Supporting Information

On-DNA Hydroalkylation of *N*-Vinyl Heterocycles via Photoinduced EDA-Complex Activation

Mohammed Sharique,[‡a] Bianca Matsuo,[‡a] Albert Granados,[‡a] Saegun Kim,[a] Mahwish Arshad,[a] Hyunjung Oh,[a] Victoria E. Wu,[b] Minxue Huang,[b] Adam Csakai,[b] Lisa A. Marcaurelle,[b] and Gary A. Molander[a]*

[a] Roy and Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, Pennsylvania 19104-6323, United States

[b] Encoded Library Technologies/NCE Molecular Discovery, R&D Medicinal Science and Technology, GlaxoSmithKline, 200 Cambridge Park Drive, Cambridge, MA 02140, United States

*To whom correspondence should be addressed. E-mail: gmolandr@sas.upenn.edu

[‡]These authors contributed equally

Table of Contents

1 General Considerations

1.1 General

All reagents were purchased and used as received from their respective suppliers unless otherwise noted. The synthesis of new compounds used in the study are described in the appropriate sections in this Supporting Information. The reactions were monitored by ^{1}H NMR or TLC using silica gel F254 plates (60 Å porosity, 250 μm thickness). TLC analysis was performed using EtOAc/hexanes and CH_2Cl_2/MeOH, and visualized using permanganate stain, ceric ammonium molybdate (Hanessian's) stain, and/or UV light. Flash chromatography was accomplished using an automated system (monitoring at 254 nm and 280 nm in conjunction with an evaporative light scattering detector) with silica cartridges (60 Å porosity, 20-40 μm). Accurate mass measurement analyses were conducted using electrospray ionization (ESI). The signals were mass-measured against an internal lock mass reference of leucine enkephalin for ESI-LCMS. The utilized software calibrates the instruments and reports measurements by use of neutral atomic masses. The mass of the electron is not included. IR spectra were recorded using either neat oil or solid products. Data are presented as follows: wavenumber (cm^{-1}) peak shape/intensity (w = weak, m = medium, s = strong, vs = very strong, br = broad). Melting points (°C) are uncorrected. ^{1}H NMR (600 MHz) chemical shifts are referenced to residual, non-deuterated $CHCl_3$ (δ 7.26), CH_3CN (δ 1.94), or $(CH_3)_2SO$ (δ 2.50). ^{13}C (^{1}H decoupled) NMR (151 MHz) chemical shifts are reported relative to $CDCl_3$ (δ 77.2), CD_3CN (δ 1.3, 118.3), or $(CD_3)_2SO$ (δ 39.5). Data are presented as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, td = triplet of doublets, dt = doublet of triplets), coupling constant *J* (Hz), and integration.

1.2 Materials for On-DNA Synthesis

The DNA headpiece (DNA-HP-NH_2) with PEG4 linker (5'-/Phos/GAGTCA/iSp9-PEG/iAm C7_CO-PEG4-NH2/iSp9-PEG/TGACTCCC-3') was obtained from Biosearch Technologies. Novato, CA. The spacer-elongated AOP-Headpiece (**Figure S1**) was prepared via HATU coupling following the general procedure described later in this document. After the reaction was deemed complete by LC/MS analysis, the reaction was precipitated following an EtOH precipitation protocol, and the product on-DNA is typically pure enough to be used without further purification.

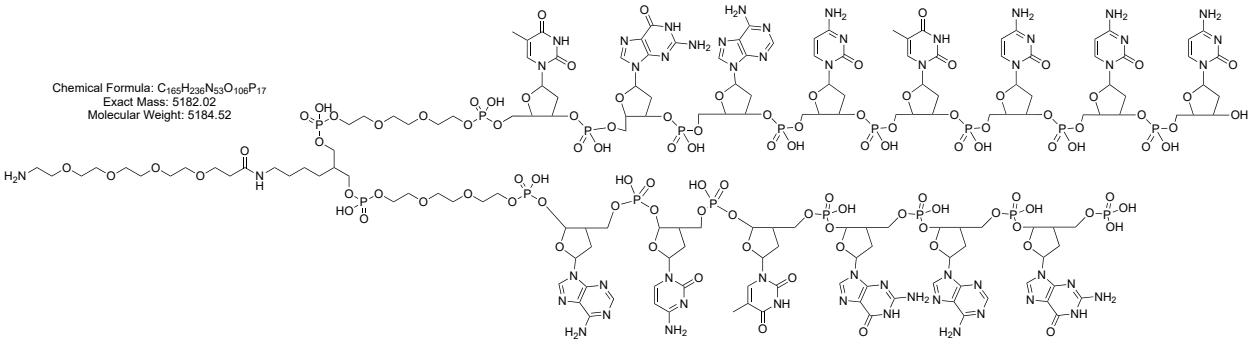


Figure S1. Molecular structure of the DNA-HP-NH_2 employed in this study.

1.3 On-DNA Photoreactions

For the visible light-mediated photoreactions, one Kessil H150 456 nm lamp (19 V DC 40 W Max) was placed 1.5 inches away from PCR tubes with the light intensity set to 100%. The photoreactions on-DNA were performed in Eppendorf PCR 8-strip microcentrifuge tubes (Fisher Ref. 781320) with PCR strips of 8 caps (Fisher Ref. 781340). HyPure™ Molecular Biology Grade water was purchased and used as received without further manipulation. Reaction mixtures were vortexed with a Fisherbrand™ Mini Vortex Mixer.

1.4 Analysis of "on-DNA" reactions

Analysis of on-DNA reactions was performed by LC/MS. After reaction completion, an aliquot of the reaction mixture was diluted with H_2O to approximately 0.2 mM. At this point, 3 µL aliquots of the LC/MS sample was injected onto reverse-phase chromatography columns (for analysis performed at GSK: Halo 400 Å, ES-C18, 3.4 µ, 2.1 x 30 mm; for analysis performed at UPenn: Cortecs T3 2.7 µm, 2.1x30 mm, Waters) and eluted (10-90% B over 4 min at 0.5 mL/min flow rate; Solvent A: 0.75% v/v/ HFIP / 0.038% TEA / 5 µM EDTA in H_2O; Solvent B: 0.75% HFIP, 0.038% TEA, 5 µM EDTA in 90/10 MeOH/deionized H_2O) with monitoring at UV 254 nm (UPenn) and UV-Multiple Wavelength Detector = 260 and 280 nm (GSK). Effluent was analyzed on a Waters SQ Detector 2 ACQUITY UPLC System in negative ion mode (UPenn) or a Bruker qTOF negative ion mode: Scan 600-3000 amu (GSK). For the functionalized headpiece samples (on-DNA), % conversion was determined based on reported peak intensities following deconvolution (between 3,000-10,000 Da) of the DNA charge states using Intact MassTM by

Protein Metrics Inc. (v4.5-53x64 intact). For the photoredox scope reactions, % conversion was determined using Intact MassTM by Protein Metrics Inc. (v4.5-53x64 intact) (GSK) or using MassLynx at UPenn. Data was scanned between 0.3-2.2 min and deconvoluted between 4,000-6,000 Da, with a mass tolerance window of 2 Da, with 5% of base peak threshold was set for reporting (GSK). Alternatively, data was scanned between 1.0- 5.0 min and deconvoluted between 3,000-8,000 Da, with a mass tolerance window of 2-4 Da, with 10% of base peak threshold was set for reporting (UPenn). Na, K, NH_4, and HFIP adducts were included in the product percentage. Detailed parameters can be found later in the Supporting Information

An ethanol precipitation protocol was employed upon reaction completion for the evaluation of the scope of the transformation (described in the following sections of this supporting information). The samples were then submitted to centrifugation using a Corning™ Mini Microcentrifuge (max. speed: 6,000 rpm). The samples for LC-MS analysis were prepared in HyPure™ Molecular Biology Grade H_2O at approximately 0.2 mM. At this point, the samples were injected onto reverse-phase chromatography columns (for analysis performed at GSK: Halo ES-C18, 2 µm particle size, 160A pore size, 2.1x30 mm; for analysis performed at UPenn: Cortecs T3 2.7 µm, 2.1x30 mm, Waters) and eluted (10-90% B over 4 min at 0.5 mL/min flow rate; Solvent A: 0.75% v/v/ HFIP/0.038% TEA/5 µM EDTA in H_2O; Solvent B: 0.75% HFIP, 0.038% TEA, 5 µM EDTA in 90/10 MeOH/deionized H_2O) with monitoring at UV 260 nm (GSK) and UV 254 nm (UPenn). Effluent was analyzed on a Bruker microTOF in negative ion mode (GSK) or Waters SQ Detector 2 ACQUITY UPLC System in negative ion mode (UPenn). For the functionalized headpiece samples (the on-DNA aryl halides/alkenes), % conversion was determined based on reported peak intensities following deconvolution (between 3,000-10,000 Da) of the DNA charge states using the Bruker Compass DataAnalysis software version 4.2 (build 383.1). An intensity of 5% or 10% of the maximum peak intensity observed for a given spectra was set as the reporting threshold. The maximum intensity peak for each distribution of peaks was manually selected as the representative peak for reporting. % Conversion was then calculated by dividing the peak intensity of the product peak by the sum of the reported peaks for the spectra. For the photoredox scope reactions, % conversion was determined using Intact Mass™ by Protein Metrics Inc. (version v3.3-421 x 64) (GSK). For the photoredox scope reactions, % conversion was determined using MassLynx at UPenn. Data was scanned between 1.9-3.0 min and deconvoluted between

4,000-6,000 Da, with a mass tolerance window of 2 Da, with 5% of base peak threshold set for reporting (GSK). Alternatively, data was scanned between 1.0-3.0 min and deconvoluted between 3,000-8,000 Da, with a mass tolerance window of 1 Da, with 10% of base peak threshold set for reporting (UPenn). Na, K, NH_4, and HFIP adducts were included in the product percentage. Detailed parameters can be found later in the Supporting Information.

2 Synthetic Protocols

2.1 Preparation of the DNA-tagged Olefin Headpieces (HP-1a to HP-1g)

The DNA-tagged olefin headpieces employed in the scope of the reported transformation (**Figure S2**) were prepared according to **General Procedure I** from the coupling between the DNA-HP-NH_2 tag and the corresponding carboxylic acids.

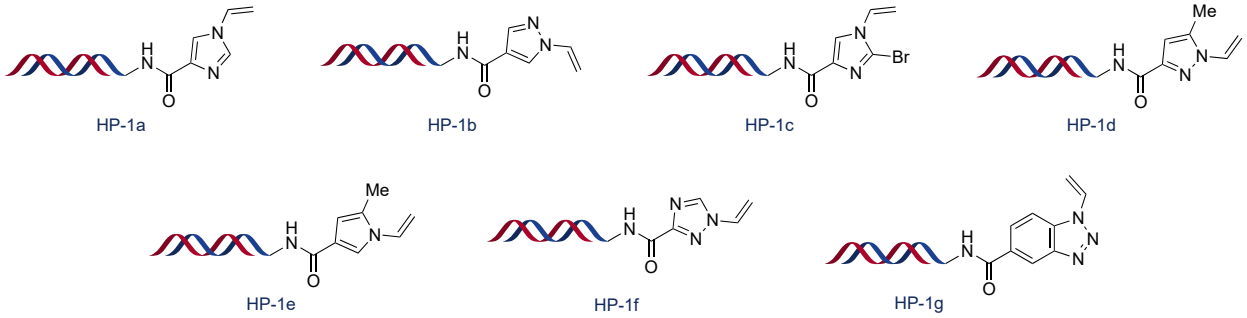
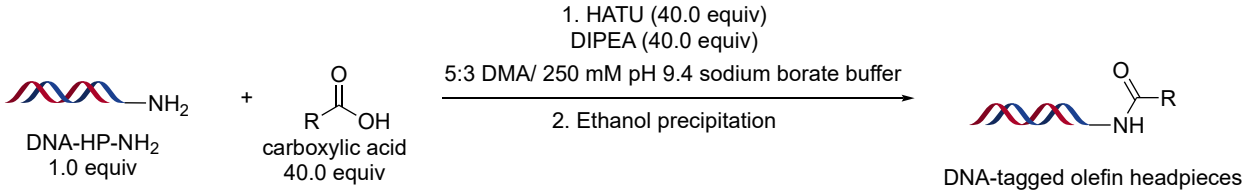


Figure S2. Molecular structures of the DNA-tagged olefin headpieces employed in the scope.

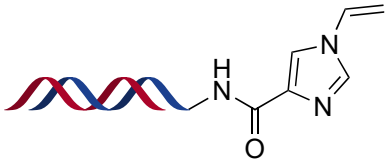
2.1.1 General Procedure I (GP-I): Acylation of DNA-HP-NH_2



Solutions of HATU (200 mM in DMA, 40.0 equiv), DIPEA (200 mM in DMA, 40.0 equiv), and the corresponding carboxylic acid (200 mM in DMA, 40.0 equiv) were cooled to 4 ºC. Subsequently, the solutions of carboxylic acid, DIPEA, and HATU were combined and vortexed

with a Fisherbrand™ Mini Vortex Mixer. The mixture was allowed to react at 4 ºC for 20 min. To this solution, the DNA-HP-NH_2 in sodium borate buffer (1 mM in 250 mM pH 9.4) was added and vigorously vortexed.[1] The reaction mixture was left to react at rt for 1 h, and after this period, worked-up by EtOH precipitation method as follow:

A solution of 5 M NaCl in H_2O (10% of the reaction volume) was added, followed by the addition of cold absolute EtOH (200 Proof, 2.5 reaction volumes). The mixture was vortexed and kept overnight at −20 °C in the freezer before being centrifuged a 4,000 rpm for 30 min at 4 ºC. The supernatant was discarded, and the obtained solid was dried under reduced pressure in the lyophilizer for 1 h. The DNA pellet was then re-dissolved in HyPure™ Molecular Biology Grade H_2O to a final concentration of 5 mM. The title compounds were analyzed via LC/MS and used for the visible light-mediated reactions without further purification.

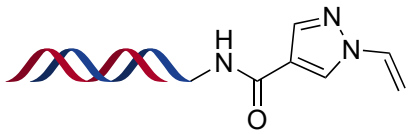


HP-1a

Chemical Formula: $C_{171}H_{240}N_{55}O_{107}P_{17}$
Exact Mass: 5302.0569
Molecular Weight: 5304.6330

Prepared according to **GP-I** using DNA-HP-NH_2 (0.013 g, 2.5 µmol, 1.0 equiv), HATU (0.037 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA), DIPEA (0.017 mL, 0.1 mmol, 40.0 equiv, 200 mM in DMA), and 1-vinyl-1*H*-imidazole-4-carboxylic acid (0.014 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA). **HP-1a** was obtained with > 95% conversion.

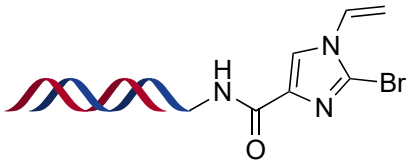
[1] Phelan, J. P.; Lang, S. B.; Sim, J.; Berrit, S.; Peat, A. J.; Billings, K. Molander, G. A. *J. Am. Chem. Soc.* 2019, **141**, 8, 3723-3732.



HP-1b

Chemical Formula: $C_{171}H_{240}N_{55}O_{107}P_{17}$
Exact Mass: 5302.0569
Molecular Weight: 5304.6330

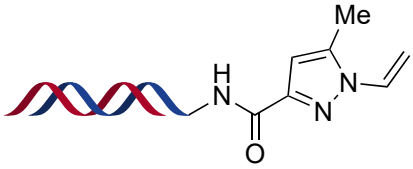
Prepared according to **GP-I** using DNA-HP-NH_2 (0.0259 g, 5.0 µmol, 1.0 equiv), HATU (0.076 g, 0.2 mmol, 40.0 equiv, 200 mM in DMA), DIPEA (0.035 mL, 0.2 mmol, 40.0 equiv, 200 mM in DMA), and 1-vinyl-1*H*-pyrazole-4-carboxylic acid (0.027 g, 0.2 mmol, 40.0 equiv, 200 mM in DMA). **HP-1b** was obtained with 93% conversion.



HP-1c

Chemical Formula: $C_{171}H_{239}BrN_{55}O_{107}P_{17}$
Exact Mass: 5379.9674
Molecular Weight: 5383.5290

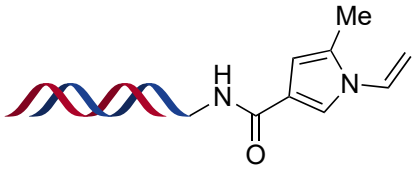
Prepared according to **GP-I** using DNA-HP-NH_2 (0.013 g, 2.5 µmol, 1.0 equiv), HATU (0.037 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA), DIPEA (0.017 mL, 0.1 mmol, 40.0 equiv, 200 mM in DMA), and 2-bromo-1-vinyl-1*H*-imidazole-4-carboxylic acid (0.021 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA). **HP-1c** was obtained with > 95% conversion.



HP-1d

Chemical Formula: $C_{172}H_{242}N_{55}O_{107}P_{17}$
Exact Mass: 5316.0725
Molecular Weight: 5318.6600

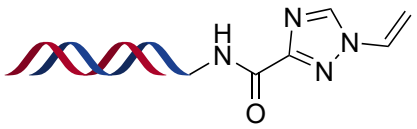
Prepared according to **GP-I** using DNA-HP-NH_2 (0.013 g, 2.5 µmol, 1.0 equiv), HATU (0.037 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA), DIPEA (0.017 mL, 0.1 mmol, 40.0 equiv, 200 mM in DMA), and 5-methyl-1-vinyl-1*H*-pyrazole-3-carboxylic acid (0.015 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA). **HP-1c** was obtained with > 95% conversion.



HP-1e

Chemical Formula: $C_{173}H_{243}N_{54}O_{107}P_{17}$
Exact Mass: 5315.0773
Molecular Weight: 5317.6720

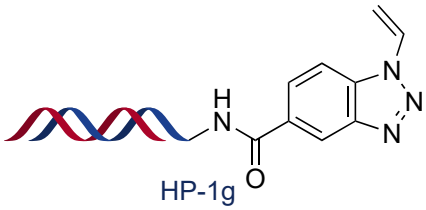
Prepared according to **GP-I** using DNA-HP-NH_2 (0.013 g, 2.5 µmol, 1.0 equiv), HATU (0.037 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA), DIPEA (0.017 mL, 0.1 mmol, 40.0 equiv, 200 mM in DMA), and 5-methyl-1-vinyl-1*H*-pyrrole-3-carboxylic acid (0.015 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA). **HP-1e** was obtained with > 95% conversion.



HP-1f

Chemical Formula: $C_{170}H_{239}N_{56}O_{107}P_{17}$
Exact Mass: 5303.0521
Molecular Weight: 5305.6210

Prepared according to **GP-I** using DNA-HP-NH_2 (0.013 g, 2.5 µmol, 1.0 equiv), HATU (0.037 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA), DIPEA (0.017 mL, 0.1 mmol, 40.0 equiv, 200 mM in DMA), and 1-vinyl-1*H*-imidazole-4-carboxylic acid (0.014 g, 0.1 mmol, 40.0 equiv, 200 mM in DMA). **HP-1f** was obtained with > 95% conversion.



HP-1g

Chemical Formula: $C_{174}H_{241}N_{56}O_{107}P_{17}$
Exact Mass: 5353.0678
Molecular Weight: 5355.6810

Prepared according to **GP-I** using DNA-HP-NH_2 (0.027 g, 5.3 µmol, 1.0 equiv), HATU (0.080 g, 0.2 mmol, 40.0 equiv, 200 mM in DMA), DIPEA (0.037 mL, 0.2 mmol, 40.0 equiv, 200 mM in DMA), and 1-vinyl-1*H*-benzo[*d*][1,2,3]triazole-5-carboxylic acid (0.040 g, 0.2 mmol, 40.0 equiv, 200 mM in DMA). **HP-1g** was obtained with > 95% conversion.

2.2 Synthesis of Small Molecules (*N*-heterocyclic carboxylic acids 1a-1g)

The *N*-heterocyclic carboxylic acids **1a-1g** employed in the scope of the reported transformation (**Figure S3**) were prepared according to the **General Procedure II**. All the *N*-heterocyclic esters starting materials (**1c'-1g'**) were received from GlaxoSmithKline, Inc. The carboxylic acids **1a** and **1b** were also received and use directly in the protocols.

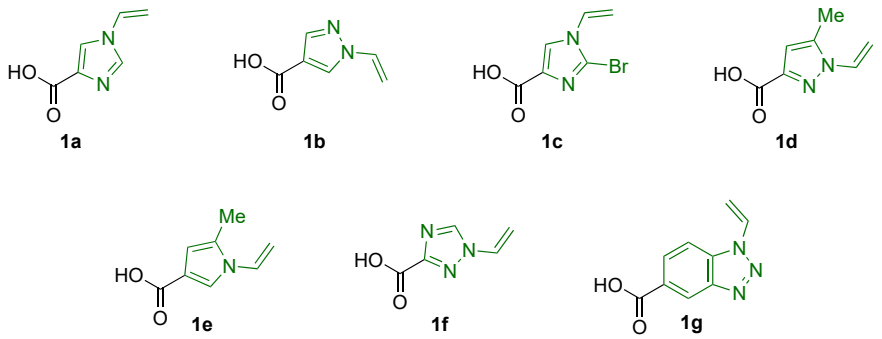
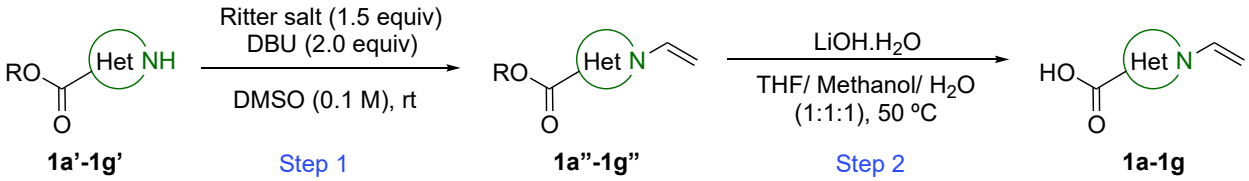
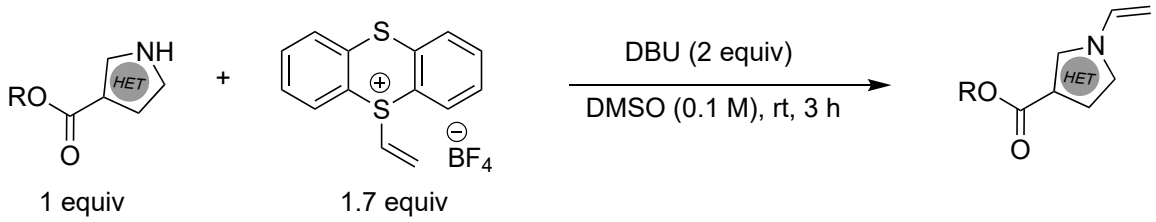


Figure S3. *N*-Heterocyclic carboxylic acids synthesized for the headpiece's preparation.

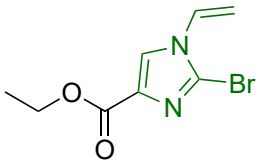
2.2.1 General Procedure II (GP-II): Preparation and Characterization of *N*-Vinylated Heterocycles



Step 1 – Vinylation of Alkyl Heterocycles



The vinylation of *N*-heterocycles was adapted from a reported procedure:[2] Under air, a vial was charged with the corresponding heterocyclic compound (1.0 equiv) and vinyl thianthrenium tetrafluoroborate (1.7 equiv). Then, DMSO (conc = 0.10 M) was added, followed by DBU (2.0 equiv), and the mixture was stirred at 25 °C for 3 h. The solvent was subsequently removed under reduced pressure, and the residue was purified by flash column chromatography to yield the corresponding product.



1c"

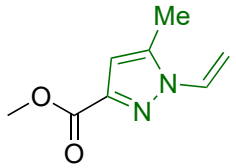
Ethyl 2-Bromo-1-vinyl-1H-imidazole-4-carboxylate (1c"): Prepared according to **GP-II (1)** using ethyl 2-bromo-1*H*-imidazole-4-carboxylate (0.100 g, 0.5 mmol, 1.0 equiv), vinyl thianthrenium tetrafluoroborate (0.28 g, 0.85 mmol, 1.7 equiv) and DBU (0.15 g, 0.15 mL, 1 mmol, 2.0 equiv) in DMSO (5 mL). Purification by flash column chromatography (EtOAC/hexane) afforded the pure title compound **1c"** as a yellow solid (0.039 g, 32%). **MP** (°C) 124 – 126.

^{1}H NMR (600 MHz, $CDCl_3$), δ (ppm) 7.86 (s, 1H), 6.92 (dd, J = 15.6, 8.8 Hz, 1H), 5.37 (dd, J = 15.7, 2.1 Hz, 1H), 5.09 (dd, J = 8.8, 2.1 Hz, 1H), 4.31 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$), δ (ppm) 161.6, 135.0, 128.7, 123.2, 120.7, 105.9, 61.1, 14.4.

FT-IR (cm^{-1}, neat, ATR) $\tilde{\nu}$ = 3136, 2982, 1728, 1644, 1552, 1458, 1382, 1364, 1311, 1290, 1222, 1196, 1133, 1094, 1025, 956, 904, 834, 762, 658.

HRMS (ESI) calcd for $C_8H_9N_2O_2Br$ $[M]^+$: 243.9847, found: 243.9828.



1d"

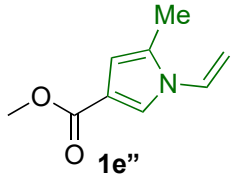
Methyl 5-Methyl-1-vinyl-1*H*-pyrazole-3-carboxylate (1d"): Prepared according to **GP-II (1)** using methyl 5-methyl-1*H*-pyrazole-3-carboxylate (0.70 g, 0.5 mmol, 1.0 equiv), vinyl thianthrenium tetrafluoroborate (0.28 g, 0.85 mmol, 1.7 equiv), and DBU (0.15 g, 0.15 mL, 1 mmol, 2.0 equiv) in DMSO (5 mL). Purification by flash column chromatography (EtOAC/hexane) afforded the pure title compound **1d"** as a clear sticky liquid (0.032 g, 39%).

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 6.99 (dd, *J* = 15.4, 8.9 Hz, 1H), 6.62 (s, 1H), 5.91 (d, *J* = 15.4 Hz, 1H), 5.04 (d, *J* = 8.9 Hz, 1H), 3.93 (s, 3H), 2.37 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$), δ (ppm) 162.8, 143.6, 139.9, 129.3, 109.2, 104.7, 52.1, 11.2.

FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 3115, 2924, 1733, 1651, 1495, 1453, 1326, 1270, 1222, 1183, 1019, 960, 904, 845, 671.

HRMS (ESI) calcd for $C_8H_{10}N_2O_2$ $[M]^+$: 166.0742, found 166.0745.



1e"

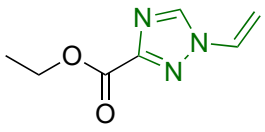
Methyl 5-Methyl-1-vinyl-1*H*-pyrazole-3-carboxylate (1e"): Prepared according to **GP-II (1)** using methyl 5-methyl-1*H*-pyrrole-3-carboxylate (0.70 g, 0.5 mmol, 1.0 equiv), vinyl thianthrenium tetrafluoroborate (0.28 g, 0.85 mmol, 1.7 equiv) and DBU (0.15 g, 0.15 mL, 1 mmol, 2.0 equiv) in DMSO (5 mL). Purification by flash column chromatography (EtOAc/hexane) afforded the pure title compound **1e"** as a yellow sticky liquid (0.030 g, 36%).

^{1}H NMR (600 MHz, $CDCl_3$), δ (ppm) 7.55 (d, *J* = 1.8 Hz, 1H), 6.83 (dd, *J* = 15.7, 8.8 Hz, 1H), 6.31 (s, 1H), 5.24 (dd, *J* = 15.7, 1.4 Hz, 1H), 4.84 (dd, *J* = 8.9, 1.4 Hz, 1H), 3.80 (s, 3H), 2.24 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$), δ (ppm) 165.1, 130.1, 121.4, 116.3, 108.9, 101.4, 51.1, 12.0.

FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 2925, 1710, 1219.

HRMS (ESI) calcd for $C_9H_{11}NO_2$ $[M]^+$: 165.0790, found 165.0788.



1f"

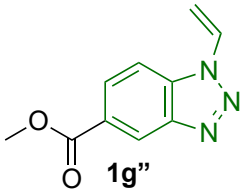
Ethyl 1-Vinyl-1H-1,2,4-triazole-3-carboxylate (1f"): Prepared according to **GP-II (1)** using methyl ethyl 1*H*-1,2,4-triazole-3-carboxylate (0.71 g, 0.5 mmol, 1.0 equiv), vinyl thianthrenium tetrafluoroborate (0.28 g, 0.85 mmol, 1.7 equiv), and DBU (0.15 g, 0.15 mL, 1 mmol, 2.0 equiv) in DMSO (5 mL). Purification by flash column chromatography (using only EtOAc) afforded the pure title compound **1f"** as a white solid (0.052 g, 62%). **MP** (°C) 66 – 68.

^{1}H NMR (600 MHz, $CDCl_3$), δ (ppm) 8.28 (s, 1H), 7.06 (dd, *J* = 15.6, 8.8 Hz, 1H), 6.02 – 5.70 (m, 1H), 5.19 (d, *J* = 8.8 Hz, 1H), 4.44 (q, *J* = 7.1 Hz, 2H), 1.38 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$), δ (ppm) 159.5, 155.3, 142.9, 129.1, 106.9, 62.2, 14.2.

FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 1730, 1651, 1495, 1470, 1451, 1391, 1325, 1270, 1218, 1180, 1162, 1095, 1041, 1018, 959, 903, 867, 845, 694, 671.

HRMS (ESI) calcd for $C_7H_{10}N_3O_2$ [M+H]: 168.0773, found 168.0775.



1g"

Methyl 1-Vinyl-1*H*-benzo[*d*][1,2,3]triazole-5-carboxylate (1g"): Prepared according to **GP-II (1)** using methyl 1*H*-benzo[*d*][1,2,3]triazole-5-carboxylate (0.89 g, 0.5 mmol, 1.0 equiv), vinyl thianthrenium tetrafluoroborate (0.28 g, 0.85 mmol, 1.7 equiv), and DBU (0.15 g, 0.15 mL, 1

mmol, 2.0 equiv) in DMSO (5 mL). Purification by flash column chromatography (using only EtOAc) afforded the pure title compound **1g"** as a white solid (0.052 g, 51%). **MP** (°C) 100 – 102.

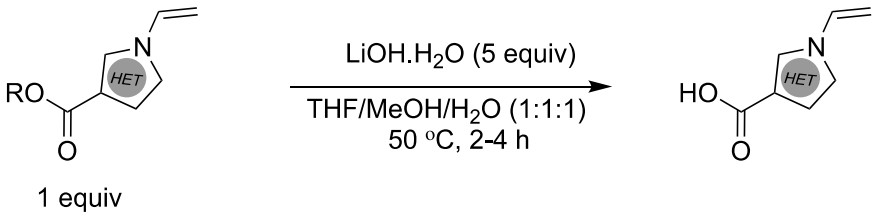
^{1}H NMR (600 MHz, $CDCl_3$), δ (ppm) δ 8.64 (s, 1H), 8.03 (d, *J* = 4.4 Hz, 1H), 7.91 – 7.86 (m, 1H), 7.59 (ddd, *J* = 14.8, 7.3, 3.2 Hz, 1H), 6.44 (dd, *J* = 15.6, 2.5 Hz, 1H), 5.49 – 5.35 (m, 1H), 3.98 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$), δ (ppm) 166.6, 146.4, 144.1, 134.2, 129.2, 127.3, 122.0, 118.3, 109.5, 52.5.

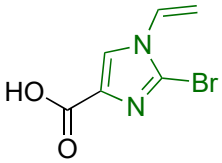
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 1720, 1709, 1461, 1436, 1361, 1321, 1288, 1267, 1235, 1219, 1181, 1137, 1082, 1045, 955, 911, 832, 811, 773, 748.

HRMS (ESI) calcd for $C_{10}H_9N_3O_2$ $[M]^+$: 203.0695, found 203.0706.

Step 2 – Hydrolysis of Alkyl *N*-Vinylated Heterocycles



The ester hydrolysis of vinylated *N*-heterocycles was carried out according to a modified literature procedure.[4] Under air, a vial was charged with the corresponding vinylated *N*-heterocyclic compound (1.0 equiv). A solution of THF/MeOH/H_2O (1:1:1) was added followed by the addition of LiOH.H_2O (5.0 equiv), and the reaction was stirred at 50 °C until completion (monitored by TLC). The reaction mixture is then evaporated *in vacuo*, and the crude is taken up with H_2O, acidified with 1 N HCl to pH = ~1, and extracted 3 times with EtOAc. The combined organic layers were evaporated, and the crude product was used directly in the next step.



1c

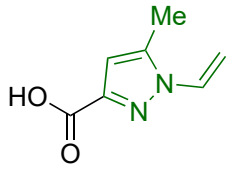
2-Bromo-1H-imidazole-4-carboxylic Acid (1c): Prepared according to **GP-II (2)** using **1c"** (0.025 g, 0.10 mmol, 1.0 equiv), LiOH•H_2O (0.021 g, 0.50 mmol, 5.0 equiv) in a mixture of THF/MeOH/H_2O (1:1:1, 3 mL). The pure title compound **1c** was obtained as a yellow solid (0.018 g, 83%). **MP** (°C) 124 – 126.

^{1}H NMR (600 MHz, CD_3CN) δ 8.11 (s, 1H), 7.29 (br, 1H), 7.02 (dd, *J* = 15.6, 8.9 Hz, 1H), 5.59 (dd, *J* = 15.5, 2.0 Hz, 1H), 5.18 (dd, *J* = 8.8, 1.9 Hz, 1H).

^{13}C NMR (151 MHz, CD_3CN) δ 162.6, 134.7, 129.8, 125.5, 121.4, 107.5.

FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 2923, 1709, 1458, 1144, 1025, 904, 747.

HRMS (ESI) calcd for $C_6H_5N_2O_2Br$ [M+]: 216.9613, found 216.9630.



1d

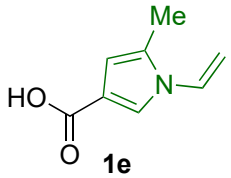
5-Methyl-1-vinyl-1*H*-pyrazole-3-carboxylic Acid (1d): Prepared according to **GP-II (2)** using **1d"** (0.025 g, 0.15 mmol, 1.0 equiv), LiOH•H_2O (0.032 g, 0.75 mmol, 5.0 equiv) in a mixture of THF/MeOH/H_2O (1:1:1, 3 mL). The pure title compound **1d** was obtained as a white solid (0.016 g, 70%). **MP** (°C) 129 – 131.

^{1}H NMR (400 MHz, CD_3CN), δ (ppm) 12.22 (s, 1H), 6.70 (dd, *J* = 15.2, 8.7 Hz, 1H), 6.01 (s, 1H), 5.14 (d, *J* = 15.2 Hz, 1H), 4.46 (d, *J* = 8.7 Hz, 1H), 1.79 (s, 3H).

^{13}C NMR (101 MHz, CD_3CN), δ (ppm) 168.3, 149.2, 145.7, 135.5, 113.9, 108.3, 15.7.

FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 3110, 1701, 1491, 1290, 901, 844, 630.

HRMS (ESI) calcd for $C_7H_8N_2O$ [M+]: 152.0586, found 152.0595.

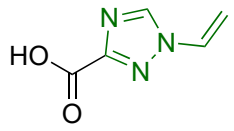


1e

5-Methyl-1-vinyl-1*H*-pyrrole-3-carboxylic Acid (1e): Prepared according to **GP-II (2)** using **1e"** (0.025 g, 0.15 mmol, 1.0 equiv), LiOH•H_2O (0.032 g, 0.75 mmol, 5.0 equiv) in a mixture of THF/MeOH/H_2O (1:1:1, 3 mL). The pure title compound **1e** was obtained as a brown solid (0.017 g, 74%). **MP** (°C) 80 – 82.

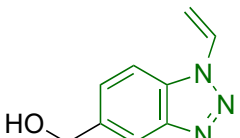
^{1}H NMR (600 MHz, CD_3CN), δ (ppm) 9.18 (br, 1H), 7.65 (d, *J* = 1.8 Hz, 1H), 6.99 (dd, *J* = 15.6, 8.9 Hz, 1H), 6.27 (s, 1H), 5.38 (dd, *J* = 15.6, 1.4 Hz, 1H), 4.90 (dd, *J* = 8.9, 1.4 Hz, 1H), 2.26 (s, 3H).

^{13}C NMR (151 MHz, CD_3CN), δ (ppm) 165.8, 131.1, 130.8, 122.3, 116.3, 109.3, 101.7, 11.6.
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 2924, 2854, 1671, 1643, 1530, 1454, 1404, 1326, 1256, 1197, 971, 790, 748, 564.
HRMS (ESI) calcd for $C_8H_9NO_2$ [M+]: 151.0633, found 151.0640.



1f

1-Vinyl-1*H*-1,2,4-triazole-3-carboxylic Acid (1f): Prepared according to **GP-II (2)** using **1f"** (0.034 g, 0.20 mmol, 1.0 equiv), LiOH•H_2O (0.042 g, 1.0 mmol, 5.0 equiv) in a mixture of THF/MeOH/H_2O (1:1:1, 3 mL). The pure title compound **1f** was obtained as a white solid (0.021 g, 75%). **MP** (°C) 192 – 194.
^{1}H NMR (600 MHz, DMSO-d_6), δ (ppm) 13.47 (s, 1H), 8.95 (s, 1H), 7.42 (dd, *J* = 15.5, 8.8 Hz, 1H), 5.85 (dd, *J* = 15.5, 1.3 Hz, 1H), 5.23 (dd, *J* = 8.8, 1.3 Hz, 1H).
^{13}C NMR (151 MHz, DMSO-d_6), δ (ppm) 161.1, 155.6, 145.3, 130.4, 106.0.
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 2923, 2800, 1790, 1450, 1255, 950, 910.
HRMS (ESI) calcd for $C_5H_5N_3O_2$ [M+]: 140.0460, found 140.0459.



1g

1-Vinyl-1H-benzo[d][1,2,3]triazole-5-carboxylic Acid (1g): Prepared according to **GP-II (2)** using **1g"** (0.040 g, 0.20 mmol, 1.0 equiv), LiOH•H_2O (0.042 g, 1.0 mmol, 5.0 equiv) in a mixture of THF/MeOH/H_2O (1:1:1, 3 mL). The pure title compound **1g** was obtained as a white solid (0.024 g, 63%). **MP** (°C) 206 – 208.

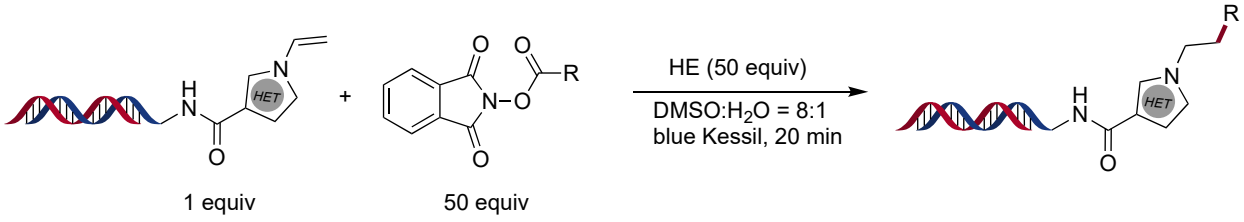
^{1}H NMR (600 MHz, d_6-DMSO), δ (ppm) 13.30 (s, 1H), 8.56 (s, 1H), 8.13 – 7.95 (m, 2H), 7.86 (dd, J = 15.4, 8.6 Hz, 1H), 6.39 (d, J = 15.4 Hz, 1H), 5.60 (d, J = 8.6 Hz, 1H).

^{13}C NMR (151 MHz, $CDCl_3$), δ (ppm) 167.3, 146.2, 144.0, 134.9, 130.5, 127.7, 121.8, 118.8, 110.8.

FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 3469, 2933, 1709, 1626, 1551, 1499, 1412, 1395, 1357, 1263, 1187, 1058, 1012, 960, 849, 778, 754, 669, 590, 473.

HRMS (ESI) calcd for $C_9H_7N_3O_2$ [M+]: 227.0884, found: 227.0881.

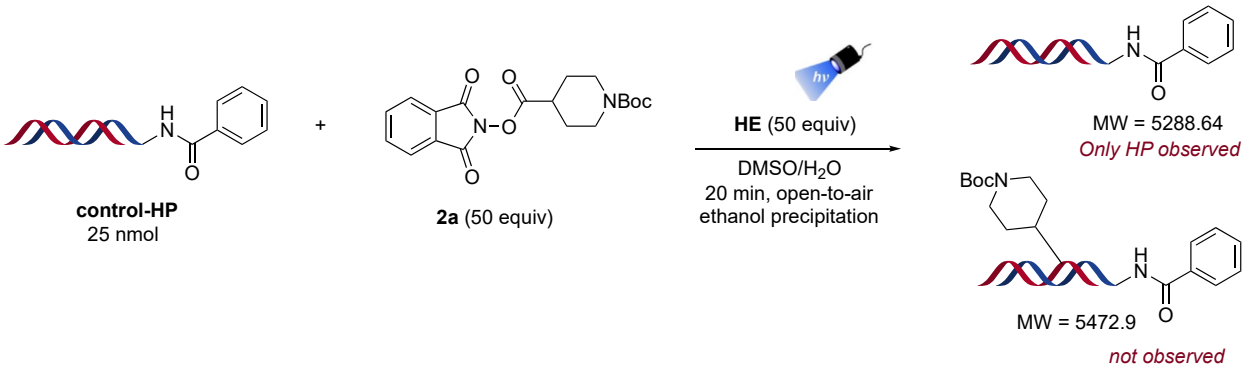
3 General Procedure III (GP-III): Hydroalkylation of *N*-Vinyl Heterocycles

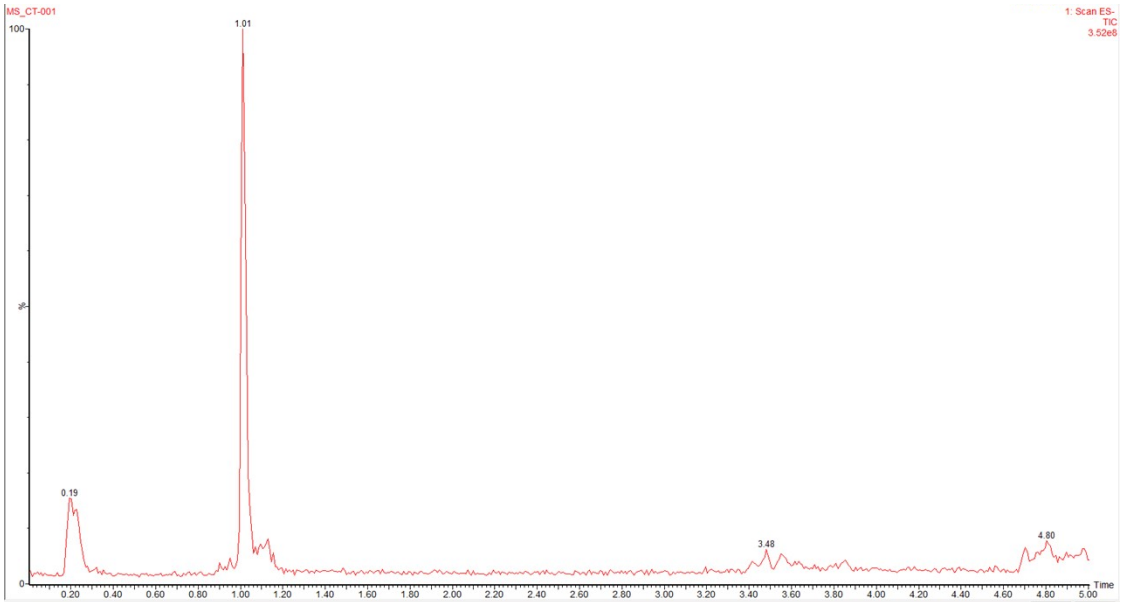


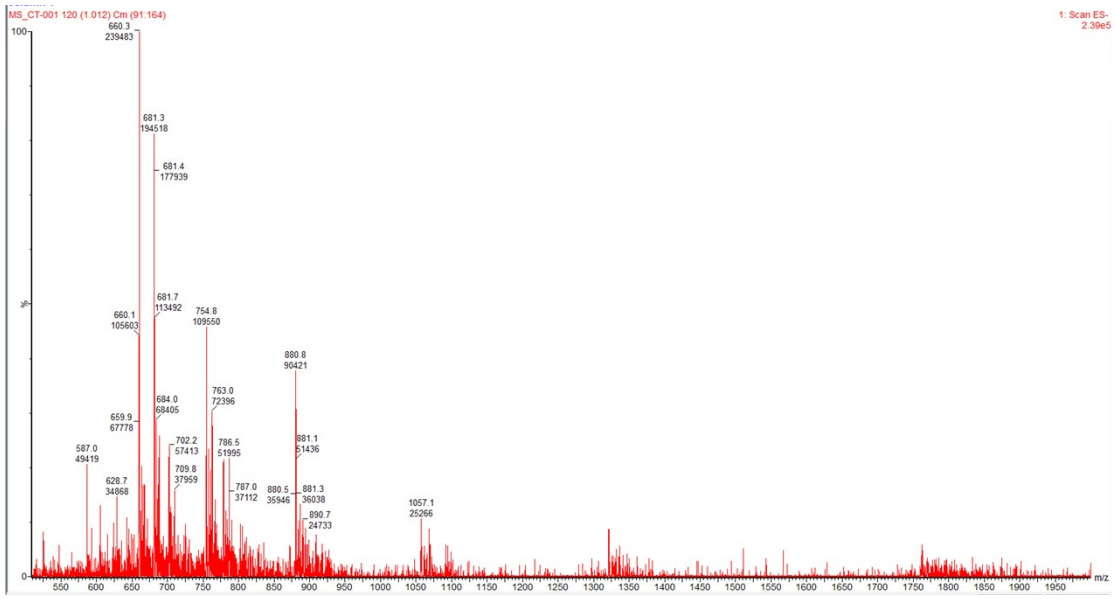
To an Eppendorf PCR microcentrifuge tube were added RAE (30 µL of a 41.7 nmol/µL soln in DMSO, 1250 nmol, 50 equiv), Hantzch Ester (HE) (10 µL of a 125 nmol/µL soln in DMSO, 1250 nmol, 50 equiv), and the DNA-tethered *N*-vinyl heterocycle (5 µL of a 5 nmol/µL soln in H_2O, 25 nmol, 1.0 equiv). The PCR tube was capped, and the reaction mixture was vortexed and placed at 1.5 inches from the irradiation source (Kessil H150-456 nm blue lamp) for 20 min. For the work-up, a solution of 5 M NaCl in HyPure™ Molecular Biology Grade H_2O (4.5 µL) was added, followed by the addition of cold EtOH (160 µL). The mixture was left at −20 °C for 1 h and then centrifuged at 6,000 rpm for 15 min. The supernatant was discarded, and the DNA pellet was re-dissolved in H_2O (150 µL), and analyzed via LC/MS.

4 Control Experiment

The control experiment was performed following the **GP-III** with a **control-HP** derived from benzoic acid. As result, no product from a side reaction with the RAE **2g** was observed, and only the control-HP was recovered. This result indicates that there is no evidence of reaction on the structure of the DNA- tag, and the only reactive site toward the hydroalkylation reaction is the attached olefin.







5 DNA Damage Assessment

qPCR, PCR, and Sequencing

4-Cycle tag mimic sequence

4-Cycle tag synthesis (pyrazole)

The top and bottom strands (purchased from IDT as lyophilized powders) of a control 4-cycle tag were annealed by combining 562.5 nmol of each strand (2 mM in H_2O), heating to 95 °C for 5 min, then cooling to rt. The annealed tag solution (1.2 equiv) was then added to the pyrazole headpiece (250 µL, 2 mM in H_2O), followed by 200 µL 10x T4 ligation buffer, 4 mL of H_2O, and 20 µL T4 DNA ligase purchased from Syngene. The ligation solution was vortexed and let sit at rt overnight. LCMS was taken to confirm full conversion to desired product. The ligation was precipitated for 30 min at -80 °C following addition of 500 µL of 5 M NaCl (aq) and 12 mL of cold EtOH. The precipitated solution was then centrifuged at 3,500 rpm at 4 °C for 30 min, and the solvent was decanted to afford the DNA pellet, which was dried on a lyophilizer overnight. The crude pellet was resuspended in 1 mL of H_2O and purified by HPLC (Column: Gemini C18, 5 µm, 21.2x100 mm; Gradient: 5 to 50% B in 25 min, 20 mL/min; UV at 260 nm; Solvent A: 50 mM TEAA, pH 7.5; Solvent B: 1% H_2O in MeCN) to afford the desired product. The lyophilized product was analyzed by optical density using a composite extinction coefficient of 1176468 L/(mol-cm) to determine isolated yield (142 nmol, 28.45%). LCMS calcd: 34,378, found: 34,379.

Closing primer ligation on reacted material

Top Strand: 5'-/5Phos/ACG ATG CCC GGT CTA CNN NNN NNN NNN NCT GAT GGC GCG AGG GAG GC3'

Bottom Strand: 5'-GTA GAC CGG GCA TCG TAA-3'

The exemplar reaction samples received from UPenn were transferred to PCR vials, frozen, and lyophilized. Once dry, the resulting pellet was redissolved in H_2O to obtain an OD. 10 nmol (1 mM in H_2O) of the sample was transferred to another PCR tube, and to that was added 0.5 µL of ligase, followed by 5 µL of 10x T4 ligation buffer, 12.5 µL of annealed closing tag (1.25 equiv), and 24.5 µL of H_2O. The vials were vortexed and left at rt overnight. LCMS was taken but still

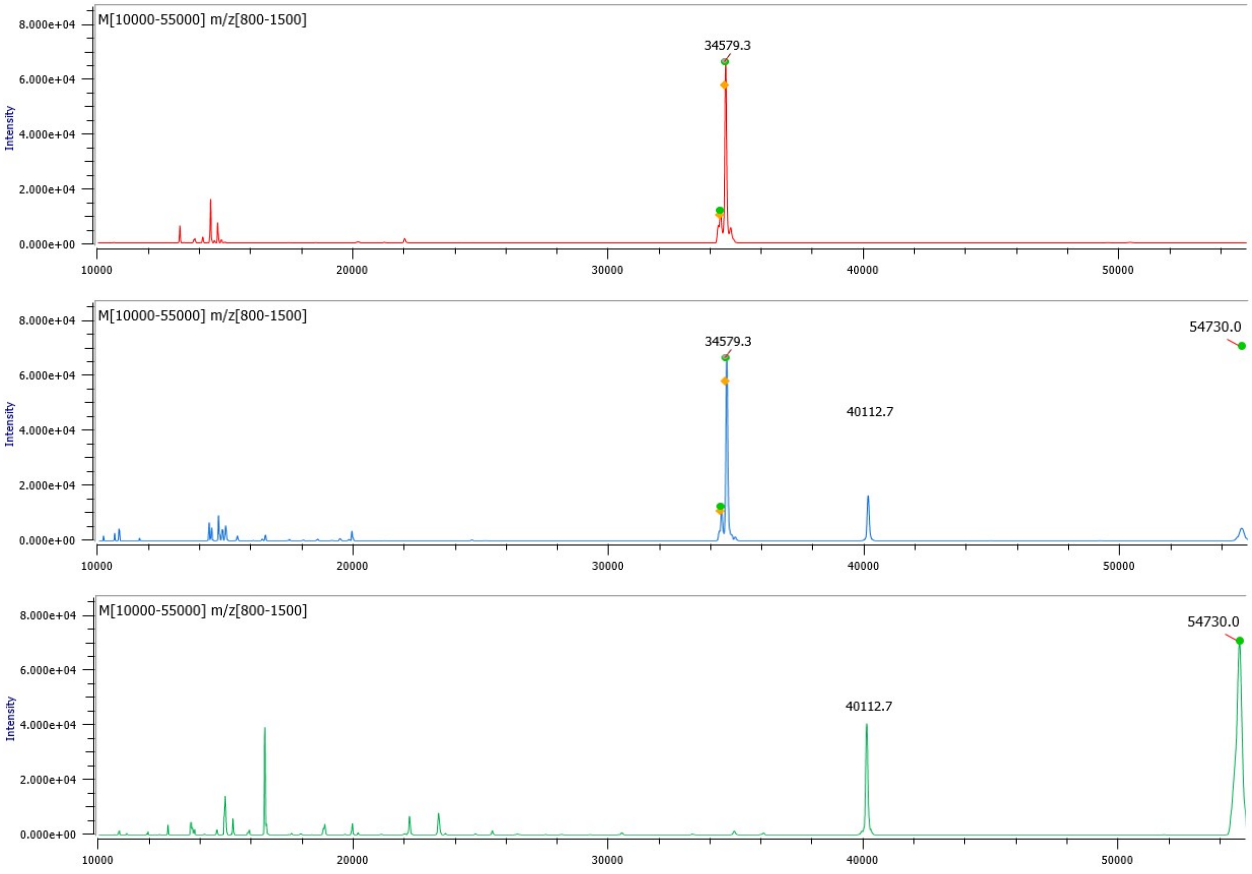
showed some starting material and minimal closing tag ligation product. An additional 1 µL of ligase, 12.5 µL of annealed closing tag, 10 µL of 10x T4 ligation buffer, and 20 µL of H_2O was added to each vial and again allowed to continue at rt overnight. LCMS was taken suggesting closing tag ligation had gone to completion.

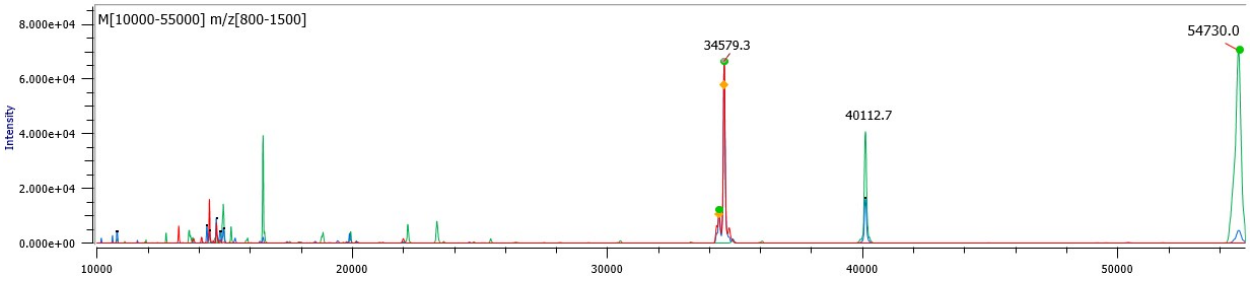
Closing Tag Ligation Sample A

Red: 4 Cycle Starting Material

Blue: Closing Tag Ligation Overnight

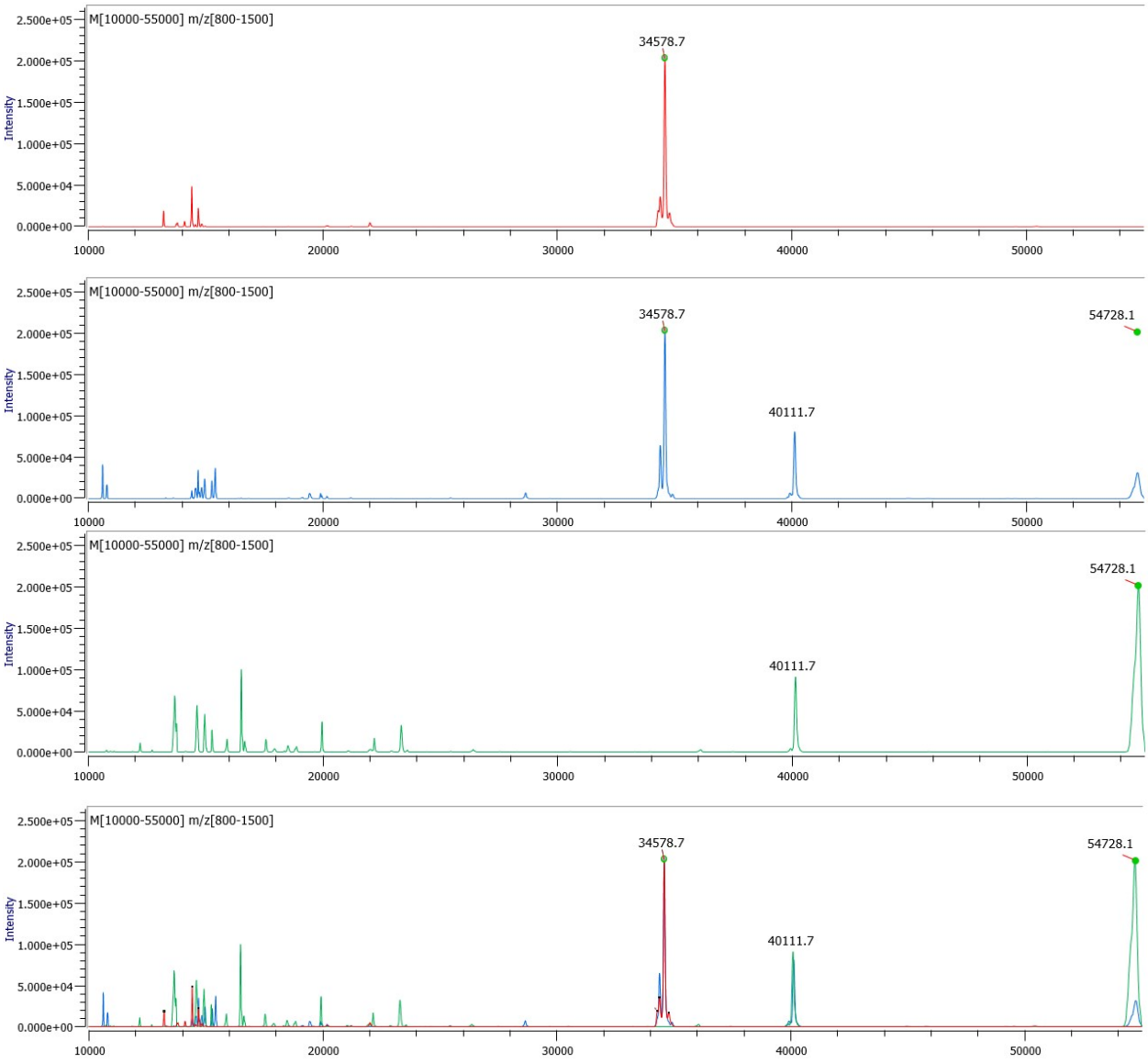
Green: Closing Tag Ligation Push Overnight





Closing Tag Ligation Sample B

Red: 4 Cycle Starting Material
Blue: Closing Tag Ligation Overnight
Green: Closing Tag Ligation Push Overnight



qPCR

qFor: 5'-GCT ACC TCT GAC TCC CAA ATC GAT GT -3'
qRev: 5'-ATA TTA GCC TCC CTC GCG CCA TCA -3'

Quantitative PCR was performed on a Roche LightCycler 480 II PCR system with SYBR Green I as the detection dye. A bulk master mix solution was prepared by combining 1 mL of SYBR green, 60 µL of 10 µM PCR primer 565 Cla, 60 µL of 10 µM PCR primer 454 short, and 680 µL of H_2O. To 2 µL of sample was then added 18 µL of master mix. Samples were subjected to qPCR:

Stage	Temperature/Time	Number of Cycle
HotStart	95 °C/5 min	1
Amplification	95 °C/10 sec 55 °C/15 sec 72 °C/15 sec	40
Melt	95 °C/1 sec 70 °C/1 sec 95 °C	1
Cool	45 °C/30 sec	0

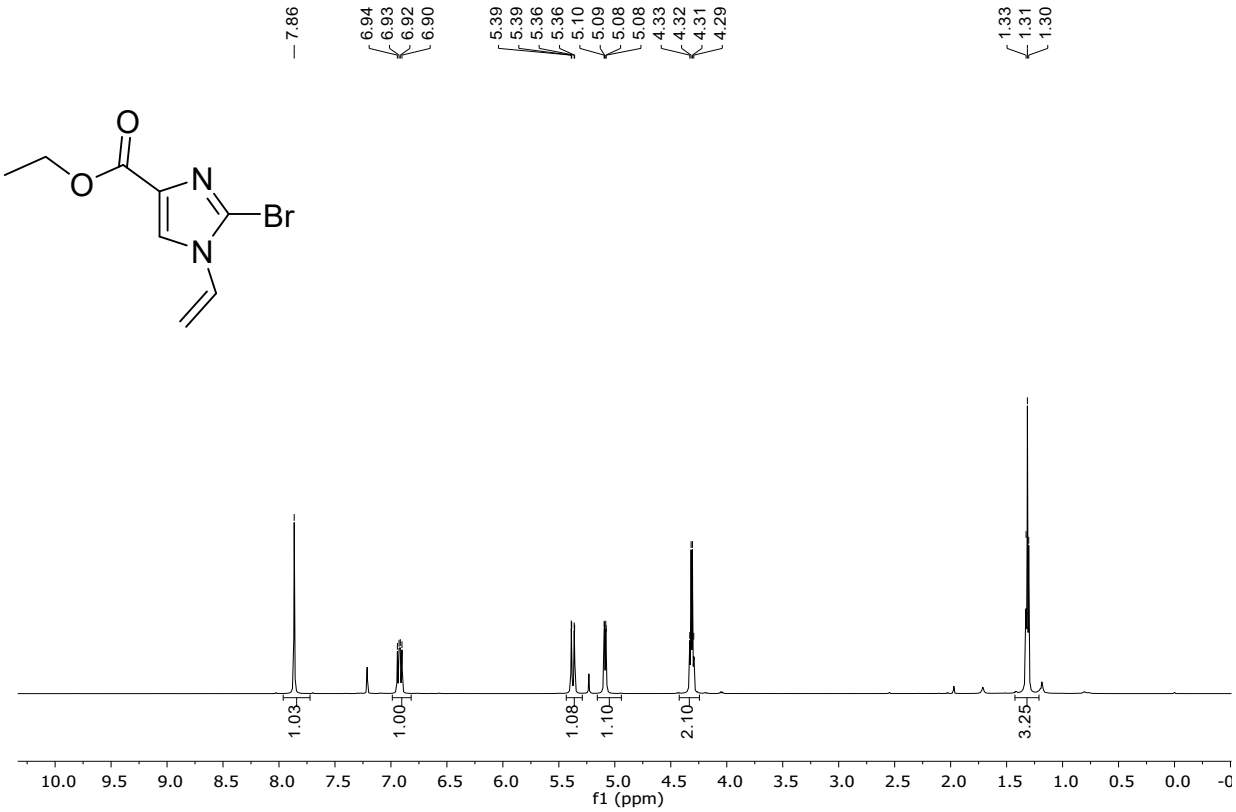
Samples were then analyzed using the 2nd derivative maximum standard protocol on the instrument to determine how many molecules were present per µL sample. Samples achieved acceptable consistency, suggesting that the conditions developed are not impacting the amount of amplifiable DNA present in a significant way.

qPCR Raw Data

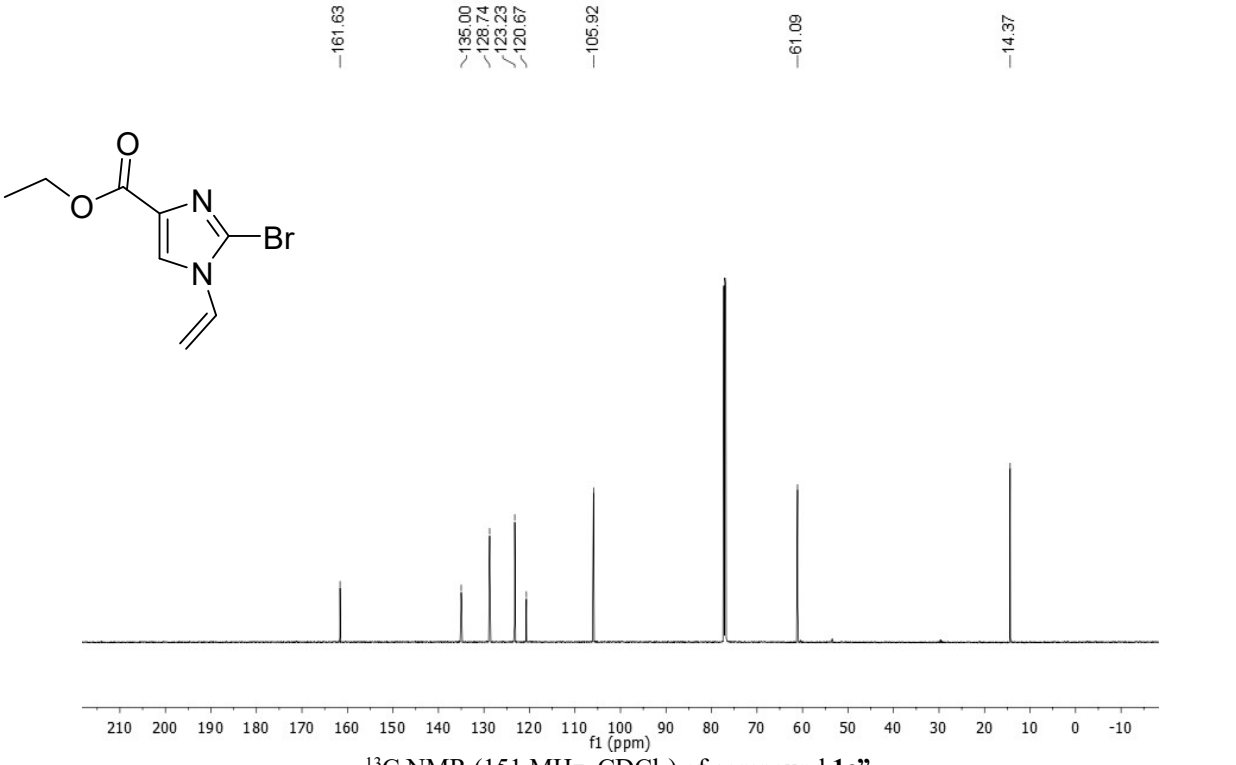
Include	Pos	Name	Cp	Concentration	Standard
TRUE	A1	Sample 2	7.86	1.23E+09	1000000000
TRUE	A2	Sample 2	8.03	1.10E+09	1000000000
TRUE	B1	Sample 14	11.67	9.94E+07	100000000

TRUE	B2	Sample 14	11.63	1.02E+08	100000000
TRUE	B3	Sample 15	12.57	5.47E+07	0
TRUE	B4	Sample 16	13.03	4.04E+07	0
TRUE	B5	Sample 17	13.75	2.52E+07	0
TRUE	B6	Sample 18	13.84	2.37E+07	0
TRUE	C1	Sample 26	15.55	7.66E+06	10000000
TRUE	C2	Sample 26	15.38	8.57E+06	10000000
TRUE	C3	Sample 27	16.4	4.36E+06	0
TRUE	C4	Sample 28	16.43	4.27E+06	0
TRUE	C5	Sample 29	17.25	2.49E+06	0
TRUE	C6	Sample 30	17.32	2.38E+06	0
TRUE	D1	Sample 38	18.74	9.34E+05	1000000
TRUE	D2	Sample 38	18.84	8.76E+05	1000000
TRUE	D3	Sample 39	19.91	4.32E+05	0
TRUE	D4	Sample 40	19.58	5.36E+05	0
TRUE	D5	Sample 41	20.51	2.90E+05	0
TRUE	D6	Sample 42	20.63	2.69E+05	0
TRUE	E1	Sample 50	22.09	1.03E+05	100000
TRUE	E2	Sample 50	21.97	1.11E+05	100000
TRUE	F1	Sample 62	25.58	1.02E+04	10000
TRUE	F2	Sample 62	25.36	1.18E+04	10000
TRUE	G1	Sample 73	28.66	1.34E+03	0
TRUE	G2	Sample 74	28.55	1.44E+03	0

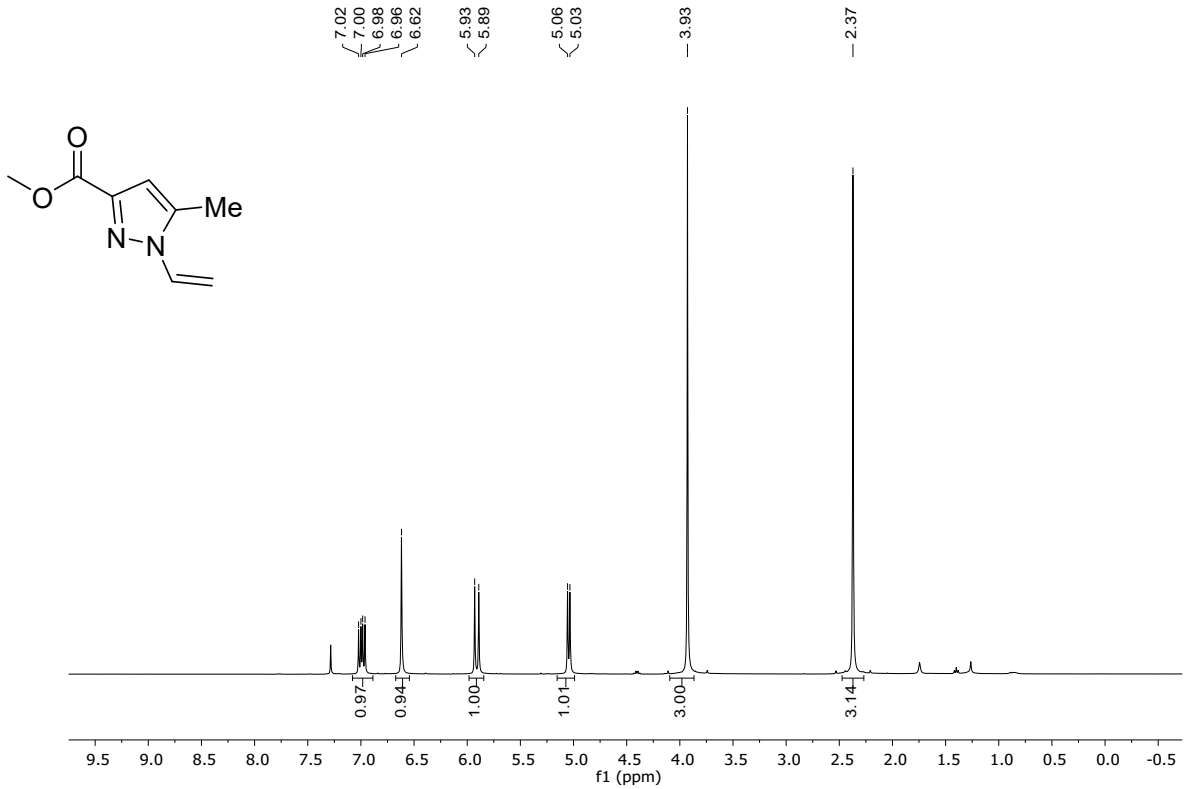
6 NMR Spectra: Small Molecules



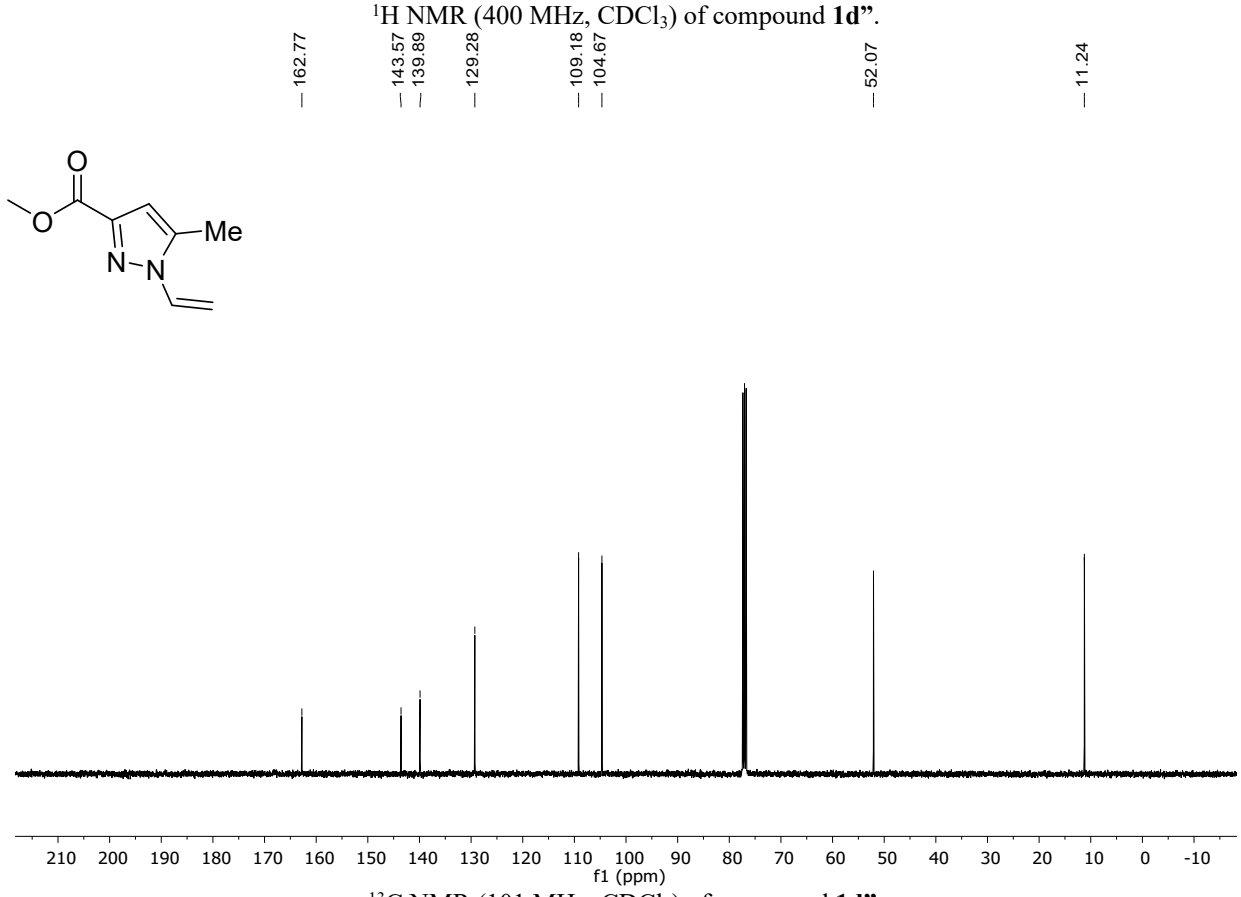
^{1}H NMR (600 MHz, $CDCl_3$) of compound **1c”**.



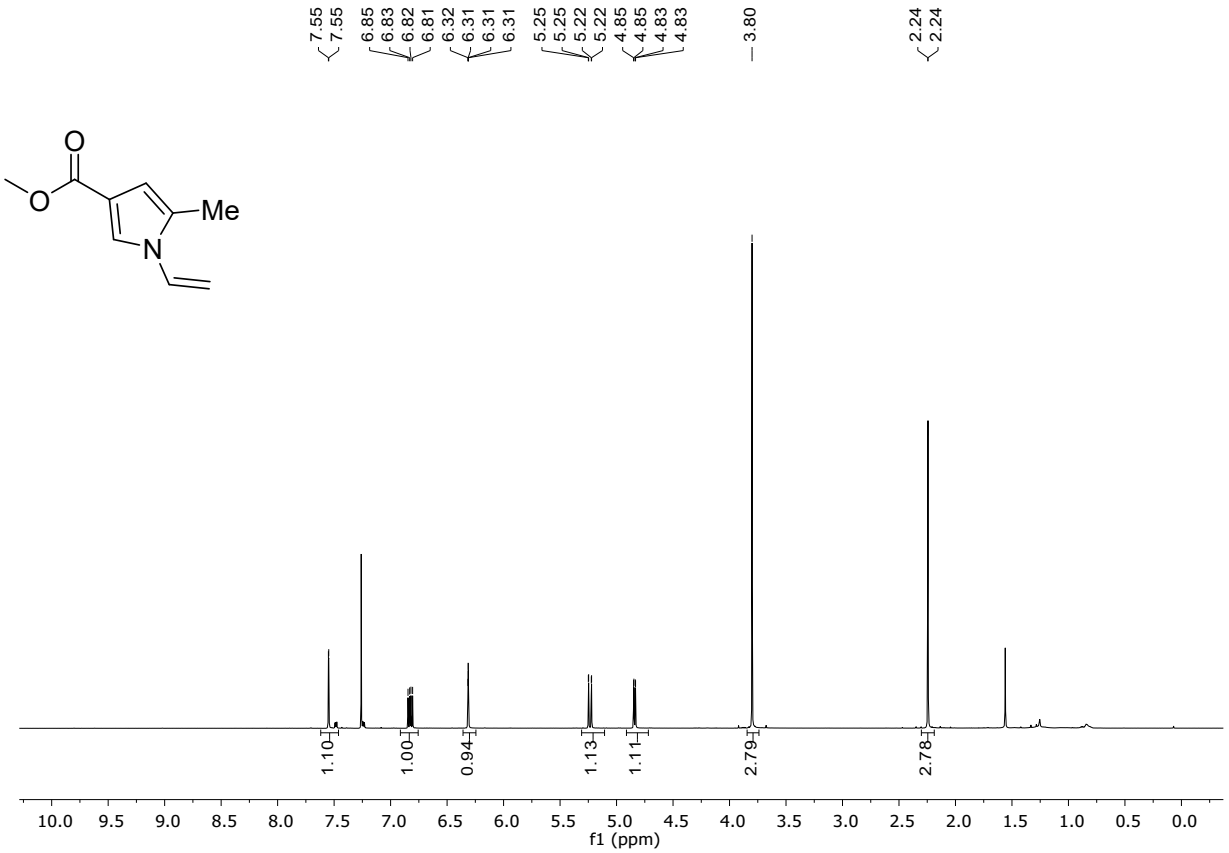
^{13}C NMR (151 MHz, $CDCl_3$) of compound **1c”.**



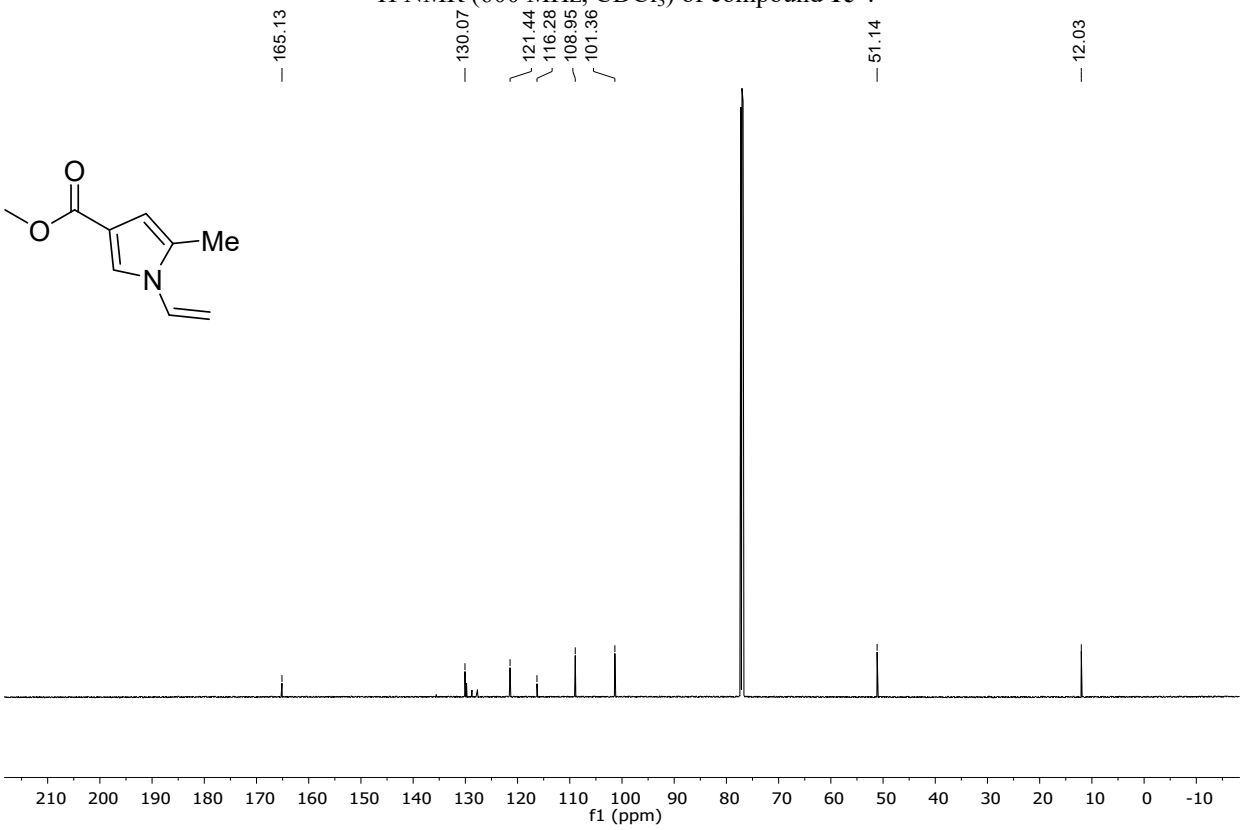
^{1}H NMR (400 MHz, $CDCl_3$) of compound **1d"**.



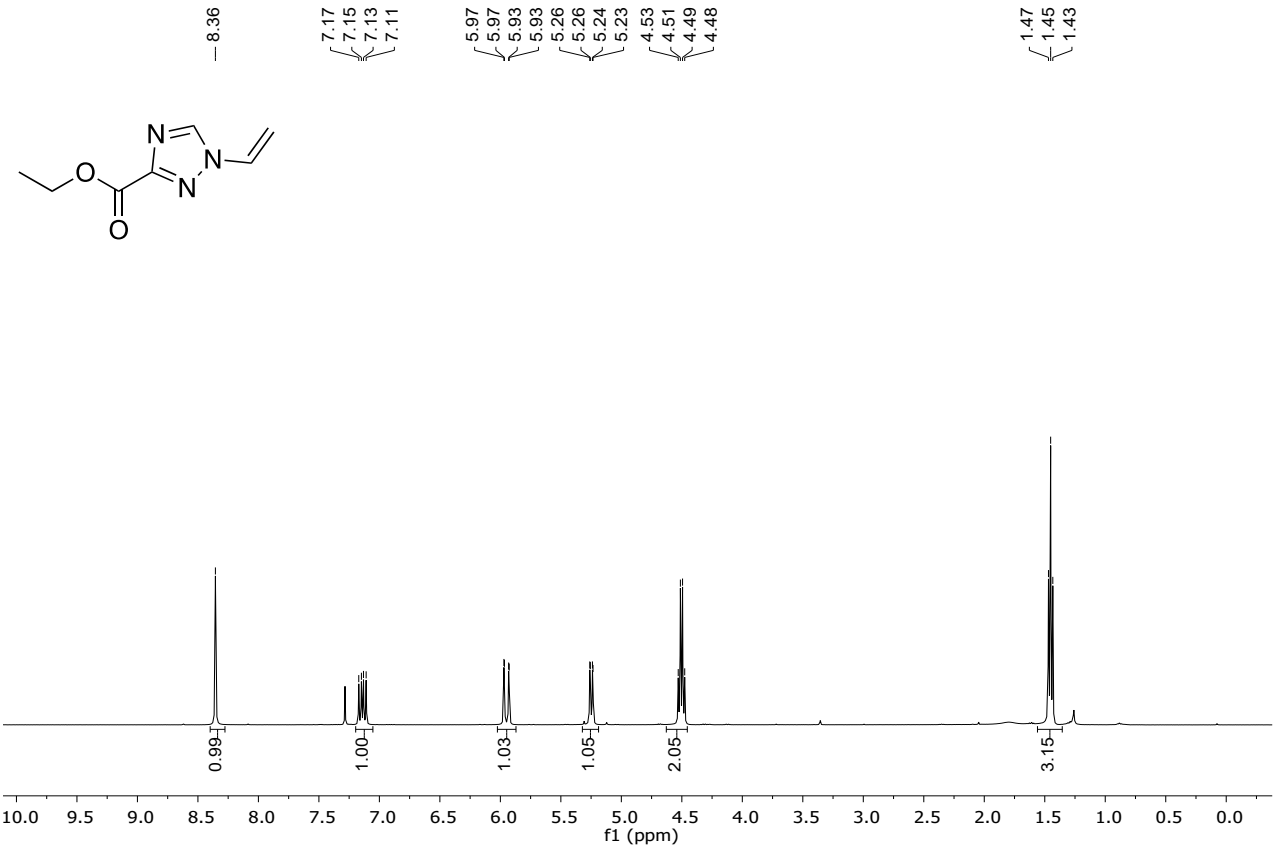
^{13}C NMR (101 MHz, $CDCl_3$) of compound **1d".**



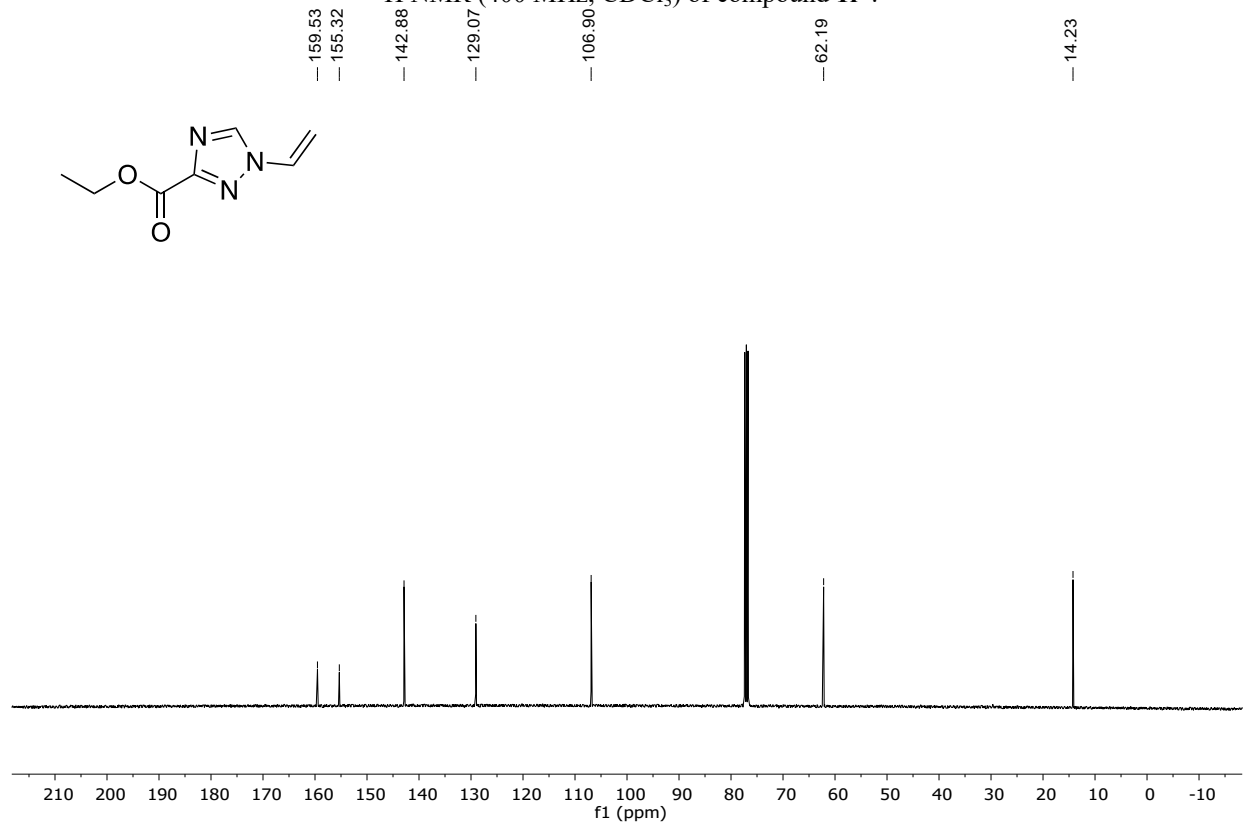
^{1}H NMR (600 MHz, $CDCl_3$) of compound **1e”**.



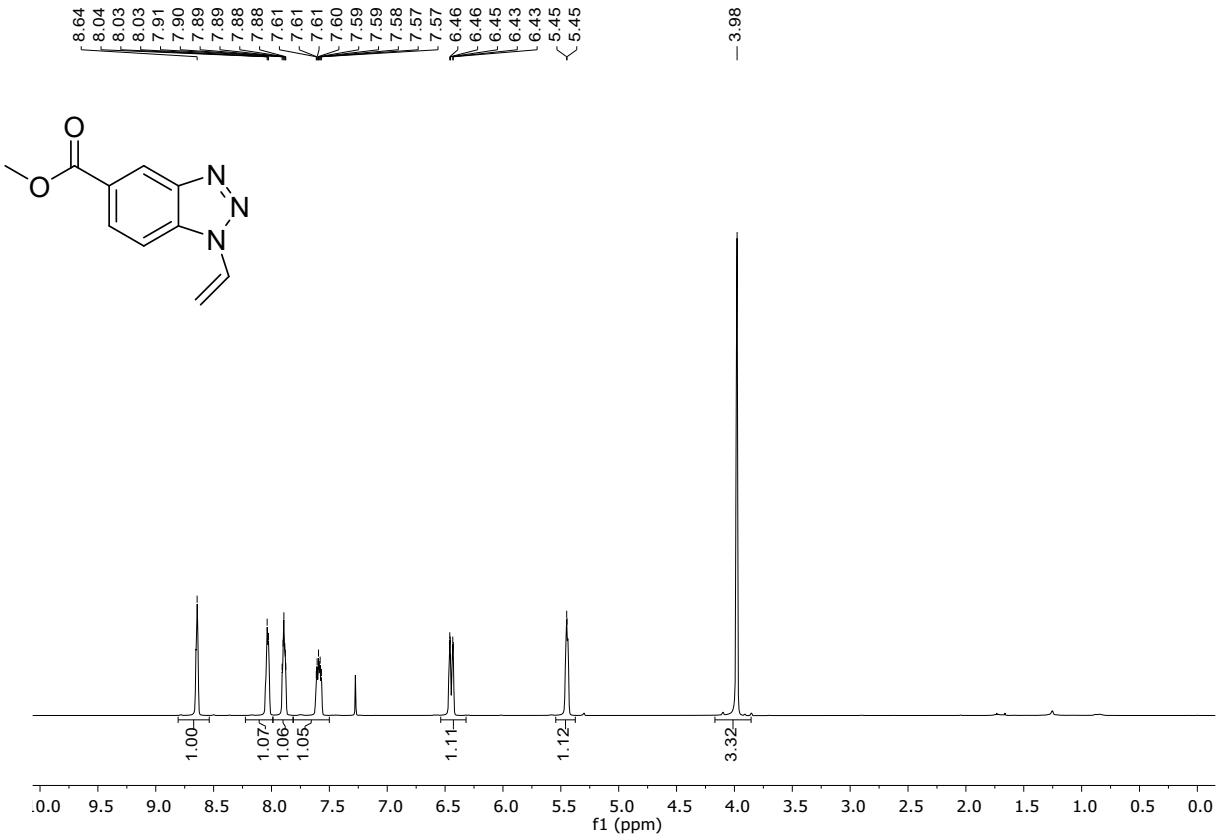
^{13}C NMR (151 MHz, $CDCl_3$) of compound **1e”.**



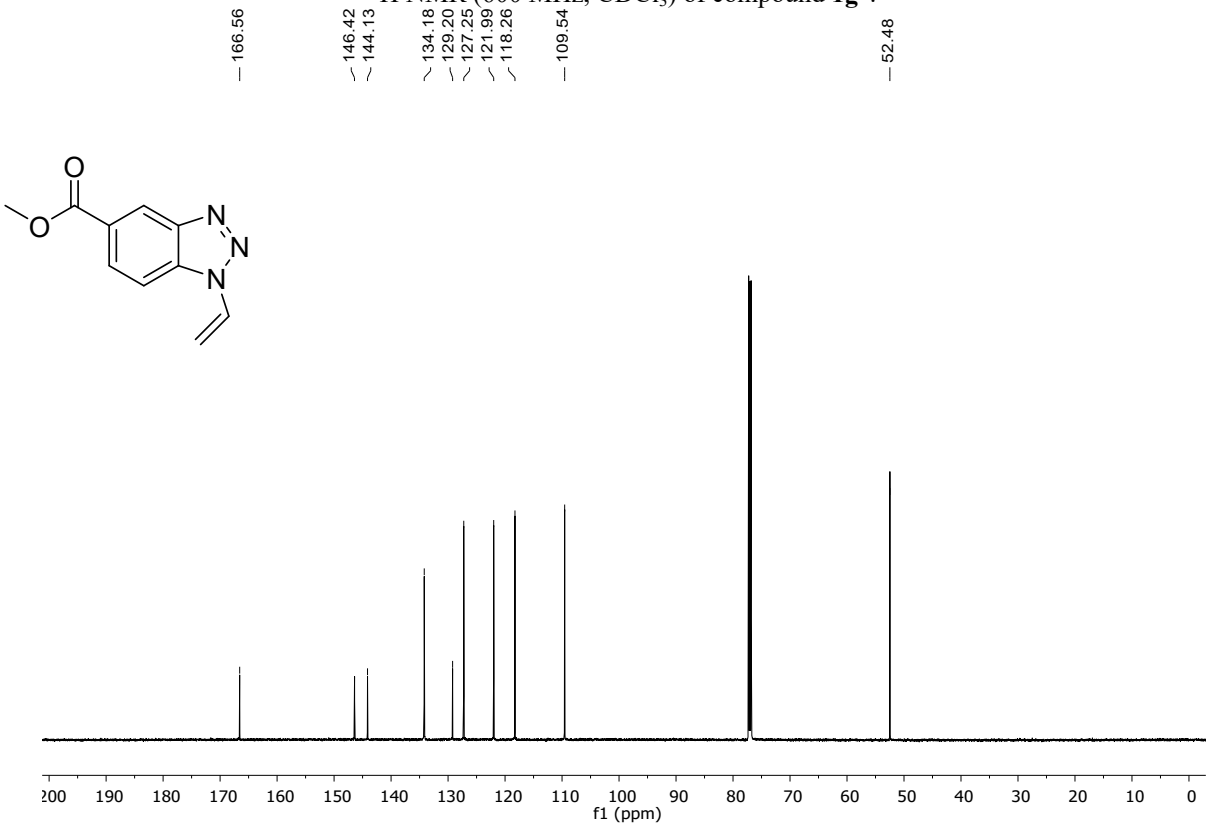
^{1}H NMR (400 MHz, $CDCl_3$) of compound **1f”**.



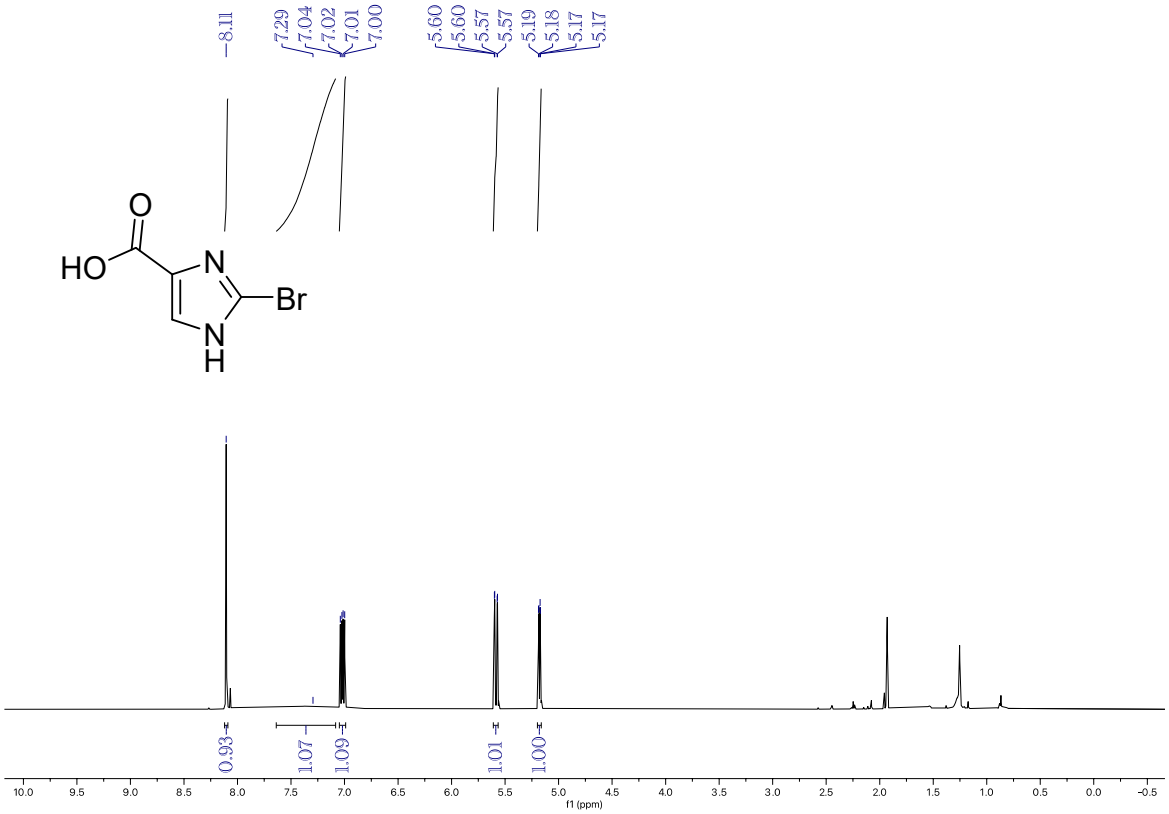
^{13}C NMR (101 MHz, $CDCl_3$) of compound **1f”.**



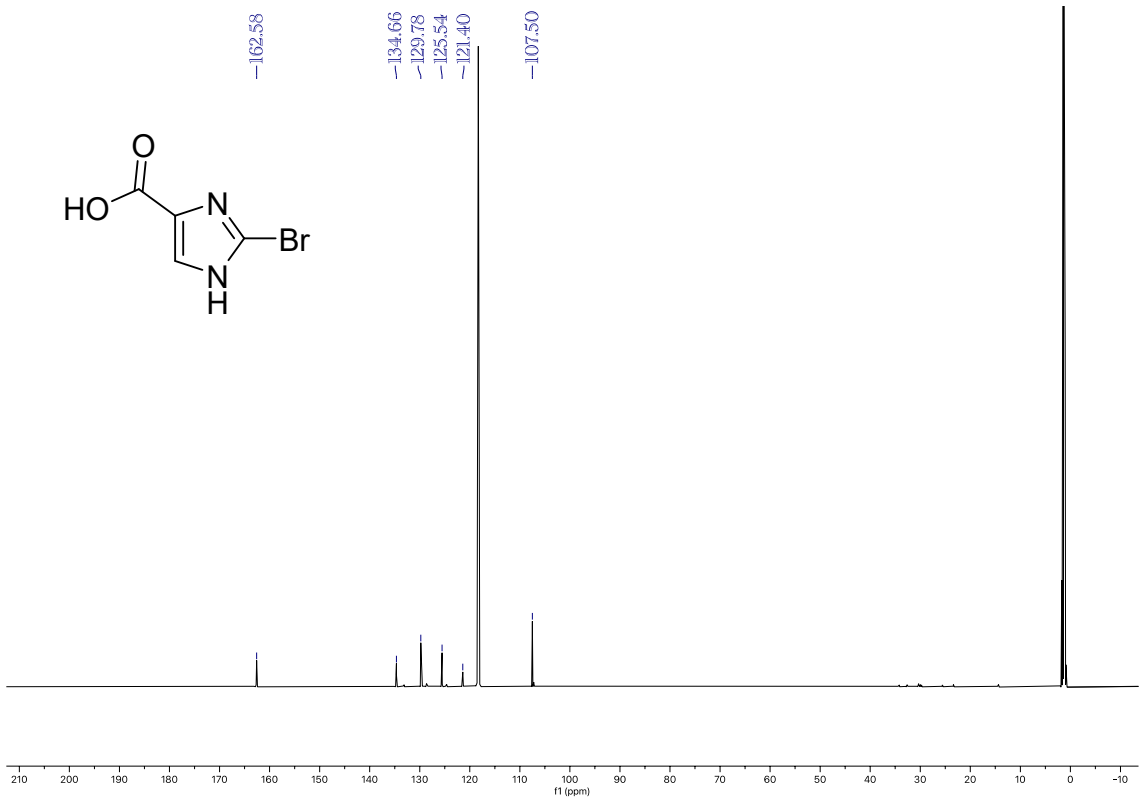
^{1}H NMR (600 MHz, $CDCl_3$) of compound **1g**".



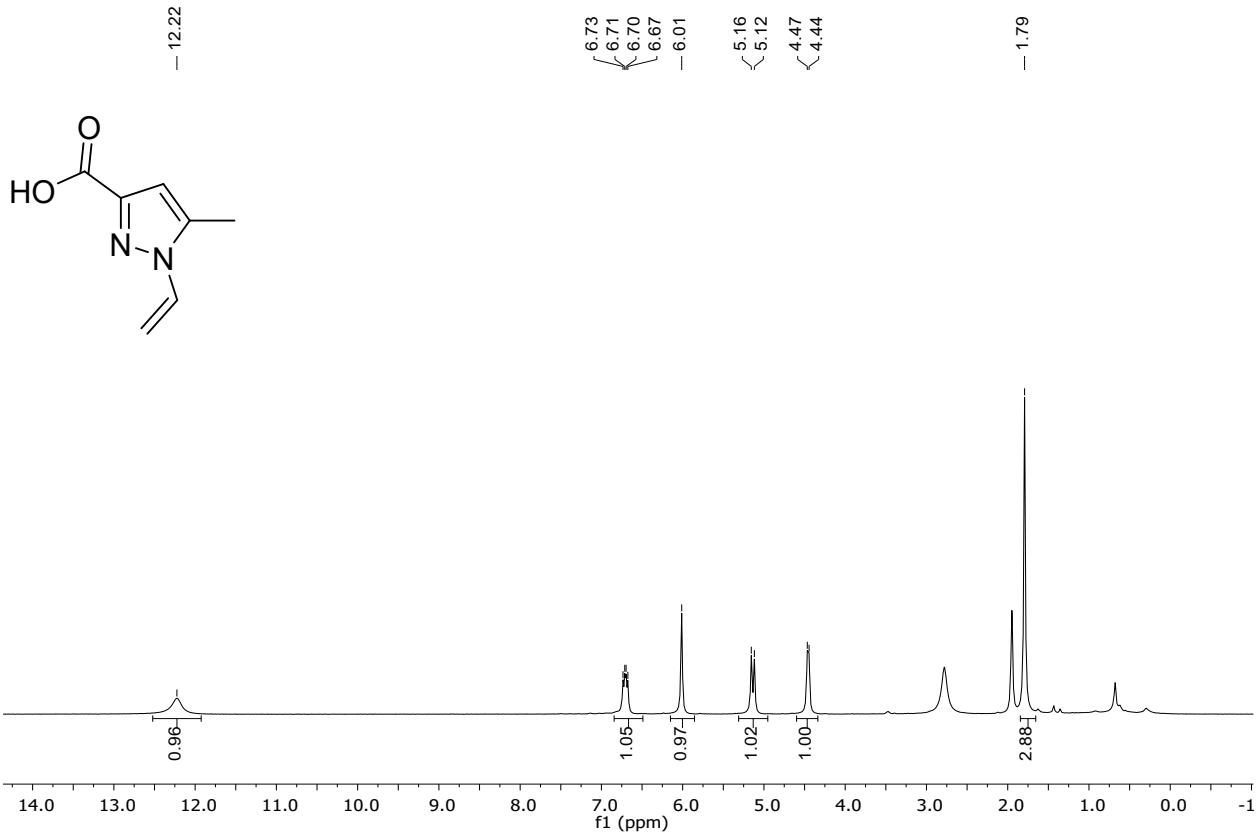
^{13}C NMR (151 MHz, $CDCl_3$) of compound **1g".**



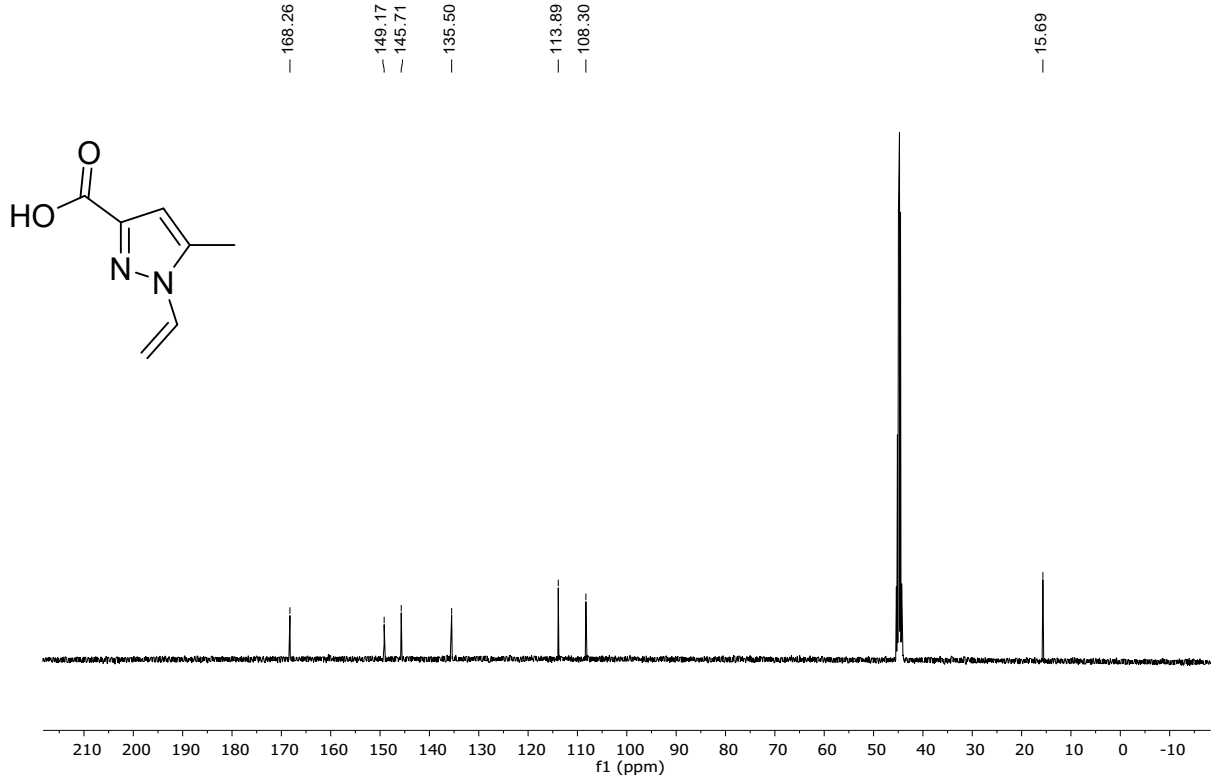
^{1}H NMR (600 MHz, $CDCl_3$) of compound **1c**.



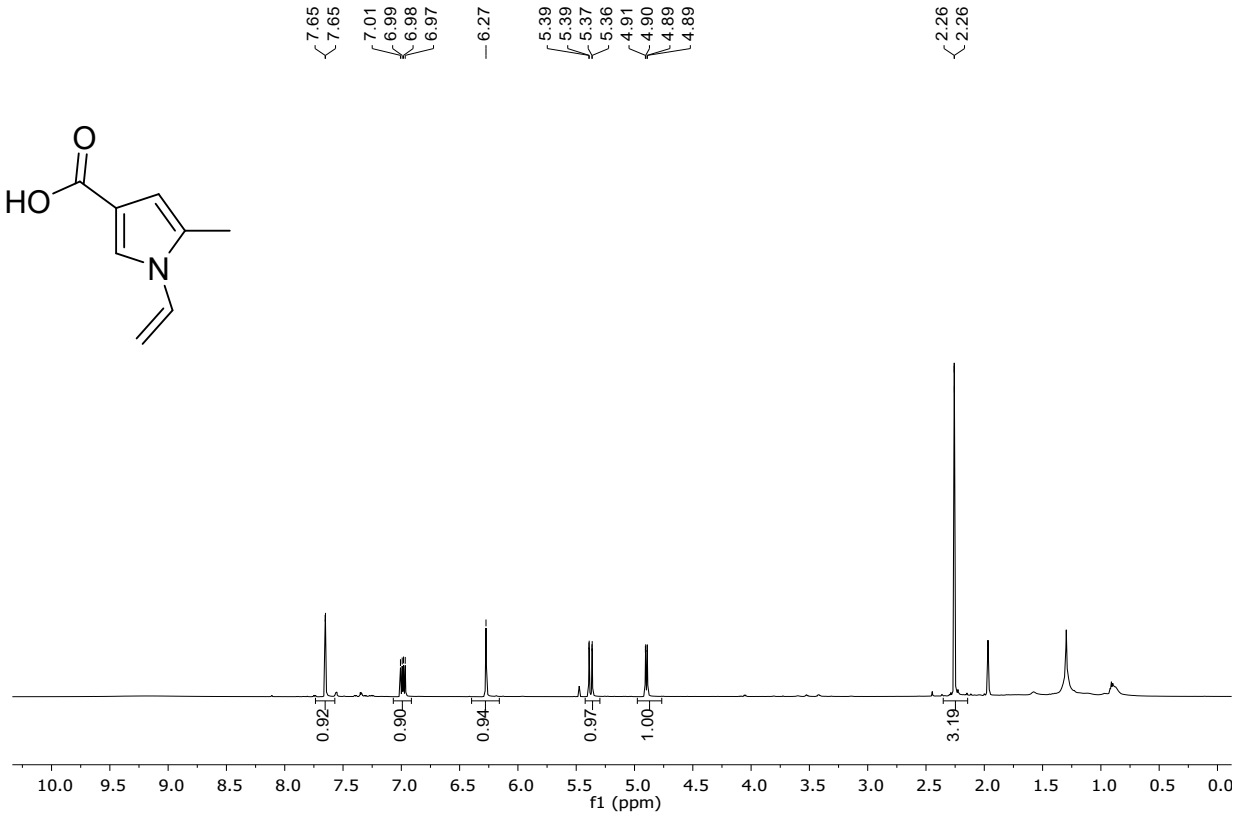
^{13}C NMR (151 MHz, $CDCl_3$) of compound **1c.**



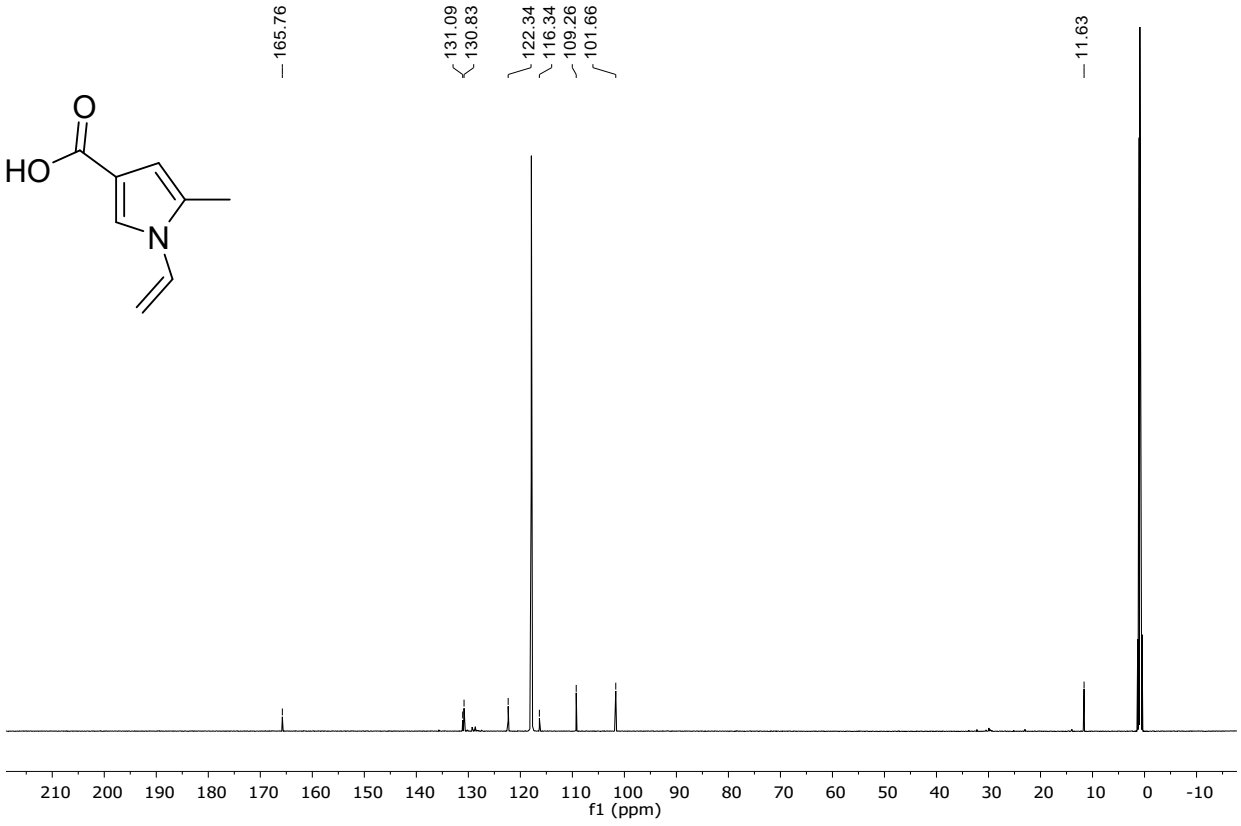
^{1}H NMR (400 MHz, CD_3CN) of compound **1d**.



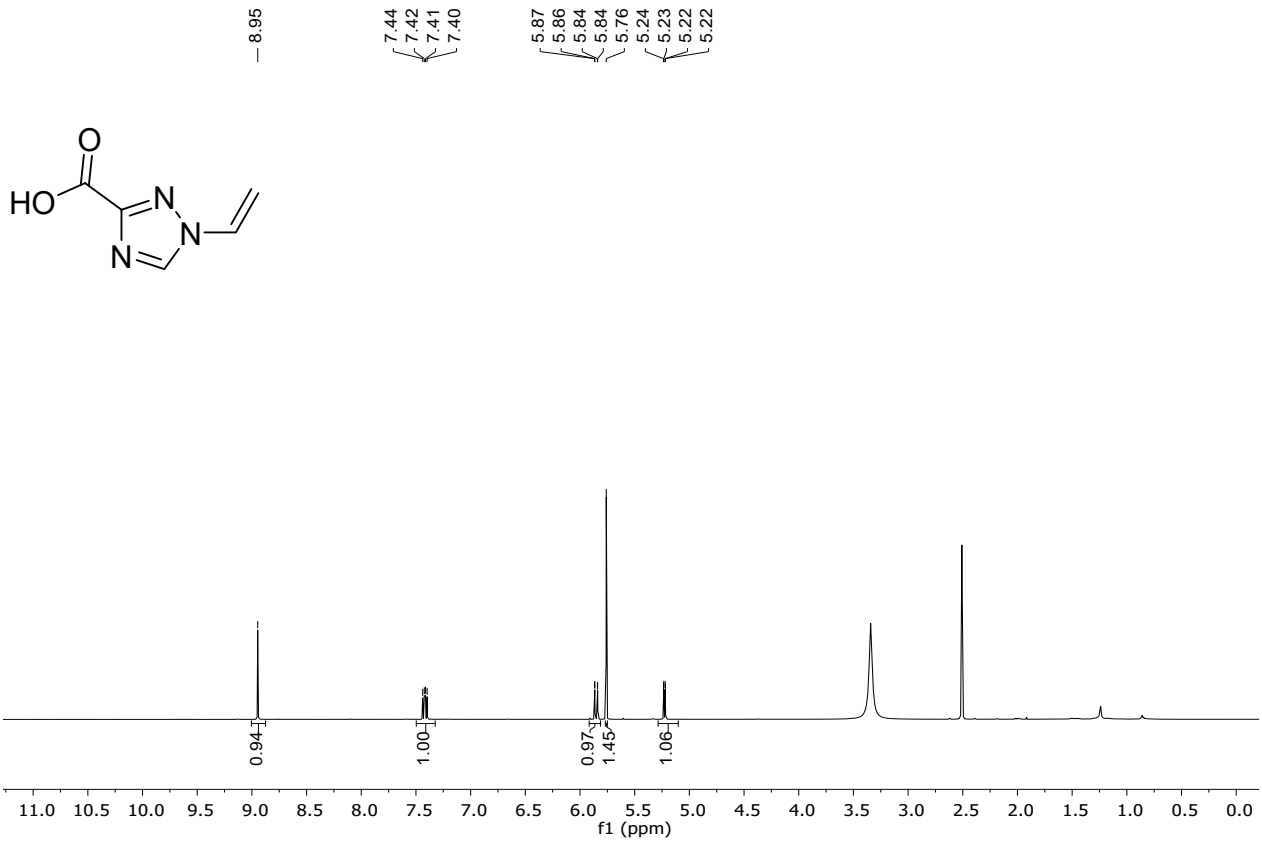
^{13}C NMR (101 MHz, CD_3CN) of compound **1d.**



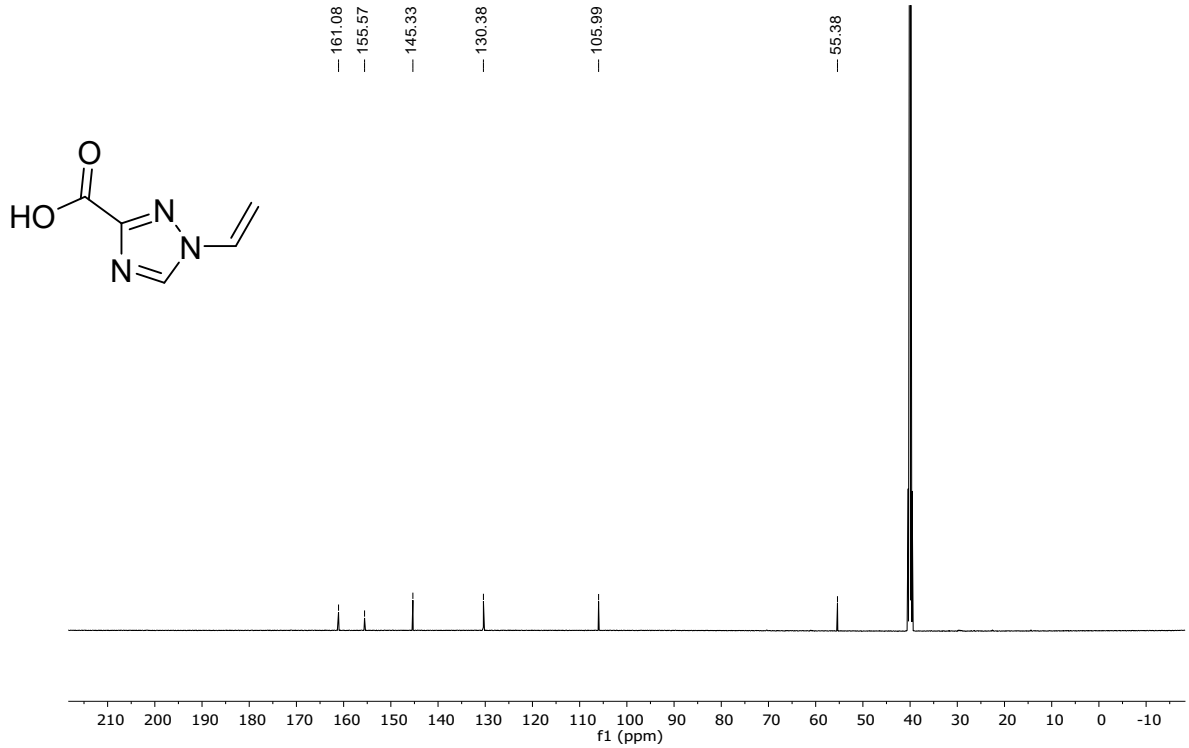
^{1}H NMR (600 MHz, CD_3CN) of compound **1e**.



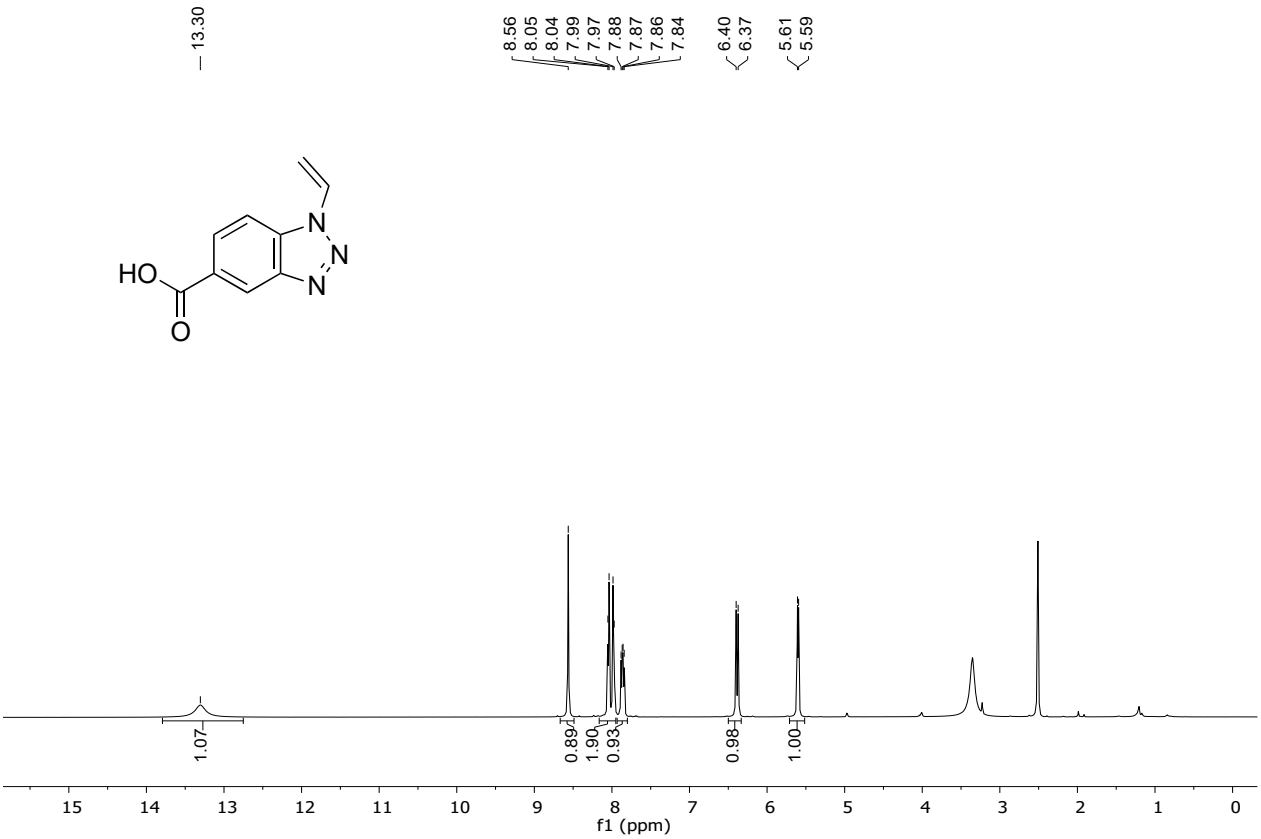
^{13}C NMR (151 MHz, CD_3CN) of compound **1e.**



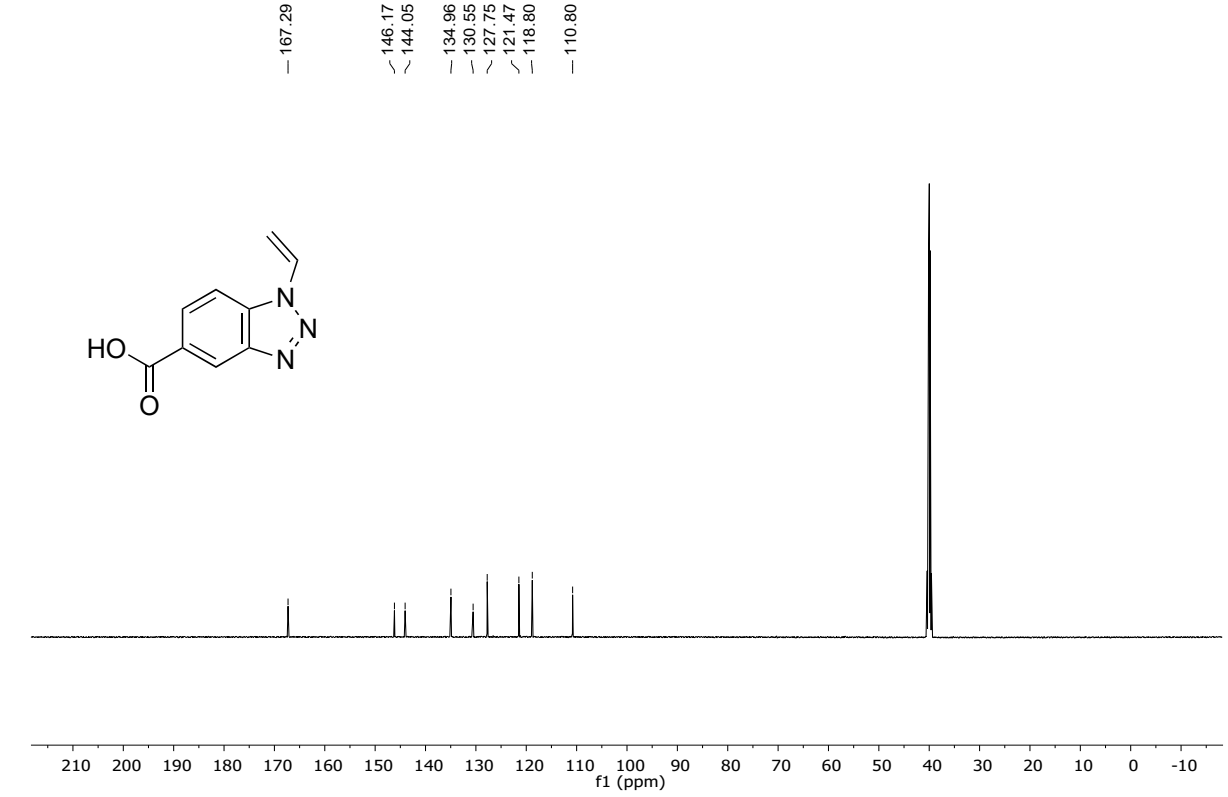
^{1}H NMR (600 MHz, CD_3CN) of compound **1f**.



^{13}C NMR (151 MHz, CD_3CN) of compound **1f.**



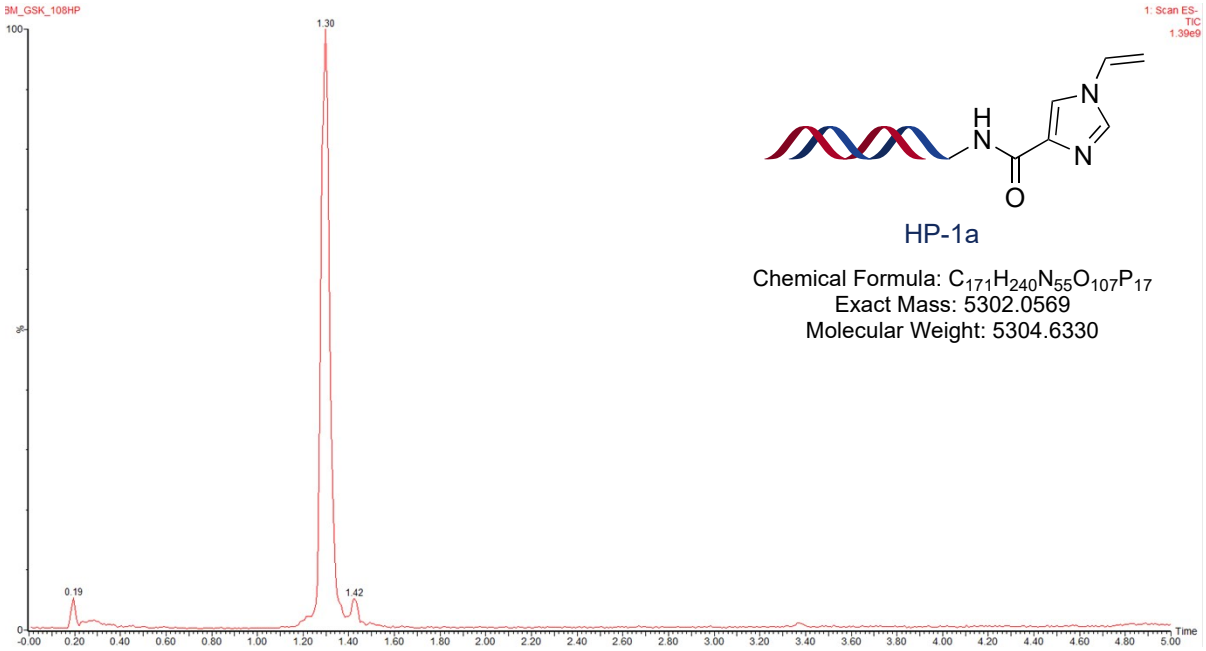
^{1}H NMR (600 MHz, d_6-DMSO) of compound **1g**.



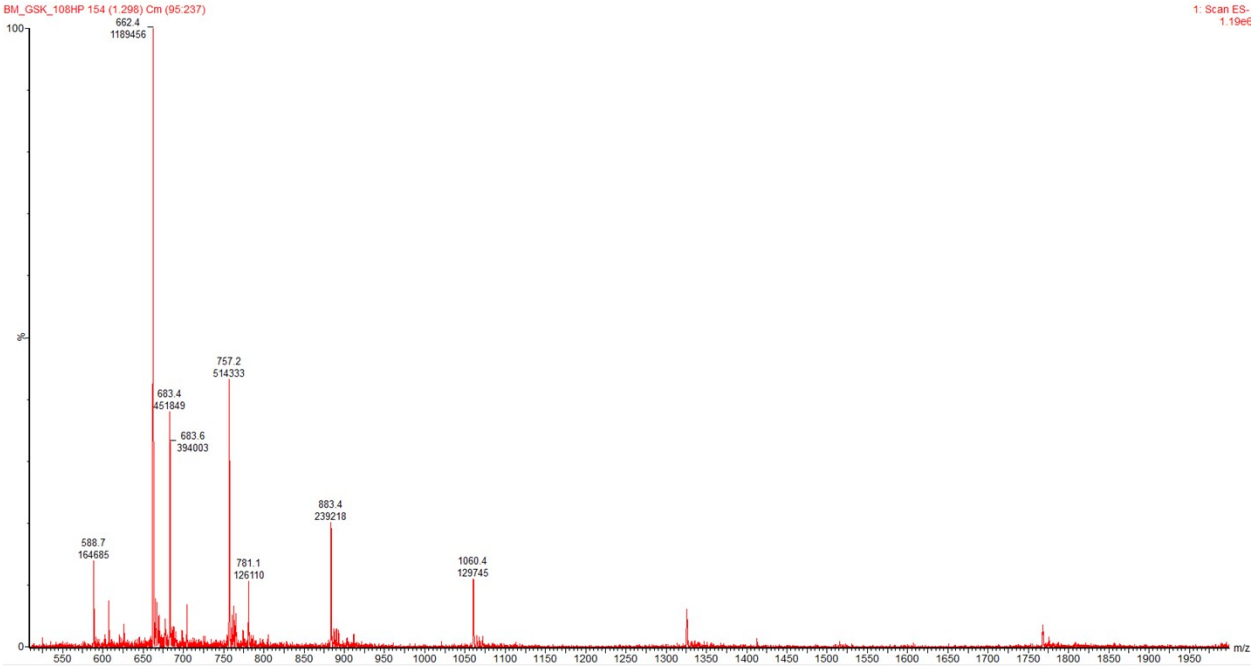
^{13}C NMR (151 MHz, d_6-DMSO) of compound **1g.**

7 UPLC/MS Spectra

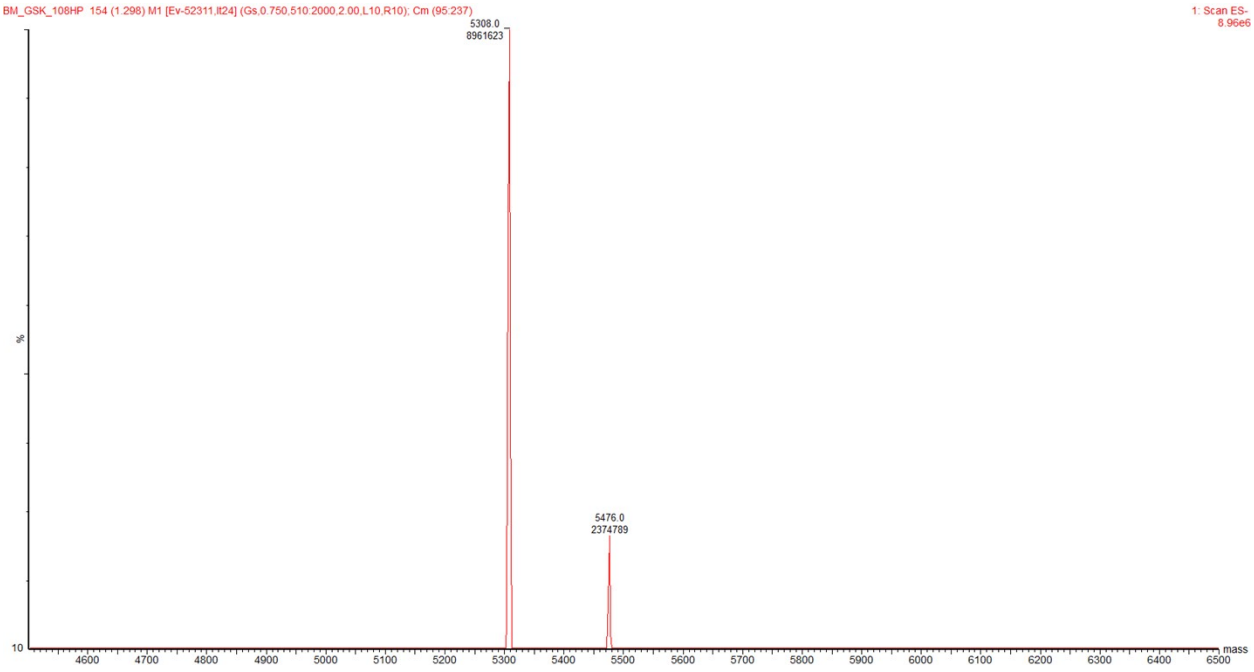
7.1 Functionalized DNA Headpieces



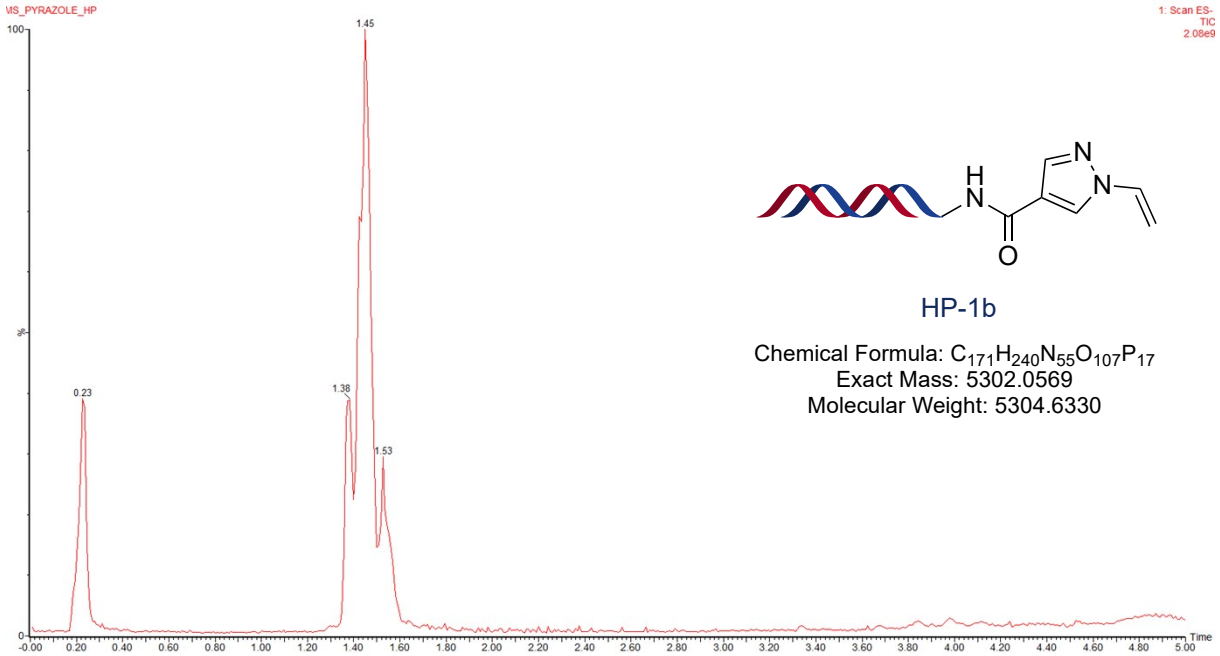
TIC of HP-1a.



Raw MS of **HP-1a**.



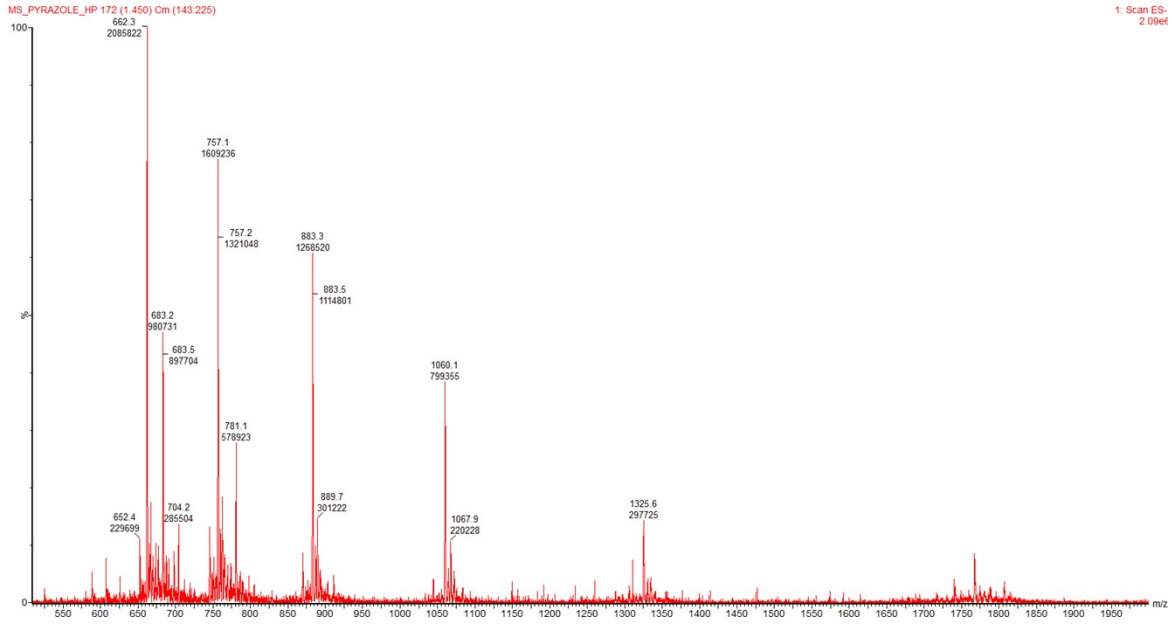
Deconvoluted MS of **HP-1a**. (mass signals >10% of peak height).



HP-1b

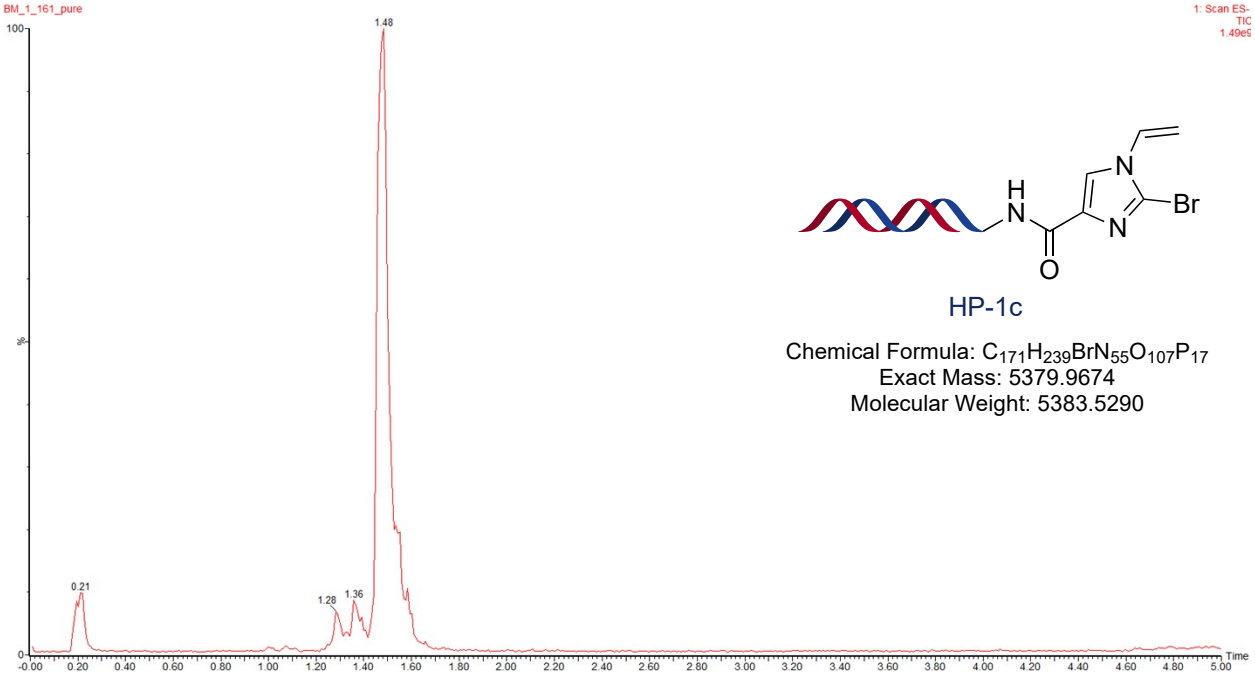
Chemical Formula: $C_{171}H_{240}N_{55}O_{107}P_{17}$
Exact Mass: 5302.0569
Molecular Weight: 5304.6330

TIC of **HP-1b**.

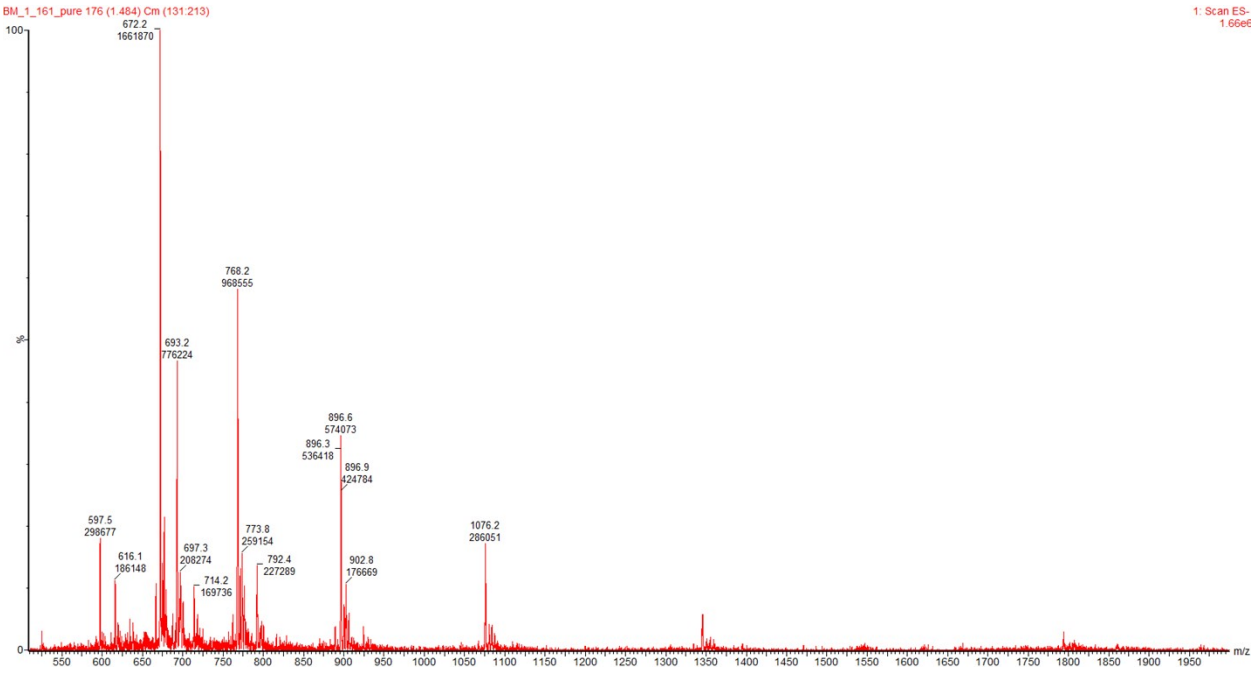


Raw MS of **HP-1b**.

Deconvoluted MS of **HP-1b**. (mass signals >10% of peak height).

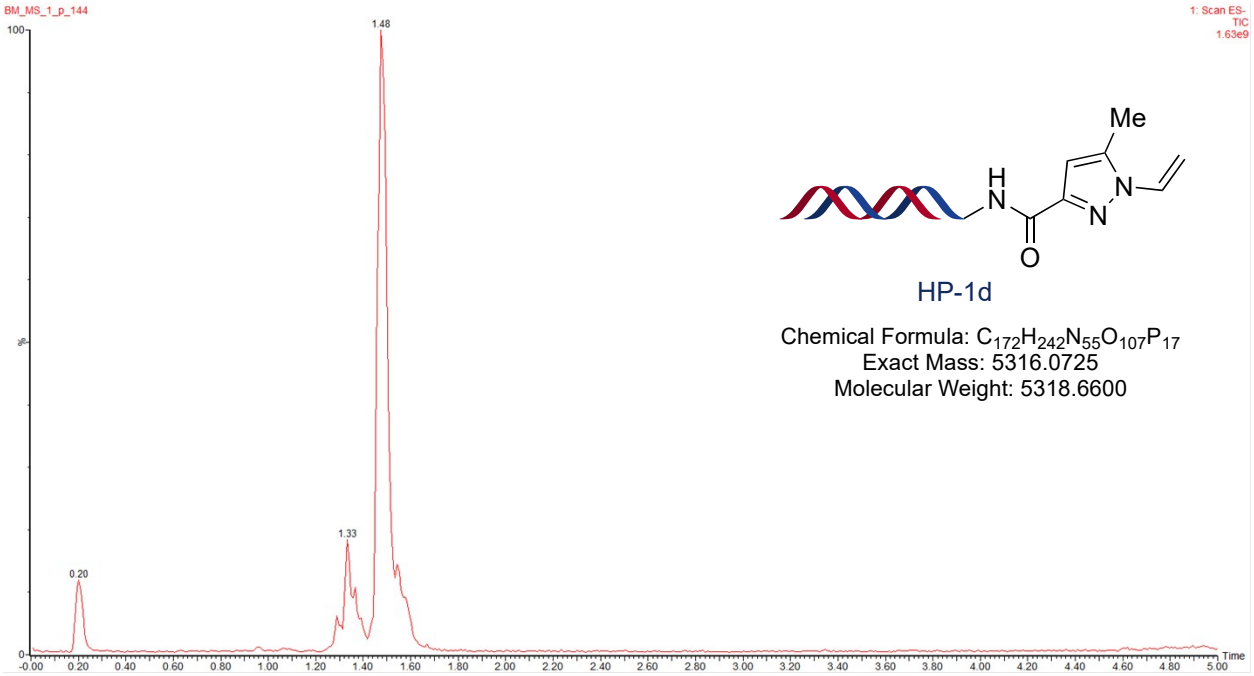


TIC of **HP-1c**.

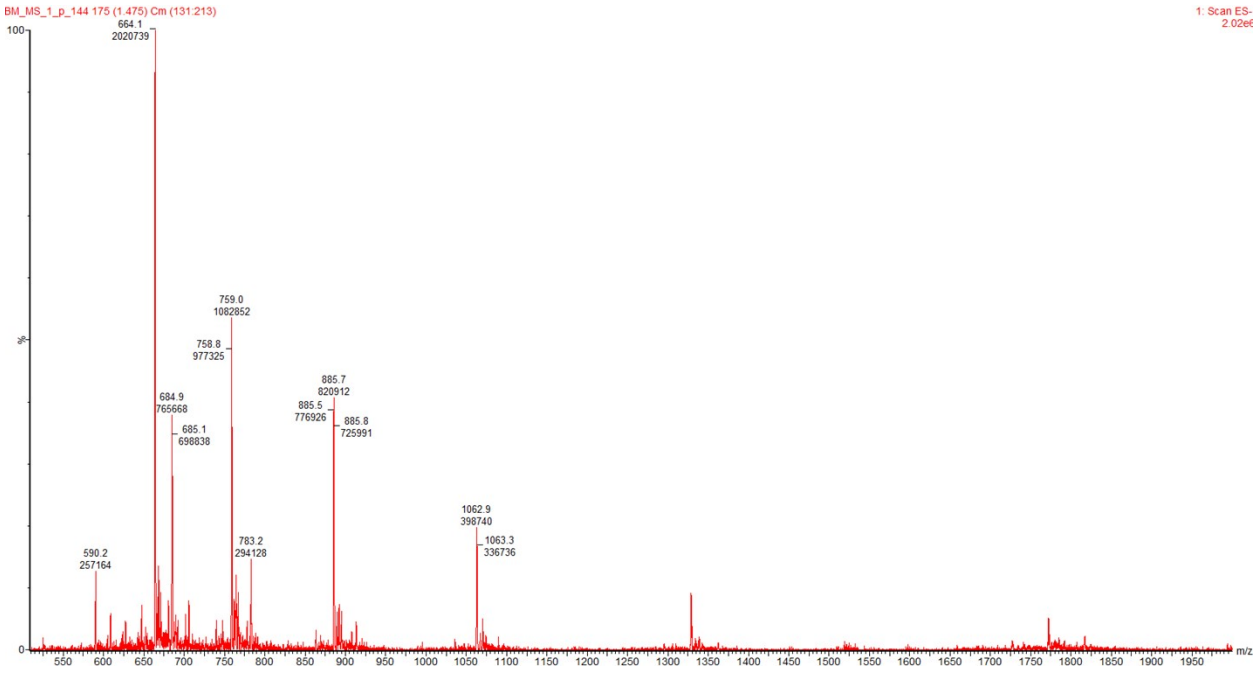


Raw MS of **HP-1c**.

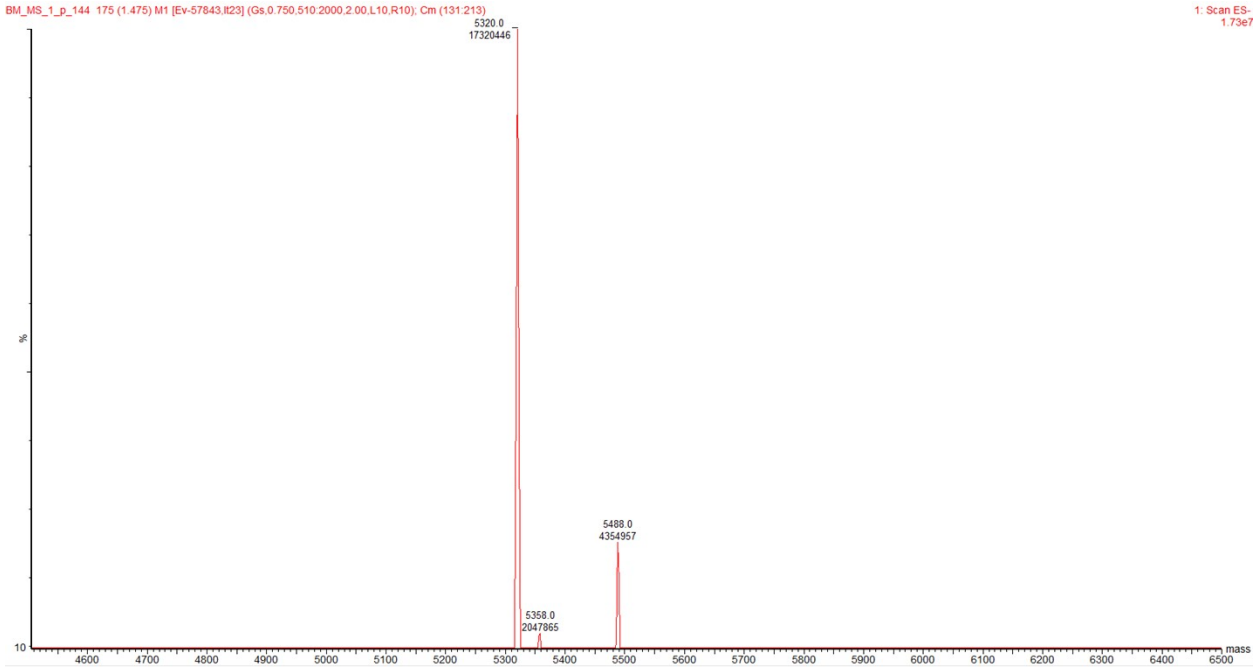
Deconvoluted MS of **HP-1c**. (mass signals >10% of peak height).



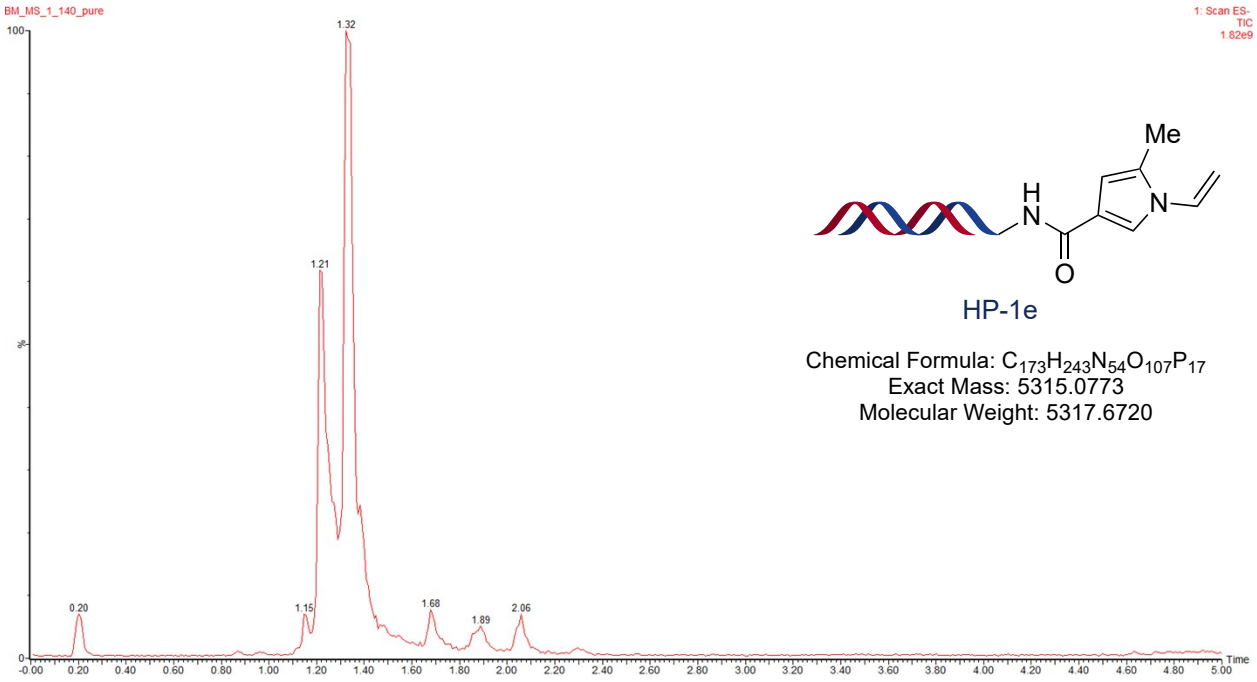
TIC of **HP-1d**.



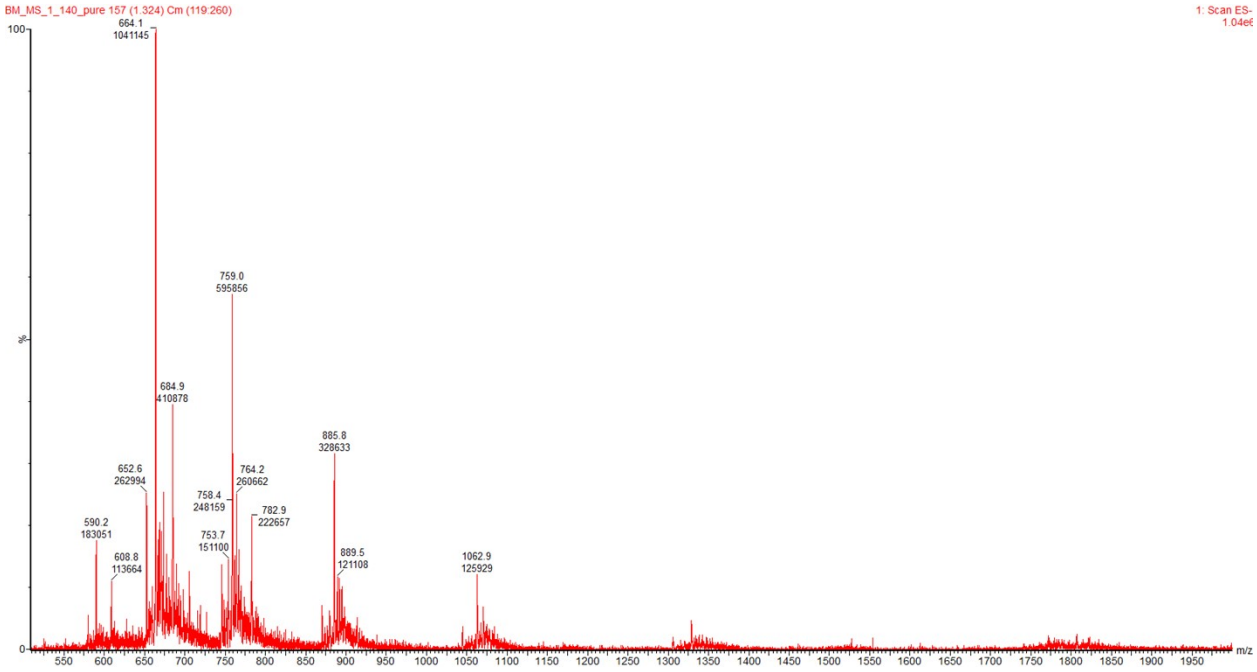
Raw MS of **HP-1d**.



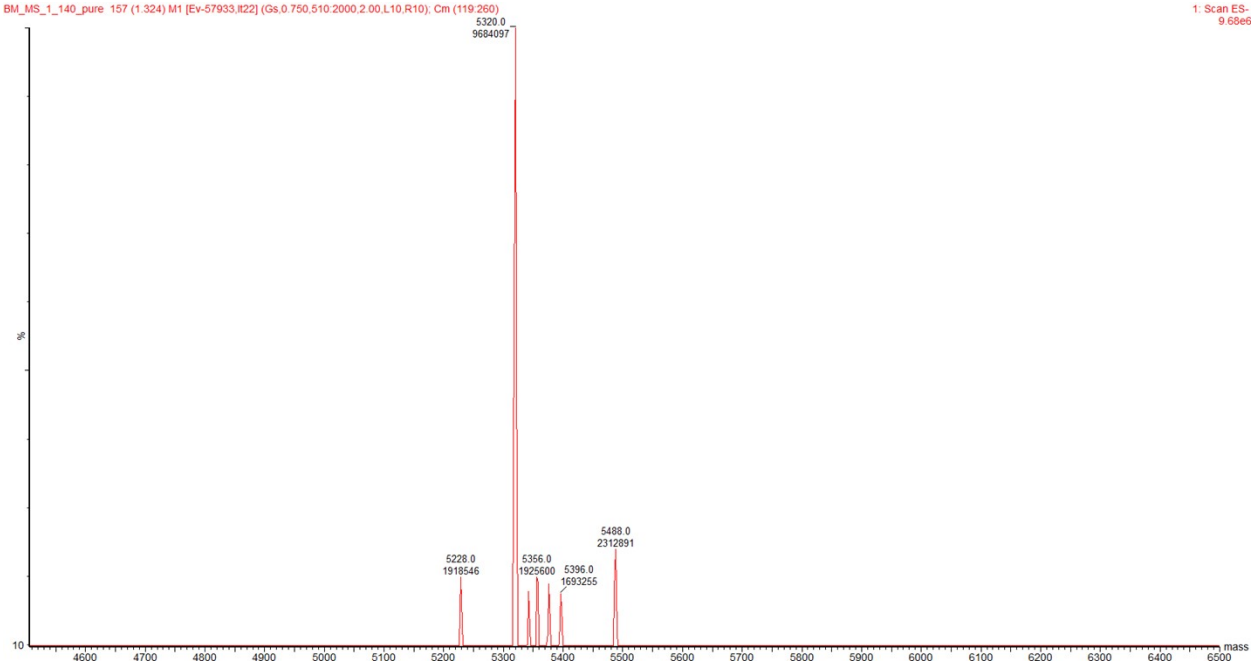
Deconvoluted MS of **HP-1d**. (mass signals >10% of peak height).



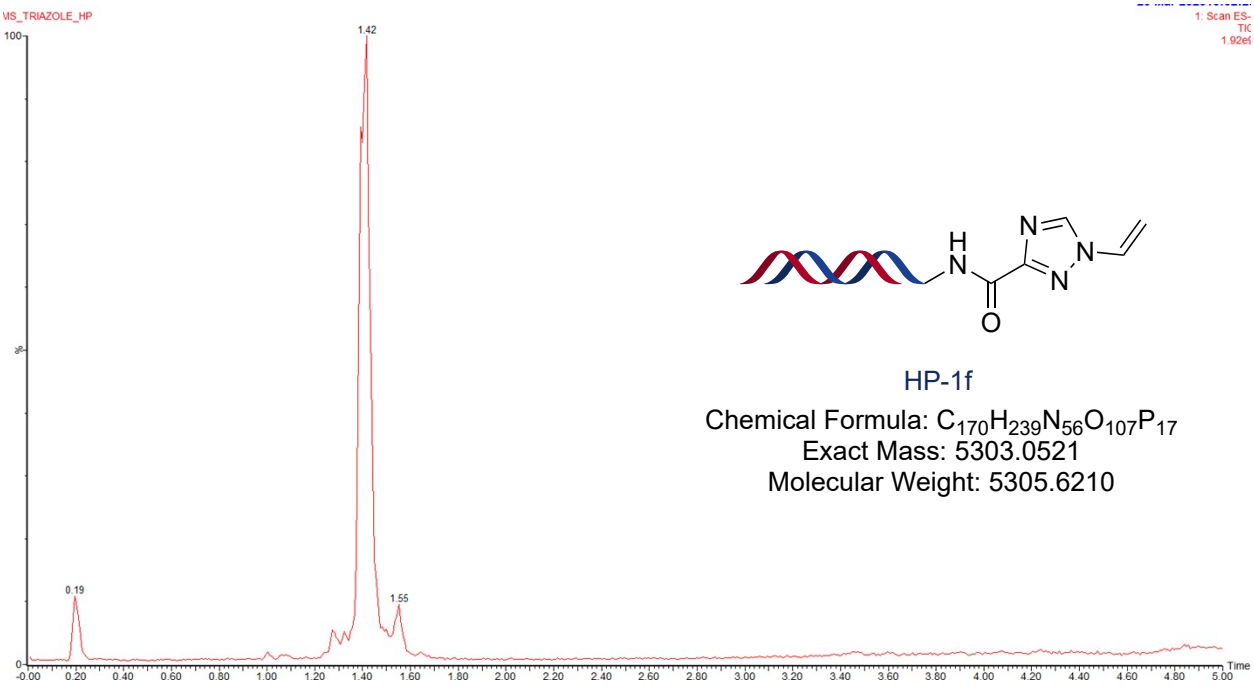
TIC of **HP-1e**.



Raw MS of **HP-1e**.



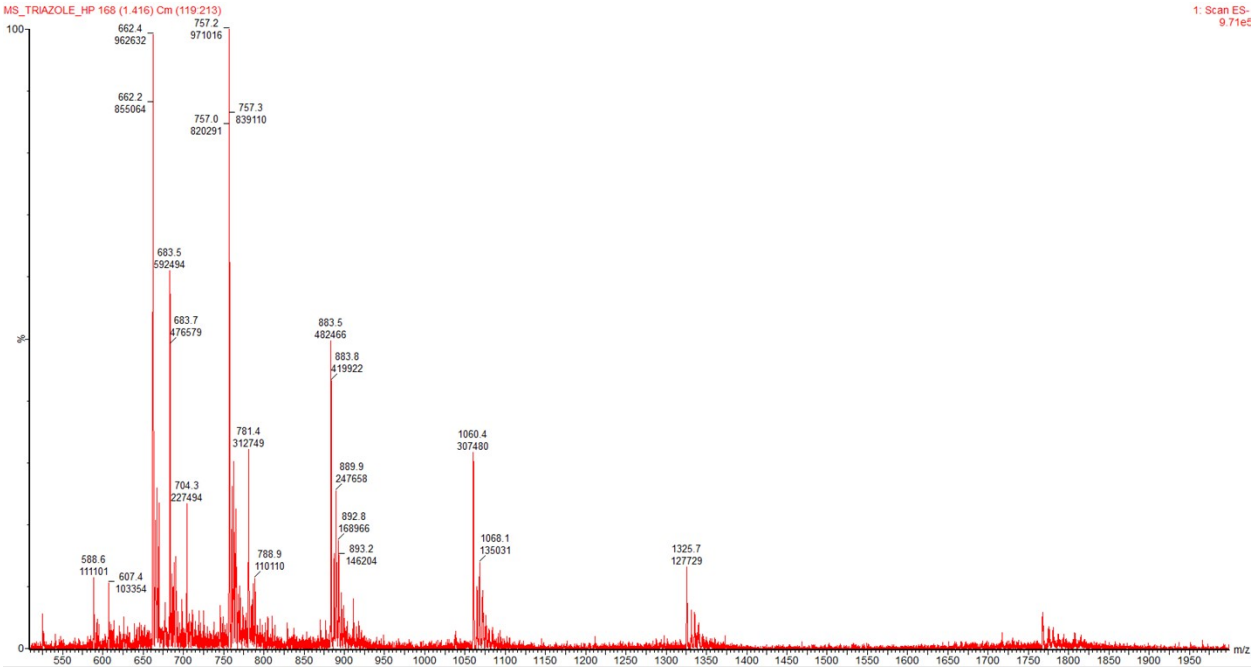
Deconvoluted MS of **HP-1e**. (mass signals >10% of peak height).



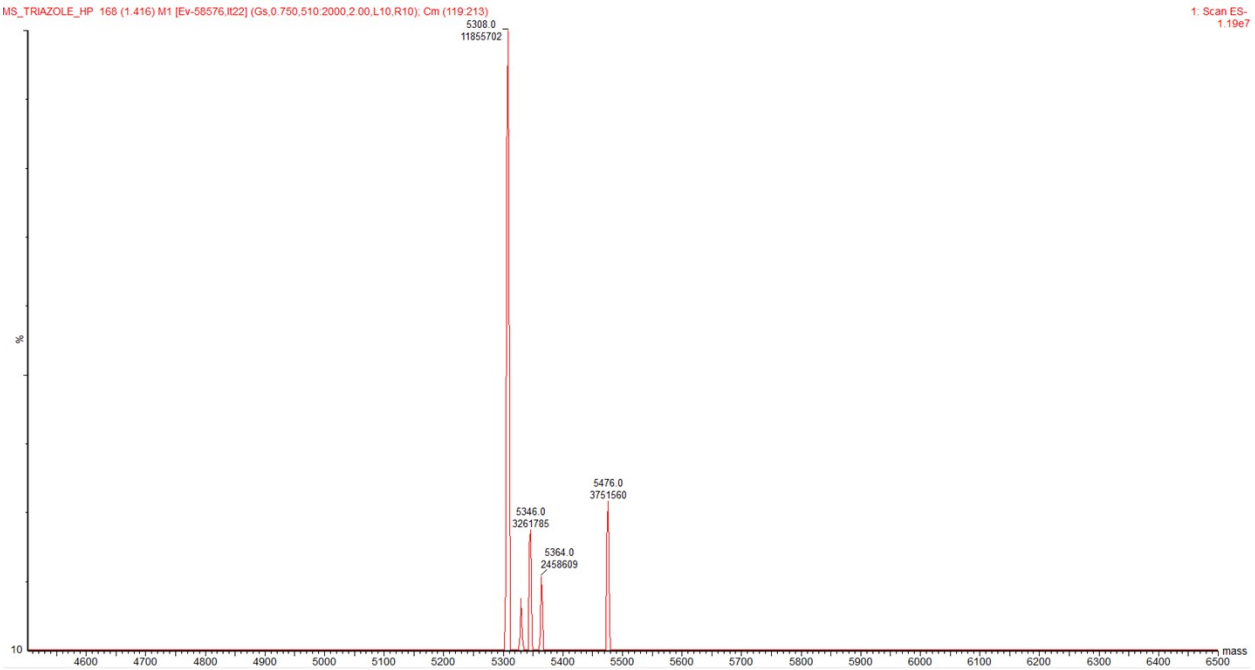
HP-1f

Chemical Formula: $C_{170}H_{239}N_{56}O_{107}P_{17}$
Exact Mass: 5303.0521
Molecular Weight: 5305.6210

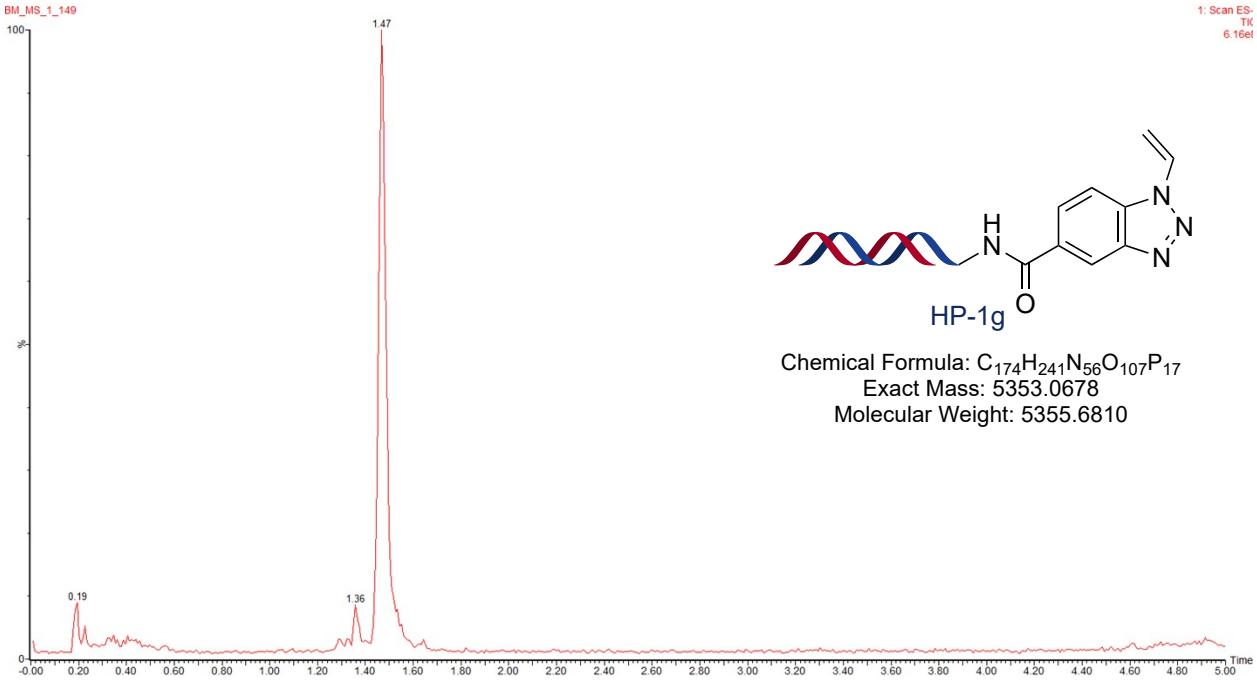
TIC of **HP-1f**.



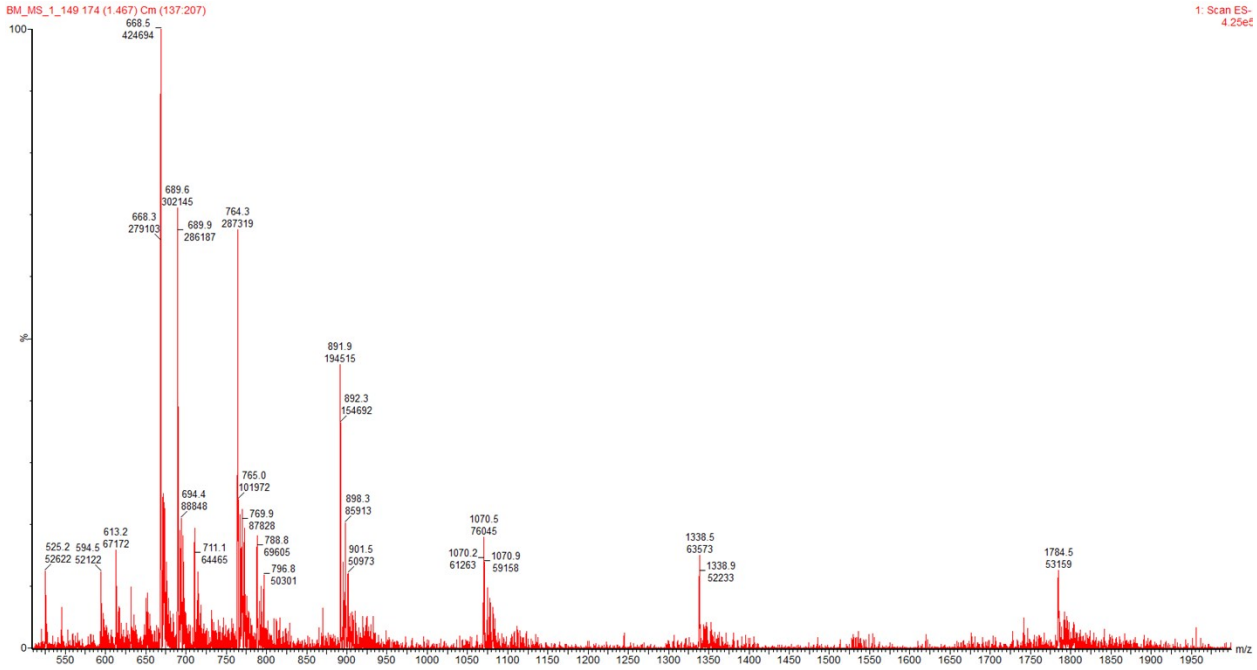
Raw MS of **HP-1f**.



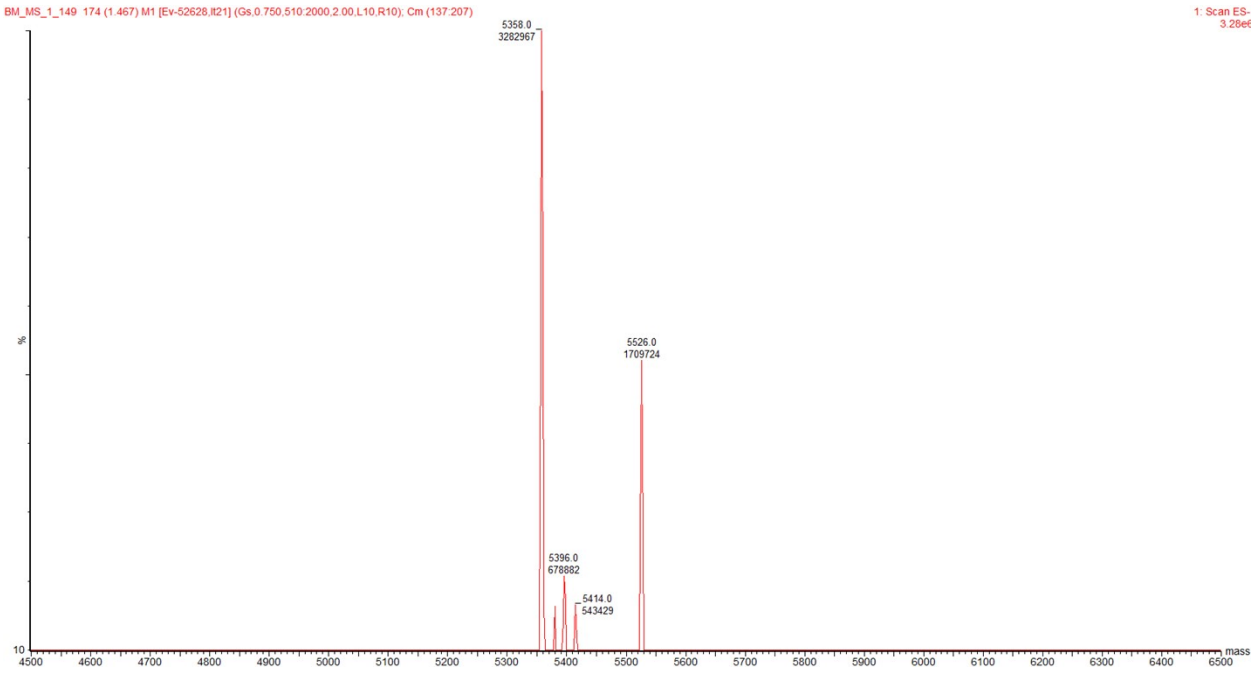
Deconvoluted MS of **HP-1f**. (mass signals >10% of peak height).



TIC of **HP-1g**.



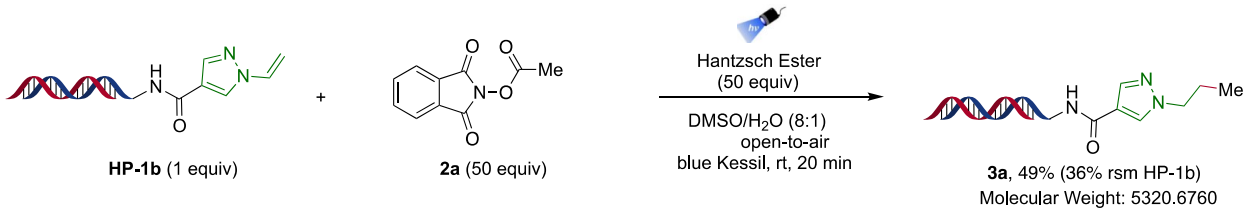
Raw MS of **HP-1g**.



Deconvoluted MS of **HP-1g**. (mass signals >10% of peak height).

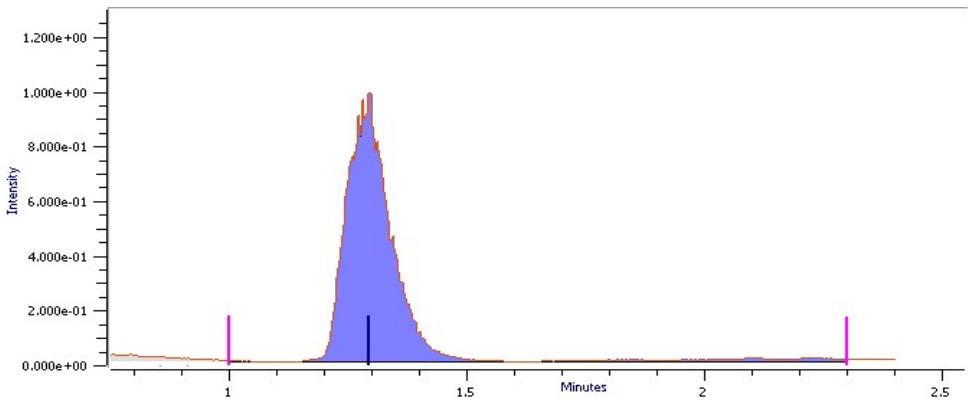
7.2 Hydroalkylated Compounds on-DNA

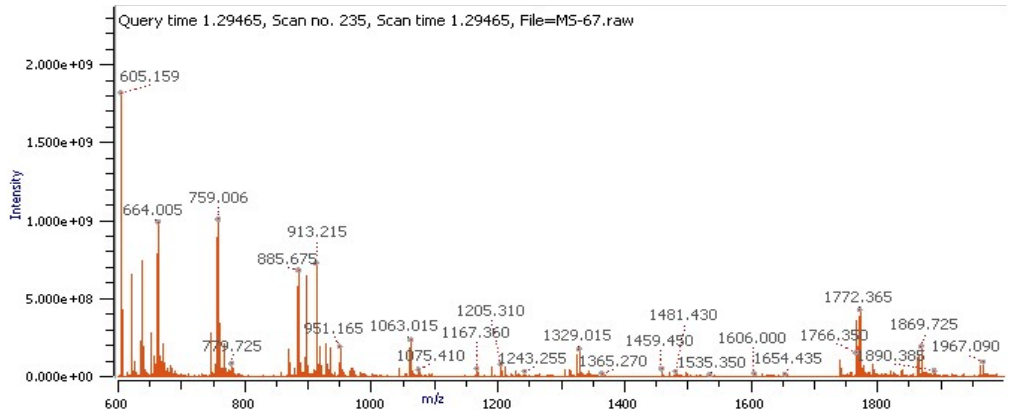
For most of the examples in the scope, the alkene-headpiece starting materials were recovered along with the major formation of the desired alkylated compound on-DNA. The recovered starting materials percentages (rsm) are also included in parenthesis for each of the examples.

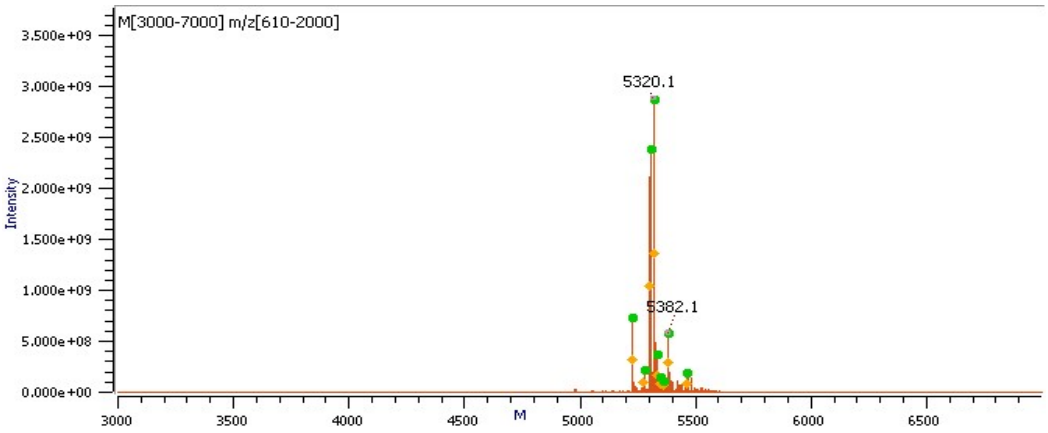


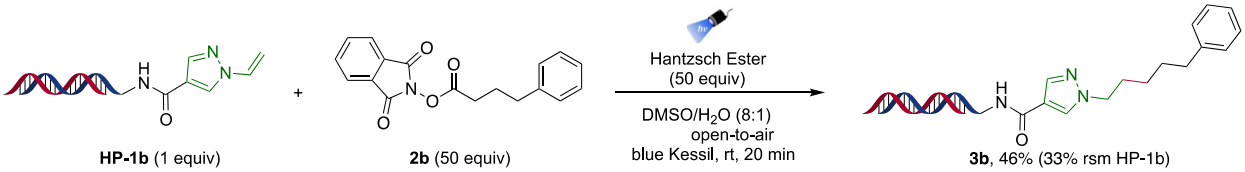
HP-1b (1 equiv) + **2a** (50 equiv)

3a, 49% (36% rsm HP-1b)
Molecular Weight: 5320.6760





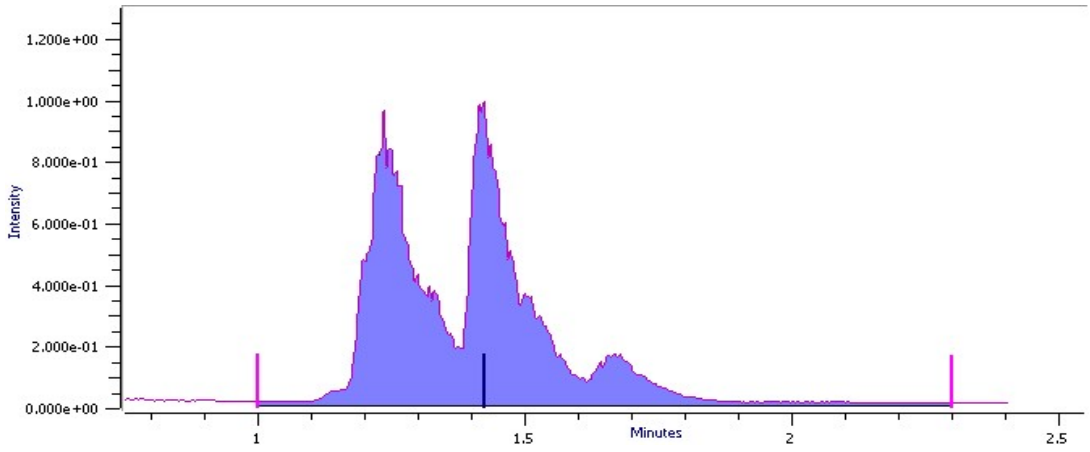


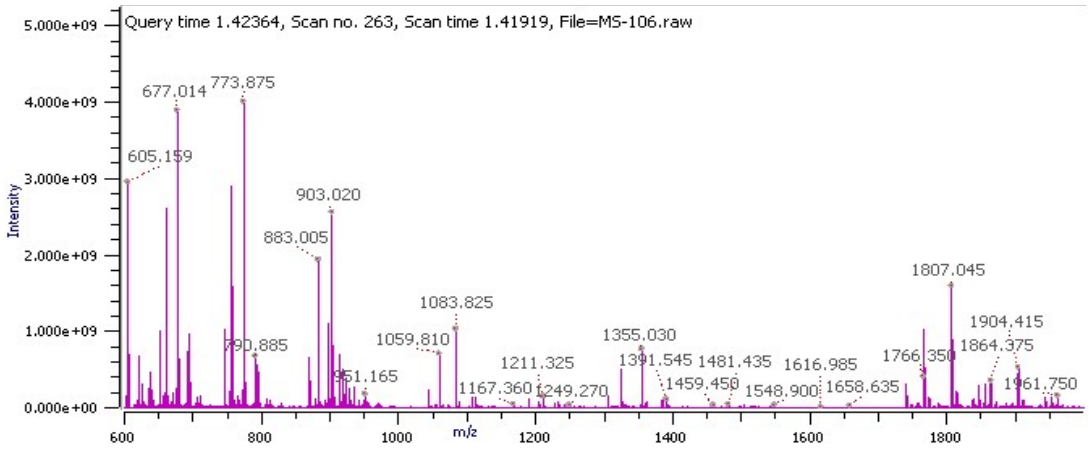


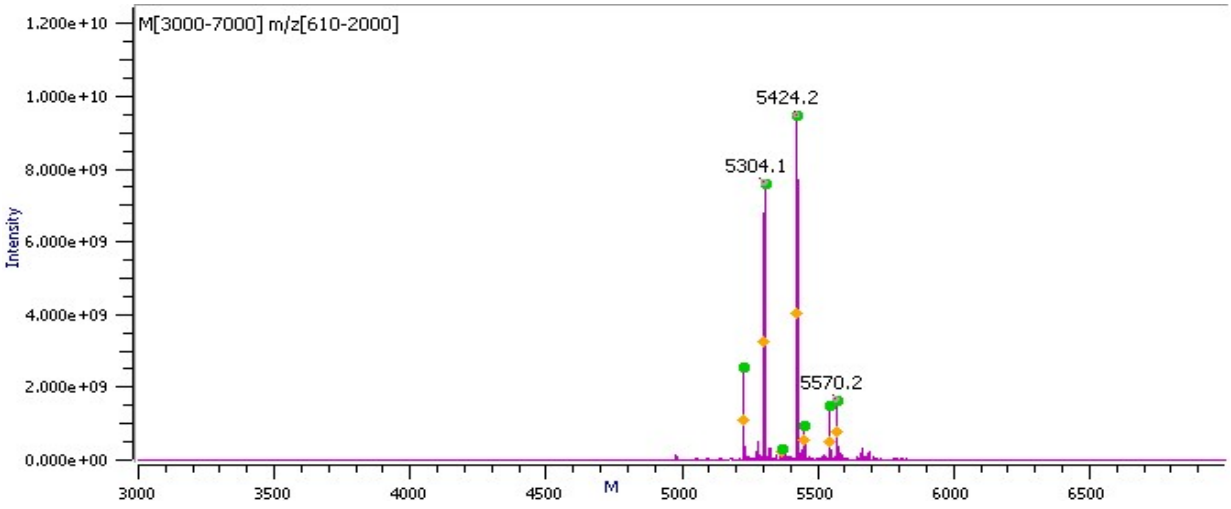
HP-1b (1 equiv) + **2b** (50 equiv)

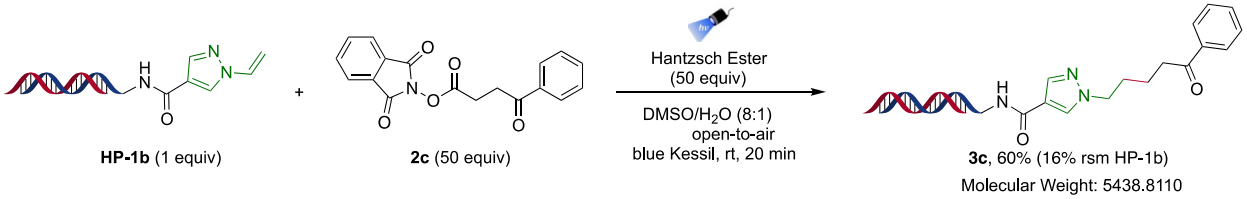
3b, 46% (33% rsm HP-1b)

Molecular Weight: 5424.8280




Query time 1.42364, Scan no. 263, Scan time 1.41919, File=MS-106.raw
Intensity
m/z
677.014
773.875
605.159
903.020
883.005
1083.825
1059.810
790.885
951.165
1211.325
1167.360
1249.270
1355.030
1391.545
1481.435
1459.450
1548.900
1616.985
1658.635
1807.045
1766.350
1904.415
1864.375
1961.750

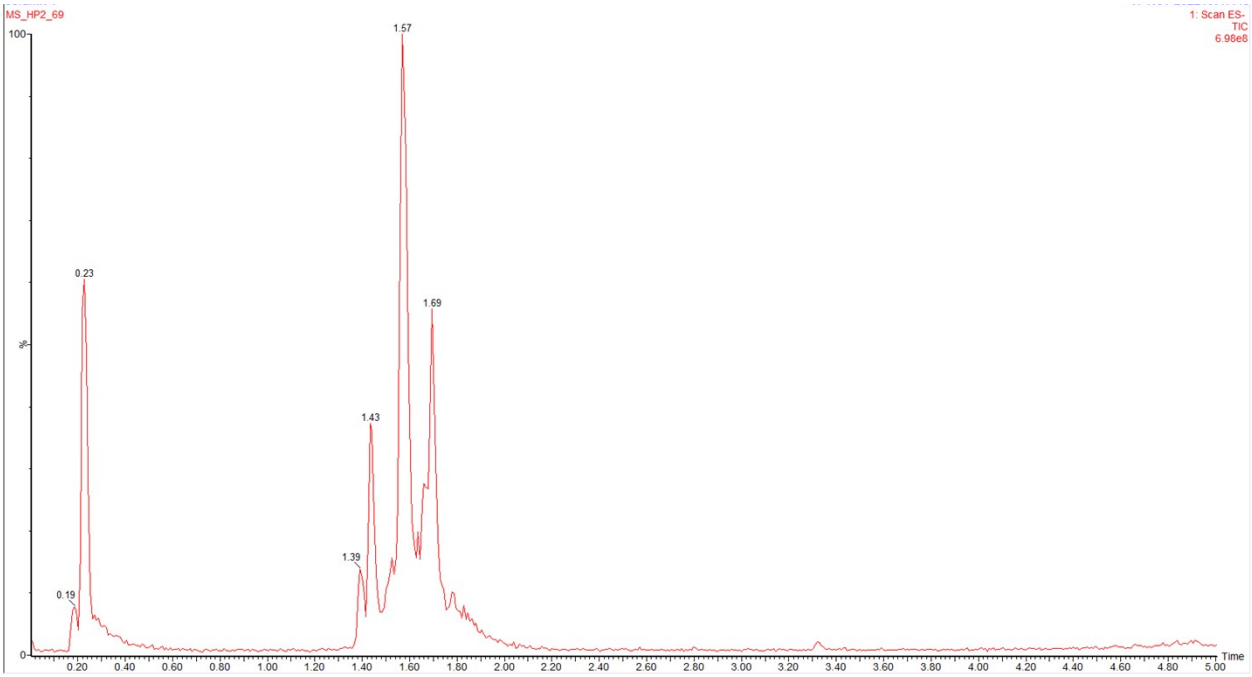

M[3000-7000] m/z[610-2000]
Intensity
M
5424.2
5304.1
5570.2

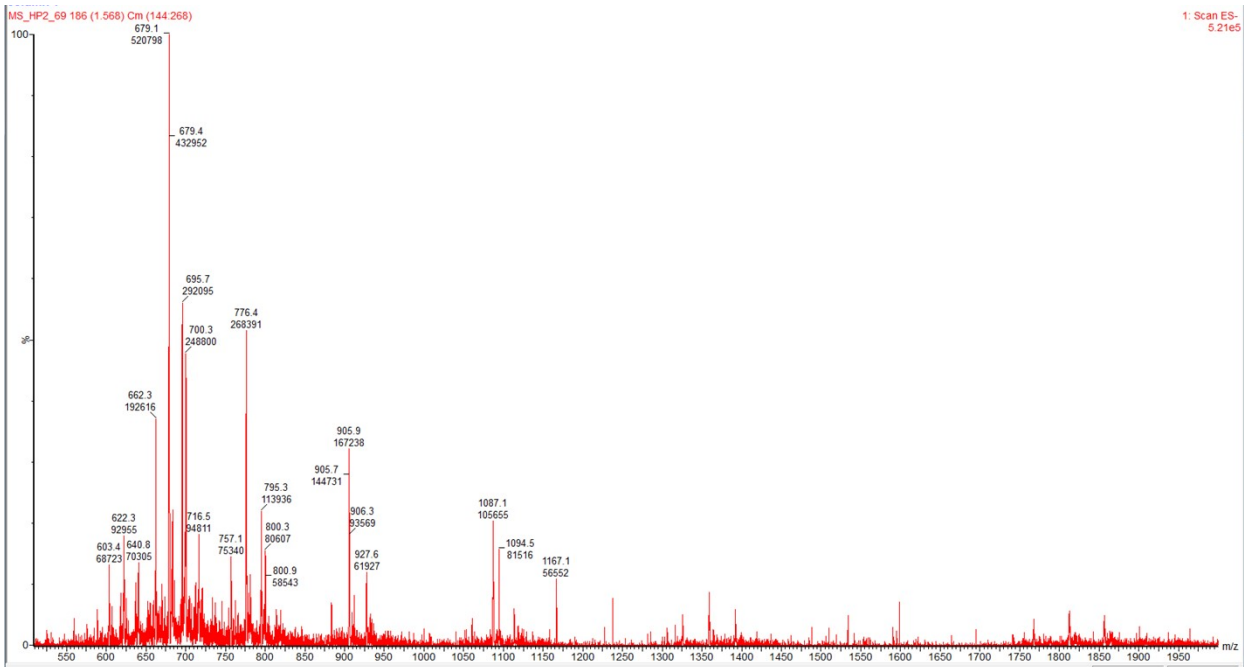


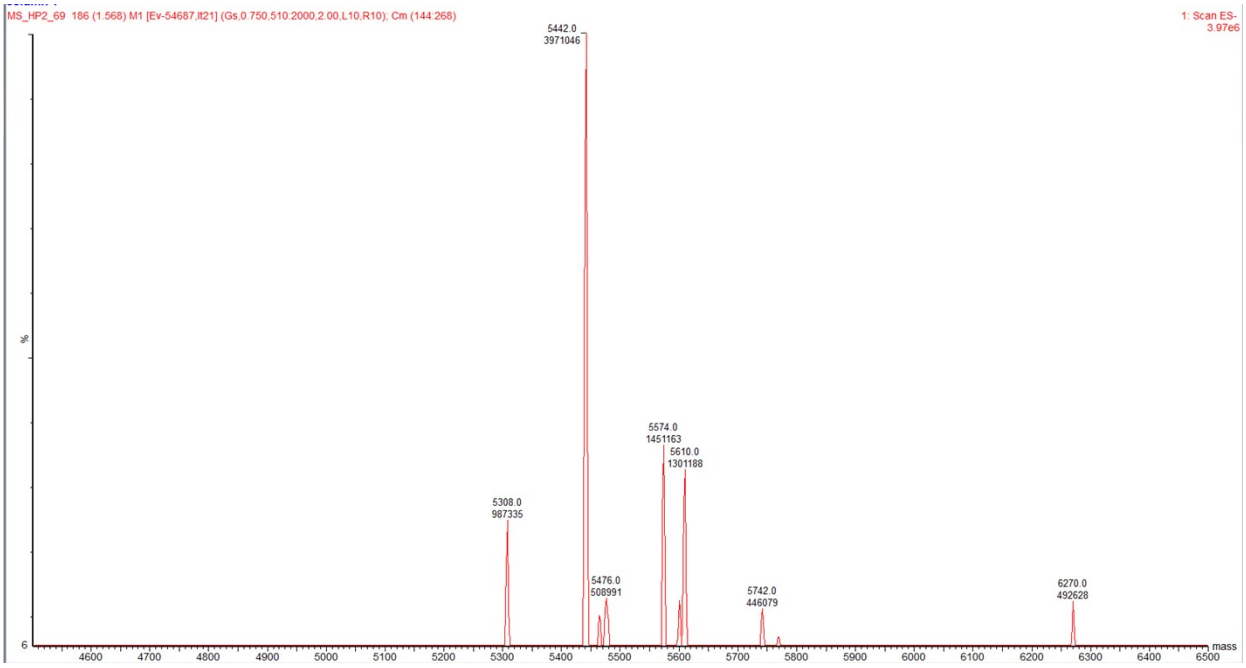
HP-1b (1 equiv) + **2c** (50 equiv)

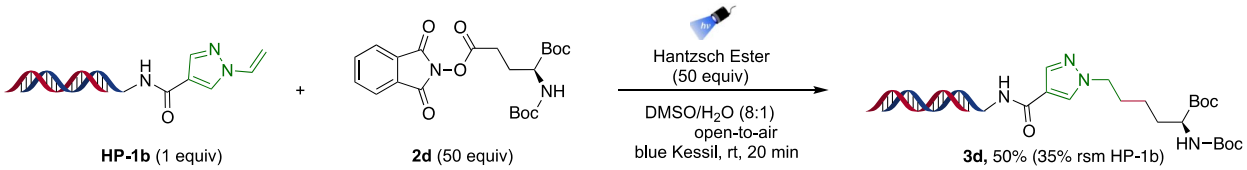
3c, 60% (16% rsm HP-1b)

Molecular Weight: 5438.8110





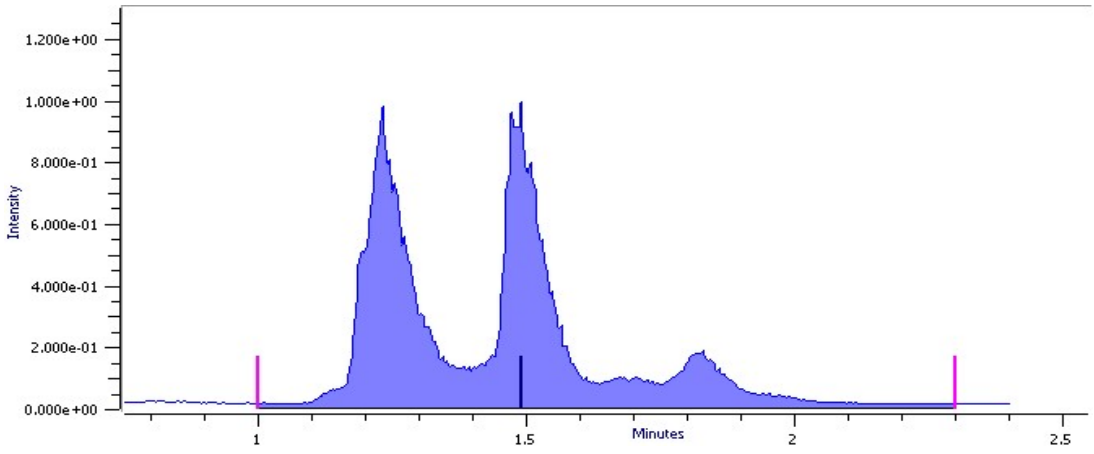


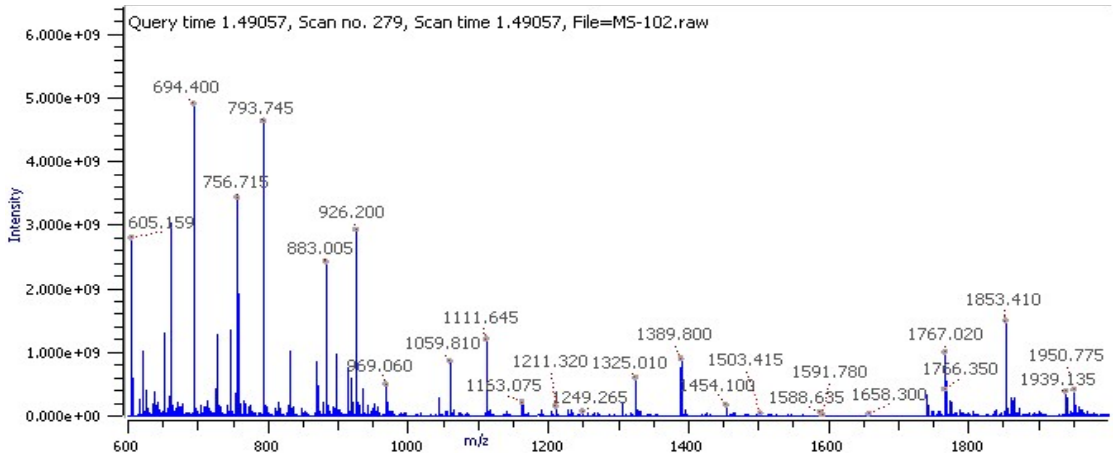


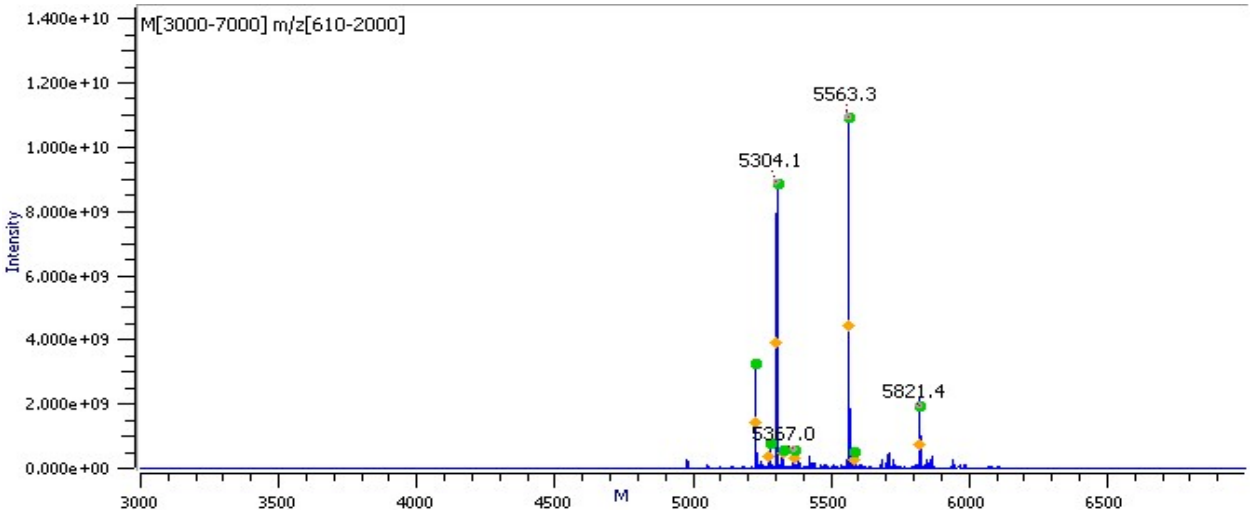
HP-1b (1 equiv) + **2d** (50 equiv)

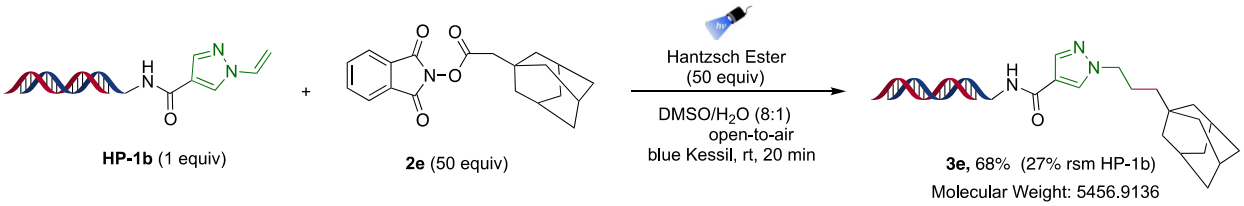
3d, 50% (35% rsm HP-1b)

Molecular Weight: 5563.9790





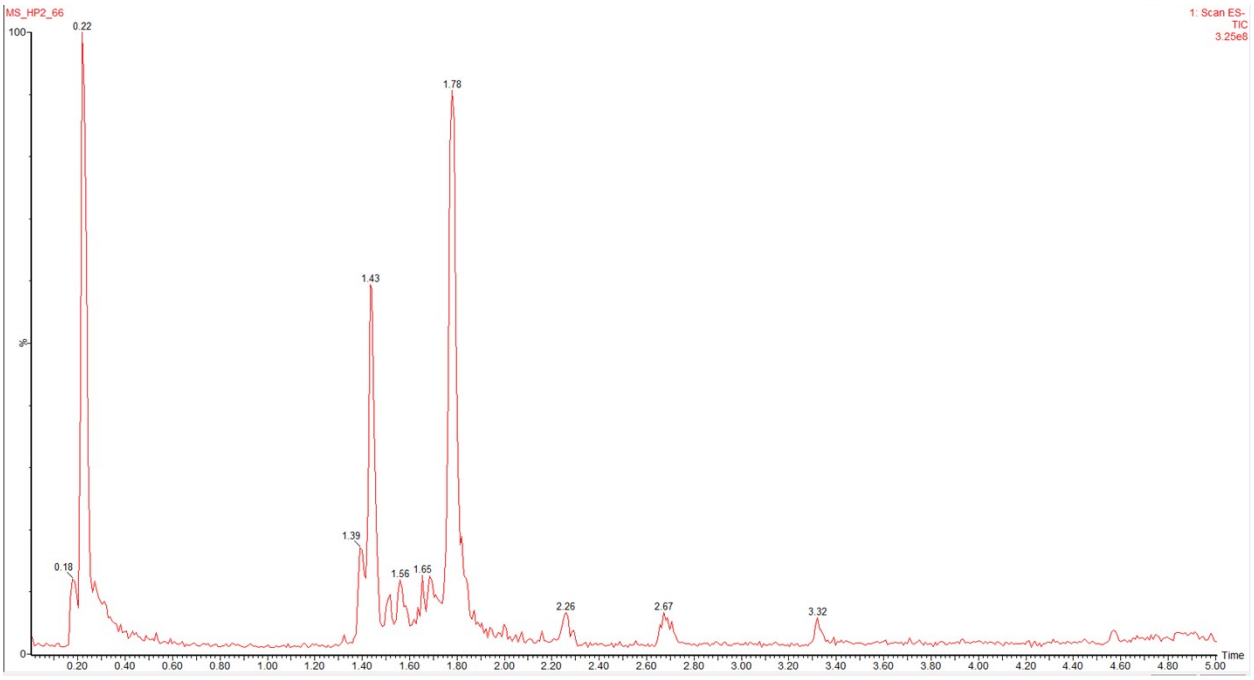


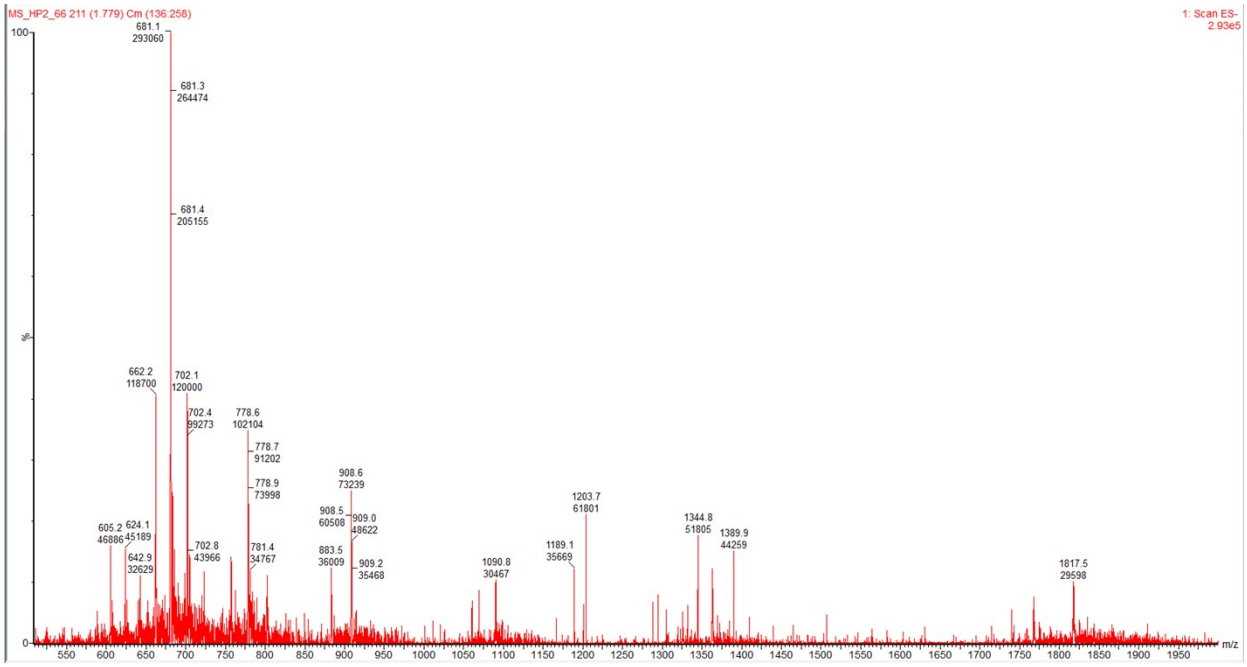


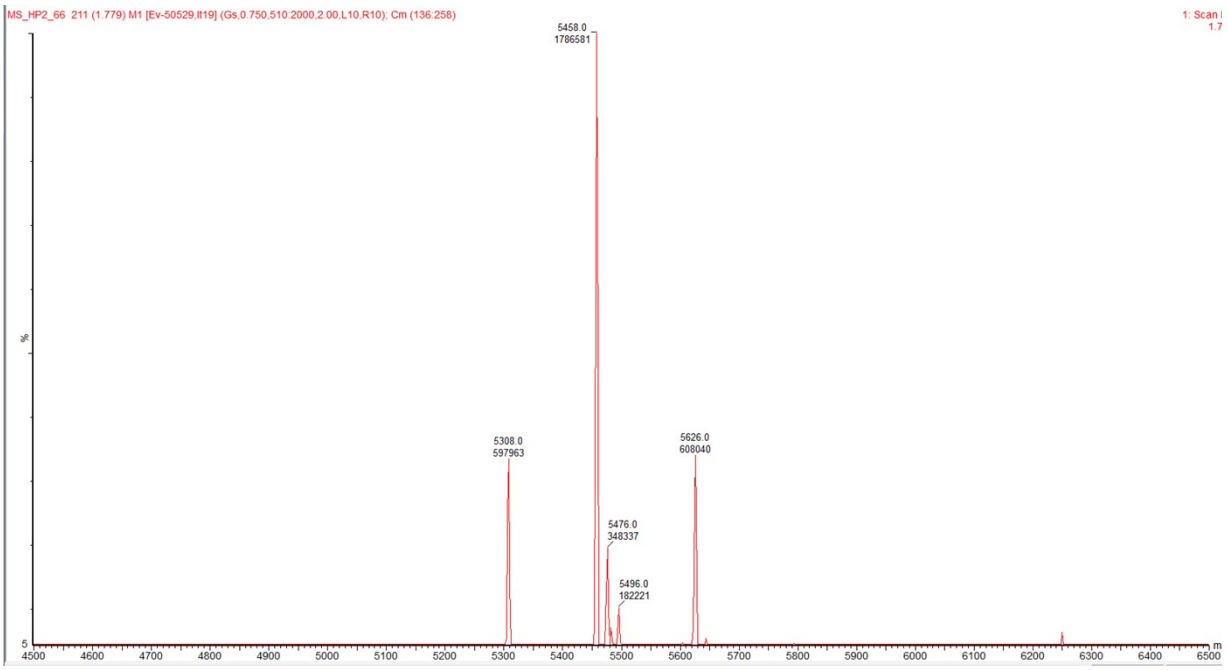
HP-1b (1 equiv) + **2e** (50 equiv)

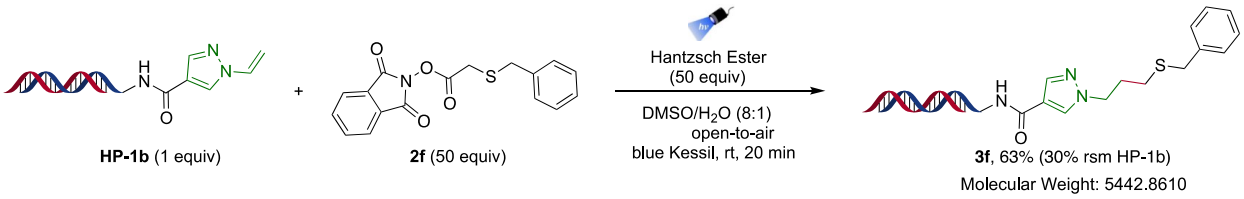
3e, 68% (27% rsm HP-1b)

Molecular Weight: 5456.9136


MS_HP2_66
1: Scan ES-
TIC
3.25e8
0.22
1.78
1.43
1.39
0.18
1.56
1.65
2.26
2.67
3.32
%
Time


MS_HP2_66 211 (1.779) Cm (136:258)
1: Scan ES-
2.93e5
681.1
293060
681.3
264474
681.4
205155
662.2
118700
702.1
120000
702.4
99273
778.6
102104
778.7
91202
778.9
73998
908.6
73239
908.5
60508
909.0
48622
909.2
35468
605.2
46886
624.1
45189
642.9
32629
702.8
43966
781.4
34767
883.5
36009
1090.8
30467
1189.1
35669
1203.7
61801
1344.8
51805
1389.9
44259
1817.5
29598
%
m/z

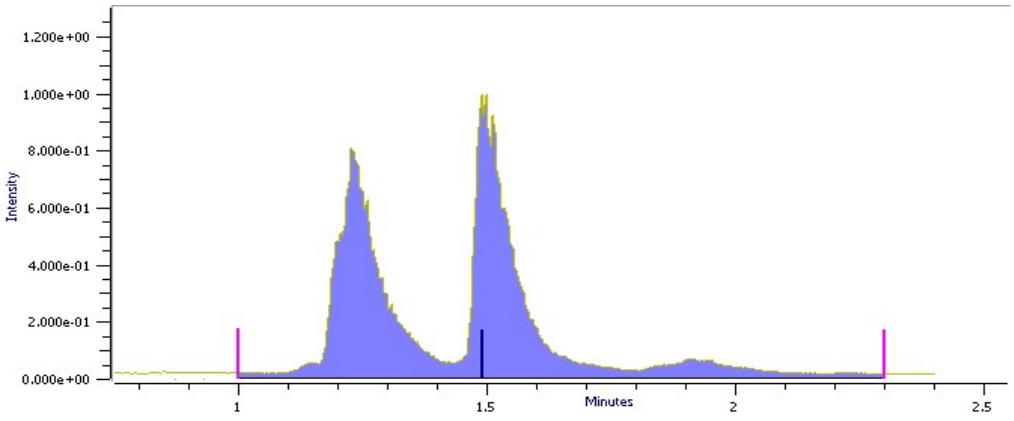


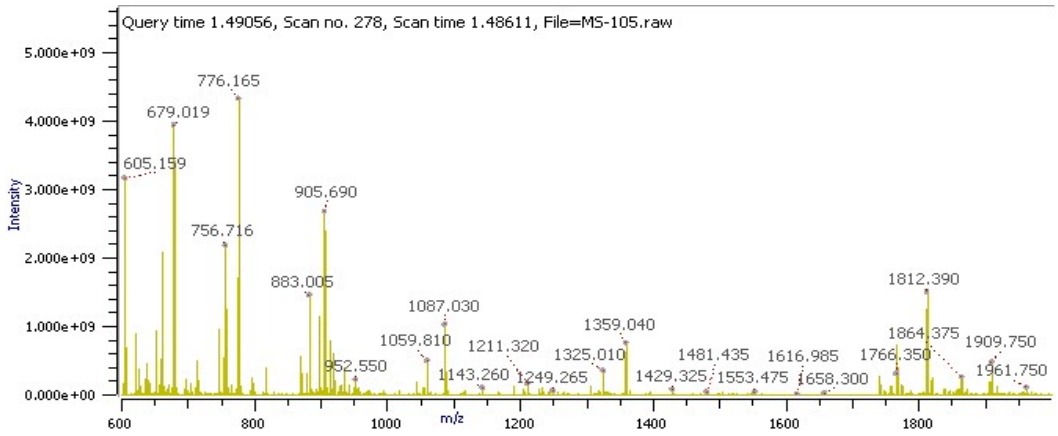


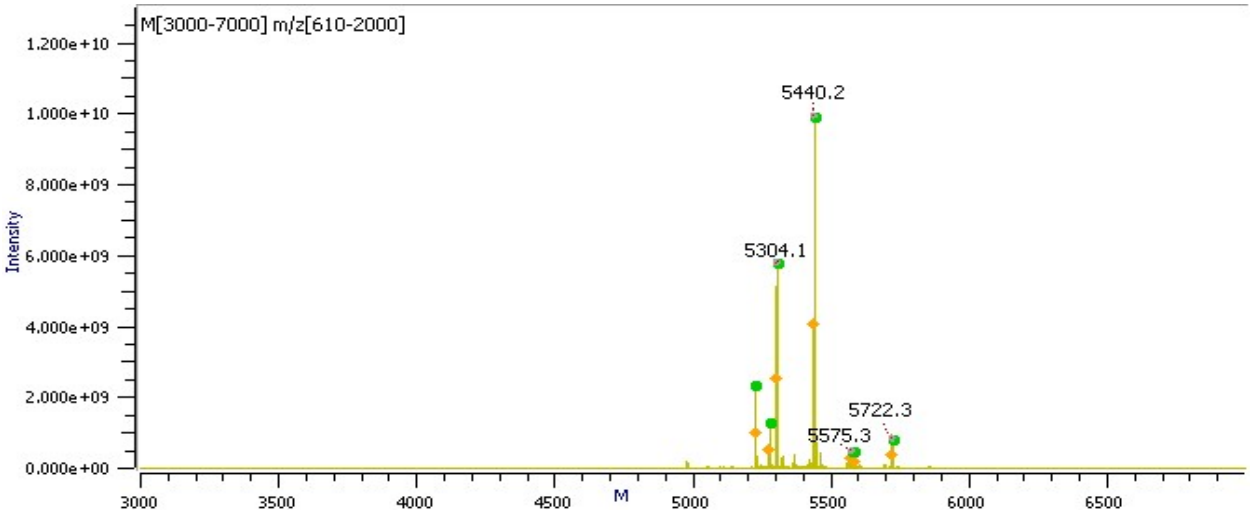
HP-1b (1 equiv) + **2f** (50 equiv)

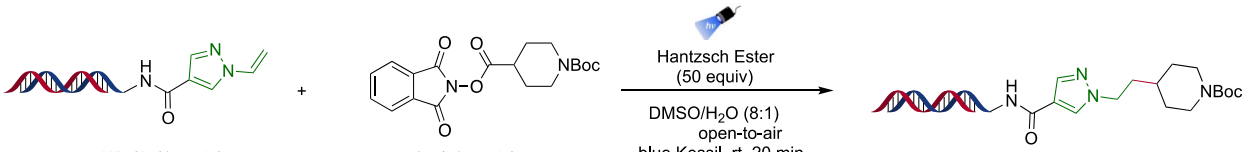
3f, 63% (30% rsm HP-1b)

Molecular Weight: 5442.8610





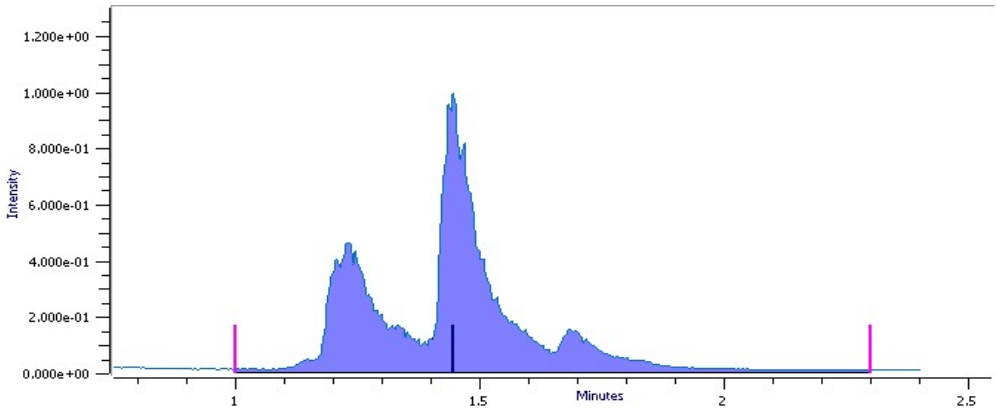


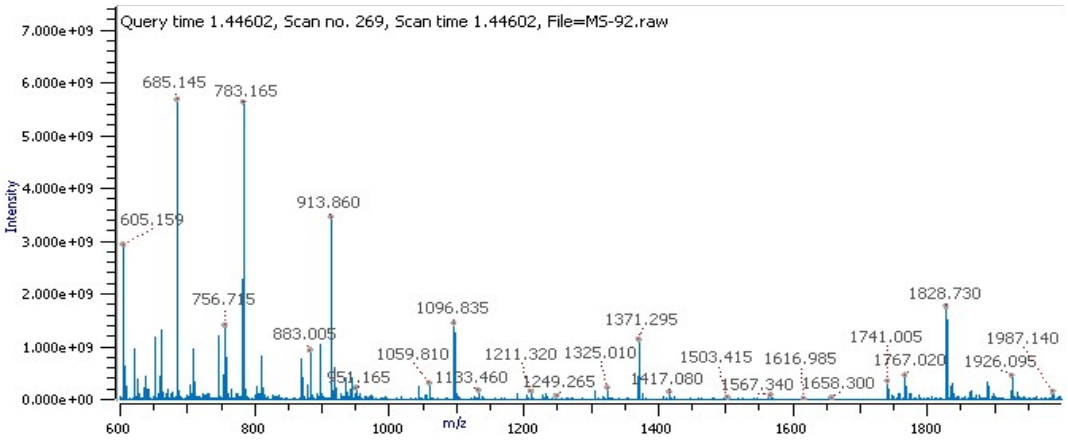


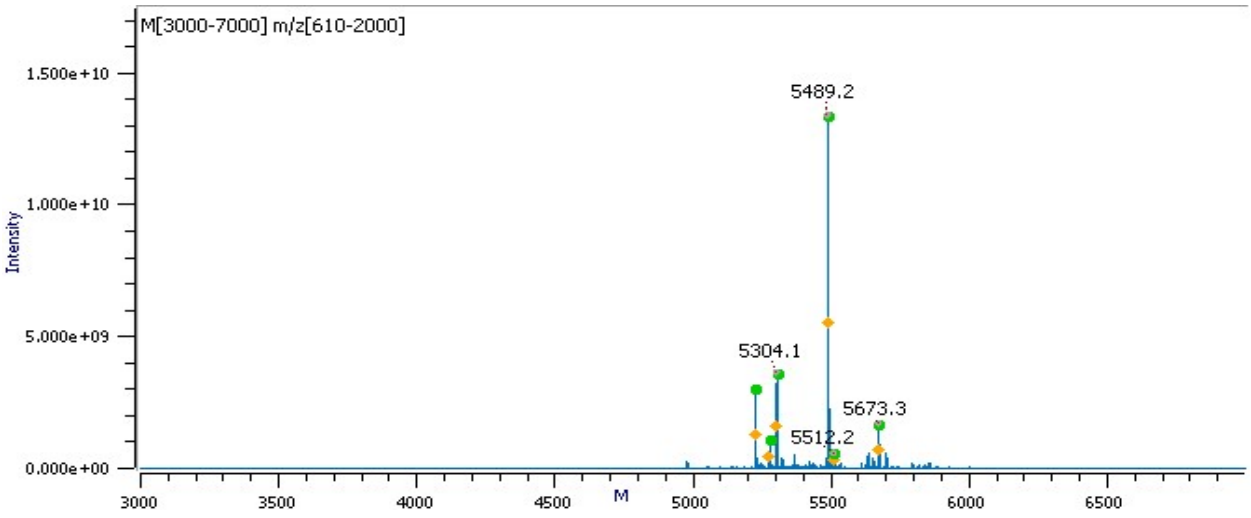
HP-1b (1 equiv) + **2g** (50 equiv)

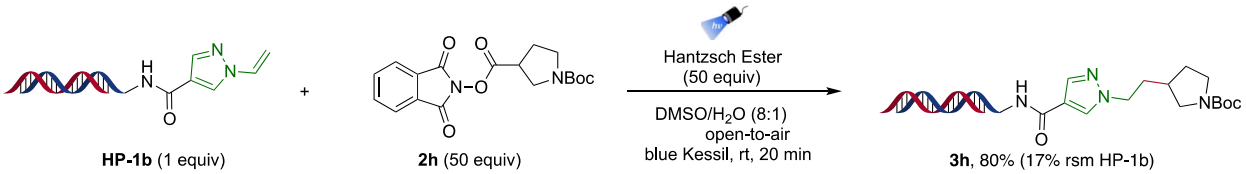
3g, 81% (17% rsm HP-1b)

Molecular Weight: 5489.9000





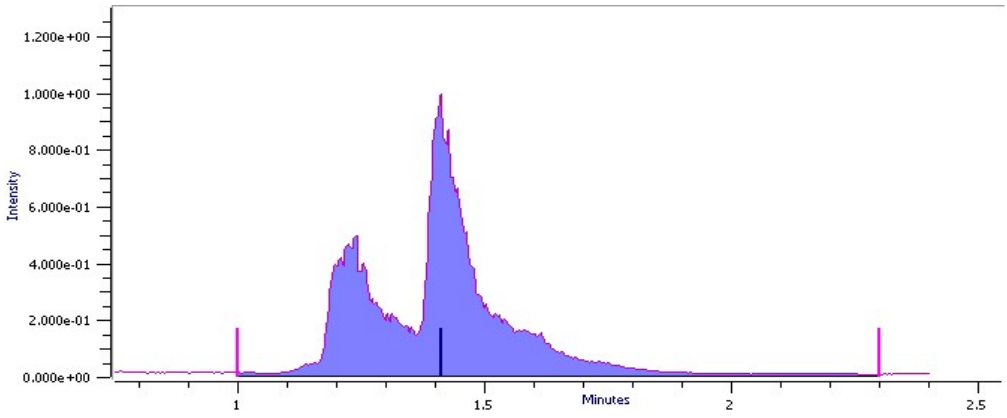


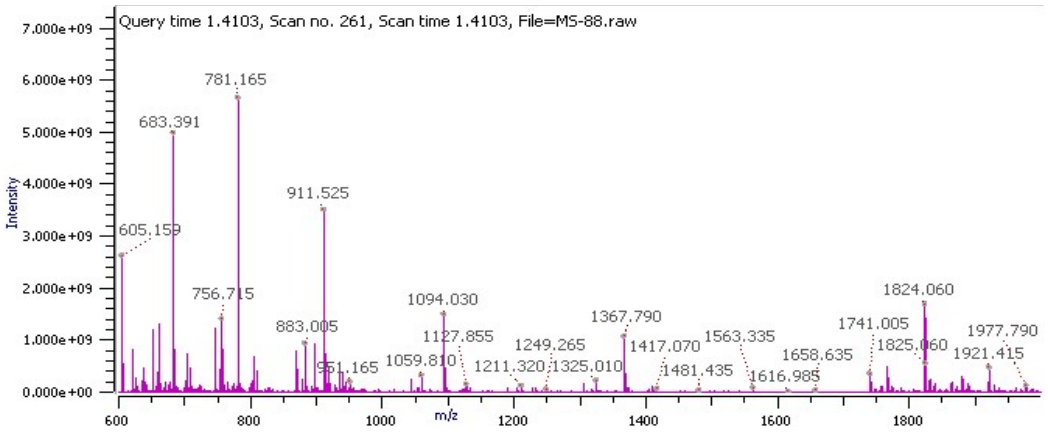


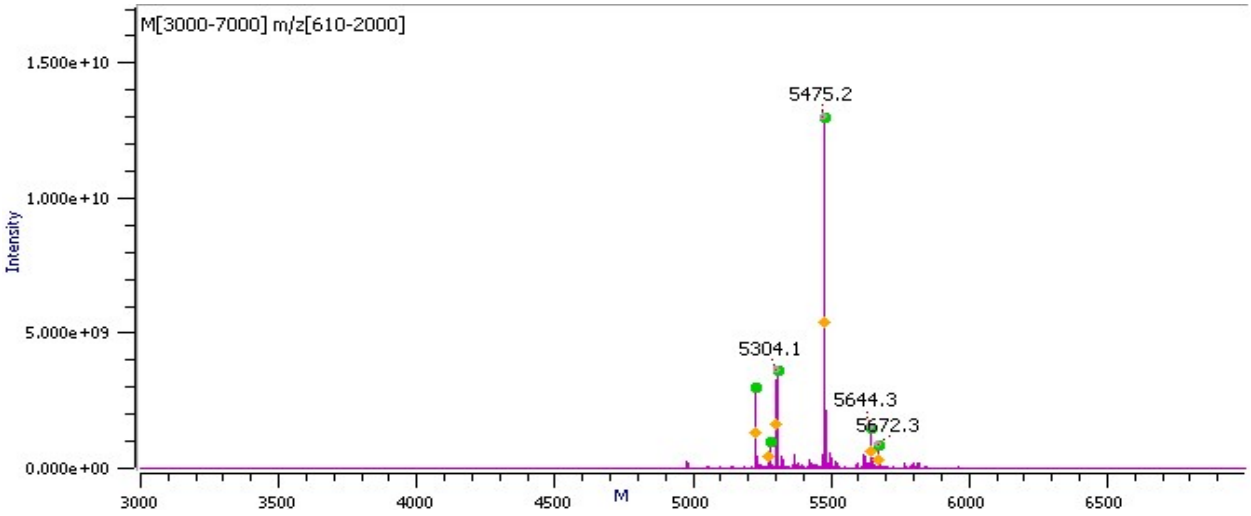
HP-1b (1 equiv) + **2h** (50 equiv)

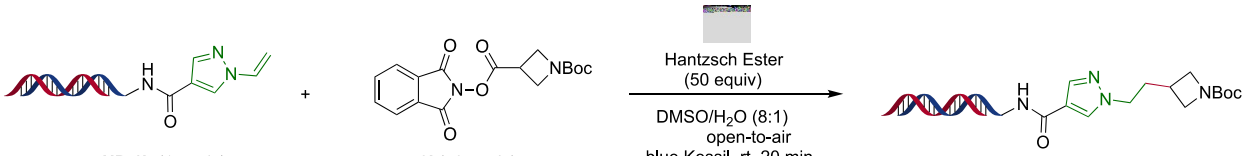
3h, 80% (17% rsm HP-1b)

Molecular Weight: 5475.8730





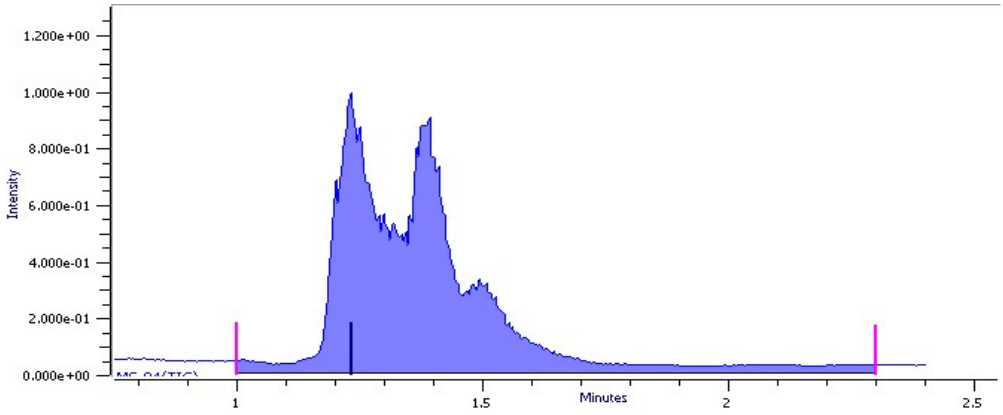


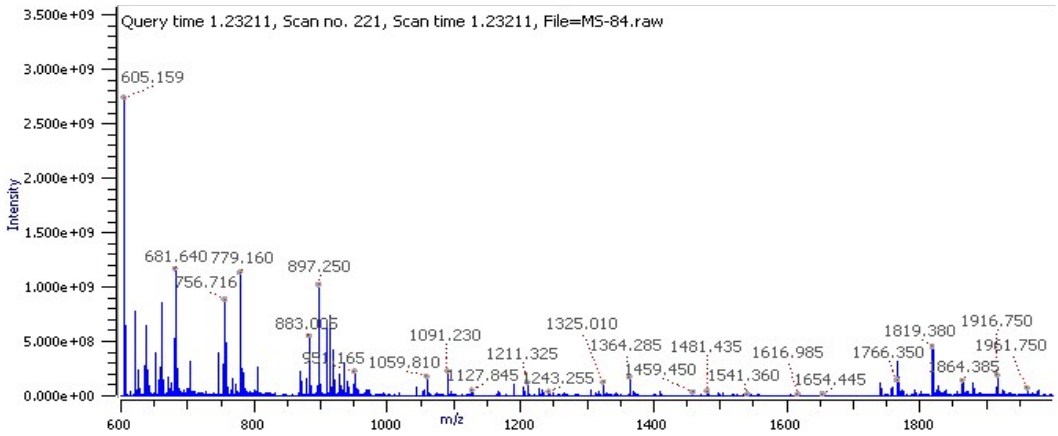


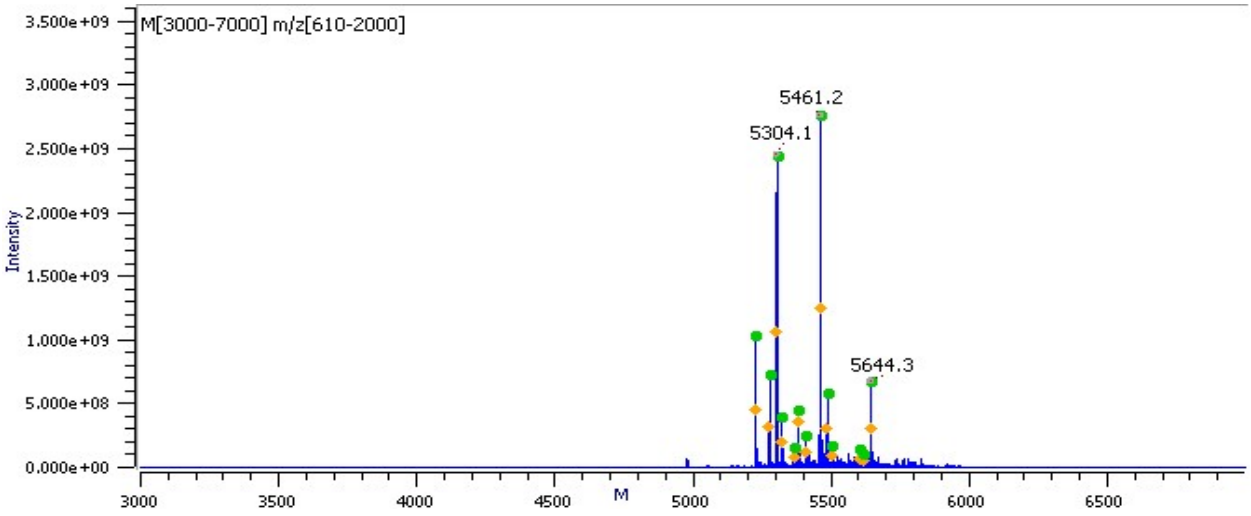
HP-1b (1 equiv) + **2i** (50 equiv)

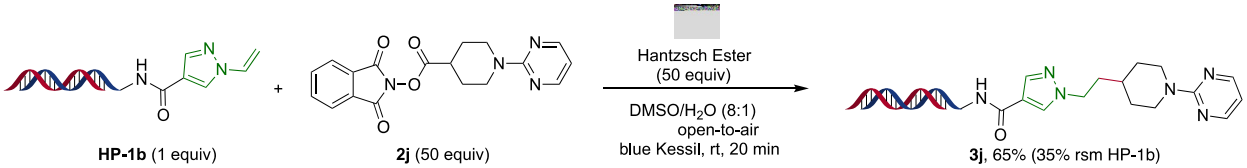
3i, 52% (30% rsm HP-1b)

Molecular Weight: 5461.8460





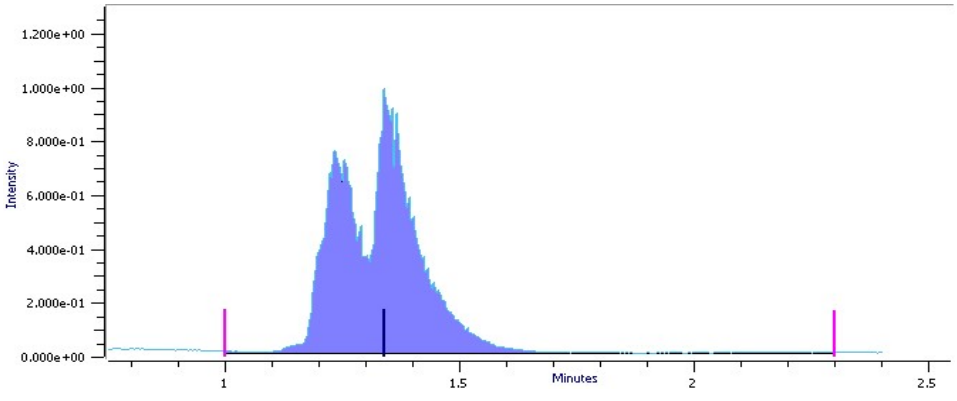


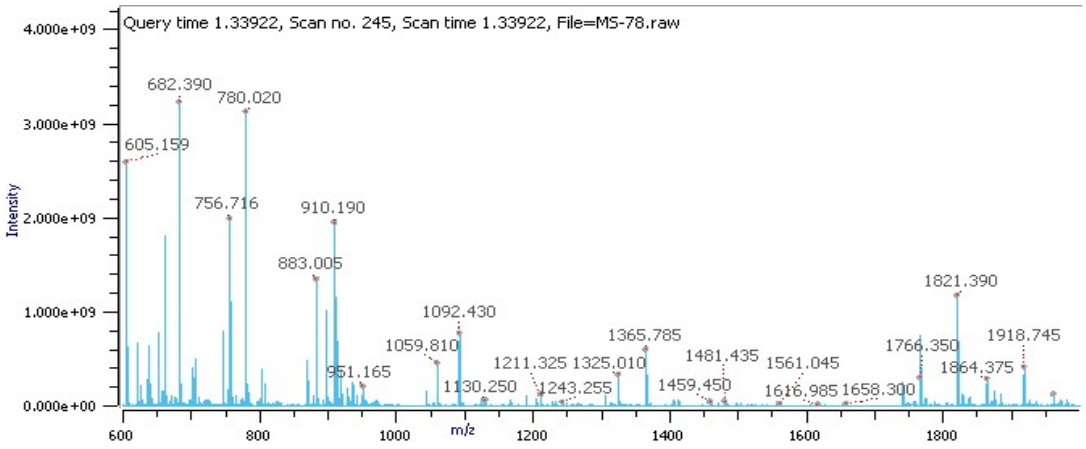


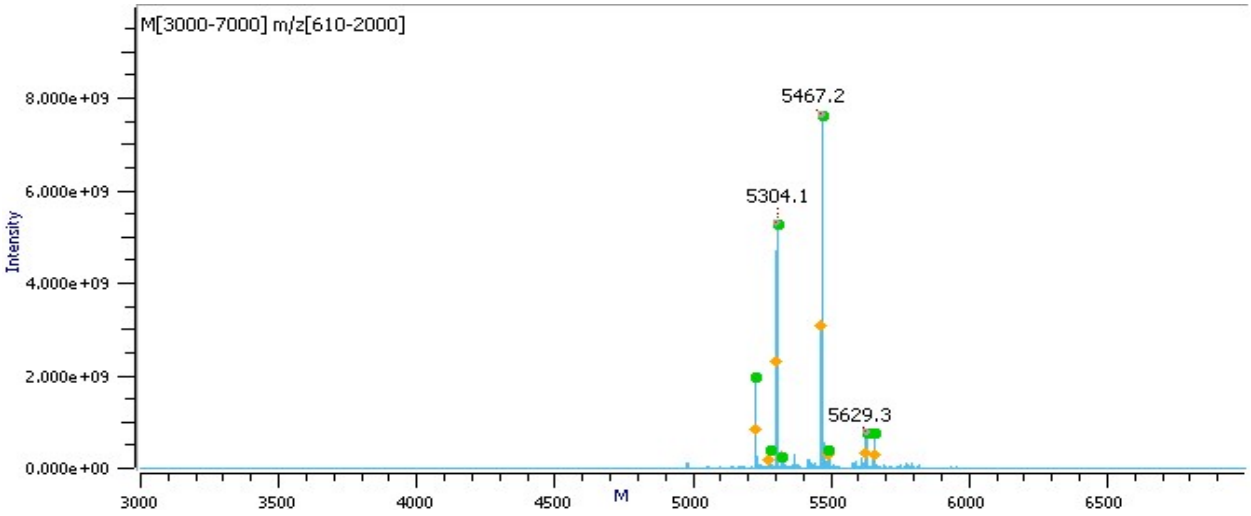
HP-1b (1 equiv) + **2j** (50 equiv)

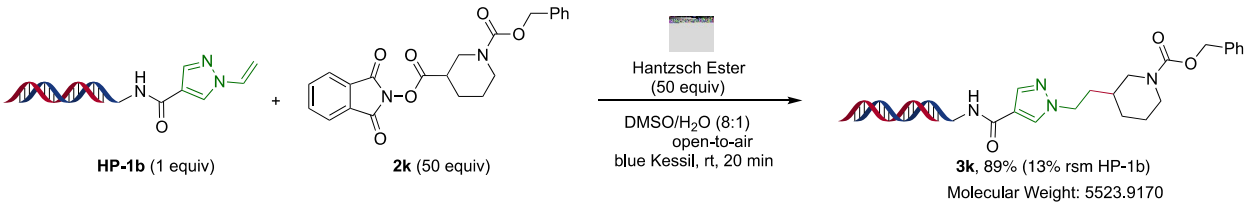
3j, 65% (35% rsm HP-1b)

Molecular Weight: 5467.8570





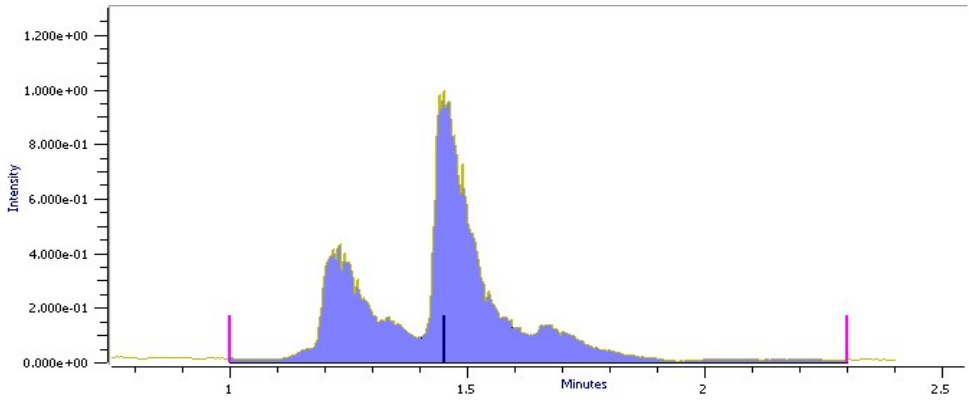


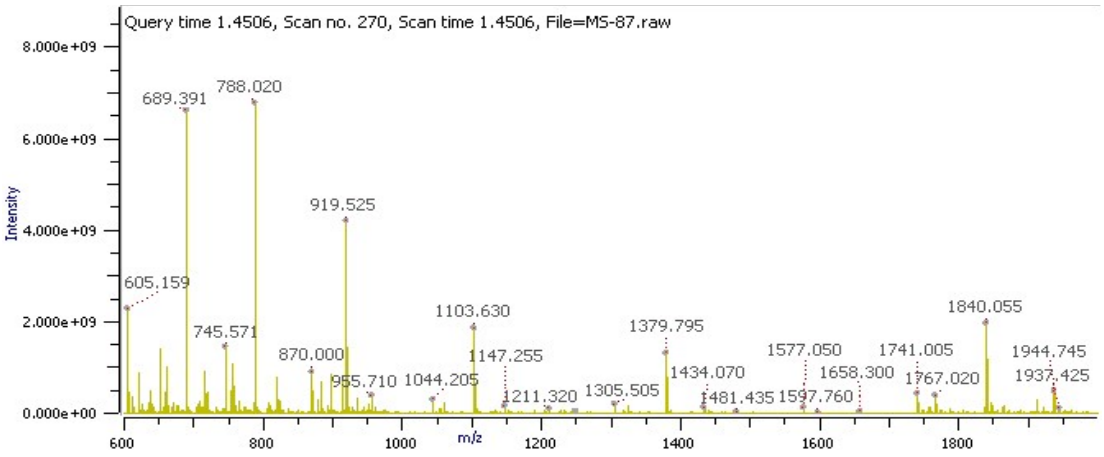


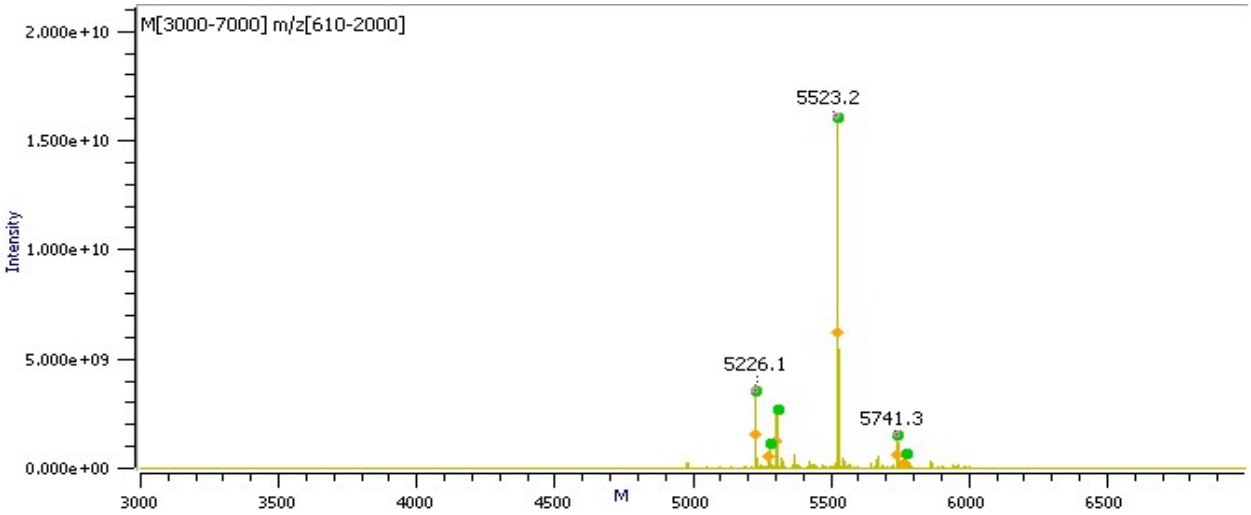
HP-1b (1 equiv) + **2k** (50 equiv)

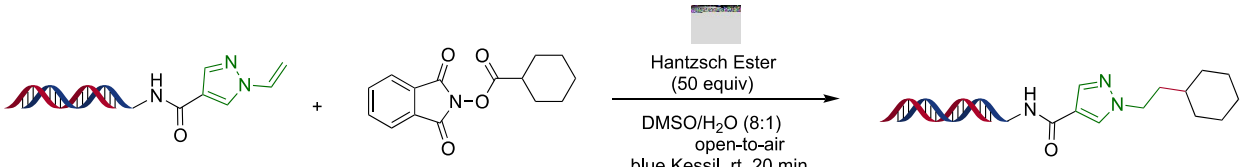
3k, 89% (13% rsm HP-1b)

Molecular Weight: 5523.9170



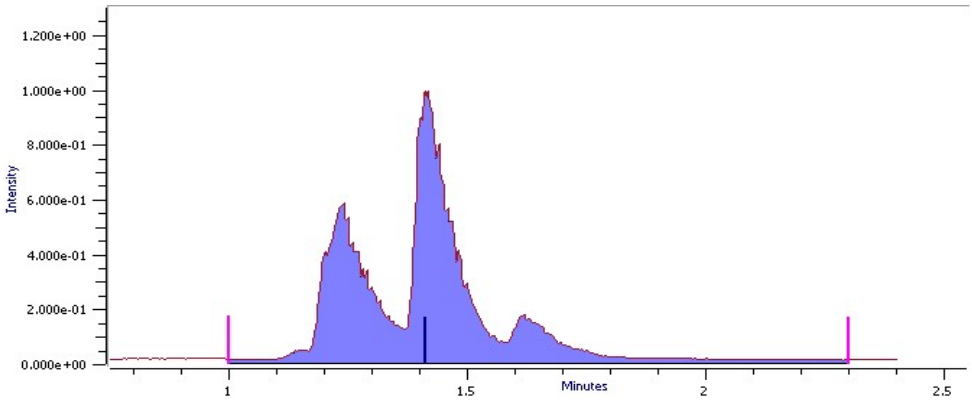


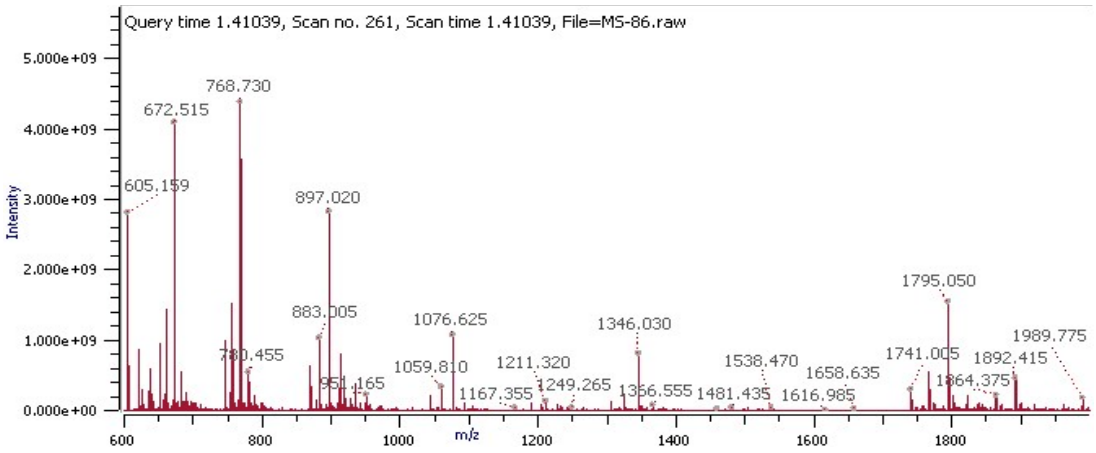


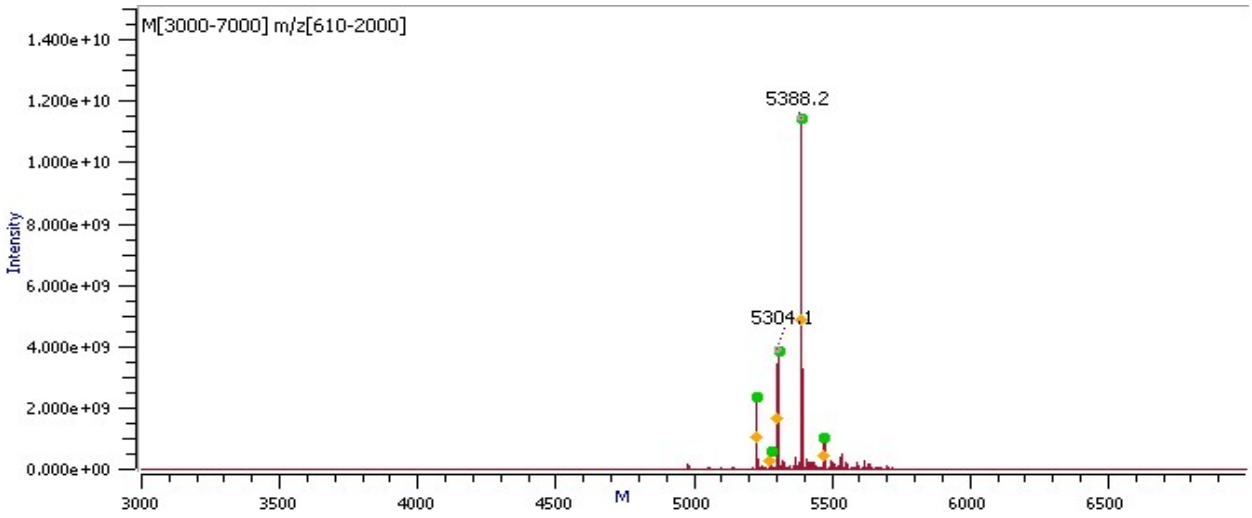


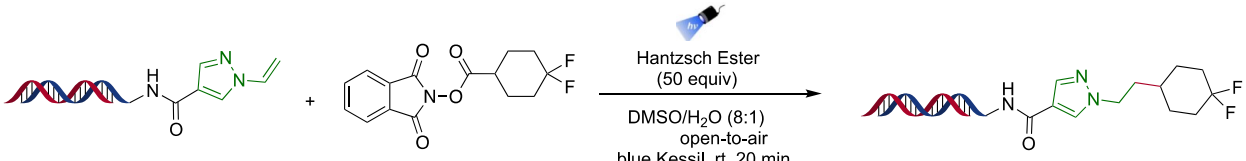
HP-1b (1 equiv) + **2l** (50 equiv)

3l, 70% (21% rsm HP-1b)
Molecular Weight: 5388.7950



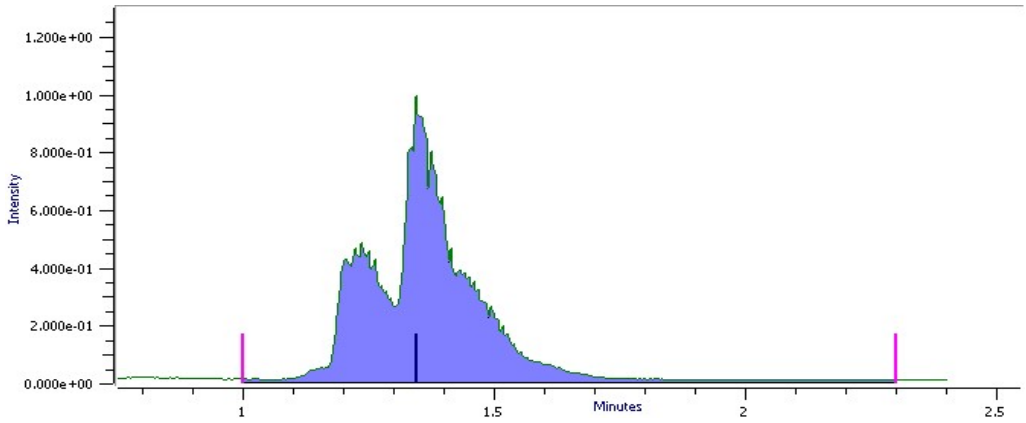


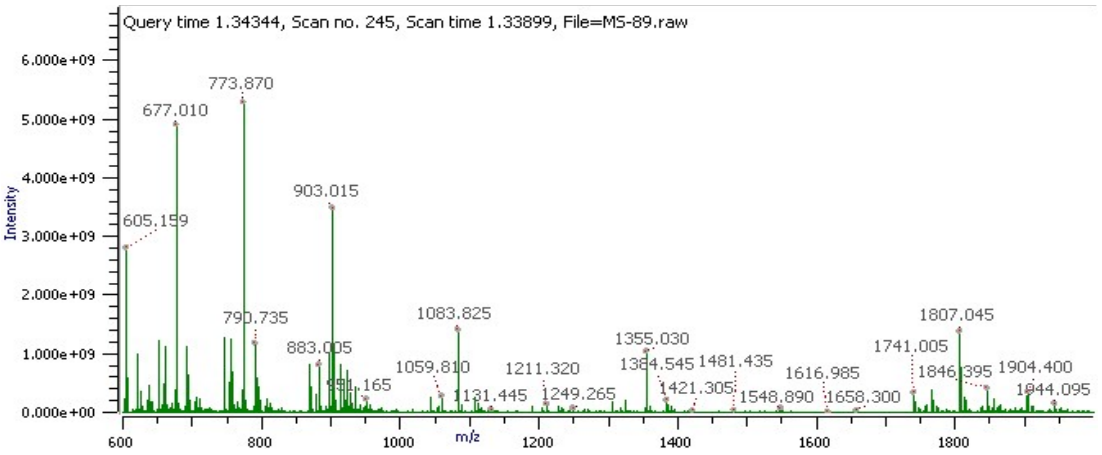


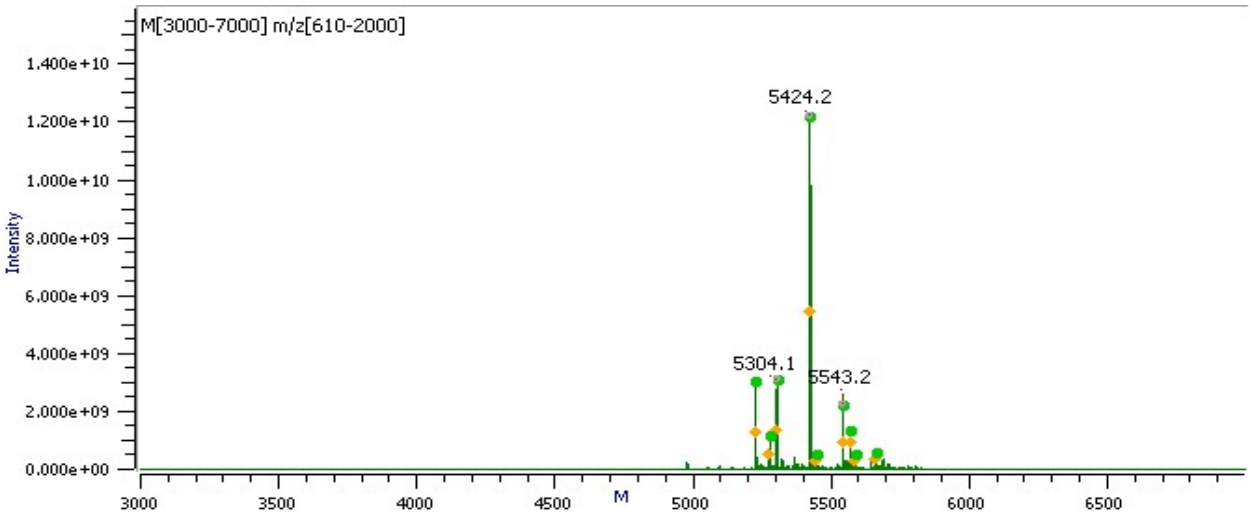


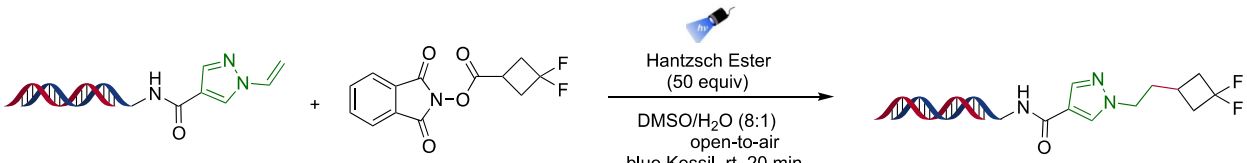
HP-1b (1 equiv) + **2m** (50 equiv)

3m, 64% (14% rsm HP-1b)
Molecular Weight: 5424.7758



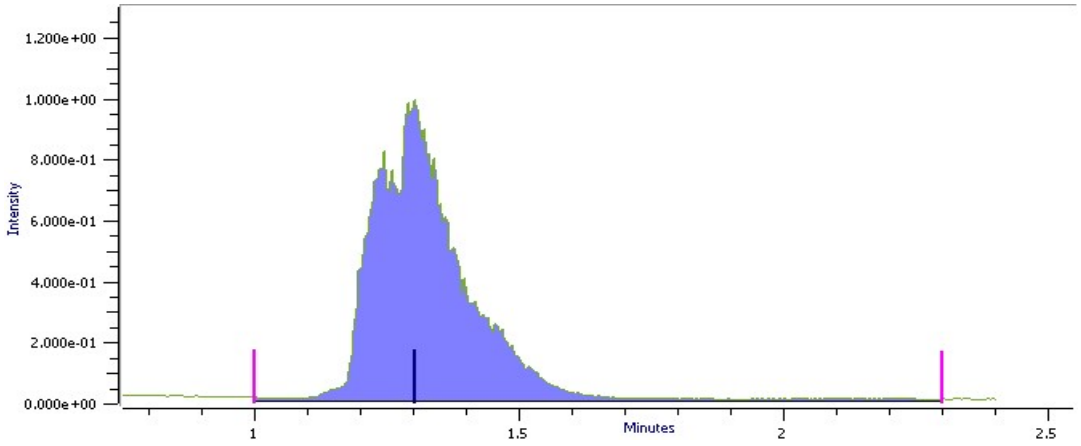


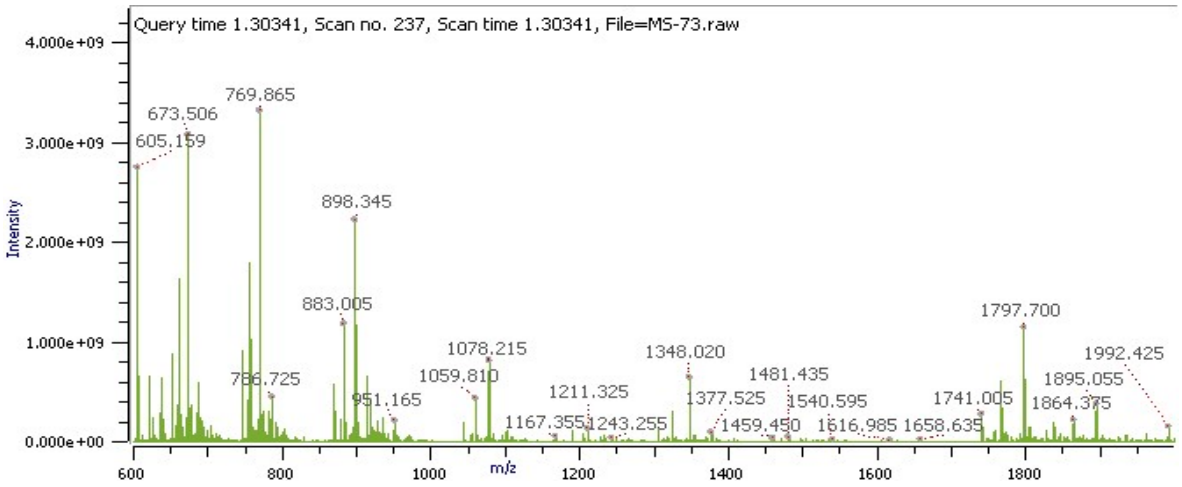


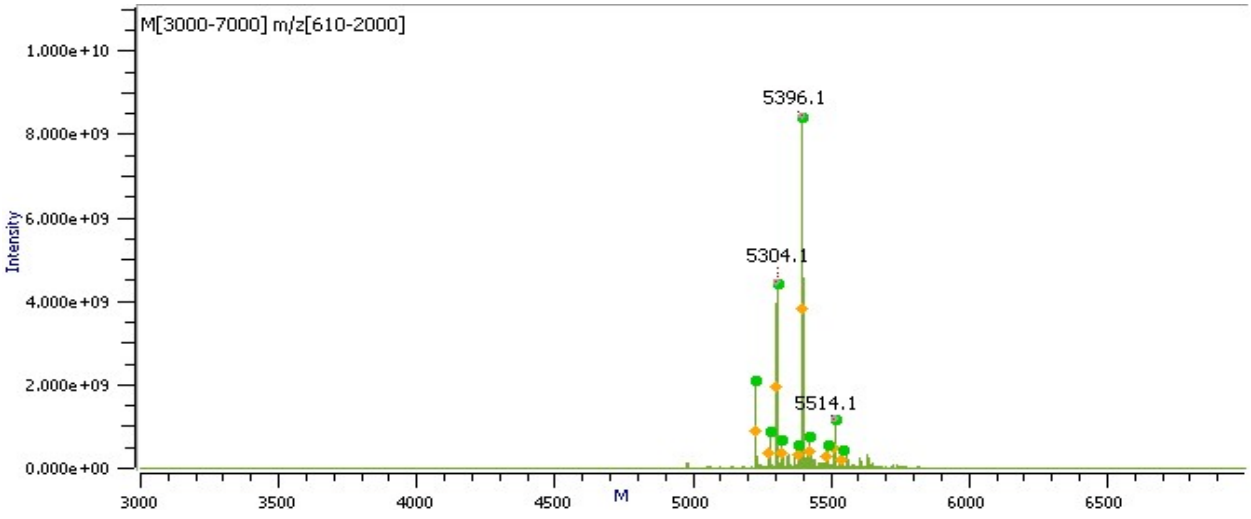


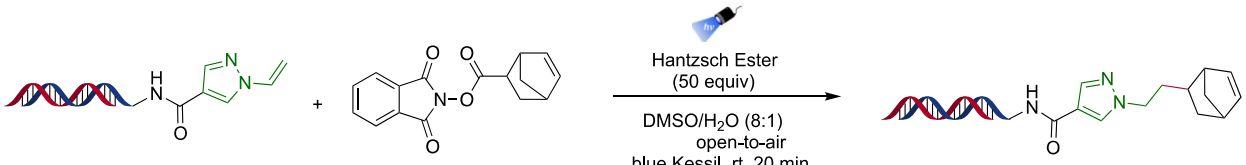
HP-1b (1 equiv) + **2n** (50 equiv)

3n, 60% (27% rsm HP-1b)
Molecular Weight: 5396.7218



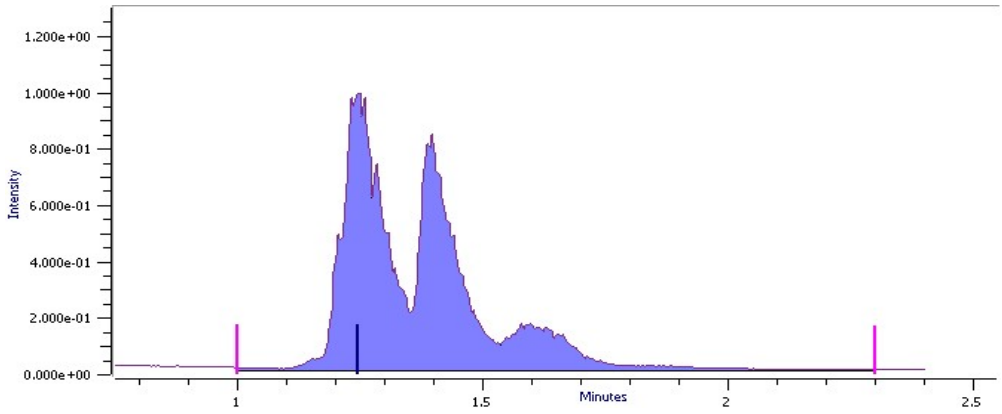


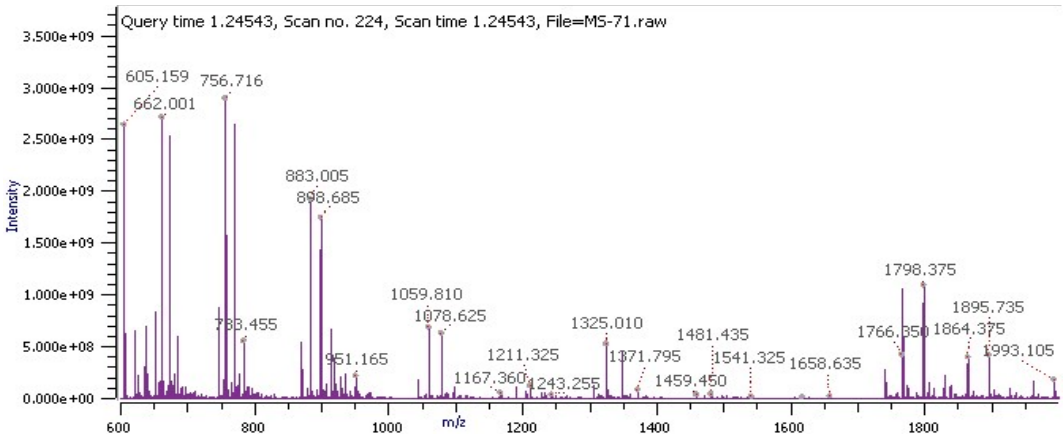


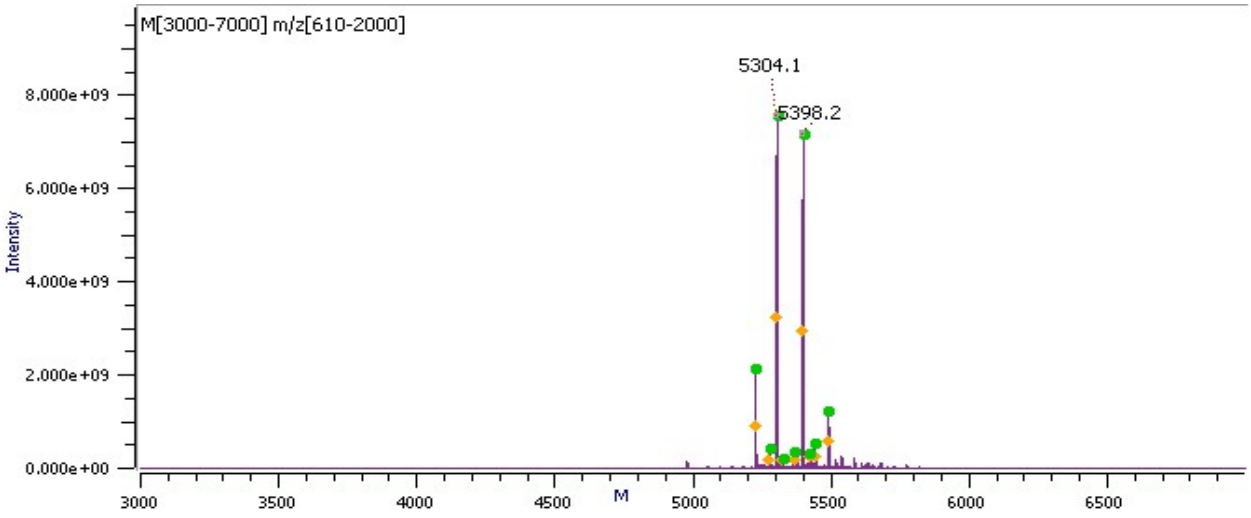


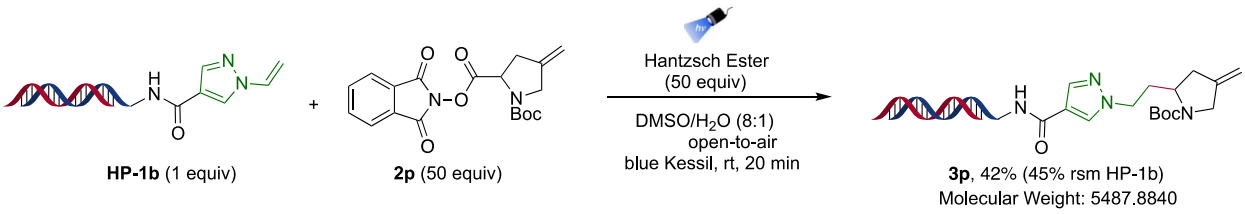
HP-1b (1 equiv) + **2o** (50 equiv)

3o, 45% (41% rsm HP-1b)
Molecular Weight: 5398.7900



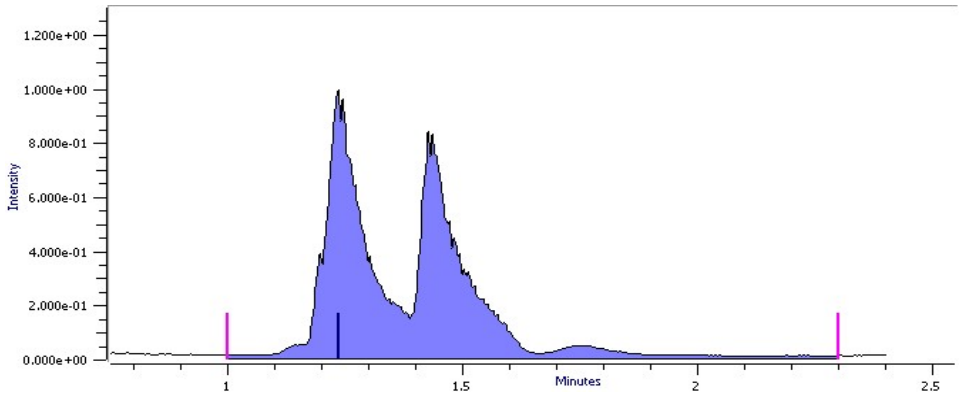


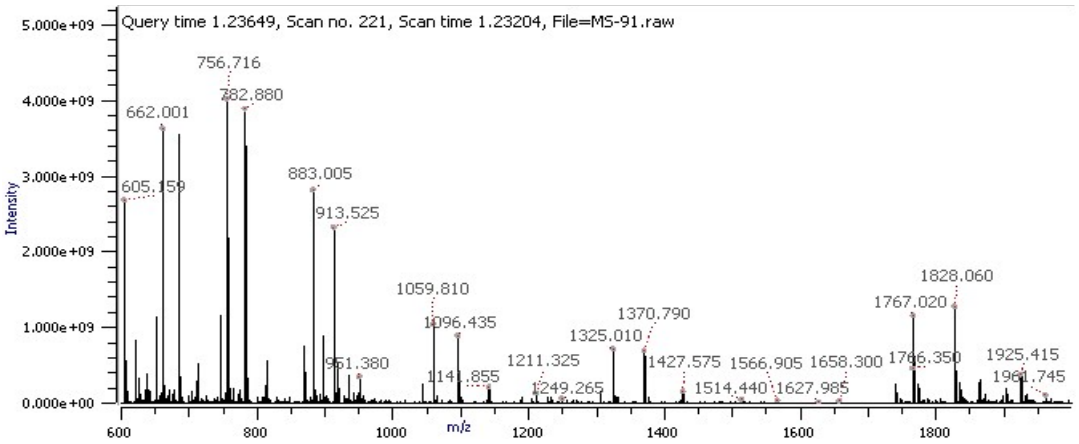


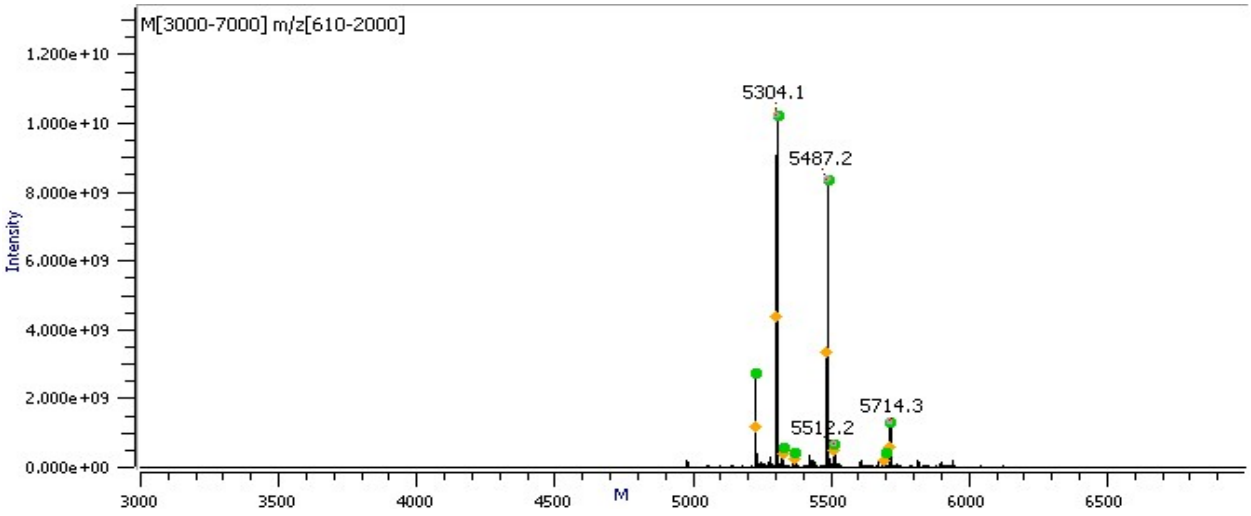


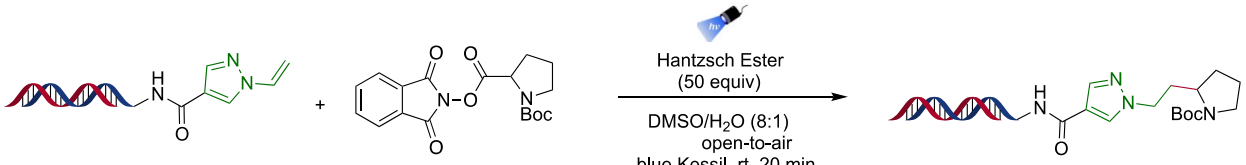
HP-1b (1 equiv) + **2p** (50 equiv)

3p, 42% (45% rsm HP-1b)
Molecular Weight: 5487.8840



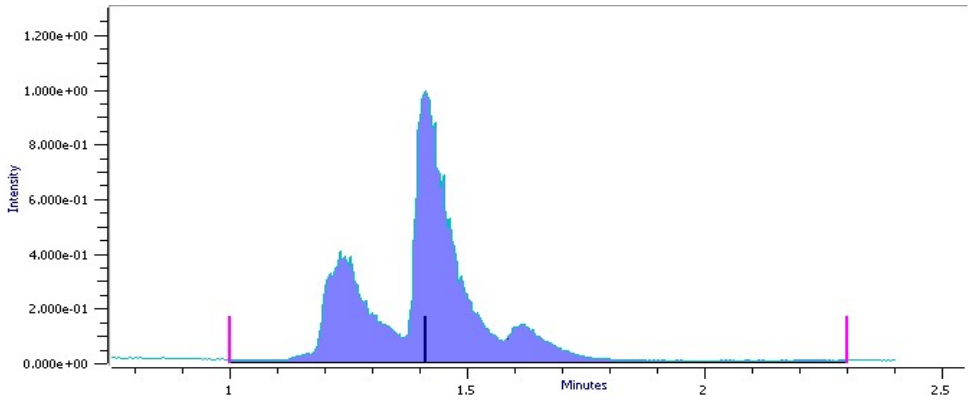


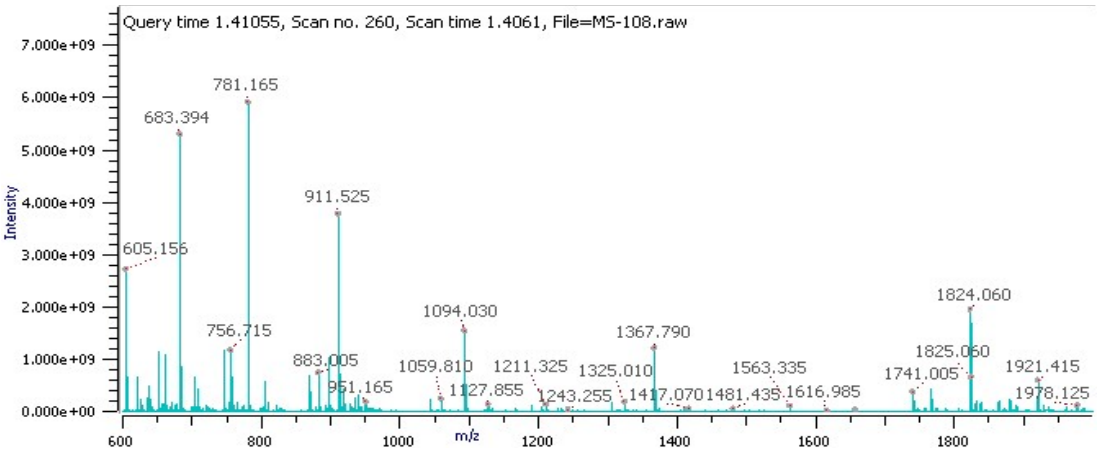


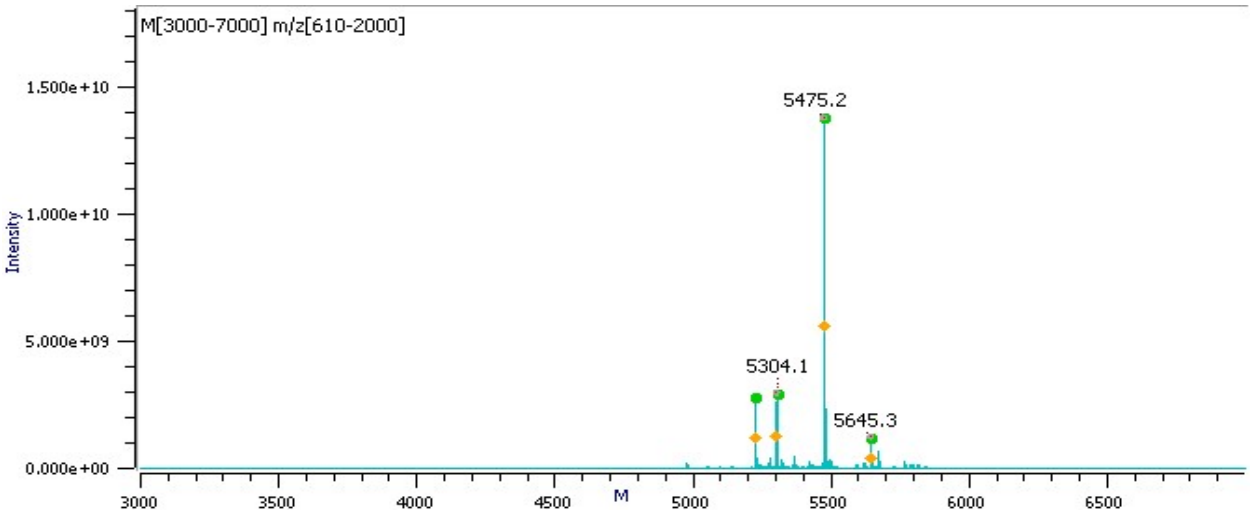


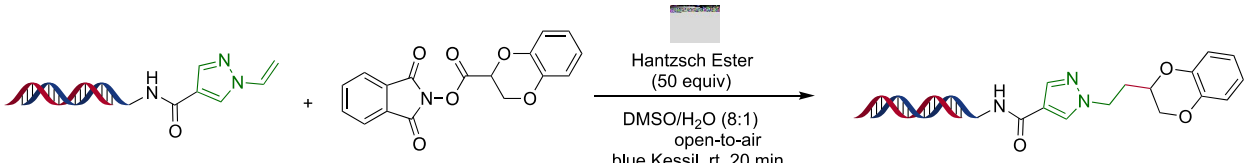
HP-1b (1 equiv) + **2q** (50 equiv)

3q, 82% (15% rsm HP-1b)
Molecular Weight: 5475.8730



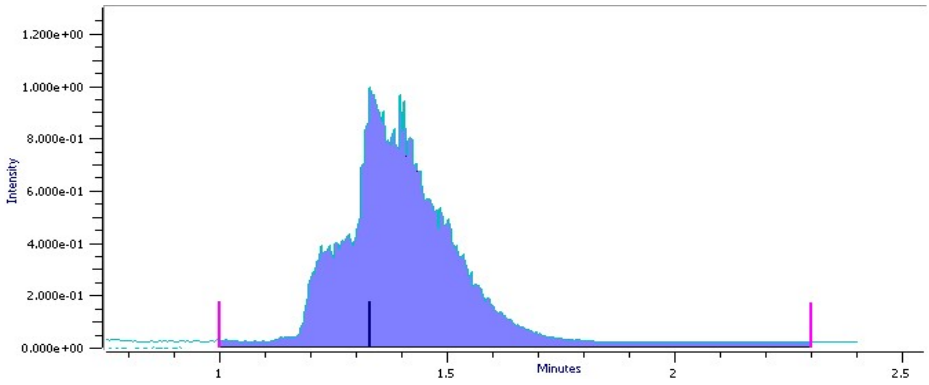


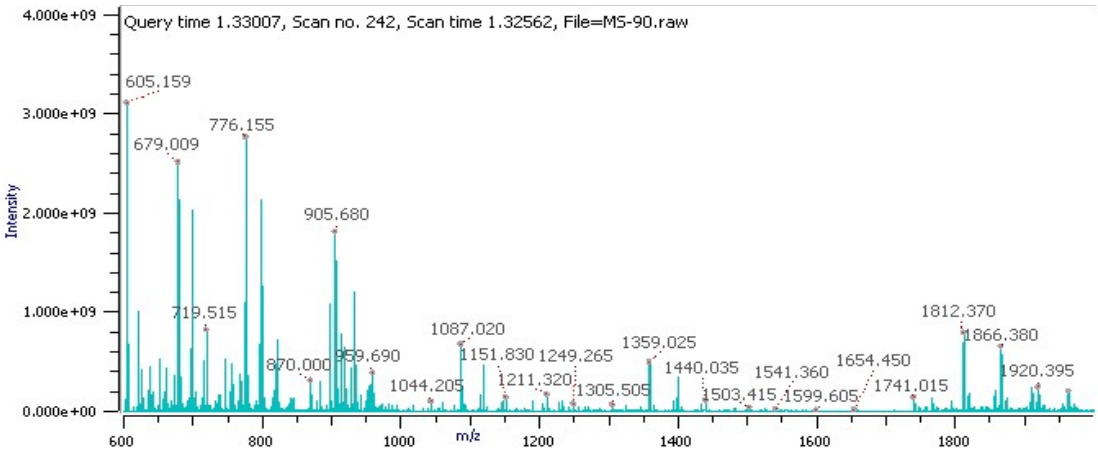


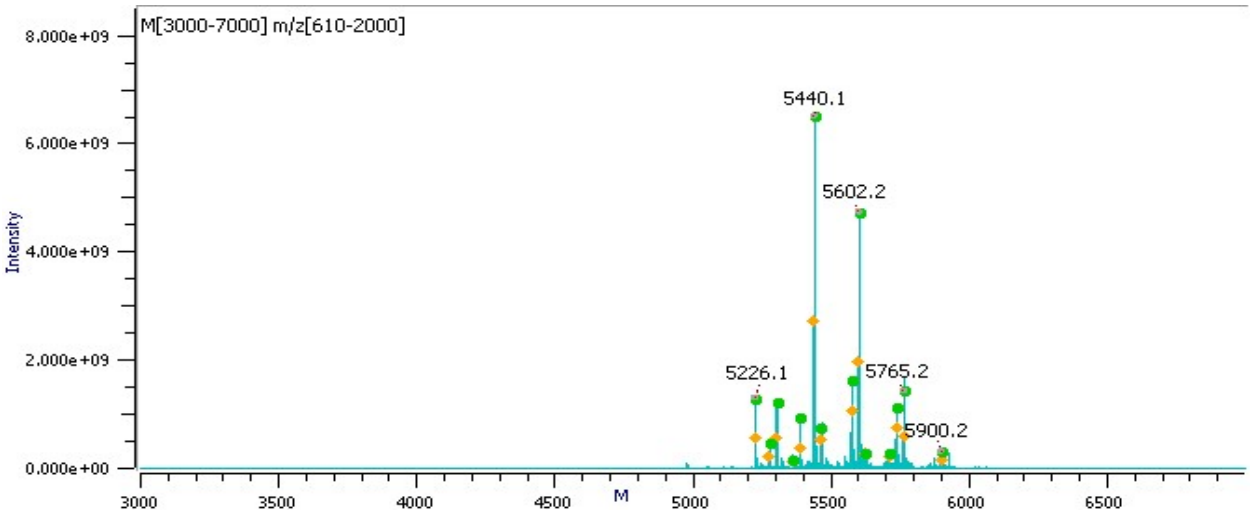


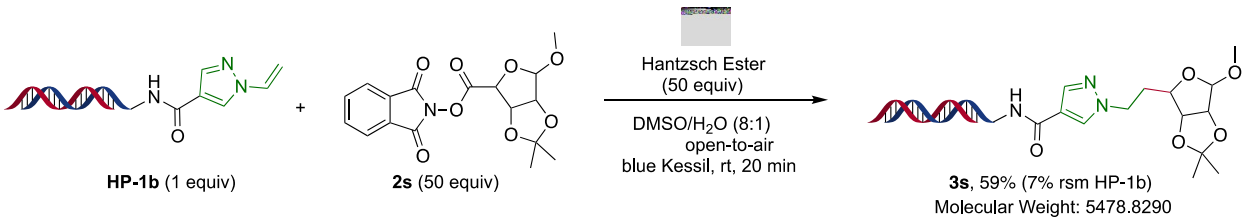
HP-1b (1 equiv) + **2r** (50 equiv)

3r, 67% (7% rsm HP-1b)
Molecular Weight: 5440.7830



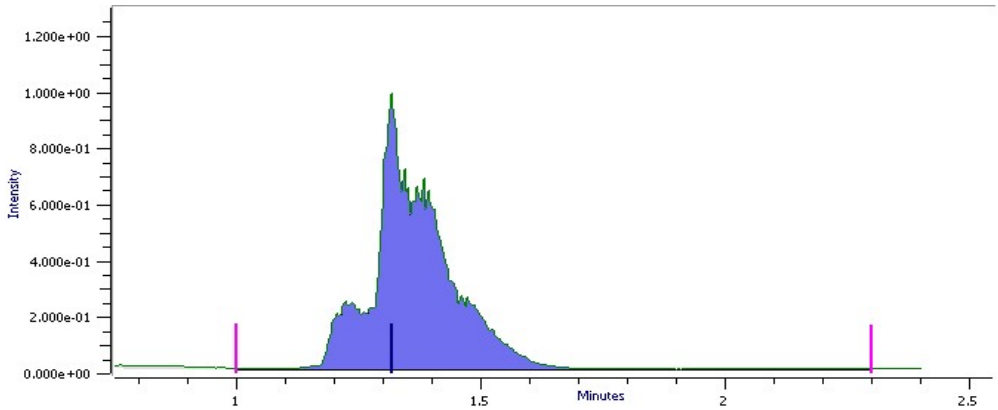


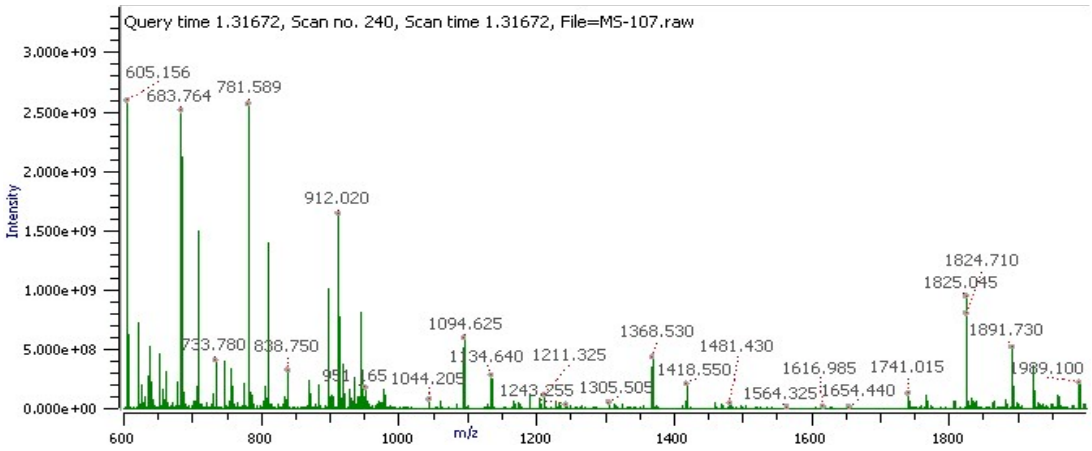


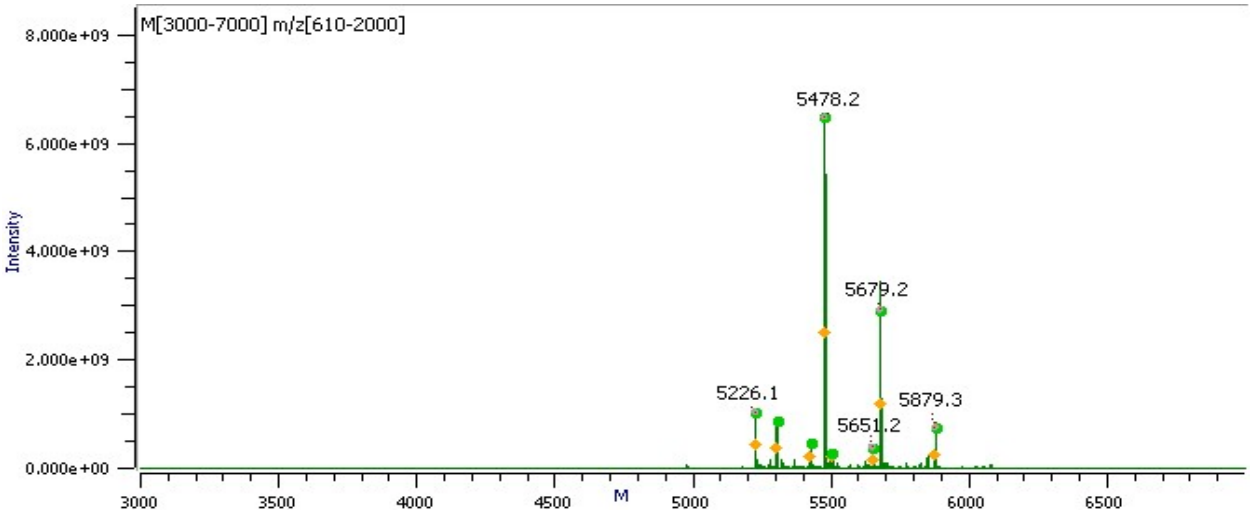


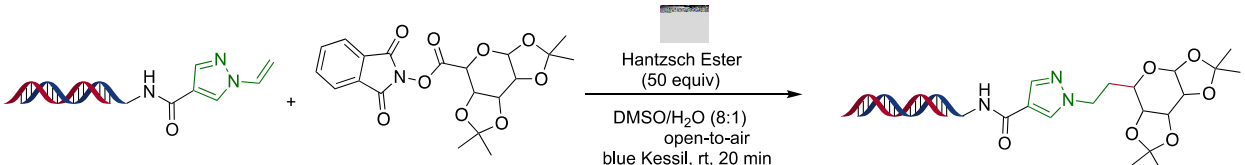
HP-1b (1 equiv) + **2s** (50 equiv)

3s, 59% (7% rsm HP-1b)
Molecular Weight: 5478.8290



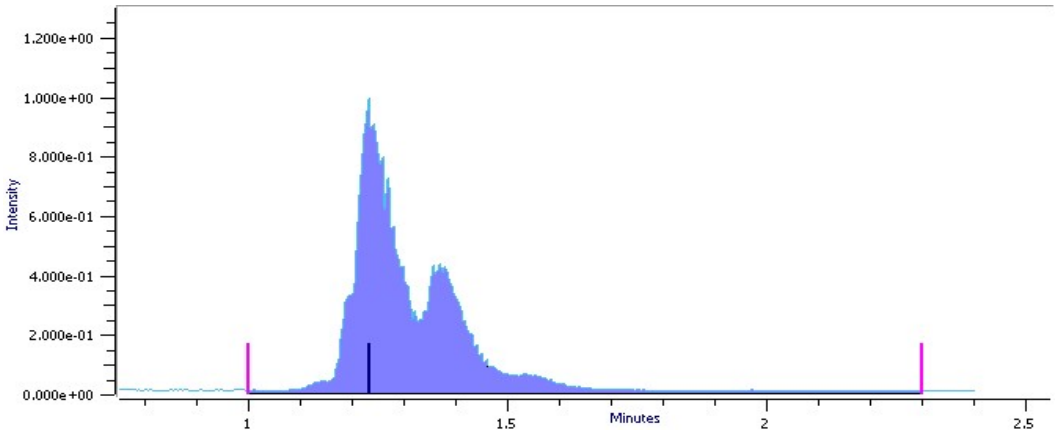


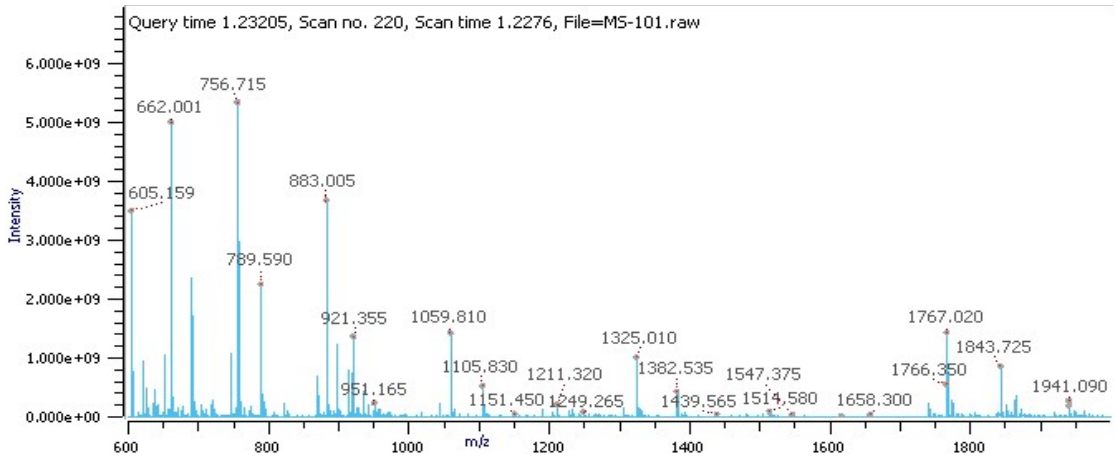


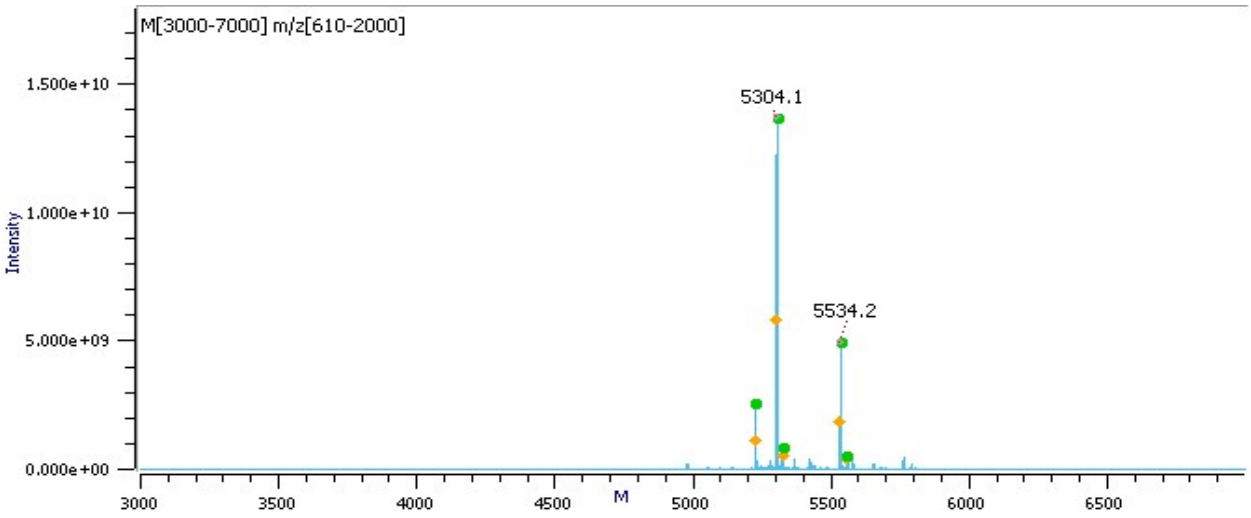


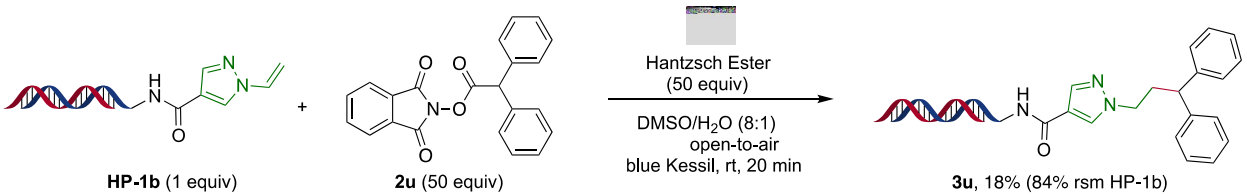
HP-1b (1 equiv) + **2t** (50 equiv)

3t, 26% (65% rsm HP-1b)
Molecular Weight: 5534.8930



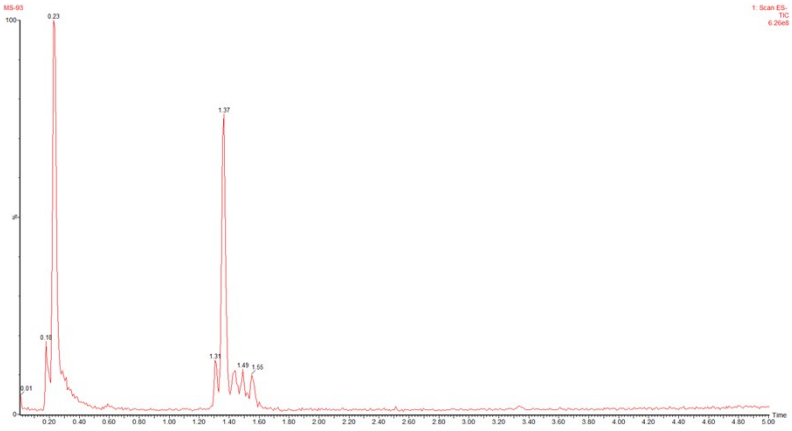


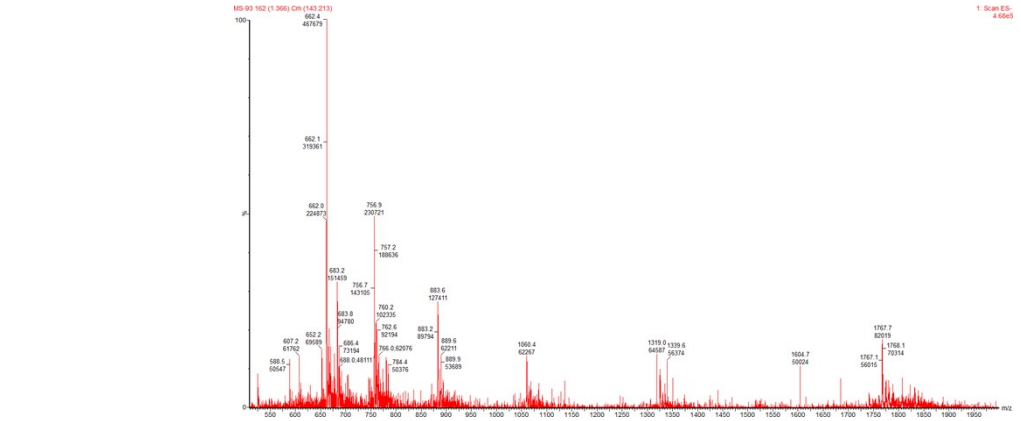


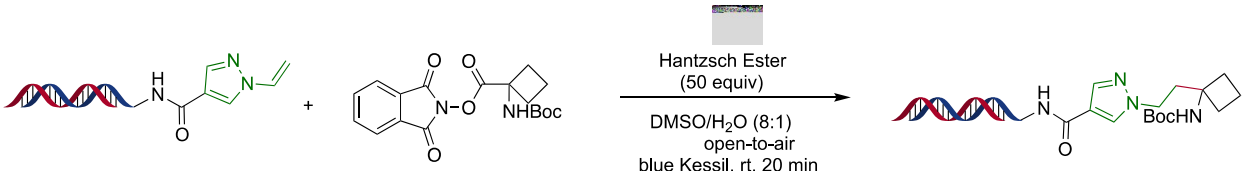


HP-1b (1 equiv) + **2u** (50 equiv)

3u, 18% (84% rsm HP-1b)
Molecular Weight: 5472.8720



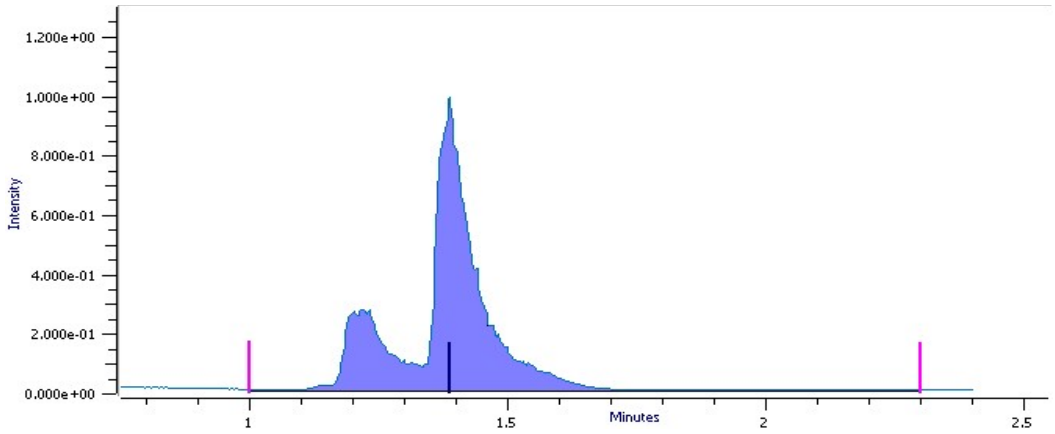


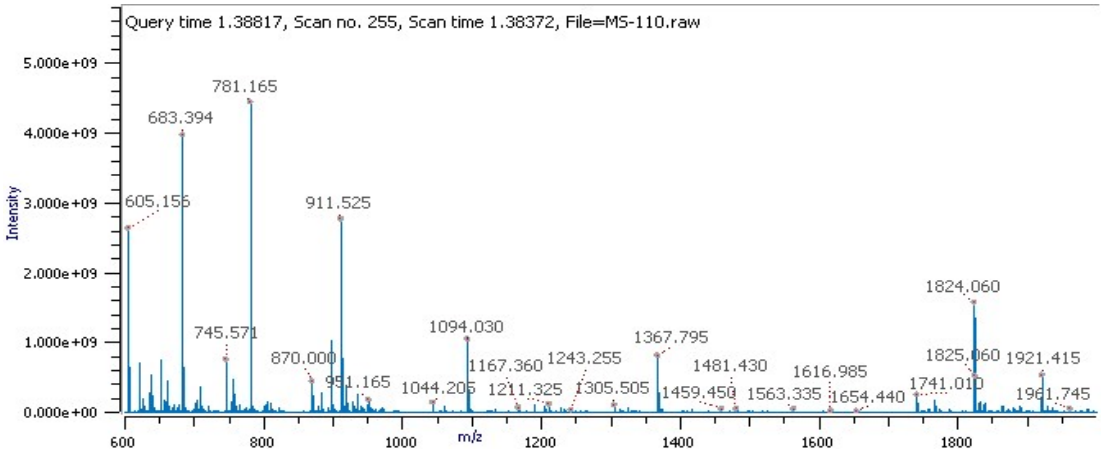


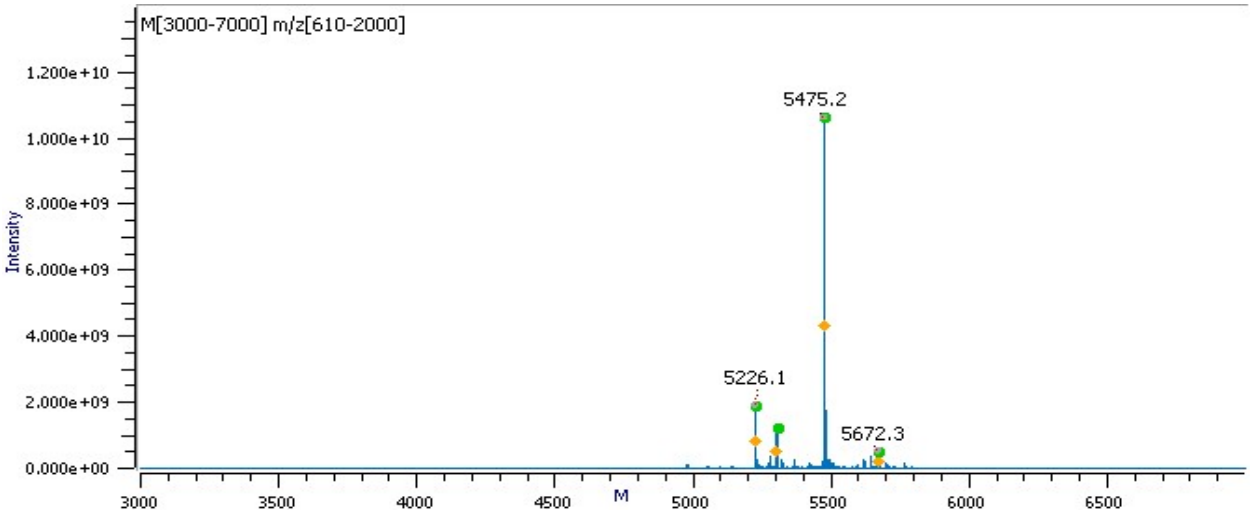
HP-1b (1 equiv)

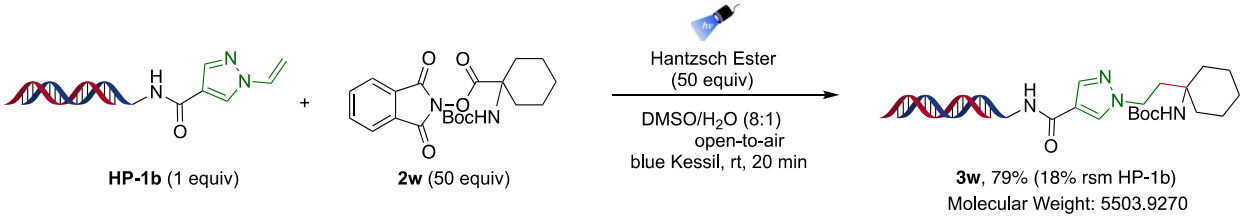
2v (50 equiv)

3v, >95%
Molecular Weight: 5475.8730



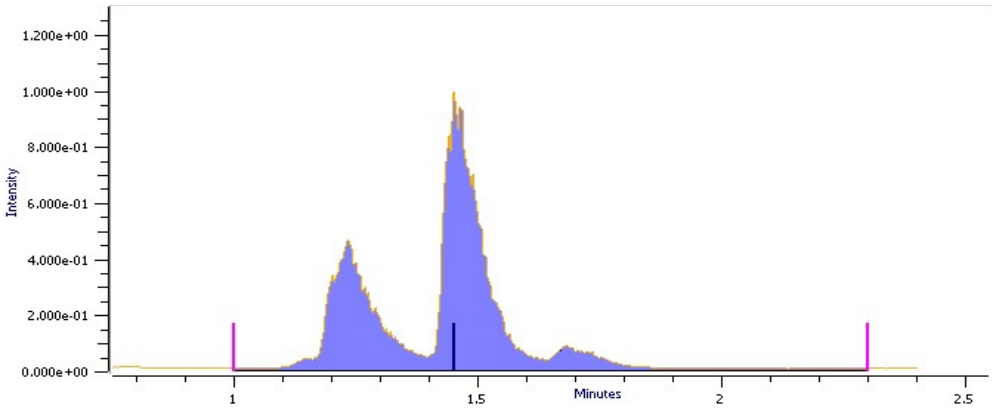


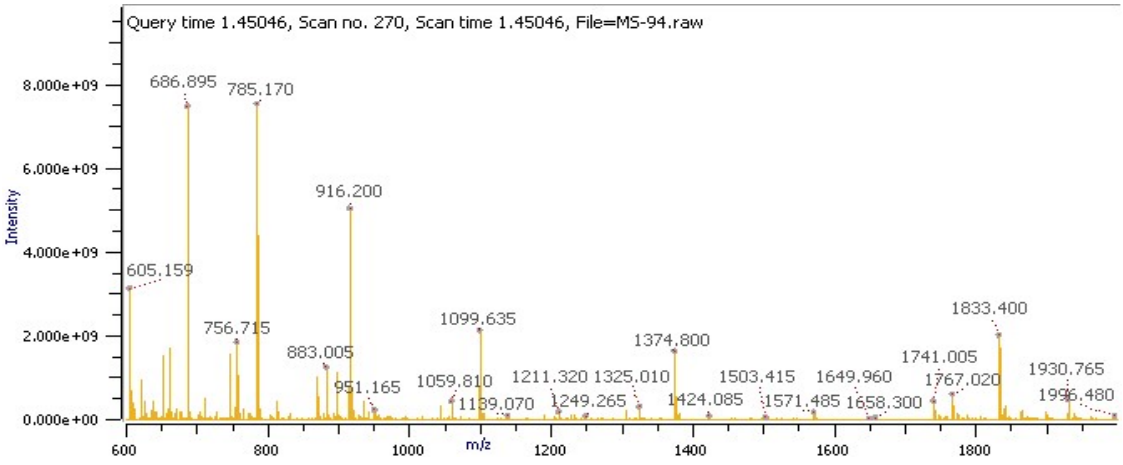


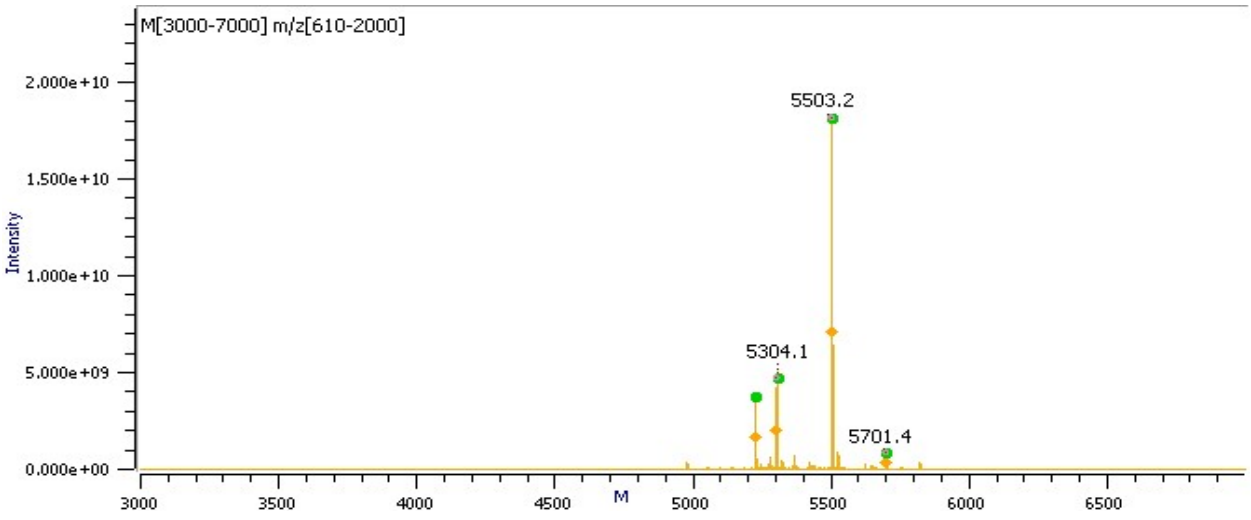


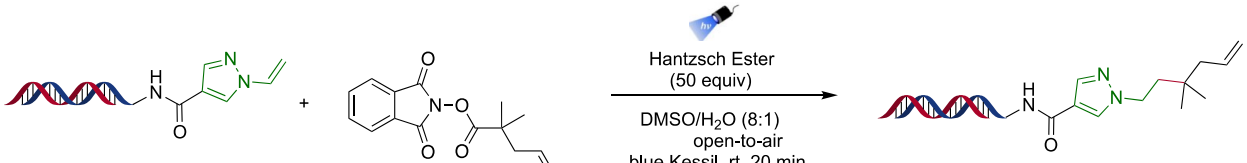
HP-1b (1 equiv) + **2w** (50 equiv)

3w, 79% (18% rsm HP-1b)
Molecular Weight: 5503.9270



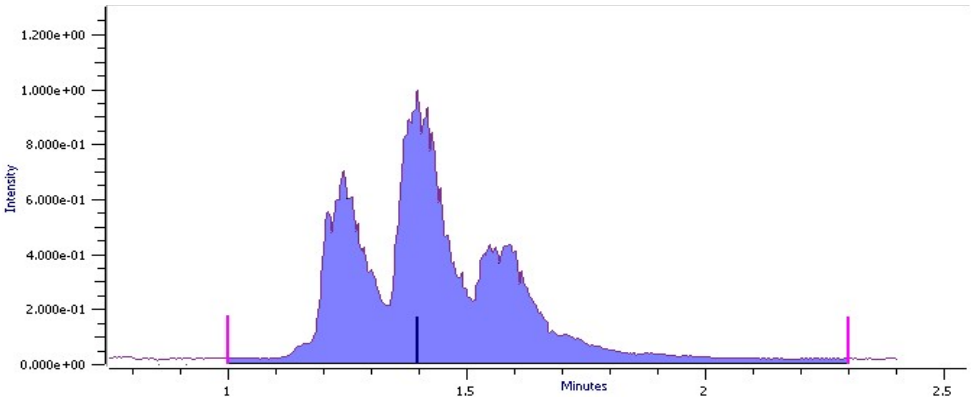


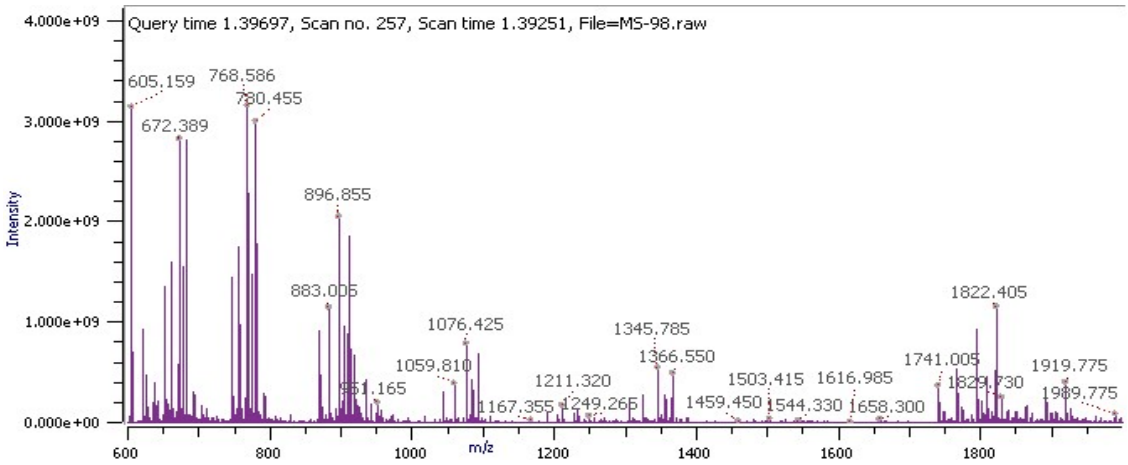


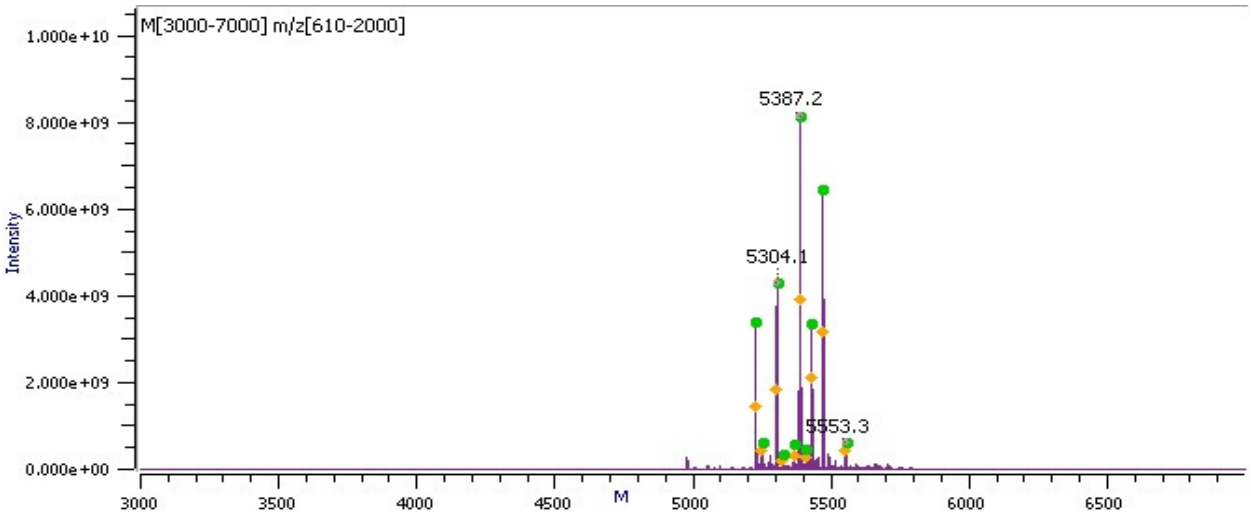


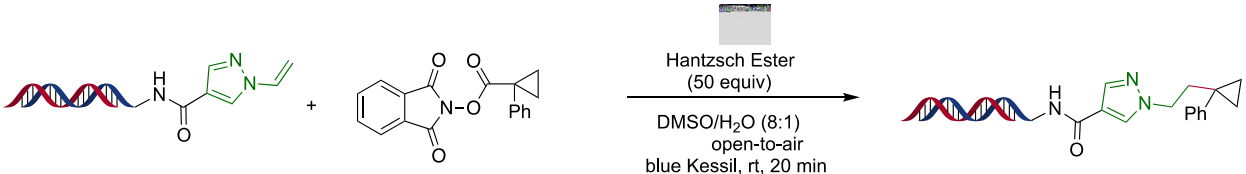
HP-1b (1 equiv) + **2x** (50 equiv)

3x, 52% (17% rsm HP-1b)
Molecular Weight: 5388.7950



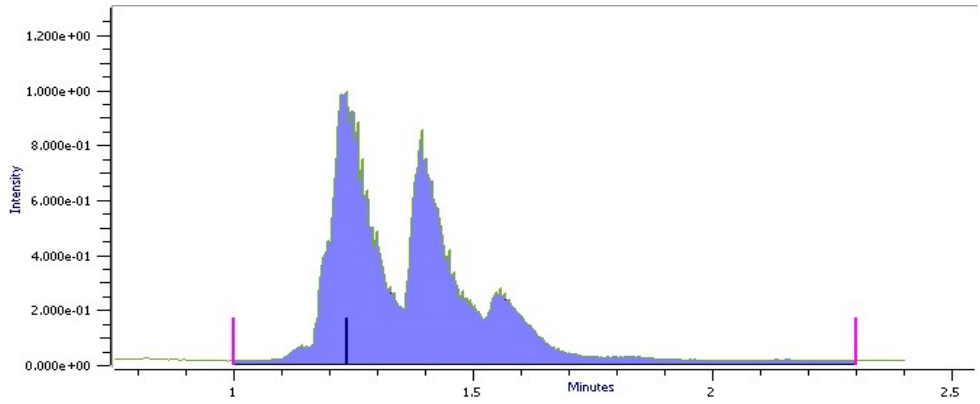


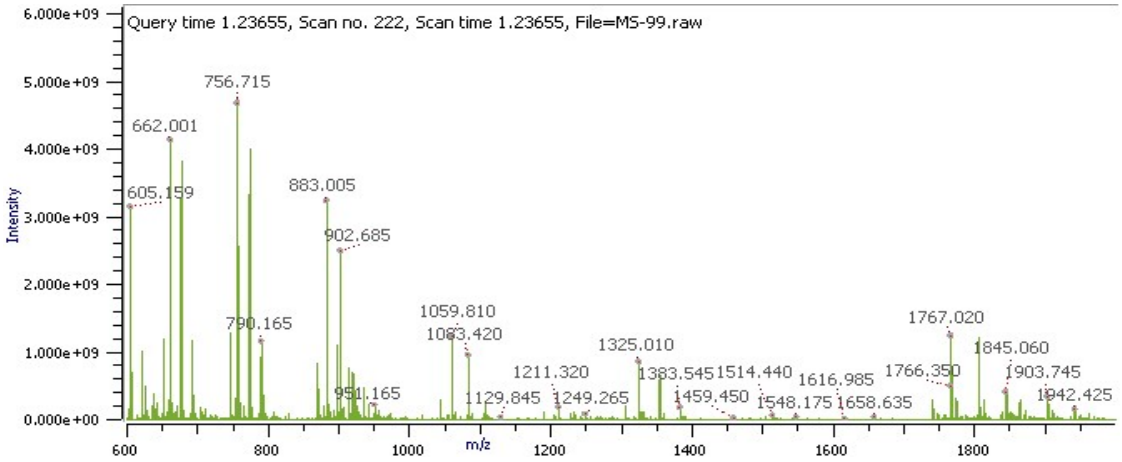


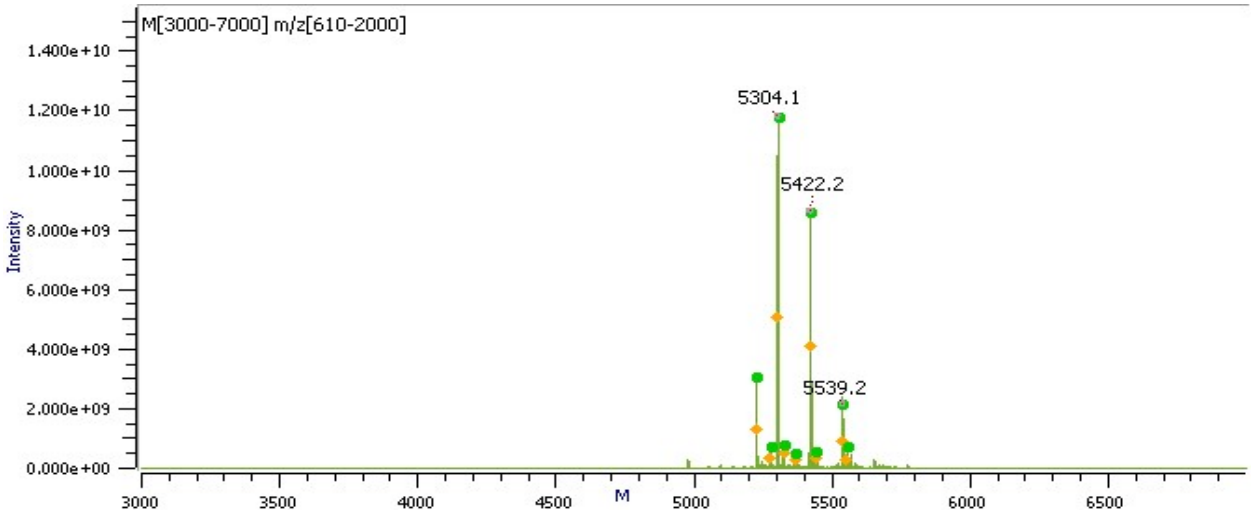


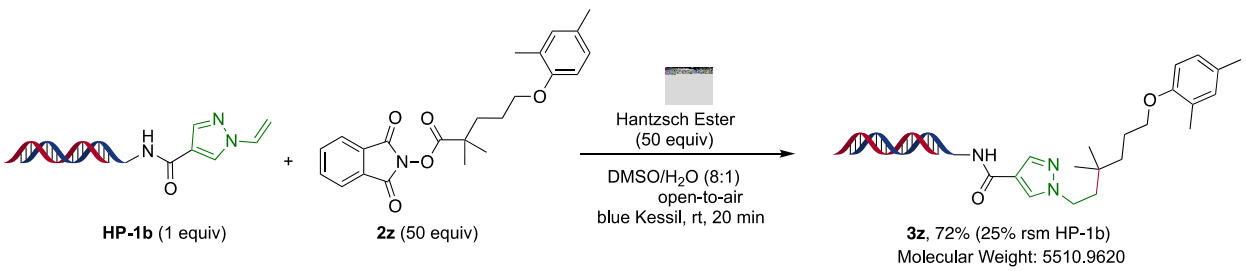
HP-1b (1 equiv) + **2y**(50 equiv)

3y, 38% (46% rsm HP-1b)
Molecular Weight: 5422.8120



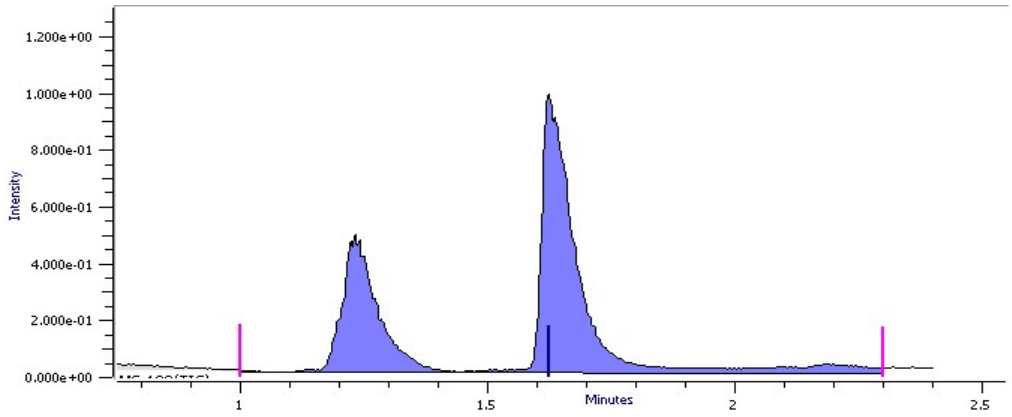


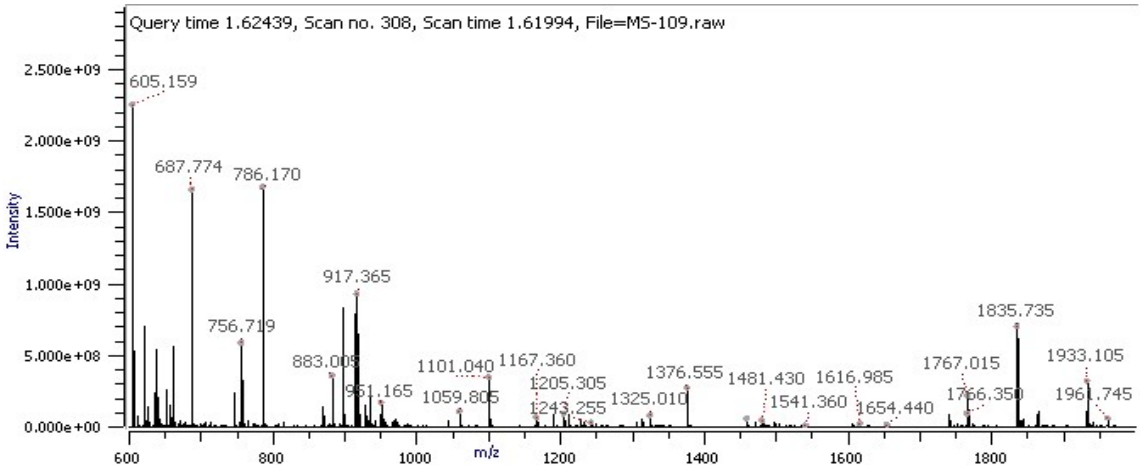


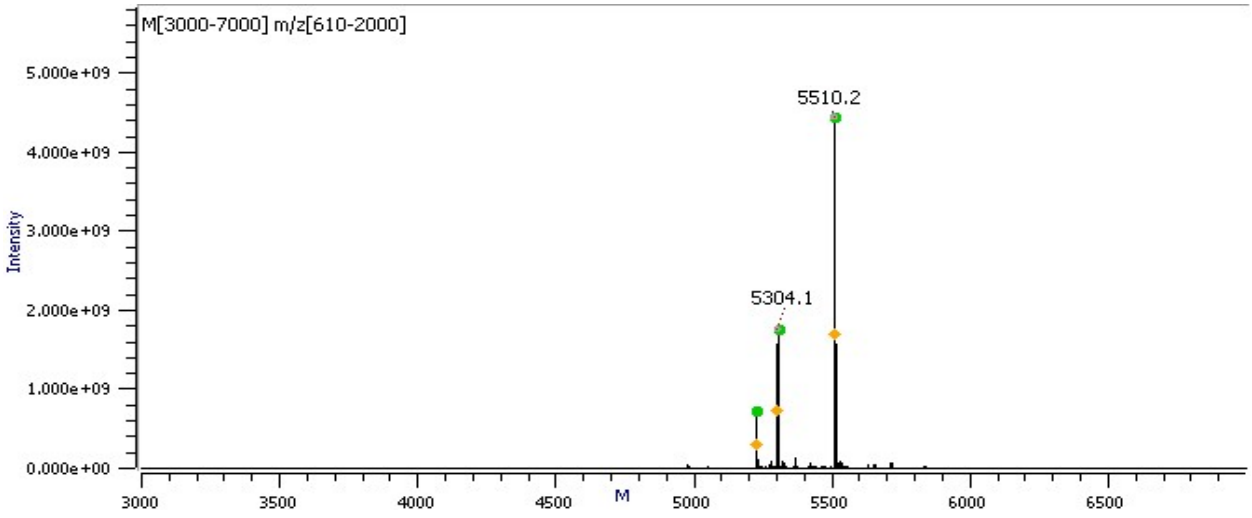


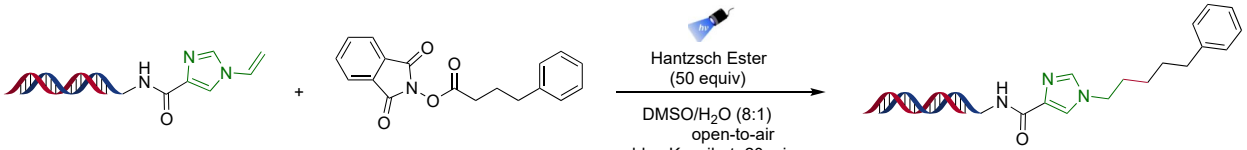
HP-1b (1 equiv) + **2z** (50 equiv)

3z, 72% (25% rsm HP-1b)
Molecular Weight: 5510.9620





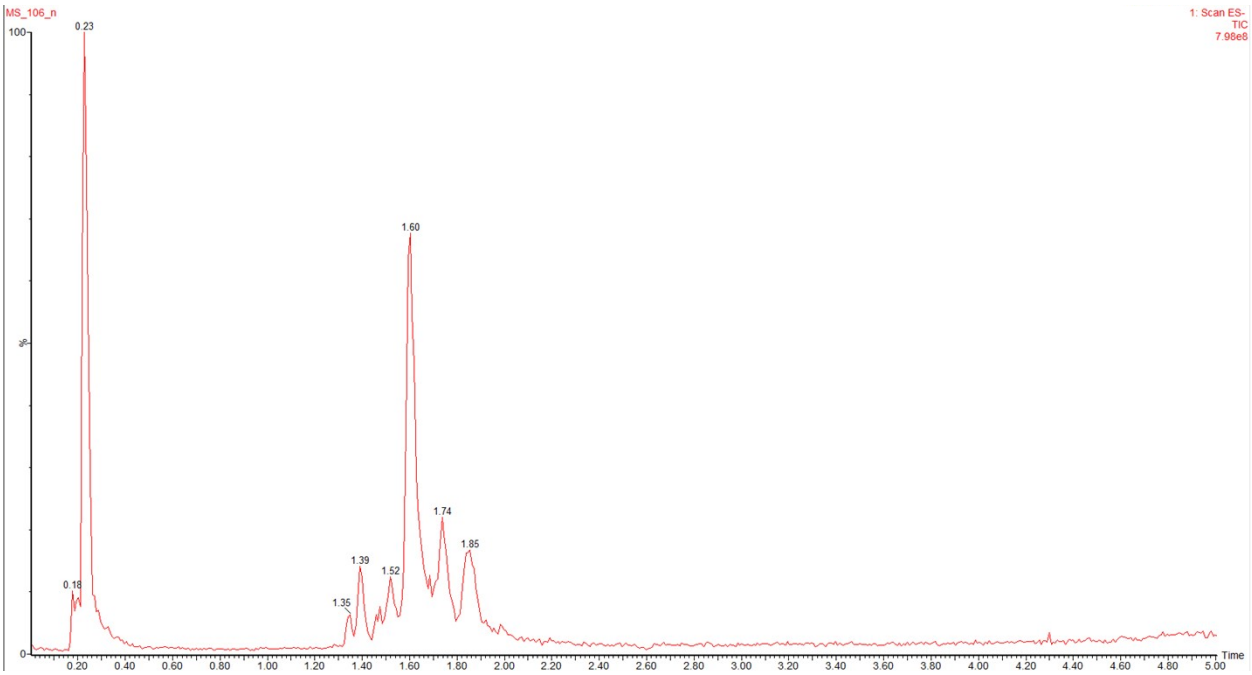


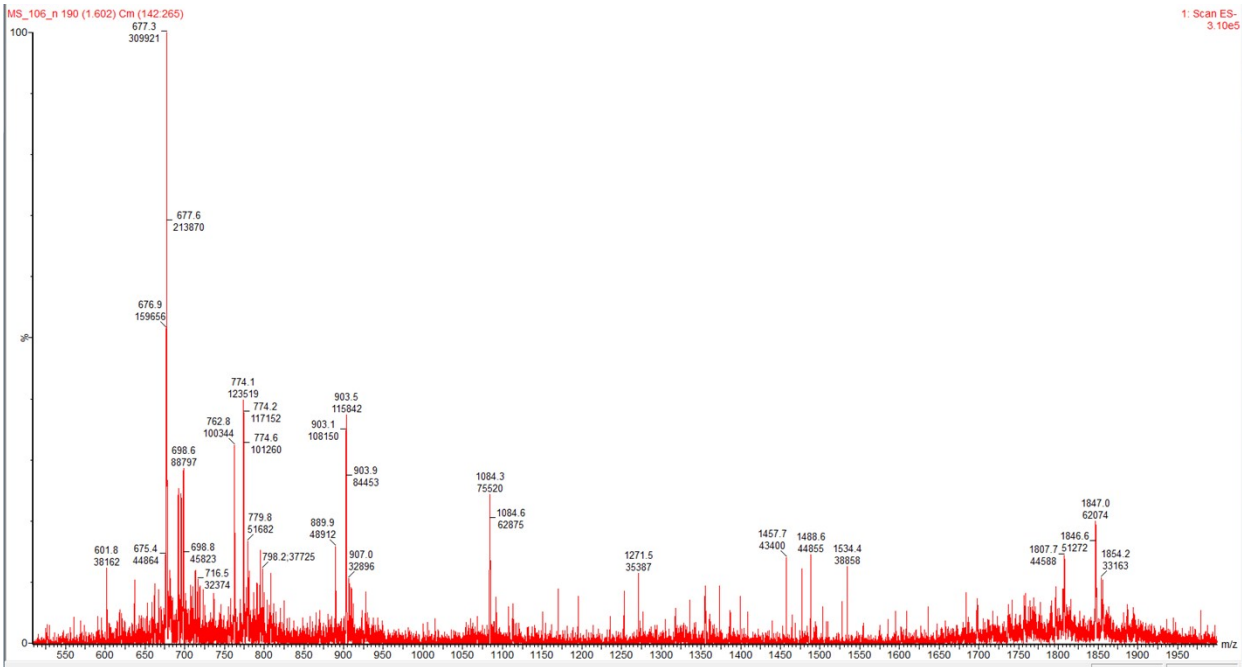


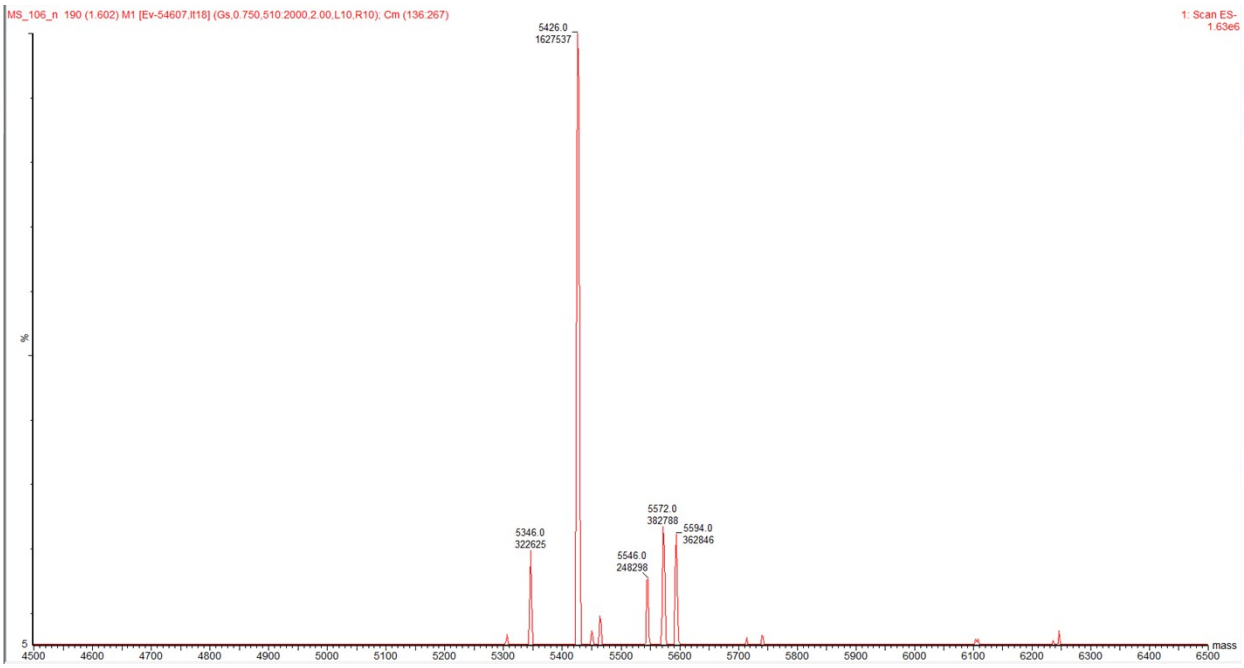
HP-1a (1 equiv) + **2b** (50 equiv)

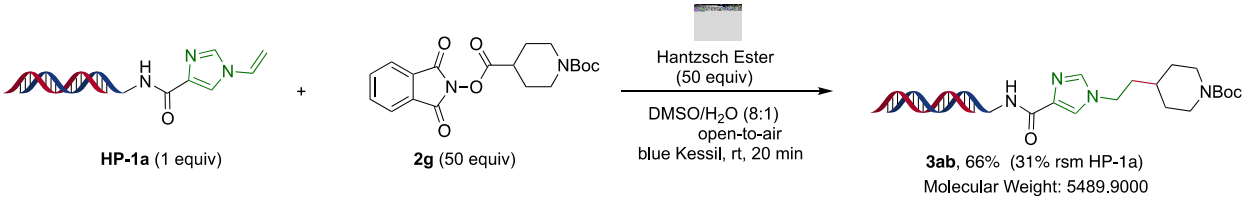
3aa, 68%

Molecular Weight: 5424.8280


MS_106_n
1: Scan ES-
TIC
7.96e8
100
%
0
0.23
0.18
1.35
1.39
1.52
1.60
1.74
1.85
Time
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00

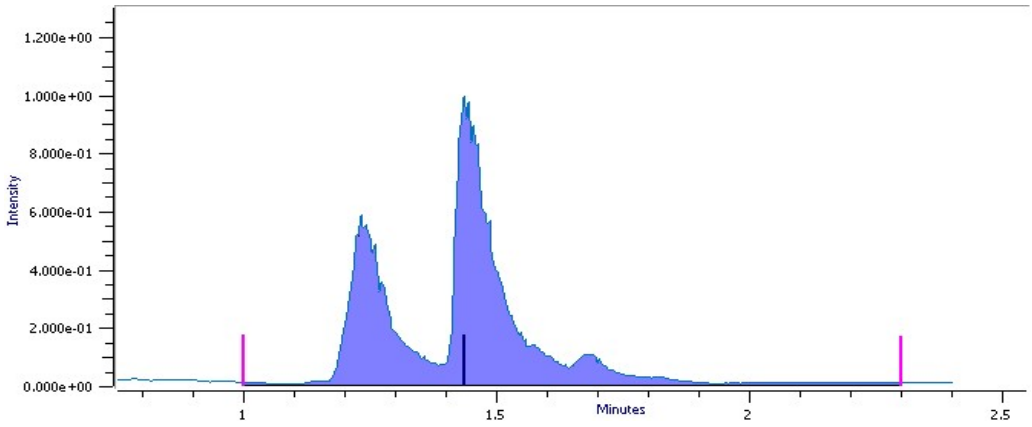

MS_106_n 190 (1.602) Cm (142:265)
1: Scan ES-
3.10e5
100
%
0
677.3
309921
677.6
213870
676.9
159656
774.1
123519
774.2
117152
762.8
100344
774.6
101260
698.6
88797
903.5
115842
903.1
108150
903.9
84453
1084.3
75520
1084.6
62875
779.8
51682
889.9
48912
907.0
32896
601.8
38162
675.4
44864
698.8
45823
716.5
32374
798.2
37725
1271.5
35387
1457.7
43400
1488.6
44855
1534.4
38858
1847.0
62074
1846.6
51272
1807.7
44588
1854.2
33163
m/z
550 600 650 700 750 800 850 900 950 1000 1050 1100 1150 1200 1250 1300 1350 1400 1450 1500 1550 1600 1650 1700 1750 1800 1850 1900 1950

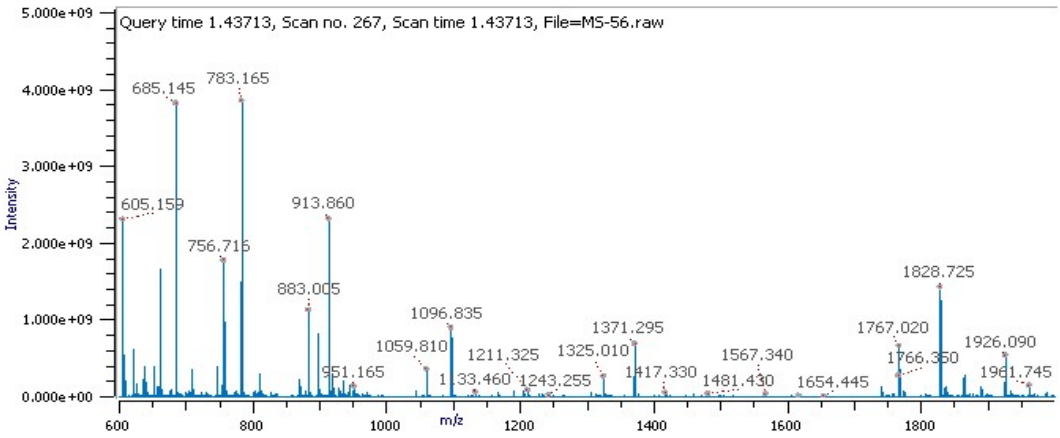


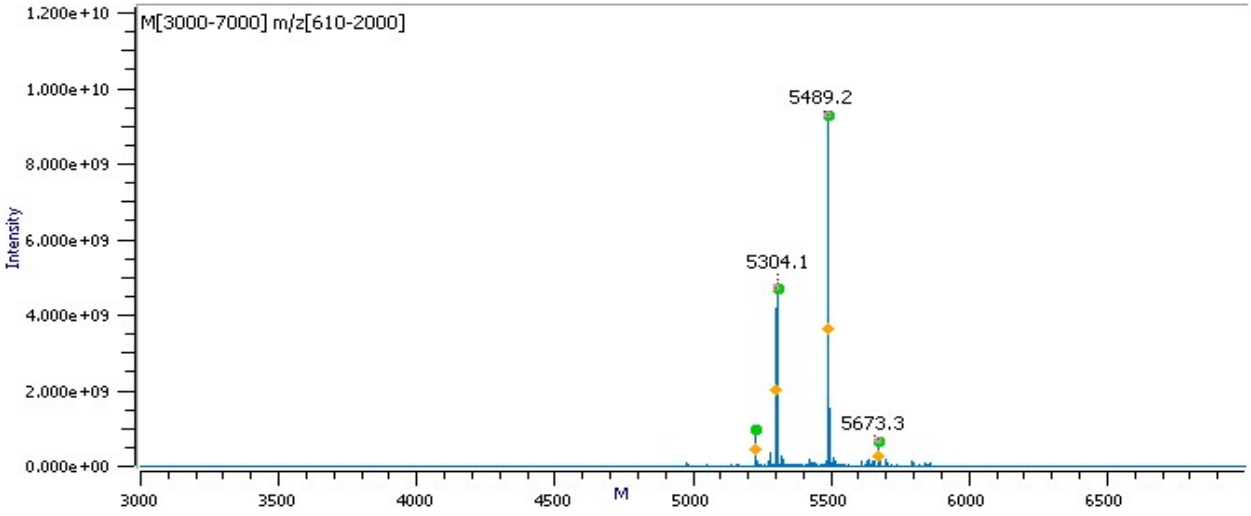


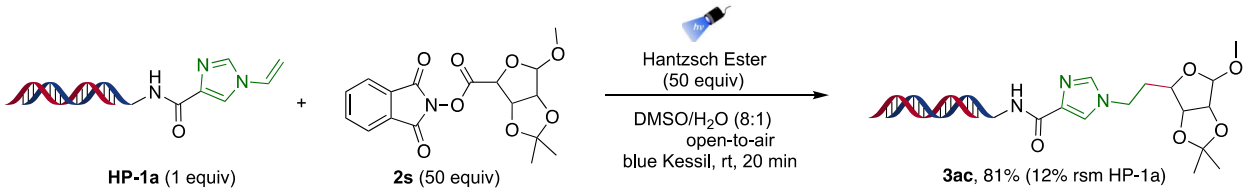
HP-1a (1 equiv) + **2g** (50 equiv)

3ab, 66% (31% rsm HP-1a)
Molecular Weight: 5489.9000



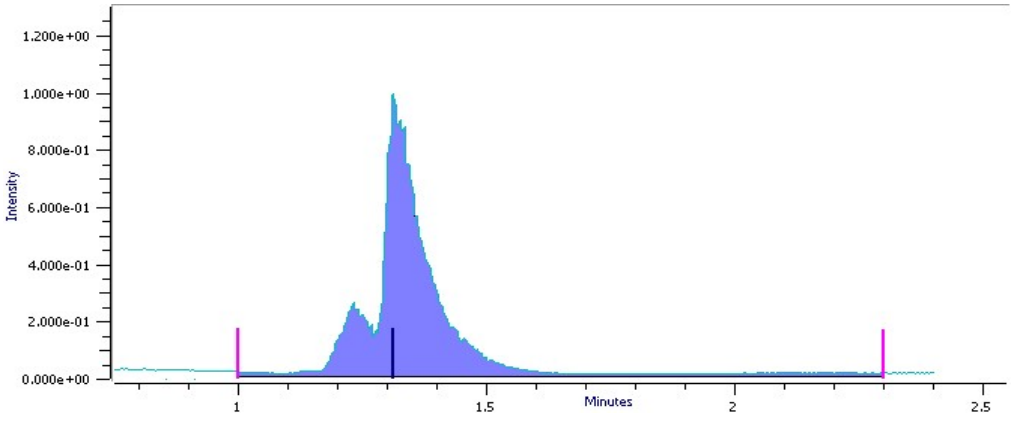


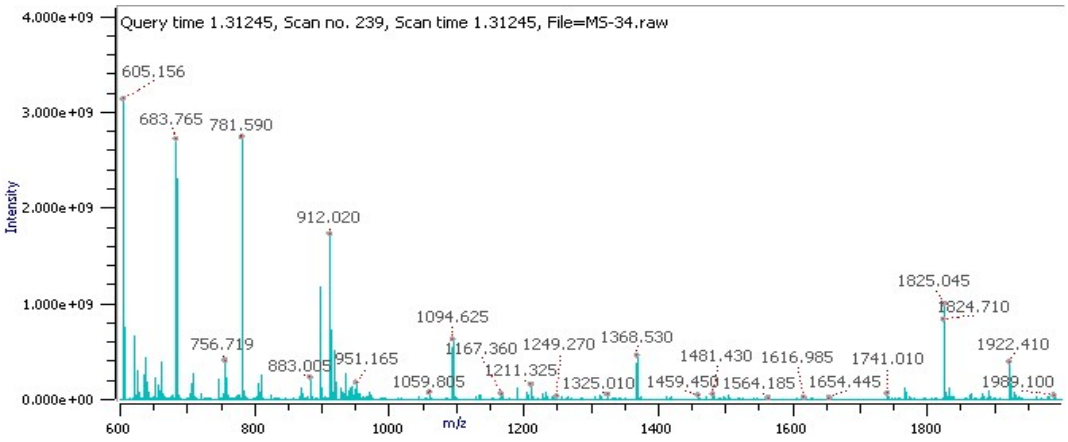


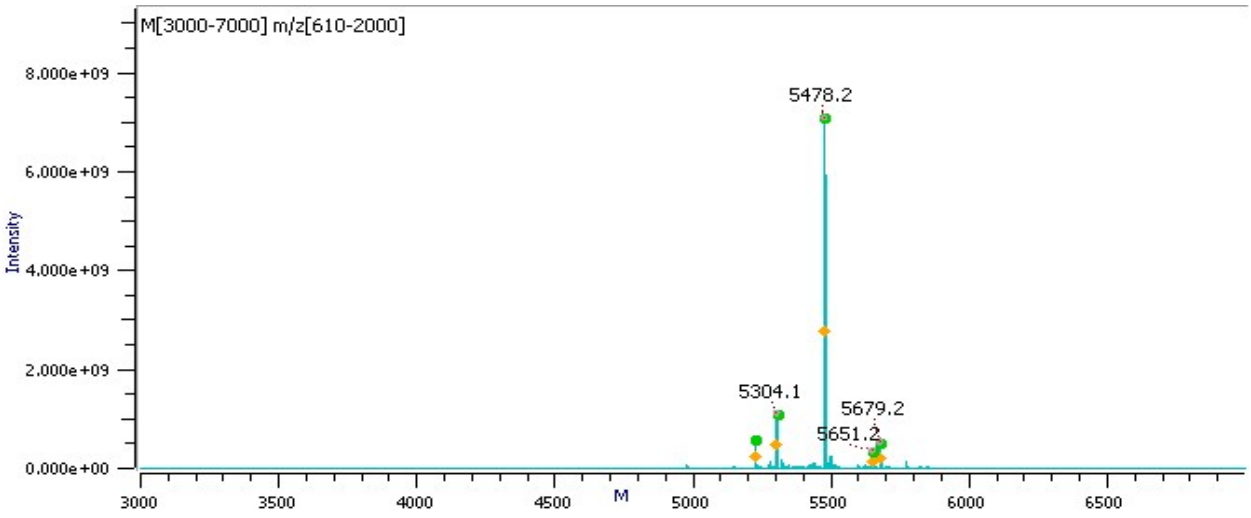


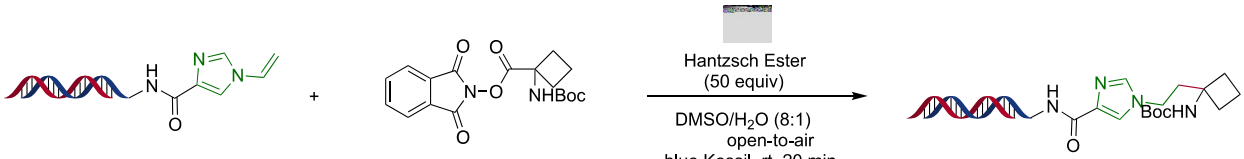
HP-1a (1 equiv) + **2s** (50 equiv)

3ac, 81% (12% rsm HP-1a)
Molecular Weight: 5478.8290



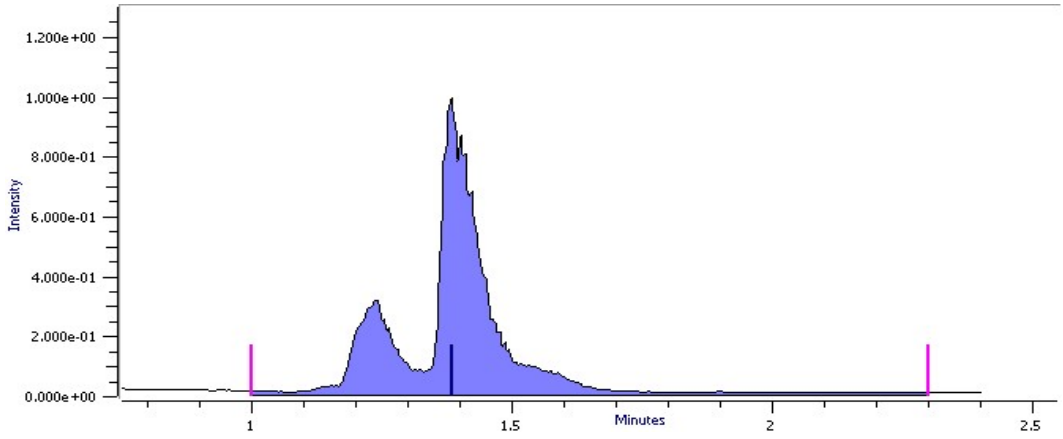


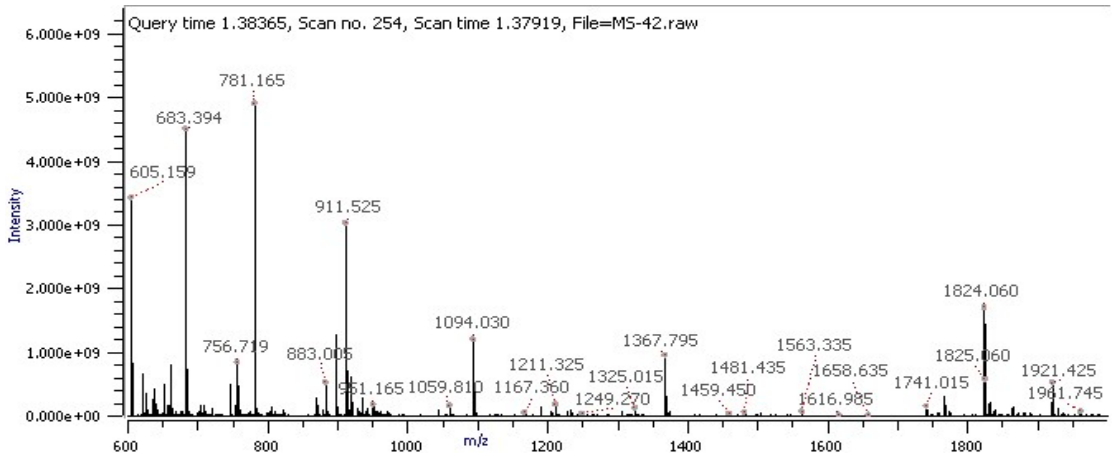


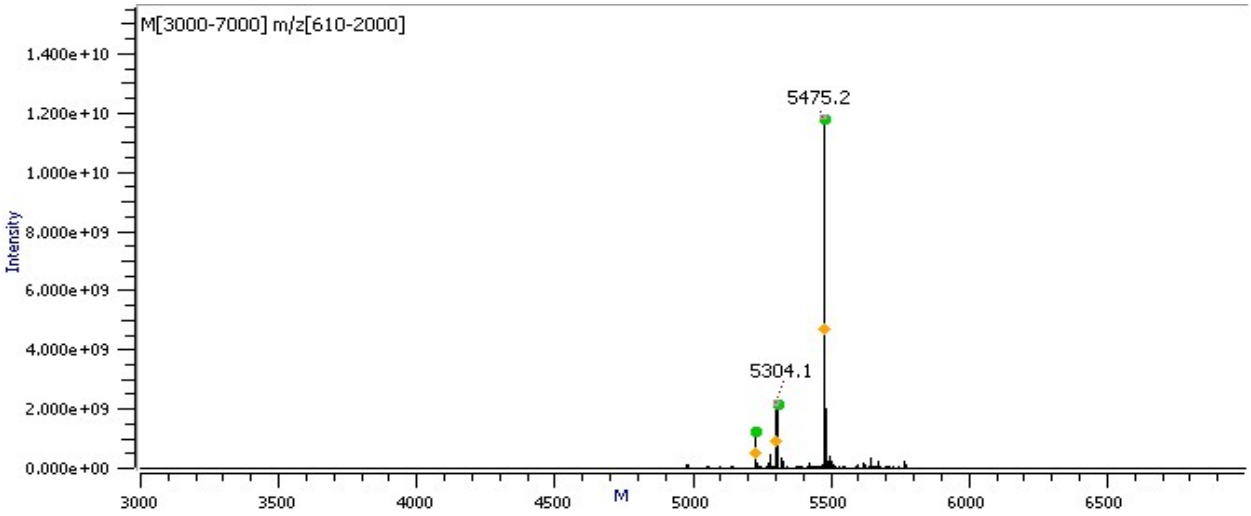


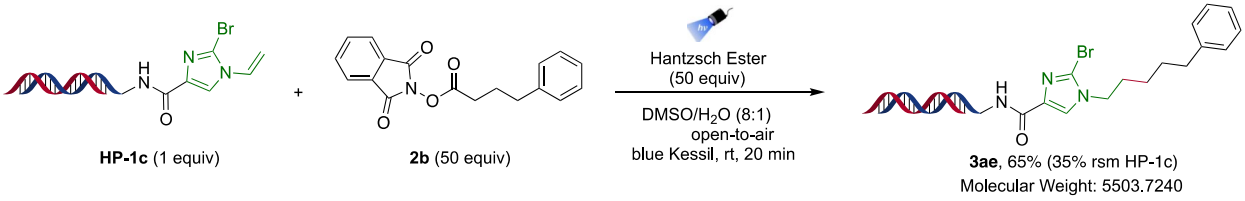
HP-1a (1 equiv) + **2i** (50 equiv)

3ad, 84% (14% rsm HP-1a)
Molecular Weight: 5475.8730



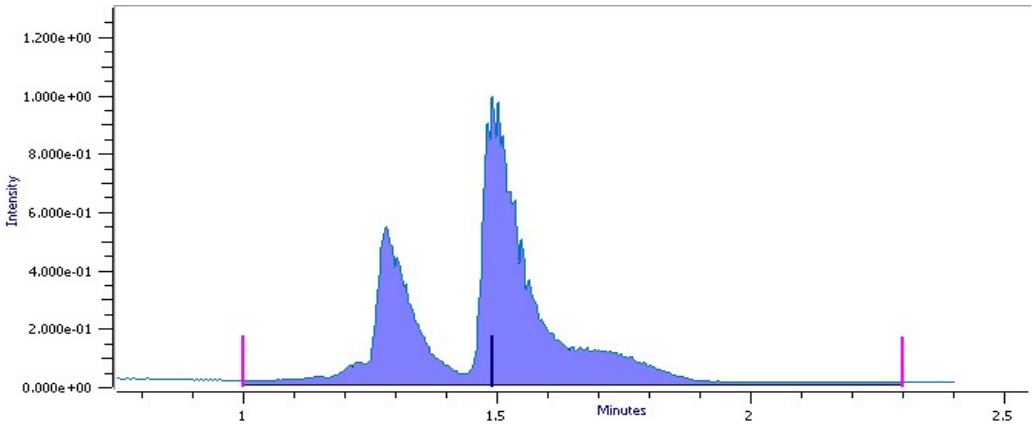


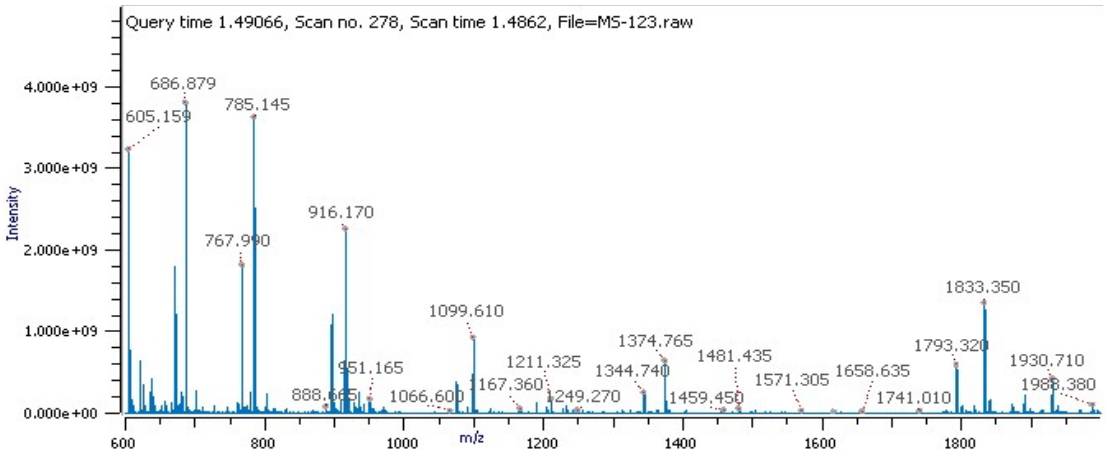


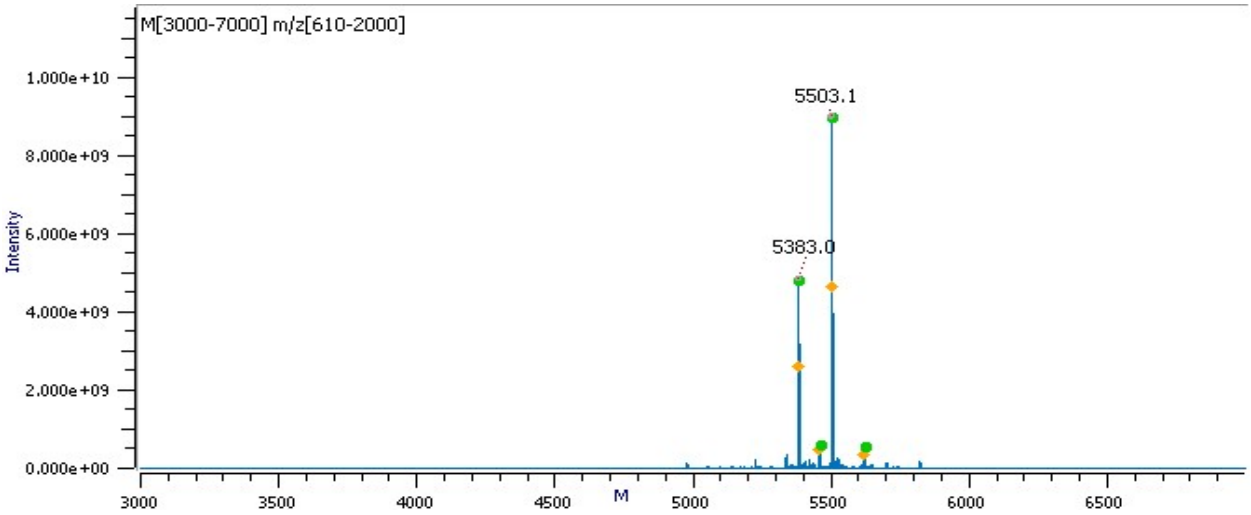


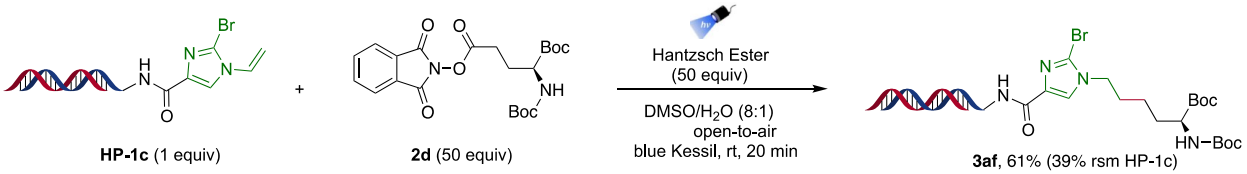
HP-1c (1 equiv) + **2b** (50 equiv)

3ae, 65% (35% rsm HP-1c)
Molecular Weight: 5503.7240



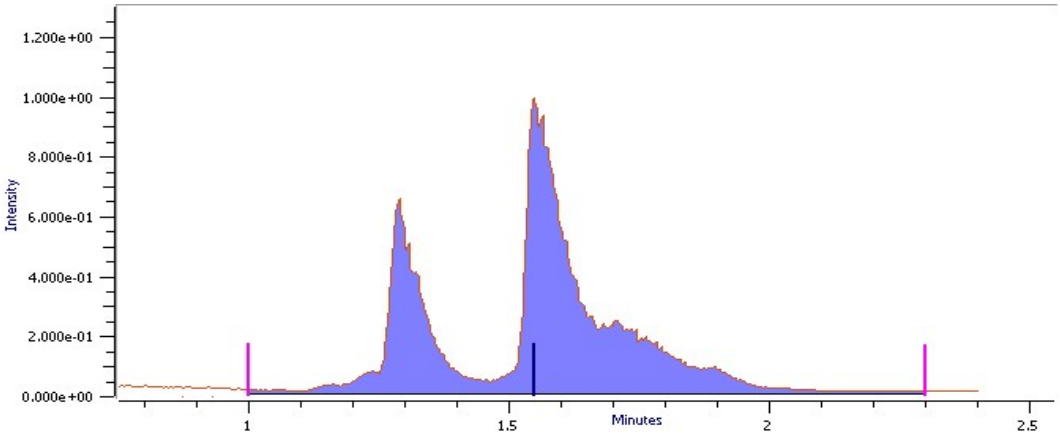


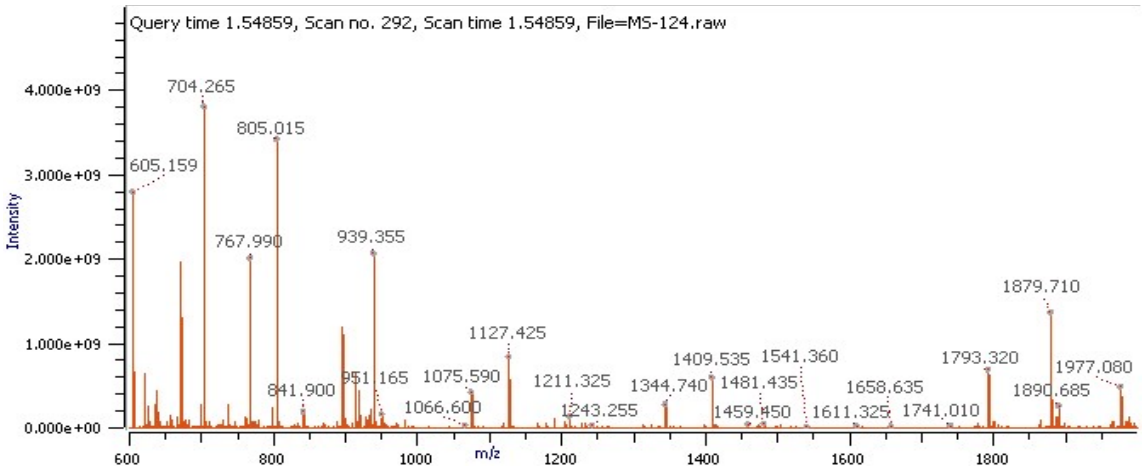


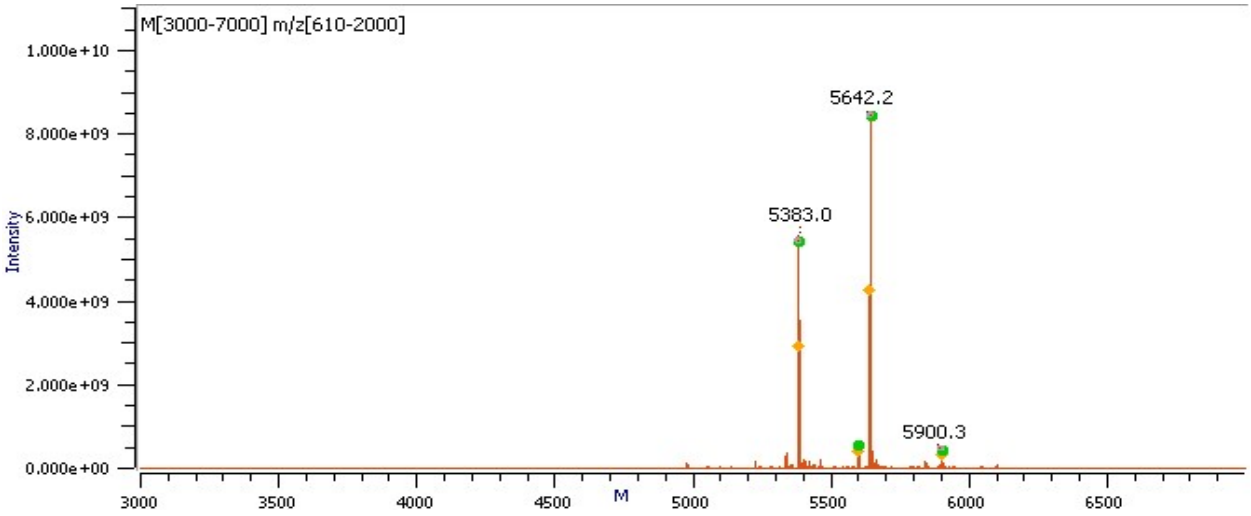


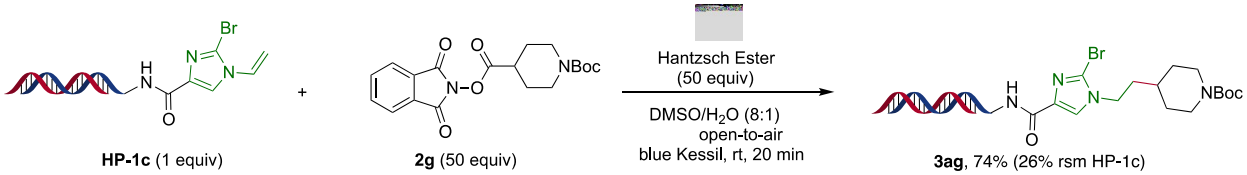
HP-1c (1 equiv) + **2d** (50 equiv)

3af, 61% (39% rsm HP-1c)
Molecular Weight: 5642.8750



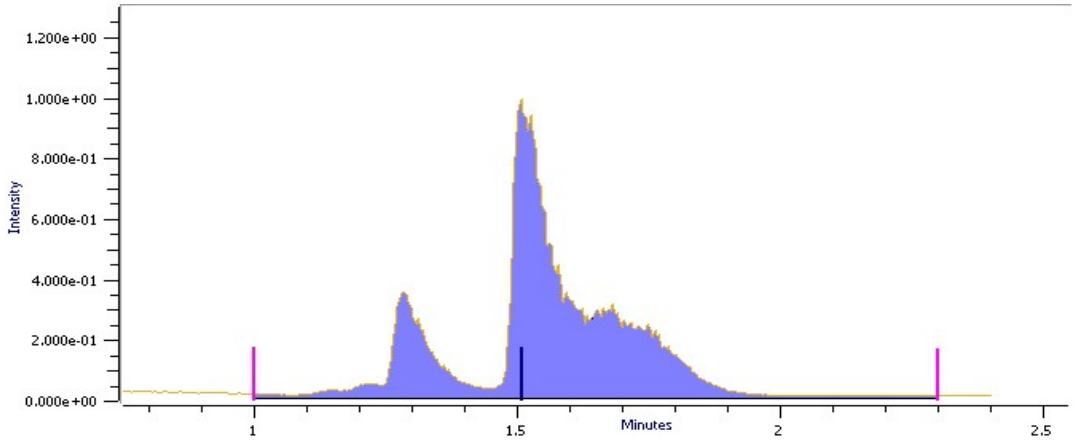


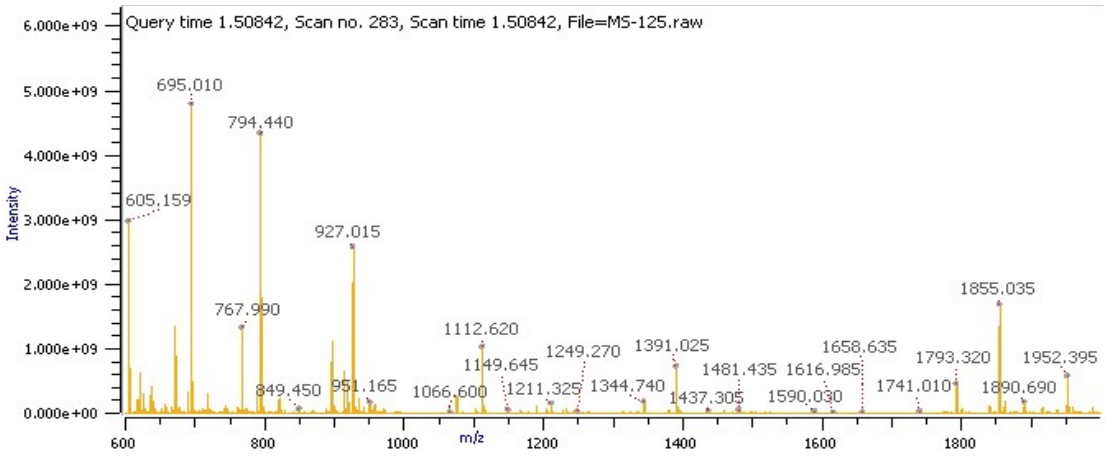


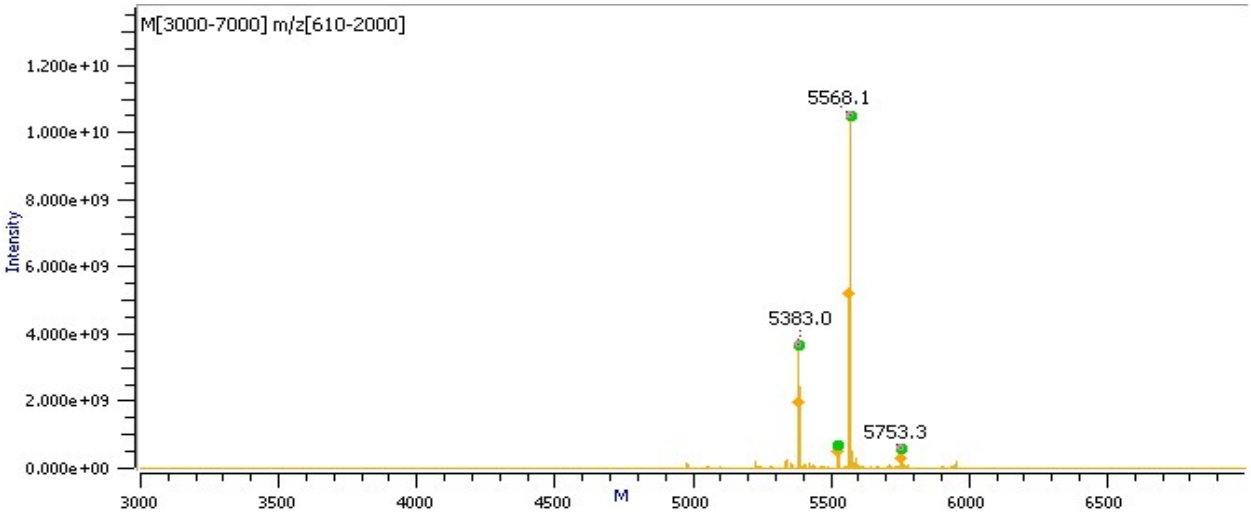


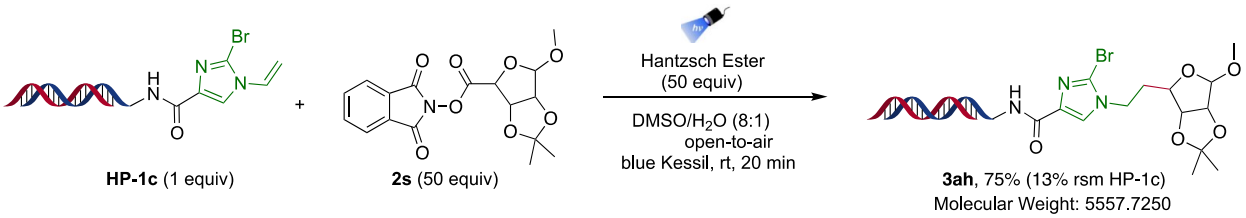
HP-1c (1 equiv) + **2g** (50 equiv)

3ag, 74% (26% rsm HP-1c)
Molecular Weight: 5568.7960



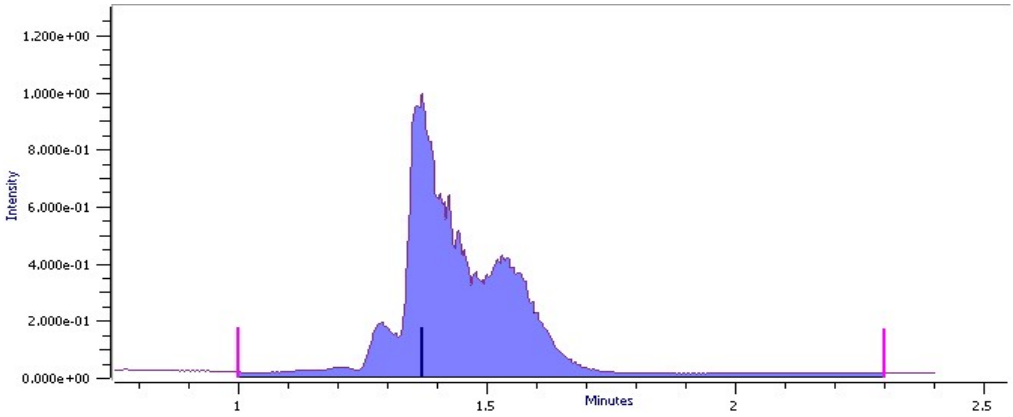


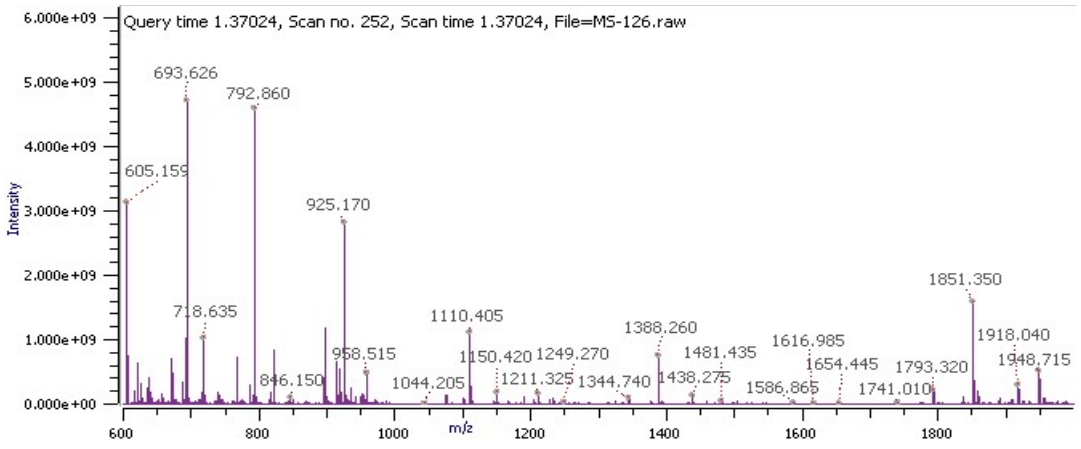


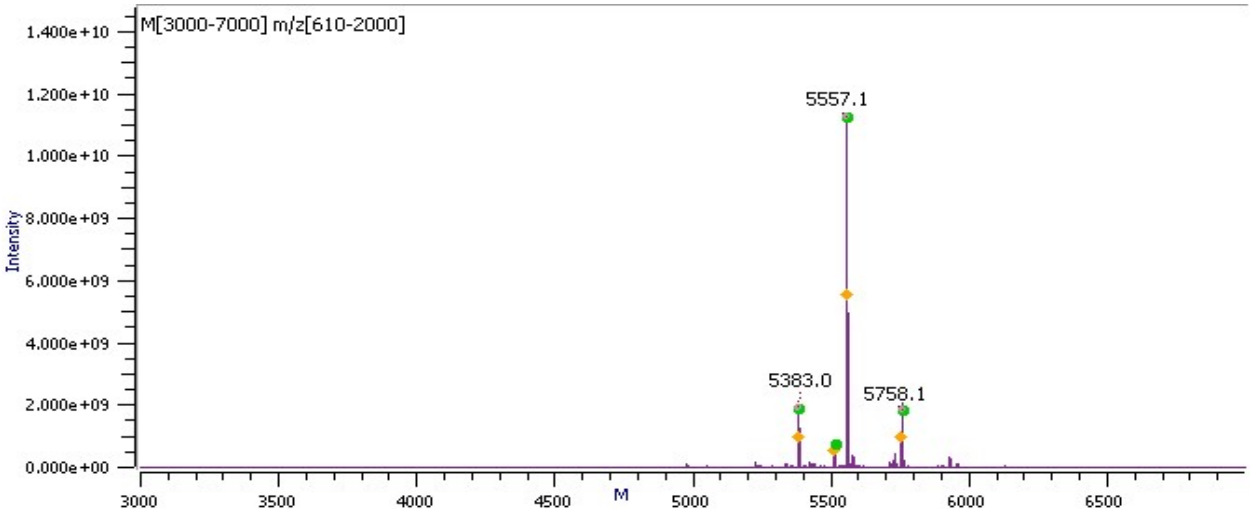


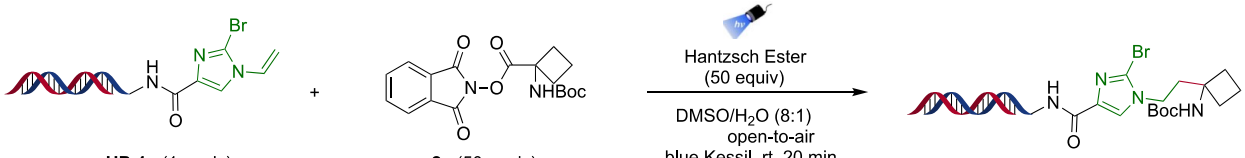
HP-1c (1 equiv) + **2s** (50 equiv)

3ah, 75% (13% rsm HP-1c)
Molecular Weight: 5557.7250





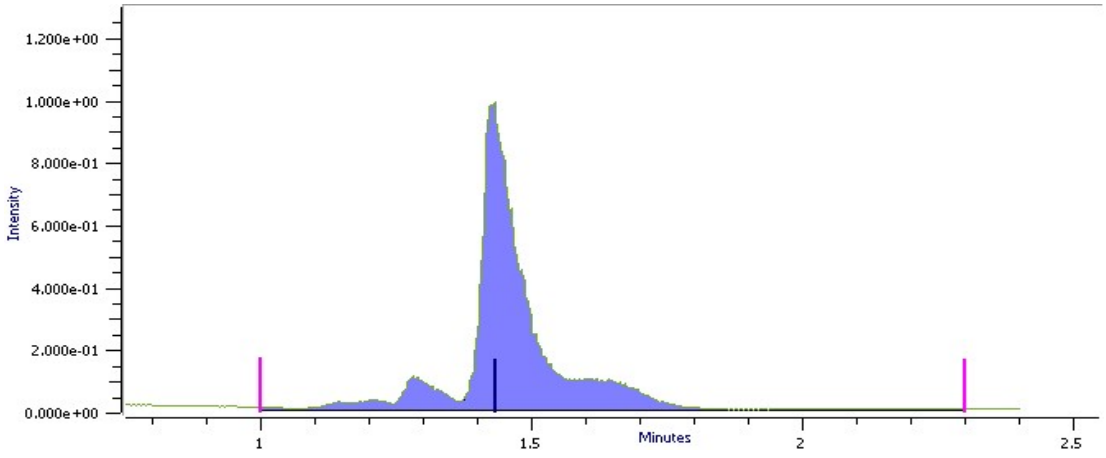


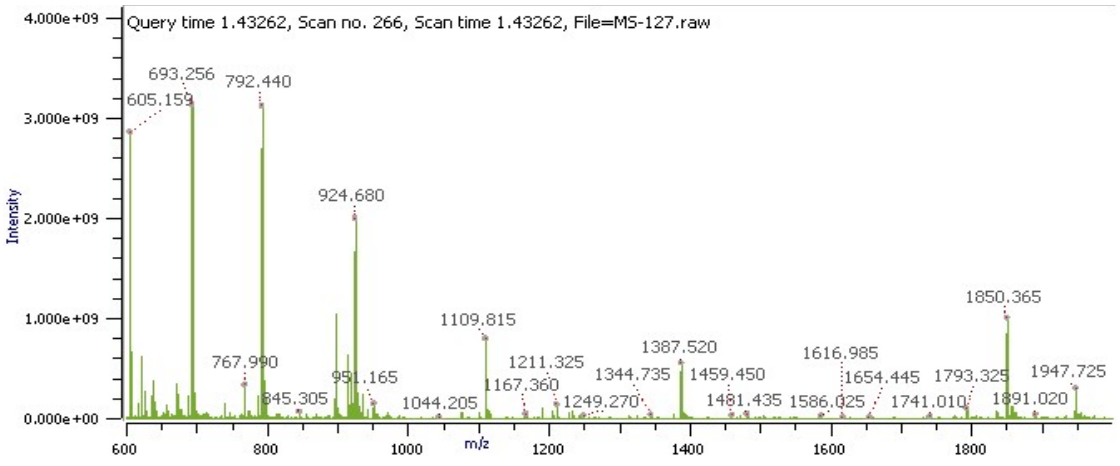


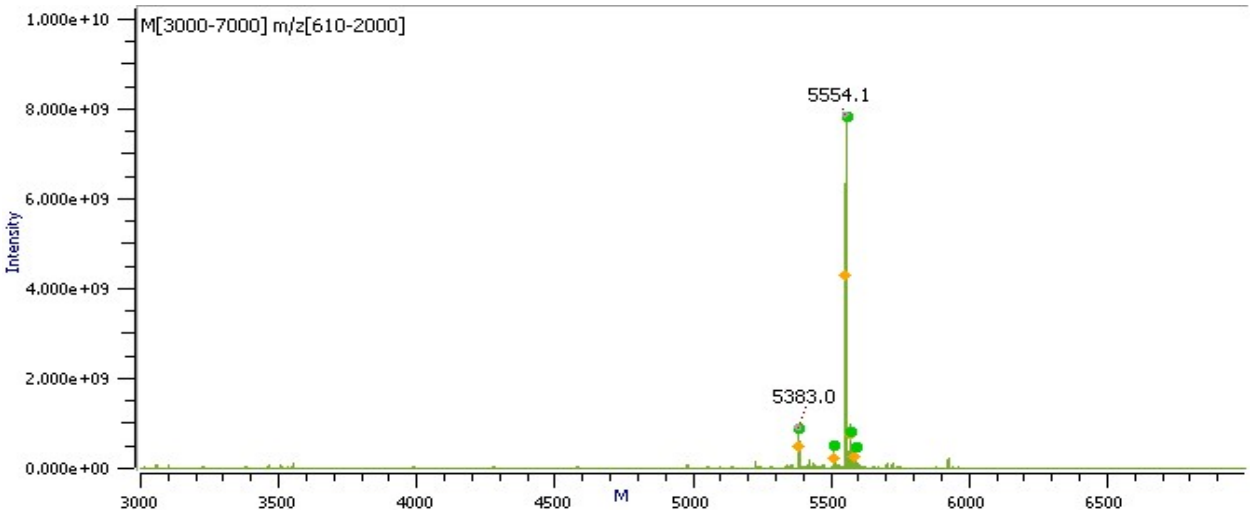
HP-1c (1 equiv) + **2v** (50 equiv)

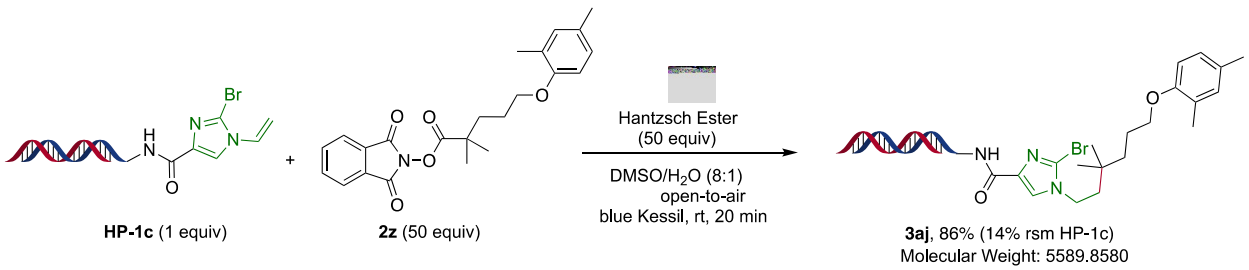
3ai, 74% (9% rsm HP-1c)

Molecular Weight: 5554.7690



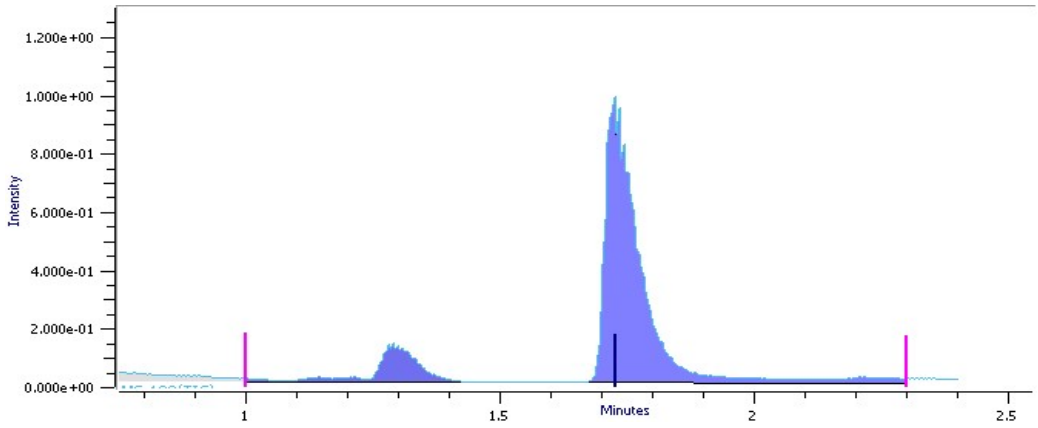


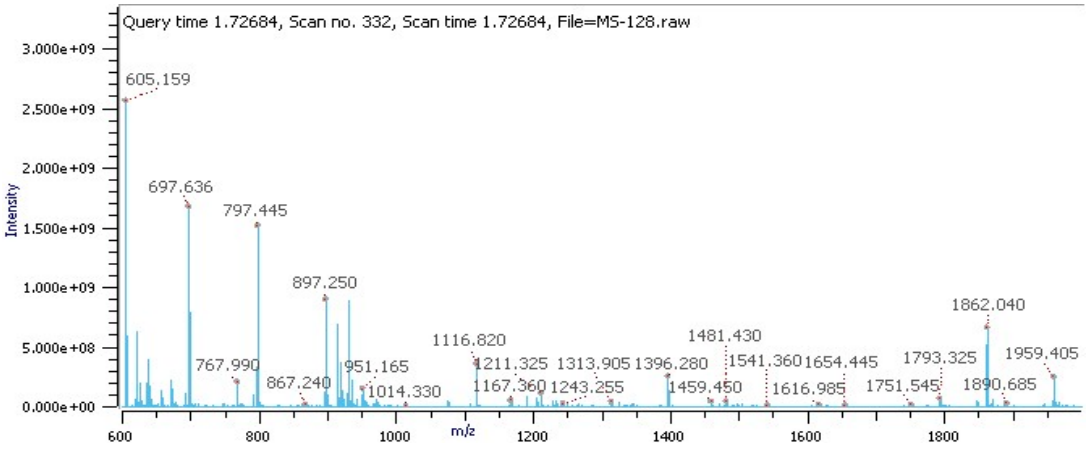


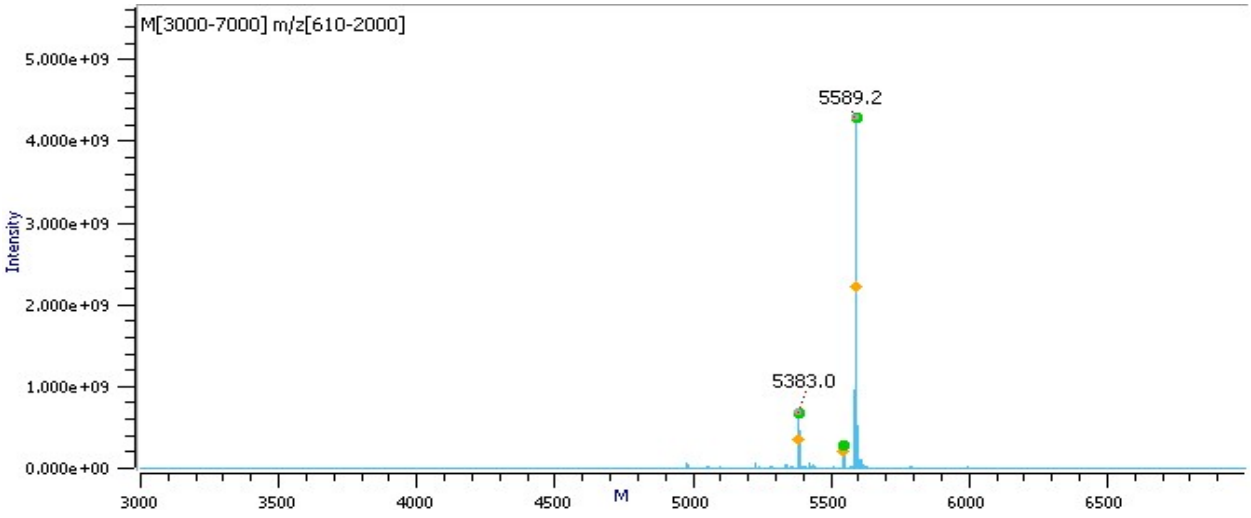


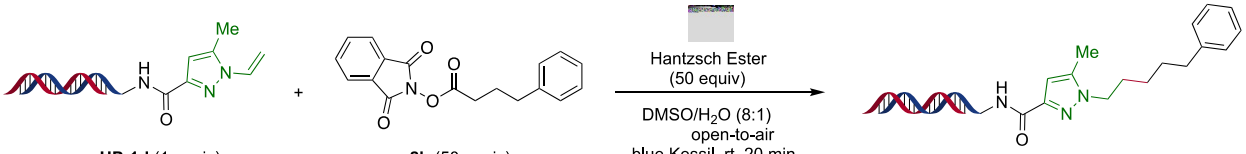
HP-1c (1 equiv) + **2z** (50 equiv)

3aj, 86% (14% rsm HP-1c)
Molecular Weight: 5589.8580



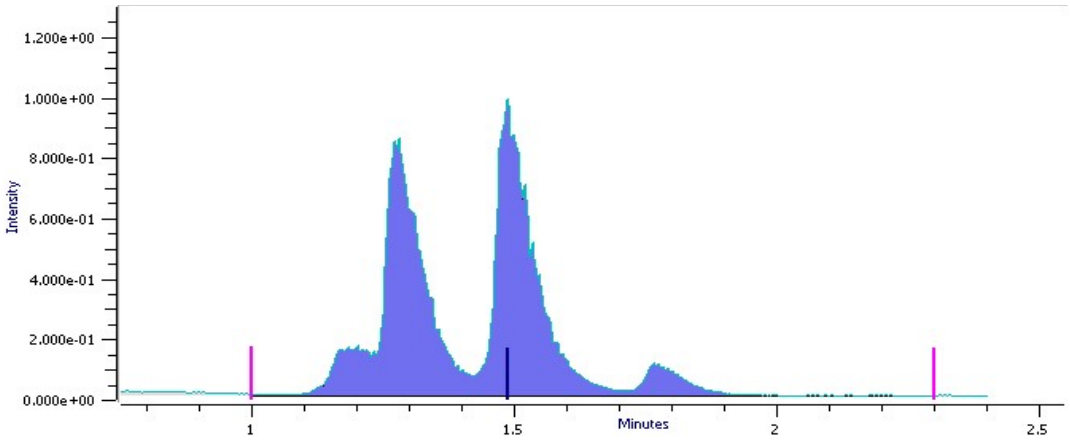


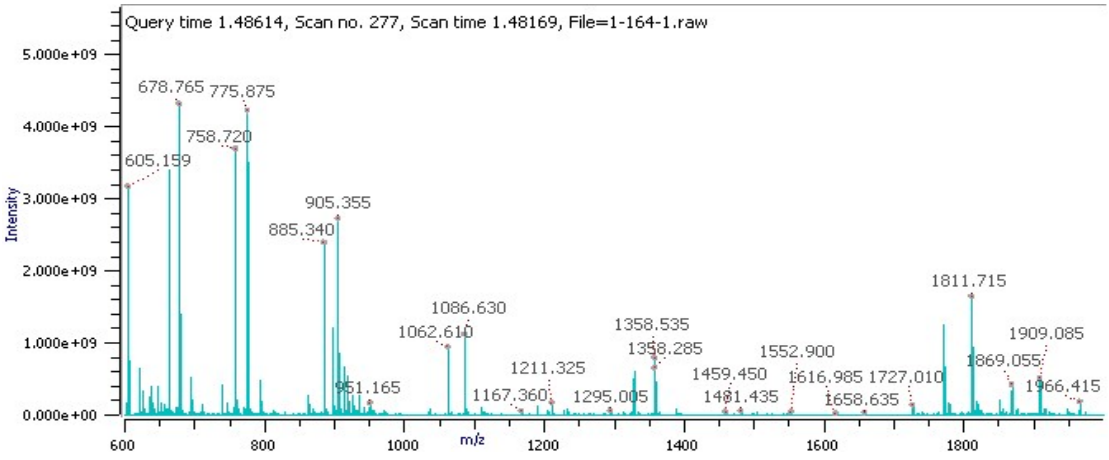


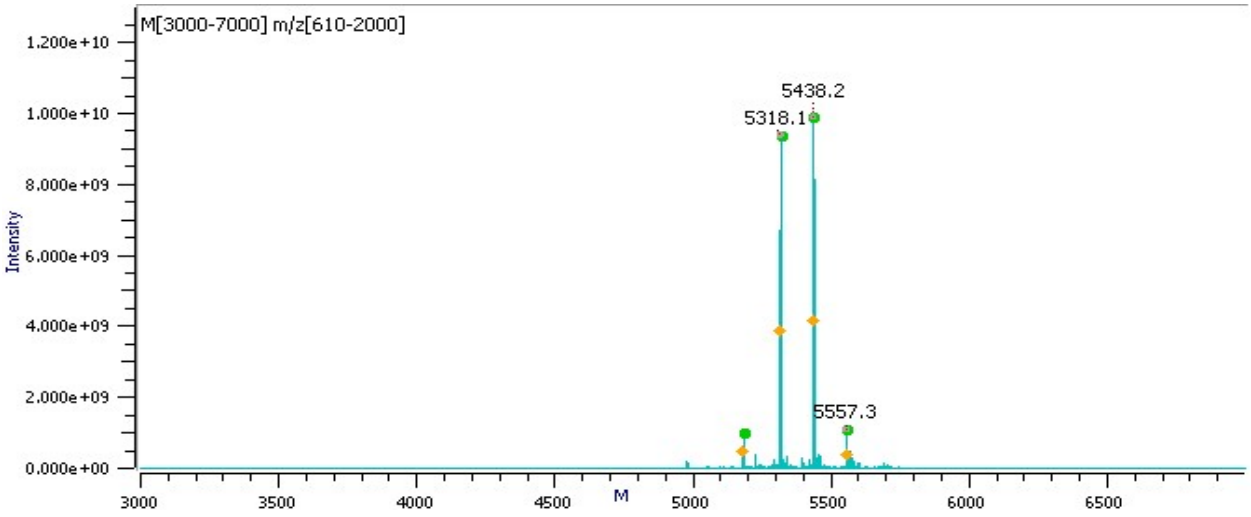


HP-1d (1 equiv) + **2b** (50 equiv)

3ak, 48% (44% rsm HP-1d)
Molecular Weight: 5438.8550



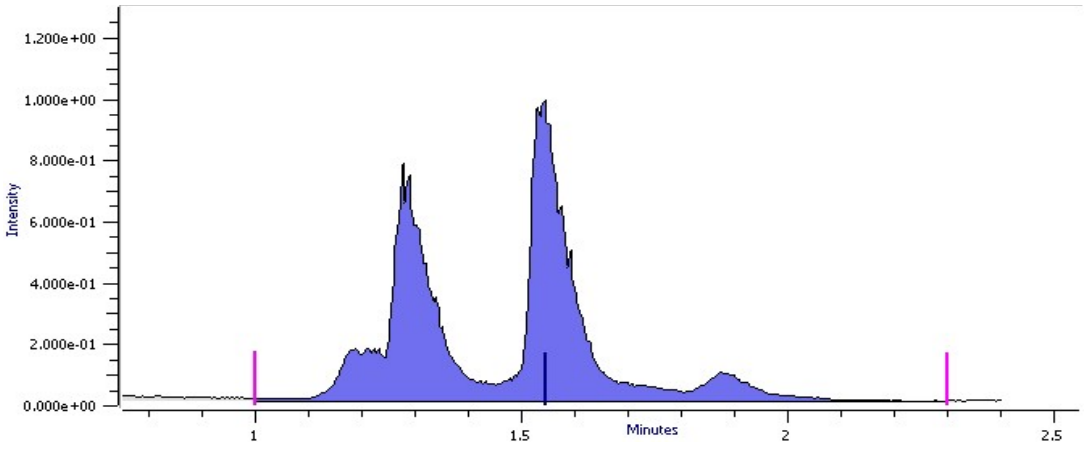


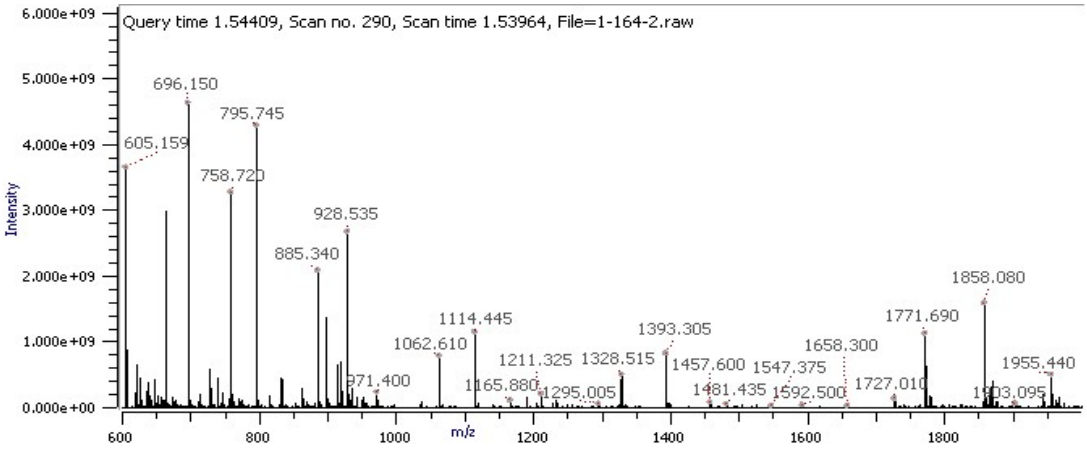


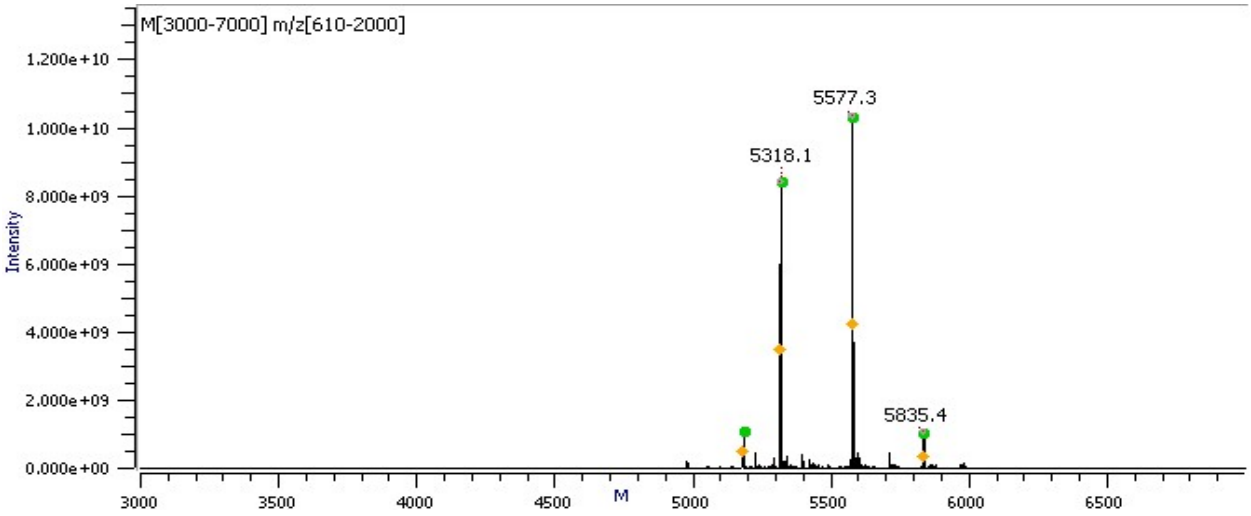
HP-1d (1 equiv) + **2d** (50 equiv)

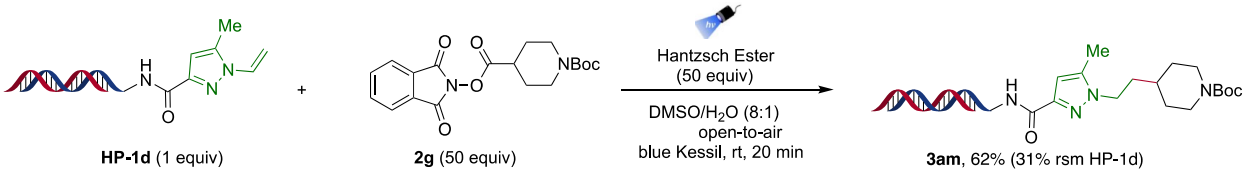
3al, 52% (40% rsm HP-1d)

Molecular Weight: 5578.0060





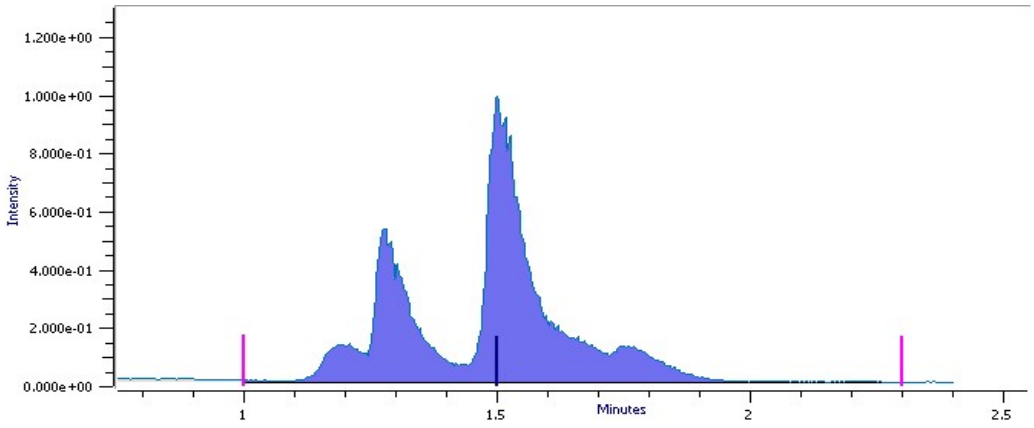


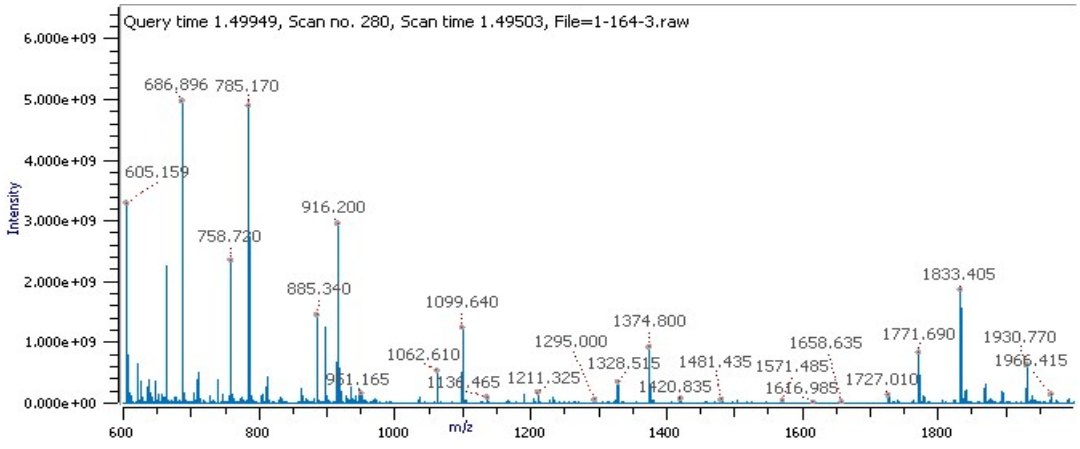


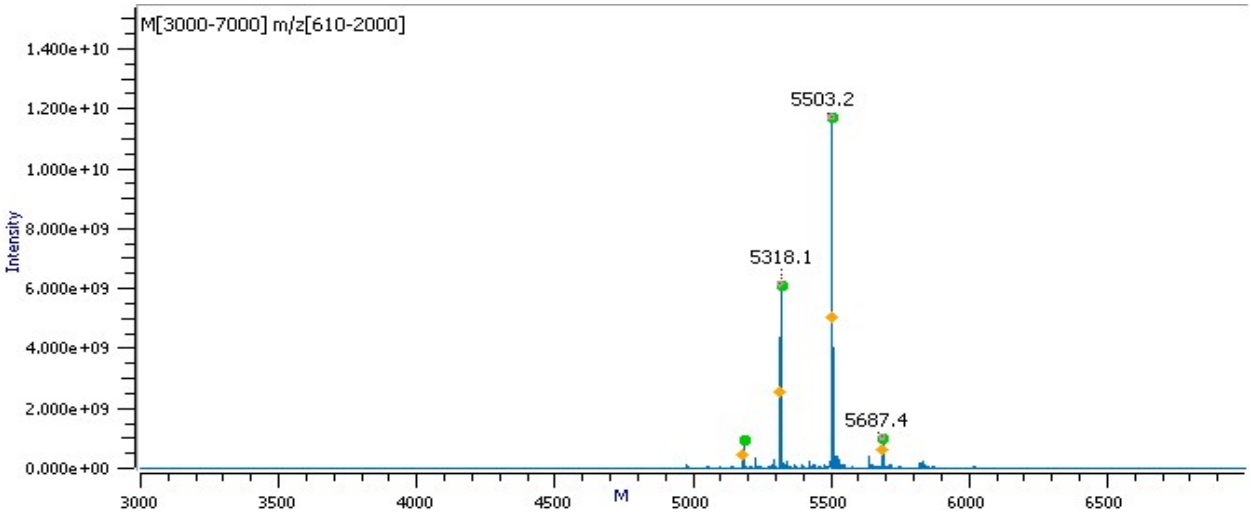
HP-1d (1 equiv) + **2g** (50 equiv)

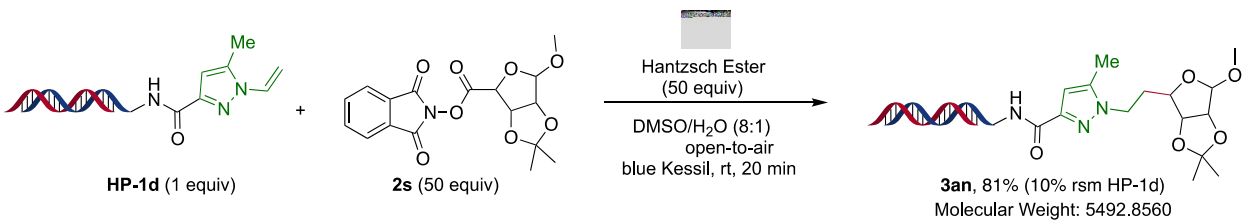
3am, 62% (31% rsm HP-1d)

Molecular Weight: 5503.9270



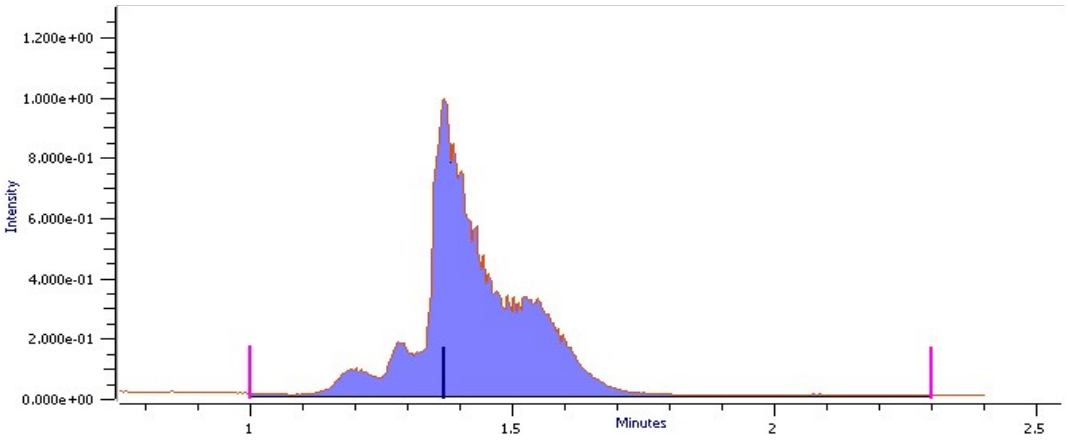


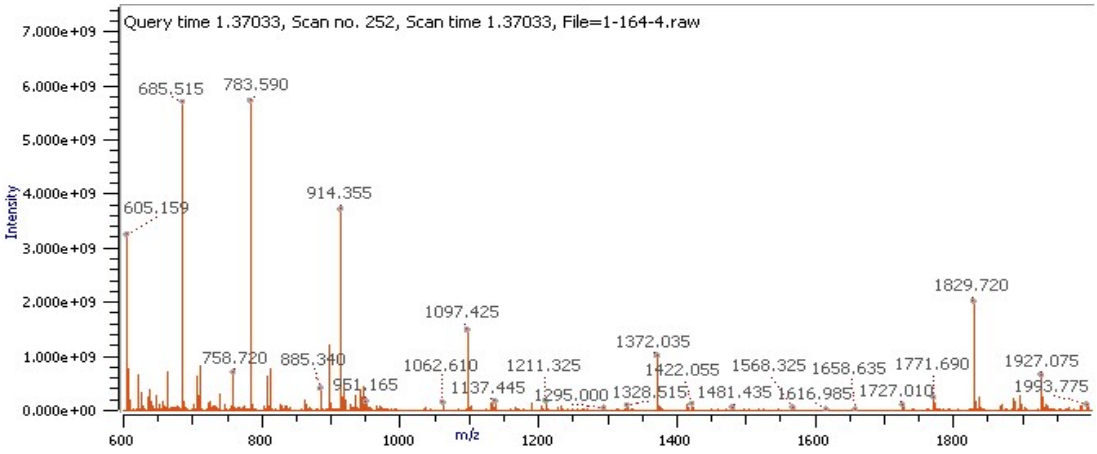


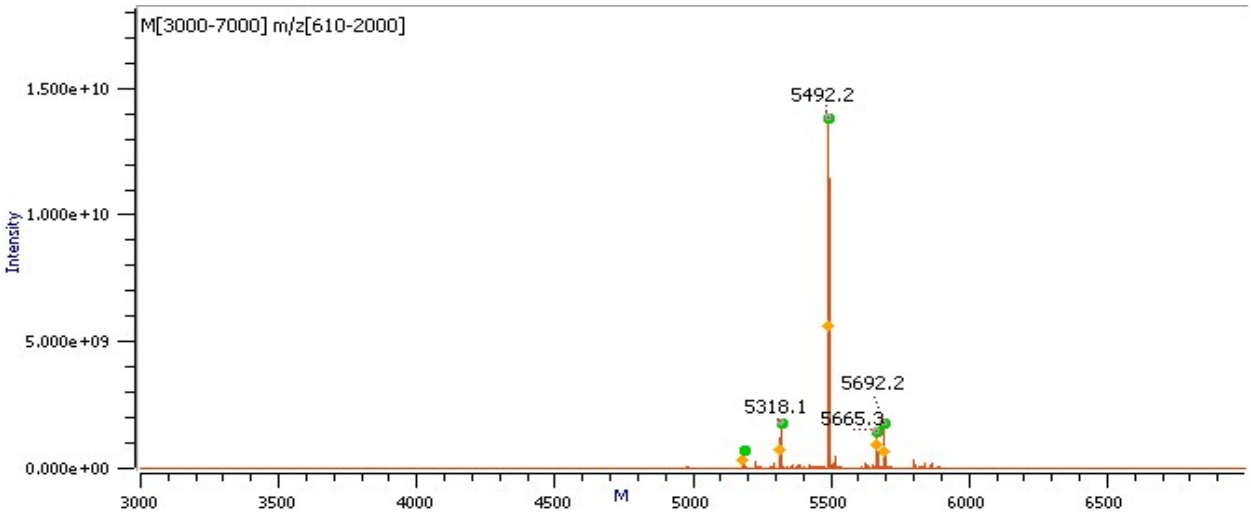


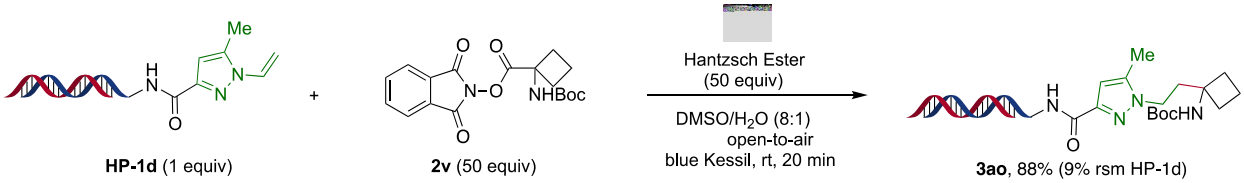
HP-1d (1 equiv) + **2s** (50 equiv)

3an, 81% (10% rsm HP-1d)
Molecular Weight: 5492.8560







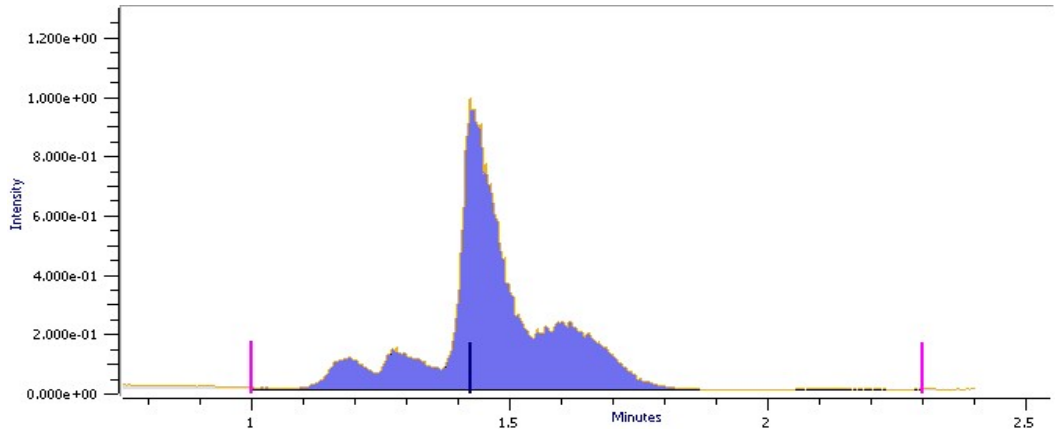


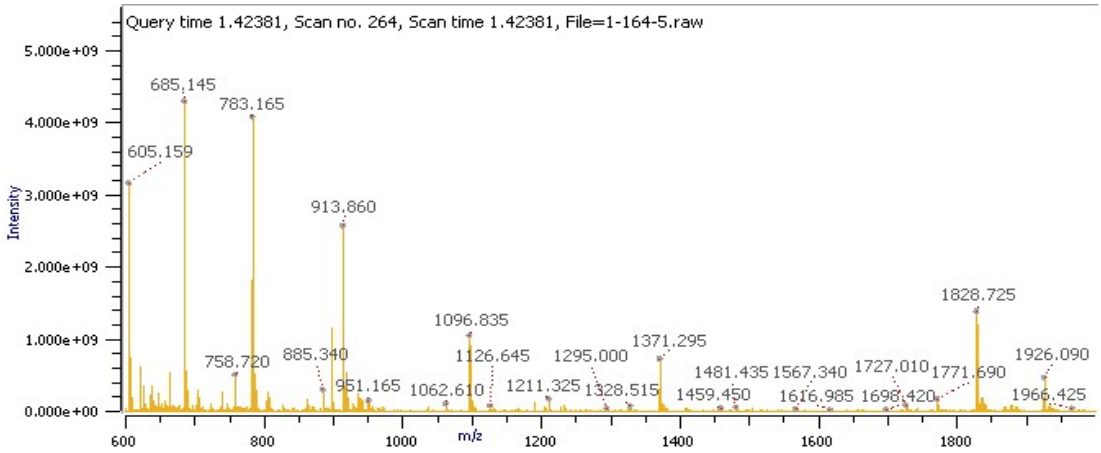
HP-1d (1 equiv)

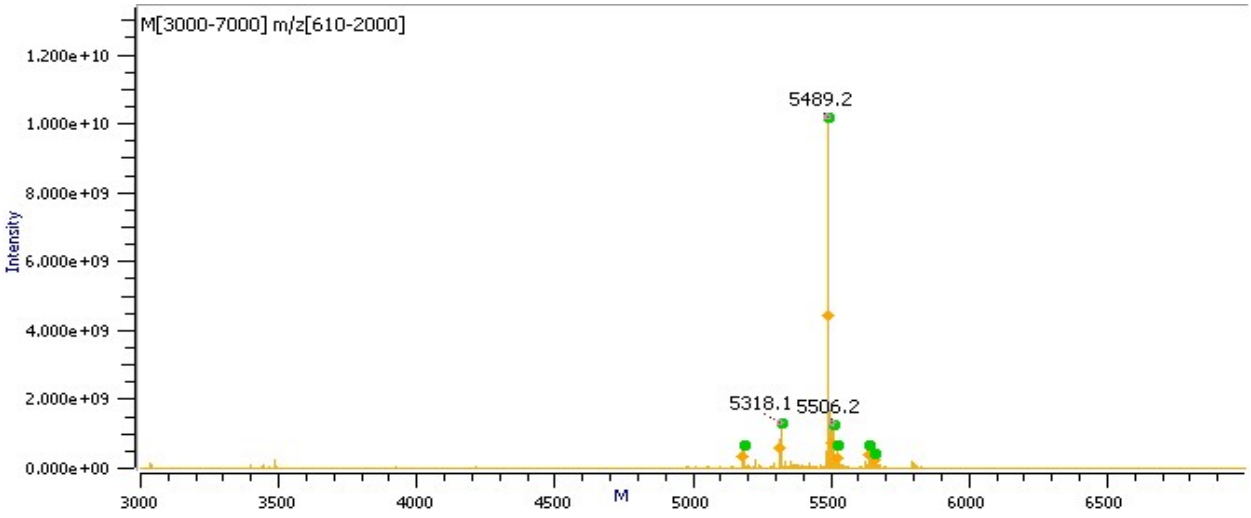
2v (50 equiv)

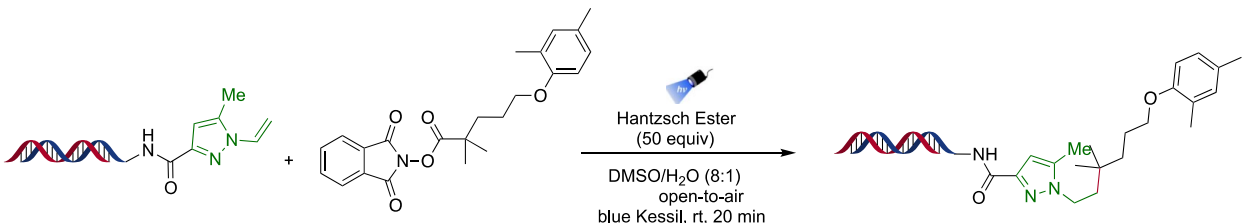
3ao, 88% (9% rsm HP-1d)

Molecular Weight: 5489.9000


Intensity
Minutes

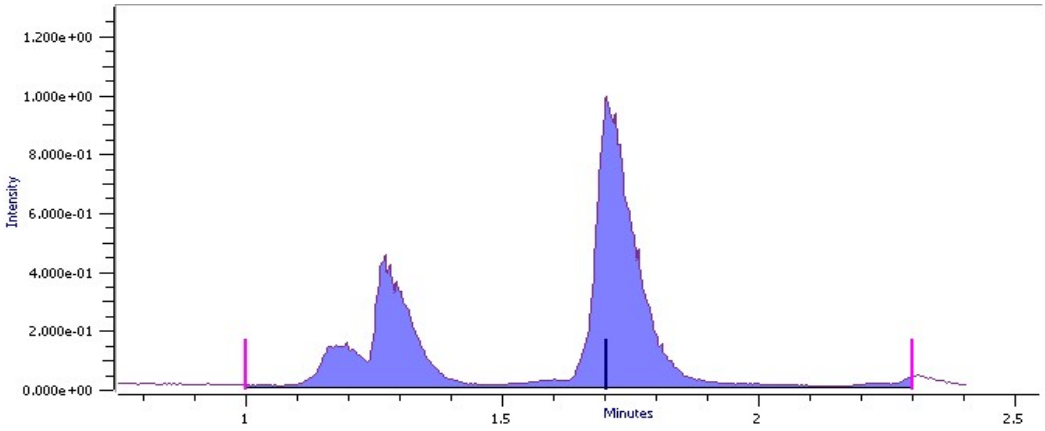

Query time 1.42381, Scan no. 264, Scan time 1.42381, File=1-164-5.raw
Intensity
m/z
685.145
783.165
605.159
913.860
1096.835
1828.725
758.720
885.340
1126.645
1295.000
1371.295
1926.090
951.165
1062.610
1211.325
1328.515
1481.435
1567.340
1727.010
1771.690
1459.450
1616.985
1698.420
1966.425

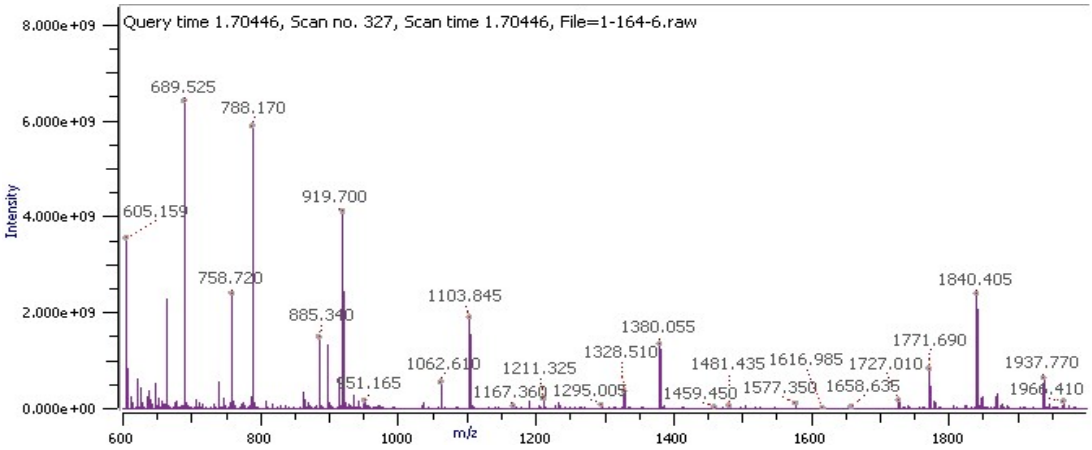

M[3000-7000] m/z[610-2000]
Intensity
M
5489.2
5318.1
5506.2

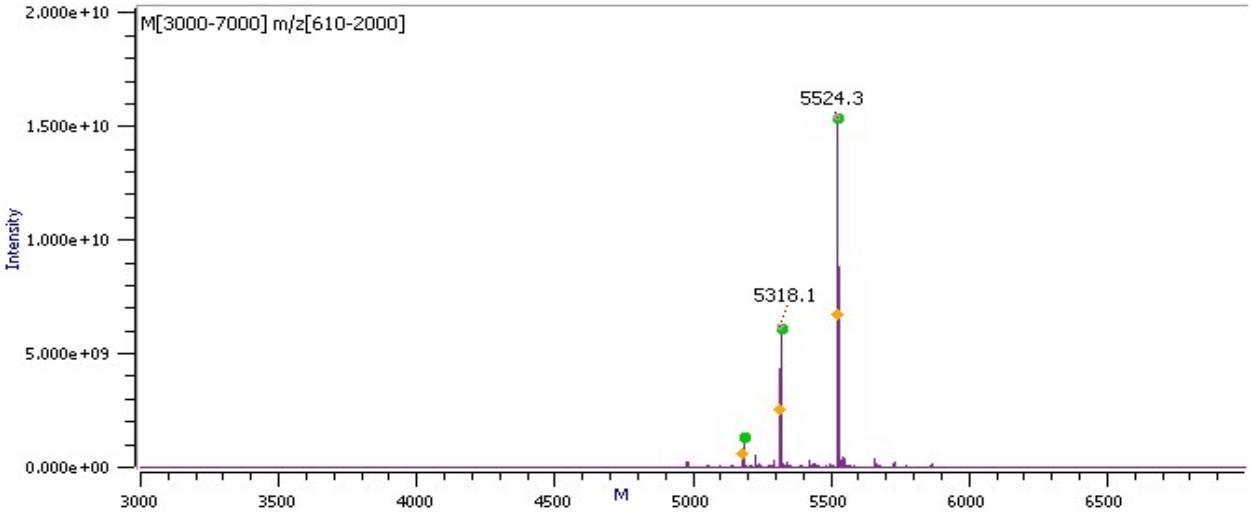


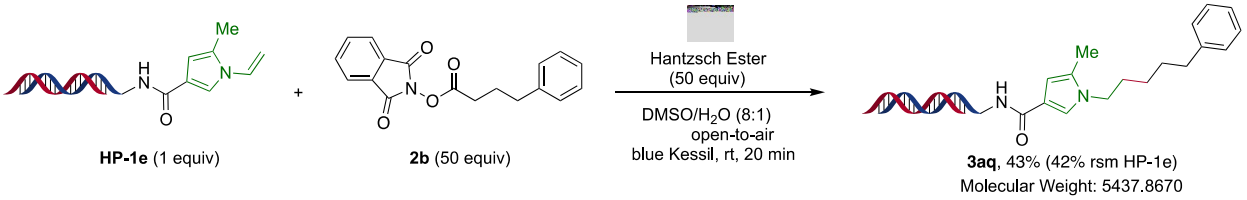
HP-1d (1 equiv) **2z** (50 equiv)

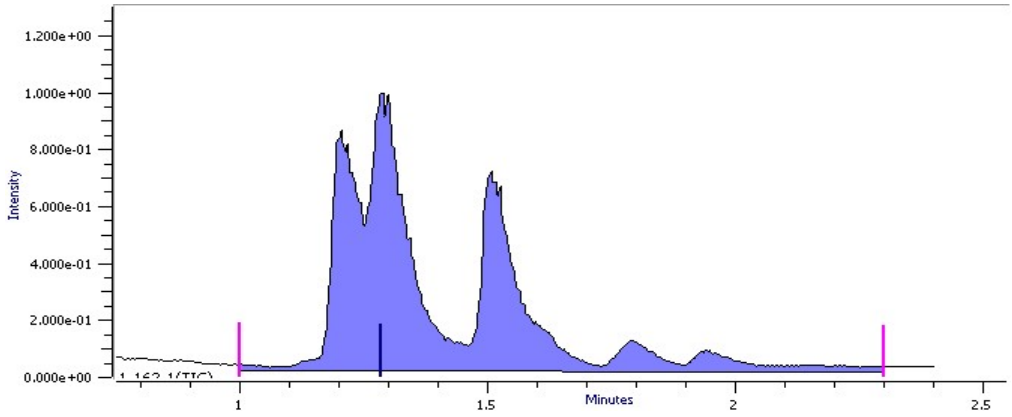
3ap, 71% (27% rsm HP-1d)
Molecular Weight: 5524.9890

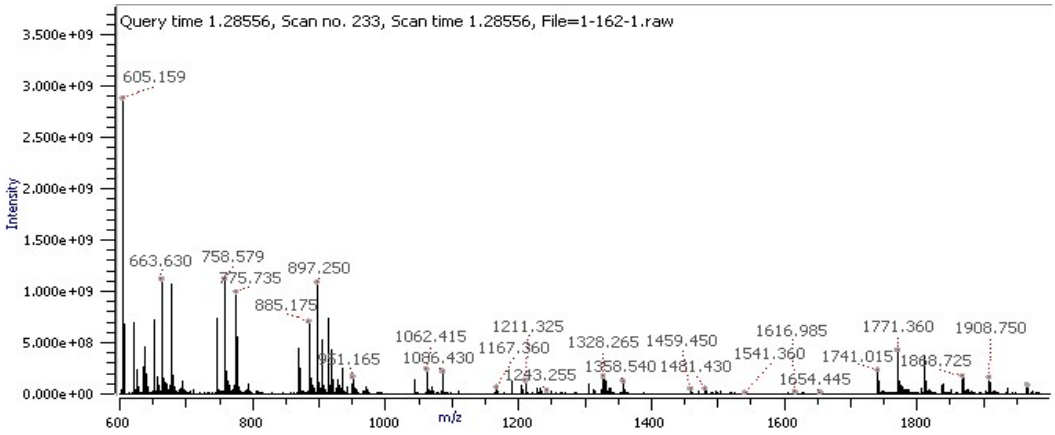


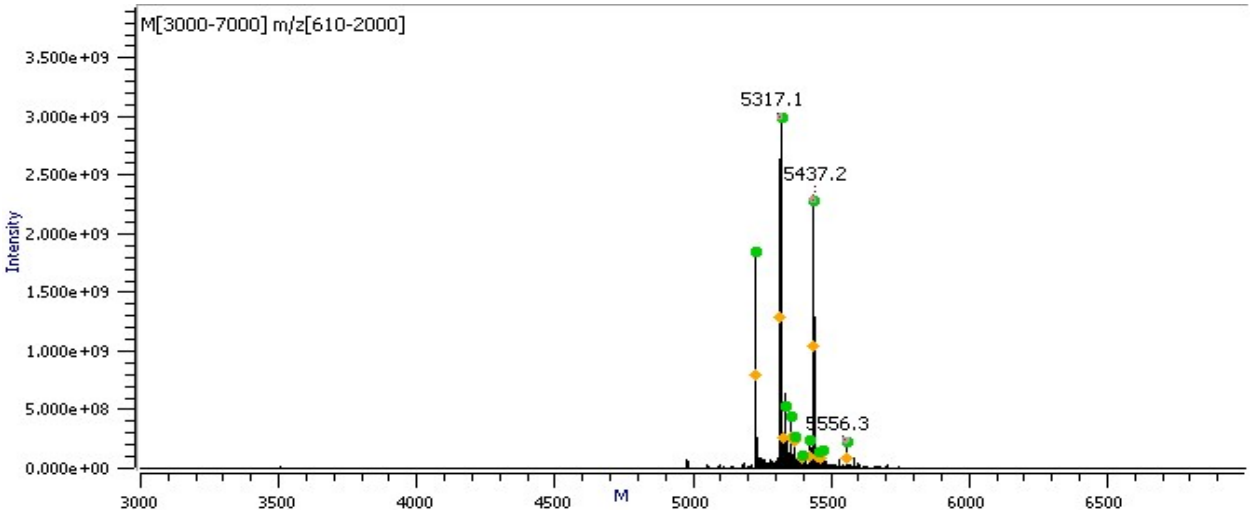


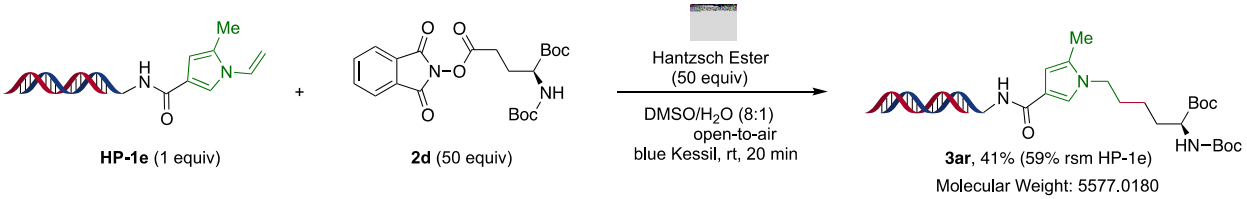



Me
H
N
O
HP-1e (1 equiv)
+
2b (50 equiv)
Hantzsch Ester
(50 equiv)
DMSO/H2O (8:1)
open-to-air
blue Kessil, rt, 20 min
Me
3aq, 43% (42% rsm HP-1e)
Molecular Weight: 5437.8670


Intensity
Minutes


Query time 1.28556, Scan no. 233, Scan time 1.28556, File=1-162-1.raw
605.159
663.630
758.579
775.735
897.250
885.175
1062.415
1086.430
1211.325
1167.360
1328.265
1459.450
1616.985
1771.360
1908.750
Intensity
m/z

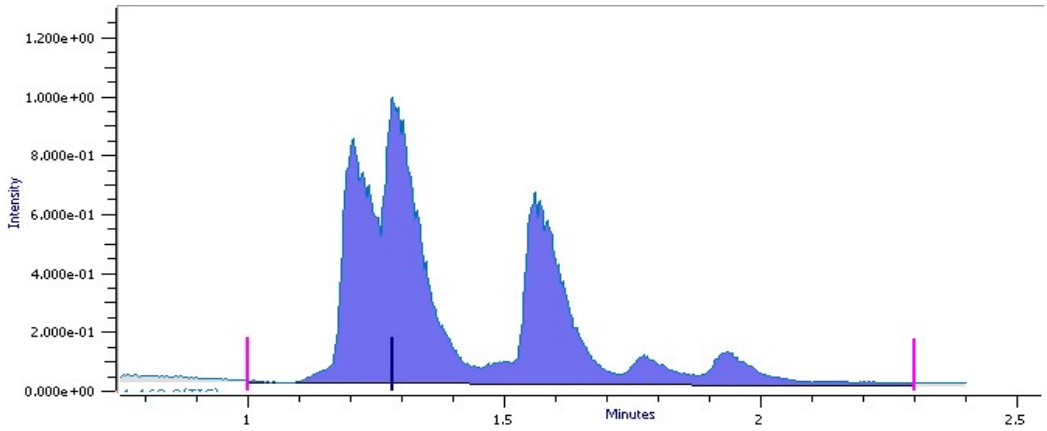

M[3000-7000] m/z[610-2000]
5317.1
5437.2
5556.3
Intensity
M

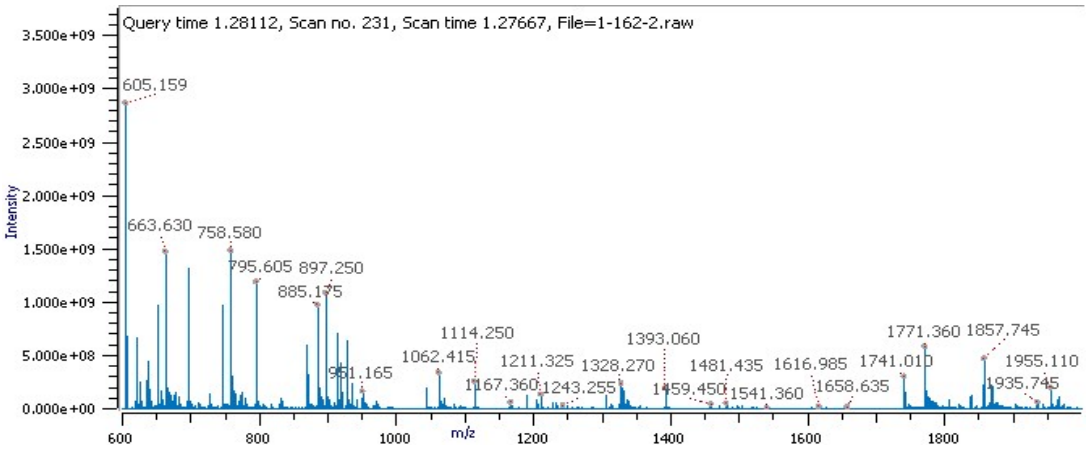


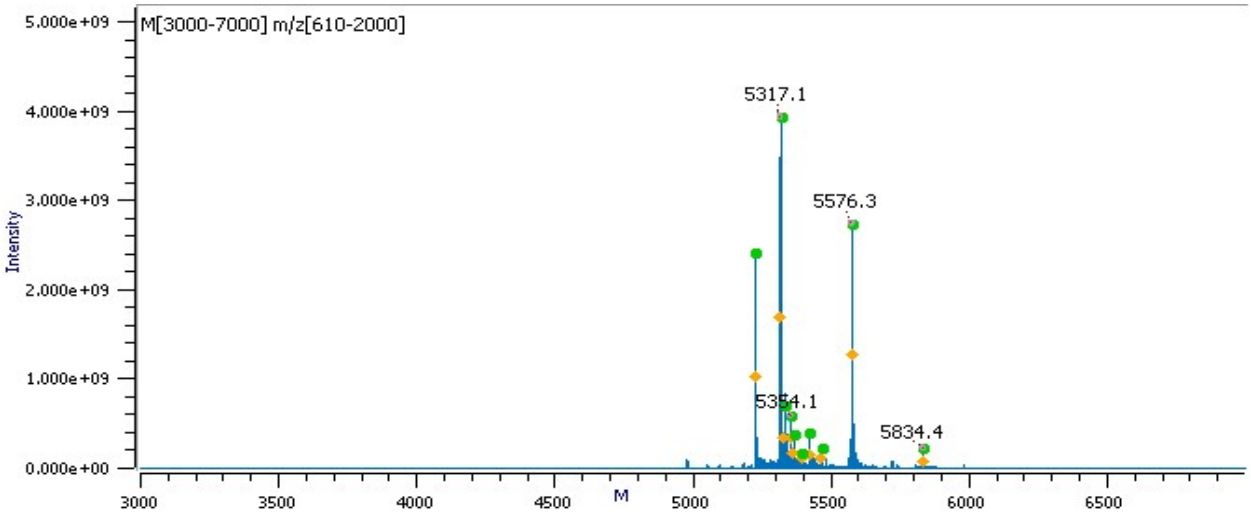
HP-1e (1 equiv) + **2d** (50 equiv)

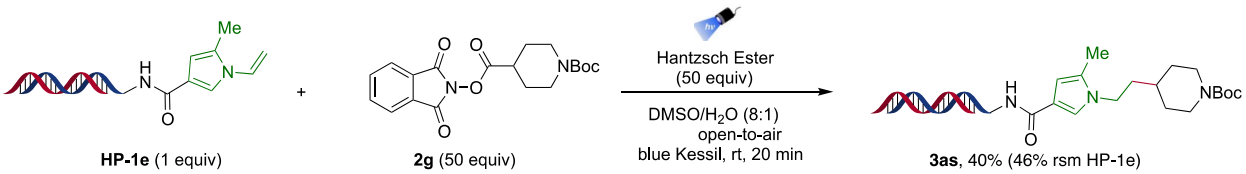
3ar, 41% (59% rsm HP-1e)

Molecular Weight: 5577.0180





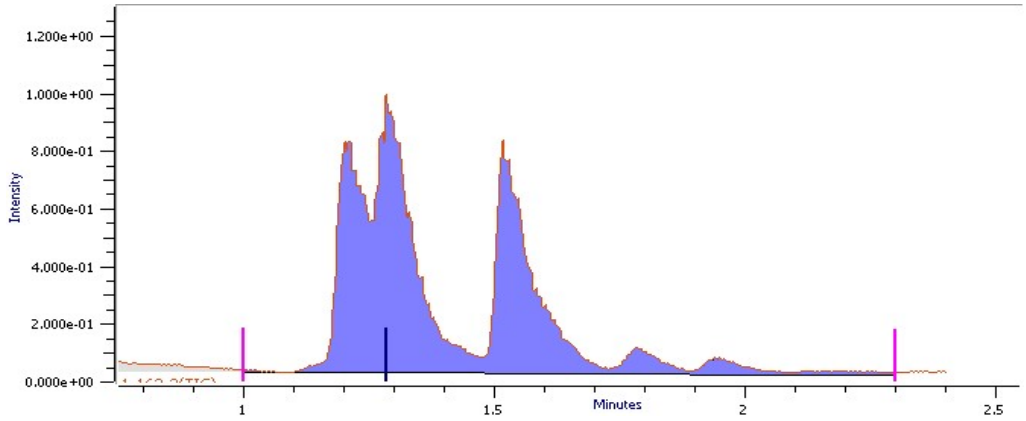


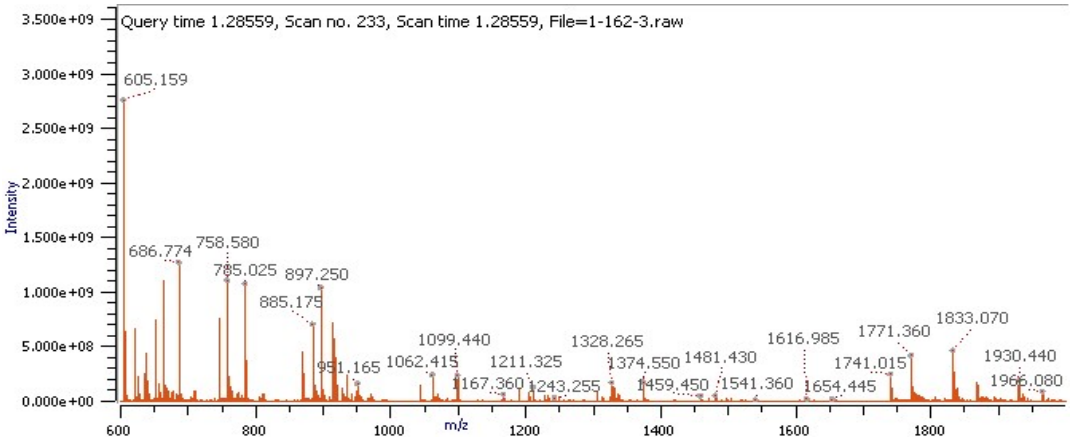


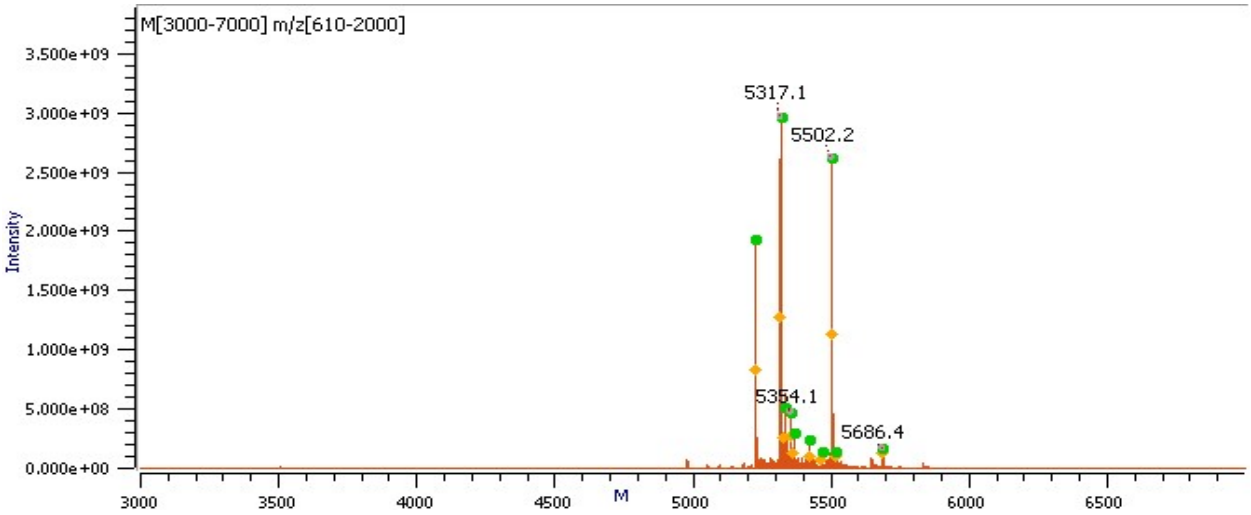
HP-1e (1 equiv) + **2g** (50 equiv)

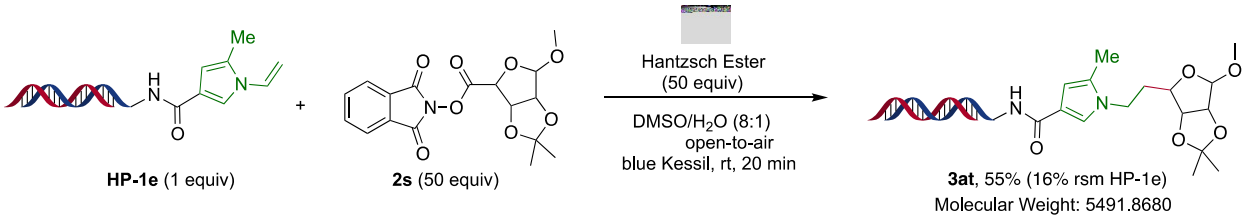
3as, 40% (46% rsm HP-1e)

Molecular Weight: 5502.9390



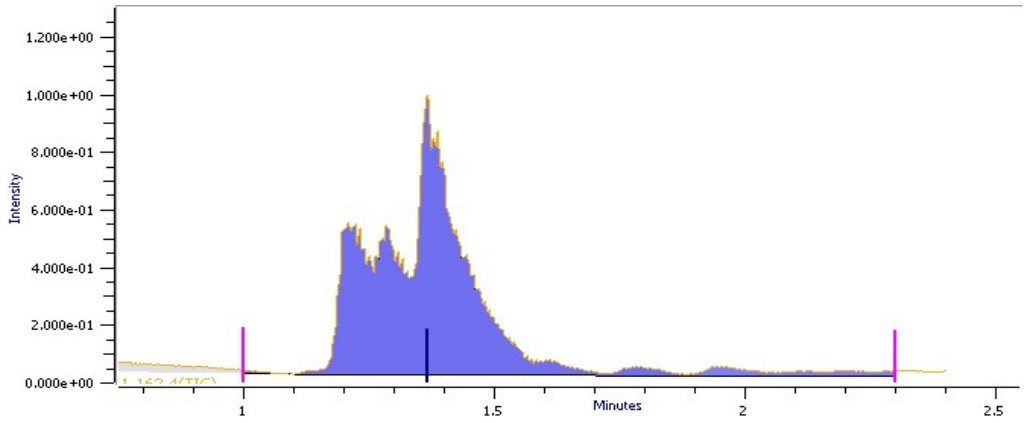


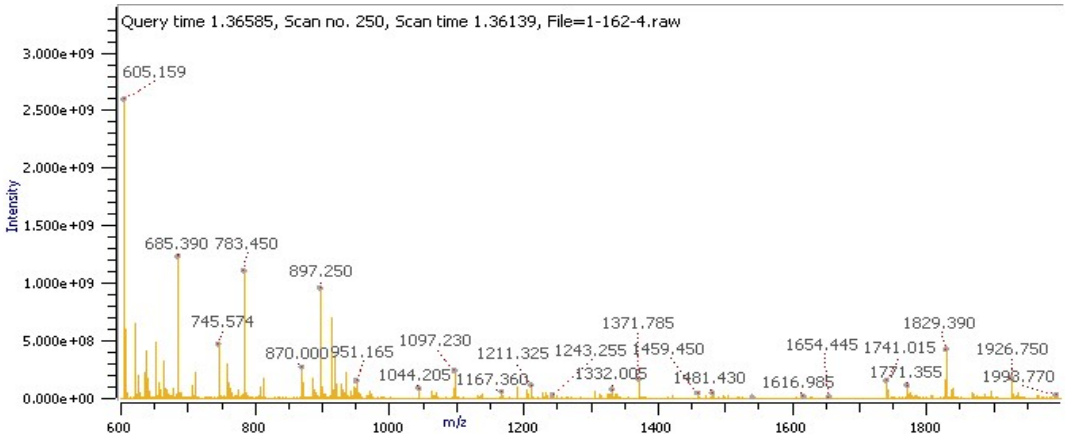


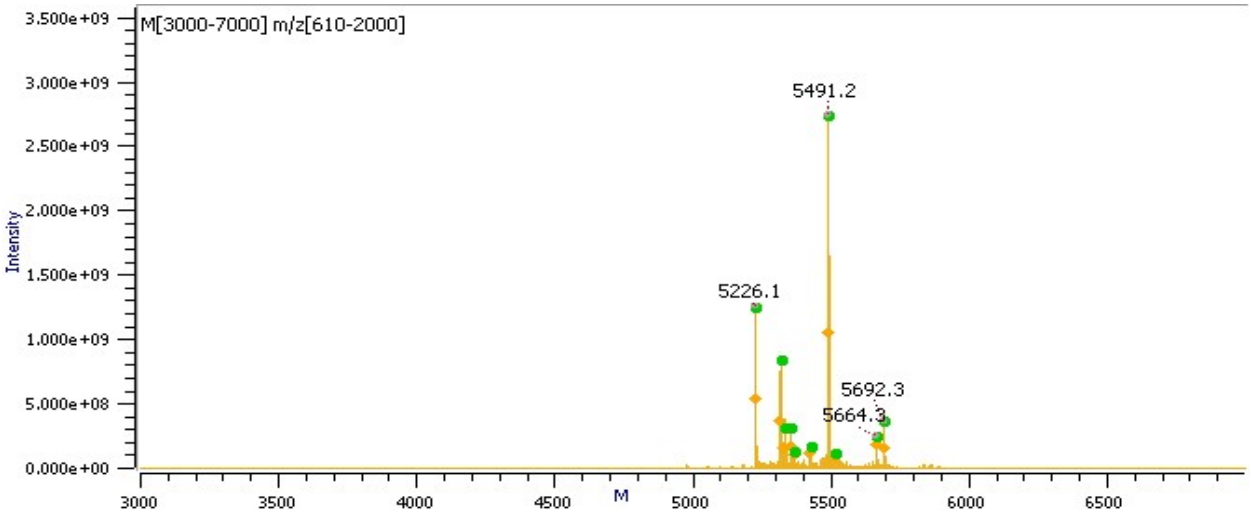


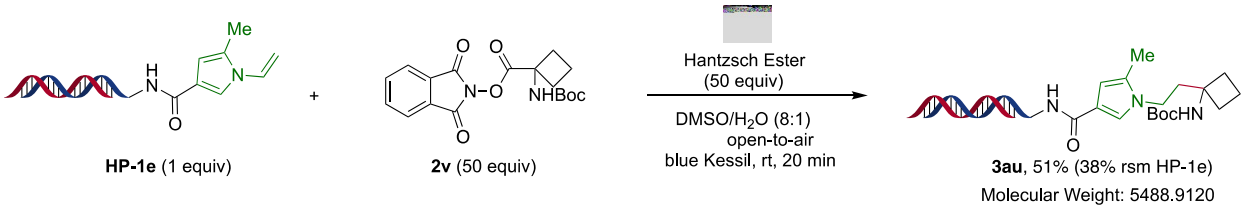
HP-1e (1 equiv) + **2s** (50 equiv)

3at, 55% (16% rsm HP-1e)
Molecular Weight: 5491.8680





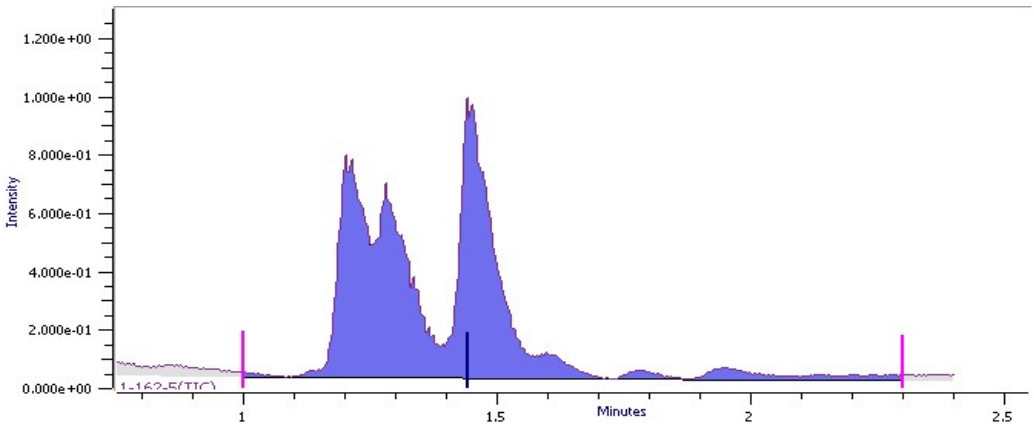


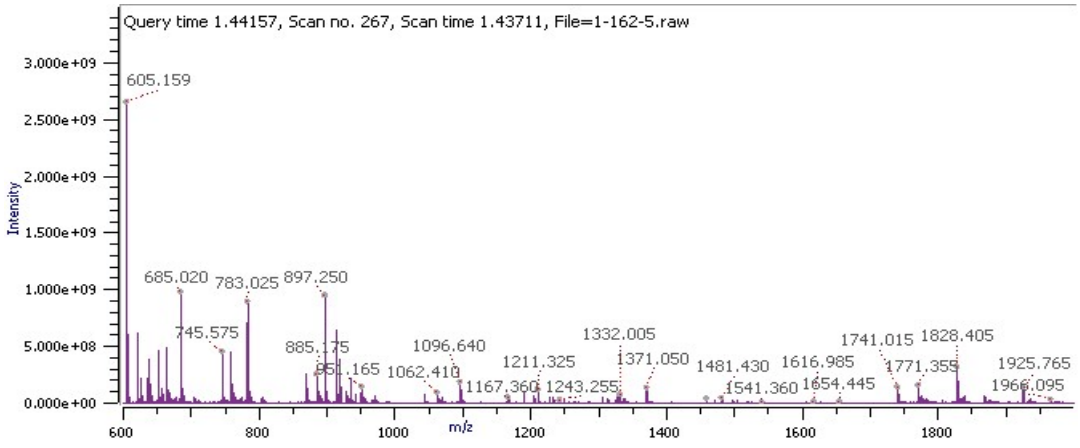


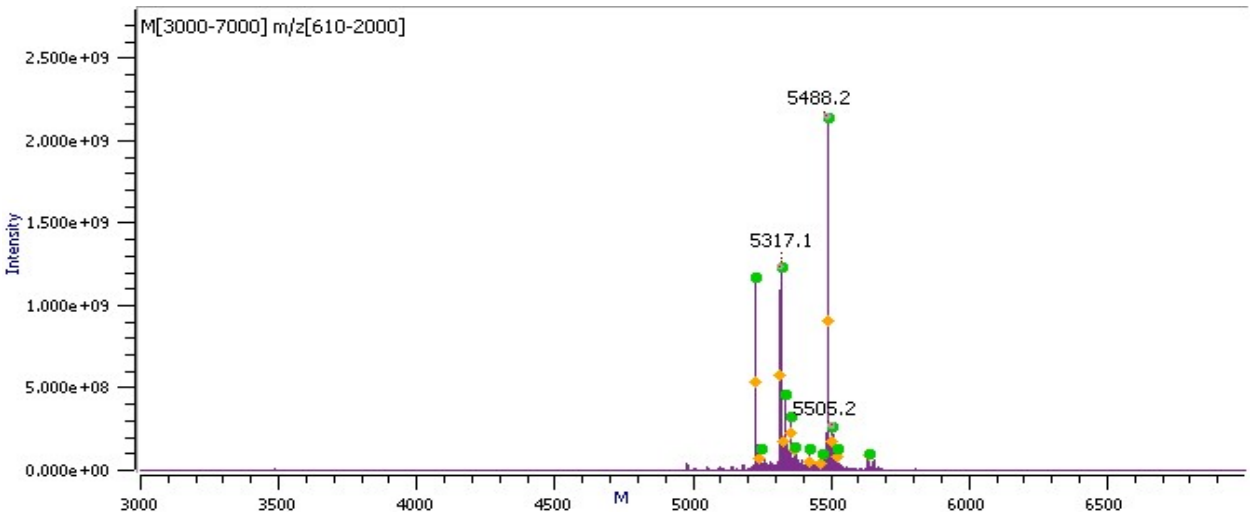
HP-1e (1 equiv) + **2v** (50 equiv)

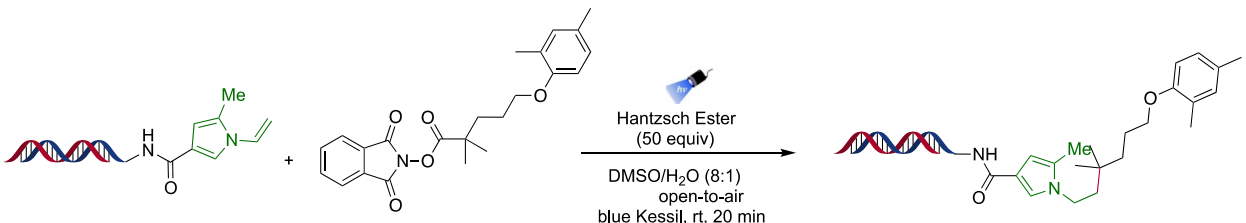
3au, 51% (38% rsm HP-1e)

Molecular Weight: 5488.9120



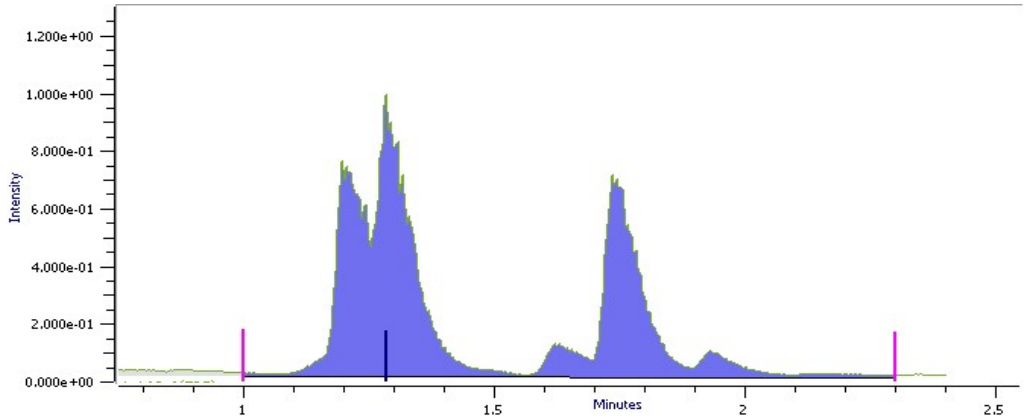


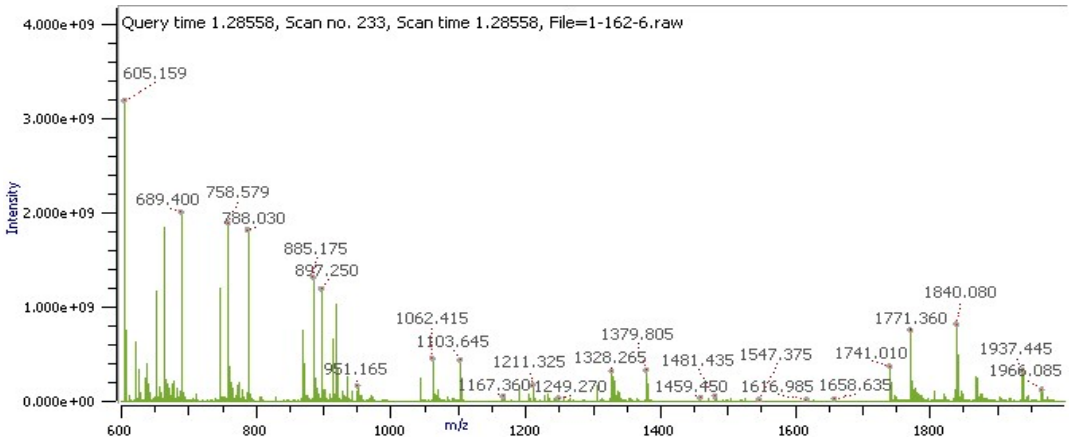


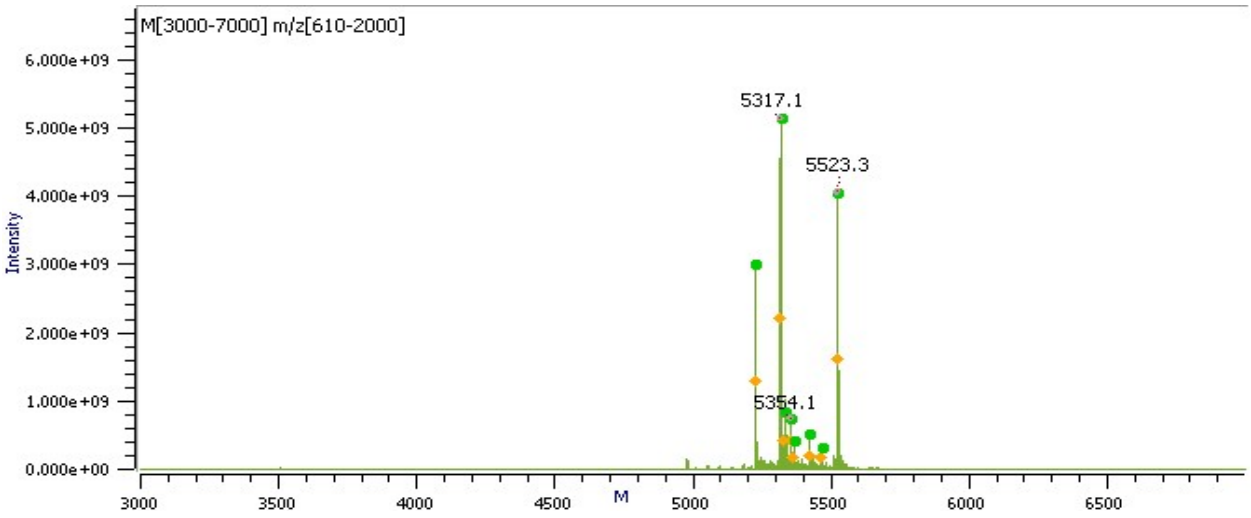


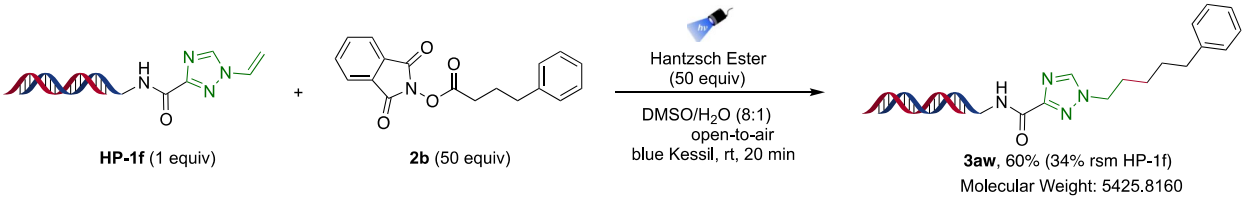
HP-1e (1 equiv) + **2z** (50 equiv)

3av, 40% (45% rsm HP-1e)
Molecular Weight: 5524.0010



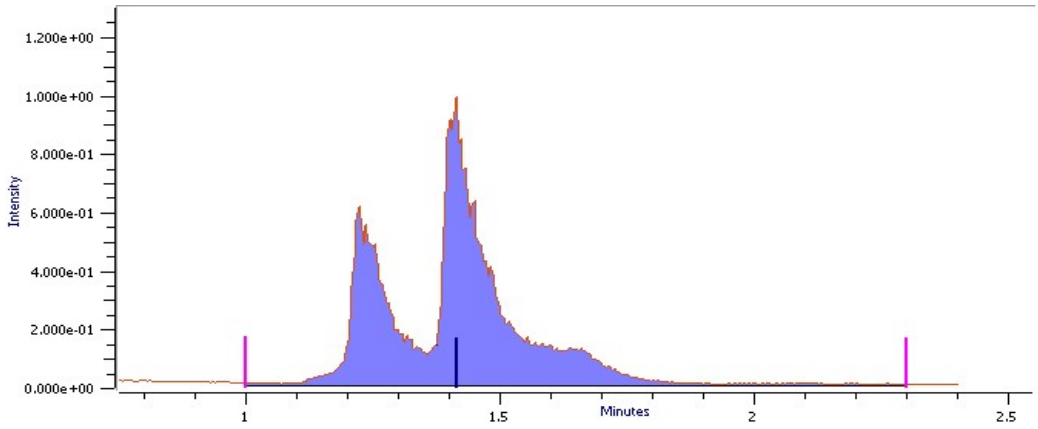


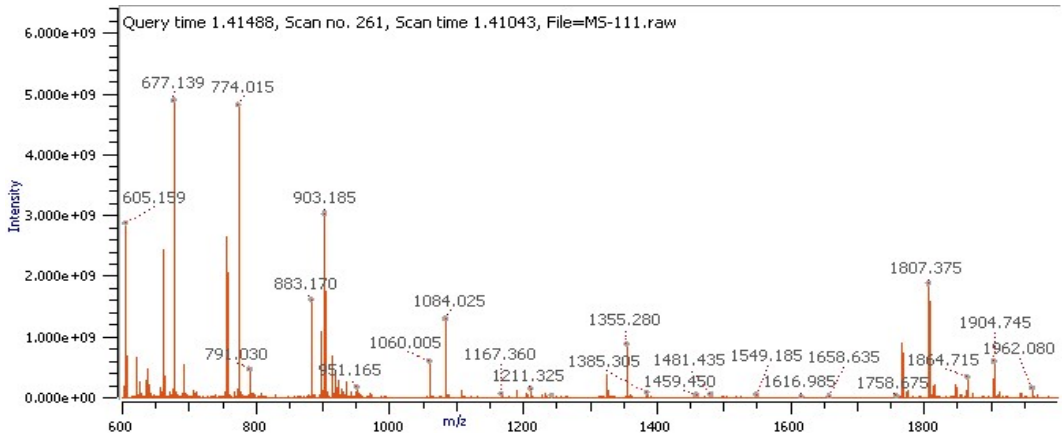


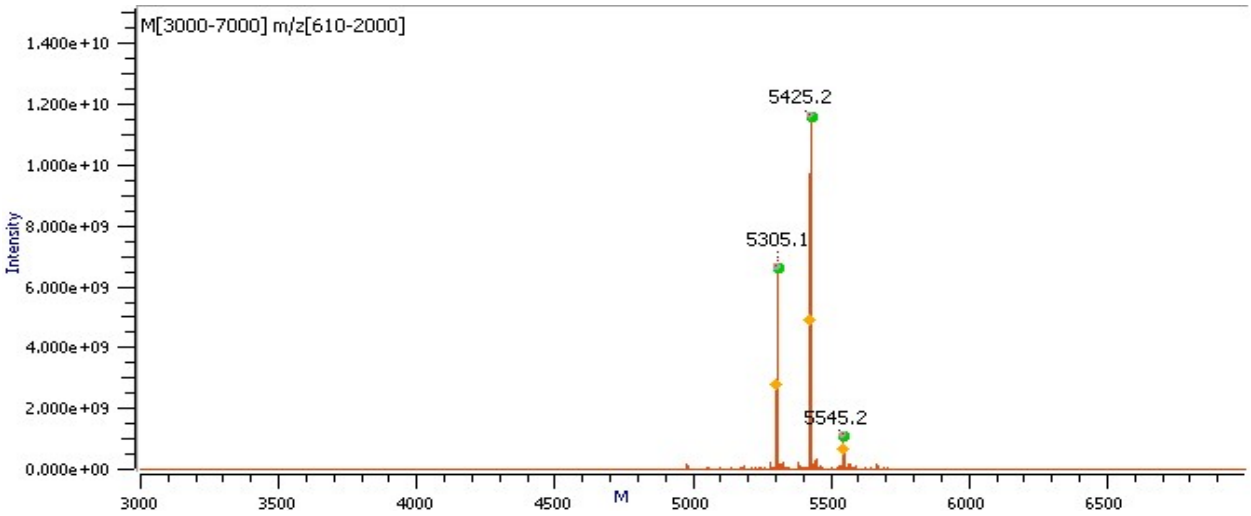


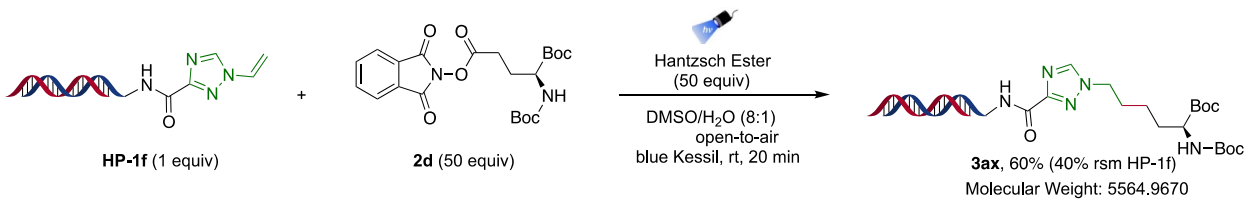
HP-1f (1 equiv) + **2b** (50 equiv)

3aw, 60% (34% rsm HP-1f)
Molecular Weight: 5425.8160





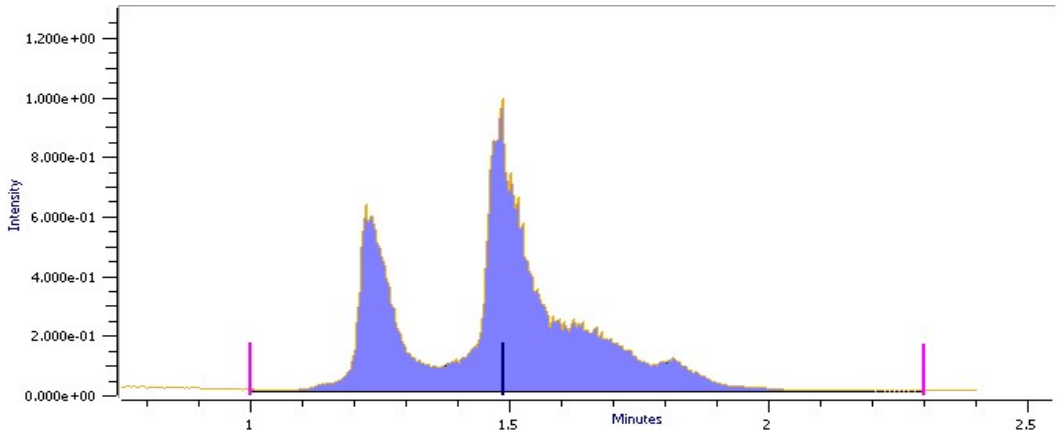


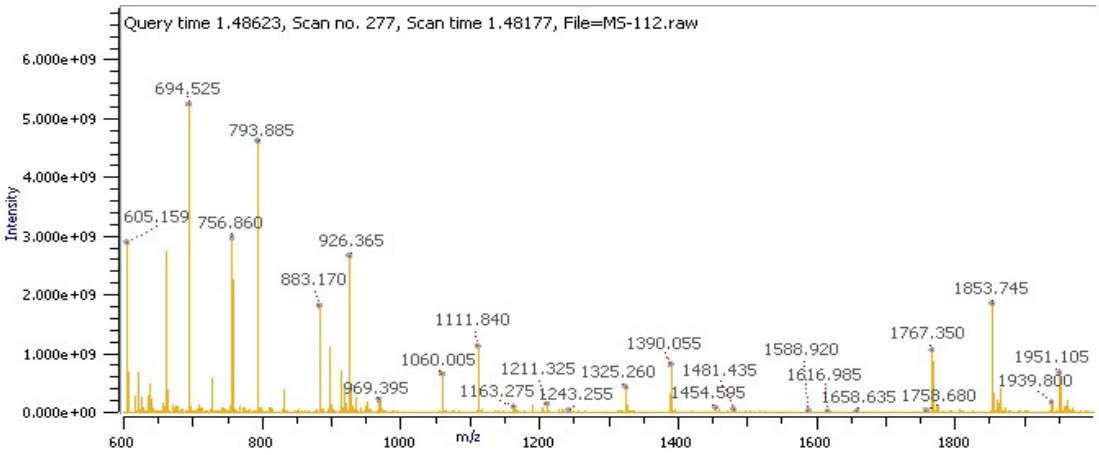


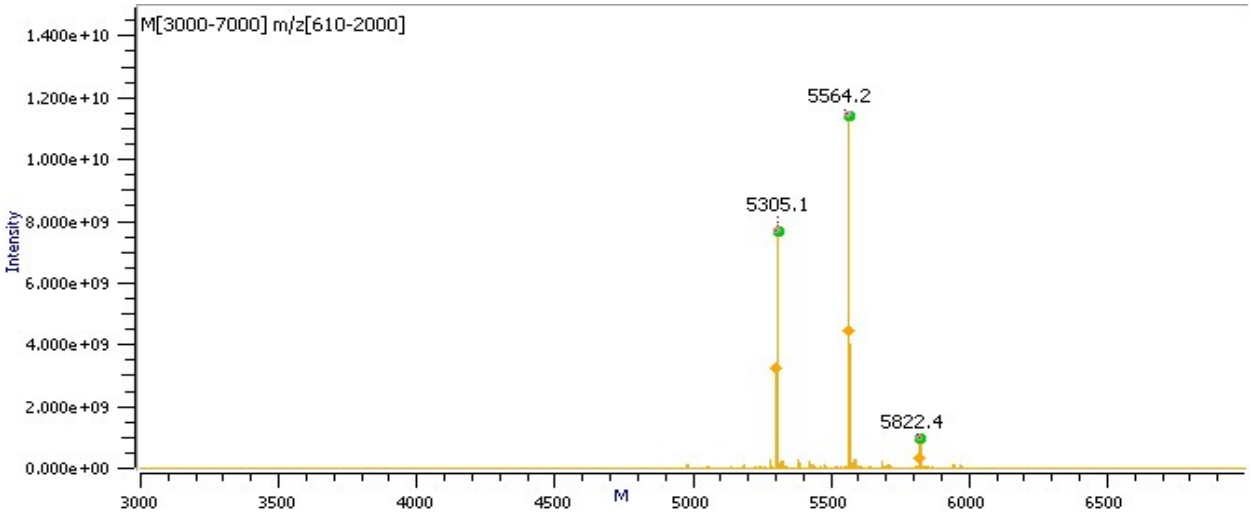
HP-1f (1 equiv) + **2d** (50 equiv)

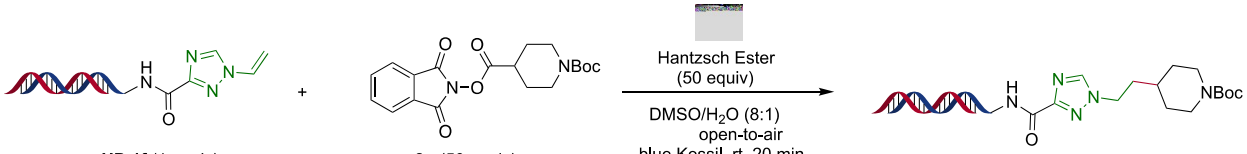
3ax, 60% (40% rsm HP-1f)

Molecular Weight: 5564.9670





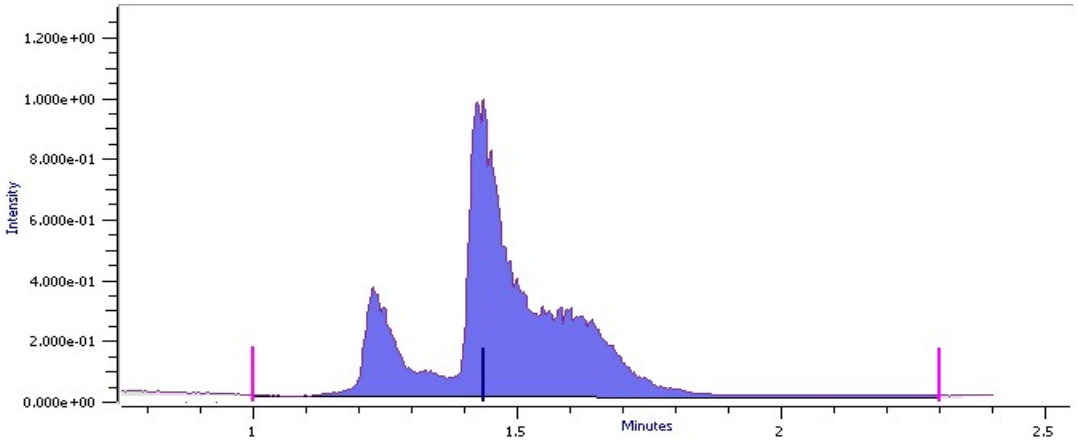


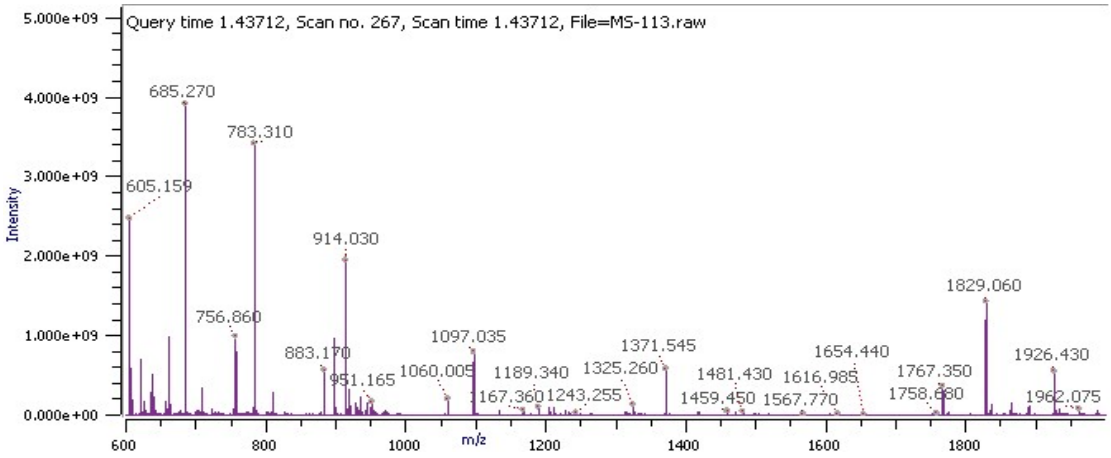


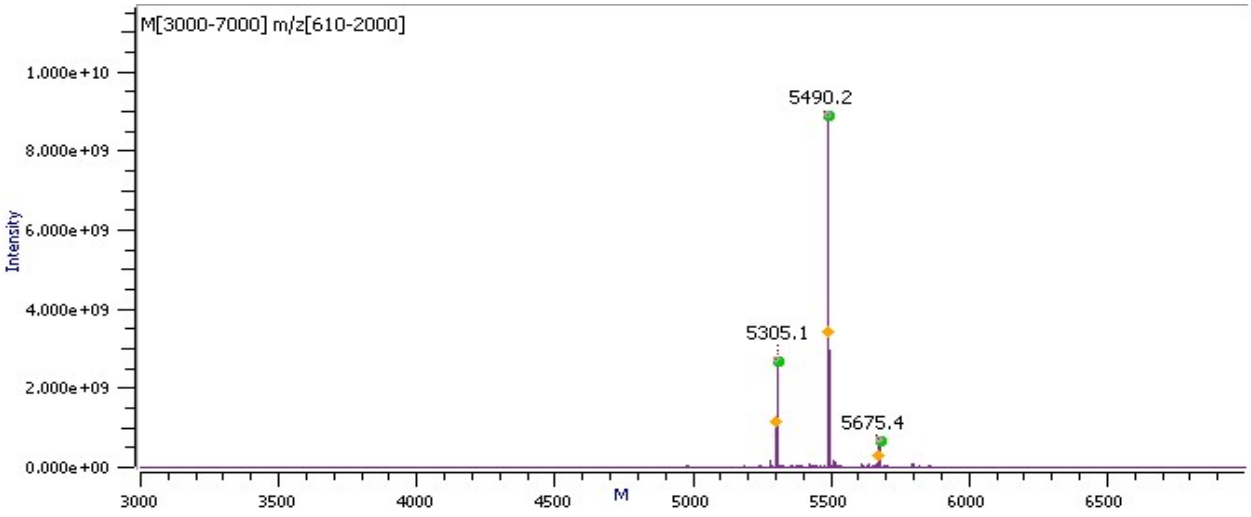
HP-1f (1 equiv) + **2g** (50 equiv)

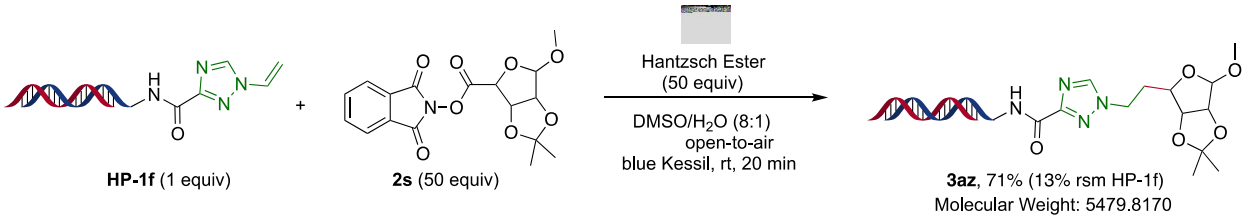
3ay, 72% (22% rsm HP-1f)

Molecular Weight: 5490.8880



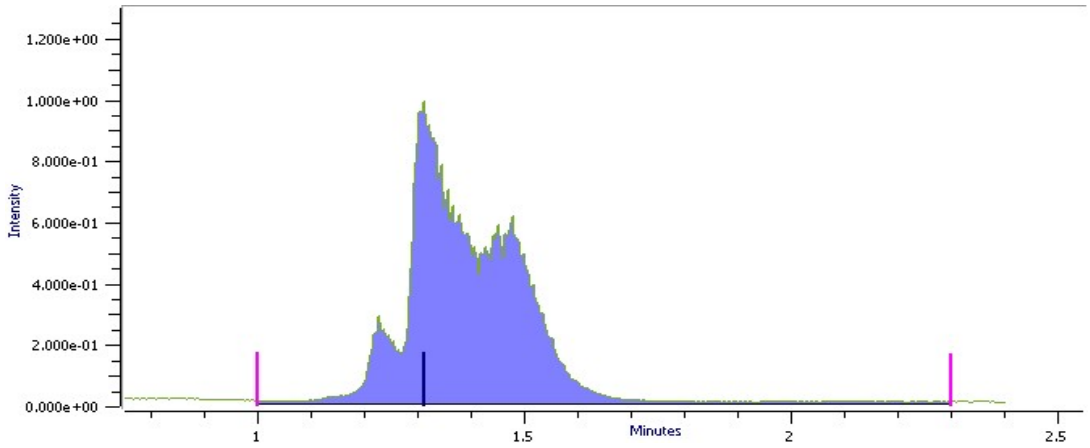


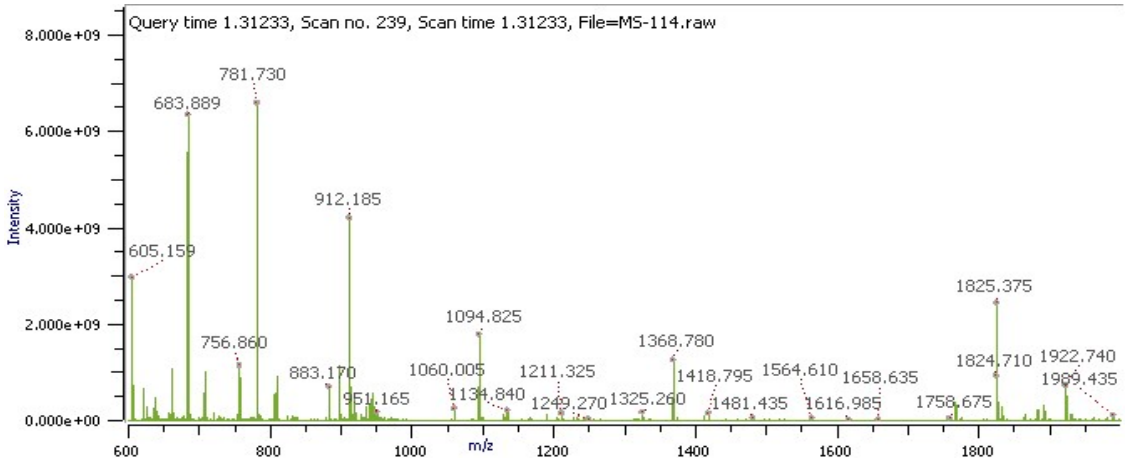


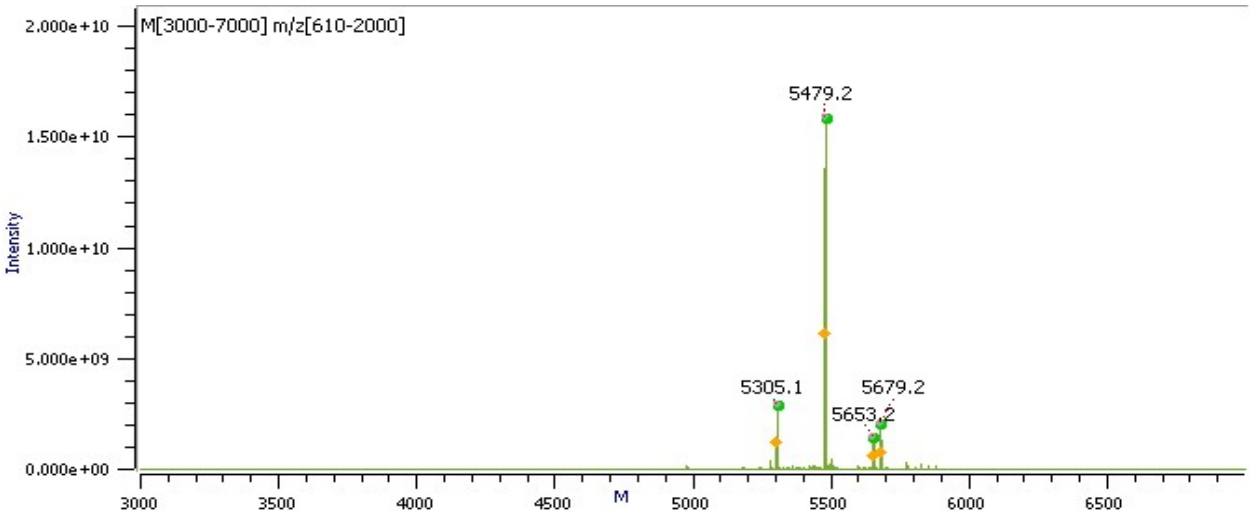


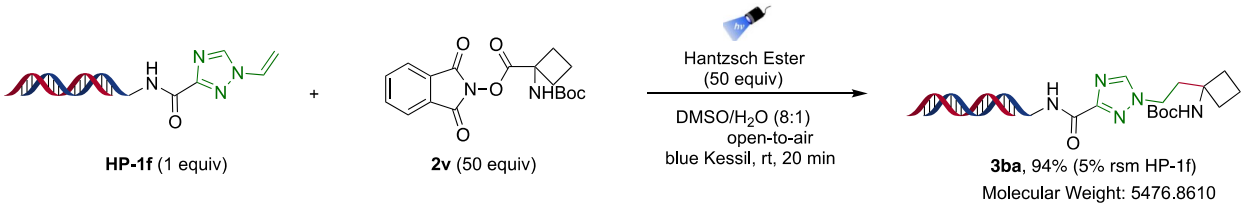
HP-1f (1 equiv) + **2s** (50 equiv)

3az, 71% (13% rsm HP-1f)
Molecular Weight: 5479.8170





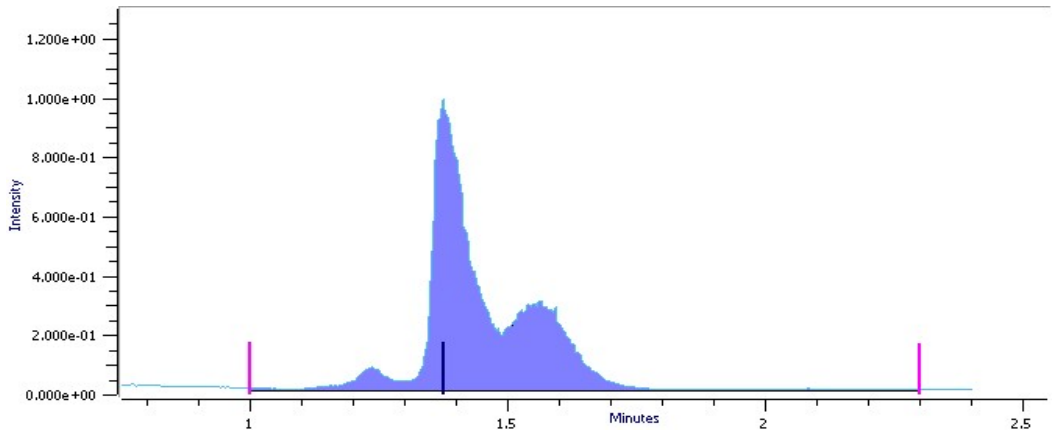


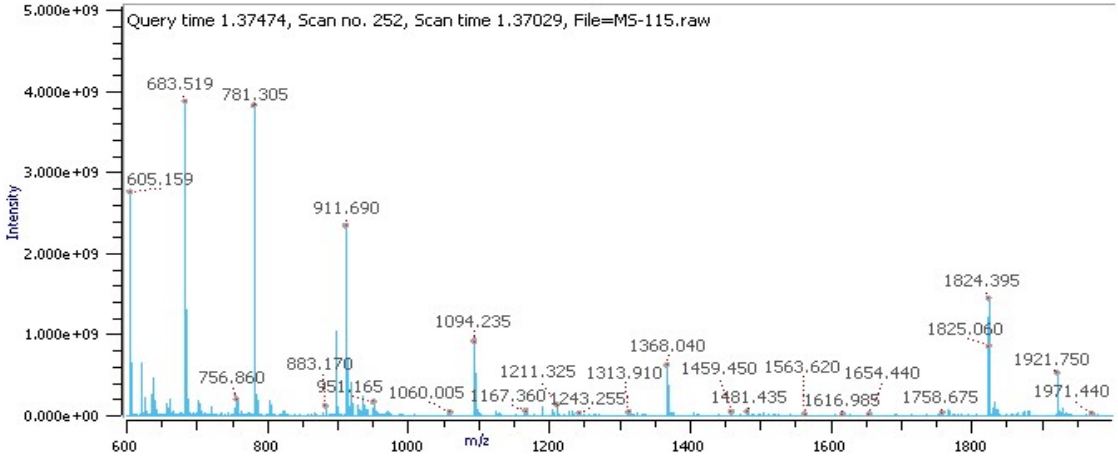


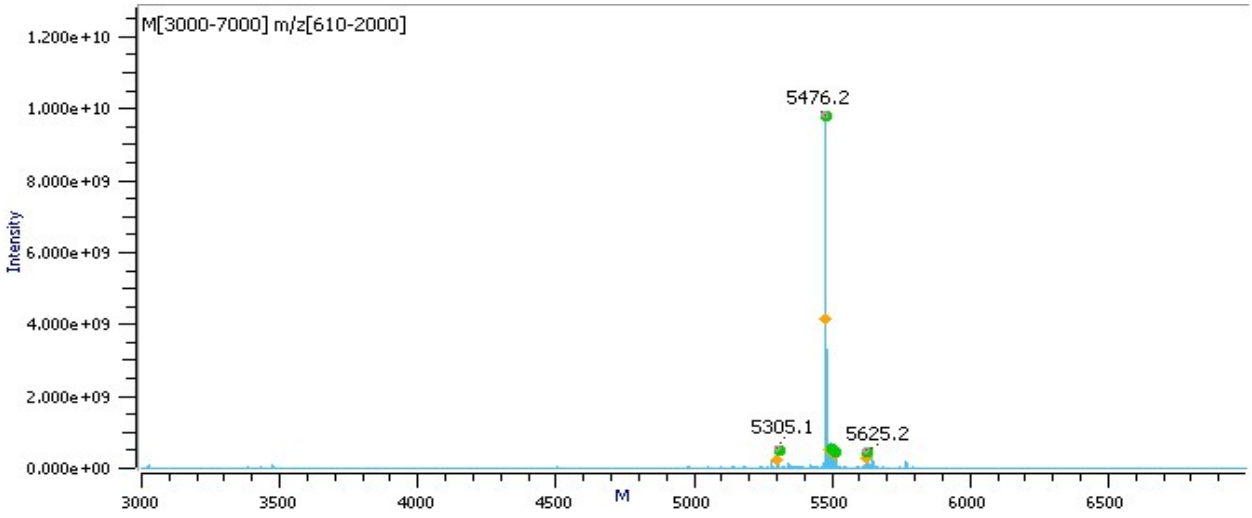
HP-1f (1 equiv) + **2v** (50 equiv)

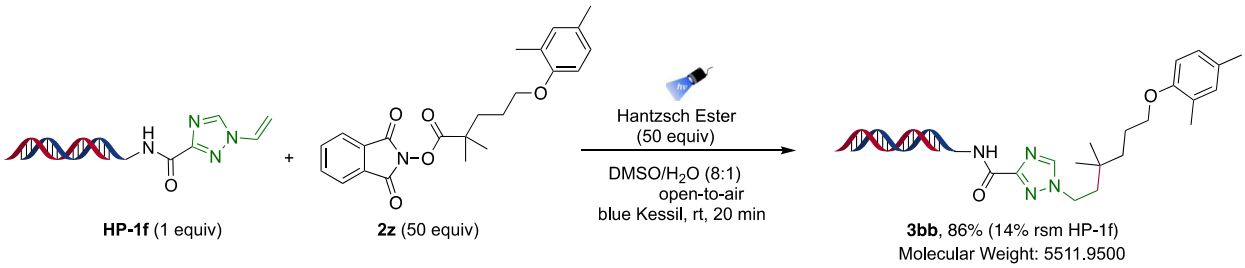
3ba, 94% (5% rsm HP-1f)

Molecular Weight: 5476.8610



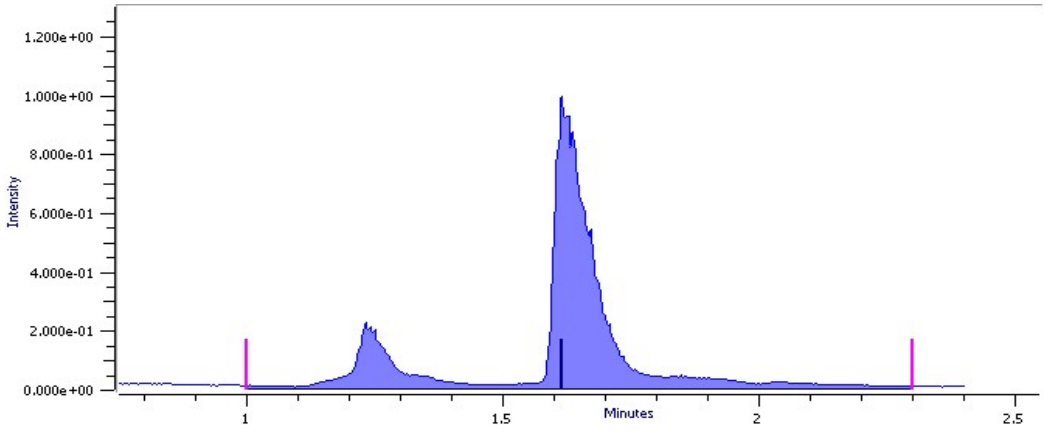


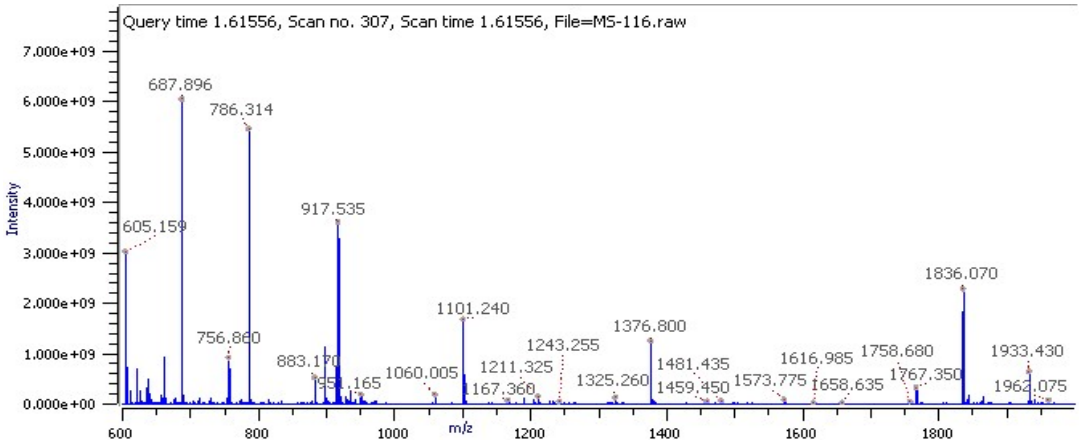


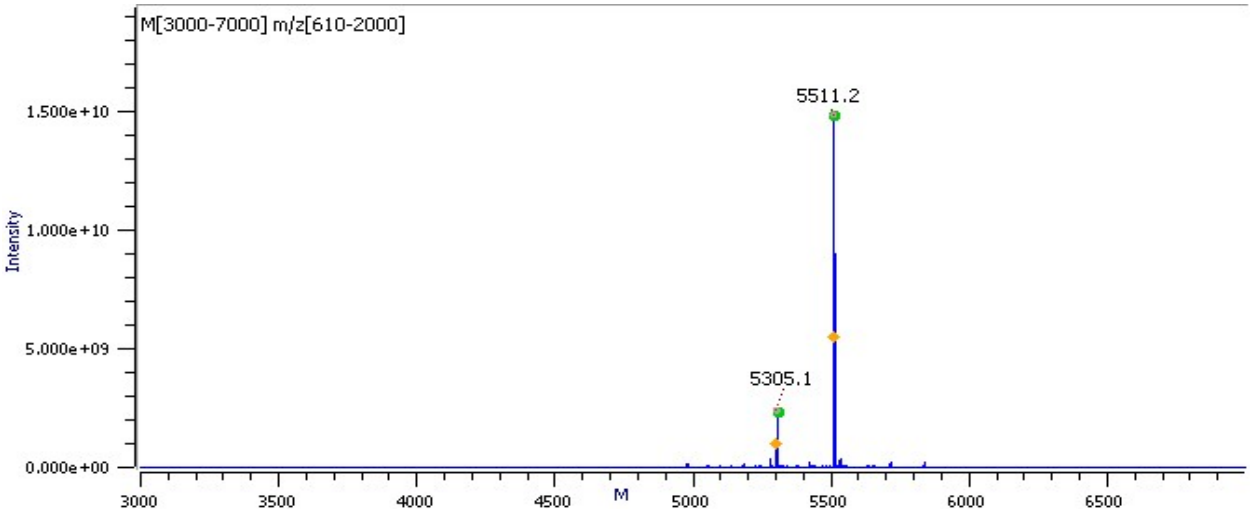


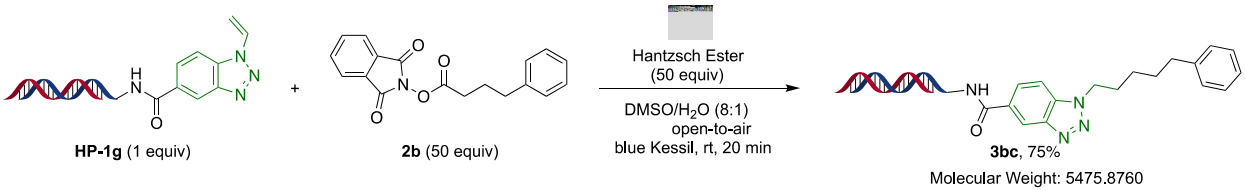
HP-1f (1 equiv) + **2z** (50 equiv)

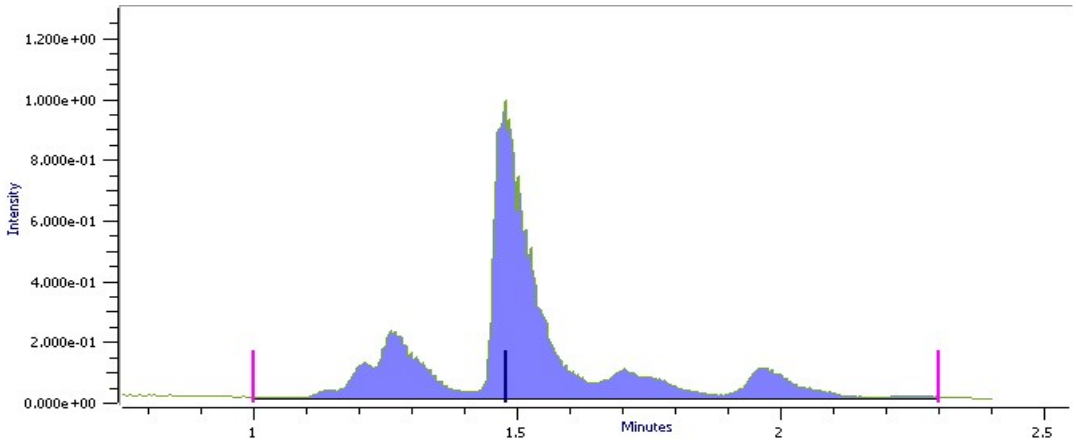
3bb, 86% (14% rsm HP-1f)
Molecular Weight: 5511.9500

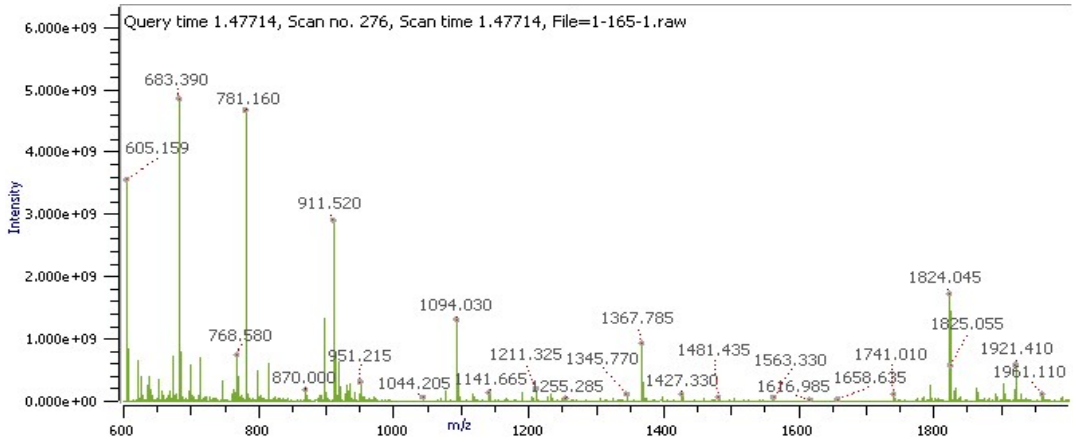


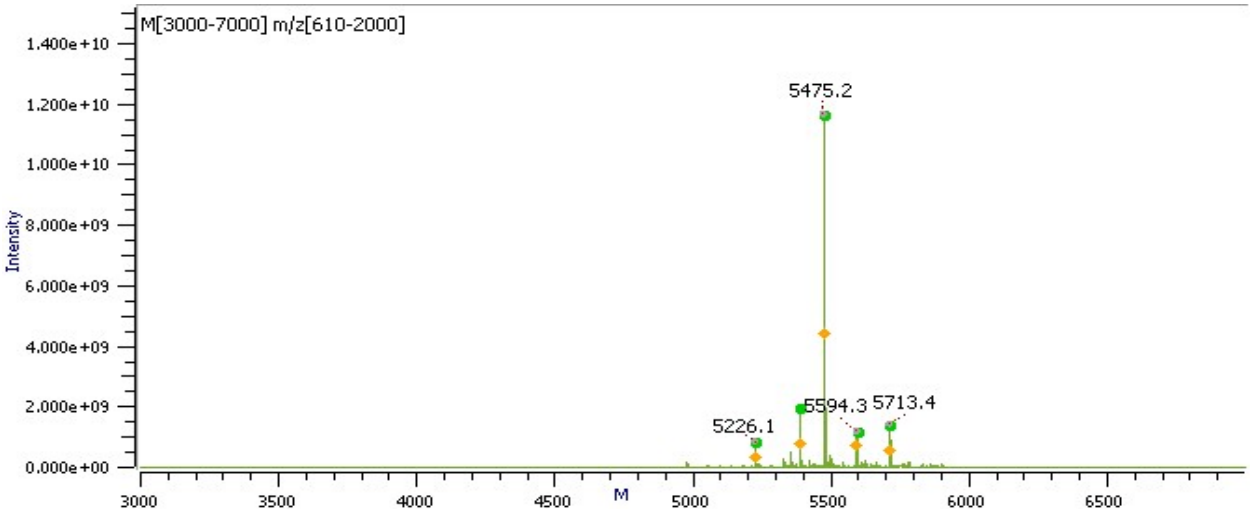


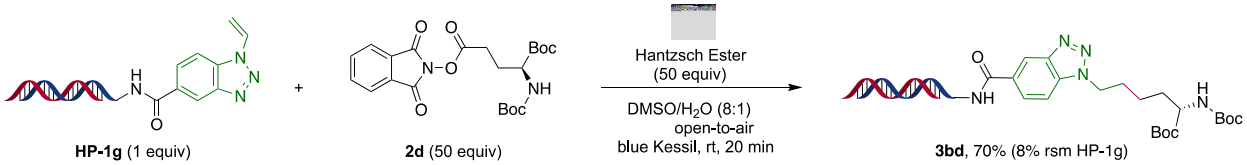



HP-1g (1 equiv)
+
2b (50 equiv)
Hantzsch Ester
(50 equiv)
DMSO/H2O (8:1)
open-to-air
blue Kessil, rt, 20 min
3bc, 75%
Molecular Weight: 5475.8760


Intensity
Minutes


Query time 1.47714, Scan no. 276, Scan time 1.47714, File=1-165-1.raw
683.390
781.160
605.159
911.520
1094.030
768.580
951.215
870.000
1044.205
1141.665
1211.325
1255.285
1345.770
1367.785
1427.330
1481.435
1563.330
1616.985
1658.635
1741.010
1824.045
1825.055
1921.410
1961.110
Intensity
m/z

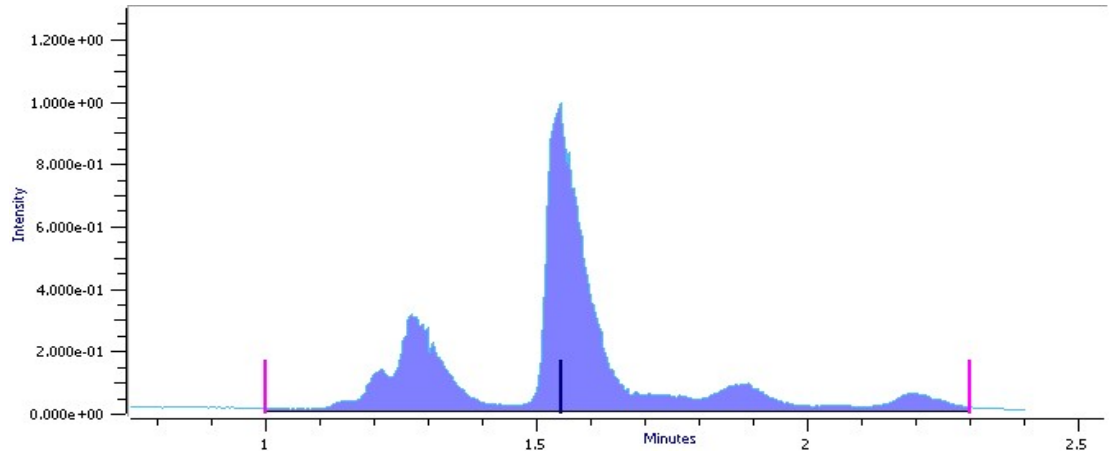

M[3000-7000] m/z[610-2000]
5475.2
5594.3
5713.4
5226.1
Intensity
M

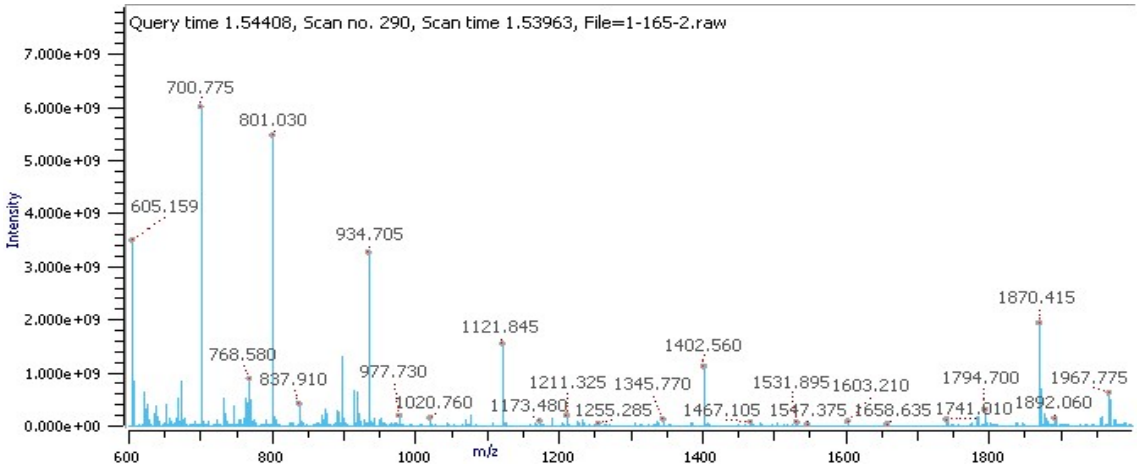


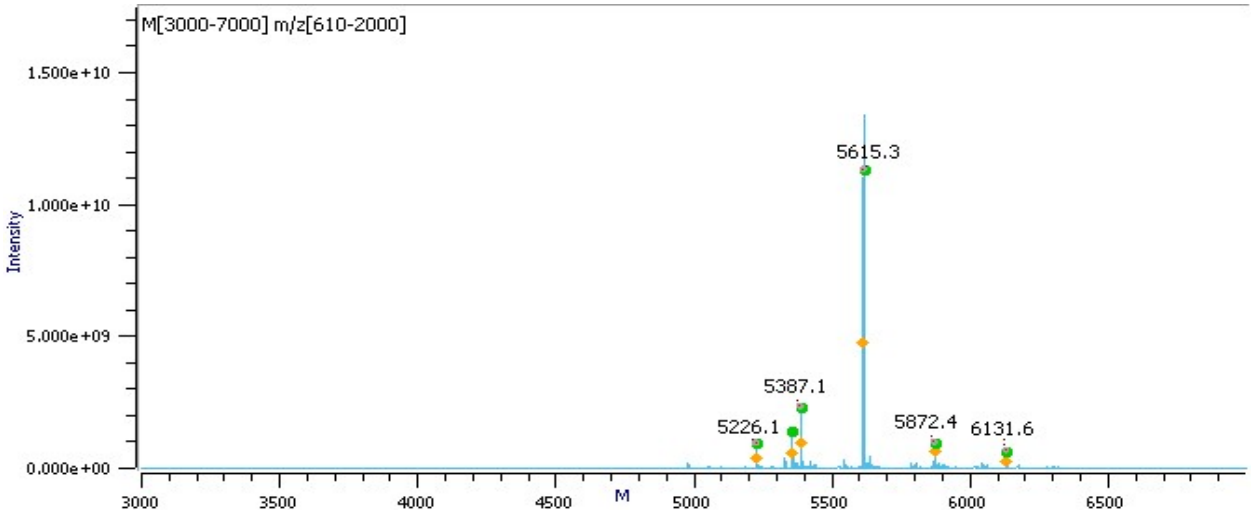
HP-1g (1 equiv) + **2d** (50 equiv)

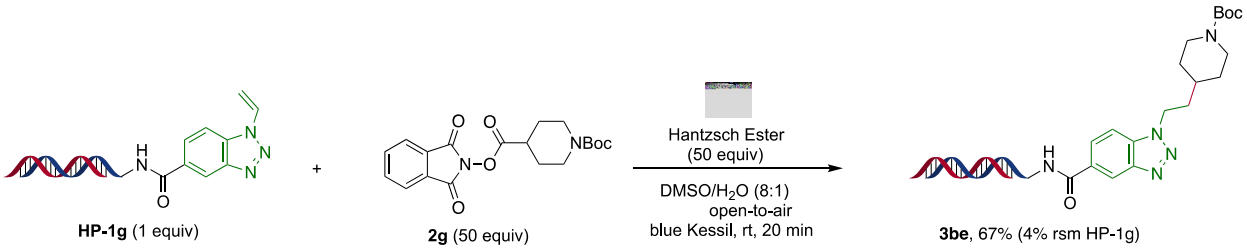
3bd, 70% (8% rsm HP-1g)

Molecular Weight: 5615.0270





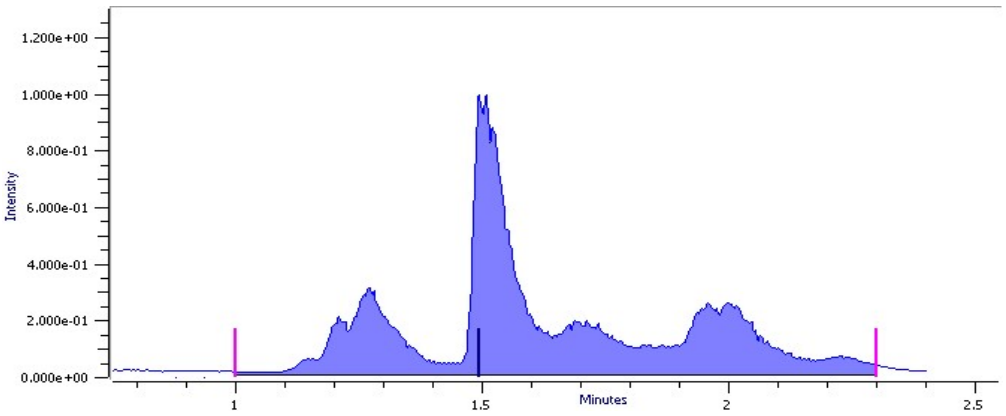


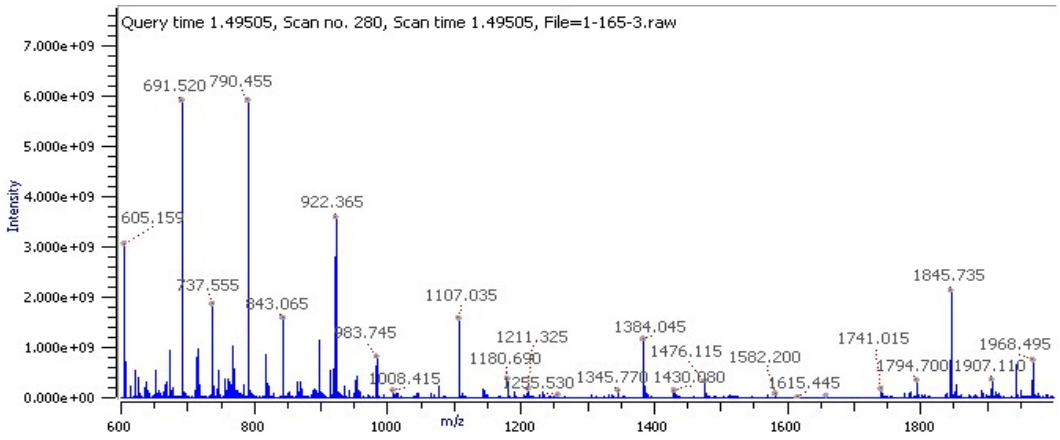


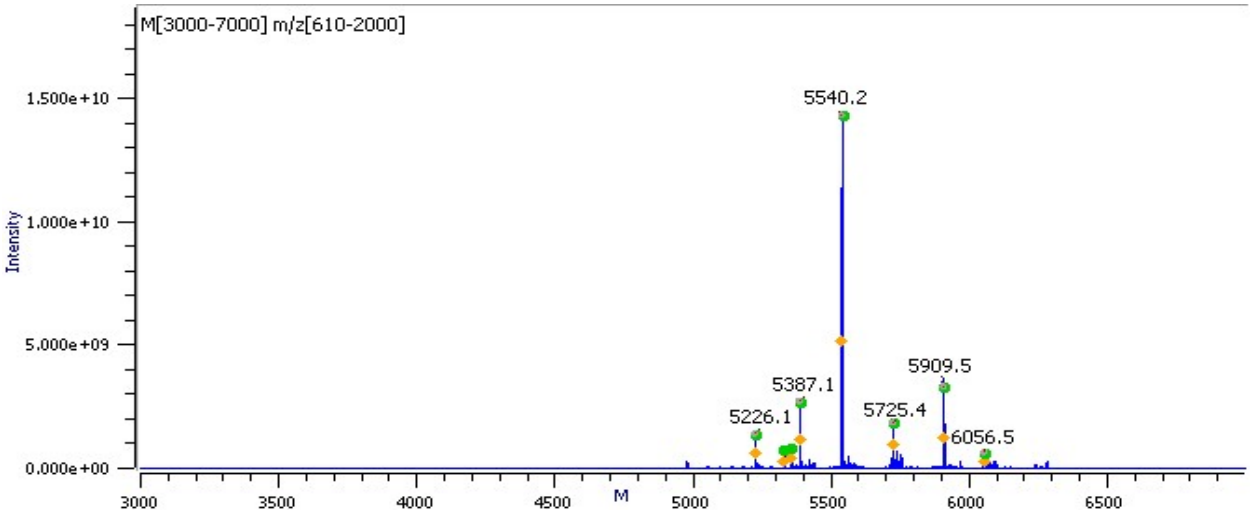
HP-1g (1 equiv) + **2g** (50 equiv)

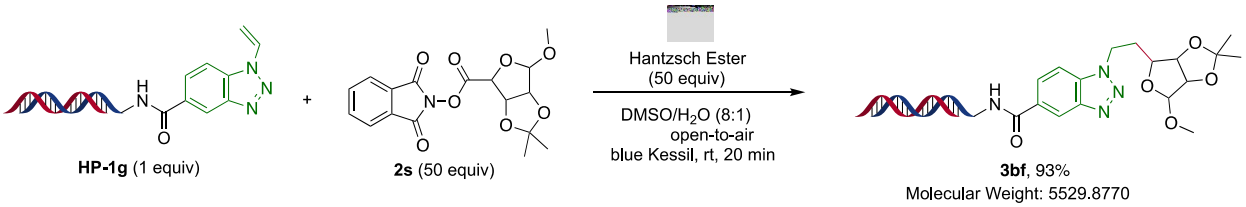
3be, 67% (4% rsm HP-1g)

Molecular Weight: 5540.9480



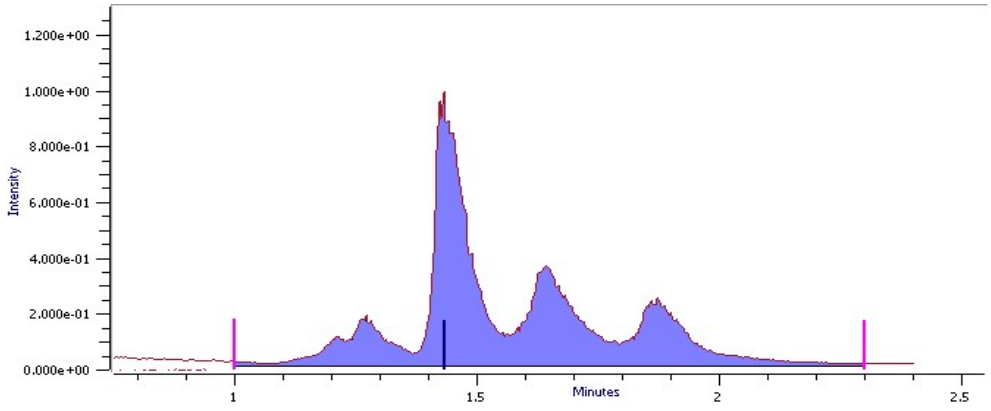


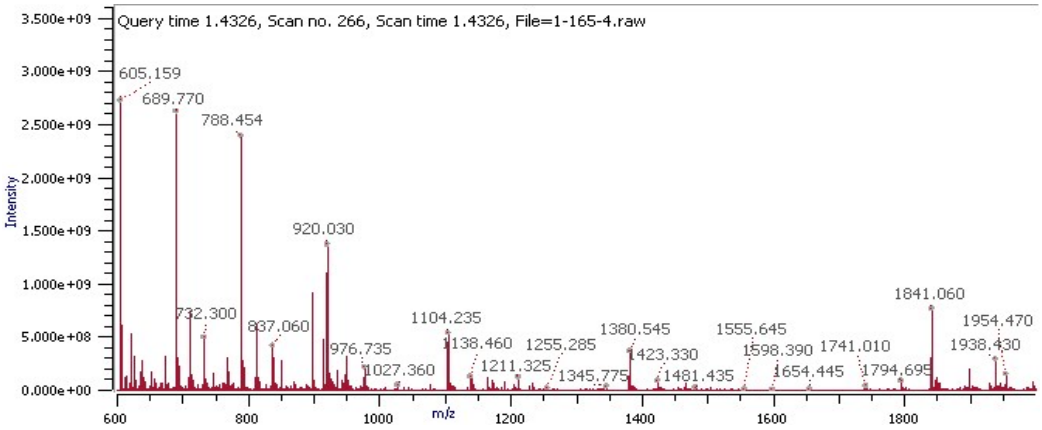


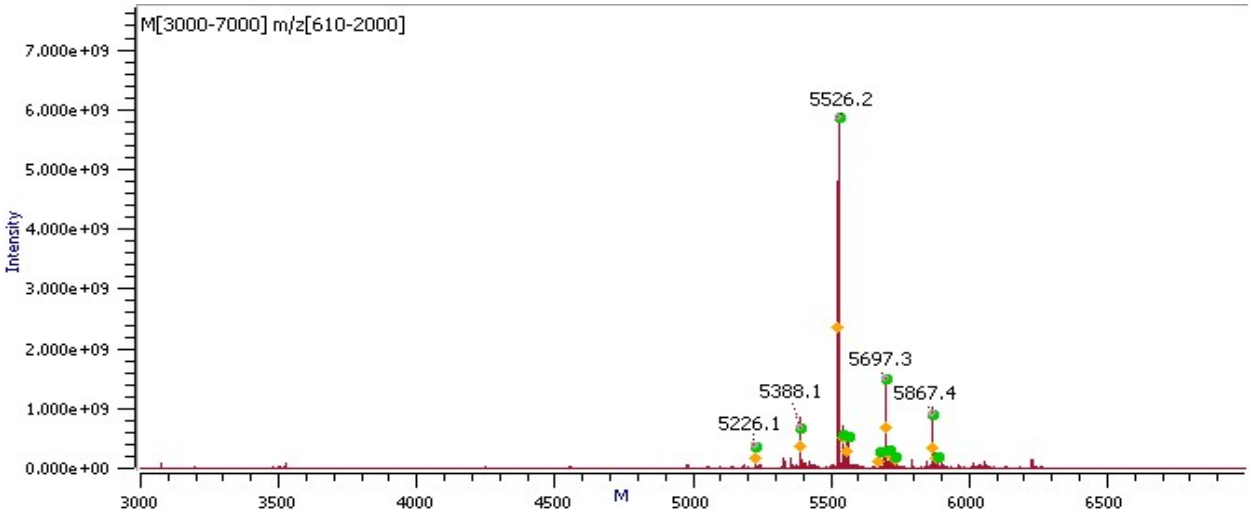


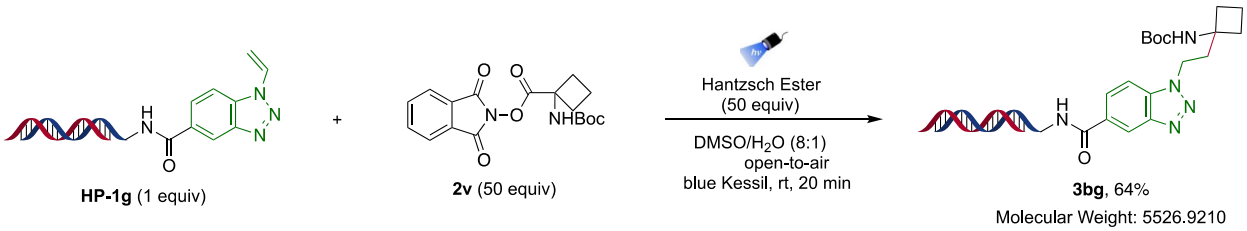
HP-1g (1 equiv) + **2s** (50 equiv)

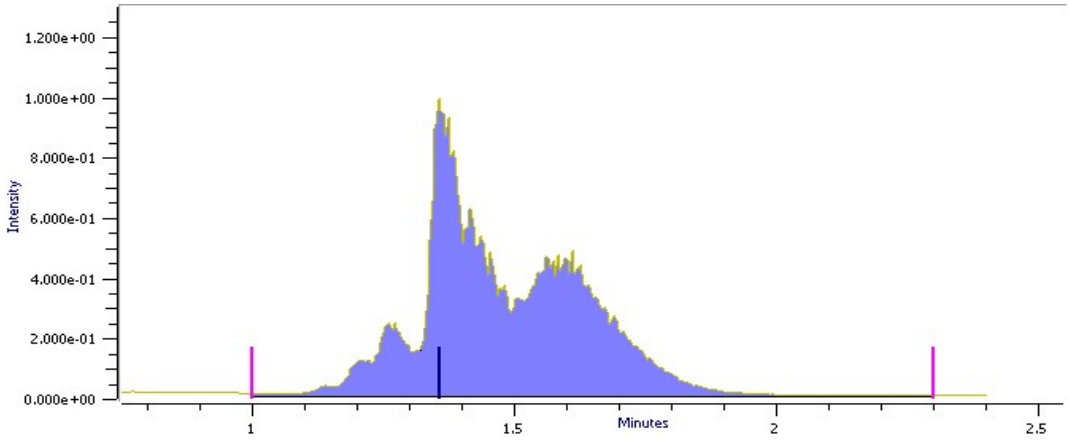
3bf, 93%
Molecular Weight: 5529.8770

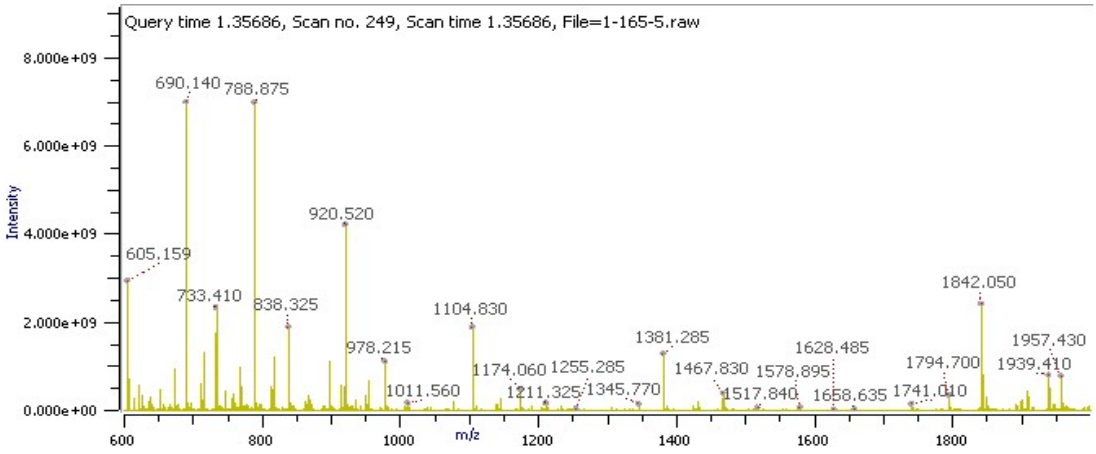


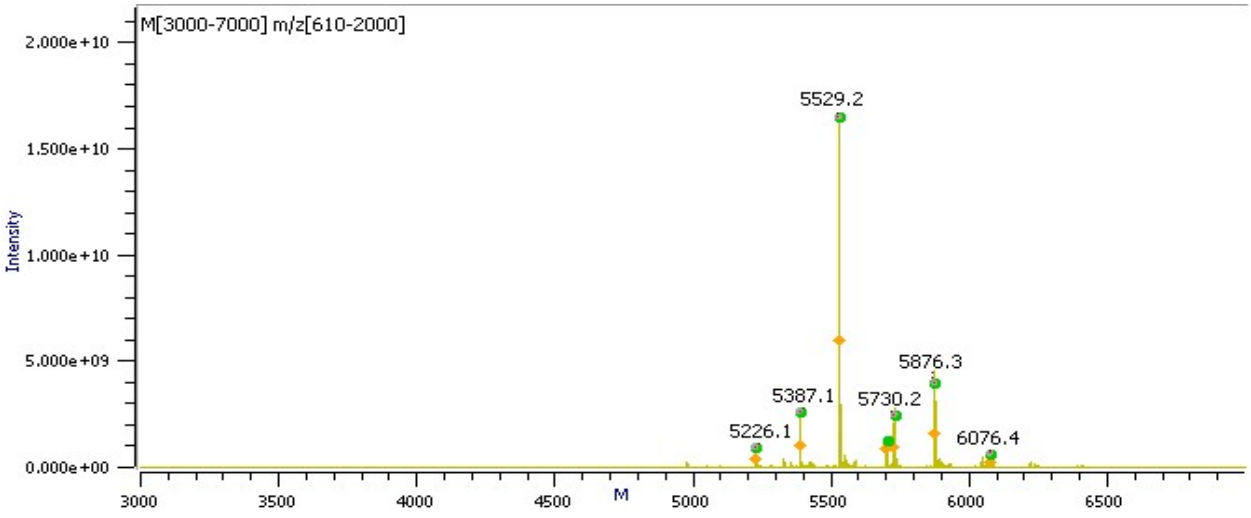


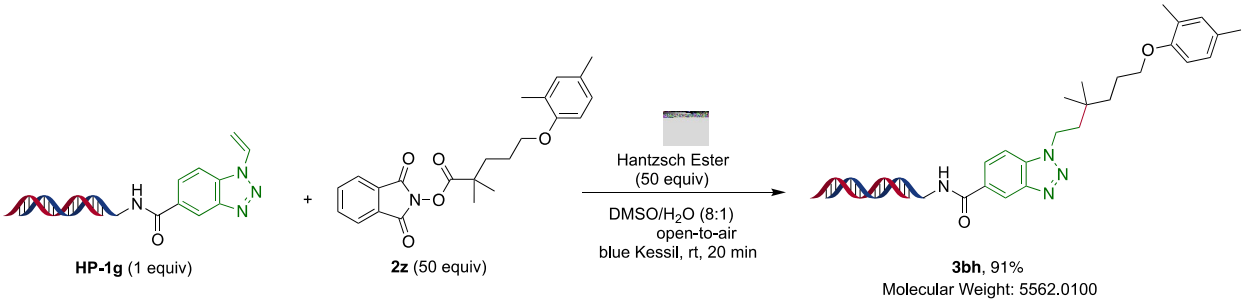



HP-1g (1 equiv)
+
2v (50 equiv)
Hantzsch Ester
(50 equiv)
DMSO/H2O (8:1)
open-to-air
blue Kessil, rt, 20 min
BocHN
3bg, 64%
Molecular Weight: 5526.9210

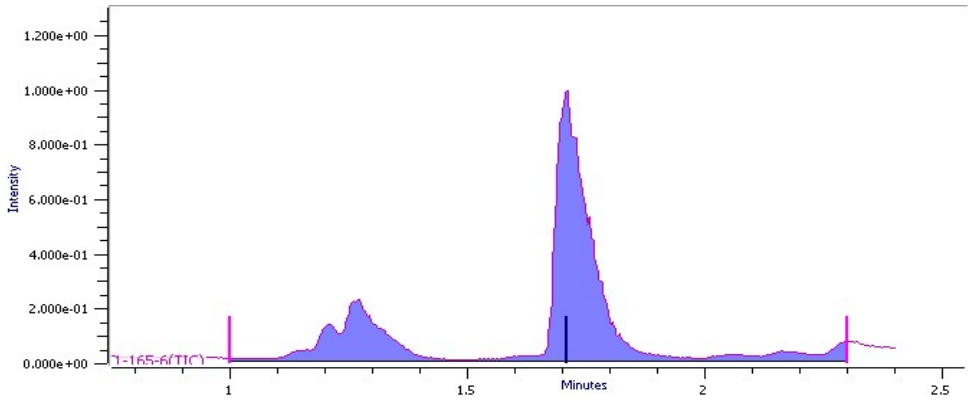

Intensity
Minutes

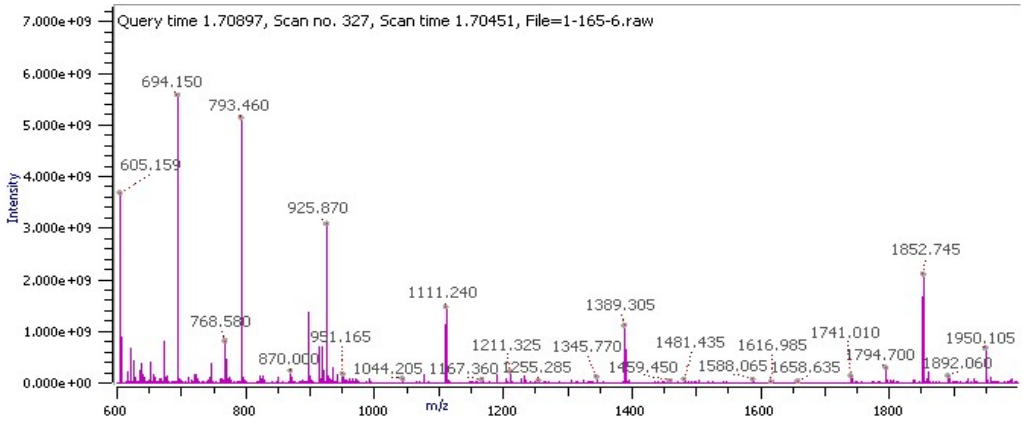

Query time 1.35686, Scan no. 249, Scan time 1.35686, File=1-165-5.raw
Intensity
m/z
690.140
788.875
920.520
605.159
733.410
838.325
1104.830
978.215
1011.560
1174.060
1255.285
1211.325
1345.770
1381.285
1467.830
1517.840
1578.895
1628.485
1658.635
1794.700
1741.010
1842.050
1939.410
1957.430

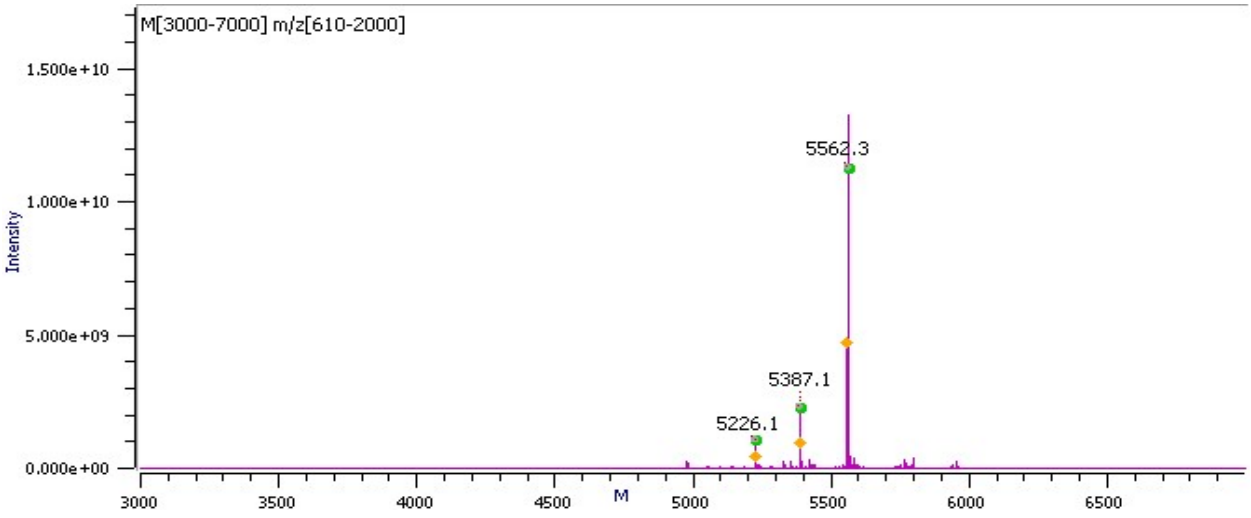

M[3000-7000] m/z[610-2000]
Intensity
M
5529.2
5876.3
5387.1
5730.2
5226.1
6076.4



HP-1g (1 equiv) + **2z** (50 equiv) → **3bh**, 91%
Molecular Weight: 5562.0100







8 References

Phelan, J. P.; Lang, S. B.; Sim, J.; Berrit, S.; Peat, A. J.; Billings, K.; Molander, G. A. *J. Am. Chem. Soc.* **2019**, *141*, 3723-3732.

[2] Juliá, F.; Yan, J.; Paulus, F.; Ritter, T. *J. Am. Chem. Soc.* **2021**, *143*, 12992-12998.

[3] Pieroni, M.; Azzali, E.; Basilico, N.; Parapini, S.; Zolkiewski, M.; Beato, C.; Annunziato, G.; Bruno, A.; Vacondio, F.; Costantino, G. *J. Med. Chem.* **2017**, *60*, 1959-1970.