Supporting Information

Nitroxide-labeled pyrimidines for noncovalent spin-labeling of abasic sites in DNA and RNA duplexes

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General

All commercial reagents were purchased from Sigma-Aldrich and used without further purification and all air and moisture sensitive reactions were performed in oven-dried reaction flasks, under an argon atmosphere. CH_2Cl_2, acetonitrile and pyridine were freshly distilled over calcium hydride before use; triethylamine was purchased anhydrous and stored over KOH pellets. Thin layer chromatography (TLC) was performed on glass backed TLC with extra hard layer (Kieselgel 60 F_{254}, 250μm, Silicycle) and compounds visualized by UV light. Silica gel (230-400 mesh, 60 Å) was purchased from Silicycle and used for flash column chromatography. The petroleum ether used for column chromatography was a fraction from distillation between 60 and 90 °C. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 400 MHz spectrometer and the chemical shifts were reported in parts per million (ppm) relative to the residual proton signal (for ^{1}H NMR) and the carbon signal (for ^{13}C NMR) of the deuterated solvents used [d_6-DMSO (2.50 ppm), $CDCl_3$ (7.26 ppm), d_4-MeOH (4.84 and 3.31 ppm)] for ^{1}H NMR; [d_6-DMSO (39.52 ppm), $CDCl_3$ (77.0 ppm), d_4-MeOH (49.05 ppm)] for ^{13}C NMR. All coupling constants were reported in Hertz. Molecular mass of organic compounds were determined by HR-ESI/ESI-MS (Bruker, MicroTof-Q). EPR samples were placed in 50 μL quartz capillary (BLAUBRAND intraMARK) and the EPR spectra recorded on a MiniScope MS200 (Magnettech Germany) spectrometer (100 kHz modulation frequency, 1.0 G modulation amplitude, and 2.0 mW microwave power). DNA oligonucleotides that contain abasic sites and unmodified DNAs were prepared by phosphoramidite chemistry, on an automated ASM800 DNA synthesizer (Biosset) using a trityl-off protocol and phosphoramidites with standard protecting groups on a 1.0 μmol scale (1000 Å CPG columns) and purified by following previously reported protocol.[1] The RNA oligonucleotides containing abasic sites and unmodified RNAs were purchased from RiboTask Aps (Denmark).

Note: Because of the paramagnetic nature of nitroxides, the NMR spectra of nitroxide compounds shows significant broadening of the signals and some peaks, particularly of nuclei close to the radical, are not seen in the spectra.

List of abbreviations

DCE	1,2-dichloroethane
DMAP	4-dimethylaminopyridine
DMSO	dimethylsulfoxide
HMDS	1,1,1,3,3,3-hexamethyldisilazane
HR-ESI-MS	high-resolution electrospray ionization mass spectrometry
*m*CPBA	*meta*-chloroperbenzoic acid
TMS-Cl	trimethylsilyl chloride
TPS-Cl	2,4,6-triisopropylbenzenesulfonyl chloride

Sample preparation for EPR measurements

A stock solution of a spin label (200 μM) and a DNA or RNA (400 μM) oligomer containing an abasic site and its complementary strand (1:1.2) were mixed together and evaporated in SpeedVac. The resulting residue was dissolved in phosphate buffer (10 μL; 10 mM $NaHPO_4$, 100 mM NaCl, 0.1 mM Na_2EDTA, pH 7.0), annealed (annealing protocol: 90 °C for 2 min, 60 °C for 5 min, 50 °C for 5 min, 40 °C for 5 min, 22 °C for 15 min) and dried in SpeedVac. The residue was dissolved in an aqueous 30% ethylene glycol solution containing 2% DMSO (10 μL) and placed in a 50 μL quartz capillary (BLAUBRAND intraMARK) for the EPR measurements.

Binding of spin labels to an unmodified DNA duplex

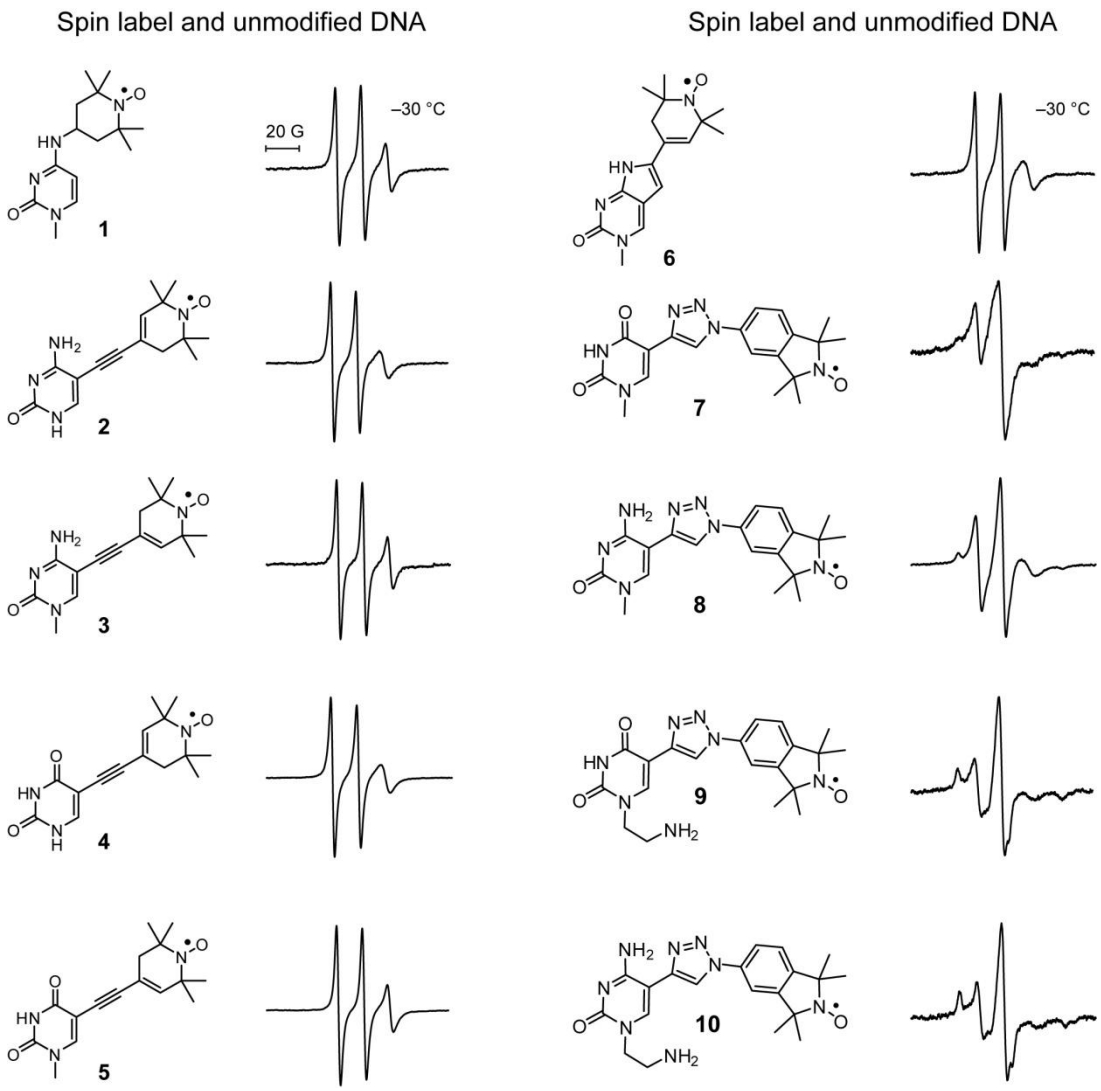


Figure S1: EPR spectra of pyrimidine-derived spin labels in the presence of an unmodified 14-mer DNA duplex. 5'-d(GAC CTC GCA TCG TG)-3' : 5'-d(CAC GAT GCG AGG TC)-3'. EPR spectra were recorded in a phosphate buffer pH 7.0, 30% ethylene glycol, 2% DMSO at −30 °C.

Binding of triazole-linked spin labels to a DNA duplex containing either A, C, T or G as an orphan base opposite the abasic site

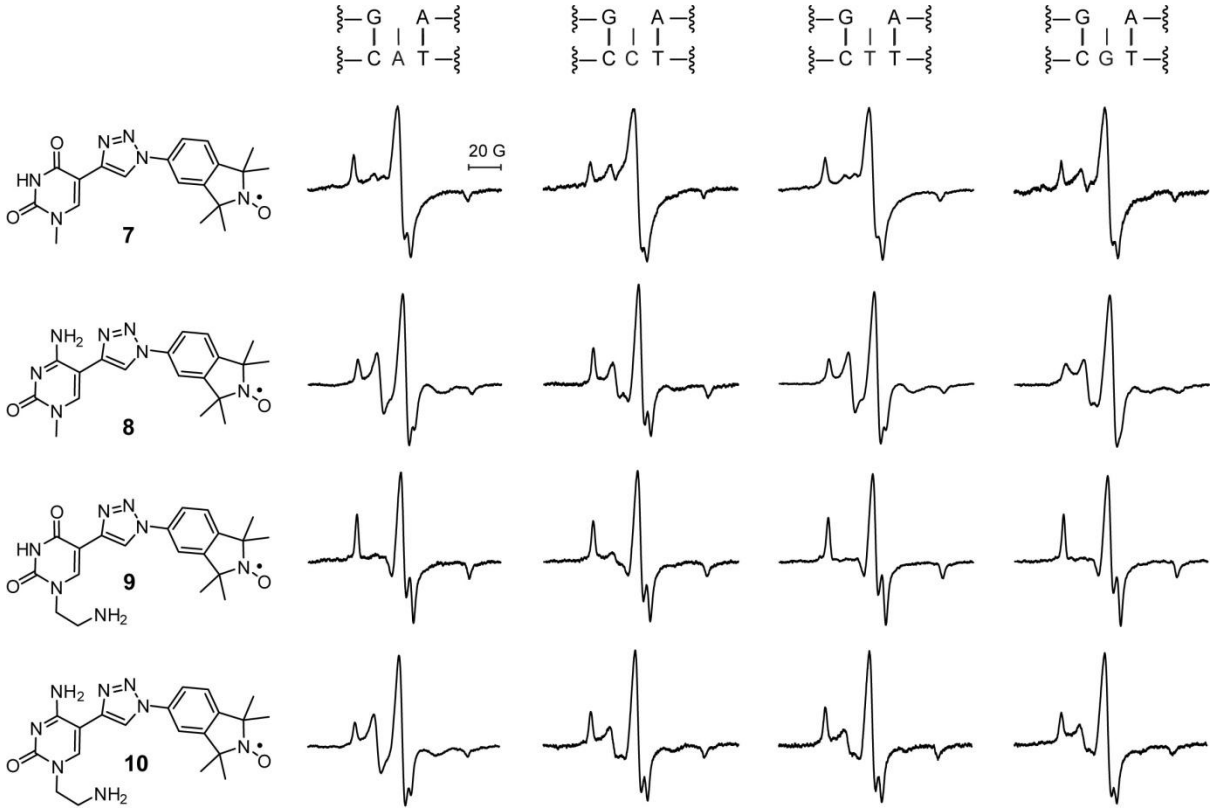
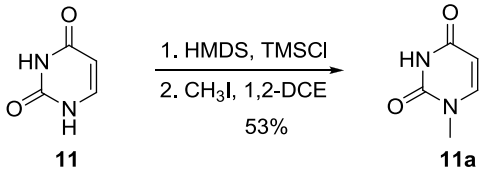


Figure S2: EPR spectra of triazole-linked spin labels in the presence of a 14-mer DNA duplex containing an abasic site. 5'-d(GAC CTC G__A TCG TG)-3' : 5'-d(CAC GAT **X**CG AGG TC)-3', where __ is the abasic site and **X** is either A, C, T or G. EPR spectra were recorded in a phosphate buffer pH 7.0, 30% ethylene glycol, 2% DMSO at −30 °C.

Experimental protocols for synthetic compounds



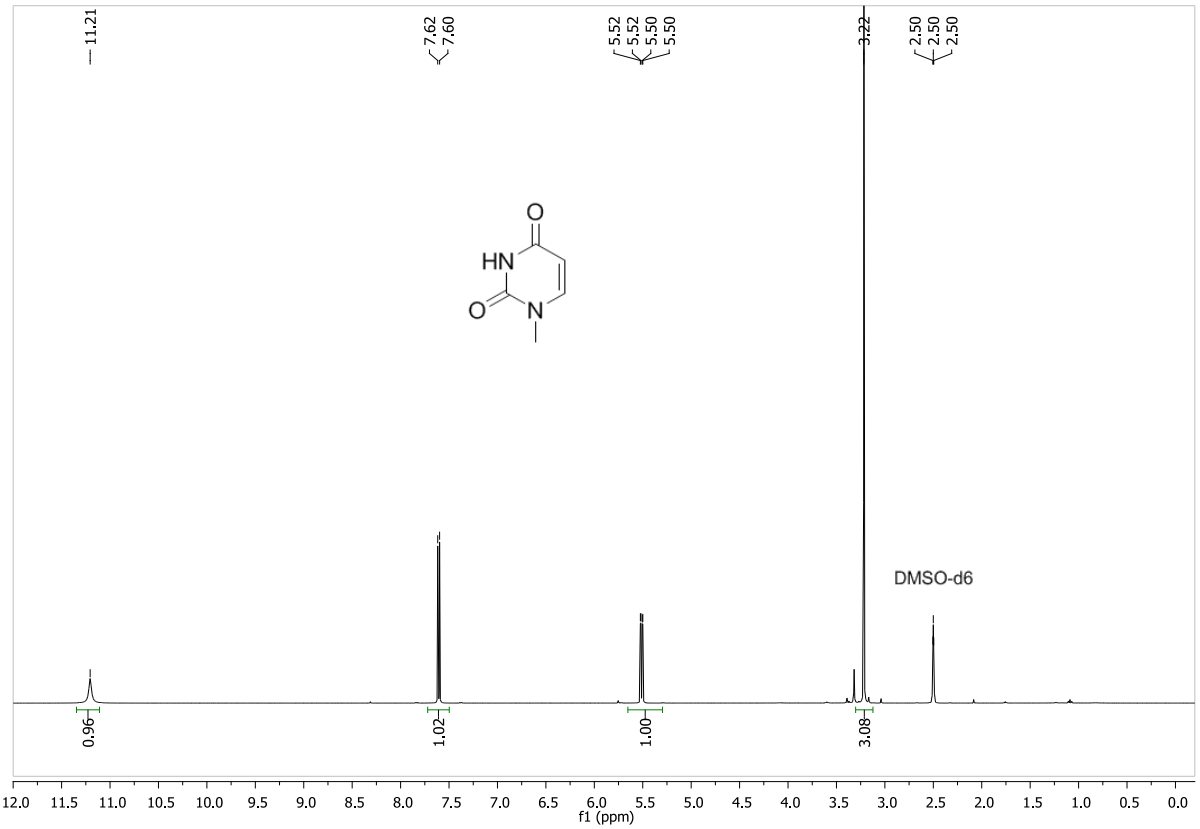
Compound 11a. To a suspension of uracil **11** (0.500 g, 4.46 mmol) in 1,2-dichloroethane (10 mL), HMDS (3.8 mL, 18.1 mmol) and TMSCl (0.3 mL, 2.4 mmol) were added and refluxed for 4 h. The resulting reaction mixture was cooled to 50 °C, the solvent was removed under reduced pressure and 1,2-dichloroethane (10 mL) was added. The reaction mixture was cooled 24 °C and CH_3I (1.1 mL, 17.7 mmol) and I_2 (5 mg, 0.0197 mmol) were added and the solution refluxed for 24 h. The solvent was removed *in vacuo* and the residue purified by column chromatography using CH_3OH:CH_2Cl_2 (0:100 to 10:90) to afford **11a** as a white solid (256 mg, 53% yield).

^{1}H-NMR (d_6-DMSO): δ 3.22 (s, 3H, C*H*$_3$), 5.51 (dd, *J* = 7.81, 2.29 Hz, 1H, C*H*), 7.61 (d, *J* = 7.81 Hz, 1H, C*H*), 11.21 (s, 1H, N*H*) ppm.

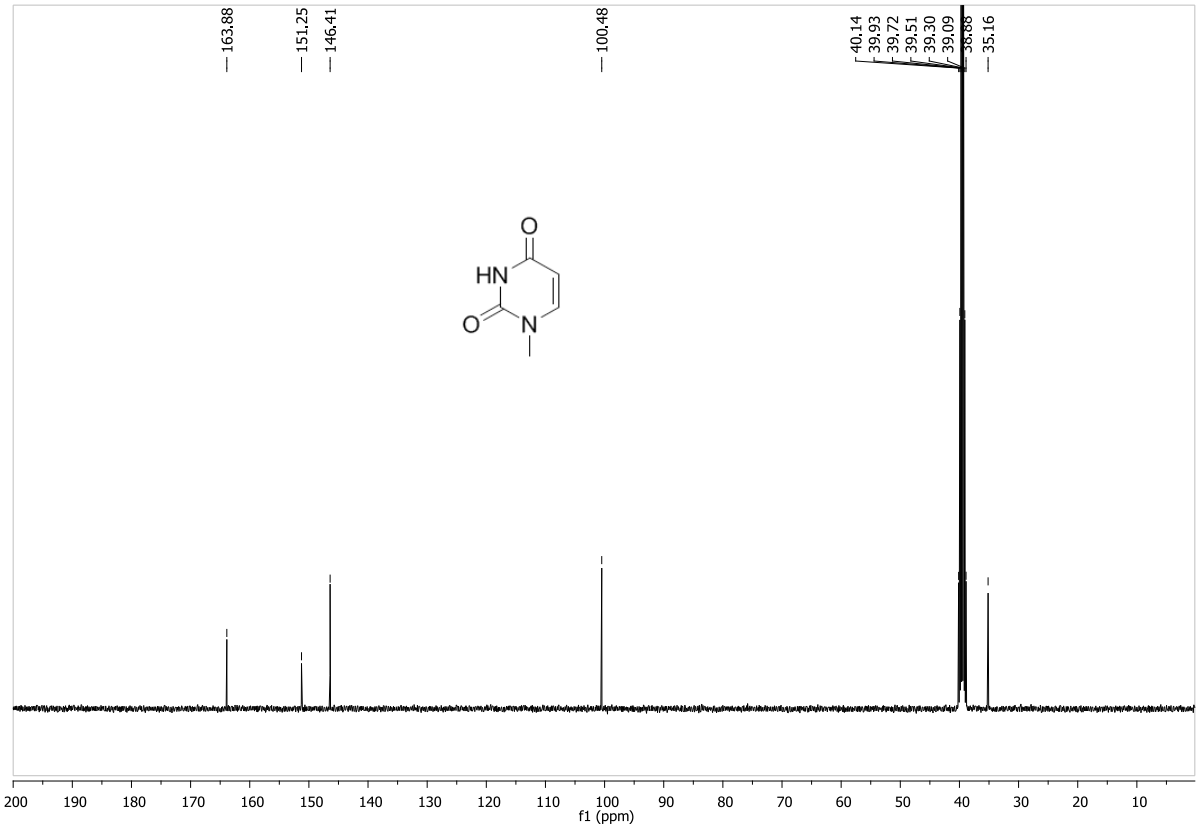
^{13}C-NMR (d_6-DMSO): δ 163.88, 151.25, 146.41, 100.48, 35.16 ppm.

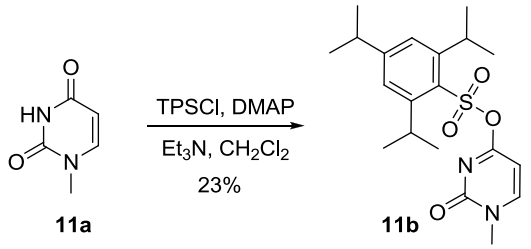
HR-ESI-MS: Calculated for $C_5H_6N_2O_2$ 126.0429 *m/z* found 149.0323 $[M + Na]^+$.

^{1}H NMR spectrum of **11a:**



^{13}C NMR spectrum of **11a:**





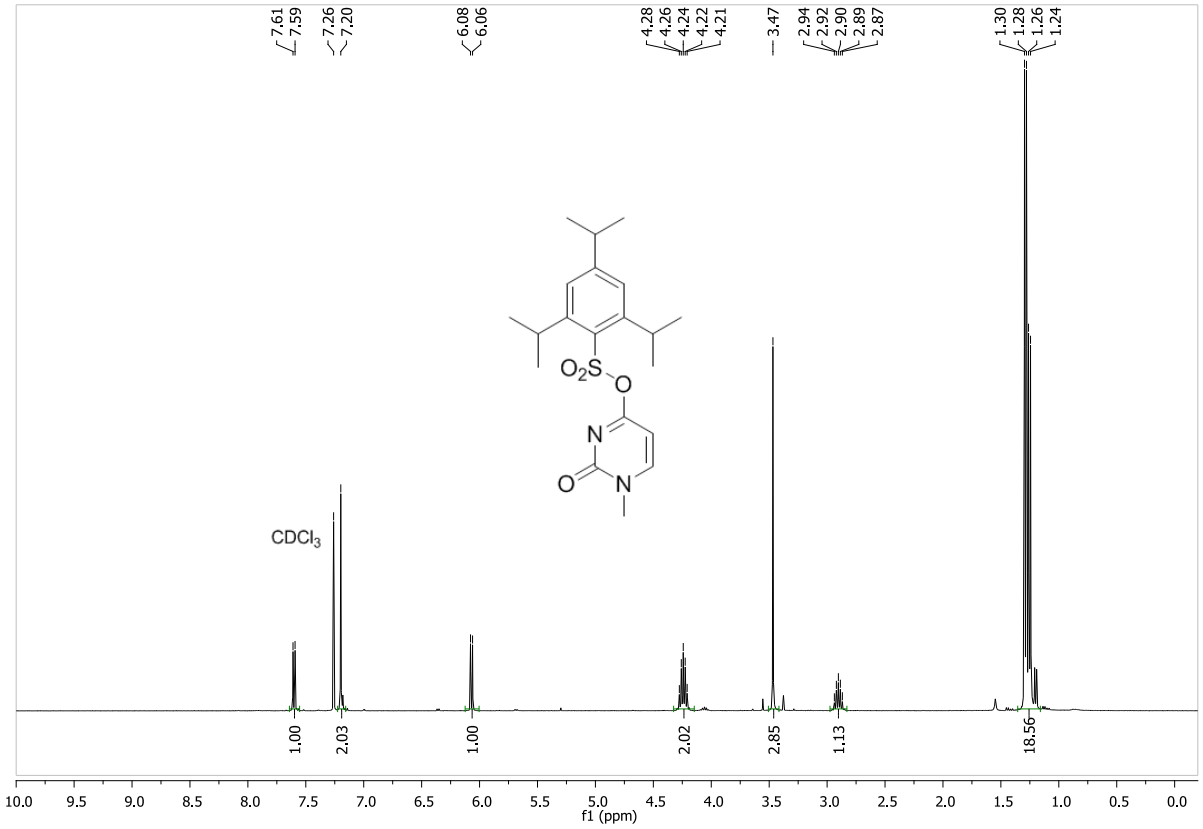
11a 11b

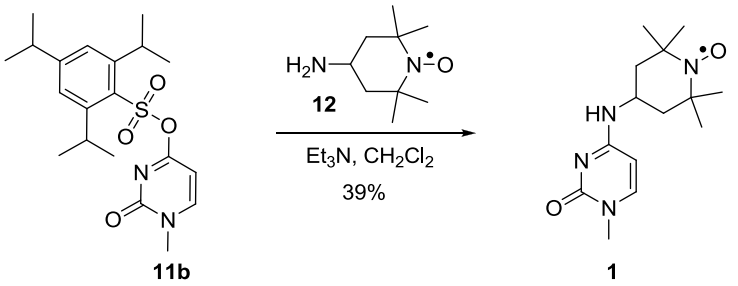
Compound 11b. To a solution of **11a** (199 mg, 1.58 mmol) in anhydrous CH_2Cl_2 (10 mL), TPS-Cl (360 mg, 2.39 mmol) and DMAP (19 mg, 0.16 mmol) and Et_3N (0.9 mL, 6.42 mmol) were added at 0 °C and the resulting reaction mixture was stirred for 4 h at 24 °C. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed subsequently with water, aqueous $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The residue was purified by column chromatography using CH_3OH:CH_2Cl_2 (0:100 to 2:98) to get **11b** as a white solid (142.5 mg, 23% yield).

^{1}H-NMR ($CDCl_3$): δ 7.60 (d, *J* = 7.04 Hz, 1H, ArC*H*), 7.20 (s, 2H, ArC*H*), 6.07 (d, *J* = 7.03 Hz, 1H, ArC*H*), 4.24 (m, 2H, 2C*H*), 3.47 (s, 3H, C*H*$_3$), 2.90 (m, 1H, C*H*), 1.29 (d, *J* = 8 Hz, 12H, 4C*H*$_3$) 1.29 (d, *J* = 8 Hz, 12H, 4C*H*$_3$) ppm.

HR-ESI-MS: Calculated for $C_{20}H_{28}N_2O_4S$ 392.17 not found, compound is unstable.

^{1}H NMR spectrum of **11b:**



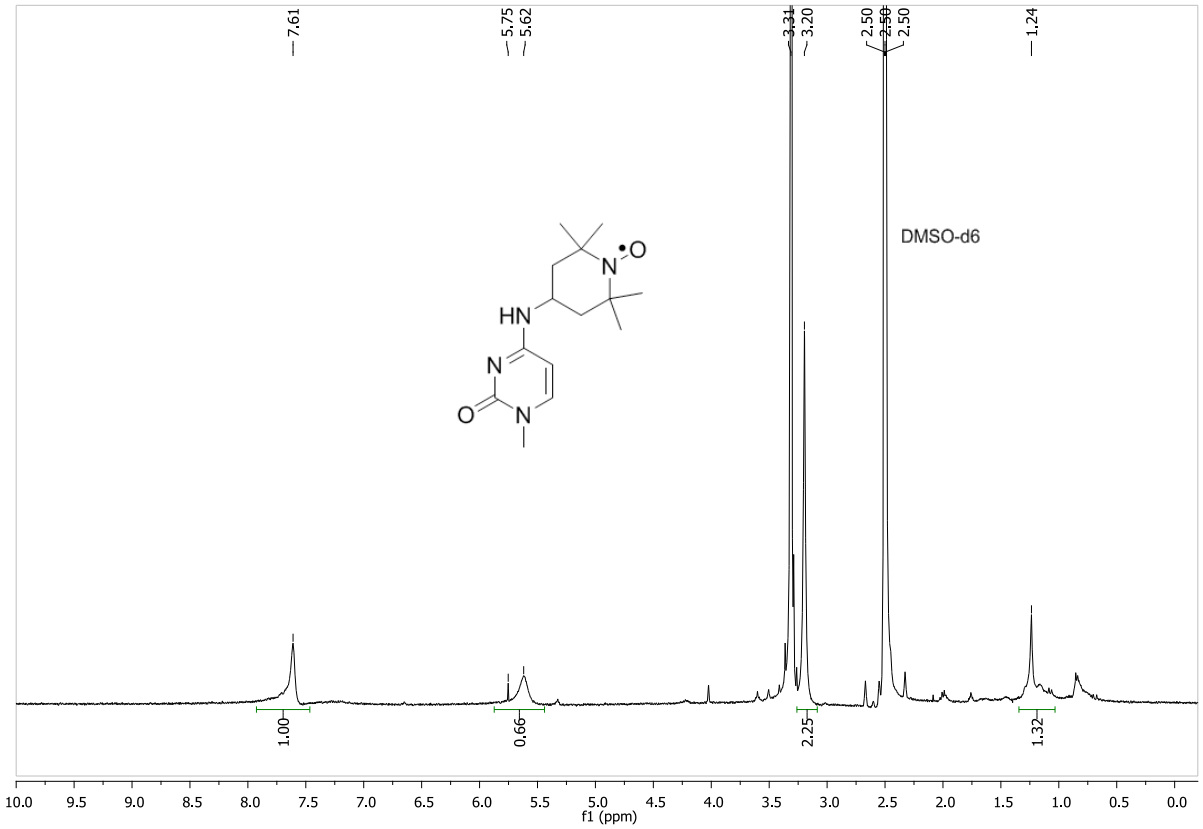


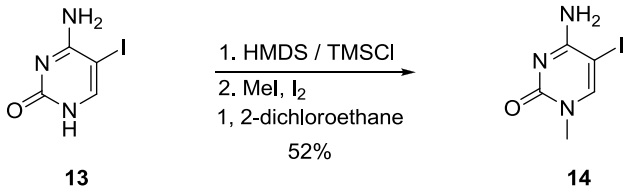
Spin label 1. A solution of **11b** (42.5 mg, 0.108 mmol) and 4-amino-TEMPO (29.6 mg, 0.173 mmol) and Et_3N (0.2 mL, 1.43 mmol) in anhydrous CH_2Cl_2 (10 mL) were refluxed for 12 h and diluted with CH_2Cl_2 (10 mL). The reaction mixture was washed with water and organic layer was dried over Na_2SO_4 and the solvent removed *in vacuo* to afford **1** as a white solid (11.9 mg, 39% yield).

^{1}H-NMR ($CDCl_3$): δ 1.24 (s, 1H), 3.2 (s, 3H, C*H*$_3$), 5.62 (bs, 1H, C*H*), 7.61(bs, 1H, C*H*) ppm.

HR-ESI-MS: Calculated for $C_{14}H_{23}N_4O_2$ 279.1821 found *m/z* 302.1677 $[M + Na]^+$.

^{1}H NMR spectrum of **1:**





13 **14**

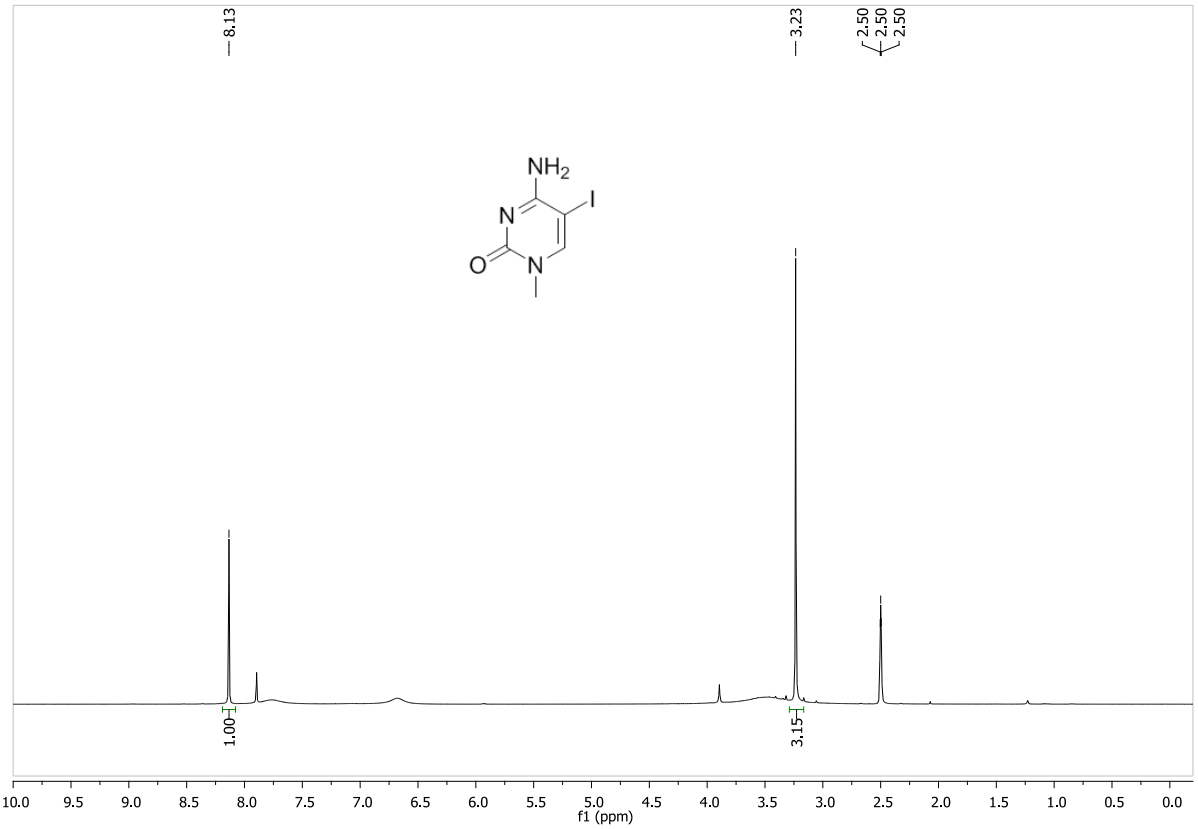
Compound 14: A suspension of 5-iodocytosine (2 g, 8.43 mmol) in 1,2-dichloroethane (1,2-DCE) (20 ml) was treated with hexamethyldisilazane (HMDS) (5.3 ml, 25.31 mmol) and trimethylsilyl chloride (TMS-Cl) (0.53 ml, 4.21 mmol). The reaction mixture was refluxed until it became clear, cooled to 60 °C and the solvent was removed *in vacuo* to yield a colorless oil. The residue was dissolved in 1,2-DCE (20 ml) and treated with iodomethane (0.8 ml, 12.62 mmol) and a catalytic amount of I_2 (0.02 g, 0.08 mmol) at 25 °C. The reaction mixture was refluxed for 12 h and cooled to 25 °C prior to addition of H_2O (15 ml). The precipitate was filtered off and washed with water to give **14** as a white solid (1.1 g, 52% yield).

^{1}H NMR (DMSO-d_6): δ 3.23 (s, 3H, CH_3), 8.13 (s, 1H, C*H*) ppm.

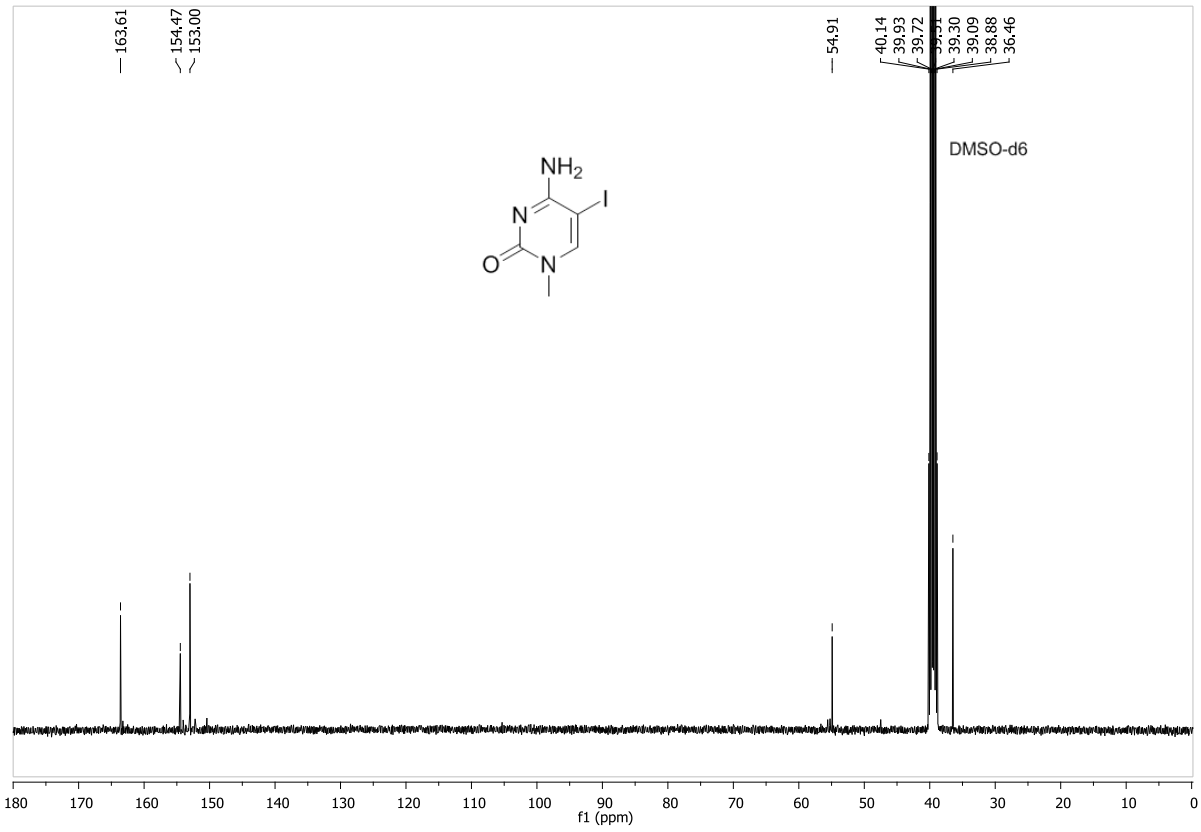
^{13}C NMR (DMSO-d_6): δ 163.61, 154.47, 153, 54.91, 36.46 ppm.

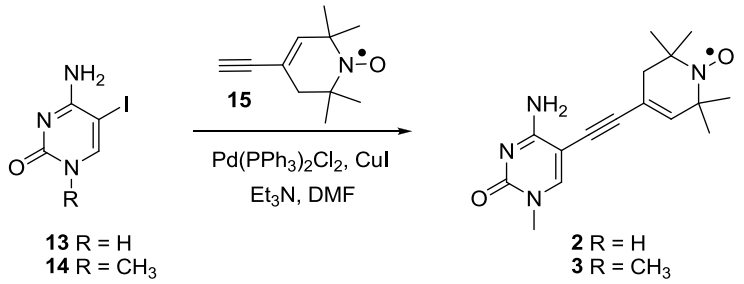
HR-ESI-MS: Calculated for $C_5H_6IN_3O$ 250.9556 found *m/z* 273.9433 $[M + Na]^+$.

^{1}H NMR spectrum of **14:**



^{13}C NMR spectrum of **14:**



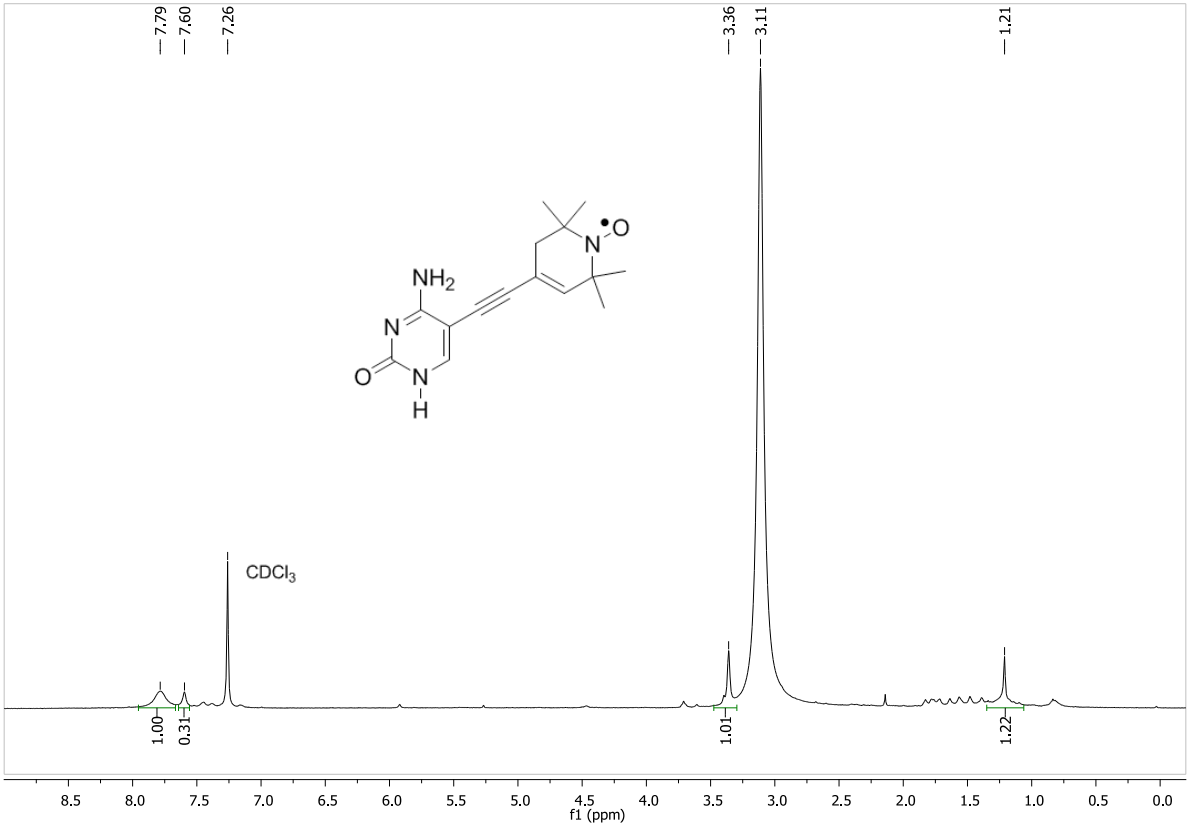


Spin labels 2 and 3. A solution of **13** or **14** (0.084 mmol) and nitroxide **15** (44 mg, 0.25 mmol) in DMF (2 mL) and Et_3N (2 mL) was degassed by bubbling argon through the solution for 5 min. $Pd(PPh_3)_2Cl_2$ (6 mg, 0.0084 mmol) and CuI (1.5 mg, 0.0084 mmol) were added and the resulting reaction mixture was stirred at 50 °C for 3 h. The solvent was removed *in vacuo* and the residue was purified preparative TLC using 5% CH_3OH in CH_2Cl_2 to give the spin labels as pale yellow solids (**2**, 7 mg, 30% yield; **3**, 5 mg, 25% yield).

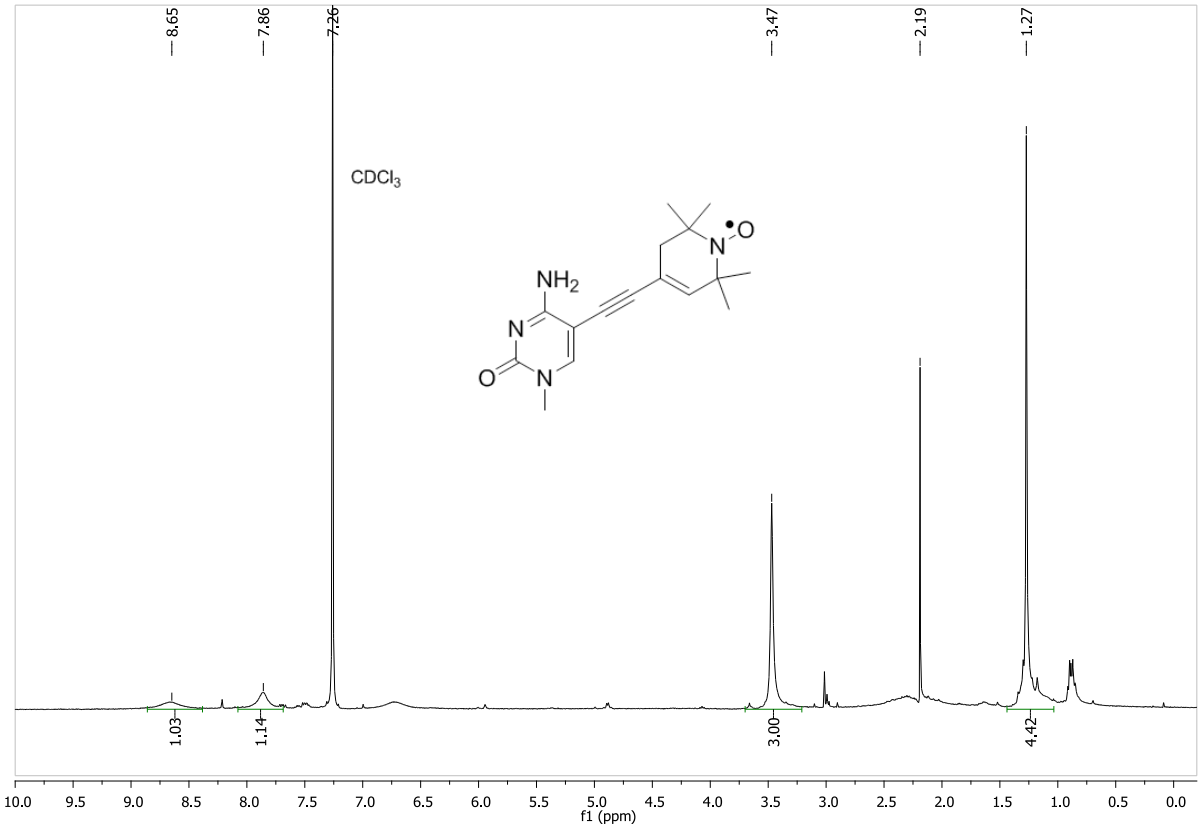
^{1}H-NMR (10% $CD_3OD/CDCl_3$) (**2**): δ 1.21 (bs, 1H, C*H*), 3.36 (s, 1H, C*H*), 7.60 (s, C*H*), 7.79 (s, N*H*) ppm.
HR-ESI-MS (**2**): Calculated for $C_{15}H_{20}N_4O_2$ 287.1508 *m/z* found 288.1581 $[M + H]^+$.

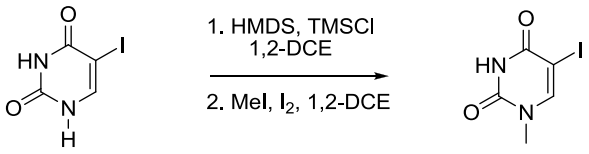
^{1}H-NMR ($CDCl_3$) (**3**): δ 1.27 (s, 4H, C*H*$_3$), 3.47 (s, 3H, C*H*$_3$), 7.86 (s, C*H*) 8.65 (s, N*H*) ppm.
HR-ESI-MS (**3**): Calculated for $C_{16}H_{21}N_4O_2$ 301.1665 *m/z* found 302.1755 $[M + H]^+$.

^{1}H NMR spectrum of **2:**



^{1}H NMR spectrum of **3:**





16 **17**

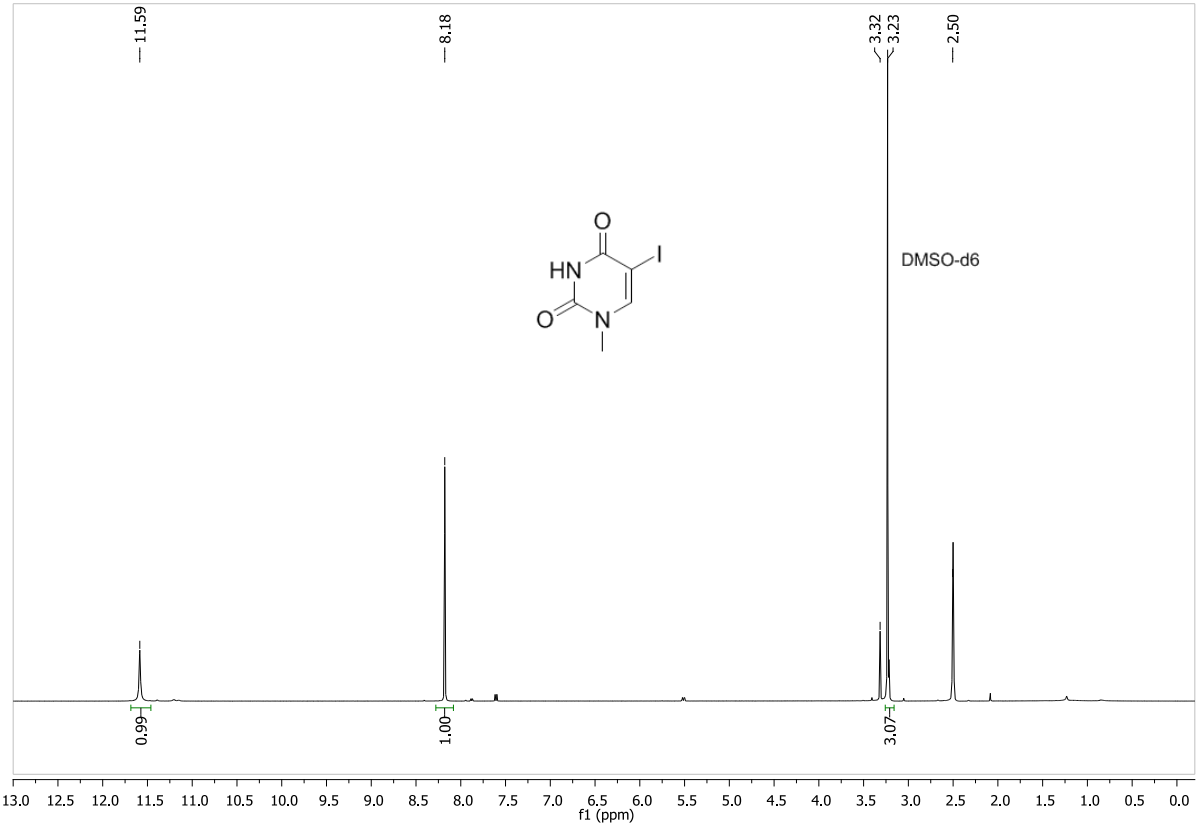
Compound 17. To a suspension of 5-iodo-uracil (**16**) (800 mg, 3.36 mmol) in 1,2-dichloroethane (10 mL), HMDS (2.0 mL, 8.4 mmol) and TMSCl (0.2 mL, 1.7 mmol) were added and the solution refluxed for 3 h under argon atmosphere. The reaction mixture was cooled to 50 °C, the solvent and excess silyl reagent was removed under reduced pressure. 1,2-Dichloroethane (10 mL) was added, followed by CH_3I (0.8 mL, 12.84 mmol) and I_2 (10 mg, 0.03 mmol) and the resulting reaction mixture was refluxed for 24 h. Solvent was removed under high vacuum and the residue purified by column chromatography using CH_3OH:CH_2Cl_2 (0:100 to 4:96) to afford **17** as a white solid (700 mg, 60% yield).

^{1}H-NMR (d_6-DMSO): δ 11.59 (s, 1H N*H*), 8.18 (s, 1H, C*H*), 3.23 (s, 3H, C*H*$_3$) ppm.

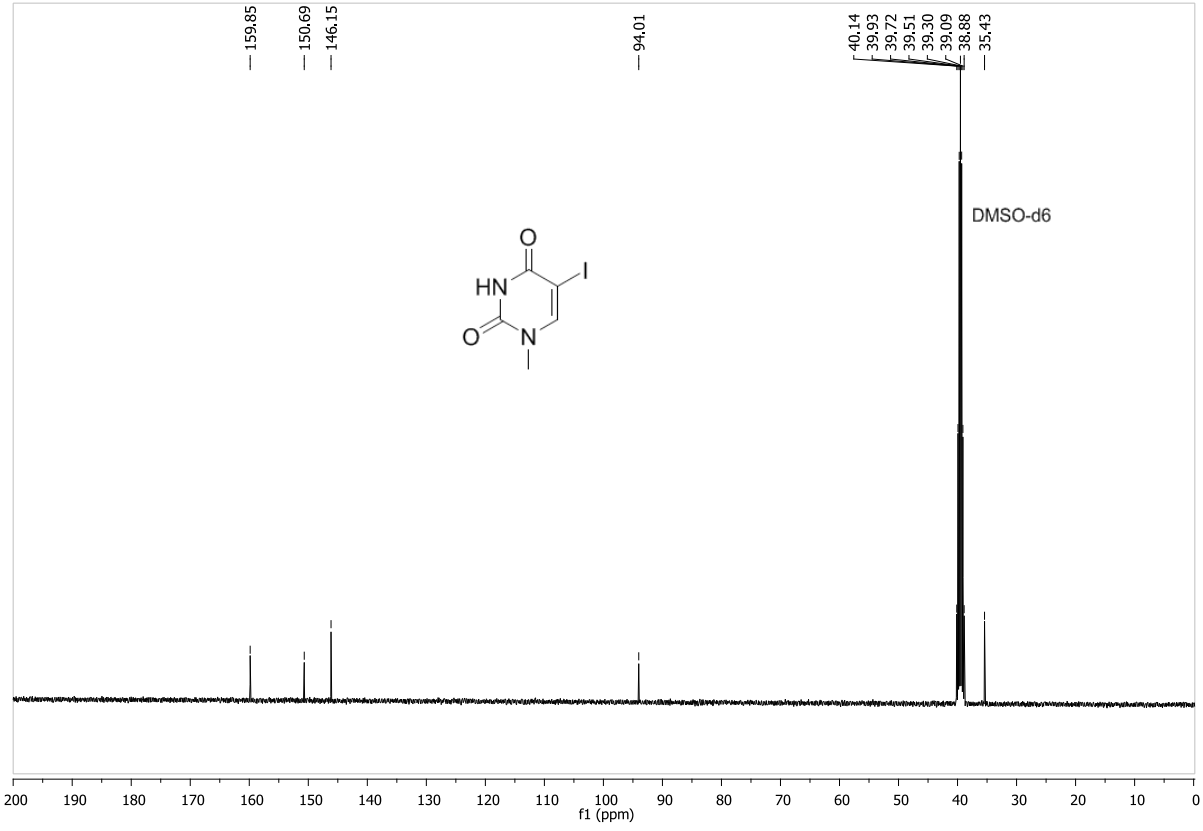
^{13}C-NMR (d_6-DMSO): δ 159.85, 150.69, 146.15, 94.01, 35.43 ppm.

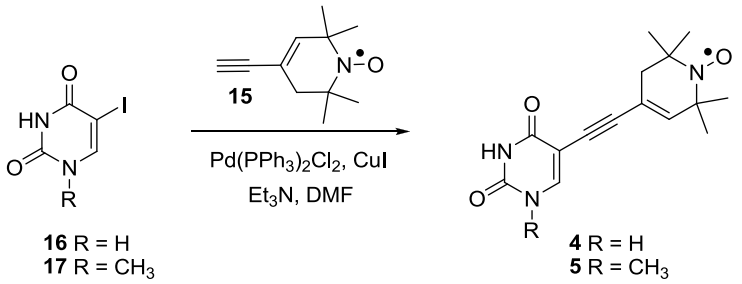
HR-ESI-MS: Calculated for $C_5H_5IN_2O_2$ 251.9396 *m/z* found 274.9341 $[M + Na]^+$.

^{1}H NMR spectrum of **17:**



^{13}C NMR spectrum of **17:**



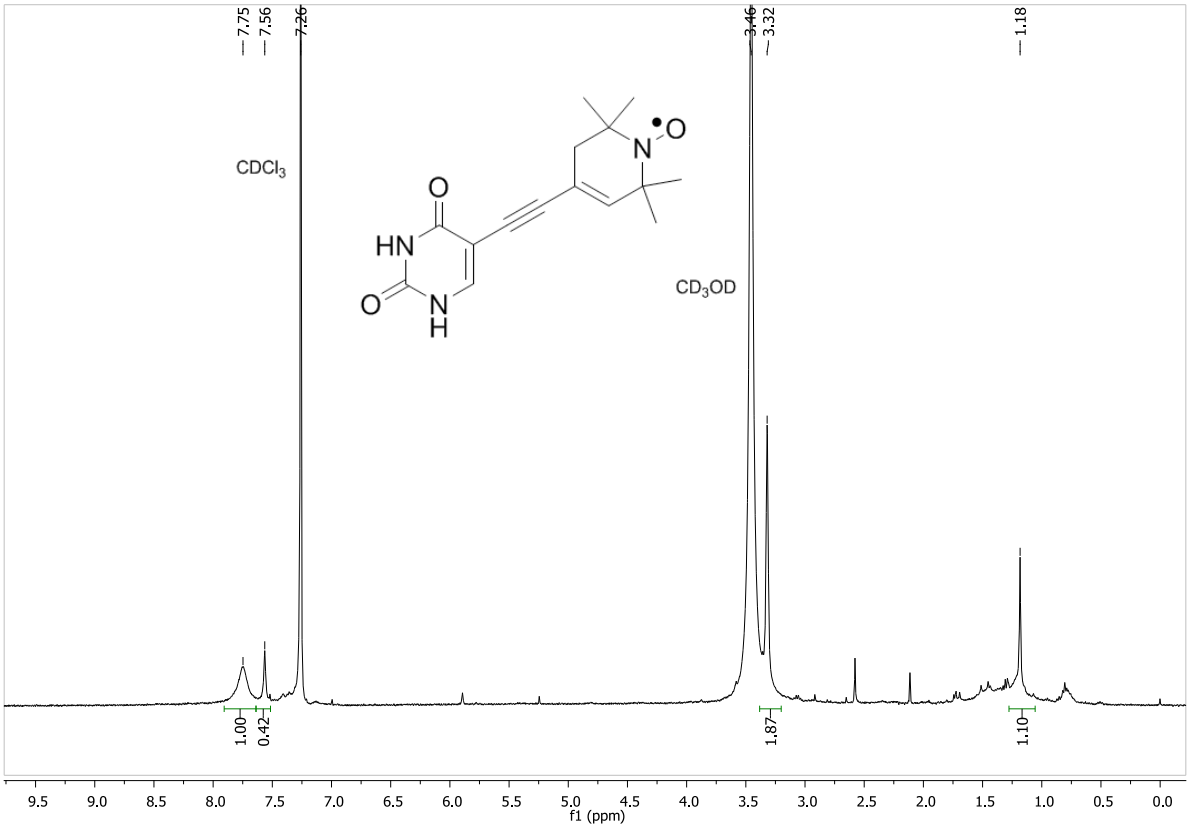


Spin labels 4 and 5. A solution of **16** or **17** (0.084 mmol) and nitroxide **15** (44 mg, 0.25 mmol) in DMF (2 mL) and Et_3N (2 mL) was degassed by bubbling argon through the solution for 5 min. $Pd(PPh_3)_2Cl_2$ (6 mg, 0.0084 mmol) and CuI (1.5 mg, 0.0084 mmol) were added and the resulting reaction mixture was stirred at 50 °C for 3 h. The solvent was removed *in vacuo* and the residue was purified by preparative TLC using 5% CH_3OH in CH_2Cl_2 to give spin labels as pale yellow solids (**4**, 7 mg, 30% yield; **5**, 5 mg, 25% yield).

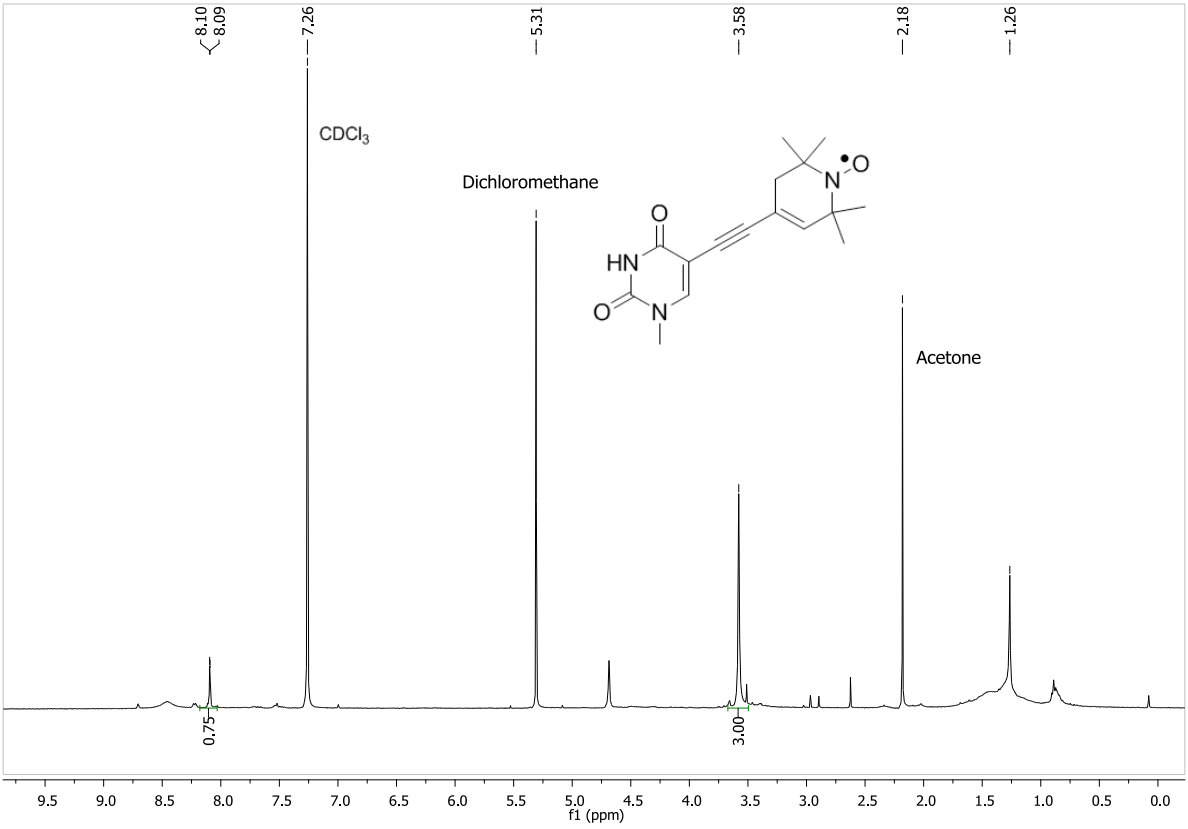
^{1}H-NMR (10% $CD_3OD/CDCl_3$) (**4**): δ 1.18 (s, 1H, C*H*), 3.32 (s, 2H, C*H*), 7.56 (s, 1H, C*H*), 7.75 (bs, ArC*H*) ppm.
HR-ESI-MS (**4**): Calculated for $C_{15}H_{18}N_3O_3$ 288.1348 *m/z* found 311.1240 $[M + Na]^+$.

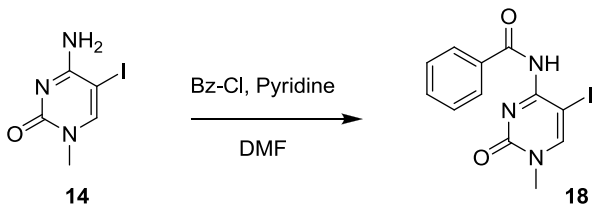
^{1}H-NMR ($CDCl_3$) (**5**): δ 1.26 (s, H, CH_3), 3.56 (s, 3H, CH_3), 8.10 (s, C*H*) ppm.
HR-ESI-MS (**5**): Calculated for $C_{16}H_{20}N_3O_3$ 302.1505 *m/z* found 325.1393 $[M + Na]^+$.

^{1}H NMR spectrum of **4:**



^{1}H NMR spectrum of **5:**





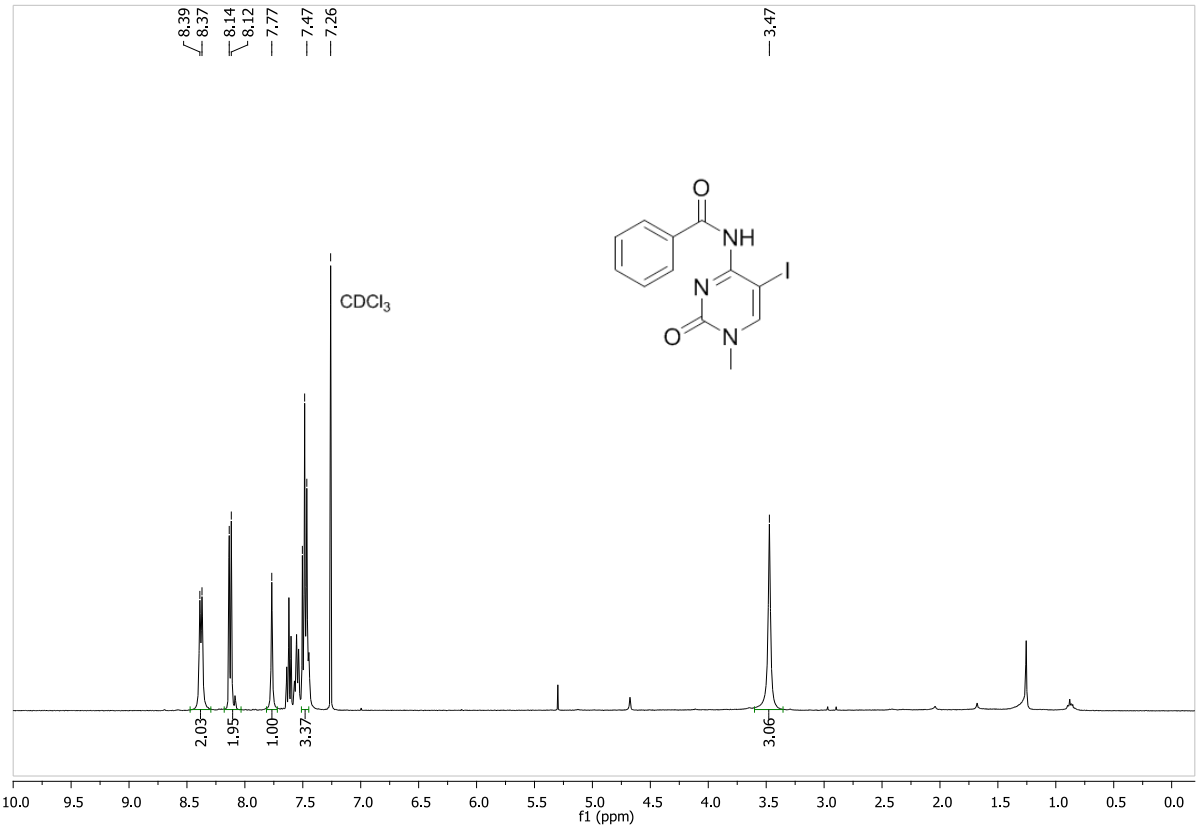
Compound 18: A solution of **14** (250 mg, 0.99 mmol) in DMF (5 mL), pyridine (5 mL) was added benzoyl chloride (0.14 mL, 1.19 mmol) and the resulting reaction mixture was stirred for 12 h at 70 °C. The reaction mixture was cooled to 24 °C, diluted with CH_2Cl_2 (20 mL) and washed with water (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo.* The residue was purified by column chromatography using 1% MeOH in CH_2Cl_2 to yield **18** as a pale yellow solid (100 mg, 30% yield).

^{1}H-NMR ($CDCl_3$): δ 3.47 (s, 3H, C*H*$_3$), 7.47-7.50 (m, 3H, ArC*H*), 7.77 (s, 1H, ArC*H*), 8.12 (d, *J* = 8, 2H, ArC*H*) 8.37 (d, *J* = 8, 2H, ArC*H*) ppm.

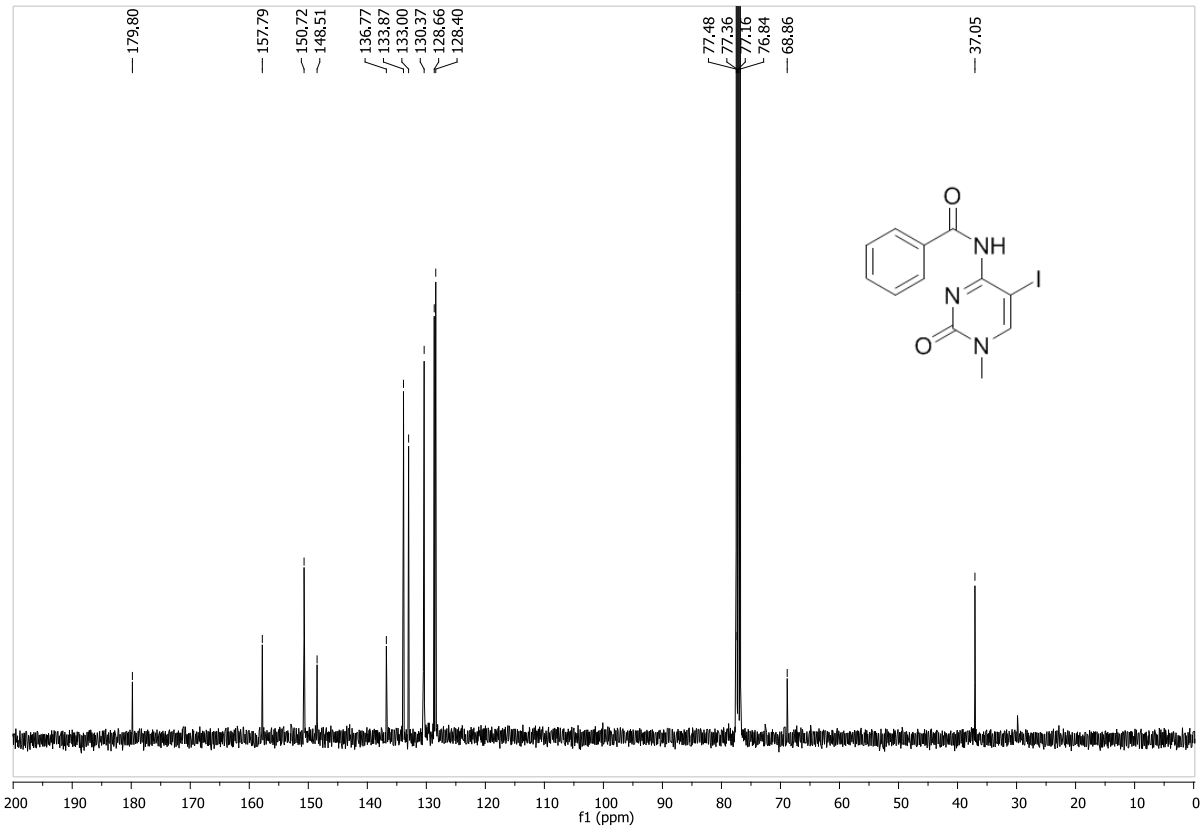
^{13}C-NMR ($CDCl_3$): δ 179.80, 157.79, 150.72, 148.51, 136.77, 133.87, 133.00, 130.37, 128.66, 128.40, 68.86, 37.05 ppm.

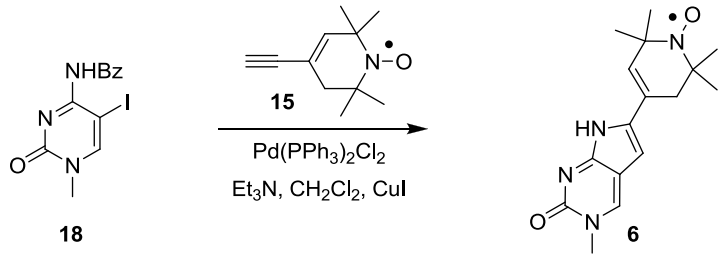
HR-ESI-MS: Calculated for $C_{12}H_{10}IN_3O_2$ 354.9818 found 377.9696 $[M + Na]^+$.

^{1}H NMR spectrum of **18:**



^{13}C NMR spectrum of **18:**



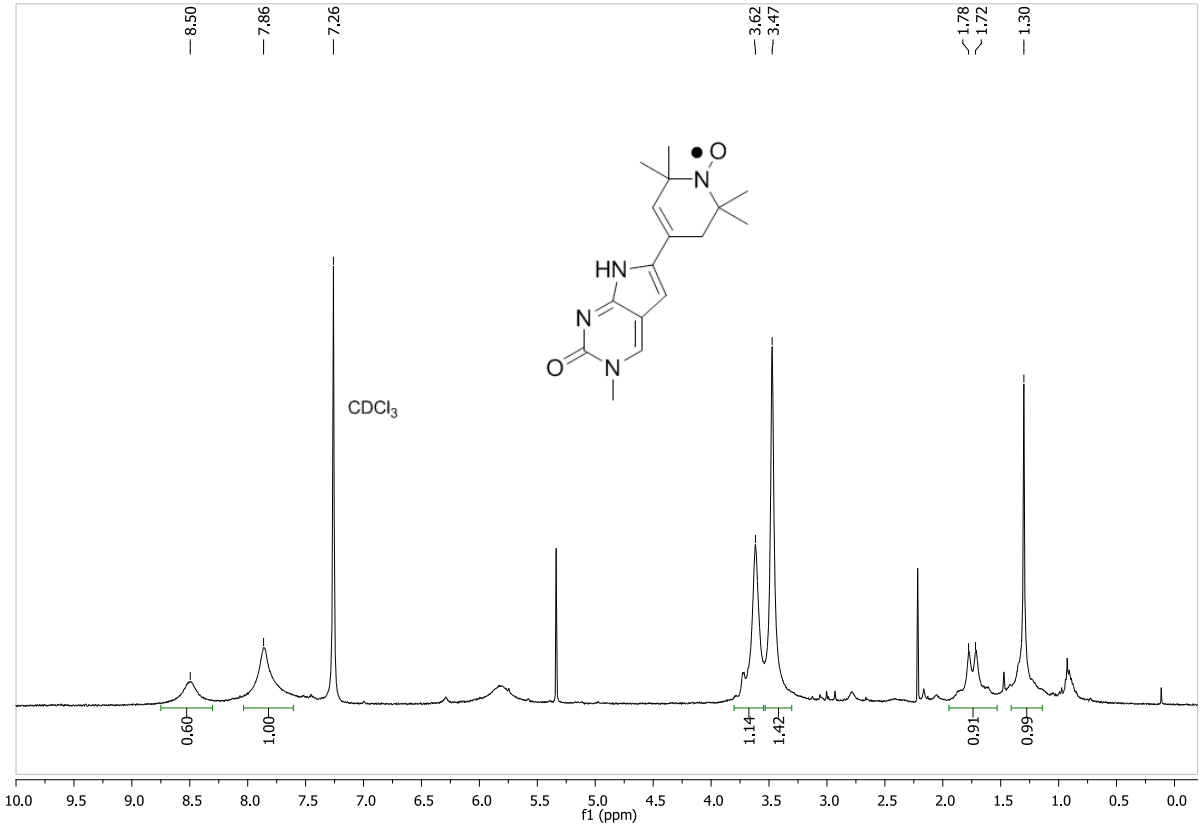


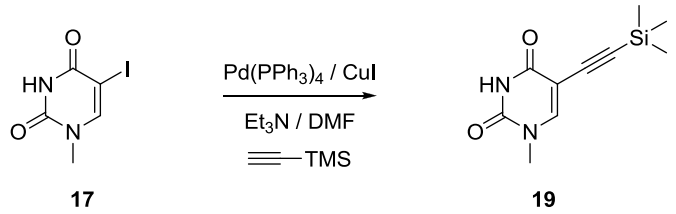
Spin label 6: To a solution of **18** (20 mg, 0.056 mmol) in DMF (2 mL) and Et_3N (2 mL), $Pd(PPh_3)_2Cl_2$ (4 mg, 0.0056 mmol) and CuI (2.1 mg, 0.011 mmol) were added. The resulting reaction mixture was treated with **15** (15 mg 0.084 mmol) and stirred for 12 h at 50 °C. The reaction mixture was diluted with absolute ethanol (2 mL) and stirred for 60 °C for 12 h. The resulting reaction mixture was cooled and the solvent was removed *in vacuo.* The residue was purified by preparative TLC to yield **6** as a pale yellow solid (12 mg, 70% yield).

^{1}H-NMR ($CDCl_3$): δ 1.30 (bs, 1H, C*H*$_3$), 1.72-1.78 (bd, 1H, C*H*$_2$), 3.47 (s, 2H, C*H*$_3$), 3.62 (s, 1H, C*H*) 7.86 (s, 1H, C*H*), 8.5 (s, 1H, C*H*) ppm.

HR-ESI-MS: Calculated for $C_{16}H_{21}N_4O_2$ 301.1665 found 302.1740 $[M + 1]^+$.

^{1}H NMR spectrum of **6:**





17 **19**

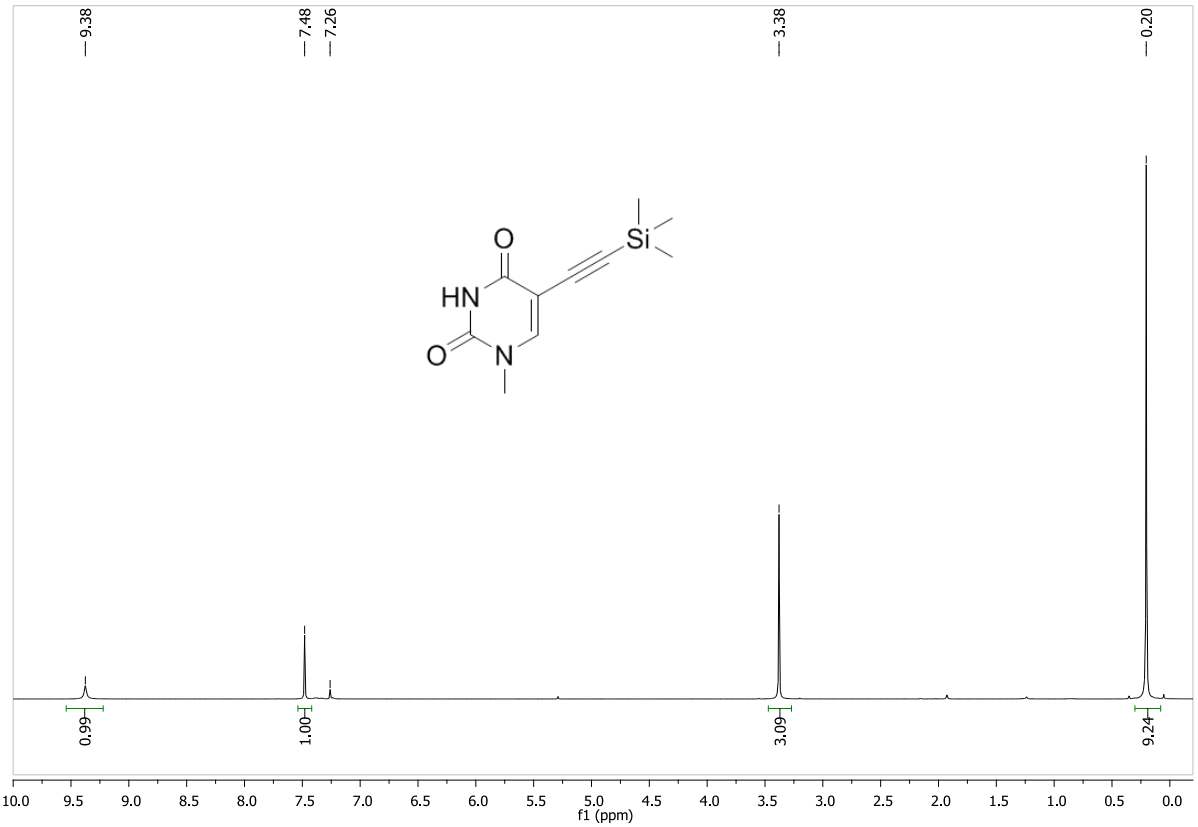
Compound 19: To a solution of **17** (160 mg, 0.78 mmol) in DMF (2 mL) and Et_3N (2 mL), $Pd(PPh_3)_4$ (85 mg, 0.078 mmol) and CuI (14 mg, 0.078 mmol) was added. The resulting reaction mixture was treated with trimethylsilyl acetylene (0.27 mL 1.95 mmol) and stirred for 2 h at 50 °C. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed subsequently with H_2O (10 mL x 2), aqueous $NaHCO_3$ solution and brine (10 mL each). The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by column chromatography using a 99.5:0.5 to 98:2 CH_2Cl_2/MeOH gradient to yield **19** as a white solid (120 mg, 70% yield).

^{1}H-NMR ($CDCl_3$): δ 0.20 (s, 9H, 3xC*H*$_3$), 3.38 (s, 3H, C*H*$_3$), 7.48 (s, C*H*), 9.38 (s, N*H*) ppm.

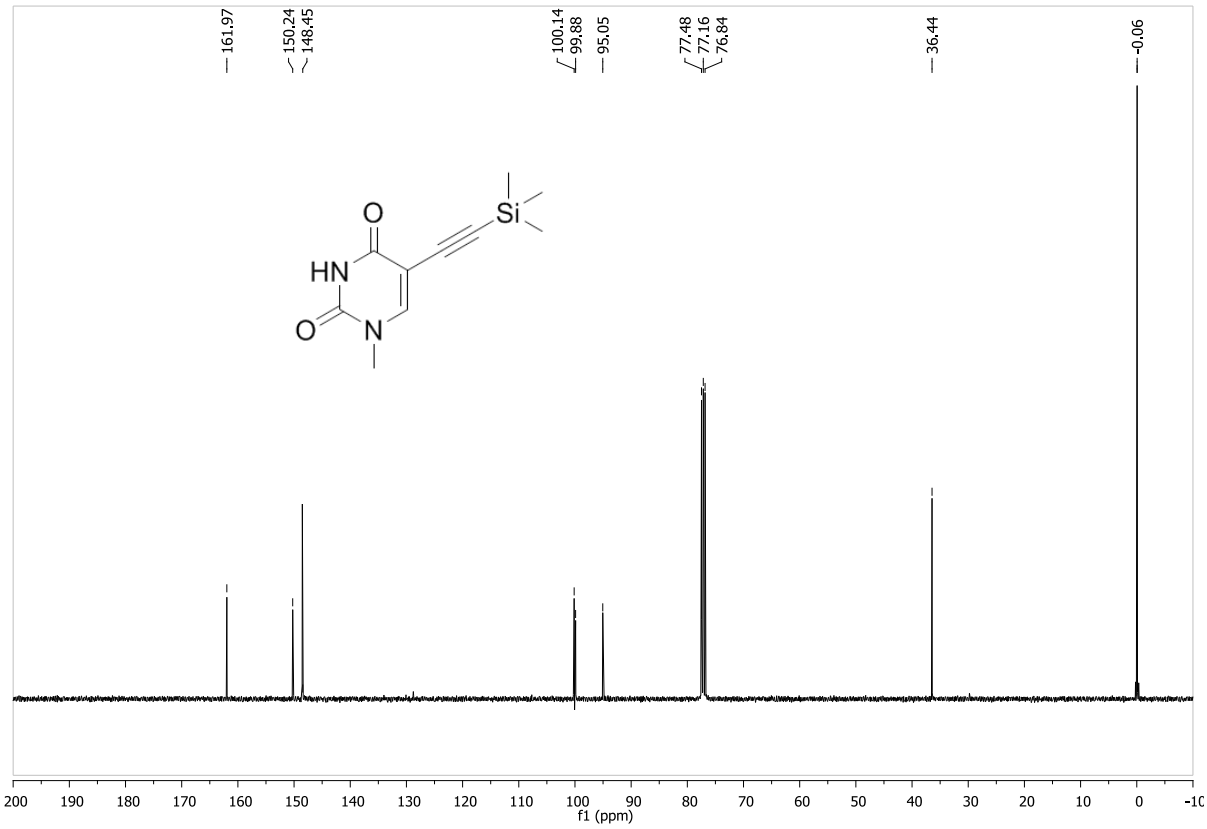
^{13}C-NMR ($CDCl_3$): δ –0.06, 36.44, 95.05, 99.88, 100.14, 148.45, 150.24, 161.97 ppm.

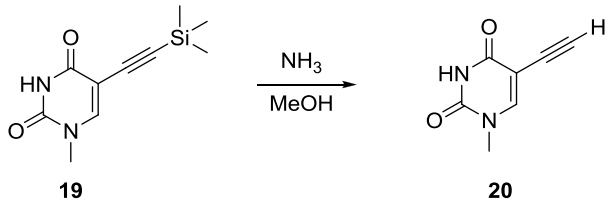
HR-ESI-MS: Calculated for $C_{10}H_{14}N_2O_2Si$ 222.0825 *m/z* found 223.0818 $[M + 1]^+$.

^{1}H NMR spectrum of **19:**



^{13}C NMR spectrum of **19:**





19 **20**

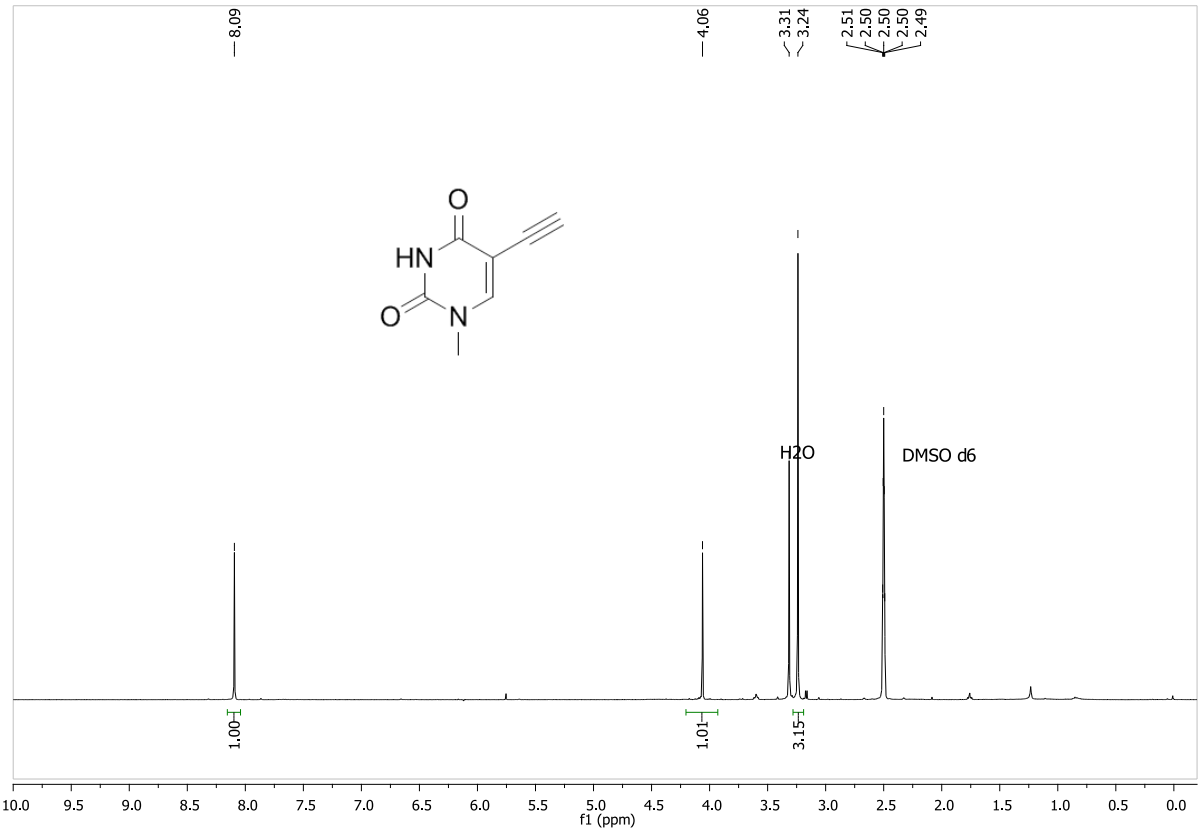
Compound 20: To a solution of **19** (80 mg, 0.36 mmol) in conc. methanolic ammonia (10 mL) was stirred for 12 h at 24 °C. The solvent was removed *in vacuo* and the crude product was purified by column chromatography using a gradient of 99:1 to 95:5 CH_2Cl_2/MeOH to yield **20** as a pale yellow solid (30 mg, 55% yield).

^{1}H-NMR (d_6-DMSO): δ 3.24 (s, 3H, C*H*$_3$), 4.06 (s, ≡C*H*), 8.09 (s, C*H*) ppm.

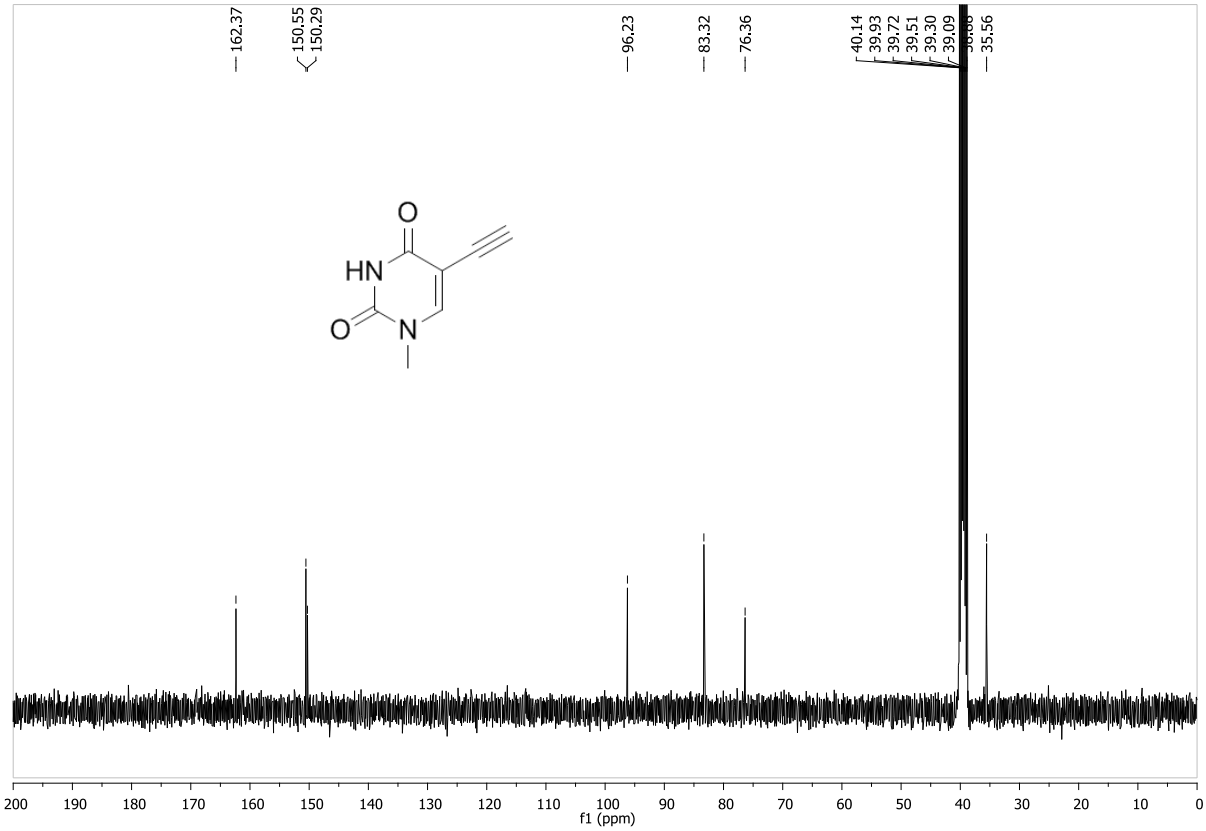
^{13}C-NMR (d_6-DMSO): δ 35.56, 76.36, 83.32, 96.23, 150.29, 150.55, 162.37 ppm.

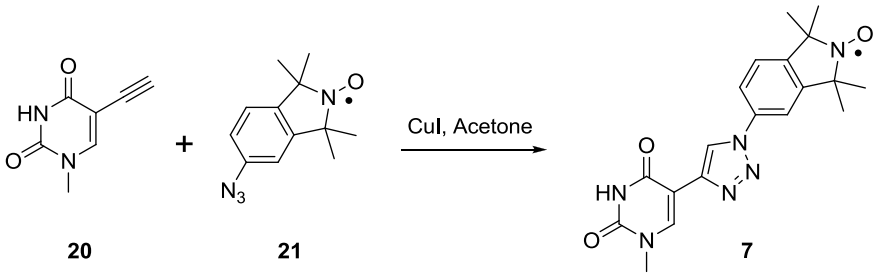
HR-ESI-MS: Calculated for $C_7H_6N_2O_2Si$ 150.0429 found *m/z* 173.0319$[M + Na]^+$.

^{1}H NMR spectrum of **20:**



^{13}C NMR spectrum of **20:**





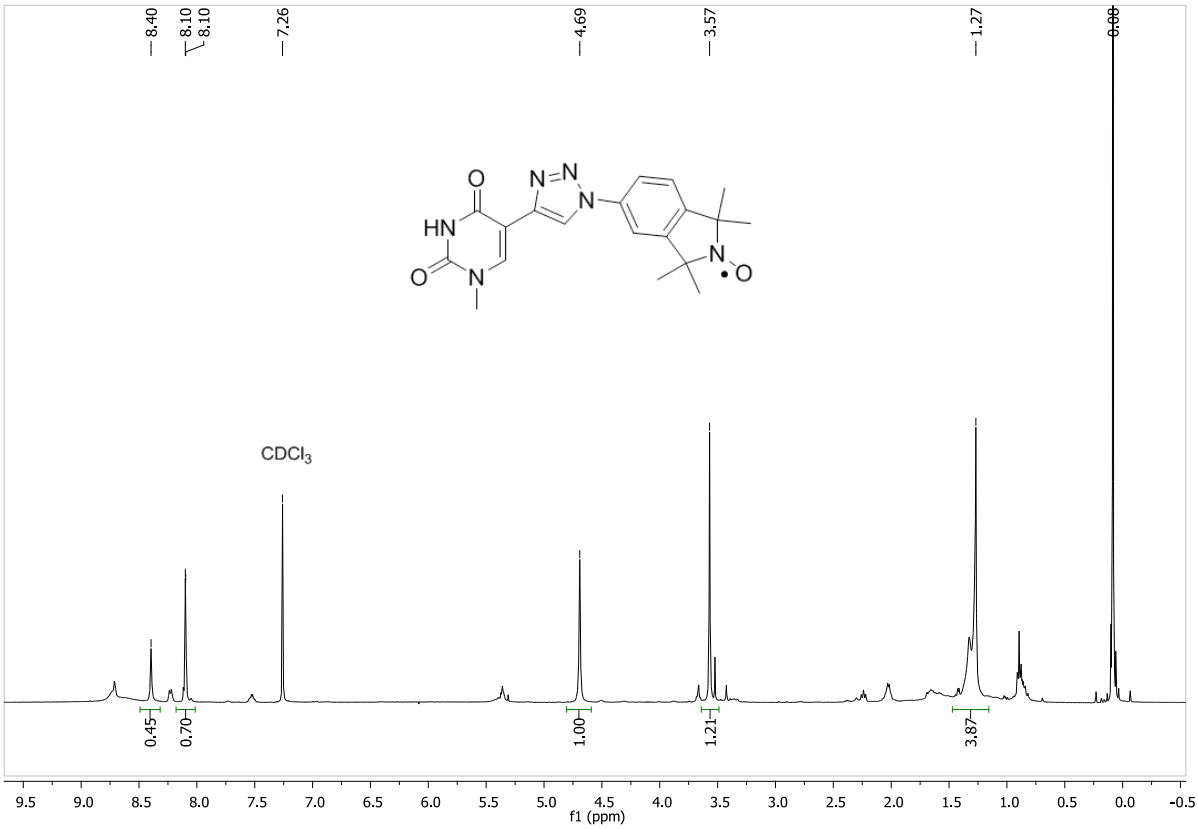
20 **21** **7**

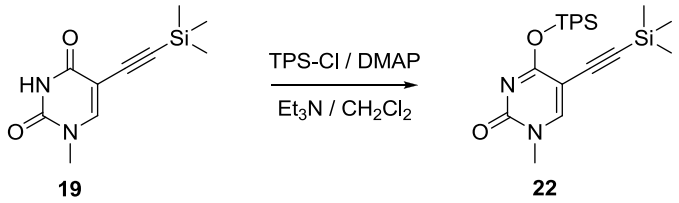
Spin label 7: To a suspension of **20** (7 mg, 0.046 mmol) in acetone (5 mL), CuI (2 mg, 0.01 mmol) and **21** (5 mg, 0.021 mmol) were added. Few drops of DMSO were subsequently added to completely dissolve **20**. The reaction mixture was refluxed for 2 h and the solvent was removed *in vacuo*. The residue was purified by preparative TLC using 10% MeOH/CH_2Cl_2 to afford **7** as a pale yellow solid (10 mg, 65% yield).

^{1}H NMR ($CDCl_3$): δ 1.27 (bs, 4H), 3.57 (bs, 1H, C*H*$_3$), 4.69 (bs, 1H, C*H*), 8.10 (s, 1H, ArC*H*), 8.40 (s, 1H, ArC*H*) ppm.

HR-ESI-MS: Calculated for $C_{19}H_{21}N_6O_3$ 381.1675 and found *m/z* 404.1574 $[M + Na]^+$.

^{1}H NMR spectrum of **7:**





19 22

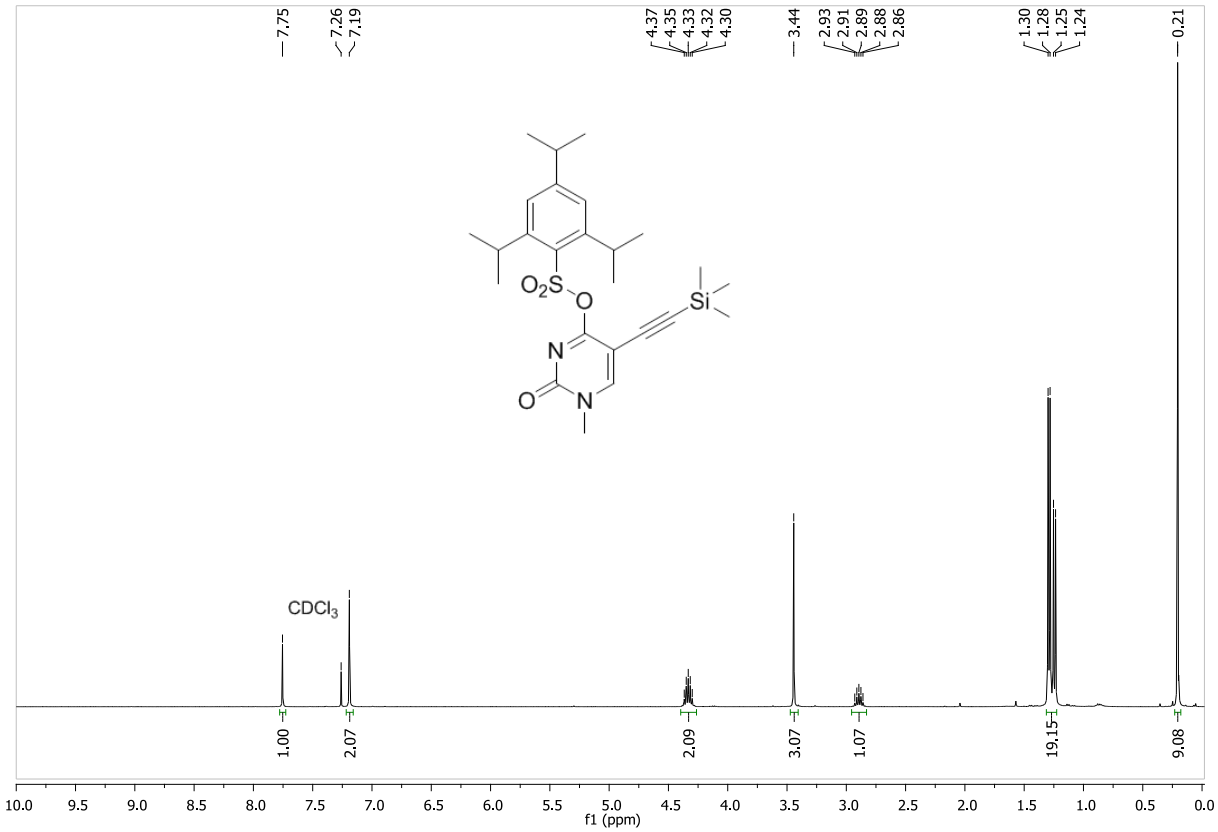
Compound 22: To a solution of **19** (50 mg, 0.2249 mmol) in dry CH_2Cl_2 (5 mL), TPS-Cl (136 mg, 0.4498 mmol) was added at 0 °C. The resulting solution was treated with DMAP (3 mg, 0.0224 mmol) and Et_3N (0.13 mL, 0.8996 mmol) and stirred for 2 h at 0 °C. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with water, aqueous $NaHCO_3$ and brine (10 mL). The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. The crude product was purified by flash column chromatography using EtOAc/petroleum ether (10:90) to yield **22** as a white solid (55 mg, 55% yield).

^{1}H-NMR ($CDCl_3$): δ -0.21 (s, 9H, 3C*H*$_3$), 1.24 & 1.28 (2d, 18H, 6C*H*$_3$), 2.89 (m, 1H, C*H*), 3.44 (s, 3H, C*H*$_3$), 4.30 - 4.37 (m, 2H, C*H*), 7.19 (s, 2H, ArC*H*), 7.75 (s, 1H, C*H*) ppm.

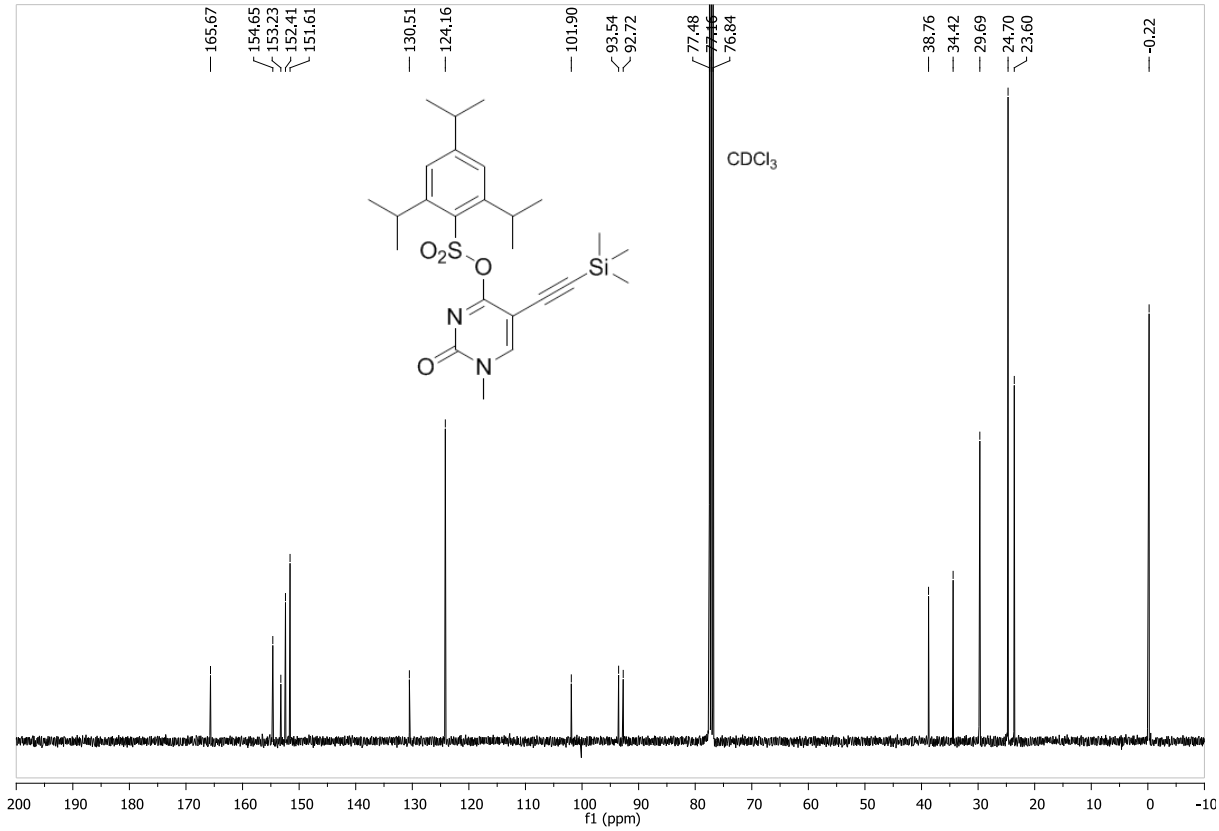
^{13}C-NMR ($CDCl_3$): δ –0.22, 23.60, 24.70, 29.69, 34.42, 38.76, 92.72, 93.54, 101.90, 124.16, 130.51, 151.61, 152.41, 153.23, 154.65, 165.67 ppm.

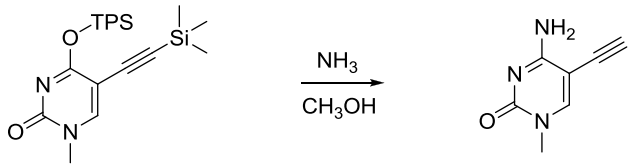
HR-ESI-MS: Calculated for $C_{25}H_{36}N_2O_4SSi$ 488.2165 found *m/z* 511.2016 $[M + Na]^+$.

^{1}H NMR spectrum of **22:**



^{13}C NMR spectrum of **22:**





22 **23**

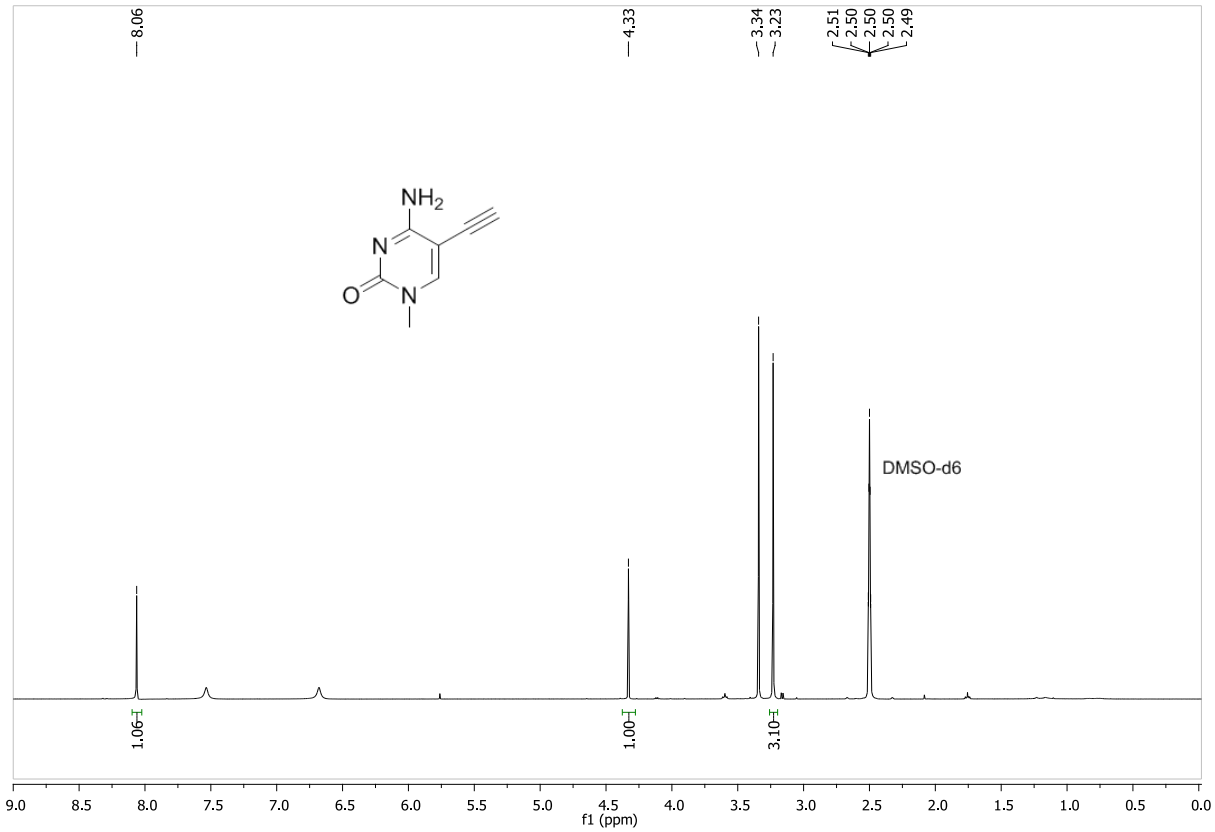
Compound 23: Compound **22** (100 mg, 0.2046 mmol) was dissolved in conc. methanolic ammonia (5 mL) and stirred for 12 h at 24 °C. The reaction mixture was concentrated and the crude material was purified by flash column chromatography using MeOH/CH_2Cl_2 (1:99 to 3:97) to afford **23** as a white solid (50 mg, 90% yield).

^{1}H-NMR (d_6-DMSO): δ 3.23 (s, 3H, C*H*$_3$), 4.33 (s, 1H, C*H*), 8.06 (s, 1H, ArC*H*) ppm.

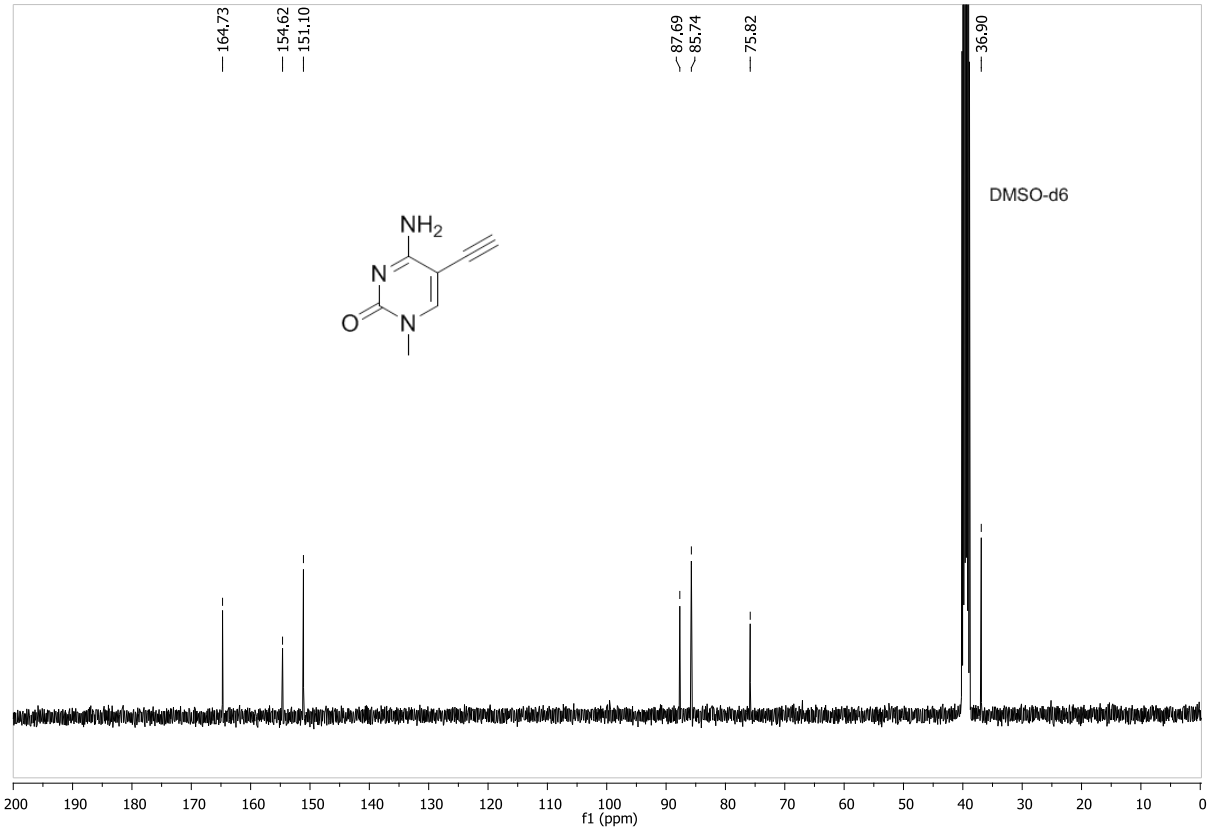
^{13}C-NMR (d_6-DMSO): δ 36.90, 75.82, 85.74, 87.69, 151.10, 154.62, 164.73 ppm.

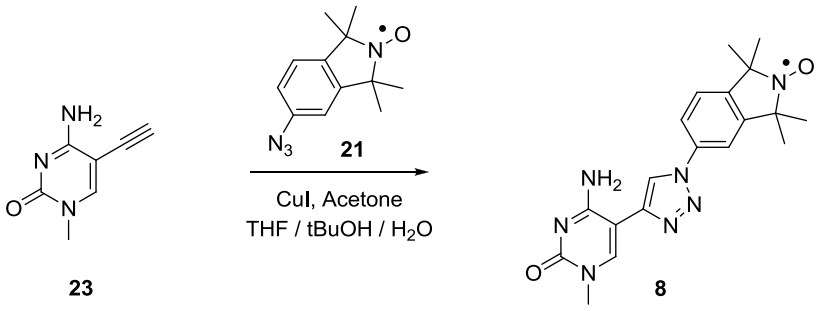
HR-ESI-MS: Calculated for $C_7H_7N_3O$ 149.0589 found m/z 150.0662 $[M + 1]^+$.

^{1}H NMR spectrum of **23:**



^{13}C NMR spectrum of **23:**



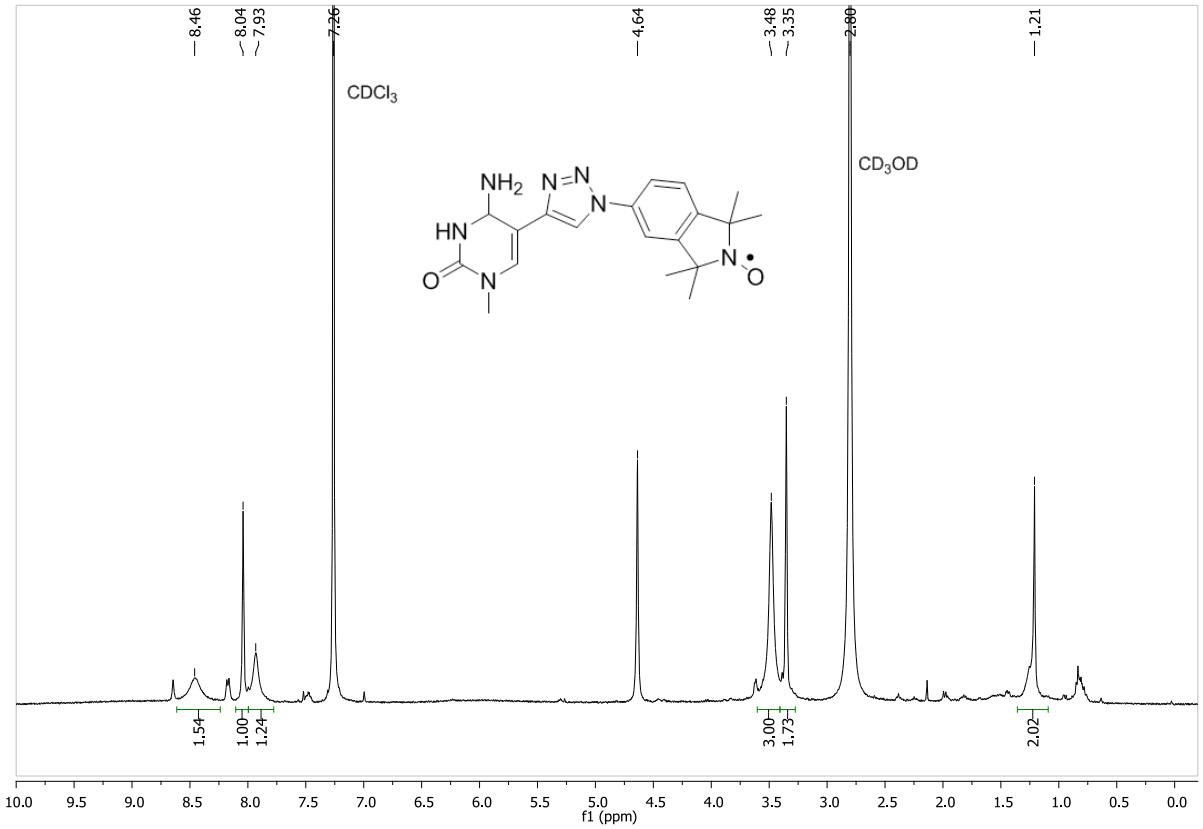


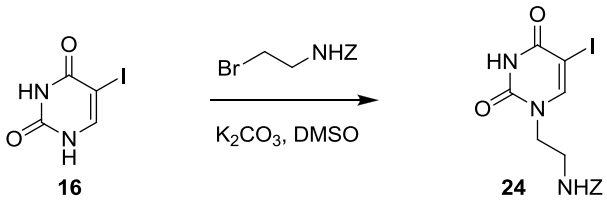
Spin label 8: To a solution of **23** (5 mg, 0.0335 mmol) in THF:H_2O:tBuOH:acetone (0.6 mL: 0.2 mL: 0.2 mL: 1 mL), **21** (7.6 mg, 0.335 mmol) was added, followed by CuI (2 mg, 0.0105 mmol). The resulting reaction mixture was stirred for 12 h at 70 °C and concentrated. The residue was purified by preparative TLC using 8% MeOH in CH_2Cl_2, giving **8** as a white solid (5 mg, 65% yield).

^{1}H NMR ($CDCl_3$ 5% CD_3OD): δ 1.21 (bs, 2H), 3.35 (bs, 1H, C*H*), 3.48 (bs, 3H, C*H*$_3$), 7.93 (s, 1H, ArC*H*), 8.04 (s, 1H, ArC*H*), 8.46 (s, 1H, ArC*H*) ppm.

HR-ESI-MS: Calculated for $C_{19}H_{22}N_7O_2$ 380.1835 found m/z 381.1908 $[M + 1]^+$.

^{1}H NMR spectrum of **8:**





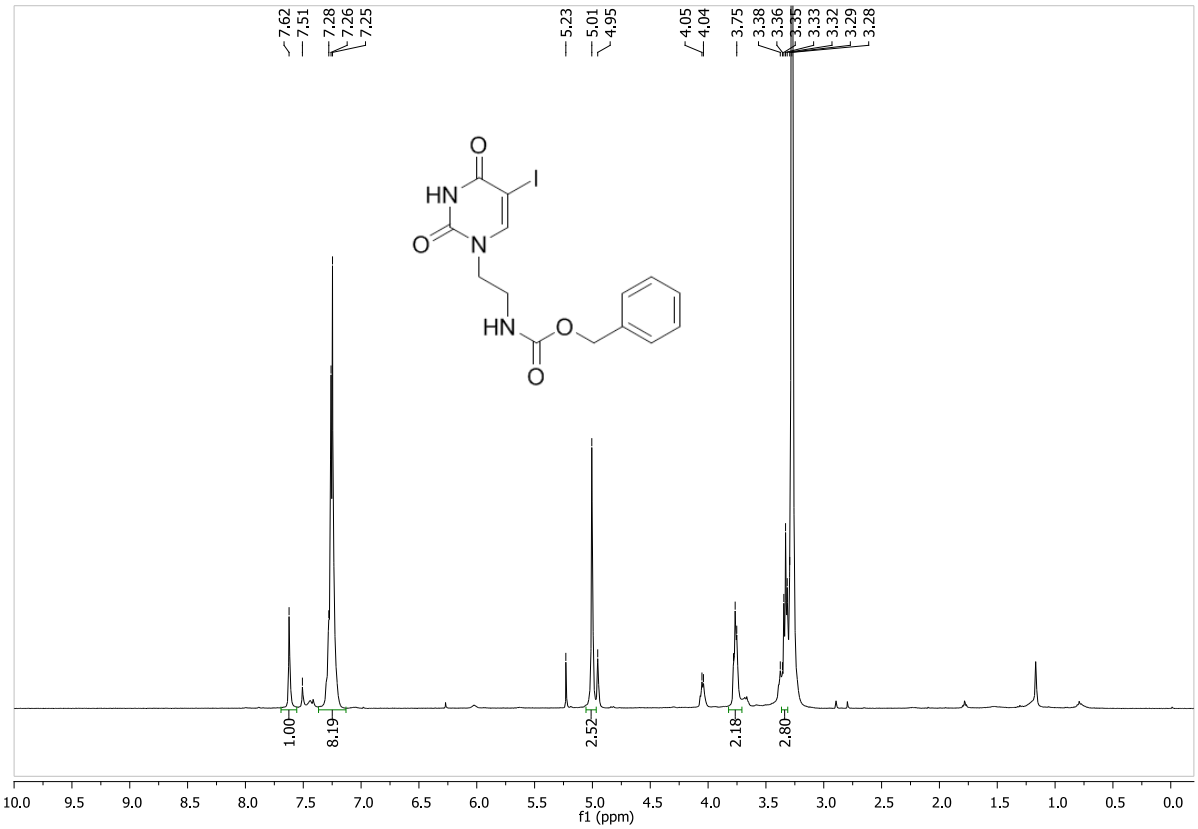
Compound 24: A solution of 5-iodouracil (**16**) (1 g, 4.20 mmol) and K_2CO_3 (0.35 g, 2.5 mmol) in DMSO (10 ml) was stirred for an hour prior to addition of a solution of Z-protected 2-bromoethylamine (0.54 g, 2.10 mmol) in DMSO (50 ml). The resulting reaction mixture was stirred for 12 h at 23 °C, diluted with H_2O (50 ml) in an ice-bath, stirred for 5 min and extracted with CH_2Cl_2 (5 times x 50 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. The residue was purified by column chromatography (1.5% MeOH in CH_2Cl_2) to yield the **24** as a white solid (0.45 g, 35% yield).

^{1}H NMR ($CDCl_3$, 10% CD_3OD): δ 3.33 (t, *J* = 8 Hz, 2H, C*H*$_2$), 3.77 (t, *J* = 8 Hz, 2H, C*H*$_2$), 5.01 (s, 2H, C*H*$_2$), 7.25 (m, 5H, ArC*H*), 7.62 (s, 1H, ArC*H*) ppm.

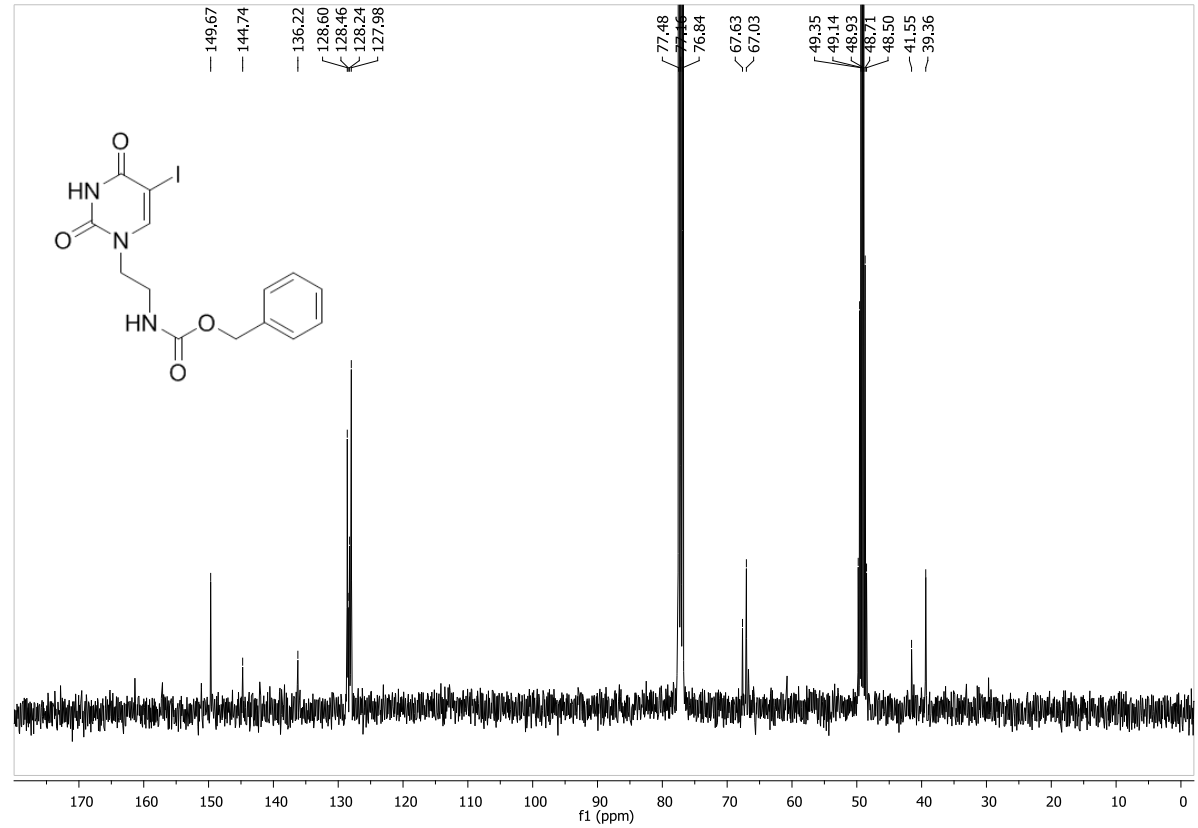
^{13}C NMR ($CDCl_3$, 10% CD_3OD): δ 149.67, 144.74, 136.22, 128.60, 128.46, 128.24, 127.98, 67.63, 67.03, 49.78, 49.57, 41.55, 39.36 ppm.

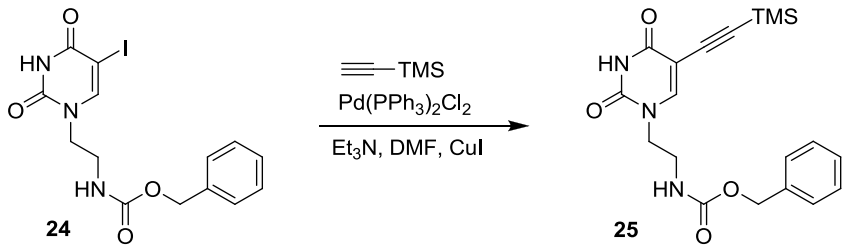
HR-ESI-MS: Calculated for $C_{14}H_{14}IN_3O_4$ 415.0029 found *m/z* 437.9901 $[M + Na]^+$.

^{1}H NMR spectrum of **24:**



^{13}C NMR spectrum of **24:**





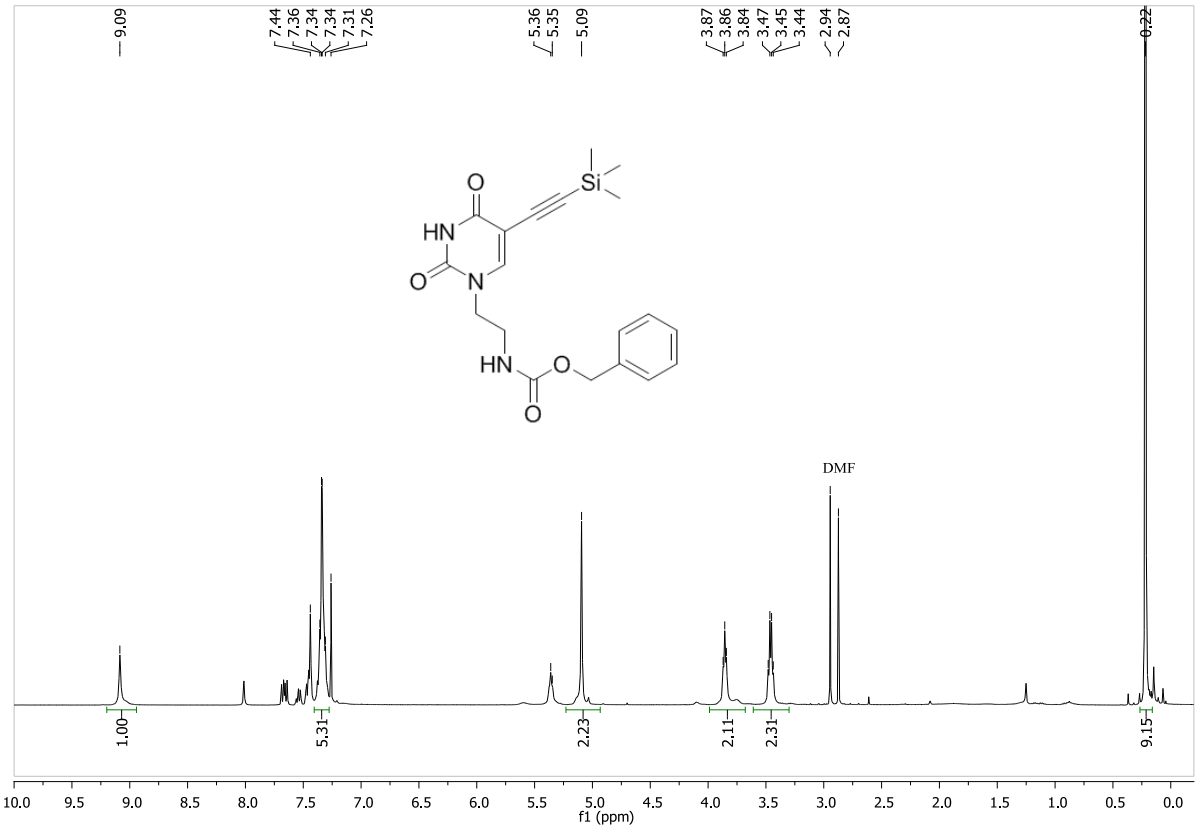
Compound 25: To a mixture of **24** (840 mg, 2.02 mmol), $Pd(PPh_3)_2Cl_2$ (142 mg, 0.20 mmol) and CuI (76 mg, 0.40 mmol) was added DMF (10 mL) and Et_3N (10 mL) and the solution degassed by bubbling argon through it for 10 min. The resulting reaction mixture was treated with trimethylsilyl acetylene (0.57 mL 4.04 mmol) and stirred for 12 h at 25 °C. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography using 99.5:0.5 to 99:1 CH_2Cl_2/MeOH to yield **25** as a pale brown solid (750 mg, 70% yield).

^{1}H NMR ($CDCl_3$): δ 0.22 (s, 9H, 3C*H*$_3$), 3.45 (t, *J* = 8 Hz, 2H, C*H*$_2$), 3.86 (t, *J* = 8 Hz, 2H, C*H*$_2$), 5.09 (s, 2H, C*H*$_2$), 7.34 (m, 5H, ArC*H*), 9.09 (s, 1H, ArC*H*) ppm.

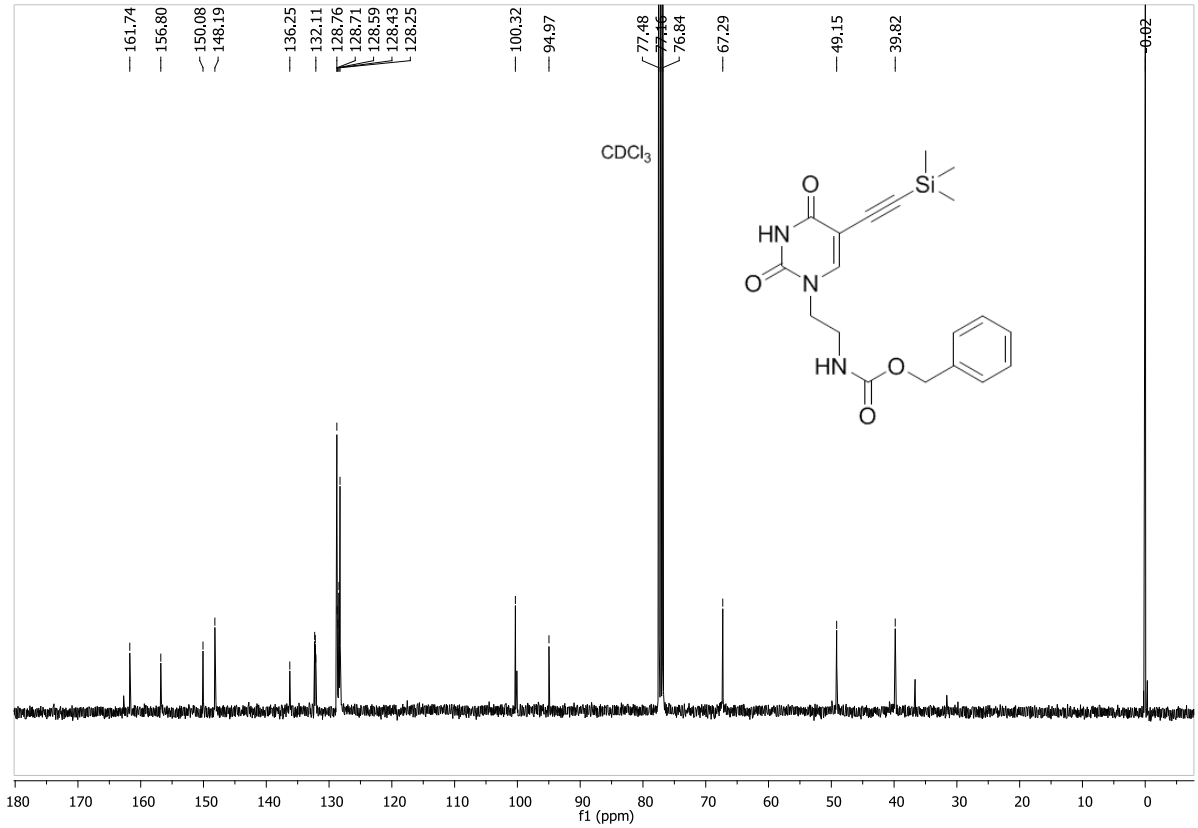
^{13}C NMR ($CDCl_3$): δ 161.74, 156.80, 150.08, 148.19, 136.25, 132.29, 132.19, 132.11, 128.76, 128.71, 128.59, 128.43, 128.25, 100.32, 94.97, 67.29, 49.15, 39.82, -0.02 ppm.

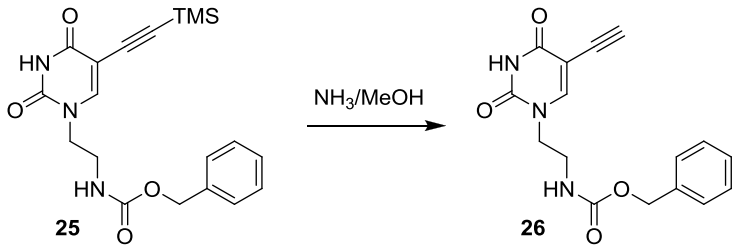
HR-ESI-MS: Calculated for $C_{19}H_{23}N_3O_4Si$ 385.1458 found *m/z* 408.1340 $[M + Na]^+$.

^{1}H NMR spectrum of **25:**



^{13}C NMR spectrum of **25:**





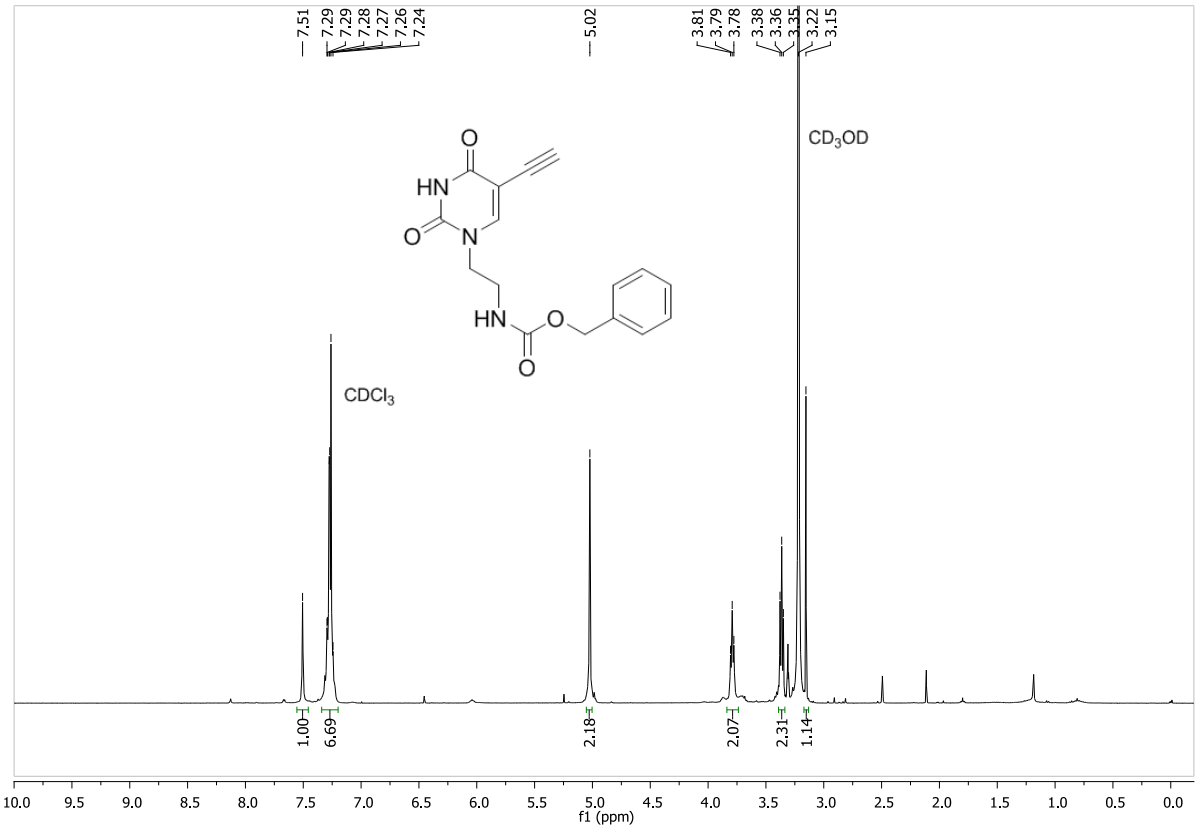
Compound 26: Compound **25** (150 mg, 0.38 mmol) was dissolved in conc. methanolic ammonia (10 mL) and resulting solution was stirred for 12 h at 25 °C in a sealed tube. The solvent was removed *in vacuo* and the residue was purified by column chromatography using 0.5% - 2% MeOH in CH_2Cl_2 to yield **26** a pale yellow solid (80 mg, 90% yield).

^{1}H NMR ($CDCl_3$): δ 3.15 (s, 1H, C*H*), 3.36 (t, *J* = 8 Hz, 2H, C*H*$_2$), 3.89 (t, *J* = 8 Hz, 2H, C*H*$_2$), 5.02 (s, 2H, C*H*$_2$), 7.28 (m, 5H, ArC*H*), 7.51 (s, 1H, ArC*H*) ppm.

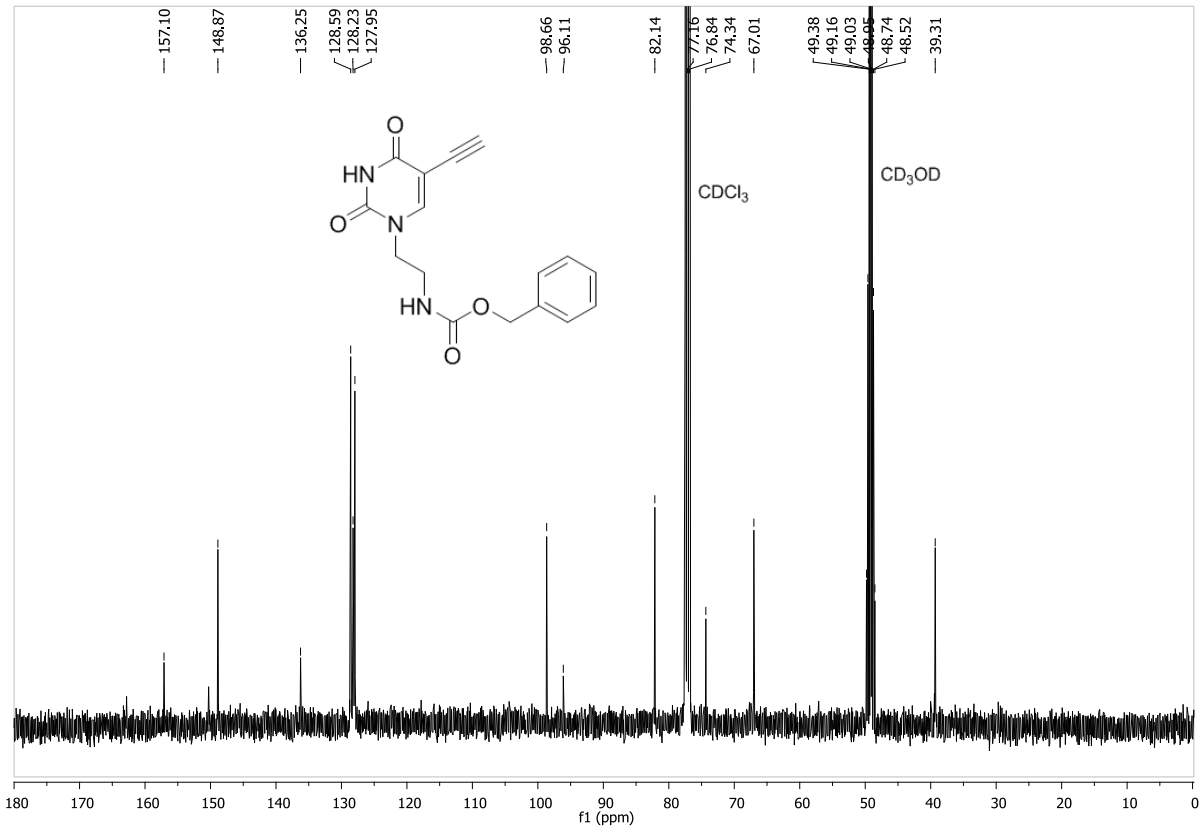
^{13}C NMR ($CDCl_3$): δ 157.10, 148.87, 136.25, 128.59, 128.23, 127.95, 98.66, 96.11, 82.14, 74.34, 67.01, 39.31 ppm.

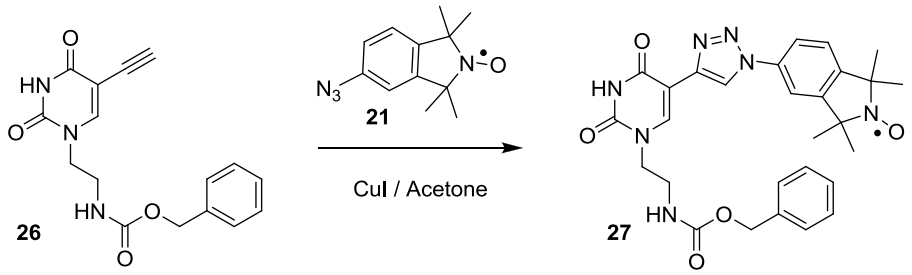
HR-ESI-MS: Calculated for $C_{16}H_{15}N_3O_4$ 313.1063 found *m/z* 336.0935 $[M + Na]^+$.

^{1}H NMR spectrum of **26:**



^{13}C NMR spectrum of **26:**





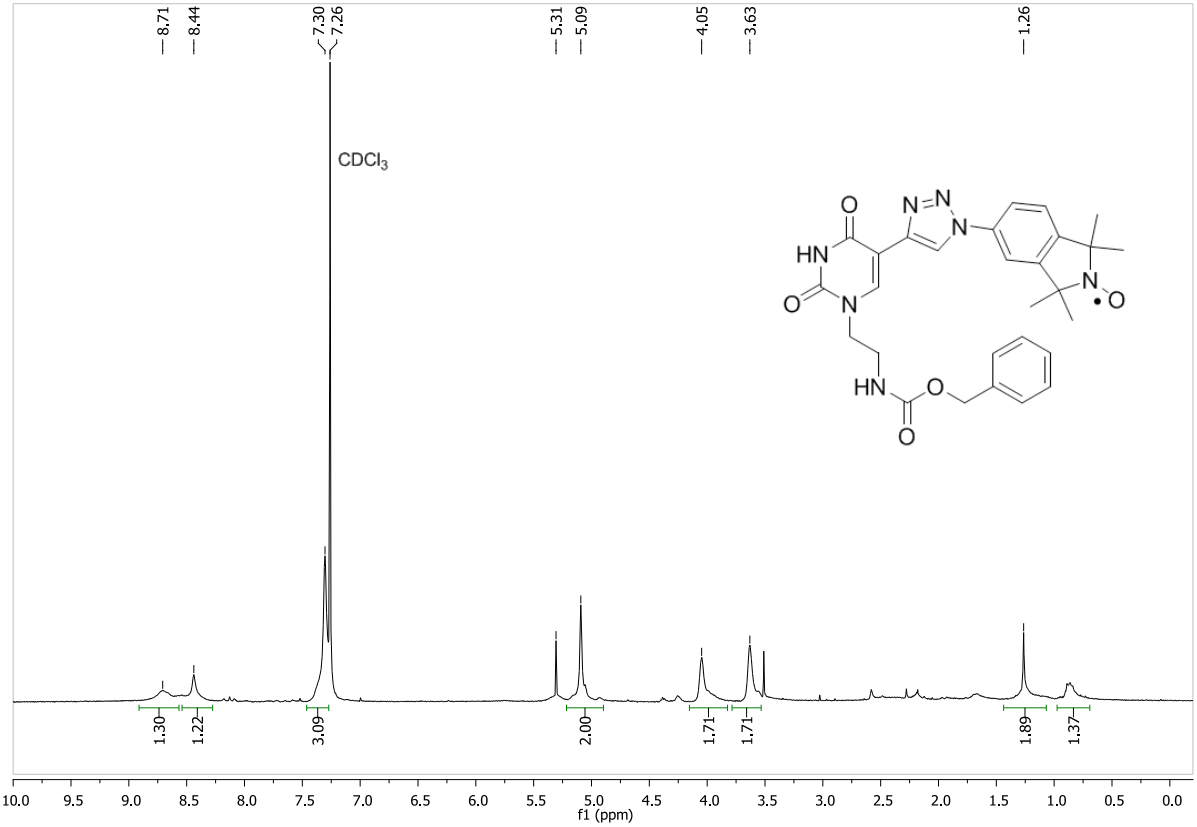
Compound 27: To a solution of **26** (50 mg, 0.15 mmol) in acetone (5 mL) was added **21** (37 mg, 0.16 mmol) followed by CuI (13 mg, 0.06 mmol). The resulting reaction mixture was stirred for 12 h at 60 °C and concentrated. The residue was purified by flash column chromatography (2% MeOH in CH_2Cl_2) to afford **27** as a pale yellow solid (51 mg, 65% yield).

^{1}H NMR ($CDCl_3$): δ 1.26 (bs, 1H, C*H*), 3.63 (bs, 2H, CH_2), 4.05 (bs, 2H, CH_2), 5.09 (s, 2H, CH_2), 7.30 (m, 3H, ArC*H*), 8.44 (s, 1H, ArC*H*), 8.71 (s, 1H, ArC*H*) ppm.

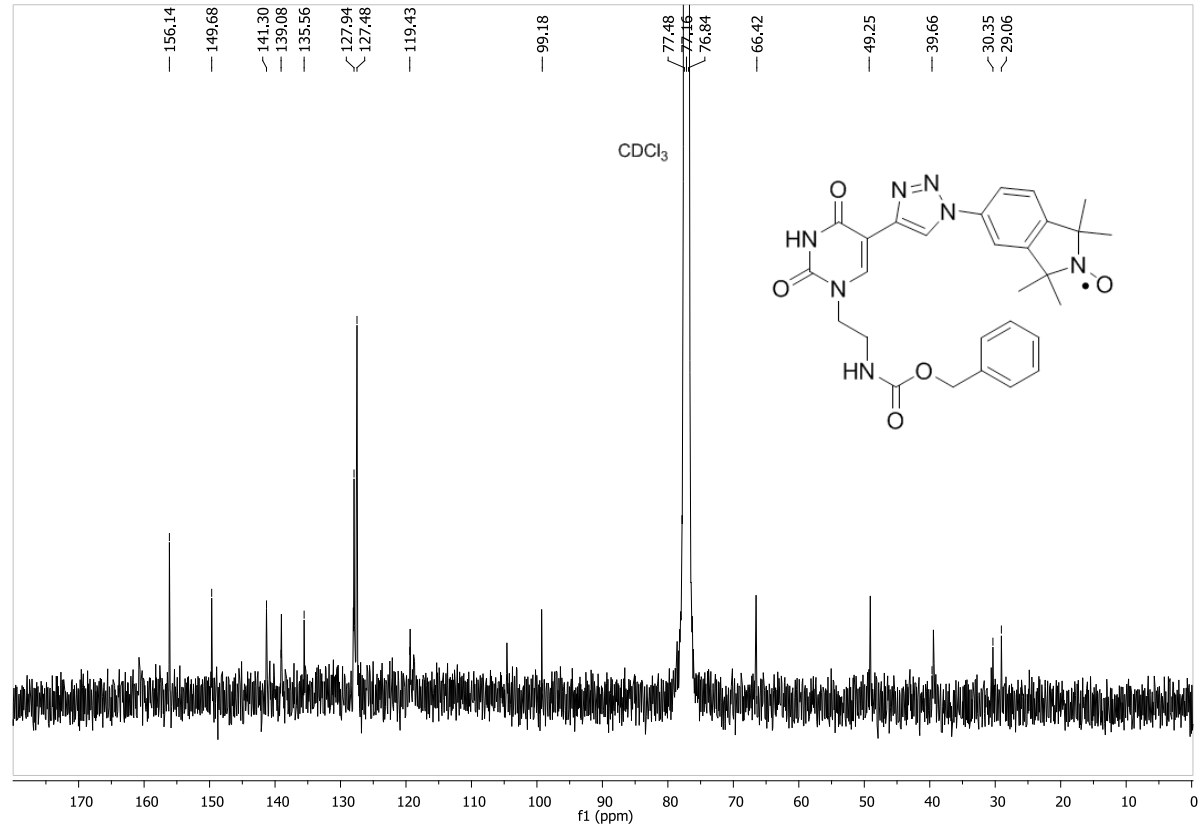
^{13}C NMR ($CDCl_3$): δ156.14, 149.68, 141.30, 139.08, 135.56, 127.94, 127.48, 119.43, 99.18, 66.42, 49.25, 39.66, 30.35, 29.06 ppm.

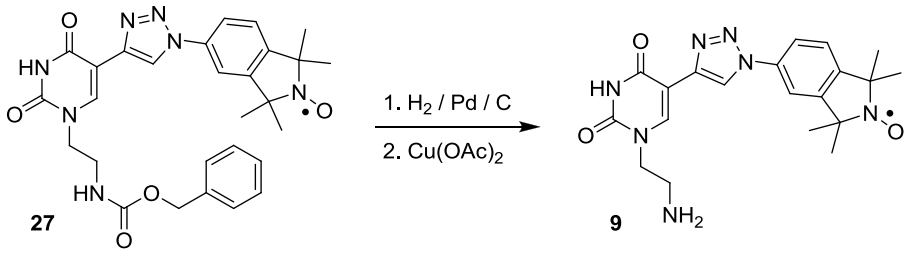
HR-ESI-MS: Calculated for $C_{28}H_{30}N_7O_5$ 544.2308 found *m/z* 567.2202 $[M + Na]^+$.

^{1}H NMR spectrum of **27:**



^{13}C NMR spectrum of **27:**





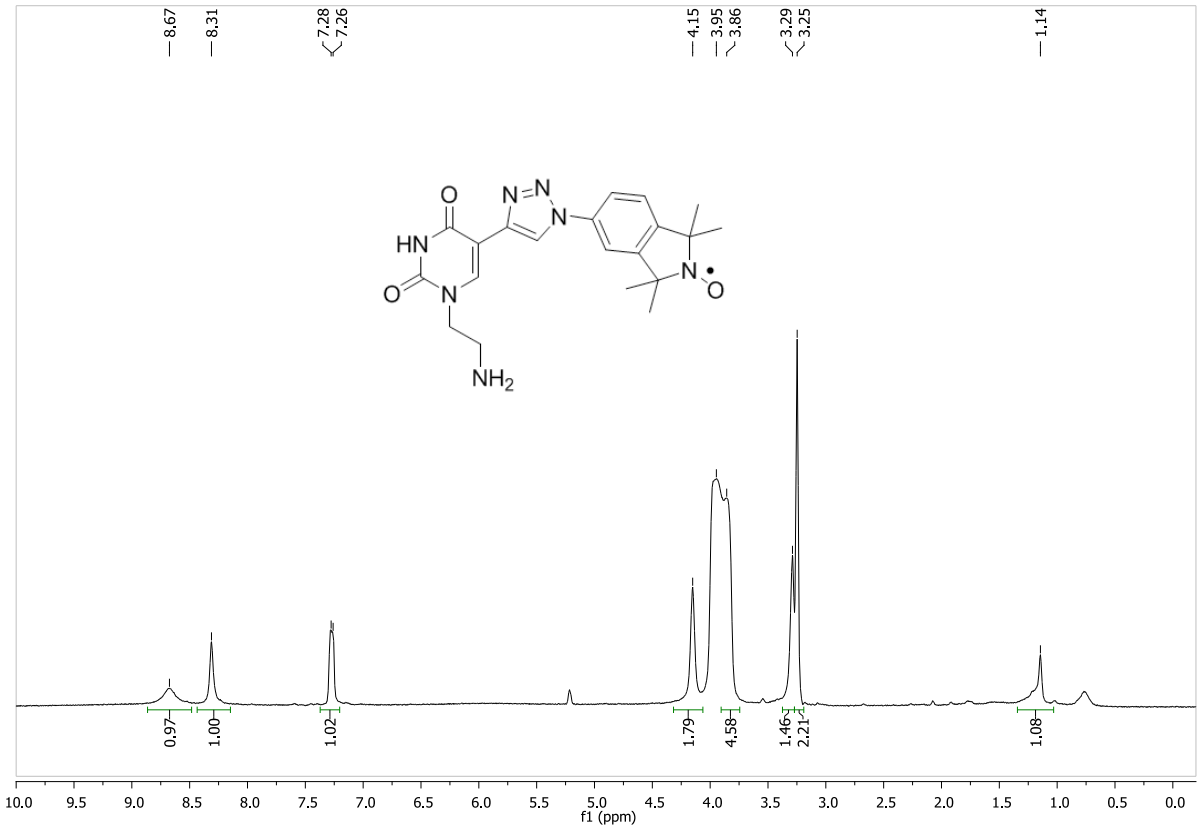
27 9

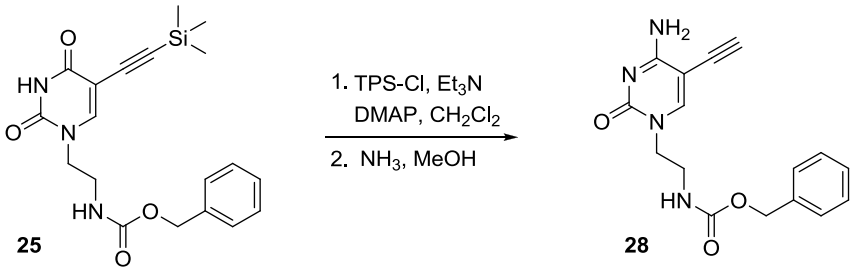
Spin label 9: A solution of **27** (36 mg, 0.06 mmol) in MeOH (6 mL) was hydrogenated over 10% Pd/C (10 mg) for 2 h at 30 psi pressure. The resulting reaction mixture was filtered through celite bed and solvent was removed *in vacuo*. The residue was dissolved in MeOH (5 mL) and treated with Cu(OAc)2•H_2O (5 mg, 0.002 mmol) and stirred for 1 h at 24 °C in the presence of air. The reaction mixture was concentrated and residue was purified by preparative TLC using 10% MeOH in CH_2Cl_2 containing 1% aq. ammonia to yield **9** as a white solid (19 mg, 75% yield).

^{1}H NMR ($CDCl_3$, 5% CD_3OD): δ 1.14 (bs, 1H), 3.25 (bs, 2H, C*H*$_2$), 3.29 (bs, 1H, C*H*), 3.86-3.95 (bs, 2H, C*H*$_2$), 4.15 (s, 2H, C*H*$_2$), 7.28 (bs, 1H, ArC*H*), 8.31 (s, 1H, ArC*H*), 8.67 (s, 1H, ArC*H*) ppm.

HR-ESI-MS: Calculated for $C_{20}H_{24}N_7O_3$ 410.1914 found *m/z* 411.1946 $[M + 1]^+$.

^{1}H NMR spectrum of **9:**





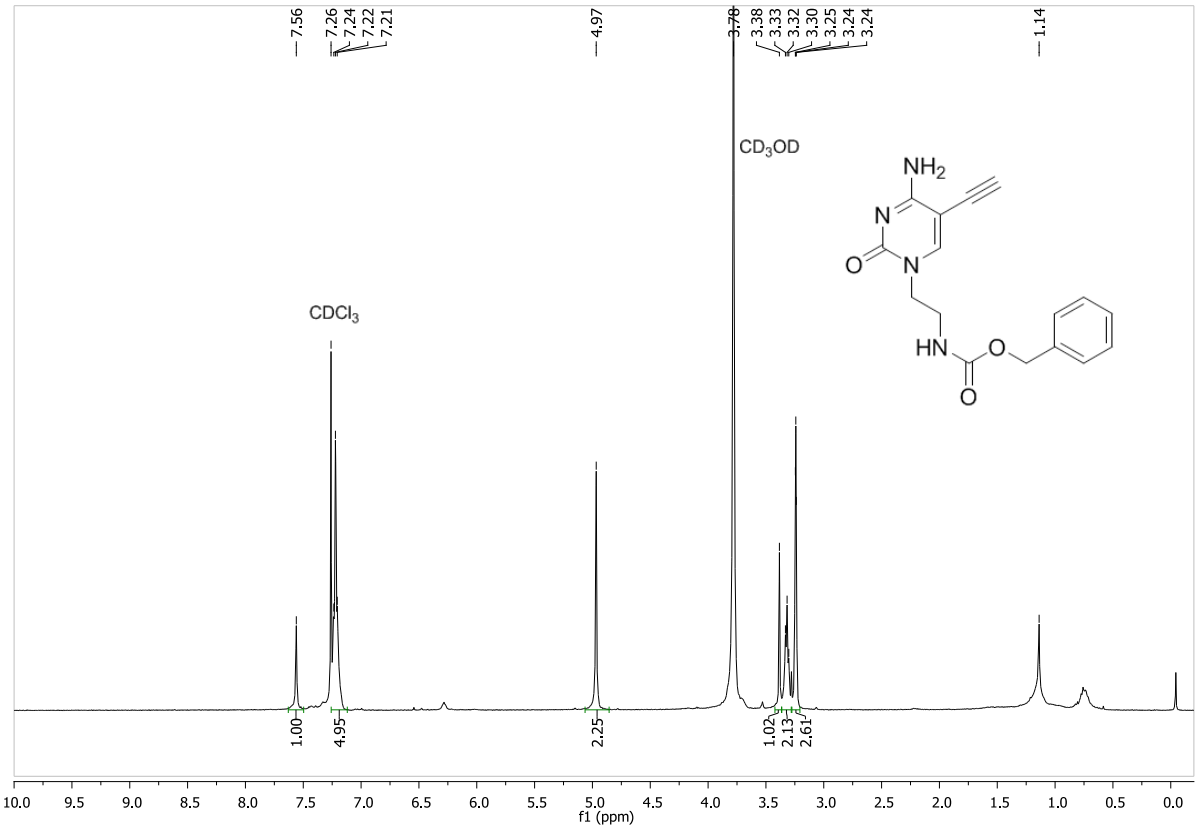
Compound 28: A solution of **25** (250 mg, 0.46 mmol) in dry CH_2Cl_2 (20 mL) treated with TPS-Cl (589 mg, 1.94 mmol) and DMAP (8 mg, 0.064 mmol) at 0 °C. The resulting reaction mixture was stirred for 5 min and Et_3N (0.36 mL, 2.59 mmol) was added. The resulting solution was stirred for 2 h at 24 °C before dilution with CH_2Cl_2 (20 mL) and washed with water (20 mL), aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried over sodium sulphate and the solvent was removed under reduced pressure. The residue was dissolved in conc. methanolic NH_3 (7 mL) and stirred for 12 h in a sealed tube. The solvent was removed *in vacuo* and the residue was purified by column chromatography using 3% MeOH in CH_2Cl_2 to afford **28** as a pale yellow solid (50 mg, 25-30% yield).

^{1}H-NMR ($CDCl_3$): δ 3.24 (t, *J* = 4 Hz, 2H, C*H*$_2$), 3.32 (t, *J* = 4 Hz, 2H, C*H*$_2$), 3.38 (s, 1H, C*H*), 4.97 (s, 2H, C*H*$_2$), 7.22 (m, 5H, ArC*H*), 7.56 (s, 1H, ArC*H*) ppm.

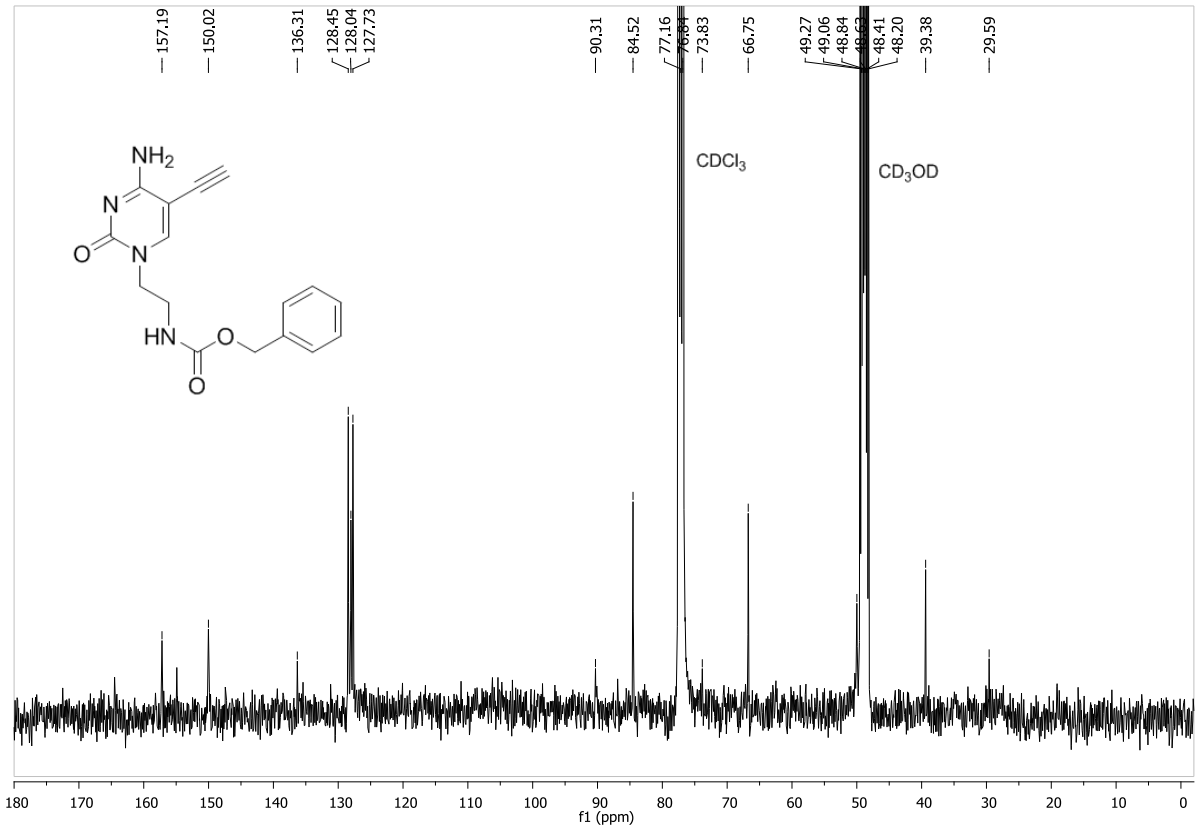
^{13}C-NMR ($CDCl_3$): δ 157.19, 150.02, 136.31, 128.45, 128.04, 127.73, 90.31, 84.52, 73.83, 66.75, 49.99, 39.38 ppm.

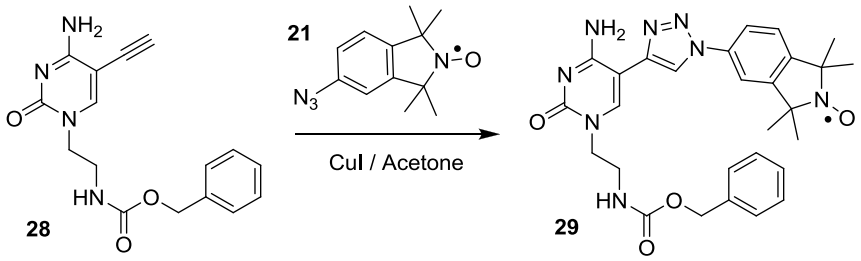
HR-ESI-MS: Calculated for $C_{16}H_{16}N_4O_3$ 312.1222 found *m/z* 313.1257 $[M + 1]^+$.

^{1}H NMR spectrum of **28:**



^{13}C NMR spectrum of **28:**



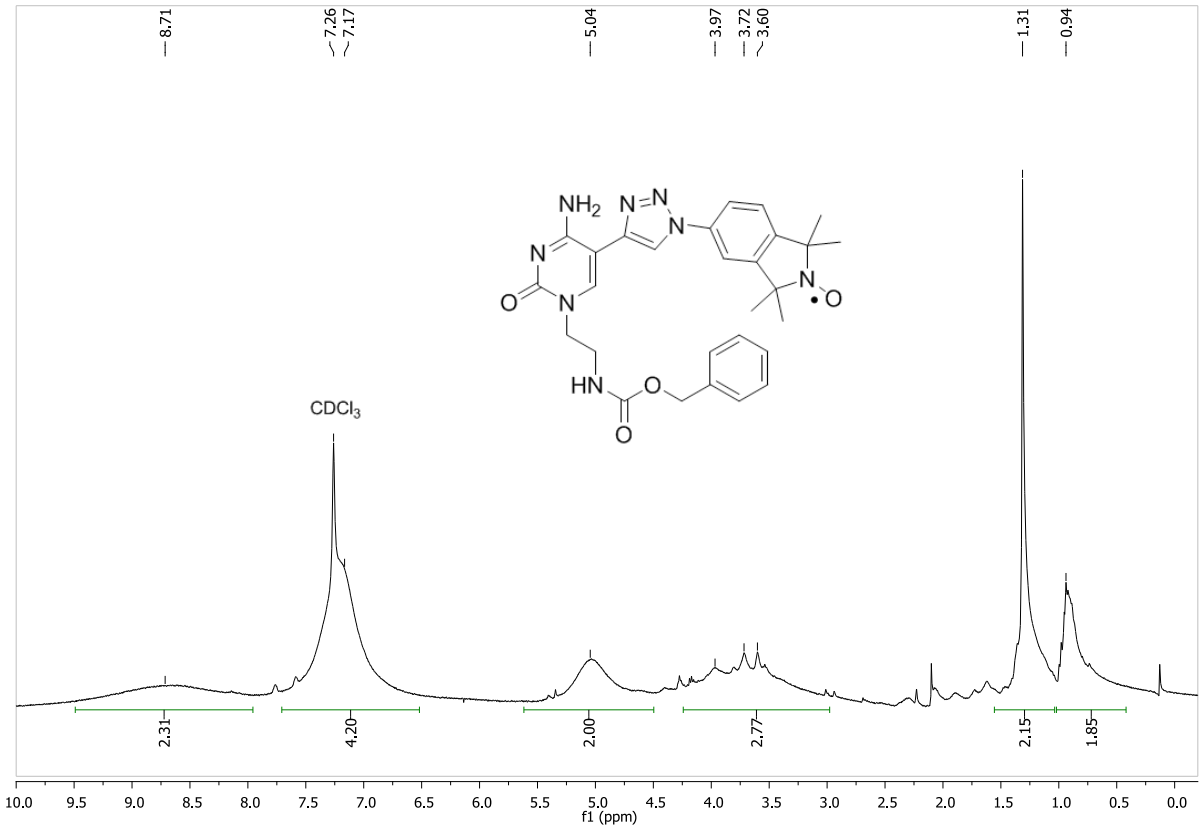


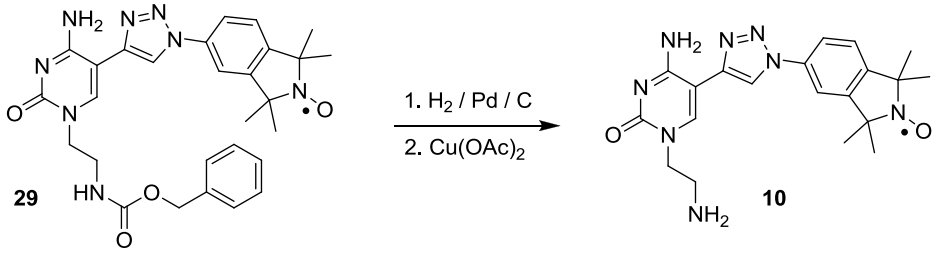
Compound 29: To a solution of **28** (41 mg, 0.13 mmol) in acetone (10 mL), compound **21** (40 mg, 0.17 mmol) was added followed by CuI (12.5 mg, 0.06 mmol). The resulting reaction mixture was stirred for 12 h at 60 °C and concentrated *in vacuo*. The residue was purified by flash column chromatography (3% MeOH in CH_2Cl_2) to afford **29** as a pale yellow solid (55 mg, 65% yield).

^{1}H NMR ($CDCl_3$): δ 0.94 (bs, 1H, C*H*), 1.31 (bs, 2H, CH_2), 3.6-3.97 (bs, 2H, CH_2), 5.04 (bs, 2H, CH_2), 7.17 (m, 4H, ArC*H*), 8.71 (s, 2H, ArC*H*) ppm.

HR-ESI-MS: Calculated for $C_{28}H_{31}N_8O_4$ 543.2468 found *m/z* 566.2202 $[M + Na]^+$.

^{1}H NMR spectrum of **29:**



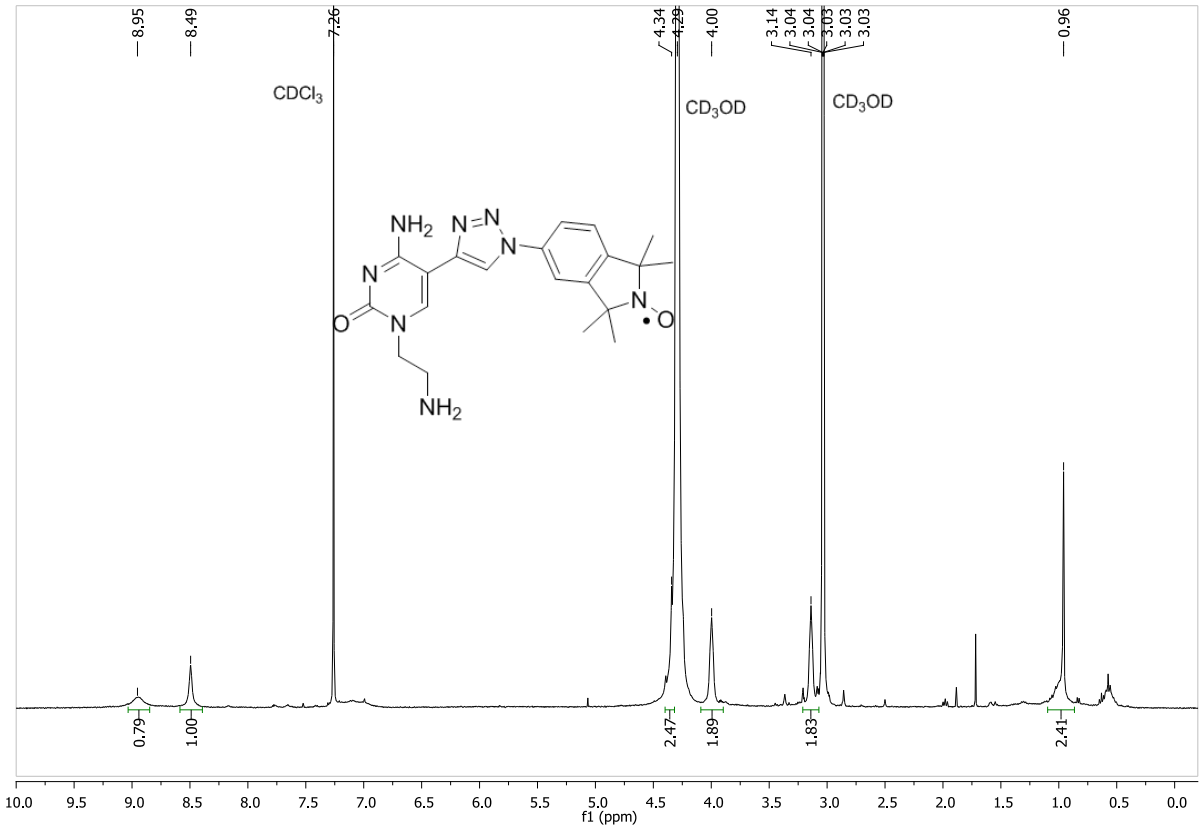


Spin label 10: A solution of **29** (30 mg, 0.05 mmol) in MeOH (20 mL) was hydrogenated over 10% Pd/C (10 mg) for 2 h at 30 psi. The resulting reaction mixture was filtered through a celite bed and the solvent was removed *in vacuo*. The residue was dissolved in MeOH (10 mL) and treated with $Cu(OAc)_2 \bullet H_2O$ (5 mg, 0.002 mmol) and stirred for 1 h at 24 °C. The reaction mixture was concentrated and the residue was purified by preparative TLC using 10% MeOH in CH_2Cl_2 containing 1% aq. NH_3 to yield **10** as a white solid (20 mg, 75% yield).

^{1}H NMR ($CDCl_3$, 10% CD_3OD): δ 0.96 (bs, 1H), 3.14 (bs, 2H, C*H*$_2$), 4.00 (bs, 2H, C*H*$_2$), 4.34 (bs, 2H, C*H*$_2$), 8.49 (bs, 1H, ArC*H*), 8.95 (s, 1H, ArC*H*) ppm.

HR-ESI-MS: Calculated for $C_{20}H_{25}N_8O_2$ 409.2100 found *m/z* 410.2173 $[M + 1]^+$.

^{1}H NMR spectrum of **10:**



References

[1] S. A. Shelke, S. T. Sigurdsson, *ChemBioChem* **2012**, *13*, 684-690.