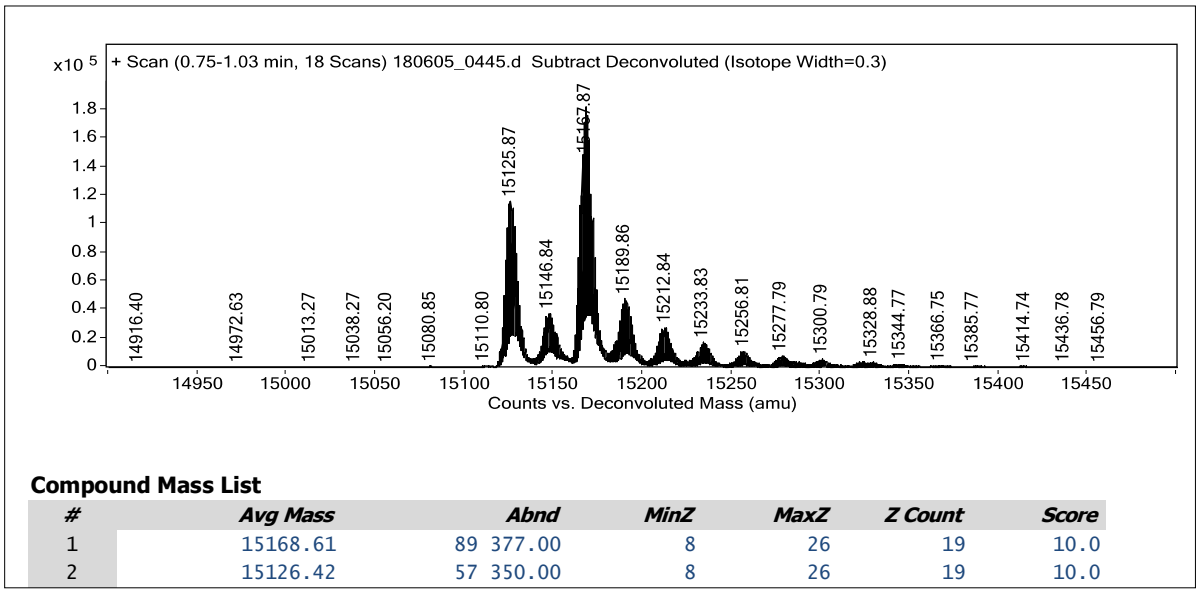
Supporting Information

Mass spectral data, additional C4 and size-exclusion chromatograms, oxygen binding data, ICP-OES data, synthetic protocol of reagents, ^{1}H NMRs, ^{13}C NMRs, ^{31}P NMRs.

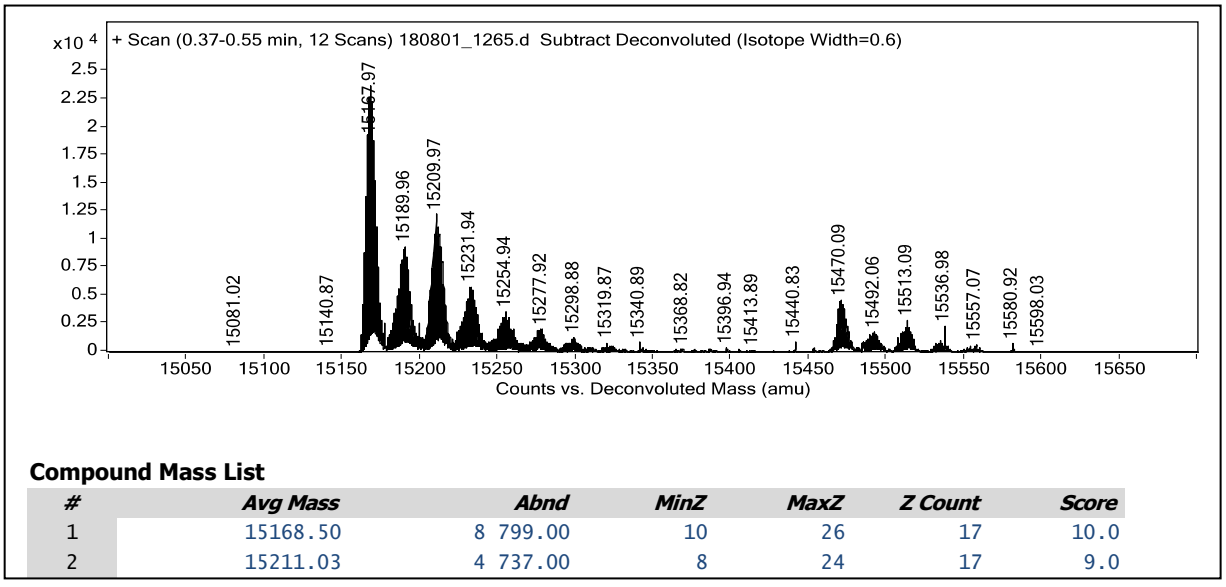
1. Mass Spectral Data:



Compound Mass List

#	Avg Mass	Abnd	MinZ	MaxZ	Z Count	Score
1	15168.61	89 377.00	8	26	19	10.0
2	15126.42	57 350.00	8	26	19	10.0

Figure S1. ESI(+)-MS of Peak 2+3 from Figure 3 (MAP acetylation). Peaks identified: α subunit (15126.42 Da; calculated mass = 15126 Da), mono-acetylated α subunit (15168.61 Da; calculated mass = 15168 Da).

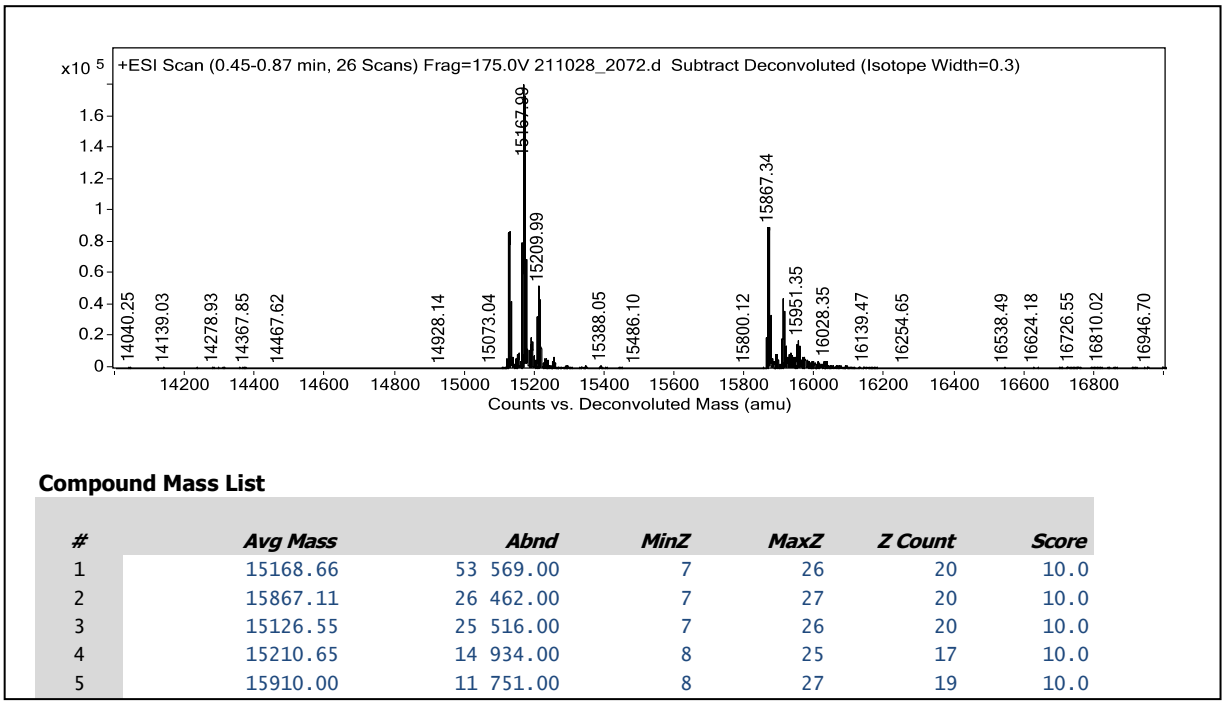


Compound Mass List

#	Avg Mass	Abnd	MinZ	MaxZ	Z Count	Score
1	15168.50	8 799.00	10	26	17	10.0
2	15211.03	4 737.00	8	24	17	9.0

Figure S2. ESI(+)-MS of Peak 4+5 from Figure 3 (ADBS acetylation). Peaks identified: Monoacetylated α-subunit (15168.50 Da; calculated mass = 15168 Da), bisacetylated α-subunit (15210.03 Da; calculated mass = 15210 Da). The ADBS acetylated chromatogram was used to distinguish the identities of peak 4 and 5 (separation of the two peaks was difficult due to

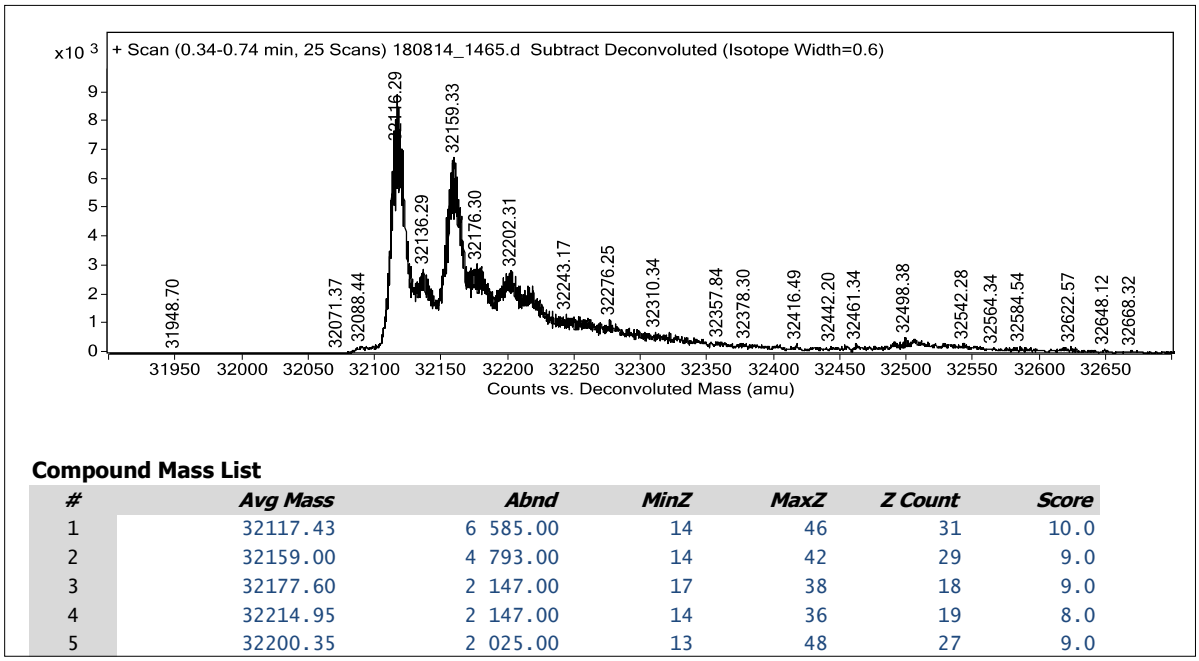
significant overlap). The difference in absorbance of the two peaks is significant with ADBS compared with MAP acetylation, thus the major peak in the ESI-MS spectrum would be peak 4.



Compound Mass List

#	Avg Mass	Abnd	MinZ	MaxZ	Z Count	Score
1	15168.66	53 569.00	7	26	20	10.0
2	15867.11	26 462.00	7	27	20	10.0
3	15126.55	25 516.00	7	26	20	10.0
4	15210.65	14 934.00	8	25	17	10.0
5	15910.00	11 751.00	8	27	19	10.0

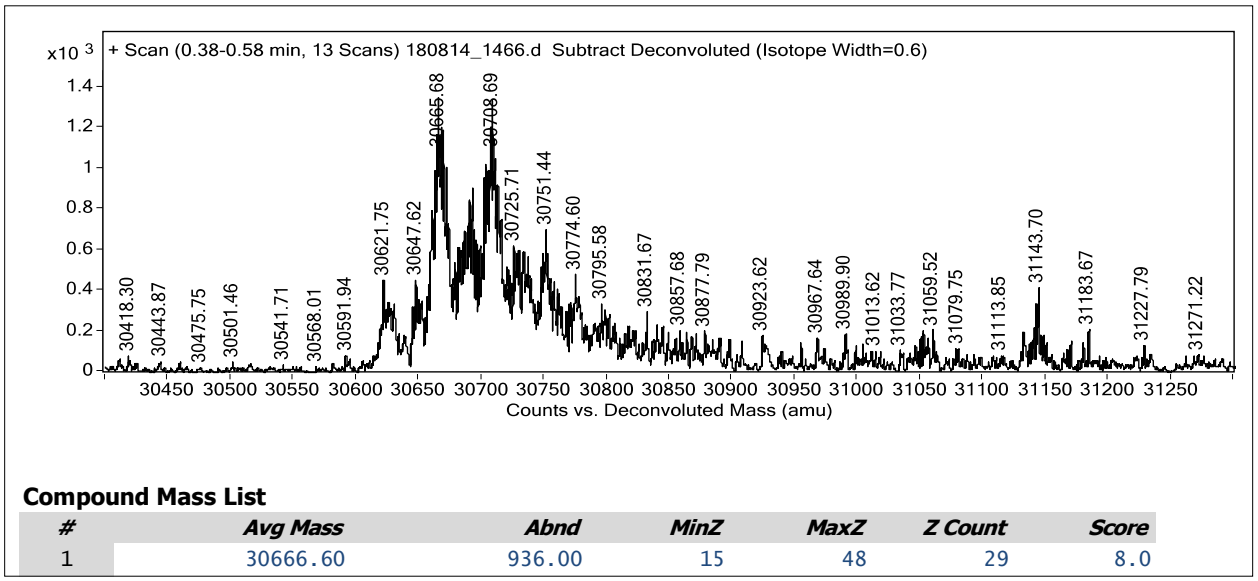
Figure S3. ESI(+)-MS of all peaks from MAP acetylated Hb in Figure 3. Peaks identified: α-subunit (15126.55 Da; calculated mass = 15126 Da), monoacetylated α-subunit (15168.66 Da; calculated mass = 15168 Da), bisacetylated α-subunit (15210.65 Da; calculated mass = 15210 Da), β-subunit (15867.11 Da; calculated mass = 15867 Da), monoacetylated β-subunit (15910.00 Da; calculated mass = 15909 Da).



Compound Mass List

#	Avg Mass	Abnd	MinZ	MaxZ	Z Count	Score
1	32117.43	6 585.00	14	46	31	10.0
2	32159.00	4 793.00	14	42	29	9.0
3	32177.60	2 147.00	17	38	18	9.0
4	32214.95	2 147.00	14	36	19	8.0
5	32200.35	2 025.00	13	48	27	9.0

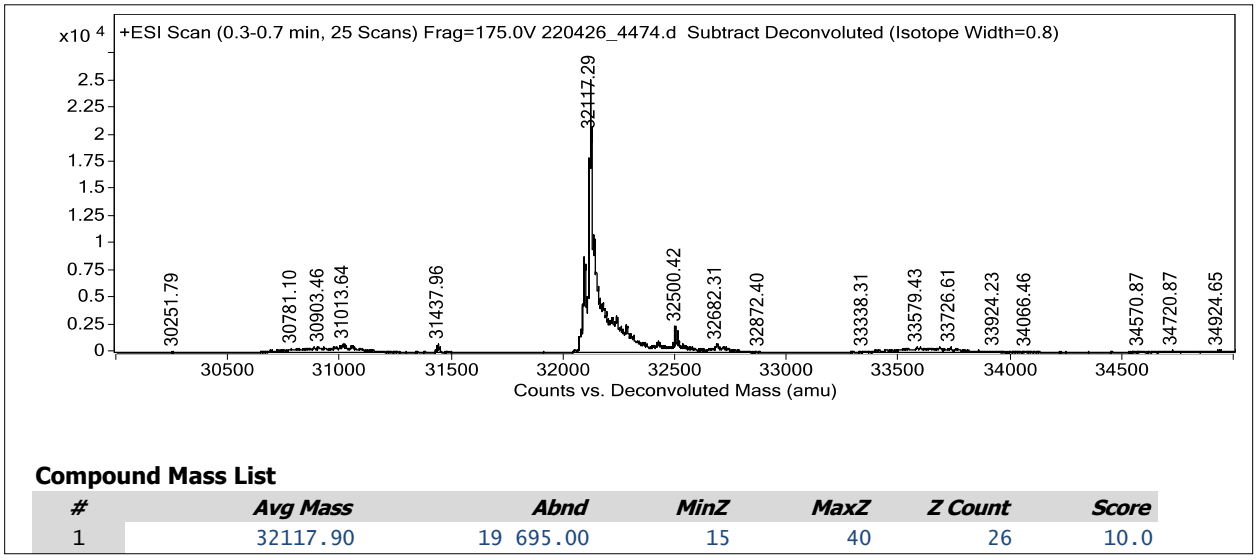
Figure S4. ESI(+)-MS of β-subunit cross-linked peaks (Figure 5B, elution time: 47 to 50 minutes). Peaks identified: β-subunit cross-linked Hb (32117.43 Da; calculated mass = 32117 Da (*calculated by the following formula: 2*(MW of β-subunit) + (MW of azide cross-linker) – 2*(MW of hydroxyl group)*)), monoacetylated β-cross-linked Hb (32159.00 Da; calculated mass = 32159 Da), bisacetylated β-cross-linked Hb (32200.35 Da; calculated mass = 32201 Da).



Compound Mass List

#	Avg Mass	Abnd	MinZ	MaxZ	Z Count	Score
1	30666.60	936.00	15	48	29	8.0

Figure S5. ESI(+)-MS of α-subunit cross-linked peaks (Figure 5B, elution time: 56 minutes). Peaks identified: α-subunit cross-linked Hb (30666.60 Da, calculated mass = 30636 Da)



Compound Mass List

#	Avg Mass	Abnd	MinZ	MaxZ	Z Count	Score
1	32117.90	19 695.00	15	40	26	10.0

Figure S6. ESI(+)-MS of β-subunit cross-linked peak from the cross-linking reaction of native Hb and the azide cross-linker (elution time: 47 minutes) Peaks identified: β-subunit cross-linked Hb (32117.90 Da; calculated mass = 32117 Da).

2. Additional HPLC Chromatograms

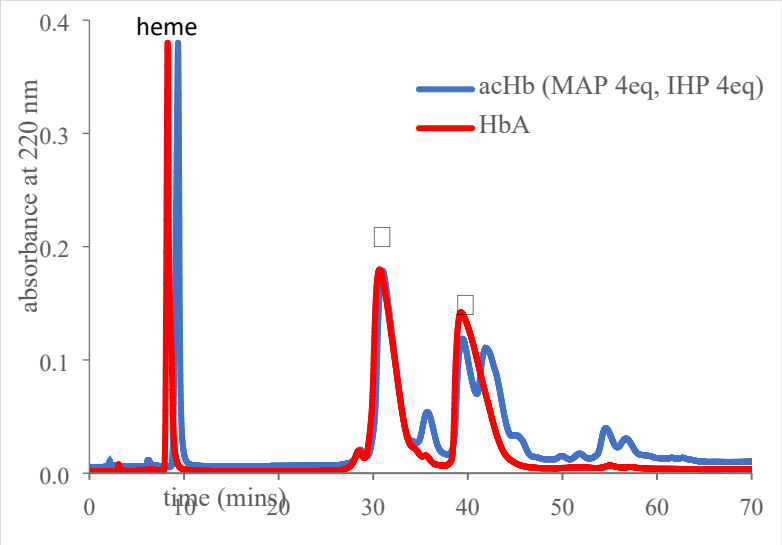


Figure S7. Full C4 reverse-phase HPLC chromatogram of MAP (4 equiv) acetylated Hb and native Hb to confirm the identities of the native β and α subunit peaks.

Table S1. Relative peak areas of all peaks from Figure S7 determined by simple splice.

Peak	**Relative peak area with respect to all peaks (au, raw data)**		**% β subunits[a]**		**% α subunits[b]**	
	MAP	ADBS	MAP	ADBS	MAP	ADBS
β	38.59	40.78	84.8	92.79	-	-
1	6.94	3.17	15.2	7.21	-	-
α	18.66	19.79	-	-	34.24	36.96
2	24.55	14.41	-	-	45.05	26.97
3	4.29	1.13	-	-	7.87	2.11
4	4.22	13.96	-	-	7.74	26.13
5	2.77	4.18	-	-	5.08	7.82

[a]*calculated via the formula: (area of peak)/(sum of all β-subunit peak areas), assuming that any potential changes to the extinction coefficient are negligible upon acetylation.*
[b]*calculated via the formula: (area of peak)/(sum of all α-subunit peak areas), assuming that any potential changes to the extinction coefficient are negligible upon acetylation.*

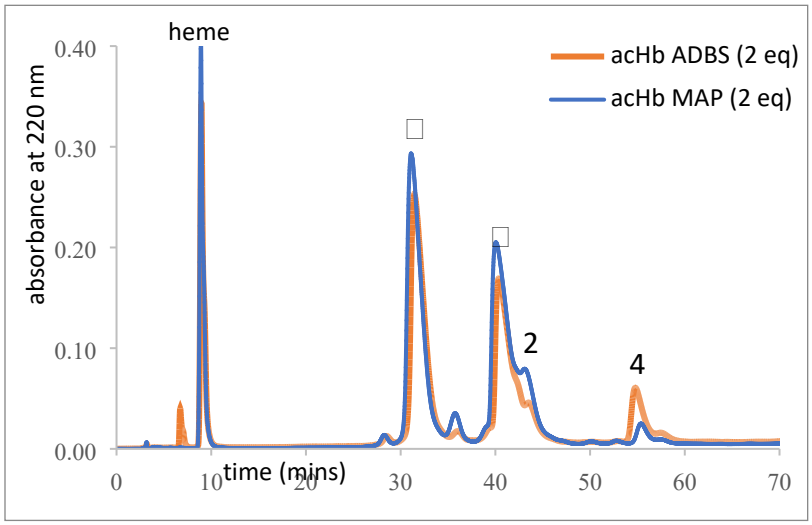


Figure S8. Analytical C4 reverse-phase chromatogram of Hb acetylated with either MAP (2 equiv) or ADBS (2 equiv). Evidently, 2 equivalents of acetylating agent was insufficient in blocking alpha subunit amino groups.

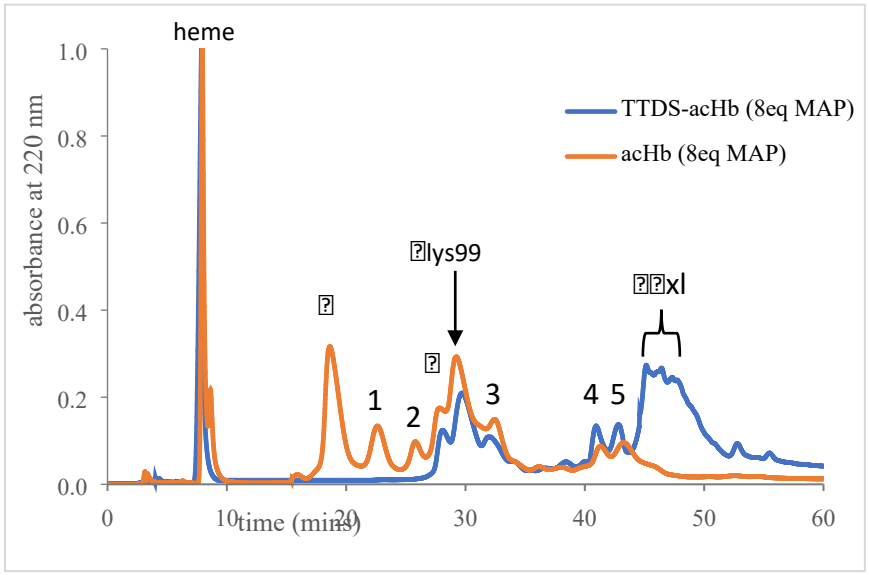


Figure S9. Reverse-phase C4 HPLC analysis of the reaction between TTDS and Hb acetylated with 8 equivalents (12 mM) of MAP. All beta subunit peaks (β, 1, 2) disappear upon reacting with TTDS, and re-emerge as a ββ-crosslinked peak eluting around 48 minutes. This confirms that IHP effectively blocks the 2,3-DPG site from acetylation, leaving it fully available for cross-linking.

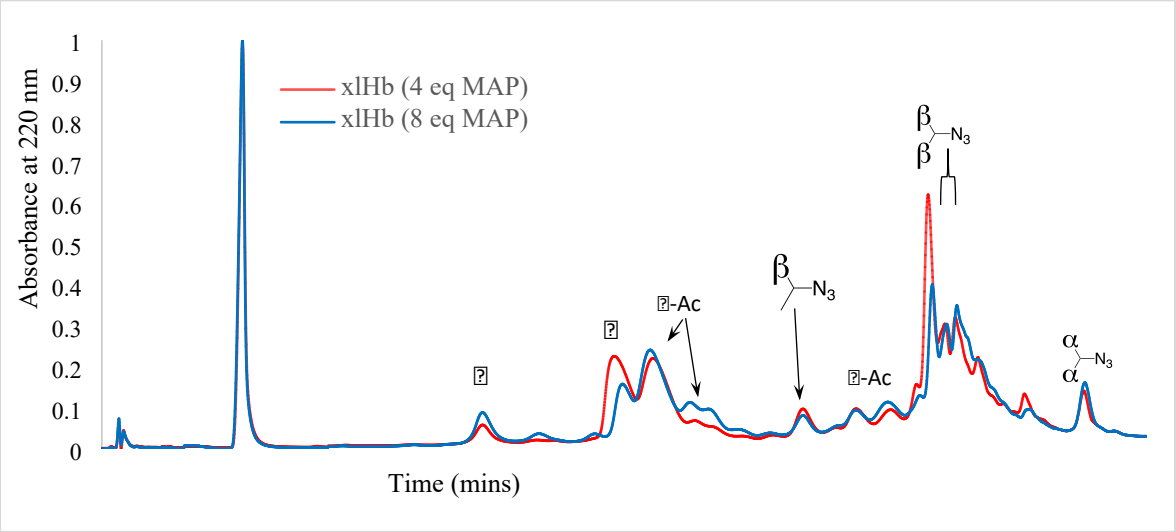


Figure S10. reverse-phase C4 HPLC analysis of the cross-linking reactions between acetylated Hbs (4 equiv and 8 equiv MAP) and 5 equivalents of the azide functionalized cross-linker. β and α subunit cross-linked peaks were determined by ESI(+)-MS analysis and ββ:αα selectivity was determined based on their relative peak areas. Increasing the extent of acetylation decreases the selectivity of the cross-linking towards the β-subunits.

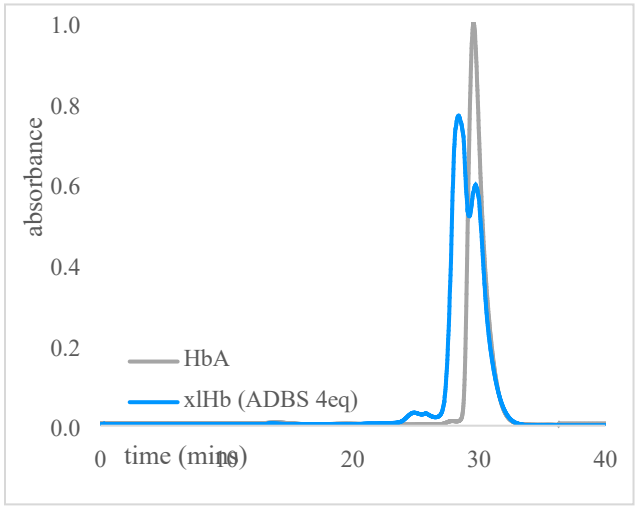


Figure S11. Analytical size-exclusion chromatographic analysis of the cross-linking reaction between ADBS acetylated Hb (4 equiv) and 5 equivalents of the azide cross-linker. The 60% yield obtained is consistent with previous reports.

3. Oxygen Binding Data

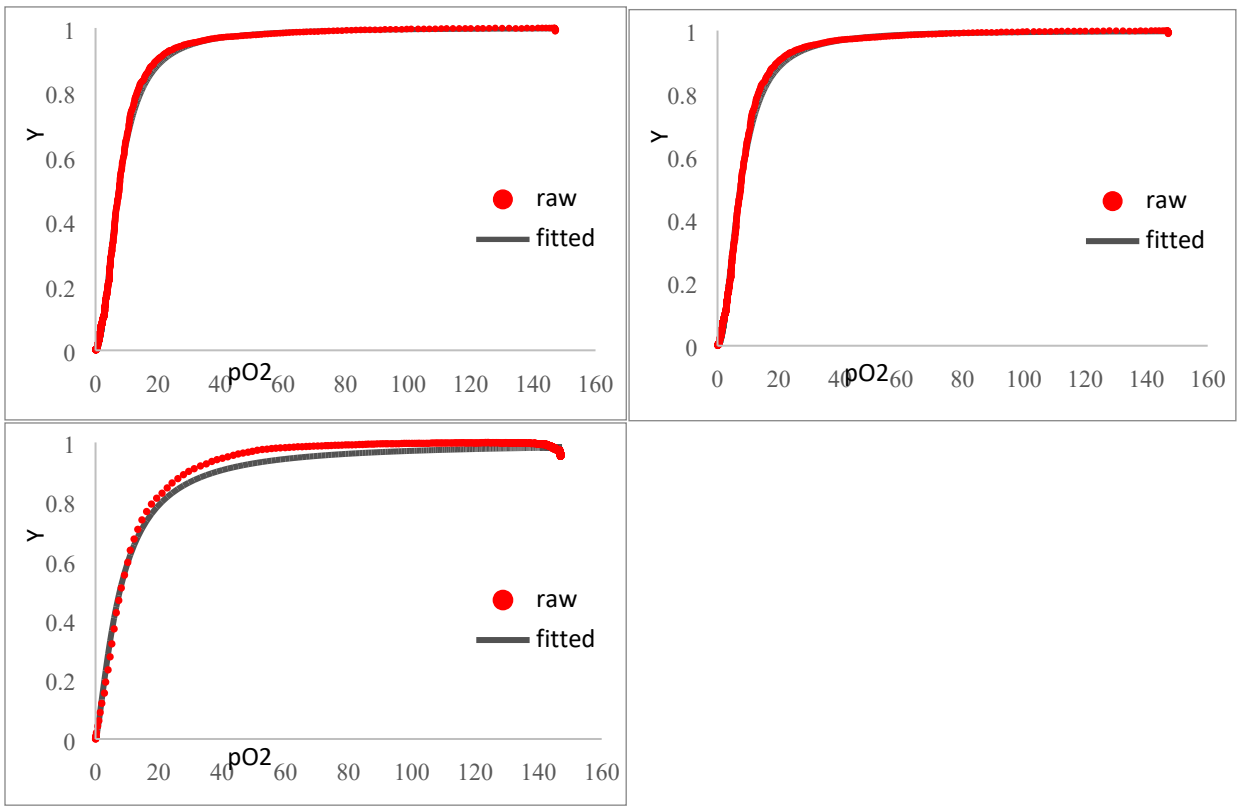


Figure S11. Oxygen binding curves of native Hb performed in triplicate. Data was fit to the fill equation using excel solver. The oxygen affinity (p50) was determined by the pO_2 value at half saturation (Y=0.5).

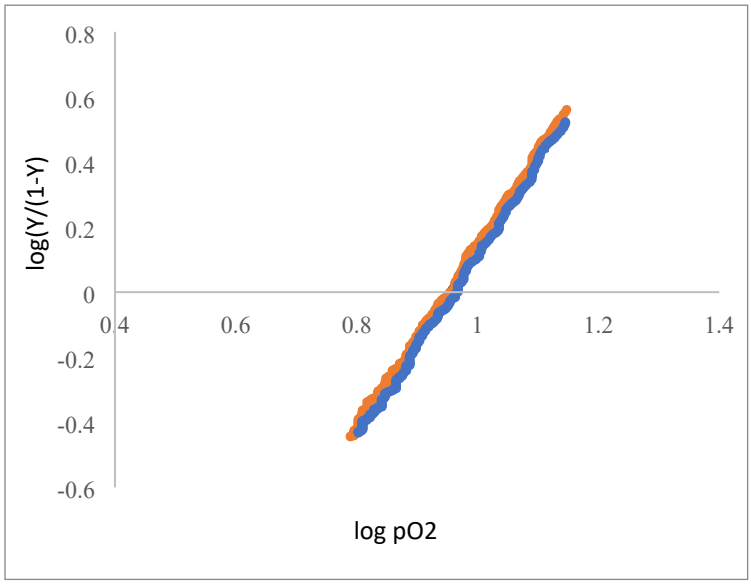


Figure S12. Hill plots of native Hb derived from its associated oxygen binding curve. Cooperativity (n_{50}) is determined by the slop of the plot near log(Y/(1-Y))=0.

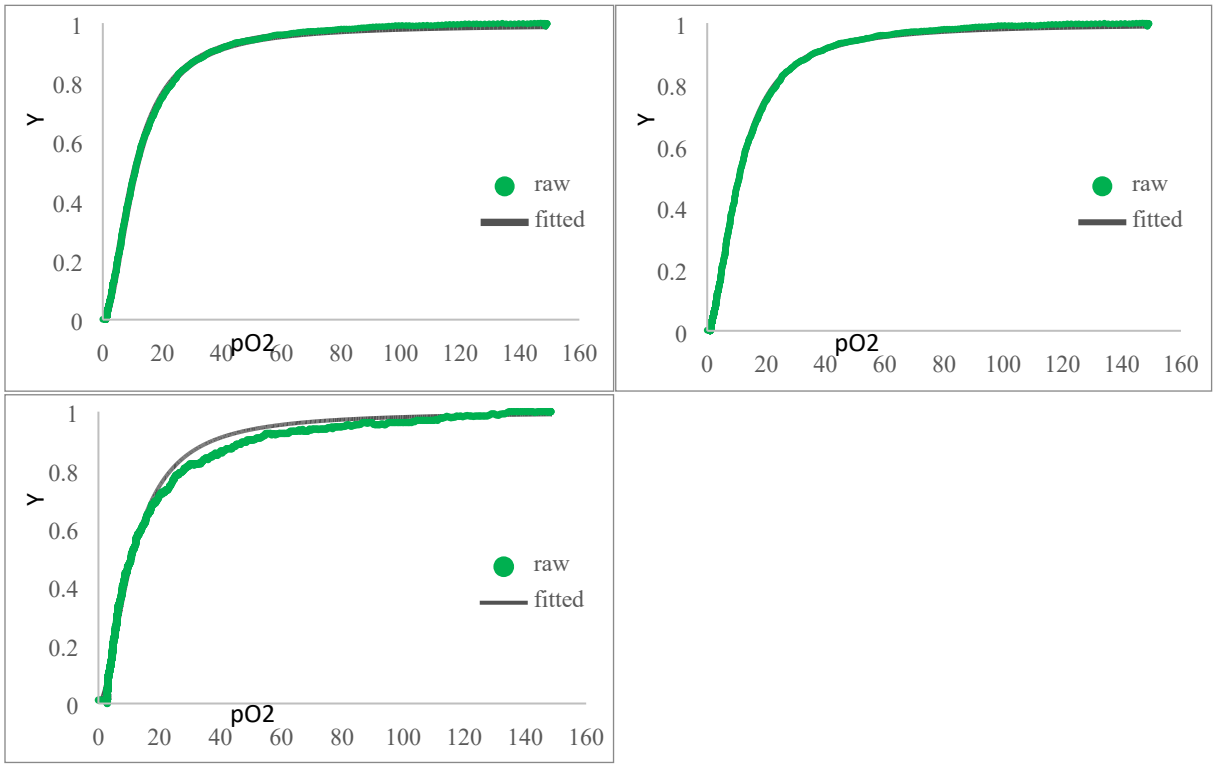


Figure S13. Oxygen binding curves of MAP acetylated Hb (4 equiv) performed in triplicate. Data was fit to the fill equation using excel solver. The oxygen affinity (p50) was determined by the pO_2 value at half saturation (Y=0.5).

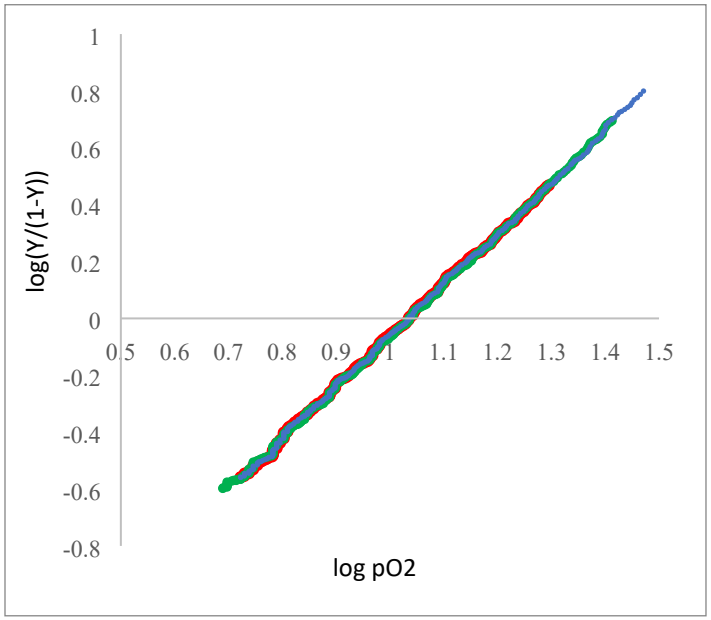


Figure S14. Hill plots of MAP (4 equiv) acetylated Hb derived from its associated oxygen binding curve. Cooperativity (n_{50}) is determined by the slop of the plot near log(Y/(1-Y))=0.

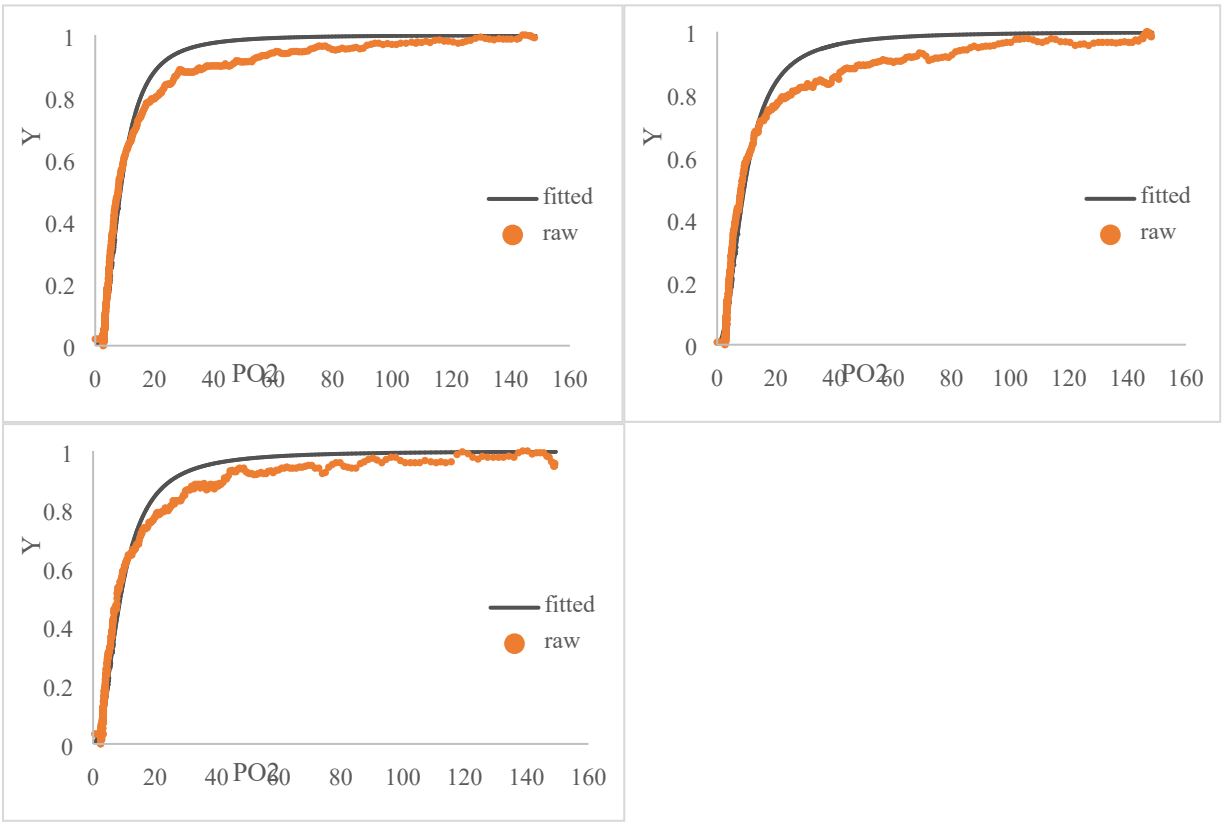


Figure S15. Oxygen binding curves of MAP acetylated Bis-tetramers performed in triplicate. Data was fit to the fill equation using excel solver. The oxygen affinity (p50) was determined by the pO_2 value at half saturation (Y=0.5).

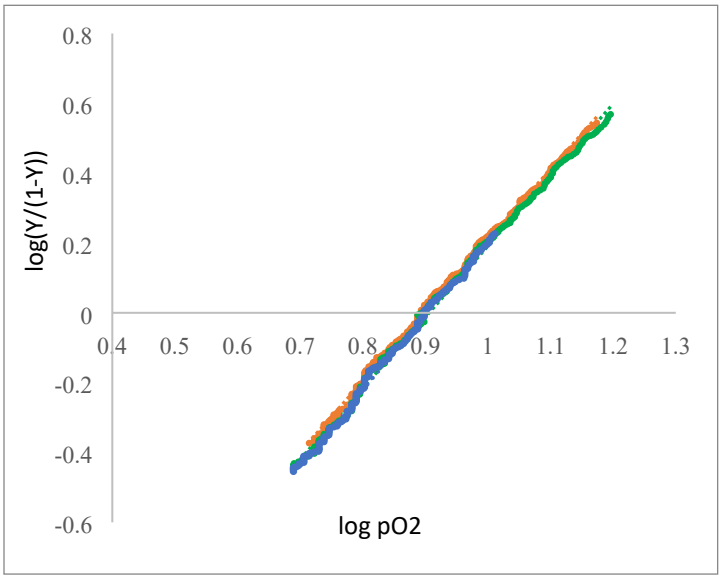


Figure S16. Hill plots of acetylated bis-tetramers derived from its associated oxygen binding curve. Cooperativity (n_{50}) is determined by the slop of the plot near log(Y/(1-Y))=0.

4. ICP-OES Data

Table S2. Average measured concentration of copper in bis-tetramer solutions prior to purification (pre-BT) and after purification (post-BT). ICP-OES experiments were performed in triplicate.

	Average Measured Concentration	Average Measured Concentration	Average Measured Concentration	Average Measured Concentration	Average Measured Concentration
analyte	Cu 327.396 (Aqueous-Axial-iFR)	Cu 224.700 (Aqueous-Axial-iFR)	Cu 324.754 (Aqueous-Axial-iFR)	Cu 219.958 (Aqueous-Axial-iFR)	Cu 221.810 (Aqueous-Axial-iFR)
	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)
Std1 (0.01 ppm)	0.012585317	0.013062302	0.012933984	0.013941876	-0.010298832
Std2 (0.1 ppm)	0.116525588	0.119363813	0.118411312	0.119691681	0.094288047
Std3 (1 ppm)	1.072472884	1.096170719	1.091836371	1.089049023	1.057738901
Std4 (10 ppm)	9.992583964	9.990185368	9.990628433	9.990893526	9.99430488
Hb	0.010950616	0.014470326	0.009061592	0.011485811	-0.029613562
pre-BT	4.389569479	4.33949147	4.437706266	4.13679881	4.38092053
post-BT	1.026998997	1.009787854	1.042938359	0.979196867	1.022145524

Table S3. Average raw intensity of copper in bis-tetramer solutions prior to purification (pre-BT) and after purification (post-BT). ICP-OES experiments were performed in triplicate.

	Average Raw Intensity	Average Raw Intensity	Average Raw Intensity	Average Raw Intensity	Average Raw Intensity
analyte	Cu 327.396 (Aqueous-Axial-iFR)	Cu 224.700 (Aqueous-Axial-iFR)	Cu 324.754 (Aqueous-Axial-iFR)	Cu 219.958 (Aqueous-Axial-iFR)	Cu 221.810 (Aqueous-Axial-iFR)
	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)
Std1 (0.01 ppm)	52.92793336	19.44747754	169.212326	11.38022796	8.526011464
Std2 (0.1 ppm)	589.9637579	149.2646474	1101.294346	64.75631683	64.07918321
Std3 (1 ppm)	5529.126813	1342.157455	9703.25656	554.0291214	575.8331533
Std4 (10 ppm)	51617.31939	12203.67577	88340.09913	5047.140421	5322.648314
Hb	44.4818021	21.16698031	134.9927772	10.14055507	-1.733348982
pre-BT	22667.81324	5302.954589	39270.03848	2092.348399	2340.999883
post-BT	5294.17354	1236.665266	9271.154624	498.5824144	556.9271012

Table S4. Blank-corrected intensity average of copper in bis-tetramer solutions prior to purification (pre-BT) and after purification (post-BT). ICP-OES experiments were performed in triplicate.

	Blank Corrected Intensity Average	Blank Corrected Intensity Average	Blank Corrected Intensity Average	Blank Corrected Intensity Average	Blank Corrected Intensity Average
analyte	Cu 327.396 (Aqueous-Axial-iFR)	Cu 224.700 (Aqueous-Axial-iFR)	Cu 324.754 (Aqueous-Axial-iFR)	Cu 219.958 (Aqueous-Axial-iFR)	Cu 221.810 (Aqueous-Axial-iFR)
	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)
Std1 (0.01 ppm)	65.02548301	15.95189997	114.2950231	7.037013558	-5.470406892
Std2 (0.1 ppm)	602.0613075	145.7690698	1046.377043	60.41310243	50.08276485
Std3 (1 ppm)	5541.224363	1338.661877	9648.339257	549.685907	561.8367349
Std4 (10 ppm)	51629.41694	12200.18019	88285.18183	5042.797207	5308.651896
Hb	56.57935176	17.67140273	80.07547425	5.797340675	-15.72976734
pre-BT	22679.91079	5299.459012	39215.12117	2088.005185	2327.003464
post-BT	5306.271089	1233.169688	9216.237321	494.2392	542.9306828

Table S5. Raw standard deviation data from ICP-OES experiments, performed in triplicate.

	Raw STD	Raw STD	Raw STD	Raw STD	Raw STD
analyte	Cu 327.396 (Aqueous-Axial-iFR)	Cu 224.700 (Aqueous-Axial-iFR)	Cu 324.754 (Aqueous-Axial-iFR)	Cu 219.958 (Aqueous-Axial-iFR)	Cu 221.810 (Aqueous-Axial-iFR)
	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)
Std1 (0.01 ppm)	5.26182438	1.40100887	5.16460198	1.44576214	0.70985764
Std2 (0.1 ppm)	5.53988144	1.49636665	14.1292722	1.39151665	0.67568045
Std3 (1 ppm)	10.3108131	3.34268143	27.6536686	2.77235087	1.14800517
Std4 (10 ppm)	221.59185	78.0589016	687.86851	62.2994897	31.1599319
Hb	1.72860056	0.45983524	4.56032146	1.27754686	0.4852309
pre-BT	82.0922685	20.2055573	116.558573	23.8896237	8.99599105
post-BT	17.2320066	7.96744056	64.0375834	1.36812346	4.97054355

5. Syntheses

Synthesis of the azide cross-linker:

Diethyl 5-((4-methyl-2-bromophenyl)carbamoyl)isophthalate (1). Diethyl-1,3,5-benzenetricarboxylate (1.36 g, 5.1 mmol) was refluxed in $SOCl_2$ (6 mL) for 3 h. This mixture was concentrated to give an oil which was subsequently dissolved in dry THF (15 mL, dried over $MgSO_4$). The solution was cooled to 0 °C then added to a solution of 2-bromo-4-methylaniline (0.93 g, 5.0 mmol) and DMAP (0.61 g, 5.0 mmol) in dry THF (10 mL, dried over $MgSO_4$) also at 0 °C. The mixture was stirred overnight at room temperature then filtered. EtOAc was added and the organic phase was washed with H_2O and brine then dried over $MgSO_4$, filtered, and concentrated. Purification by column chromatography (25% EtOAc:hexanes) gave compound **1** as a white solid in 78% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.80 (1H, t, J = 1.6 Hz, Ar), 8.70 (2H, d, J = 1.6 Hz, Ar), 8.31 (2H, m, Ar+ NH), 7.37 (1H, d, J = 2.1 Hz, Ar), 7.13 (1H, dd, J = 8.5, 2.1 Hz, Ar), 4.39 (4H, q, J = 7.1 Hz, CH_2-O), 2.28 (3H, s, CH_3), 1.39 (6H, t, J = 7.1 Hz, CH_3CH_2-O). ^{13}C NMR ($CDCl_3$, 500 MHz): 164.93 (CO_2Et), 163.40 (CONH), 136.08 (Ar), 135.42 (Ar), 133.61 (Ar), 132.74 (Ar), 132.62 (Ar), 131.98 (Ar), 131.96 (Ar), 131.86 (Ar), 122.07 (Ar), 114.13 (Ar), 61.82 (CH_2-O), 61.82 (CH_3), 14.30 (CH_3CH_2-O). ESIMS calculated for $C_{20}H_{20}BrNO_5$: 434.39, found 436.06.

Diethyl 5-((4-bromomethyl-2-bromophenyl)carbamoyl)isophthalate (2). Compound **1** (1.69, 3.9 mmol) was dissolved in DCM (10 mL). NBS (0.71 g, 4.0 mmol) and benzoyl peroxide (24 mg, 0.1 mmol) were added. The mixture was heated to reflux and stirred for 4 h. The organic phase was washed with saturated aq. $NaHCO_3$, dried with $MgSO_4$, filtered, then concentrated to give the crude brominated product **2**.

Diethyl 5-((4-azidomethyl-2-bromophenyl)carbamoyl)isophthalate (3). The crude brominated construct **2** was dissolved in anhydrous acetonitrile (5 mL). NaN_3 (0.78 g, 12 mmol) was added and this mixture was stirred overnight at 65 °C. EtOAc was added (50 mL) and the organic phase was washed with H_2O (50 mL), dried over $MgSO_4$, filtered then concentrated. The crude mixture was purification by column chromatography (25% EtOAc:hexanes) to give the product **3** (80% over 2 steps). ^{1}H NMR ($CDCl_3$, 400 MHz): 8.91 (1H, t, J = 1.6 Hz, Ar), 8.80 (2H, d, J = 1.6 Hz,

Ar), 8.57 (2H, m, Ar+ NH), 7.63 (1H, d, J = 2.1 Hz, Ar), 7.39 (1H, dd, J = 8.5, 2.1 Hz, Ar), 4.50 (4H, q, J = 7.1 Hz, CH_2-O), 4.38 (2H, s, CH_2-N_3), 1.48 (6H, t, J = 7.1 Hz, CH_3CH_2-O). ^{13}C NMR ($CDCl_3$, 500 MHz): 166.52 (CO_2Et), 164.50 (CONH), 136.49 (Ar), 135.35 (Ar), 133.22 (Ar), 132.94 (Ar), 132.84 (Ar), 132.26 (Ar), 129.75 (Ar), 128.6 (Ar), 121.32 (Ar), 114.38 (Ar), 62.04 (CH_2-O), 52.78 (CH_2-N_3), 14.46 (CH_3CH_2-O). ESIMS calculated for $C_{20}H_{19}BrN_4O_5$: 475.3, found 477.06.

5-((4-(azidomethyl)-2-bromophenyl)carbamoyl)isophthalic acid (4). The protected ester **3** (1.47 g, 3.1 mmol) was dissolved in MeOH/THF (20 mL, 1:1 by volume). A solution of KOH in H_2O (0.5 g/mL, 6 mL) was added. The mixture was stirred at room temperature for 1 h. HCl (2.0 M) was added until the solution became acidic (pH 2-3). EtOAc (30 mL) was added and the organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated to give the deprotected diacid **4** as a white solid (96%). ^{1}H NMR (DMSO-d_6, 400 MHz): 13.52 (2H, br s, COOH), 10.58 (1H, s, NH), 8.78 (2H, d, J = 1.6 Hz, Ar), 8.56 (1H, t, J = 1.6 Hz, Ar), 8.57 (1H, s, Ar), 7.47 (1H, d, J = 2.1 Hz, Ar), 7.45 (1H, dd, J = 8.5, 2.1 Hz, Ar), 4.53 (2H, s, CH_2-N_3). ^{13}C NMR (DMSO-d_6, 500 MHz): 164.88 (CO_2H), 163.54 (CONH), 135.28 (Ar), 135.14 (Ar), 133.81 (Ar), 133.24 (Ar), 132.00 (Ar), 131.95 (Ar), 131.89 (Ar), 128.35 (Ar), 122.21 (Ar), 114.34 (Ar), 53.63 (CH_2-N_3). ESIMS calculated for $C_{16}H_{11}BrN_4O_5$: 419.19, found 418.98.

1,3-bis(2,4-Dibromo-6-(tert-butoxycarbonyl)phenyl)-5-((4-(azidomethyl)-2bromophenyl)carbamoyl)isophthalate (5). The diacid **4** (1.26 g, 3.0 mmol), tert-butyl 3,5dibromosalicylic acid (2.15 g, 6.1 mmol) and DMAP (36.6 mg, 0.3 mmol) were dissolved in dry THF (20 mL, dried over $MgSO_4$) and cooled in an ice/water bath. A solution of EDC (1.01 g, 6.5 mmol) dissolved in DCM (5 mL) was added and the mixture was stirred initially at 0 ^{o}C then at room temperature overnight. EtOAc was added and the organic layer was washed with saturated $NaHCO_3$ (aq), dried over $MgSO_4$, and concentrated. Purification by column chromatography (25% EtOAc:hexanes) gave the protected diester **5** in 72% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): 9.27 (1H, t, J = 1.6 Hz, Ar), 9.09 (2H, d, J = 1.6 Hz, Ar), 8.66 (1H, Br-s, NH), 8.58 (1H, d, J = 8.4 Hz, Ar), 8.09 (2H, d, J = 2.4 Hz, Ar), 7.99 (2H, d, J =2.4 Hz, Ar), 7.63 (1H, d, J = 2.1 Hz, Ar), 7.40 (1H, dd, J = 8.4, 2.1 Hz, Ar), 4.39 (2H, s, CH_2-N_3), 1.47 (18H, s, tBu). ^{13}C NMR ($CDCl_3$, 500 MHz): 163.03 (CONH), 162.02 (CO_2R), 161.75 (CO_2tBu), 146.64 (Ar), 139.08 (Ar), 136.20 (Ar), 135.43 (Ar), 135.30 (Ar), 133.88 (Ar), 133.84 (Ar), 133.53 (Ar), 132.05 (Ar), 130.98 (Ar), 128.73 (Ar), 128.56 (Ar), 122.24 (Ar), 119.95 (Ar), 119.30 (Ar), 114.41 (Ar), 83.41 (CO_2tBu), 53.79 (CH_2-N_3), 28.15 (tBu). ESIMS calculated for $C_{38}H_{31}Br_5N_4O_9$: 1081.8, found 1081.7.

1,3-bis(2,4-Dibromo-6-(carboxyphenyl)-5-((4-(azidomethyl)-2bromophenyl)carbamoyl)isophthalate (6). The tert-butyl protected ester **5** (2.39 g, 2.2 mmol) was dissolved in DCM (15 mL). TFA (3 mL) was added and the mixture was stirred at room temperature for 1 h then concentrated to give the pure azide cross-linker in 97% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): 10.84 (1H, s, NH), 9.12 (2H, d, J = 1.6 Hz, Ar), 8.99 (1H, t, J = 1.6 Hz, Ar), 8.39 (1H, d, J = 8.4 Hz, Ar), 8.15 (2H, d, J = 2.4 Hz, Ar), 7.80 (2H, d, J =2.4 Hz, Ar), 7.61 (1H, d, J = 2.1 Hz, Ar), 7.48 (1H, dd, J = 8.4, 2.1 Hz, Ar), 4.54 (2H, s, CH_2-N_3). ^{13}C NMR ($CDCl_3$, 500 MHz): 162.88 (CONH), 161.86 (CO_2R), 161.56 (CO_2tBu), 146.48 (Ar), 138.89 (Ar), 136.05 (Ar), 135.80 (Ar), 135.21 (Ar), 133.79 (Ar), 133.71 (Ar), 133.57 (Ar), 131.90 (Ar), 130.76 (Ar), 128.56 (Ar), 128.31 (Ar), 122.52 (Ar), 119.77 (Ar), 119.14 (Ar), 114.66 (Ar), 83.20 (CO_2tBu), 53.56 (CH_2-N_3). ESIMS calculated for $C_{30}H_{15}Br_5N_4O_9$: 964.99, found 964.66.

Synthesis of MAP:

Sodium dimethyl phosphate. Trimethyl phosphate (1.16 mL, 10 mmol) and NaI (1.50 g, 10 mmol) in acetone (15 mL) was stirred at room temperature for 3 days. The resulting precipitate of sodium dimethyl phosphate was vacuum-filtered, rinsed with acetone, and air-dried. No further purification was required. The isolated yield was 85%.

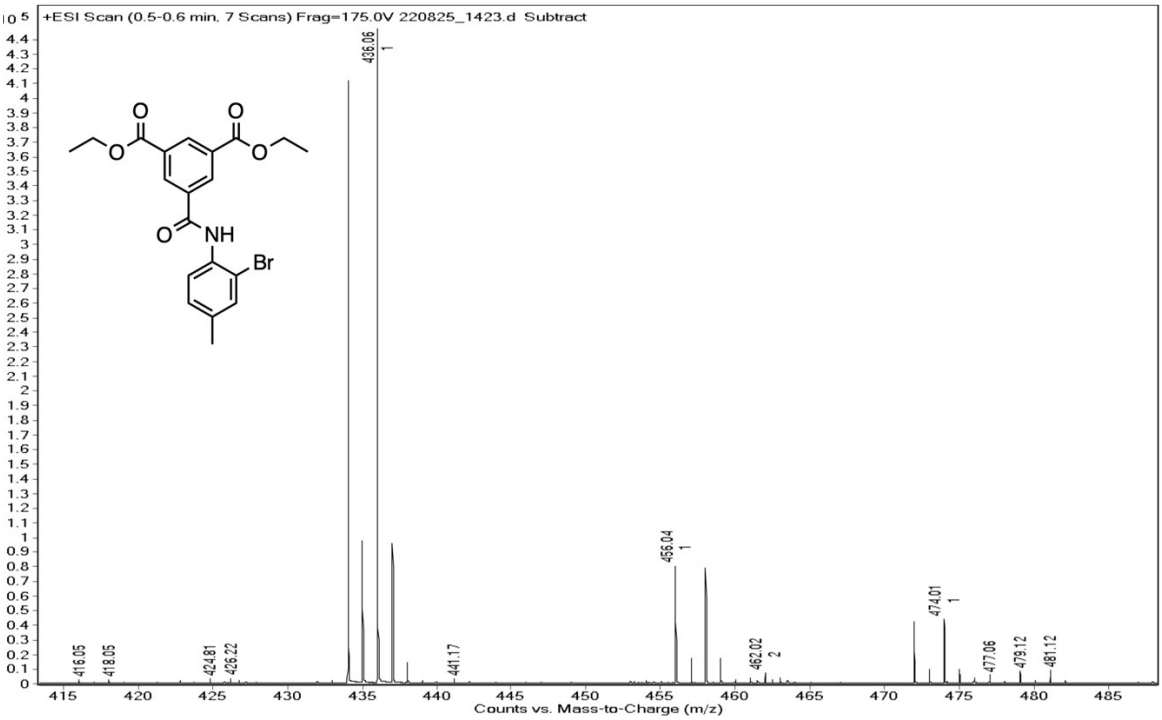
Acetyl dimethyl phosphate. Sodium dimethyl phosphate (1.26g, 8.5 mmol), and acetyl chloride (8.5 mmol, 0.63 mL) were added to 20 mL THF that had been dried over 3 Å molecular sieves and stirred at room temperature for 24 h under nitrogen. The solution was filtered and THF was evaporated, leaving dimethyl acetyl phosphate as an oil. crude yield = 80%. ^{1}H NMR (400 MHz, D_2O): δ 3.52 (6H, d, 10.7 Hz). ^{31}P NMR (400 MHz, D_2O): δ 3.05.

Sodium methyl acetyl phosphate. A solution of sodium iodide (1.02 g, 6.8 mmol) in dry acetone (10 mL) was added to the crude solution of dimethyl acetyl phosphate (1.14 g, 6.8 mmol) in dry acetone (10 mL). The mixture was allowed to stand for 12 h. The resulting precipitate was vacuum-filtered, rinsed with dry acetone and dried. yield = 70%. ^{1}H NMR (400 MHz, D_2O): δ 3.53 (6H, d, 10.7 Hz). ^{31}P NMR (400 MHz, D_2O): δ 6.03. ESIMS calculated = 172.94, found = 172.94.

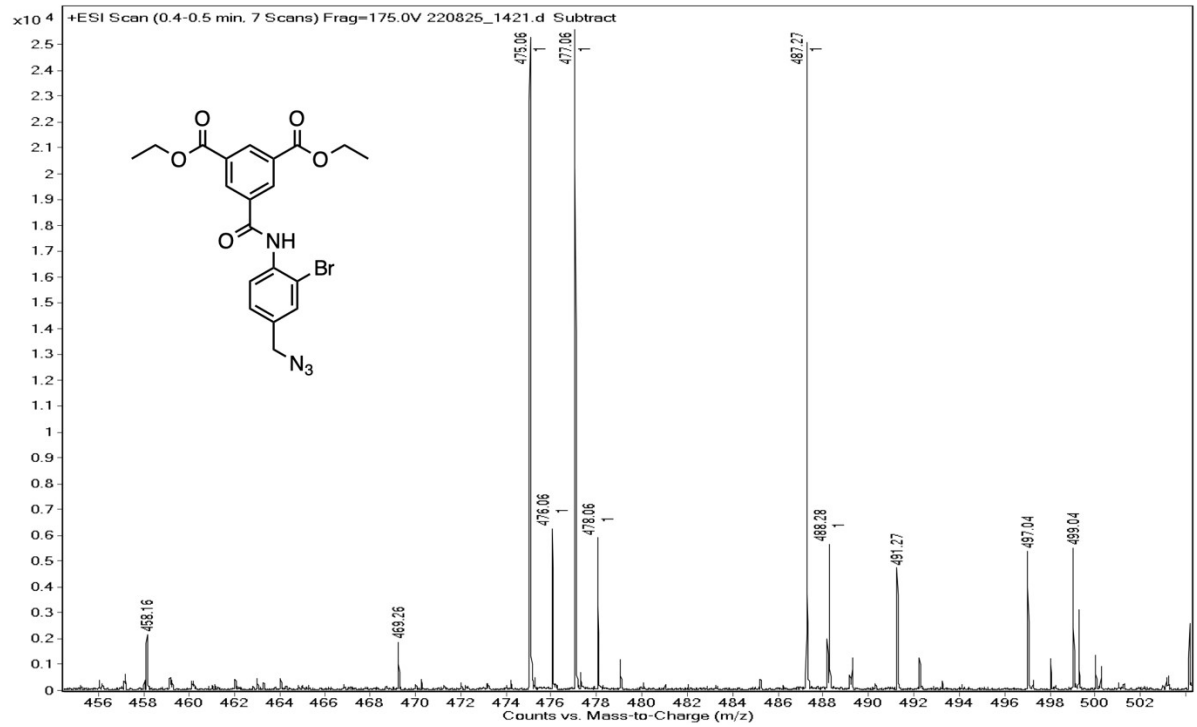
Synthesis of ADBS:

2-acetoxy-3,5-dibromobenzoic acid. 3,5-dibromosalicylic acid (2.0 mmol, 0.59 g) was dissolved in acetic anhydride (6.0 mmol, 0.57 mL). 10-15 drops of 48% (*v/v*) sulfuric acid was added, and the reaction was left to stir for 1 hr. Cold distilled water was added and the precipitate was vacuum-filtered. Recrystallization in toluene afforded the pure product in 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (d, J = 2.4Hz, 1H), 8.02 (d, J = 2.4 Hz, 1H), 2.31 (s, 3H). ESIMS calculated = 337.95, found = 336.85 Da.

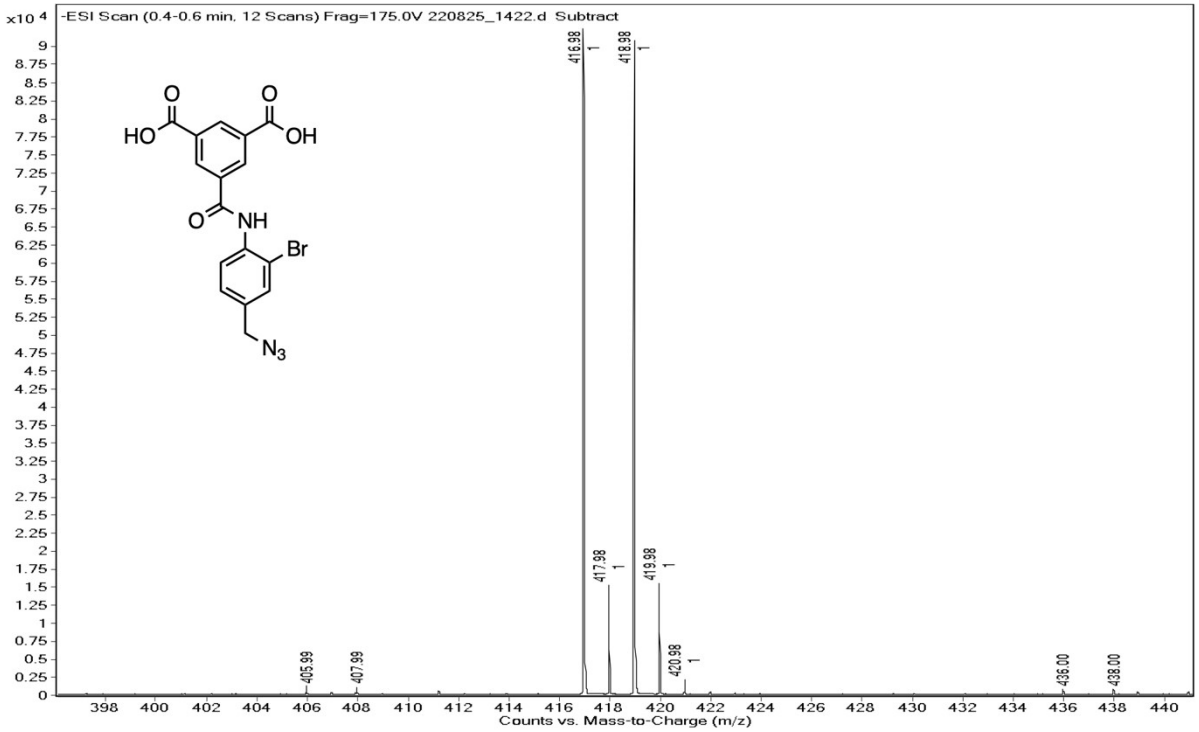
6. ESI-MS Mass Spectra of Synthesized Compounds:



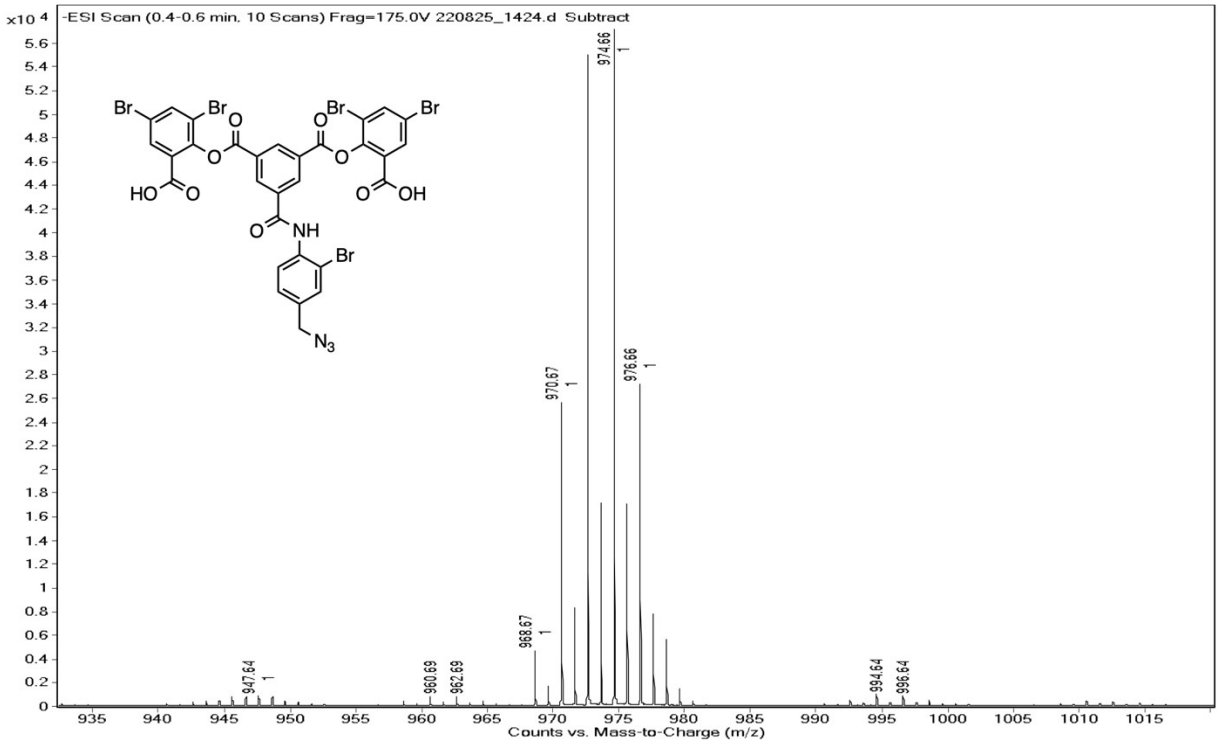
Mass calculated = 434.29 Da, ESI(+)MS mass found = 436.06 Da



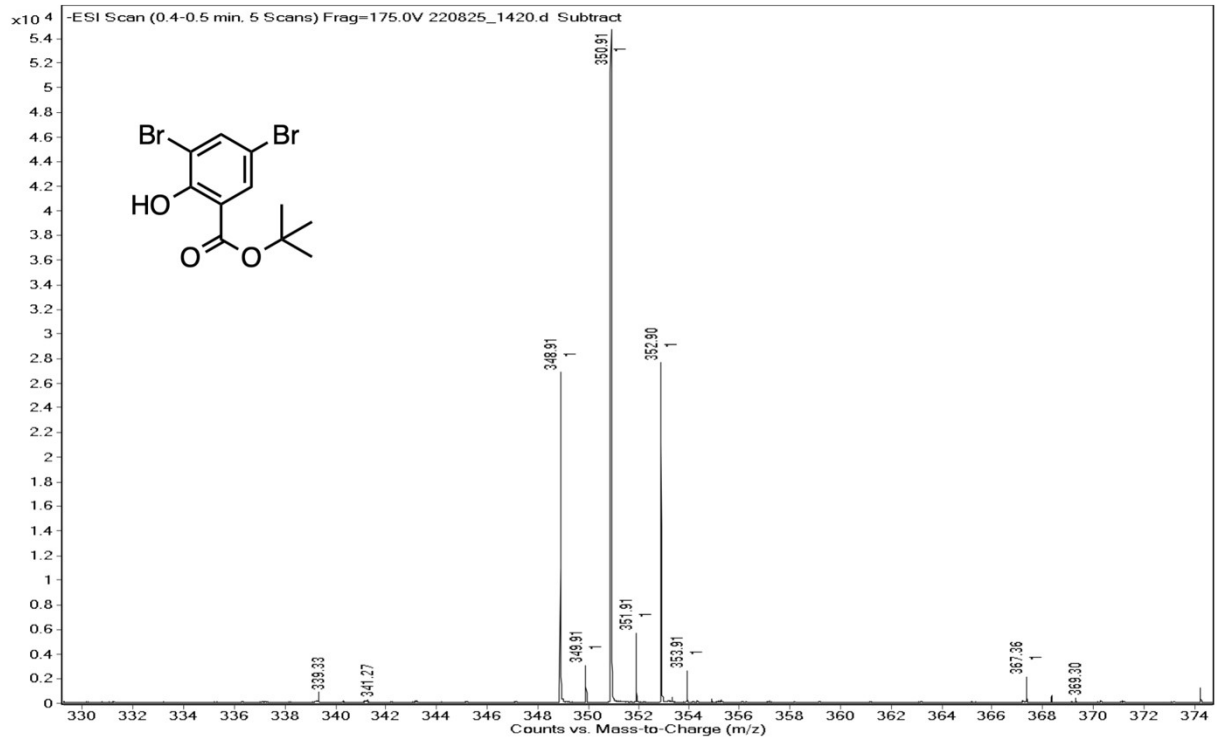
Mass calculated = 475.30 Da, ESI(+)MS mass found = 477.06 Da



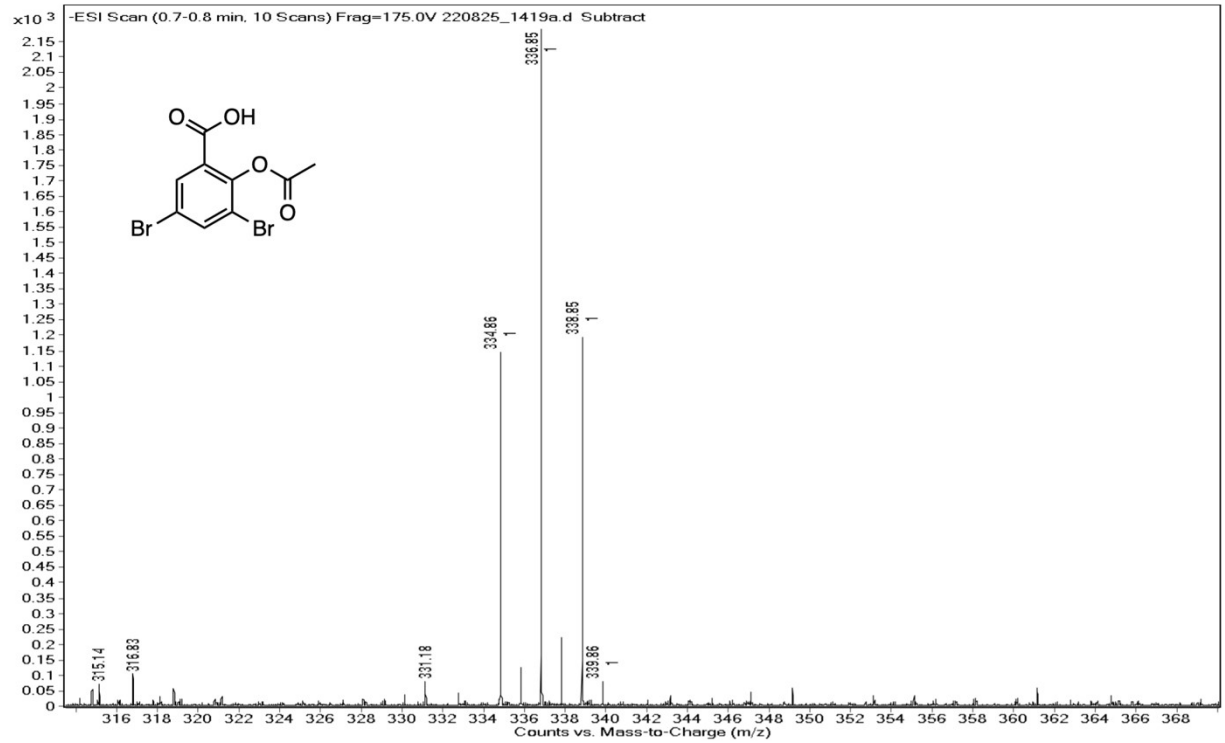
Mass calculated = 419.19 Da, ESI(-)MS mass found = 418.98 Da



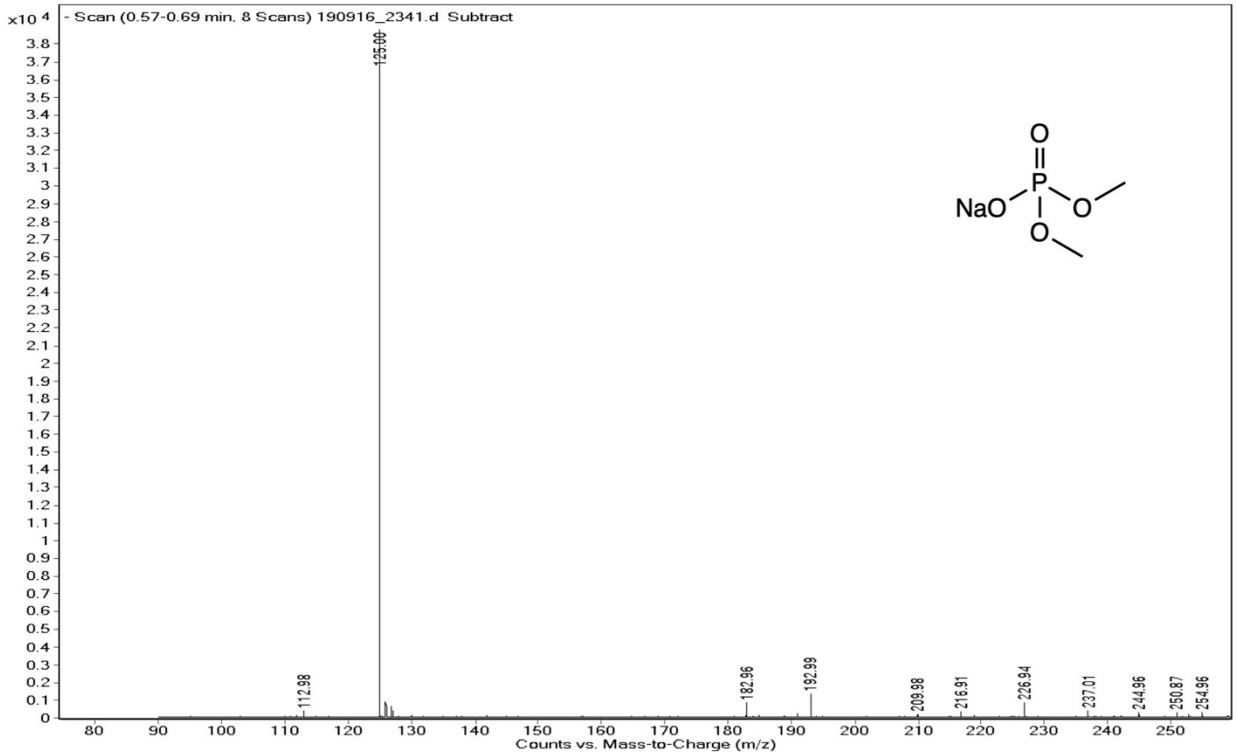
Mass calculated = 974.99 Da, ESI(-)MS mass found = 974.66 Da



Mass calculated = 352.02 Da, ESI(-)MS mass found = 350.91 Da

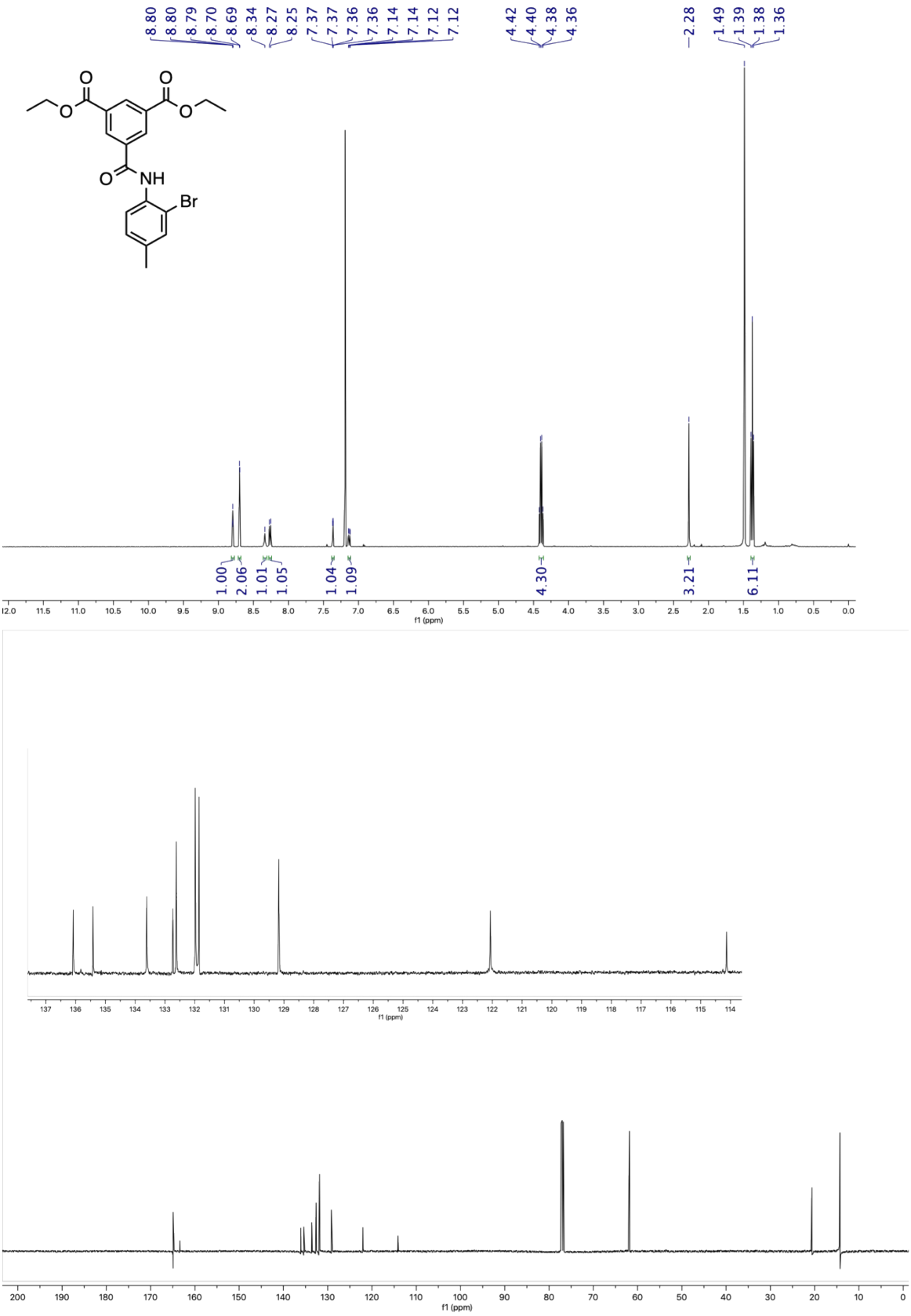


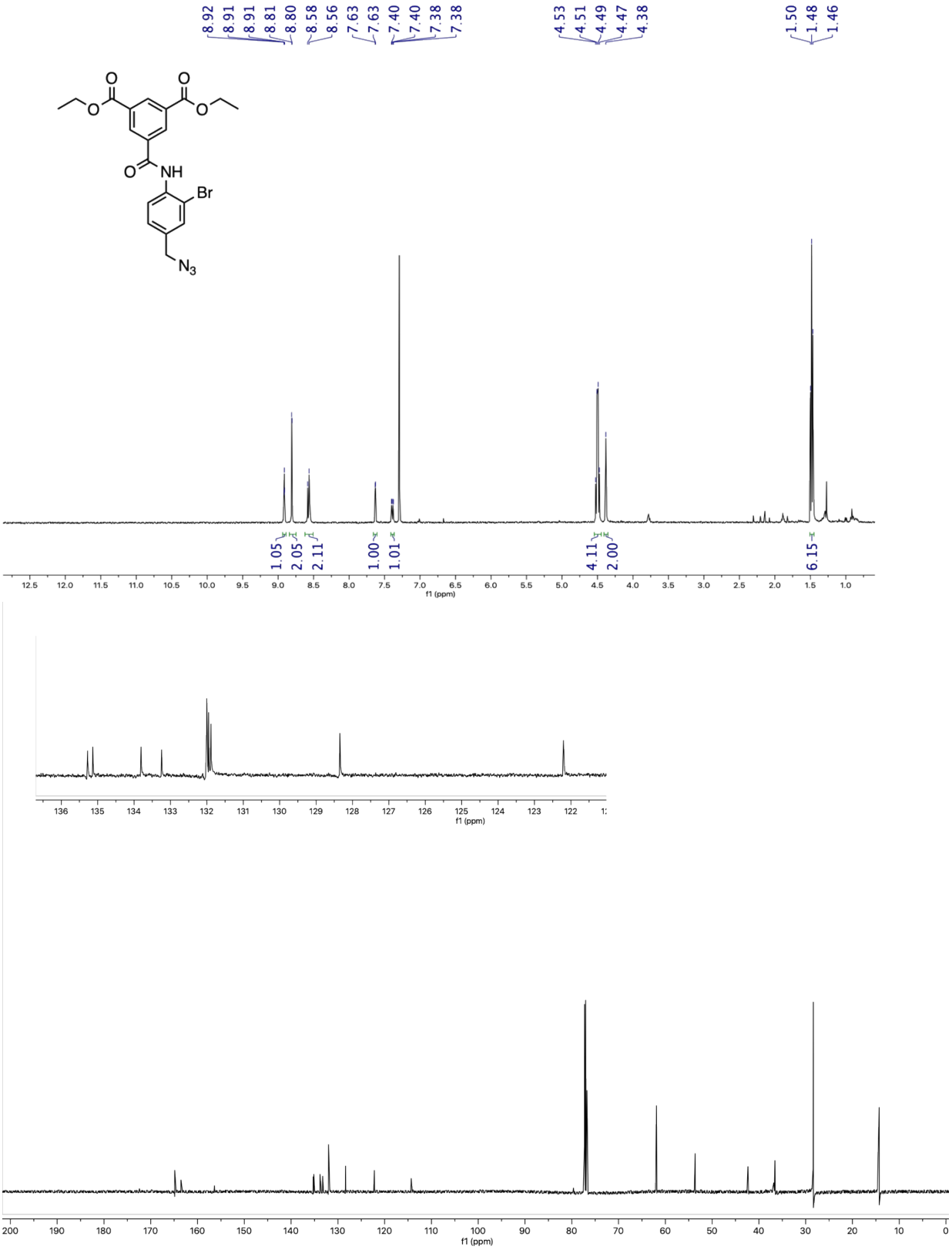
Mass calculated = 337.95 Da, ESI(-)MS mass found = 336.85 Da

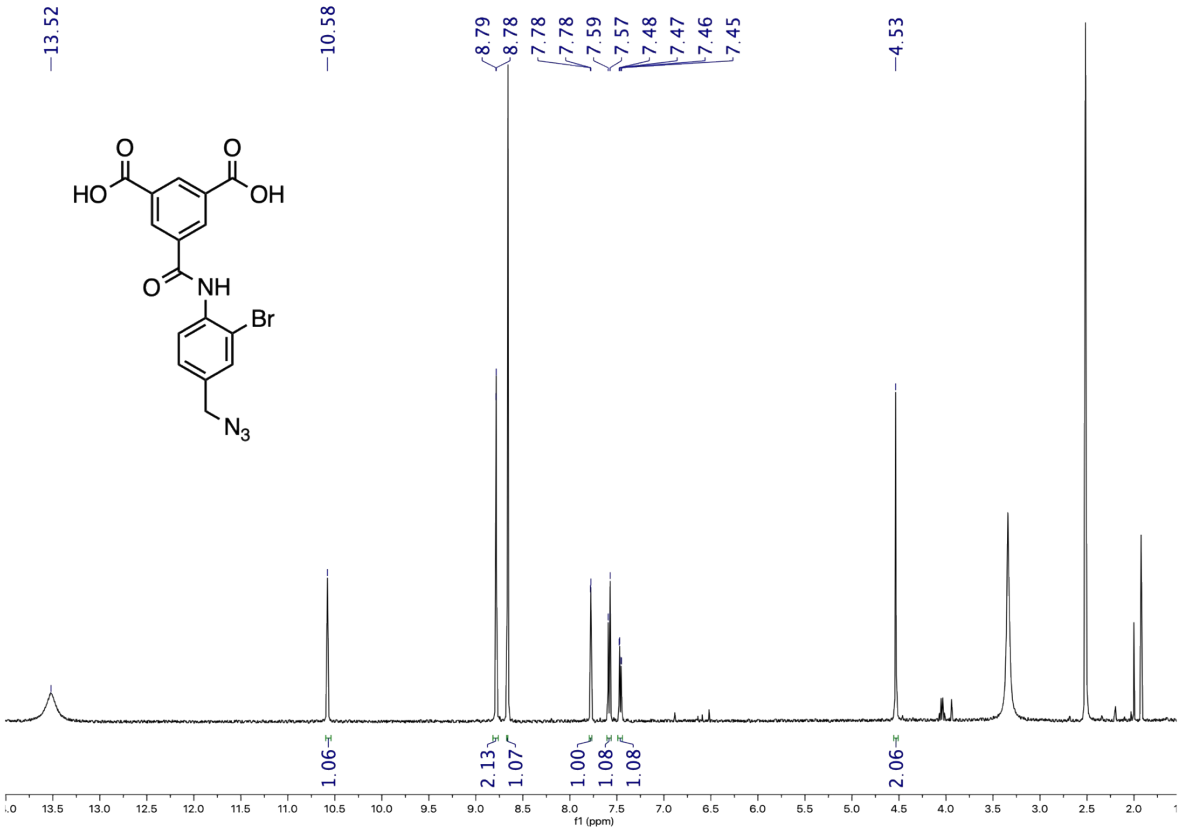


Mass calculated = 125.00 Da, ESI(-)MS mass found = 125.00 Da

7. ^{1}H and ^{13}C NMR Spectra:




8.92
8.91
8.91
8.81
8.80
8.58
8.56
7.63
7.63
7.40
7.40
7.38
7.38
4.53
4.51
4.49
4.47
4.38
1.50
1.48
1.46
O
O
O
O
NH
Br
N3
1.05
2.05
2.11
1.00
1.01
4.11
2.00
6.15
f1 (ppm)
f1 (ppm)
f1 (ppm)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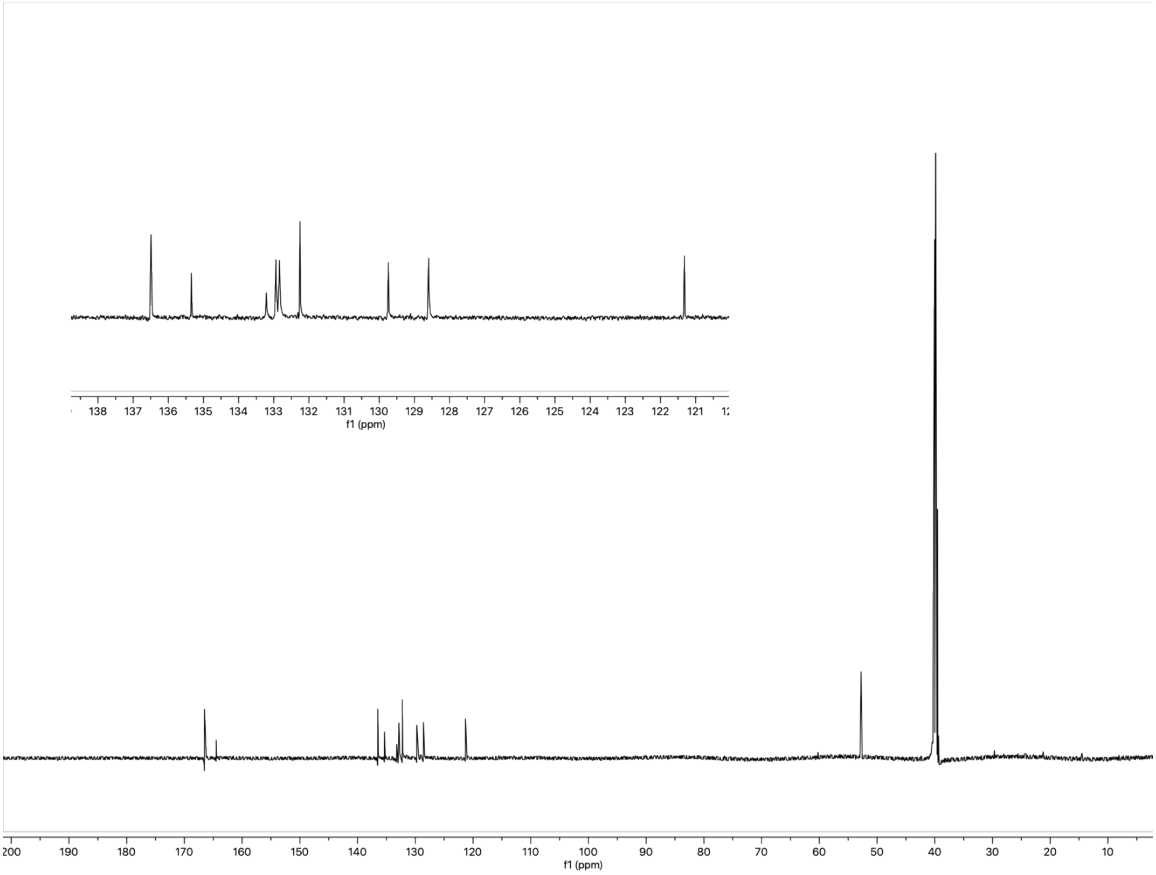
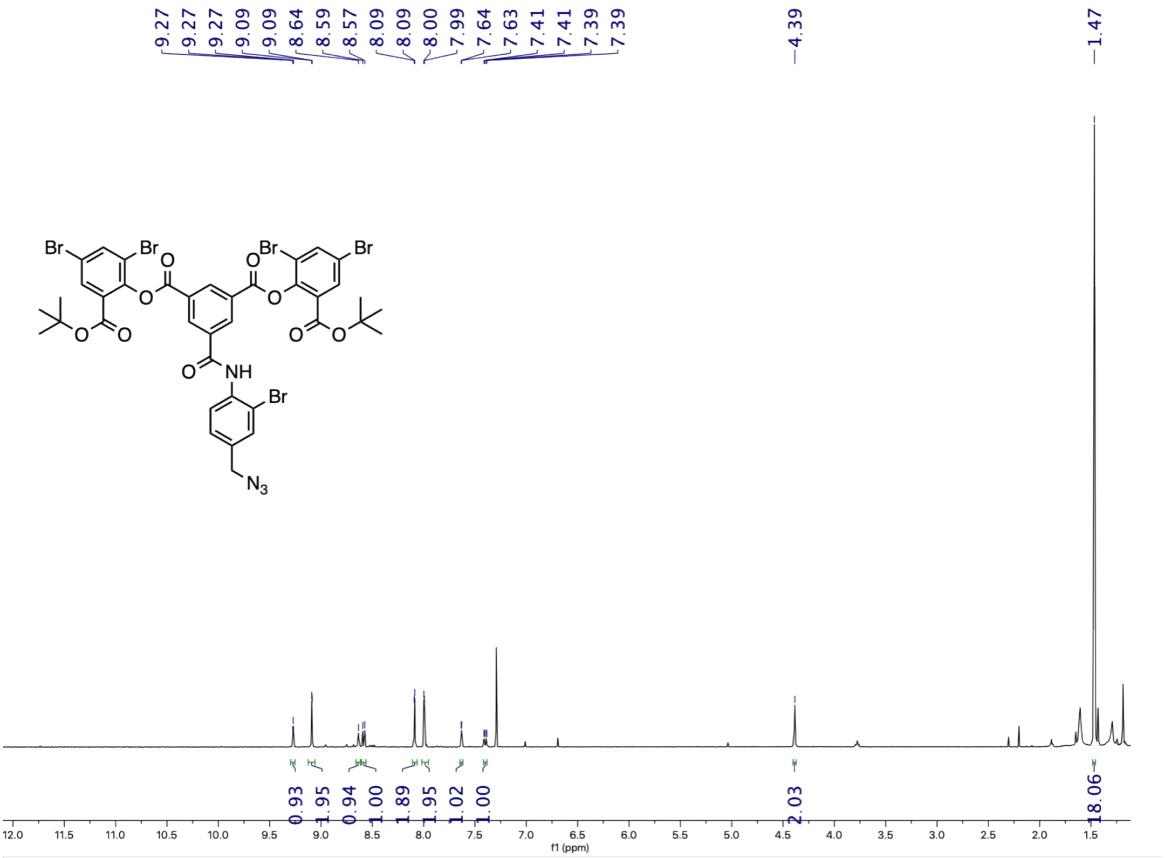
8.78
7.78
7.78
7.59
7.57
7.48
7.47
7.46
7.45
4.53
HO
O
O
OH
O
NH
Br
N3
1.06
2.13
1.07
1.00
1.08
1.08
2.06
f1 (ppm)


f1 (ppm)
f1 (ppm)


9.27
9.27
9.27
9.09
9.09
8.64
8.59
8.57
8.09
8.09
8.00
7.99
7.64
7.63
7.41
7.41
7.39
7.39
4.39
1.47
Br
Br
Br
Br
O
O
O
O
O
O
O
O
NH
Br
N3
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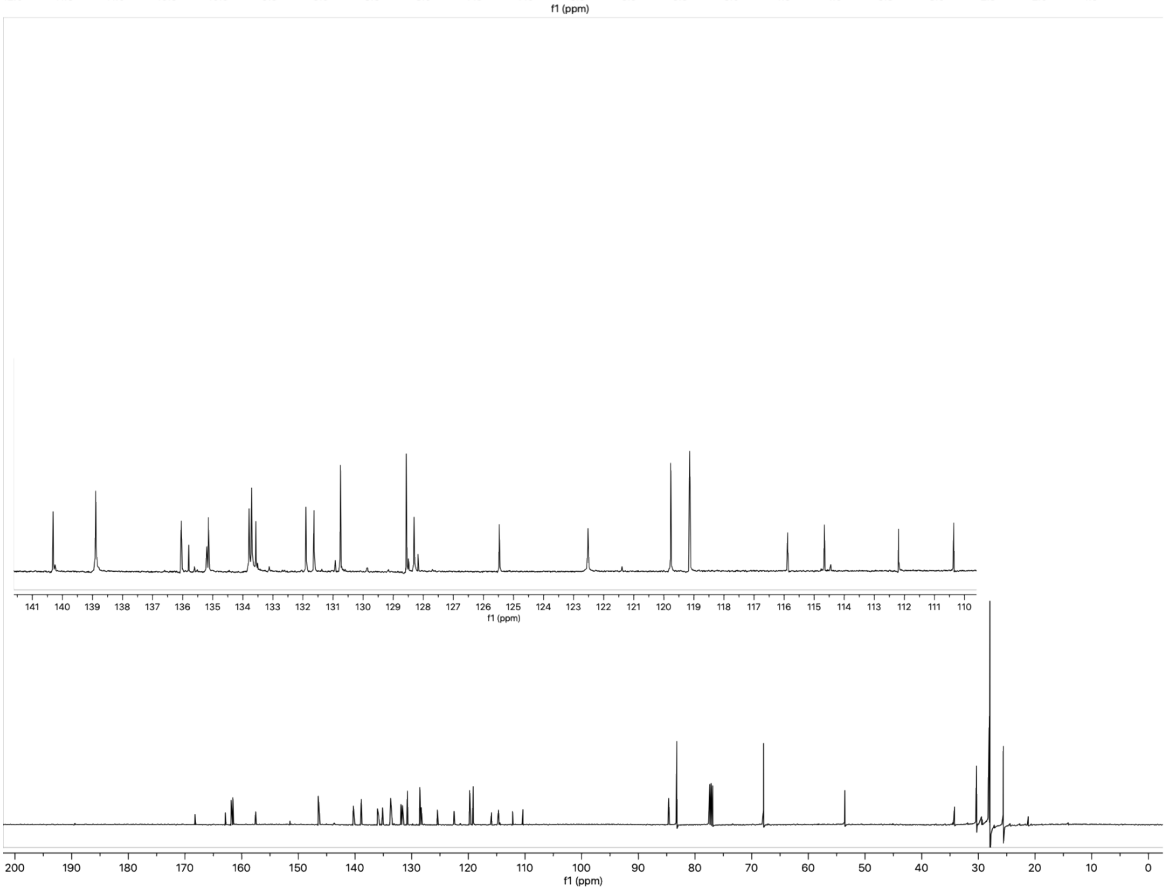
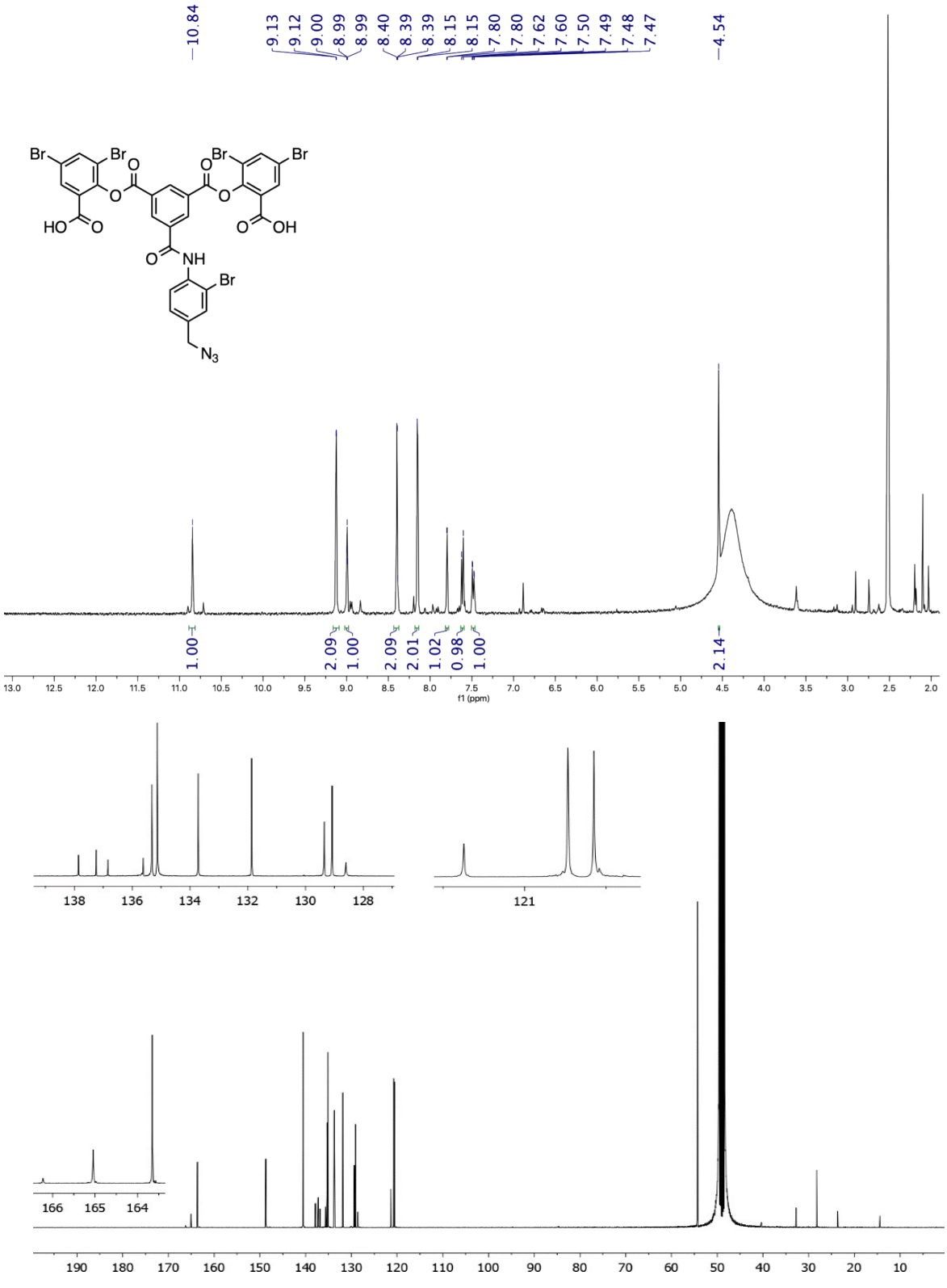
1.95
0.94
1.00
1.89
1.95
1.02
1.00
2.03
18.06
f1 (ppm)


f1 (ppm)
f1 (ppm)


10.84
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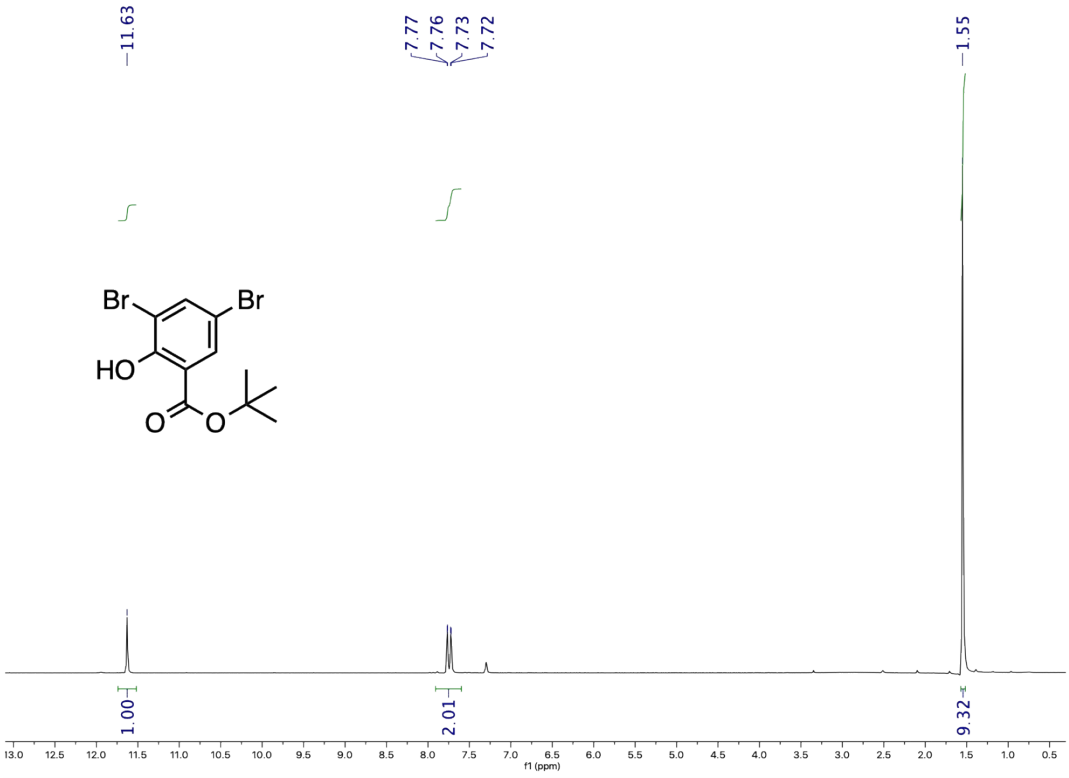
9.12
9.00
8.99
8.99
8.40
8.39
8.39
8.15
8.15
7.80
7.80
7.62
7.60
7.50
7.49
7.48
7.47
4.54
Br
Br
Br
Br
O
O
O
O
HO
O
O
OH
NH
Br
N3
1.00
2.09
1.00
2.09
2.01
1.02
0.98
1.00
2.14
f1 (ppm)
138
136
134
132
130
128
121
166
165
164


11.63
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7.76
7.73
7.72
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Br
HO
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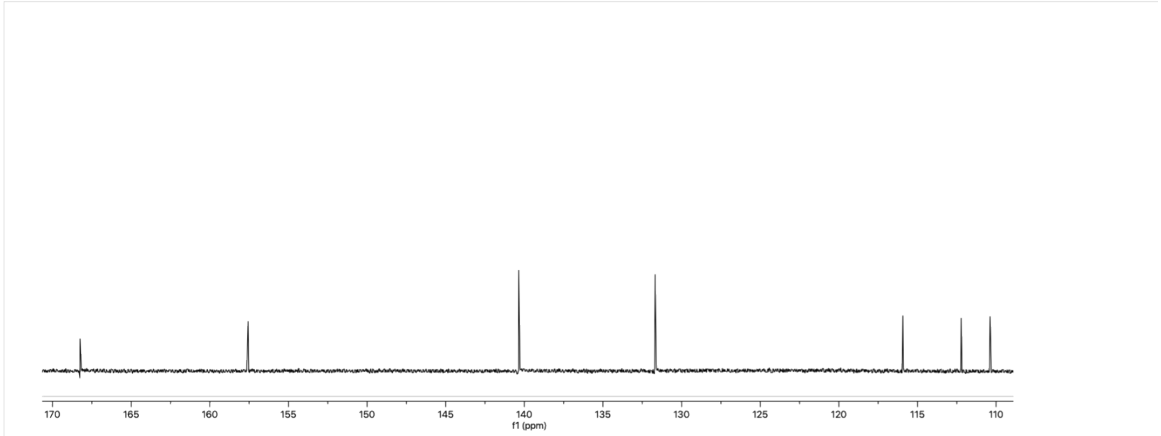
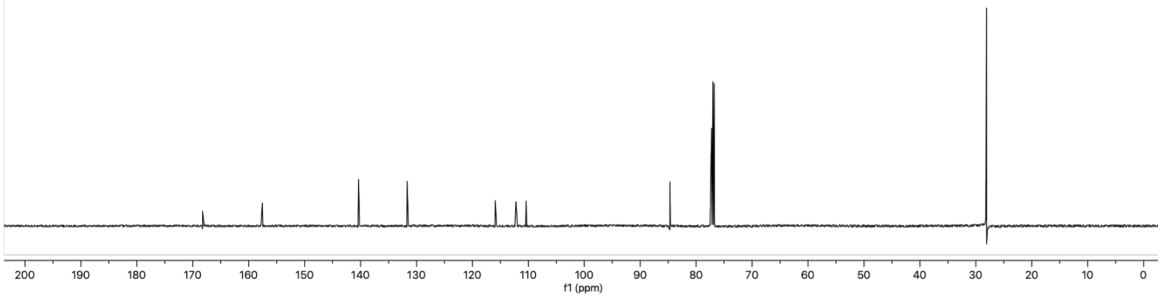
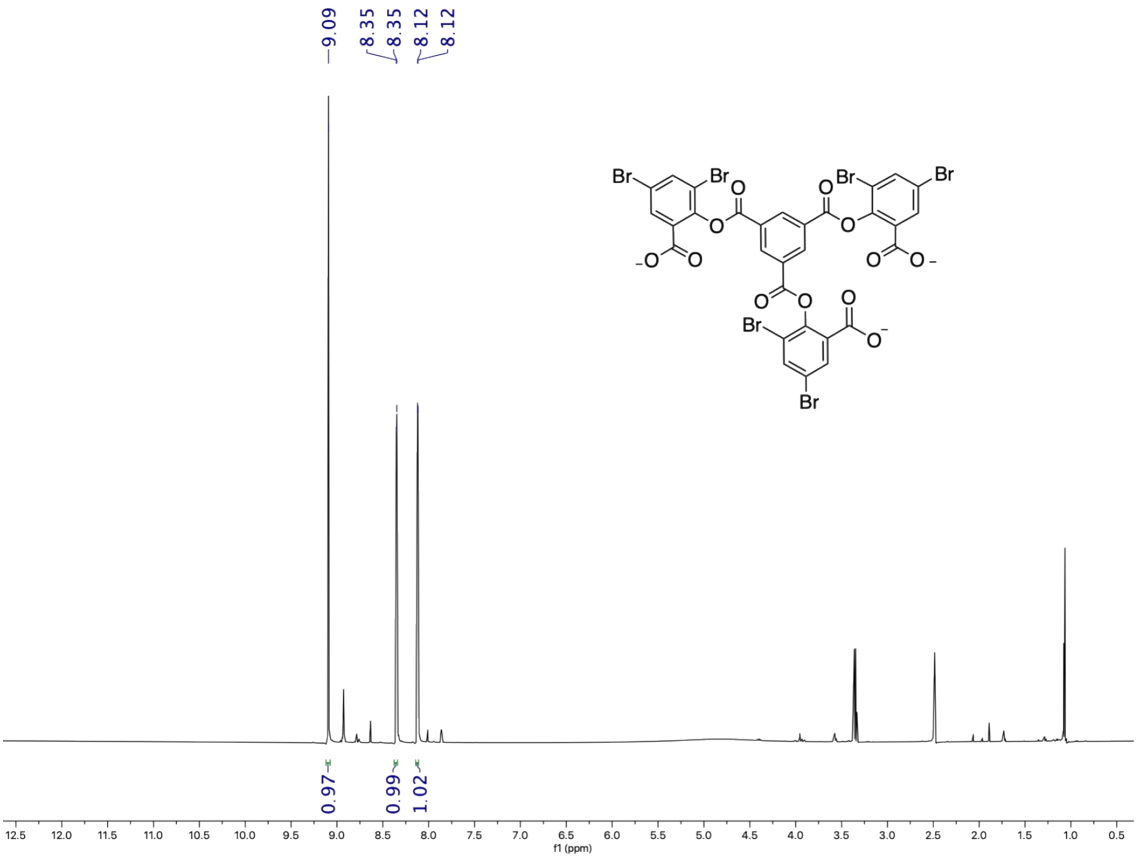
O
1.00
2.01
9.32
f1 (ppm)


f1 (ppm)


f1 (ppm)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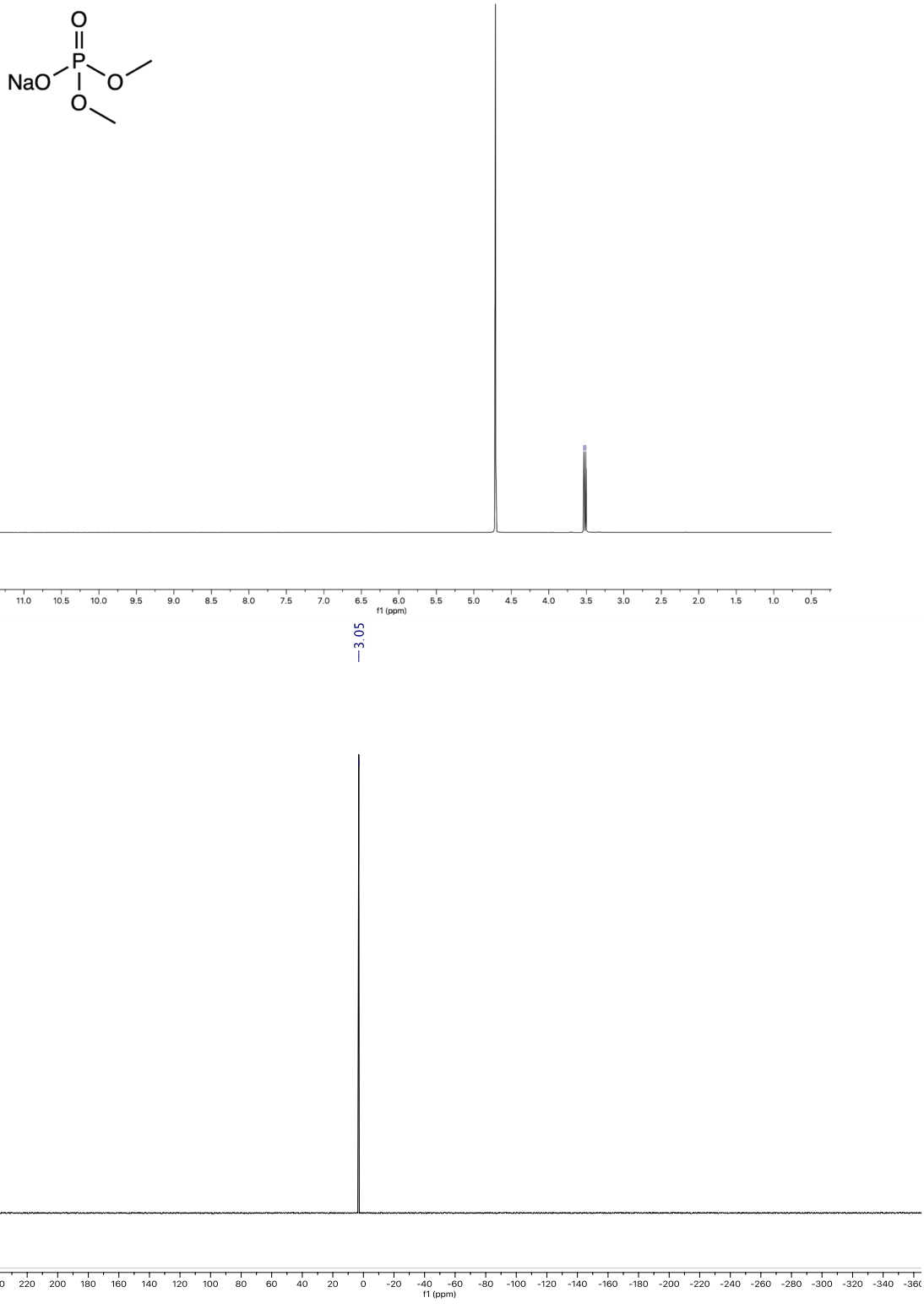
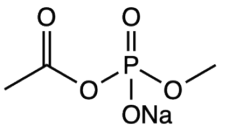
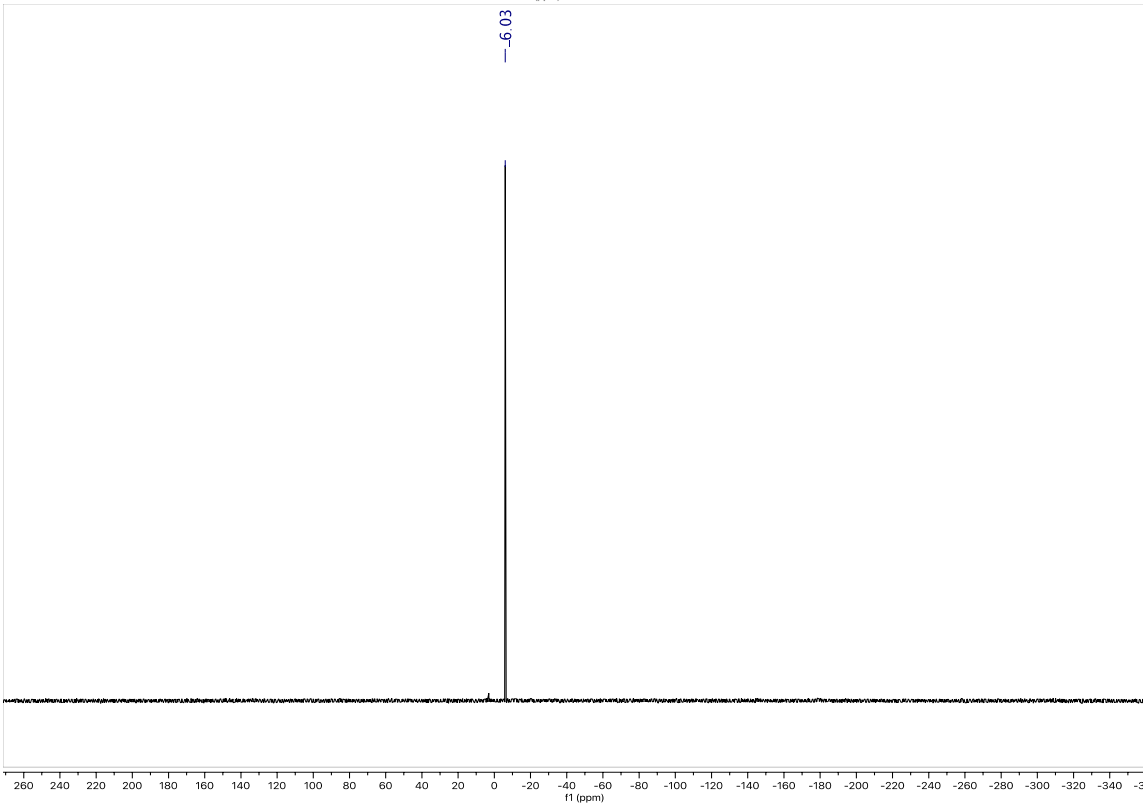
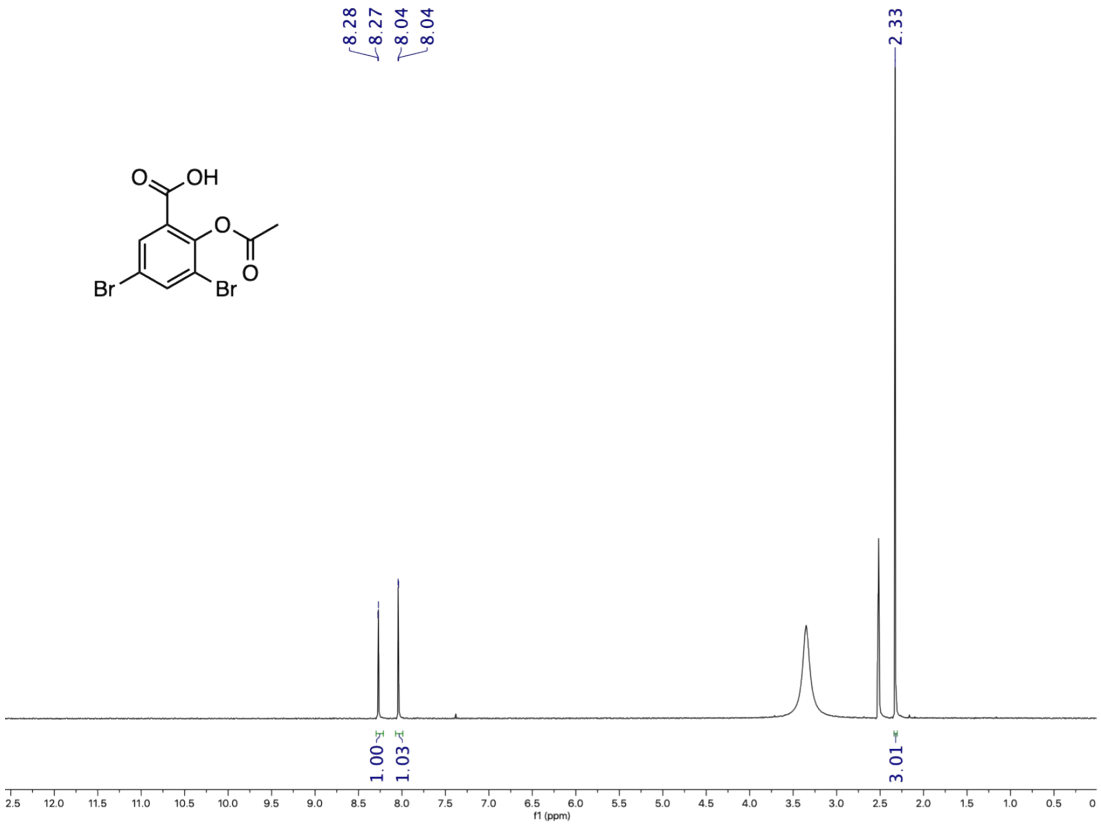
8.35
8.35
8.12
8.12
0.97
0.99
1.02
f1 (ppm)


NaO
O
O
O
3.53
3.50
f1 (ppm)
3.05
f1 (ppm)


O
O
P
O
O
ONa

3.54
3.51
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


6.03
260 240 220 200 180 160 140 120 100 80 60 40 20 0 -20 -40 -60 -80 -100 -120 -140 -160 -180 -200 -220 -240 -260 -280 -300 -320 -340
f1 (ppm)


8.28
8.27
8.04
8.04
2.33
O
OH
O
O
Br
Br
1.00
1.03
3.01
2.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)