SUPPORTING INFORMATION

Click, release, and fluoresce: a chemical strategy for a cascade prodrug system for co-delivery of carbon monoxide, a drug payload, and a fluorescent reporter

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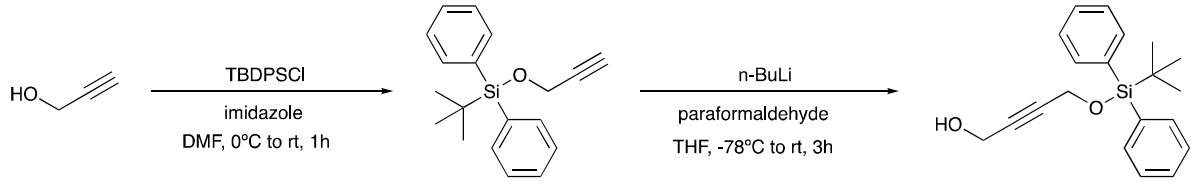
General experimental details

Unless otherwise noted, all reactions were carried out under nitrogen atmosphere using glassware that was previously oven-dried overnight with magnetic stirring and, all reagents were obtained from commercial suppliers (Sigma Aldrich, VWR International, and Oakwood Chemicals) and used without further purification. Thin layer chromatography was performed on glass-backed silica gel TLC plates (250 μm) using mixtures of hexanes/ethyl acetate as eluent, and using either UV light, iodine powder, or potassium permanganate stain for visualization. Column chromatography was done using Silica Flash P60 silica gel (230-400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on Bruker-400 spectrometers (400 MHz and 100 MHz, respectively). Chemical shifts were reported in ppm relative to residual solvent peaks (δ7.26 for ^{1}H, 77.1 for ^{13}C, $CHCl_3$/$CDCl_3$) and (δ 2.49 for ^{1}H, and 39.1 for ^{13}C, DMSO/DMSO-d_6). Data are reported as follows: bs= broad singlet, s= singlet, d= doublet, t= triplet, q= quartet, m= multiplet, dd= doublet of doublets, ddd= doublet of doublet of doublets, ddt= doublet of doublets of triplets, td= triplet of doublets; coupling constants in Hz; integration. Accurate mass measurements were acquired at the Mass Spectrometry Facilities at Georgia State University. For spectrophotometric studies, Shimadzu PharmaSpec UV-1700 was used as UV-Vis spectrophotometer; while Shimadzu RF-5301PC fluorimeter was used for fluorescent studies.

Synthesis of CO-drug conjugates



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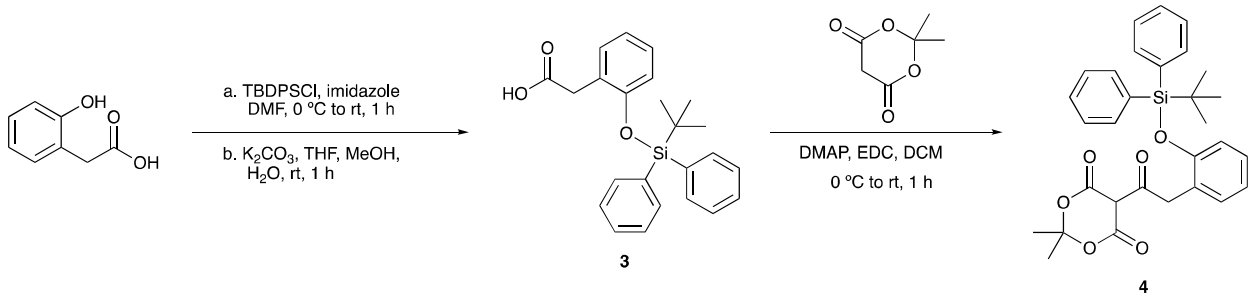
Compound 1: ***tert*-butyldiphenyl(prop-2-yn-1-yloxy)silane**. To a solution of prop-2-yn-1-ol (0.52 mL, 8.92 mmol) and imidazole (2.3 g, 26.8 mmol) in dry DMF (1.5 mL) cooled over an ice-bath, *tert*-butyldiphenylsilyl chloride (2.70 g, 9.81 mmol) was slowly added. Then, the reaction mixture was warmed to room temperature, and stirred for 1 h. Ethyl acetate was added followed by washing with saturated ammonium chloride solution (3 × 15 mL). The aqueous solution was extracted using ethyl acetate (2 × 25 mL). The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a yellow oil. The crude product was purified by silica gel column chromatography with an eluent consisting of hexanes only to give white solid (2.52 g, 96%). ^{1}H NMR ($CDCl_3$): δ = 7.79-7.76 (m, 4H), 7.51-7.42 (m, 6H), 4.37 (d, *J* = 2.4 Hz, 2H), 2.40 (t, *J* = 2.4 Hz, 1H), 1.13 (s, 9H). ^{13}C NMR ($CDCl_3$): δ =135.7,

133.1, 130.0, 127.9, 82.1, 73.2, 52.6, 26.8, 19.3. HRMS (ESI) calculated for $C_{19}H_{23}OSi$ $[M+H]^+$: m/z 295.1518, found 295.1511.

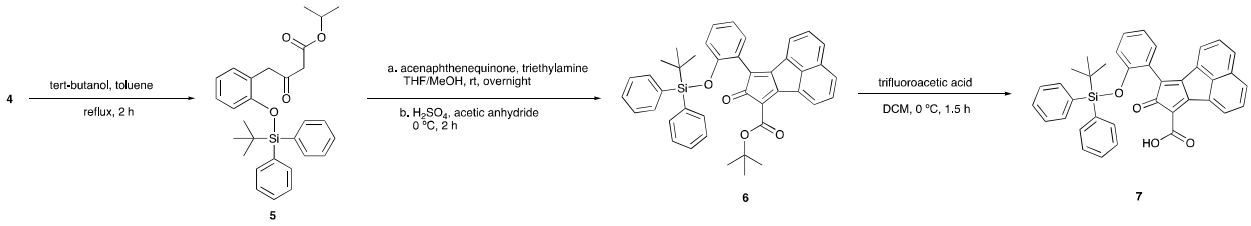
Compound 2: ***4*-((*tert*-butyldiphenylsilyl)oxy)but-2-yn-1-ol**. To a solution of compound **1** (1.32 g, 4.47 mmol) in dry THF was added dropwise *n*-butyllithium solution in THF (2.68 mL, 6.71 mmol, 2.5 M) at -78 °C under N_2. After stirring for 1 h, paraformaldehyde (403 mg, 13.41 mmol) was added with stirring at -78 °C. After 30 min, the reaction mixture was allowed to stir at room temperature for 3 h. After reaction completion, saturated ammonium chloride solution was added followed by extraction with ethyl acetate (3 × 30 mL). The combined organic layer was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure to yield a colorless oil. The crude product was purified by silica gel column chromatography with an eluent consisting of hexanes:ethyl acetate 100:30 to give clear and colorless oil (1.12 g, 77%). ^{1}H NMR ($CDCl_3$): δ = 7.76-7.74 (m, 4H), 7.48-7.40 (m, 6H), 4.40-4.39 (m, 2H), 4.22-4.21 (m, 2H), 1.88 (bs, 1H), 1.10 (s, 9H). ^{13}C NMR ($CDCl_3$): δ = 135.7, 133.1, 129.9, 127.8, 84.1, 83.6, 52.7, 51.1, 26.8, 19.2. HRMS (ESI) calculated for $C_{20}H_{24}O_2Si$ $[M+H]^+$: m/z 323.1462, found 323.1454.



Compound 3: **2-(2-((*tert*-butyldiphenylsilyl)oxy)phenyl)acetic acid**. To a solution of phenylacetic acid (3.0 g, 19.7 mmol) in dry DMF (6 mL) cooled over an ice-bath, imidazole (4 g, 58.8 mmol) and *tert*-butyldiphenylsilyl chloride (11.4 g, 41.5 mmol) were slowly added. After addition was complete, the reaction mixture was warmed to room temperature, and stirred for 12 h. Diethyl ether was added followed by washing with saturated ammonium chloride solution. The aqueous solution was extracted using diethyl ether (3 x 25 mL). The combined organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give yellowish solids. The crude product was dissolved in THF followed by addition of K_2CO_3 (5.45 g, 39.4 mmol). A small amount of water was added to dissolve K_2CO_3. Then methanol was added to make a homogenous solution. After overnight stirring, THF and methanol were removed by rotary evaporation to give a cloudy residue. Water was added and the organic layer was separated. The aqueous layer was extracted using ethyl acetate (3 × 30 mL). The organic layer was saved while the aqueous layer was acidified and extracted with ethyl acetate (3 × 15 mL). The two organic layers were combined, washed with water and then brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with an eluent consisting of hexanes and ethyl acetate in a 100:15 ratio to give creamy white solid (4.14 g, 54%). ^{1}H NMR ($CDCl_3$): δ = 7.77-7.75 (m, 4H), 7.46-7.37 (m, 6H), 7.24-7.22 (m, 1H), 6.87-6.85 (m, 2H), 6.44-6.43 (m, 1H), 3.89 (s, 2H), 1.09 (s, 9H). ^{13}C NMR ($CDCl_3$): δ = 178.4, 153.8, 135.5, 132.4, 131.1, 130.1, 128.4, 128.0, 124.1, 121.1, 119.0,

36.4, 26.4, 19.4. HRMS (ESI) calculated for $C_{24}H_{26}O_3SiNa$ $[M+Na]^+$: m/z 413.1549, found 413.1546.

Compound 4: **5-(2-(2-((*tert*-butyldiphenylsilyl)oxy)phenyl)acetyl)-2,2-dimethyl-1,3-dioxane-4,6-dione**. To a solution of compound **3** (2.0 g, 5.1 mmol), meldrum's acid (1.1 g, 7.68 mmol), and dimethylaminopyridine (1.6 g, 7.68 mmol) in dichloromethane was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in small portions while the reaction was being stirred at 0 °C. After stirring for 2 h, the reaction mixture was diluted with 0.5 N HCl (50 mL) and then extracted with dichloromethane (3 × 30 mL). The organic layer was washed with water (2 × 25 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with an eluent consisting of hexanes and ethyl acetate in a 100:2 ratio to give yellow oil (1.40 g, 53%). ^{1}H NMR ($CDCl_3$): δ = 15.77 (s, 1H), 7.76-7.74 (m, 4H), 7.45-7.38 (m, 6H), 7.21 (d, *J* = 4.4 Hz, 1H), 6.87 (t, *J* = 4.0 Hz, 2H), 6.46 (d, *J* = 9.2 Hz, 1H), 4.72 (s, 2H), 1.79 (s, 6H), 1.10 (s, 9H). ^{13}C NMR ($CDCl_3$): δ = 196.5, 170.9, 160.5, 153.8, 135.4, 132.1, 131.1, 130.1, 128.5, 128.0, 123.8, 121.1, 118.9, 105.1, 91.2, 38.0, 27.0, 26.4, 19.4. HRMS (ESI) calculated for $C_{30}H_{32}O_6SiNa$ $[M+Na]^+$: m/z 539.1866, found 539.1851.

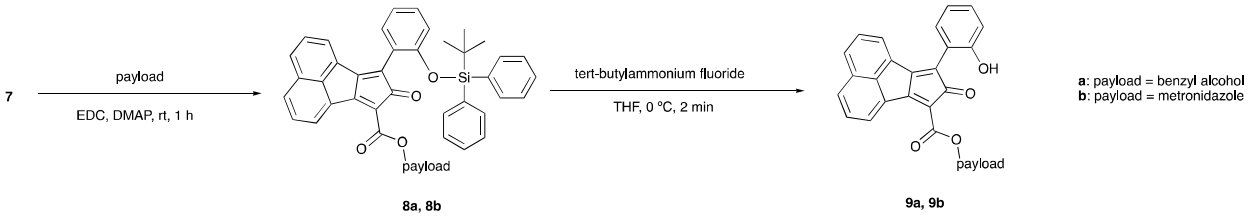


Compound 5: ***tert*-butyl 4-(2-((*tert*-butyldiphenylsilyl)oxy)phenyl)-3-oxobutanoate.** To a solution of compound **4** (3.00 g, 5.8 mmol) in toluene (15 mL) was added isopropyl alcohol (547 mg, 11.61 mmol). The reaction mixture was allowed to stir for 30 min under reflux at 110 °C. Toluene was removed by rotary evaporation and the colorless oily crude product (a mixture of keto-enol tautomers) was used without purification in the next step. ^{1}H NMR ($CDCl_3$): δ = 12.59 (s, 0.15H), 7.84-7.82 (m, 4.19H), 7.49-7.41 (m, 6.28H), 7.25-7.22 (m, 1.11H), 6.91-6.84 (m, 2.09H), 6.56-6.51 (m, 1.05H), 4.91 (s, 0.156), 4.07 (s, 1.71H), 3.81 (s, 0.325H), 3.56-3.54 (m, 1.71H), 1.57-1.54 (m, 9H), 1.18-1.17 (m, 9.33H). ^{13}C NMR ($CDCl_3$): δ = 200.7, 177.0, 172.7, 170.8, 166.4, 153.6, 153.5, 135.3, 132.3, 132.1, 131.4, 131.2, 130.0, 129.9, 128.2, 127.9, 127.8, 125.4, 124.2, 121.2, 120.9, 119.0, 118.9, 90.9, 81.5, 80.5, 60.2, 49.6, 45.4, 36.2, 28.3, 27.9, 26.4, 26.2, 20.9, 19.3, 14.1. HRMS (ESI) calculated for $C_{30}H_{36}O_4SiNa$ $[M+Na]^+$: m/z 511.2281, found 511.2297.

Compound 6: ***tert*-butyl 9-(2-((*tert*-butyldiphenylsilyl)oxy)phenyl)-8-oxo-8*H*-cyclopenta[*a*]acenaphthylene-7-carboxylate**. To a solution of compound **5** (2.3 g, 4.66 mmol) in THF (30 mL) was added acenaphthylene-1,2-dione (934 mg, 5.13 mmol). Then methanol (10 mL) was added followed by triethylamine (711 mg, 6.99 mmol). After 24 h, the reaction mixture was dried in the rotary evaporator. The residue was dissolved in acetic anhydride (20 mL), allowed to stir in an ice-bath; and then four drops of sulfuric acid was added. After stirring for 5 min, methanol and diethyl ether was added while stirring over an ice-bath. Then the mixture was filtered via

suction filtration. The purple filtrate was concentrated *in vacuo* and then the residue was dissolved in ethyl acetate, washed with water, saturated $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give purple solid, which was used without further purification (2.0 g, 67%). ^{1}H NMR ($CDCl_3$): δ = 8.74 (d, J = 7.2 Hz, 1H), 8.01-7.99 (m, 1H), 7.84-7.82 (m, 1H), 7.77-7.73 (m, 1H), 7.67-7.55 (m, 5H), 7.54-7.50 (m, 1H), 7.43-7.27 (m, 5H), 7.19-7.16 (m, 2H), 7.03-6.98 (m, 2H), 6.68-6.66 (m, 1H), 1.69 (s, 9H), 0.77 (s, 9H). ^{13}C NMR ($CDCl_3$): δ = 197.0, 168.1, 162.3, 154.4, 145.3, 135.8, 135.5, 132.5, 131.6, 131.5, 130.8, 130.3, 129.7, 128.9, 128.5, 127.9, 127.7, 127.3, 122.9, 122.4, 121.3, 120.3, 81.6, 28.6, 26.6, 19.4. HRMS (ESI) calculated for $C_{42}H_{39}O_4Si$ $[M+H]^+$: m/z 635.2618, found 635.2622.

Compound 7: **9-(2-((*tert*-butyldiphenylsilyl)oxy)phenyl)-8-oxo-8*H*-cyclopenta[*a*]acenaphthylene-7-carboxylic acid**. To a solution of compound **6** (1.50 g, 2.36 mmol) in dichloromethane (24 mL) was added trifluoroacetic acid (12 mL) while the reaction was being stirred at 0°C. The reaction mixture was allowed to stir for 1.5 h and then concentrated *in vacuo* to give a green residue. The crude product was purified by silica gel column chromatography with an eluent consisting of dichloromethane only to give green solid (1.17 g, 85%). ^{1}H NMR ($CDCl_3$): δ = 10.44 (bs, 1H), 8.84 (d, *J* = 7.2 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.83-7.79 (m, 1H), 7.68 (d, *J* = 6.8 Hz, 1H), 7.63-7.59-7.54 (m, 5H), 7.43-7.40 (m, 2H), 7.31 (bs, 3H), 7.18 (bs, 2H), 7.11-7.07 (m, 1H), 7.04-7.00 (m, 1H), 6.76-6.74 (m, 1H), 0.82 (s, 9H). ^{13}C NMR ($CDCl_3$): δ = 203.2, 171.2, 161.5, 155.0, 154.4, 146.7, 135.6, 132.1, 131.6, 131.0, 130.6, 130.4, 130.1, 129.7, 129.6, 129.5, 128.7, 128.2, 127.9, 123.4, 123.0, 121.6, 121.3, 120.7, 107.7, 26.6,19.5. HRMS (ESI) calculated for $C_{38}H_{30}O_4SiNa$ $[M+Na]^+$: m/z 601.1811, found 601.1833.



General procedure for synthesis of compound 8: To a solution of compound **7** (1 eq), drug (1.5 eq), and *N*,*N*-dimethylaminopyridine (0.3 eq) in dry dichloromethane (10 mL), was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.3 eq) while the mixture was being stirred at room temperature. For work-up, the reaction mixture was diluted with ethyl acetate, washed with 10% HCl and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give **8a** and **8b**.

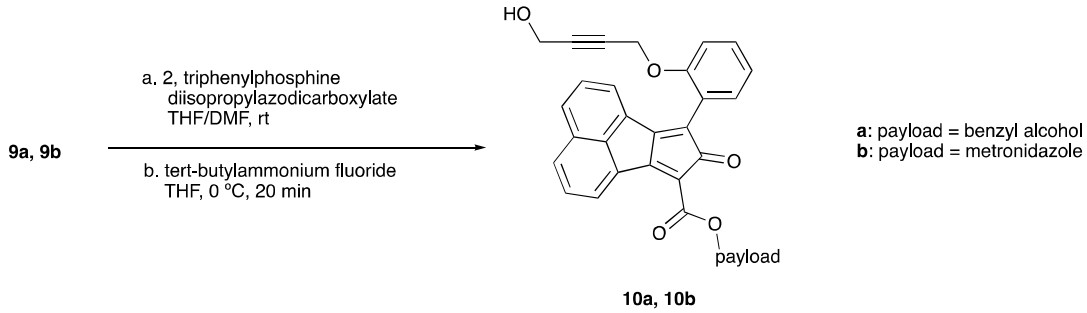
Compound **8a**: **benzyl 9-(2-((*tert*-butyldiphenylsilyl)oxy)phenyl)-8-oxo-8*H*-cyclopenta[*a*]acenaphthylene-7-carboxylate.** Purple solid (382 mg, 85%). ^{1}H NMR ($CDCl_3$): δ = 8.65 (d, *J* = 7.2 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.70-7.63 (m, 8H), 7.56-7.52 (m, 1H), 7.49-7.41 (m, 3H), 7.39-7.31 (m, 5H), 7.24-7.20 (m, 2H), 7.08-7.04 (m, 2H), 6.77-6.74 (m, 1H), 5.58-5.49 (m, 2H), 0.80 (s, 9H). ^{13}C NMR ($CDCl_3$): δ = 196.7, 169.7, 162.5, 154.4, 152.8, 145.3, 136.3, 135.7, 135.4, 133.0, 132.3, 131.4, 130.8, 130.3, 129.9, 128.9, 128.7, 128.5, 128.3, 128.2, 128.2, 127.8, 127.7, 127.4, 123.0, 122.6, 122.2, 121.3, 120.3, 110.4, 66.2, 26.5, 19.3. HRMS (ESI) calculated for $C_{45}H_{37}O_4Si$ $[M+H]^+$: m/z 669.2461, found 669.2478.

Compound **8b**: **2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl9-(2((*tert*butyldiphenylsilyl)oxy) phenyl)-8-oxo-8*H*-cyclopenta[*a*]acenaphthylene-7-carboxylate.** Purple solid (108 mg, 85%). ^{1}H NMR ($CDCl_3$): δ = 8.60 (d, J = 7.2 Hz, 1H), 8.03-798 (m, 2H), 7.83 (d, J = 8.4 Hz, 1H), 7.723 (t, *J* = 8.0 Hz, 1H), 7.65-7.61 (m, 3H), 7.57-7.496 (m, 3H), 7.40-7.36 (m, 2H), 7.32-7.26 (m, 3H), 7.19-7.15 (m, 2H), 7.04-6.98 (m, 2H), 6.68 (d, *J* = 7.6 Hz, 1H), 4.79-4.68 (m, 4H), 2.62 (s, 3H), 0.75 (s, 9H). ^{13}C NMR (DMSO-d_6): δ = 196.3, 171.8, 162.4, 154.4, 152.7, 152.1, 145.4, 138.5, 135.7, 135.4, 133.6, 132.8, 132.4, 131.5, 131.4, 130.1, 129.99, 129.85, 129.1, 128.6, 127.9, 127.7, 127.6, 123.1, 122.9, 122.93, 121.98, 121.4, 120.3, 109.5, 63.0, 45.6, 26.5, 19.40, 14.6. HRMS (ESI) calculated for $C_{44}H_{37}N_3O_6SiNa$ $[M+Na]^+$: m/z 754.2349, found 754.2329.

General procedure for the synthesis of compound 9. To a solution of compound **8** (1 eq) in dry THF protected with a nitrogen balloon was added *tert*-butylammonium fluoride (1.05 eq, 1.0 M in THF) with stirring at room temperature. After a few minutes, reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was washed with a small amount of cold methanol and then filtered to give **9a** and **9b**.

Compound **9a**: **benzyl 9-(2-hydroxyphenyl)-8-oxo-8*H*-cyclopenta[*a*]acenaphthylene-7-carboxylate.** Green solid (93 mg, 72%). ^{1}H NMR (DMSO-d_6): δ = 10.02 (s, 1H), 8.46 (d, *J* = 7.2 Hz, 1H), 8.17 (d, *J* = 8.4 Hz, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.73-7.68 (m, 2H), 7.55-7.41 (m, 3H), 7.39-7.30 (m, 5H), 7.04 (d, *J* = 8.0 Hz, 1H), 6.94 (t, *J* = 7.6 Hz, 1H), 5.41 (s, 2H). ^{13}C NMR (DMSO-d_6): δ = 196.6, 169.4, 161.4, 156.4, 150.9, 144.3, 136.3, 131.6, 131.5, 131.0, 130.4, 130.2, 129.2, 128.9, 128.5, 128.1, 127.8, 127.3, 124.0, 120.9, 118.8, 117.1, 115.9, 109.1, 65.5. HRMS (ESI) calculated for $C_{29}H_{18}O_4$ $[M+H]^+$: m/z 431.1283, found 431.1278.

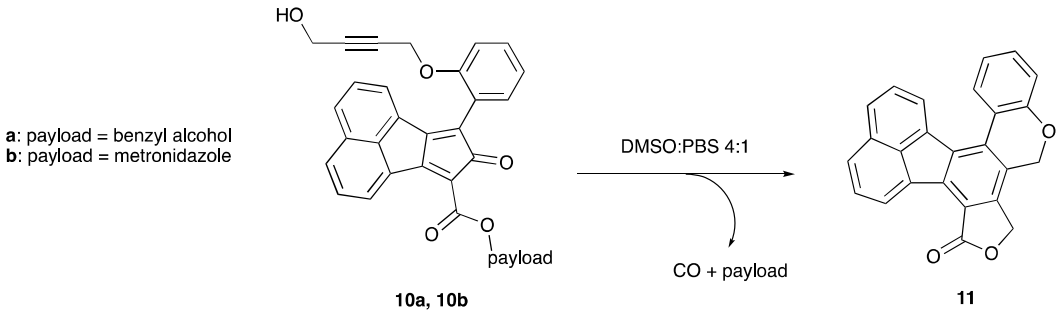
Compound **9b**: **2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 9-(2-hydroxyphenyl)-8-oxo-8*H*-cyclopenta[*a*]acenaphthylene-7-carboxylate.** Green solid (183 mg, 87%). ^{1}H NMR (DMSO-d_6): δ = 10.07 (s, 1H), 8.46 (d, *J* = 7.2 Hz, 1H), 8.24 (d, *J* = 8.4 Hz, 1H), 8.05-8.03 (m, 2H), 7.82-7.78 (m, 1H), 7.75-7.71 (m, 1H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.35-7.30 (m, 2H), 7.05 (d, *J* = 8.0 Hz, 1H), 6.95 (t, *J* = 7.6 Hz, 1H), 4.72 (s, 4H), 2.51 (s, 3H). ^{13}C NMR (DMSO-d_6): δ = 196.3, 170.4, 161.4, 156.4, 151.9, 150.7, 144.3, 138.6, 133.2, 131.7, 131.6, 131.0, 130.4, 130.2, 129.1, 129.0, 127.9, 127.4, 124.1, 120.8, 118.8, 117.1, 116.0, 108.8, 62.3, 45.1. HRMS (ESI) calculated for $C_{28}H_{20}N_3O_6$ $[M+H]^+$: m/z 494.1352, found 494.1337.



General procedure for the synthesis of compound 10: To a solution of compound **9** (1 eq), compound **2** (1.5 eq), and triphenylphosphine (1.5 eq) dissolved in dry DMF:THF 3:10 was added diisopropylazodicarboxylate (1.5 eq) dropwise with stirring at room temperature. After 15 min, the reaction mixture was concentrated *in vacuo* and then dissolved in dichloromethane, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo* at below 20 °C. The residue was loaded into a short column and then eluted using dichloromethane only first and then CH_2Cl_2:EtOAc 100:30. The violet fractions were combined and then concentrated *in vacuo* to give purple solids. These solids were dissolved in THF (3 mL) and then *tert*-butylammonium fluoride (1.1 eq, 1.0 M in THF) was added while the mixture was stirred at room temperature. After 20 min, the reaction mixture was concentrated *in vacuo* and directly loaded into a silica gel column using CH_2Cl_2:EtOAc 100:50 as eluent.

Compound **10a**: **benzyl 9-(2-((4-hydroxybut-2-yn-1-yl)oxy)phenyl)-8-oxo-8*H*cyclopenta[*a*]acenaphthylene-7-carboxylate.** Dark purple solid (35 mg, 32%).^{1}H NMR (DMSO-d_6): δ = 8.49 (d, *J* = 7.2 Hz, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.75-7.70 (m, 2H), 7.56-7.48 (m, 4H), 7.45-7.35 (m, 4H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.13 (t, *J* = 7.6 Hz, 1H), 5.41 (s, 2H), 5.23 (t, *J* = 6.0 Hz, 1H), 4.86 (s, 2H), 4.05 (d, *J* = 6.0 Hz, 2H), 4.87 (s, 2H), 4.72 (s, 4H), 4.07 (s, 2H). ^{13}C NMR (DMSO-d_6): δ = 169.2, 161.3, 155.8, 151.7, 144.4, 136.2, 131.9, 131.6, 131.1, 130.4, 129.8, 129.1, 128.9, 128.6, 128.1, 127.8, 127.7, 124.1, 120.7, 119.0, 112.8, 109.2, 87.6, 78.9, 65.6, 55.8, 48.9. HRMS (ESI) calculated for $C_{33}H_{23}O_5$ $[M+H]^+$: m/z 499.1545, found 499.1561.

Compound **10b**: **2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 9-(2-((4-hydroxybut-2-yn-1-yl)oxy)phenyl)-8-oxo-8*H*-cyclopenta[*a*]acenaphthylene-7-carboxylate.** Dark purple solid (35 mg, 32%). ^{1}H NMR (DMSO-d_6): δ = 8.44 (d, *J* = 7.2 Hz, 1H), 8.23 (d, *J* = 8.0 Hz, 1H), 8.06-8.04 (m, 2H), 7.81-7.78 (m, 1H), 7.74-7.70 (m, 1H), 7.56 (d, *J* = 6.8 Hz, 1H), 7.54-7.49 (m, 1H), 7.39 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.16-7.12 (m, 1H), 5.26 (s, 1H), 4.87 (s, 2H), 4.72 (s, 4H), 4.07 (s, 2H), 2.51 (s, 3H). ^{13}C NMR (DMSO-d_6): δ = 195.9, 170.2, 161.3, 155.8, 151.9, 151.6, 144.4, 138.6, 133.2, 131.9, 131.8, 131.1, 130.5, 130.0, 129.2, 129.1, 129.0, 127.9, 127.7, 124.1, 120.8, 120.0, 118.9, 112.8, 108.9, 87.6, 78.9, 62.4, 55.8, 48.9, 45.1, 14.0. HRMS (ESI) calculated for $C_{32}H_{24}N_3O_7$ $[M+H]^+$: m/z 562.1614, found 562.1581.



Characterization of cyclized product 11 from prodrugs 10a and 10b

Compound **11**: **6,7-dihydro-9*H*-furo[3',4':9,10]fluorantheno[8,7-*c*]chromen-9-one.** Compounds **10a** or **10b** (10 mg) were dissolved in DMSO and then diluted with PBS. The solution was incubated at 37 °C. After 24 h, the purple color of the solution disappeared and the reaction mixture exhibited blue fluorescence under 365 nm of light. Water was added to precipitate the cyclized product fully followed by ethyl acetate extraction. The organic layer was washed with water, dried over sodium sulfate, and concentrated under reduced pressure to give a yellow solid. The crude solid was purified via column chromatography using hexane:EtOAc as eluent to give yellow solids. ^{1}H NMR ($CDCl_3$): δ = 9.25 (d, *J* = 7.2 Hz, 1H), 8.46 (d, *J* = 7.2 Hz, 1H), 8.42 (d, *J* = 7.6 Hz, 1H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.80-7.76 (m, 1H), 7.60-7.56 (m, 1H), 7.47-7.43 (m, 1H), 7.23-7.21 (m, 2H), 5.42 (s, 2H), 5.04 (s, 2H). ^{13}C NMR ($CDCl_3$): δ = 157.0, 140.7, 139.6,135.9, 135.2, 133.7, 132.6, 131.3, 130.0, 129.1, 128.7, 128.7, 128.4, 128.3, 127.4, 124.3, 123.3, 122.1, 119.6, 118.4, 68.9, 65.9. HRMS (ESI) calculated for $C_{25}H_{15}O_3$ $[M+H]^+$: m/z 363.1021, found 363.1013.

CO-release kinetics: spectrofluorometric studies

Because the by-product after CO release is fluorescent, the release kinetics of the CO-drug conjugate can be conveniently studied by monitoring the fluorescence intensity of the solution. The kinetic studies were performed using slit width of 3 nm. First, the emission and excitation spectra and wavelength of cyclized product **11** were obtained (Figure S1a). Using 355 nm and 460 nm as excitation and emission wavelengths, respectively, the CO release rate of model prodrug **10a** (25 μM, 4:1 DMSO:PBS) was measured. Data points were collected every 15 min for the first hour to every 30 min in the succeeding hours. To determine the first order reaction rate constant, the fluorescence intensity was plotted against time and the obtained curve was processed using Sigmaplot using the exponential growth to maximum function with 3 parameters.

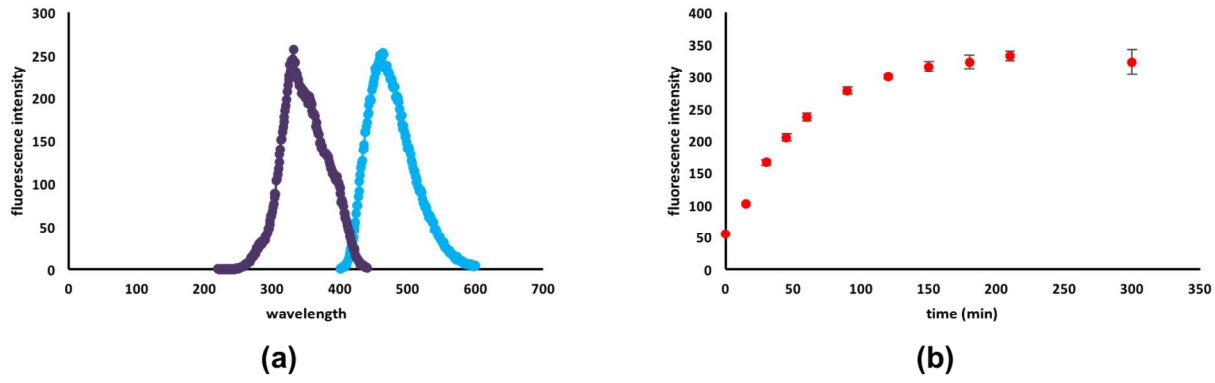


Fig. S1 *Increase in fluorescence intensity as cyclized product is formed. (n=3)*

Detection of CO release: the CO-myoglobin assay

Direct detection of carbon monoxide release was done through a "two-compartment" Mb-CO assay developed earlier.[1] The set-up was assembled by putting a small vial inside a bigger vial and sealing the system. The bigger vial contains the deoxy-Mb solution while the small vial contains the CO-drug conjugate in DMSO/PBS. The deoxy-Mb solution was prepared by degassing a solution of myoglobin in PBS (1 mg/mL, pH = 7.4) with nitrogen for at least 20 min, and then converted to deoxy-Mb by adding freshly prepared solution of sodium dithionite (22 mg/mL). Then a solution of CO-drug conjugate in DMSO was added to the inner vial containing PBS via a syringe. The whole set-up was then incubated at 37 ° C. At the end of 6 h, the set-up was cooled in an ice-bath for 10 min to increase the solubility of CO gas in water, after which, the incubated solution was immediately transferred into a cuvette for UV-Vis spectral measurements.

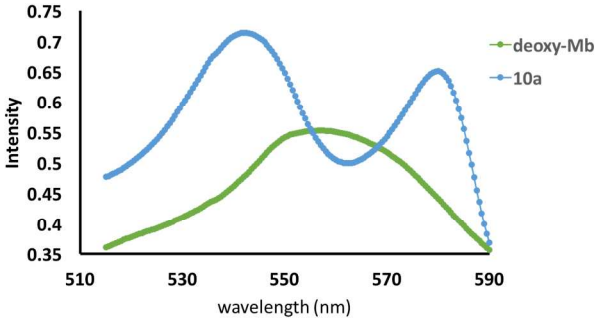


Fig. S2 Conversion of deoxy-Mb to CO-Mb in the presence of compound 10a. (n=3)

Initial studies on the mechanism of release: NMR studies

As initial mechanistic studies, the release of a model prodrug from **10a** was studied using NMR. At time 0, only DMSO was used as solvent to prevent cyclization. Then deuterated PBS was

added to make a final concentration of approximately 10 mM. The prodrug solution was incubated at 37 °C for 24 h. At indicated time points, the proton NMR spectrum of the solution was taken. After 24 h, most of the cyclized product precipitated out so more DMSO was added for a well-resolved NMR spectrum. Shown below are the full NMR spectra of the prodrug **10a** solution taken at different time points.

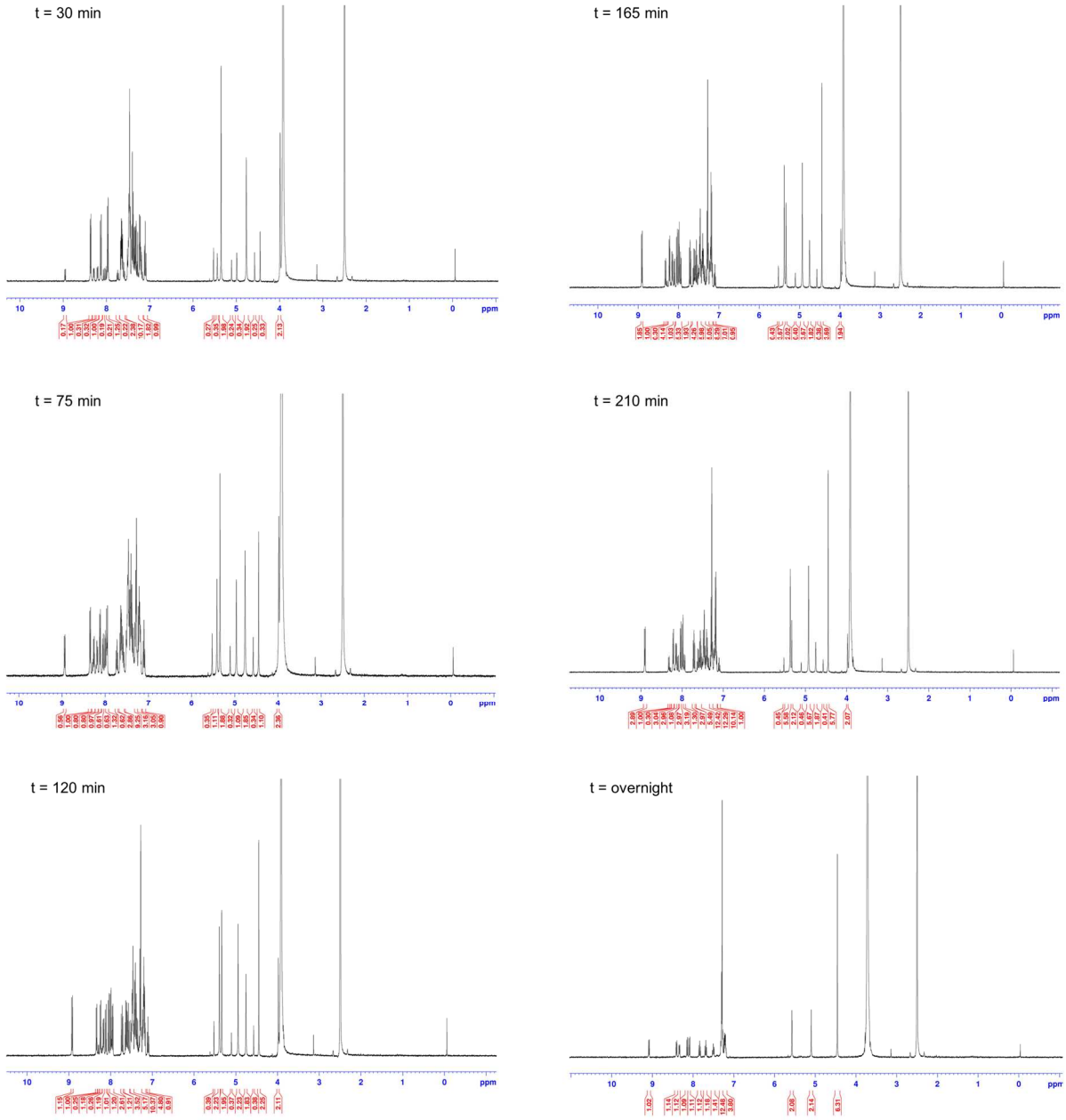


*Fig. S3 NMR spectra of the incubated prodrug **10a** solution at different time points.*

Verification of the release of the three components (CO, drug, and 11): RP-HPLC
Samples injected:

- mixture containing 25 μM each of prodrug **10b**, metronidazole, and cyclized product **11**
- incubated solution of prodrug **10b** (25 μM) dissolved in 4:1 DMSO:PBS at 37 °C at different time points
- different concentration of the drug metronidazole (0, 5, 10, 15, 20, 25, and 30 μM) for the calibration curve

A 200 μL aliquot of incubated sample was placed inside a 0.5 mL vial followed by dilution with 200 μL of acetonitrile. The study was performed using the Shimadzu Prominence UFLC with a reversed-phase analytical column (Waters C18 3.5 μM, 4.6 x 100 mm) at 25 °C. The flow rate was set at 0.5 mL/min. Gradient elution using acetonitrile and deionized water was used to elute out the components of the sample. Elution conditions: 0-6 min 5% acetonitrile, 6-12 min 70% acetonitrile, 12-30 min 70% acetonitrile; injection volume of 20 μL; detection wavelength of 254 and 280 nm. Experiments were done in triplicates.

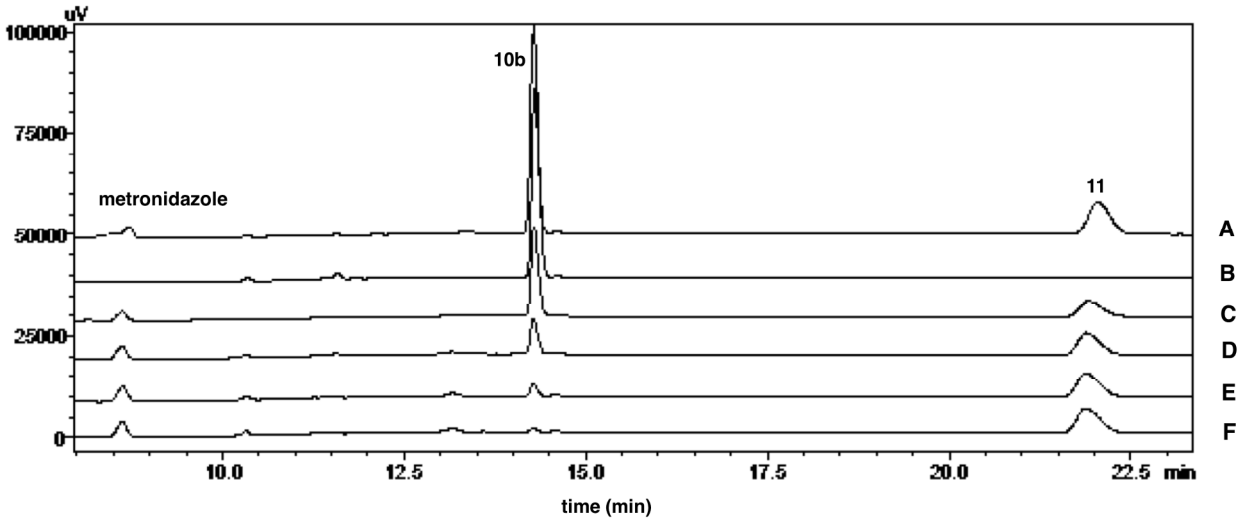


Fig. S4 (A) Mixture of 25 μM each of prodrug 10b, 11, and metronidazole. (B-F) HPLC chromatogram after incubation of 25 μM of 10b in PBS buffer, pH 7, 37 ℃ at time 0 h (B), 1 h (C), 2 h (D), 4 h (E), 6 h (F).

Verification of CO release: fluorescent imaging in cell culture using either the fluorescent by-product or the probe COP-1

Raw 264.7 cells were seeded on coverslips in 6-well plates one day before the experiment. The CO-drug conjugate dissolved in DMSO was added to the cell culture media to give a final concentration of 20 μM or 40 μM with 1% DMSO. In two other wells, the same was done with the addition of COP-1 probe with a final concentration of 1 μM. The cells were incubated with CO-drug conjugate for 6 hours at 37 °C. Then, the cells were washed with PBS twice and fixed with 4% paraformaldehyde for 30 minutes at room temperature. The cells were then washed with PBS again twice and the coverslips with cells were immersed in DI water. The coverslips were mounted onto glass slides using the mounting media without DAPI (ProLong® Live Antifade Reagent; P36974). The fluorescent imaging was performed on a Zeiss fluorescent microscope, using DAPI imaging channel (excitation: 358 nm, emission: 461 nm) and FITC channel (excitation: 490 nm, emission: 525 nm) for the fluorescence of COP-1. The

concentration-dependent images were taken using the oil objective (40x). Experiments for compound 11 and COP-1 probe were done side-by-side.

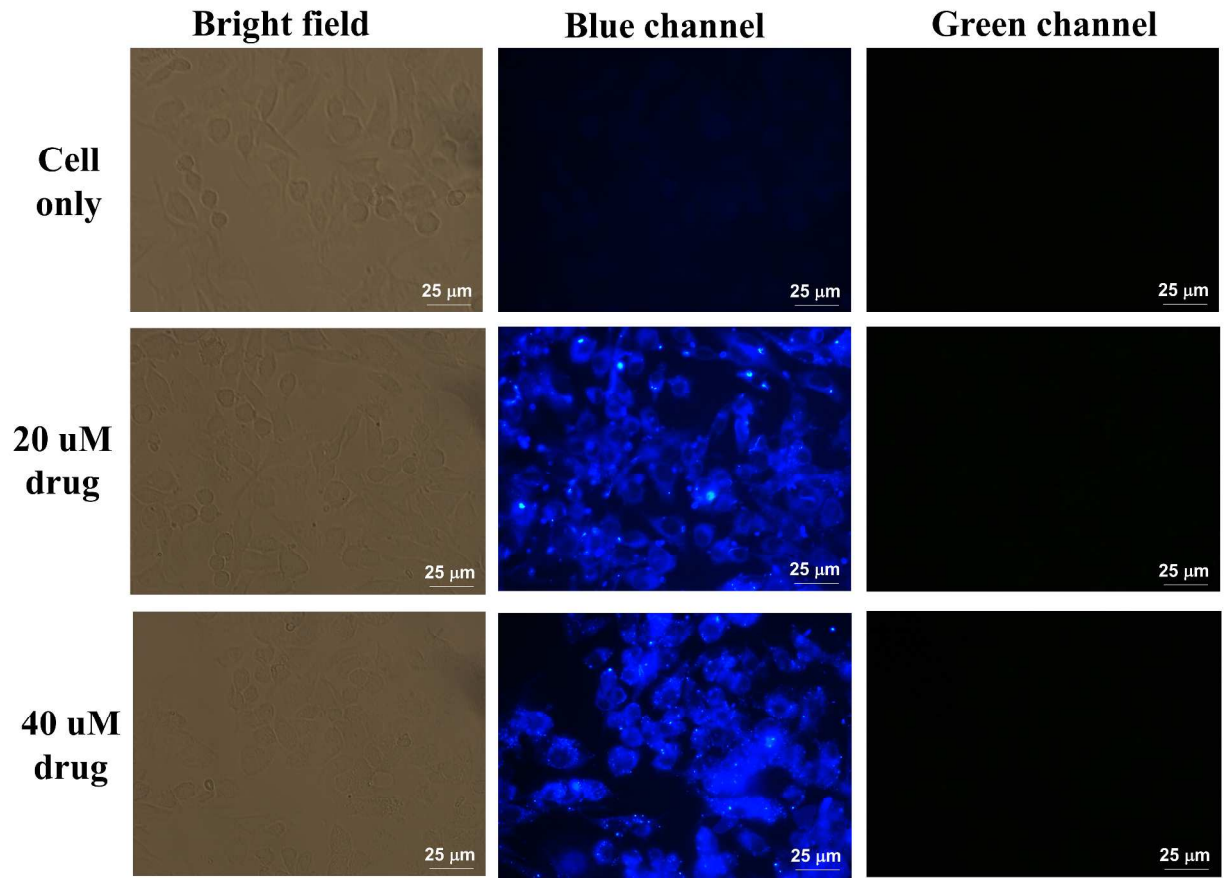


*Fig. S5 Concentration-dependent increase in fluorescence intensity of **11** (blue channel) after 6 h incubation of prodrug **10b**.*

Cytotoxicity of CO-drug conjugates and cyclized product

For the crystal violet assay and cell-imaging studies, RAW 264.7 (ATCC® TIB-71™) within 10 passages was used. The cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum (MidSci; S01520HI) and 1% penicillin-streptomycin (Sigma-Aldrich; P4333) at 37 °C with 5% CO_2. Fresh medium was replenished every other day. The cells were treated with the compounds (0–100 μM) using 1% DMSO in DMEM for 24 h.

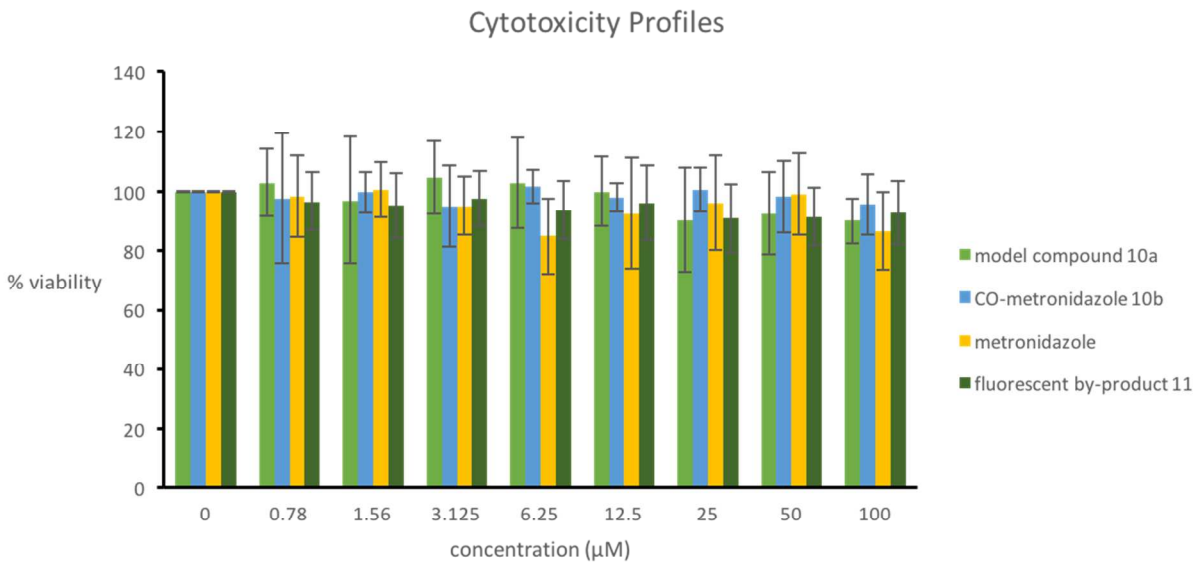
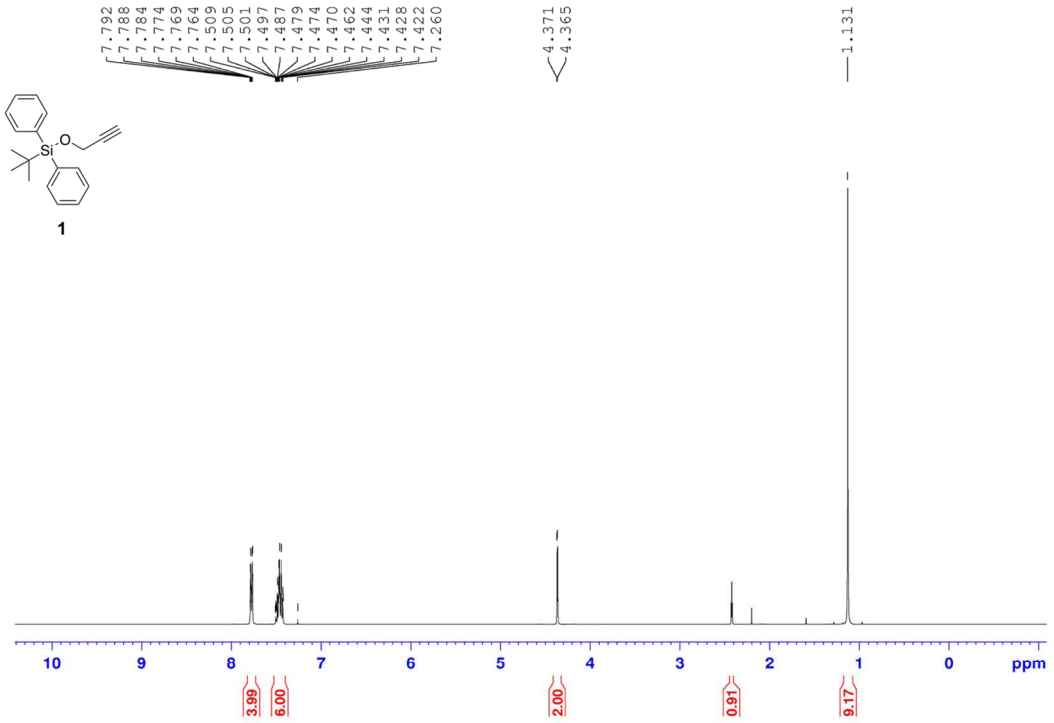


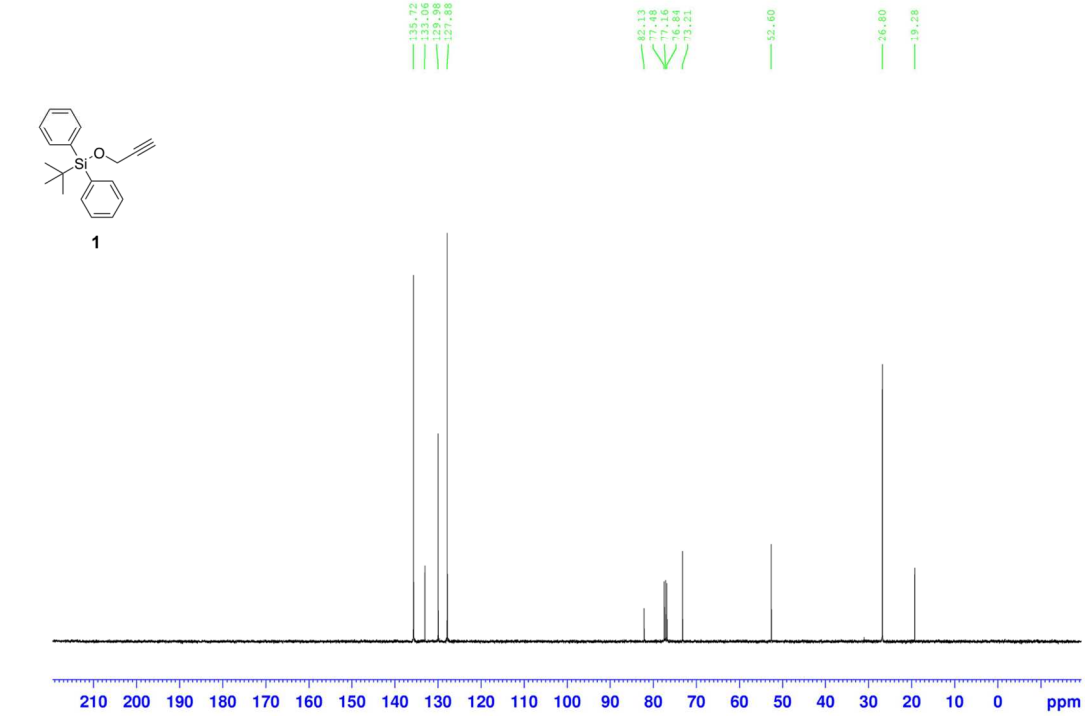
Fig. S6 Cytotoxicity profiles of prodrugs **10**a and **10b**, the antibiotic metronidazole, and the fluorescent by product **11**. (n=6)

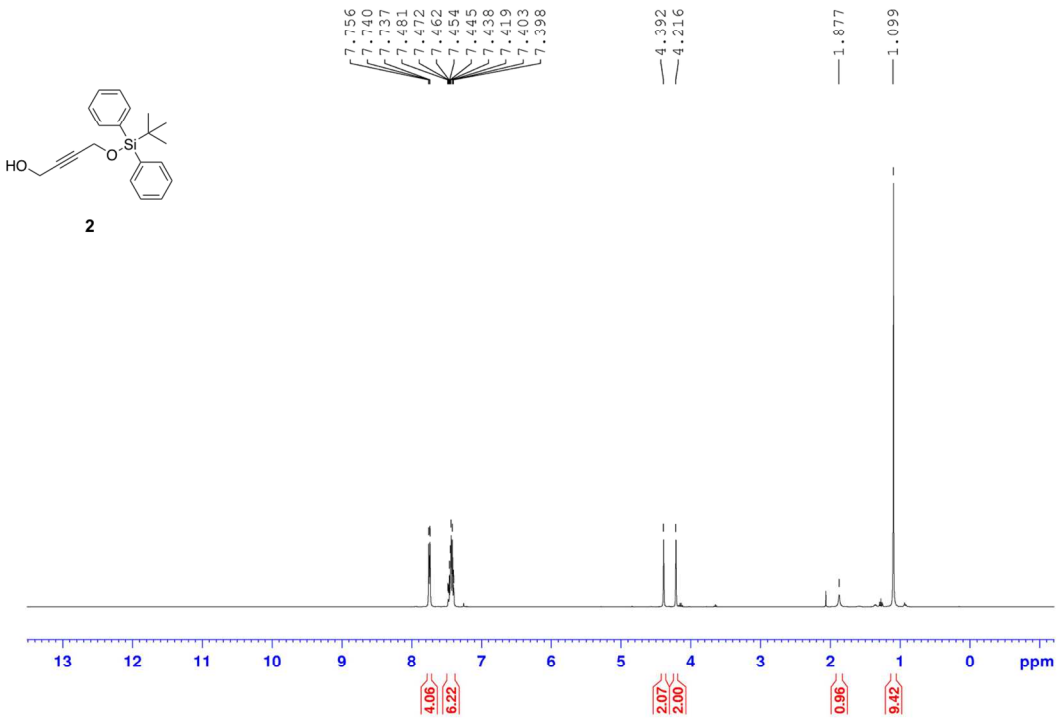
Helicobacter pylori growth inhibition

H. pylori strain 26695[2] cells were grown on Brucella agar plates supplemented with 10% defibrinated sheep blood at 37°C under microaerophilic conditions (5% CO_2, 4% O_2 and 91% N_2) and resuspended in Brain-Heart Infusion supplemented with 0.4% ß-cyclodextrin (BHI- ßc) to a final OD_{600} of 4 -5. The inoculum was 1:100 fold diluted inoculated in 10 mL of BHI- ßc (starting OD_{600}: 0.04 to 0.05) in bottles with 150 mL head space filled with 10% H_2, 5% CO_2 , 5% O_2 80% N_2. Right before inoculating *H. pylori*, each compound (metronidazole, **10a**, **10b**, or **11**) was suspended and serially diluted in DMSO, to achieve final concentrations ranging from 250 to 16 µg/mL. Then, 0.1 mL of each diluted compound was added in one bottle to achieve final concentrations ranging from 2.5 to 0.16 µg/mL (and 1% DMSO) in 10 mL of BHI- ßc. *H. pylori* cells were grown for 24 h at 37 °C, 200 rpm shaking. Results represent data from growth experiments done in triplicate and are expressed as (mean and standard deviation) percentages of OD_{600} at 24h for each compound compared to OD_{600} of control bottles (*H. pylori* grown in BHI- ßc with 1% DMSO).

Spectroscopic Data of Synthesized Compounds



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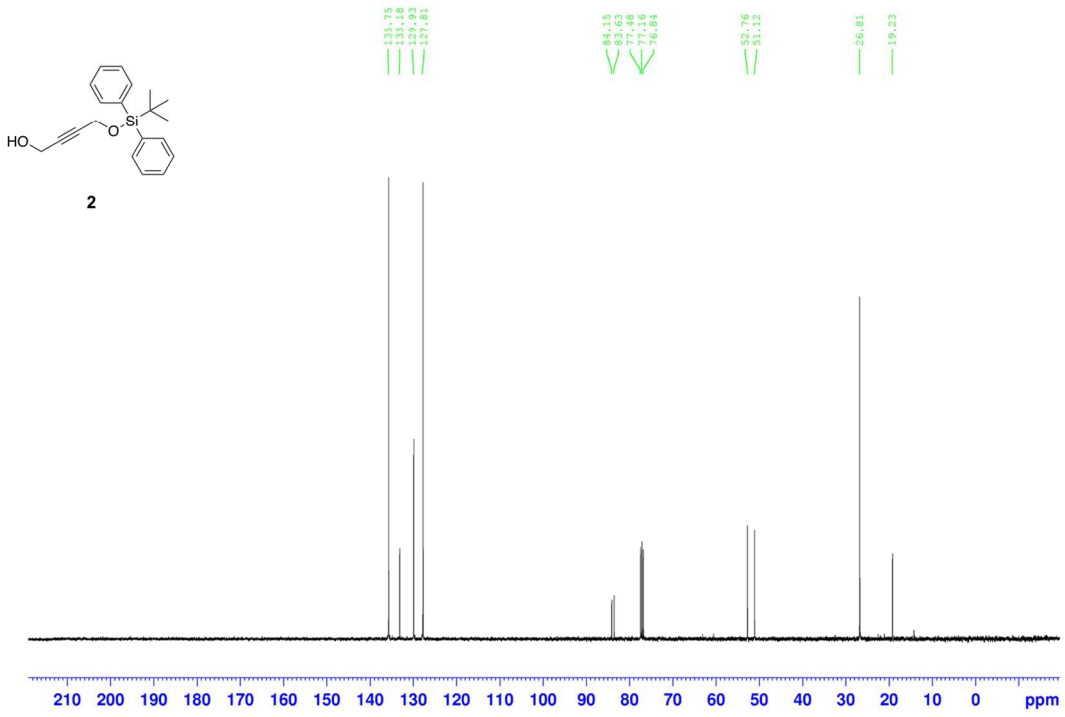
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77.16
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O
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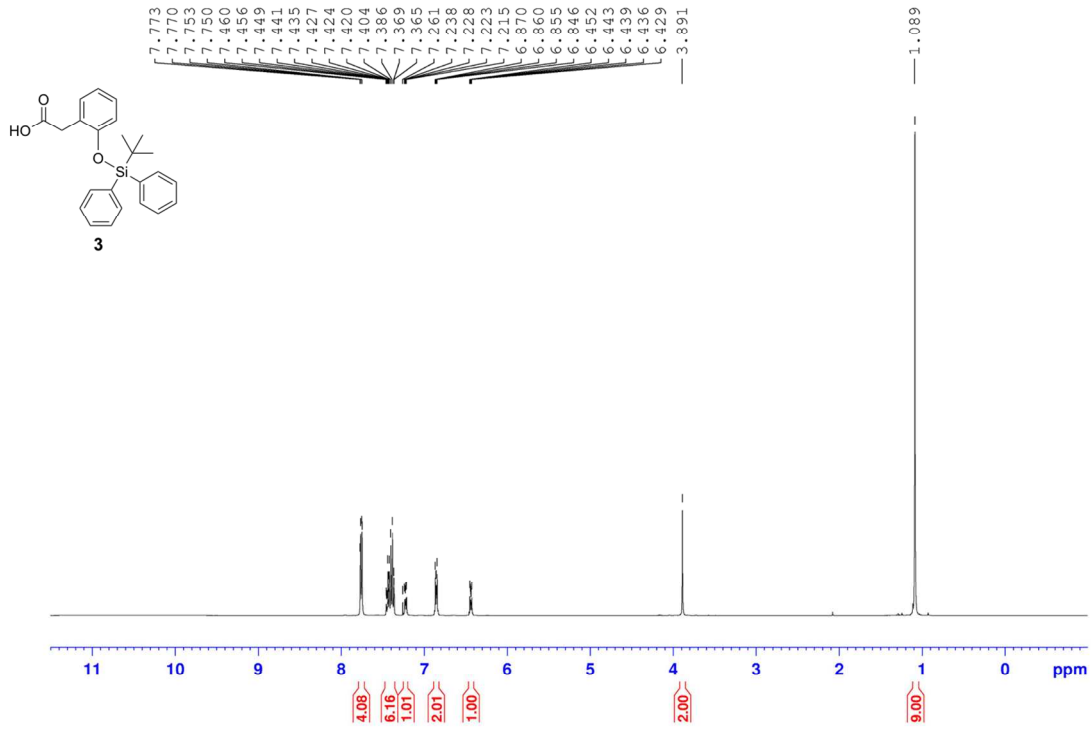
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6.846
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6.439
6.436
6.429
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1.089
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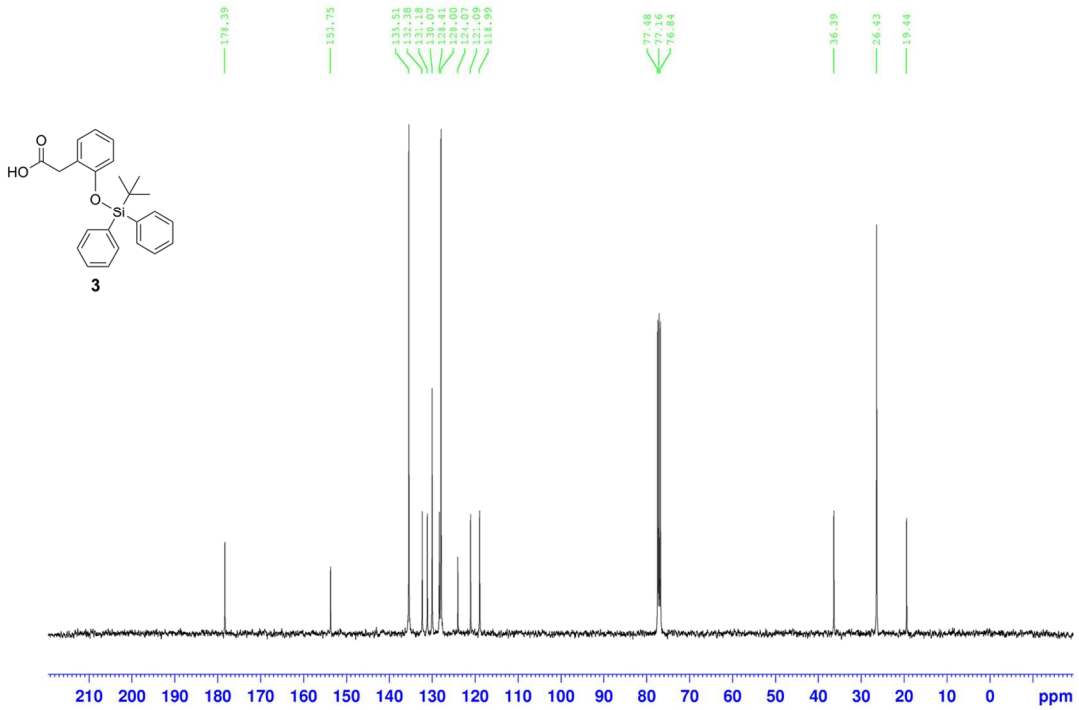
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HO
O
Si
3
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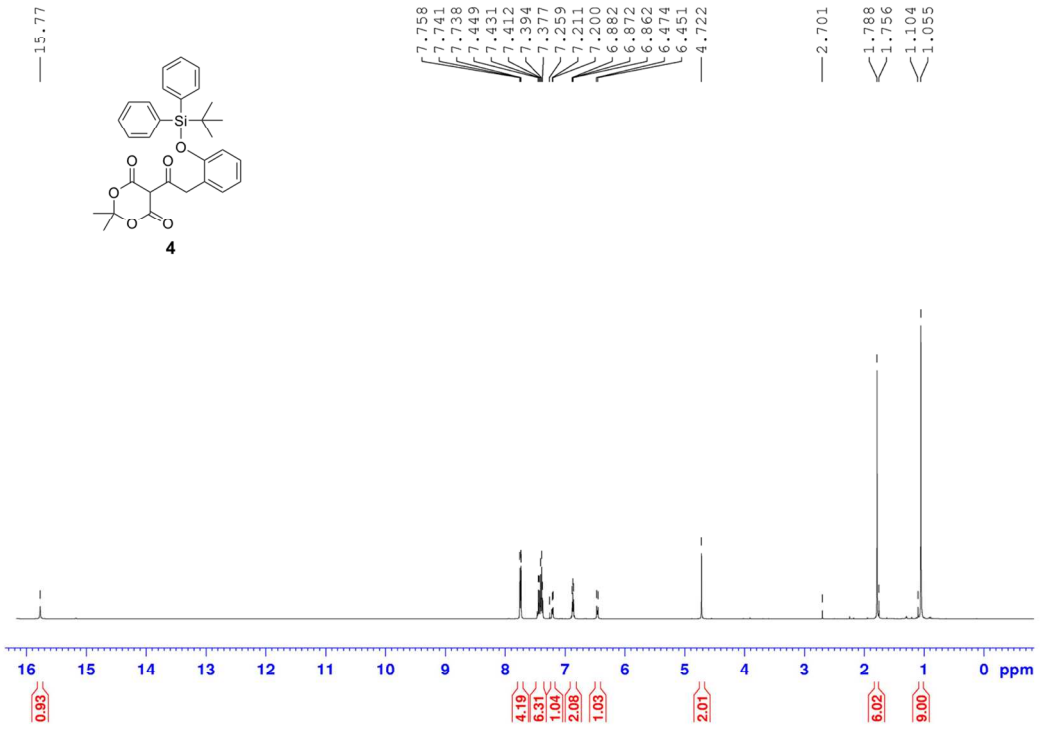
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2.701
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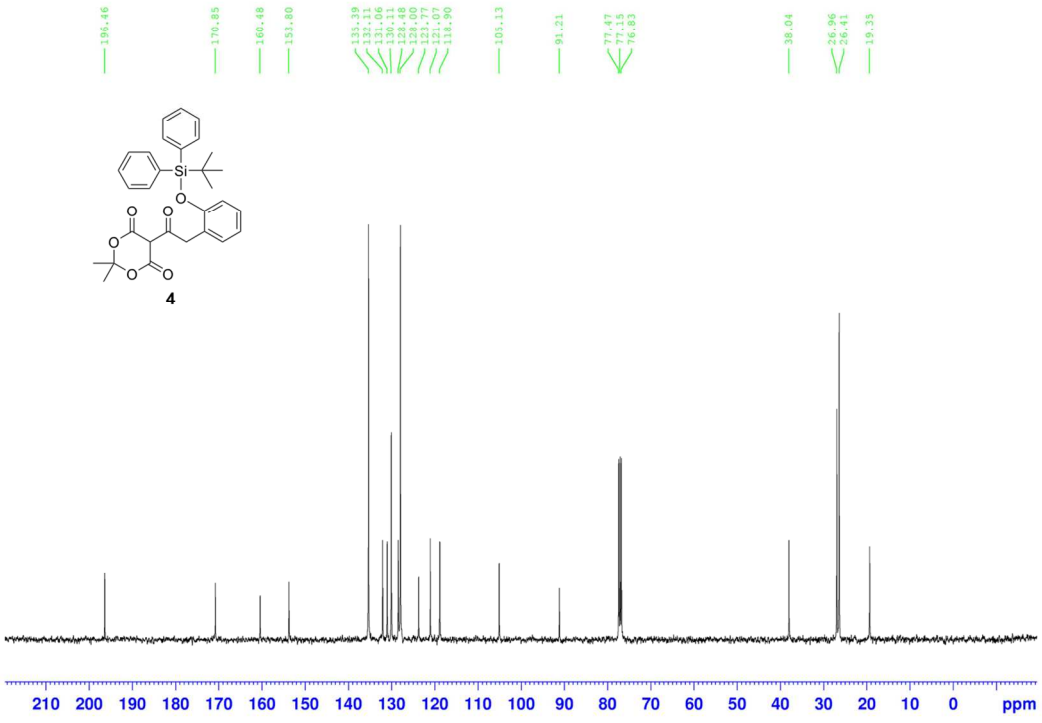
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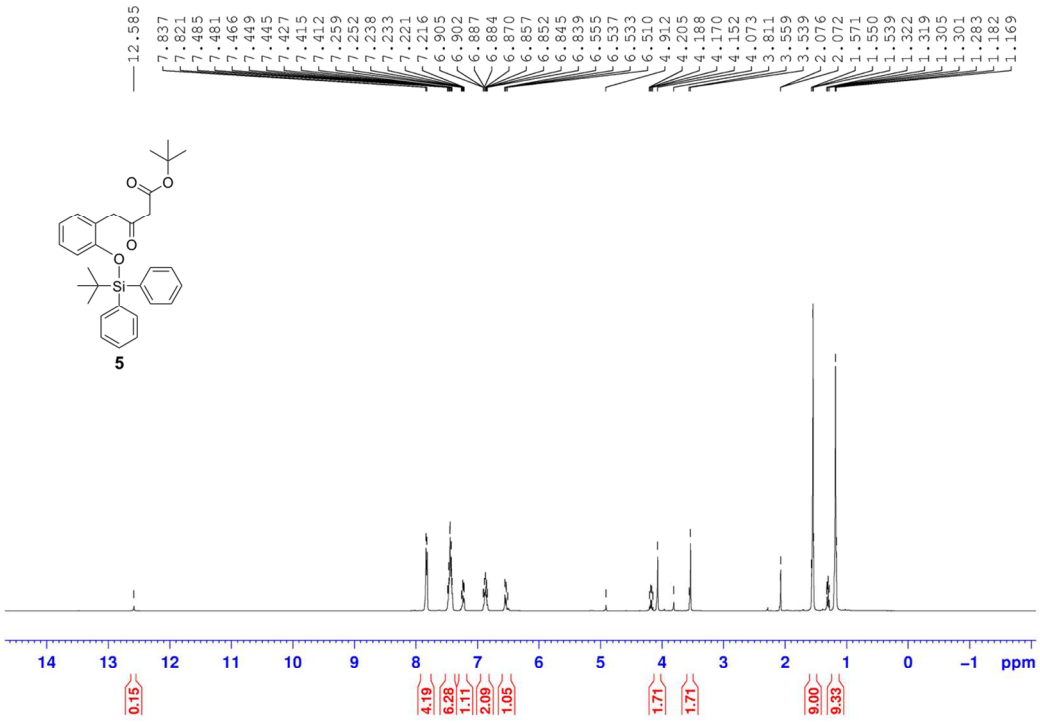
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7.216
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6.902
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6.870
6.857
6.852
6.845
6.839
6.555
6.537
6.533
6.510
4.912
4.205
4.188
4.170
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3.539
2.076
2.072
1.571
1.550
1.539
1.322
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1.305
1.301
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1.182
1.169
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14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 ppm
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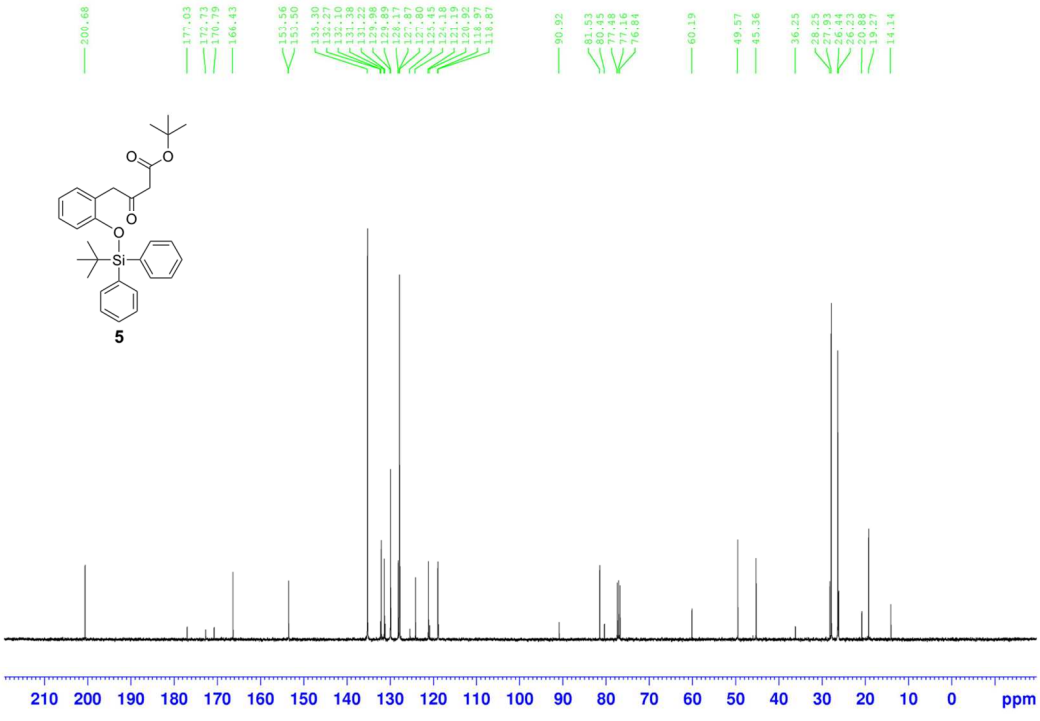
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76.84
60.19
49.57
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36.25
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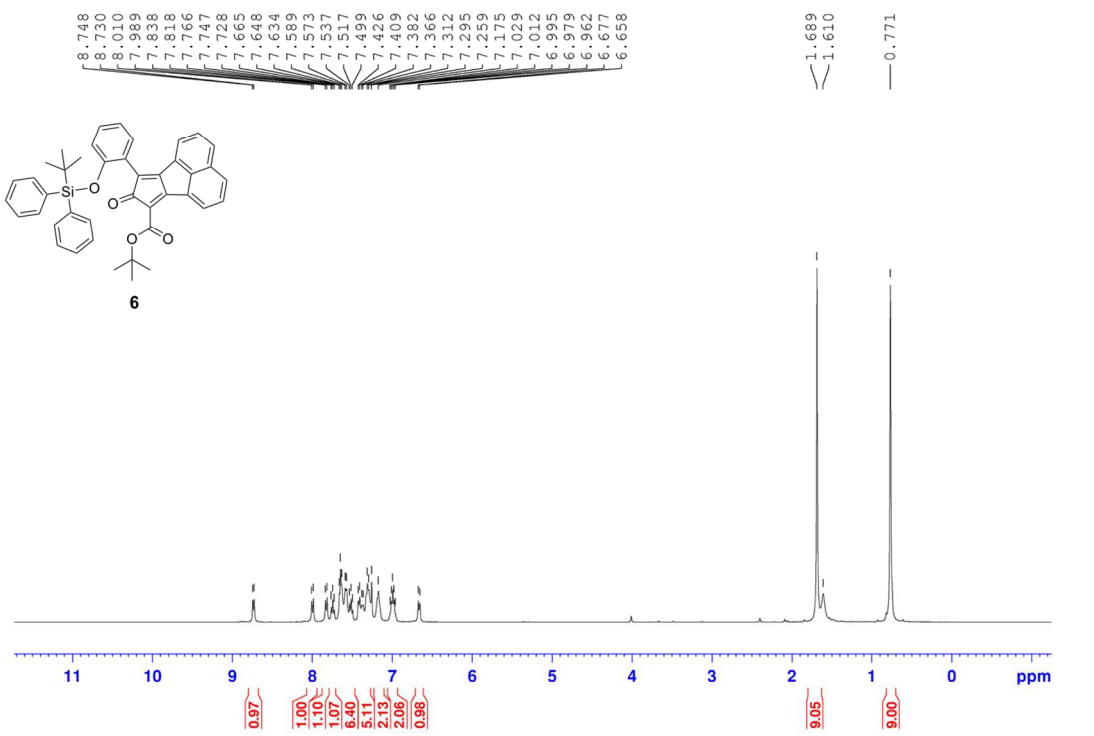
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7.366
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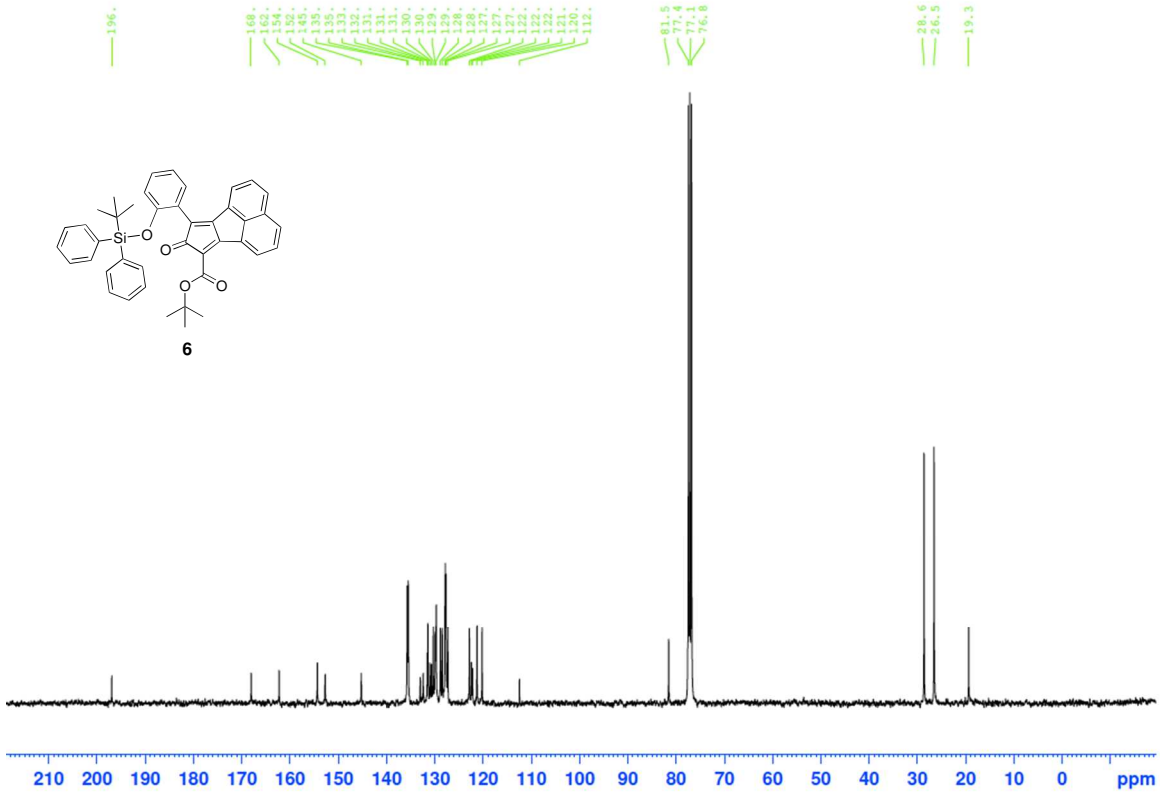
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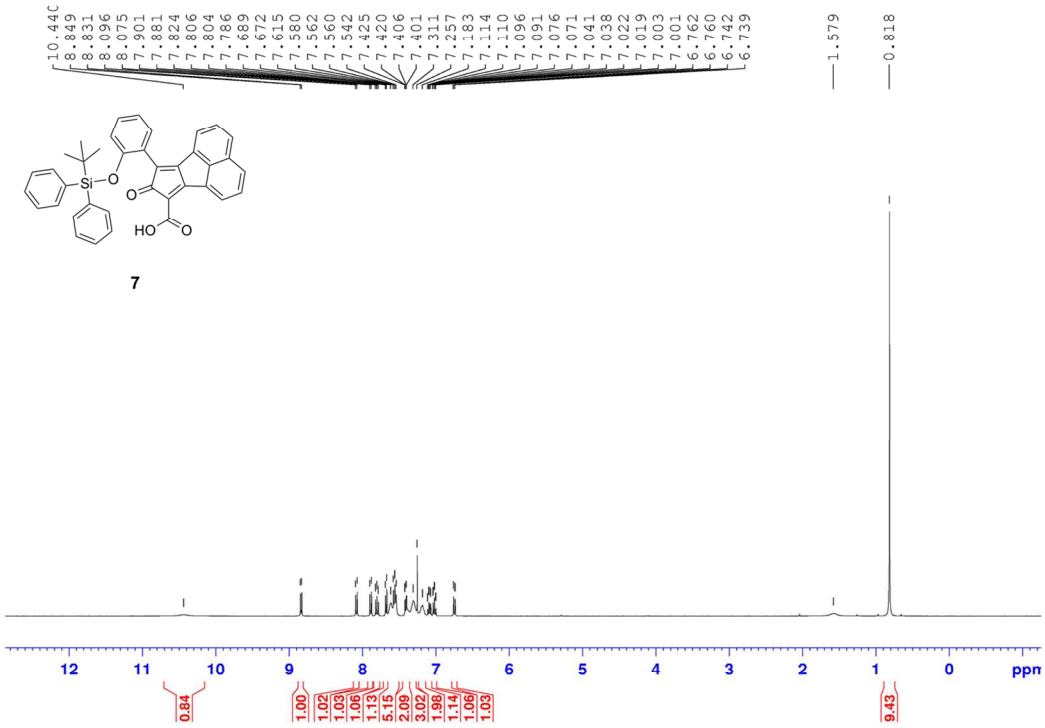
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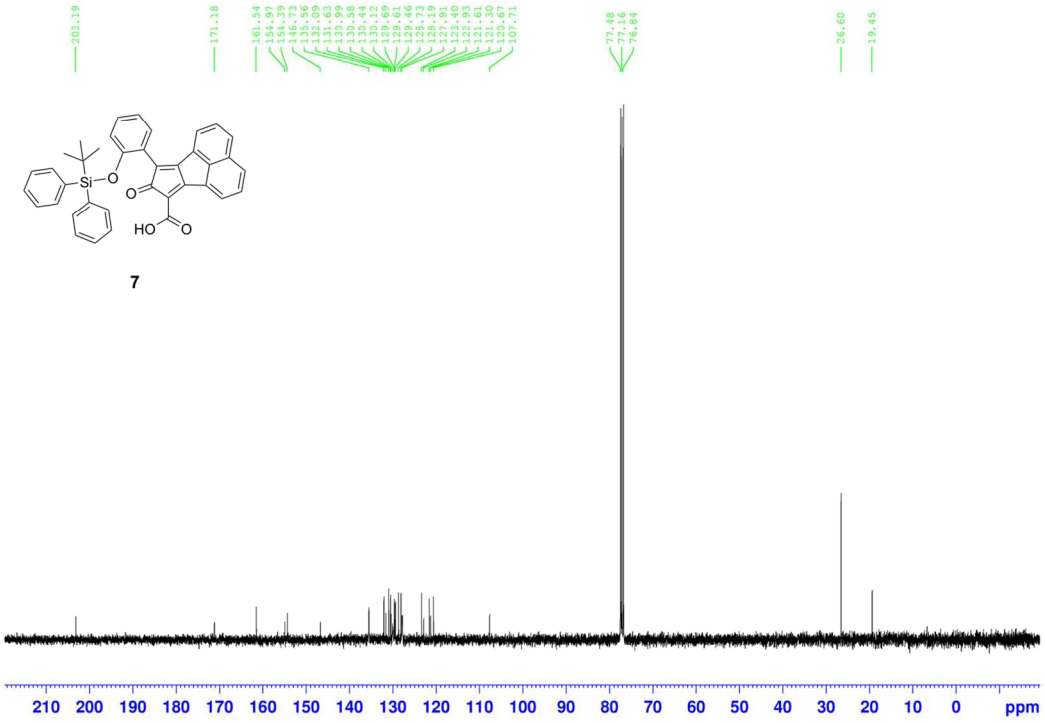
7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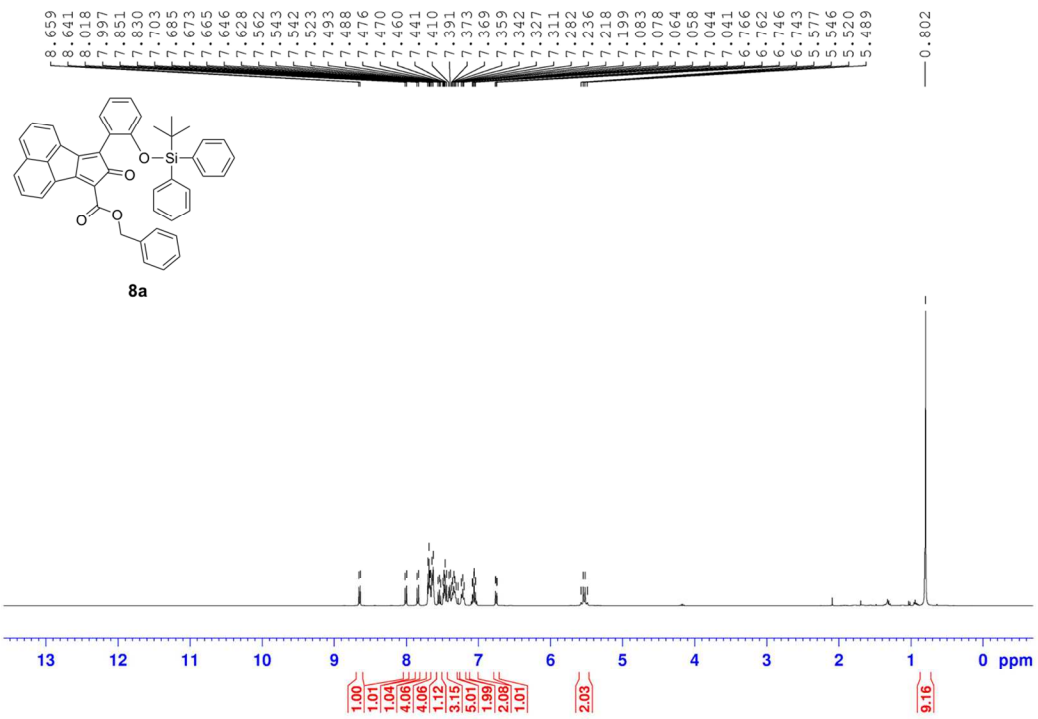
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

7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7.665
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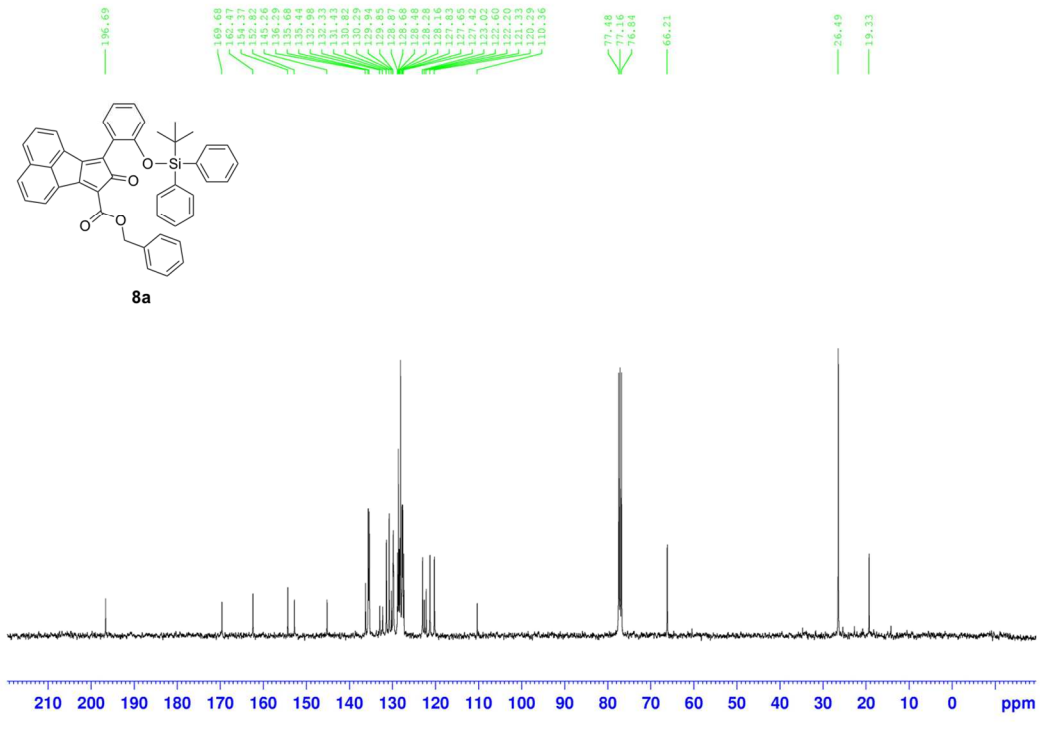
7.562
7.543
7.542
7.523
7.493
7.488
7.476
7.470
7.460
7.441
7.410
7.391
7.373
7.369
7.359
7.342
7.327
7.311
7.282
7.236
7.218
7.199
7.083
7.078
7.064
7.058
7.044
7.041
6.766
6.762
6.746
6.743
5.577
5.546
5.520
5.489
0.802
O
Si
O
O
O
13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
1.00 1.01 1.04 4.06 4.06 1.12 3.15 5.01 1.99 2.08 1.01
2.03
9.16

8a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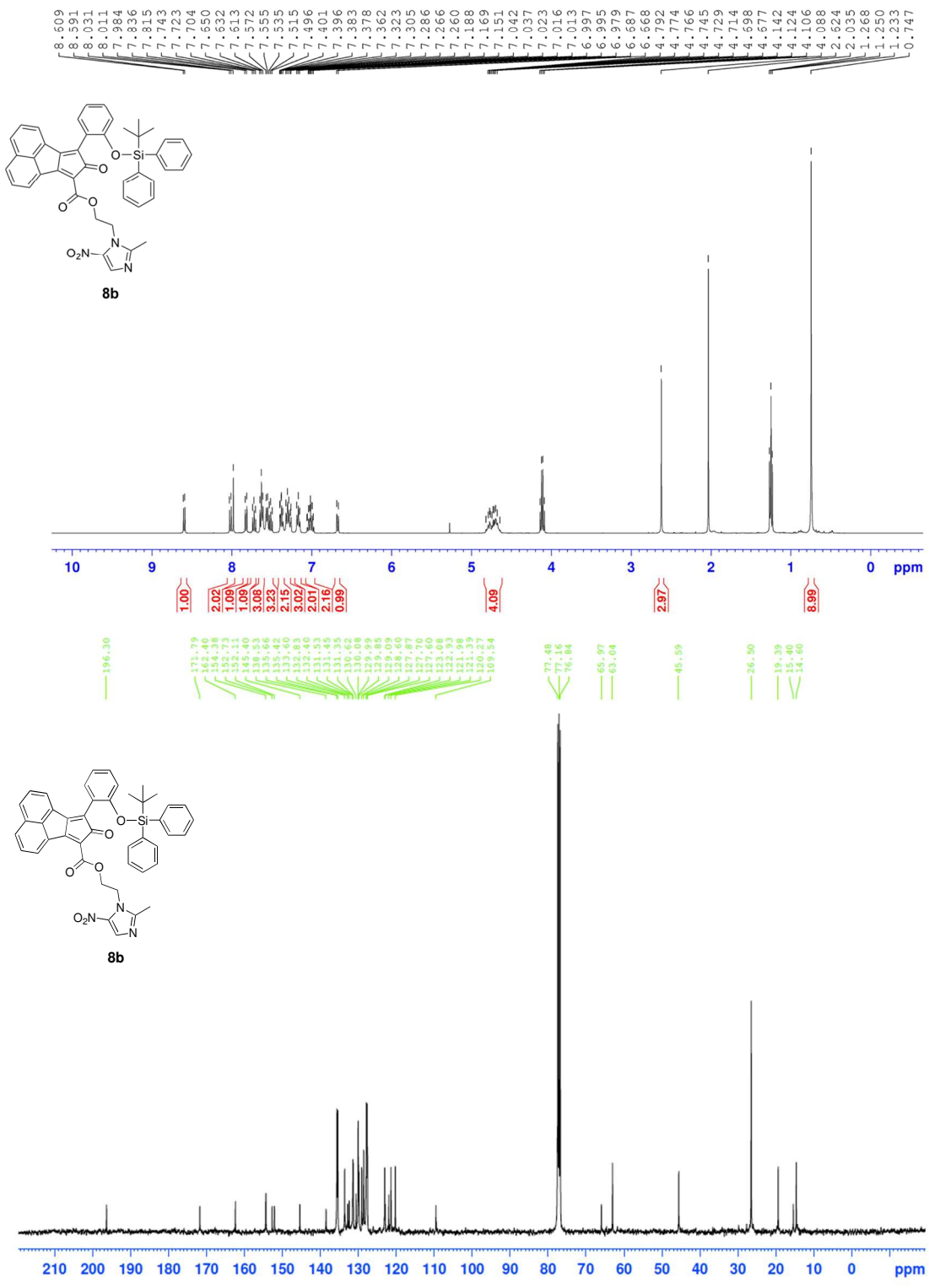
169.68
162.47
154.37
152.80
145.26
136.29
135.68
135.44
132.98
132.33
131.43
130.82
130.29
129.94
129.55
128.87
128.68
128.48
128.28
128.16
127.85
127.65
127.42
123.02
122.60
122.10
121.31
120.29
110.36
77.48
77.16
76.84
66.21
26.49
19.33
O
Si
O
O
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

8a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8.591
8.031
8.011
7.984
7.836
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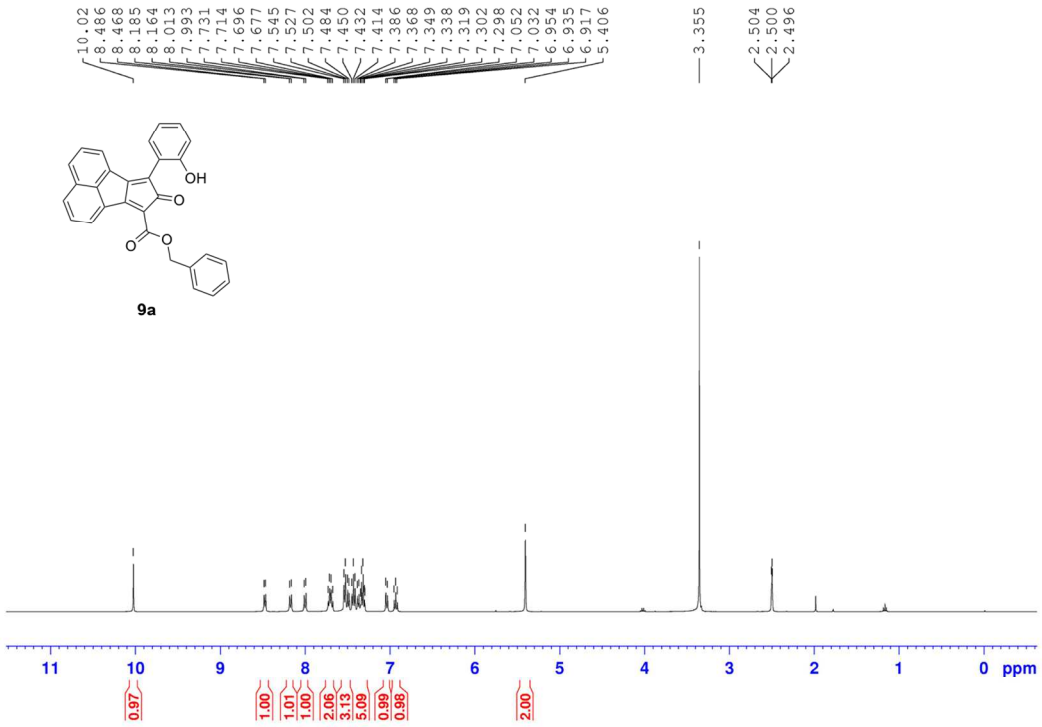
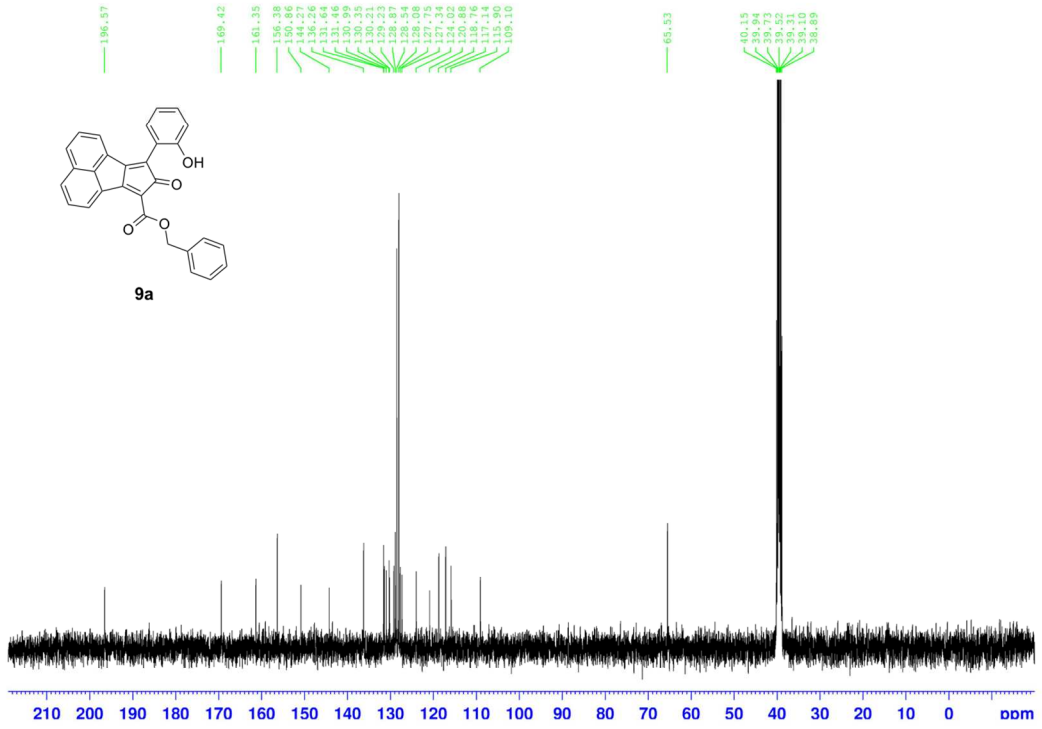
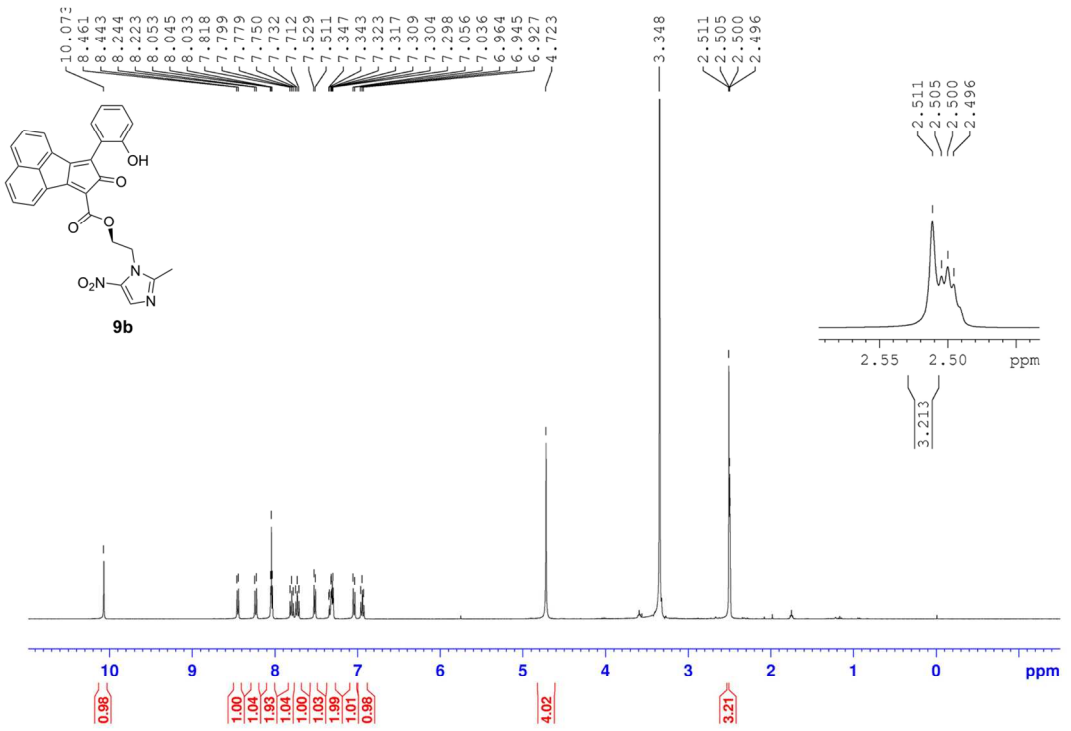
7.743
7.723
7.704
7.650
7.632
7.613
7.572
7.555
7.535
7.515
7.496
7.401
7.396
7.383
7.378
7.362
7.323
7.305
7.286
7.266
7.260
7.188
7.169
7.151
7.042
7.037
7.023
7.016
7.013
6.997
6.995
6.979
6.687
6.668
4.792
4.774
4.766
4.745
4.729
4.714
4.698
4.677
4.142
4.124
4.106
4.088
2.624
2.035
1.268
1.250
1.233
0.747
8b
10 9 8 7 6 5 4 3 2 1 0 ppm
1.00 2.02 1.09 1.09 3.08 3.23 2.15 3.02 2.01 2.16 0.99 4.09 2.97 8.99
196.30
171.79
162.40
154.38
152.73
152.11
145.40
138.53
135.66
135.42
133.60
133.43
132.40
131.53
131.45
131.35
130.62
130.08
129.98
129.85
129.09
128.60
127.87
127.70
127.60
123.98
123.53
121.98
121.39
120.27
109.54
77.48
77.16
76.84
65.97
63.04
45.59
26.50
19.39
15.40
14.60
8b
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


10.02
8.486
8.468
8.185
8.164
8.013
7.993
7.731
7.714
7.696
7.677
7.545
7.527
7.502
7.484
7.450
7.432
7.414
7.386
7.368
7.349
7.338
7.319
7.302
7.298
7.052
7.032
6.954
6.935
6.917
5.406
3.355
2.504
2.500
2.496
OH
O
O
O
9a
11
10
9
8
7
6
5
4
3
2
1
0 ppm
0.97
1.00
1.01
1.00
2.06
3.13
5.09
0.99
0.98
2.00


196.57
169.42
161.35
156.38
150.86
144.27
136.26
131.64
131.46
130.99
130.35
130.21
129.23
128.97
128.54
128.08
127.75
127.34
124.02
120.68
118.76
117.14
115.90
109.10
65.53
40.15
39.94
39.73
39.52
39.31
39.10
38.89
OH
O
O
O
9a
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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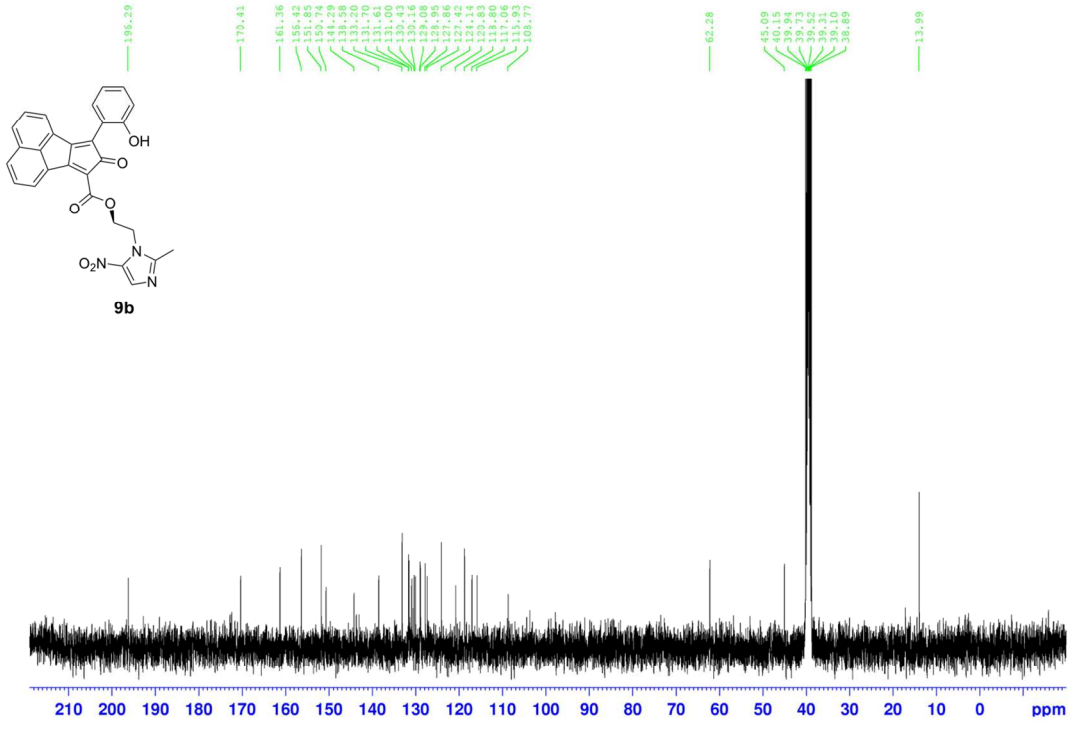
8.443
8.244
8.223
8.053
8.045
8.033
7.818
7.799
7.779
7.750
7.732
7.712
7.529
7.511
7.347
7.343
7.323
7.317
7.309
7.304
7.298
7.056
7.036
6.964
6.945
6.927
4.723
3.348
2.511
2.505
2.500
2.496
OH
O
O
O
N
O_2N
N
9b
2.55
2.50
ppm
3.213
10
9
8
7
6
5
4
3
2
1
0
ppm
0.98
1.00
1.04
1.93
1.04
1.00
1.02
1.99
1.01
0.98
4.02
3.21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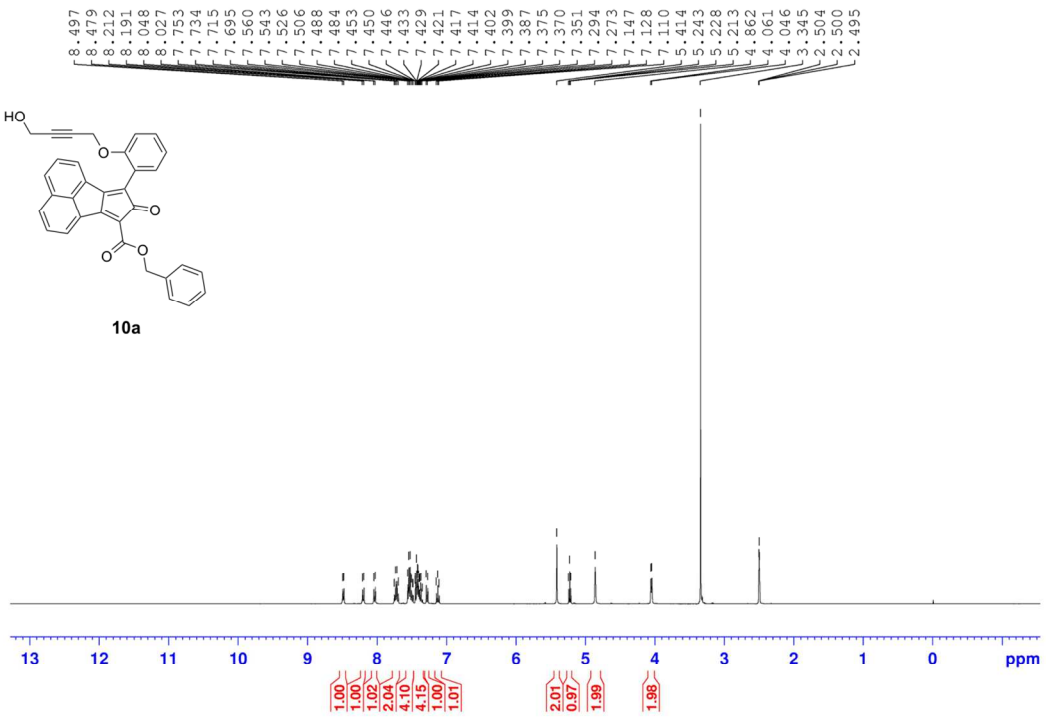
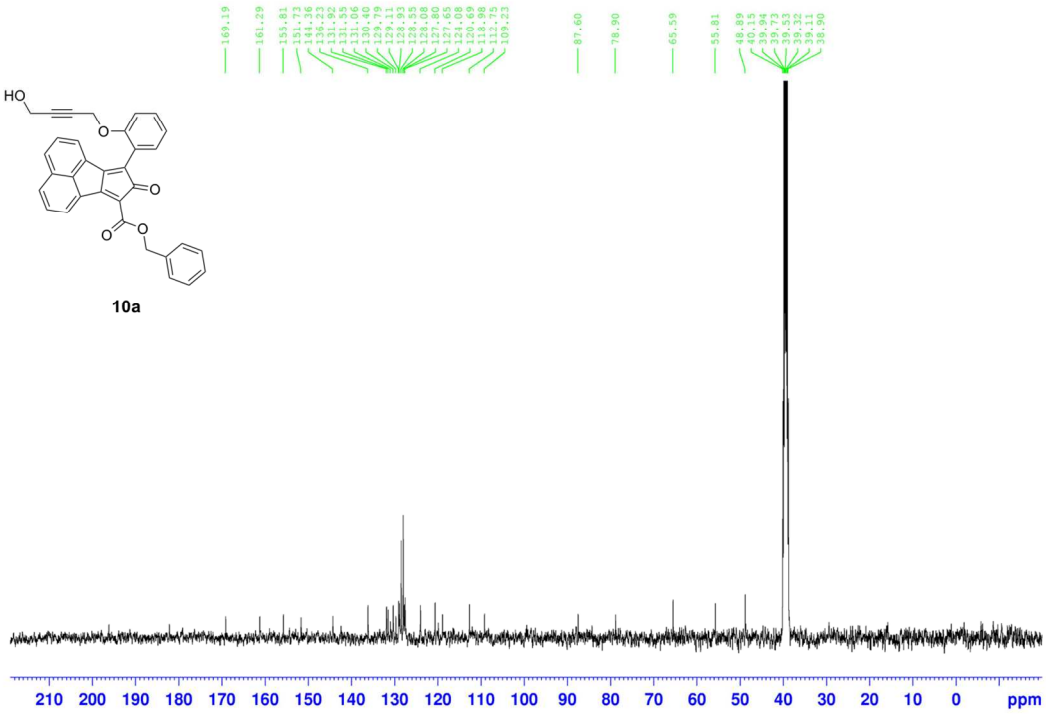
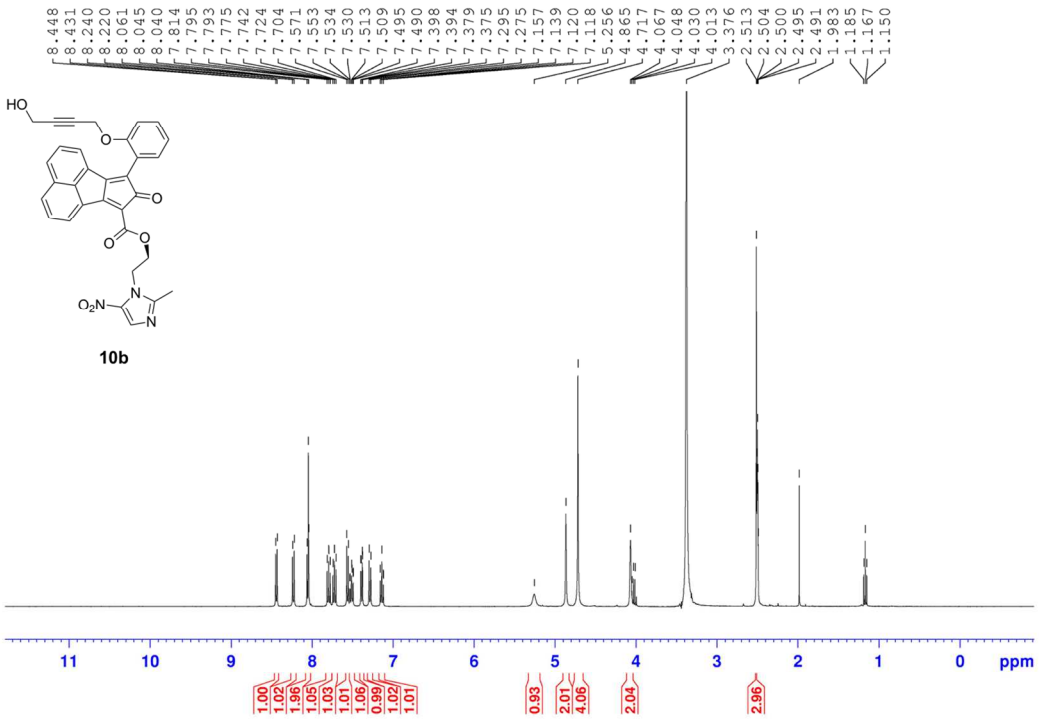
170.41
161.36
156.42
151.85
150.74
149.29
138.58
133.20
131.70
131.61
131.00
130.45
130.16
129.08
128.95
127.66
127.42
124.14
120.83
118.80
117.06
115.93
109.77
62.28
45.09
40.15
39.94
39.73
39.52
39.31
39.10
38.89
13.99
OH
O
O
O
N
O_2N
N
9b
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


8.497
8.479
8.212
8.191
8.048
8.027
7.753
7.734
7.715
7.695
7.560
7.543
7.526
7.506
7.488
7.484
7.453
7.450
7.446
7.433
7.429
7.421
7.417
7.414
7.402
7.399
7.387
7.375
7.370
7.351
7.294
7.273
7.147
7.128
7.110
5.414
5.243
5.228
5.213
4.862
4.061
4.046
3.345
2.504
2.500
2.495
HO
O
O
O
O
13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
1.00 1.00 1.02 2.04 4.10 4.15 1.00 1.01
2.01 0.97 1.99 1.98

10a


169.19
161.29
155.81
151.73
144.36
136.23
131.92
131.55
131.06
130.40
129.79
129.11
128.93
128.55
128.08
127.80
127.65
124.08
120.69
118.88
112.75
109.23
87.60
78.90
65.59
55.81
48.89
40.15
39.94
39.73
39.53
39.32
39.11
38.90
HO
O
O
O
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

10a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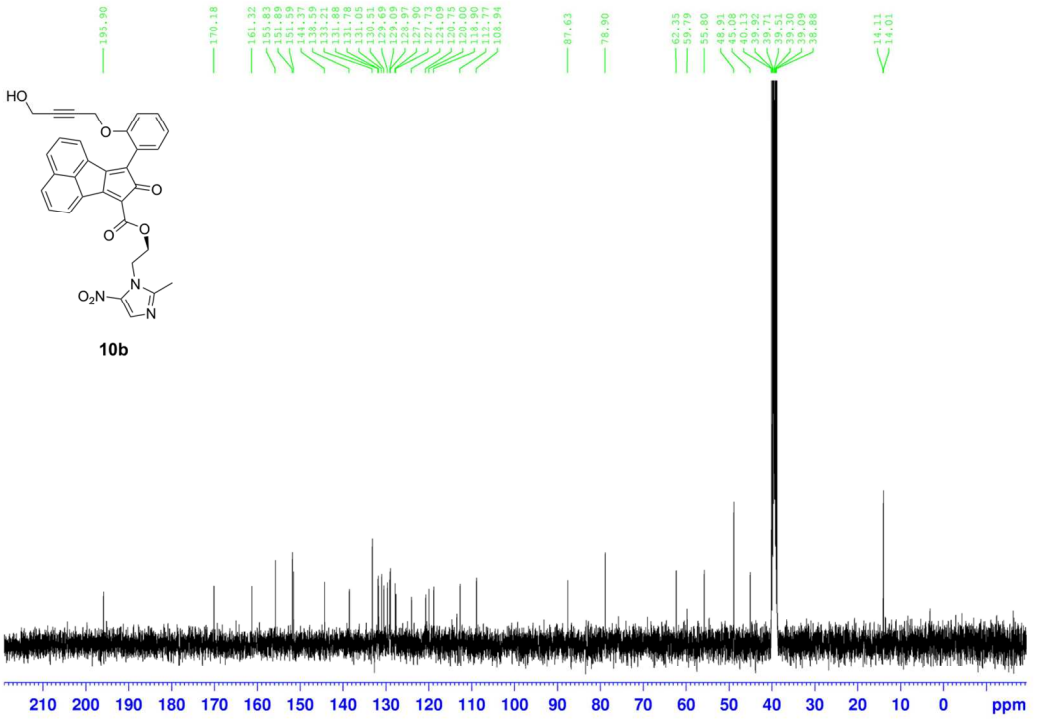
8.240
8.220
8.061
8.045
8.040
7.814
7.795
7.793
7.775
7.742
7.724
7.704
7.571
7.553
7.534
7.530
7.513
7.509
7.495
7.490
7.398
7.394
7.379
7.375
7.295
7.275
7.157
7.139
7.120
7.118
5.256
4.865
4.717
4.067
4.048
4.030
4.013
3.376
2.513
2.504
2.500
2.495
2.491
1.983
1.185
1.167
1.150
HO
O
O
O
O
O
N
O_2N
N
10b
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
1.02
1.96
1.05
1.03
1.01
1.06
0.99
1.02
1.01
0.93
2.01
4.06
2.04
2.96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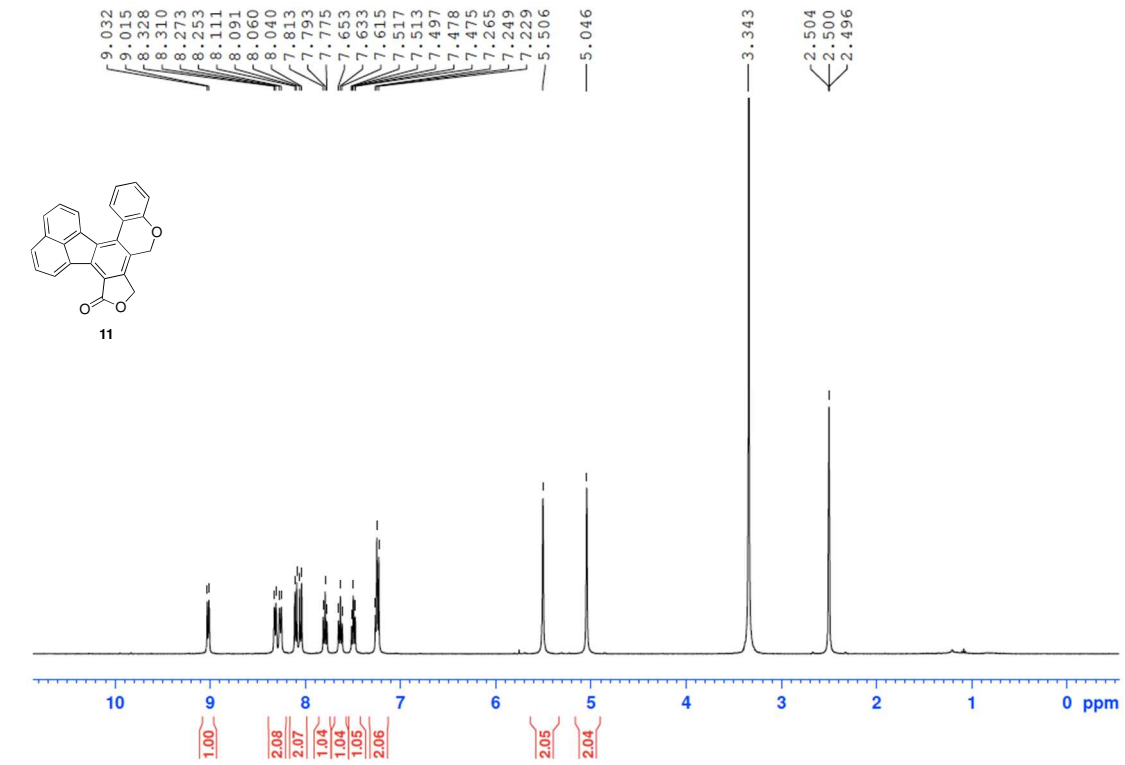
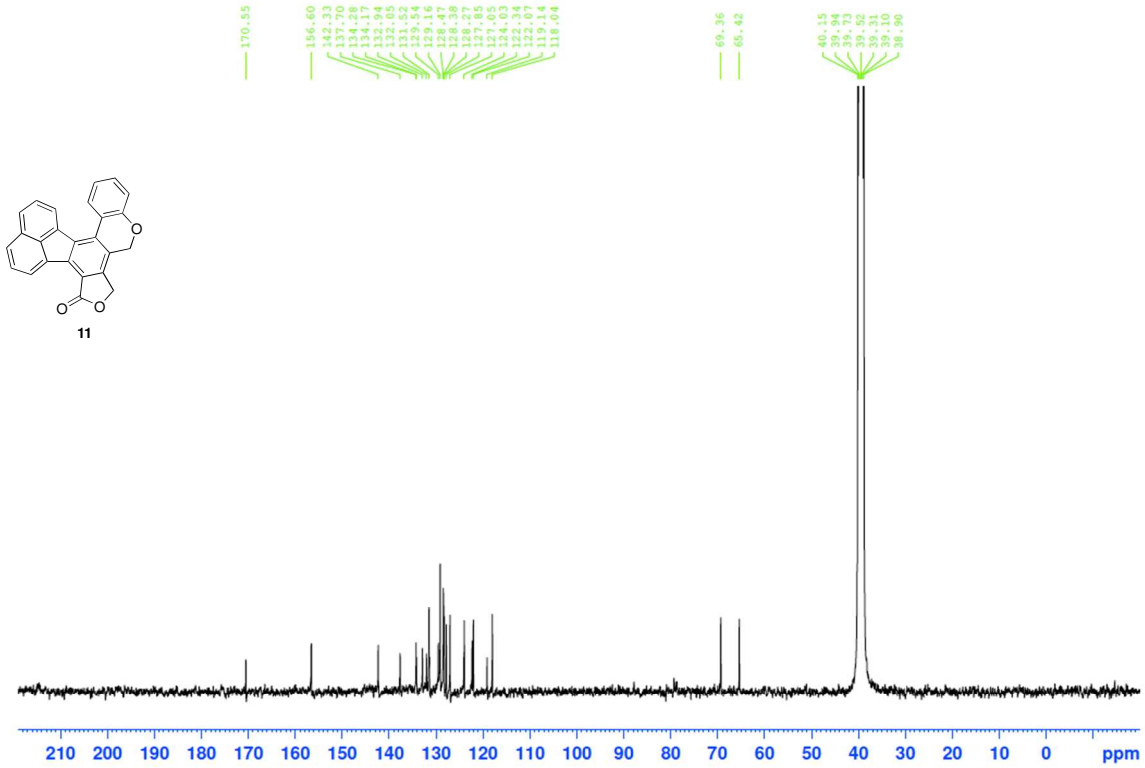
151.59
144.37
138.59
133.21
131.88
131.78
131.05
130.51
129.69
129.09
128.97
127.90
127.73
124.09
120.75
120.00
118.90
112.77
108.94
87.63
78.90
63.35
59.79
55.80
48.91
45.08
40.13
39.92
39.71
39.51
39.30
39.09
38.88
14.11
14.01
HO
O
O
O
O
O
N
O_2N
N
10b
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


9.032
9.015
8.328
8.310
8.273
8.253
8.111
8.091
8.060
8.040
7.813
7.793
7.775
7.653
7.633
7.615
7.517
7.513
7.497
7.478
7.475
7.265
7.249
7.229
5.506
5.046
3.343
2.504
2.500
2.496
11
10 9 8 7 6 5 4 3 2 1 0 ppm
1.00
2.08
2.07
1.04
1.04
1.05
2.06
2.05
2.04


170.55
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142.33
137.70
134.28
134.17
132.94
132.05
131.52
131.34
129.16
128.47
128.38
128.27
127.85
127.05
124.03
122.34
122.07
119.14
119.04
69.36
65.42
40.15
39.94
39.73
39.52
39.31
39.10
38.90
11
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

References:

1. Ji, X.; Zhou, C.; Ji, K.; Aghoghovbia, R. E.; Pan, Z.; Chittavong, V.; Ke, B.; Wang, B. *Angew. Chem. Int. Ed.* **2016,** *55*, 15846-15851.
2. Tomb, J.-F.; White, O.; Kerlavage, A. R.; Clayton, R. A.; Sutton, G. G.; Fleischmann, R. D.; Ketchum, K. A.; Klenk, H. P.; Gill, S.; Dougherty, B. A.; Nelson, K.; Quackenbush, J.; Zhou, L.; Kirkness, E. F.; Peterson, S.; Loftus, B.; Richardson, D.; Dodson, R.; Khalak, H. G.; Glodek, A.; McKenney, K.; Fitzegerald, L. M.; Lee, N.; Adams, M. D.; Hickey, E. K.; Berg, D. E.; Gocayne, J. D.; Utterback, T. R.; Peterson, J. D.; Kelley, J. M.; Cotton, M. D.; Weidman, J. M.; Fujii, C.; Bowman, C.; Watthey, L.; Wallin, E.; Hayes, W. S.; Borodovsky, M.; Karp, P. D.; Smith, H. O.; Fraser, C. M.; Venter, J. C. *Nature* **1997,** *388*, 539.