Supplemental Information for

Enrichment-triggered Prodrug Activation Demonstrated through Mitochondria-targeted Delivery of Doxorubicin and Carbon Monoxide

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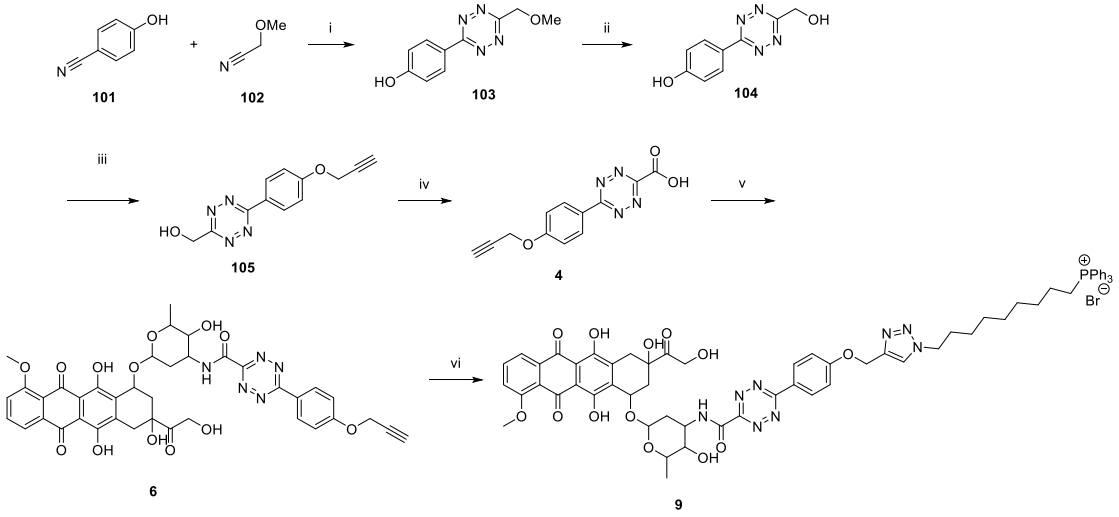
1. Chemical synthesis

General Information.

All reagents and solvents were of reagent grade and were purchased from Aldrich. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. Mass spectral analyses were performed on an ABI API 3200 (ESI-Triple Quadruple) instrument. HPLC was performed on a Shimadzu Prominence UFLC (column: Waters C18 3.5 μM, 4.6×100 mm). UV-Vis absorption spectra were recorded on a Shimadzu PharmaSpec UV-1700 UV-Visible spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301PC fluorometer. 96-Well plates were read and recorded on a PerkinElmer 1420 multi-label counter.

Synthesis of TPP-Dox-prodrug.



Reagents and conditions (i). (1) N_2H_4, $Zn(OTf)_2$, 60 ℃, 24 h, (2) $NaNO_2$, H_2O, HCl; (ii). dichloromethane (DCM), BBr_3, 0 ℃, 0.5 h; (iii) propargyl bromide, acetonitrile, K_2CO_3, 60 ℃, 3 h; (iv). (1) DCM, Dess–Martin periodinane, room temperature (r.t.), 10 min; (2) $NaClO_2/NaH_2PO_4$, 2-methylbut-2-ene, *t*-BuOH, r.t, 2h; (v). (1) $C_2O_2Cl_2$, DMF, DCM. r.t., (2) NHS, Et_3N, DCM, r.t. 1h (3) Dox, Et_3N, DMF, DCM.r.t.. (vi). $CuSO_4$ $5H_2O$, sodium ascorbate, DMSO, *t*-BuOH, $N_3(CH_2)_9PPh_3Br$, TBTA, 6 h r.t.

Supplementary Scheme 1. Synthesis of TPP-Dox-prodrug **9**

Synthesis of 4-(6-(methoxymethyl)-1,2,4,5-tetrazin-3-yl)phenol (**103**): To a solution of 4-hydroxybenzonitrile (**101**, 1.785 g, 15.0 mmol) and 2-methoxyacetonitrile (**102**, 3.195 g, 45.0 mmol) in N_2H_4 (13.5 ml) was added Zn(OTf) (1.812 g, 6 mmol). The reaction mixture was stirred at 60 ℃ for 24 h and cooled down to the room temperature (r.t). Then 40 mL of ethyl acetate (EtOAc), 20mL H_2O, and $NaNO_2$ (10 g, 145 mmol) were added to the mixture. HCl (10 M, 10 ml) was added slowly to the mixture over a period of 1 h. The reaction mixture was extracted with EtOAc (3 × 40 ml). The combined organic layer was washed with brine (50 mL), and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the crude product. The final product was obtained as a purple solid by recrystallization with hexane and EtOAc (2.18 g, 67%). ^{1}H NMR (CD_3OD): δ 8.41 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 4.99 (s, 2H), 3.56 (s, 3H). ^{13}C NMR (CD_3OD): δ166.8, 166.0, 163.5, 131.1, 124.0, 117.2, 73.2, 59.6. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{10}H_{11}N_4O_2$ 219.0882; found, 219.0898.

Synthesis of 4-(6-(hydroxymethyl)-1,2,4,5-tetrazin-3-yl)phenol (**104**): To a solution of compound **103** (410 mg, 1.9 mmol) in dichloromethane (DCM, 20 ml) was added BBr_3 solution (1 M, 5 ml, 5.0 mmol) dropwise. The mixture was stirred at 0 ℃ for 30 min, and then the reaction was quenched with water (20 ml) and extracted with EtOAc (3 × 30 ml). The combined organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The pure product was achieved by chromatography as a purple solid (230 mg, 60%). ^{1}H NMR (CD_3OD): δ8.45 (d, J = 8.8 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 5.14 (s, 2H). ^{13}C NMR (CD_3OD): δ168.8, 166.1, 163.4, 131.0, 124.2, 117.2, 63.5. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_9H_9N_4O_2$, 205.0726; found, 205.0756.

Synthesis of (6-(4-(prop-2-yn-1-yloxy)phenyl)-1,2,4,5-tetrazin-3-yl)methanol (**105**): To a solution of compound **104** (230 mg, 1.1 mmol) in acetonitrile (ACN) (10 ml), 3-bromoprop-1-yne (250 mg, 2.1 mmol) and K_2CO_3 (690 mg, 5.0 mmol) were added

at r. t. The reaction was stirred at 60 ^{o}C for 2 h, cooled down to r.t, quenched with the HCl solution (1M, 10 ml), and then extracted with EtOAC (2 × 50 ml). The combined organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The pure product was achieved by chromatography as a purple solid (230 mg, 86%). ^{1}H NMR (CD_3OD): δ 8.46 (d, *J* = 8.8 Hz, 2H), 7.26 (d, *J* = 8.8 Hz, 2H), 5.99 (t, *J* = 6.4 Hz, 1H), 5.02 (d, *J* = 6.4 Hz, 2H), 4.95 (d, *J* = 2.4 Hz, 2H), 3.64 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (CD_3OD): δ168.0, 163.7, 160.8, 129.5, 124.7, 115.8, 78.8, 78.7, 62.0, 55.8. $[M + H]^+$ calcd for $C_{12}H_{11}N_4O_2$, 243.0882; found, 243.0895.

Synthesis of 6-(4-(prop-2-yn-1-yloxy)phenyl)-1,2,4,5-tetrazine-3-carboxylic acid (**4**): To a solution of compound **105** (100 mg, 0.41 mmol) in DCM (5 ml) was added Dess–Martin periodinane (260 mg, 0.62 mmol) at r.t. After 20 min, the mixture was loaded into a silica column and eluted with DCM/EtOAC (2/1) to afford a purple solid (98 mg). The solid was dissolved in a solution of *t*-BuOH (3 mL) and 2-methylbut -2-ene (0.5 mL). Then a solution of $NaClO_2$ (74 mg, 0.82 mmol) in 0.67M NaH_2PO_4 (0.7 mL) was added slowly to the reaction mixture at r.t. After 2 h, the reaction mixture was quenched with HCl (1 M, 10 mL), and extracted with ethyl acetate (2 × 20 mL). The combined organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography to yield a purple solid (73 mg, 70%). ^{1}H NMR (DMSO-D6): δ 8.53 (d, *J* = 8.8 Hz, 2H), 7.29 (d, *J* = 8.8 Hz, 2H), 4.97 (d, *J* = 2.4 Hz, 2H), 3.63 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (DMSO-D6): δ 163.5, 163.2., 161.3, 160.2, 130.2, 124.6, 116.0, 78.9, 78.8, 55.9. $[M + H]^+$ calcd for $C_{12}H_9N_4O_3$, 257.0675, found; 257.0689.

Synthesis of N-(3-hydroxy-2-methyl-6-((3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-1-yl)oxy)tetrahydro-2H-pyran-4-yl)-6-(4-(prop-2-yn-1-yloxy)phenyl)-1,2,4,5-tetrazine-3-carboxamide (**6**): To a solution of **4** (32 mg, 0.125 mmol) in 1.5 ml DCM was added oxalyl chloride (32 mg, 0.25 mmol); then DMF (2 μL) was added. The reaction was stirred at r.t. for 20 min. The solvent was

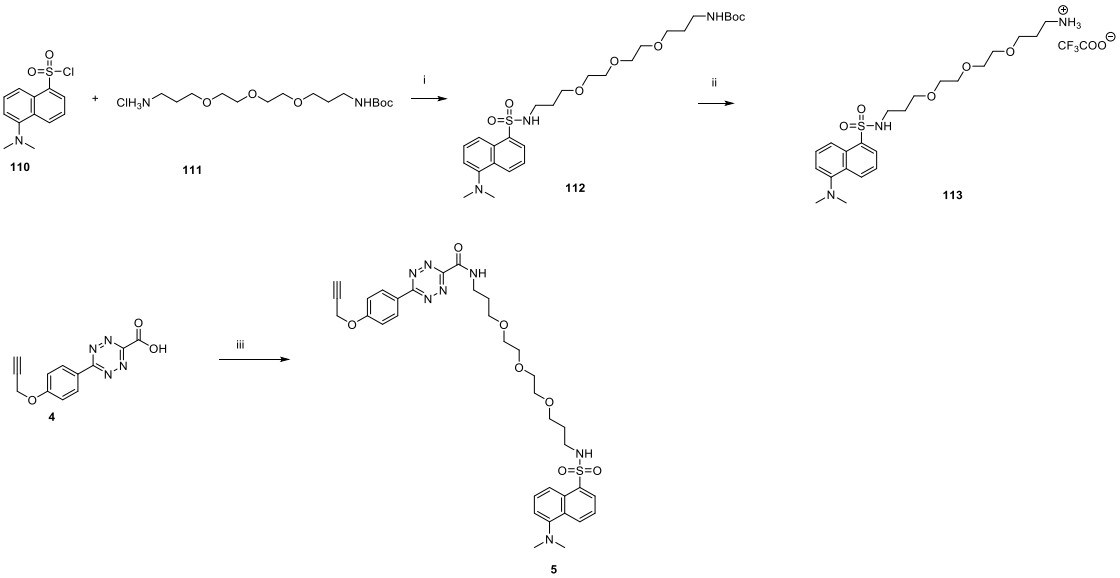
removed by rotavapor. The residue was dissolved in 1 ml DCM. A solution of *N*-hydroxysuccinimide (NHS, 29 mg, 0.25 mmol) in 2 mL DCM was added to the reaction mixture, followed by triethylamine (Et_3N, 16 μL). The reaction mixture was stirred at r.t. for 1 h; then a solution of doxorubicin hydrochloride (68 mg, 0.125 mmol) in 2 mL DMF was added to the reaction mixture. Then Et_3N (16 μL) was added to the mixture. The reaction was stirred at r.t. for 20 min and diluted with 20 mL DCM, and washed with H_2O (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography to yield a red solid (50 mg, 51%). ^{1}H NMR ($CDCl_3$): δ 13.97 (s, 1H), 13.21 (s, 1H), 8.56 (d, J = 8.8 Hz, 2H), 8.19 (d, J = 8.8 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.76 (t, J = 8.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.8 Hz, 2H), 5.57 (d, J = 3.2 Hz, 1H), 5.30 (d, J = 1.6 Hz, 1H), 4.80-4.79 (m, 4H), 4.54 (s, 1H), 4.52-4.48 (m, 1H), 4.27 (q, J = 6.4 Hz, 1H), 4.05 (s, 3H), 3.84 (d, J = 5.6 Hz, 1H), 3.26-3.28 (m 1H), 2.98-3.02 (m, 2H), 2.59 (t, J = 2.4 Hz, 1H), 2.42-2.37 (m, 2H), 2.22-1.99 (m, 3H), 1.34 (d, J = 9.6 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 214.0, 187.2, 186.8, 164.9, 162.3, 161.2, 158.7, 157.4, 156.3, 155.8, 135.9, 135.6, 133.8, 133.6, 131.0, 124.1, 121.0 120.0, 118.6, 116.0, 111.8, 111.6, 100.7, 100.1, 77.7, 77.4, 76.8, 76.6, 70.0, 69.3, 67.3, 65.7, 56.8, 56.1, 46.3, 35.8, 34.1, 29.9, 17.0. $[M - H]^-$ calcd for $C_{39}H_{34}N_5O_{13}$. 780.2159. found; 780.2140.

*Synthesis of (9-azidononyl)triphenylphosphonium bromide (***109***).* To a solution of 1, 9-dibromononane (2 ml, 10 mmol) in toluene (5 ml) was added triphenylphosphine (262 mg). The reaction with stirred at 110 °C for 24 h, and then cooled down to r.t. The solvent was removed under reduce pressure, and the residue was purified by column (DCM/MeOH= 50/1) to afford colorless oil (400 mg). Then the oil was dissolved in ethanol (5 ml), and followed by addition of NaN_3 (325 mg, 5 mmol). The reaction was stirred at 70 °C for 48 h, and cooled down to r.t. The solvent was removed under reduce pressure, and the residue was washed with H_2O (10 ml), and extracted with EtOAc (3 × 20 ml). The combined organic phase was washed with brine (10 ml) and dried over Na_2SO_4, and then evaporated under reduced pressure to

give the crude product, which was purified by column chromatography (DCM/MeOH= 20/1) to yield a red solid (300 mg, 62%). 1H NMR ($CDCl_3$): δ 7.65-7.85 (m, 15H), 3.78-3.70 (m, 2H), 3.20 (t, J = 7.0 Hz, 2H), 1.46-1.58 (m, 6H), 1.13-1.25 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 135.0 (d, J = 3 Hz), 133.7 (d, J = 10 Hz), 130.5 (d, J = 12 Hz), 118.4 (d, J = 85 Hz), 51.4, 30.4 (d, J = 15 Hz), 29.0, 28.9, 28.8, 26.6, 22.7, 22.6 (d, J = 50 Hz) 22.6, $[M - Br]^+$ calcd for $C_{27}H_{33}N_3P$, 430.2407. found: 430.2406.

*Synthesis of (7-(4-((4-(6-((3-hydroxy-2-methyl-6-((3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-1-yl)oxy)tetrahydro-2H-pyran-4-yl)carbamoyl)-1,2,4,5-tetrazin-3-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)heptyl)triphenyl phosphonium bromide(**9**):* To a solution of **6** (30 mg, 0.038 mmol), **109** (39 mg, 0.076 mmol), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 1 mg) in *t*-BuOH (1.5 mL), and DMSO (0.5 mL) was added a solution of $CuSO_4$ $5H_2O$ (1 mg) and sodium ascorbate (1.5 mg) in 0.5 mL H_2O. The reaction mixture was stirred at r.t. for 6 h, diluted with DCM (20 mL) and H_2O (10 mL), and extracted with DCM (2 × 20 ml). The combined organic layer was washed with EDTA (20 mM, 10 mL) and brine (10 mL), dried over Na_2SO_4, and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography (DCM/MeOH= 9/1) to yield a red solid (28 mg, 57%). ^1H NMR (DMSO-D6): δ 14.07 (s, 1H), 13.29 (s, 1H), 8.63 (d, J = 8.4 Hz, 1H), 8.48 (d, J = 8.0 Hz, 2H), 8.29 (s,1H), 7.60-7.90 (m, 18H), 7.33 (d, J = 8.0 Hz, 2H), 5.57 (s, 1H), 5.25-5.30 (m, 3H), 5.20 (d, J = 6.0 Hz, 1H), 4.94-5.02 (m, 1H), 4.89 (t, J = 6.0 Hz, 1H), 4.61 (d, J = 6.0 Hz, 2H), 4.30-4.37 (m, 3H), 3.97 (s, 3H), 3.50-3.60 (m, 2H), 2.94-3.07 (m, 2H), 2.00-2.33 (m, 4H), 1.71-1.78 (m, 4H), 1.41-1.50 (m, 4H), 1.16-1.23 (m, 11H). ^{13}C NMR (DMSO-D6): δ 213.9, 186.5, 186.5, 163.9, 162.3, 160.8, 158.9, 158.1, 156.2, 154.6, 142.0, 136.2, 135.5, 134.9 (d, J = 3.0Hz), 134.7, 134.2, 133.6 (d, J = 10 Hz), 133.4, 130.2 (d, J = 12 Hz), 130.2, 124.7, 123.7, 120.0, 119.8, 118.6 (d, J = 85 Hz), 115.9, 110.8, 110.7, 100.1,

75.0, 70.1, 67.8, 66.6, 63.7, 61.5, 56.6, 49.4, 46.0, 36.7, 32.1, 29.8, 29.8, 29.6, 28.5, 28.2, 27.9, 25.7, 21.6(d, J = 4 Hz), 20.2 (d, J = 50 Hz), 17.0. $[M - Br]^+$ calcd for $C_{66}H_{68}N_8O_{13}P$,1211.4638 found: 1211.4692.



Reagents and conditions: (i) DCM, Et_3N, r.t 1 h; (ii) DCM, TFA, 30 min. r.t.; (iii) (1) $C_2O_2Cl_2$, DMF, DCM. r.t. 30 min; (2) NHS, Et_3N, DCM, 1 h, r.t., (3) **113**, Et_3N, DCM, r.t. 20 min.

Supplementary Scheme 2. Synthesis of Dansylamine prodrugs.

*Synthesis of tert-butyl(3-(2-(2-(3-((5-(dimethylamino)naphthalene)-1-sulfonamido)propoxy)-ethoxy)ethoxy)propyl)carbamate (**112**).* To a solution of dansyl chloride (**110**, 270 mg, 1 mmol) in DCM (2 mL) was add a solution of NH_2-PEG-NHBoc (**111**, 352 mg, 1.1 mmol) and then Et_3N (180 uL). The reaction mixture was stirred at r.t. for 30 min and evaporated under reduced pressure to give the crude product, which was purified by column chromatography (DCM/MeOH= 50/1) to give a yellow oil (590 mg). 1H NMR ($CDCl_3$): δ 8.51 (d, J = 8.4 Hz, 1H), 8.31 (d, J = 8.4 Hz, 1H), 8.22 (dd, J1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.50-7.54 (m, 2H), 7.16 (d, J = 7.6 Hz, 1H), 5.70-5.78 (m, 1H), 4.90-4.98(m, 1H), 3.40-3.65 (m, 12H), 3.13-3.23 (m 2H), 3.02(q, *J* = 6.4 Hz), 2.87 (s, 6H), 1.65-1.75 (m, 2H), 1.58-1.64 (m, 2H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 156.14

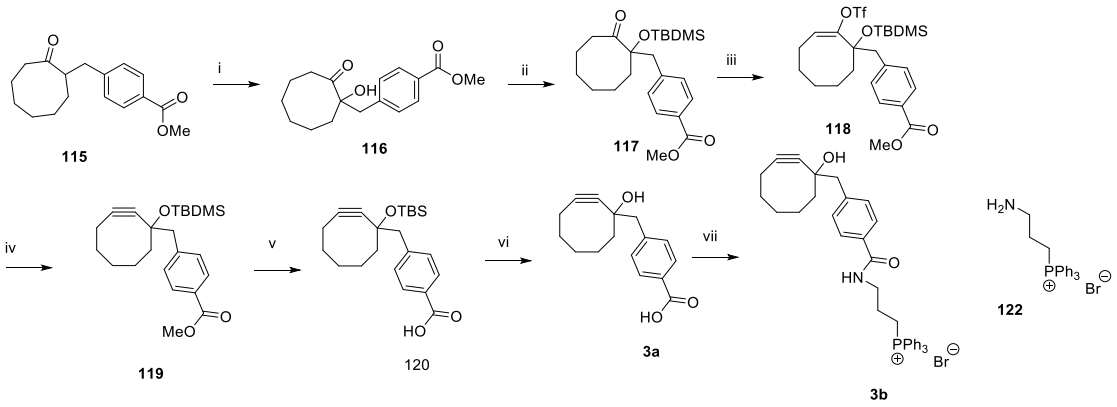
152.0, 135.0, 130.2, 130.0, 129.8, 129.6, 128.3, 123.3, 119.2, 115.2, 78.9, 70.7, 70.7, 70.6, 70.4, 70.2, 70.0, 45.5, 42.2, 38.6, 29.7, 28.8, 28.6. $[M+H]^+$ calcd for $C_{27}H_{44}N_3O_7S$. 554.2894 found: 554.2890.

Synthesis of N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**113**). To a solution of **112** (300 mg) in DCM (2 mL) was added TFA (2 mL) at r.t. The reaction mixture was stirred at r.t for 30 min and then the solvent was evaporated under reduced pressure to give the crude product, which was purified by column chromatography (DCM/MeOH= 10/1) to afford a green oil (292 mg, 95%). 1H NMR ($CDCl_3$): δ 8.51 (d, J = 8.4 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 8.21 (dd, J1 = 7.2 Hz, J2= 0.8Hz, 1H), 7.82-7.95 (m, 2H), 7.48-7.56 (m, 2H), 7.18 (d, J = 7.6 Hz, 1H) 6.48-6.55 (m, 1H), 3.55-3.68 (m, 8H), 3.44-3.50 (m, 4H), 3.15-3.22 (m, 2h), 2.95-3.00 (m 2H), 2.88(s, 6H), 1.90-1.95(m, 2H), 1.62-1.69(m, 2H). ^{13}C NMR ($CDCl_3$) δ 151.6, 135.0, 130.2, 129.9, 129.8, 129.5, 128.4, 123.4, 119.5, 115.5, 70.4, 70.4, 70.0, 69.9, 69.8, 69.6, 45.6, 41.7, 40.0, 28.8, 26.4. $[M+H]^+$ calcd for $C_{22}H_{36}N_3O_5S$ 454.2370 found: 454.2359.

Synthesis of N-(3-(2-(2-(3-((5-(dimethylamino)naphthalene)-1-sulfonamido)propoxy)ethoxy)-ethoxy)propyl)-6-(4-(prop-2-yn-1-yloxy)phenyl)-1,2,4,5-tetrazine-3-carboxamide(**5**). To a solution of **4** (16 mg, 0.0625 mmol) in 1ml DCM was added oxalyl chloride (16 mg, 0.125 mmol) and DMF (2 μL). The reaction was stirred at r.t for 20 min. Then the solvent was removed by rotavapor. The residue was dissolved in 1 mL DCM. A solution of NHS in 2 mL DCM was added to the reaction mixture, followed by the addition of Et_3N (16 μL). The reaction mixture was stirred at r.t for 1 h; then a solution of dansyzlamine (96 mg, 0.125 mmol) in 2 mL DCM was added, followed by the addition of Et_3N (32 μL). The reaction was stirred at r.t for 20 min, diluted with 20 mL DCM, and washed with H_2O (10 mL) and brine (10 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated under reduced pressure. The residue

was purified by column (DCM/MeOH= 50/1) to afford a purple solid 25 mg (60%). ^{1}H NMR ($CDCl_3$): δ 8.63 (dd, J_1 = 6.8 Hz, J_2 = 2 Hz, 2H), 8.55-8.61 (m 1H), 8.49 (d, J = 8.4 Hz, 1H), 8.31 (d, J = 8.4 Hz, 1H), 8.21 (dd, J_1 = 7.2 Hz, J_2= 1.2 Hz, 1H), 7.46-7.54 (m, 2H), 7.18 (dd, J_1 = 6.8 Hz, J_2 = 2 Hz, 2H), 7.14 (d, J = 7.2, 1H), 5.73-5.78 (m, 1H), 4.82 (d, J = 2.4 Hz, 2H), 3.64-3.76 (m, 10H), 3.47-3.49 (m 2H), 3.40 (t, J = 6.4 Hz 2H), 3.02 (q, J = 6.0 Hz, 2H), 2.87 (s, 6H), 2.59 (t, J = 2.4 Hz, 1H), 1.92-1.99 (m, 2H), 1.58-1.64 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 164.9, 162.2, 159.32, 157.8, 152.0, 135.1, 131.0, 130.3, 123.0, 129.8, 129.6, 128.3, 124.3, 123.3, 119.3, 116.0, 115.2, 77.4, 76.5, 70.9, 70.7, 70.6, 70.3, 70.2, 70.1, 56.1, 45.5, 42.3, 39.0, 28.9, 28.7. $[M+H]^+$ calcd for $C_{34}H_{42}N_7O_7S$, 692.2861 found: 692.2846.

Synthesis the Alkynes.



Reagents and conditions: (i) Cs_2CO_3, $P(OMe)_3$, DMSO, O_2, r.t, 24 h; (ii) TBDMSOTf, Et_3N, DCM, r.t, 6 h; (iii) (1) KHMDS, THF, -78 ^{o}C, 1 h; (2) Tf_2NPh, THF, -78^{o}C-r.t., 1h; (iv). LDA, THF, 0 ^{o}C, 2.5 h. (v) LiOH, dioxane/H_2O (5:1), 60 ^{o}C, 3 h; (vi). TBAF, THF, r.t, 2 h; (vii) (1) EDC, NHS, DCM, r.t., 1 h; (2) **122**, DCM, Et3N, r.t., 3 h.

Supplementary Scheme 3. Synthesis of TPP-Alkyne.

Compound **115**[1] and **122**[2] are known compounds.

Synthesis of methyl 4-((1-hydroxy-2-oxocyclooctyl)methyl)benzoate (**116**): To the solution of **115** (4.6 g, 16.8 mmol) in 20 ml of DMSO was added Cs_2CO_3 (821 mg, 2.52 mmol) and $P(OMe)_3$ (5.2 g, 42 mmol). The reaction was stirred at r.t under O_2 for 24 h, quenched with H_2O (40 mL), and extracted with EtOAc (2 × 200 ml). The combined organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography to yield a white solid (3.1 g, 64%).[3] ^{1}H NMR ($CDCl_3$): δ 7.91 (d, *J* = 8.3 Hz, 2H), 7.18 (d, *J* = 8.3 Hz, 2H), 3.88 (s, 3H), 3.84 (d, *J* = 1.2 Hz, 1H), 2.94 – 2.82 (m, 3H), 2.42 – 2.31 (m, 1H), 2.22-2.27 (m, 1H), 2.00 – 1.86 (m, 2H), 1.83 – 1.54 (m, 4H), 1.47 – 1.20 (m, 2H), 0.98 – 0.78 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 218.1, 167.1, 140.9, 1302, 129.5, 128.9, 81.0, 52.1, 46.3, 36.8, 33.3, 30.5, 25.5, 24.5, 23.0. [M +H]+ calcd for $C_{17}H_{23}O_4$, 291.1591 found: 291.1586.

Synthesis of methyl 4-((1-((tert-butyldimethylsilyl)oxy)-2-oxocyclooctyl)methyl)-benzoate(**117**). To a solution of **116** (3.1 g, 10.7 mmol) in 50 ml of DCM, was added TBDMSOTf (3.4 g, 12.84 mmol) and Et_3N (1.3 g, 12.84 mmol). The reaction was stirred at r.t for 6 h. Then solvent was evaporated under reduced pressure to give the crude product, which was purified by column chromatography to yield a white solid (1.7 g, 33%). ^{1}H NMR ($CDCl_3$): δ 7.91 (d, *J* = 7.2 Hz, 2H), 7.16 (d, *J* = 7.6 Hz, 2H), 3.90 (d, *J* = 1.0 Hz, 3H), 3.05 (d, *J* = 13.6 Hz, 1H), 2.90 (d, *J* = 13.6 Hz, 1H), 2.56 – 2.15 (m, 3H), 1.92 – 1.20 (m, 8H), 1.11 – 1.01 (m, 1H), 0.86 (d, *J* = 0.9 Hz, 9H), 0.05 (s, 3H), -0.12 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 216.3, 167.2, 141.6, 130.8, 129.4, 128.8, 85.1, 52.2, 47.8, 38.7, 35.9, 30.5, 26.6, 25.7, 24.8, 23.4, 19.4, -2.1, -2.4. [M +H]+ calcd for $C_{23}H_{37}O_4Si$, 405.2456 found: 405.2468.

Synthesis of methyl (E)-4-((1-((tert-butyldimethylsilyl)oxy)-2-(((trifluoromethyl)-sulfonyl)oxy)cyclooct-2-en-1-yl)methyl)benzoate(**118**). To a solution of **117**(1.7 g, 4.2 mmol) in 50 ml of THF was added a solution of potassium bis(trimethylsilyl)amide(KHMDS) in THF (0.5 M,

9.2 ml) over a period of 10 min under the protection of argon at -78 ^{o}C. The reaction was stirred at -78 ^{o}C for 1 h, and a solution of *N*-phenyl-bis(trifluoromethanesulfonimide) (Tf_2NPh, 1.6 g, 4.62 mmol) in 20 ml of THF was added slowly over 10 min. The reaction was stirred for another 10 min at -78 ^{o}C, then warmed to r.t, and then stirred for another 30 min. The solvent was removed by a rotavapor and the residue was purified by chromatography to give a colorless oil (1.0 g, 47%). ^{1}H NMR ($CDCl_3$): δ 7.93 (d, *J* = 8.1 Hz, 2H), 7.22 (d, *J* = 8.1 Hz, 2H), 5.57 (t, *J* = 9.4 Hz, 1H), 3.91 (s, 3H), 3.36 (d, *J* = 12.4 Hz, 1H), 2.91 (d, *J* = 12.4 Hz, 1H), 1.69-2.01 (m, 6H), 1.53 – 1.32 (m, 4H), 0.96 (s, 9H), 0.27 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 167.1, 152.3, 141.6, 130.5, 129.4, 128.7, 120.5, 118. 6(q, J= 317 Hz), 80.1, 52.2, 35.9, 26.5, 25.4, 23.5, 23.4, 22.1, 19.0, -1.3, -1.5. [M +H]+ calcd for $C_{24}H_{36}F_3O_6SSi$, 537.1948 found: 537.1970.

Synthesis of methyl 4-((1-((tert-butyldimethylsilyl)oxy)cyclooct-2-yn-1-yl)methyl)benzoate(**119**) To a solution of **118** (450 mg, 0.84 mmol) in 10 ml of THF was added a solution of LDA in THF (2M, 0.53 ml) drop-wise over a period of 2.5 h under the protection of argon at 0 ^{o}C. Then the reaction was quenched with H_2O (40 mL), and extracted with ethyl acetate (2 × 50 ml). The combined organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography to yield a colorless oil (200 mg, 62%). ^{1}H NMR ($CDCl_3$): δ 7.93 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 3.89 (s. 3H), 2.97 (d, *J* = 13.0 Hz, 1H), 2.78 (d, *J* = 13.0 Hz, 1H), 1.36-2.32 (m, 10H), 0.83 (s, 9H), 0.12 (s, 3H), -0.26 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 167.6, 143.6, 131.2, 129.0, 128.3, 99.5, 94.9, 75.9, 52.1, 52.0, 48.3, 34.5, 30.1, 27.2, 26.1, 20.7, 18.3, -2.7, -3.6. [M +H]+ calcd $C_{23}H_{35}O_3Si$ 387.2350 found: 387.2365.

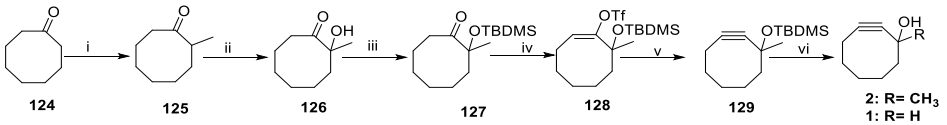
Synthesis of *4-((1-((tert-butyldimethylsilyl)oxy)cyclooct-2-yn-1-yl)methyl)benzoic acid* **(120)**. To a solution of **119** (100 mg, 0.26 mmol) in dioxane (3 mL) and H_2O (0.75 mL) was added finely crushed LiOH (200 mg, 8.3 mmol). The suspension was

heated to 50 ºC and then stirred for 3 h. The dioxane was removed on a rotary evaporator and the reaction mixture was diluted with DCM (20 mL). The organic layer was washed with 1 N HCl (2 × 10 mL), H_2O (3 x 10 mL), and brine (1 × 10 mL), and dried over Na_2SO_4, yielding a white solid (86 mg 89%). ^{1}H NMR ($CDCl_3$): δ 8.02 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 3.00 (d, J = 13Hz, 1H), 2.82 (d, J = 13Hz, 1H), 2.05- 2.28 (m, 2H), 1.51-2.01 (m, 6H), 1.26-1.50 (m, 2H), 0.84 (s, 9H), 0.13 (s, 3H), -0.25 (s, 3H) ^{13}C NMR ($CDCl_3$): δ 172.4, 144.6, 131.3, 129.6, 127.4, 99.6, 94.6, 75.7, 52.1, 48.4, 34.5, 30.1, 27.2, 26.0, 20.7, 18.2, -2.7, -3.6. $[M+H]^+$ calcd $C_{22}H_{33}O_3Si$ 373.2193 found: 373.2204.

Synthesis of *4-((1-hydroxycyclooct-2-yn-1-yl)methyl)benzoic acid* (**3a**). To a solution of **120** (68 mg, 0.18 mmol) in 0.2 ml of THF was added tetra-*n*-butylammonium fluoride (TBAF) solution (2M in THF/hexane, 1 mL). The reaction was stirred at r.t for 2 h, and then the solvent was removed under reduced pressure. The residue was purified by chromatography (DCM/EtOAc=2/1) to give a white sticky solid (46 mg, 99%). ^{1}H NMR ($CDCl_3$): δ 8.03 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 3.01 (d, *J*=13 Hz, 1H), 2.88 (d, *J*=13 Hz, 1H), 2.08-2.21(m, 3H), 1.72-2.03 (m, 6H), 1.36-1.44 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 172.0, 143.6, 130.7, 130.1, 127.9, 100.5, 94.4, 74.4, 50.7, 46.9, 34.5, 29.9, 26.5, 20.7. $[M+H]^+$ calcd $C_{16}H_{19}O_3$ 259.1329 found: 259.1325.

Synthesis of *(3-(4-((1-hydroxycyclooct-2-yn-1-yl)methyl)benzamido)propyl)-triphenylphosphonium bromide* (**3b**). To a solution of **3a** (38 mg, 0.15 mmol) in DCM, was added *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC 42 mg, 0.225 mmol) and NHS (35 mg, 0.3 mmol). The reaction was stirred at r.t for 1 h, diluted with DCM (10 mL), and then washed with H_2O (5 mL) and brine (5 mL). The organic phase was dried over Na_2SO_4, and evaporated under reduced pressure to afford a white solid. The solid was dissolved in 2mL DCM, followed by the addition of a solution of **122** (90 mg, 0.225 mmol) in 2mL DCM. Then Et_3N (30 mg, 0.3 mmol) was added into the reaction mixture. The reaction was stirred at r.t for 3 h, and diluted

with DCM (15 mL). The organic layer was washed with H_2O (5 mL) and brine (5 mL), dried over Na_2SO_4, and evaporated under reduced pressure to afford the crude product, which was purified by chromatography (DCM/MeOH =10/1) to give a white solid (57 mg, 60 %). ^{1}H NMR ($CDCl_3$): δ 9.43 (t, J = 6.0 Hz, 1H), 8.14 (d, J = 8.0 Hz, 2H) 7.69-7.74 (m, 9H), 7.56-7.61 (m, 6H), 7.39 (d, J = 8.0 Hz, 2H), 3.89-3.82 (m, 2H), 3.71-3.70 (m, 2H), 2.97 (d, J=13 Hz, 1H), 2.82 (d, J=13 Hz, 1H), 2.16 (t, J = 6.0 Hz, 2H), 2.12-1.67 (m, 10H), 1.43-1.38 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 167.8, 140.6, 135.1 (d, *J* = 3 Hz), 133.5 (d, *J* = 10 Hz), 132.4, 130.6, 130.6 (d, *J* = 12 Hz), 127.9, 118.4 (d, *J* = 85 Hz), 100.1, 99.7, 94.9, 74.3, 50.8, 46.9, 39.4 (d, *J* = 17 Hz), 34.6, 30.0, 26.7, 22.6 (d, *J* = 4 Hz), 20.7, 20.6 (d, *J* = 52 Hz). $[M+H]^+$ calcd : $C_{37}H_{39}NO_2P$, 560.2713 found: 560.2717



Reagents and conditions: (i) 1) LDA, THF, -78 °C, 1h; 2) methyliodide, THF, r.t, 40 min; (ii) Cs_2CO_3, $P(OEt)_3$, DMSO, O_2 (1.0 atm), r.t, 36 h; (iii) TBDMSOTf, Et_3N, DCM, r.t, 6 h; (iv) 1) KHMDS, THF, 46 °C, 1 h; 2) Tf_2NPh, THF, r.t, 1 h; (v) LDA, THF, 0 °C, 2.5 h, 89%; (vi) TBAF, THF, r.t, 2 h.

Supplementary Scheme 4. Synthesis of Alkyne **2** and the structure of Alkyne **1**[4]

Compound **124** is commercially available, and compound **1**[4] is a known compound.

Synthesis of 2-methylcyclooctan-1-one (**125**). To a solution of cyclooctanone (**124**, 3.2g, 25.3 mmol) in 50 ml of THF, a solution of LDA in THF (2M, 15.1 mL) was added dropwise under the protection of argon at -78 °C. The reaction was stirred at -78 °C for 1 h, and then methyliodide (1.6 ml, 25.7 mmol) was added slowly over 10 min. The reaction was stirred for another 30 min after being warmed to r.t. The solvent was removed on a rotavapor, and the residue was purified by chromatography to give a colorless oil (1.9 g, 56%). ^{1}H NMR ($CDCl_3$): δ2.63-2.52 (m, 1H), 2.43-2.30 (m, 2H), 1.33-1.95 (m, 9H), 1.25-1.12 (m, 1H), 1.01 (d, *J*=6.8 Hz, 3H). ^{13}C NMR

($CDCl_3$): δ 220.4, 45.3, 40.4, 33.1, 26.9, 26.6, 25.7, 24.6, 16.6. $[M+H]^+$ calcd: $C_9H_{17}O$ 141.1274 found: 141.1286

Synthesis of 2-hydroxy-2-methylcyclooctan-1-one (**126**). The mixture of **125** (1.9 g, 13.5 mmol), Cs_2CO_3 (1.3 g, 4.0 mmol) and $P(OEt)_3$ (6.9 ml, 40.5 mmol) in 20 ml of DMSO was stirred at r.t under O_2 for 36 h. Then the reaction was quenched with H_2O (40 mL), and extracted with EtOAc (2 × 200 ml). The combined organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography to yield a colorless liquid (0.75 g, 35%). ^1H NMR ($CDCl_3$): δ 3.91 (s, 1H), 2.83-2.72 (m, 1H), 2.35-2.24 (m, 2H), 1.99-1.83 (m, 2H), 1.82-1.67 (m, 3H), 1.67-1.56 (m, 1H), 1.39-1.28 (m, 2H), 1.27 (s, 3H), 0.96-0.83 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 219.8, 77.8, 35.8, 34.4, 30.3, 27.4, 25.4, 24.3, 23.1. $[M+Na]^+$ calcd: $C_9H_{16}NaO_2$, 179.1048 found:179.1055.

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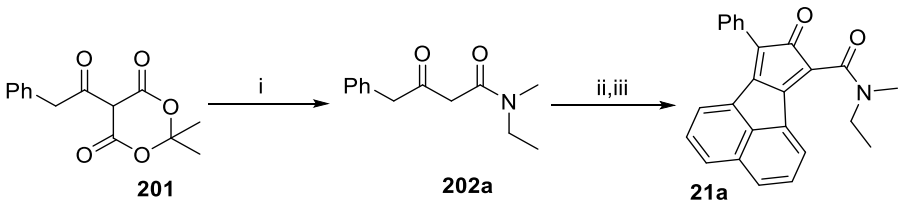
Synthesis of 2-((tert-butyldimethylsilyl)oxy)-2-methylcyclooctan-1-one (**127**). To a solution of **126** (0.75 g, 4.8 mmol) in 50 ml of DCM were added TBDMSOTf (1.6ml, 7.2 mmol) and Et_3N (0.73 ml, 5.3 mmol). The reaction was stirred at r.t for 6 h before solvent evaporation under reduced pressure to give the crude product, which was purified by column chromatography to yield a white solid (0.42 g, 33%). ^1H NMR ($CDCl_3$): δ 2.71-2.61 (m, 1H), 2.44-2.35 (m, 1H), 2.08-1.99 (m, 1H), 1.96-1.78 (m, 3H), 1.77-1.54 (m, 1H), 1.53-1.37 (m, 2H), 1.37-1.29 (m, 5H), 0.90 (s, 9H), 0.14 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 217.4, 80.8, 38.6, 36.9, 29.3, 26.2, 25.9, 25.6, 24.6, 23.1, 18.5, -2.3, -2.6. $[M+Na]^+$ calcd: $C_{15}H_{30}NaO_2Si$, 293.1913 found: 293.1913.

Synthesis of (E)-8-((tert-butyldimethylsilyl)oxy)-8-methylcyclooct-1-en-1-yl trifluoromethanesulfonate (**128**). To a solution of **127** (0.42 g, 1.58 mmol) in 50 ml of THF was added a solution of KHMDS in THF (0.5 M, 3.4 mL) slowly over 10 min under the protection of argon at -78 °C. After the reaction was stirred at -78 °C for 1 h, a solution of Tf_2NPh, (0.62 g, 1.73 mmol) in 10 ml of THF was added slowly over 10

min. The reaction was stirred for another 1 h after warming to r.t. The solvent was removed on a rotavapor and the residue was purified by column chromatography to give a colorless oil (0.32 g, 50%). ^{1}H NMR ($CDCl_3$): δ6.18 (dd, *J* =12.8, 4.7 Hz, 1H), 2.65-2.47 (m, 1H), 2.30-2.20 (m, 1H), 2.11-1.99 (m, 1H), 1.92-1.65 (m, 4H), 1.57-1.42 (m, 4H), 1.24-1.08 (m, 1H), 0.95 (s, 9H), 0.84-0.71 (m, 1H), 0.15 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 155.5, 118.6 (q, *J* = 318 Hz), 118.0, 76.6, 40.1, 28.8, 26.3, 26.2, 23.7, 23.5, 22.1, 18.7, -2.0, -2.0. $[M+H]^+$ calcd: $C_{16}H_{30}F_3O_4SSi$, 403.1586; found 403.1575.

Synthesis of tert-butyldimethyl((1-methylcyclooct-2-yn-1-yl)oxy)silane (**129**). To a solution of **128** (0.32, 0.80 mmol) in 10 ml of THF, a solution of LDA in THF (2 M, 0.8 ml) was added drop-wise over a period of 20 minutes under the protection of argon at 0 °C. After being warmed to r.t., the reaction was quenched with H_2O (10 mL), and extracted with ethyl acetate (2 × 30 ml). The combined organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure to give the crude product, which was purified by column chromatography to yield a colorless oil (179 mg, 89%).^{1}H NMR ($CDCl_3$): δ 2.29-2.10 (m, 2H), 2.08-1.98 (m, 1H), 1.92-1.82 (m, 3H), 1.80-1.68 (m, 2H), 1.61-1.51 (m, 2H), 1.36 (s, 3H), 0.88 (s, 9H), 0.19 (d, J=16.6Hz, 6H). ^{13}C NMR ($CDCl_3$): δ97.7, 96.8, 71.9, 53.9, 34.8, 30.2, 29.6, 27.1, 25.9, 20.8, 18.1, -3.0, -3.0. $[M+H]^+$ calcd: $C_{15}H_{29}OSi$, 253.1988, found: 253.1972

Synthesis of *1-methylcyclooct-2-yn-1-ol (**2**).* To a solution of **129** (179 mg, 0.71 mmol), TBAF (1 M, 2.1 ml) was added. After stirring at r.t. for 2 h, the solvent was removed under reduced pressure and was purified by chromatography to give a colorless oil (51 mg, 52%). ^{1}H NMR ($CDCl_3$): δ2.29-2.12 (m, 2H), 2.10-2.01 (m, 1H), 1.99-1.75 (m, 5H), 1.74-1.61 (m, 2H), 1.58-1.47 (m, 1H), 1.42(s, 3H). ^{13}C NMR ($CDCl_3$): δ 98.3, 96.1, 70.9, 52.5, 34.7, 30.0, 28.2, 27.0, 20.7. $M+H]^+$ calcd: $C_9H_{15}O$, 139.1117 found 139.1109.



Supplementary Scheme 5. **The synthesis of dienone 21a**. Reagents and conditions: i) *N*-methylethylamine, $MeSiCl_3$, toluene, reflux, 1 h; ii) acenaphthylene-1,2-dione, Et_3N, THF/MeOH, r.t.; iii) Ac_2O, H_2SO_4, 0 ^oC.

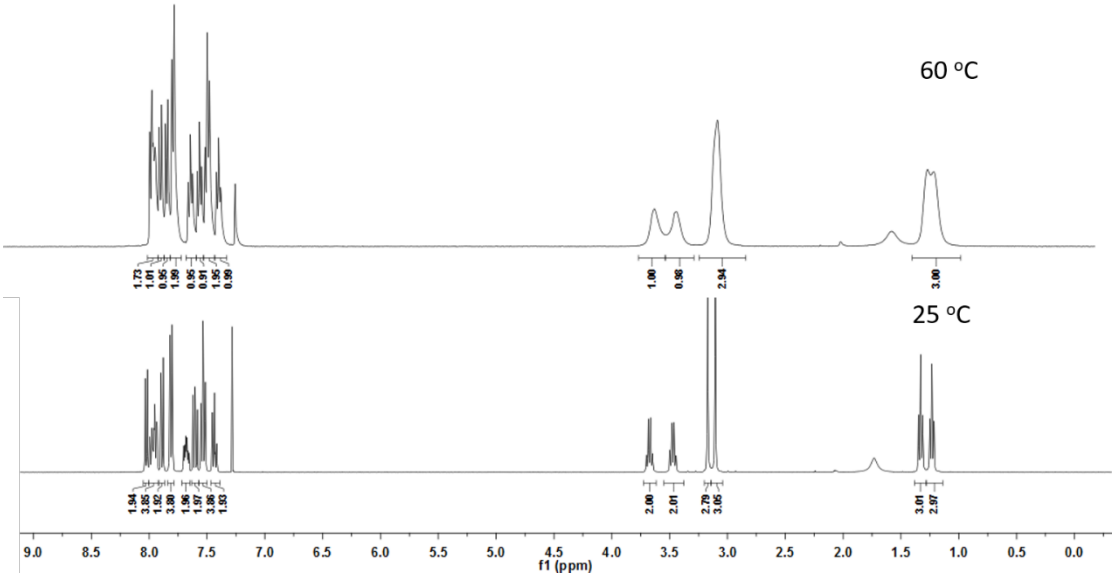
The synthesis of compound 202a

A solution of compound **201** (260 mg, 0.99 mmol), *N*-methylethylamine (117 mg, 1.98 mmol) and $MeSiCl_3$ (324 mg, 3.0 mmol) in toluene (10 mL) was heated under reflux for 1 h. Then the reaction mixture was diluted with ethyl acetate (30 mL), and was washed with $NaHCO_3$ and brine successively. The organic layer was dried over Na_2SO_4. After concentration, the obtained residue was purified over a silica gel column to afford the title compound as a mixture of keto-enol tautomers and amidic rotamers (185 mg, yield: 84%). ^1H NMR ($CDCl_3$) 14.97 (br, 0.2H), 7.37-7.25 (m, 5H), 5.06 (s, 0.27 H), 3.89 (s, 1.5 H), 3.54-3.52 (m, 2H), 3.43 (q, J = 7.2 Hz, 1H), 3.18 (q, J = 7.2 Hz, 1H), 2.93 (s, 2H), 2.85 (s, 1H), 1.14-1.06 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 202.3, 202.2, 171.5, 166.3, 166.2, 136.5, 133.7, 133.7, 129.6, 129.6, 129.4, 129.2, 128.7, 128.5, 127.2, 126.9, 126.8, 49.9, 48.4, 47.6, 45.0, 42.6, 42.3, 35.2, 32.7, 13.3, 12.1.

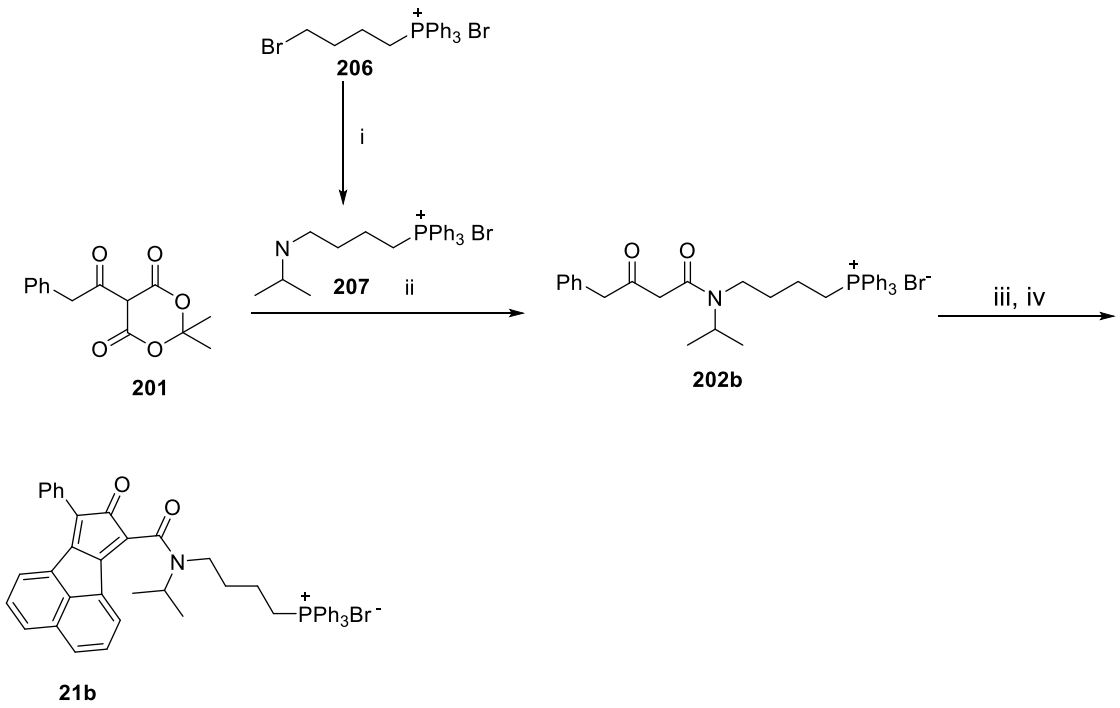
The synthesis of compound 21a

A solution of compound **202a** (200 mg, 0.91 mmol), acenaphthylene-1,2-dione (166 mg, 0.91 mmol), and Et_3N (138 mg, 1.36 mmol) in MeOH/THF (1:2, 4 mL) was stirred at room temperature overnight. Then the reaction mixture was dried under vacuum, and the obtained residue was taken into Ac_2O (2 mL). The resulting solution was cooled to 0 ^oC, and 3-4 drops of concentrated sulfuric acid was added. Then the reaction mixture was stirred for another 10 min at 0 ^oC. The reaction mixture was diluted with ethyl acetate, and washed with water, saturated $NaHCO_3$ solution, and

brine successively. The organic layer was dried over anhydrous Na_2SO_4. After filtration and concentration, the obtained solid was washed with MeOH to give the title compound as a mixture of amide rotamers (dark solid 200 mg, yield: 60%).^{1}H NMR ($CDCl_3$): δ 8.02 (d, *J* = 7.2 Hz, 1H), 8.00 – 7.92 (m, 2H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.82-7.80 (m, 2H), 7.69-7.65 (m, 1H), 7.62-7.58 (m, 1H), 7.53 (t, *J* = 7.6 Hz, 2H), 7.47 – 7.39 (m, 1H), 3.67 (q, *J* = 7.1 Hz, 2H), 3.47 (q, *J* = 7.2 Hz, 2H), 3.17 (s, 3H), 3.10 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 199.1, 199.0, 164.1, 163.7, 161.1, 152.1, 145.1, 131.9, 131.1, 130.9, 130.1, 129.2, 129.1, 129.0, 128.7, 128.6, 128.5, 127.8, 124.4, 124.1, 121.2, 116.6, 45.8, 42.2, 36.1, 32.1, 14.0, 12.3. HRMS(ESI) Cacld. for $C_{25}H_{20}NO_2$ $[M+H]^+$ 366.1489, found 366.1484.



Supplementary Figure 1. The ^{1}H NMR spectra of compound **21a** in $CDCl_3$ at different temperature.



Supplementary Scheme 6. The synthesis of dineone compound 21b. Reagents and conditions: i) isopropylamine, K_2CO_3, ACN, reflux, overnight; ii) $MeSiCl_3$, toluene, reflux, 1 h; iii) acenaphthylene-1,2-dione, Et_3N, THF/MeOH, r.t.; iv) Ac_2O, H_2SO_4, 0 ^oC.

The synthesis of the TPP functionalized amine 207

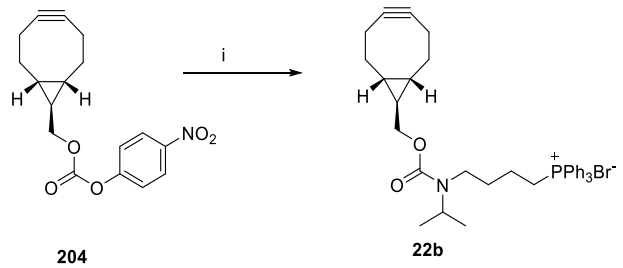
A suspension of compound **206** (2.0 g, 4.2 mmol), isopropylamine (1.5 g, 25.2 mmol), and K_2CO_3 (0.87 g, 6.3 mmol) in CH_3CN (100 mL) was heated under reflux under N_2 atmosphere overnight. Then the reaction mixture was filtered, and the filtrate was concentrated to give a colorless oil, which was purified over silica gel column to afford the title compound as a sticky solid (0.95 g, yield: 50%). ^1H NMR ($CDCl_3$): δ 7.81-7.70 (m, 15H), 3.69 (m, 2H), 3.43-3.39 (m, 1H), 3.10 (br, 2H), 2.39 (m, 2H), 1.99 (m, 2H), 1.48 (d, J = 6.4 Hz, 6H). ^{13}C NMR ($CDCl_3$) 135.2, 135.2, 133.8, 133.7, 130.7, 130.6, 118.2, 117.4, 51.9, 44.2, 27.1, 23.1, 20.1, 18.9. HRMS(ESI) Cacld. for $C_{25}H_{31}NP$ $[M\text{-}Br]^+$ 376.2189, found 376.2185.

The synthesis of compound 202b

Using a method similar for the synthesis of compound **202a**, compound **202b** was obtained as a yellowish oil as a mixture of keto-enol tautomers (yield: 60%). ^{1}H NMR ($CDCl_3$): 15.02 (s, 0.1H), 14.96 (s, 0.2H), 7.86-7.72 (m, 11.2H), 7.69-7.60 (m, 8.1H), 7.31-7.28 (m, 1.9H), 7.24-7.14 (m, 3.7H), 3.95-3.81 (m, 3.2H), 3.76-3.73 (m, 1.8H), 3.61-3.54 (m, J = 8.0 Hz, 0.8H), 3.47-3.40 (m, 2.7H), 3.36-3.27 (m, 1.9H), 3.23-3.13 (m, 0.9H), 1.92-1.86 (m, 2.4H), 1.71-1.51 (m, 2.7H), 1.22-1.14 (m, 3.5H), 1.09-1.08 (d, J = 6.4 Hz, 6H). ^{13}C NMR ($CDCl_3$): 202.3, 166.9, 166.5, 135.0, 134.9, 134.9, 133.8, 133.7, 130.6, 130.5, 130.4, 130.4, 130.0, 129.6, 129.2, 128.8, 128.5, 128.4, 127.3, 126.8, 118.8, 118.0, 117.8, 65.8, 50.0, 49.7, 48.1, 39.7, 30.2, 30.1, 22.5, 22.0, 21.2, 20.4, 20.2, 15.3. HRMS (ESI+): m/z $[M\text{-}Br]^+$ calcd. for $C_{35}H_{39}O_2NP$: 536.2713, found: 536.2714.

The synthesis of compound 21b

Using a method similar for the synthesis of compounds **21a**, compound **21b** was obtained as a mixture of amidic rotamers (yield 60%).^{1}H NMR ($CDCl_3$): 8.05 (d, J = 7.2 Hz, 1H), 7.91-7.87 (m, 8H), 7.82-7.78 (m, 3H), 7.72-7.60 (m, 11H), 7.54 (t, J = 7.6 Hz, 2H), 7.45 (t, J = 7.6 Hz, 1H), 411-4.04 (m, 3H), 3.61 (t, J = 6.8 Hz, 2H), 2.22-2.17 (m, 2H), 1.91-1.86 (m, 2H), 1.23 (d, J = 6.7 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 199.0, 164.1, 163.7, 161.1, 152.1, 145.1, 131.9, 131.1, 130.9, 130.1, 129.2, 129.1, 129.0, 128.7, 128.6, 128.5, 127.8, 124.4, 124.1, 121.2, 116.6, 45.9, 42.2, 36.1, 32.1, 14.0, 12.3. HRMS(ESI) Calcd. for $C_{47}H_{41}NO_2P$ $[M\text{-}Br]^+$ 682.2869, found 682.2856.

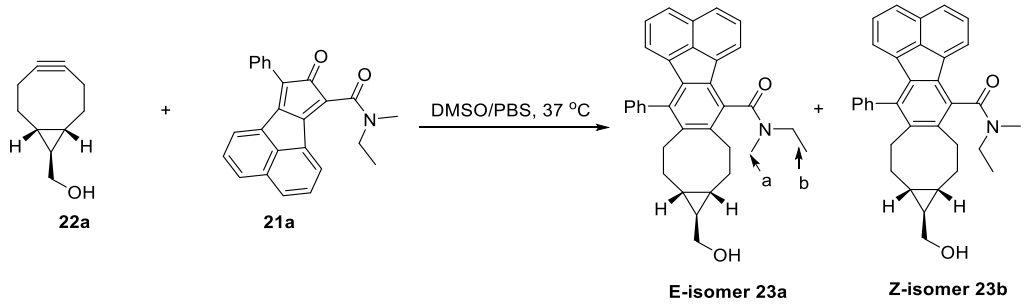


Supplementary Scheme 7. The synthesis of compound **22b**. Reagents and conditions: i) DMF, DIPEA, 60 ^{o}C, overnight.

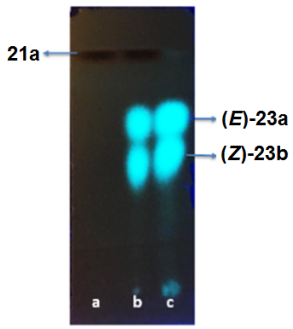
The synthesis of compound 22b

The activated ester of **22a. 204** (100 mg, 0.32 mmol) was dissolved in dry DMF (1 mL), followed by the addition of compound **7** (218 mg, 0.48 mmol) and DIPEA (62 mg, 0.48 mmol). The resulting solution was heated to 60 ^{o}C, and stirred overnight at this temperature. Then the reaction mixture was concentration under high vacuum, and the residue was purified on a silica gel column to afford compound **5** as a stick solid (90mg, yield: 45%). ^{1}H NMR ($CDCl_3$): 7.89-7.84 (m, 6H), 7.80-7.76 (m, 3H), 7.71-7.66 (m, 6H), 3.96 (br s, 3H), 3.90-3.89 (d, 2H), 3.23 (br s, 2H), 2.35-2.24 (m, 4H), 2.16-2.12 (m, 2H), 1.97 (br s, 2H), 1.70-1.63 (m, 2H), 1.34 (br s, 2H), 1.30-1.25 (m, 1H), 1.21 (s, 6H). ^{13}C NMR ($CDCl_3$): 135.0, 133.8, 133.6, 130.5, 130.4, 118.6, 117.8, 98.7, 69.2, 60.4, 53.5, 48.3, 42.7, 33.3, 23.8, 22.9, 21.4, 21.0, 14.2. HRMS (ESI+): m/z $[M-Br]^+$ calcd. for $C_{35}H_{39}O_2NP$ 552.3031, found: 552.3028.

The click reaction between **22a** and **21a**



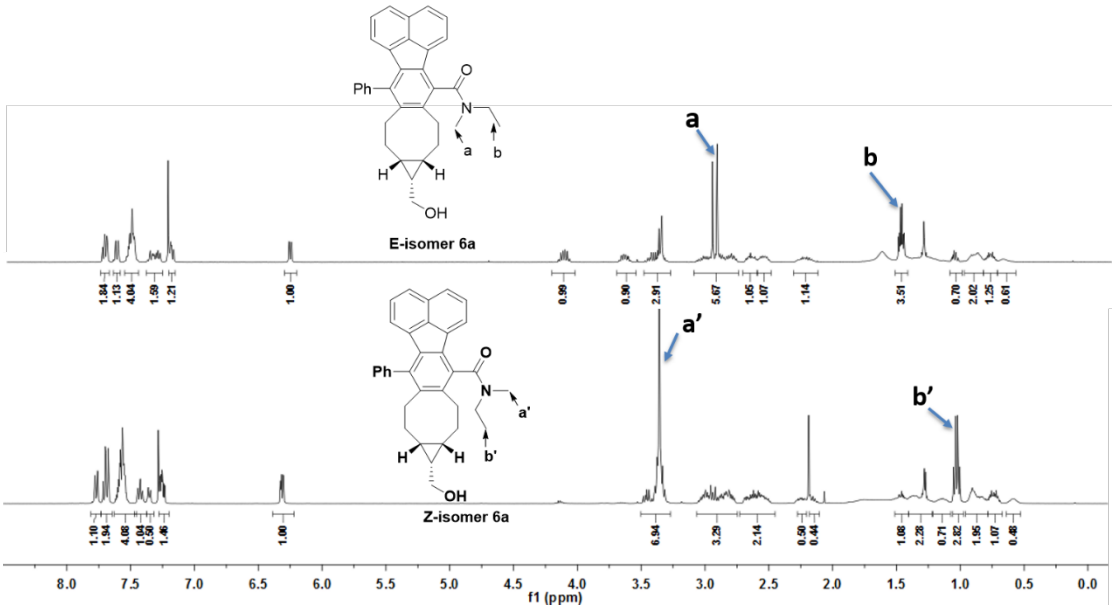
A solution of compound **21a** (60 mg, 0.16 mmol) and **22a** (36 mg, 0.24 mmol) in DMSO/PBS (1:1, 10 mL) was stirred at 37 ^{o}C overnight. Then water (30 mL) was added, and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with water and brine, and was dried over anhydrous Na_2SO_4. After filtration and concentration, the residue was purified on a silica gel column using DCM/acetone = 8: 1 as eluent to afford the E and Z isomers of the click product, both of which exist as a mixture of boat and chair conformational isomers.



(The TLC plate of the reaction between **21a** and **22a**. CH_2Cl_2/acetone = 4:1. The TLC was run twice)

(*E*)-23a (34 mg, yield: 42%).^{1}H NMR ($CDCl_3$) δ 7.78 (m, 2H), 7.69 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.58 (m, 4H), 7.38 (m, 2H), 7.26-7.23 (m, 1H), 6.31 (dd, *J* = 7.0, 1.6 Hz, 1H), 4.14 (m, 1H), 3.66-3.62 (m, 1H), 3.41-3.33 (m, 3H), 3.03-2.78 (m, 6H), 2.66-2.61 (m, 1H), 2.56-2.52 (m, 1H), 2.25-2.17 (m, 1H), 1.46-1.43 (m, 3H), 1.04-1.00 (m, 1H), 0.91-0.85 (m, 2H), 0.77-0.72 (m, 1H), 0.65 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 170.6, 140.6, 140.4, 138.3, 136.7, 135.2, 132.8, 131.9, 129.7, 129.3, 129.0, 128.9, 128.9, 128.8, 127.9, 127.7, 127.5, 126.7, 122.7, 121.3, 66.5, 60.4, 41.5, 35.4, 35.3, 31.6, 30.7, 30.2, 29.5, 28.9, 21.1, 14.2, 12.2, 12.1. HRMS (ESI+): m/z [M+H]+ calcd. for $C_{34}H_{34}NO_2$: 488.2584, found: 488.2592.

(Z)-23b (32 mg, yield: 40%): ^{1}H NMR ($CDCl_3$): δ 7.77 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.71-7.67 (m, 2H), 7.61-7.53 (m, 4H), 7.42 (t, *J* = 7.2 Hz, 1H), 7.36-7.34 (m, 0.5 H), 7.27-7.23 (m, 1.5 H), 6.31 (dd, *J* = 7.2, 2.5 Hz, 1H), 3.48-3.31 (m, 7H), 3.01-2.91 (m, 3H), 2.64-2.54 (m, 2H), 2.27-2.21 (m, 1H), 1.51-1.44 (m, 1H), 1.36-1.26 (m, 2H), 1.14-1.09 (m, 1H), 1.03 (t, *J* = 7.2 Hz, 3H), 0.95-0.80 (m, 2H), 0.76-0.70 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 170.8, 141.8, 140.6, 138.4, 137.2, 136.6, 136.0, 135.2, 132.8, 131.7, 129.7, 129.1, 129.0, 128.9, 127.9, 127.8, 127.7, 127.5, 126.7, 126.3, 122.7, 121.6, 121.5, 66.5, 45.6, 31.4, 31.4, 30.9, 30.9, 30.3, 30.0, 29.3, 29.2, 13.7. HRMS (ESI+): m/z $[M+H]^+$ calcd. for $C_{34}H_{34}NO_2$: 488.2584, found: 488.2582.



Supplementary Figure 2. The ^{1}H NMR differences between compound **(*E*)-23a** and **(*Z*)-23b.**

2. Kinetic studies of the CCR system

Stock solution preparation.

Each of the tetrazine prodrugs (compounds **5**, **6** and **9**) was dissolved in DMSO to afford a 500-μM stock solution. Each of the alkynes (compounds **1**, **2**, **3a** and **3b**) was dissolved in DMSO to afford a 50-mM stock solution. Doxorubicin (Dox) and dansylamino compound (DA) **113** were each dissolved in DMSO to afford 1-mM stock solutions

HPLC method A: mobile phase A (10 mM NaH_2PO_4 in water, pH = 5.0) and mobile phase B (ACN), flow rate: 1 mL/min, running time: 30 min, the gradient elution method: 25% B from 0 to 6 min, 25% to 50 % B from 6 to 8 min, 50% B from 8 to 15 min, 50% to 99% B from 15 to 20 min, 99% B from 20 to 25 min, 99% to 25% B from 25 to 30 min. Detection wavelength: 256 nm. Column: Waters C18 3.5 μM, 4.6 × 100 mm. Injection volume: 20 μL.

HPLC method B: mobile phase A (10 mM NaH_2PO_4 in water, pH = 5.0) and mobile phase B (ACN), flow rate: 1 mL/min, running time: 30 min, the gradient elution method: 30% B from 0 to 6 min, 30% to 50 % B from 6 to 8 min, 50% B from 8 to 15

min, 50% to 99% B from 15 to 20 min, 99% B from 20 to 25 min. 99% to 30 % B from 25 to 30 min. Detection wavelength 1: 256, detection wavelength 2: 337 nm. Column: Waters C18 3.5 μM, 4.6 × 100 mm. Injection volume: 20 μL.

Standard curve of Dox concentration measurement.

To four HPLC vials containing 0.9 mL PBS (size: 1.5 mL) were added 25, 15, 20, 10 μL Dox stock solution (1 mM) respectively. Then defined amounts DMSO (975, 985, 980, and 990 μL) were added to each vial to afford final concentration of four vials were 25, 15, 20, 10 μM in PBS (10% DMSO), respectively. The samples were analyzed with HPLC method A. Dox retention time: 4.0 min.

Standard curve for DA concentration measurement.

To four HPLC vials containing 0.9 mL PBS (size: 1.5 mL) were added 25, 15, 20, 10 μL Dox stock solution (1 mM) respectively. Then defined amounts DMSO (975, 985, 980, and 990 μL) were added to each vial to afford final concentration of four vials were 25, 15, 20, 10 μM in PBS (10% DMSO), respectively. The samples were analyzed with HPLC method B. DA retention time: 5.8 min.

General procedures HPLC studies of reaction kinetic.

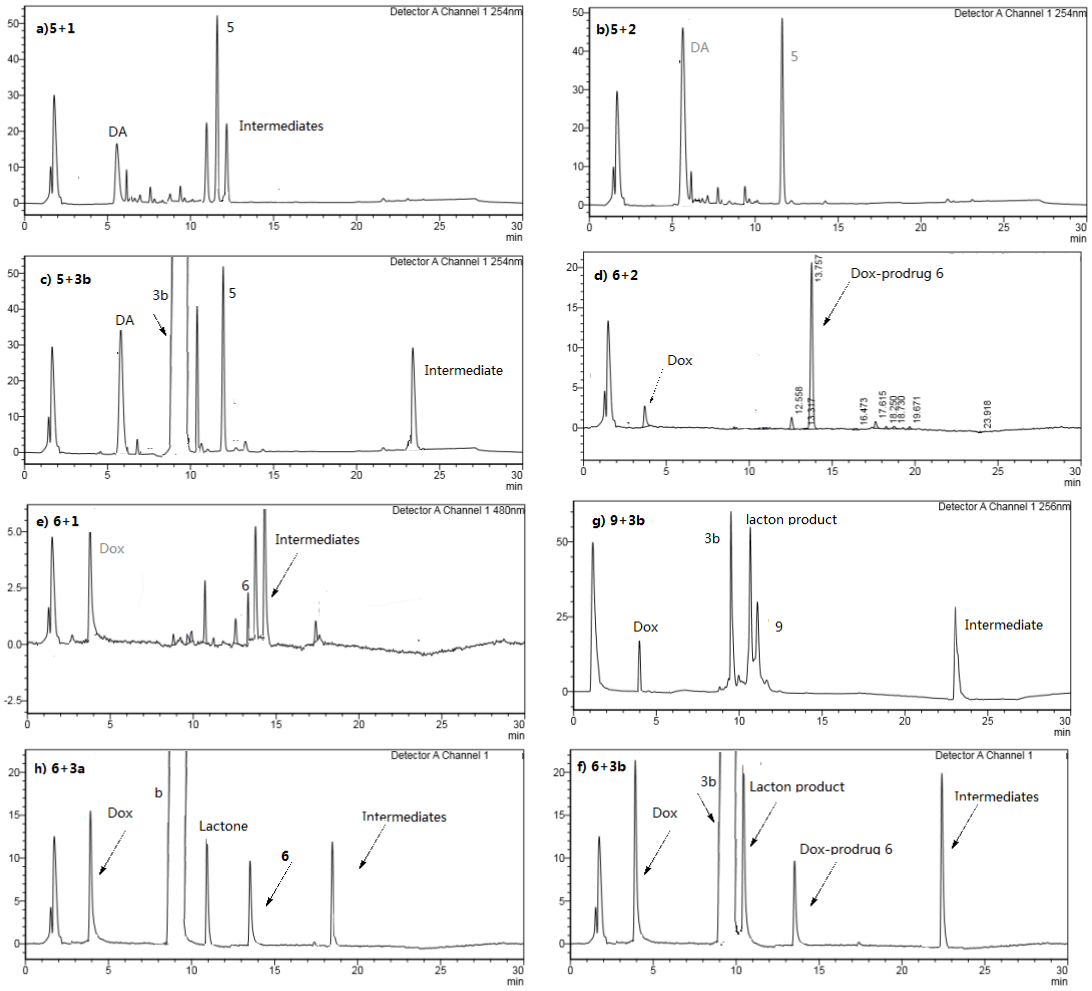
To three 20-ml vials with 9 mL PBS were added a solution of tetrazine prodrug (500 μL, 500μM). 350 μL, 300 μL and 250 μL DMSO were added to vial respectively, and then 150 μL, 200 μL and 250 μL of the alkyne solution (50 mM in DMSO) were added to the vials to afford final concentrations of alkyne 0.75 mM, 1 mM and 1.25 mM in PBS (10% DMSO, 25 μM prodrug) respectively. The reaction was stirred at r.t or 37 ºC respectively. Every 30 min about 500 μL reaction mixtures were taken out and 20 μL of them were injected into the HPLC by the autosampler; the rest of them were poured back to the reaction mixture. (Note: In the cases of prodrugs **5** or **6** reacting with alkyne **1** at 37 °C, the final concentrations of **1** in the kinetic studies were 250 μM, 300 μM and 350 μM).

Method	Prodrugs	Alkyne	Prodrugs	Dox/DA	Intermediates

			retention time (min)	retention time (min)	retention time (min)
A	**6**	**1**	13.7	3.9	13.9/14.5
		2	13.7	3.9	NO
		3a	13.7	4.0	18.5
		3b	13.7	4.0	22.8
	9	**3b**	11.0	4.0	23.1
B	**5**	**1**	11.7	5.7	11.5/12.1
		2	11.7	5.7	no
		3b	11.7	5.7	23.5

Supplementary Table 1. Retention time from the kinetic studies.

HPLC chromatograms



Determination of second-order rate constants:

The reaction rate constant, k_{obs}, was calculated for each concentration of the alkyne by fitting the prodrug areas versus time using eq. 1

$Y=Aexp(-k_{obs}t)$ eq. 1

Where Y is the prodrug area, and t is time. The pseudo-first-order rate constant, k_{obs}, was then plotted against the concentration of alkyne to yield the second-order rate constant using eq 2.

$k_{obs} = k_2[Alkyne]$ eq. 1

where k_2 is the second-order rate constant. The results are shown in Table 2.

Temp.	Prodrugs (25 μM)	Alkyne	Concentration of Alkyne (mM)	Expt. 1	Expt. 2	Expt. 3
				K_{obs} (h^{-1})		
r.t	**5**	**1**	0.75	0.752	0.704	0.756
		1	1.00	0.980	0.802	1.01
		1	1.25	1.20	1.10	1.26
37 °C	**5**	**1**	0.25	1.82	1.64	1.90
		1	0.3	2.15	1.94	2.26
		1	0.35	2.53	2.25	2.65
r.t	**5**	**2**	0.75	0.0203	0.0201	0.0217
		2	1.00	0.0273	0.0269	0.0289
		2	1.25	0.0339	0.0324	0.0359
37 °C	**5**	**2**	0.75	0.0506	0.0584	0.0599
		2	1.00	0.0670	0.0781	0.0801
		2	1.25	0.083	0.0978	0.101
r.t	**5**	**3b**	0.75	0.125	0.115	0.13
		3b	1.00	0.155	0.148	0.175
		3b	1.25	0.199	0.180	0.22
37 °C	**5**	**3b**	0.75	0.411	0.388	0.353
		3b	1.00	0.548	0.511	0.453

		3b	1.25	0.695	0.636	0.561
r.t	**6**	**1**	0.75	0.894	0.976	0.993
		1	1.00	1.18	1.30	1.34
		1	1.25	1.47	1.62	1.69
37 °C	**6**	**1**	0.25	1.90	1.81	1.97
		1	0.3	2.28	2.15	2.38
		1	0.35	2.65	2.48	2.78
r.t	**6**	**2**	0.75	0.0202	0.0209	0.0207
		2	1.00	0.0282	0.0262	0.0278
		2	1.25	0.0353	0.0341	0.0344
37 °C	**6**	**2**	0.75	0.0607	0.0671	0.0696
		2	1.00	0.0798	0.0895	0.0941
		2	1.25	0.0986	0.112	0.119
r.t	**6**	**3b**	0.75	0.201	0.195	0.197
		3b	1.00	0.261	0.257	0.261
		3b	1.25	0.309	0.318	0.326
37 °C	**6**	**3b**	0.75	0.486	0.503	0.533
		3b	1.00	0.635	0.672	0.622
		3b	1.25	0.783	0.846	0.905
r.t	**6**	**3a**	0.75	0.183	0.191	0.185
		3a	1.00	0.235	0.246	0.247
		3a	1.25	0.289	0.295	0.307
37 °C	**6**	**3a**	0.75	0.418	0.438	0.495
		3a	1.00	0.546	0.598	0.678
		3a	1.25	0.673	0.739	0.857
r.t	**9**	**3b**	0.75	0.296	0.305	0.291
		3b	1.00	0.331	0.356	0.359
		3b	1.25	0.445	0.465	0.474
37 °C	**9**	**3b**	0.75	0.711	0.701	0.714

		3b	1.00	0.921	0.902	0.871
		3b	1.25	1.21	1.15	1.09

Supplementary Table 2. K_{obs} table from the kinetic studies.

3. General procedure for the determination of the lactonization rate constants.

To a 9 mL PBS in a 20-mL vial was added a solution of the respective tetrazine prodrug (500 μL, 500 μM). Then 1 mL of the respective alkyne (50 mM in DMSO) was added to afford a final concentration of 5 mM in PBS (15% DMSO). The reaction was stirred at r.t. Every 30 min about 500 μL the reaction mixture was taken out and 20 μL of them was injected into the HPLC by an autosampler; the rest of them was poured back to the reaction mixture. The reaction rate constant, k_1, was determined by fitting the area in HPLC chromatogram of the intermediate versus time using eq. 3.

$Y=Aexp(-k_1 t)$ eq. 3

Where Y is the intermediate areas, and t is the time. The results are shown in Table 3.

Tetrazines prodrugs	Alkyne	First Data point time*	k_1 (h^{-1})		
			Expt 1	Exp 2	Expt. 3
5	**1**	1 h	0.0253	0.0291	0.0332
5	**3b**	4 h	0.162	0.184	0.142
6	**3b**	4 h	0.223	0.179	0.196
6	**3a**	4 h	0.185	0.206	0.229
9	**3b**	4h	0.225	0.191	0.265

*: The time point that more that 90% prodrug were consumed.

Supplementary Table 3. Lactonization rate constants.

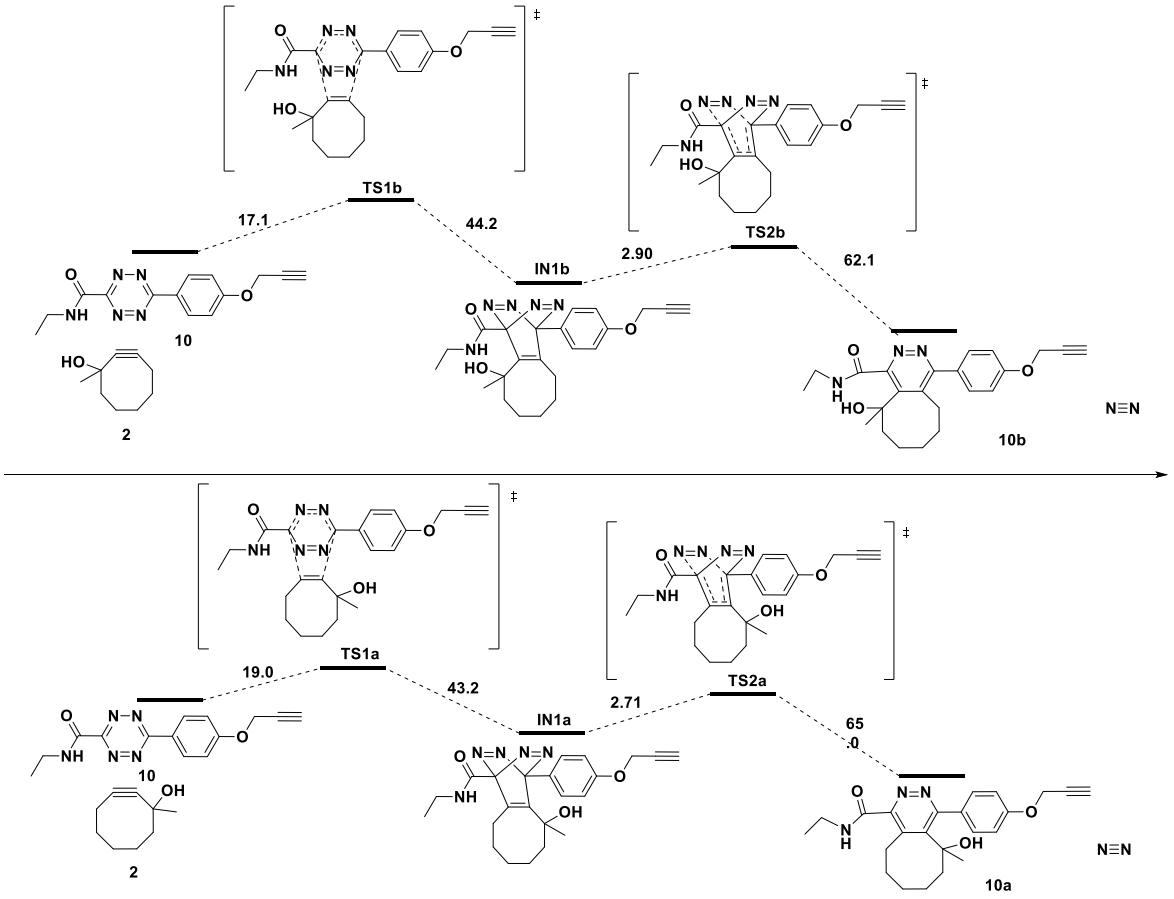
4. General procedure of prodrug stability studies.

To a 9.5 mL of PBS or 1 mM cysteine solution in PBS in a 20-mL vial was added a solution of tetrazine prodrug (500 μL, 500 μM). The solution was stirred at r.t. or 37 ºC for 24 h. The reaction solution was injected into HPLC, and analyzed with Method A for prodrugs **6** and **9**, and Method B for prodrug **5**.

Prodrug (**5** or **6**, 25 μM in PBS alone or in the presence of 1 mM cysteine (Cys) in PBS) was incubated at r.t or 37 °C for 24 h. Then HPLC was used to monitor the concentration of the remaining prodrug (Table 4). (Note: there are literature reports of Dox decomposition by itself[5]).[6]

Prodrugs remaining	24 h in PBS at r.t (%)	24 h in PBS at 37 °C (%)	24 h in PBS with 1mM Cys at r.t (%)	24 h in PBS with 1mM Cys at 37 °C (%)
5	91±5	85±5	88±5	81±5
6	85±5	78±5	80±5	76±5

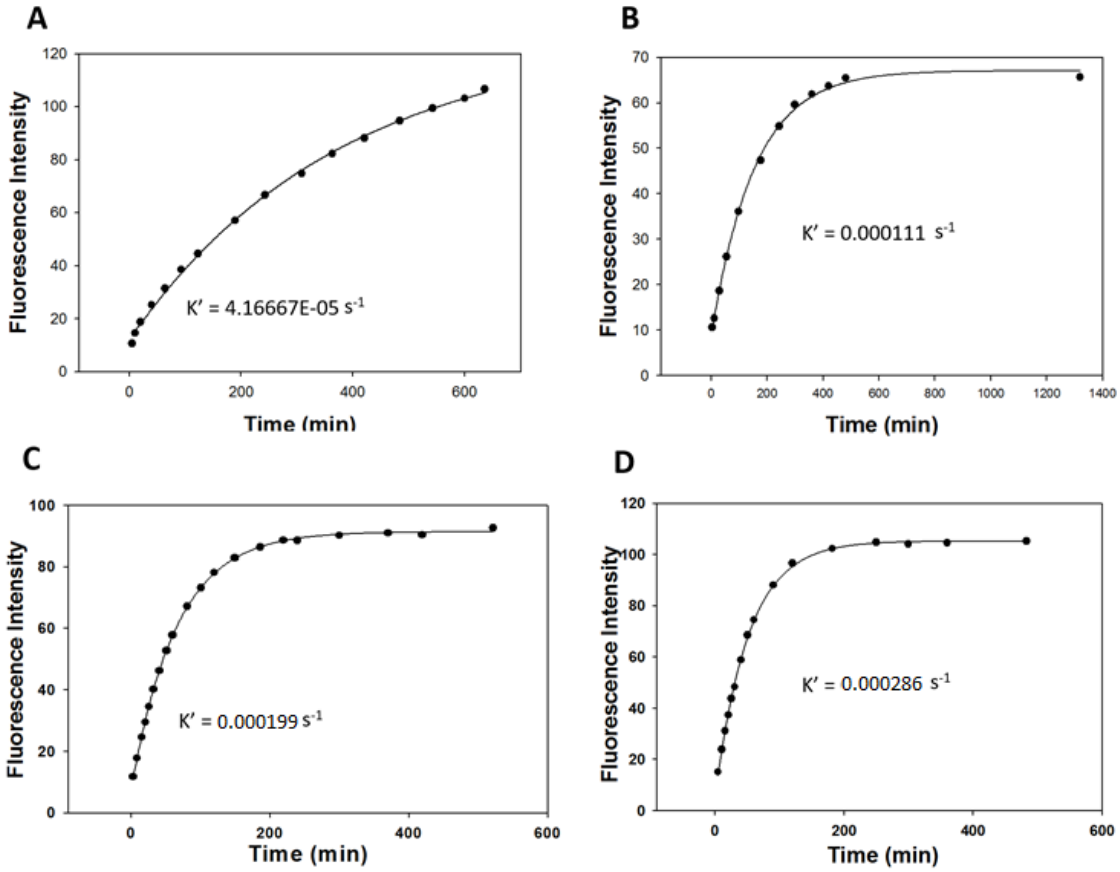
Supplementary Table 4. Stability studies of prodrugs **5** and **6** in PBS (5% DMSO, n = 4, p = 0.95).



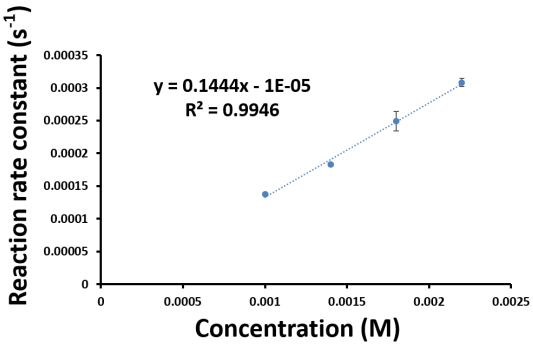
Supplementary Figure 3. Schematic representations of the reaction between tetrazine **10** and alkyne **2** (kcal·mol^{-1})

5. Determination of the reaction rate constants between 21a and 22a

A solution of **21a** (50 μM) and **22a** (1 mM, 1.4 mM, 1.8 mM or 2.2 mM) in 20% of DMSO in PBS (pH 7.4) was incubated at 37 ^{o}C. The fluorescence intensity at 461 nm (λ_{ex} = 370 nm) was recorded at intervals. The fluorescence intensity was then plotted against the time, and the obtained curve was fitted using Sigmaplot to give the pseudo first order reaction rate constant k' (Figure 3). The k' values were then plotted against the concentrations of **22a** to obtain the second order reaction rate constant (Figure 4).



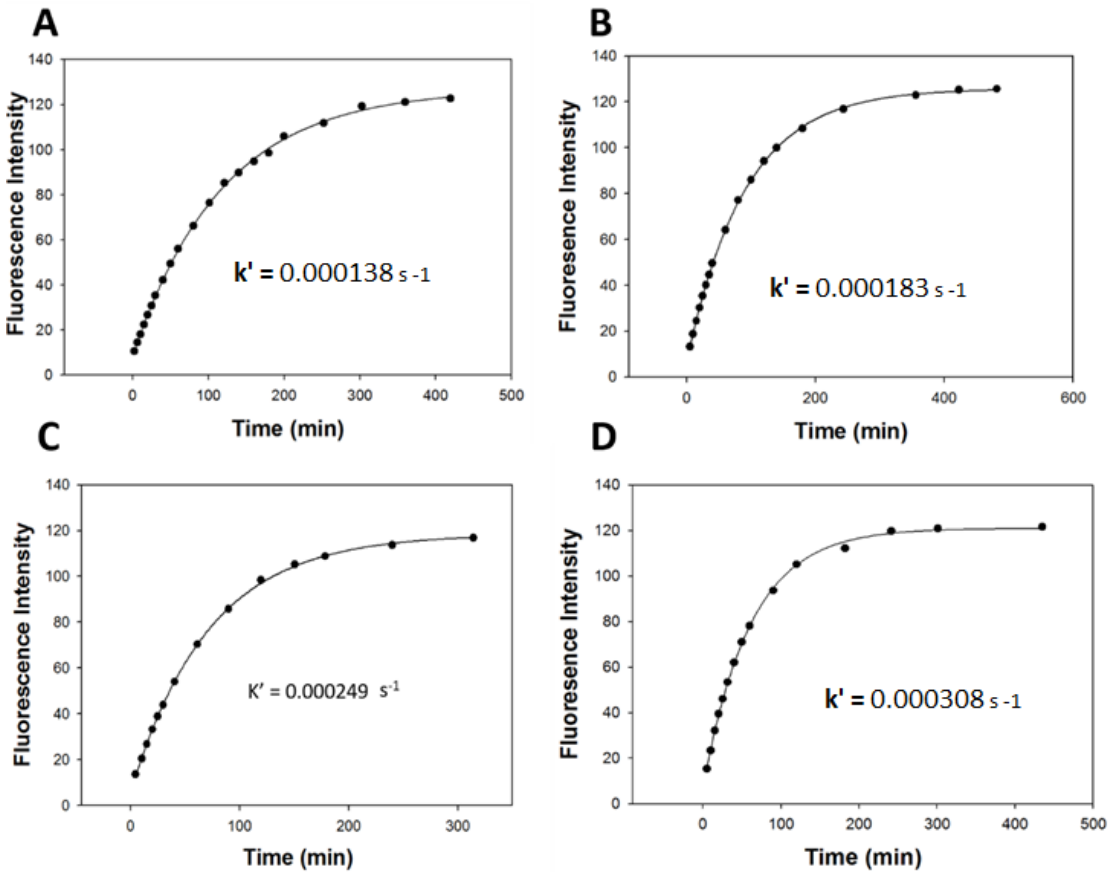
Supplementary Figure 4, The pseudo first reaction rate constant between **22a** (A: 1 mM; B: 1.4 mM; C: 1.8 mM; D: 2.2 mM) and **21a** (50 μM). The fluorescent intensity was taken with emission and excitation wavelength being 461 and 370 nm, respectively.



Supplementary Figure 5. The second order reaction rate constant between **22a** and **21a**.

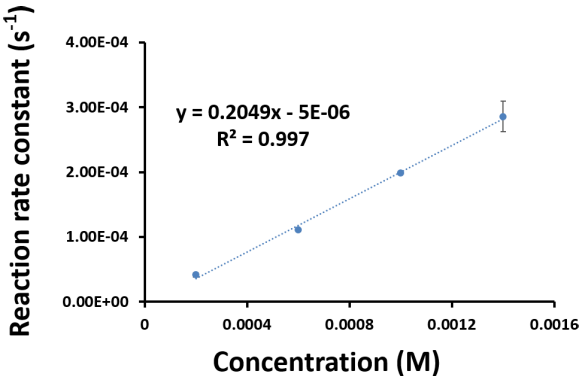
6. The determination of the reaction rate constant between 21b and 22b

By a similar strategy used for the determination of reaction constant between **22a** and **21a**, the reaction rate constant between **21b** and **22b** was determined to be 0.20 $M^{-1}s^{-1}$.



Supplementary Figure 6. The pseudo first reaction rate constant between **22b** (A: 0.2 mM; B: 0.4 mM; C: 1 mM; D: 1.4 mM) and **21b** (50 μM). The fluorescent

intensity was taken with emission and excitation wavelength being 461 and 370 nm, respectively.

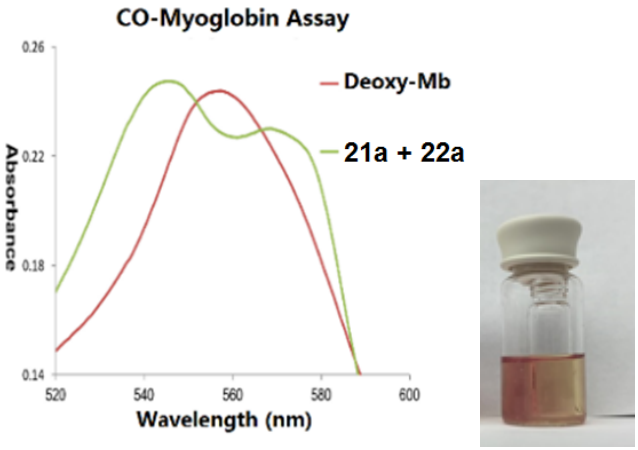


Supplementary Figure 7. The second order reaction rate constant between **22b** and **21b**.

7. CO-myoglobin Assay

Compound **21a** was unstable in the presence of sodium dithionite (DTT), which is needed for the CO-deoxy-Mb assay. Therefore, a "two compartment" CO-deoxy-Mb assay was developed.[7] As shown in Figure 7, the inner vial is for the solution of **21a** and **22a** in DMSO/PBS (10:1), and the bigger outer vial contains the deoxy-Mb solution.

A myoglobin solution in PBS (0.01 M, pH = 7.4) (0.5 mg/ml) was degassed by bubbling with nitrogen for at least 20 min. To this degassed solution was added a freshly prepared solution of sodium dithionite (0.1%), which gave a 27 μM solution of deoxy-Mb. The obtained deoxy-Mb solution was then transferred into the bigger vial (under an inert N_2 atmosphere), and a solution of **21a** in DMSO/PBS (10:1, 10 mM) and **22a** in DMSO/PBS (10:1, 5 mM) was transferred into the inner vial sequentially by using a syringe. Then the obtained solution was incubated at 37 ^{o}C for 10 min. Then the solution was cooled with an ice bath for 10 min, in order to increase the solubility of CO in water. Subsequently, UV-Vis spectra of the deoxy-Mb solution were taken.



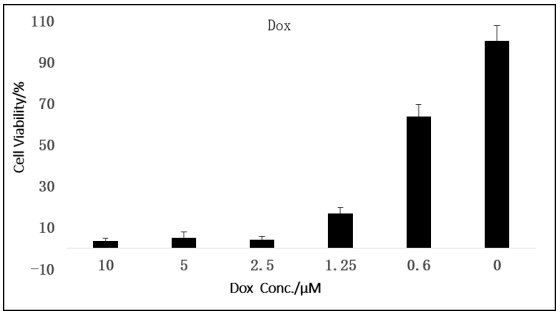
Supplementary Figure 8. The "two compartment" Mb-CO assay

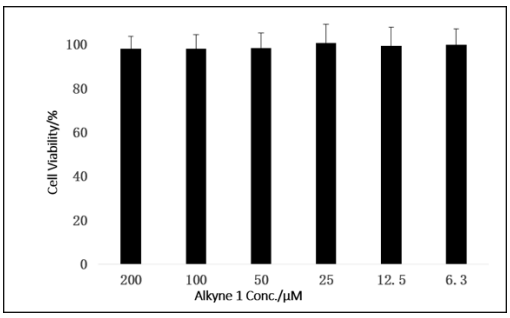
8. Biology studies

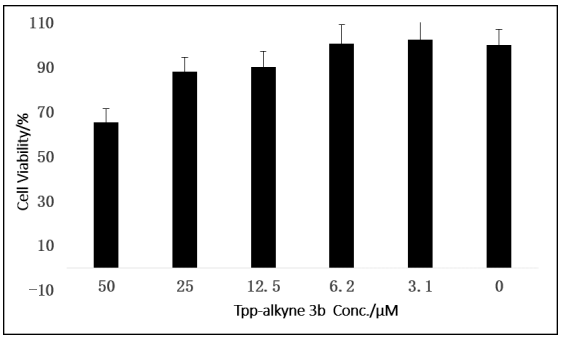
Hela cells (ATCC) were used in the studies. Hela cells were maintained in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum (MidSci; S01520HI), and 1% penicillin-streptomycin (Sigma-Aldrich; P4333) at 37 °C with 5% CO_2.

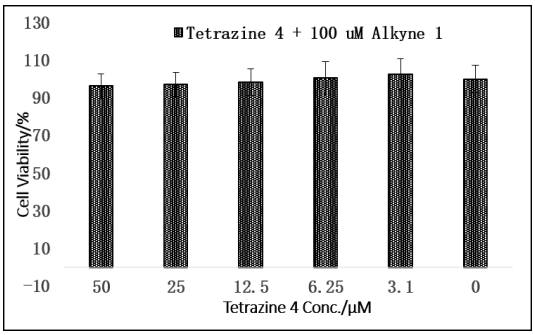
8.1 Studies of cytotoxicity

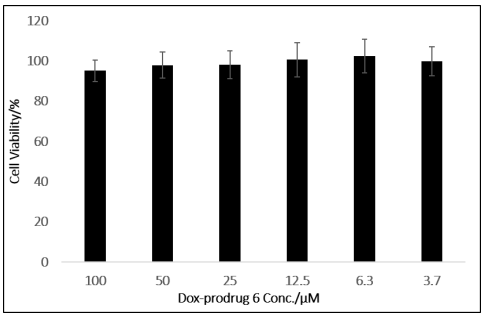
Test compound was dissolved in DMSO to afford a stock solution. The final concentration of DMSO in cell culture was 1% (v/v) in PBS. Hela cells were seeded in 96-well plates one day before the experiment. Different concentrations of prodrug was added into the cell culture. The cells were then incubated for 48 h at 37 °C with 5% CO_2. The cell viability was tested by the MTT assay.[8] Specifically, after 48 h of incubation, 5 mg/mL MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was added into the cell culture. After incubation for 4 h, the supernatant was removed and 100 μL DMSO was added into the wells containing the cells. After shaking gently for 3 min, absorbance at 605 nm was read by a plate reader. Some of the result were shown in Figure 9.

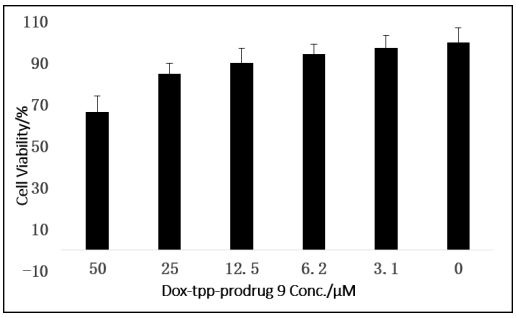

Dox
Cell Viability/%
110
90
70
50
30
10
−10
10
5
2.5
1.25
0.6
0
Dox Conc./μM

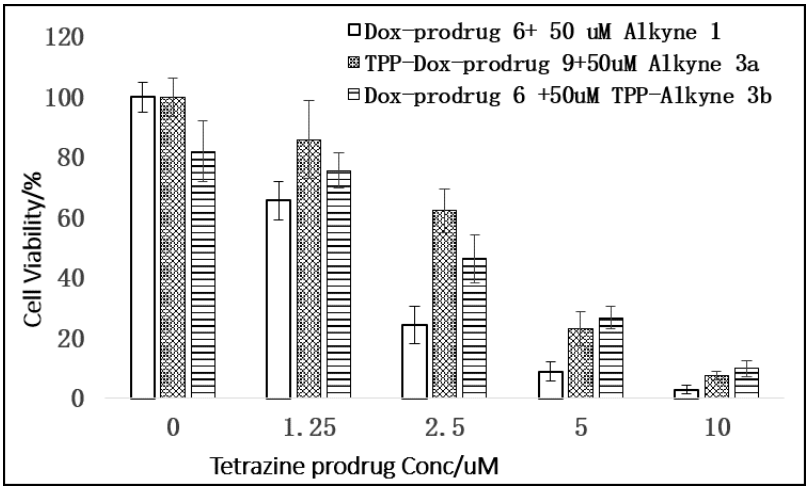

Cell Viability/%
100
80
60
40
20
0
200
100
50
25
12.5
6.3
Alkyne 1 Conc./μM

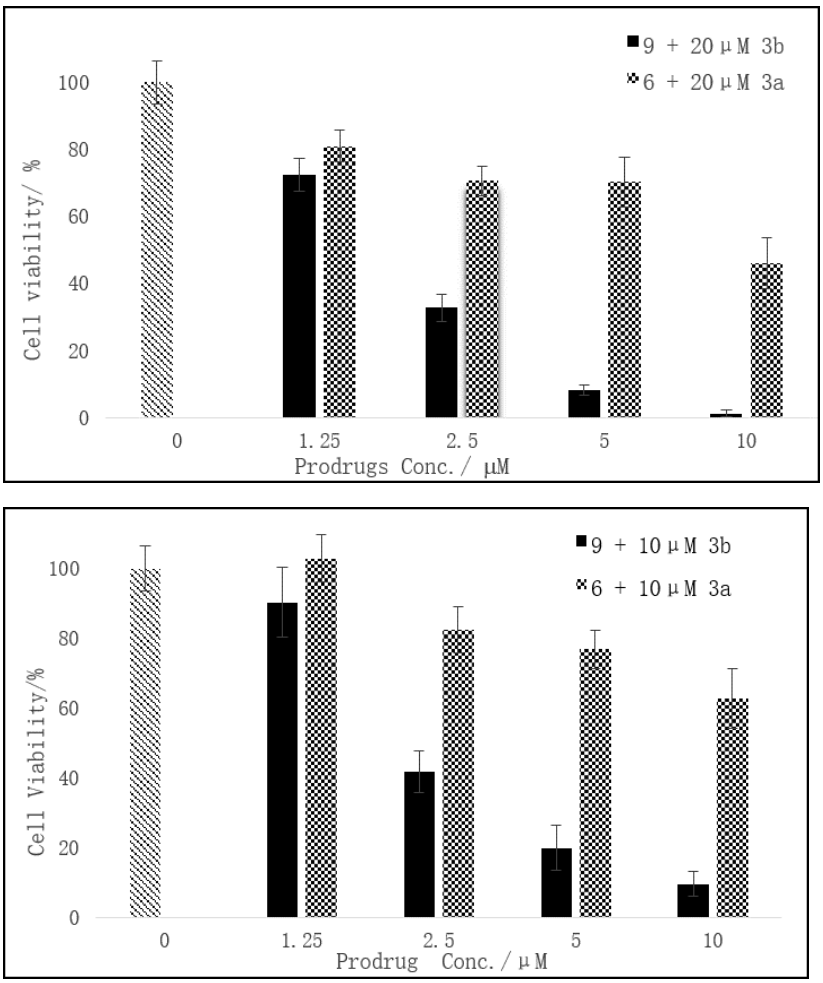

Cell Viability/%
110
90
70
50
30
10
−10
50
25
12.5
6.2
3.1
0
Tpp-alkyne 3b Conc./μM


Tetrazine 4 + 100 uM Alkyne 1
Cell Viability/%
130
110
90
70
50
30
10
−10
50
25
12.5
6.25
3.1
0
Tetrazine 4 Conc./μM


Cell Viability/%
120
100
80
60
40
20
0
100
50
25
12.5
6.3
3.7
Dox-prodrug 6 Conc./μM


Cell Viability/%
110
90
70
50
30
10
−10
50
25
12.5
6.2
3.1
0
Dox-tpp-prodrug 9 Conc./μM


Dox−prodrug 6+ 50 uM Alkyne 1
TPP−Dox−prodrug 9+50uM Alkyne 3a
Dox−prodrug 6 +50uM TPP−Alkyne 3b
Cell Viability/%
120
100
80
60
40
20
0
0
1.25
2.5
5
10
Tetrazine prodrug Conc/uM

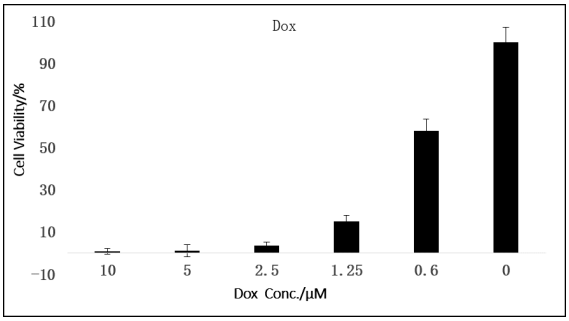


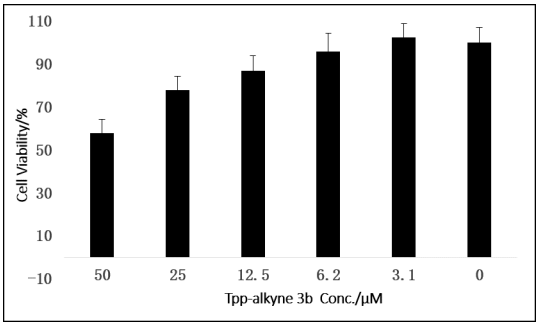
Supplementary Figure 9. Studies of cytotoxicity (n = 4).

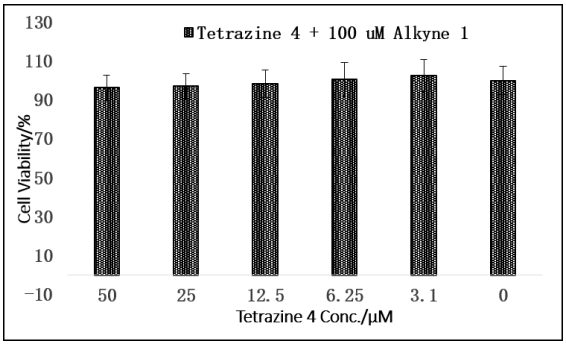
Studies of cytotoxicity (crystal violet assay)

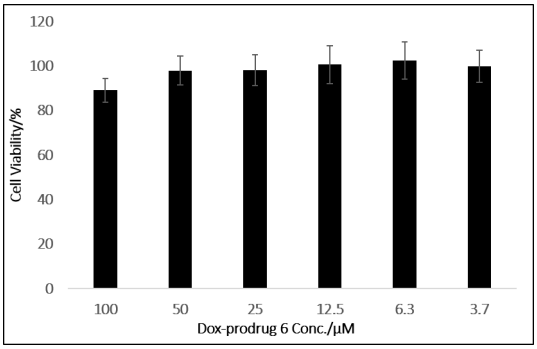
The test compound was dissolved in DMSO to afford a stock solution. The final concentration of DMSO in cell culture was 1% (v/v) in PBS. Hela cells were seeded in 96-well plates one day before the experiment. Different concentrations of the test compound were added into the cell culture. The cells were then incubated for 48 h at 37 °C with 5% CO_2. Cell viability was tested by the crystal violet assay. Specifically, after 48 h of incubation, the cells were fixed fixed in 100% methanol for 10 min and stained for 20 min with a 1% crystal violet solution in 50% methanol, followed by 5 repeated washings with PBS. 50 μL of DMSO was added to each well. After 20 min

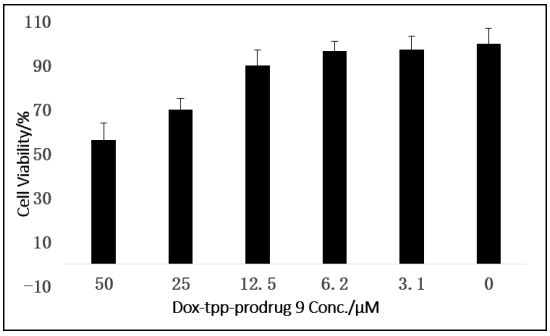
of incubation, the plates were analyzed in a microplate reader at 595 nm. Some of the results are shown in Figure 10.

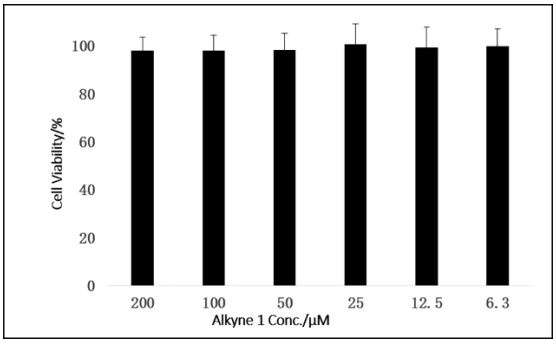


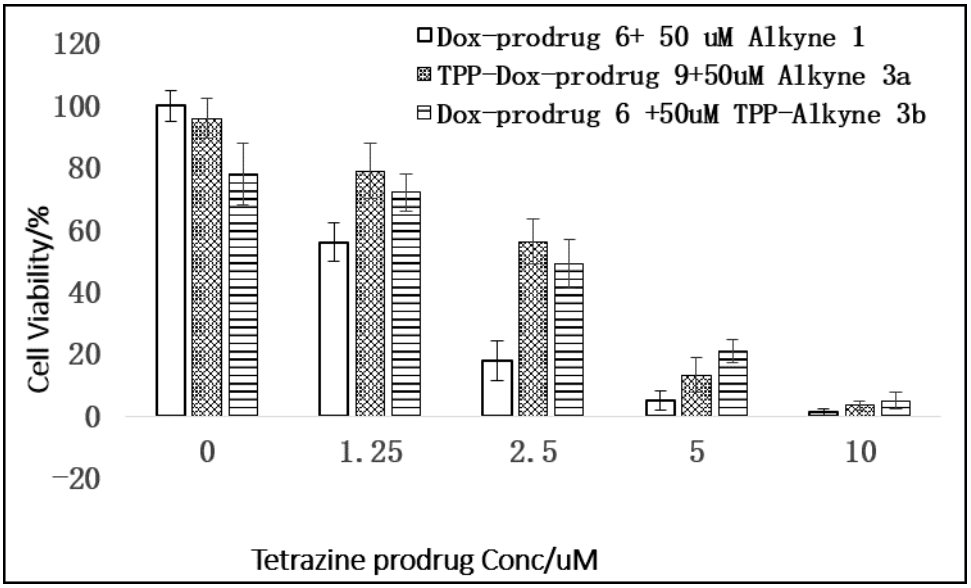


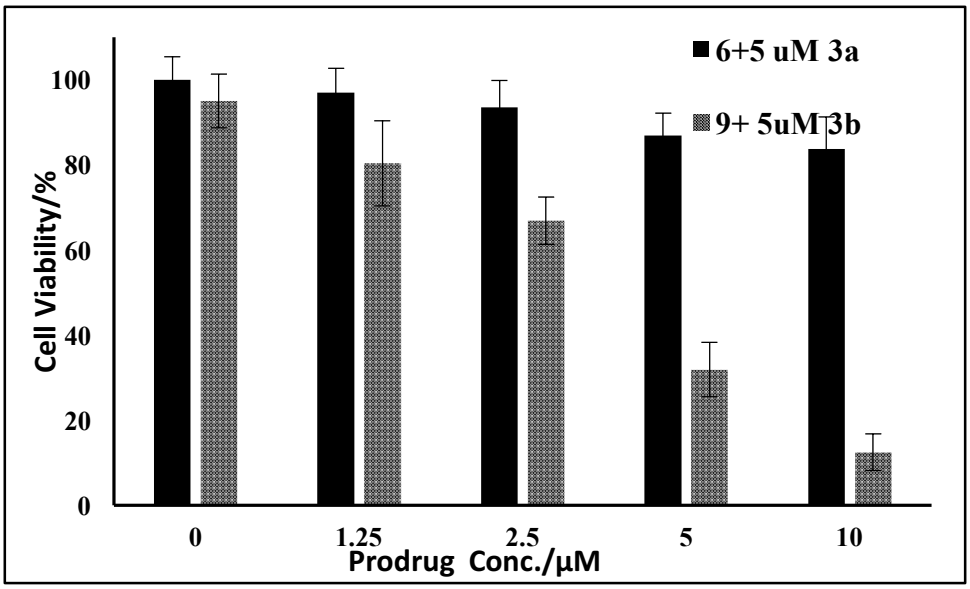












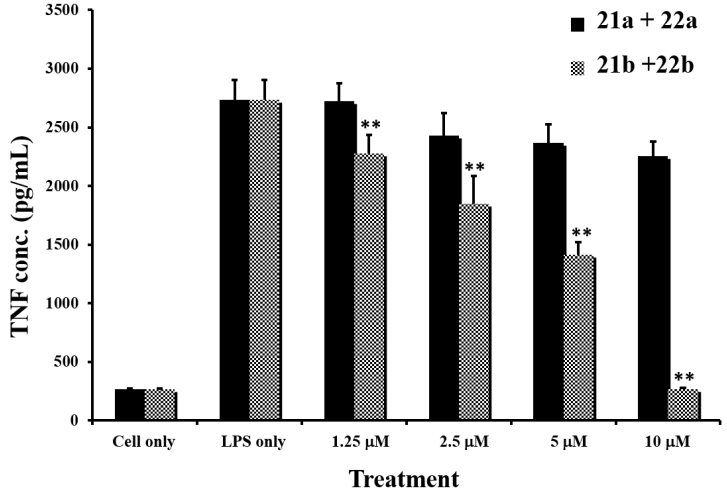
Supplementary Figure 10. Studies of cytotoxicity by the crystal violet assay (n=4)

8.2 Cell imaging studies

Raw264.7 cells (ATCC® CCL-2™) were maintained 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. The media was changed every other day. All the experiments were done within 10 passages. The cells were seeded in the 6-well plate one day before the imaging experiment. Test compound was dissolved in DMSO to prepare a stock solution. Compounds **21b** and **22b** were added into the cell culture separately to give a final concentration of 1 or 5 μM for each compound. The cells treated with **21a** and **22a**, **21b** or **22b** only were used as controls. After adding the compound, the cells were incubated at 37 °C for 4 h. The cell samples were then washed with PBS three times and covered with fresh DMEM medium. For co-localization experiment, the cells were treated with 50 nM of MT-deep red for additional 30 mins. The fluorescent images were obtained using the DAPI channel (excitation: 358 nm, emission: 461 nm) on a Zeiss fluorescent microscope or Olympus FV1000 confocal laser-scanning microscope.

8.3 Anti-inflammatory effect using the ELISA assay

RAW 264.7 cells were seeded in 96-well plates one day before the experiment. LPS was used to initiate the inflammatory response. Cells were pre-treated with different concentrations of compound **21b** and **22b** for 4 h. Thereafter, 1 μg/mL LPS was added into the cell culture media. The cells treated with **21a** and **22a, 21b** or **22b** only were used as negative controls. For the TNF test, the cell culture supernatant was collected 1 h after LPS treatment. Cell culture without LPS treatment was used as a control. The concentrations of the cytokine in the cell culture supernatant were measurement by a commercial ELISA kit (ELISA Ready-SET-Go!®-eBioscience).



Supplementary Figure 11. CO prodrugs inhibit LPS-induced TNF production in macrophages. The anti-inflammatory effects of biorthogonal CO prodrugs. Results represent mean ±SD of 3 samples from 3 independent experiments. The mean of each concentration of CO prodrug treated group was compared with LPS-only group by two-sample t test. **: $p<0.01$.

8.4 Animal Studies

Male CD-1 mice (Jackson Laboratory, ME) were used for all experimentation. Mice were acclimated for 1 week and provided food and water *ad libitum*. All animal experimentation was approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee.

Acute Liver Injury: Mice were fasted overnight before being administered Acetaminophen (APAP, Sigma, MO) at 300 mg/kg, i.p. Four hours after APAP the prodrugs were administered intravenously at the doses indicated in Dimethyl Sulfoxide (DMSO). Twenty-four hours after APAP mice were anesthetized and blood was collected via cardiac puncture and the liver was collected and fixed in 10% paraformaldehyde (PFA) and frozen in liquid nitrogen for immunopathology. Serum was analyzed for Alanine Aminotransferase (ALT) using an IDEXX veterinary chemistry analyzer (IDEXX, TX).

Blood Carboxyhemoglobin Measurements: Whole blood (50-75µl) was collected via an indwelling femoral catheter in anesthetized mice over time in heparinized syringes and analyzed immediately by co-oximetry (Radiometer, CA).

Histologic Analyses

During animal sacrifice, liver specimens were harvested from different lobes. The tissue was sectioned (5-7um) and stained with hematoxylin and eosin (H&E) to evaluate architectural changes, and in separate sections with TUNEL for apoptosis as previously reported. [9, 10] For TUNEL staining, sections were permeabilized with 0.1% Triton X for 2 min and stained per kit instructions per manufacturer's instructions (Promega, Madison, WI). Areas of TUNEL positive staining or loss of architecture identified by H&E staining were demarcated. Four to five sections from each liver were assessed from each mouse in a blinded fashion. Images were acquired using a Zeiss Apotome microscope (Jena, Germany).

9. Computational studies

All calculations were performed using the Gaussian 09 program.[11] Initial geometry optimizations were carried out by DFT calculations with use of the B3LYP[12, 13] with the standard 6-31G(d,p) basis set. The transition state geometries were obtained using the QST3 method. Energies are shown in Table 5. The stationary points are characterized by frequency calculations to verify that TSs has one and only one

imaginary frequency (Scheme 8). All calculations were conducted on Georgia State University cluster Orion with 8 CPU cores.[14]

Coordinates and connections of **10**

N	-3.33510000	1.54220000	-0.14430000
C	4.67560000	0.52310000	-0.31910000
C	5.21030000	-0.72150000	0.01340000
C	4.36190000	-1.79170000	0.29720000
C	2.97890000	-1.61720000	0.24860000
C	2.44420000	-0.37260000	-0.08380000
C	3.29260000	0.69760000	-0.36770000
C	1.11850000	-0.20540000	-0.13030000
N	0.63990000	1.04560000	-0.00410000
N	-0.69490000	1.21400000	-0.05090000
C	-1.45900000	0.11980000	-0.22080000
N	-0.98040000	-1.13120000	-0.34700000
N	0.35440000	-1.29960000	-0.30010000
C	-2.79850000	0.28880000	-0.26790000
O	6.55380000	-0.89100000	0.06070000
C	6.87730000	-2.20900000	0.41250000
C	8.33880000	-2.36090000	0.45530000
C	9.54380000	-2.48610000	0.49060000
O	-3.52290000	-0.66600000	-0.41910000
C	-4.77280000	1.72360000	-0.19480000
C	-5.10480000	3.20130000	-0.03400000
H	-2.72830000	2.34220000	-0.01760000
H	5.34460000	1.36700000	-0.54300000
H	4.78350000	-2.77330000	0.55940000
H	2.30990000	-2.46120000	0.47240000
H	2.87090000	1.67910000	-0.62990000
H	6.45130000	-2.44030000	1.41440000

H	6.45370000	-2.90890000	-0.34230000
H	10.62750000	-2.59870000	0.52230000
H	-5.15760000	1.36200000	-1.17460000
H	-5.24880000	1.14490000	0.62820000
H	-6.20830000	3.34050000	-0.07280000
H	-4.72000000	3.56290000	0.94580000
H	-4.62880000	3.78000000	-0.85700000

1 14 1.0 20 1.0 22 1.0

2 3 1.0 7 2.0 23 1.0

3 4 2.0 15 1.0

4 5 1.0 24 1.0

5 6 2.0 25 1.0

6 7 1.0 8 1.0

7 26 1.0

8 9 1.0 13 2.0

9 10 2.0

10 11 1.0

11 12 2.0 14 1.0

12 13 1.0

13

14 19 2.0

15 16 1.0

16 17 1.0 27 1.0 28 1.0

17 18 3.0

18 29 1.0

19

20 21 1.0 30 1.0 31 1.0

21 32 1.0 33 1.0 34 1.0

22

23

24

25

26

27

28

29

30

31

32

33

34

Coordinates and connections of **2**

C	-4.18010000	-2.30160000	0.09260000
C	-5.19150000	-1.18990000	-0.25300000
C	-4.97230000	0.16130000	0.47260000
C	-4.06550000	1.19260000	-0.25130000
C	-2.63920000	1.37760000	0.32480000
C	-1.55430000	0.46390000	-0.28050000
C	-2.01330000	-0.91580000	-0.20460000
C	-2.83370000	-1.77920000	-0.09320000
C	-0.25860000	0.61690000	0.50520000
O	-1.32060000	0.81810000	-1.61670000
H	-4.33910000	-3.17460000	-0.57920000
H	-4.31920000	-2.62770000	1.14760000
H	-6.20830000	-1.55850000	0.00950000
H	-5.05560000	-0.97980000	-1.33750000
H	-4.51330000	-0.05680000	1.46290000
H	-5.97760000	0.63600000	0.52550000
H	-3.96310000	0.86830000	-1.31100000
H	-4.56920000	2.17600000	-0.11720000

H	-2.68330000	1.17830000	1.41890000
H	-2.33920000	2.41850000	0.06930000
H	0.54860000	0.97060000	-0.17450000
H	-0.40610000	1.35810000	1.32230000
H	0.02850000	-0.36580000	0.94190000
H	-2.13710000	0.71350000	-2.07460000

1 2 1.0 8 1.0 11 1.0 12 1.0

2 3 1.0 13 1.0 14 1.0

3 4 1.0 15 1.0 16 1.0

4 5 1.0 17 1.0 18 1.0

5 6 1.0 19 1.0 20 1.0

6 7 1.0 9 1.0 10 1.0

7 8 3.0

8

9 21 1.0 22 1.0 23 1.0

10 24 1.0

11

12

13

14

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16

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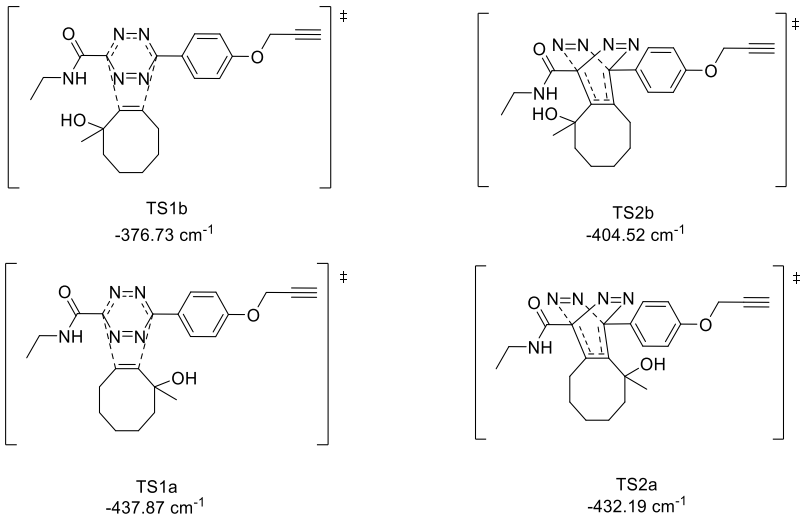
24

Supplementary Scheme 8. Unique imaginary frequencies.



Supplementary Table 5. Calculated energies.

Structure	Energy (Hartree)
10	-965.3956
2	-426.5493
TS1b:	-1391.9178
IN1b:	-1391.9879
TS2b:	-1391.9833
10b	-1282.5577
N2:	-109.5241
TS1a:	-1391.9147
IN1a:	-1391.9832
TS2a:	-1391.9789
10a	-1282.5579

10. Acknowledgement

We gratefully acknowledge the use of Orion that is supported by Georgia State University's Research Solutions.

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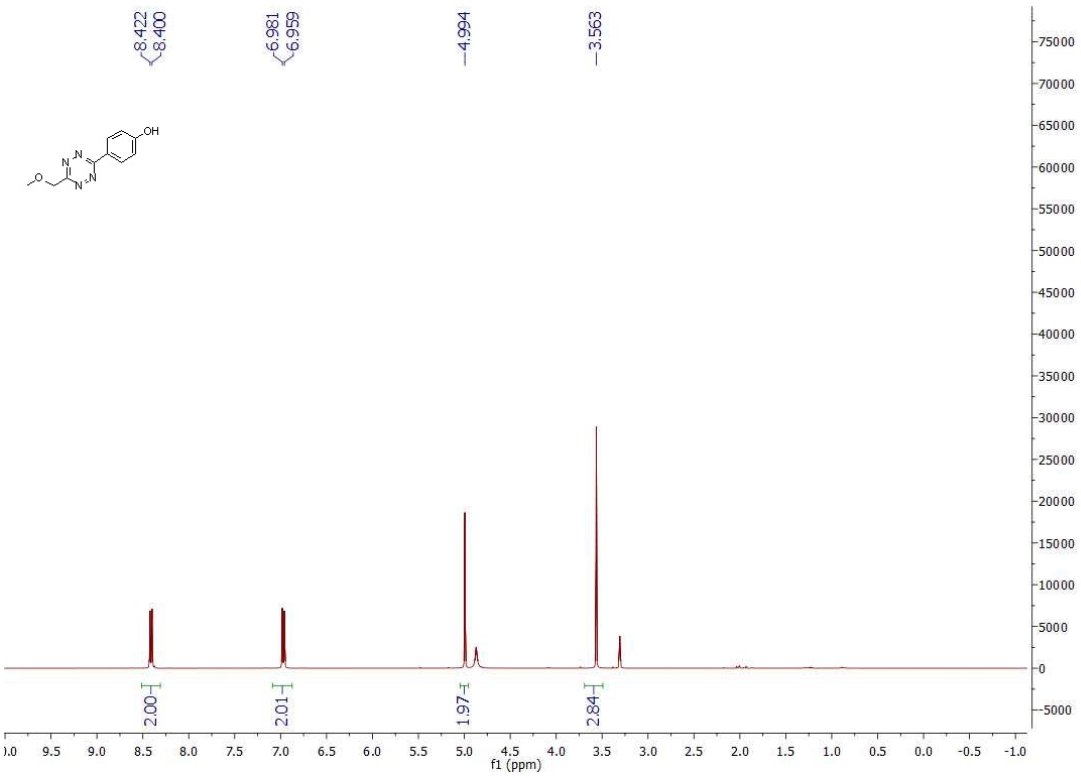
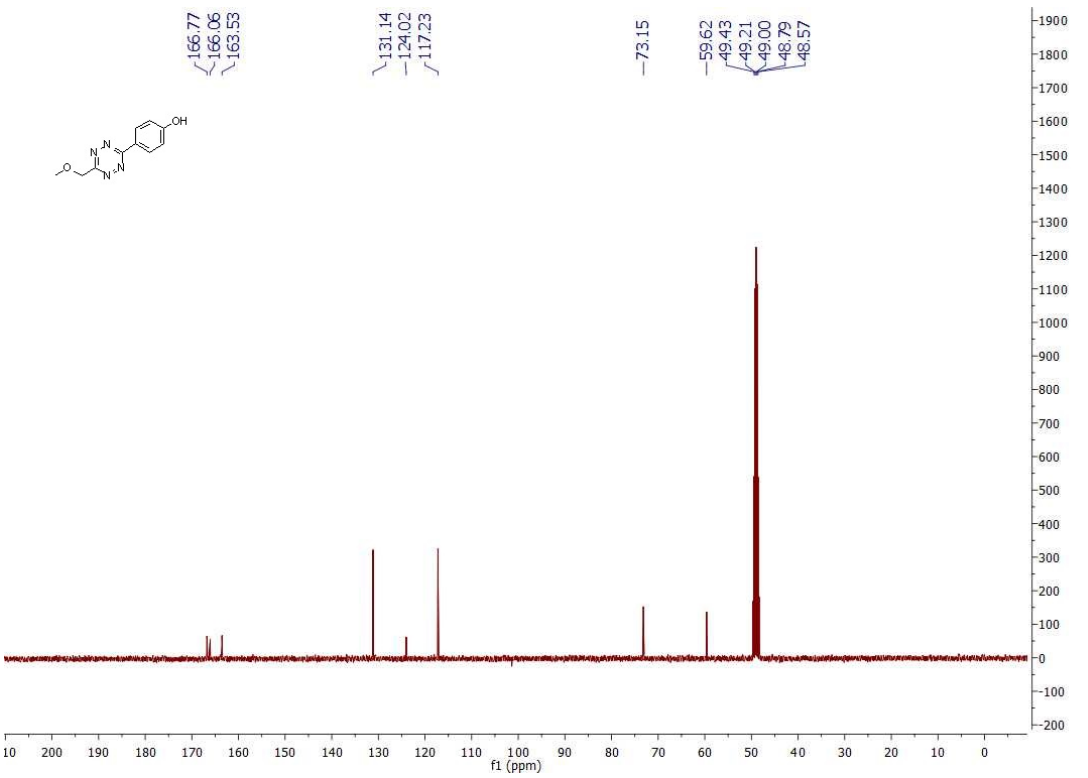
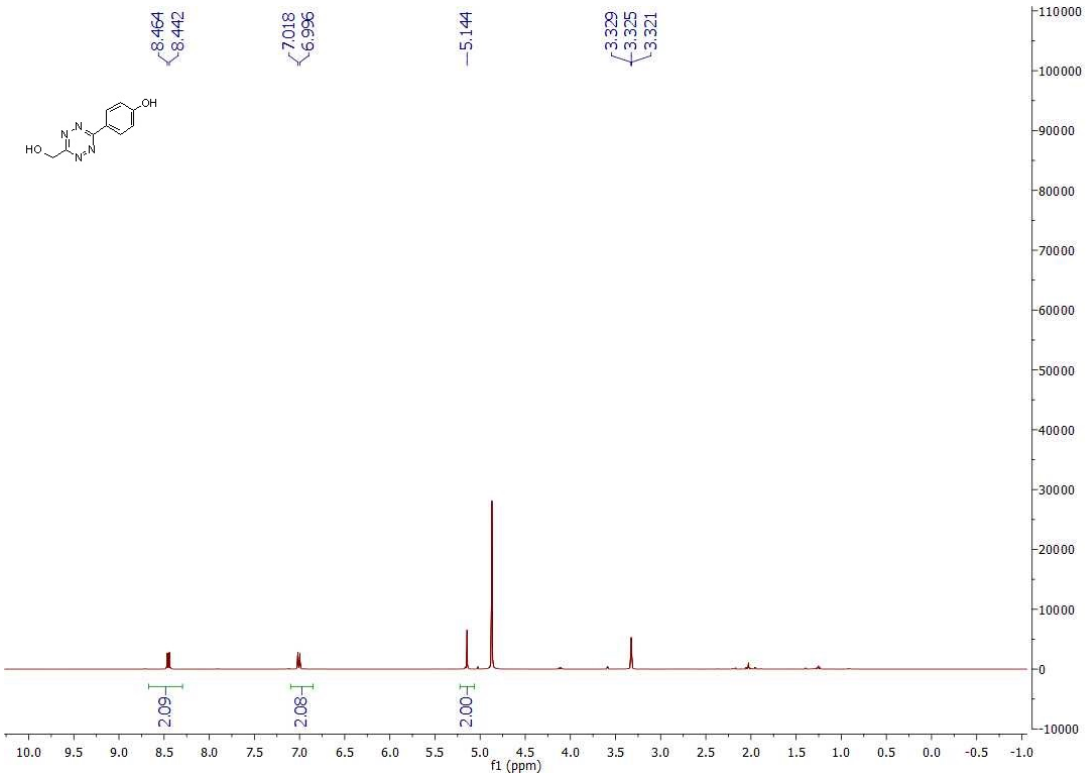



166.77
166.06
163.53
131.14
124.02
117.23
73.15
59.62
49.43
49.21
49.00
48.79
48.57
OH
f1 (ppm)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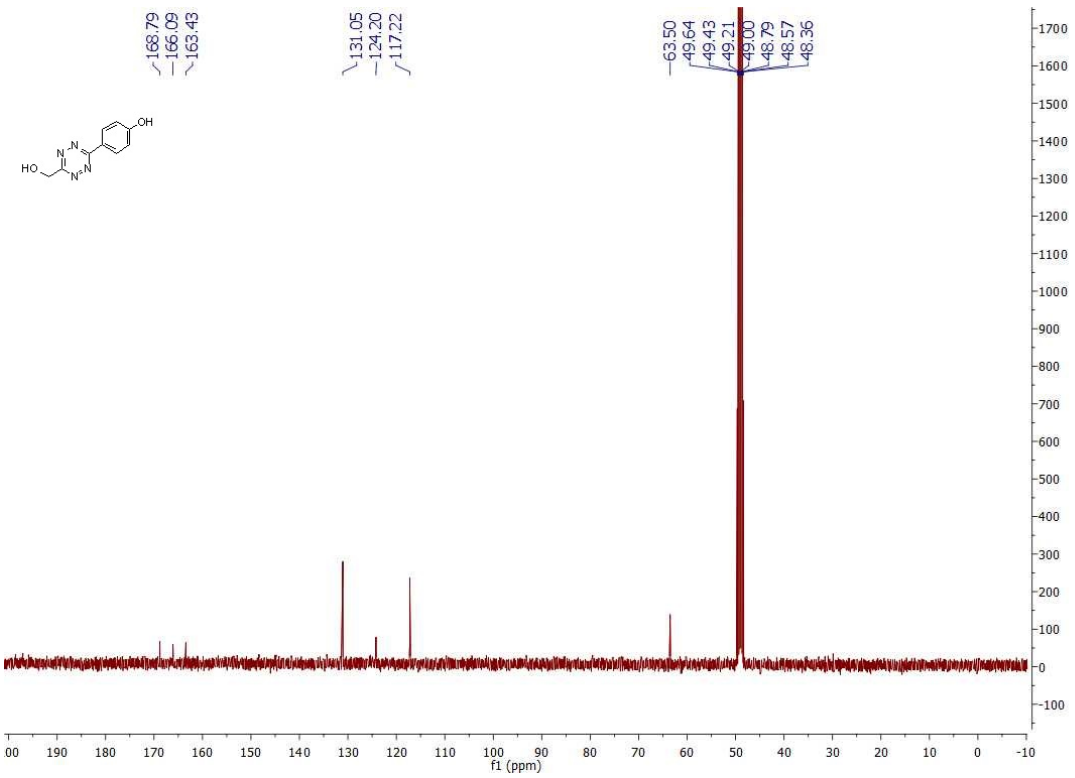
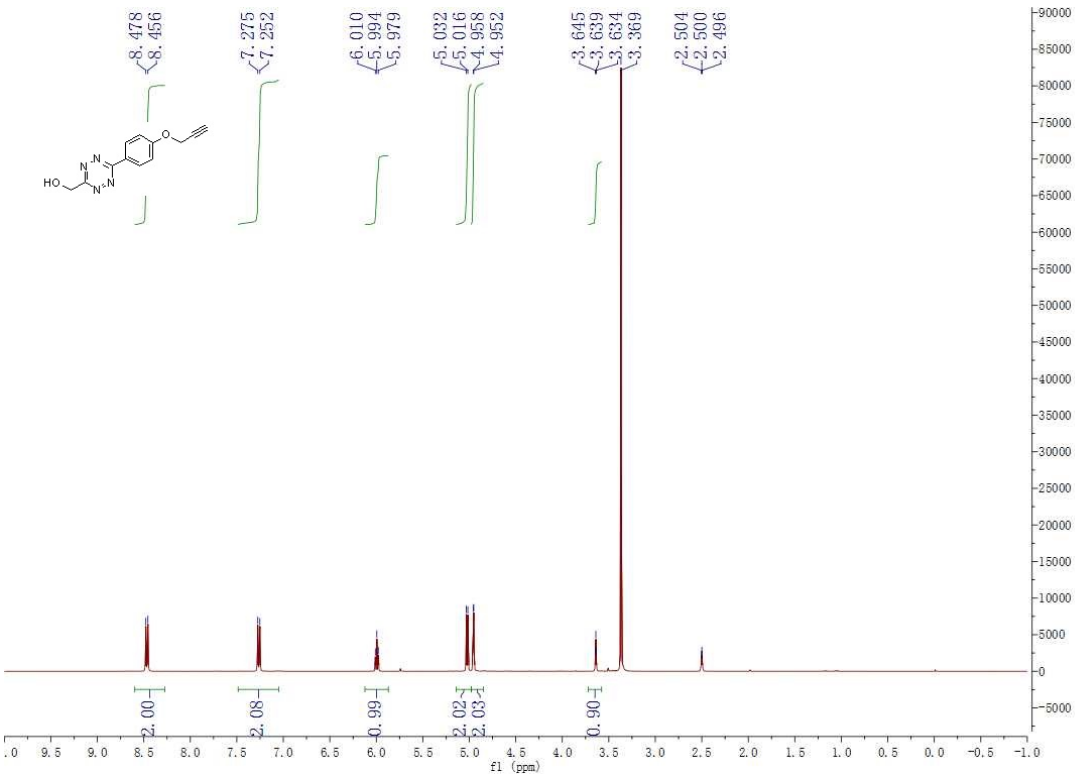
5.144
3.329
3.325
3.321
OH
HO
2.09
2.08
2.00
f1 (ppm)


168.79
166.09
163.43
131.05
124.20
117.22
63.50
49.64
49.43
49.21
49.00
48.79
48.57
48.36
OH
HO
f1 (ppm)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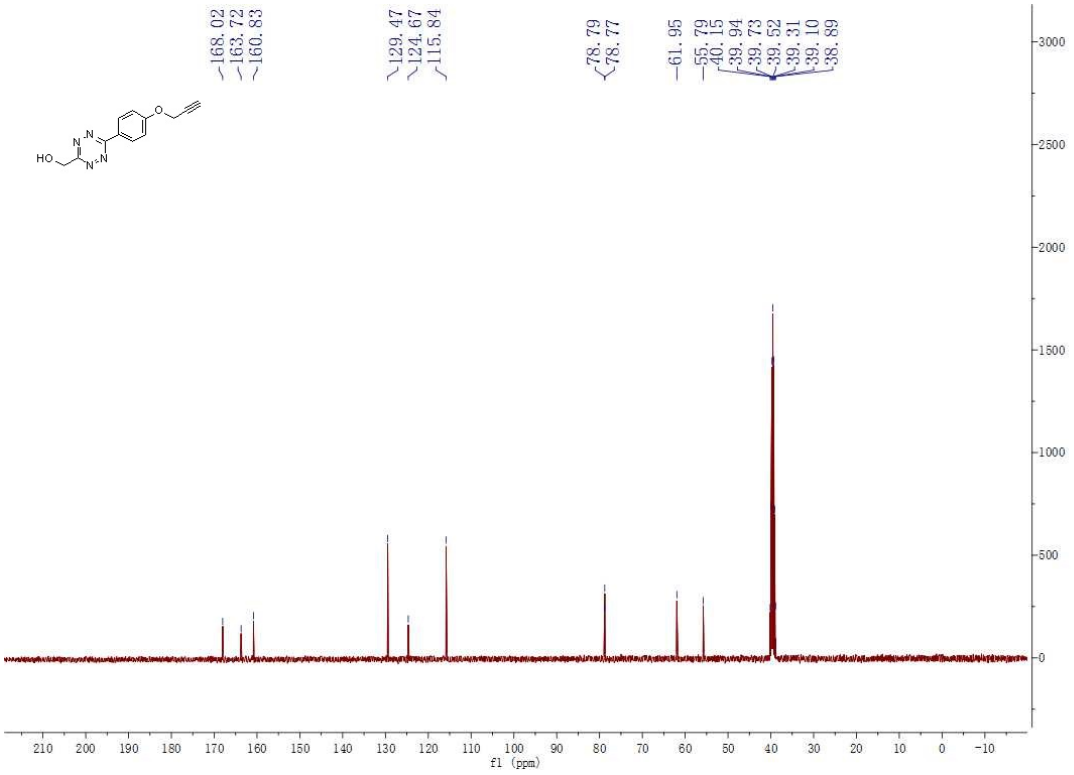
8.456
7.275
7.252
6.010
5.994
5.979
5.032
5.016
4.958
4.952
3.645
3.639
3.634
3.369
2.504
2.500
2.496
HO
2.00
2.08
0.99
2.02
2.03
0.90
f1 (ppm)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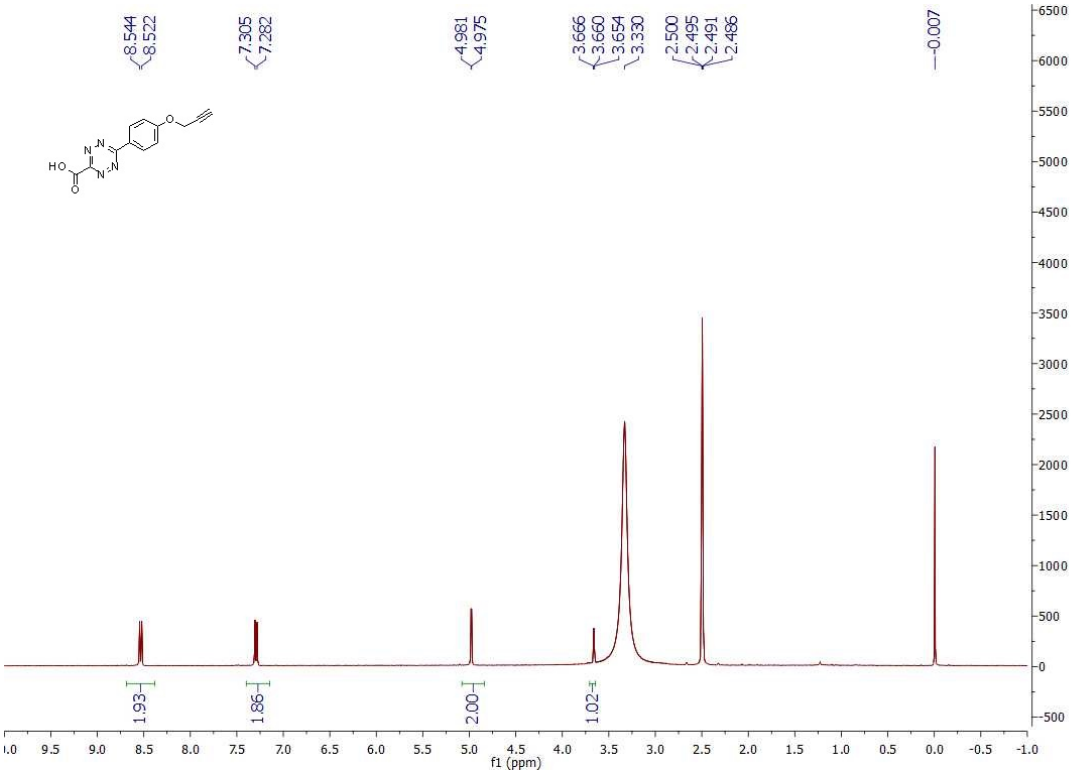
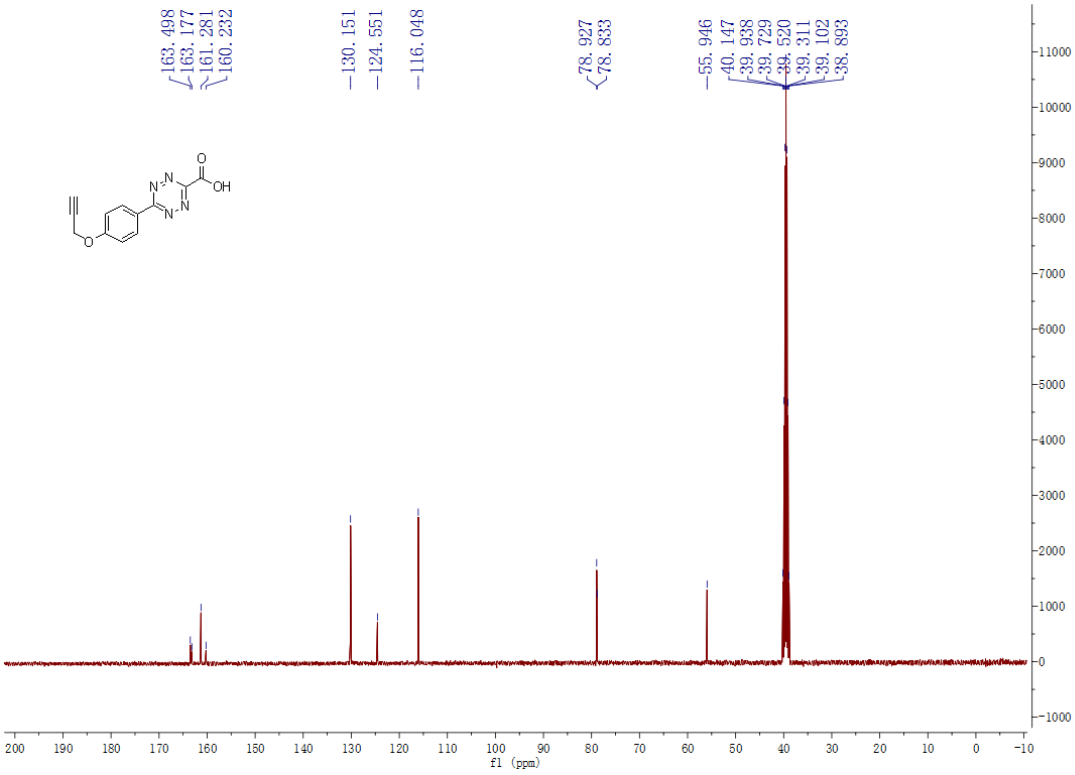
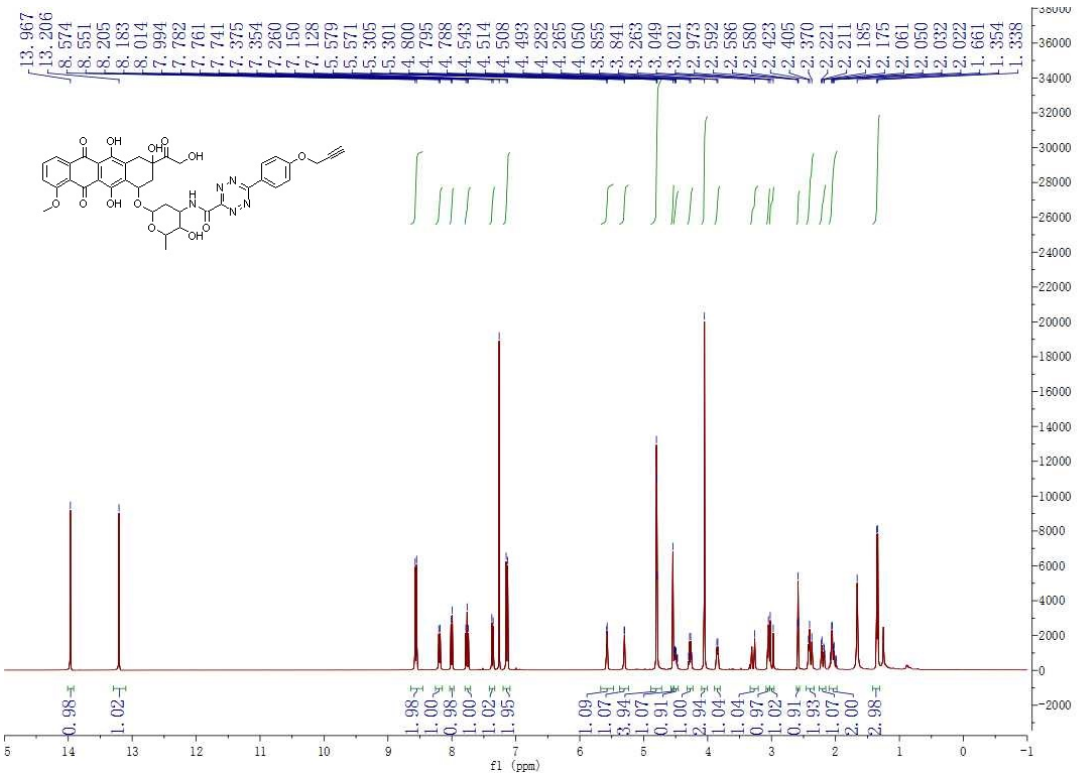
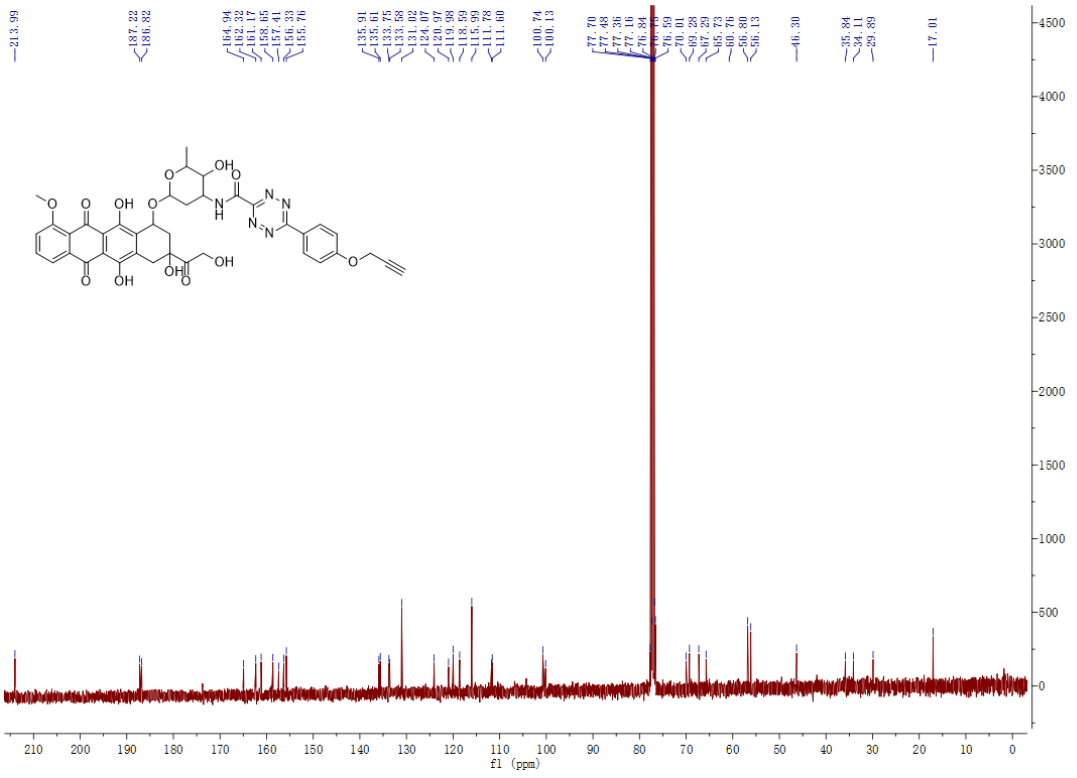
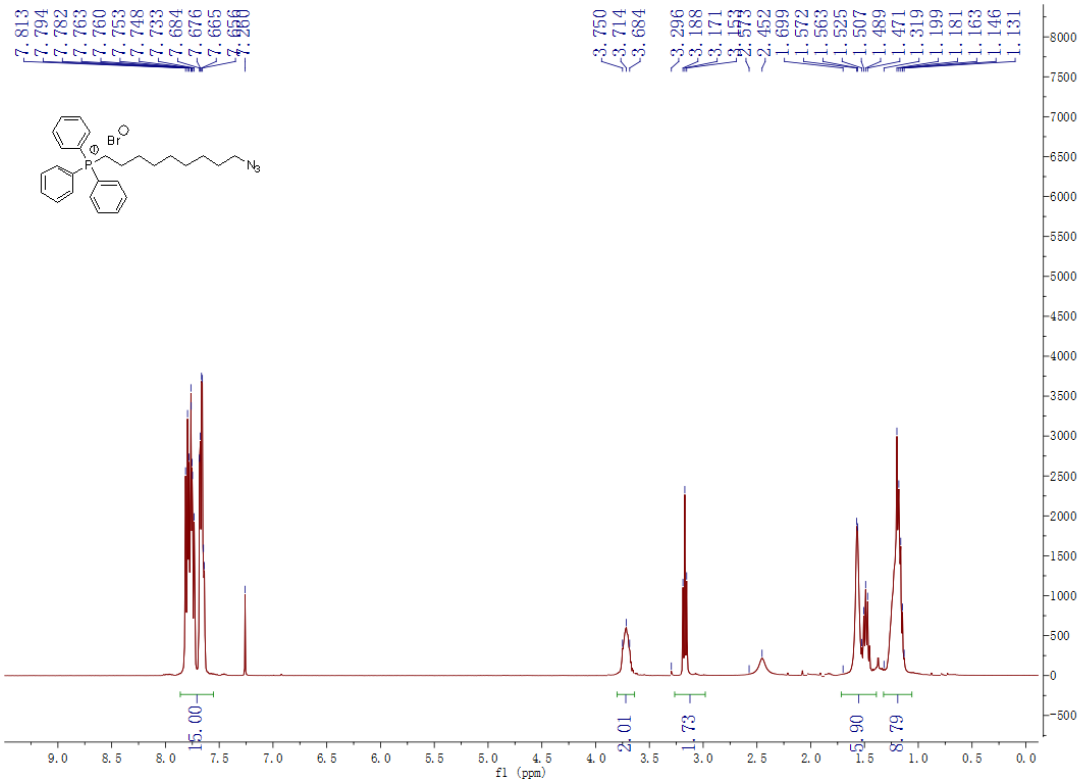
163.72
160.83
129.47
124.67
115.84
78.79
78.77
61.95
55.79
40.15
39.94
39.73
39.52
39.31
39.10
38.89
HO
O
N
N
N
3000
2500
2000
1500
1000
500
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


8.544
8.522
7.305
7.282
4.981
4.975
3.666
3.660
3.654
3.330
2.500
2.495
2.491
2.486
-0.007
1.93
1.86
2.00
1.02
f1 (ppm)


163.498
163.177
161.281
160.232
130.151
124.551
116.048
78.927
78.833
55.946
40.147
39.938
39.729
39.520
39.311
39.102
38.893
f1 (ppm)


f1 (ppm)


f1 (ppm)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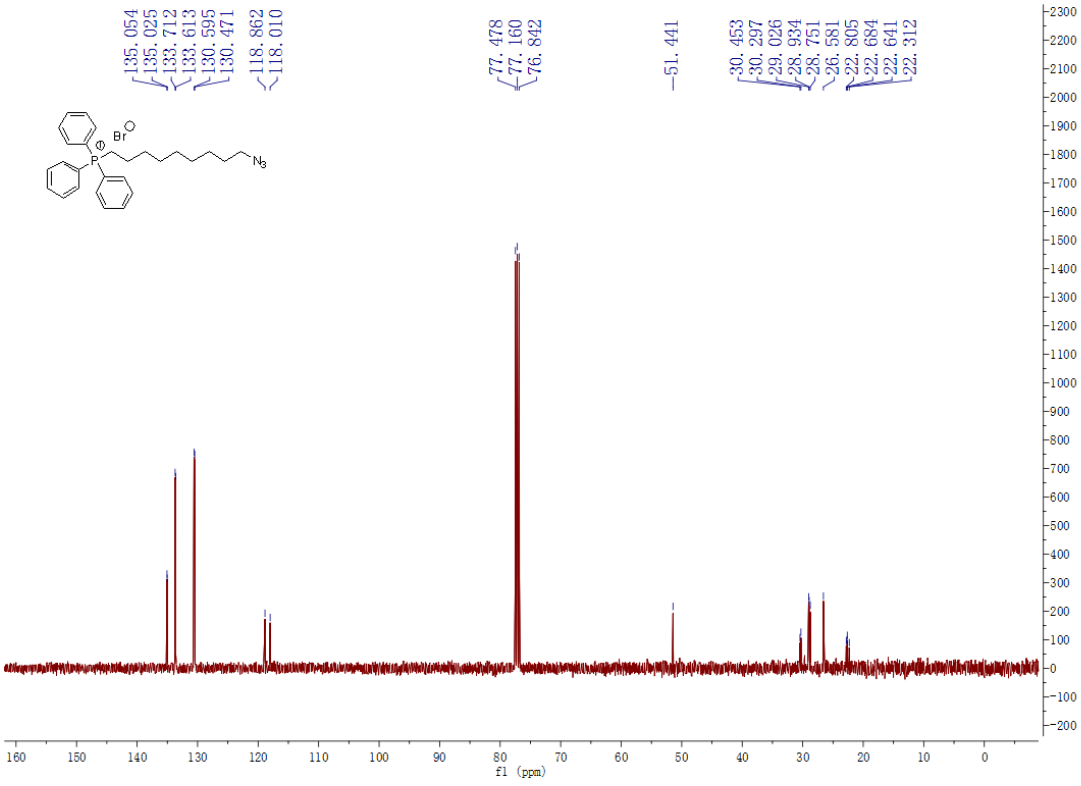
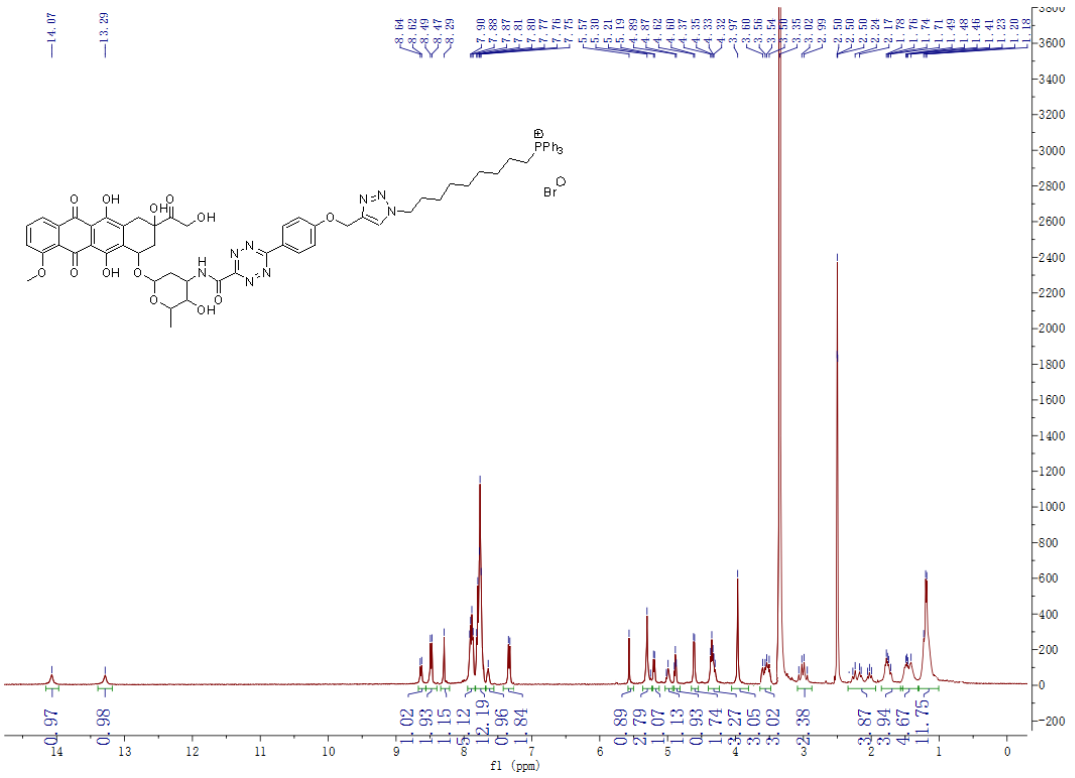
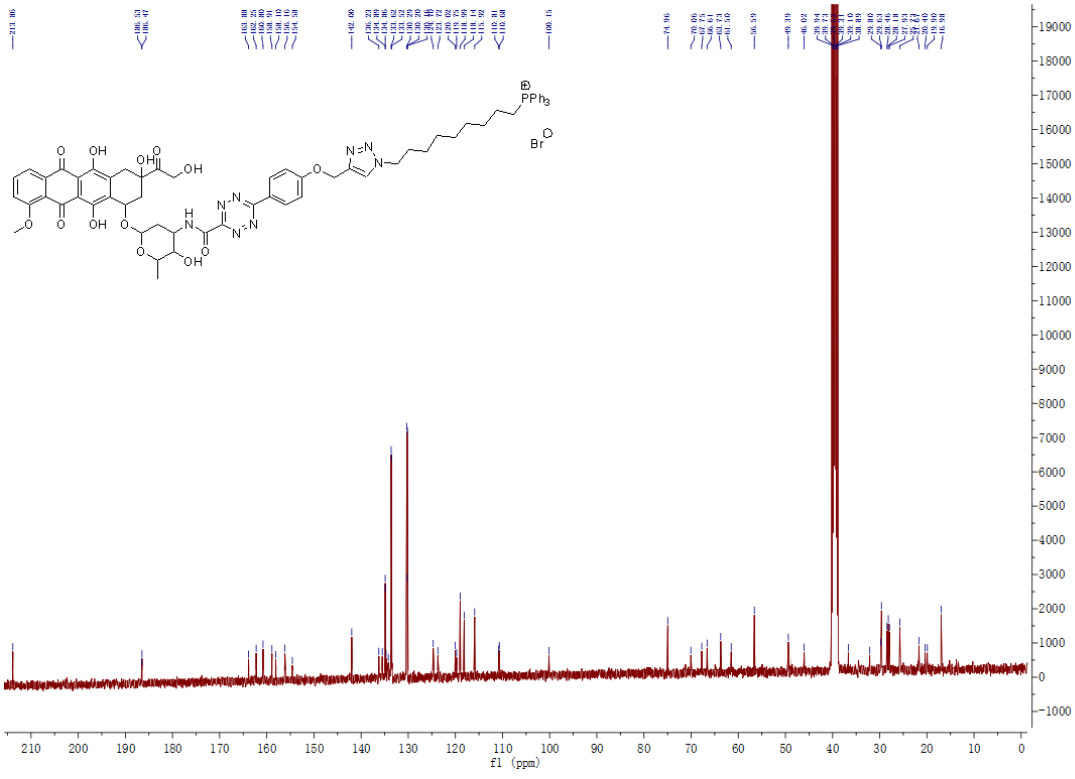
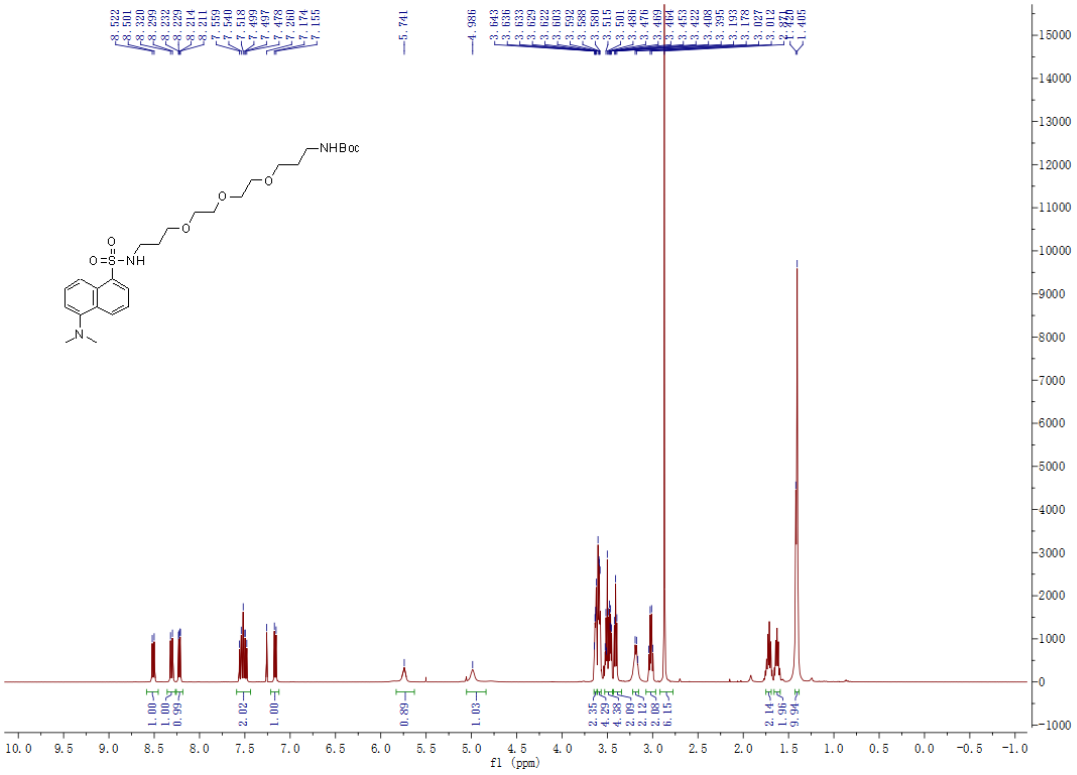
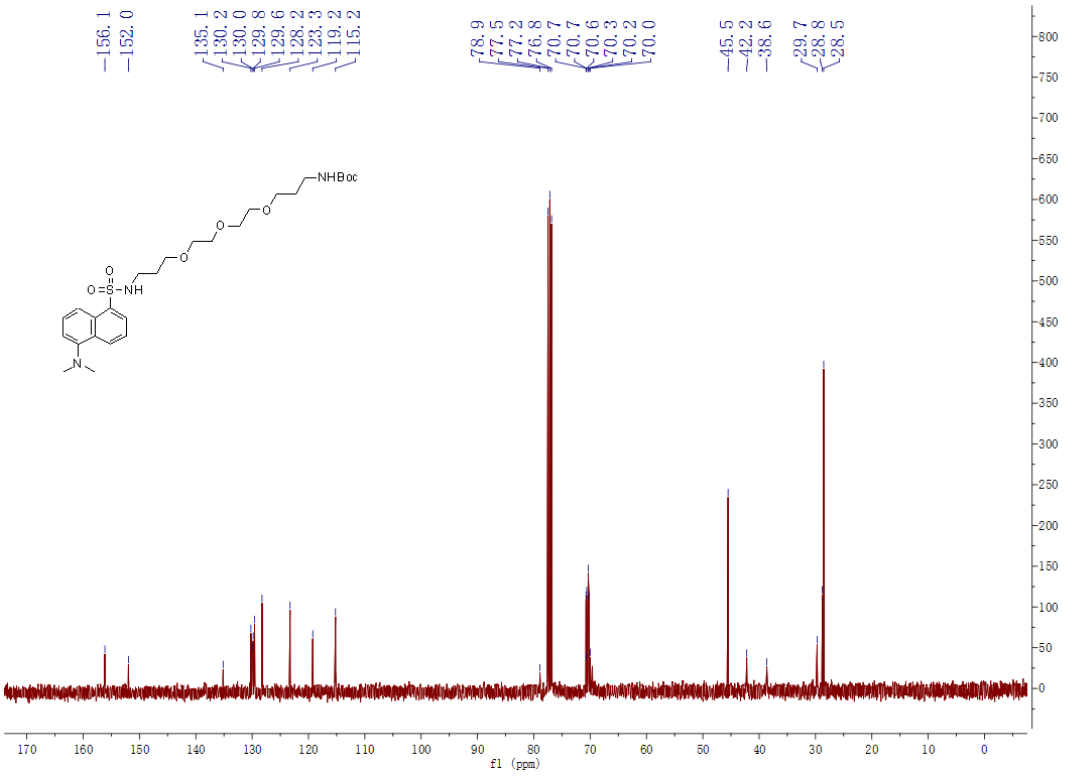
7.794
7.782
7.763
7.760
7.753
7.748
7.733
7.684
7.676
7.665
3.750
3.714
3.684
3.296
3.188
3.171
2.573
2.452
1.699
1.572
1.563
1.525
1.507
1.489
1.471
1.319
1.199
1.181
1.163
1.146
1.131
Br
N3
15.00
2.01
1.73
5.90
8.79
f1 (ppm)

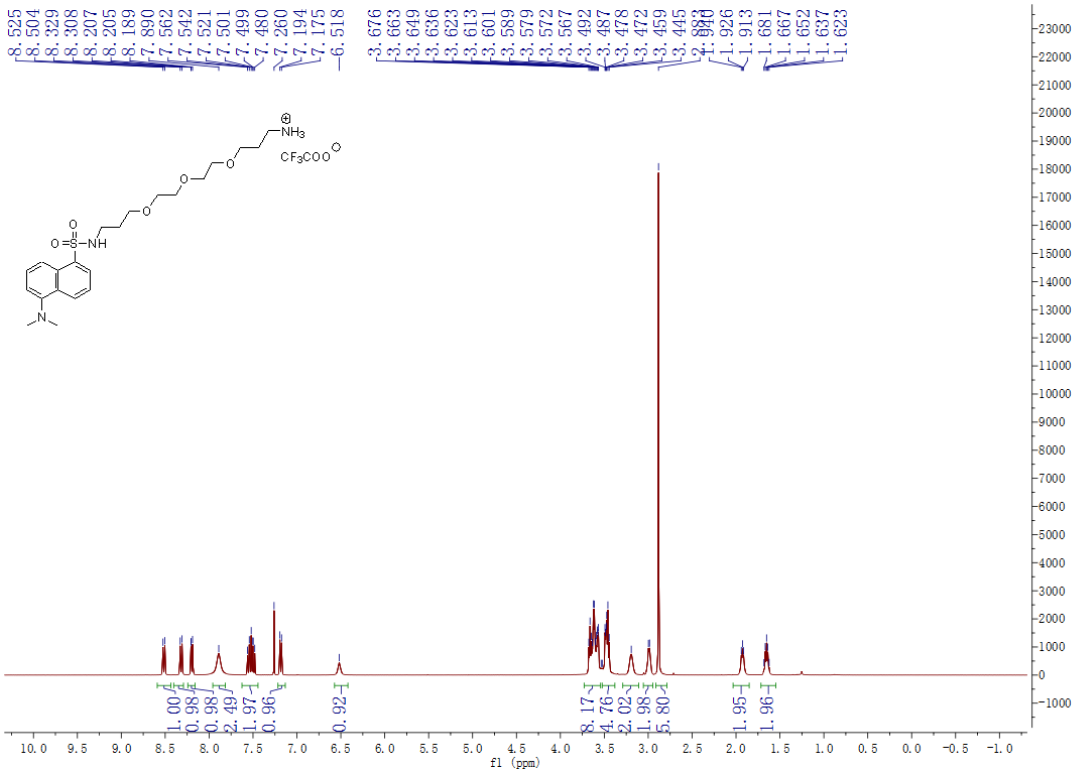

135.054
135.025
133.712
133.613
130.595
130.471
118.862
118.010
77.478
77.160
76.842
51.441
30.453
30.297
29.026
28.934
28.751
26.581
22.805
22.684
22.641
22.312
Br
N3
f1 (ppm)

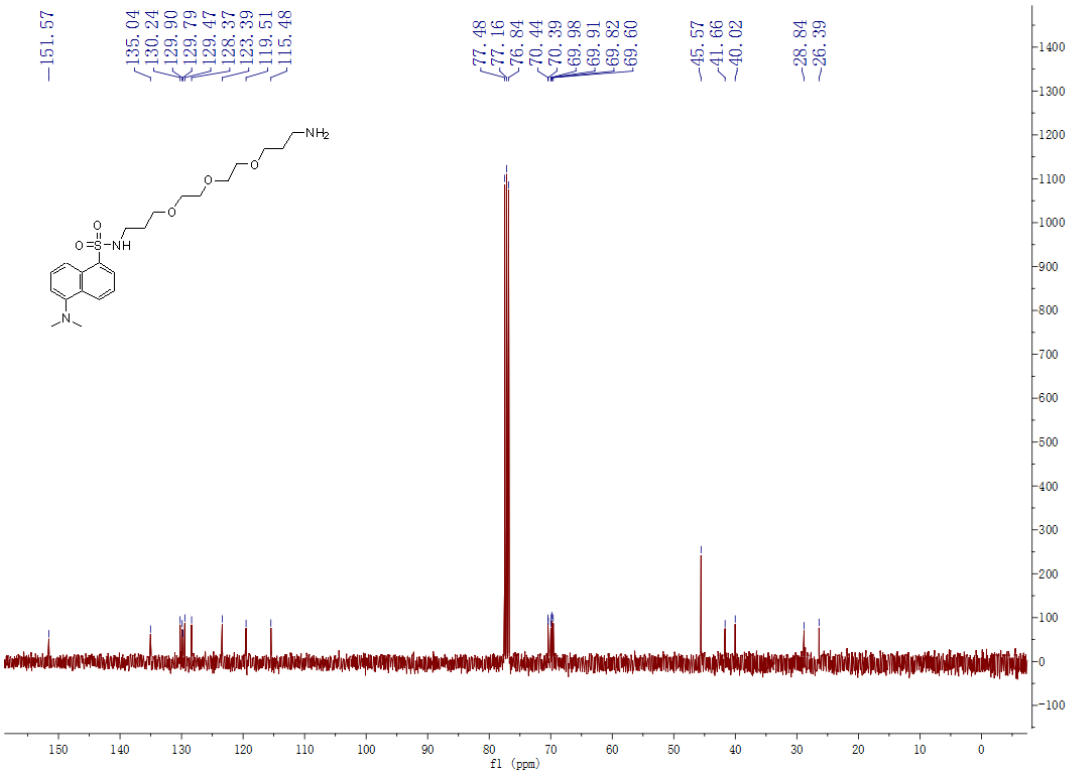

14.07
13.29
0.97
0.98
1.02
1.93
1.15
5.12
12.19
0.96
1.84
0.89
2.79
1.07
1.13
0.93
1.74
3.27
3.05
3.02
2.38
3.87
3.94
4.67
11.75
f1 (ppm)

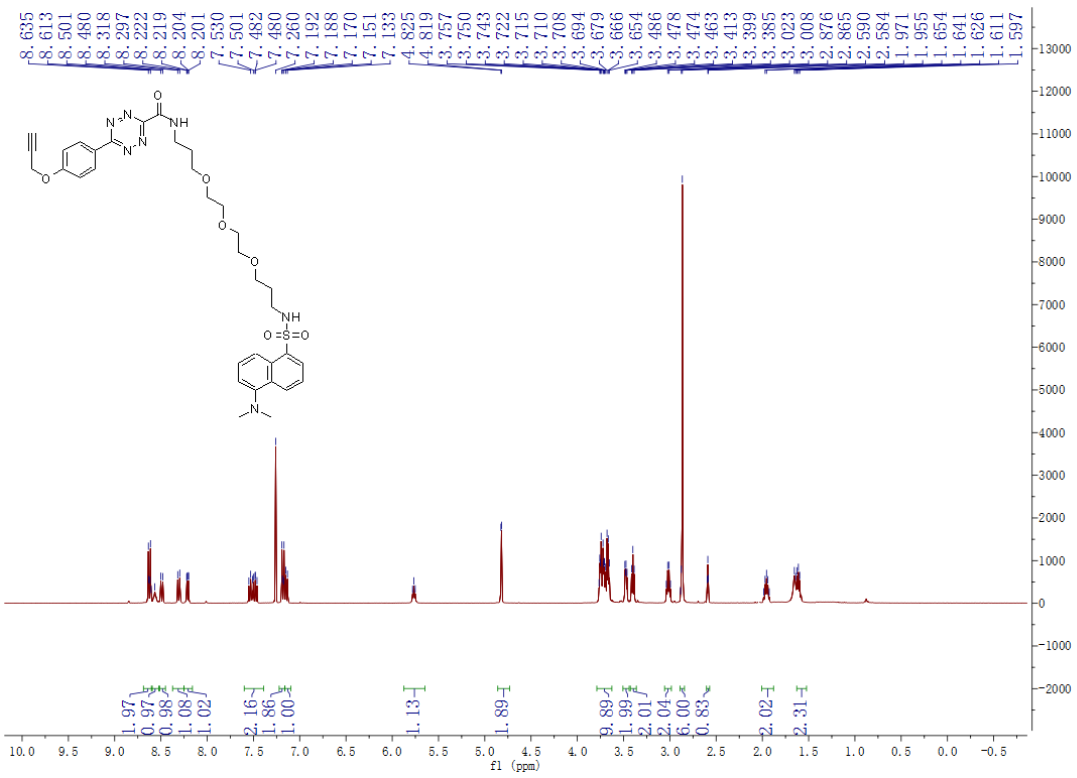

f1 (ppm)

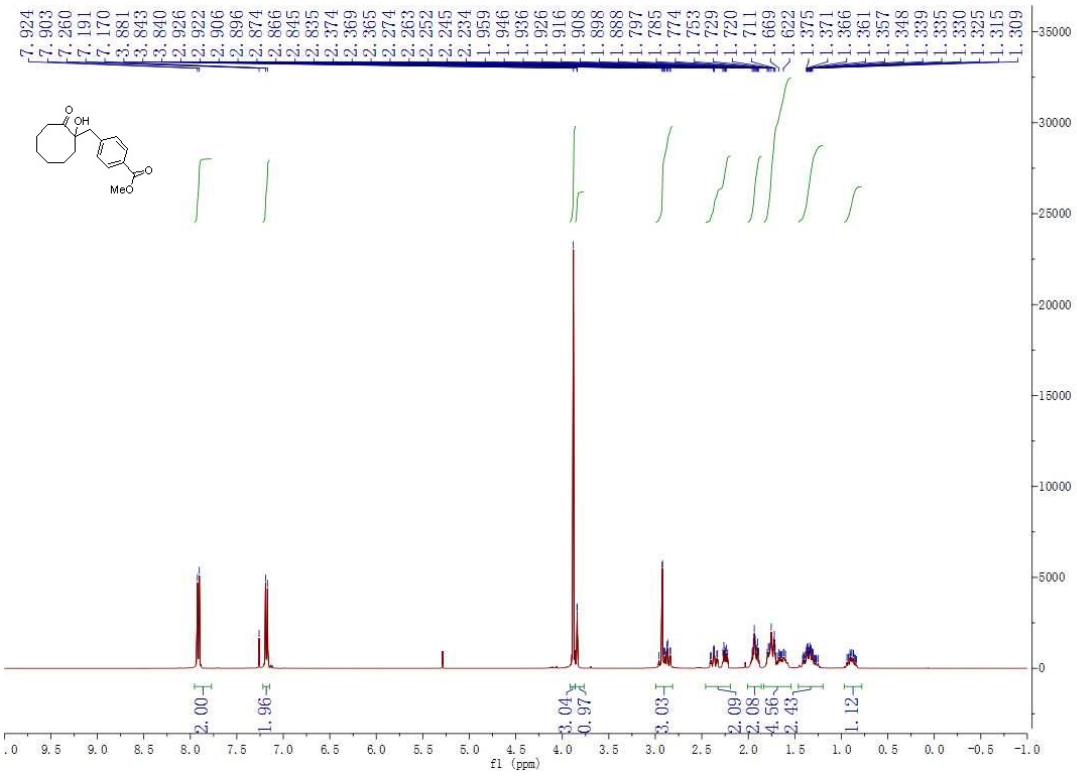

NHBoc
O
O
O
O
O=S-NH
N
fl (ppm)

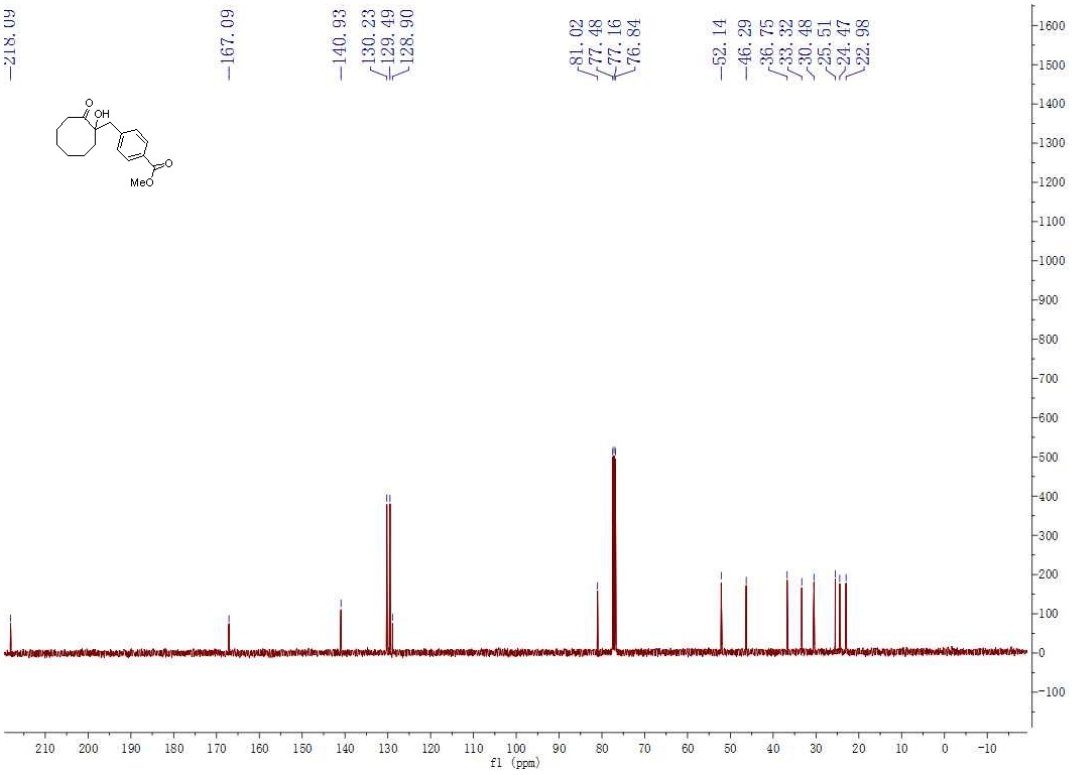

NHBoc
O
O
O
O
O=S-NH
N
fl (ppm)

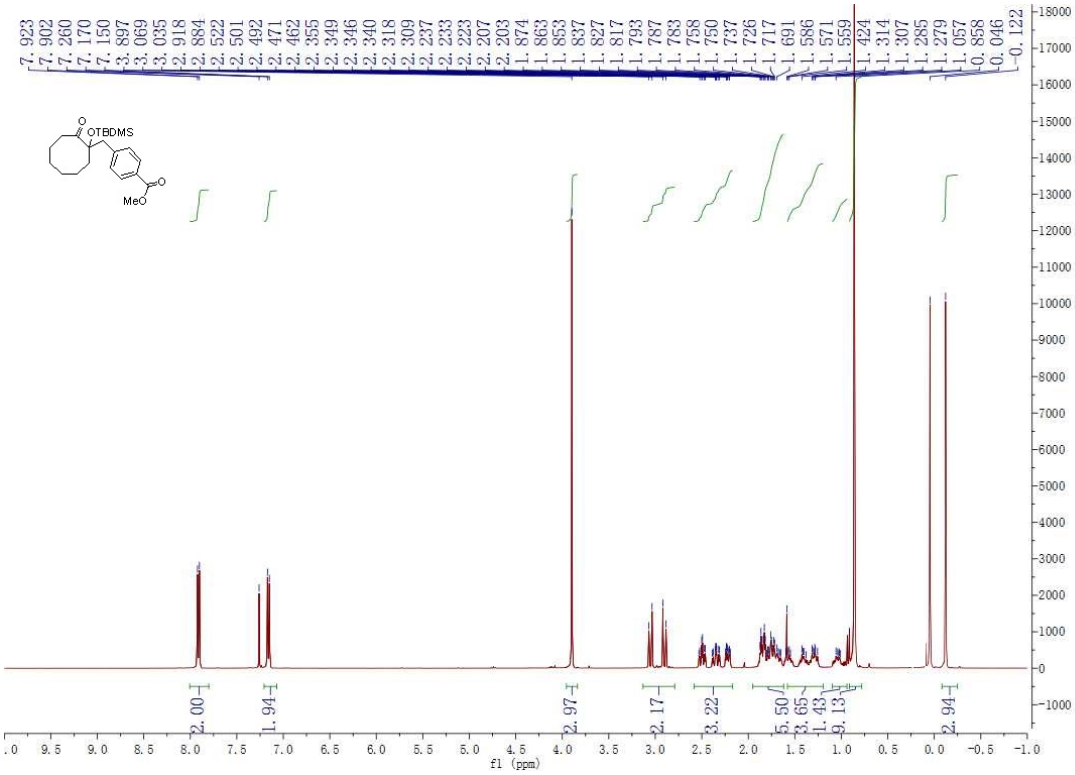

8.525
8.504
8.329
8.308
8.207
8.205
8.189
7.890
7.562
7.542
7.521
7.501
7.499
7.480
7.260
7.194
7.175
6.518
3.676
3.663
3.649
3.636
3.623
3.613
3.601
3.589
3.579
3.572
3.567
3.492
3.487
3.478
3.472
3.459
3.445
1.926
1.913
1.681
1.667
1.652
1.637
1.623
NH3
CF3COO
NH
N
1.00
0.98
0.98
2.49
1.97
0.96
0.92
8.17
4.76
2.02
1.98
5.80
1.95
1.96
f1 (ppm)

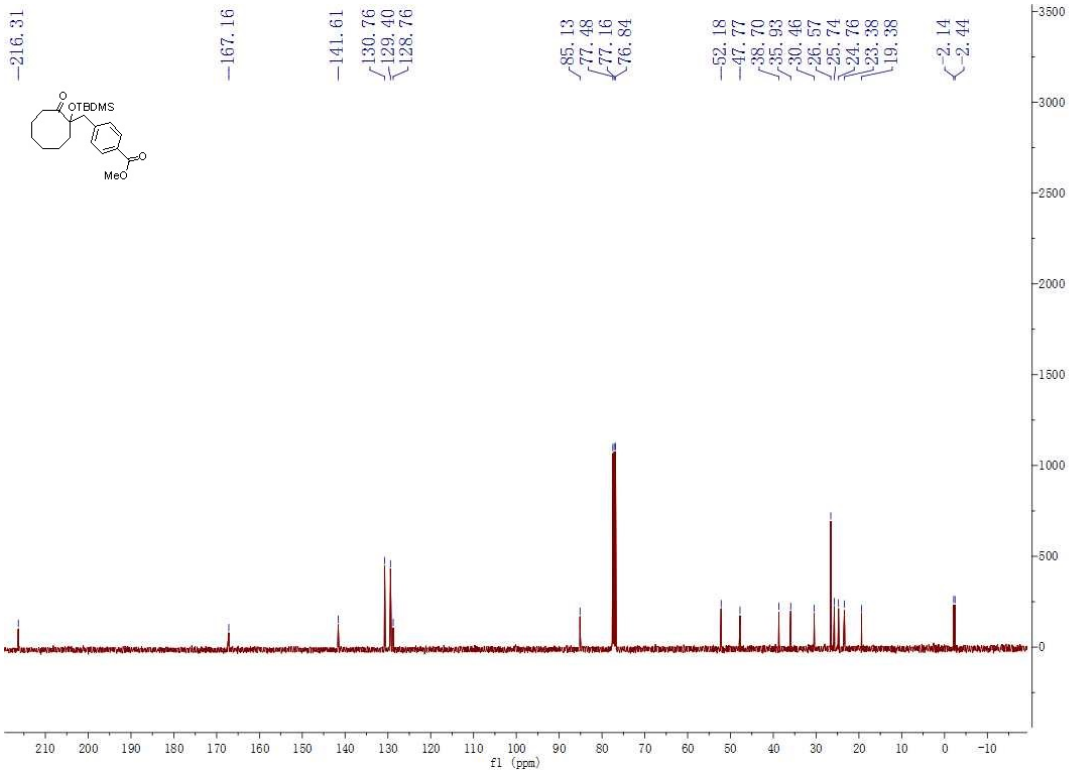

151.57
135.04
130.24
129.90
129.79
129.47
128.37
123.39
119.51
115.48
77.48
77.16
76.84
70.44
70.39
69.98
69.91
69.82
69.60
45.57
41.66
40.02
28.84
26.39
NH2
NH
N
f1 (ppm)

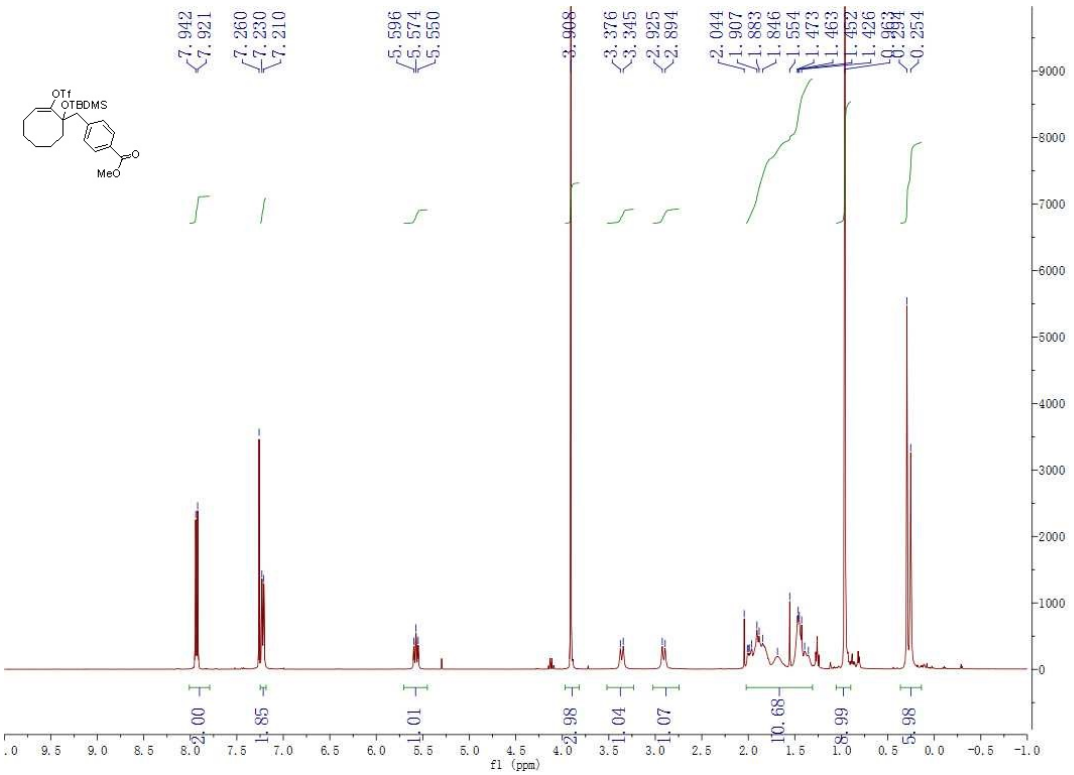





218.09
167.09
140.93
130.23
129.49
128.90
81.02
77.48
77.16
76.84
52.14
46.29
36.75
33.32
30.48
25.51
24.47
22.98
OH
MeO
f1 (ppm)


OTBDMS
MeO
2.00
1.94
2.97
2.17
3.22
5.50
3.65
1.43
9.13
2.94
f1 (ppm)


216.31
167.16
141.61
130.76
129.40
128.76
85.13
77.48
77.16
76.84
52.18
47.77
38.70
35.93
30.46
26.57
25.74
24.76
23.38
19.38
2.14
2.44
OTBDMS
MeO
f1 (ppm)


7.942
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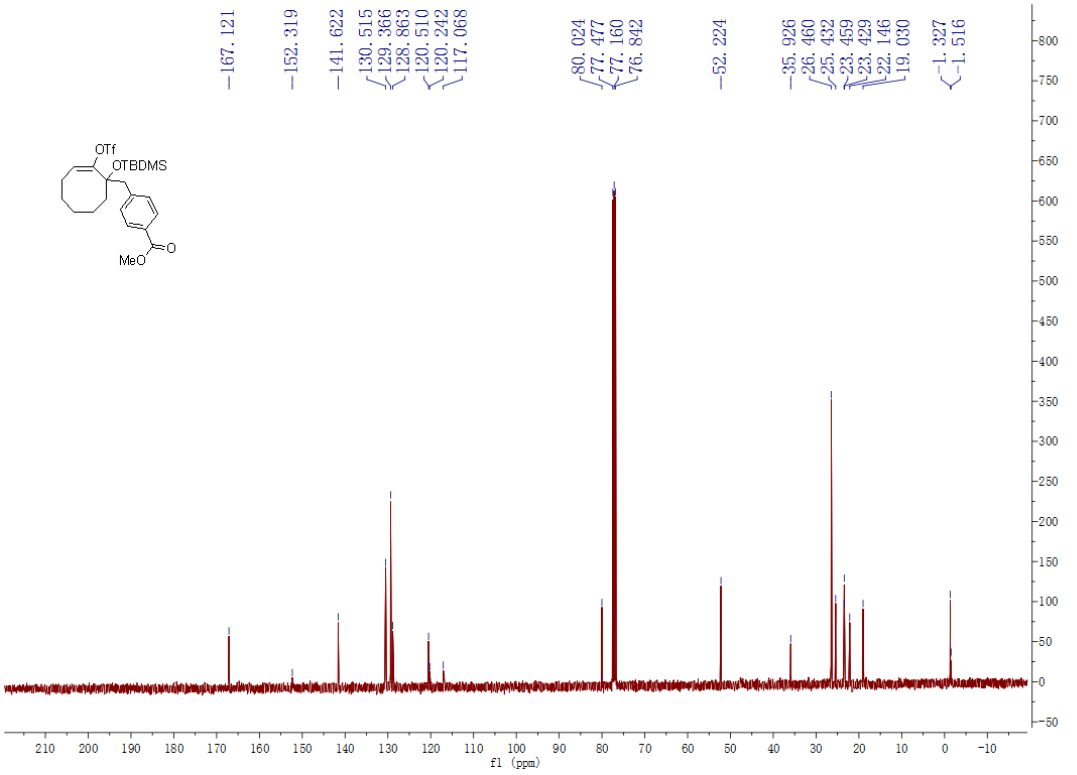
7.260
7.230
7.210
5.596
5.574
5.550
3.908
3.376
3.345
2.925
2.894
2.044
1.907
1.883
1.846
1.554
1.473
1.463
1.452
1.426
0.904
0.254
OTf
OTBDMS
MeO
2.00
1.85
1.01
2.98
1.04
1.07
10.68
8.99
5.98
f1 (ppm)


OTf
OTBDMS
MeO
O
167.121
152.319
141.622
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129.366
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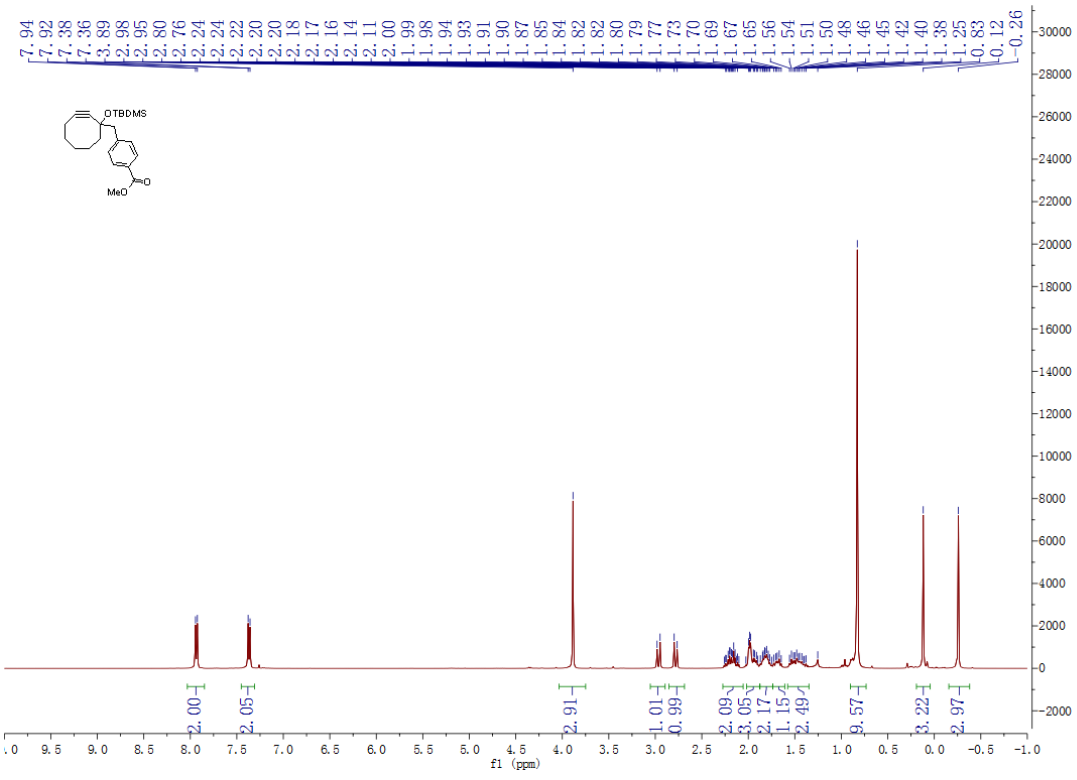
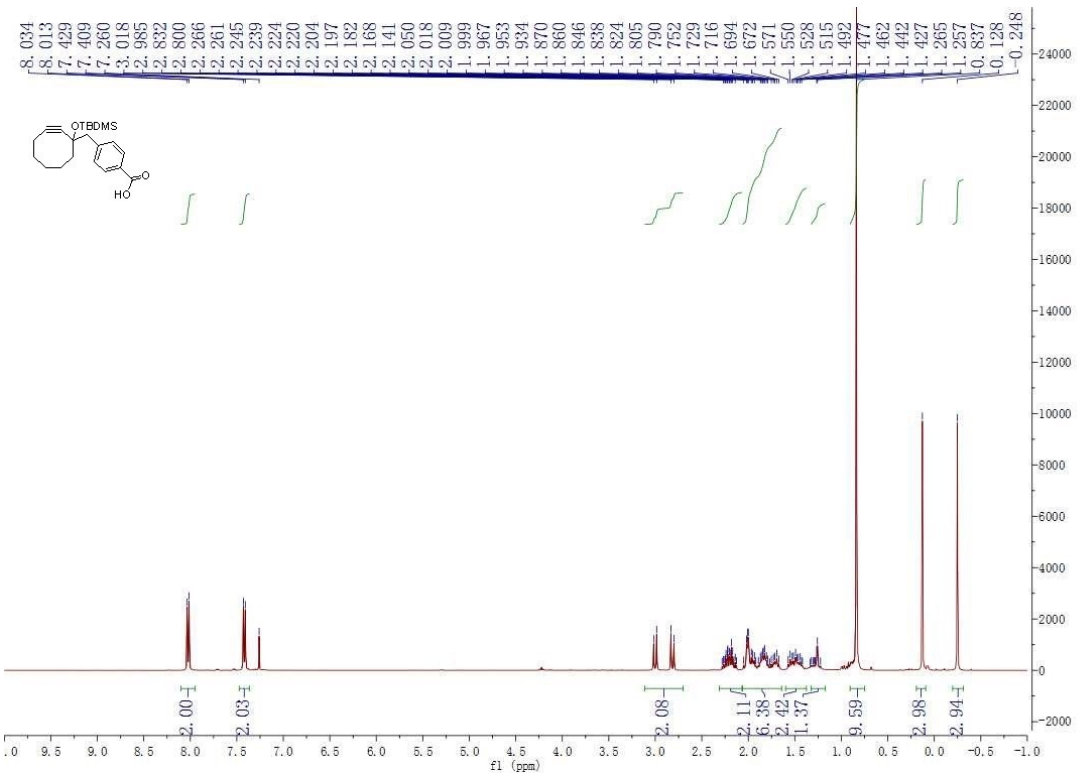
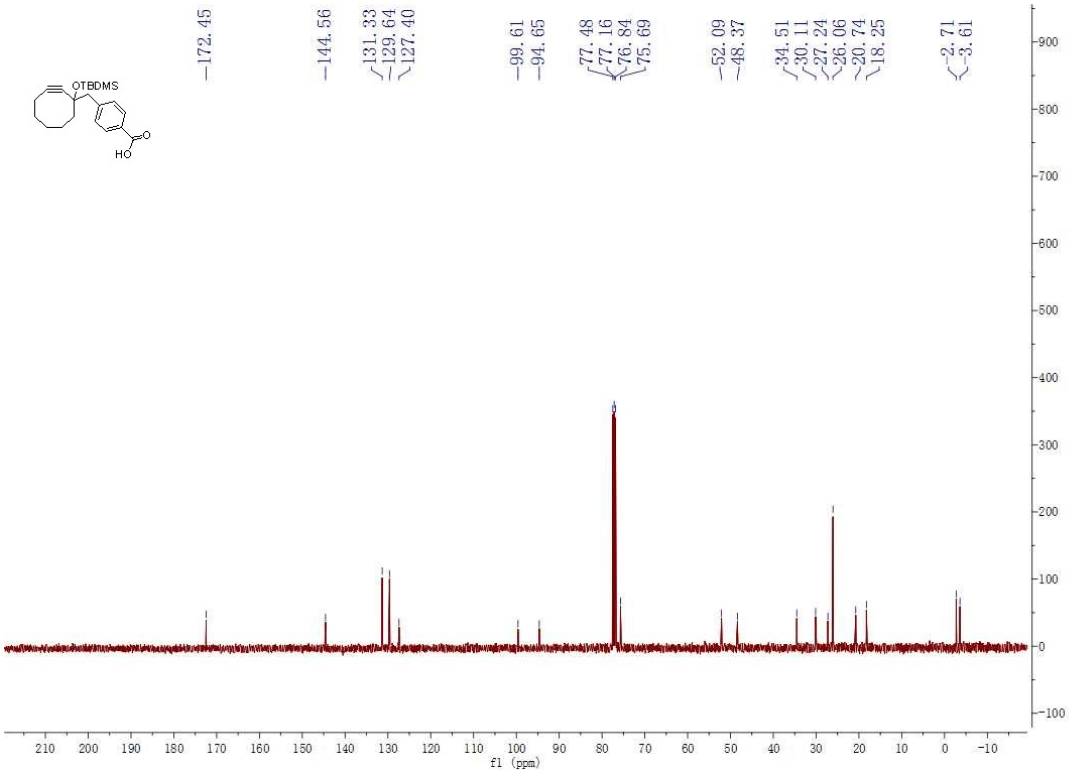
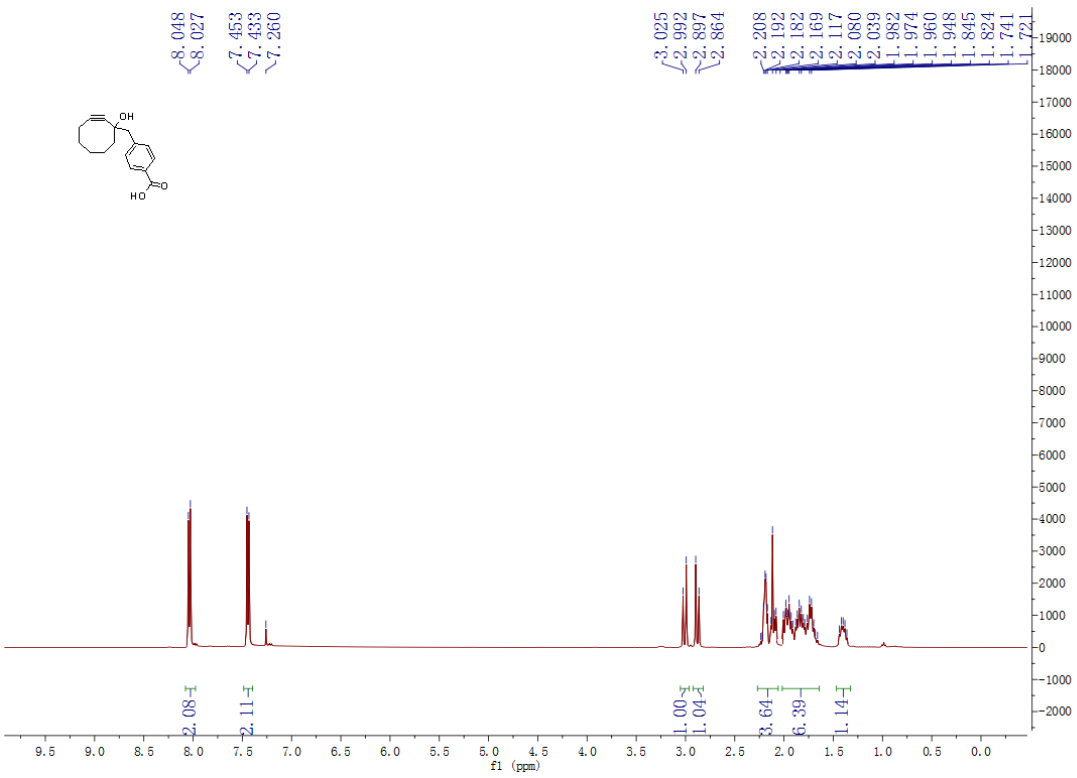
120.510
120.242
117.068
80.024
77.477
77.160
76.842
52.224
35.926
26.460
25.432
23.459
23.429
22.146
19.030
-1.327
-1.516
f1 (ppm)


7.94
7.92
7.38
7.36
3.89
2.98
2.95
2.80
2.76
2.24
2.24
2.22
2.20
2.20
2.18
2.17
2.16
2.14
2.11
2.00
1.99
1.98
1.94
1.93
1.91
1.90
1.87
1.85
1.84
1.82
1.82
1.80
1.79
1.77
1.73
1.70
1.69
1.67
1.65
1.56
1.54
1.51
1.50
1.48
1.46
1.45
1.42
1.40
1.38
1.25
0.83
0.12
0.26
OTBDMS
MeO
O
2.00
2.05
2.91
1.01
0.99
2.09
3.05
2.17
1.15
2.49
9.57
3.22
2.97
f1 (ppm)


OTBDMS
HO
O
f1 (ppm)


OTBDMS
HO
O
f1 (ppm)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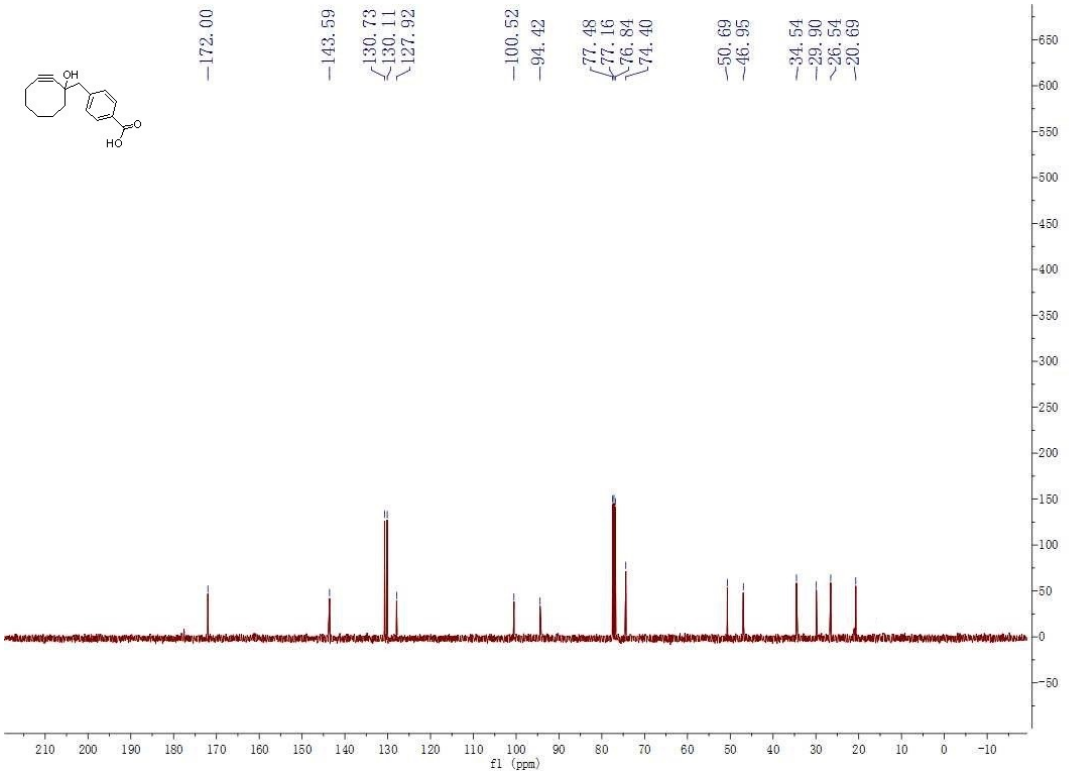
2.182
2.169
2.117
2.080
2.039
1.982
1.974
1.960
1.948
1.845
1.824
1.741
1.721
OH
HO
O
2.08
2.11
1.00
1.04
3.64
6.39
1.14
f1 (ppm)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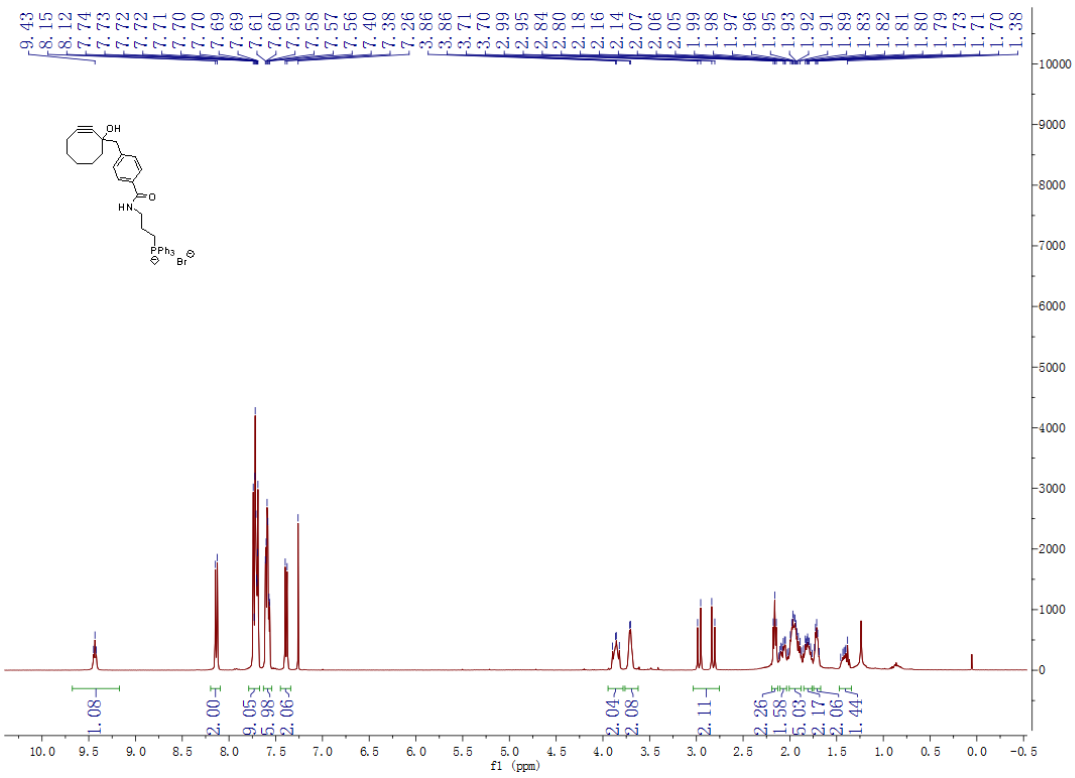
143.59
130.73
130.11
127.92
100.52
94.42
77.48
77.16
76.84
74.40
50.69
46.95
34.54
29.90
26.54
20.69
OH
HO
O
f1 (ppm)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8.12
7.74
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7.72
7.72
7.71
7.70
7.70
7.69
7.69
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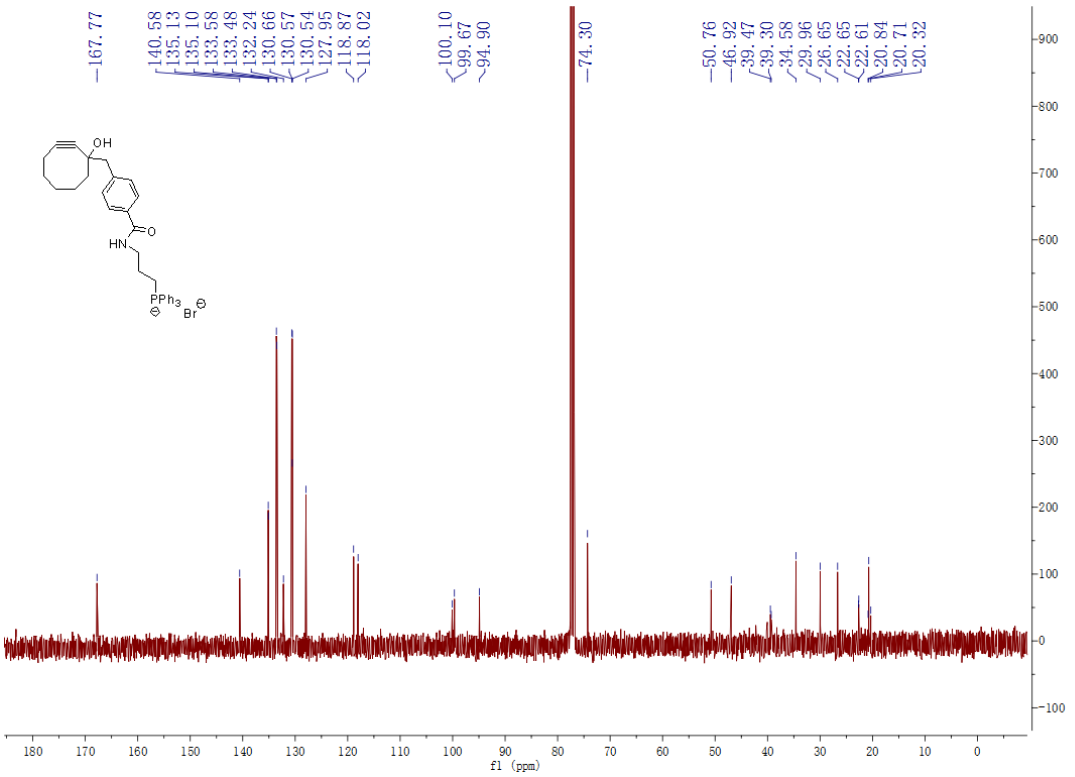
7.60
7.59
7.58
7.57
7.56
7.40
7.38
7.26
3.86
3.71
3.70
2.99
2.95
2.84
2.80
2.18
2.16
2.14
2.07
2.06
2.05
1.99
1.98
1.97
1.96
1.95
1.93
1.92
1.91
1.89
1.83
1.82
1.81
1.80
1.79
1.73
1.71
1.70
1.38
OH
HN
O
PPh3
Br
1.08
2.00
9.05
5.98
2.06
2.04
2.08
2.11
2.26
1.58
5.03
2.17
2.06
1.44
f1 (ppm)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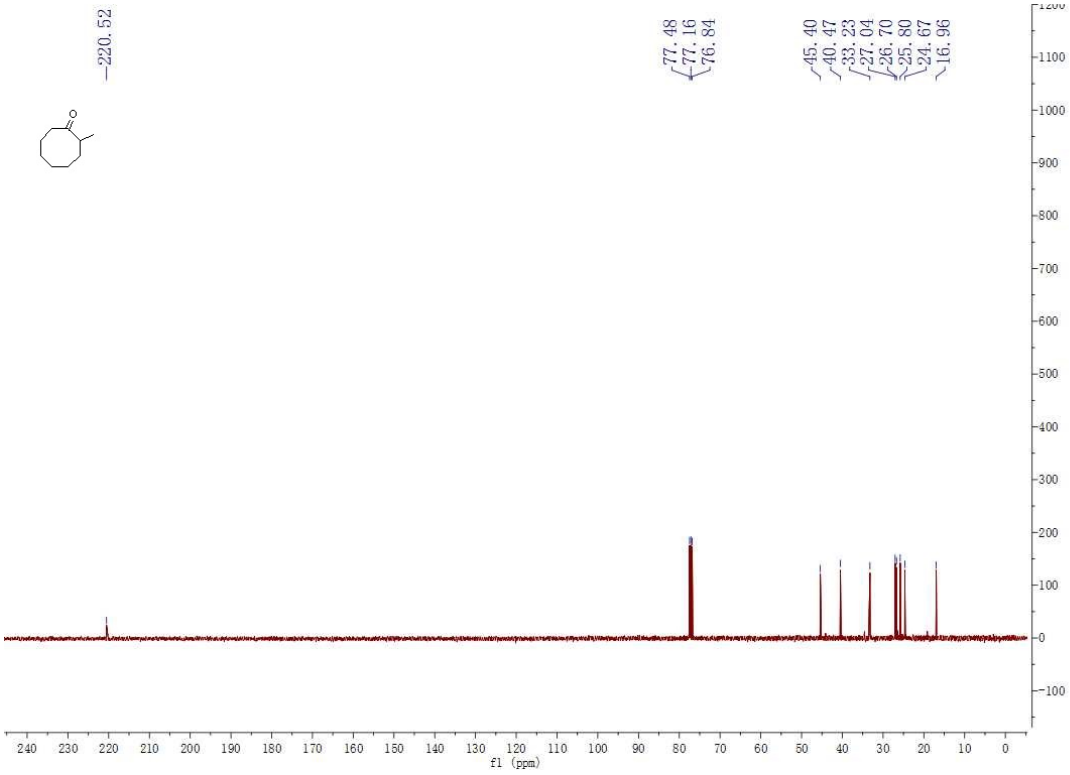
135.10
133.58
133.48
132.24
130.66
130.57
130.54
127.95
118.87
118.02
100.10
99.67
94.90
74.30
50.76
46.92
39.47
39.30
34.58
29.96
26.65
22.65
22.61
20.84
20.71
20.32
OH
HN
O
PPh3
Br
f1 (ppm)


220.52
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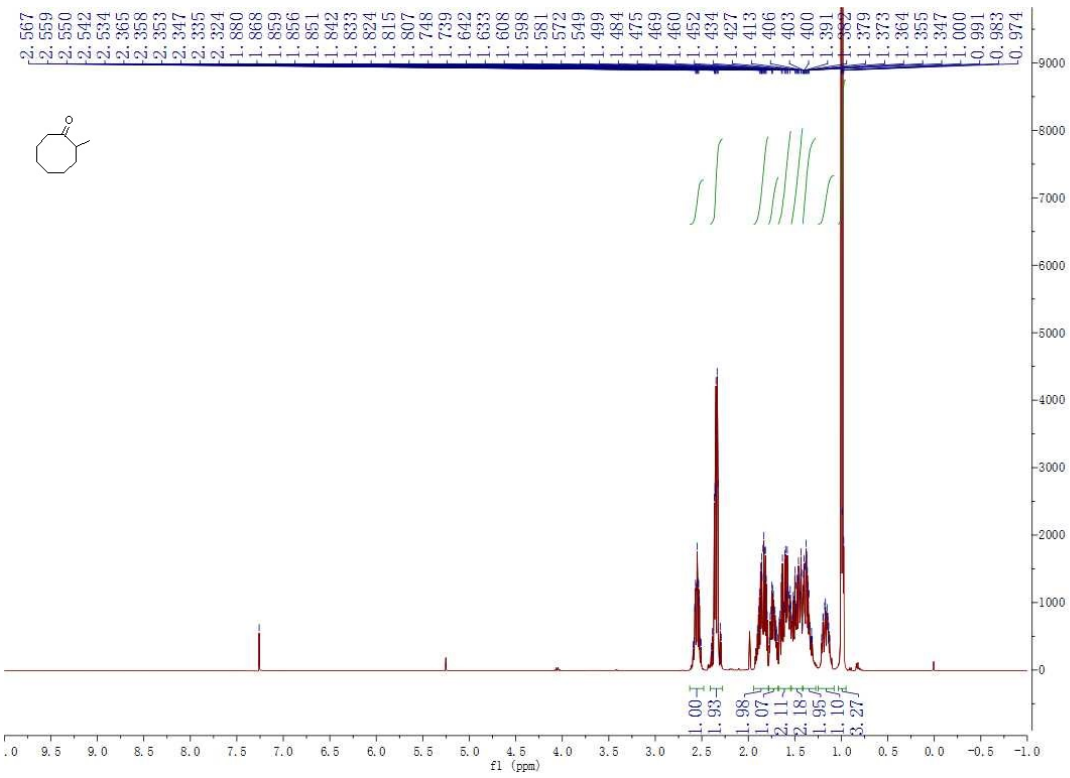
40.47
33.23
27.04
26.70
25.80
24.67
16.96
f1 (ppm)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2.358
2.353
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2.335
2.324
1.880
1.868
1.859
1.856
1.851
1.842
1.833
1.824
1.815
1.807
1.748
1.739
1.642
1.633
1.608
1.598
1.581
1.572
1.549
1.499
1.484
1.475
1.469
1.460
1.452
1.434
1.427
1.413
1.406
1.403
1.400
1.391
1.382
1.379
1.373
1.364
1.355
1.347
1.000
0.991
0.983
0.974
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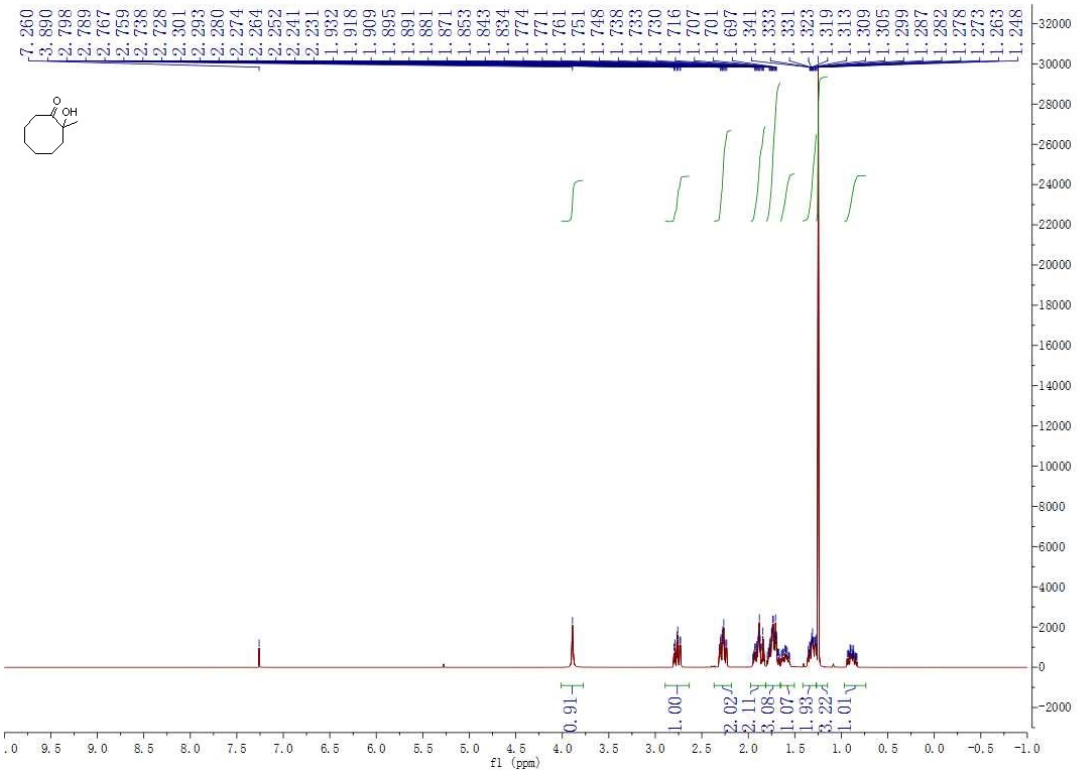
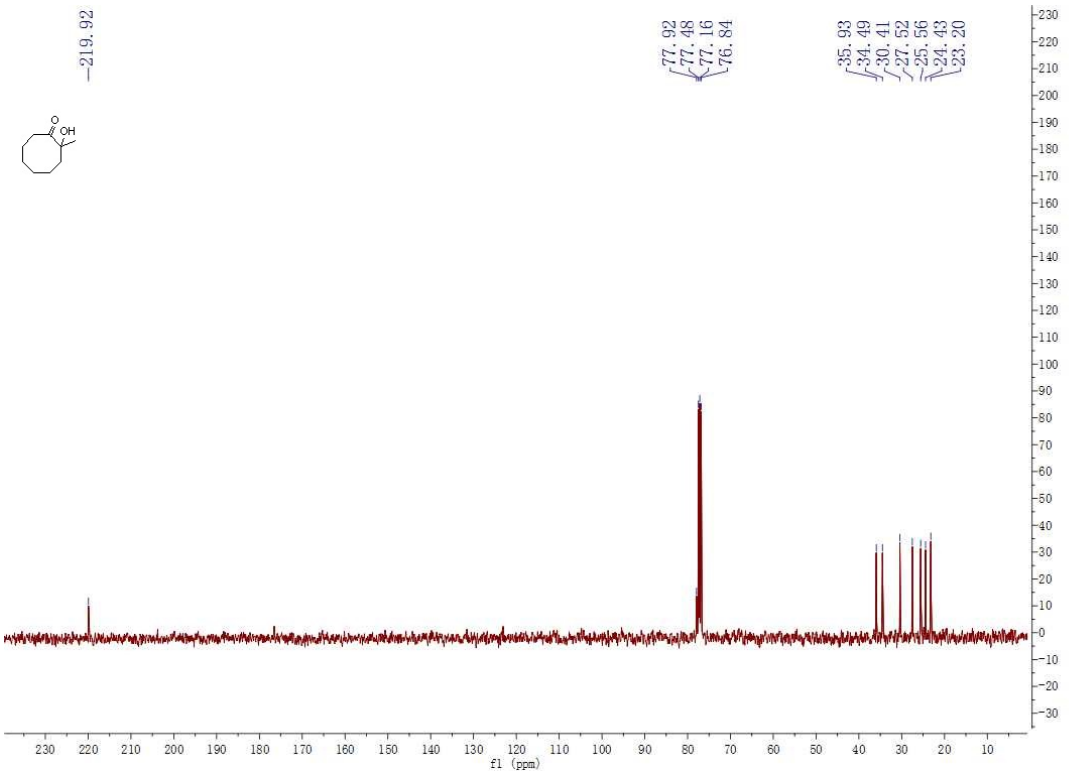
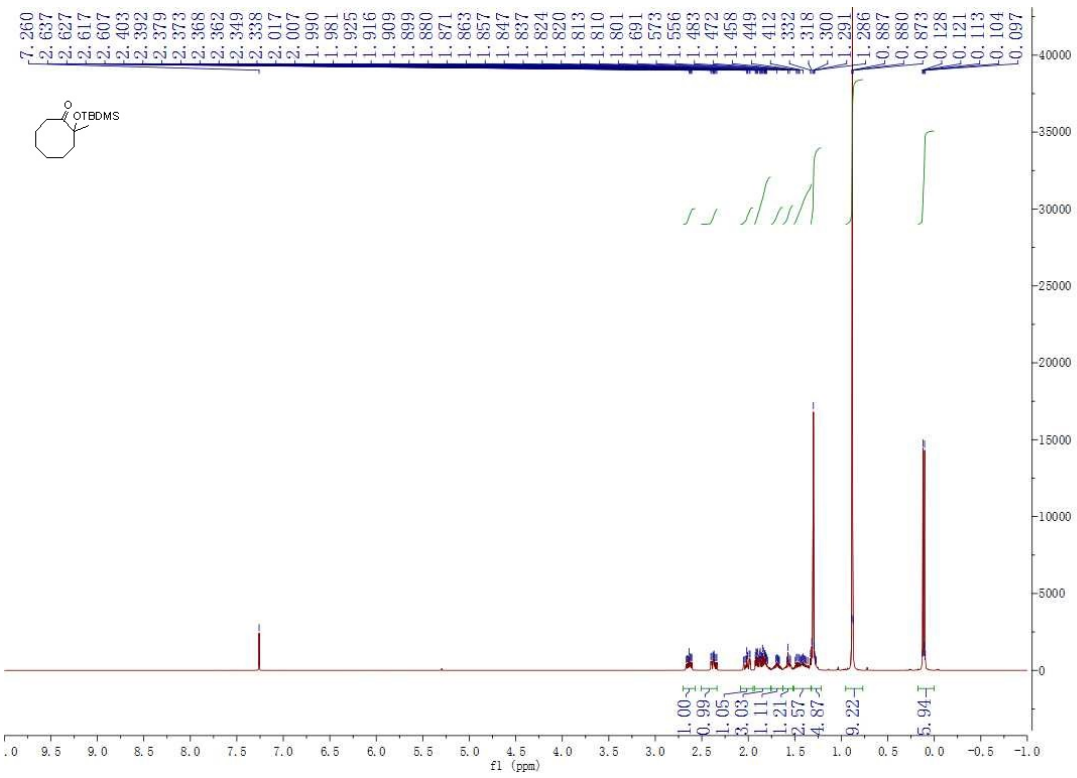
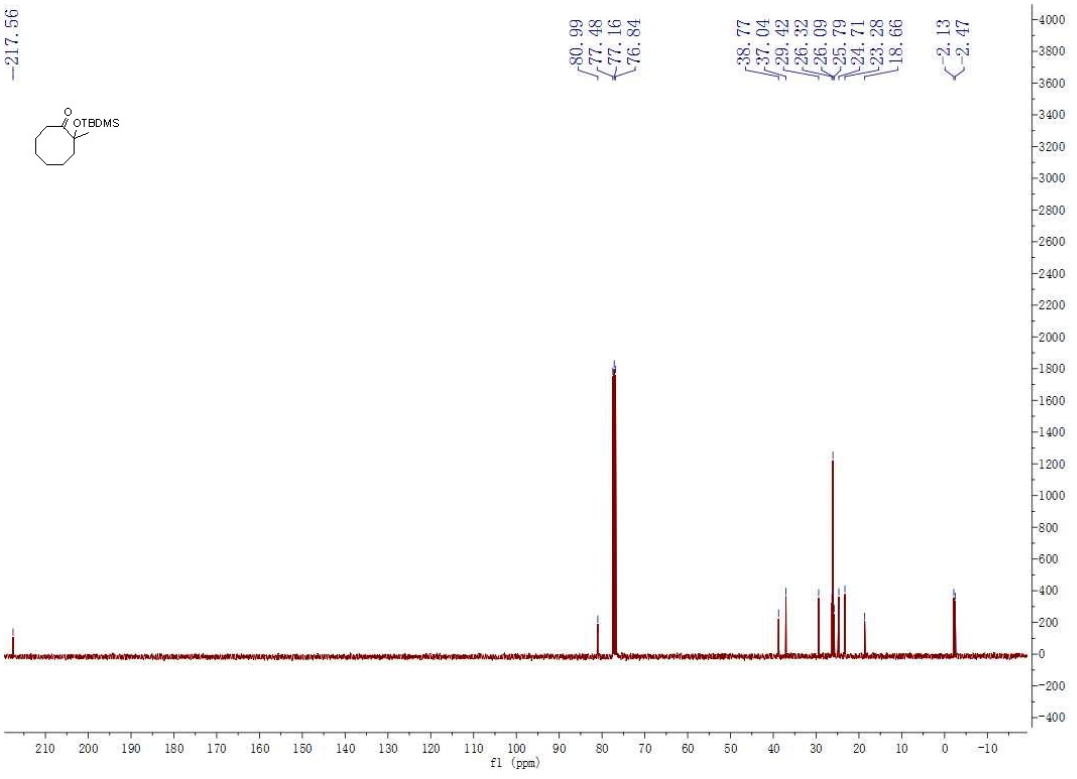
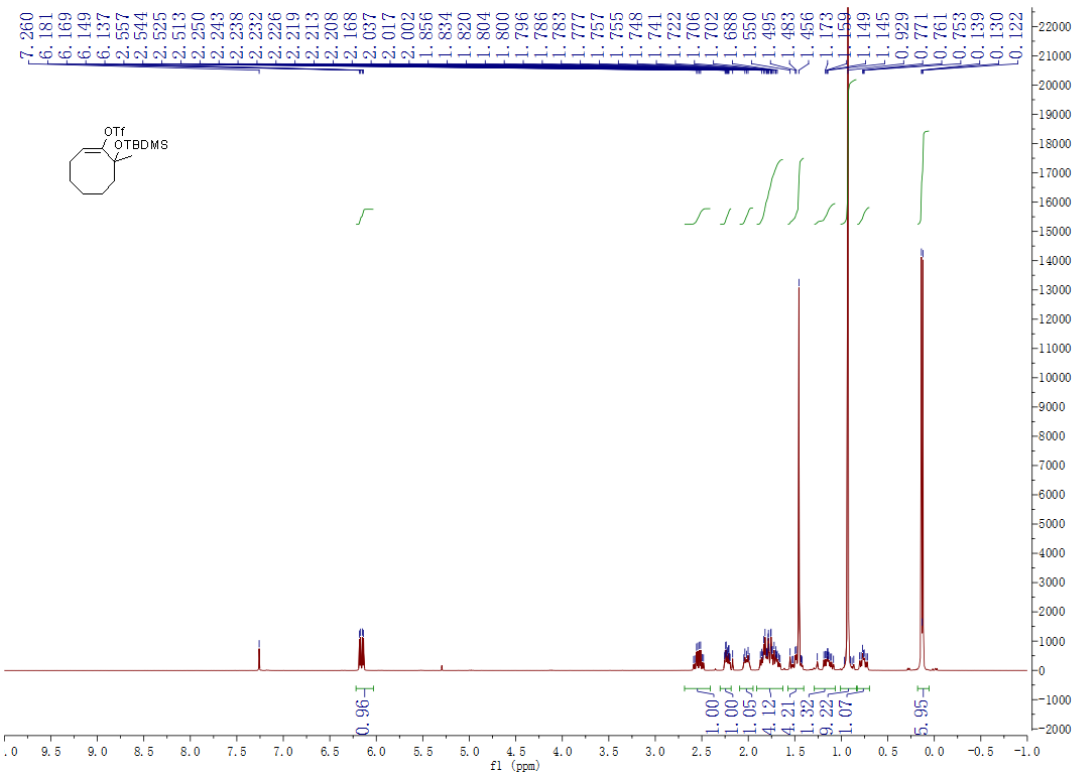
1.98
1.07
2.11
2.18
1.95
1.10
3.27
f1 (ppm)

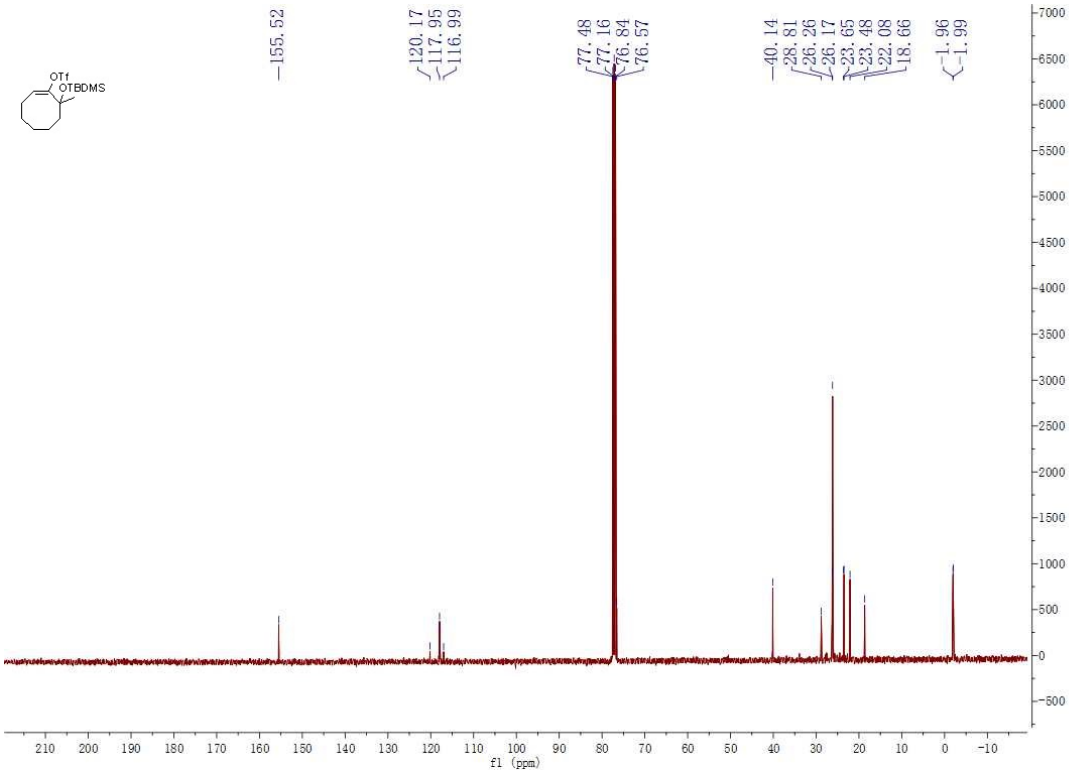

7.260
3.890
2.798
2.789
2.767
2.759
2.738
2.728
2.301
2.293
2.280
2.274
2.264
2.252
2.241
2.231
1.932
1.918
1.909
1.895
1.891
1.881
1.871
1.853
1.843
1.834
1.774
1.771
1.761
1.751
1.748
1.738
1.733
1.730
1.716
1.707
1.701
1.697
1.341
1.333
1.331
1.323
1.319
1.313
1.309
1.305
1.299
1.287
1.282
1.278
1.273
1.263
1.248
OH
0.91
1.00
2.02
2.11
3.08
1.07
1.93
3.22
1.01
f1 (ppm)

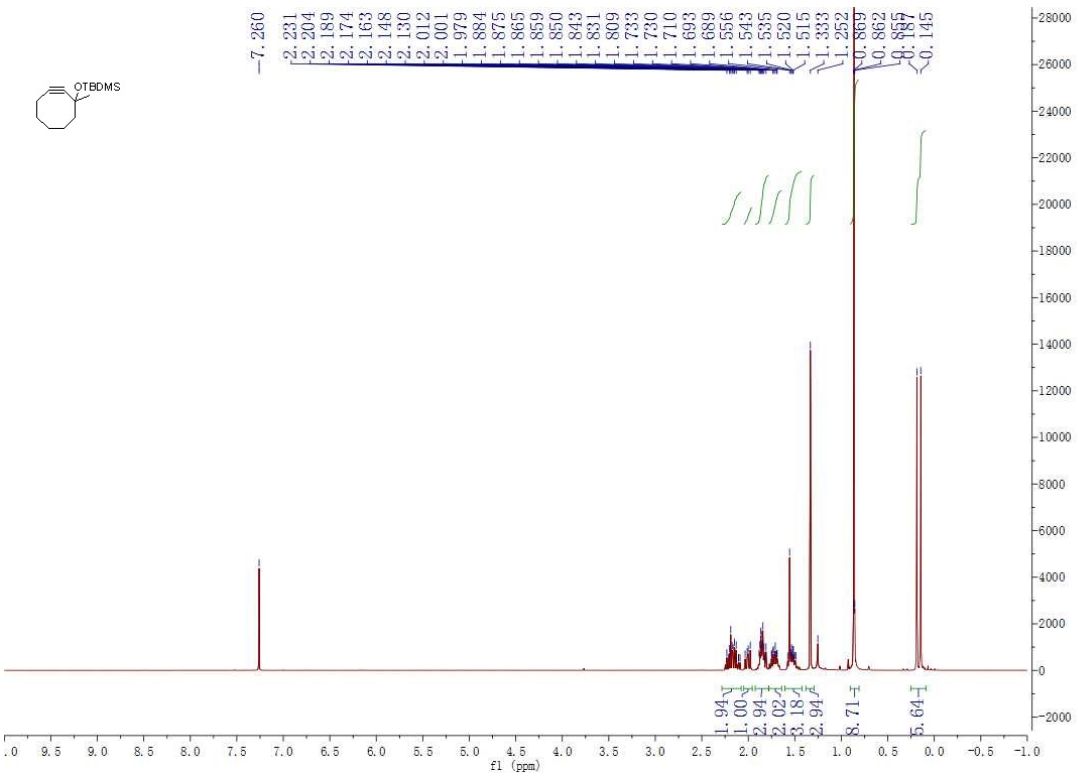

219.92
77.92
77.48
77.16
76.84
35.93
34.49
30.41
27.52
25.56
24.43
23.20
OH
f1 (ppm)

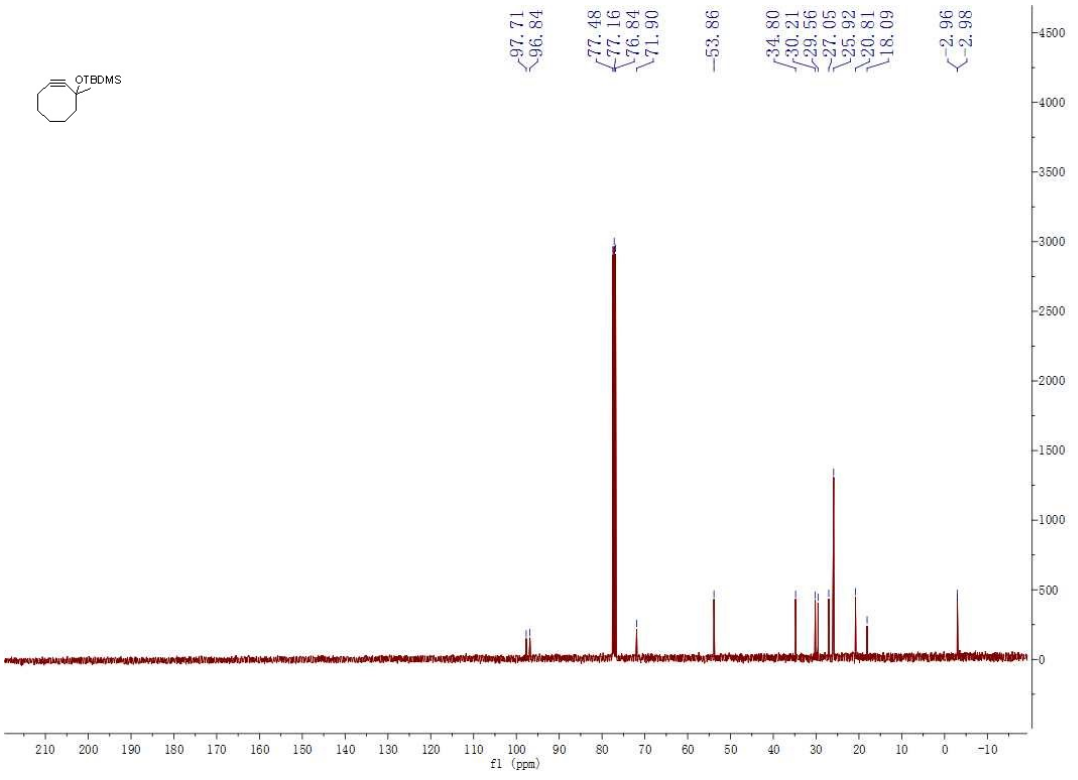

7.260
2.637
2.627
2.617
2.607
2.403
2.392
2.379
2.373
2.368
2.362
2.349
2.338
2.017
2.007
1.990
1.981
1.925
1.916
1.909
1.899
1.880
1.871
1.863
1.857
1.847
1.837
1.824
1.820
1.813
1.810
1.801
1.691
1.573
1.556
1.483
1.472
1.458
1.449
1.412
1.332
1.318
1.300
1.291
1.286
0.887
0.880
0.873
0.128
0.121
0.113
0.104
0.097
OTBDMS
1.00
0.99
1.05
3.03
1.11
1.21
2.57
4.87
9.22
5.94
f1 (ppm)

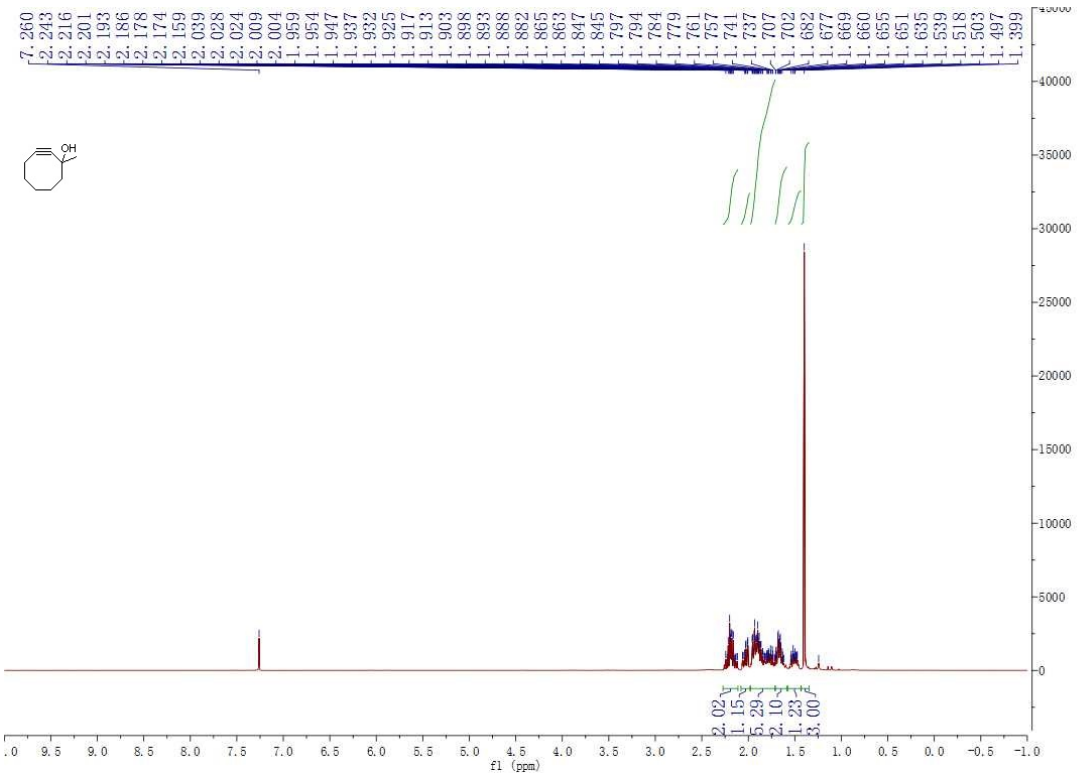

217.56
80.99
77.48
77.16
76.84
38.77
37.04
29.42
26.32
26.09
25.79
24.71
23.28
18.66
2.13
2.47
OTBDMS
f1 (ppm)

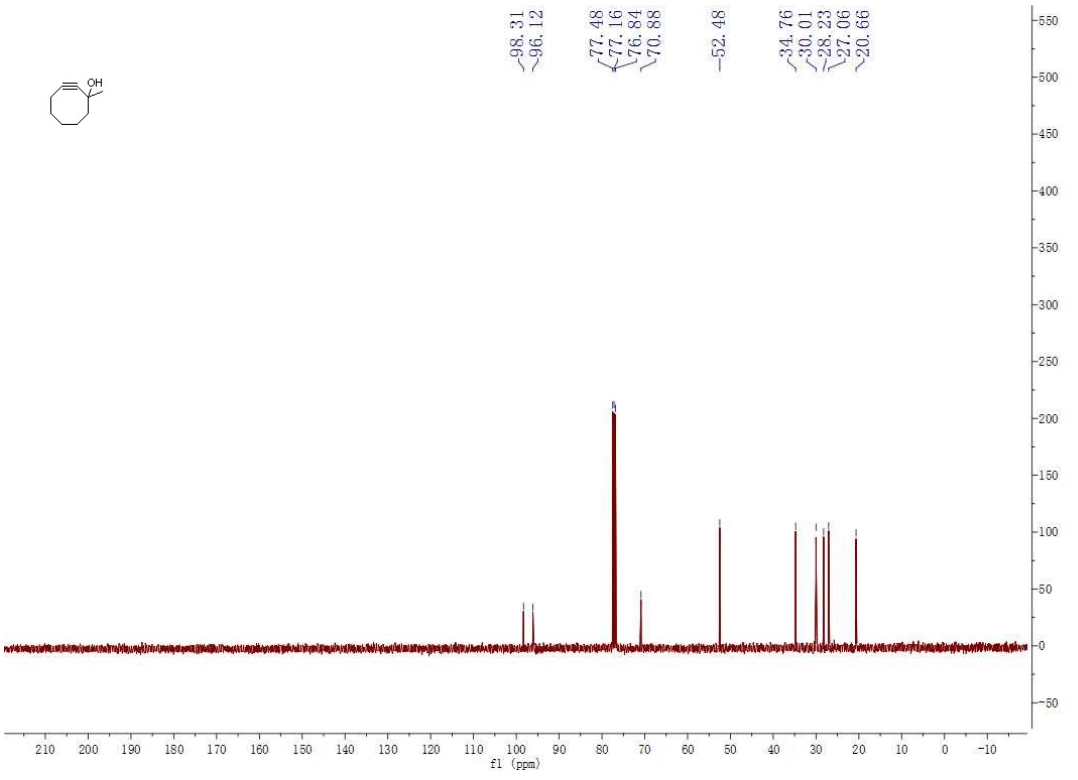

OTf
OTBDMS
f1 (ppm)

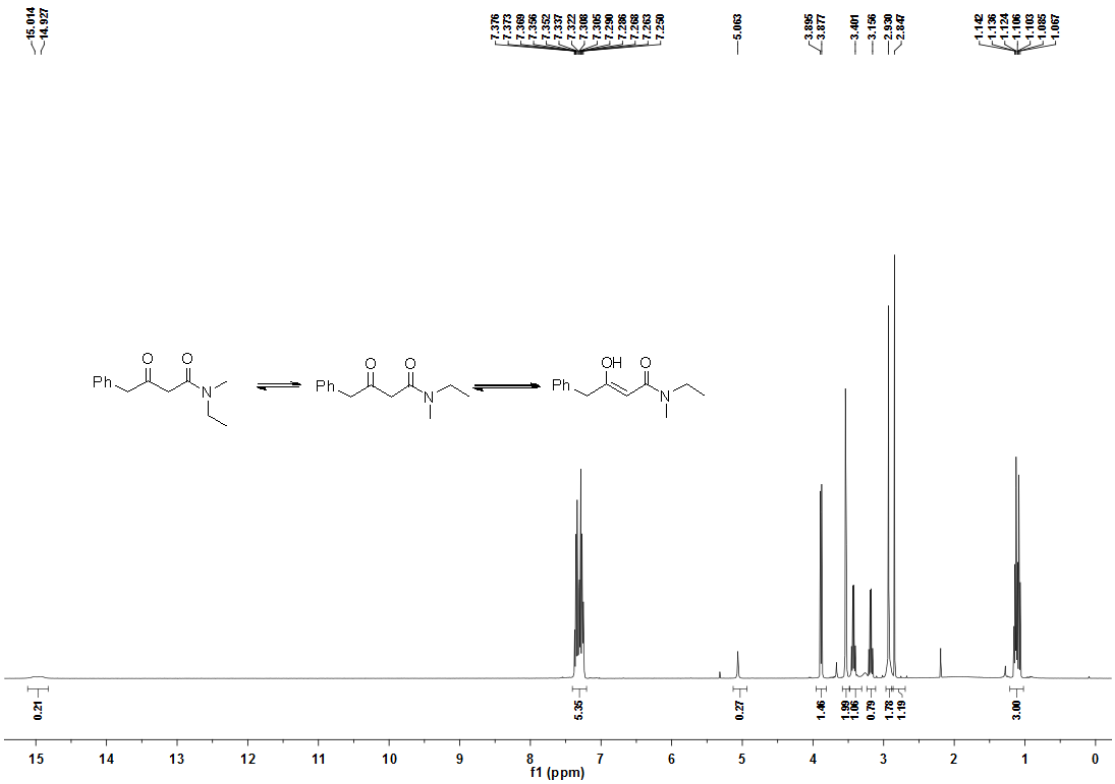

OTf
OTBDMS
f1 (ppm)

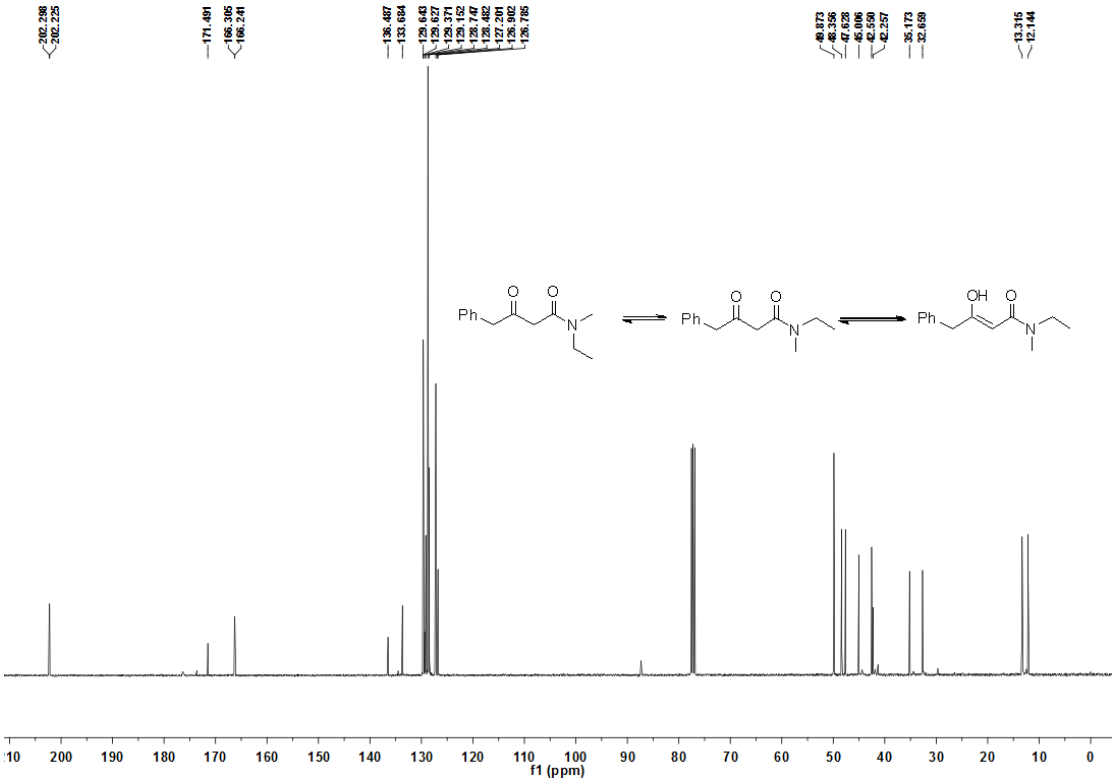

OTBDMS
7.260
2.231
2.204
2.189
2.174
2.163
2.148
2.130
2.012
2.001
1.979
1.884
1.875
1.865
1.859
1.850
1.843
1.831
1.809
1.733
1.730
1.710
1.693
1.689
1.556
1.543
1.535
1.520
1.515
1.333
1.252
0.869
0.862
0.855
0.847
0.145
1.94
1.00
2.94
2.02
3.18
2.94
8.71
5.64
f1 (ppm)

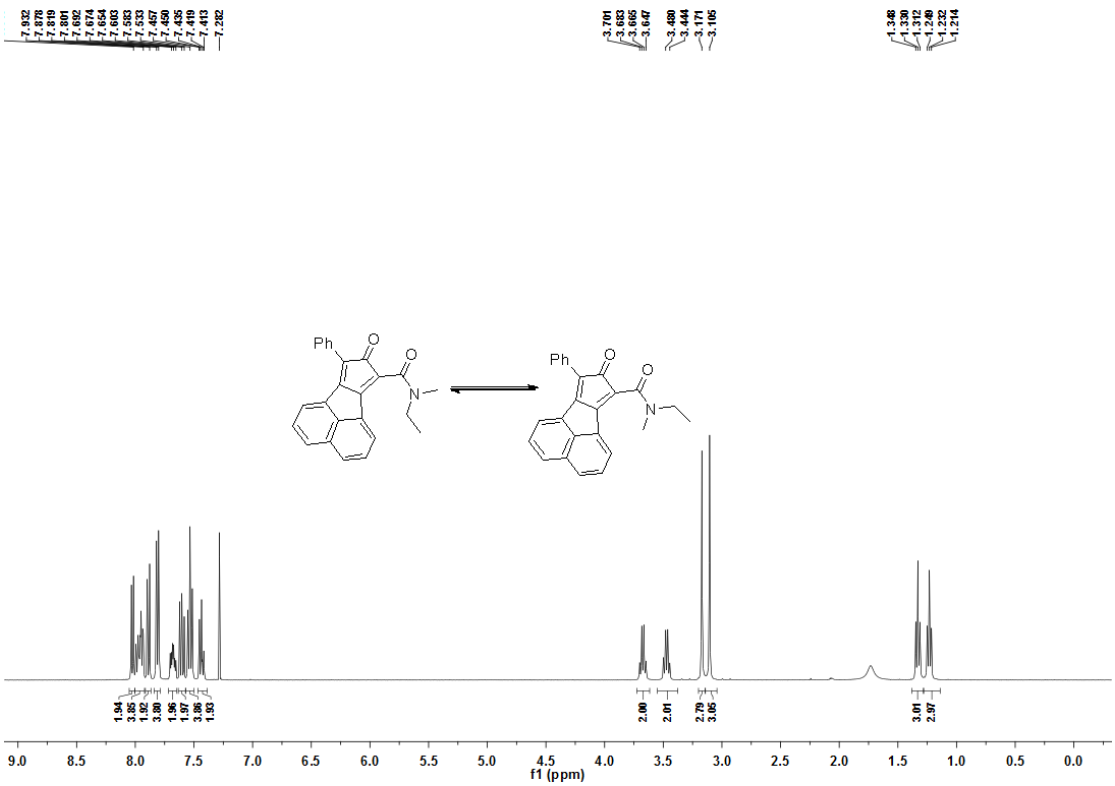

OTBDMS
97.71
96.84
77.48
77.16
76.84
71.90
53.86
34.80
30.21
29.56
27.05
25.92
20.81
18.09
2.96
2.98
f1 (ppm)

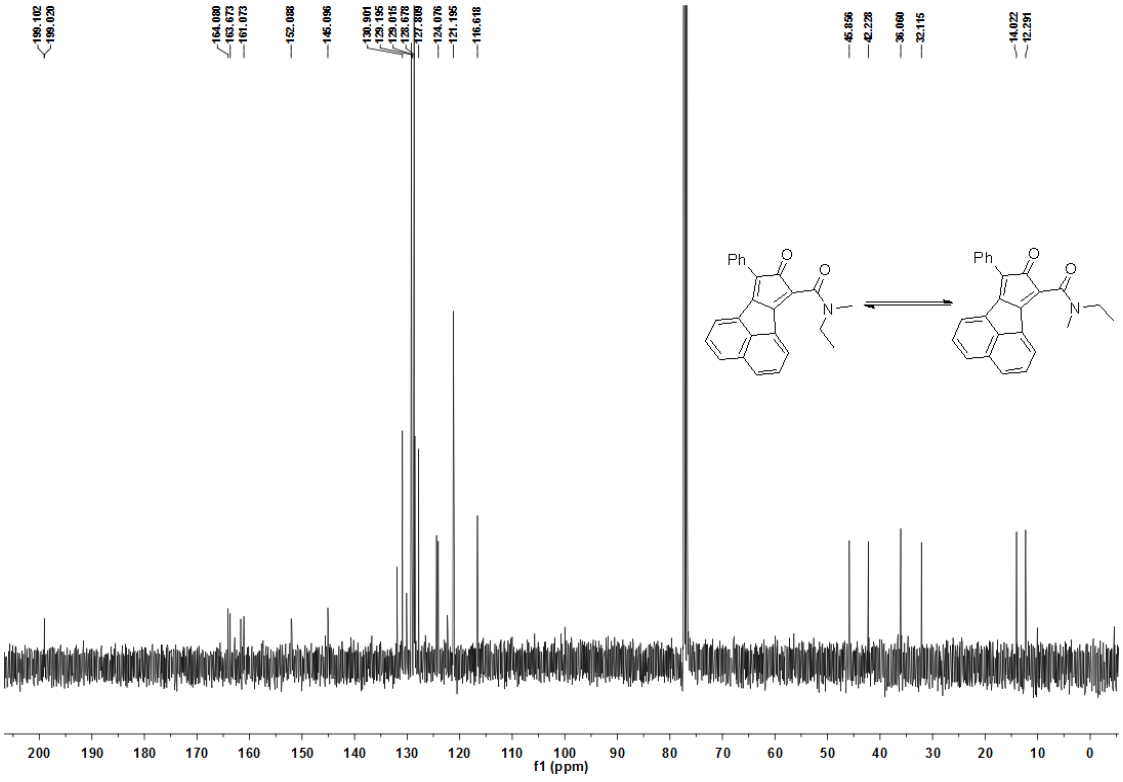

7.260
2.243
2.216
2.201
2.193
2.186
2.178
2.174
2.159
2.039
2.028
2.024
2.009
2.004
1.959
1.954
1.947
1.937
1.932
1.925
1.917
1.913
1.903
1.898
1.893
1.888
1.882
1.865
1.863
1.847
1.845
1.797
1.794
1.784
1.779
1.761
1.757
1.741
1.737
1.707
1.702
1.682
1.677
1.669
1.660
1.655
1.651
1.635
1.539
1.518
1.503
1.497
1.399
OH
2.02
1.15
5.29
2.10
1.23
3.00
f1 (ppm)

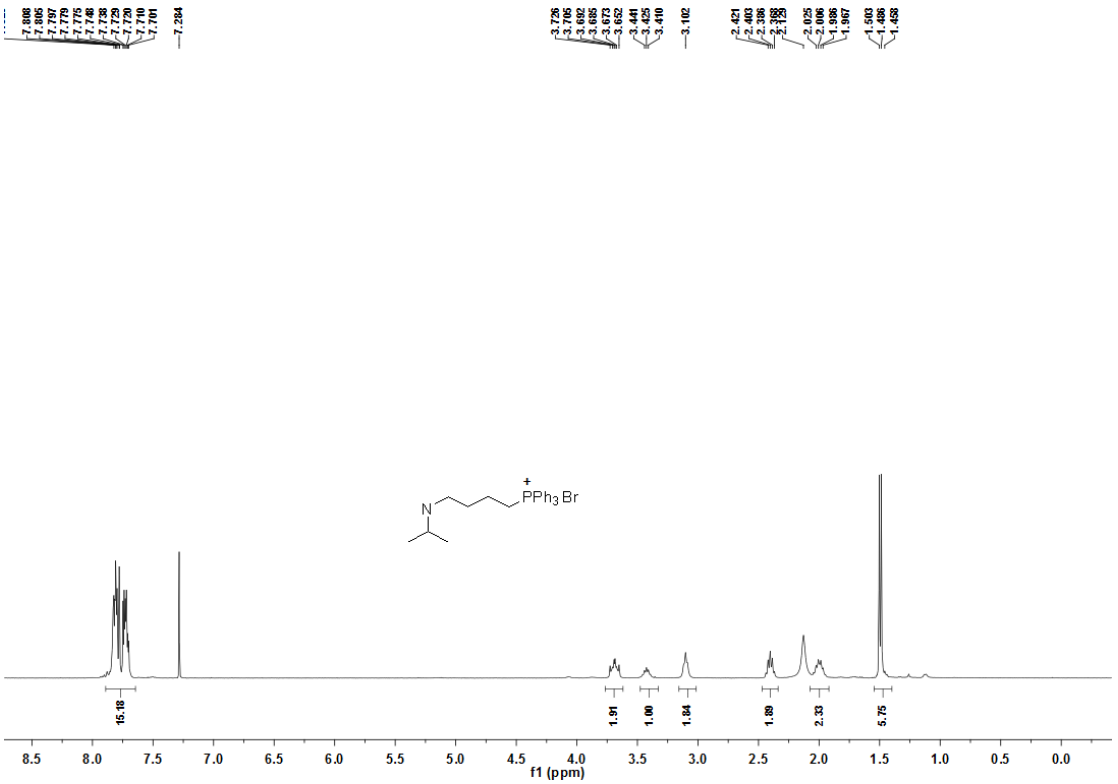

98.31
96.12
77.48
77.16
76.84
70.88
52.48
34.76
30.01
28.23
27.06
20.66
OH
f1 (ppm)

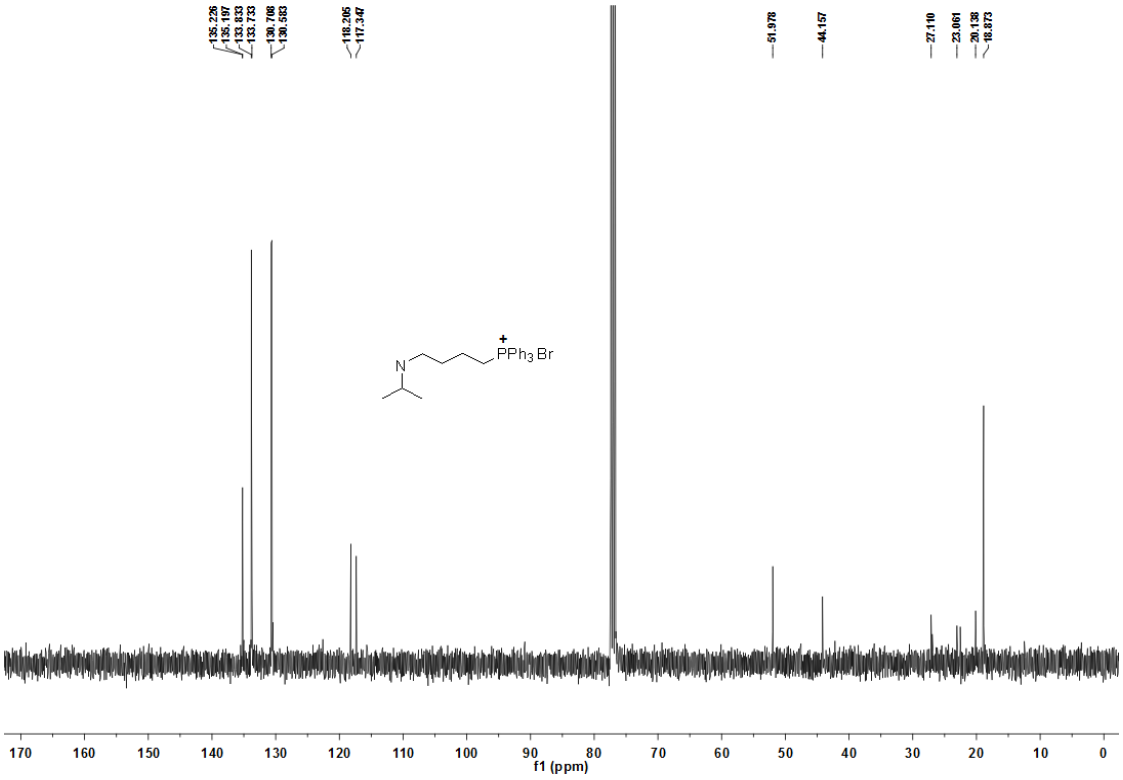

15.014
14.927
7.376
7.373
7.369
7.366
7.352
7.337
7.322
7.308
7.305
7.290
7.286
7.268
7.253
7.230
5.063
3.895
3.877
3.491
3.156
2.930
2.847
1.142
1.136
1.124
1.106
1.103
1.085
1.067
Ph
O
O
N
OH
0.21
5.35
0.27
1.46
1.88
1.86
0.79
1.78
1.19
3.00
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


202.298
202.225
171.491
166.305
166.241
136.487
133.684
129.643
129.627
129.371
129.152
128.747
128.482
127.201
126.902
126.785
49.873
48.356
47.628
45.906
42.150
42.257
35.173
32.659
13.315
12.144
Ph
O
O
N
OH
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

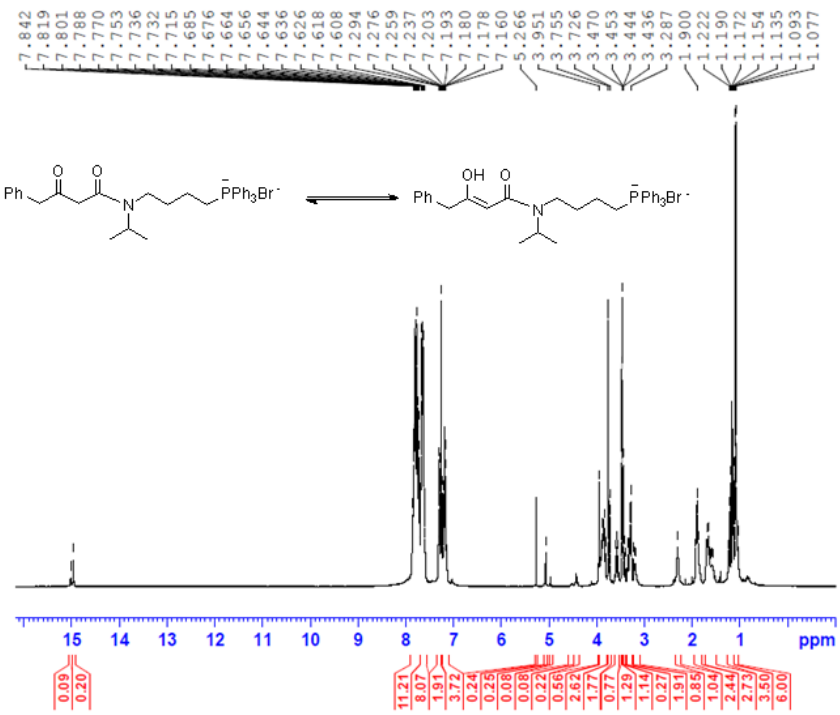

7.932
7.878
7.819
7.801
7.692
7.674
7.654
7.645
7.565
7.533
7.457
7.450
7.435
7.419
7.413
7.282
3.701
3.683
3.665
3.647
3.480
3.444
3.171
3.105
1.348
1.330
1.312
1.249
1.232
1.214
Ph
O
N
1.94
3.85
1.92
3.00
1.96
1.97
3.86
1.93
2.00
2.01
2.79
3.05
3.01
2.97
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


199.102
199.020
164.080
163.673
161.073
152.088
145.096
130.901
129.195
129.015
128.678
127.309
124.076
121.195
116.618
45.856
42.228
36.060
32.115
14.022
12.291
Ph
O
N
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.808
7.805
7.797
7.779
7.775
7.748
7.738
7.729
7.720
7.710
7.701
7.284
3.726
3.705
3.692
3.685
3.673
3.652
3.441
3.435
3.410
3.102
2.421
2.403
2.386
2.025
2.006
1.996
1.967
1.503
1.486
1.458
PPh3 Br
N
15.18
1.91
1.00
1.84
1.89
2.33
5.75
f1 (ppm)


135.226
135.197
133.833
133.733
130.708
130.583
118.205
117.347
51.978
44.157
27.110
23.061
20.138
18.873
PPh3 Br
N
f1 (ppm)

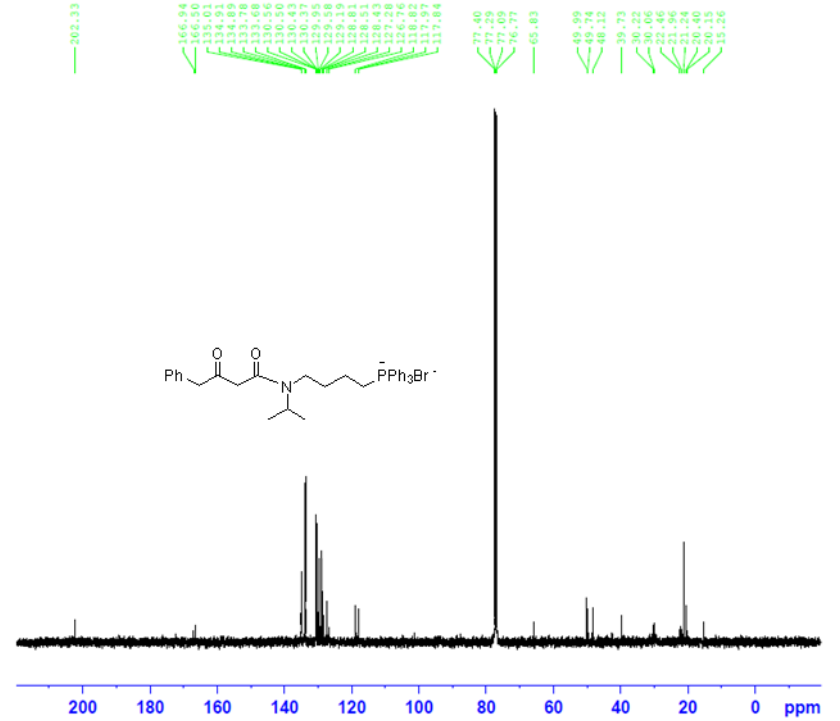
KCD-II-76 H



BRUKER

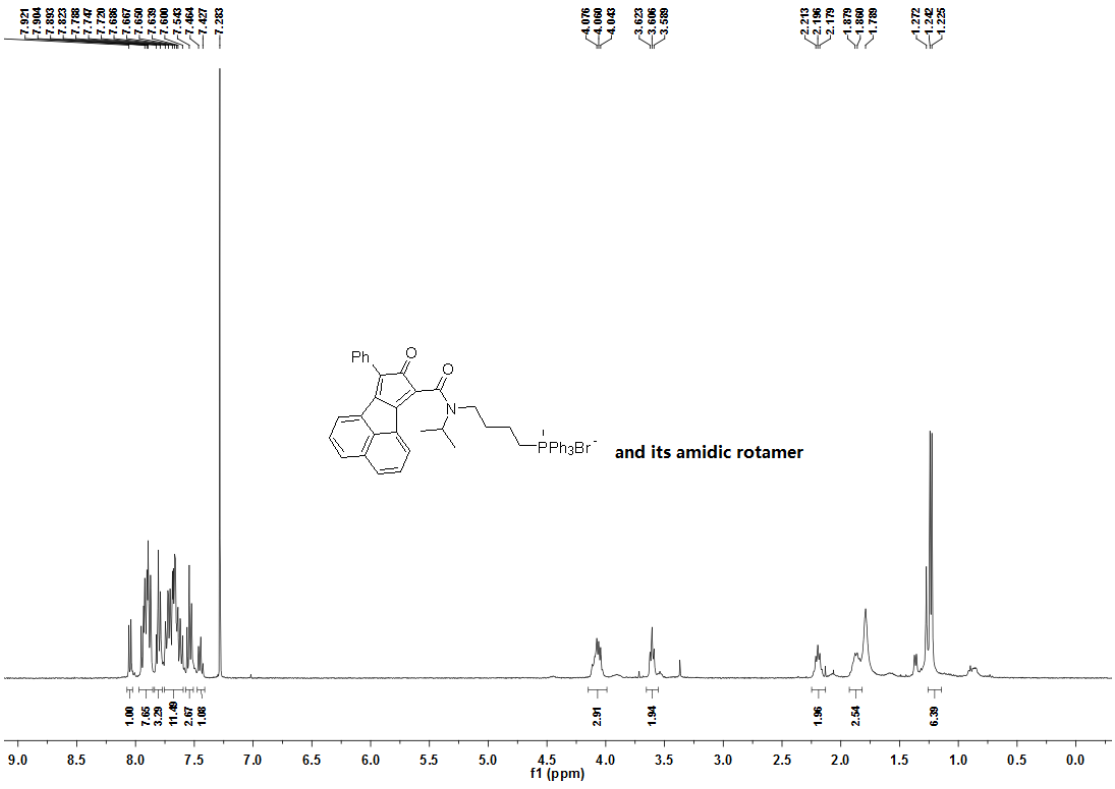
```
Current Data Parameters
NAME          KCD-II-76 H
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_              20160829
Time                 13.56
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              8012.820 Hz
FIDRES           0.122266 Hz
AQ               4.0894465 sec
RG                   90.5
DW                62.400 usec
DE                 6.50 usec
TE                296.5 K
D1             1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          400.1424710 MHz
NUC1                   1H
P1                  13.55 usec
PLW1          16.00000000 W

F2 - Processing parameters
SI                  65536
SF            400.1400093 MHz
WDW                    EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

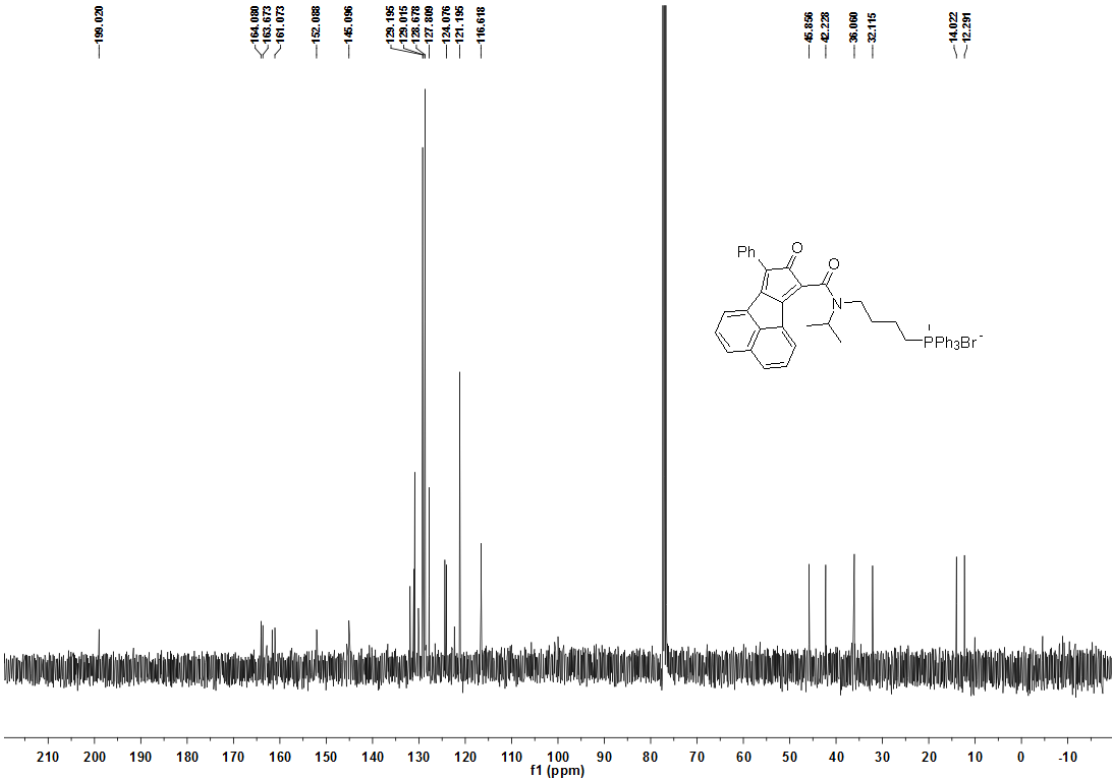
KCD-II-76 C



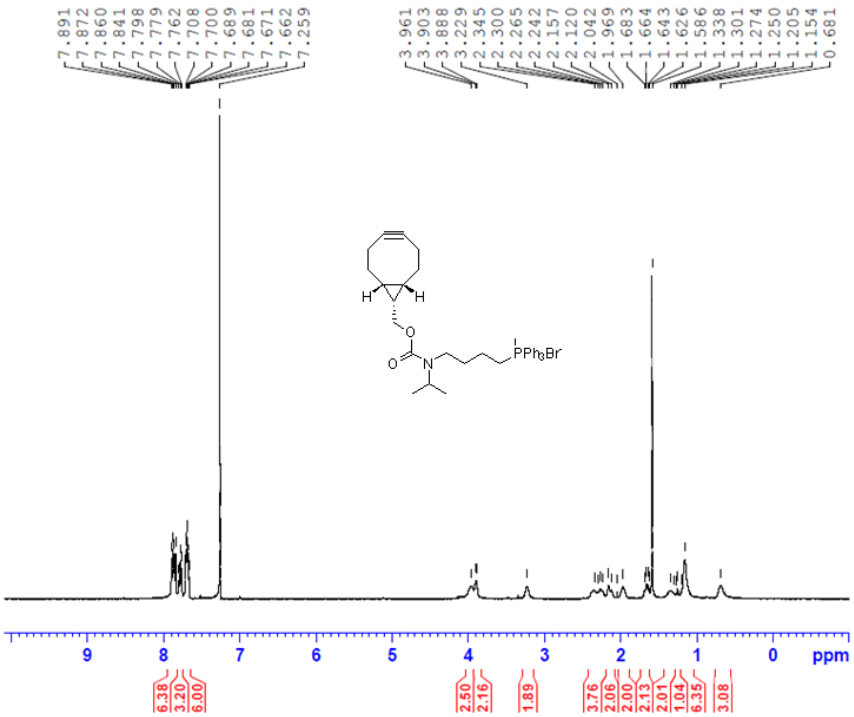
BRUKER

```
Current Data Parameters
NAME          KCD-II-76 C
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_              20160829
Time                 13.48
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                    419
DS                      4
SWH             24038.461 Hz
FIDRES           0.366798 Hz
AQ               1.3631488 sec
RG                    203
DW                20.800 usec
DE                 6.50 usec
TE                297.4 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          100.6253441 MHz
NUC1                   13C
P1                  9.25 usec
PLW1          62.00000000 W

======== CHANNEL f2 ========
SFO2          400.1416006 MHz
NUC2                   1H
CPDPRG[2           waltz16
PCPD2               90.00 usec
PLW2          16.00000000 W
PLW12          0.36267000 W
PLW13          0.29376000 W

F2 - Processing parameters
SI                  32768
SF            100.6152830 MHz
WDW                    EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```


7.921
7.904
7.883
7.823
7.788
7.747
7.730
7.688
7.667
7.650
7.639
7.600
7.543
7.464
7.427
7.283
4.076
4.060
4.043
3.623
3.606
3.589
2.213
2.196
2.179
1.879
1.860
1.789
1.272
1.242
1.225
Ph
O
O
N
PPh3Br-
and its amidic rotamer
1.00
7.65
3.29
11.49
2.67
1.08
2.91
1.94
1.96
2.54
6.39
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

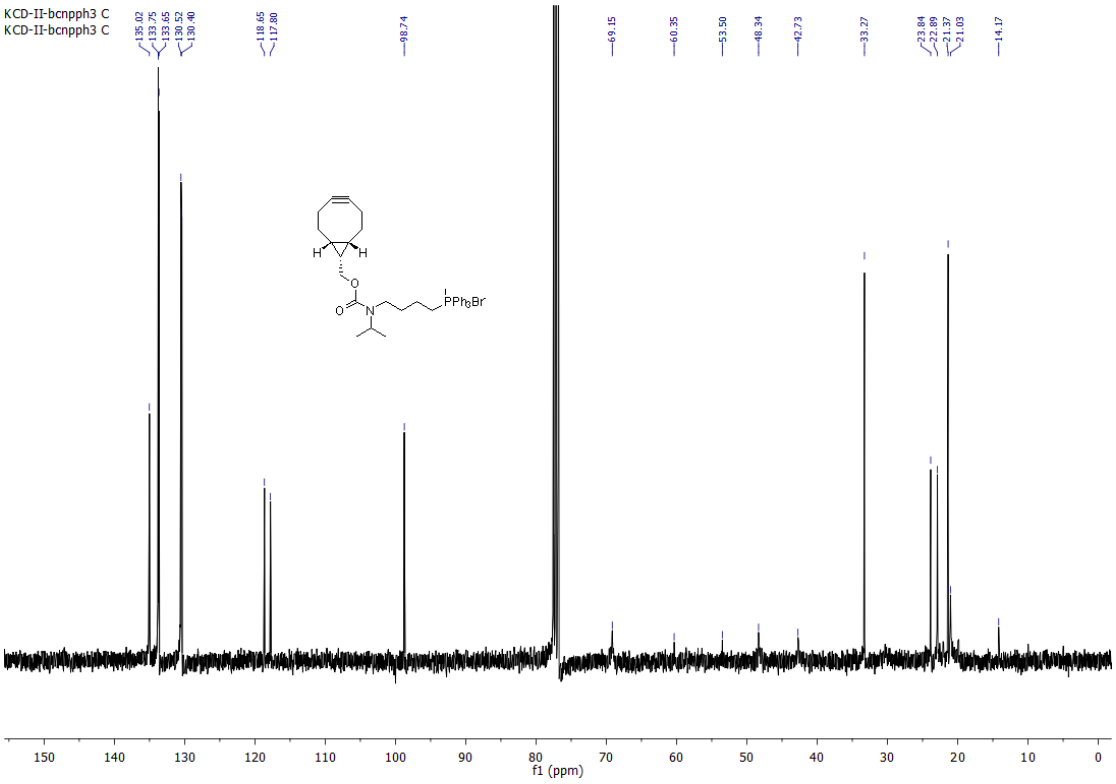

199.020
164.080
163.673
161.073
152.088
145.096
129.195
129.015
128.678
127.389
124.076
121.195
116.618
45.856
42.229
36.060
32.115
14.022
12.291
Ph
O
O
N
PPh3Br-
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

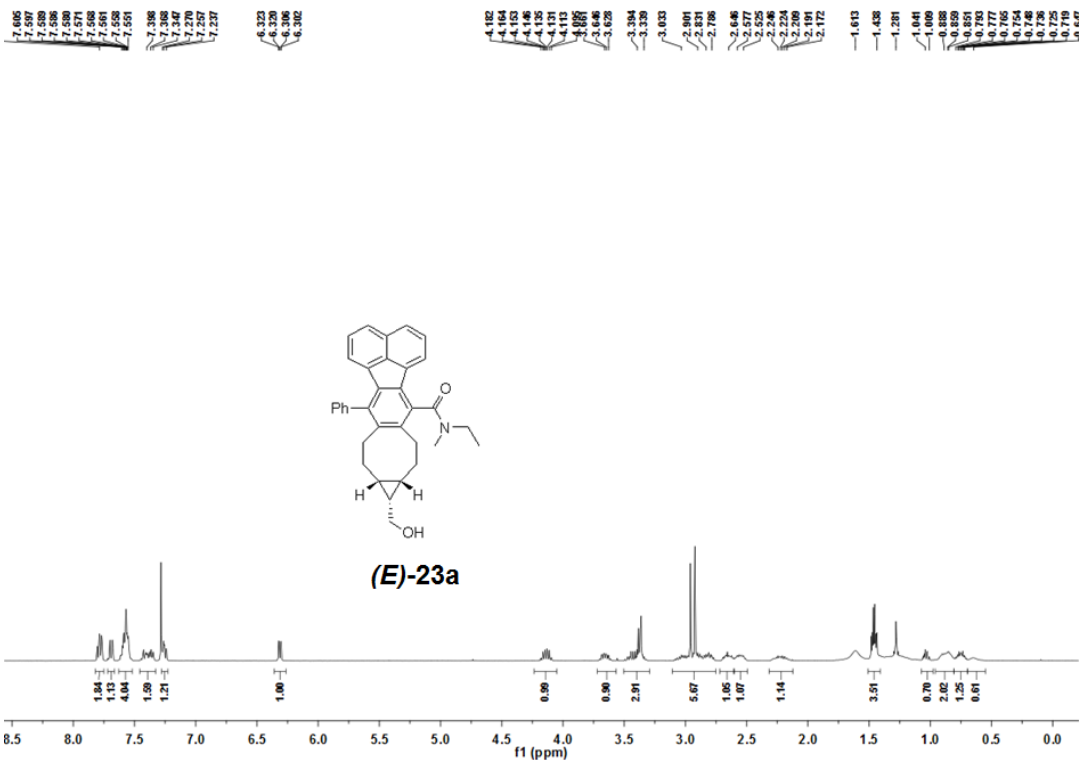
KCD-II-bcnpph3 Ha

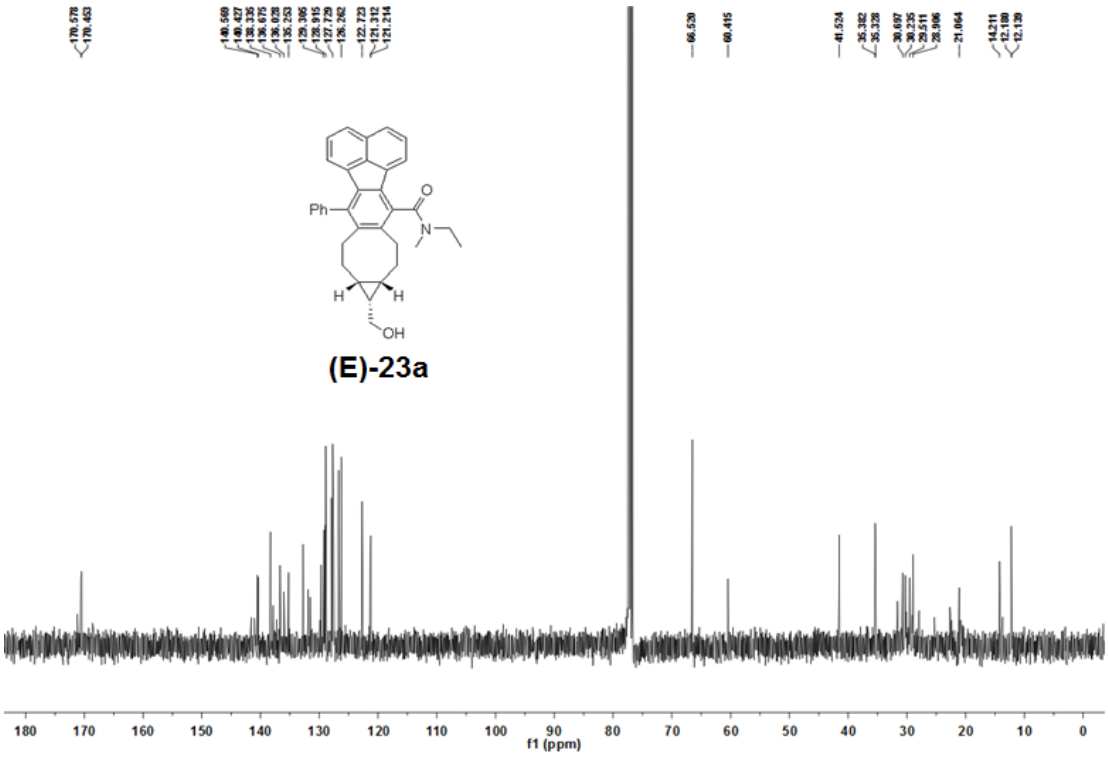


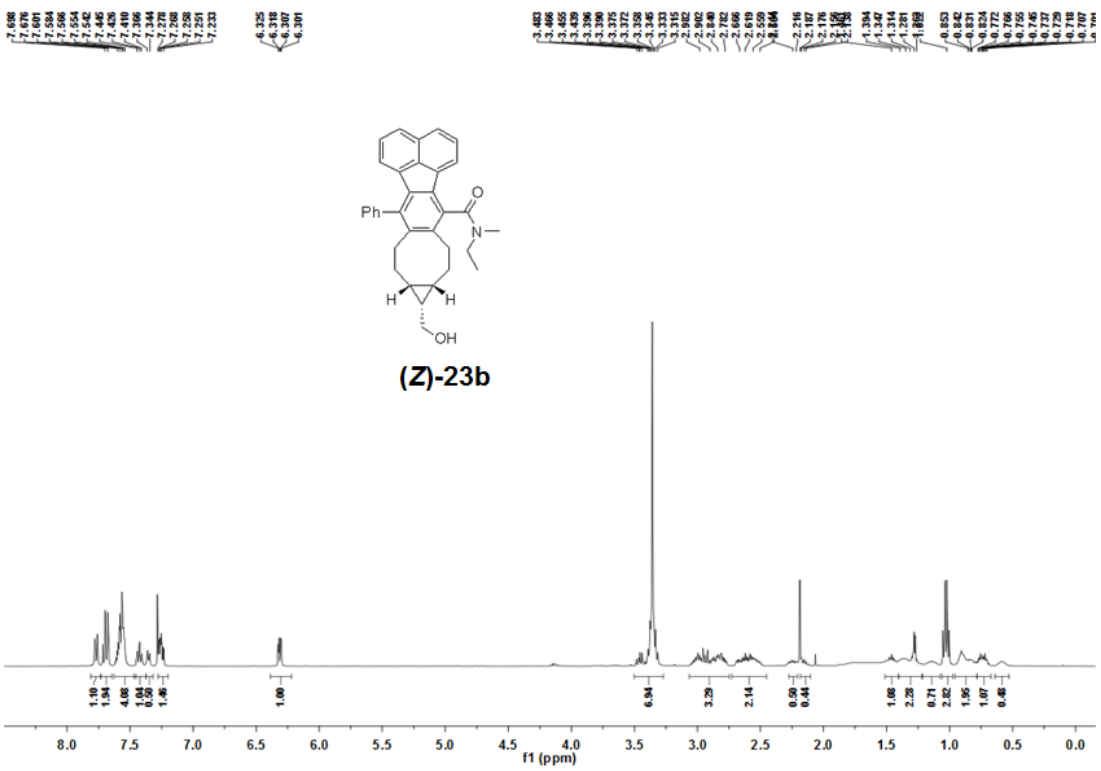
BRUKER

```
Current Data Parameters
NAME      KCD-II-bcnpph3 Ha
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20160823
Time_              16.26
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH              8012.820 Hz
FIDRES           0.122266 Hz
AQ               4.0894465 sec
RG                   203
DW                62.400 usec
DE                 6.50 usec
TE                296.3 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.1424710 MHz
NUC1                   1H
P1                 13.55 usec
PLW1         16.00000000 W

F2 - Processing parameters
SI                 65536
SF           400.1400097 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

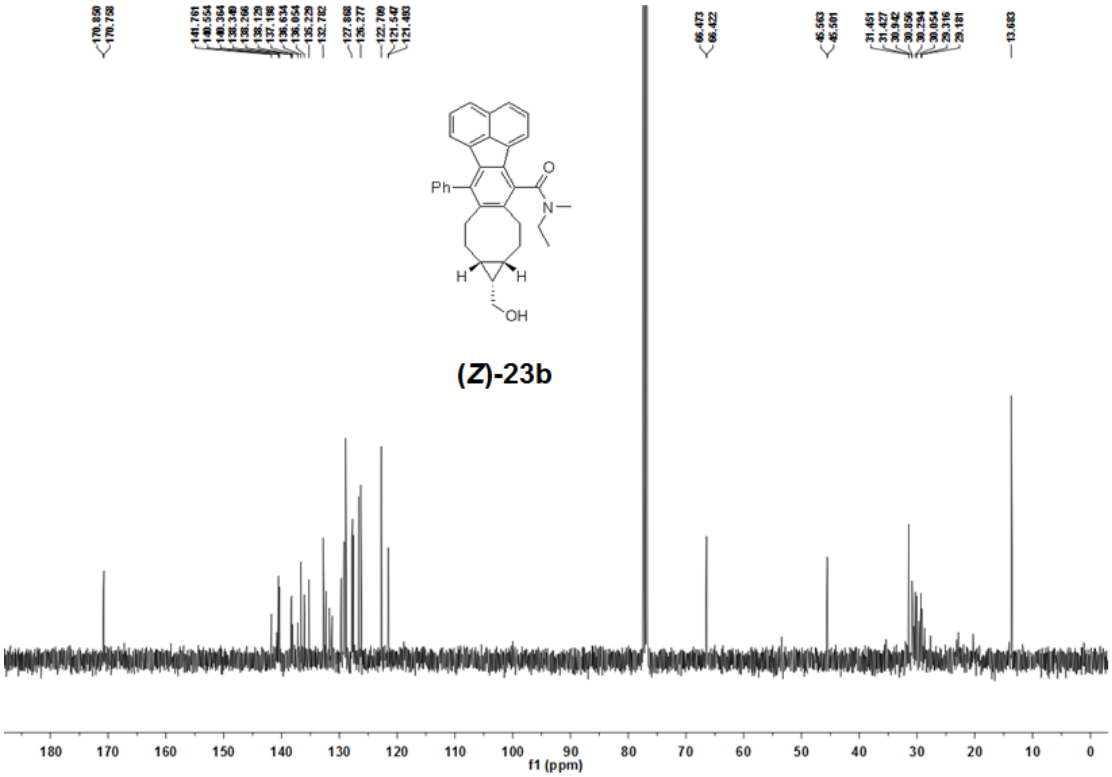



Ph
O
N
H
H
OH
(E)-23a
f1 (ppm)


Ph
O
N
H
H
OH
(E)-23a
f1 (ppm)



(*Z*)-23b



(*Z*)-23b