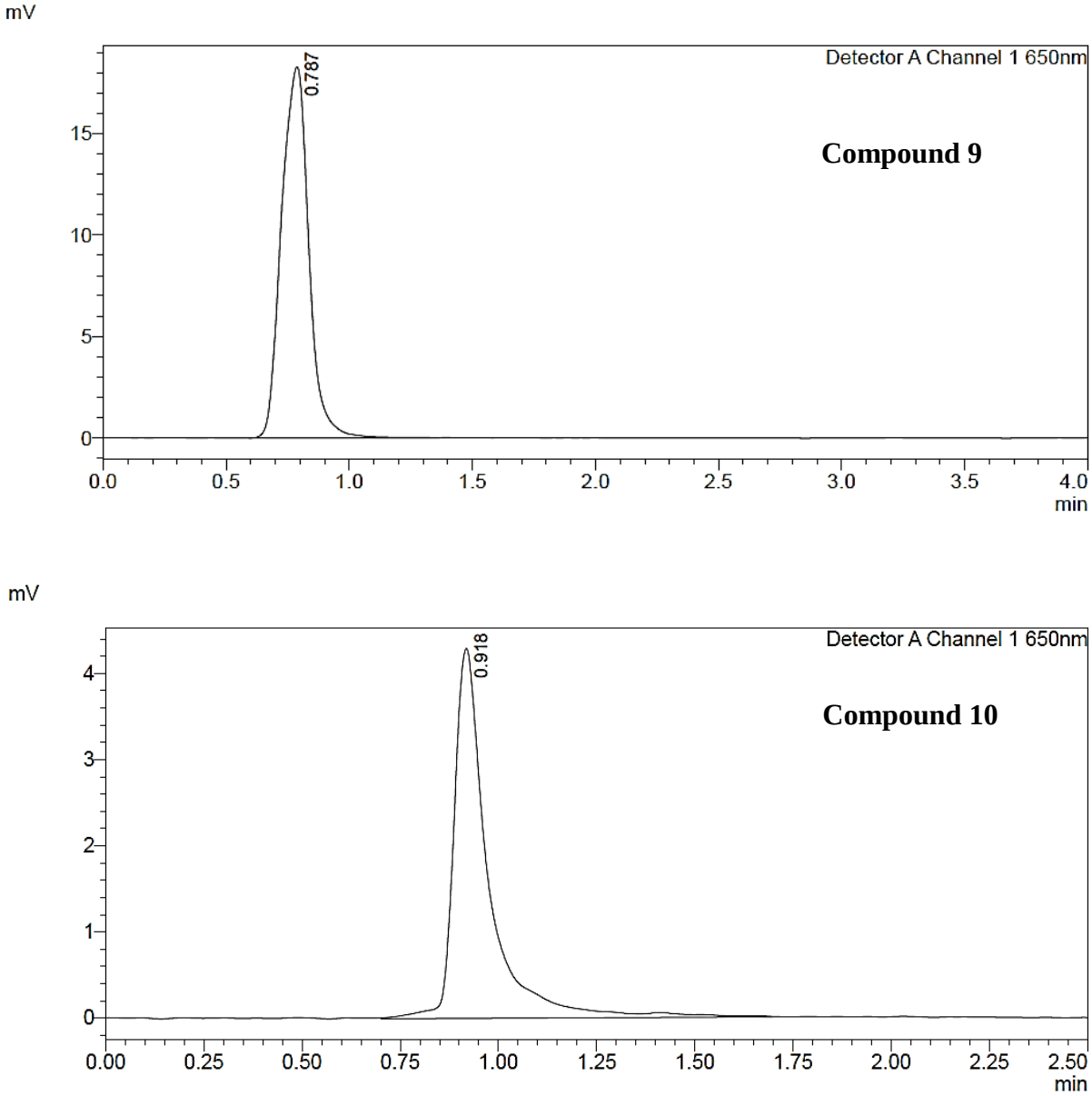


Fig. S5. *In vivo* imaging of **10** using a human esophageal cancer cell line Eca-109 subcutaneous tumour model in mice. NIR fluorescence imaging at 1, 2, 4, 6, 8, 10, 12, 24 and 48 h post intravenous (i.v.) administration of **10** (excit. 640 nm, emis. 700 nm).

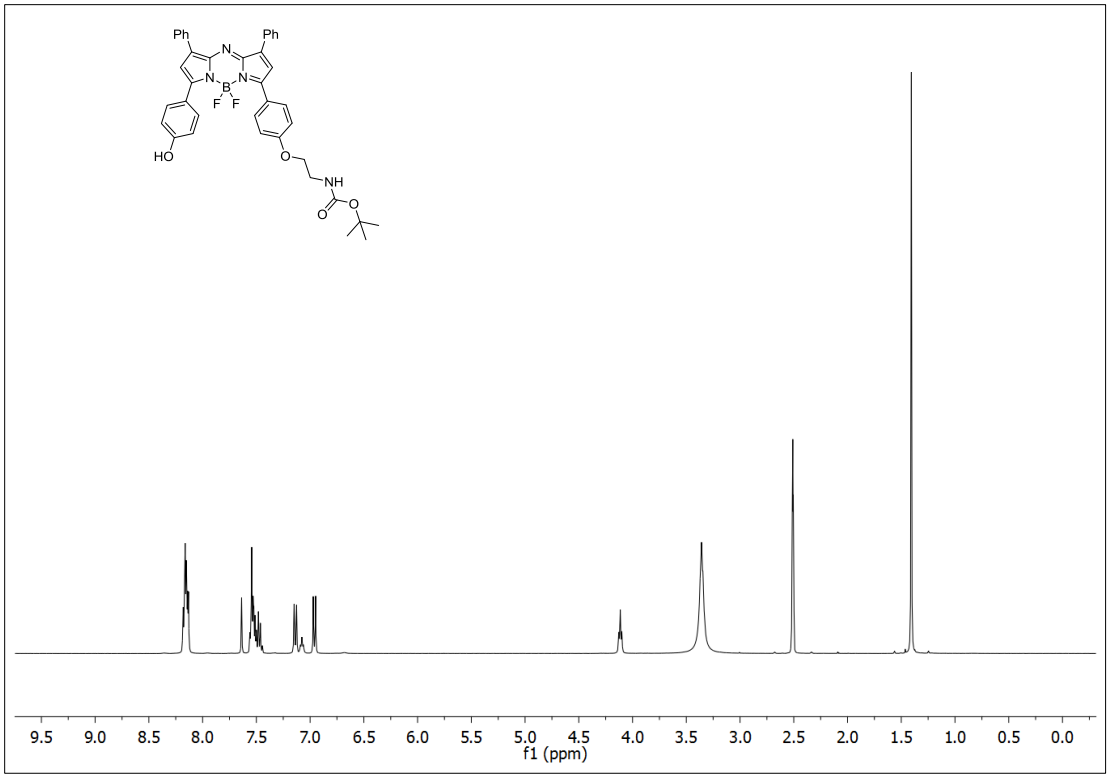
Fig. S6. HPLC trace of compound 9 and compound 10.



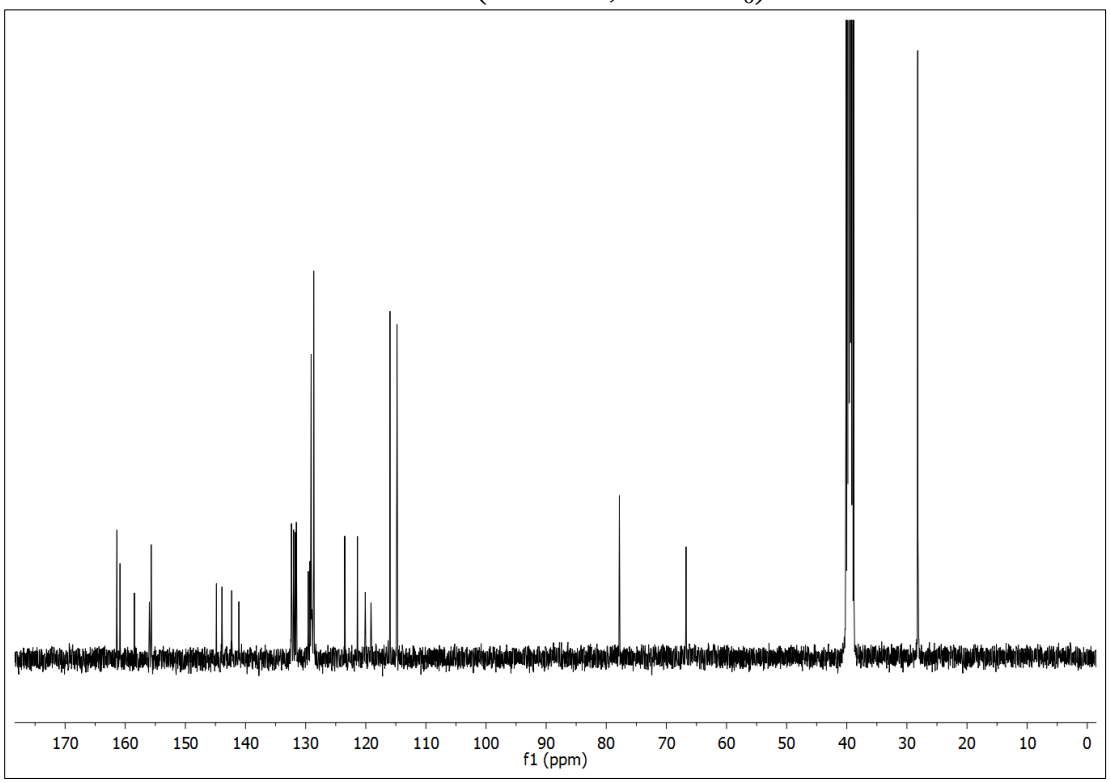
Column: reverse phase HLPC with YMC triart phenyl column and size: 150 × 4.6 mm I.D., particle size: S-5 µm, 12 nm hole, detection method: UV-Vis and wavelength for detection: 650 nm. Eluent CH_3CN : H_2O=70:30.

Compound 5

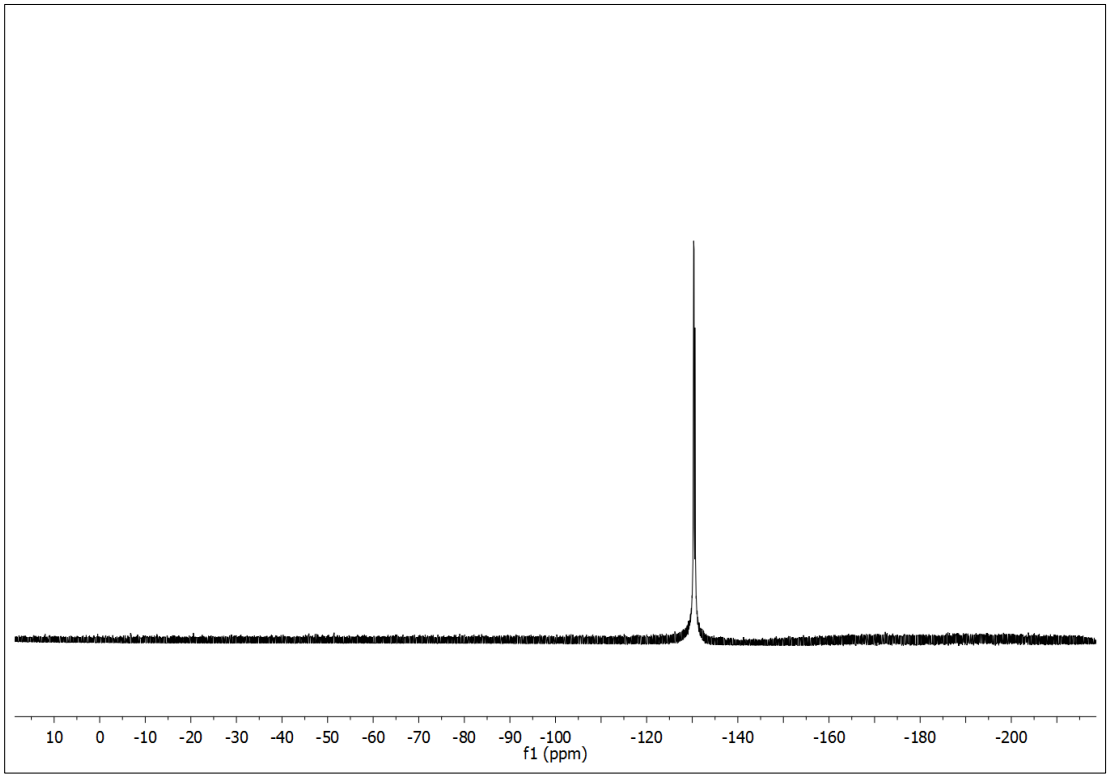
^{1}H NMR (400 MHz, DMSO-d_6)



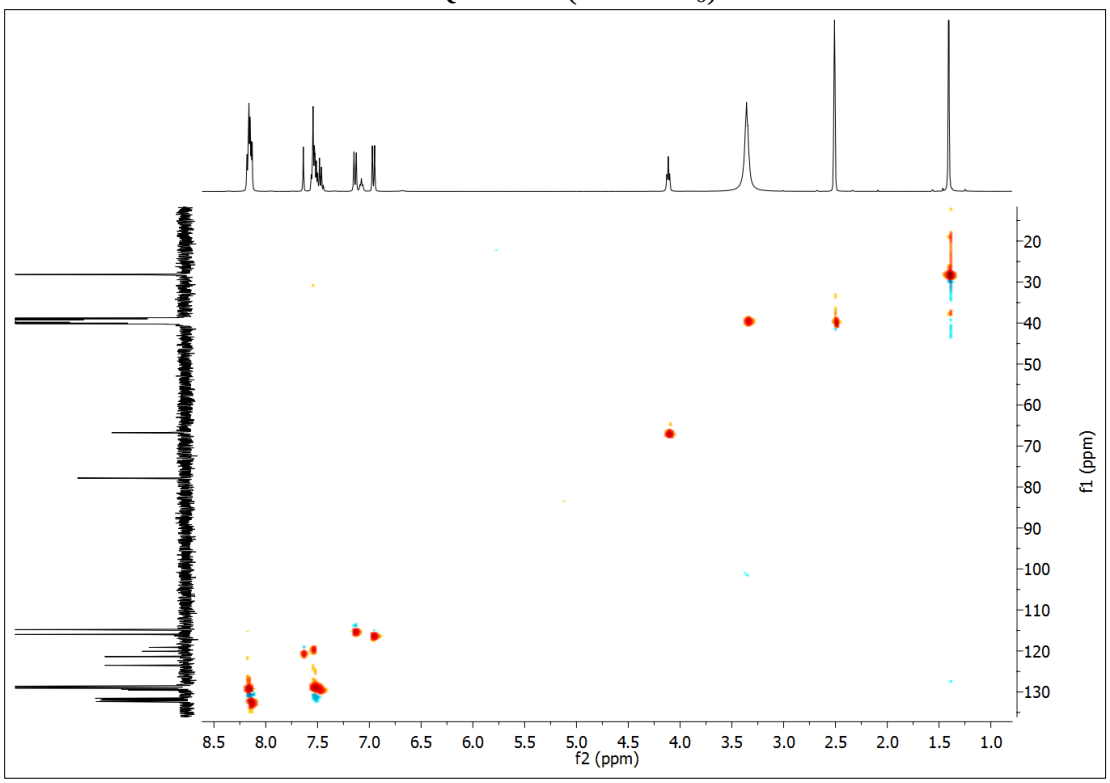
^{13}C NMR (100 MHz, DMSO-d_6)



^{19}F NMR (375 MHz, DMSO-d_6)

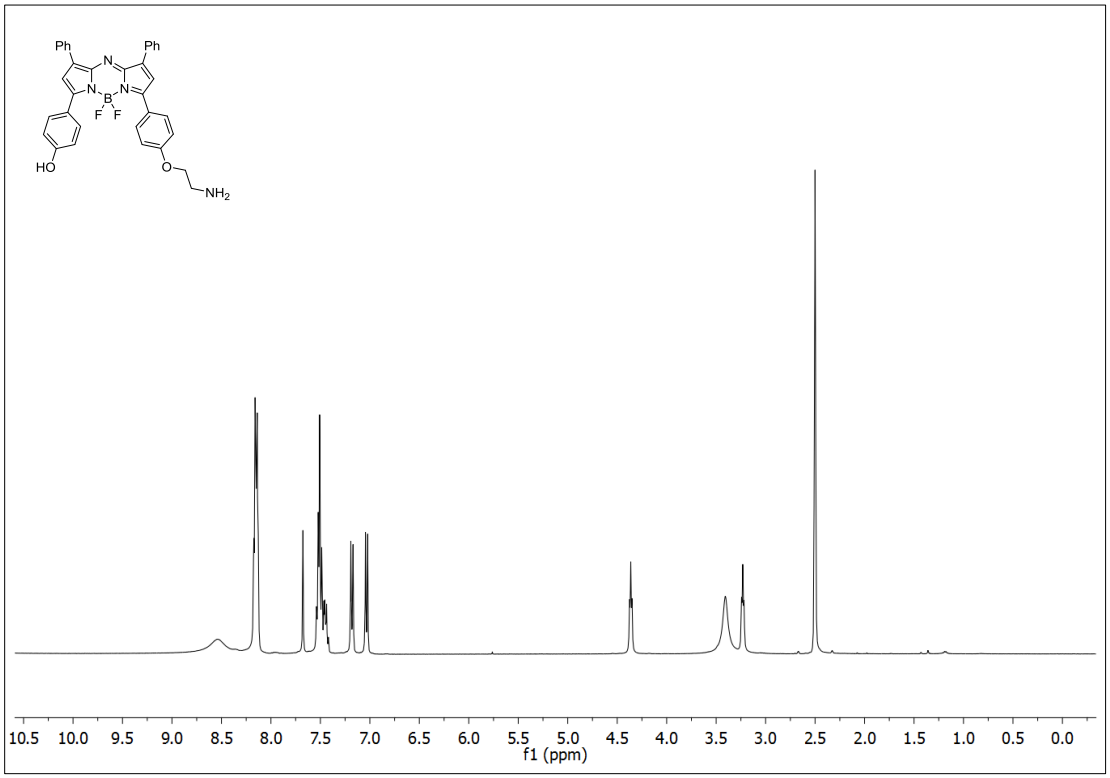


HSQC NMR (DMSO-d_6)

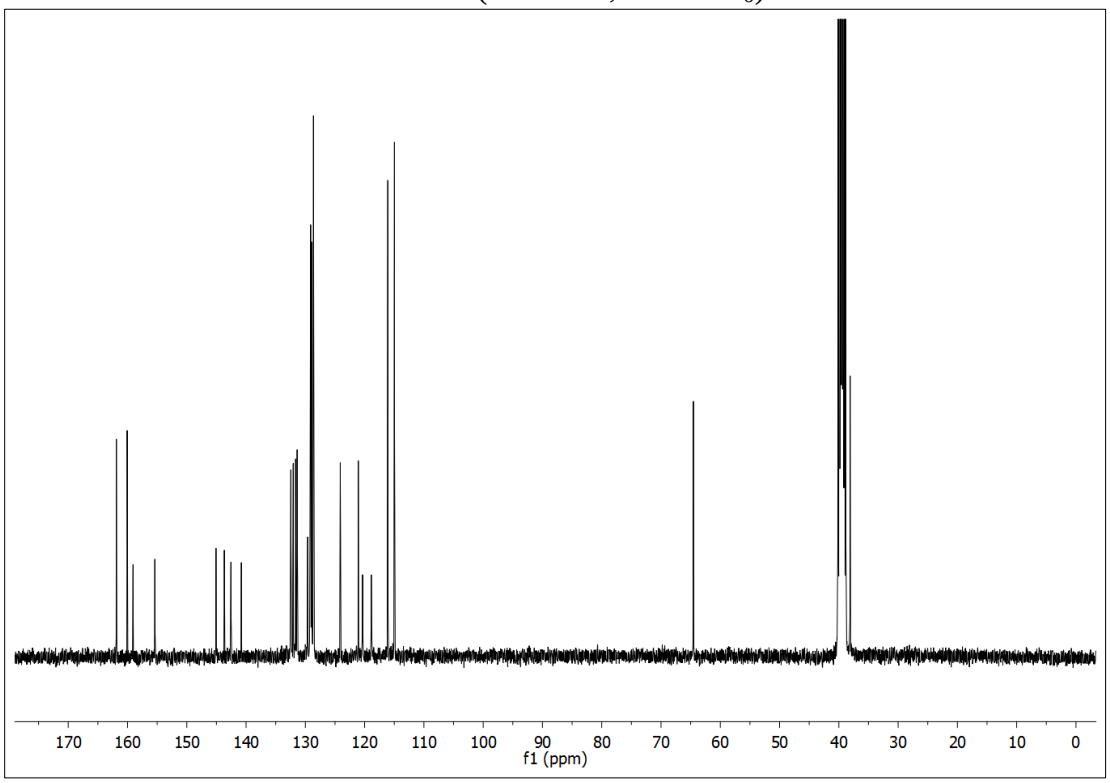


Compound 6

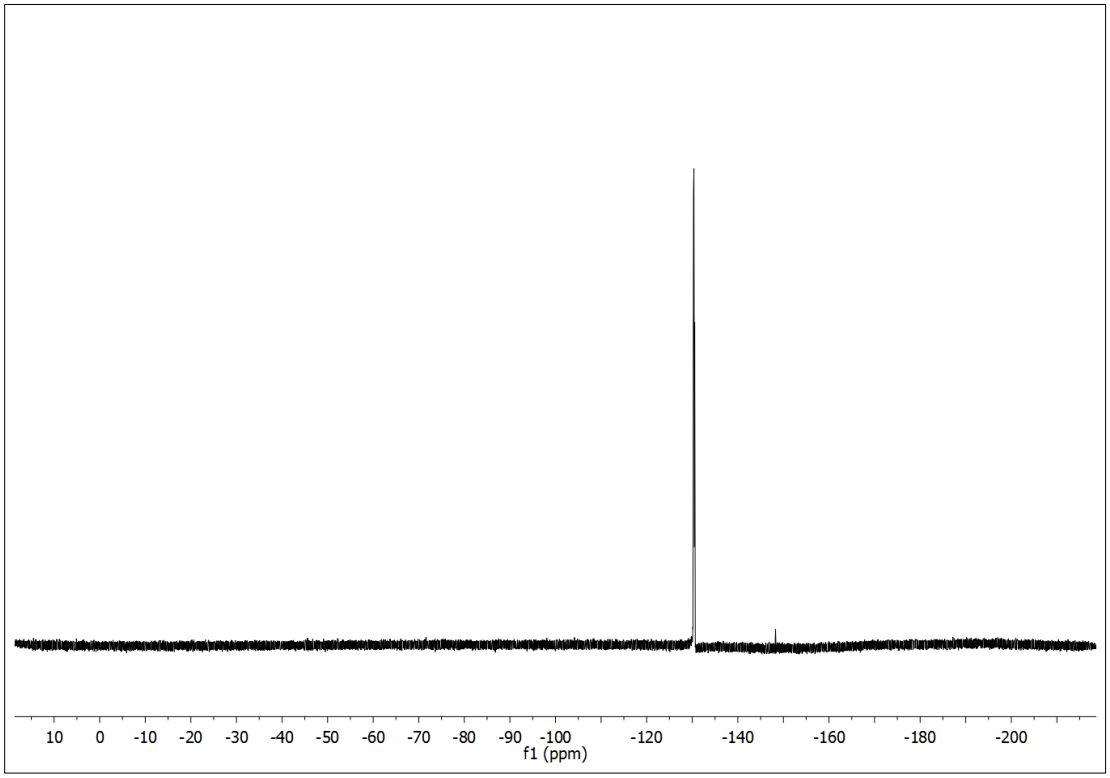
^{1}H NMR (400 MHz, DMSO-d_6)



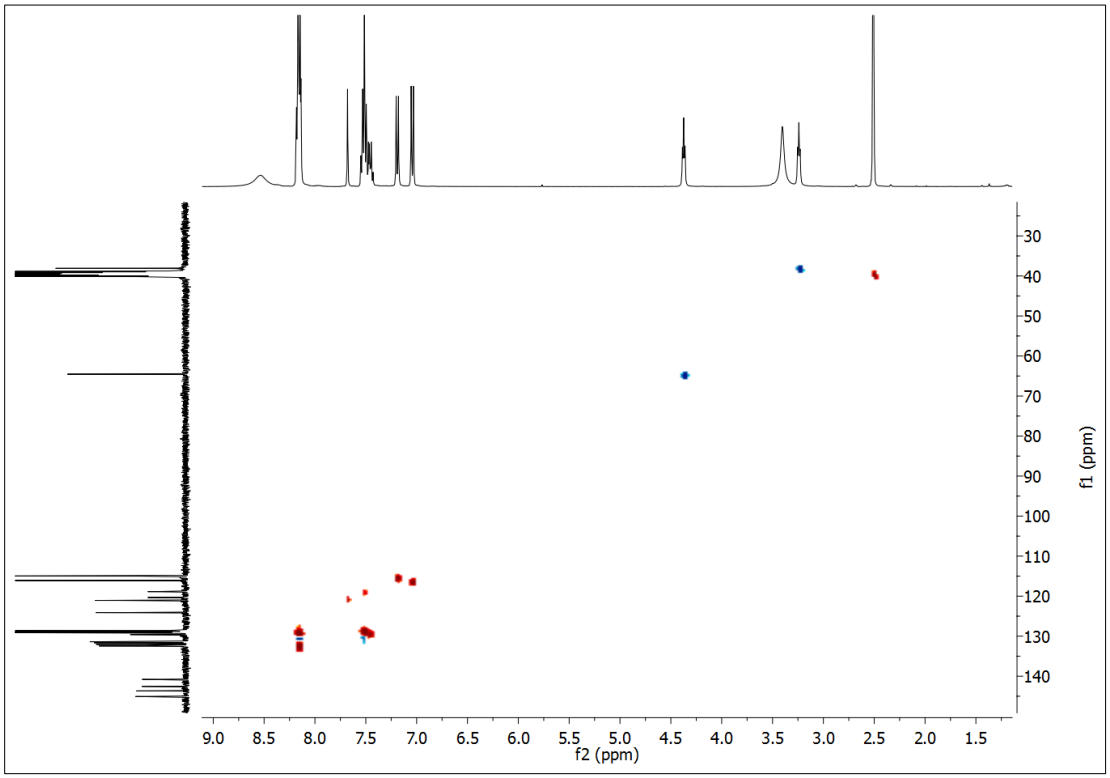
^{13}C NMR (100 MHz, DMSO-d_6)



^{19}F NMR (375 MHz, DMSO-d_6)

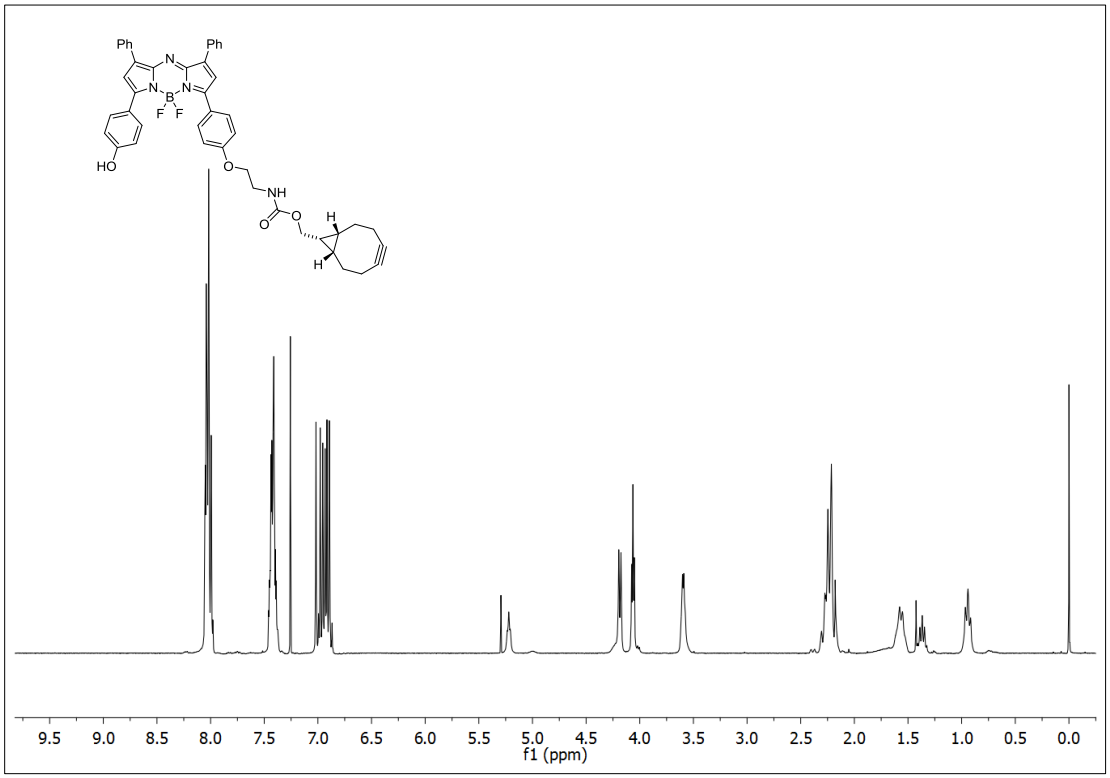


HSQC NMR (DMSO-d_6)

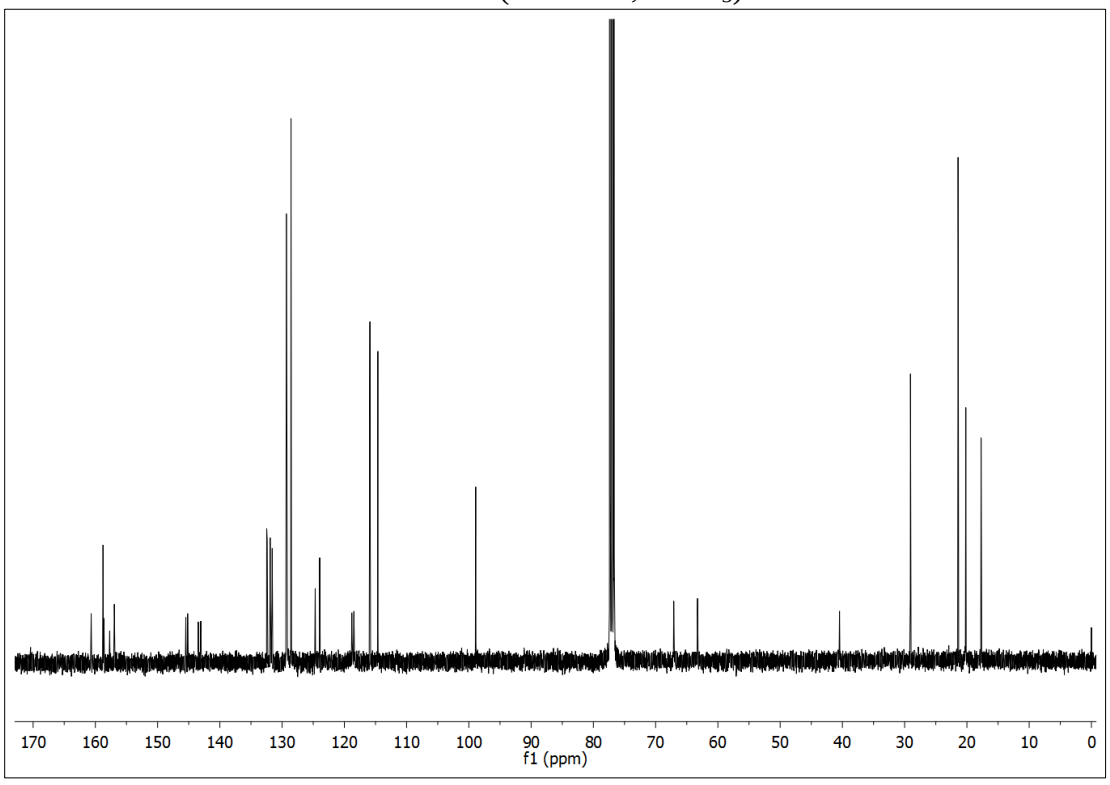


Compound 8

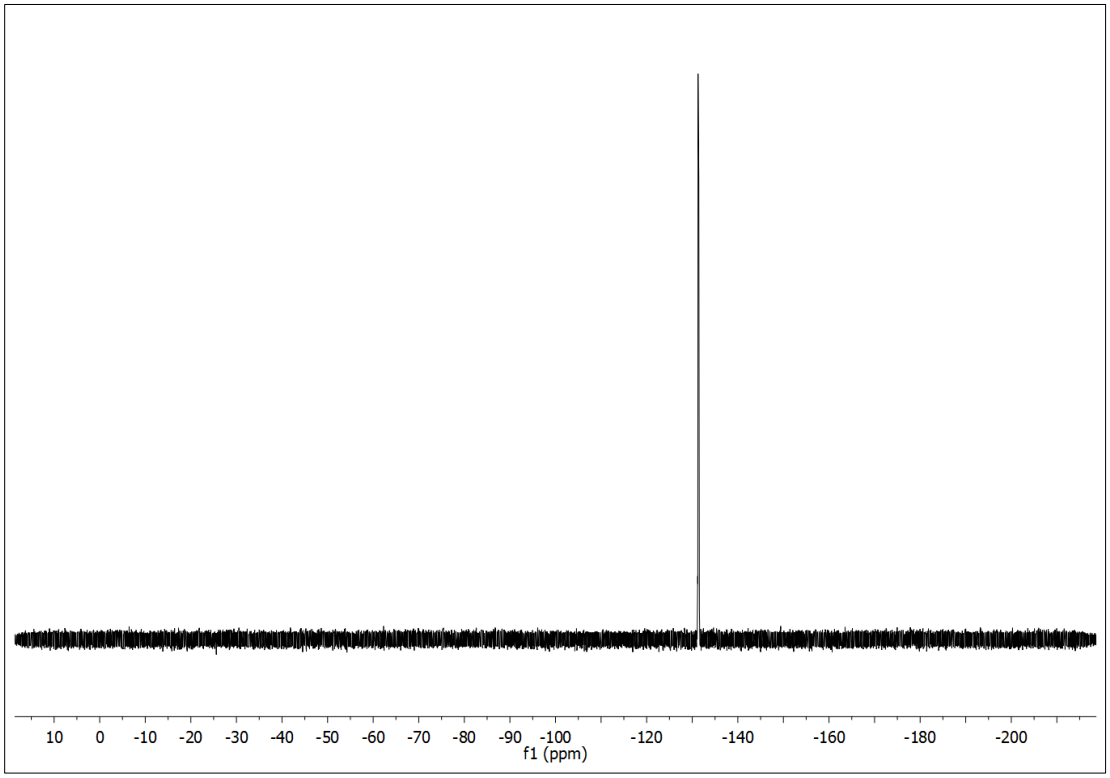
^{1}H NMR (400 MHz, $CDCl_3$)



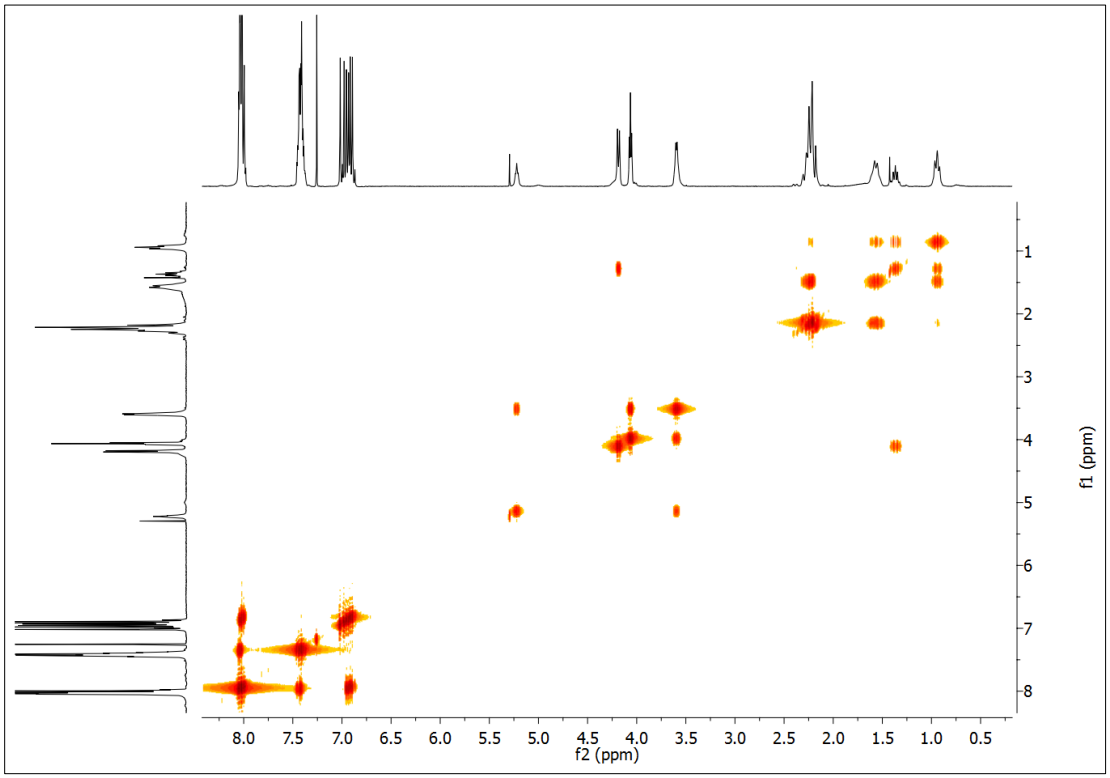
^{13}C NMR (100 MHz, $CDCl_3$)



^{19}F NMR (375 MHz, $CDCl_3$)

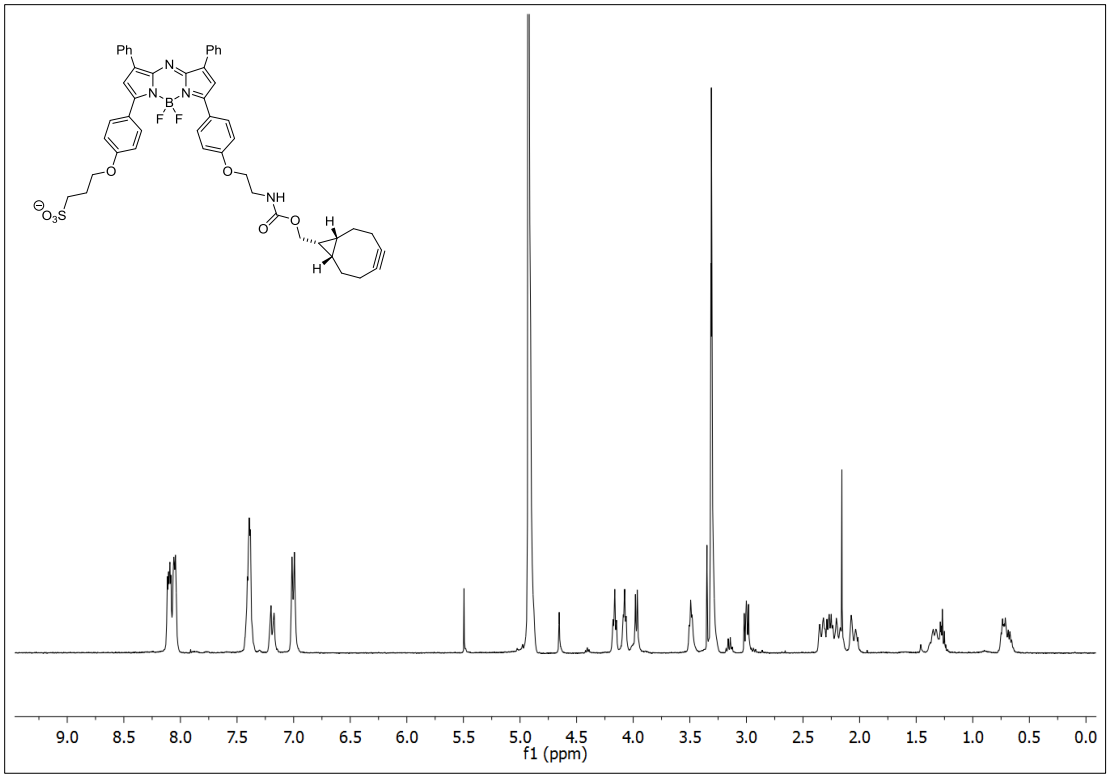


^{1}H COSY NMR ($CDCl_3$)

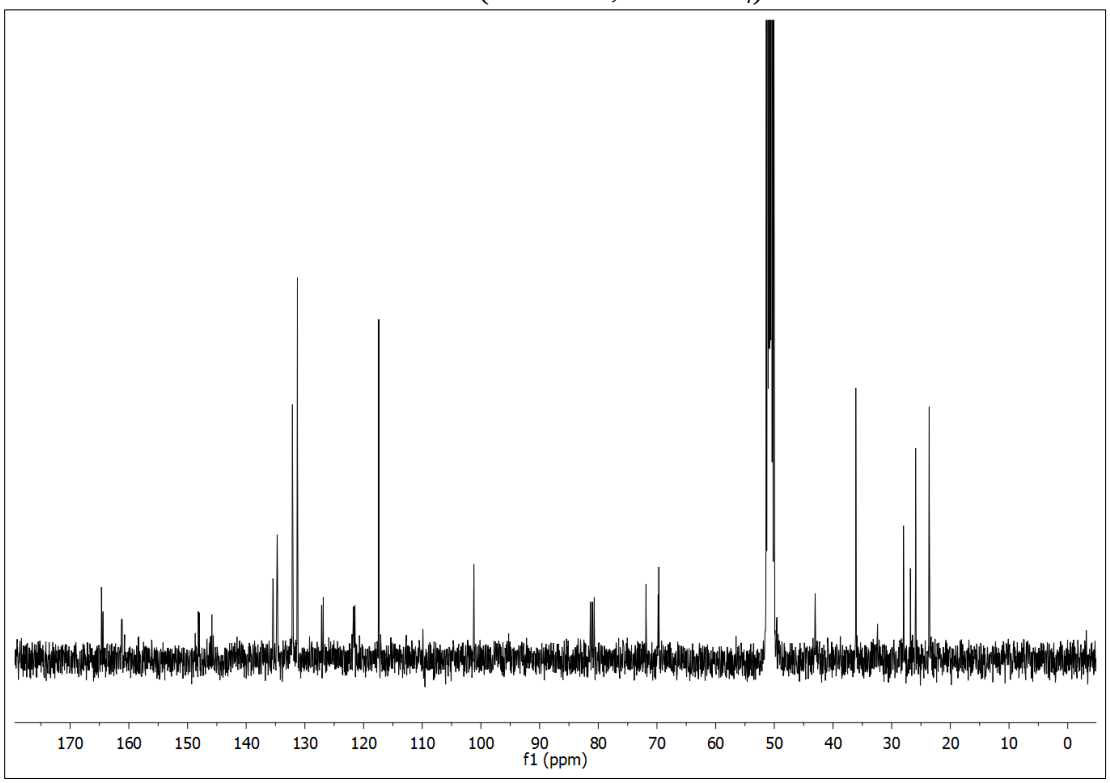


Compound 4

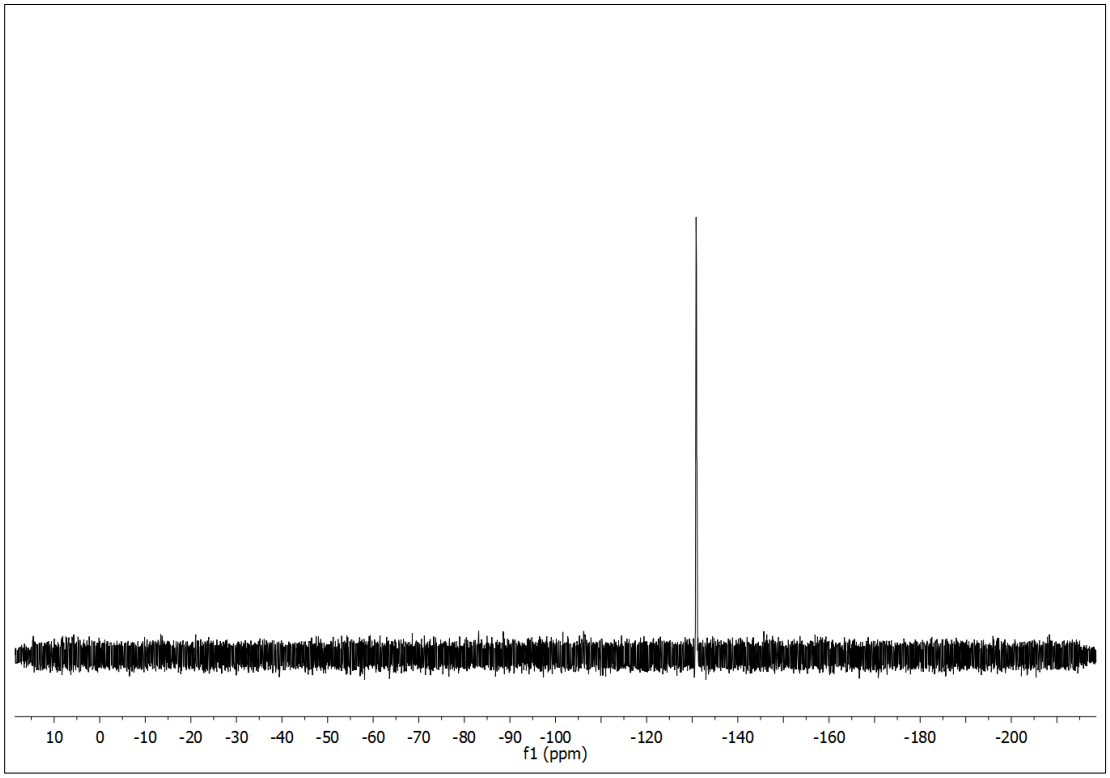
^{1}H NMR (400 MHz, MeOD-d_4)



^{13}C NMR (100 MHz, MeOD-d_4)



^{19}F NMR (375 MHz, MeOD-d_4)



^{1}H COSY NMR (MeOD-d_4)