

# Large-scale, Protection-free Synthesis of Se-Adenosyl-*L*-selenomethionine Analogues and Their Application as Cofactor Surrogates of Methyltransferases

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## Supporting Information

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## 1. Materials and General Methods.

All chemical reagents were purchased from Aldrich Chemical or Acros Organics and used without additional purification unless noted otherwise. Optima grade acetonitrile was obtained from Fisher Scientific and degassed under vacuum prior to HPLC purification of SAM and SeAM analogues. All reactions for the preparation of SAM and SeAM analogues were carried out in capped vials (4 mL) stirred with Teflon®-coated magnetic stir bars. Reaction solvents were removed by a Büchi rotary evaporator equipped with a dry ice-acetone condenser. Preparative HPLC purification of SAM and SeAM analogues was carried out using a Waters 6000 Controller HPLC/2998 diode array detector with an XBridge™ Prep C18 5 µm OBD™ 19x150 mm reverse phase column with UV detection at 260 nm. For preparative HPLC purification, linear gradients of H<sub>2</sub>O containing 0.1% TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B) were used at a flow rate of 10 mL/min (0–70% solvent B in 20 min, then 100% solvent A for 4 min). Lyophilization and concentration of aqueous HPLC-purified samples was performed using a Flexi-Dry™ mP Freeze-Dryer (FTS™ System).

Nuclear magnetic resonance and mass spectral data were collected at MSKCC Analytical Core Facility. Nuclear magnetic resonance spectra were recorded on a Bruker UltraShield™ Plus 600 MHz instrument. NMR samples were performed in D<sub>2</sub>O, except in the case of SeAH, SAM, and SeAM analogues, which were performed in D<sub>2</sub>O containing 0.1% deuterated trifluoacetic acid to suppress their decomposition. High-resolution mass spectrometry (HRMS) was conducted using a Waters Acuity SQD LC-MS with electrospray ionization (ESI).

Histone H3(aa1-21) (ARTKQTARKSTGGKAPRKQLAGGK) and RGG-biotin (GGRGGFGGRGGFGGRGGFGGGK-[Biotin]) peptides were obtained from the Proteomics Resource Center of the Rockefeller University and purified by HPLC. MALDI-MS analysis was performed on a Voyager-DE STR (Applied Biosystems, Framingham, MA, USA) MALDI-TOF mass spectrometer as described previously.<sup>1, 2</sup> In order to analyze peptide samples, 1  $\mu$ L of reaction mixture was added to 1  $\mu$ L of a saturated  $\alpha$ -cyano-hydroxy-cinnamic acid (Protease Biosciences) on a MALDI plate and allowed to dry at ambient temperature (22 °C). Mass spectra were gathered using delayed extraction in a positive ion mode. Desorption was obtained using a 337-nm nitrogen laser with a 3 ns pulse width.

## 2. Synthetic Details

**Synthesis of  $\alpha$ -amino-4-hydroxybutanoic acid (4).** L-Methionine (15.0 g, 100 mmol) was suspended in a mixture of 140 mL distilled deionized H<sub>2</sub>O (ddH<sub>2</sub>O) and 200 mL methanol. Iodomethane (15 mL, 240 mmol) was then added and the resultant mixture was stirred vigorously for 48 h at ambient temperature (22 °C). Bulk solvent was then removed by rotary evaporation to yield a white solid. The reaction product was dissolved in 100 mL ddH<sub>2</sub>O. After adding sodium bicarbonate (8.4 g, 100 mmol), the solution was brought to 100 °C with reflux for 24 h. After cooling the reaction mixture to ambient temperature (22 °C), the solvent was removed by rotary evaporation, affording the crude product as golden oil. This crude product was dissolved in 10 mL H<sub>2</sub>O and 25 mL acetone. Ethanol (200 mL) was then slowly added while stirring the mixture, resulting in the preci-

precipitation of  $\alpha$ -amino-4-hydroxybutanoic acid **4** as a white solid. This product was then filtered and dried under high vacuum overnight to give 8.8 g product (74.0 mmol, 74 % yield). **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O):**  $\delta$  ppm 1.87-2.06 (m; 2H; H $\beta$ ), 3.62-3.69 (m; 2H; H $\gamma$ ), 3.73 (dd; 1H; J<sub>a</sub> = 7.56 Hz, J<sub>b</sub> = 4.8 Hz; H $\alpha$ ). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O):**  $\delta$  ppm 174.38, 58.57, 53.31, 32.06.

**Synthesis of  $\alpha$ -amino-4-bromobutanoic acid hydrobromide (5).** In a 75 mL glass high-pressure reaction vessel,  $\alpha$ -amino-4-hydroxybutanoic acid **4** (5.1 g, 42.9 mmol) was suspended in 25 mL hydrobromic acid (33 wt.% in acetic acid). The reaction vessel was sealed and heated to 80 °C for 5 h, and then allowed to cool down overnight to ambient temperature (22 °C). The reaction mixture was isolated by vacuum filtration and washed with 75 mL diethyl ether. The final product  $\alpha$ -amino-4-bromobutanoic acid hydrobromide **5** was obtained by vacuum filtration and dried under high vacuum (7.1 g, 26.8 mmol, 62 % yield). **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O):**  $\delta$  ppm 2.26-2.48 (m; 2H; H $\beta$ ), 3.47-3.56 (m; 2H; H $\gamma$ ), 4.09 (t; 1H; J = 6.66 Hz; H $\alpha$ ). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O):**  $\delta$  ppm 173.06, 68.64, 49.83, 29.32.

**Synthesis of Selenohomocystine (6).** In a 250 mL round bottom flask, 200-mesh selenium powder (3.6 g, 46.0 mmol) was suspended in 70 mL ethanol (200-proof grade). After adding sodium borohydride (1.74 g, 46.0 mmol), the mixture was heated to reflux at 80 °C for 2 h (the solution became dark maroon in color). Into the reaction vessel was added  $\alpha$ -amino-4-bromobutanoic acid hy-

drobromide **5** (6.0 g, 23.0 mmol), resulting in a rapid formation of an opaque yellow mixture. The reaction was allowed to proceed under reflux at 80 °C overnight and then quenched with 10 mL 2 M HCl. After removing bulk solvent by rotary evaporation, the resulting residue was mixed with 25 mL 5% HCl. This aqueous solution was then washed three times with 75 mL diethyl ether and subjected to vacuum filtration to remove insoluble materials. A yellow solid was obtained after removing aqueous solvent by rotary evaporation. This semi-crude product was then dissolved in 15 mL 1 M HCl and purified over Dowex® 50WX8 ion exchange resin according to manufacturer's specifications. In brief, activated resin (5.0 cm x 2.5 cm) was washed with 100 mL H<sub>2</sub>O, followed by the loading of semi-crude product. The resin was then washed with an additional 100 mL H<sub>2</sub>O to remove unbound impurities. Product was eluted from the resin using a 5% ammonium hydroxide solution. Fractions containing the desired product (bright yellow solution) were combined. After removing bulk solvent by rotary evaporation, the resulting yellow solid was placed under high vacuum overnight to afford 3.4 g selenohomocystine **6** (9.5 mmol, 84% yield). **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d)**: δ ppm 2.21-2.34 (m; 2H; Hβ), 2.87-2.92 (m; 2H; Hγ), 3.98 (t; 1H; J = 6.30 Hz; Hα). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d)**: δ ppm 172.25, 52.97, 31.39, 22.60. **HRMS**: Calculated for C<sub>8</sub>H<sub>17</sub>N<sub>2</sub>O<sub>4</sub>Se<sub>2</sub>: 364.9519; obtained: 364.9518.

**Synthesis of 5'-Iodo-5'-deoxyadenosine (7)**. Adenosine (10 g, 37.4 mmol) was suspended in 25 mL pyridine in a 100 mL round-bottom flask. Triphenylphos-

phine (15.0 g, 57.2 mmol) was added to the reaction vessel and stirred vigorously. Iodine (15.0 g, 59.1 mmol) was then slowly added to the mixture, which rapidly became brown in color. The vessel was protected from light and the reaction was allowed to proceed for 18 h at ambient temperature (22 °C). Saturated sodium thiosulfate solution (3 mL) was then added to the reaction vessel and allowed to mix for 30 min in order to quench the reaction. Bulk solvent was removed by rotary evaporation. The residue was dissolved in 50 mL H<sub>2</sub>O and washed with 75 mL chloroform three times. The aqueous phase was collected and the solvent was removed by rotary evaporation. The resulting off-white solid was stored at -20 °C as a crude product prior to further purification using Amberlite® XAD4 resin. In brief, the crude product was dissolved in 100 mL 0.5 M NaOH and loaded onto a 3 x 29 cm column of Amberlite XAD4 resin prewashed with 200 mL H<sub>2</sub>O. The column was then washed with 200 mL 0.2 M ammonium acetate buffer (pH 5.0) followed by 300 mL H<sub>2</sub>O. The desired product was eluted from the resin with 350 mL methanol. After removing the solvent by rotary evaporation followed by high vacuum overnight, 5'-iodo-5'-deoxyadenosine **7** (5.7 g, 15.0 mmol) was obtained with a yield of 40%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O):** δ ppm 3.46 (ddd; 2H; J<sub>a</sub> = 27.54 Hz, J<sub>b</sub> = 11.22 Hz, J<sub>c</sub> = 4.98 Hz; H5'), 4.03 (dt; 1H; J<sub>a</sub> = 4.98 Hz, J<sub>b</sub> = 4.92 Hz; H4'), 4.27 (dd; 1H; J<sub>a</sub> = 5.10 Hz, J<sub>b</sub> = 5.04 Hz; H3'), 4.77 (dd; 1H; J<sub>a</sub> = 5.28 Hz, J<sub>b</sub> = 5.28 Hz; H2'), 5.99 (d; 1H; J = 5.1 Hz; H1'), 8.13 (s; 1H; arom. H), 8.28 (s; 1H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O):** δ ppm 155.63, 152.91, 148.96, 140.09, 118.72, 87.26, 82.93, 73.20, 73.11, 5.90. **HRMS:** Calculated for C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>3</sub>I: 378.0063; obtained: 378.0063.

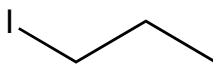
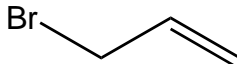
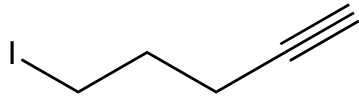
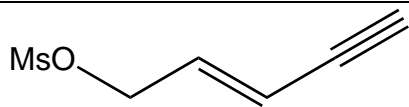
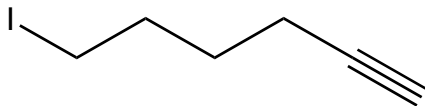
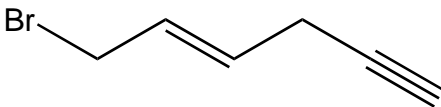
**Synthesis of Se-adenosylselenohomocysteine (SeAH, 3).** Selenohomocysteine **6** (1.4 g, 3.9 mmol) and sodium borohydride (1.47 g, 38.6 mmol) were suspended in 60 mL anhydrous ethanol in a 100 mL round-bottom flask. The mixture was stirred vigorously for 15 min under a steady flow of argon (the mixture became opaque white in color). 5'-Iodo-5'-deoxyadenosine **7** (3.5 g, 9.3 mmol) was then added, the reaction vessel purged, and the reaction was allowed to proceed under argon overnight. After removal of bulk solvent by rotary evaporation, the residue was dissolved in 30 mL dilute HCl. The aqueous phase was washed three times with 50 mL diethyl ether, collected and dried by rotary evaporation to afford an off-white solid. This crude product was subject to further purification using Amberlite® XAD4 resin. In brief, the crude product was dissolved in 100 mL 0.5 M NaOH and then loaded onto a 3 x 29 cm column of Amberlite® XAD4 resin prewashed with 200 mL H<sub>2</sub>O. The column was washed with 200 mL 0.2 M ammonium acetate buffer (pH 5.0) followed by 300 mL H<sub>2</sub>O. The desired product was eluted from the resin with 200 mL 1:1 H<sub>2</sub>O:methanol. Fractions (25 mL each) were collected and analyzed by LCMS. Fractions containing the desired material were combined. Removal of solvent by rotary evaporation and the subsequent high vacuum overnight afforded Se-adenosylselenohomocysteine **3** with 58% yield (1.94 g, 4.49 mmol). **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d):** δ ppm 2.05-2.20 (m; 2H; Hβ), 2.60 (t; 2H; J = 7.86 Hz; Hγ), 2.95 (ddd; 2H; J<sub>a</sub> = 28.86 Hz, J<sub>b</sub> = 13.38 Hz, J<sub>c</sub> = 5.22 Hz; H5'), 3.92 (t; 1H; J = 6.42 Hz; Hα), 4.26(dt; 1H; J<sub>a</sub> = 6.78 Hz, J<sub>b</sub> = 5.1 Hz; H4'), 4.32 (dd; 1H; J<sub>a</sub> = 5.07 Hz, J<sub>b</sub> = 5.04 Hz; H3'), 4.77 (dd; 1H;

$J_a = 5.07$  Hz,  $J_b = 5.04$  Hz; H<sup>2'</sup>), 6.03 (d; 1H;  $J = 4.86$  Hz; H<sup>1'</sup>), 8.33 (s; 1H; arom. H), 8.41 (s; 1H; arom. H). **<sup>13</sup>C- NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d)**:  $\delta$  ppm 171.36, 149.18, 147.50, 143.73, 142.05, 118.11, 87.50, 83.30, 72.79, 72.07, 52.26, 30.01, 24.52, 17.90. **HRMS**: Calculated for C<sub>14</sub>H<sub>21</sub>N<sub>6</sub>O<sub>5</sub>Se: 433.0739; obtained: 433.0743.

**Synthesis and purification of SAM and SeAM analogues (9–14).** A list of activated electrophiles used for the synthesis of SAM and SeAM analogues is presented in Table S1. These electrophiles were either purchased directly from Sigma-Aldrich and used without further purification or freshly prepared in house (Table S1).<sup>3, 4</sup> Briefly, SAH (20 mg, 51.9  $\mu$ mol) or SeAH **3** (20 mg, 46.3  $\mu$ mol) was dissolved in 500  $\mu$ L 1:1 formic acid and acetic acid. Respective electrophiles (Table S1, 50 equivalents) and AgClO<sub>4</sub> (1 equivalent dissolved in 250  $\mu$ L 1:1 formic:acetic acid) were then added to the reaction mixture. Reactions were allowed to proceed for 8 h at ambient temperature (22 °C) and then quenched by addition of 3 mL distilled water containing 0.1% TFA. The aqueous phases were washed with diethyl ether (3x10 mL). Trace organic solvent in the crude sample was removed by rotary evaporation. After passing the sample through a Nalgene 0.22  $\mu$ m syringe filter, the crude product was then purified by preparative reversed-phase HPLC as described in General Methods. Desired products were collected, lyophilized, dissolved in a small volume of 0.1% TFA and stored at -80 °C. Concentrations of SAM and SeAM analogues were determined by their UV absorption ( $\epsilon_{260} = 15,400$  L $\cdot$ mol<sup>-1</sup> $\cdot$ cm<sup>-1</sup>).



**Table S1.** Electrophiles used in the preparation of SAM and SeAM analogues.

#	Name	Structure	Source
1,2	Iodomethane	CH <sub>3</sub> I	Sigma-Aldrich
9	1-Iodopropane		Sigma-Aldrich
10	Allyl bromide		Sigma-Aldrich
11	5-Iodo-1-pentyne		Sigma-Aldrich
12	(E)-Pent-2-en-4-yn-1-methanesulfonate		In-house <sup>S2</sup>
13	6-Iodo-1-hexyne		Sigma-Aldrich
14	(E)-6-bromohex-5-en-1-yne		In-house <sup>S1</sup>

**Spectral Data:**

**S-Adenosyl-L-methionine (SAM, 1).** Purification by HPLC (retention time = 6.0 min) afforded **1** as a mixture of sulfonium-*R,S*-epimers with a yield of 50%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d):** δ ppm 2.29-2.41 (m, 2H, Hβ), 2.96/2.93 (s, 3H, 4:1 S-epimers, 1''), 3.42-3.68 (m; 2H; Hγ), 3.87-4.00 (m; 3H; H5', Hα), 4.50-4.53 (m; 1H; H4'), 4.57 (dd; 1H; J<sub>a</sub> = 5.88 Hz, J<sub>b</sub> = 5.73 Hz; H3'), 4.79 (solvent overlap; H2'), 6.12 (d; 1H; J = 3.90 Hz; H1'), 8.40 (s; 1H; arom. H), 8.41 (s; 1H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d):** δ ppm 170.90, 149.96, 147.94, 144.41, 143.41, 119.32, 90.00, 78.42, 73.05, 72.60, 51.58, 44.22, 38.48, 24.81, 23.45. **HRMS:** Calculated for C<sub>15</sub>H<sub>23</sub>N<sub>6</sub>O<sub>5</sub>S: 399.1451; obtained: 399.1442.

**Propyl-SAM (9b)**. Purification by HPLC (retention time = 7.8 min) afforded **9b** as a mixture of sulfonium-*R,S*-epimers as reported previously.<sup>S3</sup> **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d)**: δ ppm 0.84/0.87 (t; 3H; 1:1 epimers; J = 7.32 Hz; H<sub>3</sub>"), 1.64-1.74 (m; 2H; H<sub>2</sub>"), 2.23-2.32 (m; 2H; H<sub>β</sub>), 3.29-3.31 (t; 2H; 1:1 *Se*-epimers; H<sub>1</sub>"), 3.39-3.57 (m; 2H; H<sub>γ</sub>), 3.83-3.86 (m; 2H; H<sub>5</sub>'), 3.88-3.90 (m; 1H; H<sub>α</sub>), 4.43-4.46 (m; 1H; H<sub>4</sub>'), 4.50-4.52 (m; 1H; H<sub>3</sub>'), 4.72-4.77 (m; 1H; solvent overlap; H<sub>2</sub>'), 6.07 (m; 1H; H<sub>1</sub>'), 8.35 (s; 2H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d)**: δ ppm 171.00, 149.98, 147.95, 144.47, 143.39, 119.28, 89.99, 78.49, 73.01, 72.71, 51.67, 42.58, 41.84, 36.26, 25.08, 17.37, 11.79. **HRMS**: Calculated for C<sub>17</sub>H<sub>27</sub>N<sub>6</sub>O<sub>5</sub>S: 427.1764; obtained: 427.1758.

**Allyl-SAM (10b)**. Purification by HPLC yielded **9b** (retention time = 7.5 min) as a mixture of sulfonium-*R,S*-epimers as reported previously.<sup>S3</sup> **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d)**: δ ppm 2.18-2.29 (m; 2H; H<sub>β</sub>), 3.31-3.48 (m; 2H; H<sub>γ</sub>), 3.75-3.79 (m; 2H; H<sub>5</sub>'), 3.81-3.88 (m; 1H; H<sub>α</sub>), 4.00-4.04 (m; 2H; H<sub>1</sub>"), 4.39-4.43 (m; 1H; H<sub>4</sub>'), 4.47-4.50 (m; 1H; H<sub>3</sub>'), 4.69 (m; 1H; solvent overlap; H<sub>2</sub>'), 5.52 (d; 1H; J = 17 Hz; H<sub>3</sub>"<sub>a</sub>), 5.57 (d; 1H; J = 10 Hz; H<sub>3</sub>"<sub>b</sub>), 5.74 (ddt; 1H; J = 17 Hz, J = 10 Hz, J = 7.5 Hz; H<sub>2</sub>"), 6.02-6.03 (m; 1H, H<sub>1</sub>'), 8.30 (s; 2H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d)**: δ ppm 170.90, 149.97, 147.93, 144.41, 143.46, 128.83, 122.31, 119.33, 90.03, 78.58, 73.03, 72.66, 51.53, 42.48, 40.88, 35.25, 25.04. **HRMS**: Calculated for C<sub>17</sub>H<sub>25</sub>N<sub>6</sub>O<sub>5</sub>S: 425.1607; obtained: 425.1592.

**Pentyne-SAM (11b)**. Purification by HPLC (retention time = 8.1 min) afforded **11b** as sulfonium-*S*-epimer with a yield of 25%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d)**: δ ppm 1.76-1.87 (m; 2H; H<sub>3</sub>"), 2.08-2.18 (m; 4H; H<sub>5</sub>"; H<sub>2</sub>"), 2.21-2.34

(m; 2H; H $\beta$ ), 3.37 (t; 2H; 7.80 Hz; H1''), 3.39-3.51 (m; 2H; H $\gamma$ ), 3.84-3.89 (m; 3H; H5'; H $\alpha$ ), 4.42-4.45 (m; 1H; H4'), 4.49-4.53 (m; 1H; H3'), 4.69-4.76 (m; 1H; solvent overlap; H2'), 6.03-6.04 (m; 1H; H1'), 8.34 (m; 2H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d)**:  $\delta$  ppm 170.96, 149.99, 147.93, 144.51, 143.54, 119.38, 90.10, 82.0, 78.86, 72.92, 72.60, 70.94, 51.63, 41.95, 39.98, 36.53, 25.03, 22.43, 16.35. **HRMS**: Calculated for C<sub>19</sub>H<sub>27</sub>N<sub>6</sub>O<sub>5</sub>S: 451.1764; obtained: 451.1743.

**Enyne-SAM (12b)**. Purification by HPLC (retention time = 8.1 min) afforded **12b** as a mixture of sulfonium-*R,S*-epimers with a yield of 30%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d)**:  $\delta$  ppm 2.21-2.35 (m; 2H; H $\beta$ ), 3.35/3.4 (d; 1H; 40:60 epimers; J = 2.2 Hz; H5''), 3.37-3.54 (m; 2H; H $\gamma$ ), 3.71-3.83 (m; 2H; H5'), 3.89-3.97 (m; 1H; H $\alpha$ ), 4.07/4.12 (d; 2H; 40:60 epimers; J = 7.7 Hz; H1''), 4.38-4.44 (m; 1H; H4'), 4.53-5.62 (m; 1H; H3'), 4.67 (m; 1H; solvent overlap; H2'), 5.60/5.80 (dd; 1H; 40:60 epimers; J<sub>a</sub> = 15.8 Hz, J<sub>b</sub> = 1.6 Hz; H3''), 5.94/6.04 (dt; 1H; 40:60 epimers; J<sub>a</sub> = 15.8 Hz, J<sub>b</sub> = 7.8 Hz; H2''), 6.04 (d; 1H; J = 6.04 Hz; H1'), 8.32-8.34 (m; 2H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d)**:  $\delta$  ppm 169.81, 149.12, 147.05, 143.64, 142.99, 127.87, 120.36, 118.65, 89.68, 81.64, 78.18, 77.70, 72.21, 71.90, 50.49, 41.77, 40.35, 34.93, 24.20. **HRMS**: Calculated for C<sub>19</sub>H<sub>25</sub>N<sub>6</sub>O<sub>5</sub>S: 449.1607; obtained: 449.1598.

**Hexyne-SAM (13b)**. Purification by HPLC (retention time = 9.5 min) afforded **13b** as a mixture of sulfonium-*R,S*-epimers with a yield of 35%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d)**:  $\delta$  ppm 1.33-1.46 (m; 2H; H3''), 1.68-1.79 (m; 2H; H2''), 1.96/2.03 (dt; 2H; 40:60 epimers; J<sub>a</sub> = 6.84 Hz, J<sub>b</sub> = 2.5 Hz; H4''), 2.17/2.20 (t;

1H; 40:60 epimers;  $J = 2.5$  Hz; H6''), 2.24-2.39 (m; 2H; H $\beta$ ), 3.31-3.38 (m; 2H H1''), 3.43-3.60 (m; 2H; H $\gamma$ ), 3.84-3.87 (m; 2H; H5'), 3.94-4.00 (m; 1H; H $\alpha$ ), 4.43-4.46 (m; 1H; H4'), 4.50-4.54 (m; 1H; H3'), 4.72-4.77 (dd; 1H;  $J_a = 5.46$  Hz,  $J_b = 4.02$  Hz; solvent overlap; H2'), 6.06 (d; 1H;  $J = 3.36$  Hz; H1'), 8.36 (m; 2H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d):**  $\delta$  ppm 170.44, 149.94, 147.92, 144.48, 143.47, 119.26, 90.00, 84.20, 78.24, 72.91, 72.61, 69.85, 51.22, 41.54, 40.49, 36.18, 25.94, 24.91, 22.56, 16.76. **HRMS:** Calculated for C<sub>20</sub>H<sub>29</sub>N<sub>6</sub>O<sub>5</sub>S: 465.1920; obtained: 465.1919.

**Hey-SAM (14b).** Purification by HPLC (retention time = 9.5 min) afforded **14b** as a mixture of sulfonium-*R,S*-epimers with a yield of 35%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d):**  $\delta$  ppm 2.21-2.33 (m; 2H; H $\beta$ ), 2.40/2.44 (t; 1H;  $J = 2.58$  Hz; 30:70 isomers; H6''), 2.89/2.97 (m; 2H; 30:70 isomers; H4''), 3.32-3.50 (m; 2H; H $\gamma$ ), 3.69-3.91 (m; 3H; H5'; H $\alpha$ ), 4.04-4.08 (m; 2H; H1''), 4.43-4.46 (m; 1H; H4'), 4.51-4.53 (m; 1H; H3'), 4.72-4.74 (m; 1H; solvent overlap; H2'), 5.62/5.72 (dt; 1H;  $J_a = 15.3$  Hz,  $J_b = 7.7$  Hz; 30:70 isomers; H2''), 5.86/6.01 (dt; 1H;  $J_a = 15.3$  Hz,  $J_b = 5.1$  Hz; 30:70 isomers; H3''), 6.05-6.06 (m; 1H; H1'), 8.34-8.36 (m; 2H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d):**  $\delta$  ppm 170.88, 149.98, 147.89, 144.50, 143.50, 139.15, 119.33 115.64, 90.08, 80.51, 78.59, 73.00, 72.62, 72.29, 51.60, 42.32, 41.07, 35.46, 25.06, 21.00. **HRMS:** Calculated for C<sub>20</sub>H<sub>27</sub>N<sub>6</sub>O<sub>5</sub>S: 463.1764; obtained: 463.1745.

**Se-Adenosyl-L-methionine (SeAM, 2).** Purification by HPLC (retention time = 6.0 min) afforded **2** as a mixture of sulfonium-*R,S*-epimers with a yield of 24%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d):**  $\delta$  ppm 2.22-2.31 (m; 2H; H $\beta$ ), 2.72/2.69

(s; 3H; 45:55 Se-epimers; H1''), 3.28-3.50 (m; 2H; H $\gamma$ ), 3.74-3.90 (m; 3H; H5'; H $\alpha$ ), 4.41-4.45 (m; 1H; H4'), 4.48-4.49 (m; 1H; H3'), 4.72 (solvent overlap; H2'), 6.05 (d; 1H; J = 3.84 Hz; H1'), 8.34 (s; 2H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d)**:  $\delta$  ppm 171.00, 149.96, 147.93, 144.41, 143.38, 119.29, 89.85, 79.05, 73.24, 72.95, 52.17, 41.31, 35.44, 25.51, 19.44. **HRMS**: Calculated for C<sub>15</sub>H<sub>23</sub>N<sub>6</sub>O<sub>5</sub>Se: 447.0895; obtained: 447.0898.

**Propyl-SeAM (9a)**. Purification by HPLC (retention time = 7.8 min) afforded **9a** as a mixture of sulfonium-*R,S*-epimers with a yield of 25%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d)**:  $\delta$  ppm 0.79/0.85 (t; 3H; 55: 45 epimers; J = 7.32 Hz; H3''), 1.62-1.73 (m; 2H; H2''), 2.22-2.34 (m; 2H; H $\beta$ ), 3.24-3.47 (m; 4H; H1''; H $\gamma$ ), 3.74-3.81 (m; 2H; H5'), 3.84-3.94 (m; 1H; H $\alpha$ ), 4.41-4.44 (m; 1H; H4'), 4.47-4.49 (m; 1H; H3'), 4.72-4.77 (m; 1H; solvent overlap; H2'), 6.04-6.05 (m; 1H; H1'), 8.34-8.35 (m; 2H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d)**:  $\delta$  ppm 170.68, 149.94, 147.92, 144.45, 143.43, 119.24, 89.86, 79.07, 73.34, 73.01, 52.01, 41.91, 39.98, 33.84, 25.78, 18.34, 12.73. **HRMS**: Calculated for C<sub>17</sub>H<sub>27</sub>N<sub>6</sub>O<sub>5</sub>Se: 475.1208; obtained: 475.1199.

**Allyl-SeAM (10a)**. Purification by HPLC (retention time = 7.5 min) afforded **10a** as a mixture of sulfonium-*R,S*-epimers with a yield of 23%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d)**:  $\delta$  ppm 2.21-2.33 (m; 2H; H $\beta$ ), 3.23-3.37 (m; 2H; H $\gamma$ ), 3.67-3.76 (m; 2H; H5'), 3.81-3.89 (m; 1H; H $\alpha$ ), 3.96-4.0 (m; 2H; H1''), 4.40-4.43 (m; 1H; H4'), 4.47-4.51 (m; 1H; H3'), 4.73 (m; 1H; solvent overlap; H2'), 5.45 (d; 1H; J = 17 Hz; H3''<sub>a</sub>), 5.49 (d; 1H; J = 10 Hz; H3''<sub>b</sub>), 5.79 (ddt; 1H; J = 17 Hz, J = 10 Hz, J = 8 Hz; H2''), 6.04 (d; 1H, J = 3.66 Hz; H1'), 8.34 (s; 2H; arom. H). **<sup>13</sup>C-**

**NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d):**  $\delta$  ppm 170.86, 149.95, 147.95, 144.44, 143.44, 127.63, 124.42, 119.26, 89.94, 79.04, 73.38, 72.97, 52.19, 41.17, 39.63, 33.42, 25.70. **HRMS:** Calculated for C<sub>17</sub>H<sub>25</sub>N<sub>6</sub>O<sub>5</sub>Se: 473.1052; obtained: 473.1068.

**Pentyne-SeAM (11a).** Purification by HPLC (retention time = 8.2 min) afforded **11a** as a mixture of sulfonium-*R,S*-epimers with a yield of 12%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d):**  $\delta$  ppm 1.79-1.87 (m; 2H; 3''), 2.09-2.17 (m; 3H; H5''), H2''), 2.22-2.34 (m; 2H; H $\beta$ ), 3.33-3.52 (m; 4H; H1''; H $\gamma$ ), 3.79-3.9 (m; 3H; H5'; H $\alpha$ ), 4.43-4.46 (m; 1H; H4'), 4.50-4.54 (m; 1H; H3'), 4.72-4.78 (m; 1H; H2'), 6.05-6.06 (m; 1H; H1'), 8.37 (s; 2H; arom. H). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d):**  $\delta$  ppm 170.65, 149.96, 147.91, 144.49, 143.59, 119.31, 90.00, 84.43, 78.88, 73.08, 72.89, 71.11, 51.96, 40.15, 39.03, 34.07, 25.74, 23.39, 17.14. **HRMS:** Calculated for C<sub>19</sub>H<sub>27</sub>N<sub>6</sub>O<sub>5</sub>Se: 499.1208; obtained: 499.1204.

**Enyne-SeAM (12a).** Purification by HPLC (retention time = 8.2 min) afforded **12a** as a mixture of sulfonium-*R,S*-epimers with a yield of 34%. **<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O + 0.1% TFA-d):**  $\delta$  ppm 2.26-2.32 (m; 2H; H $\beta$ ), 3.28-3.48 (m; 2H; H $\gamma$ ), 3.36/3.40 (d; 1H; 30:70 epimers; J = 2.3 Hz; H5''), 3.73-3.87 (m; 3H; H5'; H $\alpha$ ), 4.02/4.06 (d; 2H; 30:70 epimers; J = 8.0 Hz; H1''), 4.40-4.44 (m; 1H; H4'), 4.52-4.59 (m; 1H; H3'), 4.68-4.72 (m; 1H; solvent overlap; H2'), 5.64/5.79 (dd; 1H; 30:70 epimers; J<sub>a</sub> = 1.9 Hz, J<sub>b</sub> = 15.8 Hz; H3''), 5.99-6.14 (m; 1H; H2''), 6.05 (d; 1H; J = 3.6 Hz; H1'), 8.36-8.37 (m; 2H; arom. H.). **<sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O + 0.1% TFA-d):**  $\delta$  ppm 170.65, 149.96, 147.86, 143.66, 142.84, 131.08, 119.83,

119.36, 90.10, 82.00, 78.90, 77.95, 72.89, 72.84, 51.92, 40.84, 39.89, 33.58, 24.71. **HRMS:** Calculated for  $C_{15}H_{25}N_6O_5Se$ : 497.1052; obtained: 497.1034.

**Hexyne-SeAM (13a).** Purification by HPLC (retention time = 9.4 min) afforded **13a** as sulfonium-S-epimer with a yield of 16%.  **$^1H$ -NMR (600 MHz,  $D_2O$  + 0.1% TFA-d):**  $\delta$  ppm 1.39 (p; 2H;  $J = 7.32$  Hz;  $H3''$ ), 1.77 (dp; 2H;  $J_a = 7.5$  Hz,  $J_b = 3.18$  Hz;  $H2''$ ), 1.99 (dt; 2H;  $J_a = 6.96$  Hz,  $J_b = 2.58$  Hz;  $H4''$ ), 2.25 (t; 1H;  $J = 2.58$  Hz;  $H6''$ ), 2.35-2.39 (m; 2H;  $H\beta$ ), 3.34 (t; 2H;  $J = 8.0$  Hz;  $H1''$ ), 3.45-3.55 (m; 2H;  $H\gamma$ ), 3.80-3.89 (m; 2H;  $H5'$ ), 3.98 (t; 1H;  $J = 6.84$  Hz;  $H\alpha$ ), 4.49-4.52 (m; 1H;  $H4'$ ), 4.54-4.57 (m; 1H;  $H3'$ ), 4.85 (dd; 1H;  $J_a = 5.46$  Hz,  $J_b = 4.77$  Hz;  $H2'$ ), 6.12 (d; 1H;  $J = 4.02$  Hz;  $H1'$ ), 8.41-8.45 (2H, arom. H).  **$^{13}C$ -NMR (150 MHz,  $D_2O$  + 0.1% TFA-d):**  $\delta$  ppm 171.03, 150.00, 147.99, 144.61, 143.44, 119.23, 89.59, 84.14, 79.23, 73.15, 73.08, 69.75, 52.17, 40.23, 39.01, 34.15, 26.78, 25.71, 23.8, 16.63. **HRMS:** Calculated for  $C_{20}H_{29}N_6O_5Se$ : 513.1365; obtained: 513.1356.

**Hey-SeAM (14a).** Purification by HPLC (retention time = 9.4 min) yielded **14a** as sulfonium-S-epimer with a yield of 23%.  **$^1H$ -NMR (600 MHz,  $D_2O$  + 0.1% TFA-d):**  $\delta$  ppm 2.24-2.31 (m; 2H;  $H\beta$ ), 2.43 (t; 1H;  $J = 2.64$  Hz;  $H6''$ ), 2.94-2.95 (m; 2H;  $H4''$ ), 3.23-3.37 (m; 2H;  $H\gamma$ ), 3.65-3.76 (m; 2H;  $H5'$ ), 3.83 (dd; 1H;  $J_a = 7.62$  Hz,  $J_b = 5.46$  Hz;  $H\alpha$ ), 4.02 (d; 2H;  $J = 7.92$  Hz;  $H1''$ ), 4.40-4.43 (m; 1H;  $H4'$ ), 4.50 (dd; 1H;  $J_a = 6.06$  Hz,  $J_b = 3.36$  Hz;  $H3'$ ), 4.68 (dd; 1H;  $J_a = 5.58$  Hz,  $J_b = 3.48$  Hz; solvent overlap;  $H2'$ ), 5.76 (dt; 1H;  $J_a = 15.24$  Hz,  $J_b = 8.04$  Hz;  $H3''$ ), 5.96 (dt; 1H;  $J_a = 15.24$  Hz,  $J_b = 5.22$  Hz;  $H2''$ ), 6.04 (d; 1H;  $J = 3.66$  Hz;  $H1'$ ), 8.35 (s; 1H; arom. H), 8.36 (s; 1H, arom. H).  **$^{13}C$ -NMR (150 MHz,  $D_2O$  + 0.1% TFA-d):**  $\delta$  ppm 171.00, 149.98, 147.90, 144.46, 143.47, 137.94, 119.32, 117.89, 90.02, 80.75,

78.98, 73.33, 72.92, 72.14, 52.22, 40.71, 39.46, 33.36, 25.76, 20.92. **HRMS:** Calculated for  $C_{20}H_{27}N_6O_5Se$ : 511.1208; obtained: 511.1199.



### 3. MALDI-MS Analysis of Methyltransferase Reactions

**Enzyme Assays.** All enzymes were expressed and purified according to previously reported procedures.<sup>5-8</sup> For the assays of native G9a, G9a Y1154A, native GLP1, and GLP1 Y1211A, 1  $\mu\text{M}$  enzyme and 25  $\mu\text{M}$  H3K9 (aa1-21) peptide were used. For the assays of native PRMT1 and PRMT1 Y39FM48G, 2  $\mu\text{M}$  enzyme and 20  $\mu\text{M}$  RGG-Biotin peptide were used. For the assays of native PRMT3 and PRMT3 M233G, 1  $\mu\text{M}$  enzyme and 25  $\mu\text{M}$  RGG-biotin peptide were used. The concentration of cofactor analogue was 100  $\mu\text{M}$  for all samples. All reactions (10  $\mu\text{L}$ ) were carried out in 50 mM Tris HCl pH 8.0 for 2 h at ambient temperature (22  $^{\circ}\text{C}$ ). Reactions were quenched by the addition of 1  $\mu\text{L}$   $\text{H}_2\text{O}$  containing 10% TFA and processed with C18 ZipTip® (Millipore) pipette tips according to manufacturer's instructions prior to MALDI-MS analysis as described previously.<sup>1, 2</sup> Degrees of transalkylation (equivalent to *units of cofactor consumed*) per unit peptide for each analogue were quantified by MALDI-MS according to the following formula:

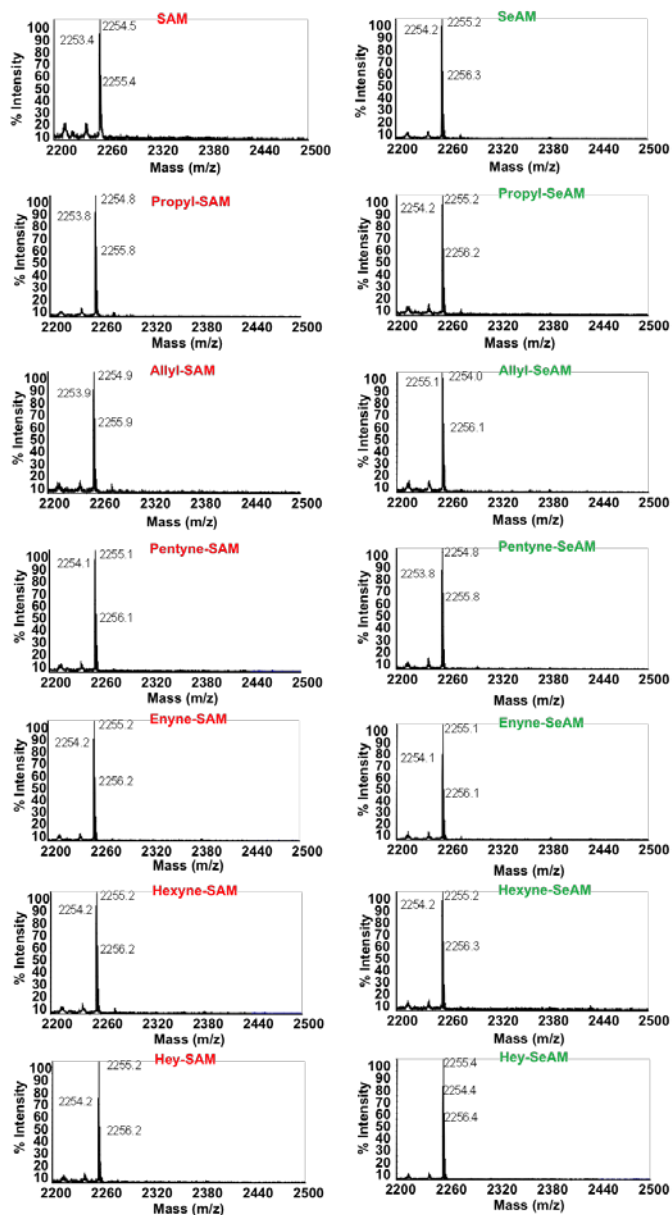
$$\frac{\text{Units Cofactor Consumed}}{\text{Unit Peptide}} = \frac{(I_{\text{alk}^1}) + (I_{\text{alk}^2} \times 2) + (I_{\text{alk}^3} \times 3) \dots}{(I_{\text{alk}^0}) + (I_{\text{alk}^1}) + (I_{\text{alk}^2}) + (I_{\text{alk}^3}) \dots}$$

where  $I$  represents peak intensities for unmodified ( $\text{alk}^0$ ), mono- ( $\text{alk}^1$ ), di- ( $\text{alk}^2$ ), and tri-alkylated ( $\text{alk}^3$ ) products peptides, etc. Relative transalkylation activities for sulfur and selenium analogues with equivalent R-groups were then compared and categorized for each examined enzyme. These data were summarized in Table S2.

**Table S2.** Summary of relative efficiency of transalkylation with SAM analogues versus equivalent SeAM analogues. Degree of transalkylation was defined as the equivalents of alkylation (or cofactor consumption) per unit peptide substrate according to relative peak intensity of MALDI-MS spectra. For instance, if 50% peptide is converted into a mono-alkylated product, the degree of the transalkylation will be “0.5”; if all the peptide is converted into a di-alkylated product, the degree of the transalkylation will be “2.0”. The relative efficiency between of SeAM and their equivalent SAM analogues as cofactors were categorized as “=” for 0~0.1 change of the degree of alkylation; “+” or “-” for increase or decrease of 0.1 ~ 0.5 degree of transalkylation, respectively; “+ +” or “-” for increase or decrease of >0.5 degree of transalkylation, respectively; NR for no reaction observed for SeAM or SAM analogues. These values are shown in parentheses as (S-analogue, Se-analogue). nG9a, nGLP1, nPRMT1 and nPRMT3 stand as native enzymes.

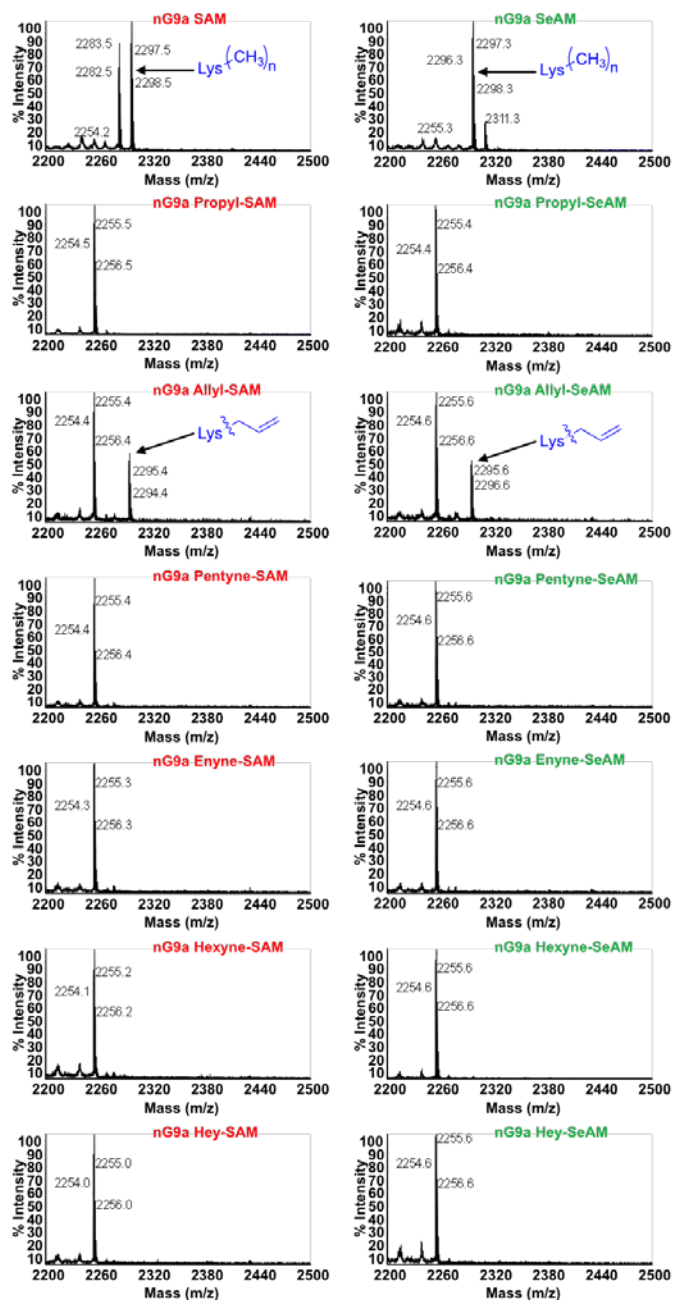
S vs. Se	nG9a	G9a Y1154A	nGLP1	GLP1 Y1244A	nPRMT1	PRMT1 Y39FM48G	nPRMT3	PRMT3 M233G
<b>Methyl (1, 2)</b>	+	++	+	+	--	+	-	=
	(2.36, 2.84)	(0.15, 1.03)	(2.70, 2.96)	(0.06, 0.34)	(3.16, 2.50)	(0.00, 0.13)	(3.37, 3.01)	(0.00, 0.08)
<b>Propyl (9b, a)</b>	NR	NR	NR	NR	NR	NR	NR	NR
<b>Allyl (10b, a)</b>	=	+	=	+	-	=	=	=
	(0.35, 0.32)	(0.78, 0.91)	(0.77, 0.82)	(0.46, 0.69)	(0.13, 0.00)	(0.07, 0.10)	(0.04, 0.06)	(0.00, 0.09)
<b>Pentyne (11b, a)</b>	NR	NR	NR	NR	NR	NR	NR	NR
<b>Enyne (12b, a)</b>	NR	++	NR	+	NR	-	NR	=
		(0.23, 0.71)		(0.06, 0.46)		(0.74, 0.24)		(0.34, 0.39)
<b>Hexyne (13b, a)</b>	NR	NR	NR	NR	NR	NR	NR	+
								(0.00, 0.13)
<b>Hey (14b, a)</b>	NR	-	NR	+	NR	--	NR	--
		(0.96, 0.85)		(0.77, 0.91)		(1.31, 0.32)		(2.29, 0.62)

### H3 Peptide (aa1-20) – No Enzyme



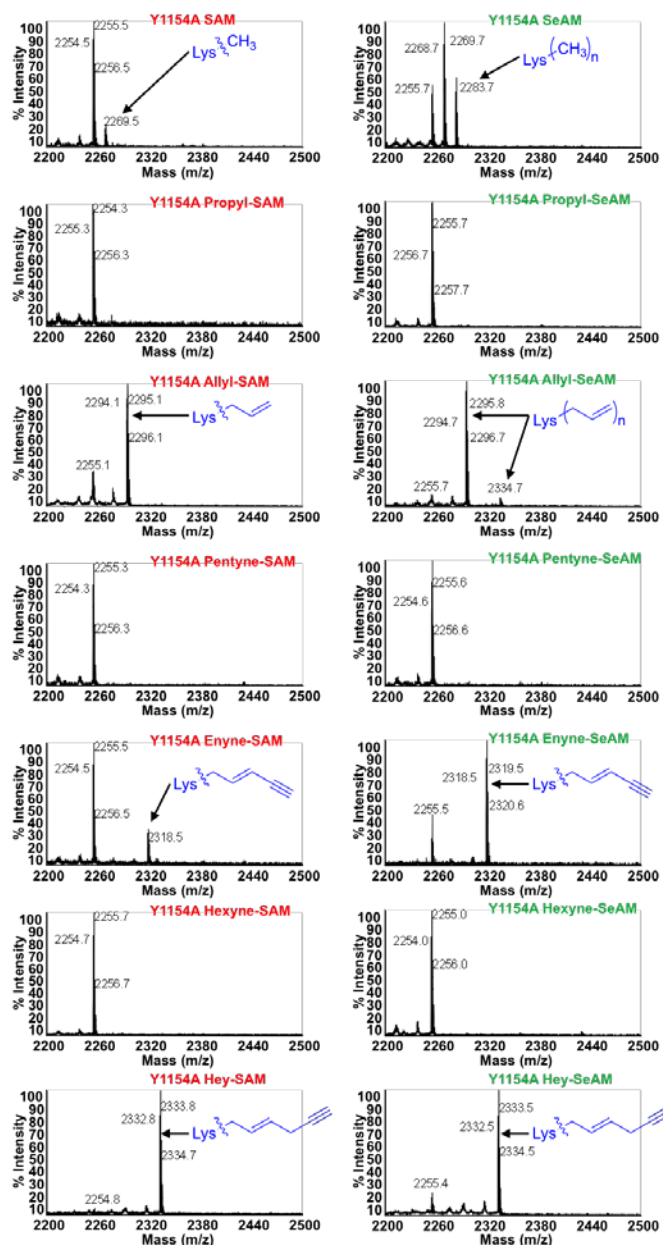
**Figure S1.** MALDI-MS spectra demonstrating a lack of modification of H3 peptide substrates by cofactor analogues in the absence of methyltransferase. Spectra in the left hand column are derived from reactions containing sulfur-based analogues (red labels); spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

### H3 Peptide (aa1-21) - nG9a



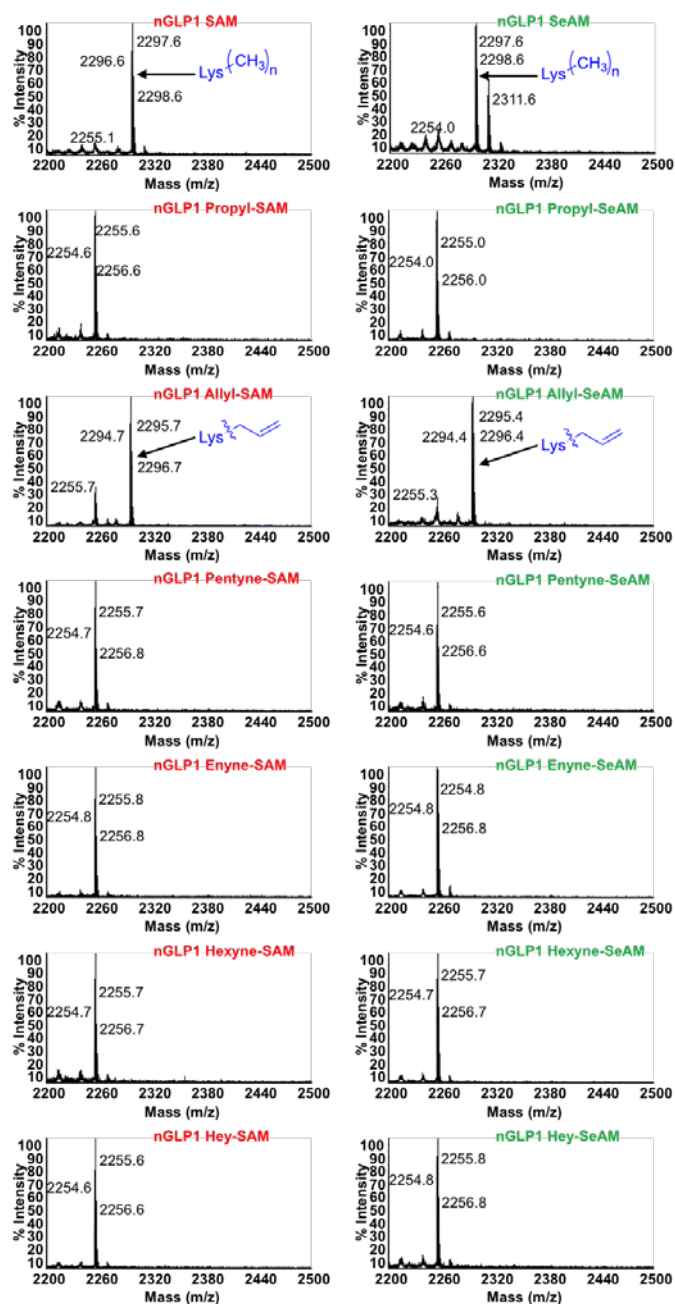
**Figure S2.** MALDI-MS spectra demonstrating sulfur and selenium-based cofactor analogue compatibility with native G9a. Spectra in the left hand column are derived from the reactions containing sulfur-based analogues (red labels); spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

### H3 Peptide (aa1-21) - G9a Y1154A



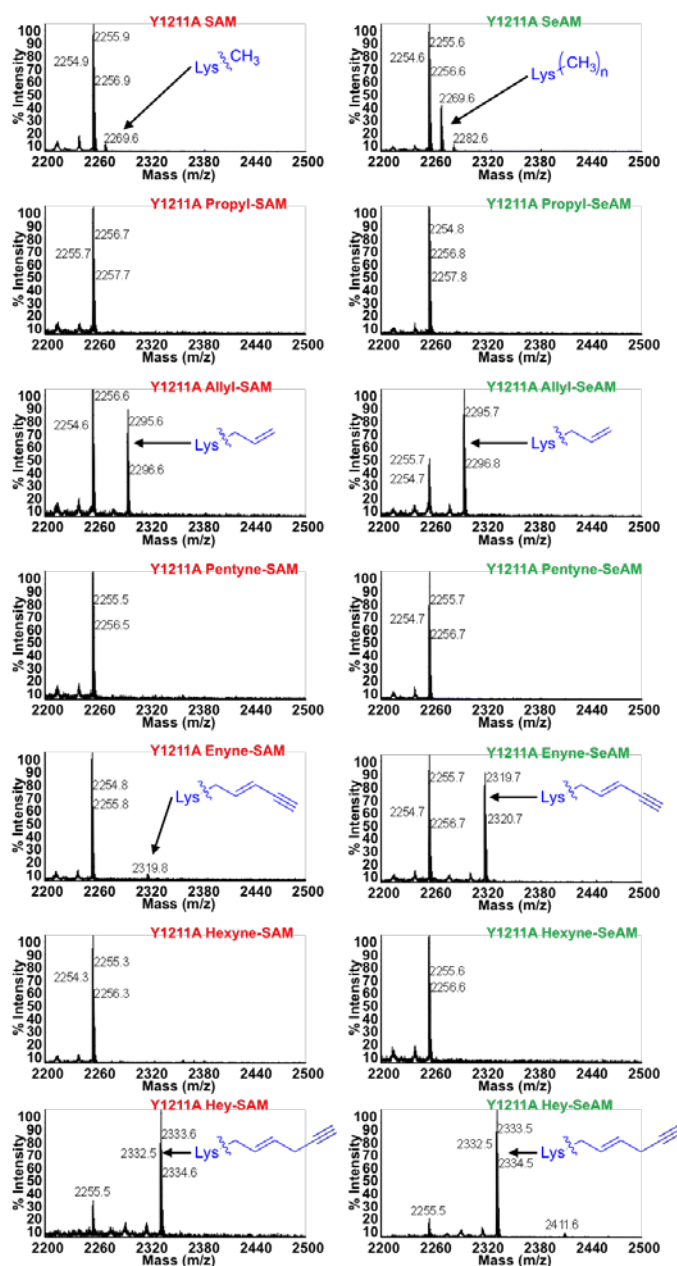
**Figure S3.** MALDI-MS spectra demonstrating sulfur and selenium-based cofactor analogue compatibility with the G9a Y1154A mutant. Spectra in the left hand column are derived from the reactions containing sulfur-based analogues (red labels), while spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

### H3 Peptide (aa1-21) - nGLP1

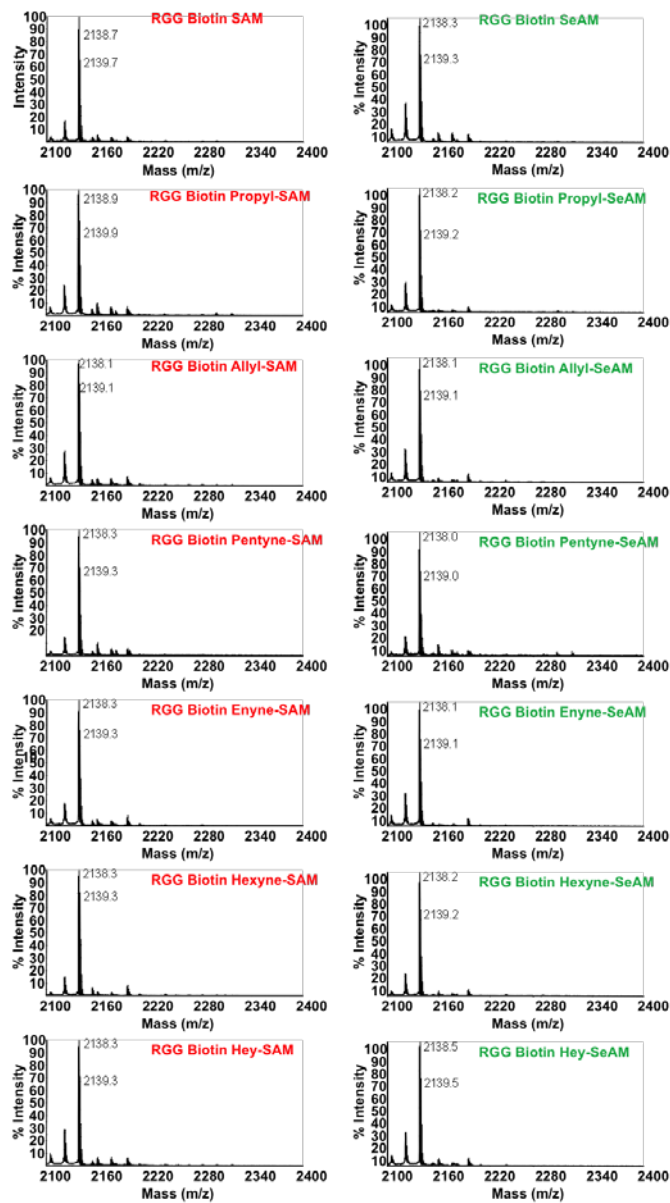


**Figure S4.** MALDI-MS spectra demonstrating sulfur and selenium-based cofactor analogue compatibility with native GLP1. Spectra in the left hand column are derived from the reactions containing sulfur-based analogues (red labels); spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

### H3 Peptide (aa1-21) - GLP1 Y1211A



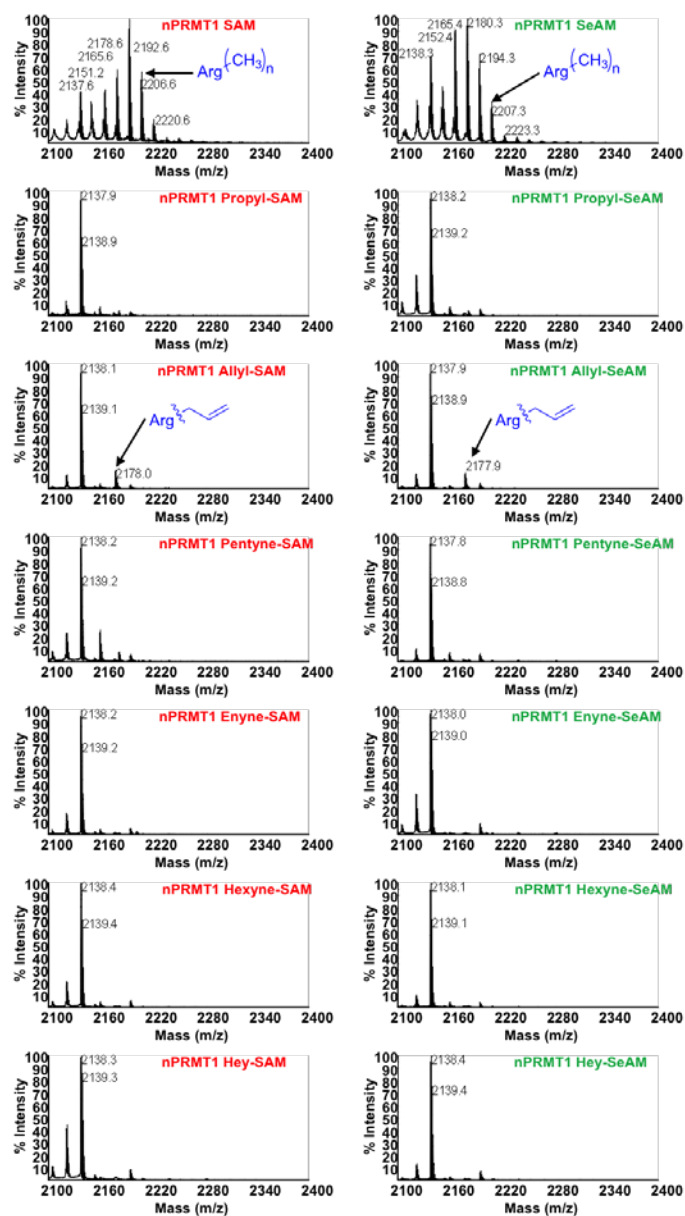
**Figure S5.** MALDI-MS spectra demonstrating sulfur and selenium-based cofactor analogue compatibility with the GLP1 Y1211A mutant. Spectra in the left hand column are derived from the reactions containing sulfur-based analogues (red labels); spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

**RGG Biotin – No Enzyme**

**Figure S6.** MALDI-MS spectra demonstrating a lack of modification of RGG-Biotin peptide substrates by cofactor analogues in the absence of methyltransferase. Spectra in the left hand column are derived from the reactions containing sulfur-based analogues (red labels); spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

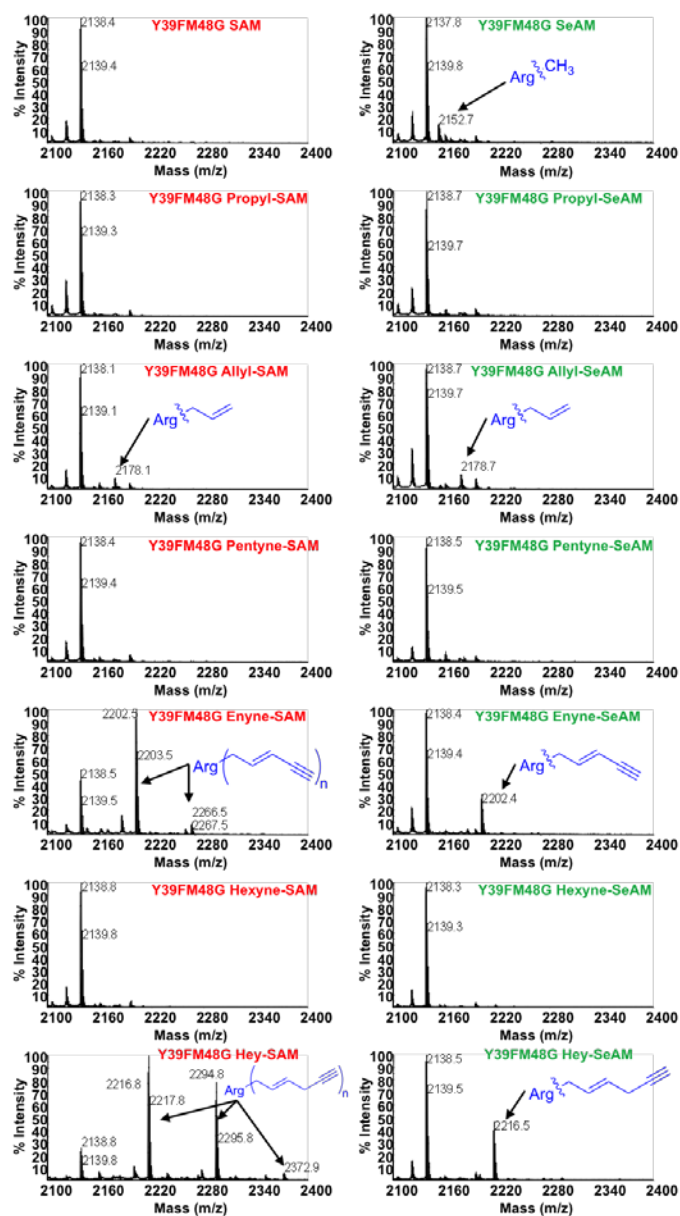


### RGG Biotin – nPRMT1



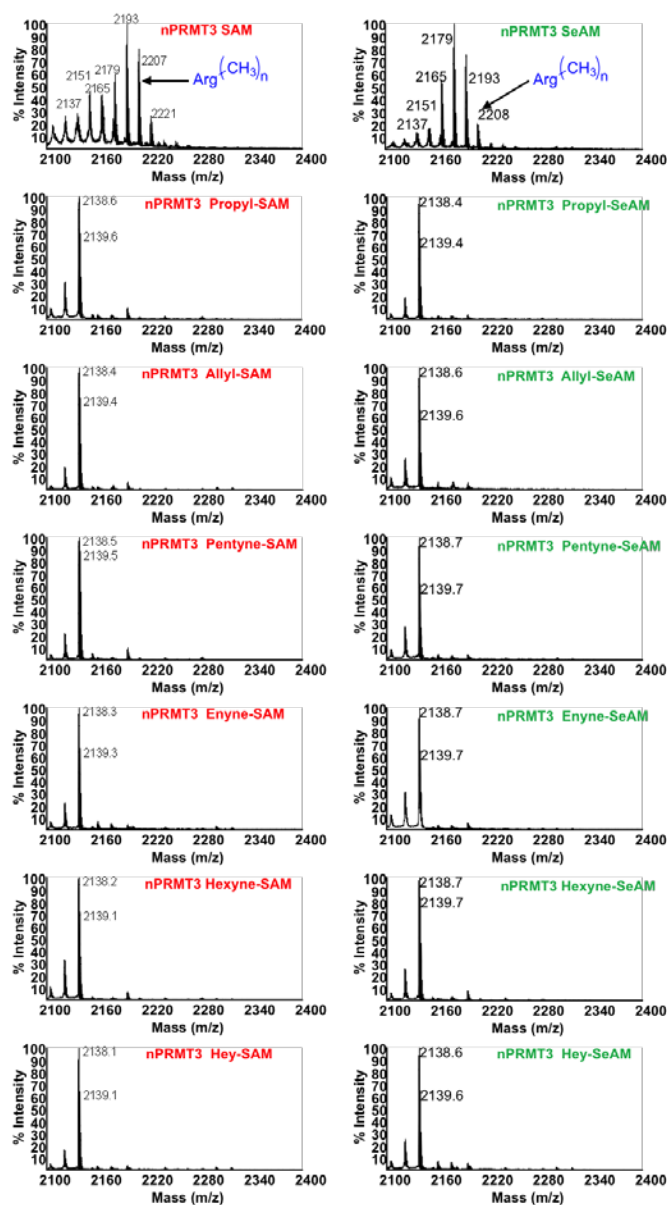
**Figure S7.** MALDI-MS spectra demonstrating sulfur and selenium-based cofactor analogue compatibility with native PRMT1. Spectra in the left hand column are derived from the reactions containing sulfur-based analogues (red labels); spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

### RGG Biotin – PRMT1 Y39FM48G



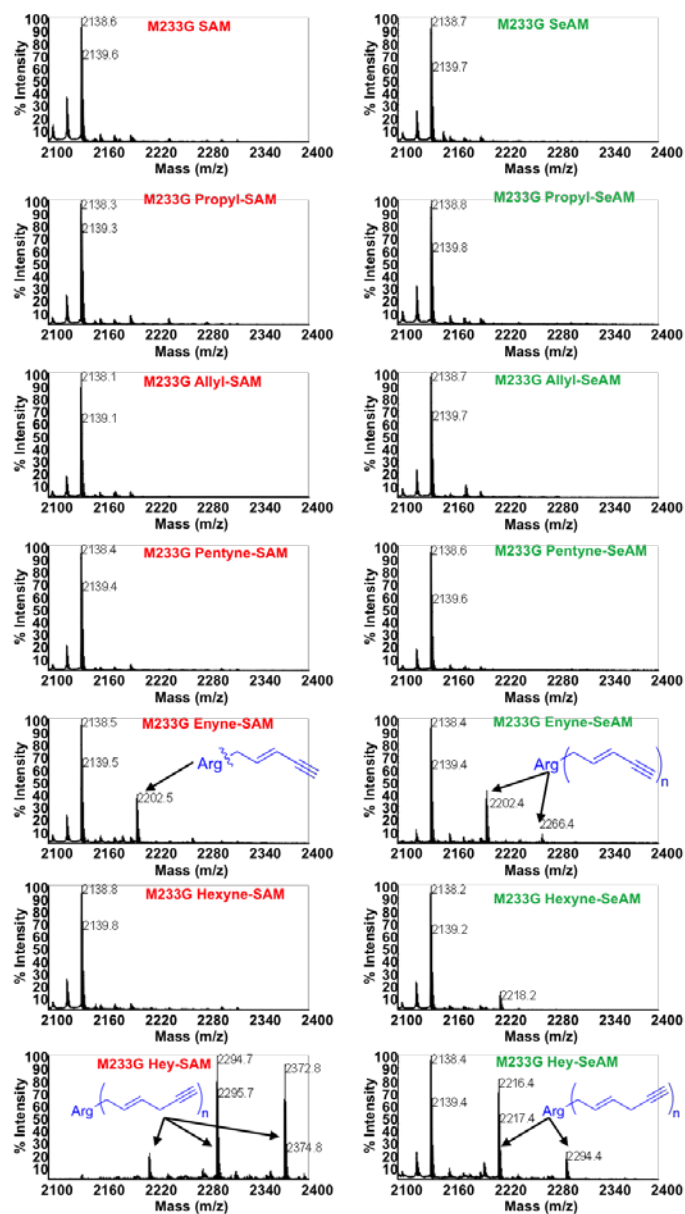
**Figure S8.** MALDI-MS spectra demonstrating sulfur and selenium-based cofactor analogue compatibility with the PRMT1 Y39FM48G mutant. Spectra in the left hand column are derived from the reactions containing sulfur-based analogues (red labels); spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

### RGG Biotin – nPRMT3



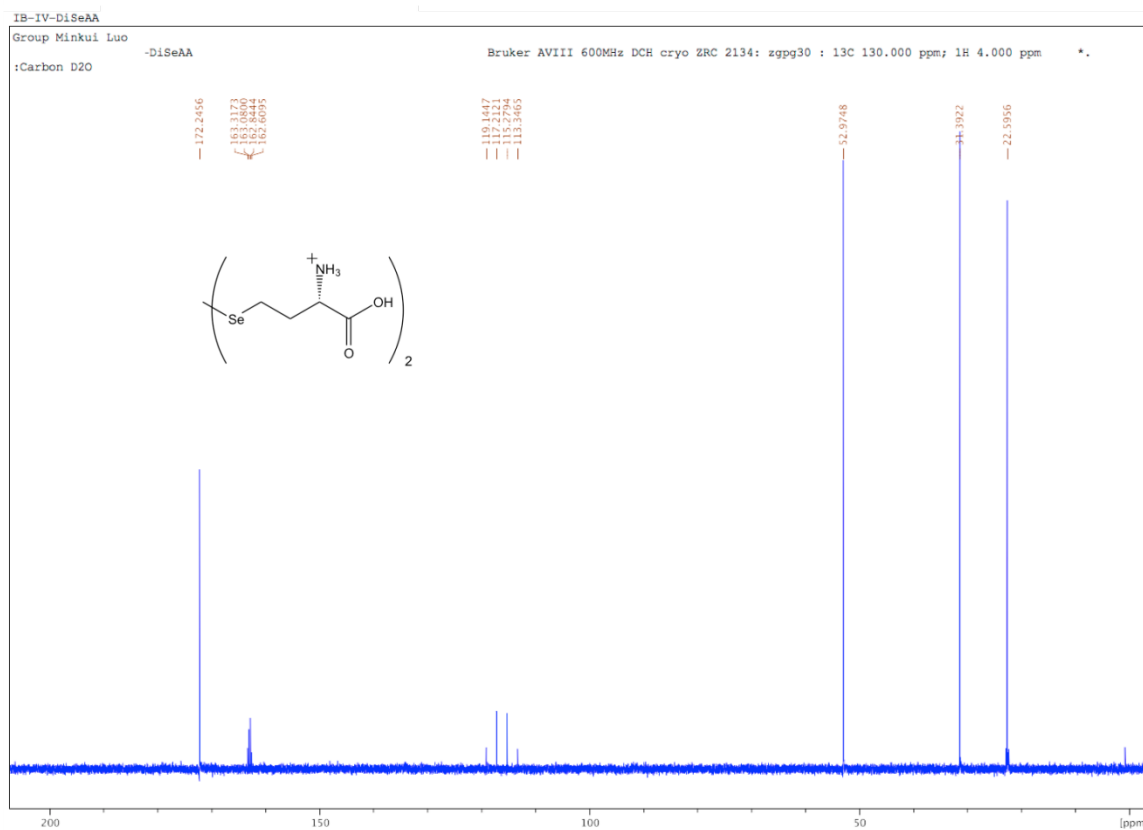
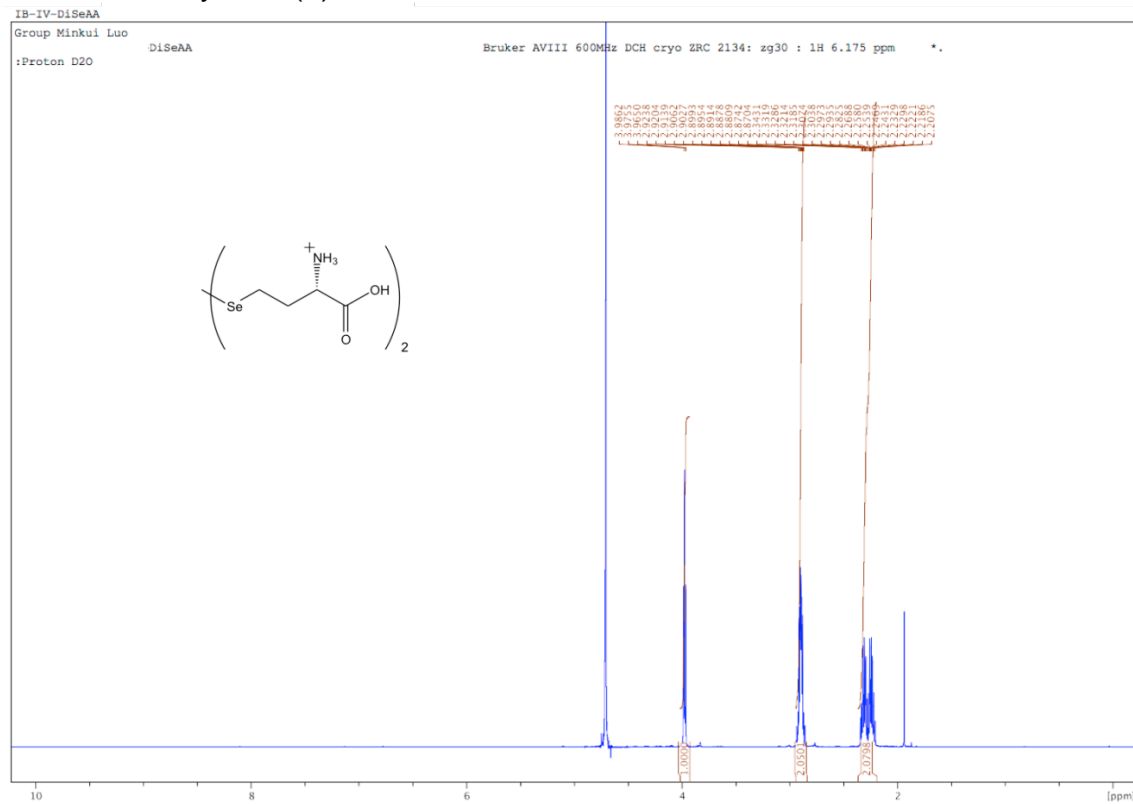
**Figure S9.** MALDI-MS spectra demonstrating sulfur and selenium-based cofactor analogue compatibility with native PRMT3. Spectra in the left hand column are derived from the reactions containing sulfur-based analogues (red labels), while spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

### RGG Biotin – PRMT3 M233G

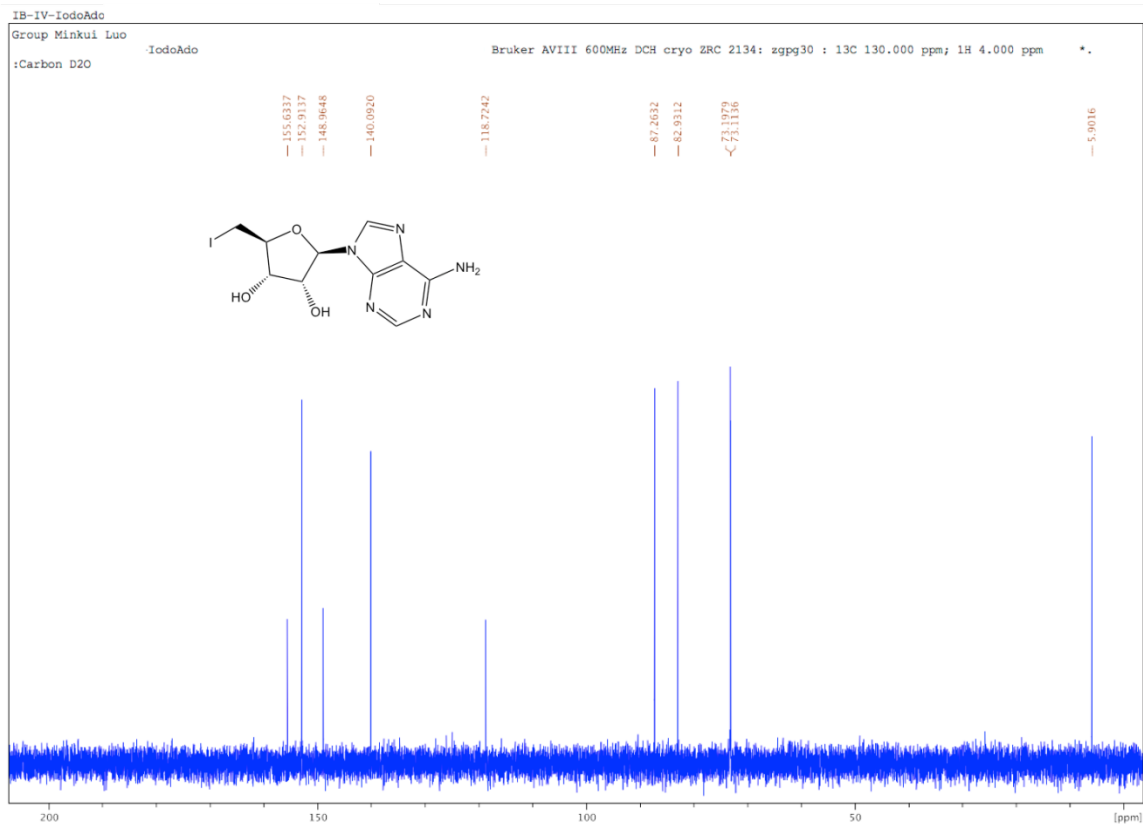
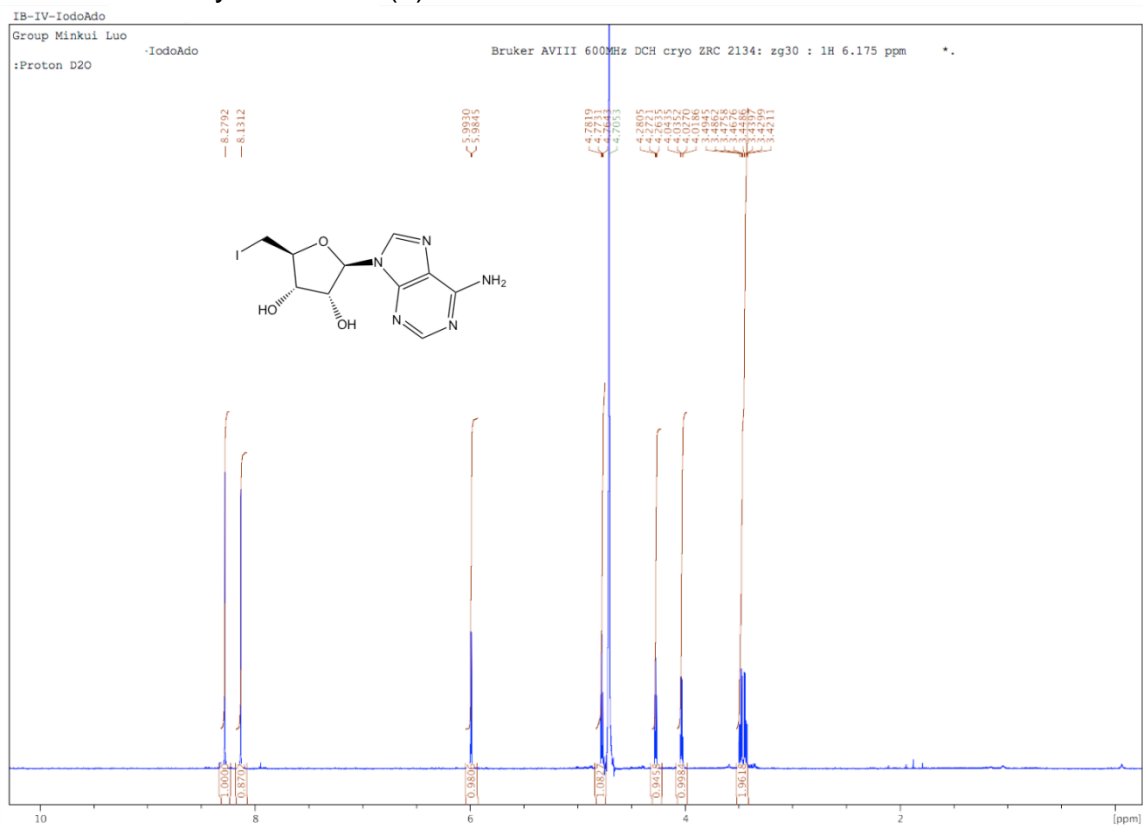


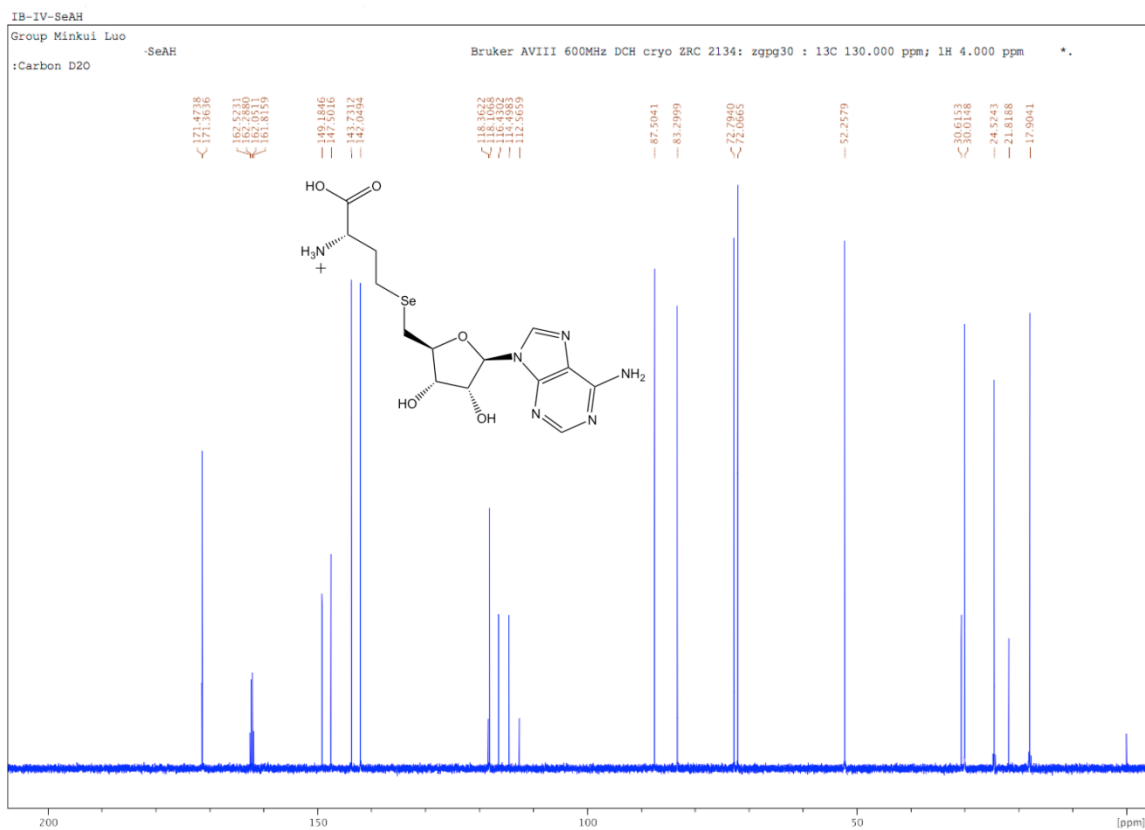
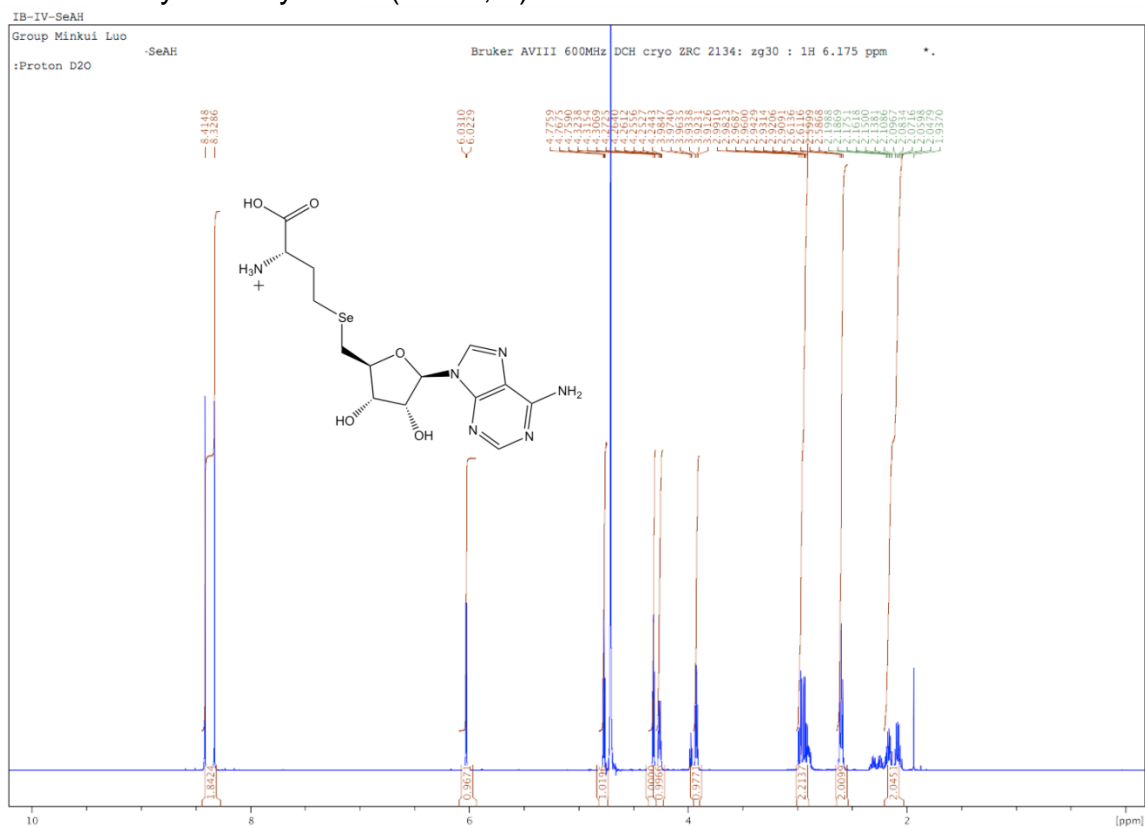
**Figure S10.** MALDI-MS spectra demonstrating sulfur and selenium-based cofactor analogue compatibility with the PRMT3 M233G mutant. Spectra in the left hand column are derived from the reactions containing sulfur-based analogues (red labels); spectra in the right hand column are from the reactions containing selenium-based analogues (green labels).

# NMR Spectra: Selenohomocystine (6):



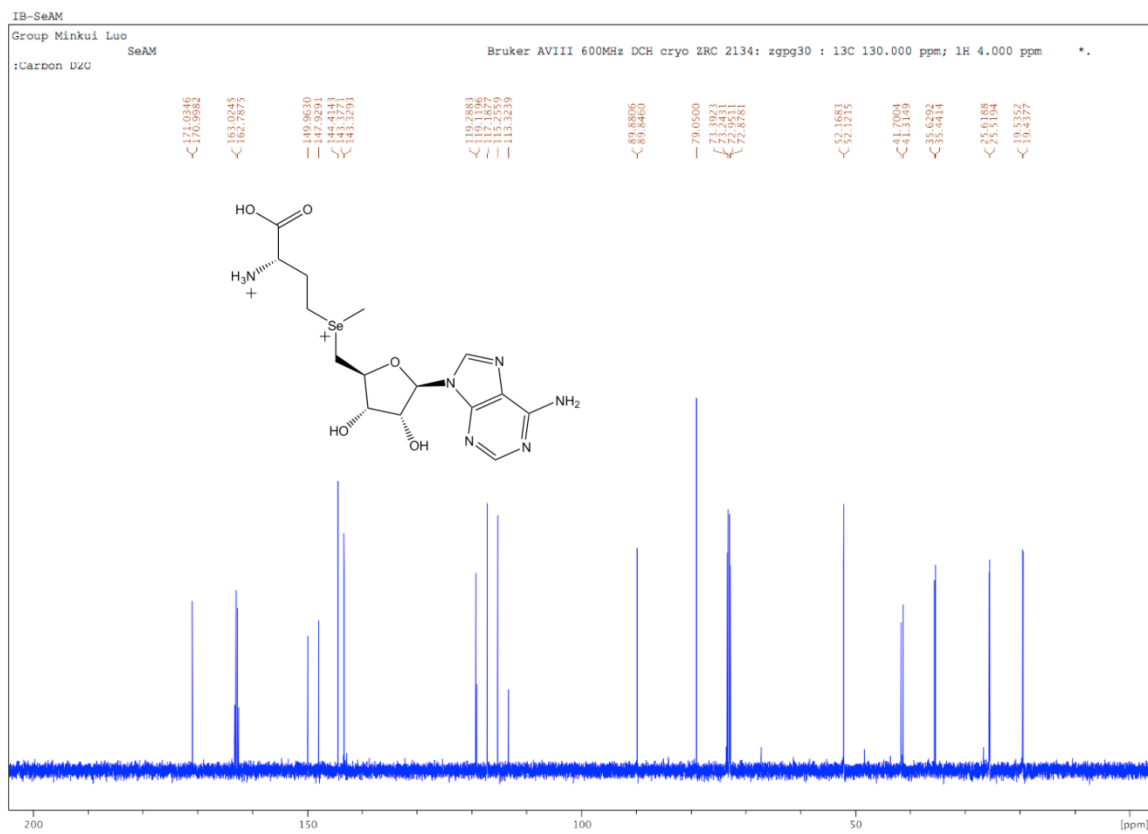
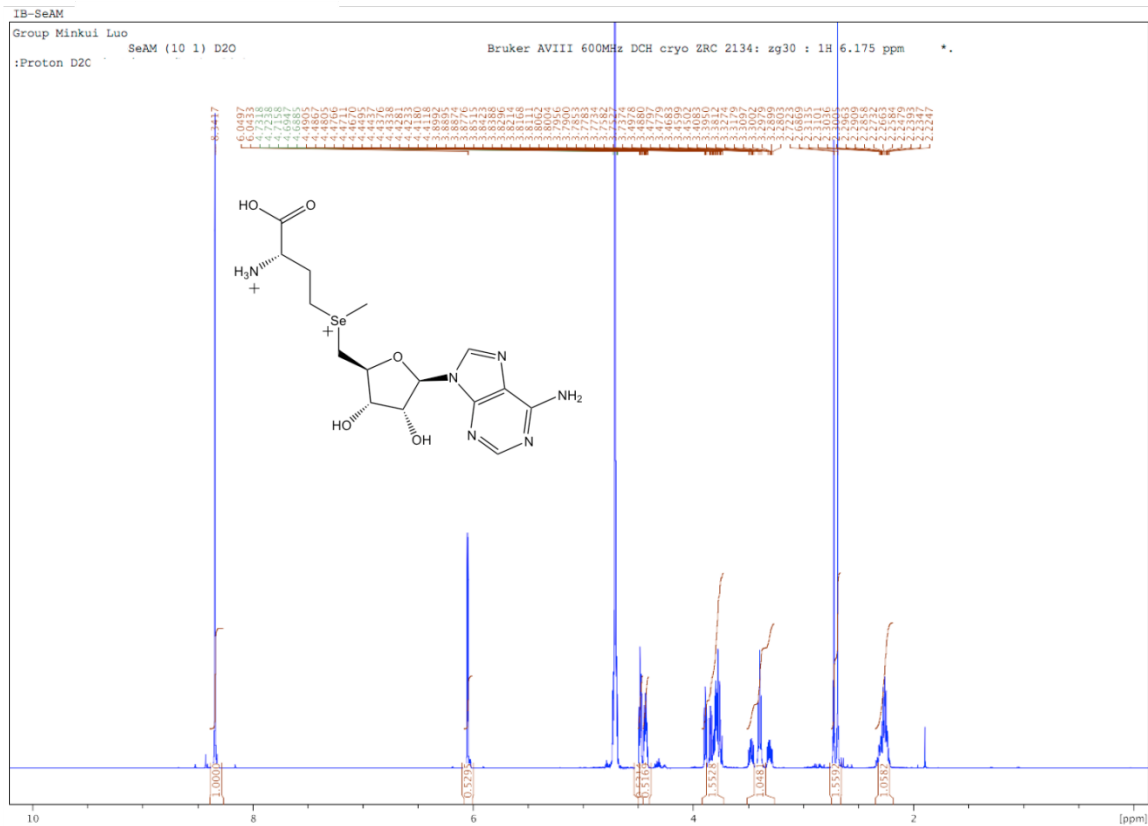
## 5'-Iodo-5'-deoxyadenosine (7):



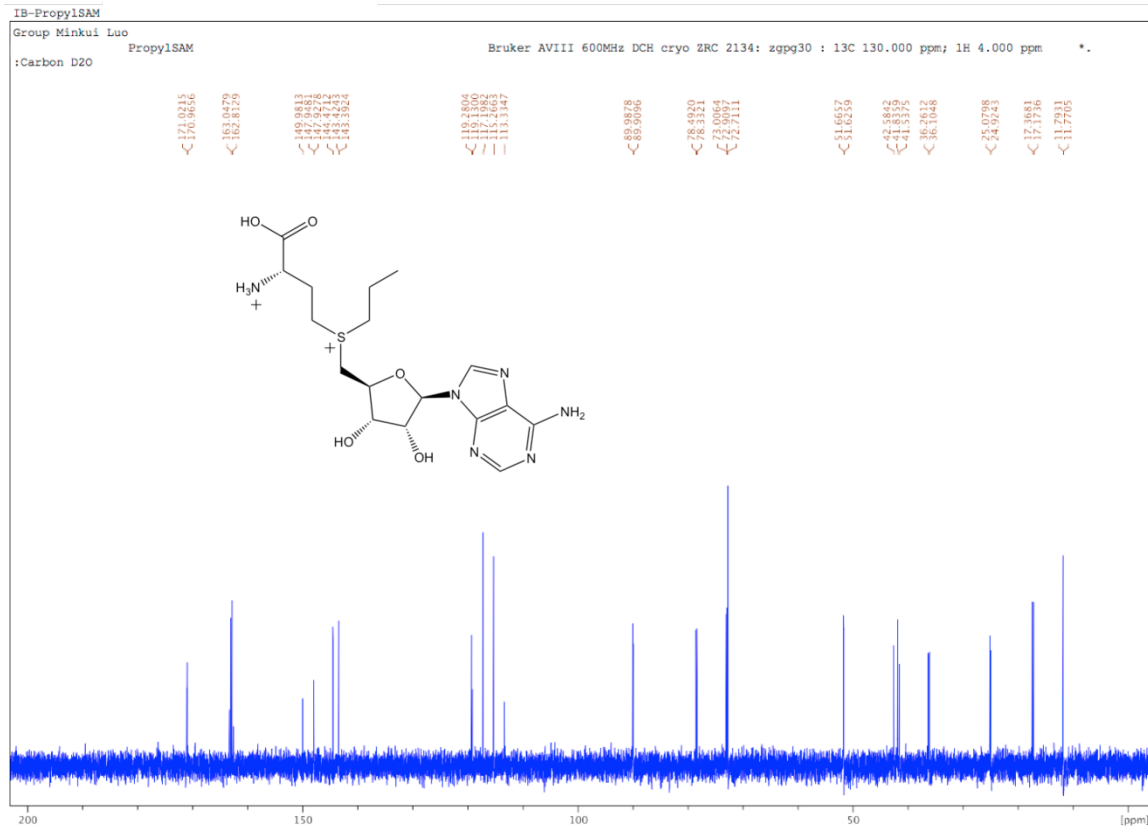
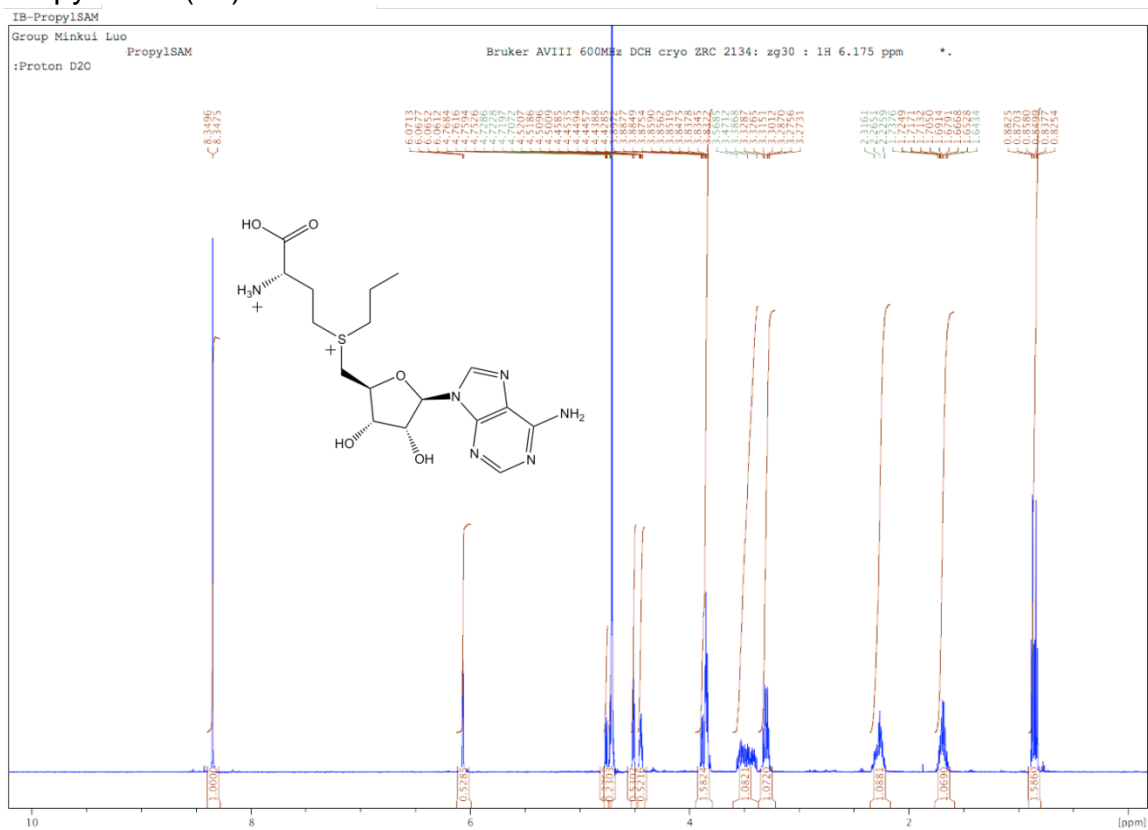
Se-Adenosylhomocysteine (SeAH, **3**):



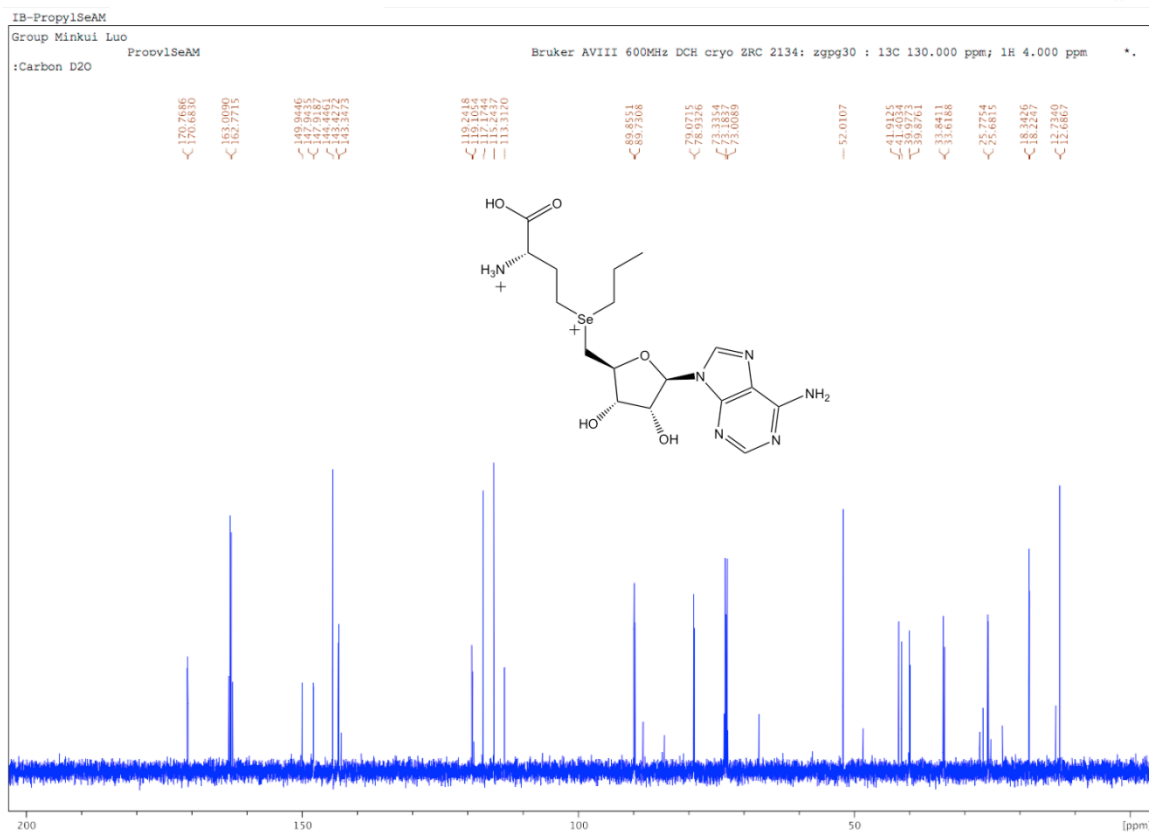
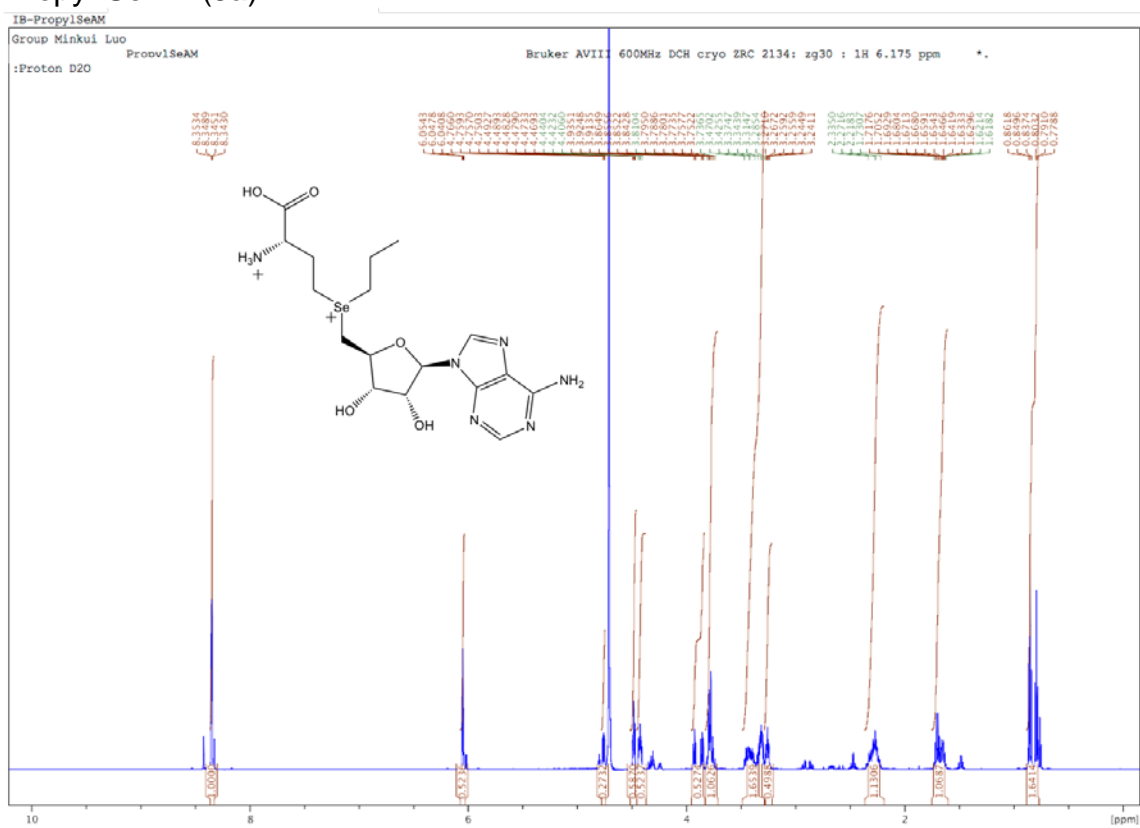




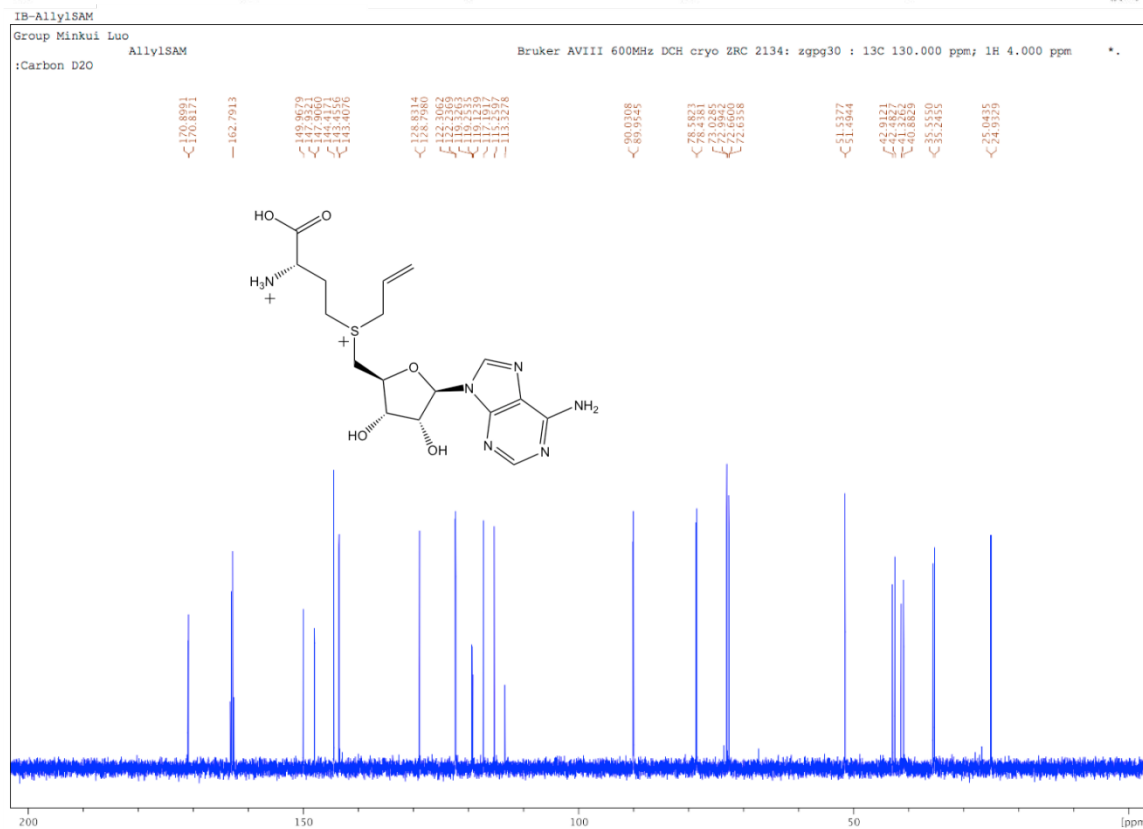
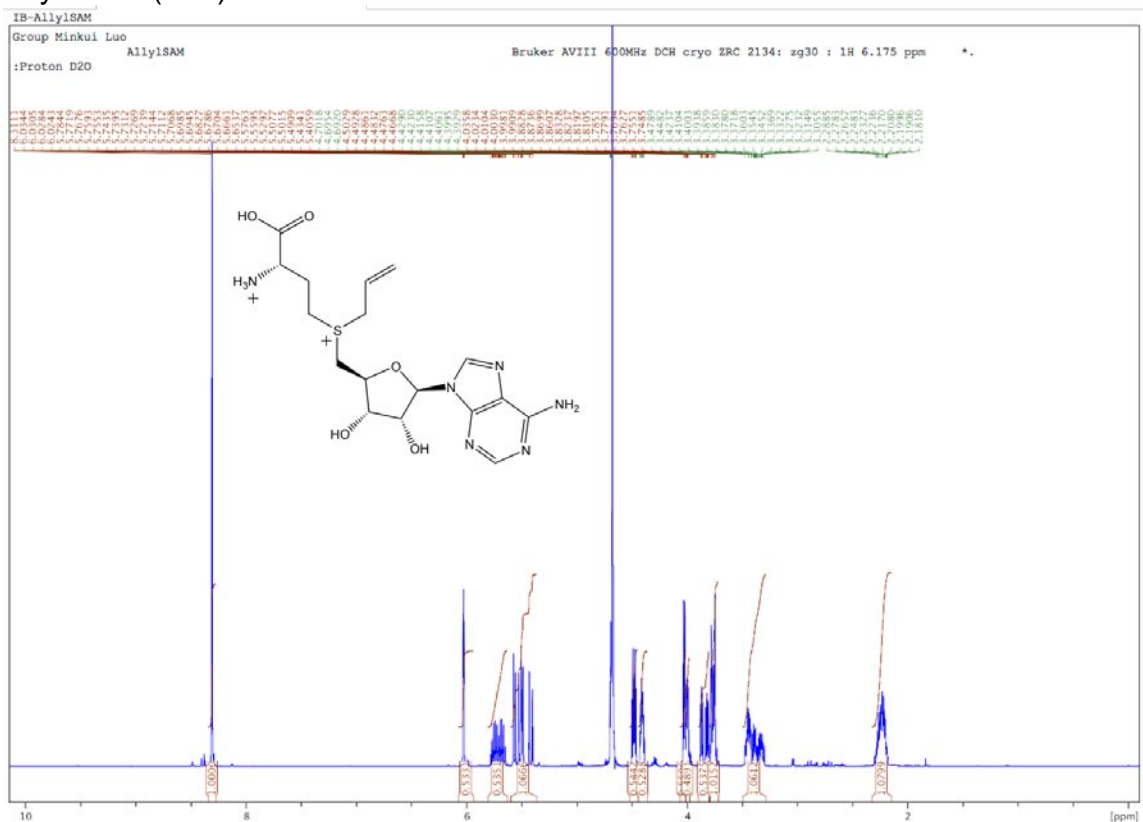
## Propyl-SAM (9b):



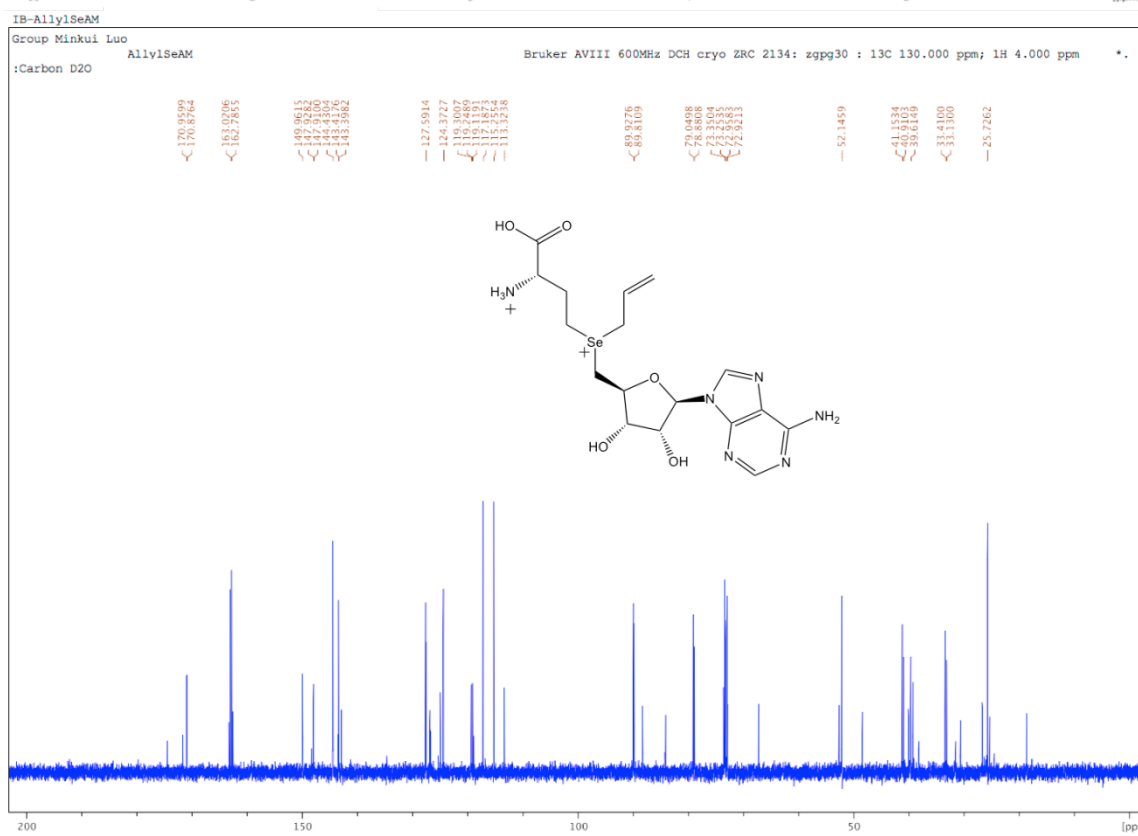
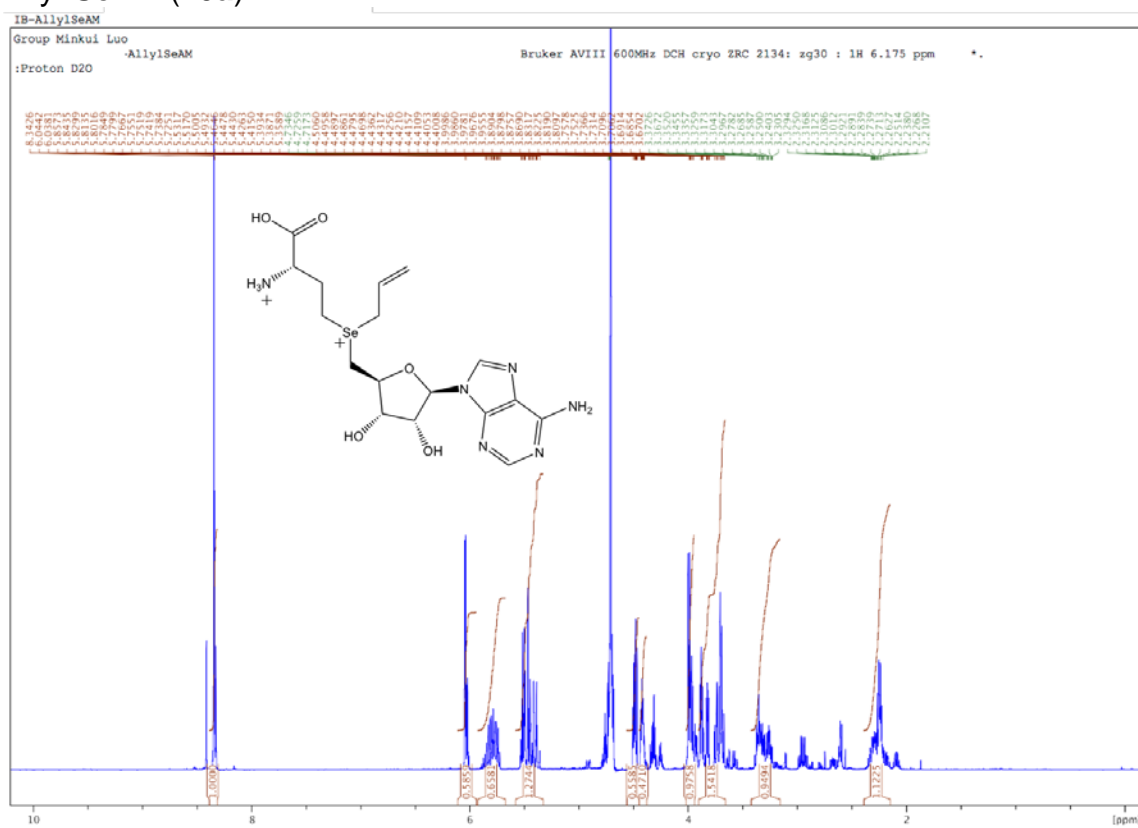
## Propyl-SeAM (9a):



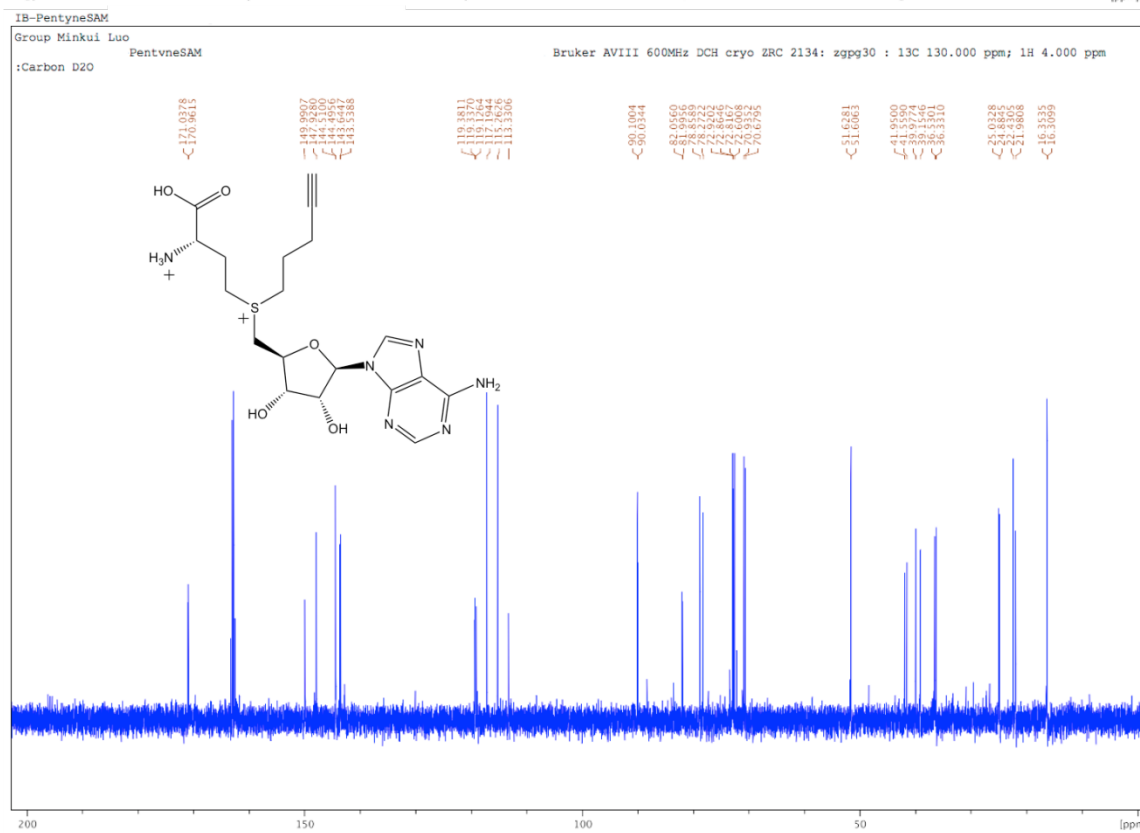
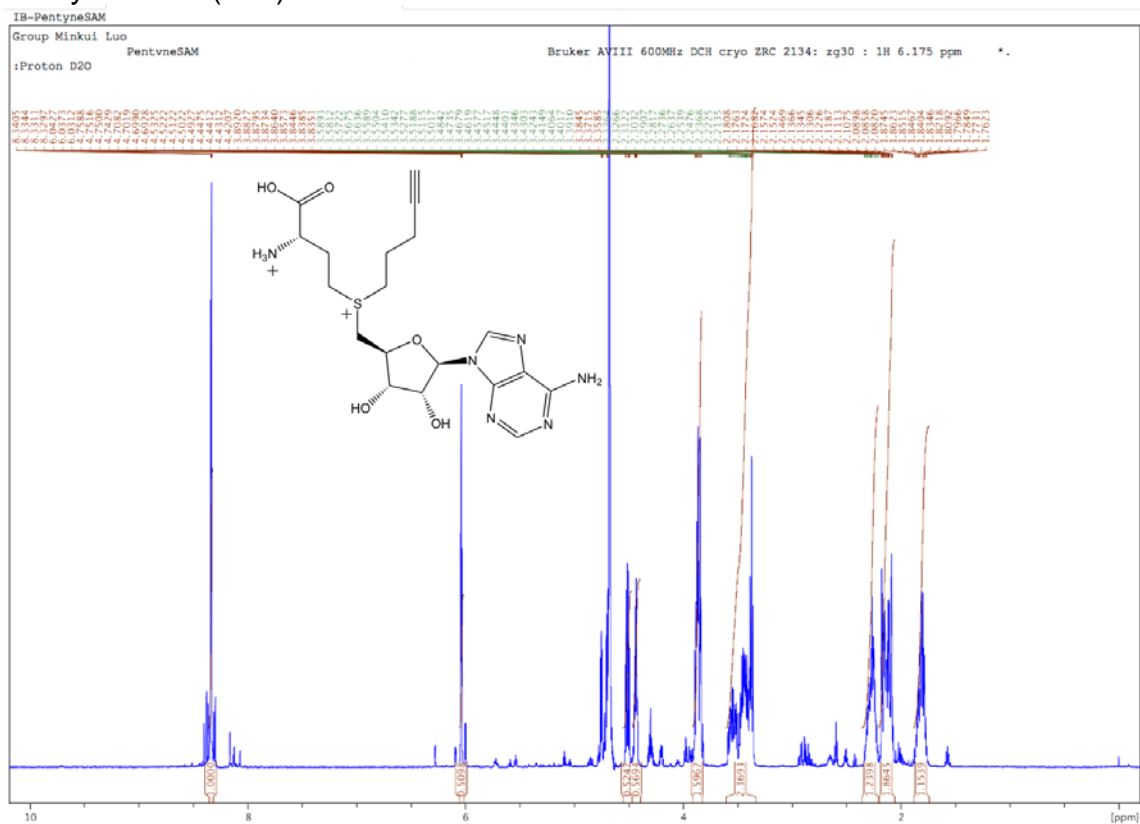
### Allyl-SAM (10b):



## Allyl-SeAM (10a):

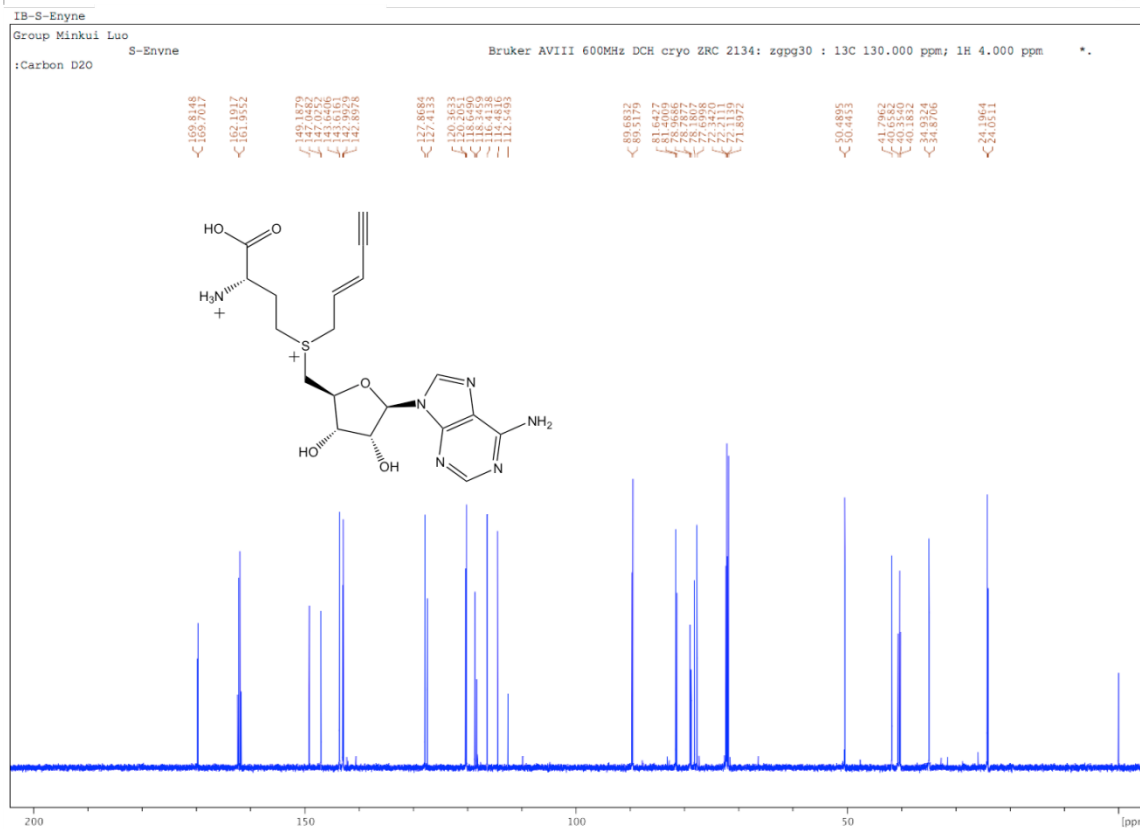
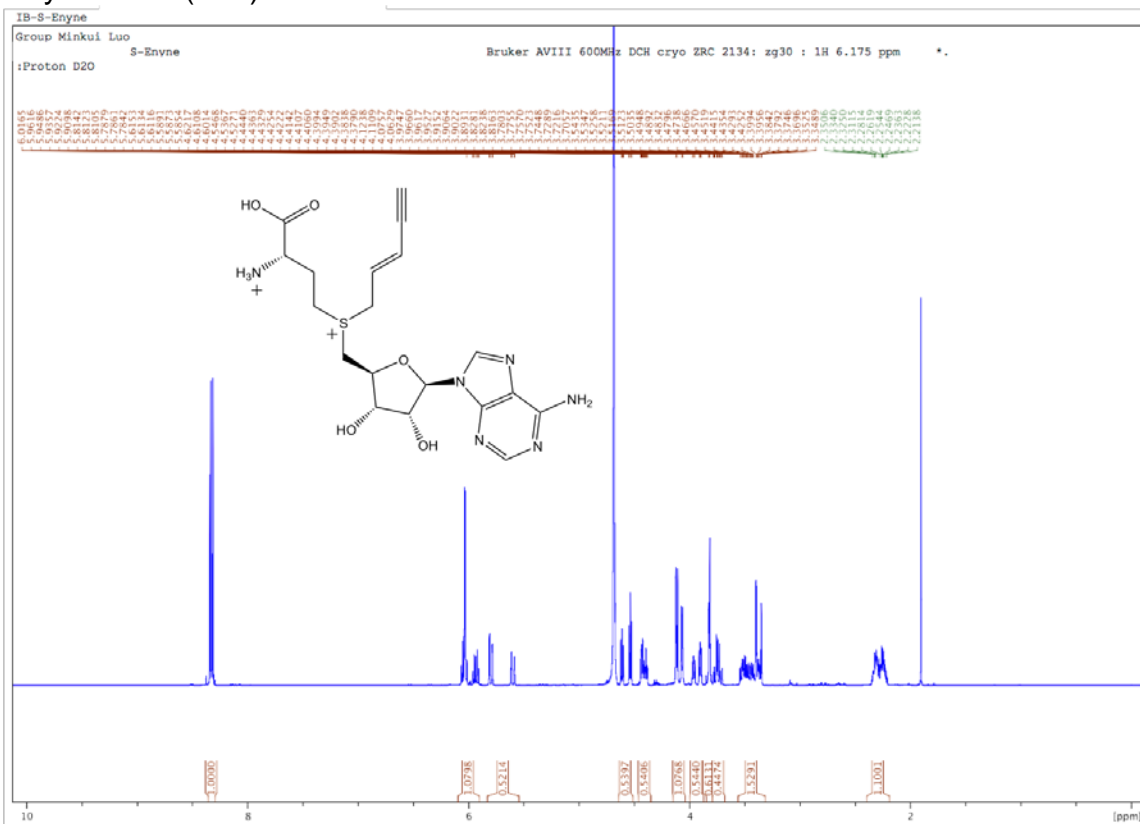


## Pentyne-SAM (11b):



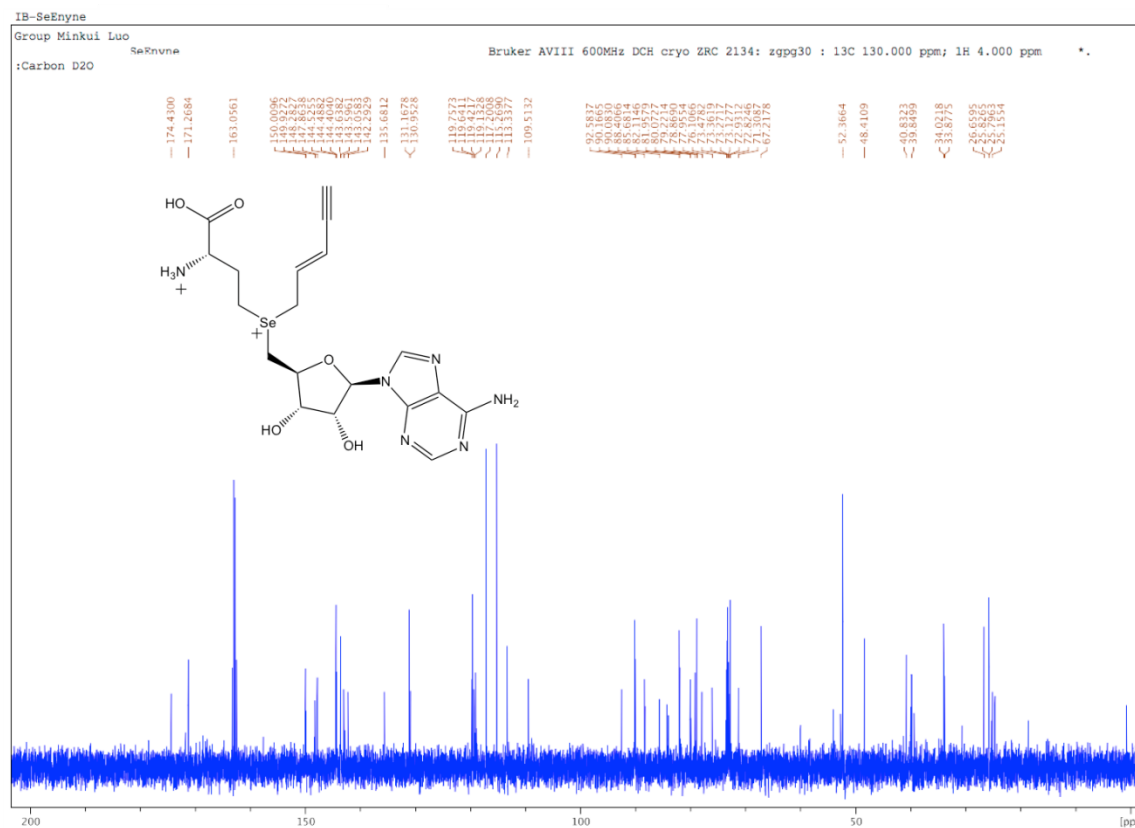
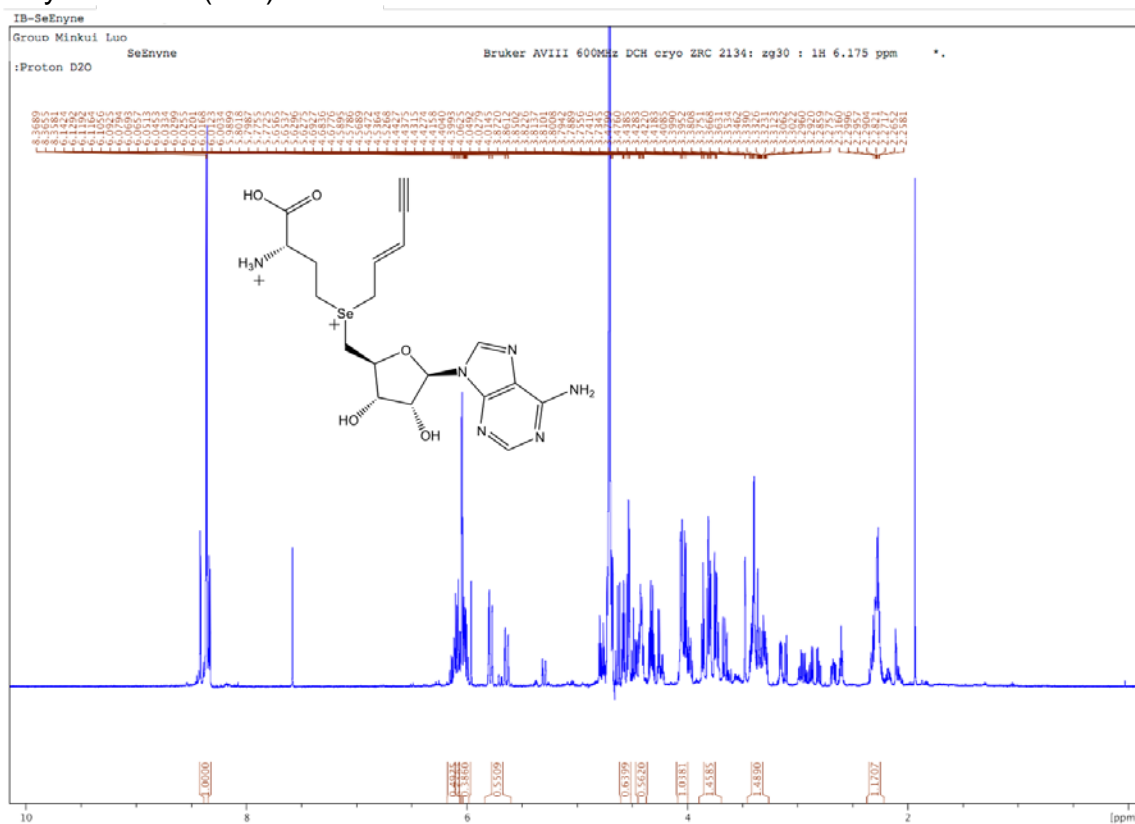


## Enyne-SAM (12b):

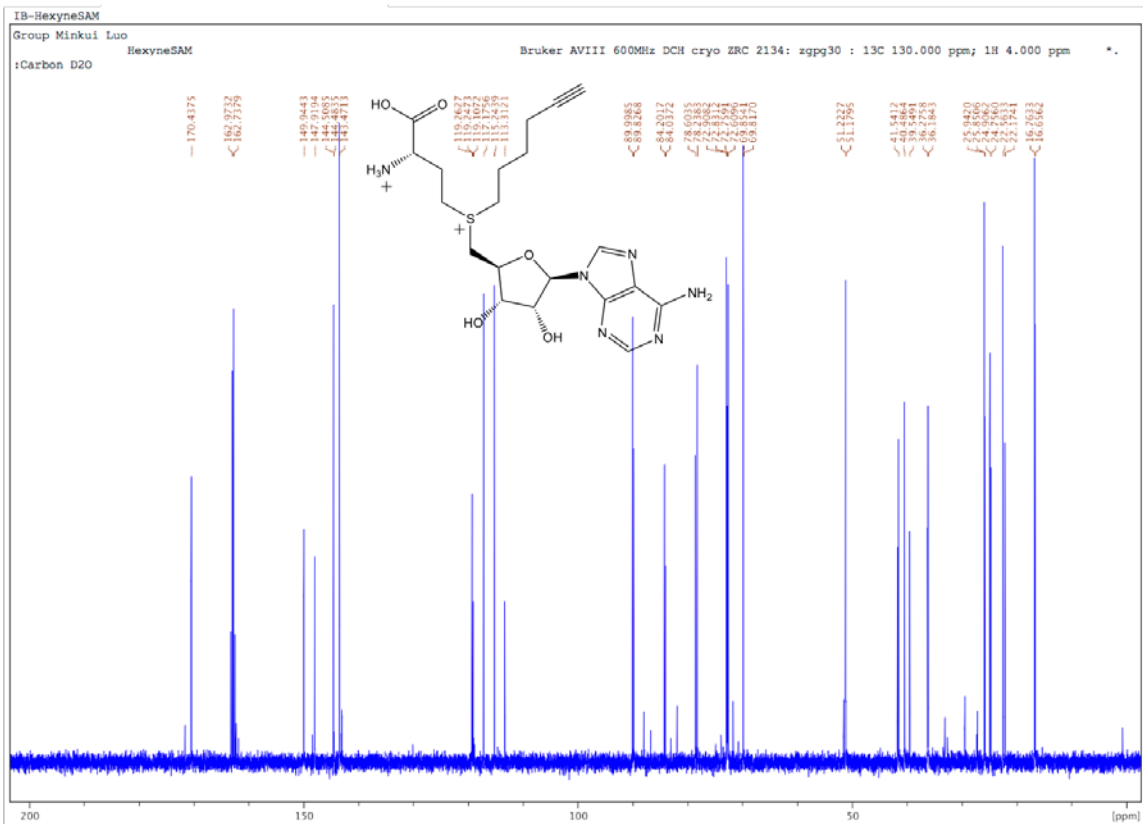
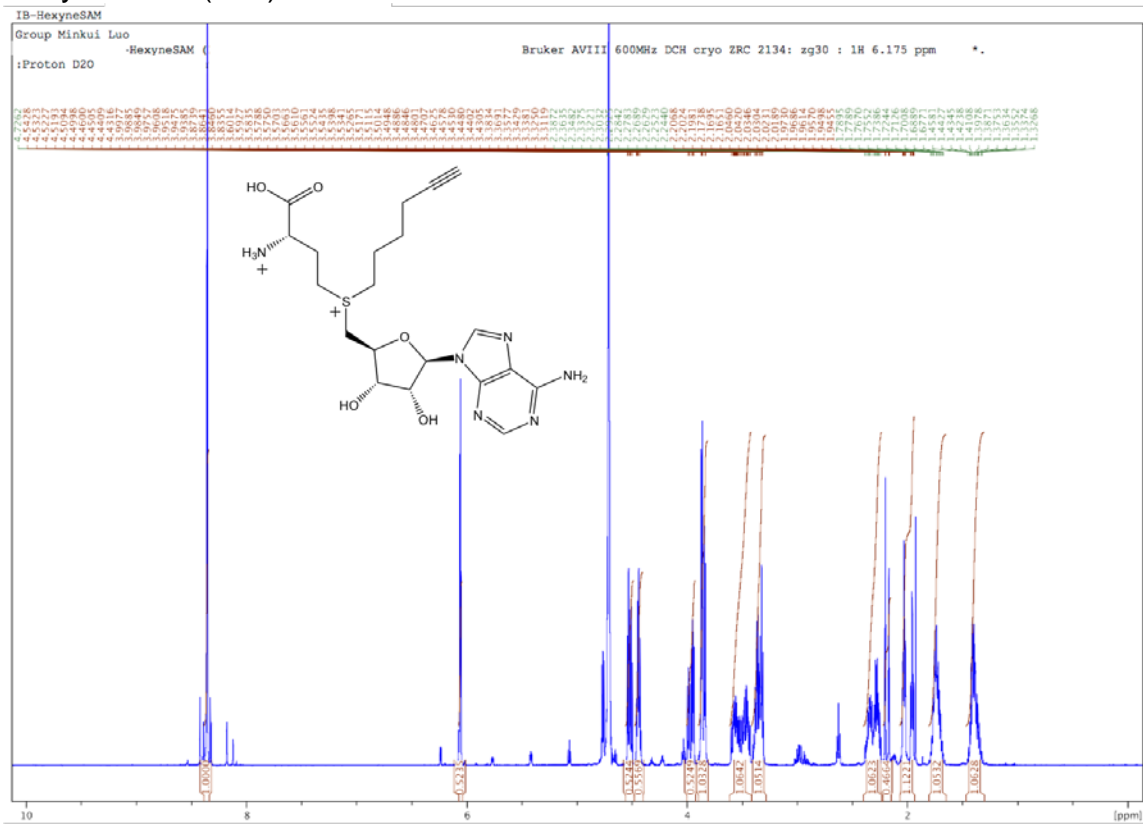




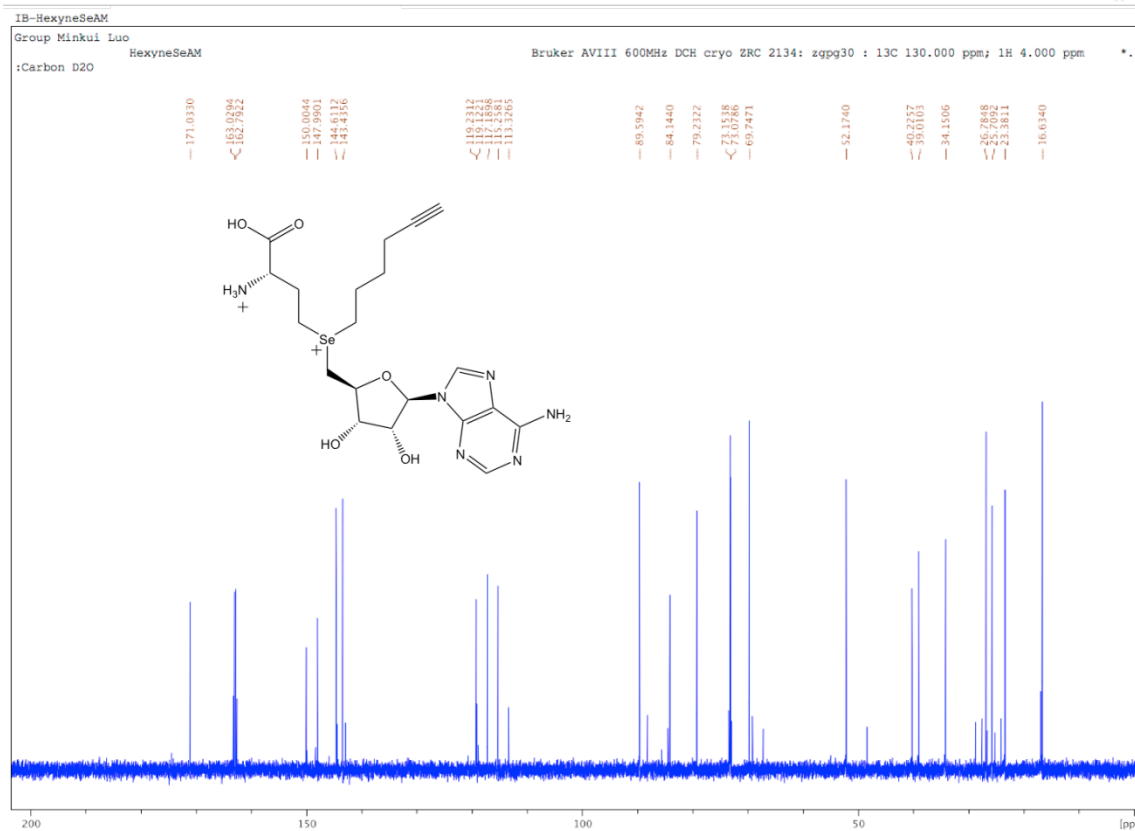
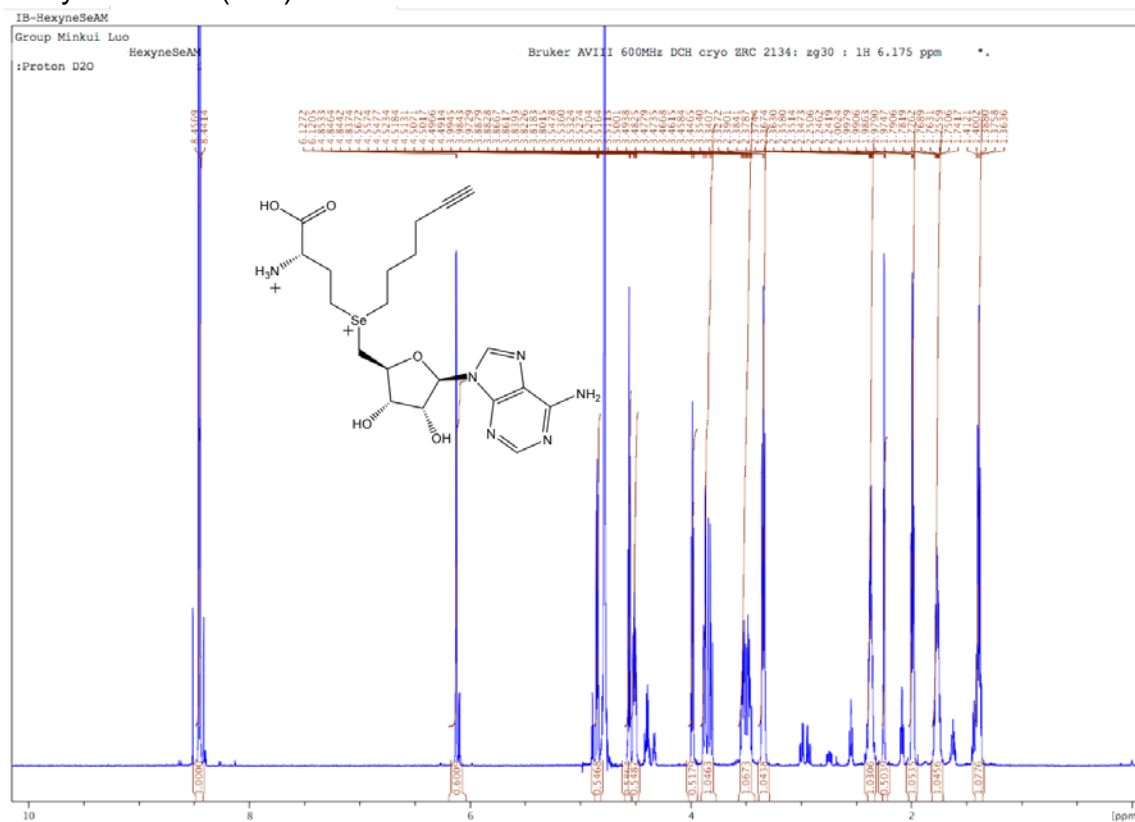
## Enyne-SeAM (12a)



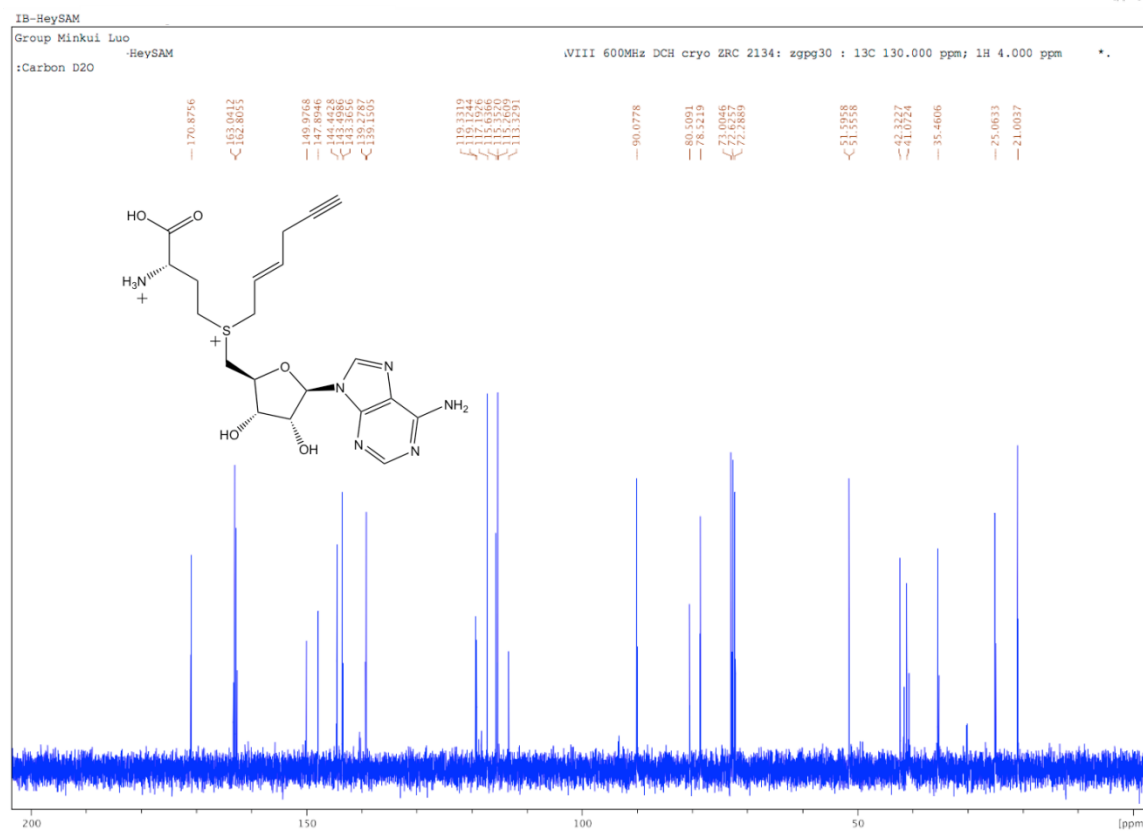
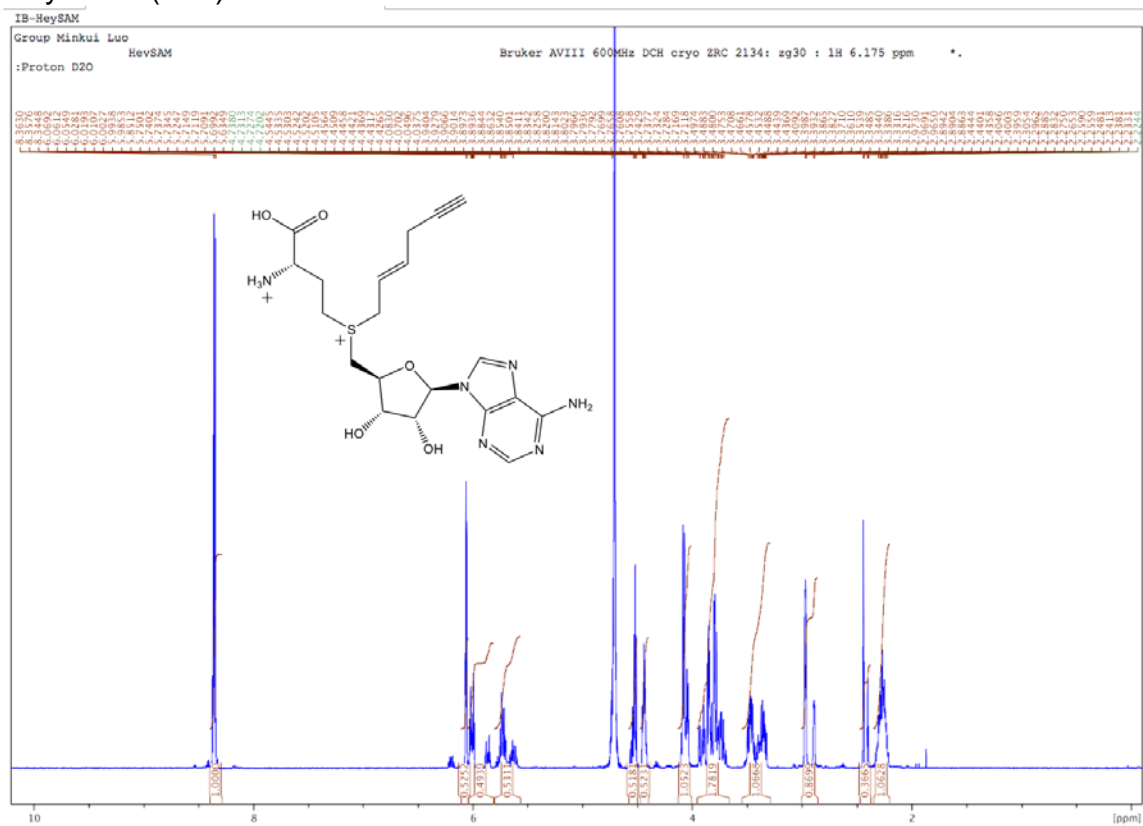
## Hexyne-SAM (13b):



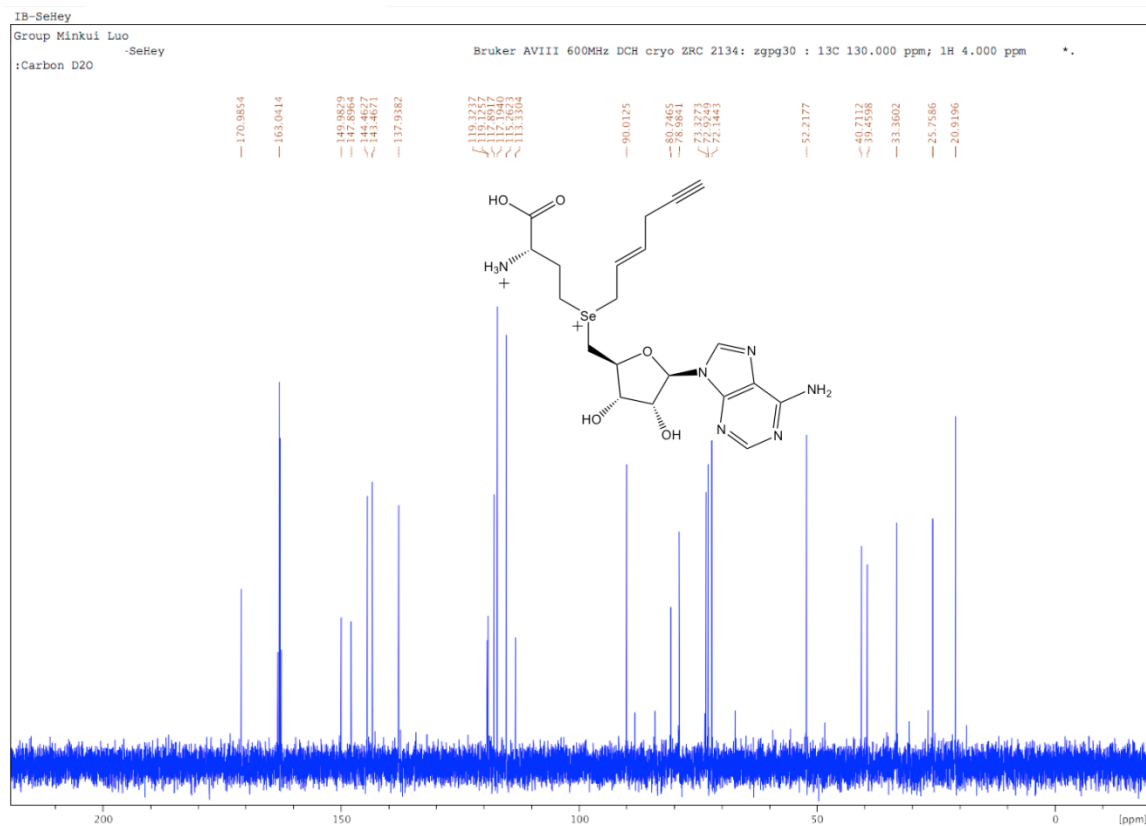
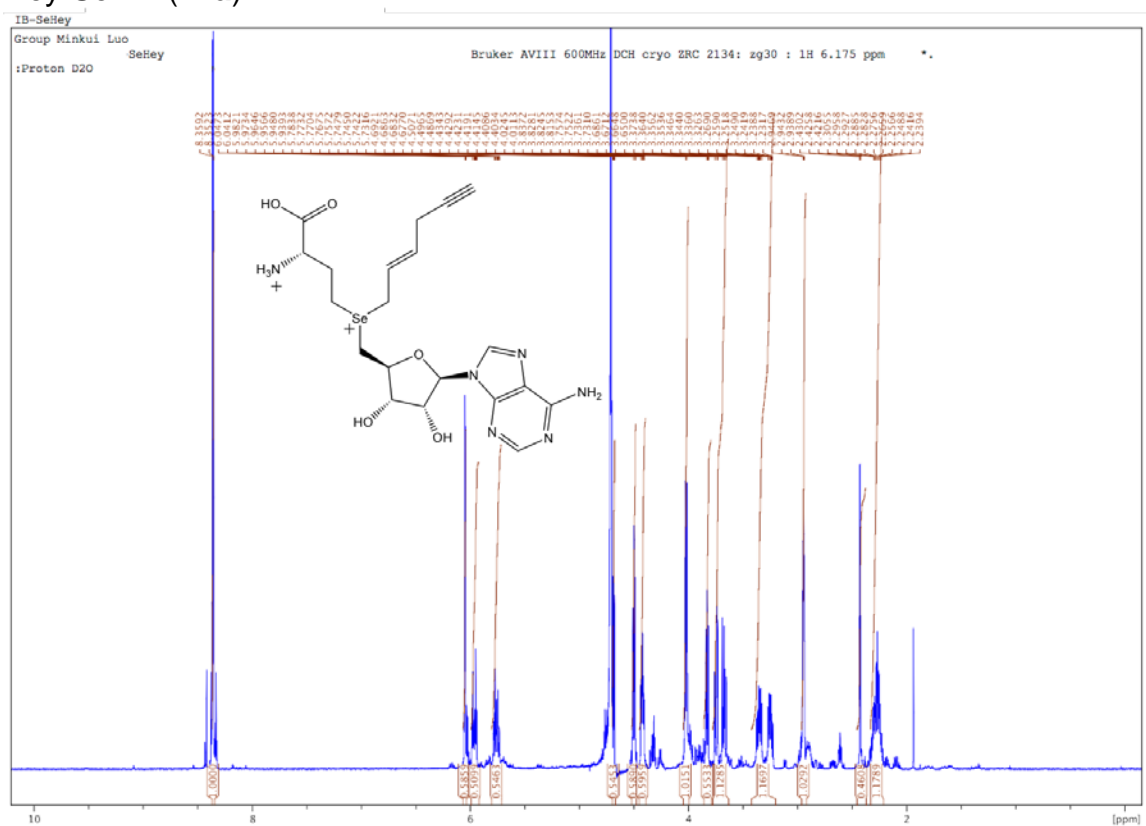
## Hexyne-SeAM (13a):



## Hey-SAM (14b):



## Hey-SeAM (14a):



## Supplementary References

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