

Electronic Supplementary Information for

Storable *N*-Phenylcarbamate Palladacycles for Rapid Functionalization of An Alkyne-Encoded Protein

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Supplemental Tables and Figures

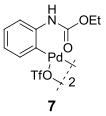
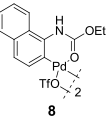
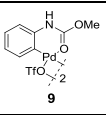
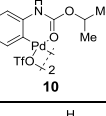
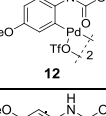
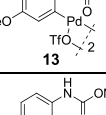
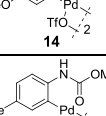
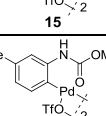
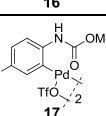
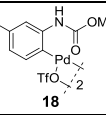
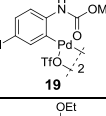
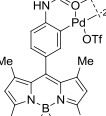
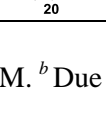
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Table S1. Preparation of the palladacycles ^a

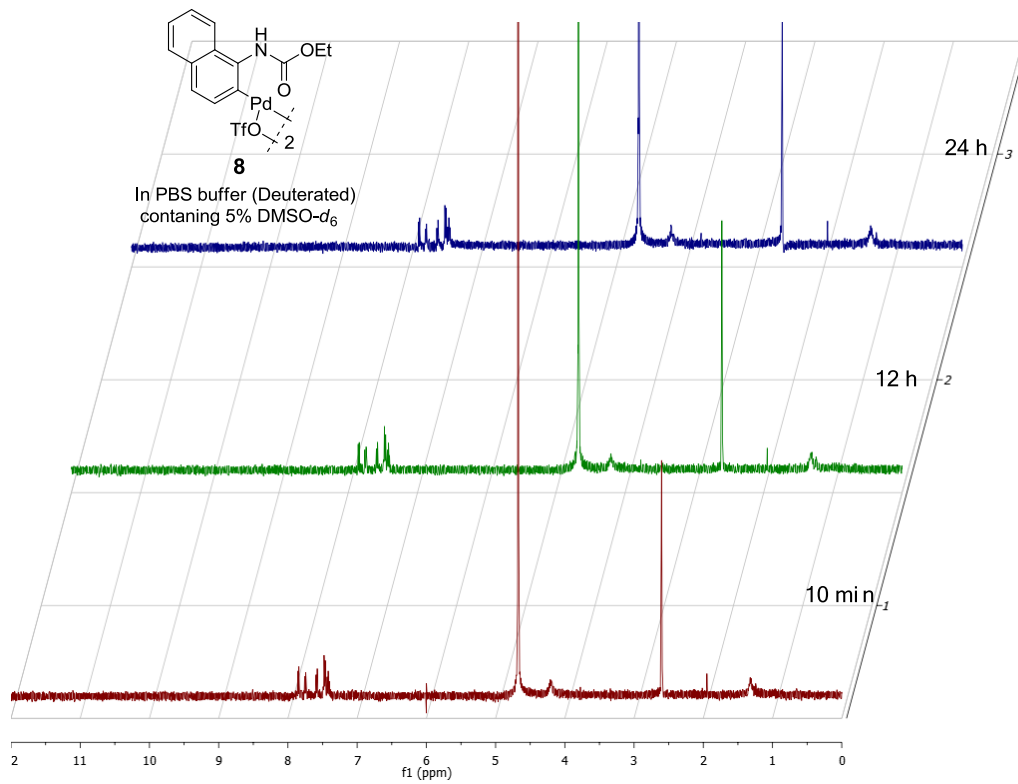
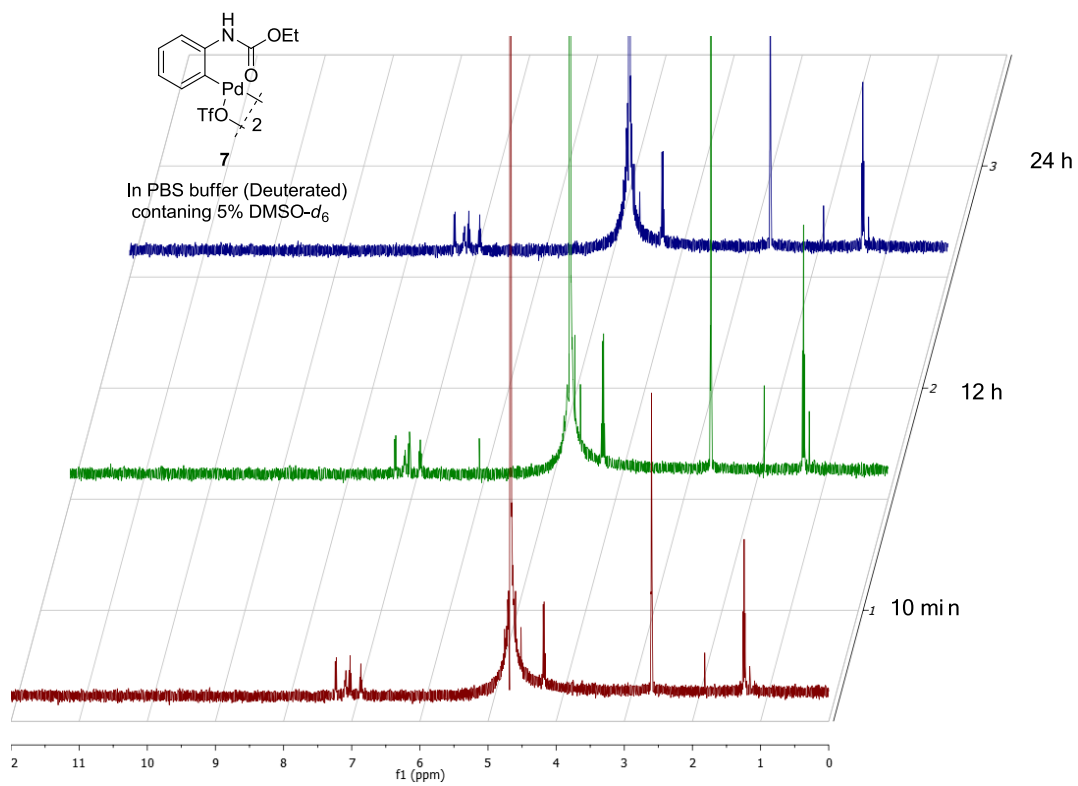
Entry	Substrate	Palladacycle	Yield (%)	Entry	Substrate	Palladacycle	Yield (%)
1			76	10			70
2			87	11			30
3			51	12			65
4			73	13			75
5			75	14			45
6			60	15			72
7			80	16			70
8			80	17			60
9			90	18			17

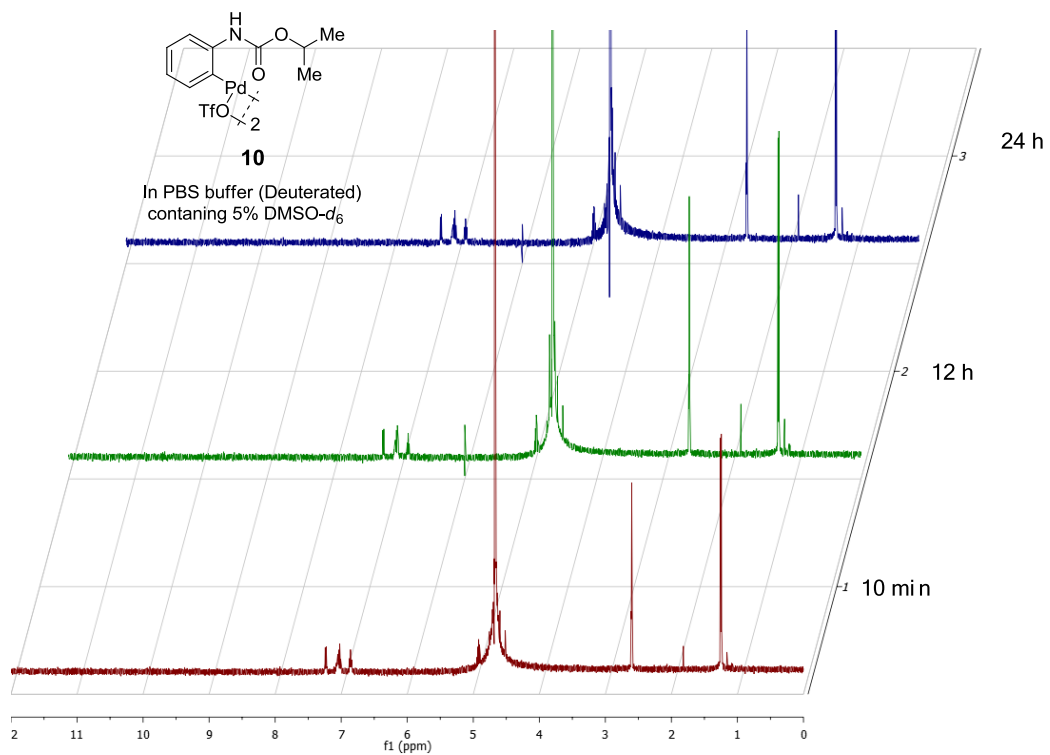
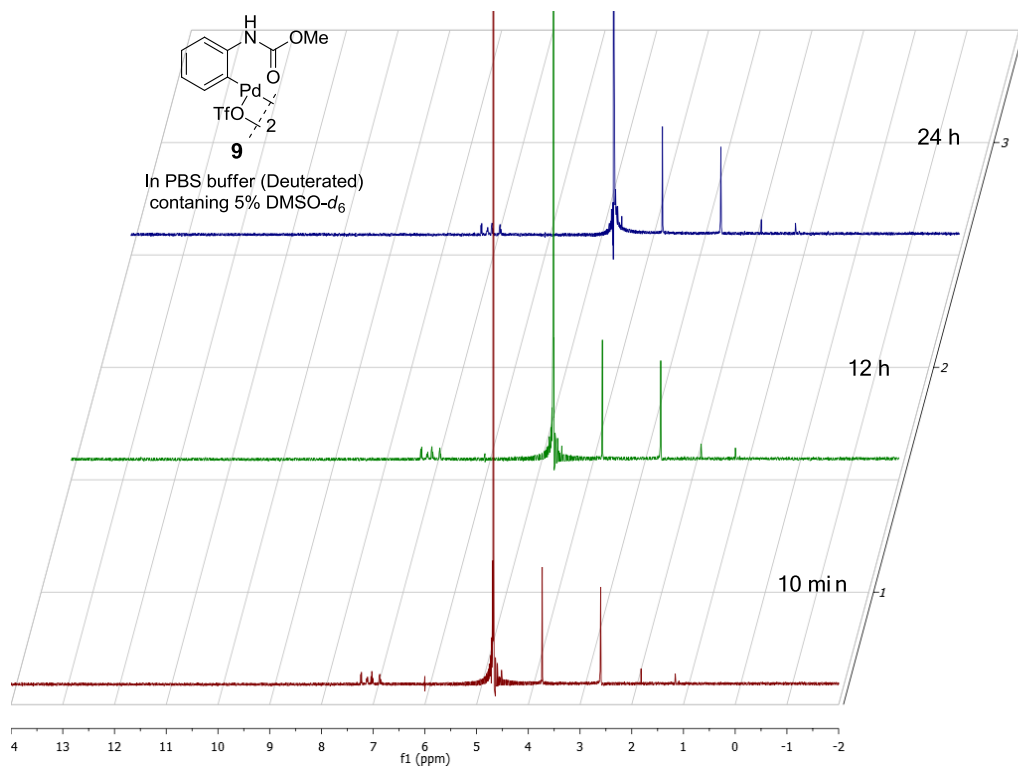
^a Palladacycle was prepared using either Procedure A or B; see Experimental section for details.

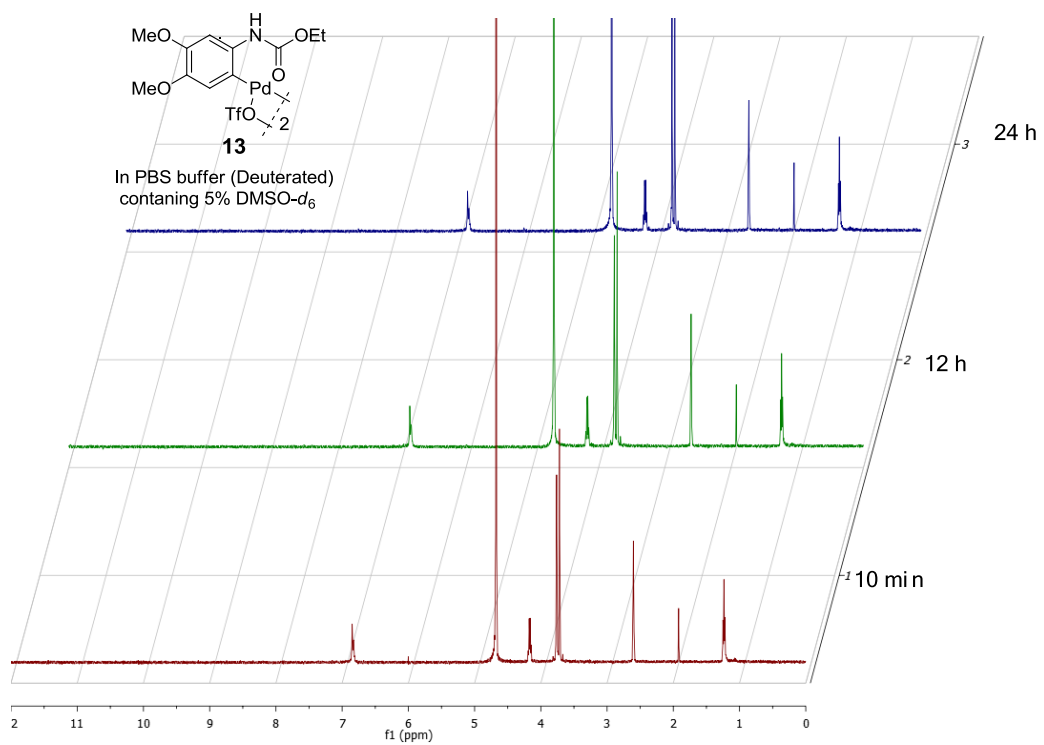
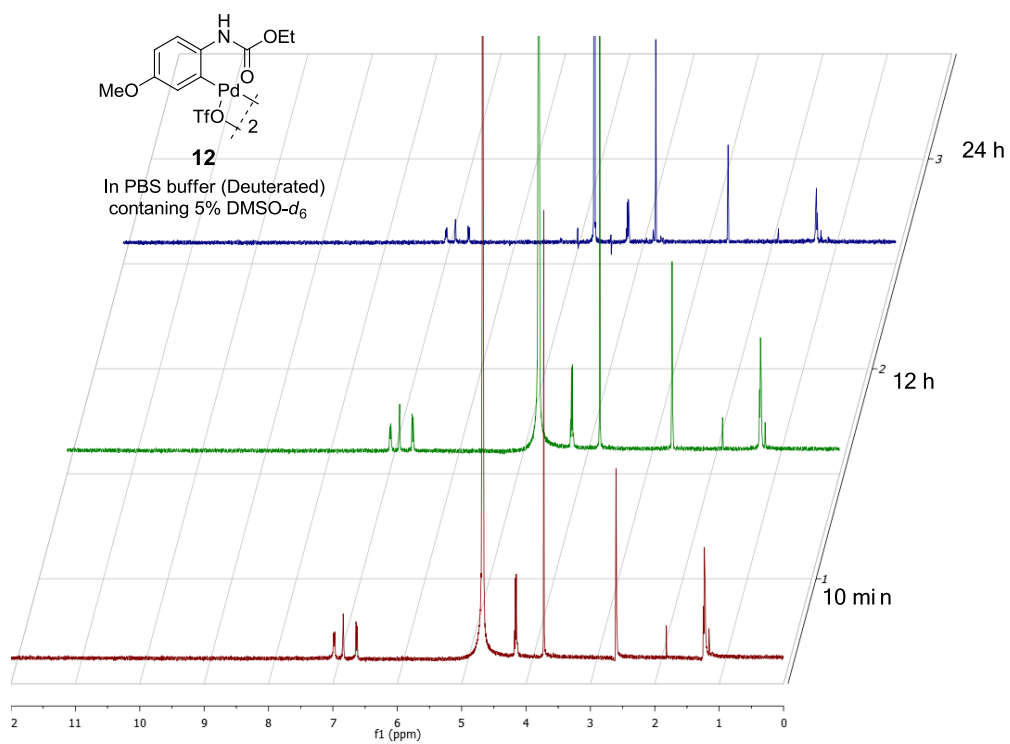
Table S2. Evaluation of palladacycle stability in PBS buffer by ^1H NMR^a

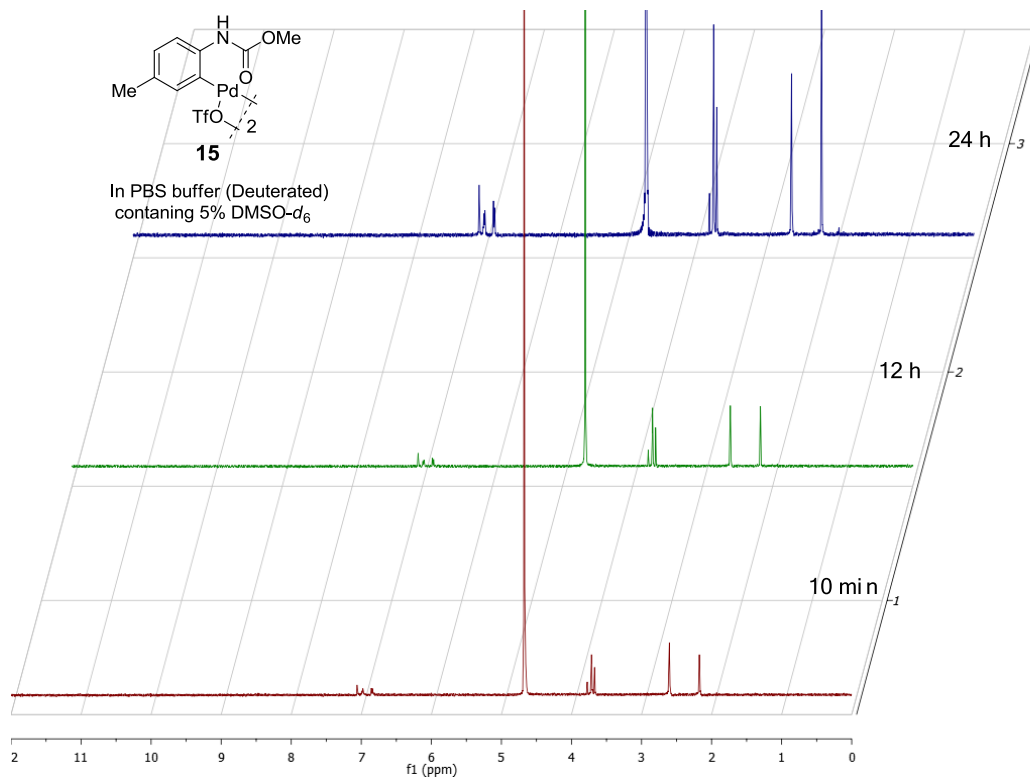
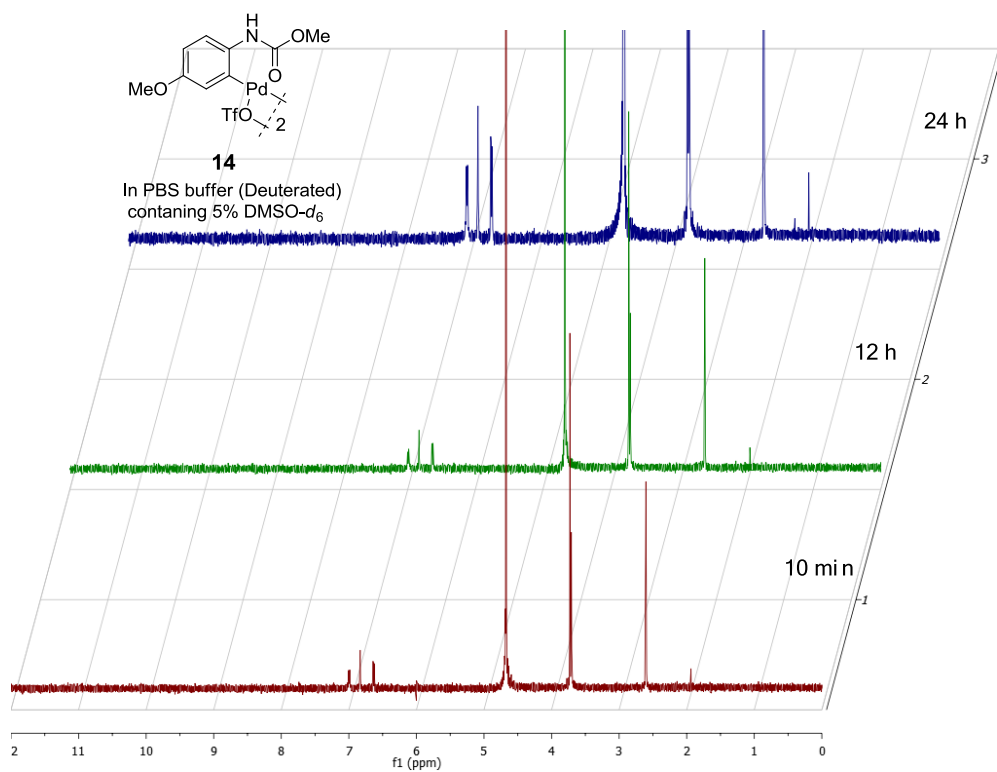
Entry	Palladacycles	Solvent	Stability
1	 7	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
2	 8	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
3	 9	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
4	 10	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
5	 12	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
6	 13	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
7	 14	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
8	 15	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
9	 16	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
10	 17	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
11	 18	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
12	 19	95% PBS + 5% DMSO- <i>d</i> ₆	Stable for 24 h
13	 20	40% PBS + 60% DMSO- <i>d</i> ₆ ^b	Stable for 24 h

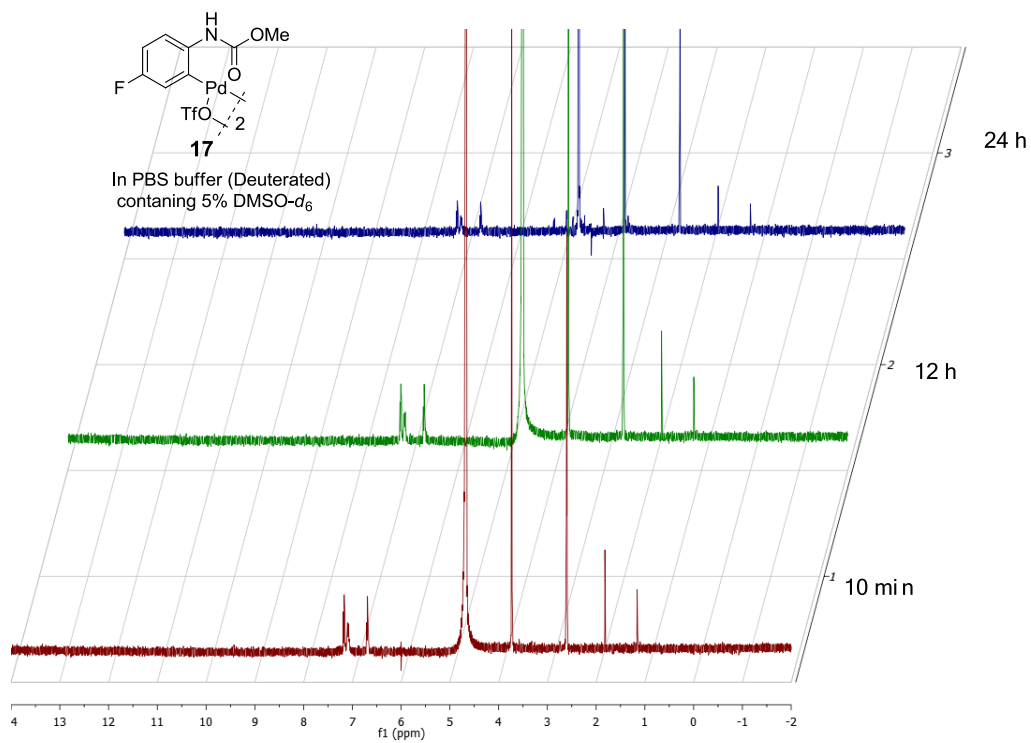
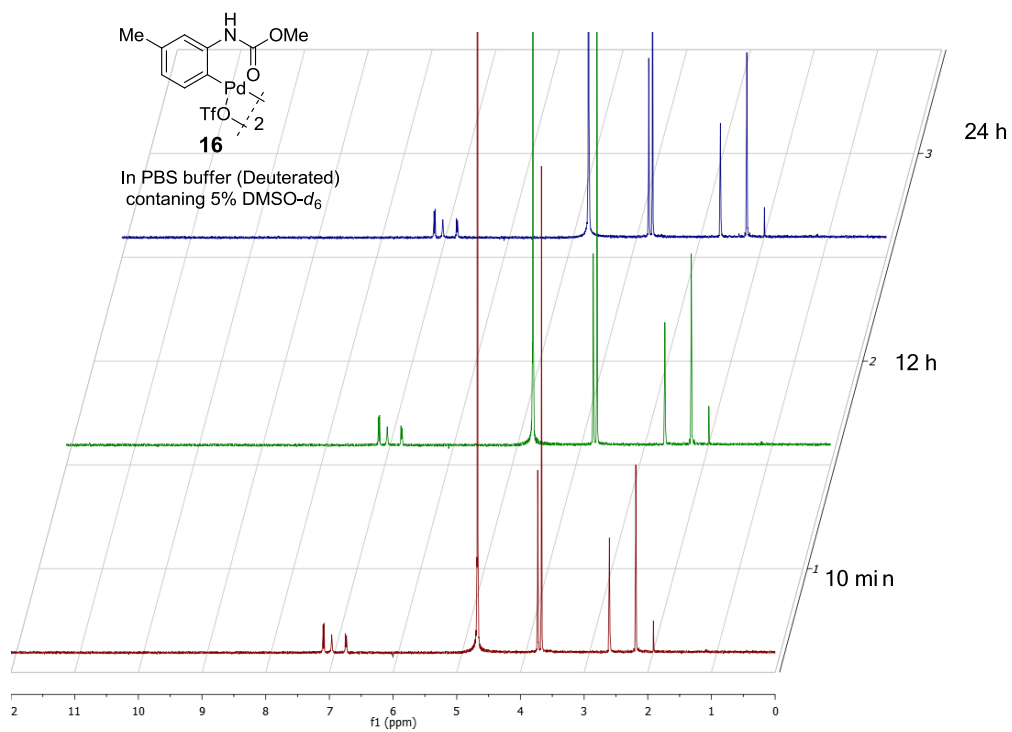
^a Sample concentration was 1 mM. ^b Due to limited solubility, DMSO-*d*₆ content was increased to 60%.

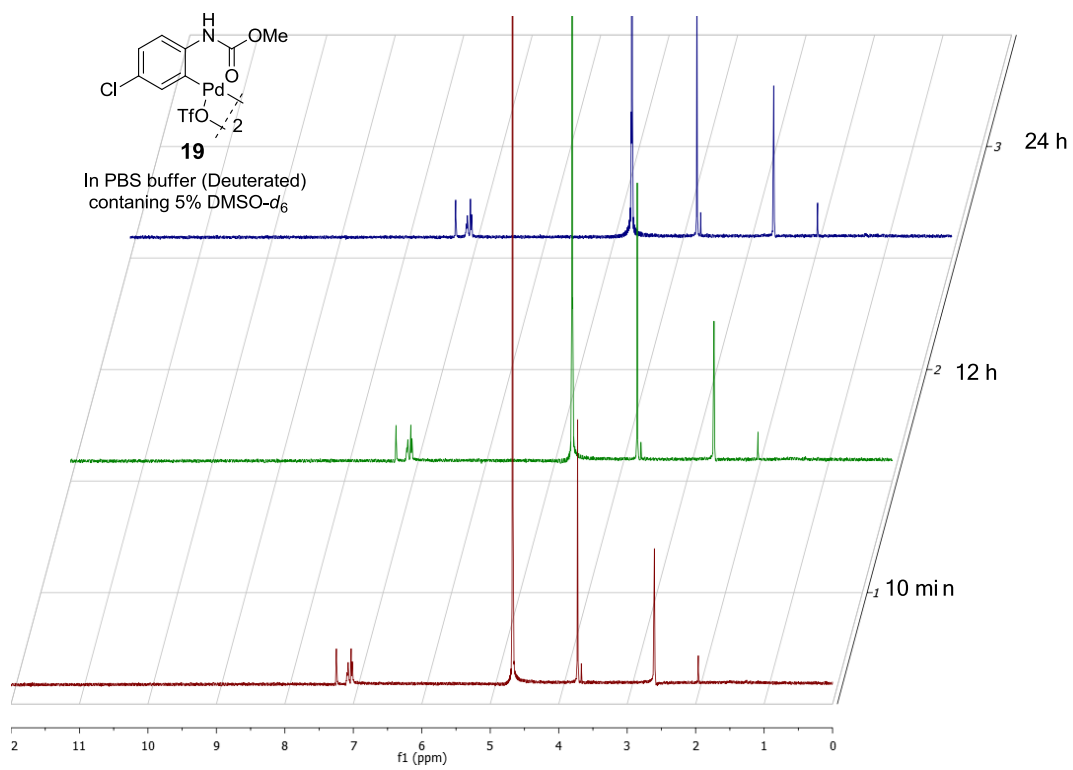
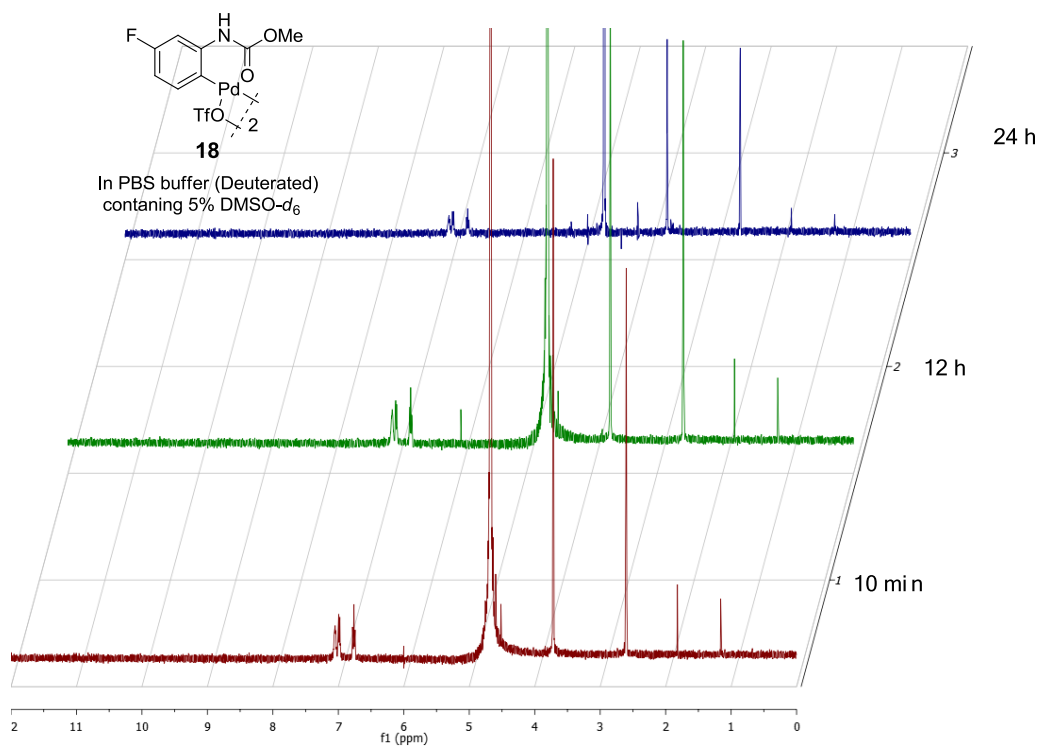












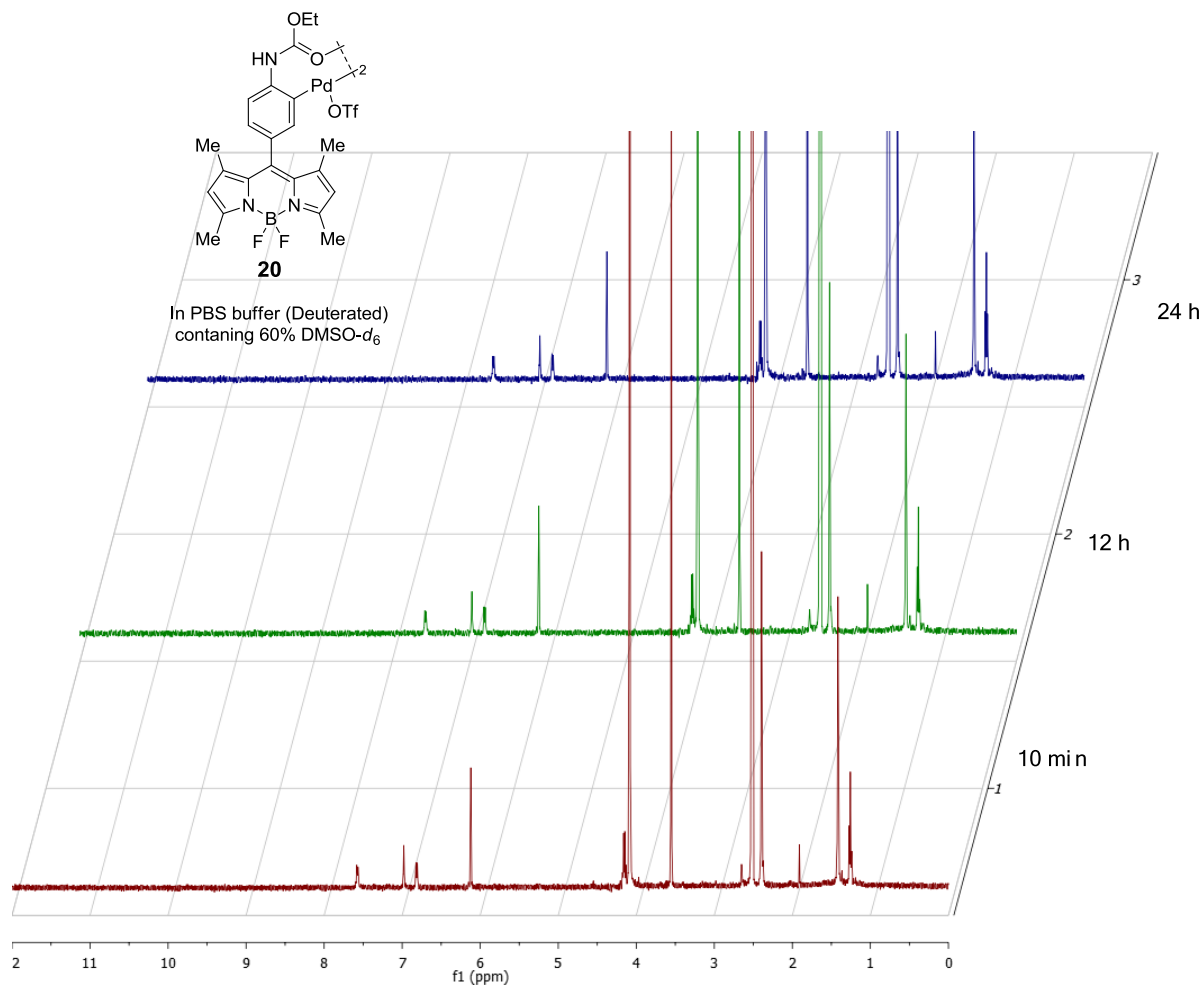


Figure S1. Time course of ¹H NMR spectra of the palladacycles in deuterated PBS at 10 min, 12 hours, and 24 hours.

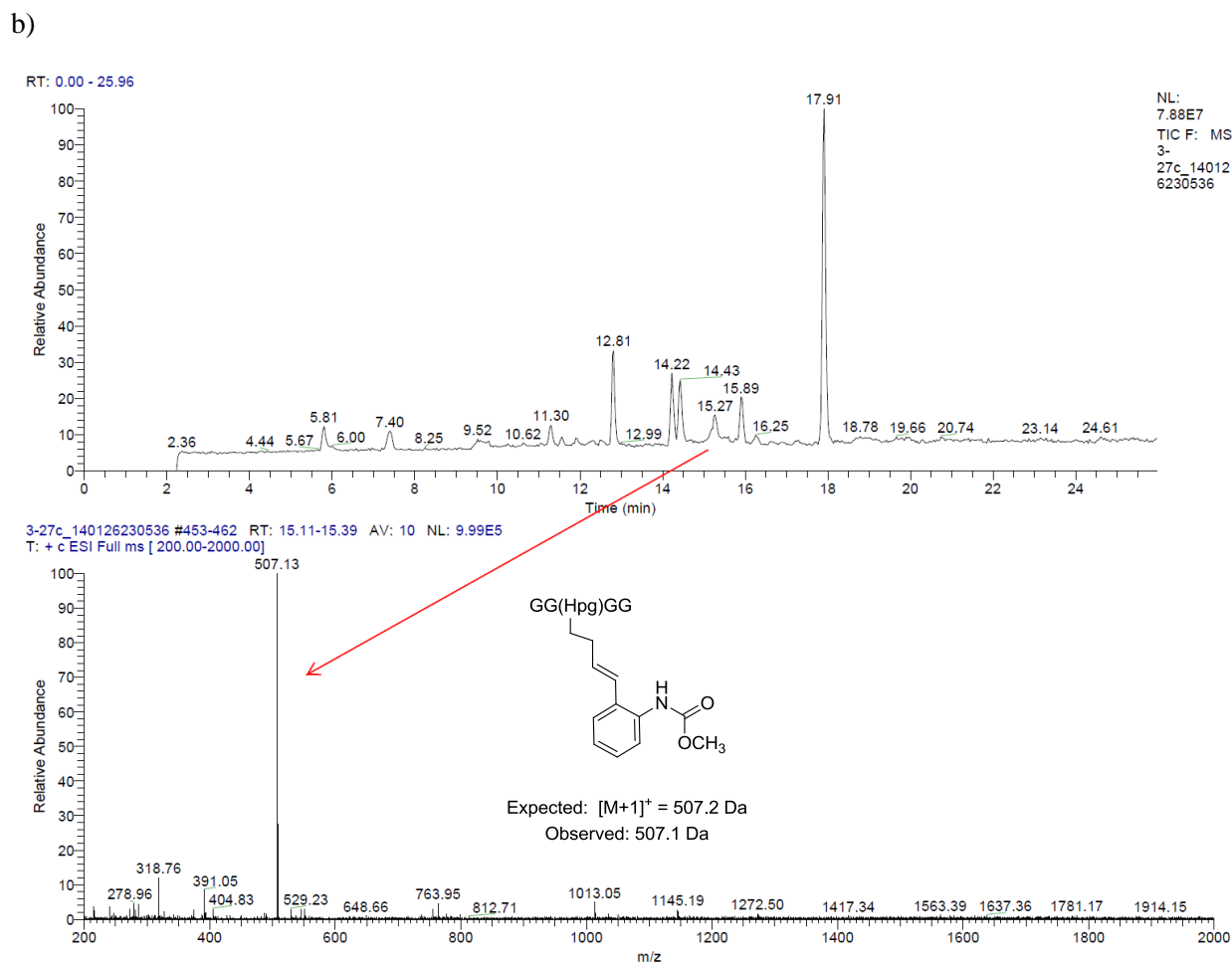
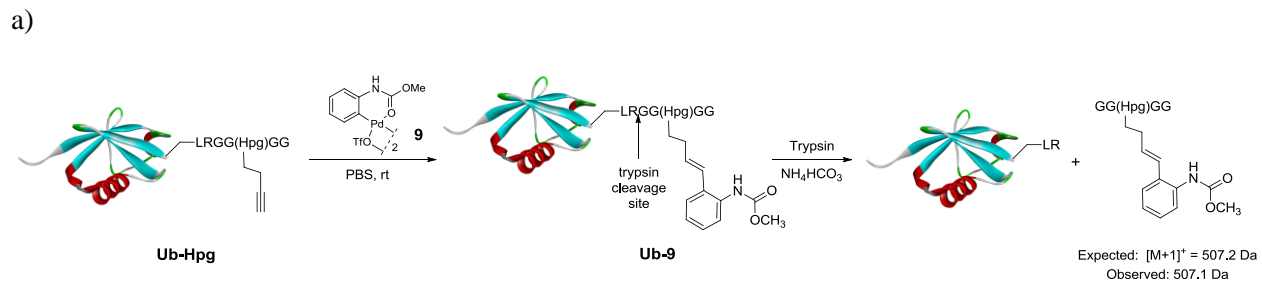
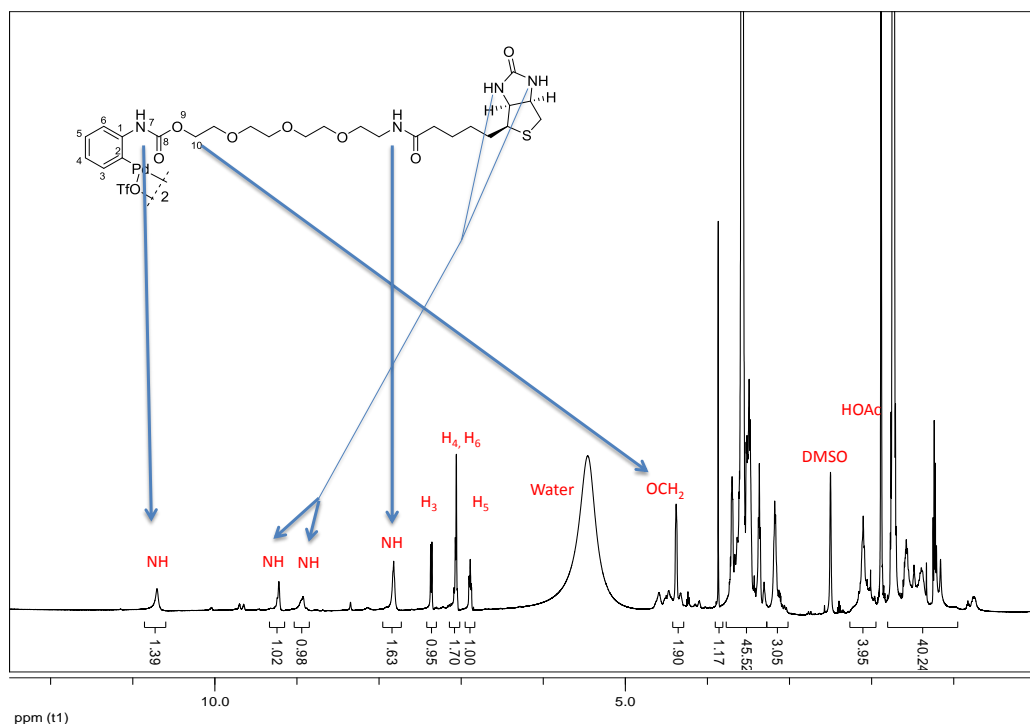


Figure S2. Structural analysis of the cross-coupling product derived from palladacycle **9** via trypsinization followed by LC-MS. (a) Scheme showing the bioconjugation reaction with Ub-Hpg and the subsequent trypsin digestion and LC-MS analysis of the digested fragments. (b) The LC-MS chromatogram and mass spectrum showing the positive identification of the C-terminal fragment containing the styrenyl moiety.

a)



b)

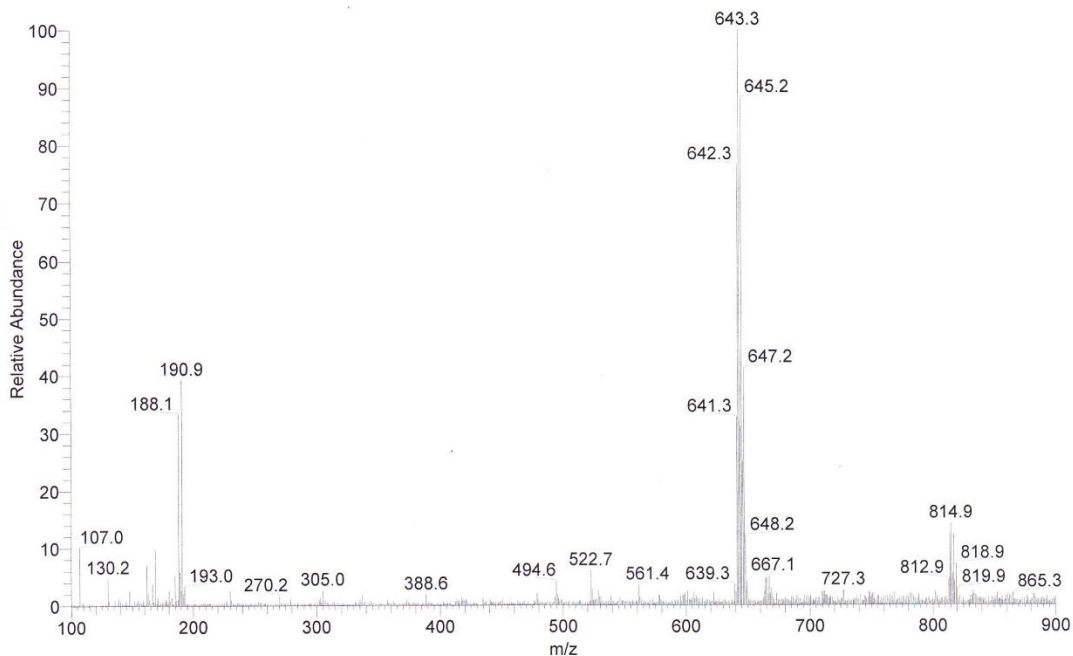
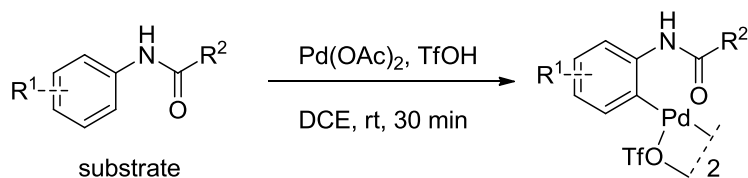


Figure S3. (a) ^1H NMR of the biotin-containing palladacycle dissolved in $\text{DMSO-}d_6$. (b) Electrospray mass spectrum of the biotin-containing palladacycle showing the desired mass: calcd for $[\text{M}/2 - \text{OTf}]^+$ 643.14, found 643.3.

General Information

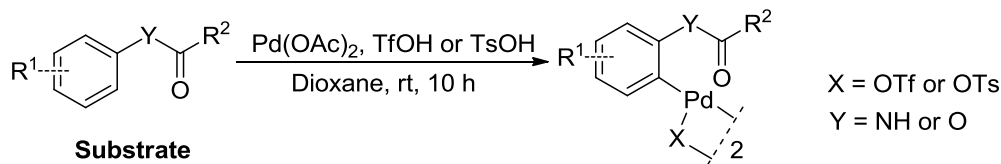
Solvents and chemicals were purchased from commercial sources and used directly without further purification. Flash chromatography was performed with SiliCycle P60 silica gel (40-63 μm , 60 \AA). ^1H NMR spectra were recorded with Inova-300, -400 or -500 MHz spectrometers and chemical shifts were reported in ppm using either TMS or deuterated solvents as internal standards (CDCl_3 , 7.26; $\text{DMSO}-d_6$, 2.50). Multiplicity was reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad singlet. ^{13}C NMR spectra were recorded at 75 MHz, and chemical shifts were reported in ppm using the deuterated solvents as internal standards (CDCl_3 , 77.0; $\text{DMSO}-d_6$, 39.5). Electrospray LC-MS analysis was performed using a Finnigan LCQ Advantage IonTrap mass spectrometry coupled with a Surveyor HPLC system. Protein liquid chromatography was performed using a Phenomenex Jupiter C4 column (5 μm , 300 \AA , 2.00 \times 50 mm) with a flow rate of 200 $\mu\text{L}/\text{min}$ and a linear gradient of 10-90% $\text{ACN}/\text{H}_2\text{O}$ containing 0.1% HCOOH . High resolution mass spectrometry was performed on a Bruker solariX XR Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS).

General procedure for the preparation of palladacycles 5-9, 11-19 (Procedure A)



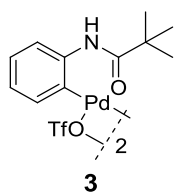
To a 5 mL vial was sequentially added $\text{Pd}(\text{OAc})_2$ (22.4 mg, 0.1 mmol), substrate (0.2 mmol), and DCE (0.5 mL). The vial was stirred at room temperature for 5 min and then triflic acid (18 μL , 0.2 mmol) was added. The reaction mixture was stirred at room temperature under open air for 30 min. The reaction mixture was filtered and washed with DCE and dioxane to give the desired palladacycle product.

General procedure for the preparation of palladacycles 3, 4, 20 and 21 (Procedure B)

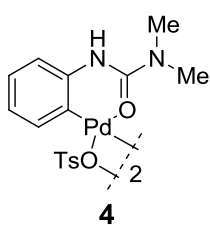


A solution of $\text{Pd}(\text{OAc})_2$ (11.2 mg, 0.05 mmol) in dioxane (400 μL) was added triflic acid (5.3 μL , 0.06 mmol) or tosylic acid (0.06 mmol), and substrate (0.05 mmol). The mixture was stirred for 10 h at room temperature. Afterwards, the mixture was either filtered or concentrated to afford the desired palladacycle.

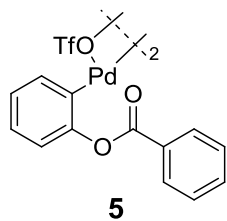
Palladacycles **1**, **2**^[1] and **6**^[2] were prepared as reported previously.



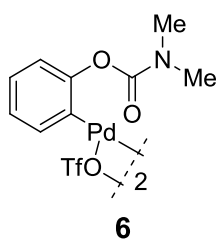
Di- μ -triflyloxy-bis(2-pivalamido-phenyl-2C,O)dipalladium(II) (3) (Table 1, entry 3): The title compound was obtained as a yellow solid in 76% yield according to general procedure B: ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.9 (s, 1H), 7.57 (d, $J = 7.5$ Hz, 1H), 7.27 (d, $J = 7.5$ Hz, 1H), 7.19 (t, $J = 7.2$ Hz, 1H), 7.02 (t, $J = 7.2$ Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 176.9, 134.7, 132.2, 126.8, 125.4, 121.3, 121.1 (q, $^1J_{\text{C-F}} = 320.3$ Hz), 118.8, 27.7; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{17}\text{N}_2\text{OPd}$ [$\text{M}/2 - (\text{OTf}) + (\text{CH}_3\text{CN})$] $^+$ 323.0376, found 323.0375.



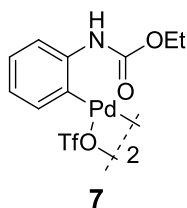
Di- μ -tosyloxy-bis(3,3-dimethylureido-phenyl-2C,O)dipalladium(II) (4) (Table 1, entry 4): The title compound was obtained as a yellow solid in 87% yield according to general procedure B: ^1H NMR (DMSO- d_6 , 300 MHz) δ 9.51 (s, 1H), 7.47 (d, $J = 7.5$ Hz, 2H), 7.38 (d, $J = 8.0$ Hz, 1H), 7.13-7.00 (m, 4H), 7.38 (t, $J = 8.0$ Hz, 1H), 3.09 (s, 6H), 2.26 (s, 3H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.4, 146.1, 138.1, 136.3, 134.4, 128.5, 126.5, 125.9, 123.9, 123.3, 118.4, 37.9, 21.2; MS (ESI) calcd for $\text{C}_{11}\text{H}_{14}\text{N}_3\text{OPd}$ [$\text{M}/2 - (\text{OTs}) + (\text{CH}_3\text{CN})$] $^+$ 310.0172, found 310.0167.



Di- μ -tosyloxy-bis(2-benzoyloxy-phenyl-2C,O)dipalladium(II)(5) (Table 1, entry 5): The title compound was obtained as a yellow solid in 51% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 300 MHz) δ 8.31 (d, $J = 7.5$ Hz, 2H), 7.78 (t, $J = 7.5$ Hz, 1H), 7.65 (t, $J = 7.5$ Hz, 2H), 7.43 (d, $J = 7.5$ Hz, 1H), 7.20 (t, $J = 7.5$ Hz, 1H), 7.12-7.00 (m, 2H); ^{13}C NMR (DMSO- d_6 , 300 MHz) δ 165.7, 153.5, 136.5, 134.5, 132.1, 130.5, 129.9, 129.5, 127.0, 126.2, 122.9; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{12}\text{NO}_2\text{Pd}$ [$\text{M}/2 - (\text{OTf}) + (\text{CH}_3\text{CN})$] $^+$ 343.9903, found 343.9897.

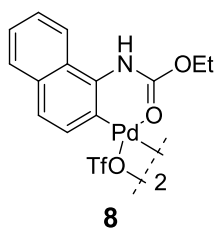


Di- μ -triflyloxy-bis(2-(dimethylcarbamoyl)oxy-phenyl-2C,O) dipalladium(II) (6)² (Table 1, entry 6): The title compound was obtained as a yellow solid in 73% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.36 (d, $J = 7.5$ Hz, 1H), 7.14 (t, $J = 7.5$ Hz, 1H), 7.03 (t, $J = 7.5$ Hz, 1H), 6.89 (d, $J = 7.5$ Hz, 1H), 3.25 (s, 3H), 3.02 (s, 3H).

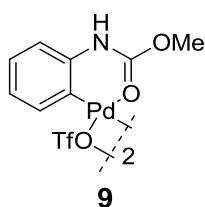


Di- μ -triflyloxy-bis(2-ethoxycarbonylamino-phenyl-2C,O)dipalladium(II) (7) (Table 1, entry 7): The title compound was obtained as a yellow solid in 75% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.8 (s, 1H), 7.38 (d, $J = 8.0$ Hz, 1H), 7.10-6.97 (m, 2H), 6.91 (t, $J = 8.0$ Hz, 1H), 4.32 (q, $J = 7.2$ Hz, 2H), 1.32 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.5, 138.2, 134.9, 126.7, 124.9, 124.2, 121.1 (q, $^1J_{\text{C-F}} = 320.6$ Hz), 119.0,

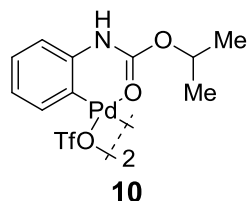
63.6, 14.9; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{11}\text{H}_{13}\text{N}_2\text{O}_2\text{Pd}$ [M/2 - (OTf) + (CH₃CN)]⁺ 311.0012, found 311.0008.



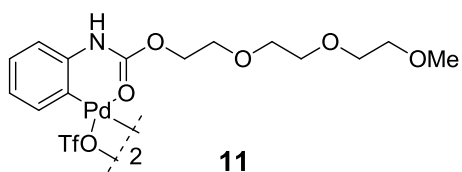
Di- μ -triflyloxy-bis(1-(ethoxycarbonylamino)naphthalen-2-yl-2C,O) dipalladium(II) (8) (Table 1, entry 8): The title compound was obtained as a black solid in 60% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 500 MHz) δ 9.75 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 7.5 Hz, 1H), 7.63-7.60 (m, 2H), 7.50-7.43 (m, 2H), 4.26 (brs, 2H), 1.35 (brs, 3H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.9, 136.2, 135.2, 132.8, 132.1, 130.1, 128.3, 126.3, 125.9, 125.6, 122.6, 61.5, 15.2; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{15}\text{N}_2\text{O}_2\text{Pd}$ [M/2 - (OTf) + (CH₃CN)]⁺ 361.0168, found 361.0174.



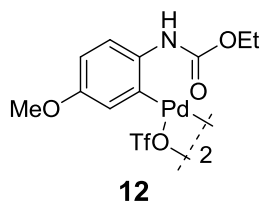
Di- μ -triflyloxy-bis(2-methoxycarbonylamino-phenyl-2C,O) dipalladium(II) (9) (Table 2, entry 1): The title compound was obtained as a yellow solid in 80% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.7 (s, 1H), 7.37 (d, J = 7.5 Hz, 1H), 7.12-7.03 (m, 2H), 6.98-6.93 (m, 1H), 3.85 (s, 3H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.7, 138.7, 134.9, 126.7, 125.8, 124.2, 121.1 (q, $^1J_{\text{C-F}}$ = 320.6 Hz), 119.6, 54.2; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{10}\text{H}_{11}\text{N}_2\text{O}_2\text{Pd}$ [M/2 - (OTf) + (CH₃CN)]⁺ 296.9855, found 296.9850.



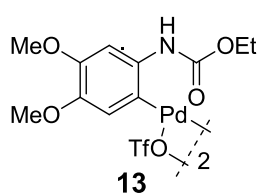
Di- μ -triflyloxy-bis(2-isopropoxycarbonylamino-phenyl-2C,O) dipalladium(II) (10) (Table 2, entry 2): The title compound was obtained as a black solid in 80% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.7 (s, 1H), 7.37 (d, J = 7.8 Hz, 1H), 7.10-6.97 (m, 2H), 6.90 (t, J = 7.8 Hz, 1H), 5.0 (m, 1H), 1.33 (d, J = 6.3 Hz, 6H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.2, 137.9, 134.9, 126.7, 124.3, 124.2, 119.0, 72.0, 22.4; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{15}\text{N}_2\text{O}_2\text{Pd}$ [M/2 - (OTf) + (CH₃CN)]⁺ 325.0168, found 325.0162.



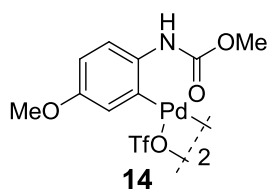
Palladacycle 11 (Table 2, entry 3): Following general procedure B, the mixture was concentrated and dried under vacuum to give the title compound as a black solid in 90% yield: ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.7 (s, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.11-7.03 (m, 2H), 6.98-6.95 (m, 1H), 4.41 (m, 2H), 3.72 (m, 2H), 3.64-3.48 (m, 6H), 3.42 (m, 2H), 3.24 (m, 3H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.4, 138.5, 134.9, 126.7, 125.7, 124.3, 119.6, 71.7, 70.2(2C), 70.1, 68.8, 66.8, 66.6, 21.5; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{20}\text{NO}_5\text{Pd}$ [M/2 - (OTf)]⁺ 388.0376, found 388.0378.



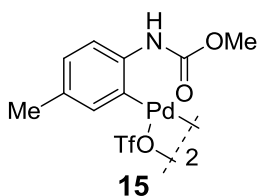
Di- μ -triflyloxy-bis(5-methoxy-2-ethoxycarbonylamino-phenyl-2C,O) dipalladium(II) (12) (Table 2, entry 4): The title compound was obtained as a yellow solid in 70% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.5 (s, 1H), 6.96 (s, 1H), 6.91 (d, $J = 8.4$ Hz, 1H), 6.68 (d, $J = 8.4$ Hz, 1H), 4.29 (q, $J = 6.9$ Hz, 2H), 3.68 (s, 3H), 1.30 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.5, 155.1, 131.5, 126.3, 121.1 (q, $^1J_{\text{C-F}} = 320.6$ Hz), 119.9, 119.4, 112.1, 63.6, 55.7, 14.9; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{15}\text{N}_2\text{O}_3\text{Pd}$ [M/2 - (OTf) + (CH₃CN)]⁺ 341.0118, found 341.0125.



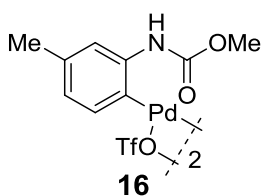
Di- μ -triflyloxy-bis(4,5-dimethoxy-2-methoxycarbonylamino-phenyl-2C,O) dipalladium(II) (13) (Table 2, entry 5): The title compound was obtained as a black solid in 30% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.4 (s, 1H), 6.99 (s, 1H), 6.71 (s, 1H), 4.31 (q, $J = 6.9$ Hz, 2H), 3.69 (s, 3H), 3.67 (s, 3H), 1.31 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.4, 147.8, 144.7, 130.8, 121.1 (q, $J = 320.6$ Hz), 117.1, 112.8, 107.2, 63.6, 56.3, 55.9, 14.9; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{17}\text{N}_2\text{O}_4\text{Pd}$ [M/2 - (OTf) + (CH₃CN)]⁺ 371.0223, found 371.0226.



Di- μ -triflyloxy-bis(4-methoxy-2-methoxycarbonylamino-phenyl-2C,O) dipalladium(II) (14) (Table 2, entry 6): The title compound was obtained as a yellow solid in 65% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.4 (s, 1H), 6.98-6.90 (m, 2H), 6.68 (d, $J = 8.1$ Hz, 1H), 3.84 (s, 3H), 3.68 (s, 3H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.8, 155.1, 132.0, 126.7, 121.1 (q, $J = 320.6$ Hz), 120.3, 119.5, 112.0, 55.7, 54.1; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{11}\text{H}_{13}\text{N}_2\text{O}_3\text{Pd}$ [M/2 - (OTf) + (CH₃CN)]⁺ 326.9961, found 326.9957.

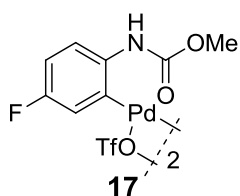


Di- μ -triflyloxy-bis(5-methyl-2-methoxycarbonylamino-phenyl-2C,O) dipalladium(II) (15) (Table 2, entry 7): The title compound was obtained as a yellow solid in 75% yield according to general procedure A: ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.4 (s, 1H), 7.17 (d, 1H), 6.96 (d, $J = 7.8$ Hz, 1H), 6.88 (d, $J = 7.8$ Hz, 1H), 3.84 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ 156.7, 136.2, 135.1, 133.2, 127.3, 126.1, 119.4, 54.1, 21.0; ^{19}F NMR (DMSO- d_6 , 282.4 MHz) δ -77.8; HRMS (ESI) calcd for $\text{C}_{11}\text{H}_{13}\text{N}_2\text{O}_2\text{Pd}$ [M/2 - (OTf) + (CH₃CN)]⁺ 311.0012, found 311.0016.

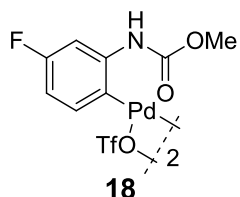


Di- μ -triflyloxy-bis(4-methyl-2-methoxycarbonylamino-phenyl-2C,O) dipalladium(II) (16) (Table 2, entry 8): The title compound was obtained as a black solid in 45% yield according to general procedure A: ^1H NMR

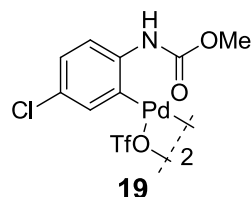
(DMSO-*d*₆, 300 MHz) δ 10.5 (s, 1H), 7.23 (d, J = 7.5 Hz, 1H), 6.89 (s, 1H), 6.73 (d, J = 7.5 Hz, 1H), 3.84 (s, 3H), 2.20 (s, 3H); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 156.8, 138.2, 135.9, 134.6, 125.2, 121.2, 120.0, 54.4, 20.7; ¹⁹F NMR (DMSO-*d*₆, 282.4 MHz) δ -77.8; HRMS (ESI) calcd for C₁₁H₁₃N₂O₂Pd [M/2 - (OTf) + (CH₃CN)]⁺ 311.0012, found 311.0016.



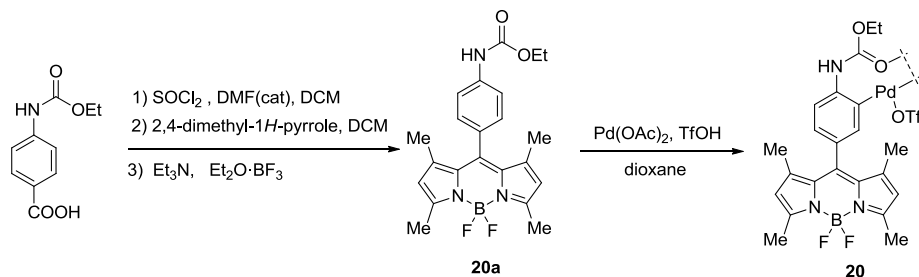
Di- μ -triflyloxy-bis(5-fluoro-2-methoxycarbonylamino-phenyl-2C,*O*) dipalladium(II) (17) (Table 2, entry 9): The title compound was obtained as a yellow solid in 72% yield according to general procedure A: ¹H NMR (DMSO-*d*₆, 300 MHz) δ 10.4 (s, 1H), 7.33 (t, J = 8.4 Hz, 1H), 7.12 (dd, J = 8.7, 2.4 Hz, 1H), 6.77 (td, J = 8.7, 2.4 Hz, 1H), 3.81 (s, 3H); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 161.7 (d, ¹ J_{C-F} = 238.1 Hz), 156.1, 140.8 (d, ³ J_{C-F} = 9.2 Hz), 135.8 (d, ³ J_{C-F} = 9.2 Hz), 121.1 (q, ¹ J_{C-F} = 320.6 Hz), 120.1, 110.2 (d, ² J_{C-F} = 20.6 Hz), 106.5 (d, ² J_{C-F} = 25.2 Hz), 53.7; ¹⁹F NMR (DMSO-*d*₆, 282.4 MHz) δ -77.8, -118.1; HRMS (ESI) calcd for C₁₀H₁₀FN₂O₂Pd [M/2 - (OTf) + (CH₃CN)]⁺ 314.9761, found 314.9765.



Di- μ -triflyloxy-bis(4-fluoro-2-methoxycarbonylamino-phenyl-2C,*O*) dipalladium(II) (18) (Table 2, entry 10): The title compound was obtained as a yellow solid in 70% yield according to general procedure A: ¹H NMR (DMSO-*d*₆, 500 MHz) δ 10.5 (s, 1H), 7.21 (dd, J = 10.0, 3.0 Hz, 1H), 7.09 (dd, J = 9.0, 5.5 Hz, 1H), 6.96 (td, J = 8.5, 3.0 Hz, 1H), 3.86 (s, 3H); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 157.3 (d, ¹ J_{C-F} = 243.8 Hz), 156.7, 135.6, 128.2, 121.1 (q, ¹ J_{C-F} = 320.6 Hz), 120.8 (d, ² J_{C-F} = 21.8 Hz), 120.3, 113.1 (d, ² J_{C-F} = 21.8 Hz), 54.2; ¹⁹F NMR (DMSO-*d*₆, 282.4 MHz) δ -77.8, -118.3; HRMS (ESI) calcd for C₁₀H₁₀FN₂O₂Pd [M/2 - (OTf) + (CH₃CN)]⁺ 314.9761, found 314.9766.

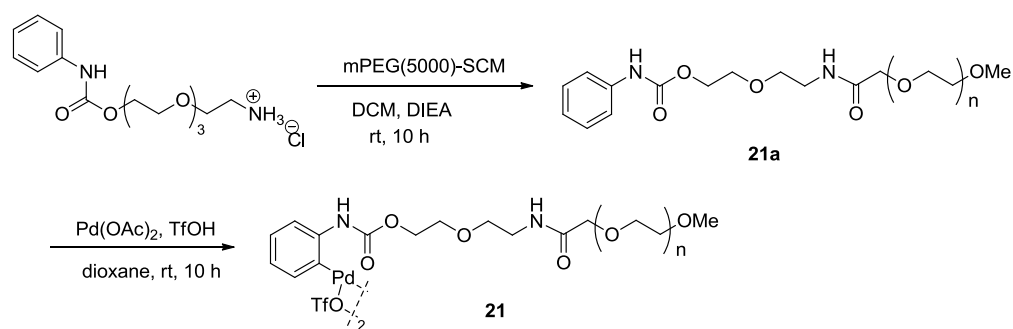


Di- μ -triflyloxy-bis(5-chloro-2-methoxycarbonylamino-phenyl-2C,*O*) dipalladium(II) (19) (Table 2, entry 11): The title compound was obtained as a yellow solid in 60% yield according to general procedure A: ¹H NMR (DMSO-*d*₆, 300 MHz) δ 10.5 (s, 1H), 7.38 (s, 1H), 7.13 (m, 2H), 3.83 (s, 3H); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 156.5, 138.4, 133.8, 128.0, 127.0, 126.3, 120.5, 54.1; ¹⁹F NMR (DMSO-*d*₆, 282.4 MHz) δ -77.8; HRMS (ESI) calcd for C₁₀H₁₀ClN₂O₂Pd [M/2 - (OTs) + (CH₃CN)]⁺ 330.9466, found 330.9469.



To a slurry of 4-(ethoxycarbonyl)amino-benzoic acid in 4 mL DCM, 1 mL thionyl chloride was added dropwise at 0°C under argon, followed by the addition of 6 μL DMF. This mixture was stirred at room temperature for 4 h and a clear solution was obtained. The solvent and excess amount of thionyl chloride was removed under reduced pressure to afford a yellow solid. The yellow solid was re-dissolved in 10 mL DCM, and to the solution was added 2, 4-dimethyl pyrrole under argon. After stirring at room temperature for 4 h, the reaction mixture was cooled to 0°C, and Et₃N and BF₃·OEt₂ was added at 0°C. The mixture was stirred overnight at room temperature under argon. Afterwards, the solvent was removed under reduced pressure and the residue was purified through column chromatography on silica gel (1:2 hexanes/DCM) to afford an orange powder **20a** (210 mg, 26% yield): ¹H NMR (CDCl₃, 300 MHz) δ 7.54 (d, *J* = 7.8 Hz, 2H), 7.21 (d, *J* = 7.8 Hz, 2H), 6.77 (s, 1H), 5.98 (s, 2H), 4.26 (q, *J* = 6.9 Hz, 2H), 2.56 (s, 6H), 1.43 (s, 6H), 1.33 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 155.4, 153.4, 143.1, 141.4, 138.8, 131.6, 129.6, 128.8, 121.2, 118.7, 61.5, 14.6, 14.5; ¹⁹F NMR (DMSO-*d*₆, 282.4 MHz) δ -146.1 (q, *J* = 32.7 Hz); HRMS (ESI) calcd for C₂₂H₂₄BF₂N₃NaO₂ [M+Na]⁺ 434.1827, found 434.1828.

Following the general procedure B, a solution of Pd(OAc)₂ (11.2 mg, 0.05 mmol) in dioxane (400 μL) was added triflic acid (5.3 μL, 0.06 mmol), followed by *N*-phenylcarbamate (0.05 mmol). The mixture was stirred at room temperature for 10 h. Afterward, the mixture was filtered and washed with dioxane (300 μL) to afford palladacycle **20** as a brown powder (11 mg, 17% yield): ¹H NMR (DMSO-*d*₆, 300 MHz) δ 11.0 (s, 1H), 7.24 (m, 2H), 7.08 (d, *J* = 7.8 Hz, 1H), 6.14 (s, 1H), 4.37 (q, *J* = 6.9 Hz, 2H), 2.42 (s, 6H), 1.39 (s, 6H), 1.34 (t, *J* = 6.6 Hz, 3H); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 156.5, 155.1, 143.4, 142.5, 138.9, 133.8, 131.3, 129.1, 126.1, 125.0, 121.6, 118.4, 64.0, 14.9, 14.7, 14.6; ¹⁹F NMR (DMSO-*d*₆, 282.4 MHz) δ -77.8, -127 (brs); HRMS (ESI) calcd for C₂₄H₂₆BF₂N₄O₂Pd [M/2 - (OTf) + (CH₃CN)]⁺ 557.1152, found 557.1185.



A solution of 2-(2-((phenylcarbamoyl)oxy)ethoxy)ethanaminium chloride (4.9 mg, 0.2 mmol), mPEG-SCM (~5 kDa; 30 mg, 0.006 mmol) and DIEA (15 μL) in 2 mL DCM was stirred at room temperature for 10 h. The solvent was removed under the reduced pressure, and the residue was purified through preparative HPLC to give the PEGylated carbamate **21a** as a white solid (10 mg, 30% yield): ¹H NMR (CDCl₃, 500 MHz) δ 8.20 (brs, 1H), 7.45 (d, *J* = 7.2 Hz, 2H), 7.31 (t, *J* =

7.2 Hz, 2H), 7.24 (brs, 1H), 7.07 (t, $J = 7.0$ Hz, 1H), 4.34-4.30 (m, 2H), 4.03 (s, 3H), 3.81 (m, 2H), 3.79-3.56 (m, 428H), 3.52 (m, 4H), 3.40 (s, 3H).

Palladacycle 21: Following the general procedure B, a solution of Pd(OAc)₂ (0.4 mg, 1.8 μ mol) in dioxane (200 μ L) was added triflic acid (0.2 μ L, 2.1 μ mol) and **21a** (10 mg, 1.8 μ mol). After stirring at room temperature for 10 h, the solvent was removed under the reduced pressure. The desired palladacycle was obtained as a sticky black solid after drying under vacuum overnight: ¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.7 (s, 1H), 7.60 (m, 1H), 7.35 (d, $J = 7.0$ Hz, 1H), 7.05 (m, 1H), 6.95 (m, 1H), 4.37 (m, 2H), 3.82 (s, 2H), 3.80-3.10 (m, 265H).

Expression and purification of Hpg-encoded ubiquitin (Ub-Hpg)

Ub-Hpg was expressed and purified as described previously.^[3] Finally, the protein was desalted to 1 \times PBS buffer and concentrated to 0.14 mM .

General procedures for the reaction of palladacycles with Ub-Hpg (for Table 1, Table 2, Figure 1 and Figure 2).

To a 0.6-mL microcentrifuge tube was added 48 μ L PBS buffer solution and Ub-Hpg (0.9 μ L, 0.14 mM). Then the palladacycle stock solution (2 μ L, 0.25 mM in DMSO) was added under vigorous stirring at room temperature. After reacted for the indicated time, the samples were immediately quenched with 10 μ L of 3-mercaptopropanoic acid (4% v/v in water) and injected into LC-MS for analysis.

Final concentration of this reaction before quenching:

Ub-Hpg: 2.5 μ M

Palladacycle: 10 μ M

DMSO: 4%

Buffer: 1 \times PBS, pH =7.4

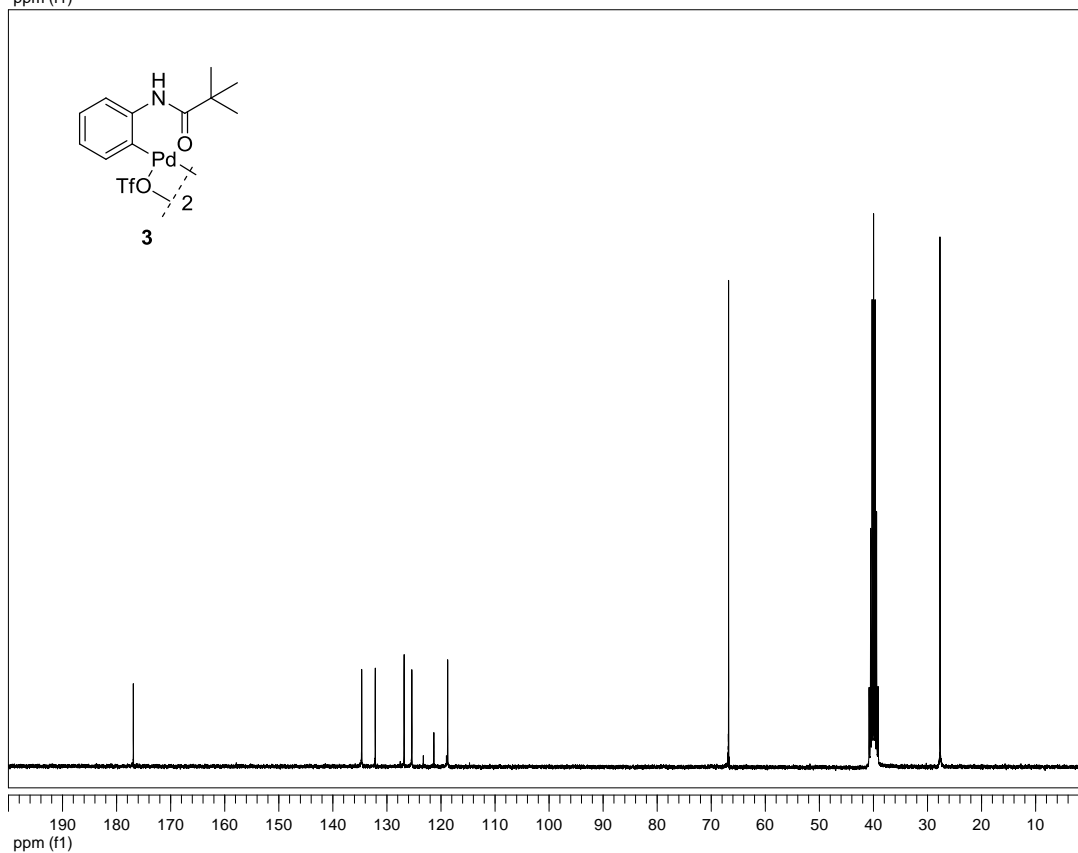
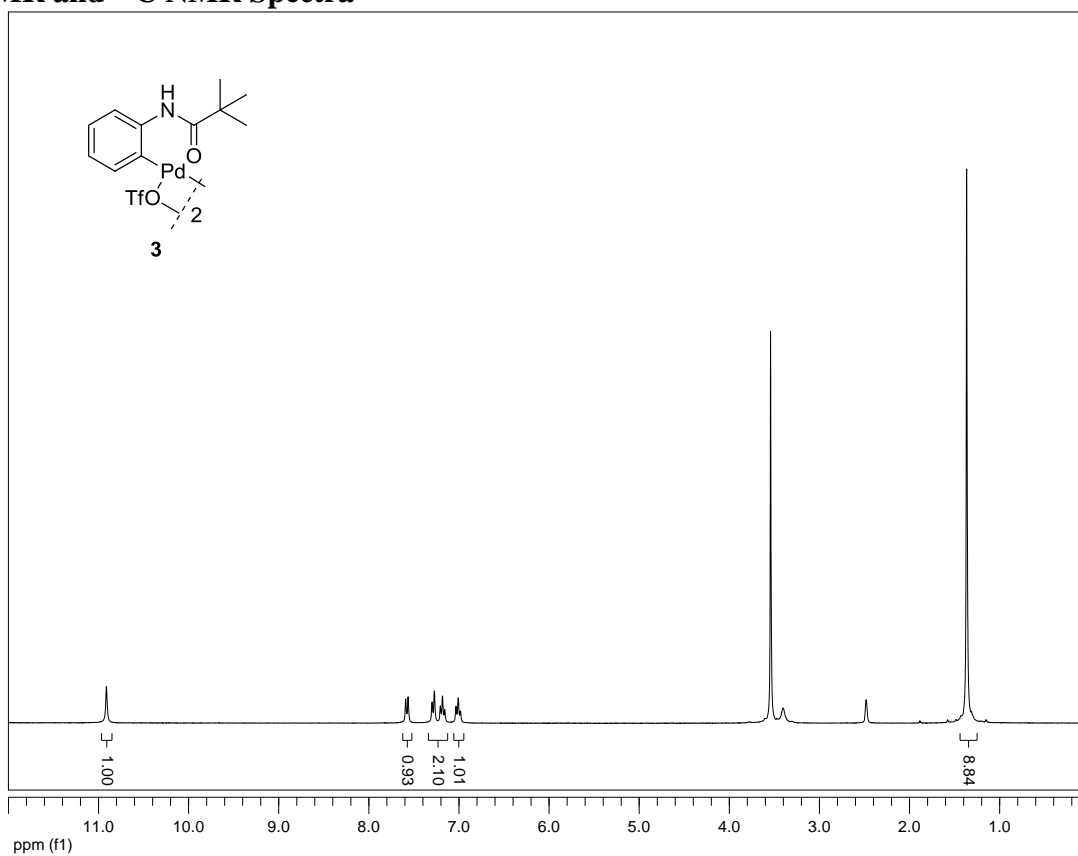
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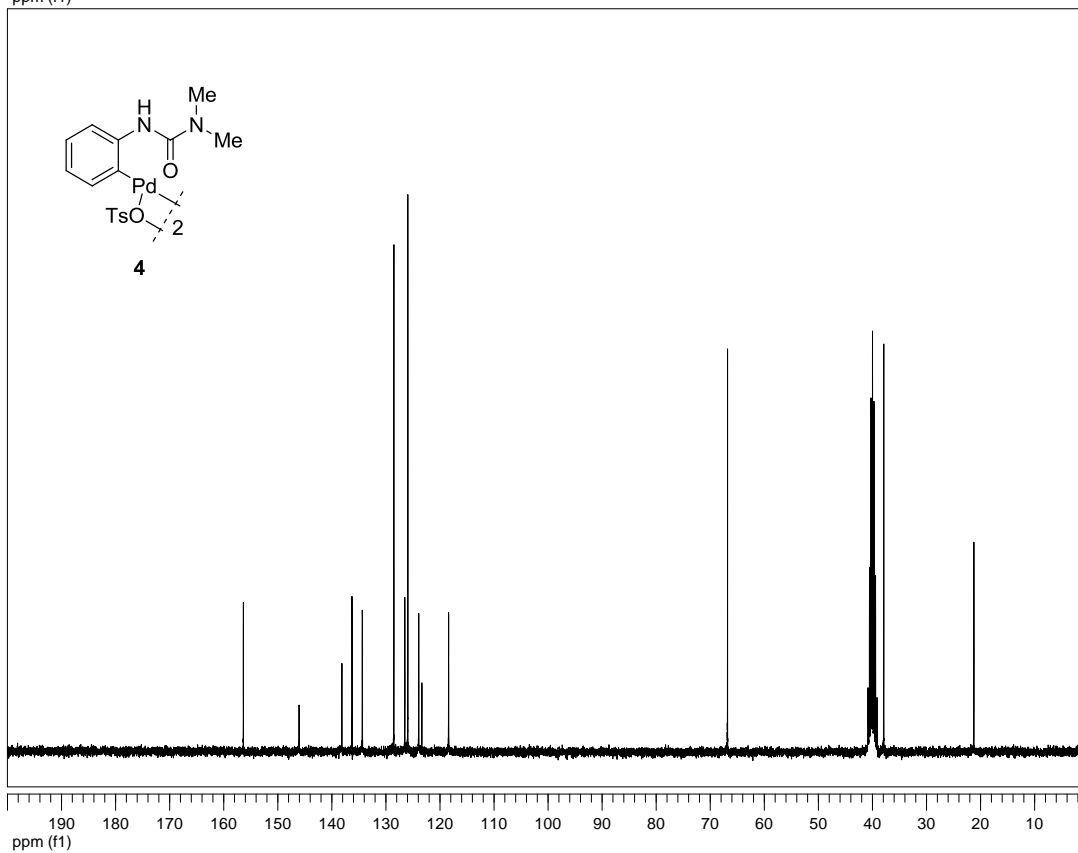
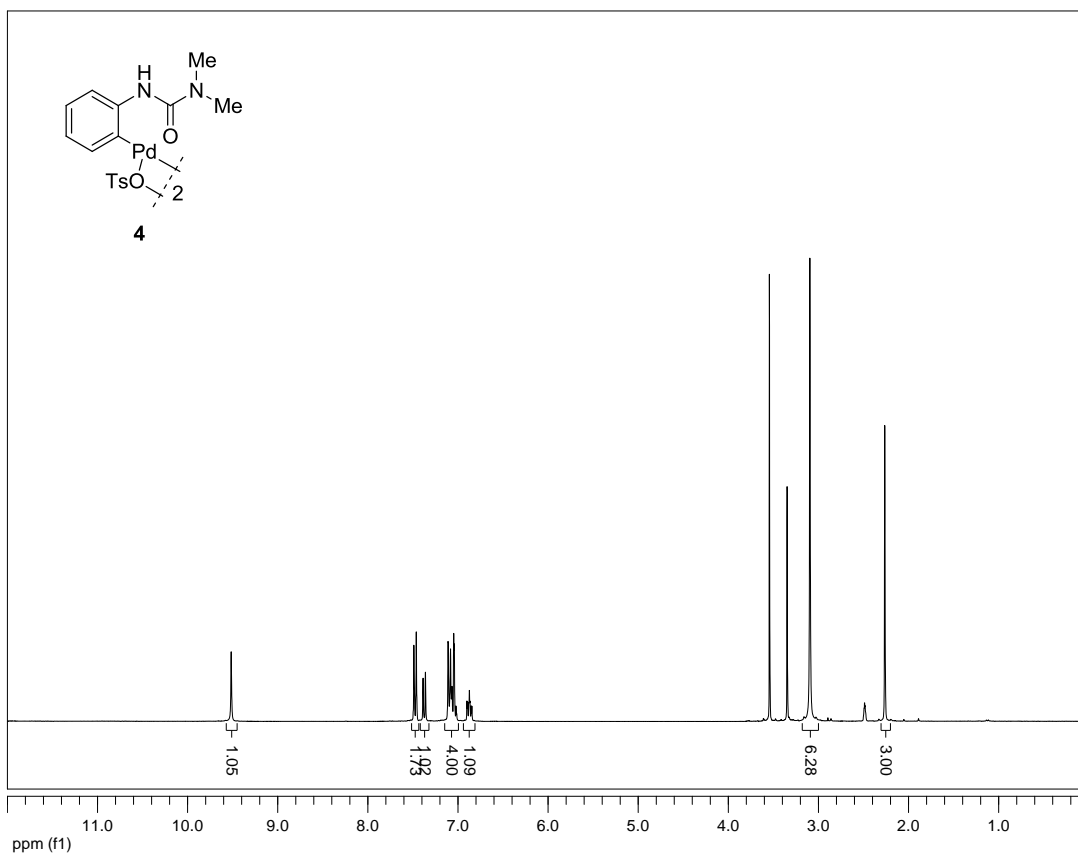
[1] Cheng, G.; Lim, R. K.; Li, N.; Lin, Q. *Chem. Commun.* **2013**, 49, 6809.

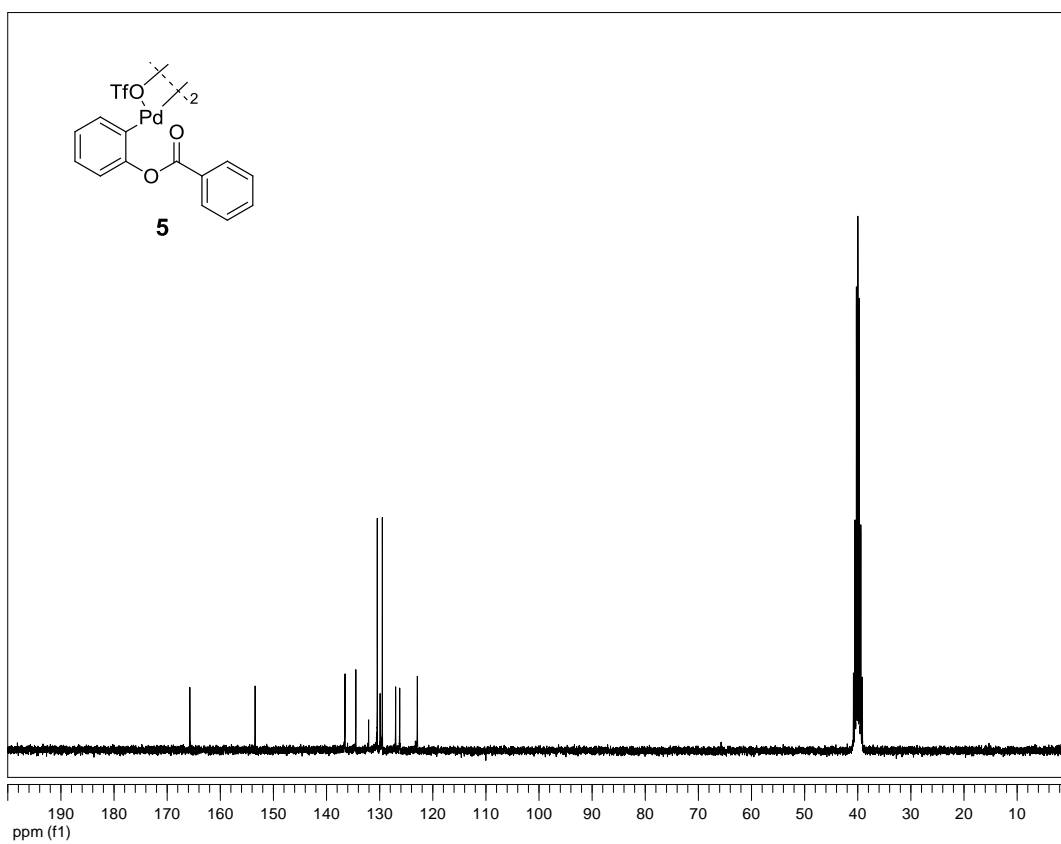
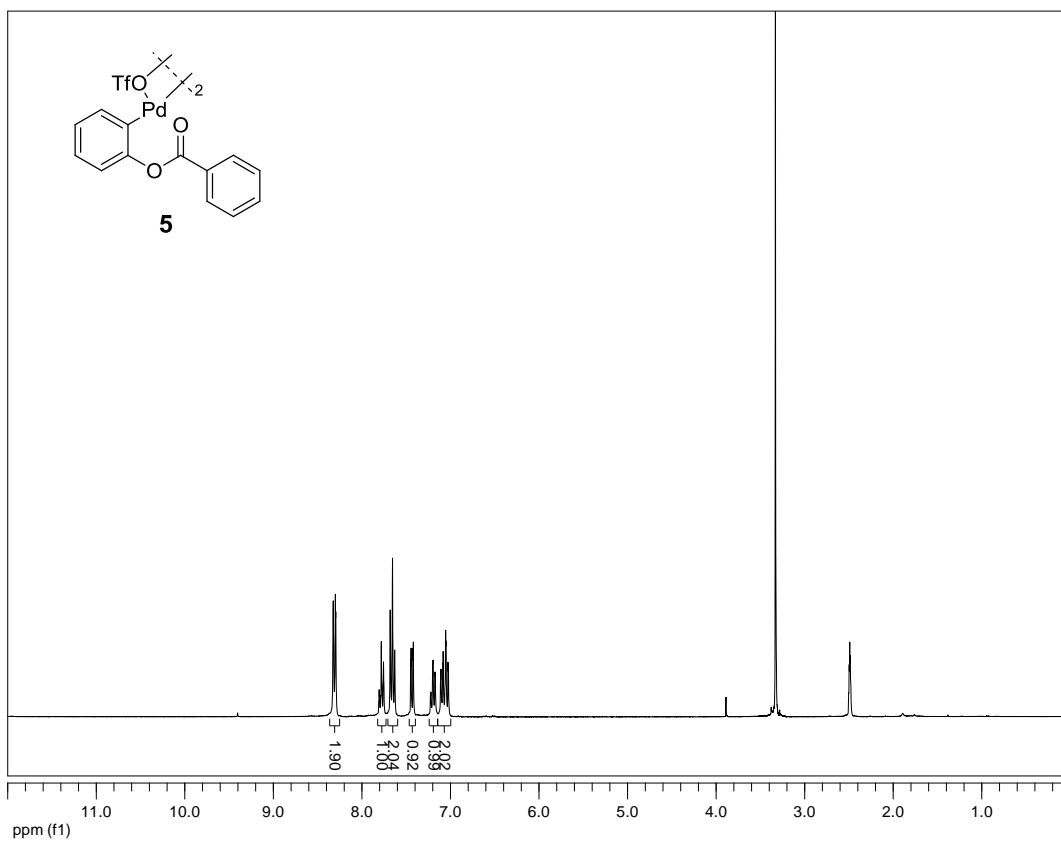
[2] John, A.; Nicholas, K. M. *J. Org. Chem.* **2012**, 77, 5600.

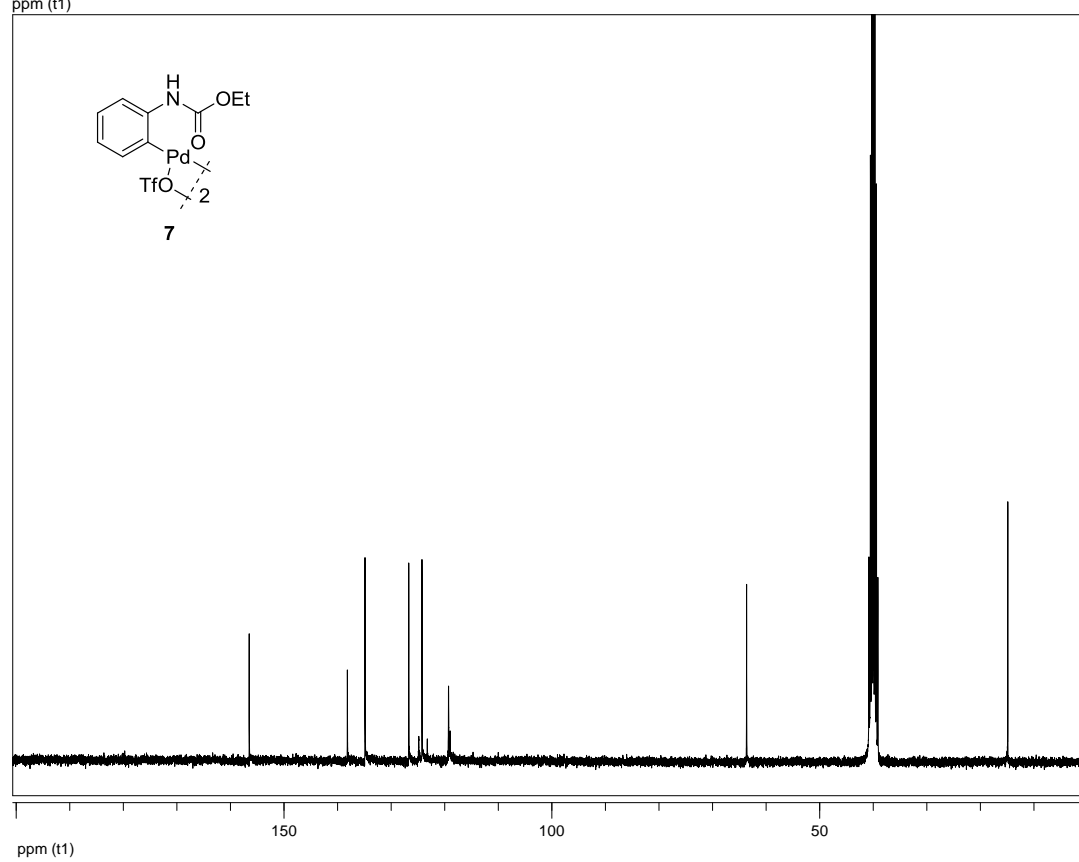
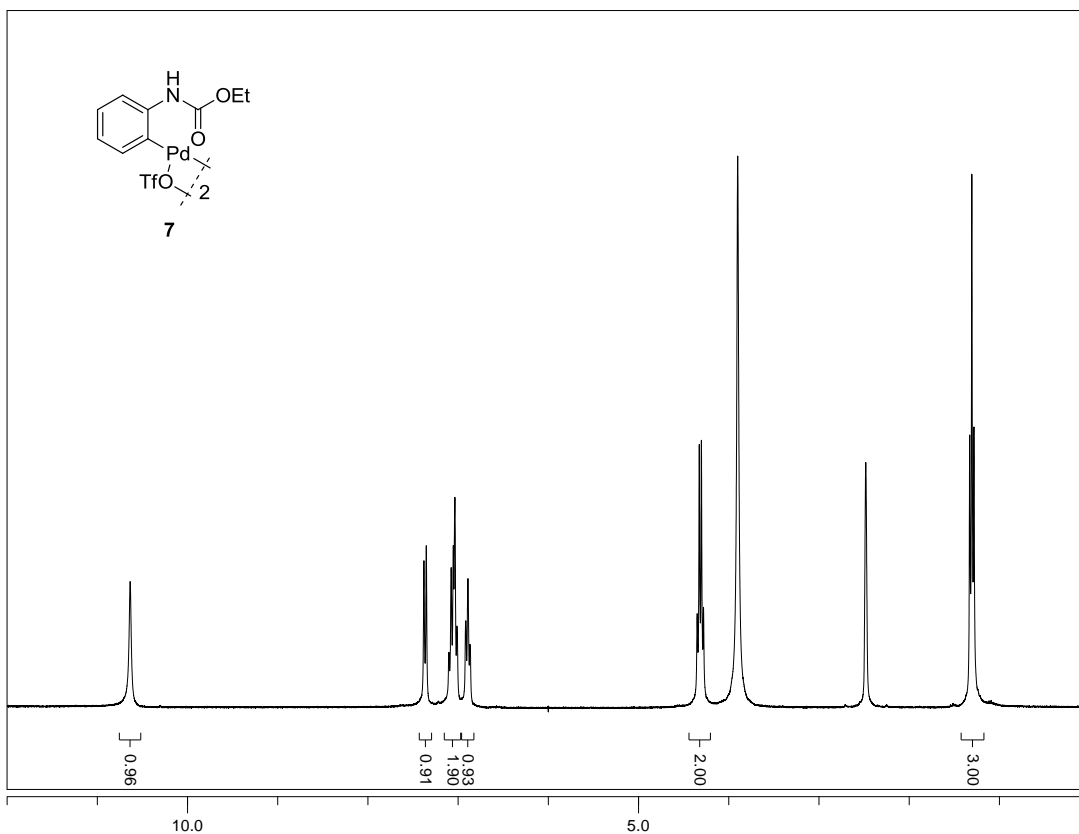
[3] Li, N.; Lim, R. K.; Edwardraja, S.; Lin, Q. *J. Am. Chem. Soc.* **2011**, 133, 15316.

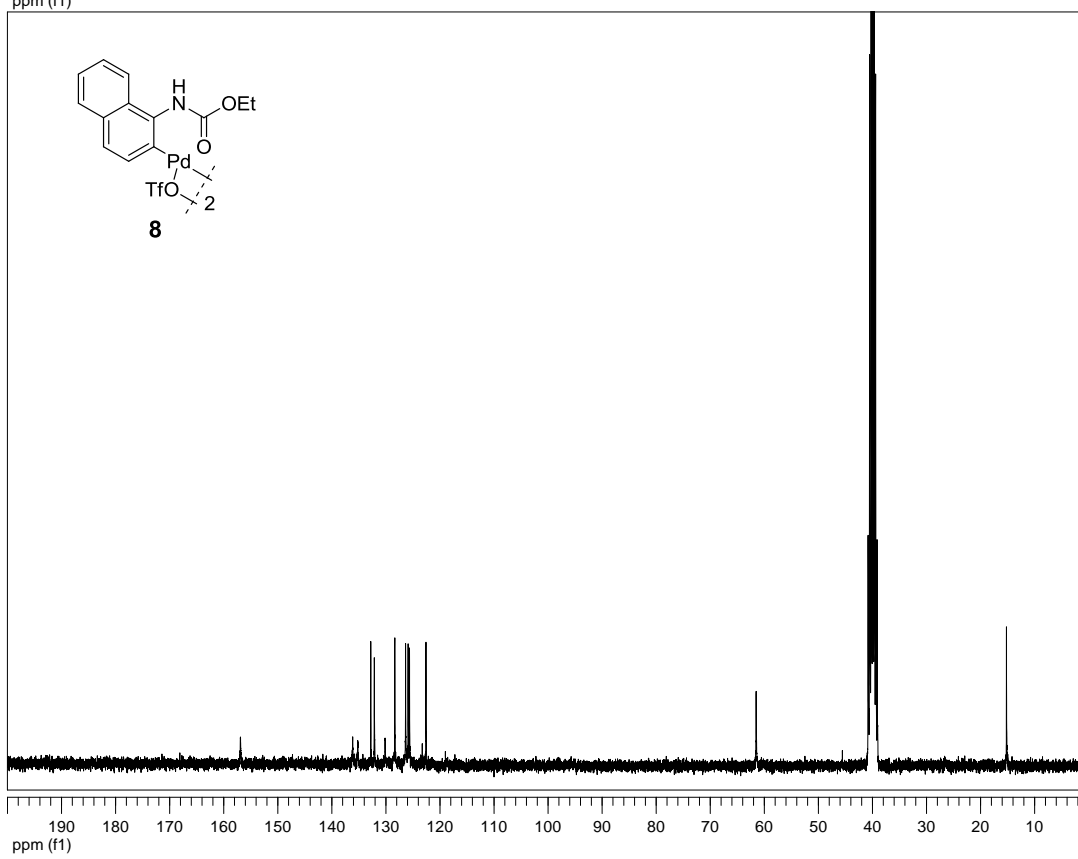
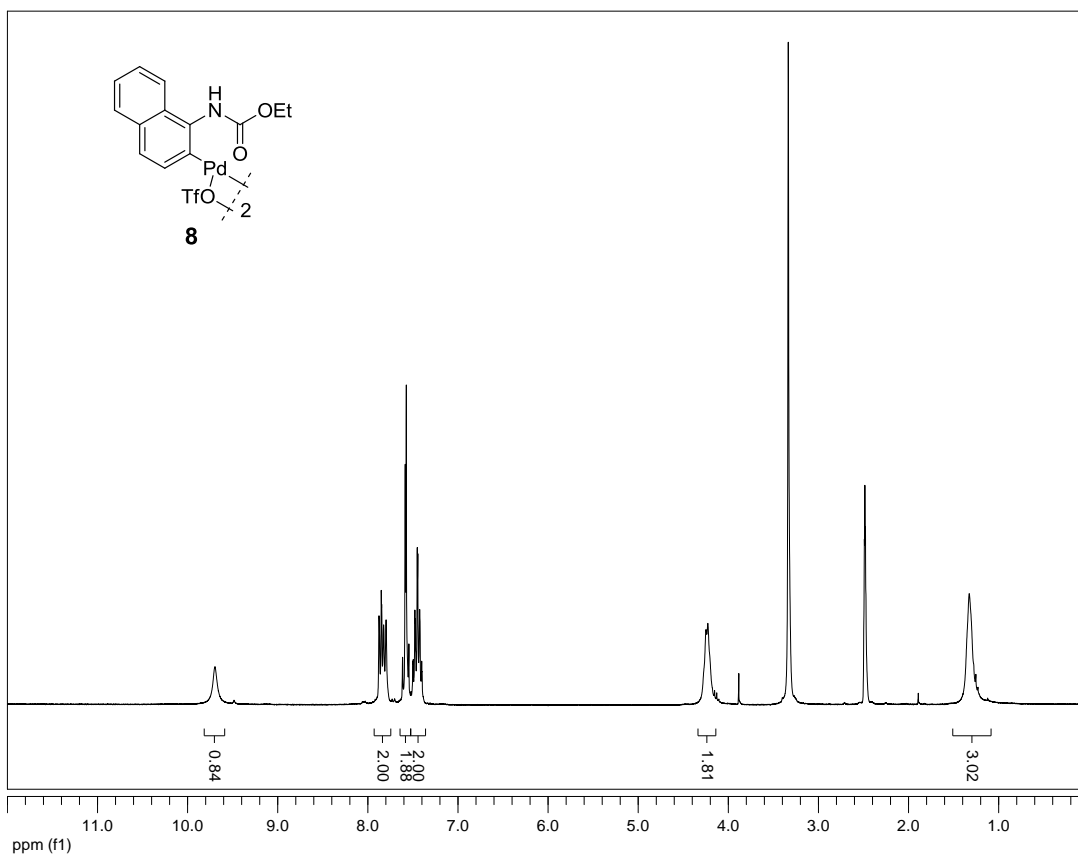
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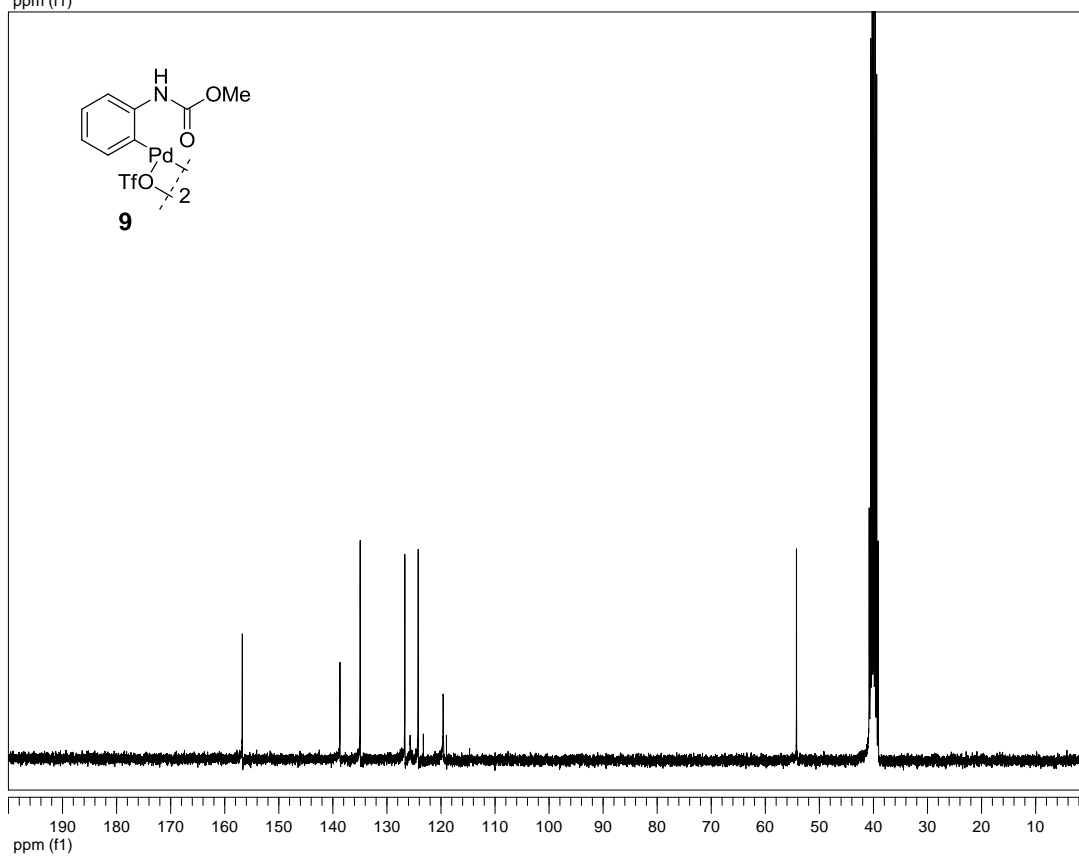
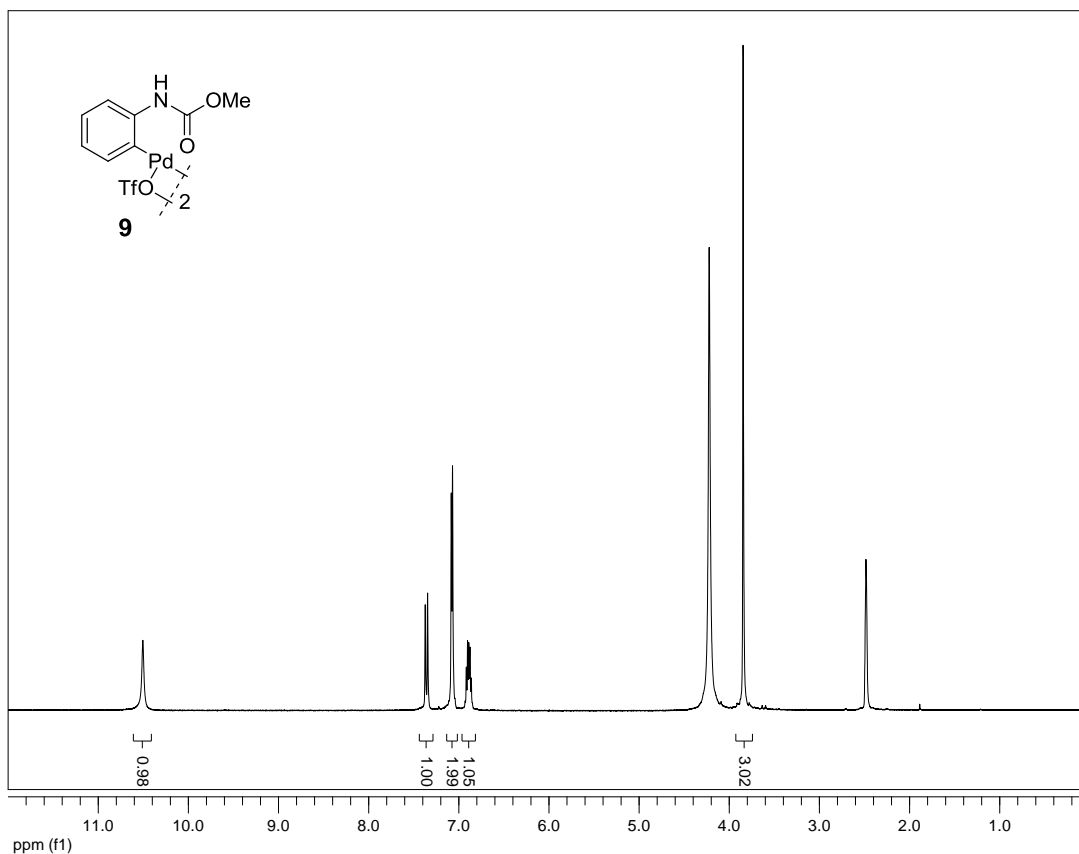


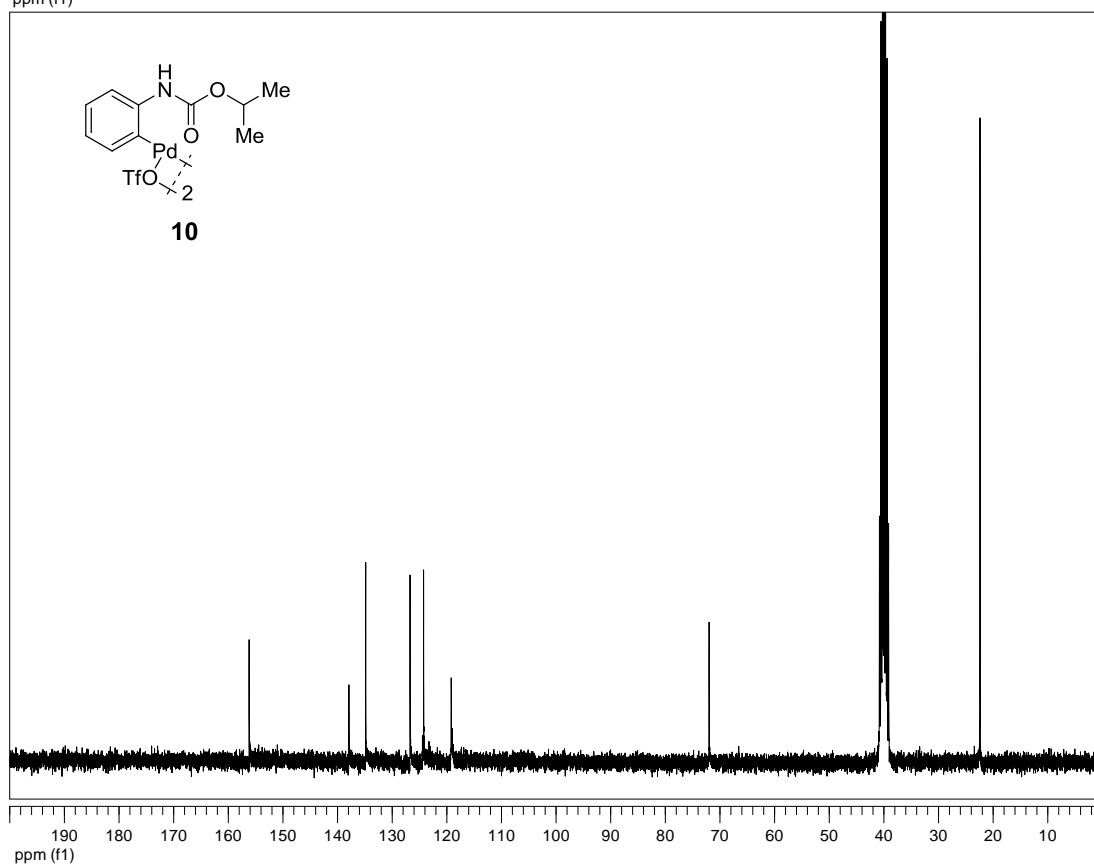
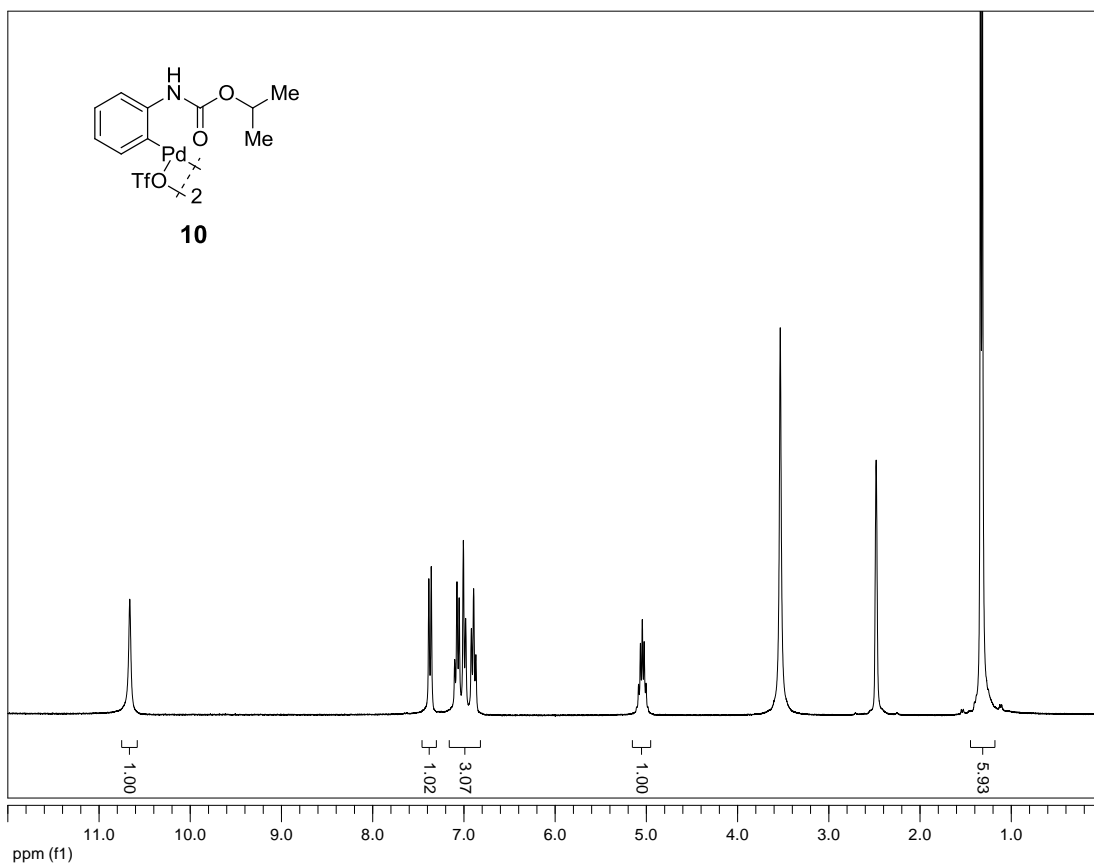


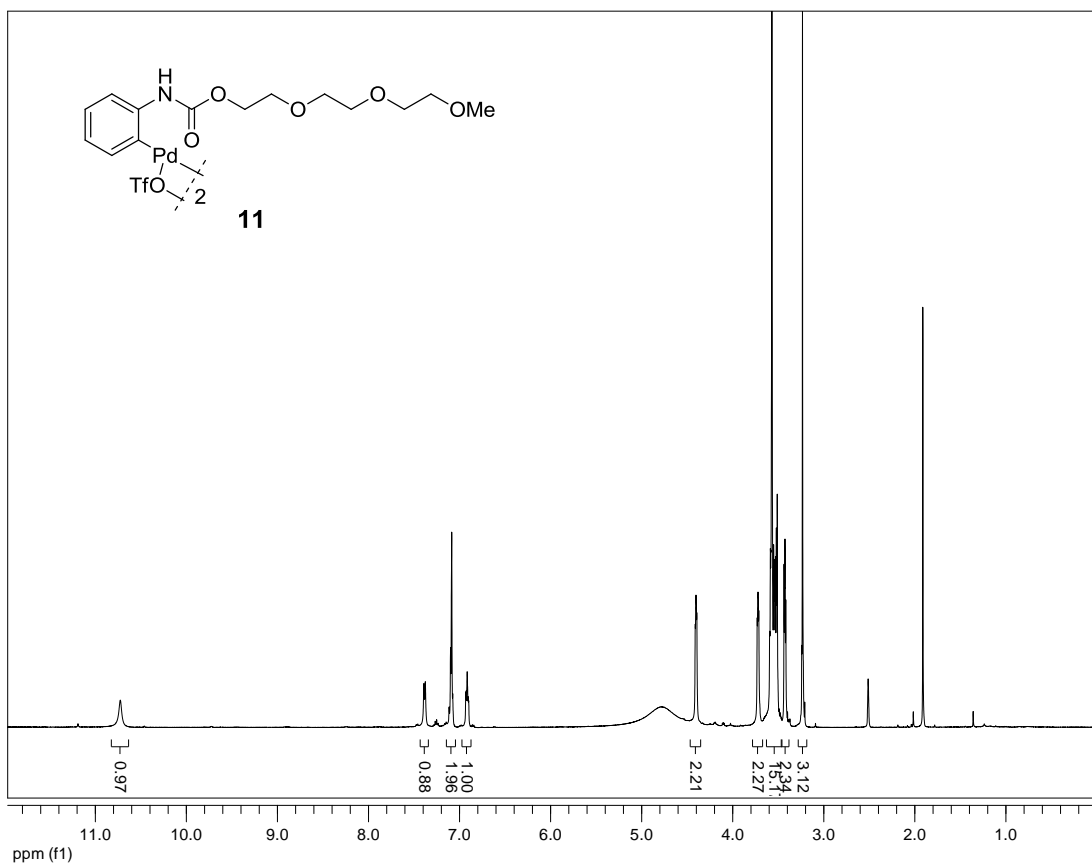












chg3-52_10mg_sub_400ul_13C_U4

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Data Collected on: nmr590c.chem.buffalo.edu-inoval500

Archive directory: /export/home/chempack/vmrsys/data

Sample directory:

Fidfile: CARBON

Pulse Sequence: CARBON (s2pul)

Solvent: dmsc

Data collected on: Feb 3 2014

Temp. 25.0 C / 298.1 K

Operator: Lin

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.042 sec

Width 31666.5 Hz

3584 repetitions

OBSERVE C13, 125.7001474 MHz

DECOUPLE H1, 499.9032648 MHz

Power 36 dB

Continuously on

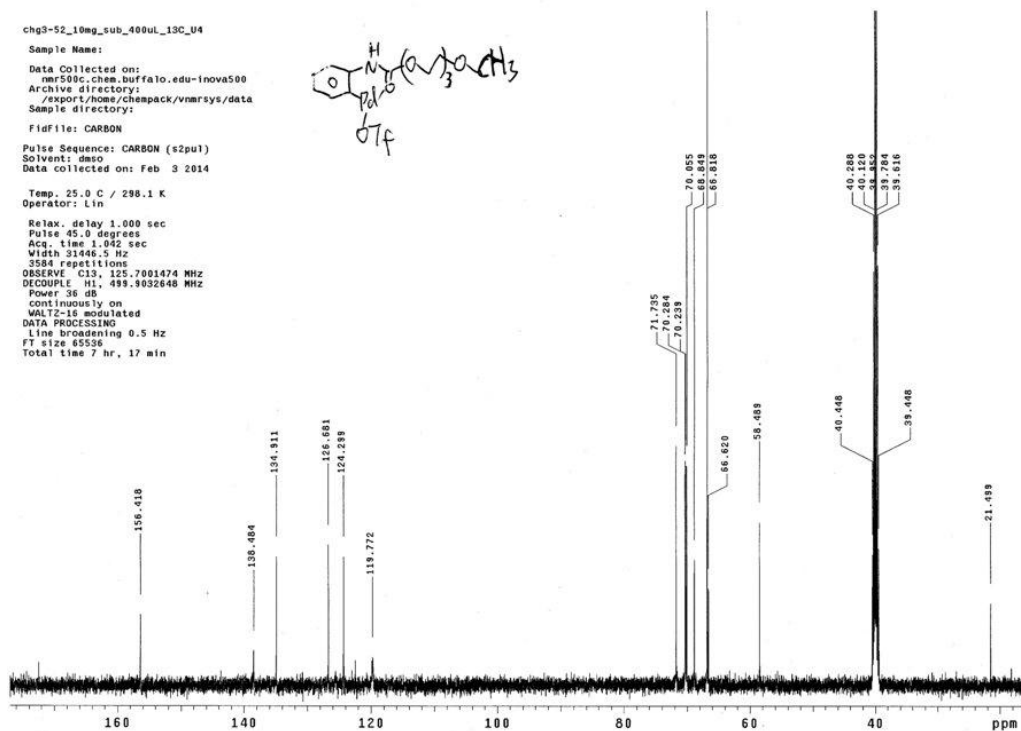
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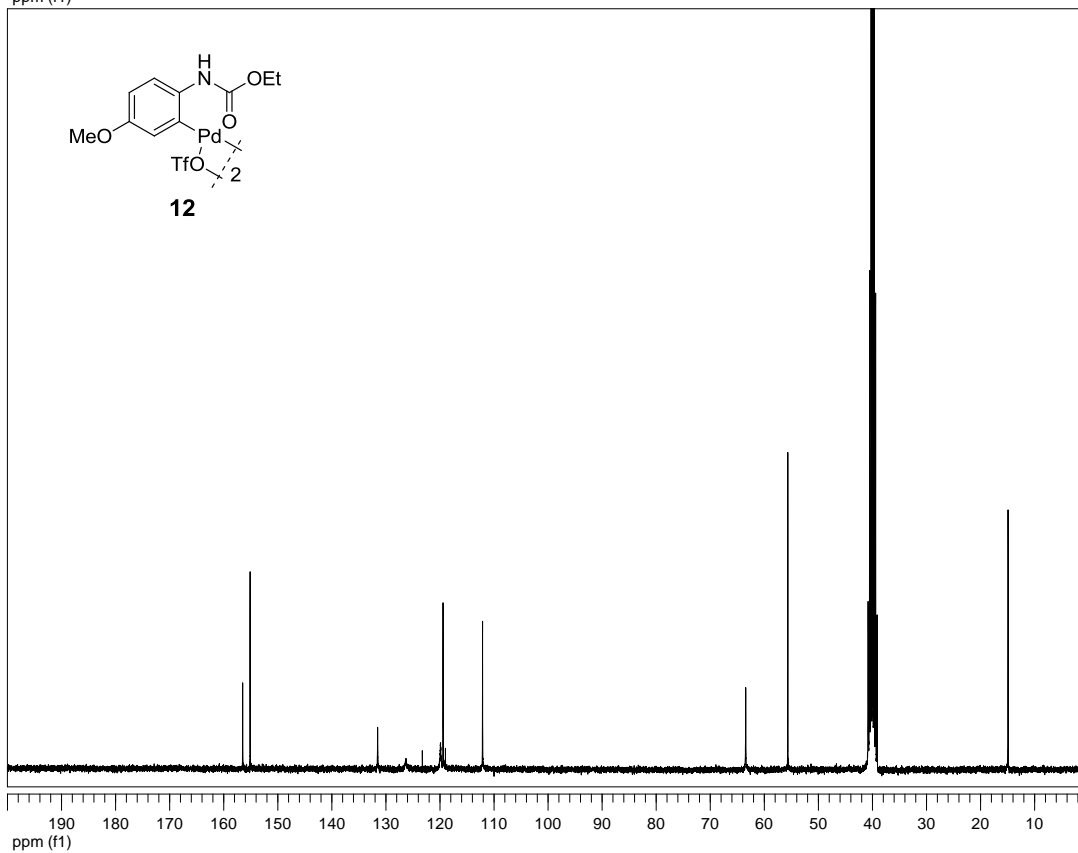
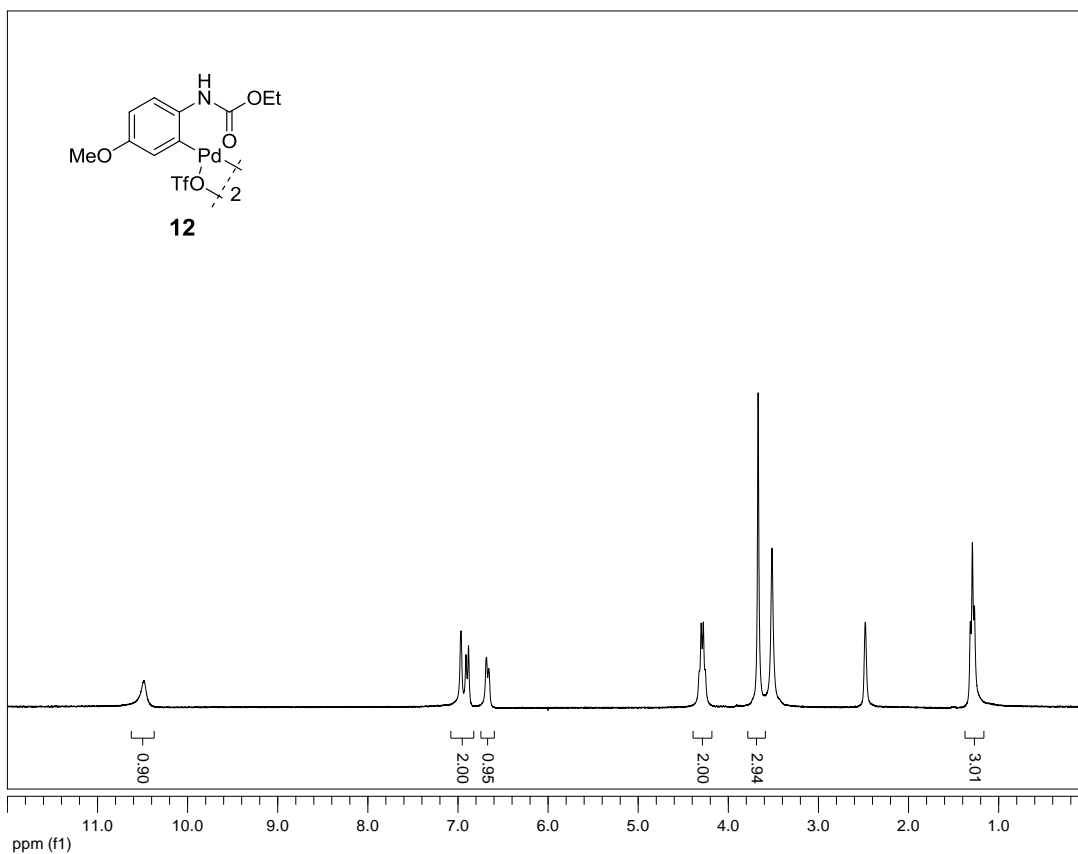
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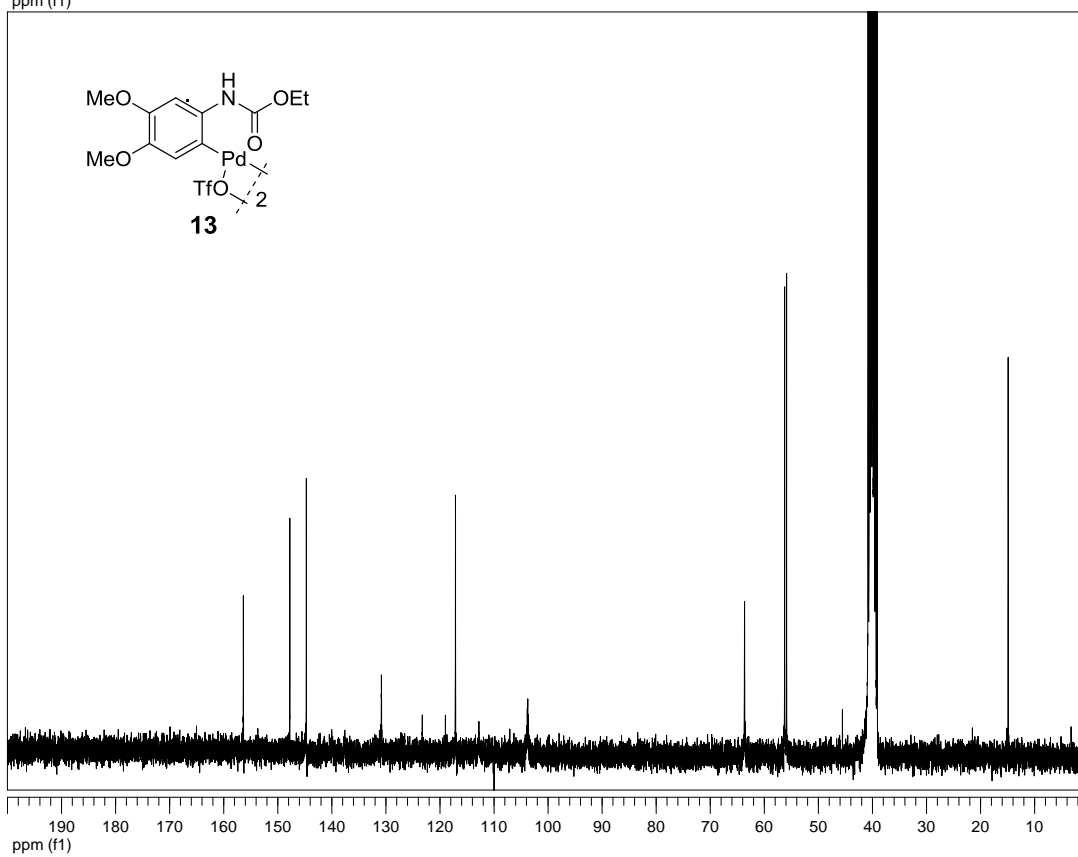
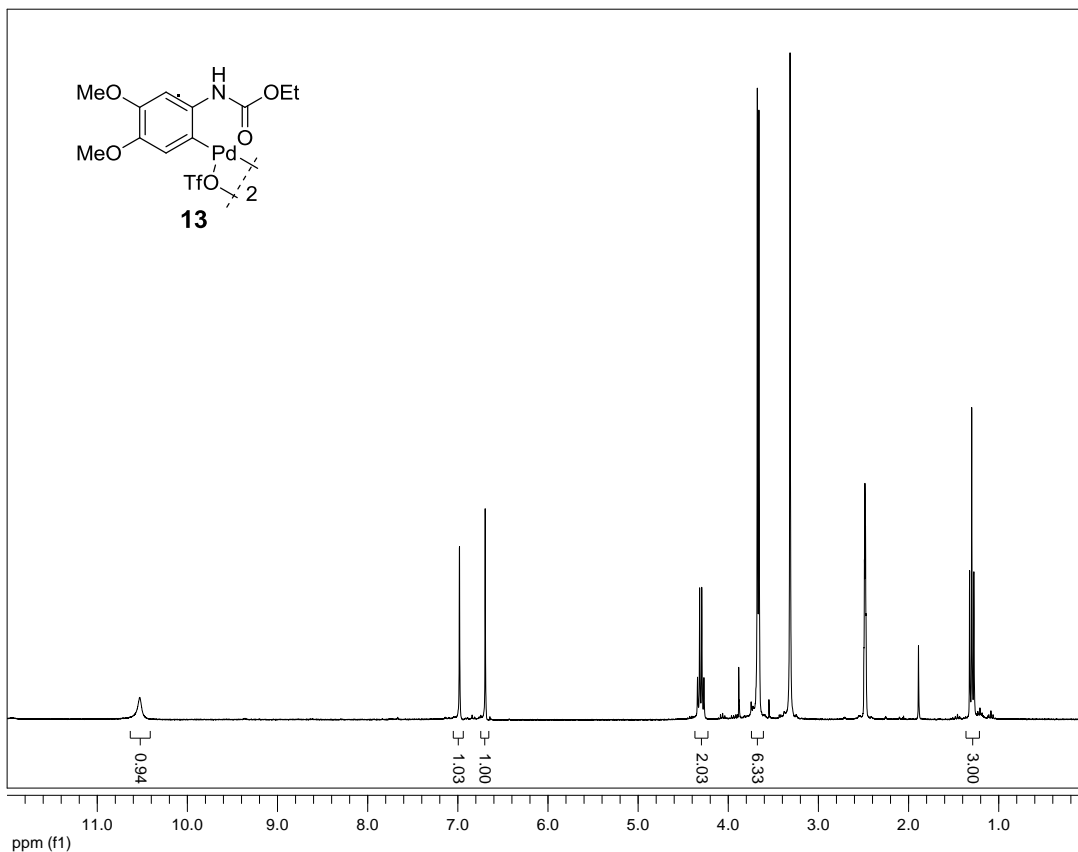
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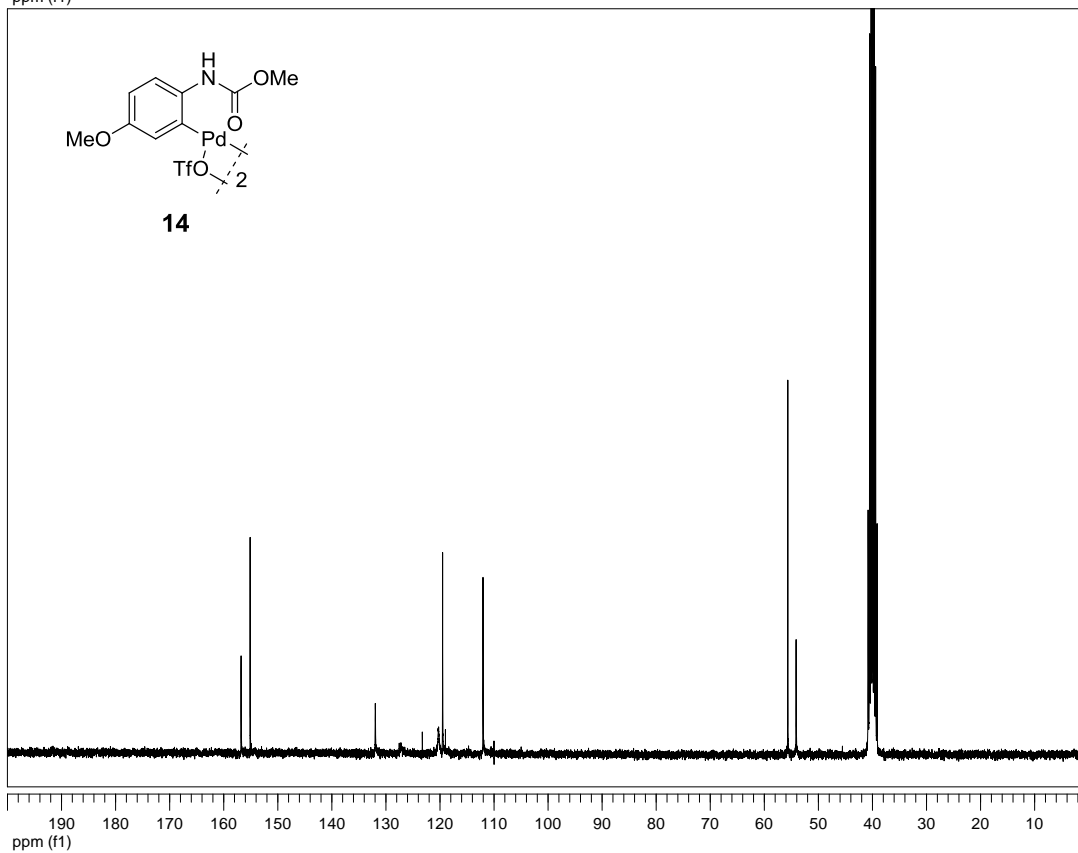
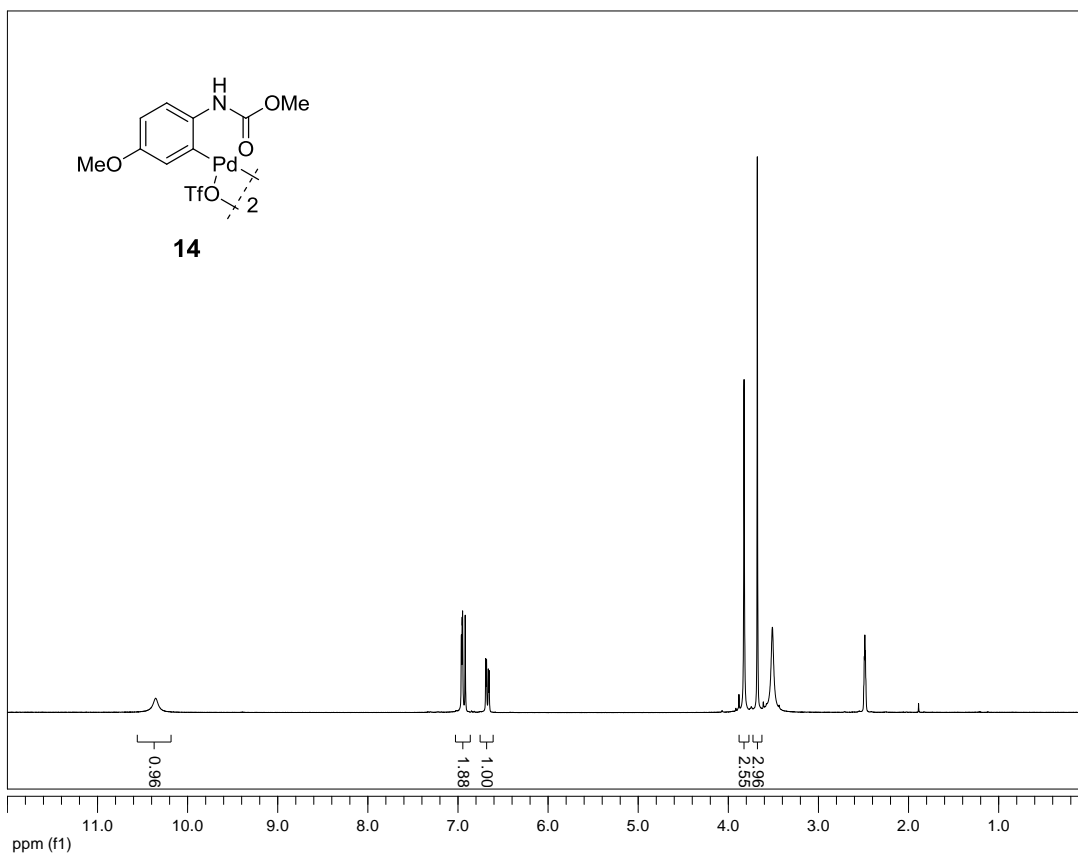
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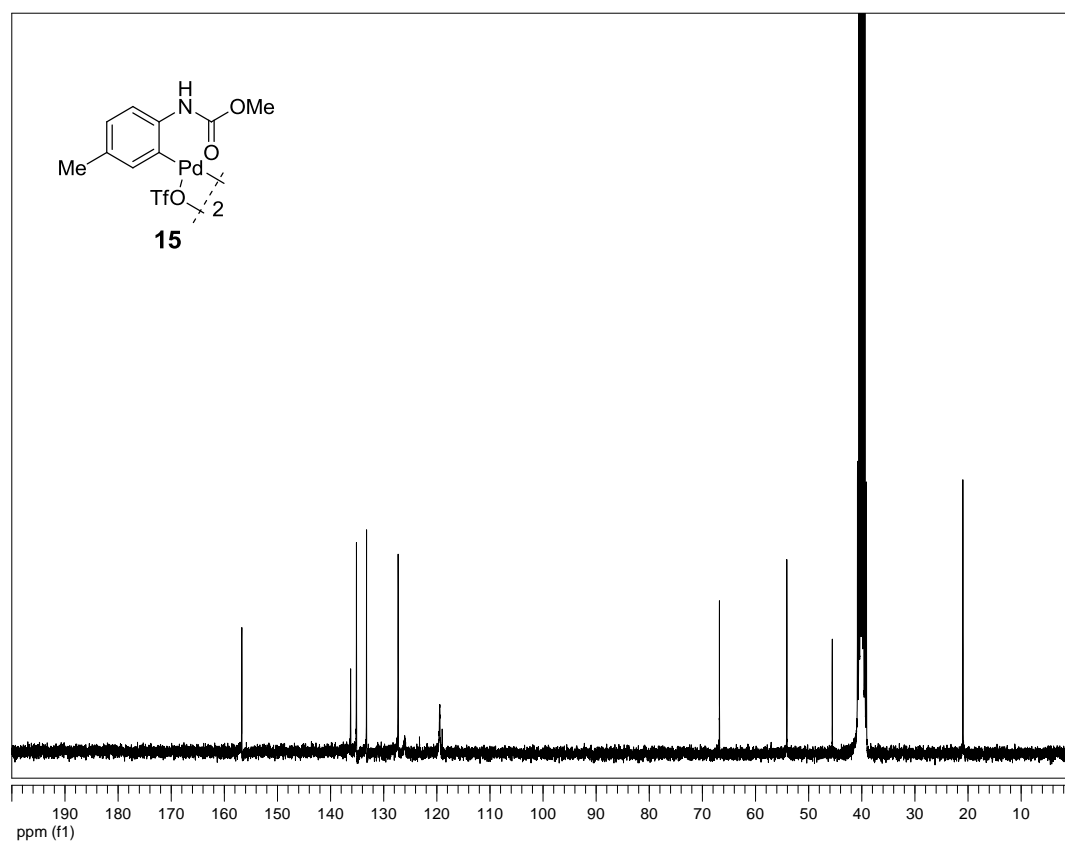
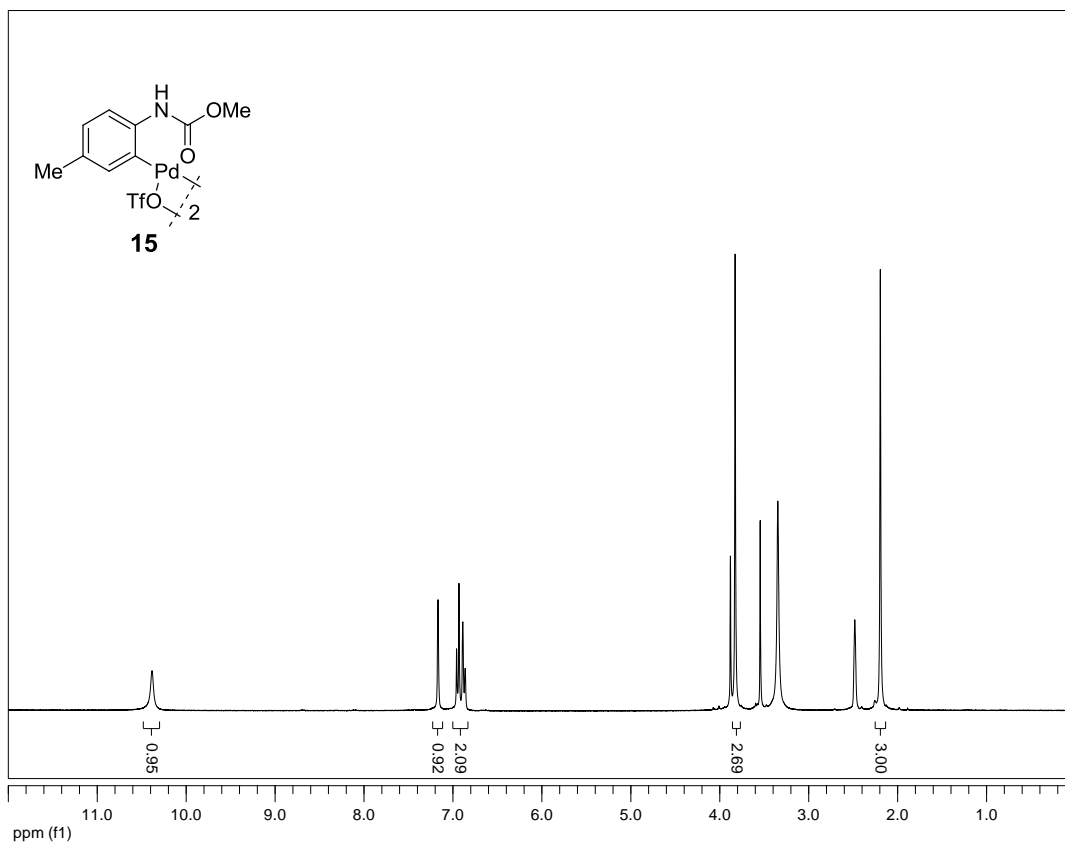
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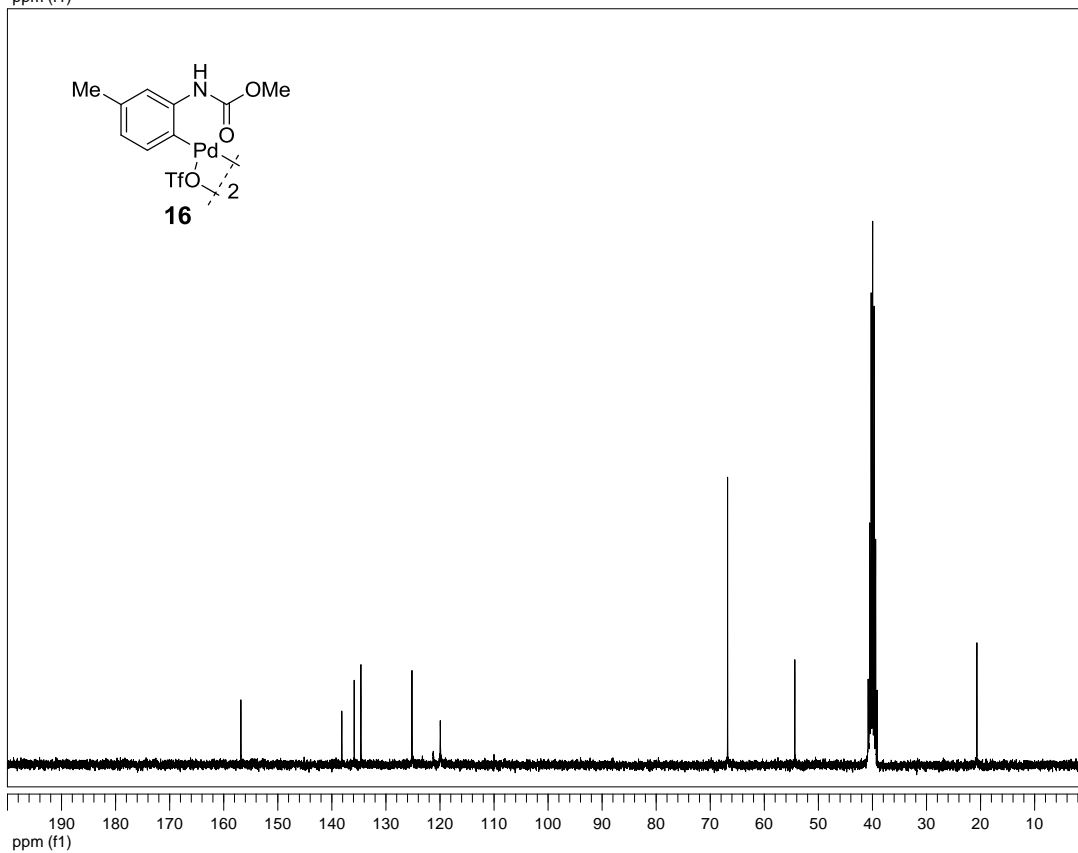
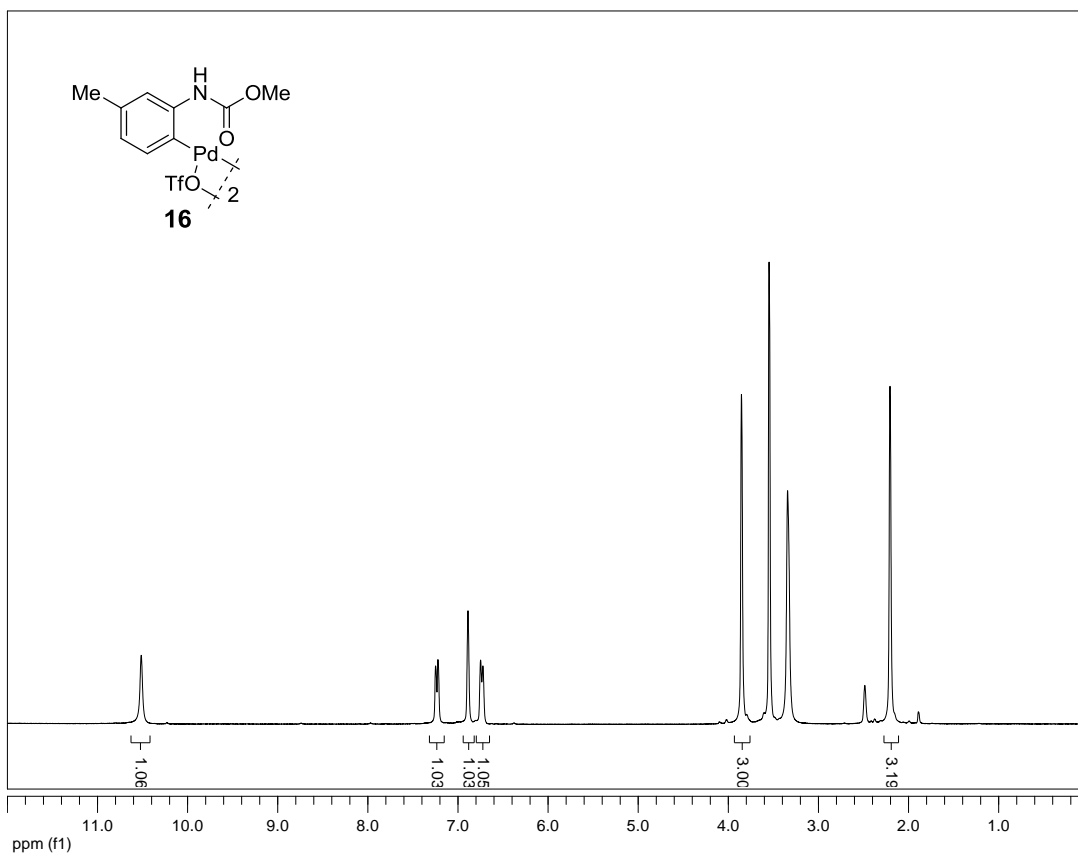


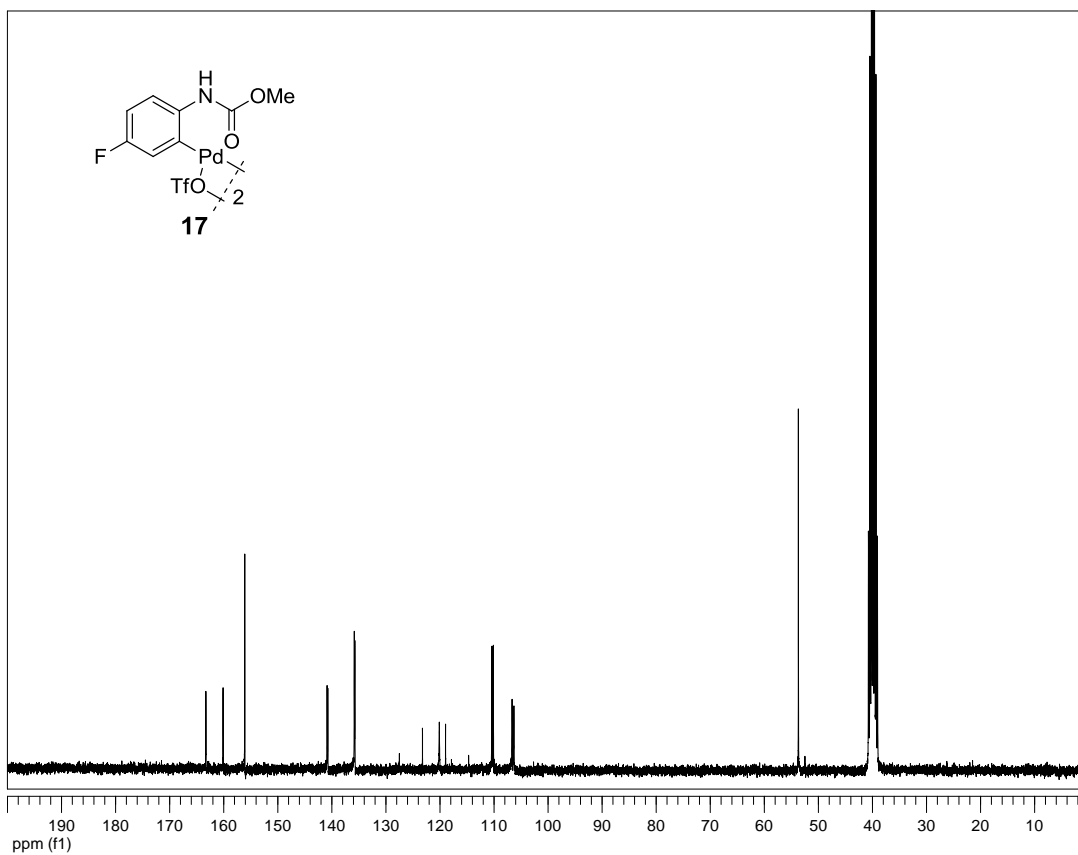
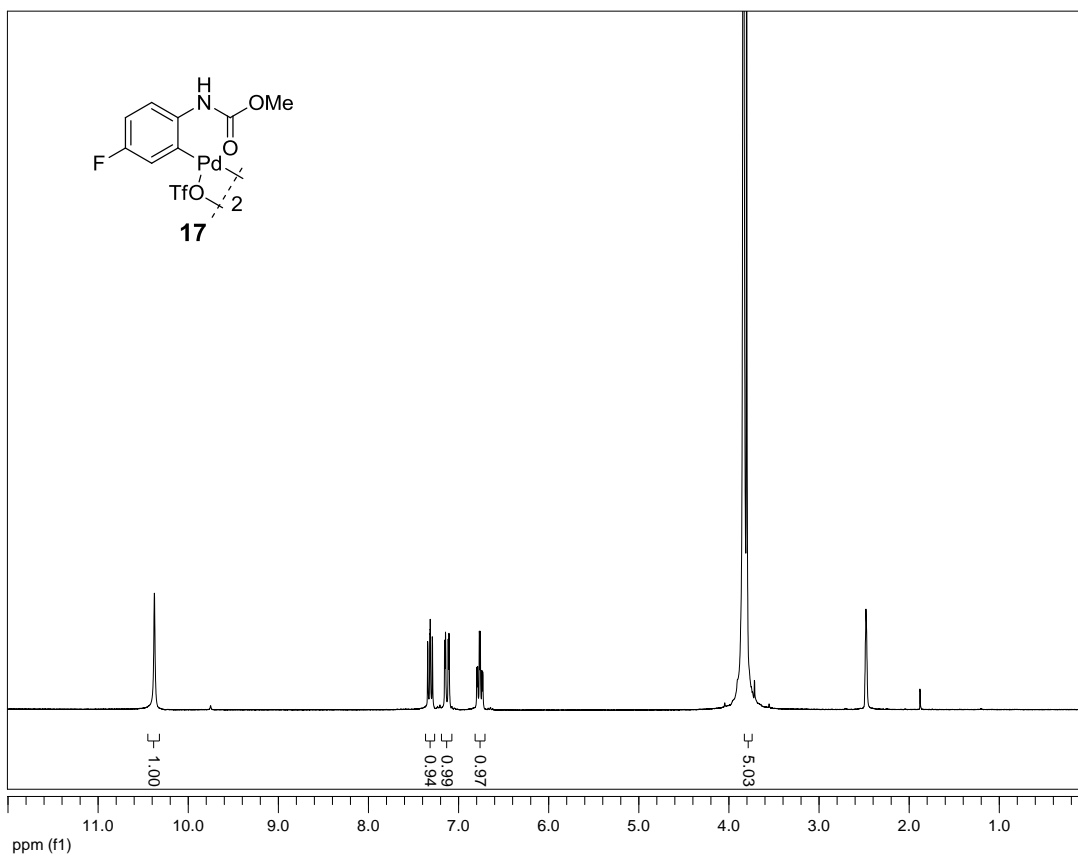


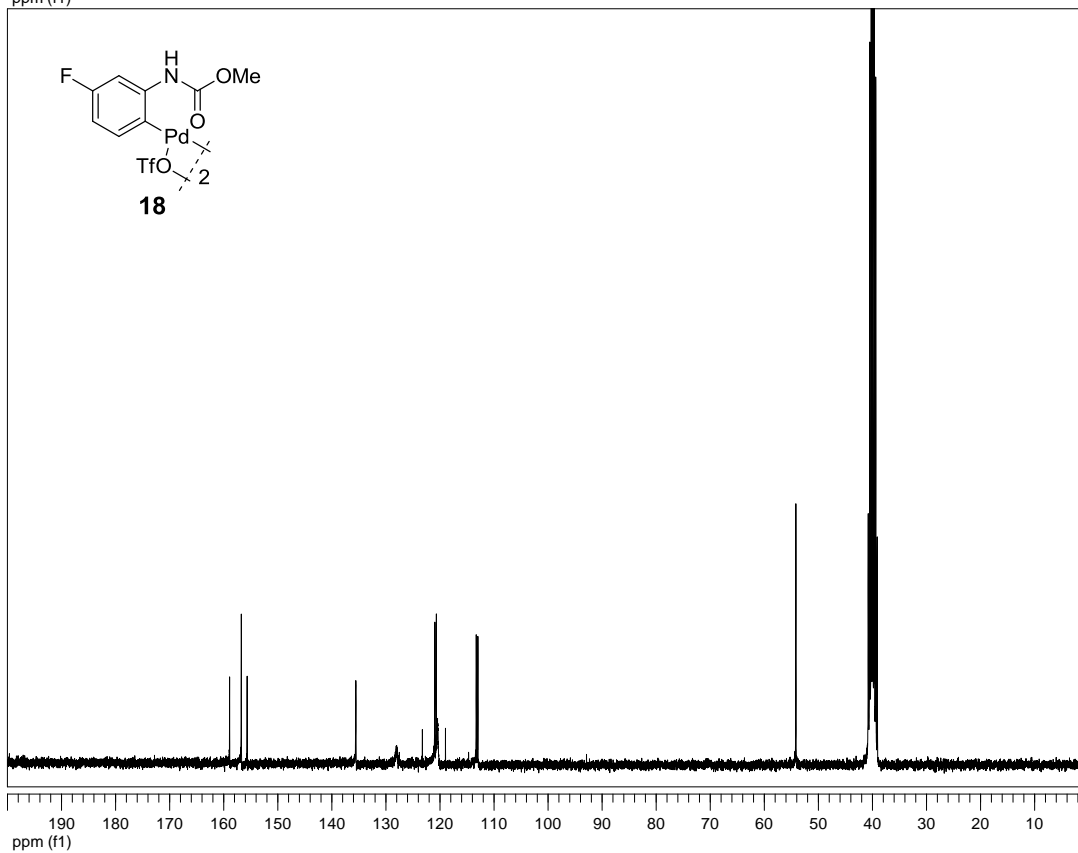
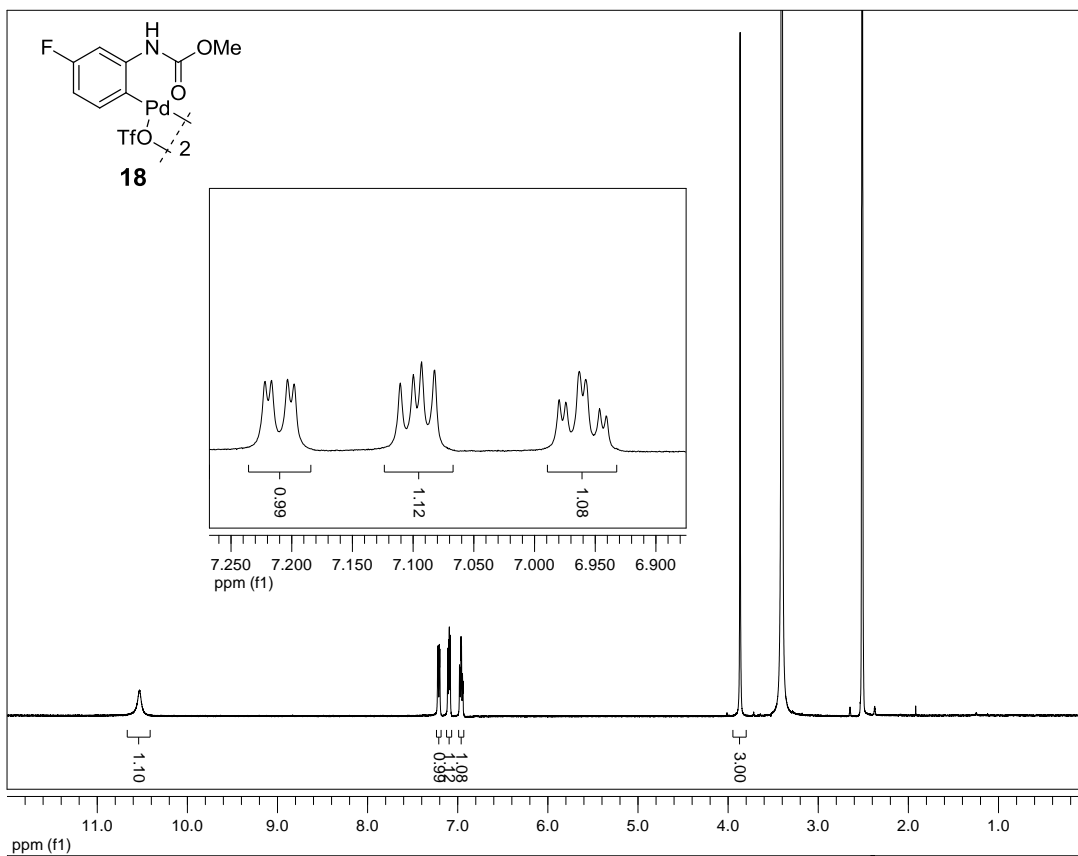


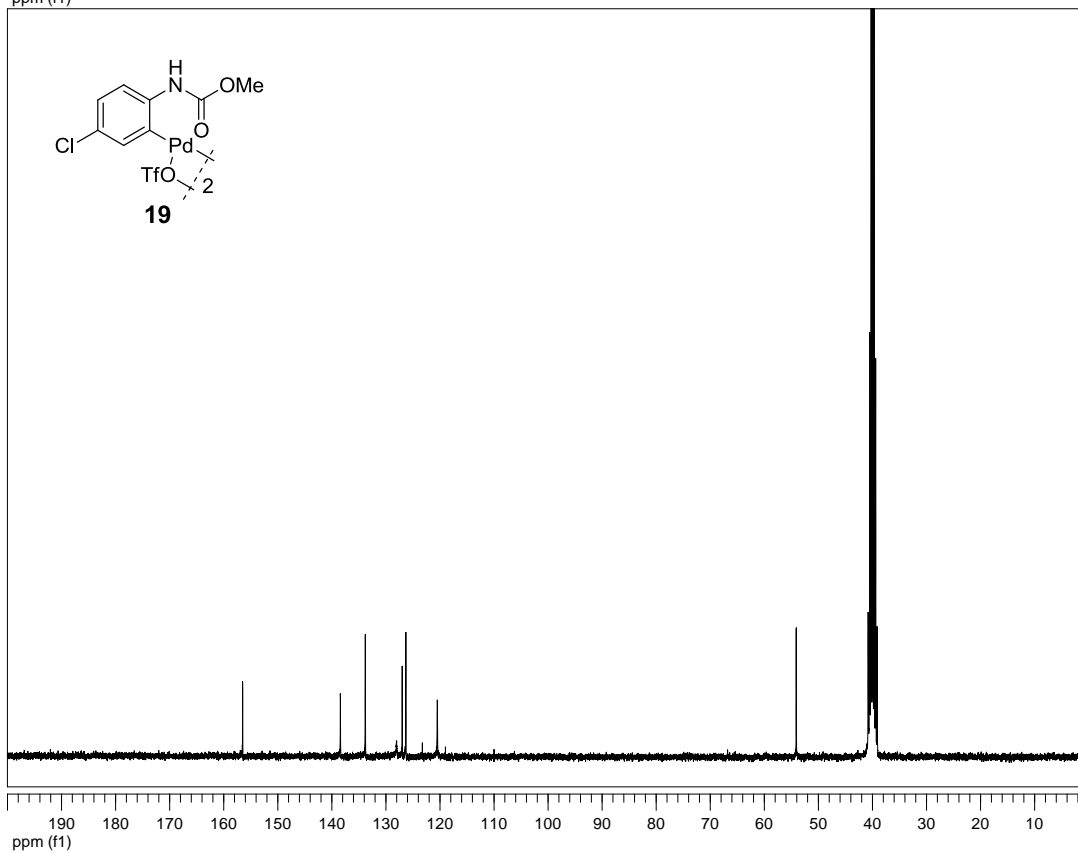
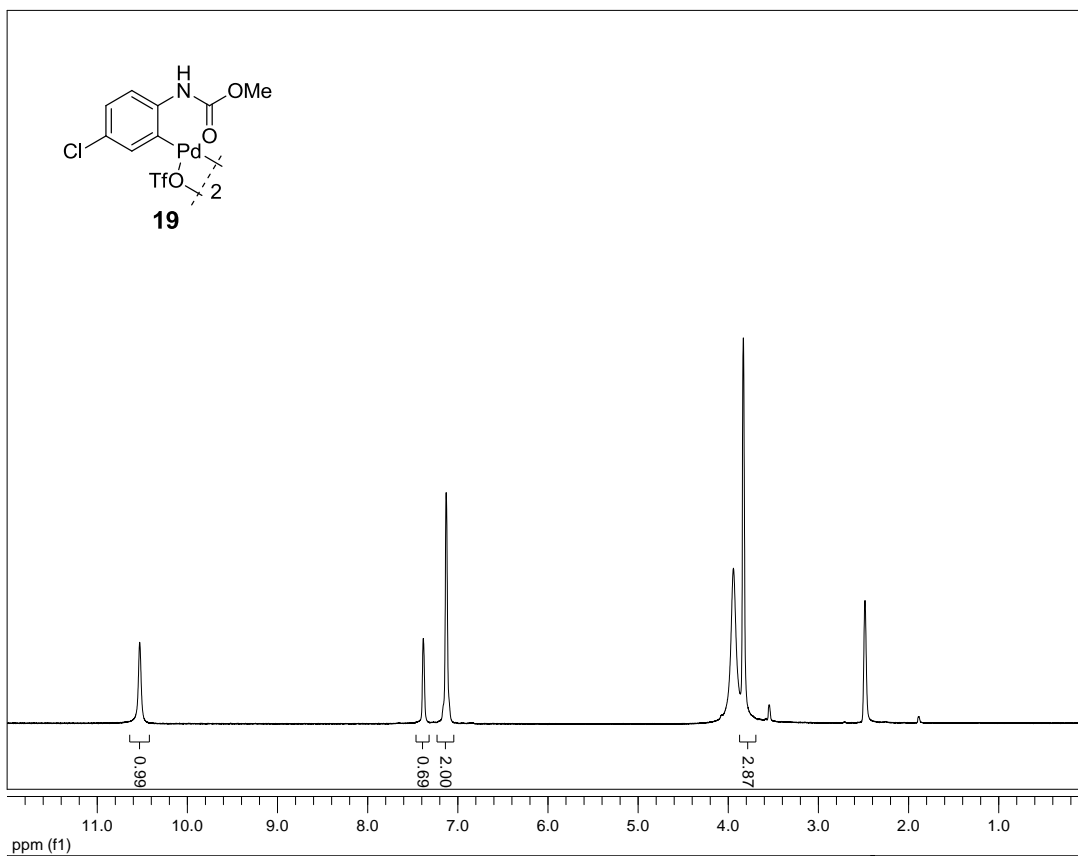


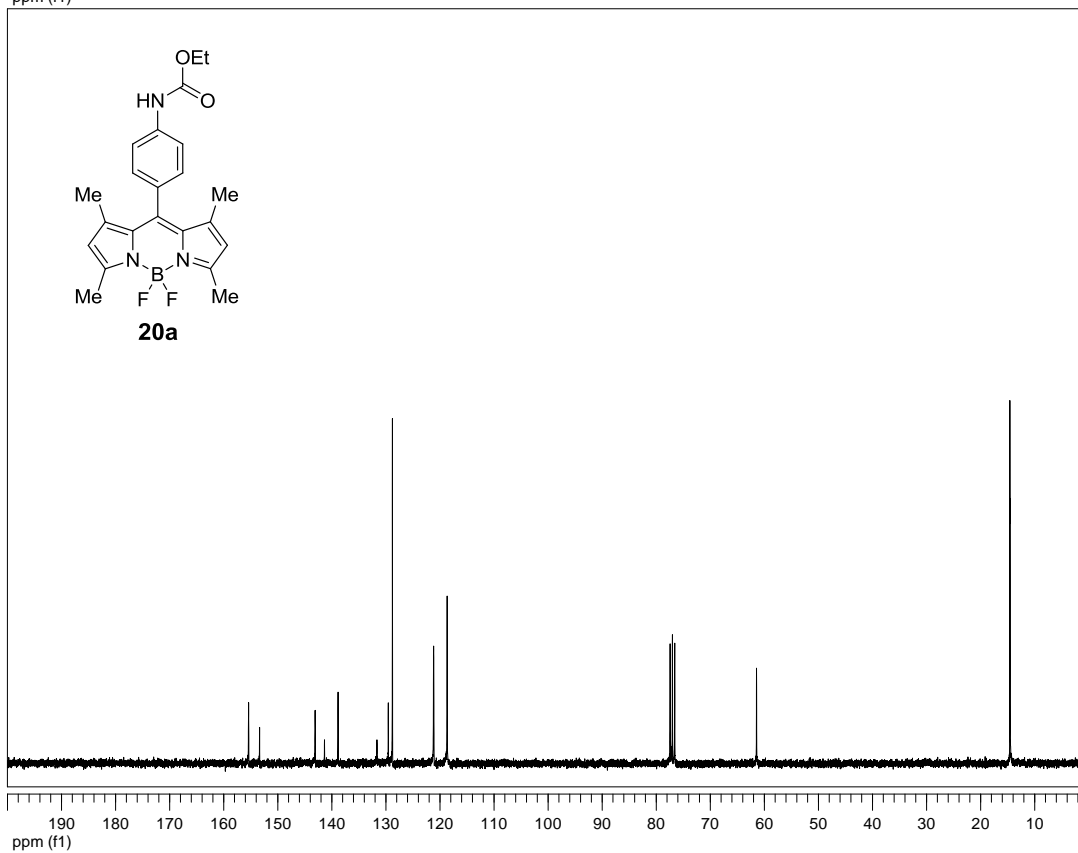
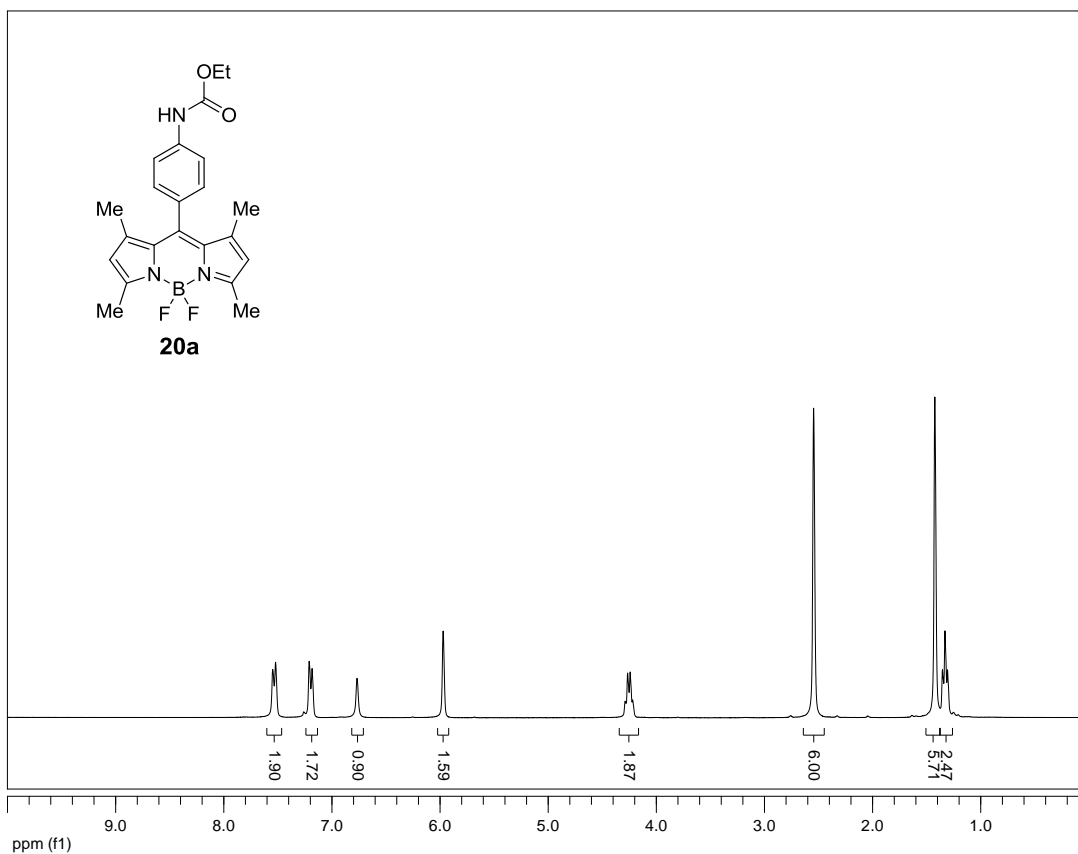


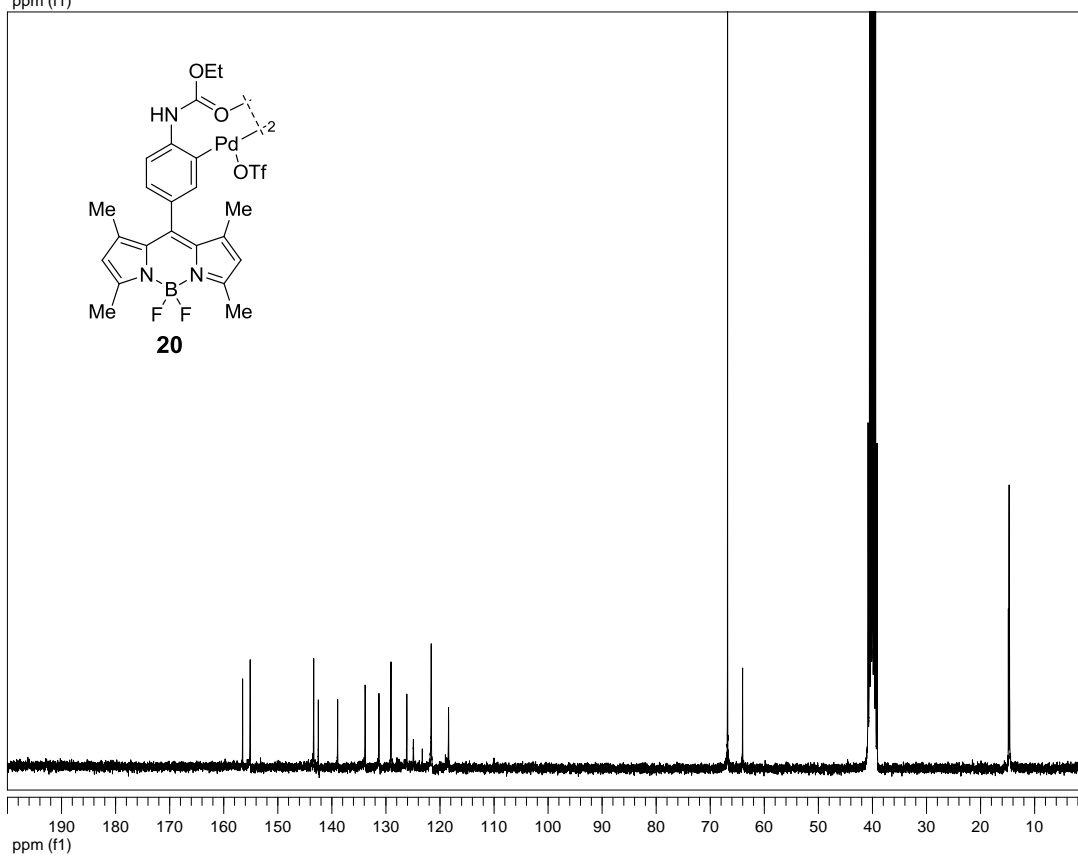
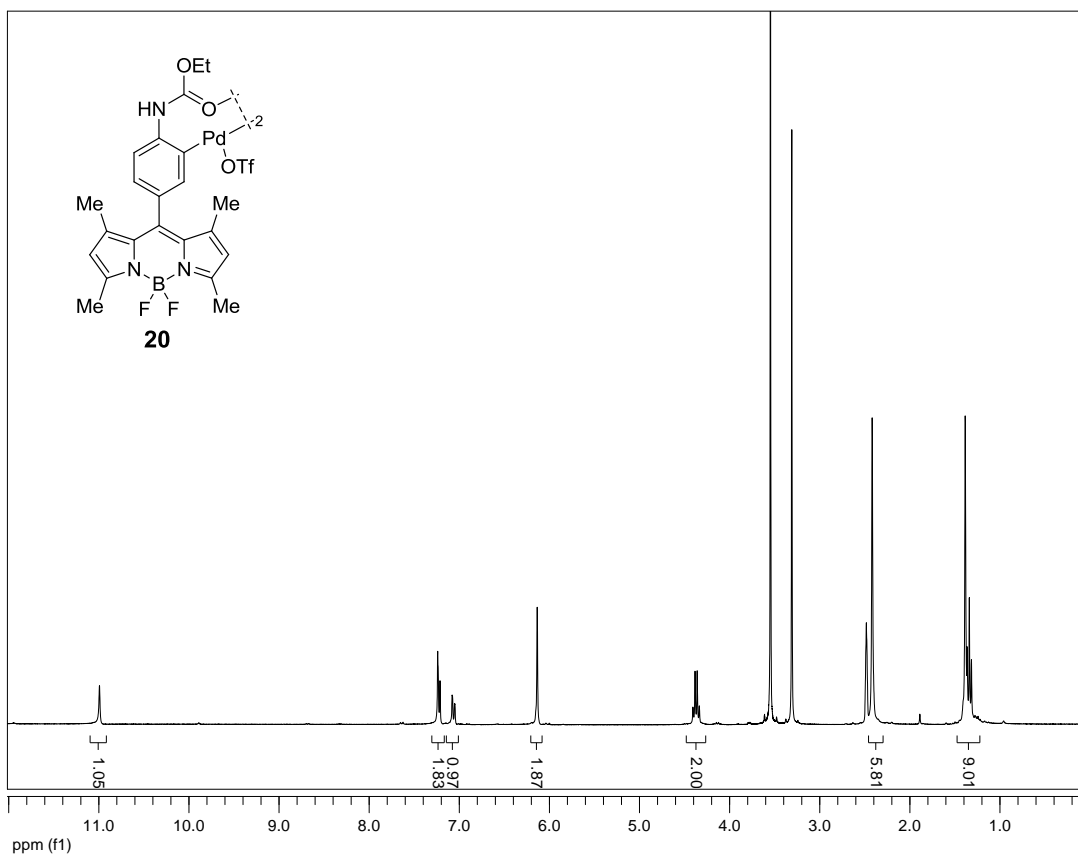


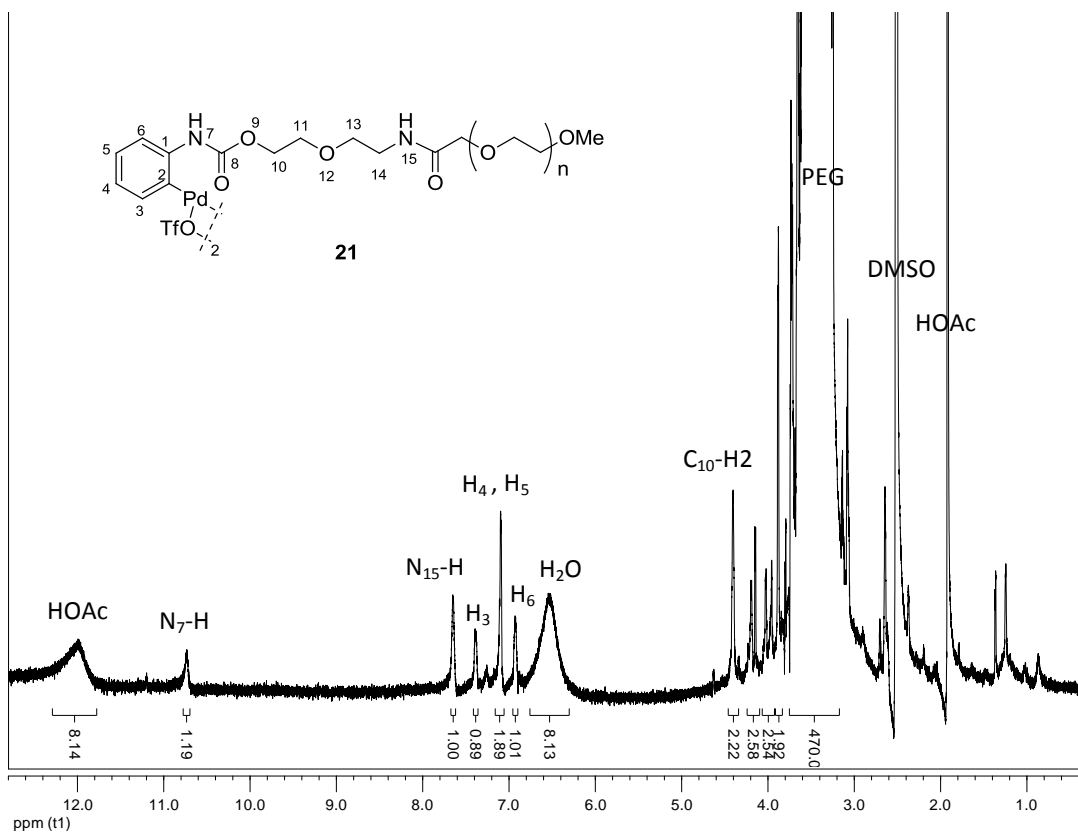
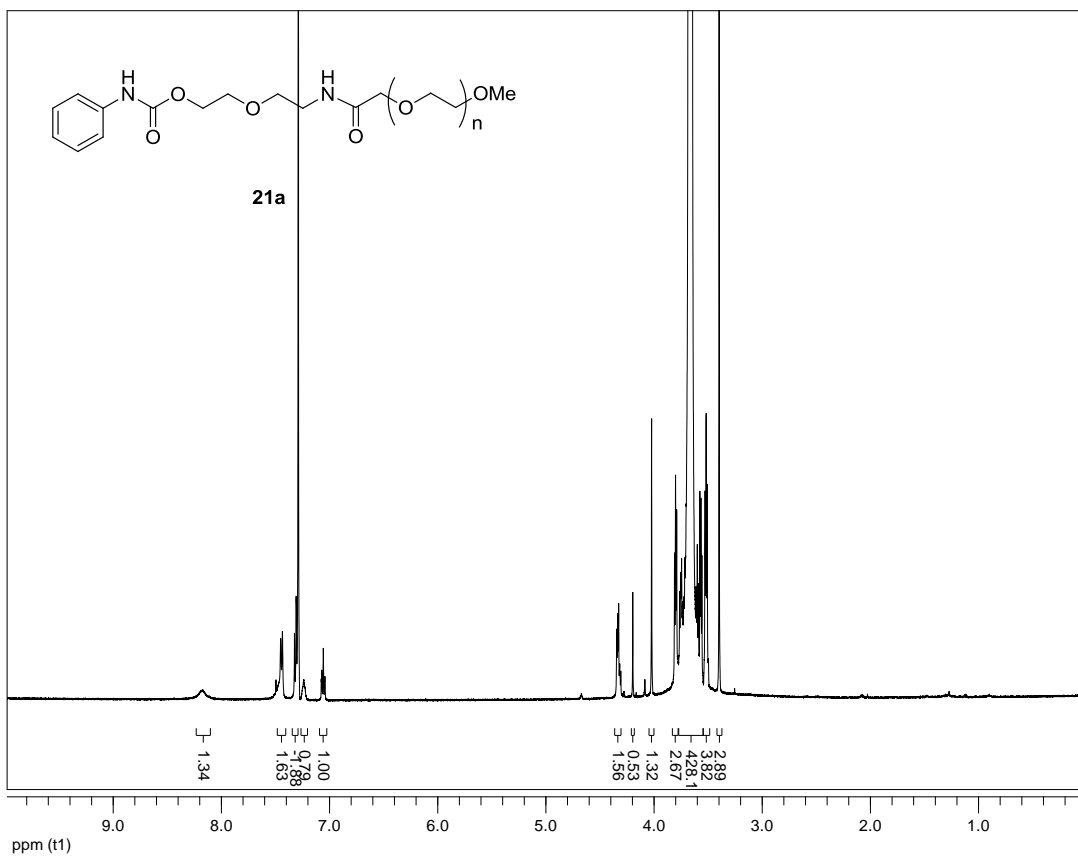








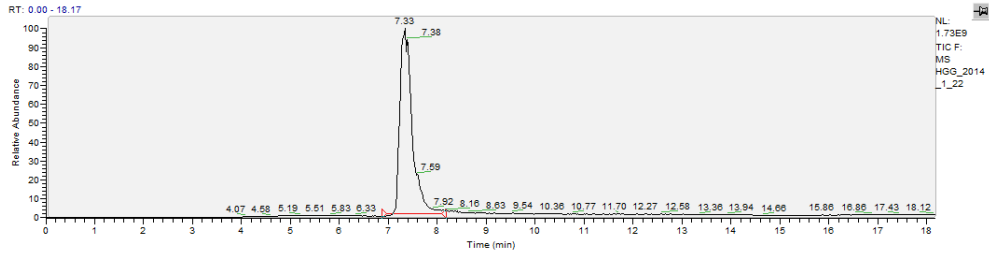




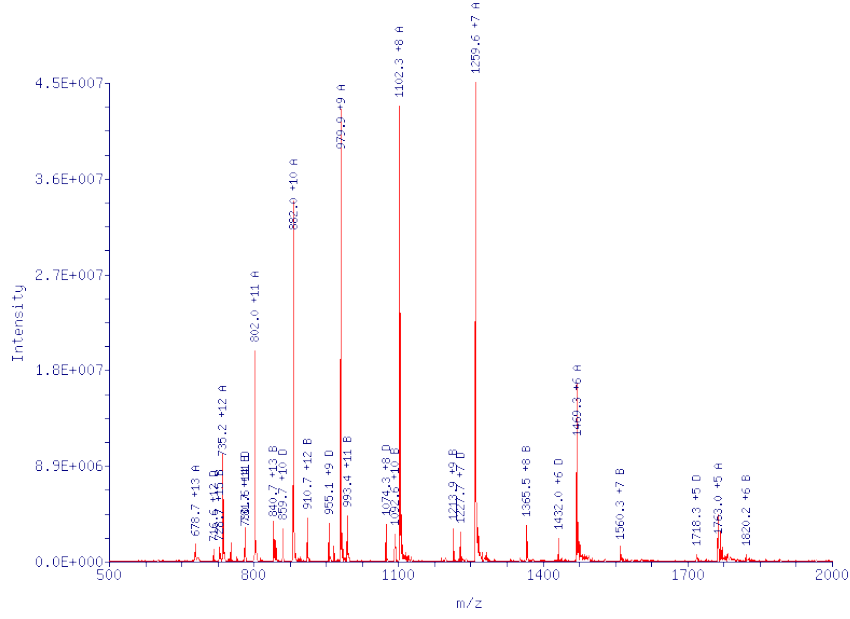
LC-MS Data

Ub-Hpg

LC-MS



Charge ladder



Deconvoluted mass

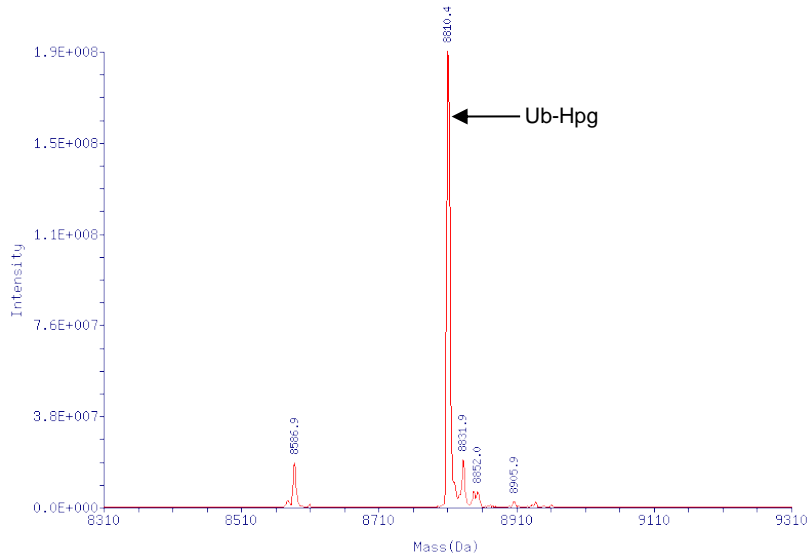


Table 1, entry 1 (10 s)

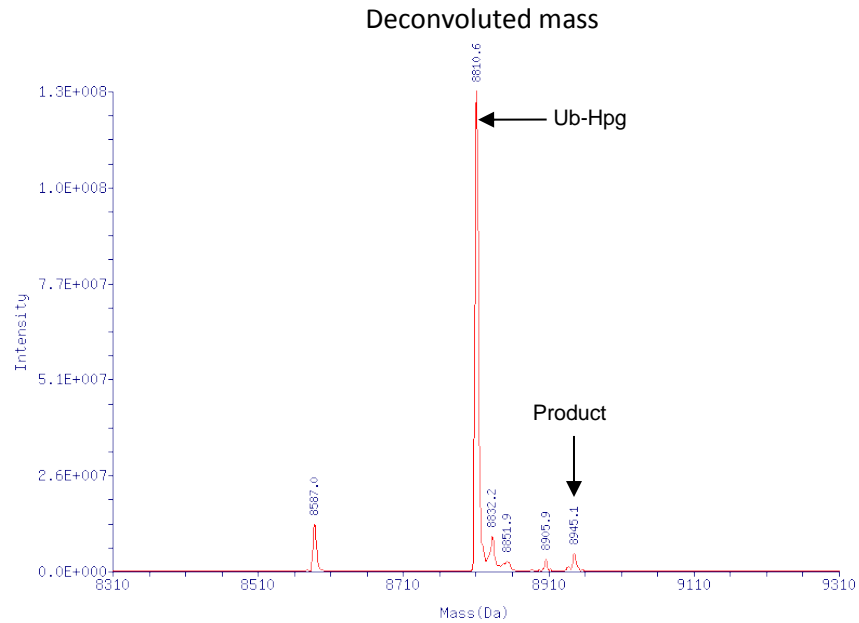
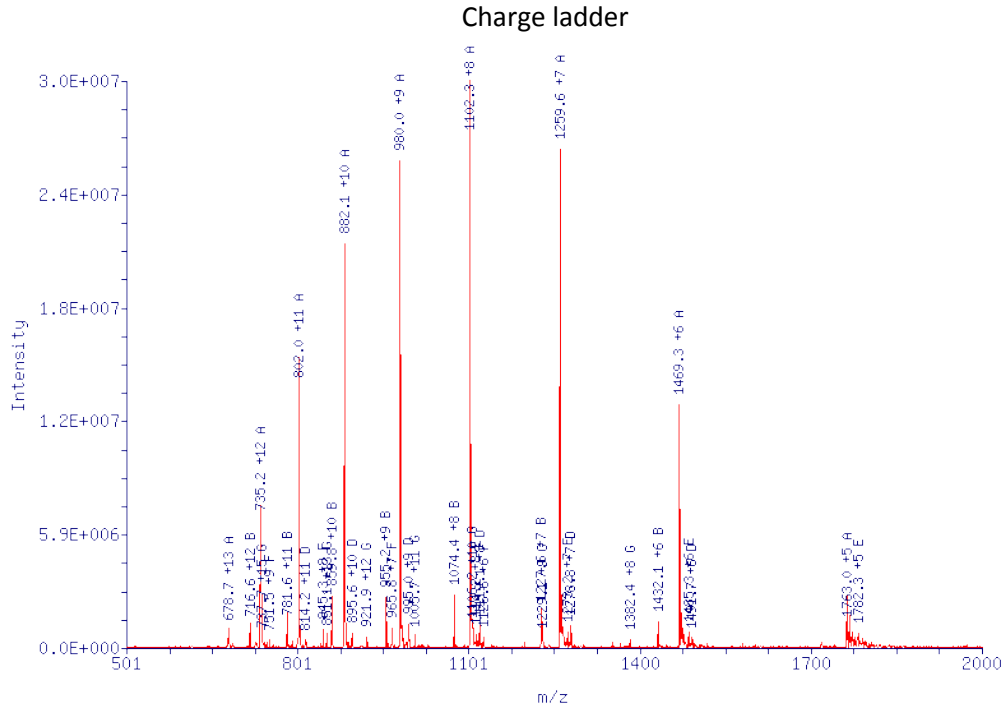
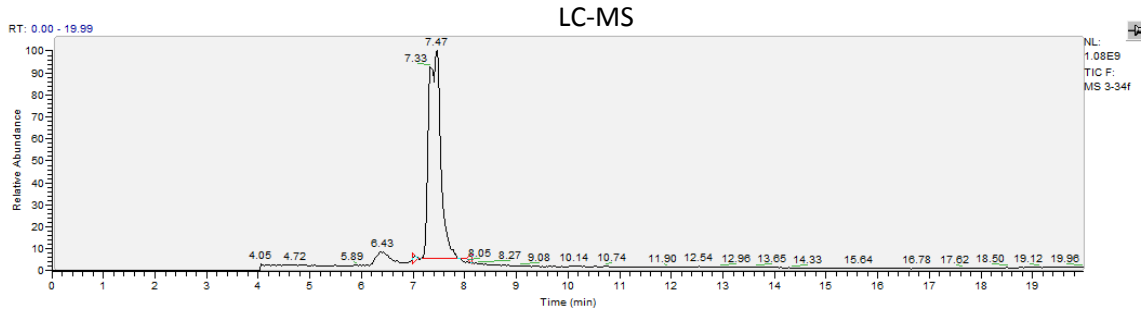


Table 1, entry 1 (3 min)

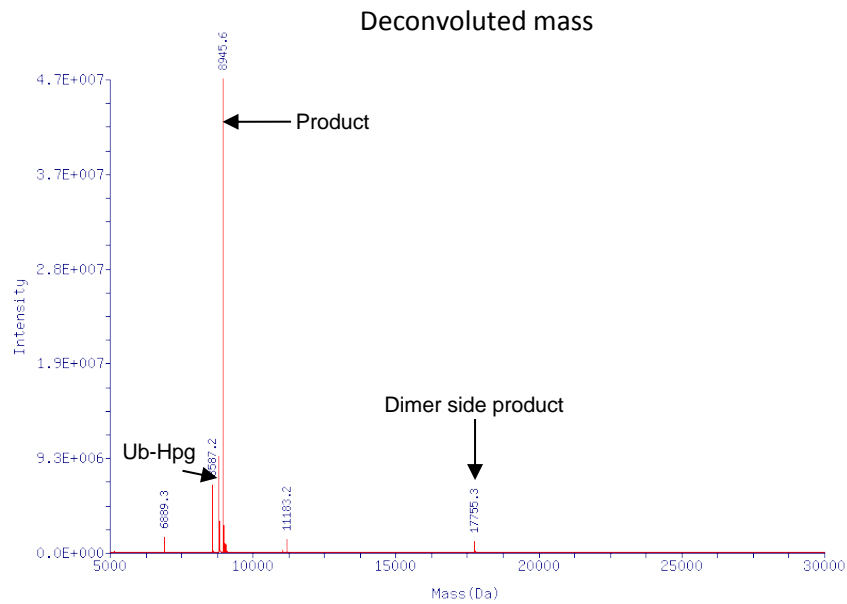
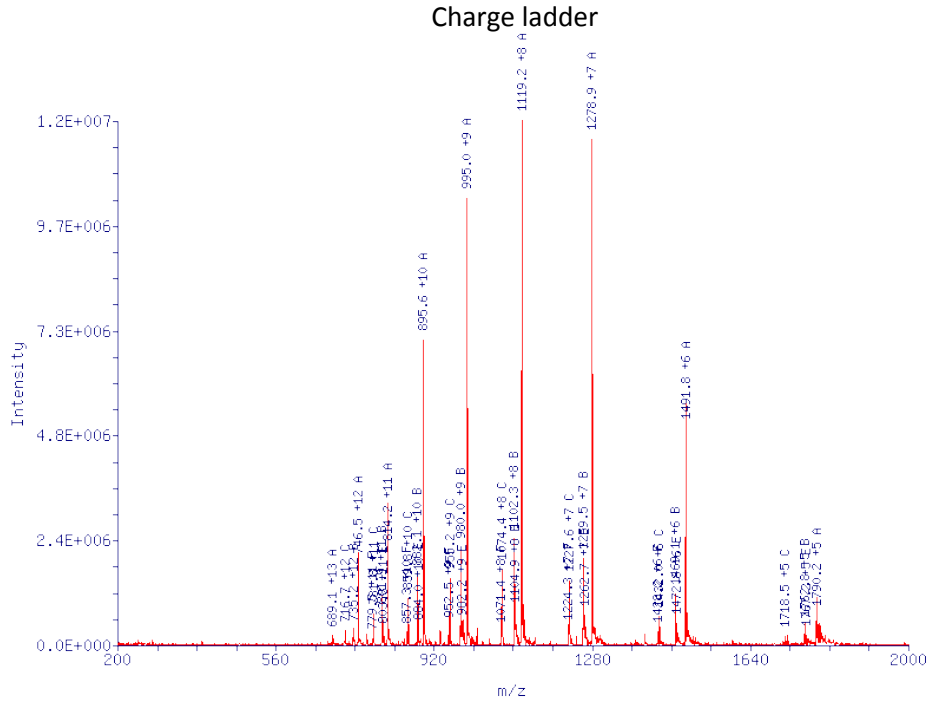
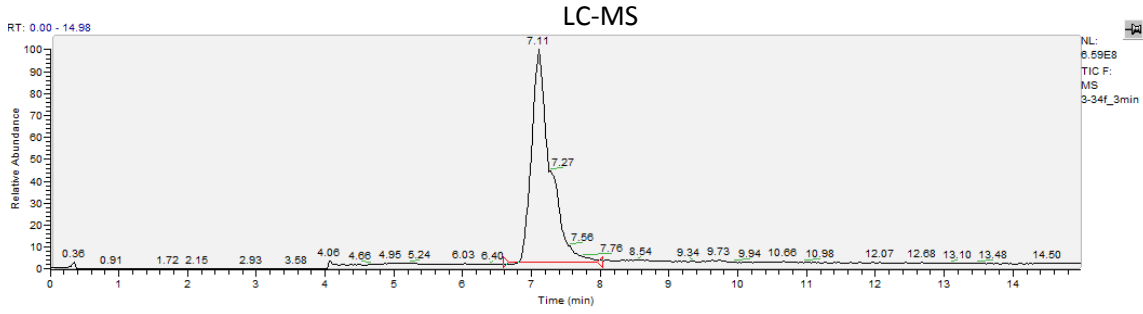


Table 1, entry 2 (10 s)

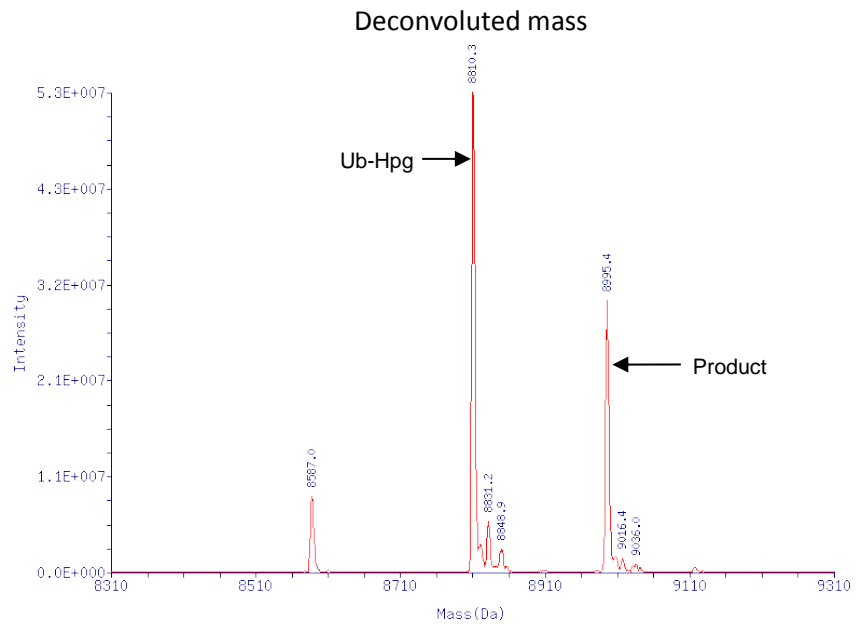
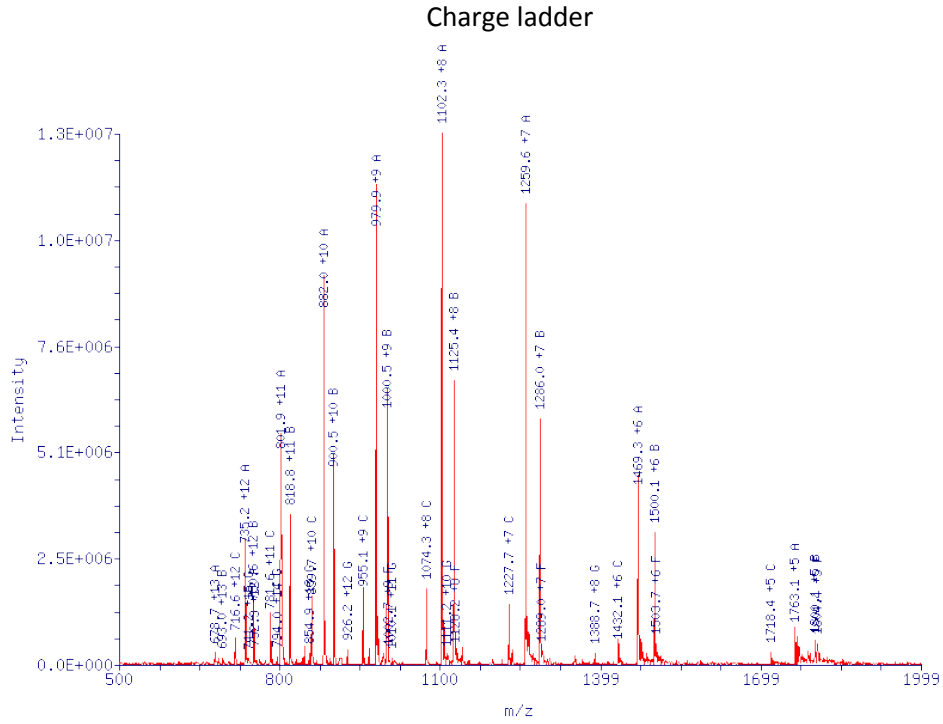
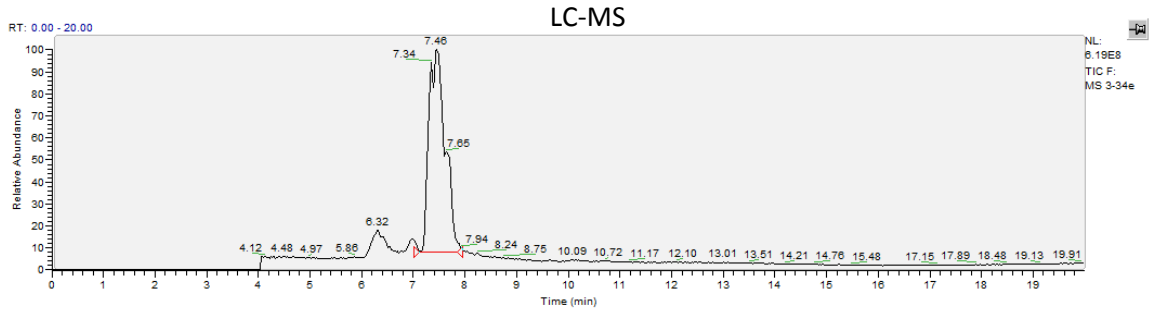
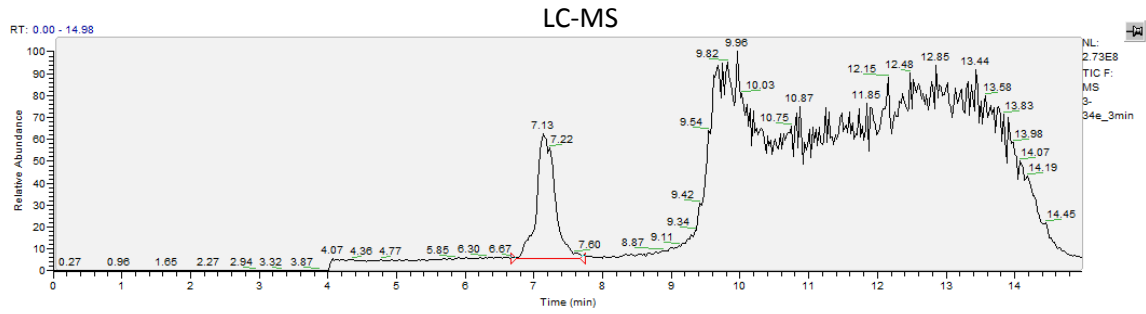
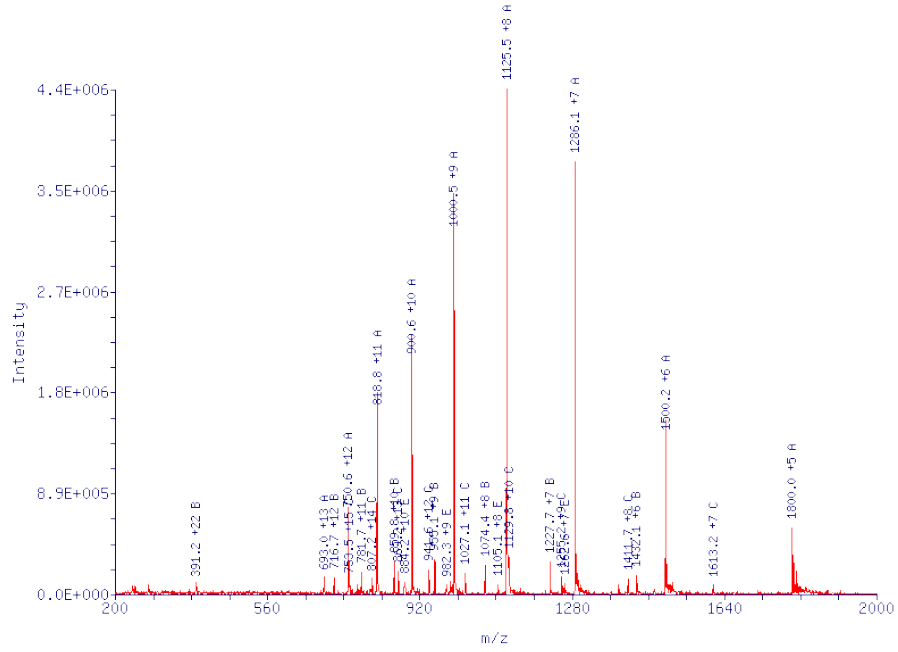


Table 1, entry 2 (3 min)



Charge ladder



Deconvoluted mass

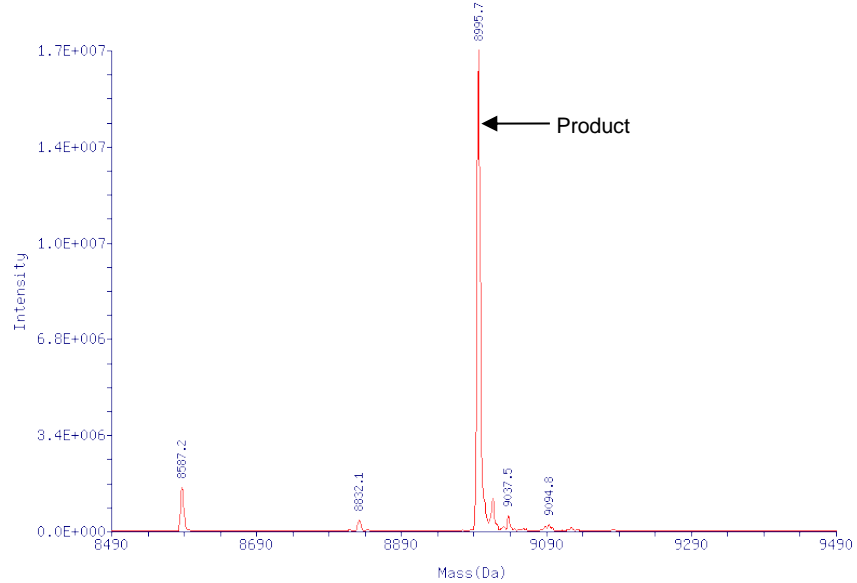


Table 1, entry 3 (10 s)

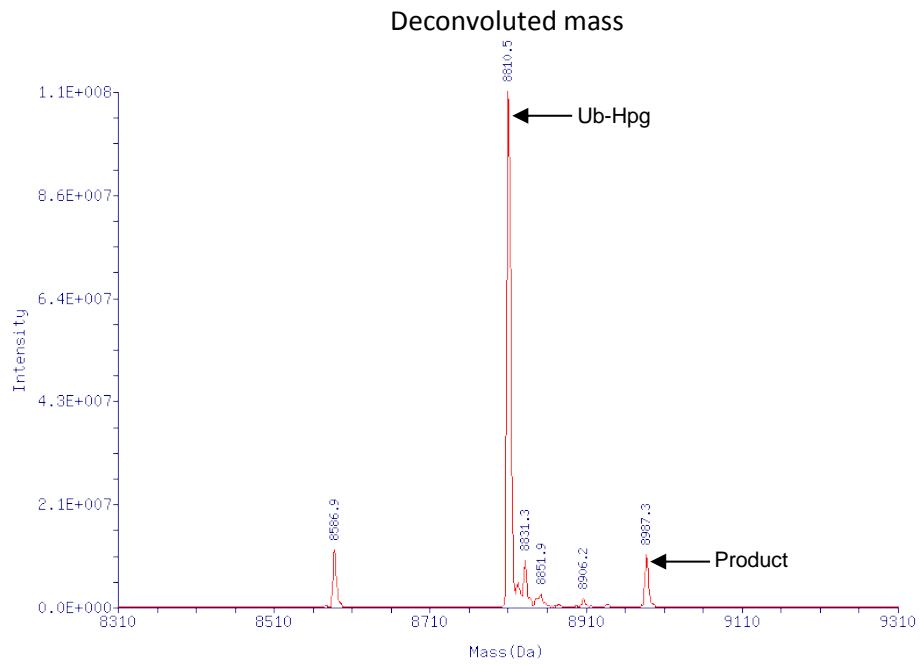
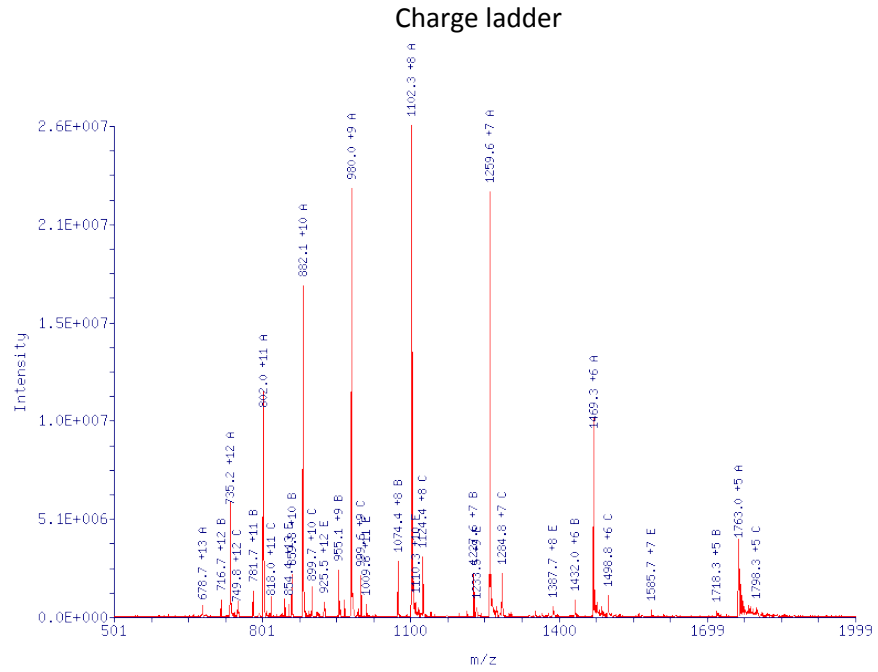
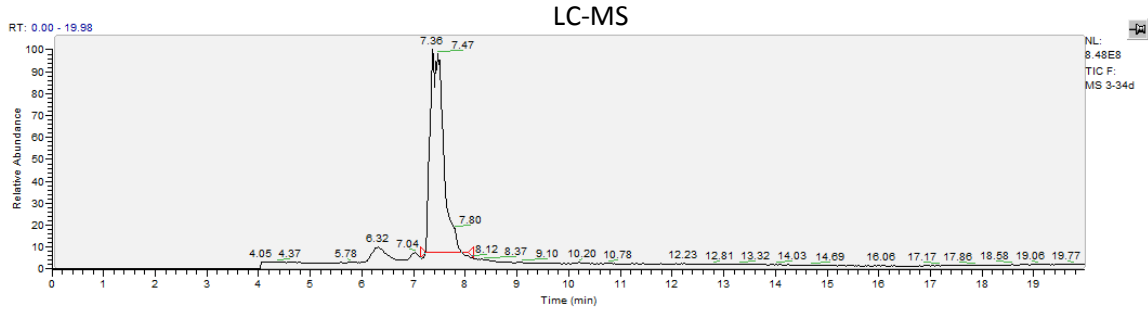


Table 1, entry 3 (3 min)

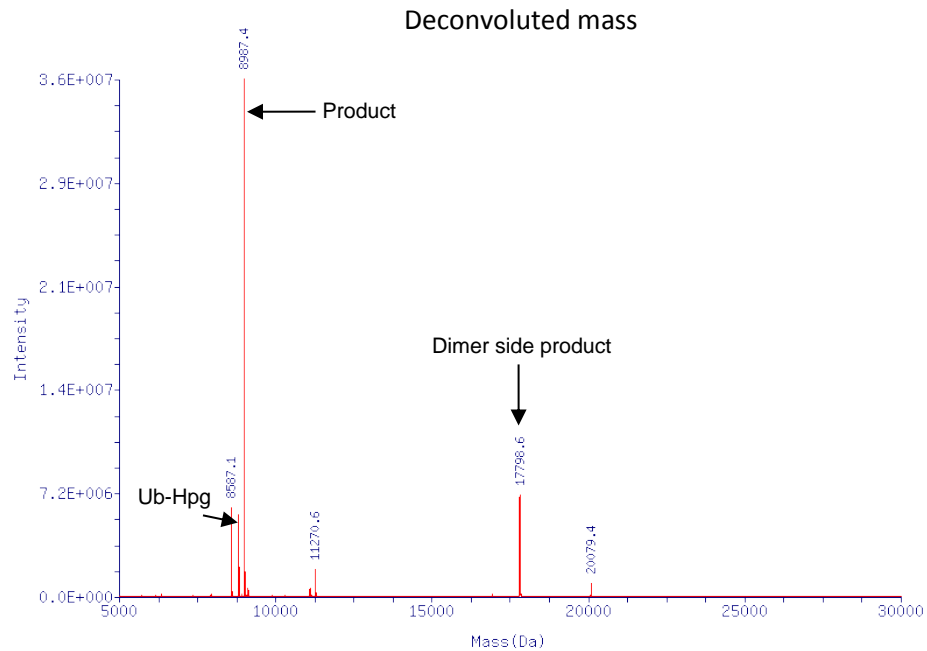
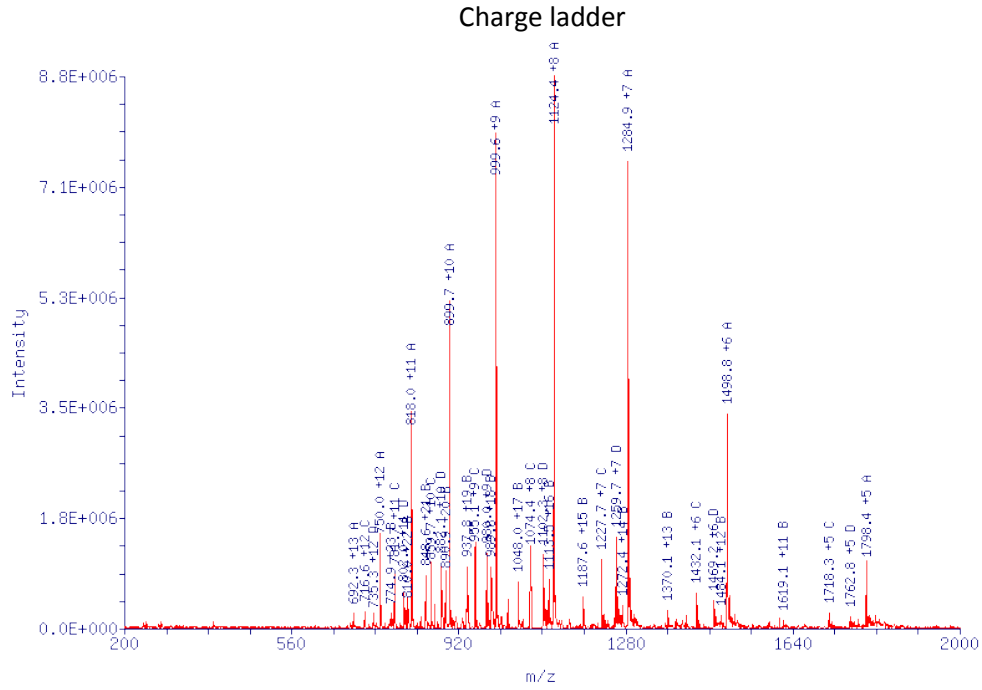
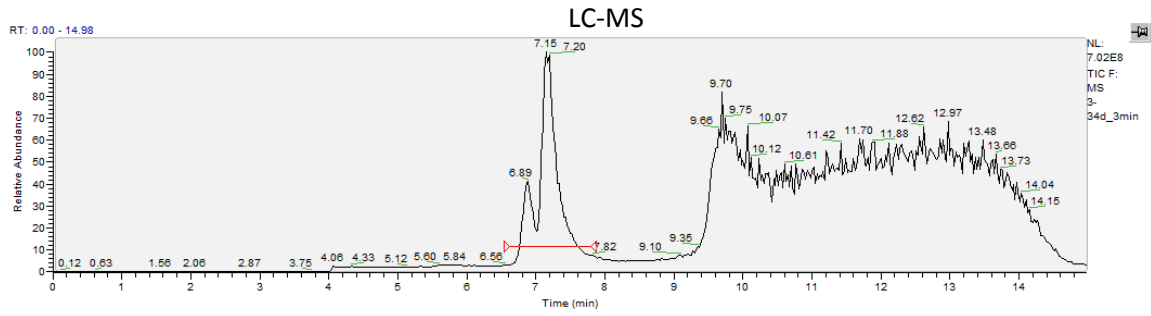
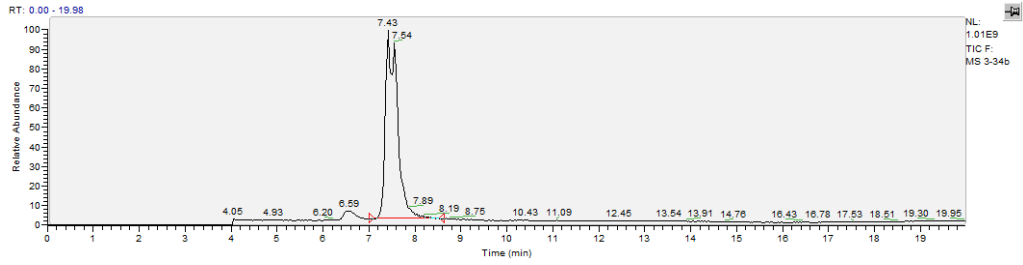
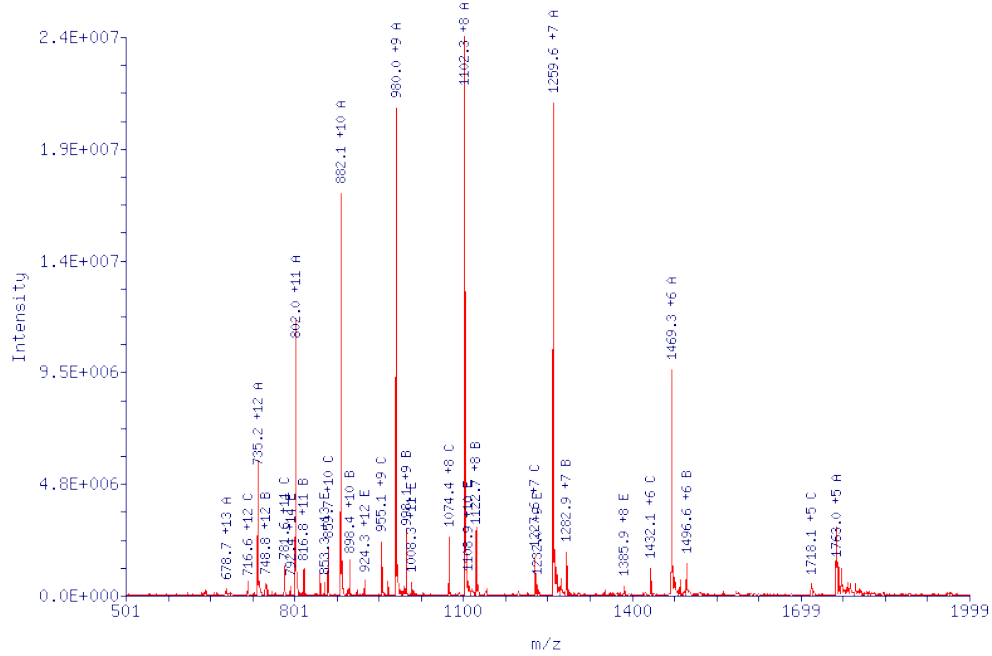


Table 1, entry 4 (10 s)

LC-MS



Charge ladder



Deconvoluted mass

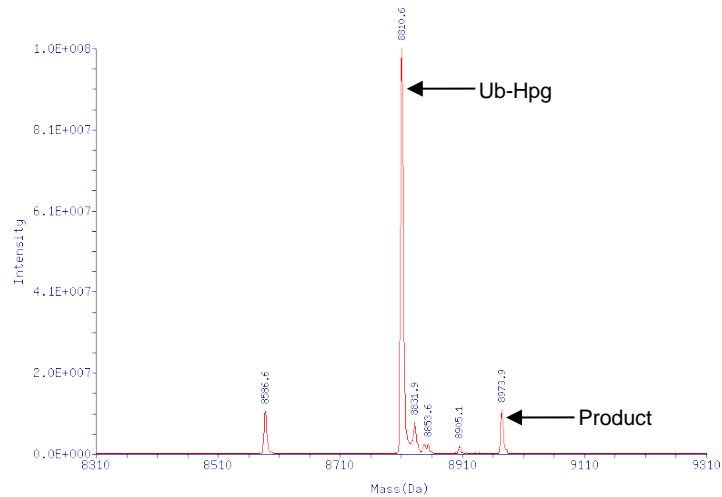


Table 1, entry 4 (3 min)

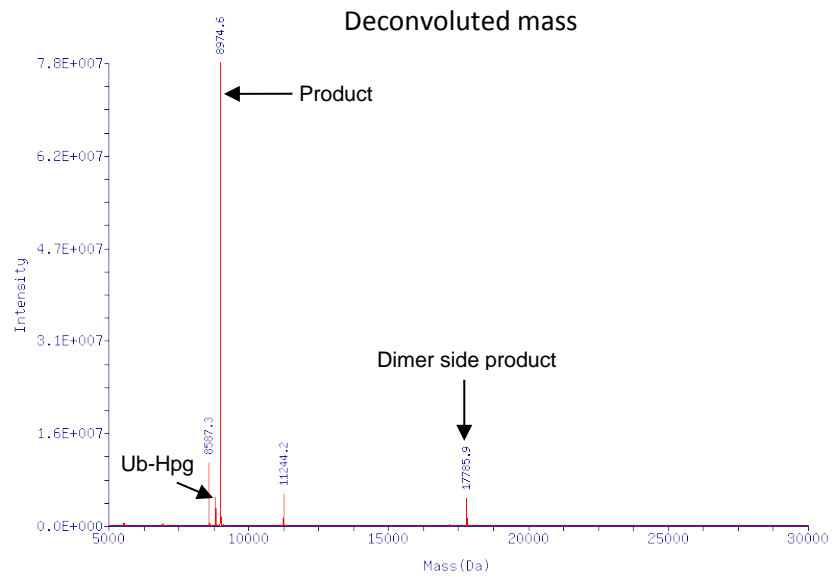
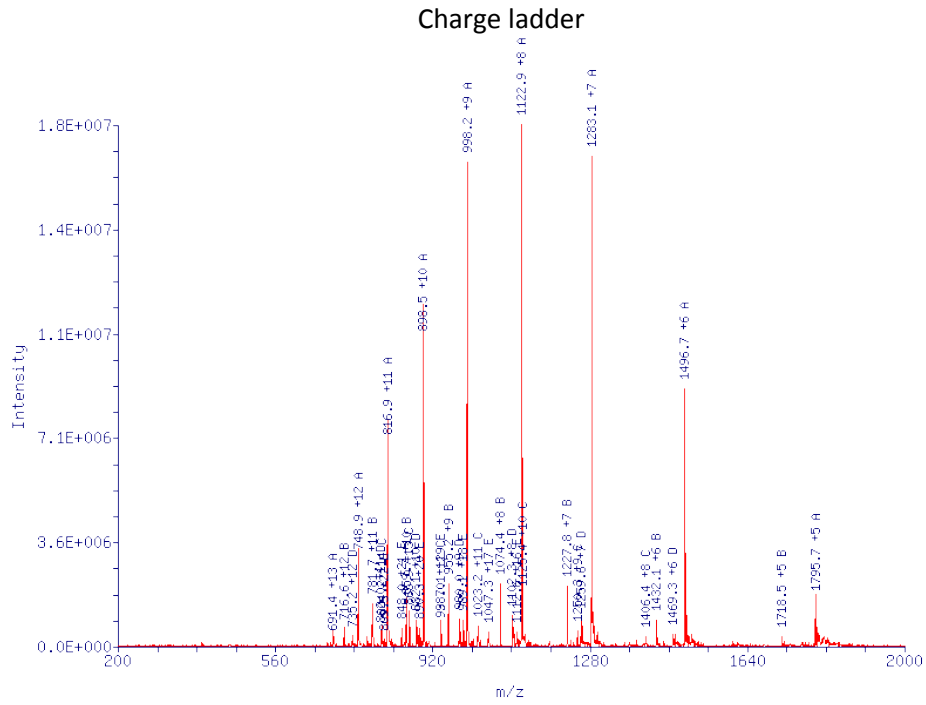
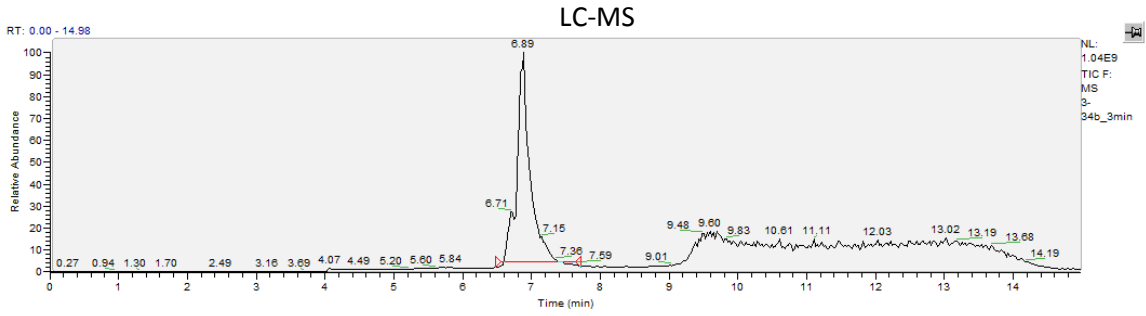


Table 1, entry 5 (10 s)

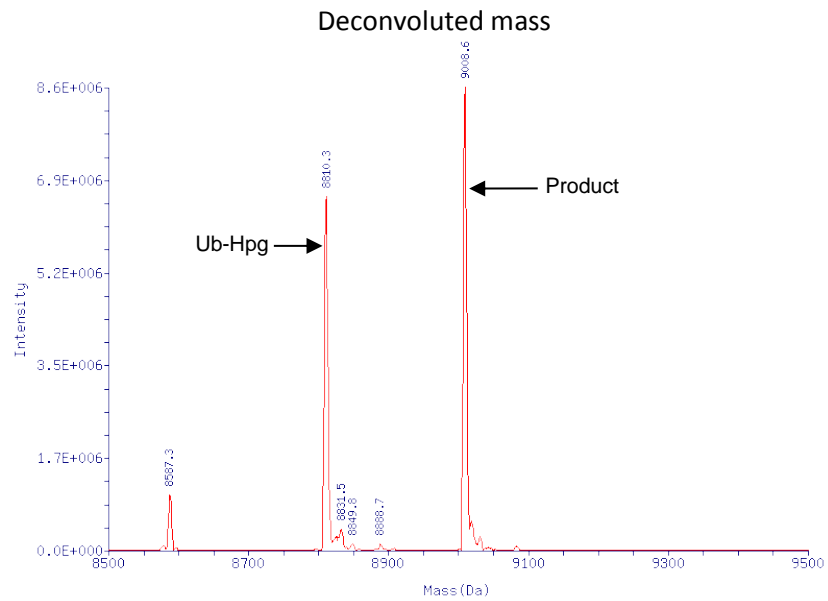
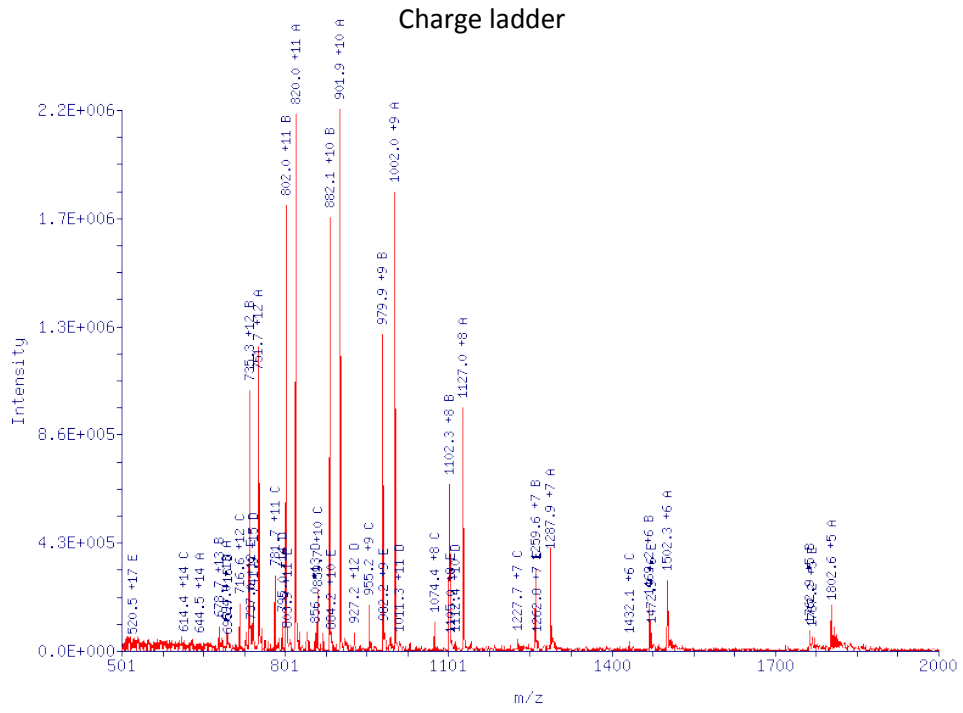
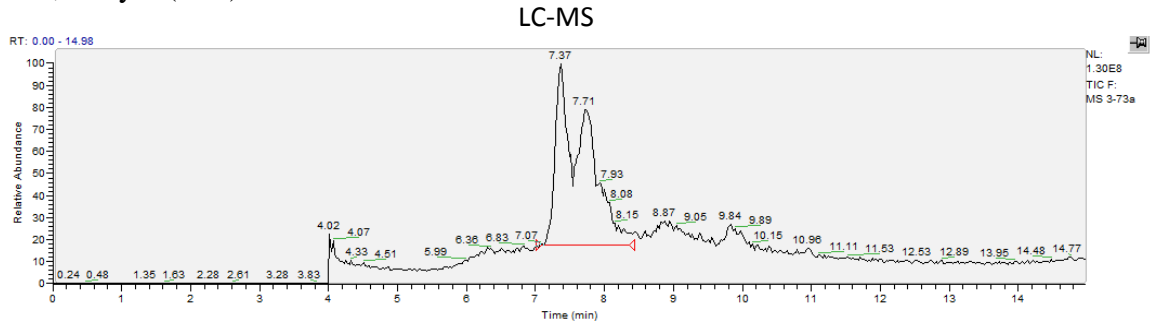


Table 1, entry 5 (3 min)

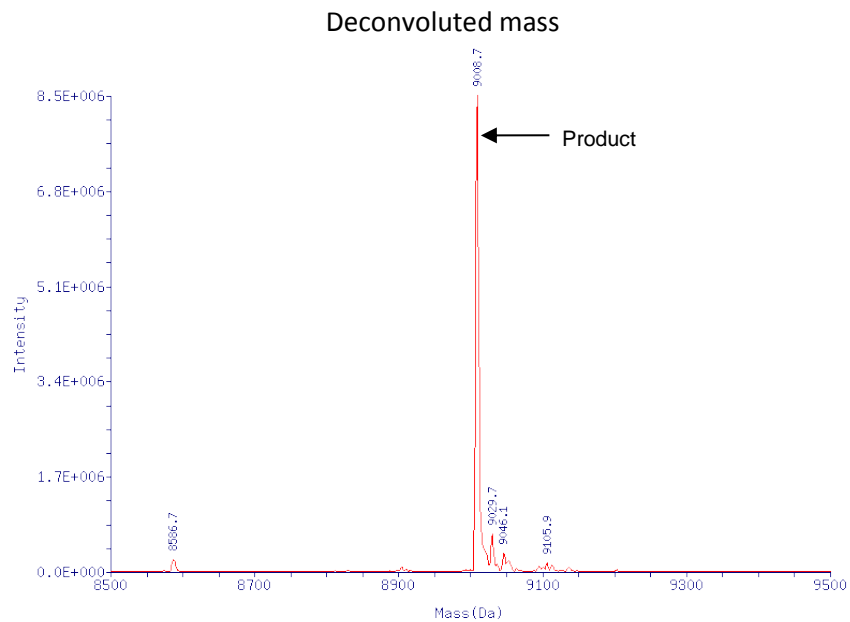
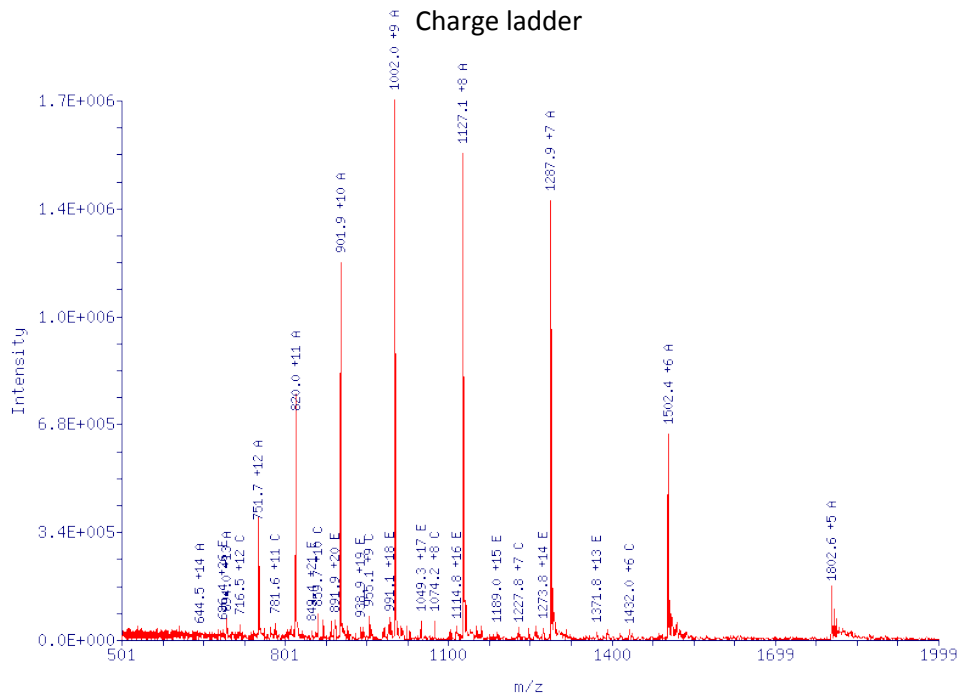
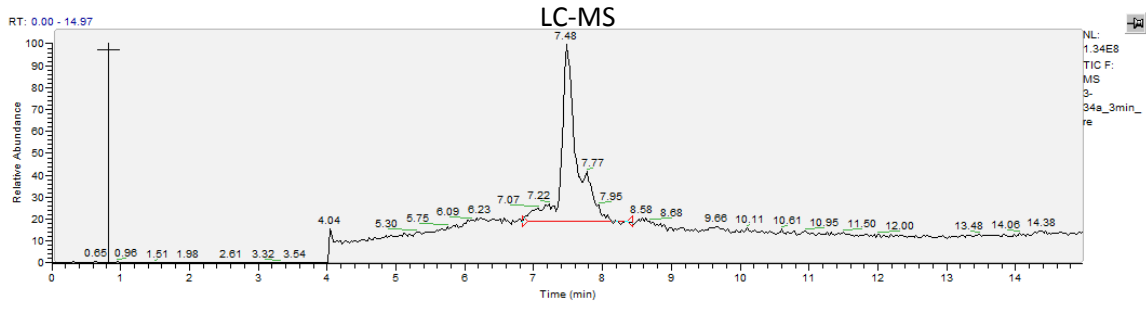


Table 1, entry 6 (10 s)

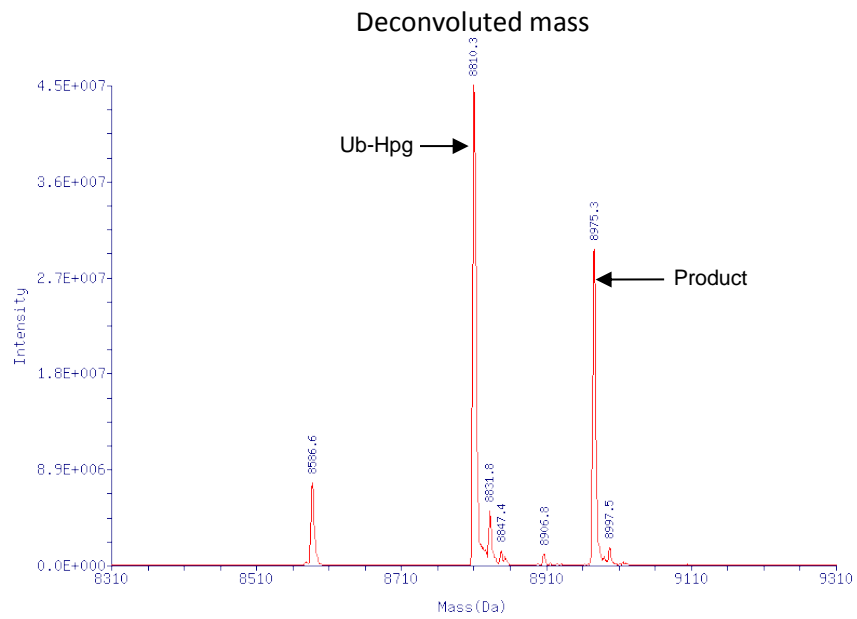
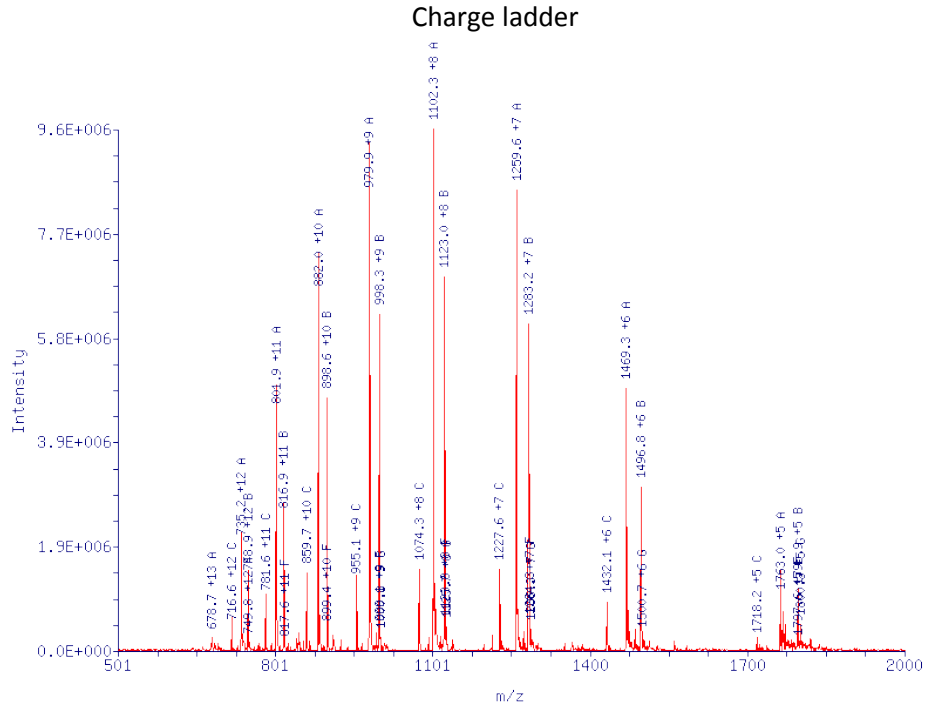
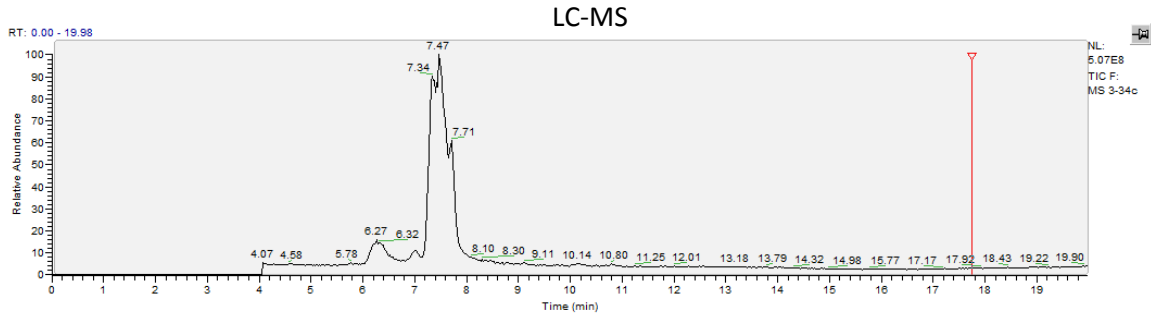


Table 1, entry 6 (3 min)

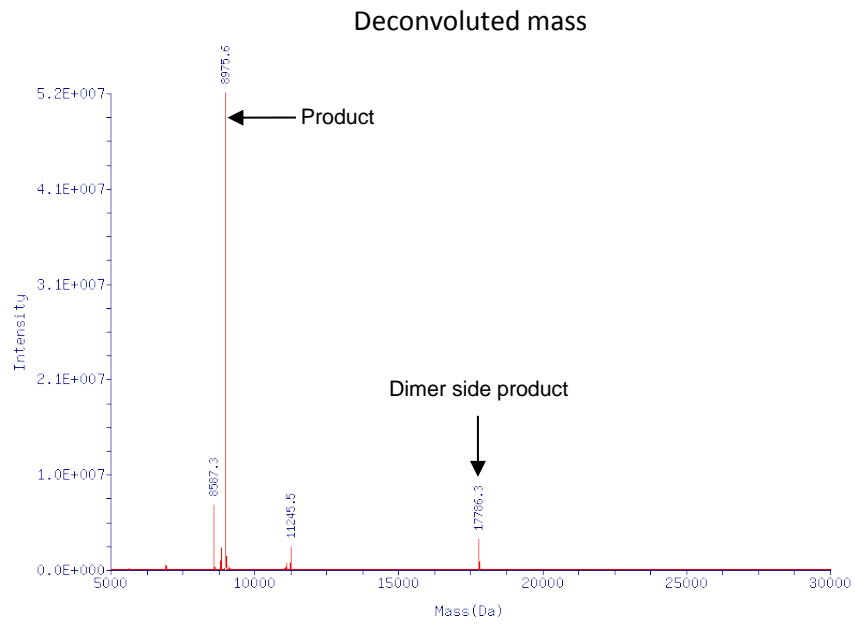
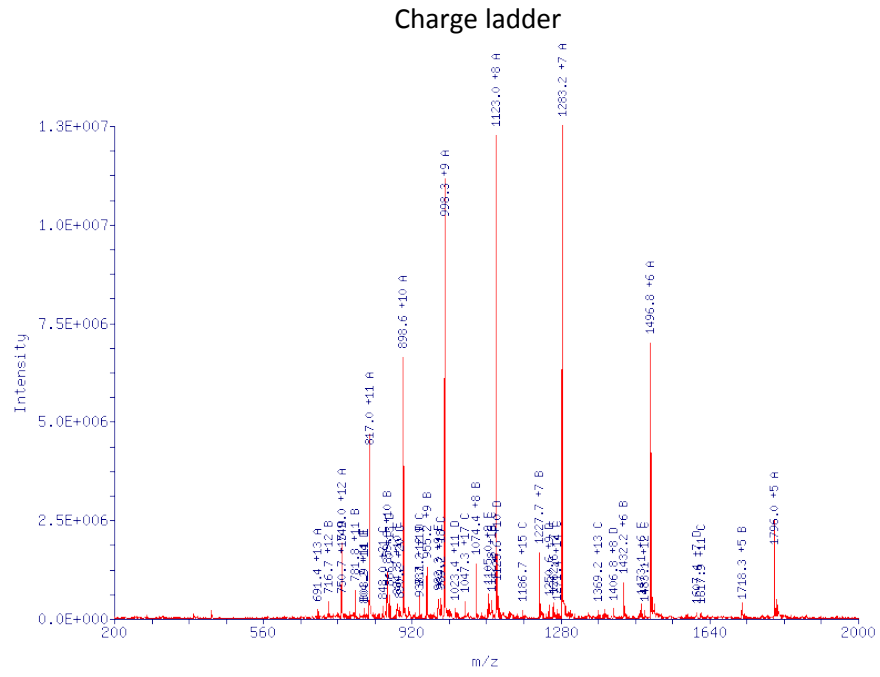
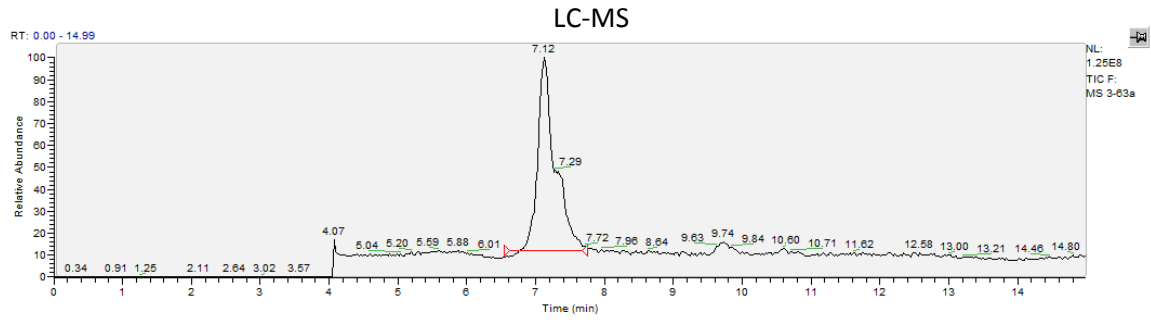


Table 1, entry 7 (10 s)

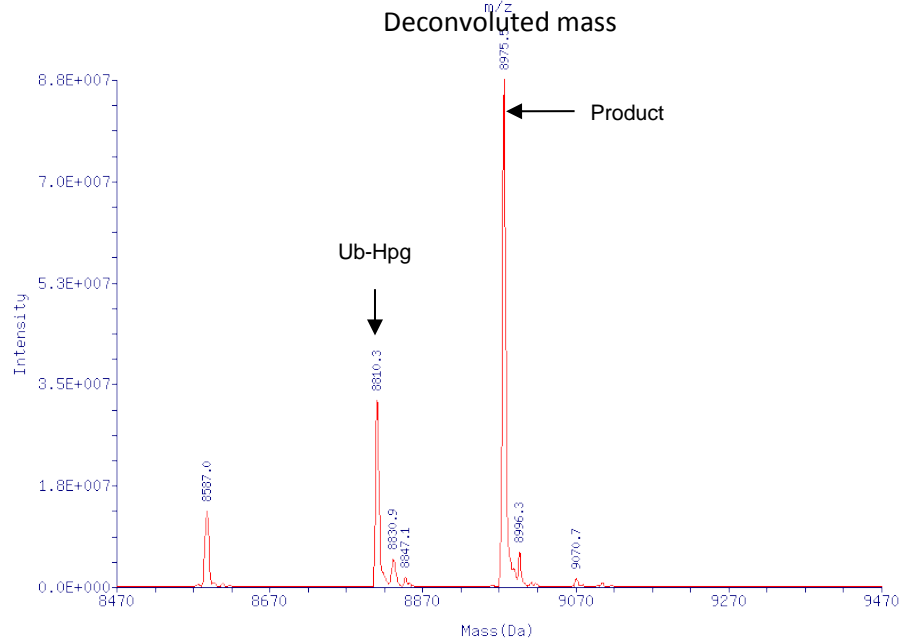
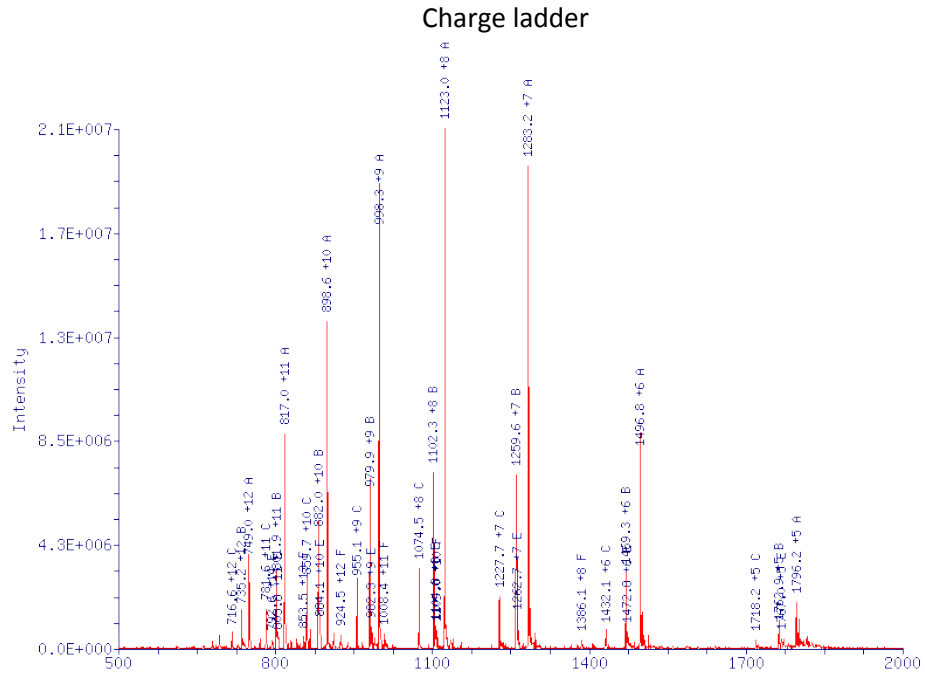
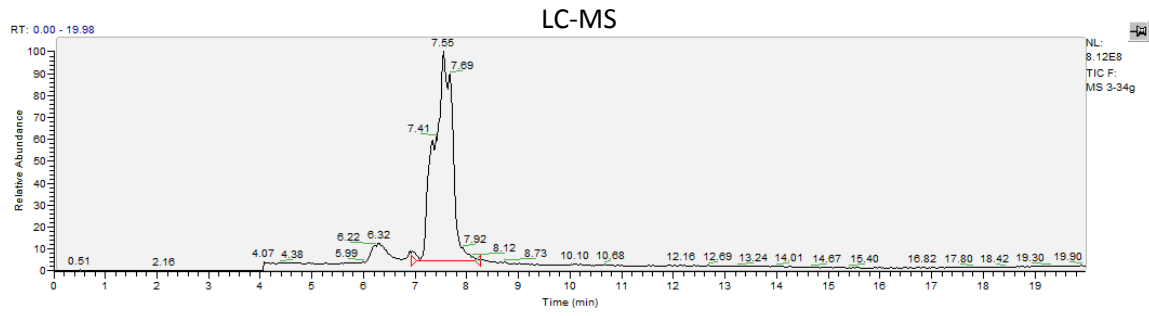


Table 1, entry 7 (3 min)

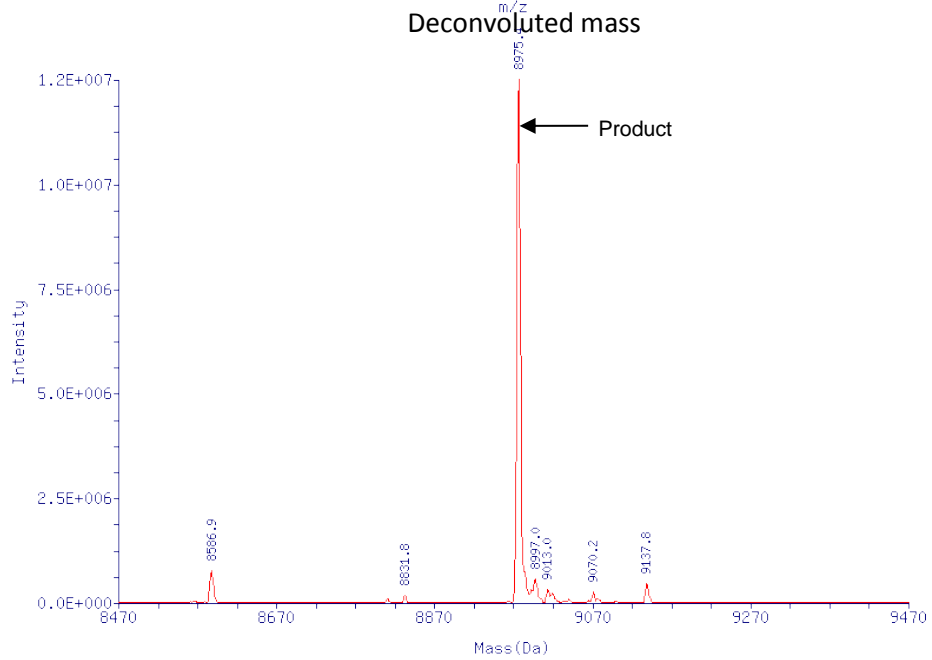
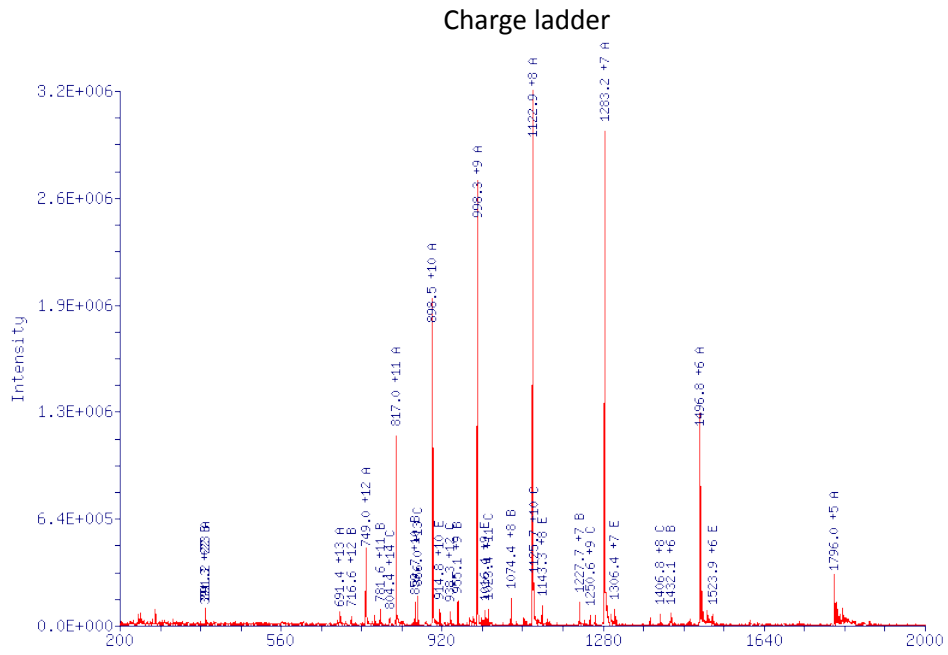
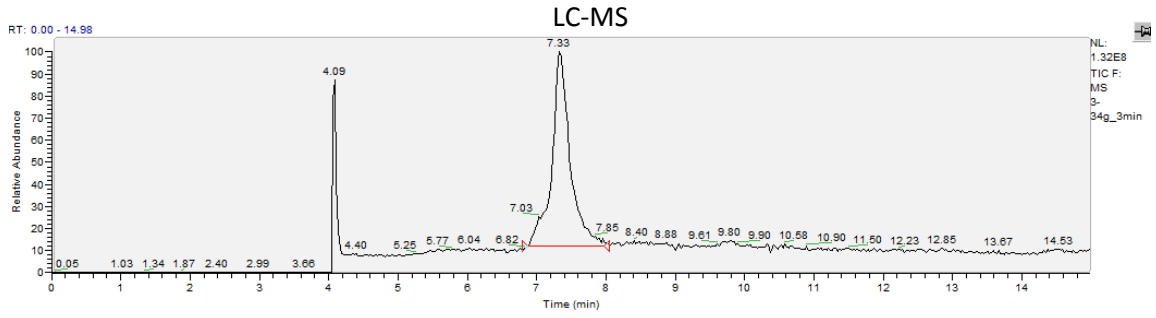


Table 1, entry 8 (10 s)

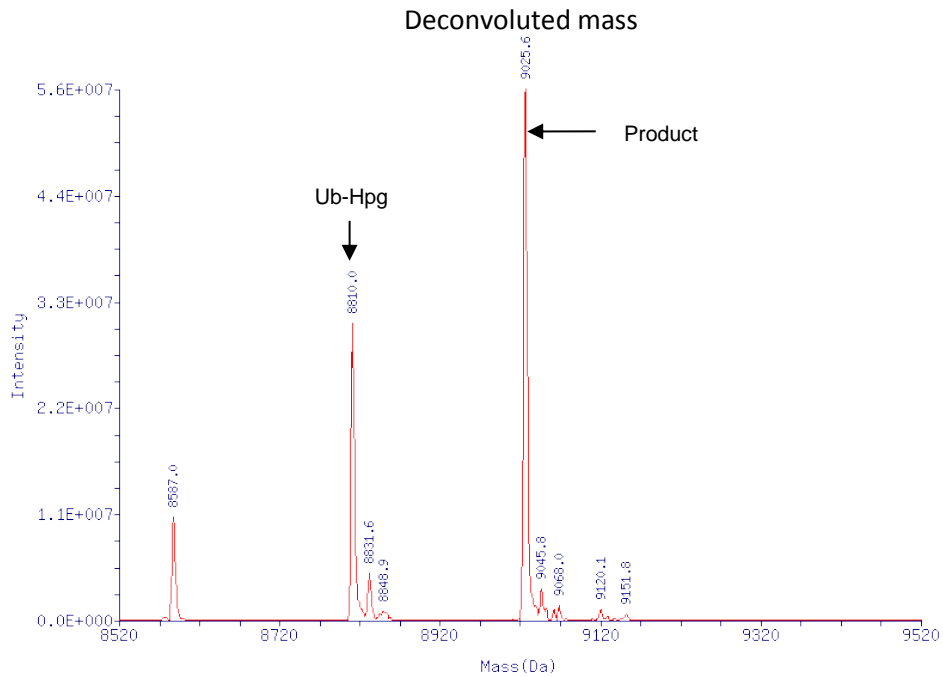
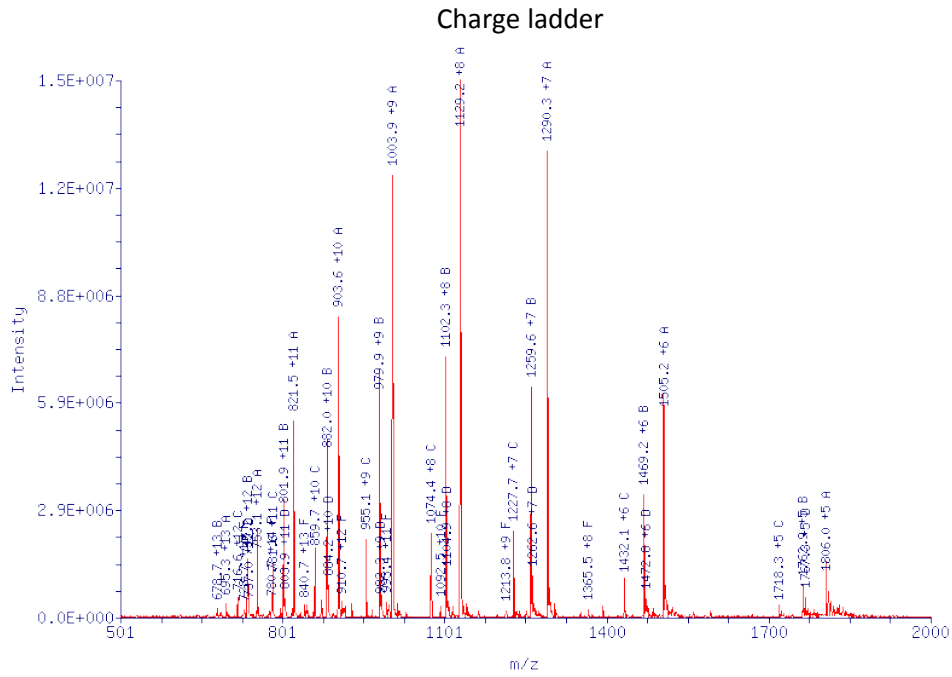
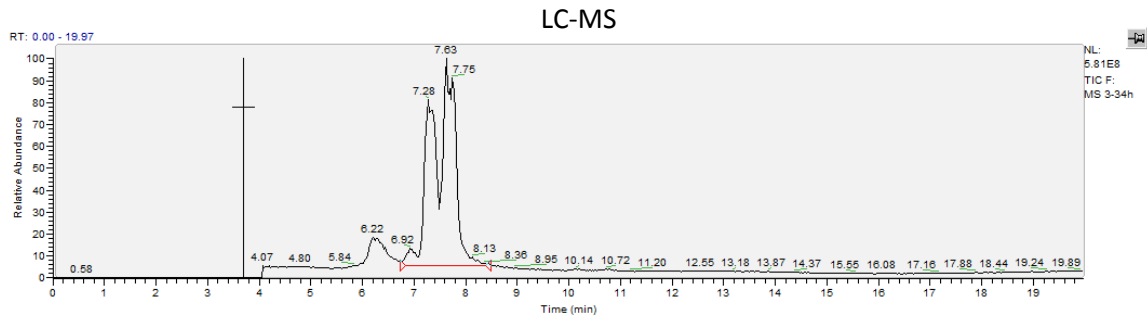


Table 1, entry 8 (3 min)

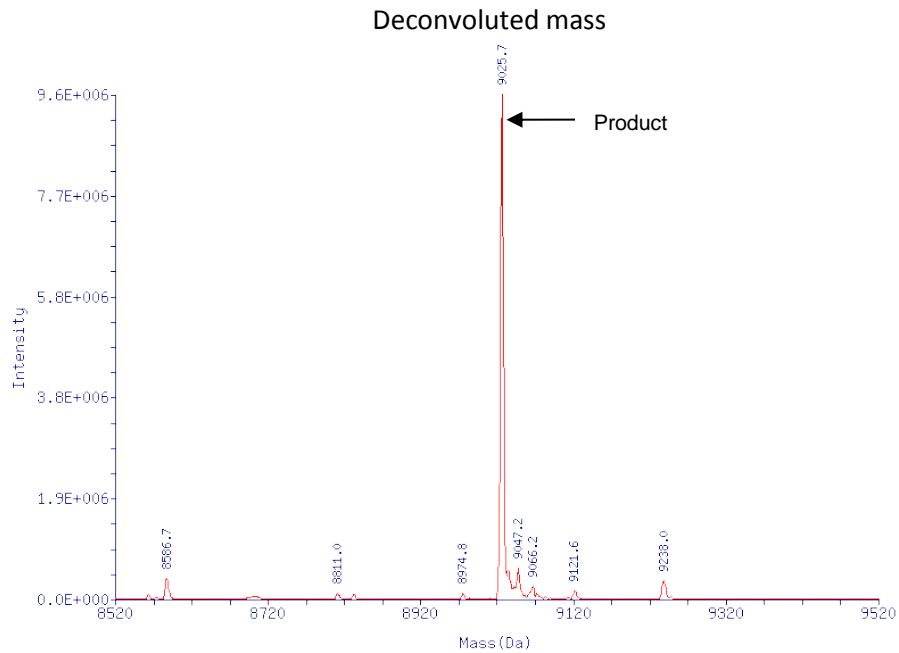
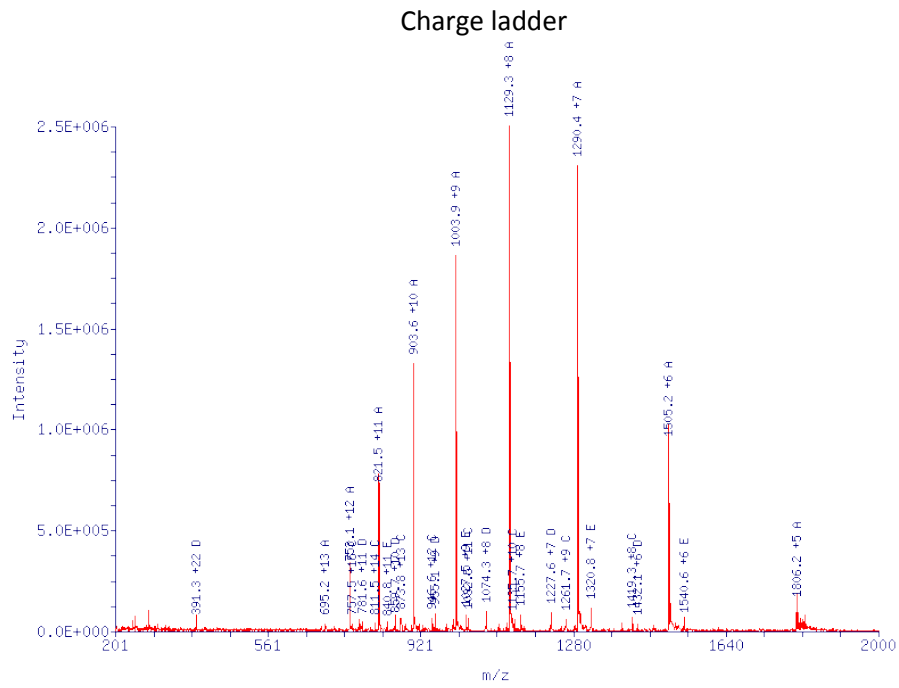
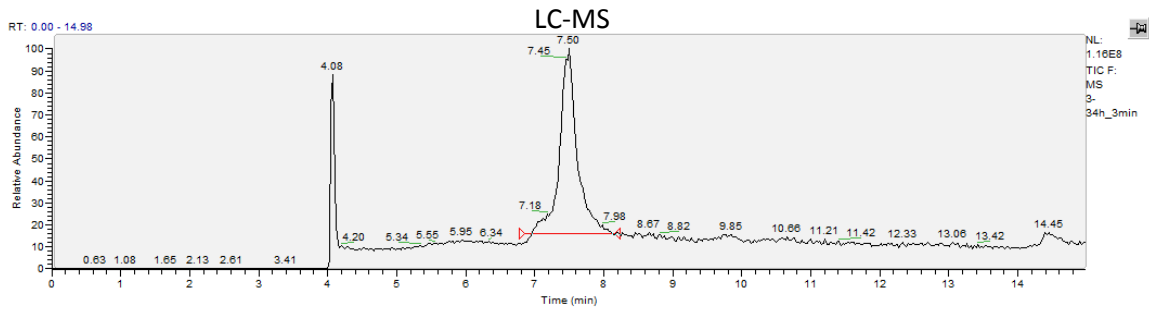


Table 2, entry 1 (10 s)

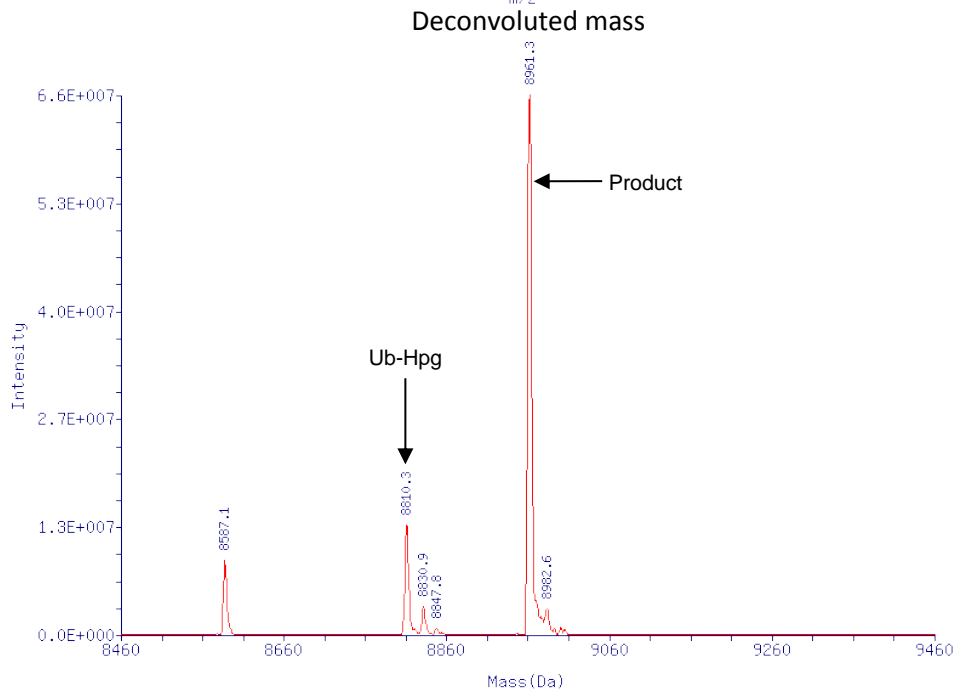
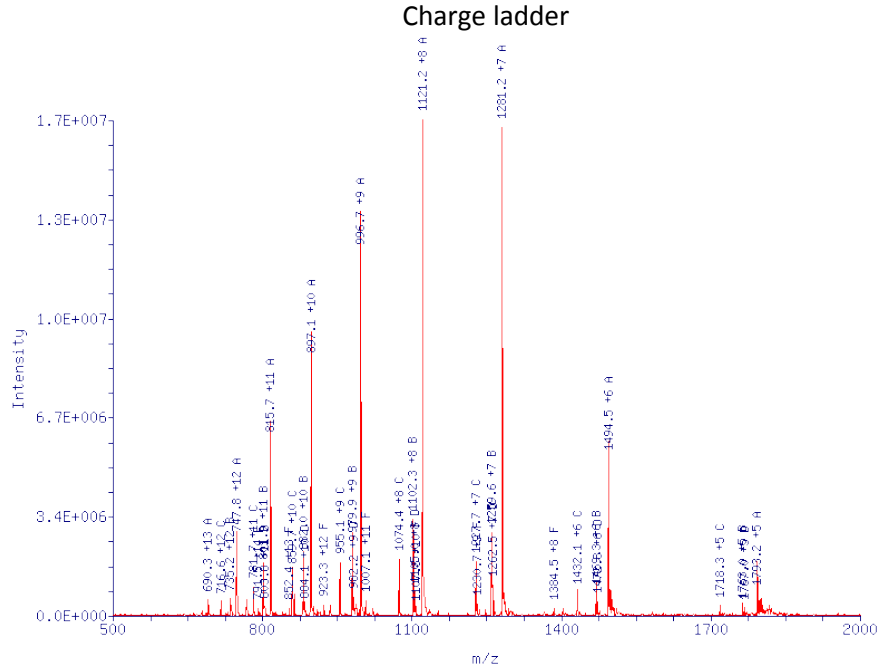
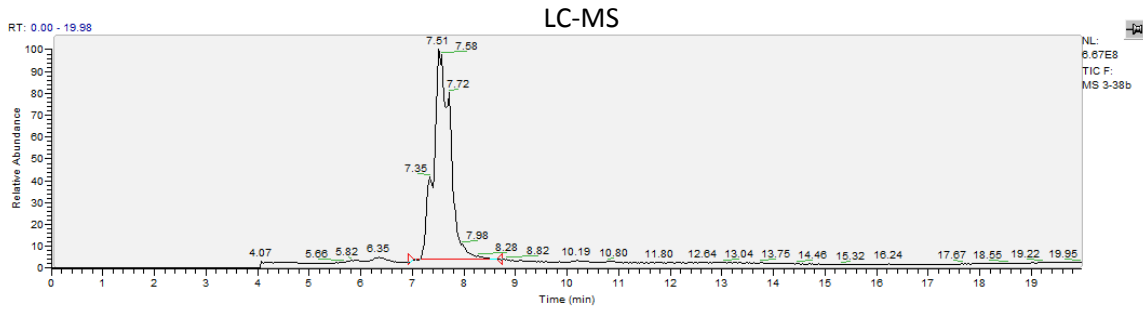


Table 2, entry 1 (3 min)

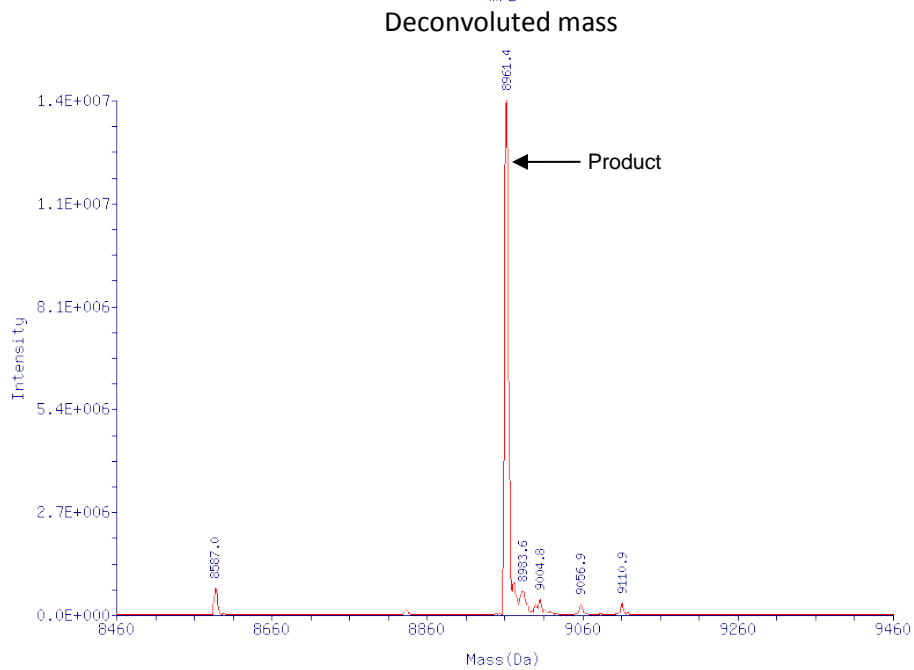
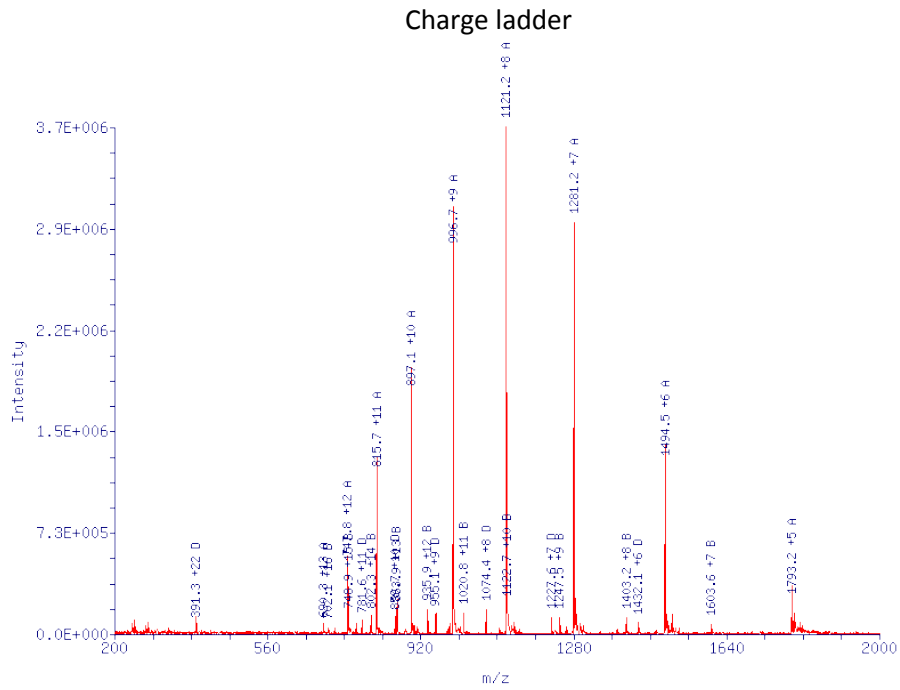
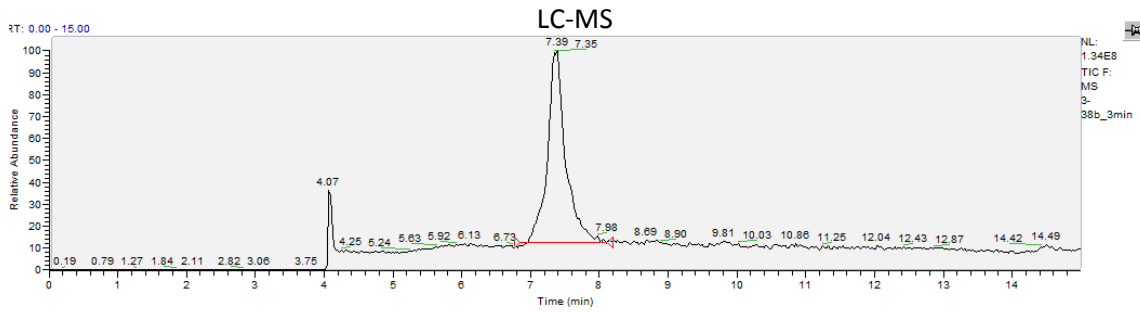


Table 2, entry 2 (10 s)

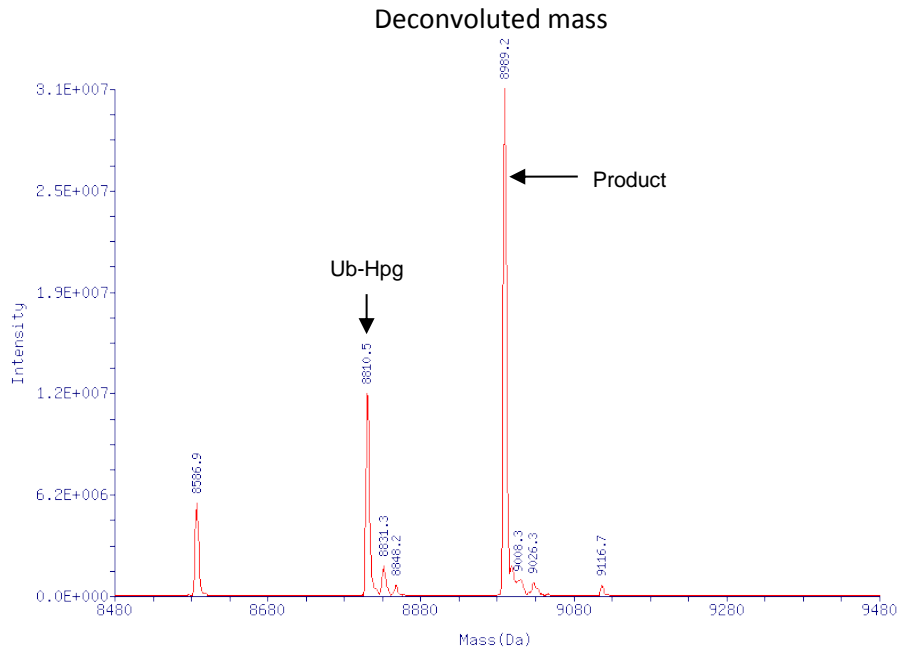
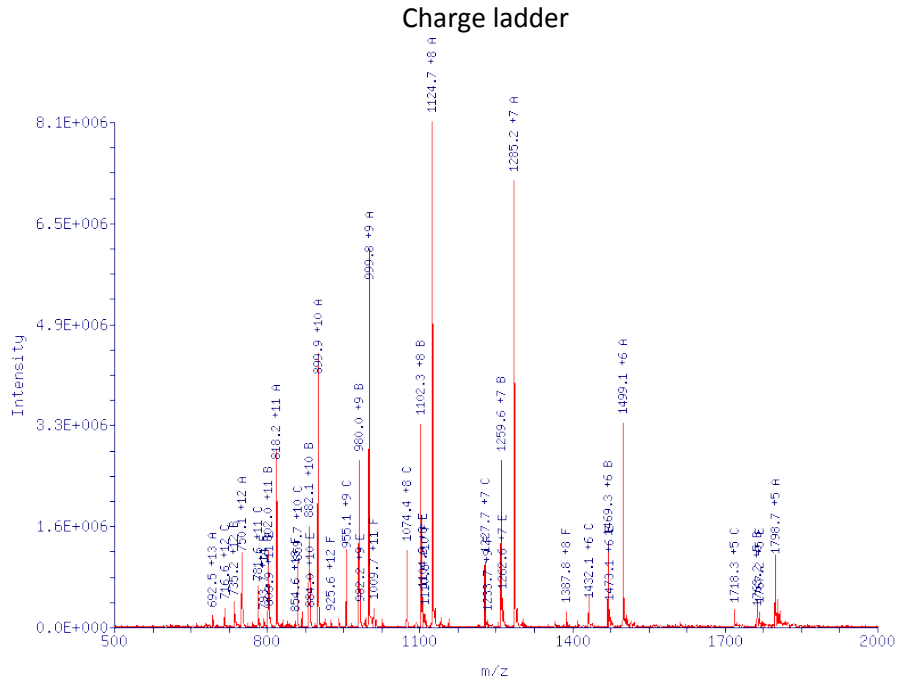
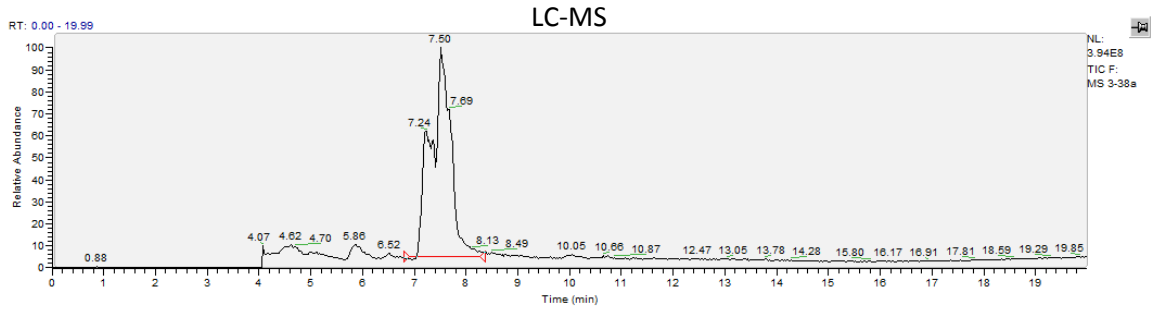


Table 2, entry 2 (3 min)

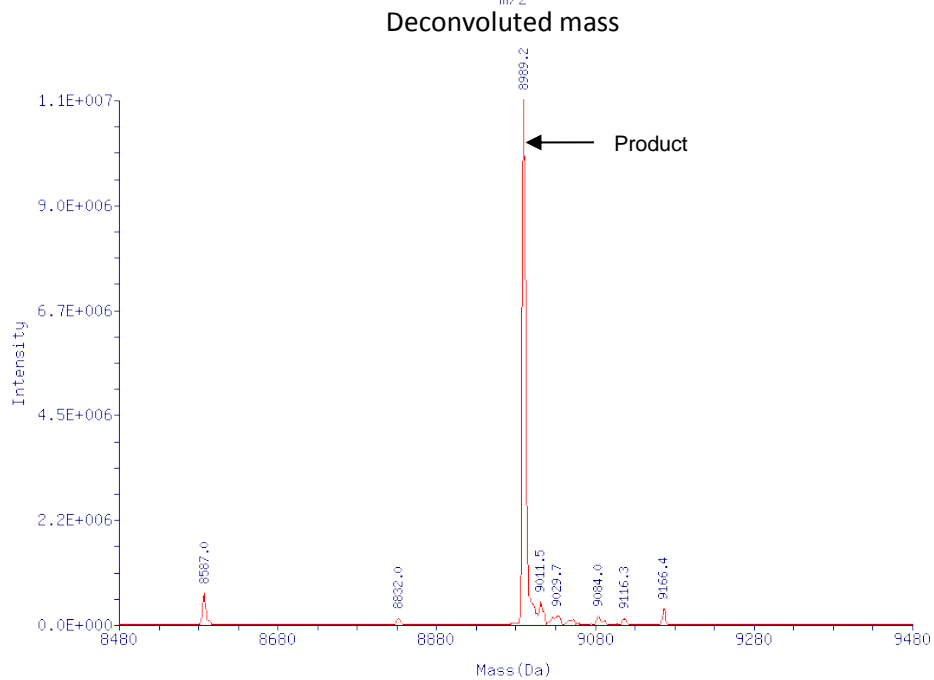
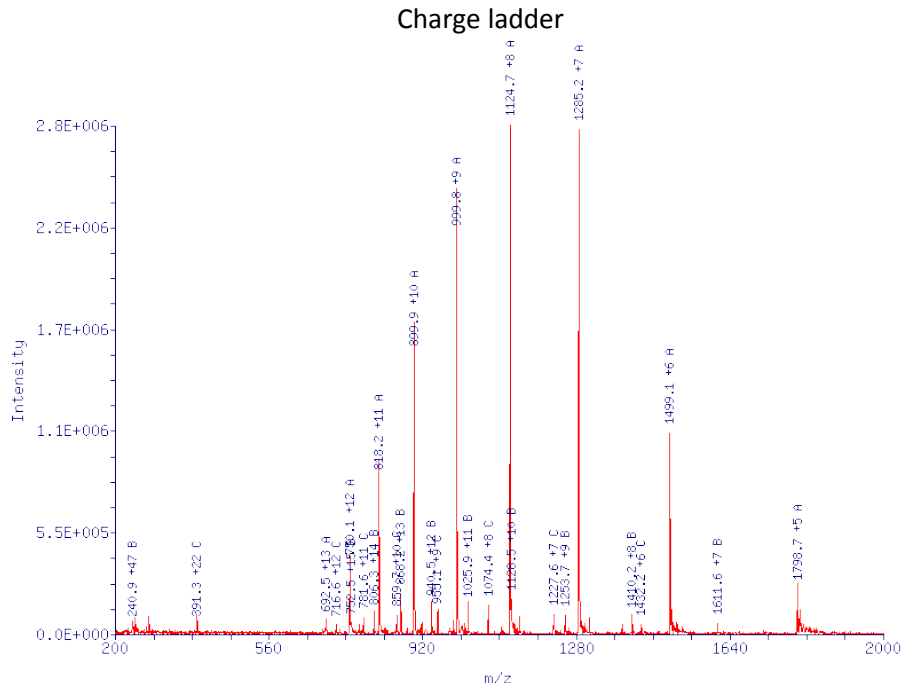
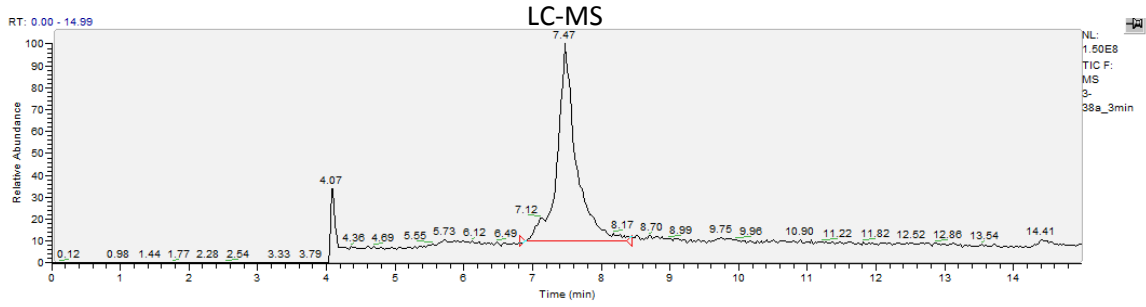
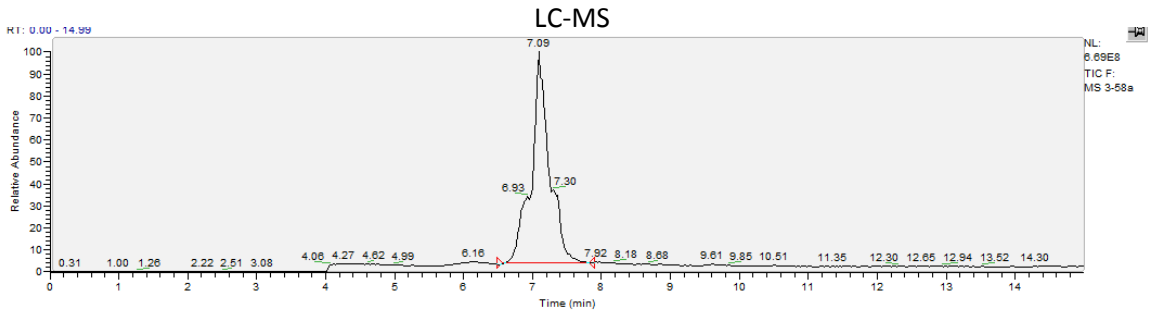
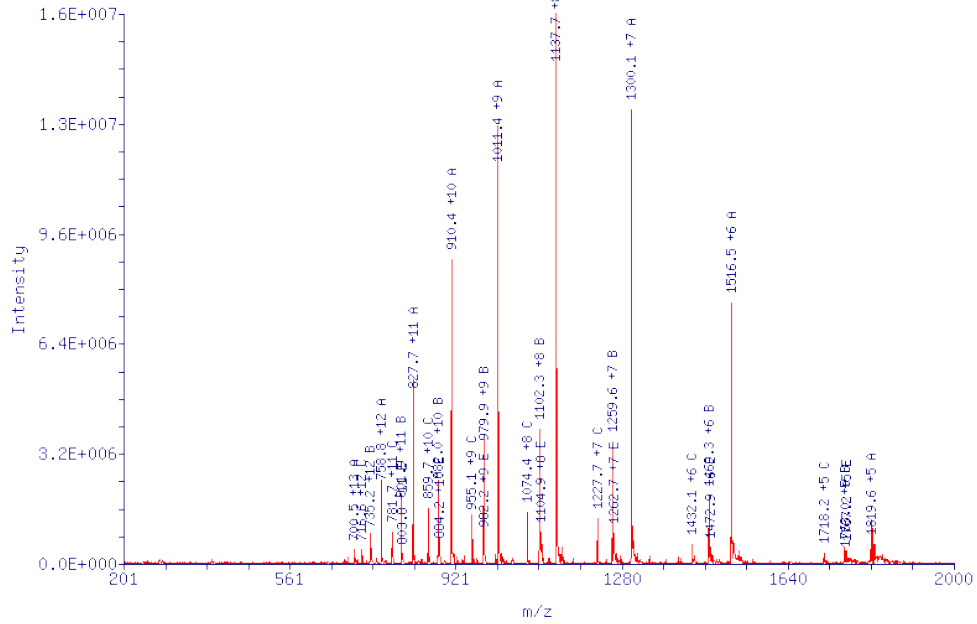


Table 2, entry 3 (10 s)



Charge ladder



Deconvoluted mass

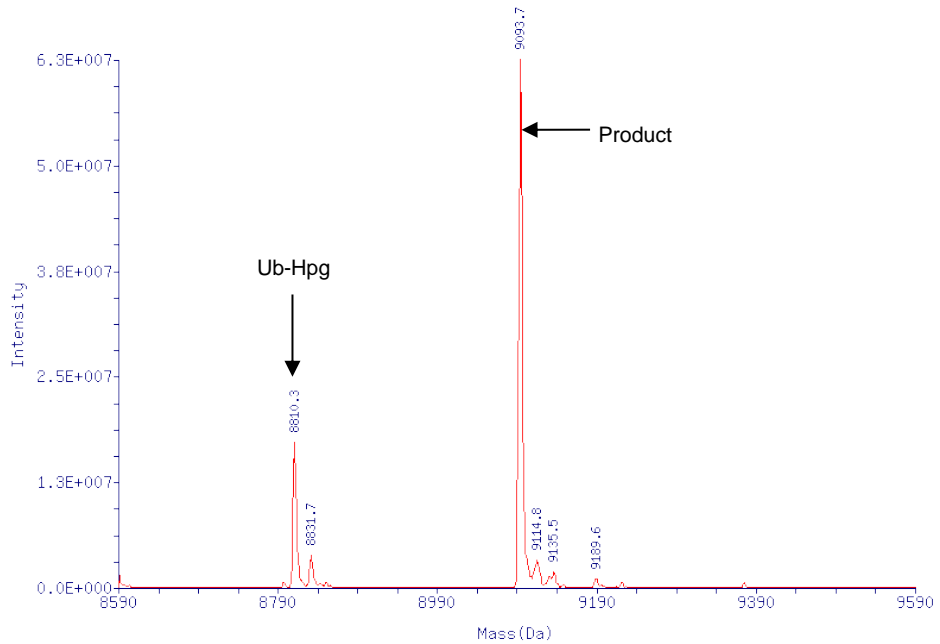


Table 2, entry 3 (3 min)

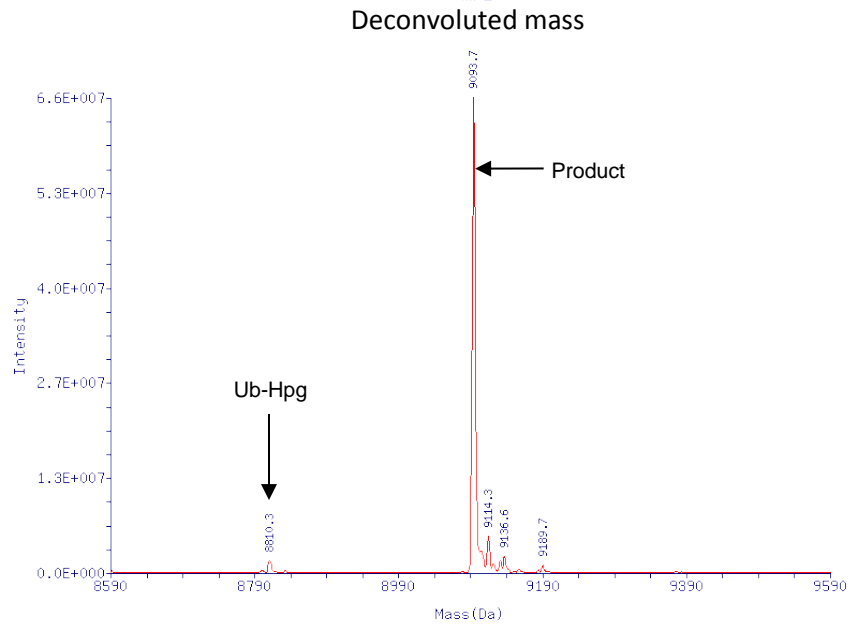
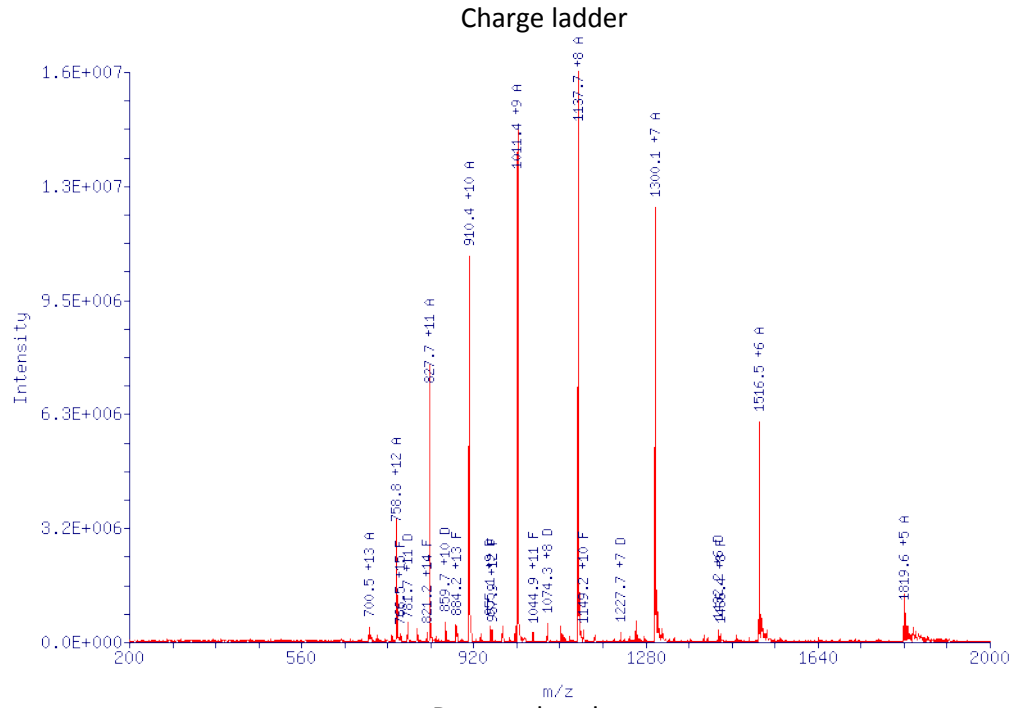
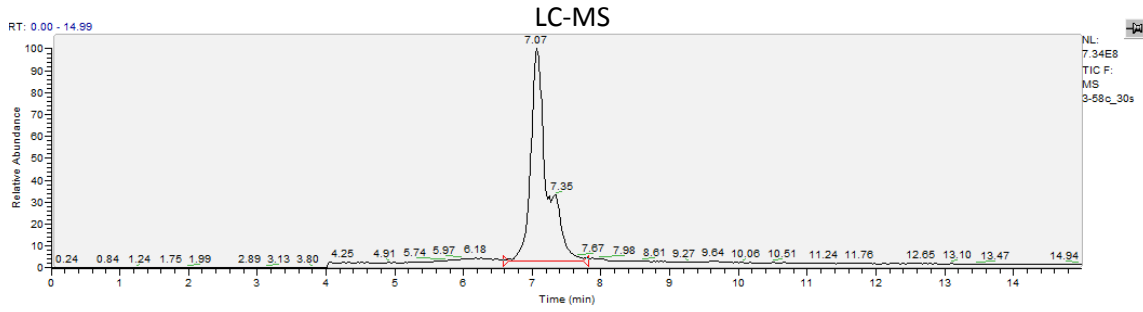


Table 2, entry 4 (10 s)

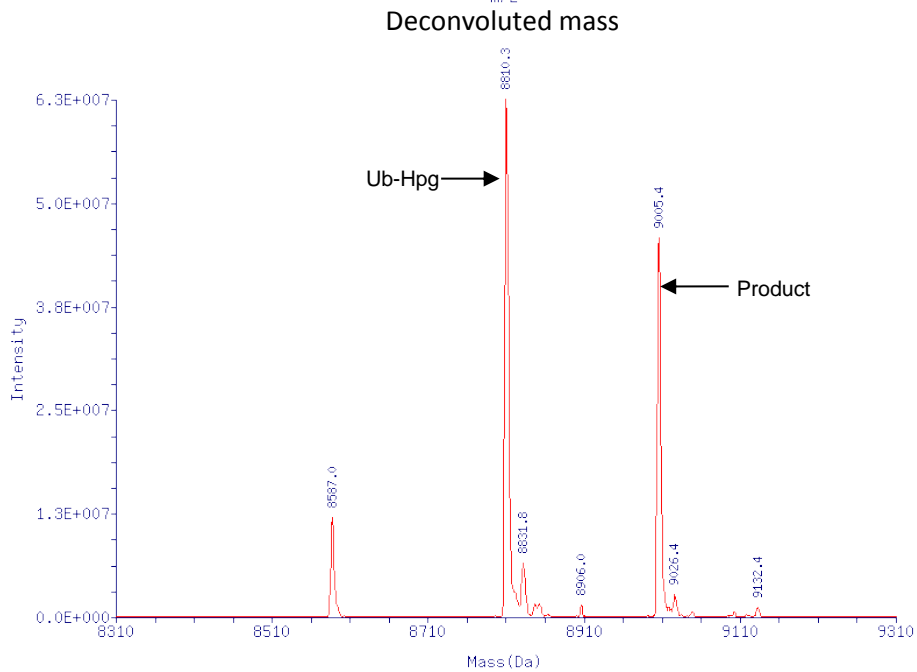
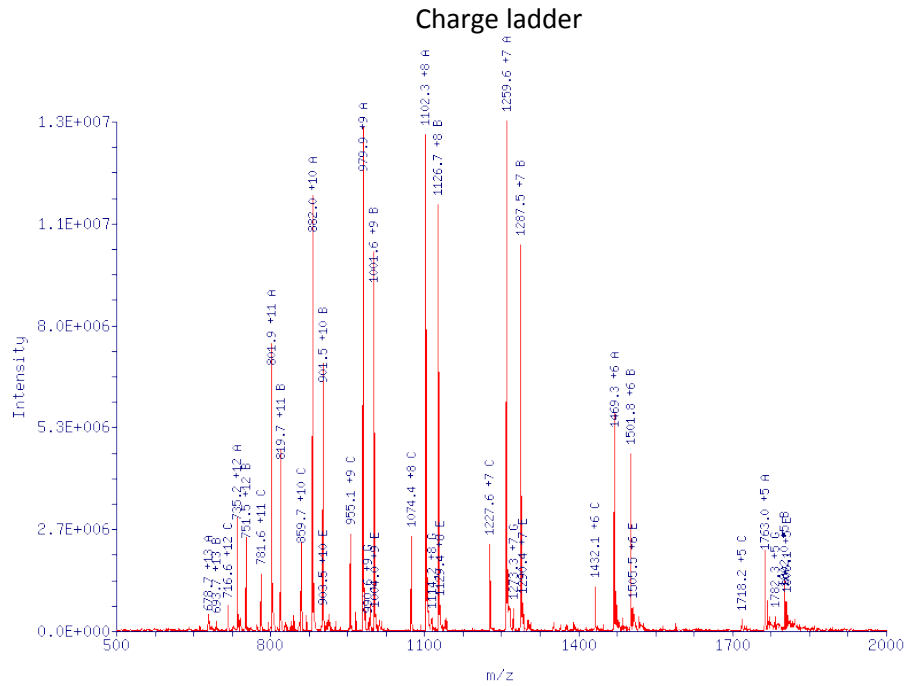
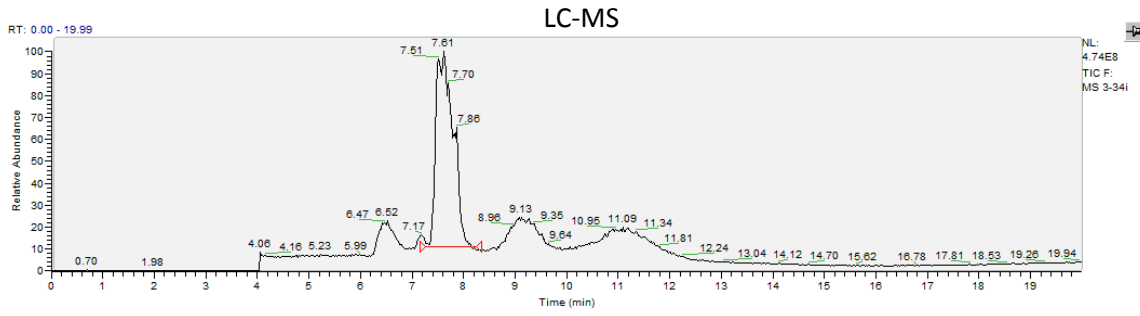


Table 2, entry 4 (3 min)

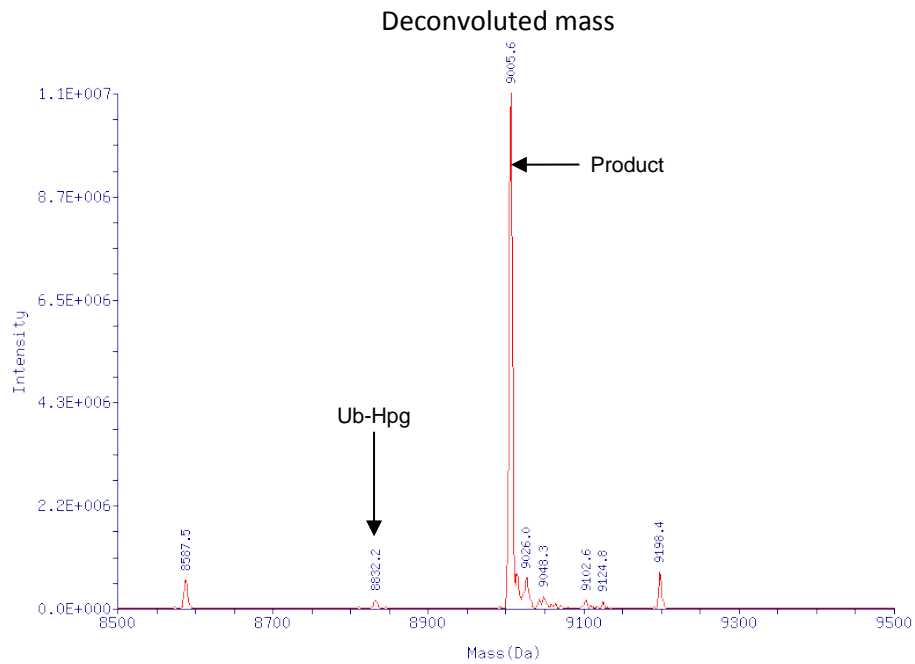
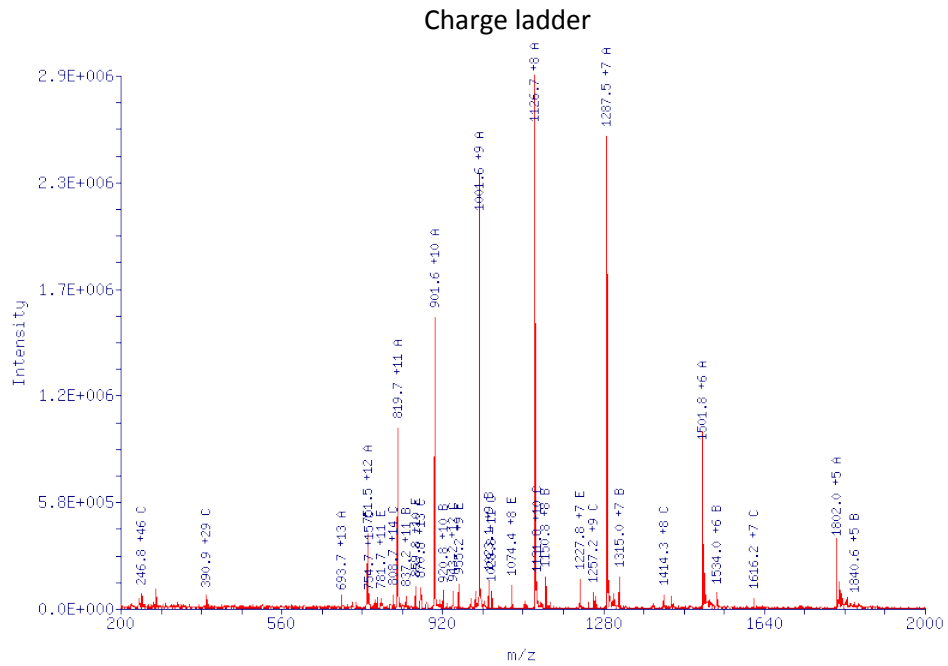
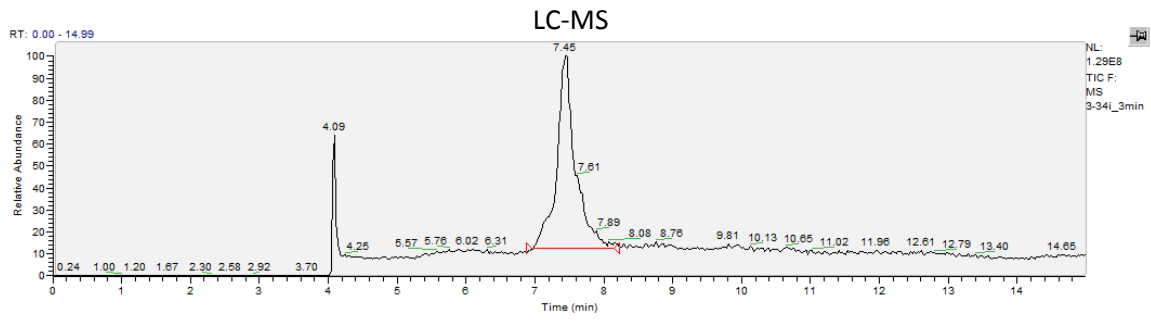


Table 2, entry 5 (10 s)

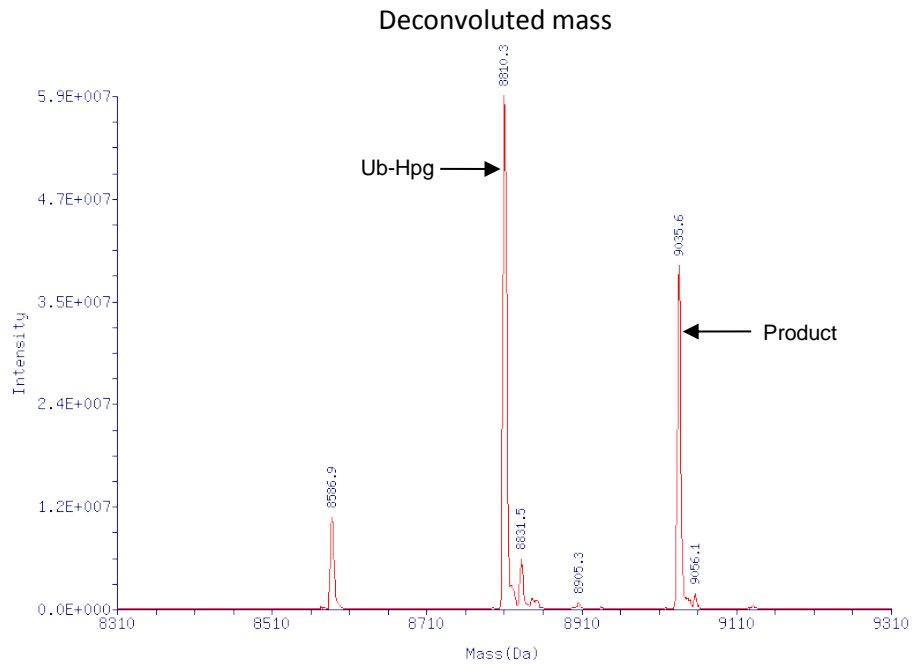
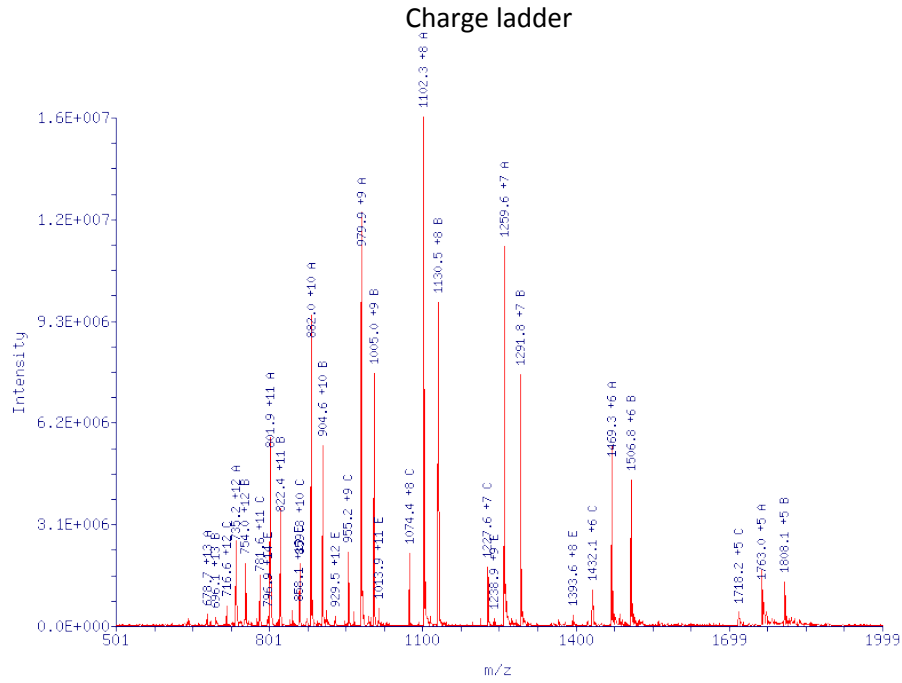
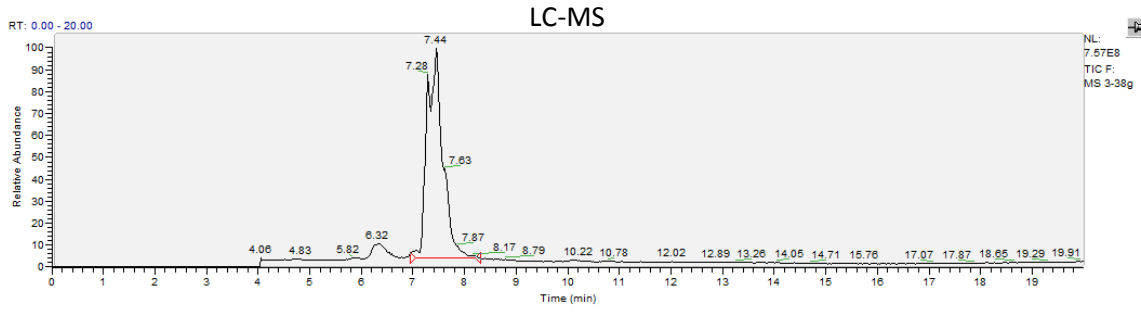


Table 2, entry 5 (3 min)

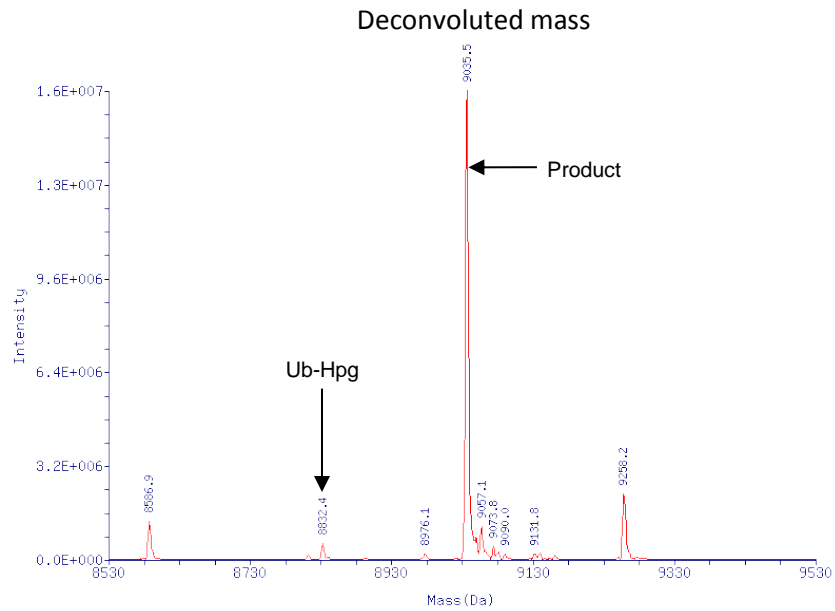
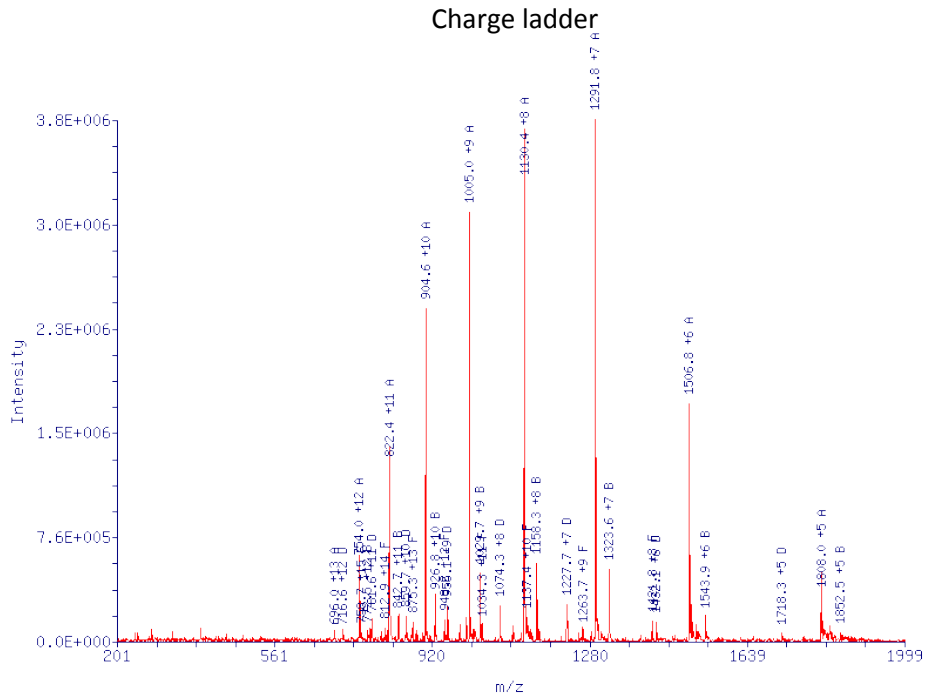
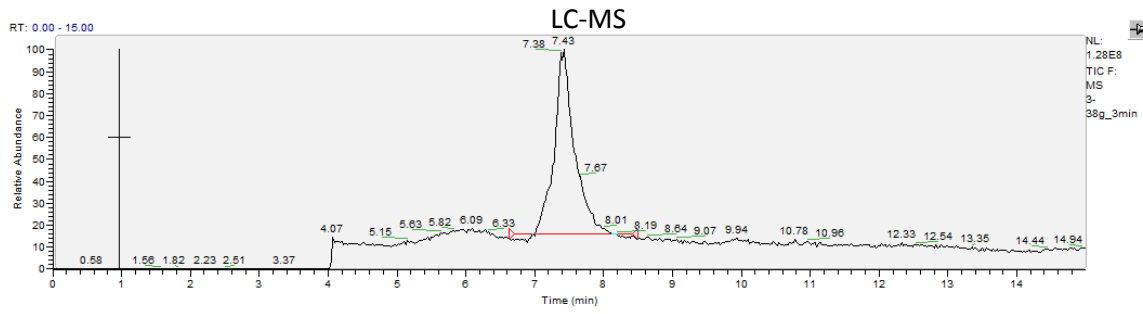


Table 2, entry 6 (10 s)

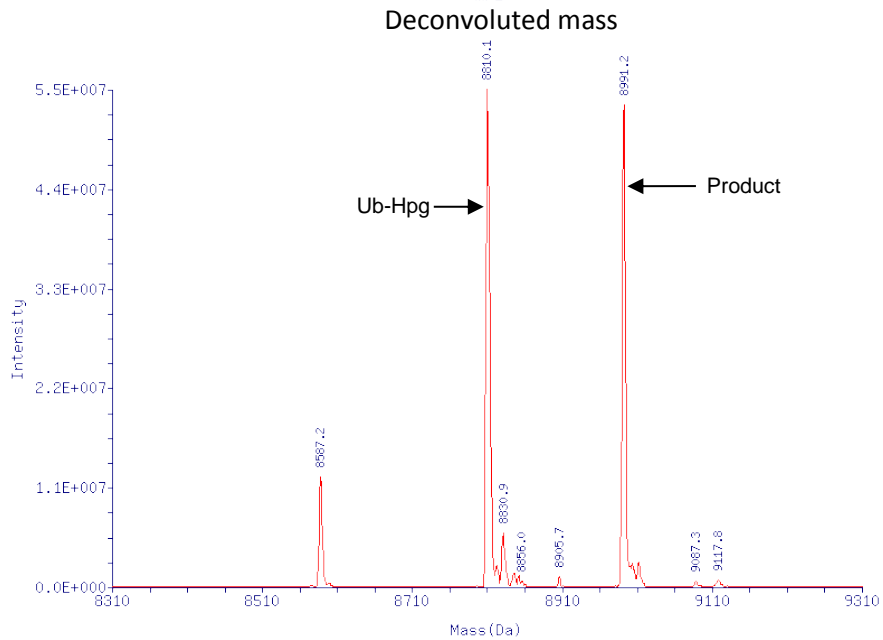
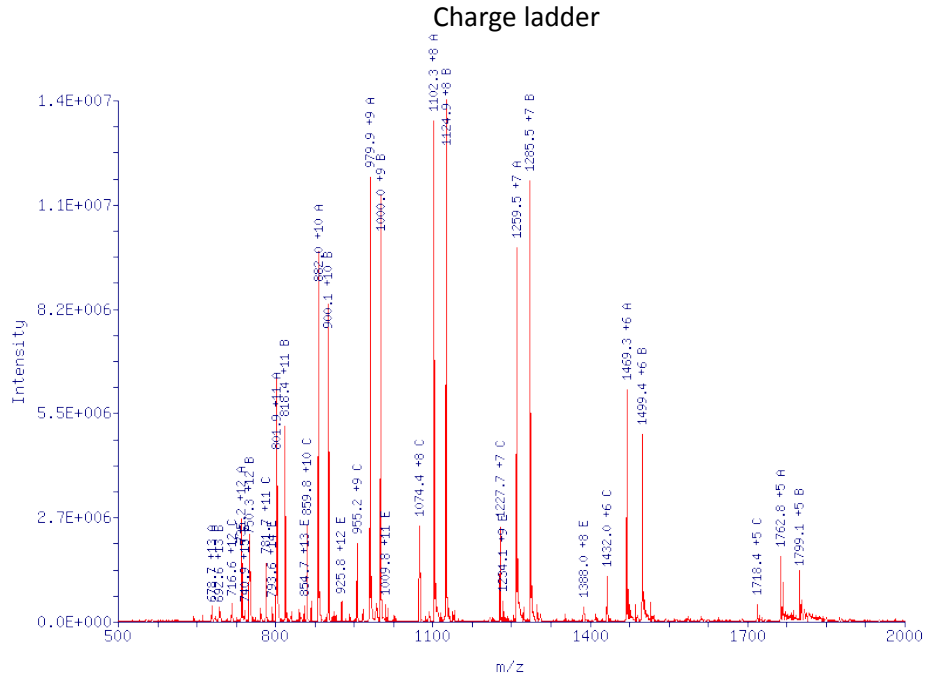
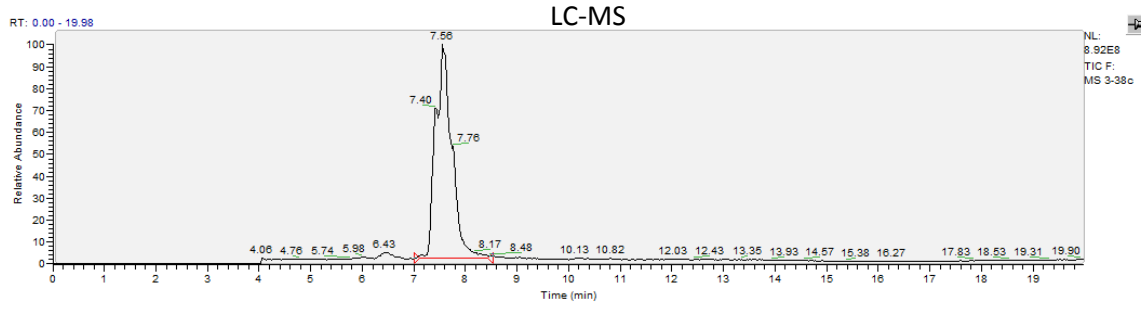


Table 2, entry 6 (3 min)

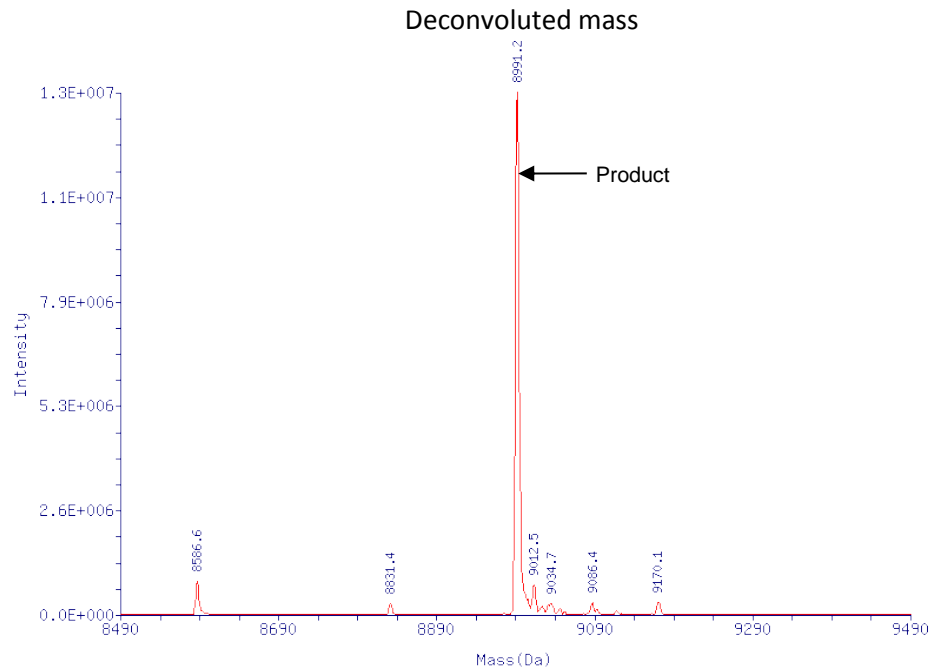
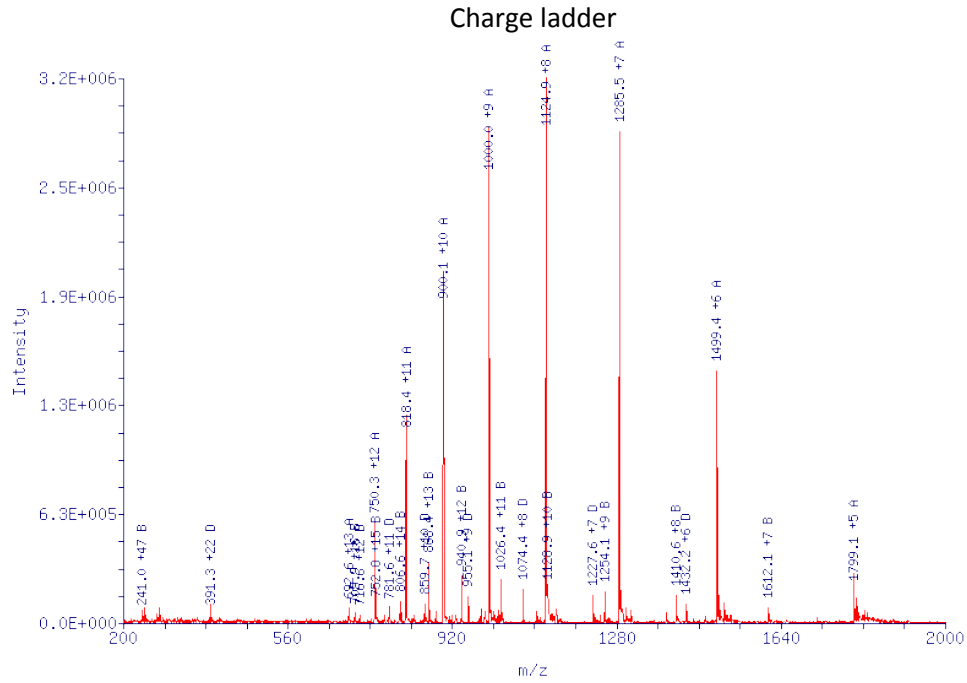
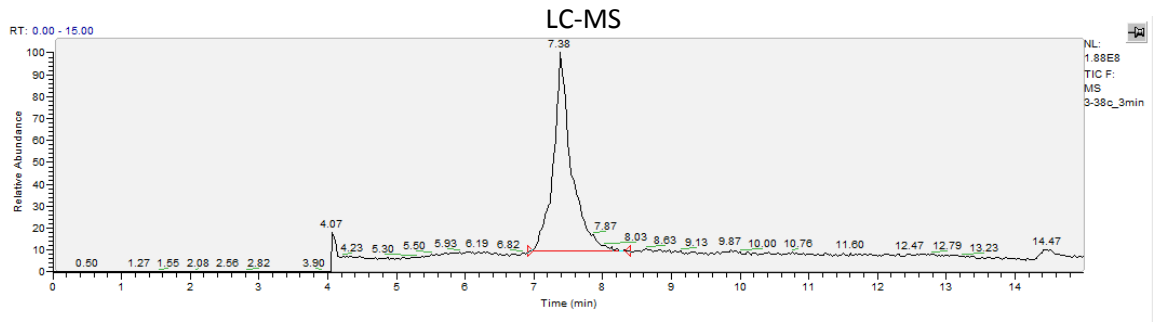


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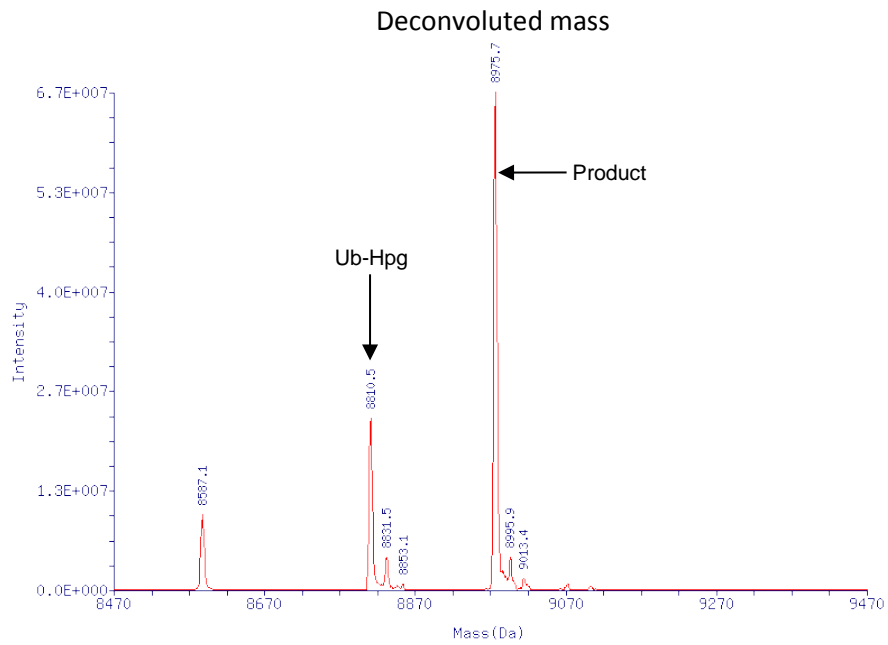
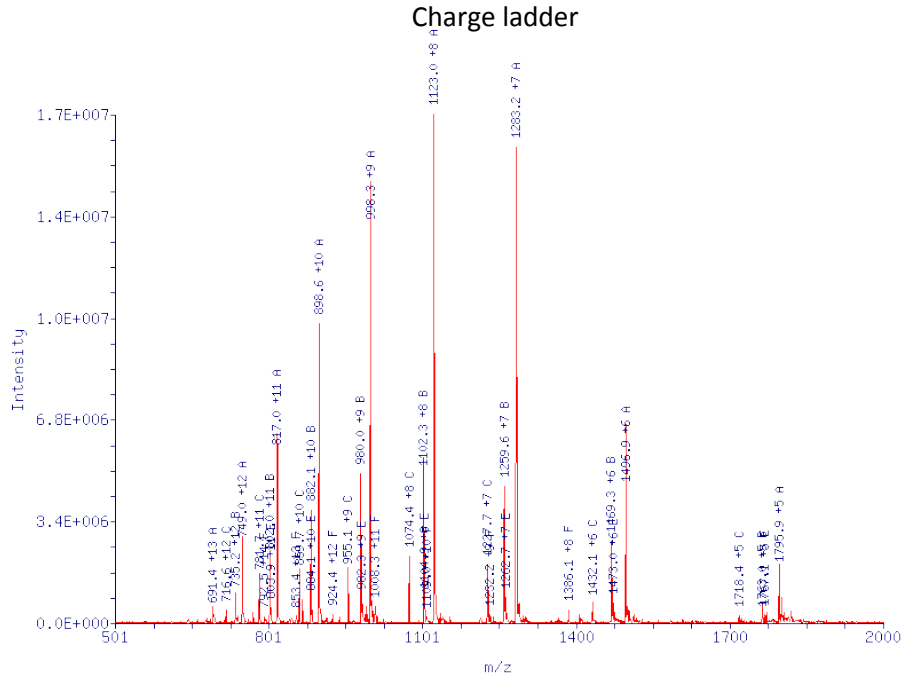
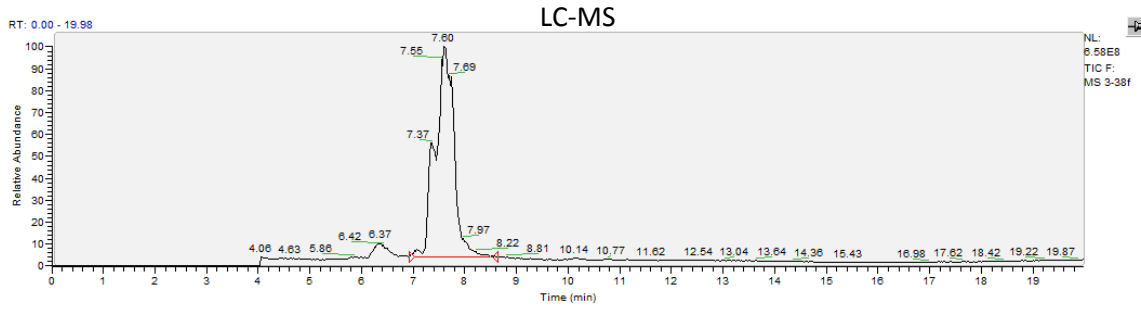


Table 2, entry 7 (3 min)

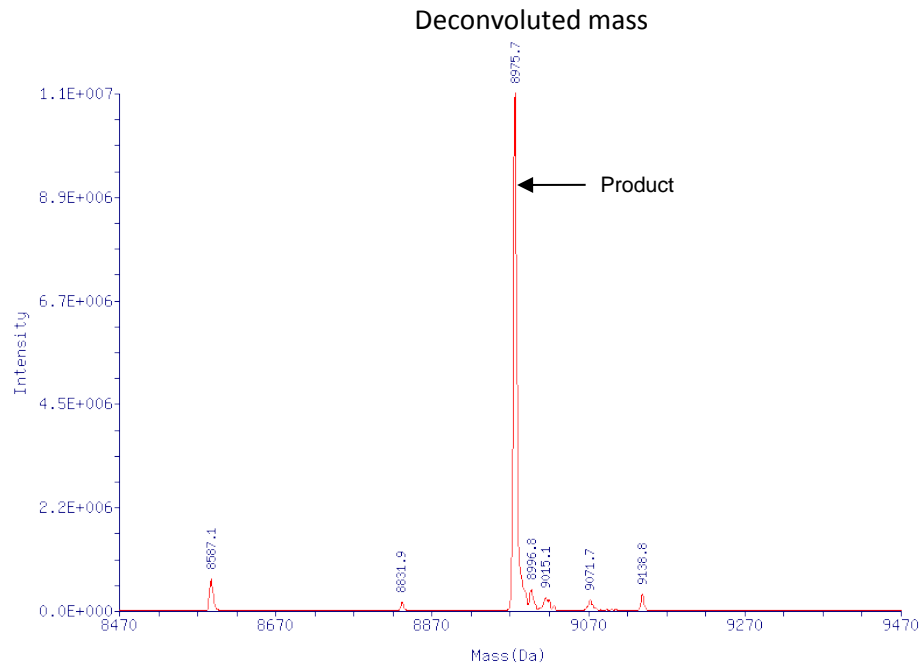
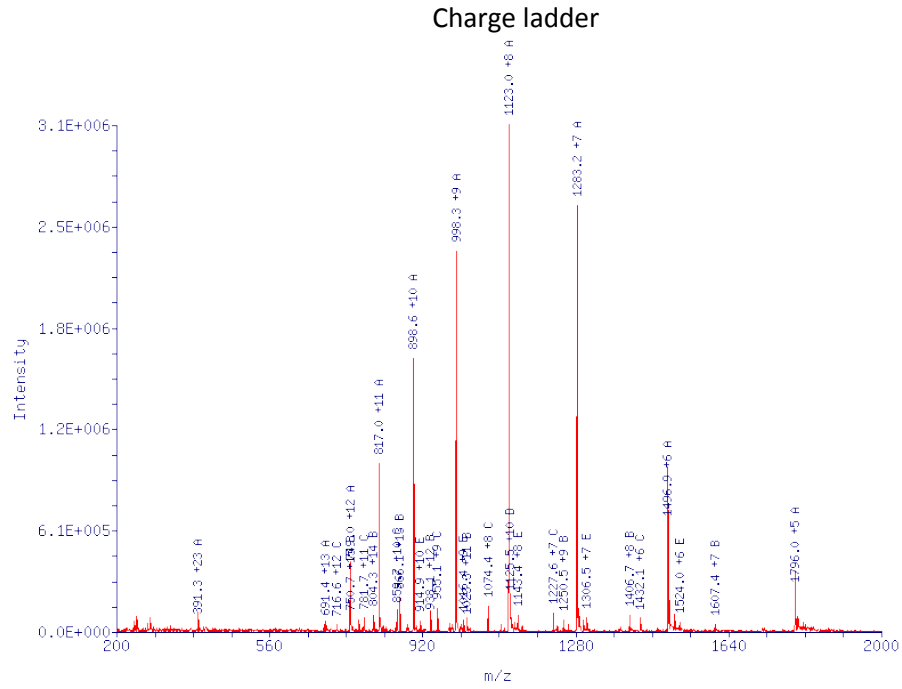
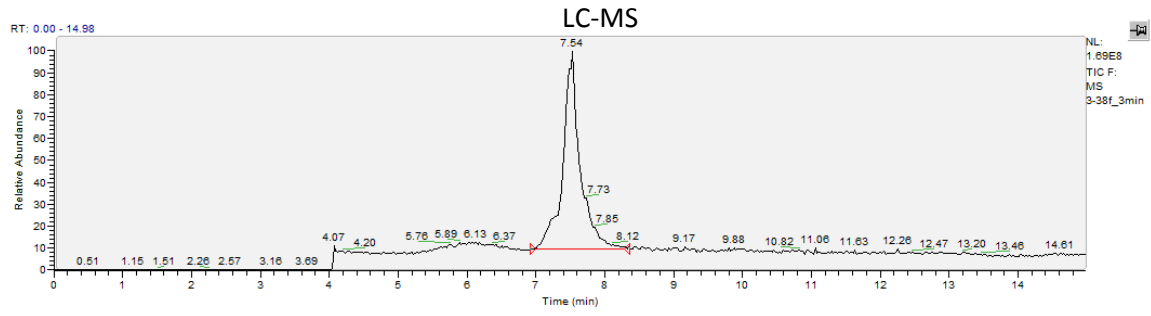


Table 2, entry 8 (10 s)

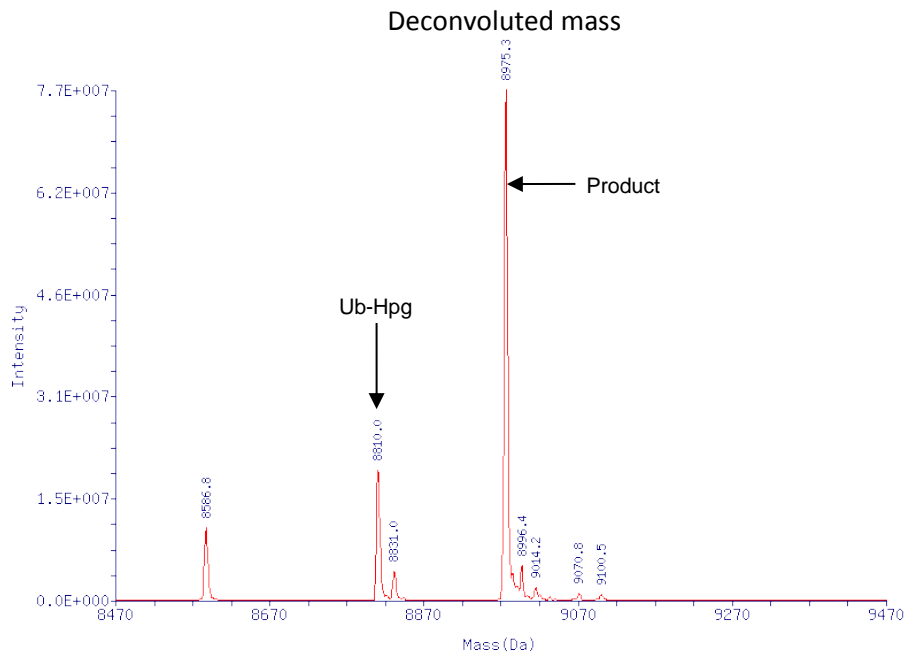
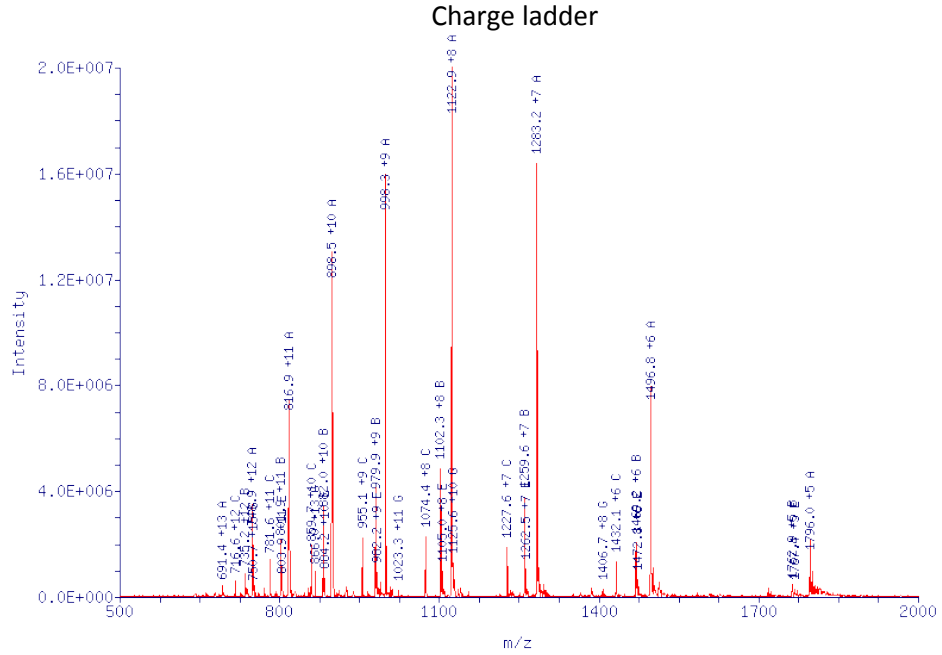
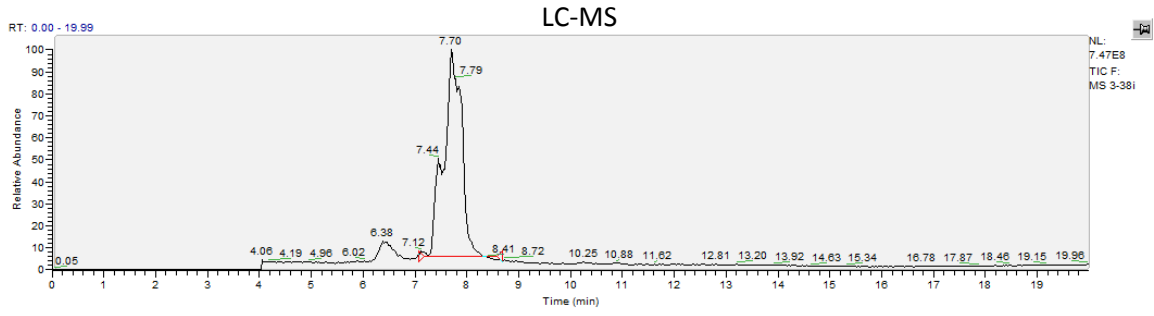


Table 2, entry 8 (3 min)

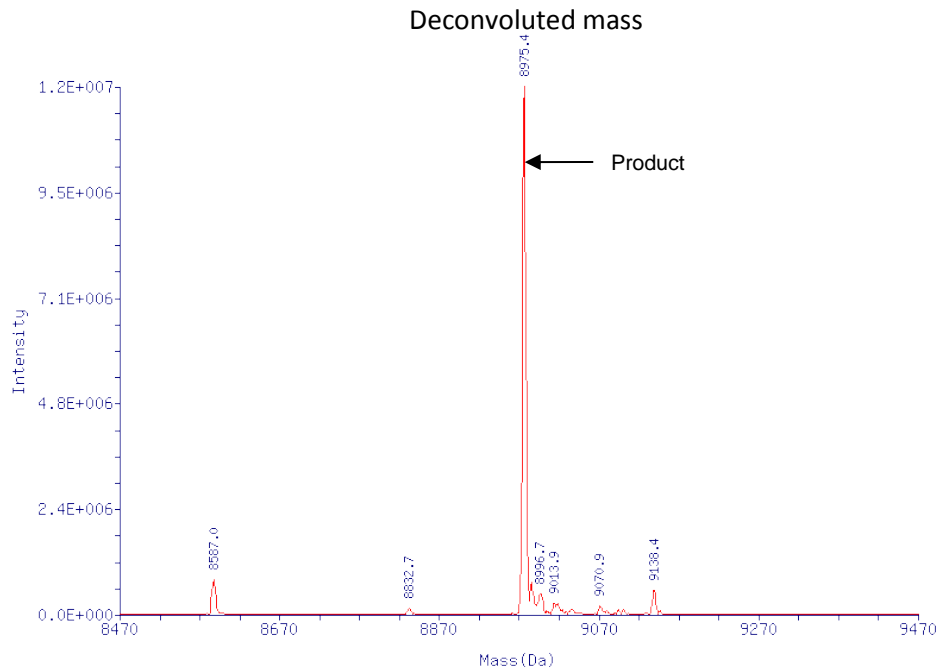
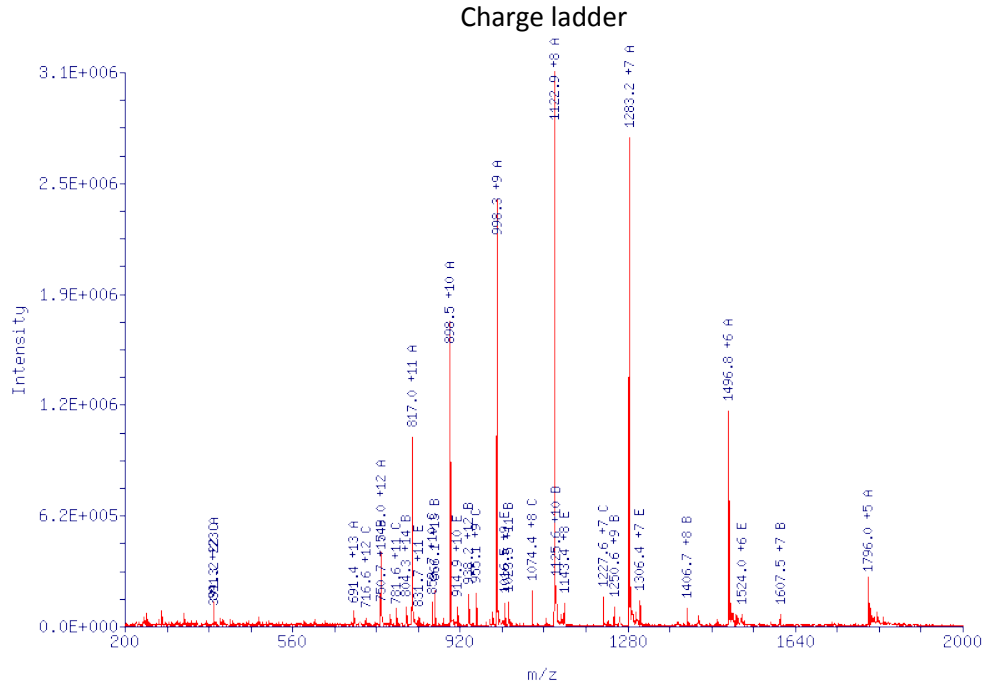
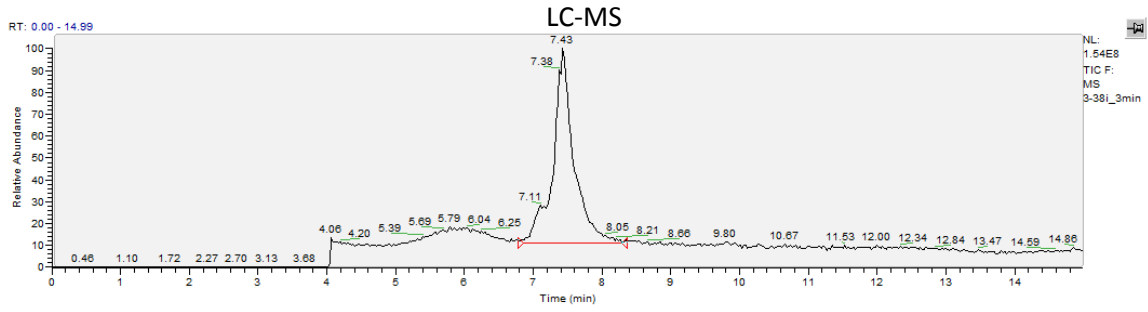


Table 2, entry 9 (10 s)

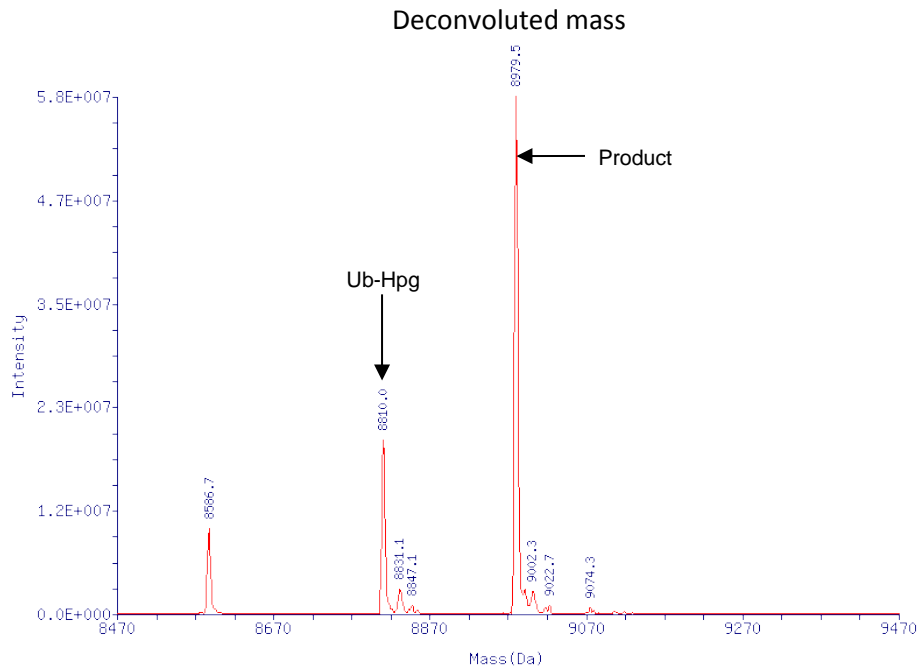
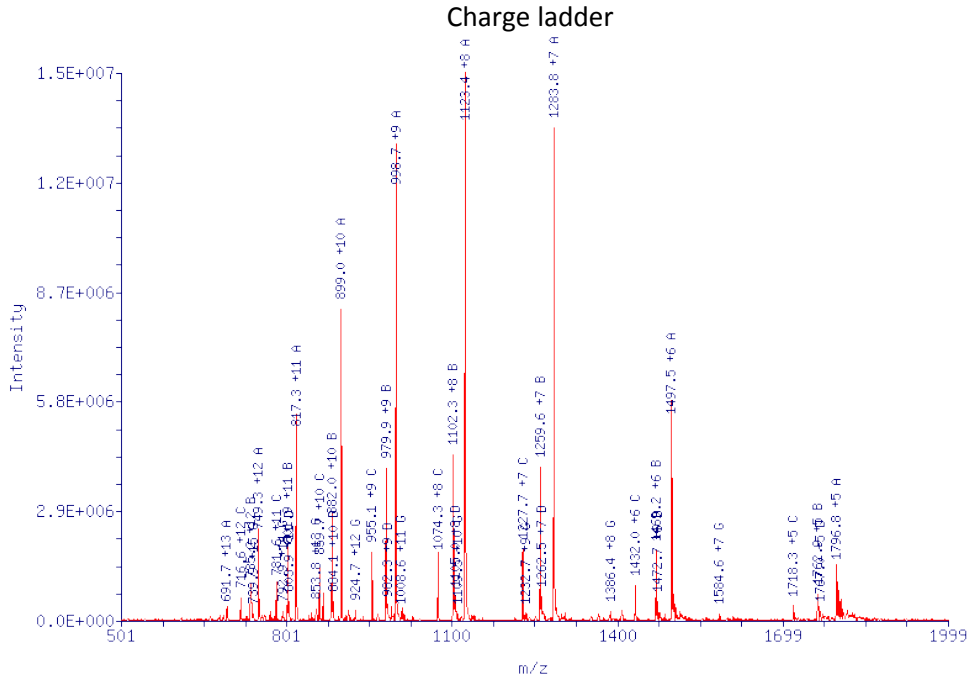
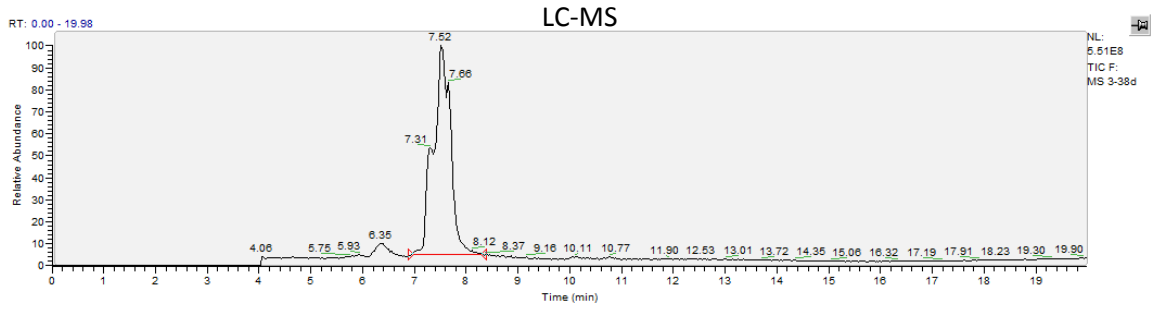


Table 2, entry 9 (3 min)

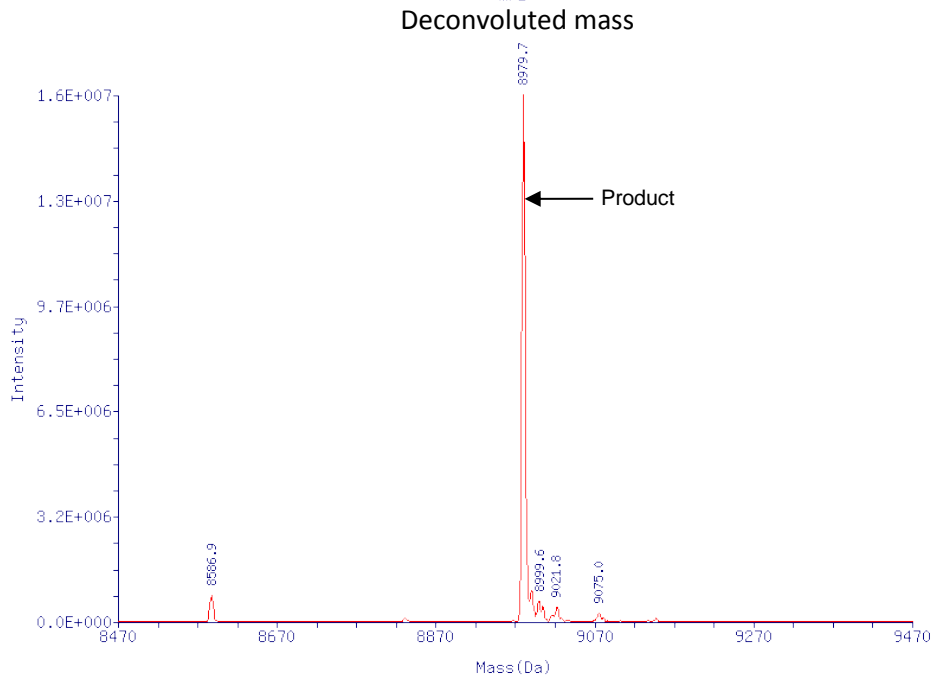
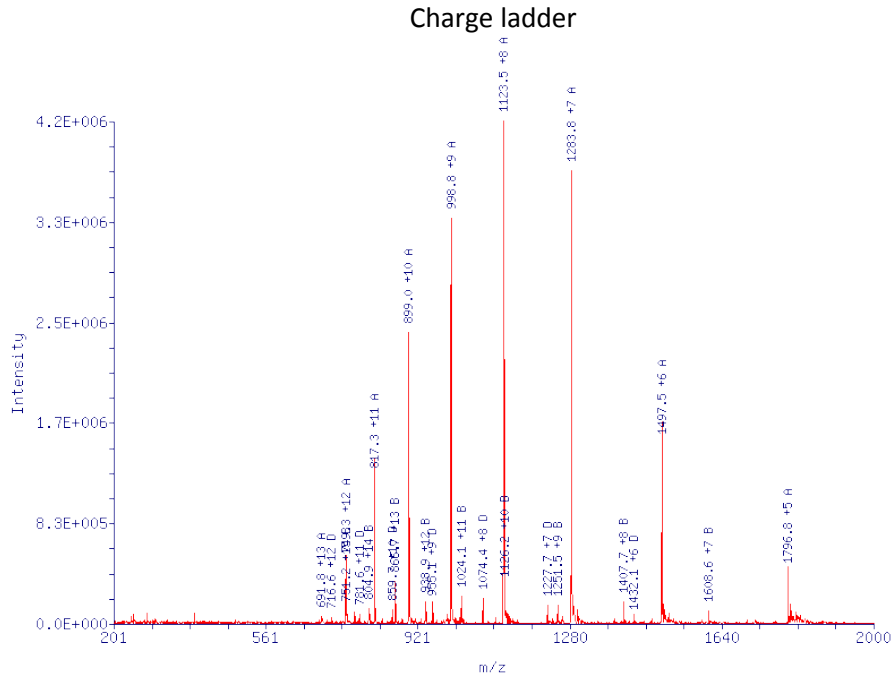
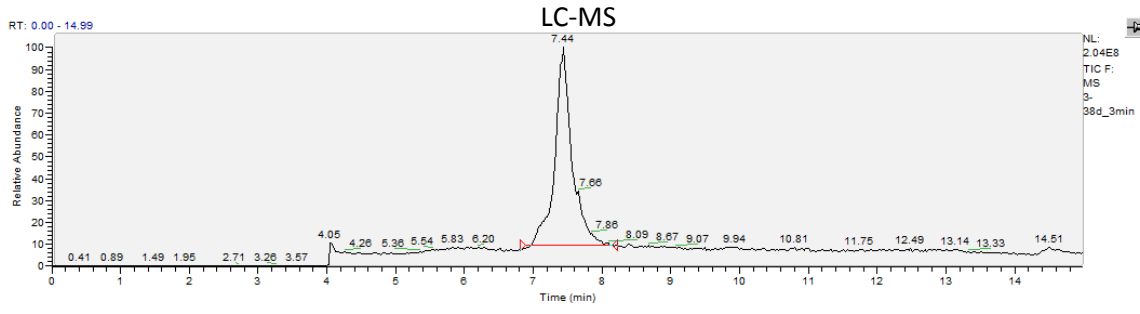


Table 2, entry 10 (10 s)

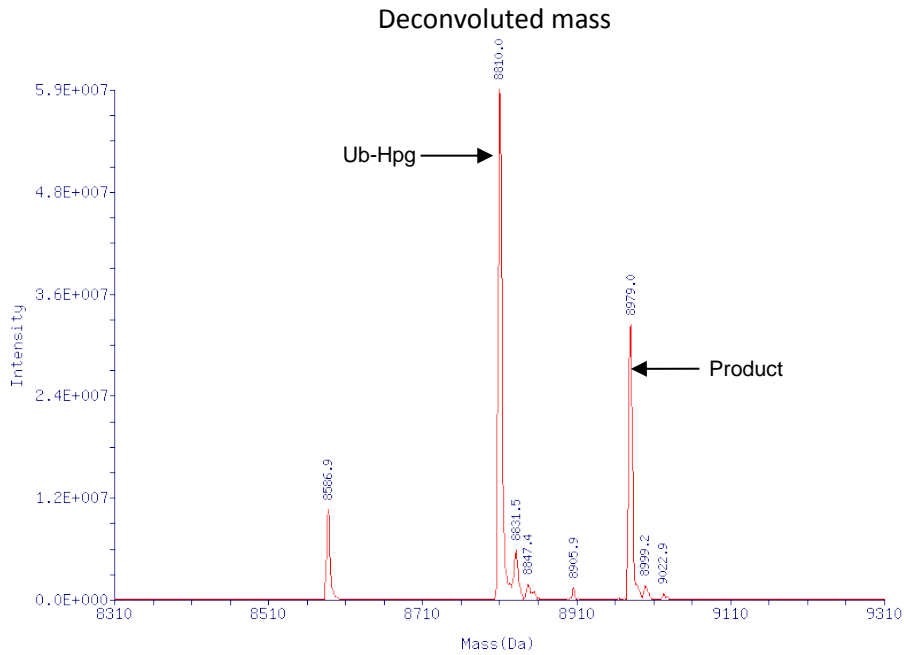
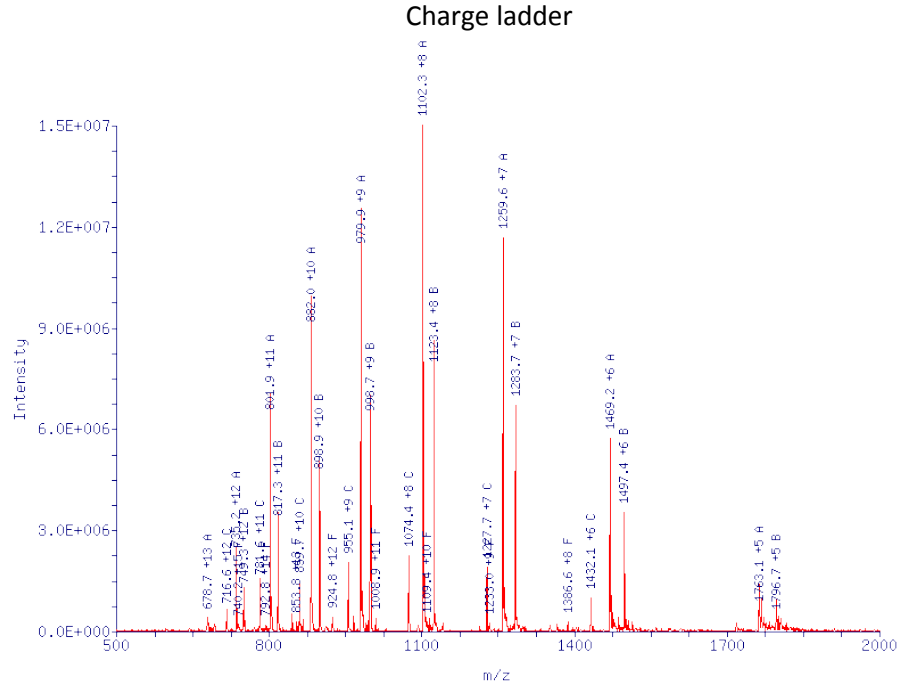
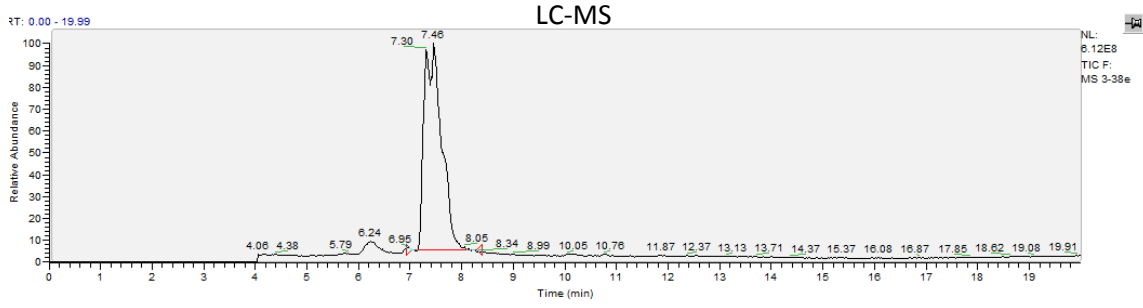


Table 2, entry 10 (3 min)

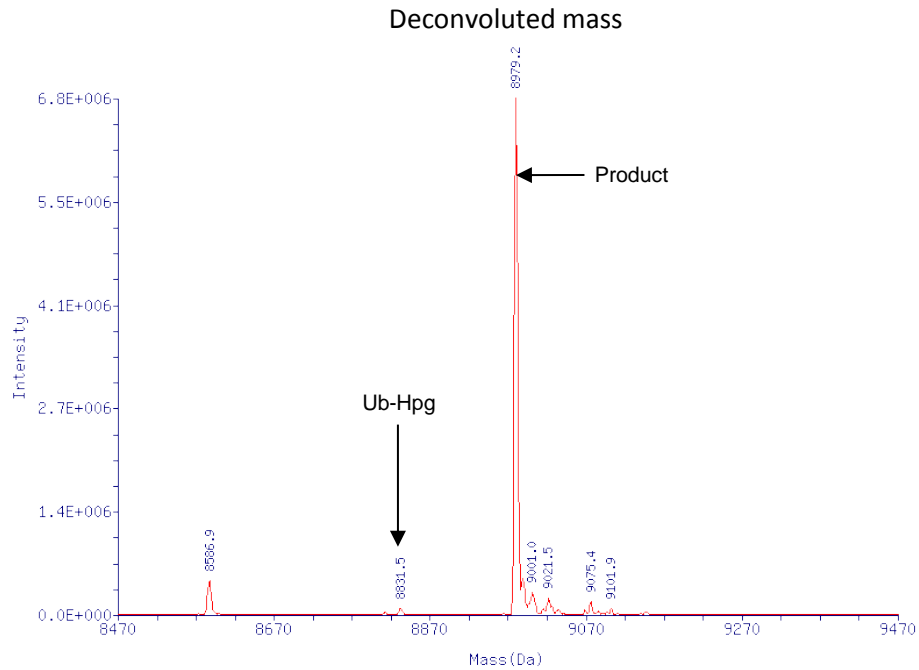
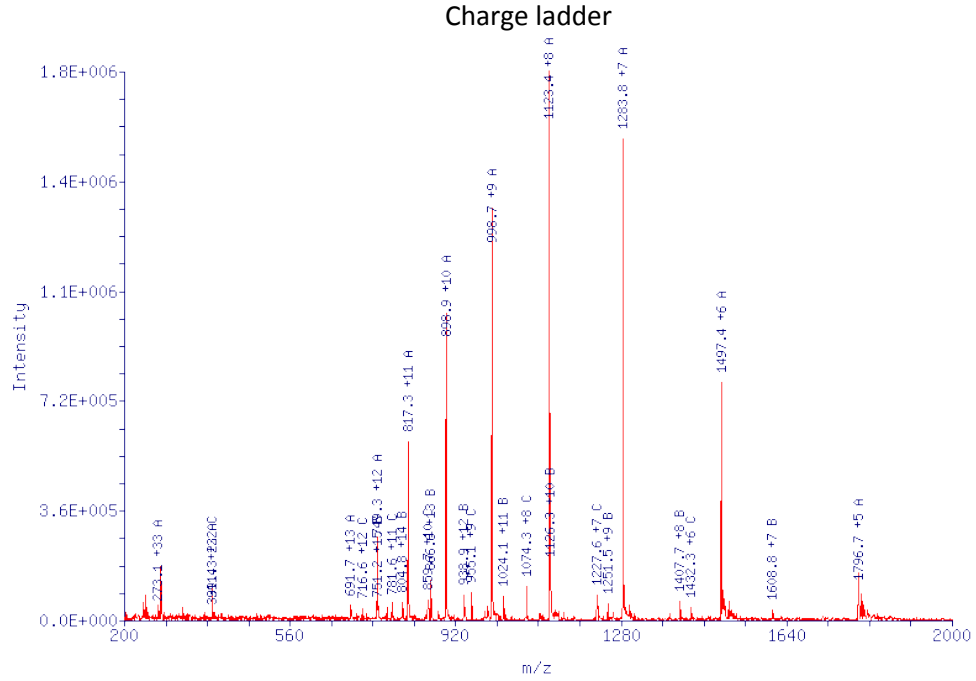
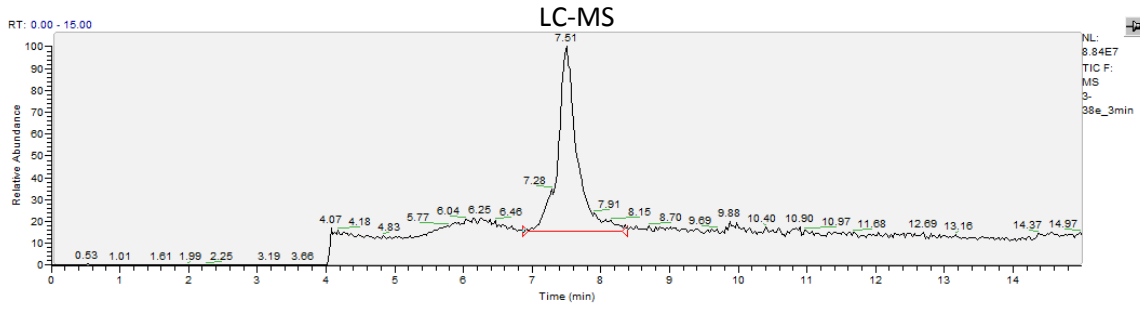


Table 2, entry 11 (10 s)

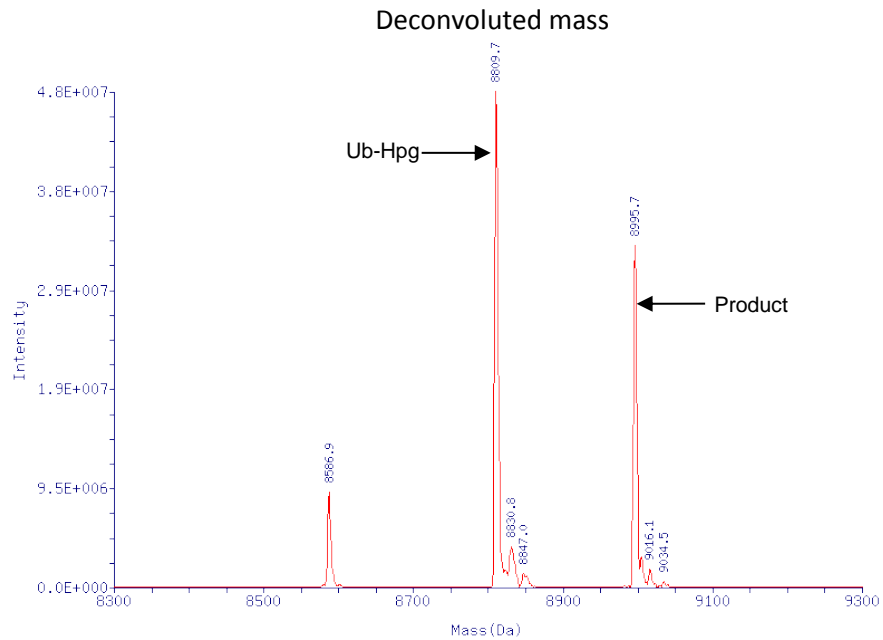
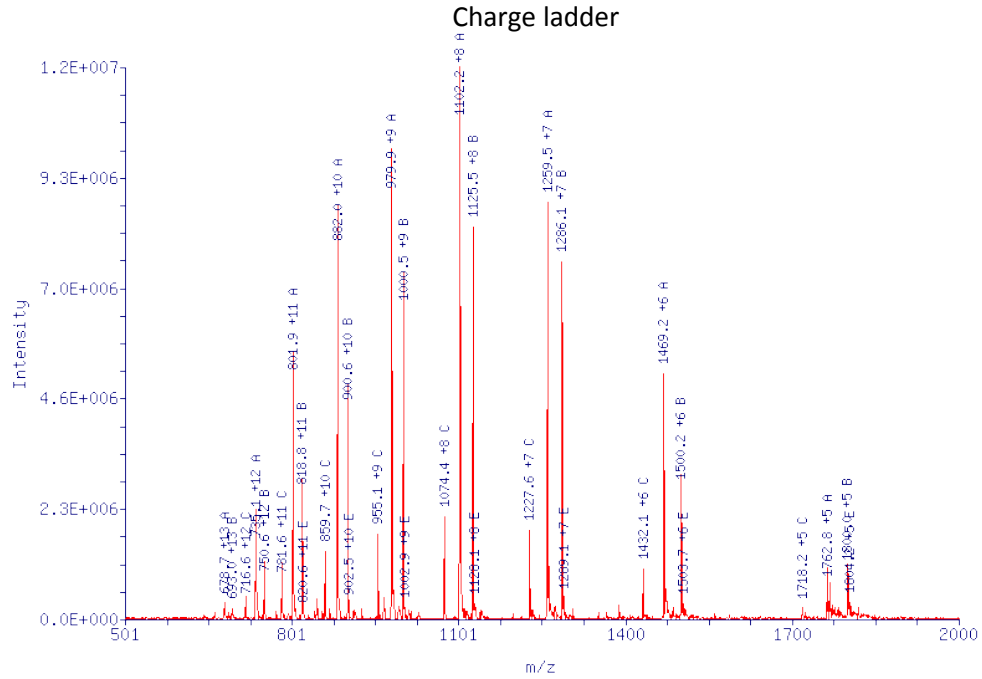
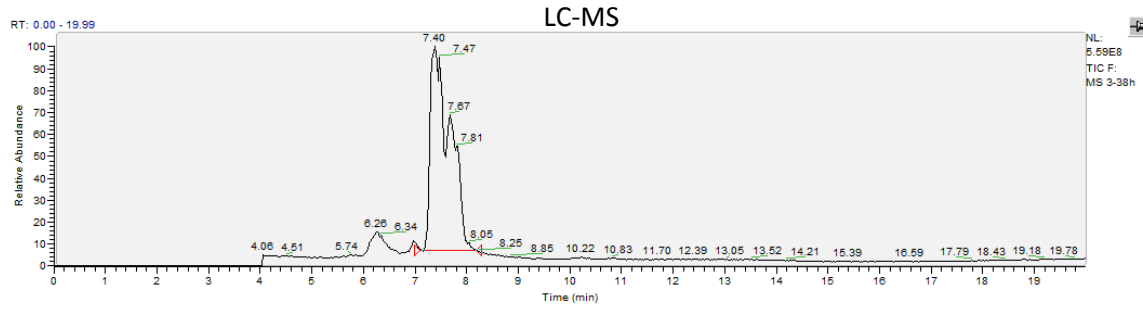


Table 2, entry 11 (3 min)

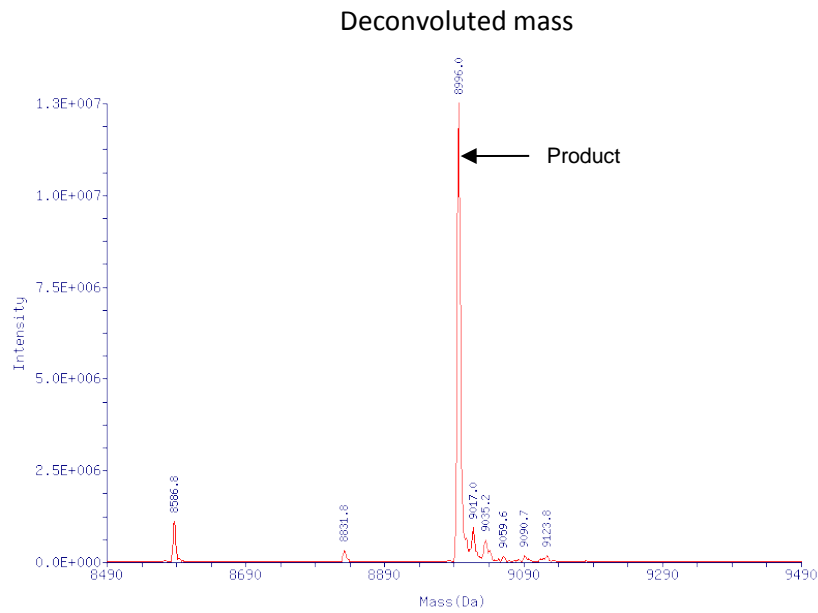
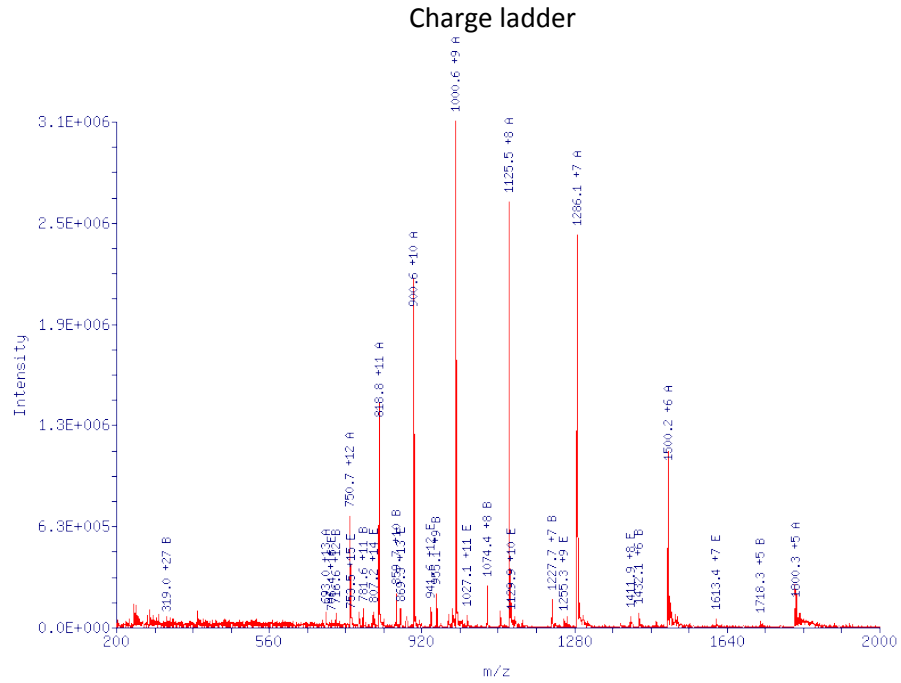
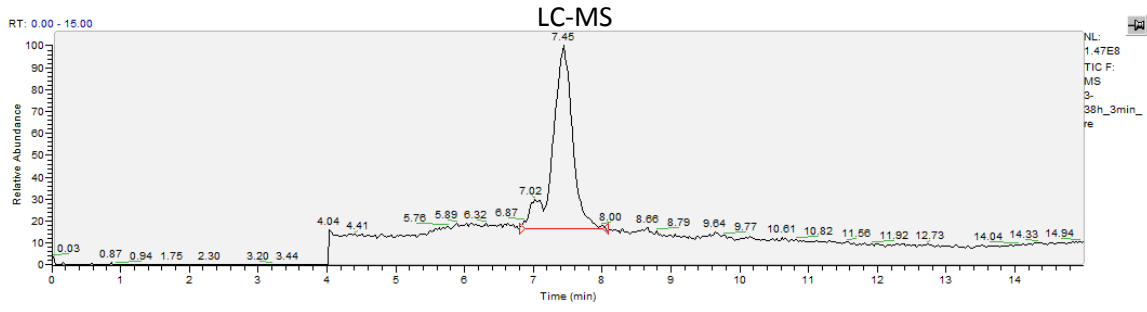


Table 2, entry 12 (10 s)

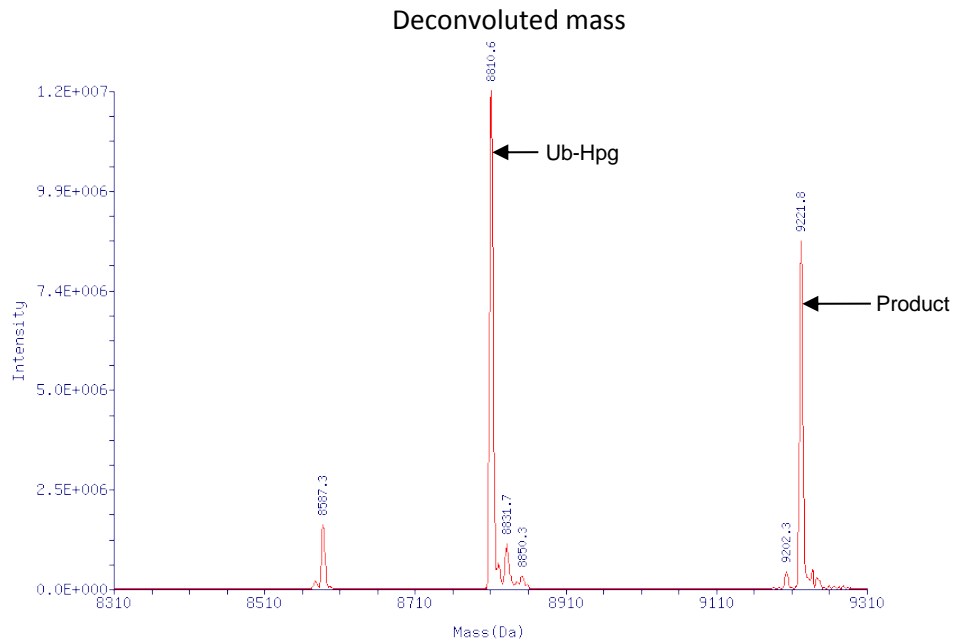
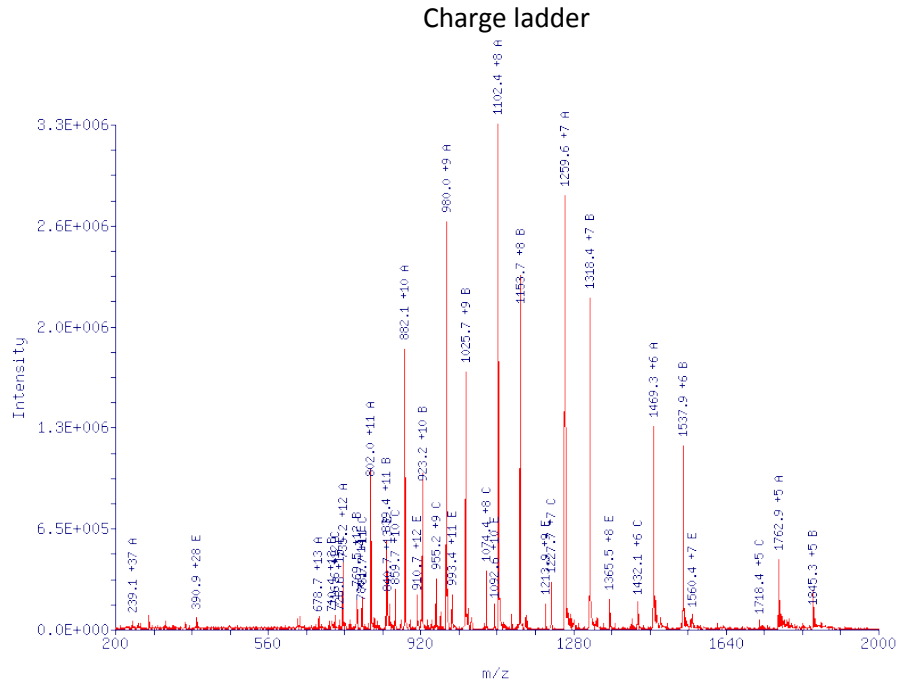
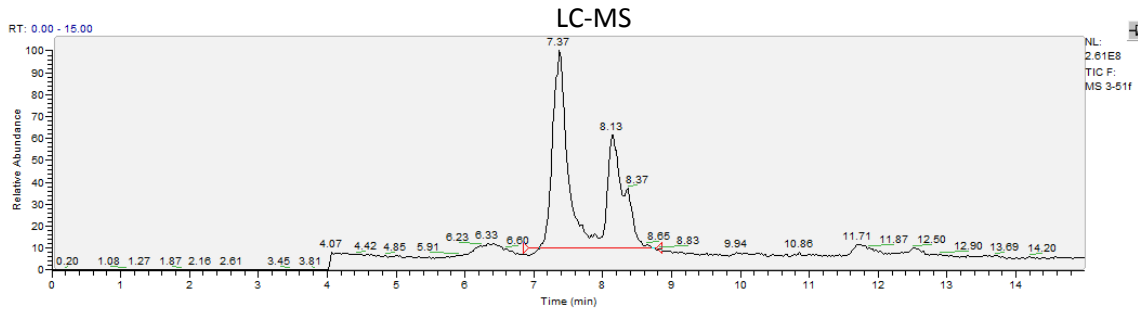


Table 2, entry 12 (3 min)

