FSupplementary Information (SI)

Optimized Aqueous Kinugasa Reactions for Bioorthogonal Chemistry Applications

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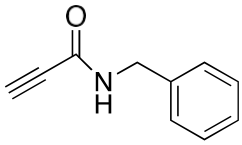
Materials and Synthetic Methods

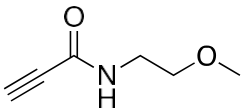
All reagents and solvents were purchased from Sigma-Aldrich, unless otherwise stated, and used without further purification. Deuterated solvents were purchased from Cambridge Isotope laboratories. Thin layer chromatography was performed on SiliCycle Siliaplate® silica gel plates (60 Å F254, layer thickness 200µm). Flash chromatography was performed using silica gel (60 Å, particle size 40–63 µm). Fluorescence microscopy was performed with a Nikon Ni-U ratiometric fluorescence microscope equipped with a LED excitation light source and Ultra-sensitive Andor iXon Ultra 897 cooled EMCCD camera. Images were acquired using a Nikon 60x oil dip objective lens and, if indicated, a 2x relay lens. Fluorescence images were obtained under strictly identical conditions of gain and exposure time, on focused beads, typically 2-5 s, and brightfield images were obtained using a 20-50 ms exposure. Images were ac- quired using Nikon NIS Elements software and processed with ImageJ. All ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 300 or 400 spectrometer using a frequency of 300 MHz or 400 MHz for ^{1}H and 100 MHz for ^{13}C and processed using iNMR 4.2.0 software. The following abbreviations were used to designate chemical shift multiplici- ties: s = singlet, d = doublet, t = triplet, m = multiplet or unresolved, br = broad signal and *J* = coupling constants in Hz.

Synthetic Methods

Synthesis of Propiolamides



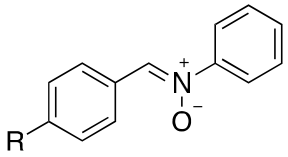
***N*-Benzylprop-2-ynamide.** 0.3 g of propiolic acid was added to 5mL DMF in a 25mL round bottom flask equipped with a stir bar. 1.71 g HATU, 0.49 mL benzylamine were added successively to reaction mixture. Lastly, 0.82 mL DIPEA was added and the reaction was let stir for 1 hour at room temperature. Following reaction completion by TLC, the reaction was concentrated in vacuo. Reaction contents were dissolved in 20 mL of ethyl acetate and washed with 10 mL 70% brine 3 times. 60% yield of pure product was recovered following column chromatography using 1:1 EtOAc: Hexanes. **^{1}H NMR** (300 MHz; $CDCl_3$): δ 7.37-7.30 (m, 5H), 6.10 (s, 1H), 4.50 (d, *J* = 5.9 Hz, 2H), 2.80 (s, 1H). Spectral data was consistent with previously reported data.[1]



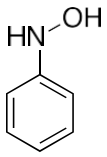
***N*-(2-methoxyethyl)prop-2-ynamide.** Under an Argon atmosphere, propiolic acid (0.3 g, 4.28 mmol) was dissolved in 10 mL of dry DMF, followed by the addition of HATU (1.71g, 4.50 mmol). 2-Methoxyethylamine (391 µL, 4.50 mmol) was slowly added over 15 minutes, followed by the addition of DIPEA (819 µL). The reaction was allowed to stir at room temperature for 6 hours, after which 10 mL of EtOAc was added. The mixture was extracted three times with 10 mL of a 50% brine solution. The organic layer was recovered, dried over Na_2SO_4, filtered and concentrated. Pure product (0.24 g) was obtained as a light yellow solid in 44% yield following flash column chromatography using 3:97 MeOH:DCM. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 6.26 (s, 1H), 3.52-3.46 (m, 4H), 3.37 (s, 3H), 2.79 (s, 1H); **^{13}C NMR** ($CDCl_3$, 100MHz) δ= 152.1, 73.2, 70.5, 58.8, 39.5. **HRMS:** for $C_6H_9NO_2$ (M+H): calculated: 128.0633; found: 128.0693.

Synthesis N-Phenyl-Nitrone Derivatives

General Nitrone Synthesis A

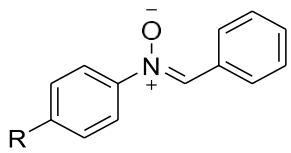


300 mg (2.8 mmol) of phenylhydroxylamine and 3 mmol of *para*-substituted benzaldehyde were added to a dry 25 mL round-bottom flask. 5 mL of dry ethanol was added to the flask and the mixture was stirred for 2 hours at 35 °C. Pure products were obtained following filtration of the precipitated crude product and recrystallization from warm ethanol and hexanes.

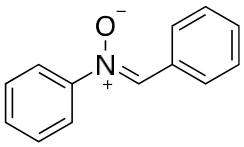


N-Phenylhydroxylamine. A mixture of nitrobenzene (10.8 mL, 0.105 mol), NH_4Cl (6.5 g, 0.12 mol) and degassed H_2O (200 mL) under argon at r.t. was stirred vigorously while zinc dust (15.4 g, 0.21 mol) was added portion wise over 20 minutes. After addition was complete, the reaction mixture was stirred for an additional 20 minutes and was filtered while still warm. The resultant filter cake was washed with hot distilled water (50 mL) and the com- bined filtrate was saturated with NaCl, and extracted with 3x100 mL of EtOAc. The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude N-phenyl hydroxylamine was recrystal- lized from petroleum ether/EtOAc (8.2 g, 72 %), dried thoroughly and stored under an atmosphere of argon at - 20°C.[1] HNMR and MS data are in agreement with that reported previously.[2] **^{1}H NMR** (300MHz; $CDCl_3$): δ 7.32- 7.26 (m, 2H), 7.02-6.97 (m, 3H), 6.77 (dq, *J* = 2.5, 0.8 Hz, 1H).

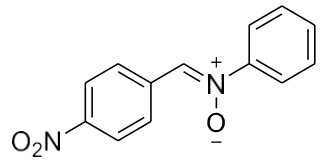
General Nitrone Synthesis B



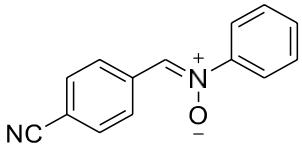
16 mmol of *para*-substituted nitrobenzene and 1.7 g (16 mmol) of benzaldehyde were added to a dry 100 mL round- bottom flask. 29 mL of EtOH and 29 mL of H_2O were added, followed by 0.9 g (18 mmol) of NH_4Cl. The reaction mixture was cooled to 0°C, and 1.9 g of Zn power (29 mmol) was added over the course of 20 minutes. The reaction was allowed to warm to room temperature and stirred for 16 hours. The reaction was then filtered through Celite, and washed 3 times with60 mL DCM. The organic layers were combined, dried over Na_2SO_4, filtered, and concen- trated. Pure product was obtained following recrystallization from warm EtOAc and Hexanes.[3]



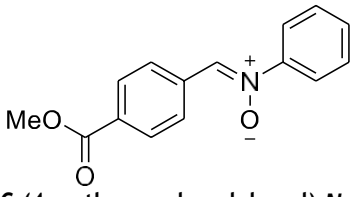
C,N-diphenylnitrone. The nitrone was synthesized according to General Nitrone Synthesis B. The product was ob- tained in 50% yield. **^{1}H NMR** (300 MHz; $CDCl_3$): δ 8.42-8.39 (m, 2H), 7.93 (s, 1H), 7.80-7.77 (m, 2H), 7.50-7.48 (m, 6H). Spectral data was consistent with previously reported data.[4]



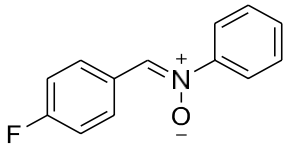
C-(4-nitrophenyl)-N-phenylnitrone. The nitrone was synthesized according to General Nitrone Synthesis A. The product was obtained in 75% yield. **^{1}H NMR** (300 MHz; $CDCl_3$): δ 8.58-8.53 (m, 2H), 8.34-8.30 (m, 2H), 8.07 (s, 1H), 7.80-7.77 (m, 2H), 7.53 (dd, *J* = 4.2, 2.5 Hz, 3H). Spectral data was consistent with previously reported data.[4]



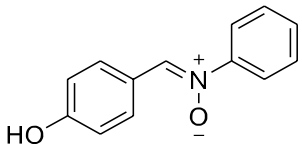
C-(4-cyanophenyl)-n-phenylnitrone. The nitrone was synthesized according to General Nitrone Synthesis A. The product was obtained in 52% yield. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 8.48 (d, *J* = 8.4 Hz, 2H), 8.00 (s, 1H), 7.78-7.74 (m, 4H), 7.52 (t, *J* = 3.3 Hz, 3H). Spectral data was consistent with previously reported data.[4]



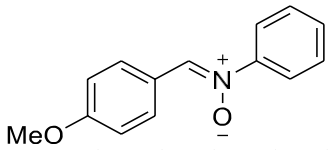
C-(4-methoxycarbonylphenyl)-*N*-phenylnitrone. The nitrone was synthesized according to General Nitrone Synthe- sis A. The product was obtained in 63% yield. **^{1}H NMR** (300 MHz; $CDCl_3$): δ 8.45 (d, *J* = 8.5 Hz, 2H), 8.14 (d, *J* = 8.7 Hz, 2H), 8.00 (s, 1H), 7.78 (dd, *J* = 6.9, 2.9 Hz, 2H), 7.52-7.49 (m, 3H), 3.95 (s, 3H). Spectral data was consistent with previously reported data.[5]



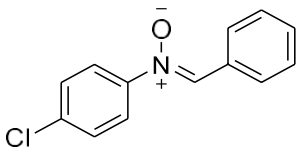
C-(4-fluorophenyl)-*N*-phenylnitrone. The nitrone was synthesized according General Nitrone Synthesis A. The prod- uct was obtained in 54% yield. **^{1}H NMR** (300 MHz; $CDCl_3$): δ 8.48-8.43 (m, 2H), 7.91 (s, 1H), 7.79-7.76 (m, 2H), 7.52-7.48 (m, 3H), 7.20-7.15 (m, 2H). Spectral data was consistent with previously reported data.[4]



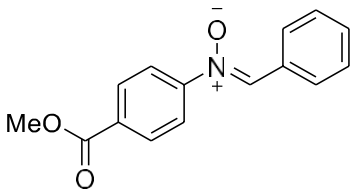
C-(4-hydroxyphenyl)-*N*-phenylnitrone. The nitrone was synthesized according to General Nitrone Synthesis A. The product was obtained in a 47% yield. **^{1}H NMR** (300 MHz; CD_3OD): δ 8.40-8.35 (m, 2H), 8.27 (s, 1H), 7.84-7.81 (m, 2H), 7.57-7.51 (m, 3H), 6.95-6.90 (m, 2H). Spectral data was consistent with previously reported data.[5]



C-(4-methoxyphenyl)-*N*-phenylnitrone. The nitrone was synthesized according to General Nitrone Synthesis A. The product was obtained in 77% yield. **^{1}H NMR** (300 MHz; $CDCl_3$): δ 8.43-8.39 (m, 2H), 7.86 (s, 1H), 7.80-7.76 (m, 2H), 7.51-7.44 (m, 3H), 7.02-6.99 (m, 2H), 3.89 (s, 3H). Spectral data was consistent with previously reported data.[3]

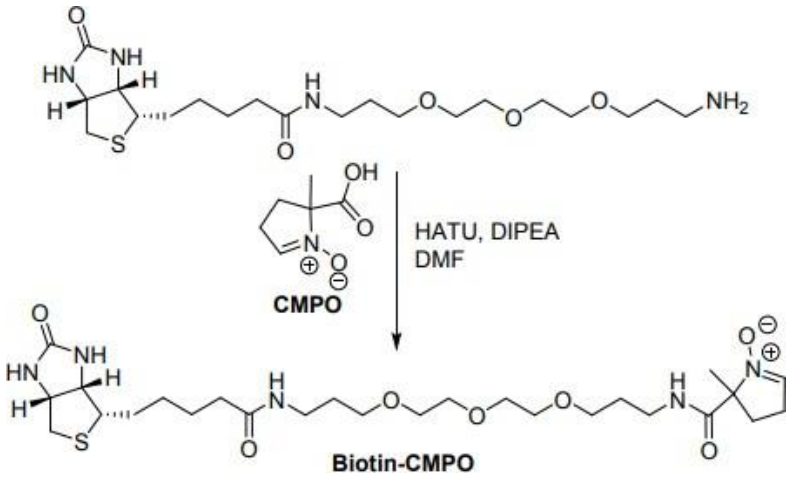


***N*-(4-chlorophenyl)-*a*-phenylnitrone**.The nitrone was synthesized according to General Nitrone Synthesis B. The product was obtained in 53% yield. **^{1}H NMR** (300 MHz; $CDCl_3$): δ 8.40-8.37 (m, 2H), 7.90 (s, 1H), 7.77-7.73 (m, 2H), 7.49 (q, J = 3.3 Hz, 4H), 7.45 (t, J = 2.5 Hz, 1H). Spectral data was consistent with previously reported data.[3]



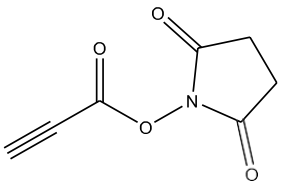
***N*-(4-methoxycarbonylphenyl)-*a*-phenylnitrone.** The nitrone was synthesized according to General Nitrone Synthe- sis B. The product was obtained in 83% yield.[5] **^{1}H NMR** (300 MHz; $CDCl_3$): δ 8.43-8.39 (m, 2H), 8.19-8.15 (m, 2H), 7.98 (s, 1H), 7.89-7.85 (m, 2H), 7.52-7.49 (m, 3H), 3.96 (s, 3H). Spectral data was consistent with previously reported data.[5]

Synthesis of Biotin-CMPO



The Biotin-CMPO was synthesized according to previously reported procedures.[9] Biotin-PEG (91 mg, 0.20 mmol, 1 eq), CMPO (35 mg, 0.24 mmol, 1.2 eq) and HATU (76 mg, 0.20 mmol, 1 eq) were dissolved in DMF (175 µL). DIPEA (52 µL, 0.30 mmol, 1.75 eq) was added all at once and the mixture was stirred for 45 minutes. Reaction progress was confirmed by LC-MS. The reaction was concentrated under reduced pressure and stored at -20 ˚C overnight. The crude was purified using preparatory HPLC with MeCN/H_2O/Formic acid (0.1%) as eluent, running gradient of 10 to 60% acetonitrile over 15 minutes. The product eluted at 7.5-8 minutes and its presence was confirmed by MS; the fractions were pooled and concentrated under reduced pressure. Some starting material was also isolated. The product was obtained as colourless oil (20.1 mg, 0.035 mmol, 17.5 % yield). MS (ESI+) calcd ($C_{26}H_{45}N_5O_7S$): 572.30 [M+H]+ , found 572.1; **^{1}H NMR** (400 MHz, MeOD-d4) δ 7.24 (s, 1H), 4.52 (dd, 1H, J=4.9, 7.7 Hz), 4.33 (dd, 1H, J=4.4,
7.8 Hz), 3.64 (m, 9H), 3.55 (dt, 4H, J=4.7, 6.0, 6.1 Hz), 3.36 (m, 2H), 3.25 (m, 3H), 2.95 (dd, 1H, J=4.9, 12.7 Hz), 2.71 (dd, 3H, J=10.6, 14.1 Hz), 2.22 (t, 3H, J=7.4, 7.4 Hz), 1.79 (ddd, 5H, J=4.0, 6.4, 12.8 Hz), 1.69 (s, 3H), 1.62 (m, 3H), 1.47 (dd, 2H, J=7.5, 15.2 9 Hz); **^{13}C NMR** (100 MHz, MeOD-d4) δ 174.6, 170.8, 141.1, 79.1, 70.1, 69.9, 69.8, 68.6, 68.5, 62.0, 60.2, 55.6, 39.7, 37.0, 36.4, 35.5, 31.0, 29.0, 28.8, 28.4, 28.1, 25.5, 25.0, 21.5. Spectral data was consistent with previously reported data.[6]

Synthesis of N-hydroxysuccinimide ester alkyne

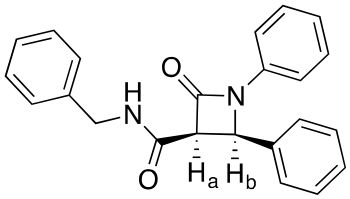


1-[(1-oxo-2-propynyl)oxy]-2,5-pyrrolidinedione. Synthesis was accomplished following a modified procedure.[7] Pro- piolic acid (500 mg, 7.14 mmol) and N-hydroxysuccinimide (822 mg, 7.14 mmol) were suspended in EtOAc (39 mL) and cooled to 0ºC. A solution of *N*,*N*′-Dicyclohexylcarbodiimide (1.47 g, 7.14 mmol) in EtOAc (13 mL) was added dropwise over the course of 1 hour. The mixture was then stirred at 0 ºC for 6 hours. The urea byproduct was re- moved by filtration and the filtrate was concentrated under reduced pressure to approximately 10 mL and then washed twice with brine (5 mL). The organic phase was dried with Na_2SO_4, then concentrated to approximately 1-2 mL. The concentrated organic phase was cooled to -5 ºC; 1-2 mL of heptanes was then added and the mixture was

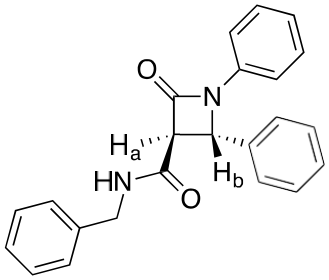
further cooled to -10 °C and the resulting solids were stirred for 2 hours. The solid precipitate was filtered, rinsed with cold heptanes and dried under vacuum to yield the product was a white solid (895 mg, 75% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 2.87 (s, 4H), 3.31 (s, 1H). Spectral data was consistent with previously reported data.[7]

Procedure for isolation of β-lactam products for characterization

The reaction was conducted in 6 mL of argon degassed H_2O. 4 mL of acetonitrile was added to help solubilize organic reagents. L-Proline (36 mg, 0.63 mmol), $CuSO_4$▪$5H_2O$ (39 mg, 0.16 mmol), sodium ascorbate (249 mg, 1.26 mmol) and pyridine (51 µL, 0.63 mmol) were then added successively. Following the addition of N-benzylprop-2- ynamide (50 mg, 0.31 mmol), C,N-diphenylnitrone (62 mg, 0.31 mmol) was added and the mixture was allowed to stir for 1 hour at 25 °C. The reaction was then extracted 3x20 mL of EtOAc. The organic fractions were then dried over Na_2SO_4 and concentrated under reduced pressure. The dried residue was then purified by flash column chro- matography using 30% EtOAc in hexanes. The product was recovered as a white solid for analytical and characteri- zation purposes.



N*-benzyl-2-oxo-1,4-diphenylazetidine-3-carboxamide, (3S, 4R) *-rel-
^{1}H NMR (400 MHz; $CDCl_3$): δ 7.38 (d, *J* = 13.6 Hz, 4H), 7.29 (d, *J* = 14.0 Hz, 6H), 7.24 (s, 4H), 7.06 (s, 1H), 6.54 (t, *J* = 0.3 Hz, 1H), 5.41 (d, *J* = 2.6 Hz, 1H), 4.56 (dd, *J* = 14.8, 6.1 Hz, 1H), 4.41 (dd, *J* = 14.8, 5.5 Hz, 1H), 3.86 (d, *J* = 2.6 Hz, 1H); **^{13}C NMR** (100MHz; $CDCl_3$): δ 163.5, 163.1, 137.6, 136.9, 133.9, 129.5, 129.25, 129.21, 129.01, 128.89, 128.82, 128.6, 127.86, 127.81, 127.5, 126.9, 126.2, 124.6, 77.3, 59.4, 58.3, 43.2, 29.9; **HRMS (ESI-TOF):** for $C_{23}H_{20}N_2O_2$ ($M+Na^+$): calculated: 379.1525; found: 379.1422.



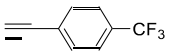
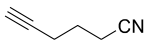
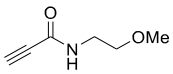
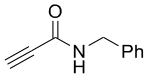
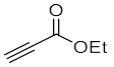
N*-benzyl-2-oxo-1,4-diphenylazetidine-3-carboxamide, (3R, 4R) *-rel-
^{1}H NMR (400 MHz; $CDCl_3$): δ 7.33 (s, 5H), 7.28 (d, *J* = 7.1 Hz, 4H), 7.25 (s, 3H), 7.08 (d, *J* = 6.9 Hz, 1H), 7.03 (d, *J* = 9.5 Hz, 2H), 6.77 (s, 1H), 5.37 (d, *J* = 6.3 Hz, 1H), 4.52 (d, *J* = 6.2 Hz, 1H), 4.39 (dd, *J* = 14.9, 6.5 Hz, 1H), 4.14 (dd, *J* = 14.8, 5.1 Hz, 1H); **^{13}C NMR** (101 MHz; $CDCl_3$): δ 163.5, 163.1, 137.6, 136.9, 133.9, 129.5, 129.25, 129.21, 129.01, 128.89, 128.82, 128.6, 127.86, 127.81, 127.5, 126.9, 126.2, 124.6, 77.3, 59.4, 58.3, 43.2, 29.9; **HRMS (ESI-TOF):** for $C_{23}H_{20}N_2O_2$ ($M+Na^+$): calculated: 379.1525; found: 379.1422.

In vitro Micelle-Assisted Kinugasa/CuANCR reactions

General Procedure

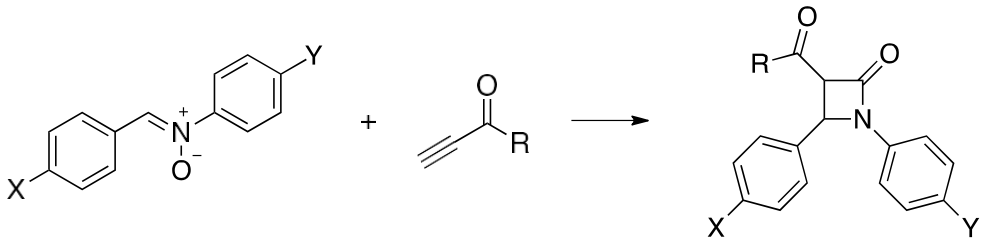
Reactions were conducted in 20 mL of argon degassed H_2O containing 10 mM sodium dodecyl sulfate (58 mg, 0.2 mmol). Sodium ascorbate (40 mg, 0.2 mmol), pyridine (8 µL, 0.1mmol), L-proline (6 mg, 0.05 mmol) and $CuSO_4$ (6 mg, 0.025 mmol) were then added successively. Following the addition of alkyne **1-8** (0.05 mmol), C,N- diphenylnitrone (10 mg, 0.05 mmol) was added and the reaction was stirred for 30 minutes at 25 °C. 3 mL of brine was then added to the mixture, followed by an extraction with 3x20 mL of EtOAc. The organic fractions were then dried over Na_2SO_4 and concentrated under reduced pressure. An internal standard, 1,4-Dimethoxybenzene, was accurately weighed (approximately 0.1 mmol) and added to the dried reaction. NMR yields were obtained by com- paring relevant new cis/trans product peaks (β-lactam doublet peaks, 4.5-5.5 ppm range, H_a from the representative product spectra) to the internal standard peak (6.83 ppm, s, 4H). Nitrone conversion was determined by comparing the remaining nitrone peak (8.41 ppm, m, 2H) to the initial amount used (0.05 mmol). The diastereomeric ratio was determined by comparing the calculated NMR yields of both the β -lactam products, while assuming that the minor product was *cis*.

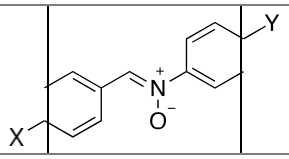
Table S1. Diastereomeric ratios for screen of alkynes used in micelle-assisted Kinugasa reactions[a]

Entry	Alkyne	Yield	trans: cis
1		22[b]	55:45
2		16[b]	70:30
3		20[b]	55:45
4		32[b]	77:23
5		60	80:20
6		64	68:32
7a/b		65/21[c]	74:26/ 55:45

[a]Isolated yields extracted from micellar emulsions. [b]Entries 1, 2, 3 and 4 were conducted in 3.5 mM CTAB (26 mg, 0.07 mmol) instead of SDS and in the absence of L-proline. [c]Entry 7b was conducted in the absence of surfactant.

Table S2. Screen of nitrones used in micelle-assisted Kinugasa Reaction



Entry		A R=OEt (%) yield	B R=NHBn (%) yield	C R=NH(CH$_2$)$_2$OCH$_3$ (%) yield
1	X= NO_2, Y=H	25	38	27
2	X=OCH_3, Y=H	53	45	50
3	X=F, Y=H	46	41	56
4	X=OH, Y=H	57	37	54
5	X=CN, Y=H	47	40	44
6	X=CO_2CH_3, Y=H	30	36	41
7	X=H, Y= CO_2CH_3	59	65	63
8	X=H, Y= Cl	31	55	24

Micelle Assisted Kinugasa/CuANCR Reaction on Alkyne Beads

5 µL of alkyne-tagged beads (corresponding to 3-5 µM of reactive alkyne groups, Click Chemistry tools) were washed in PBS prior to use. The reaction was carried out in PBS and consisted of 100 µM $CuSO_4$, 2 mM freshly solu- bilized sodium ascorbate, 200 µM L-proline, 50 µM biotin-CMPO (or vehicle DMSO) to which the indicated amount of surfactant or water solvent was added. The reaction was started by addition of washed beads and was carried out with gentle shaking at 37 °C for the indicated amount of time. Beads were washed 1x with PBS containing 0.05% Tween20, then 3x with PBS prior addition of 5 µg/mL FITC-streptavidin in PBS. The binding of streptavidin-FITC was carried out at room temperature for 30 minutes in the dark. The beads were washed three more times with PBS and resuspended in PBS containing 5% glycerol. 8 µL of this solution was applied to a microscopy slide which were imaged using the Nikon Ni-U ratiometric fluorescence microscope equipped with a LED excitation light source and Ultra- sensitive Andor iXon Ultra 897 cooled EMCCD camera. Images were acquired using a Nikon 60x oil dip objective lens and, if indicated, a 2x relay lens. Fluorescence images were obtained under strictly identical conditions of gain and exposure time, on focused beads, typically 2-5 s, and brightfield images were obtained using a 20-50 ms exposure. Images were acquired using Nikon NIS Elements software and processed with ImageJ.

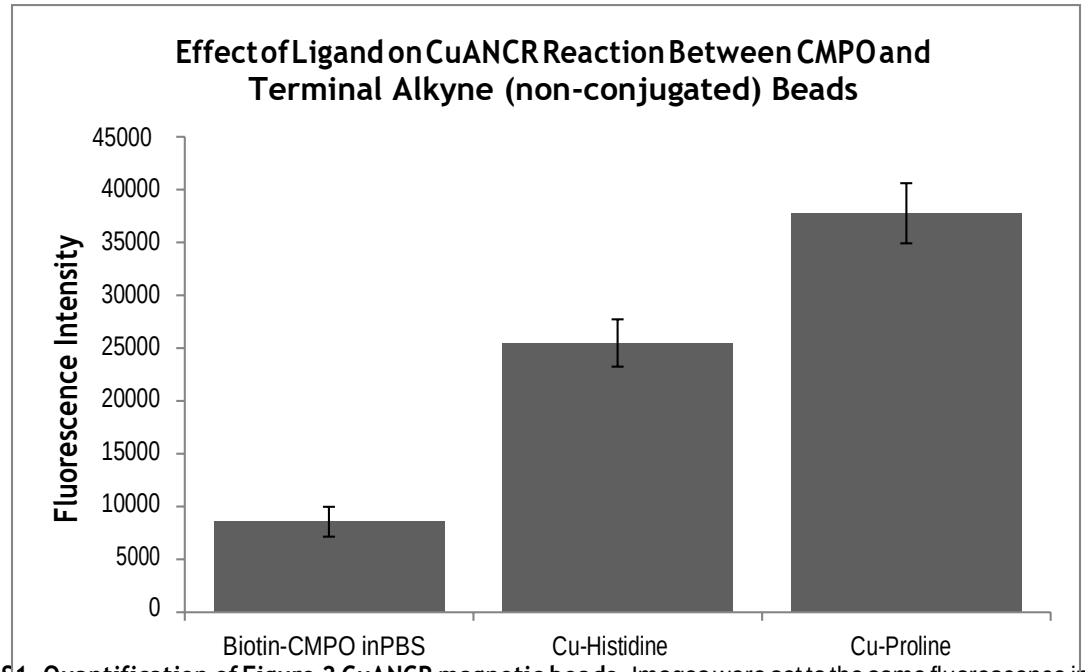


Figure S1. Quantification of Figure 2 CuANCR magnetic beads. Images were set to the same fluorescence inten- sity levels then converted to 8bit. Integrated density from the same area was then measured for 5 beads per image, which is from different fields of view for each sample

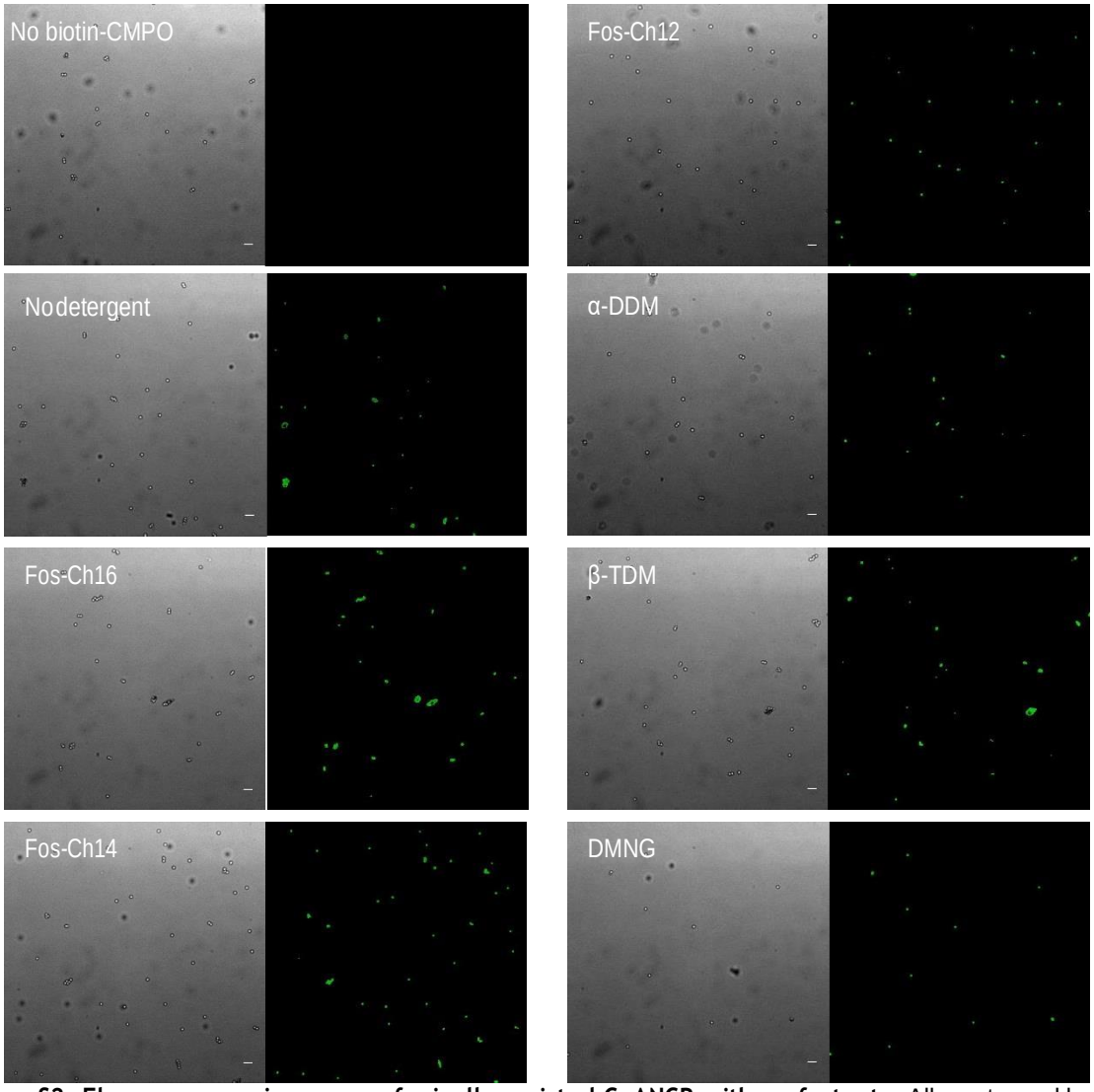
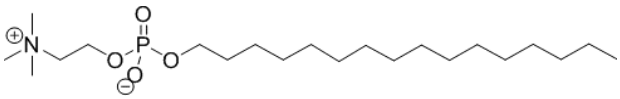
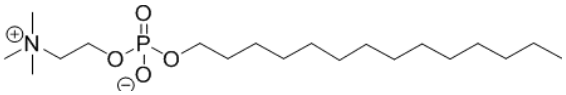
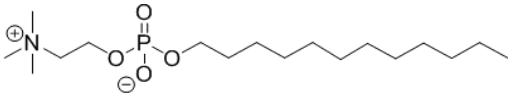
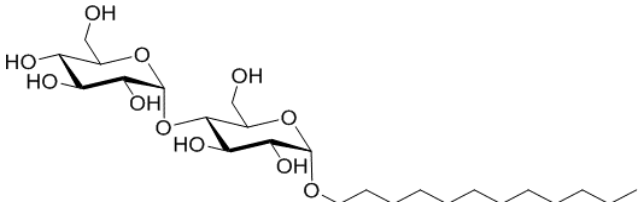
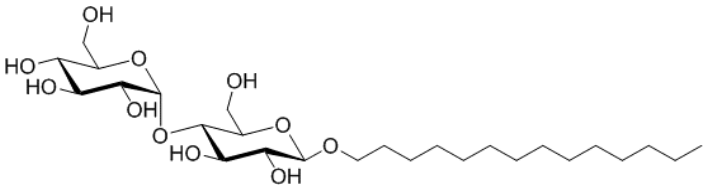
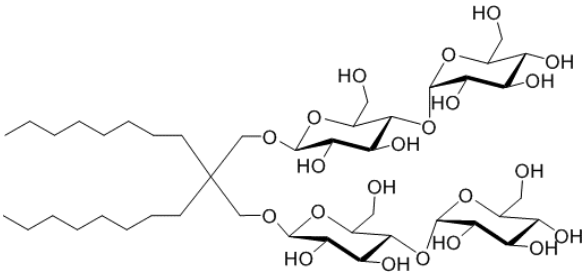


Figure S2. Fluorescence microscopy of micelle-assisted CuANCR with surfactants. Alkyne-tagged beads were labelled with 50 µM biotin-CMPO in presence of 1.5 mM of indicated surfactant (at or above CMC for all detergents) from Table S3. Labelling was carried out for 30 minutes at 37°C after which beads were washed and incubated with 5 µg/mL FITC-streptavidin for another 30 minutes at room temperature in the dark. Images were acquired as indi- cated in the labelling protocol using a 60x objective with oil dip lens and background fluorescence (sample without biotin-CMPO) was subtracted using software ImageJ. Scale bar indicates 5 µm.

Table S3. Lipids used in screening of Kinugasa reaction on alkyne-tagged beads.

Structures are shown as well as approximate critical micellar concentrations (CMC) in water for each lipid.

Name[b]	Structure	CMC in water[a]
FCH16		~ 0.013 mM
FCH14		~ 0.12 mM
FCH12		~ 1.5 mM
α-DDM		~ 0.152 mM
β-TDM		~ 0.01 mM
DMNG		~ 0.36 mM

[a]Values obtained from www.anatrace.com. [b]FCH; Fos-choline, α-DDM; n-Dodecyl–α-D-Maltopyranoside, β-TDM; N-Tetradecyl-β-D-Maltopyranoside, DMNG; Decyl Maltose Neopentyl Glycol.

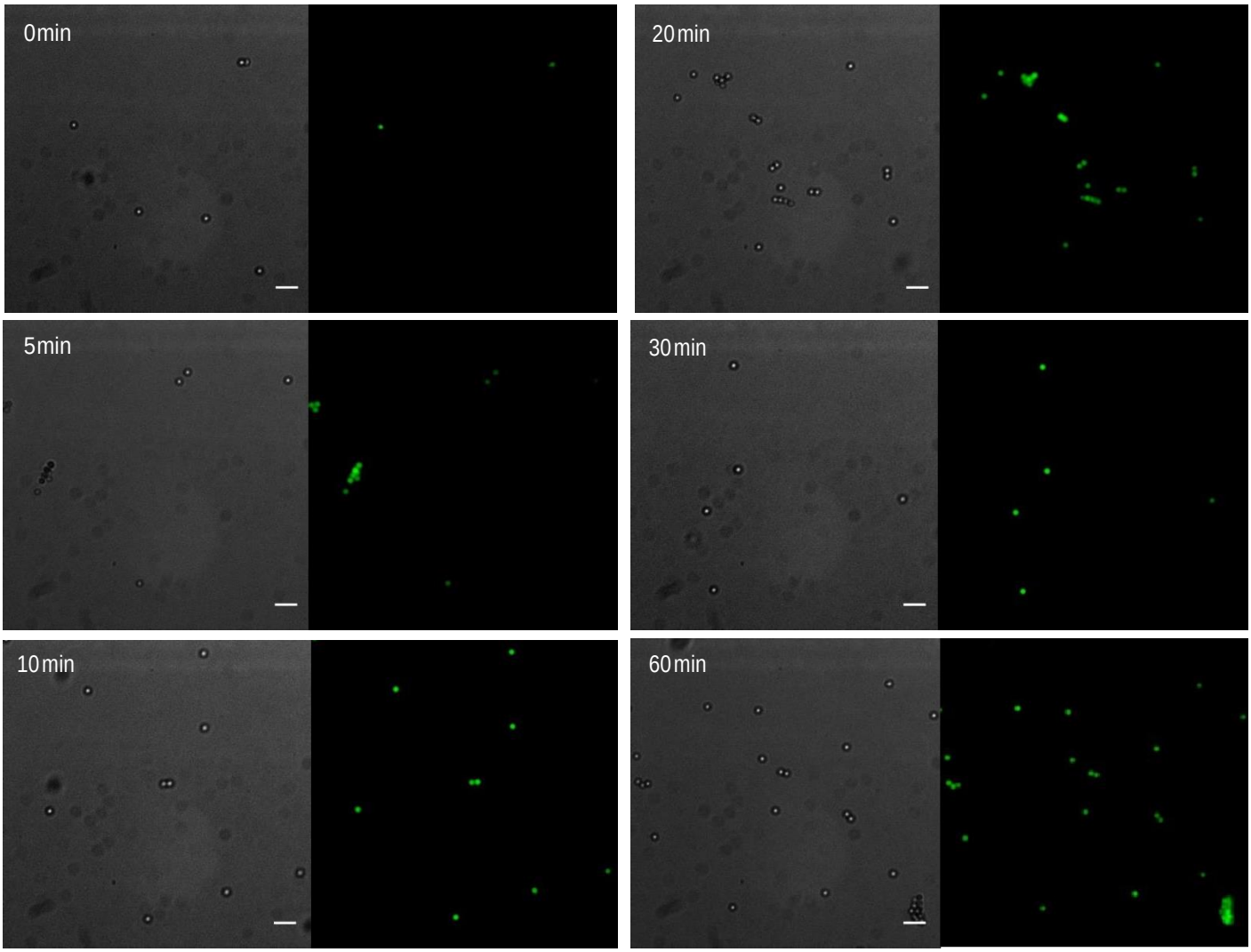


Figure S3. Time course of micelle-assisted CuANCR reaction in presence of β-TDM surfactant. Alkyne-tagged beads were labelled with 50 µM biotin-CMPO in presence of 150 µM β-TDM, 100 µM $CuSO_4$, 2 mM sodium ascor- bate and 200 µM L-proline for 0-60 min, as indicated, after which beads were washed with PBS and were then in- cubated with 5 µg/mL FITC-streptavidin in the dark. Beads were again washed with 3x PBS and imaged using Nikon Ni-U ratiometric microscope equipped with a 60x objective lens and a 2x relay lens. Average background fluores- cence obtained in absence of biotin-CMPO was subtracted from all images. Scale bar indicates 5 µm.

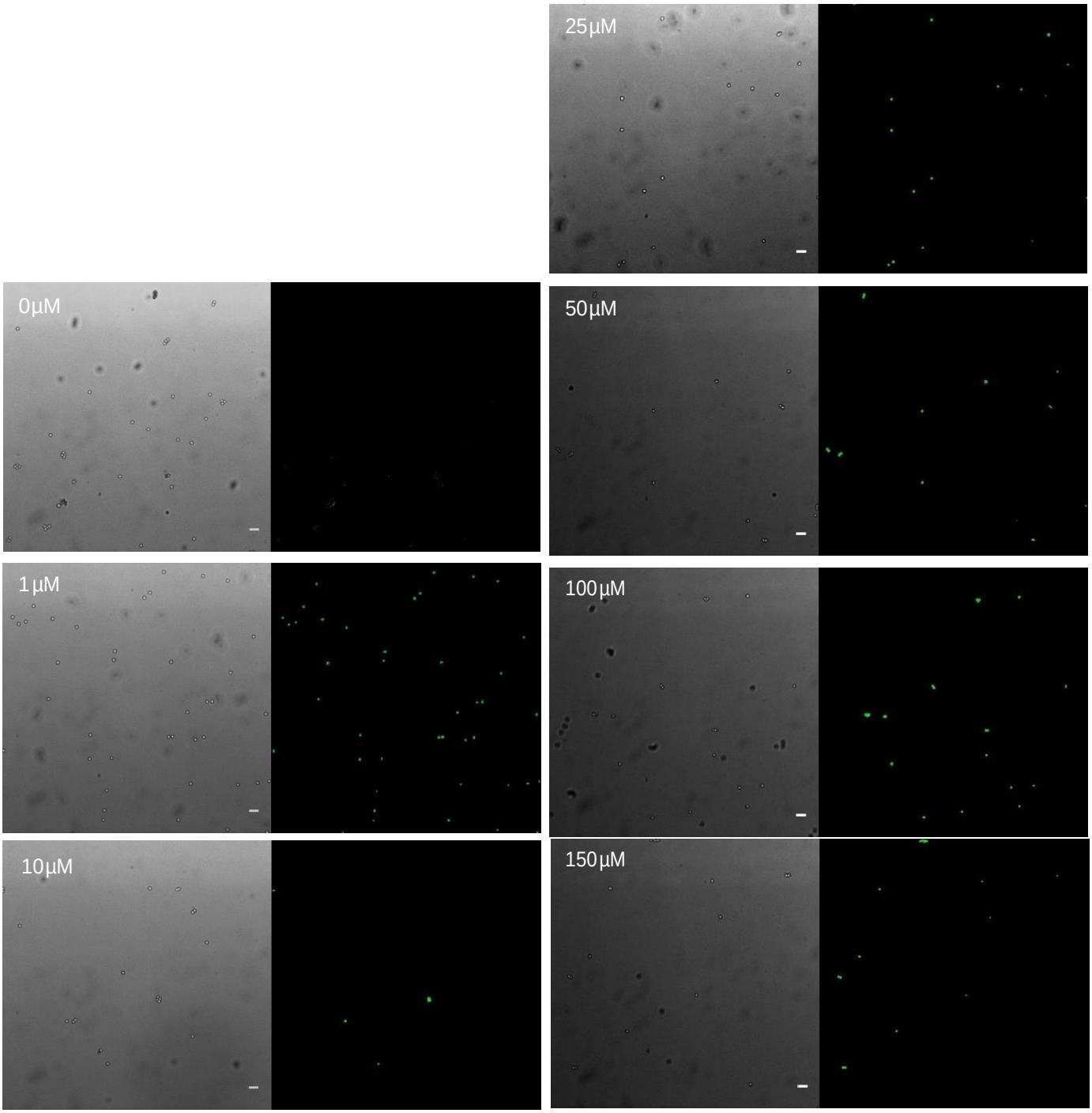


Figure S4. Micelle-dependence of aqueous CuANCR labelling reaction. Labelling of alkyne beads with biotin-CMPO was performed as indicated in the experimental section, in a range of concentrations of β-TDM, both below and above the CMC of β-TDM of 10 µM (See Table S3). Fluorescence images were acquired using the Nikon rati- ometric microscope Ni-U equipped with an oil-dip 60x objective lens. Background fluorescence was determined as average fluorescence of beads in absence of biotin-CMPO, and was subtracted from all images. Scale bar indicates 5 µm.

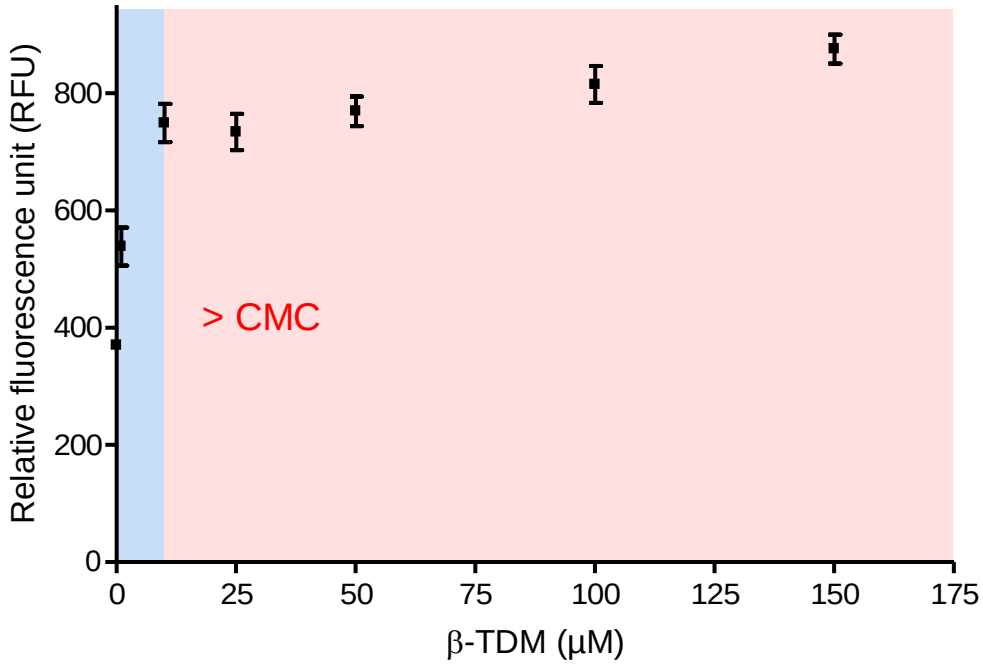


Figure S5. Micelle-dependence of aqueous CuANCR labelling reaction. Labelling of alkyne-tagged beads with 50 μM biotin-CMPO in 100 μM $CuSO_4$, 2 mM sodium ascorbate and 200 μM L-proline was performed in a range of concentrations of β-TDM, both below (blue area) and above (light red area) the CMC of β-TDM of 10 μM (See Table S3). Each data point is an average above background fluorescence determined from at least five different beads.

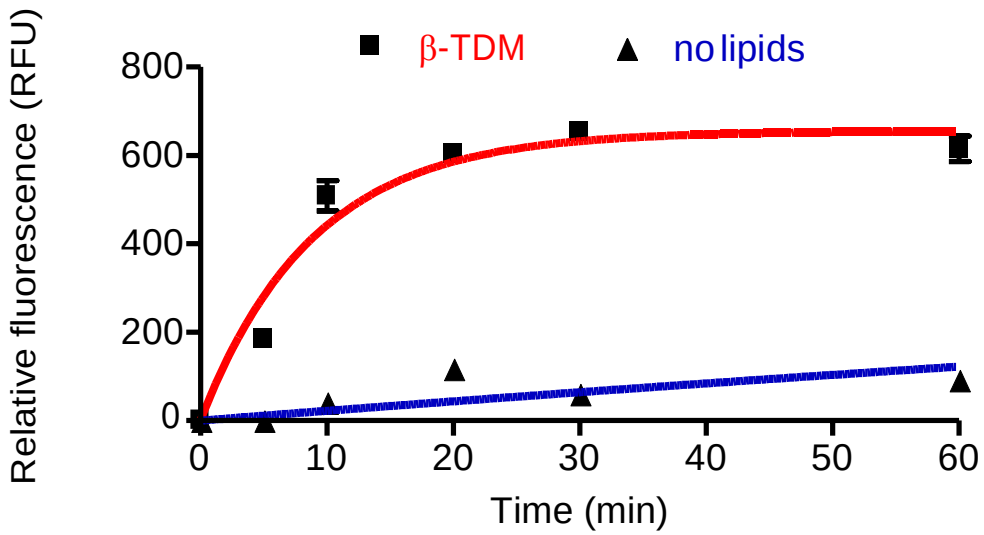


Figure S6. **Kinetics of aqueous Kinugasa on alkyne-tagged beads**. Alkyne-tagged beads with β-TDM (150 µM, red curve) and without lipid (0 µM, blue curve). Alkyne beads (3-5 µM alkyne groups) were incubated with 50 µM biotin- CMPO, 100 µM $CuSO_4$, 2 mM sodium ascorbate and 200 µM L-proline in PBS for the indicated amount of time at 37°C, and then stained with 5 µg/mL FITC-streptavidin. Beads were washed in PBS before fluorescence imaging. Above background fluorescence of five beads from two independent view fields (10 beads total) was determined using ImageJ software, normalized and plotted against time of labelling (minutes). Data points were fitted to first order kinetics equation in Prism 4. See also Figure S3 for fluorescence imaging data.

E5-TAT peptide modification procedure

Two-Step Biotin Labeling of E5-TAT peptide

E5-TAT peptide (GLFEAIAEFIENGWEGLIEGWYGGRKKRRQRRR) (GenScript) samples were diluted (20 µM) in Phos- phate-Buffered Saline (PBS) containing varying concentrations of Fos-Choline 12 (FC12) (0.01% to 0.2%, CMC=0.047%). The peptide samples were then treated with 1-[(1-oxo-2-propynyl)oxy]-2,5-pyrrolidinedione (N-hy- droxysuccinimide ester alkyne) (300 µM, DMSO stock) and allowed to stand for 1 hour at room temperature. Sodium ascorbate (300 µM), L-proline (40 µM), $CuSO_4 5H_2O$ (20 µM) and Biotin-CMPO (300 µM, DMSO stock) were then sequentially added to the samples, which were allowed to sit for an additional hour at room temperature. The sam- ples were then prepared for SDS-PAGE and Western blotting analysis.

Immunoblotting

Labeled E5-TAT peptide (GenScript) samples were loaded and analyzed using SDS-PAGE and western blotting. Sam- ples were ran using 12% stain-free polyacrylamide gel electrophoresis (TGX Stain-Free Fastcast Acrylamide kit, Bio- Rad). The proteins were then transferred to a PVDF membrane using the Trans-blot Turbo RTA Transfer Kit (Bio- Rad). Membrane was blocked using Tris-buffered saline with 0.05% Tween-20 (TBS-T) containing 3% W/V Bovine serum albumin (Sigma-Aldrich). Peptides were probed using anti-biotin antibody (1:1000, Invitrogen, MA5-11251) overnight at 4 °C. Blot was washed in TBS-T and probed for one 1 hour at room temperature with HRP-conjugated goat anti-mouse secondary antibody (1:20000, Jackson Immunoresearch Laboratories, Westgrove, PA). Bands were visualized using Clarity ECL western blotting substrate (Bio-Rad) according to the manufacturer's protocols. Inte- grated signal was calculated relative to negative control (no Biotin-CMPO) taking into account peptide loading using Image Lab software (Bio-Rad). Figure S7 shows a repeat experiment with the unmodified E5-TAT negative control.

Peptide Modification Analysis

2 mg of E5-TAT peptide dissolved in 1x phosphaste-buffered saline (pH 7.5) was treated with 15 equivalents of N-hydroxysuccinimide ester alkyne and left for one hour at room temperature. The peptide sample was then subjected to FPLC purification. The size exclusion chromatography profile was obtained for the alkyne modified E5-TAT using a Superdex 75 size exclusion column (FPLC ÄKTA pure, GE) at a concentration of 1 mg/ml in 1x phosphate-buffered saline (pH 7.5). Absorbance was recorded at 280 nm, with flow rate maintained at 0.8 mL/min (See Figure S8). 10 ug of purified modified and unmodified peptide were then subjected to desalting using C18 spin columns (Thermofisher Scientific) according to manufacturer's protocol. Samples were then subjected to mass spectrometry analysis. Pro- teome Discoverer 2.1 (Thermofisher Scientific) was used to evaluate the modification of peptide with the N-hydroxy- succinimide ester alkyne. Search engine: SEQUEST-HT implemented in Proteome Discovery was applied for all MS raw files. Search parameters were set to allow for dynamic modification of the N-hydroxysuccinimide ester alkyne (51.995 Da). The peptide-spectrum matches (PSMs) was used to evaluate the alkyne modification of peptides and only peptides for Sequest results of XCorr ≥ 2.5 were retained. The obtained PSMs showed modification of lysine residues K26 and K27 (both mono and di-substitution), modifications not found for the unmodified peptide sample.

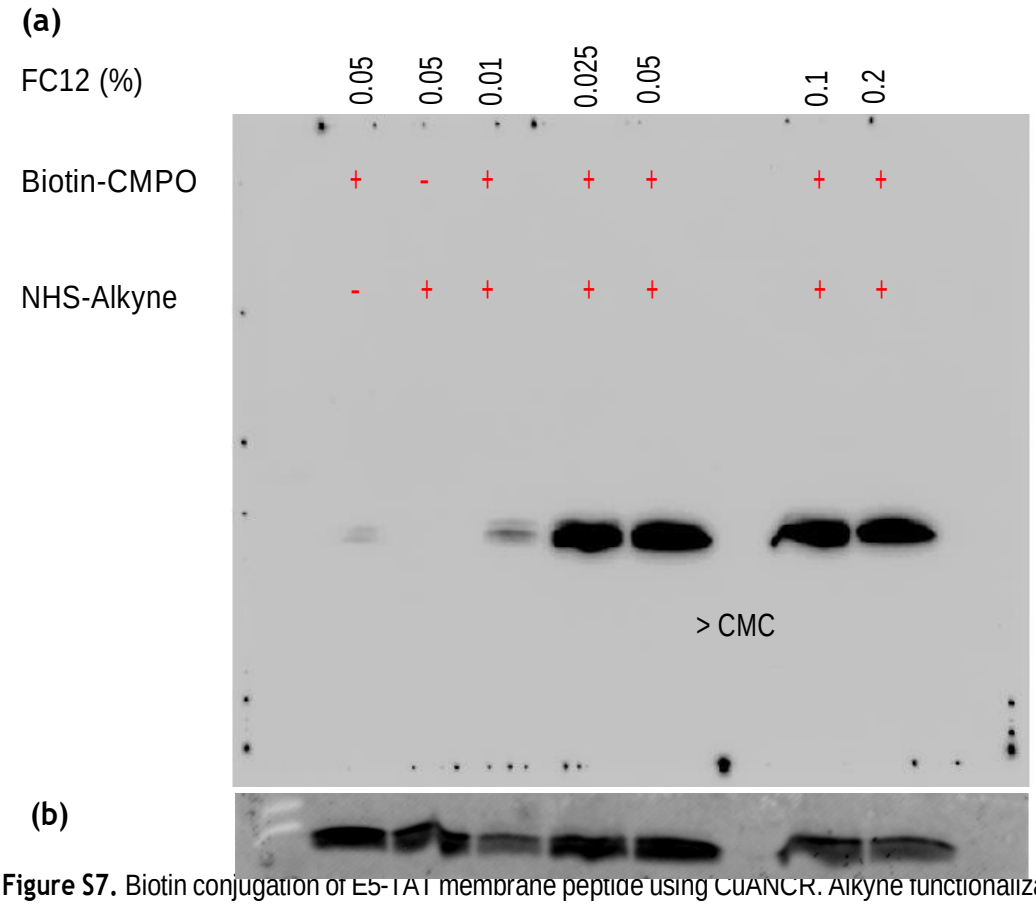


Figure S7. Biotin conjugation of E5-TAT membrane peptide using CuAAC. Alkyne functionalization of E5- TAT (20 μM) was carried out using N-hydroxysuccinimide ester alkyne (300 μM). Biotin labelling was achieved by treating modified peptide with $CuSO_4 \cdot H_2O$ (20 μM), sodium ascorbate (300 μM), L-proline (40 μM) and biotin-CMPO (300 μM). (a) Western blot analysis of biotin labelled E5-TAT samples shown under varying percentages (%W/V) of detergent (FC12) ranging from (0.01-0.2%, CMC=0.05%). (b) TGX Stain-free protein loading control.

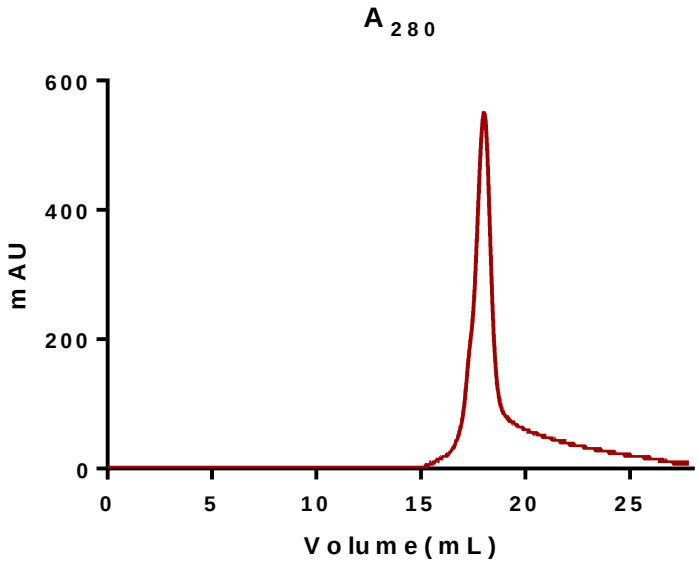


Figure S8. Size exclusion chromatography profile for modified E5-TAT run in 1x PBS, pH 7.5 on a Super- dex 75 column (GE).

Table S4. Results from SEQUEST-HT implementation in Proteome Discoverer for unmodified E5-TAT samples. XCorr values ≥ 2.5 are shown.

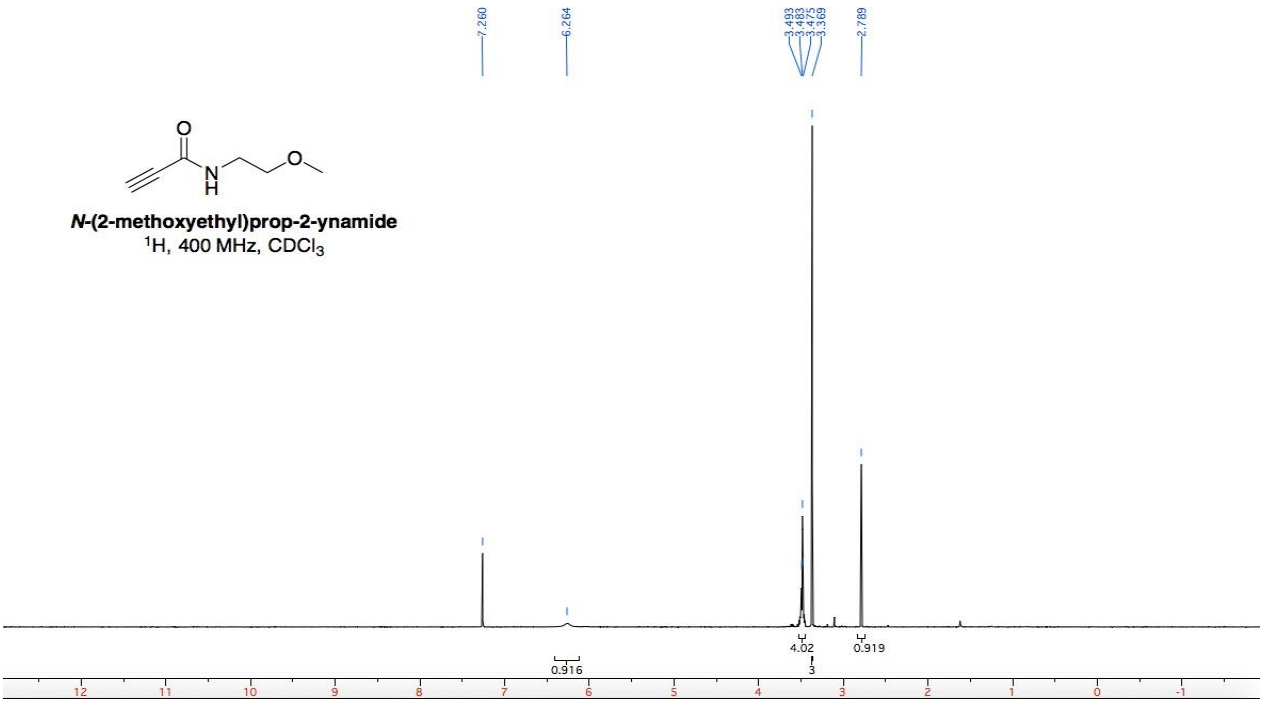
Confidenc	Identifyin	PSM Ambi	Annotated Sequence	Modifications	# Protein	# Proteins	Master Pr	Protein A	# Missed (	Charge	DeltaScor	DeltaCn	Rank	Search En	m/z [Da]	MH+ [Da]	DeltaM [p	Deltam/z	Activation	MS Order	Isolation	Ion Inject	RT [min]	First Scan	Spectrum	Ions Matc	XCorr	Area	Apex RT [min]
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2813	3978.103	-1.9104	-0.0019	CID (Collis	MS2	4	140.5454	57.92243	23691	1_unmod	0/0	4.743893	66302815	57.87073517
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2841	3978.114	0.851266	0.000847	CID (Collis	MS2	4	68.79326	38.47175	14662	1_unmod	0/0	4.411004		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2798	3978.097	-3.4441	-0.00343	CID (Collis	MS2	4	117.4296	57.72387	23612	1_unmod	0/0	4.407228	50604561	57.78352356
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1441	3978.102	-2.2921	-0.00114	CID (Collis	MS2	0.812856	14.23745	37.35387	14110	1_unmod	0/0	3.941107		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2834	3978.112	0.176181	0.000175	CID (Collis	MS2	0.67484	25.58952	38.0981	14490	1_unmod	0/0	3.925747		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2798	3978.097	-3.4441	-0.00343	CID (Collis	MS2	0.641611	11.71251	37.13908	13992	1_unmod	0/0	3.893034		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2803	3978.099	-2.9537	-0.00294	CID (Collis	MS2	4	150	38.86283	14840	1_unmod	0/0	3.851491		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1455	3978.103	0.530893	0.000264	CID (Collis	MS2	10.57674	119.5001	38.57165	14706	1_unmod	0/0	3.808585	23369333	38.63791275
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2805	3978.1	-2.7696	-0.00275	CID (Collis	MS2	0.512361	10.47783	37.33337	14096	1_unmod	0/0	3.782155		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.427	3978.106	-1.2847	-0.00102	CID (Collis	MS2	0.285902	1.987852	37.83471	14366	1_unmod	0/0	3.777211		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.279	3978.094	-4.2425	-0.00422	CID (Collis	MS2	4	4.251281	36.97043	13900	1_unmod	0/0	3.749854		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1447	3978.107	-1.1261	-0.00056	CID (Collis	MS2	3.647698	33.04153	37.95887	14424	1_unmod	0/0	3.683121		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	3	4	1	0	1	1	1326.707	3978.105	-1.4927	-0.00198	CID (Collis	MS2	8.26472	67.84008	36.87024	13835	1_unmod	0/0	3.598034	2.01E+08	36.92218018
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.165	3978.112	0.150704	8.56E-05	CID (Collis	MS2	0.79167	17.89352	38.83939	14830	1_unmod	0/0	3.580093		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1435	3978.097	-3.4582	-0.00172	CID (Collis	MS2	4.422194	150	57.99996	23722	1_unmod	0/0	3.576095		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2809	3978.102	-2.3400	-0.00233	CID (Collis	MS2	1	28.84985	37.91509	14404	1_unmod	0/0	3.573057		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2825	3978.108	-0.6830	-0.00068	CID (Collis	MS2	1.961661	37.10045	38.27891	14575	1_unmod	0/0	3.556824		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1655	3978.115	0.902499	0.000513	CID (Collis	MS2	0.874546	31.81051	58.13913	23771	1_unmod	0/0	3.471304	2.66E+08	57.86439514
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1646	3978.108	-0.7084	-0.0004	CID (Collis	MS2	1.000393	0.425447	36.92422	13866	1_unmod	0/0	3.465834		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1638	3978.103	-2.1046	-0.0012	CID (Collis	MS2	1.002694	17.83573	57.72175	23611	1_unmod	0/0	3.465371		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1645	3978.108	-0.8158	-0.00046	CID (Collis	MS2	1.203939	150	58.93848	24076	1_unmod	0/0	3.463707		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	663.8611	3978.13	4.894995	0.003245	CID (Collis	MS2	22.56742	2.478715	38.21172	14545	1_unmod	0/0	3.461434		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1456	3978.114	0.715006	0.000356	CID (Collis	MS2	3.885144	26.04707	38.16812	14522	1_unmod	0/0	3.460276		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1456	3978.114	0.776371	0.000386	CID (Collis	MS2	12.11564	51.86987	38.38544	14624	1_unmod	0/0	3.382505		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1649	3978.11	-0.1714	-9.7E-05	CID (Collis	MS2	1.049062	1.045396	37.13212	13961	1_unmod	0/0	3.374755		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.278	3978.09	-5.2244	-0.0052	CID (Collis	MS2	4	8.156715	37.53065	14210	1_unmod	0/0	3.37105		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1654	3978.114	0.795099	0.000452	CID (Collis	MS2	1.13002	3.30596	38.64684	14742	1_unmod	0/0	3.36351		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2794	3978.096	-3.8129	-0.00379	CID (Collis	MS2	0.497774	7.520233	36.78901	13796	1_unmod	0/0	3.344334		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.4268	3978.105	-1.5145	-0.00121	CID (Collis	MS2	0.370766	1.338864	37.30659	14085	1_unmod	0/0	3.342888		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1427	3978.091	-5.0538	-0.00251	CID (Collis	MS2	2.316644	21.66924	37.53523	14214	1_unmod	0/0	3.340454		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1661	3978.119	1.976491	0.001123	CID (Collis	MS2	2.343021	12.64181	57.91961	23696	1_unmod	0/0	3.339486	2.66E+08	57.86439514
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2822	3978.107	-1.0512	-0.00105	CID (Collis	MS2	1	38.68597	38.64785	14744	1_unmod	0/0	3.336351		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1653	3978.113	0.580301	0.00033	CID (Collis	MS2	0.498625	1.634333	37.91411	14401	1_unmod	0/0	3.33414		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	4	4	1	0	1	1	995.2813	3978.103	-1.9104	-0.0019	CID (Collis	MS2	0.415333	16.1498	37.72318	14306	1_unmod	0/0	3.279951		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.4258	3978.1	-2.7423	-0.00218	CID (Collis	MS2	0.107356	0.812052	36.74545	13770	1_unmod	0/0	3.271689		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1437	3978.099	-3.05	-0.00154	CID (Collis	MS2	3.90362	12.37752	37.1765	14013	1_unmod	0/0	3.17528		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1669	3978.125	3.48006	0.001978	CID (Collis	MS2	0.343771	3.301521	38.09709	14485	1_unmod	0/0	3.167206		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1664	3978.121	2.620881	0.001489	CID (Collis	MS2	1.041488	4.288625	38.47027	14661	1_unmod	0/0	3.141017		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.4261	3978.102	-2.3587	-0.00188	CID (Collis	MS2	4	2.038975	37.66141	14275	1_unmod	0/0	3.120983		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1644	3978.107	-0.9232	-0.00052	CID (Collis	MS2	0.414314	126.4324	58.40479	23876	1_unmod	0/0	3.113648	2.66E+08	57.86439514
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1425	3978.089	-5.4834	-0.00273	CID (Collis	MS2	3.559866	9.118275	36.72212	13754	1_unmod	0/0	3.049924		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1632	3978.099	-3.0712	-0.00175	CID (Collis	MS2	2.451736	150	59.16166	24161	1_unmod	0/0	3.047627		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.4284	3978.113	0.479662	0.000382	CID (Collis	MS2	1.001284	15.34885	38.97324	14888	1_unmod	0/0	3.042379		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1666	3978.122	2.835686	0.001612	CID (Collis	MS2	23.57327	1.962864	37.72219	14305	1_unmod	0/0	3.016861		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.4278	3978.11	-0.2674	-0.00023	CID (Collis	MS2	0.337998	1.46574	37.11305	13976	1_unmod	0/0	3.008665		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.145	3978.109	-0.4510	-0.00022	CID (Collis	MS2	10.04413	31.69995	37.74997	14316	1_unmod	0/0	3.003917		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1635	3978.101	-2.5342	-0.00144	CID (Collis	MS2	32.46635	88.40292	42.07001	16175	1_unmod	0/0	2.929647		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.428	3978.111	-0.0573	-4.6E-05	CID (Collis	MS2	0.457939	22.65708	38.7884	14805	1_unmod	0/0	2.91562		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1659	3978.118	1.654299	0.00094	CID (Collis	MS2	42.62211	1.078127	37.30757	14086	1_unmod	0/0	2.896043		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1638	3978.103	-1.9972	-0.00114	CID (Collis	MS2	17.6507	150	59.35185	24230	1_unmod	0/0	2.849425		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1663	3978.121	2.406086	0.001367	CID (Collis	MS2	1.048451	3.73344	38.27789	14574	1_unmod	0/0	2.839174		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1432	3978.095	-4.0719	-0.00202	CID (Collis	MS2	1.028153	7.61597	36.9714	13901	1_unmod	0/0	2.828975	92560054	36.92717743
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1646	3978.109	-0.6010	-0.00034	CID (Collis	MS2	0.893022	1.124045	37.52967	14205	1_unmod	0/0	2.810067		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	663.8583	3978.113	0.568338	0.000377	CID (Collis	MS2	0.325652	9.706686	38.78729	14806	1_unmod	0/0	2.809453		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	498.1444	3978.104	-1.7398	-0.00087	CID (Collis	MS2	7.195007	150	57.78636	23631	1_unmod	0/0	2.790236	2896647	57.78102493
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	663.8599	3978.123	2.961801	0.001964	CID (Collis	MS2	0.222724	3.231561	38.41545	14636	1_unmod	0/0	2.771056		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	7	4	1	0	1	1	569.1639	3978.104	-1.7824	-0.00101	CID (Collis	MS2	1.093216	149.0648	58.74461	24005	1_unmod	0/0	2.699229		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	6	4	1	0	1	1	663.8592	3978.119	1.949186	0.001292	CID (Collis	MS2	3.612453	10.31764	57.98531	23711	1_unmod	0/0	2.689505	2.66E+08	57.86334229
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.4266	3978.104	-1.7450	-0.00139	CID (Collis	MS2	1	11.65125	38.60648	14724	1_unmod	0/0	2.67		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.4263	3978.102	-2.2053	-0.00175	CID (Collis	MS2	3.328261	150	58.45942	23899	1_unmod	0/0	2.619301	2.9E+08	57.86603165
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.4286	3978.114	0.786511	0.000626	CID (Collis	MS2	0.56899	3.841676	38.02888	14451	1_unmod	0/0	2.537992		
High	SequestH	Unambigu	[-].GLFEAAEFENGWE		1	1	peptide	peptide	5	4	1	0	1	1	796.4274	3978.108	-0.7477	-0.00059	CID (Collis	MS2	4	150	58.96535	24088	1_unmod	0/0	2.503753		

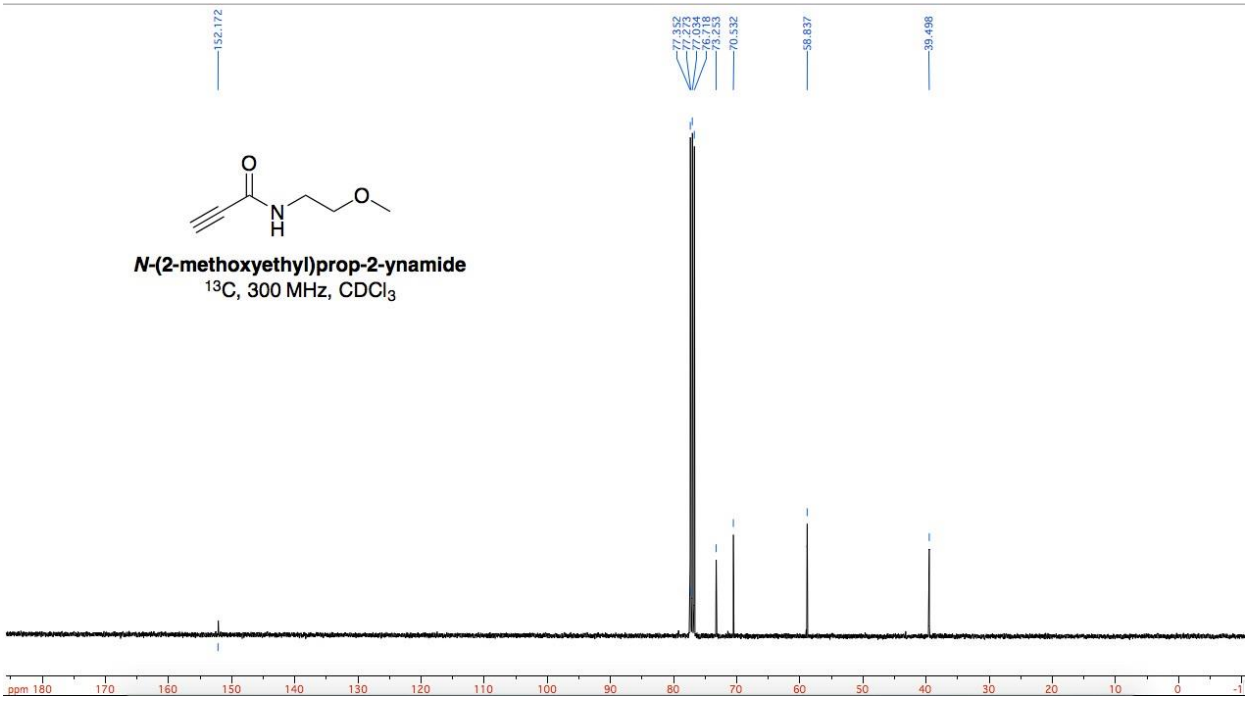
Table S5. Results from SEQUEST-HT implementation in Proteome Discoverer for modified E5-TAT samples. XCorr values ≥ 2.5 are shown.

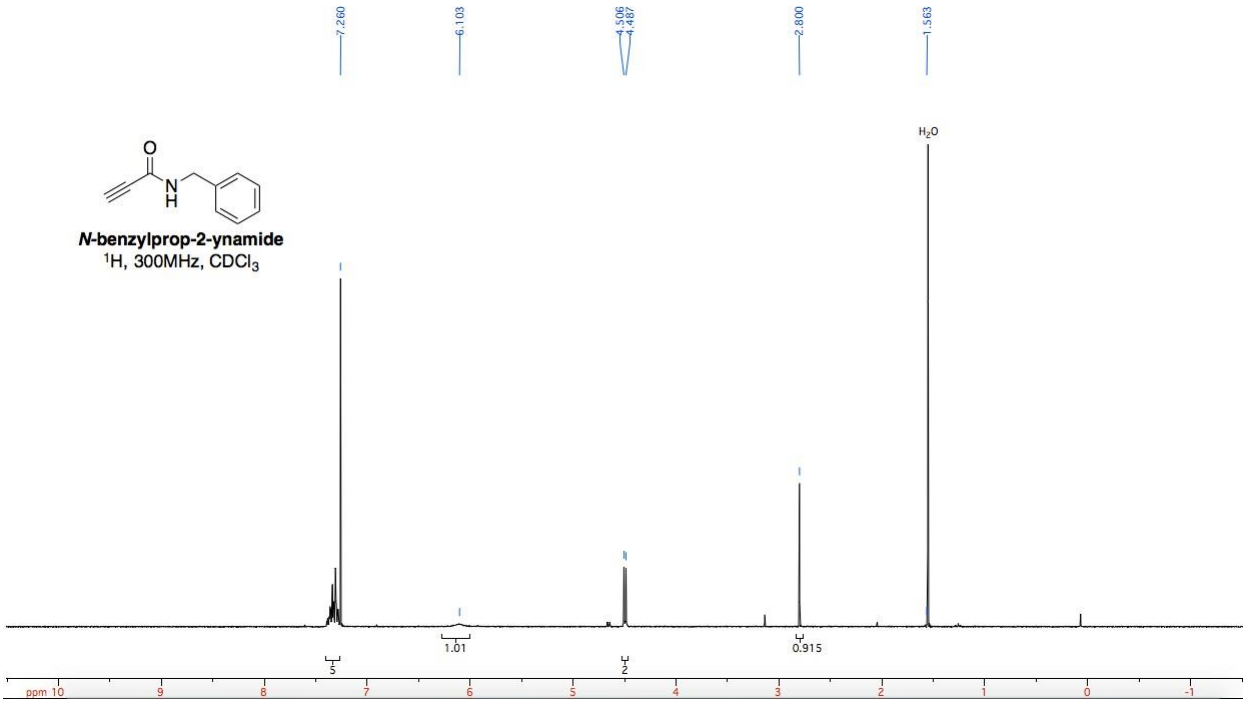
Confidenc	Identifyin	PSM Amb	Annotate	Modifications	# Protein	# Proteins	Master Pr	Protein A	# Missed (	Charge	DeltaScor	DeltaCn	Rank	Search En	m/z [Da]	MH+ [Da]	DeltaM [p	Deltam/z	Activation	MS Order	Isolation	Ion Inject	RT [min]	First Scan	Spectrum	Ions Matc	XCorr	Area	Apex RT [
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	4	1	0	1	1	995.2745	3978.076	-8.7839	-0.00874	CID (Collis	MS2	80.86436	150	40.8231	15274	2_mod_ox	0/0	4.024749	12725294	40.83605
High	SequestH	Unambigu	[-].GLFEAI	K26(alkyne); K27(alkyne	1	1	peptide	peptide	0	6	1	0	1	1	681.1879	4082.091	-2.4497	-0.00167	CID (Collis	MS2	0	34.08564	40.13075	15008	2_mod_ox	0/0	3.384698	49995360	40.1216
High	SequestH	Unambigu	[-].GLFEAI	K27(alkyne)	1	1	peptide	peptide	0	6	0.1098	0	1	1	672.5219	4030.095	-2.7775	-0.00187	CID (Collis	MS2	38.78105	98.17396	39.18972	14633	2_mod_ox	0/0	3.282524	21085314	39.15646
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	5	1	0	1	1	796.4235	3978.088	-5.7341	-0.00456	CID (Collis	MS2	1.455265	97.48626	41.52988	15543	2_mod_ox	0/0	3.023272	45186202	41.28741
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	5	1	0	1	1	796.4254	3978.098	-3.3560	-0.00267	CID (Collis	MS2	2.948861	150	41.02731	15351	2_mod_ox	0/0	3.017358		
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	7	1	0	1	1	569.1671	3978.126	3.802276	0.002161	CID (Collis	MS2	17.42871	150	59.36454	23572	2_mod_ox	0/0	2.962573		
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	4	1	0	1	1	995.284	3978.114	0.789889	0.000786	CID (Collis	MS2	1.933469	150	57.95038	23046	2_mod_ox	0/0	2.92249	6488897	57.95372
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	8	1	0	1	1	498.1432	3978.094	-4.1945	-0.00209	CID (Collis	MS2	13.46342	150	38.76765	14458	2_mod_ox	0/0	2.884229		
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	7	1	0	1	1	569.1659	3978.118	1.654293	0.00094	CID (Collis	MS2	4.582896	150	58.93892	23415	2_mod_ox	0/0	2.833701		
High	SequestH	Unambigu	[-].GLFEAI	K26(alkyne)	1	1	peptide	peptide	0	5	1	0	1	1	796.4266	3978.104	-1.7450	-0.00139	CID (Collis	MS2	2.635605	150	57.89562	23025	2_mod_ox	0/0	2.819271	30472706	57.94647
High	SequestH	Unambigu	[-].GLFEAI	K26(alkyne)	1	1	peptide	peptide	0	4	0.0679	0	1	1	1008.278	4030.089	-4.2550	-0.00429	CID (Collis	MS2	0	150	39.08386	14587	2_mod_ox	0/0	2.799827	4829720	39.09619
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	7	1	0	1	1	569.1655	3978.115	1.009884	0.000574	CID (Collis	MS2	3.54653	150	58.28882	23174	2_mod_ox	0/0	2.7794		
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	6	1	0	1	1	663.8577	3978.11	-0.3522	-0.00023	CID (Collis	MS2	2.207013	150	57.89298	23024	2_mod_ox	0/0	2.65208	41935704	57.98085
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	6	1	0	1	1	663.8596	3978.121	2.501525	0.001659	CID (Collis	MS2	46.86189	150	58.56418	23276	2_mod_ox	0/0	2.628621		
High	SequestH	Unambigu	[-].GLFEAI	K26(alkyne); K27(alkyne	1	1	peptide	peptide	0	4	1	0	1	1	1021.278	4082.089	-2.9515	-0.00301	CID (Collis	MS2	0	150	40.16831	15022	2_mod_ox	0/0	2.584297	19352082	40.06479
High	SequestH	Unambigu	[-].GLFEAI		1	1	peptide	peptide	0	5	1	0	1	1	796.4304	3978.123	3.011215	0.002396	CID (Collis	MS2	0	150	58.82111	23371	2_mod_ox	0/0	2.54528		
High	SequestH	Unambigu	[-].GLFEAI	K26(alkyne); K27(alkyne	1	1	peptide	peptide	0	5	1	0	1	1	817.2231	4082.086	-3.5528	-0.0029	CID (Collis	MS2	0	150	40.35887	15099	2_mod_ox	0/0	2.501196	65599408	40.05726

NMR Spectra

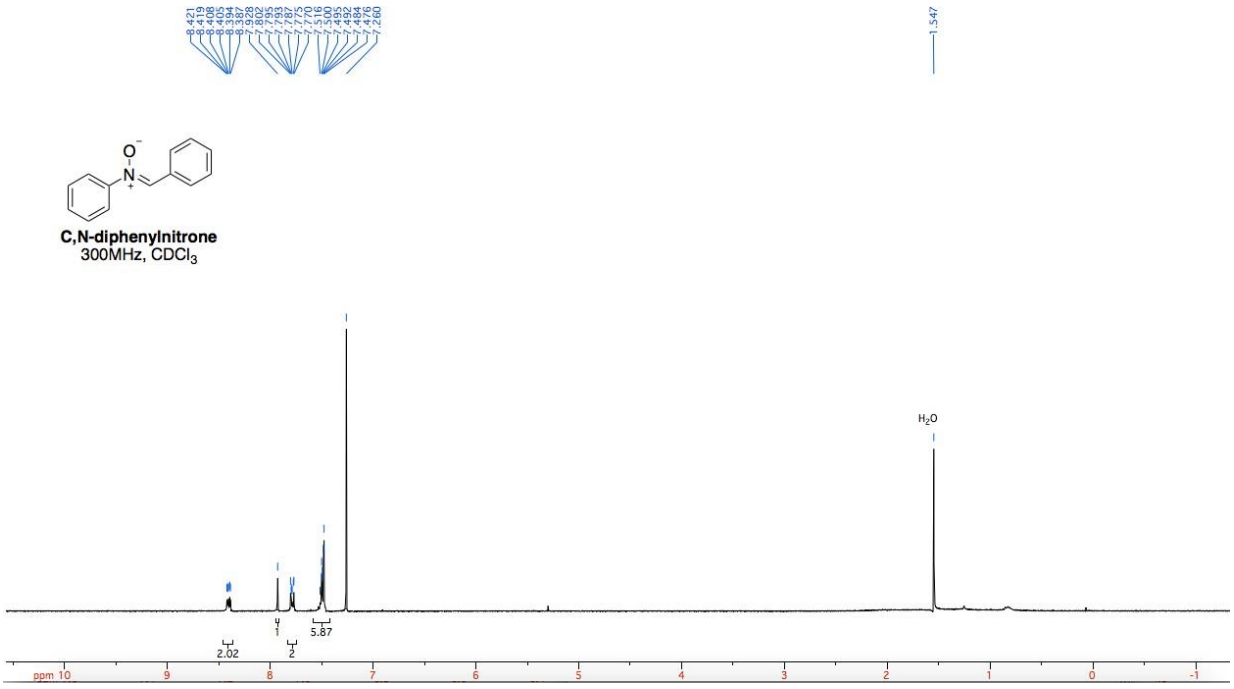
Synthesized Activated Alkynes

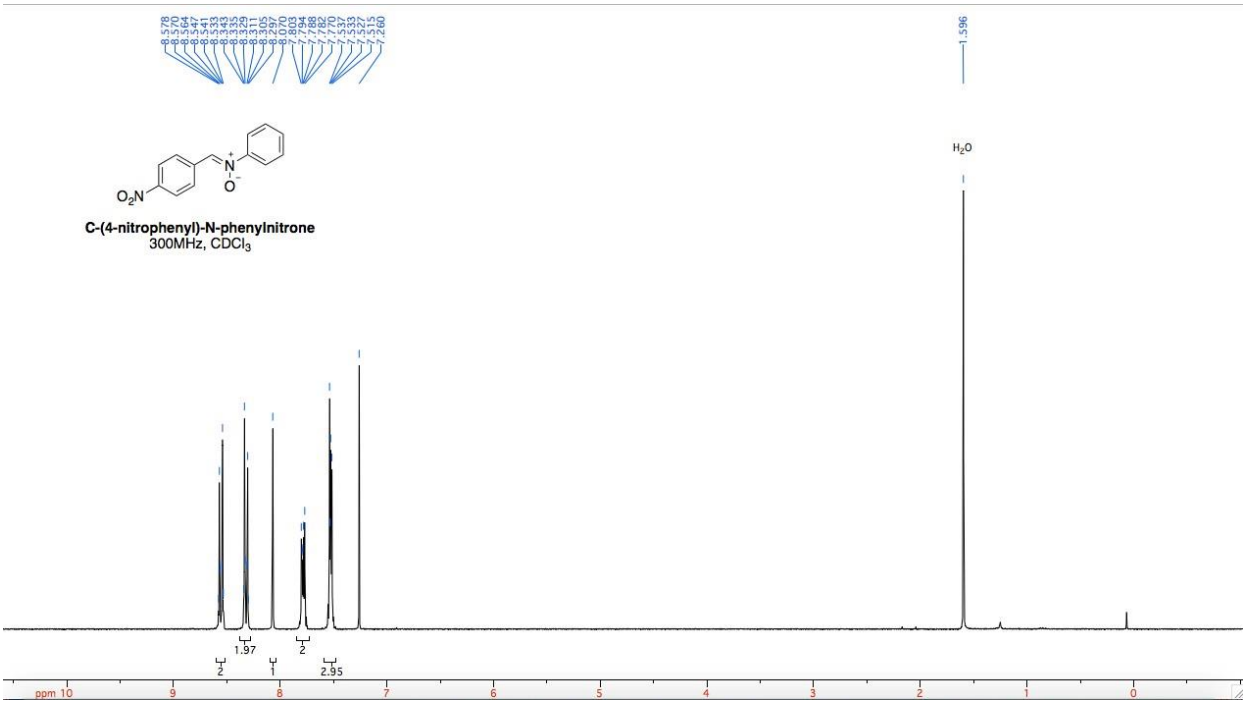






^{1}H NMR of Synthesized Nitrones

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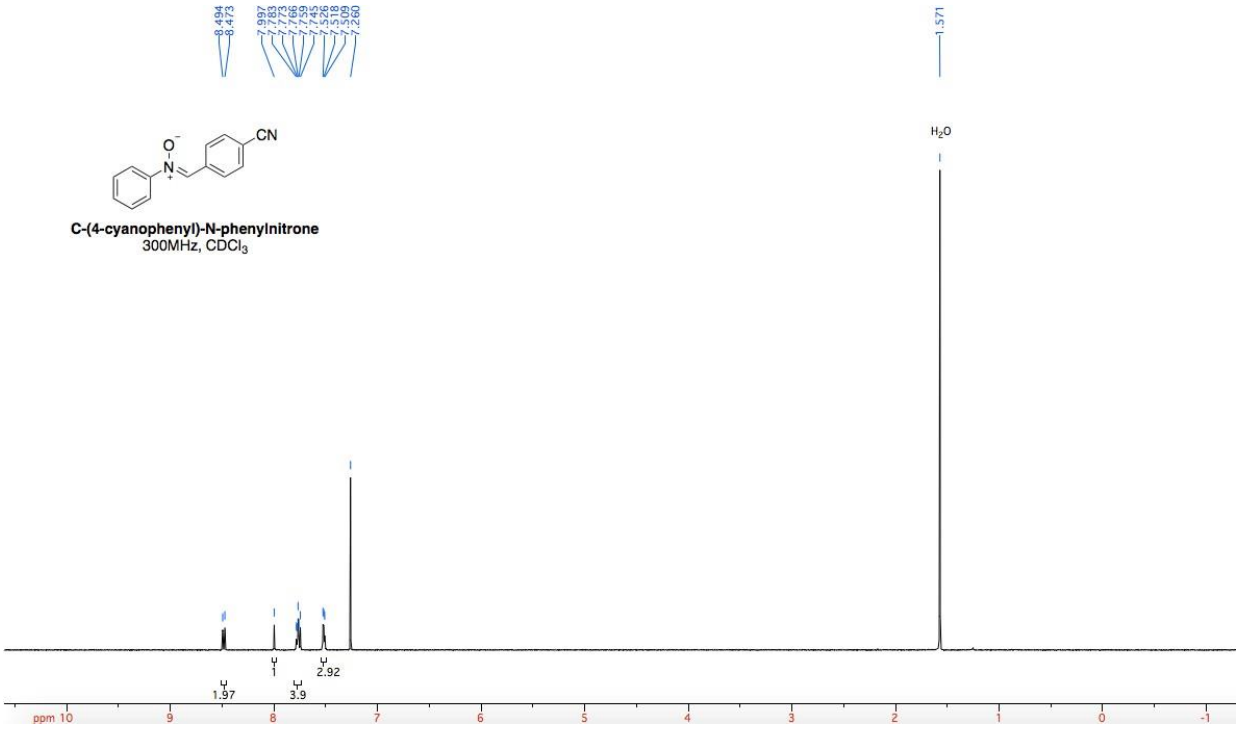
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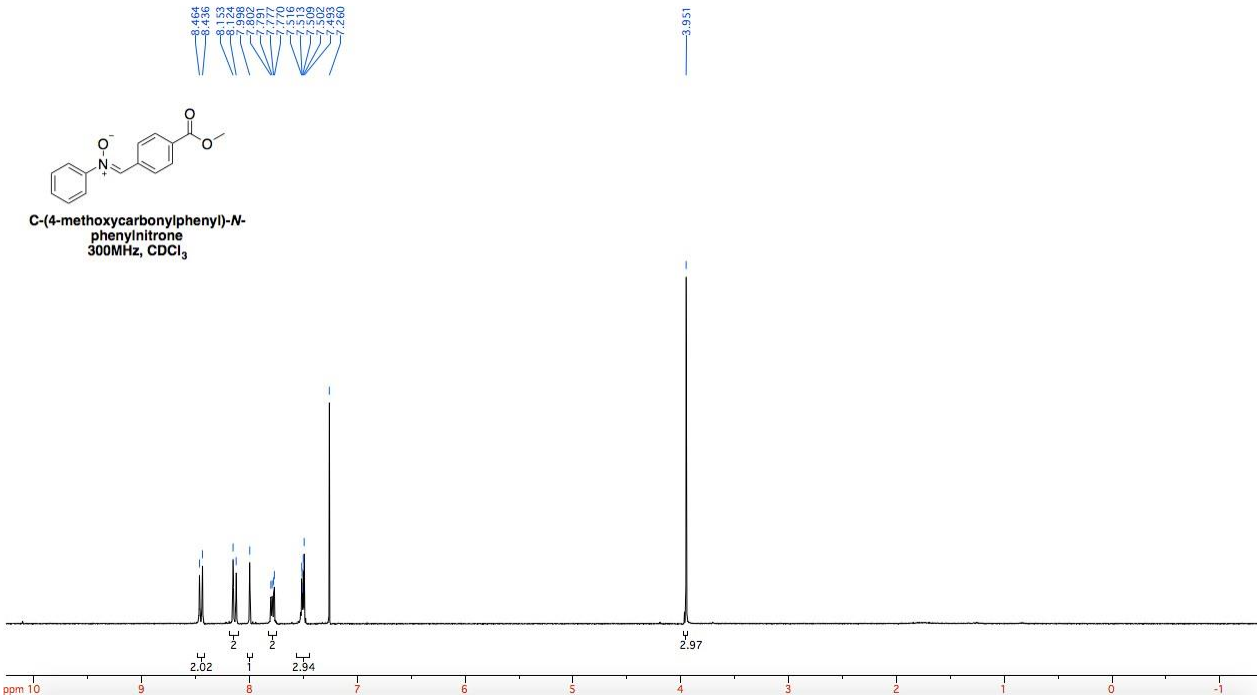
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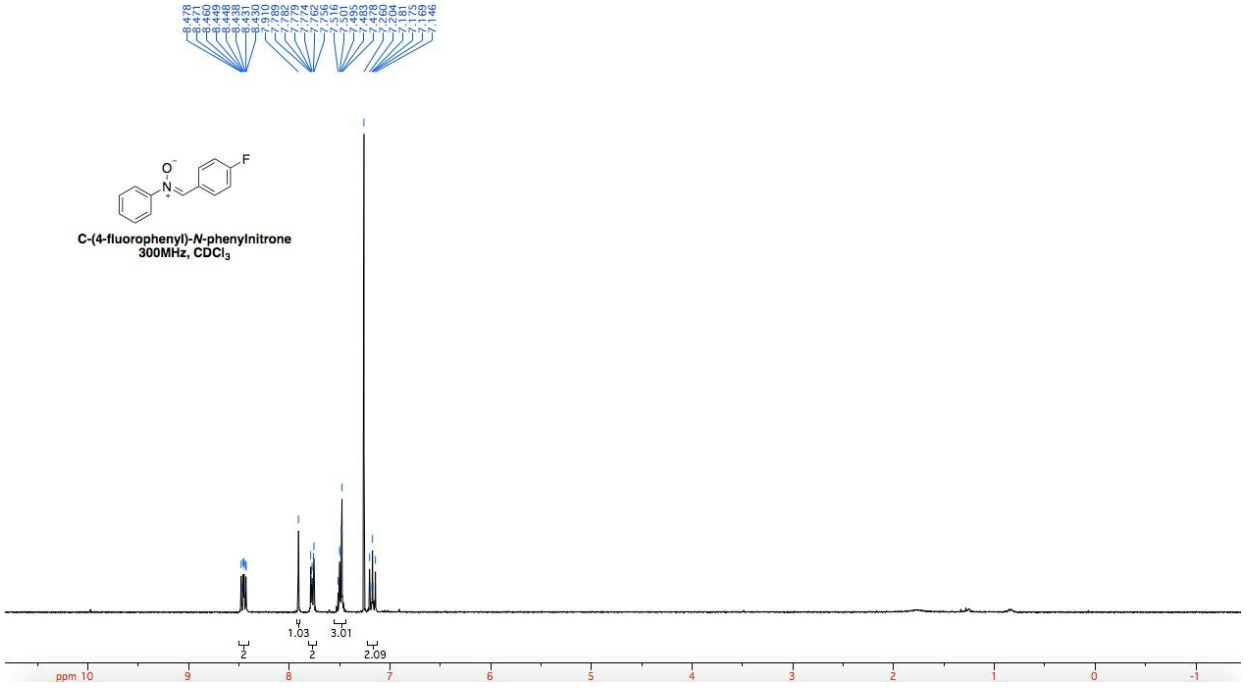
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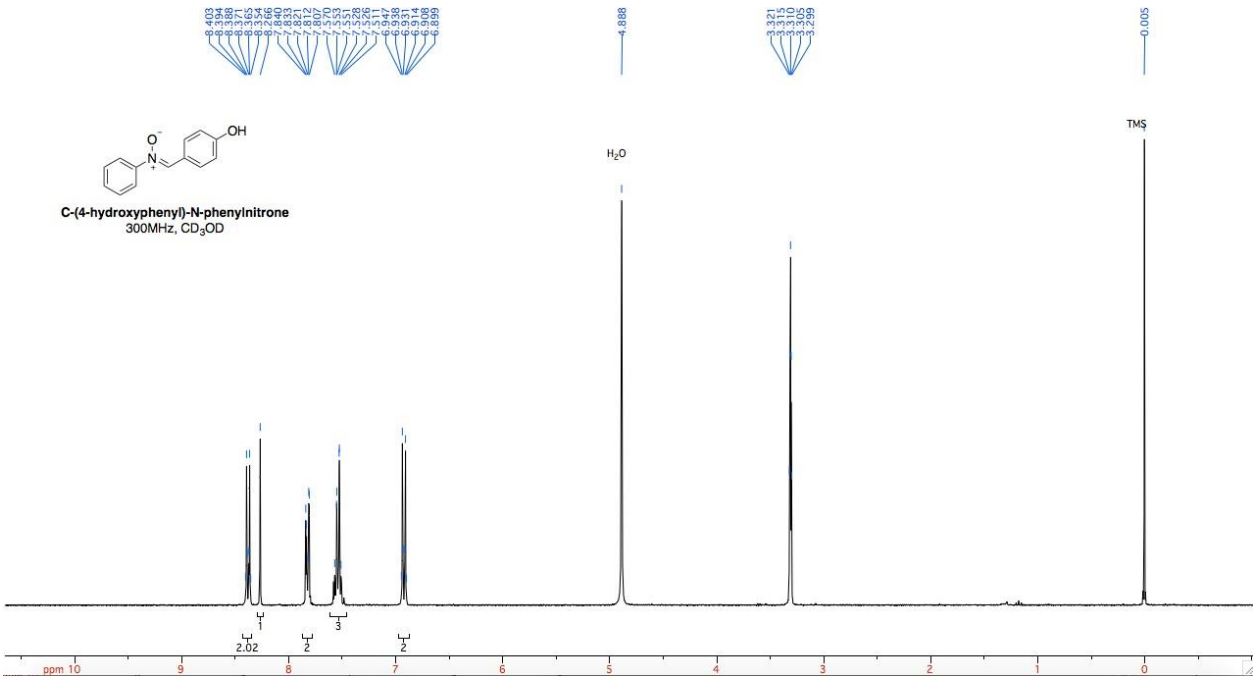
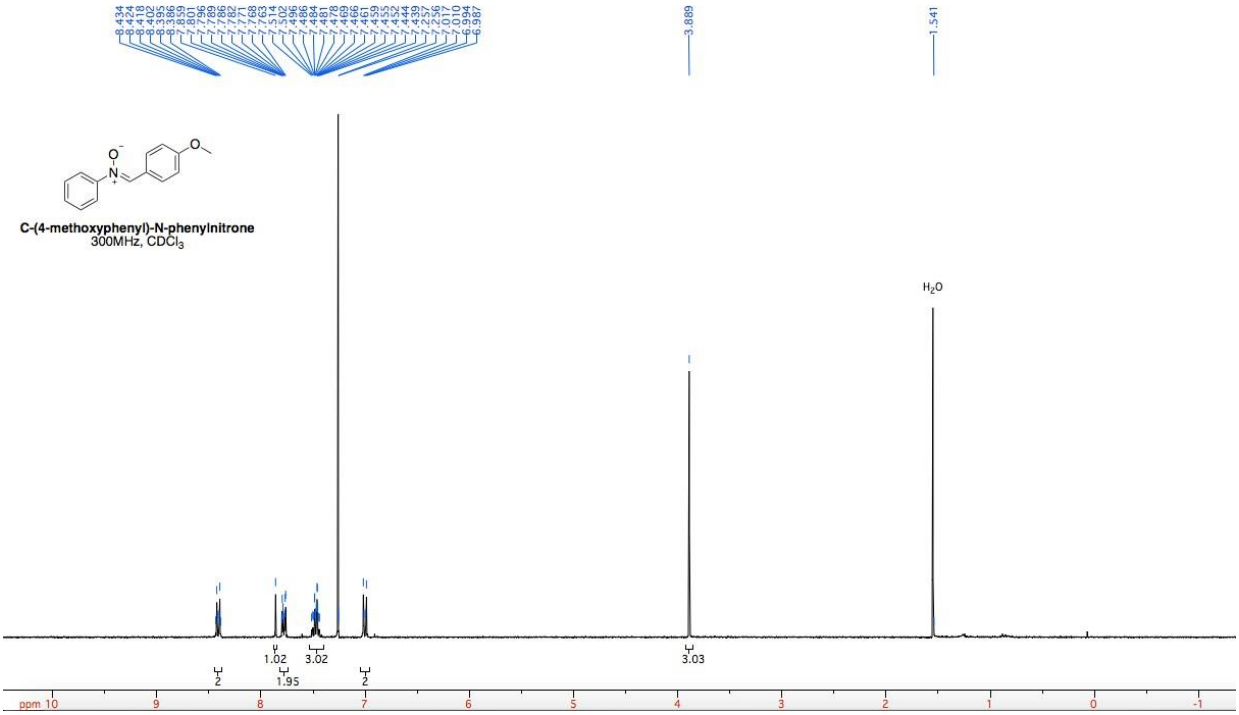
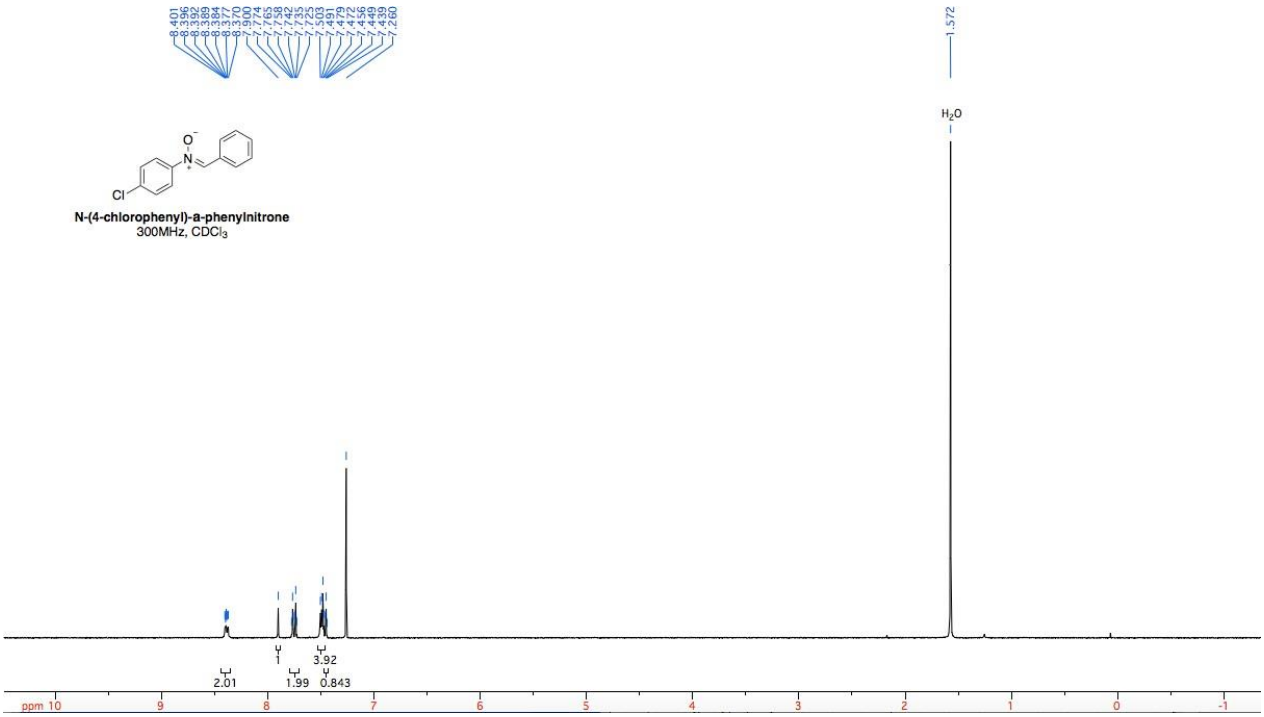
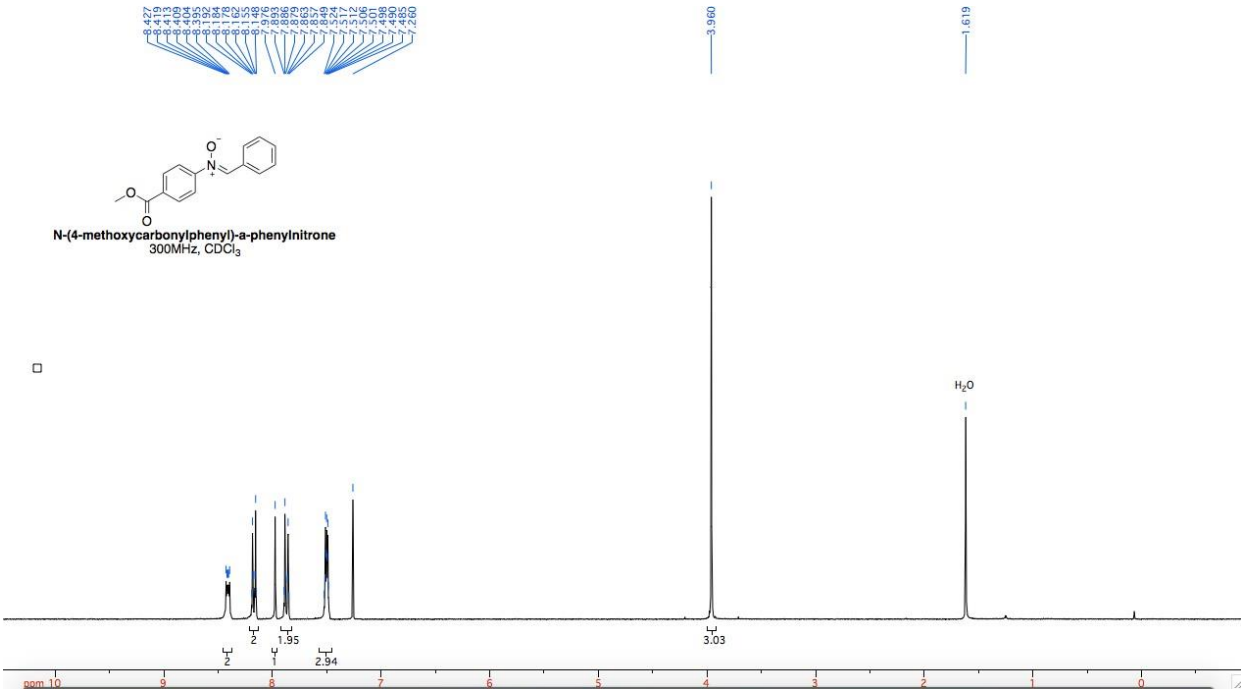
7.483
7.478
7.470
7.204
7.181
7.175
7.169
7.146
C-(4-fluorophenyl)-N-phenylnitrone
300MHz, CDCl3
F
1.03
3.01
2.09
ppm 10
9
8
7
6
5
4
3
2
1
0
-1

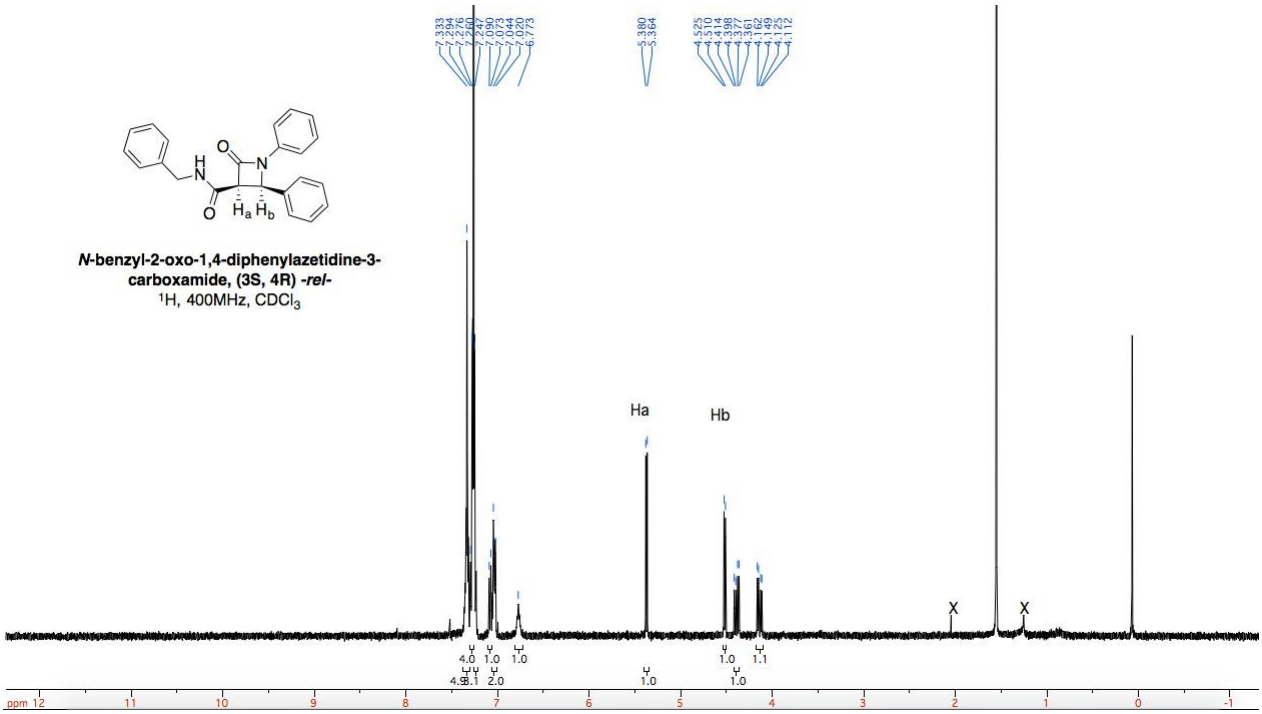

C-(4-hydroxyphenyl)-N-phenylnitrone
300MHz, CD3OD
H2O
TMS
2.02
1
2
3
2
ppm 10


C-(4-methoxyphenyl)-N-phenylnitrone
300MHz, CDCl3
H2O
3.889
1.541
1.02
3.02
2
1.95
2
3.03
ppm 10

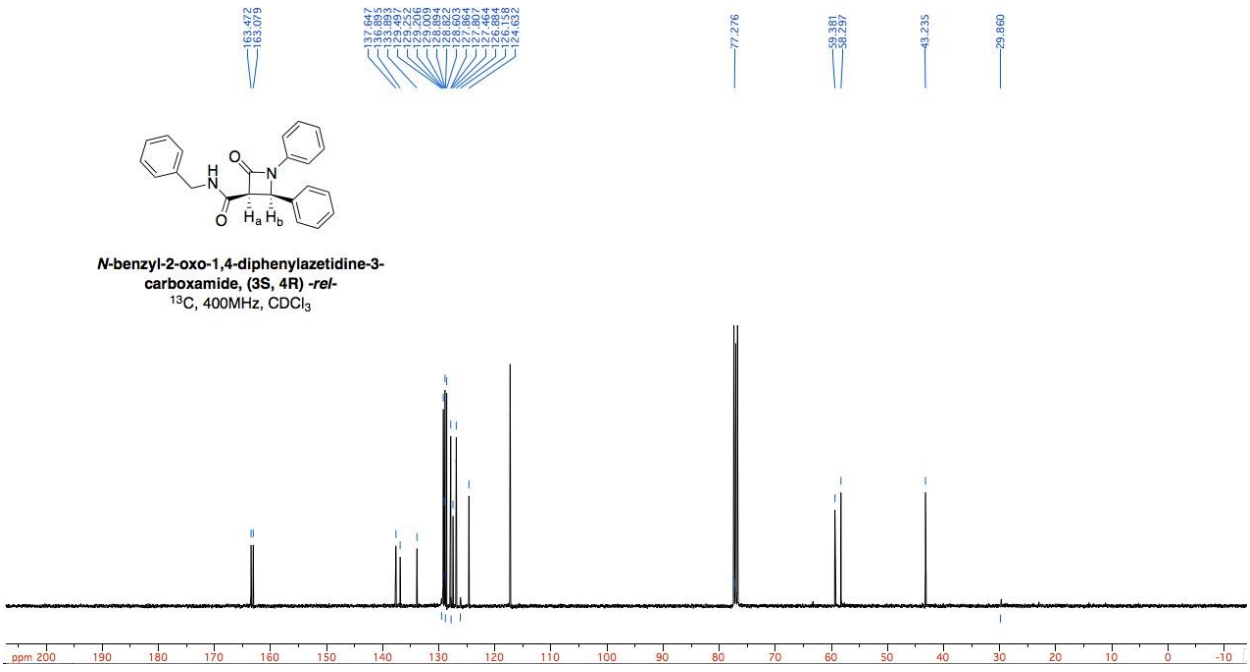

8.401
8.396
8.392
8.389
8.384
8.377
8.370
7.990
7.974
7.765
7.758
7.742
7.735
7.725
7.503
7.491
7.479
7.472
7.456
7.449
7.439
7.260
1.572
H2O
Cl
N-(4-chlorophenyl)-a-phenylnitrone
300MHz, CDCl3
2.01
1
1.99
3.92
0.843
ppm 10
9
8
7
6
5
4
3
2
1
0
-1


8.427
8.419
8.413
8.409
8.404
8.395
8.192
8.184
8.178
8.162
8.155
8.148
7.976
7.883
7.880
7.879
7.863
7.857
7.849
7.524
7.517
7.512
7.506
7.501
7.498
7.490
7.485
7.260
3.960
1.619
H2O
N-(4-methoxycarbonylphenyl)-a-phenylnitrone
300MHz, CDCl3
2
2
1
1.95
1
2.94
3.03
ppm 10
9
8
7
6
5
4
3
2
1
0

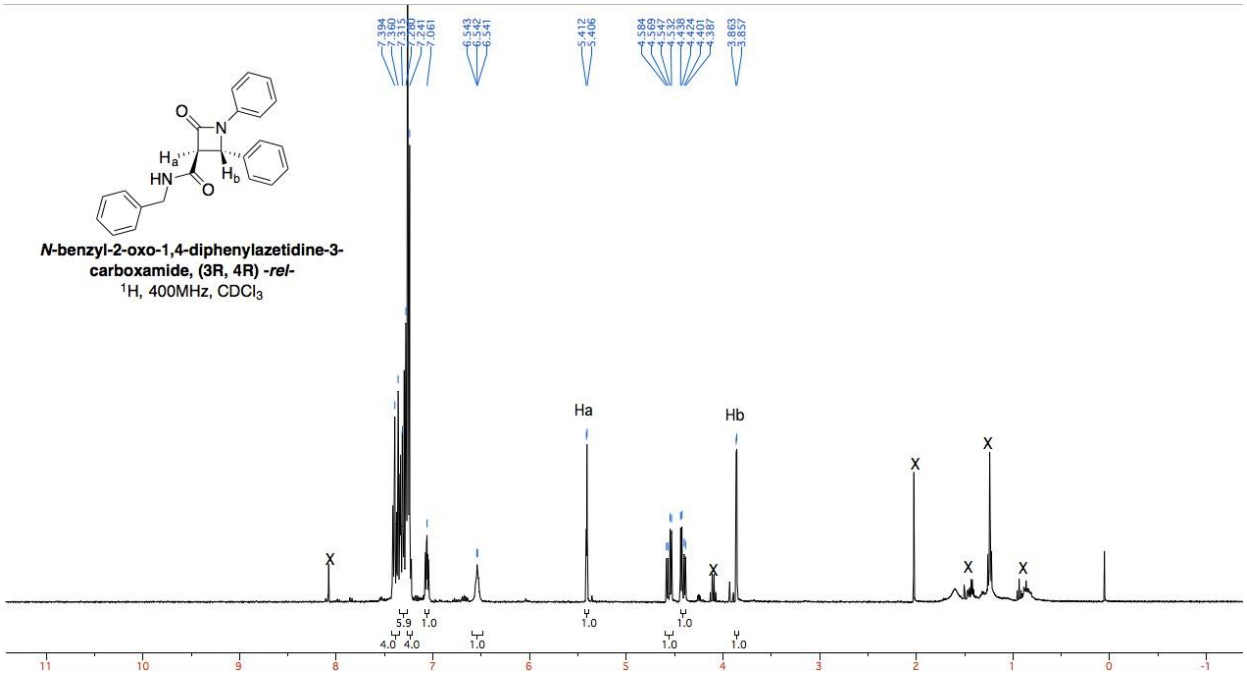
Characterization ß-Lactams for Table 1



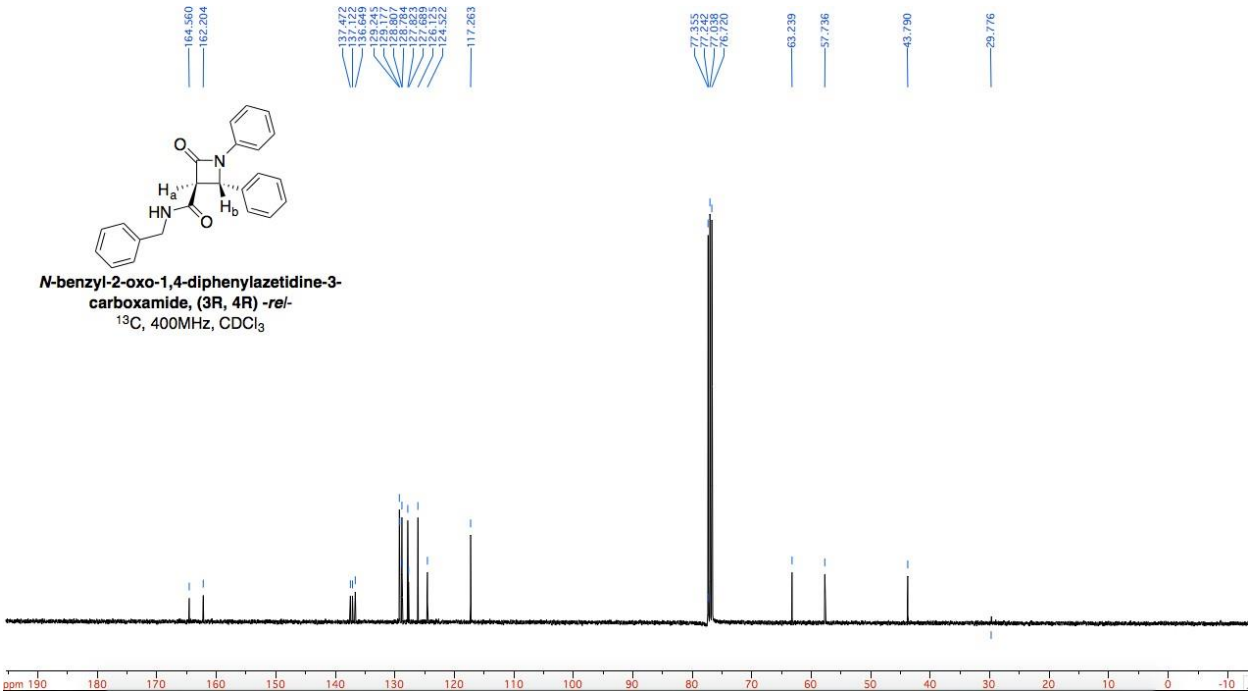
N*-benzyl-2-oxo-1,4-diphenylazetidine-3-carboxamide, (3S, 4R) *-rel-
^{1}H, 400MHz, $CDCl_3$



N*-benzyl-2-oxo-1,4-diphenylazetidine-3-carboxamide, (3S, 4R) *-rel-
^{13}C, 400MHz, $CDCl_3$

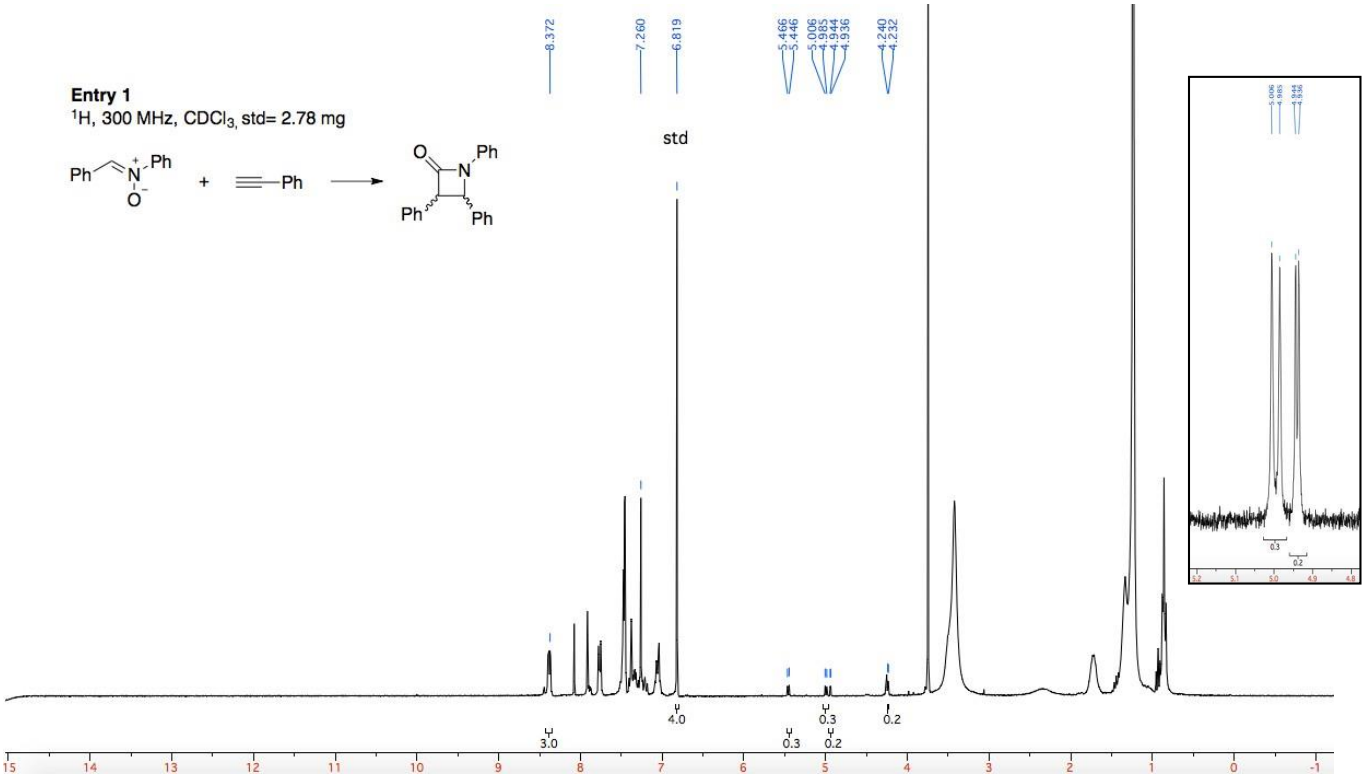


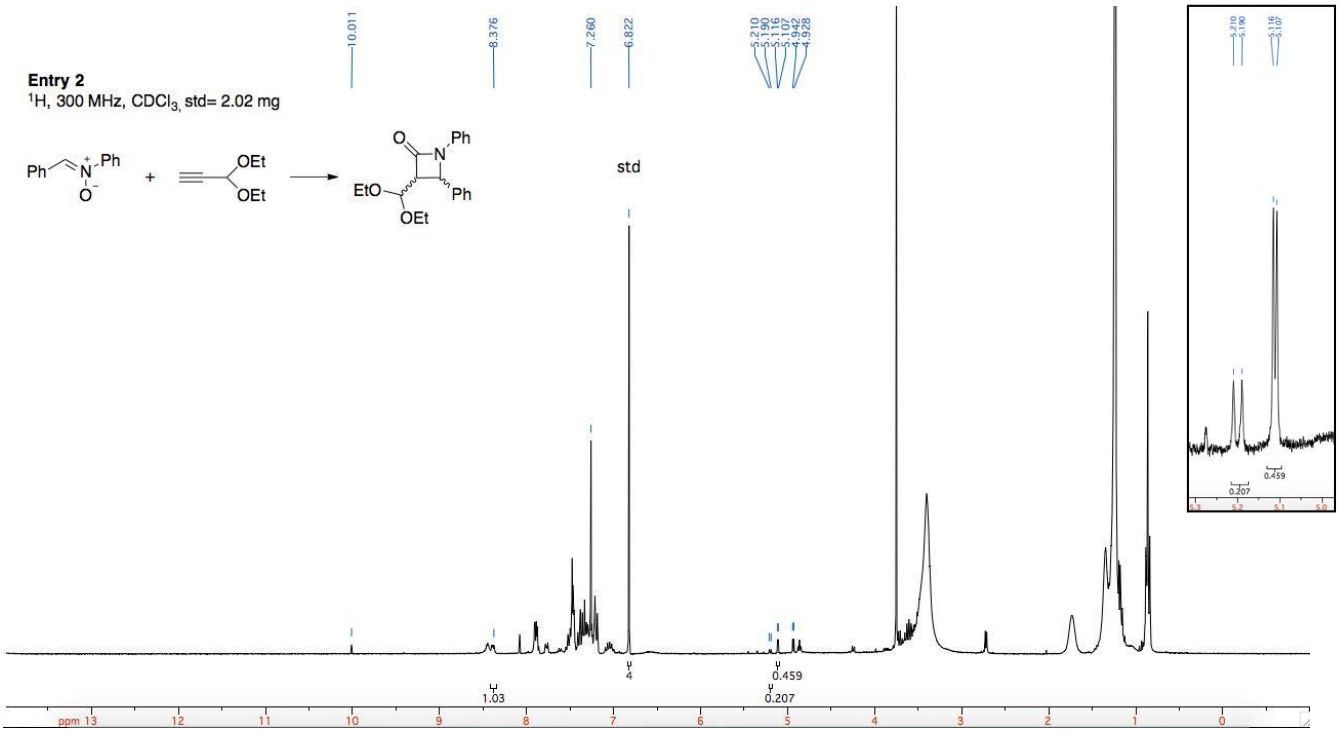
N*-benzyl-2-oxo-1,4-diphenylazetidine-3-carboxamide, (3R, 4R) *-rel-
^{1}H, 400MHz, $CDCl_3$

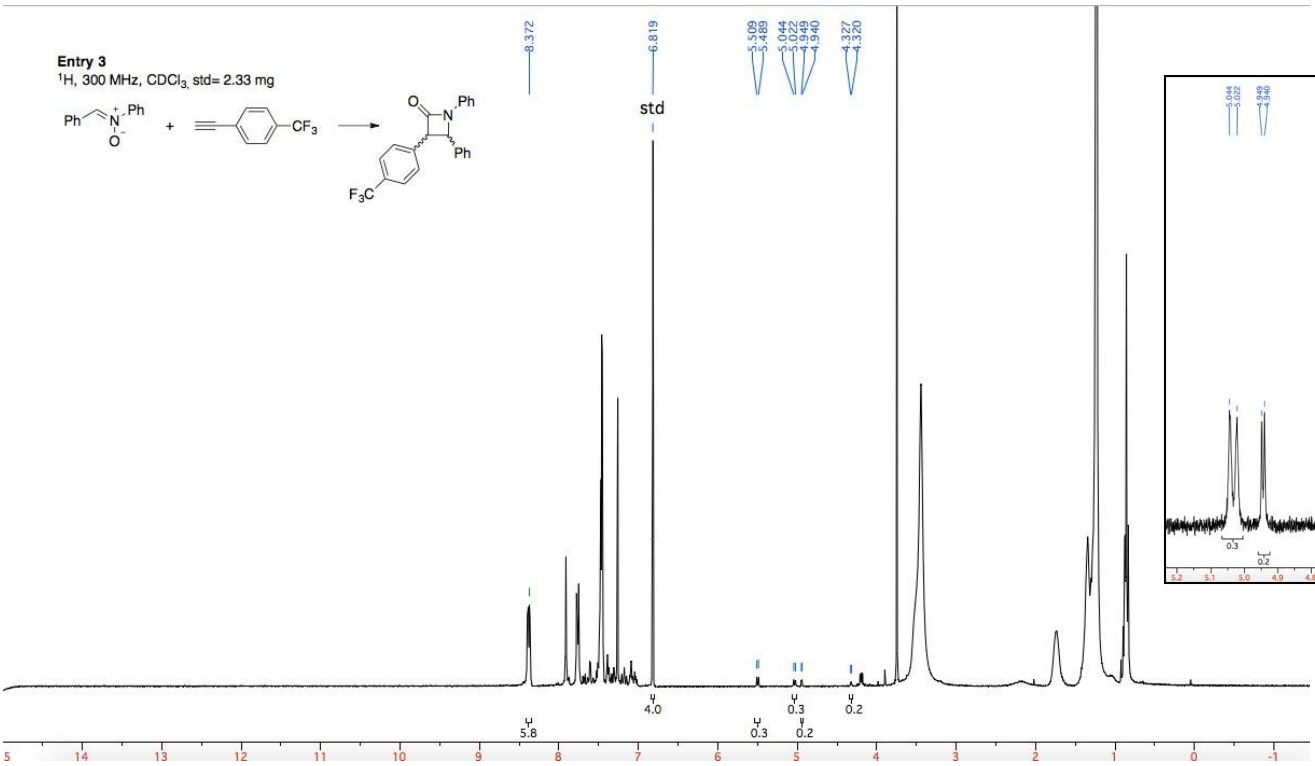


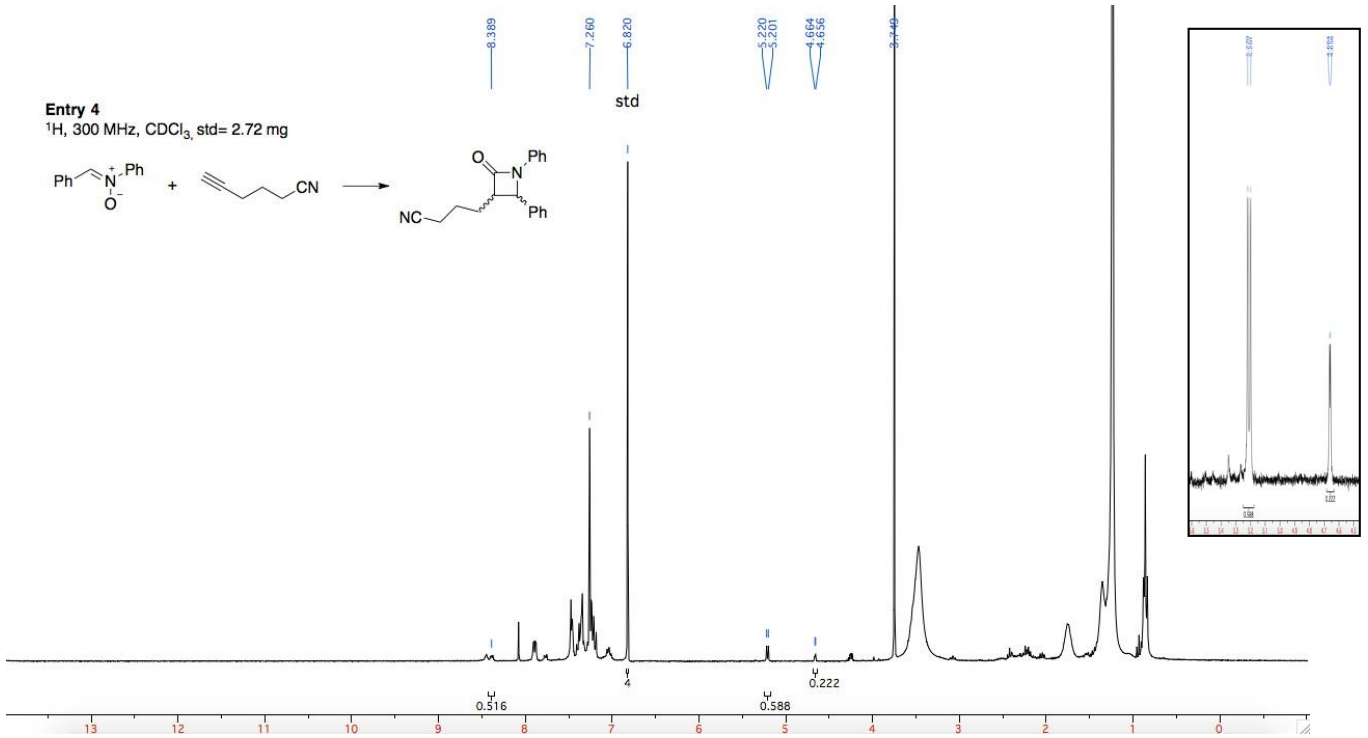
N*-benzyl-2-oxo-1,4-diphenylazetidine-3-carboxamide, (3R, 4R) *-rel-
^{13}C, 400MHz, $CDCl_3$

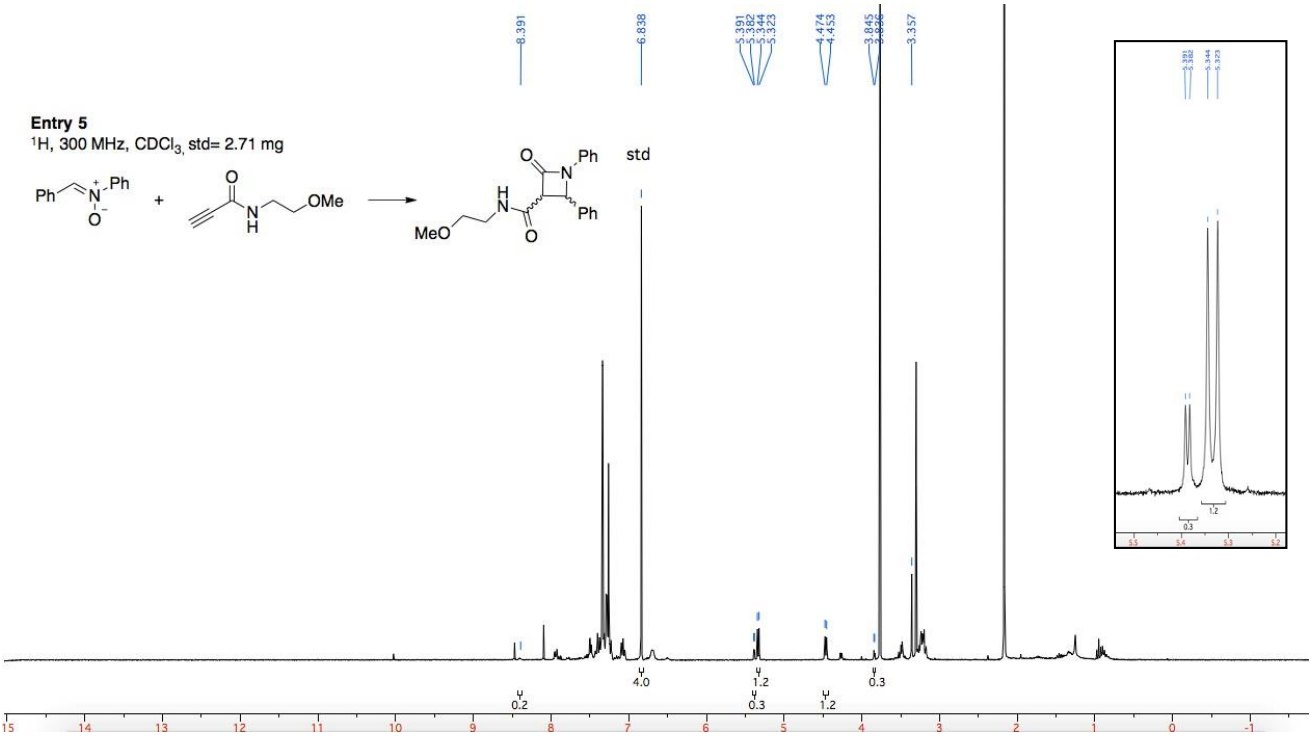
NMR Data for Table 1. Screen of Alkyne Reactivity

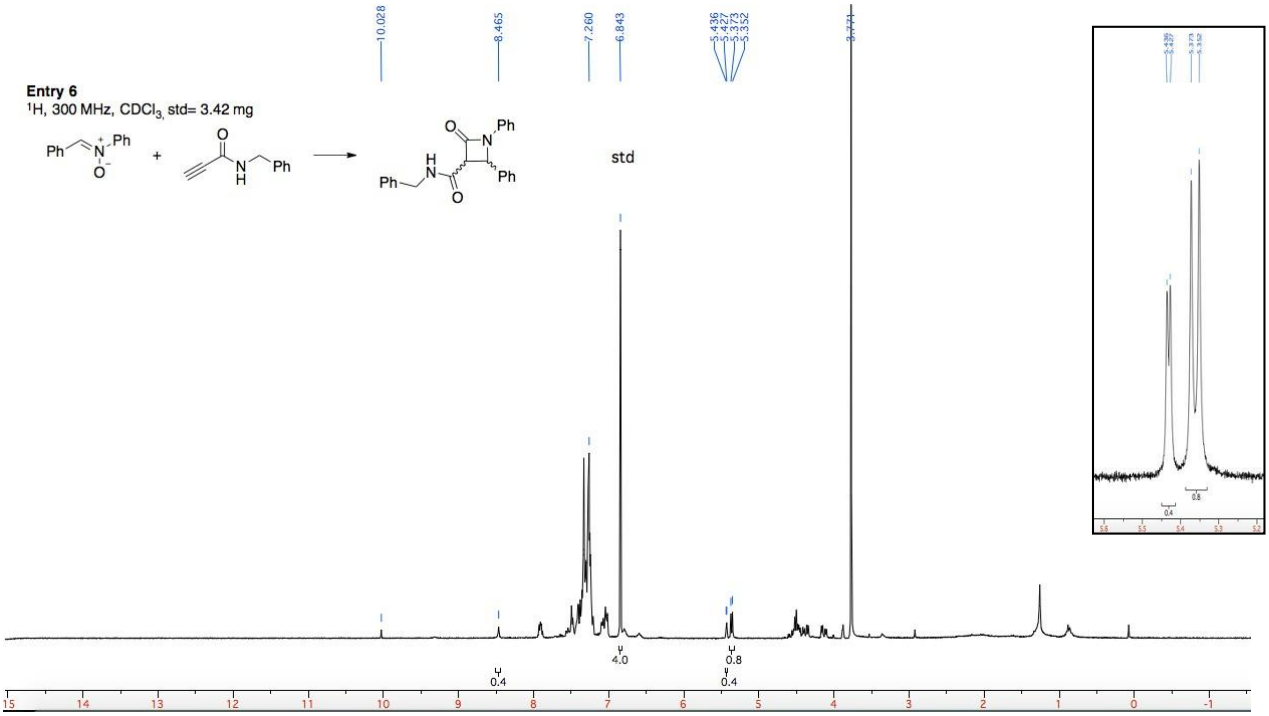


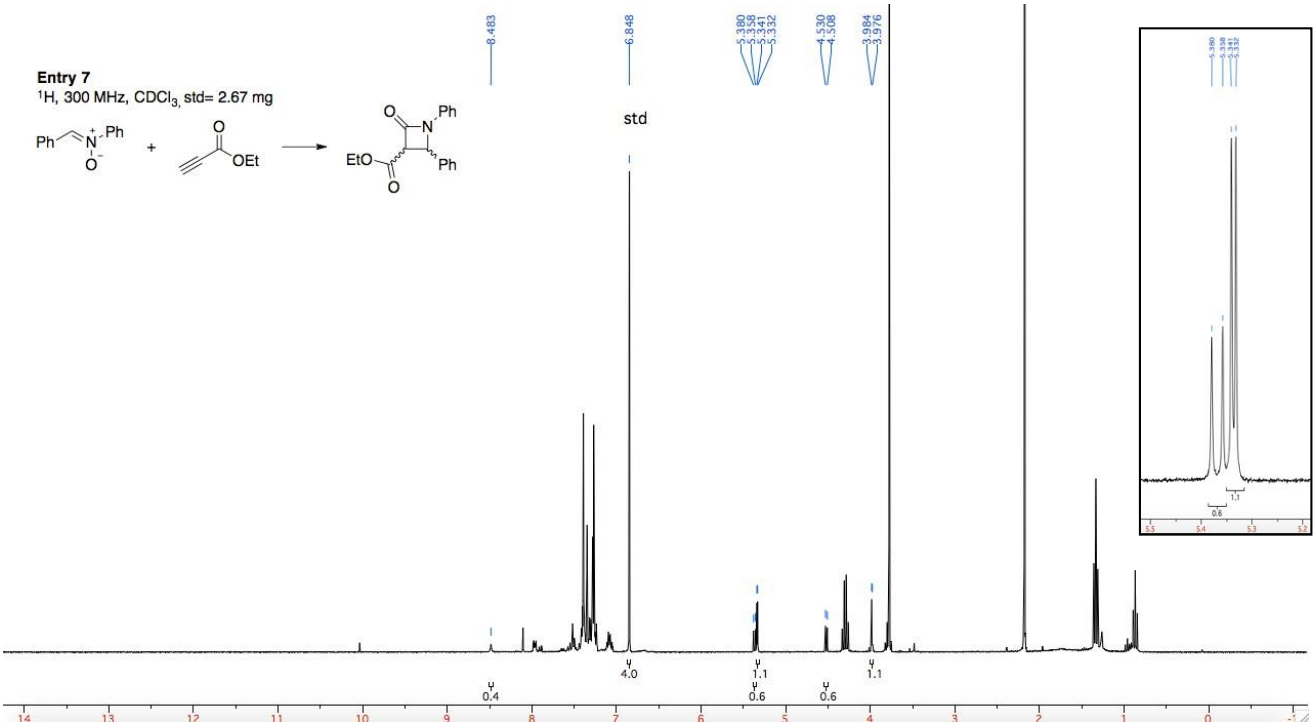


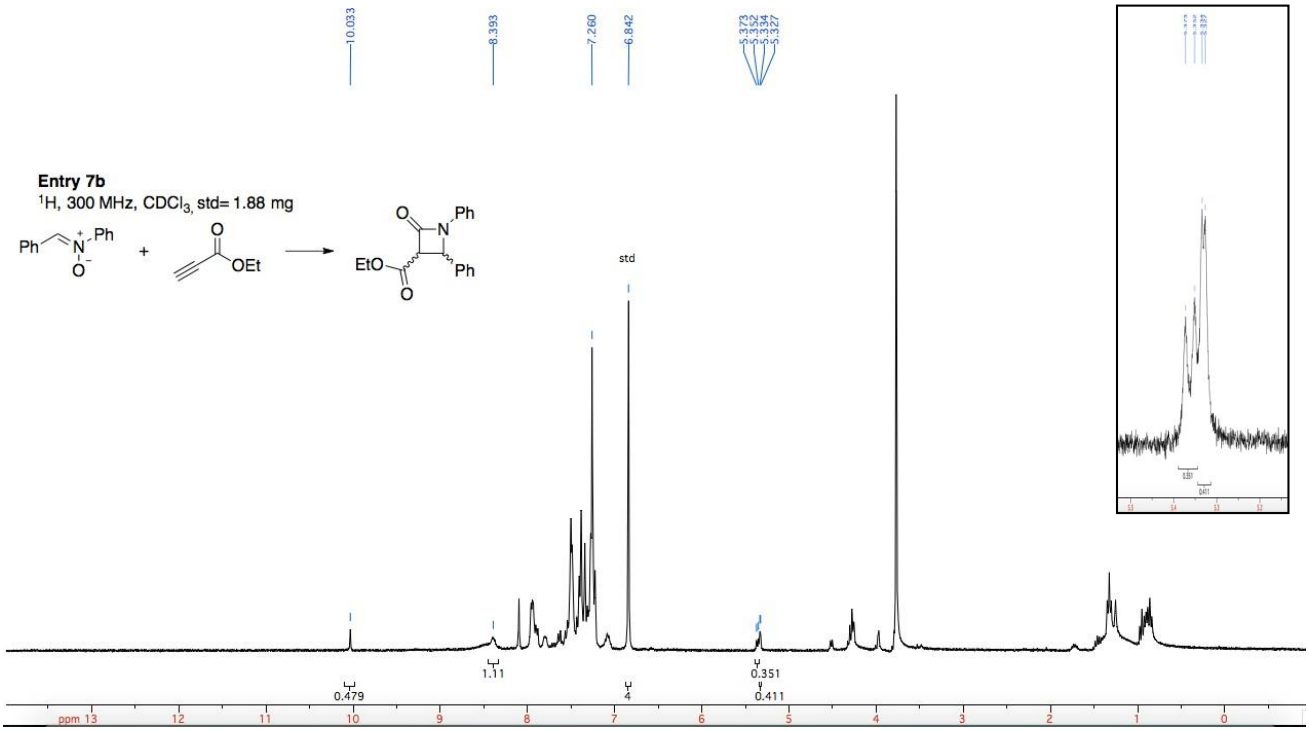

Entry 3
^{1}H, 300 MHz, $CDCl_3$, std= 2.33 mg
8.372
6.819
std
5.509
5.489
5.044
5.022
4.940
4.327
4.320
5.8
4.0
0.3
0.3
0.2
0.2


Entry 4
^{1}H, 300 MHz, $CDCl_3$, std= 2.72 mg
8.389
7.260
6.820
std
5.220
5.201
4.664
4.656
0.516
4
0.588
0.222


Entry 5
1H, 300 MHz, CDCl3, std= 2.71 mg
Ph
N
Ph
O
+
O
N
H
OMe
MeO
O
N
Ph
H
N
Ph
O
std
8.391
6.838
3.845
3.357
4.0
1.2
0.3
0.2
1.2
0.3


Entry 6
1H, 300 MHz, CDCl3, std= 3.42 mg
Ph
N
Ph
O
+
O
N
H
Ph
Ph
N
Ph
H
N
Ph
O
std
10.028
8.465
7.260
6.843
3.436
3.427
3.373
3.352
9.771
4.0
0.8
0.4
0.4


Entry 7
1H, 300 MHz, CDCl3, std= 2.67 mg
Ph
N
Ph
O
+
O
OEt
EtO
O
N
Ph
Ph
O
8.483
6.848
std
5.380
5.358
5.341
5.332
4.530
4.508
3.984
3.976
4.0
1.1
0.6
0.4
1.1
0.6


Entry 7b
1H, 300 MHz, CDCl3, std= 1.88 mg
Ph
N
Ph
O
+
O
OEt
EtO
O
N
Ph
Ph
O
10.033
8.393
7.260
6.842
std
5.373
5.352
5.344
5.327
0.479
1.11
4
0.351
0.411
ppm 13

NMR Data for Table S2. Screen of Nitrone Reactivity

Table S2 yields were calculated based on ^{1}H NMR Spectra recorded with a frequency of 300MHz. NMR yields are reported using 1,4-Dimethoxybenzene as internal standard.

Table S2. Reaction **1A.**
Std= 2.34 mg

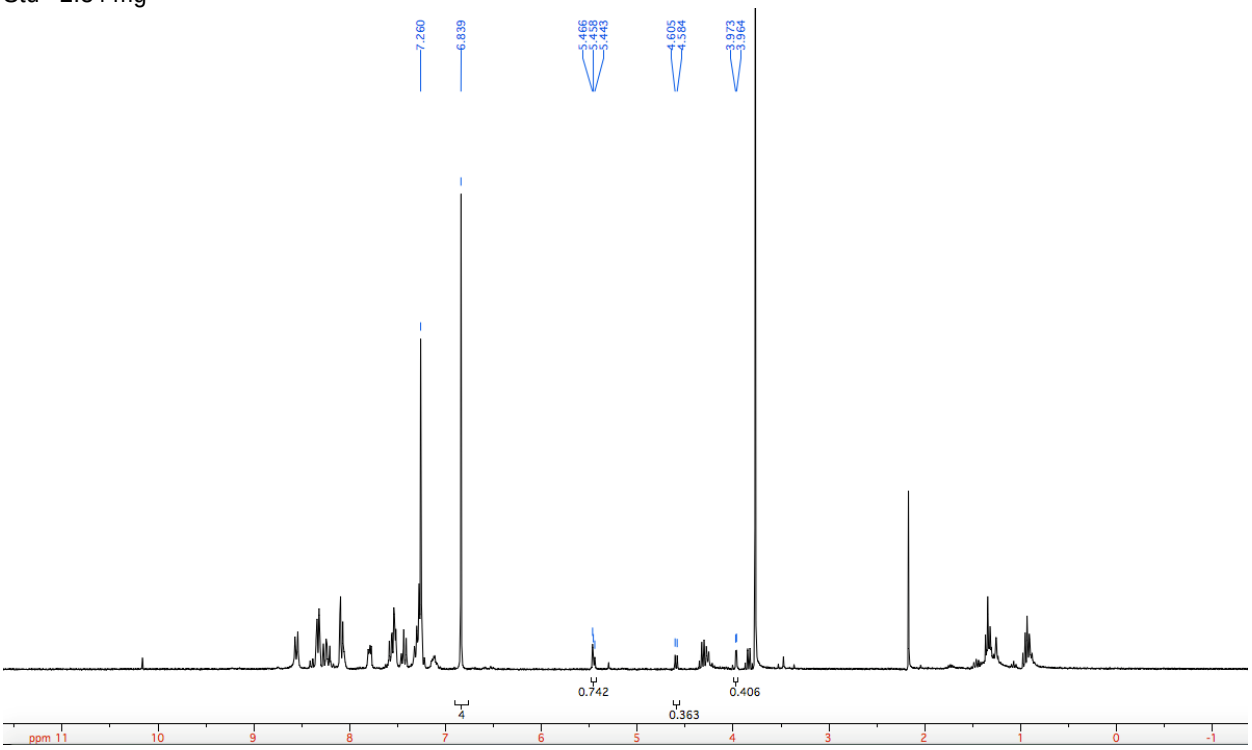


Table S2. Reaction **1B.**
Std= 2.32 mg

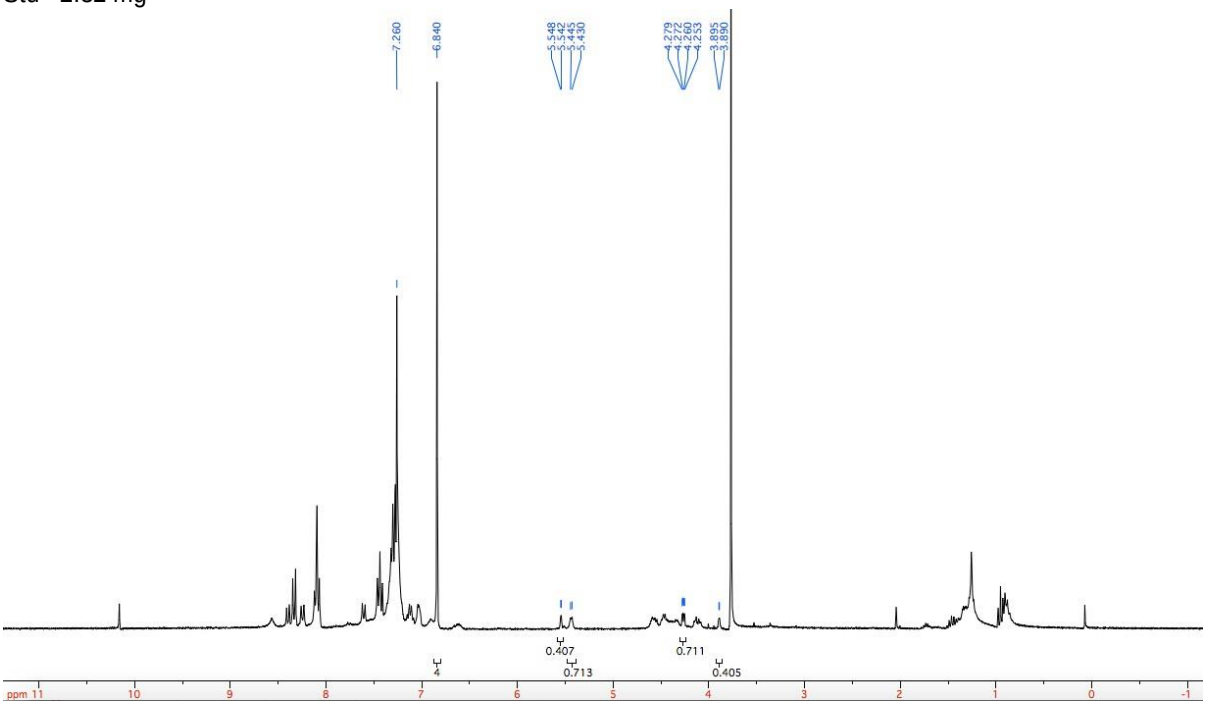


Table S2. Reaction **1C.**
Std= 2.50 mg

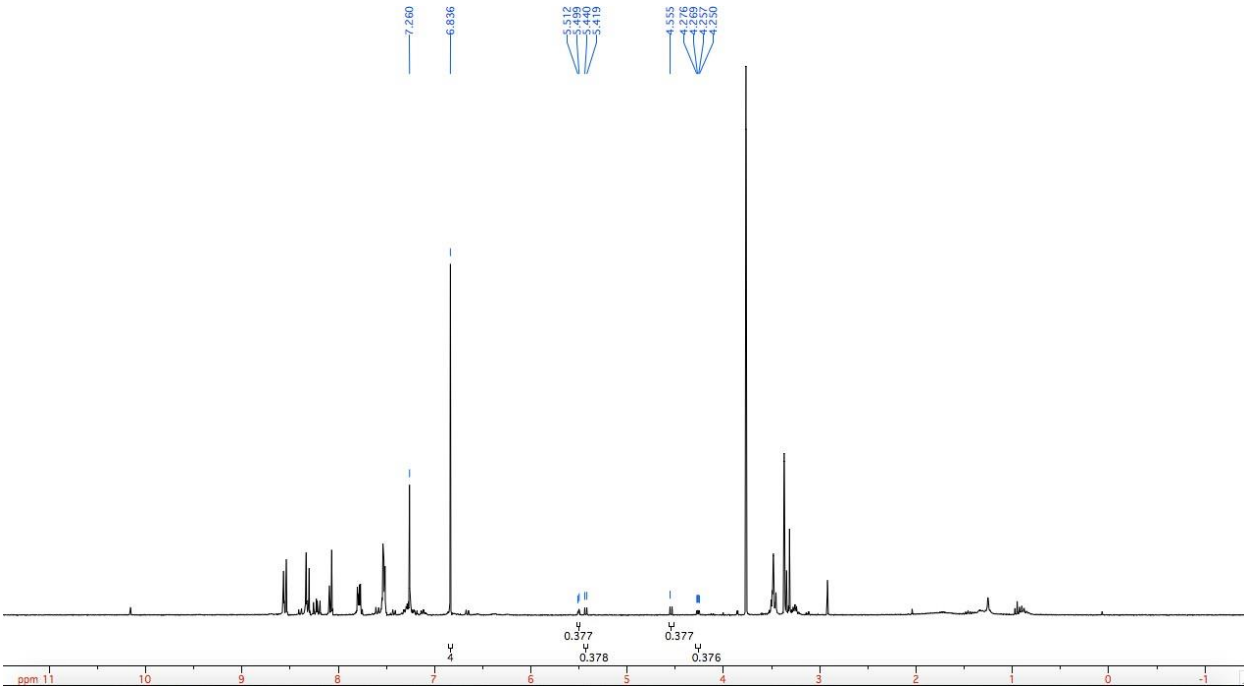


Table S2. Reaction **2A.**

Std= 1.76 mg

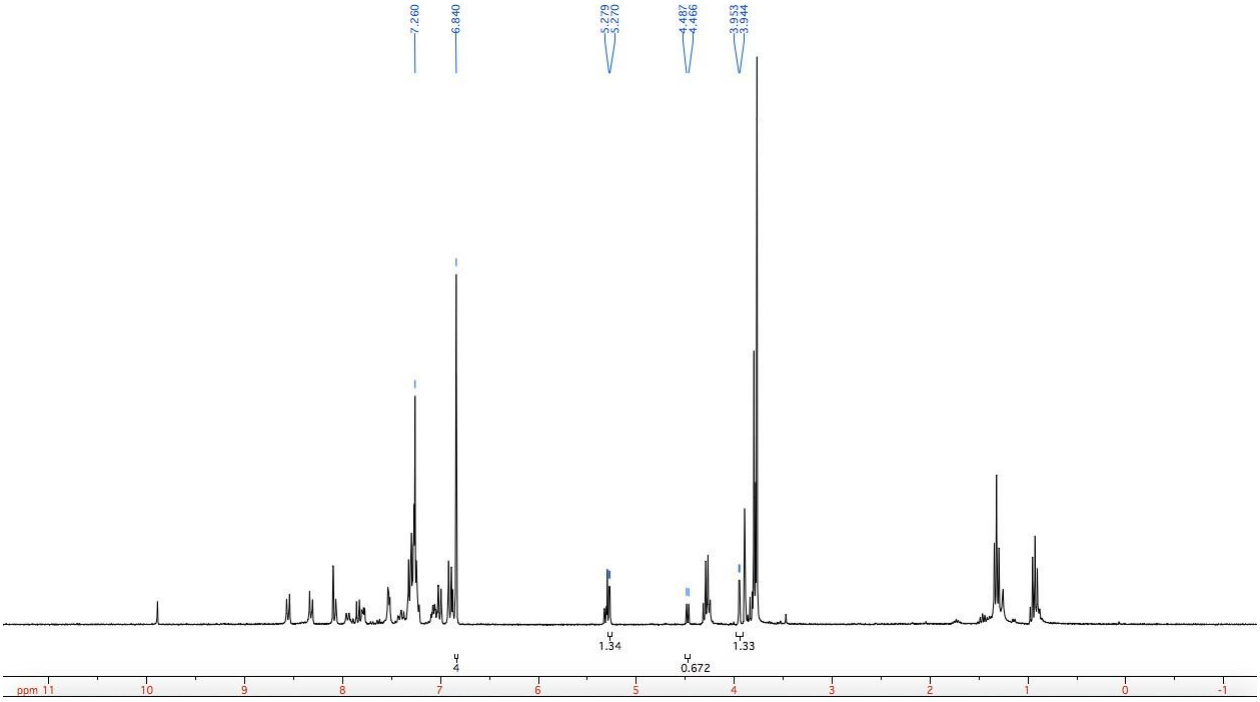


Table S2. Reaction **2B.**

Std= 2.90 mg

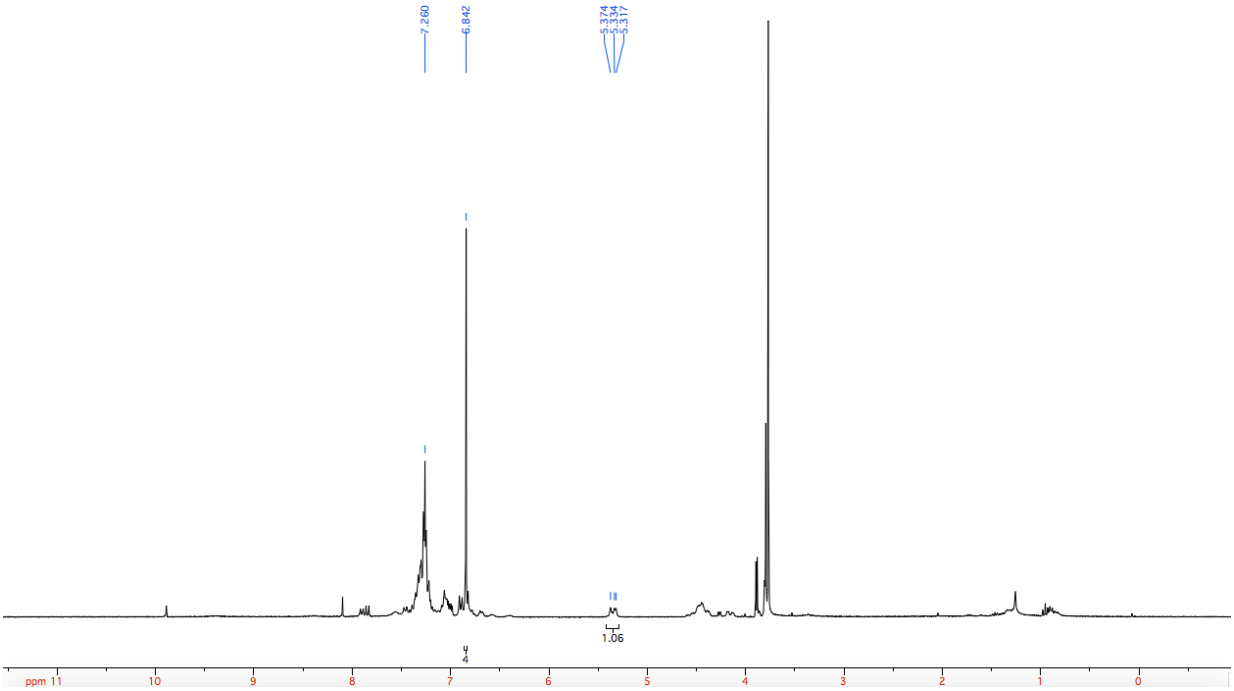


Table S2. Reaction **2C.**

Std= 2.47 mg

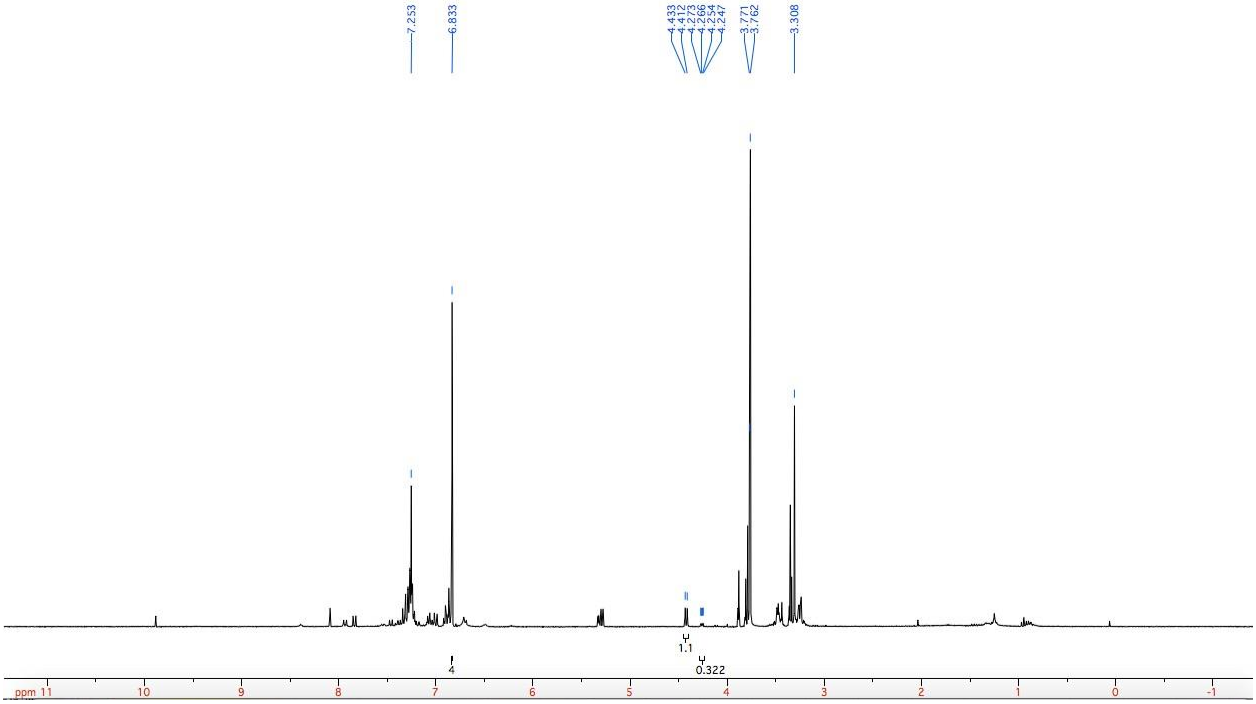


Table S2. Reaction **3A**

Std= 1.82 mg

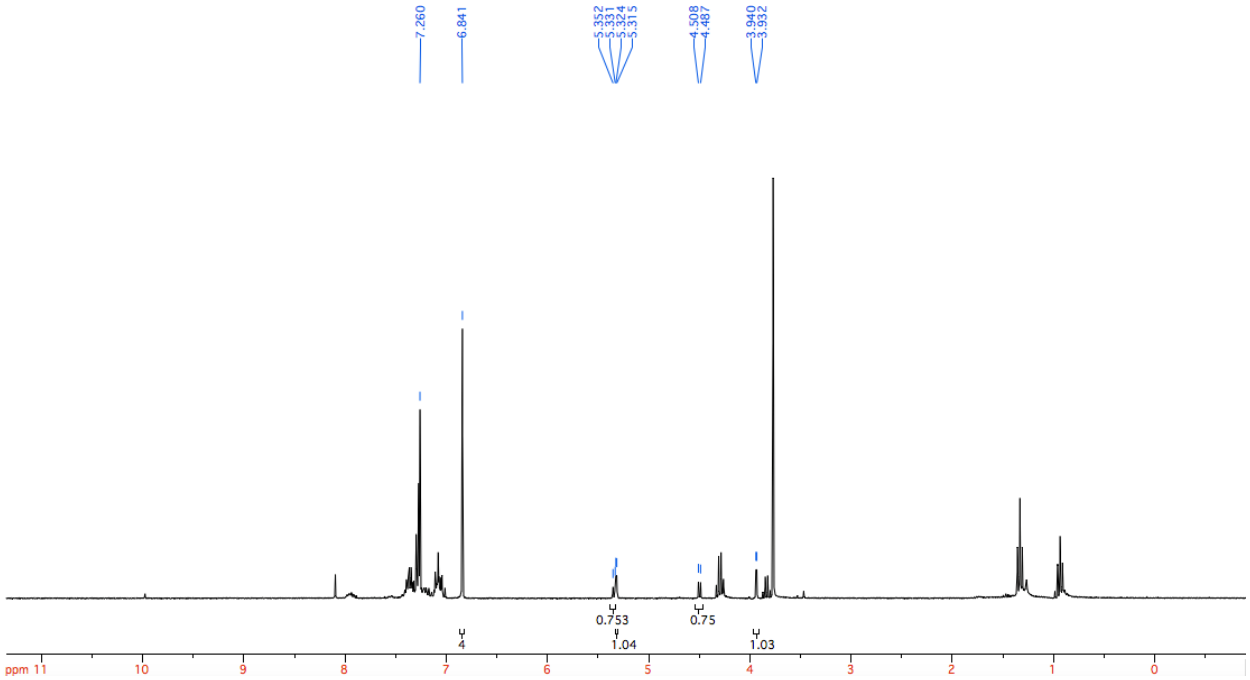


Table S2. Reaction **3B.**

Std= 1.81 mg

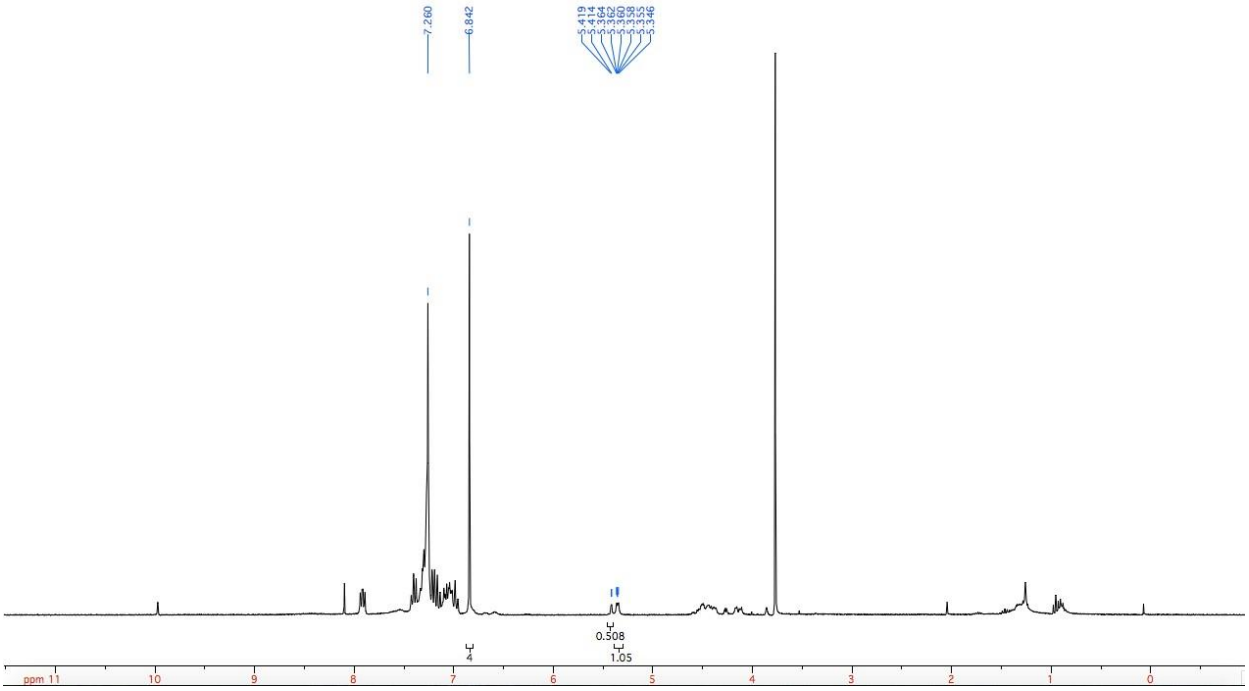


Table S2. Reaction **3C.**

Std= 1.52 mg

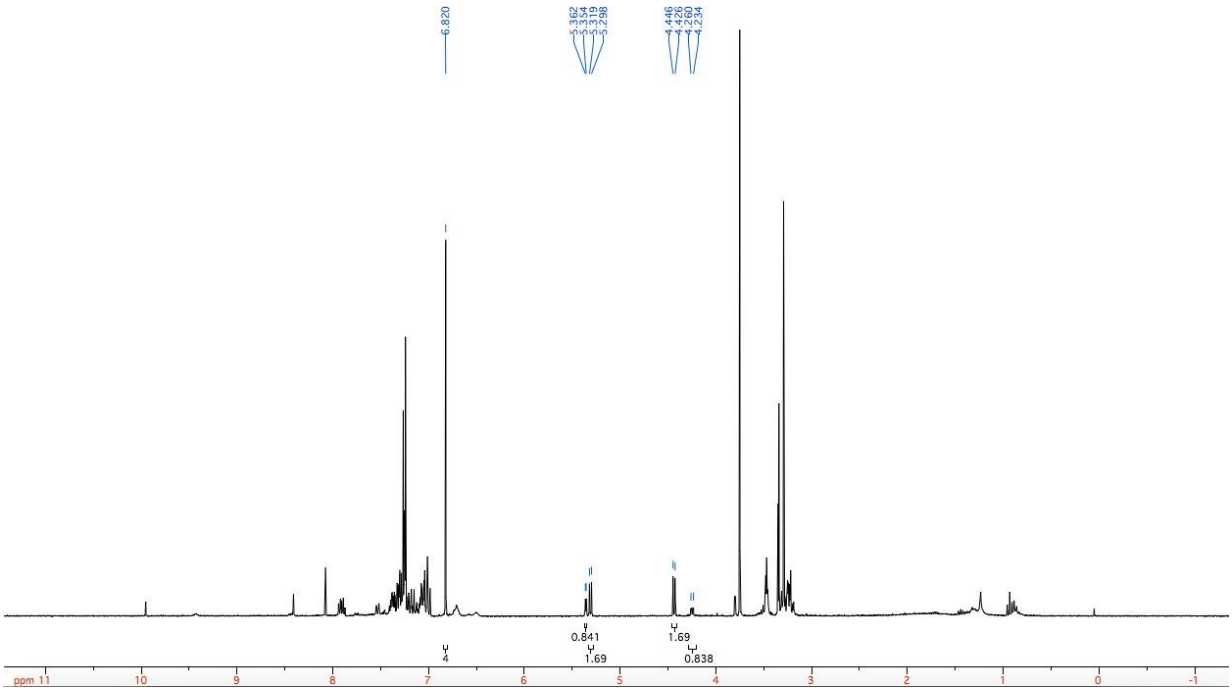


Table S2. Reaction **4A.**

Std= 1.90 mg

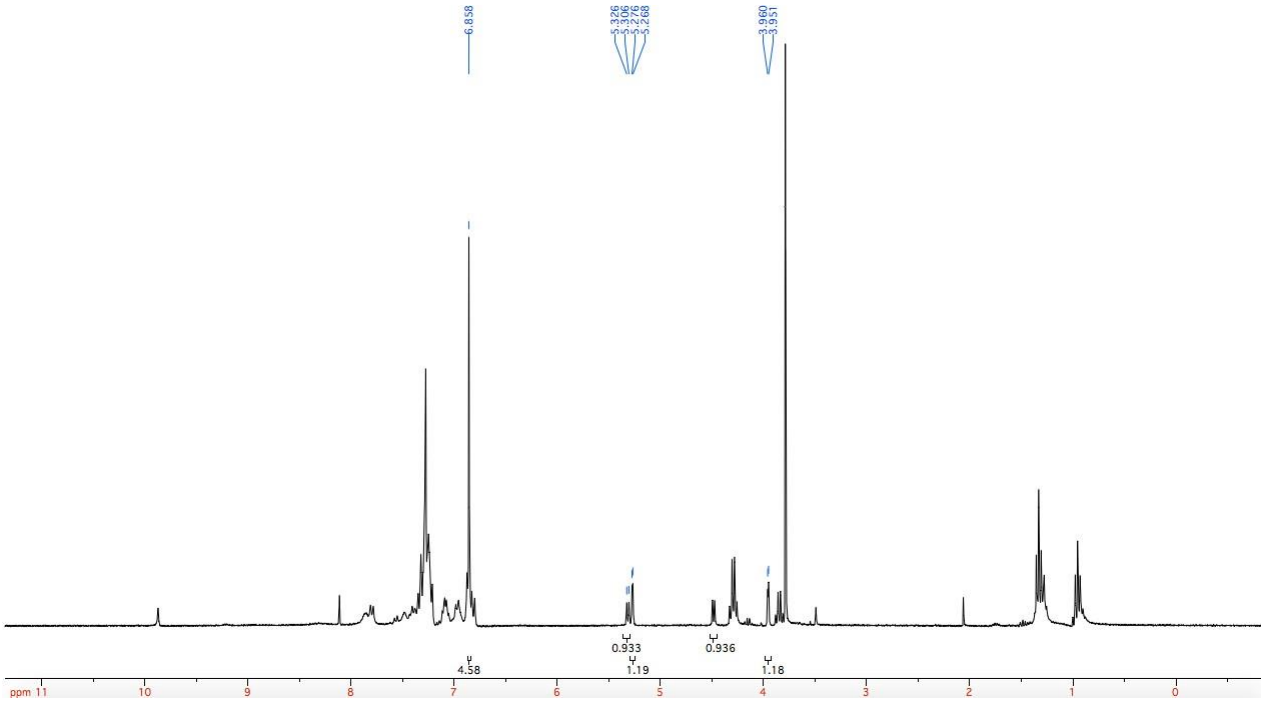


Table S2. Reaction **4B.**

Std= 1.92 mg

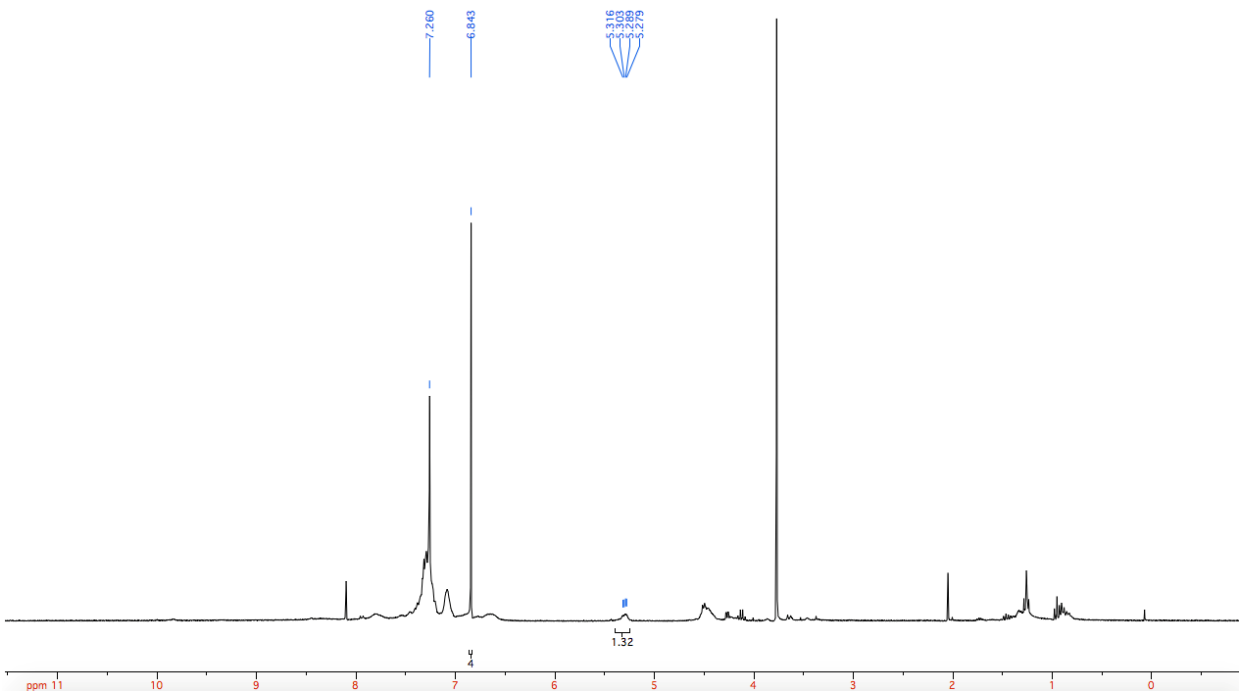


Table S2. Reaction **4C.**
Std= 2.78 mg

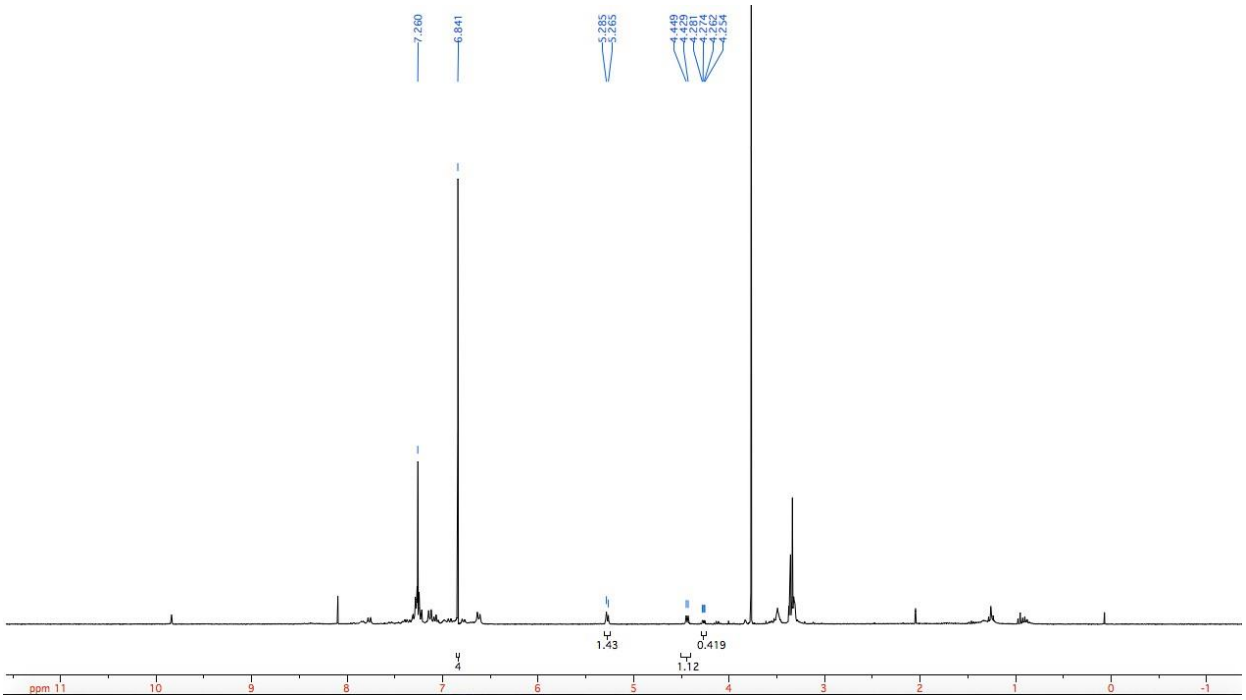


Table S2. Reaction **5A.**
Std= 2.14 mg

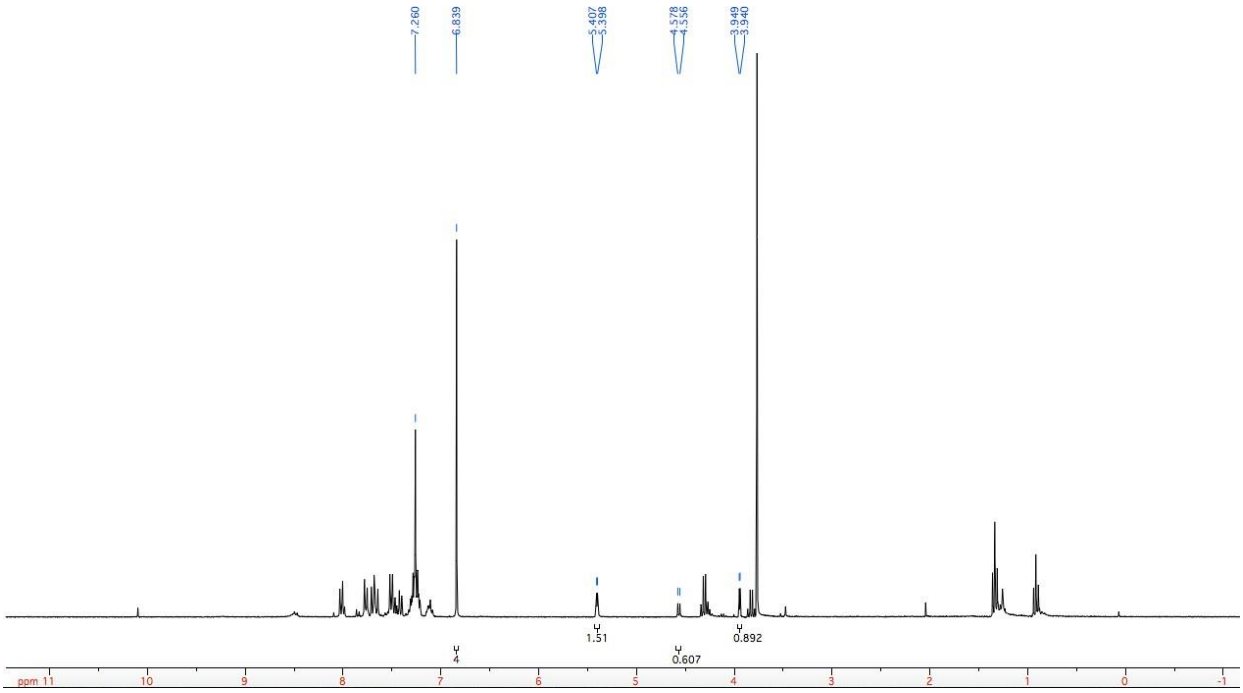


Table S2. Reaction **5B.**
Std= 2.23 mg

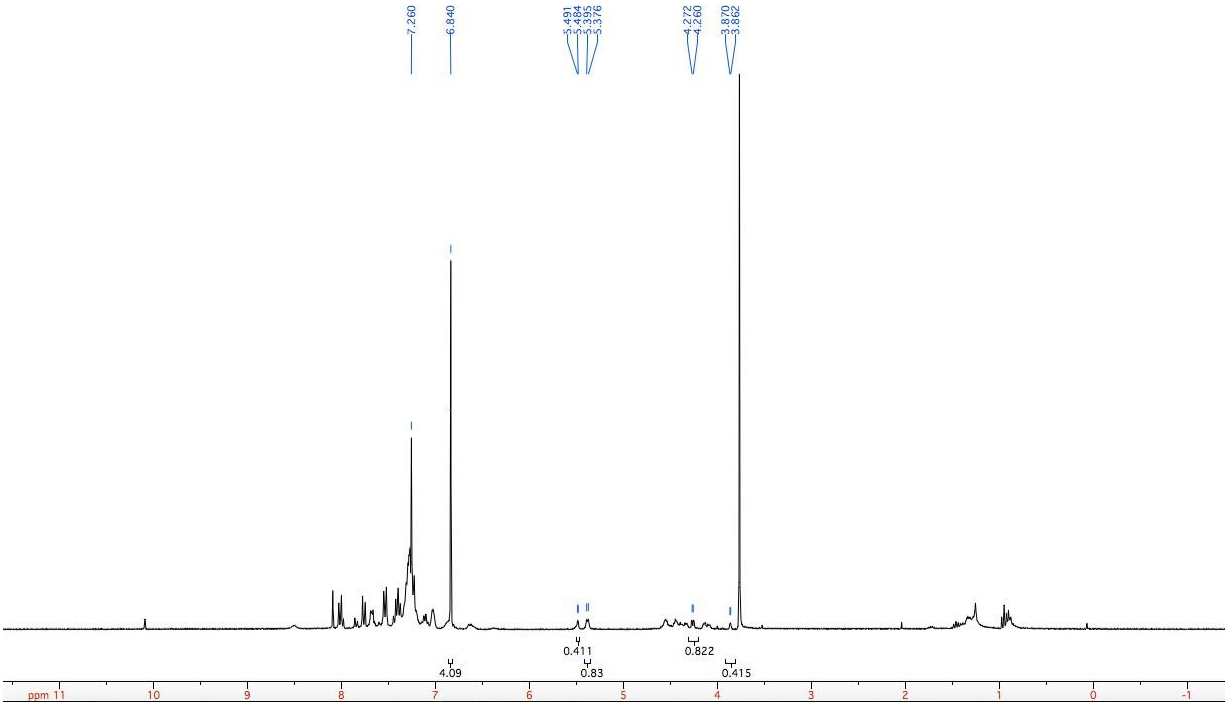


Table S2. Reaction **5C.**
Std= 1.84 mg

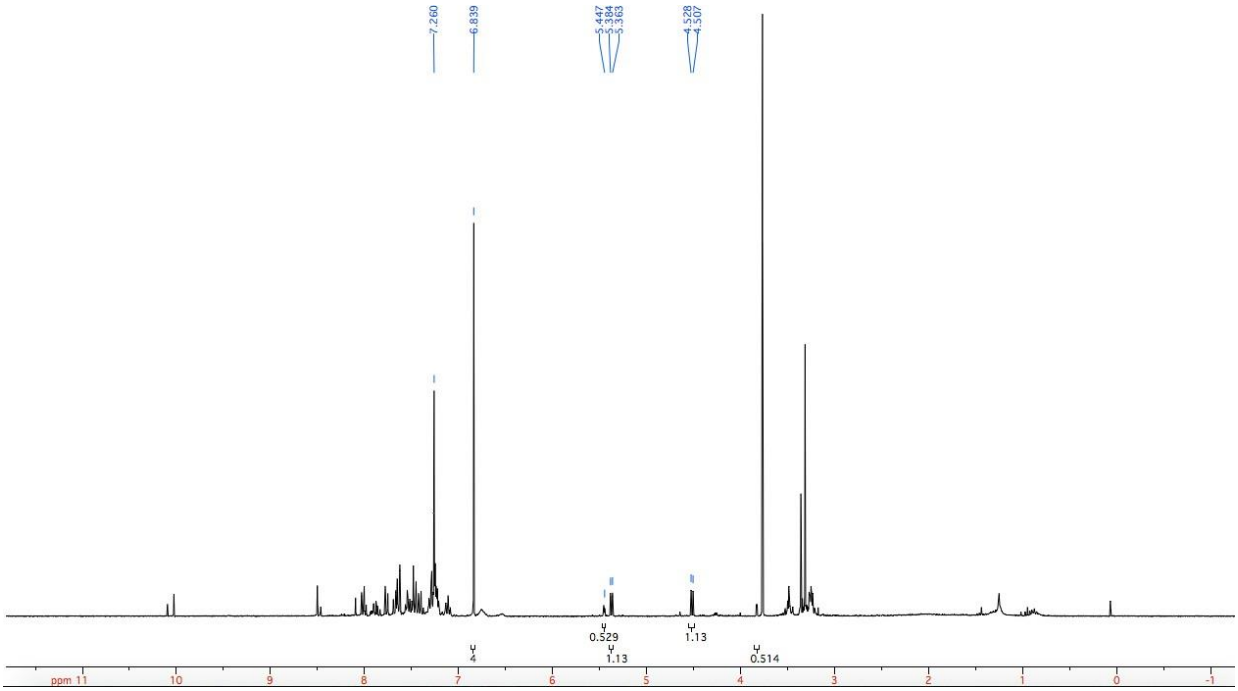


Table S2. Reaction **6A.**
Std= 2.61 mg

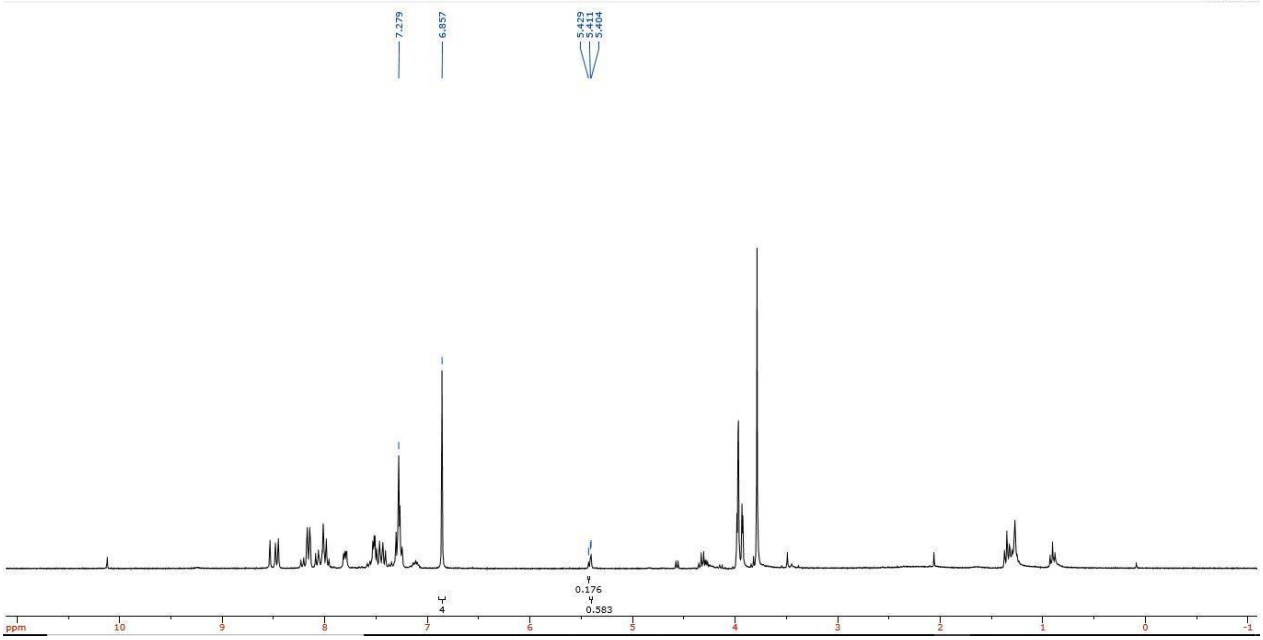


Table S2. Reaction **6B.**
Std= 1.41 mg

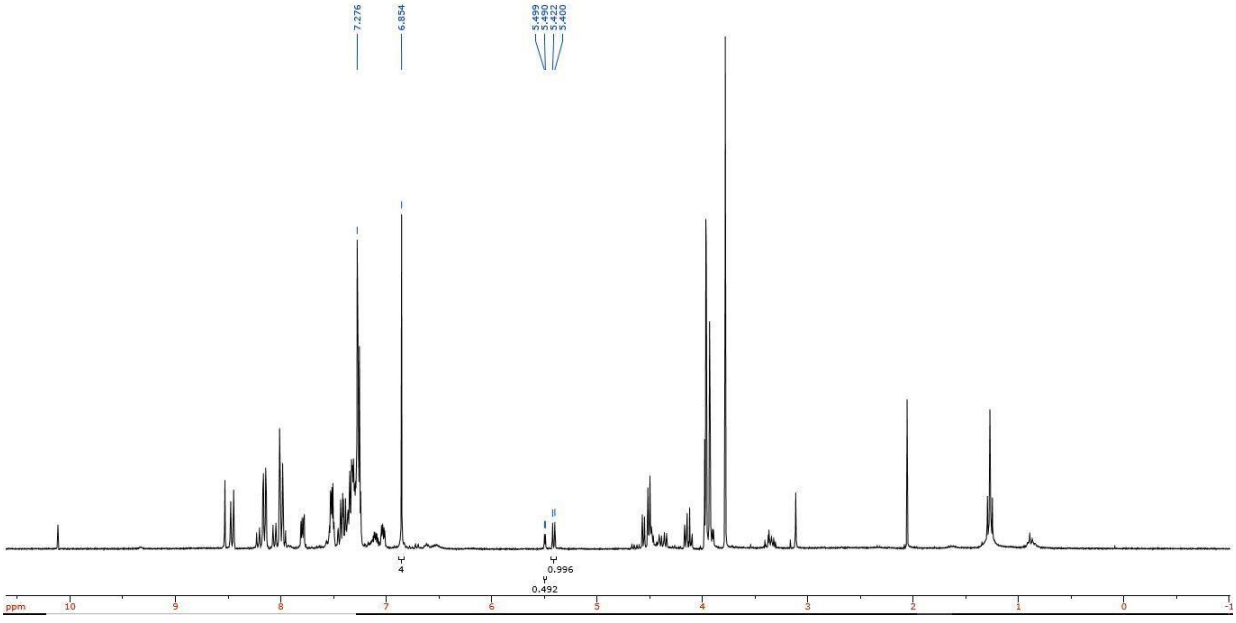


Table S2. Reaction **6C.**

Std= 2.96 mg

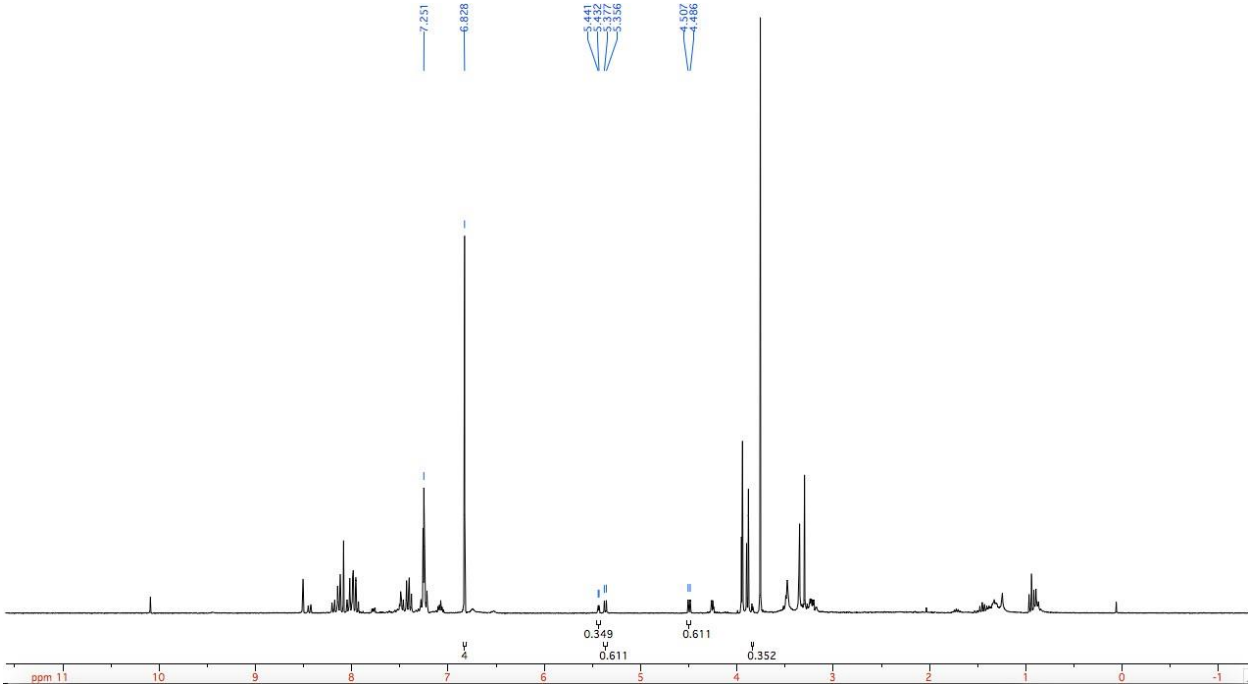


Table S2. Reaction **7A.**

Std= 1.47 mg

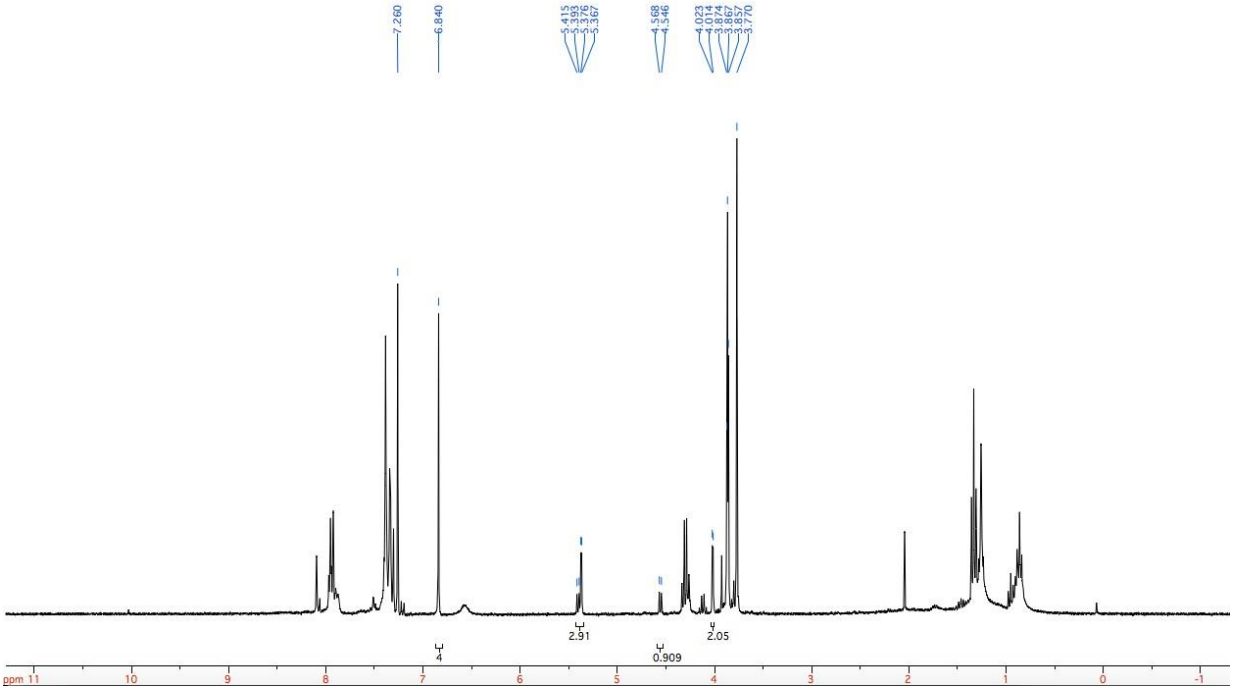


Table S2. Reaction **7B.**

Std= 3.34 mg

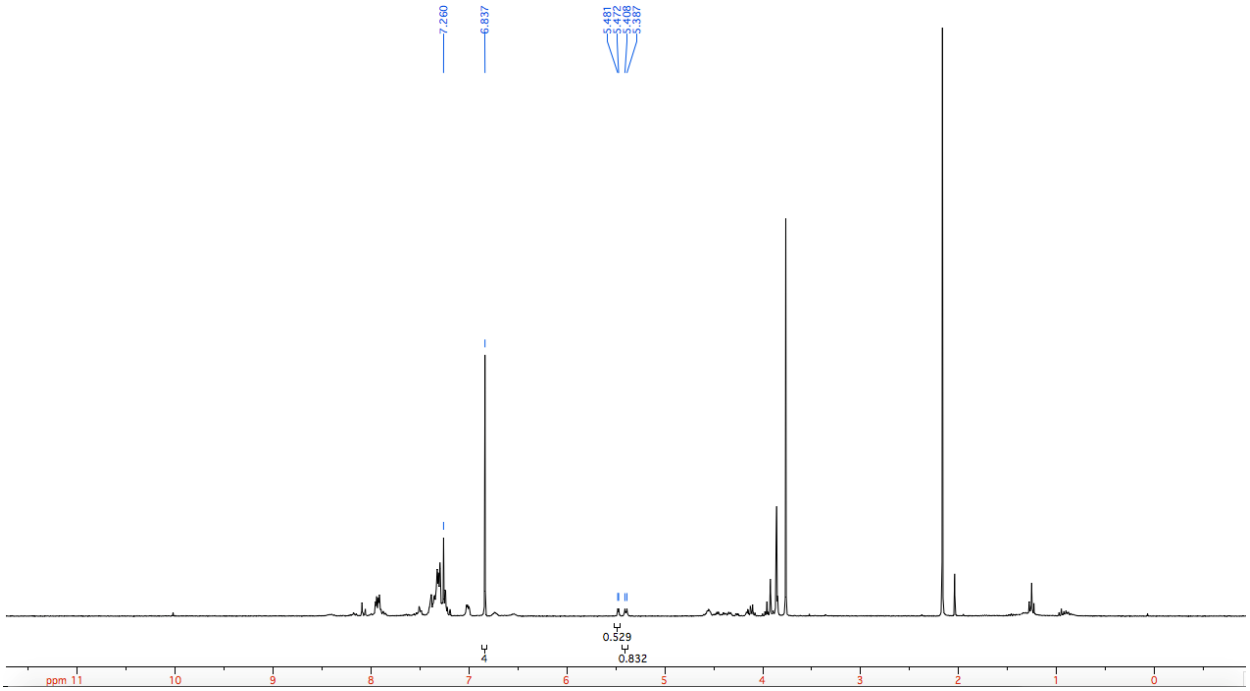


Table S2. Reaction **7C.**

Std= 2.42 mg

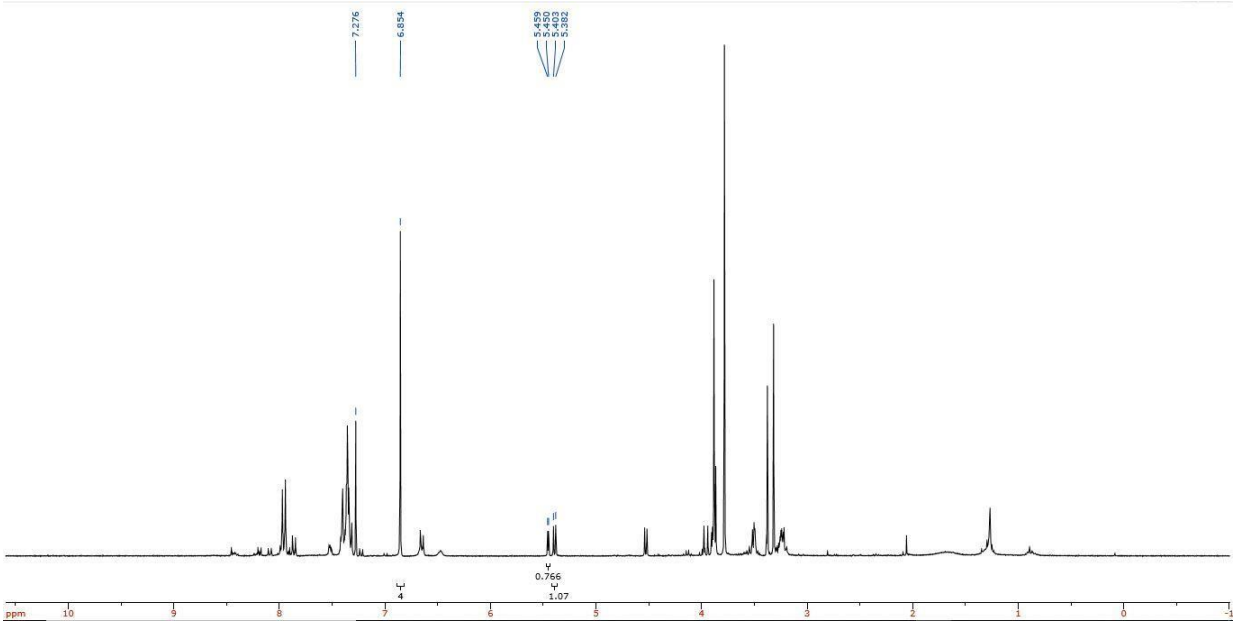


Table S2. Reaction **8A.**
Std= 3.02 mg

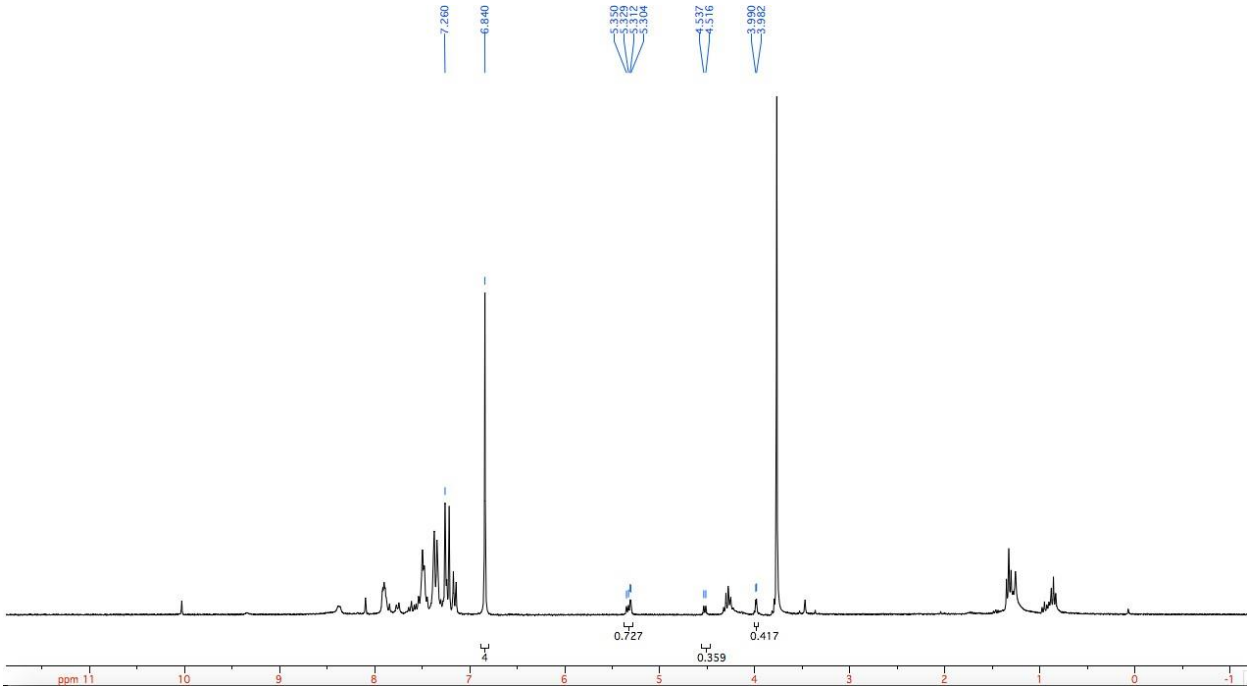


Table S2. Reaction **8B.**
Std= 3.23 mg

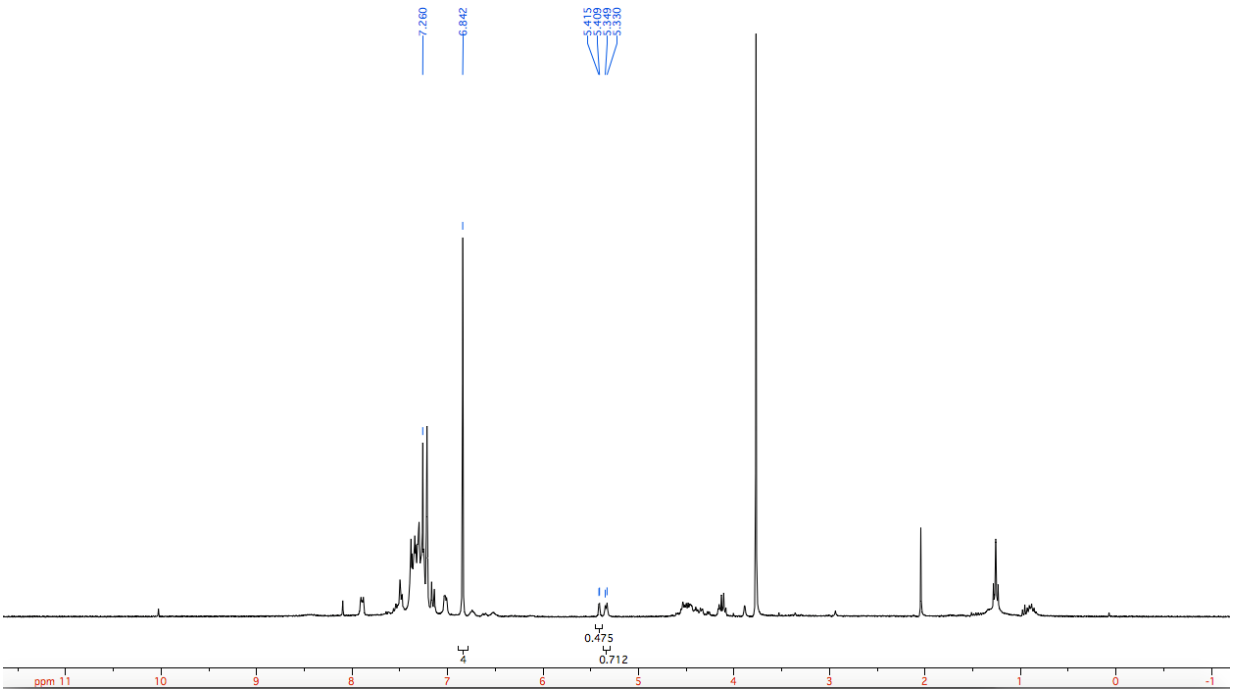
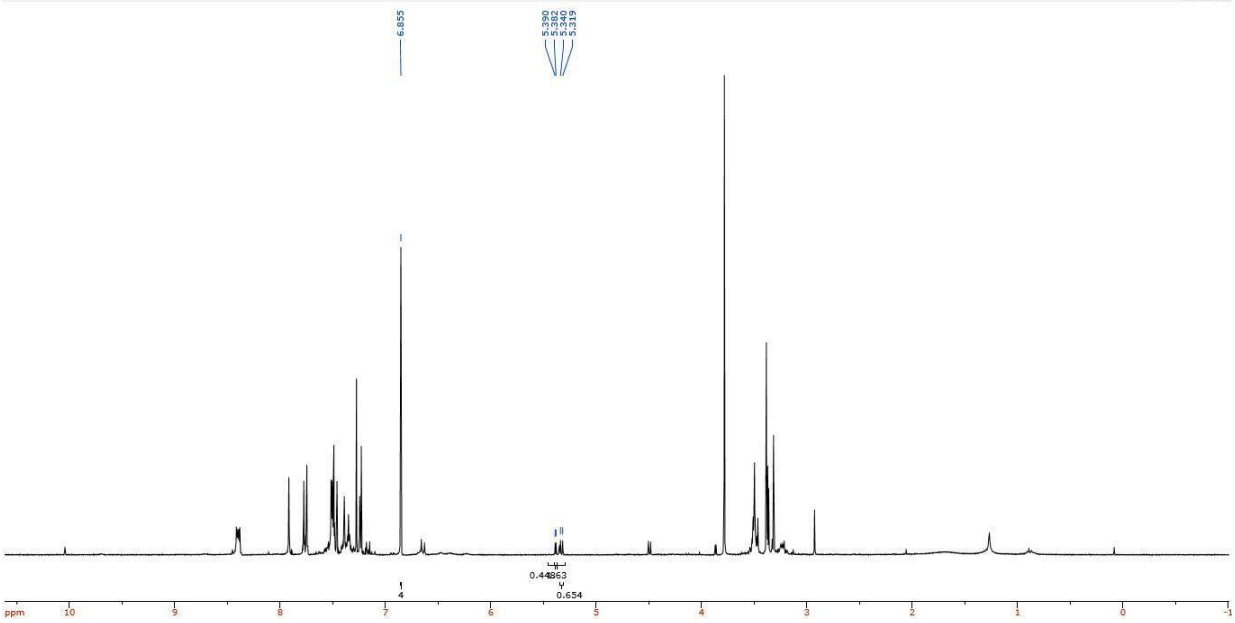


Table S2. Reaction **8C.**

Std= 1.69 mg



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