Electronic Supplementary Information for

Storable Palladacycles for Selective Functionalization of Alkyne-Containing Proteins

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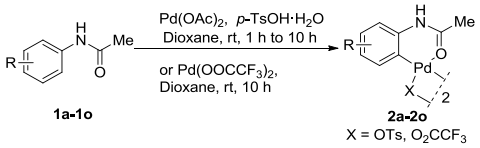
Materials and General Procedures

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, 60Å).[1]H 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; CD_3OD, 3.31; 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, 100, or 125 MHz, and chemical shifts were reported in ppm using the deuterated solvents as internal standards ($CDCl_3$, 77.0; DMSO-d_6, 39.5; CD_3OD). 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 Å, 2.00 × 50 mm) with a flow rate of 200 μL/min and a linear gradient of 5-95% ACN/H_2O containing 0.1% HCOOH.

General procedure to prepare palladacycles2a-2o:



Following Yu's procedure,[1]a solution of acetanilide (0.1 mmol), *p*-toluenesulfonic acid monohydrate (0.1 mmol), and $Pd(OAc)_2$ or ($Pd(OOCCF_3)_2$ (0.1 mmol) in dioxane (1 mL) was stirred at room temperature for 1 to 10 h. Afterwards, the mixture was filtered to afford the corresponding palladacycle as a solid, which was then dried under vacuum.

Di-*μ*-tosyloxy-bis(2-acetaminophenyl-2C,O)dipalladium(II) (2a)[1,2] (Table 1, entry 1): The titled compound was obtained as a yellow solid in 75% yield according to the general procedure: ^1H NMR (DMSO-d_6, 500 MHz) δ 12.0 (s, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.20 (t, J = 8.0 Hz, 1H), 7.11 (d, J = 8.0 Hz, 2H), 7.05-6.99 (m, 2H), 2.40 (s, 3H), 2.29 (s, 3H); ^1H NMR (CD_3OD, 500 MHz) δ 7.71 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H), 7.13 (t, J = 8.0 Hz, 1H), 6.95-6.89 (m, 2H), 6.87 (d, J = 8.0 Hz, 1H), 2.37 (s, 3H), 2.32 (s, 3H);MS (ESI) calcd for $C_{12}H_{14}N_3OPd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 322.0, found321.7.

Di-*μ*-tosyloxy-bis(4, 5-dimethoxy-2-acetaminophenyl-2C,O)dipalladium(II) (2b) (Table 1, entry 2):The title compound was prepared as a yellow solid in 93% yield according to the general procedure: ^1H NMR (DMSO-d_6, 500 MHz) δ 11.8 (s, 1H), 7.47 (d, J = 8.0 Hz, 2H), 7.27 (s, 1H), 7.10 (d, J = 8.0 Hz, 2H),

6.69 (s, 1H), 3.73 (s, 3H), 3.70 (s, 3H), 2.36 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.5, 147.9, 146.1, 145.2, 138.1, 128.5, 125.9, 125.3, 116.6, 110.1, 101.9, 56.0, 55.8, 21.4, 21.2; MS (ESI) calcd for $C_{14}H_{18}N_3O_3Pd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 382.0, found 381.6.

Di-μ-tosyloxy-bis(3, 5-dimethoxy-2-acetaminophenyl-2C,O)dipalladium(II) (2c) (Table 1, entry 3):The title compound was prepared as a greenish yellow solid in 68% yield according to the general procedure: ^{1}H NMR (CD_3OD, 500MHz) δ 7.69 (d, J = 7.5 Hz, 2H), 7.21 (d, J = 7.5 Hz, 2H), 6.45 (s, 1H), 6.03 (s, 1H), 3.88 (s, 3H), 3.74 (s, 3H), 2.34 (m, 6H); ^{13}C NMR (CD_3OD, 100MHz) δ 166.0, 154.5, 146.6, 142.1, 140.2, 128.4, 125.5, 115.3, 114.1, 106.4, 96.0, 55.1, 54.5, 19.9, 19.1; MS (ESI) calcd for $C_{14}H_{18}N_3O_3Pd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 382.0, found 381.7.

Palladacycle 2d (Table 1, entry 4): The title compound was prepared as a yellow solid in 87% yield according to the general procedure: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.22 (d, J = 9.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.71 (d, J = 8.5 Hz, 2H), 7.56 (t, J = 7.5 Hz, 1H), 7.50(t, J = 7.5 Hz, 1H), 7.46(d, J = 9.0 Hz, 1H), 7.22(d, J = 7.5 Hz, 2H),7.10 (d, J = 8.5 Hz, 1H), 2.53 (s, 3H), 2.36 (s, 3H); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.86 (s, 1H), 8.33 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.76-7.45 (m, 6H), 7.10 (d, J = 8.4 Hz, 2H), 2.61 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (DMSO-d_6,100 MHz) δ 172.6, 146.1, 138.1, 132.8, 131.9, 128.5, 128.4, 126.8, 126.7, 126.2, 126.0, 125.6, 123.6, 122.6, 121.9, 22.0, 21.2; MS (ESI) calcd for $C_{16}H_{16}N_3OPd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 372.0, found 371.6.

Di-μ-tosyloxy-bis(4, 5-dichloro-2-acetaminophenyl-2C,O)dipalladium(II) (2e) (Table 1, entry 5). The title compound was prepared as a greenish yellow solid in 54% yield according to the general procedure: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.2 (s, 1H), 7.80 (s, 1H), 7.48 (d, J = 8.1 Hz, 2H), 7.24 (s, 1H), 7.11 (d, J = 7.8 Hz, 2H), 2.40 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.8, 146.1, 138.1, 135.3, 132.7, 129.2, 128.5, 126.1, 125.9, 120.3, 118.5, 21.9, 21.2; MS (ESI) calcd for $C_{12}H_{12}C_{l2}N_3OPd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 389.9, found 389.7.

Di-μ-tosyloxy-bis(4-fluro-2-acetaminophenyl-2C,O)dipalladium(II) (2f) (Table 1, entry 6): The title compound was prepared as a yellow solid in 95% yield according to the general procedure: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.08 (s, 1H), 7.64-7.57 (m, 1H), 7.48 (d, J = 8.1 Hz, 2H), 7.11 (d, J = 8.1 Hz, 2H), 6.99-6.85 (m, 2H), 2.40 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.7, 161.2 (d, $^1J_{C-F}$ = 240 Hz, C_{Ar-F}), 146.2, 138.1, 136.1 (d, J_{C-F} = 8.0 Hz, C_{Ar-F}), 133.1 (d, J_{C-F} = 9.1 Hz, C_{Ar-F}), 128.5, 125.9, 114.67, 111.8 (d, J_{C-F} = 20.6 Hz, C_{Ar-F}), 104.5 (d, J_{C-F} = 25.2 Hz, C_{Ar-F}), 21.8, 21.2; ^{19}F NMR (DMSO-d_6, 470.4 MHz) δ -118.2 (s, 1F); MS (ESI) calcd for $C_{12}H_{13}FN_3OPd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 340.0, found 339.7.

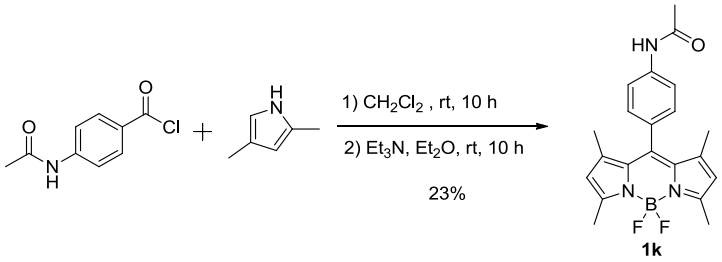
Di-μ-tosyloxy-bis(5-chloro-2-acetaminophenyl-2C,O)dipalladium(II) (2g) (Table 1, entry 7):The title compound was prepared as a yellow solid in 67% yield according to the general procedure as described above. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.06 (s, 1H), 7.65 (s, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.27 (d, J = 8.0 Hz, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.02 (d, J = 8.0 Hz, 1H), 2.40 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.1, 146.2, 138.0, 133.6, 131.4, 128.5, 126.7, 125.9, 118.9, 110.0, 21.7, 21.2; MS (ESI) calcd for $C_{12}H_{13}ClN_3OPd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 356.0, found 355.7.

Di-μ-tosyloxy-bis(5-methyl-2-acetaminophenyl-2C,O)dipalladium(II) (2h)[3](Table 1, entry 8): The title compound was prepared as a yellow solid in 70% yield according to the general procedure. ^{1}H NMR

(DMSO-d_6, 400 MHz) δ 11.9 (s, 1H), 7.48 (d, J = 7.5 Hz, 2H), 7.42 (s, 1H), 7.11 (d, J = 8.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 2.39 (s, 3H), 2.30 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.3, 146.2, 138.0, 135.1, 134.4, 129.8, 128.5, 127.6, 125.9, 121.0, 117.5, 21.5, 21.2, 21.1; MS (ESI) calcd for $C_{13}H_{16}N_3OPd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 336.0, found 335.8.

Di-μ-tosyloxy-bis(4-methyl-2-acetaminophenyl-2C,O)dipalladium(II) (2i) (Table 1, entry 9): The title compound was prepared as a yellow solid in 71% yield according to the general procedure: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.9 (s, 1H), 7.52-7.42 (m, 3H), 7.11 (d, J = 7.6 Hz, 2H), 6.89-6.82 (m, 2H), 2.39 (s, 3H), 2.28 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.8, 146.1, 138.1, 136.2, 134.6, 131.9, 128.5, 126.3, 125.9, 118.3, 117.4, 21.6, 21.2, 20.4; MS (ESI) calcd for $C_{13}H_{16}N_3OPd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 336.0, found 335.7.

Di-μ-tosyloxy-bis(4, 6-difluoro-2-acetaminophenyl-2C,O)dipalladium(II) (2j) (Table 1, entry 10): The title compound was prepared as a yellow solid in 73% yield according to the general procedure: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.07 (s, 1H), 7.47 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 6.92 (t, J = 9.0 Hz, 1H), 6.76 (d, J = 9.0 Hz, 1H), 2.41 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 171.7, 165.6 (dd, J_{C-F} = 232.5, 13.7 Hz, C_{Ar-F}), 161.3 (dd, J_{C-F} = 239.3, 15.0 Hz, C_{Ar-F}), 145.5, 137.7, 135.5 (dd, J_{C-F} = 18.3, 12.6 Hz, C_{Ar-F}), 128.0, 125.4, 161.3 (d, J_{C-F} = 33.8 Hz, C_{Ar-F}), 101.9 (m), 100.2 (m), 21.6, 20.7; ^{19}F NMR (DMSO-d_6, 470.4 MHz) δ -85.8 (s, 1F), -115.3 (m, 1F); MS (ESI) calcd for $C_{12}H_{12}F_2N_3OPd$ [M/2 - (OTs) + $(CH_3CN)_2]^+$ 358.0, found 357.7.



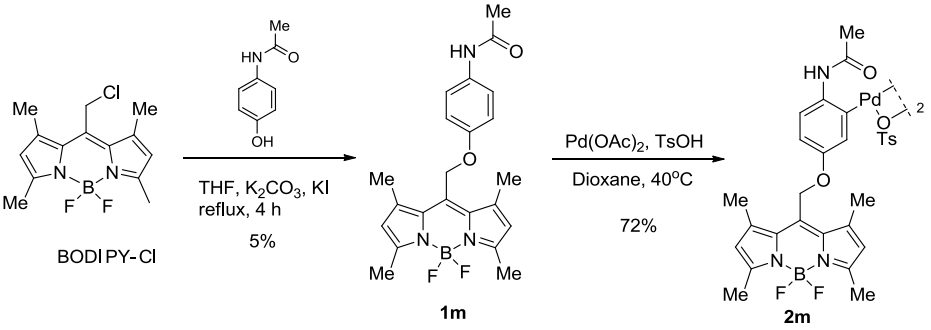
1k

Compound **1k**:4-acetamidobenzoyl chloride (197.6 mg, 1 mmol) was dissolved in CH_2Cl_2, and 2,4-dimehylpyrrole (216 µL, 21 mmol) was added under argon atmosphere at room temperature. After the reaction mixture was stirred at room temperature for 10 h, triethylamine (1 mL) and BF_3·OEt (1 mL) were added dropwise at 0 ºC, and the mixture was stirred for additional 10 h at room temperature. Afterwards, the reaction mixture was diluted with ethyl acetate (20 mL), washed with water (10 mL× 3), dried over Na_2SO_4 and concentrated. The resulting residue was purified by column chromatograph (silica gel, CH_2Cl_2/Ethyl acetate = 10:1) to afford the titled compound **1k** (86 mg, 23%) as a red powder: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.67 (d, J = 8.5 Hz, 2H), 7.41 (s, 1H), 7.21 (d, J = 8.5 Hz, 2H), 5.97 (s, 2H), 2.55 (s, 6H), 2.21 (s, 3H), 1.41 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.4, 155.5, 143.1, 141.2, 138.8, 131.6, 130.5, 128.7, 121.2, 119.8, 24.7, 14.6; MS (ESI) calcd for $C_{21}H_{22}BF_2N_3O$ $(M + H)^+$ 382.2, found: 382.3.

Di-μ-tosyloxy-bis(4-methoxy-2-acetaminophenyl-2C,O)dipalladium(II) (2k) (Table 1, entry 11): The title compound was prepared as a red solid in 35% yield according to the general procedure: ^{1}H NMR (CD_3OD, 500 M Hz) δ 11.80 (s, 1H), 7.71 (d, J = 7.5 Hz, 2H), 7.23(d, J = 8.0 Hz, 2H), 7.14 (d, J = 7.5 Hz, 1H), 7.09 (d, J = 8.0 Hz, 1H), 6.88 (s, 1H),6.08 (s, 2H), 2.49 (s, 6H), 2.38 (s, 6H), 1.48 (s, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 167.3, 155.3, 143.0, 141.8, 131.7, 131.2, 131.0, 129.3, 128.4, 125.9, 125.5, 120.8,

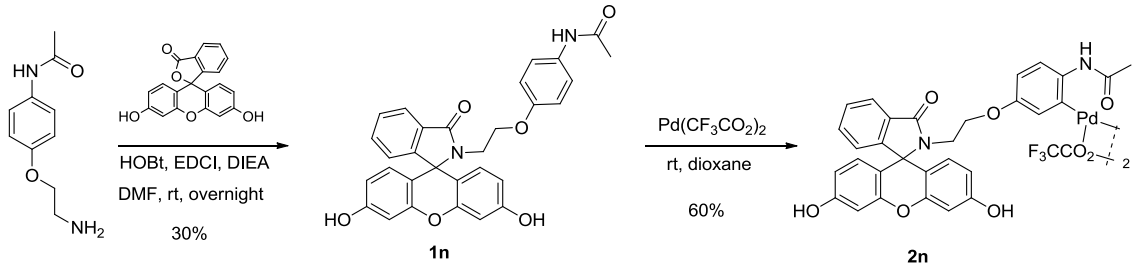
116.6, 114.0, 19.9, 19.4, 13.3, 13.1; MS (ESI) calcd for $C_{25}H_{27}BF_2N_5OPd$ [M/2 - (OTs) + $(CH_3CN)_2$]$^+$ 568.1, found 567.8.

Di-*μ*-tosyloxy-bis(4-methoxy-2-acetaminophenyl-2C,O)dipalladium(II) (2l) (Table 1, entry 12): The title compound was prepared as a yellow solid in 92% yield according to the general procedure. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.9 (s, 1H), 7.46 (d, J = 8.0 Hz, 2H), 7.23 (s, 1H), 7.10 (d, J = 8.5 Hz, 2H), 6.96 (d, J = 8.0 Hz, 1H), 6.79 (d, J = 8.0 Hz, 1H), 3.70 (s, 3H), 2.36 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.7, 155.8, 146.2, 138.1, 128.5, 126.0, 122.0, 119.1, 118.2, 112.7, 55.7, 21.4, 21.2; MS (ESI) calcd for $C_{13}H_{16}N_3O_2Pd$ [M/2 - (OTs) + $(CH_3CN)_2$]$^+$ 352.0, found 351.7.



Compound BODIPY-Cl was prepared from chloro-acetyl chloride and 2,4-dimethylpyrrole by following a reported method.[6] A mixture of compound **1m** (30mg, 0.1 mmol), *N*-(4-hydroxyphenyl)acetamide (30 mg, 0.2 mmol), K_2CO_3 (55 mg, 0.4 mg) and KI (20 mg, 0.12 mmol) in 2 mL THF was refluxed for 4 h under argon atmosphere. The reaction mixture was directly purified through column chromatograph (silica gel, CH_2Cl_2/MeOH = 20 : 1) to give compound **1m** (2 mg, 5%). ^{1}H NMR ($CDCl_3$+CD_3OD, 300 MHz) δ 7.45 (d, J = 9.0 Hz, 2H), 7.08 (s, 1H), 6.97 (d, J = 9.0 Hz, 2H), 6.09 (s, 2H), 5.15 (s, 2H), 2.56 (s, 6H), 2.30 (s, 6H), 2.20 (s, 3H).

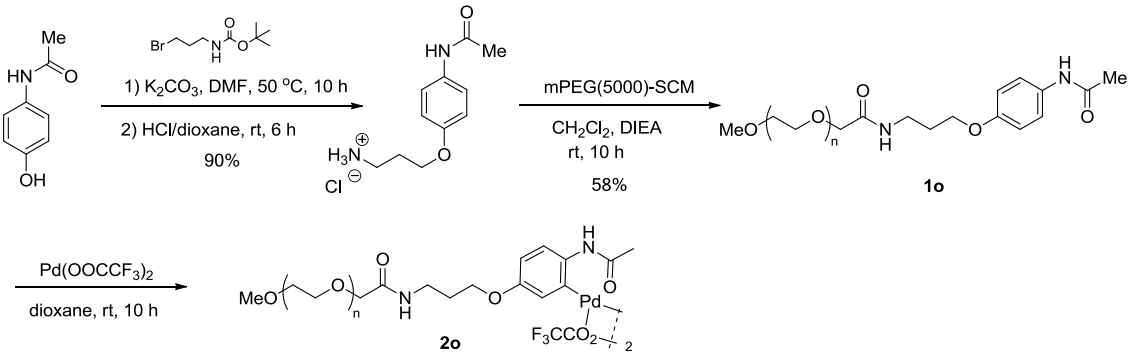
Palladacycle **2m** (Table 1, entry 13): The title compound was prepared as a red solid from **1m** in 72% yield according to the general procedure. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.9 (s, 1H), 7.47 (d, J = 8.0 Hz, 2H), 7.34 (s, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.03-6.96 (m, 2H), 6.28 (s, 1H), 5.18 (s, 1H), 2.44 (s, 6H), 2.38 (s, 3H), 2.28 (s, 3H), 2.26 (s, 6H).



A solution of uranine (75 mg, 0.2 mmol), *N*-(4-(2-aminoethoxy)phenyl)acetamide (46 mg, 0.24 mmol), HOBt (41 mg, 0.3 mmol), DIEA (173 μL, 1 mmol) and EDCI (57 mg, 0.3 mmol) in 3 mL DMF was stirred at room temperature overnight. The solvent was removed under reduced pressure and water was added. The solid was collected by filtration, and further purified through column chromatograph (silica gel, CH_2Cl_2/MeOH = 30 : 1) to give compound **1n** (30 mg, 30 %): ^{1}H NMR ($CDCl_3$+CD_3OD, 500 MHz)

δ 7.89 (m, 1H), 7.44 (m, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.00 (m, 1H), 6.57 (d, J = 2.5 Hz, 2H), 6.43 (d, J = 8.5 Hz, 2H), 6.37 (d, J = 8.5 Hz, 2H), 6.26 (dd, J = 8.5, 2.5 Hz, 2H), 3.58 (t, J = 6.0 Hz, 2H), 3.49 (t, J = 6.0 Hz, 2H), 2.11 (s, 3H).

Palladacycle **2n** (Table 1, entry 14): A mixture of compound **1n** (17 mg, 33 µmol) and $Pd(O_2CCF_3)_2$ (15 mg, 50 µmol) in 1 mL dioxane was stirred at room temperature overnight. Excess $Pd(O_2CCF_3)_2$ was removed by filtration. The filtrate was concentrated to afford palladacycle **2n** (20 mg, 80%): ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.85 (s, 1H), 9.93 (s, 2H), 7.80 (m, 1H), 7.53 (m, 2H), 7.22 (m, 2H), 6.86 (d, J = 8.5 Hz, 1H), 6.60 (m, 2H), 6.46-6.40 (m, 6H), 3.59 (t, J = 6.0 Hz, 2H), 3.33 (t, J = 6.0 Hz, 2H), 2.33 (s, 3H).

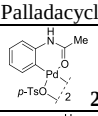
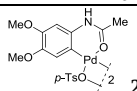
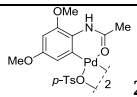
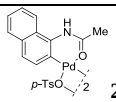
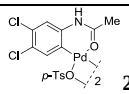
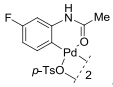
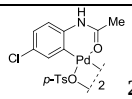
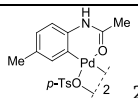
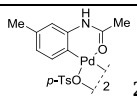
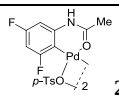
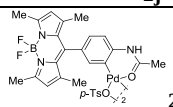
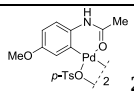
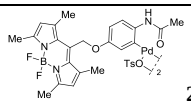
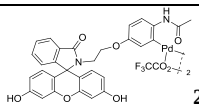


A mixture of *N*-(4-hydroxyphenyl)acetamide (453 mg, 3 mmol), *tert*-butyl (3-bromopropyl)carbamate (785 mg, 3.3 mmol) and K_2CO_3 (2.07g, 15 mmol) in 15 mL DMF was heated to 50 ºC for 8 h. Water (10 mL) was added to the reaction mixture and a white solid was collected by filtration. After drying under vacuum, the solid was treated with HCl/dioxane at room temperature for 5 h. Then the solution was concentrated and ethyl ether (10 mL) was added. The compound (4-acetamidophenoxy)propan-1-ammonium chloride was collected by filtration as a white solid (732 mg, 90%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.80 (s, 1H), 7.85 (brs, 3H), 7.48 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.0 Hz, 2H), 4.01 (t, J = 6.3 Hz, 2H), 2.96 (t, J = 6.3 Hz, 2H), 2.00 (m, 5H).

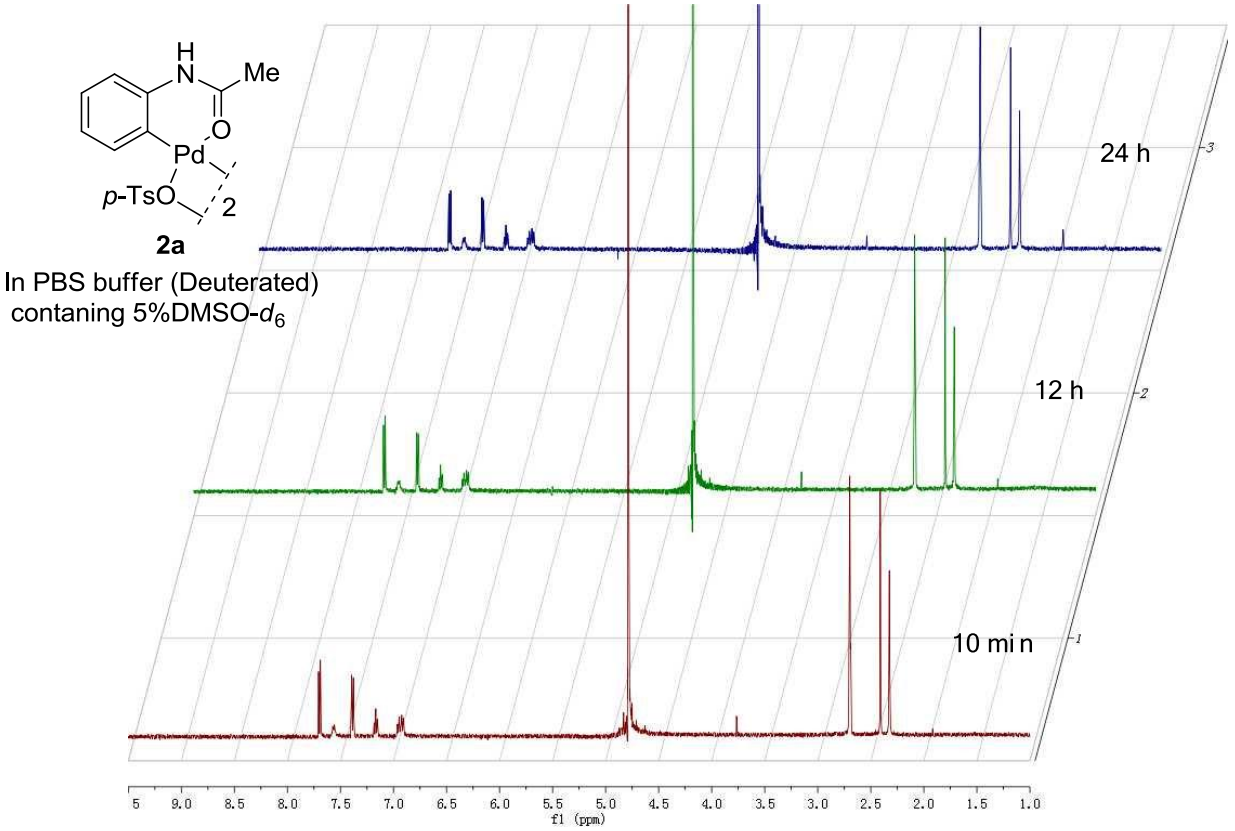
A solution of 3-(4-acetamidophenoxy)propan-1-aminium chloride (4.9 mg, 0.2 mmol), mPEG-SCM (200 mg, ~5 kDa, 0.04 mmol) and DIEA (15 µL) in 2 mL CH_2Cl_2 was stirred for 10 h at room temperature. The solvent was removed by reduced pressure. The residue was purified by preparative HPLC to give the PEGylated product **1o** as a white solid (60 mg, 58%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.88 (s, 1H), 7.45 (d, J = 9.0 Hz, 2H), 6.84 (d, J = 9.0 Hz, 2H), 4.02-3.97 (m, 4H), 3.37 (s, 3H), 2.12 (s, 3H), 2.00 (m, 2H).

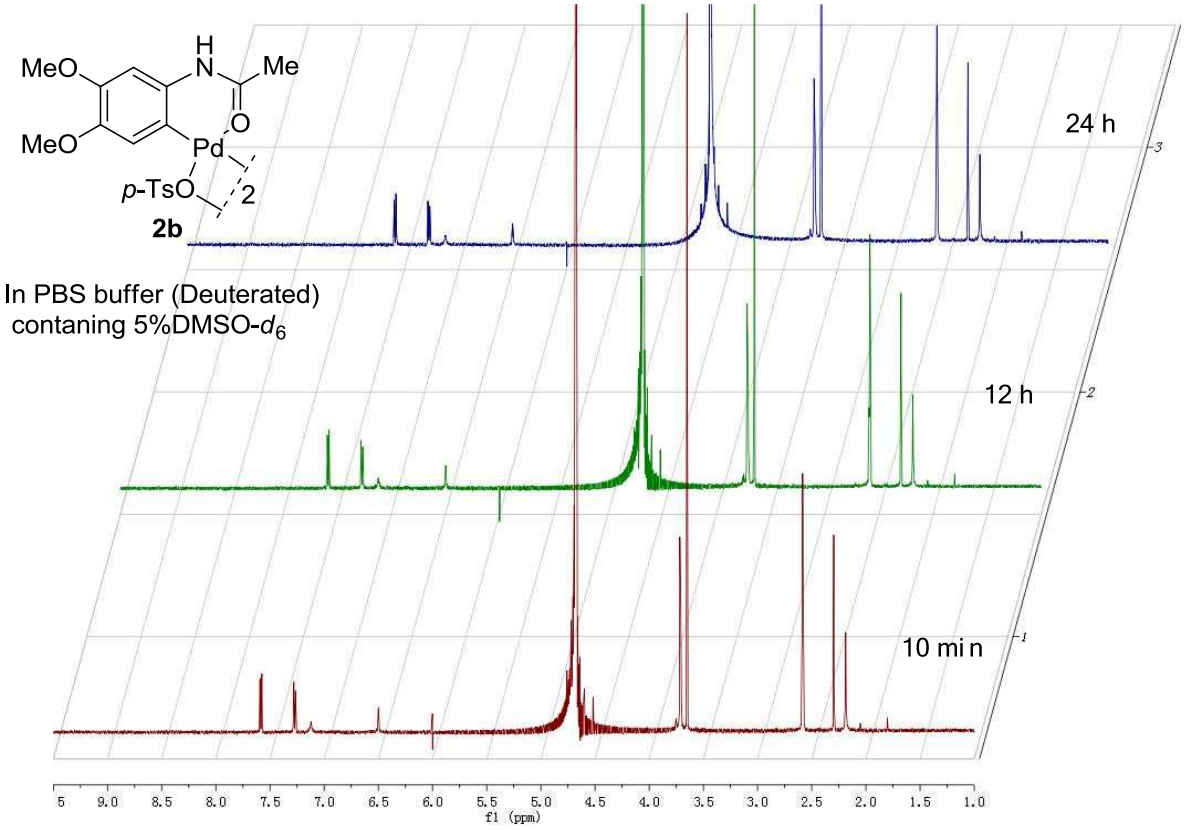
Palladacycle **2o** (Table 1, entry 15): A mixture of compound **1o** (6 mg, 1.1 µmol) and $Pd(OOCCF_3)_2$ (0.5 mg, 1.5 µmol) in 100 µL dioxane was stirred at room temperature for 10 h. The solvent was removed under reduced pressure. A light black solid (6.5 mg) was obtained after drying under vacuum. The yield was determined to be 85% based on ^{1}H NMR: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.88 (s, 1H), 7.76 (m, 1H), 7.21 (s, 1H), 6.95 (d, J = 8.0 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 3.92 (t, J = 5.5 Hz, 2H), 3.86 (s, 2H), 3.24 (s, 3H), 2.36(s, 3H), 1.85 (m, 2H).

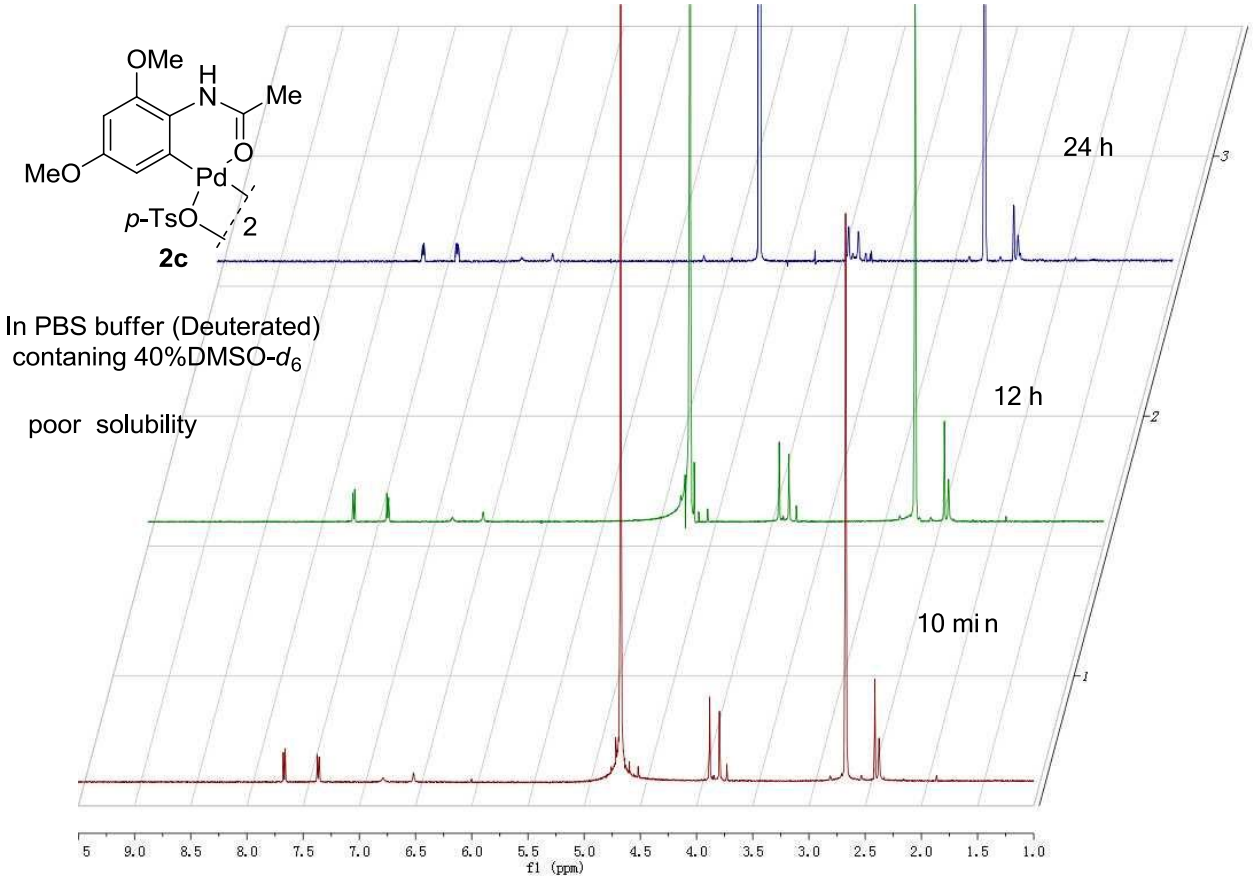
Table S1. Evaluation of palladacycle stability in PBS buffer by ^{1}H NMR.[a]

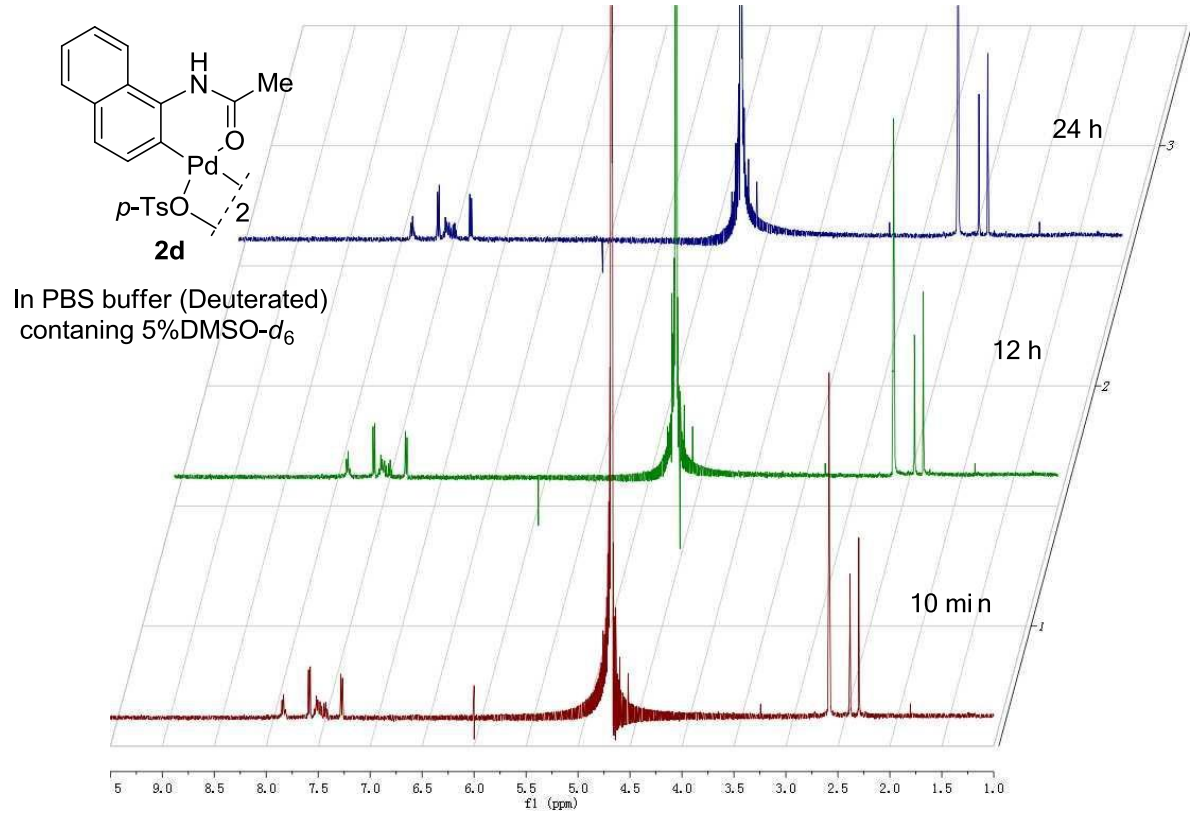
Entry	Palladacycles	Test solvent	Stability
1	 **2a**	95% PBS + 5% DMSO-d_6	Stable after 24 h
2	 **2b**	95% PBS + 5% DMSO-d_6	Stable after 24 h
3	 **2c**	60% PBS + 40% DMSO-d_6	Poor solubility. Relative stable after 24 h; Concentration decreased
4	 **2d**	95% PBS + 5% DMSO-d_6	Stable after 24 h
5	 **2e**	50% PBS + 50%DMSO-d_6	Poor solubility
6	 **2f**	95% PBS + 5% DMSO-d_6	Stable after 24 h
7	 **2g**	95% PBS + 5% DMSO-d_6	Stable after 24 h
8	 **2h**	95% PBS + 5% DMSO-d_6	Stable after 24 h
9	 **2i**	95% PBS + 5% DMSO-d_6	Stable after 24 h
10	 **2j**	95% PBS + 5% DMSO-d_6	Stable after 24 h
11	 **2k**	30% PBS + 70% DMSO-d_6	Poor solubility
12	 **2l**	95% PBS + 5% DMSO-d_6	Stable after 24 h
13	 **2m**	95% PBS + 5% DMSO-d_6	Poor solubility
14	 **2n**	95% PBS + 5% DMSO-d_6	Two sets of signals, possibly due to the presence of two tautomers

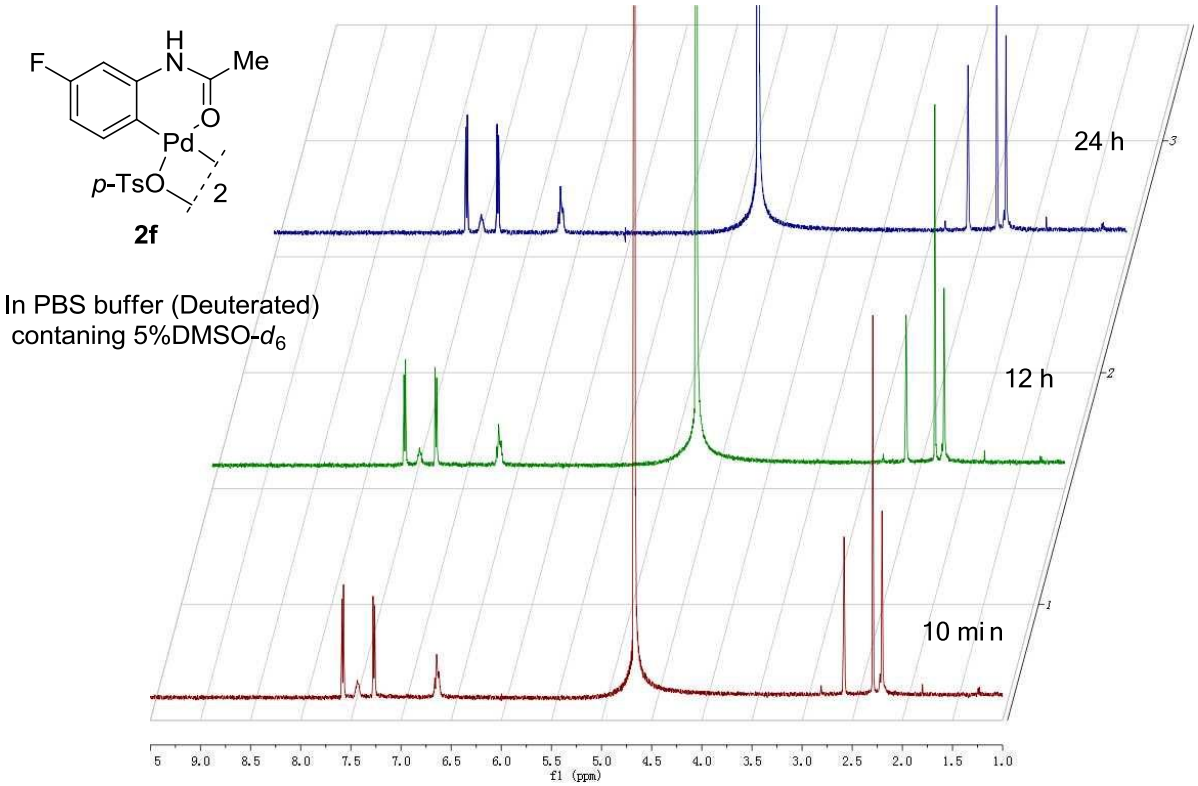
[a]Sample concentration was about 0.5 mM.

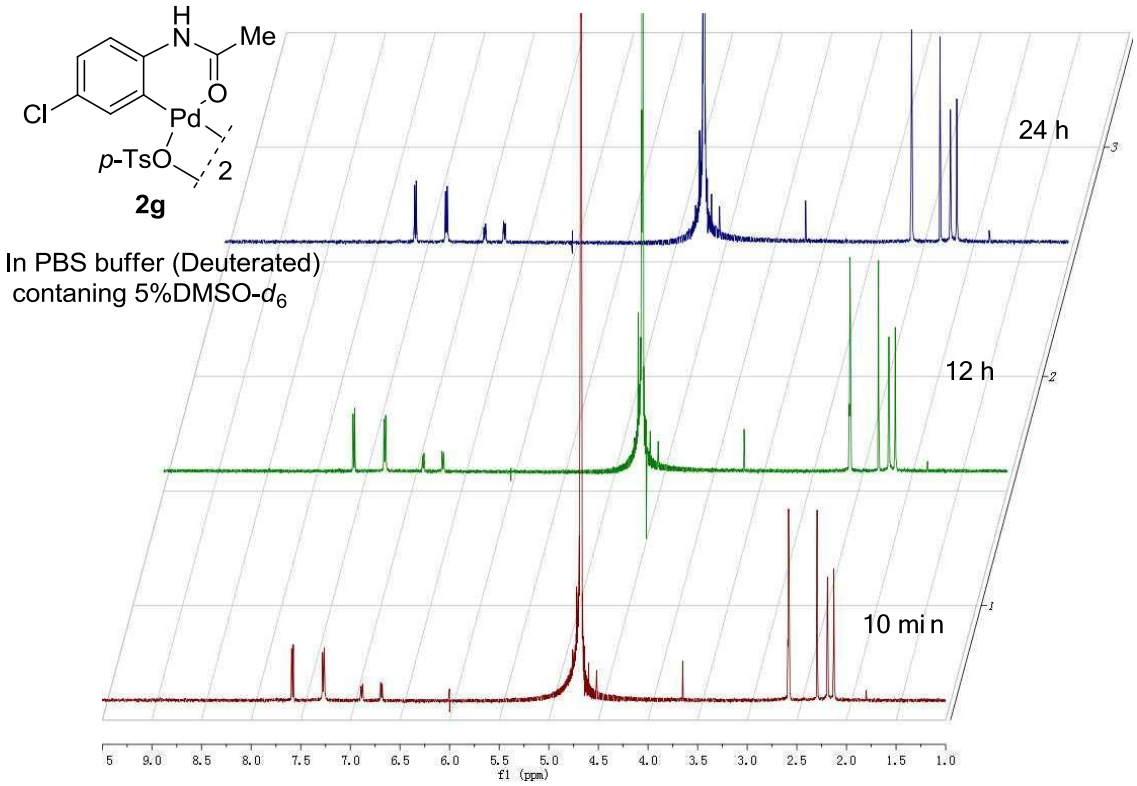

H
N
Me
O
Pd
p-TsO
2
2a
In PBS buffer (Deuterated)
containing 5%DMSO-d6
24 h
12 h
10 min
f1 (ppm)

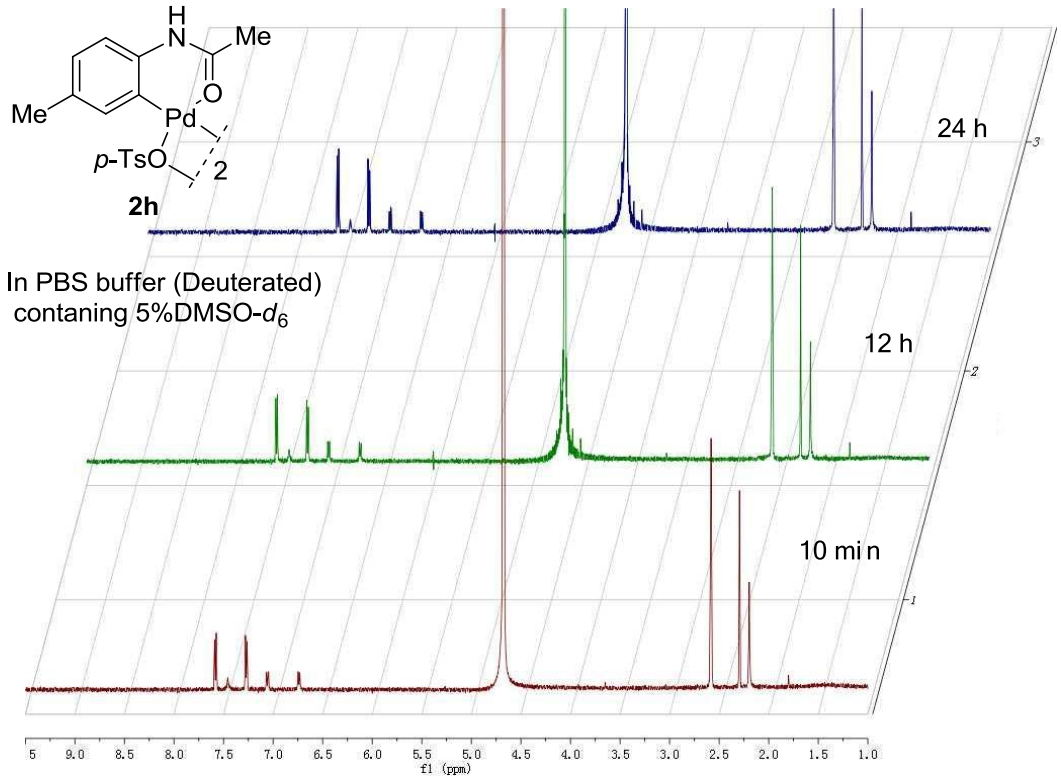

H
N
Me
MeO
MeO
O
Pd
p-TsO
2
2b
In PBS buffer (Deuterated)
containing 5%DMSO-d6
24 h
12 h
10 min
f1 (ppm)

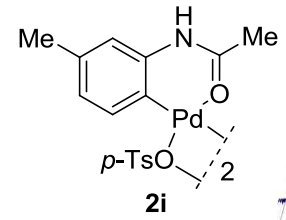

OMe
H
N
Me
O
MeO
Pd
p-TsO
2
2c
In PBS buffer (Deuterated)
containg 40%DMSO-d6
poor solubility
24 h
12 h
10 min
f1 (ppm)


H
N
Me
O
Pd
p-TsO
2
2d
In PBS buffer (Deuterated)
containg 5%DMSO-d6
24 h
12 h
10 min
f1 (ppm)


F
H
N
Me
O
Pd
p-TsO
2
2f
In PBS buffer (Deuterated)
containg 5%DMSO-d6
24 h
12 h
10 min
f1 (ppm)

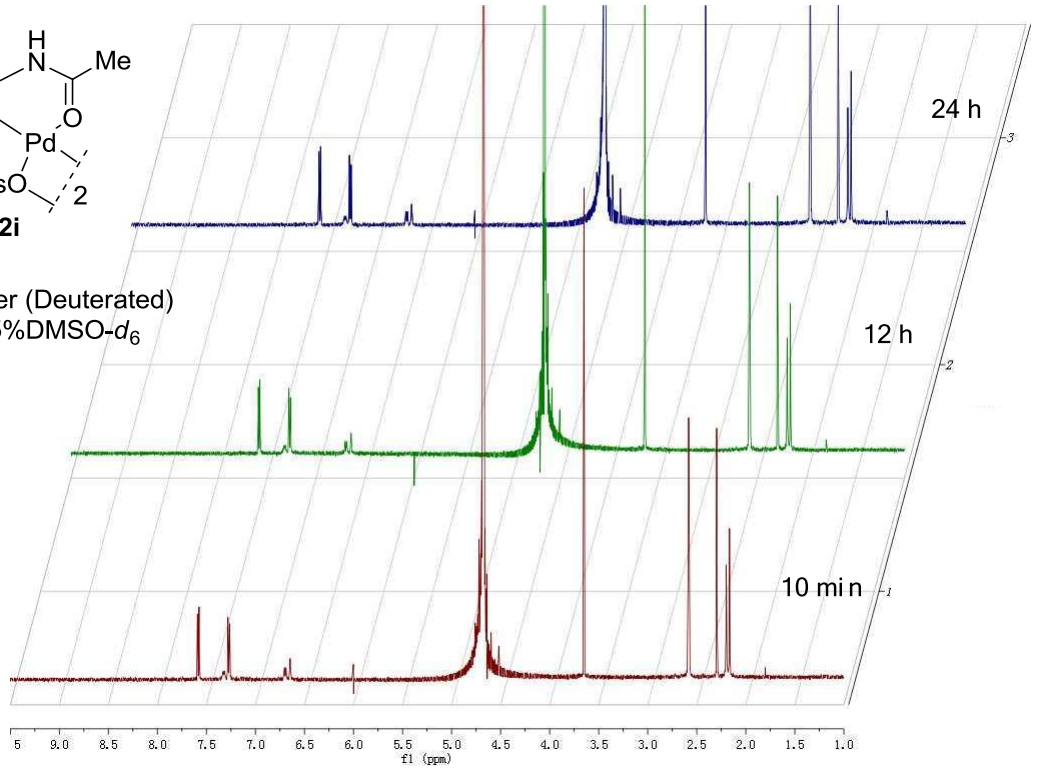

H
N
Me
O
Cl
Pd
p-TsO
2
2g
In PBS buffer (Deuterated)
containing 5%DMSO-d6
24 h
12 h
10 min
f1 (ppm)

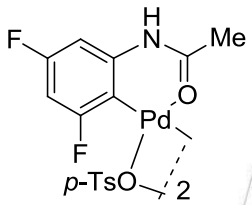

H
N
Me
O
Me
Pd
p-TsO
2
2h
In PBS buffer (Deuterated)
containing 5%DMSO-d6
24 h
12 h
10 min
f1 (ppm)



2i

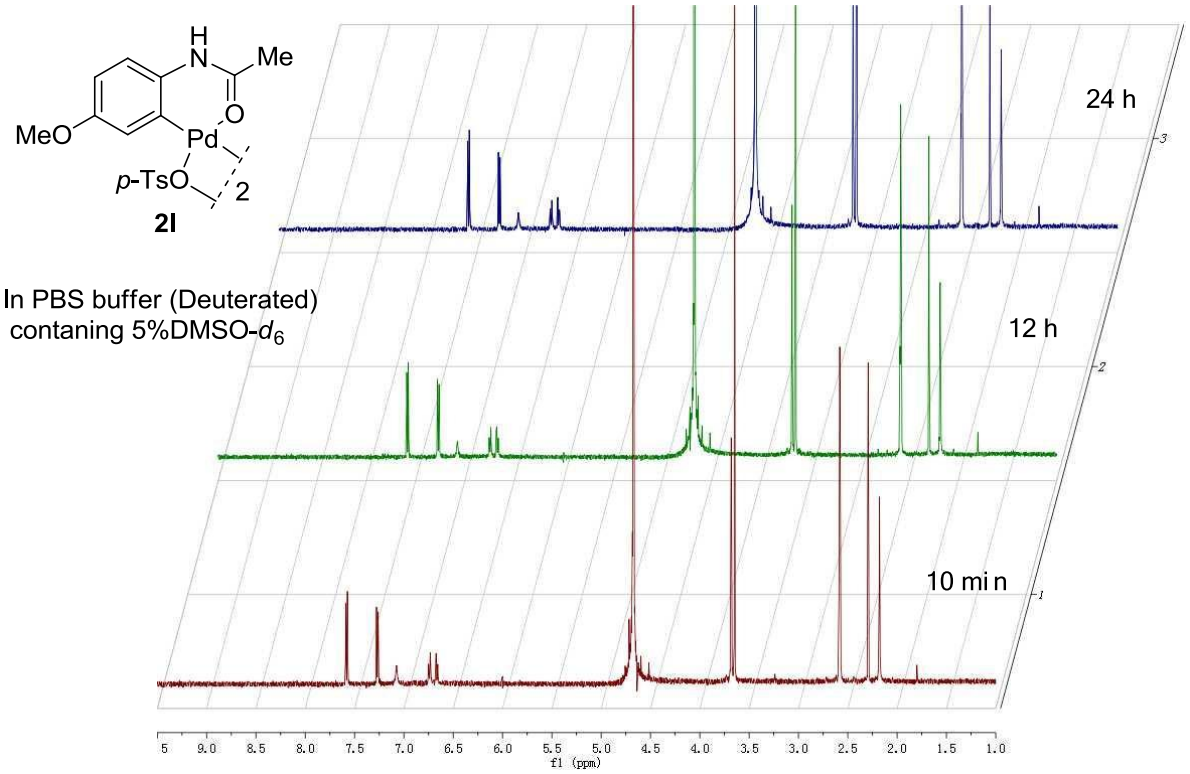
In PBS buffer (Deuterated) containg 5%DMSO-d_6





2j

In PBS buffer (Deuterated) containg 5%DMSO-d_6


H
N
Me
O
MeO
Pd
p-TsO
2
2l
In PBS buffer (Deuterated)
containing 5%DMSO-d6
24 h
12 h
10 min
f1 (ppm)

Reactions of Palladacycle with HPG-Ub

A stock solution of 250 µM palladacycle compound was prepared by dissolving the compound in DMSO. To an ice-chilled a 0.75-mL eppendorf tube containing 48 µL PBS buffer solution was added 1.8 µL of HPG-Ub protein[5] stock (69.6 µM, 0.125 nmol) and 2 µL of palladacycle (dimer) (500 µM in DMSO, 0.5 nmol), and the mixture was stirred at 37 ºC for 30 minutes. Afterwards, the reaction was quenched by addition of 10 µL of 0.5% 3-mercaptopropanoic acid solution in H_2O (0.57µmol). The reaction mixture was analyzed directly by LC-MS.

For LC-MS analysis, a Finnigan LCQ Advantage IonTrap mass spectrometry coupled with a Surveyor HPLC system was used with ion counts as readout (no UV-Vis detector was attached). Protein liquid chromatography was performed using a Phenomenex Jupiter C4 column (5 µm, 300 Å, 2.00 × 50 mm) with a flow rate of 250 µL/min and a linear gradient of 5-95% ACN/H_2O containing 0.1%HCOOH. After obtaining LC-MS raw data, the masses of individual protein species (Met-Ub, HPG-Ub, and ligation product) were identified by performing ion extraction using the respective m/z values of the base peaks followed by area integration to obtain the amounts of the individual protein ions. When an appreciable amount of side product was detected which can be attributed to HPG-Ub, the area integration of the side product was also included in the yield calculation. The following equations were used in determining the reaction yield: $= I_{product}/(I_{HPG\text{-}Ub} + I_{product})$ when there were no detectable side products, or yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product} + I_{side\ product})$ when side products were detected; where $I_{HPG\text{-}Ub}$, $I_{product}$, and $I_{side\ product}$ represent the ion counts of the remaining HPG-Ub, product, and side product, respectively.

Table 2, entry 1: The charge ladder for the desired product is shown as follows: calcd mass 8946.3 Da, found 8946.4 ± 2.6 Da. The reaction yield was determined to be 93% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

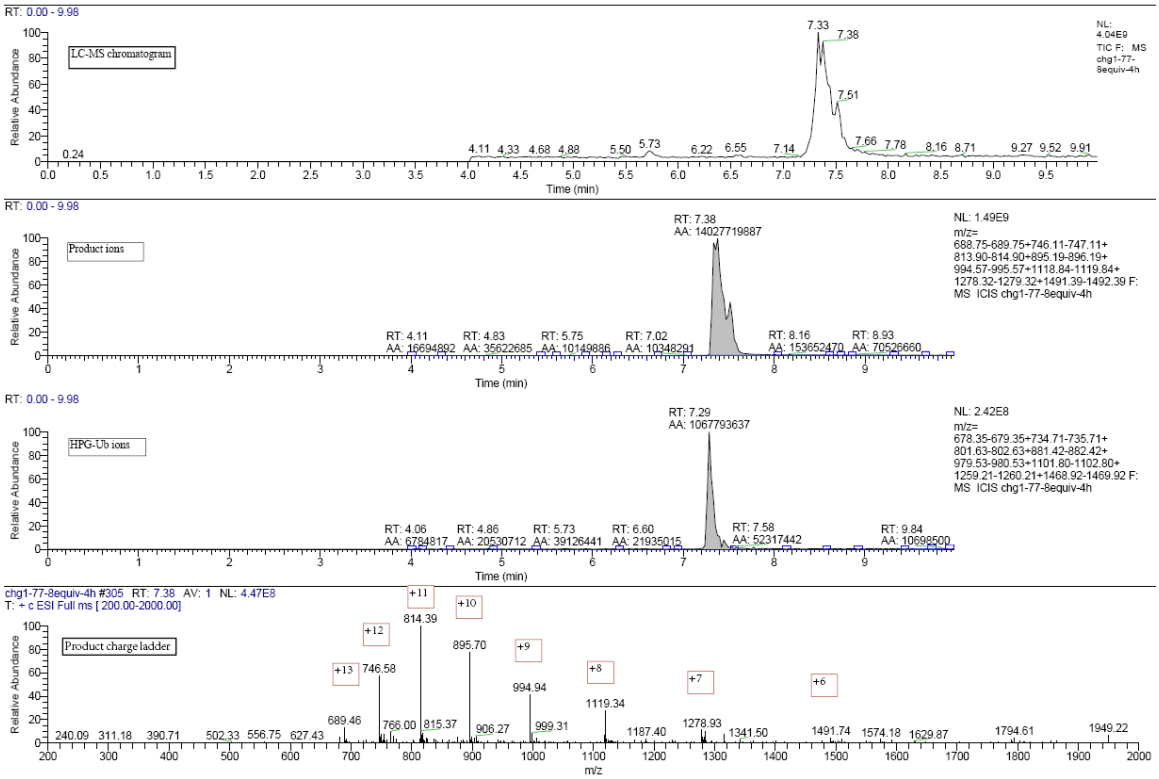


Table 2, entry 2: The charge ladder for the desired product is shown as follows: calcd mass 9006.3 Da, found 9006.5 ± 1.1 Da. The reaction yield was determined to be 93% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

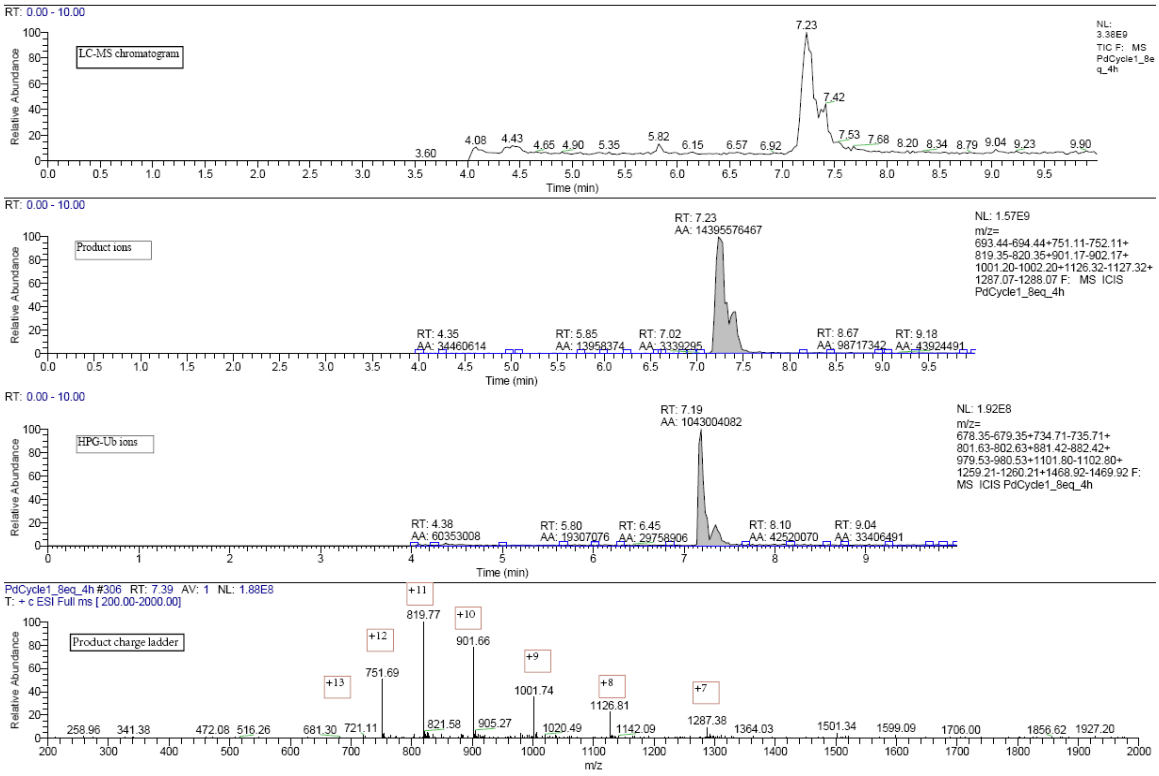


Table 2, entry 3: The charge ladder for the desired product is shown as follows: calcd mass 9006.3 Da, found 9006.1 ± 1.5 Da. The reaction yield was determined to be 80% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

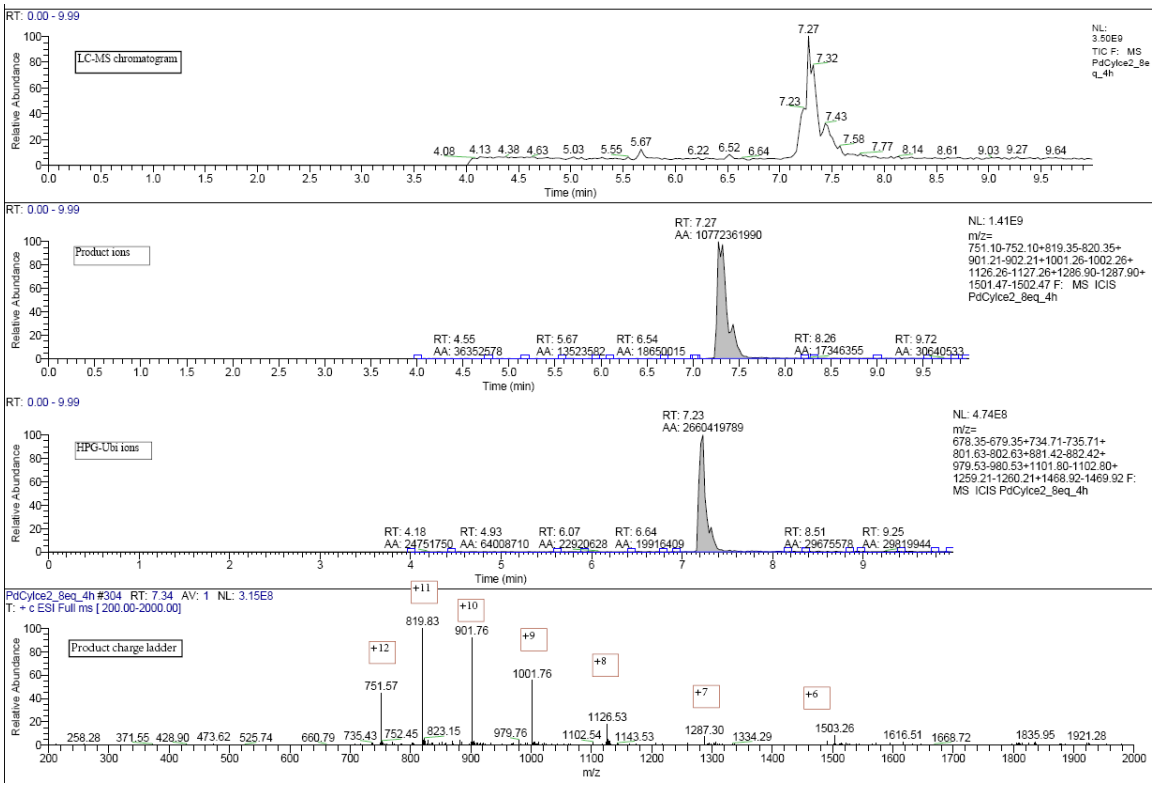


Table 2, entry 4: The charge ladder for the desired product is shown as follows: calcd mass 8996.3 Da, found 8996.6 ± 0.9 Da. The reaction yield was determined to be 99% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

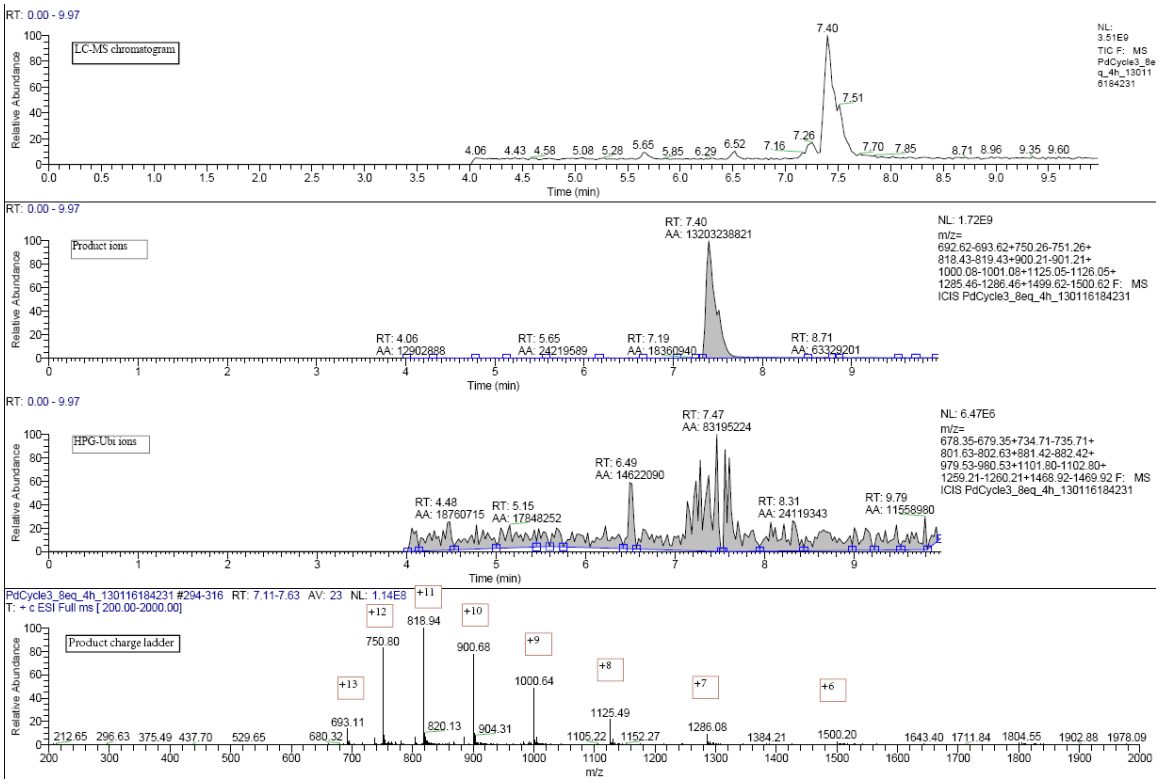


Table 2, entry 5: The charge ladder for the desired product is shown as follows: calcd mass 9015.2 Da, found 9015.5 ± 0.9 Da. The reaction yield was determined to be 42% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

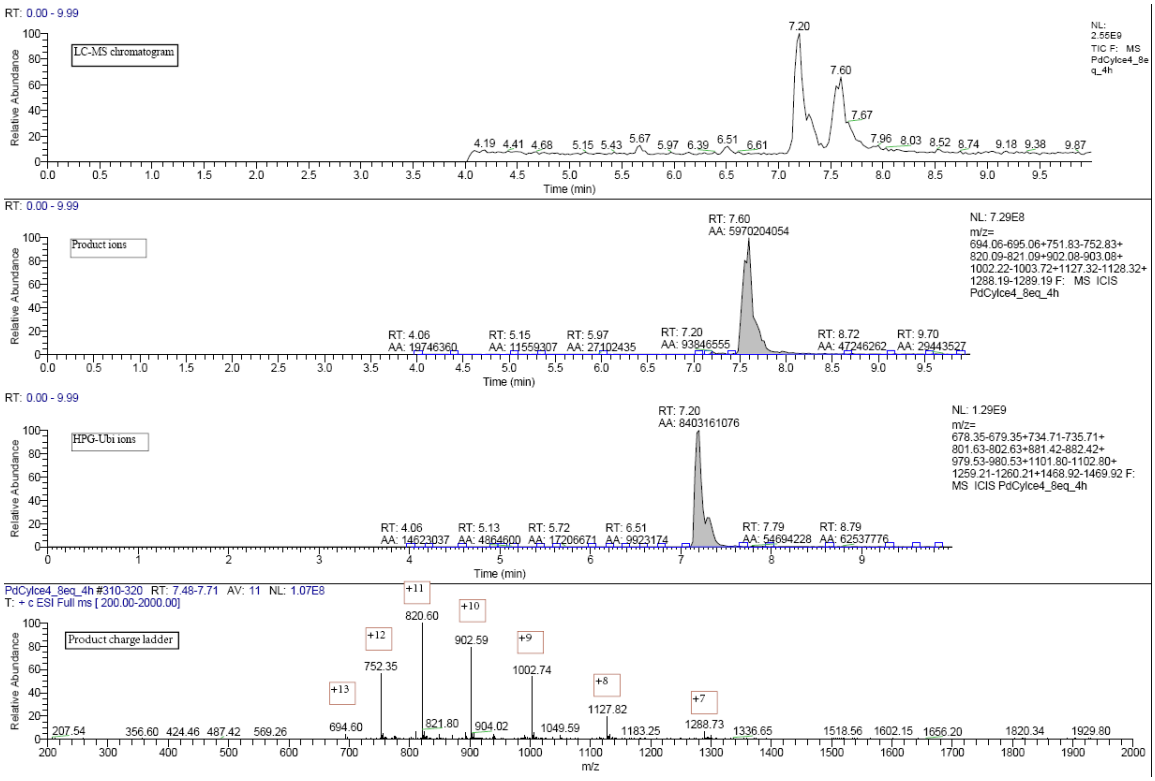


Table 2, entry 6: The charge ladder for the desired product is shown as follows: calcd mass 8964.3 Da, found 8964.3 ± 0.8 Da. The reaction yield was determined to be 89% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

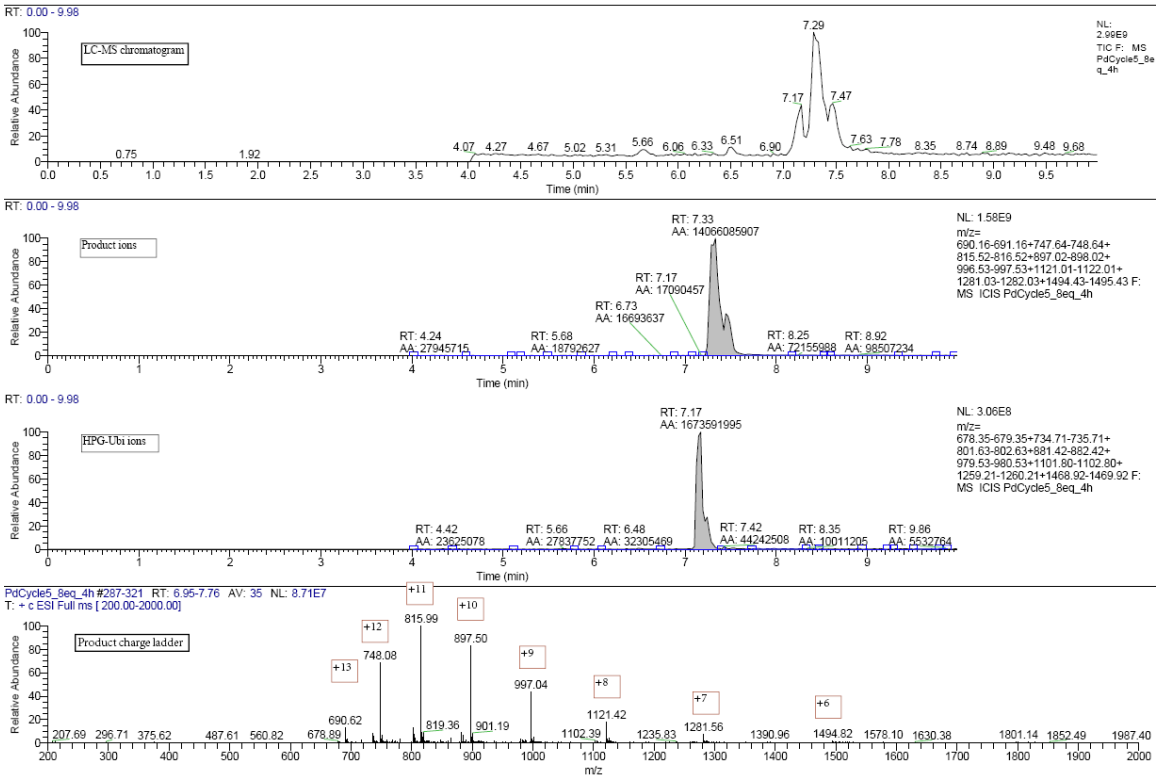


Table 2, entry 7: The charge ladder for the desired product is shown as follows: calcd mass 8980.7 Da, found 8980.7 ± 0.5 Da. The reaction yield was determined to be 61% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

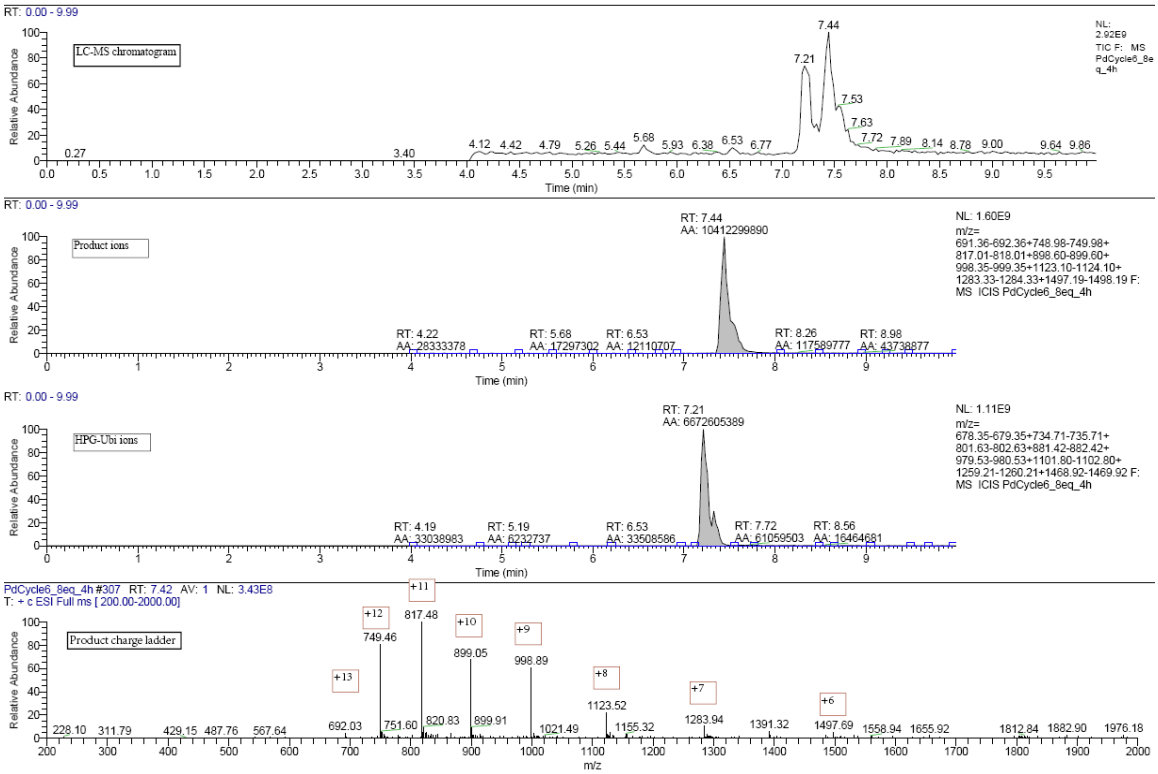


Table 2, entry 8: The charge ladder for the desired product is shown as follows: calcd mass 8960.3 Da, found 8960.7 ± 1.1 Da. The reaction yield was determined to be 98% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

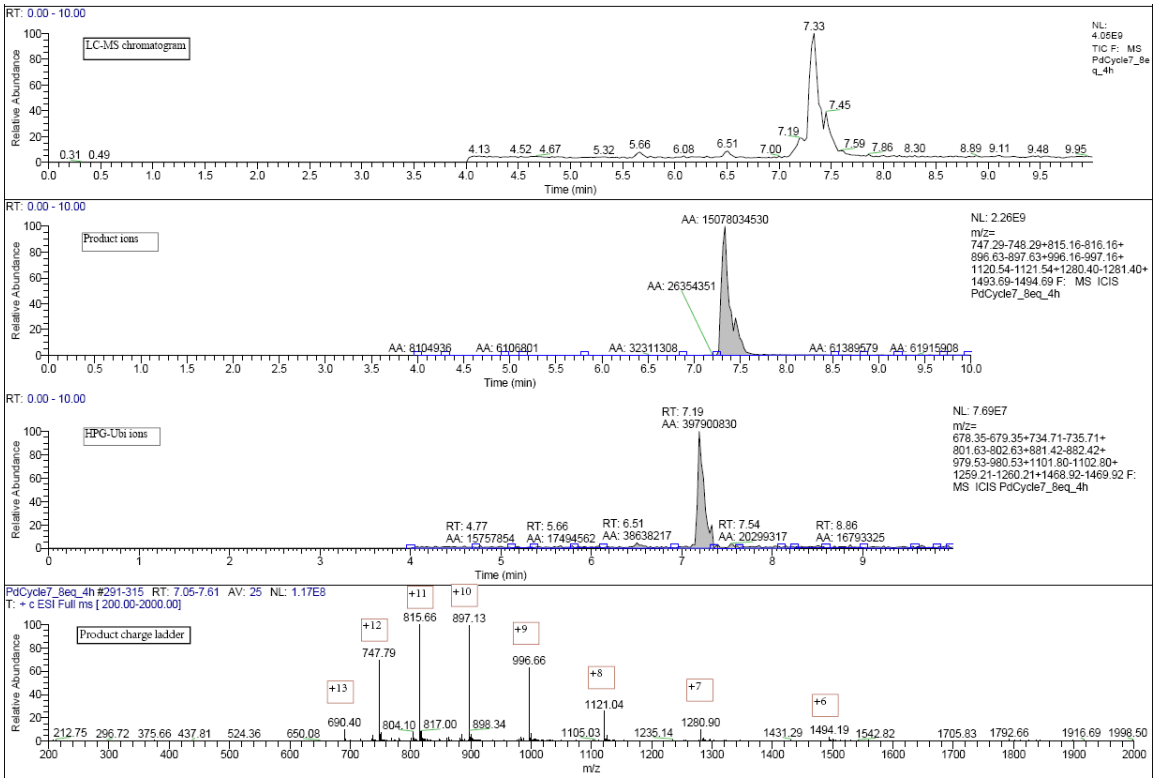


Table 2, entry 9: The charge ladder for the desired product is shown as follows: calcd mass 8960.3 Da, found 8960.3 ± 1.5 Da. The reaction yield was determined to be 92% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

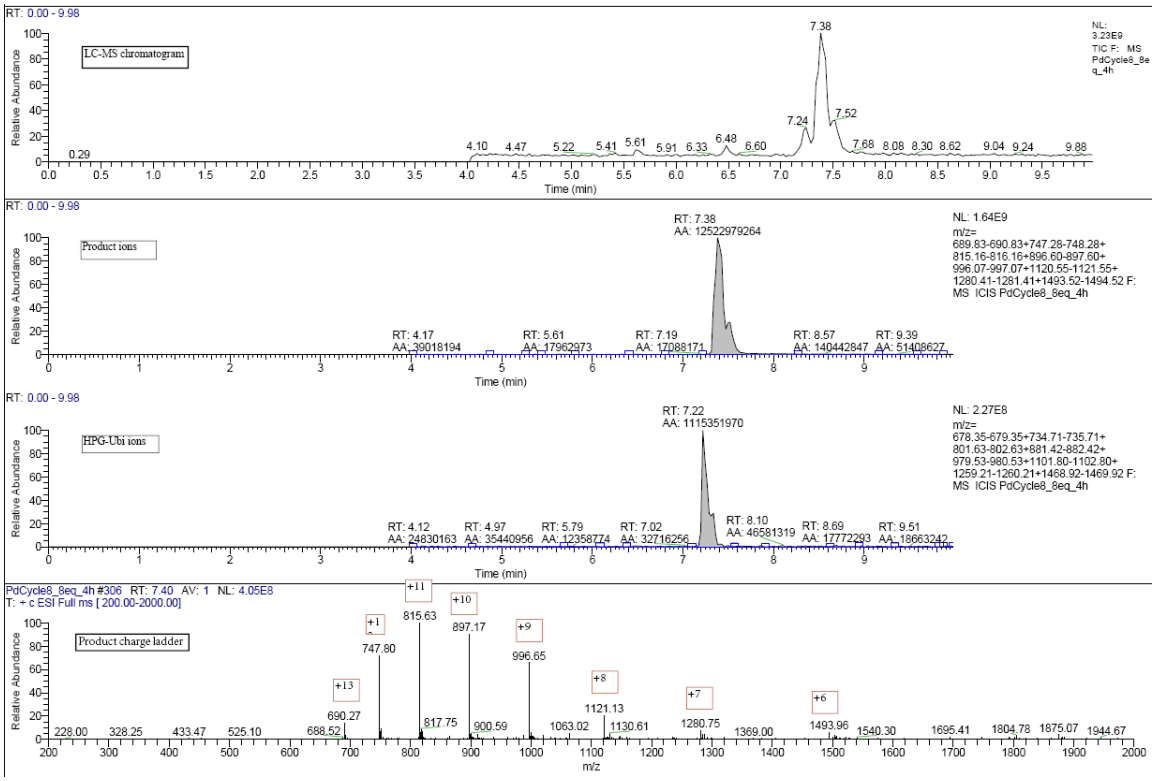


Table 2, entry 10: The charge ladder for the desired product is shown as follows: calcd mass 8982.2 Da, found 8982.3 ± 1.3 Da. The reaction yield was determined to be 80% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

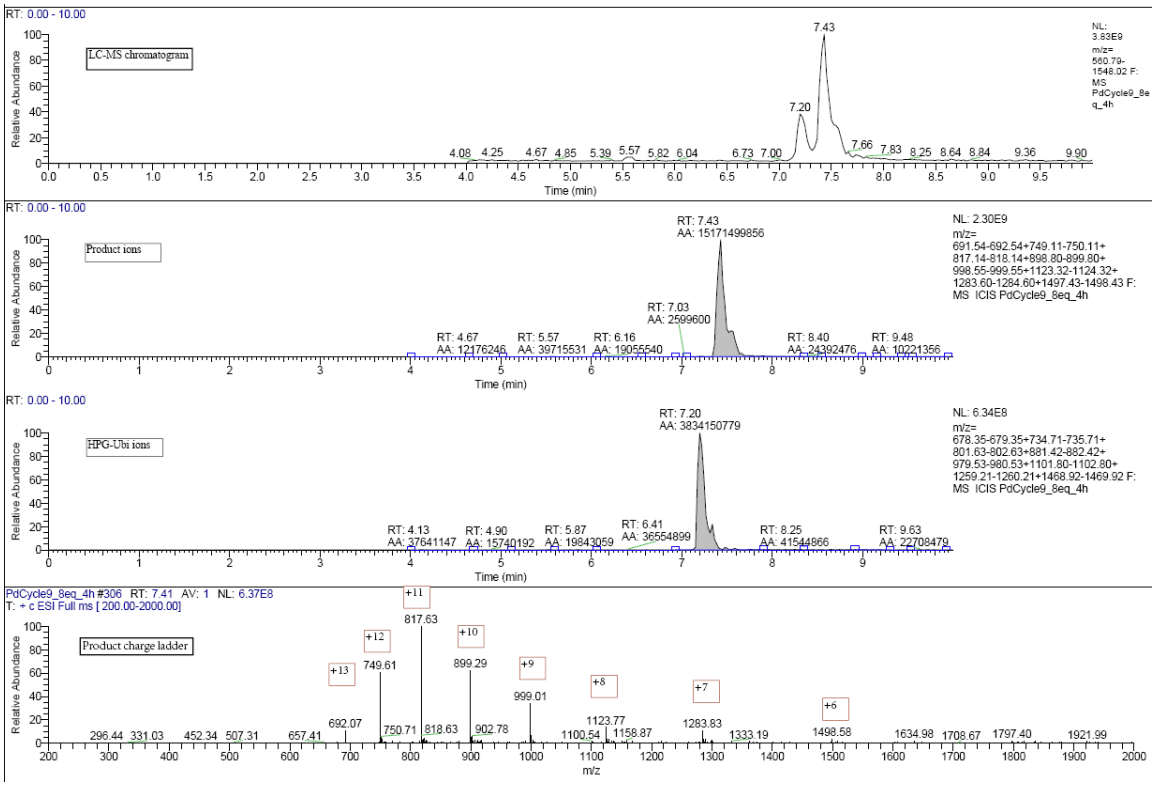


Table 2, entry 11: 25 equiv of **2k** was used, the charge ladder for the desired product is shown as follows: calcd mass 9192.3 Da, found 9192.1 ± 0.8 Da. The reaction yield was determined to be 30% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product} + I_{side\text{-}product})$.

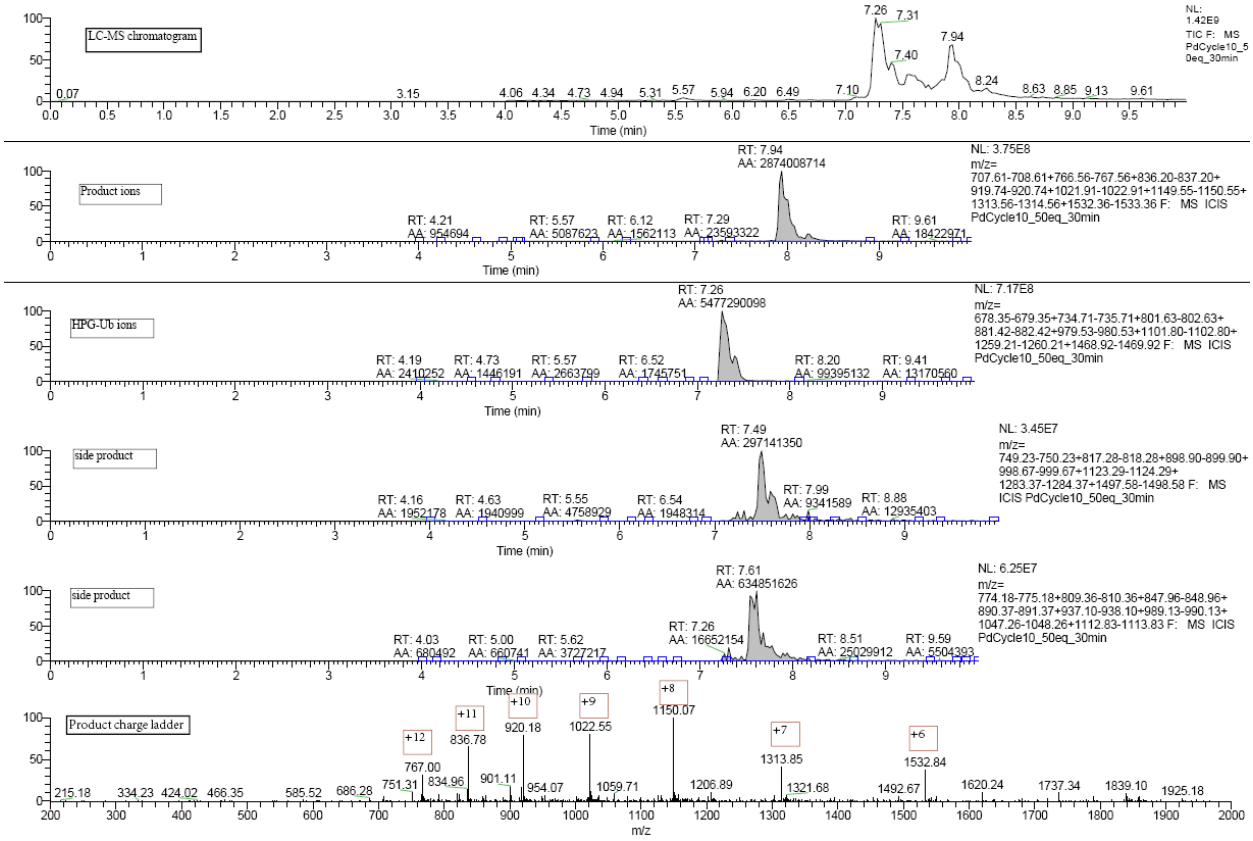


Table 2, entry 12: The charge ladder for the desired product is shown as follows: calcd mass 8976.3 Da, found 8976.5 ± 0.8 Da. The reaction yield was determined to be 85% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

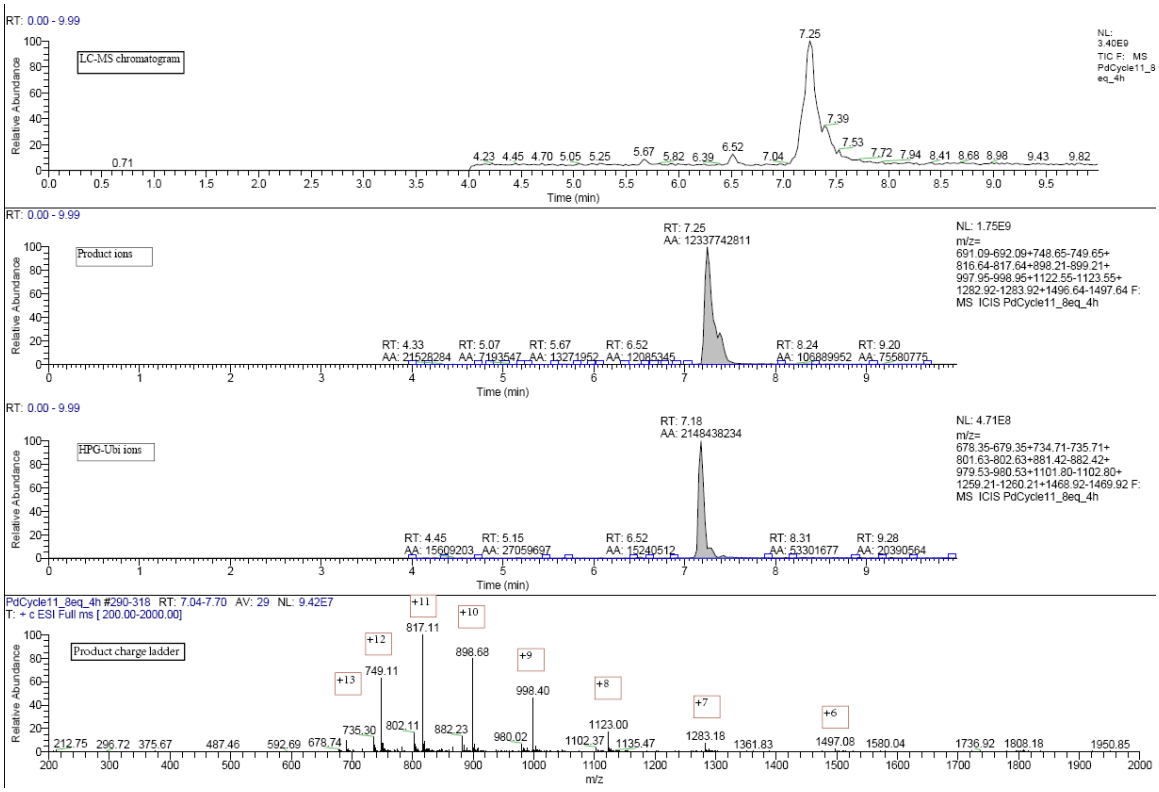


Table 2, entry 13: 25 equiv of **2m** was used; the charge ladder for the desired product is shown as follows: calcd mass 9222.4 Da, found 9221.8 ± 0.8 Da. The reaction yield was determined to be 14% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.

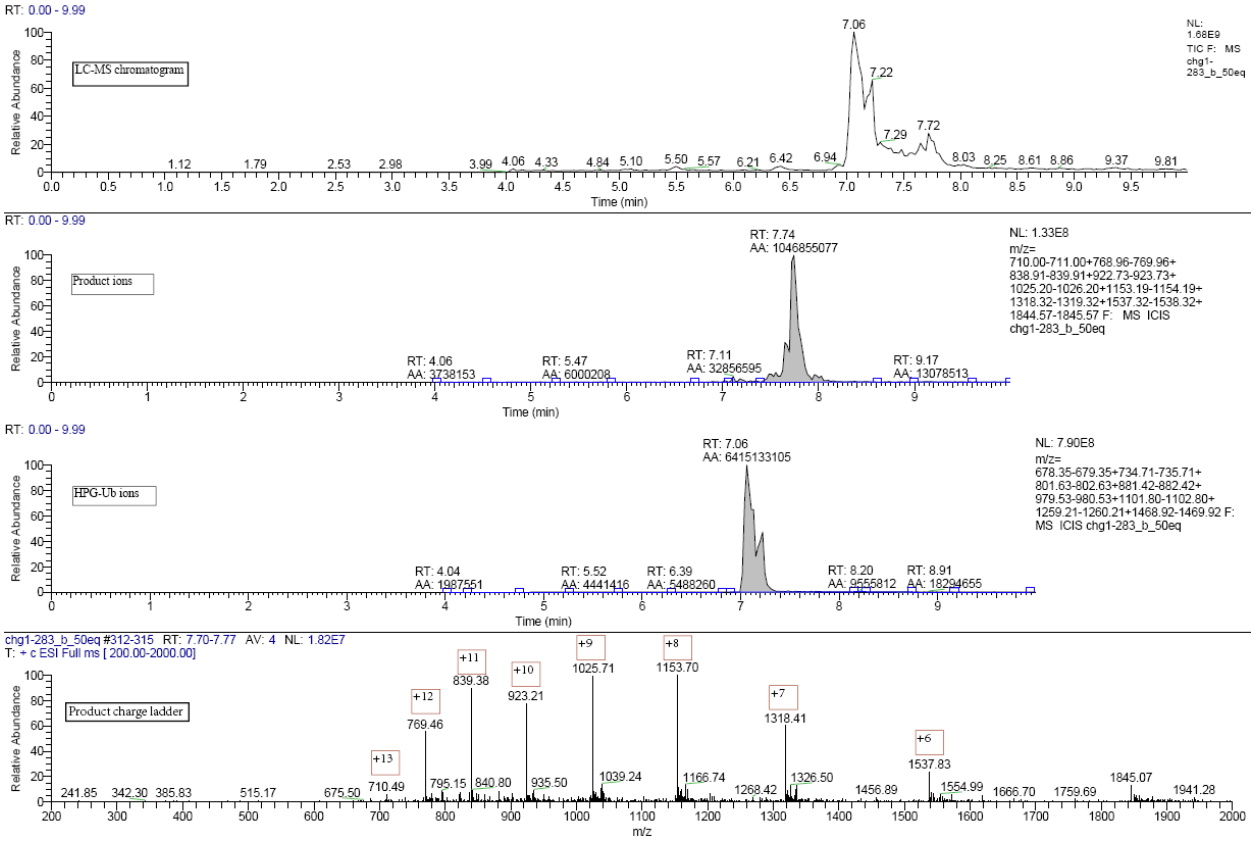
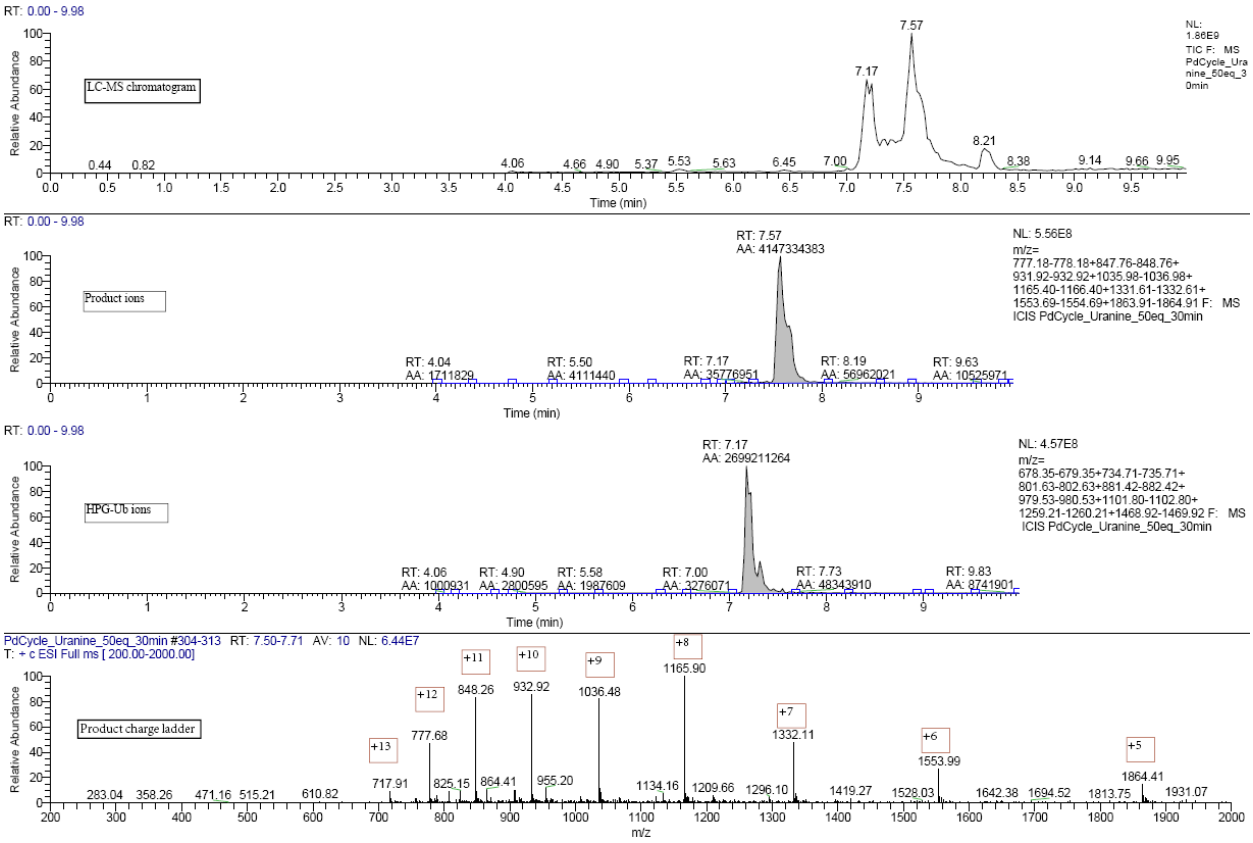


Table 2, entry 14: 25 equiv of **2n** was used; the charge ladder for the desired product is shown as follows: calcd mass 9319.6 Da, found 9319.0 ± 1.0 Da. The reaction yield was determined to be 60% by LC-MS based on the following equation: yield % = $I_{product}/(I_{HPG\text{-}Ub} + I_{product})$.



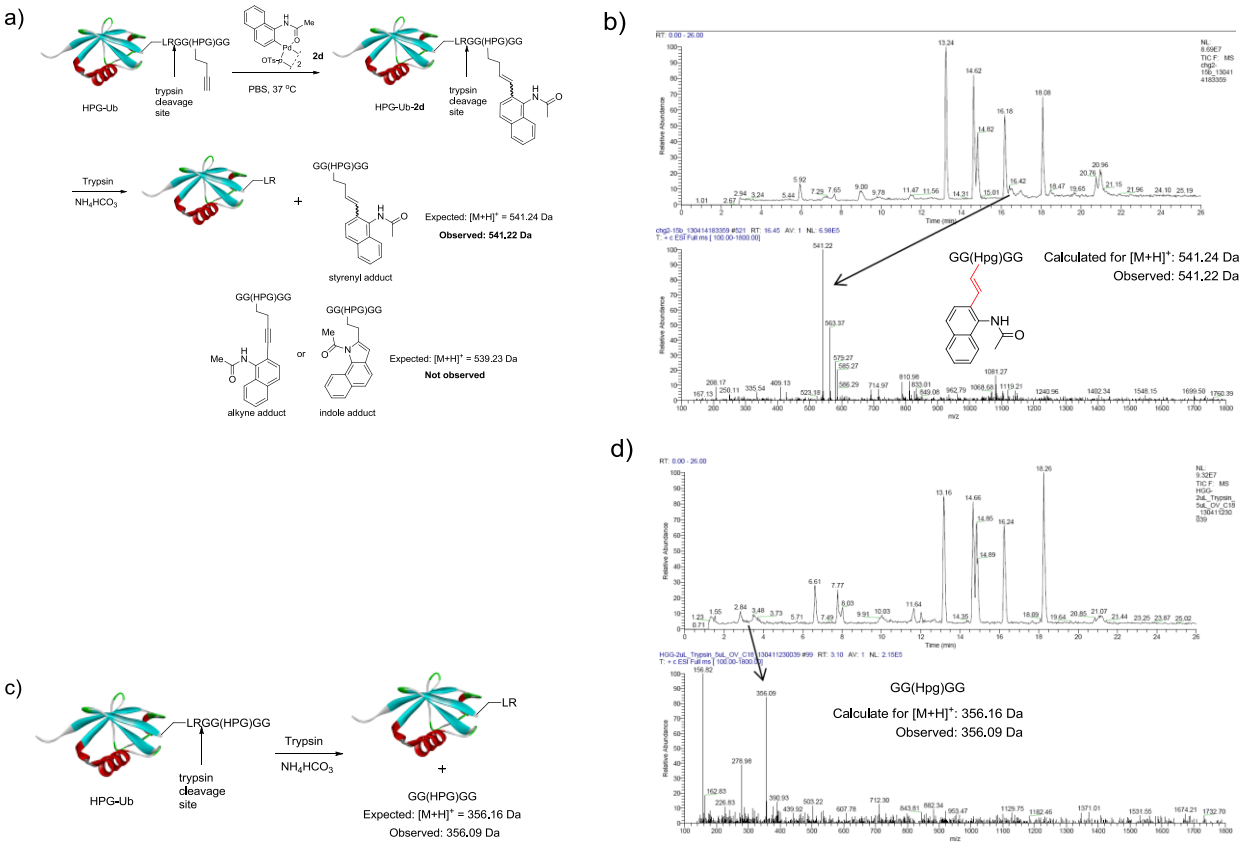


Figure S1. Trypsin digestion and LC-MS analysis of the HPG-Ub–**2d** cross-coupled product: a) Reaction scheme; b) LC-MS analysis of the tryptic reaction mixture showing the identification of the styrenyl adduct; c) Trypsin digestion of the unlabelled HPG-Ub; d) LC-MS analysis of the tryptic HPG-Ub mixture showing the identification of the the GG(Hpg)GG fragment.

Table S2. List of tryptic peptides found in the digestion mixture of ligation product HPG-Ub–**2d**

HPG-Ub sequence:
GPQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKEST LHLVLRLRGG(HPG)GG

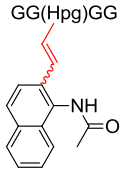
Retention time, min	Fragment	Formula	Calculated mass for $[M+H]^+$, Da	Observed mass, Da
5.9	TLTGK	$C_{22}H_{42}N_6O_8$	519.32	519.30
7.4	QLEDGR	$C_{28}H_{48}N_{10}O_{12}$	717.36	717.38
9.7	EGIPPDQQR	$C_{43}H_{70}N_{14}O_{16}$	1039.52	1039.52
13.2	TLSDY NIQK	$C_{47}H_{76}N_{12}O_{17}$	1081.56	1081.54
14.6	GPQIFVK	$C_{38}H_{61}N_9O_9$	788.47	788.48
14.8	LIFAGK	$C_{32}H_{53}N_7O_7$	648.41	648.41
16.2	ESTLHLV LR	$C_{47}H_{82}N_{14}O_{14}$	1067.62	1067.65
16.4		$C_{26}H_{33}N_6O_7$	541.24	541.22
18.1	TITLEVEPS DTIENVK	$C_{77}H_{130}N_{18}O_{30}$	1787.93	1787.85

Table S3. List of tryptic peptides found in HPG-Ub digestion mixture

Retention time, min	Fragment	Formula	Calculated mass for $[M+H]^+$, Da	Observed mass, Da
1.3	I QDK	$C_{21}H_{38}N_6O_8$	503.29	503.30
2.8	LR	$C_{12}H_{25}N_5O_3$	288.21	288.22
3.1	GG(HPG)GG	$C_{14}H_{21}N_5O_6$	356.16	356.09
6.6	TLTGK	$C_{22}H_{42}N_6O_8$	519.32	519.32
7.8	QLEDGR	$C_{28}H_{48}N_{10}O_{12}$	717.36	717.37
10.0	EGIPPDQQR	$C_{43}H_{70}N_{14}O_{16}$	1039.52	1039.52
13.2	TLSDY NIQK	$C_{47}H_{76}N_{12}O_{17}$	1081.56	1081.55
14.7	GPQIFVK	$C_{38}H_{61}N_9O_9$	788.47	788.43
14.8	LIFAGK	$C_{32}H_{53}N_7O_7$	648.41	648.38
16.2	ESTLHLV LR	$C_{47}H_{82}N_{14}O_{14}$	1067.62	1067.75
18.3	TITLEVEPS DTIENVK	$C_{77}H_{130}N_{18}O_{30}$	1787.93	1787.91

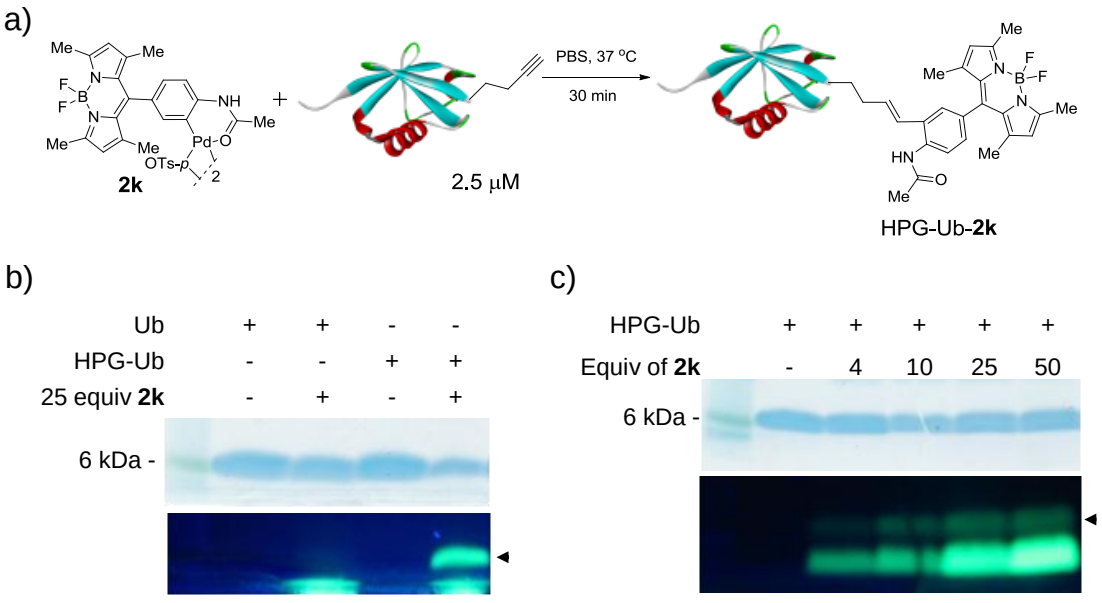


Figure S2. SDS-PAGE/in-gel fluorescence analysis of the reaction of HPG-Ub with **2k:** a) Reaction scheme; b) Evaluation of the reaction selectivity. Top panel: Coomassie blue stain; bottom panel, in-gel fluorescence of the same gel, λ_{ex} = 365 nm. The labeled product, HPG-Ub–**2k**, was indicated with an arrow. The strongly fluorescent bands in the bottom were excess small molecules generated from **2k**; c) Reactions of HPG-Ub with **2k** at 4 different concentrations**.** Top panel: Coomassie blue stain; bottom panel, in-gel fluorescence of the same gel, λ_{ex} = 365 nm. The fluorescent labeled product, HPG-Ub–**2k**, was indicated with an arrow.

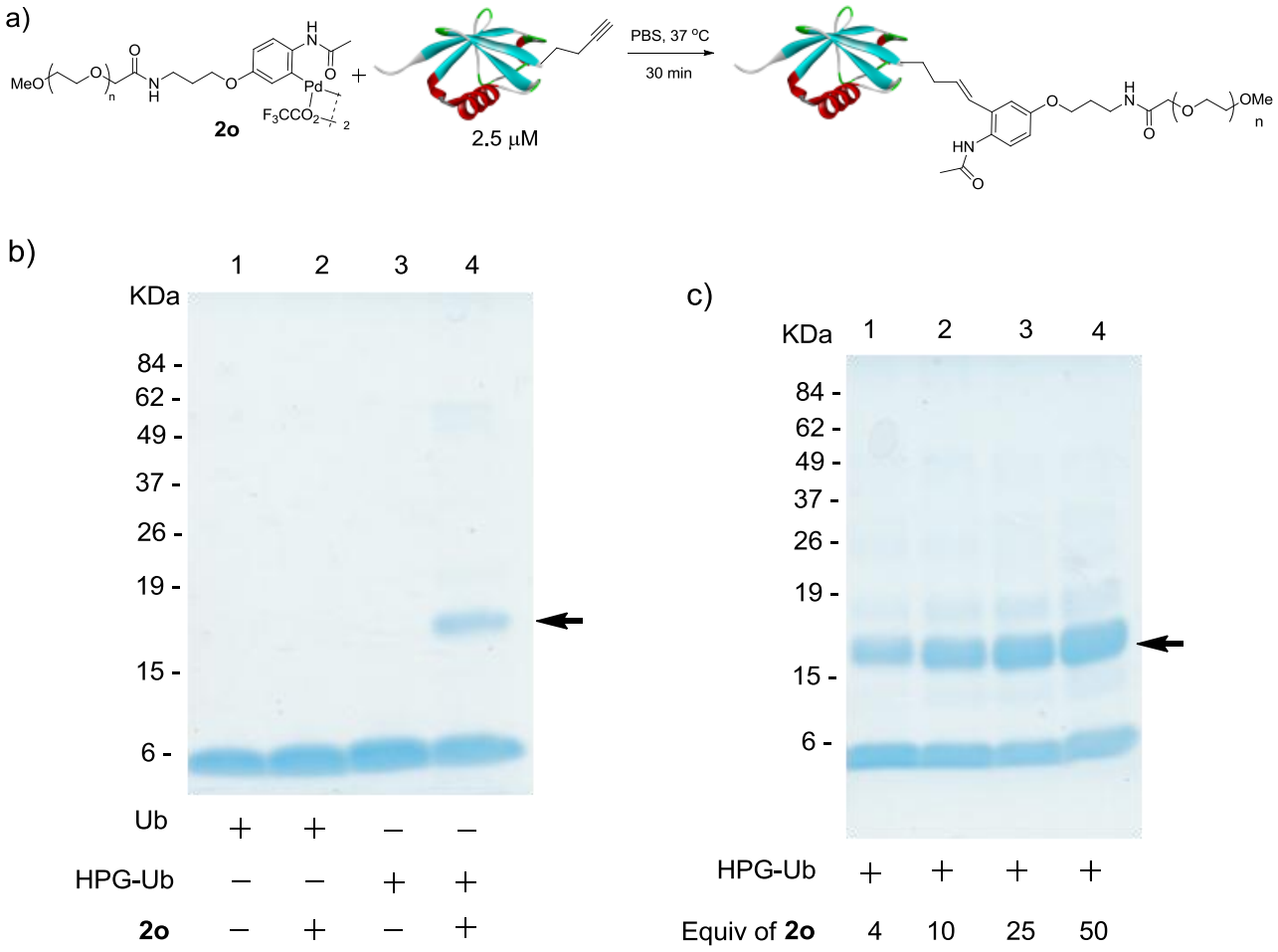


Figure S3. SDS-PAGE analysis of the reaction of HPG-Ub with **2o**: a) Reaction scheme; b) Coomassie blue stained SDS-PAGE confirms reaction selectivity. The PEGylated product, HPG-Ub–**2o**, was indicated with an arrow**;** c) Reactions of HPG-Ub with **2o** at 4 different concentrations (4, 10, 25 and 50 equiv)**.** Based on densitometry, the yields were 36%, 46%, 56% and 66%, respectively.

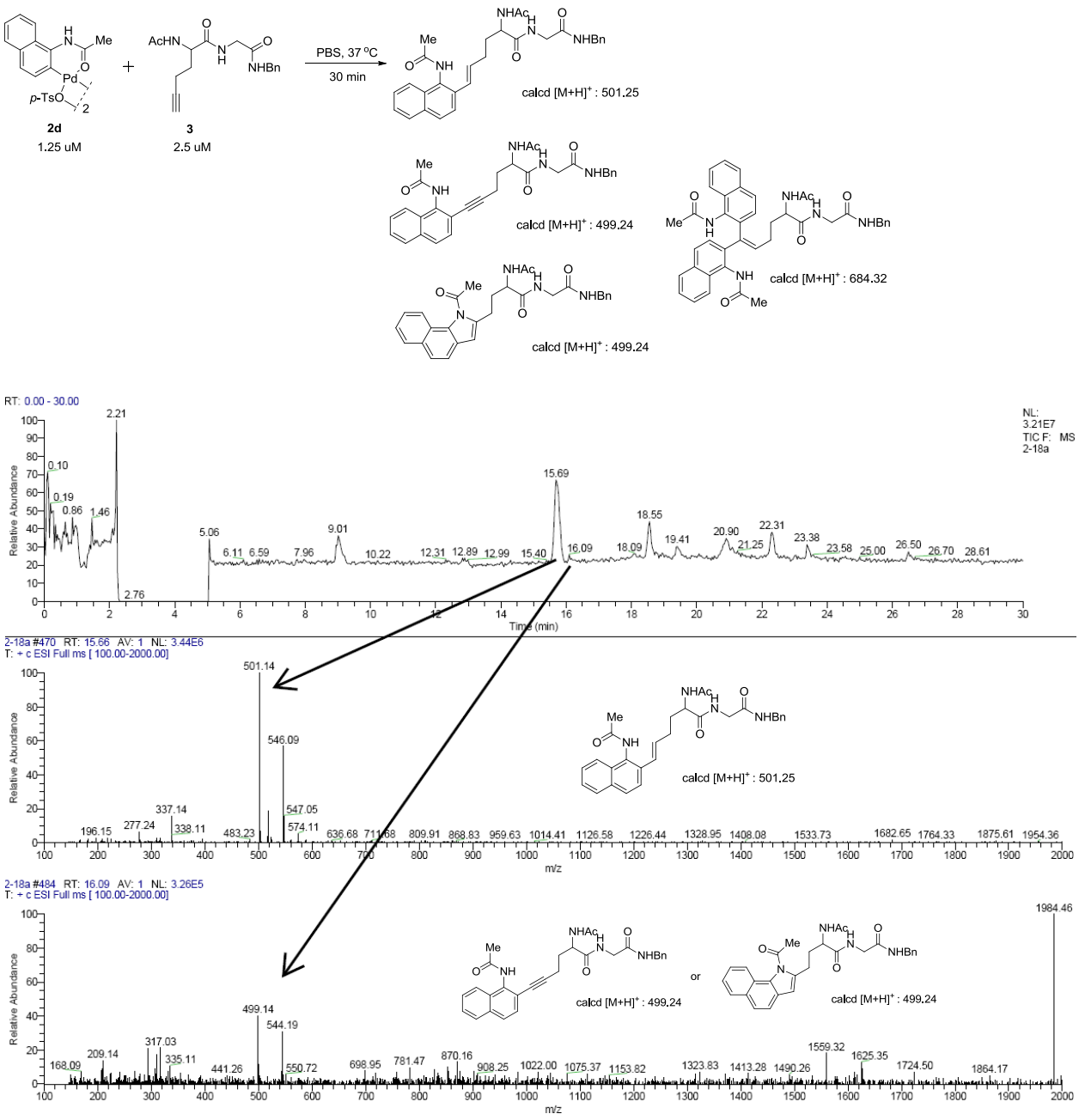
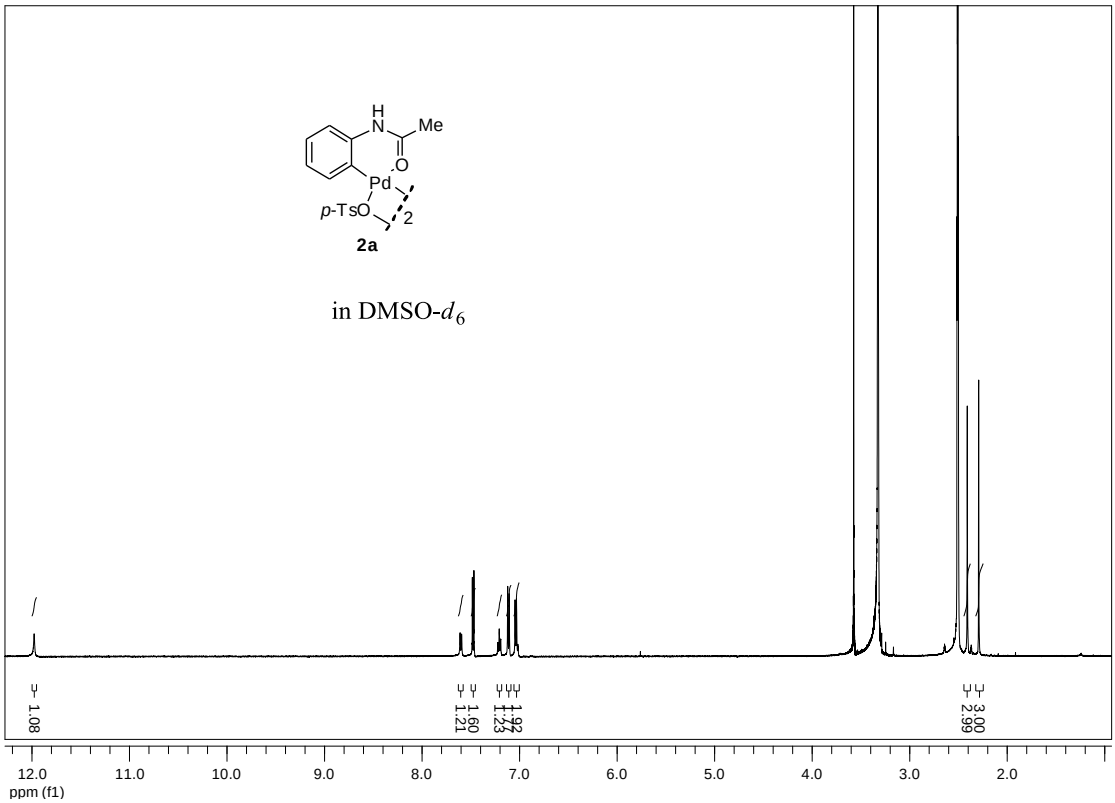
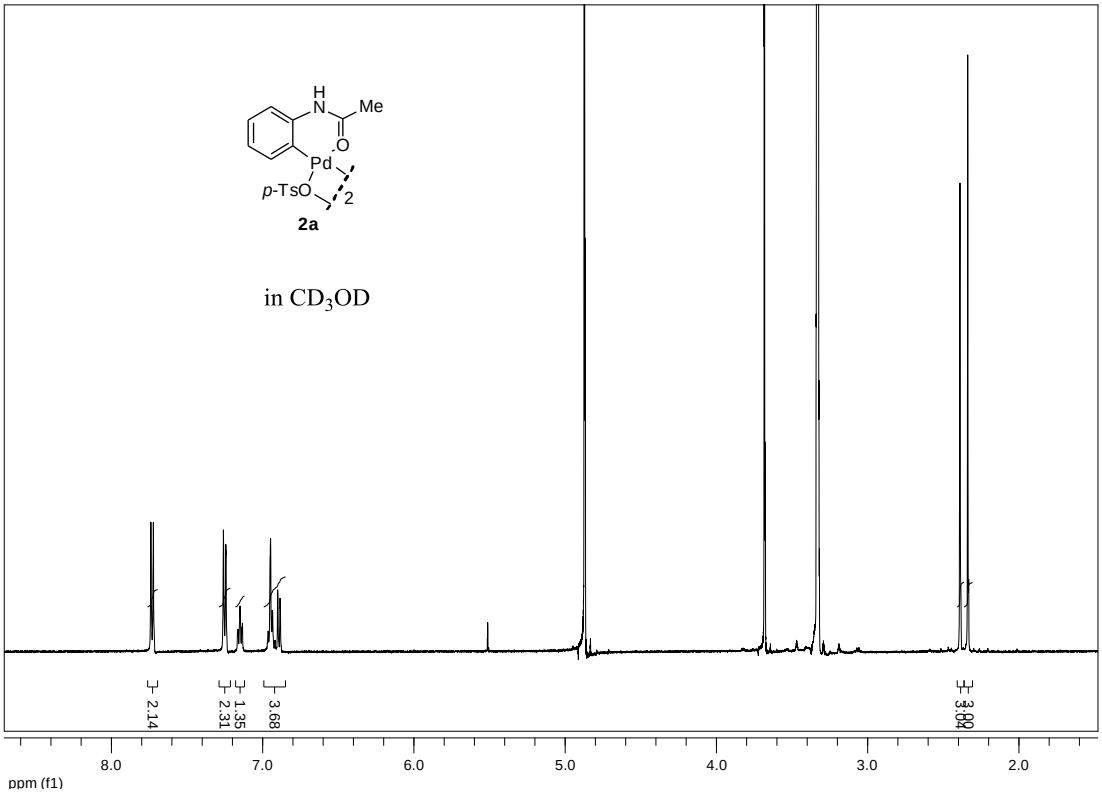


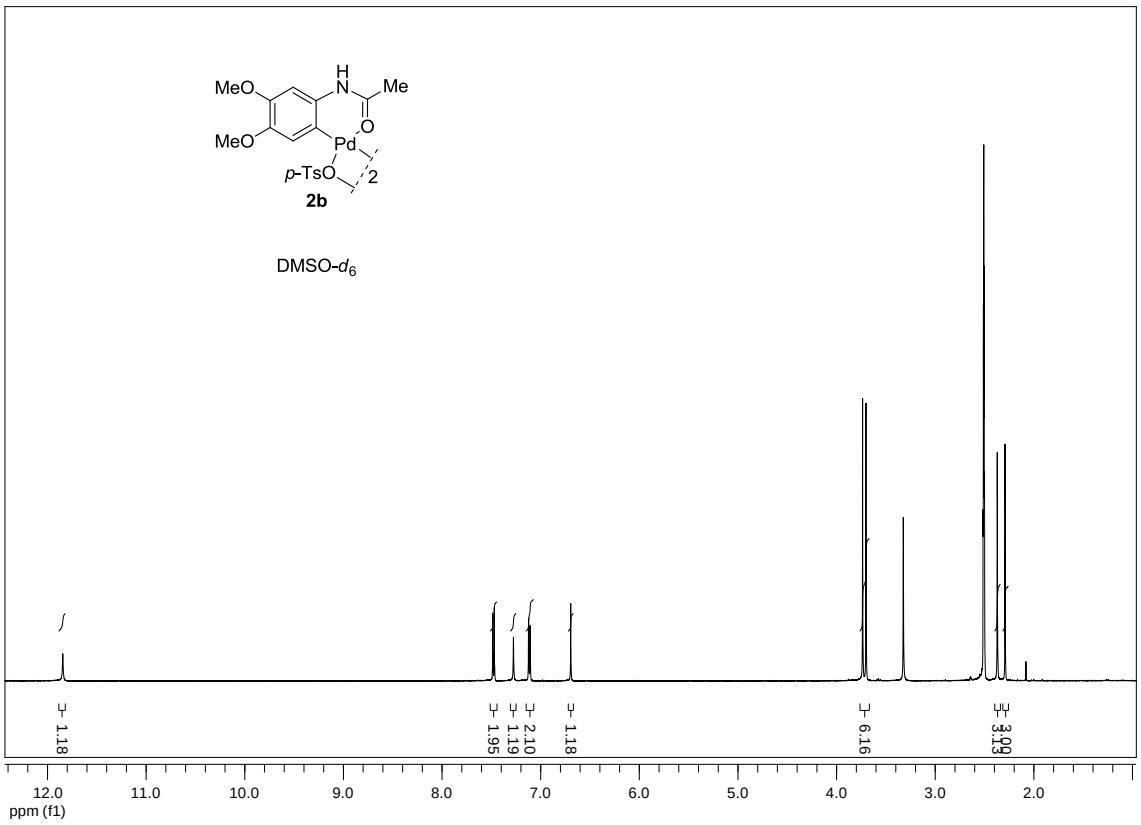
Figure S4. LC-MS analysis of the reaction of palladacycle **2d** with *N*-acylated HPG-dipeptide **3**.[5]

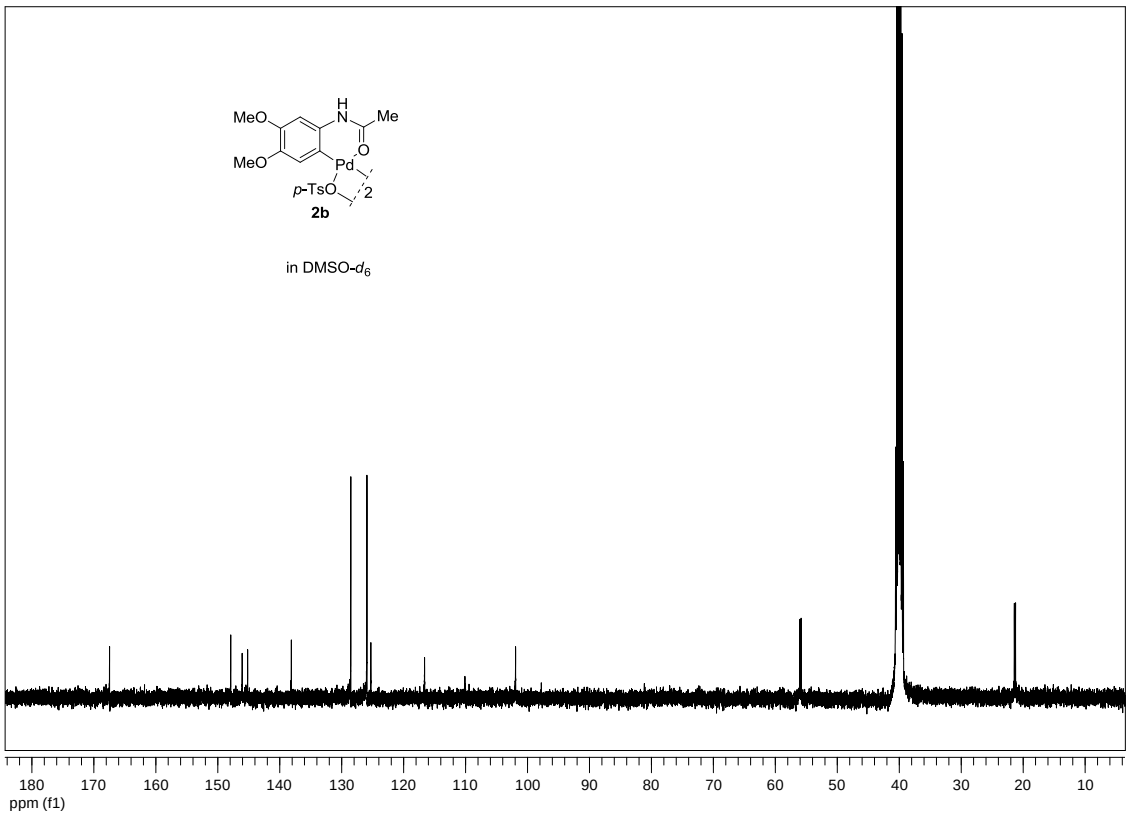
Reference:

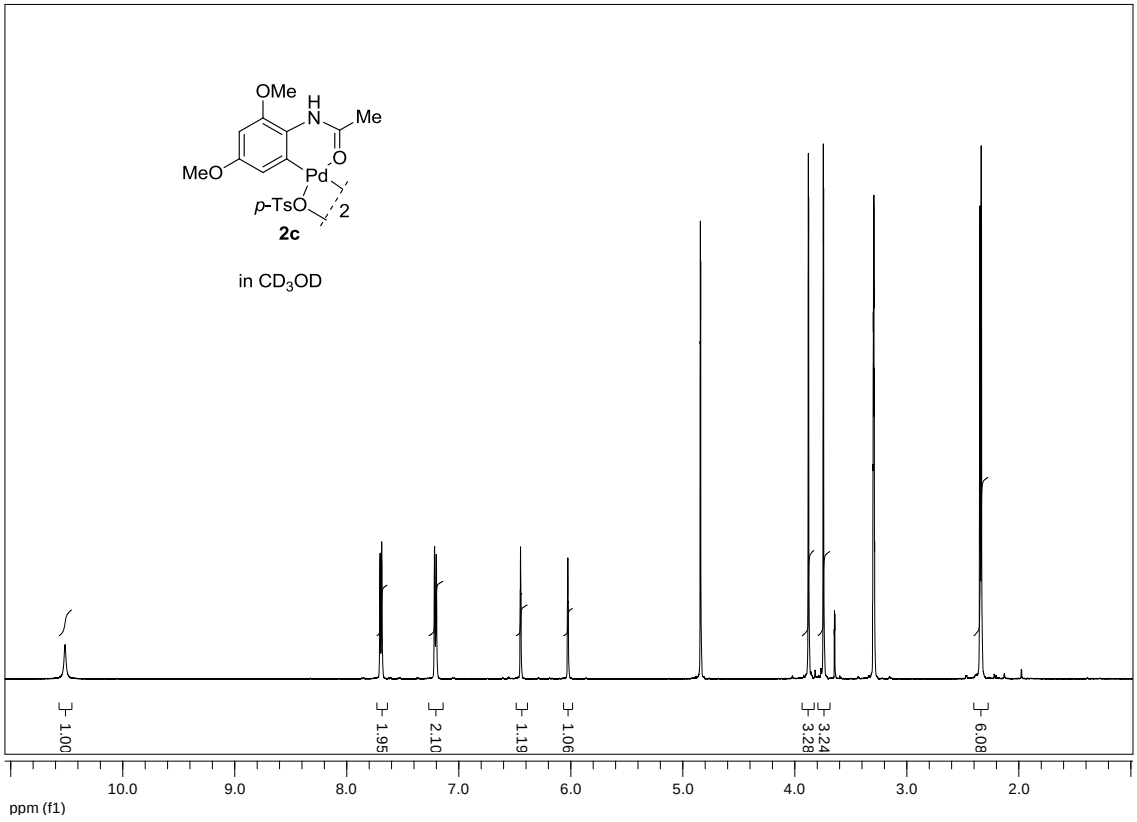
1 R. Giri, J. K. Lam and J. Q. Yu, *J. Am. Chem. Soc.*, 2010, **132**, 686-693.
2 R. B. Bedford, M. F. Haddow, C. J. Mitchell and R. L. Webster, *Angew. Chem. Int. Ed.*, **50**, 5524-5527.
3 W. Rauf, A. L. Thompson and J. M. Brown, *Dalton Trans*, 2010, **39**, 10414-10421.
4 S. Zhu, A. L. Ruchelman, N. Zhou, A. Liu, L. F. Liu and E. J. LaVoie, *Bioorg Med Chem*, 2006, **14**, 3131-3143.
5 N. Li, R. K. V. Lim, S. Edwardraja and Q. Lin,*J. Am. Chem. Soc.* 2011, **133**, 15316-15319.
6 M. Tian, X. Peng, F. Feng, S Meng, J. Fan, S. Sun; *Dyes and Pigments*, 2009, **81**, 58-62.

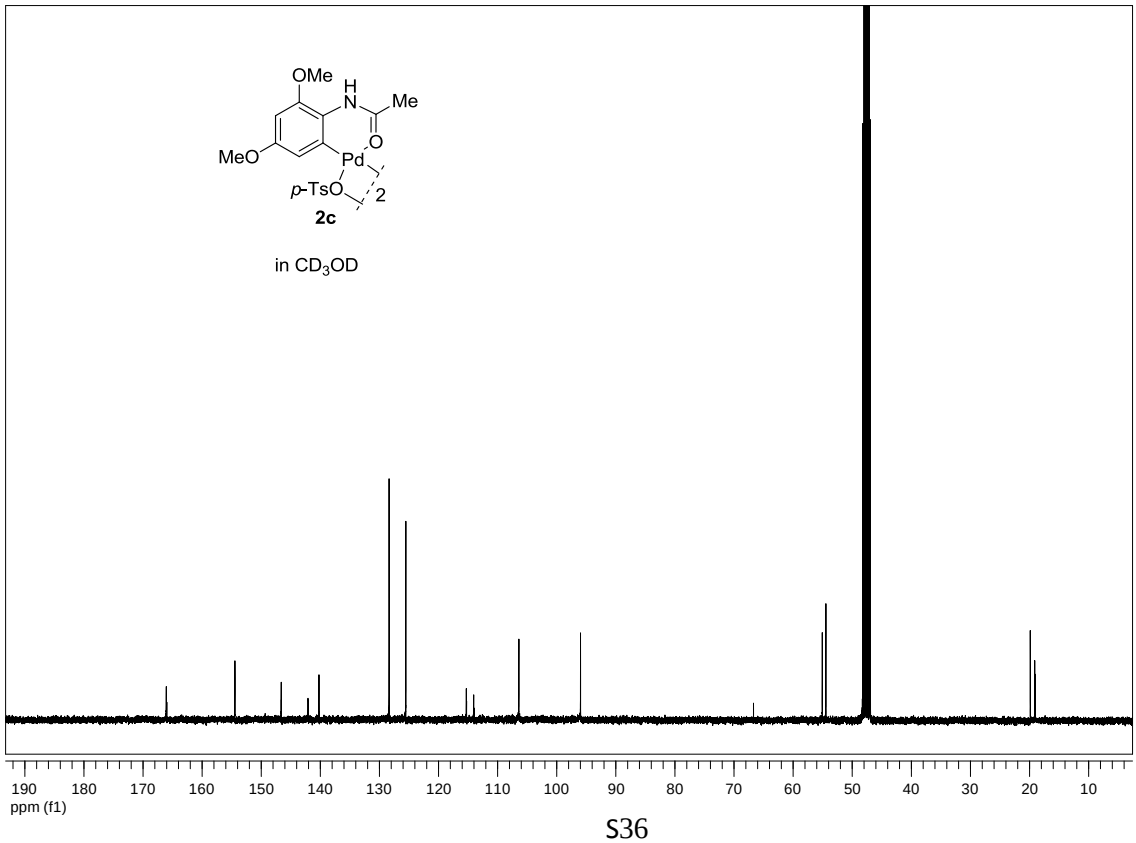

H
N
Me
O
Pd
p-TsO
2
2a
in DMSO-d6
1.08
1.60
1.21
1.23
3.00
2.99
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)

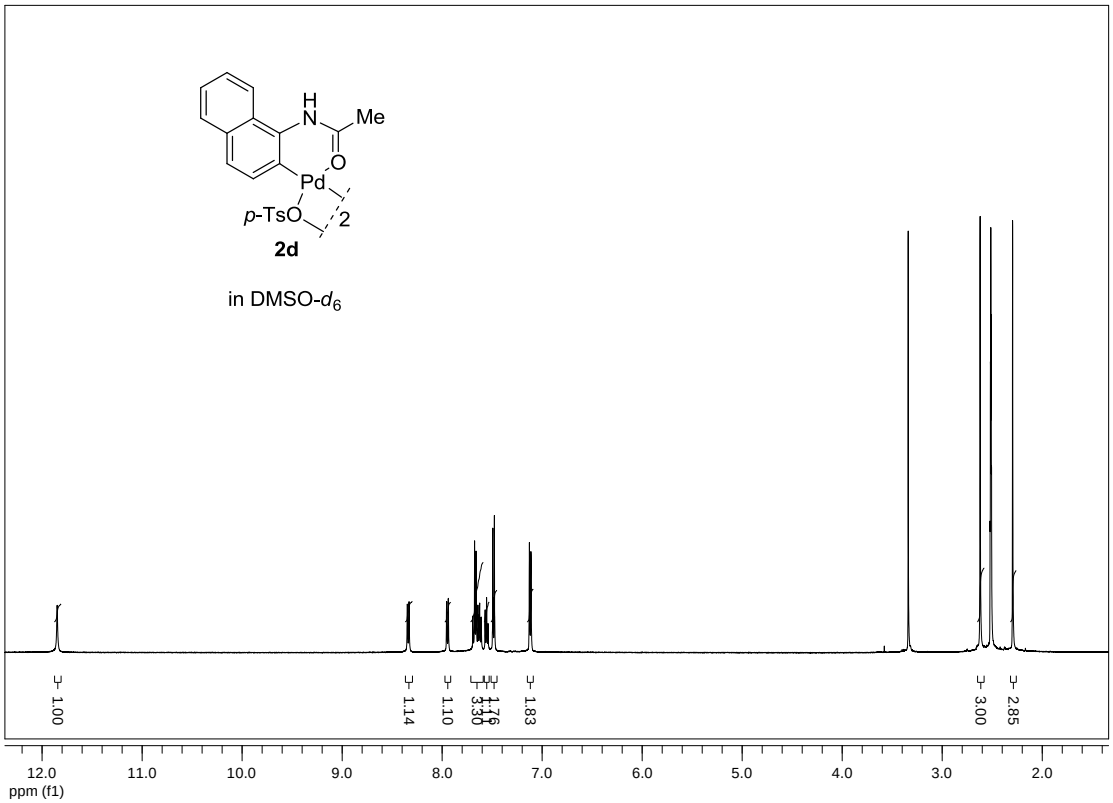

H
N
Me
O
Pd
p-TsO
2
2a
in CD3OD
2.14
2.31
1.35
3.68
3.00
3.04
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)

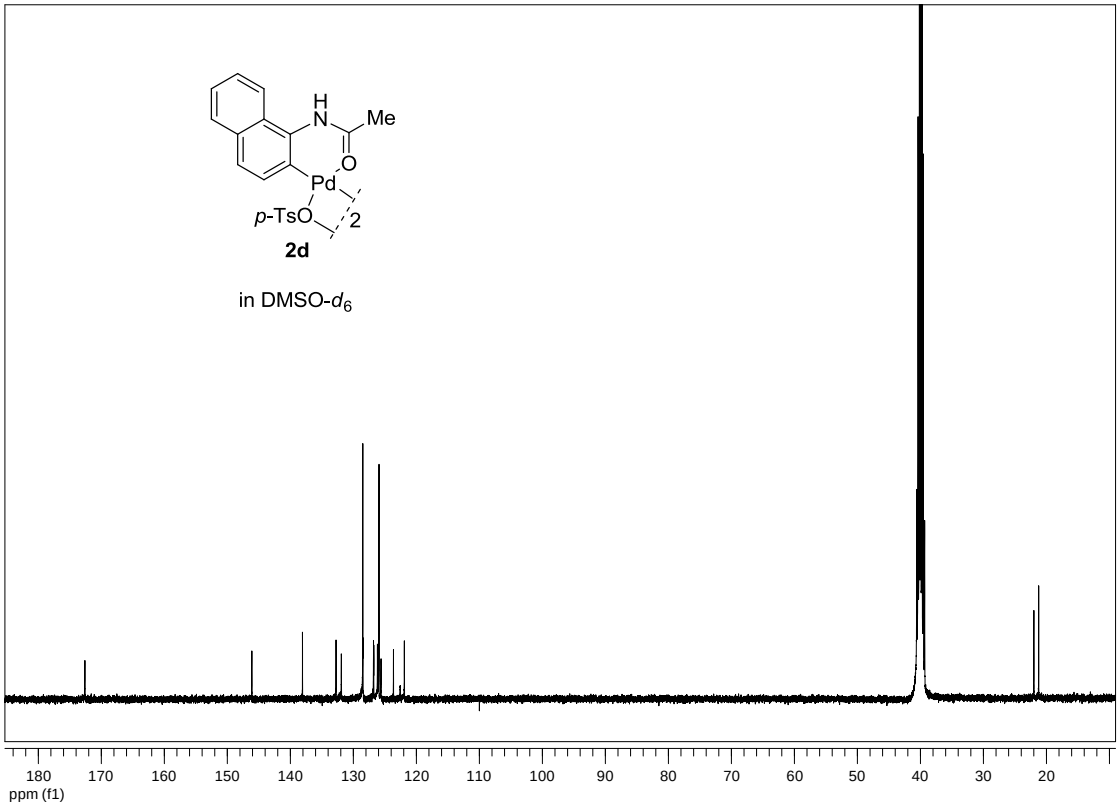

MeO
MeO
H
N
Me
O
Pd
p-TsO
2
2b
DMSO-d6
1.18
1.95
1.19
2.10
1.18
6.16
3.09
3.13
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)

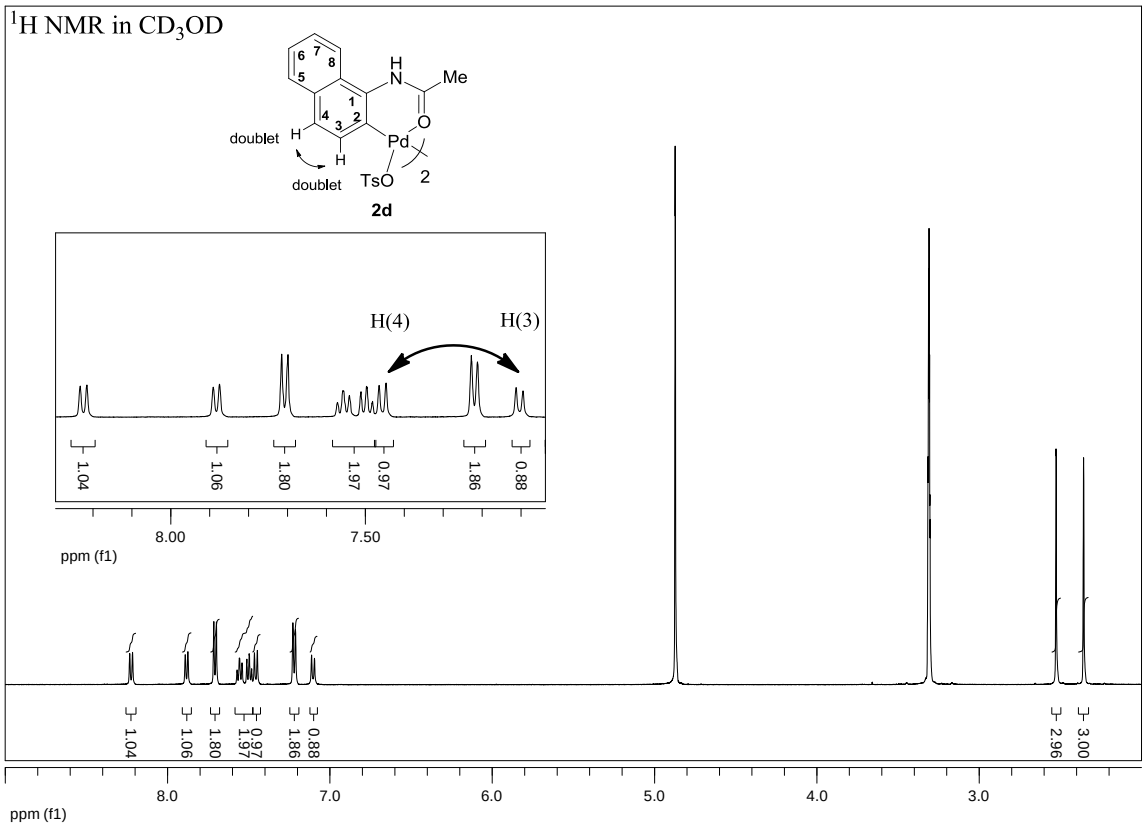

MeO
MeO
H
N
Me
O
Pd
p-TsO
2
2b
in DMSO-d6
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm (f1)

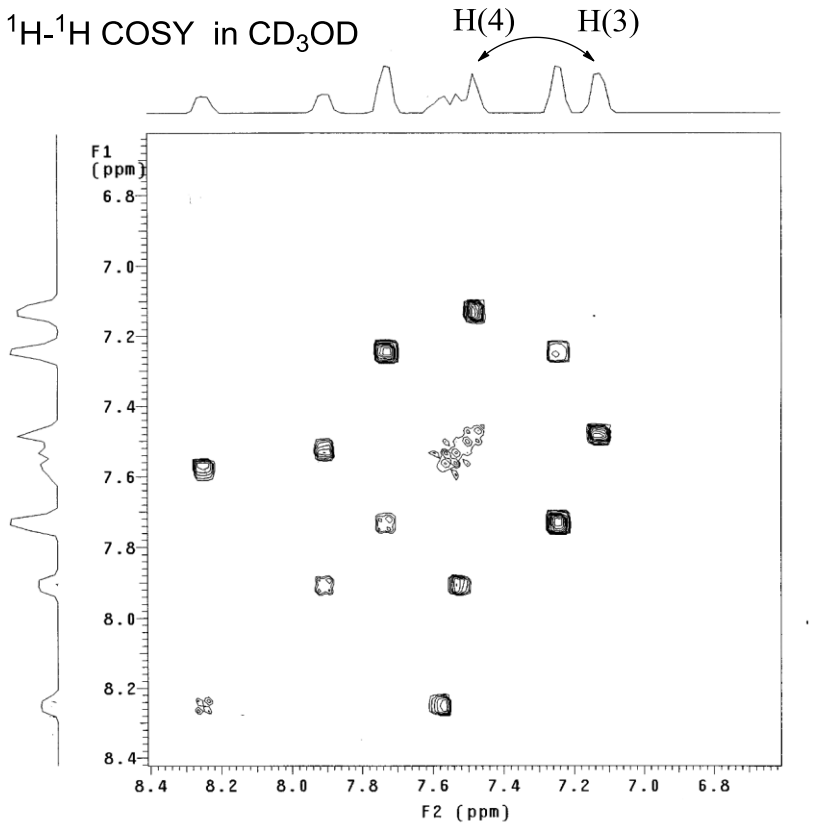

OMe
H
N
Me
O
MeO
Pd
p-TsO
2
2c
in CD3OD
1.00
1.95
2.10
1.19
1.06
3.28
3.24
6.08
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)

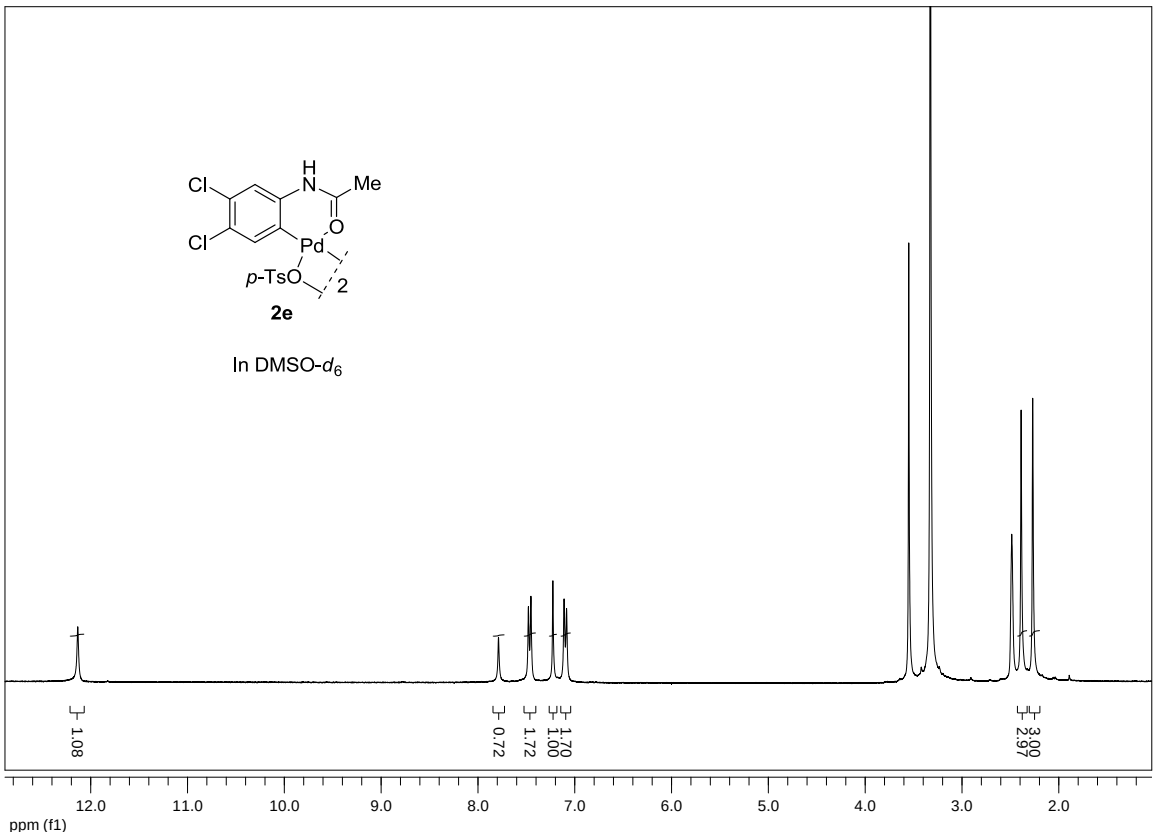

OMe
H
N
Me
O
MeO
Pd
p-TsO
2
2c
in CD3OD
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm (f1)

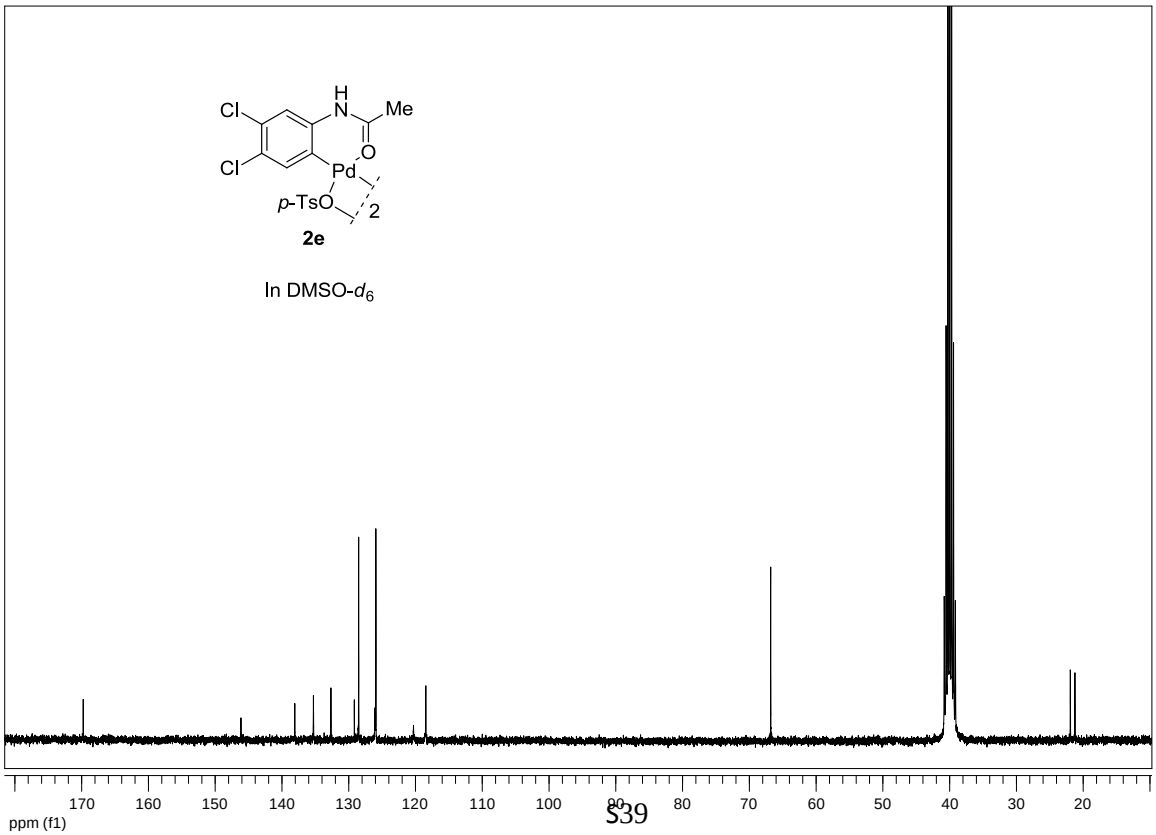

H
N
Me
O
Pd
p-TsO
2
2d
in DMSO-d6
1.00
1.14
1.10
1.76
3.30
1.83
3.00
2.85
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)

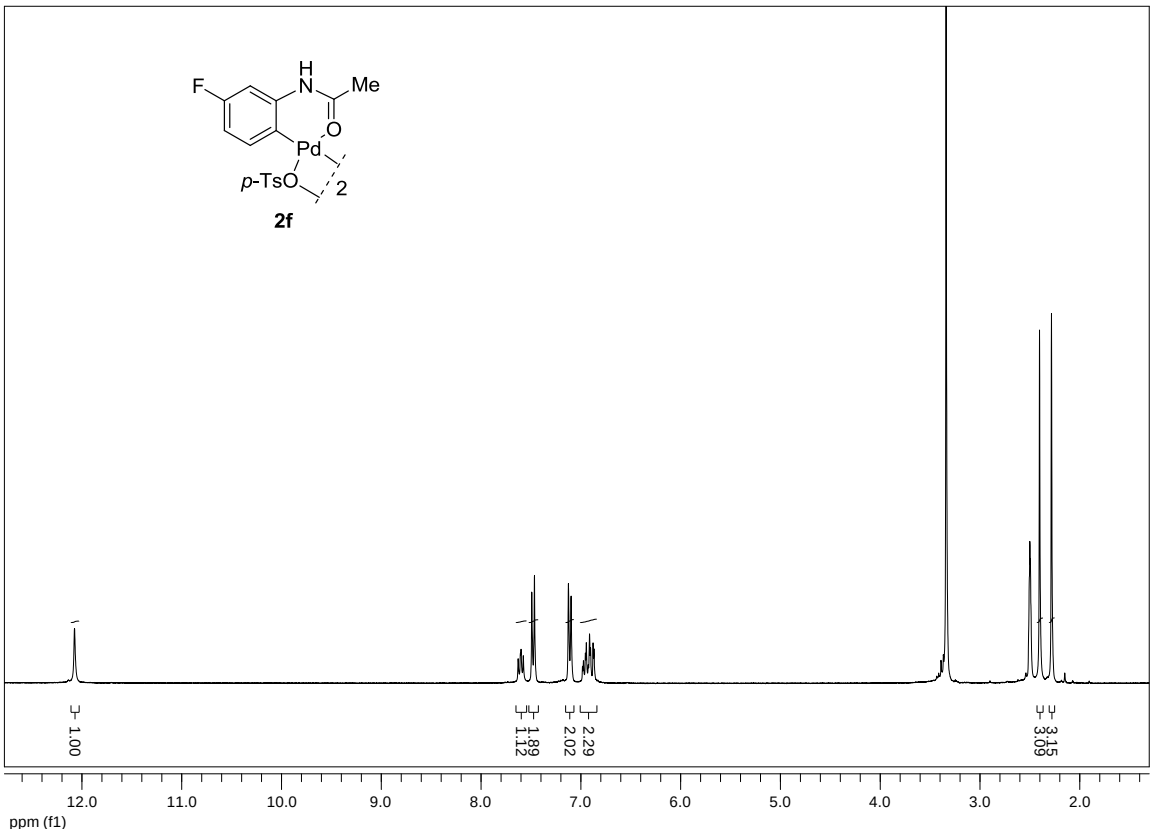

H
N
Me
O
Pd
p-TsO
2
2d
in DMSO-d6
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm (f1)

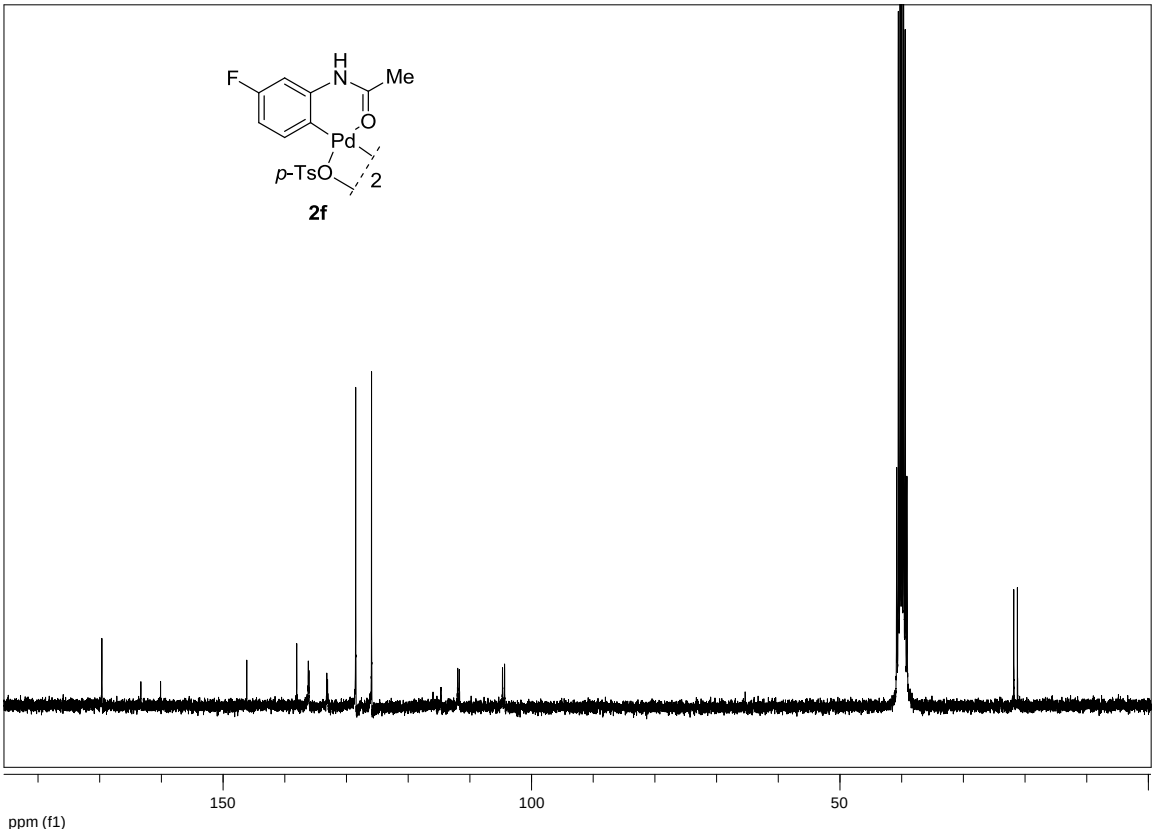


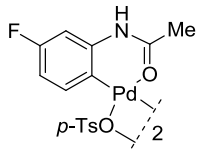






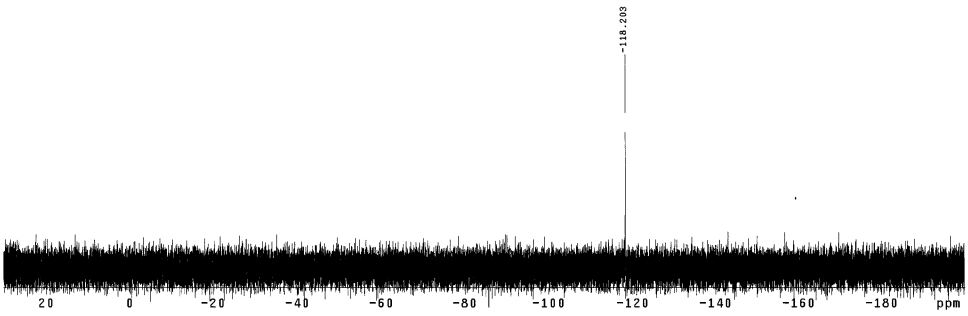

F
H
N
Me
O
Pd
p-TsO
2
2f
1.00
1.89
1.12
2.29
2.02
3.15
3.09
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)

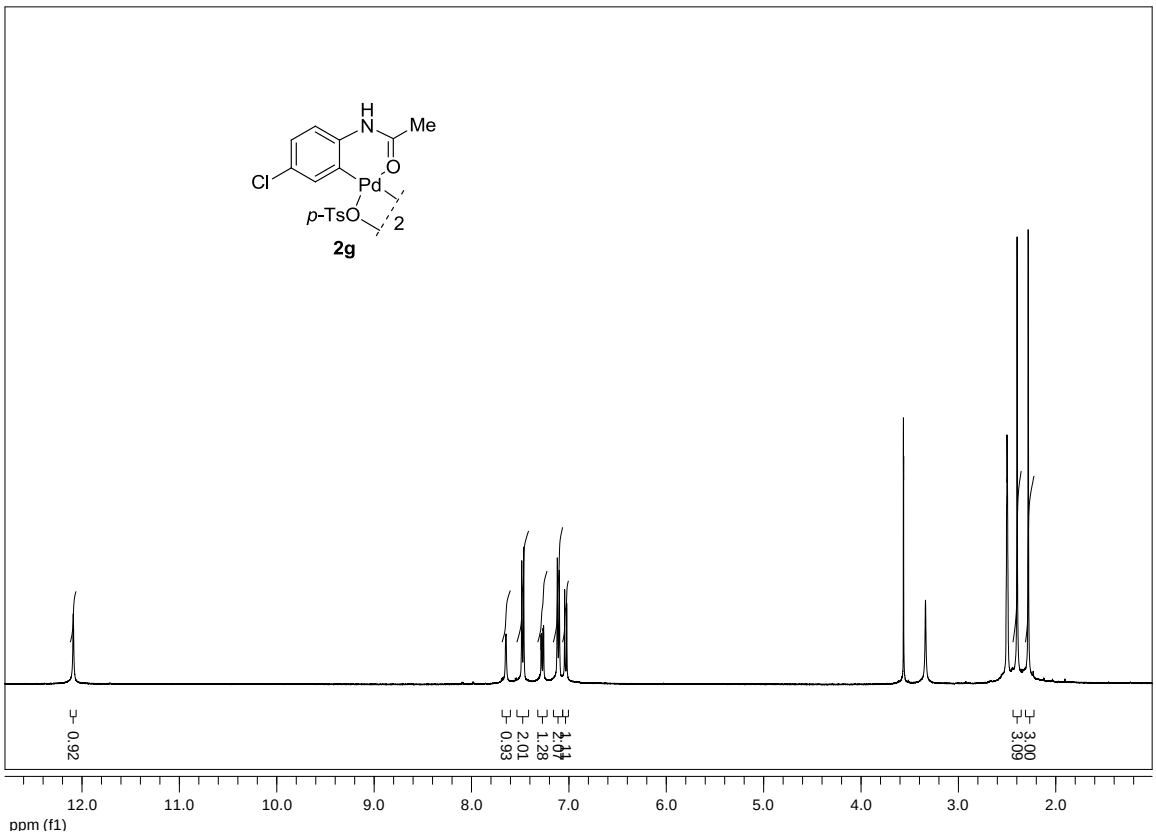

F
H
N
Me
O
Pd
p-TsO
2
2f
150
100
50
ppm (f1)

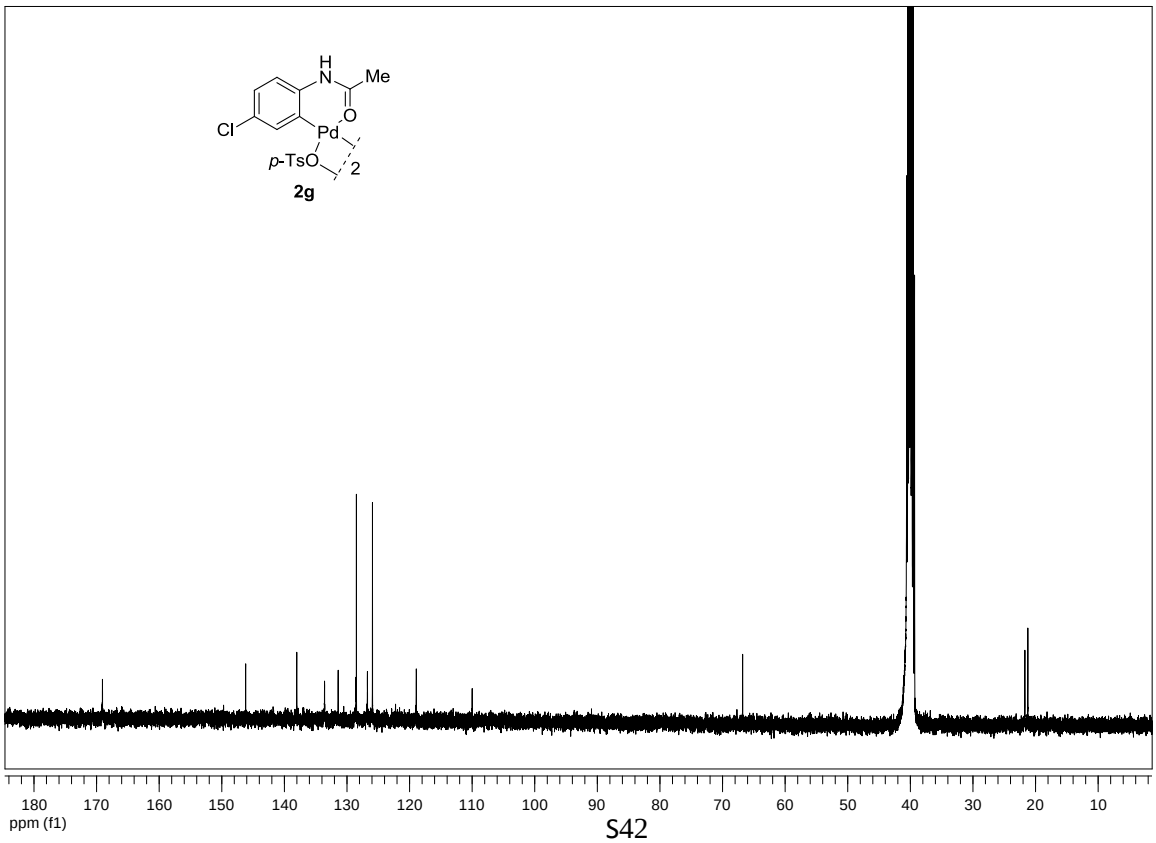


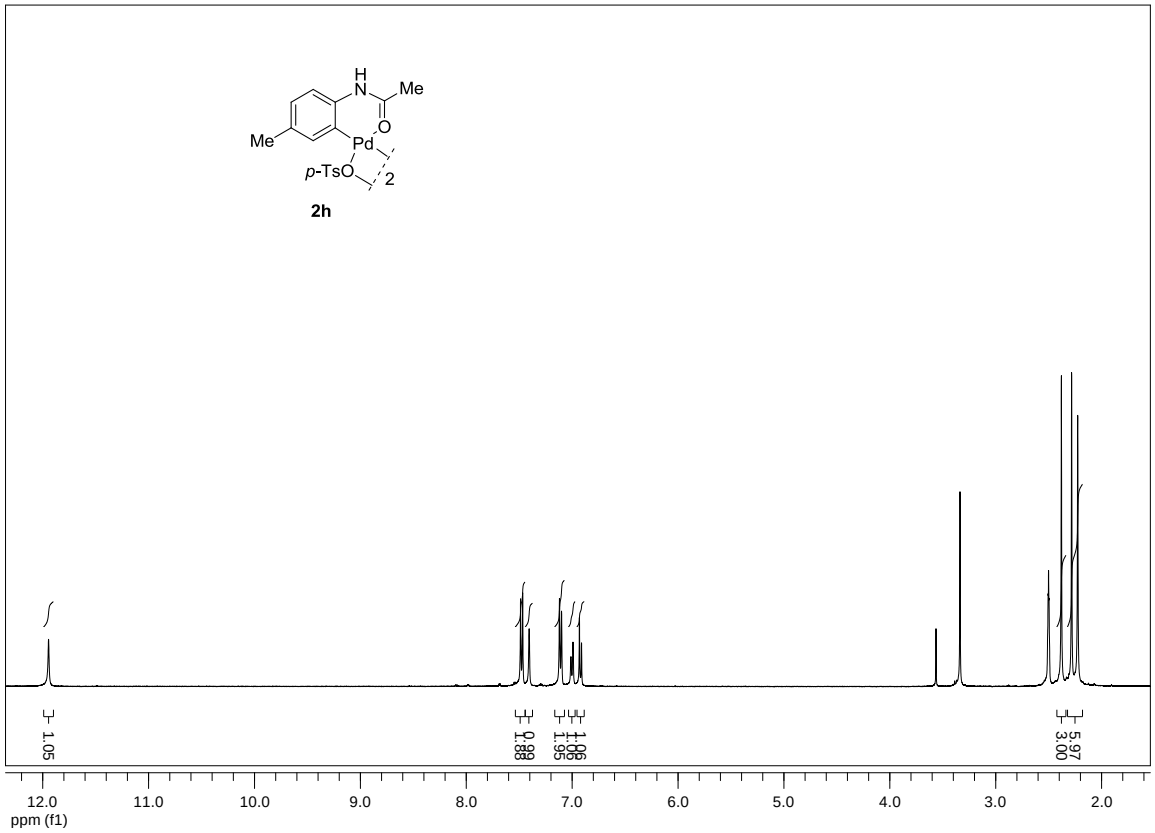
2f

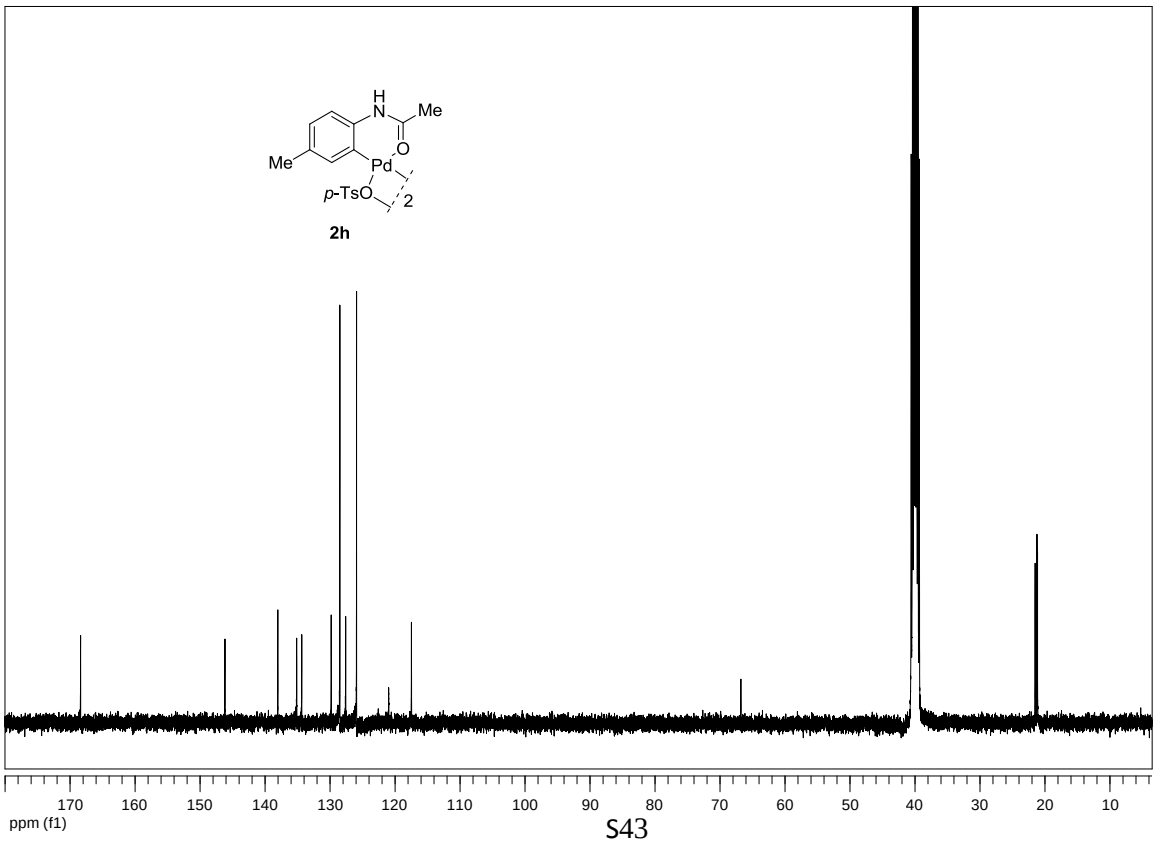
^{19}F-NMR

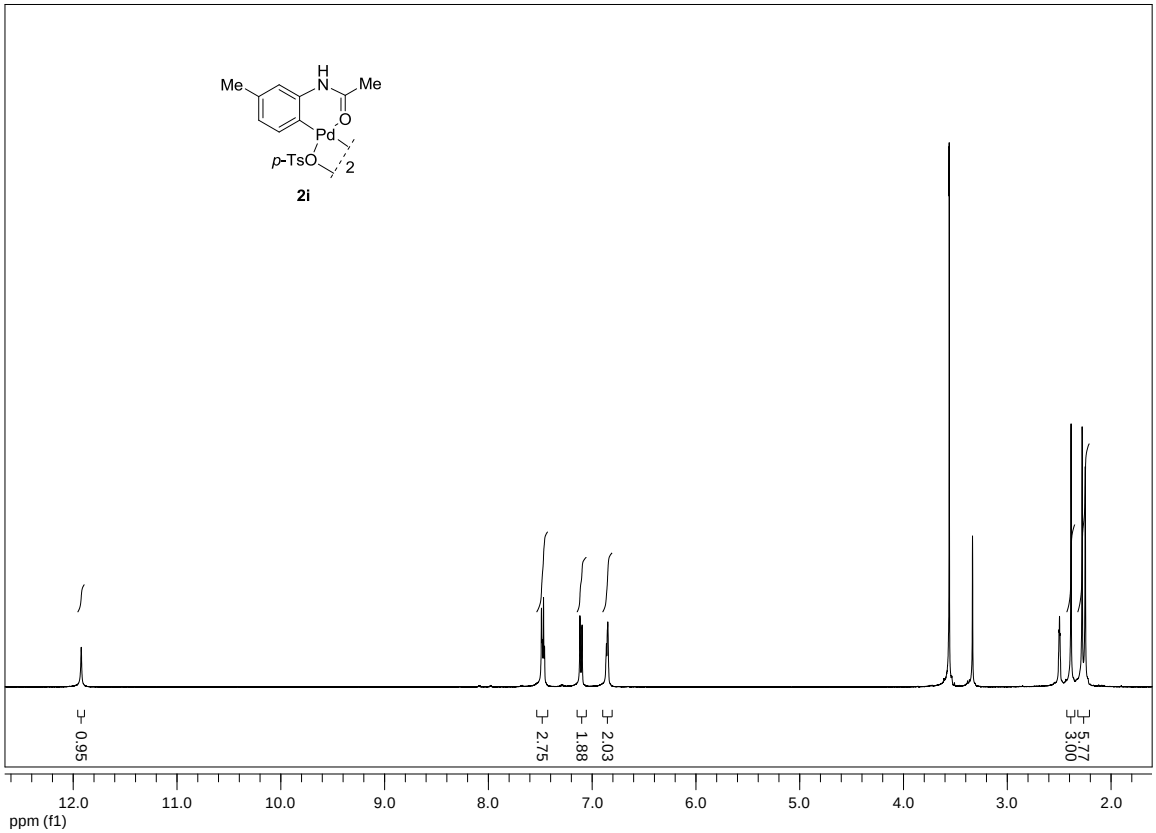


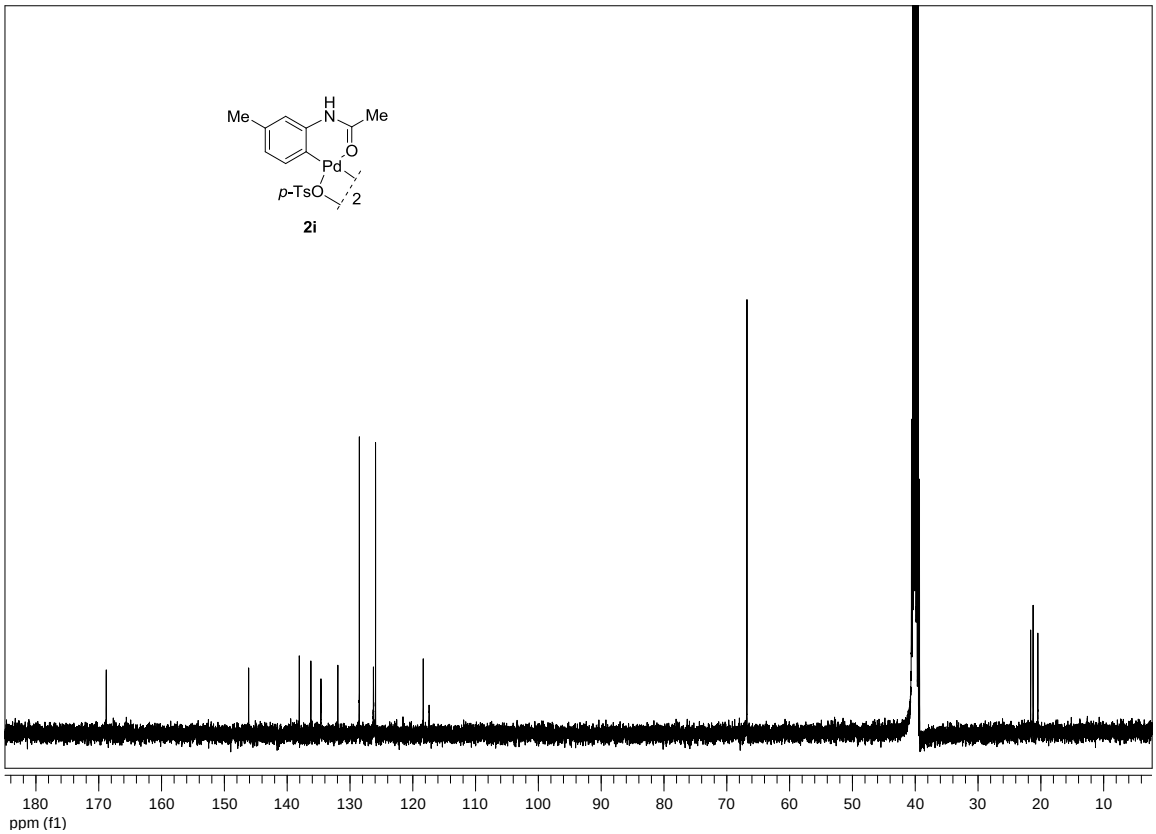

H
N
Me
O
Cl
Pd
p-TsO
2
2g
0.92
0.93
2.01
1.28
2.07
2.11
3.00
3.09
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)

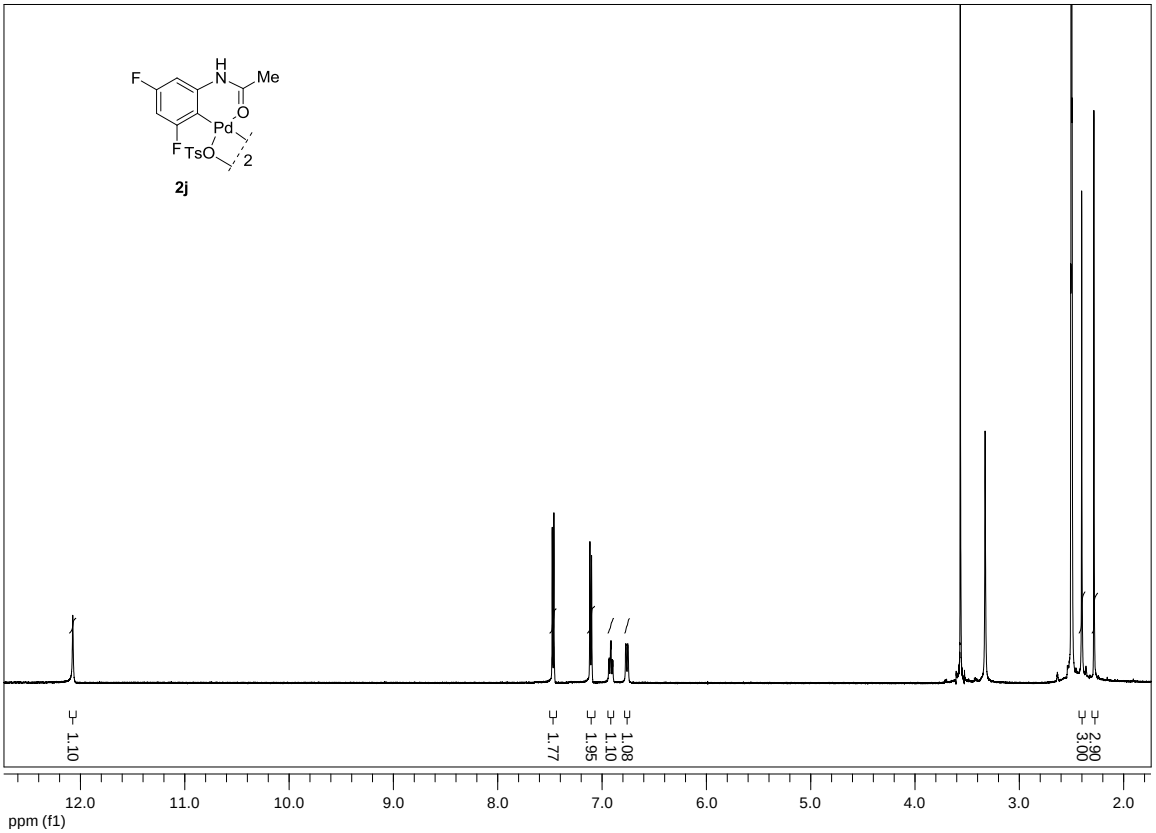

H
N
Me
O
Cl
Pd
p-TsO
2
2g
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm (f1)

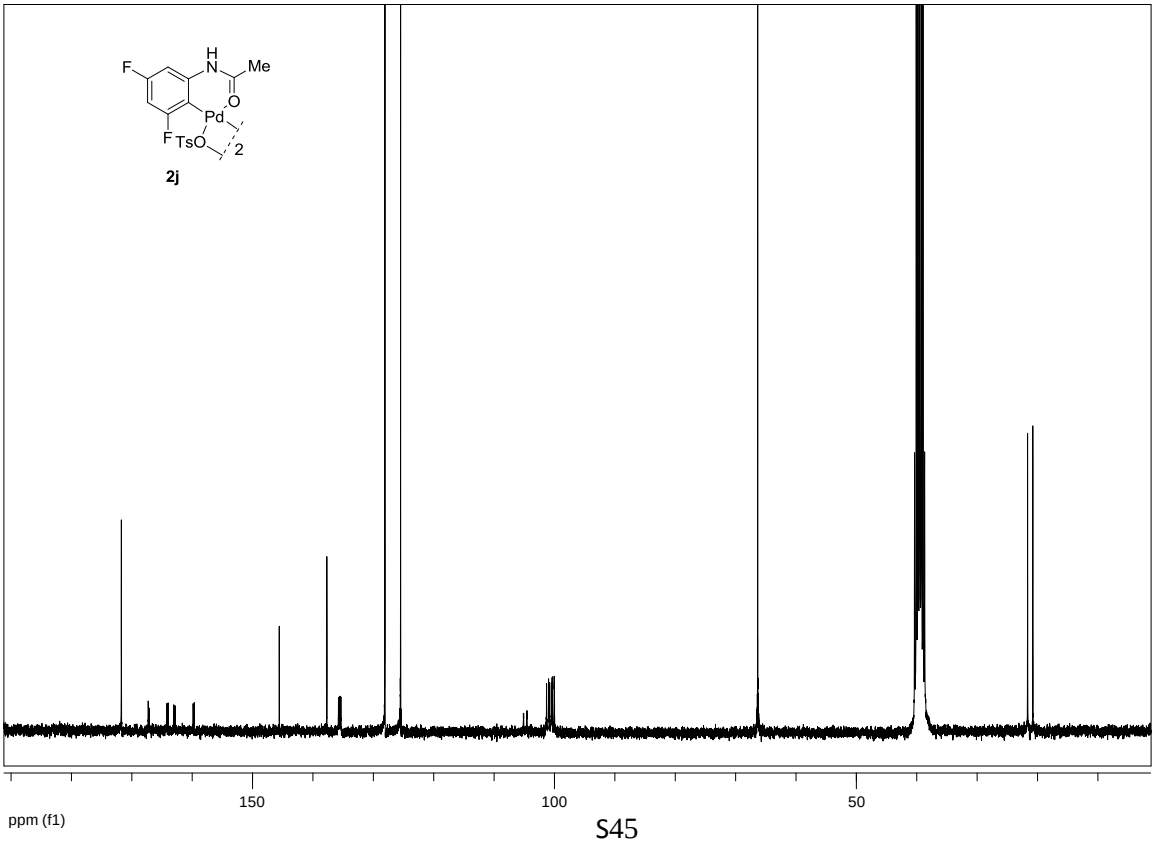


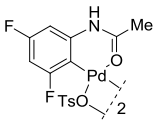



Me
H
N
Me
O
Pd
p-TsO
2
2i
0.95
2.75
1.88
2.03
5.77
3.00
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)


Me
H
N
Me
O
Pd
p-TsO
2
2i
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm (f1)

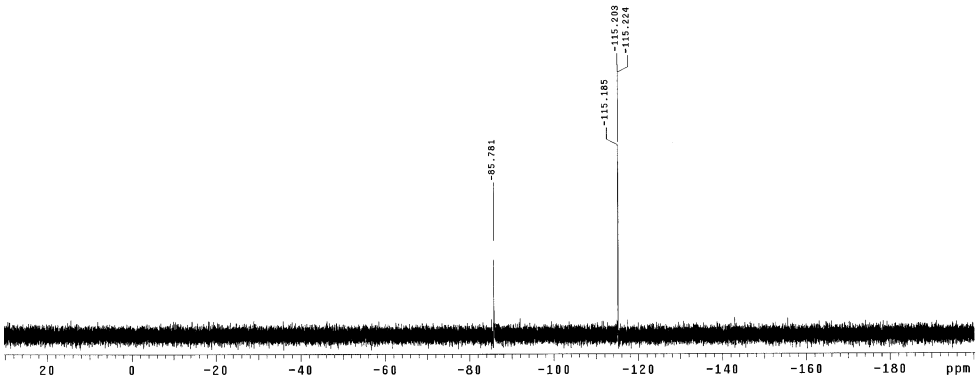


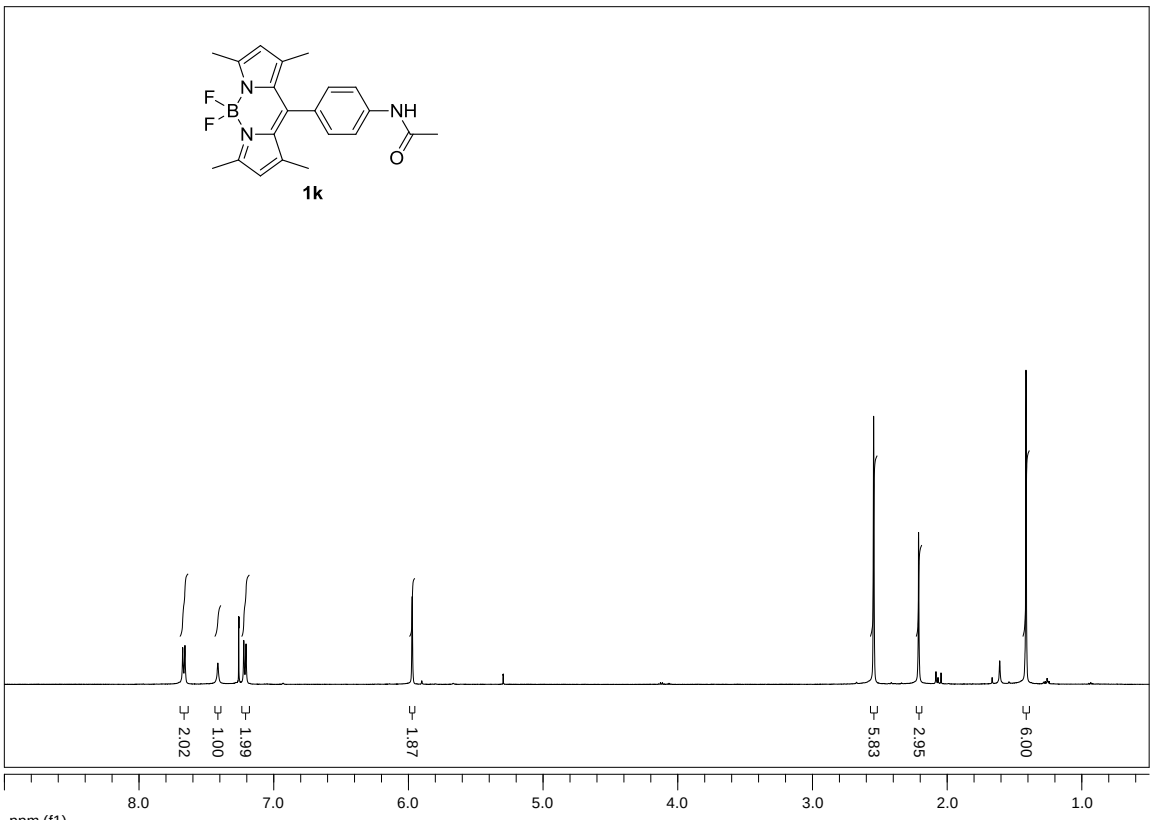


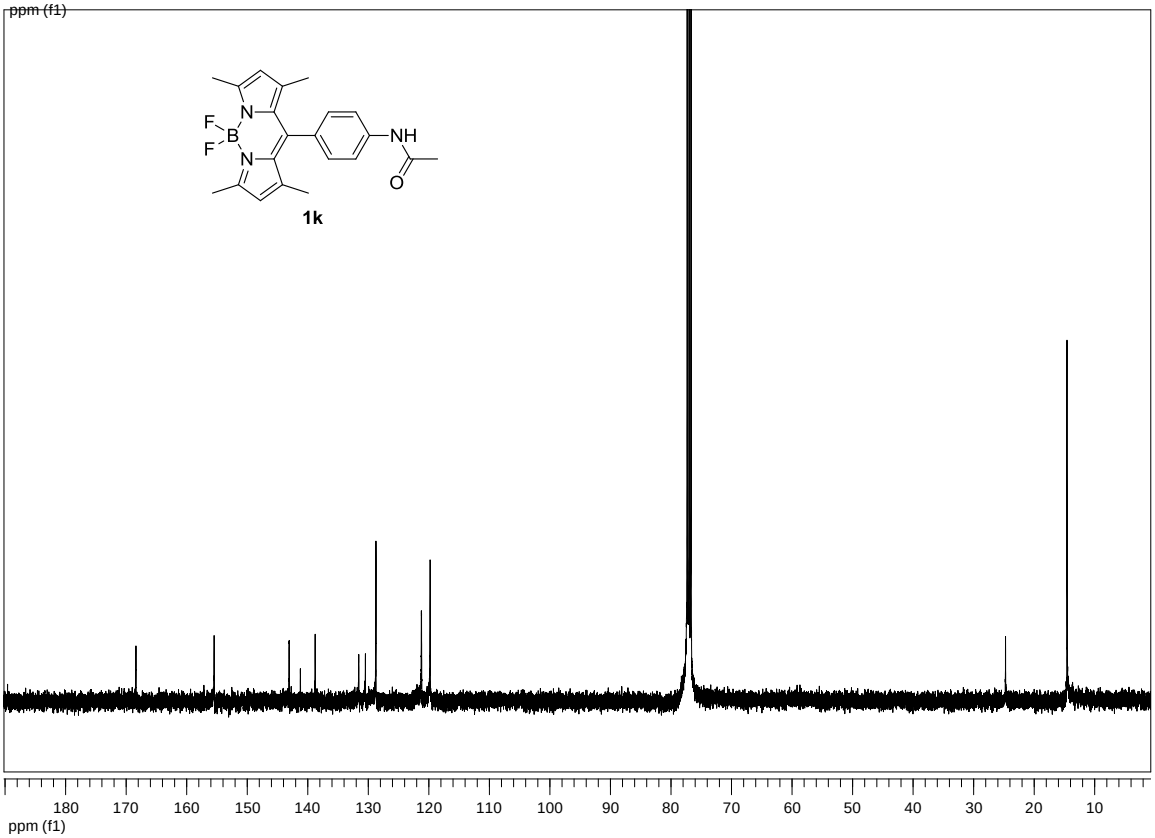

H
N
Me
F
O
Pd
F
TsO
2

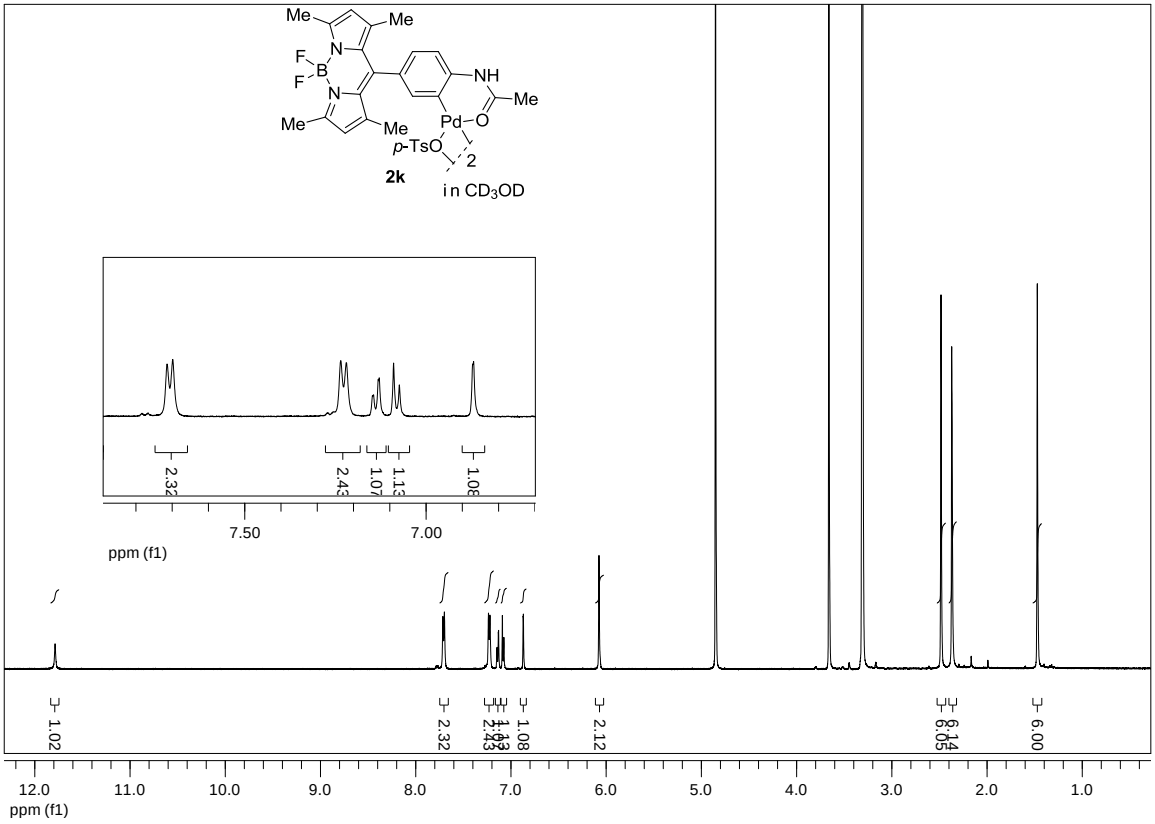
2j

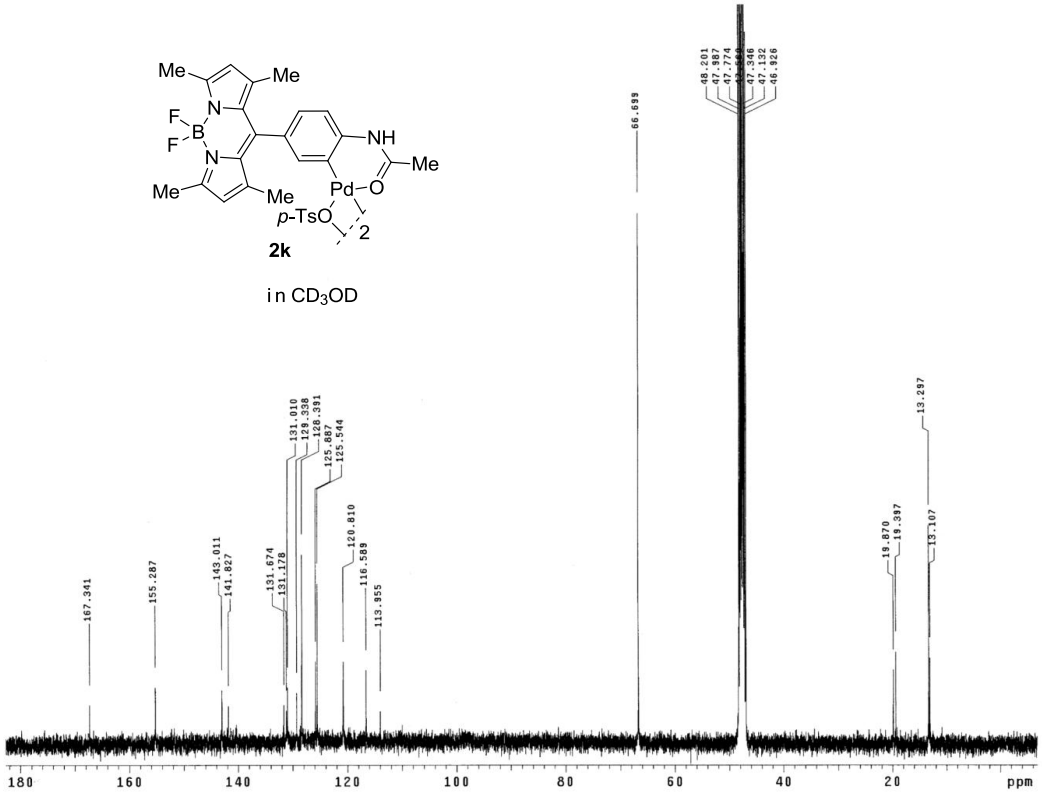
^{19}F NMR

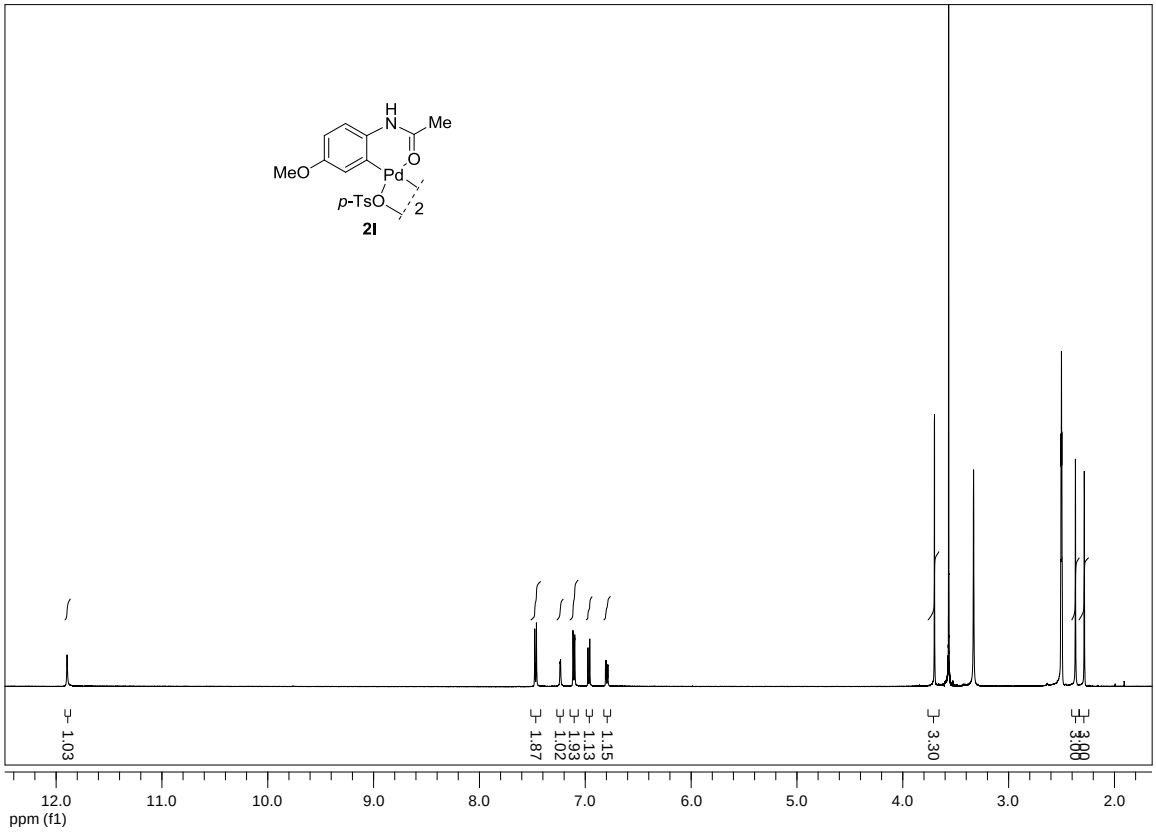

-85.781
-115.185
-115.203
-115.224
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
ppm

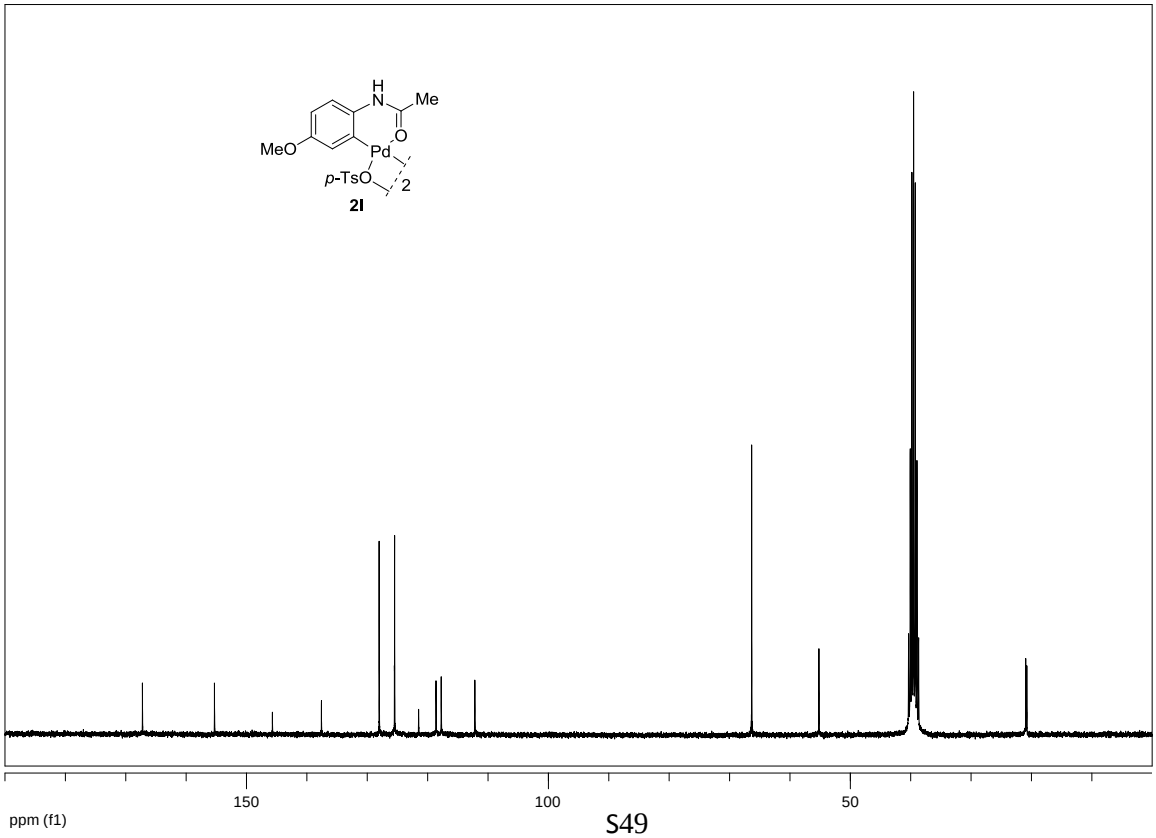

F
B
F
N
N
NH
O
1k
2.02
1.00
1.99
1.87
5.83
2.95
6.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

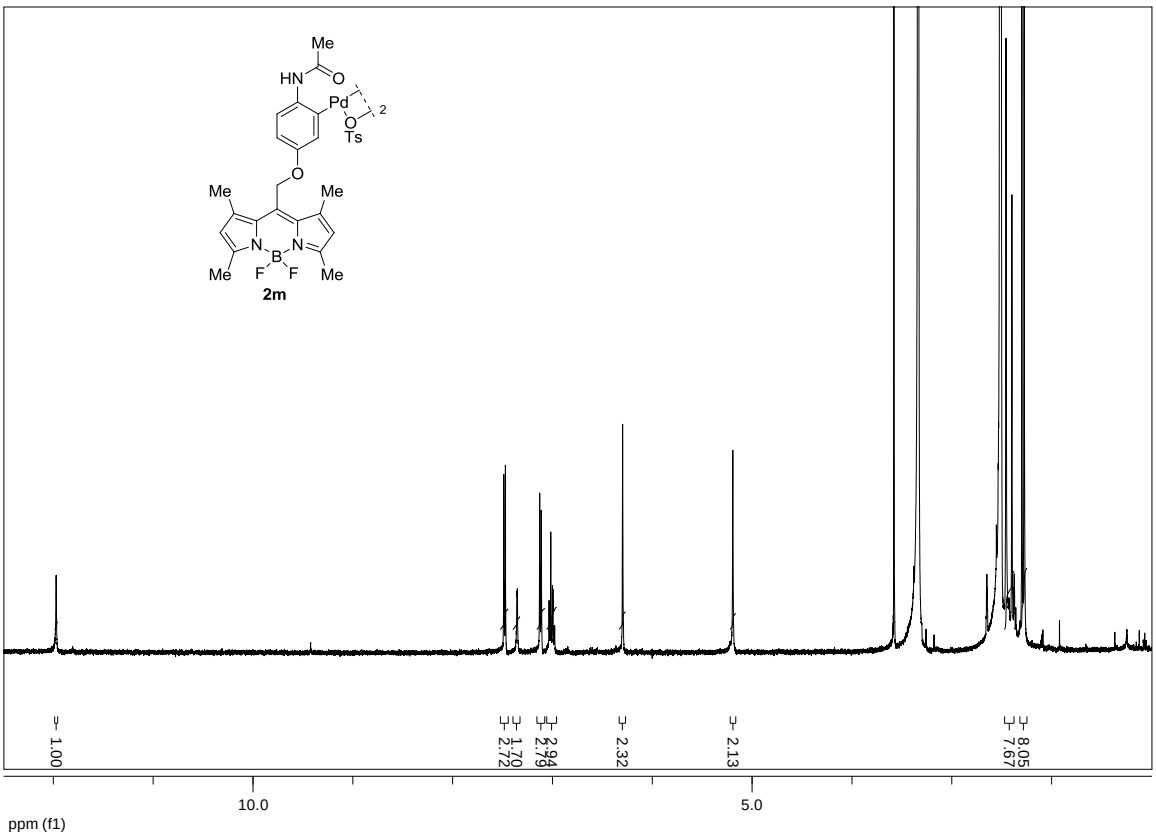

F
B
F
N
N
NH
O
1k
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm (f1)

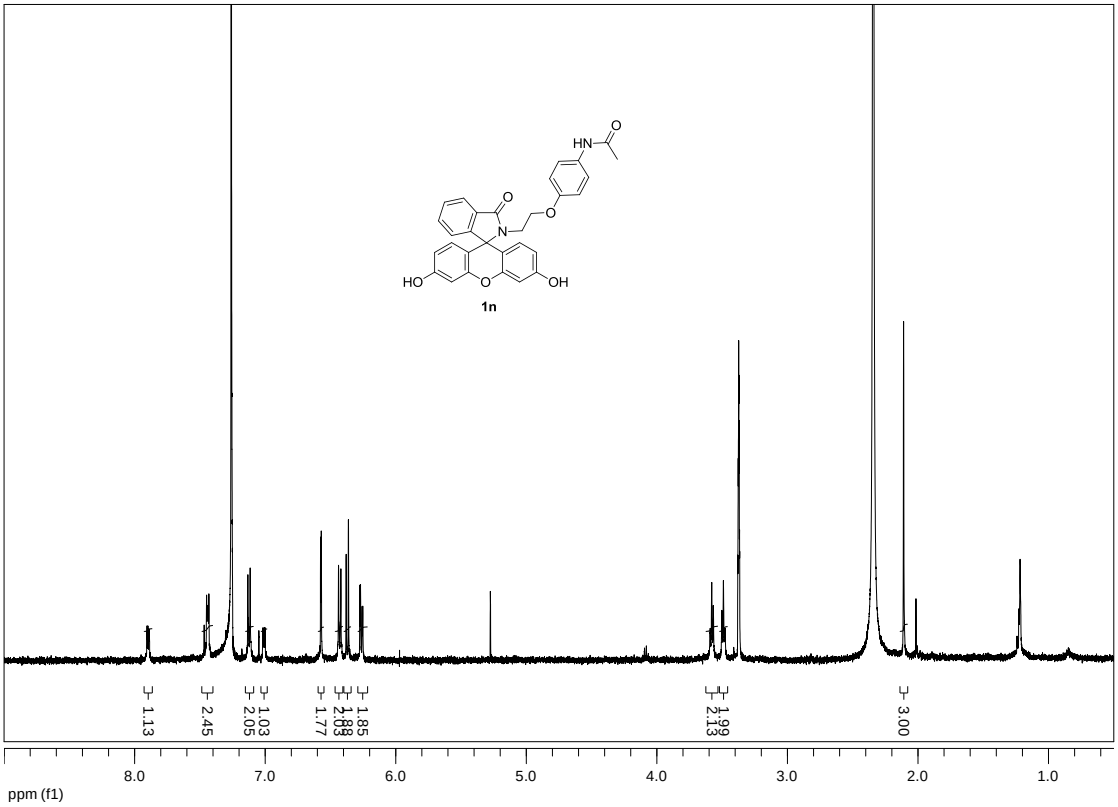

Me
Me
F
B
F
N
N
NH
Me
Me
Me
Pd
O
p-TsO
2
2k
in CD3OD
2.32
2.43
1.07
1.13
1.08
7.50
7.00
ppm (f1)
1.02
2.32
2.43
1.08
2.12
6.05
6.14
6.00
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

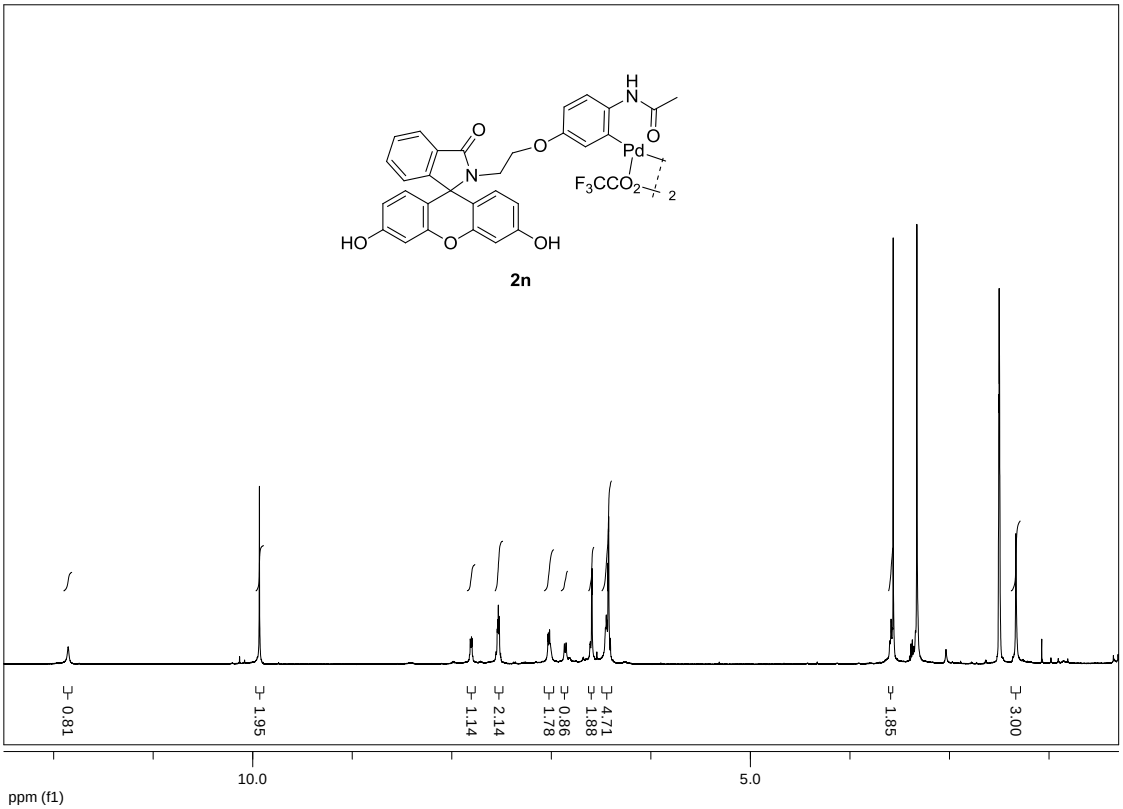

Me
Me
F
B
F
N
N
NH
Me
Me
Me
Pd
O
p-TsO
2
2k
in CD3OD
66.699
48.201
47.987
47.774
47.346
47.132
46.920
167.341
155.287
143.011
141.827
131.674
131.178
131.010
129.398
126.391
125.887
125.544
120.810
116.589
113.955
19.870
19.397
13.297
13.107
180
160
140
120
100
80
60
40
20
ppm

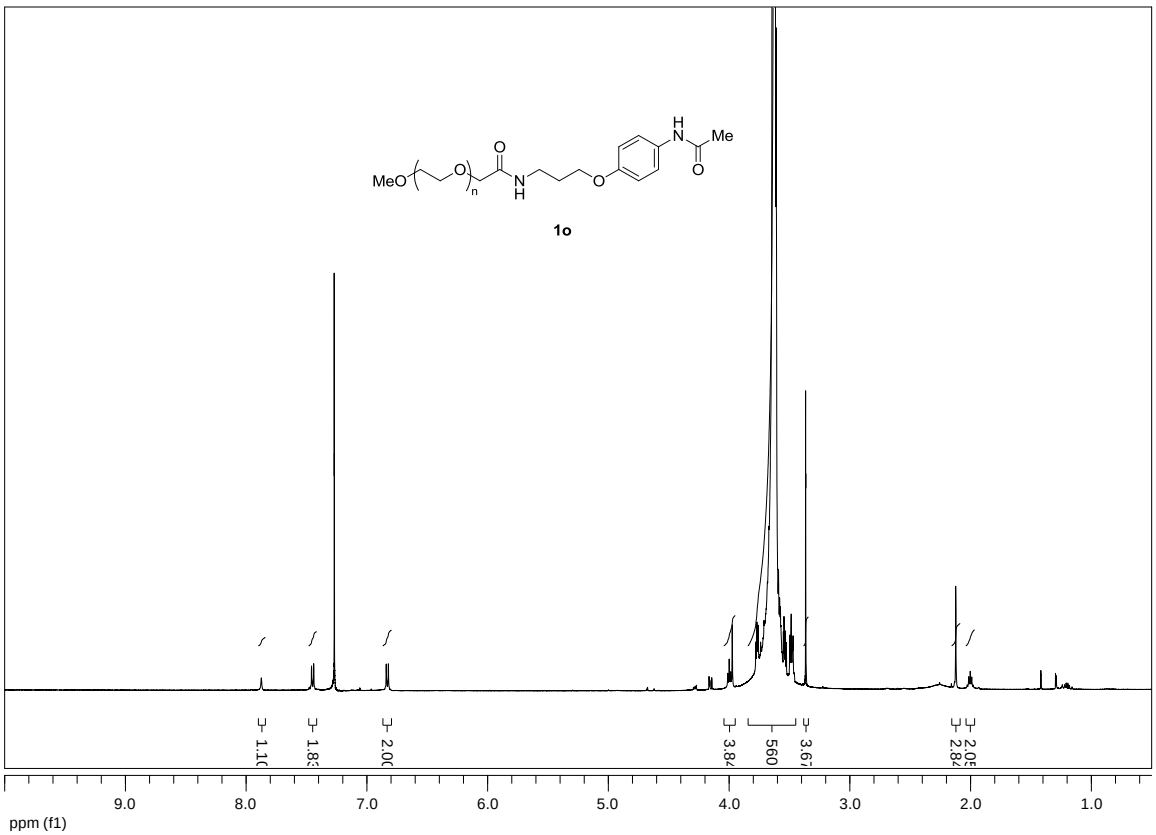


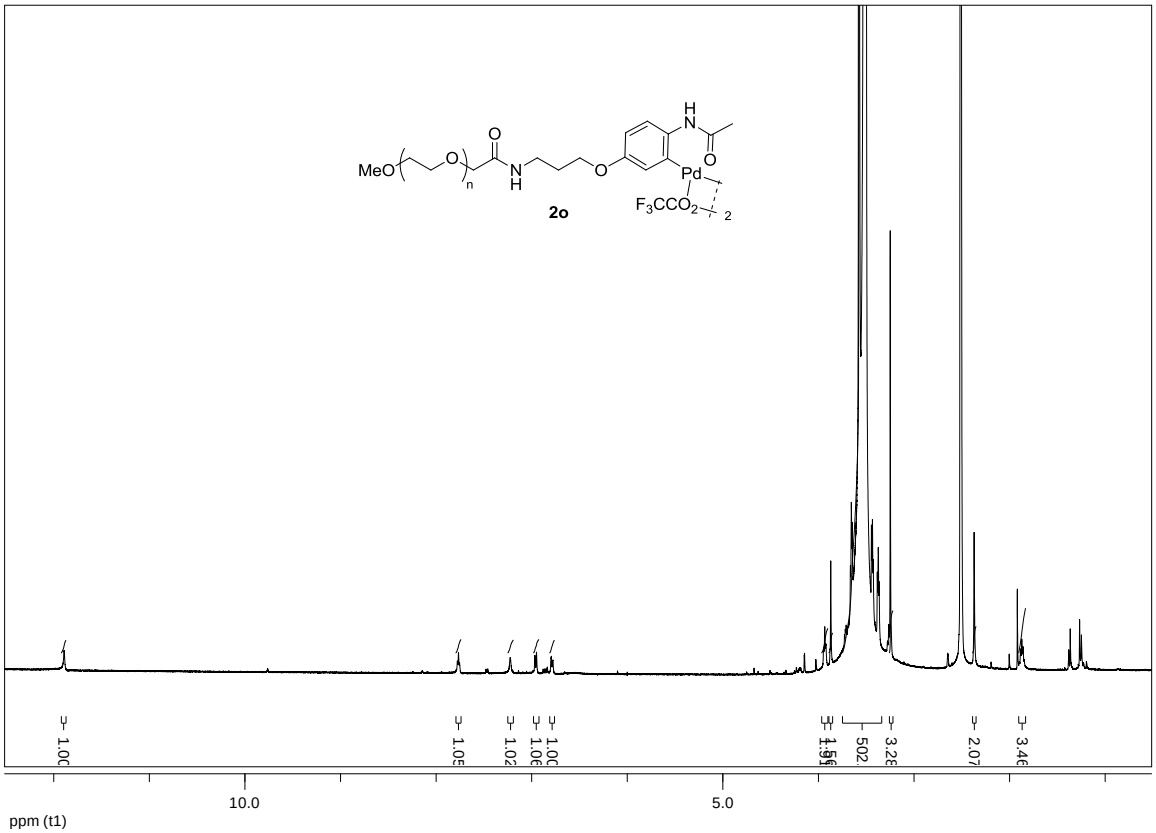



Me
HN
O
Pd
O
Ts
2
O
Me
Me
N
B
N
Me
F
F
Me
2m
1.00
2.72
1.70
2.79
2.94
2.32
2.13
7.67
8.05
10.0
5.0
ppm (f1)


HN
O
O
N
O
HO
O
OH
1n
1.13
2.45
1.03
2.05
1.77
1.85
1.99
2.13
3.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


H
N
O
O
N
O
Pd
F3CCO2
2
HO
O
OH
2n
0.81
1.95
1.14
2.14
0.86
1.78
4.71
1.88
1.85
3.00
10.0
5.0
ppm (f1)


MeO
O
n
N
H
O
H
N
Me
O
1o
1.10
1.83
2.00
3.84
5.60
3.67
2.05
2.82
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


MeO
O
n
N
H
O
H
N
O
Pd
F3CCO2
2
2o
1.00
1.05
1.02
1.06
1.00
1.56
5.02
3.28
2.07
3.46
10.0
5.0
ppm (t1)