Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

Bio-orthogonal chemistry enables solid phase synthesis and HPLC & gel-free purification of long RNA oligonucleotides

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Materials and Methods

All oligonucleotide solid phase syntheses were done on a 1.0 μmol scale using the Oligo-800 synthesizer (Azco Biotech, Oceanside, CA, USA). Solid phase syntheses were performed on control-pore glass (CPG-1000) purchased from Glen Research (Sterling, VA, USA). Other oligonucleotide solid phase synthesis reagents were obtained from ChemGenes Corporation (Wilmington, MA, USA). Phosphoramidites (TBDMS as the 2′-OH protecting group): rA was N-Bz protected, rC was N-Ac protected and rG was N-iBu protected. Coupling step was done using 5-ethylthio-1H-tetrazole solution (0.25 M) in acetonitrile for 12 min. 5'-detritylation step was done using 3% trichloroacetic acid in CH_2Cl_2. Oxidation step was done using I_2 (0.02 M) in THF/pyridine/H_2O solution. CPG modifications were carried out using native amino lcaa CPG 1000 Å, purchased from ChemGenes (Wilmington, MA, USA), Cat.# N-5100-10.

For gel electrophoresis, 10X Tris/Borate/EDTA (TBE) buffer was purchased from Fisher Scientific Company L.L.C. (Waltham, MA, USA) and used with proper dilution. 30% Arcylamide/Bis-arcylamide solution (29:1) was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA).

Chromatographic purifications of synthetic materials were conducted using SiliaSphere™ spherical silica gel with an average particle and pore size of 5 μm and 60 Å, respectively (Silicycle Inc, QC, Canada). Thin layer chromatography (TLC) was performed on SiliaPlate™ silica gel TLC plates with 250 μm thickness (Silicycle Inc, QC, Canada). Preparative TLC was performed using SiliaPlate™ silica gel TLC plates with 1000 μm thickness. ^{1}H and ^{13}C NMR spectroscopy was performed on a Bruker NMR at 500 MHz (^{1}H) and 126 MHz (^{13}C). All ^{13}C NMR spectra were proton decoupled. High resolution ESI-MS spectra was acquired using Agilent Technologies 6530 Q-TOF instrument. The RNA and DNA samples were analyzed on a Thermo Fisher Scientific (West Palm Beach, CA) LTQ Orbitrap Velos Mass spectrometer, using quartz capillary emitters. To facilitate spray optimization, 10% isopropyl alcohol was added to each sample prior to MS analysis.

Purification of all synthetic oligonucleotides were characterized by denaturing urea polyacrylamide gel electrophoresis (PAGE) (15% wt, Acrylamide:Bis-acrylamine=29:1, 1x TBE buffer). Gels were then stained with Ethidium Bromide (EthBr, 1 μg/mL) and visualized with ChemDoc Imaging System. Ultra low range DNA ladder (ThermoFisher Scientific, cat. SM1213) was used for the DNA analysis shown in Figure 2. The ladder contains DNA: 300, 200, 150, 100, 75, 50, 35, 25, 20, 15, and 10-nt long. Low range ssRNA ladder (New England Biolabs, cat# N0364S) was used for the RNA analysis shown in Figure 3B. The ladder contains RNA: 1000, 500, 300, 150, 80, 50-nt long.

Standard oligonucleotide purification procedure:
Upon completion of solid phase synthesis, the solid support was dried with a Speed-Vac concentrator. The oligonucleotides were cleaved from the solid support and were fully deprotected with AMA solution (a 1:1 aqueous solution of methylamine and concentrated ammonium hydroxide) at 65 °C for 2 h. The solution was evaporated to dryness using a Speed-Vac concentrator. DNA samples were redissolved in water and characterized by PAGE and nanodrop. For RNA samples, the solid was dissolved in DMSO (100 μL) and was desilylated using a solution of $Et_3N{\cdot}3HF$ at 65 °C for 2.5 h. After cooling to rt, the RNA was precipitated with 3M sodium acetate (25 μL) and ethanol (1 mL). The solution was cooled to -20 °C overnight before the RNA was recovered by centrifugation and finally dried under vacuum. Target oligonucleotide strands were purified by preparative gel electrophoresis.

Preparative gel purification of RNA was carried out using denaturing urea polyacrylamide gel electrophoresis (PAGE) (15% wt, Acrylamide:Bis-acrylamine=29:1, 1x TBE buffer). Target RNA was visualized by UV light (254 nm). The band containing target RNA was cut with a clean razor

blade and chopped into fine particles. Gel stabs were transferred to 1.5 mL Eppendorf tube and RNA was eluted with 800 μL elution buffer (500 mM ammonium acetate, 10 mM magnesium acetate, 2 mM EDTA) on a rotary shaker for 24 h at rt. After elution the gel fragments were spun down and the supernatant was transferred to another Eppendorf tube. The supernatant solution containing RNA was washed several times with an equal volume of n-butanol. The wash step was repeated several times until the volume of the lower aqueous portion was convenient for RNA precipitation. The RNA was precipitated after addition of ethanol (1 mL) and cooling at -20 °C for 18 h. The purified RNA was pelleted by centrifugation and resuspend in Milli-Q water for characterization.

Analytical HPLC was performed on Shimadzu LC-20 Instrument using DNAPac PA200 4× 250 mm column (Thermo Scientific Dionex, Sunnyvale, CA) using recently reported procedure [*Sci. Rep.* **2019,** *9* (1), 1019]. Prior to each injection, RNAs were diluted with milliQ water to 12-15 μM. RNAs were analyzed with a highly basic (pH=12) mobile phase, NaCl as a salt gradient, at 10 °C and with the flow rate of 0.8 mL/min. Solvent A: 10 mM NaOH in water at pH 11.8, Solvent B: 1.5 M NaCl, 10 mM NaOH in water at pH 11.7. Elution was performed with a 0-95% gradient of solvent B.

HPLC-free oligonucleotide purification procedure described in this work:

Our experimental procedure utilized a CPG-based solid support that was functionalized with photolabile o-nitrobenzyl group. Oligonucleotides were synthesized by the standard solid phase synthesis described above. Upon completion, the solid support (20-30 mg) was dried with a Speed-Vac concentrator. On support tagging with a freshly prepared Tz anhydride was carried using conditions described in the manuscript (Table 1). Successful capping with Tz resulted in CPG beads turning purple, as shown in Figure S1. Oligonucleotides were subsequently cleaved from the solid support using UV light. Cleavage was done using Rayonet RPR-200 Photochemical Reactor equipped with RPR 3500A lamps (The Southern New England Ultraviolet Co. Branford, CT, USA). CPG beads were placed in a quartz cuvette (Starna Scientific, Atascadero, CA, USA; cat.# GL14/S) a 3:1 solution of CH_3CN:H_2O (3 mL) was added and the resulting suspension was degassed with N_2 for 30 min. The photocleavage was carried out for 2 h under N_2 atmosphere. Under these conditions, there is minimal formation of pyrimidine-pyrimidine photodimers. The supernatant solution containing cleaved oligonucleotides was separated from CPG beads and concentrated to ~1 mL in a Speed-Vac concentrator. The supernatant solution was treated with TCO-functionalized CPG beads to capture the target strands. TCO-CPG beads (100 mg) were added to the supernatant solution and placed in a thermoshaker at 37 °C for 2 h. Captured target oligonucleotides were cleaved from the CPG beads and were fully deprotected with AMA solution using the standard conditions. The solution was evaporated to dryness using a Speed-Vac concentrator. For the synthetic DNA samples, the solid was redissolved in water and characterized by PAGE and ESI-MS. For the synthetic RNA samples, the solid was dissolved in DMSO (100 μL) and was desilylated using a solution of $Et_3N{\cdot}3HF$ at 65 °C for 2.5 h. After cooling to rt, the RNA was precipitated with 3M sodium acetate (25 μL) and ethanol (1 mL). The solution was cooled to -20 °C overnight before the RNA was recovered by centrifugation and finally dried under vacuum.

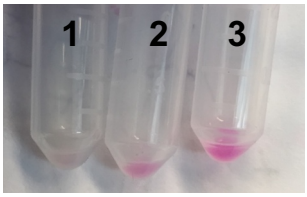


Figure S1. Picture of CPG beads after the tagging step: **1** – unsuccessful tagging; **2** – low yielding tagging; **3** – high yielding tagging. The solid support becomes pink after conjugation to Tz.

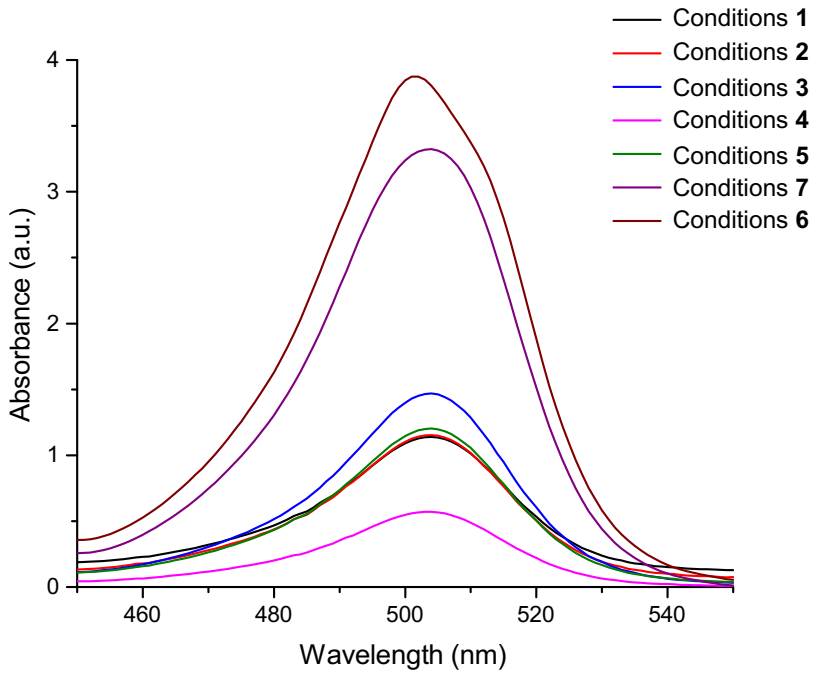


Figure S2. Determination of tagging efficiency using the detritylation process illustrated in **Scheme 1B**. The tagging conditions are summarized in **Table 1**. Absorbance at 504 nm was used to calculate the amount of cleaved DMT group ($\varepsilon = 76$ mL cm^{-1} μmol^{-1}).

Oligonucleotide strands used in this work:

20-nt DNA: 5'-TCA TTG CTG CTT AGA TTG CT-3'
17-nt DNA: 5'-TTG CTG CTT AGA TTG CT-3'
10-nt DNA: 5'-TTA GAT TGC T-3'

76-nt $tRNA^{Lys}$: 5'- rGrGrG rUrCrG rUrUrA rGrCrU rCrArG rUrUrG rGrUrA rGrArG rGrArG rUrUrG rArCrU rUrUrU rArArU rCrArA rUrUrG rGrUrC rGrCrA rGrGrU rUrCrG rArArU rCrCrU rGrCrA rCrGrA rCrCrC rArCrC T-3'

101-nt sgRNA: 5'-rGrGrG rCrGrA rGrGrA rGrCrU rGrUrU rCrArC rCrGrG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUT-3'

Determination of functional fidelity of purified sgRNA

CRISPR-Cas9 experiments, were carried out following the procedure reported by Yin, H. *et al.* [*Nat. Chem. Biol.* **2018**, *14*, 311-316]. HEK293T cells were infected by lentiviral particles to stably express EF1a-GFP-PGK-Puro (Addgene; 26777) and EFs-spCas9-Blast (Addgene; 52962). The cells were grown to 70-90% confluence in DMEM, containing 10% FBS. One set of HEK293T cells was transfected with a crRNA targeting GFP and the tracrRNA (30 nM each, final concentration) using Lipofectamine (Thermo Fisher Scientific). The second set of cells was transfected with the experimentally purified sgRNA targeting GFP (30 nM, final concentration) using Lipofectamine. After 3 days of transfection, the cells were fixed with 2% paraformaldehyde in PBS and GFP expression was analyzed by flow cytometry. Data from 10^6 cells were acquired using a FACS Aria III cell sorter equipped with a 488 nm/blue coherent sapphire solid-state laser, 20 mW (BD Biosciences, San Jose, CA, USA). Data analyses were carried out using FlowJo

software (Ashland, OR, USA), according to manufacturer's instructions. Parameters, such as MFI and the percentages of specific populations, were quantified by histogram analysis.

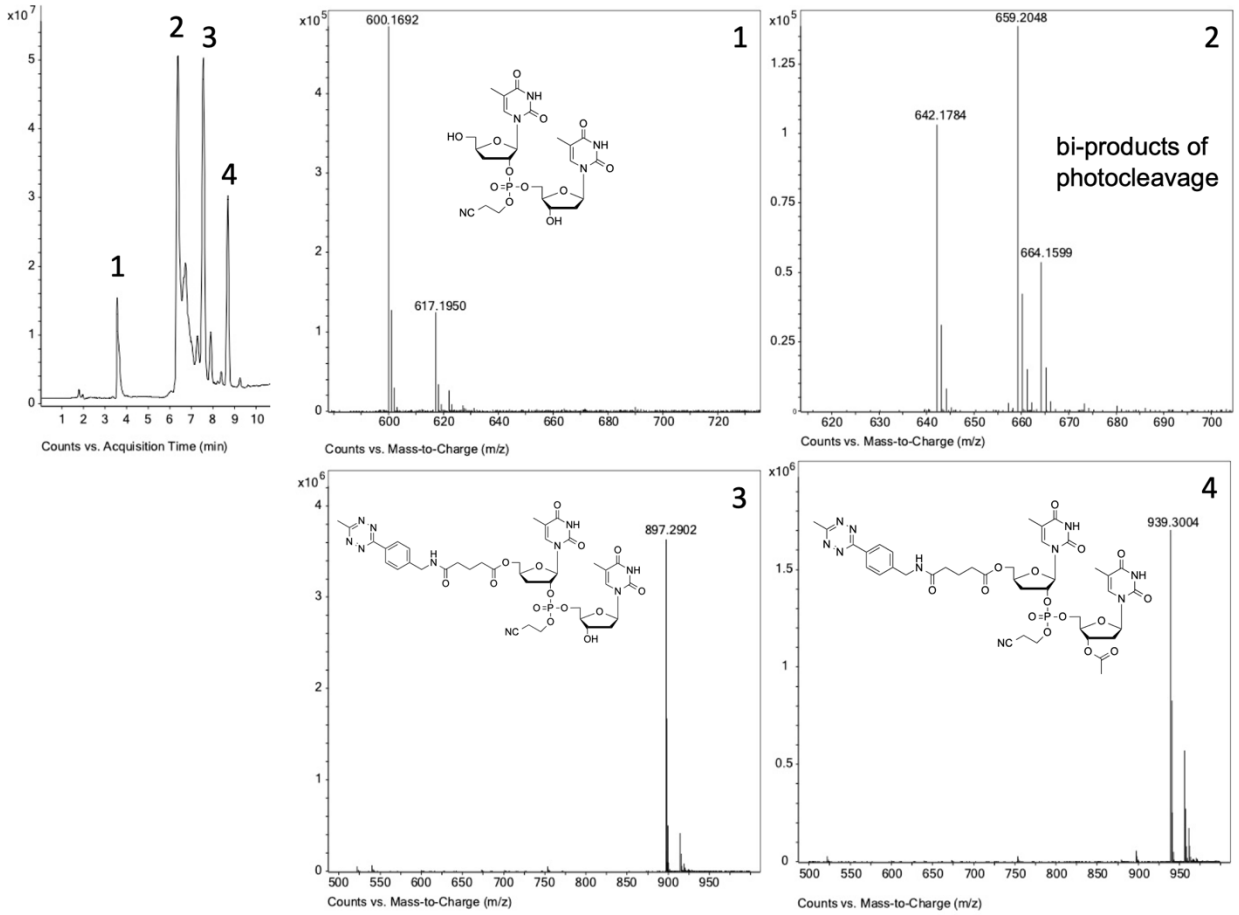


Figure S3. LC-MS analysis of a DNA dimer after photocleavage.

To investigate photocleavage, we synthesized a short DNA dimer: 5'-TT-3'. After the solid phase synthesis, the DNA was tagged with **Tz 3**. Subsequently, we carried out photocleavage using the procedure described above and analyzed the resulting supernatant solution by LC-MS. **Figure S2** illustrates the LC-MS analysis. In addition to main photocleavage products containing tagged DNA, we were able to identify a minor bi-product (1) lacking Tz. The LC-MS results imply that a small amount of Tz gets hydrolyzed from DNA during the photocleavage step. This leads to partial loss of target oligonucleotide during the HPLC-free purification procedure.

Determination of purity of the isolated RNA

Purity of the RNA isolated using our experimental method was determined relative to the RNA purified by preparative gel electrophoresis. Bands observed in lanes 2 and 4 in Figures 3 and 4 were quantified using ImageJ software (https://imagej.nih.gov/ij/). Amounts of RNA loaded in each lane was determined using nanodrop measurements.

	Tagging Reagent	Base	Coupling reagent	Catalyst	Temperature	Time	Tagging efficiency
1	**Tz 1**	Pyridine	---	N-Me-imidazole	25 ℃	2 h	30%
2	**Tz 1**	DIPEA	---	N-Me-imidazole	25 ℃	2 h	30%
3	**Tz 1**	DMAP	EDC	---	25 ℃	2 h	38%
4	**Tz 1**	DIPEA	---	DMAP	25 ℃	2 h	15%
5	**Tz 2**	DIPEA	---	DMAP	25 ℃	2 h	31%
6	**Tz 3**	DIPEA	---	DMAP	25 ℃	2 h	94%
7	**Tz 3**	DIPEA	---	DMAP	37 ℃	2 h	91%

Table S1. Optimization of DNA tagging using different Tz anhydrides.

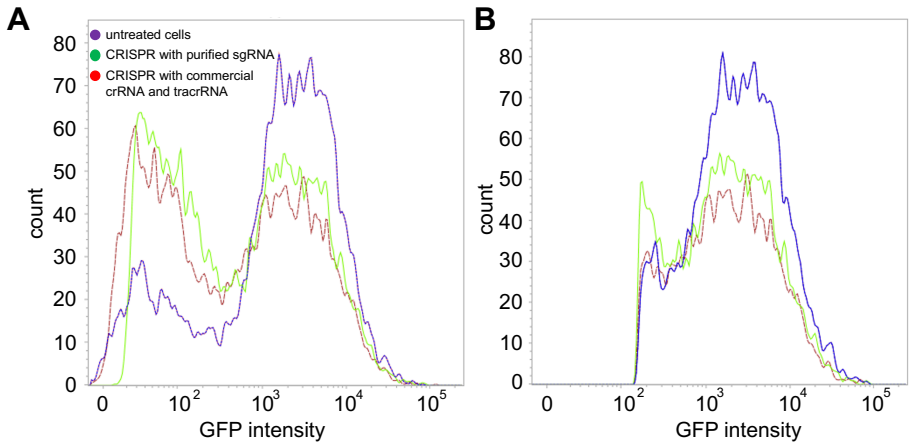


Figure S4. Flow cytometry analysis of CRISPR experiments targeting the GFP gene in HEK293T cells, expressing Cas9. (**A**) Histograms of total GFP-expressing cells: untreated cells are shown in purple, cells transfected with the commercial HPLC-purified crRNA and tracrRNA are shown in red, cells transfected with the sgRNA purified using our experimental procedure are shown in green. (**B**) Histograms of total live cells using the same coloring scheme.

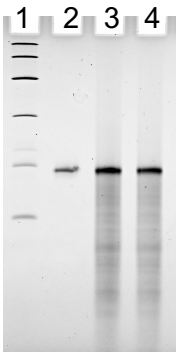
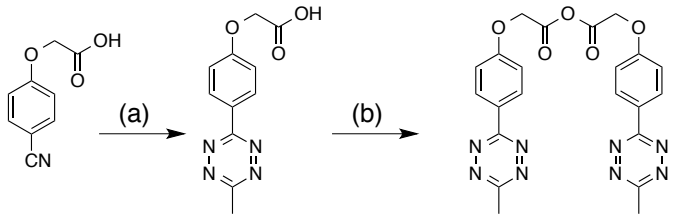
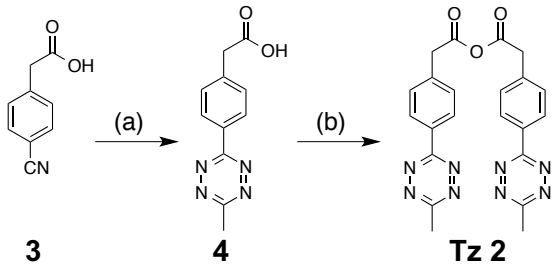


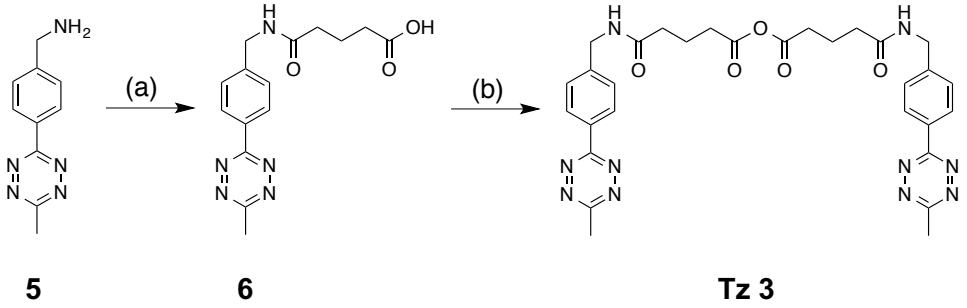
Figure S5. PAGE analysis of photocleavage efficiency. *Lane 1:* low range ssRNA ladder; *Lane 2:* previously purified $tRNA^{Lys}$; Lane 3: crude $tRNA^{Lys}$ sample obtained after AMA cleavage/deprotection and desilylation; Lane 4: crude $tRNA^{Lys}$ sample obtained after photocleavage (350 nm), deprotection and desilylation.



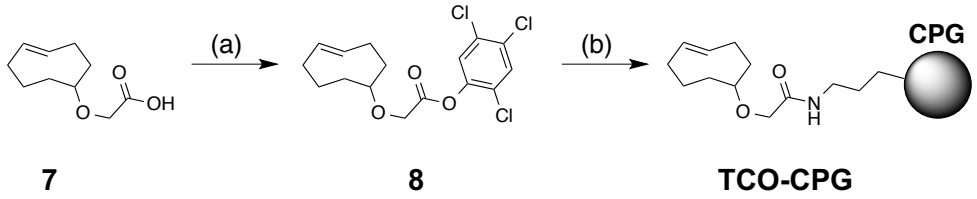
Scheme S1. Synthesis of the capping reagent **Tz 1**: (a) CH_3CN, $Ni(OTf)_2$, NH_2NH_2 60 °C, followed by $NaNO_2$ and HCl; (b) DCC, CH_2Cl_2.



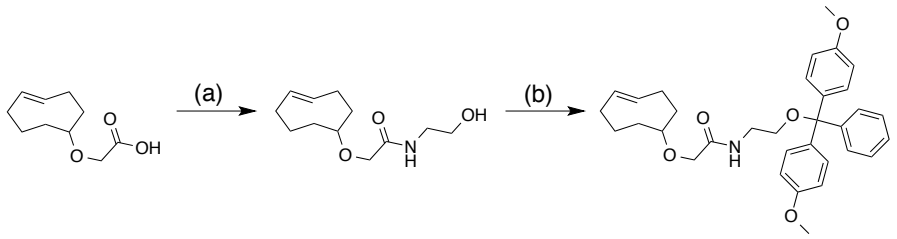
Scheme S2. Synthesis of the capping reagent **Tz 2**: (a) CH_3CN, $Ni(OTf)_2$, NH_2NH_2 60 °C, followed by $NaNO_2$ and HCl; (b) DCC, CH_2Cl_2.



Scheme S3. Synthesis of the capping reagent **Tz 3**: (a) glutaric anhydride, DIPEA, THF ; (b) DCC, CH_2Cl_2.

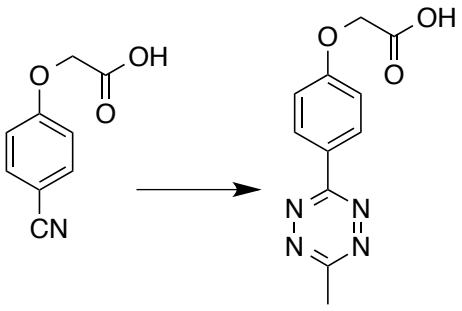


Scheme S4. Synthesis of the TCO-CPG: (a) 2,4,5-trichlorophenol, DCC, CH_2Cl_2 ; (b) LCAA-CPG, HOBt, DMF.



7 **9** **TCO-DMT**

Scheme S5. Synthesis of the **TCO-DMT** reagent: (a) aminoethanol, HATU, DMF ; (b) DMT-Cl, pyridine.



1 **2**

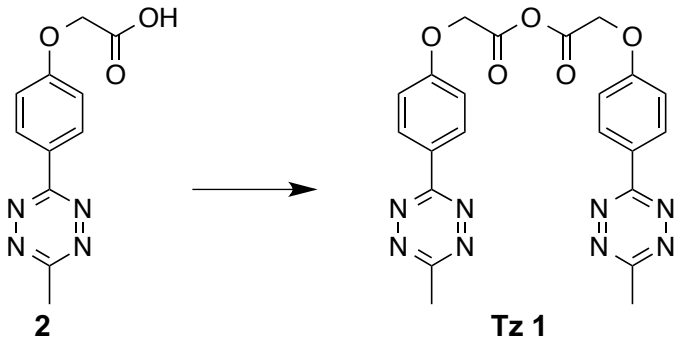
Scheme S6. Synthesis of compound **2**.

The tetrazine **2** was synthesized based on the previously described procedure [Yang, J.; Karver, M. R.; Li, W.; Sahu, S.; Devaraj, N. K. *Angew. Chem. Int. Ed.* **2012**, *51*, 5222-5225]. Combined compound **1** (1.0 g, 5.6 mmol), acetonitrile (2.31 g, 56 mmol) and $Ni(OTf)_2$ (1.0 g, 2.8 mmol). Slowly added anhydrous hydrazine (10 mL) and heated to 60 °C in the oil bath under N_2 atmosphere for 18 h. Cooled to rt and added an aqueous solution of $NaNO_2$ (8.0 g in 100 mL H_2O). Dropwise added 1N HCl until pH 3 was reached. Extracted the product with EtOAc (2 x 200 mL). The product was purified as a pink solid by flash chromatography using a gradient of EtOAc in Heptane (0-50%). Yield = 1 g (73%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.45 – 8.38 (m, 2H), 7.22 – 7.15 (m, 2H), 4.83 (s, 2H), 2.97 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 170.29, 167.08, 163.40, 161.70, 129.62, 124.98, 115.89, 65.07, 21.20.

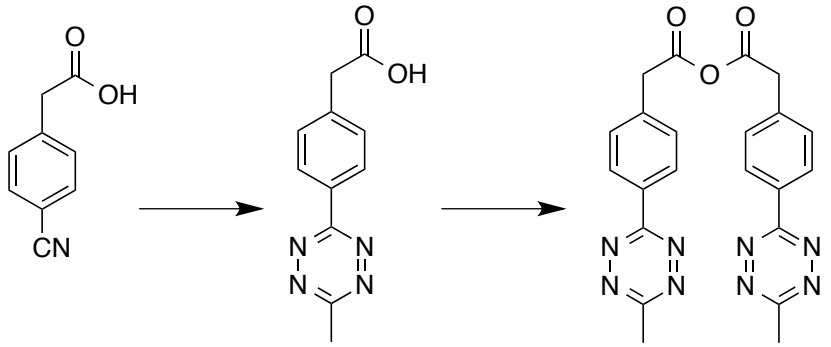
HRMS (ESI) Calc'd for $C_{11}H_{11}N_4O_3$ $[M+1]^+$ = 247.0826; found 247.0827



2 **Tz 1**

Scheme S7. Synthesis of **Tz 1**.

Tz 1 was freshly prepared in small quantity for every oligonucleotide capping experiment. Compound **2** (0.050 g, 0.20 mmol) and DCC (0.021 g, 0.1 mmol) were dissolved in CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 18 h under N_2 atmosphere. The precipitated DCU was quickly filtered through a cotton plugged Pasteur pipet. Oligonucleotide capping experiments without any further purification.



3 **4** **Tz 2**

Scheme S8. Synthesis of **Tz 2**.

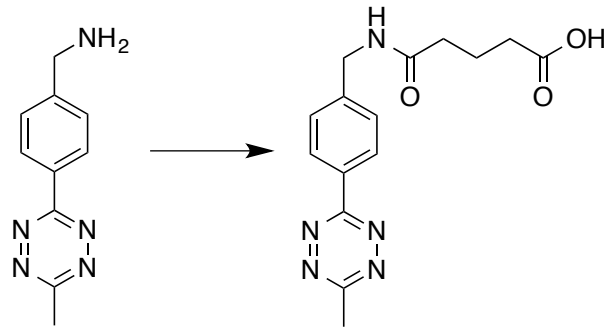
The tetrazine **4** was synthesized using previously described procedure [Yang, J.; Karver, M. R.; Li, W.; Sahu, S.; Devaraj, N. K. *Angew. Chem. Int. Ed.* **2012**, *51*, 5222-5225].
Combined compound **3** (1.6 g, 10 mmol), acetonitrile (5.2 mL, 100 mmol) and $Ni(OTf)_2$ (1.8 g, 5.0 mmol). Slowly added anhydrous hydrazine (16 mL) and heated to 60 °C in the oil bath under N_2 atmosphere for 18 h. Cooled to rt and added an aqueous solution of $NaNO_2$ (15 g in 100 mL H_2O). Dropwise added 1N HCl until pH 3 was reached. Extracted the product with EtOAc (2 x 200 mL). The product was purified as a pink solid by flash chromatography using a gradient of EtOAc in Heptane (0-50%). Yield = 1.1 g (48%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (d, *J* = 8.5 Hz, 2H), 7.59 – 7.50 (m, 2H), 3.80 (s, 2H), 3.12 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 176.41, 167.29, 163.87, 138.05, 130.38, 128.22, 40.92, 21.16.

HRMS (ESI) Calc'd for $C_{11}H_{11}N_4O_2$ $[M+1]^+$ = 231.0877; found 231.0876

Compound **4** (0.050 g, 0.22 mmol) and DCC (0.023 g, 0.11 mmol) were dissolved in CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 18 h under N_2 atmosphere. The precipitated DCU was quickly filtered through a cotton plugged Pasteur pipet. Oligonucleotide capping experiments without any further purification.



5 **6**

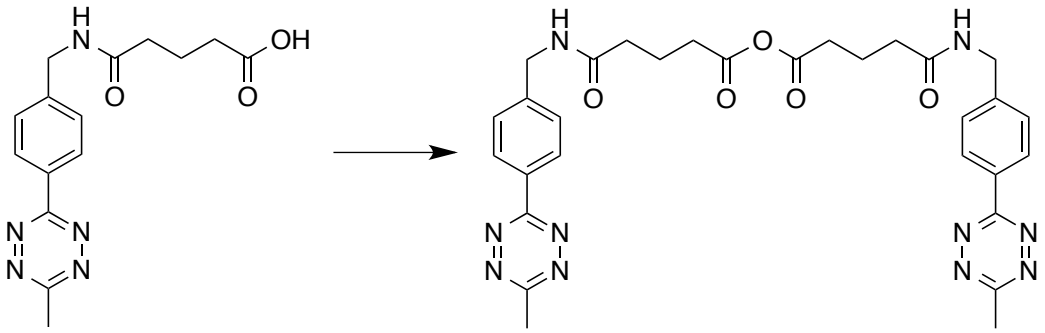
Scheme S9. Synthesis of compound **2**.

The tetrazine **5** was synthesized using previously described procedure [Yang, J.; Karver, M. R.; Li, W.; Sahu, S.; Devaraj, N. K. *Angew. Chem. Int. Ed.* **2012**, *51*, 5222-5225].
Dissolved compound **5** (0.300 g, 1.50 mmol), glutaric anhydride (0.205 g, 1.8 mmol) in THF (10 mL). DIPEA (0.775 mg, 6 mmol) was added dropwise and the reaction mixture was stirred at rt for 18 h under N_2 atmosphere. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with 5% aqueous solution of citric acid (20 mL). The aqueous layer was extracted with CH_2Cl_2 for (3 x 50 mL). The organic layers were combined, dried with Na_2SO_4 and deposited on silica. The product was purified by flash chromatography using a gradient of MeOH in DCM (0-20%). Yield = 0.330 g (70 %).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.44 – 8.38 (m, 2H), 7.59 – 7.49 (m, 2H), 4.39 (d, *J* = 6.0 Hz, 2H), 3.00 (s, 3H), 2.24 (dt, *J* = 9.4, 7.5 Hz, 4H), 1.83 – 1.70 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 174.65, 172.29, 167.54, 163.66, 145.01, 130.81, 128.51, 127.90, 34.86, 33.52, 21.30, 21.16.

HRMS (ESI) Calc'd for $C_{15}H_{18}N_5O_3$ $[M+1]^+$ = 316.1404; found 316.1400



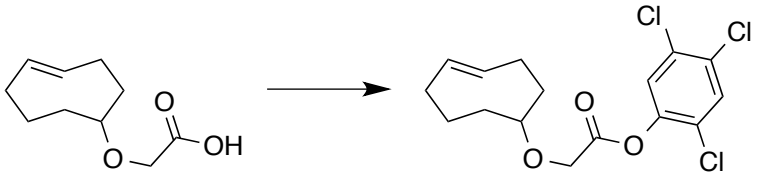
6 **Tz 3**

Scheme S10. Synthesis of compound **3**.

Tz 3 was freshly prepared in small quantity for every oligonucleotide capping experiment. Compound **6** (0.015 g, 0.048 mmol) and DCC (0.005 g, 0.024 mmol) were dissolved in CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 18 h under N_2 atmosphere. The precipitated DCU was quickly filtered through a cotton plugged Pasteur pipet. Oligonucleotide capping experiments without any further purification.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.47 – 8.40 (m, 2H), 7.59 – 7.45 (m, 2H), 4.39 (t, *J* = 5.2 Hz, 2H), 3.00 (d, *J* = 2.5 Hz, 3H), 2.40 – 2.14 (m, 3H), 1.70 – 1.56 (m, 3H)

^{13}C NMR (126 MHz, DMSO-d_6) δ 172.38, 172.00, 169.87, 167.54, 163.65, 144.92, 130.83, 128.53, 128.45, 127.90, 33.82, 25.80, 21.29



7 **8**

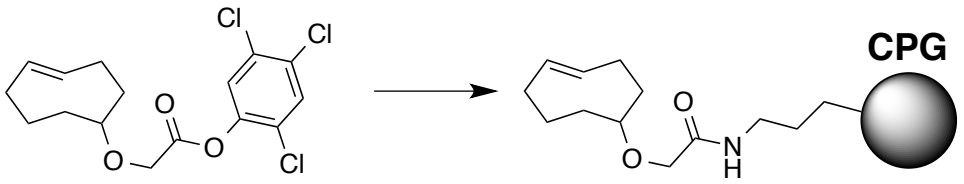
Scheme S11. Synthesis of compound **8**.

The *trans*-cyclooctene **7** was synthesized using previously described photoisomerization approach [Royzen, M.; Yap, G.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 13518-13519]. Compound **7** (0.100 g, 0.53 mmol), 2,4,5-Trichlorophenol (0.156 g, 0.79 mmol) and DCC (0.163 g, 0.79 mmol) were dissolved in CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 18 h under N_2 atomsphere. The precipitated DCU was filtered and the reaction mixture was diluted with CH_2Cl_2 (50 mL). The reaction mixture was washed with water (20 mL). The organic layer was dried with Na_2SO_4 and deposited on silica. The product was purified by flash chromatography using a gradient of EtOAc in Heptane. Yield = 0.110 g (57 %).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.62 – 7.54 (m, 1H), 7.38 – 7.32 (m, 1H), 5.77 – 5.63 (m, 1H), 5.59 – 5.46 (m, 1H), 4.42 – 4.32 (m, 2H), 3.78 (dd, *J* = 10.3, 4.8 Hz, 1H), 2.50 – 2.37 (m, 2H), 2.28 (ddd, *J* = 15.3, 9.2, 5.2 Hz, 2H), 2.00 – 1.73 (m, 4H), 1.62 – 1.55 (m, 2H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 168.26, 145.44, 135.93, 131.59, 131.15, 131.12, 125.27, 65.89, 40.08, 34.45, 32.57, 29.72, 27.62

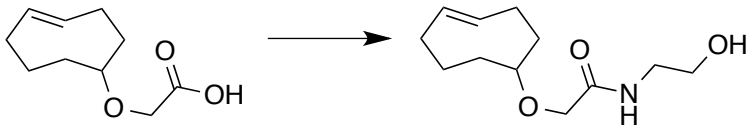
HRMS (ESI) Calc'd for $C_{16}H_{18}Cl_3O_3$ $[M+1]^+$ = 363.0316; found 363.0311



8 **TCO-CPG**

Scheme S12. Synthesis of **TCO-CPG**.

Combined compound **8** (20 mg, 0.05 mmol), native amino lcaa CPG 1000 Å (400 mg) and HOBt (51 mg, 0.38 mmol) in 3 mL anhydrous DMF. The suspension was agitated for 4 h in the dark. After that, the CPG beads were purified from the supernatant by centrifugation and washed 3-times with anhydrous DMF. The unreacted amine groups were capped by treatment with acetic anhydride (2.5 mL), DMAP (250 mg) and pyridine (20 mL) at rt for 1 h. After the treatment, the CPG beads were isolated by centrifugation, washed 3-times with EtOAc and dried under reduced pressure (speed vac). The loading was evaluated by reacting the beads with **Tz-DMT** for 1 h at rt. After the reaction, the beads were thoroughly washed with DMF and CH_2Cl_2. The CPG beads were treated with 10 mL deblocking solution (3% trichloroacetic acid in CH_2Cl_2). The amount of cleaved DMT group was measured by absorbance at 504 nm.



7 **9**

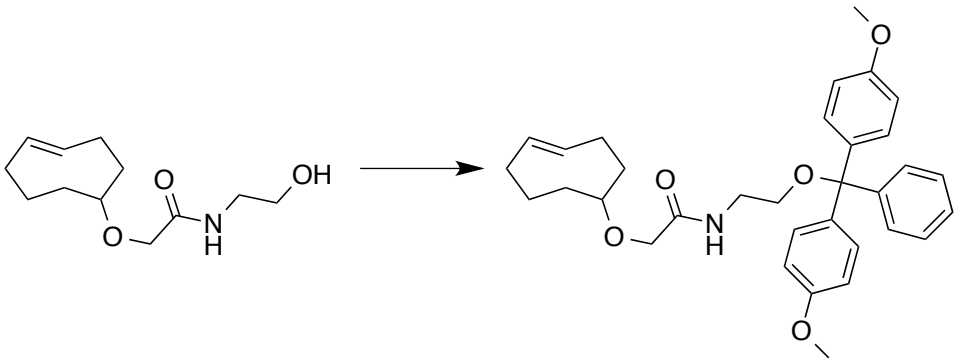
Scheme S13. Synthesis of compound **9**.

The *trans*-cyclooctene **7** was synthesized using previously described photoisomerization approach [Royzen, M.; Yap, G.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 13518-13519]. Dissolved compound **7** (0.200 g, 1.09 mmol), aminoethanol (0.132 g, 2.17 mmol) and HATU (0.621 g, 1.64 mmol) in DMF (10 mL). Stirred at rt for 18 h under N_2 atmosphere. Evaporated DMF under high vacuum. Redissolved the reaction in mixture in CH_2Cl_2 and deposited on silica. The product was purified by flash chromatography using a gradient of EtOAc in Heptane. Yield = 0.225 g (91 %).

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.72 – 5.59 (m, 2H), 4.00 – 3.93 (m, 2H), 3.83 – 3.75 (m, 2H), 3.53 – 3.48 (m, 2H), 2.41 – 2.34 (m, 1H), 2.20 – 2.15 (m, 2H), 2.01 – 1.93 (m, 2H), 1.85 – 1.80 (m, 2H), 1.72 (ddd, *J* = 12.7, 9.0, 4.4 Hz, 2H), 1.60 – 1.53 (m, 1H), 1.47 – 1.40 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.45, 131.47, 81.93, 68.25, 62.22, 41.80, 40.11, 34.01, 32.55, 27.94.

HRMS (ESI) Calc'd for $C_{12}H_{22}NO_3$ $[M+1]^+$ = 228.1594; found 228.1600



9 **TCO-DMT**

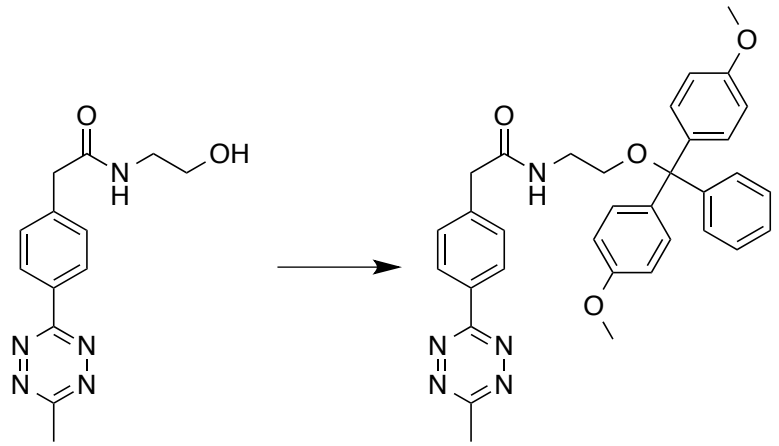
Scheme S14. Synthesis of **TCO-DMT**.

Compound **9** was azeotroped 3-times in pyridine to remove the residual moisture. Dissolved compound **9** (0.225 g, 0.991 mmol) and DMT-Cl (0.372 g, 1.10 mmol) in anhydrous pyridine (5 mL). Stirred at rt for 18 h under N_2 atmosphere. Evaporated pyridine under reduced pressure Redissolved the reaction in mixture in CH_2Cl_2 and deposited on silica. The product was purified by flash chromatography using a gradient of EtOAc in Heptane. Yield = 0.43 g (82 %).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47 – 7.41 (m, 2H), 7.36 – 7.28 (m, 6H), 7.25 – 7.20 (m, 1H), 6.87 – 6.81 (m, 4H), 5.64 – 5.43 (m, 2H), 4.02 – 3.87 (m, 2H), 3.84 – 3.77 (m, 6H), 3.68 (dd, *J* = 9.7, 4.3 Hz, 1H), 3.58 – 3.45 (m, 2H), 3.27 – 3.19 (m, 2H), 2.39 – 2.17 (m, 3H), 2.16 – 2.10 (m, 1H), 2.05 – 1.95 (m, 1H), 1.84 – 1.69 (m, 3H), 1.58 – 1.49 (m, 1H), 1.22 (ddd, *J* = 13.9, 10.1, 5.2 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 169.98, 158.47, 144.76, 135.91, 135.68, 131.20, 129.96, 129.92, 127.96, 127.82, 126.78, 113.12, 86.16, 76.06, 68.45, 62.20, 55.20, 40.11, 38.94, 34.28, 32.60, 29.80, 27.92

HRMS (ESI) Calc'd for $C_{33}H_{40}NO_5$ $[M+1]^+$ = 530.2901; found 530.2910



10 **Tz-DMT**

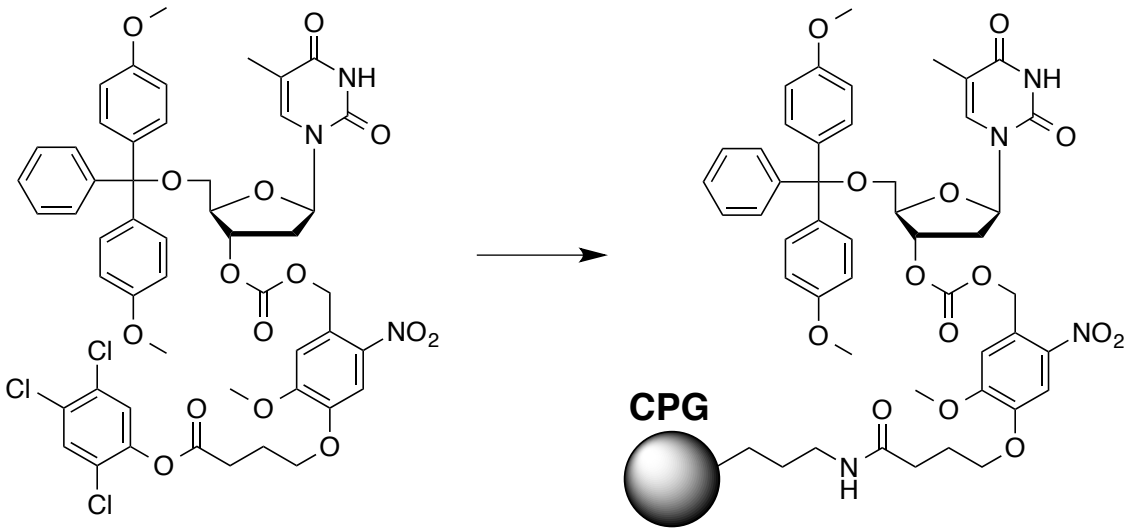
Scheme S15. Synthesis of **Tz-DMT**.

Compound **10** (0.173 g, 0.63 mmol) was azeotroped in anhydrous pyridine to remove the residual moisture. DMT-Cl (0.257 g, 0.76 mmol) dissolved in pyridine (20 mL) was added and the reaction mixture was stirred at rt for 18 h under N_2 atmosphere. The reaction was quenched with MeOH (5 mL). After stirring for 15 min, the reaction mixture was concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 (100 mL) and washed with a 5% aqueous solution of citric acid (20 mL), followed by water (20 mL). The organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The product was purified using silica prep plate and a 1:1 solution of EtOAc : Heptane as a mobile phase.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.56 (t, *J* = 6.2 Hz, 2H), 7.53 – 7.45 (m, 2H), 7.34 – 7.13 (m, 9H), 6.88 – 6.73 (m, 4H), 4.13 (p, *J* = 7.0 Hz, 1H), 3.83 – 3.74 (m, 6H), 3.72 – 3.66 (m, 3H), 3.47 – 3.40 (m, 2H), 3.20 – 3.08 (m, 5H), 2.06 (s, 2H), 1.34 – 1.22 (m, 3H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 169.87, 167.27, 163.68, 158.45, 144.48, 139.71, 135.79, 130.97, 130.42, 129.80, 129.16, 128.51, 127.94, 127.85, 127.82, 127.80, 126.79, 113.11, 86.05, 77.32, 77.07, 76.82, 61.67, 55.26, 55.21, 43.78, 39.70, 21.18

HRMS (ESI) Calc'd for $C_{34}H_{34}N_5O_4$ $[M+Na]^+$ = 598.2430; found 598.2411



13 **CPG-T**

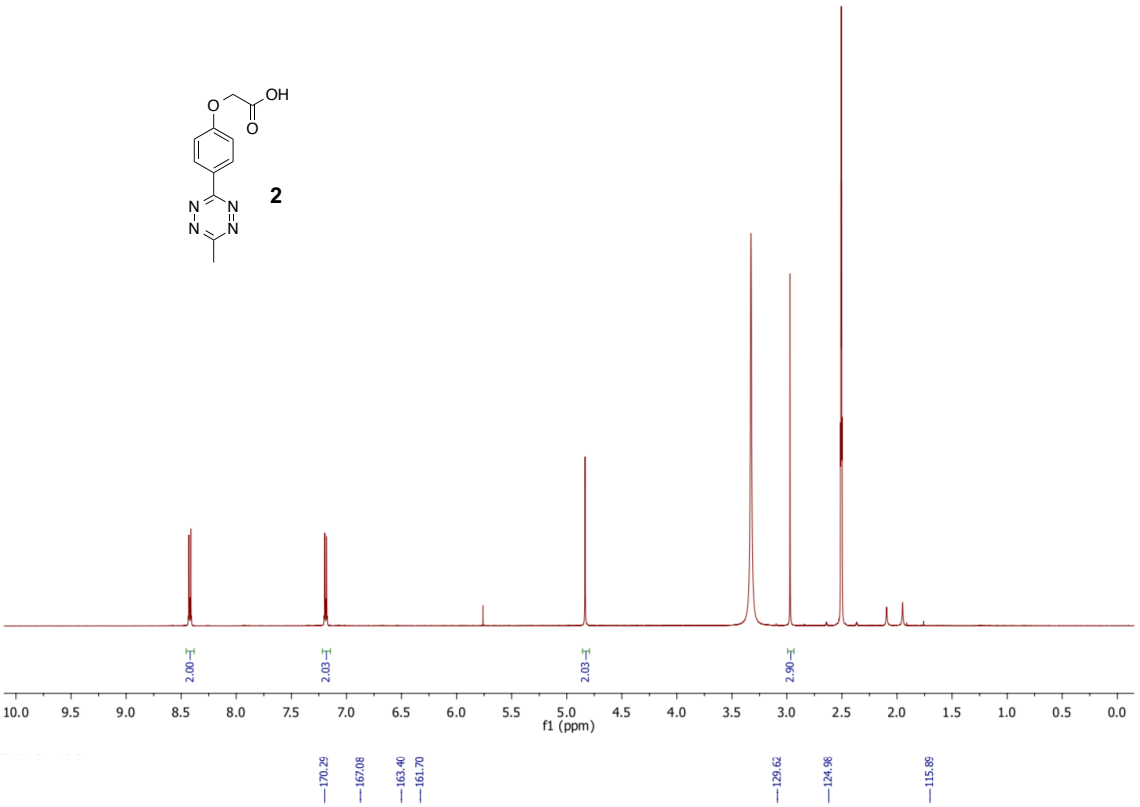
Scheme S16. Synthesis of **CPG-T**.

Compound **13** was synthesized using previously described procedure [Venkatesan, H.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 525-529].

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.58 (t, *J* = 2.4 Hz, 1H), 7.41 – 7.28 (m, 12H), 7.09 (s, 1H), 6.89 – 6.84 (m, 4H), 6.51 (dd, *J* = 9.3, 5.3 Hz, 1H), 5.61 (s, 2H), 5.43 (d, *J* = 5.7 Hz, 1H), 4.25 – 4.11 (m, 3H), 4.02 (s, 3H), 3.82 (s, 6H), 3.57 – 3.48 (m, 2H), 2.91 (t, *J* = 7.2 Hz, 2H), 2.54 – 2.30 (m, 4H), 1.40 (t, *J* = 6.1 Hz, 3H).

The ^{1}H NMR spectrum matched the one that was previously published.

Combined compound **13** (25 mg, 0.024 mmol), native amino lcaa CPG 1000Å (800 mg), HOBt (3.2 mg, 0.024 mmol) in anhydrous DMF (6 mL). Agitated at rt for 4 h in the dark. After that the CPG beads were isolated by centrifugation and washed with DMF (3 x 10 mL). Capping was carried out using acetic anhydride (5 mL), DMAP (500 mg) and pyridine (40 mL) for 1 h at rt. The CPG beads were isolated by centrifugation and washed with EtOAc (3 x 10 mL). The loading was evaluated by treatment with 10 mL deblocking solution (3% trichloroacetic acid in CH_2Cl_2). The amount of cleaved DMT group was measured by absorbance at 504 nm.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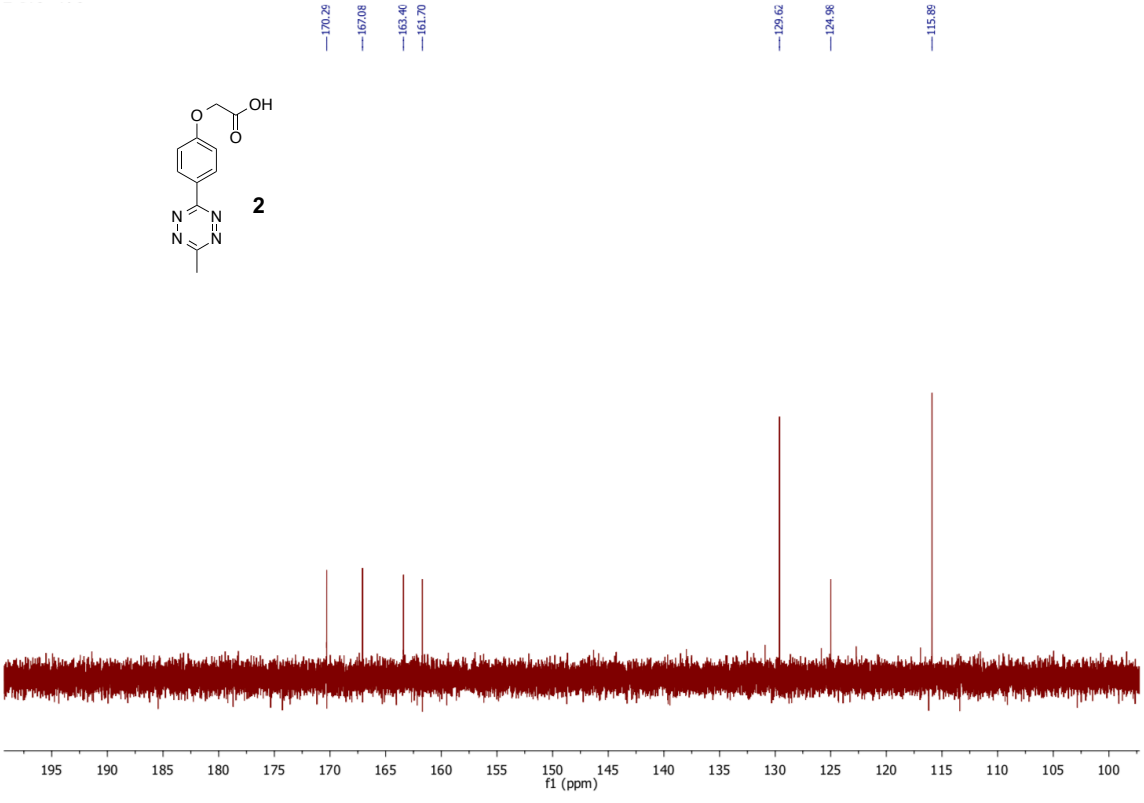
N
N
N
2
2.00
2.03
2.03
2.90
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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161.70
129.62
124.98
115.89
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O
O
N
N
N
N
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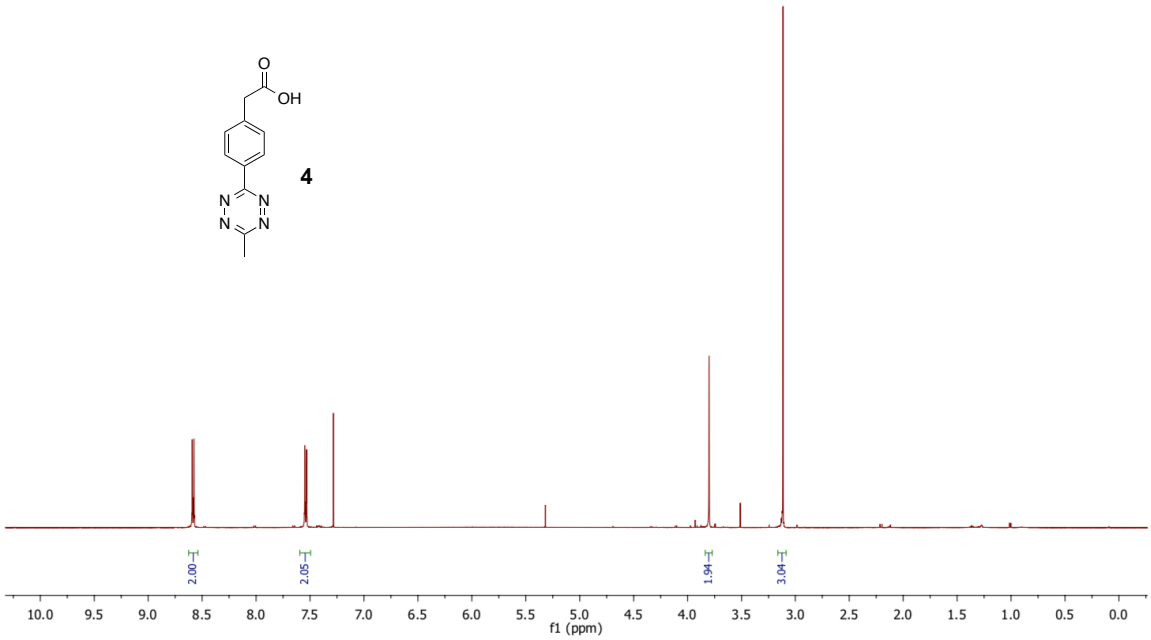
195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100
f1 (ppm)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4
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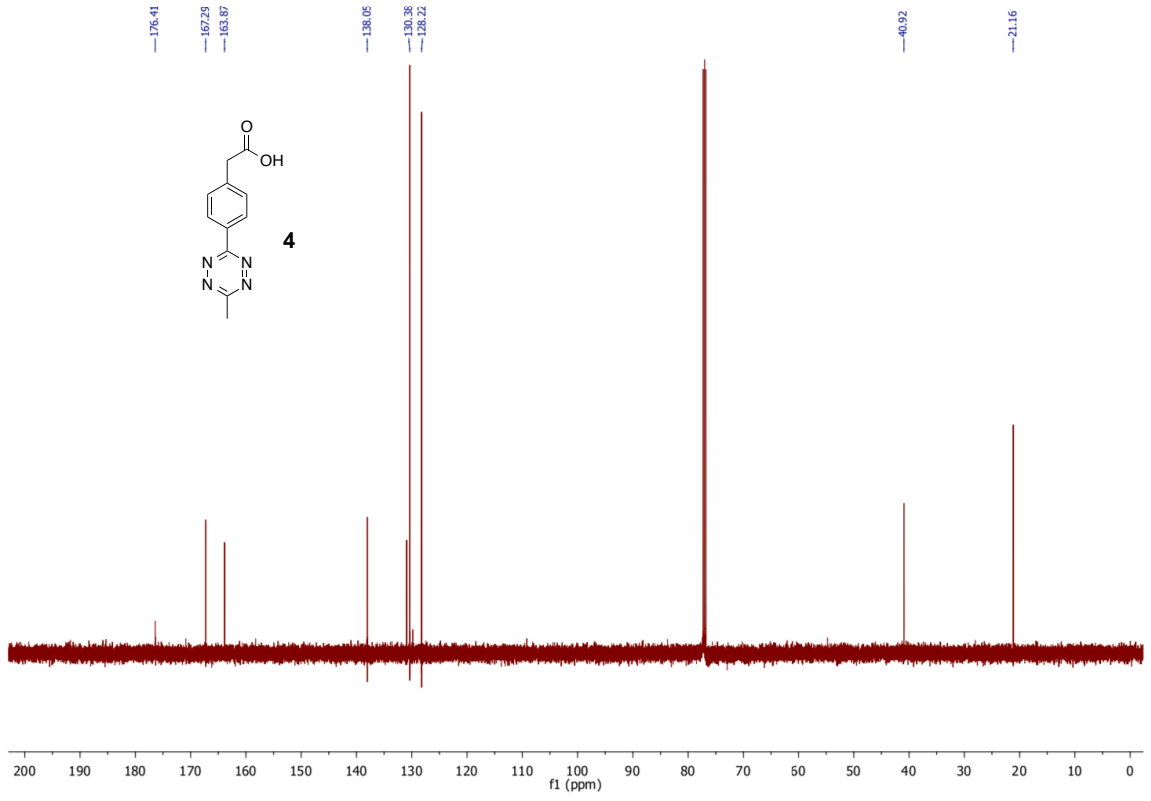
2.05
1.94
3.04
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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138.05
130.38
128.22
40.92
21.16
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OH
N
N
N
N
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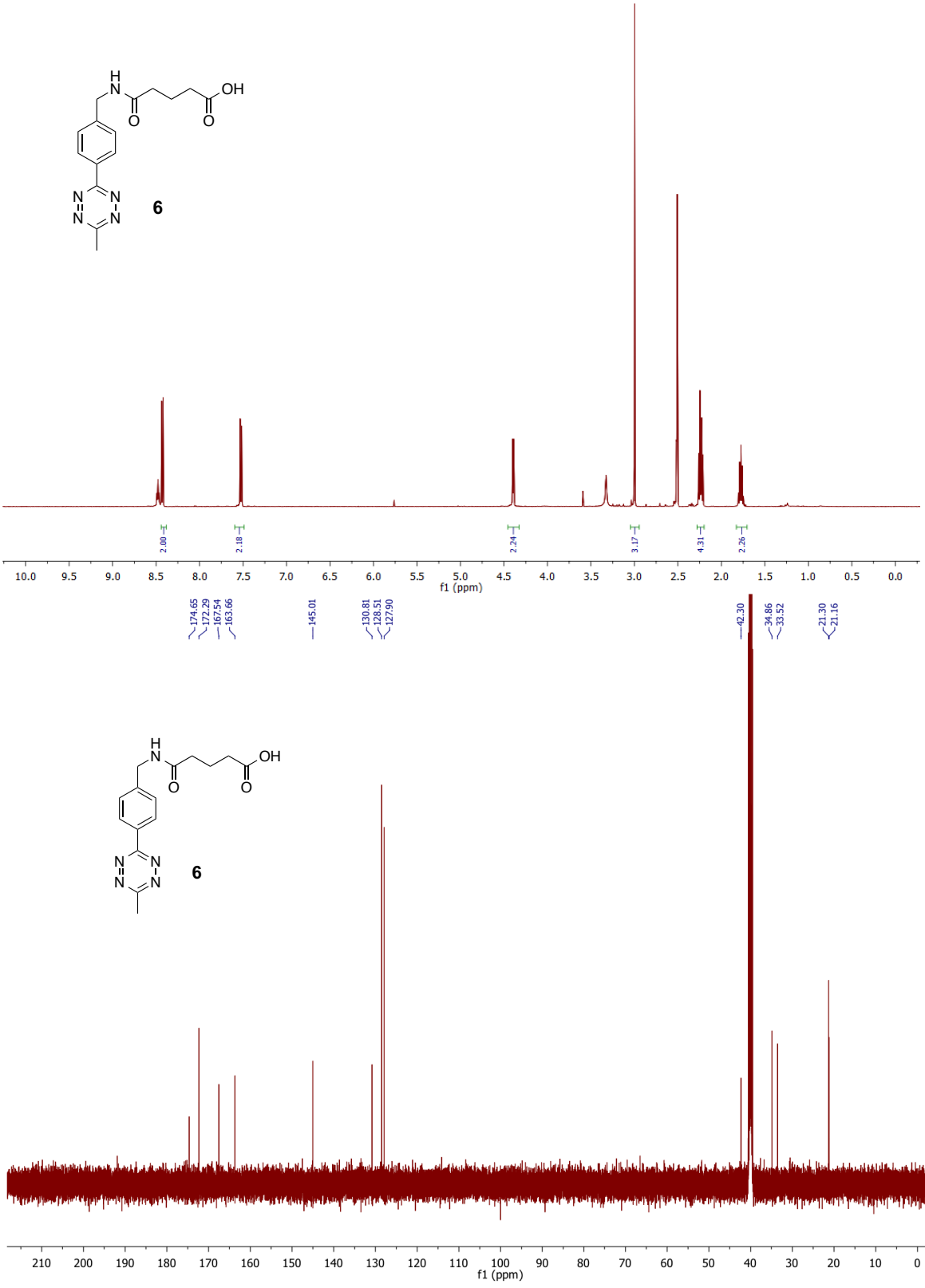
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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3.17
4.31
2.26
f1 (ppm)
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130.81
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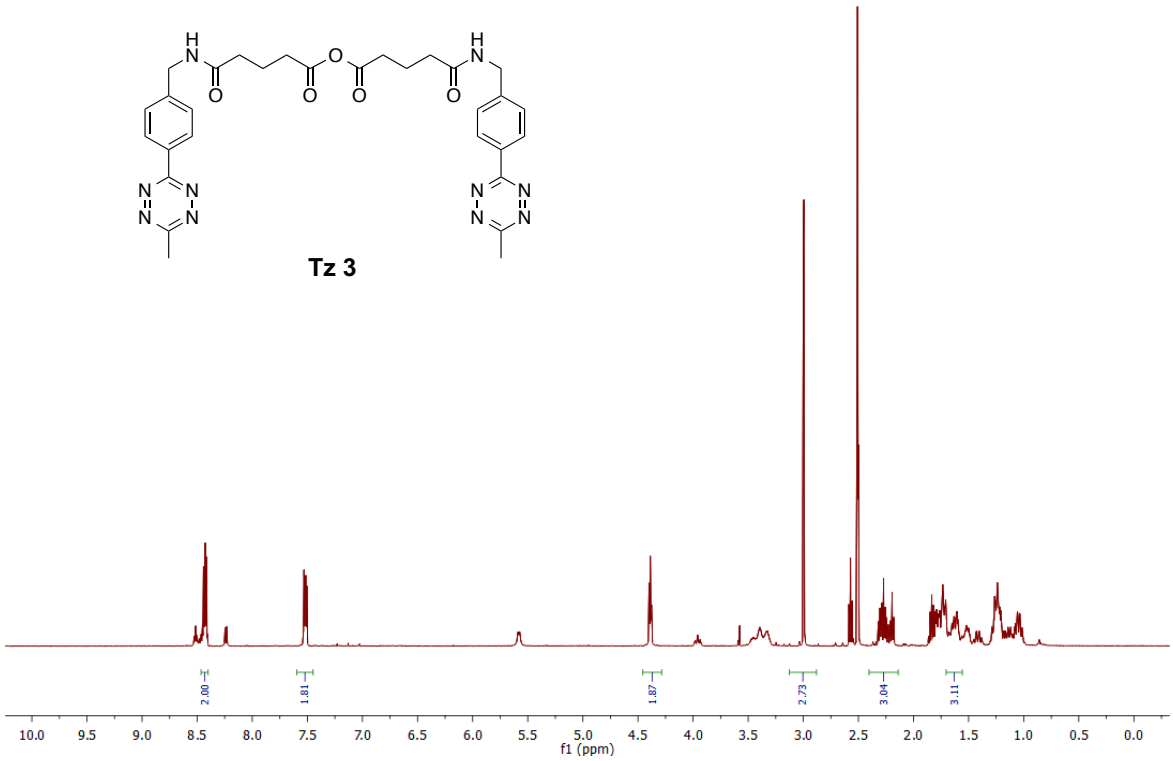
34.86
33.52
21.30
21.16
6
f1 (ppm)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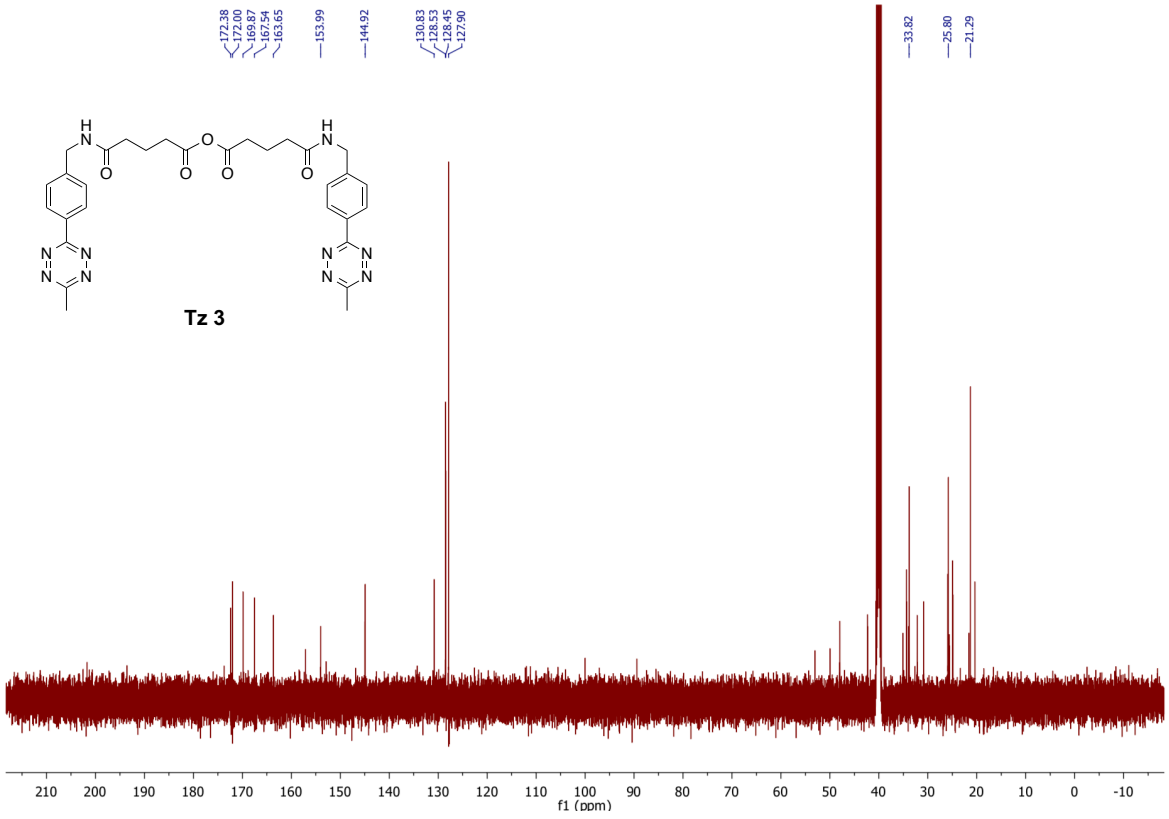
2.73
3.04
3.11
f1 (ppm)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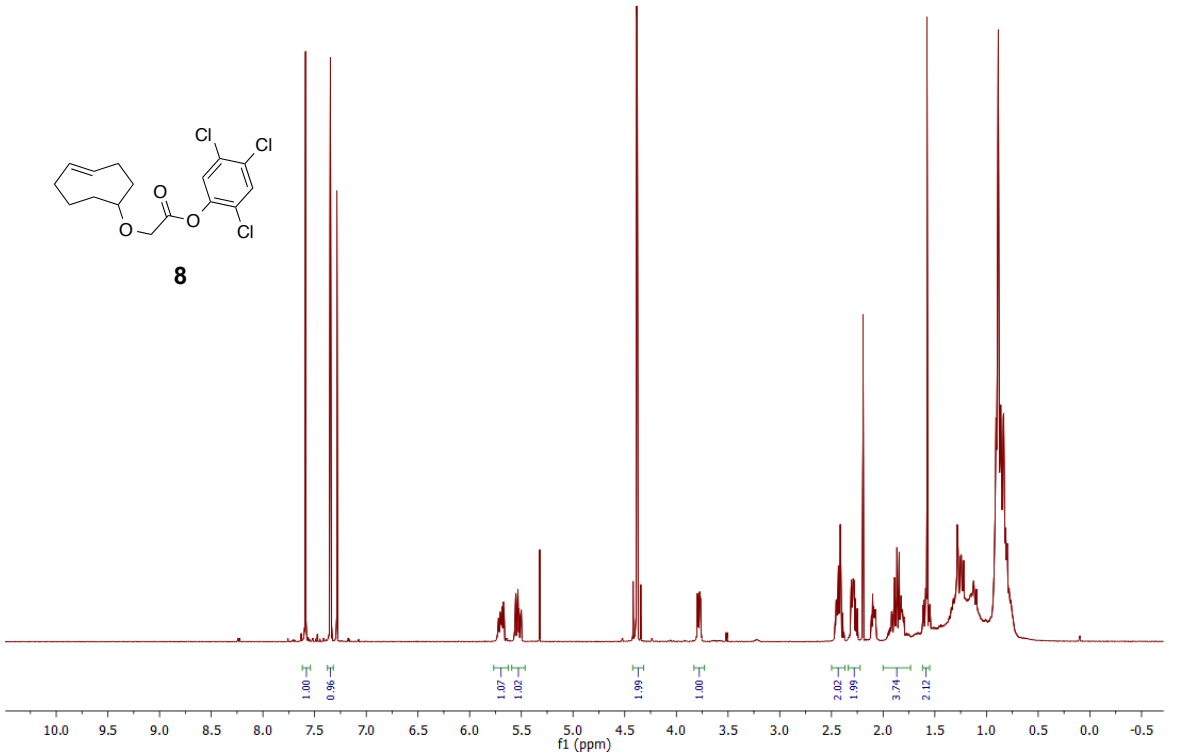
172.00
169.87
167.54
163.65
153.99
144.92
130.83
128.53
128.45
127.90
33.82
25.80
21.29
Tz 3
f1 (ppm)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O
O
Cl
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0.96
1.07
1.02
1.99
1.00
2.02
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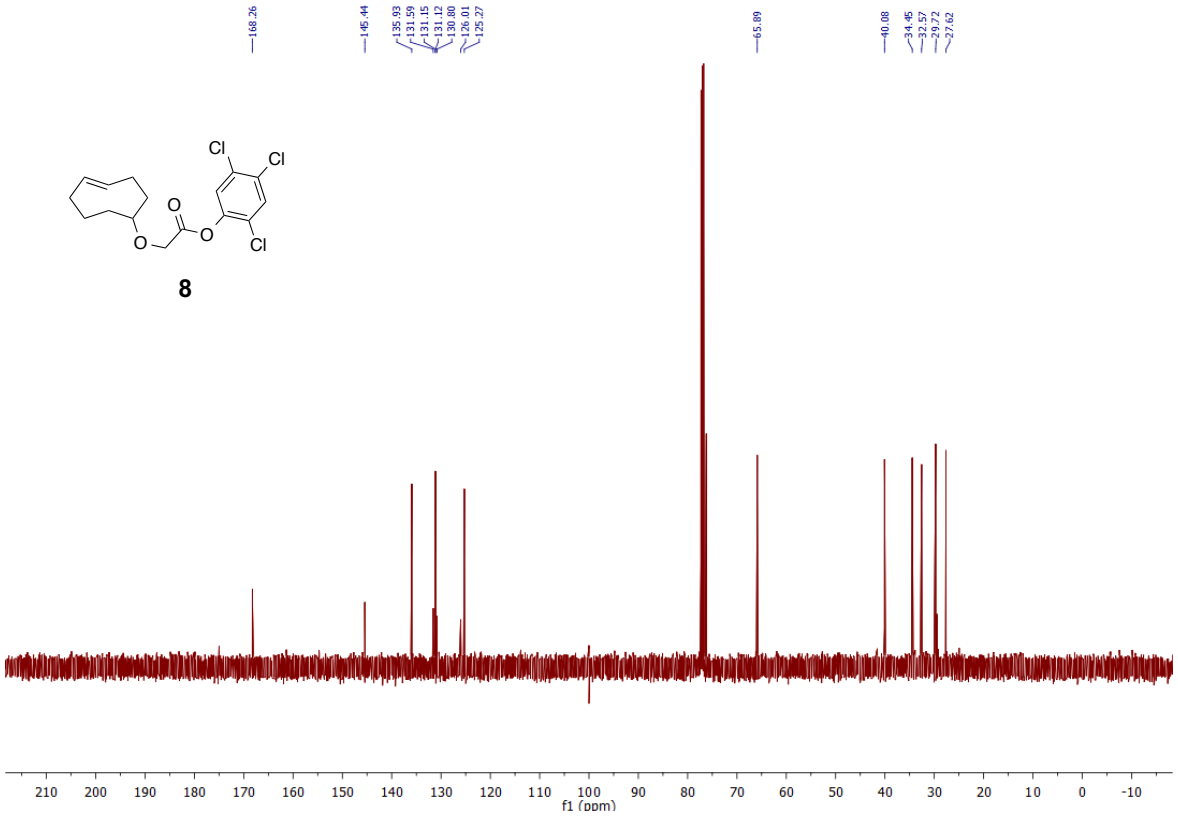
3.74
2.12
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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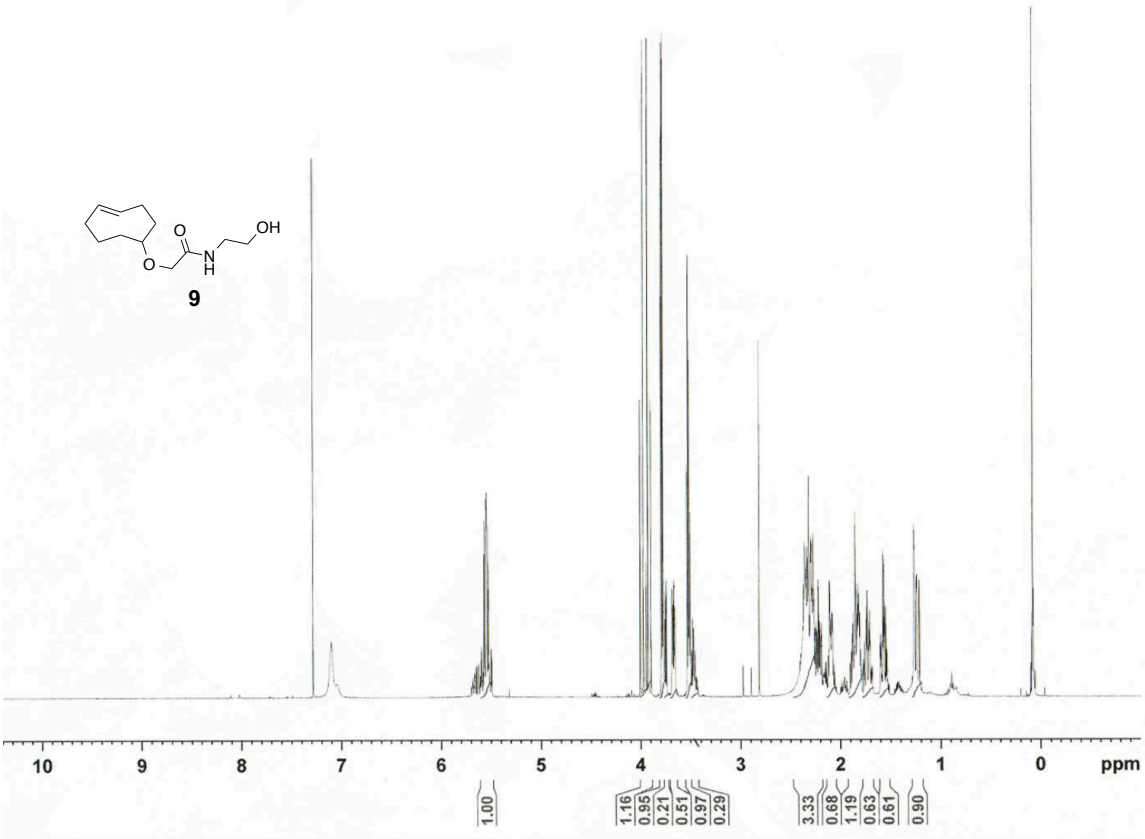
135.93
131.59
131.15
131.12
130.80
126.01
125.27
65.89
40.08
34.45
32.57
29.72
27.62
Cl
Cl
O
O
O
Cl
8
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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H
OH
O
9
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1.16
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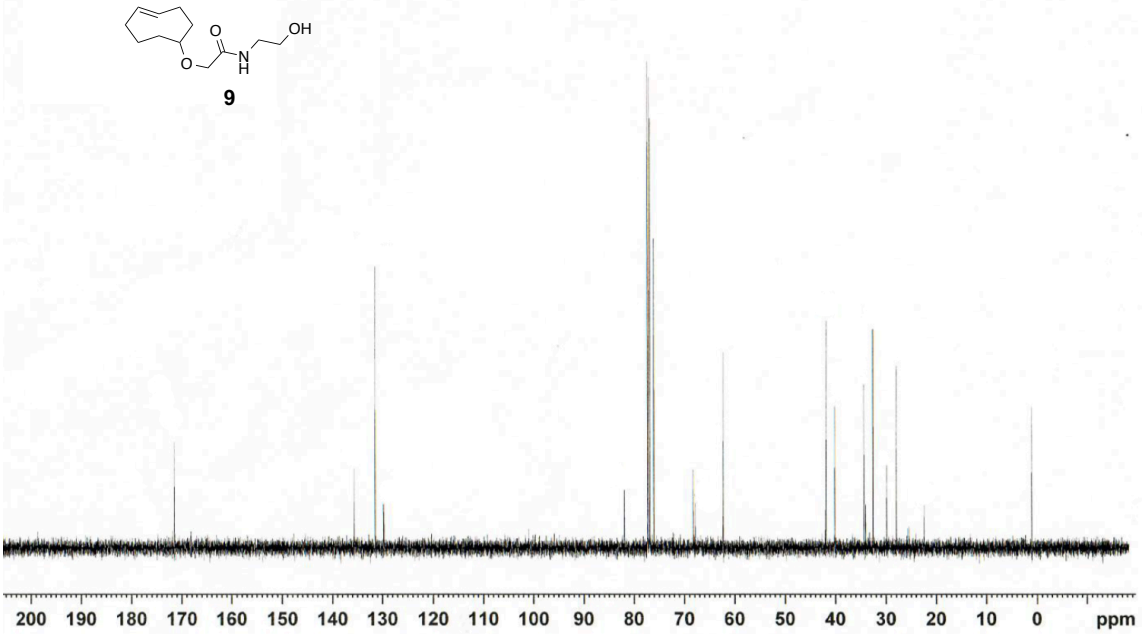
0.21
0.51
0.97
0.29
3.33
0.68
1.19
0.63
0.61
0.90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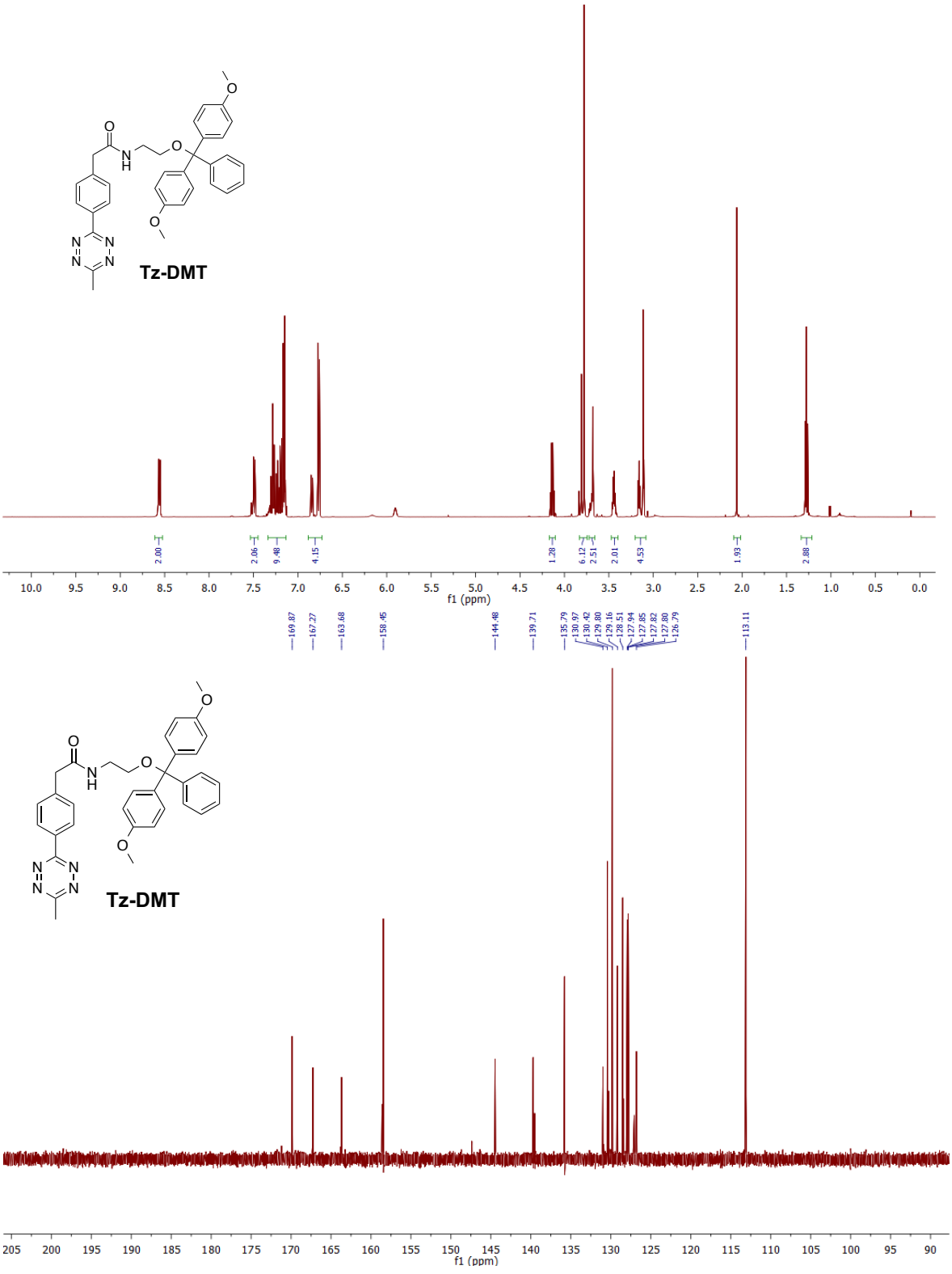
N
H
OH
O
9
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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4.53
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167.27
163.68
158.45
144.48
139.71
135.79
130.97
130.42
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128.51
127.94
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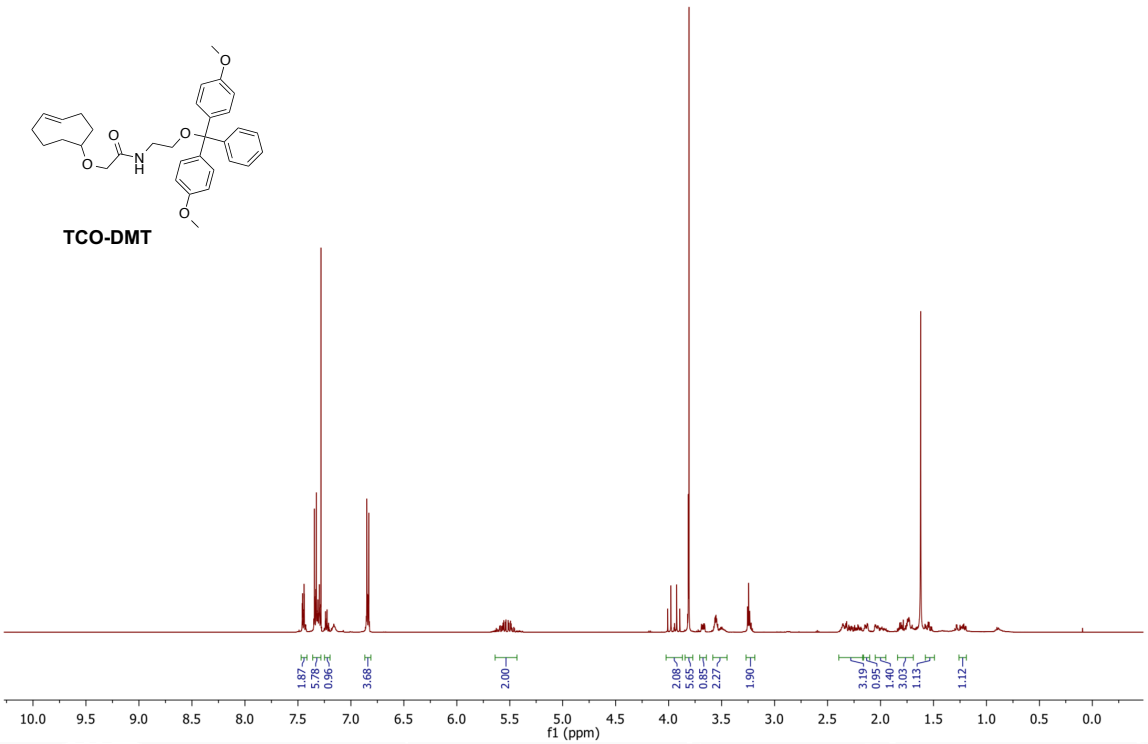
127.80
126.79
113.11
Tz-DMT
f1 (ppm)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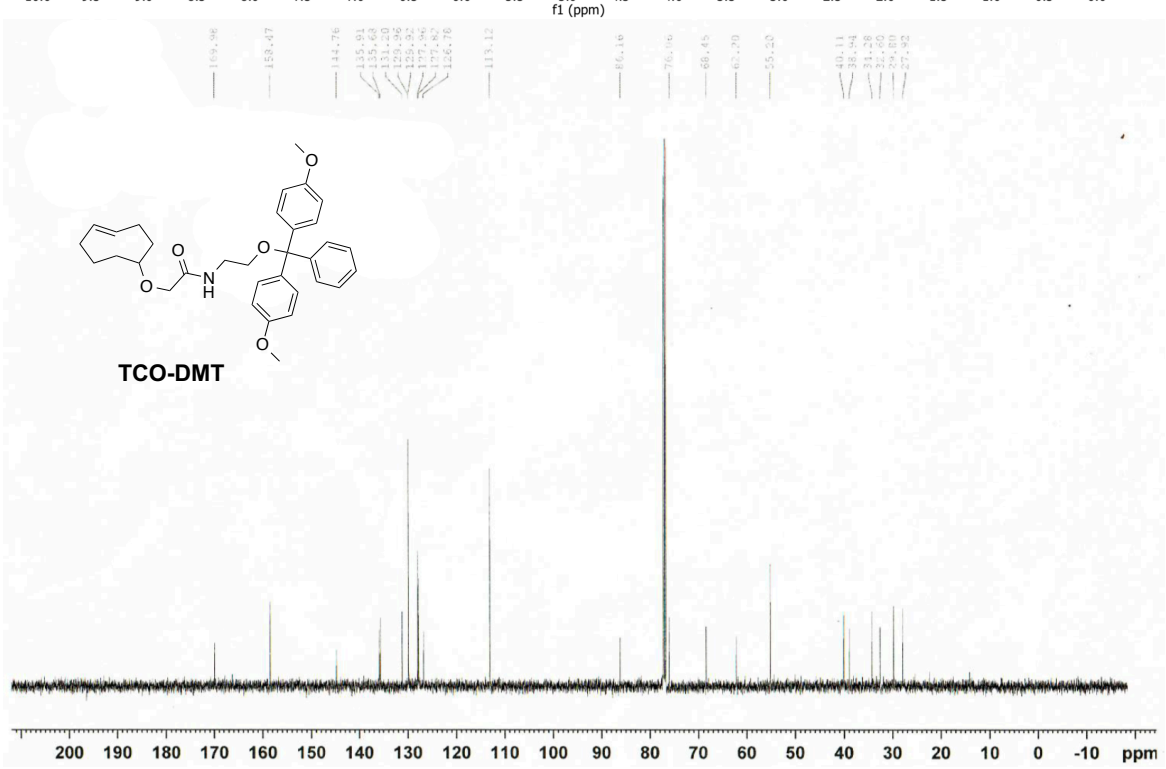
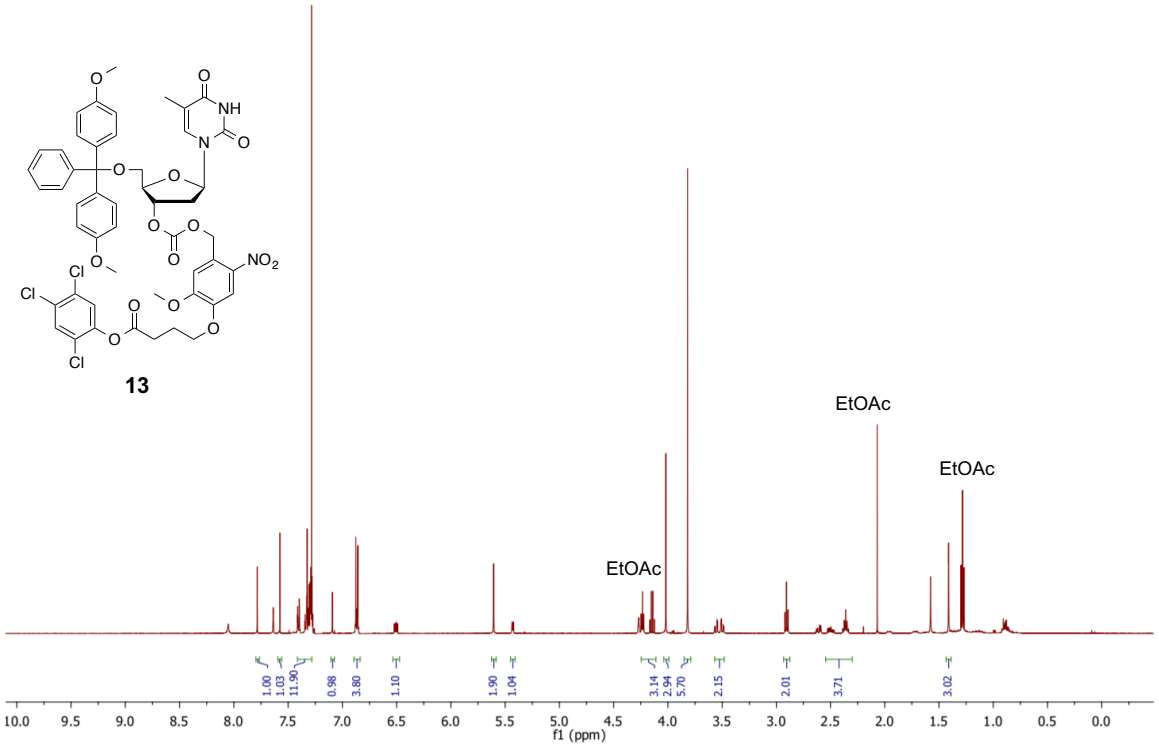
1.12
f1 (ppm)


TCO-DMT
ppm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EtOAc
EtOAc
EtOAc
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1.03
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1.10
1.90
1.04
3.14
2.94
5.70
2.15
2.01
3.71
3.02
f1 (ppm)