Supporting Information for:

Engineering the substrate specificity of ADP-ribosyltransferases for identifying direct protein targets.

Ian Carter-O'Connell[‡], Haihong Jin[‡], Rory K. Morgan[‡], Larry L. David[†], and Michael S. Cohen[‡*]

[‡]Program in Chemical Biology and Department of Physiology and Pharmacology, [†]Department of Biochemistry, Oregon Health & Science University, Portland, Oregon 97210, United States

*Email: cohenmic@ohsu.

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I. Supplemental Figures and Tables

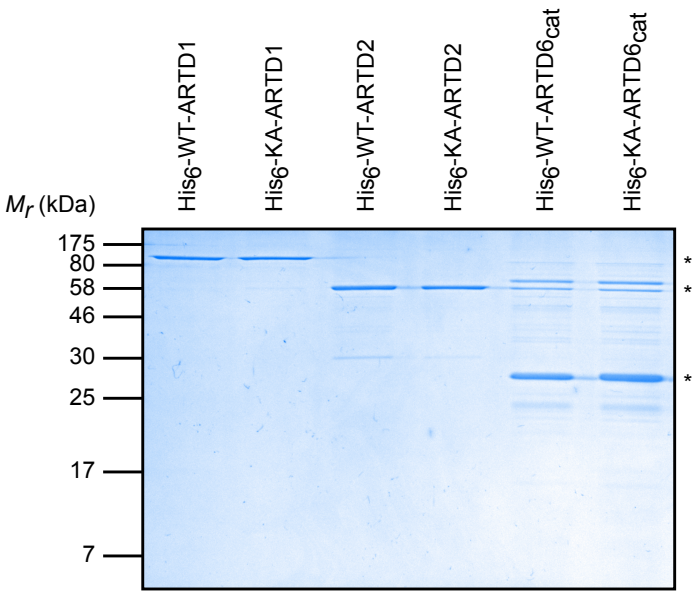


Figure S1. SDS-PAGE of recombinant WT and KA variants used in this study. 200 ng of each preparation of ARTD1, ARTD2, and ARTD6$_{cat}$ was fractionated by SDS-PAGE and developed with a coomassie stain. The asterisks indicate the expected size of ARTD1 (117 kDa, top), ARTD2 (67.4 kDa, middle), and ARTD6$_{cat}$ (29.9 kDa, bottom). Positions of the molecular-weight markers are indicated on the left of the blot.

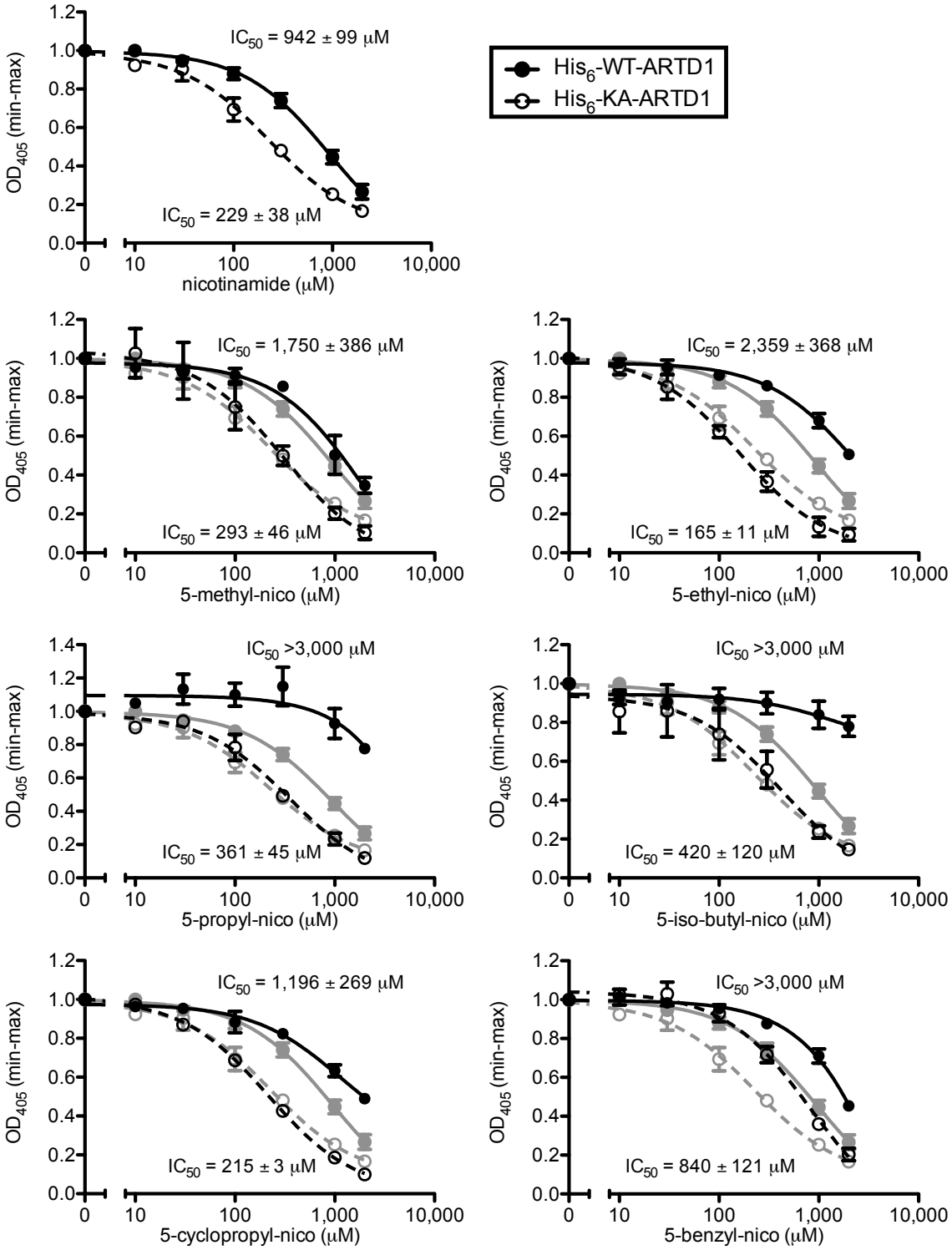


Figure S2. IC_{50} curves for the nicotinamide-based inhibitors. Inhibition of WT-ARTD1 or KA-ARTD1 by the nicotinamide-based inhibitors is represented by dose-response curves. The ARTD1 variants were incubated with indicated doses of inhibitor (0, 10, 30, 100, 300, 1,000, or 2,000 μM) for 10 min followed by an *in vitro* histone H1 modification assay. The inhibition curves for nicotinamide (gray) are included in the lower panels for comparison. IC_{50} values for WT-ARTD1 (above) and KA-ARTD1 (below) are given. Error bars represent s.d., *n*=3.

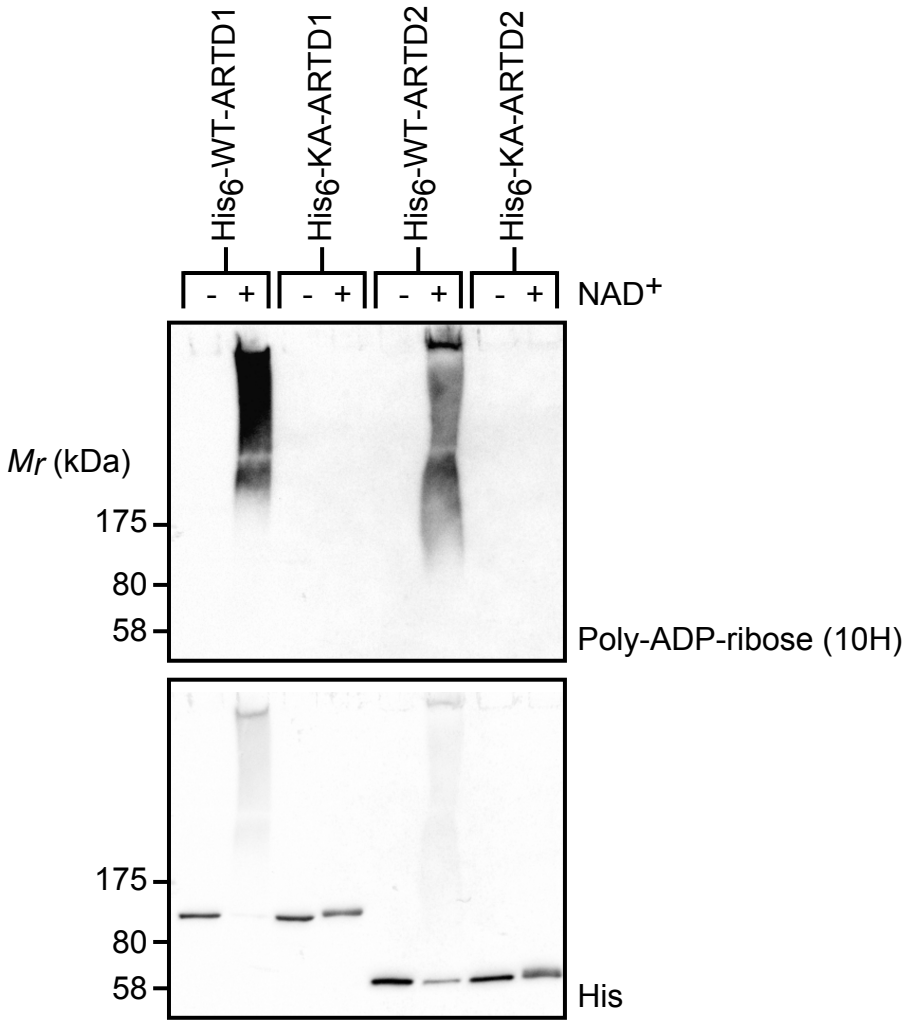


Figure S3. Auto-ADP-ribosylation of sensitized ARTD1 or ARTD2 using native NAD^+. WT or KA variants of both ARTD1 and ARTD2 were subjected to auto-modification in the presence of 250 μM NAD^+. Samples were fractionated by SDS-PAGE and poly-ADP-ribose (upper panel) and the his-tag (lower panel) were probed via immunoblot. The extensive poly-ADP-ribosylation of the WT enzyme in the presence of NAD^+ results in a smeared, faint band in the his-tag control blot. The KA variants are slightly shifted to a higher molecular weight in the presence of NAD^+ (as seen in the his blot) undergoing an auto-modification that is not detected by the poly-ADP-ribose antibody. Positions of the molecular-weight markers are indicated on the left of the blot.

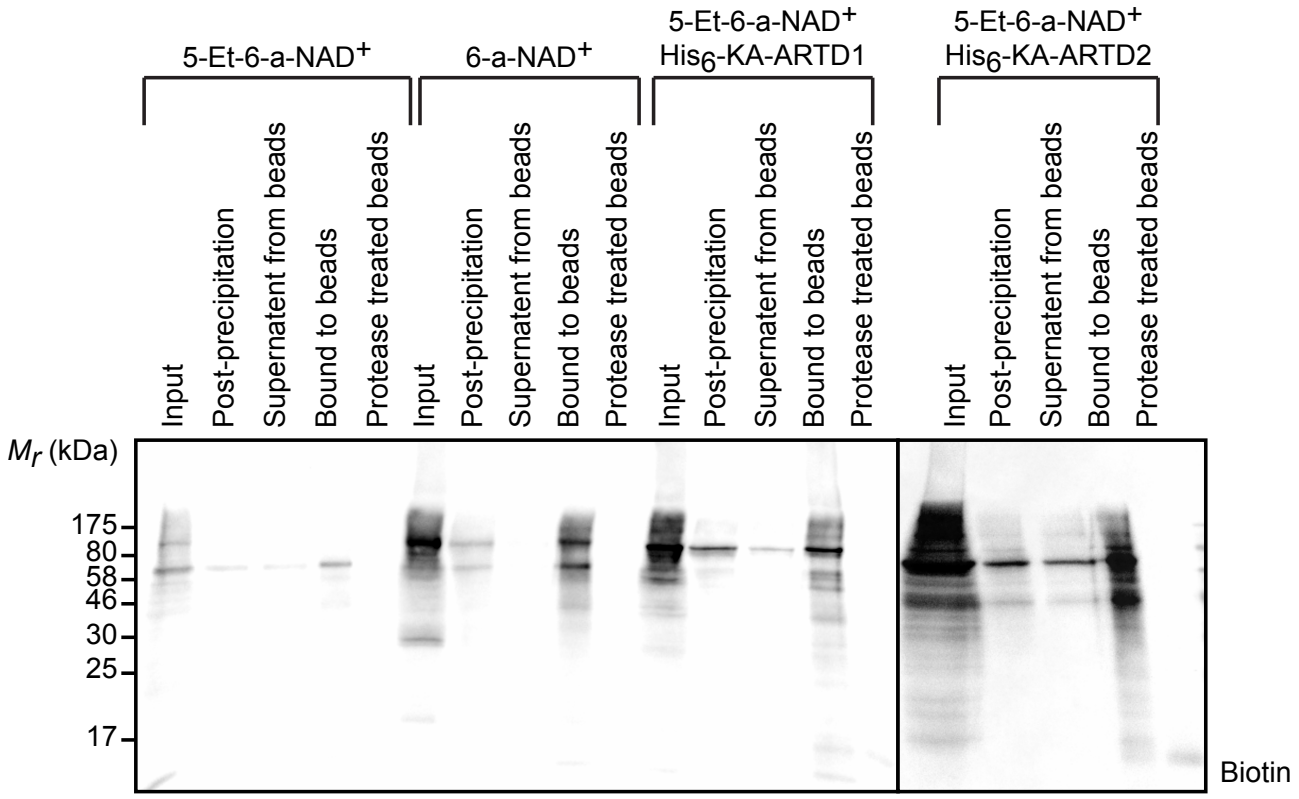


Figure S4. Immunoblot analysis of the LC-MS/MS sample preparation. The indicated fractions from the NeutrAvidin enrichment protocol were fractionated by SDS-PAGE and imaged using streptavidin-HRP. The modified NAD^+ analog was spiked into HEK 293T nuclear extract (6-a-NAD^+: 100 μM; 5-Et-6-a-NAD^+: 250 μM) both without any additional source of exogenous ARTD and with either KA-ARTD1 or KA-ARTD2, samples were labeled, conjugated to biotin, enriched, and submitted for LC-MS/MS analysis. Positions of the molecular-weight markers are indicated on the left of the blot.

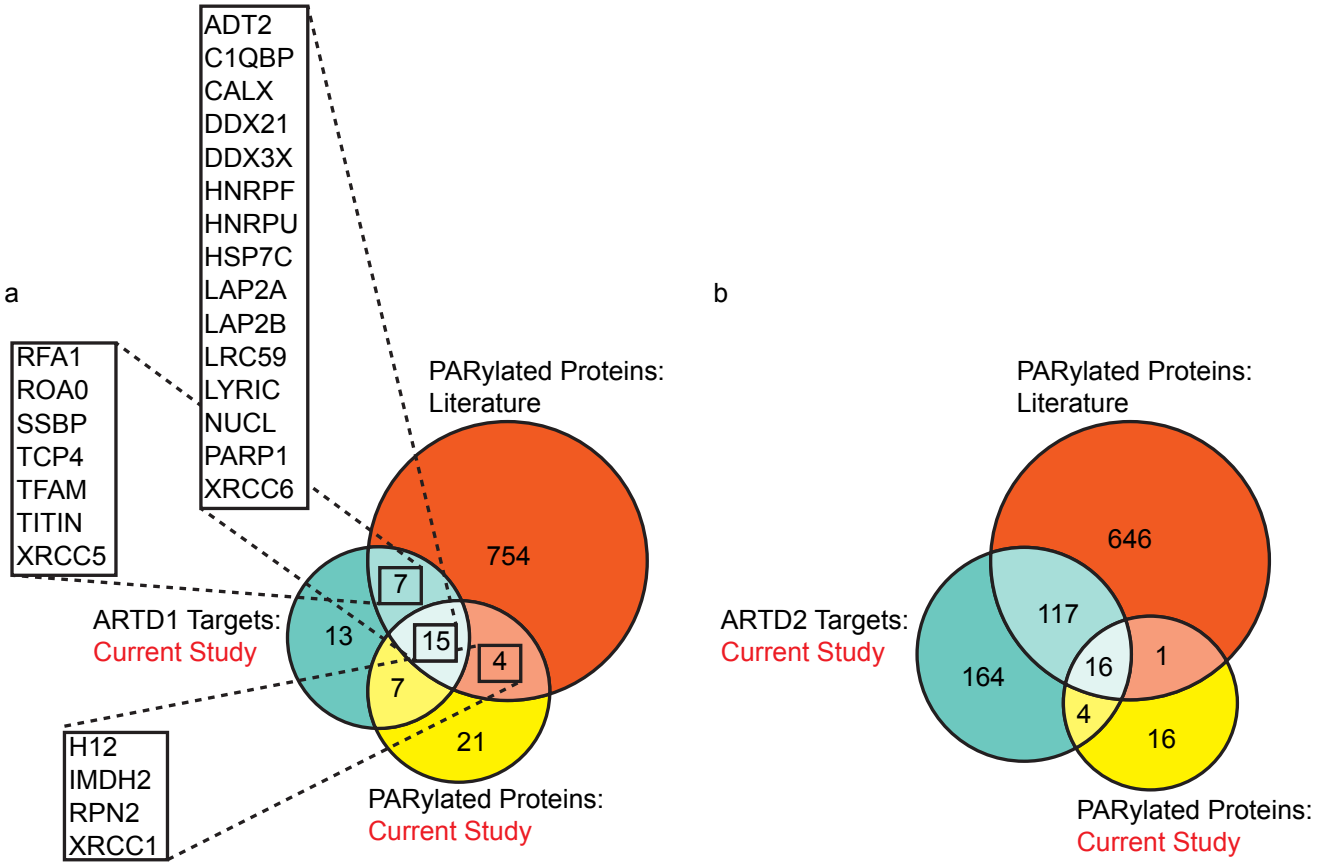


Figure S5. Comparison of specific ARTD targets identified in the current study with the previously reported poly-ADPr proteome. (a) Venn diagram for the direct ARTD1 targets identified in the current study. ARTD1 specific targets were identified as discussed in the methods section. Previously identified poly-ADPr proteins that were also identified as ARTD1 or general ARTD targets in this study are listed in the appropriate insets. (b) ARTD2 specific targets treated as in (a).

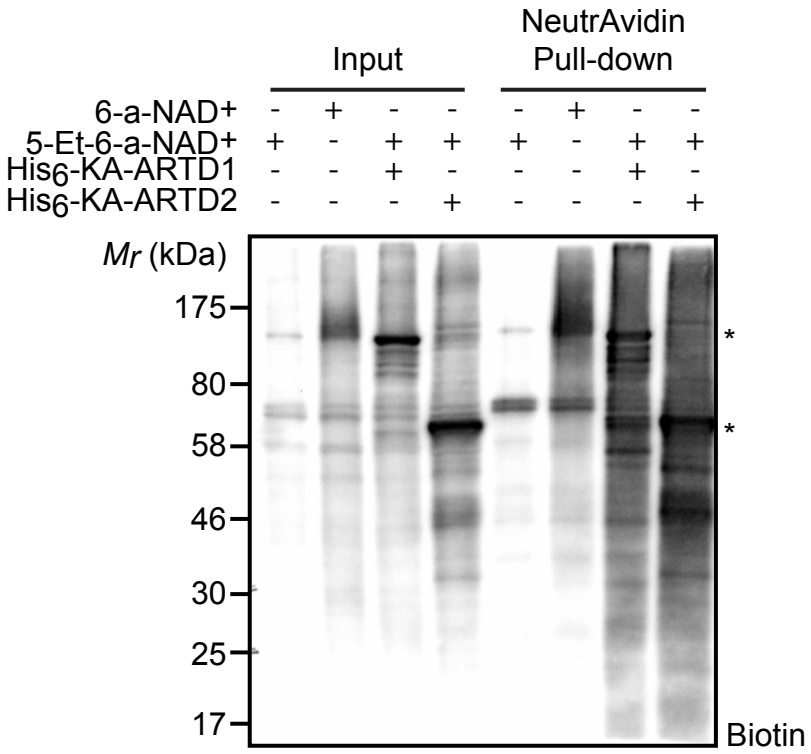


Figure S6. Detection of ADP-ribosylated ARTD1 or ARTD2 targets following NeutrAvidin enrichment. The modified NAD^+ analog was spiked into HEK 293T nuclear extract (6-a-NAD^+: 100 μM; 5-Et-6-a-NAD^+: 250 μM) both without any additional source of exogenous ARTD and with either KA-ARTD1 or KA-ARTD2, samples were labeled, conjugated to biotin, enriched, and probed with streptavidin-HRP. Immunoblot detection using antibodies for XRCC5, Catenin-δ-1, and hnRNP Q/R for this same experiment is presented in Figure 4b. The asterisks mark the KA-ARTD1 (upper) and KA-ARTD2 (lower) bands. Positions of the molecular-weight markers are indicated on the left of the blot.

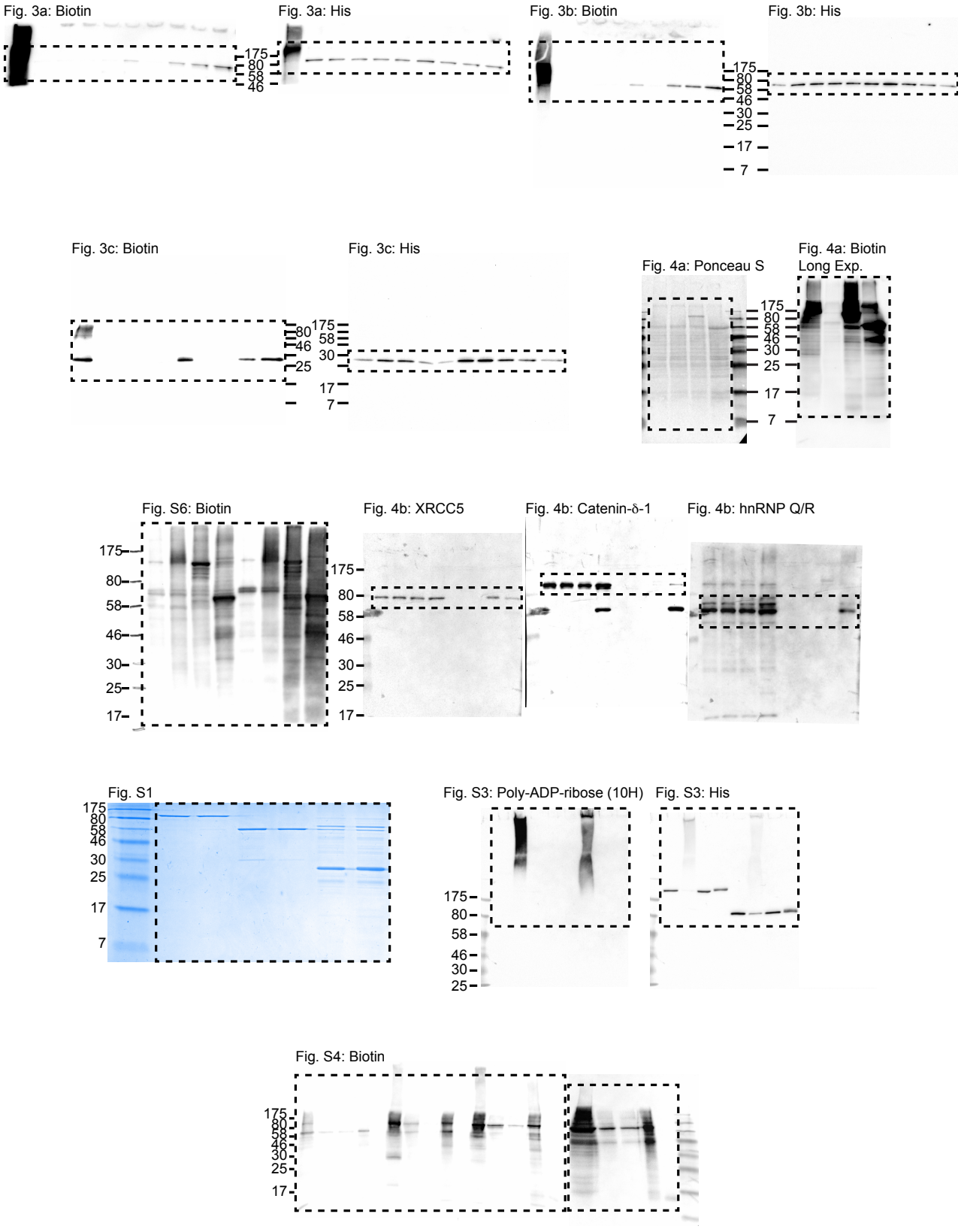
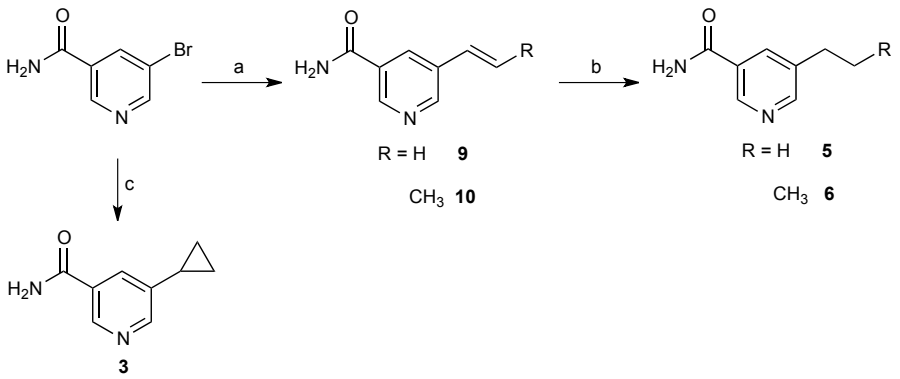


Figure S7. Data presented in the current study are presented in the uncropped form. Dashed boxes highlight the cropped data used for the indicated figures.

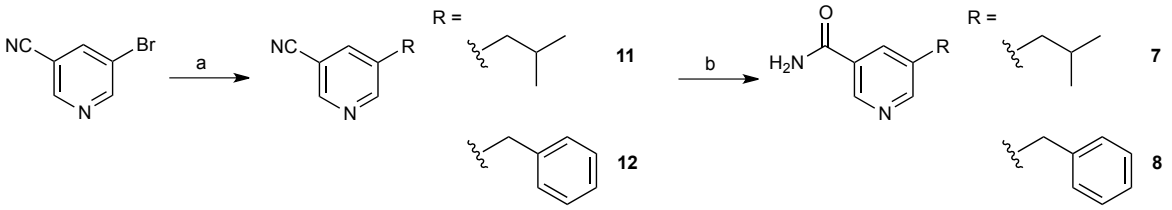
Tables included in supporting information .xls file:

Table S1. LC-MS/MS results from lysate labeling with KA-ARTD1 and/or modified analogs
Table S2. LC-MS/MS results from lysate labeling with KA-ARTD2 and/or modified analogs
Table S3. Peptides identified in lysate labeled by KA ARTD1 and/or modified analogs, 60 min MS/MS run
Table S4. Peptides identified in lysate labeled by KA ARTD1 and/or modified analogs, 140 min MS/MS run
Table S5. Peptides identified in lysate labeled by KA ARTD2 and/or modified analogs, 140 min MS/MS run
Table S6. Primers used in the current study

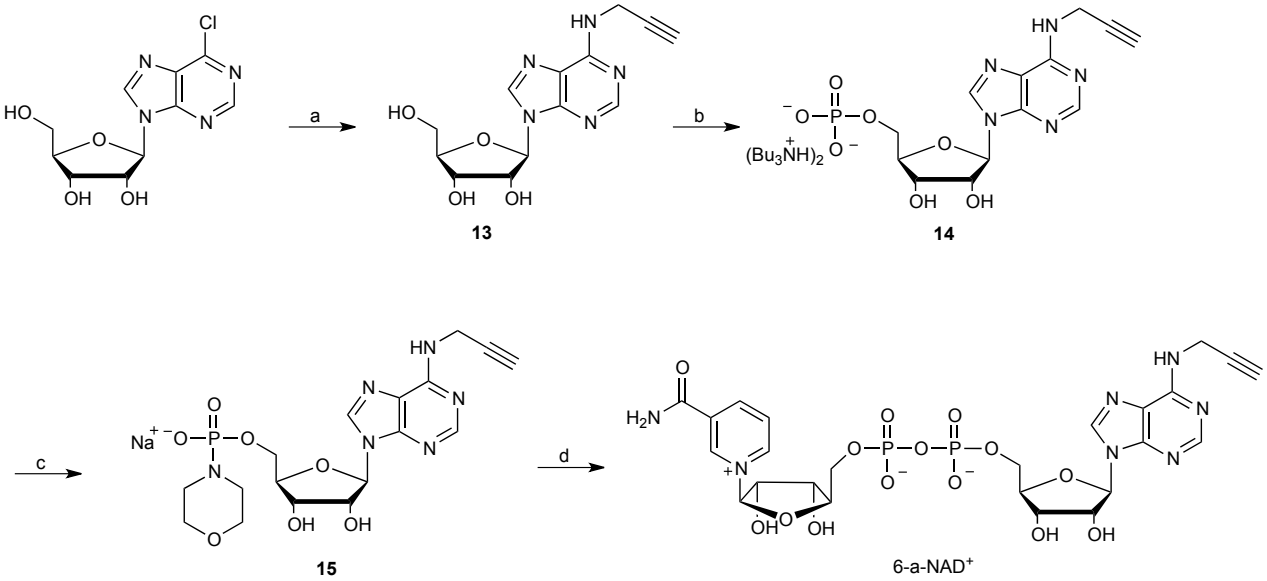
II. Supplemental Schemes



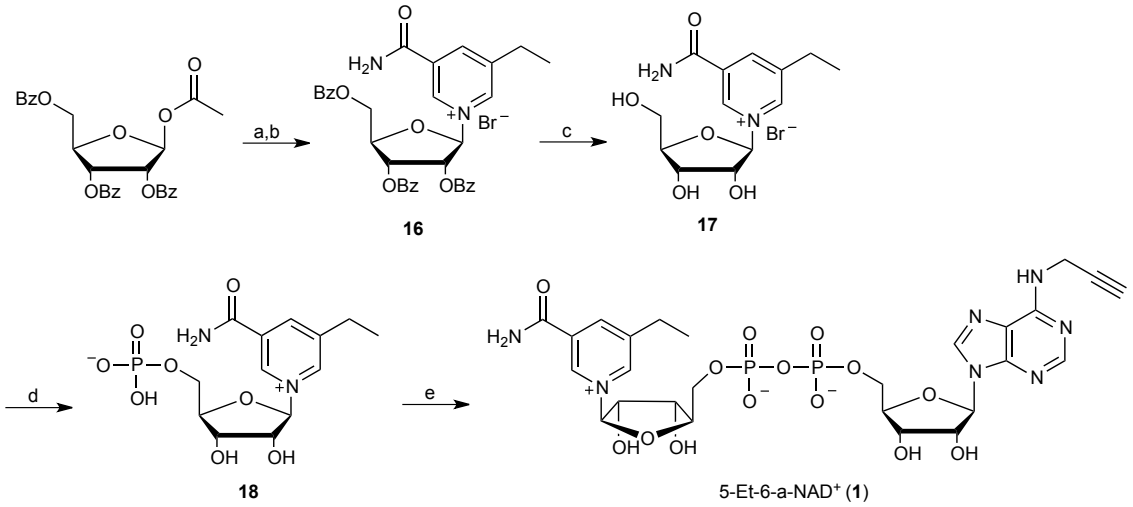
Supplemental Scheme 1. Synthesis of C-5 substituted nicotinamide analogs **3**, **5**, and **6.** Reagents and conditions: (a) potassium vinyltrifluoroborate (for **9**) or potassium *trans*-1-propenyl trifluoroborate (for **10**), Cs_2CO_3, 1 mol % $PdCl_2(PPh_3)_2$, MeCN/H_2O (10:1), 120 ^oC; (b) triethylsilane (TES),10 mol% Pd-C, MeOH, rt; (c) cyclopropylboronic acid, $PdCl_2$(dppf), K_2CO_3, dioxane/H_2O (4:1), 120 ^oC.



Supplemental Scheme 2. Synthesis of C-5 substituted nicotinamide analogs **7** and **8.** Reagents and conditions: (a) organozinc bromide (isobutyl or benzyl zincbromide), $Pd(PPh_3)_4$ (for **12**) or $PdCl_2$(dppf) (for **11**), THF, 85 ^oC; (b) Amberlite IRA 410 resin (hydroxide form), MeOH/H_2O (1:1), 120 ^oC.



Supplemental Scheme 3. Synthesis of 6-a-NAD^+. Reagents and conditions: (a) propargylamine, DIPEA, EtOH, 80 ^oC; (b) trimethyl phosphate, H_2O, 50 ^oC, then $POCl_3$, 0 ^oC; (c) morpholine, PPh_3, Aldrithiol-2, DMSO, rt; (d) β-NMN, $MnCl_2$, $MgSO_4$, formamide, rt.



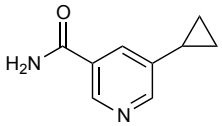
Supplemental Scheme 4. Synthesis of 6-a-NAD^+. Reagents and conditions: (a) HBr (33 wt% in acetic acid), toluene, 0 °C; (b) **5**, MeCN; (c) 7N NH_3 in MeOH, -10 °C; (d) $POCl_3$, trimethyl phosphate, H_2O, rt; (e) **15**, $MnCl_2$, $MgSO_4$, formamide, rt.

III. Supplemental Methods

Chemical Synthesis

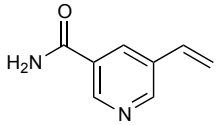
General. ^1H and ^{13}C NMR were recorded on a Bruker DPX spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are reported as parts per million (ppm) downfield from an internal tetramethylsilane standard or solvent references. High-resolution mass spectra were acquired on a ThermoElectron LTQ-Orbitrap discovery high resolution mass spectrometer with a dedicated Accela HPLC system by Andrea DeBarber at the Bioanalytical MS facility, Portland State University. For air- and water-sensitive reactions, glassware was oven-dried prior to use and reactions were performed under argon. Dichloromethane, dimethylformamide, and tetrahydrofuran were dried using the solvent purification system manufactured by Glass Contour, Inc. (Laguna Beach, CA). All other solvents were of ACS chemical grade (Fisher Scientific) and used without further purification unless otherwise indicated. Commercially available starting reagents were used without further purification. Nicotinamide (**2**) (Sigma-Aldrich, >99.5%), 5-methylnicotinamide (**4**) (Alfa Aesar, 97%), and beta-nicotinamide mononucleotide (β-NMN) (Sigma-Aldrich, >95%) were used without further purification. Analytical thin-layer chromatography was performed with silica gel 60 F_{254} glass plates (SiliCycle). Flash column chromatography was conducted with either pre-packed Redisep R_f normal/reverse phase columns (Teledyne ISCO) or self-packed columns containing 200-400 mesh silica gel (SiliCycle) on a

Combiflash Companion purification system (Teledyne ISCO). High performance liquid chromatography (HPLC) was performed on a Varian Prostar 210 (Agilent) with a flow rate of 20 ml/min using Polaris 5 C18-A columns (150 x 4.6 mm, 3 μm -analytical, 150 x 21.2 mm, 5 μm-preparative) (Agilent). HPLC analytical conditions: mobile phase (MP) A: 0.1% formic acid (aq), mobile phase (MP) B: 0.1% formic acid in acetonitrile (ACN); flow rate = 1.0 ml/min; condition **A**: 0 – 2 min: 0%B, 2 – 15 min: 0-100%B, 15 – 17 min: 100%B; condition **B**: 0 – 1 min: 0%B, 1 – 12 min: 0-40%B, 12 – 13 min: 40%B, 13 – 14 min: 0%B; UV-Vis detection: λ_1 = 254 nm, λ_2 = 280 nm. All final products were ≥95% purity as assessed by this method. Retention times (t_R) and purity refer to UV detection at 220 nm.



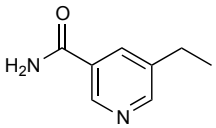
3

5-cyclopropyl-nicotinamide **3**. 5-bromo-nicotinamide (1.05g, 5mmol), cyclopropylboronic acid (558mg, 6.5mmol), K_2CO_3 (2.1g, 15 mmol), $PdCl_2$(dppf) (204mg, 0.25mmol) were dissolved in dioxane (40 ml) and water (4 ml) in a pressure glass vessel. Ar gas was then bubbled through the solution for 15 min. The reaction vessel was sealed and stirred at 100°C for 8 h. The reaction mixture was then cooled to rt and quenched with saturated aq. NH_4Cl solution (25 ml). The layers were separated and the aq. layer was extracted with EtOAc (3 x 15 ml). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified via a Combiflash Companion system (24g Redisep R_f Normal Phase; MP A: hexanes, MP B: EtOAc; 0-2 min: 40%B, 2-14 min: 40-100%B, 14-29 min: 100%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the product off white solid (681 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, *J* = 2.1 Hz, 1H), 8.52 (d, *J* = 2.2 Hz, 1H), 7.74 (t, *J* = 2.1 Hz, 1H), 6.24 (br., 1H), 6.19 (br., 1H), 2.09-1.72 (m, 1H), 1.16-0.92 (m, 2H), 0.76 (dt, *J*=6.7, 4.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.96, 151.49, 145.10, 140.07, 132.07, 128.91, 13.06, 9.60.



9

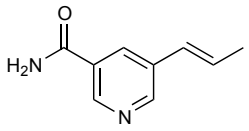
5-vinyl-nicotinamide **9**. 5-bromo-nicotinamide (1.05 g, 5 mmol), potassium vinyl trifluoroborate (871 mg, 6.5 mmol), Cs_2CO_3 (4.5g, 15 mmol) and $PdCl_2(PPh_3)_2$ (175 mg, 0.25 mmol) were added to a flask and evacuated/refilled with Ar gas (5x). The contents of the flask were suspended in ACN/H_2O (10:1; 44 ml) and evacuated/refilled with Ar (5x) and stirred at 120°C for 1.5 h. The reaction was allowed to cool to rt and quenched with saturated aq. NH_4Cl solution (25 ml). The layers were separated and the aq. layer was extracted with EtOAc (3 x 15 ml). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified via a Combiflash Companion system (24g Redisep R_f Normal Phase; MP A: hexanes, MP B: EtOAc; 0-2 min: 40%B, 2-14 min: 40-100%B, 14-29 min: 100%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the product as off white solid (700 mg, 95% yield). ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.89 (d, J = 2.1 Hz, 1H), 8.70 (d, J = 2.1 Hz, 1H), 8.37 (t, J = 2.1 Hz, 1H), 6.83 (dd, J = 17.7, 11.0 Hz, 1H), 6.02 (d, J = 17.7 Hz, 1H), 5.49 (d, J = 11.1 Hz, 1H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 169.67, 150.99, 148.38, 134.98, 133.58, 131.22, 131.22, 118.48.



5

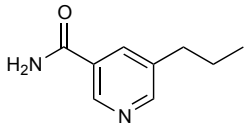
5-ethyl-nicotinamide **5**. 5-vinyl-nicotinamide **9** (550 mg, 3.7 mmol) was dissolved in anhydrous MeOH in a pressure vessel. 10% Pd/C (55 mg) was added and the reaction was stirred under H_2 gas at rt for 5 h. The reaction was filtered over a pad of Celite® 545 to remove Pd/C and the filtrate was concentrated *in vacuo*. The crude product was purified via a Combiflash Companion system (24g Redisep R_f Normal Phase; MP A: hexanes, MP B: EtOAc; 0-2 min: 40%B, 2-14 min: 40-100%B, 14-29 min: 100%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the product as an off white solid (500 mg, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.57 (s, 1H), 8.13 (s, 1H), 8.06 (s, 1H), 7.57 (s, 1H), 2.67

(q, J = 7.6 Hz, 2H), 1.22 (t, J = 7.6, 3H); ^{13}C NMR (101 MHz, DMSO) 166.65, 151.56, 146.23, 138.68, 134.29, 129.38, 25.16, 15.16. LC-HRMS (ESI) *m/z* $[M+H]^+$ calculated for $C_8H_{11}N_2O^+$ 151.0859, found 151.0852.



10

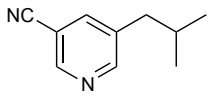
trans-5-propenyl-nicotinamide **10**. 5-bromo-nicotinamide (1.0 g, 5.0 mmol), potassium *trans*-1-propenyl trifluoroborate (961.9 mg, 6.5 mmol), $PdCl_2(PPh_3)_2$ (351 mg, 0.5 mmol), and Cs_2CO_3 (4.88g, 15.0 mmol) were added to a flask and evacuated/refilled with Ar gas (5x). The contents of the flask were suspended in ACN/H_2O (10:1; 44 ml) and evacuated/refilled with Ar (5x) and refluxed (100°C) for 3.5 h. LC analysis revealed consumption of the starting material. The reaction was allowed to cool to rt and quenched with saturated aq. NH_4Cl solution (25 ml). The layers were separated and the aq. layer was extracted with EtOAc (3 x 15 ml). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified via a Combiflash Companion system (24g Redisep R_f Normal Phase; MP A: hexanes, MP B: EtOAc; 0-2 min: 40%B, 2-14 min: 40-100%B, 14-29 min: 100%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the product as a beige solid (750 mg, 93%). Condition **A**, t_R = 8.91 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (d, J = 2.0 Hz, 1H), 8.68 (d, J = 2.1 Hz, 1H), 8.22 (t, J = 2.1 Hz, 1H), 8.15 (s, 1H), 7.60 (s, 1H), 6.69 – 6.29 (m, 2H), 1.89 (d, J = 5.1 Hz, 3H).



6

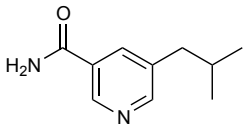
5-propyl-nicotinamide **6**. *trans*-5-(prop-1-en-1-yl)nicotinamide **10** (467 mg, 2.88 mmol) was dissolved in MeOH (20 ml) in a pressure vessel. 10% Pd/C (93.4 mg) was suspended in the reaction mixture. Triethylsilane (Et_3SiH, 25.3 ml, 158.4 mmol) was added via syringe and the reaction was capped and stirred at rt for 43 h. ^{1}H NMR analysis of a small reaction aliquot revealed consumption of the starting material. The reaction was filtered over a pad of Celite® 545 to remove Pd/C and the filtrate was concentrated *in vacuo*. The residue was purified via a

Combiflash Companion system (12g Redisep R_f Normal Phase; MPA: CH_2Cl_2, MPB: MeOH; 0-3 min: 0%B, 3-15 min: 0-30%B, 15-16 min: 30%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the product as an off-white solid (390 mg, 82%). Condition **A**, t_R = 8.54 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (d, *J* = 2.1 Hz, 1H), 8.54 (d, *J* = 2.1 Hz, 1H), 8.12 (s, 1H), 8.03 (t, *J* = 2.2 Hz, 1H), 7.56 (s, 1H), 2.62 (t, *J* = 7.6 Hz, 2H), 1.62 (m, *J* = 7.4 Hz, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.59, 151.93, 146.27, 137.06, 134.78, 129.31, 33.93, 23.66, 13.49.



11

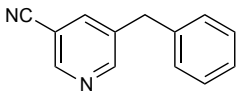
5-isobutyl-nicotinonitrile **11.** 5-bromo-nicotinonitrile (2.0 g, 10.9 mmol) and Pd(dppf)Cl_2 (400 mg, 0.55 mmol) were added to a flame-dried flask. The flask was evacuated and refilled with Ar gas (3x). Anhydrous THF (60 ml) was added via syringe along with isobutylzinc bromide (40.0 ml, 0.5 M in THF). The mixture was evacuated/refilled with Ar (4x) and heated at 85°C (reflux) for 3 h followed by overnight stirring at rt. LC analysis revealed consumption of the starting material. The reaction was quenched with saturated aqueous NH_4Cl (100 ml) and the layers were separated. The aq. layer was extracted with EtOAc (3 x 15 ml) and the organic layers were combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified via a Combiflash Companion system (24g RediSep R_f Normal Phase; MP A: hexanes, MP B: EtOAc; 0-2 min: 0%B, 2-15 min: 0-100%B, 15-17 min: 100%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the product as an amorphous off-white solid (710 mg, 41%). Condition **A**, t_R = 13.37 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, *J* = 2.0 Hz, 1H), 8.61 (d, *J* = 2.1 Hz, 1H), 7.73 (t, *J* = 2.1 Hz, 1H), 2.54 (d, *J* = 7.2 Hz, 2H), 1.89 (m, *J* = 13.6, 6.8 Hz, 1H), 0.93 (d, *J* = 6.6 Hz, 6H).



7

5-isobutyl-nicotinamide **7**. 5-isobutyl-nicotinonitrile **11** (594 mg, 3.7 mmol) was suspended in MeOH/H_2O (1:1, 50 mL). Amberlite® IRA 410 resin (6.79g, $^-$OH form) was added. The resin was prepared previously by stirring

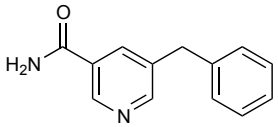
Amberlite® IRA 410 resin (Cl^- form) in 10% NaOH solution (300 ml) for 3 h, followed by extensive rinsing of the resin with degassed H_2O until rinsate pH was neutral. The reaction was heated at reflux for 4.5 h. LC analysis revealed consumption of the starting material. The reaction was cooled to rt and filtered. The resin was rinsed thoroughly with MeOH (50 ml) and the filtrates were combined and concentrated *in vacuo*. The resulting residue was redissolved in EtOAc (50 ml) to yield two layers. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 10 ml). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The solid was purified via a Combiflash Companion system (12g RediSep R_f Normal Phase; MP A: CH_2Cl_2, MP B: MeOH; 0-3 min: 0%B, 3-15 min: 0-30%B, 15-17 min: 30%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the product as a white solid (540 mg, 82%). Condition **A**, t_R = 9.52 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (d, J = 2.1 Hz, 1H), 8.51 (d, J = 2.1 Hz, 1H), 8.12 (s, 1H), 8.01 (t, J = 2.2 Hz, 1H), 7.57 (s, 1H), 2.53 (d, 2H, obscured by solvent peak), 1.87 (m, J = 13.5, 6.8 Hz, 1H), 0.86 (d, J = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.57, 152.36, 146.31, 136.04, 135.33, 129.21, 41.10, 29.34, 21.96 (2C).



12

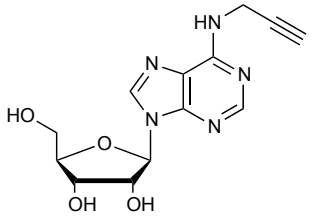
5-benzyl-nicotinonitrile **12.** 5-bromonicotinonitrile (1.0 g, 5.5 mmol) and $Pd(PPh_3)_4$ (317 mg, 0.27 mmol) were added to a flame-dried flask. The flask was evacuated and refilled with Ar gas (3x). Anhydrous THF (30 ml) was added via syringe along with benzylzinc bromide (21.9 ml, 0.5 M in THF). The mixture was evacuated/refilled with Ar (4x) and heated at 85°C (reflux) for 1.25 h. LC analysis revealed consumption of starting material. The reaction was quenched with saturated aqueous NH_4Cl (50 ml) and the layers were separated. The aqueous layer was extracted with EtOAc (3 x 10 ml) and the organic layers were combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified via a Combiflash Companion system (24g RediSep R_f Gold Normal Phase; MP A: hexanes, MP B: EtOAc; 0-2 min: 0%B, 2-13 min: 0-100%B, 13-16min: 100%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the product as an amorphous light yellow solid (900 mg g, 84%). Condition **A**, t_R = 13.36 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72

(dd, J = 13.0, 2.1 Hz, 2H), 7.71 (t, J = 2.1 Hz, 1H), 7.41 – 7.32 (m, 2H), 7.32 – 7.27 (m, 1H), 7.21 – 7.12 (m, 2H), 4.04 (s, 2H).



8

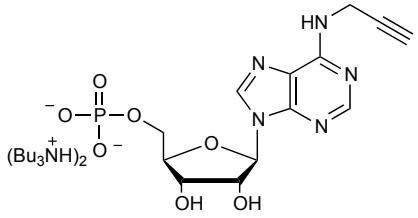
5-benzyl-nicotinamide **8.** 5-benzyl-nicotinonitrile **12** (677 mg, 3.5 mmol) was suspended in MeOH/H_2O (1:1, 48 mL). Amberlite® IRA 410 resin (6.39g, $^-$OH form) was added. The resin was prepared previously by stirring Amberlite® IRA 410 resin (Cl^- form) in 10% NaOH solution (300 ml) for 3 h, followed by extensive rinsing of the resin with degassed H_2O until rinsate pH was neutral. The reaction was heated at reflux for 3 h. LC analysis revealed consumption of the starting material. The reaction was cooled to rt and filtered. The resin was rinsed thoroughly with MeOH (50 ml) and the filtrates were combined and concentrated *in vacuo*. The resulting solid was purified via a Combiflash Companion system (12g RediSep R_f Normal Phase; MPA: CH_2Cl_2, MPB: MeOH; 0-2 min: 0%B, 2-13 min: 0-30%B, 13-15 min: 30%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the product as an off-white solid (0.53 g, 72%). Condition **A**, t_R = 10.41 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (d, J = 2.1 Hz, 1H), 8.63 (d, J = 2.1 Hz, 1H), 8.13 (s, 1H), 8.04 (t, J = 2.2 Hz, 1H), 7.57 (s, 1H), 7.36 – 7.24 (m, 4H), 7.24 – 7.16 (m, 1H), 4.02 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.40, 152.04, 146.40, 140.19, 136.62, 135.27, 129.50, 128.75 (2C), 128.67 (2C), 126.35, 37.84.



13

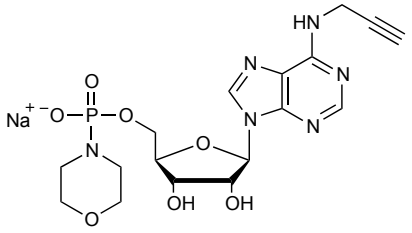
6-alkyne-adenosine **13.** 6-chloropurine riboside (1.0 g, 3.5 mmol) was added to absolute ethanol (15 ml) and heated to reflux. DIPEA (1.82 ml, 10.5 mmol) and propargylamine (0.67 ml, 10.5 mmol) were added and the mixture was refluxed for 2 h. LC analysis revealed consumption of the starting material. The reaction was cooled to rt and the precipitated product was filtered and washed with cold methanol to yield a white solid (637 mg, 60%). Condition **B**, t_R = 9.42 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (s, 1H), 8.28 (s, 1H), 5.90 (d, J =

6.1 Hz, 1H), 5.46 (d, *J* = 6.1 Hz, 1H), 5.35 (dd, *J* = 7.0, 4.6 Hz, 1H), 5.20 (d, *J* = 4.6 Hz, 1H), 4.60 (q, *J* = 5.9 Hz, 1H), 4.25 (s, 2H), 4.15 (td, *J* = 4.8, 3.1 Hz, 1H), 3.96 (q, *J* = 3.5 Hz, 1H), 3.75 – 3.48 (m, 2H), 3.04 (t, *J* = 2.4 Hz, 1H).



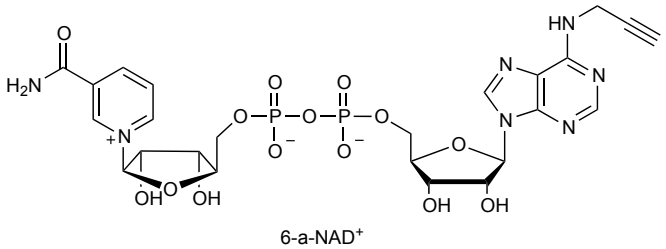
14

6-alkyne-AMP **14**. 6-alkyne adenosine **13** (500 mg, 1.64 mmol) was heated under vacuum at 60°C for 30 min and suspended in trimethyl phosphate (5.5 ml). H_2O (14.7 µl, 0.82 mmol) was added and the mixture was heated at 50 °C for 15 min with gradual clearing. The mixture was cooled to 0°C with an ice bath and $POCl_3$ (457 µl, 4.91 mmol) was added dropwise. The mixture was stirred at 0°C for 3 h. LC analysis revealed consumption of the starting material. The mixture was precipitated in cold Et_2O (150 ml) in an ice bath. The residue was dissolved in ice cold H_2O (6 ml) and stirred for 10 min. The reaction was immediately neutralized with 6N NaOH. The mixture was concentrated *in vacuo* and the solid was purified via ion-pairing reversed phase chromatography using a Combiflash Companion system (C18Aq 5.5g Redisep R_f; MP A: 10 mM tributylamine/30 mM acetic acid pH 4.4 (aq), MP B: methanol; 0-1 min: 0%B, 1-12 min: 0-50%B, 12-14 min: 100%B). Fractions containing desired product were pooled and concentrated *in vacuo* to yield the tributylammonium (TBA) salt of the product as a thick oil (632 mg, 51% yield). t_R = 7.63 min. ^{1}H NMR (400 MHz, D_2O) δ 8.54 (s, 1H), 8.26 (s, 1H), 6.09 (d, *J* = 5.9 Hz, 1H), 4.75 (t, *J* = 5.7 Hz, 1H, obscured by HOD peak), 4.47 (dd, *J* = 5.1, 3.4 Hz, 1H), 4.39 – 4.23 (m, 3H), 4.00 (dd, *J* = 4.5, 3.0 Hz, 2H), 3.15 – 3.00 (m, 12H), 2.59 (t, *J* = 2.4 Hz, 1H), 1.71 – 1.53 (m, 12H), 1.32 (m, *J* = 7.4 Hz, 12H), 0.89 (t, *J* = 7.4 Hz, 18H).



15

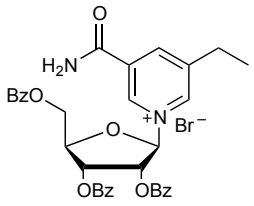
6-allkyne-AMP-morpholidate **15.** 6-alkyne-AMP TBA salt **14** (371 mg, 0.49 mmol) was dissolved in anhydrous DMSO (2.2 mL) and coevaporated with anhydrous DMF (3 x 5 ml). The resulting residue was redissolved in additional anhydrous DMSO (1.75 ml) and the following were added in sequence: PPh_3 (687 mg, 2.62 mmol), morpholine (369 µl, 4.22 mmol), and 2,2′-dipyridyldisulfide (Aldrithiol) (577 mg, 2.62 mmol). The resulting yellow solution was stirred at rt under Ar for 90 min. LC analysis revealed consumption of starting material. To the reaction was added 0.2 M NaI in acetonitrile (40 ml) dropwise to form a white precipitate (ppt). The mixture was centrifuged at 8,000g for 10 min at 4°C. The supernatant was decanted and the ppt was washed with ACN (5 ml) and centrifuged again using the same conditions. The ppt was washed with EtOAc (2 x 5 ml) or until the yellow color was removed. The ppt was redissolved in MeOH and concentrated *in vacuo* to yield the sodium salt of the product as a white solid (215 mg, 92%), which was used in subsequent coupling reactions without further purification. Condition **B**, t_R = 9.00 min. ^{1}H NMR (400 MHz, D_2O) δ 8.40 (s, 1H), 8.29 (s, 1H), 6.10 (d, *J* = 5.0 Hz, 1H), 4.83 – 4.80 (m, 1H, obscured by HOD peak), 4.52 (t, *J* = 4.8 Hz, 1H), 4.40 – 4.25 (m, 3H), 4.00 (ttd, *J* = 11.7, 8.5, 7.8, 3.8 Hz, 2H), 3.50 (t, *J* = 4.8 Hz, 4H), 2.86 (p, *J* = 4.9 Hz, 4H), 2.59 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 154.50, 153.33, 149.05, 119.80, 87.96, 84.36, 74.66, 70.87, 67.49, 64.40, 45.23, 30.81. LC-HRMS (ESI) *m/z* $[M+H+Na]^+$ calculated for $C_{17}H_{22}N_6NaO_7P$ 477.12580, observed 477.12626.



6-a-NAD$^+$

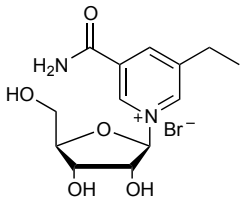
6-a-NAD$^+$. 6-a-NAD$^+$ was previously described[2]; however, we used a different synthetic procedure. 6-allkyne-AMP-morpholidate **15** (24 mg, 0.05 mmol), β-NMN (20 mg, 0.055 mmol), and $MgSO_4$ (12 mg, 0.1 mmol) were dissolved in a solution of $MnCl_2$ (0.38 ml, 0.2 M in formamide) at rt for 48 h. The reaction was then

concentrated *in vacuo* and the crude product was purified via preparative HPLC (MP A: 0.1% formic acid (aq), MP B: 0.1% formic acid in ACN; 0-5 min: 0-10%B, 5-8 min: 10-15%B, 8-10 min: 15-20%B, 10-12 min: 20-50%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the desired product (14 mg, 40% yield). ^{1}H NMR (400 MHz, D_2O) δ 9.34 (s, 1H), 9.18 (d, *J* = 6.3 Hz, 1H), 8.85 (d, *J* = 8.1 Hz, 1H), 8.53 (s, 1H), 8.34 (d, *J* = 0.8 Hz, 1H), 8.15 (m, 1H), 6.09 (d, *J* = 5.5 Hz, 2H), 4.73 (m, 1H), 4.47-4.53 (m, 3H), 4.41 (m, 2H), 4.33 (m, 3H), 4.19 (m, 3H), 2.70 (s, 1H).



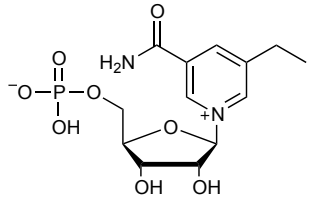
16

N'-(2,3,5-Tri-o-Benzoyl-β-D-ribofuranosyl)-3-aminocarbonyl-5-ethyl-pyridinium bromide **16**. β-D-ribofuranose-acetate-2, 3, 5-tribenzoate (504 mg, 1 mmol) was dissolved in toluene (15 ml) and cooled to 0°C. HBr (33wt% in acetic acid) (368 mg, 1.5 mmol) was added dropwise and the reaction was stirred at 0°C for 2 h. 0.5 ml of the solution mixture was taken and evaporated to dryness for NMR analysis. In the ^{1}H NMR spectrum the anomeric proton for the β-form is a singlet at 6.6 ppm and a doublet at 6.9 ppm for the α-form. After the starting material was consumed and ^{1}H NMR confirmed the formation of the β-form, the reaction was concentrated *in vacuo*. The crude β-D-ribofuranose-bromo-2, 3, 5-tribenzoate product was azeotroped with toluene (3 x 20 ml) to remove remaining acetic acid and dried *in vacuo* for 2 h. Crude β-D-ribofuranose-bromo-2, 3, 5-tribenzoate and 5-ethyl nicotinamide **5** (91 mg, 0.6 mmol) was dissolved in ACN (40 ml). The reaction was stirred under Ar gas at rt for 2 days. The reaction was concentrated *in vacuo* (temperature kept below 35°C). The crude product was dissolved in $CDCl_3$ (2 ml) and ppt by adding ethyl ether (10 ml). The entire procedure was repeated three times to yield the desired product (270 mg, 67% yield), which was used in subsequent reactions without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.66 (s, 1H), 10.43 (s, 1H), 8.95 (s, 1H), 8.87 (s, 1H), 8.47 (s, 1H), 8.08 (m, 4H), 8.01 (m, 2H), 7.68 – 7.54 (m, 3H), 7.53 – 7.34 (m, 6H), 6.08 – 5.89 (m, 2H), 5.86 – 5.71 (m, 1H), 5.14 – 5.00 (m, 2H), 4.93 – 4.79 (m, 1H), 2.66 (q, *J* = 7.6 Hz, 2H), 1.19 (t, *J* = 7.6, 3H). HRMS (ESI) *m/z* $[M+H]^+$ calculated for $C_{34}H_{32}N_2O_8^+$ 595.20749, found 595.20890.



17

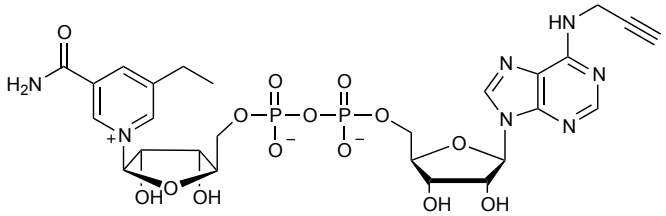
N'-(β-D-ribofuranosyl)-3-aminocarbonyl-5-ethyl-pyridinium bromide **17**. **16** (150 mg, 0.22 mmol) was dissolved in ammonia (15 m, 7 N in MeOH) and the reaction was stirred at -10°C for 36 h. The reaction was concentrated *in vacuo* and the crude product was dissolved in MeOH (1 ml). Addition of ethyl ether 10 ml) resulted in ppt of the desired product. The procedure was repeated three times to yield the desired product as an off white powder (56 mg, 71% yield), which was used in subsequent reactions without further purification. Some epimerization was observed (~5-10% α–form was present as determined by ^{1}H NMR analysis). ^{1}H NMR (400 MHz, D_2O) δ 9.40 (s, 1H), 9.13 (s, 1H), 8.82 (d, *J* = 1.7 Hz, 1H), 6.19 (d, *J* = 4.1 Hz, 1H), 4.55 – 4.39 (m, 2H), 4.34 (t, *J* = 4.8 Hz, 1H), 4.07 – 4.03 (dd, 1H, *J* = 2.80 and 12.80 Hz, 1H), 3.91-3.87 (dd, 1H, *J* = 3.2 and 12.8 Hz, 1H), 2.96 (q, *J* = 7.6 Hz, 2H), 1.31 (t, *J* = 7.6 Hz, 3H). HRMS (ESI) *m/z* $[M+H]^+$ calculated for $C_{13}H_{20}N_2O_5^+$, 283.12885, found 283.12904.



18

5-ethyl-nicotinamide mononucleotide **18**. **17** was dissolved in trimethylphosphate (0.18 ml) and the reaction was cooled to 0°C. $POCl_3$ (166 mg, 1.08 mmol) was added and the reaction was stirred at 0°C for 4 h. A few drops H_2O was then added to quench the reaction. Trimethylphosphate was removed by extraction with ethyl ether (20 ml). The remaining trimethylphosphate was removed by a second extraction with THF (5 ml). The aq layer was concentrated *in vacuo*. The crude product was dissolved in H_2O (0.5 ml) and purified via two-step ion exchange chromatography (Dowex resin 1 x 2, formate resin, eluted with water; H^+ resin, eluted with water).

Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the desired product (74 mg, 74% yield). ^{1}H NMR (400 MHz, D_2O) δ 9.31 (s, 1H), 9.06 (s, 1H), 8.84 (s, 1H), 6.14 (d, *J*=5.6 Hz, 1H), 4.56 (m, 1H), 4.43 (t, *J*=5.2 Hz, 1H), 4.41-4.40 (dd, *J*=5.0, 2.4 Hz, 1H), 4.29 (ddd, *J*=12.0, 4.4, 2.4 Hz, 1H), 4.13 (ddd, *J* = 11.9, 4.9, 2.1 Hz, 1H), 2.97 (q, *J*=7.6 Hz, 2H), 1.32 (t, *J*=7.6 Hz, 3H); ^{13}C NMR (100 MHz, D_2O/ CD_3OD (9:1) δ 167.05, 147.14, 146.59, 142.70, 138.02, 134.60, 100.99, 88.69, 78.78, 72.29, 65.11, 26.64, 14.58. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $C_{13}H_{20}N_2O_8P^+$, 363.09518, found 363.09643



5-Et-6-a-NAD$^+$ (**1**)

5-Et-6-a-NAD$^+$ **1**. 6-allkyne-AMP-morpholidate **15** (24 mg, 0.05 mmol), 5-ethyl-nicotinamide mononucleotide **18** (20 mg, 0.055 mmol), and $MgSO_4$ (12 mg, 0.1 mmol) were dissolved in a solution of $MnCl_2$ (0.38 ml, 0.2 M in formamide) at rt for 48 h. The reaction was then concentrated *in vacuo* and the crude product was purified via preparative HPLC (MP A: 0.1% formic acid (aq), MP B: 0.1% formic acid in ACN; 0-5 min: 0-10%B, 5-8 min: 10-15%B, 8-10 min: 15-20%B, 10-12 min: 20-50%B). Fractions containing the desired product were pooled and concentrated *in vacuo* to yield the desired product (11 mg, 31% yield). ^{1}H NMR (400 MHz, D_2O) δ 9.11 (s, 1H), 8.90 (s, 1H), 8.73 (s, 1H), 8.44 (s, 1H), 8.22 (s, 1H), 5.99 (dd, *J* = 13.4, 5.5 Hz, 2H), 4.71(1H, overlapping with HOD peak), 4.48 (d, *J* = 6.0 Hz, 2H), 4.47 – 4.38 (m, 1H), 4.33 (s, 3H), 4.21 (s, 3H), 2.89 (q, *J* = 7.6 Hz, 2H), 2.65 (s, 1H), 1.27 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 165.72, 147.15, 146.61, 142.71, 137.95, 132.31, 100.91, 88.16, 84.97, 80.06, 78.59, 75.30, 73.67, 71.82, 71.27, 62.64, 26.60, 14.44. LC-HRMS (ESI) *m/z* $[M+H]^+$ calculated for $C_{26}H_{33}N_7O_{14}P_2^+$, 730.16389, found 730.16502

Other Methods

Cell culture and reagents. HEK 293T cells were grown at 37°C and 5% CO_2. Cells were passaged in DMEM + 10% FBS (Gibco) + penicillin/streptomycin (Invitrogen). Antibodies used for immunoblot detection were anti-His (His.H8, Pierce, 1:1,000), anti-XRCC5 (C48E7, Cell Signaling Technology, 1:1,000), anti-Catenin-□-1 (4989, Cell Signaling Technology, 1:500), anti-hnRNP Q/R (D18B2, Cell Signaling Technology, 1:750) goat anti-rabbit IgG – HRP (Novex), and goat anti-mouse IgG – HRP (Novex). Primary antibodies (stored at 1 mg/ml) were used at the indicated concentration and secondary antibodies at 1:5,000 for immunoblot assays. Streptavidin-HRP (Jackson ImmunoResearch) was stored at -20°C in a 50% glycerol mix (0.5 mg/ml) and was used at 1:5,000 for immunoblot assays.

Cloning and mutagenesis. The coding region for human ARTD1 and ARTD6 were obtained from the DNASU plasmid repository (ARTD1: HsCD00040600, ARTD6: HsCD00080244)[7]. cDNA encoding mouse ARTD2 was obtained from a cDNA library generated for this study. RNA was isolated from mouse embryonic brain tissue using the TRIzol® reagent (Invitrogen) protocol. 5 μg of isolated RNA was reverse transcribed with SuperScript® III Reverse Transcriptase (Invitrogen) to produce cDNA. Full-length ARTD1 and ARTD2 were PCR-amplified from cDNA using primers with non-complementary restriction enzyme sites located at the 5' and 3' ends. Primers for amplification of ARTD6 were designed to amplify only the catalytic domain (residues 934-1166). Amplified products were cloned into pET-28b$^+$ (Novagen) for expression. Mutants were generated using the QuickChange® II XL site-directed mutagenesis kit (Agilent). Plasmids were sequenced from both the 5' and 3' direction to confirm the coding sequence. Primers used in this study are listed in Supplementary Table S3.

Expression and purification of ARTD1, ARTD2, and ARTD6$_{cat}$. ARTD1 and ARTD2 were expressed in the *Escherichia coli* BL21 pRARE2 strain (EMD Millipore). Cells were first cultured in LB media overnight at 225 rpm and 37°C in an Excella® E24 Incubator (New Brunswick Scientific). One liter of TB media (12 g Bacto Tryptone (BD Biosciences), 24 g Bacto Yeast Extract (BD Biosciences), 0.4% glycerol, 17 mM KH_2PO_4, 72 mM K_2HPO_4, 1% glucose, 100 μM $ZnCl_2$, 50 μg/ml kanamycin, 34 μg/ml chloramphenicol) was inoculated with the starting culture and grown to OD_{600} = 0.8 – 1.0 at 225 rpm and 37°C. The temperature was reduced to 16°C and expression was induced by adding isopropyl β-d-thiogalactoside (IPTG) to 0.4 mM. After incubation at 16°C for 18 – 24 h, cells were harvested by centrifugation at 6,000 g for 10 min. The cell pellet was re-

suspended in lysis buffer (20 mM HEPES, pH 7.5, 1 mM β-mercaptoethanol (β-Me), 1 mM benzamidine, 0.2% NP-40, 0.2% TWEEN-20, 500 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 8.3 mg/L DNAse I (Roche)) at 4°C, subjected to cell disruption using a Sonifier 450 (Branson), and lysate was clarified by centrifugation at 12,000 g for 30 min at 4°C. Lysates were incubated with pre-washed Ni-NTA agarose resin (50% slurry, Qiagen) with end-over-end rotation at 4°C for 1 h. Following extensive washing with buffer B1+20 (20 mM HEPES, pH 7.5, 1 mM β-Me, 1 mM PMSF, 1 mM benzamidine, 500 mM NaCl, 20 mM imidazole) protein was eluted in buffer B1+400 (20 mM HEPES, pH 7.5, 1 mM β-Me, 500 mM NaCl, 400 mM imidazole) and diluted with an equal volume of buffer 3AB+400 (100 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 14 mM β-Me, 0.4 M NaCl). Eluate was then loaded to a pre-washed 3-aminobenzamide-sepharose 4B chromatography column by gravity flow. The column was washed with buffer 3AB+400 followed by an equal volume of buffer 3AB+800 (100 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 14 mM β-Me, 0.8 M NaCl) and protein was eluted in buffer 3AB+MetB (100 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 14 mM β-Me, 0.4 M NaCl, 2 mM 3-methoxybenzamide). Fractions containing ARTD protein were collected and dialyzed to 50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 1 mM β-Me, 0.4 M NaCl at 4°C. Protein concentrations were determined by Bradford assay with BSA standards and purity was assessed by PageBlue staining (Pierce) after SDS-polyacrylamide gel electrophoresis (SDS-PAGE). ≥ 90% purity was achieved for each of the ARTD1 and ARTD2 variants (Figure S1). ARTD6$_{cat}$ was expressed as above, but was only purified using Ni-NTA agarose resin. ≥ 50% purity was achieved for each of the ARTD6$_{cat}$ variants (Figure S1).

Histone H1 plate assays. 250 ng of each ARTD1 variant was pre-incubated with varying concentrations (0 - 2 mM) of nicotinamide (Sigma), 5-methyl-nicotinamide (Alfa Aesar), or various nicotinamide analogs in hB buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM *tris*(2-carboxyethyl)phosphine (TCEP), 10 ng/μL activated DNA (Sigma)) in individual wells of an 8-well Histone H1 strip plate (Trevigen) at rt for 10 min. 6-a-NAD^+ pre-incubated with an identical concentration of inhibitor in plate buffer was added to the ARTD mix to a final concentration of 50 μM. The reaction proceeded for 30 min at 30°C for the WT-ARTD1 and 60 min for the mutants, the plate was washed thrice in 1X PBST (1x PBS, 1% triton X-100), thrice in 1X PBS, and click conjugation was performed in CB buffer (100 μM biotin-PEG_3-azide, 100 μM Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA, Sigma), 1 mM $CuSO_4$, 1 mM TCEP, 1X PBS) for 30 min at rt. The plates were washed

thrice in 1X PBST, thrice in 1X PBS, and incubated with streptavidin-HRP in SH buffer (150 mM KPO_4, pH 7.5, 150 mM KCl, 300 ng/μL BSA, 30 mM β-Me) for 30 min at rt. The plates were washed thrice in 1X PBST, thrice in 1X PBS, and were developed using SureBlue™ TMB Microwell Peroxidase Substrate (KPL) for 2 – 5 min prior to quenching with and equal volume of 1N HCl. Absorbance at 405 nm for each sample was read on a Fusion Universal Microplate Analyzer (Packard). Inhibitor dose-response curves were fit using linear regression in Prism 5 (GraphPad Software). The mean IC_{50} was calculated from three independent assays.

Auto-modification assays. 300 ng of either the WT or KA variant of ARTD1, ARTD2, or $ARTD6_{cat}$ were brought up in hB buffer – activated DNA was absent for assays involving $ARTD6_{cat}$ – and automodification was initiated by adding an equal volume of either hB buffer, 6-a-NAD^+, or 5-Et-6-a-NAD^+ to a final concentration of either 0 μM (hB), 50 μM (5-Et-6-a-NAD^+), 100 μM (5-Et-6-a-NAD^+ or 6-a-NAD^+), or 250 μM (5-Et-6-a-NAD^+) modified NAD^+ analog. Reactions proceeded for 1 h at 30°C, click conjugation was performed by spiking in a one-third volume of CBT buffer (1.5 mM Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, Sigma), 750 μM $CuSO_4$, 300 μM biotin-PEG_3-azide, 7.5 mM sodium ascorbate, 1X PBS) and reactions were left at rt for 1 h. Reactions were fractionated by SDS-PAGE and subsequent immunoblot analysis was performed using a ChemiDoc™ MP Imaging System (Bio-Rad). Each experiment was repeated at least twice, shown are representative images.

Auto-modification assays with native NAD^+. 300 ng of either the WT or KA variant of ARTD1 or ARTD2 were brought up in hB buffer and automodification was initiated by adding an equal volume of either hB buffer (-NAD^+) or 250 μM NAD^+ (+ NAD^+). Reactions proceeded for 1 h at 30°C and were quenched by the addition of 4X sample buffer. Reactions were fractionated by SDS-PAGE and subsequent immunoblot analysis was performed using a ChemiDoc™ MP Imaging System (Bio-Rad). Each experiment was repeated at least twice, shown are representative images.

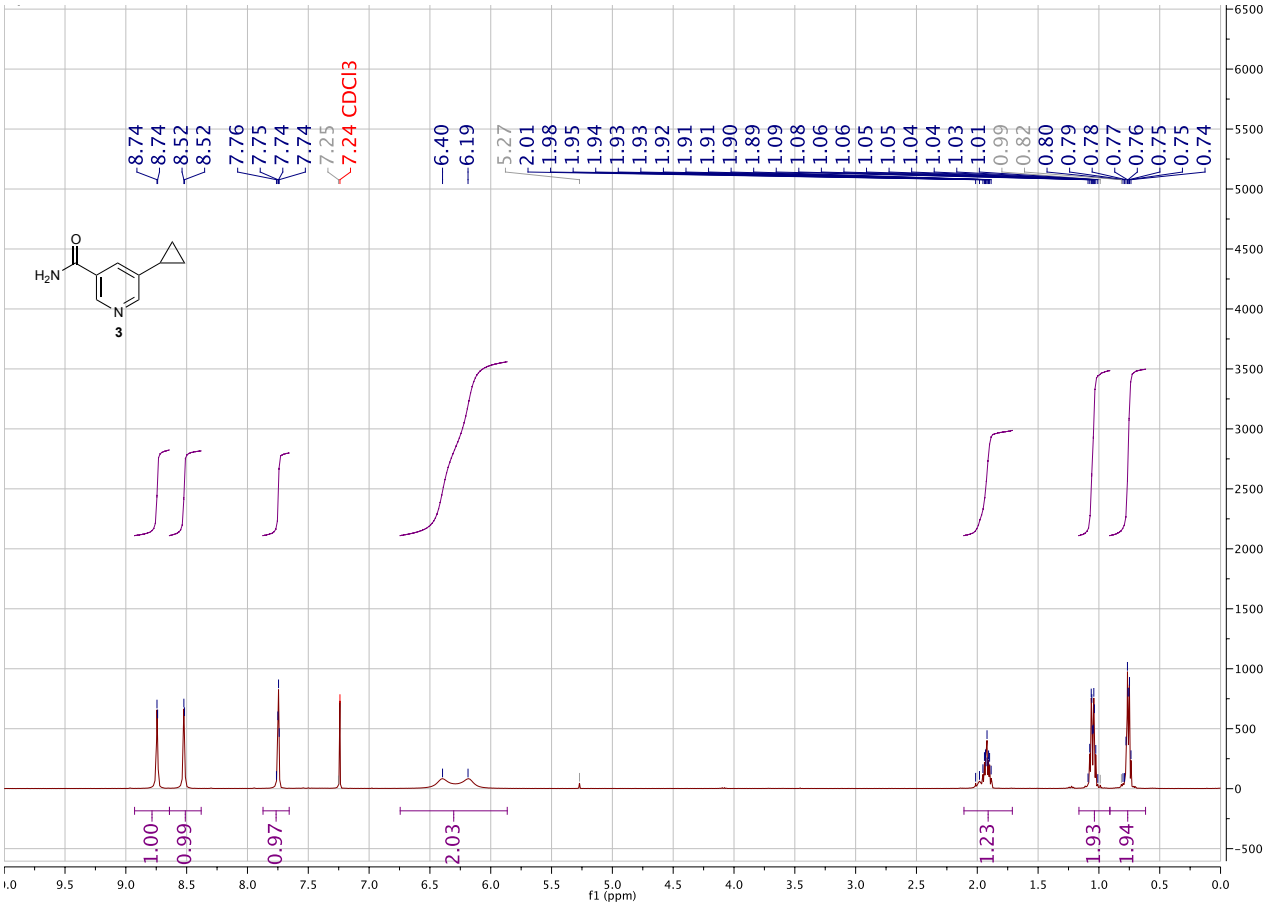
Labeling of HEK 293T nuclear lysate. HEK 293T cells were grown in 2x10 cm dishes to a confluency of 80-90%. Cells were washed with 1X PBS, treated with 1X TypLE™ Select (Invitrogen), and collected by centrifugation at 1,000 g at 4°C. The cell pellet was re-suspended in 200 μl LyB (25 mM HEPES, pH 7.5, 50 mM NaCl, 10% glycerol, 1X cOmplete EDTA-free protease inhibitor cocktail (PI, Roche)) and subjected to centrifugation at 2,000 g for 15 min at 4°C. To generate nuclear extract the supernatant was discarded, the

pellet was re-suspended in 150 μL LyB + 1% NP-40, and centrifuged at 14,000 g for 5 min at 4°C. Total protein concentration in the nuclear lysate was determined by Bradford assay with a BSA standard curve. 60 μg of nuclear extract was brought up in R buffer (47.5 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 1% NP-40), followed by addition of R buffer (No ARTD), KA-ARTD1 (3 μg), or KA-ARTD2 (3 μg) and – separately – either 6-a-NAD^+ or 5-Et-6-a-NAD^+ (to a final concentration of 100 μM or 250 μM, respectively). ADP-ribosylation proceeded for 2 h at 30°C with constant agitation, click conjugation was performed by spiking in a one-third volume of CBT buffer, and reactions were left at rt for 1 h. Reactions were fractionated by SDS-PAGE and subsequent immunoblot analysis was performed. Each experiment was repeated at least twice, shown are representative images.

NeutrAvidin enrichment and LC-MS/MS identification. Five separate HEK 293T nuclear extracts were subjected to enrichment and peptide identification by LC-MS/MS: (1) 250 μM 5-Et-6-a-NAD^+, no additional KA-ARTD (blank); (2) 100 μM 6-a-NAD^+, no additional KA-ARTD (PARylated proteins); (3) 250 μM 5-Et-6-a-NAD^+, 100 ug total KA-ARTD1 (ARTD1 targets); (4) a repeat of (1) as a control for ARTD2 specific labeling; and (5) 250 μM 5-Et-6-a-NAD^+, 100 ug total KA-ARTD2 (ARTD2 targets). ADP-ribosylation reactions as detailed above were completed in tandem (16 individual reactions per condition) to produce one mg total labeled protein. Following click conjugation, enrichment of biotinylated proteins was achieved following a modified protocol[8] using NeutrAvidin™ agarose (Pierce). Briefly, reactions for each condition were pooled, diluted in 10 volumes of chilled methanol, placed at -20°C for 1 h, and pelleted by centrifugation at 6,000 g for 30 min at 4°C. Pellets were washed thrice in one volume chilled methanol, air-dried for 15 min to remove remaining methanol, re-suspended in 2% SDS, subjected to brief heating at 95°C to solubilize the pellet to completion, and diluted with 20 volumes of DB buffer (1X PBS, 113 mM NaCl, 1% NP-40, 1X PI). 100 μL of pre-washed NeutrAvidin™ agarose was added to each sample followed by overnight incubation at 4°C with constant rotation. Agarose resin containing bound protein was washed twice with UB buffer (1X PBS, 4 M urea), thrice with NW-A buffer (1X PBS, 1% NP-40), twice with NW-B (50 mM ammonium bicarbonate), twice with 1X PBS, and twice with NW-B. The agarose resin was re-suspended in 268 μl NW-B, held at 70°C for 10 min, and urea was added to a final concentration of 2 M. TCEP was added to a final concentration of 3.125 mM and the sample was agitated at rt for 30 min. The samples were then treated with iodoacetamide at a final concentration of 11.2

mM for 30 min in the dark. $CaCl_2$ was added to a final concentration of 0.1 mM followed by addition of 2 μg sequencing grade trypsin (Promega) and overnight incubation at 37°C with constant rotation. Digested peptides were eluted from the beads using microcentrifuge spin columns (Pierce) and formic acid was added to 5% v/v. Peptides were concentrated by SpeedVac (Thermo Scientific) and subjected to LC-MS/MS using an LTQ Velos Pro linear ion trap (Thermo Scientific) equipped with a capillary HPLC system as previously described[9], except a 60 min gradient separation was initially used for samples 1-3, and 5 data-dependent MS/MS scans per survey MS scan. Samples 4-5 were analyzed as described, except a 140 min gradient separation was used. Samples 1-3 were re-analyzed using the 140 min gradient separation and both MS/MS data sets (short and long gradient separation) were utilized for samples 1-3 in subsequent data analysis. MS/MS spectra were interpreted by SEQUEST using a UniProtKB/Swiss-Prot human database amended with sequences of common contaminants and the sequence of KA-ARTD1 or KA-ARTD2. Peptide and protein false discovery rate (FDR) was controlled by also adding a complete complement of sequence-reversed entries to the database and estimating the numbers of false identifications to forward sequences as previously described[6]. Identified proteins required a minimum of 2 unique peptide matches, and the resulting FDR was below 2% for both peptide and protein identifications. PARylated or ARTD1/ARTD2 specific targets were identified based on the following criteria: (1) two unique peptides identified in the experimental condition and (2) total peptide counts for each protein were ≥ 2-fold more abundant in the experimental condition compared to the control. Previously identified MS/MS PARylated targets were collected using the thresholds defined in each study, with the exception of Gagne, et al. where proteins were collected based on being identified in 2 out of 3 experimental conditions. To confirm LC-MS/MS identification of the ARTD1/ARTD2 redundant target, XRCC5, and the ARTD2 specific targets, Catenin-□-1 and hnRNP Q/R, HEK293T nuclear extracts were enriched using NeutrAvidin™ as detailed above, except following the wash steps the beads were re-suspended in 1.5X sample buffer + 3 mM biotin (Sigma). Eluates were fractionated by SDS-PAGE and subsequent immunoblot analysis was performed. Each experiment was repeated at least twice, shown are representative images.

IV. Supplemental Compound Characterization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1.05
1.04
1.04
1.03
1.01
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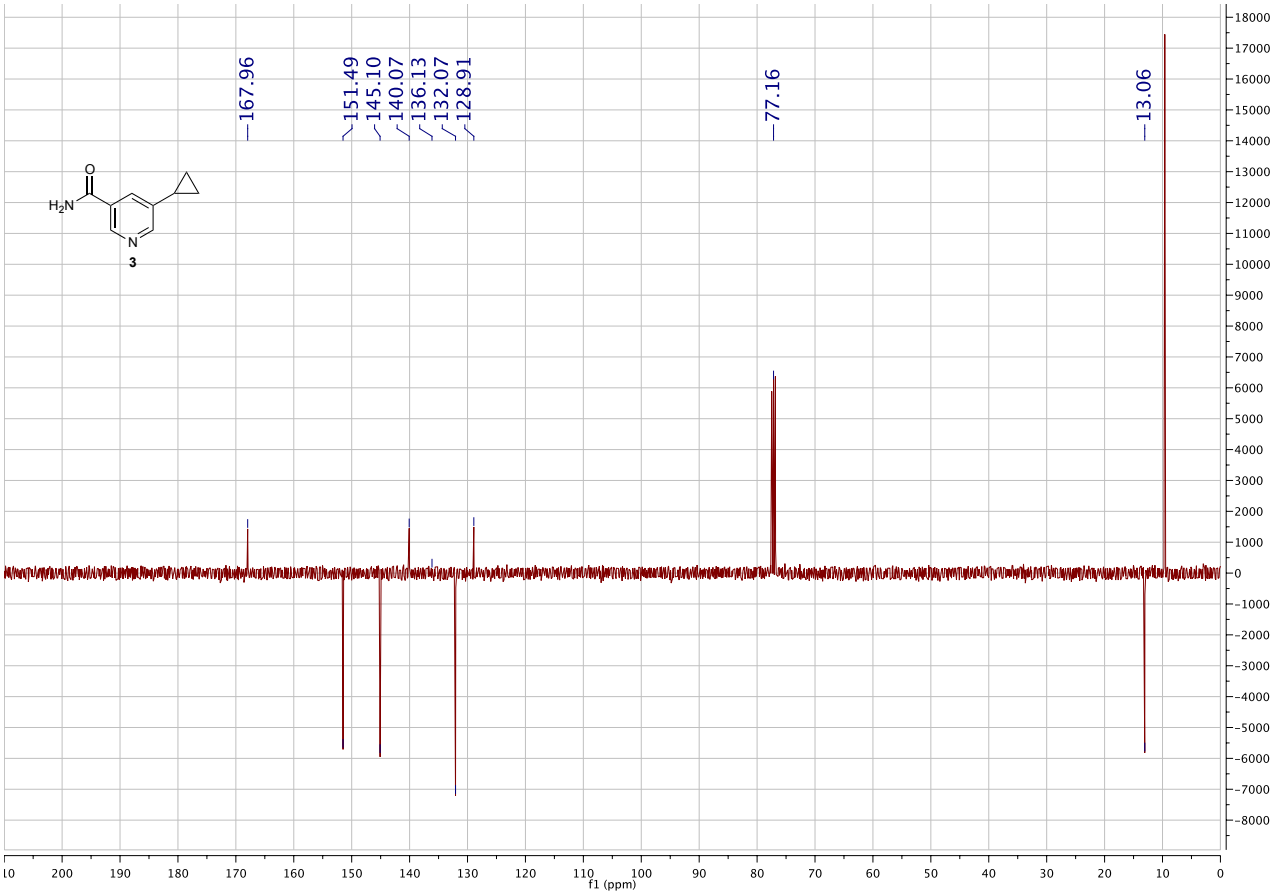
f1 (ppm)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N
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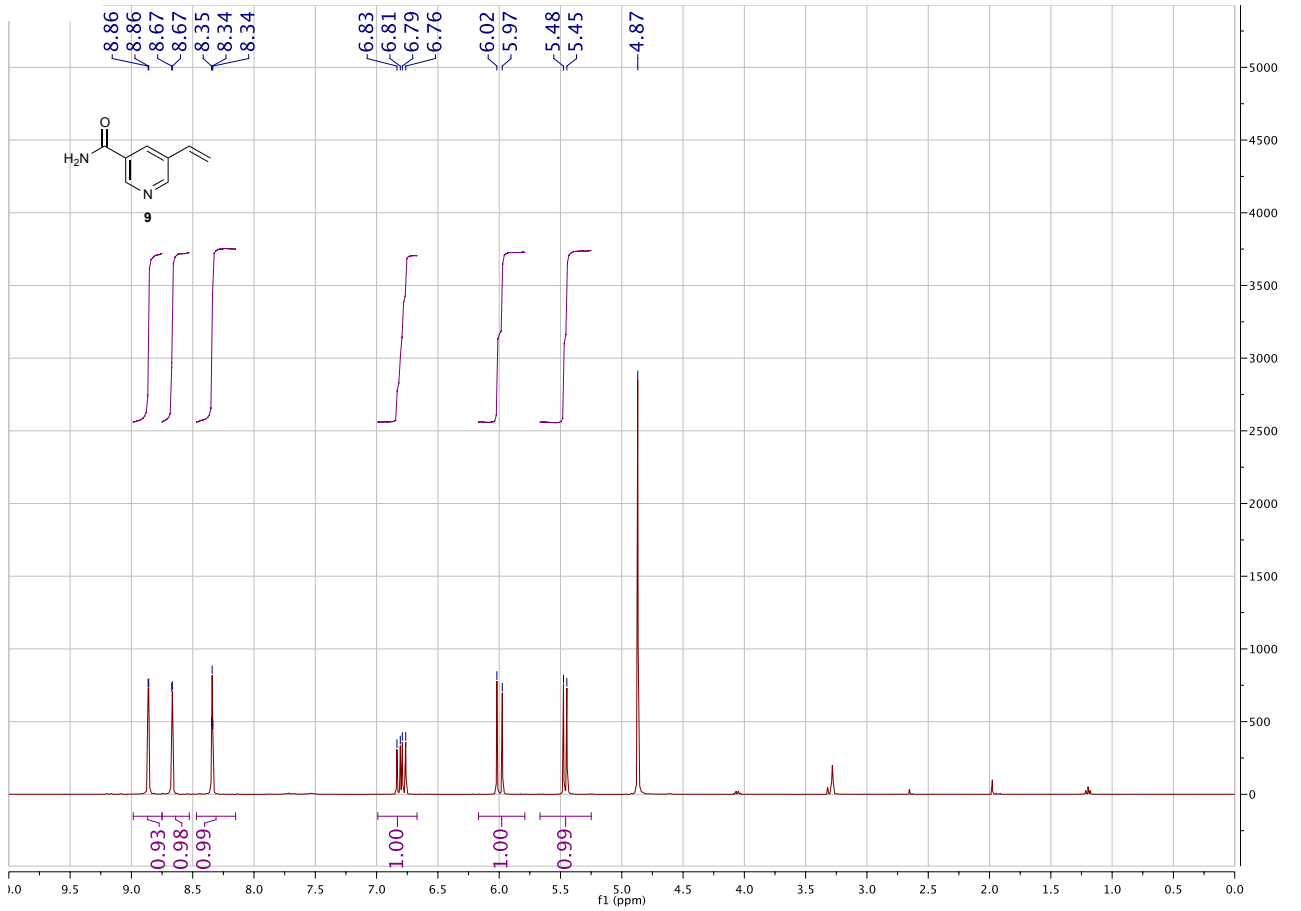
f1 (ppm)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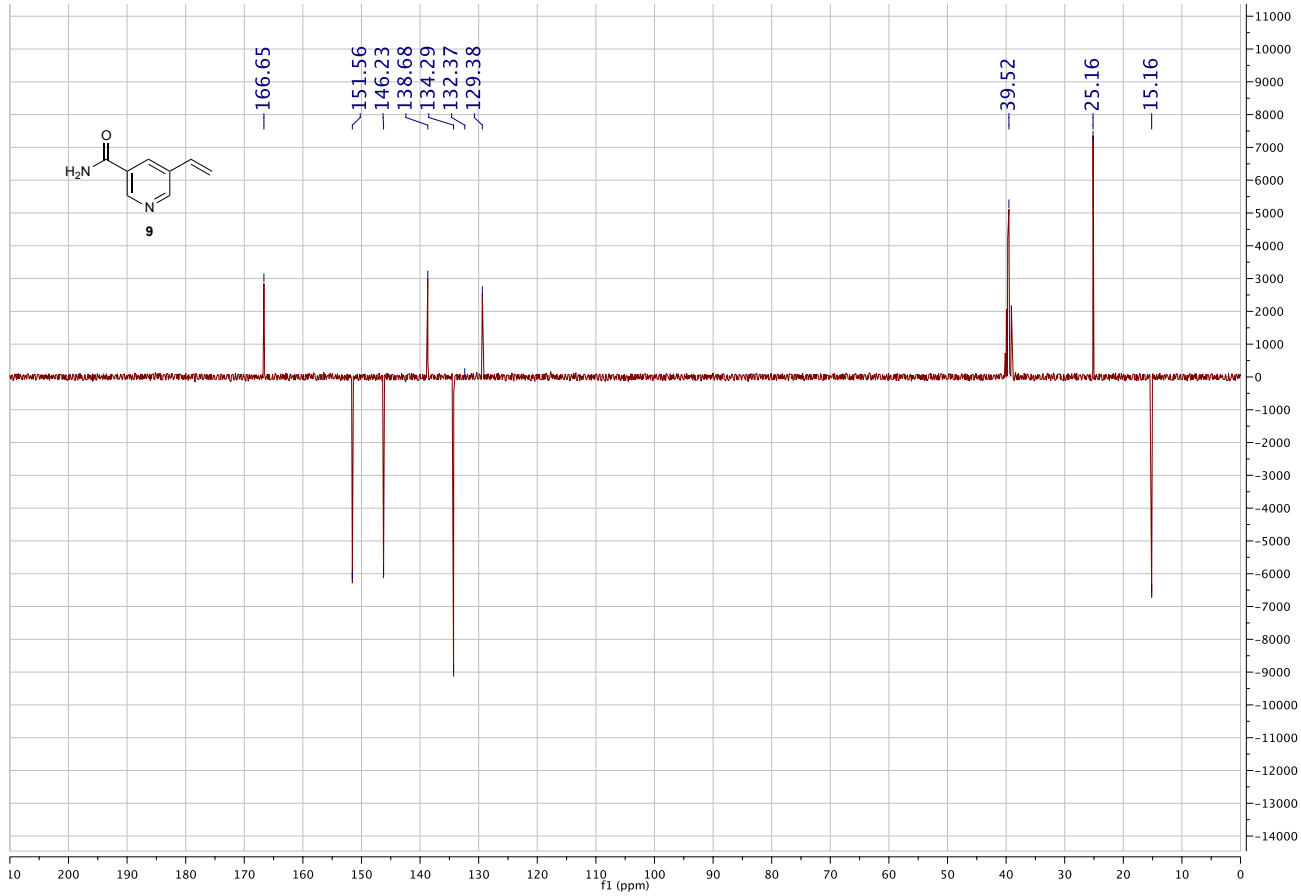
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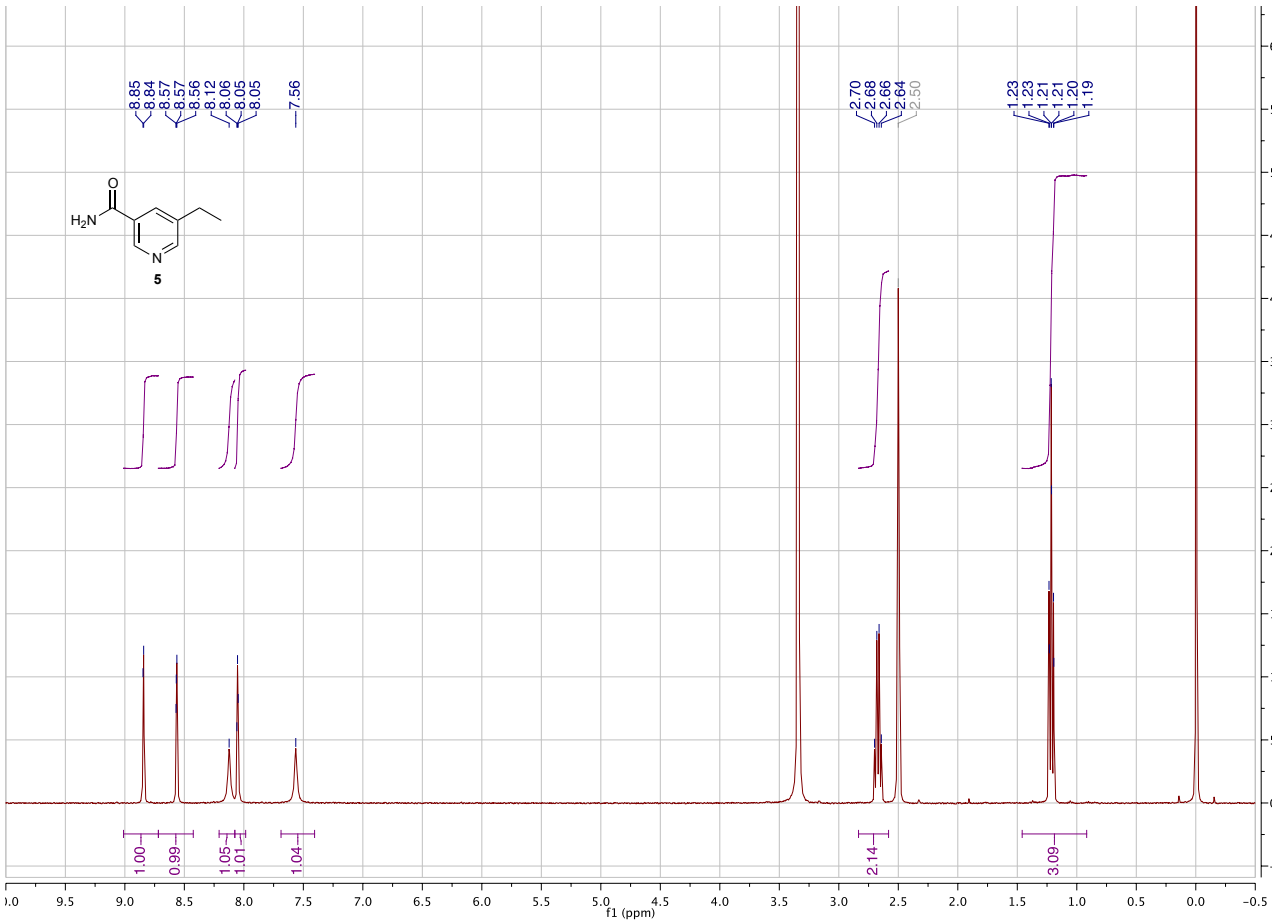
N
9
f1 (ppm)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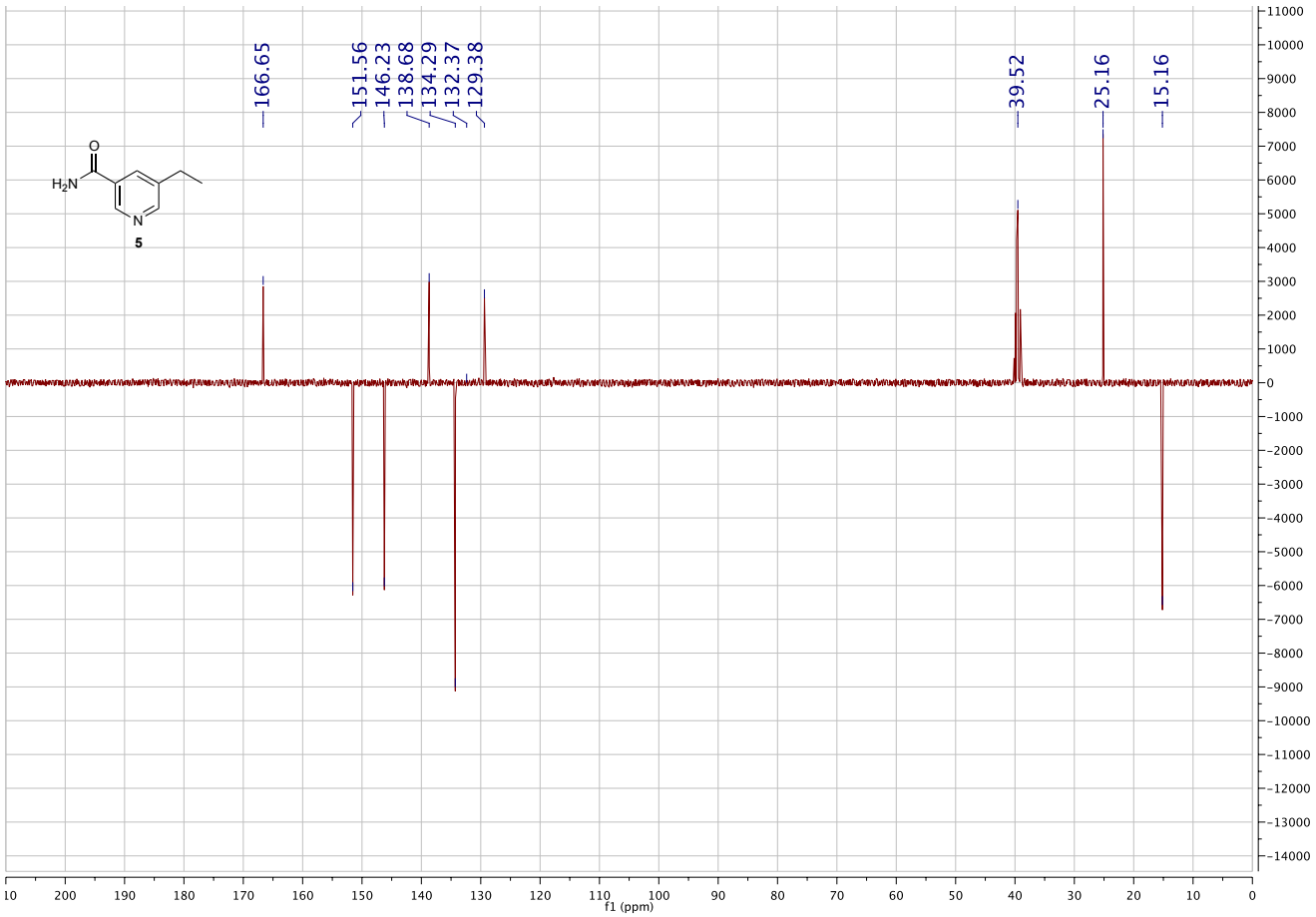
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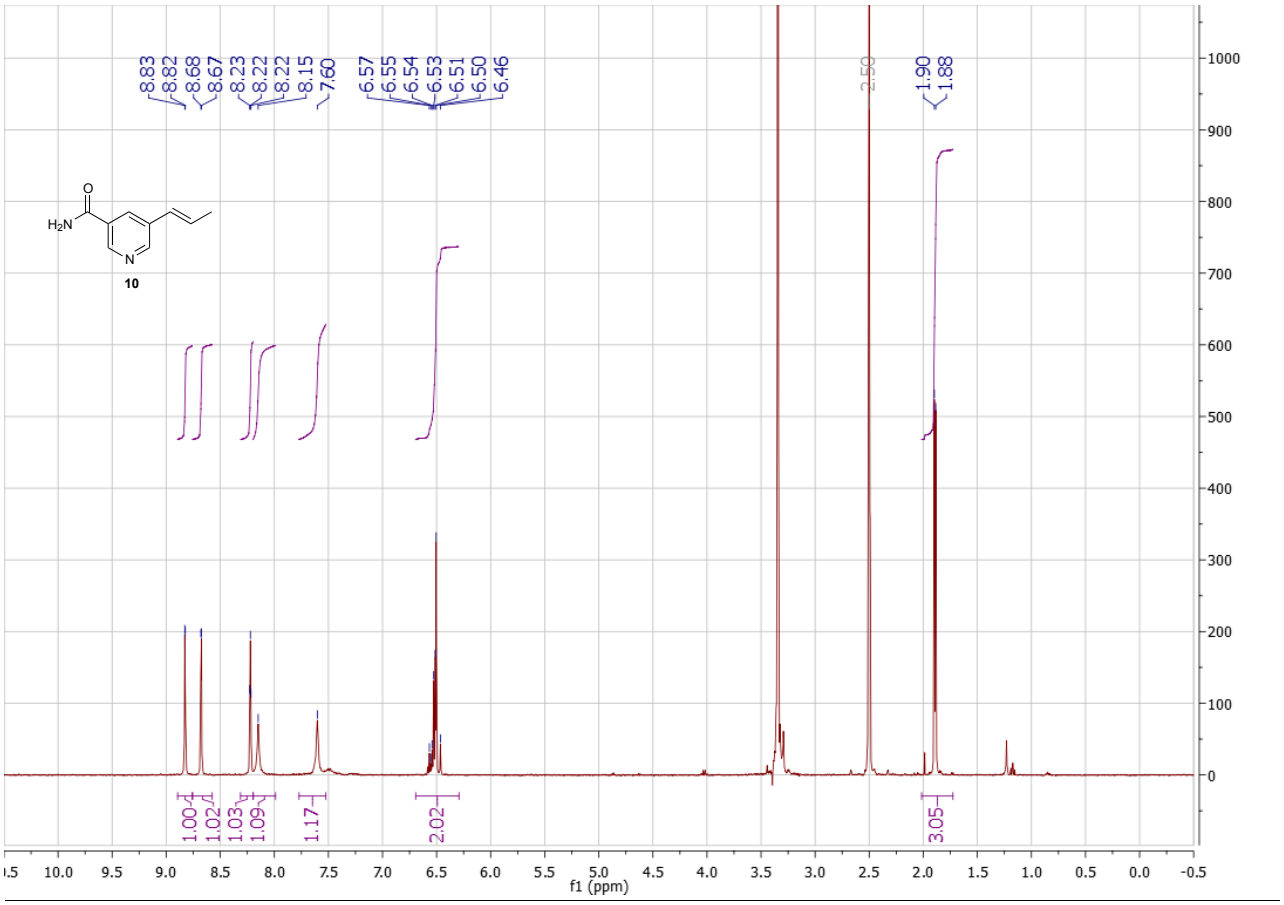
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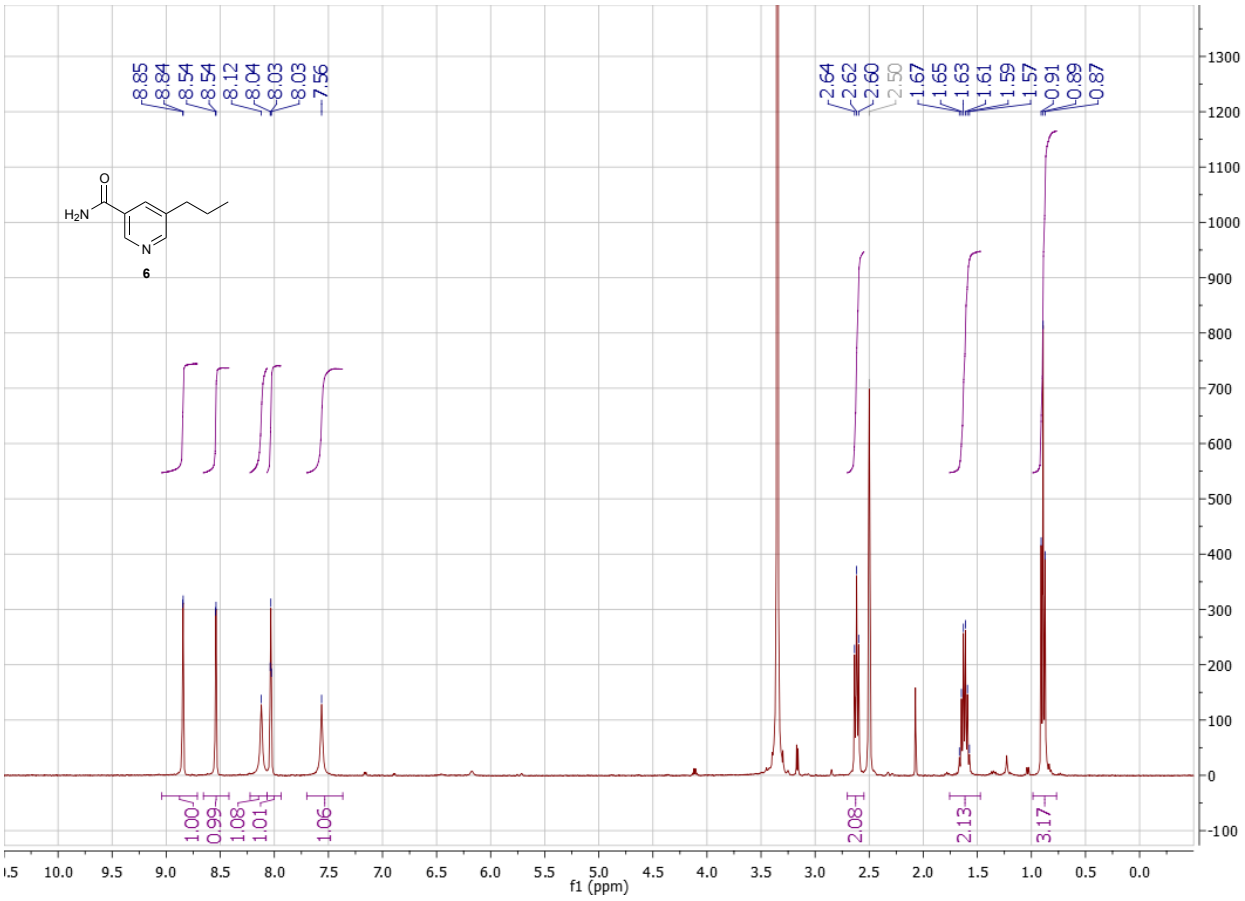
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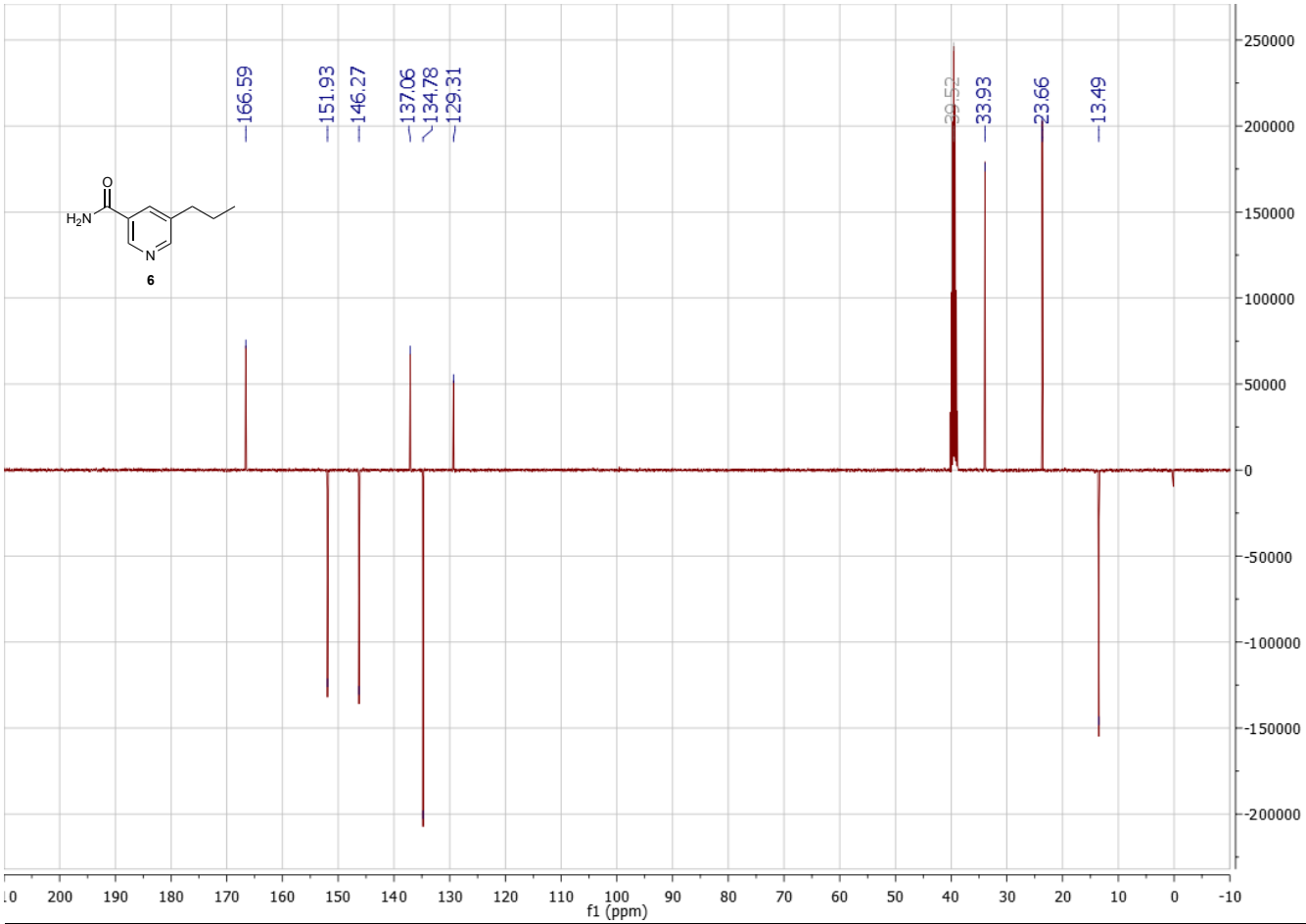
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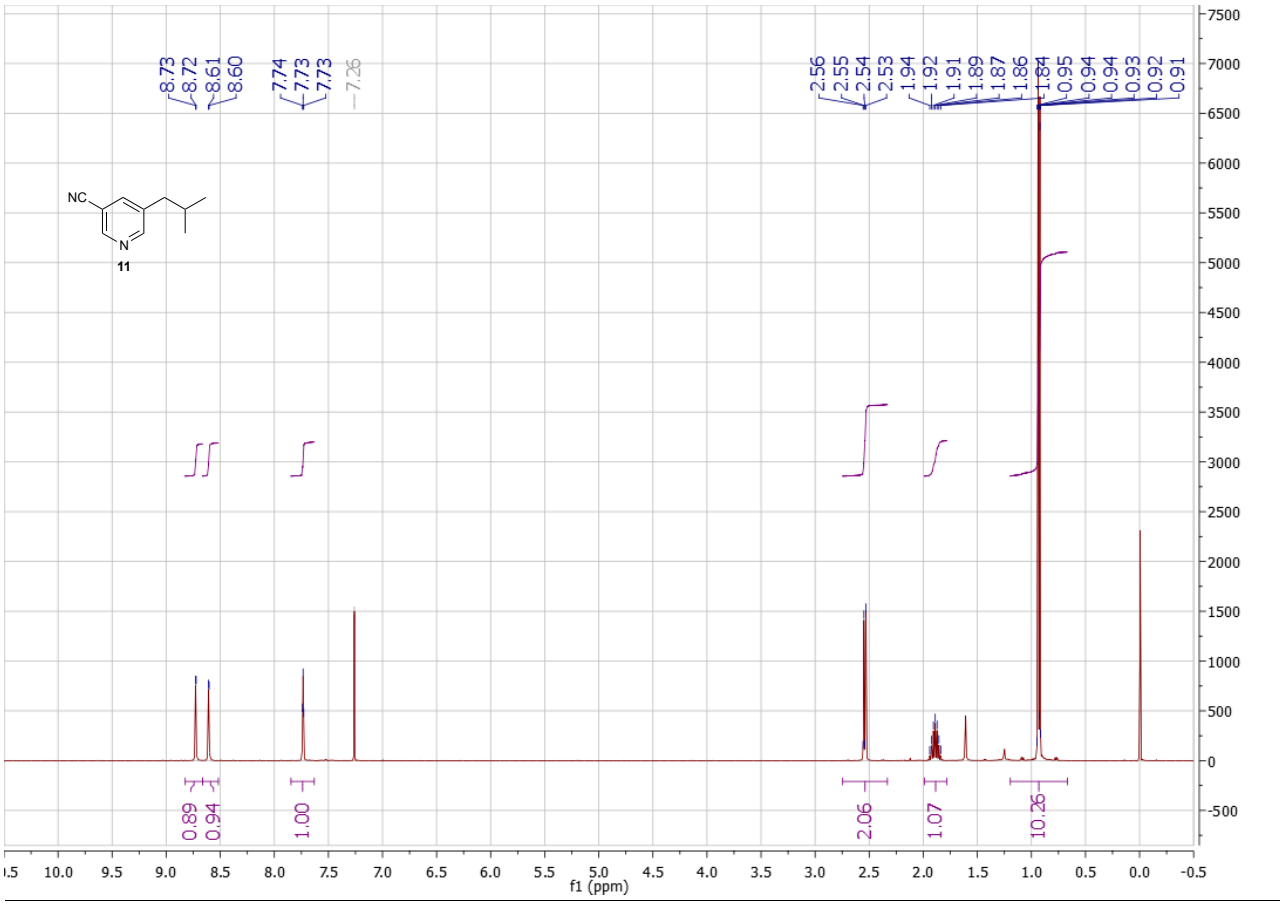
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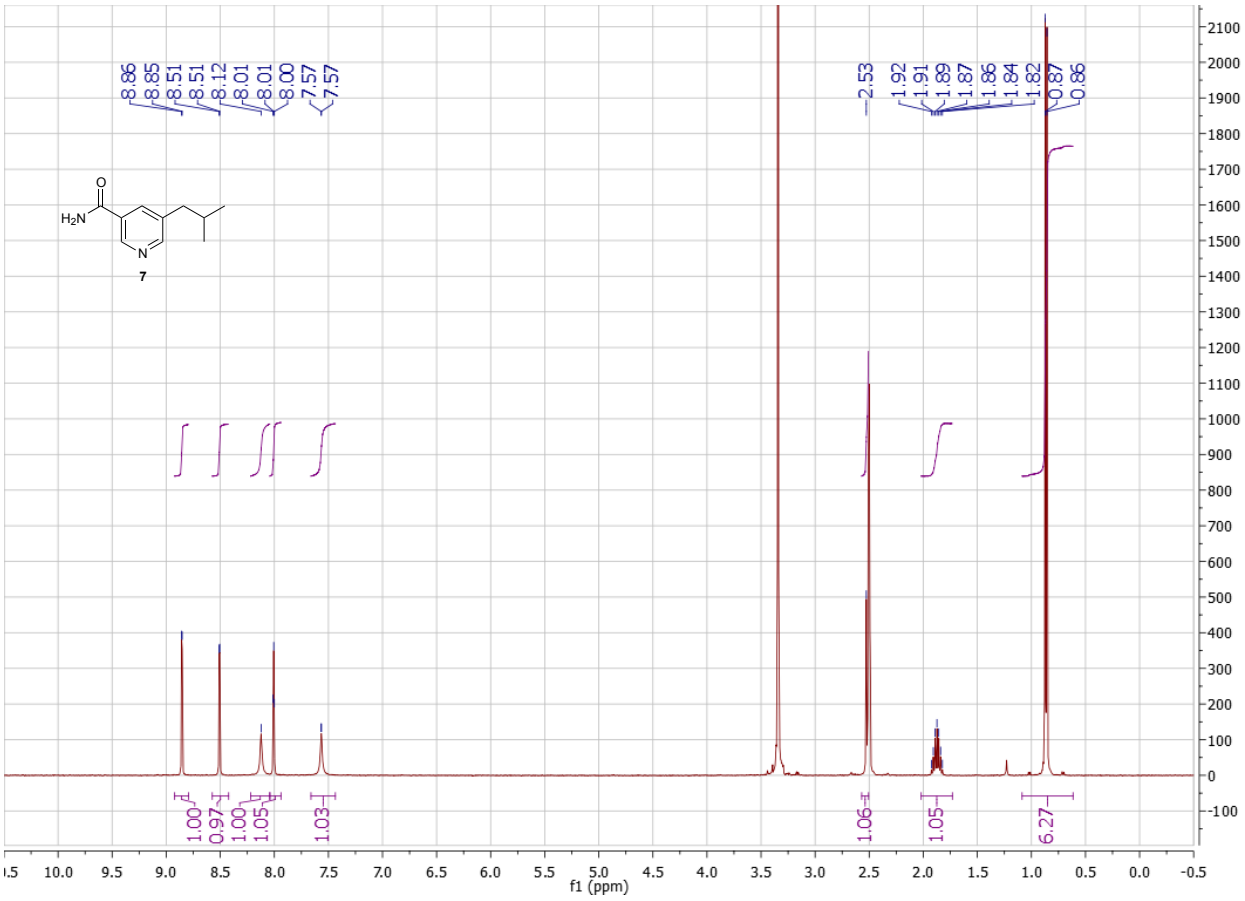
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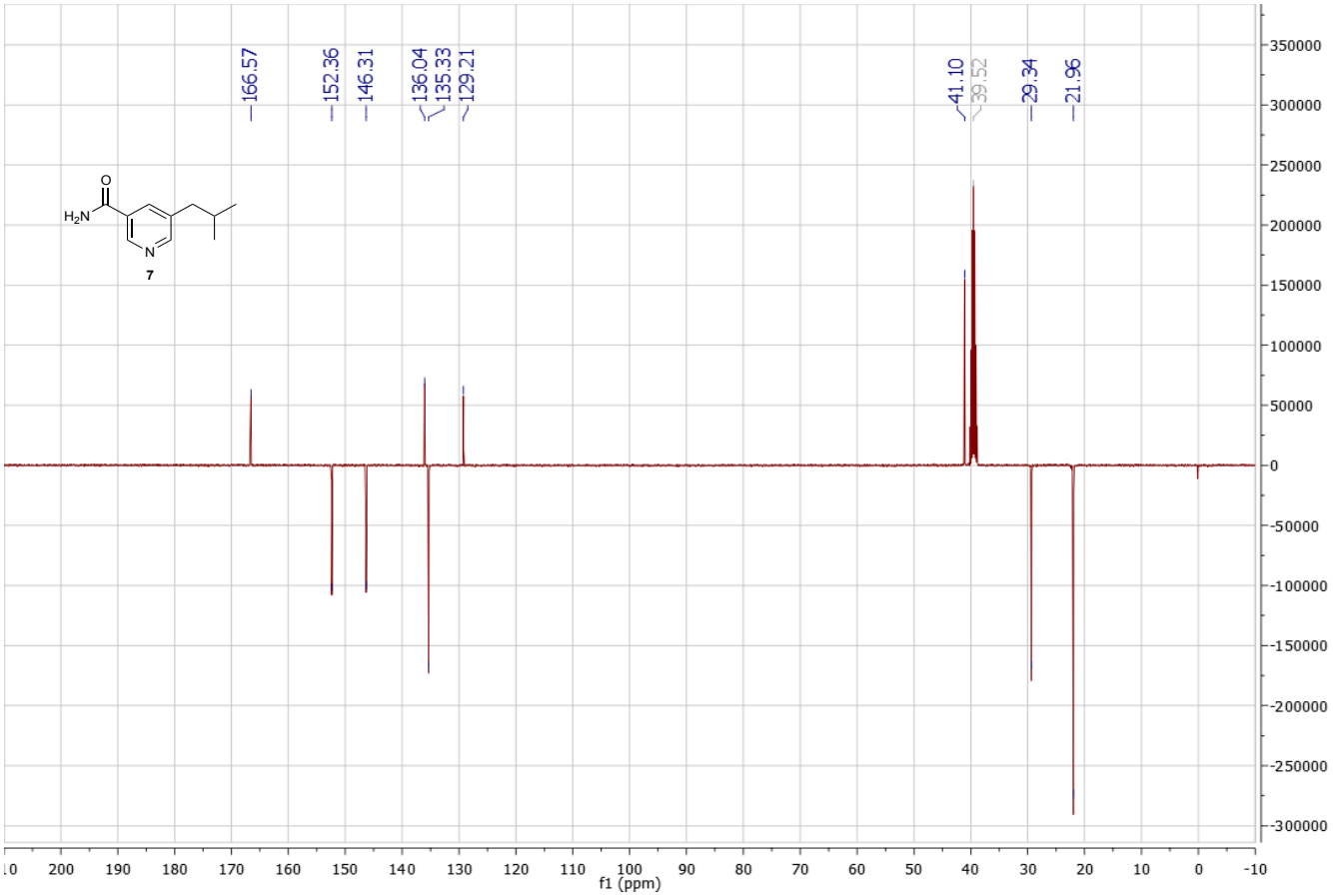
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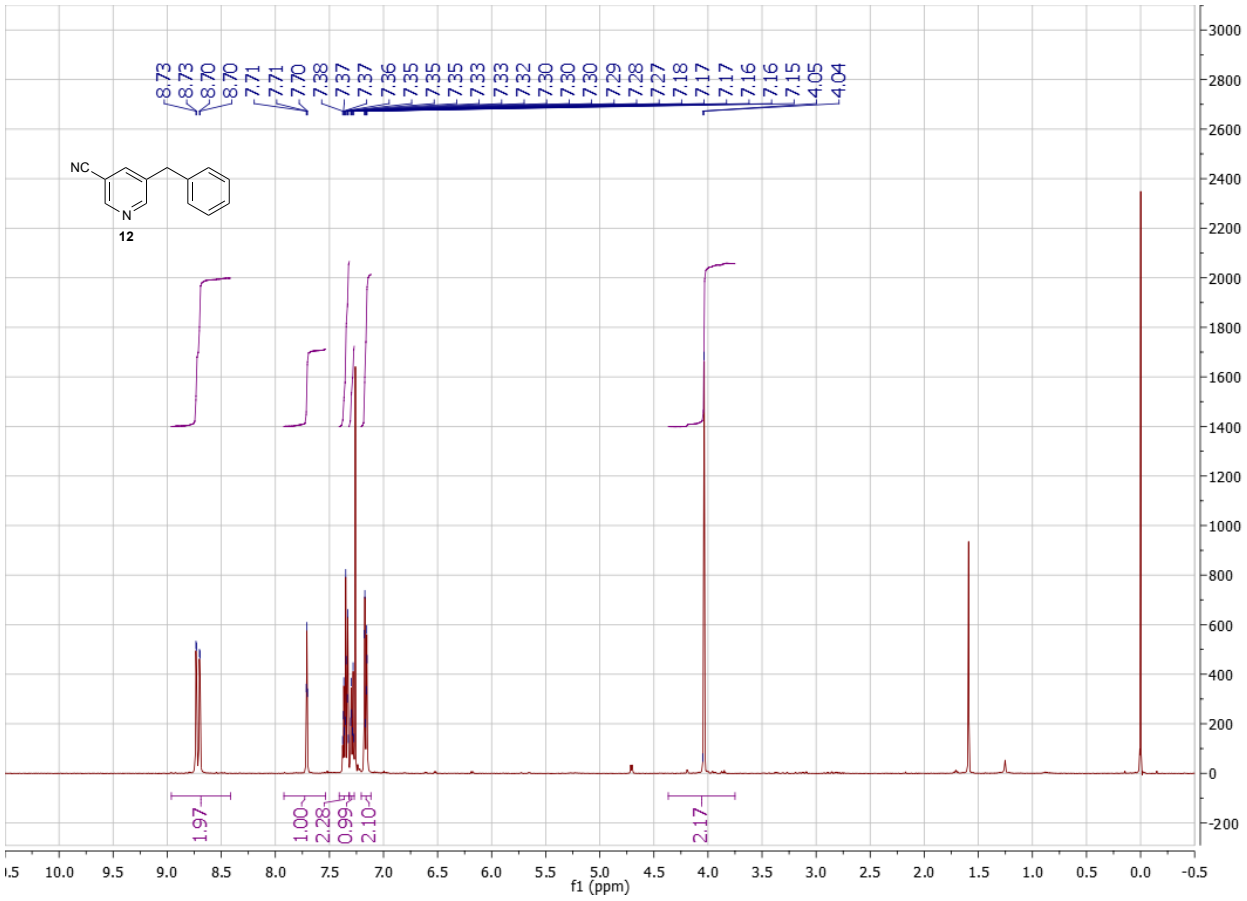
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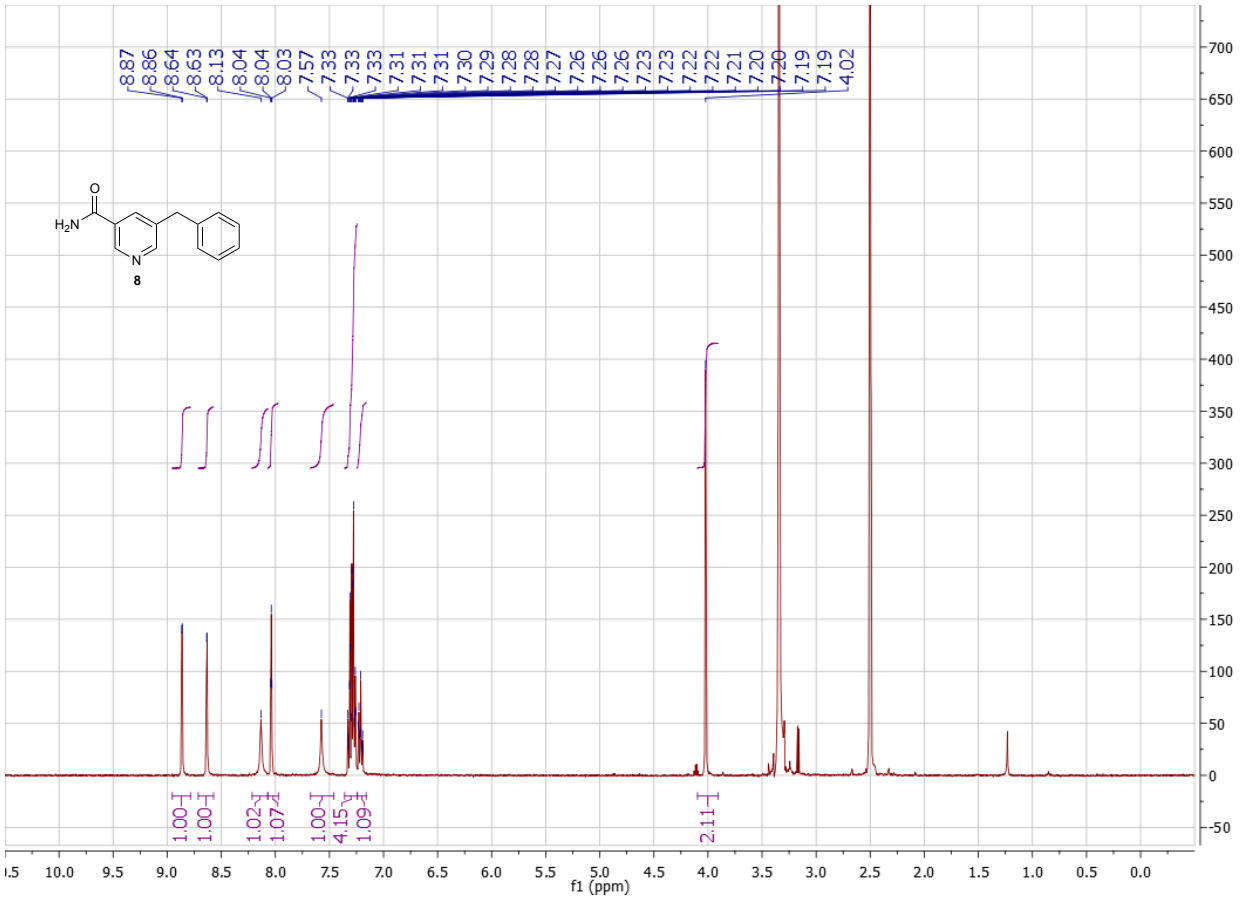
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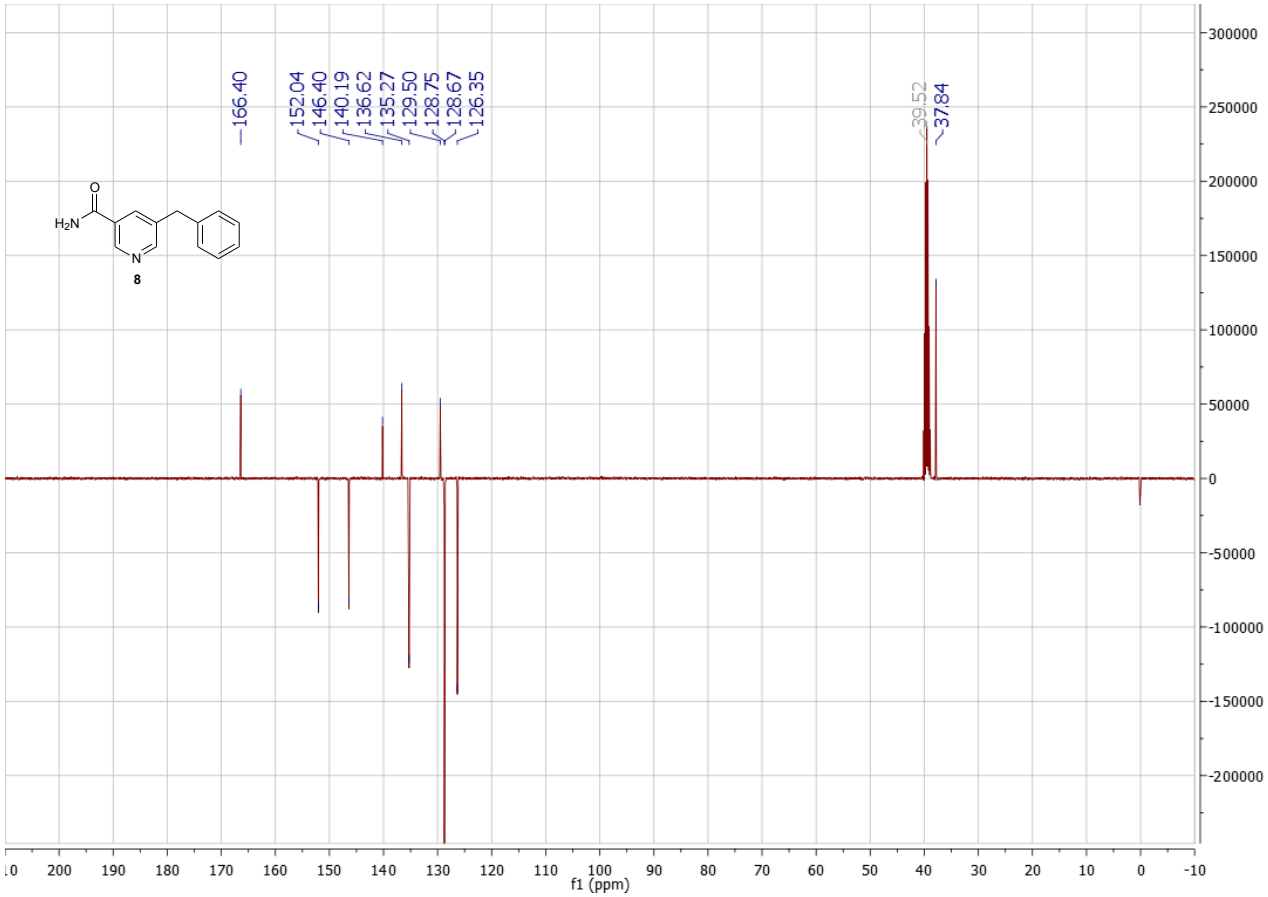
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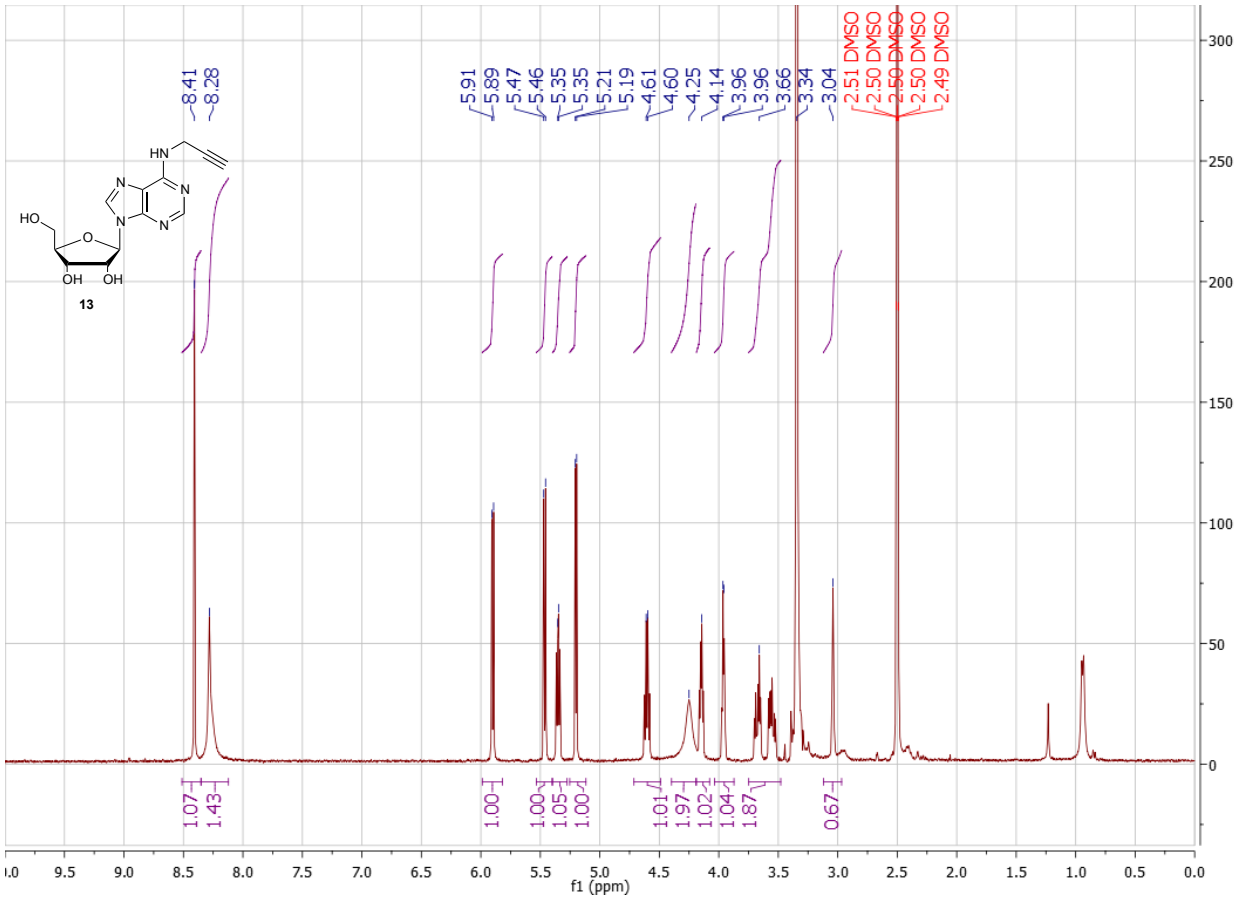
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O
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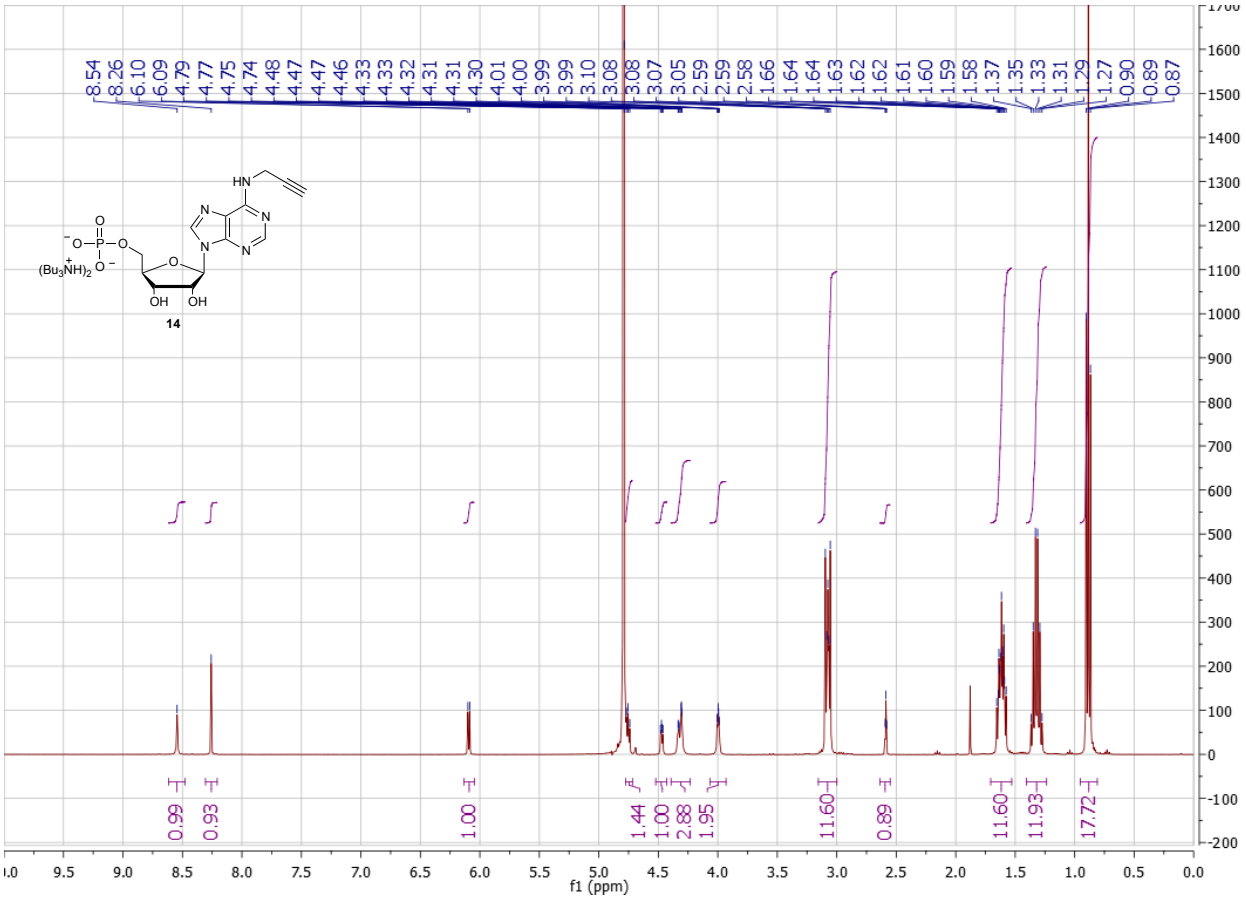
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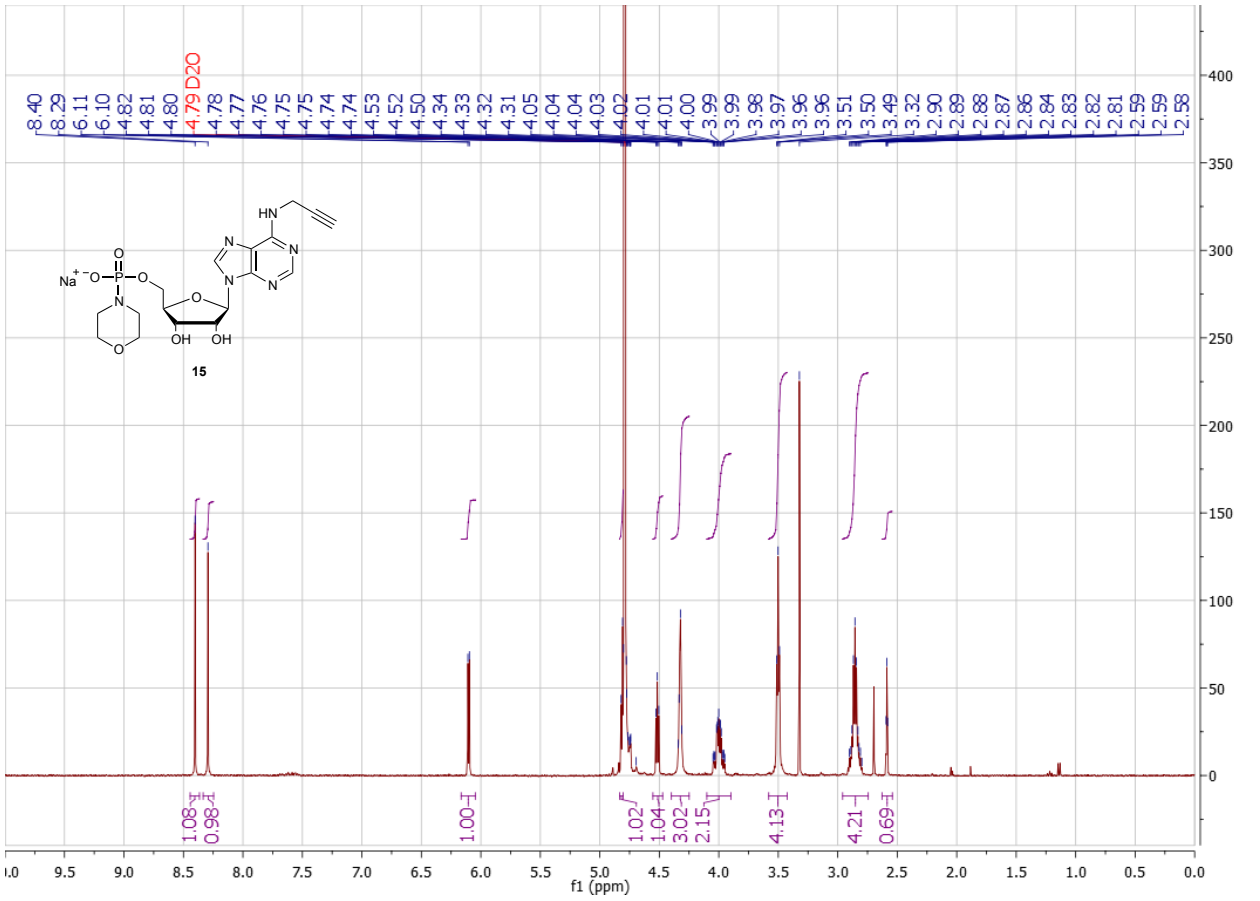
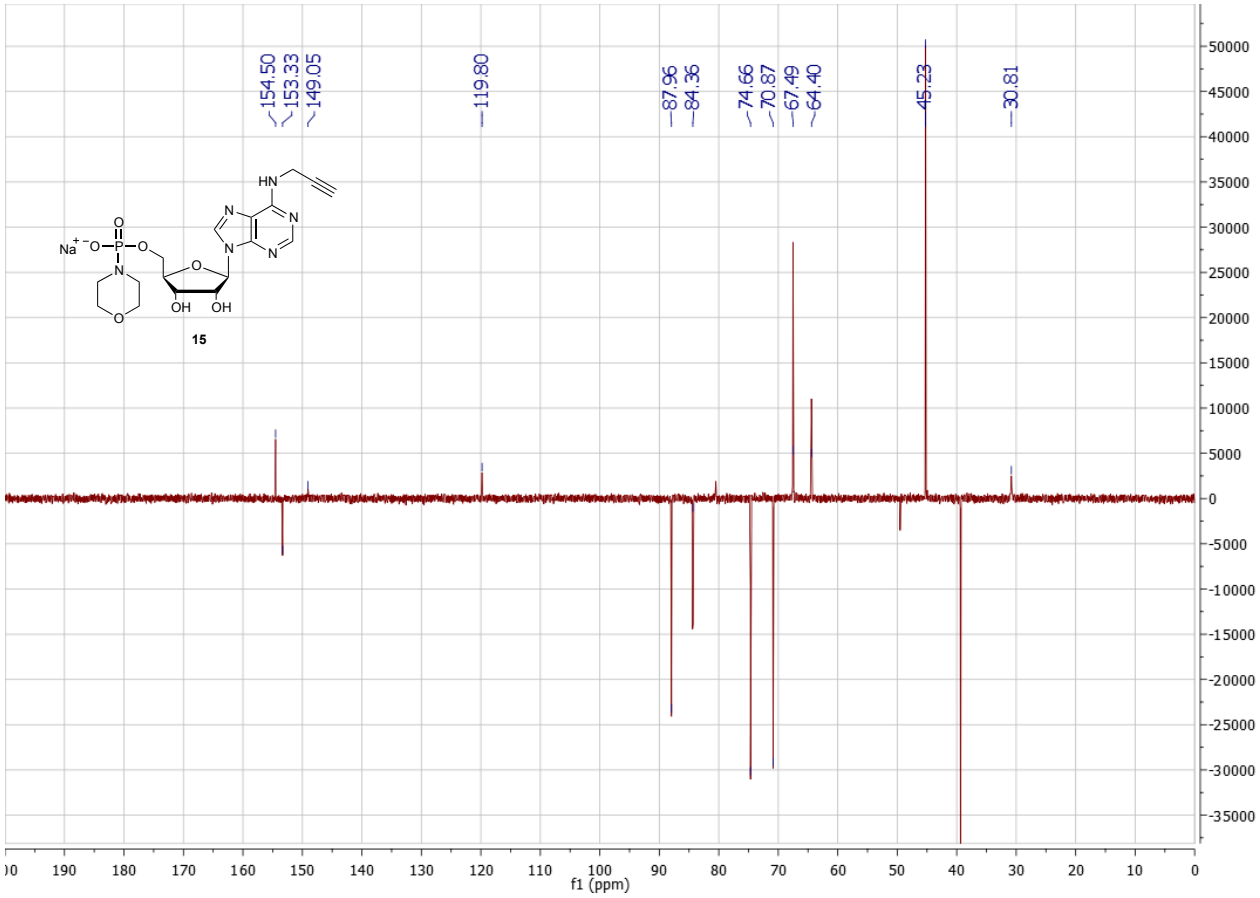
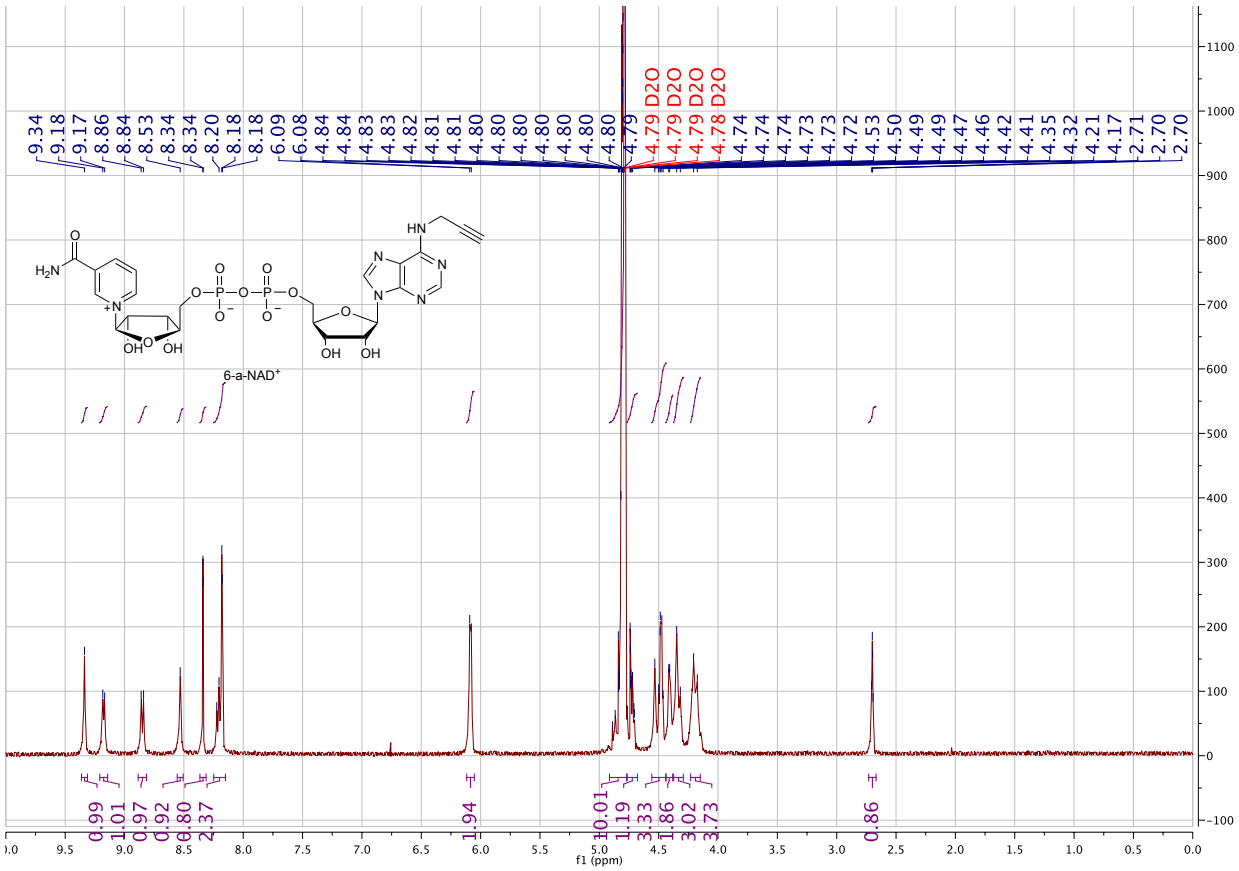
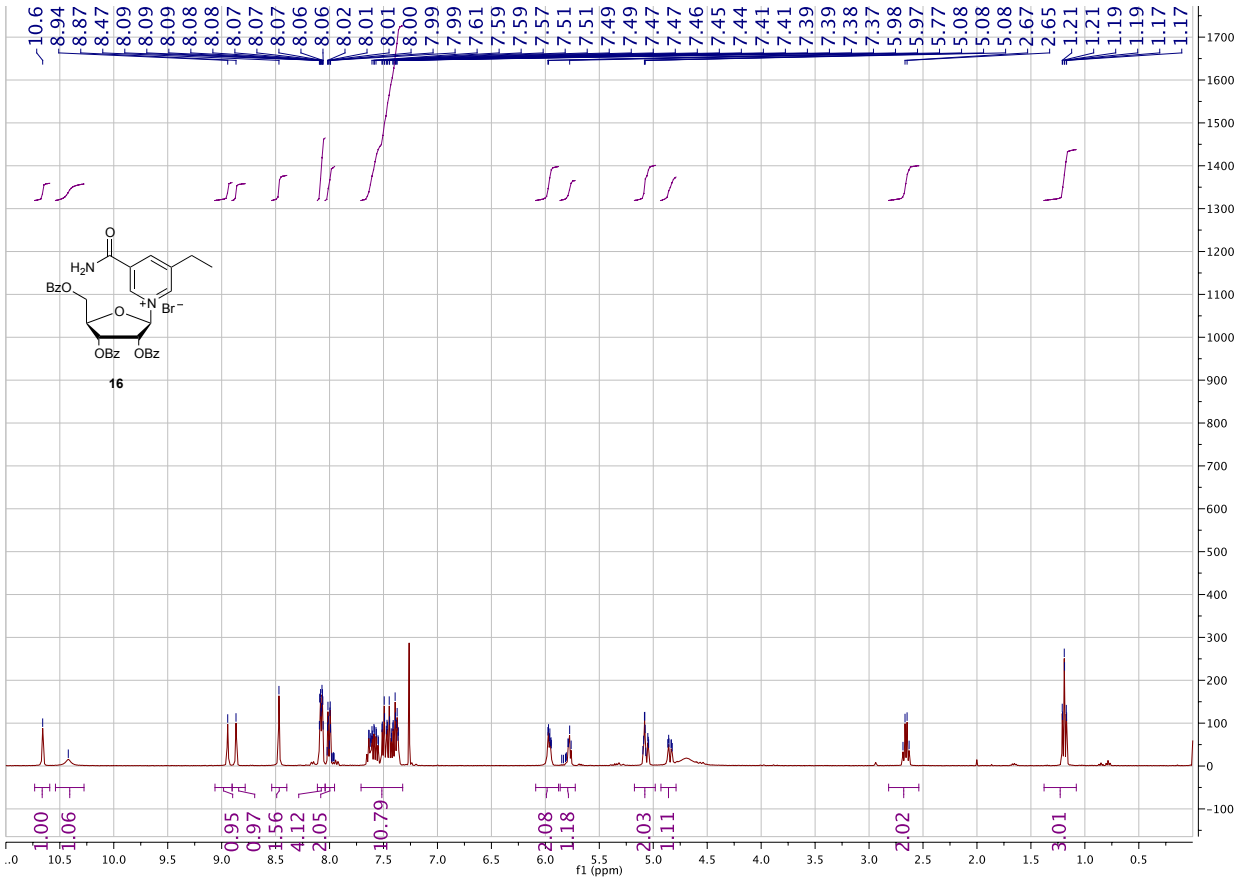
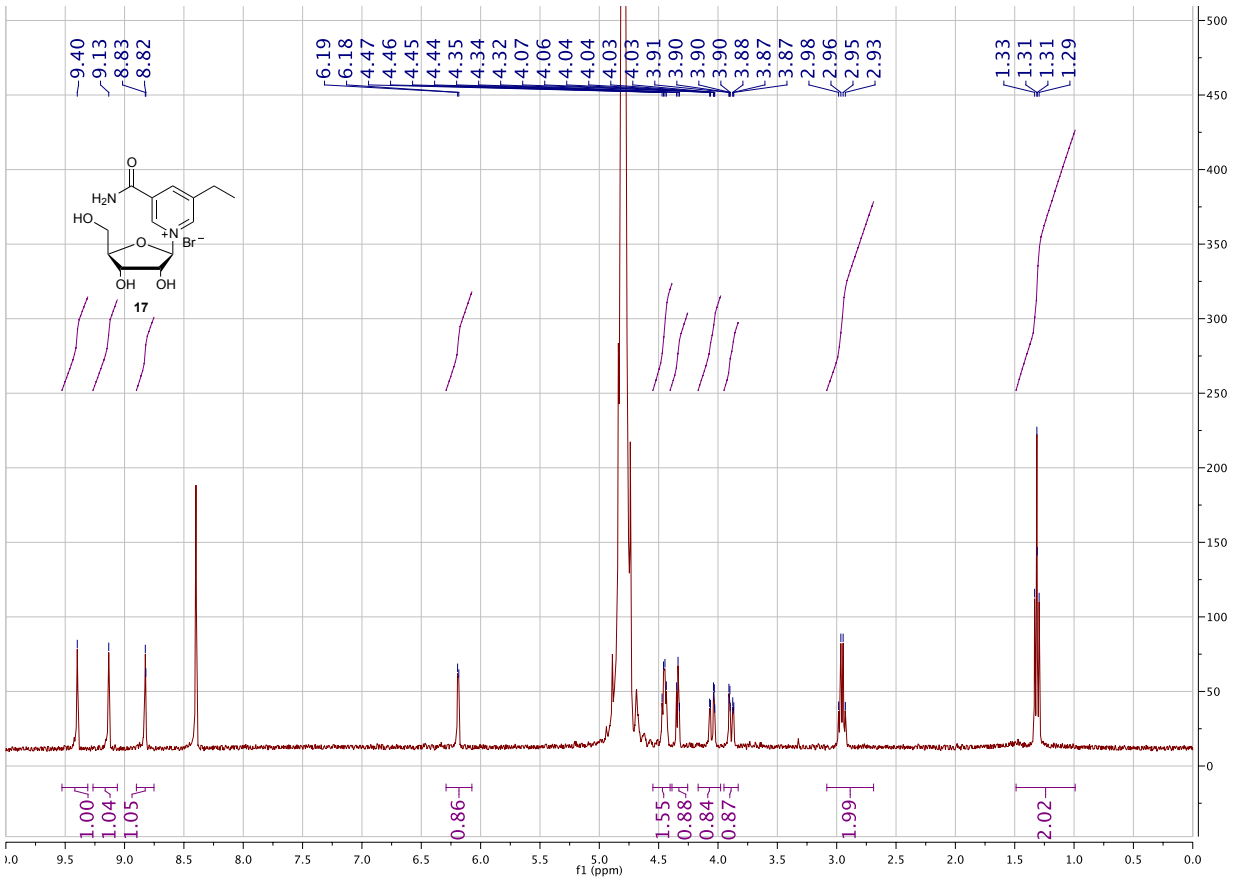
4.46
4.33
4.33
4.32
4.31
4.31
4.30
4.01
4.00
3.99
3.99
3.10
3.08
3.08
3.07
3.05
2.59
2.59
2.58
1.66
1.64
1.64
1.63
1.62
1.62
1.61
1.60
1.59
1.58
1.37
1.35
1.33
1.31
1.29
1.27
0.90
0.89
0.87
HN
N
N
N
N
O
O
O
O
O
P
(Bu3NH)2
OH
OH
14
0.99
0.93
1.00
1.44
1.00
2.88
1.95
11.60
0.89
11.60
11.93
17.72
f1 (ppm)

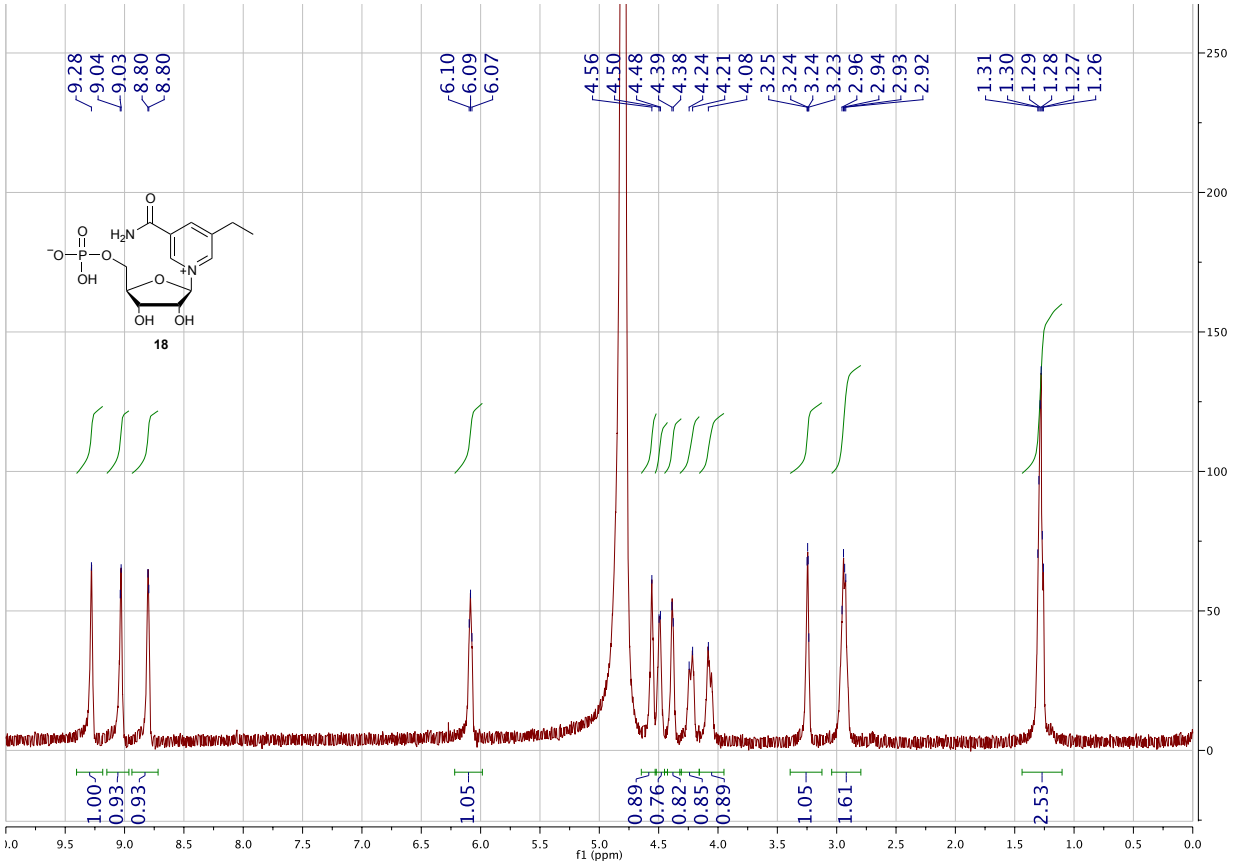

8.40
8.29
6.11
6.10
4.82
4.81
4.80
4.79 D2O
4.78
4.77
4.76
4.75
4.75
4.74
4.74
4.53
4.52
4.50
4.34
4.33
4.32
4.31
4.05
4.04
4.04
4.03
4.02
4.01
4.01
4.00
3.99
3.99
3.98
3.97
3.96
3.96
3.51
3.50
3.49
3.32
2.90
2.89
2.88
2.87
2.86
2.84
2.83
2.82
2.81
2.59
2.59
2.58
HN
N
N
N
N
Na+ -O
P
O
O
N
O
O
OH
OH
15
1.08
0.98
1.00
1.02
1.04
3.02
2.15
4.13
4.21
0.69
f1 (ppm)

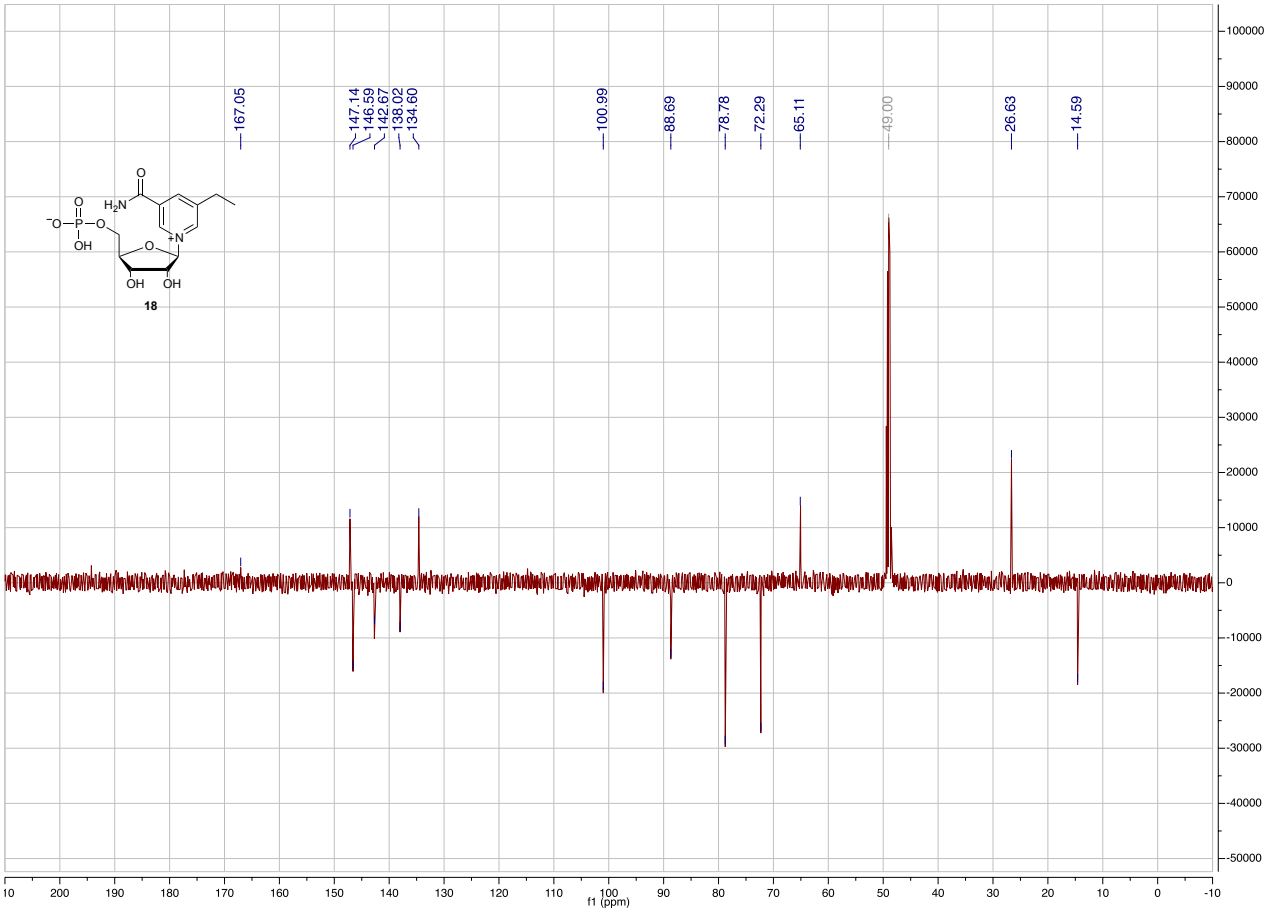

154.50
153.33
149.05
119.80
87.96
84.36
74.66
70.87
67.49
64.40
45.23
30.81
HN
N
N
N
N
Na+ -O
P
O
O
N
O
O
OH
OH
15
f1 (ppm)

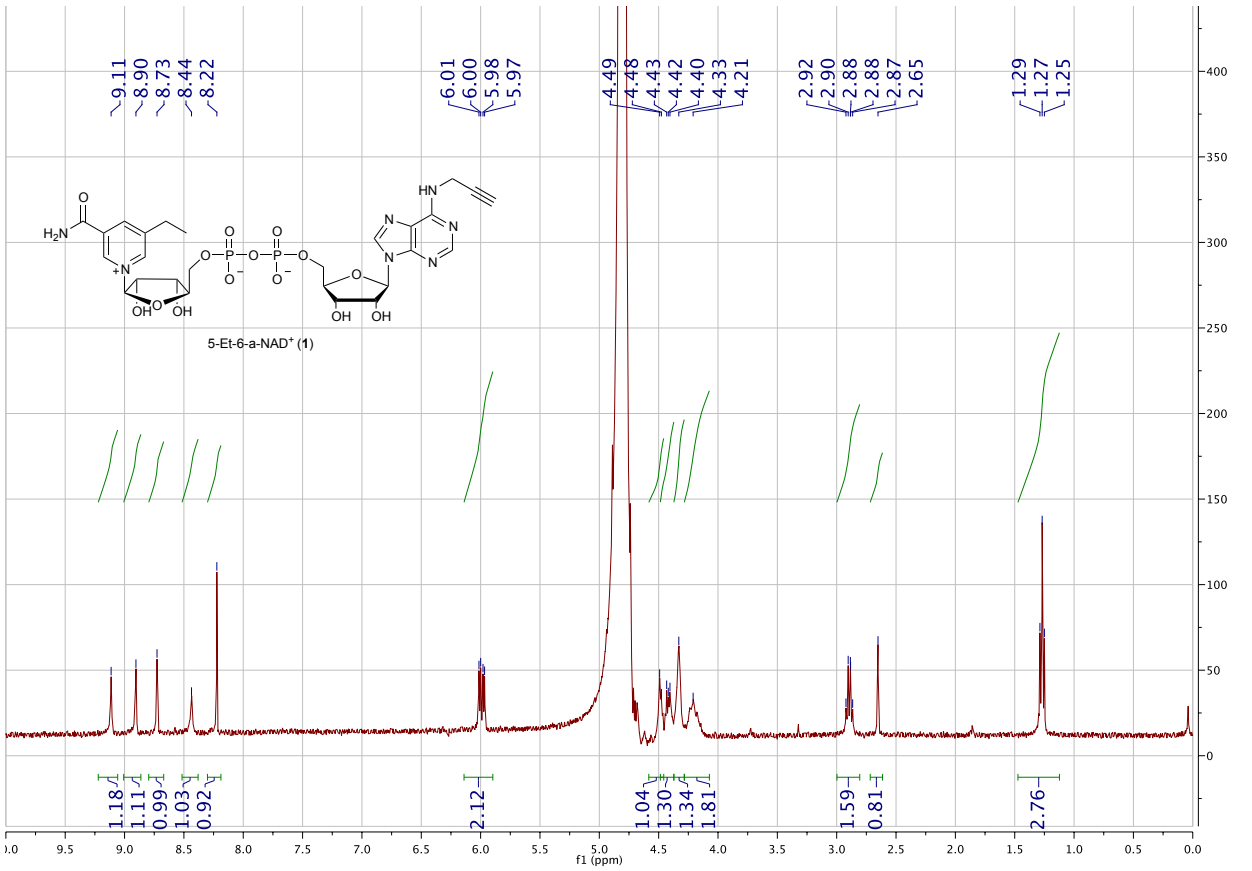

9.34 9.18 9.17 8.86 8.84 8.53 8.34 8.34 8.20 8.18 8.18 6.09 6.08 4.84 4.84 4.83 4.83 4.82 4.81 4.81 4.80 4.80 4.80 4.80 4.80 4.80 4.80 4.79 4.79 D2O 4.79 D2O 4.79 D2O 4.78 D2O 4.74 4.74 4.74 4.73 4.73 4.72 4.53 4.50 4.49 4.49 4.47 4.46 4.42 4.41 4.35 4.32 4.21 4.17 2.71 2.70 2.70
6-a-NAD+
0.99 1.01 0.97 0.92 0.80 2.37 1.94 10.01 1.19 3.33 1.86 3.02 3.73 0.86
f1 (ppm)


10.6
8.94
8.87
8.47
8.09
8.09
8.09
8.08
8.08
8.07
8.07
8.07
8.06
8.06
8.02
8.01
8.01
8.00
7.99
7.99
7.61
7.59
7.59
7.57
7.51
7.51
7.49
7.49
7.47
7.47
7.46
7.45
7.44
7.41
7.41
7.39
7.39
7.38
7.37
5.98
5.97
5.77
5.08
5.08
5.08
2.67
2.65
1.21
1.21
1.19
1.19
1.17
1.17
O
H2N
BzO
O
+N
Br−
OBz
OBz
16
1.00
1.06
0.95
0.97
1.56
4.12
2.05
10.79
2.08
1.18
2.03
1.11
2.02
3.01
f1 (ppm)

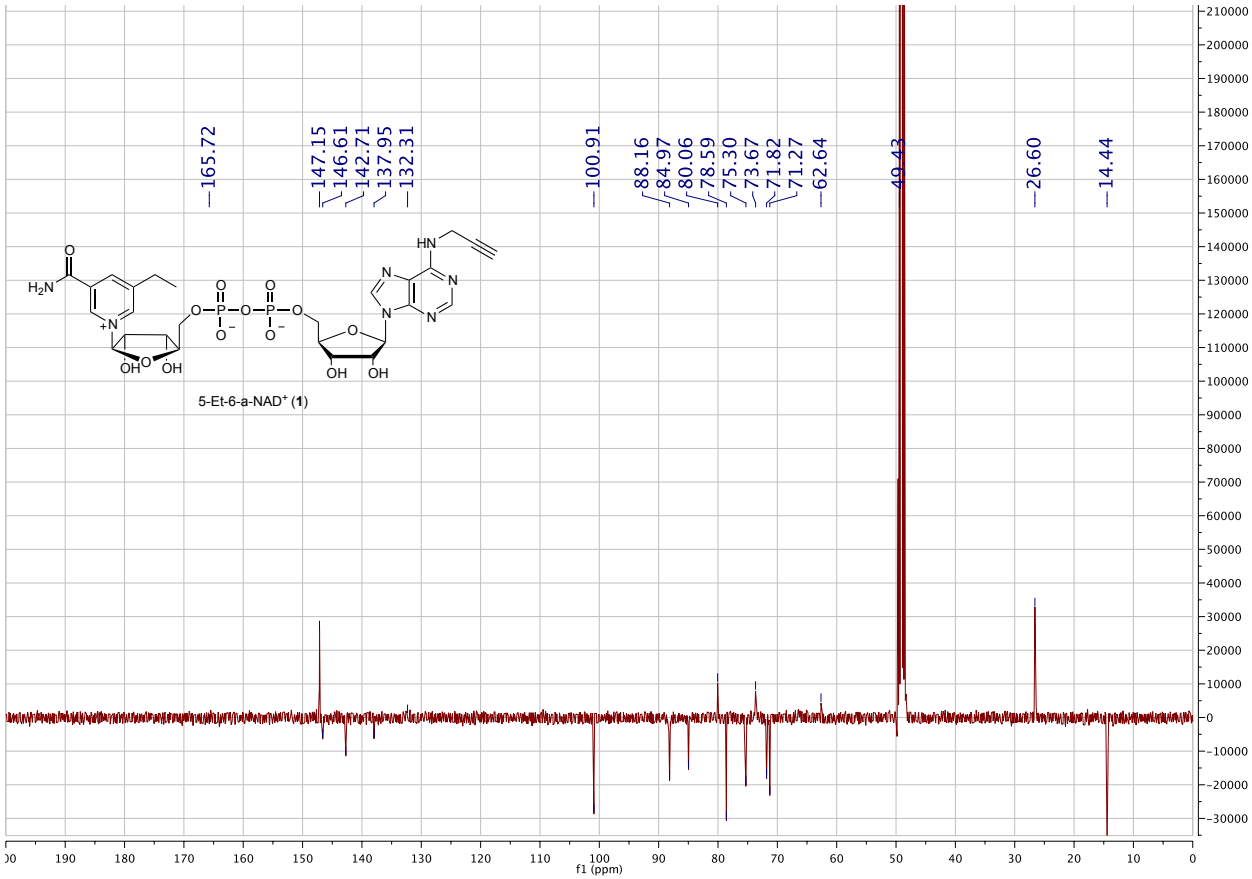

9.40
9.13
8.83
8.82
6.19
6.18
4.47
4.46
4.45
4.44
4.35
4.34
4.32
4.07
4.06
4.04
4.04
4.03
4.03
3.91
3.90
3.90
3.90
3.88
3.87
3.87
2.98
2.96
2.95
2.93
1.33
1.31
1.31
1.29
O
H2N
HO
O
+N
Br−
OH
OH
17
1.00
1.04
1.05
0.86
1.55
0.88
0.84
0.87
1.99
2.02
f1 (ppm)


9.28
9.04
9.03
8.80
8.80
6.10
6.09
6.07
4.56
4.50
4.48
4.39
4.38
4.24
4.21
4.08
3.25
3.24
3.24
3.23
2.96
2.94
2.93
2.92
1.31
1.30
1.29
1.28
1.27
1.26
1.00
0.93
0.93
1.05
0.89
0.76
0.82
0.85
0.89
1.05
1.61
2.53
f1 (ppm)
18


167.05
147.14
146.59
142.67
138.02
134.60
100.99
88.69
78.78
72.29
65.11
49.00
26.63
14.59
f1 (ppm)
18



5-Et-6-a-NAD$^+$ (**1**)



5-Et-6-a-NAD$^+$ (**1**)

V. Supplemental References

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