Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

For

Rapid and Efficient Tetrazine-Induced Drug Release from Highly Stable Benzonorbornadiene Derivatives

Minghao Xu, Julian Tu and Raphael M. Franzini*

Department of Medicinal Chemistry, College of Pharmacy, University of Utah, Salt Lake City, 84112, USA

E-mail: Raphael.franzini@utah.edu

Table of Contents

Figures

Materials and Instrumentation

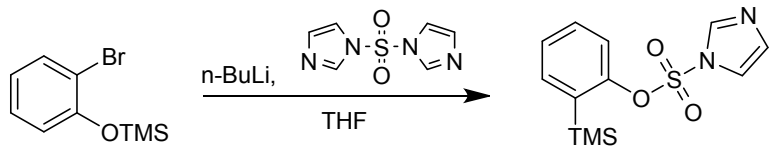
All chemical reagents and solvents were obtained from commercial sources (Sigma-Aldrich, Alfa-Aesar, Combi-Blocks, Acros-Organic, TCI) and used without further purification. Thin-layer chromatography (TLC) analysis was carried out to monitor the process of reactions. Purification of compounds was achieved by column chromatography with silica gel 300-400 mesh. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-400 spectrometer with chemical shifts expressed as ppm (in $CDCl_3$, MeOD-d_4 or DMSO-d_6) using Me_4Si (TMS) as internal standard.

Mass spectra were measured by the University of Utah Chemistry Mass Spectrometry Facility. All payload release and stability tests were performed by analytical reverse-phase HPLC (Thermo Scientific, USA) by using a LUNA C18 column (5 μM, 250×10 mm, Phenomenex, USA).

UV-VIS photospectrometic kinetic measurements were performed on a BioTek Synergy HT Microplate Reader (BioTek, USA) in a 96-well plate formate. Cell proliferation assays were performed on an Envision 2104 Multilabel Reader (PerkinElmer, USA).

Synthetic Procedures

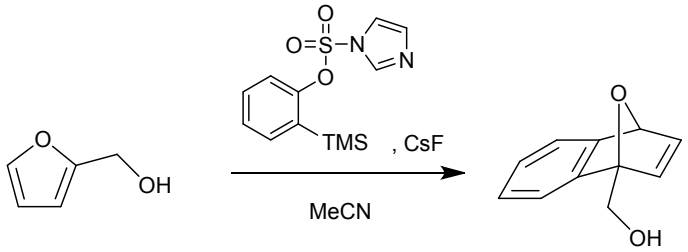
2-(Trimethylsilyl)phenyl imidazolsulfonate



2-(Trimethylsilyl)phenyl imidazolsulfonate was prepared according to a literature protocol.[1]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.54-7.53 (m, 1H), 7.39 (s, 1H), 7.32-7.22 (m, 3H), 6.50 (t, J = 4.4 Hz, 1H), 0.35 (s, 9H). The ^{1}H NMR data agreed with the reported spectrum of this compound.[1]

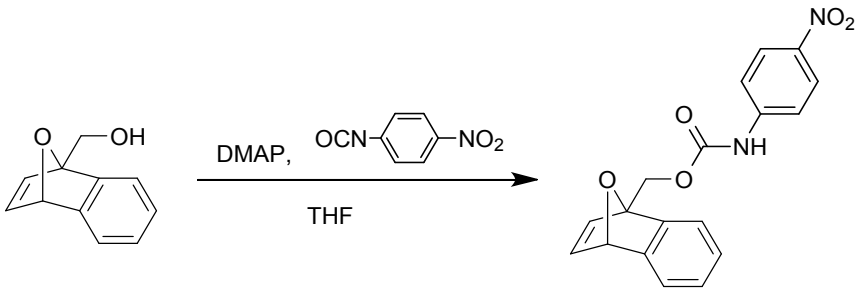
1,4-dihydro-1,4-epoxynaphthalen-1-yl)methanol (**1d**)



To a solution of 2-(trimethylsilyl)phenyl imidazolsulfonate (2.37 g, 8.0 mmol) and furan-2-ylmethanol (**1a**; 1.26 g, 12.8 mmol) in anhydrous MeCN (30 mL) was added CsF (2.43 g, 16 mmol). The reaction mixture was heated and maintained at 50 °C for 8 h. The mixture was extracted with EtOAc (250 mL) and washed with water (2×150 mL). The separated organic layer was again washed with brine (3×150 mL) and concentrated under reduced pressure. The crude was purified by column chromatography (hexane : EtOAc = 3:1, v/v) to give the desired product as yellow solid in a yield of 510 mg (36%; R_f = 0.35 in hexane : EtOAc = 1:1, v/v).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.22 (m, 1H), 7.18-7.16 (m, 1H), 7.05 (dd, J_1 = 1.6 Hz, J_2 = 5.6 Hz, 1H), 6.98-6.96 (m, 2H), 6.88 (d, J = 5.2 Hz, 1H), 5.70 (d, J = 1.6 Hz, 1H), 4.48-4.37 (m, 2H), 2.58 (t, d, J = 6.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.5, 147.8, 144.9, 142.4, 125.2, 125.1, 120.2, 119.6, 93.7, 82.2, 60.3. HRMS (ESI): calcd. for $C_{11}H_{10}O_2$ $[M+Na]^+$ 197.0578, found 197.0583.

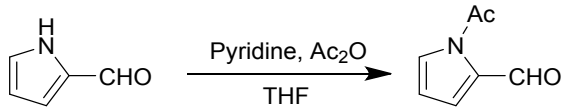
1,4-dihydro-1,4-epoxynaphthalen-1-yl)methyl (4-nitrophenyl)carbamate (**1**)



To a solution of **1d** (556 mg, 3.2 mmol) and DMAP (507 mg, 4.16 mmol) in anhydrous THF (40 mL) was added 4-nitrophenyl isocyanate (1.3 g, 8.0 mmol). The reaction mixture was stirred at 50 °C for 12 h. The mixture was diluted with EtOAc (150 mL) and washed with water (2×100 mL) and brine (3×150 mL). The separated organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography (hexane : EtOAc = 3:1, v/v) to afford the desired compound as a yellow solid in a yield of 180 mg (29%; R_f = 0.6 in hexane : EtOAc = 1:1, v/v).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 9.2 Hz, 2H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.31-7.28 (m, 1H), 7.22-7.20 (m, 1H), 7.16-7.13 (m, 2H), 7.03-7.01 (m, 2H), 6.89 (d, *J* = 5.6 Hz, 1H), 5.77 (d, *J* = 1.6 Hz, 1H), 5.16 (d, *J* = 12.8 Hz, 1H), 5.02 (d, *J* = 13.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.4, 149.8, 147.1, 145.3, 143.5, 141.6, 125.5, 125.3, 125.2, 120.5, 119.4, 117.8, 91.1, 82.4, 62.4. HRMS (ESI): calcd. for $C_{18}H_{14}N_2O_5$ $[M+Na]^+$ 361.0800, found 361.0804.

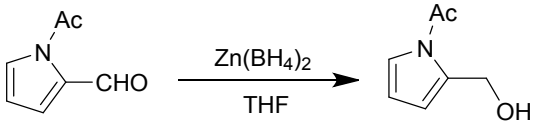
1-acetyl-1H-pyrrole-2-carbaldehyde



1-acetyl-1H-pyrrole-2-carbaldehyde was prepared according to a literature protocol.[2]

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.29 (s, 1H), 7.33 (dd, J_1 = 1.6 Hz, J_2 = 2.8 Hz, 1H), 7.21 (dd, J_1 = 1.6 Hz, J_2 = 4.0 Hz, 1H), 6.35 (t, J = 3.2 Hz, 1H), 2.66 (s, 3H). The ^{1}H NMR data agreed with the reported spectrum of this compound.[2]

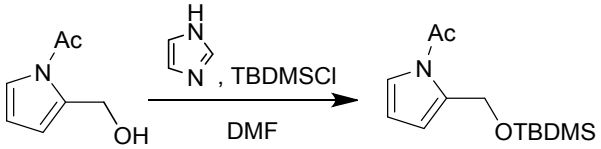
1-(2-(hydroxymethyl)-1H-pyrrol-1-yl)ethanone (**2a**)



1-(2-(hydroxymethyl)-1H-pyrrol-1-yl)ethanone (**2a**) was prepared according to a literature protocol.[3]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, J_1 = 1.6 Hz, J_2 = 3.2 Hz, 1H), 6.21-6.19 (m, 2H), 4.61 (s, 2H), 2.57 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 135.4, 121.7, 114.7, 112.2, 57.8, 23.7. The ^{1}H NMR data agreed with the reported spectrum of this compound. [3]

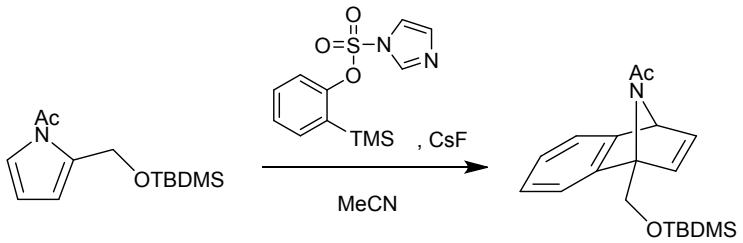
1-(2-(((tert-butyldimethylsilyl)oxy)methyl)-1H-pyrrol-1-yl)ethanone (**2b**)



To a solution of **2a** (1.2 g, 8.6 mmol) and imidazole (877 mg, 12.9 mmol) in anhydrous DMF (10 mL) was added *tert*-butylchlorodimethylsilane (1.52 g, 10.3 mmol) at 0 °C. The reaction mixture was warmed to room temperature and kept at this temperature for 3 h. The reaction was quenched with sat. aq. $NaHCO_3$ solution (200 mL), diluted with water (150 mL) and extracted with EtOAc (2×150 mL). The combined organic layers were washed with brine (150 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (hexane : EtOAc = 20:1, v/v) to give the product as a brown oil in a yield of 1.25 g (58%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06-7.04 (m, 1H), 6.31-6.30 (m, 1H), 6.22 (t, *J* = 3.2 Hz, 1H), 4.94 (brs, 2H), 2.53 (s, 3H), 0.93 (s, 9H), 0.09 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 136.9, 120.3, 112.0, 111.5, 60.7, 25.9, 23.5, 18.4, -5.4. HRMS (ESI): calcd. for $C_{13}H_{23}NO_2Si$ $[M+Na]^+$ 276.1396, found 276.1398.

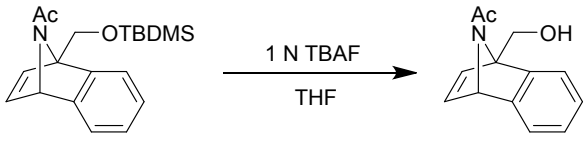
1-(((tert-butyldimethylsilyl)oxy)methyl)-1,4-dihydro-1,4-epiminonaphthalen-9-yl)ethanone (**2c**)



To a solution of 2-(trimethylsilyl)phenyl imidazolsulfonate (0.98 g, 3.3 mmol) and **2b** (1.25 g, 5 mmol) in anhydrous MeCN (20 mL) was added CsF (1 g, 6.6 mmol). The reaction mixture was heated and maintained at 60 °C for 12 h. The mixture was diluted with EtOAc (150 mL) and washed with water (2×50 mL). The separated organic layer was again washed with brine (2×150 mL) and concentrated under reduced pressure. The crude was purified by column chromatography (hexane : EtOAc = 2:1, v/v) to give the product as a brown oil in a yield of 350 mg (32%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.14 (brs, 1H), 7.00-6.93 (m, 3H), 5.41 (s, 1H), 4.96 (d, *J* = 8.0 Hz, 1H), 4.68 (d, *J* = 8.0 Hz, 1H), 1.93 (s, 3H), 0.96 (s, 9H), 0.21 (s, 3H), 0.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 151.1, 148.5, 145.5, 141.2, 125.2, 124.7, 121.2, 119.7, 77.1, 67.1, 60.9, 25.9, 22.7, 18.2, -5.3, -5.4, -5.5. HRMS (ESI): calcd. for $C_{19}H_{27}NO_2Si$ $[M+Na]^+$ 352.1709, found 352.1716.

1-(hydroxymethyl)-1,4-dihydro-1,4-epiminonaphthalen-9-yl)ethanone (**2d**)

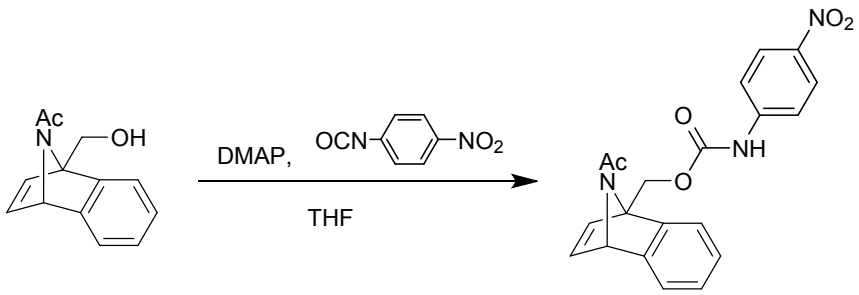


To a solution of **2c** (350 mg, 1.1 mmol) in THF (4 mL) was added 1 M tetra n-butyl ammonium fluoride (2 mL. 2 mmol) at room temperature, and the mixture was stirred for 2 h. The mixture was diluted with Et_2O/EA mixture (100 mL, 1:1) and washed with water (50 mL) and brine (2×50 mL) and the separated organic layer was dried with Na_2SO_4, and concentrated under reduced

pressure. The crude product was purified by column chromatography (hexane : EtOAc = 10:1, v/v) to afford the product as a brown oil in a yield of 200 mg (85%; R_f = 0.25 in hexane : EtOAc = 1:1, v/v).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.26 (m, 2H), 7.08-7.06 (m, 1H), 7.04-7.00 (m, 2H), 6.93 (d, *J* = 5.6 Hz, 1H), 5.51 (d, *J* = 2.4 Hz, 1H), 5.30-5.26 (m, 1H), 4.59 (dd, J_1= 3.2 Hz, J_2= 7.2 Hz, 2H), 2.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 148.2, 147.8, 145.4, 143.3, 125.5, 125.3. 120.5, 120.2, 79.0, 66.8, 58.0, 22.3. HRMS (ESI): calcd. for $C_{13}H_{13}NO_2$ $[M+Na]^+$ 238.0844, found 238.0842.

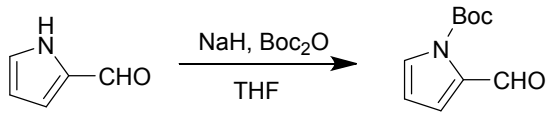
9-acetyl-1,4-dihydro-1,4-epiminonaphthalen-1-yl)methyl (4-nitrophenyl)carbamate (**2**)



To a solution of **2d** (200 mg, 0.9 mmol) and DMAP (146 mg, 1.2 mmol) in anhydrous THF (12 mL) was added 4-nitrophenyl isocyanate (300 mg, 2 mmol). The reaction mixture was stirred at 50 °C for 10 h. The mixture was diluted with EtOAc (150 mL) and washed with water (200 mL) and brine (2×150 mL). The separated organic layer was dried with Na_2SO_4, and concentrated under reduced pressure, purified by column chromatography (hexane : EtOAc = 5:1, v/v) to afford the desired compound as yellow solid in a yield of 70 mg (22%; R_f = 0.15 in hexane : EtOAc = 1:1, v/v).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.22 (d, *J* = 9.6 Hz, 2H), 7.76 (d, *J* = 9.6 Hz, 2H), 7.37-7.35 (m, 2H), 7.18-7.16 (m, 1H), 7.06 (d, *J* = 5.6 Hz, 1H), 7.01-6.99 (m, 2H), 5.84 (d, *J* = 2.4 Hz, 1H), 5.40-5.30 (m, 2H), 1.89 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.3, 153.6, 149.7, 149.3, 146.1, 144.9, 143.9, 142.2, 125.5, 125.2, 121.0, 120.6, 118.2, 75.3, 67.0, 62.2, 23.4. HRMS (ESI): calcd. for $C_{20}H_{17}N_3O_5$ $[M+Na]^+$ 402.1066, found 402.1075.

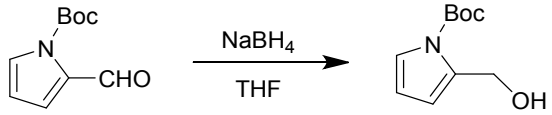
Tert-butyl 2-formyl-1H-pyrrole-1-carboxylate



Tert-butyl 2-formyl-1H-pyrrole-1-carboxylate was prepared according to a literature protocol.[4]

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.23 (s, 1H), 7.34 (brs, 1H), 7.06 (brs, 1H), 6.18 (t, *J* = 4.0 Hz, 1H), 1.55 (s, 9H). The ^{1}H NMR data agreed with the reported spectrum of this compound.[4]

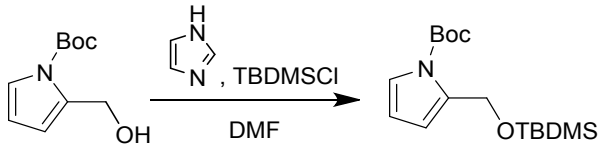
Tert-butyl 2-(hydroxymethyl)-1H-pyrrole-1-carboxylate (**3a**)



Tert-butyl 2-(hydroxymethyl)-1H-pyrrole-1-carboxylate (**3a**) was prepared according to a literature protocol.[5]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, *J* = 1.2 Hz, 1H), 6.18 (brs, 1H), 6.10 (brs, 1H), 4.64 (d, *J* = 7.6 Hz, 2H), 3.60 (d, *J* = 7.2 Hz, 1H), 1.61 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.0, 134.8, 121.9, 113.6, 110.4, 84.5, 57.7, 28.0. The ^{1}H and ^{13}C NMR data agreed with the reported spectrum of this compound. [5]

Tert-butyl 2-(((tert-butyldimethylsilyl)oxy)methyl)-1H-pyrrole-1-carboxylate (**3b**)

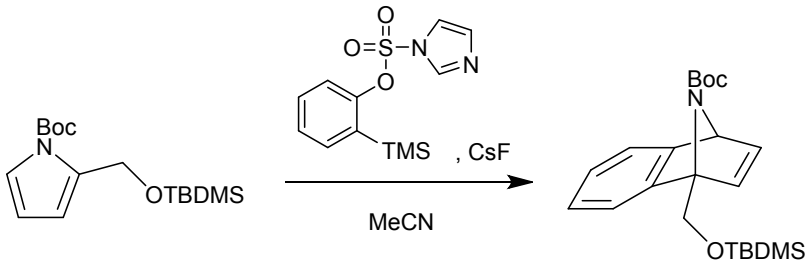


To a solution of **3a** (7.0 g, 35.5 mmol) and imidazole (3.6 g, 53 mmol) in anhydrous DMF (30 mL) was added *tert*-butylchlorodimethylsilane (6.5 g, 44 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was extracted with DCM (300 mL), quenched with sat. aq. $NaHCO_3$ solution (150 mL), and washed with water (2×200 mL). The combined organic layers were washed with brine (2×200 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (hexane : EtOAc = 20:1, v/v) to give the product as a brown oil in a yield of 8.8 g (80%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (t, *J* = 2.4 Hz, 1H), 6.23 (brs, 1H), 6.13 (t, *J* = 3.2Hz, 1H),

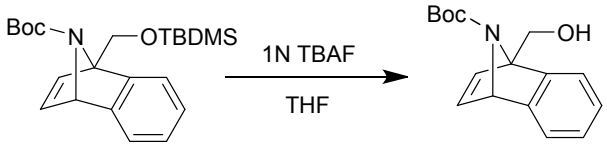
4.89 (s, 2H), 1.59 (s, 9H), 0.93 (s, 9H), 0.09 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.2, 135.4, 120.9, 111.0, 110.3, 83.5, 60.2, 27.9, 25.9, 18.4, -5.4. HRMS (ESI): calcd. for $C_{16}H_{29}NO_3Si$ $[M+Na]^+$ 334.1814, found 334.1815.

tert-butyl 1-(((tert-butyldimethylsilyl)oxy)methyl)-1,4-dihydro-1,4-epiminonaphthalene-9-carboxylate (**3c**)



To a solution of 2-(trimethylsilyl)phenyl imidazolsulfonate (4.5 g, 15 mmol) and **3b**, (7.2 g, 22.8 mmol) in anhydrous MeCN (60 mL) was added CsF (4.7 g, 30 mmol). The reaction was maintained at 60 °C for 12 h. The mixture was diluted with EtOAc (150 mL) and washed with water (100 mL). The separated organic layer was washed with brine (2×100 mL), dried with Na_2SO_4 and concentrated by reduced pressure. The crude was purified by column chromatography (hexane : EtOAc = 100:1 to 50:1, v/v) to give the product as a brown oil in a yield of 1.2 g (20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 6.0 Hz, 1H), 7.22 (d, *J* = 6.0 Hz, 1H), 7.06 (d, *J* = 5.2 Hz, 1H), 6.95 (t, *J* = 6.0 Hz, 3H), 5.44 (s, 1H), 4.78 (brs, 1H), 4.55 (d, *J* = 9.6 Hz, 1H), 1.32 (s, 9H), 0.96 (s, 9H), 0.20 (s, 3H), 0.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.8, 150.5, 149.5, 144.4, 141.6, 141.5, 124.9, 124.6, 120.9, 120.8, 120.5, 80.5, 67.4, 61.4, 29.7, 28.1, 25.9, 18.3, -5.3, -5.4. HRMS (ESI): calcd. for $C_{22}H_{33}NO_3Si$ $[M+Na]^+$ 410.2127, found 410.2138.

tert-butyl 1-(hydroxymethyl)-1,4-dihydro-1,4-epiminonaphthalene-9-carboxylate (**3d**)

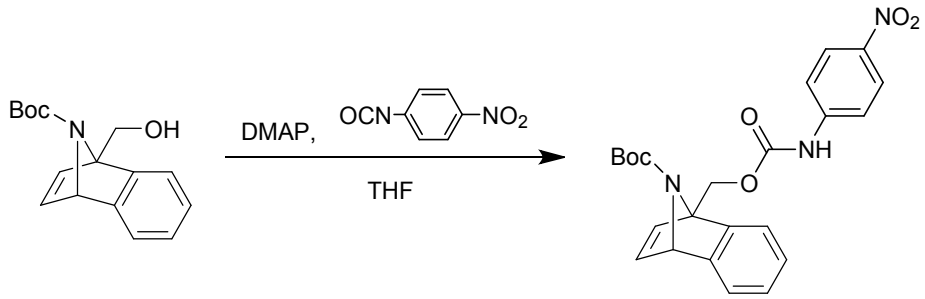


To a solution of **3c** (1.1 g, 3.0 mmol) in anhydrous THF (7 mL) was added 1 M tetra n-butyl ammonium fluoride (4.8 mL, 4.8 mmol) at room temperature and the mixture was stirred for 8 h. The mixture was extracted with Et_2O/EtOAc mixture (50 + 50 mL) and washed with water (2×50

mL). The separated organic layer was again washed with brine (2×50 mL), dried with Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography (hexane : EtOAc = 10:1, v/v) to afford the product as a brown oil in a yield of 0.7 g (85%; R_f = 0.7 in hexane : EtOAc = 1:1, v/v).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (t, *J* = 5.2 Hz, 2H), 7.04-6.98 (m, 3H), 6.88 (d, *J* = 5.2 Hz, 1H), 5.51 (brs, 1H), 4.58 (d, *J* = 6.8 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.8, 148.1, 144.4, 144.0, 125.1, 125.0, 120.5, 120.1, 81.6, 78.6, 66.9, 58.9, 28.2, 25.6. HRMS (ESI): calcd. for $C_{16}H_{19}NO_3$ $[M+Na]^+$ 296.1263, found 296.1267.

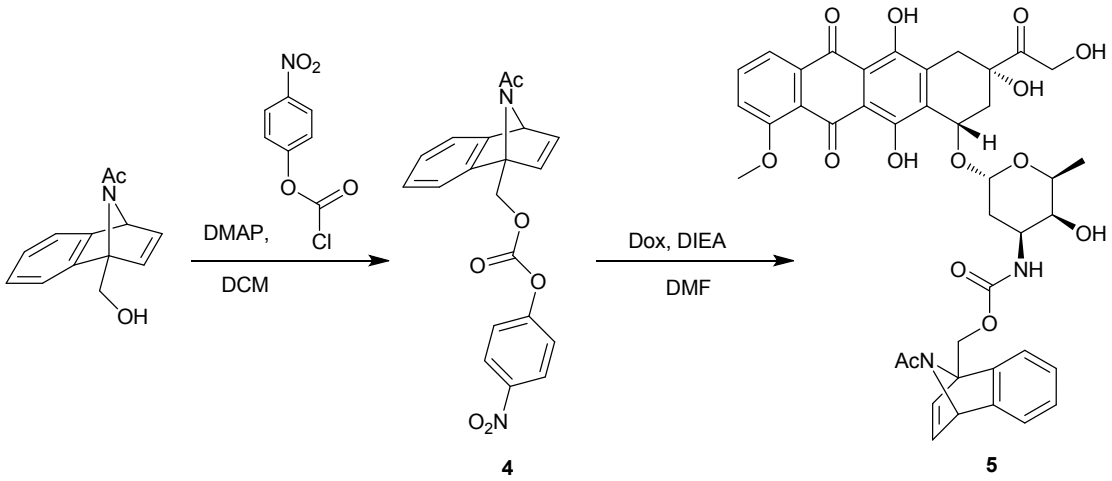
tert-butyl 1-((((4-nitrophenyl)carbamoyl)oxy)methyl)-1,4-dihydro-1,4-epiminonaphthalene-9-carboxylate (**3**)



To a solution of **3d** (408 mg, 1.5 mmol) and DMAP (244 mg, 2 mmol) in THF (25 mL) was added 4-nitrophenyl isocyanate (450 mg, 3 mmol). The reaction was stirred at 50 °C for 10 h. The mixture was diluted with EtOAc (150 mL) and washed with water (200 mL) and brine (2×150 mL). The separated organic layer was dried with Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (hexane : EtOAc = 5 :1, v/v) to afford the desired compound as a yellow solid in a yield of 405 mg (62%; R_f = 0.8 in hexane : EtOAc = 1:1, v/v

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 9.2 Hz, 2H), 7.57 (d, *J* = 9.2 Hz, 2H), 7.31-7.29 (m, 1H), 7.24-7.22 (m, 2H), 7.08-7.06 (m, 1H), 7.03-6.98 (m, 2H), 6.84 (d, *J* = 5.6 Hz, 1H), 5.55 (d, *J* = 2.0 Hz, 1H), 5.40 (brs, 2H), 1.36 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.8, 152.6, 148.6, 147.9, 143.9, 142.9, 126.3, 125.4, 125.2, 125.1, 121.1, 119.8, 117.7, 81.4, 75.6, 67.4, 62.2, 28.1. HRMS (ESI): calcd. for $C_{23}H_{23}N_3O_6$ $[M+Na]^+$ 460.1485, found 460.1495.

Doxorubicin-containing Prodrug (**5**)



To a solution of **2d** (53 mg, 0.24 mmol) in dry DCM (6 mL) was added DMAP (0.5 mmol, 70 mg) and nitrophenyl chloroformate (71 mg, 0.35 mmol) at 0 ^{o}C. The reaction was kept in the dark at 25 ^{o}C overnight. The reaction mixture was quenched with ice and extracted with DCM (2×20 mL). The combined organic layer was washed with water (3×50 mL) and brine (3×50 mL) until no more yellow color was observed in the organic phase, dried over Na_2SO_4 and concentrated to afford the carbonate intermediate (**4**) as light yellow solid in a yield of 65 mg (64%). **4** decomposed upon storage and was immediately used in the next step.

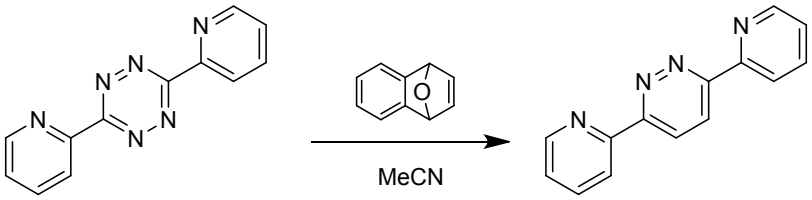
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (dd, J_1 = 2.0 Hz, J_2 = 6.8 Hz, 2H), 7.44 (dd, J_1 = 2.0 Hz, J_2 = 6.8 Hz, 2H), 7.31-7.26 (m, 2H), 7.10-7.08 (m, 1H), 7.04-6.99 (m, 3H), 5.65-5.62 (m, 1H), 5.67 (s, 1H), 5.53 (d, J = 7.6 Hz, 1H), 1.99 (s, 3H).

To a solution of **4** (64 mg, 0.17 mmol) in dry DMF (0.5 mL) was added DIEA (272 mg, 2.1 mmol), after 15 min, doxorubicin hydrochloride (120 mg, 0.2 mmol) was added and the reaction mixture was stirred in the dark and at 25 ^{o}C for 24 h. The mixture was diluted with DCM (100 mL) and washed with H_2O (50 mL) and brine (2×50 mL). The organic phase was concentrated and purified by preparatory-TLC (DCM : MeOH = 15:1, v/v) to afford the desired compound as red solid in a yield of 40 mg (30%; R_f = 0.5 in DCM : MeOH = 10:1, v/v).

^{1}H NMR (400 MHz, $CDCl_3$:MeOD-d_4 = 9:1) δ 7.97 (dd, J_1 = 1.2 Hz, J_2 = 8.0 Hz, 1H), 7.73 (t, J = 8.0 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.17 (brs, 2H), 6.95-6.90 (m, 2H), 6.81 (brs, 1H), 6.74 (brs, 1H), 5.42 (brs, 2H), 5.23 (d, J = 15.2 Hz, 2H), 5.11 (d, J = 11.6 Hz, 1H), 4.71 (s, 2H), 4.08 (d, J = 6.4 Hz, 1H), 4.01 (s, 3H), 3.79 (d, J = 12.0 Hz, 1H), 3.62 (s, 1H), 3.20 (d, J = 18.8 Hz, 1H), 2.98 (d, J = 18.8 Hz, 1H), 2.30 (d, J = 15.2 Hz, 1H), 2.09 (d, J = 15.2 Hz, 1H), 1.89 (d, J = 4.0 Hz, 3H),

1.80-1.74 (m, 2H), 1.23 (d, J = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 214.3, 186.9, 186.8, 169.3, 161.2, 156.6, 155.7, 154.9, 150.3, 144.7, 143.5, 136.6, 135.9, 135.1, 134.5, 125.3, 125.1, 120.9, 120.6, 120.4, 120.2, 119.4, 111.2, 111.0, 100.8, 75.4, 70.3, 68.4, 67.1, 67.0, 64.2, 61.3, 57.0, 55.4, 47.7, 37.0, 32.5, 30.3, 23.2, 17.5. HRMS (ESI): calcd. for $C_{41}H_{40}N_2O_{14}$ $[M+Na]^+$ 807.2377, found 807.2383.

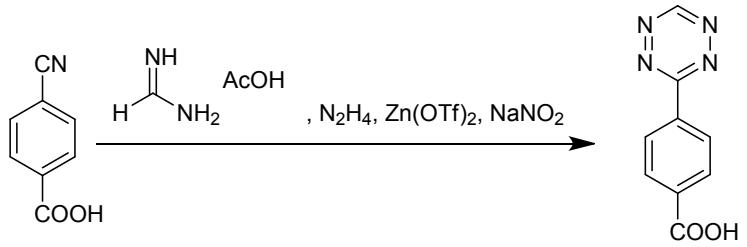
3,6-di(pyridin-2-yl)pyridazine (**DPPz**)



To a solution of 1,4-epoxynaphthalene (72 mg, 0.5 mmol) in MeCN (12 mL) was added a solution of 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (118 mg, 0.5 mmol) in MeCN (12 mL). The reaction was stirred at 40 °C for 4 h. The mixture was concentrated and purified by column chromatography (hexane : EtOAc = 1:1, v/v) to afford the desired compound as a pale solid in a yield of 60 mg (49%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (d, J = 3.6 Hz, 2H), 8.69 (s, 2H), 8.65 (d, J = 6.4 Hz, 2H), 8.08 (t, J = 6.4 Hz, 2H), 7.61-7.59 (m, 2H). The ^{1}H NMR agreed with the reported spectrum of this compound.[6]

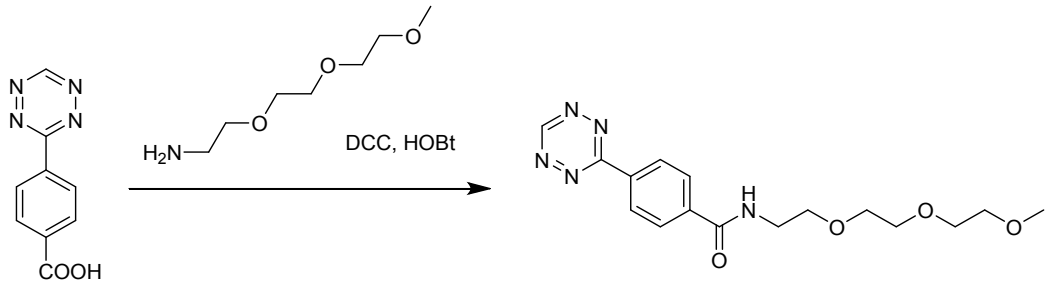
4-(1,2,4,5-tetrazin-3-yl)benzoic acid



4-(1,2,4,5-tetrazin-3-yl)benzoic acid was synthesized according to a literature protocol.[7]

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 8.64-8.57 (m, 2H), 8.24-8.17 (m, 2H). The ^{1}H NMR data agreed with the reported spectrum of this compound. [7]

N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-4-(1,2,4,5-tetrazin-3-yl) benzamide (**PEG-Tz**)



To a dry round-bottom flask was added 2-(2-(2-methoxyethoxy)ethoxy)ethan-1-amine (190 mg, 1.19 mmol) in THF (0.5 mL), dicyclohexylmethanediimine (230 mg, 1.09 mmol) in THF (1.0 mL), and hydroxybenzotriazole (170 mg, 1.09 mmol) in THF (1 mL). The solution was cooled to 0 ^{o}C in an ice bath and stirred. 4-(1,2,4,5-tetrazin-3-yl)benzoic acid (200 mg, 0.99 mmol) in THF (2.5 mL) was added to the flask while allowing the reaction mixture to return to room temperature and the resultant red reaction mixture was stirred for 20 h. After the allotted time, the reaction was diluted with 10 mL of diethyl ether to precipitate out the dicyclohexylurea byproduct, which was removed by filtration; the solution was subsequently washed with diethyl ether (2×5 mL), filtered again, and concentrated. The mixture was directly concentrated and purified by column chromatography (DCM : Acetone = 10:1, v/v) to afford the desired compound as a pink solid in a yield of 80 mg (20%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.27 (s, 1H), 8.75-8.67 (m, 2H), 8.09-8.01 (m, 2H), 7.00 (s, 1H), 3.77-3.62 (m, 9H), 3.58-3.51 (m, 2H), 3.33 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 165.9, 157.9, 138.7, 134.0, 128.4, 128.1, 71.9, 70.6, 70.5, 70.2, 69.7, 39.9. HRMS (ESI): calcd. for $C_{16}H_{21}N_5O_4$ $[M+Na]^+$ 370.1491, found 370.1496.

Photospectrometric Analysis of Reaction Kinetics

The reaction was monitored at 525 nm, which is a local absorbance maximum of tetrazine. All kinetic experiments were run in triplicates. Pseudo-first order curve fitting was performed with Origin 8.0 software using the exponential formula: $y = A_1 \times e^{kx} + y_0$.

Kinetics in DMSO and DMSO/H_2O (9:1, v/v)

Stock solutions of benzonorbornadiene derivatives **1-3** (60 mM or 12.5 mM) and tetrazine (15 mM or 2.5 mM) in DMSO were prepared. Final solutions containing tetrazine (2 mM or 0.25 mM) and **1-3** (20 mM, 30 mM, 40 mM and 50 mM or 2.5 mM, 3.75 mM, 5 mM, 6.25 mM) were prepared in 96-well plates and thoroughly mixed at 37 ^{o}C for UV-Vis measurements.

Kinetics in DMSO/PBS (3:2, v/v)

Final solutions containing tetrazine (0.05 mM) and **1-2** (0.5 mM), tetrazine alone (0.05 mM) were prepared in 96-well plates and thoroughly mixed at 37 ^{o}C for UV-Vis measurements.

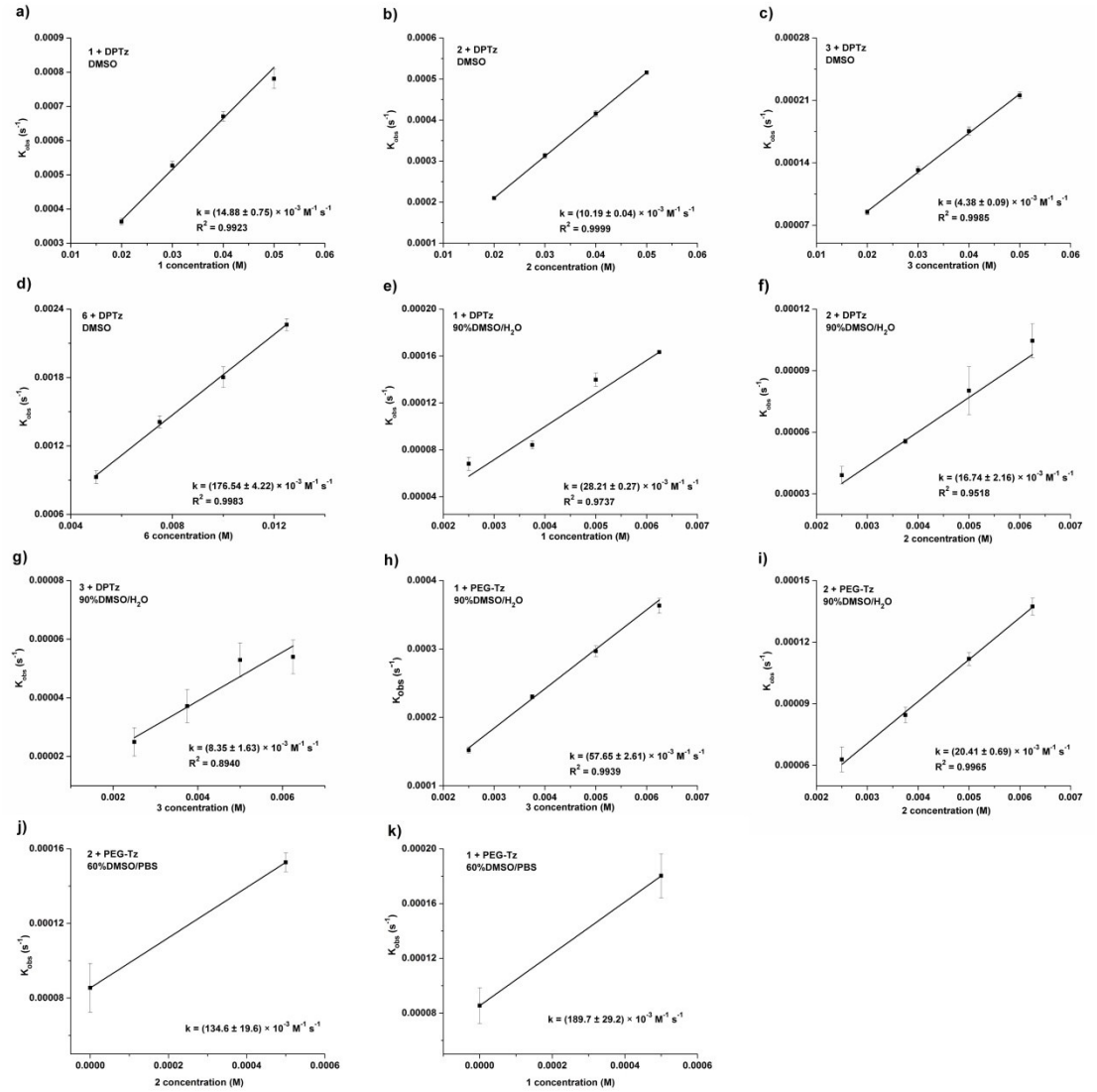


Figure S1. Second-order rate constants k_2 of the reactions with the benzonorbornadiene derivatives **1-3** and the tetrazines DPTz and PEG-Tz determined from plots of pseudo-first order k_{obs} versus concentration of **1-3.** The results are expressed as the mean ± standard deviation (n = 3).

Color change resulting from the reaction of 1 and DPTz

Stock solutions of **1** (12 mM) and DPTz (12 mM) in DMSO were prepared. Aliquots of the DPTz stock solution (500 μL) was added with DMSO (500 μL); Solution of **1** (500 μL) was added with DPTz stock solution (500 μL). Solution of **1** (500 μL) was added with DMSO (500 μL). The samples were incubated at 37 ^{o}C and the image was recorded at the time point of 24 h, shown as insert in **Figure 1**.

Analysis of Release by HPLC

Stock solutions of probes **1-3** (24 mM) and tetrazine (24 mM) in DMSO were prepared. Aliquots of the benzonorbornadiene derivatives **1-3** stock solution (125 μL) and tetrazine stock solution (375 μL) were combined to give final concentrations of 6 mM for the **1-3** and 18 mM for the DPTz. Samples were incubated at 37 °C and aliquotes were taken at five time points (5 min, 30 min, 2 h, 6 h and 24 h) and diluted by 25-fold with MeCN to quench the reaction and analyzed by HPLC monitoring. (Blue line: 317 nm channel; Red line: 378 nm channel)

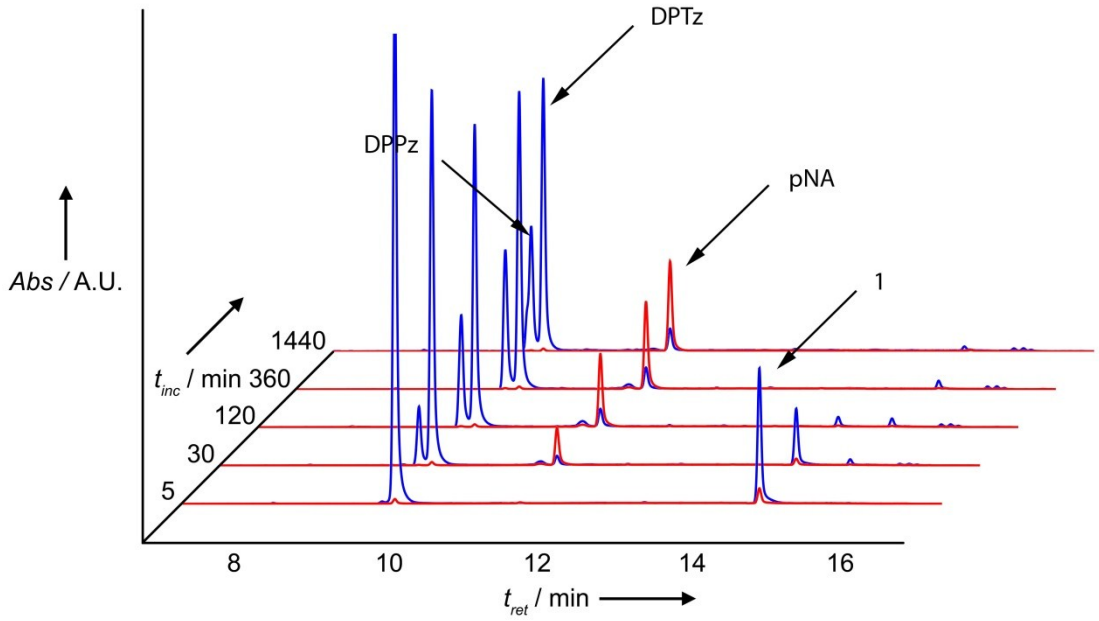


Figure S2. Analysis of reaction between **1** and DPTz at different time points. From left to right: DPPz, DPTz, pNA and **1** (Indicated with arrows). The mobile phase A was 0.1% TFA in water and mobile phase B was acetonitrile. A gradient of 0-100% B ranging from 1-15 min was run at a flow rate of 4.0 mL/min. Retention time for DPPz: 9.87-9.96 min; DPTz: 9.98-10.05 min; pNA: 11.71-11.81 min; **1**: 14.78-14.80 min.

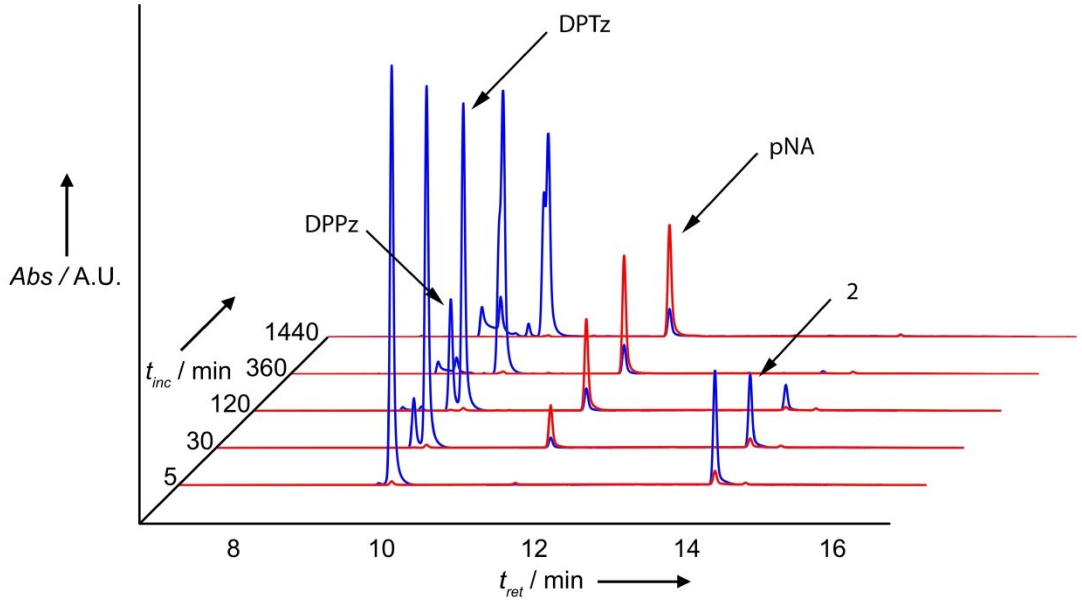


Figure S3. Analysis of reaction of **2** and DPTz at different time points. From left to right: DPPz, DPTz, pNA and **2** (Indicated with arrows). The mobile phase A was 0.1% TFA in water and mobile phase B was acetonitrile. A gradient of 0-100% B ranging from 1-15 min was run at a flow rate of 4.0 mL/min. Retention time for DPPz: 9.85-10.08 min; DPTz: 10.06-10.14 min; pNA: 11.68-11.76 min; **2**: 14.32-14.37 min.

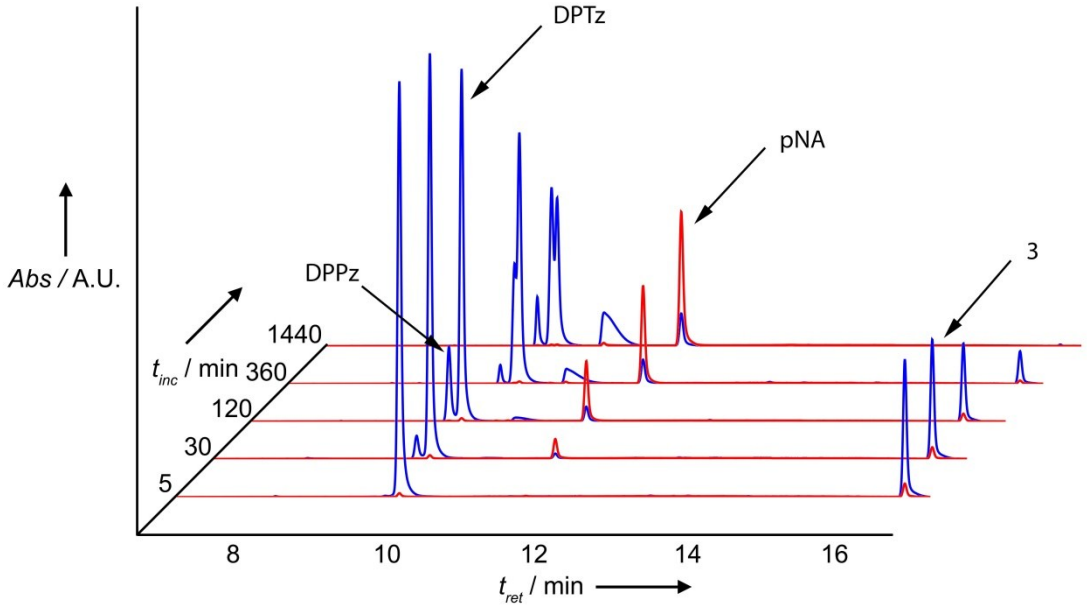


Figure S4. Release analysis of **3** with DPTz at different time points. From left to right: DPPz, DPTz, pNA and **3** (Indicated with arrows). The mobile phase A was 0.1% TFA in water and mobile phase B was acetonitrile. A gradient of 0-100% B ranging from 1-15 min was run at a flow rate of 4.0 mL/min. Retention time for DPPz: 9.83-10.18 min; DPTz: 10.00-10.23 min; pNA: 11.64-11.87 min; **3**: 16.64-16.89 min.

^{1}H NMR Mechanistic Studies and Release Products Distribution

Mechanistic studies in DMSO-d_6

Stock solutions of benzonorbornadiene derivatives **1-3** (24 mM) and DPTz (24 mM) in DMSO-d_6 were prepared. Aliquots of the benzonorbornadiene derivatives **1-3** stock solution (125 μL) and DPTz stock solution (375 μL) were combined to give final concentrations of 6 mM for the **1-3** and 18 mM for DPTz. 18-crown-6-ether or furan was added as internal standard for peak integration. The samples were incubated at 37 °C and ^{1}H NMR spectra recorded at the indicated time points (5 min, 30 min, 2 h, 6 h and 24 h) at 25 °C.

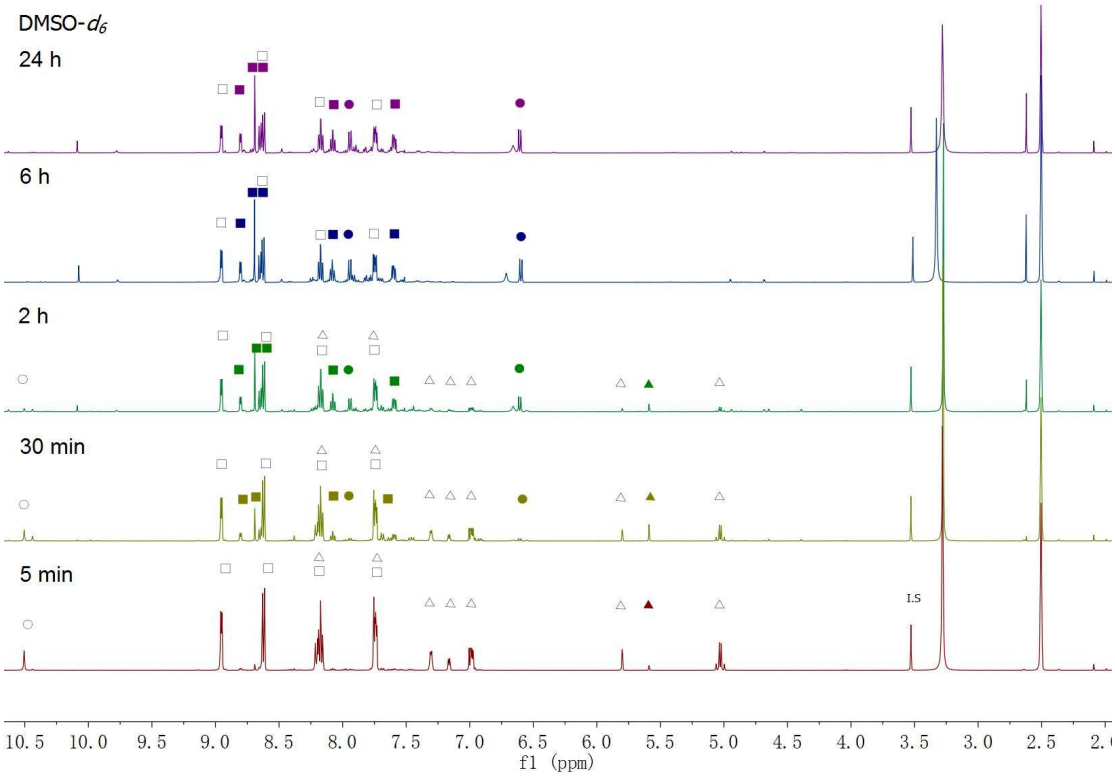


Figure S5. Full spectrum monitoring the bioorthogonal release reaction of **1** with **DPTz** in DMSO-d_6. Legend: **1**:△; **DPTz**:□; **pNA**:●; **DPPz**:■; **I3**:▲. I.S: Internal standard.

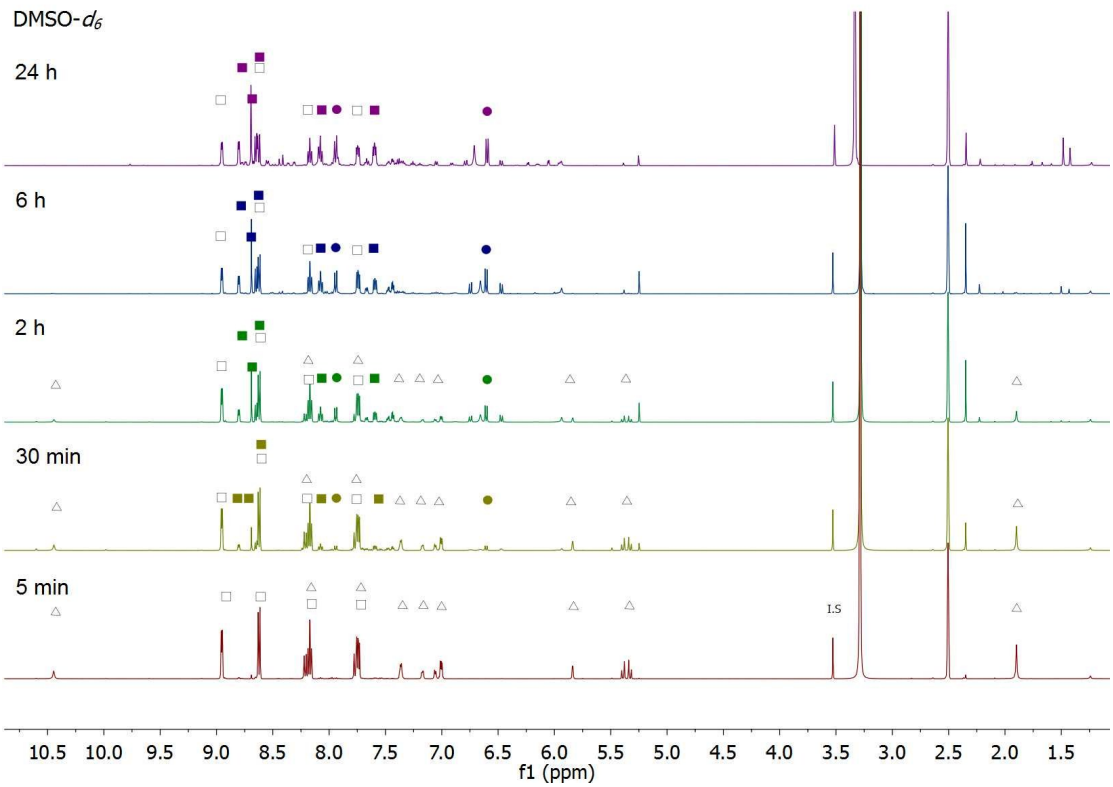


Figure S6. Full spectrum monitoring the bioorthogonal release reaction of **2** with **DPTz** in DMSO-d_6. Legend: **2**:△; **DPTz**:□; **pNA**:●; **DPPz**:■. I.S: Internal standard.

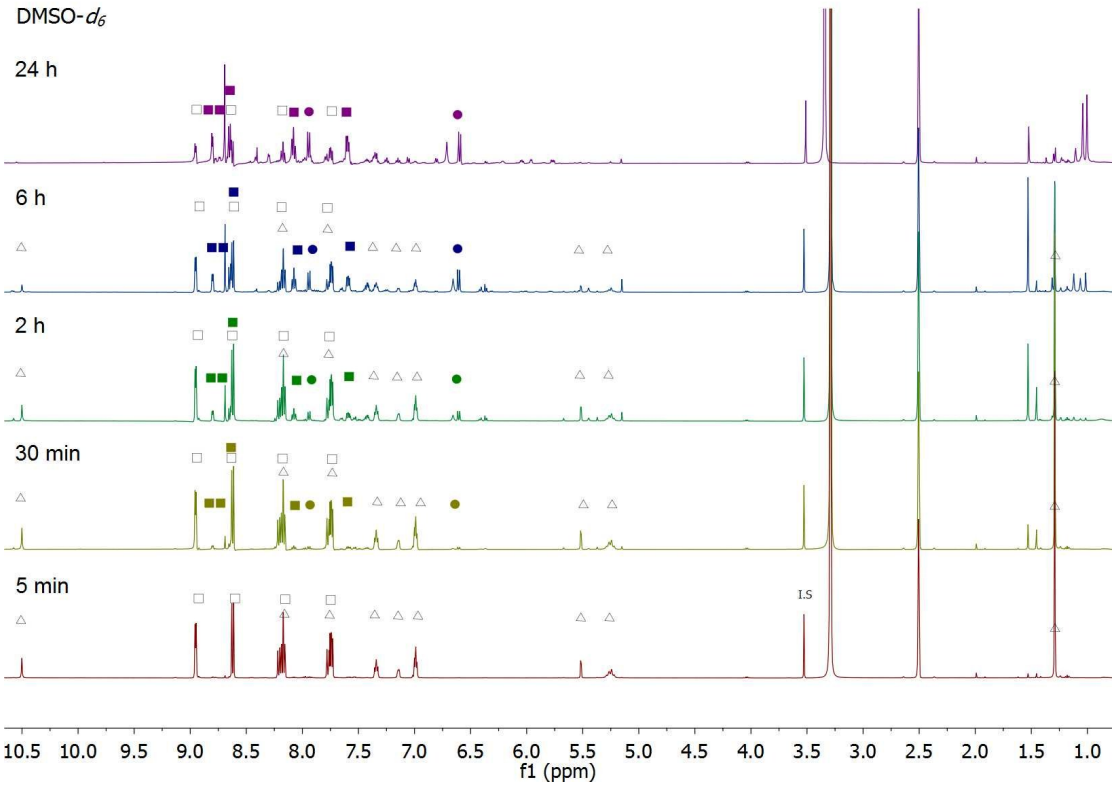


Figure S7. Full spectrum monitoring the bioorthogonal release reaction of **3** with **DPTz** in DMSO-d_6. Legend: **3**:△; **DPTz**:□; **pNA**:●; **DPPz**:■. I.S: Internal standard.

Mechanistic studies in DMSO-d_6/D_2O (9:1, v/v)

Stock solutions of benzonorbornadiene derivatives **2** (40 mM) and DPTz or PEG-Tz (24 mM) in DMSO-d_6 were prepared. Aliquots of **2** stock solution (75 μL), DPTz solution (375 μL) and D_2O (50 μL) were combined to give final concentrations of 6 mM for the **2** and 18 mM for DPTz. 18-crown-6-ether was added as internal standard for peak integration. The samples were incubated at 37 °C and ^{1}H NMR spectra recorded at the indicated time points (5 min, 30 min, 2 h, 6 h and 24 h) at 25 °C.

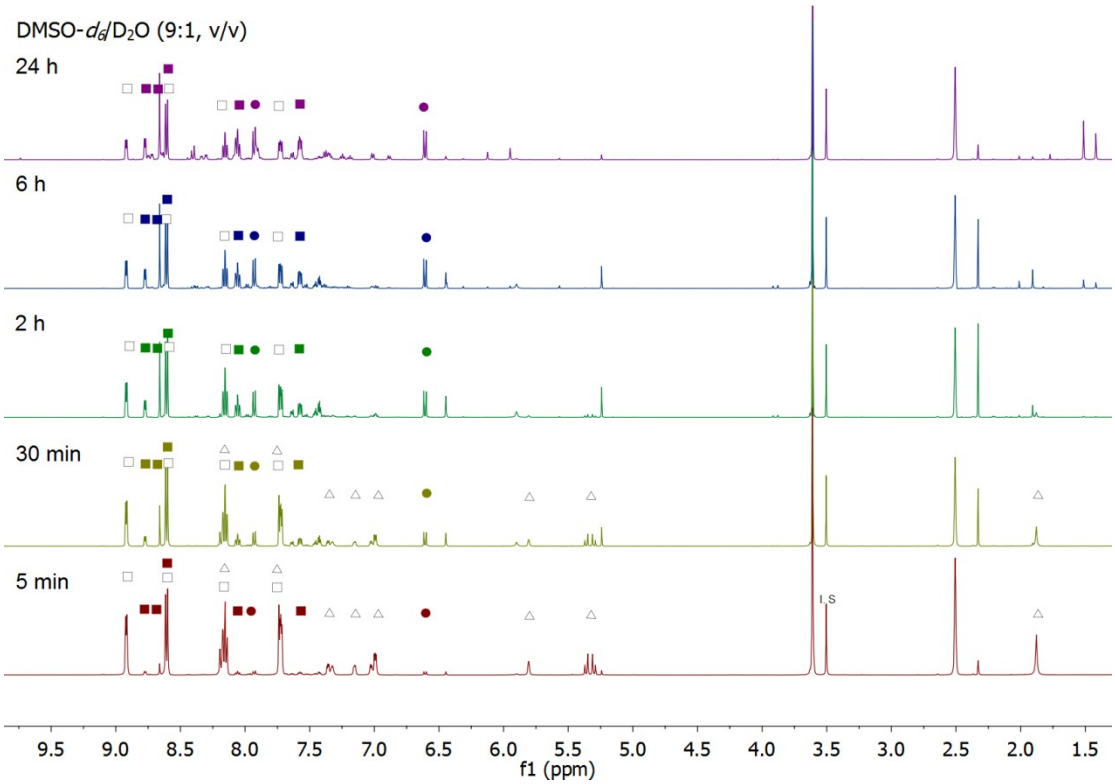


Figure S8. Full spectrum monitoring the bioorthogonal release reaction of **2** with **DPTz** in DMSO-d_6/D_2O (9:1, v/v). Legend: **2**:△; **DPTz**:□; **pNA**:●; **DPPz**:■. I.S: Internal standard.

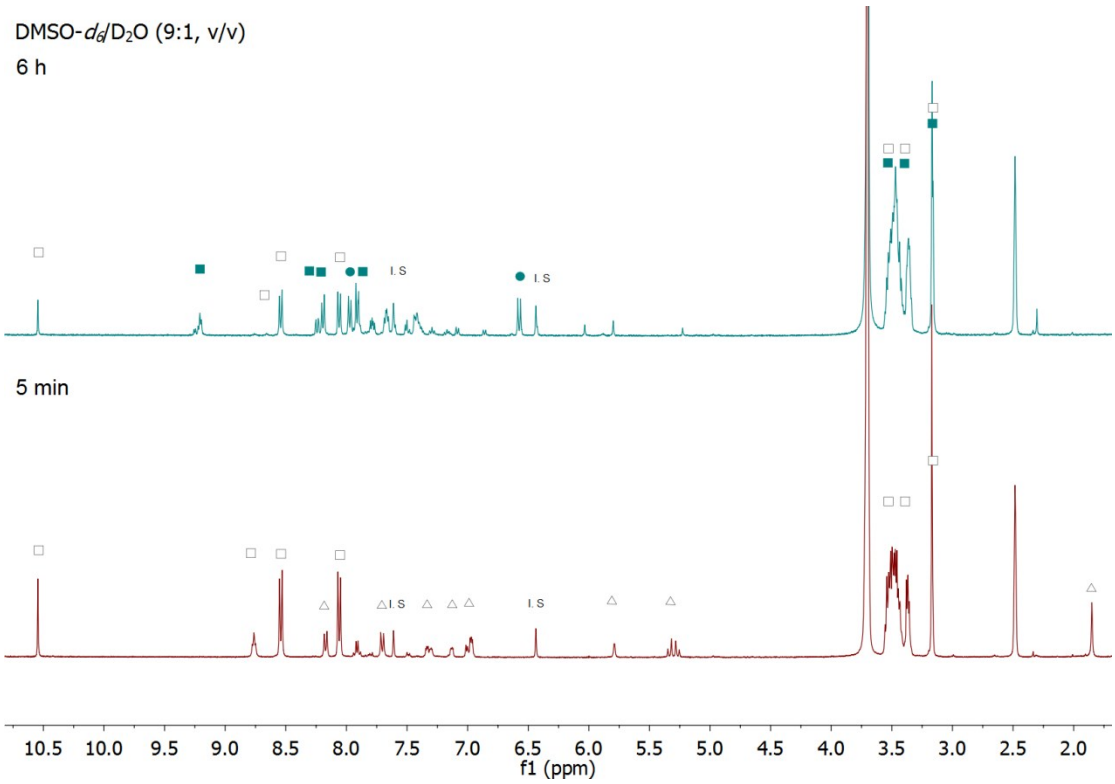


Figure S9. Full spectrum monitoring the bioorthogonal release reaction of **2** with **PEG-Tz** in DMSO-d_6/D_2O (9:1, v/v). Legend: **2**:△; **DPTz**:□; **pNA**:●; **DPPz**:■. I.S: Internal standard, furan.

Analysis of Product Distribution

Characteristic peaks in ^{1}H NMR of reaction mixtures were integrated in experiment performed as described for the ^{1}H NMR mechanistic studies in DMSO-d_6/D_2O (9:1, v/v). Data acquired by ^{1}H NMR monitoring at different time points (5 min, 30 min, 2 h, 6 h and 24 h) in triplicates.

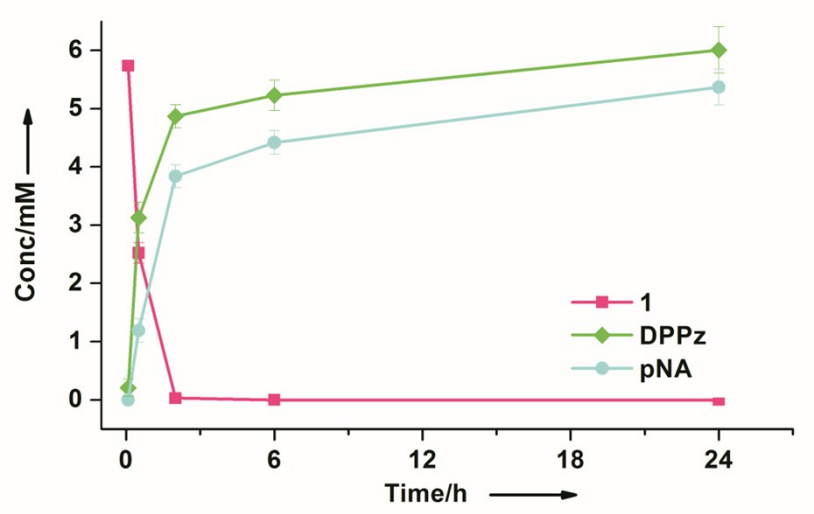


Figure S10. Products distribution as a function of time for the reaction of **1** and **DPTz** in DMSO-d_6/D_2O (9:1, v/v) at 37 ^oC. Conditions were the same as for ^{1}H NMR mechanism studies. The results are expressed as the mean ± standard deviation (n = 3).

pNA Release Studies

pNA was quantified by integration of characteristic ^{1}H NMR peaks in experiments similar to the ^{1}H NMR mechanistic studies. Aliquots of **1-3** stock solution and DPTz solution gave final concentrations of 4.5 mM for the **1-3** and 18 mM for DPTz (4 eq). 18-crown-6-ether was added as internal standard for peak integration. The samples were incubated at 37 ^{o}C and ^{1}H NMR spectra recorded at the indicated time points (6 h and 24 h) at 25 ^{o}C. The release studies were conducted in triplicate**,** shown as **Figure 2b**. The results are expressed as the mean ± standard deviation (n = 3).

Stability Studies of Prodrug 5 and Compound 2

*Stability of **5** in DMSO-PBS (1:1, v/v)*

Stock solutions of prodrug **5** (2 mM) in DMSO were prepared. Aliquots of **5** stock solution (100 μL), DMSO (400 μL) and 0.01 M PBS (500 μL) were combined to give final concentrations of 200 μM for the **5** in DMSO-PBS (1:1, v/v). The sample was incubated in the dark at 37 °C and analyzed by HPLC at the indicated time points (5 min, 6 h and 24 h) at 480 nm. No free doxorubicin or doxorubicin-containing side products were observed.

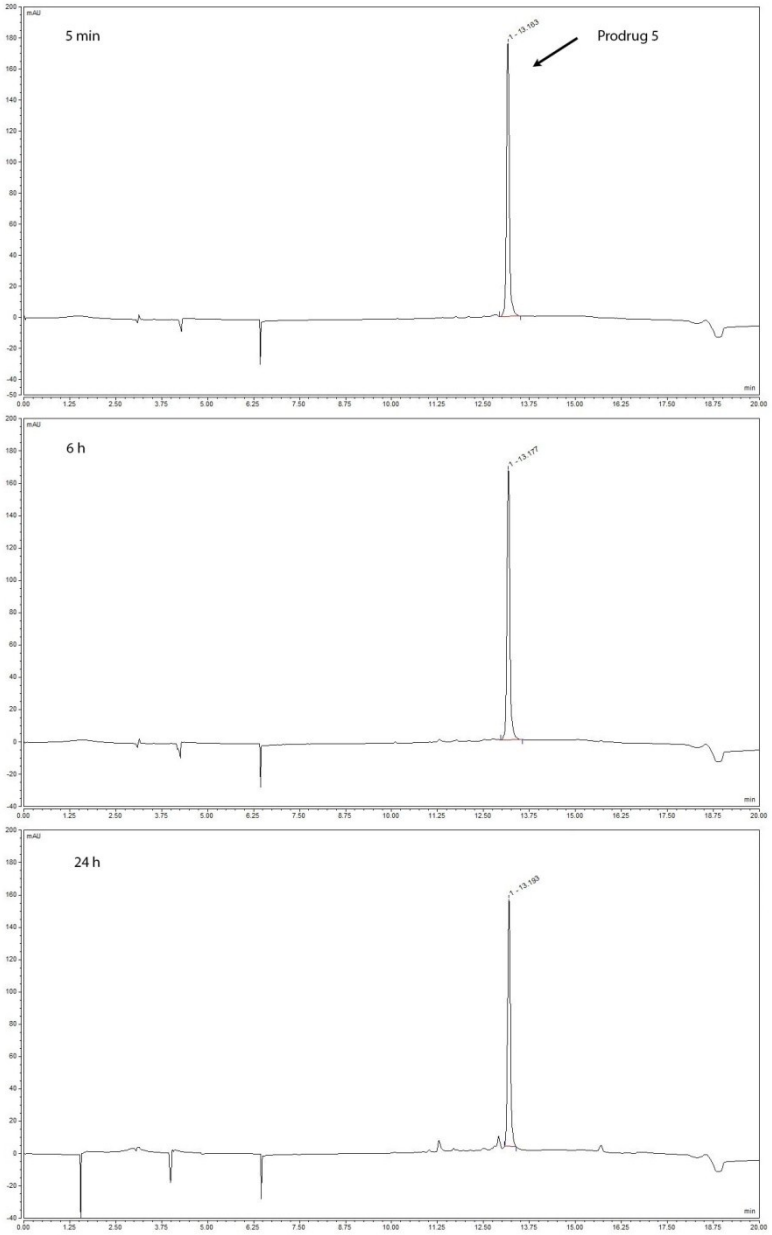


Figure S11. Representative spectra of HPLC analysis of the stability of **5** in DMSO/PBS solution at different time points. Prodrug **5** is indicated with arrow. The mobile phase A was 0.1% TFA in

water and mobile phase B was acetonitrile. A gradient of 0-100% B ranging from 1-15 min and 100%B ranging from 15-18 min was run at a flow rate of 4.0 mL/min. Retention time for **5**: 13.16-13.20 min.

Release Studies of Prodrug 5

Stock solutions of prodrug **5** (2 mM) and PEG-Tz (16 mM) in DMSO were prepared. Aliquots of **5** stock solution (100 μL), Tz stock solution (100 μL), DMSO (300 μL) and 0.01 M PBS (500 μL) were combined to give final concentrations of 200 μM for the **5** and 1.6 mM for PEG-Tz. The sample was incubated at 37 ^{o}C in the dark and HPLC spectra were recorded at the indicated time points (5 min, 30 min, 2 h and 6 h) at 480 nm.

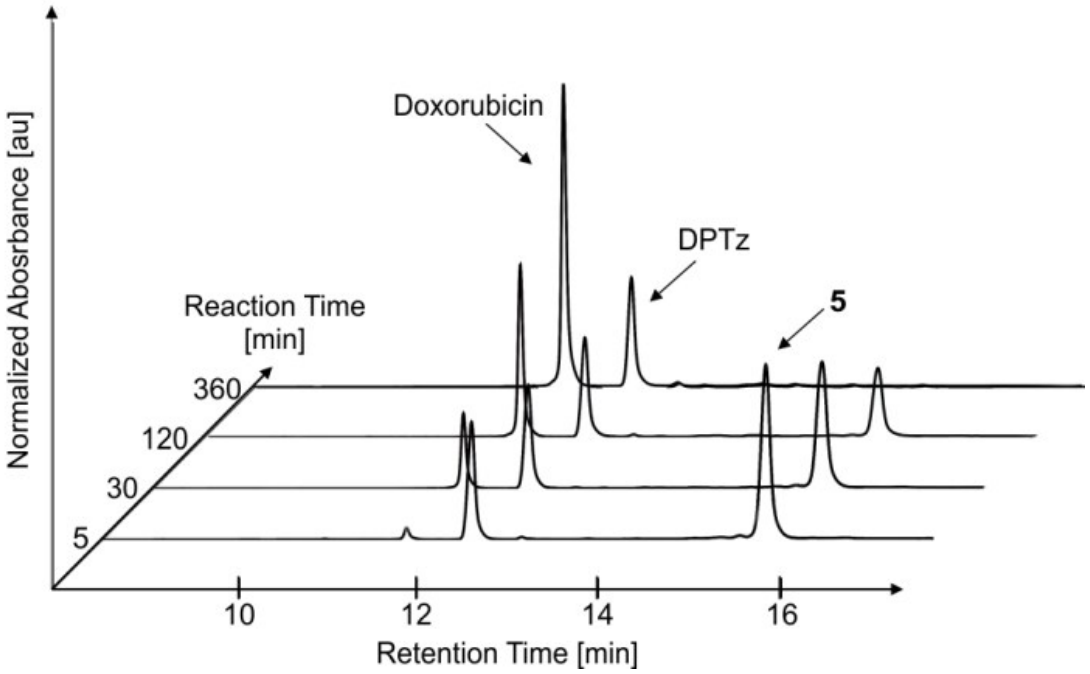


Figure S12. HPLC analysis of the reaction of **5** with **PEG-Tz** in DMSO/PBS solution (1:1, v.v) at different time points. From left to right: Released **Dox**, **PEG-Tz** and Prodrug **5** (Indicated with arrows). The mobile phase A was 0.1% TFA in water and mobile phase B was acetonitrile. A gradient of 0-75% B ranging from 1-15 min and 75%-100% B from 15-18 min and 100%B ranging from 15-18 min was run at a flow rate of 4.0 mL/min. Retention time for Free Dox: 11.80-11.87 min; PEG-Tz: 12.50-12.60; **5**: 15.68-15.74 min.

*Stability of **5** in PBS: Serum (1:1, v/v)*

Stock solutions of prodrug **5** (2.5 mM) in DMSO were prepared. Aliquots of **5** stock solution (20 μL), 0.01 M PBS (480 μL) and human serum (500 μL) (Sigma-Aldrich, USA) were combined to give final concentrations of 50 μM for the **5** in PBS: Serum (1:1, v/v). The sample was thoroughly mixed and incubated at 37 ^{o}C in the dark and subsequently a 50 μL aliquot of the sample was taken at indicated time points (5 min, 6 h, 24 h and 48 h) and quenched by 200 μL ice cold acetonitrile, followed by centrifugation at 13300 rpm for 5 min. The supernatant was injected and analyzed by HPLC at 480 nm. No free doxorubicin or doxorubicin-containing side products were observed in the stability test of **5** (**Figure S13**).

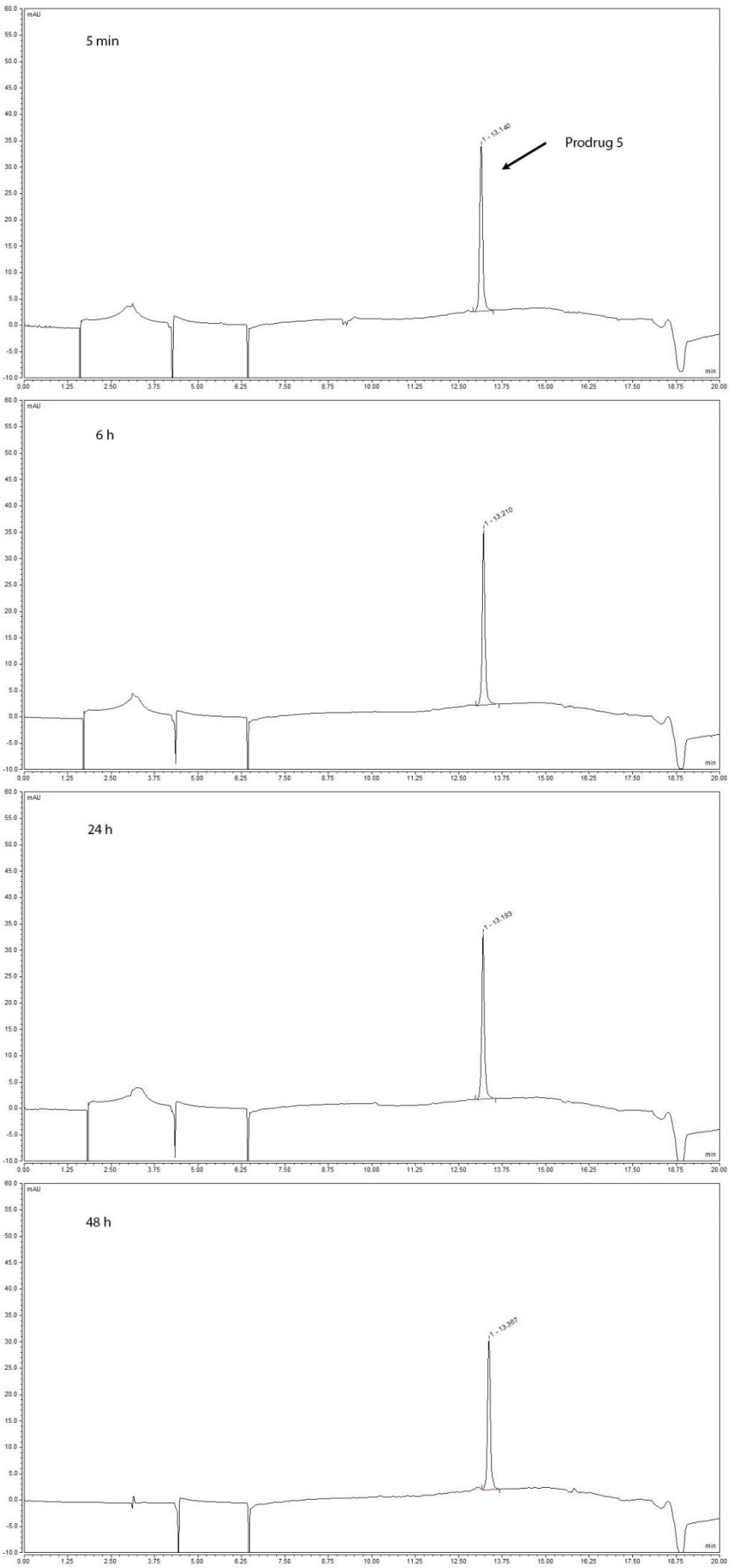

5 min
Prodrug 5
6 h
24 h
48 h
mAU
min

Figure S13. Representative spectra of HPLC analysis of the stability of **5** in human serum at different time points. Prodrug **5** is indicated with arrow. The mobile phase A was 0.1% TFA in water and mobile phase B was acetonitrile. A gradient of 0-100% B ranging from 1-15 min was run at a flow rate of 4.0 mL/min. Retention time for **5**: 13.18-13.33 min.

*Stability of **2** in PBS: Serum (1:1, v/v)*

Stock solutions of **2** (2.5 mM) in DMSO were prepared. Aliquots of **2** stock solution (20 μL), 0.01 M PBS (480 μL) and human serum (500 μL) (Sigma-Aldrich, USA) were combined to give final concentrations of 50 μM for the **2** in PBS: Serum (1:1, v/v). The sample was thoroughly mixed and incubated at 37 °C in the dark and subsequently a 50 μL aliquot of the sample was taken at indicated time points (5 min, 6 h, 24 h, 48 h, 72 h and 7 days) and quenched by 200 μL ice cold acetonitrile, followed by centrifugation at 13300 rpm for 5 min. The supernatant was injected and analyzed by HPLC at 317 nm. No free p-Nitroaniline or p-Nitroaniline-containing side products were observed in the stability test of **2** (**Figure S14**).

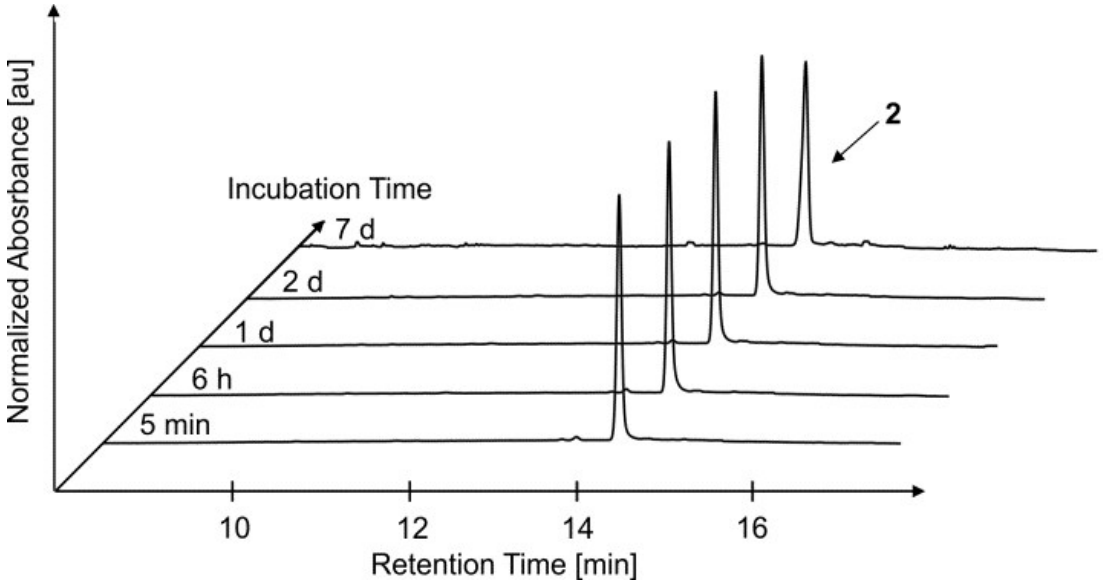


Figure S14. Representative spectra of HPLC analysis of the stability of **2** in human serum at different time points (5 min, 6 h, 1 day, 2 days and 7 days). Compound **2** is indicated with arrow. The mobile phase A was 0.1% TFA in water and mobile phase B was acetonitrile. A gradient of 0-100% B ranging from 1-15 min and 100%B ranging from 15-18 min was run at a flow rate of 4.0 mL/min. Retention time for **2**: 14.13-14.25 min.

Cytotoxicity Assay

A549 lung cancer cells (ATCC, USA) were maintained in a humidified CO_2 (5%) incubator at 37 ^oC in DMEM (Thermo Fisher, USA) supplemented with 10% fetal bovine serum in the presence of 1% Penicillin-Streptomycin-Glutamine (Thermo Fisher, USA) and 0.2% Normocin (InvivoGen, USA).

The cells were plated in 96-well TC treated plates (PerkinElmer, USA) at a 5000 cells/well density 24 h prior to the experiment. Prodrug **5**, compound **2** (2 mM in DMSO) and PEG-Tz (40 mM in DMSO) were serially diluted in pre-warmed culture medium. Prodrug **5** and compound **2** are added to the cells first (100 μL final volume per well) in a series of final concentrations ranging from 0.001 to 10 μM then followed by PEG-Tz in a series of final concentrations ranging from 200 μM, 100 μM, 50 μM, 25 μM, immediately.

Doxorubicin was used as the positive control with same series of concentrations ranging from 0.001 to 10 μM. PEG-Tz was also tested with same series of concentrations ranging from 0.02 to 200 μM, no obvious toxicity was observed. After 72 h incubation at 37 ^oC, cell proliferation was assessed by a CellTiter-Glo® viability assay. Lyophilized CellTiter Glo Substrate (Promega, USA) was dissolved in the CellTiter Glo Buffer to get CellTiter-Glo® Reagent, and from which 100 μL was added to each well. After 15 min incubation at 25 ^oC, the medium was gently measured with Envision 2104 Multilabel Reader (PerkinElmer, USA) to get the luminescent based on quantitation of the ATP present, an indicator of metabolically active cells. The proliferation assay was performed in triplicate (n = 3). EC_{50} values were derived from the normalized cell growth and corresponding sigmoidal curves were fitted and generated with Origin 8.0. Results in A549 cells are also shown in Table 2**.**

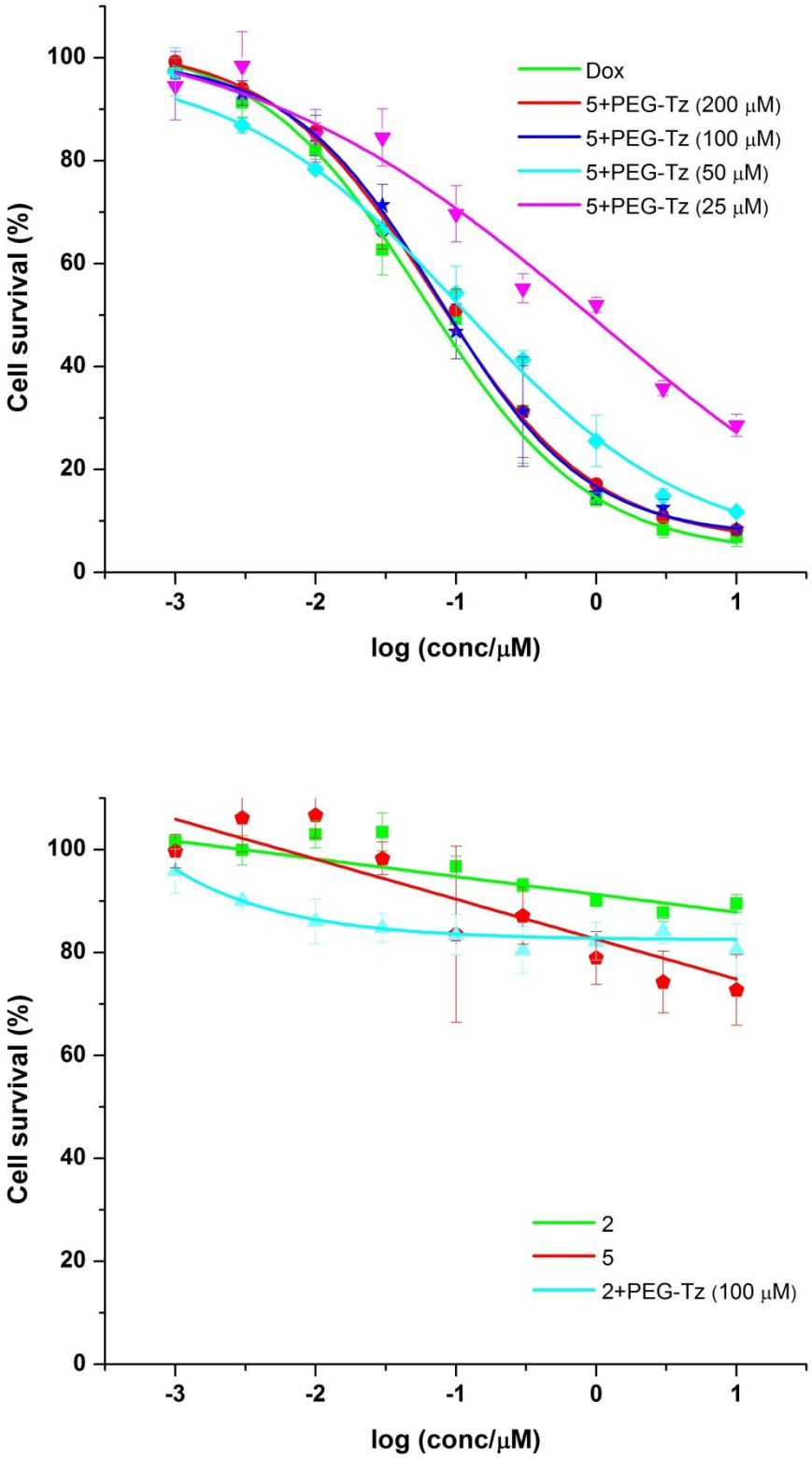


Figure S15. Cytotoxicity assay against lung cancer A549 cells. The results are expressed as the mean ± standard deviation (n = 3).

References

[1] S. Kovács, Á. I. Csincsi, T. Z. Nagy, S. Boros, G. Timári, Z. Novák, *Org. Lett.* **2012**, *14*, 2022-2025.

[2] C. Granchi, F. Rizzolio, V. Bordoni, I. Caligiuri, C. Manera, M. Macchia, F. Minutolo, A. Martinelli, A. Giordano, T. Tuccinardi, *J. Enzym. Inhibit. Med. Ch.* **2016**, *31*, 137-146.

[3] A. D. Abell, B. K. Nabbs, A. R. Battersby, *J. Am. Chem. Soc.* **1998**, *120*, 1741-1746.

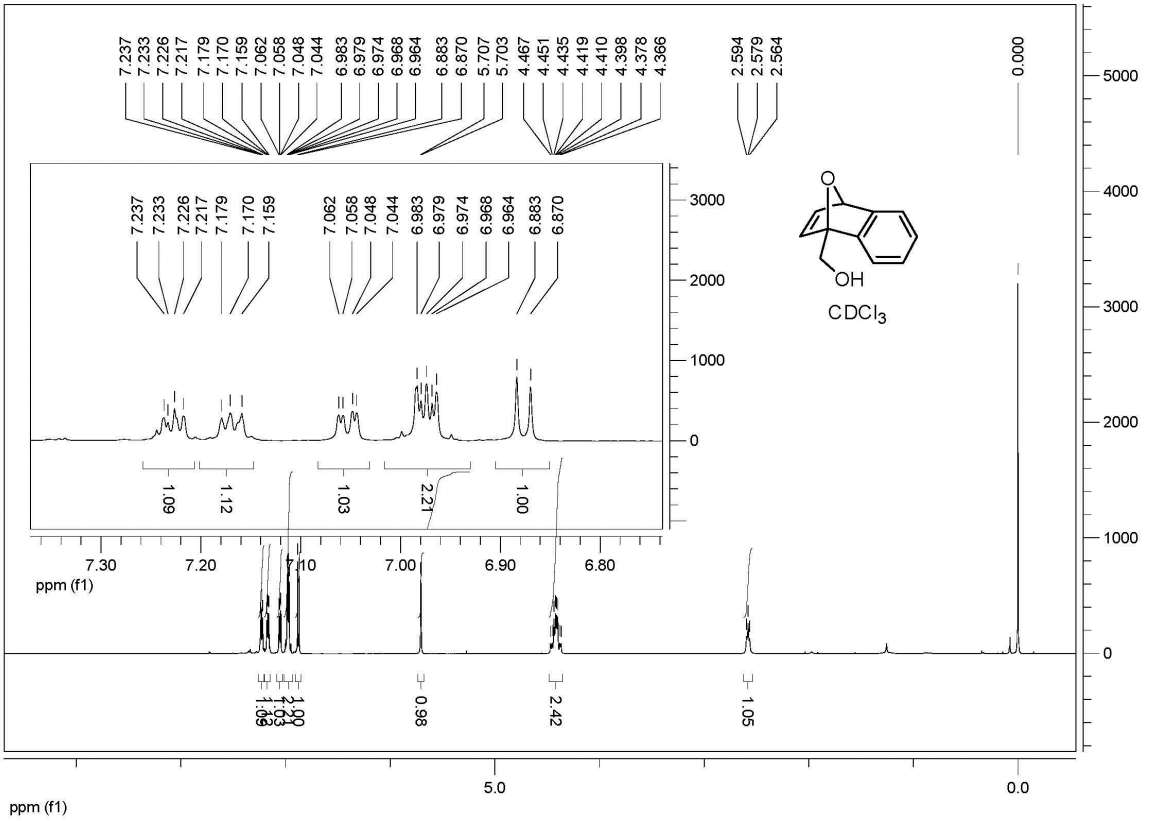
[4] J. Waser, B. Gaspar, H. Nambu, E. M. Carreira, *J. Am. Chem. Soc.* **2006**, *128*, 11693-11712.

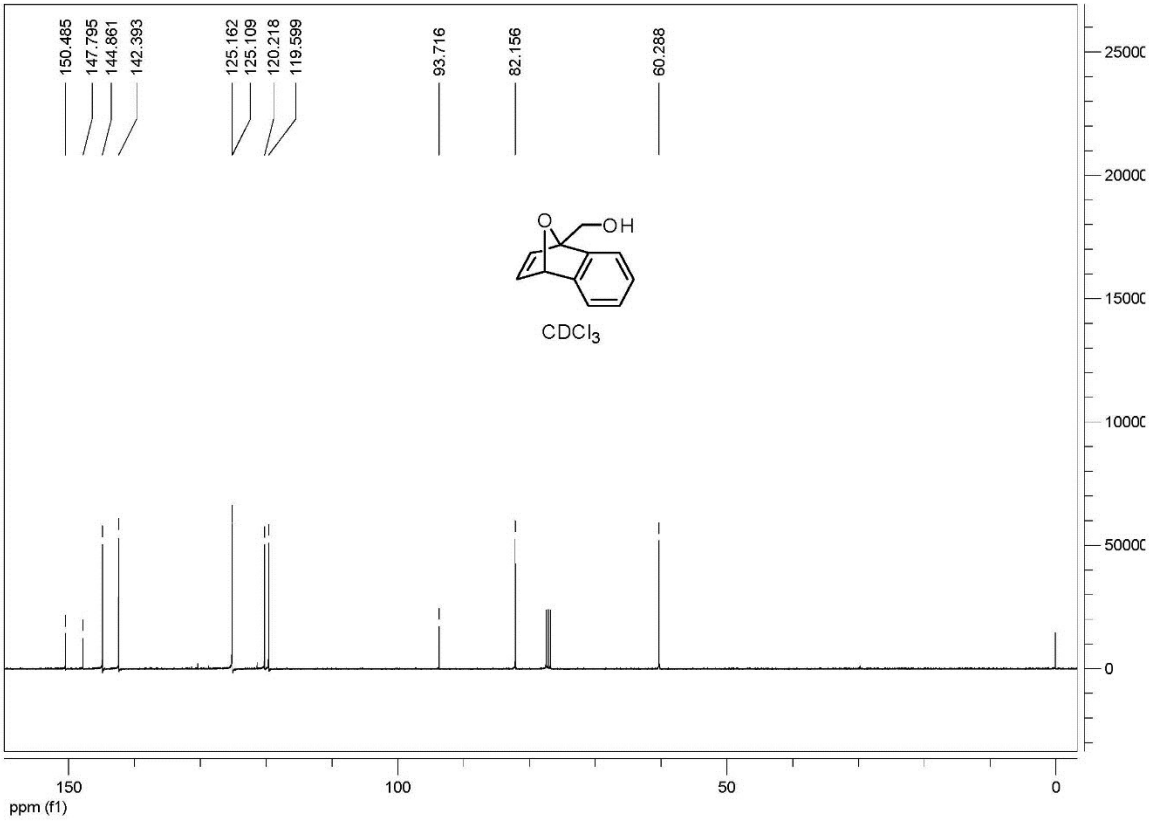
[5] H. M. L. Davies, J. J. Matasi, G. Ahmed, *J. Org. Chem.* **1996**, *61*, 2305-2313.

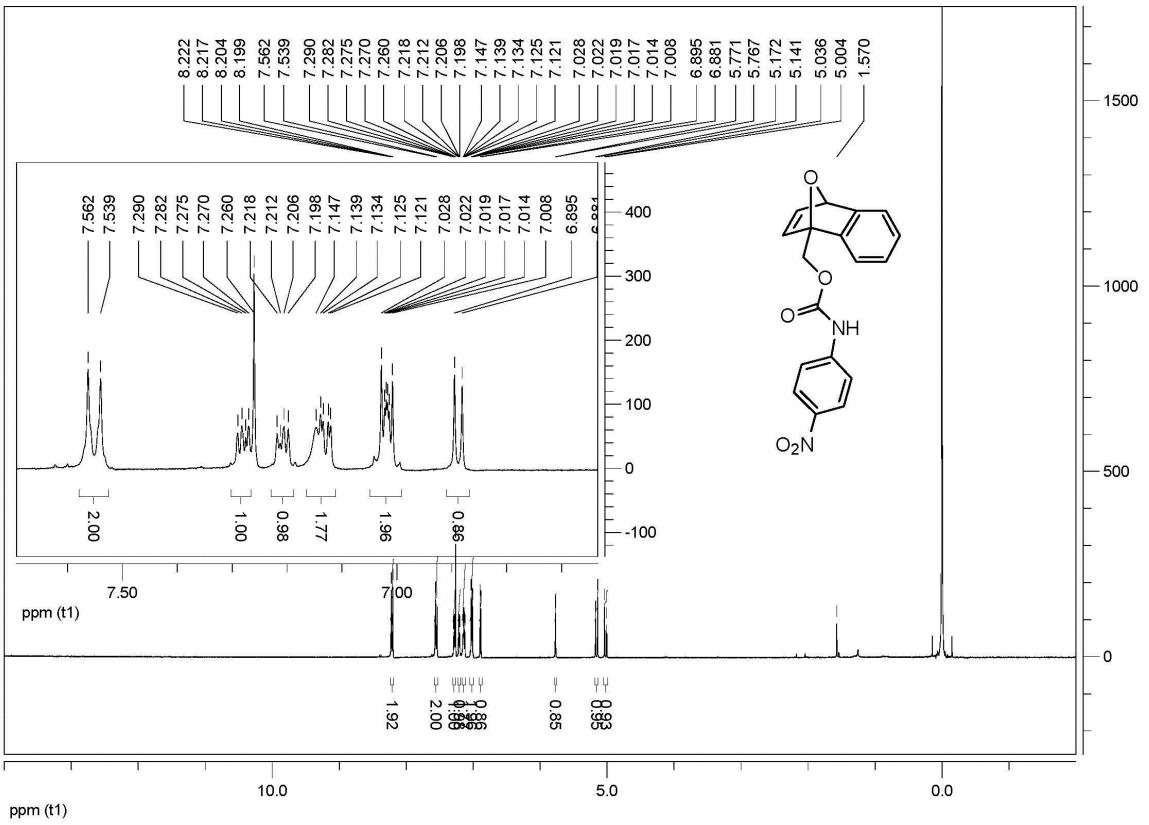
[6] H. Bakkali, C. Marie, A. Ly, C. Thobie-Gautier, J. Graton, M. Pipelier, S. Sengmany, E. Léonel, J.-Y. Nédélec, M. Evain, D. Dubreuil, *Eur. J. Org. Chem.* **2008**, *2008*, 2156-2166.

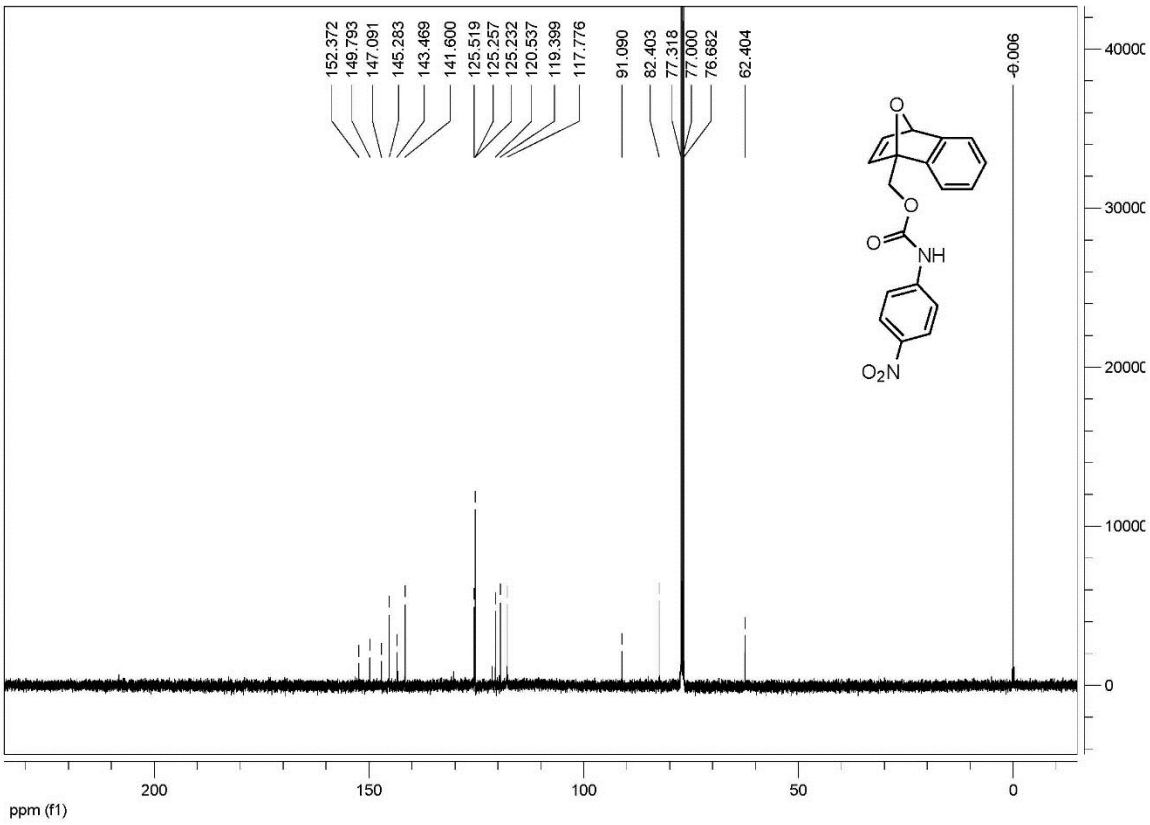
[7] Y.-J. Lee, Y. Kurra, Y. Yang, J. Torres-Kolbus, A. Deiters, W. R. Liu, *Chem. Commun.* **2014**, *50*, 13085-13088.

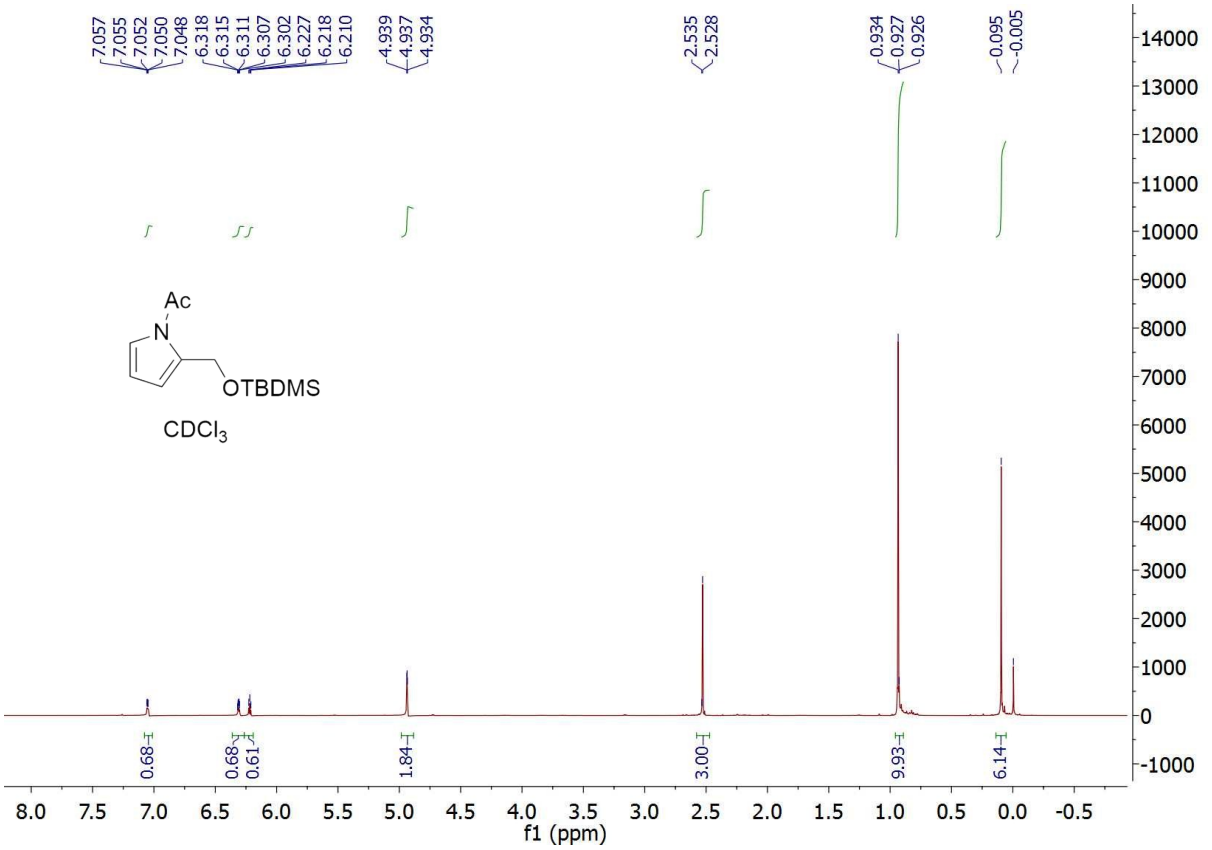
[1]H and [13]C NMR of Synthetic Compounds

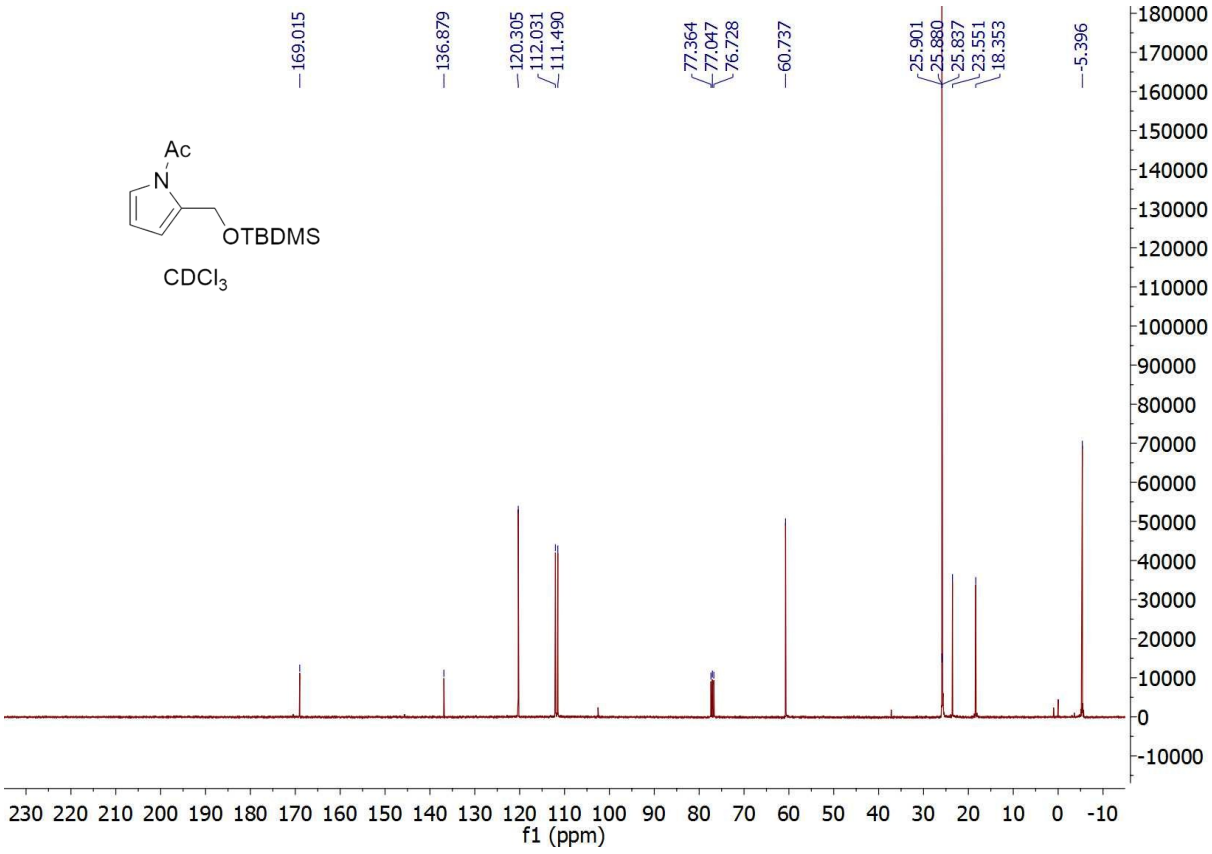




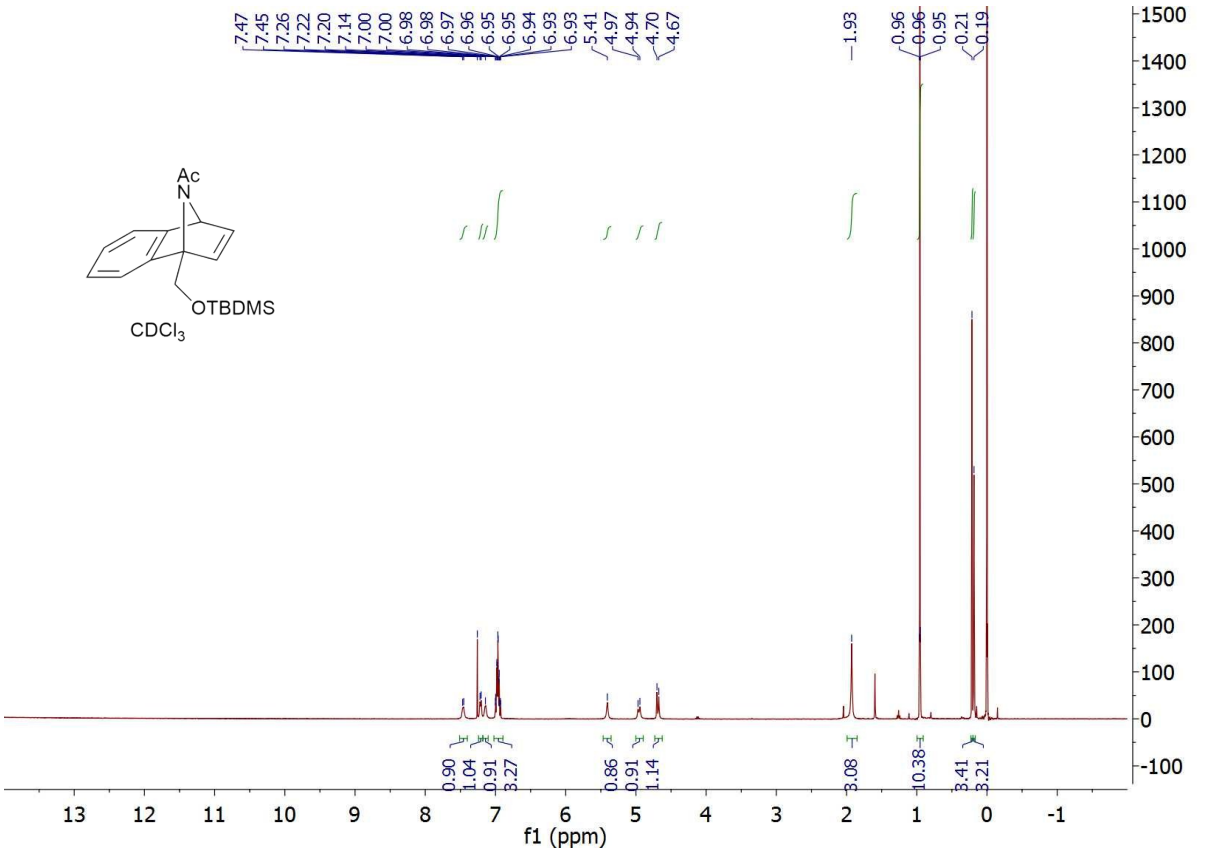

8.222
8.217
8.204
8.199
7.562
7.539
7.290
7.282
7.275
7.270
7.260
7.218
7.212
7.206
7.198
7.147
7.139
7.134
7.125
7.121
7.028
7.022
7.019
7.017
7.014
7.008
6.895
6.881
5.771
5.767
5.172
5.141
5.036
5.004
1.570
O
O
NH
O2N
2.00
1.00
0.98
1.77
1.96
0.86
1.92
2.00
0.85
ppm (t1)
ppm (t1)

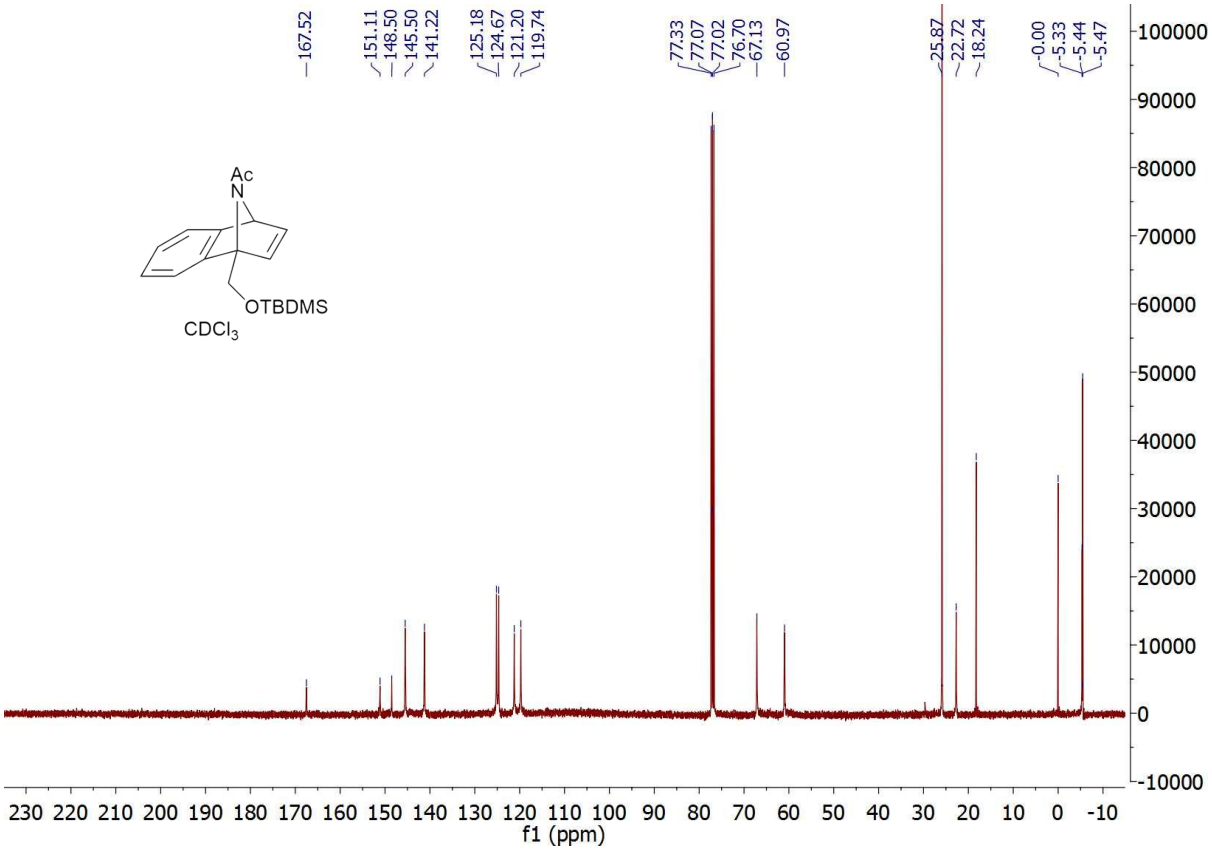

152.372
149.793
147.091
145.283
143.469
141.600
125.519
125.257
125.232
120.537
119.399
117.776
91.090
82.403
77.318
77.000
76.682
62.404
0.006
O
O
NH
O2N
ppm (f1)

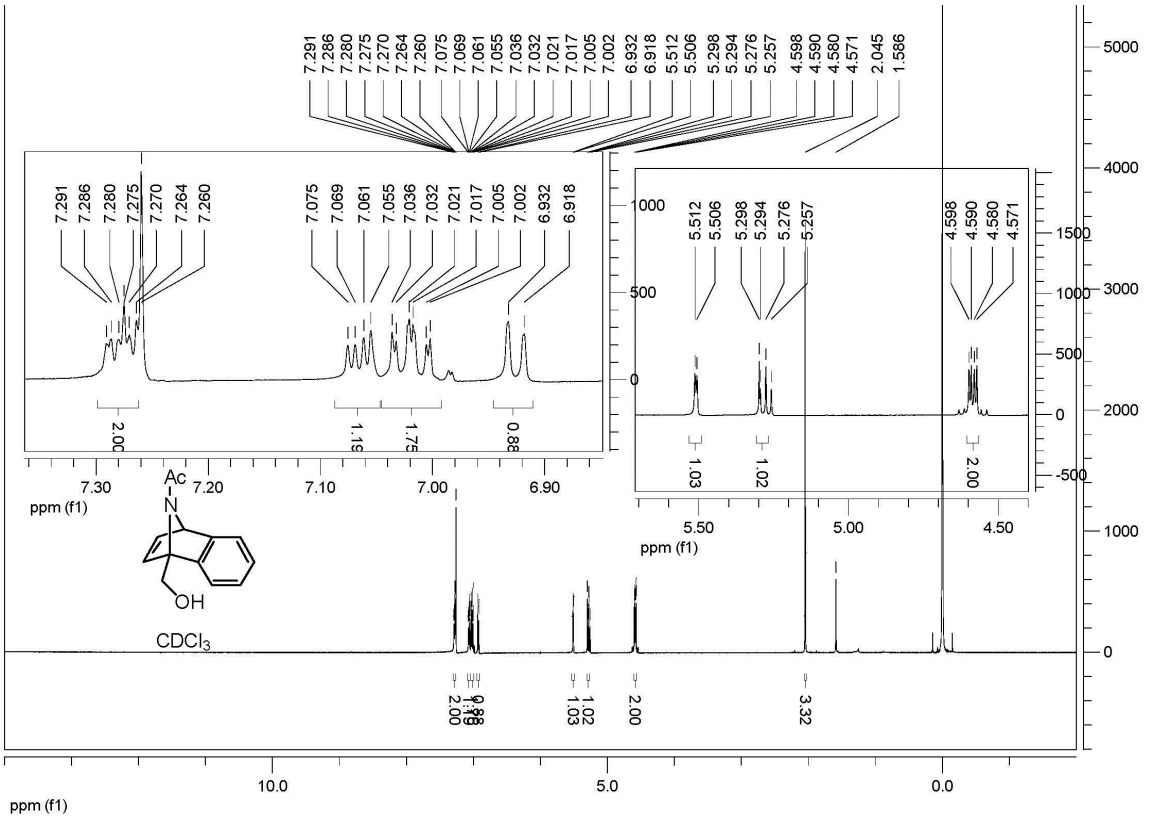

7.057
7.055
7.052
7.050
7.048
6.318
6.315
6.311
6.307
6.302
6.227
6.218
6.210
4.939
4.937
4.934
2.535
2.528
0.934
0.927
0.926
0.095
-0.005
Ac
N
OTBDMS
CDCl3
0.68
0.68
0.61
1.84
3.00
9.93
6.14
f1 (ppm)

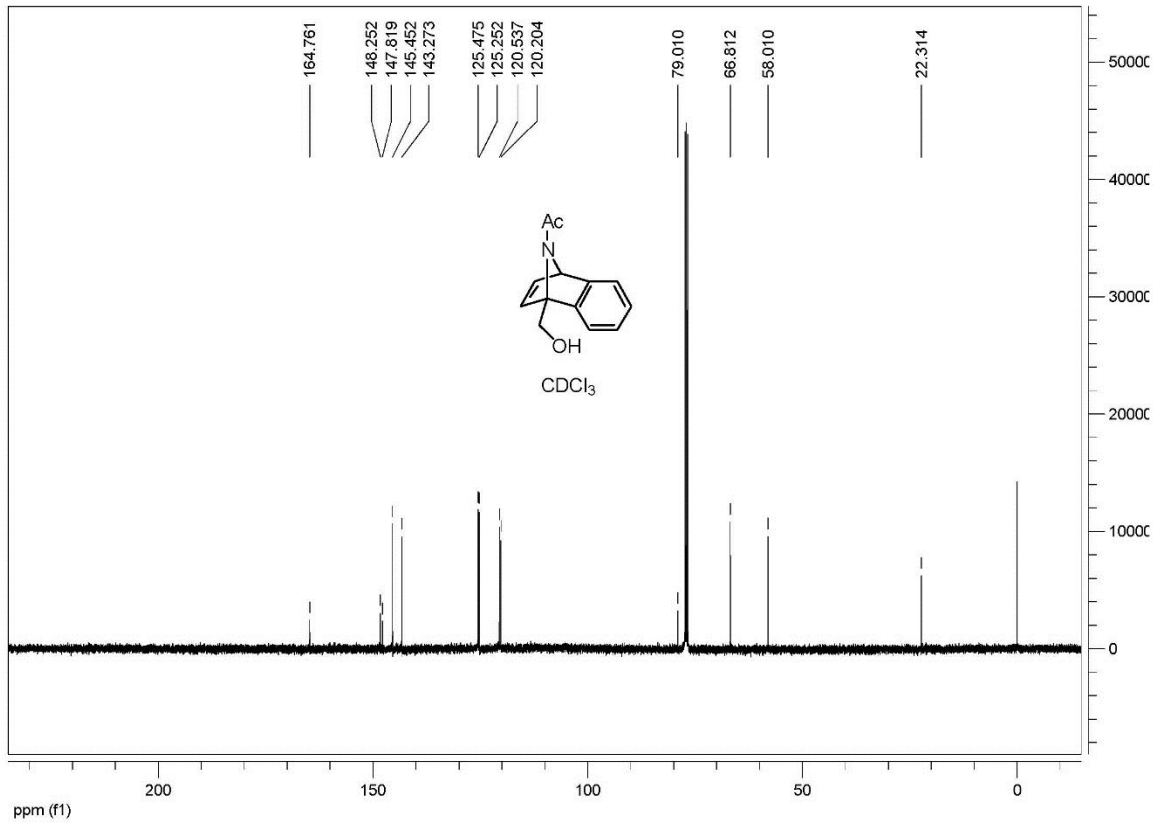

169.015
136.879
120.305
112.031
111.490
77.364
77.047
76.728
60.737
25.901
25.880
25.837
23.551
18.353
-5.396
Ac
N
OTBDMS
CDCl3
f1 (ppm)

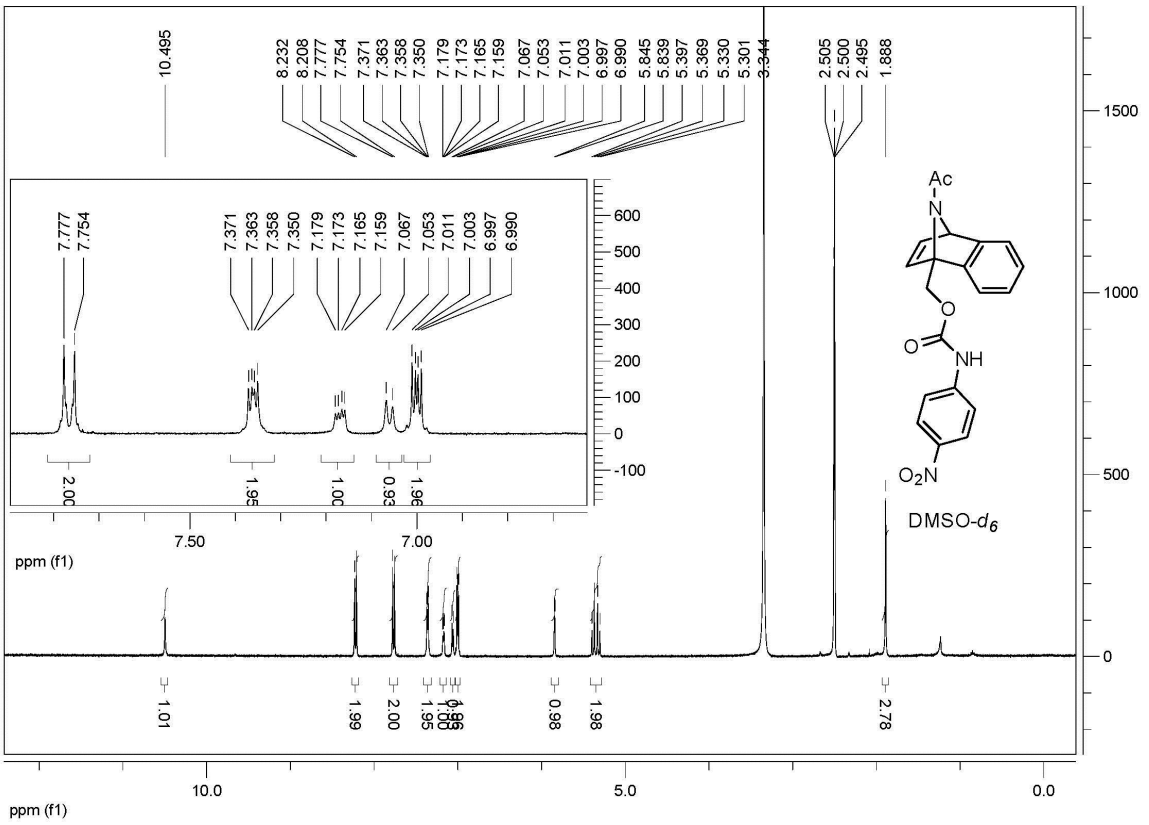
2b

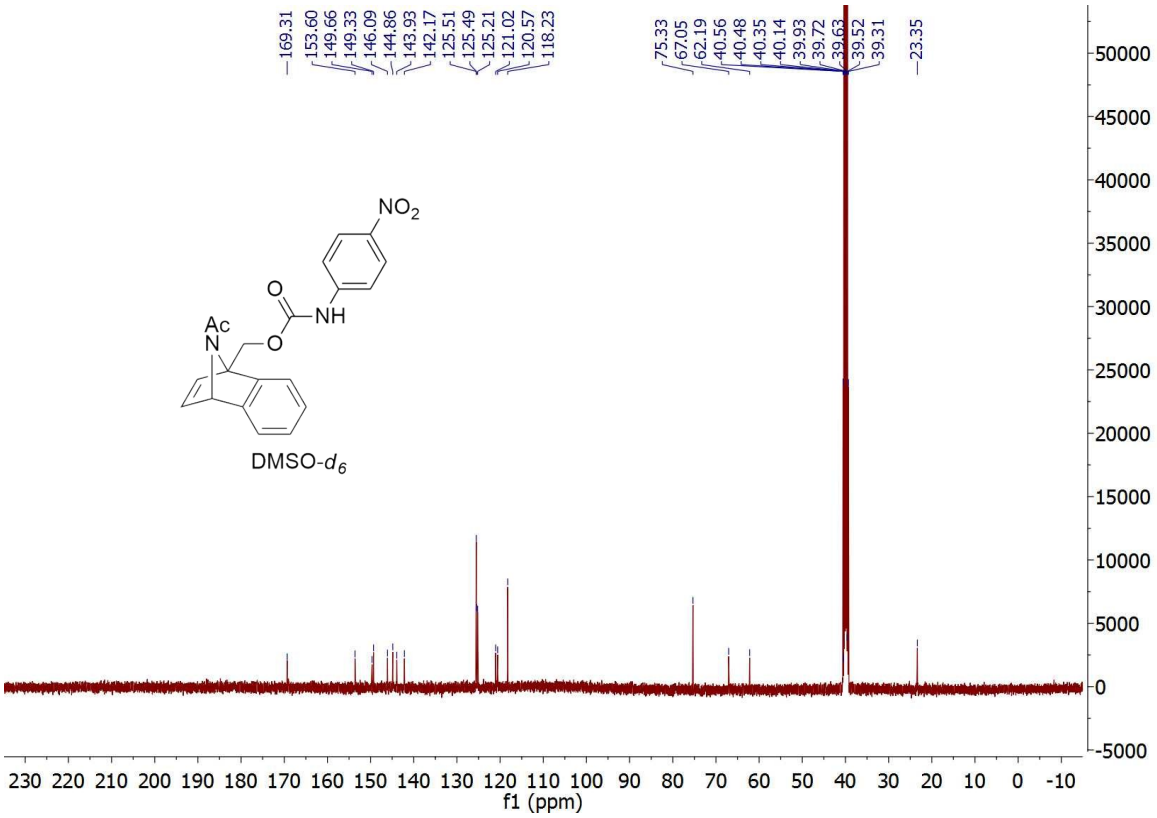

7.47
7.45
7.26
7.22
7.20
7.14
7.00
7.00
6.98
6.98
6.97
6.96
6.95
6.95
6.94
6.93
6.93
5.41
4.97
4.94
4.70
4.67
1.93
0.96
0.96
0.95
0.21
0.19
Ac
N
OTBDMS
CDCl3
0.90
1.04
0.91
3.27
0.86
0.91
1.14
3.08
10.38
3.41
3.21
f1 (ppm)

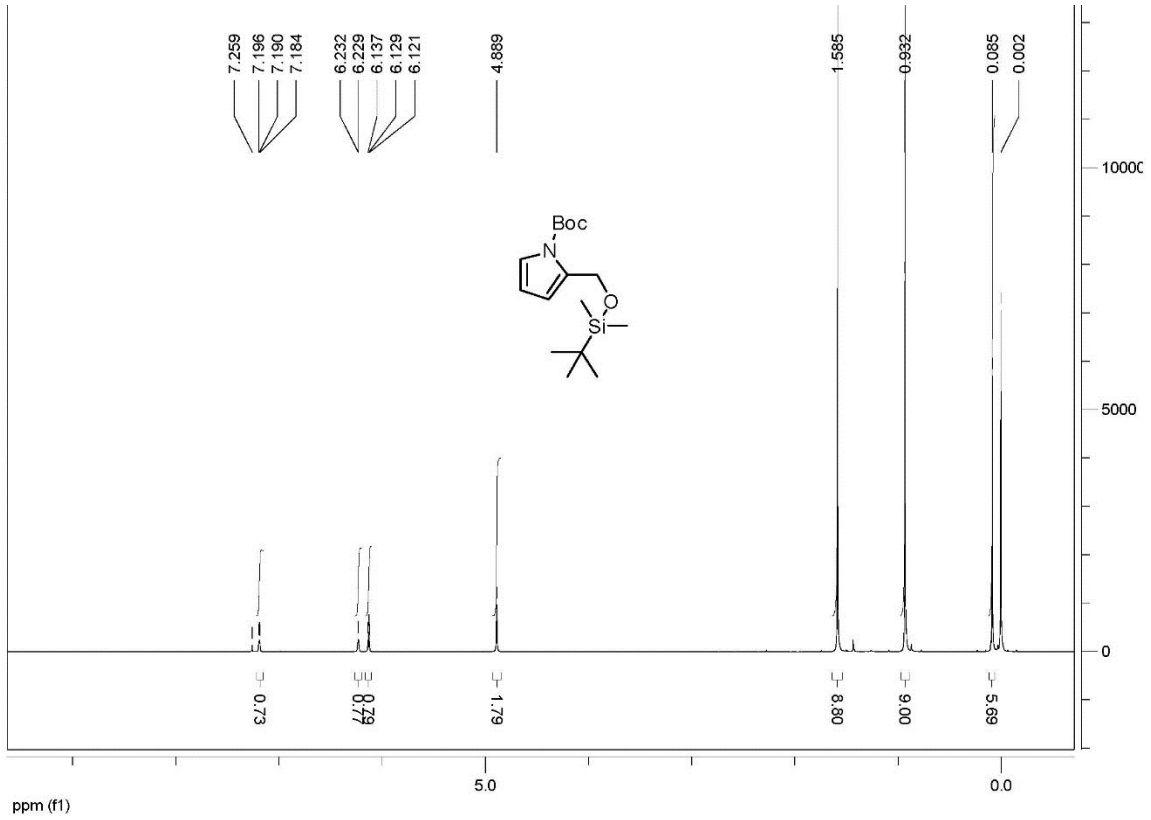

167.52
151.11
148.50
145.50
141.22
125.18
124.67
121.20
119.74
77.33
77.07
77.02
76.70
67.13
60.97
25.87
22.72
18.24
0.00
-5.33
-5.44
-5.47
Ac
N
OTBDMS
CDCl3
f1 (ppm)

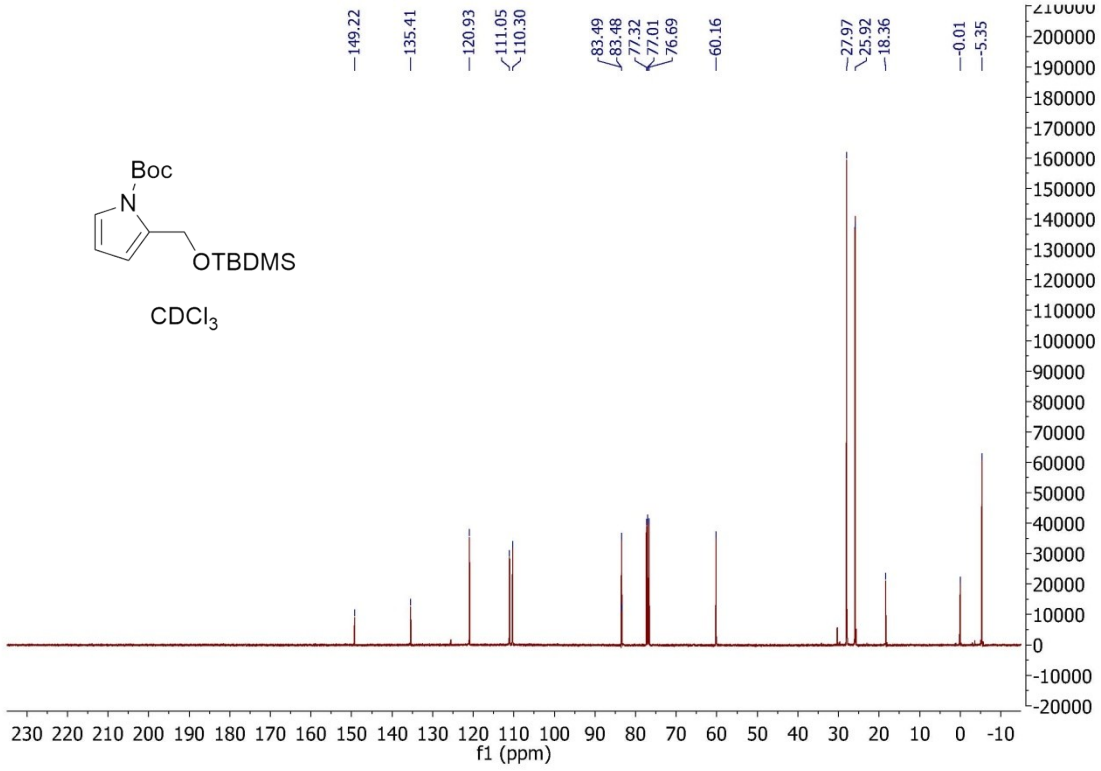

7.291
7.286
7.280
7.275
7.270
7.264
7.260
7.075
7.069
7.061
7.055
7.036
7.032
7.021
7.017
7.005
7.002
6.932
6.918
5.512
5.506
5.298
5.294
5.276
5.257
4.598
4.590
4.580
4.571
2.045
1.586
Ac
N
OH
CDCl3
ppm (f1)
2.00
1.19
1.57
0.88
1.03
1.02
2.00
3.32

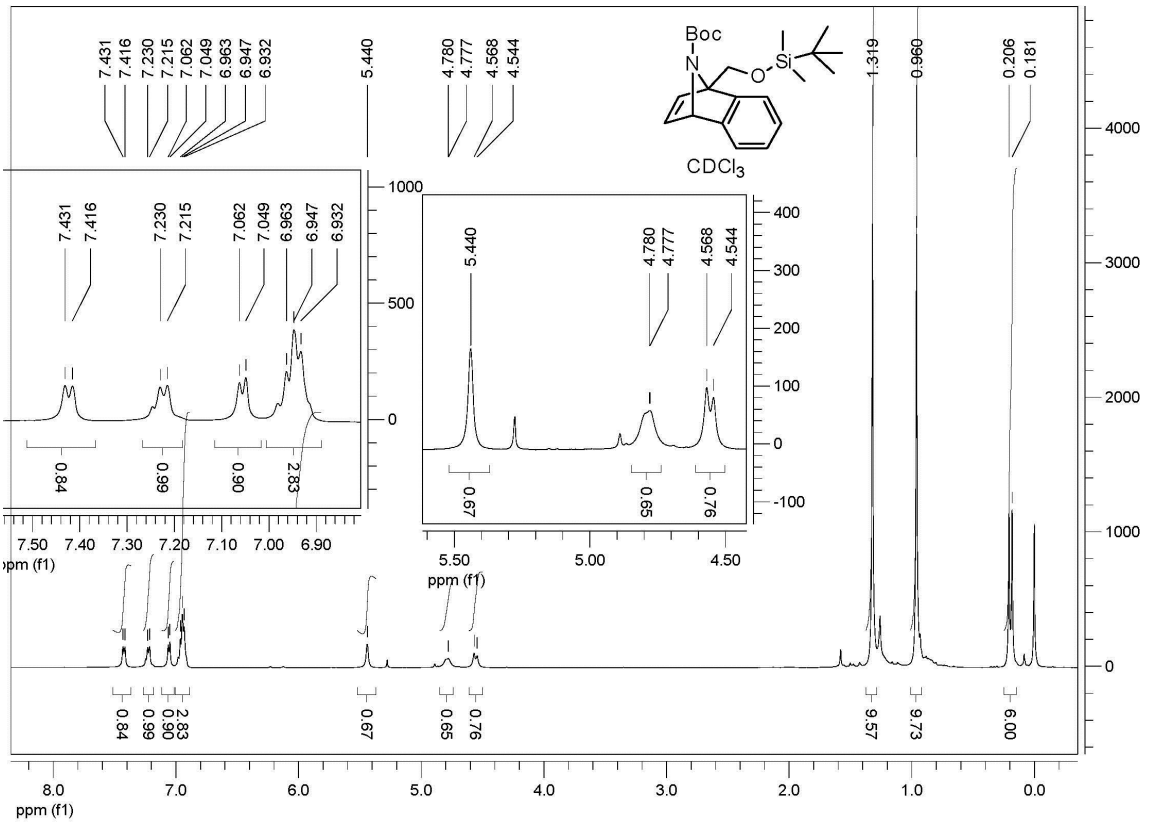

164.761
148.252
147.819
145.452
143.273
125.475
125.252
120.537
120.204
79.010
66.812
58.010
22.314
Ac
N
OH
CDCl3
ppm (f1)

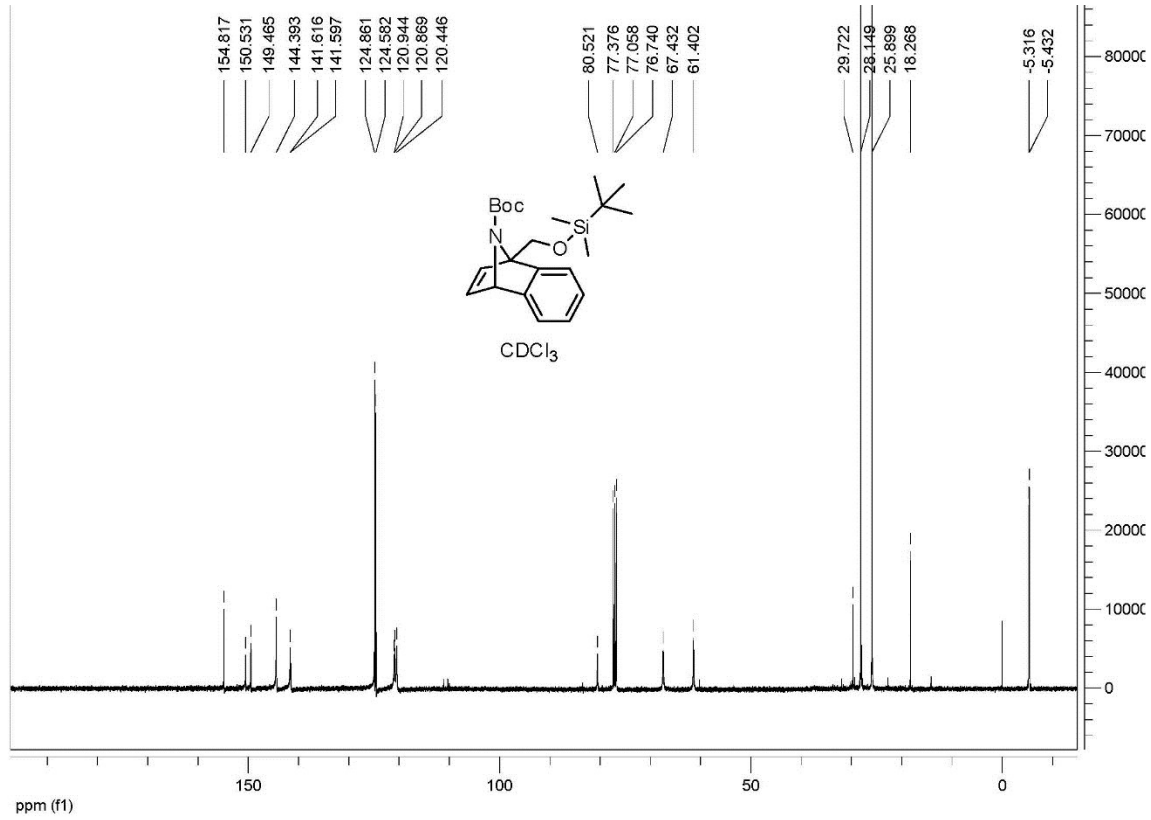

10.495
8.232
8.208
7.777
7.754
7.371
7.363
7.358
7.350
7.179
7.173
7.165
7.159
7.067
7.053
7.011
7.003
6.997
6.990
5.845
5.839
5.397
5.369
5.330
5.301
3.344
2.505
2.500
2.495
1.888
Ac
N
O
O
NH
O2N
DMSO-d6
ppm (f1)
1.01
1.99
2.00
0.98
1.98
2.78

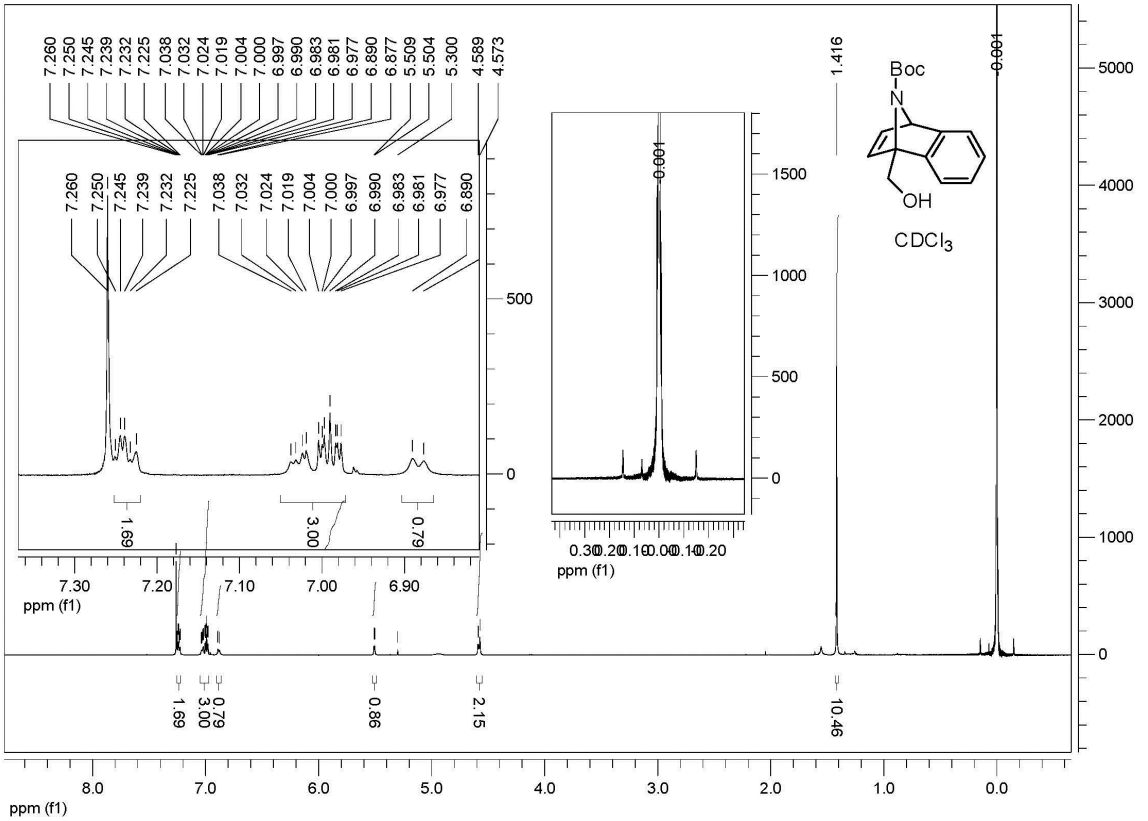

169.31
153.60
149.66
149.33
146.09
144.86
143.93
142.17
125.51
125.49
125.21
121.02
120.57
118.23
75.33
67.05
62.19
40.56
40.48
40.35
40.14
39.93
39.72
39.63
39.52
39.31
23.35
NO2
O
NH
Ac
N
O
DMSO-d6
f1 (ppm)

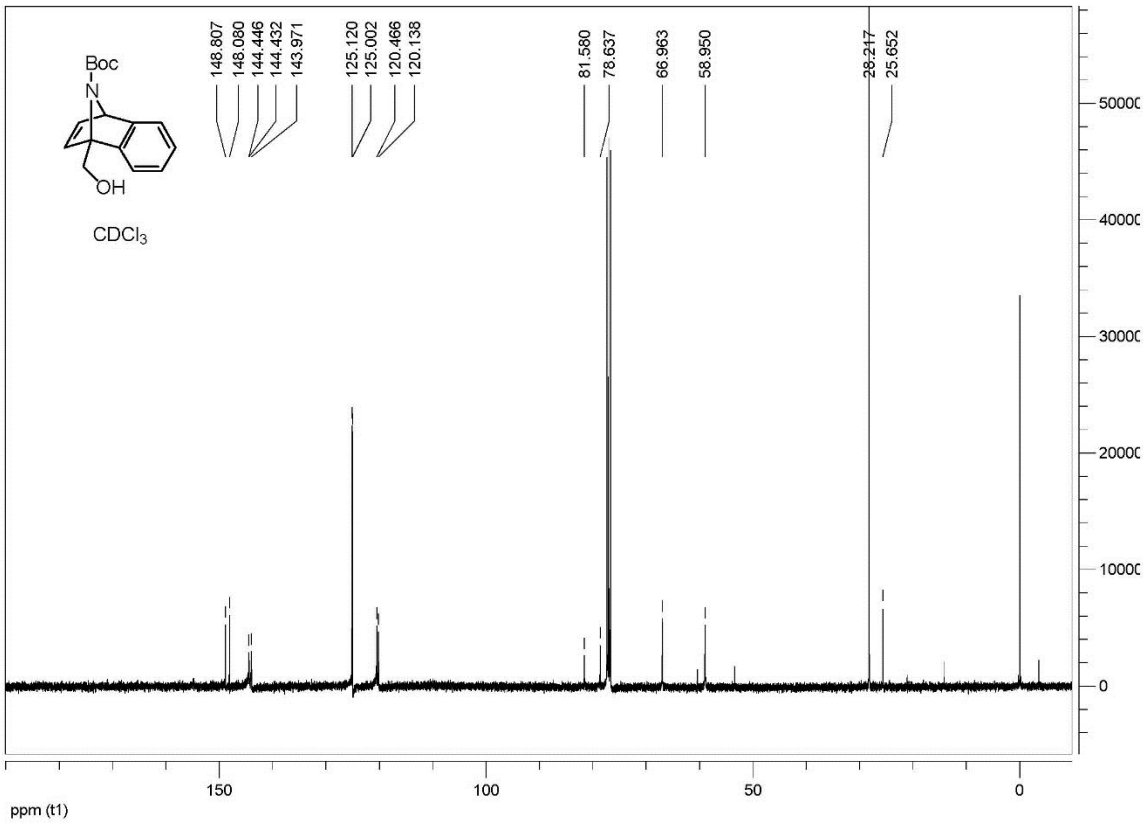

7.259
7.196
7.190
7.184
6.232
6.229
6.137
6.129
6.121
4.889
1.585
0.932
0.085
0.002
Boc
N
O
Si
10000
5000
0
0.73
0.77
0.79
1.79
8.80
9.00
5.69
5.0
0.0
ppm (f1)

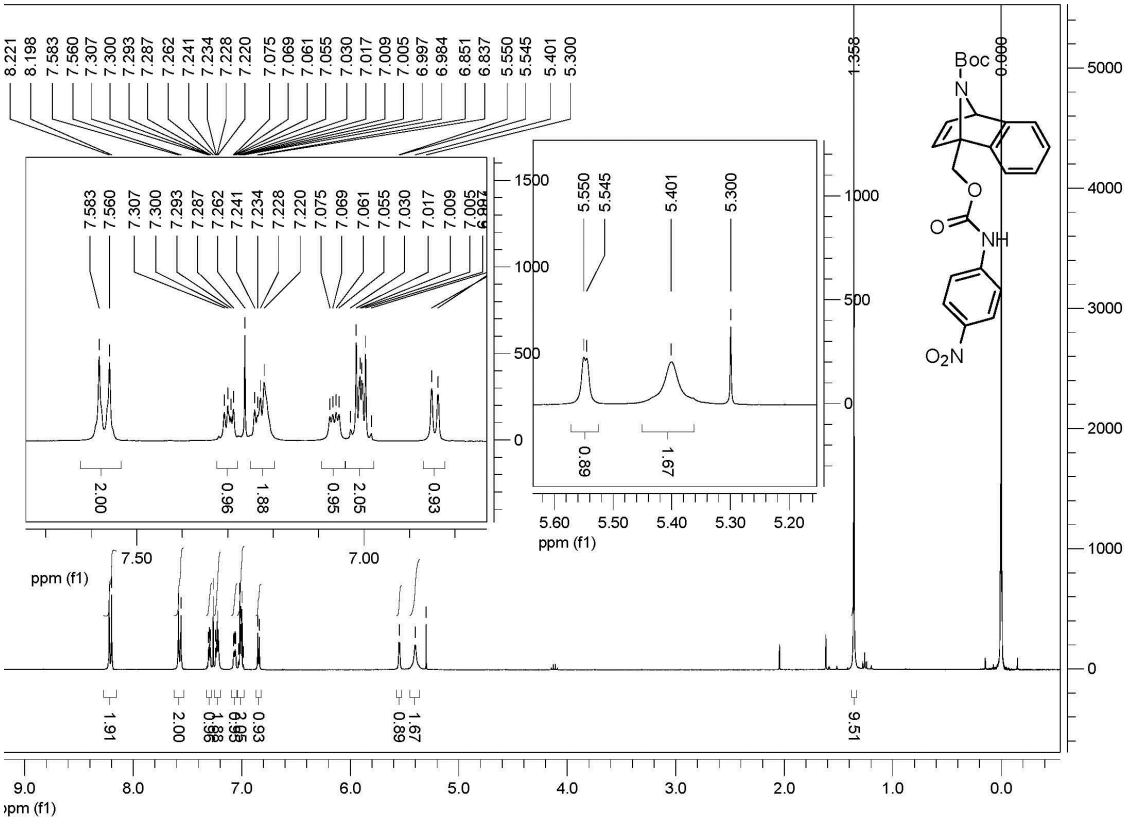

149.22
135.41
120.93
111.05
110.30
83.49
83.48
77.32
77.01
76.69
60.16
27.97
25.92
18.36
-0.01
-5.35
Boc
N
OTBDMS
CDCl3
f1 (ppm)

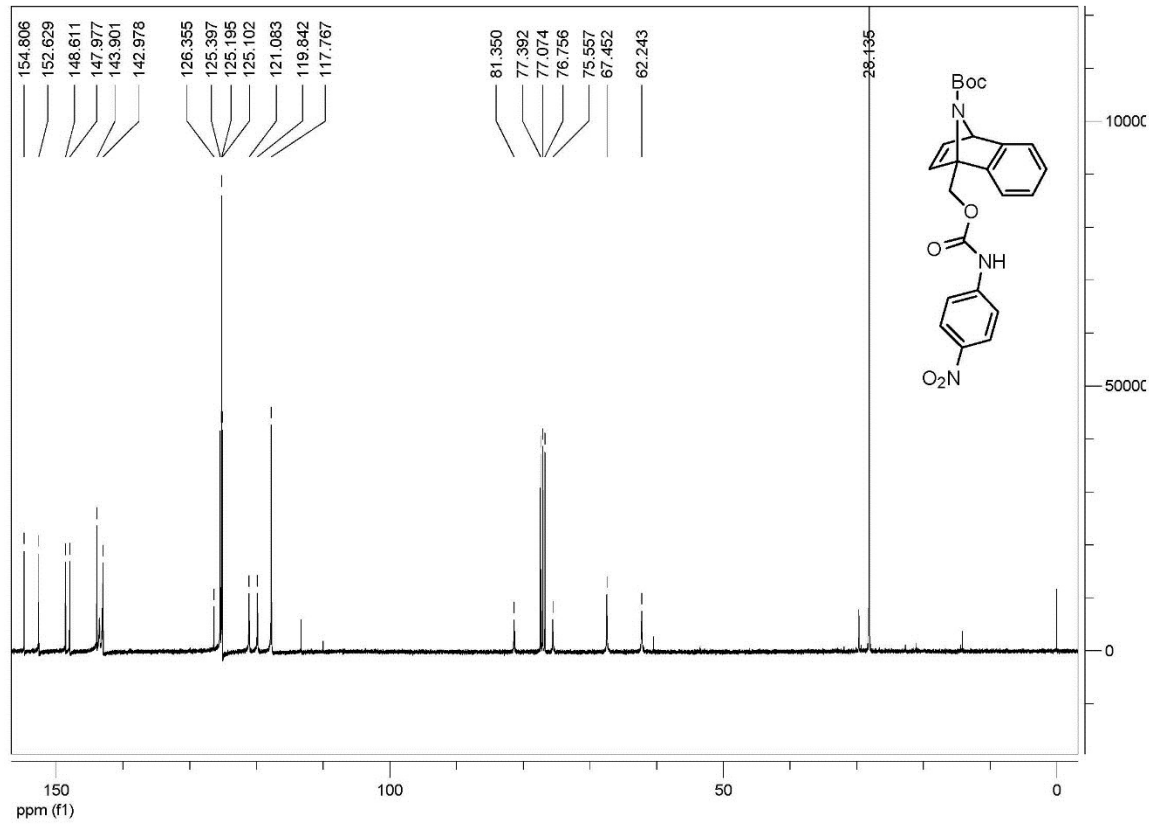

7.431
7.416
7.230
7.215
7.062
7.049
6.963
6.947
6.932
5.440
4.780
4.777
4.568
4.544
1.319
0.960
0.206
0.181
Boc
N
O
Si
CDCl3
0.84
0.99
0.90
2.83
0.67
0.65
0.76
9.57
9.73
6.00
ppm (f1)

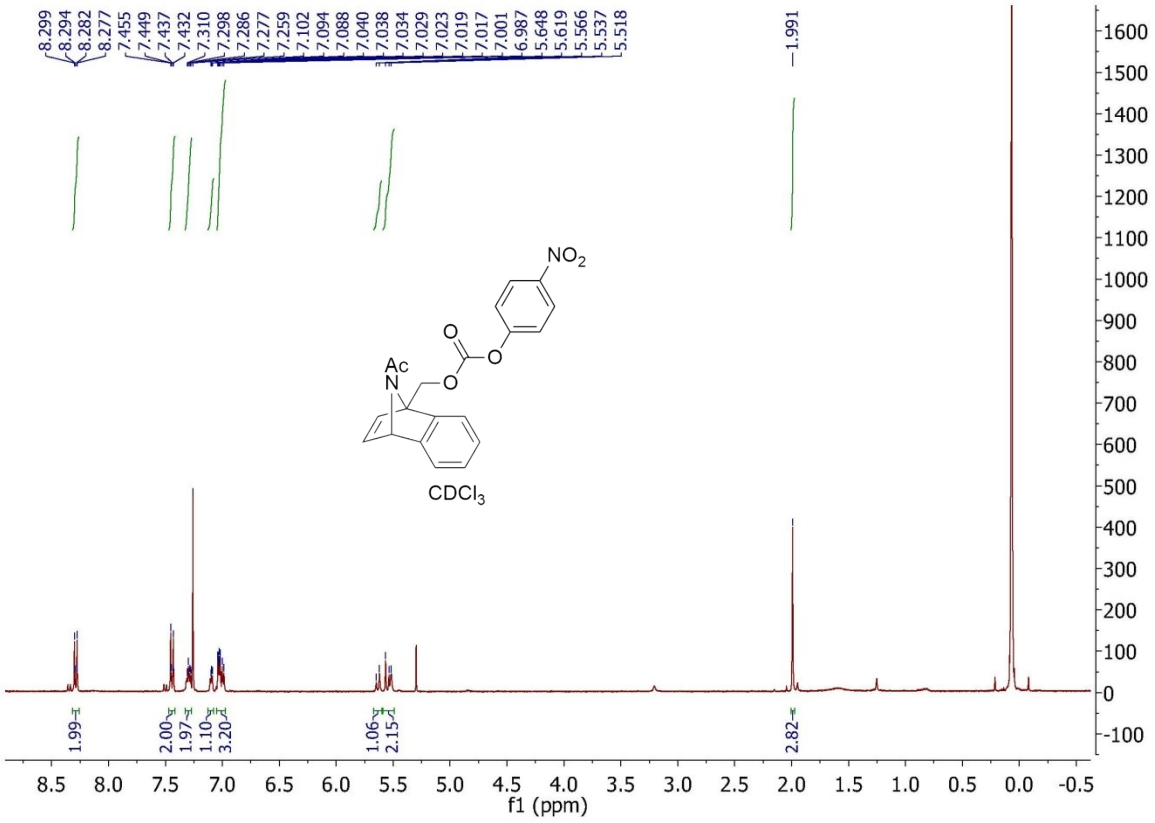

154.817
150.531
149.465
144.393
141.616
141.597
124.861
124.582
120.944
120.869
120.446
80.521
77.376
77.058
76.740
67.432
61.402
29.722
28.149
25.899
18.268
-5.316
-5.432
Boc
N
O
Si
CDCl3
ppm (f1)

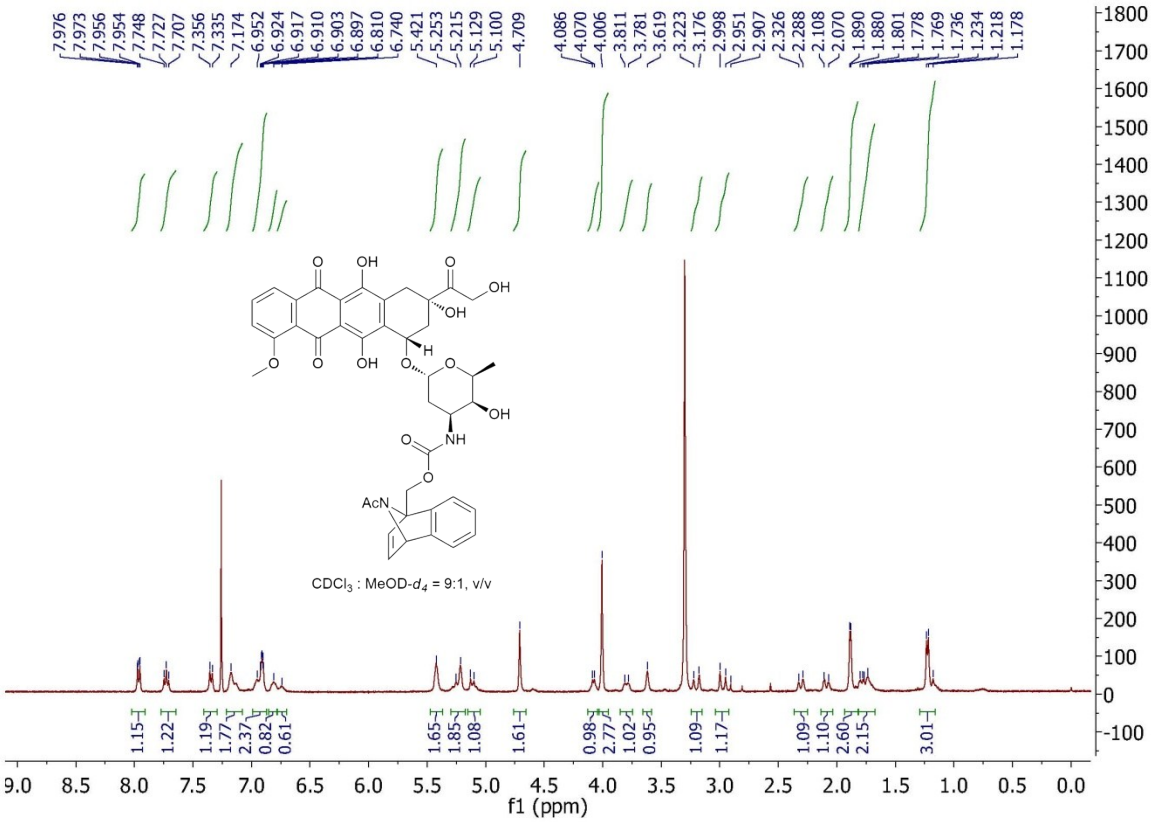

7.260
7.250
7.245
7.239
7.232
7.225
7.038
7.032
7.024
7.019
7.004
7.000
6.997
6.990
6.983
6.981
6.977
6.890
6.877
5.509
5.504
5.300
4.589
4.573
1.416
0.001
Boc
N
OH
CDCl3
1.69
3.00
0.79
0.86
2.15
10.46
ppm (f1)

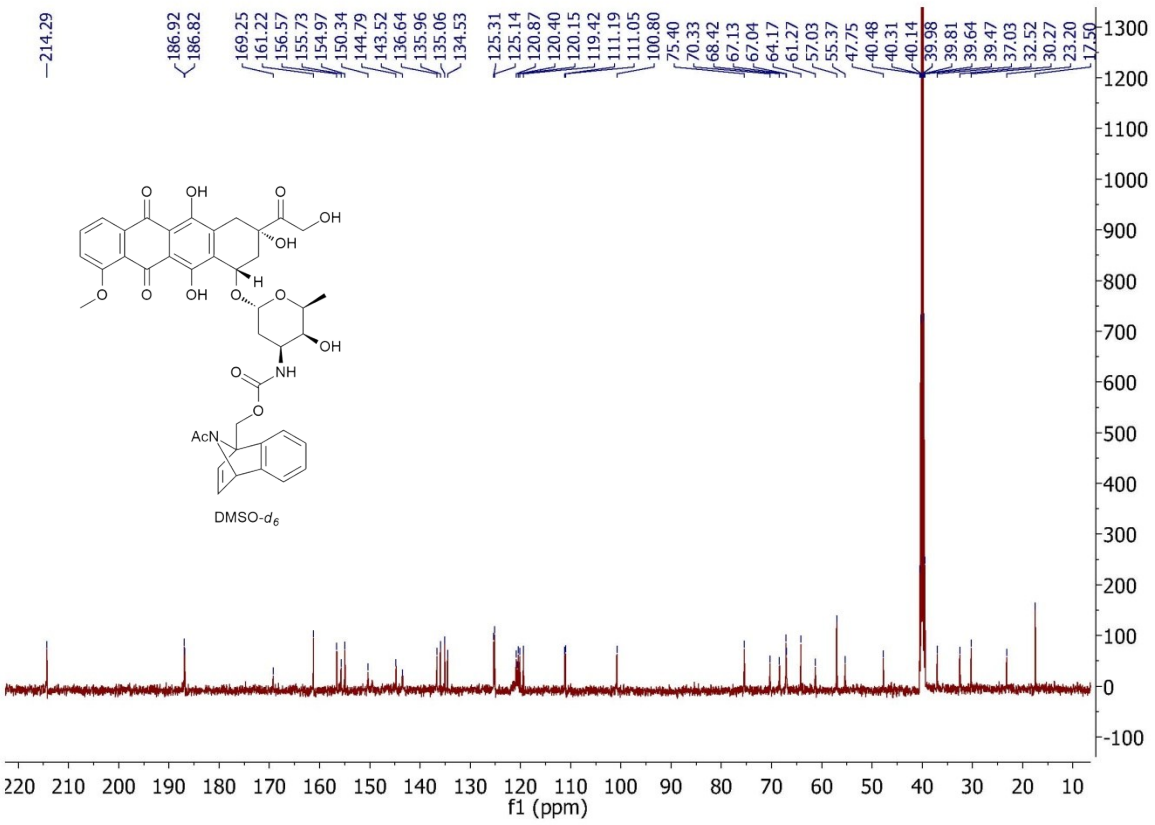

148.807
148.080
144.446
144.432
143.971
125.120
125.002
120.466
120.138
81.580
78.637
66.963
58.950
28.217
25.652
Boc
N
OH
CDCl3
ppm (t1)

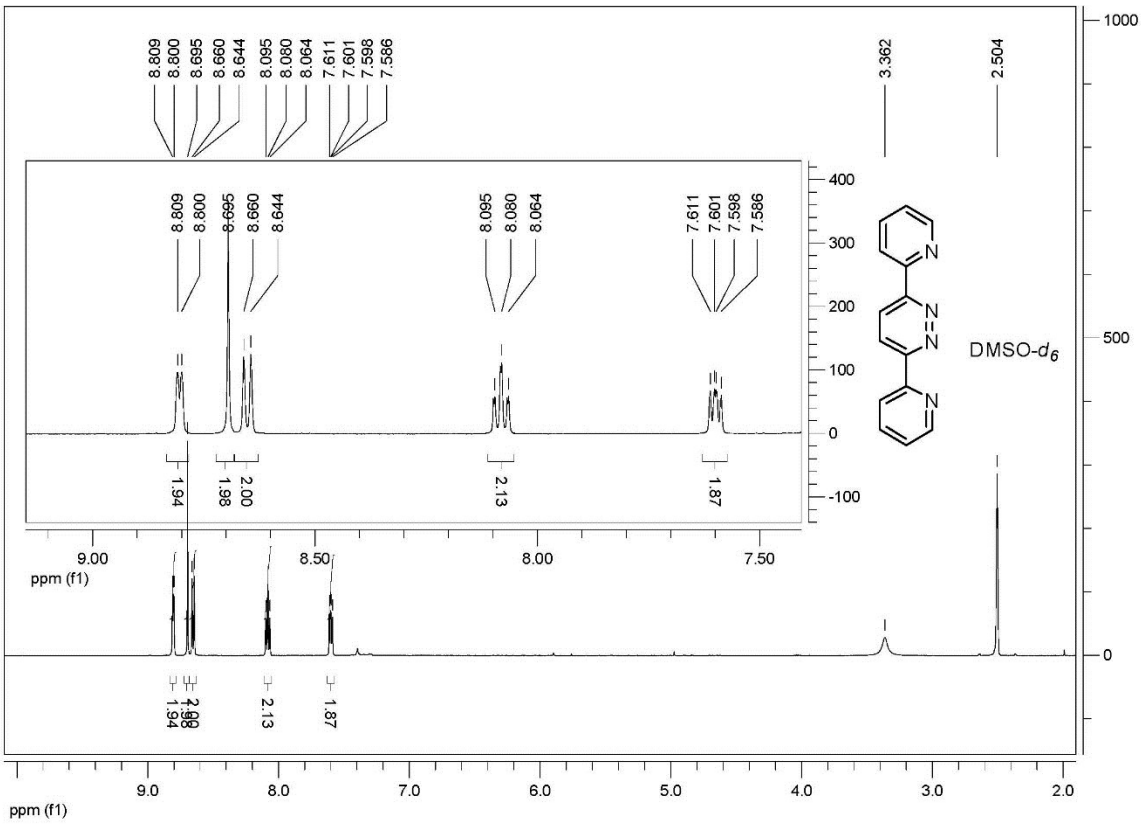

8.221
8.198
7.583
7.560
7.307
7.300
7.293
7.287
7.262
7.241
7.234
7.228
7.220
7.075
7.069
7.061
7.055
7.030
7.017
7.009
7.005
6.997
6.984
6.851
6.837
5.550
5.545
5.401
5.300
1.358
0.000
Boc
N
O
O
NH
O2N
ppm (f1)
7.50
7.00
1.91
2.00
0.93
0.89
1.97
9.51
5.60
5.50
5.40
5.30
5.20
0.89
1.67
2.00
0.96
1.88
0.95
2.05
0.93

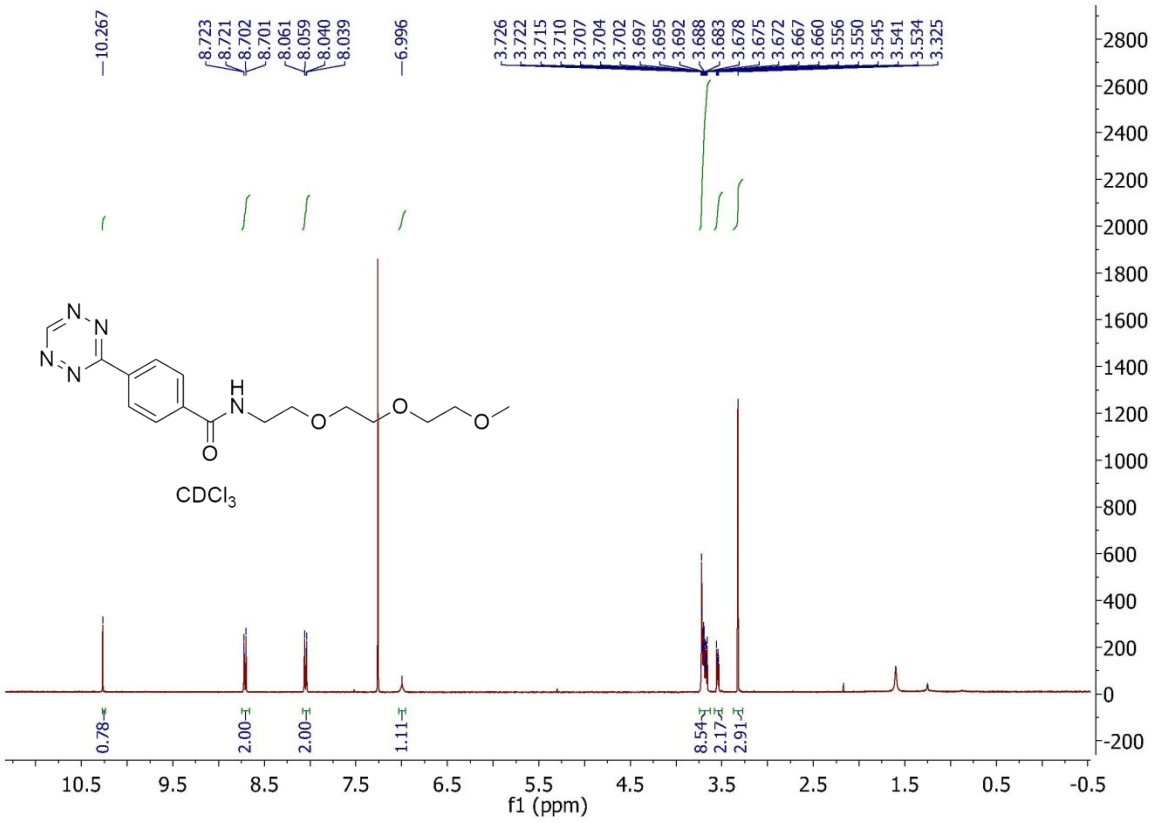

154.806
152.629
148.611
147.977
143.901
142.978
126.355
125.397
125.195
125.102
121.083
119.842
117.767
81.350
77.392
77.074
76.756
75.557
67.452
62.243
28.135
Boc
N
O
O
NH
O2N
ppm (f1)

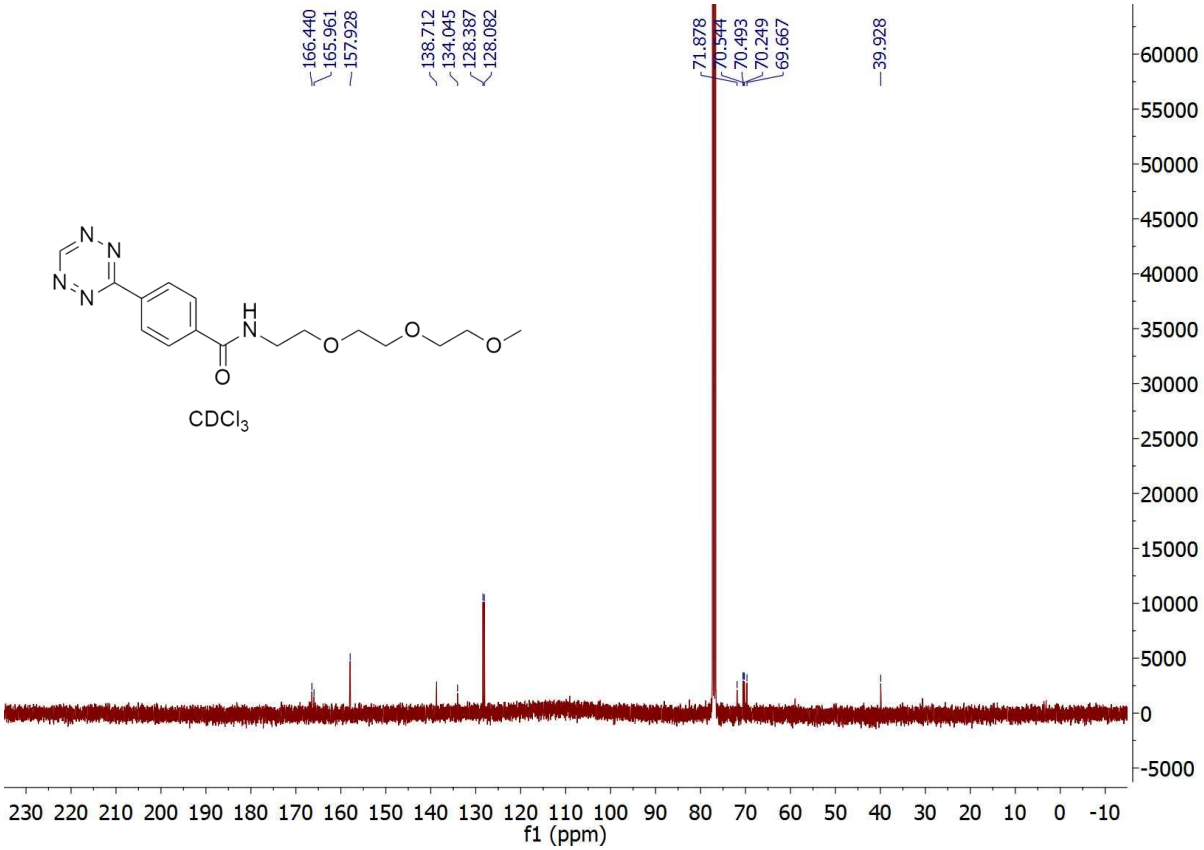

8.299
8.294
8.282
8.277
7.455
7.449
7.437
7.432
7.310
7.298
7.286
7.277
7.259
7.102
7.094
7.088
7.040
7.038
7.034
7.029
7.023
7.019
7.017
7.001
6.987
5.648
5.619
5.566
5.537
5.518
1.991
NO2
O
O
O
Ac
N
O
CDCl3
1.99
2.00
1.97
1.10
3.20
1.06
2.15
2.82
f1 (ppm)


7.976
7.973
7.956
7.954
7.748
7.727
7.707
7.356
7.335
7.174
6.952
6.924
6.917
6.910
6.903
6.897
6.810
6.740
5.421
5.253
5.215
5.129
5.100
4.709
4.086
4.070
4.006
3.811
3.781
3.619
3.223
3.176
2.998
2.951
2.907
2.326
2.288
2.108
2.070
1.890
1.880
1.801
1.778
1.769
1.736
1.234
1.218
1.178
CDCl3 : MeOD-d4 = 9:1, v/v
1.15
1.22
1.19
1.77
2.37
0.82
0.61
1.65
1.85
1.08
1.61
0.98
2.77
1.02
0.95
1.09
1.17
1.09
1.10
2.60
2.15
3.01
f1 (ppm)


214.29
186.92
186.82
169.25
161.22
156.57
155.73
154.97
150.34
144.79
143.52
136.64
135.96
135.06
134.53
125.31
125.14
120.87
120.40
120.15
119.42
111.19
111.05
100.80
75.40
70.33
68.42
67.13
67.04
64.17
61.27
57.03
55.37
47.75
40.48
40.31
40.14
39.98
39.81
39.64
39.47
37.03
32.52
30.27
23.20
17.50
DMSO-d6
f1 (ppm)


8.809
8.800
8.695
8.660
8.644
8.095
8.080
8.064
7.611
7.601
7.598
7.586
3.362
2.504
1.94
1.98
2.00
2.13
1.87
DMSO-d_6
ppm (f1)


10.267
8.723
8.721
8.702
8.701
8.061
8.059
8.040
8.039
6.996
3.726
3.722
3.715
3.710
3.707
3.704
3.702
3.697
3.695
3.692
3.688
3.683
3.678
3.675
3.672
3.667
3.660
3.556
3.550
3.545
3.541
3.534
3.325
CDCl3
0.78
2.00
2.00
1.11
8.54
2.17
2.91
f1 (ppm)


166.440
165.961
157.928
138.712
134.045
128.387
128.082
71.878
70.544
70.493
70.249
69.667
39.928
CDCl3
f1 (ppm)