Supporting Information

Ultrafast Oxime Formation Enables Efficient Fluorescence Light-up Measurement of DNA Base Excision Repair

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Table of Contents

General Information

Instrumentation: NMR spectra were acquired on a Varian Inova 400 (400 MHz) or 500 (500 MHz) spectrometer and chemical shift reported in parts per million (δ) relative to internal standard TMS (0 ppm). Small molecule mass spectra were measured on a Waters 2795 system via electrospray ionization (ESI) with a ZQ single quadrupole MS. Oligonucleotide mass spectra were acquired by MALDI-TOF using a Bruker Microflex MALDI-TOF in negative ion mode. Ultraviolet spectra were measured on a Cary 300. Fluorescence emission and excitation spectra were recorded on a Jobin Yvon-Spex Fluorolog 3 spectrometer with an external temperature controller. Fluorescence time courses were collected on a Fluoroskan Ascent Microplate Fluorometer (Thermo Fisher Scientific). Oligonucleotide concentrations were determined by UV-absorption on a NanoDrop One Microvolume UV-Vis Spectrophotometer (ThermoFisher Scientific). DNA synthesis was carried out on an Applied Biosystems 394 DNA/RNA synthesizer.

Synthesis and Chemicals: All chemicals were purchased from Acros Organics, Combi-Blocks, Sigma-Aldrich and Oakwood Chemical and used without further purification. Phosphoramidites were purchased from Glen Research. Analytical TLC was performed on ready-to-use plates with silica gel 60 (Merck, F254), Flash column chromatography was performed over Fisher Scientific silica gel (grade 60, 230-400 mesh). All reagents were weighed and handled in air and backfilled under argon at room temperature. Unless otherwise noted, all reactions were performed under an argon atmosphere.

Enzymes and Buffers: *E. coli* Uracil DNA Glycosylase (UDG), Methyl Purine Glycosylase (MPG) and Single-strand selective monofunctional uracil glycosylase 1 (SMUG1) were purchased from New England Biolabs. Endonuclease III-like protein 1 (NTH1) and 8-oxoguanine glycosylase (OGG1) were purchased from Novus biologics. UNG was purchased from OriGene and whole cell lysates were purchased from Santa Cruz Biotech. Buffers were prepared from stock solutions from Sigma Aldrich.

General procedure for creating AP site DNA: A deoxyuridine containing oligonucleotide (typically 5-20 µM) was treated with 10 U/mL *E. coli* UDG (New England Biolabs) for 10 minutes at 37 °C in buffer to create AP site containing DNA (see below for MALDI-TOF confirmation). The hairpin was then reacted with probe from a DMSO stock solution (final DMSO concentration <5%).

Procedure for assessing fluorescence response of probes: To assess the fluorescence response of the probes, 25 µM AP site containing DNA (oligo **15)** was prepared in a 20 µL solution and allowed to react with 500 µM of each probe overnight. The DNA was then precipitated by the addition of 80 µL 0.33 M

sodium acetate and 300 µL ethanol and centrifuged at 21,100 g for 1 hr at 4 °C. The resulting DNA pellet was washed twice with 70% EtOH and re-suspended in buffer to a concentration of 2 µM. A portion of the DNA pellet was used for MALDI-TOF analysis. The emission spectra of the probe-oligonucleotide conjugate was measured and compared against a 2 µM solution of the free probe

In situ lesion formation: Calf thymus DNA (ctDNA) (Sigma Aldrich) was diluted to a concentration of 0.1 mg/mL in buffer from a 1 mg/mL stock solution in water. Fenton's reagent was generated by combining equimolar amounts of iron (II) ammonium sulfate and hydrogen peroxide at varying concentrations (50-200 µM). ctDNA was treated with increasing amounts of either dimethyl sulfate (DMS) (100-1000 µM), Fenton's reagent (50-200 µM) or a buffer control at 37 °C for 2 hr. The reaction was then quenched by the addition of 1 mM 2-mercaptoethanol and allowed to stand at room temperature for 30 minutes. **CCVJ1** was added to the reaction mixture (20 µM) and 60 µL aliquots were distributed onto a 384-well microplate (60 µL). DNA repair enzymes (100 nM OGG1 or MPG) were added directly to the well and fluorescence intensity monitored for 4 hours.

UGI IC50 measurement: Initial rate velocities were measured with UNG (5 nM), Oligo **15** (20 µM) and **CCVJ1** (2 µM) and increasing amounts of UGI (0.3 to 30 nM). Initial rates were calculated as the slope of the fluorescence time course following a 25 minute delay time. Reactions were performed in triplicate and the resulting IC_{50} curve was generated by fitting the data to the Boltzmann equation in OriginPro 8.5.

Cell Growth and Lysate Preparation: HeLa cells were grown in DMEM supplemented with FBS (10%), penicillin (100 U/mL), and streptomycin (100 U/mL) in a humidified incubator at 37 °C with 5% CO_2. Cells were arrested at phase G0/G1 by serum starvation for 24 hours. To prepare lysates, cells were collected in PBS by scraping and the protocol for the CellLytic™ NuCLEAR Extraction Kit (Sigma Aldrich) was used with Roche complete mini EDTA-free protease inhibitor tablets. Briefly, cells were grown to ~90% confluency and harvested by scraping (~$5x10^7$). Cells were rinsed twice with cold PBS and swelled in hypotonic lysis buffer for 15 minutes on ice (10 mM HEPES, pH 7.9, with 1.5 mM $MgCl_2$, 10 mM NaCl, 0.1 M DTT and 1x protease inhibitor). Cells were lysed by repeated passage through a 25-gauge needle and the cytosolic fraction collected. Nuclear proteins were extracted from the nuclear pellet by shaking with a high salt nuclear extraction buffer for 60 minutes (20 mM HEPES, pH 7.9, with 1.5 mM $MgCl_2$, 420 mM NaCl, 0.1 M DTT, 25% glycerol and 1x protease inhibitor). The nuclear and cytosolic fractions were then combined and total protein was determined by Bradford assay.

Cell Lysate Experiments: HeLa or MCF-7 whole cell lysates (Santa Cruz Biotech) were used without further preparation. To assess enzymatic activity, lysates were diluted to 0.2 mg/mL in buffer (50 mM Tris buffer pH 7, 100 mM NaCl) along with **CCVJ1** (25 µM) and the appropriate oligo substrate (5 µM) to a volume of 60 µL in a 384-well plate. Fluorescence intensity was monitored over the course of 4 hours at 37 °C.

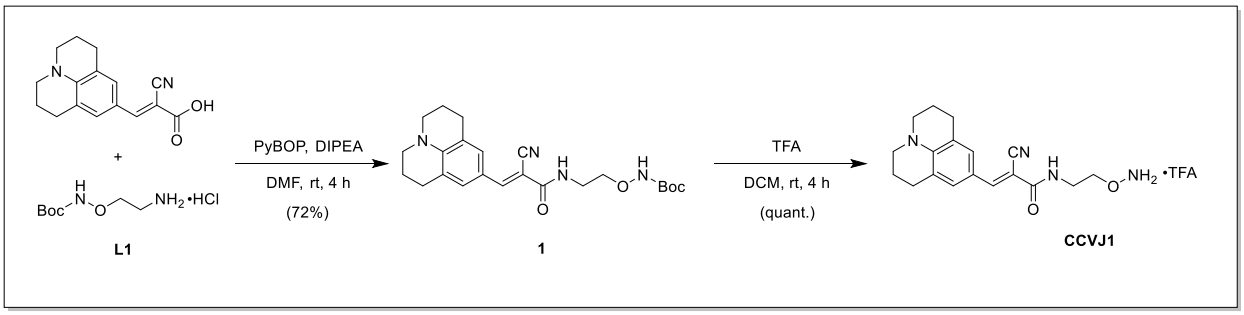
Fluorescence time courses: Unless otherwise stated, fluorescence time courses were collected on a Fluoroskan Ascent Microplate Fluorometer (Thermo Fisher Scientific) with black, non-binding 384 well plates (Greiner) at a reaction volume of 60 µL with 50 mM Tris buffer pH 7 (ionic strength adjusted to 100 mM with NaCl) with a fluorescein filter set (Ex. 485, Em. 538).

Molecular modeling: Molecular modeling was carried out using the Maestro 12.0 software package (Schrödinger, LLC).

Synthesis of UBER Probes

CCVJ Based Probes

Boc protected aminooxy linkers **L1**-**L3** were prepared as HCl salts by the method of Carrasco and coworkers[1] and 9-(2-Carboxy-2-cyanovinyl)julolidine (CCVJ) was prepared as described by Rumble and coworkers.[2]



Compound **1**

CCVJ (107 mg, 0.4 mmol), **L1** (85 mg, 0.4 mmol), PyBOP (229 mg, 0.44 mmol) and DIPEA (0.35 mL, 2 mmol) were dissolved in dry DMF (1 mL) and stirred for 1 hr. The reaction was then taken into ethyl acetate (15 mL), washed with 1M HCl and saturated brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The resulting crude was purified by FCC (EtOAc:Hexanes 2:3) yielding 123 mg (72%) of an orange foam.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.00 (s, 1H), 7.56 (s, 1H), 7.45 (s, 2H), 7.02 (s, 1H), 3.94 (t, *J* = 4.7 Hz, 2H), 3.66 (q, *J* = 5.5 Hz, 2H), 3.30 (t, *J* = 5.8 Hz, 4H), 2.74 (t, *J* = 6.3 Hz, 4H), 1.95 (p, *J* = 6.1 Hz, 4H), 1.49 (s, 9H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.55, 157.52, 152.59, 131.30, 120.94, 119.29, 118.86, 93.86, 82.24, 75.27, 50.32, 38.58, 28.44, 27.83, 21.42.

ESI-MS [M+H]: Calculated: 427.2; Observed: 427.4

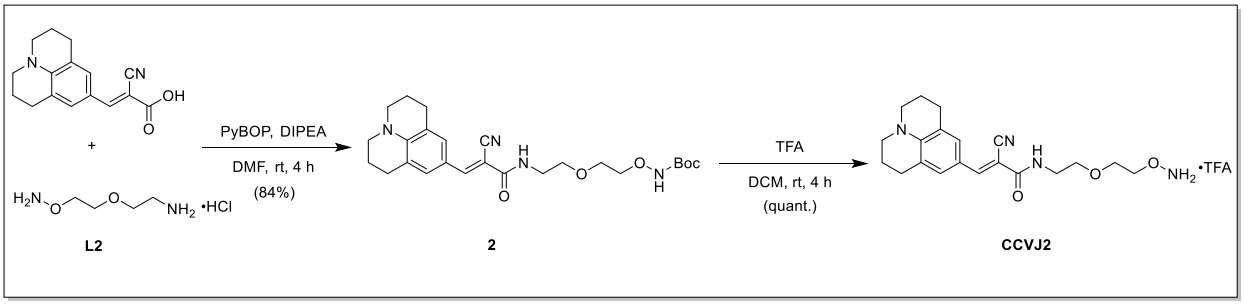
CCVJ1

Compound **1** (20.2 mg, 47.3 umol) was dissolved in 800 µL of a 1:1 solution of dry DCM and TFA. After 4 hours, the volatiles were evaporated under a continuous stream of Argon. Remaining TFA was co-evaporated by the addition of 1 drop Toluene yielding 20.0 mg (quant.) of a red-orange residue.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (t, *J* = 5.6 Hz, 1H), 7.82 (s, 1H), 7.42 (s, 2H), 4.04 (t, *J* = 5.3 Hz, 2H), 3.45 (q, *J* = 5.4 Hz, 2H), 3.28 (t, *J* = 5.7 Hz, 4H), 2.65 (t, *J* = 6.2 Hz, 4H), 1.84 (t, *J* = 6.0 Hz, 4H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 163.55, 158.93 (q, *J* = 37.5 Hz), 151.18, 147.32, 130.83, 120.92, 118.97, 117.98, 115.80 (q, *J* = 289.6 Hz), 94.14, 73.41, 49.80, 38.03, 27.52, 21.07.

ESI-MS [M+H]: Calculated: 327.2; Observed: 327.4



Compound **2**

CCVJ (107 mg, 0.4 mmol), **L2** (103 mg, 0.4 mmol), PyBOP (229 mg, 0.44 mmol) and DIPEA (0.35 mL, 2 mmol) were dissolved in dry DMF (1 mL) and stirred for 1 h. The reaction was then taken into ethyl acetate (15 mL), washed with 1M HCl and saturated brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The resulting crude was purified by FCC (EtOAc:hexanes 1:1) resulting in 158 mg (84%) of an orange foam.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.01 (s, 1H), 7.60 (s, 1H), 7.44 (s, 2H), 4.09 – 4.00 (m, 2H), 3.75 – 3.68 (m, 2H), 3.68 – 3.57 (m, 4H), 3.30 (t, *J* = 6.6 Hz, 4H), 2.74 (t, *J* = 6.3 Hz, 4H), 1.95 (p, *J* = 6.3, 5.8 Hz, 4H), 1.48 (s, 9H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.07, 157.44, 152.69, 131.30, 120.94, 119.71, 118.81, 93.54, 81.99, 75.77, 69.94, 68.76, 50.32, 40.05, 28.45, 27.83, 21.41.

ESI-MS [M+H]: Calculated: 471.3; Observed: 471.4

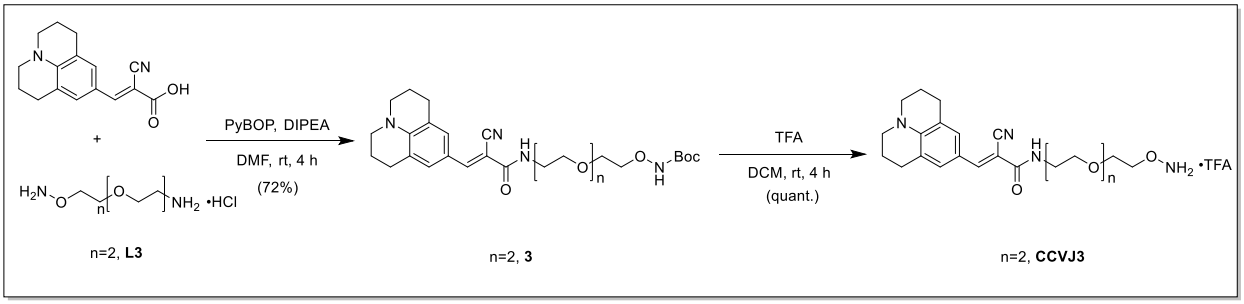
CCVJ2

Compound **2** (20.5 mg, 43.6 umol) was dissolved in 800 µL of a 1:1 solution of dry DCM and TFA. After 4 hours, the volatiles were blown off under a continuous stream of Argon. Remaining TFA was co-evaporated by the addition of 1 drop toluene yielding 20.4 mg (quant.) of a red-orange residue.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (t, *J* = 5.6 Hz, 1H), 7.80 (s, 1H), 7.40 (s, 2H), 4.08 (t, *J* = 3.9 Hz, 2H), 3.64 (t, *J* = 4.0 Hz, 2H), 3.49 (t, *J* = 6.0 Hz, 2H), 3.35 (q, *J* = 5.9 Hz, 2H), 3.28 (t, *J* = 5.8 Hz, 4H), 2.65 (t, *J* = 6.2 Hz, 4H), 1.84 (p, *J* = 6.1 Hz, 4H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 163.07, 158.91 (q, *J* = 37.6 Hz), 150.90, 147.15, 130.71, 120.89, 119.06, 118.07, 115.87 (q, *J* = 290.2 Hz), 94.80, 74.01, 69.38, 68.12, 49.78, 39.69, 27.54, 21.10.

ESI-MS [M+H]: Calculated: 371.2; Observed: 371.3



Compound **3**

CCVJ (107 mg, 0.4 mmol), **L3** (120 mg, 0.4 mmol), PyBOP (229 mg, 0.44 mmol) and DIPEA (0.35 mL, 2 mmol) were dissolved in dry DMF (1 mL) and stirred under an atmosphere of Argon for 1 hr. The reaction was then taken into ethyl acetate (15 mL), washed with 1M HCl and saturated brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The resulting crude was purified by FCC (EtOAc:hexanes 3:2) resulting in 148 mg (72%) of a red-orange foam.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.01 (s, 1H), 7.66 (s, 1H), 7.44 (s, 2H), 6.68 (s, 1H), 4.08 – 3.95 (m, 2H), 3.79 – 3.69 (m, 2H), 3.71 – 3.43 (m, 8H), 3.30 (t, *J* = 5.8 Hz, 4H), 2.74 (t, *J* = 6.3 Hz, 4H), 1.95 (p, *J* = 6.2 Hz, 5H), 1.47 (s, 9H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.05, 157.47, 152.66, 131.28, 120.93, 119.47, 118.82, 93.64, 81.84, 75.67, 70.71, 70.60, 70.02, 69.44, 50.31, 40.27, 28.47, 27.83, 21.41.

ESI-MS [M+H]: Calculated: 515.4; Observed: 515.5

CCVJ3

Compound **3** (17.0 mg, 33.0 umol) was dissolved in 800 µL of a 1:1 solution of dry DCM and TFA. After 4 hours, the volatiles were evaporated under a continuous stream of Argon. Remaining TFA was co-evaporated by the addition of 1 drop toluene yielding 17.0 mg (quant.) of a red-orange residue.

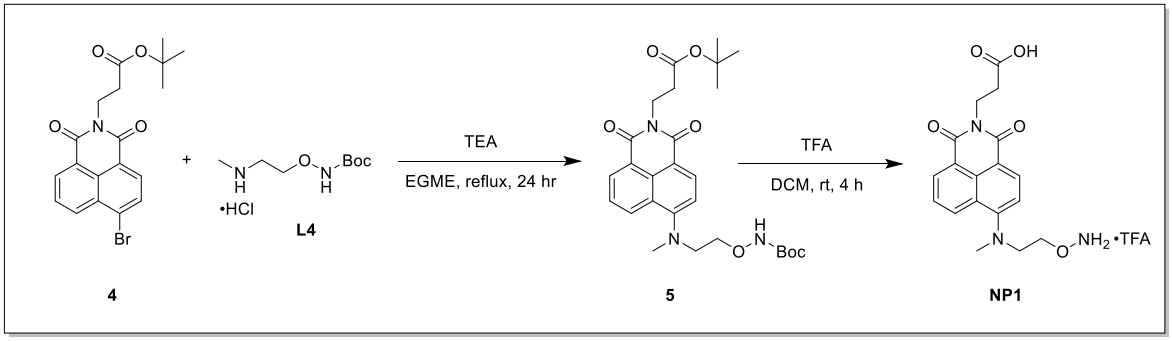
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (t, *J* = 5.6 Hz, 1H), 7.80 (s, 1H), 7.40 (s, 2H), 4.07 (t, *J* = 3.5 Hz, 2H), 3.63 (t, *J* = 3.9 Hz, 2H), 3.52 (s, 4H), 3.47 (t, *J* = 6.3, 5.8 Hz, 2H), 3.38 – 3.23 (m, 6H), 2.65 (t, *J* = 6.1 Hz, 4H), 1.84 (t, *J* = 6.0 Hz, 4H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.95, 158.99 (q, *J* = 37.5 Hz), 150.81, 147.09, 130.67, 120.85, 119.04, 118.10, 115.83 (q, *J* = 289.5 Hz), 94.86, 74.03, 70.19, 69.90, 69.21, 68.25, 49.75, 40.21, 27.51, 21.08.

ESI-MS [M+H]: Calculated: 415.2; Observed: 415.4

Naphthalimide Based Probes

Boc protected aminooxy linkers **L1** and **L4** were prepared as HCl salts by the method of Carrasco and coworkers[1] and compound **4** was prepared as described by Lee and coworkers.[3]



Compound **5**

Compound **4** (202 mg, 0.5 mmol) and **L4** (227 mg, 1.0 mmol) was dissolved in 2-methoxyethanol (1 mL) and trimethylamine (0.27 mL, 2 mmol) was added. The solution was stirred at 120 °C for 24 hours. The reaction was then cooled and concentrated *in vacuo*. The resulting crude residue was purified by FCC (1:1 EtOAc:Hexanes) yielding 48.5 mg (19%) of an orange foam.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.58 – 8.43 (m, 3H), 7.66 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.30 – 7.17 (m, 2H), 4.42 (t, *J* = 7.8, 7.4 Hz, 2H), 4.18 (t, *J* = 5.4 Hz, 2H), 3.61 (t, *J* = 5.5 Hz, 2H), 3.13 (s, 3H), 2.66 (t, *J* = 7.7 Hz, 2H), 1.46 (s, 9H), 1.42 (s, 9H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.84, 164.52, 163.95, 157.11, 132.61, 131.41, 131.19, 130.31, 126.16, 125.67, 123.17, 115.51, 82.27, 80.91, 73.98, 55.36, 41.99, 36.27, 34.13, 29.91, 28.41, 28.25.

ESI-MS [M+H]: Calculated: 514.3; Observed: 514.5

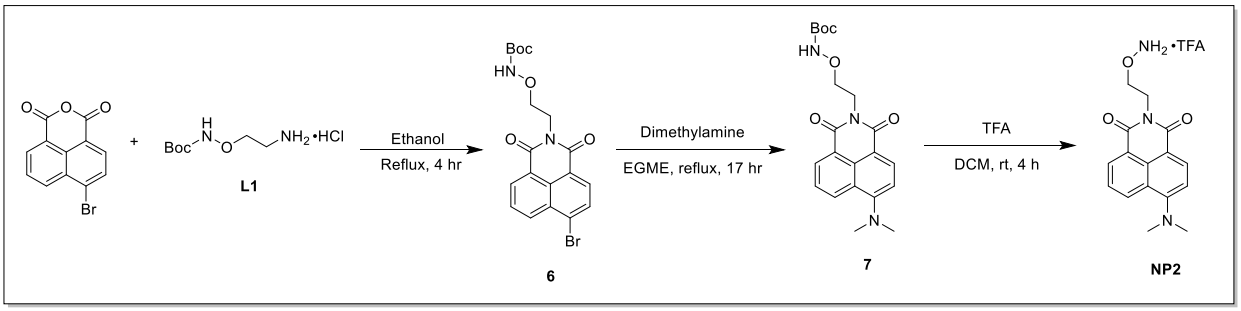
NP1

Compound **5** (19.7 mg, 38.3 umol) was dissolved in 800 µL of a 1:1 solution of dry DCM and TFA. After 4 hours, the volatiles were evaporated under a continuous stream of argon. Remaining TFA was co-evaporated by the addition of 1 drop Toluene yielding 17.5 mg (quant.) of an orange residue.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (d, *J* = 8.5 Hz, 1H), 8.45 (d, *J* = 7.1 Hz, 1H), 8.35 (d, *J* = 8.2 Hz, 1H), 7.76 (t, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 1H), 4.24 (q, *J* = 8.6, 6.7 Hz, 4H), 3.64 (t, *J* = 5.2 Hz, 2H), 3.06 (s, 3H), 2.55 (t, *J* = 7.7 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 172.95, 163.96, 163.34, 158.84 (q, *J* = 36.3 Hz), 156.44, 132.54, 131.52, 131.15, 129.90, 126.02, 125.55, 122.87, 116.22 (q, *J* = 292.1 Hz), 115.83, 115.09, 72.12, 54.62, 41.46, 36.03, 32.79.

ESI-MS [M+H]: Calculated: 358.1; Observed: 358.3



Compound **6**

4-bromo-1,8-naphthalic anhydride (277 mg, 1 mmol) and **L1** (234 mg, 1.1 mmol) were suspended in ethanol (3 mL) and heated to reflux while stirring for 4 h. The solution was cooled and the product was isolated by vacuum filtration yielding 213 mg of a grey solid (61%) that was used without further purification

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.63 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.55 (dd, *J* = 8.5, 1.1 Hz, 1H), 8.39 (d, *J* = 7.9 Hz, 1H), 8.05 (s, 1H), 8.02 (d, *J* = 7.9 Hz, 1H), 7.83 (dd, *J* = 8.5, 7.3 Hz, 1H), 4.45 (t, *J* = 4.6 Hz, 2H), 4.12 (t, *J* = 4.9 Hz, 2H), 1.47 (s, 9H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.39, 156.53, 133.87, 132.59, 131.75, 131.38, 130.95, 130.83, 129.22, 128.35, 122.94, 122.06, 113.83, 81.71, 73.36, 38.72, 28.48.

ESI-MS [M+H]: Calculated: 435.1; Observed: 435.3

Compound **7**

Compound **6** (87 mg, 0.2 mmol) was dissolved in 2-methoxyethanol (2 mL) along with dimethylamine (0.25 mL, 2 mmol). The solution was stirred at 120 °C for 2 hours. The solution was cooled and the reaction concentrated *in vacuo*. The resulting crude residue was purified by FCC (4:1 EtOAc:Hexanes) yielding 32.6 mg (41%) of an orange foam.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.55 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.44 (t, *J* = 9.7, 8.3 Hz, 2H), 8.27 (s, 1H), 7.63 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.08 (d, *J* = 8.2 Hz, 1H), 4.45 (t, *J* = 4.5 Hz, 2H), 4.12 (t, *J* = 4.9 Hz, 2H), 3.11 (s, 6H), 1.48 (s, 9H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.80, 156.48, 133.25, 131.81, 131.55, 130.59, 125.32, 125.04, 122.92, 114.58, 113.83, 113.42, 81.46, 73.47, 44.98, 38.35, 28.52.

ESI-MS [M+H]: Calculated: 400.2; Observed: 400.3

NP2

Compound **5** (32.6 mg, 81.5 umol) was dissolved in 800 µL of a 1:1 solution of dry DCM and TFA. After 4 hours, the volatiles were evaporated under a continuous stream of argon. Remaining TFA was co-evaporated by the addition of 1 drop toluene yielding 27.3 mg (quant.) of an orange residue.

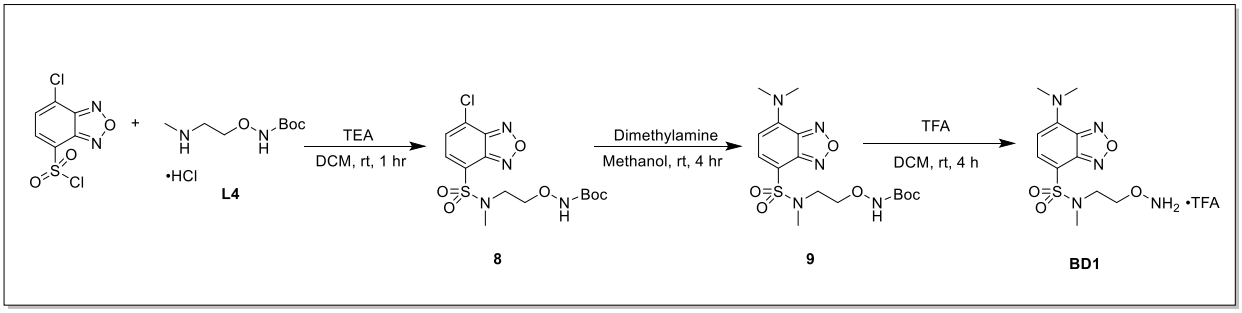
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (d, *J* = 8.6 Hz, 1H), 8.43 (d, *J* = 7.4 Hz, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 7.72 (t, *J* = 8.4, 7.4 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 4.27 (dt, *J* = 33.4, 5.4 Hz, 4H), 3.08 (s, 6H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 164.48, 163.77, 159.12 (q, *J* = 37.3 Hz), 157.41, 133.15, 132.51, 131.40, 130.40, 127.97, 125.60, 124.74, 122.73, 116.13 (q, *J* = 288.9, 287.9 Hz), 113.49, 72.52, 45.00, 37.82.

ESI-MS [M+H]: Calculated: 300.1; Observed: 300.3

Benzoxadiazole Based Probes

Boc protected aminooxy linkers **L1** and **L4** were prepared as HCl salts by the method of Carrasco and coworkers[1] and 7-chloro-*N,N*-dimethyl-*2*,1,3-benzoxadiazole-4-sulfonamide was prepared as described by Pagano and coworkers.[4]



Compound **8**

7-Chloro-2,1,3-benzoxadiazole-4-sulfonyl chloride (253 mg, 1 mmol) and **L4** (249 mg, 1.1 mmol) were dissolved in dry DCM (12 mL). Trimethylamine (0.34 mL, 2.5 mmol) was added dropwise and the solution was stirred for 1 hr. The reaction was then diluted with DCM (50 mL) and washed with 1M HCl and concentrated brine. The organic fraction was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield 342 mg (84%) of a yellow-green oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.99 (d, *J* = 7.3 Hz, 1H), 7.54 (d, *J* = 7.3 Hz, 1H), 7.35 (s, 1H), 4.01 (t, *J* = 5.0 Hz, 2H), 3.63 (t, *J* = 5.0 Hz, 2H), 3.04 (s, 3H), 1.46 (s, 9H).

ESI-MS [M+H]: Calculated: 407.1; Observed: 407.3

Compound **9**

Compound **8** (342 mg, 0.85 mmol) was dissolved in methanol (3 mL) and dimethylamine was added dropwise (0.67 mL, 8,5 mmol). The solution was stirred at room temperature for 4 hrs. The reaction was then concentrated *in vacuo*. The resulting crude residue was re-dissolved in EtOAc (25 mL) and washed with 1M HCl and concentrated brine. The organic fraction was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield 315 mg (89%) of an orange foam.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.90 (d, *J* = 8.2 Hz, 1H), 7.56 (s, 1H), 6.02 (d, *J* = 8.3 Hz, 1H), 4.01 (dd, *J* = 5.5, 4.9 Hz, 2H), 3.57 (t, *J* = 5.2 Hz, 2H), 3.50 (s, 6H), 2.91 (d, *J* = 0.4 Hz, 3H), 1.47 (d, *J* = 0.4 Hz, 9H).

^{13}C NMR (151 MHz, cdcl$_3$) δ 156.73, 146.64, 145.08, 143.27, 138.49, 108.93, 101.13, 81.63, 77.22, 77.01, 76.80, 73.66, 48.35, 42.54, 35.30, 28.16.

ESI-MS [M+H]: Calculated: 416.2; Observed: 416.3

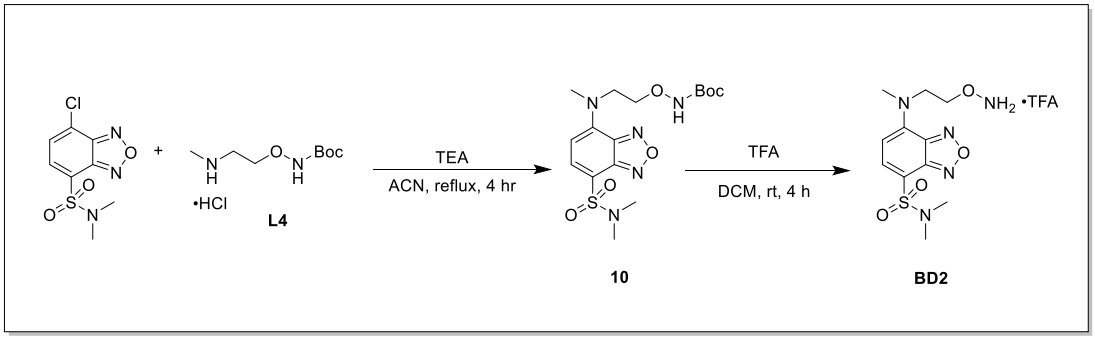
BD1

Compound **9** (21.9 mg, 52.7 umol) was dissolved in 800 µL of a 1:1 solution of dry DCM and TFA. After 4 hours, the volatiles were evaporated under a continuous stream of Argon. Remaining TFA was co-evaporated by the addition of 1 drop toluene yielding 21.8 mg (quant.) of an orange residue.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (d, J = 8.4 Hz, 1H), 6.26 (d, J = 8.4 Hz, 1H), 4.12 (t, J = 5.2 Hz, 2H), 3.49 – 3.42 (m, 8H), 2.77 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 158.96 (q, J = 36.2 Hz), 147.10, 145.35, 143.61, 139.52, 116.20 (q, J = 290.7, 289.2 Hz), 106.64, 102.06, 72.95, 48.34, 42.75, 35.78.

ESI-MS [M+H]: Calculated: 316.1; Observed: 316.3



Compound **10**

7-chloro-*N,N*-dimethyl-*2*,1,3-benzoxadiazole-4-sulfonamide (105 mg, 0.4 mmol) and **L4** (181 mg, 0.8 mmol) was dissolved in dry acetonitrile (5 mL) with trimethylamine (0.28 mL, 2 mmol). The solution was heated to reflux while stirring for 4 h. The reaction was then concentrated *in vacuo*, taken up into EtOAc (25 mL) and washed with 1M HCl and concentrated brine. The organic fraction was concentrated *in vacuo* yielding 154 mg (92%) of a yellow-green oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.90 (d, *J* = 8.3 Hz, 1H), 6.14 (d, *J* = 8.3 Hz, 1H), 4.35 (t, *J* = 5.2 Hz, 2H), 4.21 (t, *J* = 5.2 Hz, 2H), 3.40 (s, 3H), 2.90 (s, 6H), 2.12 (d, *J* = 1.0 Hz, 0H), 2.07 (s, 2H), 1.49 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 157.63, 142.43, 138.76, 123.33, 122.28, 101.98, 82.18, 75.26, 67.84, 60.40, 41.17, 37.83, 28.19.

ESI-MS [M+H]: Calculated: 416.2; Observed: 416.3

BD2

Compound **10** (22.8 mg, 54.9 umol) was dissolved in 800 µL of a 1:1 solution of dry DCM and TFA. After 4 hours, the volatiles were evaporated under a continuous stream of argon. Remaining TFA was co-evaporated by the addition of 1 drop toluene yielding 22.7 mg (quant.) of an orange residue.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.86 (d, *J* = 8.3 Hz, 1H), 6.40 (d, *J* = 8.4 Hz, 1H), 4.31 (dq, *J* = 9.6, 5.4 Hz, 4H), 3.35 (s, 3H), 2.73 (s, 6H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 158.94 (q, *J* = 36.3 Hz), 147.49, 145.02, 142.79, 139.72, 116.11 (q, *J* = 291.3 Hz), 106.87, 103.13, 73.08, 52.35, 41.29, 37.87.

ESI-MS [M+H]: Calculated: 316.1; Observed: 316.3

Table S1. List of Oligonucleotides

Unless otherwise stated, all oligonucleotides where purchased from Integrated DNA Technologies (IDT) and used without further purification. Oligonucleotides prepared in house were synthesized on an ABI 394 instrument using phosphoramidites purchased from Glen Research and purified using a Glen Pak cartridge. All sequences are comprised entirely of DNA.

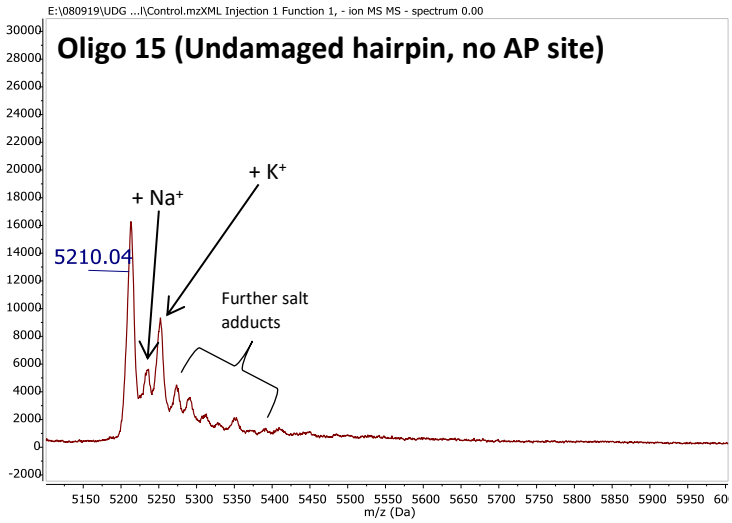
Oligo #	Source	Name	Sequence (5'->3')
1	IDT	Neighbor AA	CGA**U**AAGGAACTTATCG
2	IDT	Neighbor TA	CGT**U**AAGGAACTTAACG
3	IDT	Neighbor CA	CGC**U**AAGGAACTTAGCG
4	IDT	Neighbor GA	CGG**U**AAGGAACTTACCG
5	IDT	Neighbor AT	CGA**U**TAGGAACTAATCG
6	IDT	Neighbor TT	CGT**U**TAGGAACTAAACG
7	IDT	Neighbor CT	CGC**U**TAGGAACTAAGCG
8	IDT	Neighbor GT	CGG**U**TAGGAACTAACCG
9	IDT	Neighbor AC	CGA**U**CAGGAACTGATCG
10	IDT	Neighbor TC	CGT**U**CAGGAACTGAACG
11	IDT	Neighbor CC	CGC**U**CAGGAACTGAGCG
12	IDT	Neighbor GC	CGG**U**CAGGAACTGACCG
13	IDT	Neighbor AG	CGA**U**GAGGAACTCATCG
14	IDT	Neighbor TG	CGT**U**GAGGAACTCAACG
15	IDT	Neighbor CG	CGC**U**GAGGAACTCAGCG
16	IDT	Neighbor GG	CGG**U**GAGGAACTCACCG
17	IDT	Deoxyinosine	CGC**Hx**GAGGAACTCAGCG
18	IDT	ssDNA	CGC**U**GAGGA
19	IDT	Pseudo AP	CGC**S**GAGGAACTCAGCG
20	IDT	Control	CGCTGAGGAACTCAGCG
21	In House	NTH1	CGC**5hC**GAGGAACTCGGCG
22	In House	OGG1	CGC**8oG**GAGGAACTCCGCG

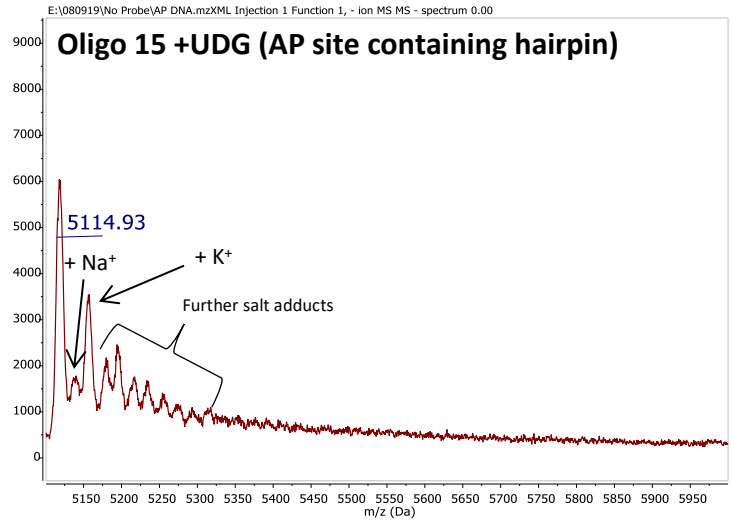
U = deoxyuridine, Hx = Deoxyinosine, S = tetrahydrofuran spacer, 5hC = 5-hydroxycytidine, 8oG = 8-oxoguanidine

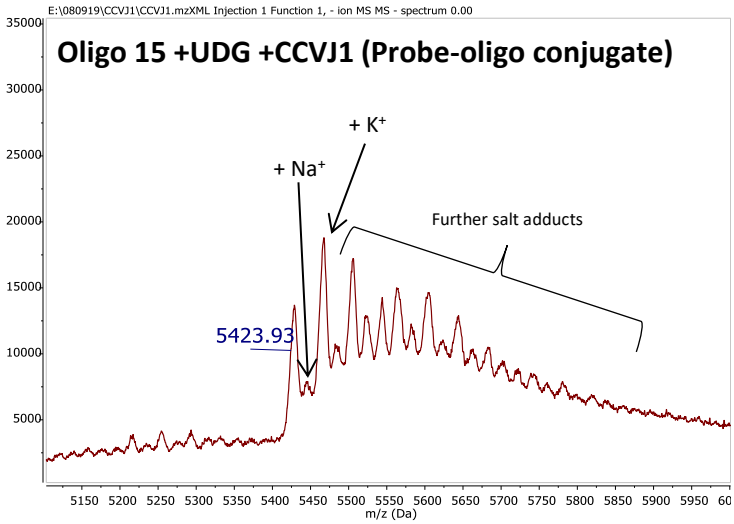
Table S2. MALDI-TOF Data

Species	**Calc'd mass**	**Observed mass**
Oligo **15**	5206.41	5210.04
Oligo **15** +UDG (AP Site)	5112.34	5114.93
CCVJ1 Conjugate	5420.72	5423.93
CCVJ2 Conjugate	5464.77	5465.05
CCVJ3 Conjugate	5508.83	5511.49
NP1 Conjugate	5451.69	5451.56
NP2 Conjugate	5393.65	5396.57
BD1 Conjugate	5409.67	5412.31
BD2 Conjugate	5409.67	5409.88
Oligo **21**	5236.44	5237.9
Oligo **22**	5221.42	5220.6

Representative MALDI Spectra of Oxime Adducts







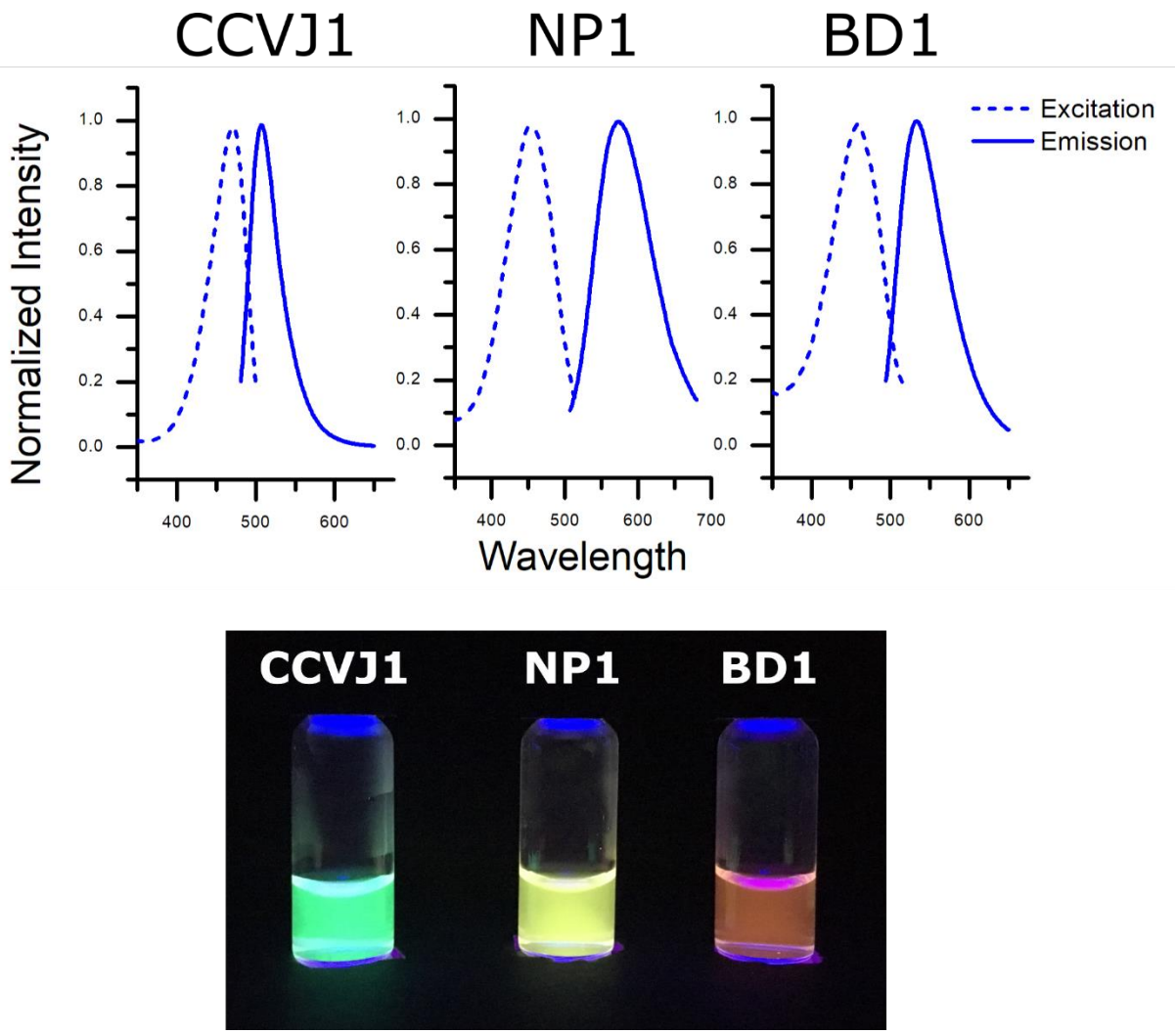


Figure S1. Excitation and emission spectra of DNA/probe conjugates (5 µM) with **CCVJ1**, **NP1** and **BD1** with oligo **15** in 50 mM Tris buffer at pH 7.0 (ionic strength adjusted to 100 mM with NaCl). Variations in linker attachment sight or length did not significantly alter either excitation or emission. Photograph taken on a gel illuminator with excitation at 365 nm (CCVJ = 5 µM, NP1 and BD1 = 10 µM)

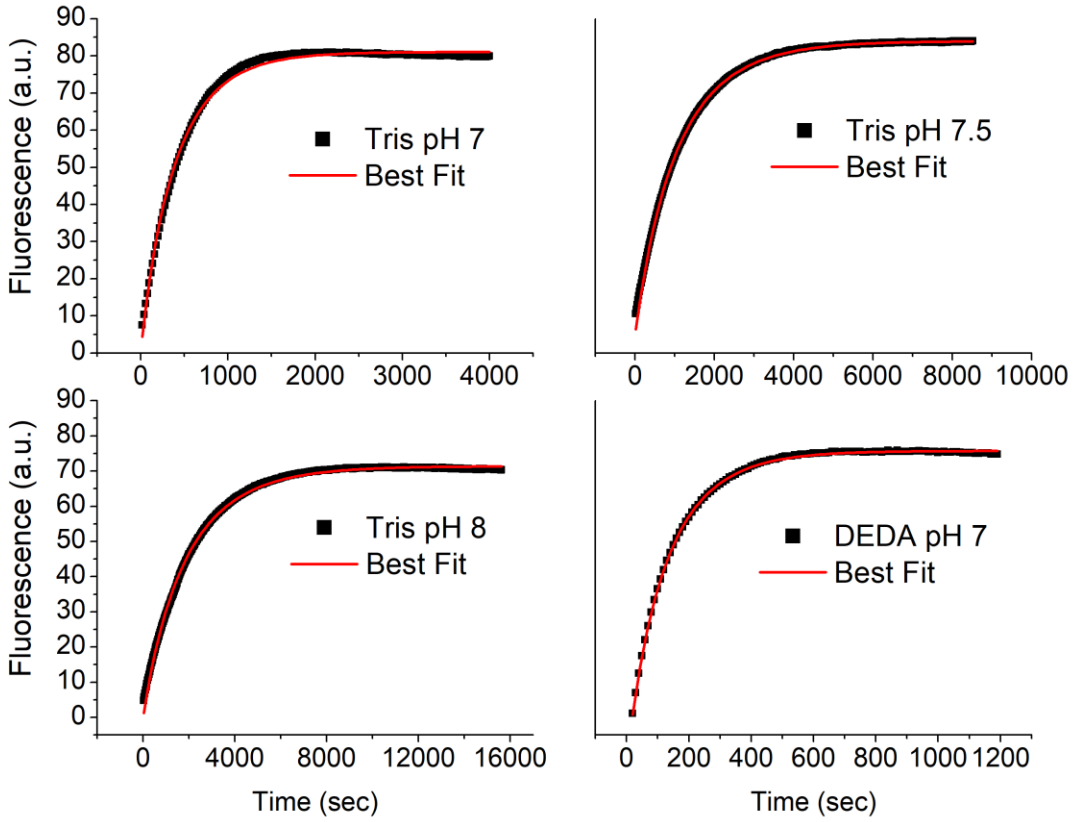


Figure S2. Representative plots for calculations of k_2 for **CCVJ1** under various buffer conditions with oligo **15**. Plots were fitted in OriginPro 8.5 according to the equation derived by Larsen *et. al.*[5] for second-order rate constants of oxime formation. Apparent second-order rate constants were calculated as the average of three replicates.

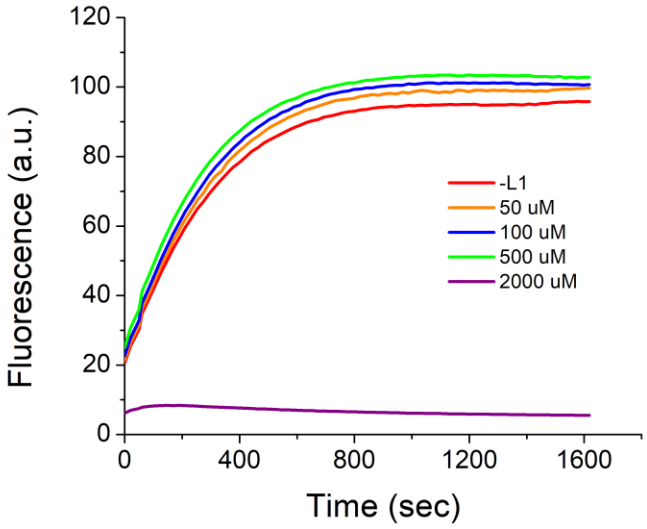


Figure S3. Effect of adding free linker **L1** to the reaction of **CCVJ1** (5 µM) with AP site containing DNA (20 µM) in buffer. The linker does not significantly compete with **CCVJ1** for oxime formation until concentrations exceed >500 µM. Interestingly, at 500 µM the linker appears to act as a "self-catalyst" by accelerating the reaction rate further.

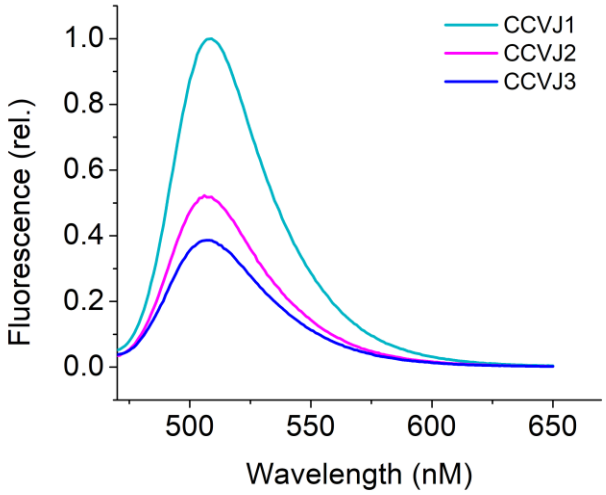


Figure S4. Relative fluorescence intensity of **CCVJ1**-**3** when bound to the AP site of Oligo **15** DNA. The emission intensity of **CCVJ2** and **CCVJ3** is significantly reduced relative to **CCVJ1**, likely due to increased linker flexibility. Excitation at 470 nm.

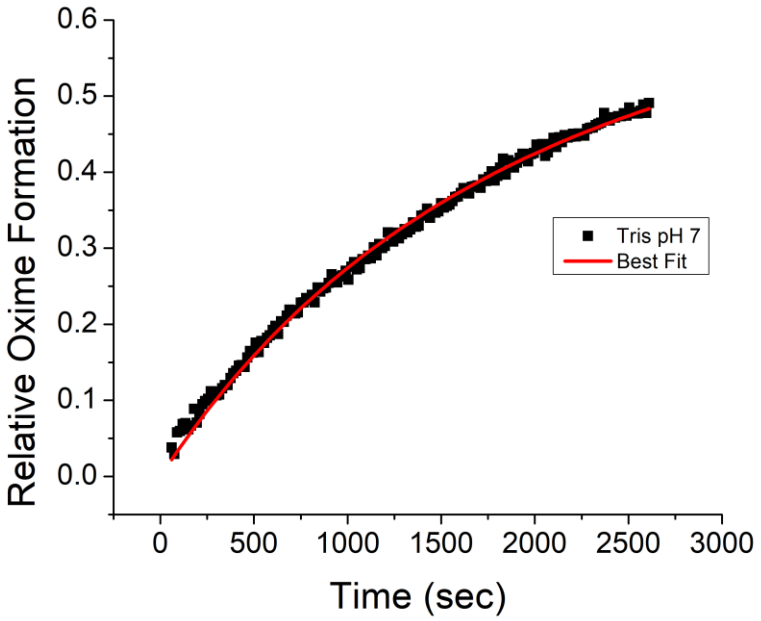


Figure S5. Representative plot for the reaction of **CCVJ1** with single stranded DNA oligonucleotide **18**. Second-order rate constant was calculated as before with the double-stranded substrate. Single-stranded DNA (oligo **18**) yielded a significantly lower overall light-up as compared to double-stranded DNA.

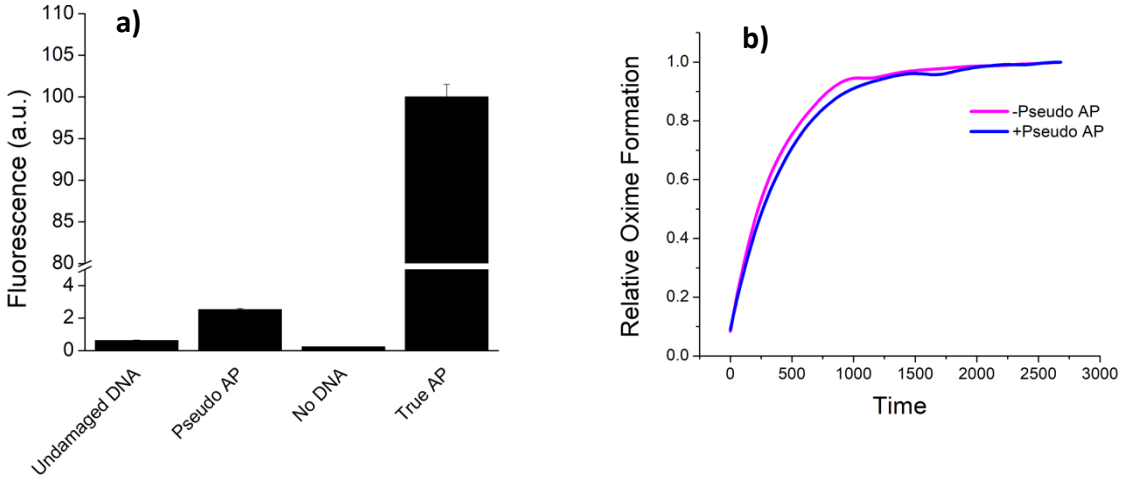


Figure S6. Interaction between **CCVJ1** and DNA. **a)** Fluorescence response of **CCVJ1** (5 µM) with 20 µM of undamaged double stranded DNA or a pseudo AP containing DNA strand compared to covalent linkage to a true AP site. The small increase in fluorescence observed between the free probe (no DNA) and the undamaged DNA is likely due to weak intercalation interactions. The pseudo AP site induces a further increase in fluorescence, likely due to increased intercalation/base stacking interactions at the pseudo AP site. However, these interactions do not appear to constrain bond rotation appreciably when compared

to the covalent linkage with a true AP site. (Ex. 485, Em. 538) **b)** Oxime formation between **CCVJ1** (5 µM) and AP site containing DNA (20 µM) with and without 20 µM pseudo AP containing hairpin. (Ex. 485, Em. 538)

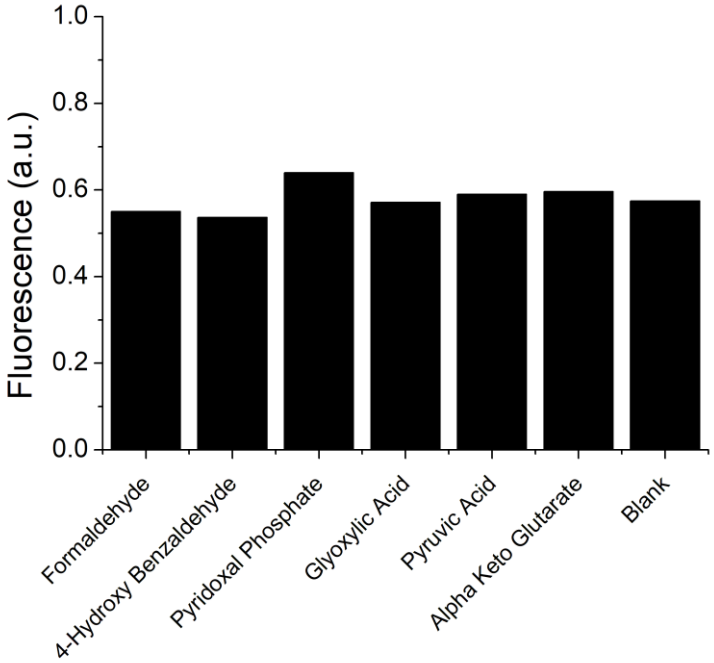


Figure S7. Fluorescence response of 5 µM **CCVJ1** with 500 µM of various biologically relevant carbonyl species after 1 h incubation in buffer at 37 °C. The absence of any light-up response suggests that oxime formation with small molecules does not appreciably constrain bond rotation. (Ex. 485, Em. 538)

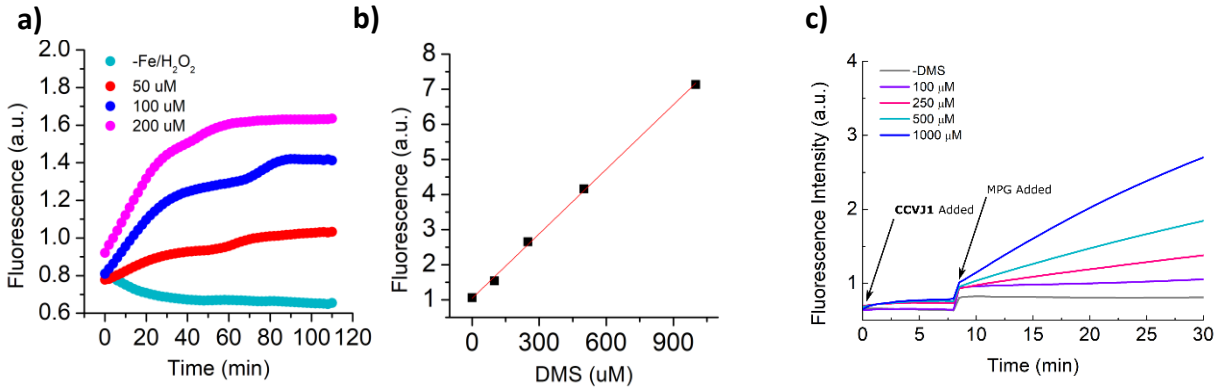


Figure S8. **a)** Measurement of OGG1 activity (100 nM) on oxidized calf thymus DNA (ctDNA) generated *in situ.* DNA was oxidized by treating ctDNA (0.1 mg/mL) with increasing amounts of Fenton's reagent (Fe/H_2O_2). **b)** Fluorescence signal observed by the repair of alkylated ctDNA with MPG demonstrates a linear relationship between final fluorescence signal and amount of DMS used to treat ctDNA from (see **Figure 7** in article text)**.** The linearity of the response suggests longer DMS treatments or higher concentrations of DMS may yield even strong signal from MPG repair of ctDNA. **c**) Pre-incubation of alkylated ctDNA with **CCVJ1** prior to addition of MPG shows stable fluorescence suggesting that the alkylated ctDNA alone does not generate a false positive through spontaneous depurination.

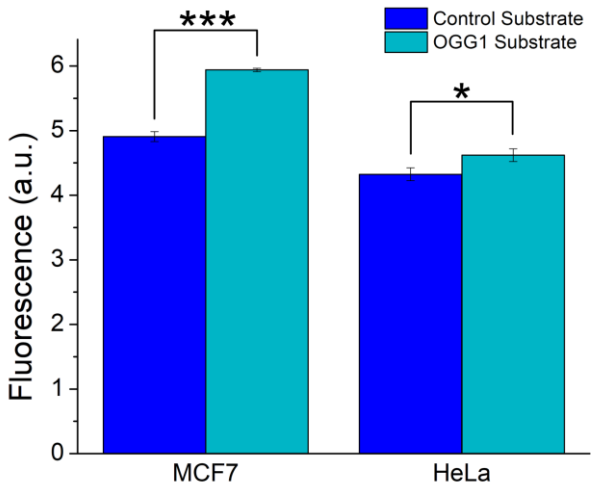


Figure S9. Measurement of OGG1 activity in HeLa cells vs activity in MCF7 cells. Lysates (0.2 mg/mL) were added to buffer containing 5 µM of either oligo **18** (non-lesion control substrate) or oligo **22** (OGG1 substrate) with 25 µM **CCVJ1** and incubated for 4 hrs. (Ex. 485, Em. 538)

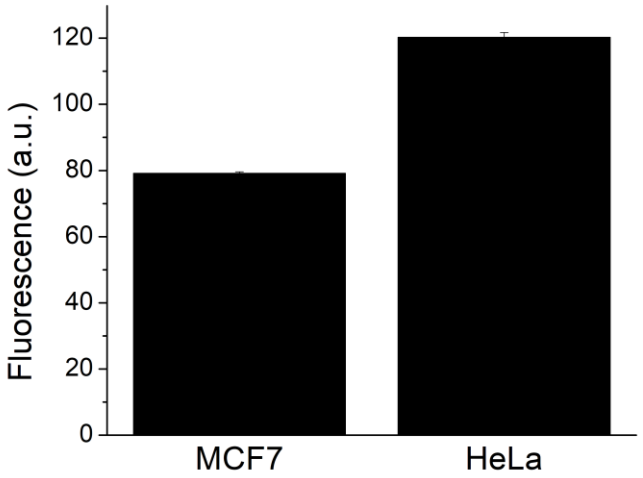


Figure S10. Measurement of UNG activity in HeLa cells vs activity in MCF7 cells. Lysates (0.2 mg/mL) were added to buffer containing 5 µM of either oligo **18** (non-lesion control substrate) or oligo **15** (UNG substrate) with 25 µM **CCVJ1** and incubated for 4 h. (Ex. 485, Em. 538)

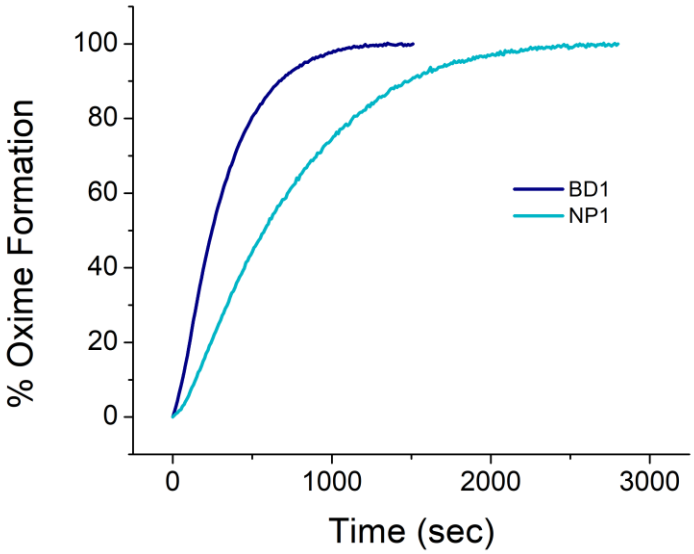


Figure S11. Time course of AP-site oxime formation of NP1 and BD1 (5 µM) with Oligo **15** (20 µM) in buffer at 37 °C. The faster relative rate of the naphthalimide probe suggests that the larger, nonpolar surface aids in intercalation and relative rate acceleration. (Ex. 485, Em. 538)

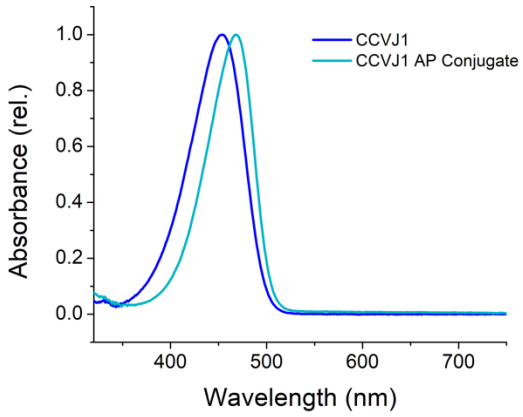


Figure S12. Normalized absorption spectra of free **CCJV1** probe and AP site bound probe in buffer. Oxime formation with the AP site red shifts absorption from a λ_{max} of 454 nm in the unbound probe to a λ_{max} of 468 nm in the bound probe, likely due to the lower-polarity environment of the DNA duplex. The extinction coefficient calculated for the unbound probe is 30,400 $M^{-1}\cdot cm^{-1}$ and 28,700 $M^{-1}\cdot cm^{-1}$ for the bound probe.

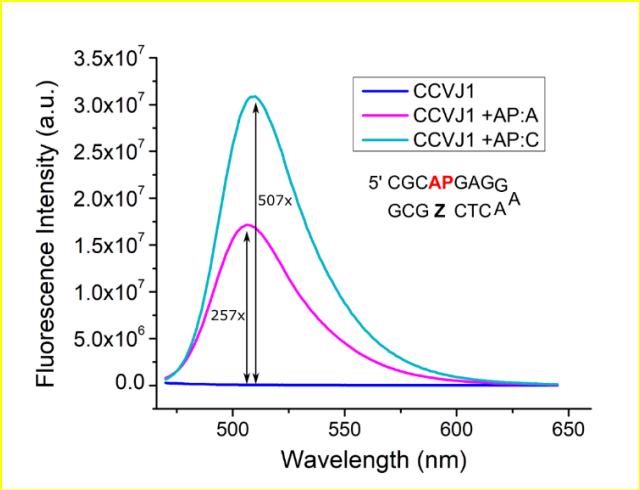


Figure S13 Comparison of oligonucleotide-probe conjugate emission spectra (Ex 470 nm) at 2 µM when the abasic site is paired with adenine or cytosine. The smaller cytosine base yields a ~2-fold increase in overall fluorescence, likely due to deeper penetration of CCVJ1 into the DNA helix. We also observed a very slight (~4 nm) red-shift in emission maxima when the AP site is base paired against a pyrimidine.

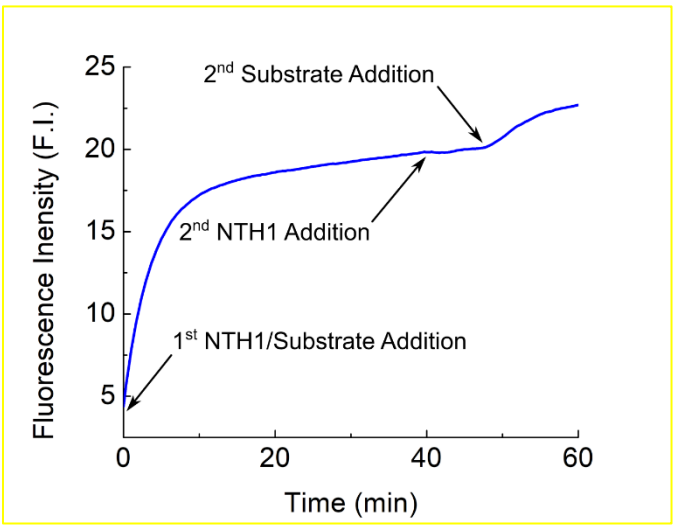


Figure S14 Timecourse of NTH1 (500 nM) with 5-hydroxycytosine containing DNA (60 µM) and **CCVJ1** (20 µM). After 40 minutes, an additional aliquot of NTH1 was added (250 nM) which did not yield any significant increase in fluorescence. Following a 10-minute incubation period, additional substrate was then added (20 µM) which yielded an increase in fluorescence. These results support the hypothesis that lyase activity competes with **CCVJ1** resulting in a lower overall fluorescence yield and the need for significantly higher substrate concentrations when assaying NTH1. Additionally these results rule out the hypothesis that NTH1 is behaving as a single-turnover enzyme under our assay conditions.

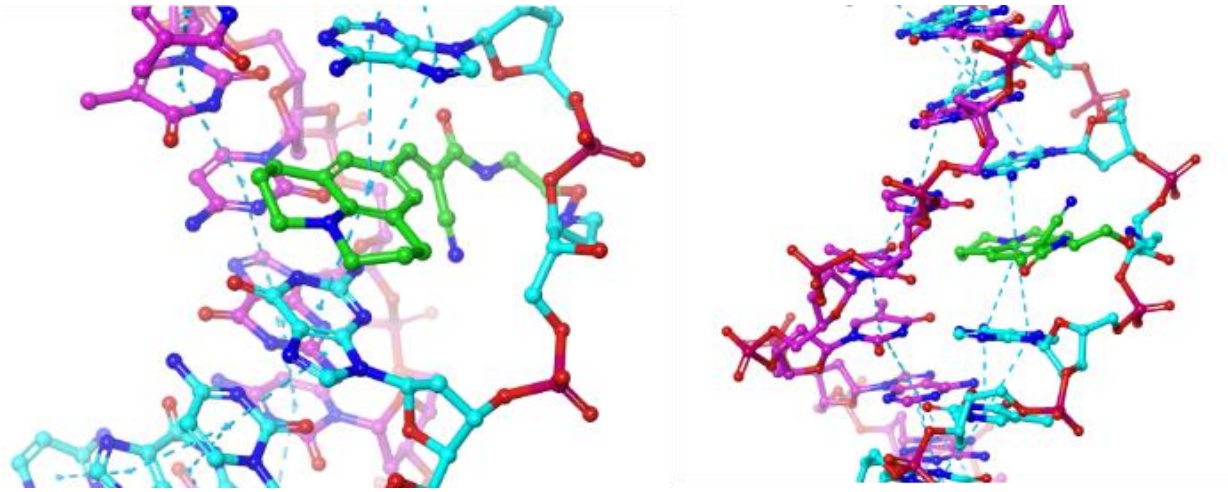


Figure S15. A computational model of CCVJ1 bound to the AP site of duplex DNA was created using the Drew-Dickerson dodecamer as a starting structure. An abasic site was created in the starting structure, **CCVJ1** was covalently bound to the hemiacetal, and the adduct was then refined using the OPLS3e force field. This model does not exclude the possibility of rotating **CCVJ1** 180 degrees about the z-axis, with the linker protruding into the major groove. Overall, the modeling is consistent with a complex that fits snugly into the enzyme-generated AP site while forming a covalent bond with the sugar, and with substantial constraint of rotatable bonds in the adduct. The extrahelical positioning of the C4' and C5' carbons of the AP site with the C3' proton facing inward is consistent with structural studies of abasic site conformation.[6]

Derivation of t_{ss} equation (adapted from McClure[7])

- The reaction scheme for a coupled reaction can be written as

$$S \xrightarrow{v_0} I \xrightarrow{v_1} P$$

- Or for our purposes

$$S \xrightarrow{v_0} I \xrightarrow{k_2 * [I]} P$$

- Where v_0 is the enzyme velocity and

$$k_2 = k_p * [probe]$$

Where k_p represents the second order rate constant of oxime formation. Given a sufficiently large excess of probe is used relative to the substrate, the value of k_2 is assumed to be a constant.

- At any given moment the rate at which [*I*] is changing may be expressed as as

$$\frac{d[I]}{dt} = v_0 - k_2[I]$$

- When t=0 the concentration of [*I*]=0. As the reaction progresses, the concentration of *I* increases causing the rate v_1 to increase as well. Eventually, the rate of v_1 will asymptotically approach the rate of v_0 and at time infinity they will become equal. When the rate $v_0 \approx v_1$, the value of d[I]/dt will be zero and the concentration of I will reach a steady state. Setting d[I]/dt to zero, we solve for $[I]_{ss}$

$$[I]_{ss} = \frac{v_0}{k_2}$$

- The rate equation given above can be integrated as

$$[I] = \frac{v_0}{k_2}\left(1 - e^{-k_2 t}\right)$$

- Or rearranged in terms of t as

$$\ln\left(1 - \frac{k_2}{v_0}[I]\right) = -k_2 t$$

- To solve for the delay time t_{ss} when steady state will be achieved, we must first define the point at which we will consider the reaction to be in steady state. As pointed out above, the value of [I] will approach $[I]_{ss}$ asymptotically and requires infinite time to reach $[I]_{ss}$. Therefore we must choose some fraction *F* of $[I]_{ss}$ at which point the concentrations are deemed sufficiently close.

Literature convention has defined the value of F as 0.99.[7,8] Therefore, to solve for the delay time t_{ss}, we solve the integrated equation above in terms of time, substituting the term $F*[I]_{ss}$ for [I]

$$\ln \frac{\left(1 - (\frac{k_2}{v_0} F * [I]_{ss})\right)}{-k_2} = t_{ss}$$

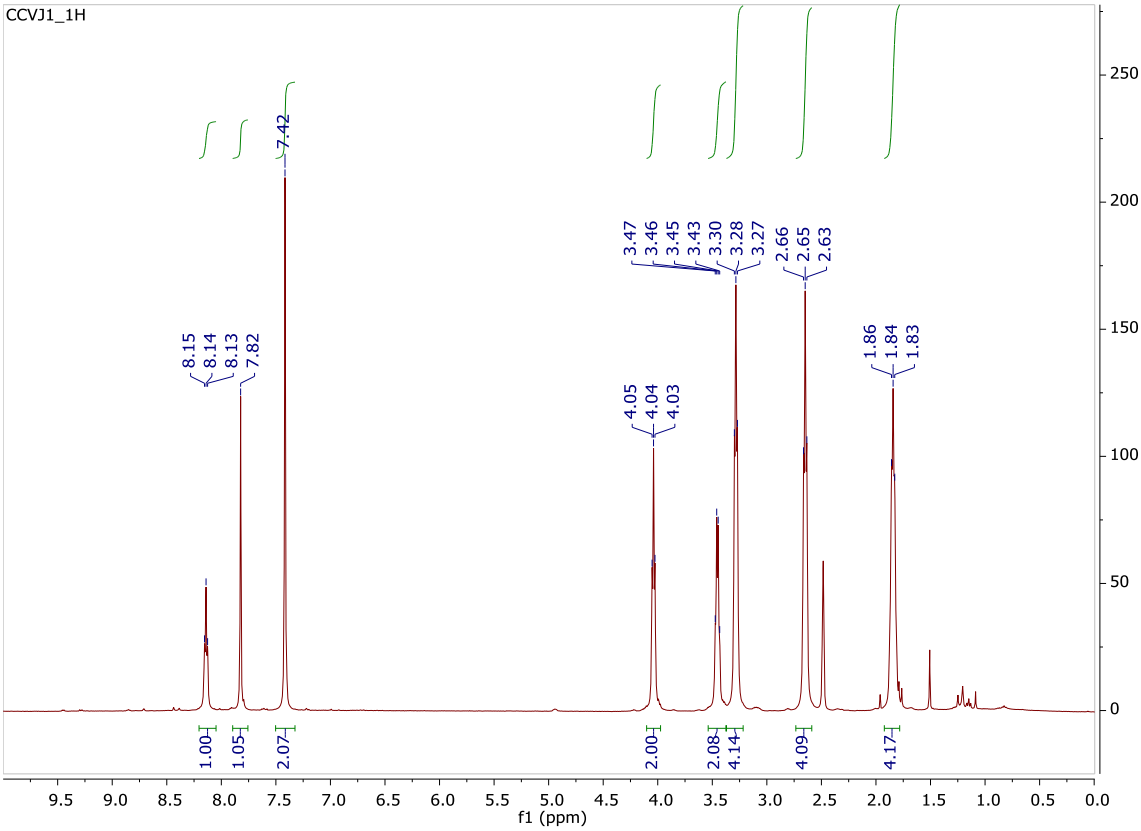
- By substituting the value

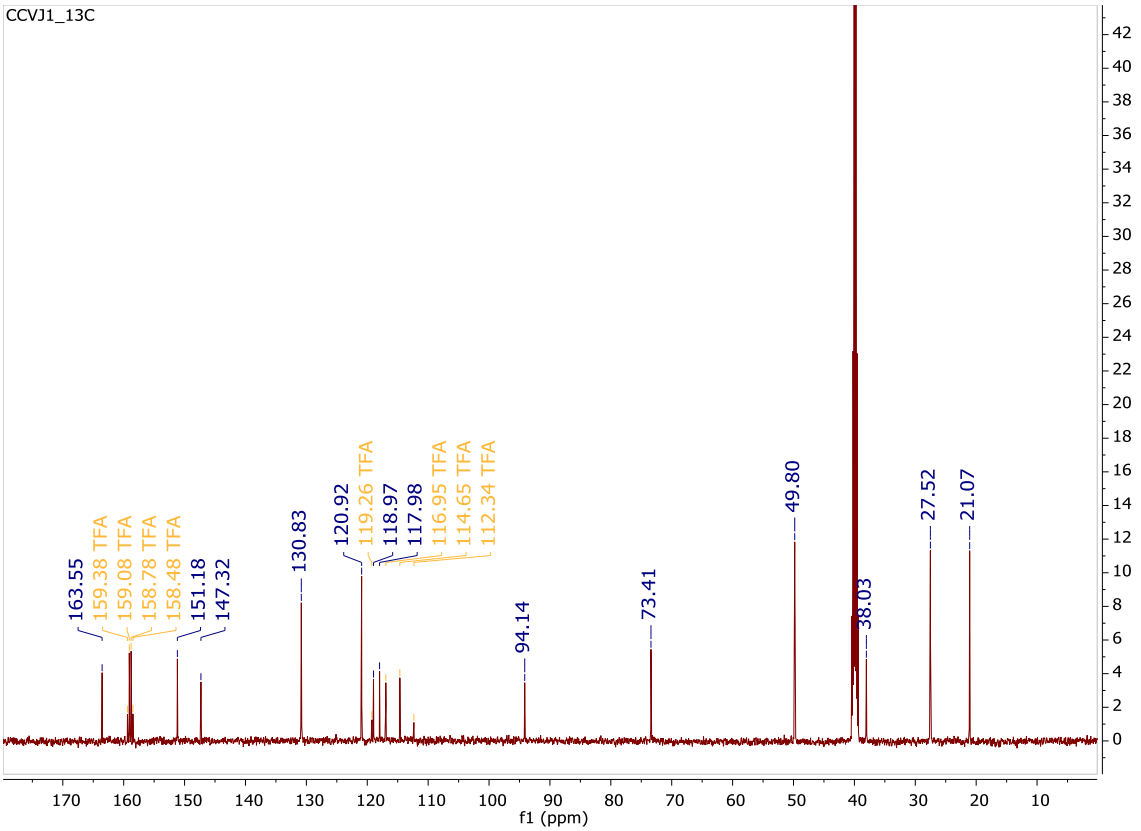
$$[I]_{ss} = \frac{v_0}{k_2}$$

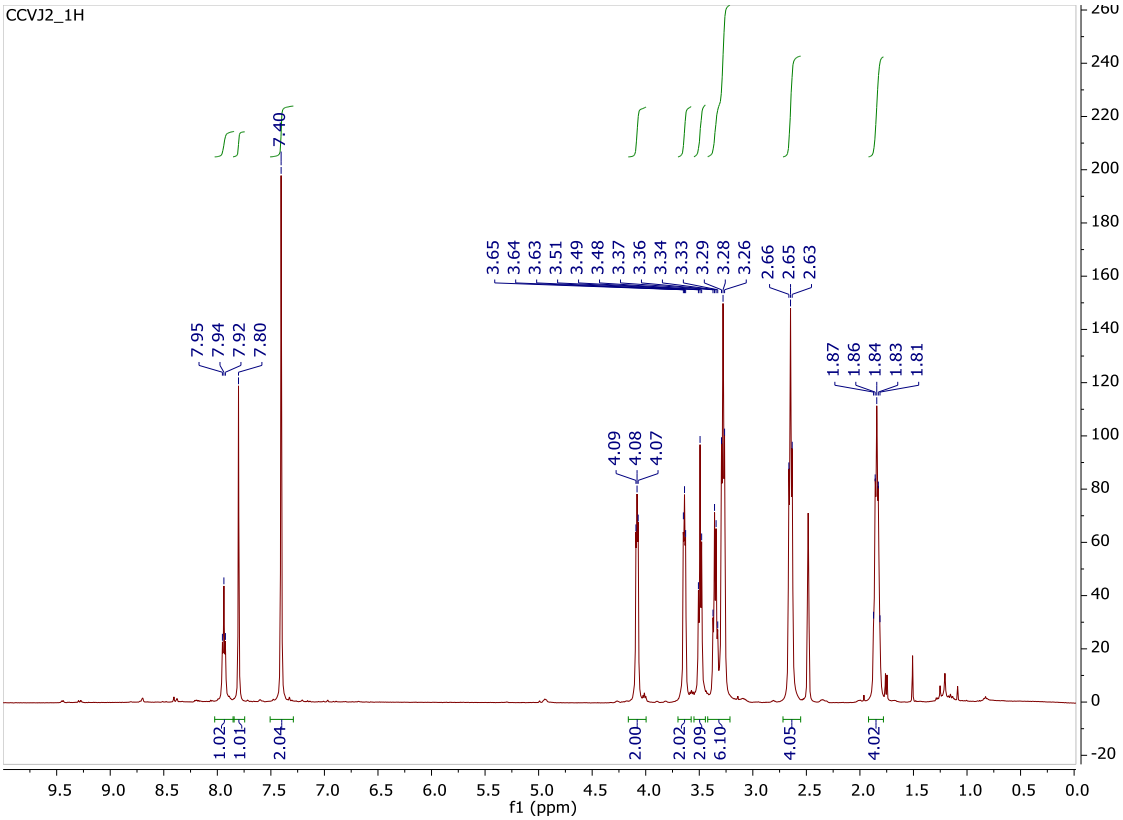
- We get the final equation for t_{ss} as

$$t_{ss} = -\frac{\ln(1-F)}{k_2} \;\; OR \;\; \boldsymbol{t_{ss} = -\frac{\ln(1-0.99)}{k_p[probe]}}$$

UBER Probe NMR Spectra



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7.94
7.92
7.80
7.40
3.65
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3.63
3.51
3.49
3.48
3.37
3.36
3.34
3.33
3.29
3.28
3.26
2.66
2.65
2.63
1.87
1.86
1.84
1.83
1.81
4.09
4.08
4.07
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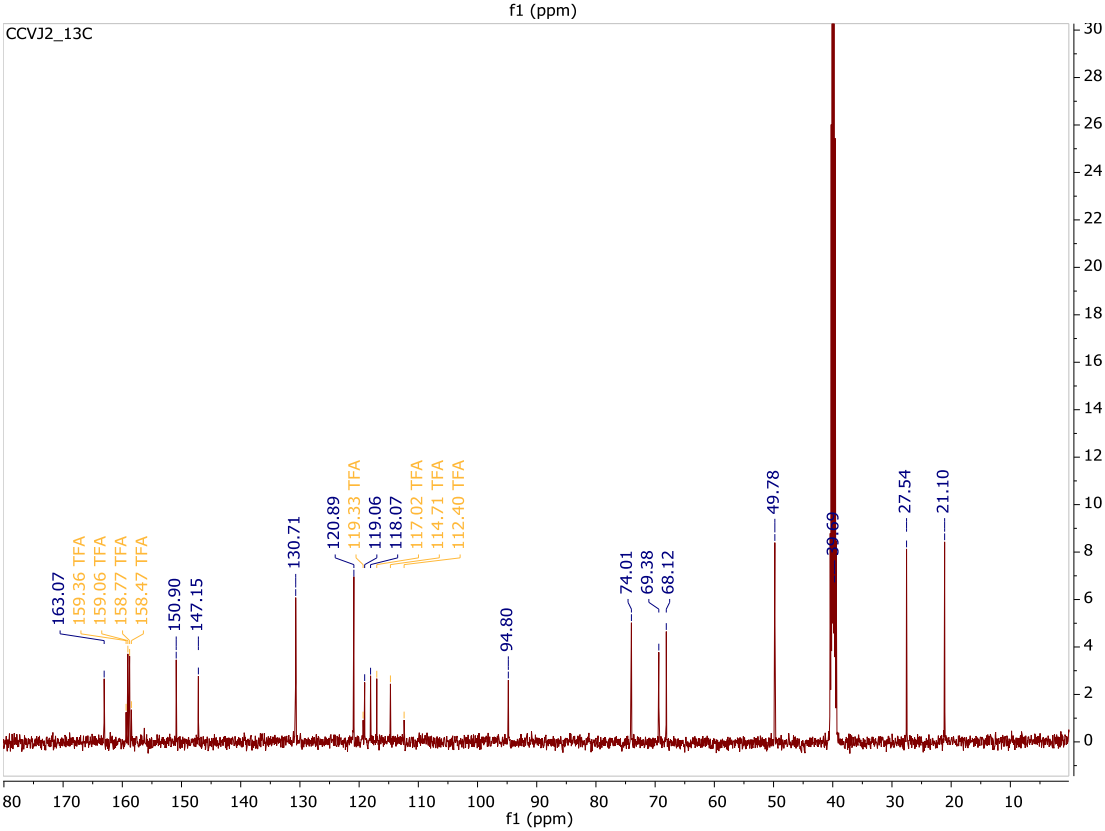
2.09
6.10
4.05
4.02
f1 (ppm)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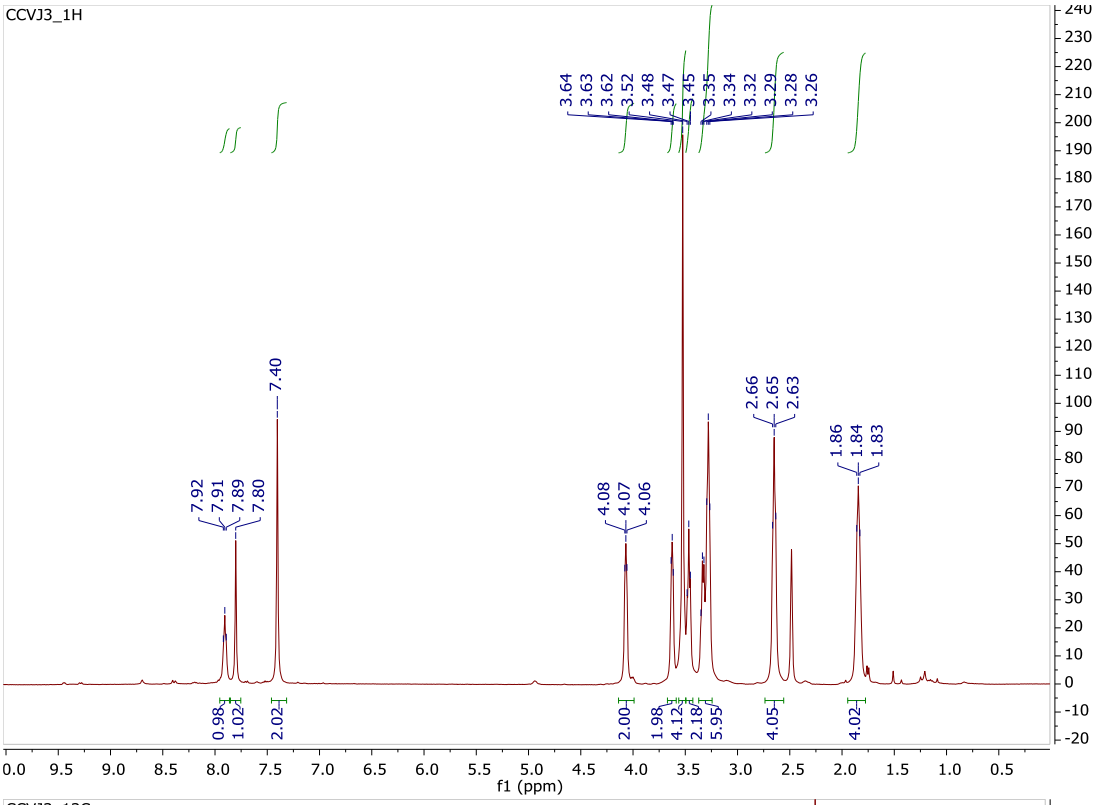
159.36 TFA
159.06 TFA
158.77 TFA
158.47 TFA
150.90
147.15
130.71
120.89
119.33 TFA
119.06
118.07
117.02 TFA
114.71 TFA
112.40 TFA
94.80
74.01
69.38
68.12
49.78
39.69
27.54
21.10
f1 (ppm)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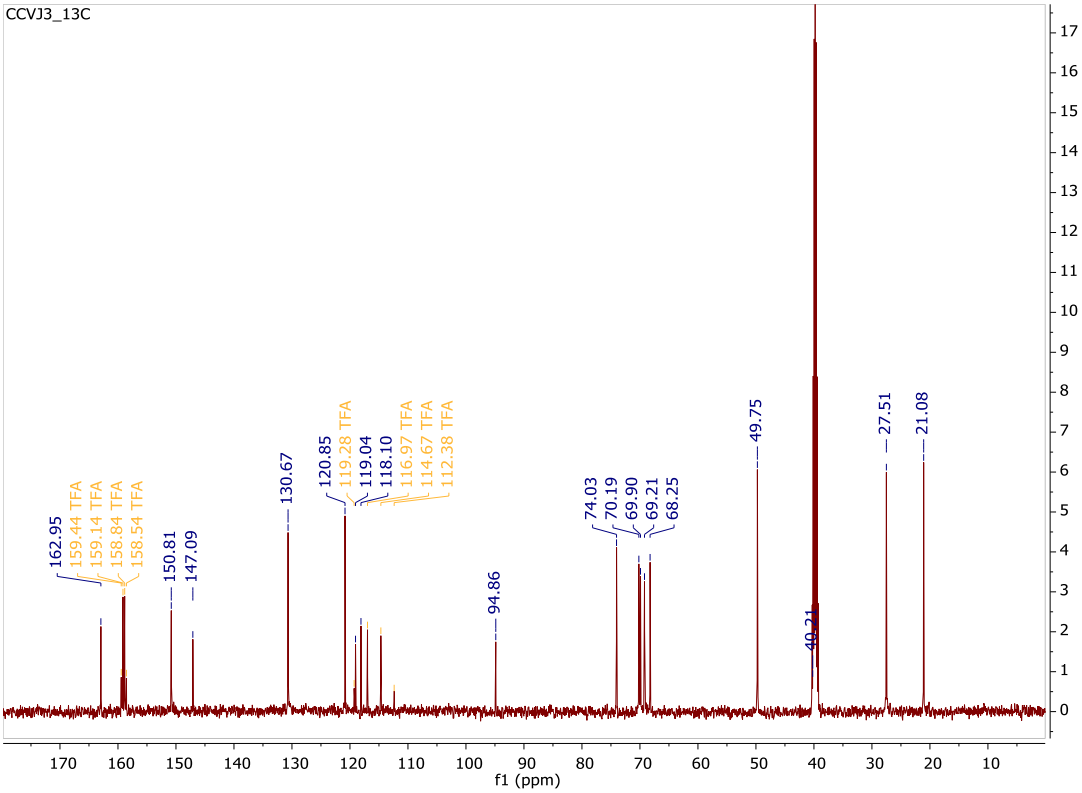
7.91
7.89
7.80
7.40
4.08
4.07
4.06
3.64
3.63
3.62
3.52
3.48
3.47
3.45
3.35
3.34
3.32
3.29
3.28
3.26
2.66
2.65
2.63
1.86
1.84
1.83
0.98
1.02
2.02
2.00
1.98
4.12
2.18
5.95
4.05
4.02
f1 (ppm)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159.14 TFA
158.84 TFA
158.54 TFA
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147.09
130.67
120.85
119.28 TFA
119.04
118.10
116.97 TFA
114.67 TFA
112.38 TFA
94.86
74.03
70.19
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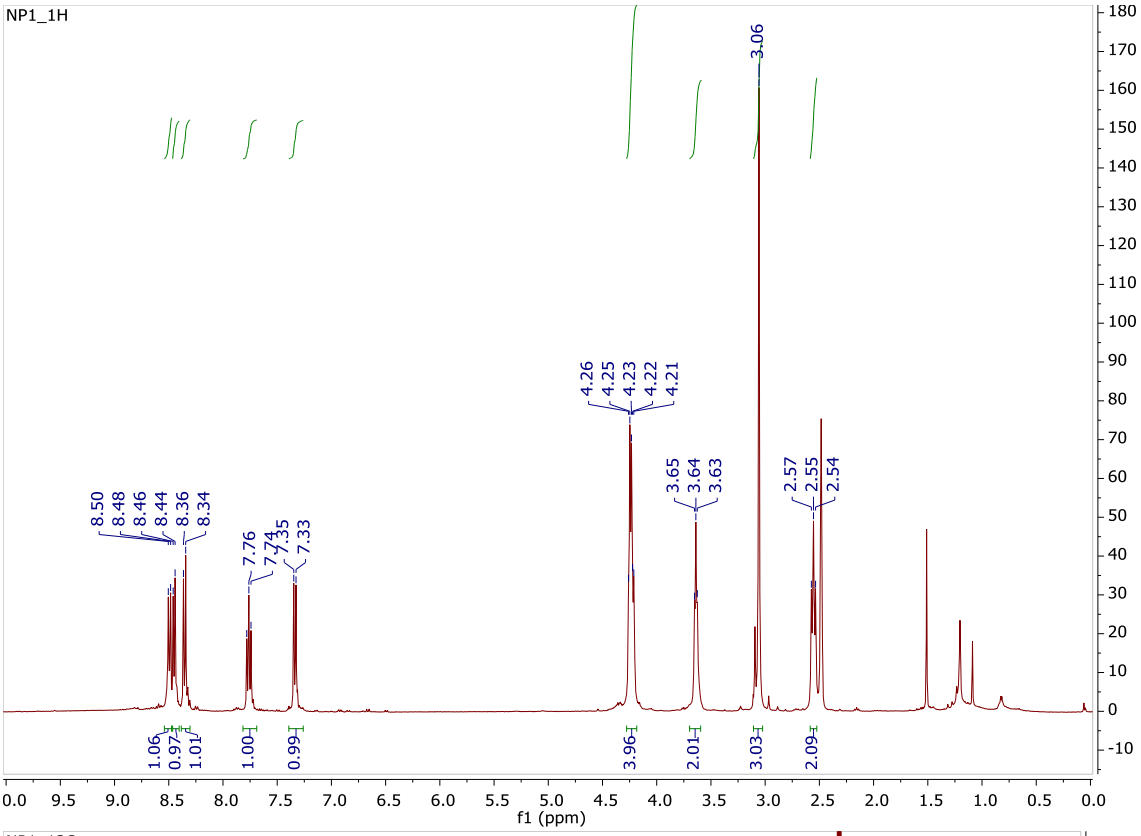
69.21
68.25
49.75
40.21
27.51
21.08
f1 (ppm)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8.34
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7.33
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4.23
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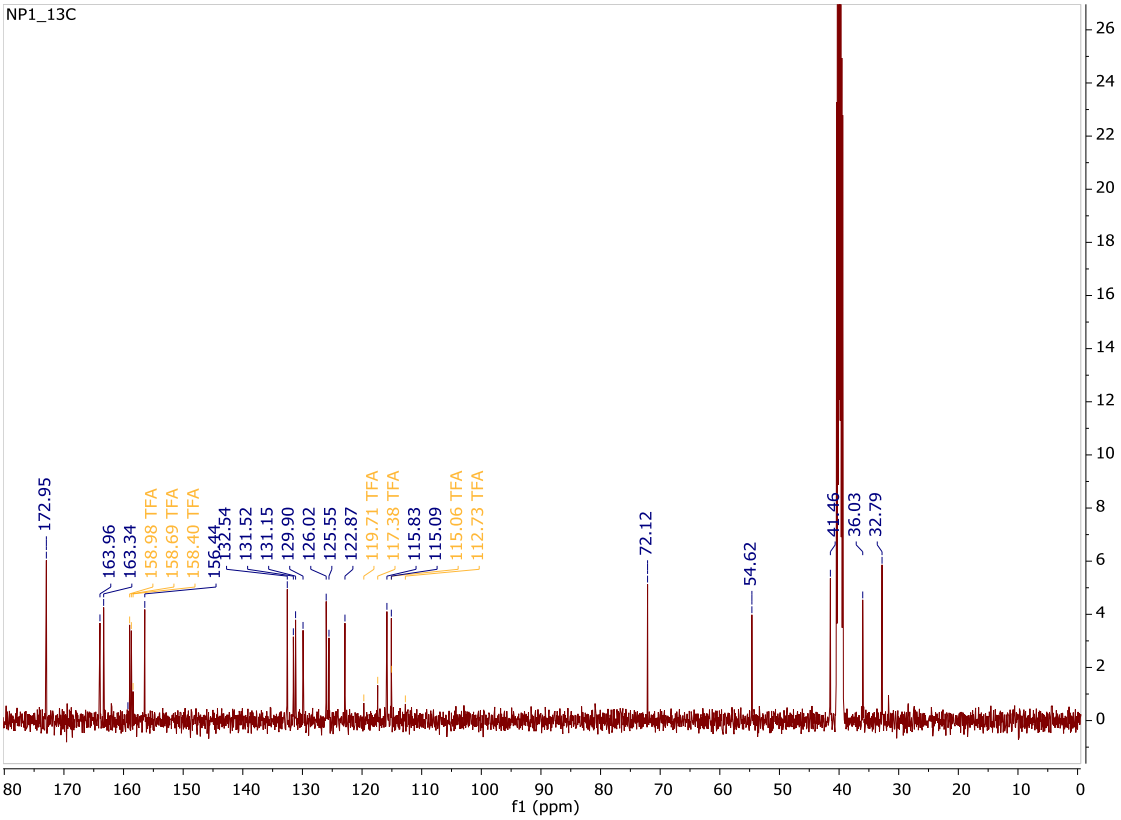
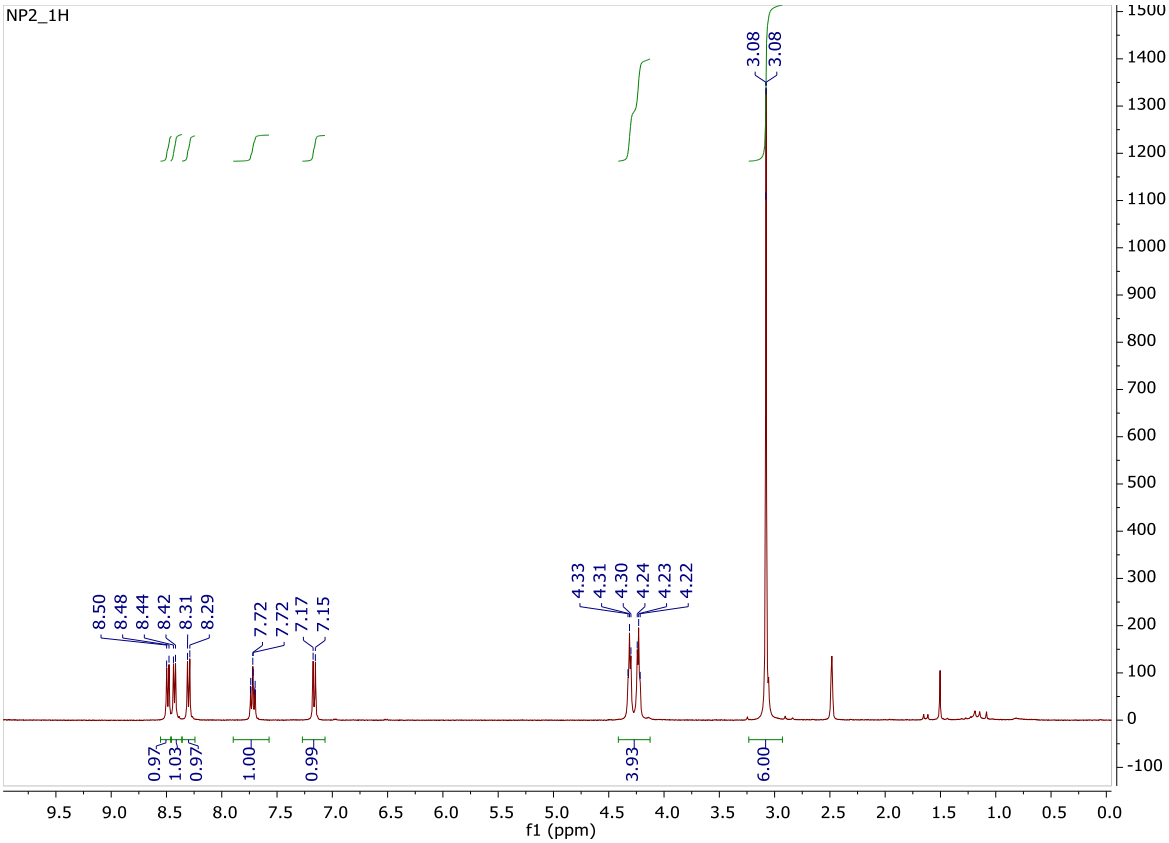
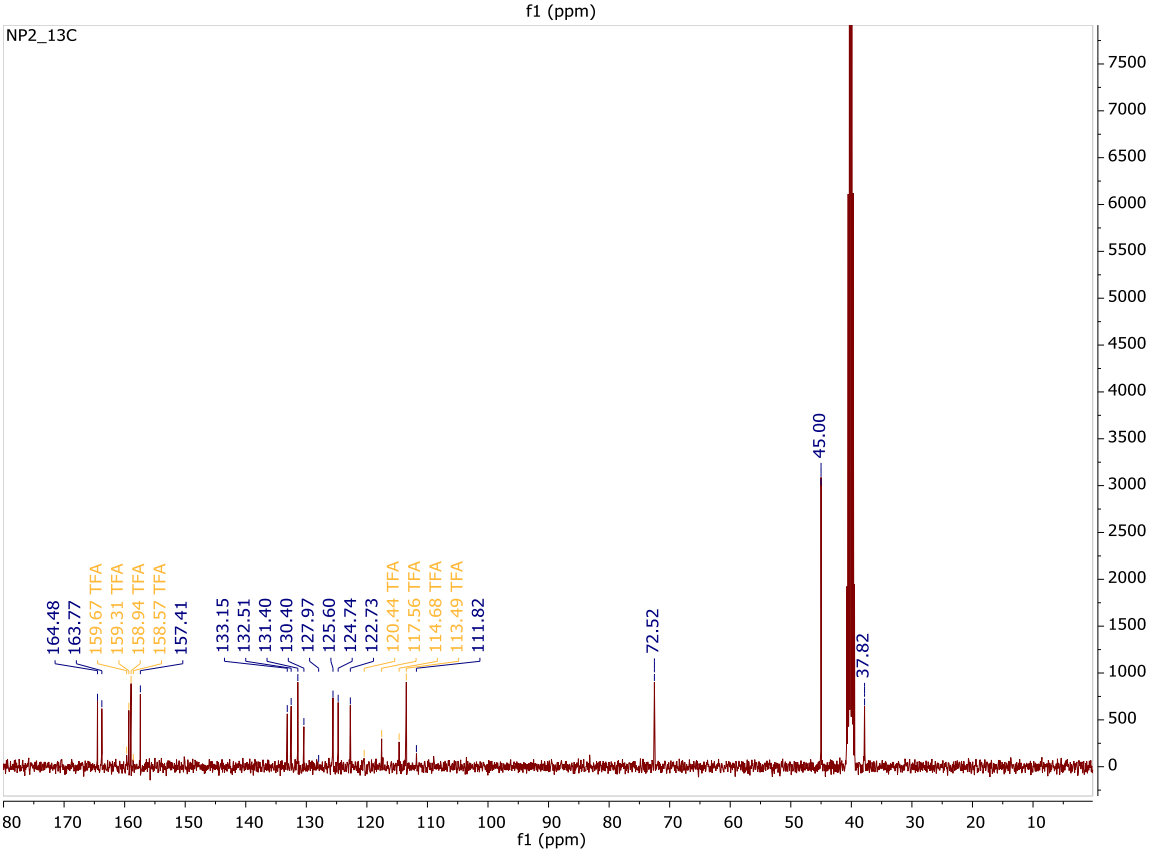
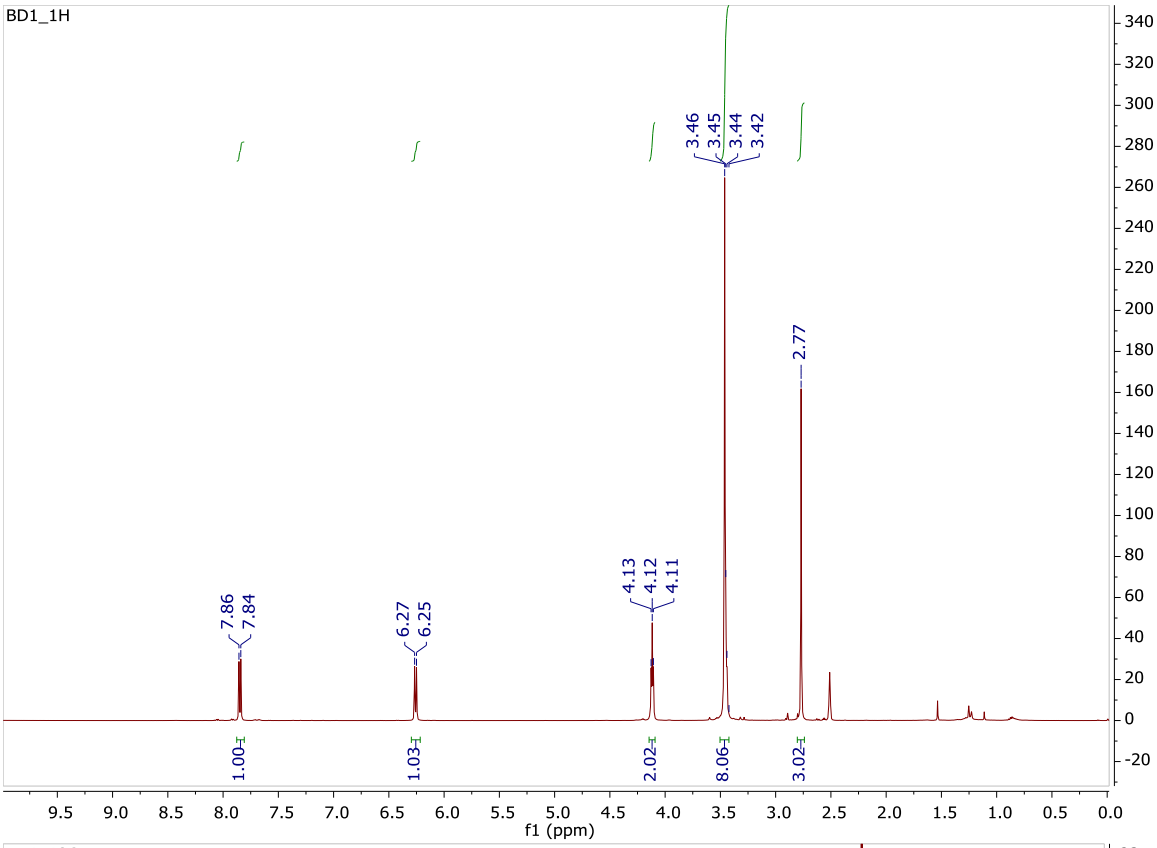
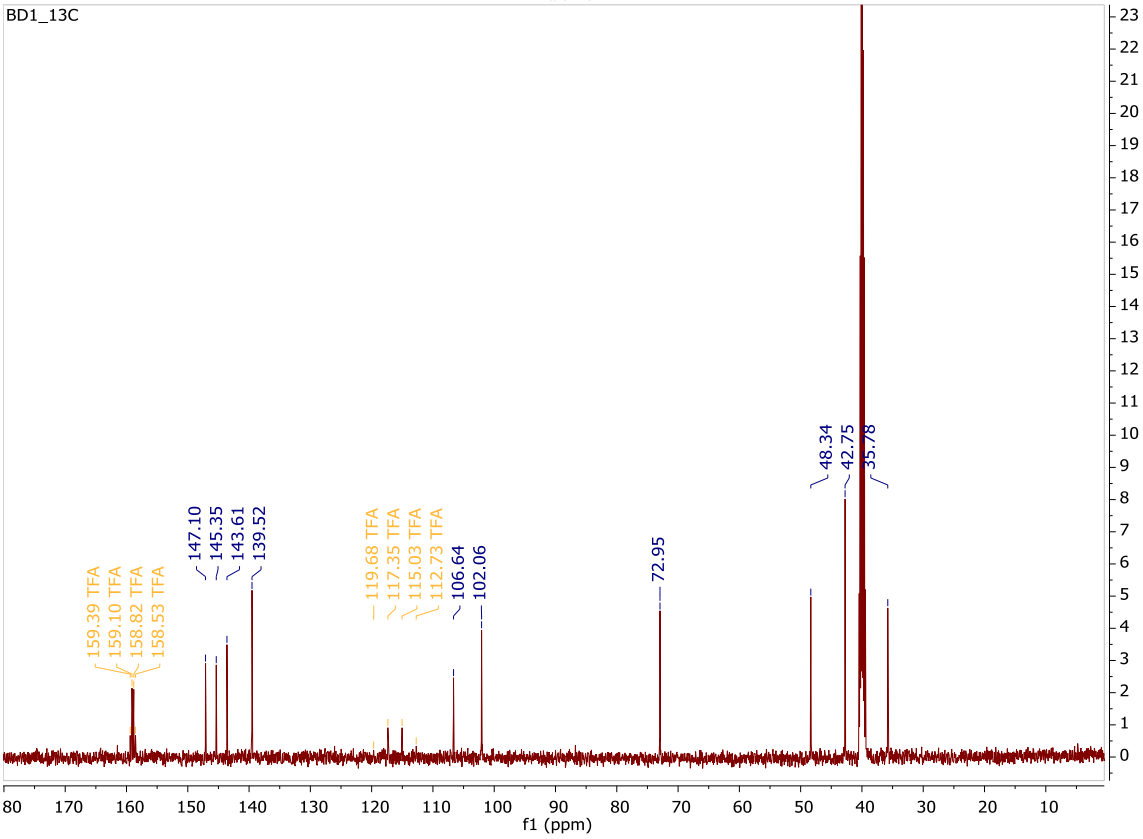
4.21
3.65
3.64
3.63
3.06
2.57
2.55
2.54
1.06
0.97
1.01
1.00
0.99
3.96
2.01
3.03
2.09
f1 (ppm)

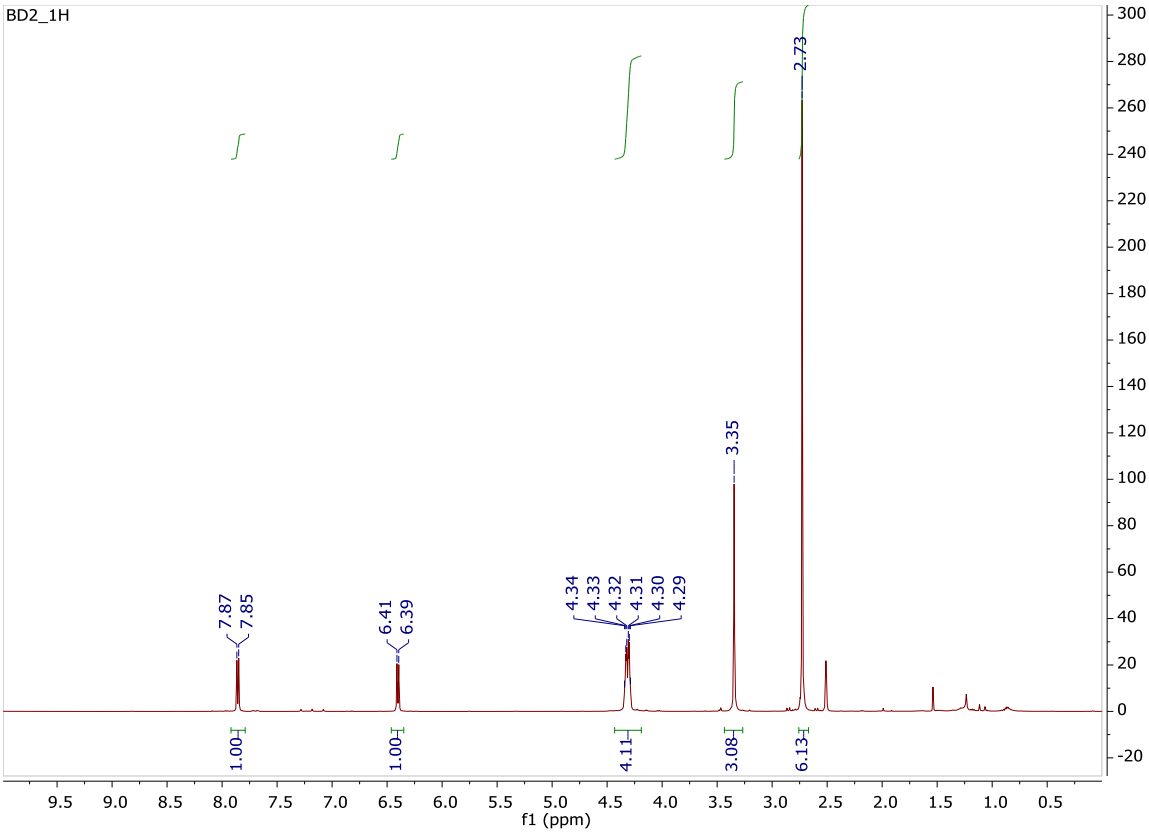

NP1_13C
172.95
163.96
163.34
158.98 TFA
158.69 TFA
158.40 TFA
156.44
132.54
131.52
131.15
129.90
126.02
125.55
122.87
119.71 TFA
117.38 TFA
115.83
115.09
115.06 TFA
112.73 TFA
72.12
54.62
41.46
36.03
32.79
f1 (ppm)

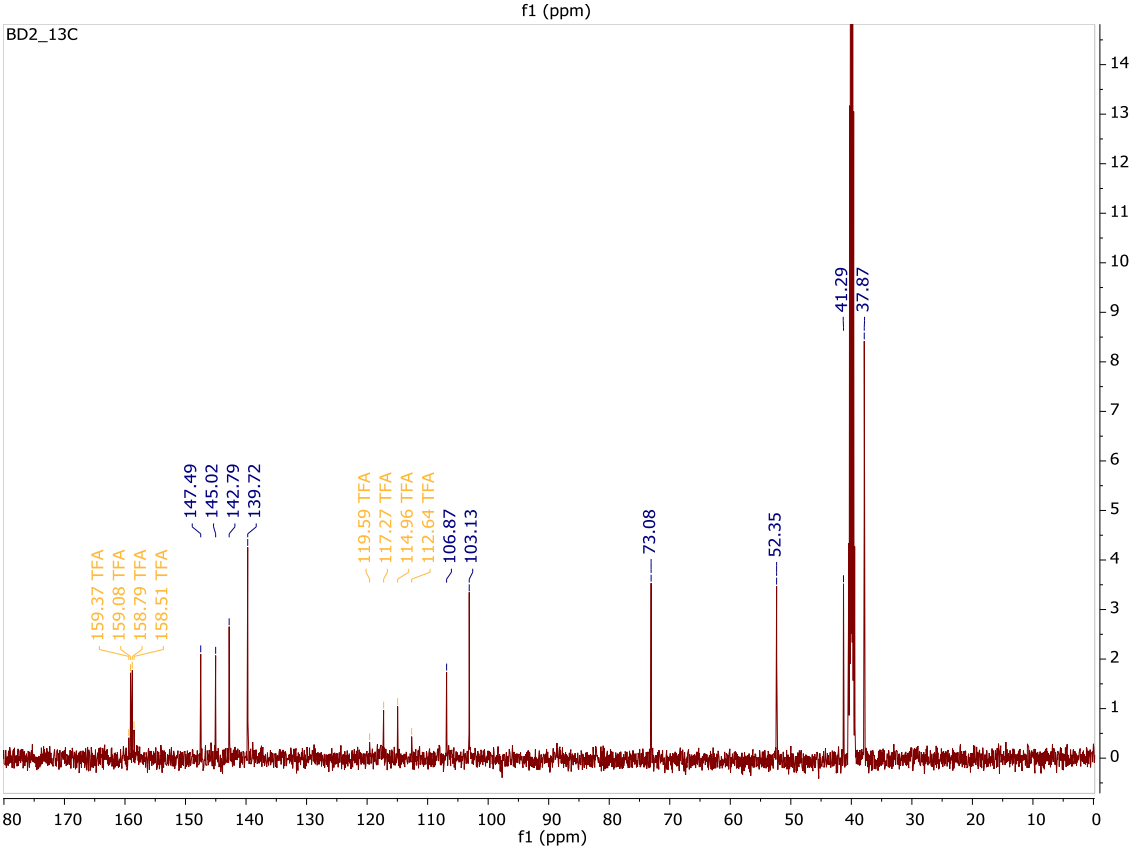

NP2_1H
8.50
8.48
8.44
8.42
8.31
8.29
7.72
7.72
7.17
7.15
4.33
4.31
4.30
4.24
4.23
4.22
3.08
3.08
0.97
1.03
0.97
1.00
0.99
3.93
6.00
f1 (ppm)


NP2_13C
164.48
163.77
159.67 TFA
159.31 TFA
158.94 TFA
158.57 TFA
157.41
133.15
132.51
131.40
130.40
127.97
125.60
124.74
122.73
120.44 TFA
117.56 TFA
114.68 TFA
113.49 TFA
111.82
72.52
45.00
37.82
f1 (ppm)


BD1_1H
7.86
7.84
6.27
6.25
4.13
4.12
4.11
3.46
3.45
3.44
3.42
2.77
1.00
1.03
2.02
8.06
3.02
f1 (ppm)


BD1_13C
159.39 TFA
159.10 TFA
158.82 TFA
158.53 TFA
147.10
145.35
143.61
139.52
119.68 TFA
117.35 TFA
115.03 TFA
112.73 TFA
106.64
102.06
72.95
48.34
42.75
35.78
f1 (ppm)


BD2_1H
7.87
7.85
6.41
6.39
4.34
4.33
4.32
4.31
4.30
4.29
3.35
2.73
1.00
1.00
4.11
3.08
6.13
f1 (ppm)


BD2_13C
159.37 TFA
159.08 TFA
158.79 TFA
158.51 TFA
147.49
145.02
142.79
139.72
119.59 TFA
117.27 TFA
114.96 TFA
112.64 TFA
106.87
103.13
73.08
52.35
41.29
37.87
f1 (ppm)

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