Supplemental Information for

A Real-Time, Click Chemistry Imaging Approach Reveals Stimulus-Specific Subcellular Locations of Phospholipase D Activity

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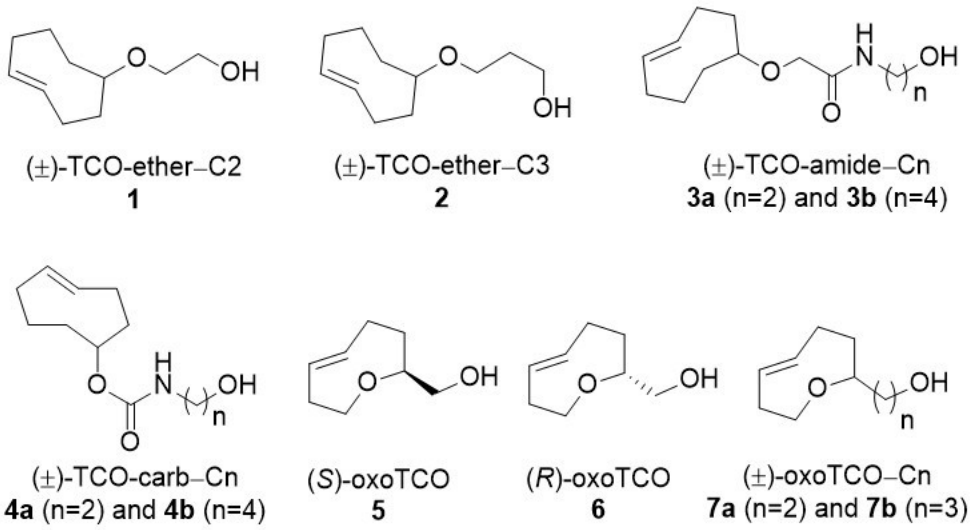


Figure S1. Structures of TCO and oxoTCO alcohols used in this study. Note: All trans-cyclooctene (TCO) and trans-5-oxocene (oxoTCO) alcohols were prepared as a mixture of equatorial and axial diastereomers. With the exception of the enantiomerically pure **5** and **6**, all compounds were isolated as racemates (±).

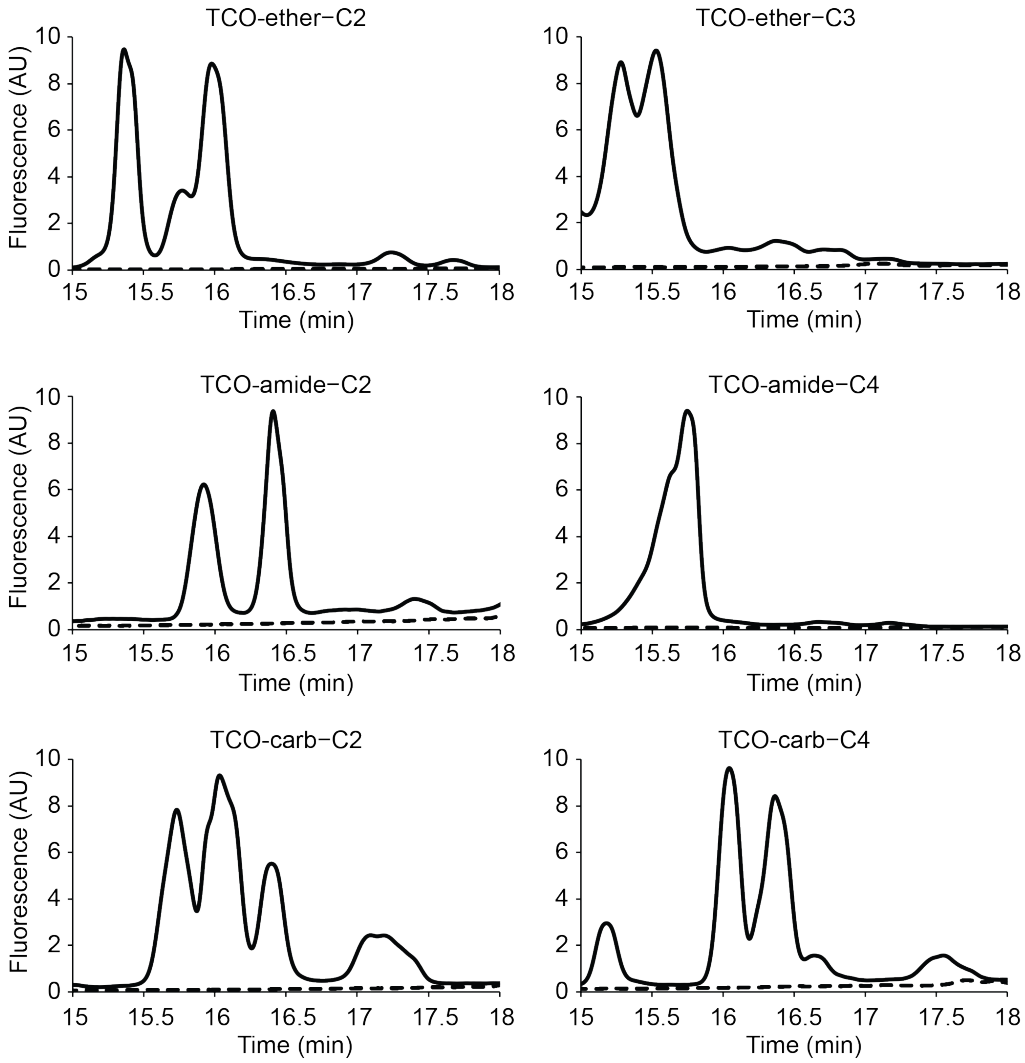


Figure S2. Carbocyclic TCO alcohols are PLD transphosphatidylation substrates *in vitro*. *In vitro* transphosphatidylation reactions catalyzed by PLD from *Streptomyces sp. PMF* were carried out with dioleoylphosphatidylcholine (DOPC) and either the indicated TCO alcohol (solid line) or no alcohol (dashed line). After a 90 min reaction at 30 ºC, lipids were extracted, dried under a stream of nitrogen, and dissolved in 100 μL of a 2:1 $CHCl_3$:MeOH mixture containing BODIPY-alkyl-Tz (25 μM) and the IEDDA reaction was performed for 5 min at 37 ºC. The reaction mixture was then analyzed by fluorescence-coupled HPLC, with fluorescence reported in arbitrary units (AU). We note the appearance of multiple peaks, which is due to a combination of TCO isomers (each alcohol is a racemic mixture of both axial and equatorial diastereomers) and the presence of several potential isomeric dihydropyridazine and/or pyridazine products of the IEDDA reaction.

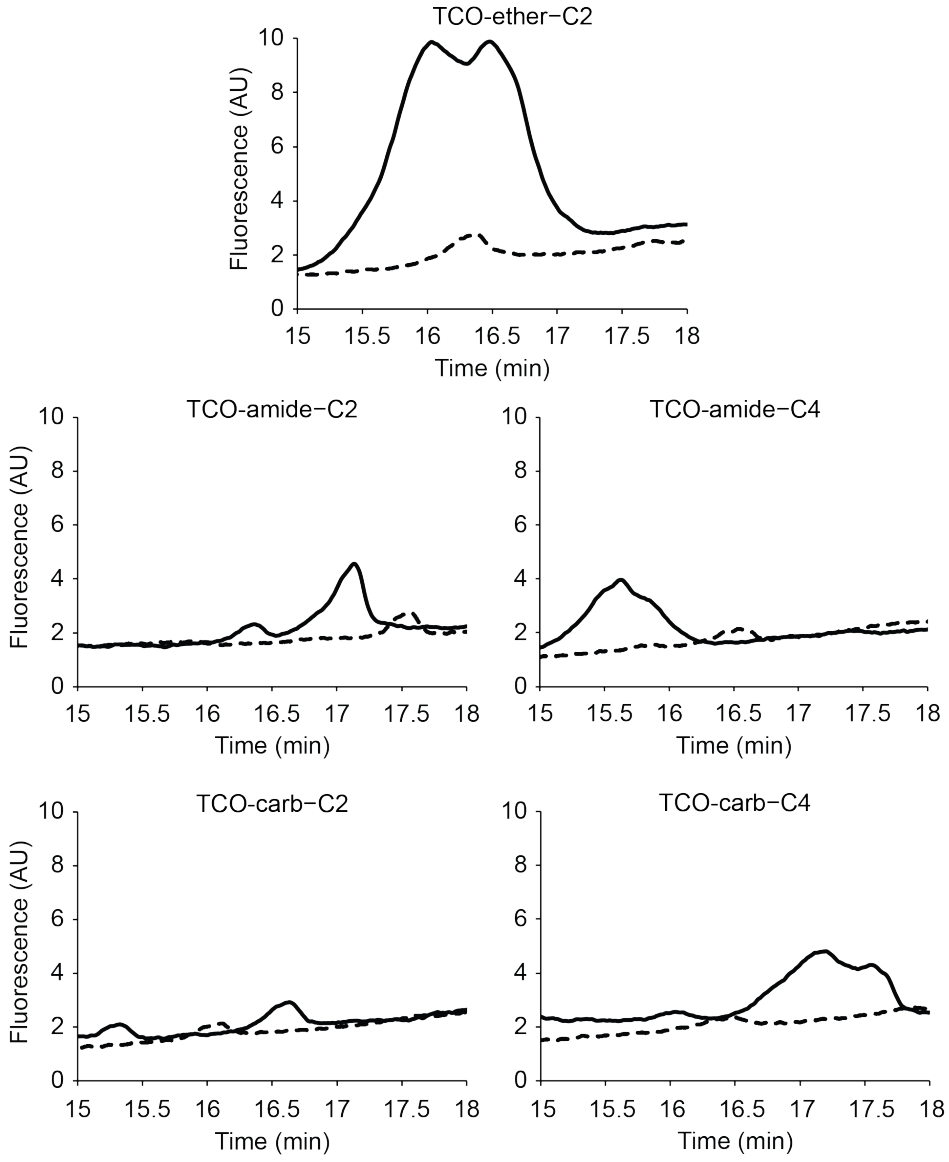


Figure S3. Carboxylic TCO alcohols are transphosphatidylation substrates for endogenous human PLDs. HeLa cells were incubated with PLDi (FIPI, dashed lines) or DMSO (solid lines) for 30 min and then treated with indicated TCO alcohols (7.5 mM for TCO-ether–C2 and 10 mM for all others) for 20 min, followed by PMA stimulation in the continued presence of the TCO alcohol for an additional 20 min. Following cell lysis, lipids extracts were generated, reacted with BODIPY-alkyl-Tz (25 μM) for 5 min at 37 ºC, and analyzed by fluorescence-coupled HPLC, with fluorescence intensity indicated in arbitrary units (AU). As in Figure S2, we note the appearance of multiple peaks, which is due to a combination of TCO isomers (each alcohol is a racemic mixture of both axial and equatorial diastereomers) and the presence of several potential isomeric dihydropyridazine and/or pyridazine products of the IEDDA reaction.

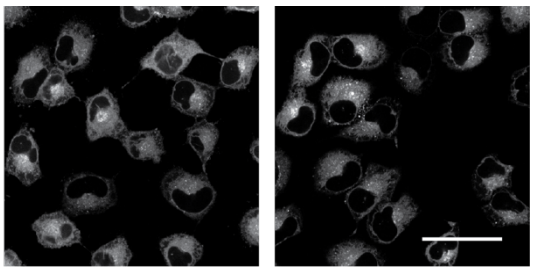


Figure S4. A representative carbocyclic TCO alcohol (TCO-ether−C2) is not a suitable probe for IMPACT due to high background labeling. HeLa cells were incubated with or without PLDi (FIPI) for 30 min and then treated with PMA for 20 min followed by treatment with TCO-ether–C2 (7.5 mM, 20 min) in the continued presence of PMA. After a 1 min rinse with PBS, cells were incubated with BODIPY-alkyl-Tz for 1 min, rinsed for 10 min at 37 ºC, and imaged by confocal microscopy. Scale bar: 50 μm.

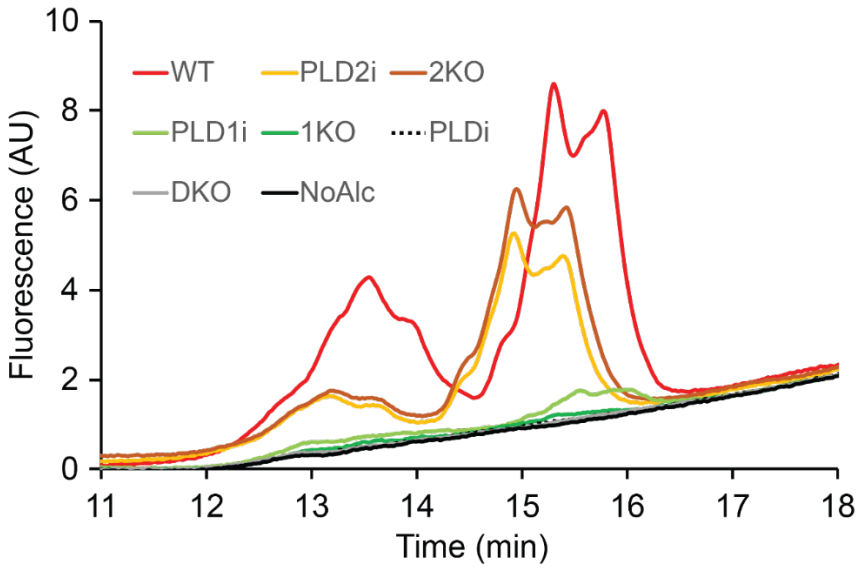


Figure S5. HPLC analysis of transphosphatidylation products of (*S*)-oxoTCO–C1 from HeLa cells. Wild-type HeLa cells were incubated with the indicated PLD inhibitor (PLDi, FIPI, dotted line; PLD1i, VU0359595, light green line; or PLD2i, VU0364739, yellow line) or DMSO (WT, wild-type, red line; 1KO, PLD1 knockout, dark green line; 2KO, PLD2 knockout, tan line; DKO, PLD1/2 double knockout, gray line) for 30 min. All cells were then stimulated with PMA for 20 min and then treated with (*S*)-oxoTCO–C1 (3 mM) for 20 min in the continued presence of PMA. Following cell lysis, lipids extracts were generated, reacted with Tz–BODIPY (25 μM) for 5 min at 37 ºC, and analyzed by fluorescence-coupled HPLC, with fluorescence intensity indicated in arbitrary units (AU). As in Figure S2, we note the appearance of multiple peaks, which is due to a combination of TCO isomers ((*S*)-oxoTCO–C1 is an enantiomerically pure mixture of both the axial and equatorial diastereomers) and the presence of several potential isomeric dihydropyridazine and/or pyridazine products of the IEDDA reaction.

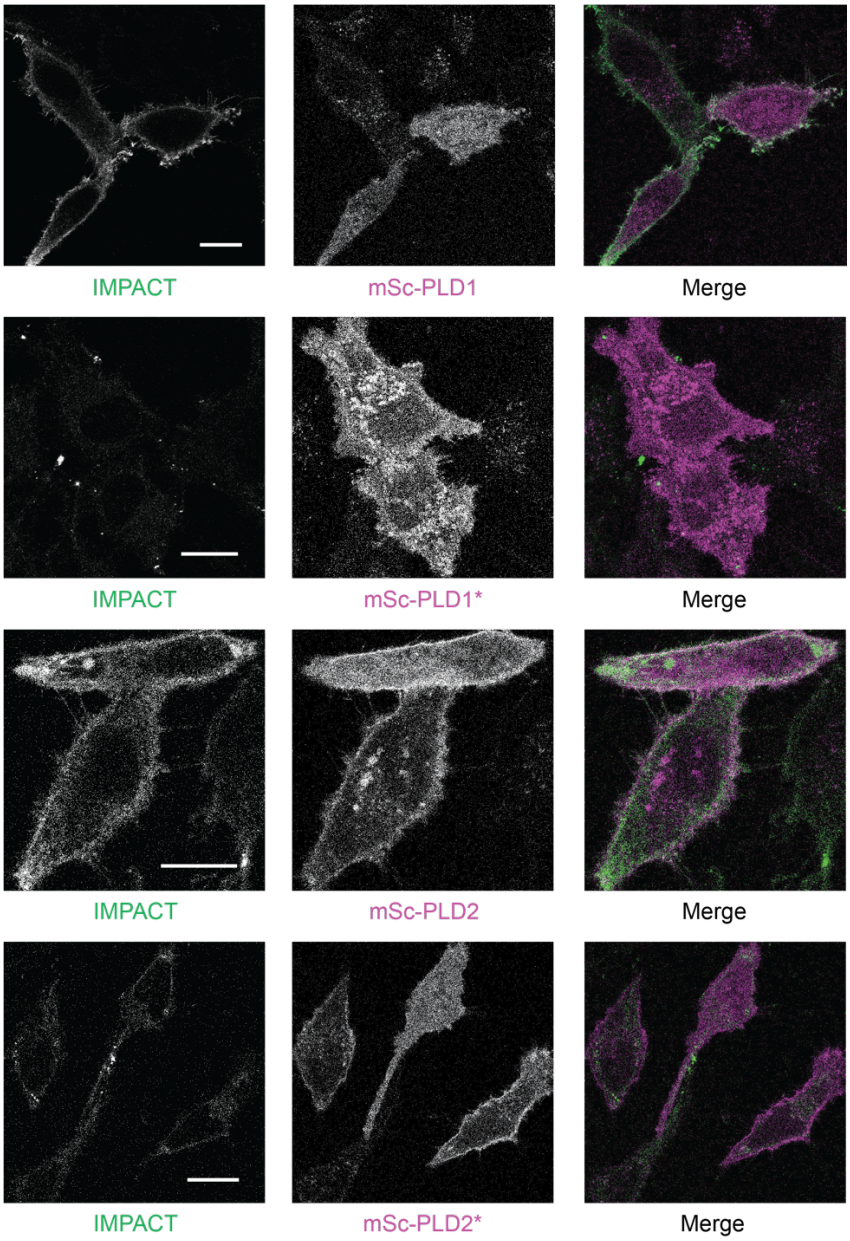


Figure S6. (*S*)-oxoTCO–C1 is a substrate of both human PLD1 and PLD2 in cells. PLD1/2 DKO HeLa cells were transfected with the indicated mScarlet-i (mSc)-tagged wild-type or catalytic dead (asterisk) variant of human PLD1 or PLD2. One day after transfection, cells were labeled via IMPACT using (*S*)-oxoTCO–C1. Briefly, cells were incubated with both (*S*)-oxoTCO–C1 (3 mM) and PMA for 5 min, rinsed for 1 min, and then Tz-BODIPY (1 μM) was added and live-cell, time-lapse confocal imaging was performed. Shown is the 9 s timepoint of the time-lapse movie. In the merged images, IMPACT is green, PLD is magenta, and colocalization appears as white. Note the appearance of IMPACT fluorescence at the plasma membrane, the site of PMA-stimulated PLD1 activity and constitutive PLD2 activity, in cells expressing active but not catalytic-dead PLD1 and PLD2, respectively. Scale bars: 20 μm.

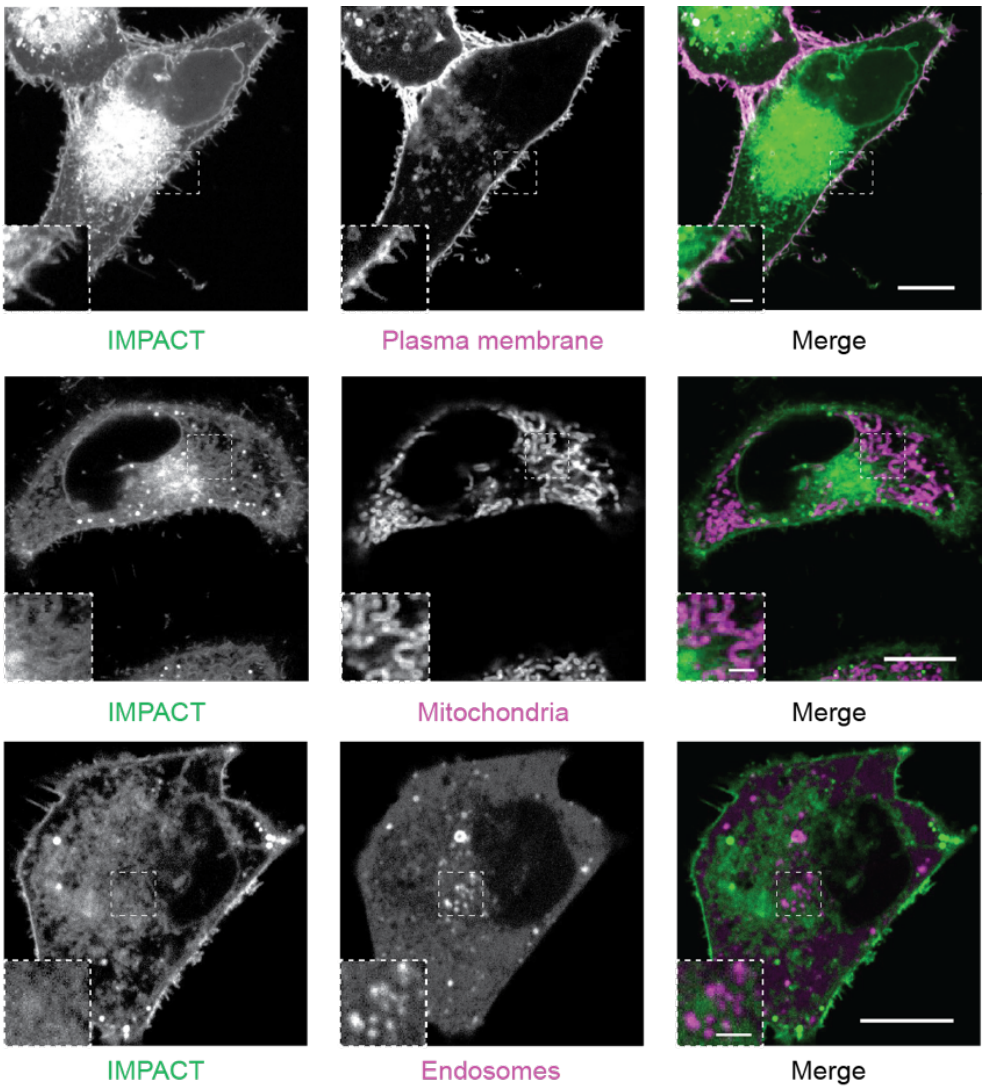


Figure S7. Colocalization of IEDDA-based IMPACT with organelle markers by confocal microscopy. HeLa cells were transfected with markers of various organelles (Plasma membrane: PM-mRFP; Mitochondria: OMP25(transmembrane domain)-mCherry; Endosomes: EEA1-mRFP). One day after transfection, cells were labeled via IMPACT using (*S*)-oxoTCO–C1. Briefly, cells were stimulated with PMA for 20 min and then treated with (*S*)-oxoTCO–C1 (3 mM) for 5 min in the continued presence of PMA, rinsed for 1 min, and then imaged by live-cell confocal microscopy. In the merged images, IMPACT is green, the organelle marker is magenta, and colocalization appears as white. Note partial colocalization under this pseudo-steady state labeling protocol with a plasma membrane marker and minimal colocalization with markers of mitochondria and endosomes. Scale bars: 10 μm for full-size images and 2 μm for zoomed-in regions.

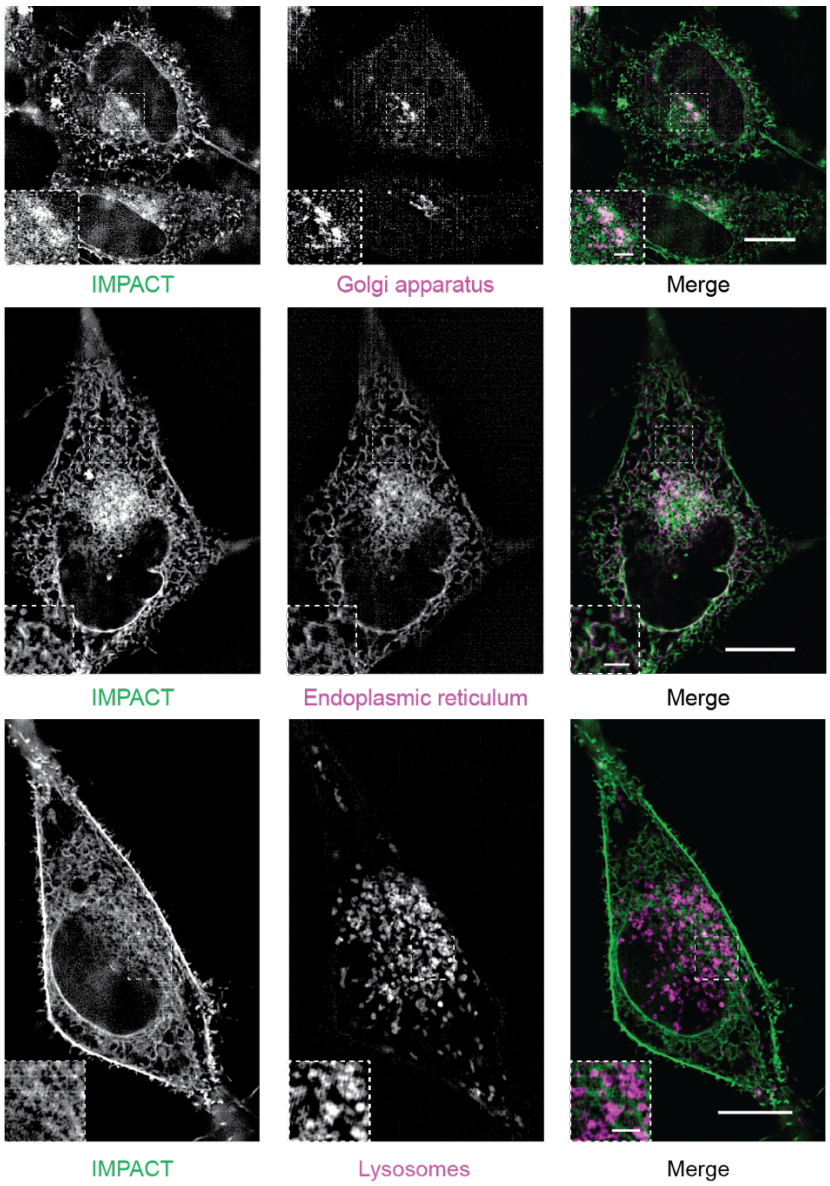


Figure S8. Colocalization of IEDDA-based IMPACT with organelle markers by super-resolution structured illumination microscopy. HeLa cells were transfected with markers of various organelles (Golgi apparatus: mCherry-P4M-SidM; Endoplasmic reticulum: STIM1-mRFP; Lysosomes: LAMP1-mRFP). One day after transfection, cells were labeled via IMPACT using (*S*)-oxoTCO–C1. Briefly, cells were stimulated with PMA for 20 min and then treated with (*S*)-oxoTCO–C1 (3 mM) for 5 min in the continued presence of PMA, rinsed for 1 min, and then imaged by live-cell super-resolution structured illumination microscopy. In the merged images, IMPACT is green, the organelle marker is magenta, and colocalization appears as white. Note partial colocalization under this pseudo-steady state labeling protocol with markers of the endoplasmic reticulum and Golgi apparatus and minimal colocalization with a marker of lysosomes. Scale bars: 10 μm for full-size images and 2 μm for zoomed-in regions.

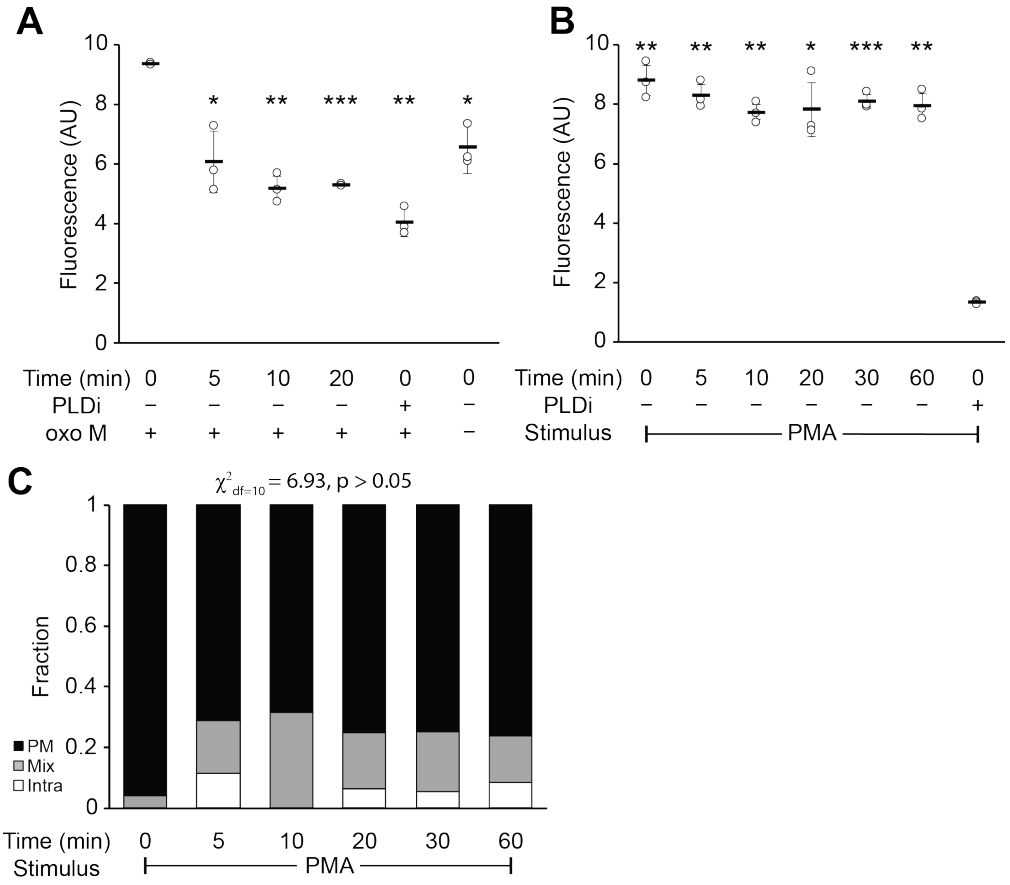


Figure S9. Muscarinic M1 receptor (M1R) stimulation or PMA treatment induces predominantly plasma membrane PLD activity in HeLa cells. (A) M1R stimulation activates PLD enzymes only within the first 5 min of M1R signaling. HeLa cells stably expressing M1R were treated with or without PLDi (FIPI) for 30 min and then treated with the M1R agonist oxotremorine-M (oxo-M) for the indicated amount of time, followed by treatment with (S)-oxoTCO (3 mM) for 5 min in the continued presence of oxo-M. Cells were then rinsed, treated with Tz–BODIPY (0.33 μM) for 1 min, and analyzed by flow cytometry. (B–C) PMA stimulation of cells leads to sustained activity (B) at the plasma membrane (C). (B) HeLa cells were labeled and analyzed as in (A), substituting oxo-M for PMA. (C) HeLa cells were labeled as in (A), except that the IEDDA reaction with Tz–BODIPY was monitored in real-time by confocal microscopy. The 9 s timepoint of the IEDDA reaction was quantified using the PM/Mix/Intra rubric described in Figure 5E–F. For (A–B), mean fluorescence intensity is in arbitrary units (AU), each data point represents a technical replicate from a single experiment (n=3), at least two biological replicate experiments were performed, each giving similar results, and error bars, where indicated, represent standard deviation. Statistical significance was assessed using a one-way ANOVA followed by Games-Howell post-hoc analysis. Asterisks directly above data points (* $p<0.05$, ** $p<0.01$, ***, $p>0.001$) denote statistical significance compared to the first sample (– PLDi, + oxo-M) for (A) and compared to the last sample (+ PLDi) for (B). For (C), data were averaged from at least three experiments. Each bar contains data from n=3–4 biological replicates with n=42–71 total cells. Statistical significance was assessed using a chi-squared test for independence, with the chi-squared value (df = degrees of freedom) and associated p value indicated.

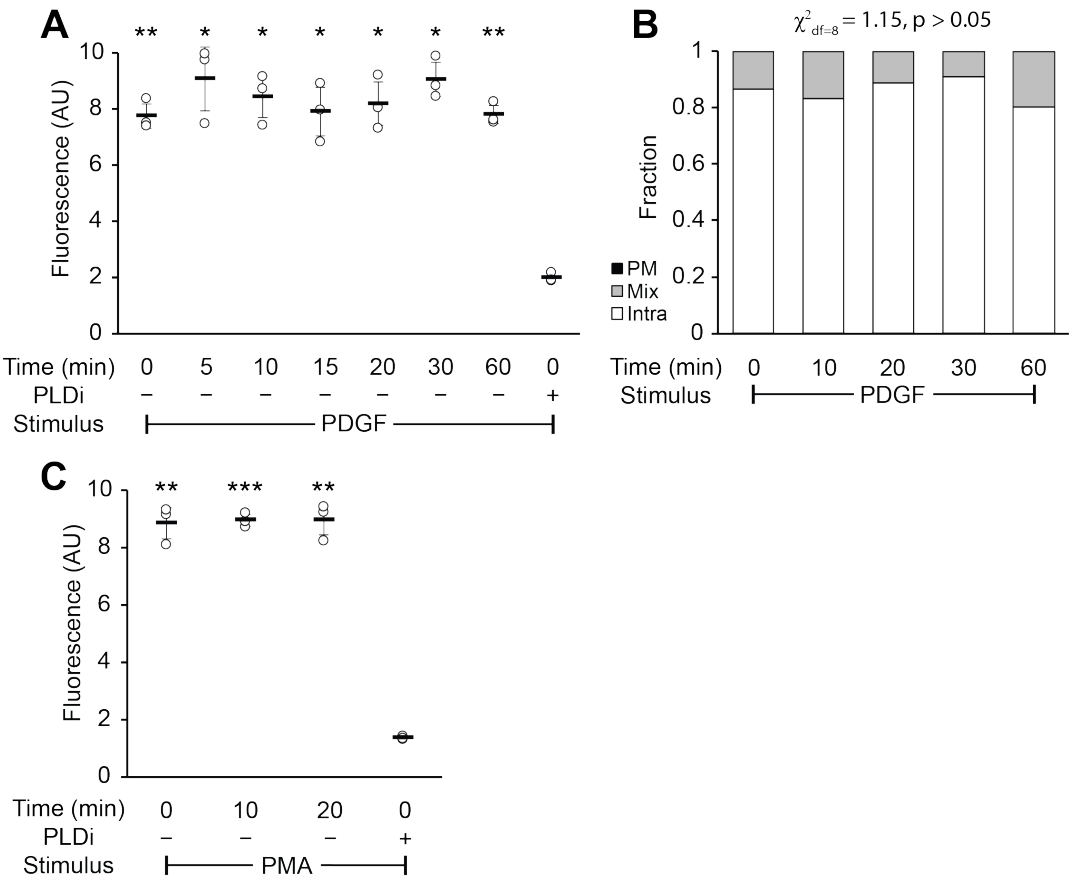
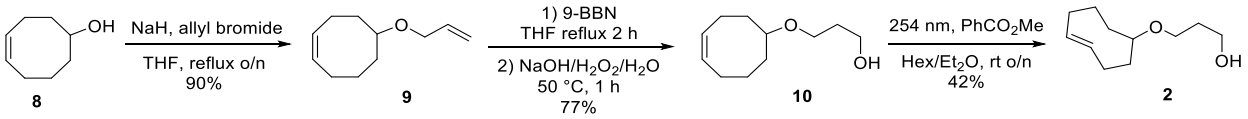


Figure S10. Platelet-derived growth factor (PDGF) receptor stimulation induces intracellular PLD activity in NIH 3T3 cells. (A) PDGF receptor stimulation activates PLD enzymes for at least 60 min of PDGF stimulation. NIH 3T3 cells were treated with or without PLDi (FIPI) for 30 min and then treated with PDGF for the indicated amount of time, followed by treatment with (S)-oxoTCO (3 mM) for 5 min in the continued presence of PDGF. Cells were then rinsed, treated with Tz–BODIPY (0.33 μM) for 1 min, and analyzed by flow cytometry. (B) PDGF stimulation over a 60 min time course leads to PLD activity at intracellular membranes. NIH 3T3 cells were labeled as in (A), except that the IEDDA reaction with Tz–BODIPY was monitored in real-time by confocal microscopy. The 9 s timepoint of the IEDDA reaction was quantified using the PM/Mix/Intra rubric described in Figure 5E–F. (C) NIH 3T3 cells exhibit sustained PLD activity over at least 20 min of PMA stimulation. Cells were labeled and analyzed as in (A), substituting PDGF for PMA. For (A) and (C), mean fluorescence intensity is in arbitrary units (AU), each data point represents a technical replicate from a single experiment (n=3), at least two biological replicate experiments were performed, each giving similar results, and error bars represent standard deviation. Statistical significance was assessed using a one-way ANOVA followed by Games-Howell post-hoc analysis. Asterisks directly above data points (* $p<0.05$, ** $p<0.01$, ***, $p>0.001$) denote statistical significance compared to the last sample in each set (+ PLDi). For (B), data were averaged from at least three experiments. Each bar contains data from n=3–4 biological replicates with n=57–78 total cells. Statistical significance was assessed using a chi-squared test for independence, with the chi-squared value (df = degrees of freedom) and associated p value indicated.

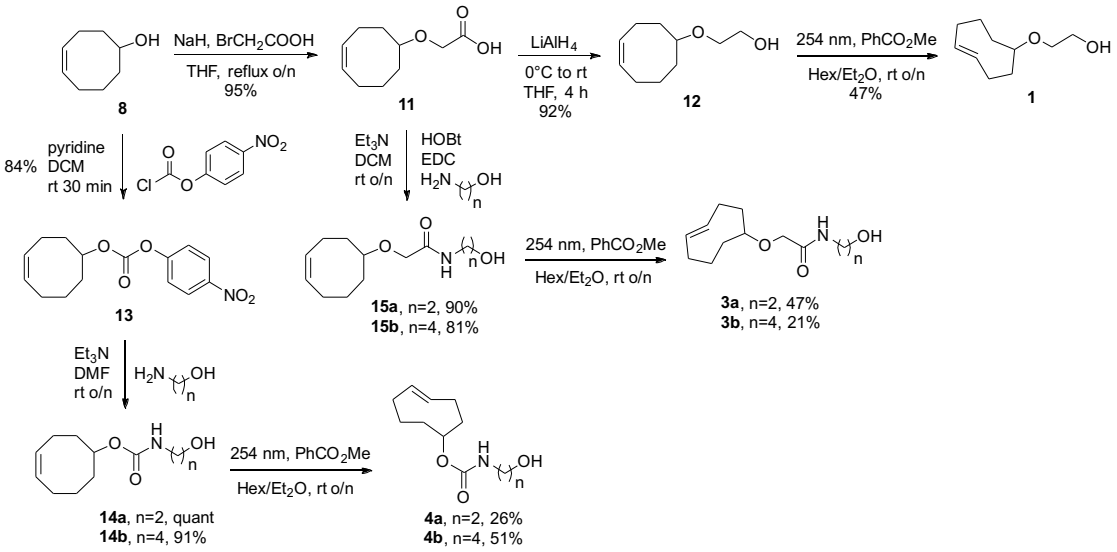
Lipid species	Calcd	Found
36:1	1000.711	1000.702
36:2	998.6957	998.6956
36:3	996.6800	996.6712
36:4	994.6643	994.6646
36:5	992.6486	992.6522
34:0	974.6958	974.6888
34:1	972.6801	972.6791
34:2	970.6644	970.6671
32:0	946.6645	946.6674
32:1	944.6488	944.6512
32:2	942.6331	942.6388
30:0	918.6332	918.635
30:1	916.6175	916.623

Table S1. Mass spectrometry analysis of functionalized phosphatidyl alcohols isolated from cells treated with (*S*)-oxoTCO–C1. HeLa cells were incubated with PMA and (*S*)-oxoTCO–C1 (5 mM) for 60 min. Cells were rinsed for 10 min before lipid extraction. Lipid extracts were derivatized by IEDDA with methyltetrazine-amine (Click Chemistry Tools, CAS # 1345955-28-3), followed by LC-TOF-MS analysis. Indicated are calculated (Calcd) and experimentally determined (Found) masses of the functionalized phosphatidyl alcohol products, with the lipid species classified by total number of carbons:number of double bonds in the fatty acyl tails.

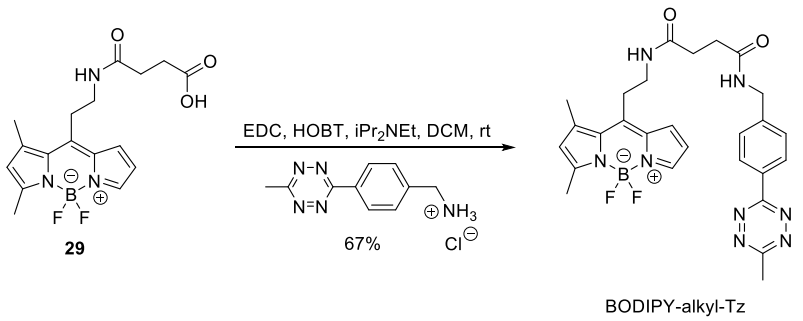
Movie S1. Time-lapse imaging of labeling and trafficking of IMPACT-derived lipids. HeLa cells were labeled via RT-IMPACT with (*S*)-oxoTCO–C1 and Tz–BODIPY as described in the legend to Figure 5B, and time-lapse imaging of the IEDDA reaction with Tz–BODIPY was performed by confocal microscopy, with images acquired every 3 s. Tz–BODIPY was added in between the first and second frames of the movie. This movie shows a population of cells, and the top and middle rows of images in Figure 5B show a representative single cell from this movie. Scale bar: 20 μm.



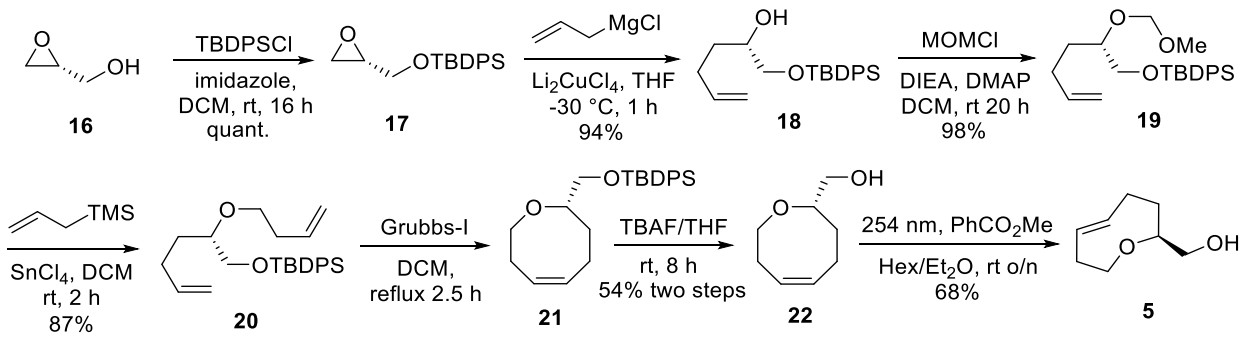
Scheme S1. Synthesis of (±)-TCO-ether–C3.



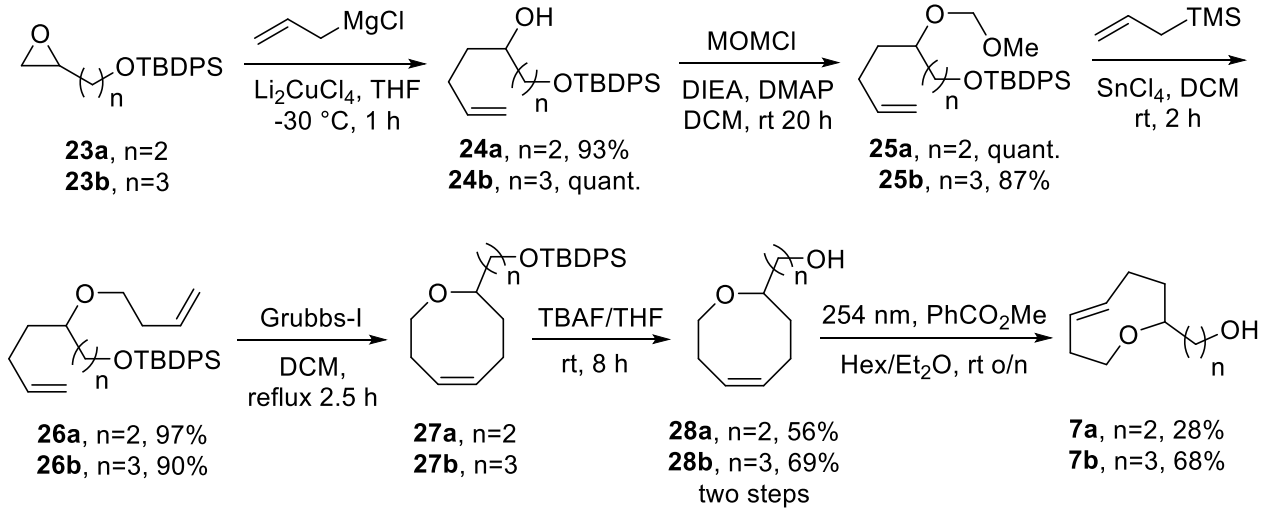
Scheme S2. Synthesis of (±)-TCO-ether–C2, (±)-TCO-amide–Cn and (±)-TCO-carb–Cn.



Scheme S3. Synthesis of BODIPY-alkyl-Tz.



Scheme S4. Synthesis of (*S*)-oxoTCO–C1.



Scheme S5. Synthesis of (±)-oxoTCO–C2 and (±)-oxoTCO–C3.

Synthetic Procedures

General synthetic methods

All reactions used ACS-grade solvents and reagents from commercial sources without further purifications. Silica gel (60 Å) was purchased from Silicycle. For ^{13}C NMR of final oxoTCO products, multiplicities were distinguished using an APT pulse sequence: typical methylene and quaternary carbons appear 'up' (u); methine and methyl carbons 'down' (dn).

General procedure for cis-to-trans photoisomerization of cyclooctenes

This general procedure for flow chemistry-based photoisomerization was adapted from work by Lambert *et al* (1). Reactions were done in a quartz flask (Chemglass) in close proximity to a Honeywell Ultraviolet Air Treatment System UV lamp (Model # RUVLAMP1, 18W bulb # UC100A 1005, 254 nm) in place of the photoreactor. All other parts were purchased and connected as described in Lambert *et al* (1). A Biotage SNAP cartridge (KP-Sil 10g, Part # FSK0-1107-0010) was used to host regular silica gel and silver-impregnated silica gel. For every 1.4 mmol cyclooctene**,** the cartridge that contained a bed of regular silica gel (~1 in.) was topped with 5.4 g of silver-impregnated silica (2.3 eq silver). Any remaining space in the cartridge was filled with cotton balls. Methyl benzoate (2 eq) was added to a solution of cis-cyclooctene in the specified percentage of diethyl ether in hexanes within the quartz reaction flask. The final concentration of cyclooctene was 0.01–0.02 M. The solution was equilibrated for 10 min through the continuous flow system at a 100 mL/min flow rate. The solution in the quartz flask was then irradiated (254 nm) under continuous flow conditions (100 mL/min) for indicated period of time (typically 16–24 h). The SNAP cartridge was flushed with ~500 mL of 1:1 ether/hexanes and then dried with compressed air. The dried silica gel was transferred to an Erlenmeyer flask. Concentrated aqueous ammonium hydroxide (100 mL) and dichloromethane (100 mL) were sequentially added to the flask. The resulting biphasic mixture was stirred vigorously for 15 min, and then filtered. The filter cake was washed with additional DCM (25 mL) and ammonium hydroxide (25 mL). This rinse was repeated three times. The combined filtrates were partitioned, and the aqueous phase was extracted with DCM (3 x 25 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography to yield the desired trans-cyclooctene (TCO or oxoTCO) as an inseparable mixture of axial and equatorial diastereomers.

Synthesis of new compounds

Cyclooct-4-enol (**8**) is reported previously (2).

Allyl ether **9**. Sodium hydride (0.476 g, 60 wt%, 11.9 mmol) was added into a flame-dried 100 mL two-neck round-bottom flask filled with nitrogen. Hexanes (7 mL) was added to thoroughly rinse the hydride then decanted to give white-colored solid. THF (9 mL) was added to the flask, followed by secondary alcohol **8** (0.5 g, 3.96 mmol) dissolved in 6 mL THF. The resulting suspension was heated to reflux for 1 h and then cooled to room temperature before addition of allyl bromide (0.34 mL, 3.96 mmol) in THF (15 mL). The mixture was heated to reflux overnight and then cooled to

room temperature. THF was removed under reduced pressure, and water was slowly added to the ice-cooled flask until bubbling ceased. The mixture was then acidified to pH 3 with 3 M HCl and extracted with ether (3 × 15 mL). The ether layers were combined and dried with $MgSO_4$ before rotary evaporation. The crude material was obtained as a yellow oil (650 mg, 99%) and used for next step without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.86-5.94 (ddt, J = 16.3 Hz, 10.6 Hz, 5.4 Hz, 1H), 5.69-5.56 (m, 2H), 5.28-5.24 (m, 1H), 5.15-5.13 (m, 1H), 3.99-3.89 (m, 2H), 3.41-3.37 (m, 1H), 2.39-2.31 (m, 1H), 2.20-2.09 (m, 2H), 2.08-2.01 (m, 1H), 1.98-1.91 (m, 1H), 1.85-1.65 (m, 3H), 1.55-1.48 (m, 1H), 1.44-1.35 (m, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 135.65, 130.22, 129.60, 116.47, 80.04, 69.38, 34.35, 33.52, 25.96, 25.74, 22.83. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{11}H_{19}O^+$ 167.1430; found 167.1425.

Ether alcohol **10**. Compound **9** (0.34 g, 2.04 mmol) was added to an oven-dried 100 mL 2-neck round-bottom flask and dissolved in THF (6 mL) under a nitrogen atmosphere. A solution of 9-BBN (0.5 M in THF, 4.1 mL, 2.04 mmol) was added slowly. The resulting mixture was heated to reflux for 2 h before cooling to room temperature, when NaOH (aq, 3 M, 0.6 mL) and H_2O_2 (30%, 0.6 mL) was added. The mixture was heated to 50 ºC for 1 h and then cooled to room temperature. Excess K_2CO_3 (s) was added to saturate the aqueous layer, and the organic layer was separated. The aqueous layer was extracted with pentane (3 × 15 mL). The organic layers were combined, and solvents were removed under reduced pressure. The crude was purified by flash column chromatography (50% ether in hexanes) to give 170 mg of compound **10** (77% with 141 mg compound **9** recovered) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.67-5.51 (m, 2H), 3.70-3.68 (m, 2H), 3.59-3.55 (m, 1H), 3.50-3.46 (m, 1H), 3.28-3.32 (m, 1H), 2.95 (br, s, 1H), 2.33-2.25 (m, 1H), 2.15-2.04 (m, 2H), 2.03-1.96 (m, 1H), 1.91-1.85 (m, 1H), 1.76-1.61 (m, 5H), 1.48-1.30 (m, 2H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 130.01, 129.45, 80.97, 67.65, 62.10, 34.18, 33.23, 32.26, 25.81, 25.61, 22.60. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{11}H_{21}O_2^+$ 185.1536; found 185.1538.

(±)-TCO-ether–C3 (**2**). The general photoisomerization procedure described above was used. 0.77 g of compound **10** in 1:1 ether/hexanes yielded compound **2** (0.36 g, 47%) as a ~2.3:1 mixture of equatorial and axial isomers after 19 h of irradiation. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.57-5.31 (m, 2H), 4.68 (br, s, 1H), 3.79-3.76 and 3.71-3.68 (q, J = 5.5 Hz, 2H), 3.61-3.39 (m, 3H), 2.93-2.76 (m, 2H), 2.37-2.26 (m, 1H), 2.25-2.11 (m, 2H), 2.05-1.85 (m, 2H), 1.85-1.70 (m, 4H), 1.51-1.42 (m, 1H) ^{13}C NMR ($CDCl_3$, 126 MHz) Peaks due to major diastereomer: δ 135.29, 132.22, 86.17, 67.51, 62.16, 40.94, 37.90, 34.55, 33.04, 32.23, 31.77. Peaks due to minor diastereomer: δ 135.23, 131.06, 75.41, 68.44, 62.42, 40.20, 34.56, 32.76, 32.20, 29.92, 27.84. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{11}H_{21}O_2^+$ 185.1536; found 185.1532.

Ether acid **11** is reported previously (3).

Ether alcohol **12**. Compound **11** (0.37 g, 2.01 mmol) was dissolved in THF (3 mL) in a flame-dried 50 mL 2-neck round bottom flask under a nitrogen atmosphere. The solution was cooled on ice before lithium aluminum hydride (1 M in THF, 6.03 mL, 6.03 mmol) was added slowly. The mixture was heated to reflux for 4 h before cooling to 0 ºC. Water (0.229 mL), NaOH (aq, 15 wt%, 0.229 mL) and water (0.687 mL) were added sequentially and slowly. The mixture was stirred vigorously for 1 h at room temperature until a large amount of white gel-like precipitate formed. The solids were filtered and washed with THF (3 × 15 mL). The filtrate was evaporated under

reduced pressure to give compound **12** (0.32g, 92%) as a colorless oil. The product was used for photoisomerization without further purification. ^{1}H NMR agreed with previous literature (3).

(±)-TCO-ether–C2 (**1**) has been reported previously (3).

Carbonate **13**. Compound **8** (0.72 g, 5.71 mmol) was dissolved in DCM (32 mL) in a 100 mL 2-neck round bottom flask under a nitrogen atmosphere. Pyridine (1.15 mL, 14.3 mmol) was added, followed by p-nitrophenyl chloroformate (1.27 g, 6.28 mmol) in DCM (8 mL). The solution was stirred at room temperature for 30 min before addition of saturated NH_4Cl (aq, 40 mL). The organic layer was separated and the aqueous layer was washed with DCM (2 × 15 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (5–10% ethyl acetate in hexanes) to give compound **13** (1.4 g, 84%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.29-8.25 (m, 2H), 7.40-7.25 (m, 2H), 5.74-5.64 (m, 2H), 4.86-4.81 (ddd, J = 10.5 Hz, 9.3 Hz, 4.6Hz, 1H), 2.45-2.37 (m, 1H), 2.21-1.90 (m, 6H), 1.80-1.68 (m, 2H), 1.64-1.57 (m, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 155.69, 151.96, 145.26, 129.99, 129.27, 125.26, 121.79, 80.27, 33.64, 33.45, 25.55, 24.81, 22.04. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{15}H_{18}NO_5^+$ 292.1179; found 292.1182.

Carbamate alcohol **14a**. Compound **13** (0.68 g, 2.33 mmol) was dissolved in anhydrous DMF (10 mL) in a flame-dried scintillation vial under a nitrogen atmosphere. Triethylamine (0.65 mL, 4.67 mmol) and ethanolamine (0.21 mL, 3.5 mmol) were added to the solution. The mixture was stirred at room temperature for 22 h before acidification with 1 M HCl until the yellow color was gone. The mixture was extracted with DCM (3 × 40 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under high vacuum. The crude product was purified by column chromatography (0–3% MeOH in DCM) to give the compound **14a** as a colorless oil (0.5 g, quant.). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.65-5.53 (m, 2H), 5.49-5.31 (br, s, 1H), 4.71-4.59 (br, s, 1H), 3.63-3.56 (m, 3H), 3.27-3.19 (m, 2H), 2.30-2.22 (m, 1H), 2.10 (q, J = 6.8 Hz, 2H), 2.06-2.00 (m, 1H), 1.88-1.66 (m, 3H), 1.60-1.46 (m, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 157.07, 129.62, 129.52, 76.32, 61.94, 43.35, 33.99, 33.74, 25.54, 24.75, 22.32. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{11}H_{20}NO_3^+$ 214.1438; found 214.1441.

Carbamate alcohol **14b**. Compound **13** (0.16 g, 0.55 mmol) was dissolved in anhydrous DMF (3 mL) in a flame-dried scintillation vial under a nitrogen atmosphere. Triethylamine (0.15 mL, 1.1 mmol) and 4-amino-1-butanol (76 μL, 0.824 mmol) was added to the solution. The mixture was stirred at room temperature for 22 h before acidification with 1 M HCl until yellow color was gone. The mixture was extracted with DCM (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under high vacuum. The crude product was purified by column chromatography (0–3% MeOH in DCM) to give compound **14b** as a colorless oil (0.12 g, 91%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.69-5.57 (m, 2H), 4.80-4.66 (m, 2H), 3.67-3.62 (m, 2H), 3.22-3.13 (m, 2H), 2.34-2.26 (m, 1H), 2.20-2.05 (m, 3H), 1.94-1.69 (m, 4H), 1.63-1.51 (m, 7H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 156.58, 129.85, 129.76, 76.11, 62.49, 40.72, 34.19, 33.99, 29.78, 26.72, 25.68, 24.89, 22.49. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{13}H_{24}NO_3^+$ 242.1751; found 242.1753.

(±)-TCO-carb–C2 (**4a**). The general photoisomerization procedure described above was used. 0.5 g of compound **14a** in 1:1 ether/hexanes generated compound **2** (0.128 g, 26%) as a ~1.5:1 mixture

of equatorial and axial isomers after 20 h of irradiation. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.61-5.32 (m, 3H), 4.93-4.80 and 4.32-4.23 (m, 1H), 3.70-3.55 (m, 3H), 3.35-3.12 (m, 2H), 2.86 (s, 1H), 2.34-2.17 (m, 3H), 2.12-1.56 (m, 5H), 1.56-1.43 (m, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz) Peaks due to major diastereomer: δ 157.05, 134.82, 132.89, 80.83, 70.29, 61.90, 41.11, 38.57, 34.25, 32.49, 30.94. Peaks due to minor diastereomer: δ 157.05, 134.82, 131.13, 61.93, 43.37, 43.31, 41.06, 34.34, 32.62, 29.94, 27.95. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{11}H_{20}NO_3^+$ 214.1438; found 214.1435.

(±)-TCO-carb–C4 (**4b**). The general photoisomerization procedure described above was used. 0.12 g of compound **10** in 1:1 ether/hexanes generated compound **2** (0.061 g, 51%) as a ~2.4:1 mixture of equatorial and axial isomers after 17 h of irradiation. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.60-5.44 (m, 2H), 5.03-4.71 and 4.33-4.24 (m, 2H), 3.67-3.59 (m, 2H), 3.25-3.10 (m, 2H), 2.38-2.18 (m, 4H), 2.18-2.03 (m, 1H), 2.02-1.83 (m, 2H), 1.80-1.64 (m, 2H), 1.64-1.47 and 1.27-1.11 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) Peaks due to major diastereomer: δ 156.53, 134.99, 133.06, 80.55, 70.13, 62.33, 41.24, 38.74, 34.37, 32.60, 31.02, 29.74, 26.66. Peaks due to minor diastereomer: δ 156.49, 135.41, 131.84, 41.16, 40.78, 40.71, 34.39, 32.75, 30.05, 28.07, 26.69. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{13}H_{24}NO_3^+$ 242.1751; found 242.1748.

Amide alcohol **15a**. Compound **11** (0.24 g, 1.28 mmol), EDC (0.4 g, 2.56 mmol), and HOBt (0.39 g, 2.56 mmol) were dissolved in DCM (13 mL) under a nitrogen atmosphere. The mixture was cooled to 0 ºC before addition of ethanolamine (0.12 mL, 1.92 mmol) and triethylamine (1.06 mL, 7.68 mmol). The reaction was stirred under room temperature overnight and diluted with DCM (100 mL). The organic layer was washed with 1 M HCl, saturated $NaHCO_3$ (aq), and brine (50 mL each). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by flash column chromatography (2:1 ethyl acetate:hexanes to 100% ethyl acetate) to give the title product as a yellow oil (0.26 g, 90%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.07-6.95 (br, s, 1H), 5.69-5.55 (m, 2H), 3.95-3.87 (m, 2H), 3.75-3.70 (m, 2H), 3.47-3.41 (m, 3H), 2.97-2.86 (br, s, 1H), 2.39-2.30 (m, 1H), 2.19-2.00 (m, 3H), 1.98-1.89 (m, 1H), 1.89-1.75 (m, 3H), 1.57-1.49 (m, 1H), 1.45-1.35 (m, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 171.76, 129.87, 129.72, 82.02, 67.94, 62.48, 42.09, 34.13, 33.35, 25.82, 25.52, 22.52. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{12}H_{22}NO_3^+$ 228.1594; found 228.1597.

Amide alcohol **15b**. Compound **11** (0.56 g, 3.05 mmol), EDC (0.95 g, 6.1 mmol), and HOBt (0.93 g, 6.1 mmol) were dissolved in DCM (26 mL) under a nitrogen atmosphere. The mixture was cooled to 0 ºC before addition of 4-amino-1-butanol (0.42 mL, 4.58 mmol) and triethylamine (2.5 mL, 18.3 mmol). The reaction was stirred under room temperature overnight and diluted with DCM (200 mL). The organic layer was washed with 1 M HCl, saturated $NaHCO_3$ (aq), and brine (100 mL each). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by flash column chromatography (3% MeOH in DCM) to give the title product as a yellow oil (0.62 g, 81%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.77-6.61 (br, s, 1H), 5.69-5.55 (m, 2H), 3.93-3.84 (m, 2H), 3.68 (q, J = 5.5 Hz, 2H), 3.42 (dq, J = 9.2 Hz, 4.3 Hz, 1H), 3.33 (q, J = 6.4 Hz, 2H), 2.40-2.30 (m, 1H), 2.18-2.01 (m, 3H), 1.97-1.89 (m, 1H), 1.81-1.49 (m, 9H), 1.45-1.35 (m, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.55, 129.90, 129.74, 81.93, 68.04, 62.54, 38.64, 34.21, 33.36, 29.85, 26.41, 25.84, 25.55, 22.54. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{14}H_{26}NO_3^+$ 256.1907; found 256.1910.

(±)-TCO-amide–C2 (**3a**). The general photoisomerization procedure described above was used. 0.68 g of compound **15a** in 1:1 ether/hexanes generated compound **3a** (0.32 g, 47%) as a ~1.5:1 mixture of equatorial and axial isomers after 19 h of irradiation. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.18-6.81 (br, s, 1H), 5.68-5.32 (m, 2H), 4.01-3.03 (m, 7H), 2.82-2.59 (m, 1H), 2.44-1.18 (m, 10H). ^{13}C NMR ($CDCl_3$, 126 MHz) Peaks due to major diastereomer: δ 171.66, 135.25, 132.55, 86.98, 67.81, 62.57, 42.15, 40.75, 37.85, 34.53, 32.94, 31.69. Peaks due to minor diastereomer: δ 171.52, 135.69, 131.59, 76.19, 68.42, 62.47, 41.99, 40.24, 34.47, 32.71, 29.96, 28.08. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{12}H_{22}NO_3^+$ 228.1594; found 228.1592.

(±)-TCO-amide–C4 (**3b**). The general photoisomerization procedure described above was used. 0.62 g of compound **15b** in 1:1 ether/hexanes generated compound **3b** (0.13 g, 21%) as a ~1.8:1 mixture of equatorial and axial isomers after 20 h of irradiation. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.79-6.56 (s, 1H), 5.61-5.33 (m, 2H), 3.97-3.76 (m, 2H), 3.74-3.01 (m, 5H), 2.43-1.44 (m, 15H). ^{13}C NMR ($CDCl_3$, 126 MHz). Peaks due to major diastereomer: δ 170.43, 135.20, 132.50, 86.91, 67.90, 62.56, 40.81, 38.66, 37.86, 34.55, 32.96, 31.70, 29.84, 26.40. Peaks due to minor diastereomer: δ 170.13, 135.31, 131.31, 75.97, 68.49, 62.53, 40.29, 38.69, 38.67, 34.49, 32.67, 30.01, 28.08, 26.47. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{14}H_{26}NO_3^+$ 256.1907; found 256.1904.

Synthesis of compounds **17**–**22** and (*S*)-oxoTCO–C1 (**5**) were adapted from routes for synthesis of (*R*)-oxoTCO (**6**) by Lambert *et al* (1) with some modifications, see below. The difference was the configuration of the initial glycidol starting material. (*R*)-glycidol was used for synthesis of (*S*)-oxoTCO–C1 (**5**), whereas (*S*)-glycidol was used for synthesis of (*R*)-oxoTCO (**6**). The intermediates for synthesis of **5** are enantiomers of corresponding intermediates for **6** and the spectra of intermediates for **5** matched those for **6** reported in the literature (1)**.** The synthetic routes adapted for **6** in this work had the same modifications as below for (*S*)-oxoTCO–C1 (**5**).

Modifications to Lambert *et al* (1) for the synthesis of (*S*)-oxoTCO–C1 (**5**) include the following:

a) For the synthesis of compound **17**, DCM was used as the solvent instead of DMF.

b) For the synthesis of compound **18**, 1.75 equivalents of allylmagnesium chloride was used instead of 1.2 equivalents.

c) For the synthesis of compound **19**, 3 equivalents of chloromethyl methyl ether was used instead of 2 equivalents and 10 mol% of 4-DMAP was used instead of 1 mol%.

d) For the synthesis of compound **20**, 0.5 equivalents of tin (IV) chloride was used instead of 5–6 equivalents.

e) For the synthesis of compound **21**, the complete removal of Ru metal from the product proved to be difficult; therefore, a two-step yield for synthesis of compound **22** was reported.

f) For the synthesis of compound **5**, the general photoisomerization procedure described above was used. 0.286 g of compound **22** in 1:1 ether/hexanes generated compound **5** (0.194 g, 68%) as a ~2.5:1 mixture of equatorial and axial isomers after 18 h of irradiation.

Compounds **23a** (4) and **23b** (5) have been reported previously.

Secondary alcohol **24a.** Compound **23a** (2.45 g, 7.50 mmol) and dilithium tetrachlorocuprate(II) (0.1 M in THF, 7.5 mL, 7.5 mmol) were dissolved in anhydrous THF (12 mL) under a nitrogen atmosphere. The solution was cooled to –30 ºC. Allylmagnesium chloride (1.7 M in THF, 7.5 mL, 12.8 mmol) was then added dropwise over 30 min. The reaction was stirred for an additional 30 min at –30 ºC before being allowed to warm to room temperature. The solution was diluted with ether (70 mL) and then quenched with saturated NH_4Cl (aq., 70 mL) slowly. The layers were separated and extracted with ether (3 × 70 mL). The organic layers were combined and washed with water and brine (100 mL each). The ether layer was dried with $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by flash column chromatography (6–7% ethyl acetate in hexanes) to give compound **24a** as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70-7.66 (m, 4H), 7.47-7.38 (m, 6H), 5.85 (ddt, *J* = 17.0 Hz, 10.2 Hz, 6.7 Hz, 1H), 5.05 (dq, *J* = 17.2 Hz, 1.7 Hz, 1H), 4.99-4.95 (m, 1H), 3.95-3.82 (m, 3H), 3.22 (d, *J* = 2.8 Hz, 1H), 2.27-2.09 (m, 2H), 1.77-1.49 (m, 4H), 1.06 (s, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 138.77, [135.72, 135.70]*, [133.17, 133.08]*, [130.00, 129.97]*, 127.93, 114.72, 71.39, 63.67, 38.47, 36.76, 30.03, 26.96, 19.18. * indicates diastereotopic carbons. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{23}H_{33}SiO_2^+$ 369.2244; found 369.2230.

Secondary alcohol **24b**. This compound was prepared analogously to **24a**. Epoxide **23b** (5.2 g, 15.3 mmol) generated 6.0 g (quant.) of compound **24b** as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.69-7.65 (m, 4H), 7.45-7.36 (m, 6H), 5.85 (ddt, *J* = 16.9 Hz, 10.1 Hz, 6.8 Hz, 1H), 5.05 (dq, *J* = 17.0 Hz, 1.7 Hz, 1H), 4.99-4.95 (m, 1H), 3.71-3.62 (m, 3H), 2.26-2.09 (m, 2H), 2.07-1.80 (br, s, 1H), 1.70-1.59 (m, 3H), 1.58-1.46 (m, 3H), 1.05 (s, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 138.85, [135.74, 135.72]*, 133.80, 129.79, 127.81, 114.81, 71.22, 64.35, 36.62, 34.51, 30.28, 28.89, 26.98, 19.32. * indicates diastereotopic carbons. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{24}H_{35}SiO_2^+$ 383.2401; found 383.2404.

Acetal **25a**. To an oven-dried scintillation vial, 4-dimethylaminopyridine (5 mg, 10 mol%), compound **24a** (146 mg, 0.40 mmol), DCM (1.5 mL), and DIEA (0.21 mL, 1.19 mmol) were added. The solution was cooled to 0 ºC, and chloromethyl methyl ether (90 μL, 1.19 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 20 h before dilution with DCM (6 mL) and quenching with water (6 mL). The layers were separated, and the aqueous layer was further extracted with DCM (2 × 6 mL). The organic layers were combined, washed with brine (10 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (6–7% ethyl acetate in hexanes) to give compound **25a** as a colorless oil (165 mg, quant.). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.69-7.64 (m, 4H), 7.45-7.36 (m, 6H), 5.81 (ddt, *J* = 16.9 Hz, 10.3 Hz, 6.6 Hz, 1H), 5.02 (dq, *J* = 17.3 Hz, 1.7 Hz, 1H), 4.98-4.94 (m, 1H), 4.62 (q, *J* = 6.9 Hz, 2H), 3.82-3.71 (m, 3H), 3.32 (s, 3H), 2.18-2.04 (m, 2H), 1.75 (q, *J* = 6.3 Hz, 2H), 1.65-1.57 (m, 2H), 1.05 (s, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 138.65, 135.71, 134.02, 129.73, 127.77, 114.72, 95.92, 74.57, 60.67, 55.66, 37.49, 34.13, 29.66, 27.01, 19.33. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{25}H_{37}SiO_3^+$ 413.2506; found 413.2516.

Acetal **25b**. This compound was prepared analogously to **25a**. Secondary alcohol **24b** (2.2 g, 5.75 mmol) generated 2.13 g (87%) of compound **25b** as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.69-7.65 (m, 4H), 7.44-7.35 (m, 6H), 5.82 (ddt, *J* = 16.8 Hz, 10.1 Hz, 6.5 Hz, 1H), 5.03 (dq, *J* = 17.1 Hz, 1.7 Hz, 1H), 4.98-4.94 (m, 1H), 4.63 (d, *J* = 1.0 Hz, 2H), 3.67 (t, *J* = 5.0 Hz, 2H), 3.57

(tt, *J* = 5.6 Hz, 5.5Hz, 1H), 3.36 (s, 3H), 2.19-2.04 (m, 2H), 1.66-1.51 (m, 6H), 1.05 (s, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 138.72, 135.72, 134.15, 129.68, 127.75, 114.71, 95.60, 76.95, 64.08, 55.68, 33.66, 30.56, 29.74, 28.35, 27.01, 19.36. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{26}H_{39}SiO_3^+$ 427.2663; found 427.2663.

Ether diene **26a**. Compound **25a** (0.25 g, 0.61 mmol) and allyltrimethylsilane (0.192 mL, 1.21 mmol) were dissolved in anhydrous DCM (2.5 mL) under a nitrogen atmosphere. The solution was cooled to 0 ºC before dropwise addition of tin (IV) chloride (1 M in DCM, 0.30 mL, 0.30 mmol). The mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was cooled to 0 ºC, diluted with DCM (10 mL), and quenched with saturated $NaHCO_3$ (aq) slowly. The layers were separated, and the aqueous layer was extracted with DCM (2 × 15 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (6–7% ethyl acetate in hexanes) to give the title compound as a colorless oil (0.248 g, 97%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71-7.64 (m, 4H), 7.45-7.36 (m, 6H), 5.80 (dtt, *J* = 17.0 Hz, 10.4 Hz, 6.6 Hz, 2H), 5.04 (ddq, *J* = 22.8 Hz, 17.1 Hz, 1.6 Hz, 2H), 5.02-4.93 (m, 2H), 3.82-3.69 (m, 2H), 3.53-3.40 (m, 3H), 2.27 (qt, J = 6.7 Hz, 1.30 Hz, 2H), 2.17-2.03 (m, 2H), 1.77-1.66 (m, 2H), 1.62-1.49 (m, 2H), 1.05 (s, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 138.86, 135.71, 135.66, 134.08, 129.71, 127.76, 116.29, 114.60, 75.91, 68.62, 60.73, 37.28, 34.77, 33.65, 29.80, 27.02, 19.35. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{27}H_{39}SiO_2^+$ 423.2714; found 423.2719.

Ether diene **26b**. This compound was prepared analogously to **26a**. Acetal **25b** (2.13 g, 5.00 mmol) generated 1.96 g (90%) of compound **26b** as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70-7.65 (m, 4H), 7.45-7.35 (m, 6H), 5.80 (ddtd, *J* = 17.3 Hz, 10.1 Hz, 6.6 Hz, 1.3 Hz, 2H), 5.18 (ddq, *J* = 30.8 Hz, 17.3 Hz, 1.6 Hz, 2H), 5.05-4.93 (m, 2H), 3.69-3.65 (m, 2H), 3.49-3.40 (m, 2H), 3.26 (quint, *J* = 5.7 Hz, 1H), 2.30 (qt, J = 6.7 Hz, 1.50 Hz, 2H), 2.24-2.02 (m, 2H), 1.64-1.51 (m, 6H), 1.05 (s, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 138.96, 135.72, 135.70, 134.20, 129.67, 127.74, 116.30, 114.56, 78.79, 68.36, 64.14, 34.82, 33.37, 30.13, 29.88, 28.45, 27.02, 19.37. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{28}H_{41}SiO_2^+$ 437.2870; found 437.2878.

Oxo-cyclooctene **27a**. Compound **26a** (1.45 g, 3.43 mmol) was dissolved in DCM (650 mL) and the solution was heated to reflux before addition of Grubb's I catalyst (0.282 g, 0.343 mmol) in one portion. The mixture was stirred for 2.5 h under reflux before removal of DCM by rotary evaporation. The crude material was partially purified by flash column chromatography (6–7% ethyl acetate in hexanes to give the title compound as a brownish oil (1.40 g, containing Ru metal). The partially purified compound was used for the next deprotection step immediately.

Oxo-cyclooctene **27b**. This compound was prepared analogously to **27a**. Ether diene **26b** (1.38 g, 3.16 mmol) generated 1.22 g of compound **27b** (containing Ru metal) as a brownish oil. The partially purified compound was used for the next deprotection step immediately.

Oxo-cyclooctene primary alcohol **28a**. Tetrabutylammonium fluoride (1 M in THF, 10.3 mL, 10.3 mmol) was added to compound **27a** (1.40 g, containing Ru) at 0 ºC. The solution was allowed to warm to room temperature and stirred overnight. The reaction mixture was diluted with ether (20 mL) and quenched slowly with saturated NH_4Cl (aq., 20 mL). The layers were separated, and the aqueous layer was extracted with ether (3 × 20 mL). The organic layers were combined, washed

with brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (6–7% ethyl acetate in hexanes) to give the title compound as a colorless oil (0.297 g, 56% over two steps). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.83-5.72 (m, 2H), 3.99-3.92 (m, 1H), 3.82-3.72 (m, 2H), 3.67-3.61 (m, 1H), 3.46-3.40 (m, 1H), 2.51-2.36 (m, 2H), 2.22-2.10 (m, 2H), 2.07-2.00 (m, 1H), 1.75-1.59 (m, 3H), 1.56-1.48 (m, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 131.72, 128.59, 78.64 (app d), 71.45, 60.75 (app d), 38.90 (app d), 36.02, 29.35, 23.79. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_9H_{17}O_2^+$ 157.1223; found 157.1220.

Oxo-cyclooctene primary alcohol **28b**. This compound was prepared analogously to **28a**. Ether diene **27b** (1.22 g, containing Ru) generated 0.37 g of compound **28b** (69% over two steps) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.82-5.70 (m, 2H), 3.99-3.92 (m, 1H), 3.68-3.59 (m, 2H), 3.41-3.31 (m, 2H), 2.52-2.36 (m, 2H), 2.14-1.97 (m, 2H), 1.94-1.79 (br, s, 1H), 1.76-1.22 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 131.75, 128.61, 79.81, 71.58, 63.21, 36.01, 33.56, 29.53, 29.15, 23.84. HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{10}H_{19}O_2^+$ 171.1380; found 171.1383.

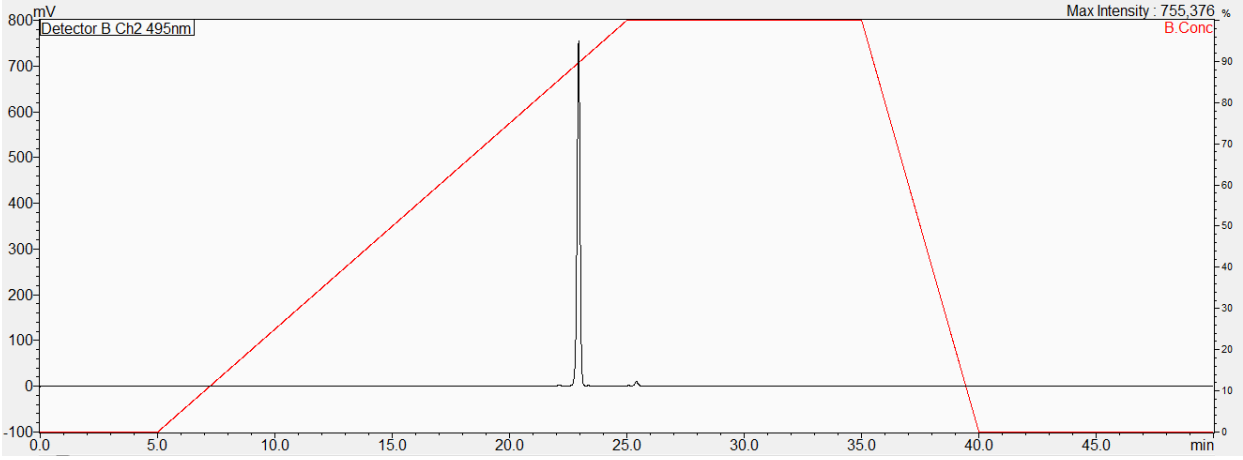
(±)-oxoTCO–C2 (**7a**). The general photoisomerization procedure described above was used. 0.315 g compound **28a** in 1:1 ether/hexanes generated compound **7a** (0.088 g, 28%) as a ~2.9:1 mixture of equatorial and axial isomers after 17 h of irradiation. ^{1}H NMR (C_6D_6, 500 MHz) δ 5.82-5.68, 5.53-5.44 and 5.21-5.12 (m, 2H), 3.82-3.76 (m, 1H), 3.67-3.27 (m, 2H, mixed with peaks from minor isomer), 2.91-2.83 (m, 2H), 2.30-2.19 (m, 2H), 2.03-1.25 (m, 7H). ^{13}C NMR (C_6D_6, 126 MHz) Peaks due to major diastereomer: δ 140.64 (dn), 126.41 (dn), 82.88 (dn), 73.26 (u), 59.08 (u), 40.94 (u), 39.16 (u), 37.98 (u), 34.29 (u). Peaks due to minor diastereomer: δ 137.07 (dn), 132.69 (dn), 80.69 (dn), 72.92 (u), 60.00 (u), 42.61 (u), 33.07 (u), 27.95 (u), 27.67 (u). HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_9H_{17}O_2^+$ 157.1223; found 157.1236.

(±)-oxoTCO–C3 (**7b**). The general photoisomerization procedure described above was used. 0.37 g compound **28b** in 1:1 ether/hexanes generated compound **7b** (0.251 g, 68%) as a ~2.7:1 mixture of equatorial and axial isomers after 17 h of irradiation. ^{1}H NMR (C_6D_6, 500 MHz) δ 5.83-5.72, 5.50-5.40 and 5.20-5.12 (m, 2H), 3.86-3.79 (m, 1H), 3.59-3.33 (m, 2H, mixed with peaks from minor isomer), 2.77-2.70 (m, 1H), 2.62-2.55 (m, 1H), 2.32-1.68 (m, 5H), 1.55-1.18 (m, 6H). ^{13}C NMR (C_6D_6, 126 MHz) Peaks due to major diastereomer: δ 140.75 (dn), 126.33 (dn), 84.84 (dn), 73.35 (u), 62.65 (u), 40.93 (u), 38.08 (u), 34.43 (u), 33.82 (u), 28.49 (u). Peaks due to minor diastereomer: δ 137.77 (dn), 131.66 (dn), 81.18 (dn), 71.20 (u), 62.28 (u), 42.32 (u), 34.31 (u), 30.96 (u), 29.44 (u), 28.26 (u). HRMS (DART) m/z: $[M+H]^+$ calcd. for $C_{10}H_{19}O_2^+$ 171.1380; found 171.1393.

Synthesis of the fluorogenic **Tz–BODIPY** has been reported previously (6).

BODIPY-alkyl-Tz. BODIPY-succinate (**29**) was synthesized as previously described (7). Compound **29** (14 mg, 39 μmol), methyltetrazine-amine (Click Chemistry Tools, 9 mg, 39 μmol), EDC-HCl (15 mg, 77 μmol), and HOBt (12 mg, 77 μmol) were added into a flame-dried scintillation vial equipped with a stir bar under a nitrogen atmosphere. Dichloromethane (3 mL) and Hunig's base (40 μL) were then added to the vial. The solution was stirred overnight at room temperature before dilution with dichloromethane. The mixture was washed with 1 M HCl, neutralized with $NaHCO_3$ (aq., saturated), and washed with brine. The organic layer was dried with $MgSO_4$ before concentration. The crude was purified with column chromatography (0–5%

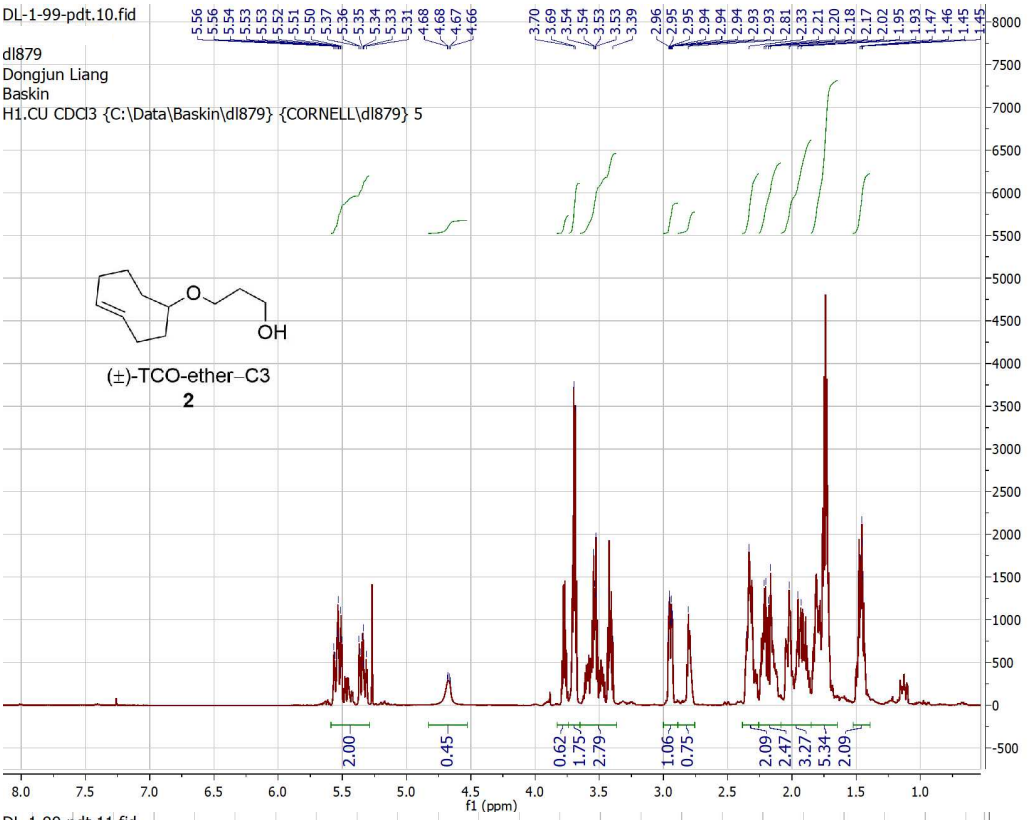
v/v MeOH in DCM) and subsequent normal-phase HPLC to give BODIPY-alkyl-Tz (14 mg, 67%) as a red solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.50 (d, 2H, J = 8.0 Hz), 7.57 (s, 1H), 7.47 (d, 2H, J = 8.1 Hz), 7.09 (d, 1H, J = 4.3 Hz), 6.48 (t, 1H, J = 5.9 Hz), 6.44-6.41 (m, 1H), 6.34 (t, 1H, J = 6.2 Hz), 6.16 (s, 1H), 4.52 (d, 2H, J = 5.9 Hz), 3.48 (q, 2H, J = 7.0 Hz), 3.10-3.05 (m, 5H), 2.59-2.49 (m, 7H), 2.44 (s, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 172.64, 172.25, 167.43, 163.84, 161.36, 145.29, 143.23, 142.49, 138.30, 134.17, 131.14, 128.49, 128.36, 124.26, 123.96, 116.18, 56.12, 43.47, 41.47, 31.69, 31.61, 21.31, 16.35, 15.17. The product was further characterized on reverse-phase HPLC (see chromatogram below) on Shimadzu LC-20AR HPLC equipped with an SPD20AV UV/Vis detector. Column: Epic Polar 5µ 120 Å, 25 cm X 4.6 mm, Catalog # 155291-EPO, Serial #271-15-84620. Channels: 495 nm and 553 nm. Solvent A: Water. Solvent B: Acetonitrile. Flow rate: 1 mL/min. The percent of solvent B is indicated by the red line.

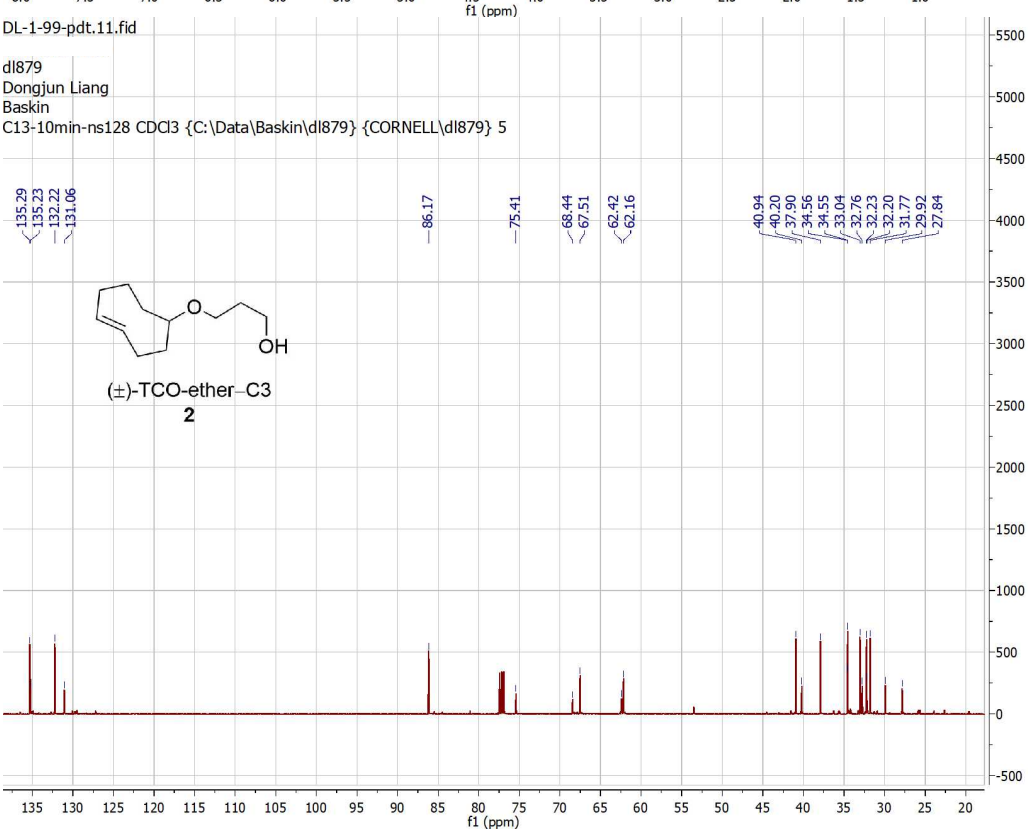


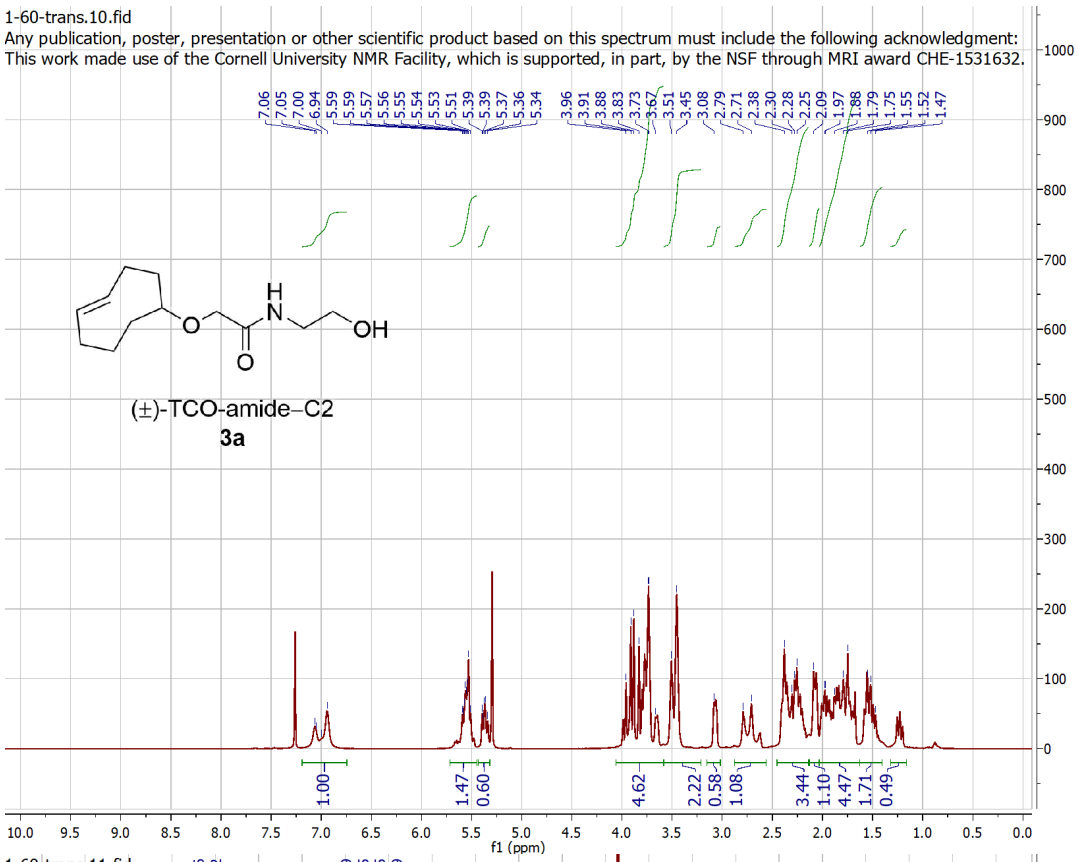
References for the Supplemental Information

1. Lambert WD, et al. (2017) Computationally guided discovery of a reactive, hydrophilic: Trans-5-oxocene dienophile for bioorthogonal labeling. *Org Biomol Chem* 15(31):6640–6644.
2. Clark PG, Guidry EN, Chan WY, Steinmetz WE, Grubbs RH (2010) Synthesis of a molecular charm bracelet via click cyclization and olefin metathesis clipping. *J Am Chem Soc* 132(10):3405–3412.
3. Li Z, et al. (2010) Tetrazine-trans-cyclooctene ligation for the rapid construction of 18F labeled probes. *Chem Commun* 46(42):8043–8045.
4. Jamieson ML, Hume PA, Furkert DP, Brimble MA (2016) Divergent reactivity via cobalt catalysis: An epoxide olefination. *Org Lett* 18(3):468–471.
5. Kim J, Jeong W, Rhee YH (2017) Flexible tetrahydropyran synthesis from homopropargylic alcohols using sequential Pd-Au catalysis. *Org Lett* 19(1):242–245.
6. Carlson JCT, Meimetis LG, Hilderbrand SA, Weissleder R (2013) BODIPY-tetrazine derivatives as superbright bioorthogonal turn-on probes. *Angew Chemie - Int Ed* 52(27):6917–6920.
7. Alamudi SH, et al. (2016) Development of background-free tame fluorescent probes for intracellular live cell imaging. *Nat Commun* 7:1–9.

^{1}H and ^{13}C NMR Spectra



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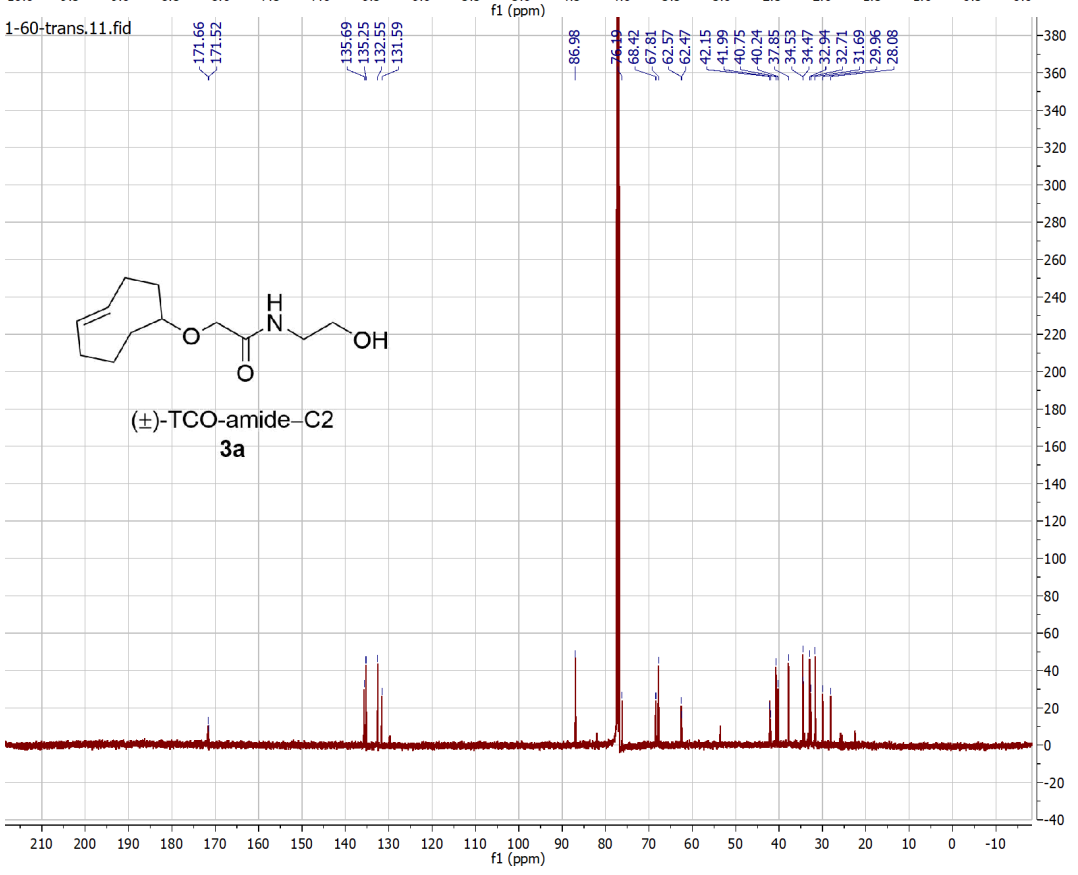
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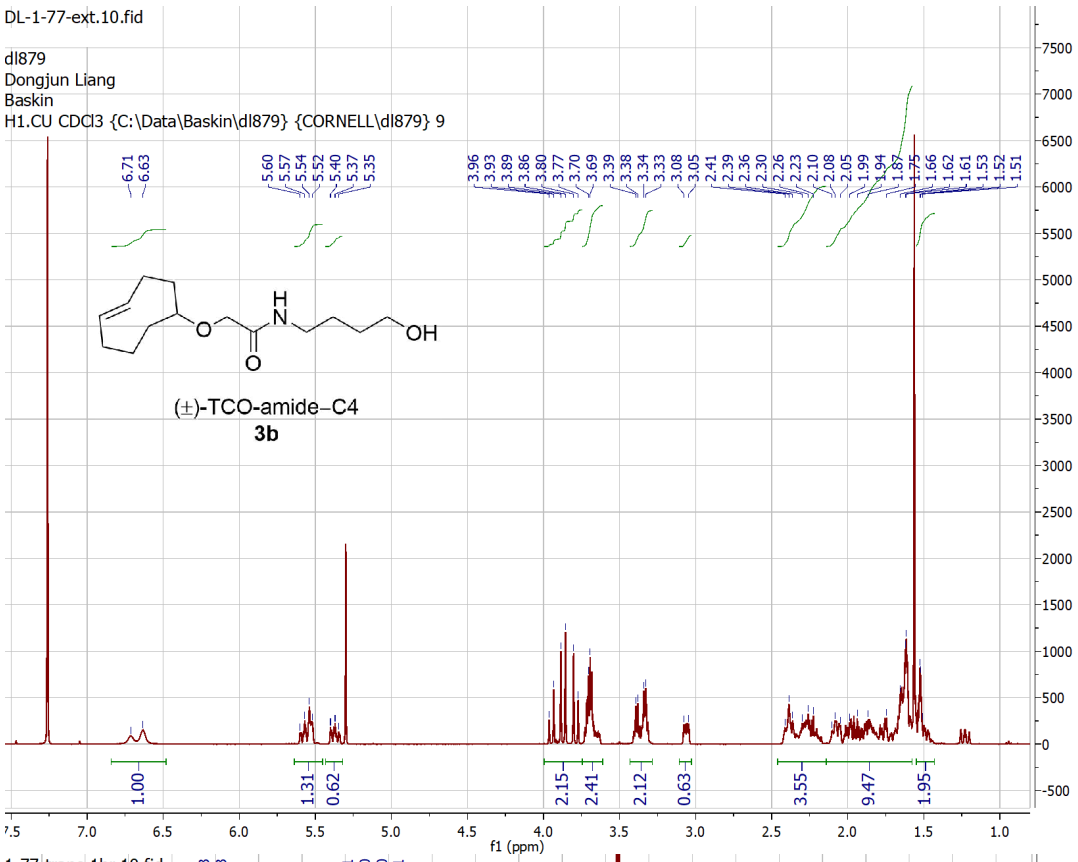
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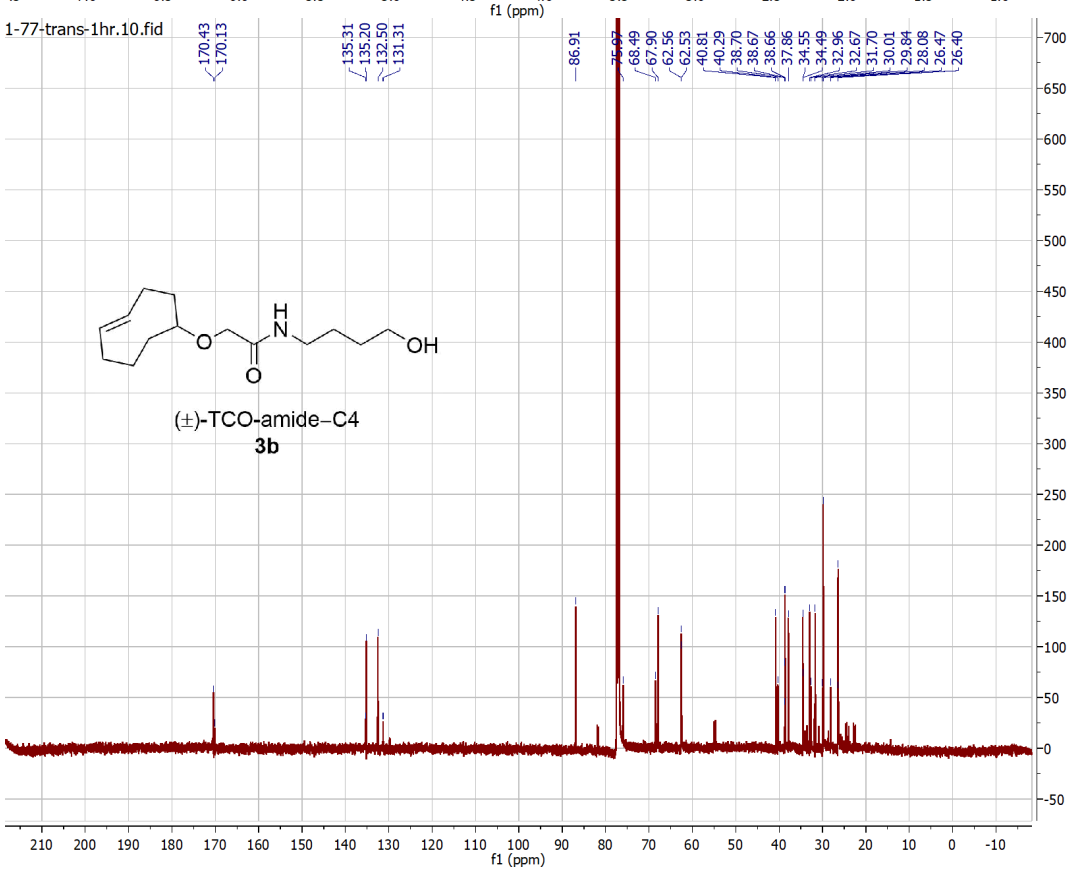
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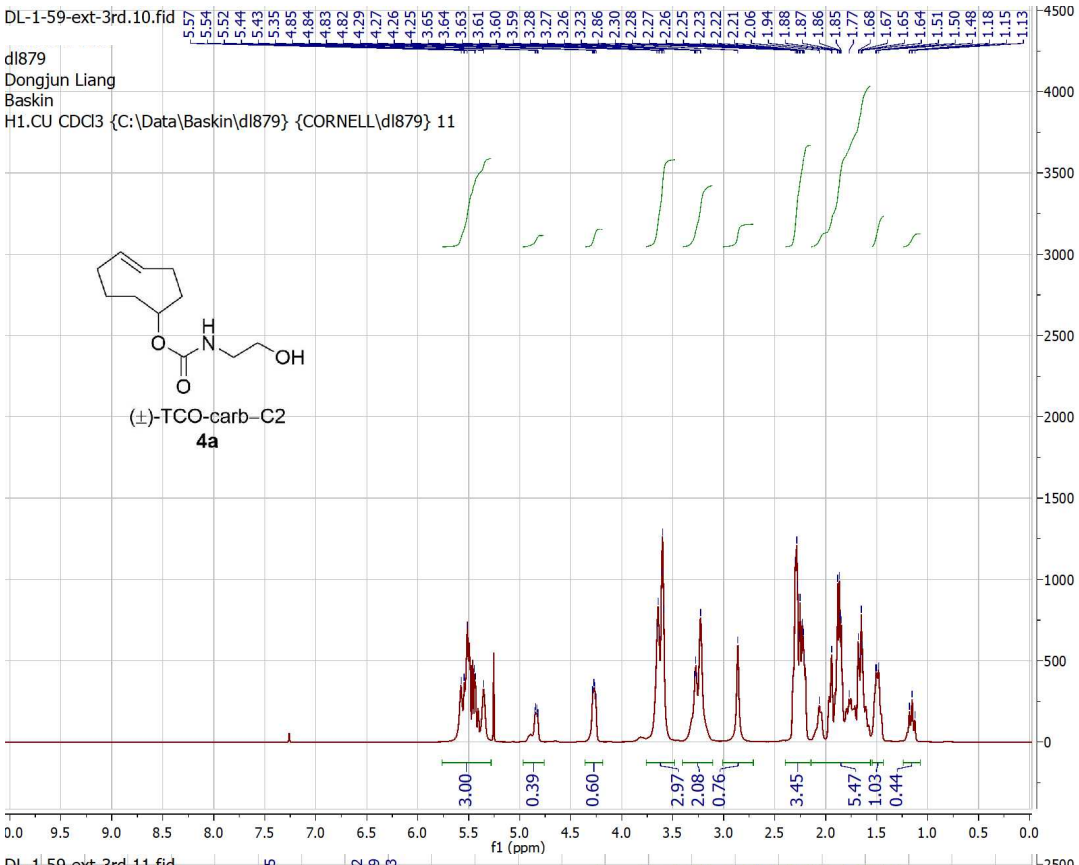
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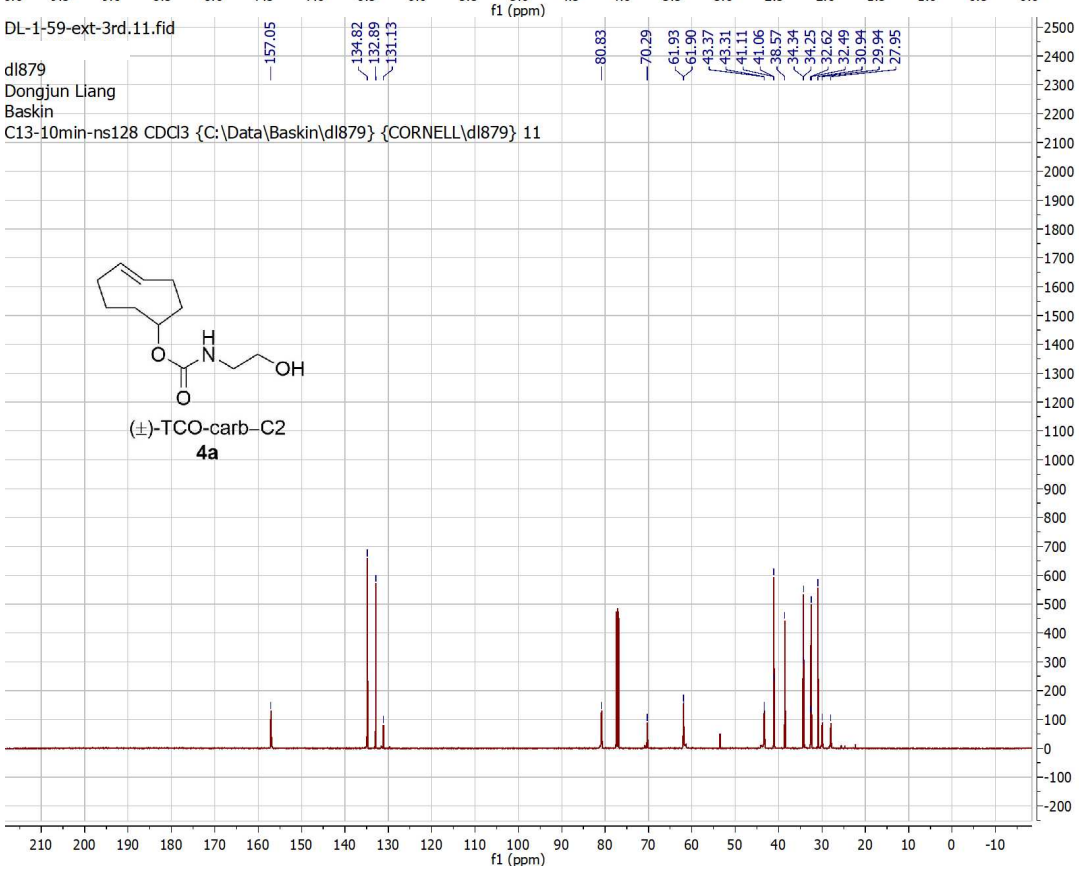
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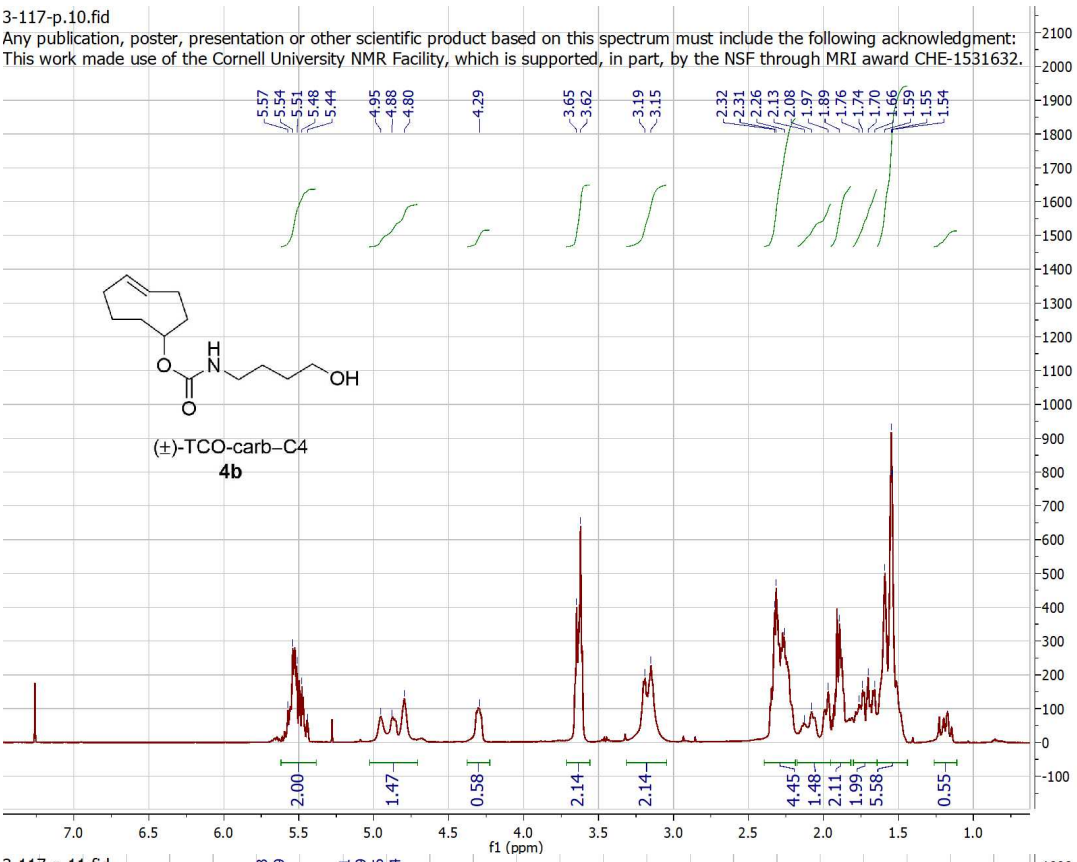
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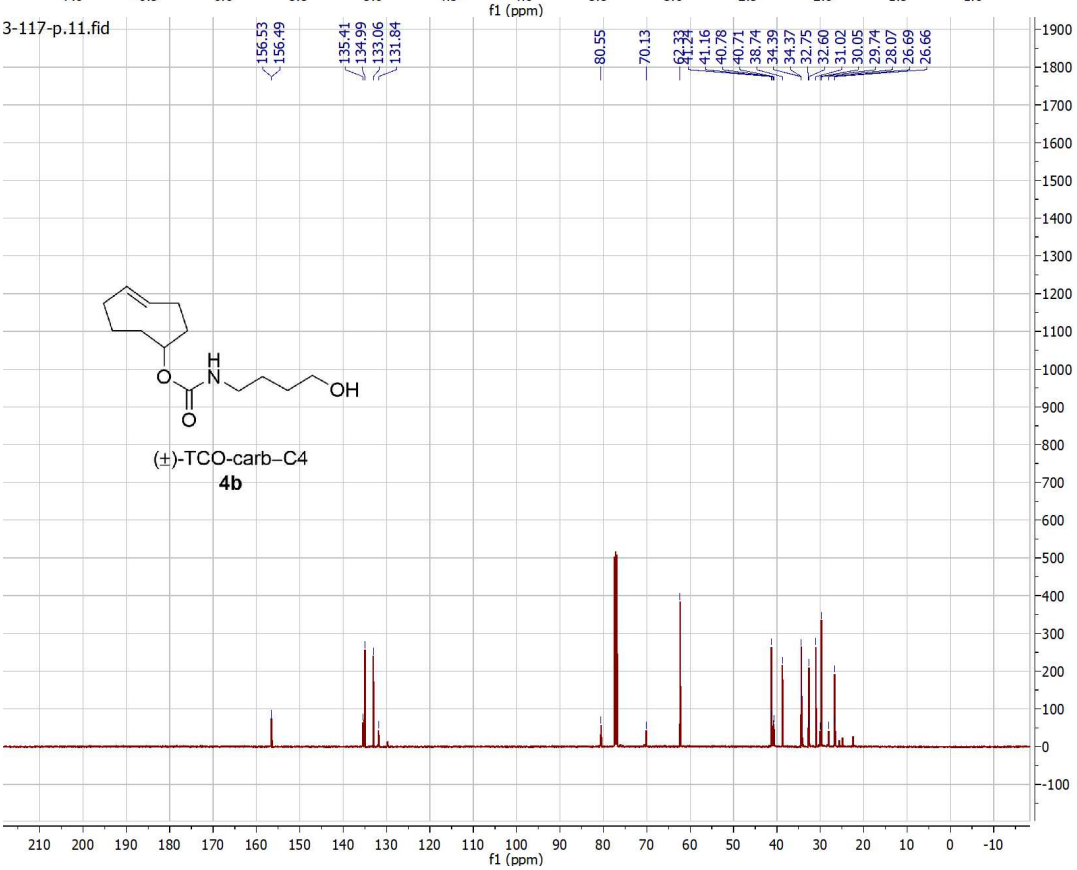
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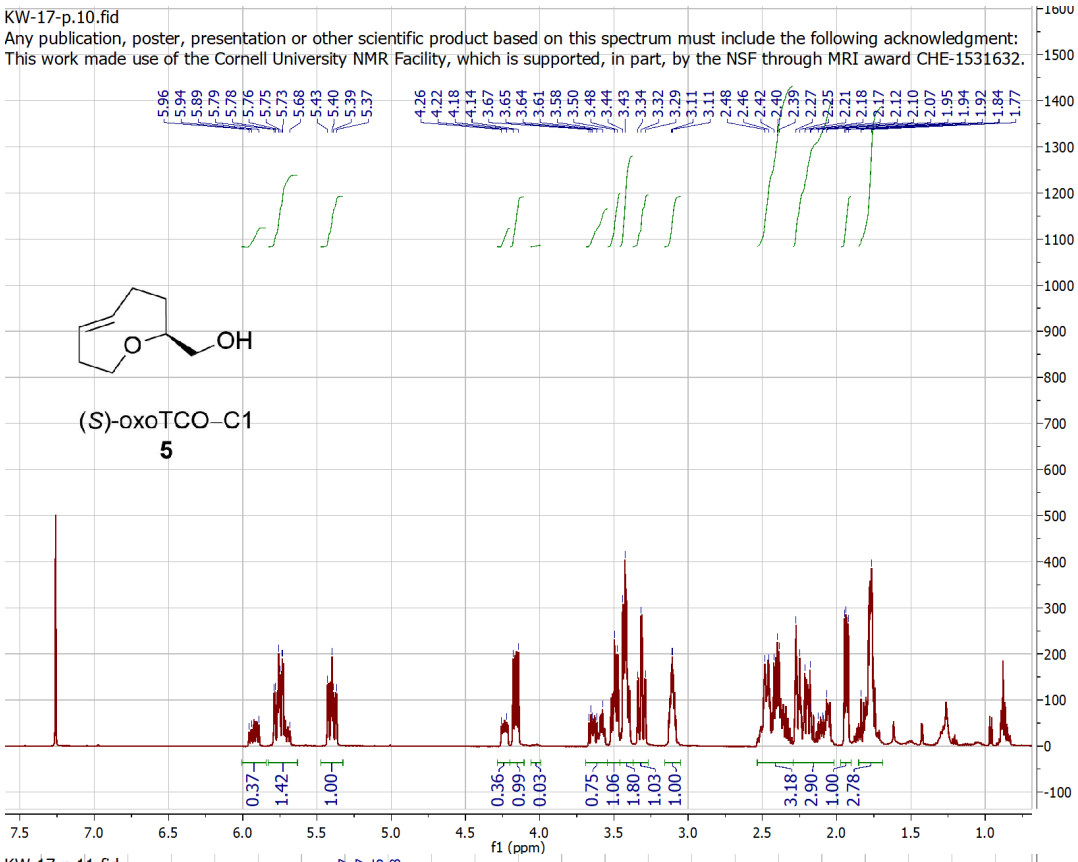
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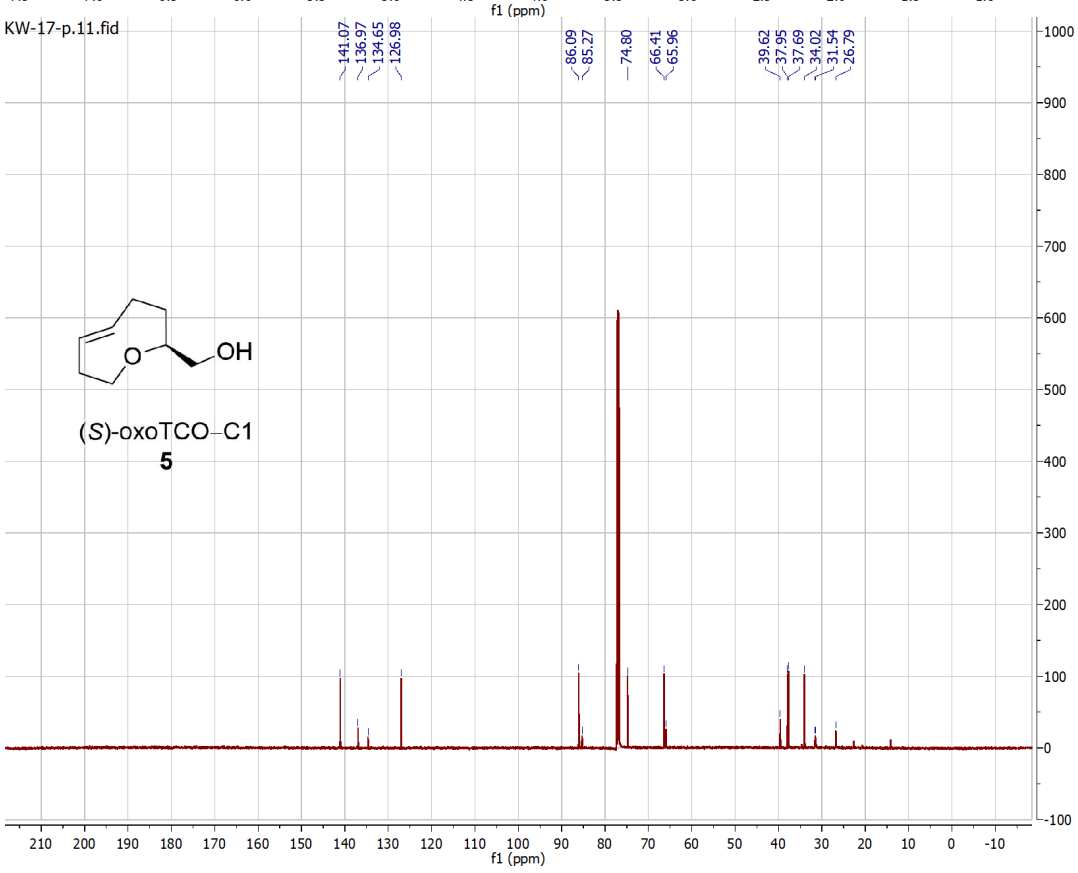
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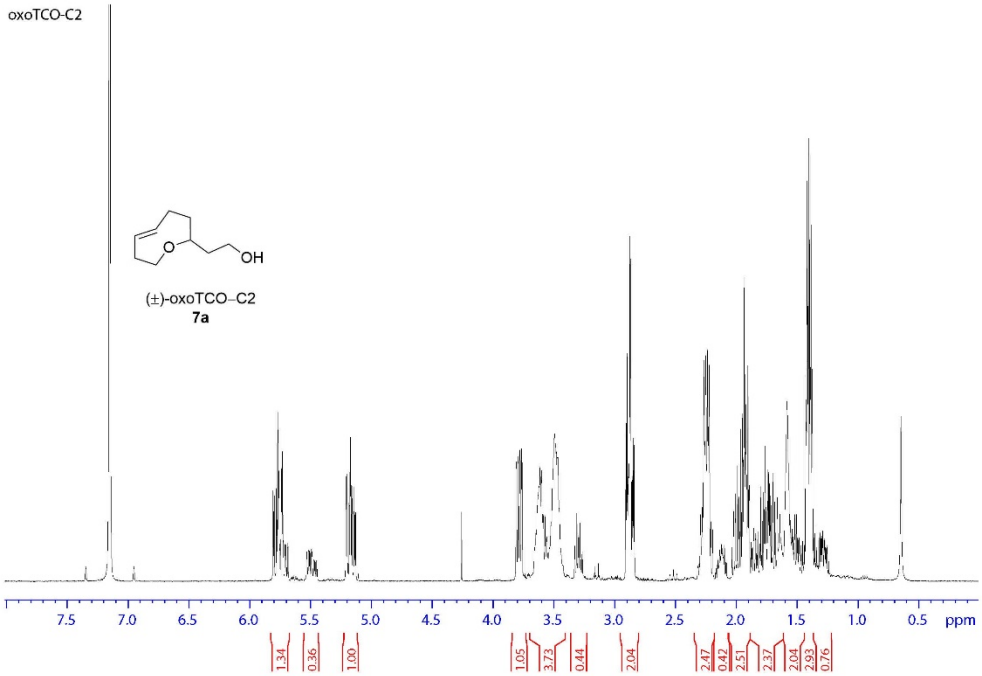
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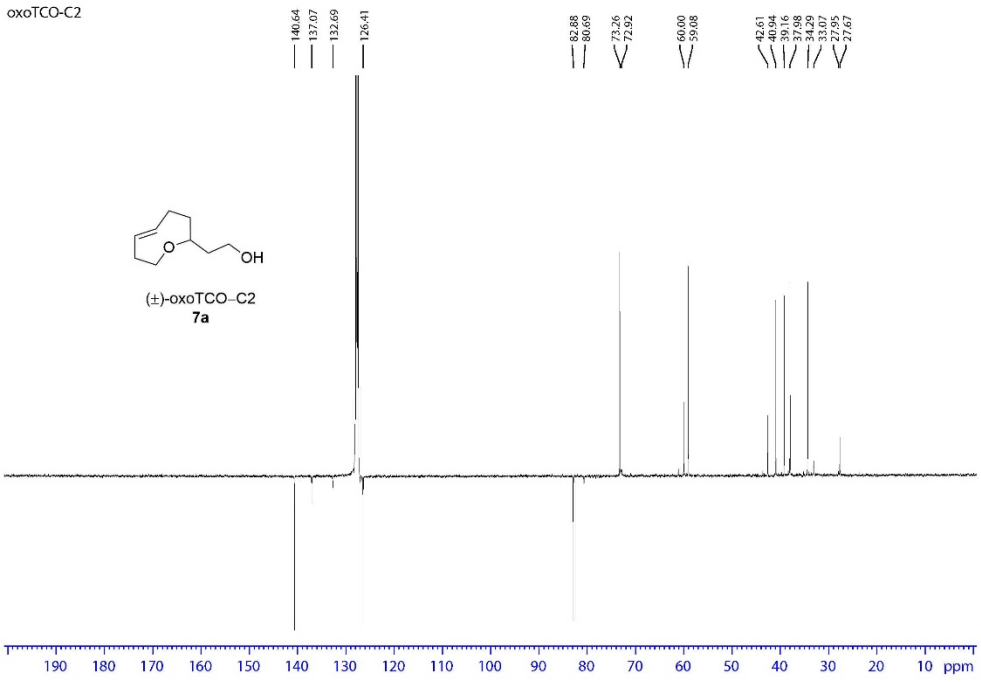
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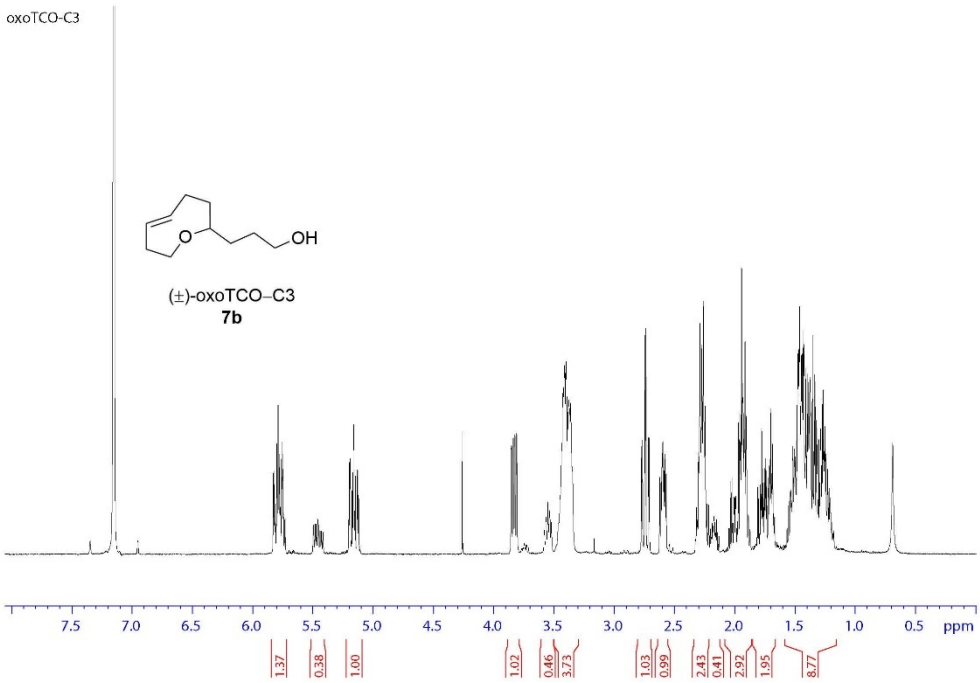
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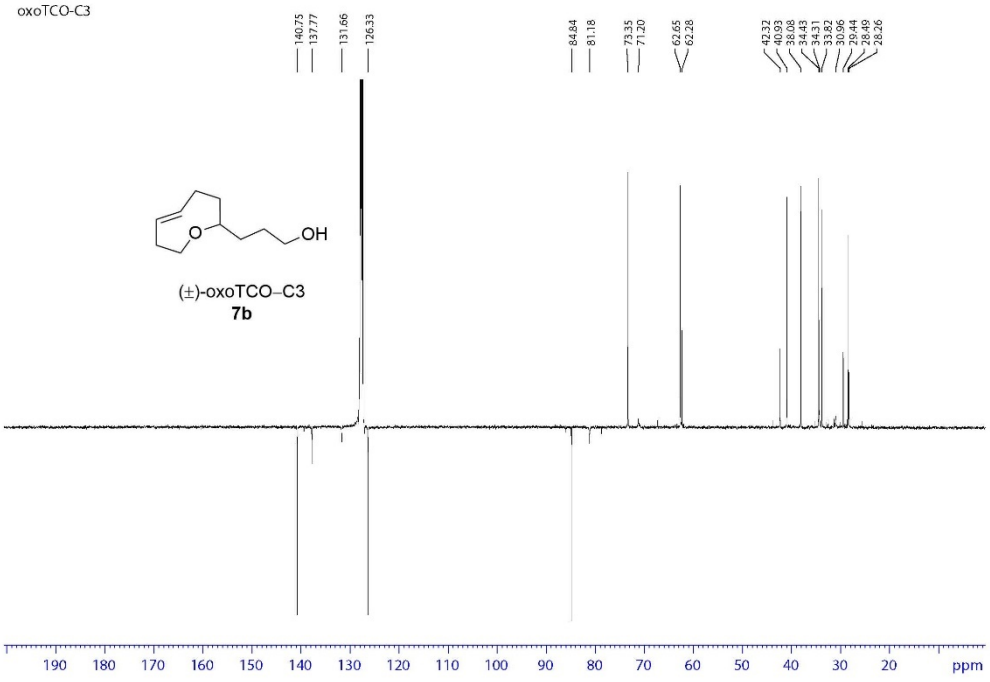
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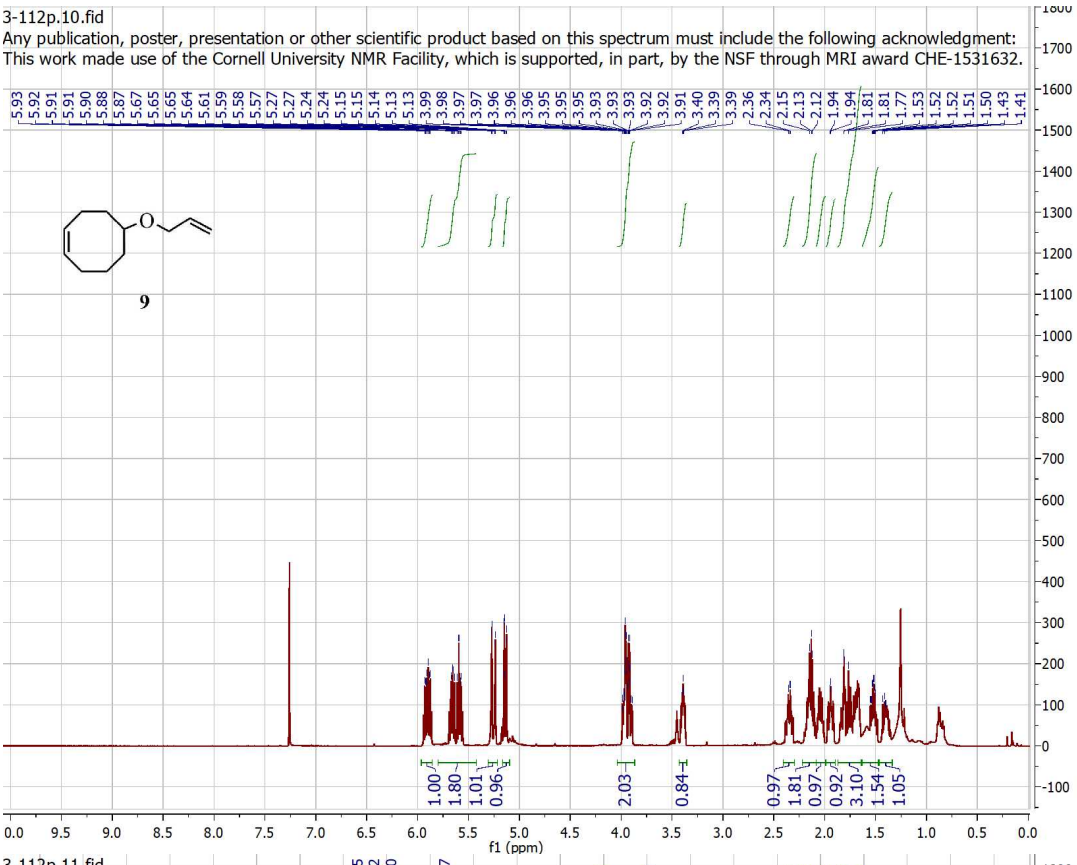
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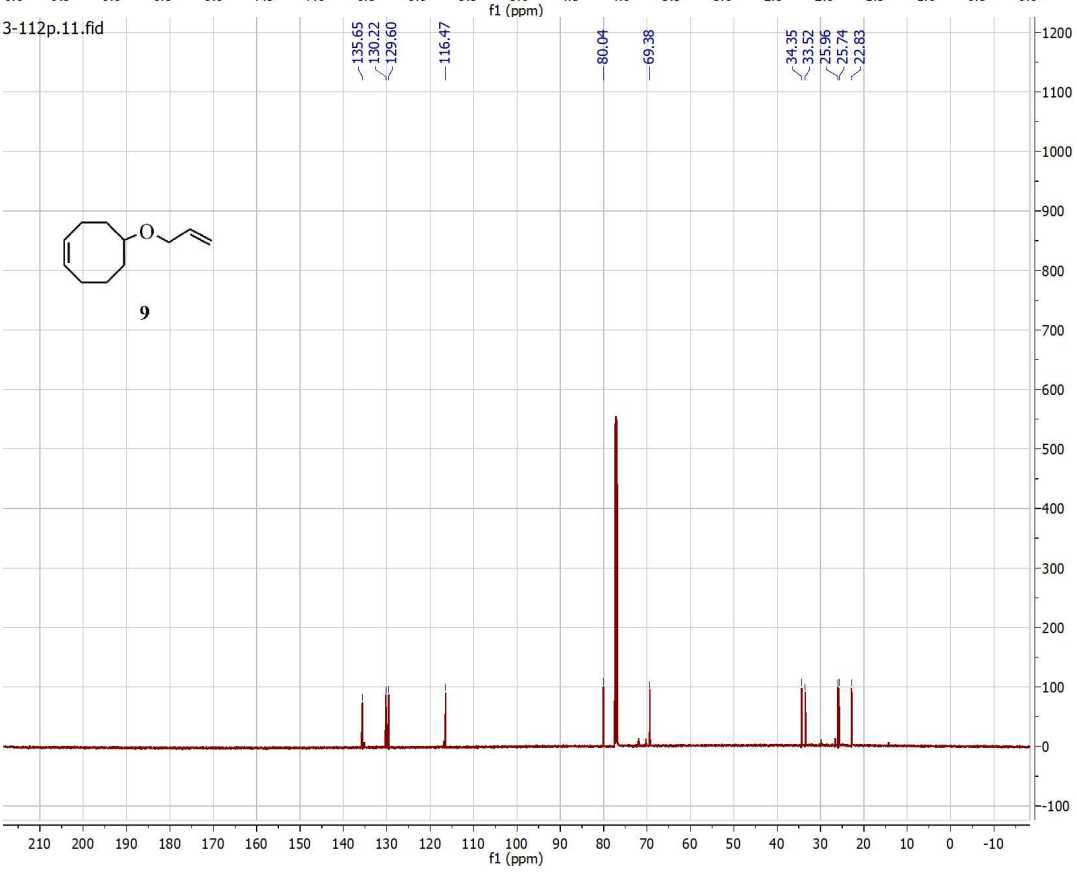
f1 (ppm)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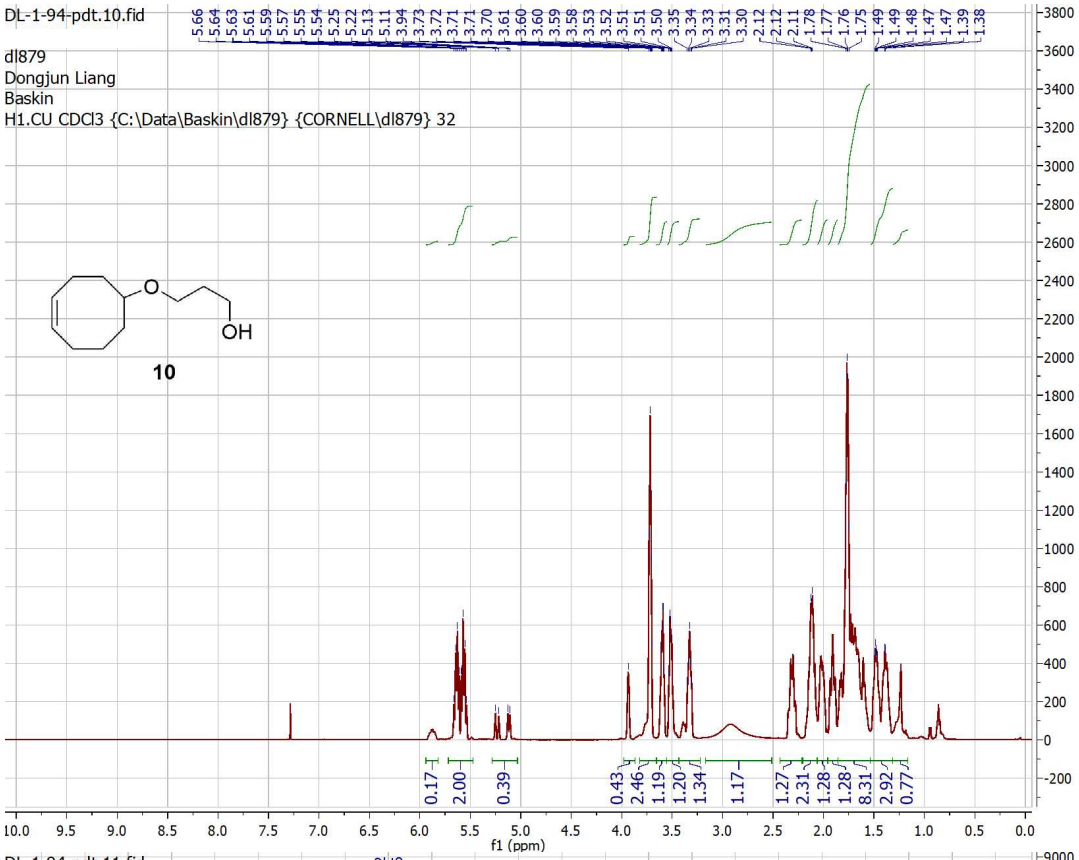
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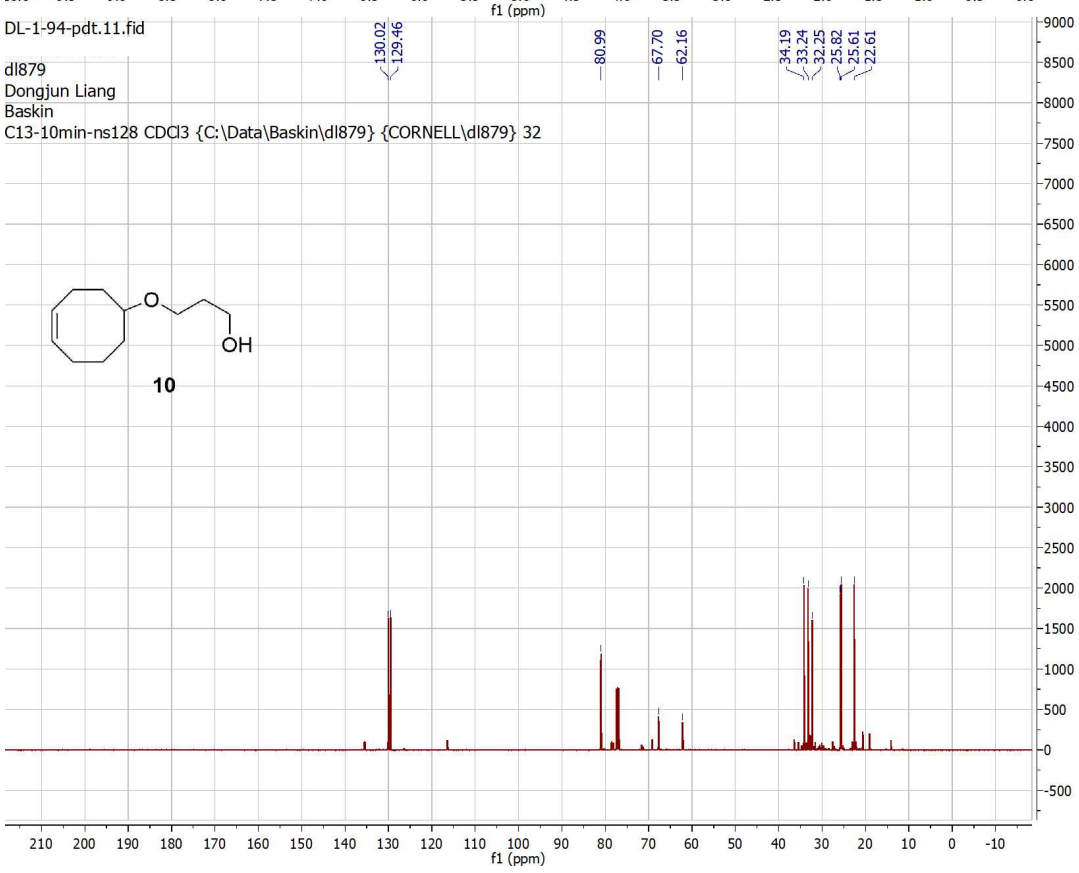
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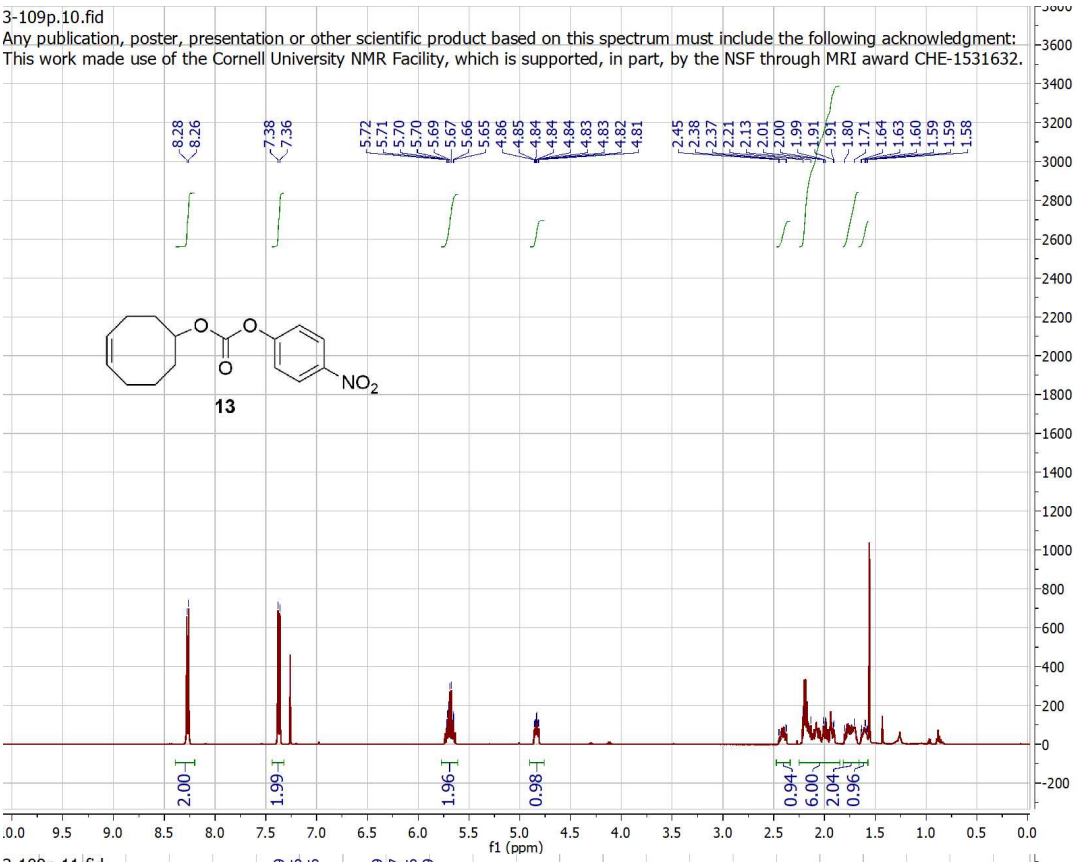
OH
10
f1 (ppm)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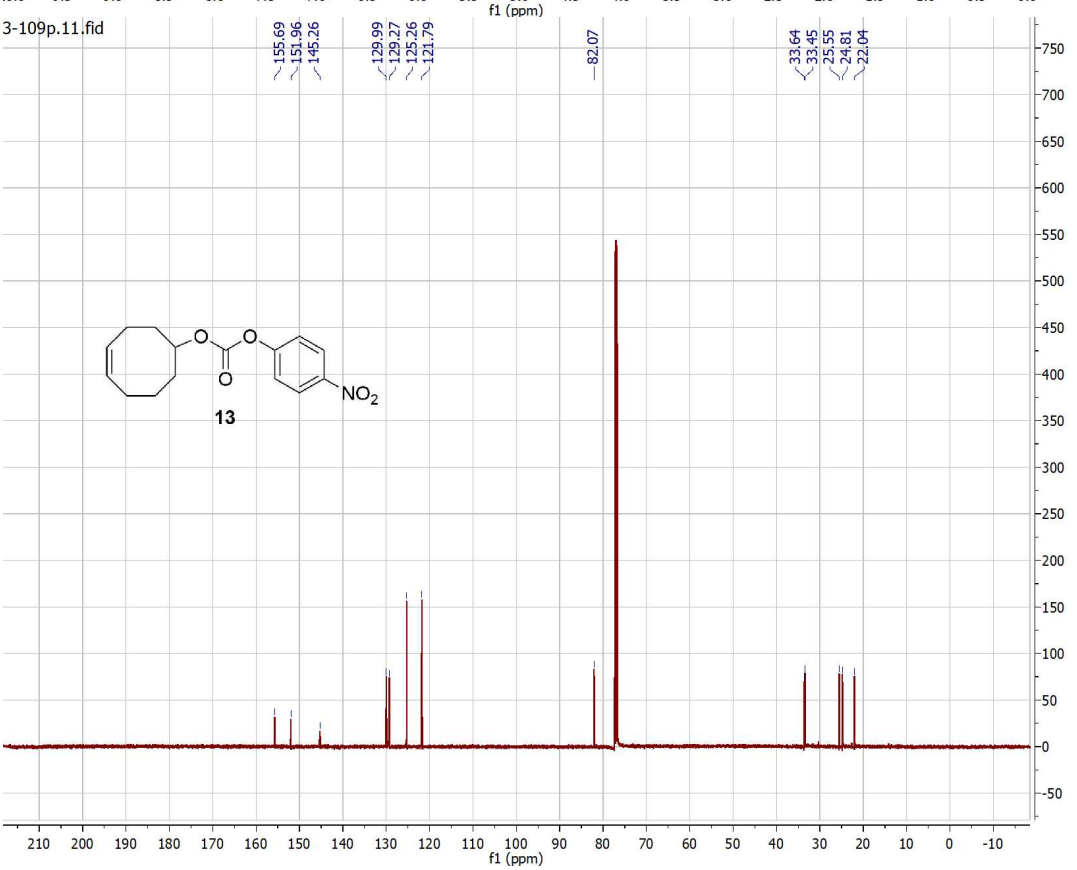
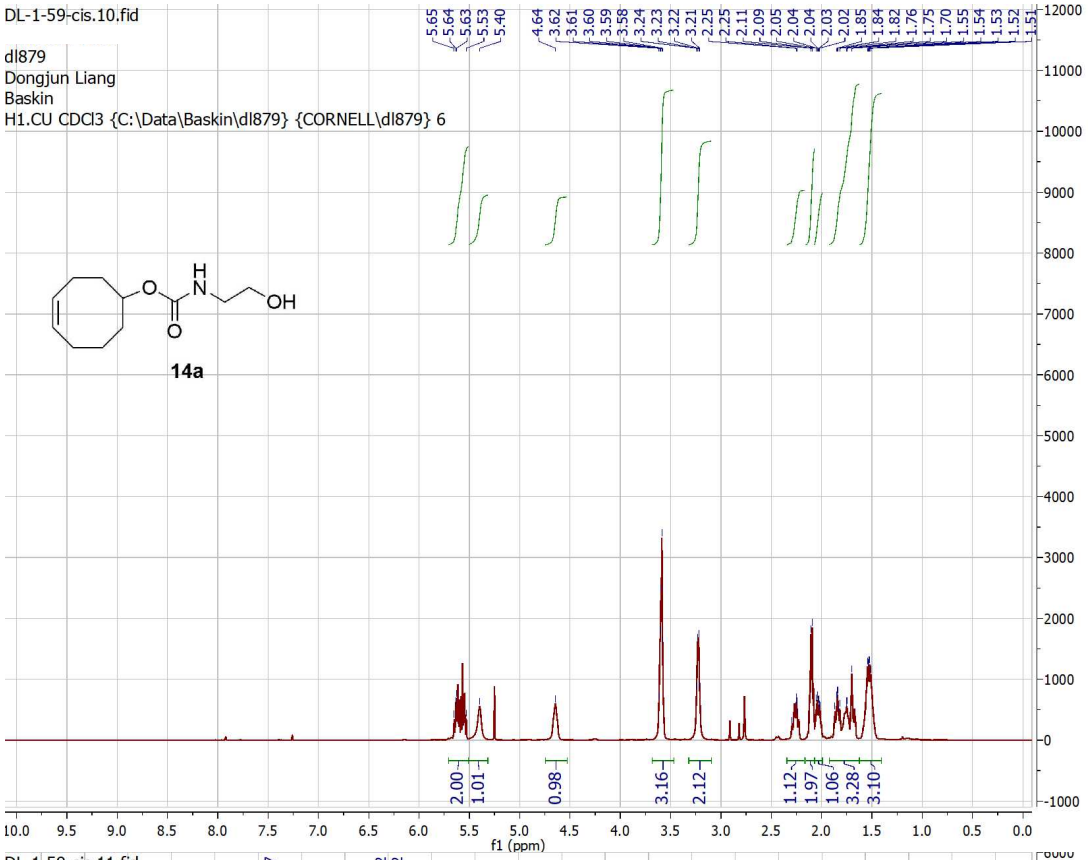
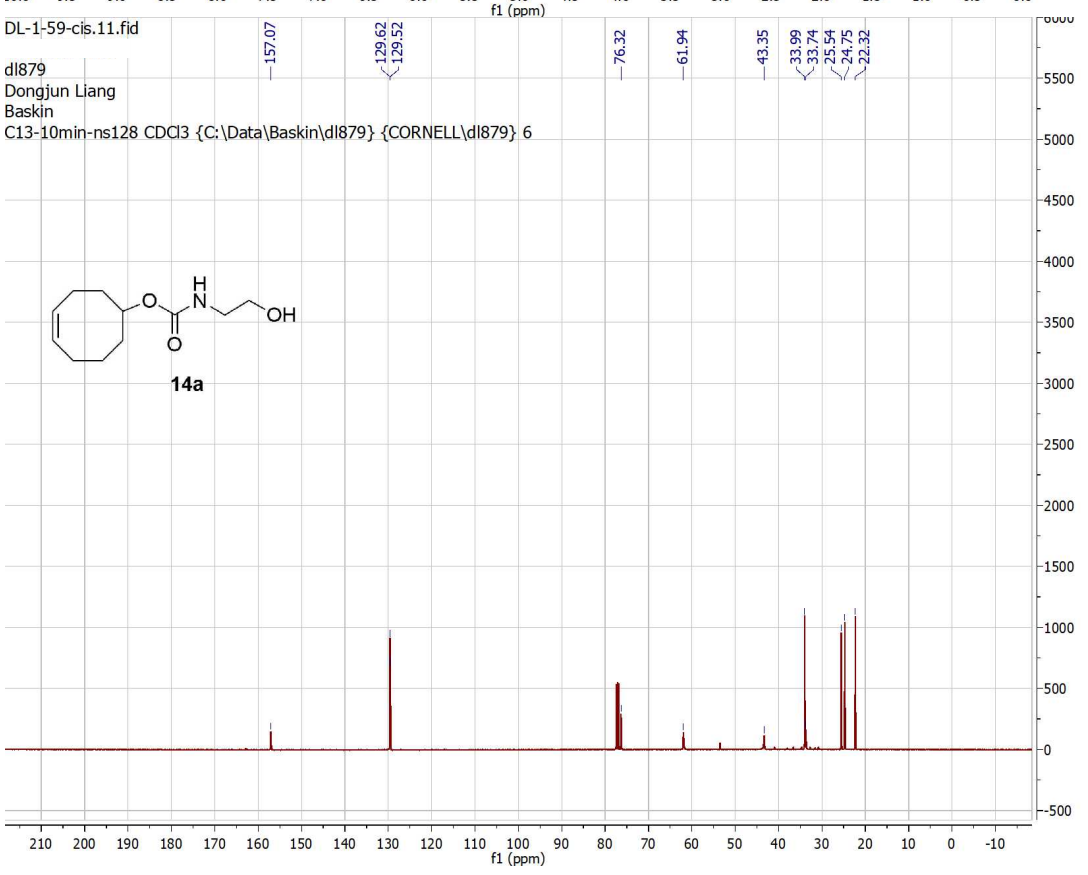
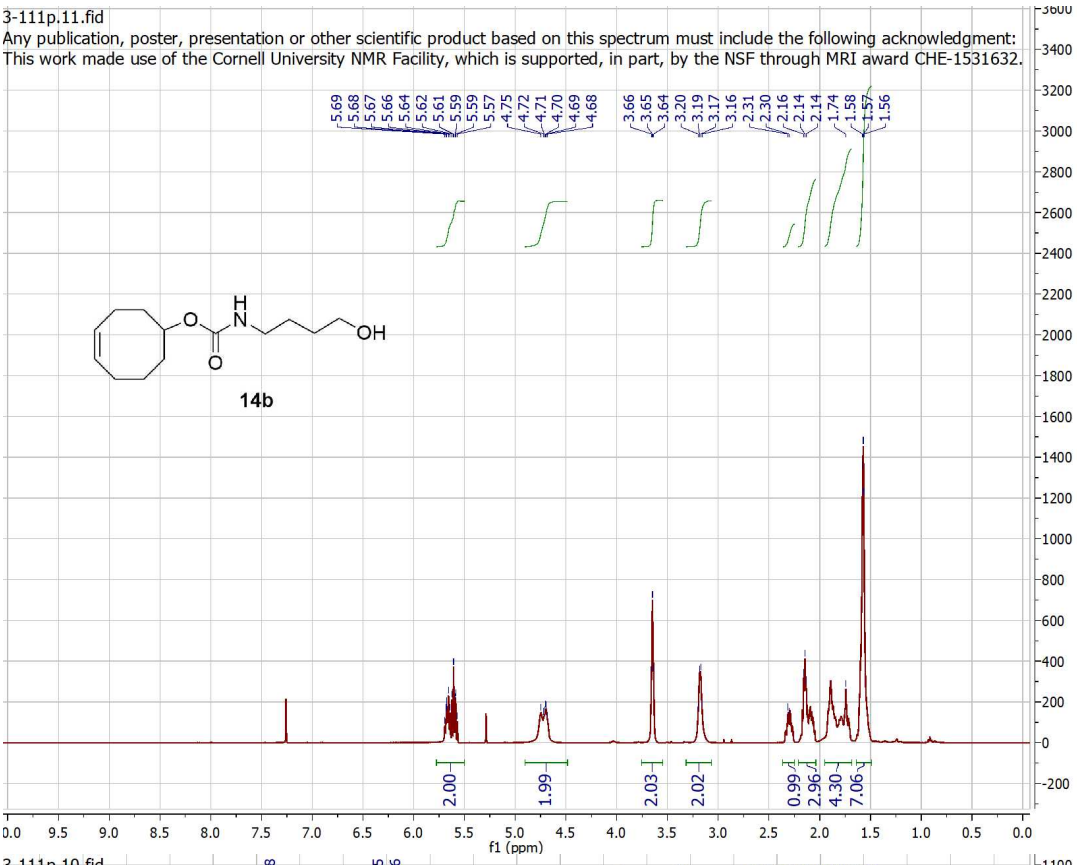
2.04
0.96
f1 (ppm)


3-109p.11.fid
155.69
151.96
145.26
129.99
129.27
125.26
121.79
82.07
33.64
33.45
25.55
24.81
22.04
O
O
O
NO2
13
f1 (ppm)


DL-1-59-cis.10.fid
dl879
Dongjun Liang
Baskin
H1.CU CDCl3 {C:\Data\Baskin\dl879} {CORNELL\dl879} 6
14a
f1 (ppm)


DL-1-59-cis.11.fid
dl879
Dongjun Liang
Baskin
C13-10min-ns128 CDCl3 {C:\Data\Baskin\dl879} {CORNELL\dl879} 6
14a
f1 (ppm)


3-111p.11.fid
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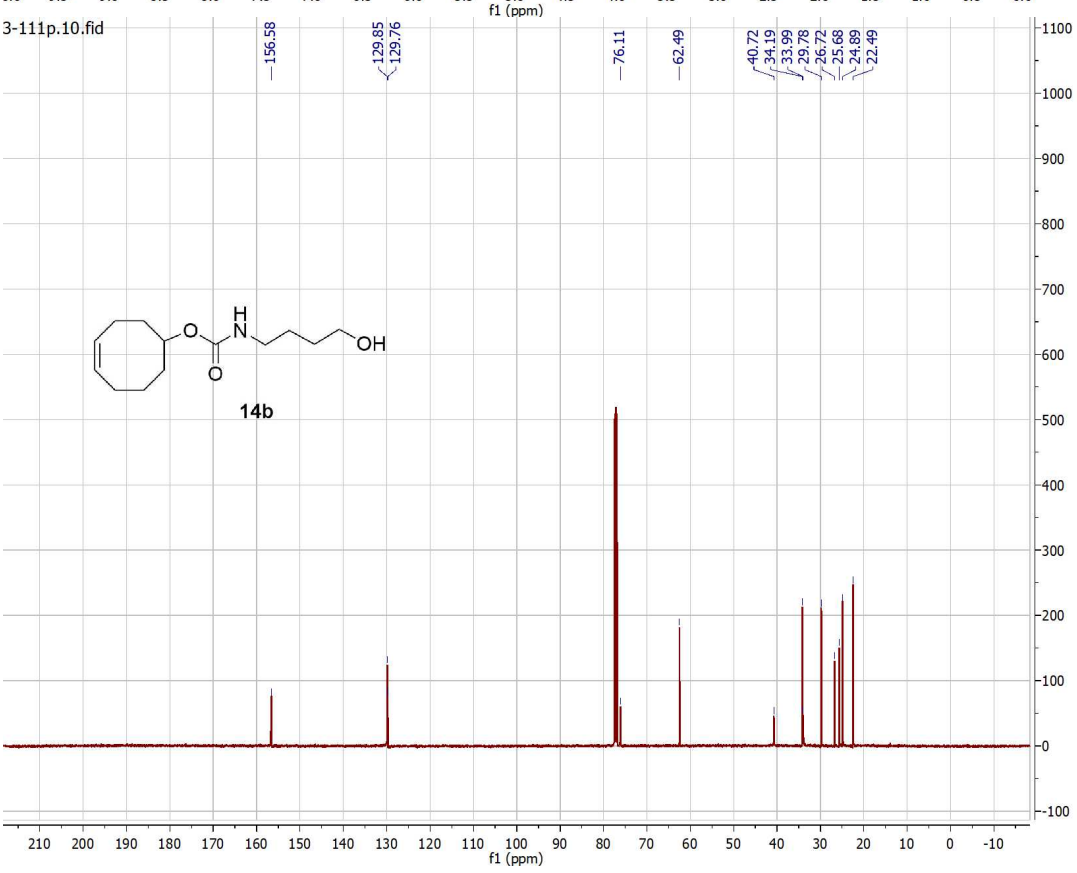
14b
f1 (ppm)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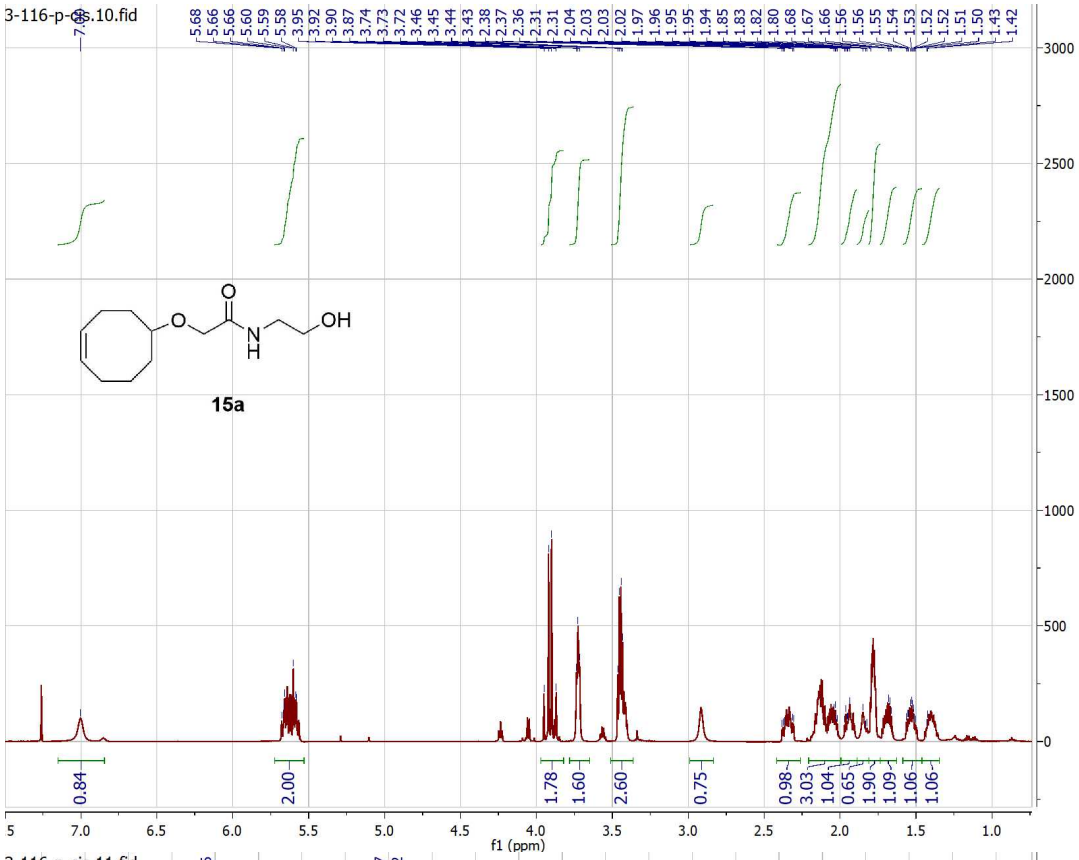
14b
f1 (ppm)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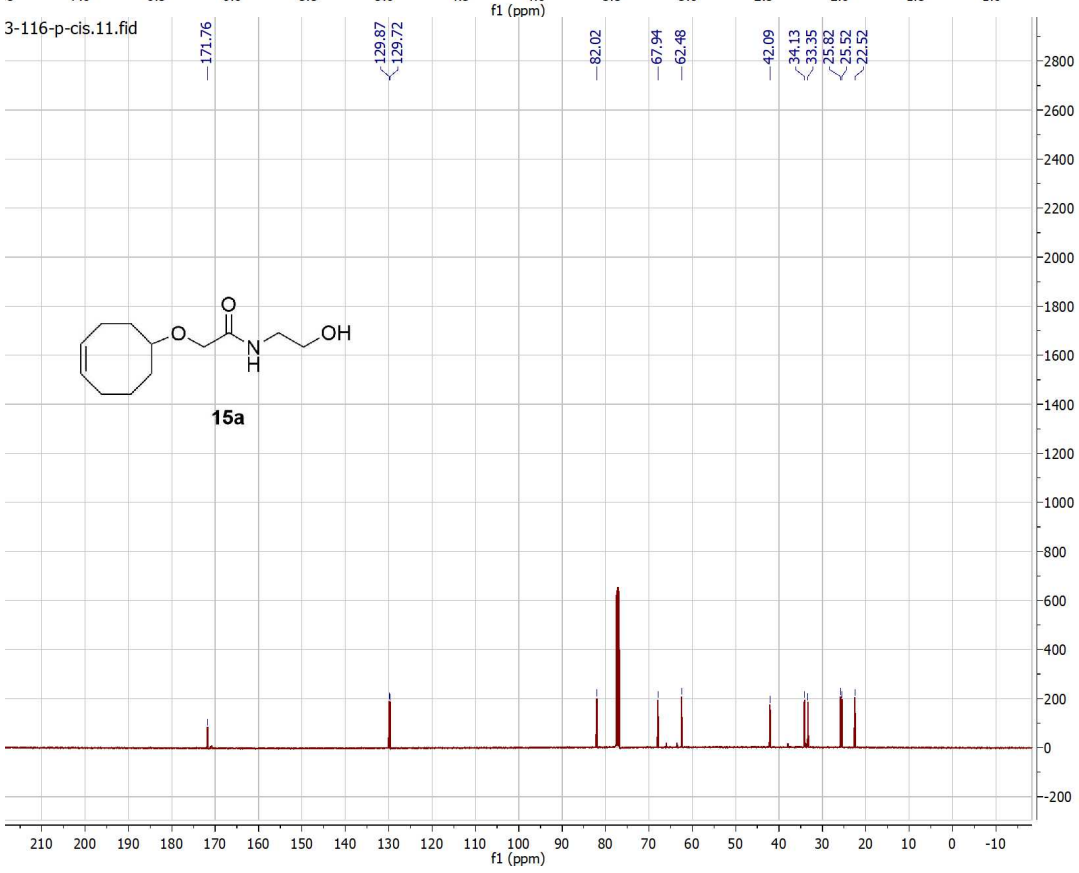
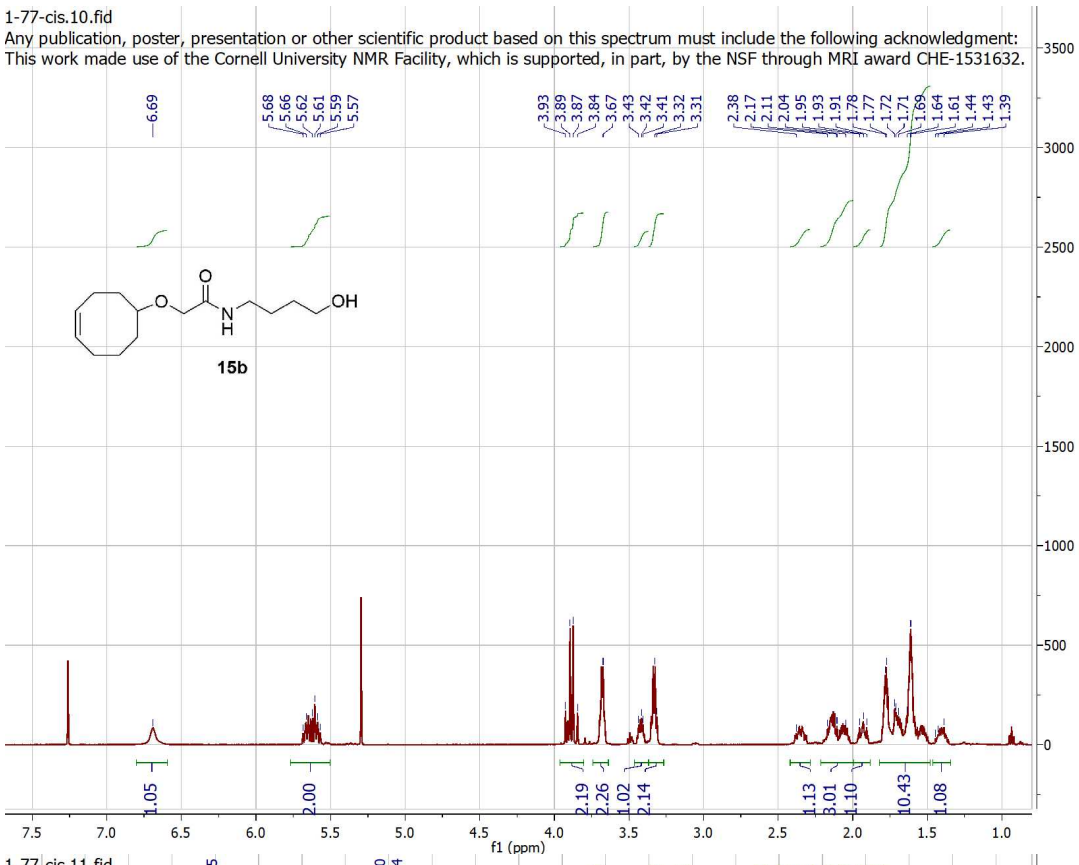
O
O
N
H
OH
15a
f1 (ppm)


3-116-p-cis.11.fid
O
O
N
H
OH
15a
f1 (ppm)


1-77-cis.10.fid
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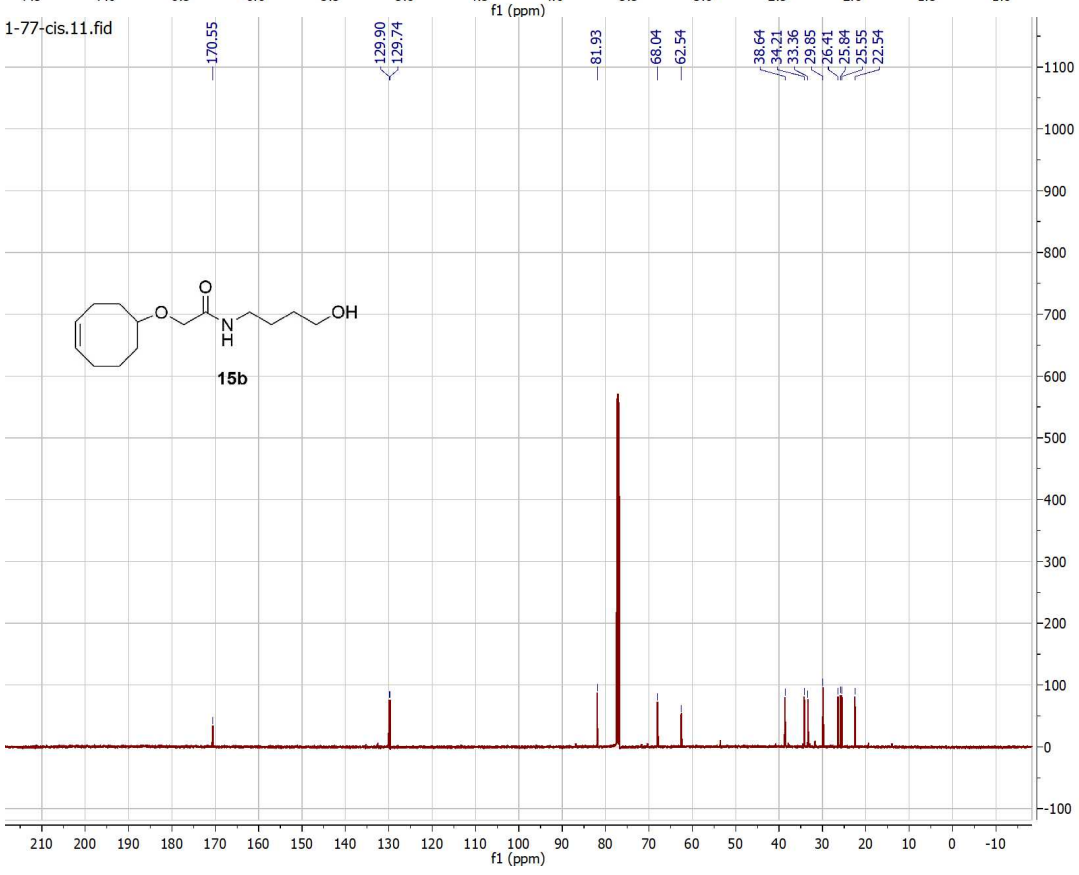
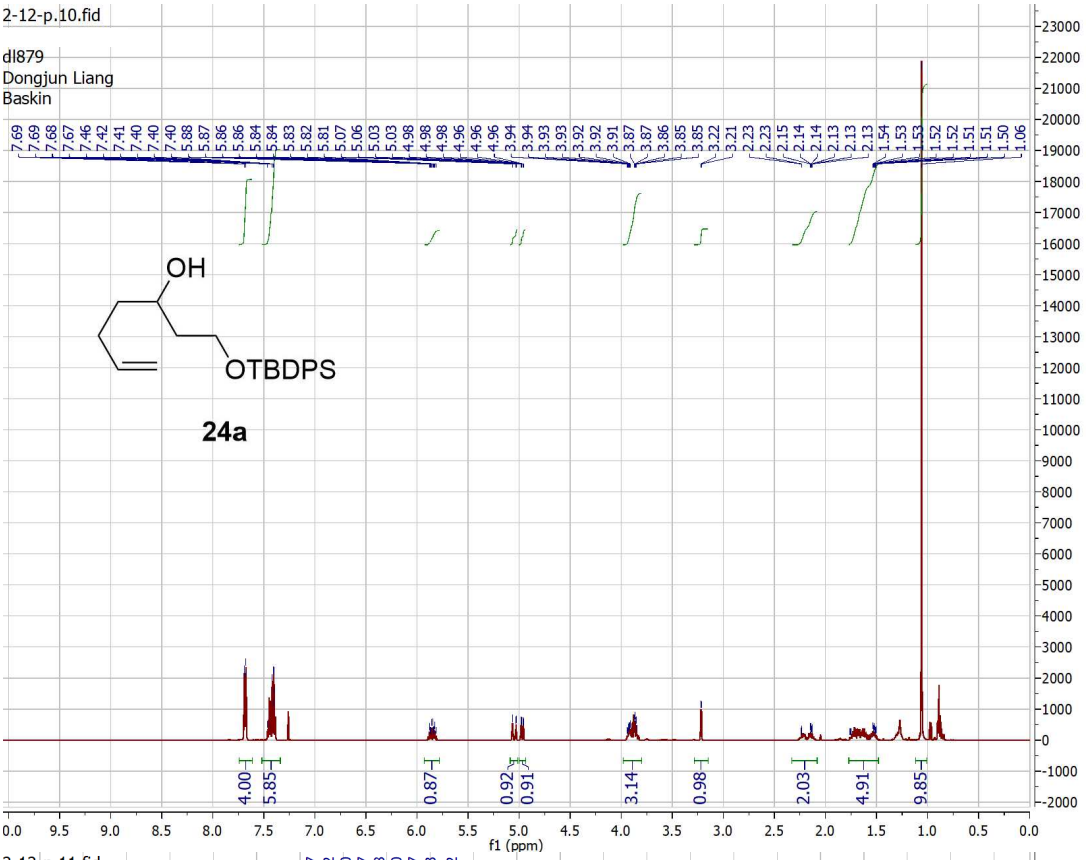
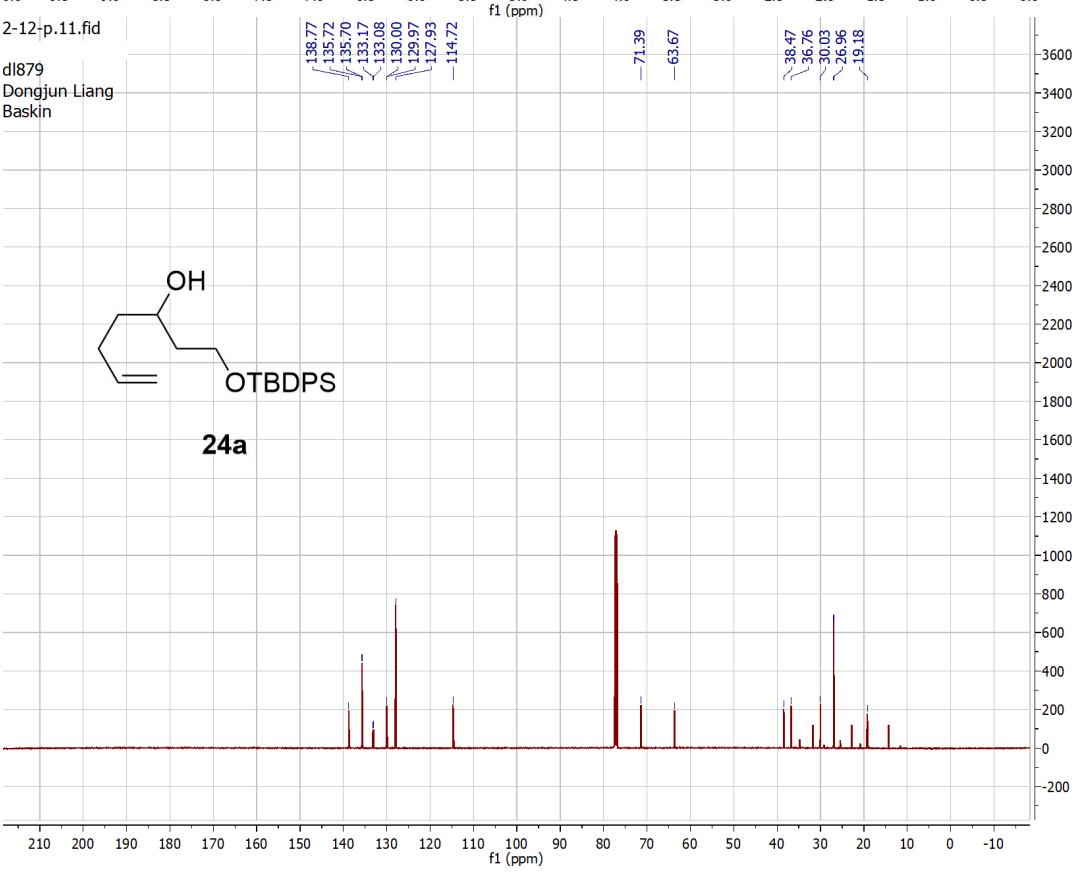
6.69
5.68
5.66
5.62
5.61
5.59
5.57
3.93
3.89
3.87
3.84
3.67
3.43
3.42
3.41
3.32
3.31
2.38
2.17
2.11
2.04
1.95
1.93
1.91
1.78
1.77
1.72
1.71
1.69
1.64
1.61
1.44
1.43
1.39
O
O
N
H
OH
15b
1.05
2.00
2.19
2.26
1.02
2.14
1.13
3.01
1.10
10.43
1.08
f1 (ppm)


1-77-cis.11.fid
170.55
129.90
129.74
81.93
68.04
62.54
38.64
34.21
33.36
29.85
26.41
25.84
25.55
22.54
O
O
N
H
OH
15b
f1 (ppm)


2-12-p.10.fid
dl879
Dongjun Liang
Baskin
OH
OTBDPS
24a
f1 (ppm)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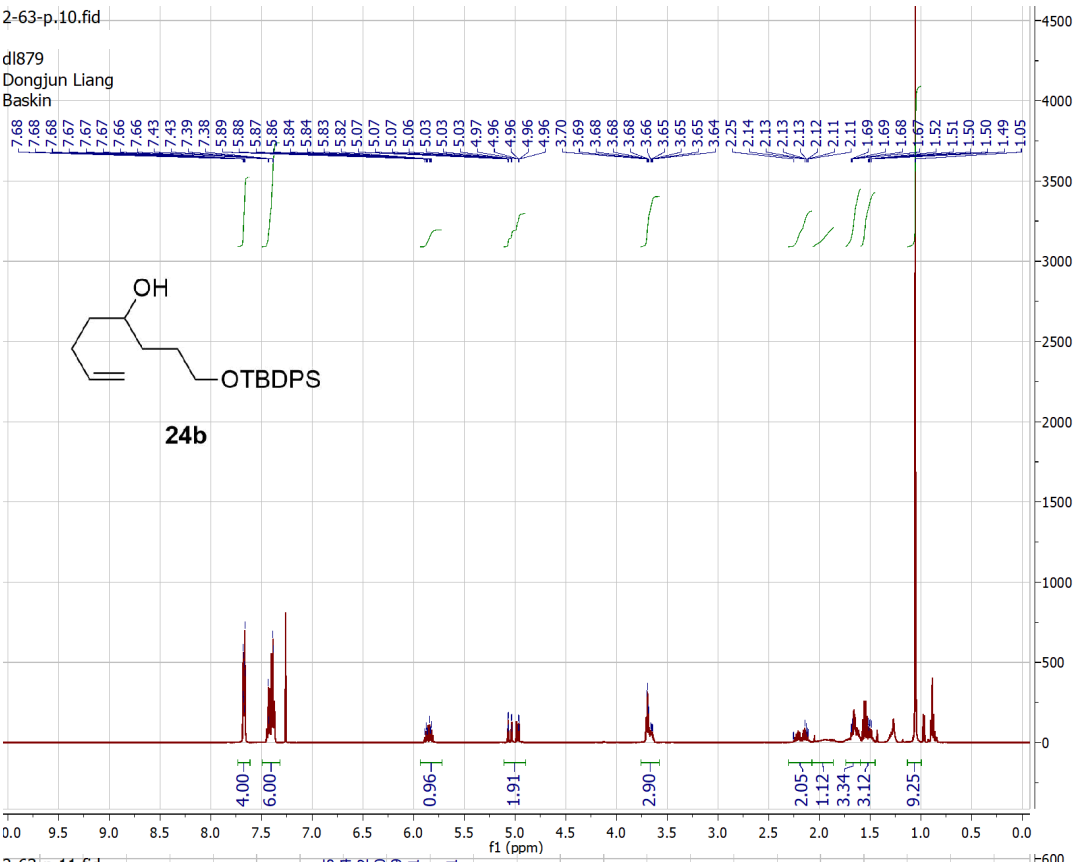
dl879
Dongjun Liang
Baskin
OH
OTBDPS
24a
f1 (ppm)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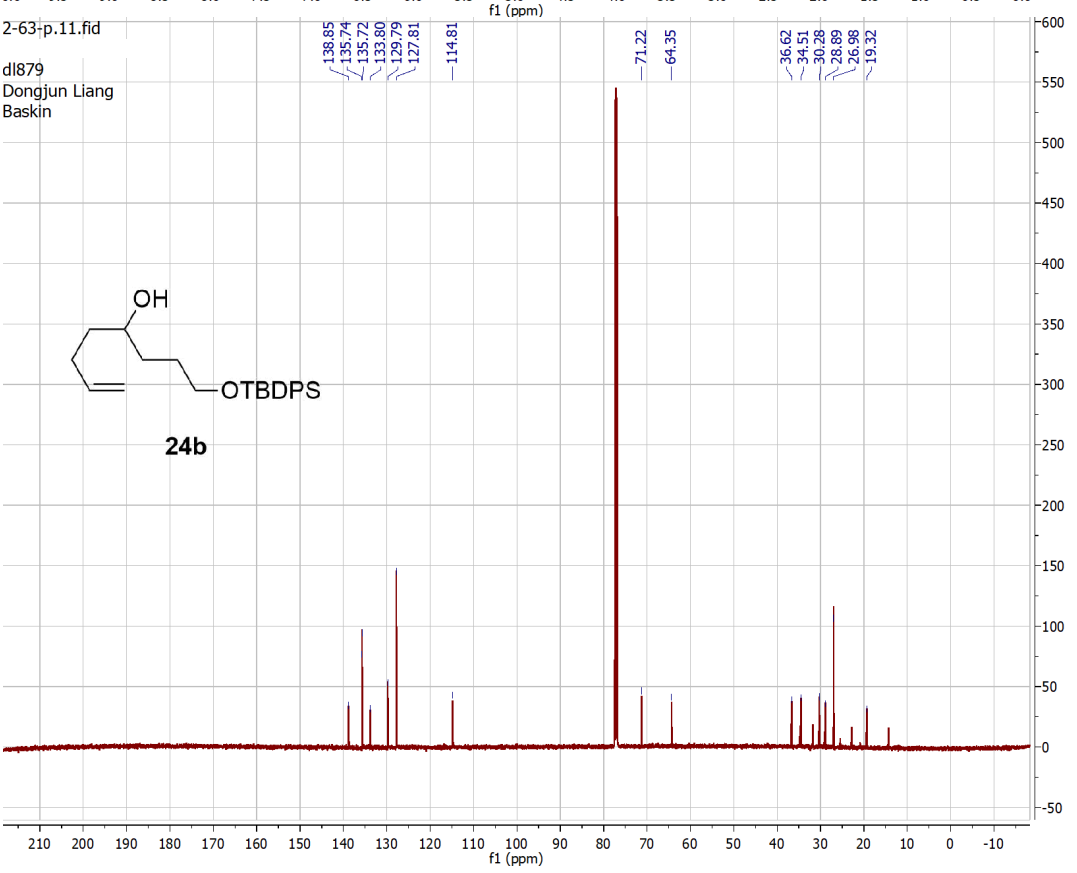
dl879
Dongjun Liang
Baskin
OH
OTBDPS
24b
f1 (ppm)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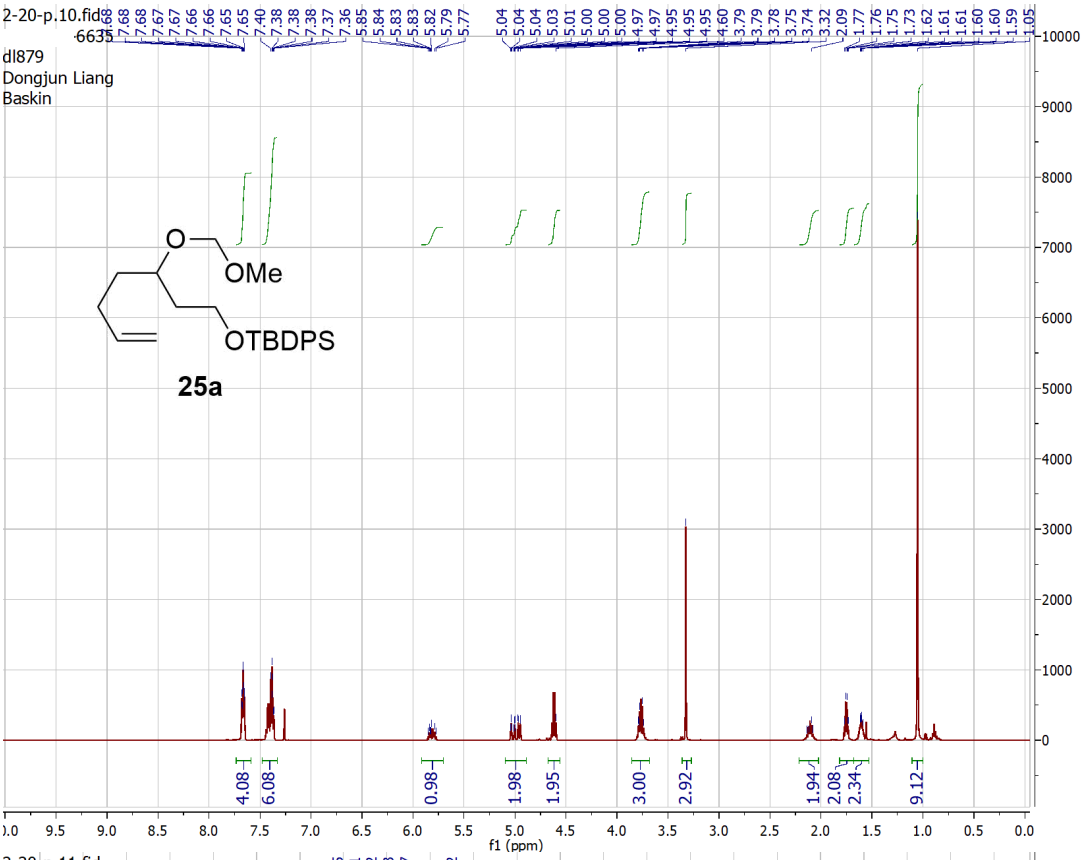
dl879
Dongjun Liang
Baskin
OH
OTBDPS
24b
f1 (ppm)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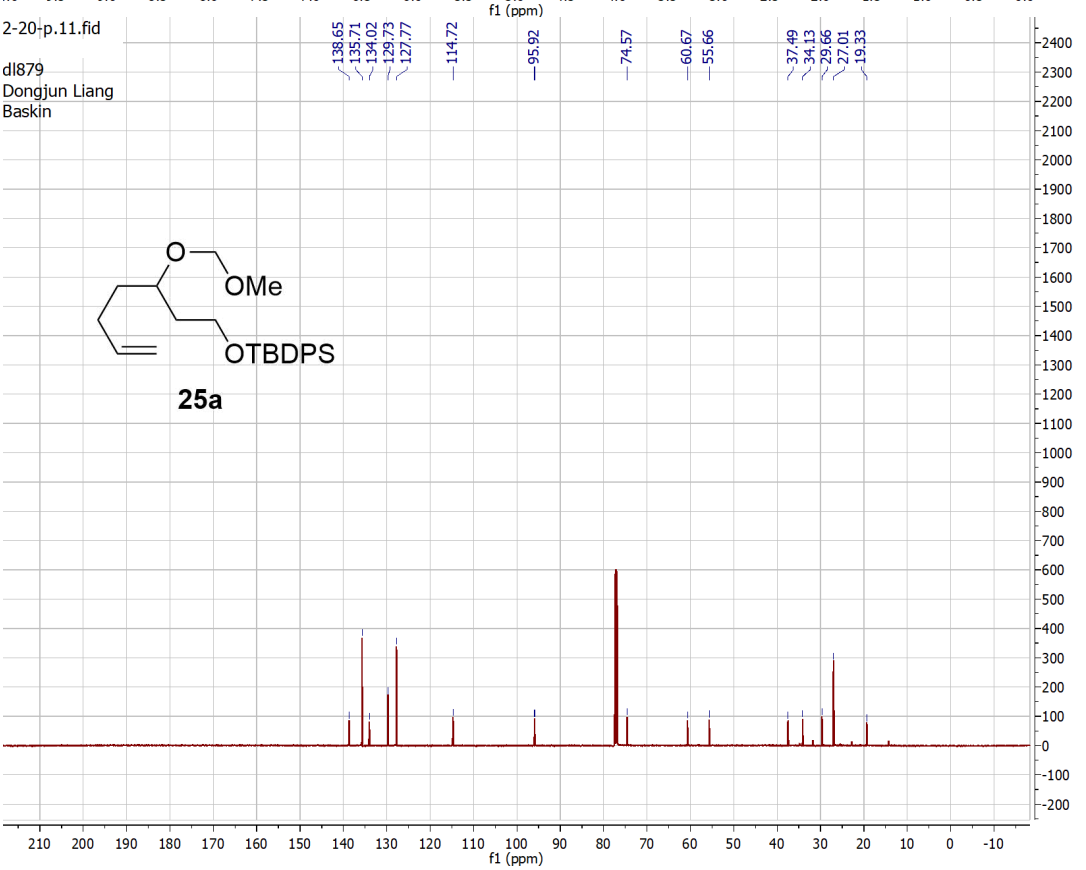
6635
dl879
Dongjun Liang
Baskin
O
OMe
OTBDPS
25a
4.08
6.08
0.98
1.98
1.95
3.00
2.92
1.94
2.08
2.34
9.12
f1 (ppm)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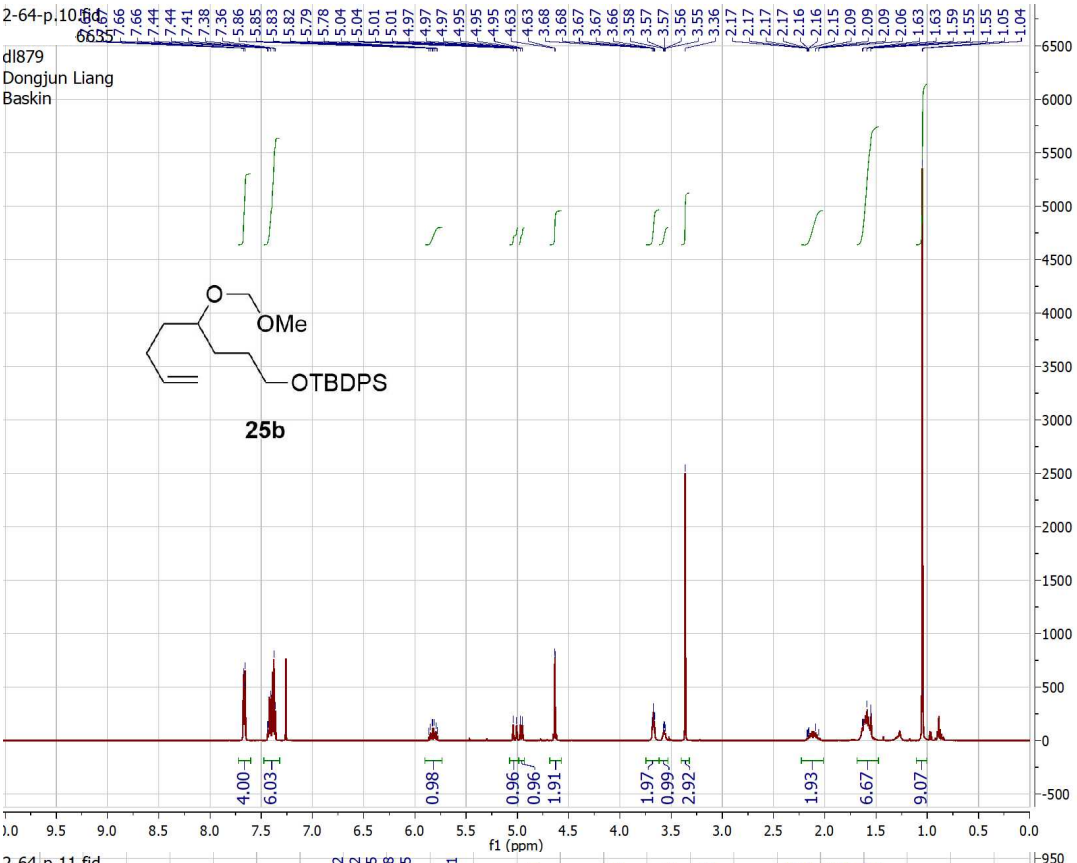
dl879
Dongjun Liang
Baskin
138.65
135.71
134.02
129.73
127.77
114.72
95.92
74.57
60.67
55.66
37.49
34.13
29.66
27.01
19.33
O
OMe
OTBDPS
25a
f1 (ppm)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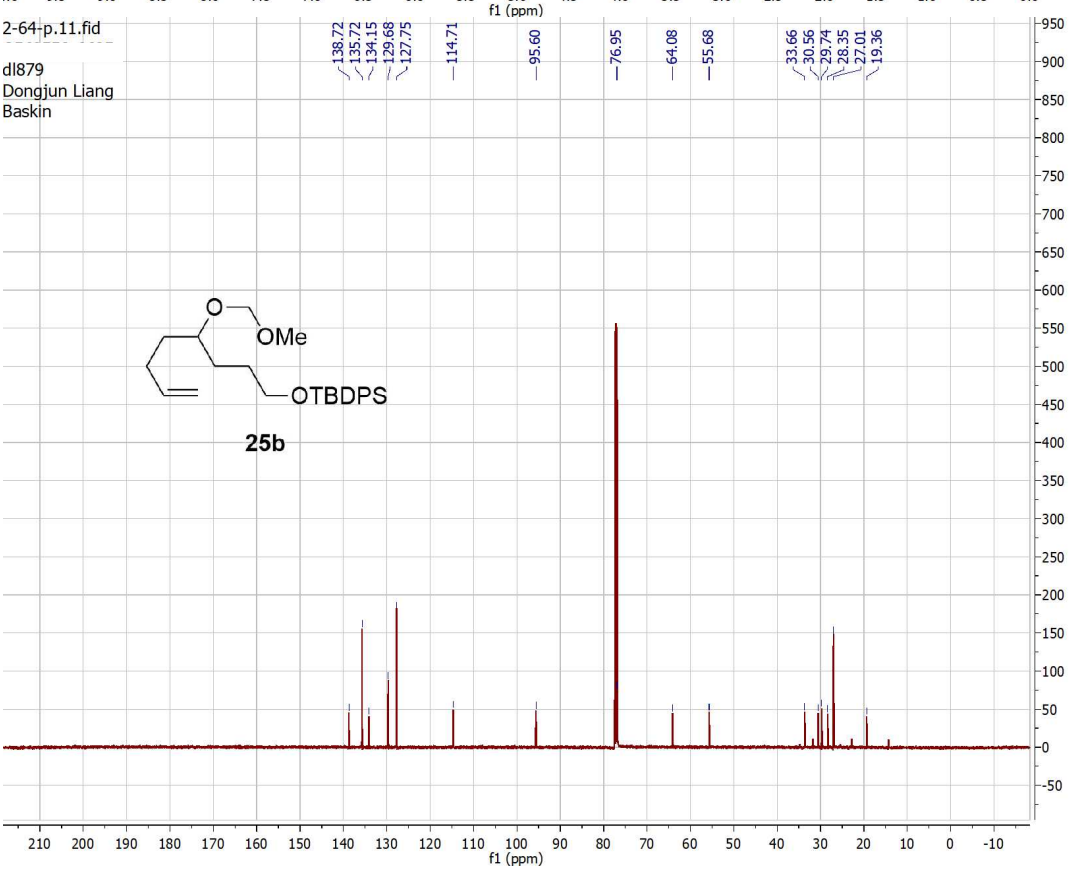
dl879
Dongjun Liang
Baskin
OMe
OTBDPS
25b
f1 (ppm)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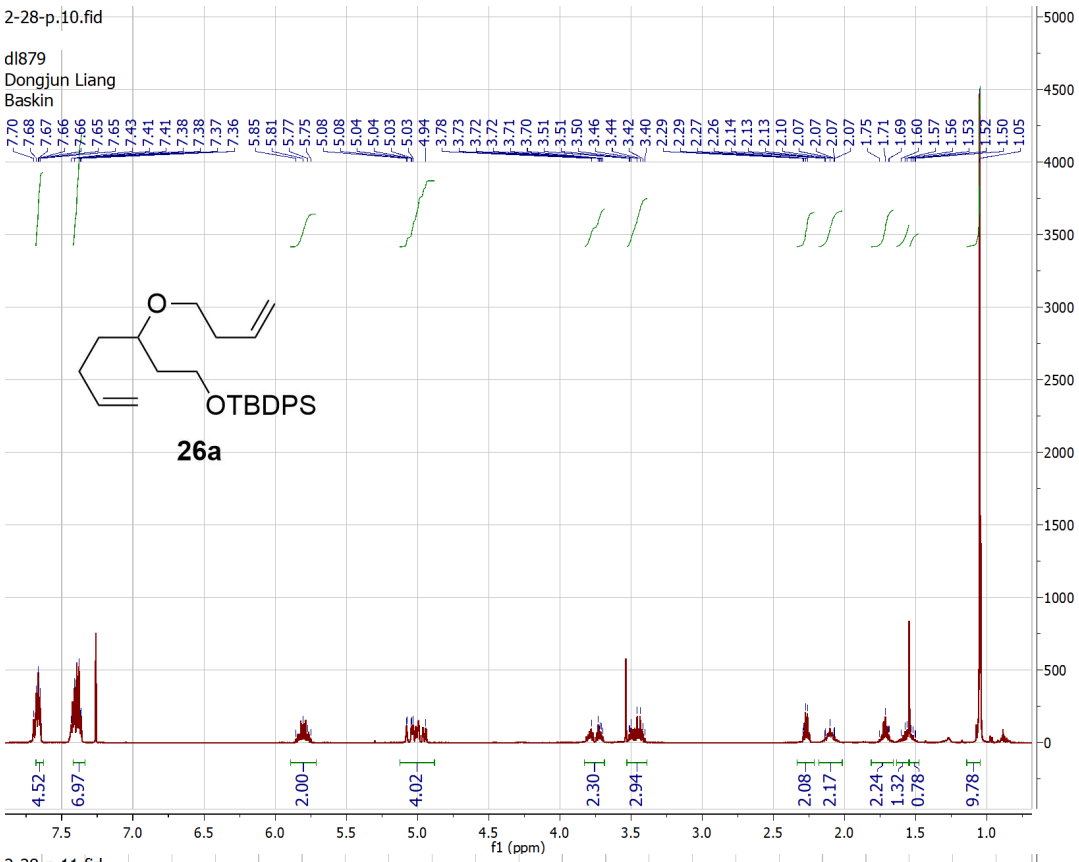
Dongjun Liang
Baskin
OMe
OTBDPS
25b
f1 (ppm)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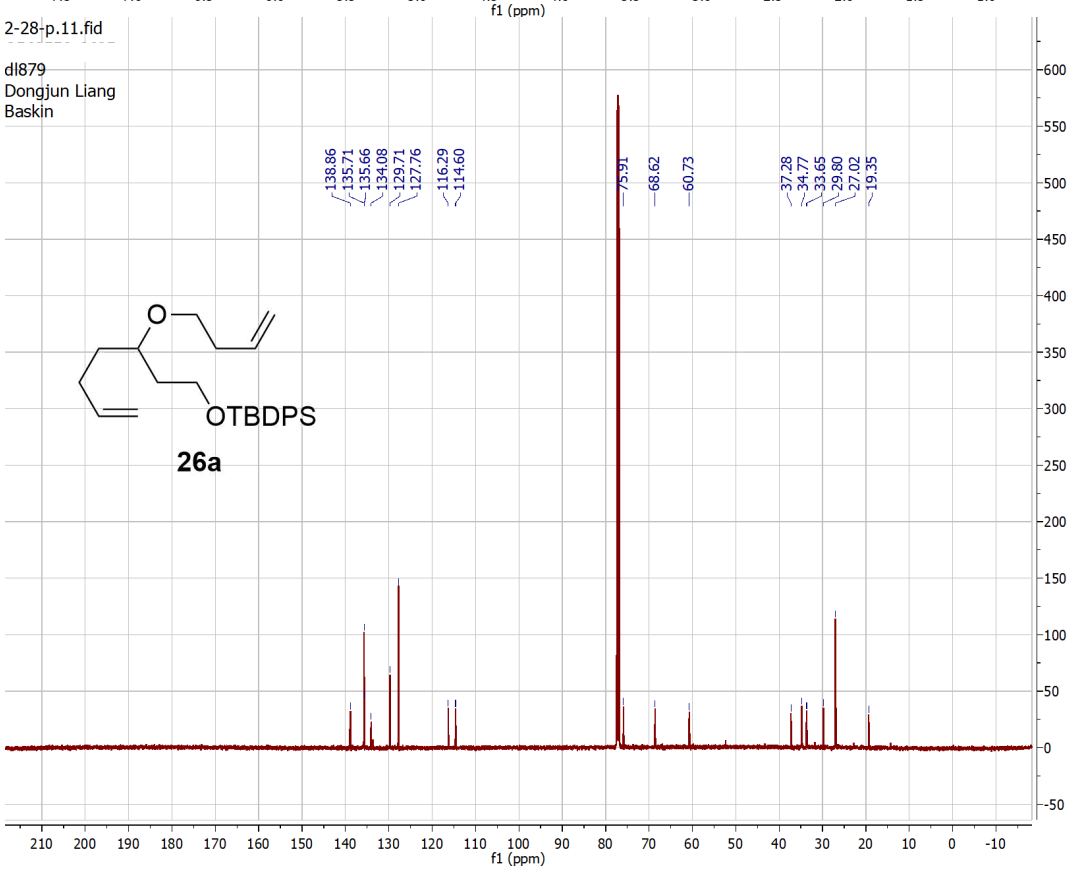
dl879
Dongjun Liang
Baskin
O
OTBDPS
26a
f1 (ppm)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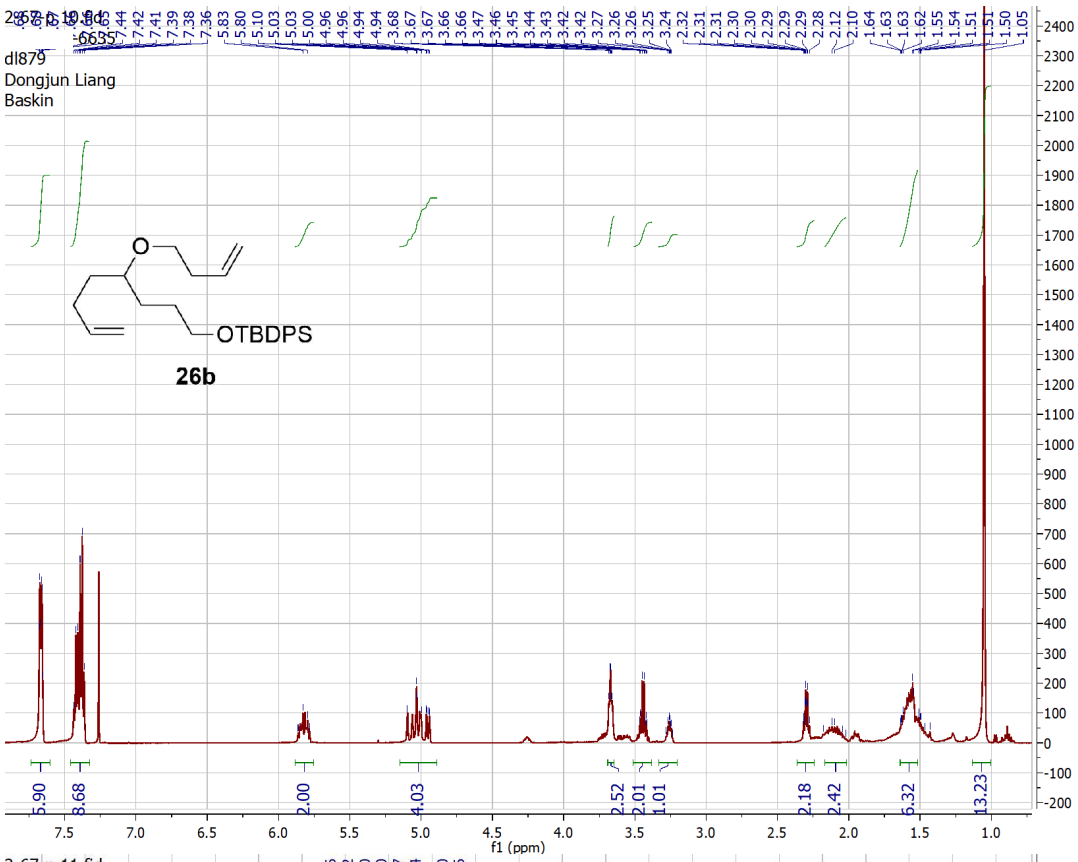
dl879
Dongjun Liang
Baskin
O
OTBDPS
26a
f1 (ppm)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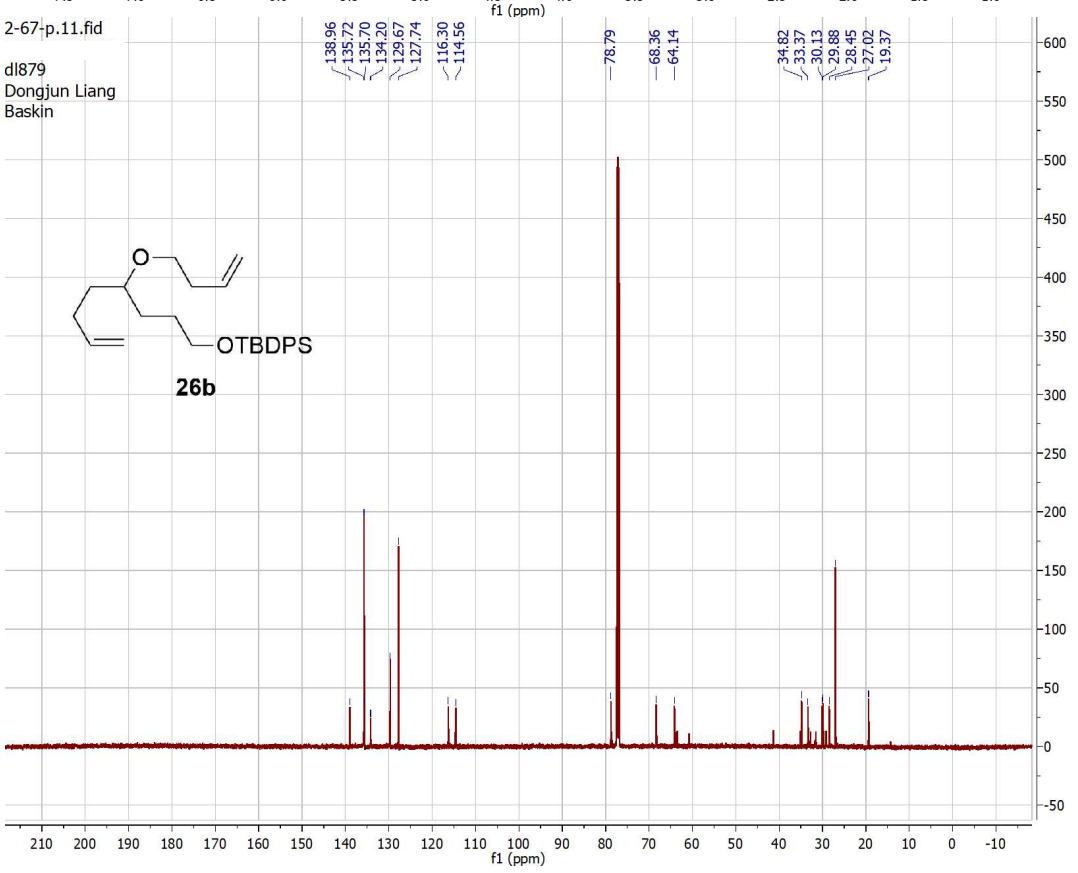
6635
dl879
Dongjun Liang
Baskin
O
OTBDPS
26b
5.90
3.68
2.00
4.03
2.52
2.01
1.01
2.18
2.42
6.32
13.23
f1 (ppm)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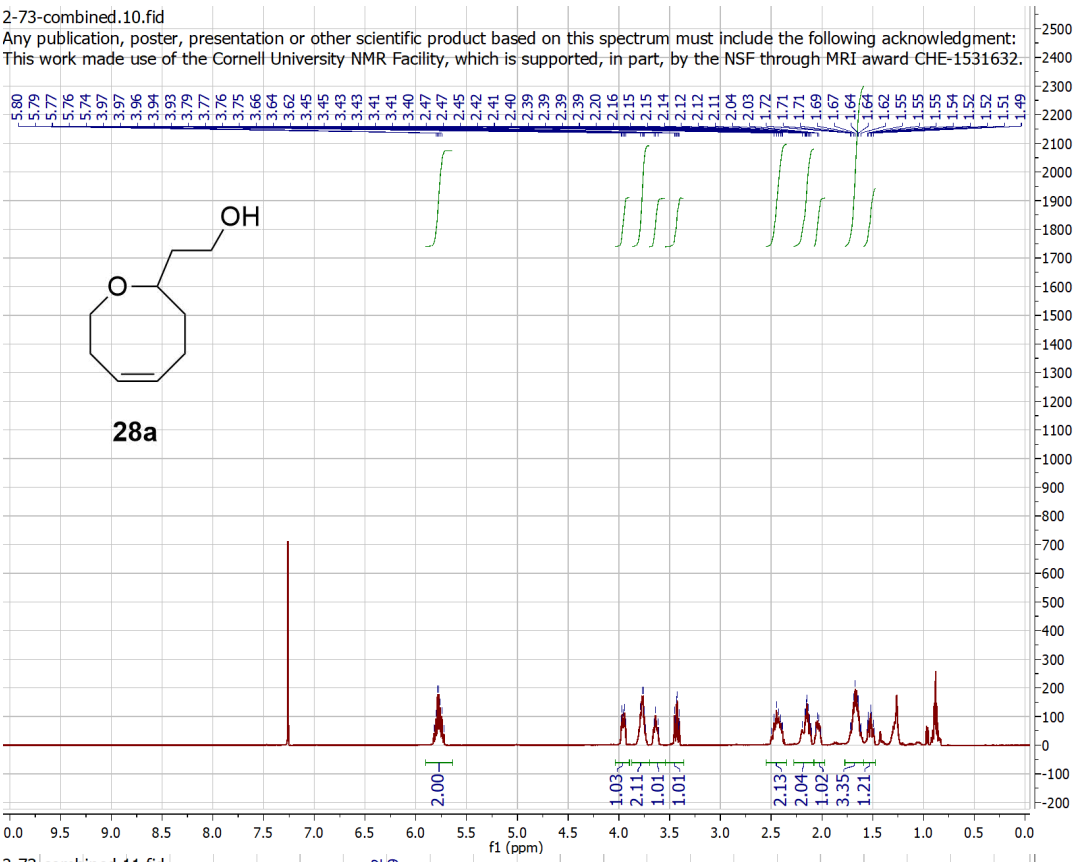
dl879
Dongjun Liang
Baskin
138.96
135.72
135.70
134.20
129.67
127.74
116.30
114.56
78.79
68.36
64.14
34.82
33.37
30.13
29.88
28.45
27.02
19.37
O
OTBDPS
26b
f1 (ppm)


2-73-combined.10.fid
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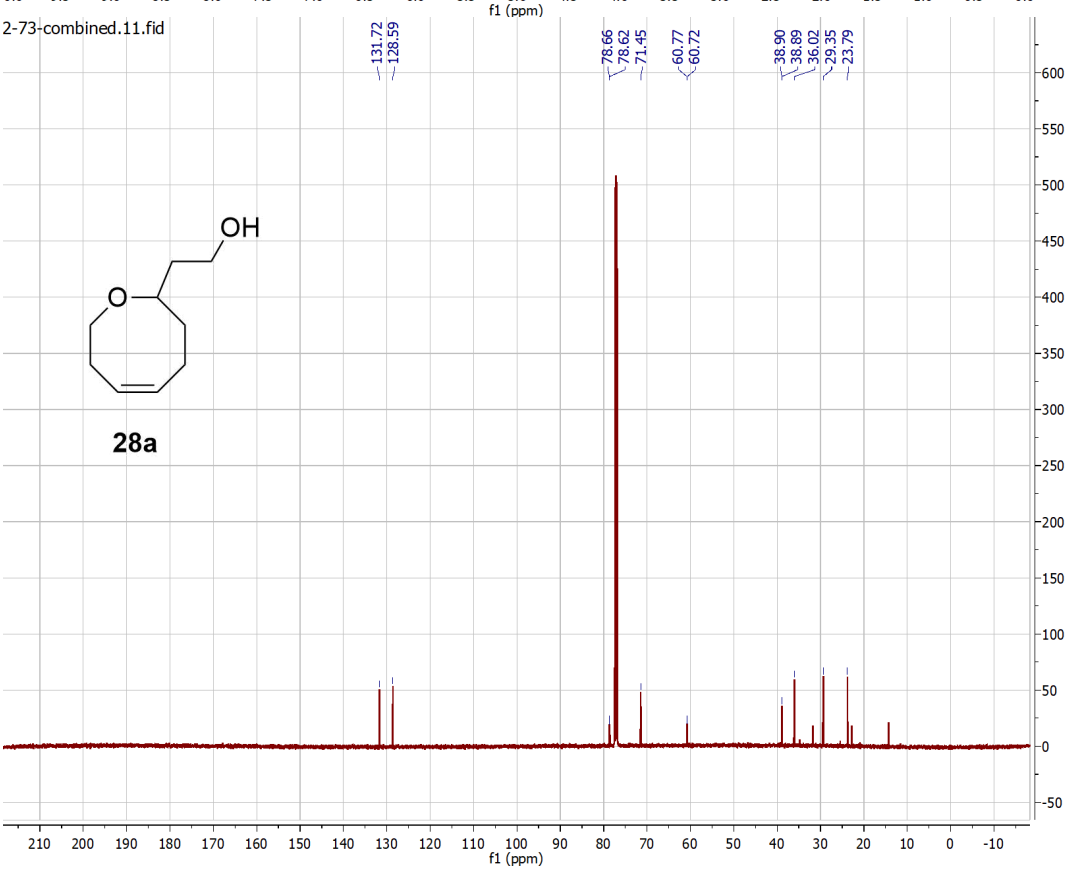
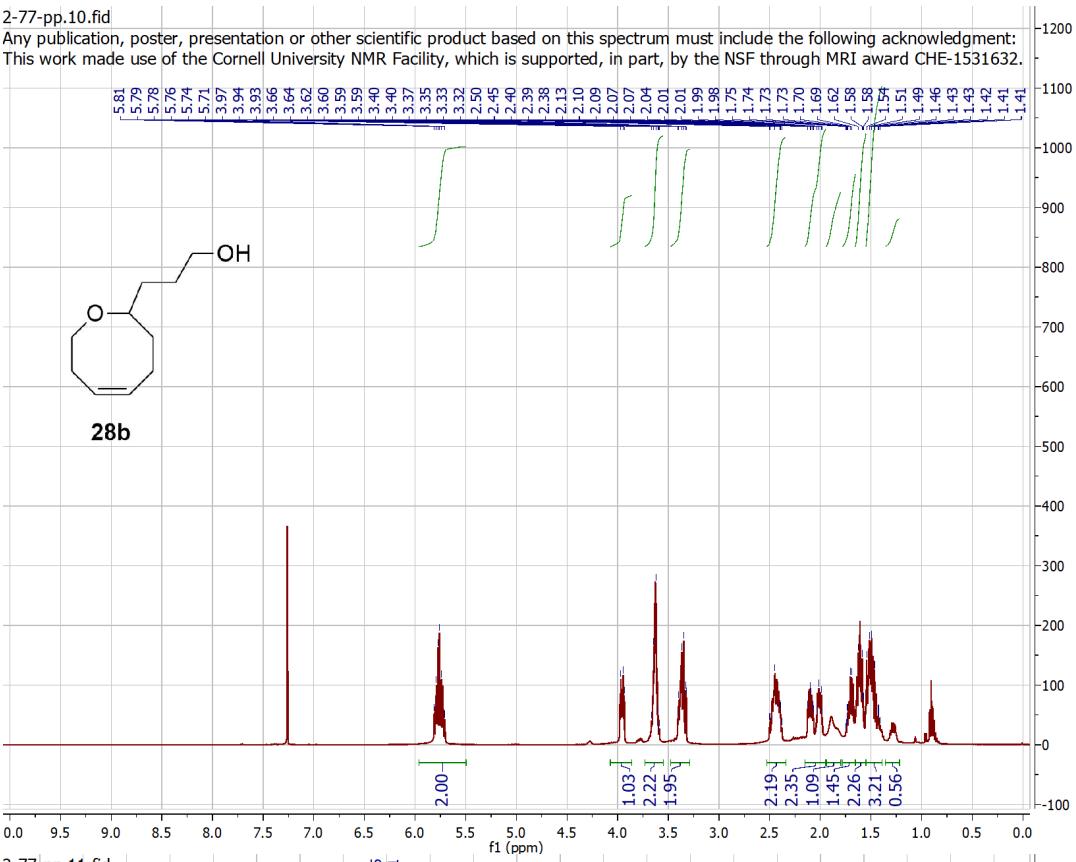
OH
O
28a
2.00
1.03
2.11
1.01
1.01
2.13
2.04
1.02
3.35
1.21
f1 (ppm)


2-73-combined.11.fid
131.72
128.59
78.66
78.62
71.45
60.77
60.72
38.90
38.89
36.02
29.35
23.79
OH
O
28a
f1 (ppm)


2-77-pp.10.fid
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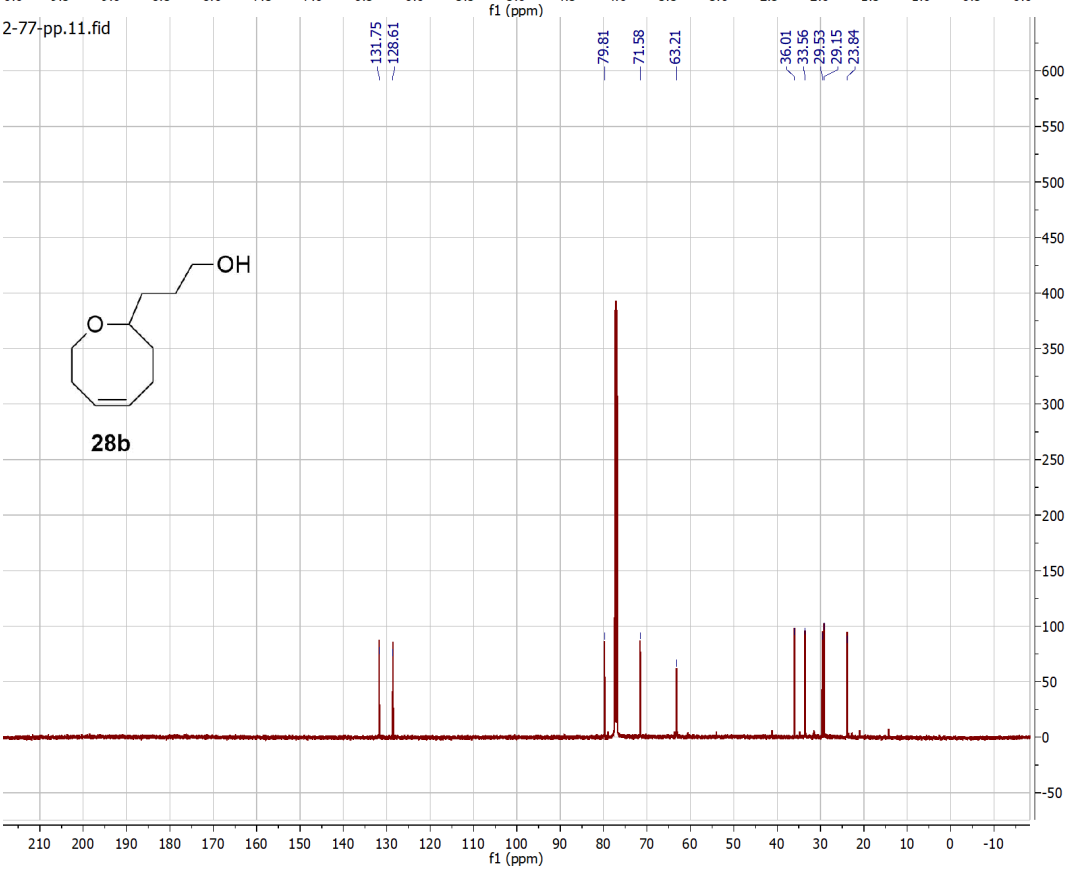
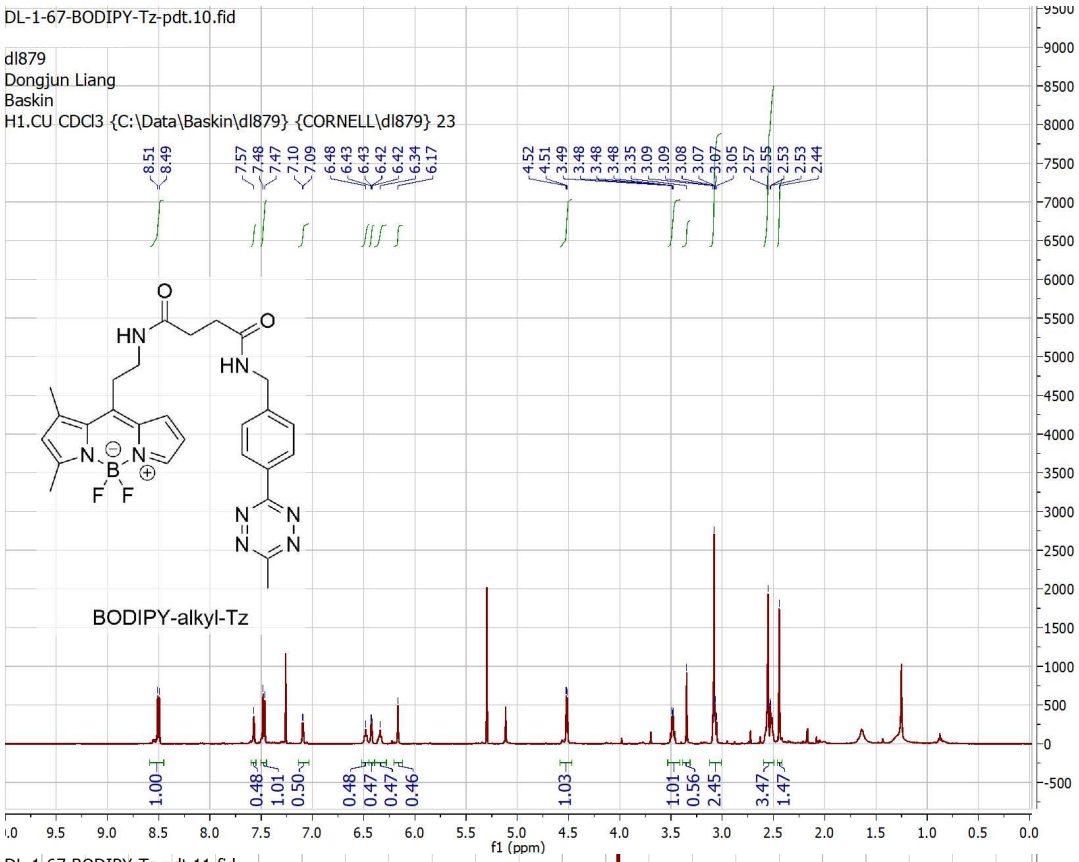
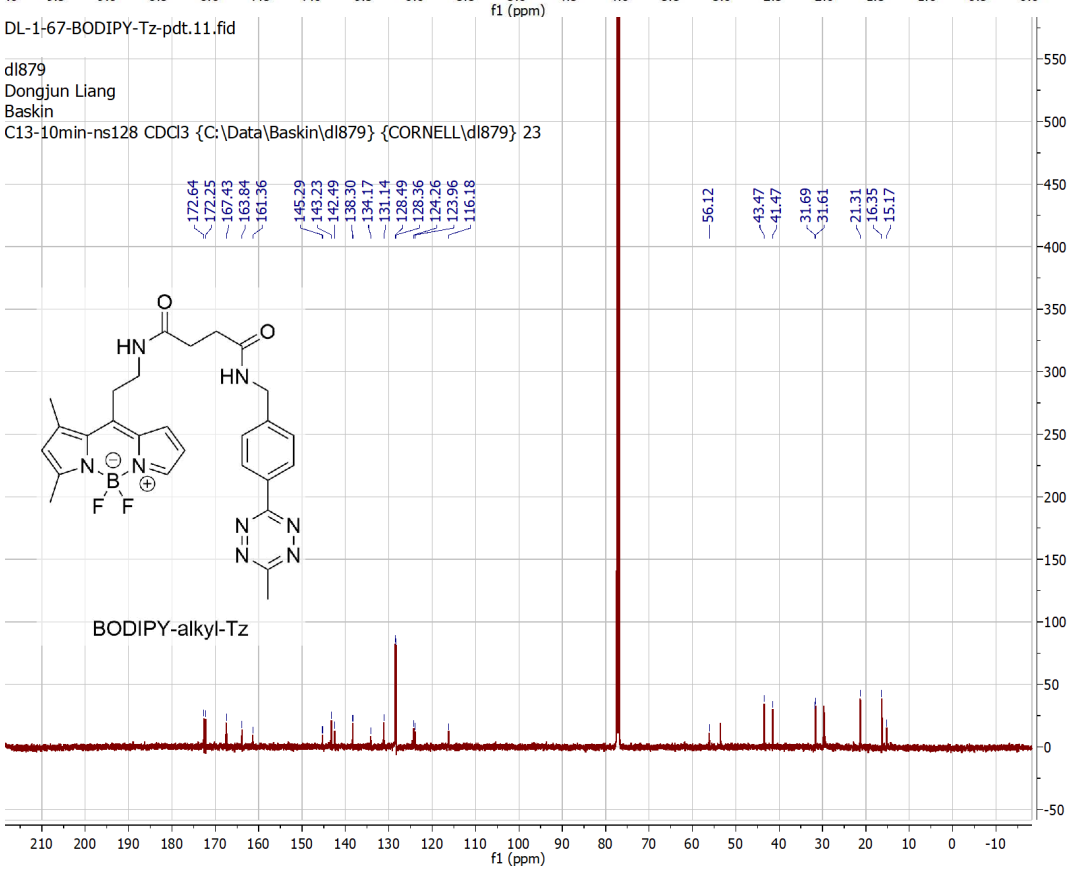
OH
O
28b
f1 (ppm)


2-77-pp.11.fid
OH
O
28b
f1 (ppm)


DL-1-67-BODIPY-Tz-pdt.10.fid
dl879
Dongjun Liang
Baskin
H1.CU CDCl3 {C:\Data\Baskin\dl879} {CORNELL\dl879} 23
BODIPY-alkyl-Tz
f1 (ppm)


DL-1-67-BODIPY-Tz-pdt.11.fid
dl879
Dongjun Liang
Baskin
C13-10min-ns128 CDCl3 {C:\Data\Baskin\dl879} {CORNELL\dl879} 23
BODIPY-alkyl-Tz
f1 (ppm)