**Supporting Information**

# Cooperative Organelle Targeting from a Single Molecular Module: Sterically Hindered Phenol–Diaminopyridine–Functionalized Cycloalkynes for Mitochondria–Endoplasmic Reticulum Localization

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## 1 General information and methods

Solvents, reagents, and chemicals (Fmoc-Cl, $Co_2(CO)_8$, 1,4-dimetoxybut-2-yne, $BF_3{\cdot}OEt_2$, $TBAF{\cdot}3H_2O$, $B_2(OH)_4$, 4,4´-bipyridine, $CSCl_2$, 3-aminopropan-1-ol, *p*-nosylchloride) used for reactions were purchased from commercial suppliers. The chemicals were used without further purification. **SHP-DAP**[1] was synthesized according to known procedures without any modifications. AF 343 (Cumarin)-X-Azid, LumiTracker® Mito Red FM (mito-tracker red), LumiTracker® ER Red (ER-tracker red), and LumiTracker® ER Green (ER-tracker green) were purchased from Lumiprobe RUS Ltd (Russia). Photophysical properties of the AF 343 (Cumarin)-X-Azid and trackers are provided by Lumiprobe at https://ru.lumiprobe.com. Solvents were purified under standard conditions. The purification and drying of DCM were carried out in accordance with the literature procedure using $CaH_2$.[2] Evaporation of solvents and concentration of reaction mixtures were performed under vacuum at 25 °C (for cycloalkynes) and at 30 °C (for other compounds) on a rotary evaporator. TLC was carried out on silica gel plates (silica gel 60, UV 254) with detection by UV (254 nm or 365 nm) or staining with a basic aqueous solution of $KMnO_4$. A normal-phase silica gel (Silica gel 60, 230−400 mesh) was used for preparative column chromatography. $^1H$ and $^{13}C\{^1H\}$, DEPT, HSQC, HMBC, COSY, NOESY NMR spectra were recorded at 400 (or 500) MHz and 101 (or 125) MHz, respectively, at 25 °C in an appropriate deuterated solvent without an internal standard. $^{31}P\{^1H\}$ NMR spectra were recorded at 162 MHz at 25 °C. The $^1H$ NMR data are reported as chemical shifts (δ), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; br, broad), coupling constants (*J*, given in Hz), and number of protons (for $^1H$ NMR). The $^{13}C\{^1H\}$ NMR and $^{31}P\{^1H\}$ NMR data are reported as chemical shifts (δ). Chemical shifts for $^1H$ and $^{13}C$ are reported as δ values (ppm) and referenced to residual solvents (δ = 7.26 ppm for $^1H$; δ = 77.16 ppm for $^{13}C$ for spectra recorded in $CDCl_3$; δ = 2.05 ppm for $^1H$; δ = 29.84 ppm for $^{13}C$ for spectra recorded in acetone-$d_6$; δ = 1.94 ppm for $^1H$; δ = 1.32 ppm for $^{13}C$ for spectra in $CD_3CN$; δ = 2.50 ppm for $^1H$; δ = 39.52 ppm for $^{13}C$ for spectra in DMSO-$d_6$). Chemical shifts of signals in $^{31}P$ NMR are referred to 85% aqueous $H_3PO_4$. High resolution mass spectra were acquired in positive mode on an AB Sciex TripleTOF 5600+ mass spectrometer equipped with DuoSpray (ESI) and Photospray (APPI) ion sources. Samples were dissolved in an appropriate solvent (MeCN or MeOH), diluted by methanol and injected in methanol flow (0.1 mL/min) via T-infusion adapter for ESI, and toluene was used in the same way for APPI.

## 2 Synthetic procedures

### 2.1 General procedures

**General procedure (I) for the Nicholas cyclization**

To a stirred cooled to 0 °C solution of sulfonamide (**1** or **2** or **5**) (1.00 equiv) and $Co_2(CO)_6$-complex of 1,4-dimethoxy-2-butyne **3** (1.00 equiv) in absolute DCM (c = 0.01 M) under an argon atmosphere $BF_3{\cdot}OEt_2$ (3.00 equiv) was added in one portion. The cooling bath was removed, and the resulting solution was allowed to warm to room temperature and then was stirred at this temperature for the corresponding time (TLC control). After reaction completion, the reaction mixture was quenched with aqueous solution of $NaHCO_3$, the organic layer was separated and the aqueous layer was extracted with DCM two times. The combine organic layers were washed with brine until pH = 7, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by column chromatography on silica gel.

**General procedure (II) for deprotection of cycloalkynes from Co**

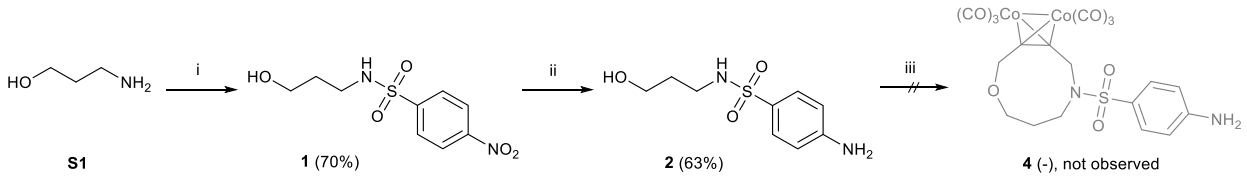
To a stirred solution of cyclic Co-complex (**4** or **6** or **7**) in a mixture of acetone/water (15:1, v/v, c = 0.006 M), tetrabutylammonium fluoride trihydrate (TBAF·3$H_2O$) (20.0 – 22.0 equiv) was added in four portions with the interval of 50-60 min. After completion of the reaction, the reaction mixture was filtered through a pad of Celite, the sorbent was washed with acetone, and the resulting solution was concentrated under reduced pressure at 25 °C up to ~ 1/5 of the original volume; the resulting mixture was mixed with ethyl acetate and brine. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed three times with brine, dried over anhydrous $Na_2SO_4$, and the solvent was evaporated under reduced pressure at 25 °C. The crude product was purified by column chromatography on silica gel.

**General procedure (III) for SPAAC**

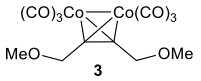
The reaction must be carried out under preventing of a direct light. To a stirred solution of cycloalkyne (**hybrid I** or **hybrid II** or **8**) (1.00 equiv) in acetonitrile or in $CDCl_3$ (c = 0.01 M) coumarin-X-azide **AF-343** (0.70 equiv) was added in one portion. The resulting solution was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel to give a mixture of isomeric triazoles.

## 2.3 Synthesis of cycloalkynes

### 2.3.1 Synthesis of OACN-$NH_2$ via path A: Nicholas cyclization for a substrate 2 with unprotected $NH_2$ group

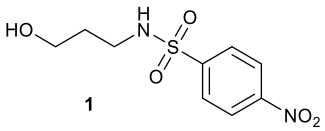



**Reagents and conditions:** i) NsCl (1.10 equiv), TEA (1.10 equiv), DCM, 0 °C to r.t., overnight; ii) Fe powder (10.0 equiv), $NH_4Cl$ (10.0 equiv), acetone/$H_2O$ 1:1, 65 °C, 24 h; iii) Co-complex **3** (1.0 equiv), $BF_3{\cdot}OEt_2$ (3.00 equiv), $DCM_{abs}$, 0 °C to r.t.,1 h.




**Scheme S1.** Synthetic procedure for the path A.

***N*-(3-hydroxypropyl)-4-nitrobenzenesulfonamide 1**

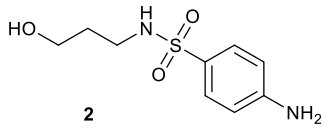



To a stirred solution of 3-aminopropan-1-ol **S1** (1.50 g, 1.53 mL, 20.0 mmol, 1.00 equiv) in DCM (350 mL), triethylamine (2.22 g, 3.06 mL, 22.0 mmol, 1.10 equiv) was added in one portion. The resulting solution was purged with argon and cooled to 0 °C. A solution of 4-nitrobenzenesulfonyl chloride (NsCl) (4.43 g, 20.0 mmol, 1.00 equiv) in DCM (10 mL) was then added dropwise during 30 minutes while maintaining the temperature at 0–5 °C. A cooling bath was immediately removed, and the resulting solution was allowed to warm to room temperature. It was then stirred at room temperature overnight. After the reaction was completed (TLC control), the reaction mixture was poured into a saturated aqueous solution of $NH_4Cl$ (200 mL) and extracted

with DCM three times (3 × 100 mL). The combined organic layers were washed with a saturated solution of $NH_4Cl$ (200 mL) and two times with brine (300 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: hexane/ethyl acetate = 1:2) that gave **1** as a white powder (3.65 g, 70%).

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.40 – 8.33 (m, 2H), 8.11 – 8.03 (m, 2H), 5.44 (br. s, 1H), 5.30 (s, 1H), 3.77 (t, *J* = 5.5 Hz, 2H), 3.20 (t, *J* = 6.1 Hz, 2H), 1.79 – 1.70 (m, 2H, overlapping with water signal). $^1$H NMR spectrum corresponds with the literature data.[3]

**4-amino-*N*-(3-hydroxypropyl)benzenesulfonamide 2**

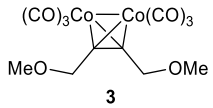


**2**

In a two-necked round bottom flask to a stirred solution of nitro compound **1** (995 mg, 3.82 mmol, 1.00 equiv) in the mixture of acetone / $H_2O$ (1:1, c = 0.02 M, 191 mL) were added iron powder (2.13 g, 38.2 mmol, 10.0 equiv) and $NH_4Cl$ (2.04 g, 38.2 mmol, 10.0 equiv). The reaction mixture was stirred at 65 °C for 24 hours (TLC control). After completion of the reaction, the reaction mixture was cooled, filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, diluted with ethyl acetate (100 mL), and then poured into saturated aqueous NaCl solution (200 mL) and extracted with ethyl acetate two times (2 × 50.0 mL). The combined organic layers were washed with brine (3 × 100 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give aminobenzenesulfonamide **2** (550 mg, 63%) as a yellow oil. The product was used in the next step without further purification.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.43 – 7.35 (m, 2H), 6.98 (t, *J* = 5.9 Hz, 1H), 6.64 – 6.57 (m, 2H), 5.88 (br. s, 2H), 4.38 (t, *J* = 5.1 Hz, 1H), 2.74 – 2.65 (m, 2H), 1.54 – 1.45 (m, 2H), one $CH_2$-group is overlapping with water signal. $^1$H NMR spectrum corresponds with the literature data.[4]

**Hexacarbonyl($\mu_2$-(3,4-$\eta^2$,$\eta^2$)-3,4-dimethoxy-2-butyne 3**



**3**

To a stirred solution of 1,4-dimethoxybut-2-yne (154 mg, 0.163 mL, 1.35 mmol, 1.00 equiv.) in absolute dichloromethane (135 mL, c = 0.01 M) under an argon atmosphere, octacarbonyl dicobalt (600 mg, 1.76 mmol, 1.30 equiv.) was added. The reaction mixture was stirred at room temperature under an argon atmosphere for 1 hour (TLC control). Then, dichloromethane was removed on a rotary evaporator, and the residue was purified by column chromatography on silica gel using hexane/ethyl acetate = 10:1 as the eluent that gave complex **3** dark red powder (0.434 mg, 80%).

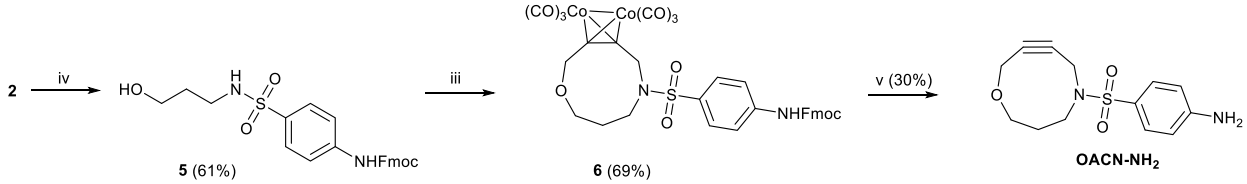
$^1$H NM (400 MHz, Acetone-$d_6$) δ 4.67 (s, 4H), 3.50 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ = 199.6, 92.2, 73.0, 59.0. NMR data corresponds with the data reported earlier.[5]

**Attempt for the synthesis of Co-complex 4 by the Nicholas cyclization**

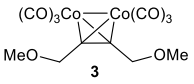
It was an attempt to obtain Co-complex **4** using the **General procedure (I)** from sulfonamide **2** (30.0 mg, 0.130 mmol, 1.00 equiv) and $Co_2(CO)_6$-complex of 1,4-dimethoxy-2-butyne **3** (44.5 mg,

0.130 mmol, 1.00 equiv) in absolute DCM (13.0 mL) using $BF_3{\cdot}OEt_2$ (55.4 mg, 48.1 µL, 0.390 mmol, 3.00 equiv). TLC control after 1 hours revealed only complex mixture of products.

2.3.2 Synthesis of OACN-$NH_2$ via path B: Nicholas cyclization for a substrate **5** with a Fmoc-protected $NH_2$ group

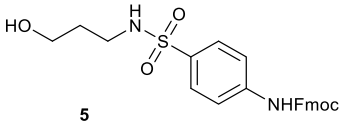



**Reagents and conditions:** iii) Co-complex **3** (1.0 equiv), $BF_3{\cdot}OEt_2$ (3.00 equiv), $DCM_{abs}$, 0 °C to r.t.,1 h; iv) Fmoc-Cl (1.00 equiv), DMAP (1.00 equiv), DCM, 0 °C then r.t. 2 h.; v) TBAF (50.0 equiv), acetone / $H_2O$ 15:1, r.t., 24 h;




**Scheme S2.** Synthetic procedure for path B.

**(9*H*-Fluoren-9-yl)methyl (4-(*N*-(3-hydroxypropyl)sulfamoyl)phenyl)carbamate 5**



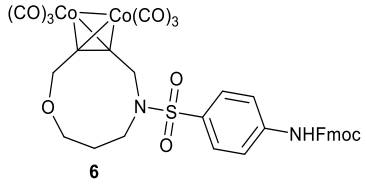

To a stirred solution of **2** (25.0 mg, 0.109 mmol, 1.00 equiv) in DCM (10.0 mL), freshly distilled pyridine (10.3 mg, 10.5 µL, 0.130 mmol, 1.20 equiv) was added in one portion. The resulting solution was purged with argon and cooled to 0 °C. A solution of fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) (33.7 mg, 0.130 mmol, 1.20 equiv) in DCM (1.00 mL) was then added dropwise during 15 minutes while maintaining the temperature at 0°C. A cooling bath was immediately removed, and the resulting solution was allowed to warm to room temperature. It was then stirred at room temperature for 2 hours. After the reaction was completed (TLC control), the reaction mixture was poured into a saturated aqueous solution of $NH_4Cl$ (10 mL) and extracted with DCM three times (3 × 10 mL). The combined organic layers were washed with a saturated solution of $NH_4Cl$ (50 mL) and two times with brine (50 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: hexane/ethyl acetate = 1:1) that gave **5** as a white powder (30 mg, 61%).

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.25 (s, 1H), 7.05 (d, *J* = 7.5 Hz, 2H), 6.89 (d, *J* = 7.4 Hz, 2H), 6.87 – 6.79 (m, 2H), 6.76 (br. s, 2H), 6.57 (t, *J* = 7.4 Hz, 2H), 6.52 – 6.48 (m, 3H), 3.69 (d, *J* = 6.4 Hz, 2H), 3.55 (t, *J* = 5.1 Hz, 1H), 3.47 (t, *J* = 6.4 Hz, 1H), 1.95 – 1.88 (m, 2H), 1.66 – 1.63 (m, 2H), 0.70 – 0.61 (m, 2H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 153.3, 143.7, 142.6, 140.8, 133.7, 127.73, 127.69, 127.2, 125.1, 120.2, 117.9, 65.9, 58.1, 46.6, 40.0 (overlapping with solvent signal, visible in DEPT), 32.3.

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{24}H_{24}N_2NaO_5S^+$: 475.1298; Found: 475.1301.

**Hexacarbonyl((9*H*-fluoren-9-yl)methyl-μ$_2$-(7,8-η$^2$,η$^2$)-{4-[(7,8-didehydro-3,4,6,9-tetrahydro-1,5-oxazonin-5(2*H*)-yl)sulfonyl)phenyl]carbamate}dicobalt 6**




6

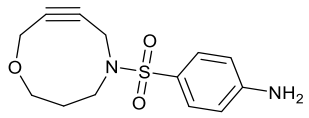
Co-complex **6** was obtained in accordance with **General procedure (I)** from Fmoc-amide **5** (23.0 mg, 0.058 mmol, 1.00 equiv), $Co_2(CO)_6$-comlex of 1,4-dimethoxy-2-butyne **3** (26.0 mg, 0.058mmol, 1.00 equiv) and $BF_3{\cdot}OEt_2$ (24.5 mg, 21.3 μL, 0.173 mmol, 3.00 equiv) in absolute DCM (5.80 mL). The reaction time was 1 h. Purification of crude product by column chromatography on silica gel (eluent: hexane/acetone = 3:1) gave cyclic Co-complex **6** as a brownish powder (31 mg, 69%).

$^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.32 (br. s, 1H), 7.93 – 7.82 (m, 4H), 7.82 – 7.72 (m, 4H), 7.48 – 7.39 (m, 2H), 7.39 – 7.30 (m, 2H), 5.09 (s, 2H), 4.71 (s, 2H), 4.59 (d, *J* = 6.4 Hz, 2H), 4.33 (t, *J* = 6.4 Hz, 1H), 4.02 – 3.92 (m, 2H), 3.42 – 3.36 (m, 2H), 2.00 – 1.91 (m, 2H).

$^{13}$C NMR (126 MHz, Acetone-$d_6$) δ 200.4 (CO signals), 154.2, 144.9, 144.4, 142.2, 132.6, 129.5, 128.7, 128.0, 126.0, 120.9, 119.0, 94.6, 93.2, 74.3, 67.9, 67.3, 56.5, 49.3, 47.9, 31.0.

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{34}H_{26}Co_2N_2O_{11}SNa^+$: 810.9814; Found: 810.9814

**4-[(7,8-didehydro-3,4,6,9-tetrahydro-1,5-oxazonin-5(2*H*)-yl)sulfonyl]aniline (OACN-NH$_2$)**




**OACN-NH$_2$**

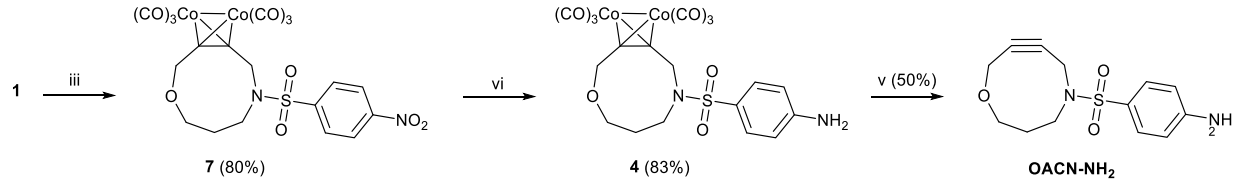
Alkyne OACN-NH$_2$ was obtained in accordance with **General procedure (II)** from cyclic Co-complex **6** (30.0 mg, 0.038 mmol, 1.00 equiv) in a mixture of acetone/water (15:1, v/v, c = 0.006 M, 6.30 mL), tetrabutylammonium fluoride trihydrate (TBAF·3H$_2$O) (120 mg, 0.380 mmol, 10.0 equiv), which was added in two portions with the interval of 40-50 min. Co-decomplexation took 3 h (TLC control). Then another portion of TBAF·3H$_2$O (120.0 mg, 0.380 mmol, 10.00 equiv) was added to the mixture in one portion to remove Fmoc grout that required overnight stirring. Purification of crude product by column chromatography on silica gel (eluent: hexane/ethyl acetate = 1:1) gave **OACN-NH$_2$** as a white powder (3.1 mg, 30%).

$^1$H NMR (500 MHz, $CD_3CN$) δ 7.50 – 7.47 (m, 2H), 6.73 – 6.69 (m, 2H), 4.86 (br. s, 2H), 4.05 (t, *J* = 2.3 Hz, 2H), 3.82 – 3.79 (m, 2H), 3.77 (t, *J* = 2.3 Hz, 2H), 3.22 – 3.17 (m, 2H), 1.81 – 1.75 (m, 2H).

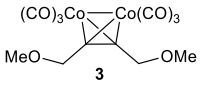
$^{13}$C NMR (126 MHz, $CD_3CN$) δ 153.5, 130.4, 124.7, 114.4, 93.1, 90.8, 65.8, 59.8, 45.3, 42.3, 34.2.

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{13}H_{17}N_2O_3S^+$: 281.0954; Found: 281.0956.

2.3.3 Synthesis of **OACN-NH$_2$** via path C: Nicholas cyclization for a $NO_2$-containing substrate **1** followed by $NO_2$ reduction before decomplexation

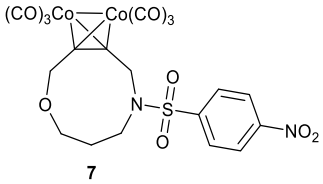



**Reagents and conditions:** iii) Co-complex **3** (1.0 equiv), $BF_3{\cdot}OEt_2$ (3.00 equiv), $DCM_{abs}$, 0 °C to r.t.,1 h; v) TBAF (21.0 equiv), acetone / $H_2O$ 15:1, r.t., 5 h; vi) $B_2(OH)_4$ (3.00 equiv), 4,4'-biPy (0.5 mol%), DMF, r.t., 1h.




**Scheme S3.** Synthetic procedure for path C.

**Hexacarbonyl(μ$_2$-(7,8-η$^2$,η$^2$)-7,8-didehydro-5-[(4-nitrophenyl)sulfonyl]-2,3,4,5,6,9-hexahydro-1,5-oxazonine)dicobalt 7**



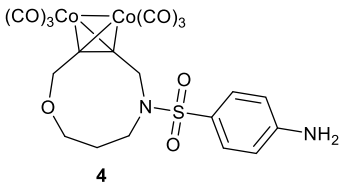

Co-complex **7** was obtained in accordance with **General procedure (I)** from *N*-Ns-3-aminopropan-1-ol **1** (201.8 mg, 0.775 mmol, 1.00 equiv), Co-complex **3** (310.0 mg, 0.775 mmol, 1.00 equiv) and $BF_3{\cdot}OEt_2$ (329.9 mg, 286.9 µL, 2.32 mmol, 3.00 equiv) in absolute DCM (c = 0.01 M, 77.5 mL). Reaction time was 1 h. Purification of crude product by column chromatography on silica gel (eluent: hexane/acetone = 4:1→ 3:1) that gave complex **7** as a brownish powder (371 mg, 80%).

$^1$H NMR (400 MHz, Acetone-$d_6$) δ 8.54 – 8.44 (m, 2H), 8.29 – 8.21 (m, 2H), 5.11 (s, 2H), 4.83 (s, 2H), 4.03 – 3.94 (m, 2H), 3.52 – 3.47 (m, 2H), 2.02 – 1.95 (m, 2H, overlapping with solvent signal).

$^{13}$C NMR (126 MHz, Acetone-$d_6$) δ 199.4, 150.4, 143.8, 128.9, 124.6, 93.8, 91.5, 73.3, 67.0, 55.6, 48.7, 30.0.

HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{19}H_{14}Co_2N_2NaO_{11}S^+$: 618.8875; Found: 618.8883

**Hexacarbonyl{μ$_2$-(7,8-η$^2$,η$^2$)-4-[(7,8-didehydro-3,4,6,9-tetrahydro-1,5-oxazonin-5(2*H*)-yl)sulfonyl]aniline}dicobalt 4**




To a stirred solution of cyclic Co-complex **4** (46.0 mg, 0.077 mmol, 1.00 equiv) in DMF (0.488 mL), 4,4'-bipyridine stock solution in DMF (c = 15 mM, 0.0609 mg, 0.00039 mmol, 0.500 mol%, 25.7 µL of stock solution) and $B_2(OH)_4$ (20.8 mg, 0.232 mmol, 3.00 equiv) were added. The resulting solution was stirred at room for 20 min (TLC control). The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate tree times(3×10 mL). The organic layers were combined, washed with brine five times (5×30 mL), dried over anhydrous $Na_2SO_4$, and concentrated under

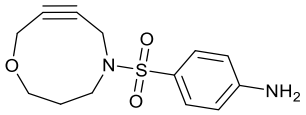
reduced pressure to give the crude product **4** as a brownish powder (36.2 mg, 83%) which was pure enough for characterization.

$^{1}$H NMR (400 MHz, Acetone-d6) δ 7.59 (d, J = 8.0 Hz, 2H), 6.80 (d, J = 8.0 Hz, 2H), 5.54 (br. s, 2H), 5.08 (s, 2H), 4.63 (s, 2H), 4.02 – 3.89 (m, 2H), 3.39 – 3.29 (m, 2H), 1.93 (br. s, 2H).

$^{13}$C NMR (126 MHz, Acetone-$d_6$) δ 200.5, **153.80, 153.75** (two distinct signals for *ortho*-C-Ar), 130.2, 125.3, **114.22, 114.18** (two distinct signals for *meta*-C-Ar), 94.6, 93.7, 74.4, 67.9, 56.6, 49.2, 31.1.

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{19}H_{17}Co_2N_2O_9S^+$: 566.9313; Found: 566.9312

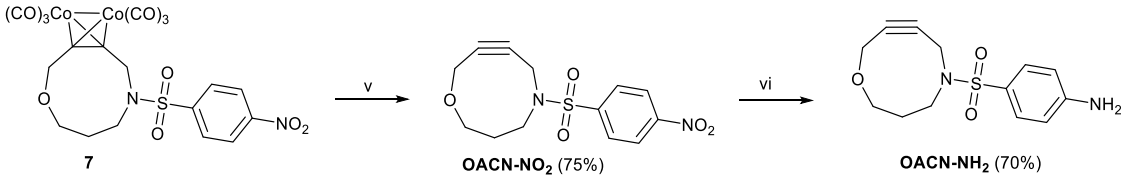
**4-((1,2-Didehydro-3,4,6,9-tetrahydro-1,5-oxazonin-5(2*H*)-yl)sulfonyl)aniline (OACN-NH$_2$)**




**OACN-NH$_2$**

Cycloalkyne **OACN-NH$_2$** was obtained according to the **General procedure (II)** from Co-complex **4** (36.2 mg, 0.0641 mmol, 1.00 equiv), TBAF·3H$_2$O (425 mg, 1.35 mmol, 21.0 equiv, added in 4 portions) in the mixture acetone / H$_2$O (15:1, 10.7 mL). The reaction time was 5 hours. Purification of the crude product by column chromatography (eluent: hexane / ethyl acetate = 1:1) gave **OACN-NH$_2$** (9.00 mg, 50%) as a white powder.

### 2.3.4 Synthesis of OACN-NH$_2$ via path D: NO$_2$ reduction on cycloalkyne

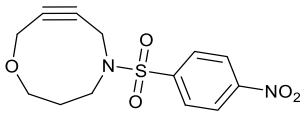



7 OACN-NO$_2$ (75%) OACN-NH$_2$ (70%)

**Reagents and conditions:** TBAF (21.0 equiv), acetone / H$_2$O 15:1, r.t., 5 h; vi) B$_2$(OH)$_4$ (3.00 equiv), 4,4'-biPy (0.5 mol%), DMF, r.t., 1h.

**Scheme S4.** Synthesis of cycloalkyne **OACN-NH$_2$** via path D.

**1,2-Didehydro-5-((4-nitrophenyl)sulfonyl)-2,3,4,5,6,9-hexahydro-1,5-oxazonine (OACN-NO$_2$)**




**OACN-NO$_2$**

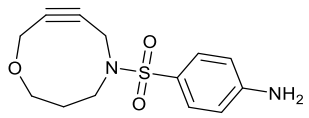
Cycloalkyne **OACN-NO$_2$** was obtained according to the **General procedure (II)** from Co-complex **7** (100 mg, 0.168 mmol, 1.00 equiv), TBAF·3H$_2$O (1.11 g, 3.52 mmol, 21.0 equiv, added in 4 portions) in the mixture acetone / H$_2$O (15:1, 28.0 mL). The reaction time was 5 hours. Purification of the crude product by column chromatography (eluent: hexane / acetone from 4:1 to 3:1) gave **OACN-NO$_2$** (39.00 mg, 75%) as a white powder. This procedure is readily scalable to >1 mmol with comparable yields. For example, the reaction performed on a 1.64 mmol scale of Co-complex **7** afforded **OACN-NO$_2$** (740 mg, 73%).

$^{1}$H NMR (500 MHz, $CD_3CN$) δ 8.39 – 8.35 (m, 2H), 8.04 – 7.99 (m, 2H), 4.06 (t, *J* = 2.3 Hz, 2H), 3.94 (t, *J* = 2.3 Hz, 2H), 3.79 – 3.76 (m, 2H), 3.34 – 3.30 (m, 2H), 1.89 – 1.81 (m, 2H).

$^{13}$C NMR (126 MHz, $CD_3CN$) δ 150.5, 143.2, 128.7, 124.6, 92.7, 88.6, 64.8, 58.8, 44.8, 41.3, 33.0.

HRMS (API) m/z: $[M+H]^+$ Calcd for $C_{19}H_{17}Co_2N_2O_9S^+$: 311.0696; Found: 311.0696

**4-((1,2-Didehydro-3,4,6,9-tetrahydro-1,5-oxazonin-5(2*H*)-yl)sulfonyl)aniline OACN-NH$_2$**

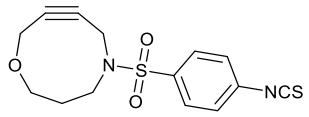



**OACN-NH$_2$**

To reduction reactions were carried out in parallel in two different vials. To a stirred solution of **OACN-NO$_2$** (365 mg, 1.18 mmol, 1.00 equiv) in DMF (7.5 mL) in a vial, 4,4'-bipyridine stock solution in DMF (c = 15 mM, 0.94 mg, 0.006 mmol, 0.50 mol%, 393 µL of stock solution) and $B_2(OH)_4$ (316 mg, 3.53 mmol, 3.00 equiv) were added. The resulting solution was stirred at room temperature for 20 min (TLC control). Then both vials were combined, diluted with water (100 mL) and extracted with ethyl acetate tree times(3×40 mL). The organic layers were combined, washed with brine five times (5×120 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give the crude product, which was then purified by column chromatography on silica gel (eluent: hexane/ethyl acetate = 1:1) that gave **OACN-NH$_2$** as a white powder (461 mg, 70%).

## 2.4 Synthesis of OACN-NCS

**1,2-Didehydro-5-((4-isothiocyanatophenyl)sulfonyl)-2,3,4,5,6,9-hexahydro-1,5-oxazonine (OACN-NCS)**




**OACN-NCS**

**OACN-NCS** was synthesised similar with cycloalkyne-isothiocyanate **BT9N-NSC**.[6] To a stirred solution of **OACN-NH$_2$** (515 mg, 1.84 mmol, 1.00 equiv) and TEA (372 mg, 512 µL, 3.67 mmol, 2.00 equiv) in absolute dichlorometane (c = 0.035 M, 53.0 mL) cooled to 0 °C, thiophosgene (232 mg, 155 µL, 0.2020 mmol, 1.10 equiv) was added in one portion under an argon atmosphere. A cooling bath was immediately removed, and the resulting solution was allowed to warm to room temperature and then was stirred at this temperature for 35 minutes (TLC control). Then the reaction mixture was poured into a saturated aqueous solution of $NH_4Cl$ (40.0 mL), the organic layer was separated and the aqueous layer was extracted with DCM three times (3 × 30.0 mL). The combined organic layers were washed with a saturated solution of $NH_4Cl$ (100 mL) and two times with brine (100 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure at 25 °C to give a crude product, which was purified by column chromatography on silica gel (eluent: hexane/acetone = 7:1) that gave **OACN-NCS** (445 mg, 75 %) as a white powder.

$^{1}$H NMR (500 MHz, $CD_3CN$) δ 7.84 – 7.80 (m, 2H), 7.50 – 7.47 (m, 2H), 4.06 (t, *J* = 2.4 Hz, 2H), 3.87 (t, *J* = 2.4 Hz, 2H), 3.80 – 3.76 (m, 2H), 3.29 – 3.24 (m, 2H), 1.87 – 1.77 (m, 2H).

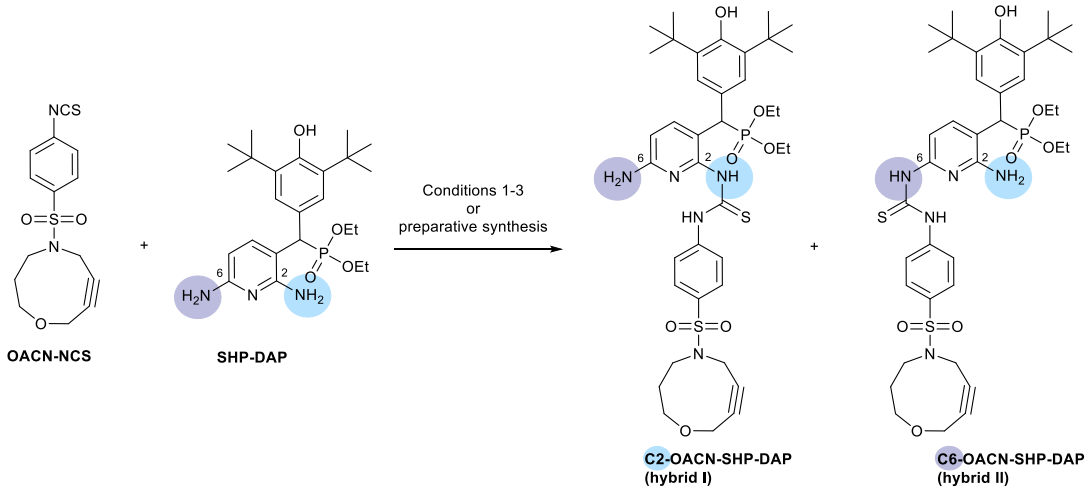
$^{13}$C NMR (126 MHz, $CD_3CN$) δ 138.2, 137.1, 136.3, 130.0, 127.7, 93.5, 89.9, 65.8, 59.8, 45.6, 42.3, 34.0.

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{14}H_{15}N_2O_3S_2^+$: 323.0519; Found: 323.0524.

Accession Code CCDC 2541034, contains the supplementary crystallographic data for this paper.

2.5 Synthesis of OACN-thiourea-linked **hybrids I, II** and **8**

2.5.1 Conditions optimization for the synthesis of **hybrid I** and **hybrid II**




**Scheme S5.** Synthesis of **hybrids I** and **II**.

The reaction conditions for synthesizing **Hybrids I** and **II** were optimized through a series of experiments (Table S1, Conditions 1–3). Reaction progress was monitored using $^1$H and $^{31}$P NMR spectroscopy to detect the formation of Hybrids I and II as the desired products and **OACN-NH$_2$** as the byproduct (Fig. S1 and S2), as well as by TLC to detect the consumption of **OACN-NCS**. TLC was used because the signals of **OACN-NCS** overlap with the signals of both hybrids in the $^1$H NMR.

It was found that the reaction does not proceed at room temperature (Table S1, Condition 1), but heating at 40 °C allows the reaction to proceed with the remaining starting materials, even after 24 hours (Table S1, Condition 2). Full consumption of the starting materials required additional heating at 50 °C for an additional 48 hours (Table S1, Condition 3). However, under these conditions, the starting NCS-alkyne produced a small amount of the hydrolyzed byproduct, OACN-NH$_2$. To avoid the formation of this byproduct, absolute acetonitrile was used as a solvent for the preparative synthesis of **hybrids I** and **II**. The amount of **SHP-DAP** was also increased to 3 equivalents. In this case, all of the starting alkyne was converted into hybrids within 72 hours at 50 °C, and only traces of **OACN-NH$_2$** were observed by TLC (Table S1, Preparative conditions and Section 2.5.2).

**Table S1.** Optimization of reaction conditions for the synthesis of **Hybrids I** and **II**

| *Control/ isolation* | OACN-NCS | SHP-DAP | Conditions | OACN-NCS (TLC) | hybrid I ($^1$H, $^{31}$P NMR) | hybrid II ($^1$H, $^{31}$P NMR) | OACN-NH$_2$ ($^1$H NMR) |
|---|---|---|---|---|---|---|---|
| *NMR monitoring, TLC* | 1.0 equiv. | 1.0 equiv. | ***Condition 1*** 25 °C, $CD_3CN$, 24 h | Consumption of OACN-NCS | Formation of hybrids I & II | | Formation of **OACN-NH$_2$** |
| | 1.0 equiv. | 1.0 equiv. | ***Condition 2*** 40 °C,$CD_3CN$, 24 h | Consumption of OACN-NCS | Formation of hybrids I & II | | Formation of **OACN-NH$_2$** |
| | 1.0 equiv. | 1.0 equiv. | ***Condition 3*** 40 °C, $CD_3CN$, 24 h then 50 °C, 48 h | Consumption of OACN-NCS | Formation of hybrids I & II | | Formation of **OACN-NH$_2$** |
| ***Preparative*** | 1.0 equiv. | 3.0 equiv. | 50 °C, **MeCN$_{abs}$**, 72 h | Consumption of OACN-NCS | 30% | 41% | Formation of **OACN-NH$_2$** |

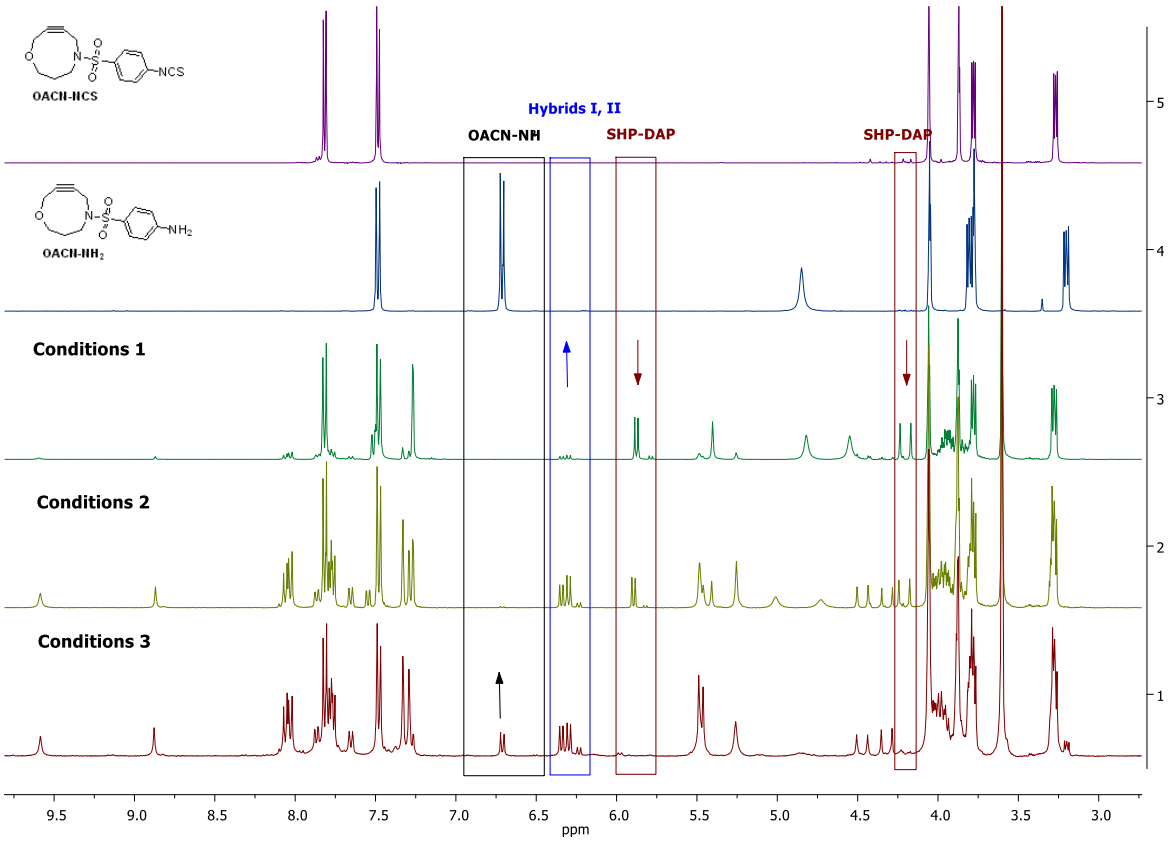



**Figure S1.** Stacked $^{1}$H NMR spectra in $CD_3CN$ for monitoring the «OACN-NCS + SHP-PAP» reaction progress (Table S1, conditions 1–3).

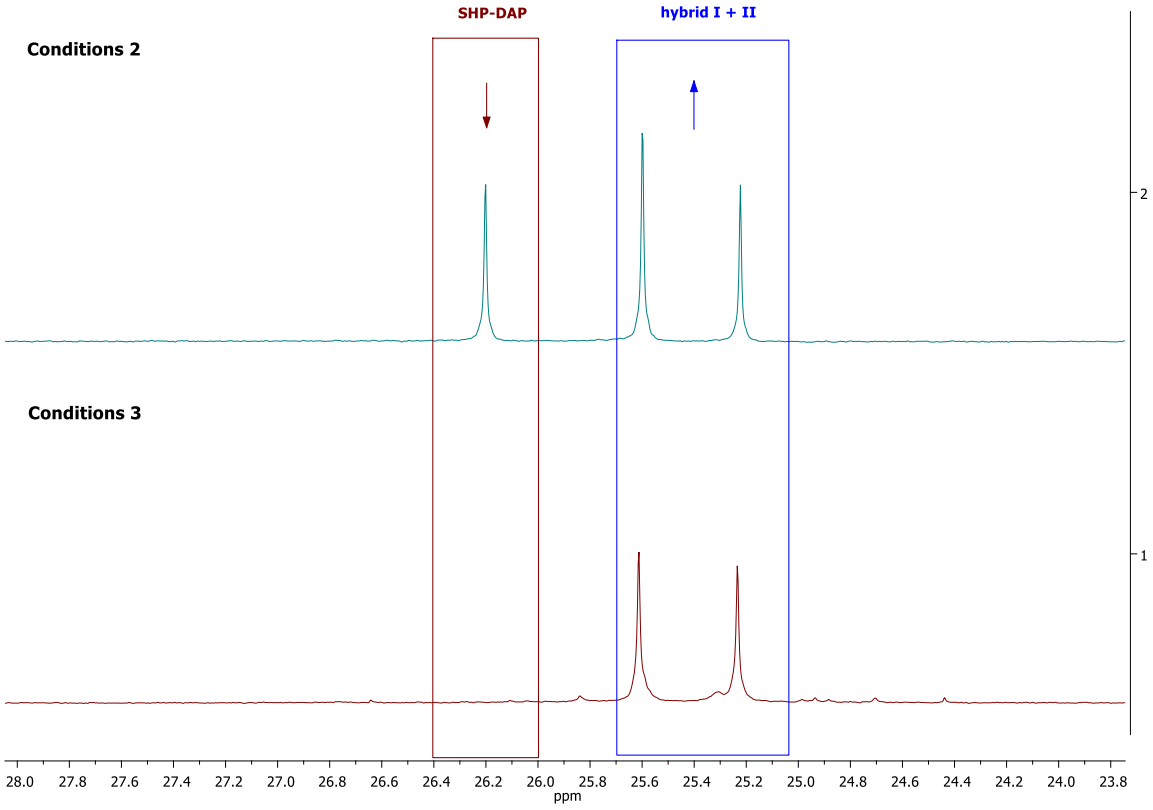



**Figure S2.** Stacked $^{31}$P NMR in $CD_3CN$ for monitoring the «OACN-NCS + SHP-PAP» reaction progress (Table S1, conditions 2,3).

2.5.2 Preparative synthesis of **hybrid I** and **hybrid II** and distinguishing between isomers by NMR

To a stirred solution of **OACN-NCS** (19.4 mg, 0.0602 mmol, 1.00 equiv) in vial in absolute acetonitrile (6.02 mL, c = 0.01 M) under an argon atmosphere **SHP-DAP** (83.7 mg, 0.181 mmol, 3.00 equiv) was added in one portion. The reaction vial sealed under argon, and the reaction mixture was stirred at 50 °C for 72 hours (TLC control). Then the reaction mixture was cooled, transferred to the flask, the solvent was evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel (eluent: benzene/acetone = 10:1 to 7:1) to yield **hybrid I** (14.4 mg, 30%) and **hybrid II** (19.4 mg, 41%) as white powders. The concentration of purified sample must be carried out at 25 °C after the addition of a portion of hexane to the sample to prevent the sample decomposition in a polar solvent under concentration.

Distinguishing between the two isomers was accomplished using a combination of 1D and 2D NMR measurements. This required an estimation of which carbon atom – C2 or C6 – the nitrogen atom of the thiourea moiety was bound to (Fig. S3). Firstly, the C2 and C6 signals in both isomers were unambiguously distinguished based on the C-P coupling constant values. $^{3}J$(C2-P) should lie within the range of 7–10 Hz in the $^{13}C\{^{1}H\}$ spectrum, while $^{5}J$(C6-P) should lie around 0–1 Hz. Found values of chemical shifts and coupling constants for the C2/C6 atom signals in both isomers are following: **Hybrid I**: C2 – 150.1 ($^{3}J_{C\text{-}P}$ = 7.4 Hz); C6 – 157.1 br s. **Hybrid II**: C2 – 155.7 ($^{3}J_{C\text{-}P}$ = 9.8 Hz); C6 – 151.7 ($^{5}J_{C\text{-}P}$ = 1.5 Hz). Next, the cross-peaks for the thiourea NH hydrogen bound to pyridine (HN-Py **Hybrid I**: 9.59, s; and HN-Py **Hybrid II**: 8.90, s), and the C2/C6 atoms in the $^{1}H$-$^{13}C$ HMBC spectra were analyzed. The hybrid with the C2/ HN-Py cross-peak (blue arrow) was assigned to the C2 isomer (**Hybrid I**), while the hybrid with the C6/HN-Py cross-peak (magenta arrow) was assigned to the C6 isomer (**Hybrid II**). Additional key cross-peaks (grey arrows) in $^{1}H$-$^{13}C$ HMBC and $^{1}H$-$^{15}N$ HMBC of both isomers found proved the choice (Fig. S3). For the copies of 1D/2D NMR with signal assignment see section7.

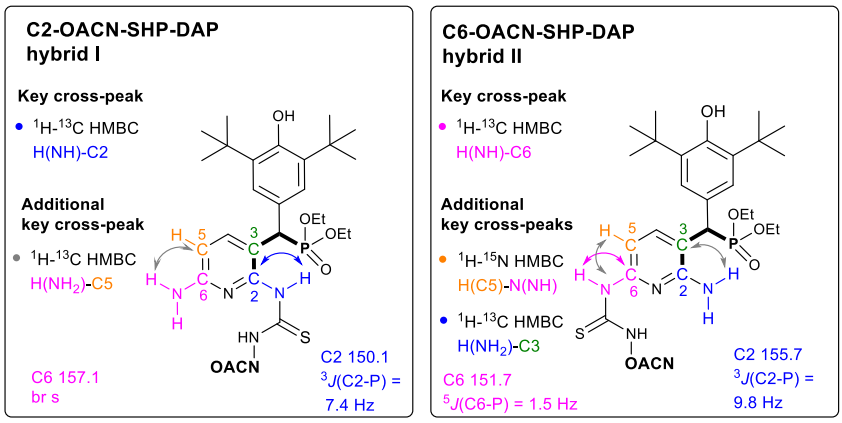
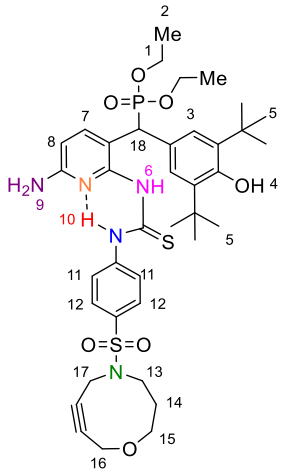


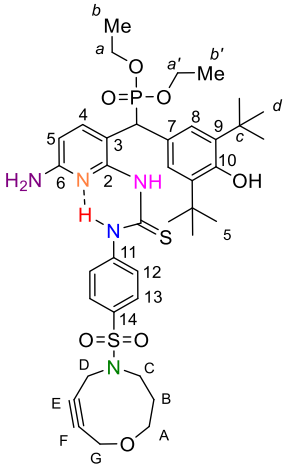

**Fig. S3.** 2D NMR cross-peaks for distinguishing between **hybrids I** and **II**.

## OACN-SHP-DAP Hybrid I

H numbering in $^{1}$H NMR



C numbering in $^{13}$C NMR



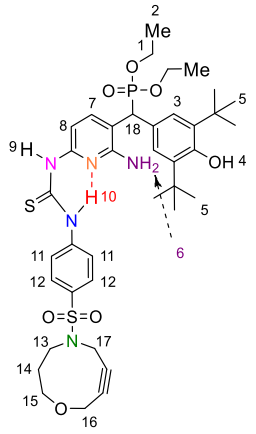
$^{1}$H NMR (400 MHz, $CD_3CN$) δ 13.87 (s, 1H, $H^{10}$), 9.59 (s, 1H, $H^{6}$), 8.03 (d, *J* = 8.5 Hz, 2H, $H^{12}$), 7.76 (d, *J* = 8.5 Hz, 2H, $H^{12}$), 7.65 (d, *J* = 8.4 Hz, 1H, $H^{7}$), 7.33 (s, 2H, $H^{3}$), 6.30 (d, *J* = 8.4 Hz, 1H, $H^{8}$), 5.48 (s, 1H, $H^{4}$), 5.25 (br. s, 2H, $H^{9}$), 4.47 (d, $^{2}J$ (H-P) = 27.3 Hz, 1H, $H^{18}$), 4.50 – 3.88 (m, 6H, $H^{16}$, $H^{1}$ – overlapping signals), 3.88 (br. s, 2H, $H^{17}$), 3.82 – 3.78 (m, 2H, $H^{15}$), 3.32 – 3.25 (m, 2H, $H^{13}$), 1.88 – 1.80 (m, 2H, $H^{14}$), 1.40 (s, 18H, $H^{5}$), 1.24 – 1.11 (m, 6H, $H^{2}$).

$^{13}$C NMR (101 MHz, $CD_3CN$) δ 179.6 (CS), 157.1 (br. s, C6), 154.1 (d, *J* = 1.8 Hz, C10), 150.1 (d, *J* = 7.4 Hz, C2), 144.6 (C11), 143.6 (d, *J* = 7.0 Hz, C4), 138.4 (*J* = 0.9 Hz, C9), 134.3 (C14), 128.7 (C13), 127.4 (d, *J* = 8.1 Hz, C8), 127.1 (d, *J* = 3.2 Hz, C7), 124.9 (C11), 109.0 (d, *J* = 3.3 Hz, C3), 104.2 (C5), 93.4 ($C^{F}$), 90.3 ($C^{E}$), 65.9 ($C^{A}$), 63.9 (d, *J* = 7.2 Hz, $C^{a/a'}$) and 63.8 (d, *J* = 7.1 Hz, $C^{a/a'}$), 59.8 ($C^{G}$), 45.9 (d, *J* = 139.5 Hz, CH-P), 45.6 ($C^{C}$), 42.3 ($C^{D}$), 35.4 ($C^{c}$), 34.1 ($C^{B}$), 30.6 ($C^{d}$), 16.8 ($C^{b/b'}$), 16.7 ($C^{b/b'}$). $^{31}$P NMR (162 MHz, $CD_3CN$) δ 25.60 (s).

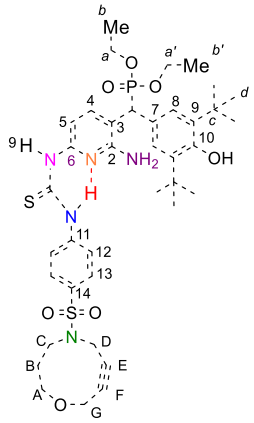
HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{38}H_{53}N_5O_7PS_2^+$: 786.3119; Found: 786.3117.

## OACN-SHP-DAP Hybrid II

H numbering in $^{1}$H NMR



C numbering in $^{13}$C NMR



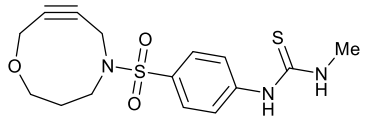
$^{1}$H NMR (400 MHz, $CD_3CN$) δ 13.87 (s, 1H, $H^{10}$), 8.90 (s, 1H, $H^{9}$), 8.10 – 8.02 (m, 2H, $H^{11}$), 7.87 (dd, *J* = 8.2, 1.5 Hz, 1H, $H^{7}$), 7.81 – 7.75 (m, 2H, $H^{12}$), 7.29 (d, 1.6 Hz, 2H, $H^{3}$), 6.35 (d, *J* = 8.2 Hz, 1H, $H^{8}$), 5.47 (s, 2H, $H^{6}$) and 5.46 (s, 1H, $H^{4}$) overlapping signals, 4.32 (d, $^{2}J$ (H-P) = 26.7 Hz, 1H, $H^{18}$), 4.06 (t, *J* = 2.3 Hz, 2H, $H^{16}$), 4.02 – 3.84 (m, 6H, $H^{1}$ and $H^{17}$ - overlapping signals), 3.82 – 3.79 (m, 2H, $H^{15}$), 3.32 – 3.27 (m, 2H, $H^{13}$), 1.87 – 1.81 (m, 2H, $H^{14}$), 1.39 (s, 18H, $H^{18}$), 1.19 – 1.09 (m, 6H, $H^{2}$).

$^{13}$C NMR (101 MHz, $CD_3CN$) δ 179.6 (C=S), 155.7 (d, *J* = 9.8 Hz, C2), 154.0 (d, *J* = 2.5 Hz, C10), 151.7 (d, *J* = 1.5 Hz, C6), 144.6 (C11), 142.0 (d, *J* = 6.2 Hz, C4), 138.3 (d, *J* = 1.4 Hz, C9), 134.5 (C14), 128.7 (C13), 127.4 (d, *J* = 4.9 Hz, C7), 127.3 (d, *J* = 7.4 Hz, C8), 125.3 (C12), 111.81 (d, *J* = 3.6 Hz, C3), 101.8 (C6), 93.4 ($C^{F}$), 90.3 ($C^{E}$), 65.9 ($C^{A}$), 63.7 (d, *J* = 7.1 Hz, $C^{a/a'}$), 63.4 (d, *J* = 7.1 Hz, $C^{a/a'}$), 59.8 ($C^{G}$), 45.6 ($C^{C}$), 44.80 (d, *J* = 139.3 Hz, CH-P), 42.3 ($C^{D}$), 35.3 ($C^{c}$), 34.1 ($C^{B}$), 30.6 ($C^{d}$), 16.7 (d, *J* = 3.0 Hz, $C^{b/b'}$), 16.7 (d, *J* = 3.3 Hz, $C^{b/b'}$). $^{31}$P NMR (162 MHz, $CD_3CN$) δ 25.23 (s).

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{38}H_{53}N_5O_7PS_2^+$: 786.3116; Found: 786.3117**.**

### 2.5.2 Synthesis of Hybrid **8**

**1-(4-((1,2-Didehydro-3,4,6,9-tetrahydro-1,5-oxazonin-5(2*H*)-yl)sulfonyl)phenyl)-3-methylthiourea (8)**




**8**

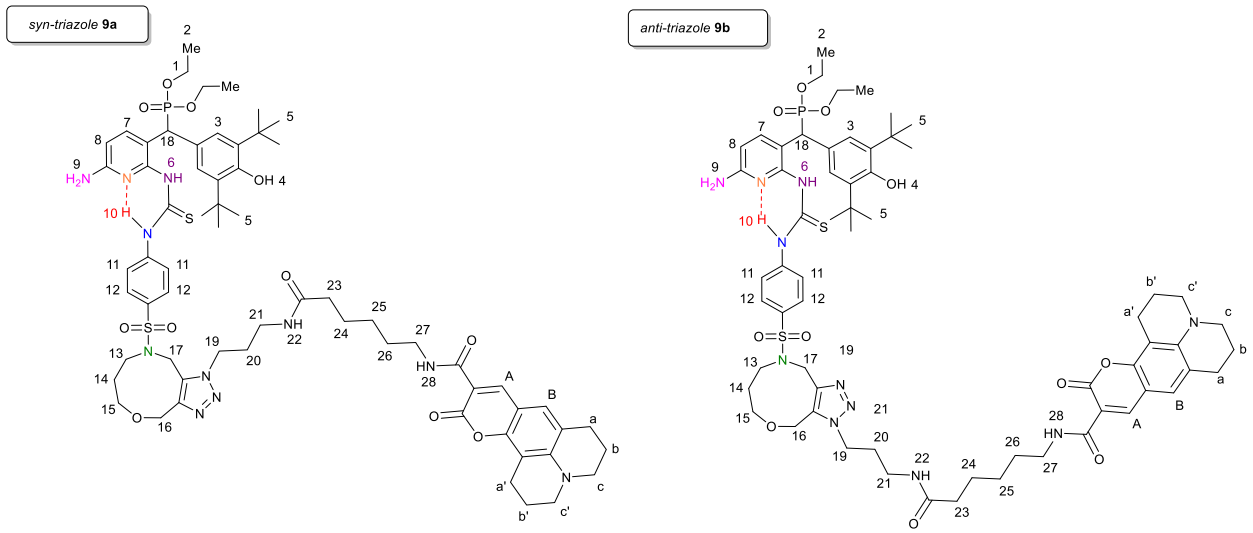
To a stirred solution of **OACN-NCS** (20.0 mg, 0.0620 mmol, 1.00 equiv) in acetonitrile (6.20 mL, c = 0.01 M), methylamine (40% aqueous solution, 9.63 mg, 11.0 µL, 0.124 mmol, 2.00 equiv) was added in one portion. The reaction was stirred for 1 h (TLC control) at room temperature. Then the reaction mixture was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (eluent: hexane/acetone = 3:2) to yield **8** (21 mg, 96%) as a white powder. The concentration of purified sample must be carried out at 25 °C after the addition of a portion of hexane to the sample to prevent the sample decomposition in a polar solvent under concentration. $^{1}$H NMR (500 MHz, Acetone-$d_6$) δ 9.27 (br. s, 1H), 7.88 – 7.82 (m, 2H), 7.79 – 7.75 (m, 2H), 7.58 (br. s, 1H), 4.08 (t, *J* = 2.4 Hz, 2H), 3.91 (t, *J* = 2.4 Hz, 2H), 3.84 – 3.80 (m, 2H), 3.31 – 3.27 (m, 2H), 3.088 (s) and 3.081 (s) (3H, $CH_3$, two rotamers), 1.86 – 1.80 (m, 2H). $^{13}$C NMR (126 MHz, Acetone-$d_6$) δ 182.8, 145.0, 132.8, 129.2, 122.6 - br.s, 93.2, 90.4, 65.5, 59.6, 45.3, 42.2, 34.2, 31.7 – br. s.

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{15}H_{20}N_3O_3S_2^+$: 354.0941; Found: 354.0950**.**

2.6 SPAAC for **hybrids I, II** and **8** with fluorescent azide

2.6.1 SPAAC of **hybrid I** and **hybrid II** with fluorescent azide coumarin-X-azide **AF-343**

**Syn- and anti-triazoles 9a,b**




Chemical Formula: $C_{63}H_{84}N_{11}O_{11}PS_2$
Exact Mass: 1265,5531

*Syn*- and *anti*-isomers **9** were obtained according to the **General Procedure (III)** from **hybrid I** (6.00 mg, 0.00763 mmol, 1.00 equiv) and coumarin-X-azide **AF-343** (2.57 mg, 0.00534 mmol, 0.70 equiv) in $CDCl_3$ (c = 0.01 M, V = 0.763 mL). Purification of crude product was performed by column chromatography on silica gel (eluent – DCM / EtOH / benzene = 15 : 1 : 1) to gave ***syn*-9a** and ***anti*-isomers 9b** as a yellow powder (8.40 mg, 86%).

$^1$H NMR (400 MHz, $CDCl_3$) δ 14.08 (s, 1H) and 13.89 (s, 1H) $H^{10}$ (*syn-, anti-*); 8.98 (s, 2H) $H^9$ (1H *syn- +* 1H *anti-*); 8.89 (s, 2H) $H^{28}$ (1H *syn- +* 1H *anti-*); 8.57 (s, 1H) and 8.54 (s, 1H) $H^A$ (*syn-, anti-*); 8.09 – 7.74 (m, 10H) $H^{11}$, $H^{12}$, $H^7$, $H^7$ (5H from *syn-* and 5H from *anti*-triazole); 7.26 (s, 4H) overlaps with the solvent signal, $H^4$ (2H *syn- +* 2H *anti-*); 7.00 (s, 1H) and 6.96 (s, 1H) $H^B$ (*syn-, anti-*); 6.28 (d, *J* = 8.5 Hz, 1H) $H^8$ (1H *syn- +* 1H *anti-*); 6.24 (br. s, 2H) $H^{22}$ (1H *syn- +* 1H *anti-*); 5.23 (s, 2H), $H^4$ (1H *syn- +* 1H *anti-*);4.92 (s, 2H) and 4.62 (s, 2H) $H^6$ (*syn-, anti-*); 4.84 (s, 2H) and 4.80 (s, 2H) $H^{16}$ (*syn-, anti-*); 4.69 – 7.46 (m, 10H), 4.32 – 4.28 (m, 2H), 4.14 – 3.92 (m, 8H), 3.78 –3.69 (m, 4H), 3.63 (br. s, 4H), 3.46 – 3.20 (m, 18H), 3.20 – 3.14 (m, 2H), 2.89 – 2.72 (m, 8H) $H^1$,$H^{13}$, $H^{15}$, $H^{17}$, $H^{18}$, $H^{21}$, $H^{23}$, $H^{27}$, $H^a$, $H^{a'}$, $H^c$, $H^{c'}$ (13$CH_2$ groups from *syn-* and 13$CH_2$ group from *anti*-triazole; CH-P from *syn-* and CH-P from *anti*-triazole); 2.19 – 2.09 (m, 10H), 1.95 – 1.83 (m, 12H), 1.66 – 1.60 (m, 6H) $H^{14}$, $H^{20}$, $H^{24}$, $H^{25}$, $H^{26}$, $H^b$, $H^{b'}$ (7$CH_2$ groups *syn-* and 7$CH_2$ group from *anti*-triazole); 1.43 (br. s, 36H), $H^5$ (18H *syn- +* 18H *anti-*); 1.27 – 1.25 (m, 6H) and 1.06 – 1.06 (m, 6H) $H^2$ (6H *syn- +* 6H *anti-*).

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{63}H_{85}N_{11}O_{11}PS_2^+$: 1266.5604; Found: 1266.5600**.**

The composition of the isomer mixture was additionally confirmed by high-performance liquid chromatography with UV and mass spectrometric detection (Fig. S4, S5). The analysis was performed using reverse-phase high-performance liquid chromatography on a NEXERA LC-30AD chromatograph (Shimadzu, Japan) equipped with an LCMS-8050 mass spectrometer and an SPD-M30A diode array detector: Shim-Pack FC-ODS column, 150 × 2 mm, 3 µm ID (C18).

Analytical conditions: column temperature 40 °C; ionisation temperature 200 °C; injection volume 10 µL; isocratic low-pressure mode, 70% acetonitrile and 30% water + 0.1% trifluoroacetic acid, flow rate 0.25 mL/min, analysis time 10 minutes.

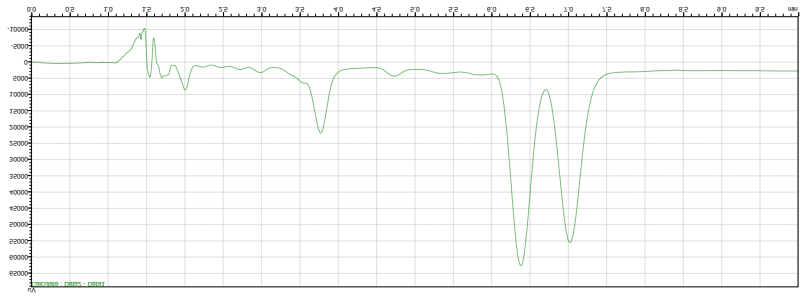


**Fig. S4.** HPLC of *syn*- and *anti*-triazoles **9a,b**, diode array detection (210 nm).

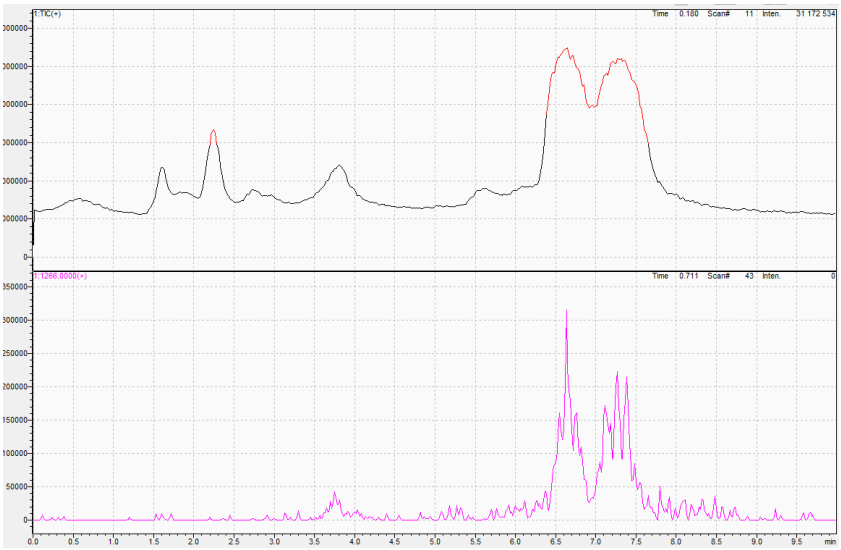
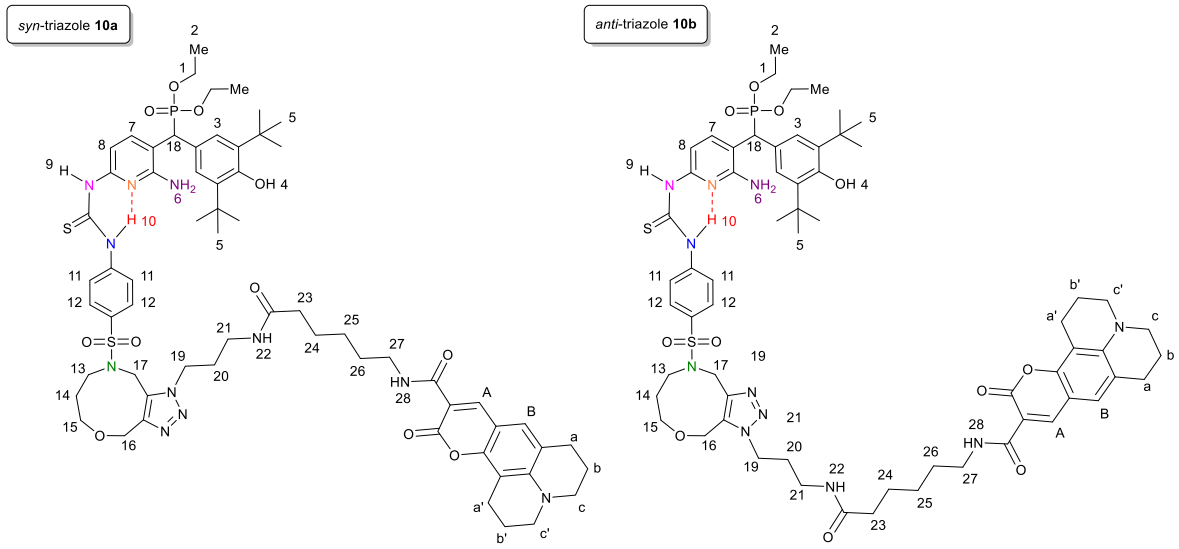


**Fig. S5.** HPLC of *syn*- and *anti*-triazoles **9a,b**, total ion current (TIC) mass spectrometry detection and mass detection at 1266.00.

### *Syn*- and anti-triazoles 10a,b




Chemical Formula: $C_{63}H_{84}N_{11}O_{11}PS_2$
Exact Mass: 1265,5531

*Syn*- and *anti*-isomers **10** were obtained according to the **General Procedure (III)** from **hybrid II** (8.00 mg, 0.0102 mmol, 1.00 equiv) and coumarin-X-azide **AF-343** (3.40 mg, 0.00713 mmol, 0.70 equiv) in $CDCl_3$ (c = 0.01 M, V = 1.02 mL). Purification of crude product was performed by column chromatography on silica gel (eluent – DCM / EtOH / benzene = 15 : 1 : 1) to gave ***syn*- 10a** and ***anti*-isomers 10b** as a yellow powder (10 mg, 77%). The ration of syn- and anti-isomers = 1 : 1.

$^1$H NMR (400 MHz, $CD_3CN$) δ 13.91 (s, 1H) and 13.82 (s, 1H) $H^{10}$ (*syn-, anti-*); 8.91 (s, 2H) $H^9$ (1H *syn- +* 1H *anti-*); 8.72 – 8.67 (m, 2H) $H^{28}$ (1H *syn- +* 1H *anti-*); 8.46 (s, 1H) and 8.43 (s, 1H) $H^A$ (*syn-, anti-*); 8.07 (*d*, 2H, *J* = 8.7 Hz) and 7.96 (*d*, 2H, *J* = 8.7 Hz) $H^{11}$ (*syn-, anti-*); 7.92 – 7.85 (m, 2H) $H^7$ (1H *syn- +* 1H *anti-*); 7.83 (*d*, 2H, *J* = 8.7 Hz) and 7.74 (*d*, 2H, *J* = 8.7 Hz) $H^{12}$ (*syn-, anti-*); 7.31 (d, $^4J_{(H\text{-}P)}$ = 1.6 Hz, 2H) and 7.30 (d, $^4J_{(H\text{-}P)}$ = 1.6 Hz, 2H) $H^3$ (*syn-, anti-*); 7.04 (s, 1H) and 6.95 (s, 1H) $H^B$ (*syn-, anti-*); 6.49 (t, *J* = 5.3 Hz, 1H) and 6.42 (t, *J* = 5.3 Hz, 1H) $H^{22}$ (*syn-, anti-*); 6.36 (d, *J* = 8.2 Hz, 1H) and 6.35 (d, *J* = 8.2 Hz, 1H) $H^8$ (*syn-, anti-*); 5.67 (s, 2H) and 5.55 (s, 2H) $H^6$ (*syn-, anti-*); 5.47 (s, 2H), $H^4$ (1H *syn- +* 1H *anti-*); 4.76 (s, 2H) and 4.70 (s, 2H) $H^{16}$ (*syn-, anti-*); 4.49 (s, 2H) and 4.49 (s, 2H) $H^{17}$ (*syn-, anti-*); 4.38 (t, $^2J_{(H\text{-}P)}$ = 26.6 Hz, 1H) and 4.34 (t, $^2J_{(H\text{-}P)}$ = 24.7 Hz, 1H) overlapping signals, $H^{18}$ (*syn-, anti-*); 4.39 – 4.35 (m, 2H) overlaps with $H^{18}$ and 4.18 (t, *J* = 7.1 Hz, 2H) $H^{19}$ (*syn-, anti-*), 4.02 – 3.83 (m, 8H) $H^1$ (4H *syn- +* 4H *anti-*), 3.60 (t, *J* = 5.4 Hz, 2H), 3.57 – 3.51 (m, 2H), 3.48 (t, *J* = 5.5 Hz, 2H), 3.40– 3.27 (m, 18H), 3.22 – 3.15 (m, 2H), 3.13 – 3.06 (m, 2H), 2.79 – 2.65 (m, 8H) $H^{13}$, $H^{15}$, $H^{21}$, $H^{23}$, $H^{27}$, $H^a$, $H^{a'}$, $H^c$, $H^{c'}$ (9$CH_2$ groups *syn*- and 9$CH_2$ group from *anti*-triazole); 2.09 – 2.01 (m, 8H), 1.91 – 1.86 (m, 8H), 1.77 – 1.72 (m, 4H), 1.59 – 1.49 (m, 8H) $H^{14}$, $H^{20}$, $H^{24}$, $H^{25}$, $H^{26}$, $H^b$, $H^{b'}$ (7$CH_2$ groups *syn*- and 7$CH_2$ group from *anti*-triazole); 1.38 (two overlapping signals, 36H), $H^5$ (18H *syn- +* 18H *anti-*); 1.19 – 1.12 (m, 12H) $H^2$ (6H *syn- +* 6H *anti-*). $^{31}$P NMR (162 MHz, $CD_3CN$) δ 25.31 (s), 25.26 (s).

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{63}H_{85}N_{11}O_{11}PS_2^+$: 1266.5604; Found: 1266.5602**.**

The composition of s*yn*- and *anti*-triazoles **10a,b** mixture was additionally confirmed by high-performance liquid chromatography with UV and mass spectrometric detection (Fig. S6, S7). For condition see the same analysis of isomers **9a,b**.

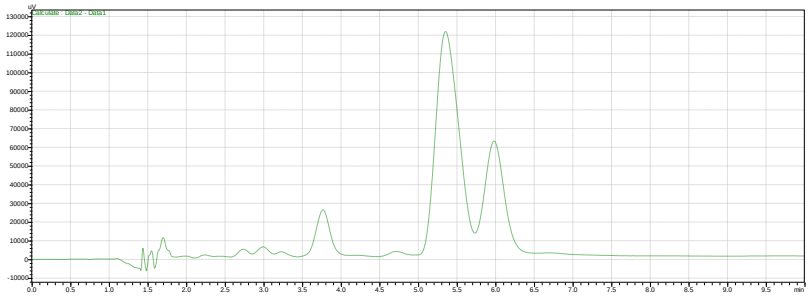


**Fig. S6.** HPLC of *syn*- and *anti*-triazoles **10a,b**, diode array detection (210 nm).

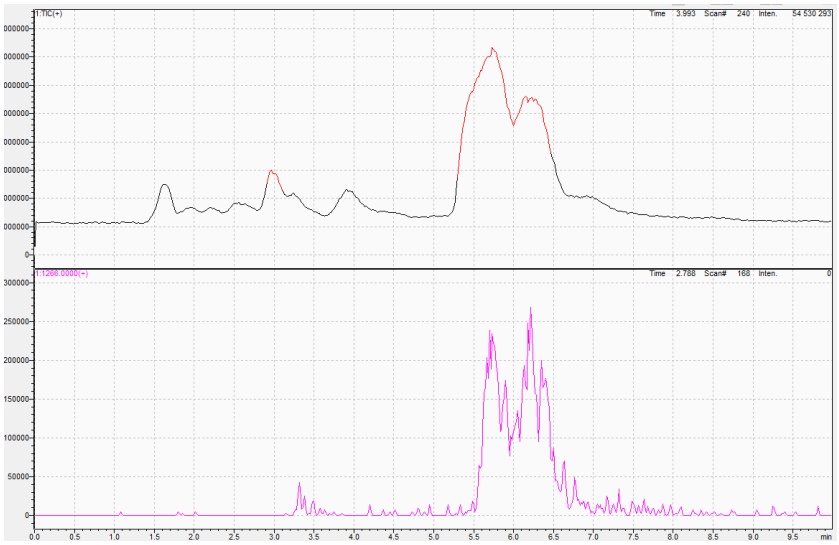
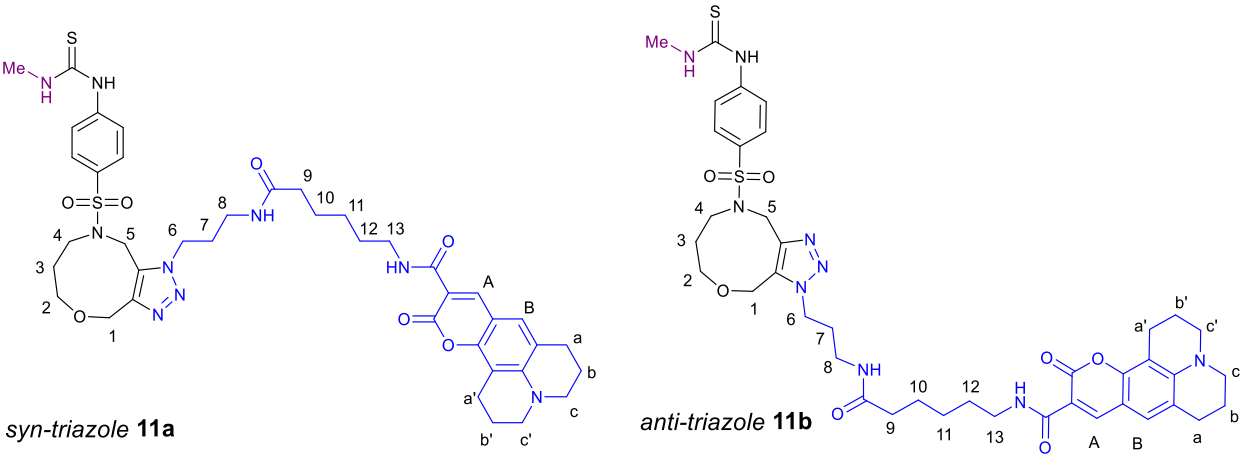


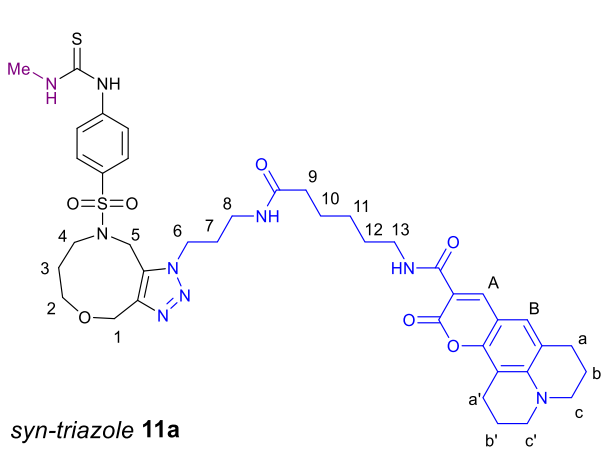
**Fig. S7**. HPLC of *syn*- and *anti*-triazoles **10a,b**, total ion current (TIC) mass spectrometry detection and mass detection at 1266.00.

2.6.2. SPAAC of **8** with fluorescent azide

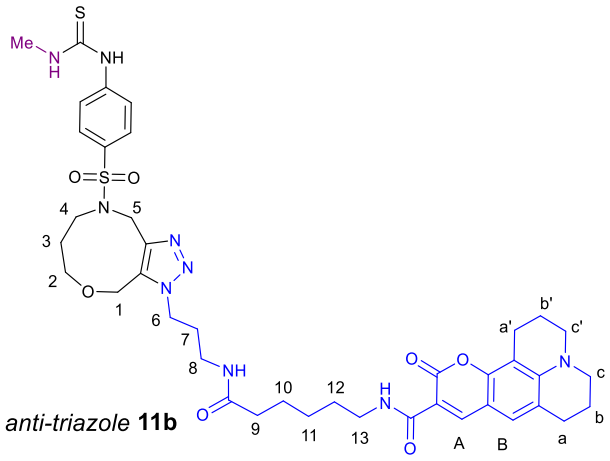
*Syn*- and *anti*-isomers **Me-OACN triazoles 11**




*Syn*-and *anti*-isomers **11** were obtained according to the **General Procedure (III)** from cycloalkyne **8** (7.40 mg, 0.0209 mmol, 1.00 equiv) and coumarin-X-azide **AF-343** (7.04 mg, 0.0147 mmol, 0.70 equiv) in MeCN (c = 0.01 M, V = 2.09 mL). Purification of crude product was performed by column chromatography on silica gel (eluent – hexane / acetone 1:1) to give ***syn*- 11a** and ***anti*-isomers 11b** as a yellow powder (14 mg, 80%).



*syn-triazole* **11a**



*anti-triazole* **11b**

$^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 10.04 (br s, 1H) and 9.99 (br s, 1H) NH (TU) of *syn*- and *anti*-isomers, 8.68 – 8.60 (m, 2H, NH amide of *syn*- and *anti*-isomers), 8.51 (s, 2H, H-coumarin (A) of *syn*- and *anti*-isomers), 8.10 (br s, 2H, NH (TU) of *syn*- and *anti*-isomers), 7.92 – 7.86 (m, 2H, NH amide of *syn*- and *anti*-isomers), 7.85 – 7.68 (m, 8H, aryl H of *syn*- and *anti*-isomers), 7.26 (s, 2H, H-coumarin (B) of *syn*- and *anti*-isomers), 4.79 (s, 2H) and 4.74 (s, 2H) $OCH_2$ (1) of *syn*- and *anti*-isomers, 4.47 (s, 2H) and 4.43 (s, 2H) $NCH_2$ (5) of *syn*- and *anti*-isomers, 4.39 (t, *J* = 6.8 Hz, 2H) and 4.24 (t, *J* = 6.9 Hz, 2H) $N_{Tr}CH_2$ (6) of *syn*- and *anti*-isomers, 3.57 (t, *J* = 5.0 Hz, 2H) and 3.52 (t, *J* = 4.9 Hz, 2H) $OCH_2$ (2) of *syn*- and *anti*-isomers, 3.31 – 3.19 (m, 16H, $4CH_2$ (c, c', 8, 13) of *syn*- and *anti*-isomers, overlapping with water signal), 3.12 – 3.03 (m, 4H, $CH_2$ (4) of *syn*- and *anti*-isomers), 2.95 (s, 6H, $CH_3$ of *syn*- and *anti*-isomers), 2.76 – 2.67 (m, 8H, $2CH_2$ (a, a') of *syn*- and *anti*-isomers), 2.09 – 2.05 (m, 4H, $CH_2$ (9) of *syn*- and *anti*-isomers, overlapping with acetone), then the residual $CH_2$ (3, 7, 10, 11, 11, c, c') can be found: 2.01 – 1.95 (m, 2H, $CH_2$ of *syn*- or *anti*-isomers), 1.94 – 1.81 (m, 10H, $1CH_2$ of *syn*- or *anti*-isomer + 2 $CH_2$ of *syn*- and *anti*-isomer), 1.76 – 1.66 (m, 4H, $CH_2$ of *syn*- and *anti*-isomers), 1.57 – 1.44 (m, 8H, $2CH_2$ of *syn*- and *anti*-isomers), 1.32 – 1.23 (m, 4H, $CH_2$ of *syn*- and *anti*-isomers, overlapping with grease).

$^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 180.95 (2 signals), 172.24 and 172.22 (CO), 162.3 (2 signals CO-N), 162.0 (2 signals CO-N), 152.1 (2 signals, C-Ar), 148.0 (2 signals, C-Ar), 147.5 (2 signals, CH-Ar coumarin), 144.3 and 143.9 (C-Ar), 143.4 and 141.1 (C-Ar), 132.6 (2 signals, C-Ar), 131.5 and 131.0 (C-Ar), 128.0 and 127.9 (br s, CH-Ar); 127.15 (2 signals, CH-Ar coumari), 121.1 (2 signals, br s, CH-Ar), 119.5 (2 signals, C-Ar), 108.0 (2 signals), 107.4 (2 signals, C-Ar), 104.7 (2 signals, C-Ar), 66.81 and 66.75 ($CH_2$-$N_{Tr}$), 64.5 and 61.3 (O-$CH_2$), 49.5 (2 signals, $CH_2$), 49.4 ($CH_2$), 49.2 ($CH_2$), 49.0 (2 signals, ($CH_2$)), 47.4 (N-$CH_2$), 45.45 and 45.42 ($CH_2$), 43.50 (N-$CH_2$), 38.7 (2 signals, $CH_2$), 35.87 and 35.73 ($CH_2$), 35.35 and 35.31 ($CH_2$),31.2 (br s, 2 signals, Me), 29.69 ($CH_2$), 29.50 and 29.48 (2 signals, $CH_2$), 29.2 ($CH_2$), 29.0 (2 signals, $CH_2$), 26.8 (2 signals, $CH_2$), 26.17 and 26.15 ($CH_2$), 25.0 (2 signals, $CH_2$), 20.6 (2 signals, $CH_2$), 19.6 (4 signals, $CH_2$).

HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{40}H_{52}N_9O_7S_2^+$: 834.3426; Found: 834.3427**.**

3 X-RAY diffraction

A single crystal of **OACN-NCS** was selected for optical microscope data collection, embedded in oil cryoprotectant and supported on glass fibers. X-ray diffraction measurements were performed using a Rigaku (Oxford Diffraction) "XtaLAB Sinergy" (Cu Kα, λ = 1.54184 Å, HyPix6000 type detector). The crystal was maintained at 100.00(10) K throughout the experimental time.

The unit cell parameters were refined by the least-squares method. Using Olex2,[7] the structure was solved with the ShelXT[8] structure solver using Intrinsic Phasing and refined with the ShelXL[9] refinement package using CGLS minimization and refined by the full-matrix least-squares method with respect to F2 in the anisotropic-isotropic approximation. Empirical absorption correction is applied in the CrysAlisPro[10] software package using spherical harmonics implemented in the SCALE3 ABSPACK scaling algorithm. All hydrogen atoms were placed in geometrically calculated positions and were refined in isotropic approximation in the riding model with the $U_{iso}$(H) parameters equal to $n \cdot U_{eq}(C_i)$ ($n$ = 1.2 for CH and $CH_2$ groups and n = 1.5 for $CH_3$ groups), where $U(C_i)$ are respectively the equivalent thermal parameters of the atoms to which corresponding H atoms are bonded.

Accession Codes CCDC 2541034, contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

**Table S2. A summary table of X-RAY diffraction experiments of OACN-NCS**

| **Crystal Data** | |
|---|---|
| Formula | $C_{14}H_{14}N_2O_3S_2$ |
| Formula Weight | 322.39 |
| Space Group | $P2_12_12_1$ |
| $Z$ | 4 |
| T, K | 100.00(10) |
| $a$, Å | 5.8114(2) |
| $b$, Å | 14.2733(5) |
| $c$, Å | 17.1408(7) |
| $\alpha$, ° | 90 |
| $\beta$, ° | 90 |
| $\gamma$, ° | 90 |
| $V$, Å$^3$ | 1421.79(9) |
| $\mu$, mm$^{-1}$ | 3.507 |
| **Data Collection** | |
| Diffractometer | «XtaLAB Sinergy», Single source at offset/far, HyPix6000 |

| | |
|---|---|
| Radiation type | Cu *K*α |
| Absorption correction | Multi-scan |
| $T_{min}$, $T_{max}$ | 0.70439, 1.00000 |
| No. of measured, independent and observed [$I > 2\sigma(I)$] reflections | 9336, 2961, 190 |
| $R_{int}$ | 0.0788 |
| $(\sin \theta/\lambda)_{max}$ ($Å^{-1}$) | 0.553 |
| **Refinement** | |
| $R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, $S$ | 0.0566, 0.0420, 1.059 |
| No. of reflections | 9336 |
| No. of parameters | 190 |
| H-atom treatment | H-atom parameters constrained |
| $\rho_{max}$, $\rho_{min}$, e/$Å^3$ | 0.32/-0.38 |
| CCDC | 2541034 |

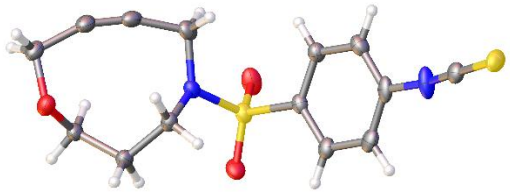


**Fig. S8**. Molecular structure of compound OACN-NCS, displacement parameters are drawn at 50% probability level

## 4 Cytotoxicity assay

The cytotoxic effect of hybrids **I, II** and corresponding triazoles **9** and **10** was assessed using the MTT colorimetric cell proliferation assay. [11]

The following human cell lines were used in the study: M-HeLa (a clone of cervical epithelioid carcinoma), HuTu 80, human duodenal adenocarcinoma, and Wi-38 (lung fibroblasts) from the Vertebrate Cell Culture Collection of the Institute of Cytology, Russian Academy of Sciences.

Cells were cultured in 96-well plates at a density of $5 \times 10^3$ cells per well in 100 µl of medium and incubated at 37°C with 5% $CO_2$ for 24 hours. All cell lines were maintained in Eagle's medium supplemented with 10% fetal bovine serum and 1% essential amino acids.

After an initial 24-hour incubation, 100 µL of the test compound solution prepared in the nutrient medium was added to each well. Cells cultured in the medium alone served as an untreated

control. Cytotoxicity measurements were performed in two independent experiments, each performed in triplicate. The results are presented in Table S3.

Table S3. Cytotoxic activity of hybrids I,II and triazoles 9a,b and 10a,b.

| Compound | $IC_{50}$ μM | | |
|---|---|---|---|
| | M-HeLa | HuTu80 | Wi38 |
| **Hybrid I** | >100 | >100 | >100 |
| **Hybrid II** | >100 | >100 | >100 |
| **9 a,b** | 65.2±0.7 | 68.4±5.2 | 69.4±8.8 |
| **10 a,b** | 61.4±7.0 | 73.5±14 | 71.1±3.8 |

5 Intracellular visualization

For studying the intracellular distribution of compounds *in vitro*, confocal live-cell microscopy with commercial trackers was used.

The HeLa cell line was obtained from the shared research facility "Vertebrate cell culture collection" of the Institute of Cytology, Russian Academy of Sciences. Cells were cultured in complete growth medium based on DMEM supplemented with L-glutamine (2 mM), gentamicin (50 μg/mL), and fetal bovine serum at a concentration of 10%. Routine cell cultivation was performed every 3-4 days by cell detachment using trypsin-EDTA solution (0.25%) and passaging at a 1:6 ratio. Cells were incubated at +37°C and 5% $CO_2$ in a humidified incubator. 48 hours prior to imaging, cells were seeded into 35 mm glass-bottom dishes with 0.17 mm optical thickness (Ibidi, Germany) specifically designed for confocal microscopy. The cell concentration was 200.000 cells per dish to achieve 70-80% confluency at the time of the experiment.

On the day of the experiment, cells were incubated with compounds at a 1 μM concentration for 4 hours at +37 °C (compounds stock solution at 10 mM concentration was prepared in dimethyl sulfoxide (DMSO); final DMSO concentration in dishes did not exceed 0.1%). After incubation, cells were washed three times with sterile Dulbecco's phosphate-buffered saline (DPBS) and replaced with fresh complete growth medium.

For visualization of subcellular compartments, commercial fluorescent trackers from Lumiprobe RUS Ltd (Russia) were used: LumiTracker Mito Red FM for mitochondrial staining (50 nM concentration and 10 min incubation at 37°C); LumiTracker ER Red for endoplasmic reticulum (ER) staining (50 nM concentration, 10 min incubation at 37°C). For the control experiments without any synthesized probes only commercial fluorescent trackers from Lumiprobe RUS Ltd (Russia) were used: LumiTracker Mito Red FM for mitochondrial staining (50 nM concentration and 10 min incubation at 37°C); LumiTracker ER Green for endoplasmic reticulum (ER) staining (50 nM concentration, 10 min incubation at 37°C) (Fig. S9). After staining, cells were washed three times with sterile DPBS and the medium was replaced with fresh medium to eliminate non-specific signal and non-specific dye binding.

Visualization was performed on a Nikon Eclipse Ti2 confocal laser scanning microscope (Nikon Corporation, Japan) using an Apo LWD 40× WI λS DIC N2 objective (NA 1.15, W.D. 0.59-0.61 mm). Fluorescence parameters: for compounds and LumiTracker ER Green in control experiment,

excitation was 406.0 nm with detection in the 500-550 nm range (FITC channel); for ER tracker Red and mitochondrial tracker Red, excitation was 561.4 nm with detection in the 570-738 nm range (TRITC-Cy5 channels). Differential interference contrast (DIC) transmission imaging was also performed to visualize cell morphology. All images were acquired at 1024 × 1024 pixel resolution with averaging over three sequential scans to improve quality and enhance signal-to-noise ratio. Laser power and detector gain were individually adjusted for each experiment to ensure detectable signal while avoiding overexposure and non-specific cellular autofluorescence. Microscopic image analysis was performed using FIJI software (ImageJ version 1.53c). Colocalization analysis was performed using the ImageJ JACoP plugin (Just Another Colocalization Plugin, version 2.1.4) to calculate Pearson's correlation coefficient and Manders' overlap coefficients (M1, M2).[12]

A Pearson's correlation coefficient above 0.7 indicates strong positive correlation and specific accumulation in the organelle, while a coefficient value below 0.5 indicates weak correlation and absence of specific accumulation. Intermediate values of 0.5-0.7 may suggest moderate correlation and partial localization.[13] Manders' coefficient M1 represents the fraction of green compound signal colocalized with the red organelle tracker signal, while Manders' coefficient M2 indicates the fraction of red organelle tracker signal colocalized with the green compound signal. Statistical analysis was performed based on a minimum of 5 independent measurements. Results are presented as mean ± standard deviation.

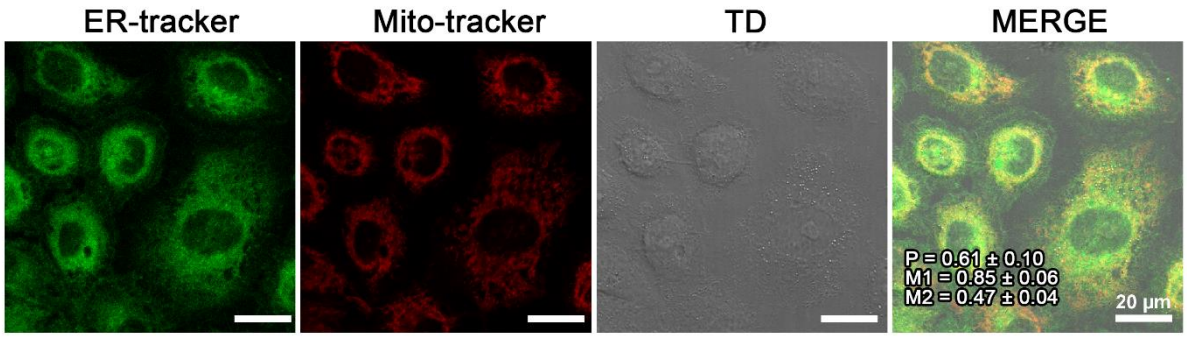



**Fig. S9.** Confocal fluorescent live-cell imaging of HeLa cells incubated with the mixture of ER-tracker Green (0.05 µM, FITC channel) and mito-tracker Red (0.05 µM, TRITC-Cy5 channels) for 10 min. Merged image and corresponding colocalization coefficients demonstrate the different localization of ER- and mito-trackers (Pearson coefficient is approximately 0.6). Scale bar: 20 µm.

## 6 References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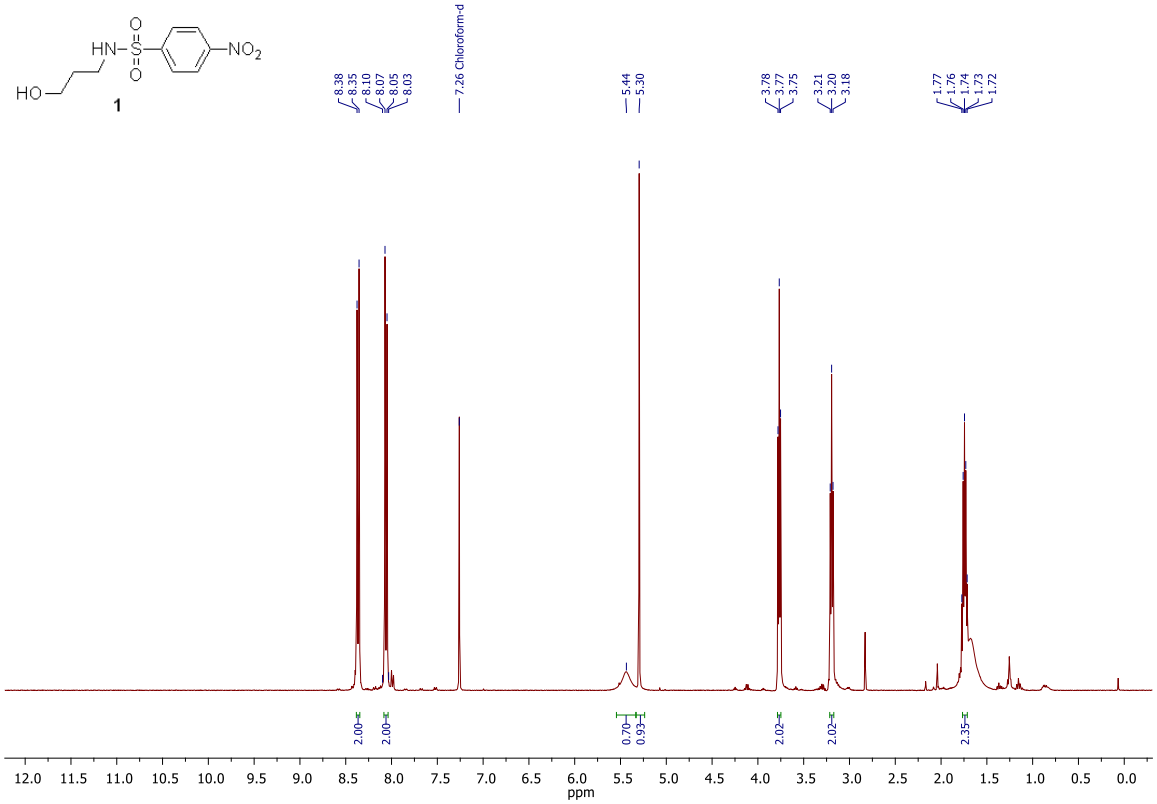
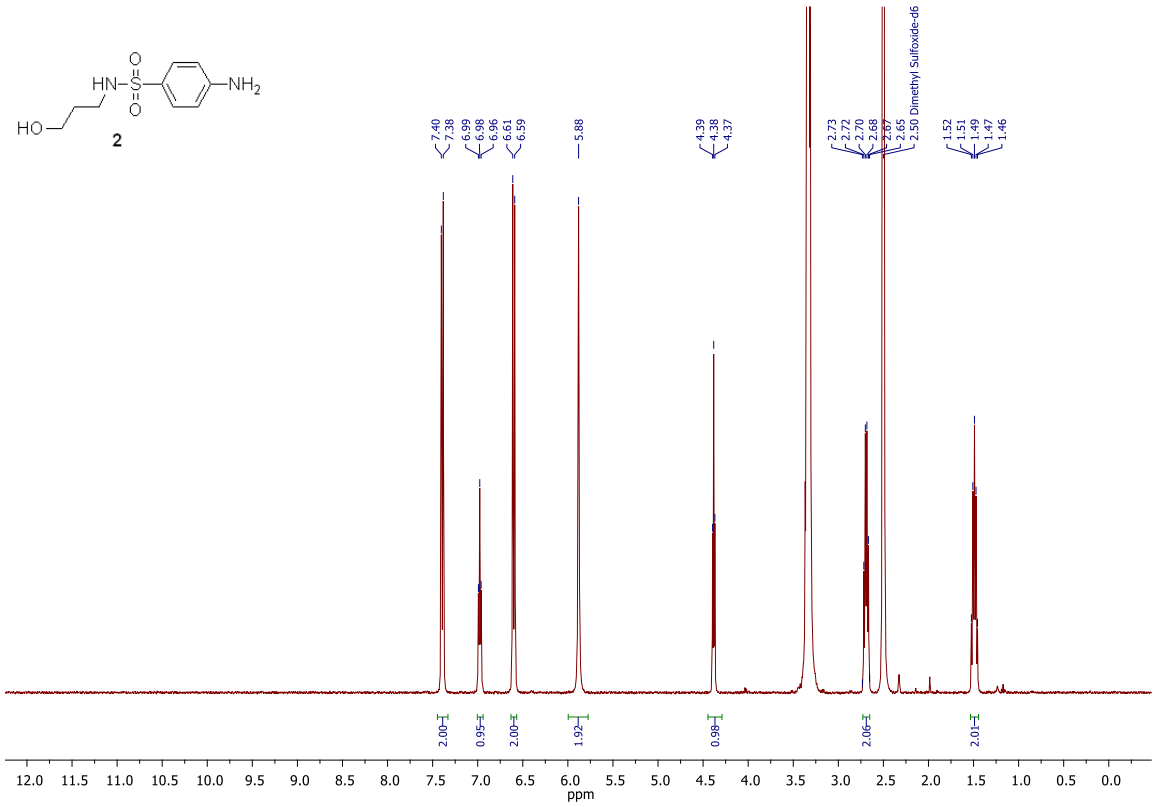
7 Copies of NMR

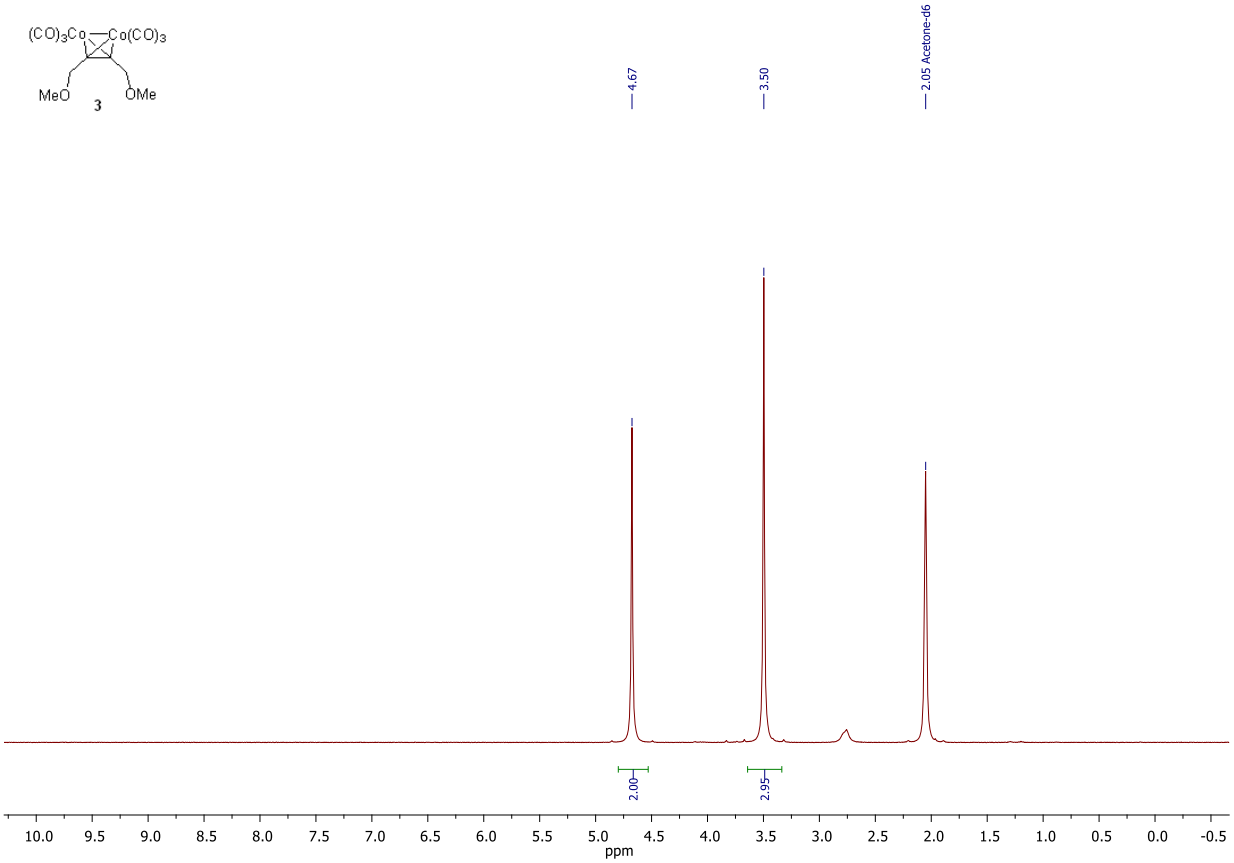
$^1$H NMR, $CDCl_3$, 400 MHz



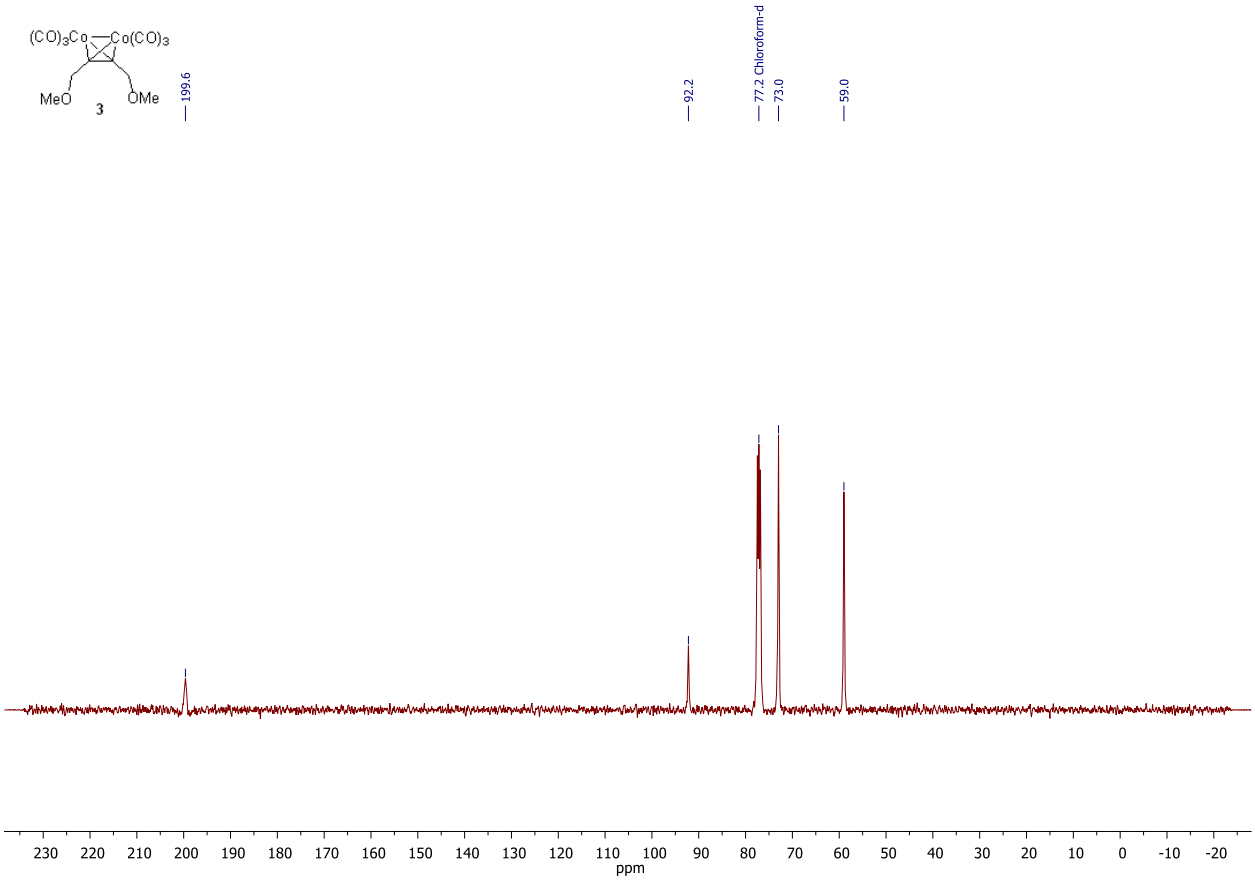

$^1$H NMR, DMSO-$d_6$, 400 MHz



$^1$H NMR, acetone-$d_6$, 400 MHz




$^1$H NMR, $CDCl_3$, 101 MHz



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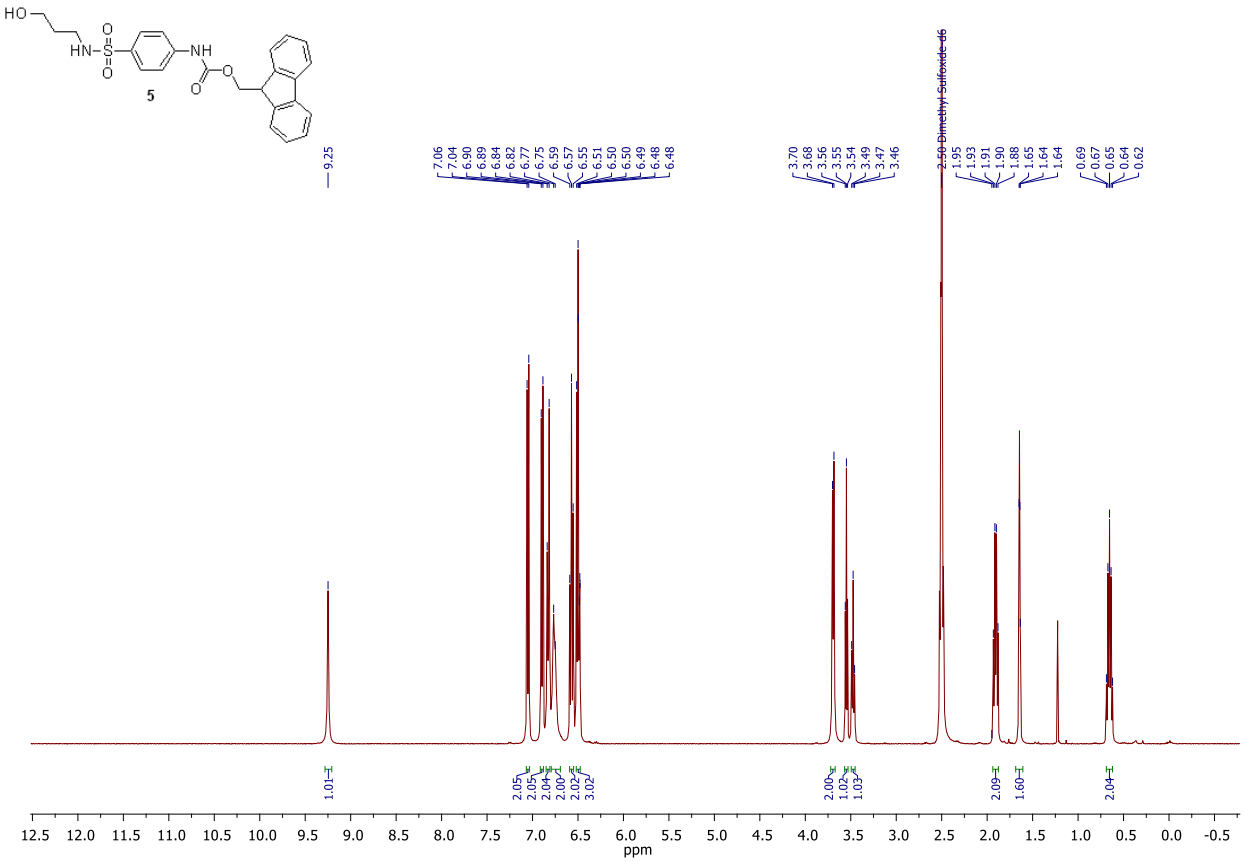
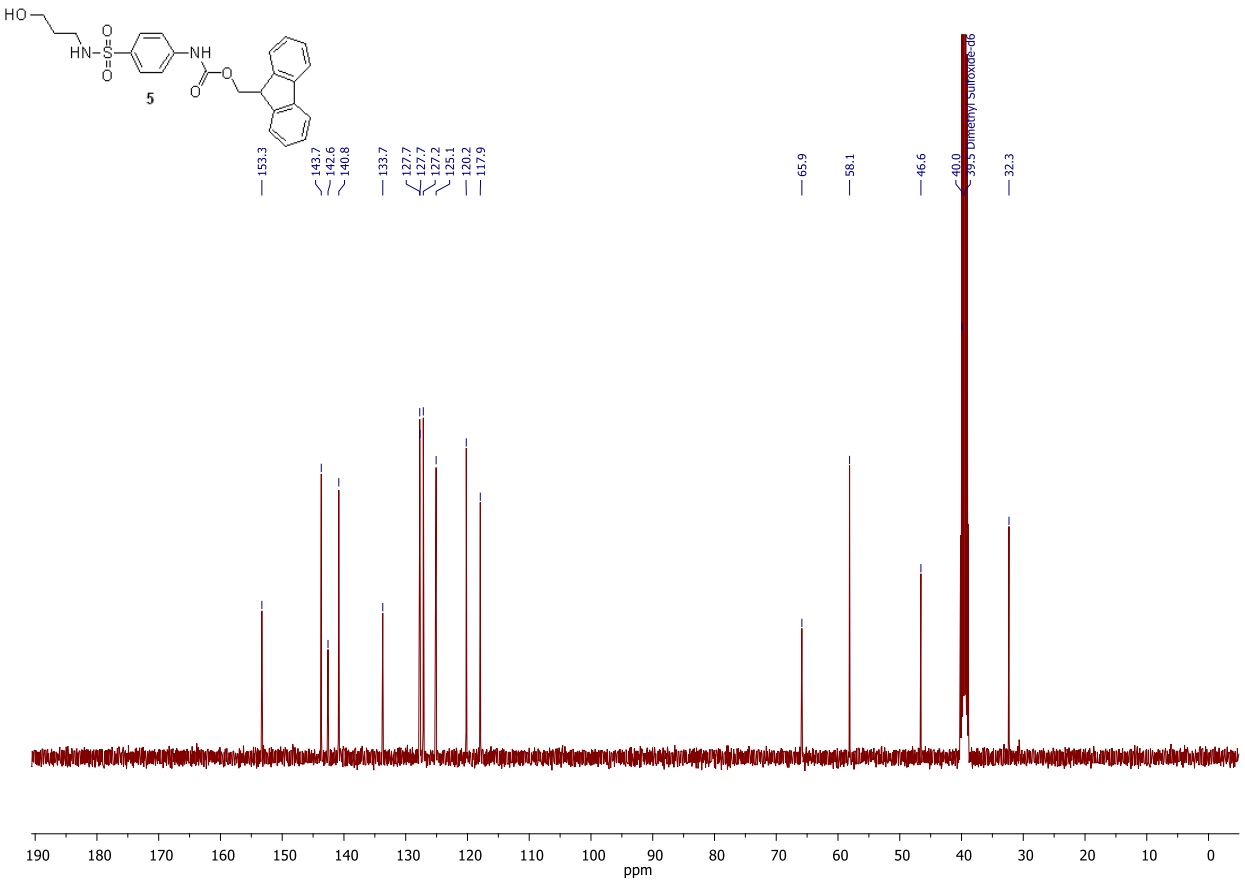
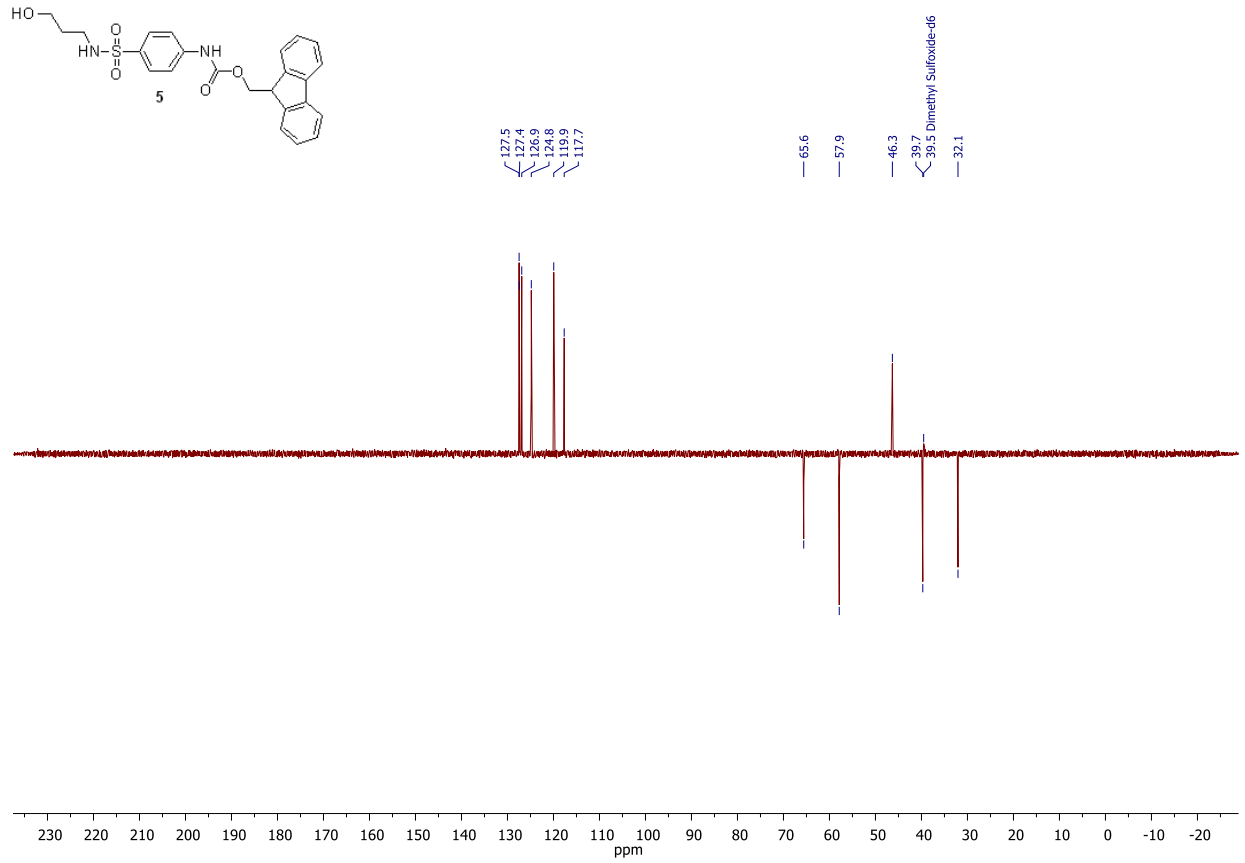



$^{13}$C NMR, DMSO-$d_6$, 101 MHz



DEPT 135, DMSO-$d_6$, 101 MHz

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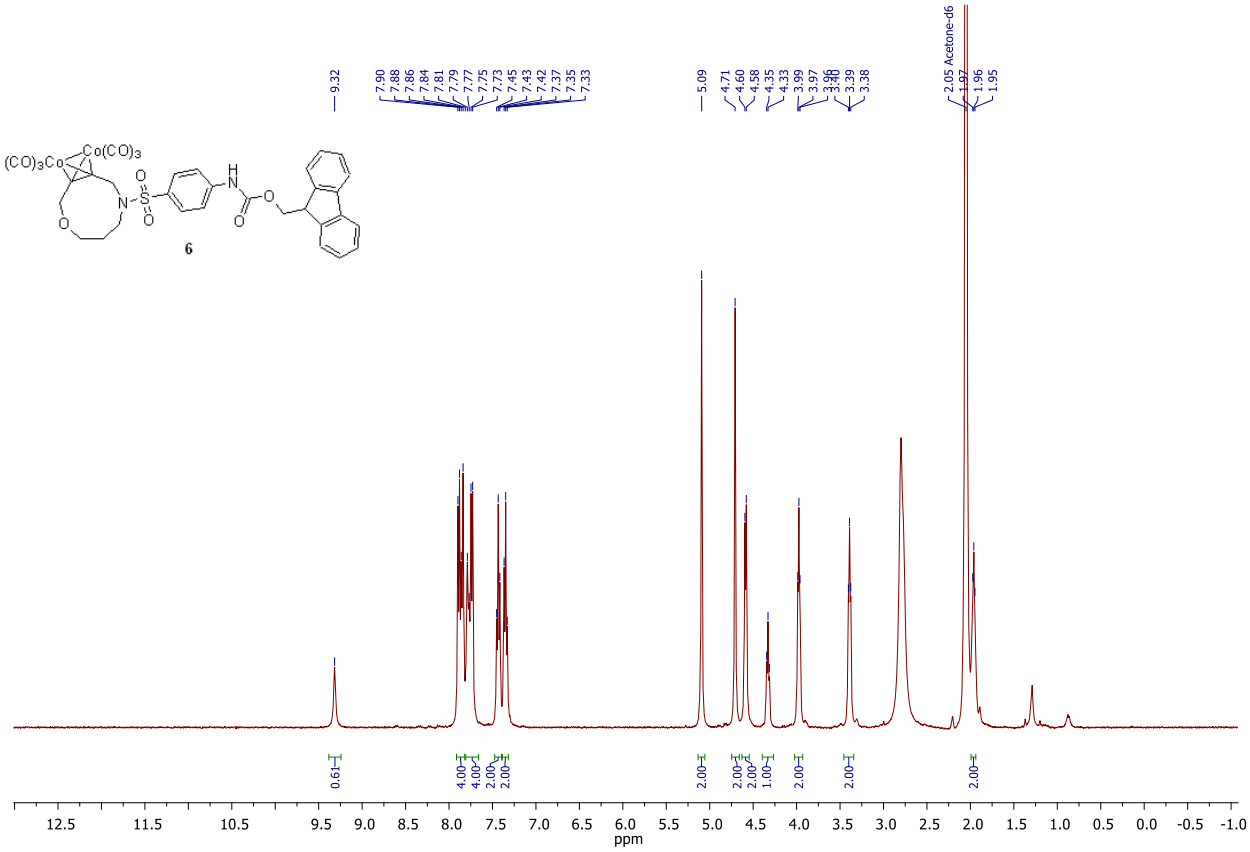
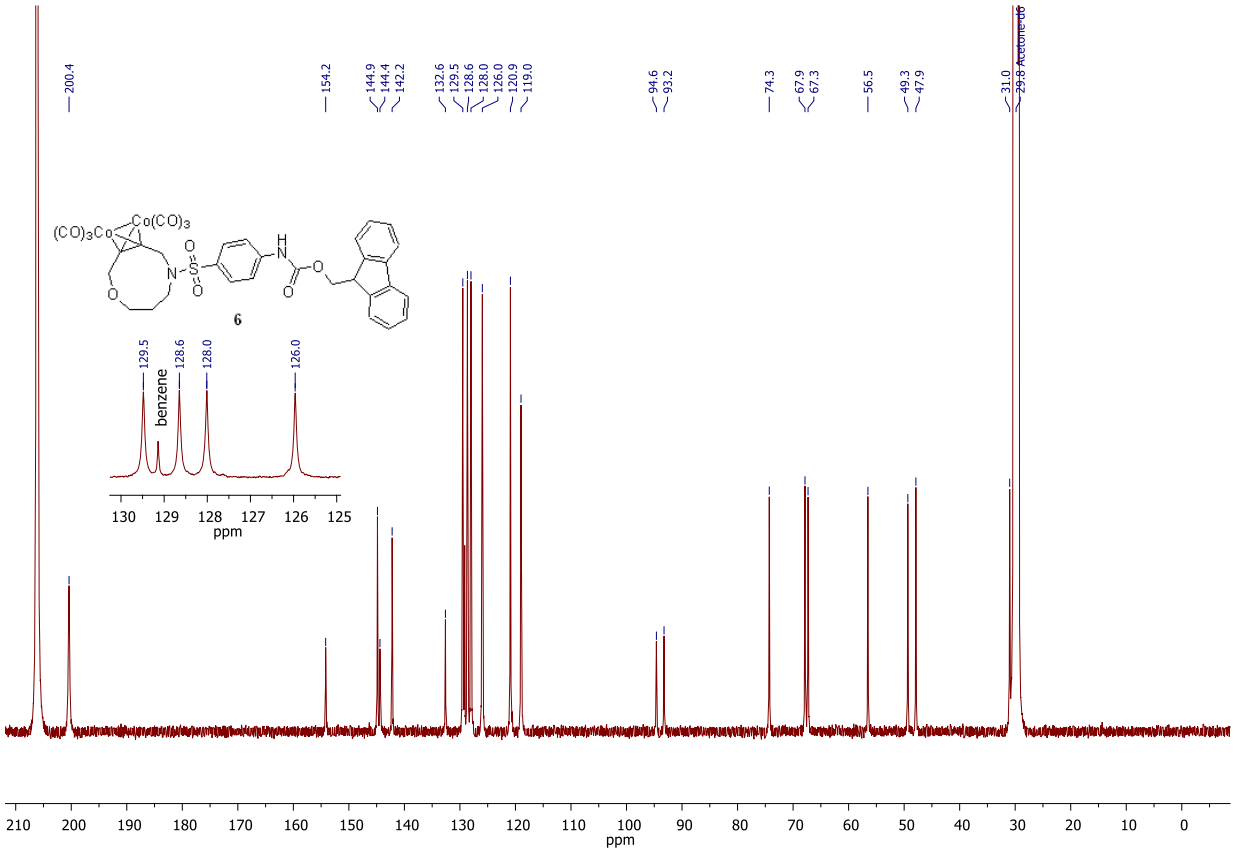


$^{13}$C NMR, acetone-$d_6$, 126 MHz

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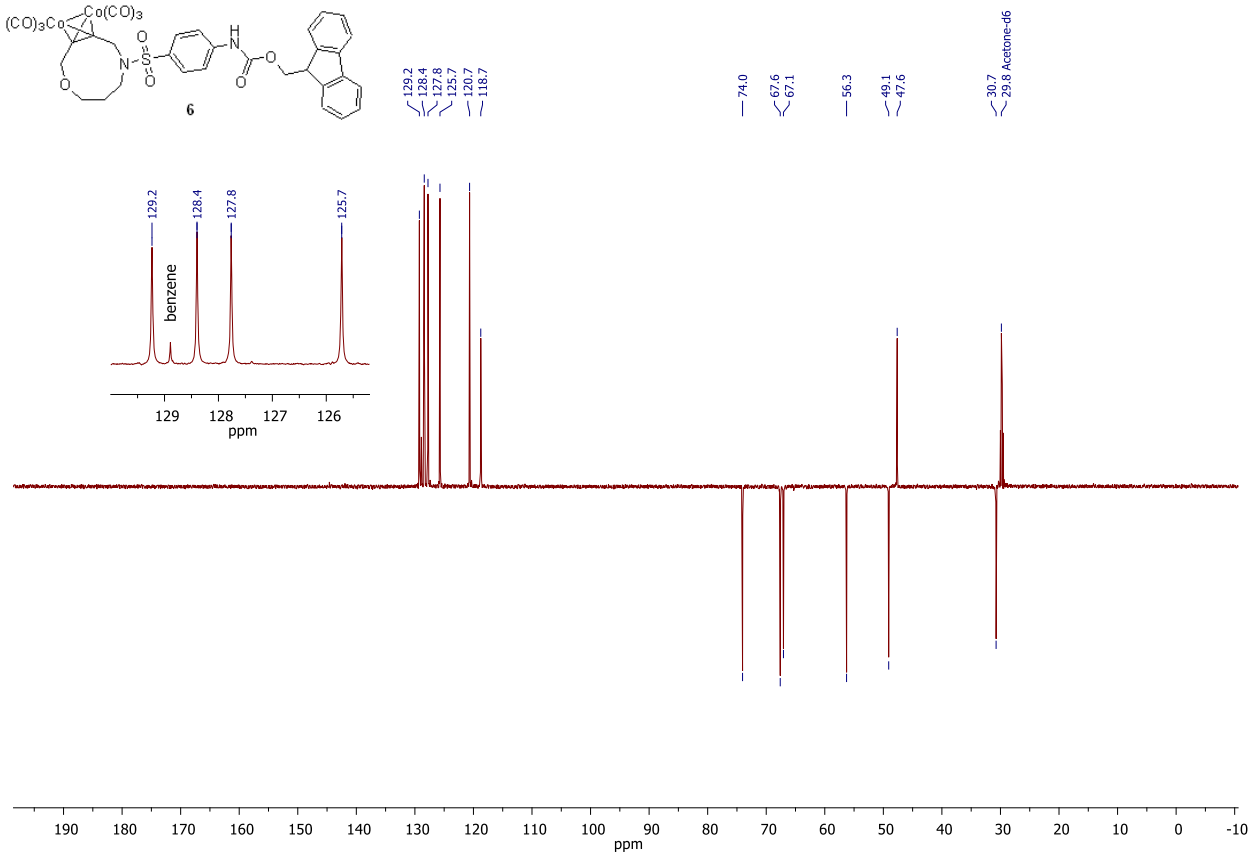
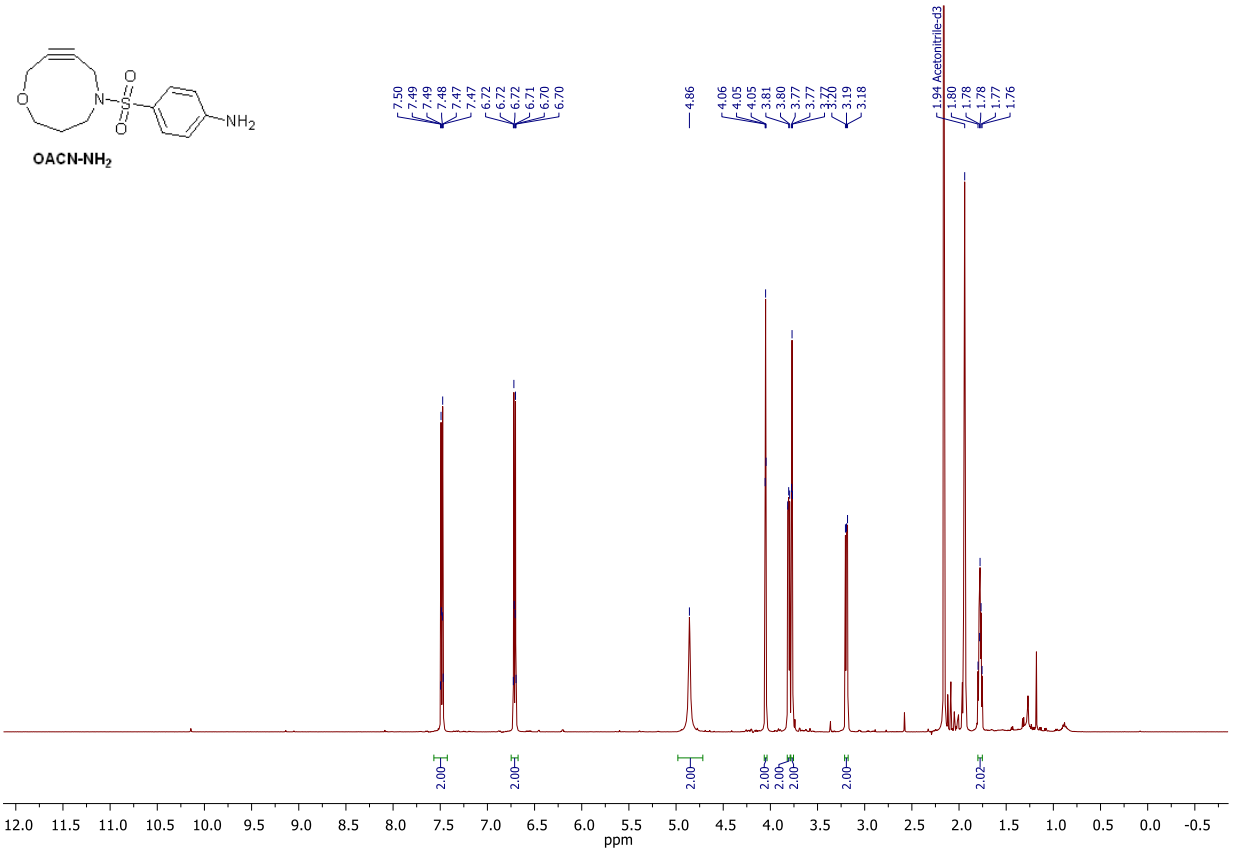
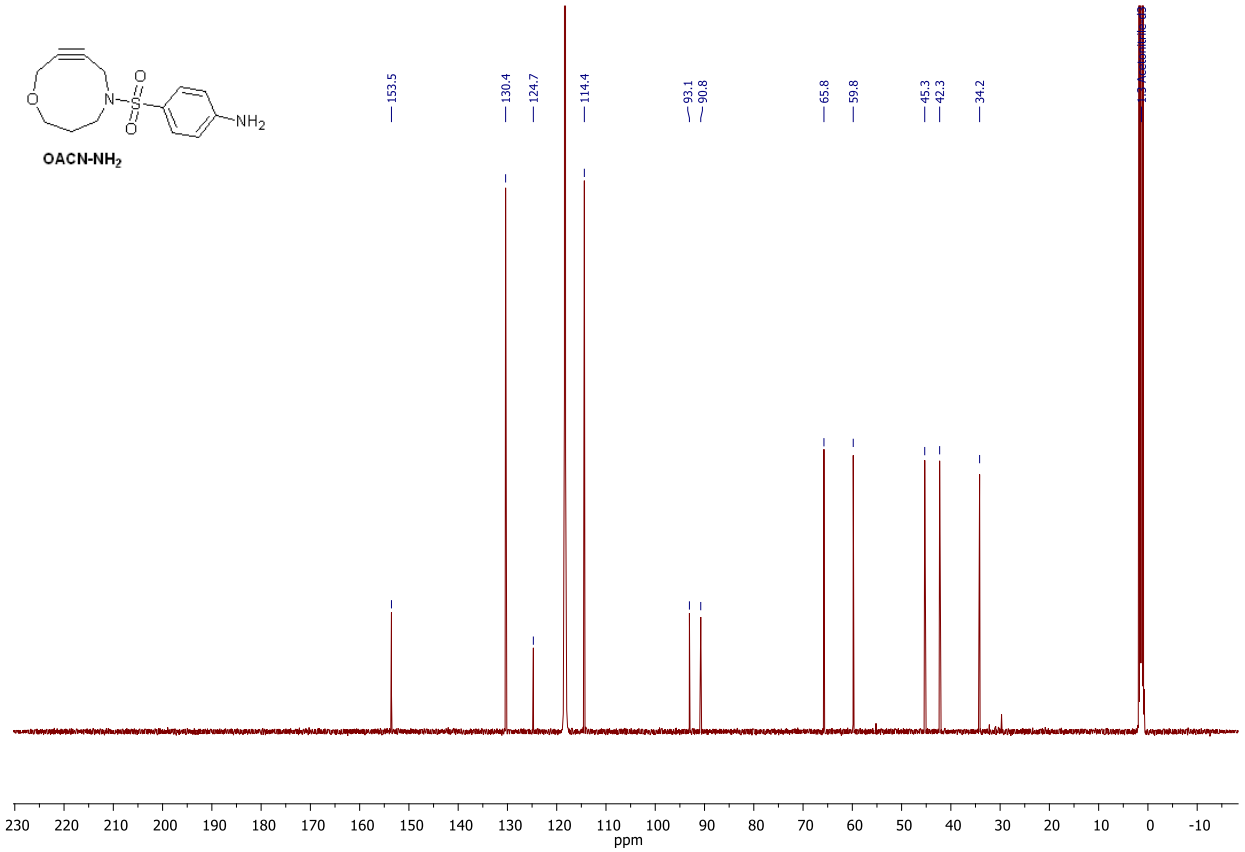


$^{1}$H NMR, $CD_3CN$, 500 MHz




$^{13}$C NMR, $CD_3CN$, 126 MHz



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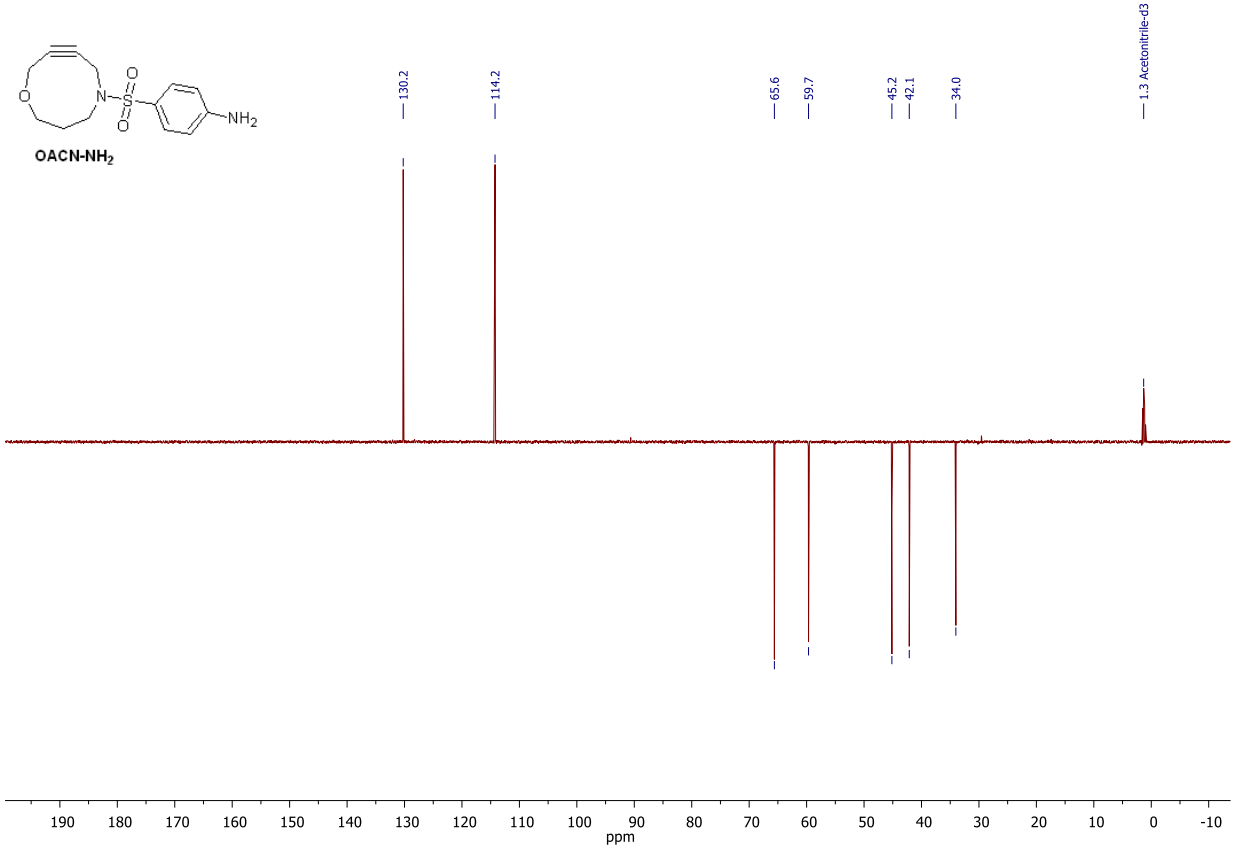
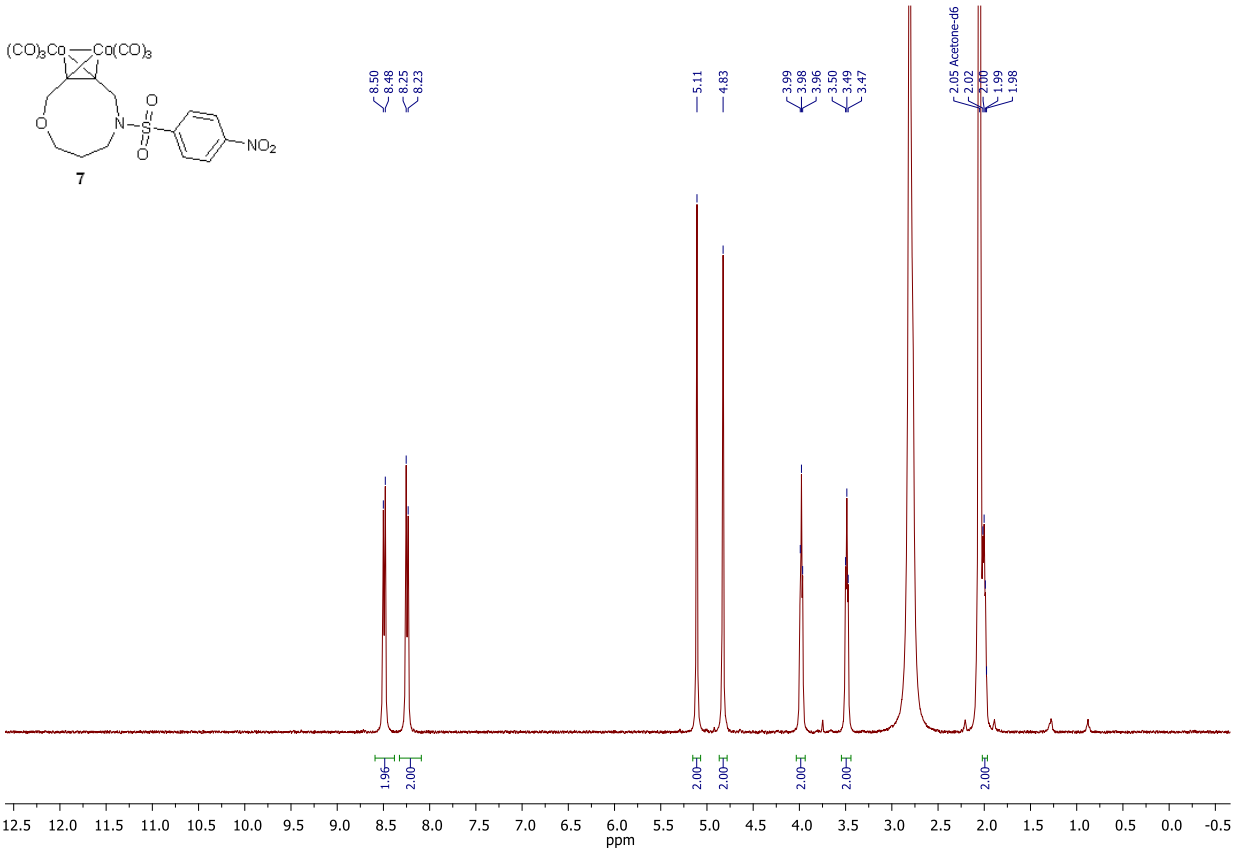
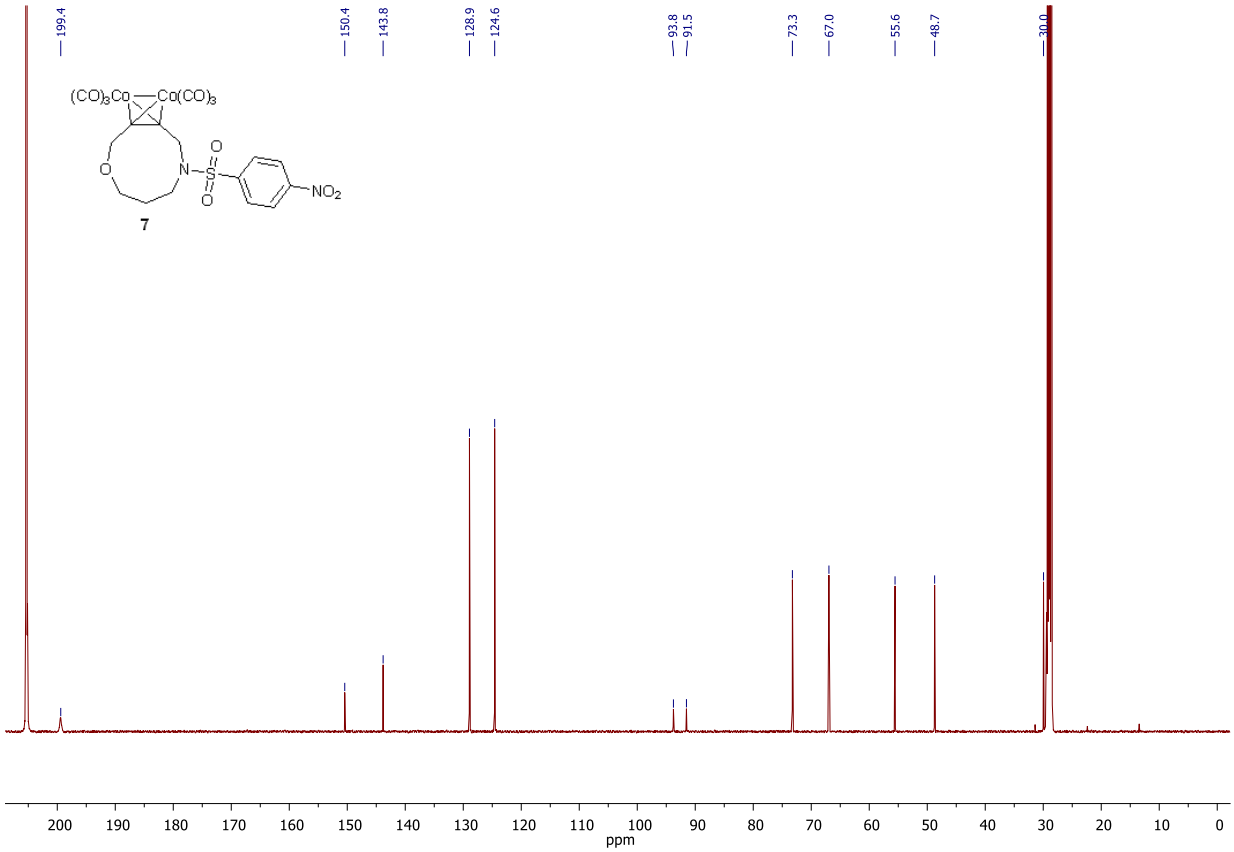


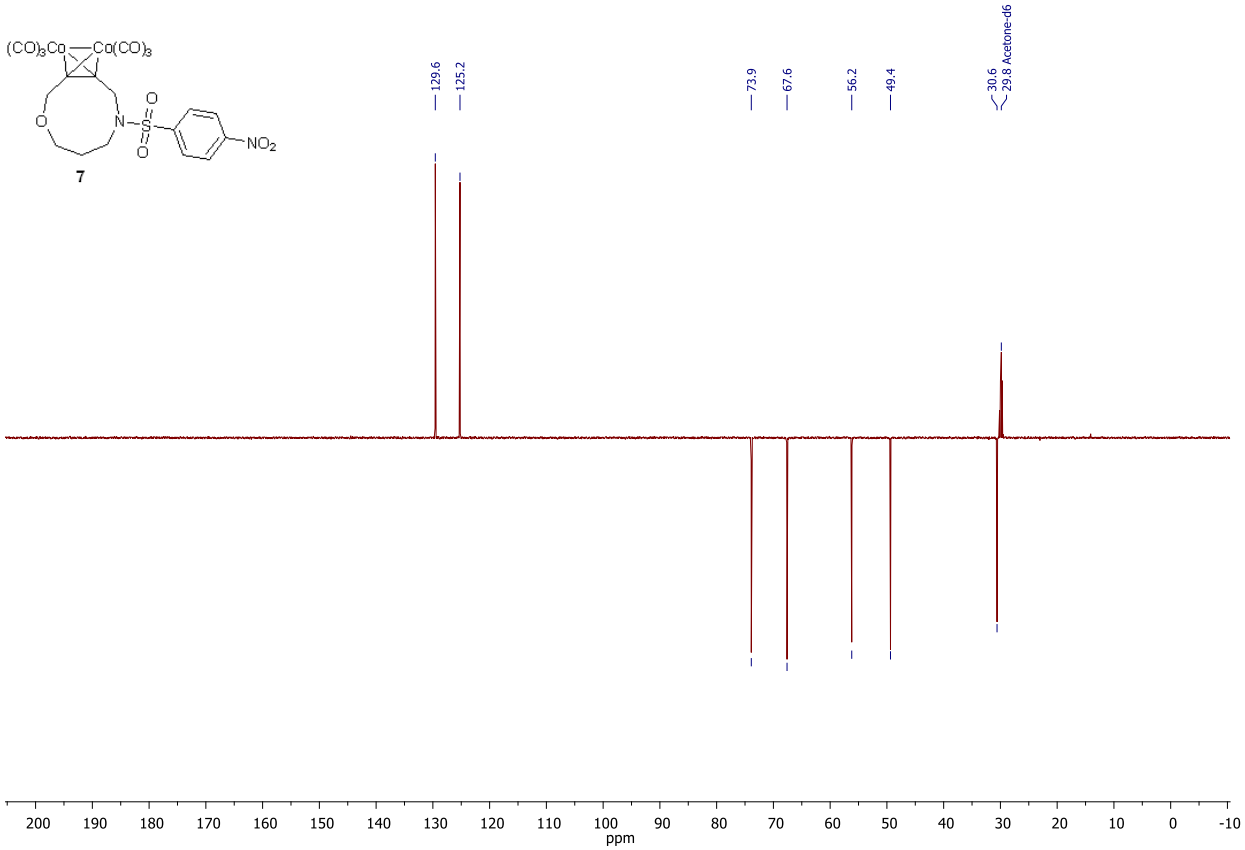

$^1H$ NMR, acetone-$d_6$, 400 MHz



$^{13}$C NMR, acetone-$d_6$, 126 MHz




DEPT 135, acetone-$d_6$, 126 MHz



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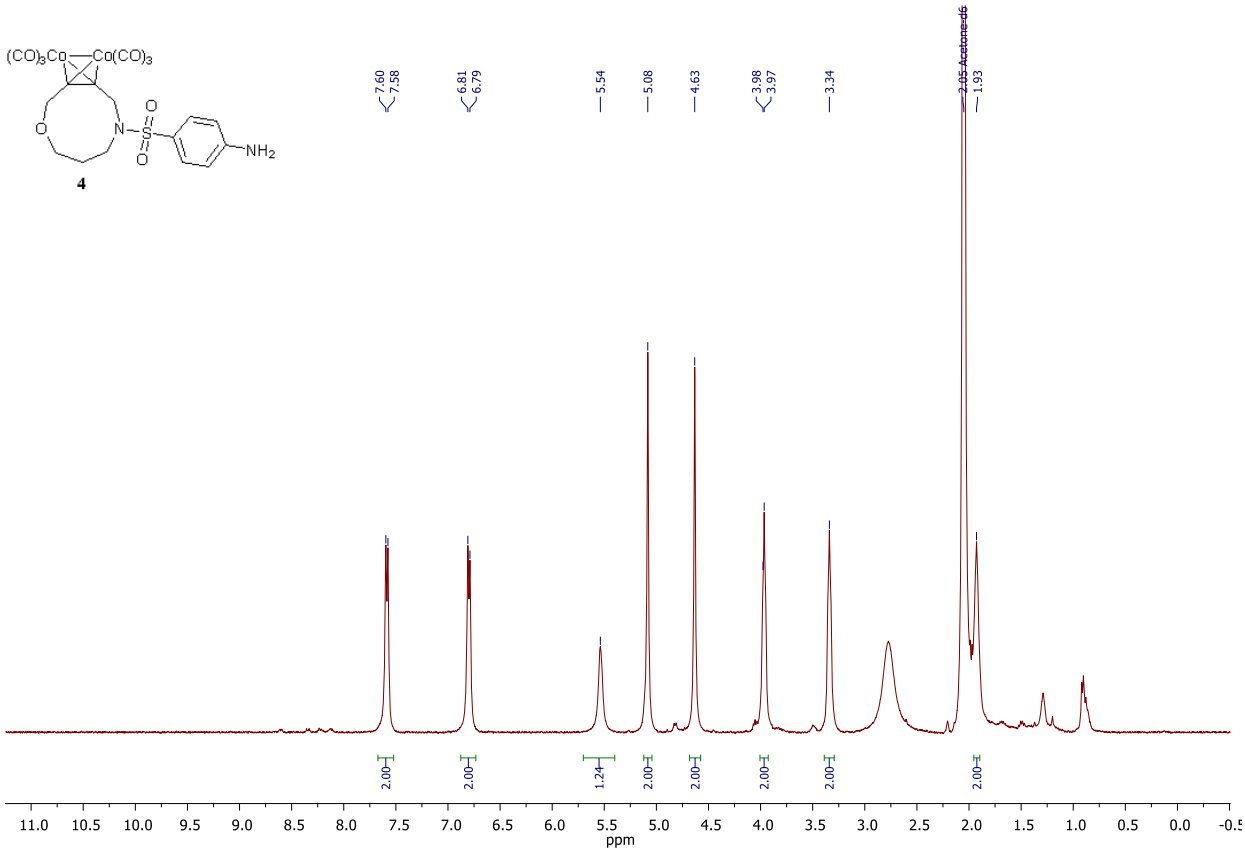


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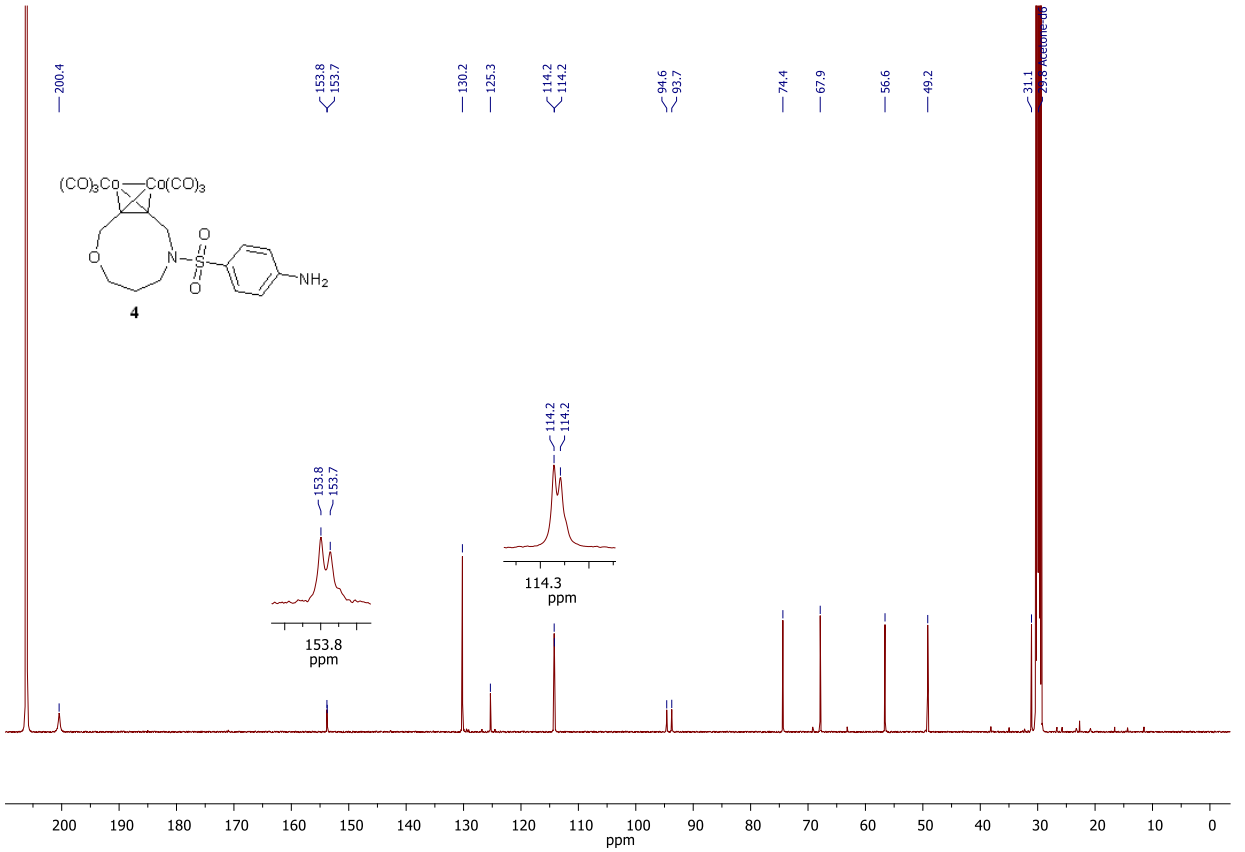
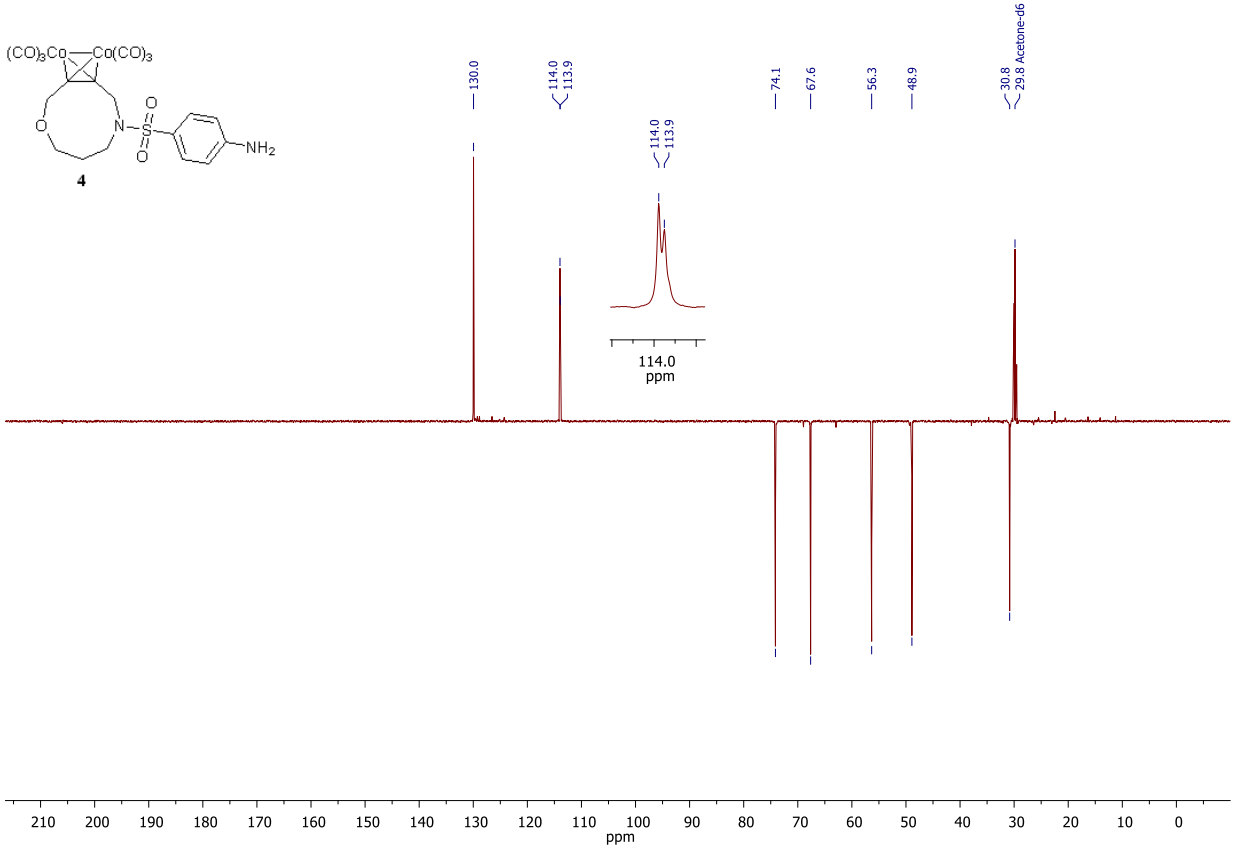
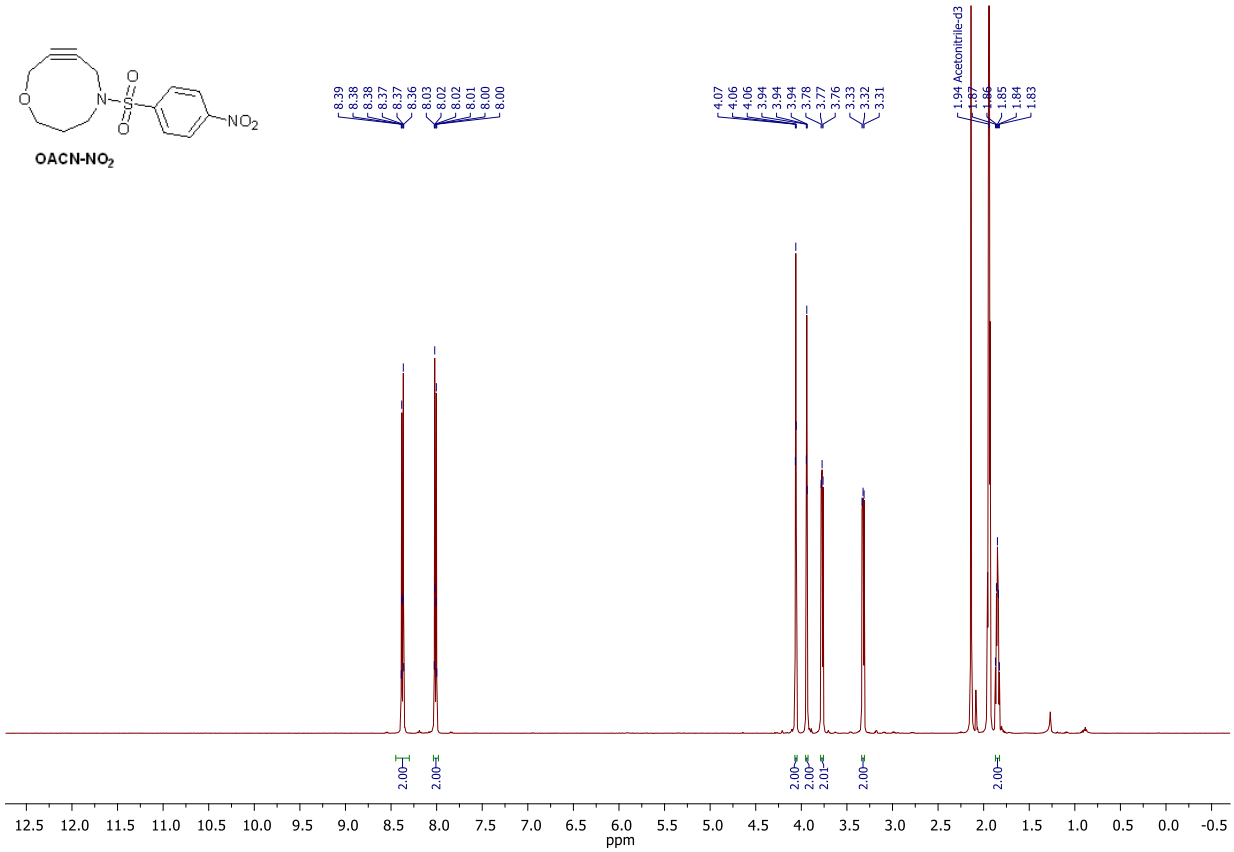


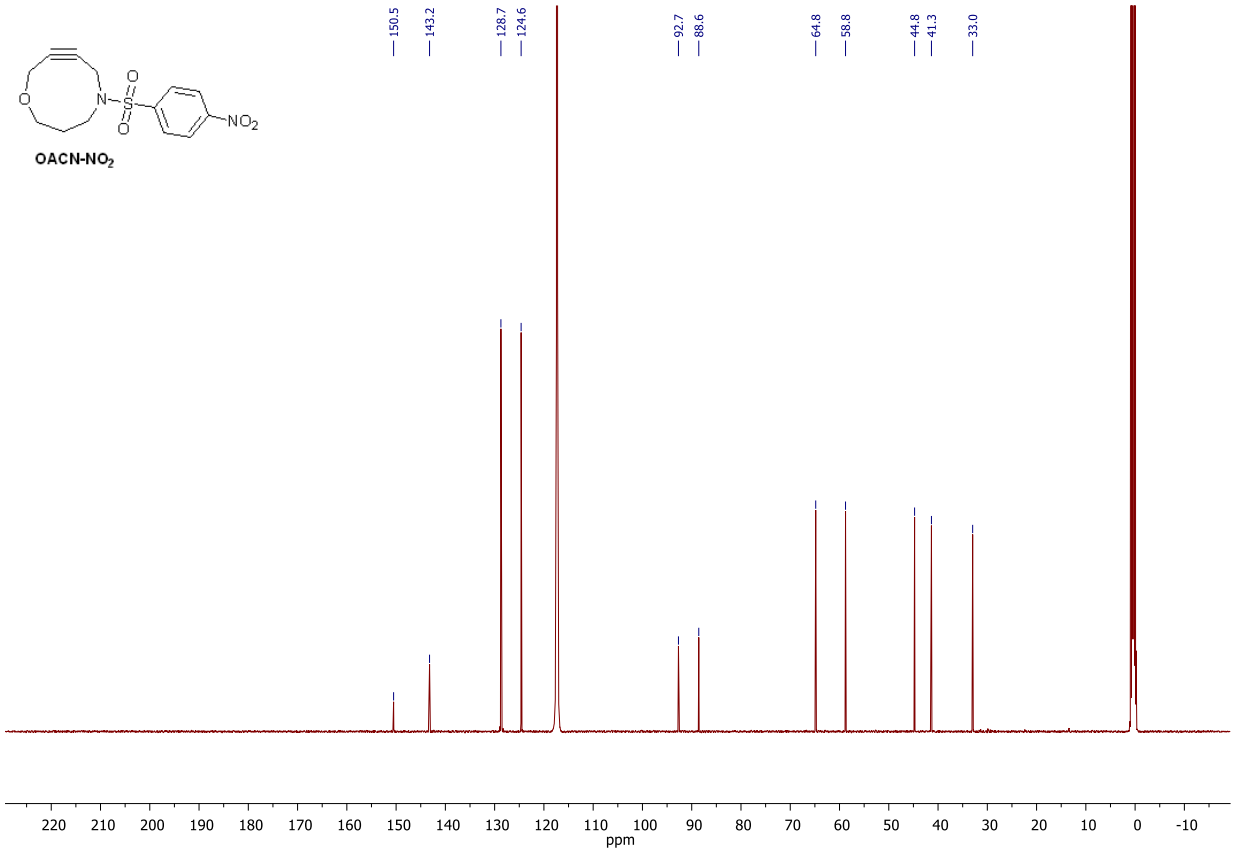
DEPT 135, acetone-$d_6$, 126 MHz




$^1$H NMR, $CD_3CN$, 500 MHz



$^{13}C$ NMR, $CD_3CN$, 126 MHz

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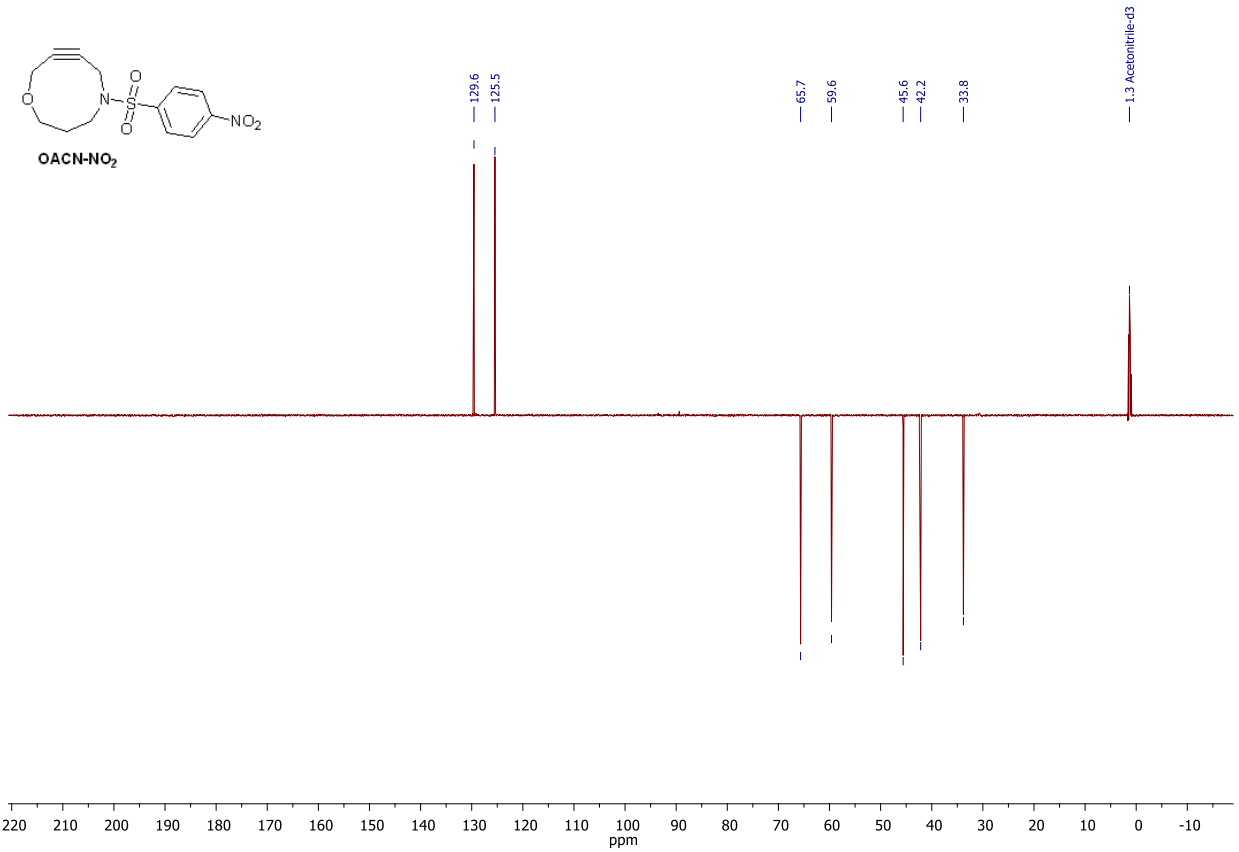

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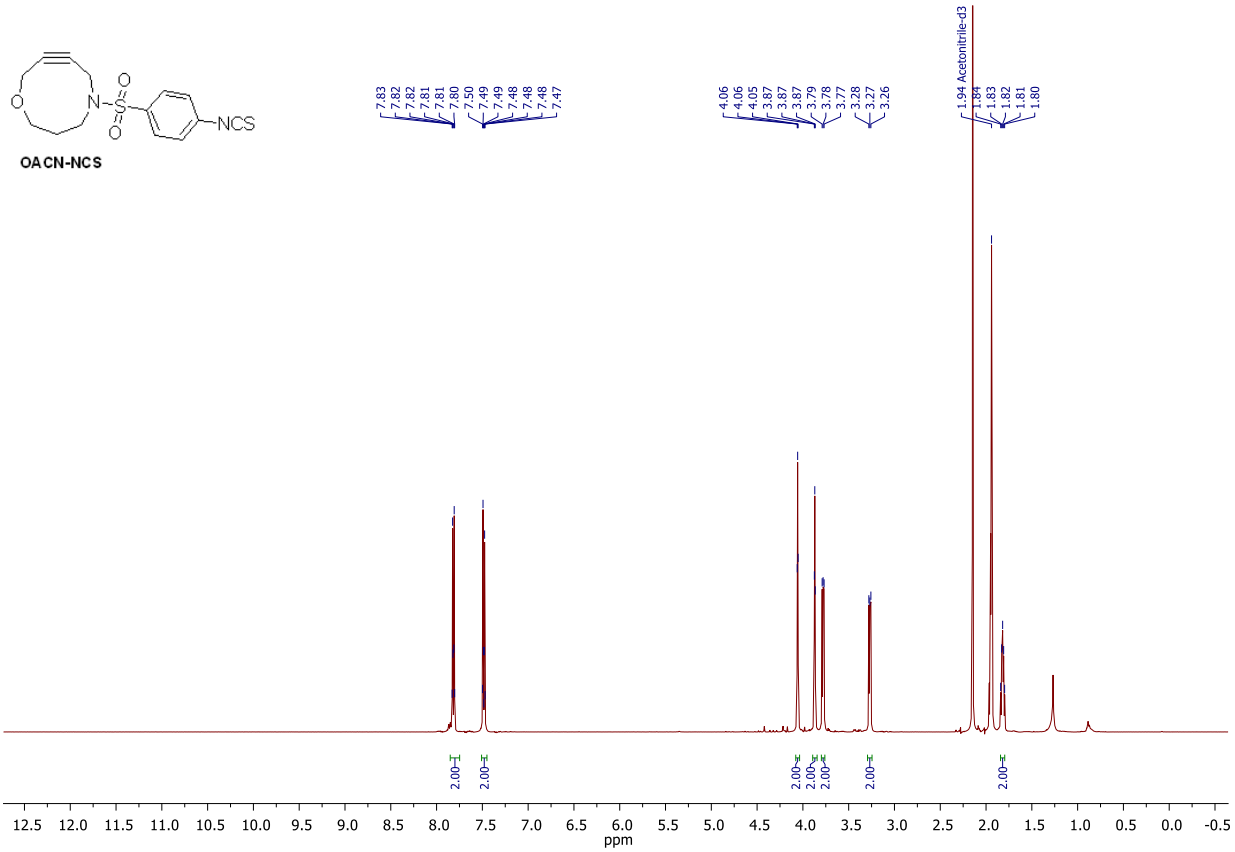


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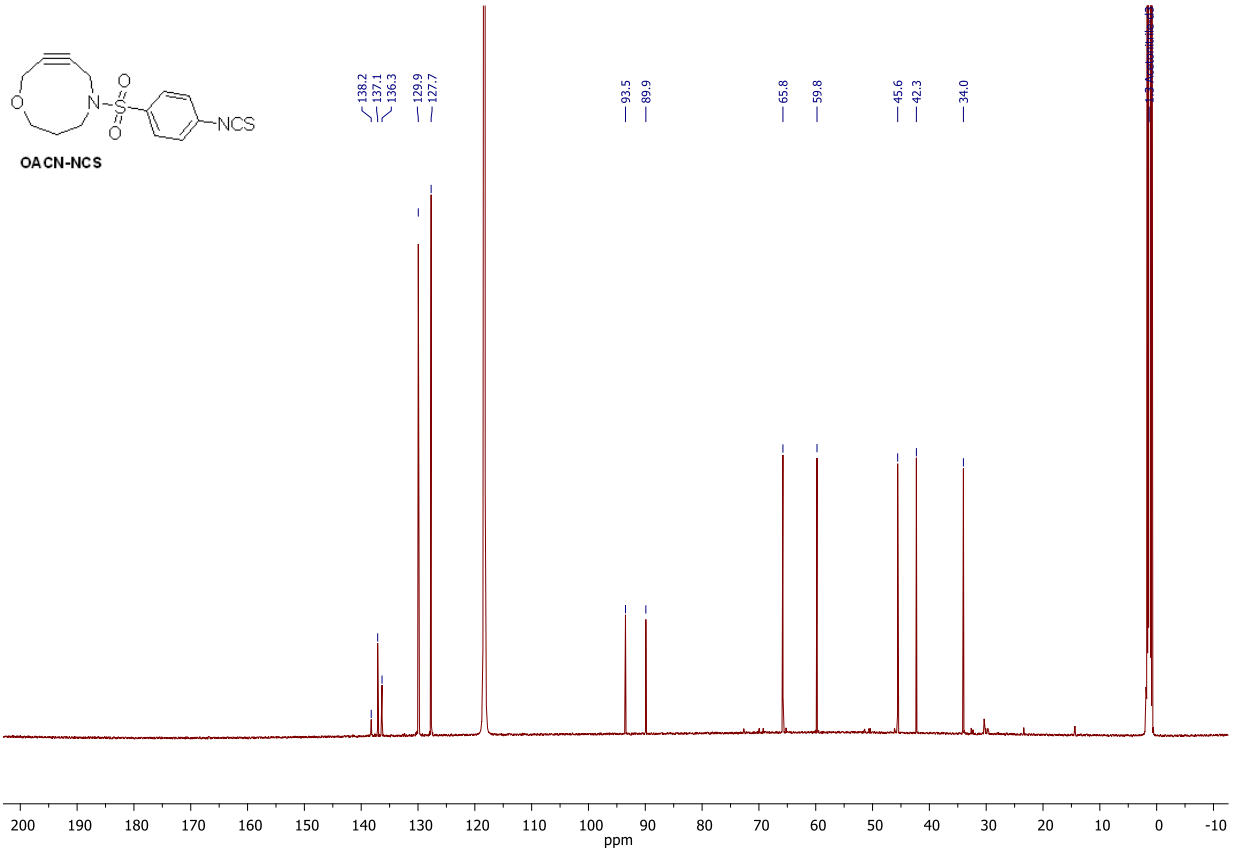
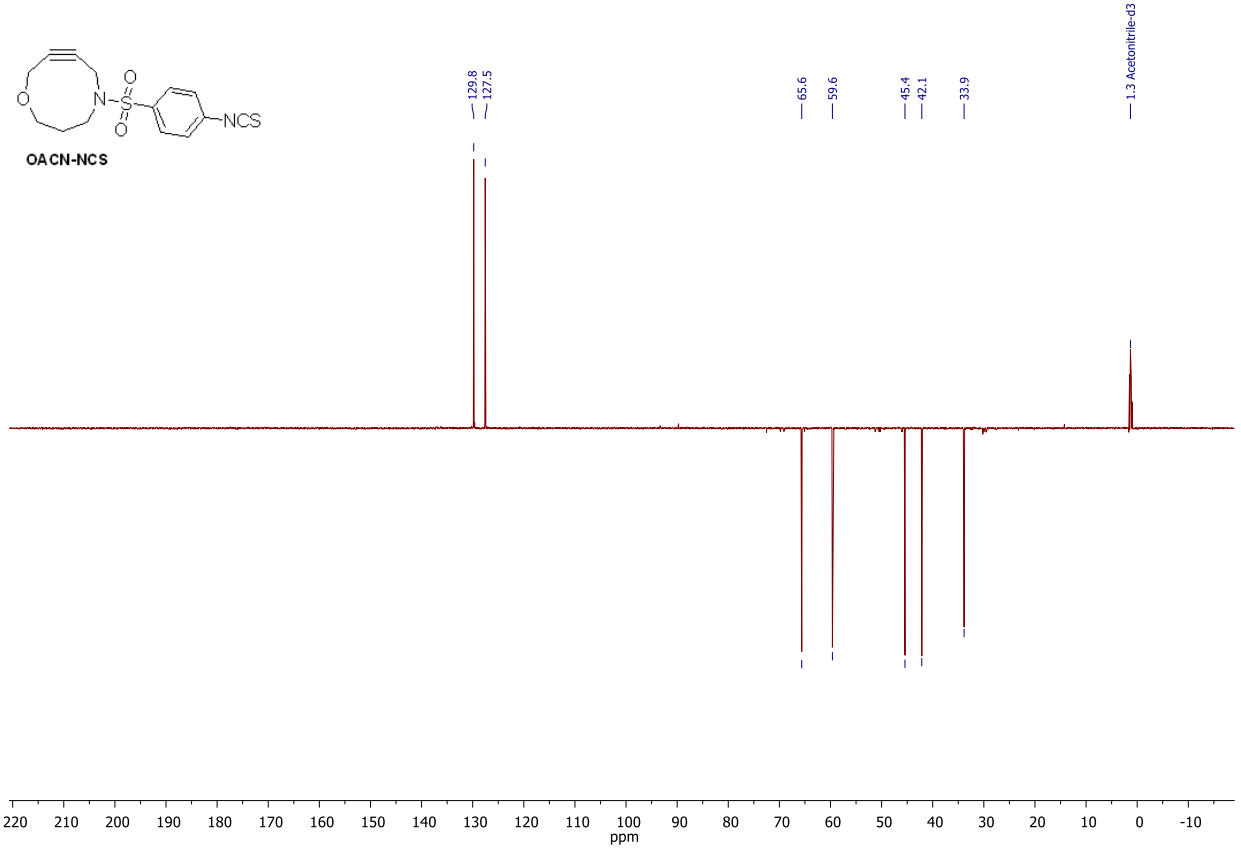
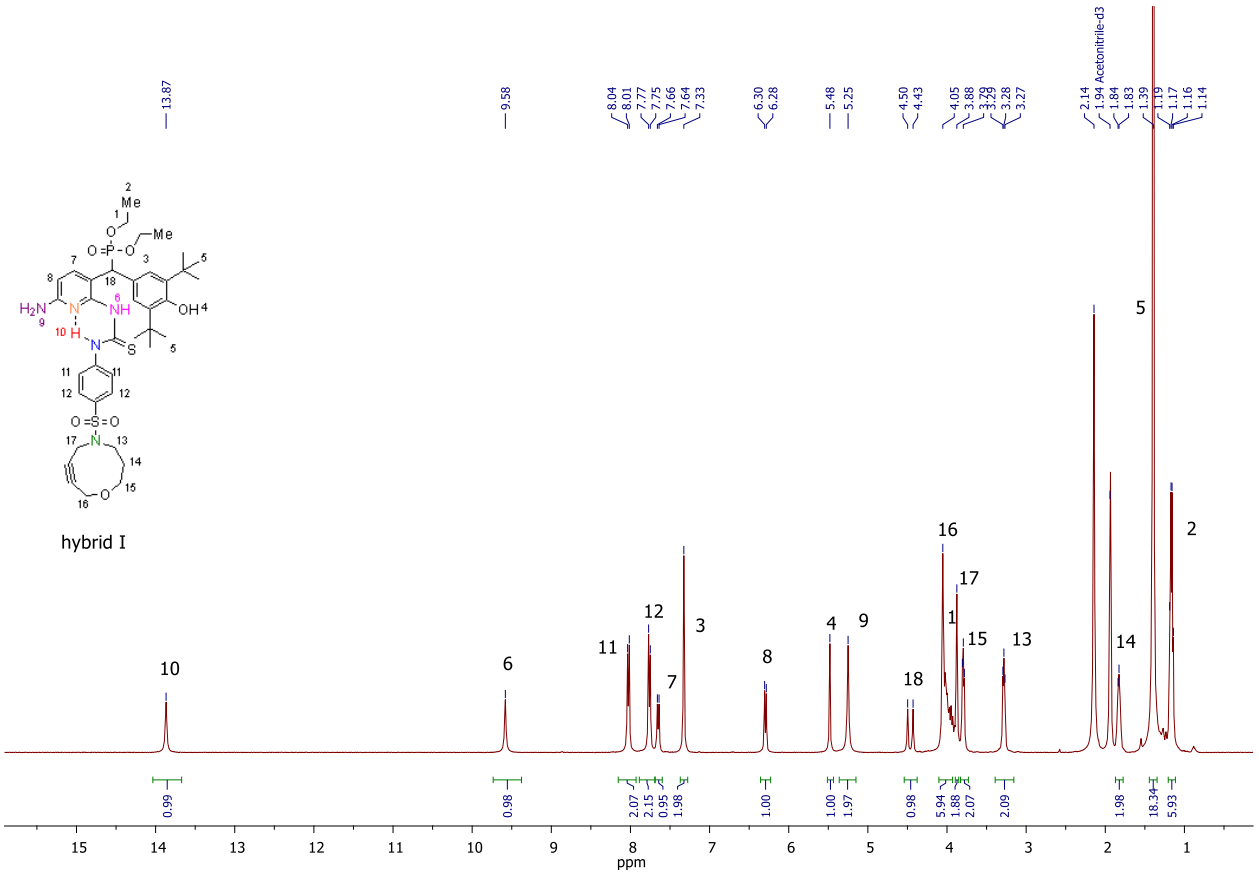


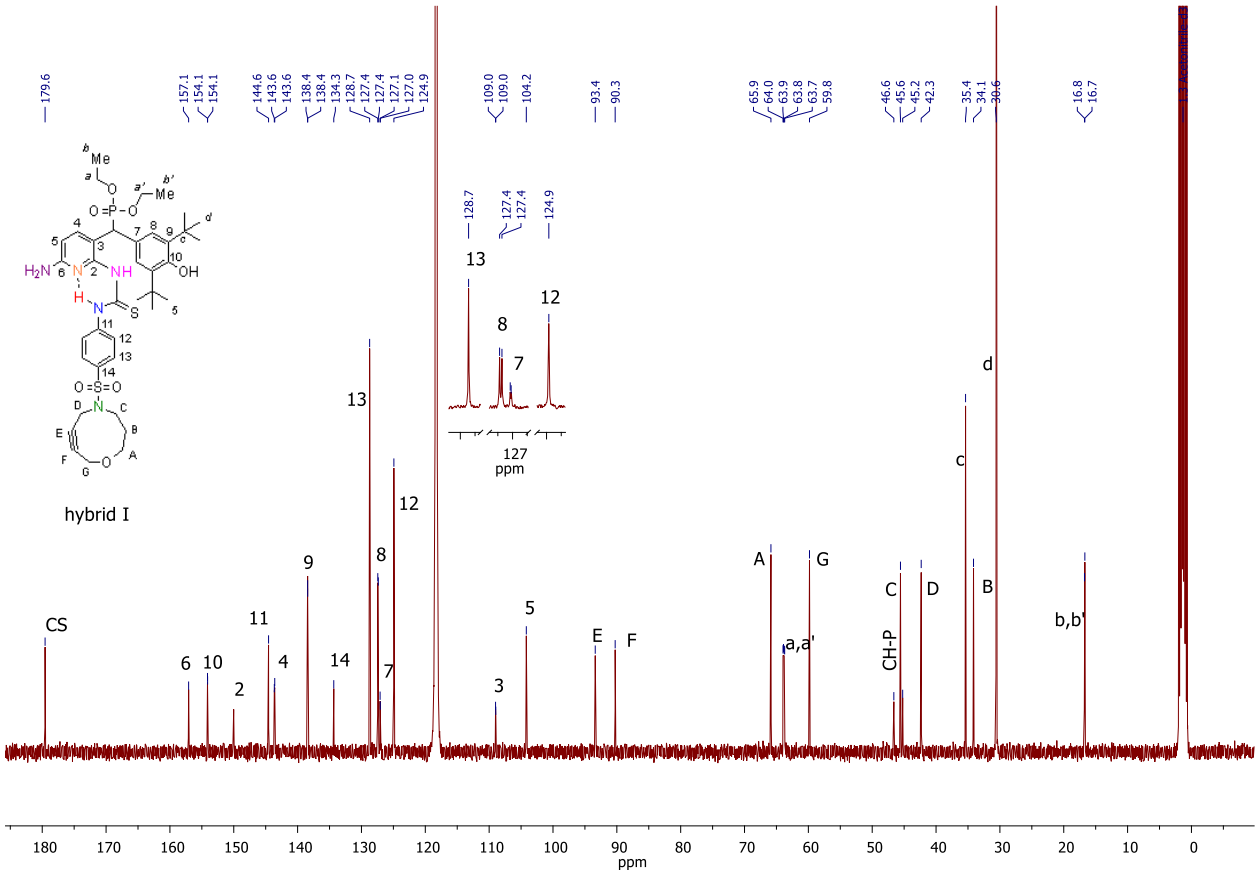
DEPT 135, $CD_3CN$, 126 MHz



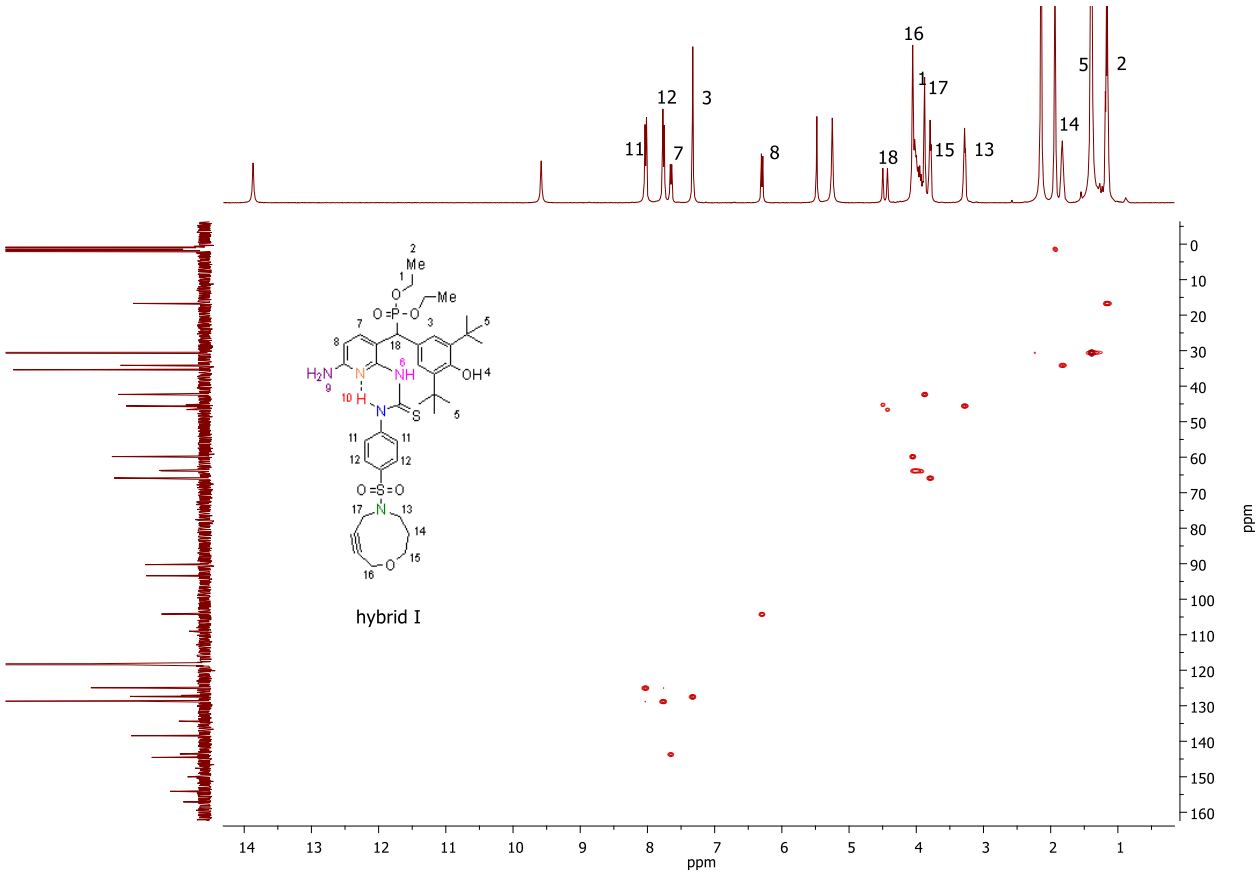

$^1H$ NMR, $CD_3CN$, 400 MHz (with hybrid I atom numbering for $^1H$ NMR assignment)



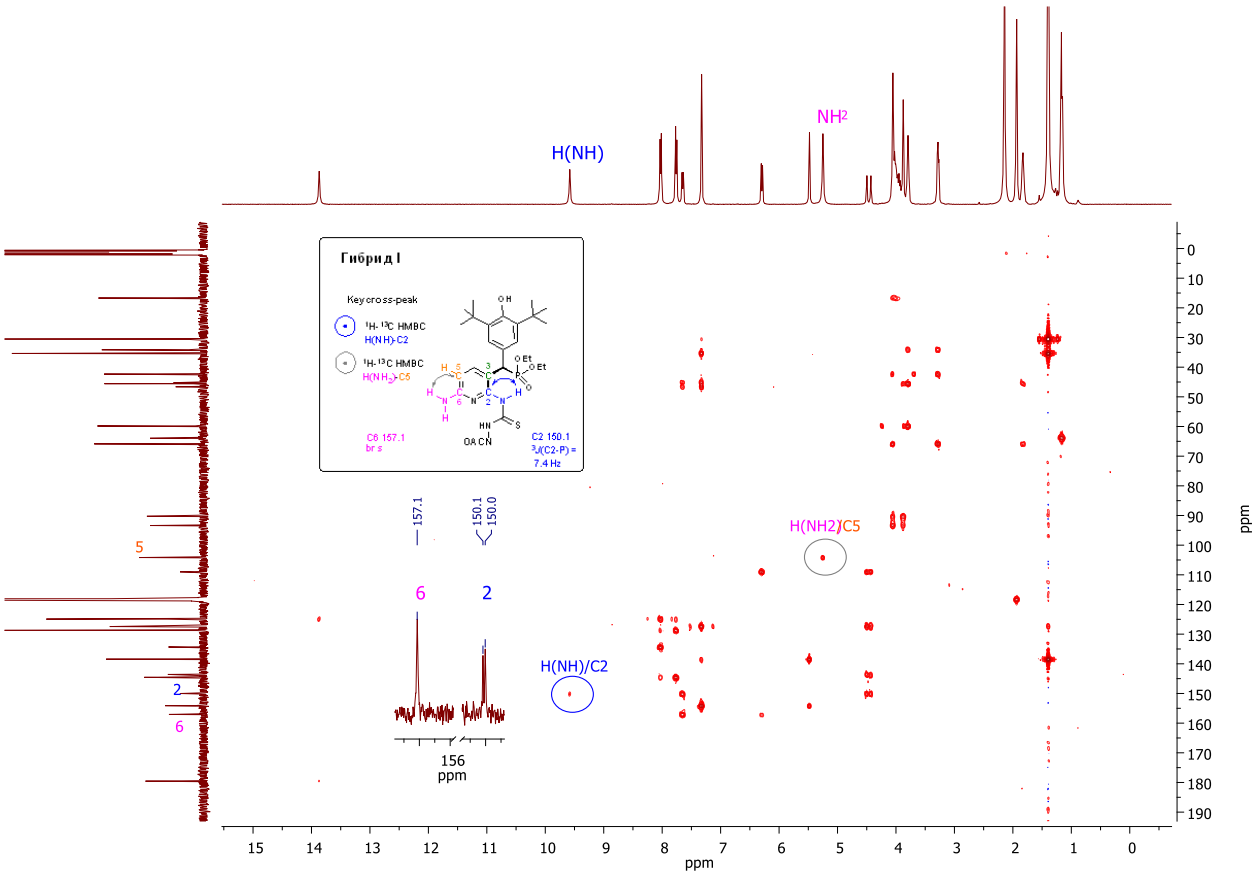
$^{13}C$ NMR, $CD_3CN$, 101 MHz (with hybrid I atom numbering for $^{13}C$ NMR assignment)



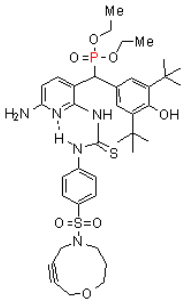

$^{1}H$-$^{13}C$ HSQC NMR, $CD_3CN$, 400 MHz (with hybrid I atom numbering for $^{1}H$ NMR assignment)



$^{1}H$-$^{13}C$ HMBC NMR, $CD_3CN$, 400 MHz

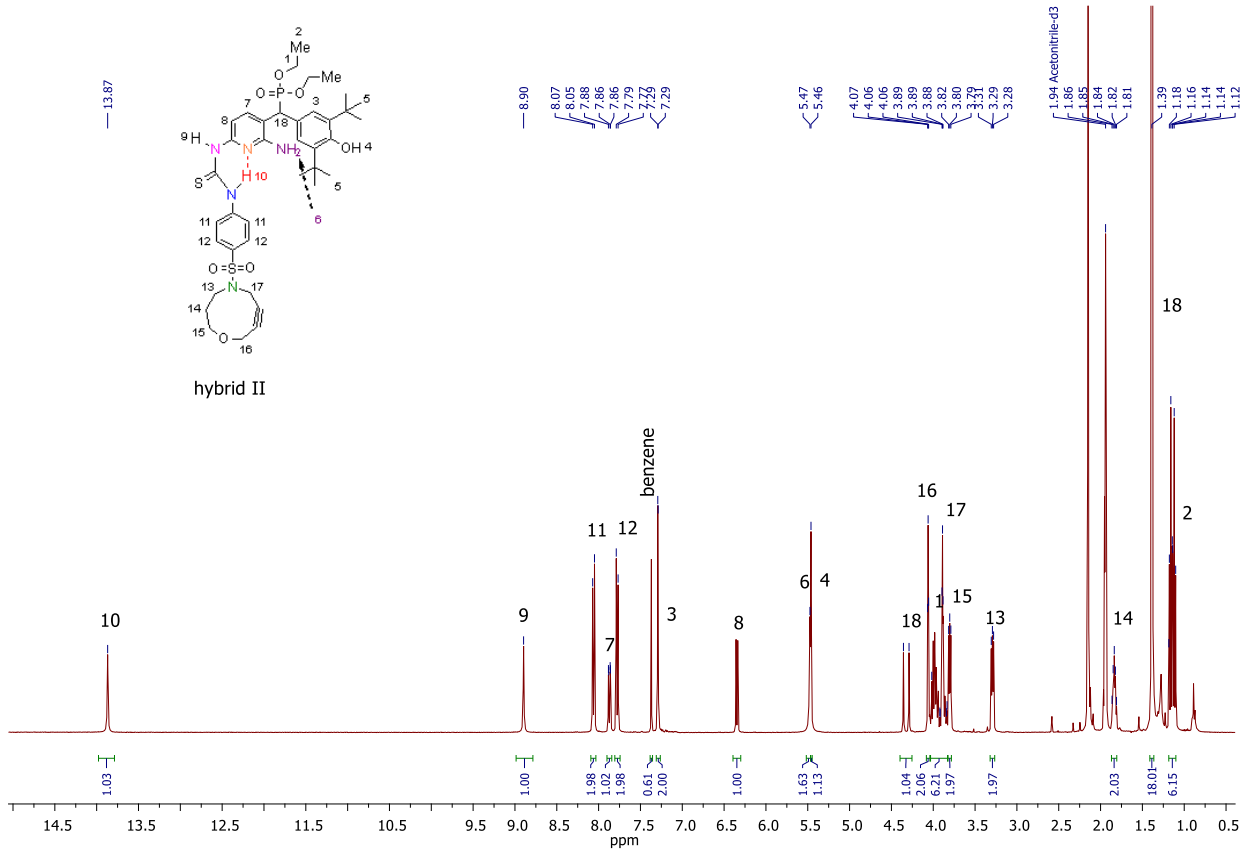



$^{31}P$ NMR, $CD_3CN$, 162 MHz

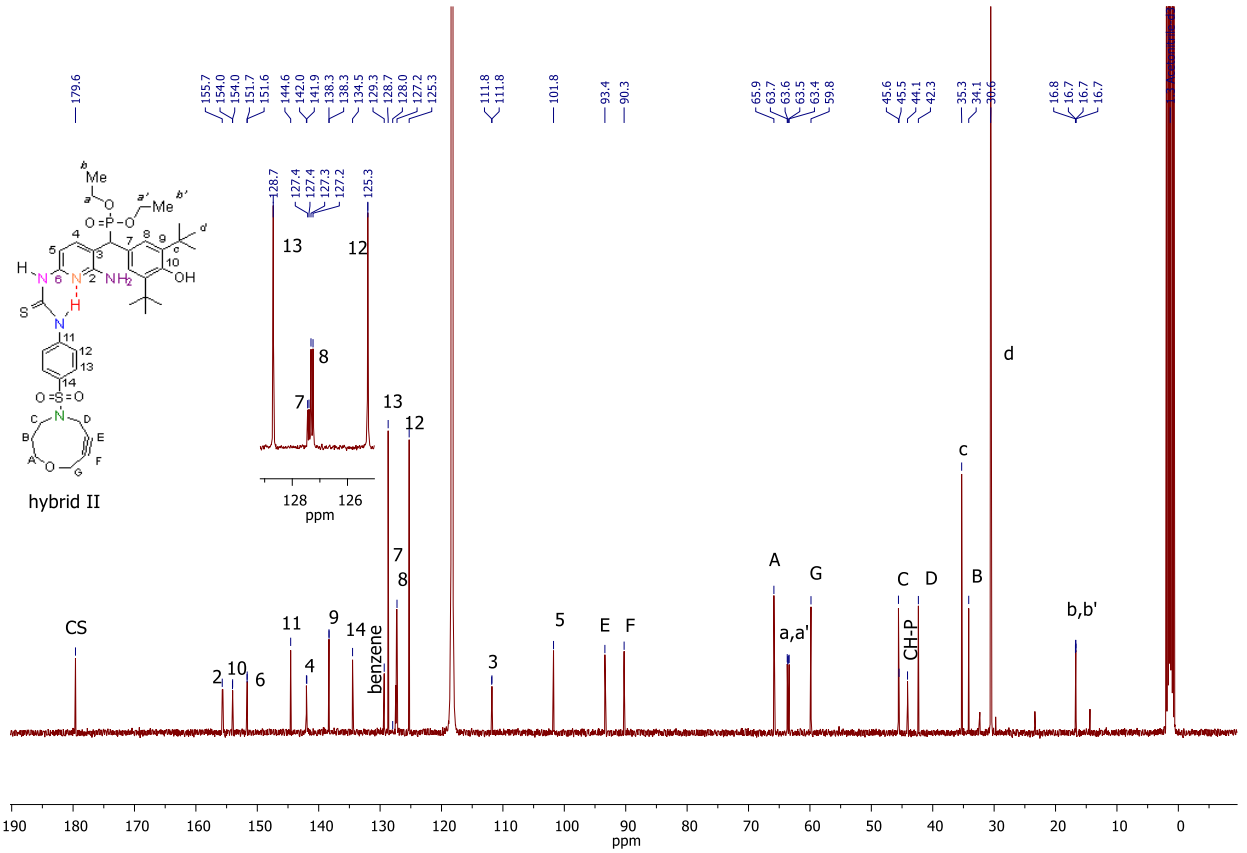


Hybrid I

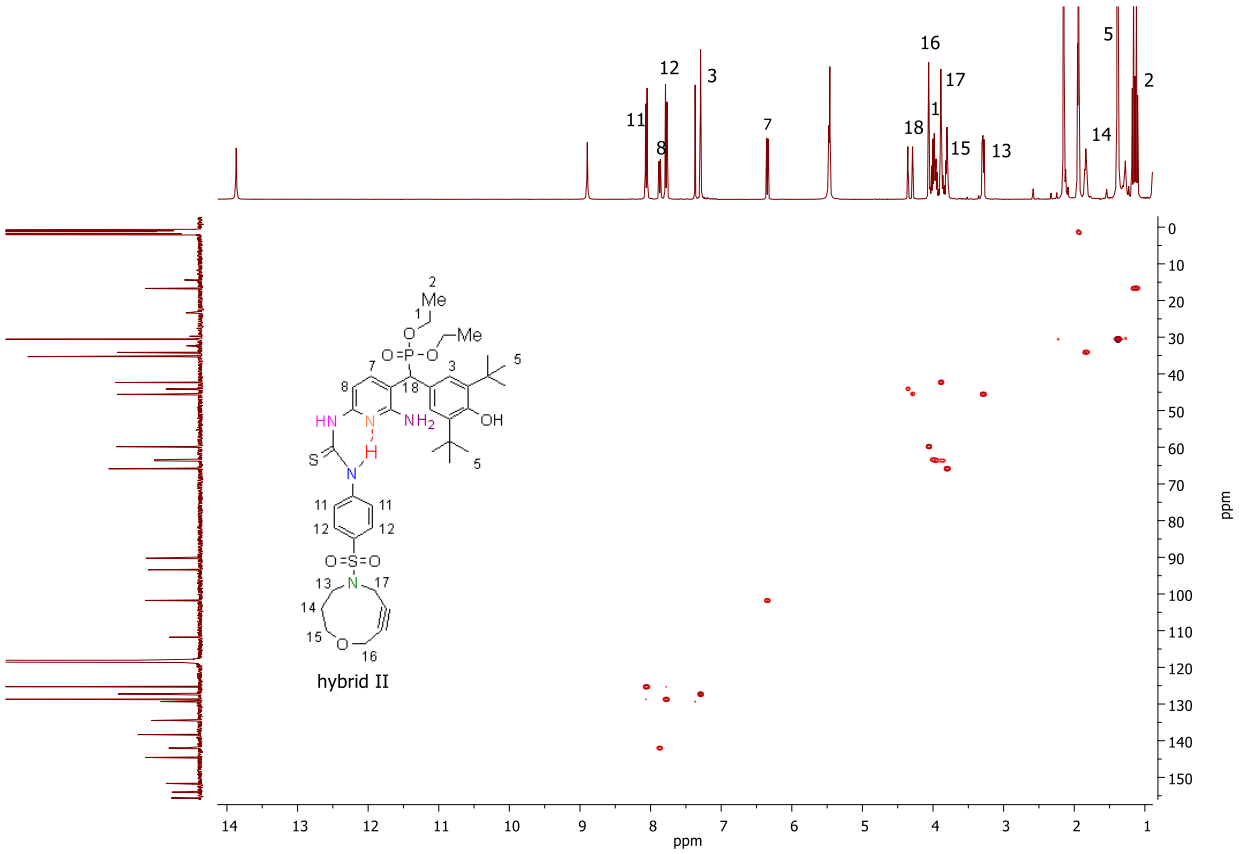
$^{1}$H NMR, $CD_3CN$, 400 MHz (with hybrid I atom numbering for $^{1}$H NMR assignment)




$^{13}$C NMR, $CD_3CN$, 101 MHz (with hybrid I atom numbering for $^{13}$C NMR assignment)



$^{1}H$-$^{13}C$ HSQC NMR, $CD_3CN$, 400 MHz (with hybrid I atom numbering for $^{1}H$ NMR assignment)

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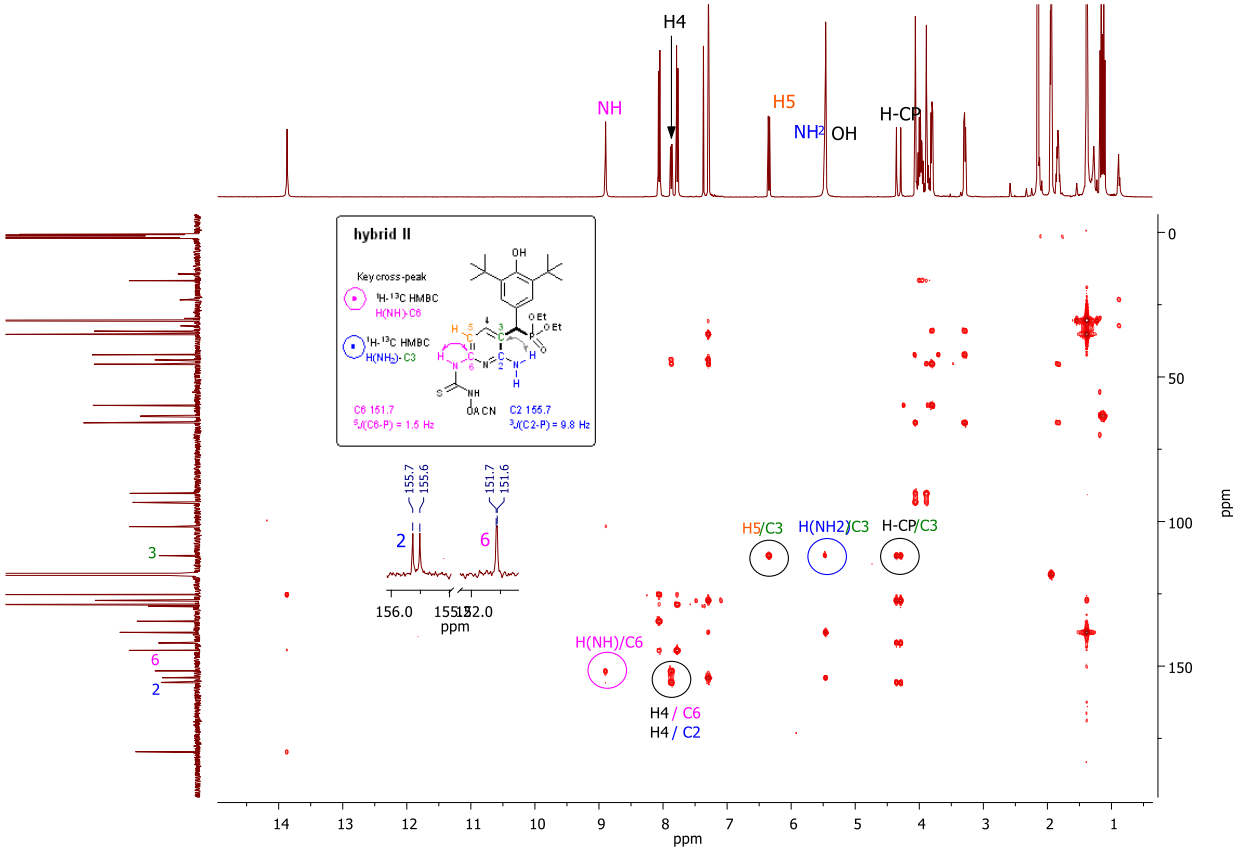

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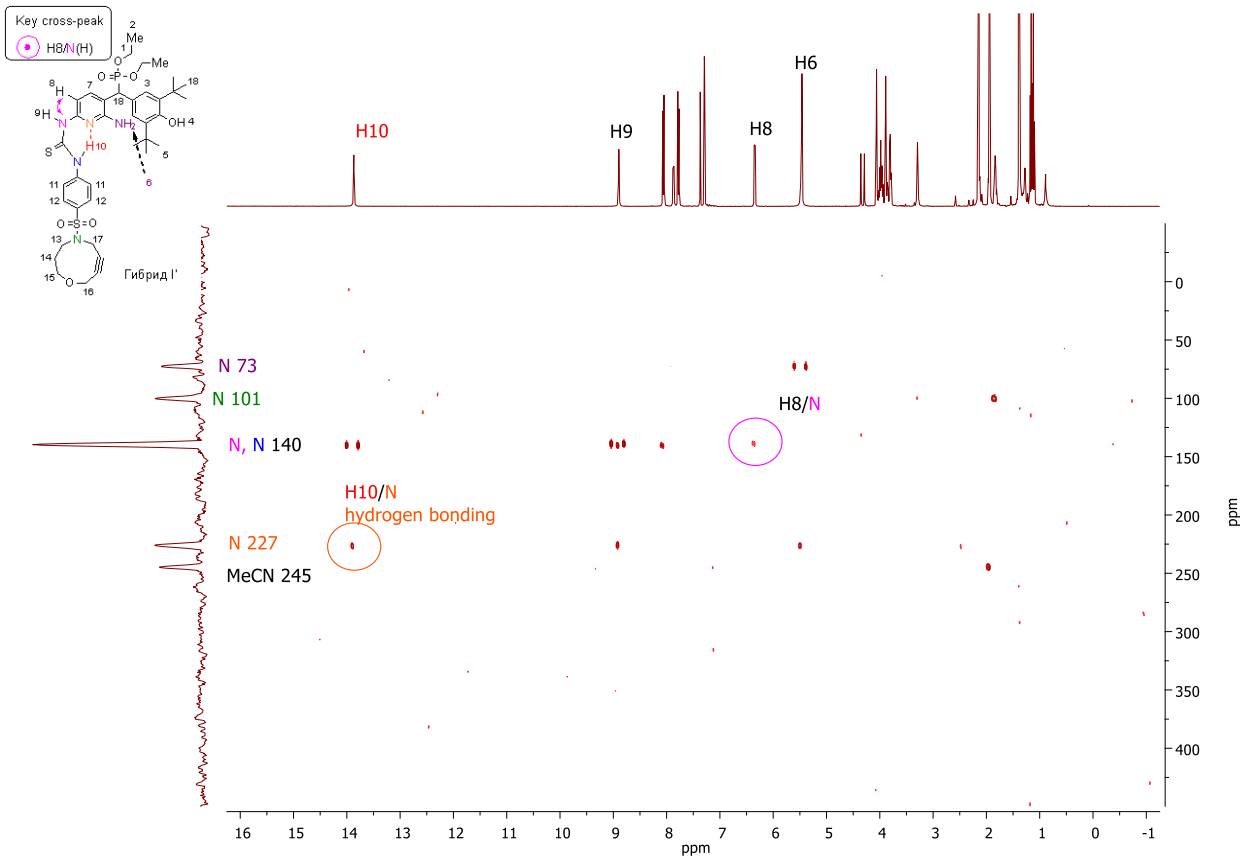
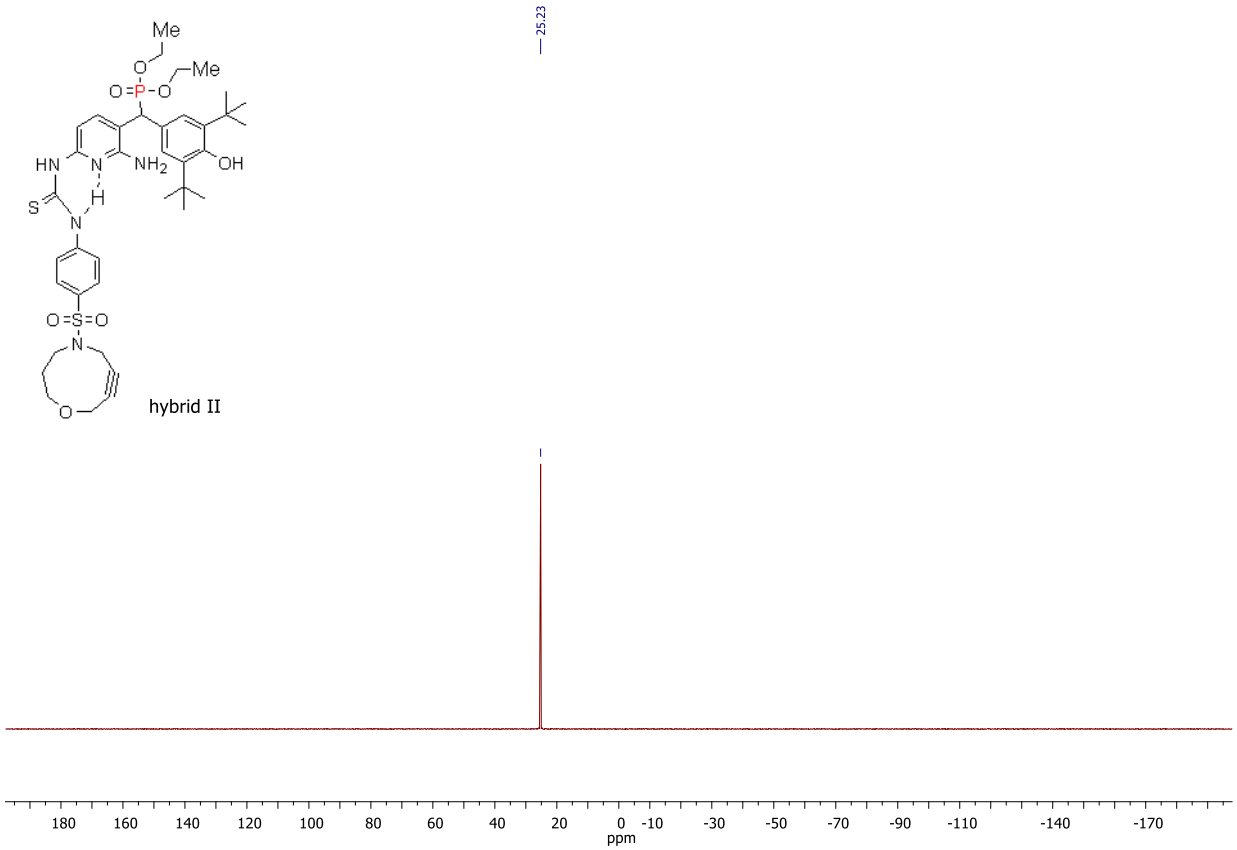
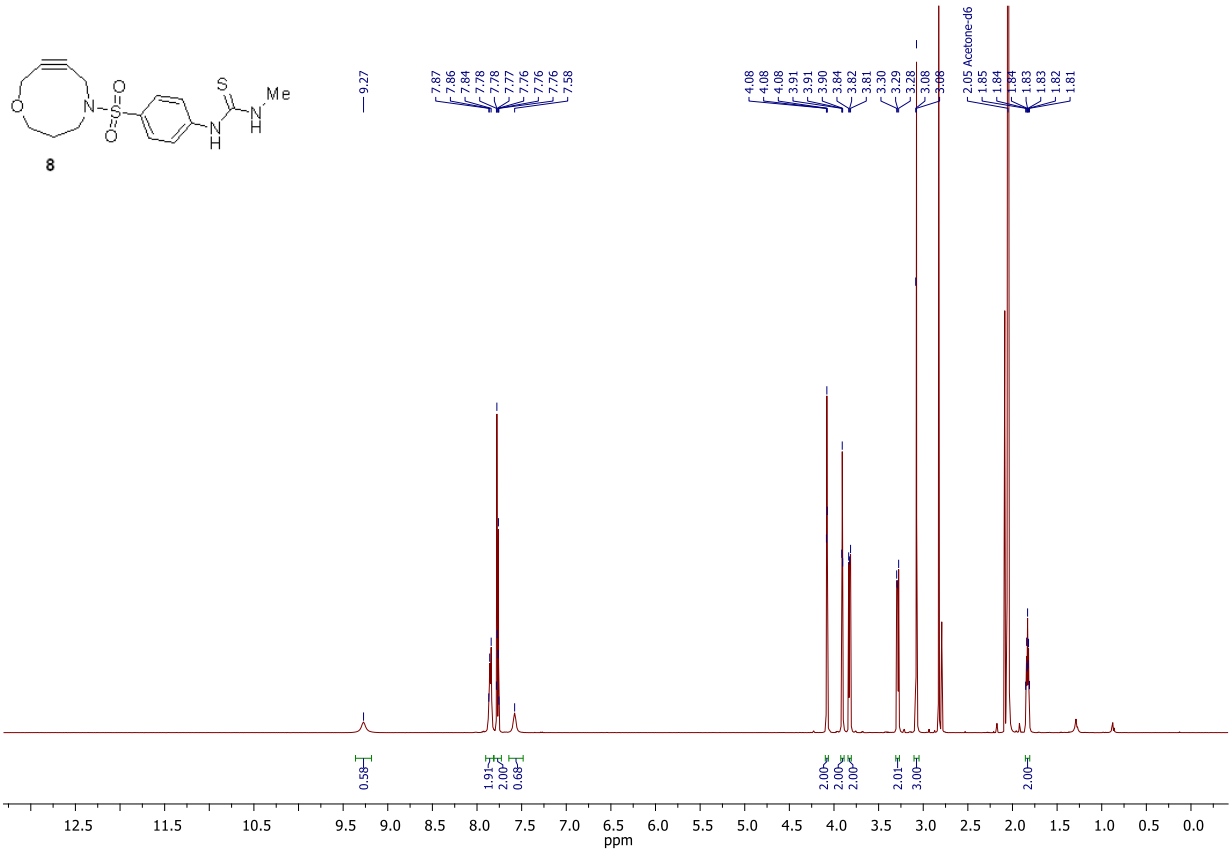


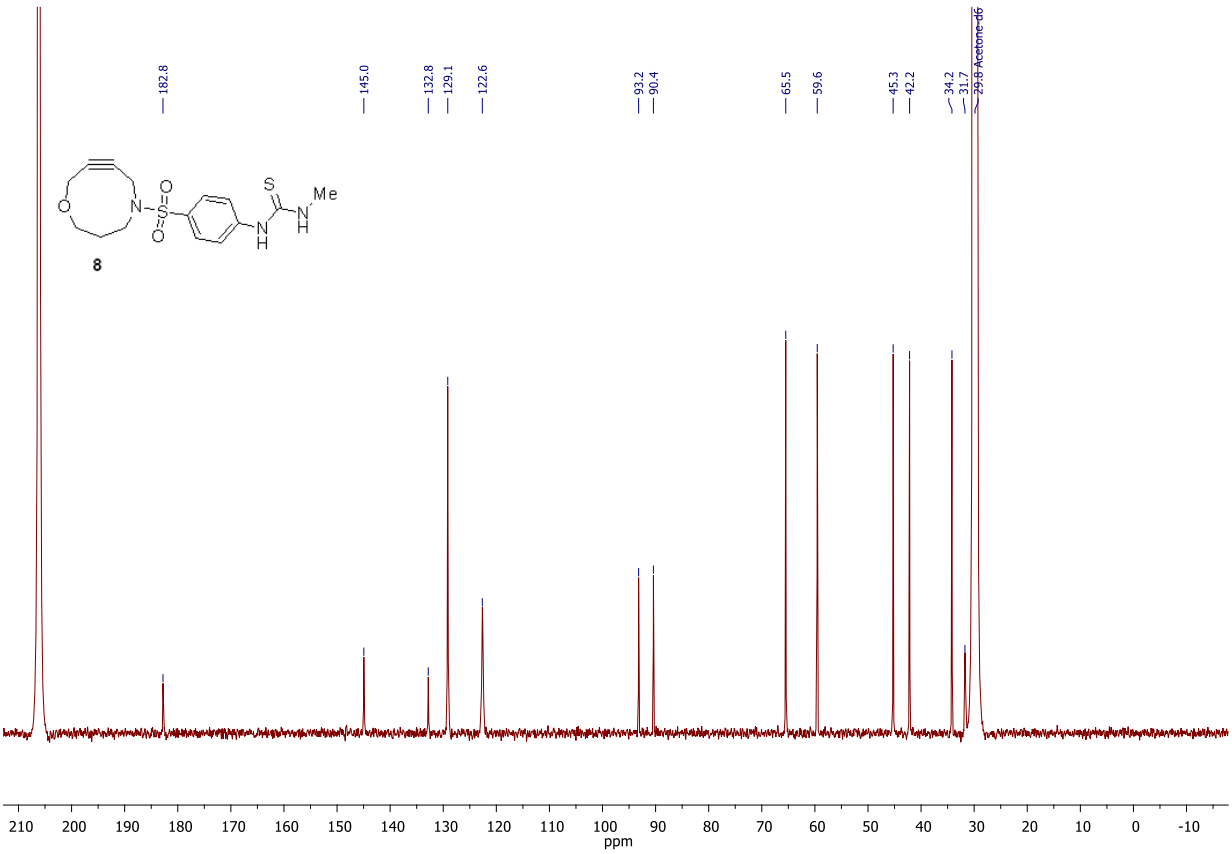

$^{31}P$ NMR, $CD_3CN$, 162 MHz



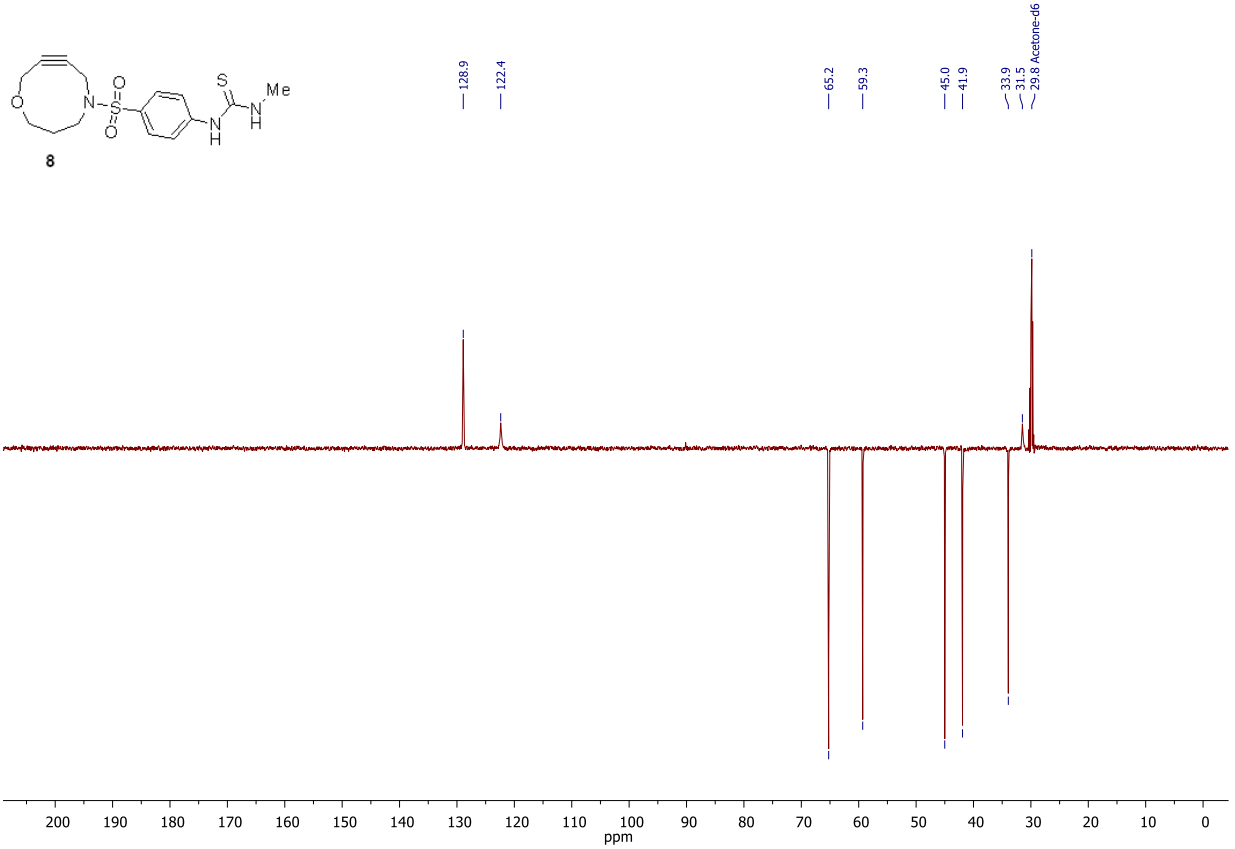
$^{1}$H NMR, acetone-$d_6$, 500 MHz




$^{13}$C NMR, acetone-$d_6$, 126 MHz



DEPT 135, acetone-$d_6$, 126 MHz

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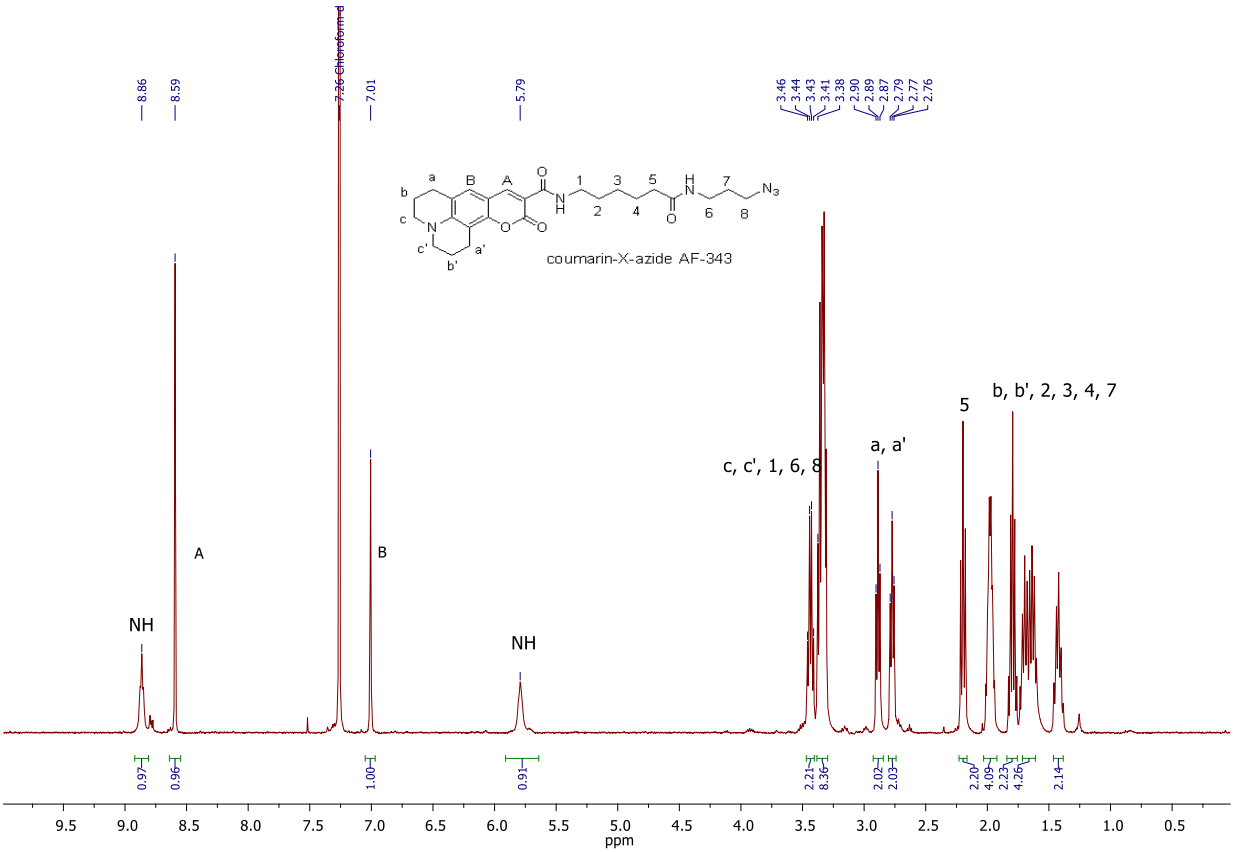
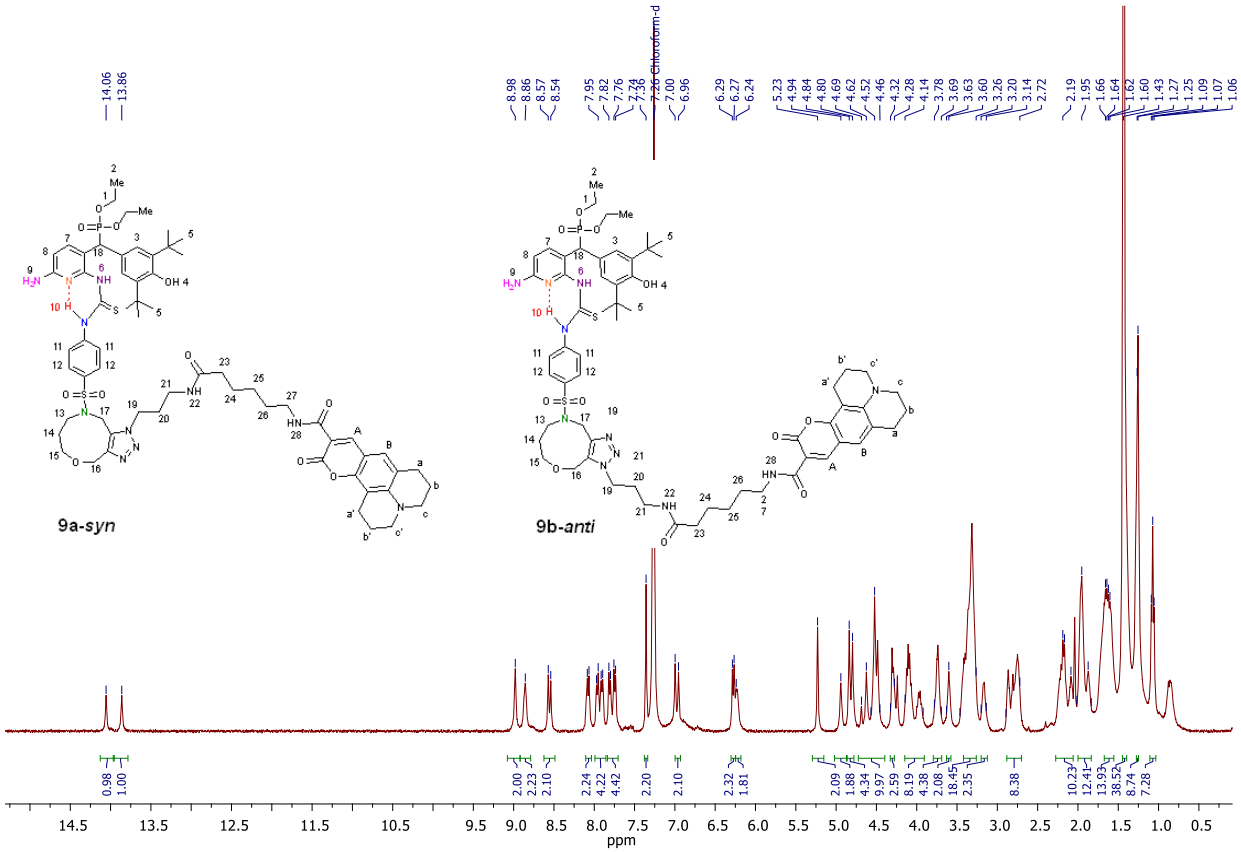
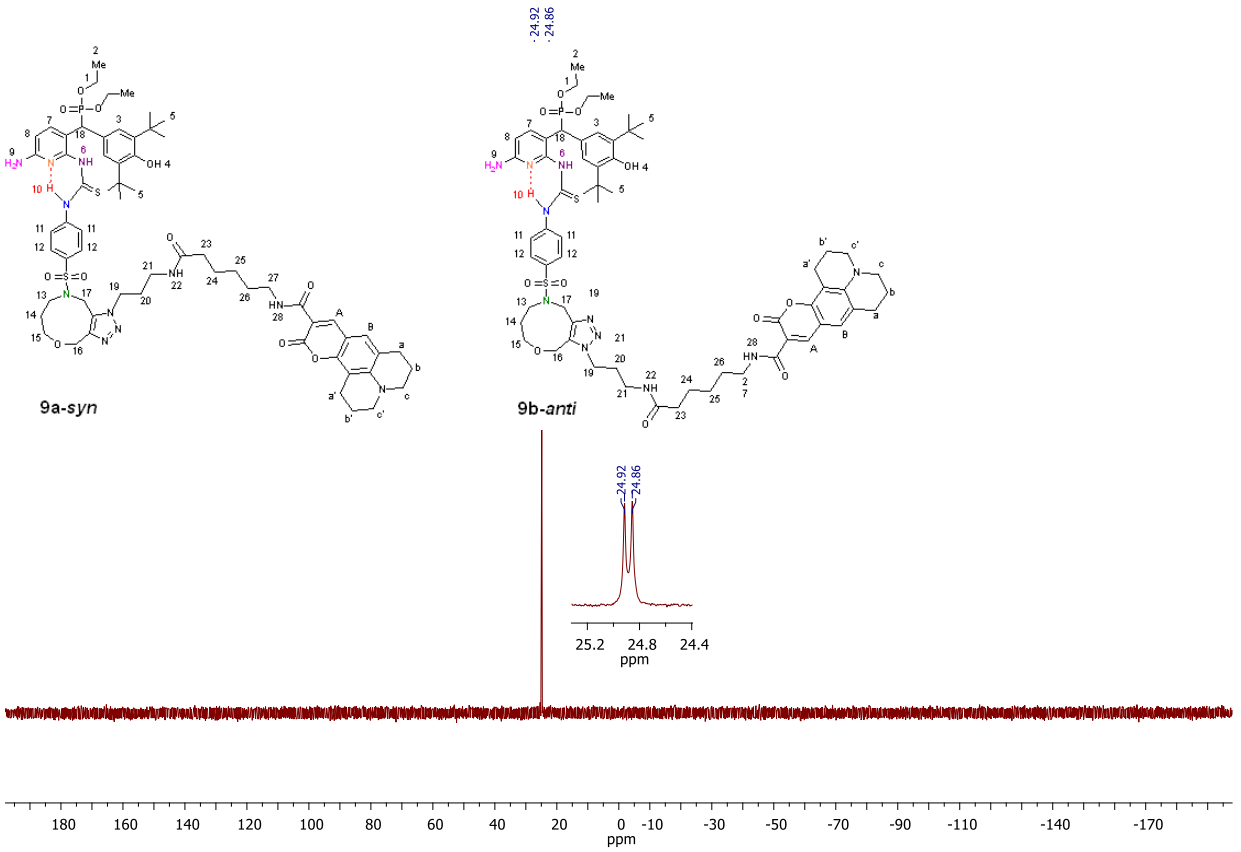


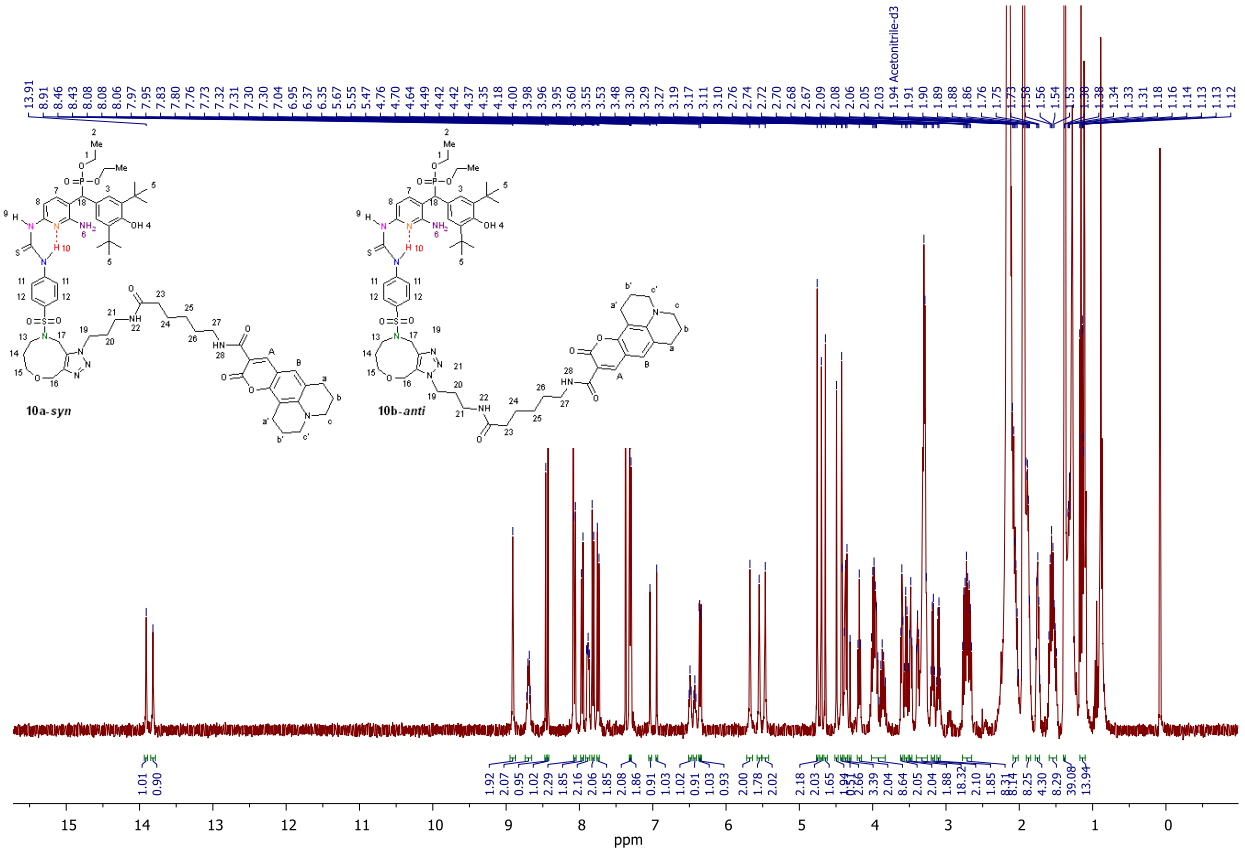
$^{1}$H NMR, $CDCl_3$, 400 MHz



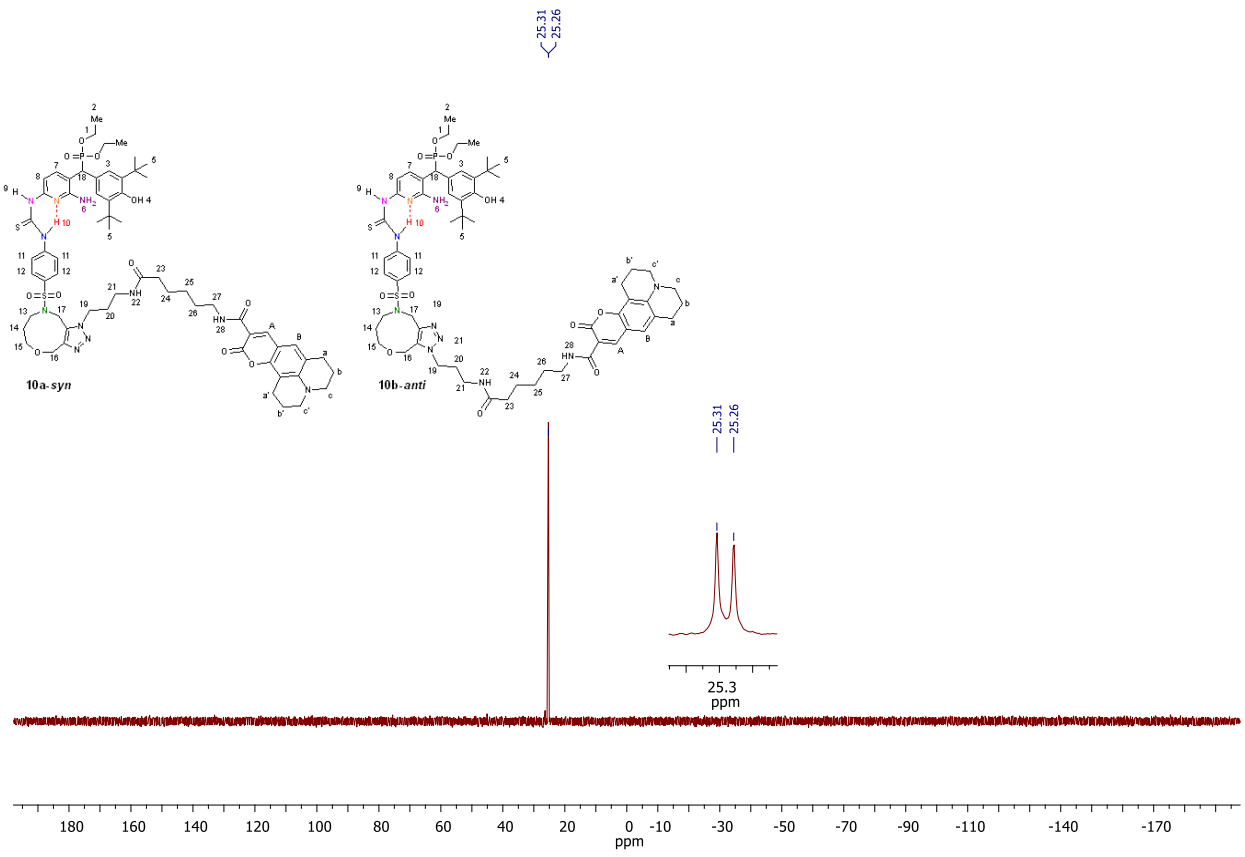

$^{31}$P NMR, $CD_3CN$, 162 MHz



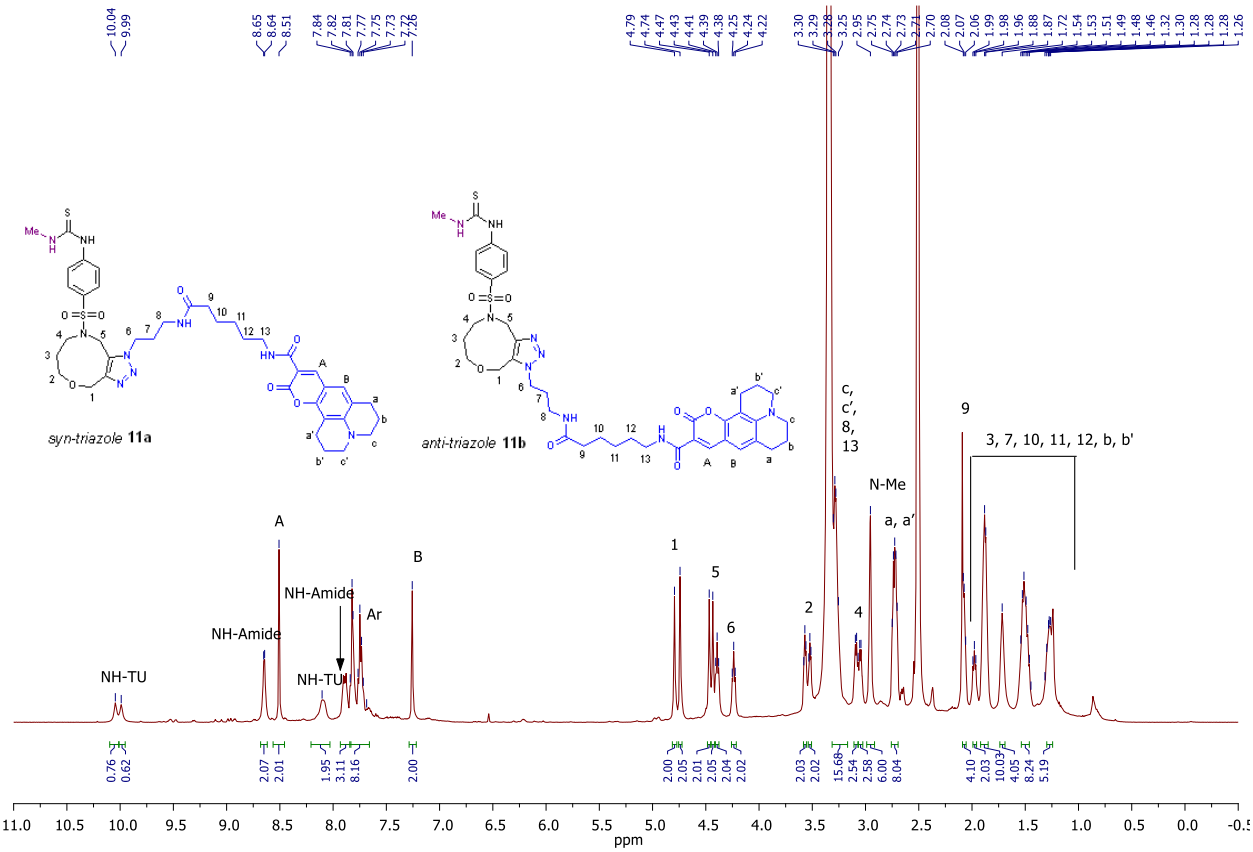
$^{1}$H NMR, $CDCl_3$, 400 MHz



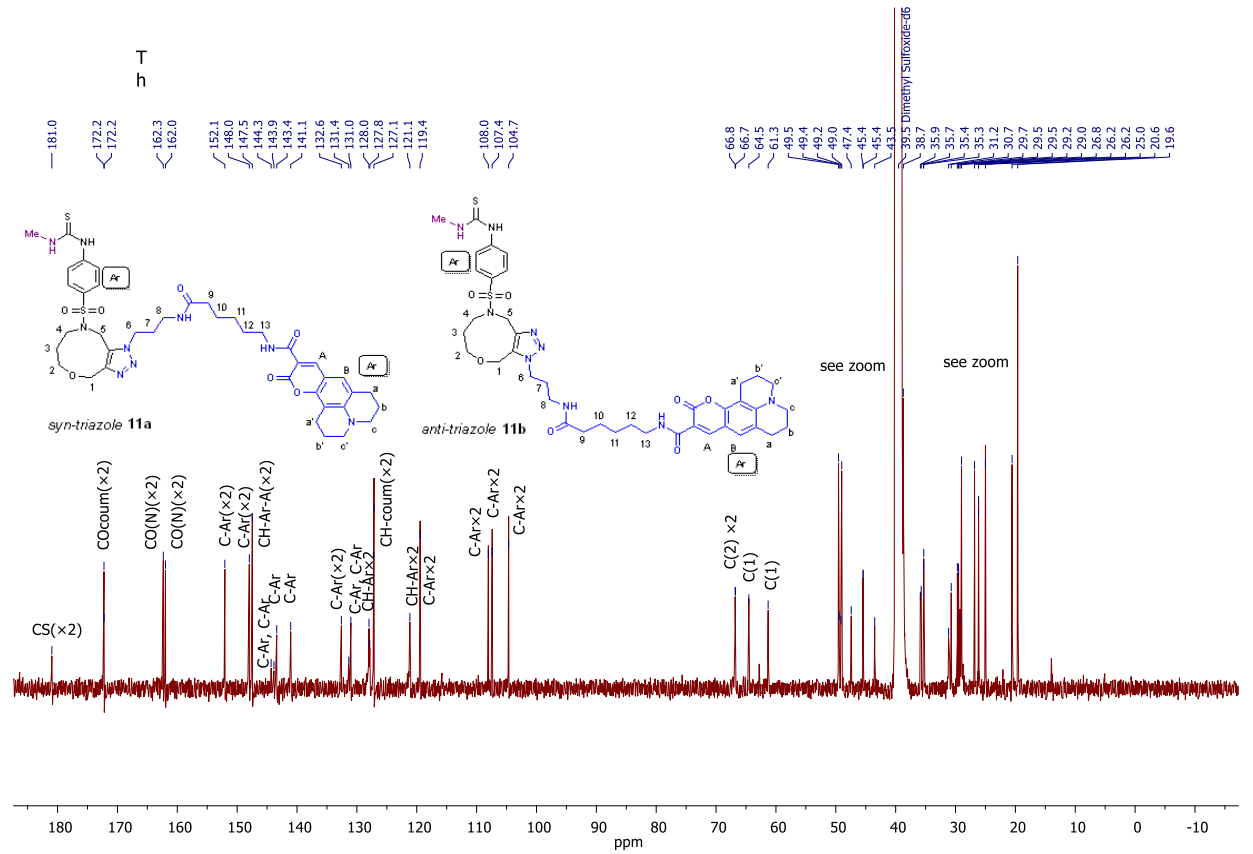

$^{31}$P NMR, $CD_3CN$, 162 MHz



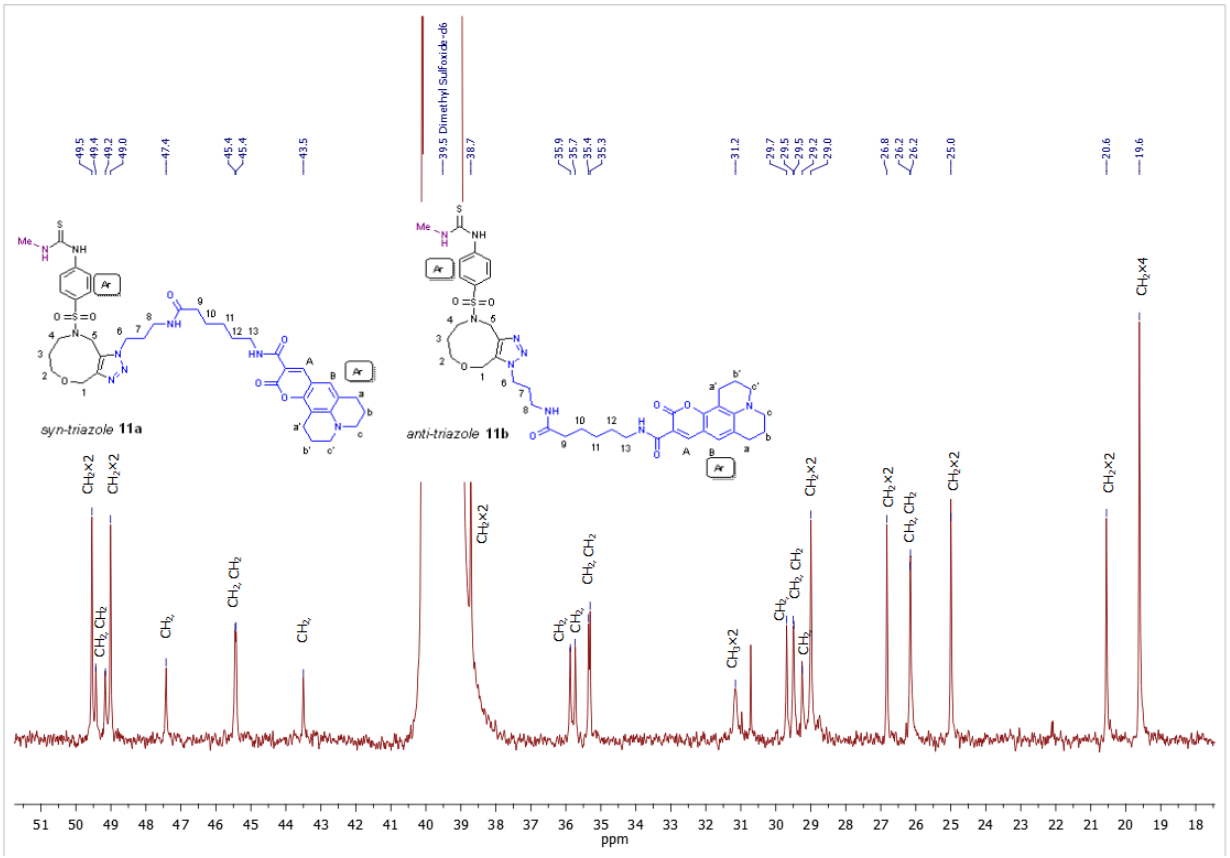
$^{1}$H NMR, DMSO-$d_6$, 400 MHz



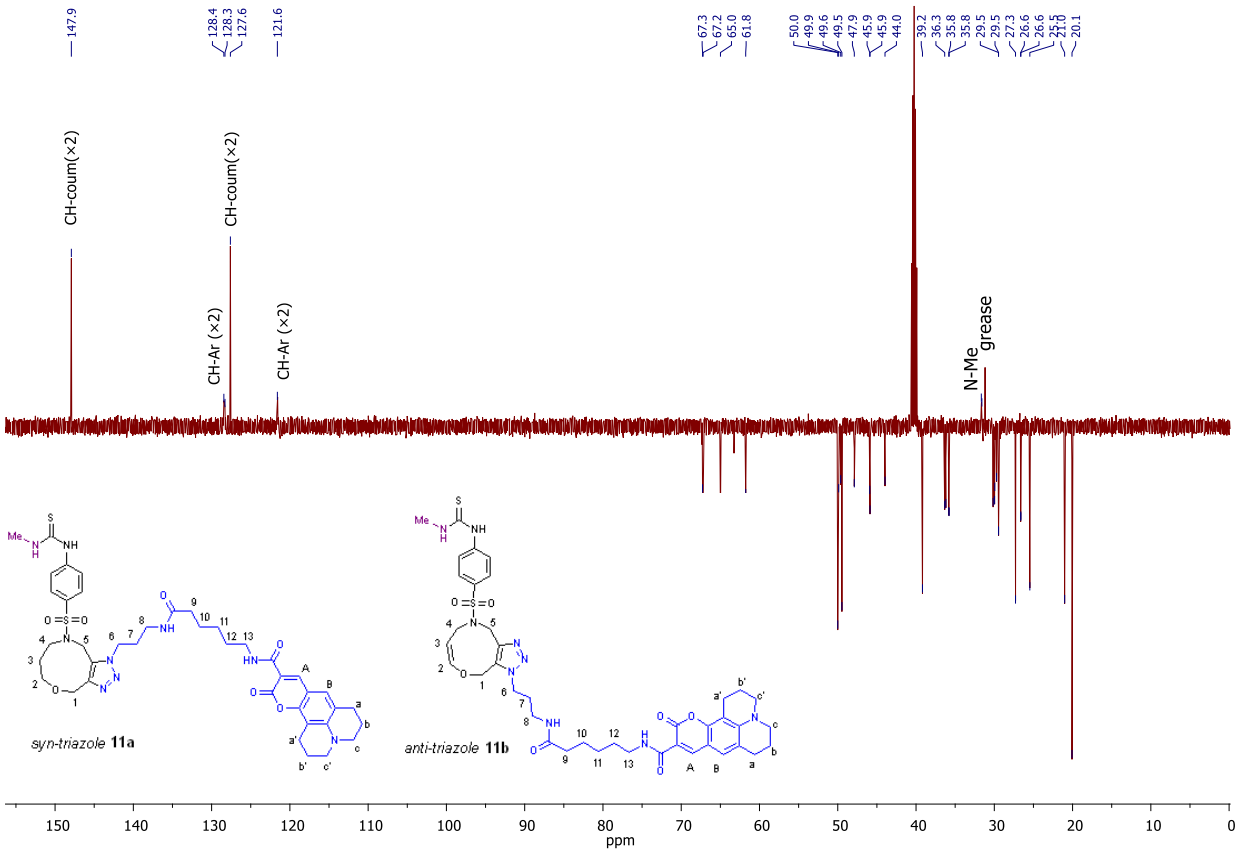

$^{13}$C NMR, DMSO-$d_6$, 126 MHz



$^{13}$C NMR, DMSO-$d_6$, 126 MHz (Zoom)




DEPT 135, DMSO-$d_6$, 126 MHz



$^{1}H$-$^{13}C$ HSQC NMR, DMSO-$d_6$, 500 MHz