Supporting Information

Biocompatible conjugation of Tris base to 2-acetyl and 2-formyl phenylboronic acid

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General Information

L-cysteine, L-serine, L-lysine, glutathione, glucose, 2-formylphenylboronic acid (2-FPBA), 2-acetylphenylboronic acid (2-APBA) and fetal bovine serum were purchased from Fisher Scientific or Sigma-Aldrich. All Fmoc protected amino acids HBTU were purchased from Chem-Impex International or Advanced Chemtech. APBA-IA was synthesized according to previously published protocol[1]. UV spectra were collected on a Nanodrop UV-vis spectrometer. NMR data were collected on a VNMRS 500 MHz NMR spectrometer. Mass-spec data were collected on an Agilent 6230 LC-TOF mass spectrometer. Peptide synthesis was carried out on a Tribute peptide synthesizer (Protein Technologies) and purified by reverse phase HPLC (Waters Prep LC, Jupiter C18 Column).

Method for RP-HPLC: isocratic 100% buffer A for 10min, 100% buffer A to 60% buffer A in 30 min, 60% to 5% buffer A in 2 min, isocratic 5% buffer A for 5 min, 5% to 100% buffer A in 5 min and isocratic buffer A for 1 min.
Buffer A: 95% nano pure water with 5% acetonitrile and 0.1% trifluoroacitic acid.
Buffer B: 5% nano pure water with 95% acetonitrile and 0.1% trifluoroacitic acid.

Method for LC/MS: isocratic 95% buffer A for 5min, 95% buffer A to 5% buffer A in 15 min, isocratic 5% buffer A for 5 min, 5% buffer A to 95% buffer A in 1 min and then isocratic 95% buffer A for 7 min.
Buffer A: nano pure water with 0.1% formic acid
Buffer B: acetonitrile with 0.1% formic acid.

Crystallographic Information

100 mM 2-FPBA or 2-APBA and 100 mM Tris were dissolved in 75% Acetonitrile/25% water in a loosely capped 5 mL glass vial at room temperature. After a few days, crystals were observed for both complex.

Table 1. Crystallography data and structure refinement for APBA-Tris.

Identification code	C12H16BNO4	
Empirical formula	C12 H16 B N O4	
Formula weight	249.07	
Temperature	100(2) K	
Wavelength	1.54178 ≈	
Crystal system	Orthorhombic	
Space group	$Pca2_1$	
Unit cell dimensions	a = 11.3174(2) ≈	a= 90∞.
	b = 13.2547(3) ≈	b= 90∞.
	c = 7.8913(2) ≈	g = 90∞.
Volume	1183.76(5) $≈^3$	
Z	4	
Density (calculated)	1.398 Mg/m^3	
Absorption coefficient	0.851 mm^{-1}	
F(000)	528	
Crystal size	0.220 x 0.130 x 0.050 mm^3	
Theta range for data collection	3.334 to 69.741∞.	
Index ranges	-12<=h<=13, -15<=k<=16, -9<=l<=9	
Reflections collected	7352	
Independent reflections	2045 [R(int) = 0.0299]	
Completeness to theta = 67.679∞	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7533 and 0.6610	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2045 / 4 / 173	
Goodness-of-fit on F^2	1.066	
Final R indices [I>2sigma(I)]	R1 = 0.0276, wR2 = 0.0729	
R indices (all data)	R1 = 0.0287, wR2 = 0.0740	
Absolute structure parameter	0.00(8)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.250 and -0.166 $e.≈^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($\approx^2$x 10^3)for C12H16BNO4. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	3606(1)	7334(1)	5203(2)	17(1)
O(2)	7568(1)	8463(1)	4306(2)	14(1)
O(3)	6132(1)	8179(1)	2222(2)	14(1)
O(4)	3767(1)	10207(1)	2494(2)	16(1)
N(1)	5346(1)	8291(1)	5079(2)	11(1)
C(1)	4764(2)	7402(2)	5937(3)	13(1)
C(2)	5500(2)	6492(2)	5452(3)	14(1)
C(3)	5274(2)	5522(2)	6033(3)	18(1)
C(4)	6059(2)	4760(2)	5588(3)	21(1)
C(5)	7038(2)	4967(2)	4578(3)	20(1)
C(6)	7234(2)	5940(2)	3990(3)	17(1)
C(7)	6465(2)	6724(1)	4432(3)	14(1)
C(8)	4878(2)	8178(1)	2174(3)	15(1)
C(9)	4479(2)	8686(2)	3818(3)	13(1)
C(10)	3311(2)	8287(2)	4477(3)	18(1)
C(11)	4677(2)	7567(2)	7833(3)	16(1)
C(12)	4569(2)	9833(2)	3731(3)	16(1)
B(1)	6528(2)	7896(2)	3923(3)	12(1)

Table 3. Crystallography data and structure refinement for FPBA-Tris.

Identification code	C11H14BNO4	
Empirical formula	C11 H14 B N O4	
Formula weight	235.04	
Temperature	100(2) K	
Wavelength	1.54178 ≈	
Crystal system	Monoclinic	
Space group	$P2_1/c$	
Unit cell dimensions	a = 13.3622(6) ≈	a= 90∞.
	b = 9.2625(4) ≈	b= 94.734(2)∞.
	c = 8.6950(4) ≈	g = 90∞.
Volume	1072.49(8) $≈^3$	
Z	4	
Density (calculated)	1.456 Mg/m^3	
Absorption coefficient	0.906 mm^{-1}	
F(000)	496	
Crystal size	0.420 x 0.180 x 0.100 mm^3	
Theta range for data collection	5.819 to 69.802∞.	
Index ranges	0<=h<=16, -11<=k<=11, -10<=l<=10	
Reflections collected	3453	
Independent reflections	3453 [R(int) = ?]	
Completeness to theta = 67.679∞	99.2 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7533 and 0.5156	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3453 / 3 / 164	
Goodness-of-fit on F^2	1.049	
Final R indices [I>2sigma(I)]	R1 = 0.0543, wR2 = 0.1538	
R indices (all data)	R1 = 0.0598, wR2 = 0.1598	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.397 and -0.217 $e.≈^{-3}$	

Table 4. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (≈2 x 10^3) for C11H14BNO4. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	6562(1)	3964(2)	6685(2)	30(1)
O(2)	9305(1)	6946(2)	5558(2)	28(1)
O(3)	8930(1)	6269(2)	8096(2)	27(1)
O(4)	8811(1)	1611(2)	8371(2)	27(1)
N(1)	8079(1)	4893(2)	5997(2)	24(1)
B(1)	8528(2)	6541(3)	6501(3)	26(1)
C(1)	6953(2)	4978(2)	5669(3)	28(1)
C(2)	6677(2)	6530(3)	5952(3)	29(1)
C(3)	5693(2)	7044(3)	5810(3)	35(1)
C(4)	5527(2)	8482(3)	6126(3)	37(1)
C(5)	6329(2)	9393(3)	6561(3)	33(1)
C(6)	7305(2)	8868(2)	6690(3)	30(1)
C(7)	7490(2)	7419(2)	6376(3)	27(1)
C(8)	9098(2)	4784(2)	8381(3)	26(1)
C(9)	8276(2)	3965(2)	7408(2)	25(1)
C(10)	7247(2)	3992(3)	8047(3)	29(1)
C(11)	8593(2)	2440(2)	7003(3)	26(1)

Relaxation Kinetics

The forward reaction kinetics data were fitted according to the equations of second order relaxation. The following equations were used to calculate the relaxation constant. Equation 1 describe the reaction mechanism, equation2 describes the second order relaxation kinetics, equation 3 describes the relaxation time constant τ, equation 4-7 correlates the concentration of the reactants and the reaction rates. $[A]_0 = 2\,mM$ for FPBA and 8 mM for APBA. $\overline{[AB]}$ is the concentration of the conjugates at equilibrium, which equals 1.6 mM for FPBA-Tris and 4.6 mM for APBA-Tris according to the integration of NMR spectrum. $\overline{[A]}$ and $\overline{[B]}$ represent the reactant concentrations at equilibrium.

[1] $A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P$

[2] $y = y_0 + Ae^{(-t/\tau)}$

[3] $\tau = \dfrac{1}{k_{-1} + k_1(\overline{[A]} + \overline{[B]})}$

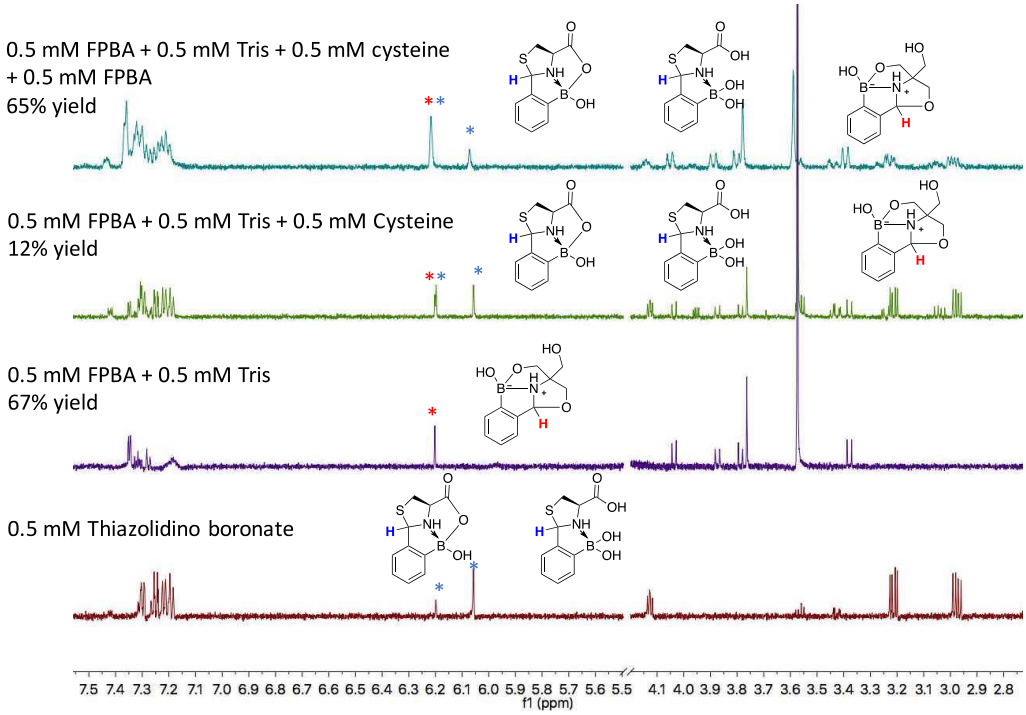
[4] $\overline{[A]} = \overline{[B]}$

[5] $\overline{[A]} + \overline{[AB]} = [A]_0$

[6] $K_d = \dfrac{\overline{[A][B]}}{\overline{[AB]}} = \dfrac{k_{-1}}{k_1}$

2-FPBA/APBA-Tris conjugation in presence and absence of cysteine

A.



B.

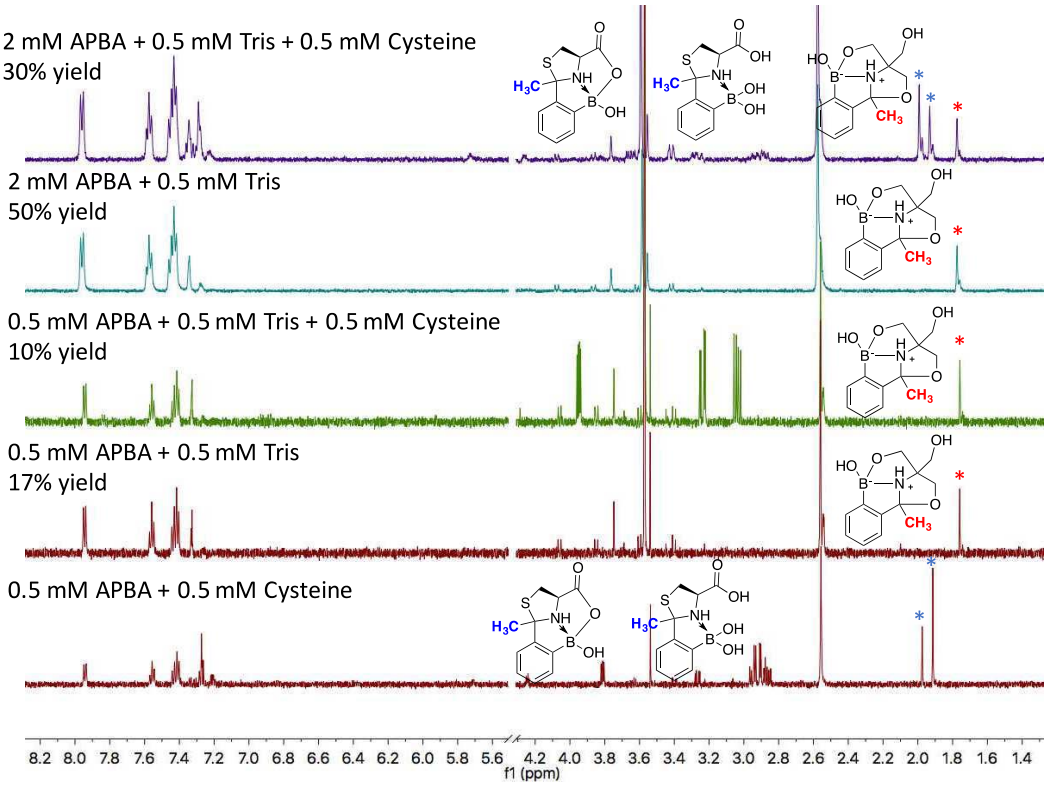


Figure S1. Tris conjugation with A) 2-FPBA and B）2-APBA in the presence and absence of free cysteine.

Toxicity of 2-APBA and 2-FPBA towards *E.coli* cells

E.coli cells (strain ER2738) were grown in LB media to an OD_{600}~ 1.0. Cells were spun down and resuspended in PBS pH 7.4). 100uL of cells were incubated with 2-APBA (2 mM), 2-FPBA (0.5 mM), or just PBS overnight at 4°C with gentle rocking. Cells were plated before overnight incubation to get an initial cell count and after overnight incubation to monitor cell death. All the experiments were done in triplicates.

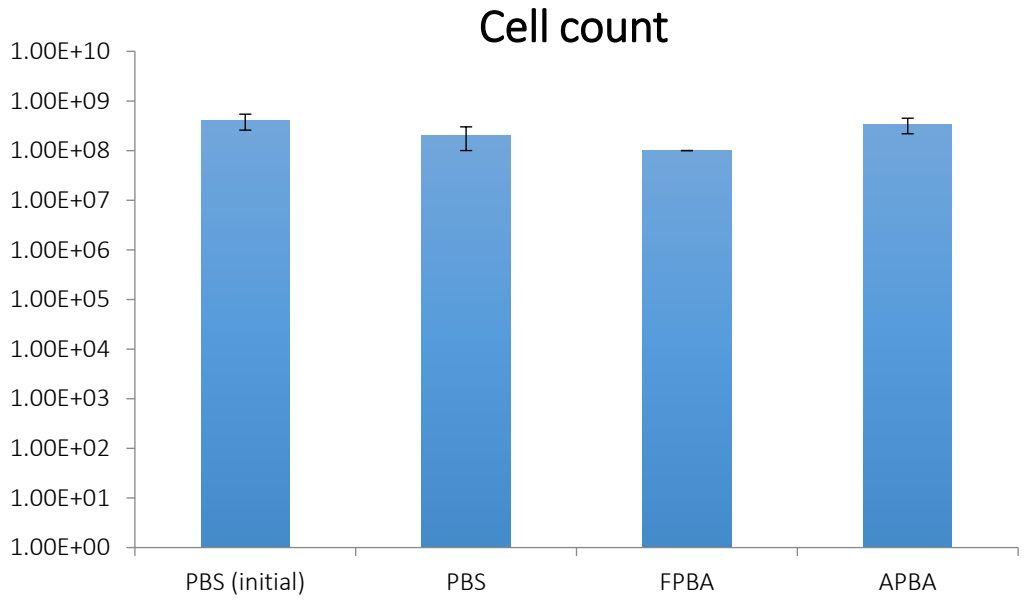


Figure S2. Toxicity of 2-APBA/FPBA towards *E. coli* cells. Neither showed significant cell killing at the concentrations needed for efficient conjugation with Tris.

Synthesis of Tris-Alkyne

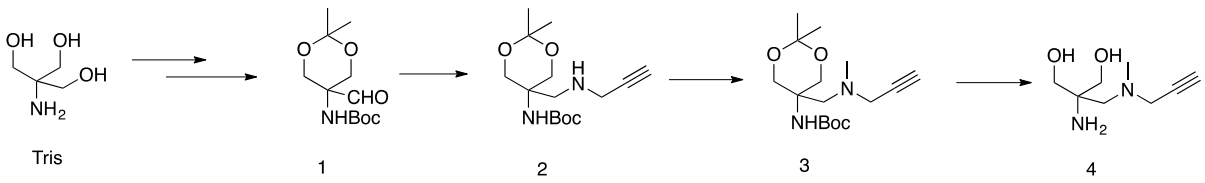


Figure S3. Synthesis of an alkyne derivative of Tris.

Synthesis of *tert*-butyl (2,2-dimethyl-5-((prop-2-yn-1-ylamino)methyl)-1,3-dioxan-5-yl)carbamate (**2)**

1 was synthesized according to a previously reported procedure[2]. **1** (259 mg, 1 mmol) was dissolved in 5 mL of ethanol, to which was added 55 mg of propargylamine. The mixture was allowed to stir for 2 hours at room temperature before the addition of 75.4 mg of $NaCNBH_3$. The reaction was stirred overnight and quenched with 30 mL of water. The product was extracted by EtOAc (2 × 50 mL). The organic layer was combined, washed with 50 mL of saturated sodium chloride and dried over sodium sulfate. After solvent removal, the product was purified by silica column (30% EtOAc/Hexane, 1% triethylamine) to give a yellow solid (153 mg, 51% yield). ^{1}H-NMR data suggest the product exists as an ethanol adduct.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.01 (s, 1H), 4.06 (d, *J* = 11.7 Hz, 2H), 3.72 – 3.61 (m, 2H), 3.39 (d, *J* = 2.5 Hz, 2H), 2.94 (s, 2H), 2.20 (t, *J* = 2.4 Hz, 1H), 1.47 – 1.31 (m, 15H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 154.94, 98.60, 98.45, 81.68, 71.66, 64.46, 64.03, 53.18, 51.85, 49.92, 38.77, 28.31, 28.27, 24.30, 22.89.

MS-ESI$^+$: m/z calculated for $C_{15}H_{27}N_2O_4$ $[M+H]^+$ 299.1965, found 299.1910.

Synthesis of *tert*-butyl (2,2-dimethyl-5-((methyl(prop-2-yn-1-yl)amino)methyl)-1,3-dioxan-5-yl)carbamate (**3)**

2 (90 mg, 0.3 mmol) was dissolved in 1 mL of Acetone and placed on ice bath. K_2CO_3 (210 mg) was added to the solution and the mixture was stirred for 10 min. Iodomethane (42.5 mg) was added and the reaction was allowed to stir overnight at room temperature and then quenched with 50 mL of water. The product was extracted by EtOAc (3 × 50 mL). The organic layer was combined, washed with 50 mL of saturated sodium chloride and dried over sodium sulfate. After solvent removal, the product was purified by silica column (20% EtOAc/Hexane) to give a white solid (60 mg, 64% yield).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.88 (s, 1H), 4.05 (d, *J* = 10.4 Hz, 2H), 3.74 (d, *J* = 11.6 Hz, 2H), 3.31 (d, *J* = 2.4 Hz, 2H), 2.85 (s, 2H), 2.40 (s, 3H), 1.42 (t, *J* = 15.4 Hz, 15H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 154.85, 98.30, 79.56, 72.68, 64.30, 56.39, 52.41, 48.14, 44.59, 24.12, 23.28.

MS-ESI$^+$: m/z calculated for $C_{16}H_{29}N_2O_4$ $[M+H]^+$ 313.2122, found 313.2067.

Synthesis of 2-amino-2-((methyl(prop-2-yn-1-yl)amino)methyl)propane-1,3-diol (**4)**

3 (31 mg, 0.1 mmol) was treated with 2 mL of 95% trifluoroacetic acid and 5% water for 1 h, twice. After removing TFA and water over vacuum, the product was lyophilized and used without purification.

^{1}H NMR (500 MHz, Methanol-d_4) δ 3.72 (d, *J* = 2.3 Hz, 2H), 3.69 (d, *J* = 2.0 Hz, 4H), 3.08 (s, 2H), 2.90 (t, *J* = 2.3 Hz, 1H), 2.70 (s, 3H).

^{13}C NMR (126 MHz, Methanol-d_4) δ 75.78, 75.60, 60.67, 60.34, 55.60, 47.67, 43.21.a

MS-ESI$^+$: m/z calculated for $C_8H_{17}N_2O_2$ $[M+H]^+$173.1285, found 173.1217.

Peptide Synthesis

KL31 precursor (sequence: AzidoAla-Leu-Val-Ala-Ala-Gly-Cys-NH_2) was synthesized by standard solid phase peptide synthesis on rink amide resin. Fmoc-AzidoAla-OH was synthesized according to literature[3]. The peptide was cleaved off resin using 85% TFA, 5% H2O, 5% phenol and 5% thioanisole and precipitated by cold ether. The crude product was purified via RP-HPLC. **4** was clicked onto the purified peptide with copper catalyst in water. Briefly, 4 mg of peptide was dissolved in 0.5 mL of DI water, to which was added 3.5 mg of $CuSO_4 \bullet 5H_2O$ and 2.7 mg of sodium L-ascorbate. 7 µL of **4** from 1 M stock in DMF was added to the mixture and the reaction was incubated for 30 min at room temperature. The purified peptides were confirmed by LC/MS to confirm the identity and purity.

CD Spectrum of KL31

All the samples were filtered before experiments. The peptides were dissolve in PBS to make a 1 mM solution. The spectra were taken at 25℃, 1 mm path length, scan 195-260 nm with 1 nm step, 10 sec averaging time, 0.33 sec settling time. The spectra are presented as the average of triplicates with PBS blank subtracted.

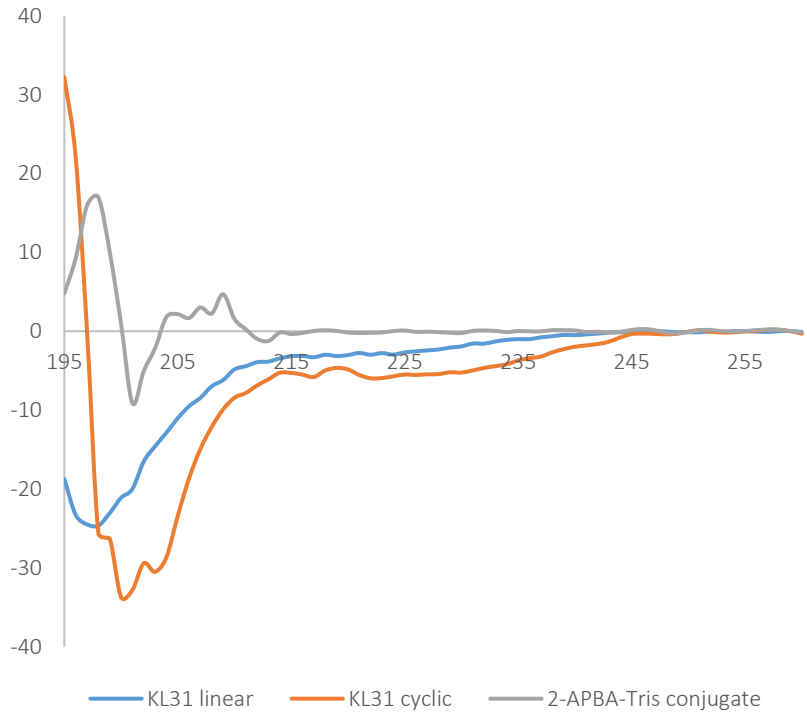


Figure S4. Circular dichroism (CD) spectra of KL31 before and after APBA-IA mediated cyclization. The spectrum of the small molecule APBA-Tris conjugate is included as a control. The peptides' spectra

show minima at 198 nm and 200 nm respectively, suggesting both the linear precursor and the cyclic product primarily adopt random coil conformations.

Oxidation kinetics of 2-APBA/FPBA

The samples were prepared in PBS buffer with 10% D_2O. The concentrations of the small molecules and H_2O_2 were all set at 0.5 mM. The conversion was monitored at different time points using ^{1}H-NMR.

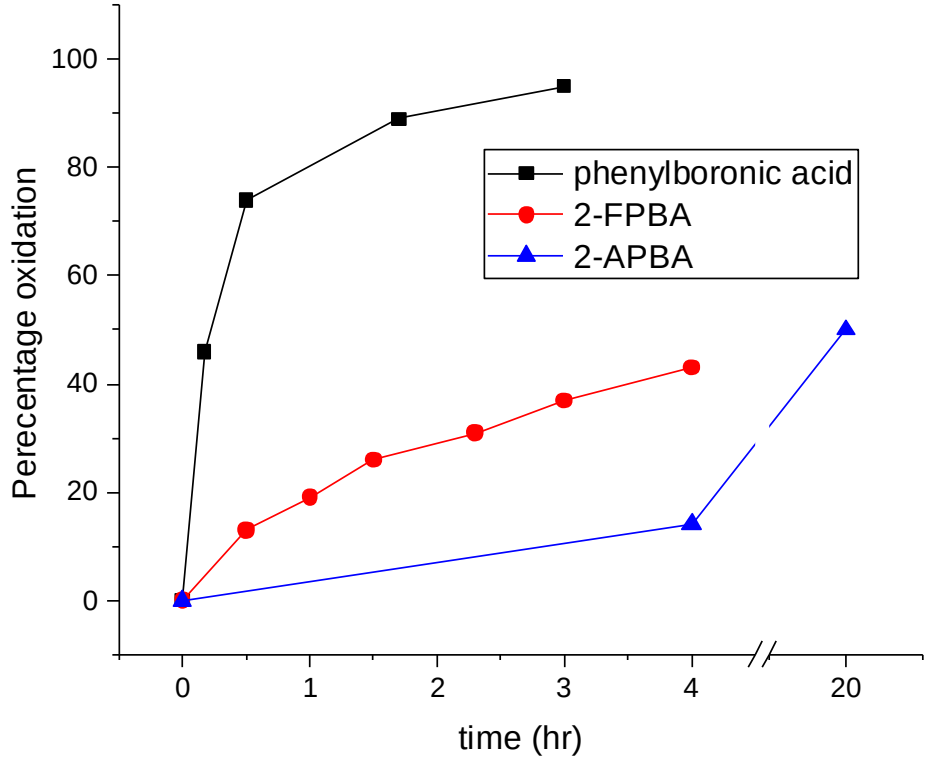


Figure S5. Oxidation kinetics of 2-APBA/FPBA in comparison to that of phenylboronic acid. Based on these results, the apparent half-lives of phenylboronic acid, 2-FPBA, and 2-APBA are estimated to be 0.5 hr, 4 hrs and 20 hrs respectively.

Conjugation of 2-APBA/FPBA in Presence of H_2O_2

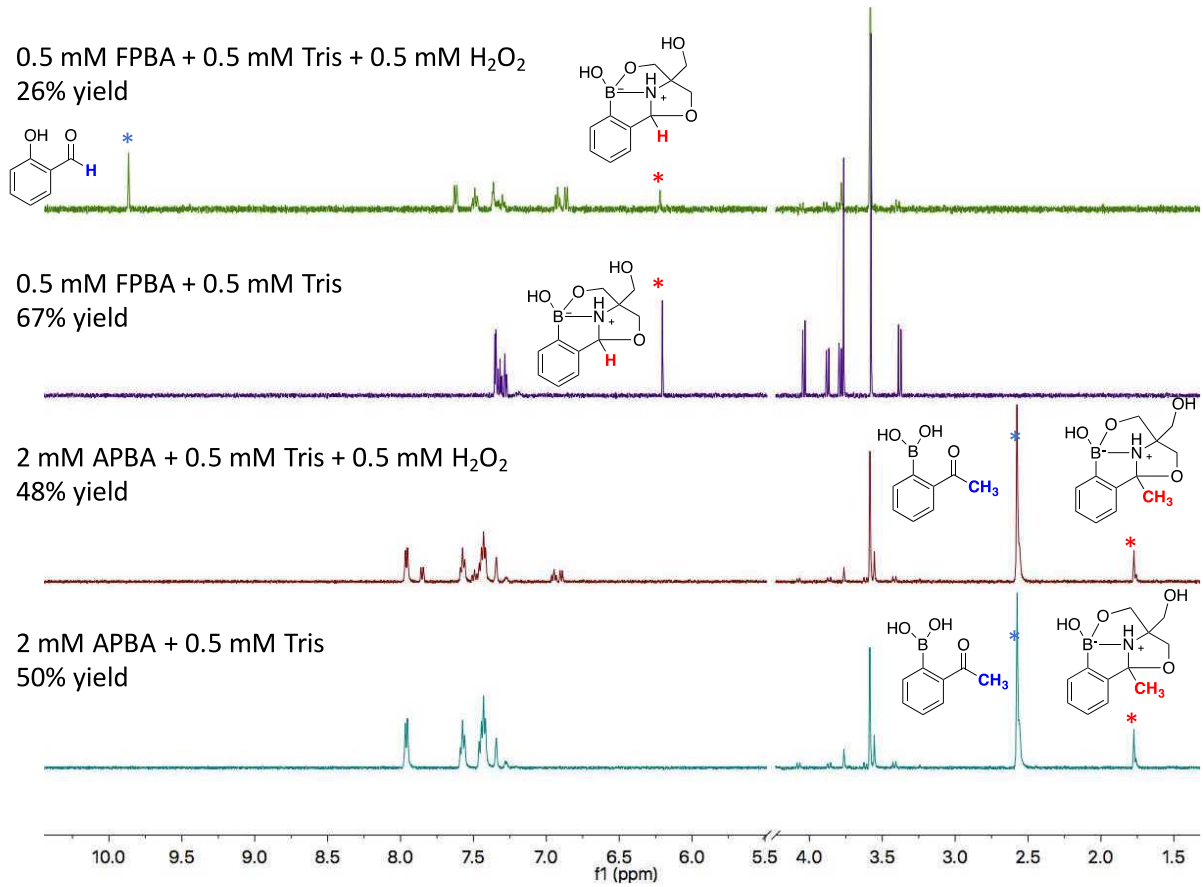
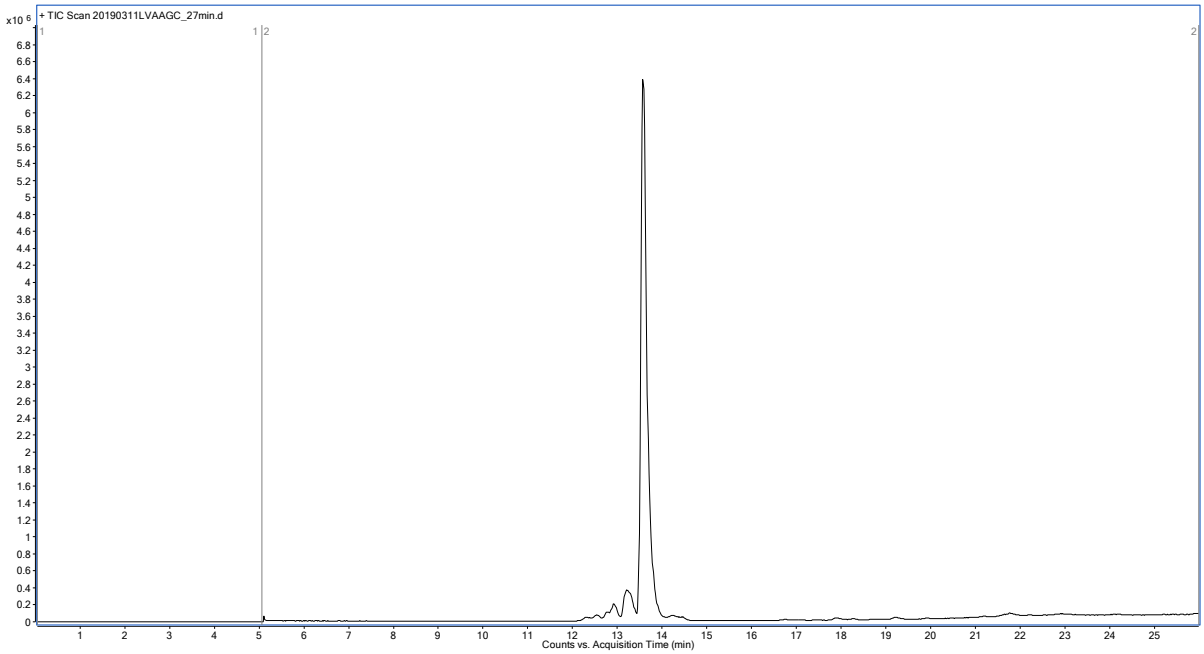
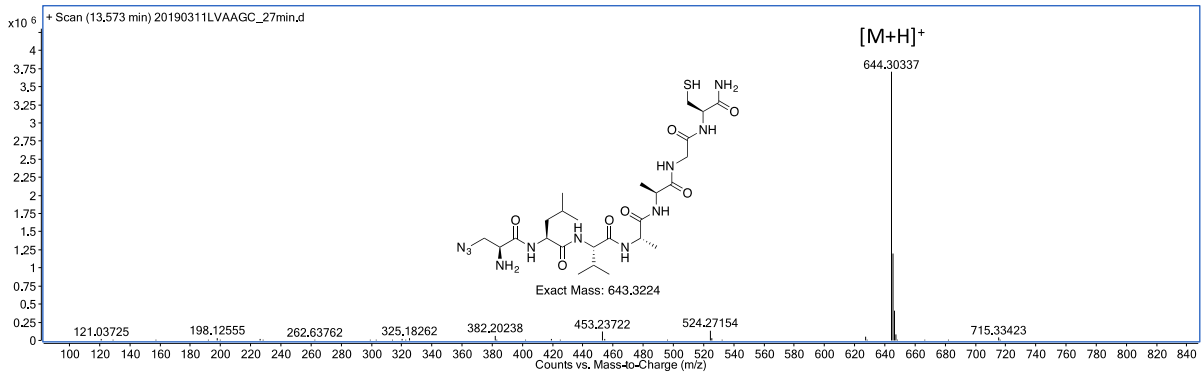


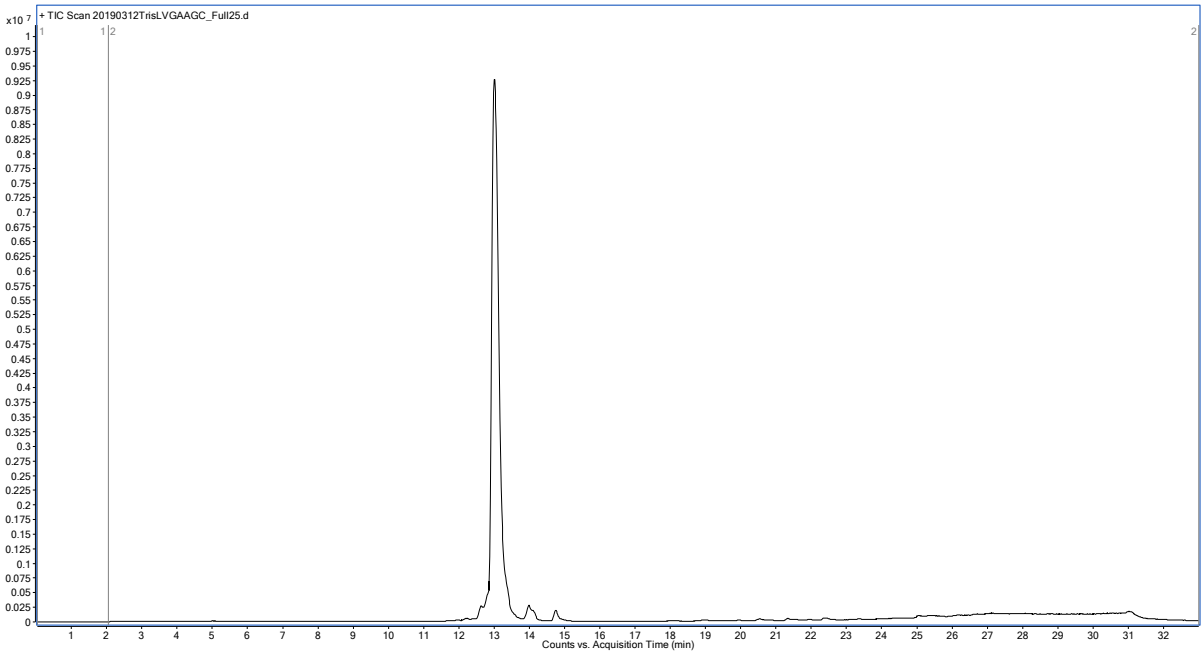
Figure S6. Tris conjugation with 2-APBA or 2-FPBA in the presence of H_2O_2. All reactions were allowed to incubate in a phosphate buffer (pH 7.4) for 20 hrs before the ^{1}H-NMR spectra were recorded.

LC-MS of AzidoAla-Leu-Val-Ala-Gly-Cys-NH_2





LC-MS of KL31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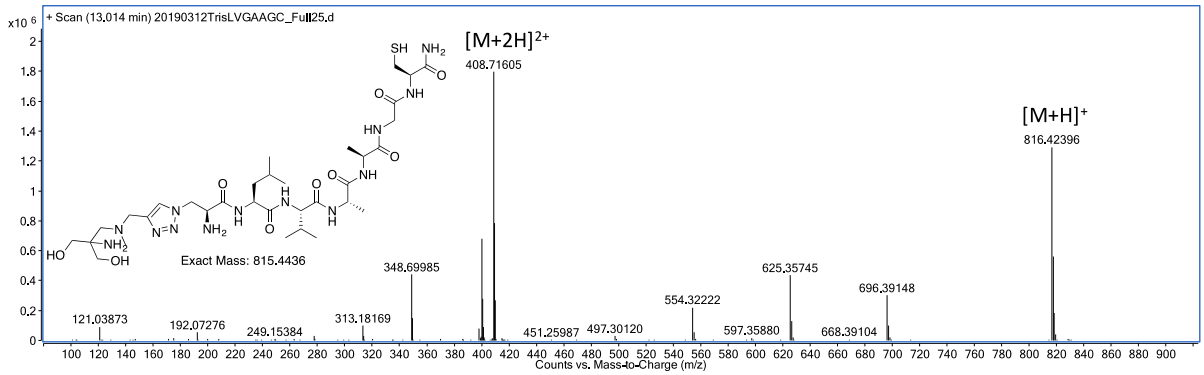
x10 7
Counts vs. Acquisition Time (min)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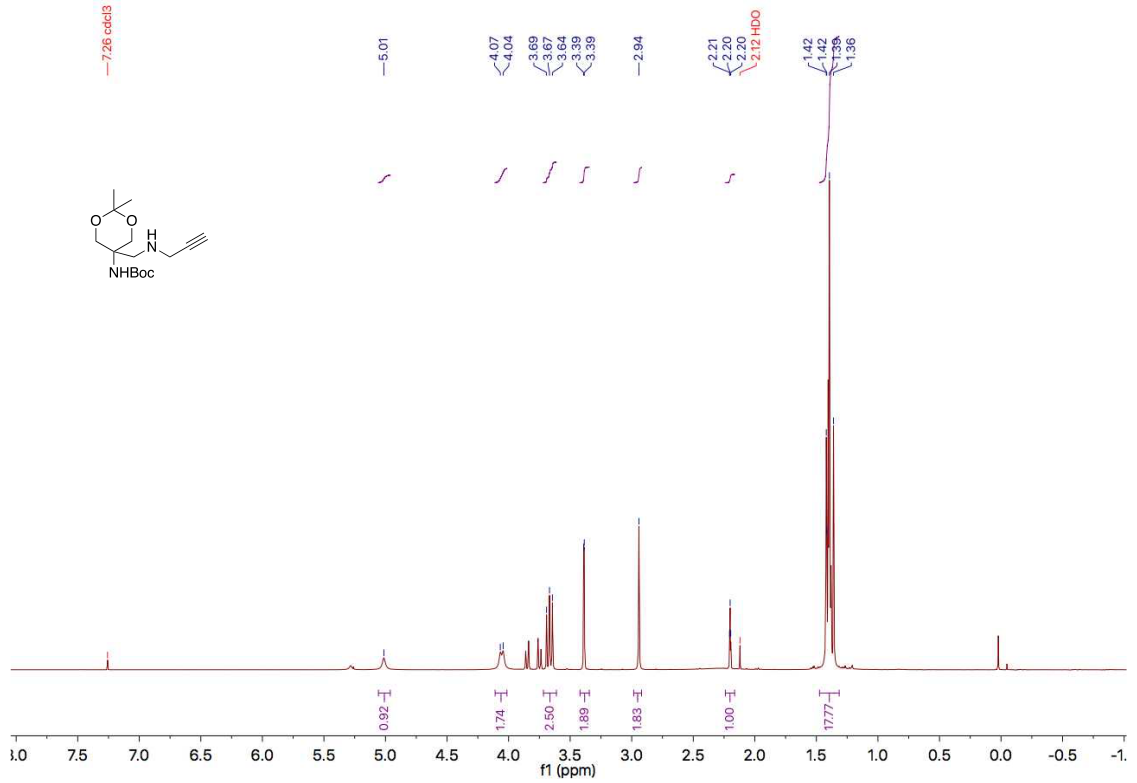
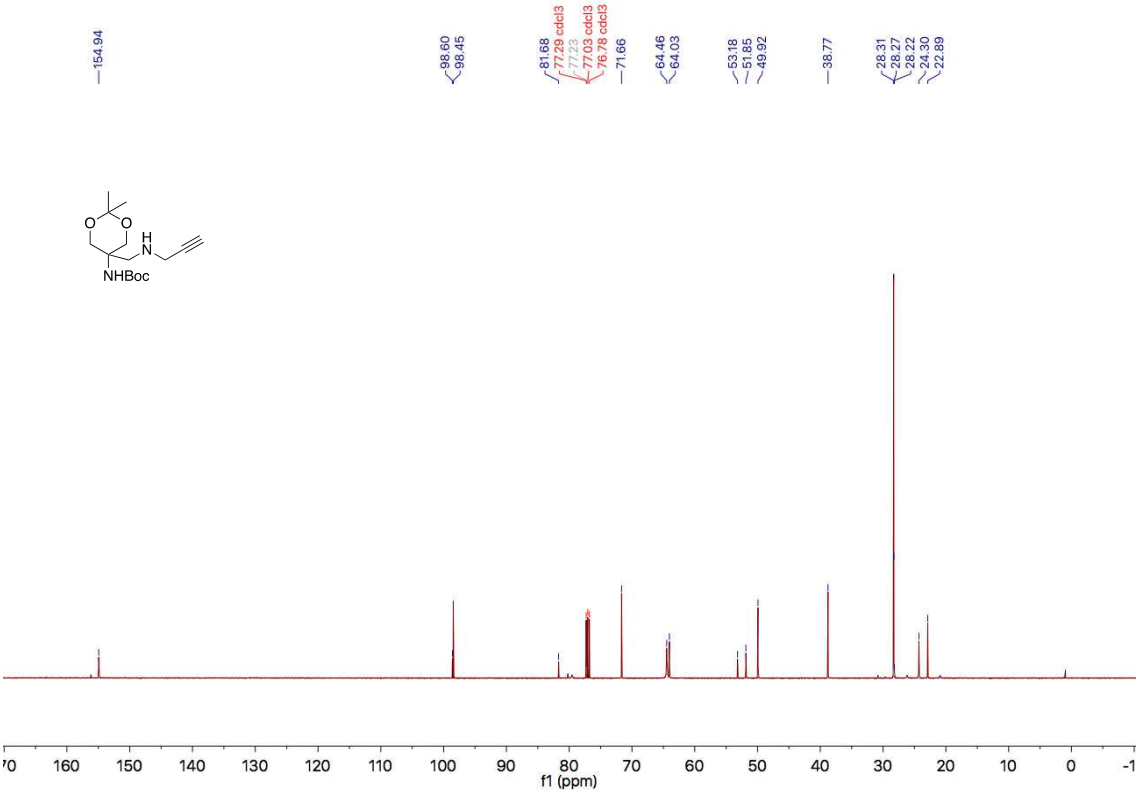
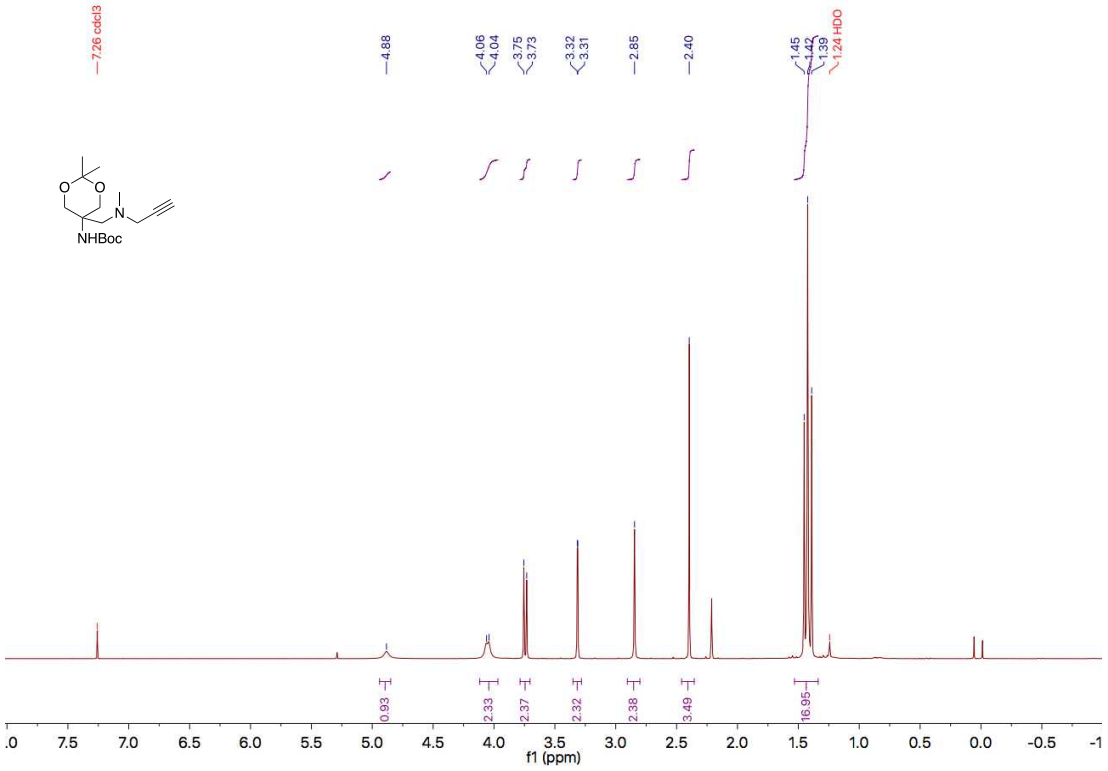
Counts vs. Mass-to-Charge (m/z)

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



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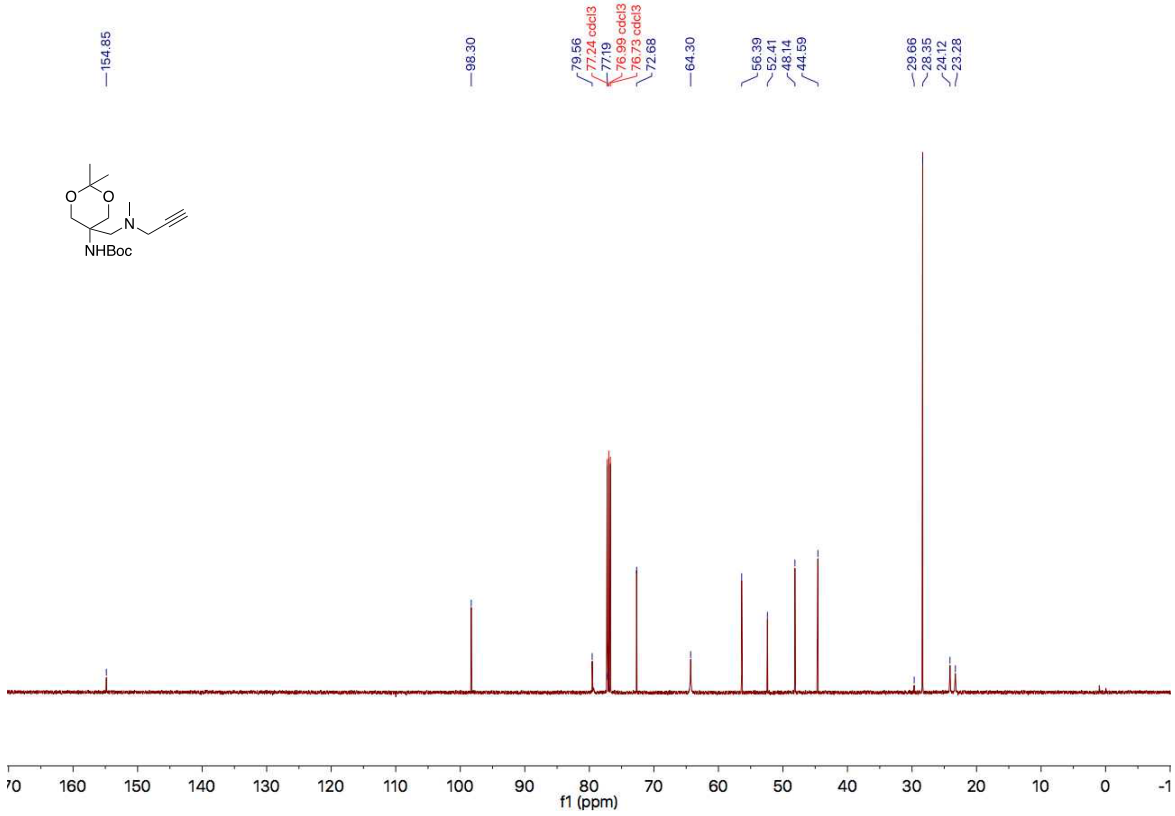
f1 (ppm)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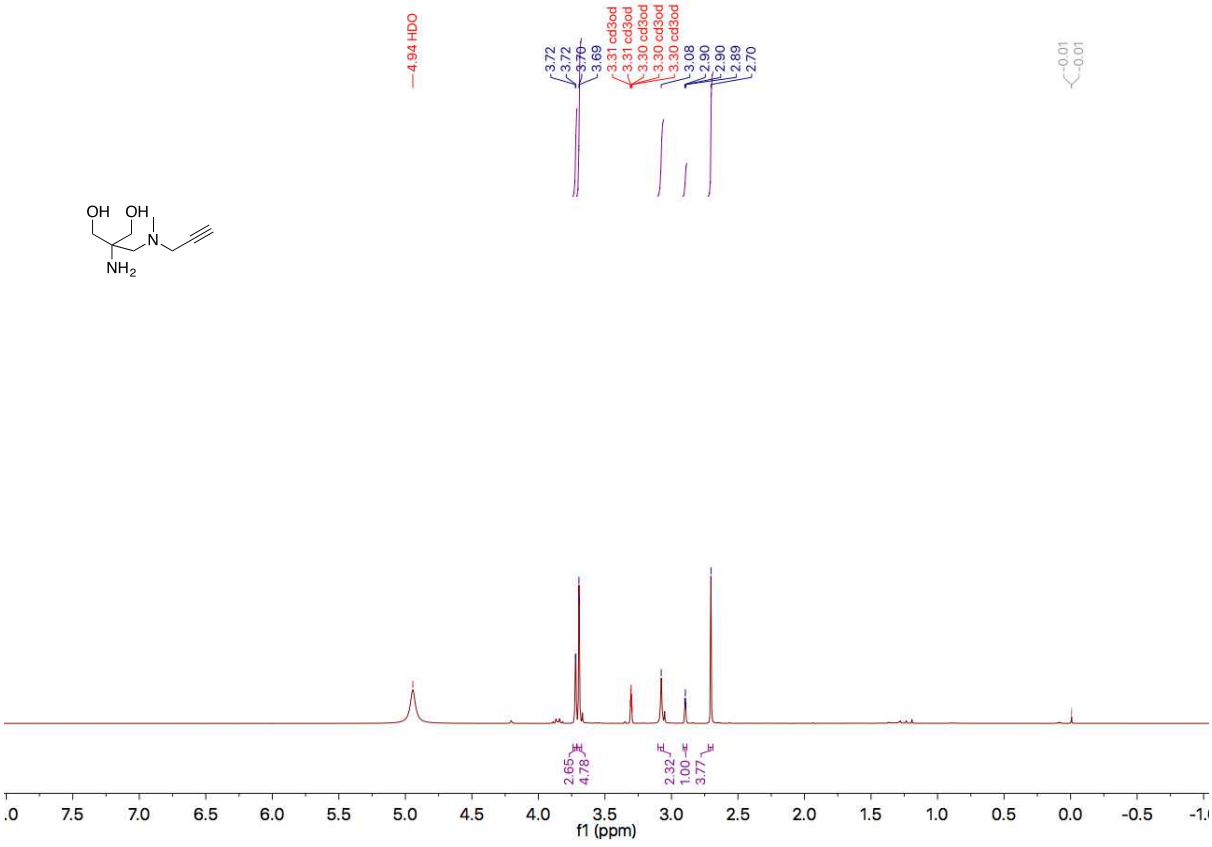
NHBoc
f1 (ppm)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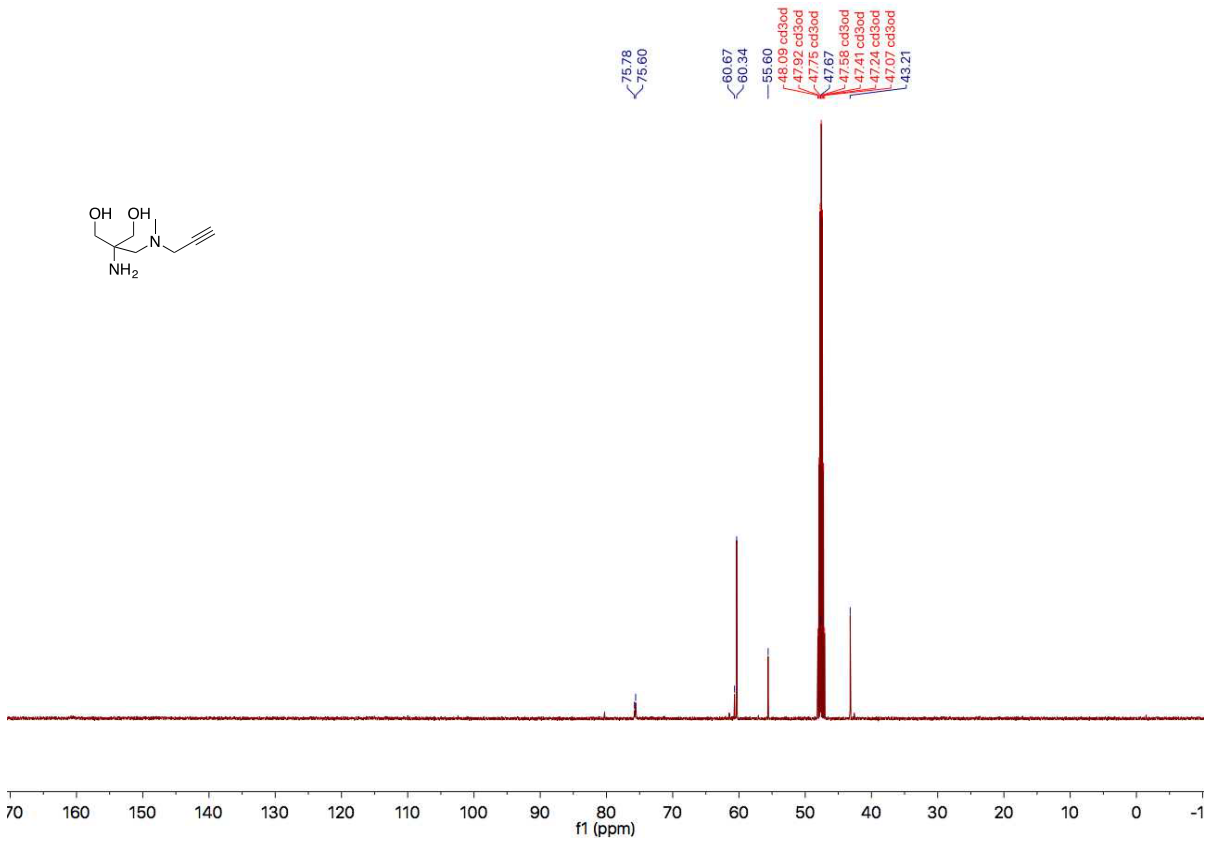
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47.07 cd3od
43.21
OH
OH
N
NH2
f1 (ppm)

References

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