Divergent unprotected peptide macrocyclisation by palladium-mediated cysteine arylation

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SUPPORTING INFORMATION

1. GENERAL EXPERIMENTAL DETAILS

General Reagent Information

1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), D-Biotin, Fmoc-Rink amide linker, Fmoc-L-Gly-OH, Fmoc-L-Leu-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Ala-OH, Fmoc-L-Cys(Trt)-OH, Fmoc-L-Gln(Trt)-OH, Fmoc-L-Asn(Trt)-OH, Fmoc-L-Glu(O*t*Bu)-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Phe-OH, Fmoc-L-Ser(*t*Bu)-OH, Fmoc-L-Thr(*t*Bu)-OH, Fmoc-L-Tyr(*t*Bu)-OH, and Fmoc-L-His(Trt)-OH were purchased from Chem-Impex International (Wood Dale, IL). Peptide synthesis-grade *N,N*-dimethylformamide (DMF), dichloromethane (CH_2Cl_2), diethyl ether, HPLC-grade acetonitrile, and guanidine hydrochloride were obtained from VWR International (Philadelphia, PA). Aryl halides and aryl trifluoromethanesulfonates were purchased from Aldrich

Chemical Co., Alfa Aesar, or Matrix Scientific and were used without additional purification. All deuterated solvents were purchased from Cambridge Isotopes and used without further purification. All other reagents were purchased from Sigma-Aldrich and used as received.

All reactions with peptides were set up on the bench top and carried out under ambient conditions. For procedures carried out in the nitrogen-filled glovebox, the dry degassed THF was obtained by passage through activated alumina columns followed by purging with argon. Anhydrous pentane, cyclohexane, and acetonitrile were purchased from Aldrich Chemical Company in Sureseal® bottles and were purged with argon before use.

All small-molecule organic and organometallic compounds were characterized by ^{1}H, ^{13}C NMR, and IR spectroscopy, as well as elemental analysis or high resolution mass spectrometry (unless otherwise noted). ^{19}F NMR spectroscopy was used for organometallic complexes containing fluorine atoms. ^{31}P NMR spectroscopy was used for characterization of palladium complexes. Copies of the ^{1}H, ^{13}C, ^{31}P, and ^{19}F NMR spectra can be found at the end of the Supporting Information. Nuclear Magnetic Resonance spectra were recorded on a Bruker 400 MHz instrument and a Varian 300 MHz instrument. Unless otherwise stated, all ^{1}H NMR experiments are reported in δ units, parts per million (ppm), and were measured relative to the signals of the residual proton resonances CH_2Cl_2 (5.32 ppm) or CH_3CN (1.94 ppm) in the deuterated solvents. All ^{13}C NMR spectra are measured decoupled from ^{1}H nuclei and are reported in δ units (ppm) relative to CD_2Cl_2 (54.00 ppm) or CD_3CN (118.69 ppm), unless otherwise stated. All ^{31}P NMR spectra are measured decoupled from ^{1}H nuclei and are reported relative to H_3PO_4 (0.00 ppm). ^{19}F NMR spectra are measured decoupled from ^{1}H nuclei and are reported in ppm relative to $CFCl_3$ (0.00 ppm) or α,α,α-trifluorotoluene (-63.72 ppm). All FT-IR spectra were recorded on a Thermo Scientific – Nicolet iS5 spectrometer (iD5 ATR – diamond). Elemental analyses were performed by Atlantic Microlabs Inc., Norcross, GA.

LC-MS chromatograms and associated mass spectra were acquired using Agilent 6520 ESI-Q-TOF mass spectrometer. Solvent compositions used in the LC-MS are 0.1% formic acid in H_2O (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The following LC-MS method was used:

Method A LC conditions: Zorbax 300SB C3 column: 2.1 x 150 mm, 5 μm, column temperature: 40 °C, gradient: 0-3 min 5% B, 3-8 min 5-95% B, 8-9 min 95% B, flow rate: 0.8 mL/min. MS conditions: positive electrospray ionization (ESI) extended dynamic mode in mass range 300 – 3000 *m/z*, temperature of drying gas = 350 °C, flow rate of drying gas = 11 L/min, pressure of nebulizer gas = 60 psi, the capillary, fragmentor, and octapole rf voltages were set at 4000, 175, and 750, respectively.

Determination of Macrocyclisation Yields

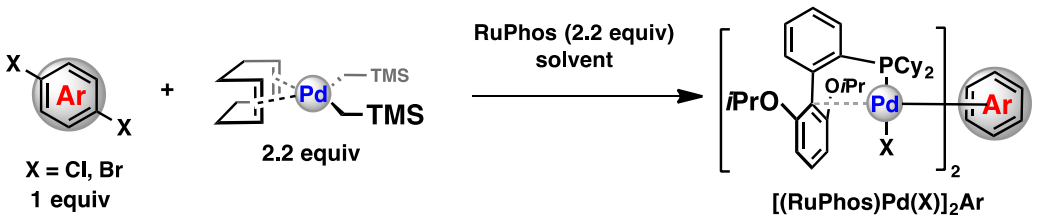
Data were processed using Agilent MassHunter software package. All reported yields were determined by integrating total ion current (TIC) spectra. First, the peak areas for all relevant peptide-containing species on the chromatogram were integrated using Agilent MassHunter software package. Since no peptide-based side products were generated in the experiments, the yields shown in **Table 2** were determined as follows: %yield = S_{pr}/S_{total} where S_{pr} is the peak area of the product and S_{total} is the peak area of combined peptide-containing species (product and starting material).

2. Oxidative Addition Complexes and Synthetic Procedures

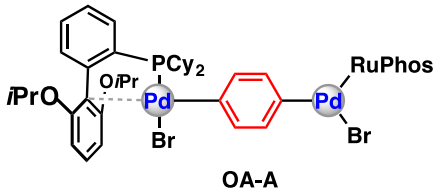
Synthesis of Oxidative Addition Complexes

Synthesis of [(1,5-COD)Pd($CH_2TMS)_2$]. A flame-dried Schlenk flask (100 mL), equipped with a magnetic stir bar, was filled with argon and charged with (1,5-COD)$PdCl_2$ (3.15g, 11.05 mmol) made according to literature reports.[1] The flask was put under vacuum and backfilled with argon (the procedure was repeated 3 consecutive times). Diethyl ether (49.3 mL) was added via syringe, the reaction was cooled to –40 °C (acetonitrile/dry ice bath) and $TMSCH_2MgCl$ (23.4 mL, 1.0 M) purchased from Sigma-Aldrich was added dropwise over 10–20 min. The reaction mixture was stirred at –40 °C for 1 h and then at 0 °C (ice/water bath) for an additional 20 min. Acetone (1.3 mL) was added at 0 °C, the reaction mixture was stirred for 5 min, after which time the solvent was removed under vacuum using an external trap (the flask was kept at 0 °C). The flask was then opened to air, pentane (100 mL) was added and the crude material was filtered through a pad of Celite into a new round-bottom flask (500 mL) at 0 °C. The filter cake was washed with pentane (50 mL × 2). Pentane from the combined washes was removed with the aid of a rotary evaporator at 0 °C (ice/water bath). The resulting white solid was dried under vacuum for 2 h at 0 °C, and transferred into a 20 mL scintillation vial in the glovebox (3.00 g, 70%). The ^{1}H and ^{13}C NMR spectra of the obtained material are identical to those reported in the literature.[1] The title compound was stored in the glovebox at –20 °C.

General Procedure for the Synthesis of bis-Palladium Oxidative Addition Complexes.



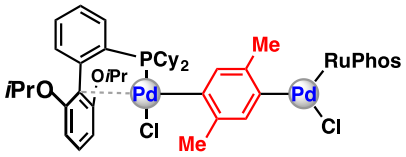
In a nitrogen-filled glovebox, an oven-dried scintillation vial (10 mL), equipped with a magnetic stir bar, was charged with RuPhos (2.2 equiv), Ar–X_2 (1 equiv), and cyclohexane. Solid (1,5-COD)Pd(CH_2SiMe_3)$_2$ (2.2 equiv) was added rapidly in one portion and the resulting solution was stirred for 16 h at rt. After this time, pentane (3 mL) was added and the resulting mixture was placed into a –20 °C freezer for 3 h. The vial was removed from the glovebox and, in the air, the resulting precipitate was filtered, washed with pentane (5 × 3 mL), and dried under reduced pressure to afford the oxidative addition complex.



OA-A

Following the general procedure, a mixture containing 1,4-dibromobenzene (29.4 mg, 0.125 mmol), RuPhos (128 mg, 0.275 mmol), and (1,5-COD)Pd(CH_2TMS)$_2$ (107 mg, 0.275 mmol) was stirred at rt in cyclohexane (1.5 mL) for 12 h. General work-up afforded **OA-A** as a grey solid (172 mg, 99%).

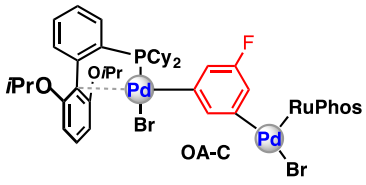
^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.72 – 7.59 (m, 1H), 7.54 – 7.37 (m, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 7.02 (dd, *J* = 8.5, 1.9 Hz, 1H), 6.92 (dd, *J* = 7.0, 2.5 Hz, 1H), 6.70 (d, *J* = 8.5 Hz, 1H), 6.64 (t, *J* = 1.6 Hz, 1H), 4.66 (dt, *J* = 12.1, 6.1 Hz, 1H), 2.23 – 2.12 (m, 1H), 1.80 (br, 4H), 1.59 (br, 1H), 1.48 (s, 1H), 1.42 (d, *J* = 6.0 Hz, 3H), 1.32 – 1.12 (m, 2H), 1.05 (dd, *J* = 6.0, 3.7 Hz, 4H), 0.93 (t, *J* = 7.1 Hz, 1H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 159.08, 144.66, 144.48, 138.97, 138.93, 135.89, 135.12, 134.64, 134.15, 133.01, 132.66, 132.62, 132.51, 131.09, 130.96, 130.62, 130.60, 130.20, 129.17, 129.15, 126.40, 126.34, 126.06, 117.33, 107.47, 106.97, 71.09, 70.64, 34.12, 33.90, 33.63, 33.26, 28.23, 28.02, 27.71, 27.69, 27.35, 27.16, 27.03, 26.91, 26.84, 26.81, 26.72, 26.69, 26.03, 26.02, 22.33, 21.87, 21.38, 21.30, 13.81. (observed complexity is due to *C–P* coupling**). ^{31}P NMR (121 MHz, CD_2Cl_2)** δ 31.99. **FT-IR (neat, cm^{-1}):** 2929.76, 2851.34, 1453.14, 1383.05, 1373.23, 1269.20, 1227.14, 1105.13, 1066.00, 1037.87, 997.86, 850.63, 816.23, 742.05, 575.3. **Anal. Calcd. for $C_{66}H_{90}Br_2O_4P_2Pd_2$:** C, 57.36; H, 6.56. Found: C, 57.27; H, 6.61.



OA-B

Following the general procedure, a mixture containing 1,4-dichloro-2,5-dimethylbenzene (20.0 mg, 0.125 mmol), RuPhos (133.3 mg, 0.275 mmol), and (1,5-COD)Pd($CH_2TMS)_2$ (111.0 mg, 0.275 mmol) was stirred at rt in THF (1.0 mL) for 12 h. General work-up afforded **OA-B** as a light grey solid (133.5 mg, 89 %).

^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.71 (t, J = 8.4 Hz, 2H), 7.64 (t, J = 7.3 Hz, 2H), 7.45 (m, 2H), 7.40 (m, 2H), 6.87 (ddd, J = 7.5, 2.9, 1.5 Hz, 3H), 6.65 (d, J = 8.5 Hz, 2H), 6.60 (d, J = 8.4 Hz, 2H), 6.27 (br, 2H), 4.61 (m, 4H), 2.54 (s, 6H), 2.27 (m, 4H), 1.83 (m, 18H), 1.43 (m, 18H), 1.16 (m, 17H), 1.05 (m, 5H), 0.91 (d, J = 6.1 Hz, 6H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 160.66, 159.65, 146.01, 145.83, 140.48, 135.81, 135.75, 135.38, 134.08, 134.03, 133.89, 132.78, 132.67, 131.14, 130.94, 126.78, 126.72, 111.18, 111.14, 107.07, 106.80, 71.69, 70.45, 35.57, 35.29, 32.47, 32.21, 31.12, 31.07, 29.61, 29.56, 27.95, 27.88, 27.80, 27.70, 27.53, 27.45, 27.10, 26.99, 26.77, 26.30, 22.77, 22.35, 22.25, 22.04 (observed complexity is due to *C–P* coupling). **^{31}P NMR (121 MHz, CD_2Cl_2)** δ 32.03. **FT-IR (neat, cm^{-1}):** 2972.34, 2927.52, 2851.98, 1590.82, 1450.73, 1382.13, 1370.98, 1264.81, 1249.18, 1135.64, 1104.19, 1064.08, 1041.81, 1002.1, 898.23, 865.21, 848.18, 812.89, 767.71, 757.24, 744.88, 732.59, 667.8. **HRMS (ESI) m/z calcd. for $C_{68}H_{94}Cl_2O_4P_2Pd_2$ $[M-Cl]^+$:** 1285.4386. Found: 1285.4389.

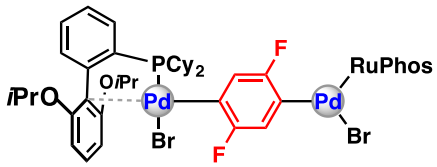


OA-C

Following the general procedure, a mixture containing 1,3-Dibromo-5-fluorobenzene (32 mg, 0.125 mmol), RuPhos (128 mg, 0.275 mmol), and (1,5-COD)Pd($CH_2TMS)_2$ (107 mg, 0.275 mmol) was stirred at rt in cyclohexane (1.5 mL) for 12 h. General work-up afforded **OA-C** as a grey solid (157 mg, 90%).

^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.54 (t, *J* = 6.9 Hz, 1H), 7.48 (t, *J* = 8.4 Hz, 1H), 7.29 (dt, *J* = 21.5, 7.4 Hz, 2H), 6.77 (ddd, *J* = 7.6, 3.0, 1.5 Hz, 1H), 6.54 (dd, *J* = 41.6, 8.4 Hz, 2H), 6.35 (d, *J* = 9.4 Hz, 1H), 6.27 (s, 1H), 4.48 (ddt, *J* = 35.1, 12.2, 6.2 Hz, 2H), 2.36 (s, 0H), 2.06 (s, 1H), 1.44 (s, 5H), 1.36 (d, *J* = 6.2 Hz, 3H),

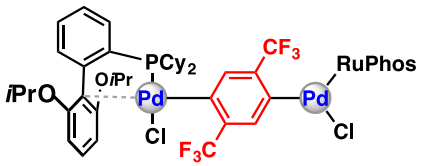
1.18 (d, *J* = 6.0 Hz, 2H), 0.96 (d, *J* = 6.0 Hz, 2H), 0.85 (d, *J* = 6.1 Hz, 2H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 159.48, 158.46, 156.64, 144.44, 140.63, 135.28, 135.12, 132.74, 132.65, 132.54, 132.37, 130.96, 130.81, 130.79, 128.84, 126.52, 126.46, 123.09, 122.87, 113.35, 113.11, 110.48, 107.55, 107.07, 71.28, 71.04, 34.40, 29.10, 28.19, 27.21, 27.08, 26.91, 26.80, 26.68, 26.28, 26.03, 21.85, 21.78, 21.39, 21.26, 13.81. (observed complexity is due to *C–P* coupling). **^{31}P NMR (121 MHz, CD_2Cl_2)** δ 31.10. **^{19}F NMR (376 MHz, CD_2Cl_2)** δ -119.28. **FT-IR (neat, cm^{-1}):** 2927.02, 2849.68, 1590.32, 1569.92, 1536.95, 1464.97, 1448.21, 1387.36, 1376.81, 1276.85, 1250.56, 1184.18, 1112.72, 1062.63, 861.73, 831.04, 766.54, 748.1, 737.28, 709.96, 576.55. **HRMS (ESI) m/z calcd. for $C_{66}H_{89}Cl_2FO_4P_2Pd_2$ [M-Cl]$^+$:** 1319.3496. Found: 1319.3490.



OA-D

Following the general procedure, a mixture containing 1,4-Dibromo-2,5-difluorobenzene (36 mg, 0.125 mmol), RuPhos (128 mg, 0.275 mmol), and (1,5-COD)Pd(CH_2TMS)$_2$ (107 mg, 0.275 mmol) was stirred at rt in cyclohexane (1.5 mL) for 12 h. General work-up afforded **OA-D** as a grey solid (150 mg, 85%).

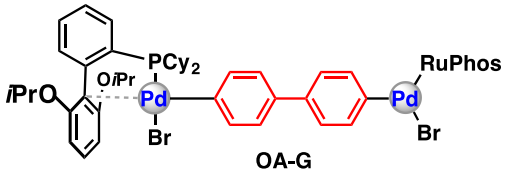
^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.74 (q, *J* = 8.1 Hz, 1H), 7.66 (t, *J* = 7.5 Hz, 1H), 7.48 (dd, *J* = 18.1, 8.6 Hz, 2H), 7.25 (t, *J* = 8.3 Hz, 1H), 7.08 (dd, *J* = 6.5, 2.3 Hz, 1H), 6.94 – 6.83 (m, 1H), 6.76 (d, *J* = 9.8 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 6.62 (t, *J* = 8.9 Hz, 1H), 4.64 (dt, *J* = 12.2, 5.8 Hz, 2H), 4.45 (dt, *J* = 12.1, 6.0 Hz, 1H), 2.32 – 2.18 (m, 1H), 1.83 (br, 1H), 1.63 (s, 4H), 1.47 (br, 8H), 1.37 (d, *J* = 6.0 Hz, 3H), 1.13 (d, *J* = 6.0 Hz, 3H), 1.05 (d, *J* = 6.0 Hz, 2H), 0.98 (d, *J* = 6.0 Hz, 2H), 0.13 (s, 1H).**^{13}C NMR (101 MHz, CD_2Cl_2)** δ 156.71, 136.37, 130.79, 128.21, 71.88, 71.12, 70.58, 37.54, 36.88, 26.91, 26.65, 26.52, 26.24, 25.95, 22.28, 21.82, 21.62, 21.43, 21.24, 0.75 (observed complexity is due to *C–P* coupling). **^{31}P NMR (121 MHz, CD_2Cl_2)** δ 31.81. **^{19}F NMR (376 MHz, CD_2Cl_2)** δ -117.91. **FT-IR (neat, cm^{-1}):** 2927.34, 2850.02, 1590.07, 1451.45, 1385.02, 1374.65, 1277.87, 1251.54, 1135.22, 1110.91, 1061.23, 866.25, 850.62, 765.31, 753.05, 737.00, 609.04. **Anal. Calcd. for $C_{66}H_{88}Br_2F_2O_4P_2Pd_2$:** C, 55.90; H, 6.26. Found: C, 55.90; H, 6.37.



OA-E

Following the general procedure, a mixture containing 1,4-Bis(trifluoromethyl)-2,5-dichlorobenzene (32 mg, 0.125 mmol), RuPhos (128 mg, 0.275 mmol), and (1,5-COD)Pd(CH_2TMS)$_2$ (107 mg, 0.275 mmol) was stirred at rt in cyclohexane (1.5 mL) for 12 h. General work-up afforded **OA-E** as a grey solid (132 mg, 83%).

^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.84 – 7.71 (m, 1H), 7.62 (q, *J* = 7.6 Hz, 1H), 7.45 (dq, *J* = 21.5, 7.1 Hz, 3H), 7.09 (s, 1H), 7.00 (s, 1H), 6.84 (dd, *J* = 16.4, 7.6 Hz, 1H), 6.65 (dd, *J* = 23.7, 8.5 Hz, 2H), 4.63 (dp, *J* = 18.1, 6.0 Hz, 3H), 1.82 (br, 8H), 1.48 (br 3H), 1.35 (s, 2H), 1.17 (s, 2H), 0.91 (dd, *J* = 17.4, 6.0 Hz, 4H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ δ 161.59, 161.35, 161.00, 160.94, 145.63, 145.45, 136.82, 136.30, 134.86, 134.46, 133.90, 132.23, 132.11, 131.90, 130.76, 130.73, 130.58, 126.43, 126.37, 107.85, 106.75, 106.65, 106.52, 106.36, 72.20, 71.63, 70.25, 70.16, 35.06, 34.78, 31.80, 31.54, 30.18, 27.41, 27.05, 26.91, 26.79, 26.72, 26.55, 26.50, 26.43, 26.34, 26.00, 25.86, 25.53, 25.45, 21.95, 21.81, 21.74, 21.35, 21.27, 21.23, 21.10. (observed complexity is due to *C–P* coupling). **^{31}P NMR (121 MHz, CD_2Cl_2)** δ 32.03. **^{19}F NMR (376 MHz, CD_2Cl_2)** δ -54.53. **FT-IR (neat, cm^{-1}):** 3855.14, 3745.16, 2923.80, 2856.75, 1589.64, 1560.57, 1444.39, 1334.44, 1252.70, 1154.35, 1093.12, 1067.17, 1047.83, 1020.47, 766.65**. HRMS (ESI) m/z calcd. for $C_{68}H_{89}Cl_2O_4P_2F_6Pd_2$ [M-Cl]$^+$:** 1391.3820. Found: 1391.3881.

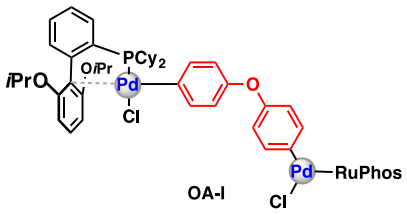


OA-G

Following the general procedure, a mixture containing 4,4-Dibromobiphenyl (39 mg, 0.125 mmol), RuPhos (128 mg, 0.275 mmol), and (1,5-COD)Pd(CH_2TMS)$_2$ (107 mg, 0.275 mmol) was stirred at rt in cyclohexane (1.5 mL) for 12 h. General work-up afforded **OA-G** as a grey solid (209 mg, 98%).

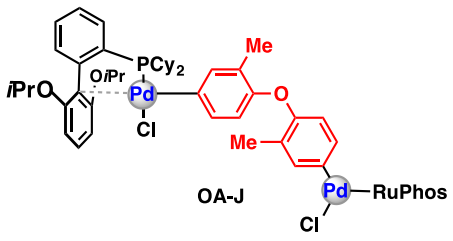
^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.63 (t, *J* = 8.4 Hz, 1H), 7.41 (dt, *J* = 23.5, 7.4 Hz, 1H), 7.16 (d, *J* = 8.1 Hz, 1H), 7.09 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.87 (dd, *J* = 7.8, 2.9 Hz, 0H), 6.65 (d, *J* = 8.5 Hz, 1H), 4.62 (p, *J* = 6.0 Hz, 1H), 2.18 (q, *J* = 12.3, 11.8 Hz, 1H), 1.71 (d, *J* = 52.0 Hz, 1H), 1.52 (s, 1H), 1.40 (d, *J* = 6.0 Hz, 3H), 1.21 (b, 1H), 1.03 (d, *J* = 6.0 Hz, 3H), 0.78 (q, *J* = 13.4 Hz, 1H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 159.02, 144.84, 144.67, 137.58, 137.55, 136.27, 134.46, 134.39,

133.71, 133.36, 132.48, 132.38, 131.04, 130.47, 130.44, 126.26, 126.21, 124.85, 124.83, 111.52, 111.48, 107.25, 70.86, 33.85, 33.59, 28.14, 27.99, 27.61, 27.59, 27.23, 27.10, 26.92, 26.88, 26.77, 26.02, 21.89, 21.38 observed complexity is due to *C–P* coupling**).** **^{31}P NMR (121 MHz, CD_2Cl_2)** δ 31.80. **FT-IR (neat, cm^{-1}):** 2976.53, 2920.64, 2849.62, 2354.67, 1588.59, 1455.66, 1242.77, 1110.89, 1059.83, 996.47, 797.70, 731.52, 667.91, 610.43, 577.71. **HRMS (ESI) m/z calcd. for $C_{72}H_{94}Br_2O_4P_2Pd_2$ [M-2Br]$^{2+}$:** 649.2364. Found: 649.2390.



Following the general procedure, a mixture containing 4-chlorophenyl ether (60 mg, 0.125 mmol), RuPhos (292.8 mg, 0.275 mmol), and (1,5-COD)Pd(CH_2TMS)$_2$ (244 mg, 0.275 mmol) was stirred at rt in cyclohexane (2.4 mL) for 12 h. General work-up afforded **OA-I** as a light brown solid (304.3 mg, 88 %).

^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.67 (q, *J* = 7.7, 7.0 Hz, 1H), 7.45 (dt, *J* = 23.0, 7.4 Hz, 1H), 7.01 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.90 (ddd, *J* = 7.6, 3.0, 1.4 Hz, 1H), 6.67 (dd, *J* = 8.5, 3.7 Hz, 3H), 4.66 (p, *J* = 6.0 Hz, 1H), 2.20 (q, *J* = 12.1 Hz, 2H), 1.81 (br, 3H), 1.61 (br, 1H), 1.42 (d, *J* = 6.0 Hz, 3H), 1.27 (b, 2H), 1.05 (d, *J* = 6.0 Hz, 3H), 0.78 (q, *J* = 12.9 Hz, 1H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 159.16, 154.65 145.05, 144.88, 137.00, 136.96, 134.81, 133.90, 133.54, 132.37, 132.26, 130.95, 130.55, 130.52, 129.45, 126.28, 126.22, 117.46, 117.43, 110.90, 110.87, 106.91, 70.75, 33.67, 33.40, 28.12, 27.71, 27.69, 27.24, 27.11, 26.84, 26.72, 26.00, 21.83, 21.34 (observed complexity is due to *C–P* coupling). **^{31}P NMR (121 MHz, CD_2Cl_2)** δ 33.45. **FT-IR (neat, cm^{-1}):** 2923.66, 2849.80, 1591.49, 1471.28, 1456.00, 1435.99, 1383.09, 1371.62, 1225.85, 1164.77, 1111.35, 1050.67, 1004.19, 848.86, 809.35, 784.21, 758.68, 731.48, 609.16, 575.02, 564.82. **Anal. Calcd. for $C_{72}H_{94}Cl_2O_5P_2Pd_2$:** C, 62.43; H, 6.84. Found: C, 62.33; H, 6.82.



Following the general procedure, a mixture containing 4,4'-oxybis(1-chloro-3-

methylbenzene) (21.9 mg, 0.125 mmol), RuPhos (96.0 mg, 0.275 mmol), and (1,5-COD)Pd($CH_2TMS)_2$ (80.0 mg, 0.275 mmol) was stirred at rt in cyclohexane (0.75 mL) for 12 h. General work-up afforded **OA-J** as a brown solid (86.6 mg, 75 %).

^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.67 (q, *J* = 7.6, 6.7 Hz, 1H), 7.45 (dt, *J* = 23.0, 7.4 Hz, 1H), 6.95 (s, 1H), 6.90 (ddd, *J* = 7.6, 2.9, 1.3 Hz, 1H), 6.78 (d, *J* = 8.3 Hz, 1H), 6.67 (d, *J* = 8.5 Hz, 1H), 6.37 (d, *J* = 8.4 Hz, 1H), 4.66 (p, *J* = 6.0 Hz, 1H), 2.18 (s, 2H), 1.78 (s, 4H), 1.47 (s, 1H), 1.25 (s, 1H), 1.05 (d, *J* = 6.0 Hz, 3H), 0.78 (br, 1H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 159.61, 153.10, 145.62, 145.45, 139.16, 135.24, 134.91, 134.88, 134.86, 134.55, 134.19, 132.94, 132.84, 131.51, 131.06, 131.04, 129.47, 127.70, 126.82, 126.77, 116.76, 111.74, 107.51, 71.32, 34.22, 33.93, 28.69, 27.90, 27.77, 27.48, 27.45, 27.34, 26.64, 22.43, 21.91, 16.36 (observed complexity is due to *C–P* coupling). **^{31}P NMR (121 MHz, CD_2Cl_2)** δ 33.23. **FT-IR (neat, cm^{-1}):** 2972.95, 2927.54, 2852.92, 1592.71, 1453.1, 1382.83, 1371.1, 1267.8, 1230.18, 1173.8, 1112.72, 1054.44, 1002.37, 898.56, 848.98, 803.64, 785.06, 756.54, 735.1, 667.84. **Anal. Calcd. for $C_{74}H_{98}Cl_2O_5P_2Pd_2$:** C, 62.89; H, 6.99. Found: C, 62.77; H, 6.94.

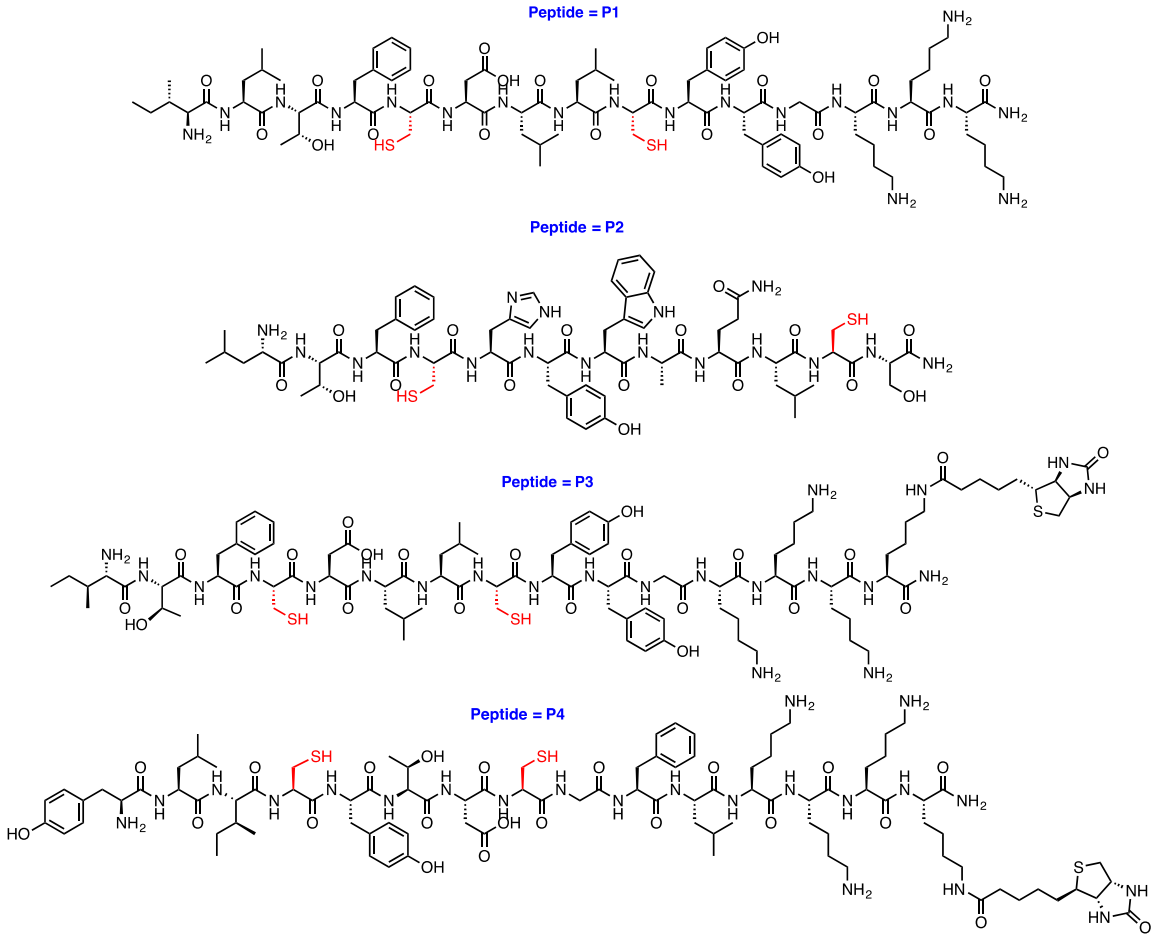
3. Peptide Synthesis and LC-MS Characterization

Linear Peptide Synthesis

General Linear Peptide Synthesis Procedure:

The peptides (**P1**-**P4**) were synthesized on a 0.9 mmol scale using manual Fmoc-SPPS (Solid Phase Peptide Synthesis) chemistry. Specifically, the resin was pre-wetted with DMF in a peptide synthesis vessel equipped with a T-bore stopcock connected to a vacuum pump for faster drainage. Each amino acid (5 mmol) was pre-dissolved in HBTU (0.2 M, 25 mL). The procedure for each amino acid coupling cycle included: 1) pre-activation of the amino acid with DIEA (2.5 mL; note, 0.9 mL was used in the case of cysteine to prevent racemization); 2) 10 min coupling time; 3) Wash with DMF (4 × 30 mL; or enough DMF to cover the resin); 4) Deprotection with 20% (v/v) piperidine in DMF (2 × 3 min; 30 mL each time, or enough of the solution to cover the resin); 5) Wash with DMF (4 × 30 mL; or enough DMF to cover the resin). After completion of the stepwise SPPS, the resin was washed thoroughly with CH_2Cl_2 and dried under vacuum. Peptides **P1, P2, P3,** and **P4** were cleaved from the resin and deprotected on the side-chains by treatment with 2.5% (v/v) water, 2.5% (v/v) 1,2-ethanedithiol (EDT), and 1% (v/v) triisopropylsilane in neat trifluoroacetic acid (TFA) for 7 min at 60 °C (water bath, 5 mL cleavage solution/250 mg of final resin). Cold (–80 °C) diethyl ether was added to the solution and the

resulting precipitate was centrifuged at 4000 rpm for 5 min. The residue was washed with cold ether (2x). The obtained solid was dissolved in 50% H_2O : 50% acetonitrile containing 0.1% TFA, the resin was removed via filtration and the final solution was lyophilized to provide the crude peptide material. For peptides containing biotin, the unnatural amino acid Fmoc-Lys(biotin)-OH was used as the coupling amino acid. The following peptides were all synthesized using this procedure:



Peptide Purification

Solvent compositions for RP-HPLC purification are water with 0.1% TFA (solvent C) and acetonitrile with 0.1% TFA (solvent D). The crude peptide was dissolved in 50% C : 50% Dand purified by semi-preparative RP-HPLC (Agilent Zorbax 300SB C_{18} column: 21.2 x 250 mm, 7 µm, linear gradient: 5-50% B over 65 min, flow rate: 5 mL/min). Each HPLC fraction was analyzed by mass-directed preparative LC-MS. HPLC fractions containing pure product were further confirmed by LC-MS, combined, and lyophilized.

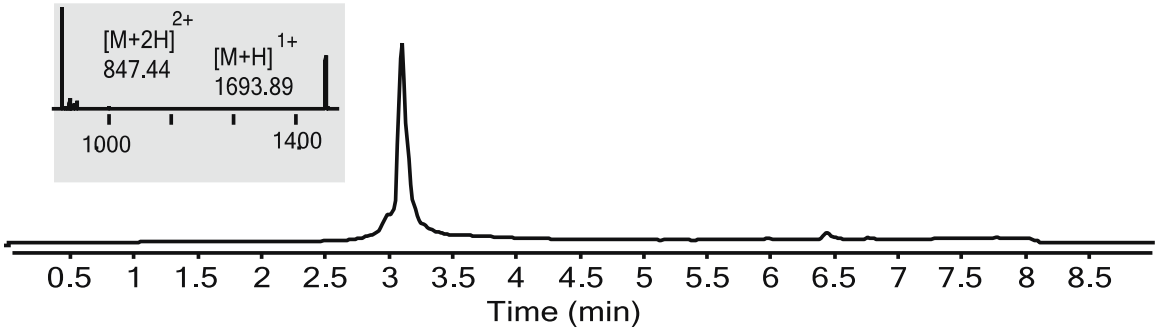
Peptides synthesized using manual SPPS and purified by RP-HPLC are listed in Table S1.

Table S1. **Sequences and masses of peptides synthesized by manual fast flow SPPS.**

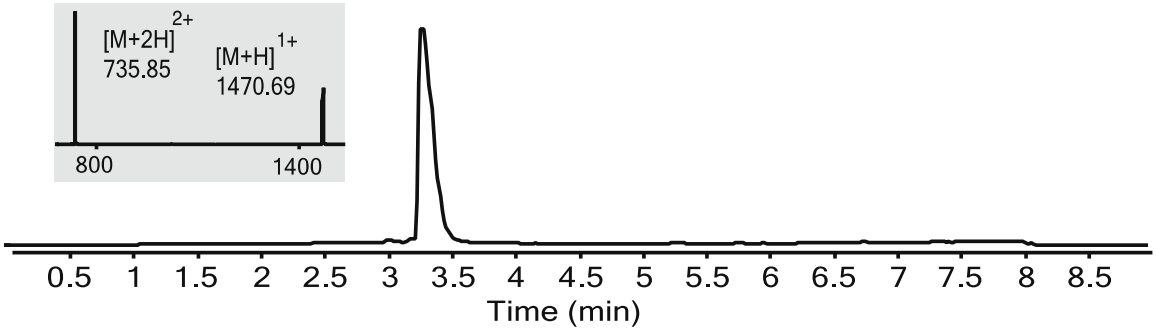
Peptide	Sequence[a]	Calculated mass	Observed Mass $[M+H]^+$ or $[M+H]^{2+}$
P1	NH_2-ITF**C**DLL**C**YYGKKK-$CONH_2$	1692.87	1693.89
P2	NH_2-LTF**C**HYWAQL**C**S-$CONH_2$	1469.66	1470.69
P3	NH_2-ITF**C**DLL**C**YYGKKKK(Biotin)-$CONH_2$	2047.05	1025.04
P4	NH_2-YLI**C**YTD**C**GFLKKKK(Biotin)-$CONH_2$	2047.05	1025.05

[a] Cysteine residues are highlighted in red.

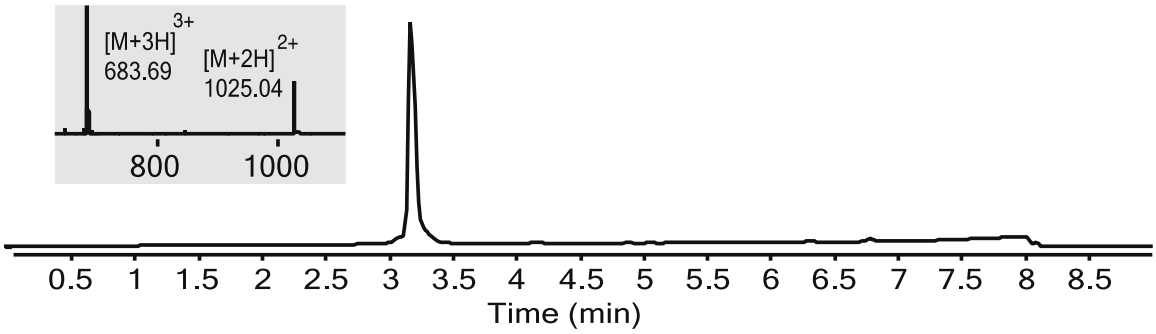
P1:



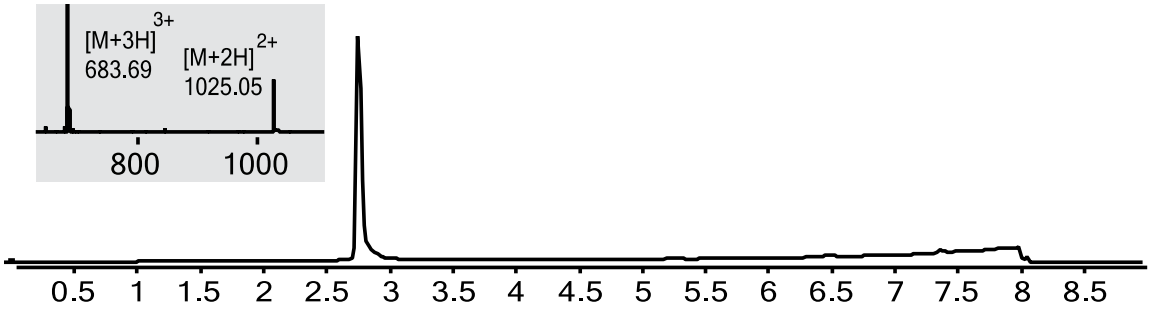
P2:



P3:

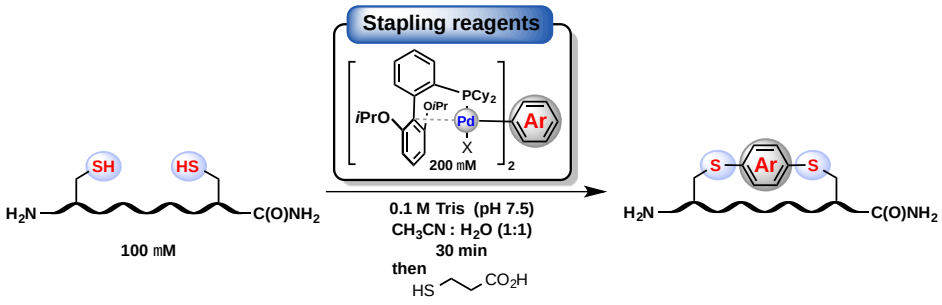


P4:



4. MACROCYCLISATION REACTIONS AND LC-MS CHARACTERIZATION

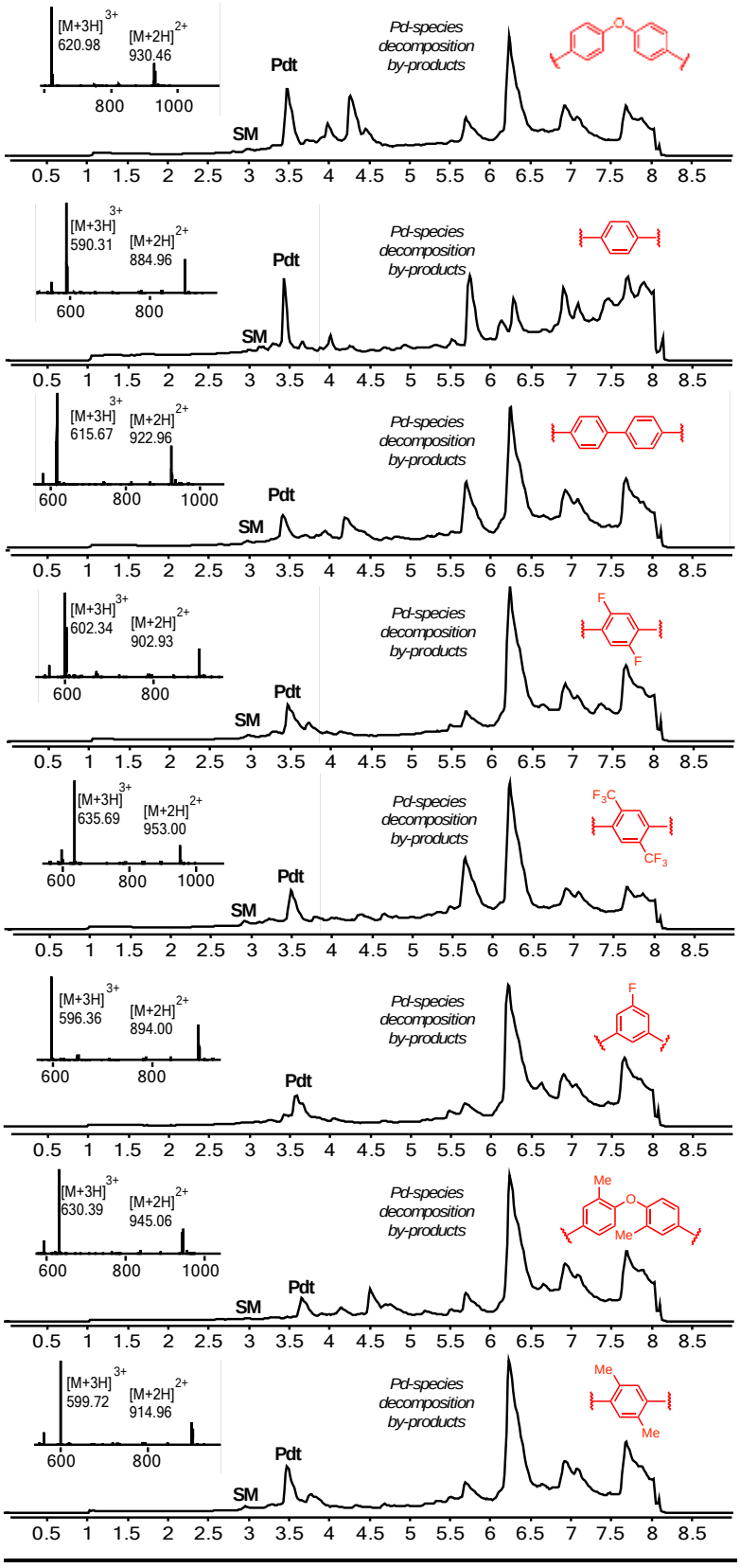
General Macrocyclisation Procedure



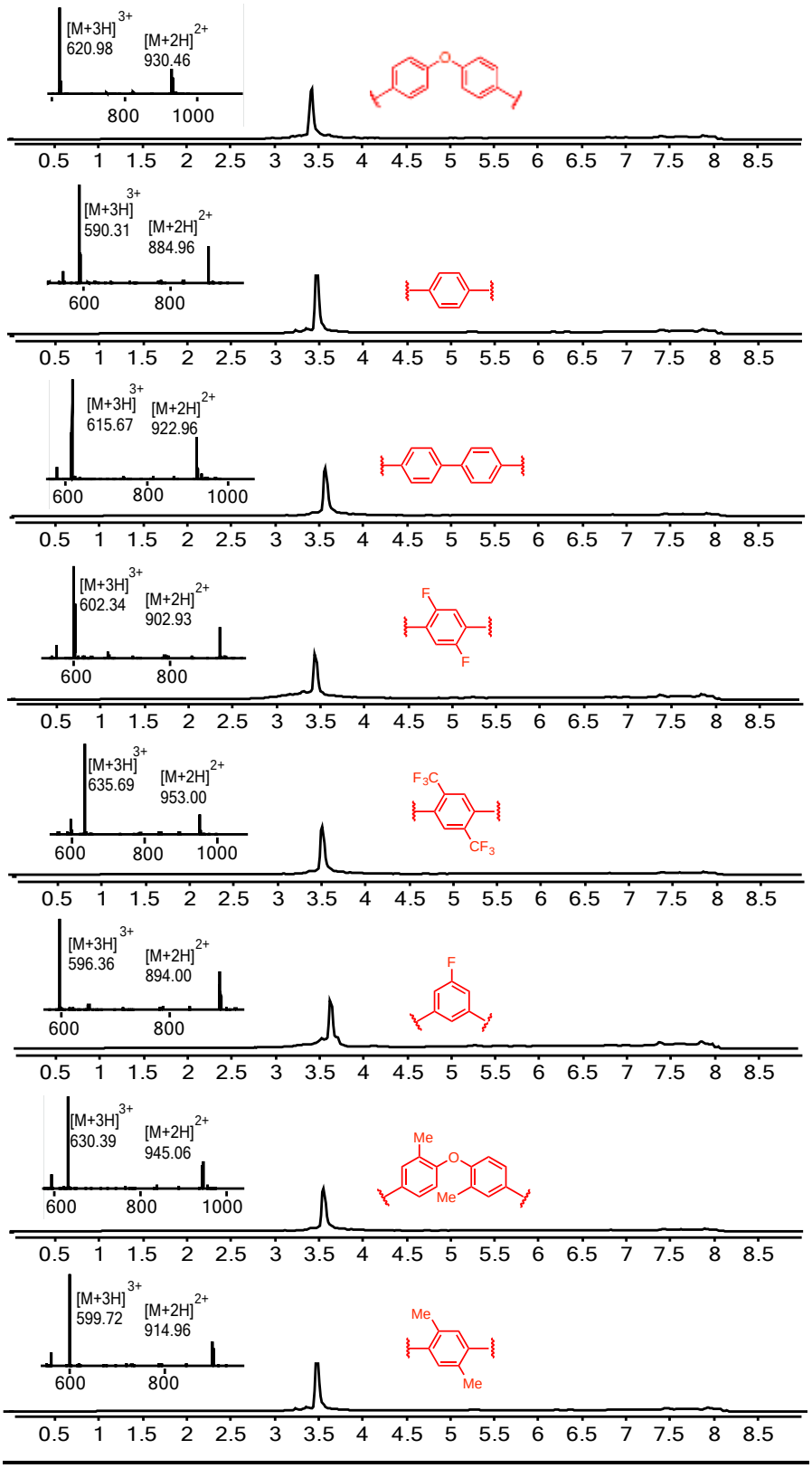
A solution of the Pd-stapling reagent in acetonitrile (400 μM) was added to a solution of peptide (200 μM) in Tris buffer (0.1 M, pH 7.5). Note: if the palladium reagent was

not readily soluble in CH_3CN, the slurry was sonicated for 30 s to facilitate this process. Final conditions: [Pd] = 200 μM; [peptide] = 100 μM; H_2O : CH_3CN = 1 : 1. After 30 min stirring at rt, 3-mercaptopropionic acid (3 equiv to the palladium complex, solution in 1 mL of H_2O) was added to the reaction mixture to quench and remove some of the remaining palladium species. The reaction was allowed to stand for 1 h for precipitate formation, the solids were centrifuged at 4000 rpm for 5 min followed by washing with an additional 5 mL of 50 % : 50 % CH_3CN : H_2O (v/v). The supernatant was collected via decantation and the acetonitrile was removed via rotary evaporation and the resulting crude material was lyophilized. The crude peptide was purified using preparative HPLC as described above.

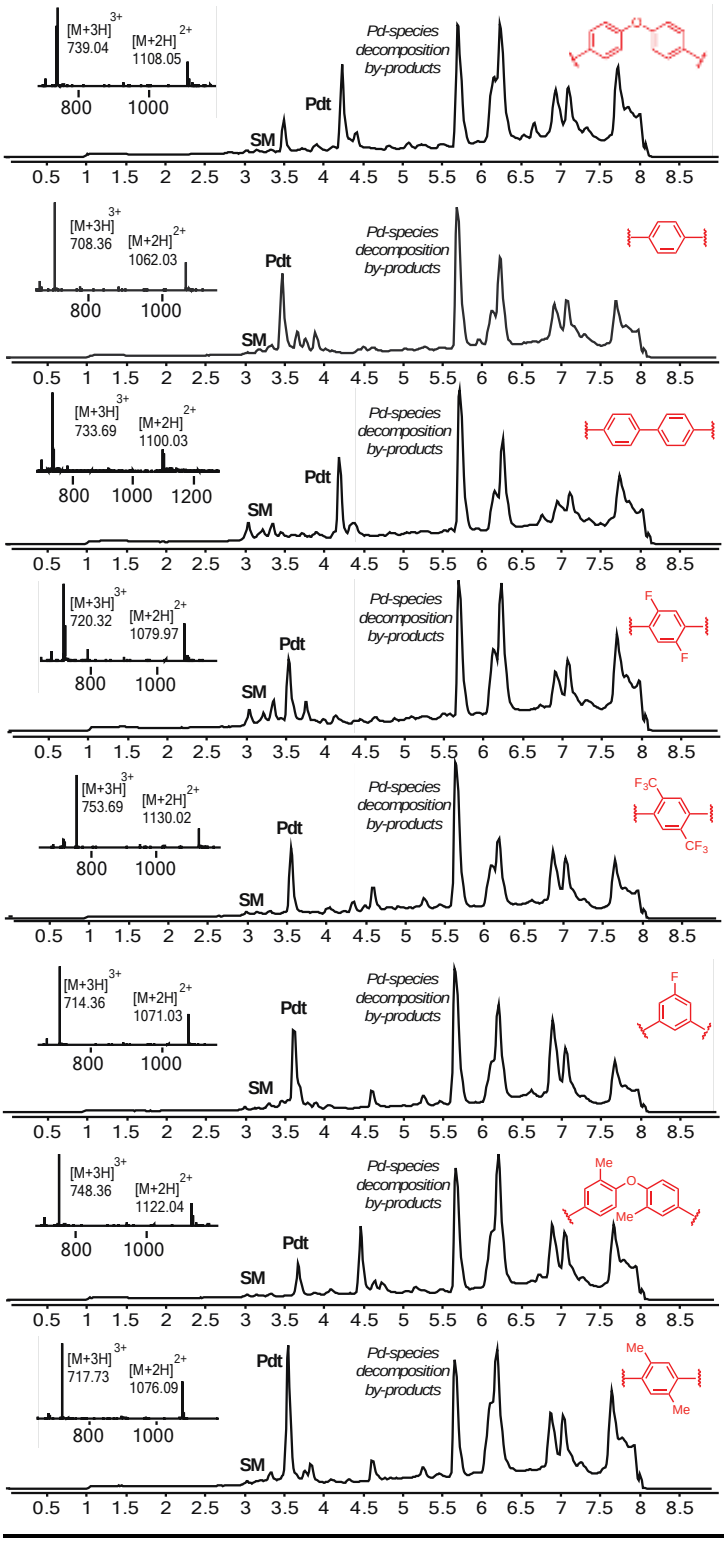
LC-MS Traces of Crude Reaction Mixtures of Macrocyclisation Experiments (for P1)



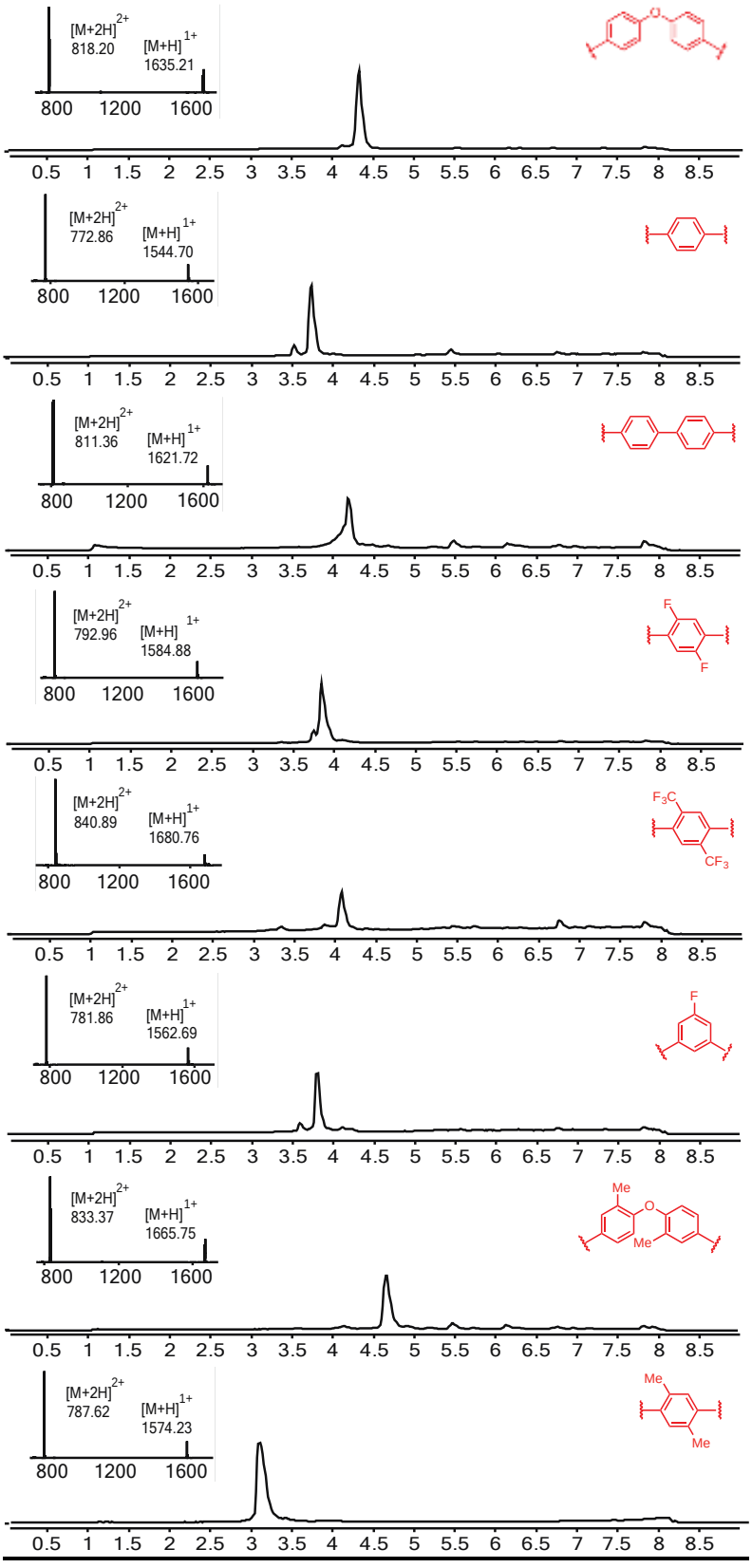
LC-MS Traces for Isolated Pure Macrocyclic Peptides (for P1)



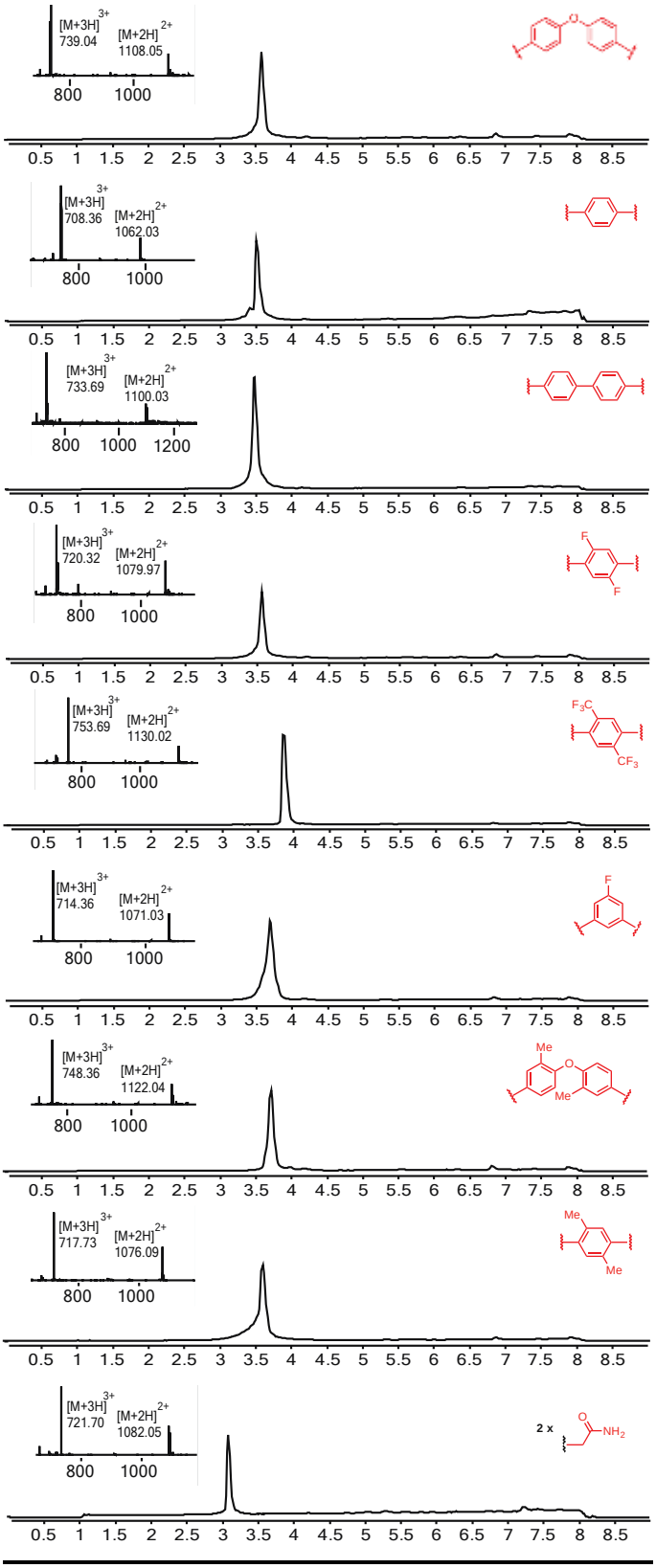
LC-MS Traces of Crude Reaction Mixtures of Macrocyclisation Experiments (for P2)



LC-MS Traces for Isolated Pure Macrocyclic Peptides (for P2)

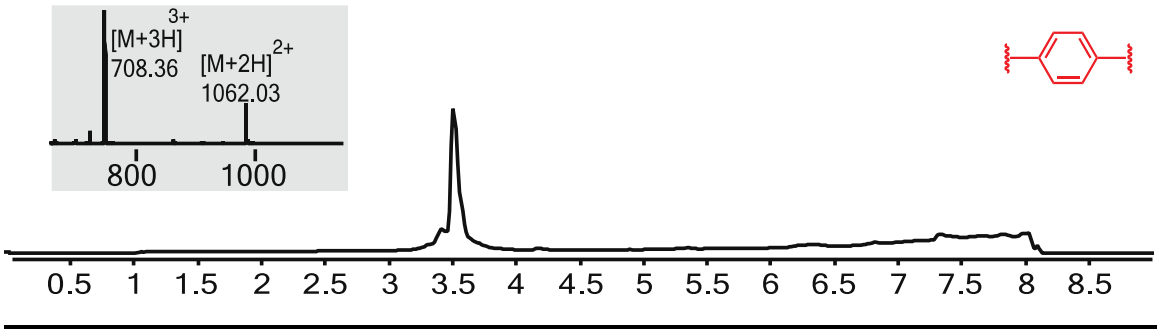


LC-MS Traces for Isolated Pure Macrocyclic Peptides (for P3)

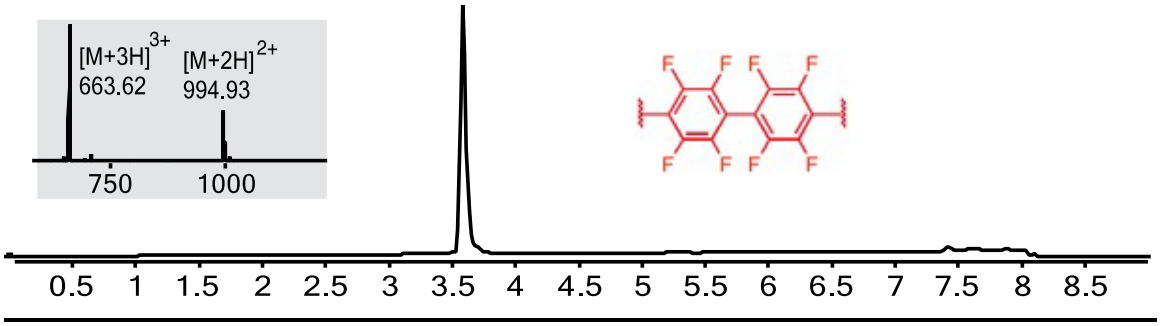


LC-MS Traces for Isolated Pure Macrocyclic Scrambled Peptides (for P4)

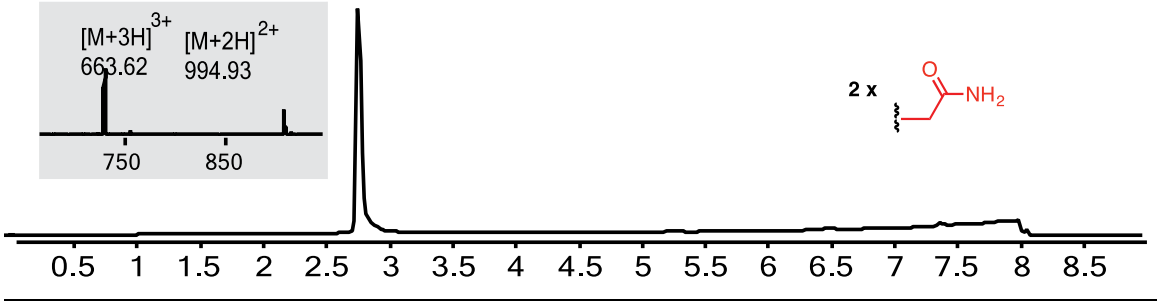
P4-A



P4-H



P4-K



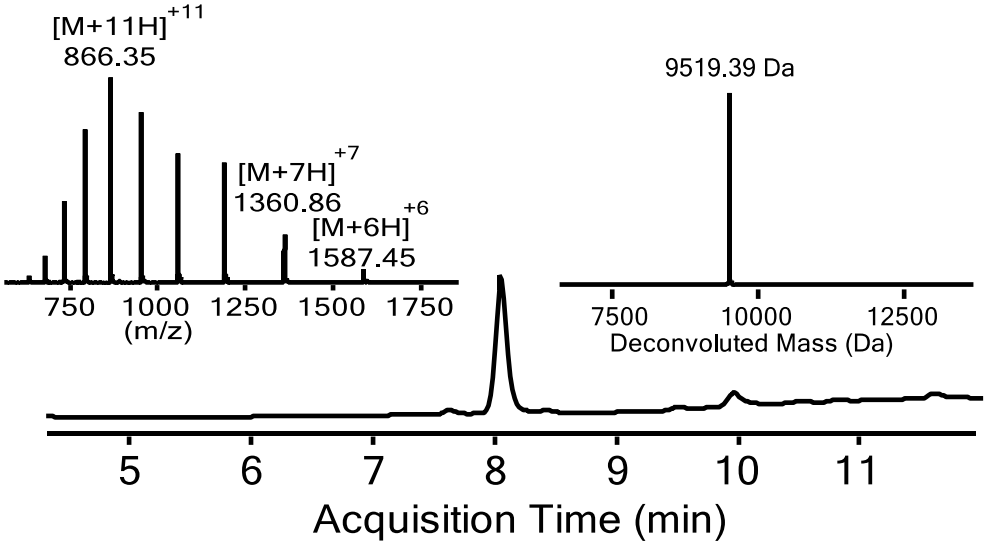
5. C-CA PROTEIN EXPRESSION

Full-length HIV1 Core Antigen was obtained from Invitrogen. C-CA sequence was cloned into pET-SUMO plasmid and ligated with T4 ligase overnight at 4 °C. The resulting construct was transformed into DH5α cells and then purified with the QIAprep Spin Miniprep Kit. BL21 DE3 competent cells were transformed via heat shock with 100 ng of plasmid containing properly oriented C-CA inserts. Single transformed colonies were grown in 1L LB broth containing 30 μg/mL kanamycin. The cultures were induced with 1 mL 0.4 M IPTG upon reaching OD_{600} = 0.6.

Following overnight expression, cells were pelleted and resuspended in 50 mL Tris buffer (50 mM Tris, 150 mM NaCl, pH 7.4) containing 20 mg lysozyme, 2 mg DNAse I, and a half tablet of cOmplete Mini protease inhibitor cocktail (Roche Life Science). Following sonication, the suspension was pelleted (25 min, 17000 rpm) and supernatant loaded onto a Ni-NTA column (HisTrap FF crude, GE Healthcare). The column was washed with 30 mL of Tris buffer (pH 8.0) and 40 mM imidazole in Tris buffer (pH 8.0) each and the SUMO-CCA fusion protein was eluted in 10 mL 150 mM imidazole in Tris buffer (pH 8.0). The protein was then subject to buffer exchange on Akta FPLC into Tris buffer at pH 7.4.

Protein concentration was determined by measuring absorbance at 280 nm (MW = 22916.9 Da, ε = 9970 as found on ExPASy ProtParam (http://web.expasy.org/protparam/)); SUMO cleavage was subsequently performed by incubating SUMO-CCA overnight at 4 °C with 1 ng SUMO protease per 1 mg protein. The resulting mixture was loaded onto a Ni-NTA column and washed with 10 mL 40 mM imidazole in Tris buffer (pH 8.0) to isolate C-CA. The protein was subjected to buffer exchange into Tris buffer at pH 7.4, concentrated over a 3000 Da Amicon Ultra-15 Centrifugal Filter Unit (EMD Millipore), analyzed by HPLC-MS, and flash frozen in liquid nitrogen for long-term storage at -80 °C. This procedure was obtained based on a previously reported synthesis.[3]

Figure S2: Deconvoluted spectrum of CCA protein after purification



Calculated molecular weight: 9518.9 Da. Found: 9519.39 Da.

6. BIOLAYER INTERFEROMETRY BINDING

Experimental Design

Binding kinetics for the **C-CA/P3-A – P3-K** interaction were measured on Octet RED96 instrument from ForteBio. All measurements were performed at 30° C in a 96-well plate shaking at 1000 rpm. In general, streptavidin-coated tips (from ForteBio) were dipped in binding buffer (1x phosphate buffered saline, 0.1% w/v bovine serum albumin, 0.02% tween-20, pH 7.4) for 10 min to remove sucrose coating. A typical experiment consisted of five steps:

- Baseline reading. Tips were dipped into a well containing fresh binding buffer for 60 sec.

- Ligand loading. ~45 μM solutions of **P3,4-A – P3,4-K** in binding buffer were associated onto the tips for 90-300 sec.

- Baseline reading #2. Tips were dipped into a well containing fresh binding buffer for 90-120 sec.

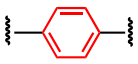
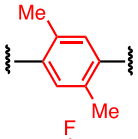
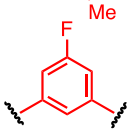
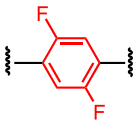
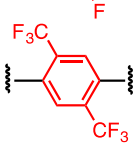
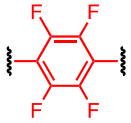
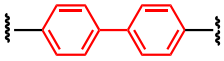
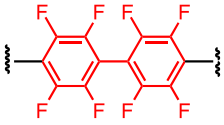
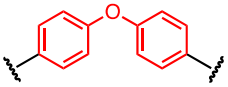
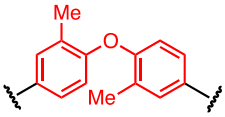
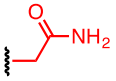
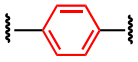
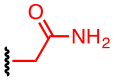
- Association. **P3** and **P4** loaded tips were dipped into wells containing variable concentrations of **C- CA** (5 μM – 100 μM) in binding buffer for 90-300 sec.

- Dissociation. The tips were dipped back into wells containing binding buffer only for 300 sec or until the response decreased back to the base line level. Double referencing using wells containing no **C-CA** and tips loaded with no ligands was used to subtract the background.

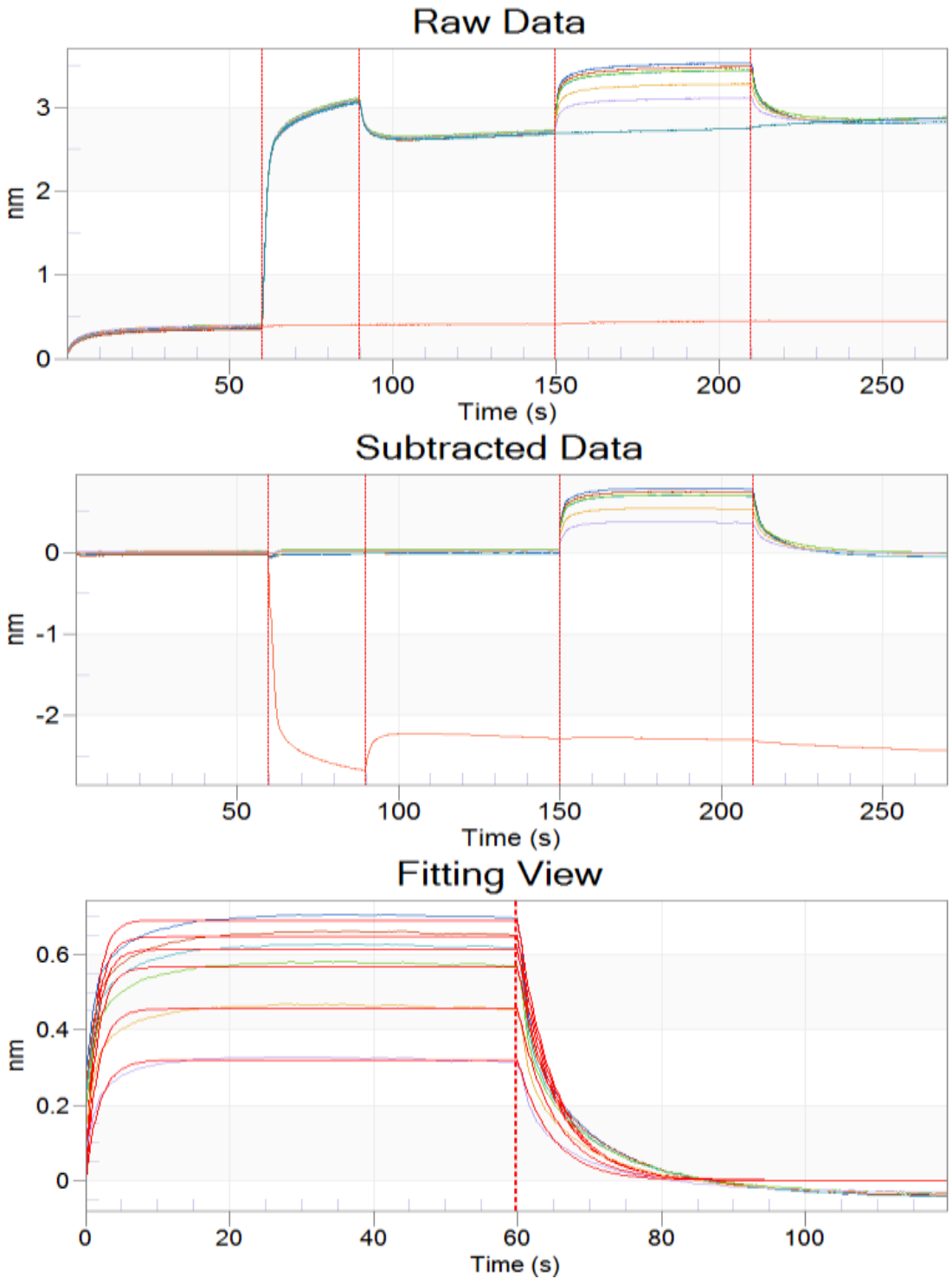
Data Analysis

Binding data collected for peptides **P3-A-K** and **P4-A, K** according to the procedure described in the section above was processed as follows. Reference readings were subtracted universally from both association and dissociation curves. Association curves were aligned to the last five sec of the base line, and dissociation curves were aligned to the last value of association curves. No curve-smoothing filtering was performed. The K_D values reported were calculated using the automated software for the Forte Bio-Layer Interferrometry software manual. The curves and subsequent fittings are provided below for each peptide, as well as a table with the calculated binding values.

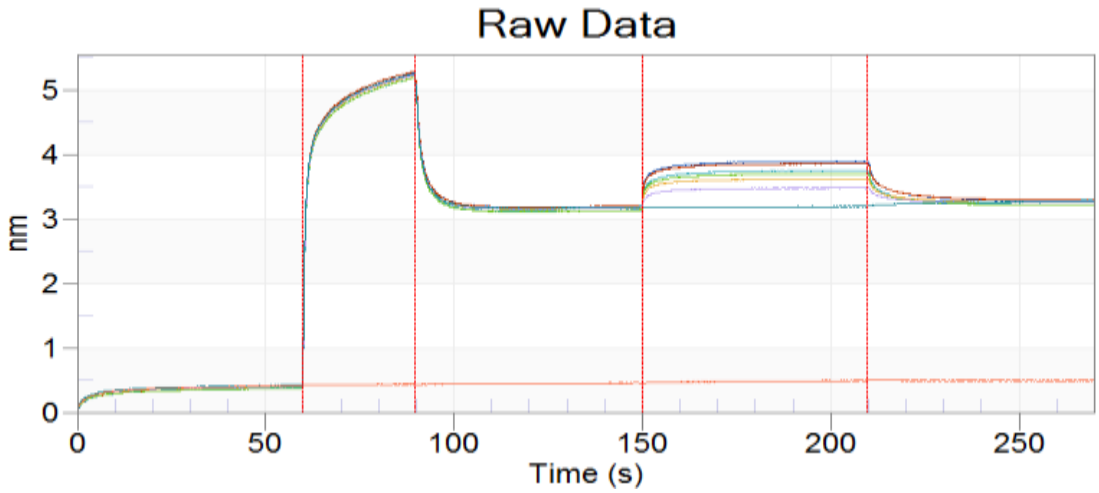
Table S2. Binding kinetics for the **C-CA/P3-A – P3-K** interaction were measured on Octet RED96 instrument from ForteBio.

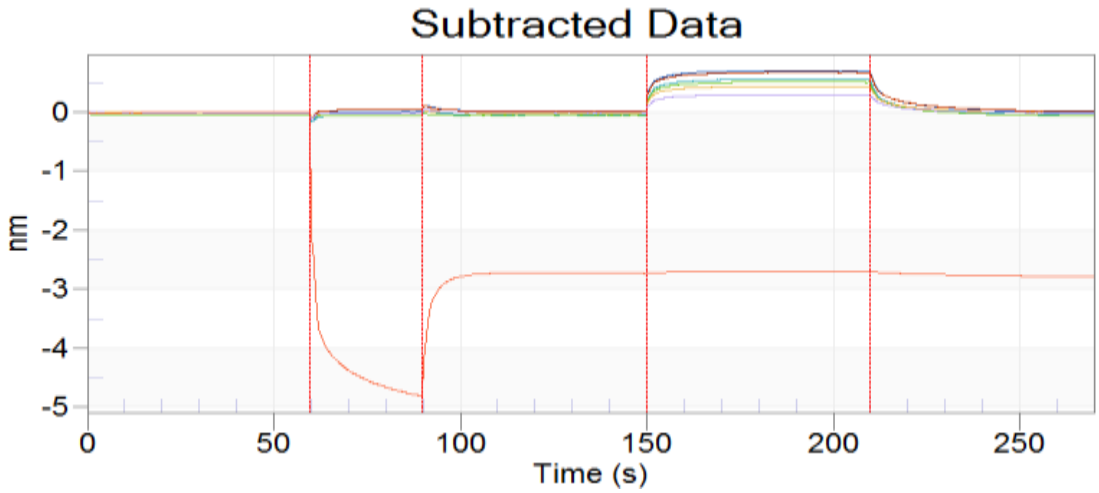
Peptide	Cross-linker	K_D (μM)
P1-A		9.2± 0.4
P1-B		9.7 ± 0.4
P1-C		21.0 ± 2.4
P1-D		9.5 ± 0.5
P1-E		12.0 ± 0.8
P1-F		12.0 ± 0.6
P1-G		61 ± 35
P1-H		>100
P1-I		56 ± 21
P1-J		>100
P1-K	2 x 	5.5 ± 0.6
P4-A		>100
P4-K	2 x 	>100

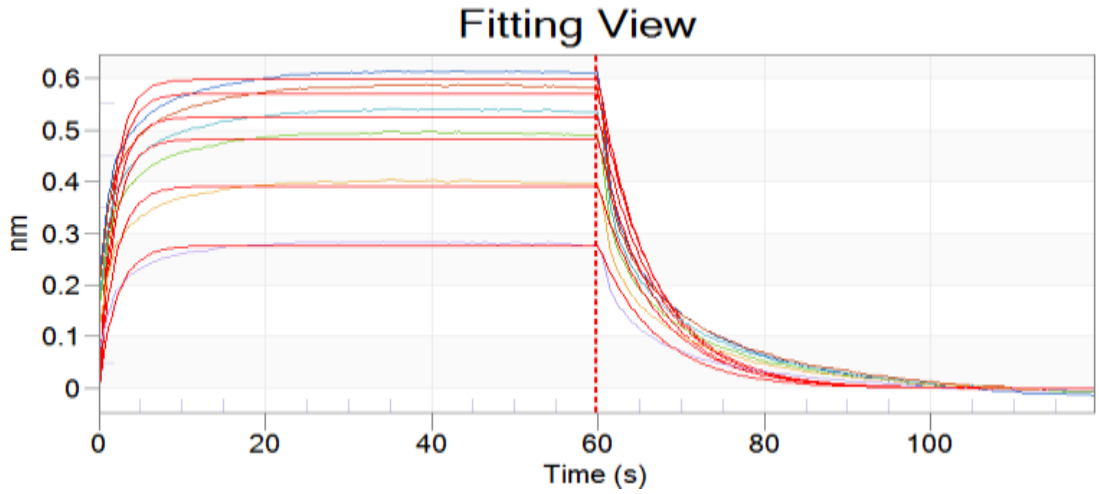
P1-A


Raw Data
nm
Time (s)
Subtracted Data
nm
Time (s)
Fitting View
nm
Time (s)

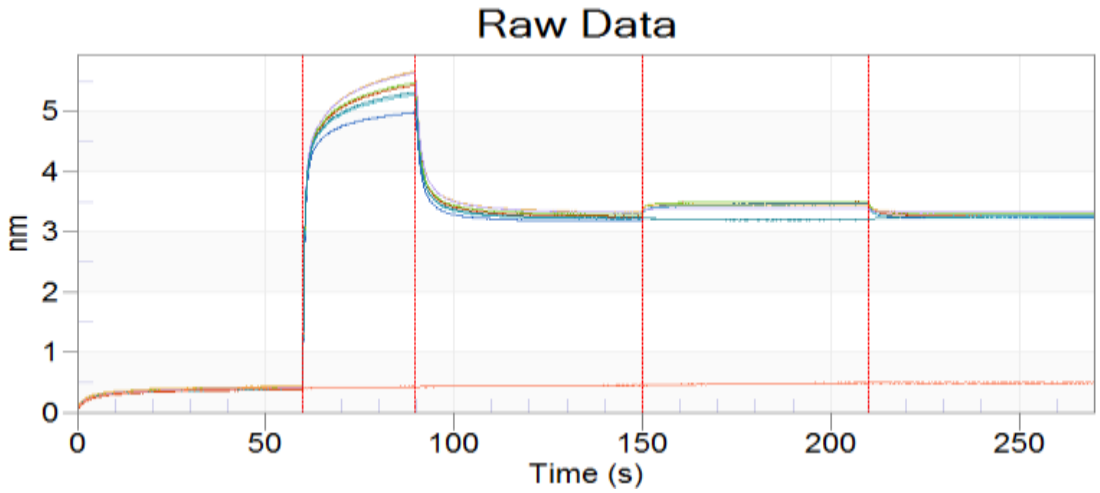
P1-B


Raw Data
nm
Time (s)


Subtracted Data
nm
Time (s)


Fitting View
nm
Time (s)

P1-C


Raw Data
nm
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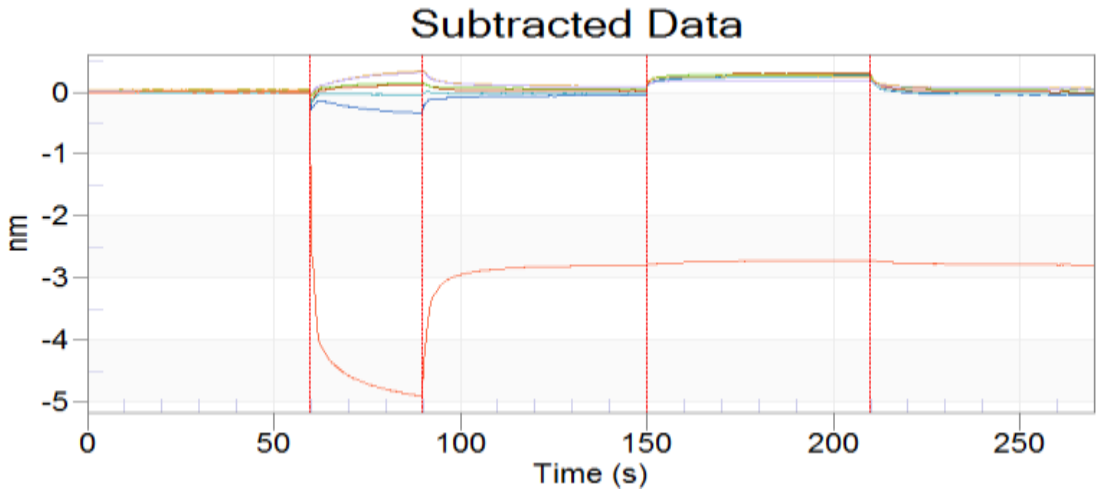
4
3
2
1
0
0
50
100
150
200
250
Time (s)


Subtracted Data
nm
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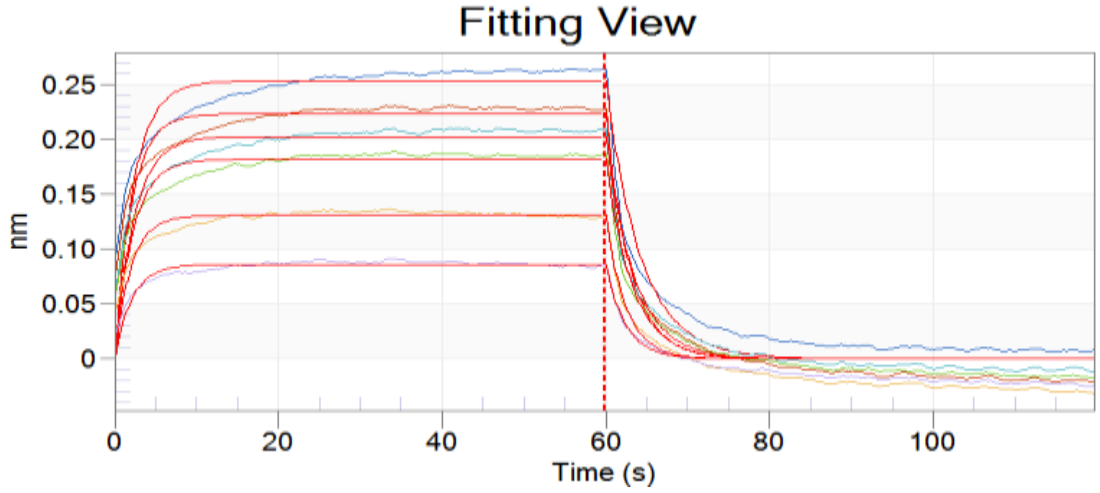
-1
-2
-3
-4
-5
0
50
100
150
200
250
Time (s)


Fitting View
nm
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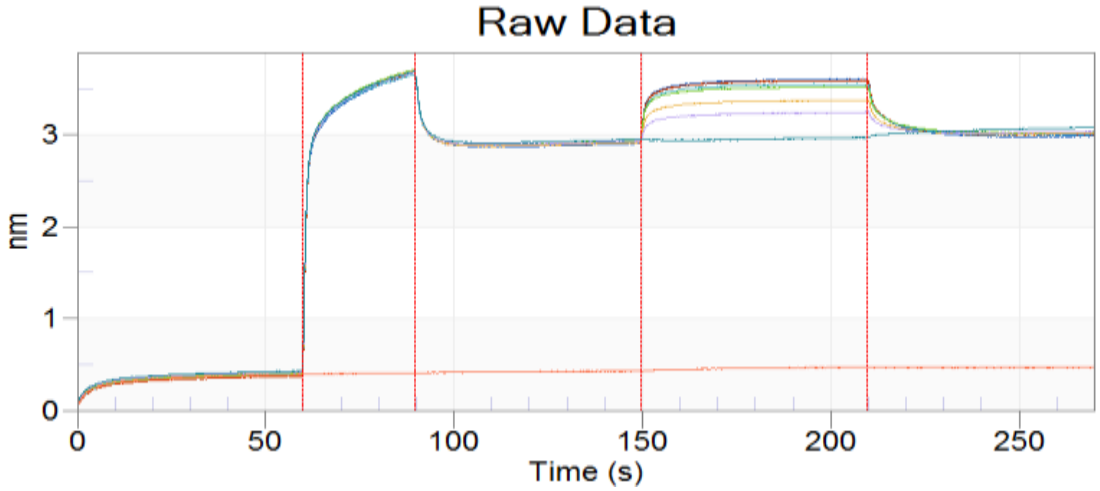
0.20
0.15
0.10
0.05
0
0
20
40
60
80
100
Time (s)

P1-D


Raw Data
nm
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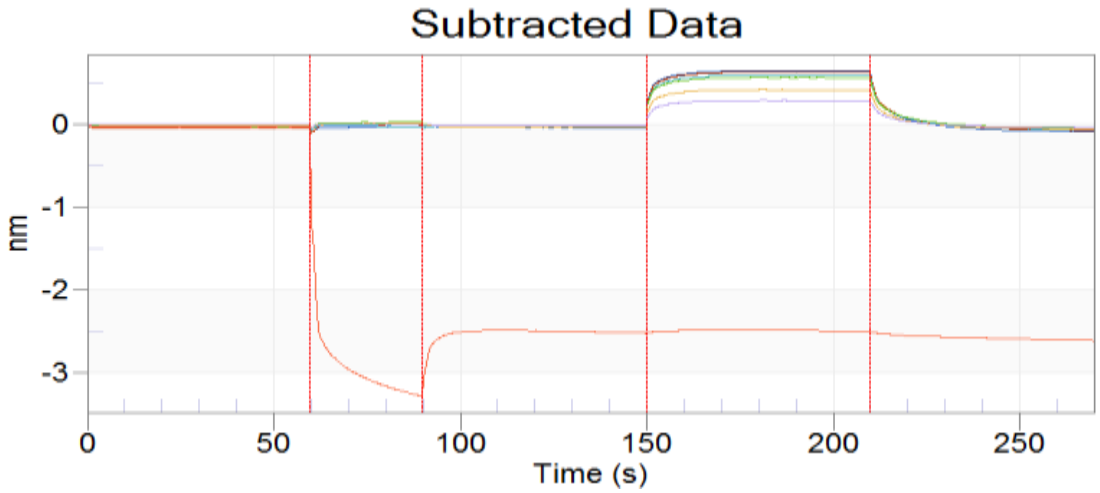
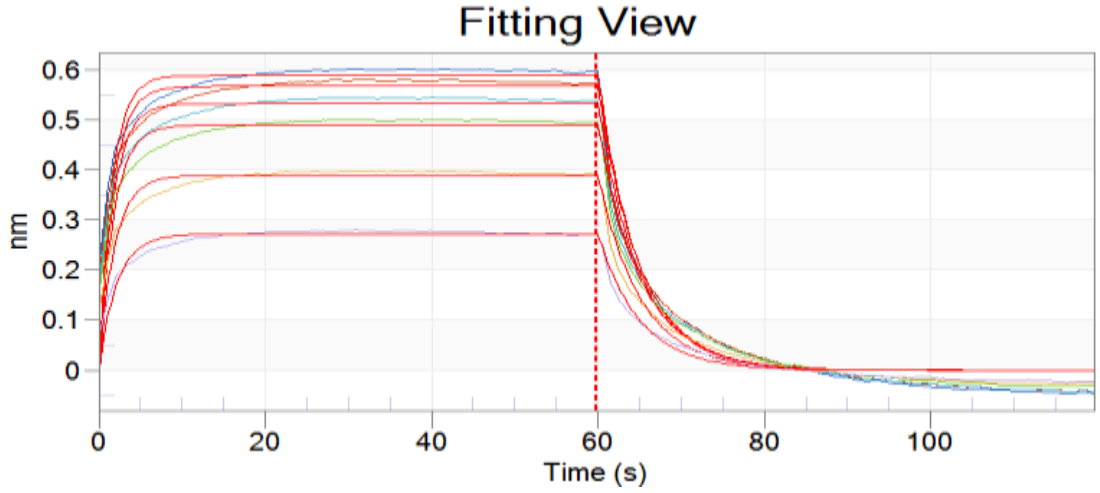
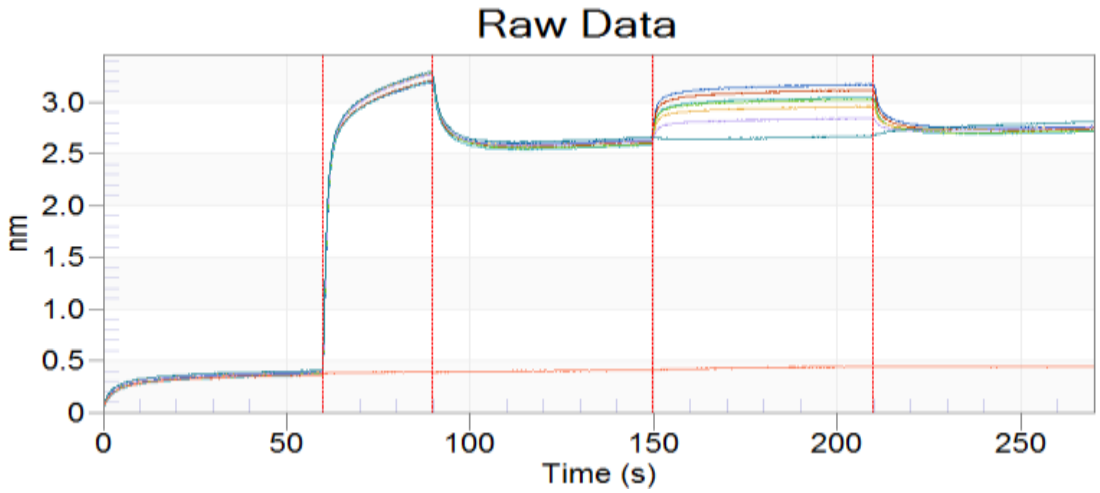
2
1
0
0
50
100
150
200
250
Time (s)


Subtracted Data
nm
0
-1
-2
-3
0
50
100
150
200
250
Time (s)


Fitting View
nm
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
Time (s)

P1-E


Raw Data
nm
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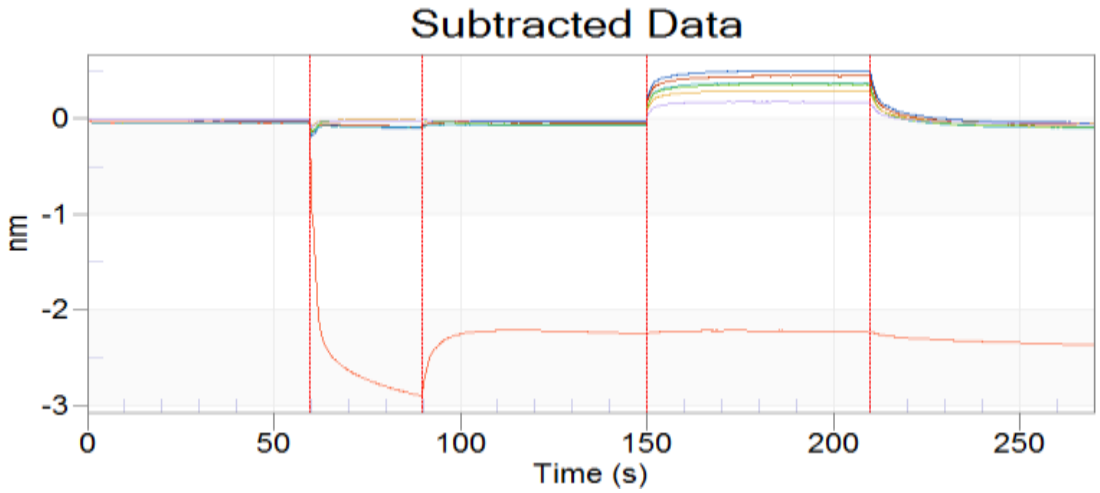
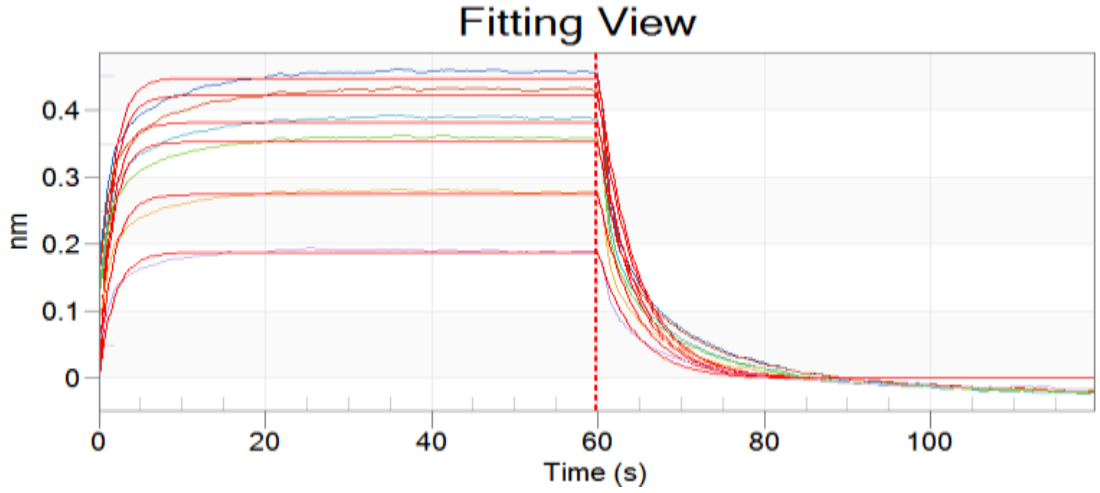
2.5
2.0
1.5
1.0
0.5
0
0
50
100
150
200
250
Time (s)


Subtracted Data
nm
0
-1
-2
-3
0
50
100
150
200
250
Time (s)


Fitting View
nm
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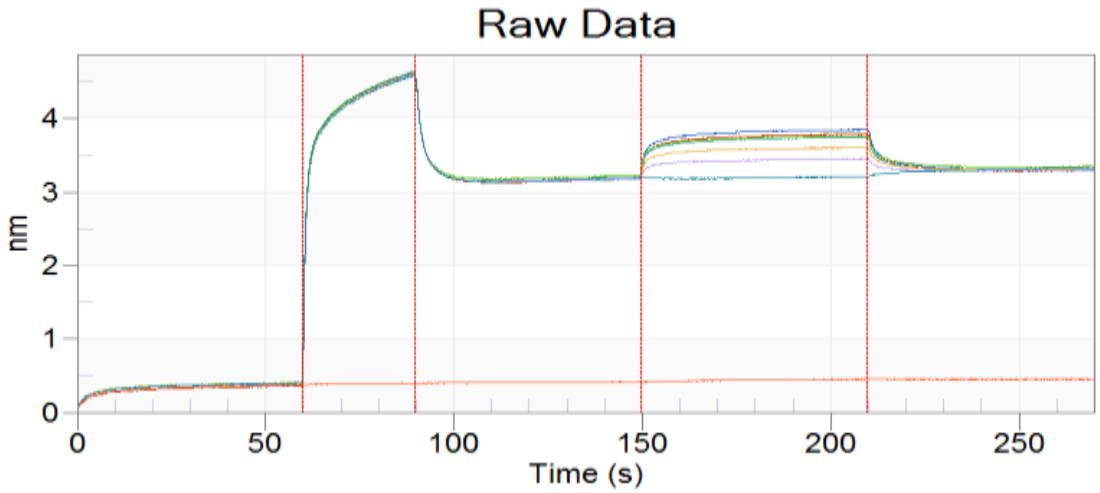
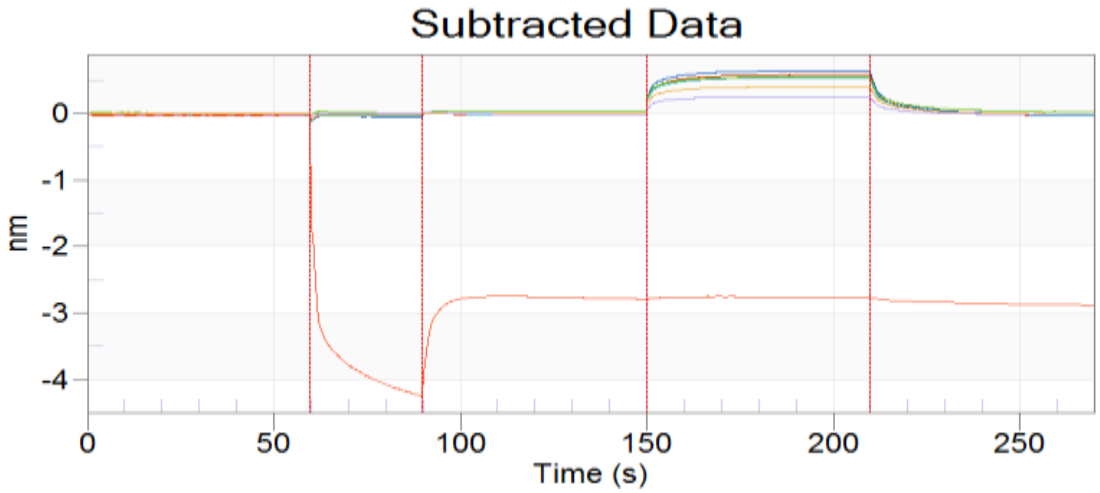
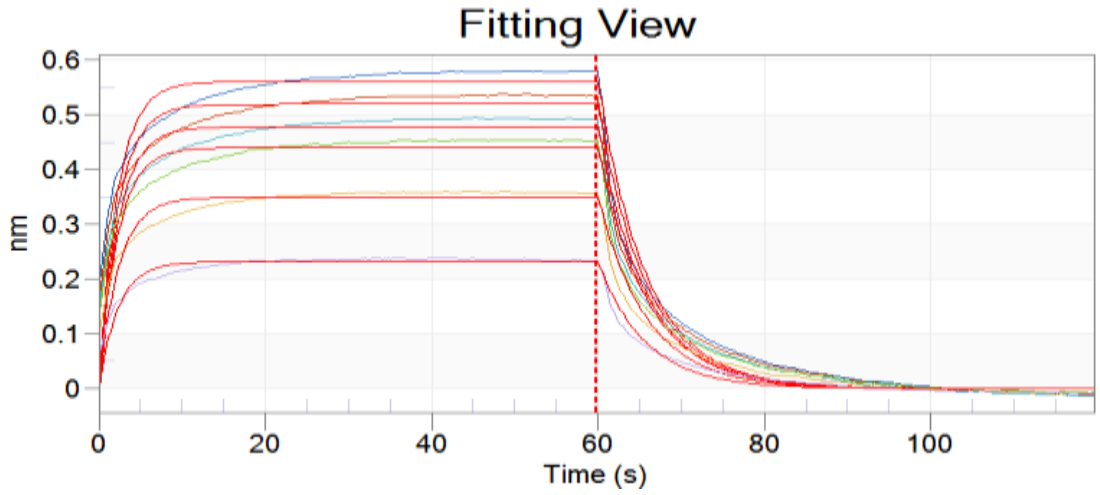
0.3
0.2
0.1
0
0
20
40
60
80
100
Time (s)

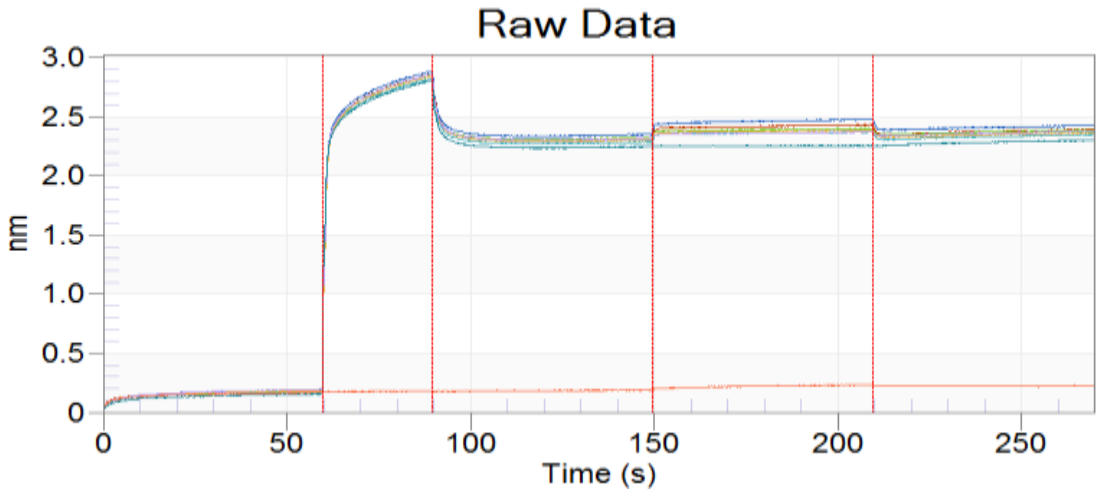
P1-F

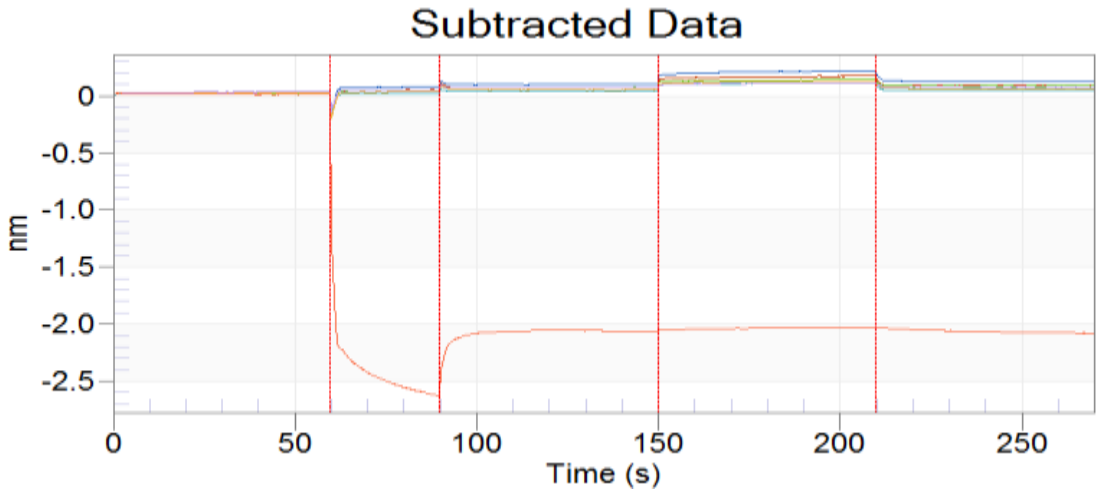

Raw Data
nm
Time (s)

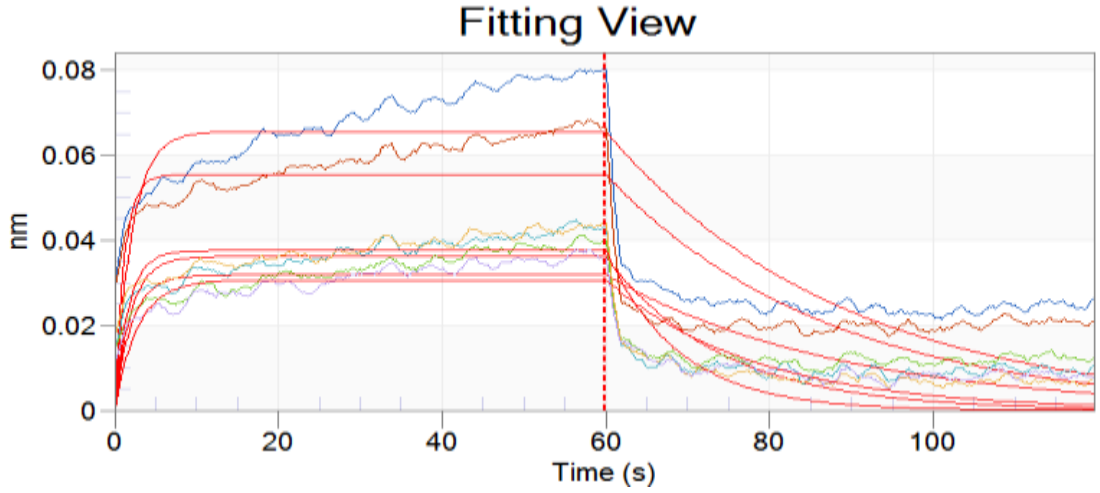

Subtracted Data
nm
Time (s)


Fitting View
nm
Time (s)

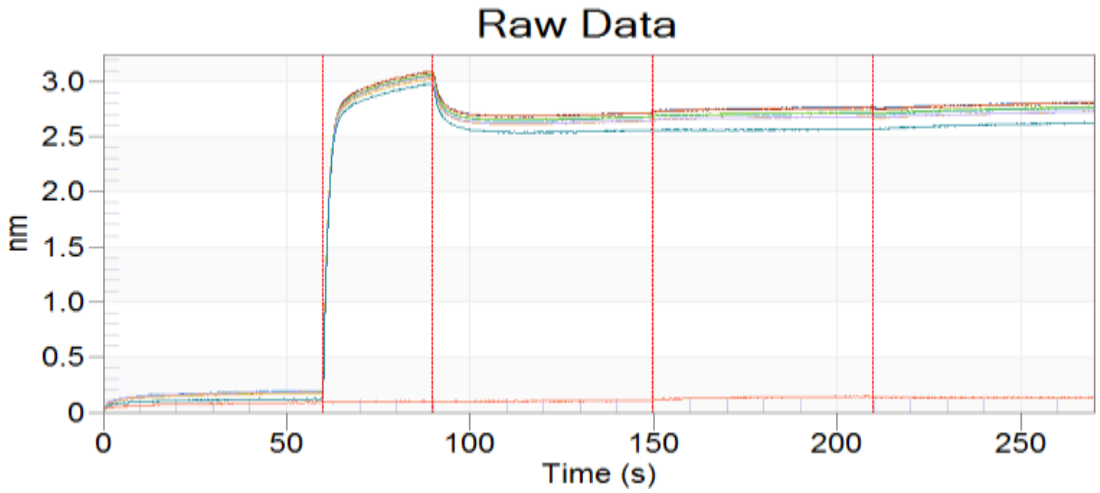
P1-G

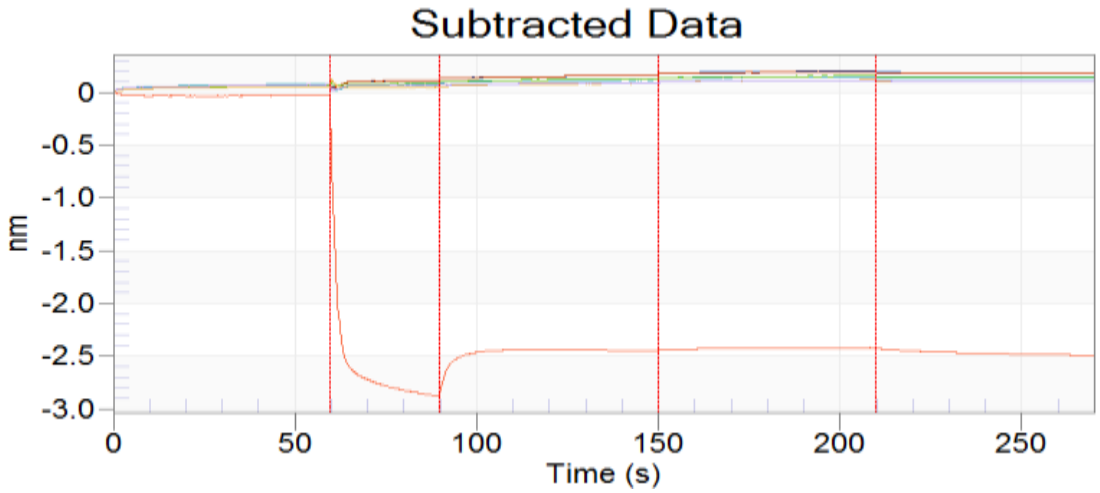

Raw Data
3.0
2.5
2.0
1.5
1.0
0.5
0
nm
0
50
100
150
200
250
Time (s)

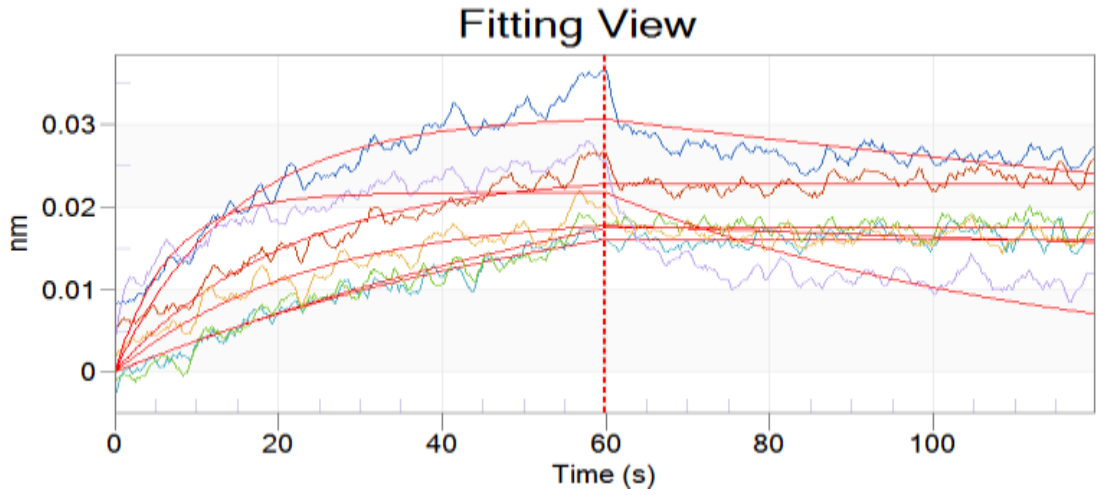

Subtracted Data
0
-0.5
-1.0
-1.5
-2.0
-2.5
nm
0
50
100
150
200
250
Time (s)


Fitting View
0.08
0.06
0.04
0.02
0
nm
0
20
40
60
80
100
Time (s)

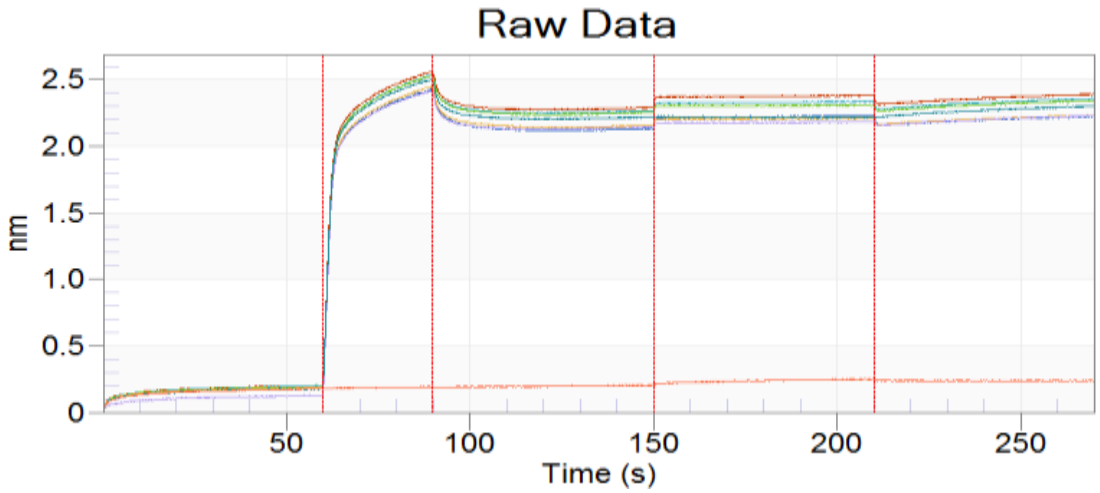
P1-H

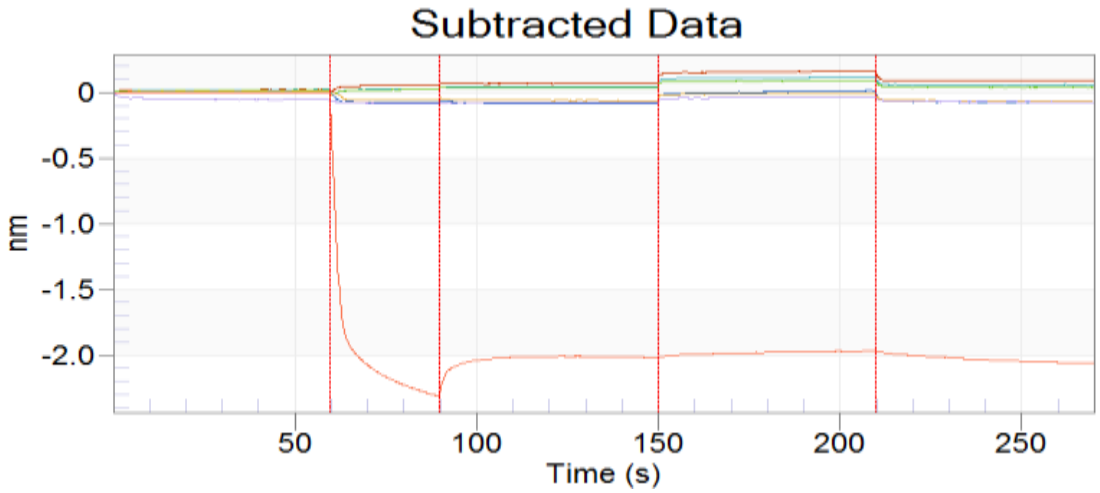

Raw Data
nm
3.0
2.5
2.0
1.5
1.0
0.5
0
0
50
100
150
200
250
Time (s)

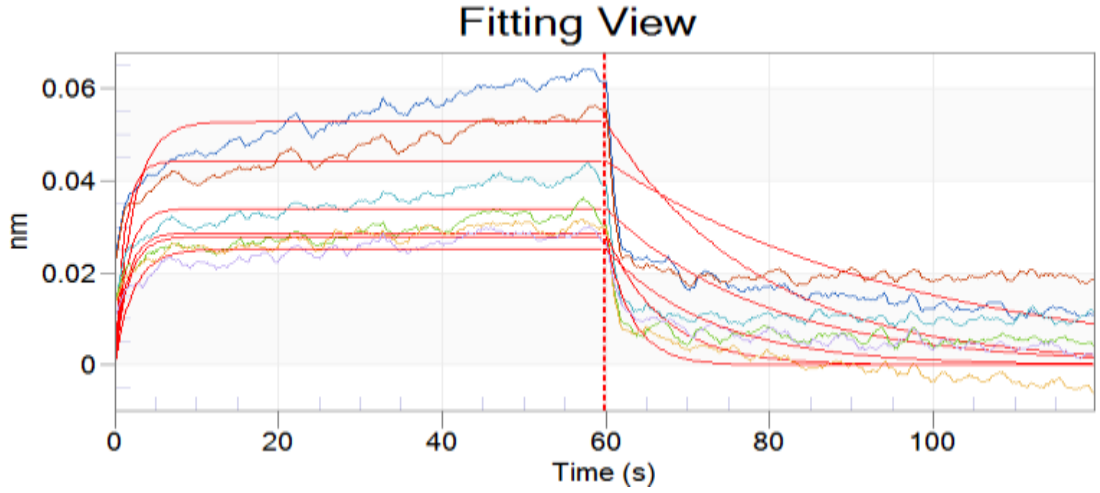

Subtracted Data
nm
0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
0
50
100
150
200
250
Time (s)


Fitting View
nm
0.03
0.02
0.01
0
0
20
40
60
80
100
Time (s)

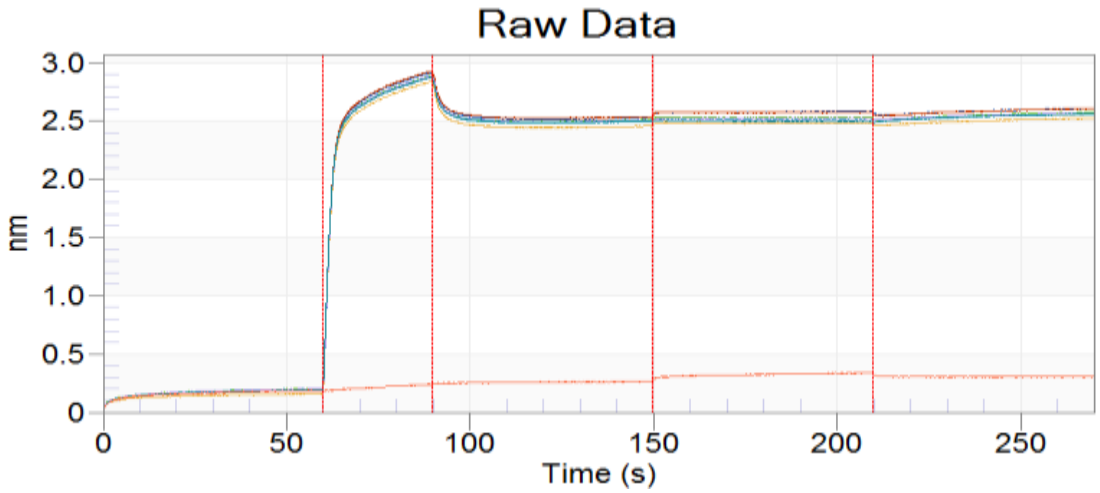
P1-I

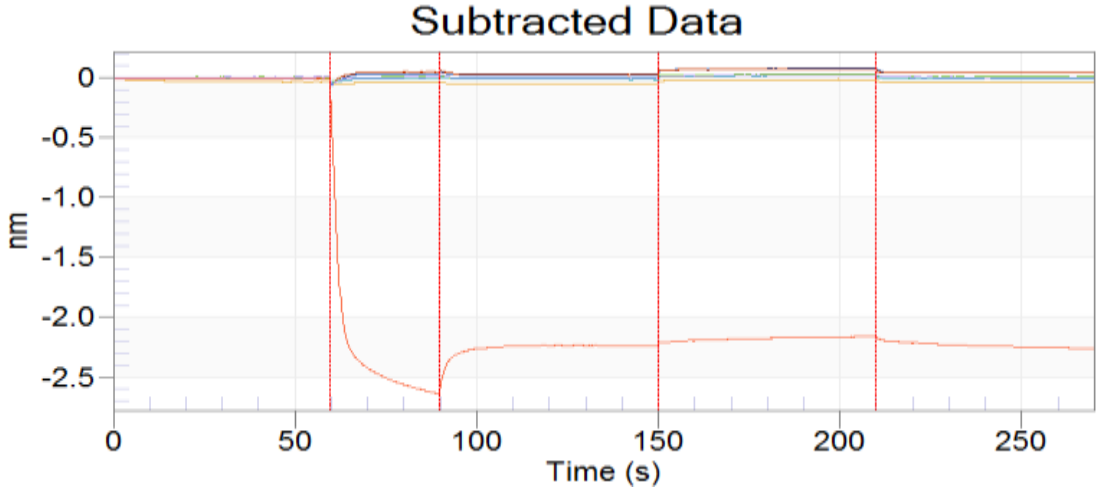

Raw Data
2.5
2.0
1.5
1.0
0.5
0
nm
50
100
150
200
250
Time (s)

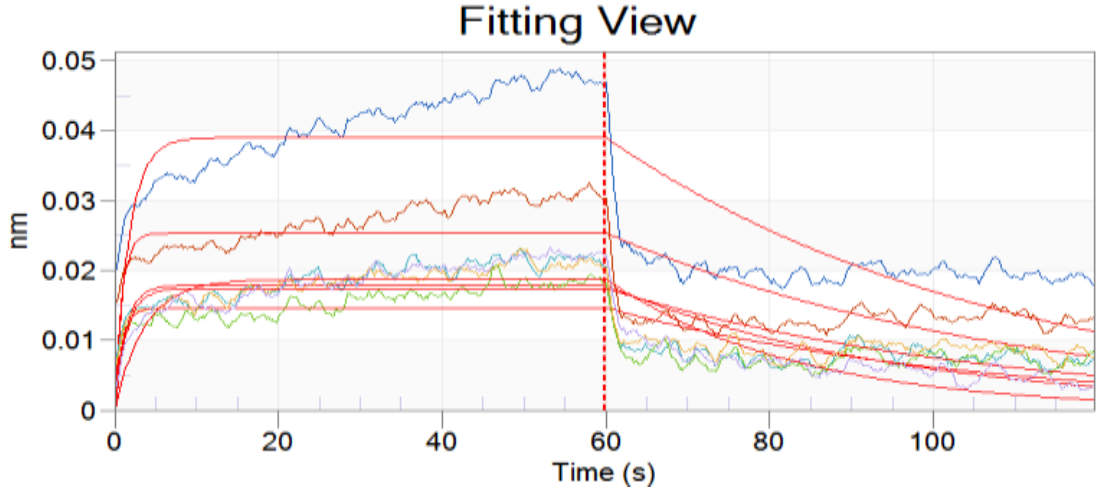

Subtracted Data
0
-0.5
-1.0
-1.5
-2.0
nm
50
100
150
200
250
Time (s)


Fitting View
0.06
0.04
0.02
0
nm
0
20
40
60
80
100
Time (s)

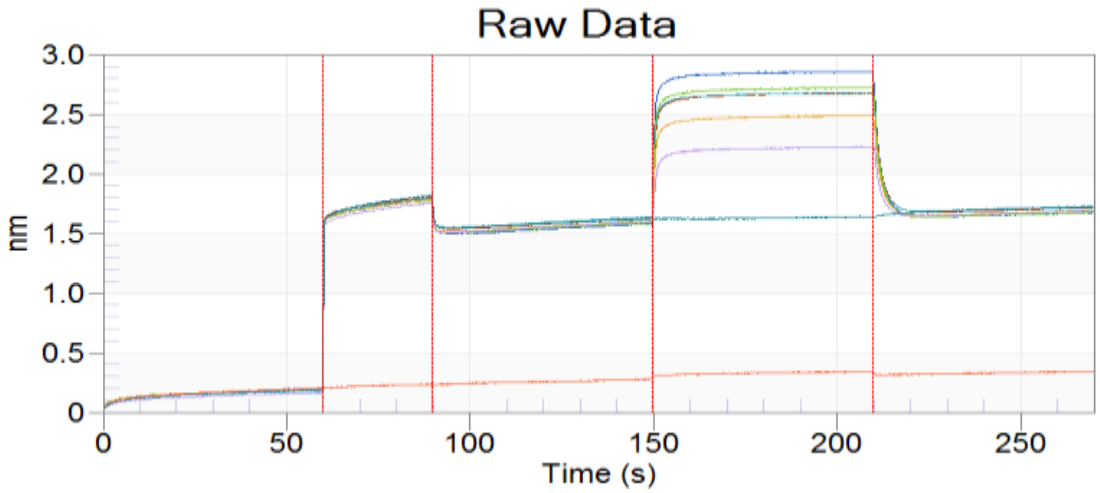
P1-J

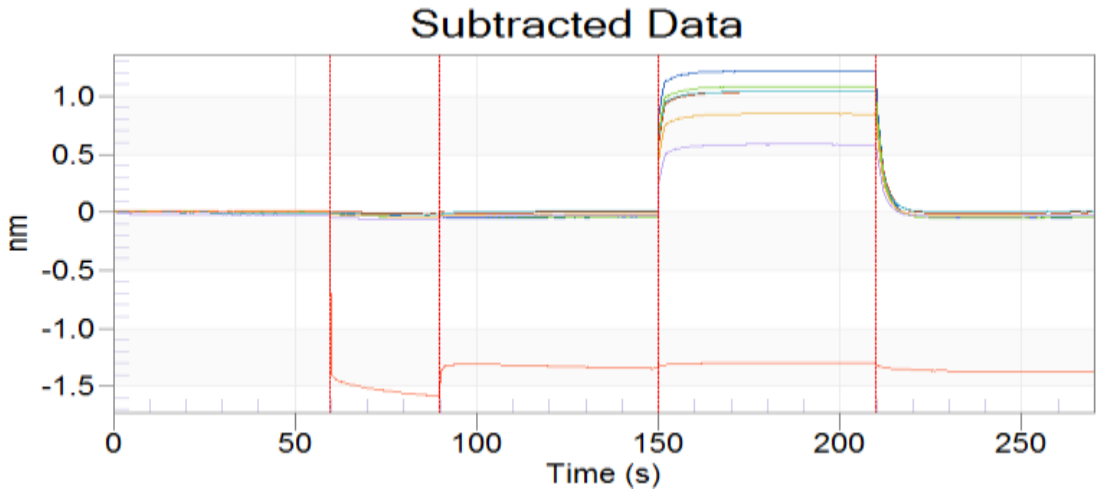

Raw Data
3.0
2.5
2.0
1.5
1.0
0.5
0
nm
0
50
100
150
200
250
Time (s)

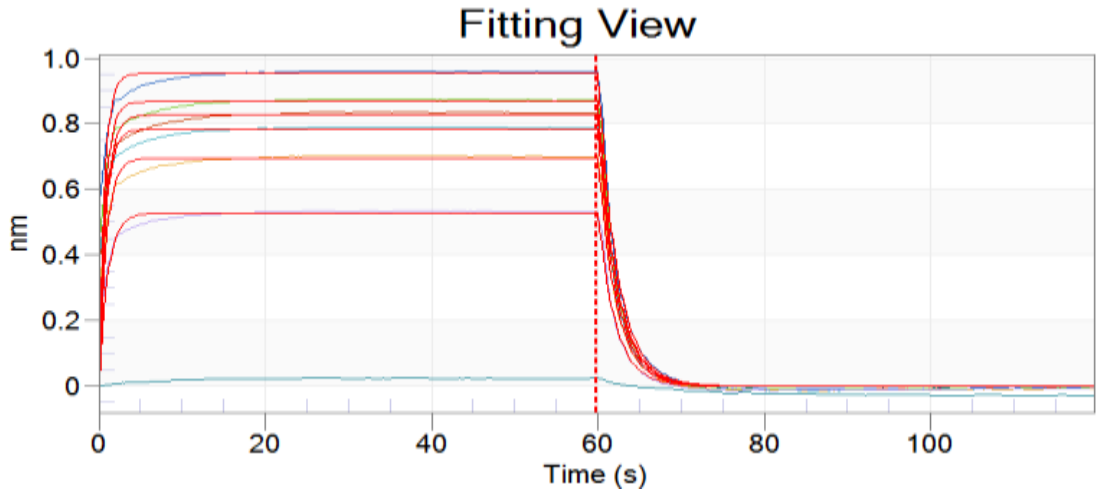

Subtracted Data
0
-0.5
-1.0
-1.5
-2.0
-2.5
nm
0
50
100
150
200
250
Time (s)


Fitting View
0.05
0.04
0.03
0.02
0.01
0
nm
0
20
40
60
80
100
Time (s)

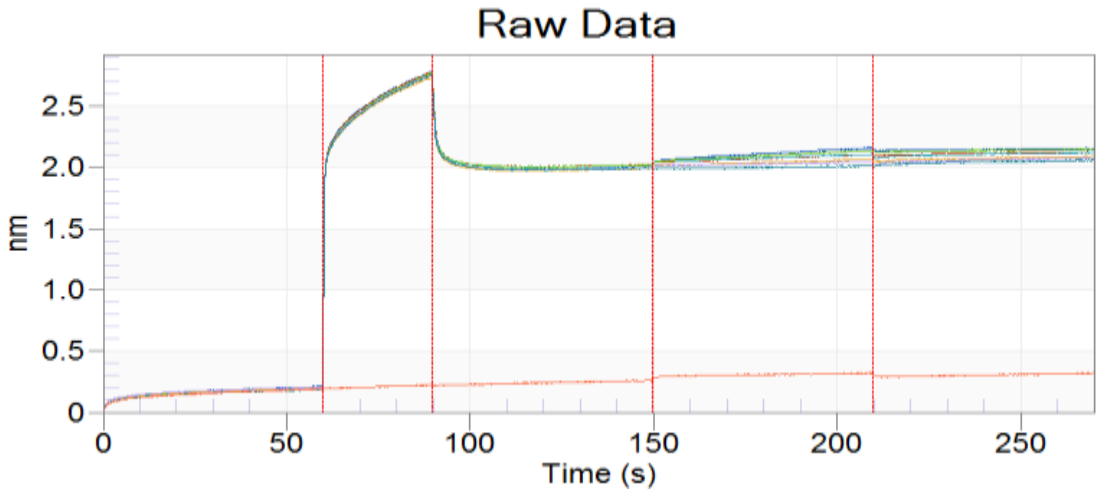
P1-K


Raw Data
nm
3.0
2.5
2.0
1.5
1.0
0.5
0
0
50
100
150
200
250
Time (s)


Subtracted Data
nm
1.0
0.5
0
-0.5
-1.0
-1.5
0
50
100
150
200
250
Time (s)


Fitting View
nm
1.0
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
Time (s)

P4-A


Raw Data
nm
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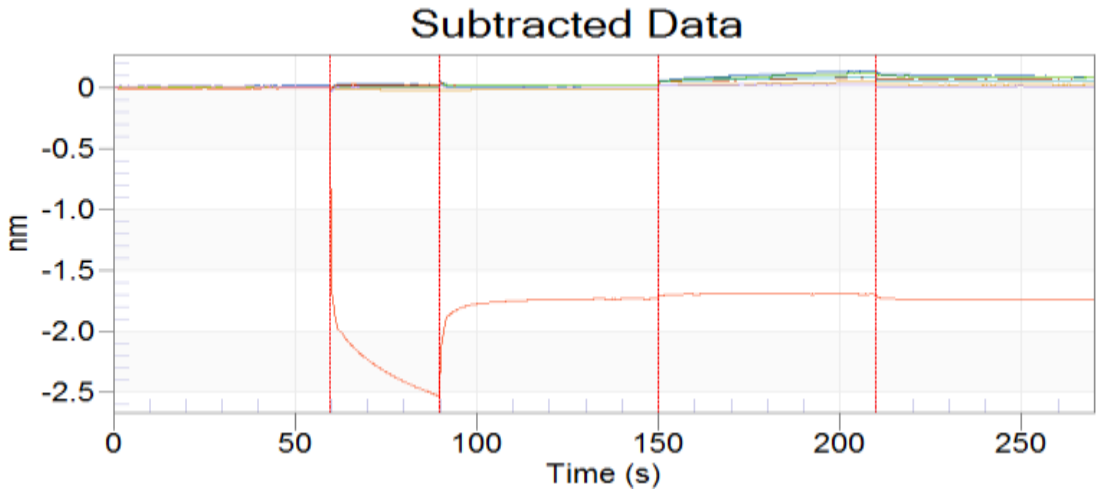
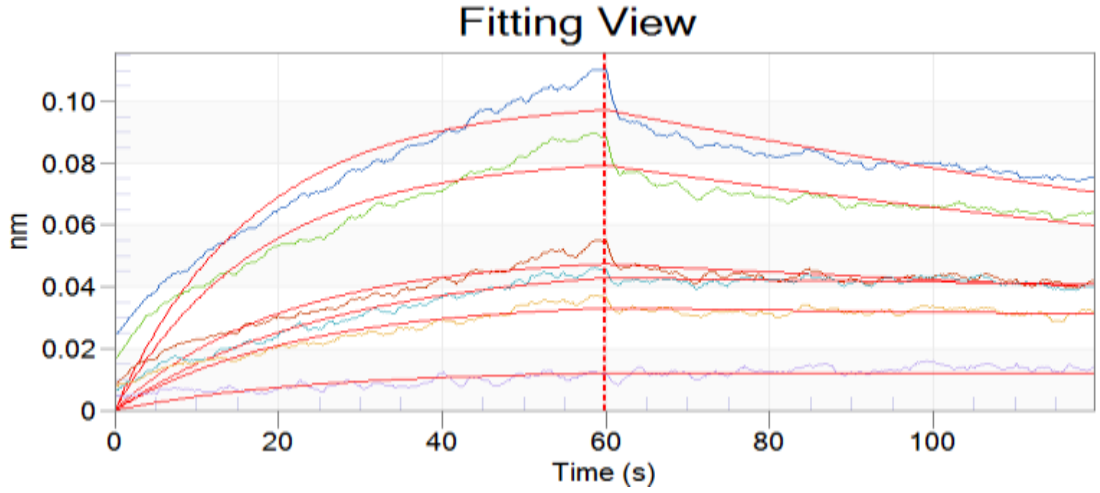
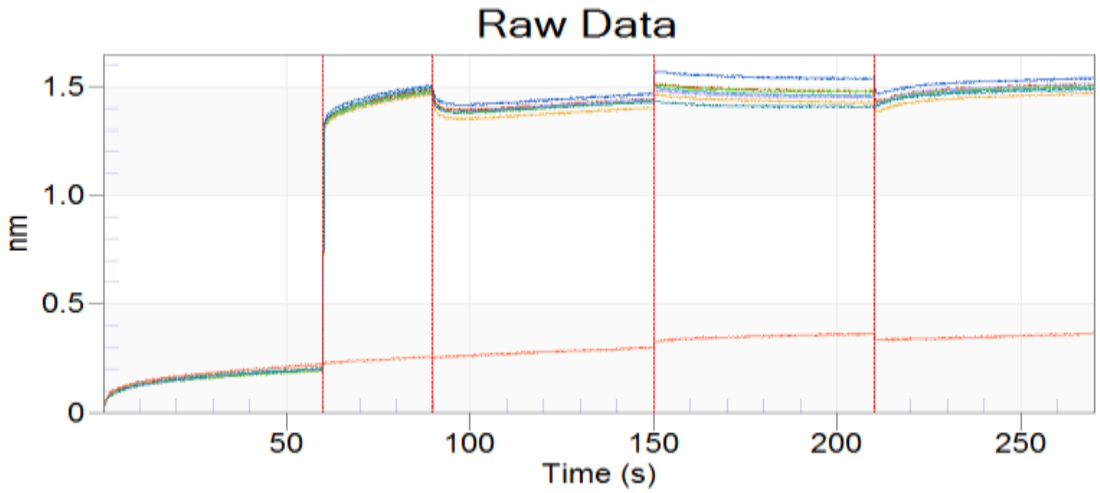
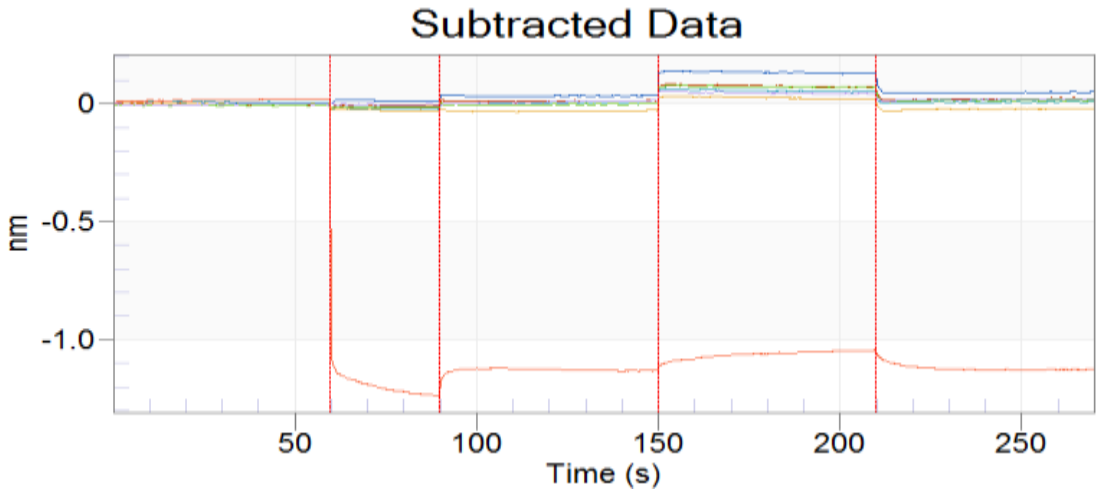
2.0
1.5
1.0
0.5
0
0
50
100
150
200
250
Time (s)

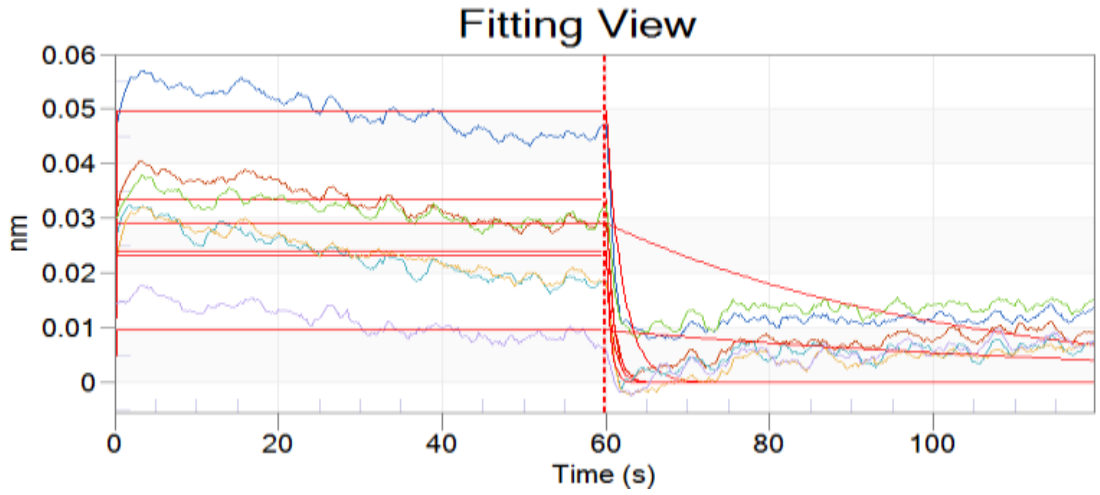

Subtracted Data
nm
0
-0.5
-1.0
-1.5
-2.0
-2.5
0
50
100
150
200
250
Time (s)


Fitting View
nm
0.10
0.08
0.06
0.04
0.02
0
0
20
40
60
80
100
Time (s)

P4-K


Raw Data
1.5
1.0
0.5
0
nm
50
100
150
200
250
Time (s)


Subtracted Data
0
-0.5
-1.0
nm
50
100
150
200
250
Time (s)


Fitting View
0.06
0.05
0.04
0.03
0.02
0.01
0
nm
0
20
40
60
80
100
Time (s)

7. Lipophilicity (Log D), Phospholipid Affinity (CHI IAM), Predicted Volume of Distribution (Log VD), and Human Serum Albumin Binding (HSA%) Data

The peptides (~1 mg) were dissolved in 1 mL of DMSO and injected onto various HPLCs for biomimetic analysis of the physicochemical properties presented below. Relevant information regarding each method has been described below.

Lipophilicity measurements: A method has been described by Kerns *et al*[4] for a quick HPLC method to estimate the logarithm of the octanol-water partition coefficient at pH 7.4.

Instrument

Agilent HP1000 HPLC

Materials:

HPLC grade acetonitrile

HPLC grade Water

Ammonium Acetate

pH 7.0 Buffer solution

50 mM Ammonium acetate pH 7.4

1 M NaOH solution

Diluent:

70% MeCN / 30% water

Analytical Column:

5cm x 4.6mm Polaris C18 5μm HPLC column

Detection:

UV - 230nm

Experimental:

Mobile Phase
Line A: 100% MeCN

Line B: 50mM ammonium acetate adjusted to pH 7.4 using 1M NaOH solution.

HPLC Gradient

Time	% Mobile Phase A	% Mobile Phase B	Flow Rate (mL min^{-1})
0	0	100	1.0
2.5	95	5	1.0
4.0	95	5	1.0
4.1	0	100	1.0
6.1	0	100	1.0

Column Temperature: 40°C

Injection volume: 2 µL

Phospholipid affinity measurements: A method has been described by Valko, *et al*[5] for a quick HPLC method to characterize the interactions of drugs with immobilized artificial membrane (IAM). With a set of standards, the gradient retention times can be converted to Chromatographic Hydrophobicity Index values referring to IAM chromatography (CHIIAM) that approximates an acetonitrile concentration with which an equal distribution of compound can be achieved between the mobile phase and IAM.

Calibration standards:

Acetophenone, Propriopheneone, Valerophenone and Octanophenone made up at concentration of ~1 mg mL^{-1} using the diluent.

Diluent:

70% MeCN / 30% water

Analytical Column:

Regis 10cm x 4.6mm IAM PC 10 µm HPLC column

Detection:

UV - 230 nm

Experimental:

Mobile Phase
Line A: 100% MeCN

Line B: 50 mM ammonium acetate adjusted to pH 7.4 using 1M NaOH solution.

HPLC Gradient

Time	% Mobile Phase A	% Mobile Phase B	Flow Rate (mL min^{-1})
0	0	100	1.0
6.0	100	0	1.0
6.5	100	0	1.0
7.0	0	100	1.0
9.0	0	100	1.0

Column Temperature: 40°C

Injection volume: 2 µL

Human Serum Albumin (HSA) binding measurements: A fast gradient HPLC method (cycle time 15 min) is described by Valko, *et al*[6] to determine Human Serum Albumin (HSA) binding of discovery compounds using chemically bonded protein stationary phases. The HSA binding values were derived from the gradient retention times that were converted to the logarithm of the equilibrium constants (logKHSA) using data from a calibration set of molecules. A calibration graph is plotted with logarithm of the retention time vs logKHSA (literature).

Calibration standards:

Carbamazapine, Nicardipine, Warfarin, Diclofenac and Ketoprofen sample solutions made up at concentration of ~1 mg mL^{-1} using the diluent, respectively.

Diluent:

70% MeCN / 30% water

Analytical Column:

5cm x 3.0 mm HSA 5µm HPLC column

Detection:

UV - 280 nm or 230 nm

Experimental:

Mobile Phase

Line A: 50 mM ammonium acetate adjusted to pH 7.4 using 1M NaOH solution.

Line B: 100% Isopropyl alcohol

HPLC Gradient

Time	% Mobile Phase A	% Mobile Phase B	Flow Rate (mL min^{-1})
0	100	0	1.2
4.5	70	30	1.2
9.0	70	30	1.2
9.5	100	0	1.2
14	100	0	1.2

Column Temperature: 40°C

Injection volume: 2 µL

Figure 1: Lipophilicity (Log $D_{7.4}$) values for each peptide variant.

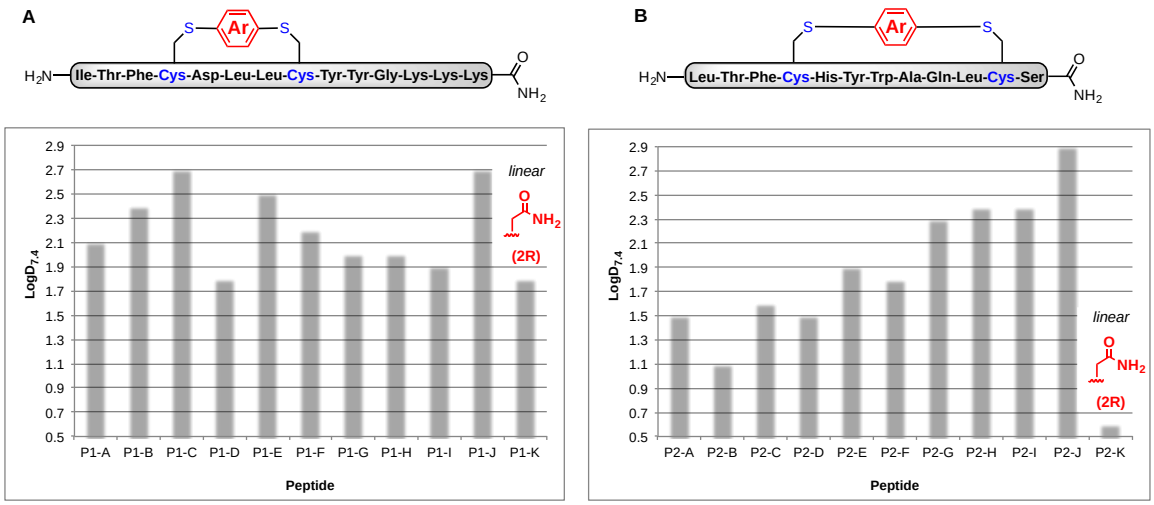


Table S3. Human Serum Albumin binding for each peptide variant.

Peptide	HSA Binding	Peptide	HSA Binding
P1-A	100%	**P1-G**	99.5%
P1-B	100%	**P1-H**	99.7%
P1-C	98.8%	**P1-I**	97.7%
P1-D	97.6%	**P1-J**	98.7%
P1-E	99.2%	**P1-K**	98.1%
P1-F	100%		

Peptide	HSA Binding	Peptide	HSA Binding
P2-A	97.2%	**P2-G**	98.3%
P2-B	97.7%	**P2-H**	98.4%
P2-C	97.5%	**P2-I**	98.5%
P2-D	97.4%	**P2-J**	98.5%
P2-E	97.9%	**P2-K**	78.5%
P2-F	97.4%		

Table S4. Phospholipid affinity (CHI IAM pH 7.4) and Volume of Distribution (Log VD) values.

Peptide	CHI IAM pH 7.4	Log VD
P2-A	47.3	0.46
P2-B	36.7	-0.26
P2-C	48.2	0.49
P2-D	46.1	0.35
P2-E	49.9	0.59
P2-F	49.2	0.59
P2-G	64.5	2.37
P2-H	59.2	1.54
P2-I	61.8	1.90
P2-J	70.0	3.42
P2-K	32.4	0.13

The predicted volume of distribution was calculated from the following equation based on HSA binding and phospholipid affinity values7:

$$\text{Log VD} = 0.44(\pm 0.02) \log K\,(\text{IAM}) - \; 0.22(\pm 0.02) \log K\,(\text{HSA}) - 0.66$$

8. ICP-MS Analysis

Two distinct macrocyclic peptides, chosen at random (**P2-C** and **P1-A**), were dissolved in 0.4 mL of concentrated nitric acid. This solution was sonicated and diluted with MilliQ pure water to 0.2% nitric acid concentration. ICP-MS was performed on the resulting mixtures. Calibration curves were generated using Pd ICP-MS standards for a range between 1000 ppm and 100 ppb. There was no palladium found to be remaining in the peptide after purification. Initial palladium content was ~300 ppm used to perform the macrocyclisation reactions indicating over 99% palladium removal.

9. References

1. Vinogradova, E. V., *et al.* Organometallic palladium reagents for cysteine

bioconjugation. *Nature*, 526, 687–691 (2015).

2. Vinogradov, A. A., *et al*. Macrocyclization of unprotected peptide isocyanates. *Org. Lett.*, 18, 1226–1229, (2016).

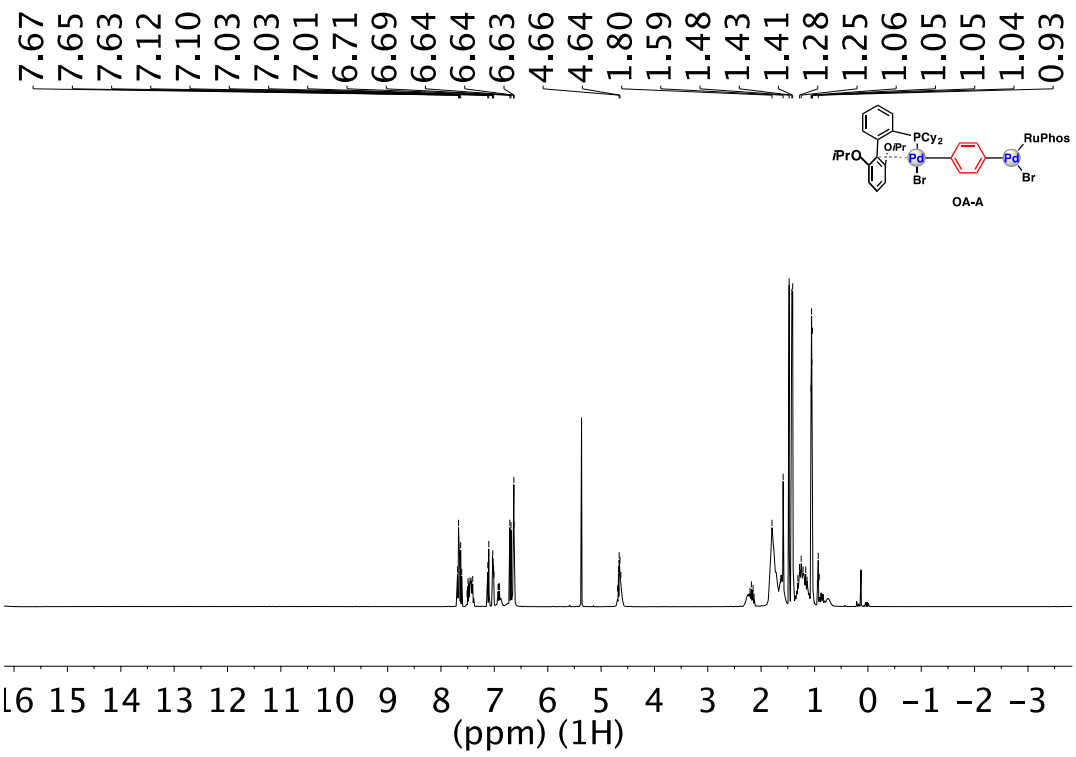
3. Kerns, E. H., *et al*. Pharmaceutical profiling method for lipophilicity and integrity using liquid chromatography-mass spectrometry. *J. of Chromatogr. B*, 791, 381–388 (2003).

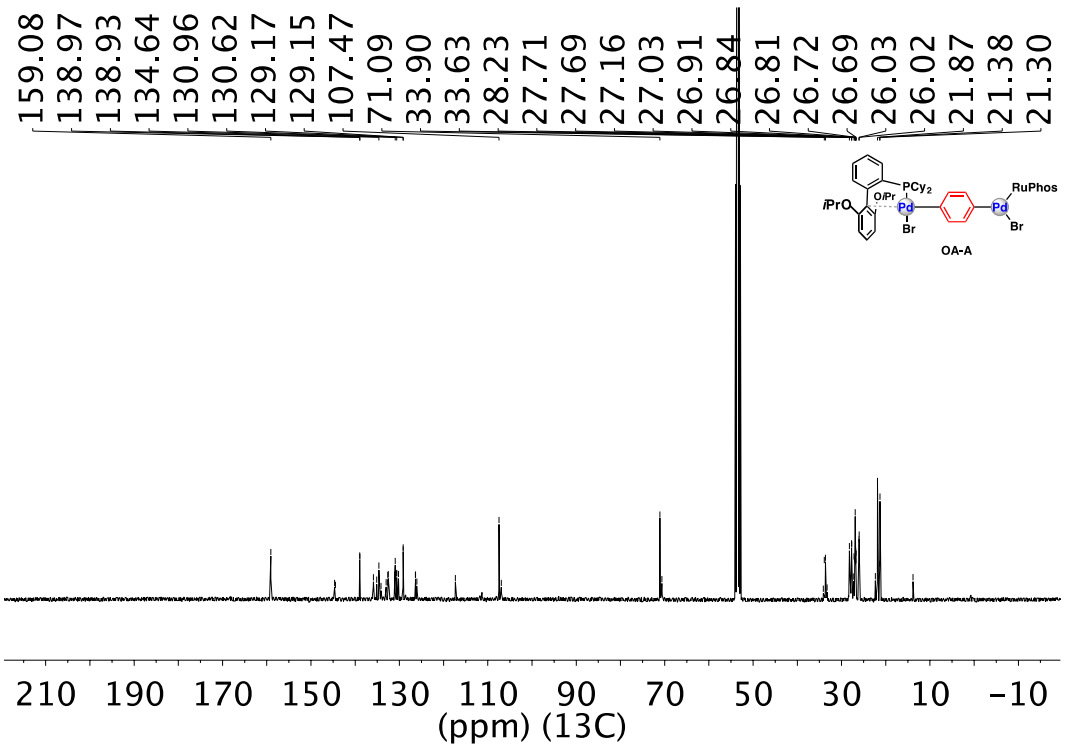
4. Valko, K., *et al*. Rapid gradient HPLC method for measuring drug interactions with immobilized artificial membrane: Comparison with other lipophilicity measures. *J. of Pharm. Sci.*, 89, 1085–1096 (2000).

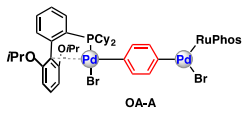
5. Valko, K., *et al*. Fast gradient HPLC method to determine compounds binding to human serum albumin. Relationships with Octanol/Water and immobilized Artificial Membrane Lipophilicity. *J. Of Pharm. Sci.*, 92, 2236–2248 (2003).

6. Hollosy, *et al*. Estimation of Volume of Distribution in Humans from HPLC Measurements of Human Serum Albumin Binding and Immobilized Artificial Membrane Partitioning, *J. Med. Chem.*, 49, 6958–6971 (2006).

10. NMR SPECTRA



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O/Pr
iPrO
Pd
Br
RuPhos
Pd
Br

OA-A

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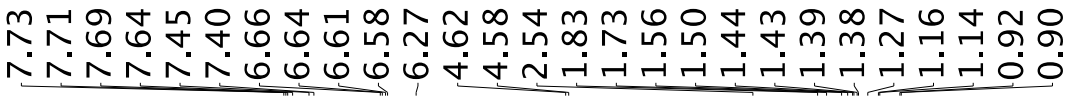
80
40
0
-40
-80
-120
-160
-20
(ppm) (31P)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6.58
6.27
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2.54
1.83
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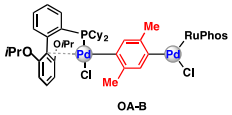
1.50
1.44
1.43
1.39
1.38
1.27
1.16
1.14
0.92
0.90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O/Pr
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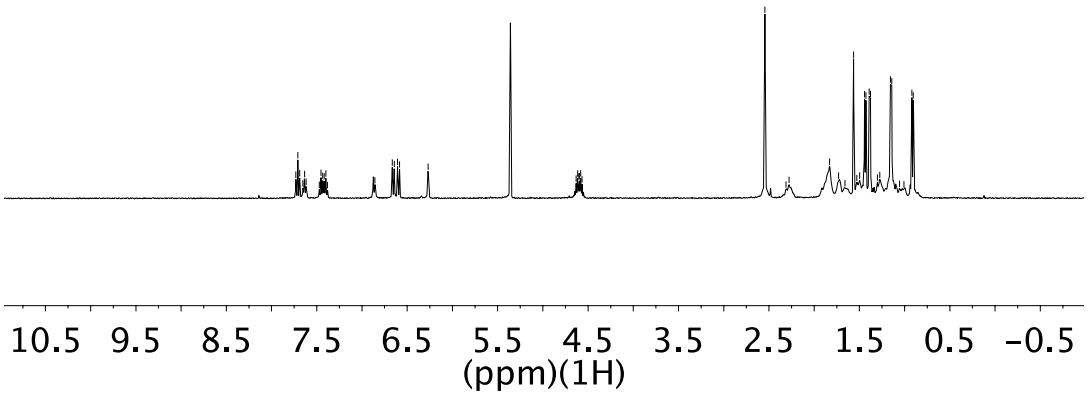
Cl
Me
Me
RuPhos
Pd
Cl

OA-B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4.5
3.5
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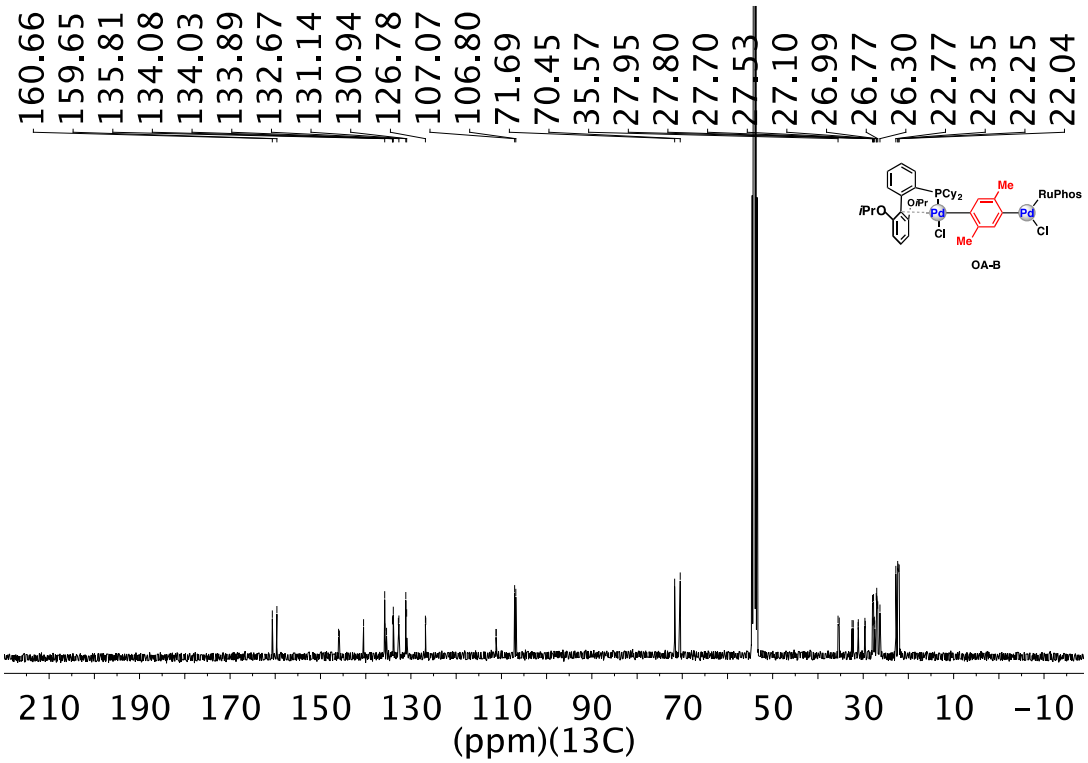
(ppm)(1H)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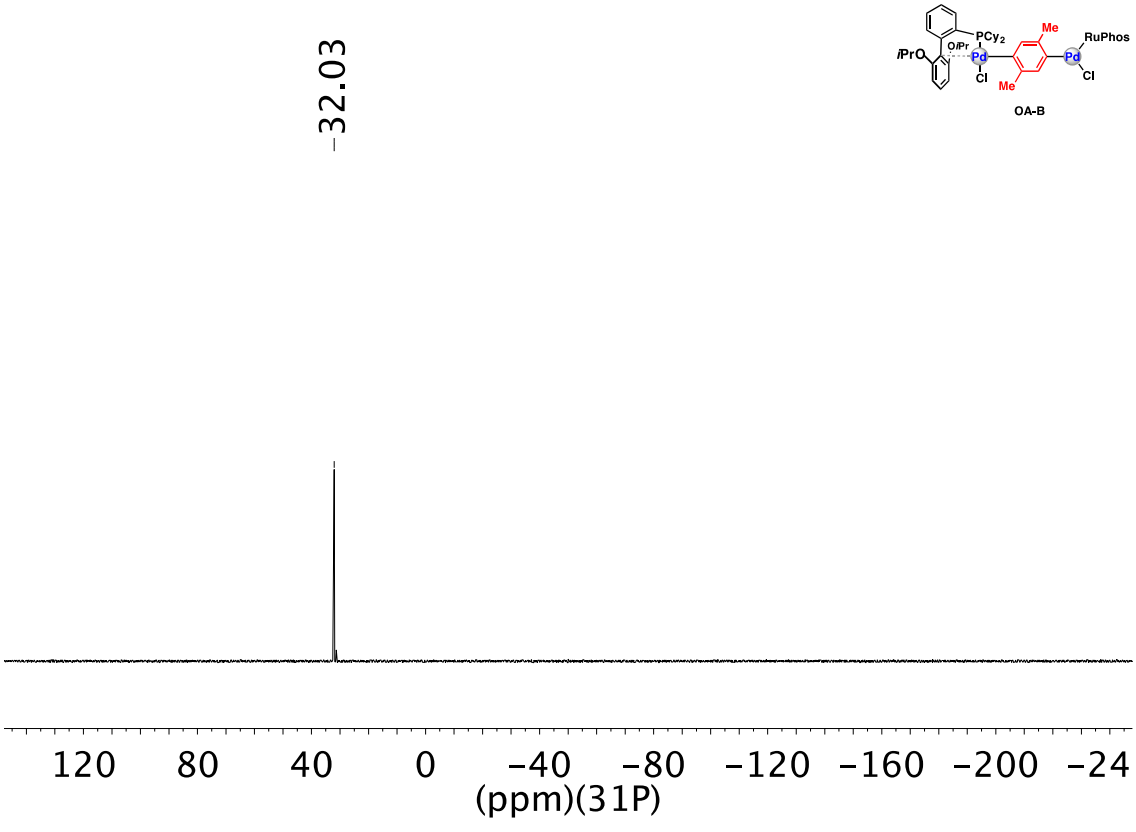
134.08
134.03
133.89
132.67
131.14
130.94
126.78
107.07
106.80
71.69
70.45
35.57
27.95
27.80
27.70
27.53
27.10
26.99
26.77
26.30
22.77
22.35
22.25
22.04
PCy2
O/Pr
iPrO
Pd
Cl
Me
RuPhos
OA-B
210 190 170 150 130 110 90 70 50 30 10 −10
(ppm)(13C)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Pd
Cl
Me
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OA-B
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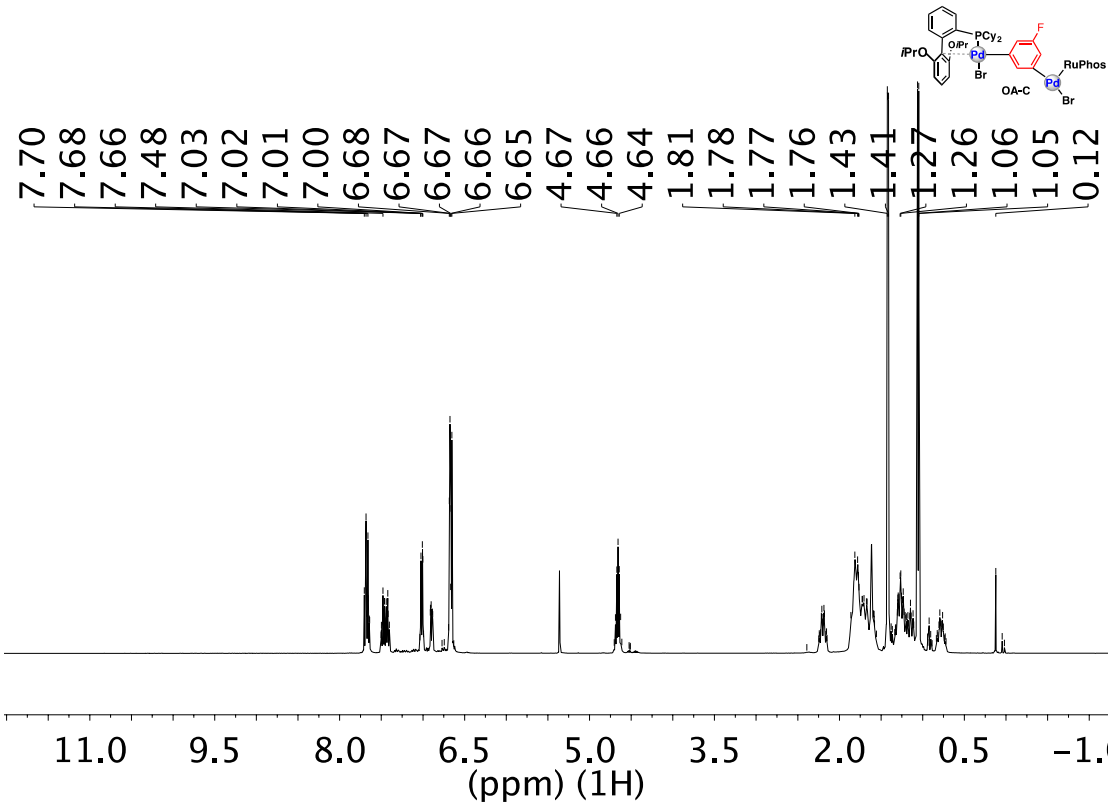
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7.00
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4.66
4.64
1.81
1.78
1.77
1.76
1.43
1.41
1.27
1.26
1.06
1.05
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3.5
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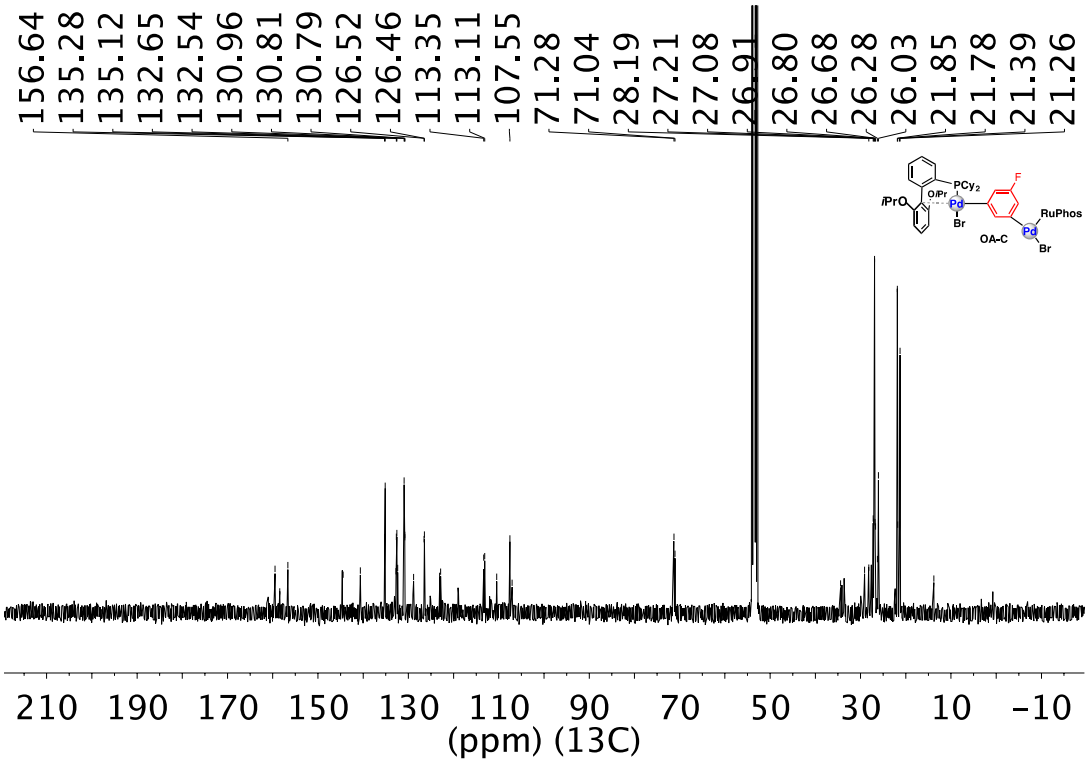
(ppm) (1H)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126.46
113.35
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107.55
71.28
71.04
28.19
27.21
27.08
26.91
26.80
26.68
26.28
26.03
21.85
21.78
21.39
21.26
210
190
170
150
130
110
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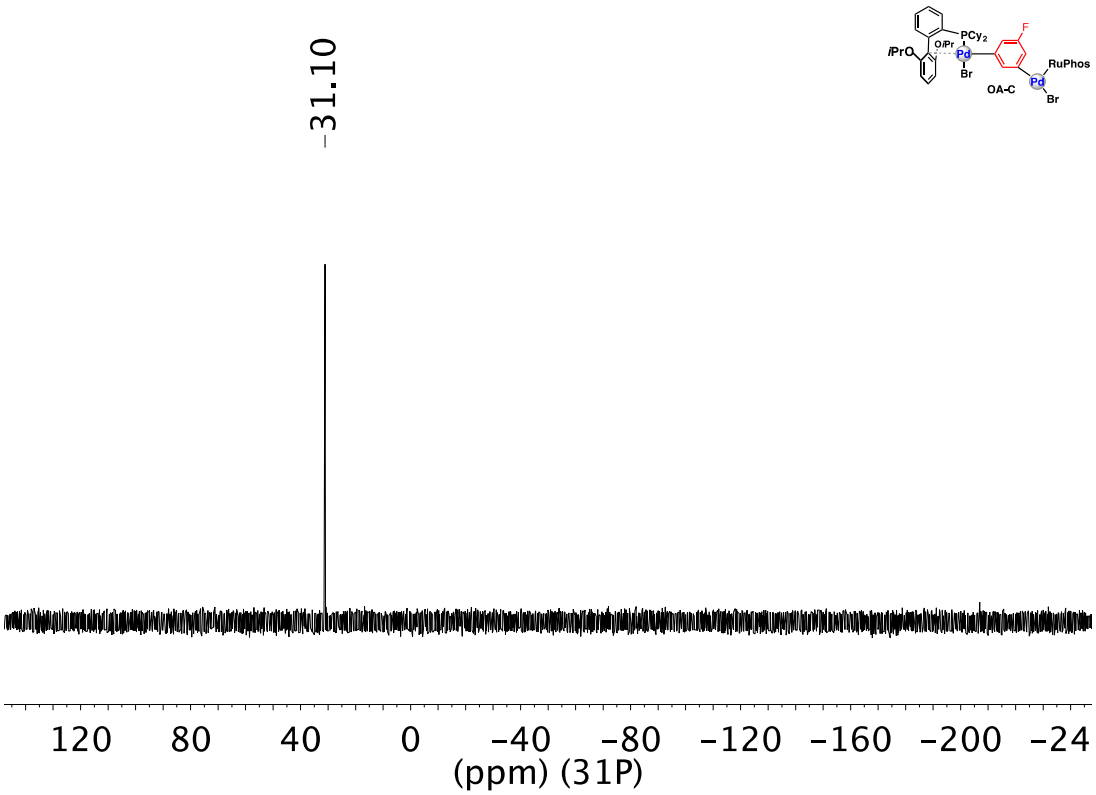
50
30
10
-10
(ppm) (13C)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F
iPrO
O/Pr
Pd
Br
RuPhos
OA-C
Pd
Br
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40
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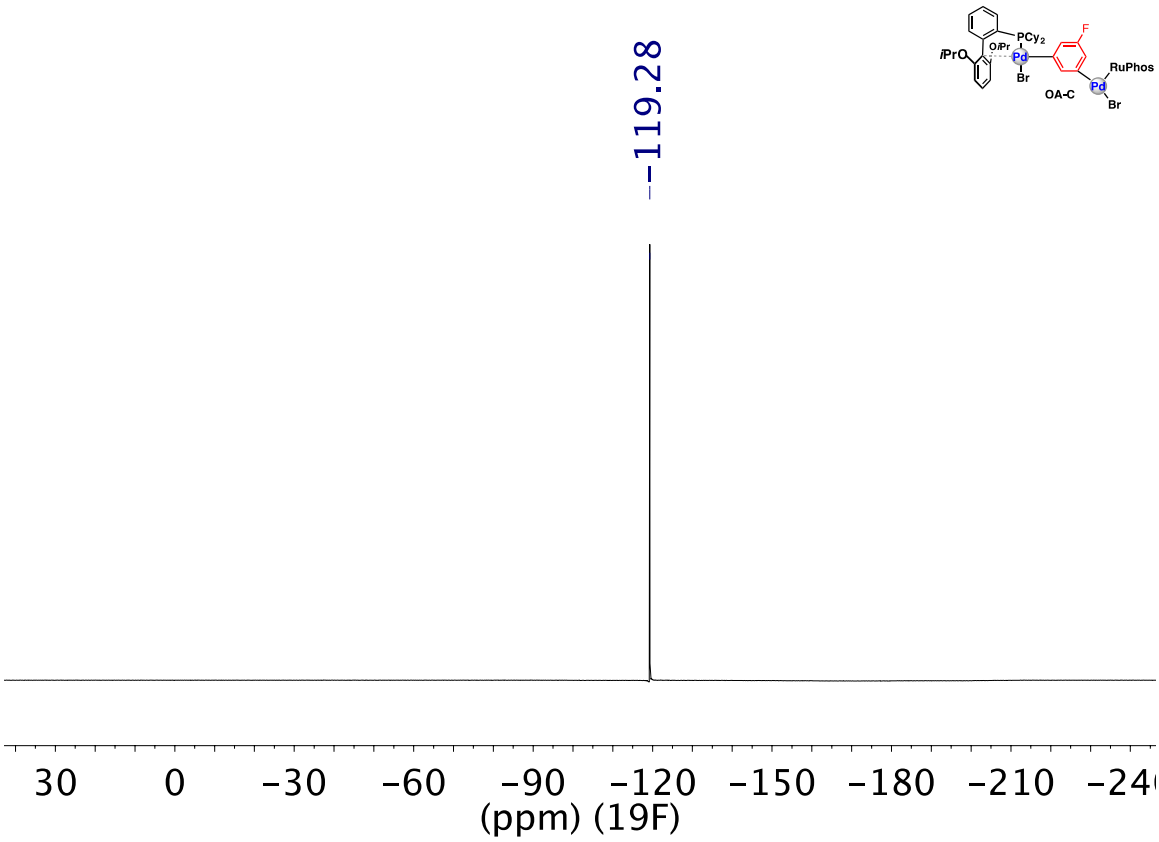
−120
−160
−200
−24
(ppm) (31P)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iPrO
OiPr
Pd
Br
F
RuPhos
OA-C
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Br
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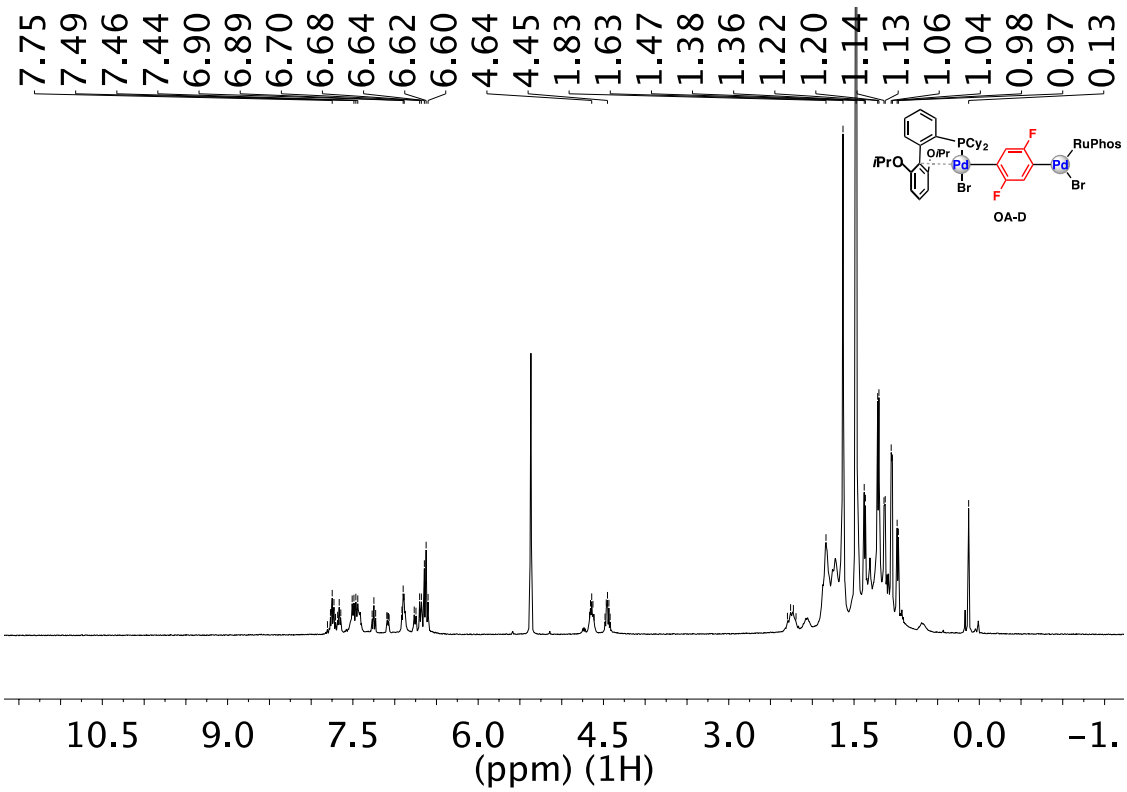
−210
(ppm) (19F)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1.83
1.63
1.47
1.38
1.36
1.22
1.20
1.14
1.13
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1.04
0.98
0.97
0.13
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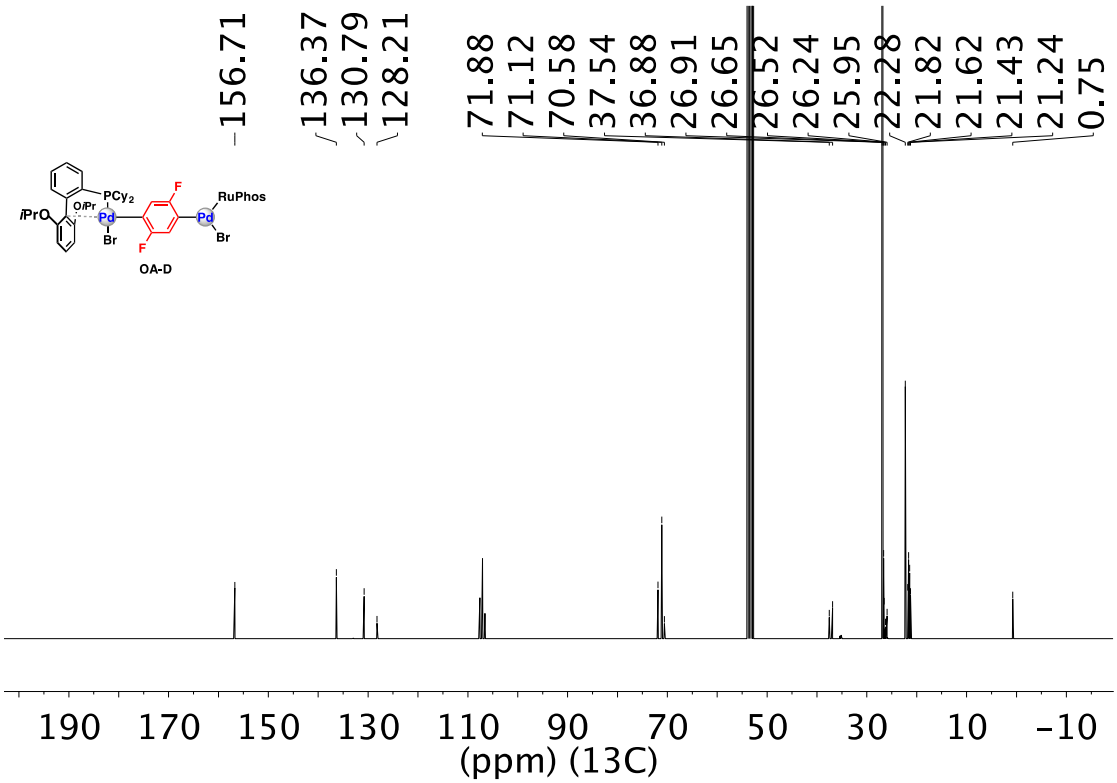
-1.
(ppm) (1H)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26.24
25.95
22.28
21.82
21.62
21.43
21.24
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OA-D
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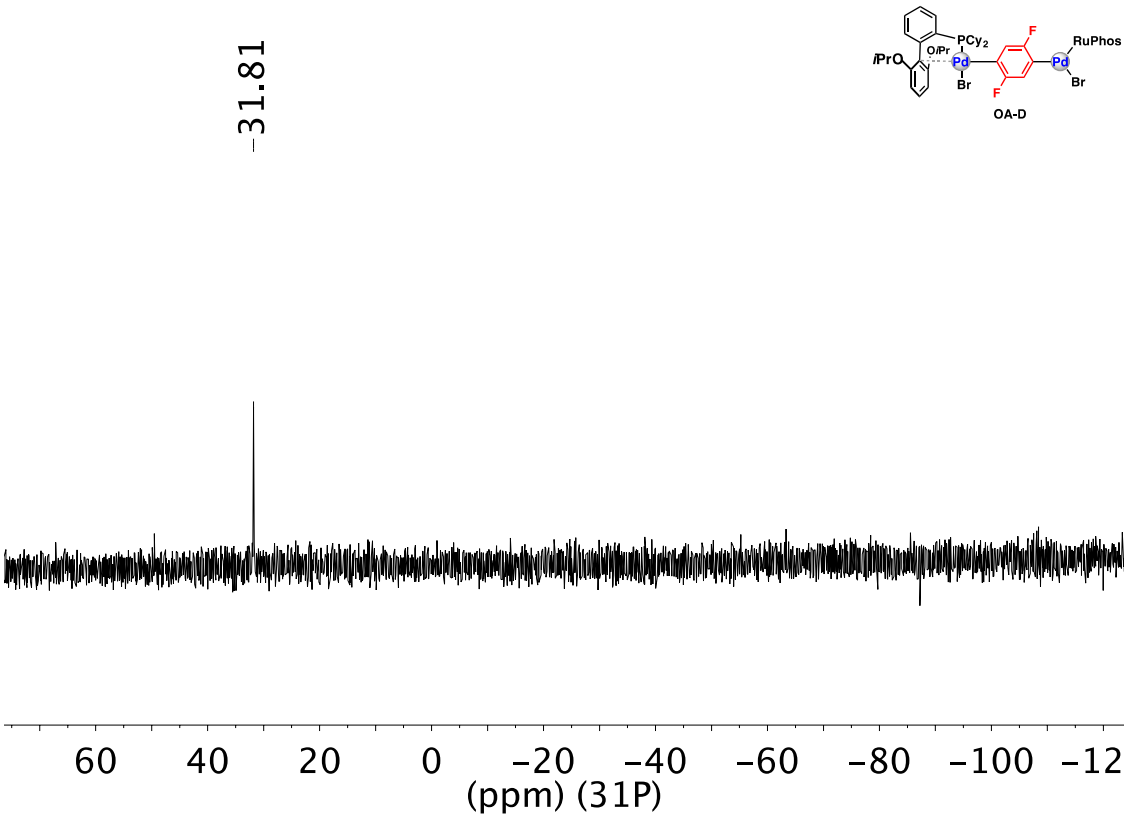
170
150
130
110
90
70
50
30
10
-10
(ppm) (13C)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iPrO
Pd
Br
F
RuPhos
OA-D
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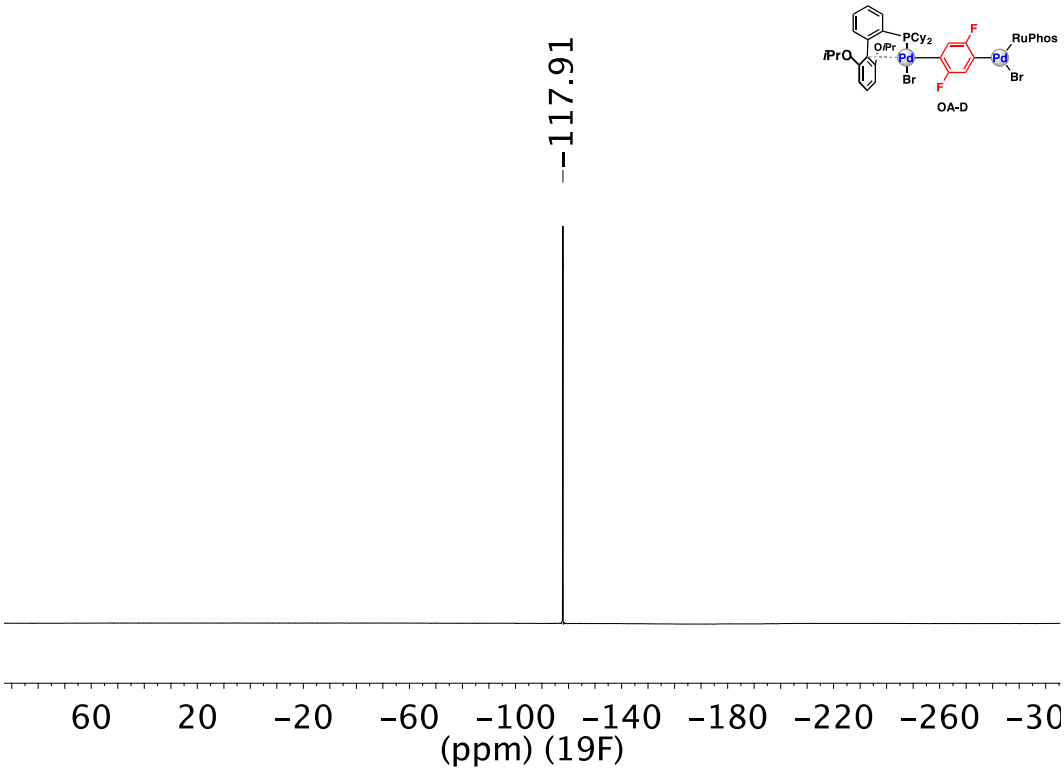
20
0
-20
-40
-60
-80
-100
-12
(ppm) (31P)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O/Pr
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Pd
Br
F
RuPhos
OA-D
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20
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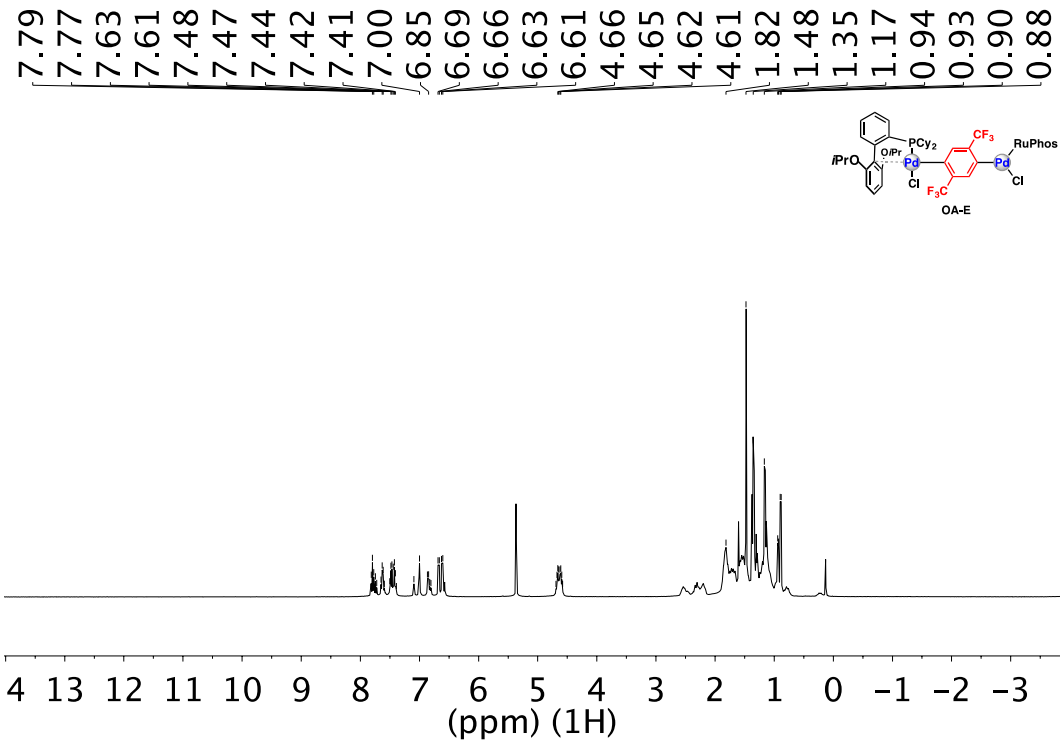
-260
-30
(ppm) (19F)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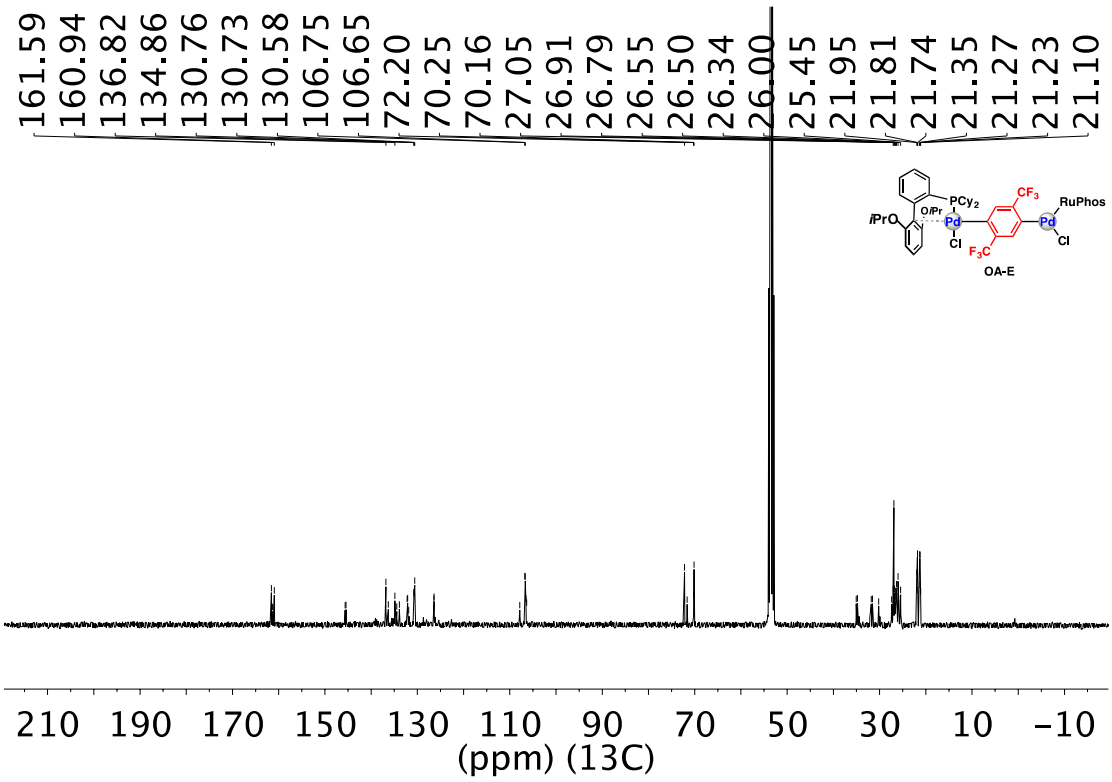
6.63
6.61
4.66
4.65
4.62
4.61
1.82
1.48
1.35
1.17
0.94
0.93
0.90
0.88
PCy2
CF3
RuPhos
iPrO
O/Pr
Pd
Cl
F3C
OA-E
4 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3
(ppm) (1H)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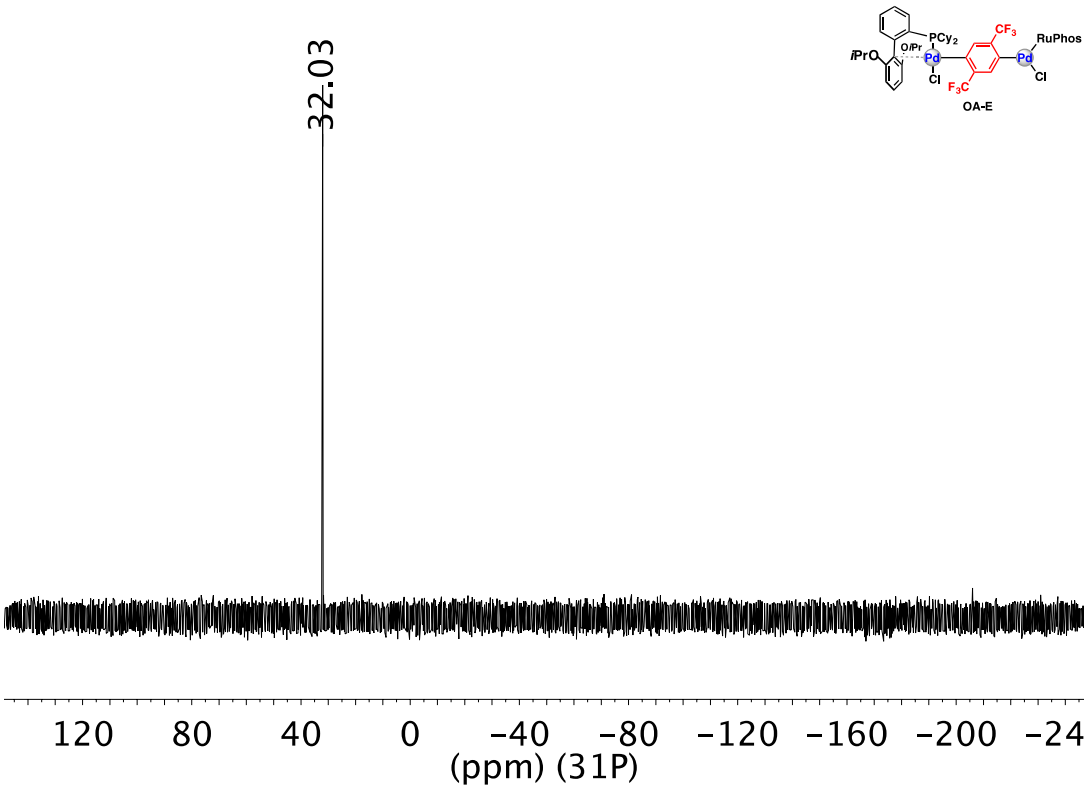
26.55
26.50
26.34
26.00
25.45
21.95
21.81
21.74
21.35
21.27
21.23
21.10
PCy2
CF3
RuPhos
iPrO
O/Pr
Pd
Cl
F3C
OA-E
210 190 170 150 130 110 90 70 50 30 10 −10
(ppm) (13C)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0
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-80
-120
-160
-200
-24
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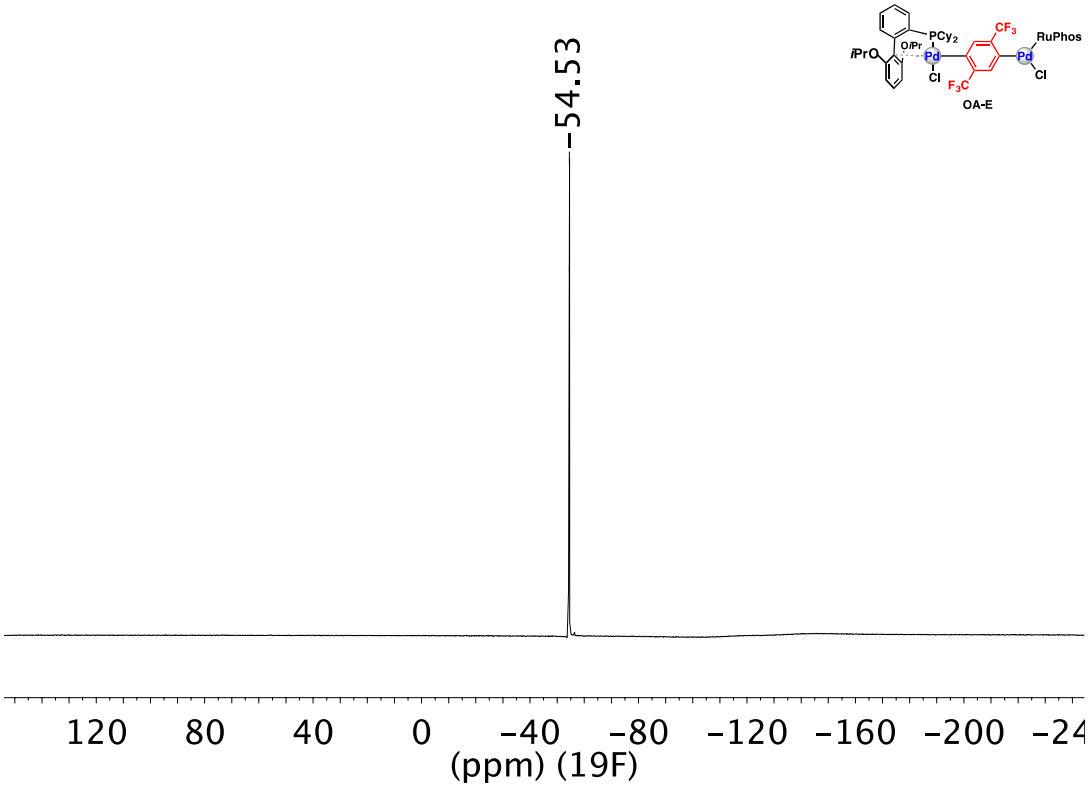
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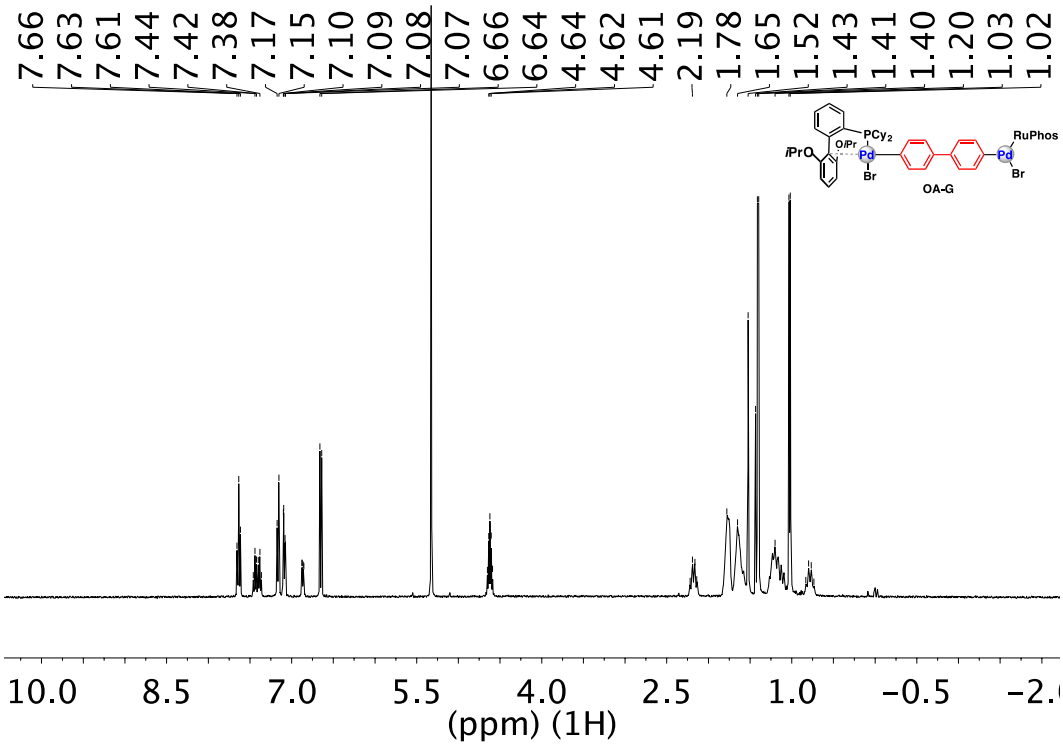
-200
-24
(ppm) (19F)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7.07
6.66
6.64
4.64
4.62
4.61
2.19
1.78
1.65
1.52
1.43
1.41
1.40
1.20
1.03
1.02
iPrO
O/Pr
PCy2
Pd
Br
RuPhos
OA-G
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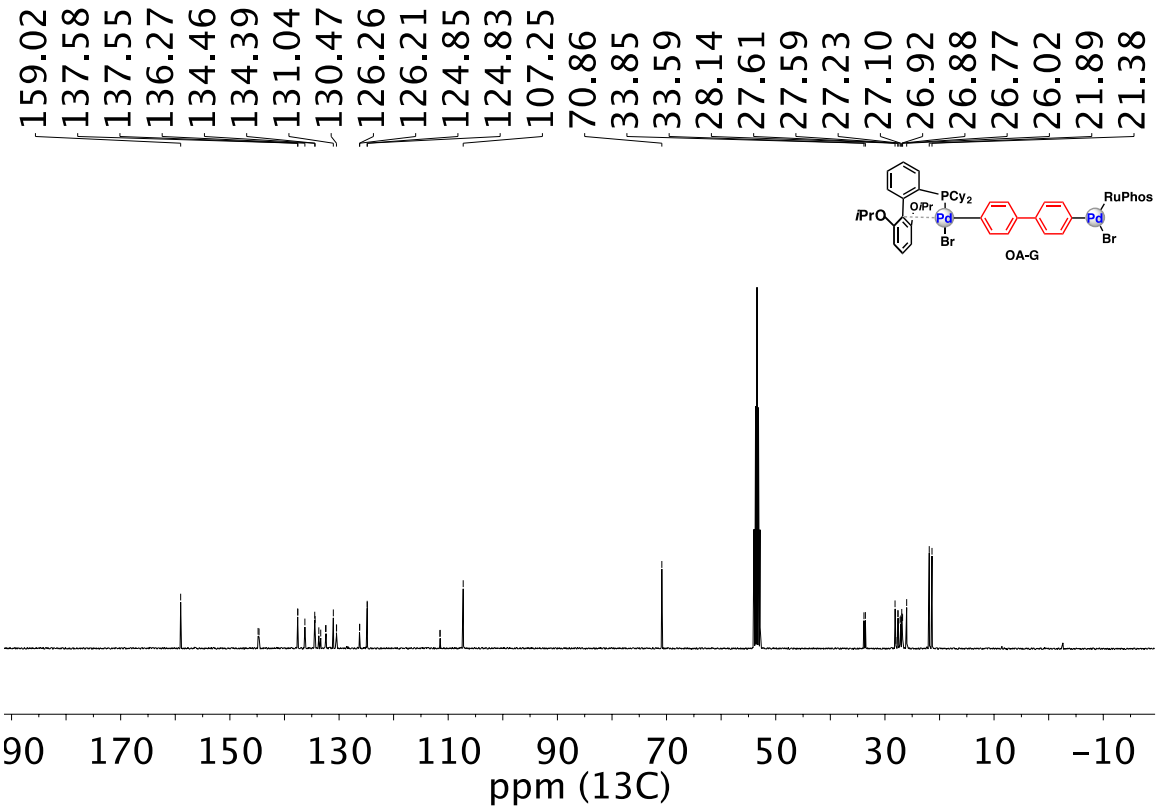
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107.25
70.86
33.85
33.59
28.14
27.61
27.59
27.23
27.10
26.92
26.88
26.77
26.02
21.89
21.38
iPrO
O/Pr
PCy2
Pd
Br
RuPhos
OA-G
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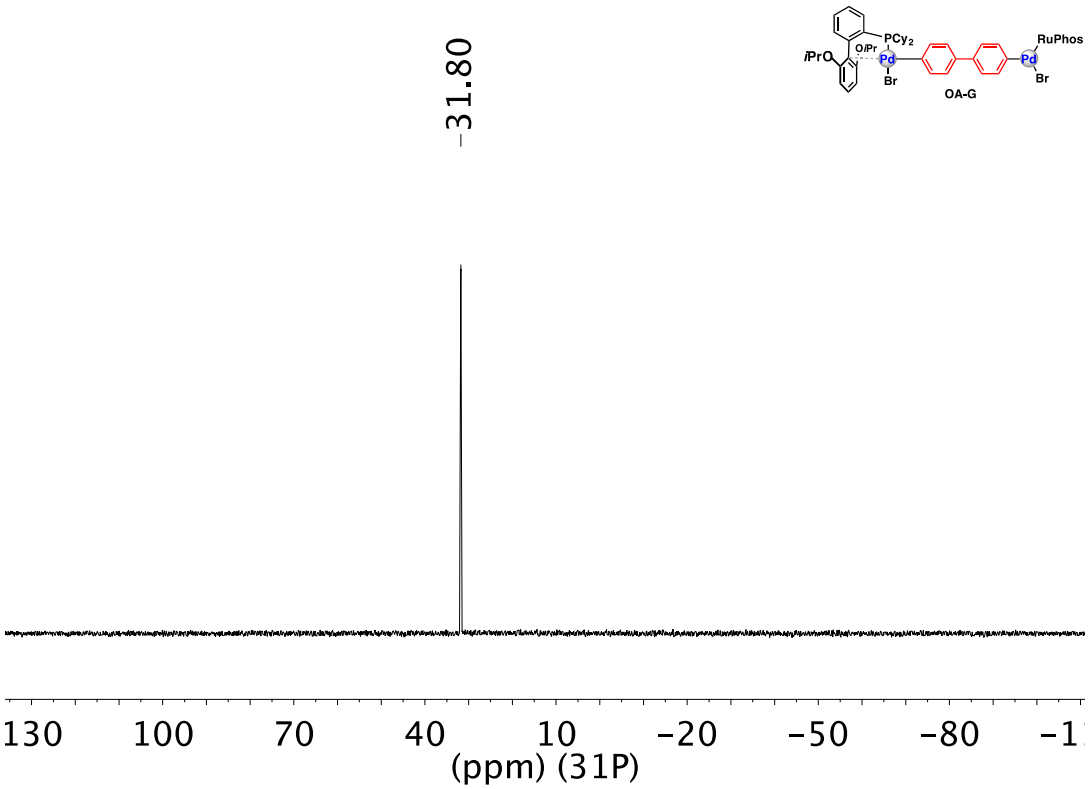
170
150
130
110
90
70
50
30
10
−10
ppm (13C)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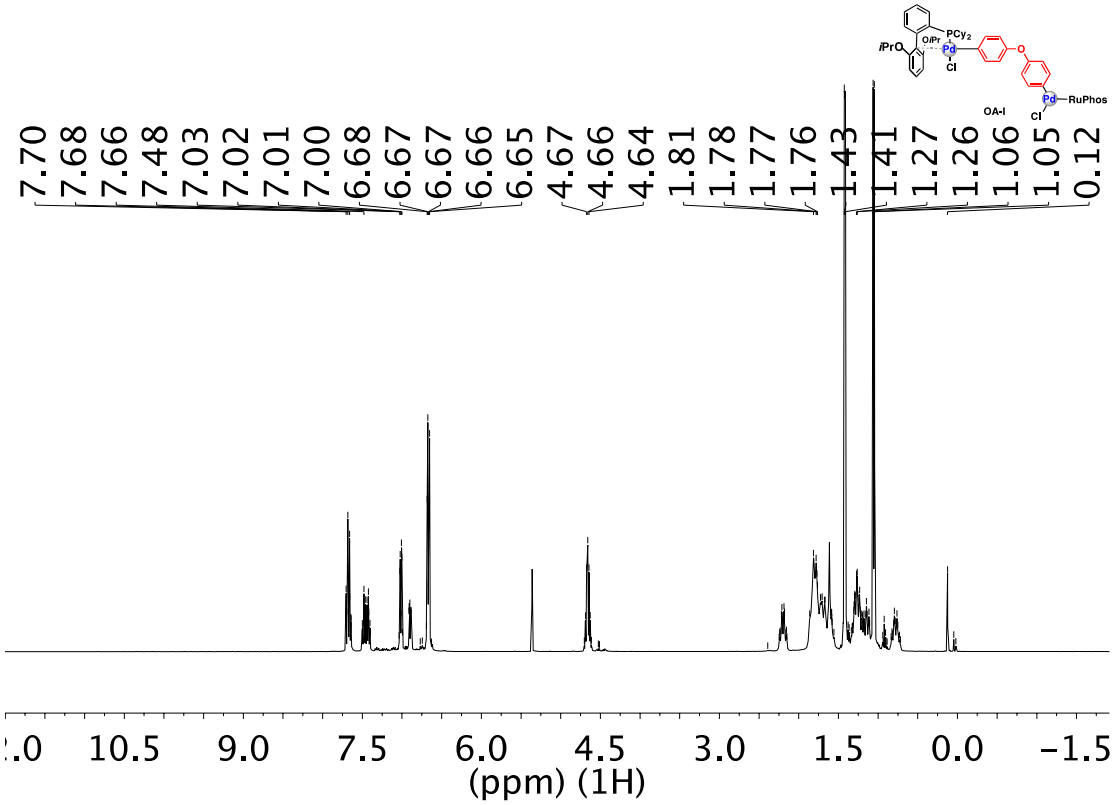
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4.66
4.64
1.81
1.78
1.77
1.76
1.43
1.41
1.27
1.26
1.06
1.05
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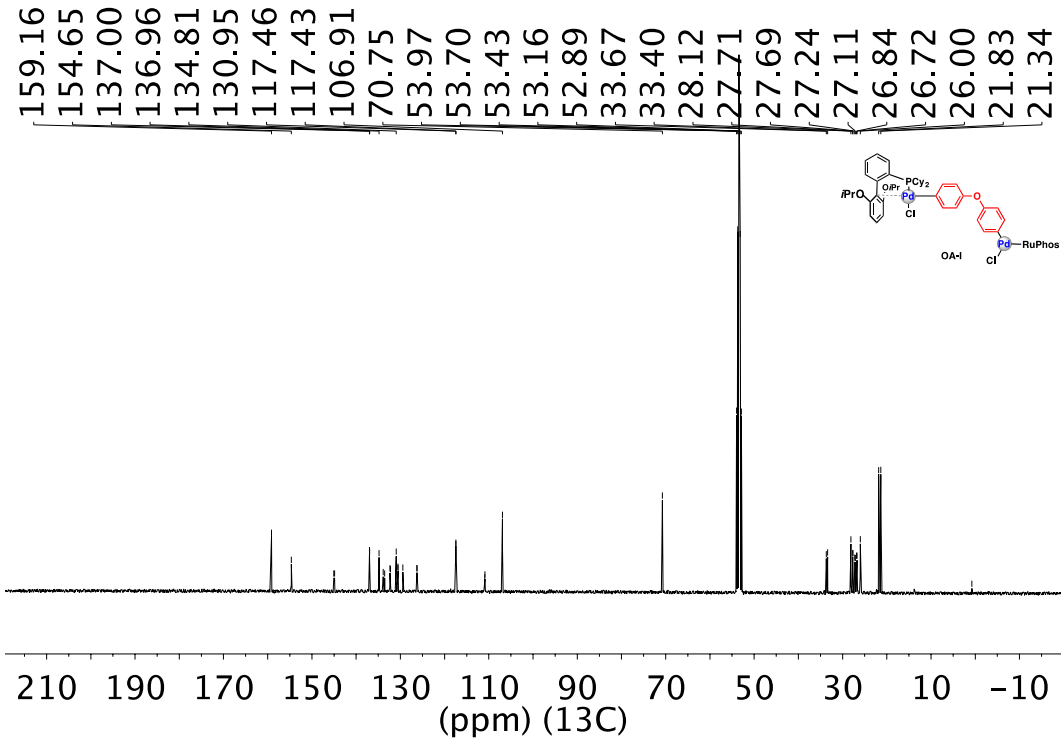
(ppm) (1H)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53.43
53.16
52.89
33.67
33.40
28.12
27.71
27.69
27.24
27.11
26.84
26.72
26.00
21.83
21.34
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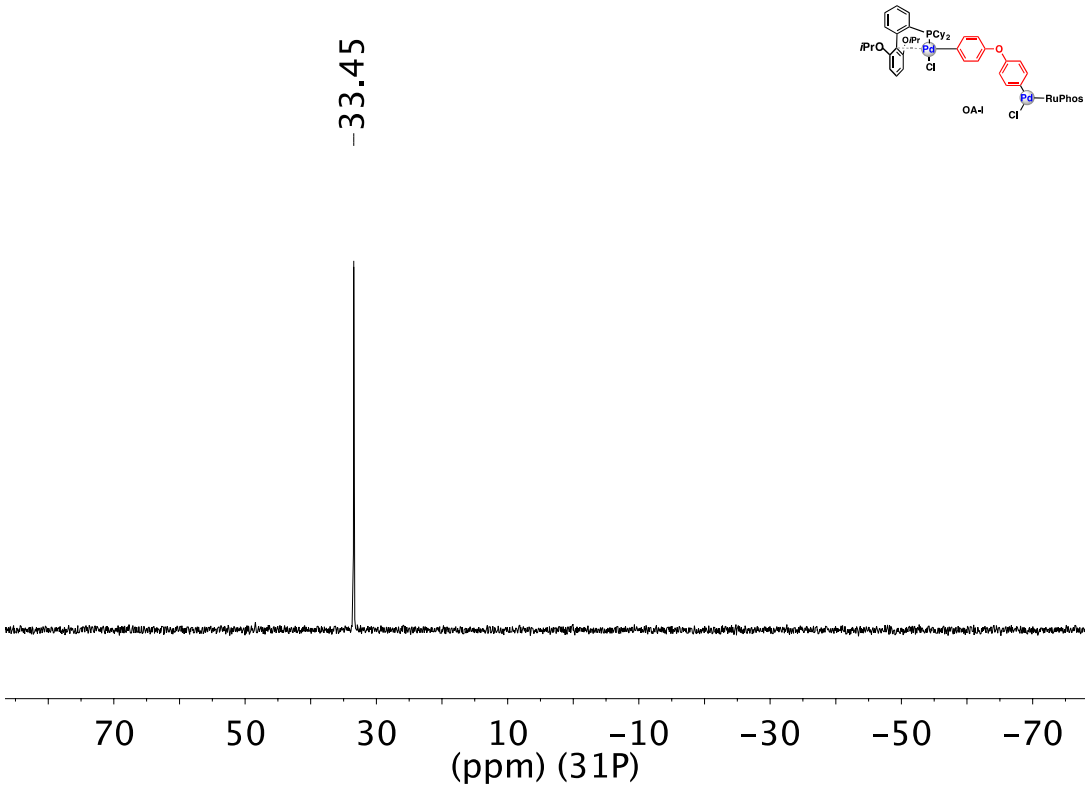
OiPr
PCy2
Pd
Cl
OA-I
RuPhos
210 190 170 150 130 110 90 70 50 30 10 -10
(ppm) (13C)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PCy2
Pd
Cl
OA-I
RuPhos
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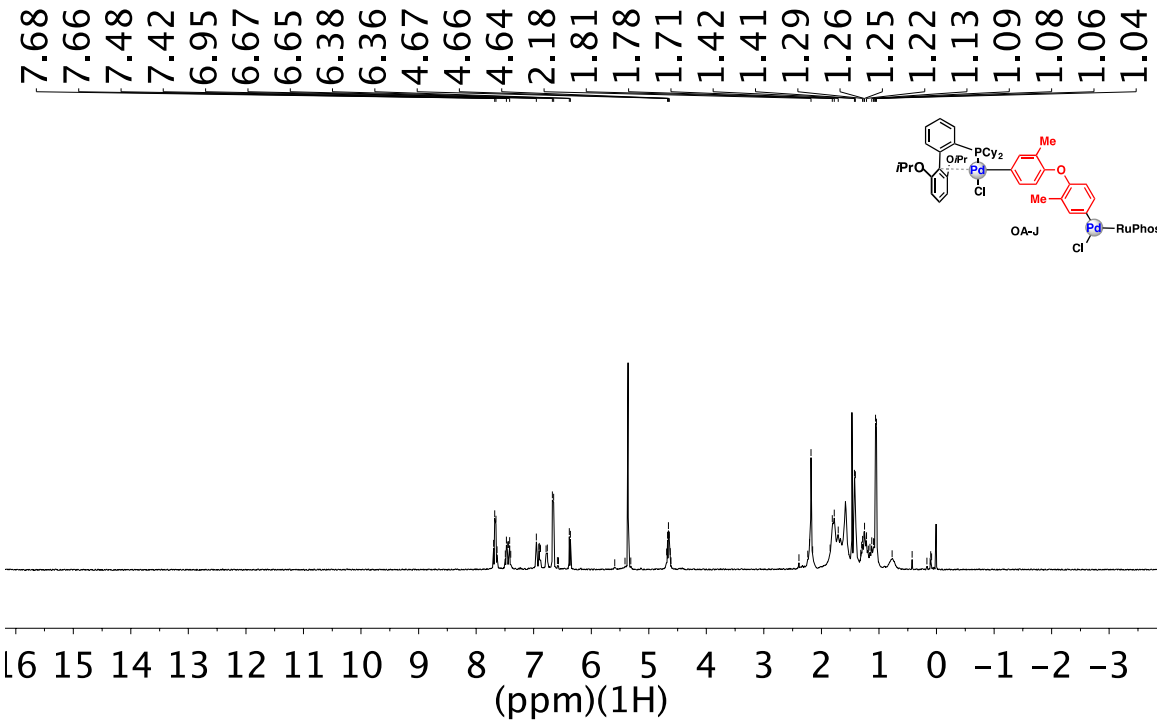
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7.48
7.42
6.95
6.67
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6.38
6.36
4.67
4.66
4.64
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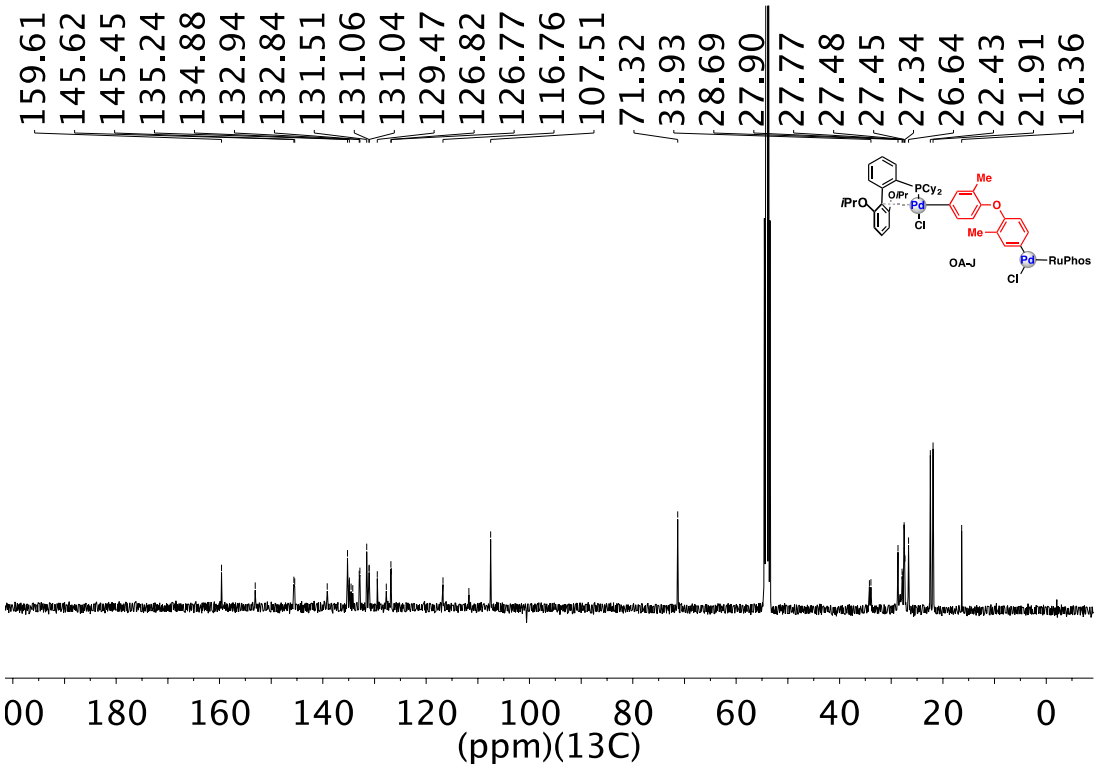
1.81
1.78
1.71
1.42
1.41
1.29
1.26
1.25
1.22
1.13
1.09
1.08
1.06
1.04
iPrO
OiPr
PCy2
Pd
Cl
Me
O
Me
OA-J
Pd
RuPhos
Cl
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3
(ppm)(1H)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33.93
28.69
27.90
27.77
27.48
27.45
27.34
26.64
22.43
21.91
16.36
iPrO
OiPr
PCy2
Pd
Cl
Me
O
Me
OA-J
Pd
RuPhos
Cl
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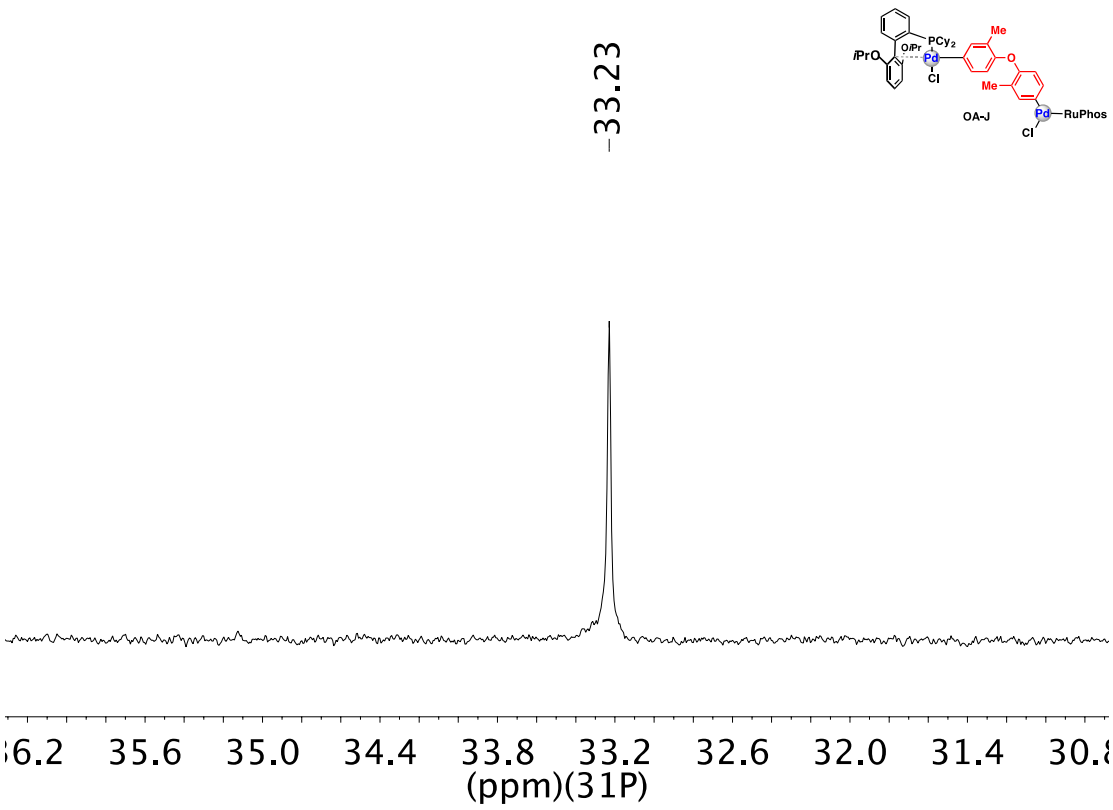
(ppm)(13C)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PCy2
Pd
Cl
Me
O
Me
OA-J
Pd
RuPhos
Cl
6.2
35.6
35.0
34.4
33.8
33.2
32.6
32.0
31.4
30.8
(ppm)(31P)