# Storable Arylpalladium(II) Reagents for Alkene Labeling in Aqueous Media

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**Supporting Information**

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**General Experimental Procedures.** All reactions were performed in round-bottom flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation (house vacuum, ca. 25–40 Torr) at ambient temperature, unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore-size, 230–400 mesh, Merck KGA) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light, then were stained with either a solution of ceric ammonium molybdate (CAM) in aqueous sulfuric acid, a solution of 2,4-dinitrophenylhydrazine (DNP) in ethanol–aqueous sulfuric acid, or a solution of potassium permanganate ($KMnO_4$) in aqueous sodium hydroxide–potassium carbonate then briefly heated on

a hot plate. Flash-column chromatography was performed as described by Still et al.,[1] employing silica gel (60 Å, 32–63 μM, standard grade, Dynamic Adsorbents, Inc.).

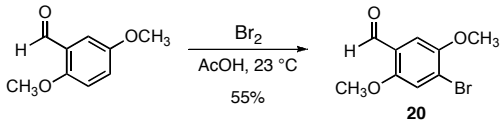
**Materials.** Commercial solvents and reagents were used as received with the following exceptions. Triethylamine and *N,N*-diisopropylethylamine were distilled from calcium hydride under an atmosphere of dinitrogen. Dimethylsulfoxide-$d_6$ was twice dried over 4 Å molecular sieves (72 h each time; the sieves were activated by heating at 200 ºC under reduced pressure for 24 h at 200 ºC). Dichloromethane, benzene, tetrahydrofuran, *N,N*-dimethylformamide, acetonitrile, toluene, and ether were purified by the method of Pangborn et al.[2] Taxol and FK506 were purchased from LC Laboratories

**Instrumentation.** Proton magnetic resonance ($^1$H NMR) spectra were recorded on Varian INOVA 500 (500 MHz) or 600 (600 MHz) NMR spectrometers at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26; $C_6D_5H$, δ 7.15). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, and coupling constant (*J*) in Hertz. Carbon nuclear magnetic resonance spectra ($^{13}$C NMR) were recorded on Varian INOVA 500 (125 MHz) NMR spectrometers at 23 °C. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$, δ 77.0; $C_6D_6$, δ 128.0). Infrared (IR) spectra were obtained using a Shimadzu 8400S FT-IR spectrometer and were referenced to a polystyrene standard. Data are represented as follows: frequency of absorption ($cm^{-1}$), intensity of absorption (s = strong, m = medium, w = weak, br = broad). High-resolution mass spectra were obtained at the Harvard University Mass Spectrometry Facility. Analytical high performance liquid chromatography was performed using a Beckman Coulter System Gold HPLC system equipped with a model 168 detector.

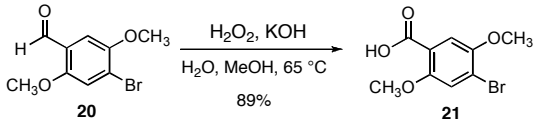
*(For clarity, intermediates that have not been assigned numbers in the text are numbered sequentially in the Supporting Information beginning with* ***20****.)*

[1] Still, W. C.; Khan, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
[2] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518–1520.

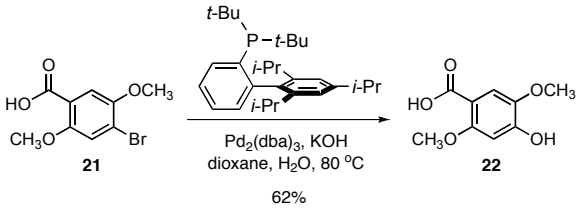
**Synthesis of the Biotin-Linkeded Arylpalladium(II) Reagent 2**



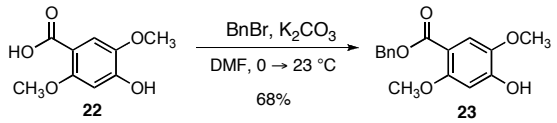

4-Bromo-2,5-dimethoxybenzaldehyde (**20**). Bromine (15.7 mL, 307 mmol, 1.02 equiv) was added by syringe to a solution of 2,5-dimethoxybenzaldehyde (50.0 g, 301 mmol, 1 equiv) in acetic acid (150 mL) at 23 °C. After 48 h, the solution was concentrated at 50 °C. The solid residue was dissolved in ethyl acetate (350 mL). The solution was cooled to –20 °C, inducing crystallization of the product. After 15 h, the yellow prisms were collected on a sintered-glass funnel. The solids were dried in vacuo (0.1 mmHg) to provide 40.7 g of 4-Bromo-2,5-dimethoxybenzaldehyde (**20**) as a yellow solid (55%). TLC: (10% acetonitrile–benzene) $R_f$ = 0.53 (CAM). $^1$H NMR (500 MHz, $CDCl_3$) δ: 10.38 (s, 1H), 7.32 (s, 1H), 7.24 (s, 1H), 3.89 (s, 3H), 3.88 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 189.0, 156.4, 150.6, 124.4, 120.5, 117.9, 109.8, 57.0, 56.6. FTIR (neat), cm$^{-1}$: 3077 (w), 2942 (w), 2864 (w), 1733 (w), 1677 (s). HRMS (ESI): calcd for $(C_9H_9BrO_3 + H)^+$ 244.9808, found 244.9810.




4-Bromo-2,5-dimethoxybenzoic acid (**21**). A solution of potassium hydroxide (34.7 g, 0.619 mol, 4.0 equiv) in water (47 mL) was added to a suspension of 4-bromo-2,5-dimethoxybenzaldehyde **20** (37.9 g, 0.155 mol, 1 equiv) in methanol (262 mL). The reaction flask was equipped with a pressure-equalizing addition funnel and the reaction assembly was placed in an oil bath at 65 °C. Aqueous hydrogen peroxide solution (30% w/v, 158 mL, 1.53 mol, 10.0 equiv) was added dropwise via the addition funnel over the course of 90 min. During this time, the solid starting material dissolved and an orange solution formed. Vigorous bubbling was observed throughout the addition. After 30 min, the heating bath was removed and the solution was allowed to cool to 23 °C. The solution was acidified to pH 2 with aqueous sulfuric acid solution (6 N). The resulting suspension was extracted with dichloromethane (3 x 1 L) and the combined organic layers were dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide 35.9 g of 4-bromo-2,5-dimethoxybenzoic acid (**21**) as a white solid (89%). TLC: (25% acetonitrile–benzene) $R_f$ = 0.33 (CAM); $^1$H NMR (500 MHz, $CDCl_3$) δ: 10.76 (br s, 1H), 7.68 (s, 1H), 7.31 (s, 1H), 4.07 (s, 3H), 3.93 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 165.0, 152.1, 151.5, 118.9, 117.8, 117.5, 115.4, 57.8, 57.1. FTIR (neat), cm$^{-1}$: 2947 (w), 2847 (w), 1674 (s), 1495 (s). HRMS (ESI): calcd for $(C_9H_9BrO_4 + H)^+$ 260.9757, found, 260.9760.



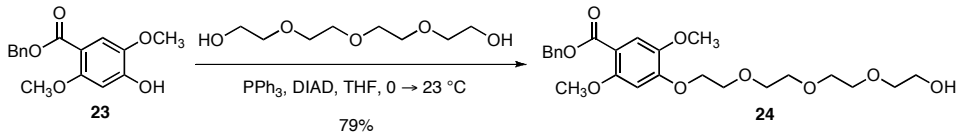

4-Hydroxy-2,5-dimethoxybenzoic acid (**22**). 4-Hydroxy-2,5-dimethoxybenzoic acid **22** was prepared according to the method developed by Anderson et al.[3] Dioxane (134 mL, degassed by sparging with argon gas for 3 h prior to use) and water (134 mL, degassed by sparging with argon gas for 3 h prior to use) were added to a 1-L reaction flask containing 4-bromo-2,5-dimethoxybenzoic acid **21** (35.0 g, 134 mmol, 1 equiv), tris(dibenzylidene)dipalladium(0) (1.11 g, 1.21 mmol, 0.010 equiv), potassium hydroxide (30.1 g, 536 mmol, 4.0 equiv), and 2-di-tert-butylphosphino-2',4',6'-triisopropylbiphenyl (1.03 g, 2.42 mmol, 0.020 equiv). The reaction flask was heated in an oil bath at 80 °C. After 15 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The product solution was partitioned between aqueous hydrochloric acid solution (1.0 N, 500 mL) and dichloromethane (200 mL). The aqueous layer was extracted with dichloromethane (4 x 500 mL). The combined organic layers were dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (50% hexanes–ethyl acetate initially, grading to 100% ethyl acetate) to provide 16.6 g of 4-hydroxy-2,5-dimethoxybenzoic acid (**22**) as a pale yellow solid (62%). 4-Hydroxy-2,5-dimethoxybenzoic acid **22** could also be purified by crystallization from a solution of 15% dichloromethane–ethyl acetate at –20 °C. TLC: (ethyl acetate) $R_f$ = 0.48 (CAM); $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.66 (s, 1H), 6.68 (s, 1H), 6.20 (br s, 1H), 4.04 (s, 3H), 3.94 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 165.6, 154.2, 151.8, 141.8, 114.6, 109.0, 99.4, 57.4, 56.7. FTIR (neat), cm$^{-1}$: 3140 (br), 3049 (s), 2947 (m), 2844 (m), 2769 (m), 1685 (s). HRMS (ESI): calcd for $(C_9H_{10}O_5 + H)^+$ 199.0606, found 199.0615.




4-Hydroxy-2,5-dimethoxybenzoic acid benzyl ester (**23**). Benzyl bromide (2.65 mL, 22.3 mmol, 1 equiv) followed by potassium carbonate (7.69 g, 55.8 mmol, 2.5 equiv) were added to an ice-cooled solution of 4-hydroxy-2,5-dimethoxybenzoic acid **22** (6.63 g, 33.5 mmol, 1.5 equiv) in *N*,*N*-dimethylformamide (22.3 mL). The cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 18 h, the reaction mixture was diluted with water (100

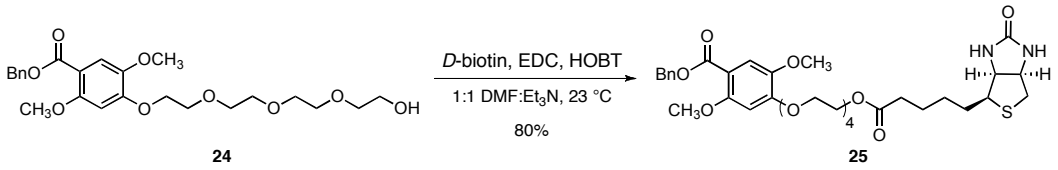
[3] Anderson, K. W.; Ikawa, T.; Tundel, R. E.; Buchwald, S. L. *J. Am. Chem. Soc.* **2006**, *128*, 10694–10695.

mL) and the mixture was brought to pH 7 with aqueous sulfuric acid solution (6 N). The resulting solution was extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with brine (75 mL). The washed solution was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (15% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–hexanes) to provide 4.37 g of 4-hydroxy-2,5-dimethoxybenzoic acid benzyl ester (**23**) as a white solid (68%). Unreacted starting material **22** was recovered by acidifying the aqueous layer to pH 2 with aqueous sulfuric acid solution (6 N) followed by extraction with ethyl acetate (3 x 100 mL). The combined organic layers were dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The solid residue was triturated with dichloromethane (40 mL) then with ethyl acetate (40 mL) to provide 2.25 g of 2,5-dimethoxy-4-hydroxybenzoic acid (**22**) as a white solid. TLC: (50% ethyl acetate–hexanes) $R_f$ = 0.42 (CAM). $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 10.01 (s, 1H), 7.43–7.30 (m, 5H), 7.29 (s, 1H), 6.56 (s, 1H), 5.24 (s, 2H), 3.72 (s, 3H), 3.71 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 165.5, 156.0, 153.0, 141.7, 137.5, 129.1, 128.4, 128.2, 115.9, 109.2, 101.9, 66.0, 57.0, 56.8. FTIR (neat), cm$^{-1}$: 3359 (w), 2933 (w), 1694 (s), 1586 (s). HRMS (ESI): calcd for $(C_{16}H_{16}O_5 + H)^+$ 289.1070, found 289.1068.

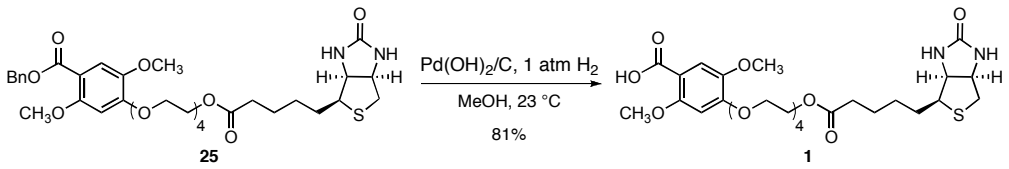



Alcohol **24**. Diisopropyl azodicarboxylate (2.16 mL, 11.2 mmol, 1.2 equiv) was added dropwise to an ice-cooled solution of benzyl 4-hydroxy-2,5-dimethoxybenzoic acid benzyl ester **23** (2.68 g, 9.30 mmol, 1 equiv), triphenylphosphine (2.92 g, 11.2 mmol, 1.2 equiv), and tetraethylene glycol (4.51 g, 23.2 mmol, 2.5 equiv) in tetrahydrofuran (46 mL). The cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 18 h, the reaction solution was poured into a 1:1 mixture of brine and saturated aqueous sodium bicarbonate solution (100 mL). The resulting solution was extracted with ethyl acetate (3 x 75 mL). The combined organic layers were dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (0.5% methanol–dichloromethane initially, grading to 4% methanol–dichloromethane) to provide 3.41 g of the alcohol **24** as a colorless oil (79%). TLC: (5% methanol–dichloromethane) $R_f$ = 0.50 (CAM). $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.45–7.29 (m, 6H), 6.62 (s, 1H), 5.33 (s, 2H), 4.24 (t, 2H, $J$ = 5.0 Hz), 3.89 (t, 2H, $J$ = 5.0 Hz), 3.86 (s, 3H), 3.81 (s, 3H), 3.73–3.63 (m, 10H), 3.58 (t, 2H, $J$ = 5.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 165.6, 156.0, 153.4, 143.1, 136.7, 128.7, 128.2, 115.6, 111.2, 100.0, 72.7, 71.1, 70.9, 70.8, 70.6,

69.8, 68.9, 66.5, 61.9, 57.2, 56.9. FTIR (neat), $cm^{-1}$: 3488 (br), 2932 (m), 2871 (m), 1718 (s), 1701 (s). HRMS (ESI): calcd for $(C_{24}H_{32}O_9 + H)^+$ 465.2124, found 465.2109.

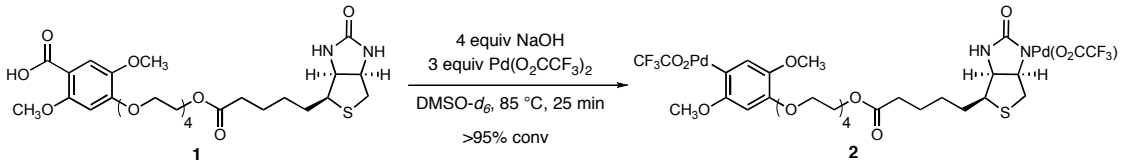



Benzyl ester **25**. *N,N*-Dimethylformamide (37 mL) was added to a reaction flask containing alcohol **24** (3.41 g, 7.34 mmol, 1 equiv), *D*-biotin (2.33 g, 9.54 mmol, 1.3 equiv), 1-hydroxybenzotriazole hydrate (1.19 g, 8.81 mmol, 1.2 equiv), and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (2.11 g, 11.0 mmol, 1.5 equiv) at 23 °C. After 10 min, triethylamine (37 mL) was added. After 48 h, the reaction mixture was poured into saturated aqueous ammonium chloride solution (100 mL). The resulting solution was extracted with dichloromethane (3 x 75 mL). The organic layers were combined and dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (1% methanol–dichloromethane initially, grading to 5% methanol–dichloromethane) to provide 4.04 g of the benzyl ester **25** as a colorless oil (80%). TLC: (10% methanol–dichloromethane) $R_f$ = 0.48 (CAM). $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.48–7.33 (m, 6H), 6.64 (s, 1H), 5.35 (s, 2H), 4.53 (dd, 1H, *J* = 7.8, 5.2 Hz), 4.33 (dd, 1H, *J* = 8.0, 4.5 Hz), 4.28–4.22 (m, 4H), 3.92 (t, 2H, *J* = 5.0 Hz), 3.89 (s, 3H), 3.84 (s, 3H), 3.75–3.66 (m, 10H), 3.18–3.14 (m, 1H), 2.92 (dd, 1H, *J* = 12.5, 5.2 Hz), 2.75 (d, 1H, *J* = 13.0 Hz), 2.36 (t, 2H, *J* = 7.5 Hz), 1.74–1.64 (m, 4H), 1.50–1.43 (m, 2H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 173.9, 165.6, 164.0, 156.0, 153.4, 143.1, 136.7, 128.7, 128.2, 128.1, 115.6, 111.2, 100.0, 71.1, 70.8(4), 70.8(0), 70.7, 69.8, 69.4, 68.9, 66.5, 63.6, 62.2, 60.4, 57.2, 56.9, 55.8, 40.7, 34.0, 28.6, 28.4, 25.0. FTIR (neat), $cm^{-1}$: 3371 (w), 3240 (w), 2935 (m), 2868 (m), 1703 (s), 1699 (s), 1610 (m), 1516 (m). HRMS (ESI): calcd for $(C_{34}H_{46}N_2O_{11}S + H)^+$ 691.2895, found 691.2901.



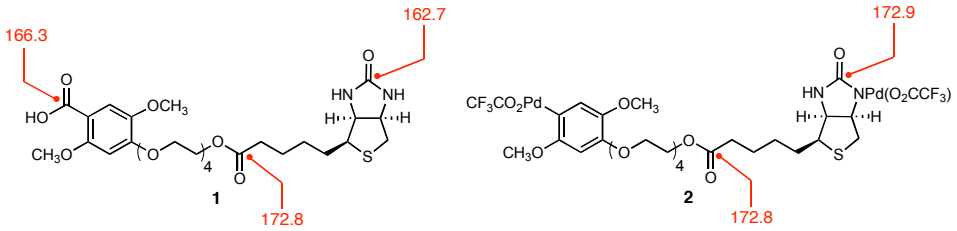

Carboxylic acid **1**. Palladium(II) hydroxide on carbon (20% w/w, 457 mg, 0.653 mmol, 1 equiv) was added to a solution of benzyl ester **25** (451 mg, 0.653 mmol, 1 equiv) in methanol (13.1 mL) at 23 °C. The reaction flask was evacuated, then filled with hydrogen (repeated twice more). A hydrogen balloon (1 atm) was affixed. After 4 h, the reaction suspension was filtered

through a pad of Celite, rinsing with dichloromethane (60 mL). The filtrate was concentrated. The residue was purified by flash-column chromatography (5% methanol–dichloromethane initially, grading to 10% methanol–dichloromethane) to provide 319 mg of the carboxylic acid **1** as a white solid (81%). TLC: (10% methanol–dichloromethane) $R_f$ = 0.44 (CAM). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 7.26 (s, 1H), 6.73 (s, 1H), 6.42 (br s, 1H), 6.34 (br s, 1H), 4.28 (dd, 1H, $J$ = 7.5, 5.0 Hz), 4.19 (t, 2H, $J$ = 4.8 Hz), 4.12–4.08 (m, 3H), 3.79 (s, 3H), 3.76 (t, 2H, $J$ = 4.5 Hz), 3.71 (s, 3H), 3.59–3.56 (m, 6H), 3.54–3.52 (m, 4H), 3.08–3.04 (m, 1H), 2.80 (dd, 1H, $J$ = 12.5, 5.0 Hz), 2.56 (d, 1H, $J$ = 12.0 Hz), 2.28 (t, 1H, $J$ = 7.2 Hz), 1.62–1.40 (m, 4H), 1.36–1.27 (m, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 172.8 ($CH_2$**C**$O_2$), 166.3 (**C**$O_2$H), 162.7 (HN(**C**=O)NH), 154.6, 152.4, 142.1, 114.7, 110.9, 99.6, 69.9, 69.8, 69.7, 68.8, 68.3, 68.1, 63.0, 61.0, 59.2, 56.6, 56.0, 55.3, 39.5, 33.2, 28.0, 27.9, 24.5. FTIR (neat), cm$^{-1}$: 3256 (w), 2929 (m), 2855 (w), 1695 (s). HRMS (ESI): calcd for $(C_{34}H_{46}N_2O_{11}S + H)^+$ 601.2426, found 601.2420.

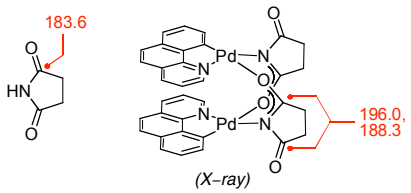



Arylpalladium(II) reagent **2**. Sodium hydride (a 60% dispersion in mineral oil, 20.1 mg, 0.503 mmol) was washed with hexanes (3 x 1.0 mL) and dried in vacuo (0.1 mmHg) prior to use. Under an atmosphere of argon, dimethylsulfoxide-$d_6$ (7.0 mL) followed by water (10.5 μL, 0.58 mmol) were added to a reaction flask containing washed and dried sodium hydride at 23 °C. The reaction flask was heated in an oil bath at 75 °C. After 15 min, the heating bath was removed. The resulting solution of sodium hydroxide in DMSO-$d_6$ (71.9 mM) was allowed to cool to 23 °C and was used immediately. The freshly prepared solution of sodium hydroxide in DMSO-$d_6$ (5.21 mL, 71.9 mM, 0.37 mmol, 4.0 equiv) was added to a reaction flask containing carboxylic acid **1** (56.3 mg, 0.094 mmol, 1.0 equiv) at 23 °C. After 10 min, palladium(II) trifluoroacetate (93.5 mg, 0.28 mmol, 3.0 equiv) was added. The reaction flask was heated in an oil bath at 85 °C. After 25 min, the heating bath was removed and the solution was allowed to cool to 23 °C. $^1$H, $^{13}$C, and $^{19}$F NMR spectroscopic analysis showed evidence of a single species fully consistent with the assigned structure **2** (>95% conversion, estimated to be 18.1 mM). The product solution, stored frozen at –20 °C, provided a reliable stock of arylpalladium(II) reagent **2** over several months, thawing just prior to use when needed. The efficiency of formation of the arylpalladium(II) reagent **2** was estimated to be >90% based on the isolated yield of the protiodepalladation product obtained upon treatment with dithiothreitol (see the next section, page 9). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 6.73 (s, 1H, Ar**H**), 6.40 (s, 1H, Ar**H**), 4.10 (br s, 2H, OCOC**H**$_2$$CH_2O$), 4.03 (br s, 2H, ArOC**H**$_2$$CH_2$), 3.79 (s, 3H, OC**H**$_3$), 3.68 (s, 5H, $ArOCH_2C$**H**$_2$, OC**H**$_3$), 3.56–3.51 (m, 10H, 5 x OC**H**$_2$$CH_2O$), 2.40 (br s, 2H,

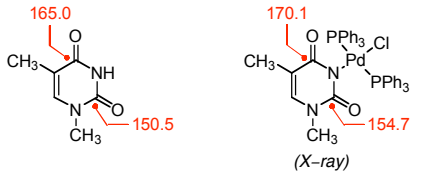
$O_2CC\mathbf{H_2}CH_2$), 1.75–1.43 (m, 6H, $O_2CCH_2C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}$). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 172.9 (N(**C**=O)N), 172.8 ($CH_2\mathbf{C}O_2$), 158.6 ($CF_3\mathbf{C}O_2$), 154.8 ($\mathbf{C}OCH_3$), 146.6 ($\mathbf{C}OCH_2CH_2$), 142.4 ($\mathbf{C}OCH_3$), 119.3 ($CH_3OCC\mathbf{H}$), 117.0 (q, $J$ = 295.3 Hz, $\mathbf{C}F_3CO_2$), 115.6 (Pd**C**), 99.3 ($CH_3OCC\mathbf{H}$), 69.9 ($O\mathbf{C}H_2CH_2O$), 69.8 ($O\mathbf{C}H_2CH_2O$), 69.2 ($O\mathbf{C}H_2CH_2O$), 68.9 ($O\mathbf{C}H_2CH_2O$), 68.3 ($OCO\mathbf{C}H_2CH_2$), 63.2 ($ArO\mathbf{C}H_2CH_2$), 56.5 ($O\mathbf{C}H_3$), 56.2 ($O\mathbf{C}H_3$), 33.1 ($O_2C\mathbf{C}H_2CH_2$), 24.4. $^{19}$F NMR (375 MHz, DMSO-$d_6$) δ: –73.9.



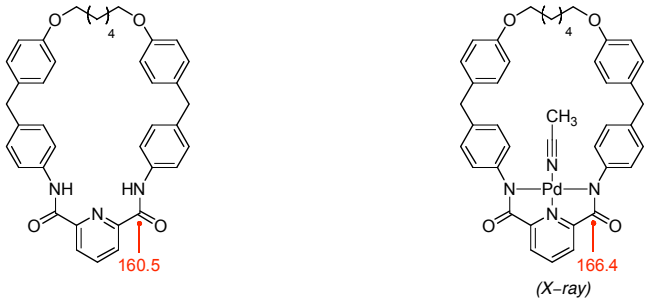

Note: Assignment of the site of palladium binding within the biotin residue is tentative, and based upon $^{13}$C NMR data from a series of related examples, shown below. We do not discount the possibility of a higher order complex such as that depicted below from the work of Powers and Ritter.




Powers, D. C. *et al.* On the Mechanism of Palladium-Catalyzed Aromatic C–H Oxidation. *J. Am. Chem. Soc.* **2010**, *132*, 14530–14536.

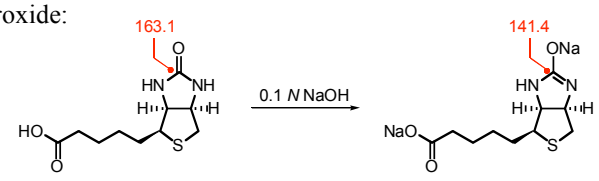



Romanelli, A. *et al.* Reactions of $Pd(PPh_3)_4$ with 3',5'-di-*O*-acetylthymidine: oxidative addition of $Pd(PPh_3)_4$ on thymidine $N_3$ and $C_4$ atoms. *Organometallics* **2005**, *24*, 3401–3406.



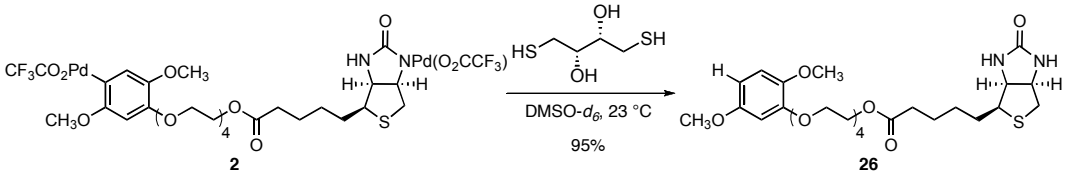

Goldup, S. M. *et al.* Active template synthesis of rotaxanes and molecular shuttles with switchable dynamics with four-component $Pd^{II}$-promoted Michael additions. *Angew. Chem. Int. Ed.* **2008**, *47*, 3381–3384.

This trend stands in contrast to the upfield shift of the carbonyl carbon of the product observed after treating D-biotin with an aqueous solution of 0.1 *N* sodium hydroxide:



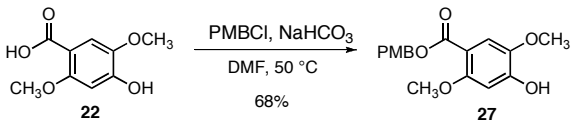

Hadjiliadis, N.; Pneumatikakis, G. Complexes of d(+)-biotin with Pd(II) and Pt(II). *J. Inorg. Biochem.* **1979**, *10*, 215–224.

**Protiodepalladation of Arylpalladium(II) Reagent 2 with Dithiothreitol.**



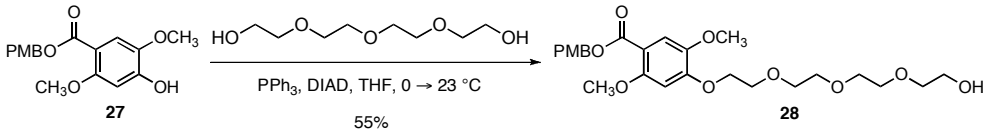

Solid DL-dithiothreitol (12.9 mg, 83.5 μmol, 5.0 equiv) was added to a solution of the arylpalladium(II) reagent **2** in DMSO-$d_6$ (1.00 mL of 16.7 mM solution, 16.7 μmol, 1 equiv) in a 20-mL scintillation vial at 23 °C. After 10 min, the reaction mixture was filtered through a pad of Celite, rinsing sequentially with ethyl acetate (33 mL), dichloromethane (33 mL), and methanol (33 mL). The filtrate was concentrated. The concentrate was partitioned between aqueous hydrochloric acid solution (1.0 N, 15 mL) and ethyl acetate (20 mL). The aqueous layer was extracted with ethyl acetate (2 x 20 mL). The combined organic layers were washed with water (3 x 15 mL) and the washed solution was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (5% methanol–ethyl acetate initially, grading to 8% methanol–ethyl acetate) to provide 8.8 mg of the protiodepalladation product **26** as a colorless oil (95%). TLC: (10% methanol–dichloromethane) $R_f$ = 0.11 (CAM). $^1$H NMR (500 MHz, $CDCl_3$) δ: 6.81 (d, 1H, *J* = 9.0 Hz), 6.57 (d, 1H, *J* = 3.0 Hz), 6.43 (dd, 1H, *J* = 9.0, 2.5 Hz), 5.40 (br s, 1H), 4.95 (br s, 1H), 4.52 (dd, 1H, *J* = 7.5, 5.0 Hz), 4.32 (dd, 1H, *J* = 7.5, 4.5 Hz), 4.25–4.22 (m, 2H), 4.18 (t, 2H, *J* = 5.2 Hz), 3.90 (t, 2H, 5.0 Hz), 3.82 (s, 3H), 3.77 (s, 3H), 3.76–3.66 (m, 10H), 3.18-3.14 (m, 1H), 2.93 (dd, 1H, *J* = 12.8, 4.8 Hz), 2.75 (d, 1H, *J* = 12.5 Hz), 2.38 (t, 2H, *J* = 7.5 Hz), 1.74–1.65 (m, 4H), 1.50–1.44 (m, 2H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 173.8, 163.2, 154.5, 149.5, 144.2, 113.2, 110.9, 104.3, 102.6, 71.1, 70.9, 70.8, 70.7, 69.9, 69.4, 68.8, 63.7, 62.2, 60.4, 57.0, 55.9, 55.5, 40.7, 34.0, 28.5, 24.9. FTIR (neat), cm$^{-1}$: 3371 (w), 3243 (w), 2928 (m), 1699 (s), 1511 (m). HRMS (ESI): calcd for $(C_{34}H_{46}N_2O_{11}S + H)^+$ 557.2527, found 557.2516.

**Synthesis of the Unsymmetrical Indocyanine-Linked Arylpalladium(II) Reagent 7.**



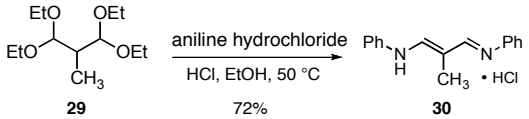

4-Methoxybenzyl 4-hydroxy-2,5-dimethoxybenzoate (**27**). 4-Methoxybenzyl chloride (1.25 mL, 9.15 mmol, 1.2 equiv) was added to a solution of sodium bicarbonate (1.28 g, 15.3 mmol, 2.0 equiv) and 4-hydroxy-2,5-dimethoxybenzoic acid **22** (1.51 g, 7.62 mmol, 1.0 equiv) in *N*,*N*-dimethylformamide (15 mL) at 23 °C. The reaction flask was heated in an oil bath at 50 °C.

After 30 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The solution was partitioned between water (100 mL) and ethyl acetate (100 mL). The aqueous layer was extracted with ethyl acetate (2 x 80 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (100 mL). The washed solution was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (1% methanol–dichloromethane initially, grading to 2% methanol–dichloromethane) to provide 1.65 g of 4-methoxybenzyl 4-hydroxy-2,5-dimethoxybenzoate (**27**) as a pale yellow solid (68%). TLC: (5% methanol–dichloromethane) $R_f$ = 0.55 ($KMnO_4$). $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.41 (s, 1H), 7.39 (d, 2H, *J* = 8.3 Hz), 6.90 (d, 2H, *J* = 8.8 Hz), 6.60 (s, 1H), 6.06 (br s, 1H), 5.27 (s, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 165.8, 159.7, 156.5, 151.0, 140.1, 130.1, 128.9, 114.4, 114.1, 110.5, 100.3, 66.3, 56.8, 56.8, 55.5. FTIR (neat), cm$^{-1}$: 3368 (m), 2940 (w), 1694 (s), 1587 (s), 1512 (s). HRMS (ESI): calcd for $(C_{17}H_{18}O_6 + Na)^+$ 341.0996, found 341.0971.



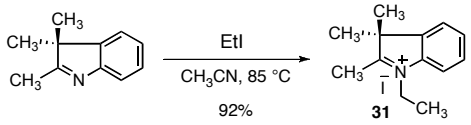

Alcohol **28**. Diisopropyl azodicarboxylate (1.18 mL, 6.11 mmol, 1.2 equiv) was added dropwise to an ice-cooled solution of 4-methoxybenzyl 4-hydroxy-2,5-dimethoxybenzoate **27** (1.62 g, 5.10 mmol, 1 equiv), triphenylphosphine (1.61 g, 6.11 mmol, 1.2 equiv), and tetraethylene glycol (2.48 g, 12.7 mmol, 2.5 equiv) in tetrahydrofuran (25 mL). The cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 18 h, the reaction solution was poured into a 1:1 mixture of brine and saturated aqueous sodium bicarbonate solution (60 mL). The resulting solution was extracted with ethyl acetate (3 x 60 mL). The combined organic layers were dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (0.5% methanol–dichloromethane initially, grading to 2% methanol–dichloromethane) to provide 1.38 g of the alcohol **28** as a colorless oil (55%). TLC: (5% methanol–dichloromethane) $R_f$ = 0.33 ($KMnO_4$). $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.40 (s, 1H), 7.39 (d, 2H, *J* = 8.8 Hz), 6.90 (d, 2H, *J* = 8.8 Hz), 6.61 (s, 1H), 5.26 (s, 2H), 4.25 (t, 2H, *J* = 4.9 Hz), 3.90 (t, 2H, *J* = 4.9 Hz), 3.86 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.74–3.64 (m, 10H), 3.59 (t, 2H, *J* = 4.8 Hz), 2.38 (br s, 1H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 165.7, 159.7, 155.9, 153.3, 143.1, 130.1, 128.9, 115.5, 114.1, 111.4, 99.9, 72.7, 71.1, 70.9, 70.8, 70.5, 69.8, 68.8, 66.3, 62.0, 57.2, 56.8,

55.5. FTIR (neat), $cm^{-1}$: 2874 (w), 1719 (s), 1694 (s), 1611 (m), 1514 (s). HRMS (ESI): calcd for $(C_{25}H_{34}O_{10} + Na)^+$ 517.20442, found 517.19719.



**29** 72% **30**

<u>*N*-((*E*)-2-Methyl-3-(phenylimino)prop-1-enyl)benzenamine hydrochloride (**30**).</u> The following experimental procedure is a modification of the protocol reported by Todoriki et al.[4] The primary modifications here are the order of addition and the use of hydrochloric acid in the reaction. Thus, 1,1,3,3-tetraethoxy-2-methylpropane[5] **29** (3.00 g, 12.8 mmol, 1.0 equiv) was added to a solution of hydrochloric acid (12 M, 1.8 mL) in ethanol (30 mL) at 23 °C. The reaction flask was fitted with a pressure-equalizing addition funnel. A solution of aniline hydrochloride (3.32 g, 25.6 mmol, 2.0 equiv) and hydrochloric acid (12 M, 2.1 mL) in ethanol (25 mL) was added dropwise via the addition funnel at 50 °C. After 17 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The resulting yellow solids were collected on a sintered-glass funnel. The solids were dried in vacuo (0.1 mmHg) to provide 2.50 g of *N*-((*E*)-2-Methyl-3-(phenylimino)prop-1-enyl)benzenamine hydrochloride (**30**) as a yellow solid (72%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 11.61 (br s, 1H), 8.91(d, 1H, $J$ = 6.9 Hz), 7.66 (d, 4H, $J$ = 7.8 Hz), 7.46 (app t, 4H, $J$ = 7.8 Hz), 7.26 (t, 2H, $J$ = 7.3 Hz), 2.24 (s, 3H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 159.7, 140.0, 130.3, 126.6, 119.1, 107.6, 10.1. FTIR (neat), $cm^{-1}$: 3323 (s), 2943 (w), 1616 (m), 1576 (s), 1491 (m). HRMS (ESI): calcd for $(C_{16}H_{16}N_2 + H)^+$ 237.1386, found 237.1392.

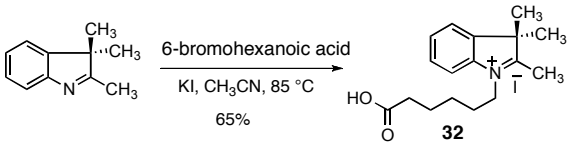


92% **31**

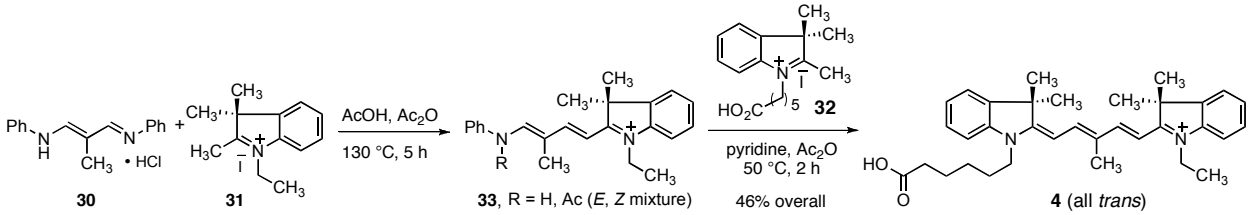
<u>1-Ethyl-2,3,3-trimethyl-3*H*-indolium iodide (**31**).</u> Ethyl iodide (3.2 mL, 18.8 mmol, 1.5 equiv) was added to a solution of 2,3,3-trimethyl-3*H*-indole (2.0 g, 12.6 mmol, 1.0 equiv) in acetonitrile (50 mL) at 23 °C. The reaction flask was fitted with a reflux condenser and the reaction assembly was placed in an oil bath (bath temperature 85 °C). After 48 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The solution was concentrated. The gummy residue was mixed with ether (100 mL) and the resulting solution was decanted. This washing process was repeated several more times until a purple solid was formed. The purple solids were collected on a sintered-glass funnel. The solids were dried in vacuo (0.1 mmHg) to provide 3.60 g of 1-Ethyl-2,3,3-trimethyl-3*H*-indolium iodide (**31**) as a purple solid (92%). $^1$H NMR (500

[4] Todoriki, R.; Ono, M.; Tamura, S. *Heterocycles* **1986**, *24*, 755–769.
[5] O'Sullivan, P. T.; Buhr, W.; Fuhry, M. A. M.; Harrison, J. R.; Davies, J. E.; Feeder, N.; Marshall, D. R.; Burton, J. W.; Holmes, A. B. *J. Am. Chem. Soc.* **2004**, *126*, 2194–2207.

MHz, DMSO-$d_6$) δ: 7.98–7.95 (m, 1H), 7.85–7.82 (m, 1H), 7.63–7.59 (m, 2H), 4.49 (q, 2H, $J$ = 7.3 Hz), 2.83 (s, 3H), 1.52 (s, 6H), 1.43 (t, 3H, $J$ = 7.3 Hz). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 196.8, 142.7, 141.5, 130.1, 129.7, 124.3, 116.0, 54.9, 43.8, 22.6, 14.6, 13.4. FTIR (neat), cm$^{-1}$: 2970 (w), 1613 (m), 1576 (m), 1460 (m). HRMS (ESI): calcd for $(C_{13}H_{18}N)^+$ 188.1434, found 188.1439.



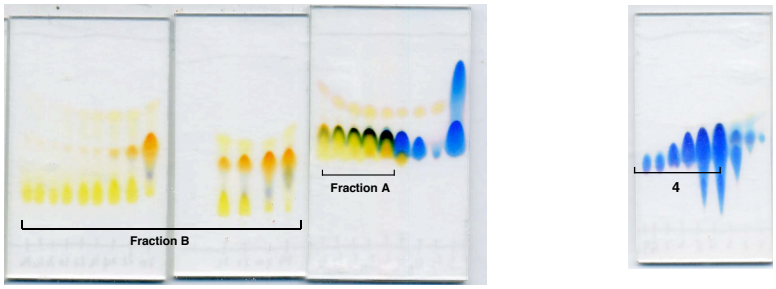

1-(5-Carboxypentyl)-2,3,3-trimethyl-3*H*-indolium iodide (**32**). Potassium iodide (2.3 g, 13.9 mmol, 1.3 equiv) was added to a solution of 2,3,3-trimethyl-3*H*-indole (1.7 g, 10.7 mmol, 1.0 equiv) and 6-bromohexanoic acid (2.7 g, 13.9 mmol, 1.3 equiv) in acetonitrile (75 mL) at 23 °C. The reaction flask was fitted with a reflux condenser and the reaction assembly was placed in an oil bath (bath temperature 85 °C). After 72 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The resulting red solution was decanted from the solid potassium bromide and concentrated. The residue was mixed with ethyl acetate (300 mL) at which point a gray solid began to form. The gray solids were collected on a sintered-glass funnel. The solids were rinsed with ethyl acetate. The rinsed solids were dried in vacuo (0.1 mmHg) to provide 2.80 g of 1-(5-Carboxypentyl)-2,3,3-trimethyl-3*H*-indolium iodide (**32**) as a gray solid (65%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 7.98–7.95 (m, 1H), 7.85–7.82 (m, 1H), 7.63–7.59 (m, 2H), 4.44 (t, 2H, $J$ = 7.8 Hz), 2.83 (s, 3H), 2.21 (t, 2H, $J$ = 7.3 Hz), 1.86–1.80 (m, 2H), 1.57–1.50 (m, 2H), 1.52 (s, 6H), 1.44–1.38 (m, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 197.2, 175.0, 142.6, 141.8, 130.1, 129.7, 124.2, 116.2, 54.9, 48.2, 34.1, 27.7, 26.1, 24.7, 22.7, 14.8. FTIR (neat), cm$^{-1}$: 3385 (w), 2942 (w), 1722 (s), 1626 (w), 1591 (w), 1460 (m). HRMS (ESI): calcd for $(C_{17}H_{24}NO_2)^+$ 274.1802, found 274.1826.




Indocyanine dye **4**.[6] *First step*: *N*-((*E*)-2-Methyl-3-(phenylimino)prop-1-enyl)benzenamine hydrochloride **30** (937 mg, 3.44 mmol, 1.1 equiv) was added to a suspension of 1-ethyl-2,3,3-trimethyl-3*H*-indolium iodide **31** (985 mg, 3.12 mmol, 1.0 equiv) in a 1:1 mixture of acetic acid (12 mL) and acetic anhydride (12 mL) at 23 °C. The reaction flask was fitted with a

[6] For a study of the effects of substitution of the polymethine chain on the optical properties of Cy5 dyes, see: Mader, O.; Reiner, K.; Egelhaaf, H.-J.; Fischer, R.; Brock, R. *Bioconjugate Chem.* **2004**, *15,* 70–78.

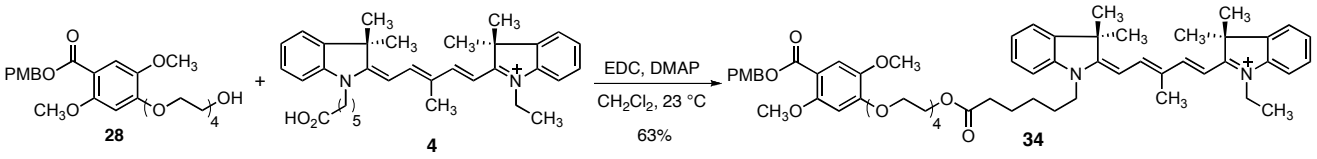
reflux condenser and the reaction assembly was placed in an oil bath at 130 °C. After 5 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The solution was concentrated at 60 °C. The residue was purified by flash-column chromatography (5% methanol–dichloromethane initially, grading to 1:15 methanol–dichloromethane, then grading to 1:8 methanol–dichloromethane) to provide the hemicyanines **33** as a green solid. Hemicyanines **33** were isolated as a mixture of at least three different forms (*E/Z* isomers, R = H or Ac). To obtain the best quality of the final product (the indocyanine dye **4**), hemicyanines **33** were isolated in two separate batches, fractions A and B (see TLC below).




Thus, fractions A were combined to provide 320 mg of hemicyanines **33** as a green solid. TLC: (1:1:7 methanol–hexanes–dichloromethane) $R_f$ = 0.58 (visible without staining, yellow). Fractions B were combined to provide 410 mg of hemicyanines **33** as a green solid. TLC: (1:1:7 methanol–hexanes–dichloromethane) $R_f$ = 0.46 (visible without staining, orange), $R_f$ = 0.25 (visible without staining, yellow).

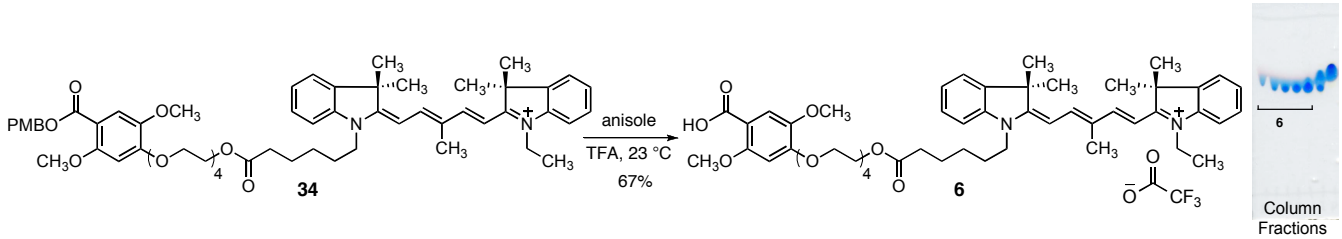
*Second step*: 1-(5-Carboxypentyl)-2,3,3-trimethyl-3*H*-indolium iodide **32** (313 mg, 0.78 mmol, 1 equiv) was added to a solution of hemicyanines **33** obtained from fractions A (319 mg, ~ 0.78 mmol, 1 equiv) in a mixture of acetic anhydride (1.0 mL) and pyridine (5 mL) at 23 °C. The reaction flask was heated in an oil bath at 50 °C. After 2 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The solution was concentrated. The residue was purified by flash-column chromatography (5% methanol–dichloromethane initially, grading to 1:8 methanol–dichloromethane) to provide 255 mg of indocyanine dye **4** as a blue solid. In a separate flask, 1-(5-Carboxypentyl)-2,3,3-trimethyl-3*H*-indolium iodide **32** (392 mg, 0.98 mmol, 1 equiv) was added to a solution of hemicyanines **33** obtained from fractions B (400 mg, ~ 0.98 mmol, 1 equiv) in a mixture of acetic anhydride (1.2 mL) and pyridine (6 mL) at 23 °C. The reaction flask was heated in an oil bath at 50 °C. After 2 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The solution was concentrated. The residue was purified by flash-column chromatography (5% methanol–dichloromethane initially, grading to 1:8 methanol–dichloromethane) to provide 523 mg of indocyanine dye **4** as a blue solid (46% combined yield). TLC: (1:1:7 methanol–hexanes–dichloromethane) $R_f$ = 0.40 (visible without staining, blue). $^1$H NMR (500 MHz,

DMSO-$d_6$) δ: 8.18 (app d, 2H, $J$ = 14.2 Hz), 7.63–7.61 (m, 2H), 7.40–7.38 (m, 4H), 7.26–7.21 (m, 2H), 6.18 (app d, 2H, $J$ = 13.9 Hz), 4.24–4.14 (m, 4H), 2.18 (t, 2H, $J$ = 7.3 Hz), 2.05 (s, 3H), 1.74–1.70 (m, 2H), 1.69 (app s, 12H), 1.57–1.52 (m, 2H), 1.41–1.36 (m, 2H), 1.28 (t, 3H, $J$ = 7.1 Hz). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 175.0, 173.3, 172.9, 154.6, 154.4, 150.3, 142.8, 142.3, 142.0, 141.8, 129.1, 125.5, 125.4, 124.6, 123.2, 123.1, 111.8, 111.6, 100.7, 100.3, 49.7, 49.6, 43.9, 39.2, 34.2, 27.7, 27.6, 27.2, 26.4, 24.9, 12.7, 11.7. FTIR (neat), cm$^{-1}$: 1711 (w), 1595 (w), 1479 (s), 1452 (s), 1370 (m). HRMS (ESI): calcd for $(C_{34}H_{43}N_2O_2)^+$ 511.3319, found 511.3323. UV/vis (DMSO) $\lambda_{abs}$ = 646 nm, $\lambda_{em}$ = 666 nm, ε = 240000 M$^{-1}$cm$^{-1}$.

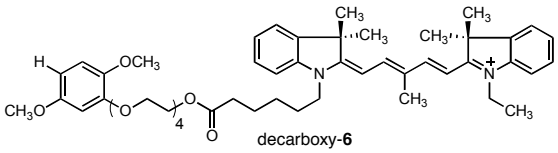


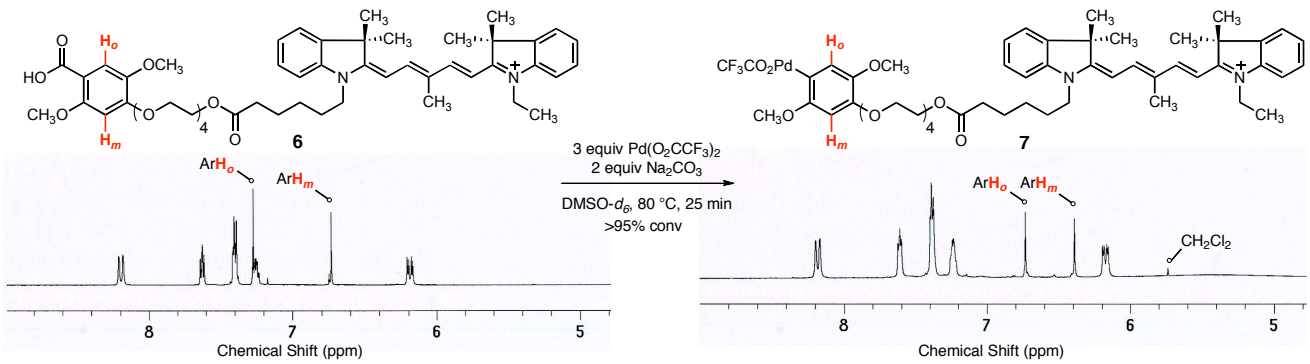

Benzyl ester **34**. *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (166 mg, 0.86 mmol, 1.2 equiv) and 4-dimethylaminopyridine (88.0 mg, 0.72 mmol, 1.0 equiv) were added to a solution of alcohol **28** (356 mg, 0.72 mmol, 1.0 equiv) and indocyanine dye **4** (473 mg, 0.86 mmol, 1.2 equiv) in dichloromethane (8.0 mL) at 23 °C. After 30 h, the solution was concentrated. The residue was purified by flash-column chromatography (3% methanol–dichloromethane initially, grading to 4% methanol–dichloromethane) to provide the benzyl ester **34** as a blue solid, contaminated with small amount of unreacted alcohol **28**. To remove the alcohol, the solid residue was mixed with ethyl acetate (8 mL). The resulting mixture was heated in an oil bath at 60 °C. After 15 min, the heating bath was removed and hexane was added (7 mL). The mixture was again heated in an oil bath at 60 °C. After 20 min, the heating bath was removed and the mixture was allowed to cool to 23 °C. After 10 min, the mixture was cooled to –20 °C. After 1 h, the ethyl acetate–hexanes supernatant containing alcohol **28** was decanted. The solid residue was dried in vacuo (0.1 mmHg) to provide 464 mg of the benzyl ester **34** as a blue solid (63%). TLC: (3% methanol–dichloromethane) $R_f$ = 0.12 (visible without staining, blue). $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.91 (d, 1H, $J$ = 14.2 Hz), 7.90 (d, 1H, $J$ = 14.2 Hz), 7.43–7.37 (m, 6H), 7.41 (s, 1H), 7.29–7.23 (m, 2H), 7.16 (d, 1H, $J$ = 7.8 Hz), 7.12 (d, 1H, $J$ = 7.8 Hz), 6.91 (d, 2H, $J$ = 8.8 Hz), 6.62 (s, 1H), 6.19 (d, 1H, $J$ = 14.2 Hz), 6.14 (d, 1H, $J$ = 14.2 Hz), 5.27 (s, 2H), 4.30–4.21 (m, 6H), 4.14 (t, 2H, $J$ = 7.3 Hz), 3.91 (t, 2H, $J$ = 4.9 Hz), 3.87 (s, 3H), 3.82 (s, 3H), 3.82 (s, 3H), 3.74–3.64 (m, 10H), 2.39 (t, 2H, $J$ = 7.1 Hz), 2.18 (s, 3H), 1.91–1.84 (m, 2H), 1.79 (app s, 12H), 1.77–1.71 (m, 2H), 1.56–1.51 (m, 2H), 1.48 (t, 3H, $J$ = 7.3 Hz). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 173.4, 173.3, 172.9, 165.5, 159.7, 155.6, 154.4, 153.5, 142.8, 142.7, 142.2, 142.0, 141.8, 130.2, 129.3, 129.2, 125.5, 125.4, 123.6, 123.2, 123.1, 119.6, 115.2, 114.5, 111.8, 111.6, 110.8, 100.6, 100.3, 70.6, 70.5, 70.4, 69.5, 69.0, 68.8, 66.0, 63.8, 57.4, 56.8, 55.8, 49.7, 49.6, 43.8, 39.2, 33.9, 27.7, 27.6, 27.1, 26.2,

24.8, 24.7, 12.7, 11.7. FTIR (neat), $cm^{-1}$: 2932 (m), 1724 (m), 1690 (m), 1611 (m), 1479 (s), 1447 (s). HRMS (ESI): calcd for $(C_{59}H_{75}N_2O_{11})^+$ 987.5365, found 987.5377.




Carboxylic acid **6**. A solution of anisole (247 μL, 2.26 mmol, 6.0 equiv) in trifluoroacetic acid (2 mL) was added dropwise to a solution of benzyl ester **34** (386 mg, 0.38 mmol, 1.0 equiv) in trifluoroacetic acid (3 mL) at 23 °C. After 20 min, toluene (10 mL) was added. The reaction mixture was concentrated. The residue was immediately purified by flash-column chromatography (5% methanol–dichloromethane initially, grading to 12% methanol–dichloromethane) to provide 250 mg of the carboxylic acid **6** as a blue solid (67%). The decarboxylated compound (decarboxy-**6**) was observed as a minor component in this reaction (a less polar compound shown on TLC; see below for the structure). TLC: (1:5 methanol–dichloromethane) $R_f$ = 0.60 (visible without staining, blue). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 8.20 (d, 1H, $J$ = 14.2 Hz), 8.19 (d, 1H, $J$ = 14.2 Hz), 7.63 (app t, 2H, $J$ = 6.4 Hz), 7.42–7.38 (m, 4H), 7.27 (s, 1H), 7.28–7.22 (m, 2H), 6.73 (s, 1H), 6.19 (d, 1H, $J$ = 14.2 Hz), 6.18 (d, 1H, $J$ = 14.2 Hz), 4.24–4.15 (m, 6H), 4.08 (t, 2H, $J$ = 4.6 Hz), 3.79 (s, 3H), 3.75–3.73 (m, 2H), 3.71 (s, 3H), 3.59–3.47 (m, 10H), 2.30 (t, 2H, $J$ = 7.1 Hz), 2.06 (s, 3H), 1.75–1.71 (m, 2H), 1.70 (app s, 12H), 1.61–1.54 (m, 2H), 1.41–1.34 (m, 2H), 1.29 (t, 3H, $J$ = 7.1 Hz). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 173.4, 173.3, 172.9, 167.1, 155.4, 154.4, 153.1, 142.8, 142.7, 142.3, 142.0, 141.8, 129.6, 129.1, 128.9, 126.0, 125.5, 125.4, 123.2, 123.1, 115.3, 111.8, 111.6, 100.6, 100.3, 100.2, 70.6, 70.5, 70.4, 69.5, 69.0, 68.8, 63.8, 57.3, 56.7, 49.7, 49.6, 43.8, 39.2, 33.9, 27.7, 27.6, 27.1, 26.2, 24.8, 12.6, 11.7. FTIR (neat), $cm^{-1}$: 2932 (m), 1726 (m), 1688 (m), 1611 (w), 1478 (s), 1447 (s). $^{19}$F NMR (375 MHz, DMSO-$d_6$) δ: –73.8. HRMS (ESI): calcd for $(C_{51}H_{67}N_2O_{10})^+$ 867.4790, found 867.4774.

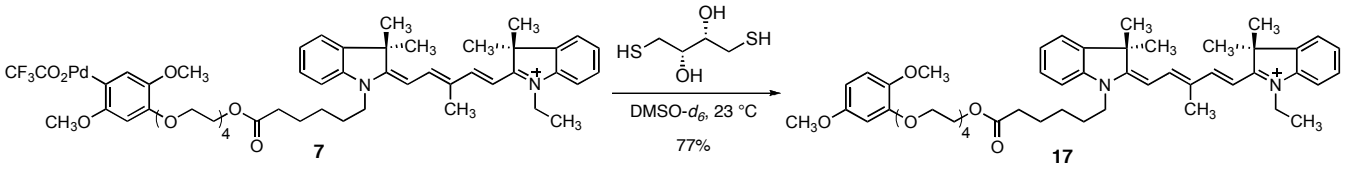





Arylpalladium(II) reagent **7**. A solution of palladium(II) trifluoroacetate (45.7 mg, 0.138 mmol, 3.0 equiv) in DMSO-$d_6$ (1.0 mL) was added to a solution of carboxylic acid **6** (45.0 mg, 0.046 mmol, 1.0 equiv) and sodium carbonate (9.72 mg, 0.092 mmol, 2.0 equiv) in DMSO-$d_6$ (2 mL) at 23 °C. The reaction flask was heated in an oil bath at 80 °C. After 25 min, the heating bath was removed and the solution was allowed to cool to 23 °C. $^1$H, $^{13}$C, and $^{19}$F NMR spectroscopic analysis showed evidence of a single species fully consistent with the assigned structure **7** (>95% conversion, estimated to be 15.3 mM). The product solution, stored frozen at –20 °C, provided a reliable stock of arylpalladium(II) reagent **7** over several months, thawing just prior to use when needed. The efficiency of formation of the arylpalladium(II) reagent **7** was estimated to be >90% based on the isolated yield of the Heck product with *N*-ethyl-4-vinylbenzamide (see the next section). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 8.18 (app d, 2H, $J$ = 14.2 Hz), 7.63–7.60 (m, 2H), 7.41–7.37 (m, 4H), 7.26–7.21 (m, 2H), 6.73 (s, 1H), 6.39 (s, 1H), 6.18 (d, 1H, $J$ = 14.2 Hz), 6.17 (d, 1H, $J$ = 14.2 Hz), 4.24–4.13 (m, 4H), 4.06 (t, 2H, $J$ = 4.6 Hz), 4.01 (t, 2H, $J$ = 4.4 Hz), 3.78 (s, 3H), 3.69–3.65 (m, 2H), 3.67 (s, 3H), 3.56–3.46 (m, 10H), 2.29 (t, 2H, $J$ = 7.3 Hz), 2.05 (s, 3H), 1.74–1.69 (m, 2H), 1.69 (app s, 12H), 1.60–1.54 (m, 2H), 1.41–1.34 (m, 2H), 1.27 (t, 3H, $J$ = 7.1 Hz). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 173.4, 173.3, 173.0, 161–159 (br $CF_3\mathbf{C}O_2$), 155.5, 154.6, 154.4, 147.4, 143.1, 142.7, 142.3, 142.0, 141.8, 129.1, 129.1, 128.9, 125.5, 125.4, 123.2, 123.1, 119.9, 115.9, 111.8, 111.6, 100.6, 100.3, 99.9, 70.6, 70.5, 70.4, 69.8 69.0, 68.9, 63.8, 57.2, 56.8, 49.7, 49.6, 43.8, 39.2, 33.9, 27.7, 27.6, 27.1, 26.2, 24.8, 12.6, 11.7. $^{19}$F NMR (375 MHz, DMSO-$d_6$) δ: –74.4.

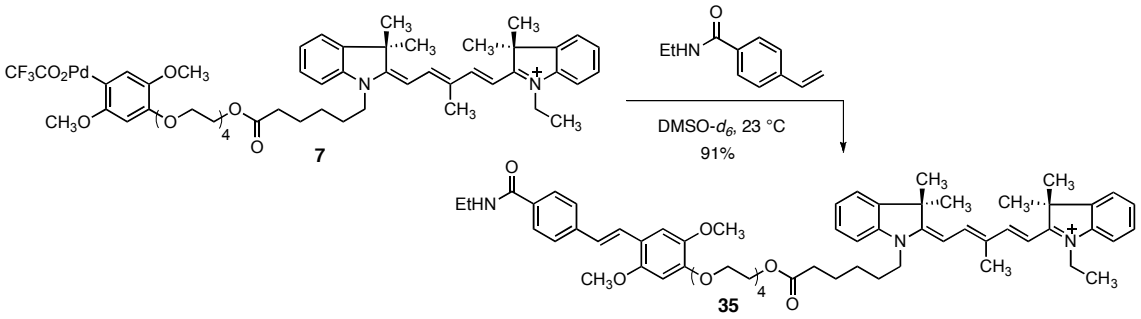
Note: Decarboxylative palladation of **6** was also found to be efficient without the use of sodium carbonate. Thus, heating a mixture of palladium(II) trifluoroacetate (45.7 mg, 0.138 mmol, 3.0 equiv) and carboxylic acid **6** (45.0 mg, 0.046 mmol, 1.0 equiv) in DMSO-$d_6$ (3 mL) at 80 °C for 25 min provided the arylpalladium(II) reagent **7** (>95% conversion, estimated to be 15.3 mM). Solutions of the arylpalladium(II) reagent as concentrated as 28.5 mM have been prepared by this alternative

protocol. However, the use of sodium carbonate was crucial to maintain a shelf-life for reagent **7**. Reagent **7** obtained in the presence of sodium carbonate displayed no decomposition by $^1$H NMR after storing for three months at –20 °C. In contrast, reagent **7** prepared in the absence of sodium carbonate had undergone partial protiodepalladation (30%) after storing for three months at –20 °C.



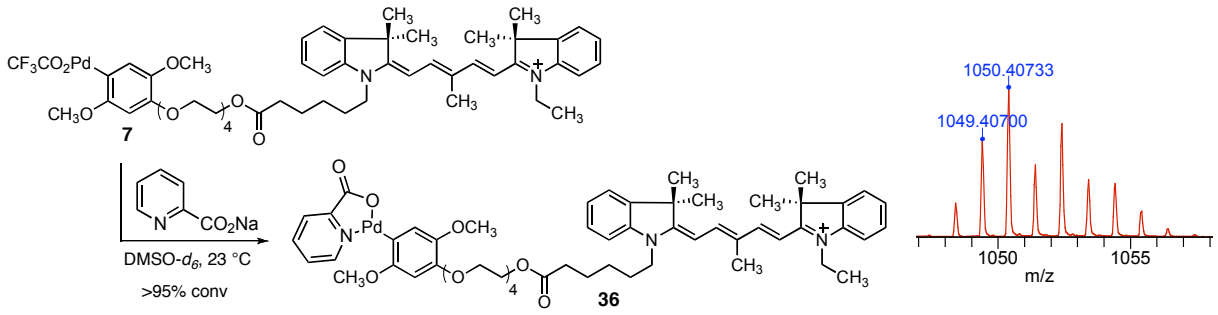

Protiodepalladation product **17**. Solid DL-dithiothreitol (8.1 mg, 52.5 μmol, 5.0 equiv) was added to a solution of the arylpalladium(II) reagent **7** in DMSO-$d_6$ (0.75 mL of 14.0 mM solution, 10.5 μmol, 1 equiv) in a 20-mL scintillation vial at 23 °C. After 30 min, the reaction mixture was diluted with dichloromethane (15 mL). The organic layer was washed sequentially with water (15 mL) and saturated aqueous sodium chloride solution (15 mL). The washed solution was dried over anhydrous sodium sulfate. The dried solution was filtered through a pad of Celite and the filtrate was concentrated. The residue was purified by flash-column chromatography (5% methanol–dichloromethane initially, grading to 9% methanol–dichloromethane) to provide 7.1 mg of the protiodepalladation product **17** as a blue solid (77%). TLC: (1:1:7 methanol–hexanes–dichloromethane) $R_f$ = 0.46 (visible without staining, blue). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.17 (d, 1H, $J$ = 13.7 Hz), 8.15 (d, 1H, $J$ = 13.7 Hz), 7.41–7.33 (m, 4H), 7.25–7.19 (m, 2H), 7.11 (d, 1H, $J$ = 8.3 Hz), 7.07 (d, 1H, $J$ = 7.8 Hz), 6.79 (1, 2H, $J$ = 8.8 Hz), 6.54 (d, 1H, $J$ = 2.4 Hz), 6.41 (dd, 1H, $J$ = 2.6, 8.8 Hz), 6.12 (d, 1H, $J$ = 13.7 Hz), 6.08 (d, 1H, $J$ = 14.1 Hz), 4.23–4.13 (m, 6H), 4.06 (t, 2H, $J$ = 7.3 Hz), 3.88–3.85 (m, 2H), 3.80 (s, 3H), 3.75 (s, 3H), 3.73–3.63 (m, 10H), 2.37 (t, 2H, $J$ = 7.3 Hz), 2.09 (s, 3H), 1.87–1.83 (m, 2H), 1.78 (app s, 12H), 1.75–1.70 (m, 2H), 1.54–1.48 (m, 2H), 1.45 (t, 3H, $J$ = 7.3 Hz). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 173.4, 173.3, 173.0, 154.5, 154.4, 149.6, 143.9, 142.7, 142.3, 142.0, 141.8, 129.1, 125.4, 123.2, 123.1, 113.9, 111.8, 111.6, 106.8, 104.5, 102.3, 100.6, 100.3, 70.6, 70.5, 70.4, 69.6, 69.0, 68.6, 63.8, 56.9, 56.0, 49.7, 49.6, 43.8, 39.2, 33.9, 27.7, 27.6, 27.1, 26.2, 24.8, 12.7, 11.7. FTIR (neat), cm$^{-1}$: 1713 (m), 1640 (m), 1601 (m), 1483 (s), 1451 (s). HRMS (ESI): calcd for $(C_{50}H_{67}N_2O_8 + Na)^{2+}$ 423.2392, found 423.2196.

**Heck Reaction of Arylpalladium(II) Reagent 7 with *N*-Ethyl-4-vinylbenzamide.**



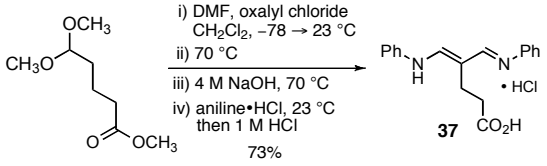

Heck product **35**. *N*-Ethyl-4-vinylbenzamide (3.7 mg, 20.9 μmol, 1.5 equiv) was added to a solution of the arylpalladium(II) reagent **7** in DMSO-$d_6$ (1.00 mL of 13.9 mM solution, 13.9 μmol, 1 equiv) in a 20-mL scintillation vial at 23 °C. After 2 h, the reaction solution was partitioned between water (15 mL) and dichloromethane (20 mL). The organic layer was washed with brine (15 mL) and the washed solution was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (2% methanol–dichloromethane initially, grading to 5% methanol–dichloromethane, then grading to 11% methanol–dichloromethane) to provide 13.0 mg of the Heck product **35** as a blue solid (91%). TLC: (1:1:7 methanol–hexanes–dichloromethane) $R_f$ = 0.48 (visible without staining, blue). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.04–7.96 (m, 2H), 7.89 (d, 2H, *J* = 8.3 Hz), 7.52 (d, 2H, *J* = 8.3 Hz), 7.46 (d, 1H, *J* = 16.2 Hz), 7.40–7.33 (m, 4H), 7.25–7.19 (m, 2H), 7.13 (d, 1H, *J* = 7.8 Hz), 7.09 (s, 1H), 7.07 (d, 1H, *J* = 8.3 Hz), 6.95 (d, 2H, *J* = 16.3 Hz), 6.61 (s, 1H), 6.11 (d, 1H, *J* = 13.2 Hz), 6.05 (d, 1H, *J* = 13.7 Hz), 4.24–4.16 (m, 6H), 4.05–4.01 (m, 2H), 3.88 (t, 2H, *J* = 5.4 Hz), 3.86 (s, 3H), 3.84 (s, 3H), 3.74–3.62 (m, 10H), 3.52 (q, 2H, *J* = 7.3 Hz), 2.34 (t, 2H, *J* = 7.3 Hz), 2.16 (s, 3H), 1.84–1.65 (m, 16H), 1.48–1.42 (m, 5H), 1.27 (t, 3H, *J* = 7.3 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 173.5, 173.2, 167.4, 154.9, 154.5, 152.2, 149.7, 144.2, 142.2, 141.8, 141.6, 141.4, 141.2, 133.0, 128.9, 128.8, 127.8, 127.5, 126.4, 126.2, 125.6, 125.4, 125.0, 122.7, 118.7, 110.7, 110.6, 100.3, 100.0, 71.1, 70.9, 70.9, 70.8, 70.1, 69.4, 69.3, 63.8, 57.1, 56.8, 49.8, 49.7, 44.5, 39.9, 35.2, 34.0, 28.4, 28.3, 27.2, 26.7, 24.7, 15.2, 12.5, 12.1. FTIR (neat), cm$^{-1}$: 2928 (m), 1732 (m), 1643 (m), 1601 (m), 1479 (s), 1451 (s). HRMS (ESI): calcd for $(C_{61}H_{78}N_3O_9)^+$ 996.5733, found 996.5751.

**Ligand Exchange of Arylpalladium(II) Reagent 7 with Sodium Picolinate.**




It was found that high-resolution mass spectra of indocyanine dye-linked arylpalladium(II) complexes can be obtained after ligand exchange with sodium picolinate: A solution of the arylpalladium(II) reagent **7** in DMSO-$d_6$ (0.5 mL 15.3 mM, 7.5 μmol, 1.0 equiv) was added to a solution of sodium picolinate monohydrate (3.7 mg, 22.5 μmol, 3.0 equiv) in DMSO-$d_6$ (0.5 mL) in a 20-mL scintillation vial at 23 °C. After 1 h, the reaction mixture was filtered through a plug of cotton (using a disposable glass pipette) to remove solids. $^1$H NMR analysis of the resulting solution showed full conversion to a new palladium complex consistent with the assigned structure **36** as well as a small amount of unbound picolinate. Arylpalladium(II) complex **36** was diluted with acetonitrile prior to mass spectroscopic analysis. $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 8.22–8.15 (m, 3H), 8.01 (d, 1H, $J$ = 7.8 Hz), 7.64–7.61 (m, 3H), 7.43–7.37 (m, 5H), 7.27–7.22 (m, 2H), 6.90 (s, 1H), 6.54 (s, 1H), 6.20 (d, 1H, $J$ = 14.2 Hz), 6.18 (d, 1H, $J$ = 14.2 Hz), 4.25–4.14 (m, 4H), 4.10–4.06 (m, 4H), 3.74–3.69 (m, 2H), 3.70 (s, 3H), 3.68 (s, 3H), 3.60–3.48 (m, 10H), 2.30 (t, 2H, $J$ = 7.3 Hz), 2.06 (s, 3H), 1.76–1.70 (m, 2H), 1.70 (app s, 12H), 1.61–1.56 (m, 2H), 1.42–1.36 (m, 2H), 1.29 (t, 3H, $J$ = 7.1 Hz). HRMS (ESI): calcd for $(C_{56}H_{70}N_3O_{10}Pd)^+$ 1050.4091, found 1050.4073; calcd for $(C_{56}H_{70}N_3O_{10}Pd + H)^{2+}$ 525.7085, found 525.7090; calcd for $(C_{56}H_{70}N_3O_{10}Pd + Na)^{2+}$ 536.6995, found 536.7009.

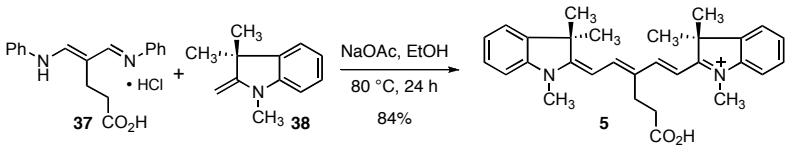
**Synthesis of the Symmetrical Indocyanine-Linked Arylpalladium(II) Reagent 9.**




(4*E*)-5-(phenylamino)-4-((phenylimino)methyl)pent-4-enoic acid hydrochloride (**37**). (4*E*)-5-(phenylamino)-4-((phenylimino)methyl)pent-4-enoic acid **37** was prepared according to the method developed by Shao et al.[7] Oxalyl chloride (0.44 mL, 5.0 mmol, 2.0 equiv) was added to a solution of *N,N*-dimethylformamide (0.51 mL, 6.5 mmol, 2.6 equiv) in

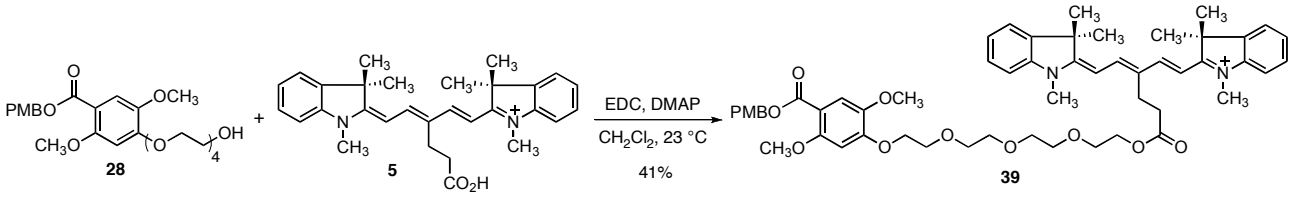
[7] Shao, F.;Weissleder, R.; Hilderbrand, S. A. *Bioconjugate Chem.* **2008**, *19*, 2487–2491.

dichloromethane (15 mL) at −78 °C (dry ice-acetone bath). After 5 min, the cooling bath was removed and the reaction mixture was allowed to warm to 23 °C over a period of 25 min. Methyl 5,5-dimethoxypentanoate (0.44 mL, 2.5 mmol, 1.0 equiv) was added dropwise to the reaction mixture at 23 °C. The reaction flask was heated in an oil bath at 70 °C (The use of a Dean-Stark trap to collect evaporating dichloromethane is recommended). After 2 h, the resulting yellow oil was dissolved in 4 M aqueous sodium hydroxide solution (2.5 mL) and the resulting solution was heated at 70 °C. After 1 h, the heating bath was removed and the reaction flask was allowed to cool to 23 °C. A solution of aniline hydrochloride (648 mg, 5.0 mmol, 2.0 equiv) in water (2.5 mL) was added slowly at 23 °C. After 15 h, 1 M aqueous hydrochloric acid solution (5 mL) was added at 23 °C. After 20 min, the yellow solids were collected on a sintered-glass funnel. The solids were dried in vacuo (0.1 mmHg) to provide 610 mg of (4*E*)-5-(phenylamino)-4-((phenylimino)methyl)pent-4-enoic acid hydrochloride (**37**) as a yellow solid (73%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 11.37 (d, 1H, $J$ = 14.2 Hz), 8.72 (d, 2H, $J$ = 14.6 Hz), 7.53 (d, 4H, $J$ = 8.3 Hz), 7.51–7.47 (m, 4H), 7.28 (t, 2H, $J$ = 7.1 Hz), 2.89 (t, 2H, $J$ = 7.8 Hz), 2.45 (t, 2H, $J$ = 7.8 Hz). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 174.5, 159.6, 140.0, 130.4, 126.7, 119.2, 110.6, 32.5, 18.1. FTIR (neat), cm$^{-1}$: 2953 (m), 1724 (w), 1614 (m), 1573 (s), 1487 (m). HRMS (ESI): calcd for $(C_{18}H_{18}N_2O_2 + H)^+$ 295.1441, found 295.1447.

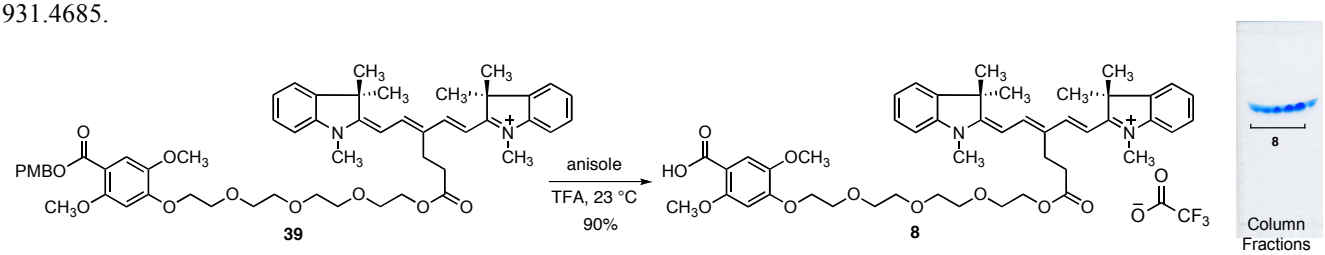



Indocyanine dye **5**. 1,3,3-trimethyl-2-methyleneindoline **38** (0.32 mL, 1.82 mmol, 2.0 equiv) followed by sodium acetate (186 mg, 2.27 mmol, 2.5 equiv) were added to a solution of (4*E*)-5-(phenylamino)-4-((phenylimino)methyl)pent-4-enoic acid hydrochloride **37** (300 mg, 0.91 mmol, 1.0 equiv) in anhydrous ethanol (17 mL) at 23 °C. The reaction flask was fitted with a reflux condenser and the reaction assembly was placed in an oil bath (bath temperature 85 °C). After 24 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The solution was concentrated. The residue was purified by flash-column chromatography (3% methanol–dichloromethane initially, grading to 1:8 methanol–dichloromethane followed by 1:5:0.5 methanol–dichloromethane–acetic acid). The product obtained by concentration of appropriate fractions from flash-column chromatography was azeotropically dried with toluene. The resulting solids were dried in vacuo (0.1 mmHg) to provide 374 mg of the indocyanine dye **5** as a blue solid (84%). TLC: (1:5 methanol–dichloromethane) $R_f$ = 0.59 (visible without staining, blue). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 8.06 (d, 2H, $J$ = 14.2 Hz), 7.58 (d, 2H, $J$ = 6.8 Hz), 7.40–7.34 (m, 4H), 7.24–7.18 (m, 2H), 6.36 (d, 2H, $J$ = 14.2 Hz), 3.62 (s, 6H), 2.83–2.76 (m, 2H), 2.18–2.12 (m, 2H), 1.67 (s, 12H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 173.7, 153.6, 143.5, 141.7, 129.0, 128.9, 125.2, 122.9, 111.6, 100.9, 49.4, 31.8,

27.7, 24.4. FTIR (neat), $cm^{-1}$: 2969 (w), 1709 (w), 1562 (m), 1539 (m), 1495 (s), 1479 (s), 1452 (s). HRMS (ESI): calcd for $(C_{30}H_{35}N_2O_2)^+$ 455.2693, found 455.2700. UV/vis (DMSO) $\lambda_{abs}$ = 642 nm, $\lambda_{em}$ = 660 nm.

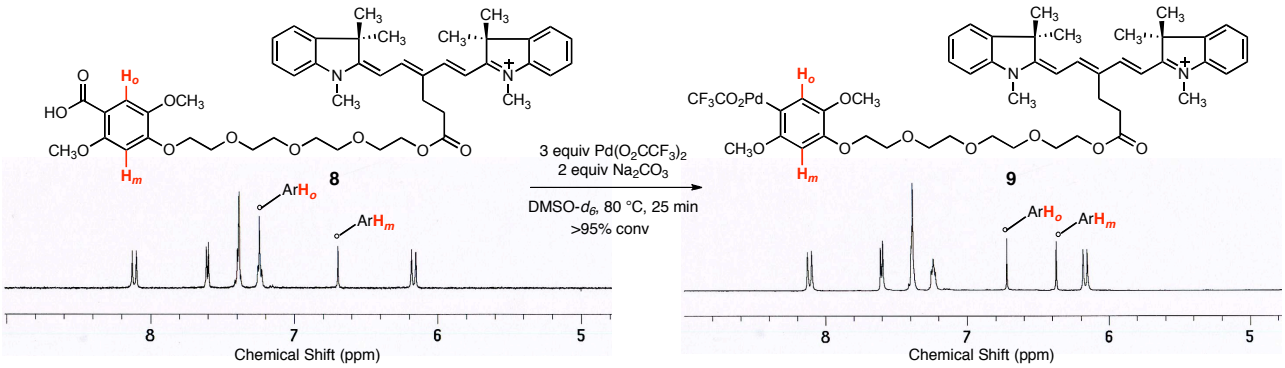



Benzyl ester **39**. *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (55.4 mg, 0.29 mmol, 1.2 equiv) followed by 4-dimethylaminopyridine (29.5 mg, 0.24 mmol, 1.0 equiv) were added to a mixture of alcohol **28** (119 mg, 0.24 mmol, 1.0 equiv) and indocyanine dye **5** (130 mg, 0.27 mmol, 1.1 equiv) in dichloromethane (3.5 mL) at 23 °C. After 30 h, the mixture was directly purified by flash-column chromatography (5% methanol–dichloromethane initially, grading to 8% methanol–dichloromethane) to provide 95.0 mg of the benzyl ester **39** as a blue solid (41%). TLC: (1:5 methanol–dichloromethane) $R_f$ = 0.57 (visible without staining, blue). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 8.13 (d, 2H, $J$ = 14.2 Hz), 7.61 (d, 2H, $J$ = 7.3 Hz), 7.43–7.35 (m, 6H), 7.26–7.24 (m, 2H), 7.23 (s, 1H), 6.94 (d, 2H, $J$ = 8.8 Hz), 6.73 (s, 1H), 6.18 (d, 2H, $J$ = 14.6 Hz), 5.17 (s, 2H), 4.18 (t, 2H, $J$ = 4.4 Hz), 4.14 (t, 2H, $J$ = 4.4 Hz), 3.77 (s, 3H), 3.75 (s, 3H), 3.75–3.72 (m, 2H), 3.68 (s, 3H), 3.64 (s, 6H), 3.60–3.54 (m, 4H), 3.53–3.48 (m, 6H), 2.91 (t, 2H, $J$ = 7.3 Hz), 2.52 (t, 2H, $J$ = 7.8 Hz), 1.69 (s, 12H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 174.2, 173.4, 165.5, 159.7, 155.6, 153.5, 143.4, 142.8, 141.8, 130.2, 129.2, 129.1, 125.5, 123.0, 115.2, 114.5, 111.7, 110.8, 100.3, 100.2, 70.6, 70.5, 70.4, 69.5, 69.0, 68.8, 66.0, 64.3, 57.4, 56.8, 55.8, 49.6, 32.9, 31.8, 27.6. FTIR (neat), $cm^{-1}$: 2931 (w), 1667 (m), 1501 (s), 1483 (s), 1464 (s). HRMS (ESI): calcd for $(C_{55}H_{67}N_2O_{11})^+$ 931.4739, found 931.4685.




Carboxylic acid **8**. Anisole (100 μL, 0.47 mmol, 6.0 equiv) was added to a solution of benzyl ester **39** (75.5 mg, 0.078 mmol, 1.0 equiv) in trifluoroacetic acid (1.5 mL) dropwise at 23 °C. After 20 min, toluene (4 mL) was added. The reaction mixture was concentrated. The residue was immediately purified by flash-column chromatography (5% methanol–dichloromethane initially, grading to 1:7 methanol–dichloromethane) to provide 65.2 mg of the carboxylic acid **8** as a blue solid (90%). TLC: (1:5 methanol–dichloromethane) $R_f$ = 0.58 (visible without staining, blue). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 8.12 (d, 2H, $J$

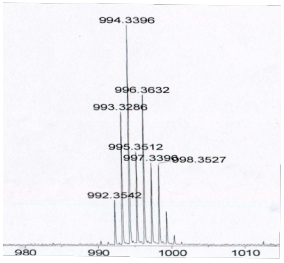
= 14.7 Hz), 7.60 (d, 2H, $J$ = 7.3 Hz), 7.42–7.37 (m, 4H), 7.26–7.22 (m, 2H), 7.24 (s, 1H), 6.70 (s, 1H), 6.17 (d, 2H, $J$ = 14.7 Hz), 4.17–4.11 (m, 4H), 3.77 (s, 3H), 3.69–3.73 (m, 2H), 3.68 (s, 3H), 3.63 (s, 6H), 3.60–3.53 (m, 4H), 3.52–3.47 (m, 6H), 2.89 (t, 2H, $J$ = 7.1 Hz), 2.51 (t, 2H, $J$ = 7.3 Hz), 1.68 (s, 12H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 174.2, 173.4, 155.3, 153.7, 153.0, 143.4, 142.8, 141.8, 129.0, 125.5, 123.0, 115.4, 111.8, 100.3, 70.6, 70.5, 70.5, 69.5, 69.0, 68.8, 64.3, 57.3, 56.7, 49.6, 33.0, 31.9, 27.6. FTIR (neat), cm$^{-1}$: 2932 (w), 1724 (m), 1690 (m), 1495 (s), 1479 (s), 1451 (s). $^{19}$F NMR (375 MHz, DMSO-$d_6$) δ: –73.9. HRMS (ESI): calcd for $(C_{47}H_{59}N_2O_{10})^+$ 811.4164, found 811.3862.



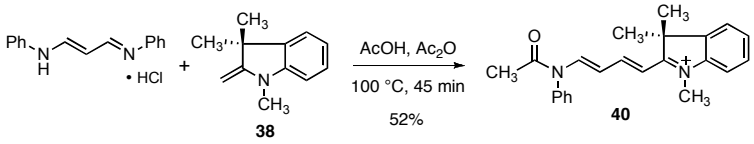

Arylpalladium(II) reagent **9**. A solution of palladium(II) trifluoroacetate (16.8 mg, 0.050 mmol, 3.0 equiv) in DMSO-$d_6$ (0.5 mL) was added to a solution of carboxylic acid **8** (15.5 mg, 0.017 mmol, 1.0 equiv) and sodium carbonate (3.56 mg, 0.034 mmol, 2.0 equiv) in DMSO-$d_6$ (0.6 mL) at 23 °C. The reaction flask was heated in an oil bath at 80 °C. After 25 min, the heating bath was removed and the product solution was allowed to cool to 23 °C. $^1$H, $^{13}$C, and $^{19}$F NMR spectroscopic analysis showed evidence of a single species fully consistent with the assigned structure **9** (>95% conversion, estimated to be 15.3 mM). The product solution, stored frozen at –20 °C, provided a reliable stock of arylpalladium(II) reagent **9** over several months, thawing just prior to use when needed. The efficiency of formation of the arylpalladium(II) reagent **9** was estimated to be >90% based on the isolated yield of the Heck product with *N*-ethyl-4-vinylbenzamide. $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 8.13 (d, 2H, $J$ = 14.2 Hz), 7.62 (d, 2H, $J$ = 7.3 Hz), 7.42–7.37 (m, 4H), 7.27–7.23 (m, 2H), 6.74 (s, 1H), 6.39 (s, 1H), 6.19 (d, 2H, $J$ = 14.6 Hz), 4.15 (t, 2H, $J$ = 4.6 Hz), 4.01 (t, 2H, $J$ = 4.6 Hz), 3.79 (s, 3H), 3.67 (s, 3H), 3.65 (s, 6H), 3.62–3.57 (m, 2H), 3.55–3.48 (m, 10H), 2.91 (t, 2H, $J$ = 7.3 Hz), 2.53 (t, 2H, $J$ = 7.3 Hz), 1.70 (s, 12H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 174.2, 173.4, 161–159 (br $CF_3CO_2$), 155.4, 153.7, 147.4, 143.4, 143.1, 141.8, 129.0, 125.5, 123.0, 119.9, 115.9, 111.7, 100.3, 99.9, 70.6, 70.5, 70.5, 70.4, 69.8, 69.0, 68.9, 64.2, 57.2, 56.8, 49.6, 32.9, 31.8, 27.6. $^{19}$F NMR (375 MHz, DMSO-$d_6$) δ: –74.2.

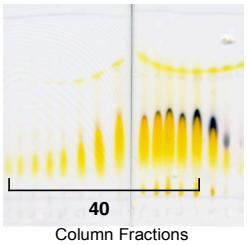
Note: Decarboxylative palladation of **8** was also found to be efficient without the use of sodium carbonate. Thus, heating a mixture of palladium(II) trifluoroacetate (16.8 mg, 0.050 mmol, 3.0 equiv) and carboxylic acid **8** (15.5 mg, 0.017 mmol, 1.0 equiv) in DMSO-$d_6$ (1.1 mL) at 80 °C for 25 min provided the arylpalladium(II) reagent **9** (>95% conversion, estimated to be 15.2 mM). Solutions of the arylpalladium(II) reagent as concentrated as 28.5 mM have been prepared by this alternative protocol. However, the use of sodium carbonate was crucial to maintain a shelf-life for reagent **9**. Reagent **9** obtained in the presence of sodium carbonate displayed no decomposition by $^1$H NMR after storing for three months at –20 °C. In contrast, reagent **9** prepared in the absence of sodium carbonate had undergone partial protiodepalladation (~35%) after storing for three months at –20 °C.

HRMS: It was found that high-resolution mass spectra of indocyanine dye-linked arylpalladium(II) complexes can be obtained after ligand exchange with sodium picolinate. A solution of the arylpalladium(II) reagent **9** in DMSO-$d_6$ (0.1 mL 15.2 mM, 1.5 μmol, 1.0 equiv) was added to a solution of sodium picolinate monohydrate (0.8 mg, 4.5 μmol, 3.0 equiv) in DMSO-$d_6$ (1.0 mL) in a 20-mL scintillation vial at 23 °C. After 1 h, the reaction mixture was filtered through a plug of cotton (using a disposable glass pipette) to remove solids. The resulting solution was diluted with acetonitrile prior to mass spectroscopic analysis. HRMS (ESI): calcd for $(C_{52}H_{62}N_3O_{10}Pd)^+$ 994.3465, found 994.3396.



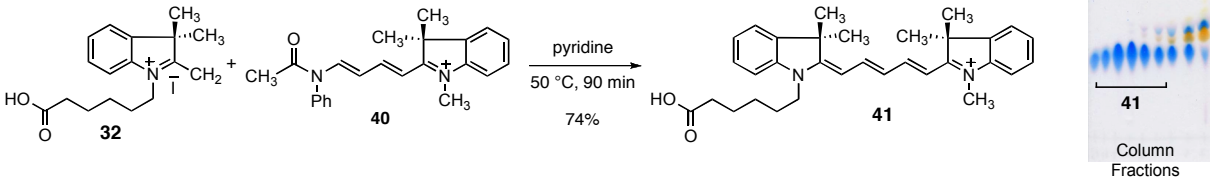

**Synthesis of Benzoic Acid Derivative 3 and Study of its Decarboxylative Palladation.**



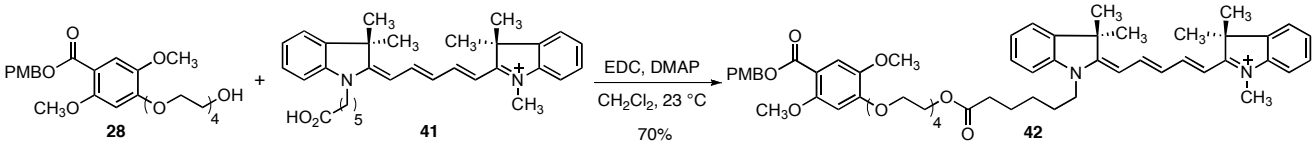

1,3,3-trimethyl-2-((1*E*,3*E*)-4-(*N*-phenylacetamido)buta-1,3-dien-1-yl)-3*H*-indolium chloride (**40**). 1,3,3-trimethyl-2-methyleneindoline **38** (0.92 mL, 5.21 mmol, 1.0 equiv) was added to a suspension of malonaldehyde dianilide hydrochloride (1.48 g, 5.74 mmol, 1.1 equiv) in a 1:1 mixture of acetic acid (7.5 mL) and acetic anhydride (7.5 mL) at 23 °C. The reaction flask was fitted with a reflux condenser and reaction assembly was placed in an oil bath (bath temperature 100 °C). After 45 min, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The solution was concentrated at

50 °C. The residue was purified by flash-column chromatography (1:10 methanol–dichloromethane initially, grading to 1:1:7 methanol–hexanes–dichloromethane, then grading to 1:1:5 methanol–hexanes–dichloromethane) to provide 1.03 g of 1,3,3-trimethyl-2-((1*E*,3*E*)-4-(*N*-phenylacetamido)buta-1,3-dien-1-yl)-3*H*-indolium chloride (**40**) as a green solid (52%). TLC: (1:1:5 methanol–hexanes–dichloromethane) $R_f$ = 0.33 (visible without staining, yellow). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 8.87 (d, 1H, *J* = 13.2 Hz), 8.49 (dd, 1H, *J* = 11.7, 15.1 Hz), 7.76 (d, 1H, *J* = 6.8 Hz), 7.70 (d, 1H, *J* = 7.8 Hz), 7.64–7.47 (m, 5H), 7.43 (d, 2H, *J* = 7.3 Hz), 6.82 (d, 1H, *J* = 15.1 Hz), 5.48 (dd, 1H, *J* = 11.7, 13.2 Hz), 3.78 (s, 3H), 2.02 (br s, 3H), 1.67 (s, 6H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 181.3, 172.7, 157.2, 143.6, 142.5, 138.6, 131.1, 130.3, 129.4, 129.1, 128.9, 123.4, 119.7, 114.8, 113.5, 112.5, 51.8, 33.9, 26.3, 24.0. FTIR (neat), cm$^{-1}$: 2970 (w), 1703 (s), 1572 (s), 1514 (s), 1474 (s). HRMS (ESI): calcd for $(C_{23}H_{25}N_2O)^+$ 345.1961, found 345.1966.

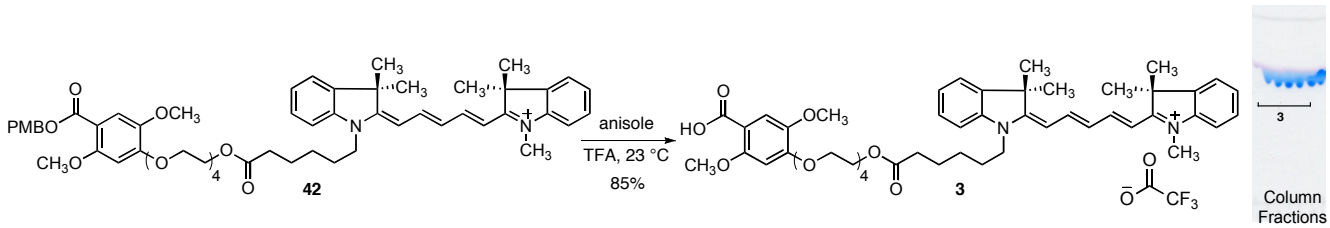



Indocyanine dye **41**. 1-(5-Carboxypentyl)-2,3,3-trimethyl-3*H*-indolium iodide (**32**, 500 mg, 1.25 mmol, 1.0 equiv) was added to a solution of 1,3,3-trimethyl-2-((1*E*,3*E*)-4-(*N*-phenylacetamido)buta-1,3-dien-1-yl)-3*H*-indolium chloride (**40**, 498 mg, 1.31 mmol, 1.1 equiv) in pyridine (8.5 mL) at 23 °C. The reaction flask was heated in an oil bath at 50 °C. After 90 min, the heating bath was removed and the reaction mixture allowed to cool to 23 °C. The solution was concentrated. The residue was purified by flash-column chromatography (1:10 methanol–dichloromethane initially, grading to 1:7 methanol–dichloromethane) to provide 478 mg of the indocyanine dye **41** as a blue solid (74%).[8] TLC: (1:5 methanol–dichloromethane) $R_f$ = 0.56 (visible without staining, blue). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.12–8.05 (m, 2H), 7.37–7.33 (m, 4H), 7.21–7.17 (m, 2H), 7.13–7.09 (m, 2H), 6.86 (app t, 1H, *J* = 12.5 Hz), 6.36 (d, 1H, *J* = 13.2 Hz), 6.30 (d, 1H, *J* = 13.6 Hz), 4.04 (t, 2H, *J* = 7.3 Hz), 3.68 (s, 3H), 2.41 (t, 2H, *J* = 7.3 Hz), 1.84–1.68 (m, 4H), 1.74 (s, 6H), 1.72 (s, 6H), 1.57–1.51 (m, 2H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.5, 173.6, 173.1, 153.8, 143.0, 142.2, 141.5, 141.2, 129.3, 128.9, 128.5, 126.7, 125.5, 125.3, 122.5, 122.4, 110.9, 110.8, 104.4, 104.0, 49.7, 49.5, 44.5, 34.8, 32.7, 28.4, 28.3, 27.1, 26.5, 24.8. FTIR (neat), cm$^{-1}$: 2930 (m), 1726 (m), 1576 (w), 1478 (s), 1443 (s), 1366 (s). HRMS (ESI): calcd for $(C_{32}H_{39}N_2O_2)^+$ 483.3006, found 483.3008. UV/vis (DMSO) $\lambda_{abs}$ = 647 nm, $\lambda_{em}$ = 663 nm, ε = 190000 M$^{-1}$cm$^{-1}$.

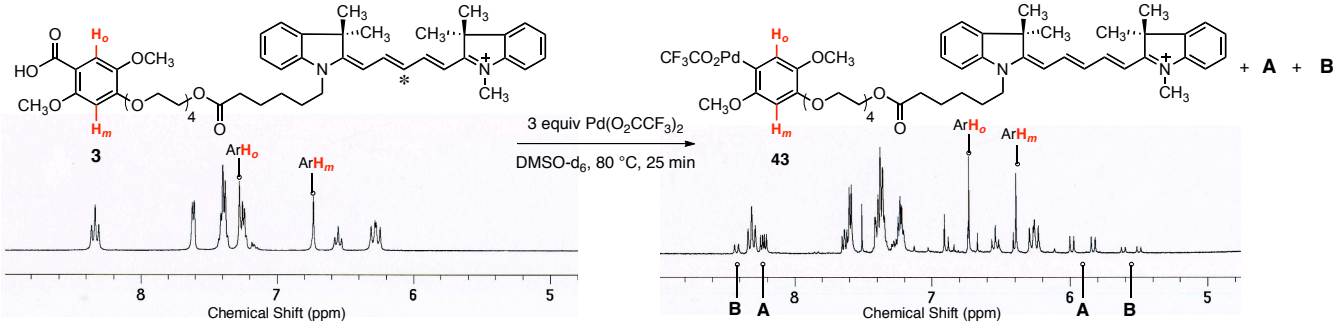
[7] For slightly different protocols to synthesize **41**, see: (a) Jung, M. E.; Kim, W.–J. *Bioorg. Med. Chem.* **2006**, *14*, 92–97. (b) Kvach, M. V.; Ustinov, A. V.; Stepanova, I. A.; Malakhov, A. D.; Skorobogatyi, M. V.; Shmanai, V. V.; Korshun, V. A. *Eur. J. Org. Chem.* **2008**, 2107–2117. The main differences were the choices of solvents to prepare either hemicyanine **40** (with acetic acid/acetic anhydride) or its de-acetyl form (with only acetic acid as solvent).




Benzyl ester **42**. *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (83.7 mg, 0.44 mmol, 1.2 equiv) and 4-dimethylaminopyridine (44.5 mg, 0.36 mmol, 1.0 equiv) were added to a solution of alcohol **28** (180 mg, 0.36 mmol, 1.0 equiv) and indocyanine dye **41** (189 mg, 0.36 mmol, 1.0 equiv) in dichloromethane (4.0 mL) at 23 °C. After 30 h, the solution was concentrated. The residue was purified by flash-column chromatography (3% methanol–dichloromethane initially, grading to 4% methanol–dichloromethane) to provide the benzyl ester **42** as a blue solid, contaminated with small amount of unreacted alcohol **28**. To remove the alcohol, the solid residue was mixed with ethyl acetate (5 mL). The resulting mixture was heated in an oil bath at 60 °C. After 15 min, the heating bath was removed and hexane was added (3.5 mL). The mixture was again heated in an oil bath at 60 °C. After 20 min, the heating bath was removed and the mixture was allowed to cool to 23 °C. After 10 min, the mixture was cooled to –20 °C. After 1 h, the ethyl acetate–hexanes supernatant containing alcohol **28** was decanted. The solid residue was dried in vacuo (0.1 mmHg) to provide 253 mg of the benzyl ester **42** as a blue solid (70%). TLC: (1:5 methanol–dichloromethane) $R_f$ = 0.59 (visible without staining, blue). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.23–8.15 (m, 2H), 7.40 (s, 1H), 7.39–7.34 (m, 6H), 7.24–7.20 (m, 2H), 7.10 (d, 1H, *J* = 7.8 Hz), 7.05 (d, 1H, *J* = 8.3 Hz), 6.93–6.86 (m, 3H), 6.61 (s, 1H), 6.38 (app t, 2H, *J* = 13.2 Hz), 5.26 (s, 2H), 4.24 (t, 2H, *J* = 5.1 Hz), 4.21 (t, 2H, *J* = 4.6 Hz), 4.09–4.04 (m, 2H), 3.90 (t, 2H, *J* = 5.1 Hz), 3.86 (s, 3H), 3.81 (app s, 6H), 3.73–3.65 (m, 10H), 3.64 (s, 3H), 2.38 (t, 2H, *J* = 7.3 Hz), 1.85–1.80 (m, 2H), 1.77 (s, 6H), 1.74 (s, 6H), 1.74–1.68 (m, 2H), 1.57–1.51 (m, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 174.0, 173.4, 173.2, 165.5, 159.7, 155.6, 154.7, 153.5, 143.5, 142.8, 142.7, 141.8, 141.7, 130.2, 129.2, 129.1, 126.1, 125.5, 125.3, 123.1, 123.0, 115.2, 114.5, 111.8, 110.9, 104.0, 103.7, 100.4, 70.6, 70.5, 70.4, 69.5, 69.0, 68.9, 66.0, 63.8, 57.4, 56.9, 55.8, 49.6, 43.9, 33.9, 33.8, 31.8, 27.9, 27.7, 27.3, 26.2, 24.8. FTIR (neat), cm$^{-1}$: 2930 (m), 1728 (m), 1690 (m), 1611 (w), 1479 (s), 1449 (s). HRMS (ESI): calcd for $(C_{57}H_{71}N_2O_{11} + Na)^{2+}$ 491.2475, found 491.2462.

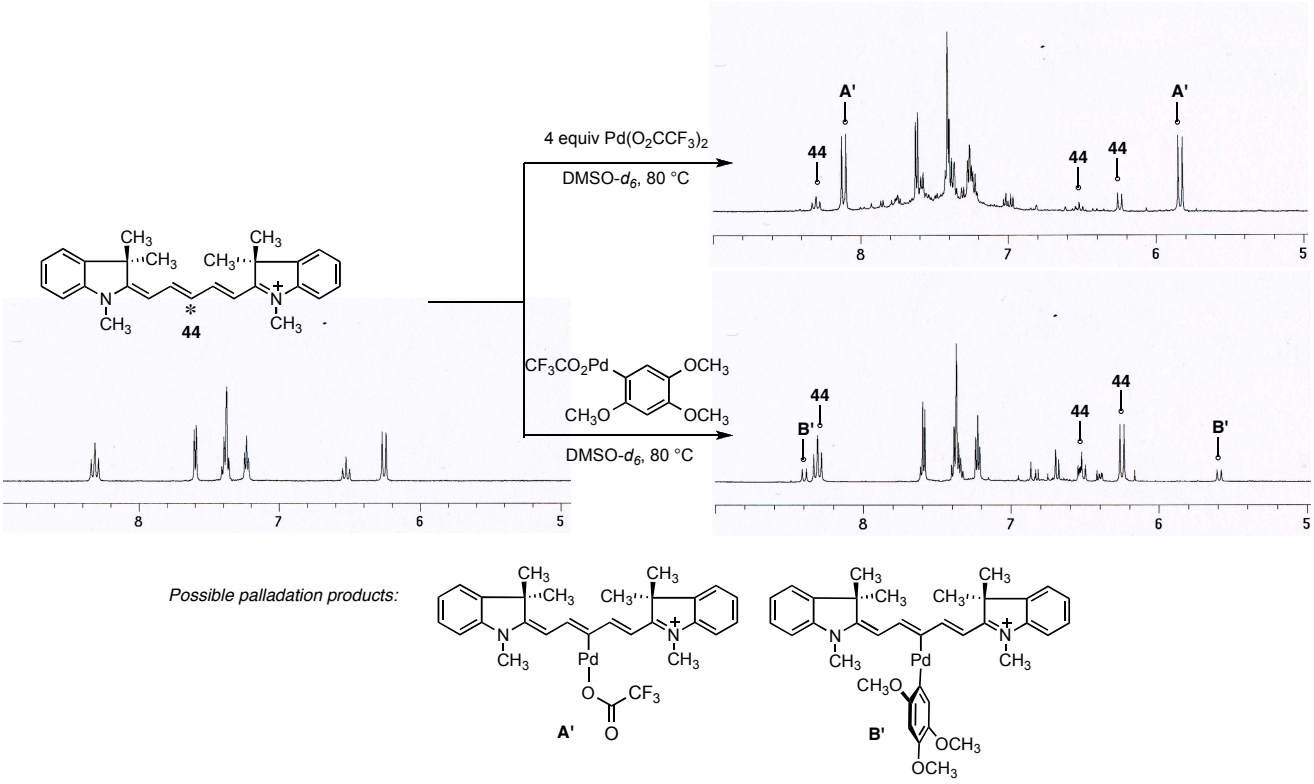



Carboxylic acid **3**. A solution of anisole (135 μL, 1.23 mmol, 6.0 equiv) in trifluoroacetic acid (1 mL) was added dropwise to a solution of benzyl ester **42** (204 mg, 0.21 mmol, 1.0 equiv) in trifluoroacetic acid (1.5 mL) at 23 °C. After 20 min, toluene (10 mL) was added. The reaction mixture was concentrated. The residue was immediately purified by flash-column chromatography (5% methanol–dichloromethane initially, grading to 13% methanol–dichloromethane) to provide 167 mg of the carboxylic acid **3** as a blue solid (85%). TLC: (1:5 methanol–dichloromethane) $R_f$ = 0.62 (visible without staining, blue). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 8.36–8.31 (m, 2H), 7.61 (app d, 2H, $J$ = 6.8 Hz), 7.43–7.36 (m, 4H), 7.27 (s, 1H), 7.27–7.22 (m, 2H), 6.73 (s, 1H), 6.55 (app t, 1H, $J$ = 12.2 Hz), 6.30 (d, 1H, $J$ = 14.2 Hz), 6.26 (d, 1H, $J$ = 14.2 Hz), 4.22–4.18 (m, 2H), 4.12–4.07 (m, 4H), 3.79 (s, 3H), 3.77–3.73 (m, 2H), 3.71 (s, 3H), 3.60 (s, 3H), 3.59–3.49 (m, 10H), 2.30 (t, 2H, $J$ = 7.1 Hz), 1.72–1.68 (m, 2H), 1.68 (app s, 12H), 1.60–1.54 (m, 2H), 1.41–1.34 (m, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 174.0, 173.5, 173.2, 155.2, 154.7, 152.9, 143.5, 142.8, 142.7, 141.8, 141.7, 129.6, 129.1, 129.1, 128.9, 126.0, 125.5, 125.3, 123.1, 123.0, 115.4, 111.8, 104.0, 103.7, 100.4, 70.6, 70.5, 70.4, 69.5, 69.0, 68.8, 63.8, 57.4, 56.7, 49.6, 43.9, 33.9, 31.8, 27.9, 27.7, 27.3, 26.2, 24.8. FTIR (neat), cm$^{-1}$: 2932 (m), 1726 (m), 1690 (m), 1611 (w), 1479 (s), 1447 (s). $^{19}$F NMR (375 MHz, DMSO-$d_6$) δ: –73.9. HRMS (ESI): calcd for $(C_{49}H_{63}N_2O_{10})^+$ 839.4477, found 839.4279.

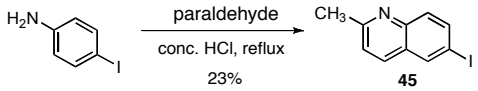



Decarboxylative palladation of carboxylic acid **3**: Heating a mixture of palladium(II) trifluoroacetate and carboxylic acid **3** in DMSO-$d_6$ at 80 °C for 25 min provided the desired arylpalladium(II) complex **43** as the major product along with two side products (**A** and **B**). The ratio of **43** to **A**+**B** was approximately 3:1 based on $^1$H NMR analysis (see below). $^1$H NMR analysis revealed that the protons adjacent to the central carbon (denoted as *) of the pentamethine group had been transformed from an apparent triplet (8.3 ppm) to two sets of peaks, a doublet (8.4 ppm) and a doublet of doublets (8.2 ppm). This suggests that two different palladation reactions are operative toward the central carbon of the pentamethine functionality.

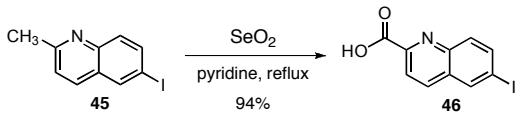
To probe these competing processes further and to shed light on what **A** and **B** might be, experiments using the symmetrical dye **44** were carried out. In the first experiment, a mixture of palladium(II) trifluoroacetate and symmetrical dye **44** in DMSO-$d_6$ was heated at 80 °C for an extended time (12 h). While the reaction was sluggish, the α–protons adjacent to the central carbon (denoted as *) of the pentamethine moiety had been transformed from a triplet (**44**, 8.3 ppm) to a doublet (**A′**, 8.1 ppm), suggesting that the carbon-hydrogen bond at the central atom had been substituted. In the second experiment, symmetrical dye **44** was heated with a pre-formed arylpalladium(II) complex derived from 2,4,5-benzoic acid at 80 °C for 5 h. Despite extensive decomposition of the arylpalladium(II) complex, new proton peaks arising from the pentamethine group were observed. Again, the α–protons adjacent to the central carbon had been transformed from a triplet (**44**, 8.3 ppm) to a doublet (**B′**, 8.4 ppm). Importantly, the chemical shifts of these protons from **A′** and **B′** match very well to **A** and **B** from the previous experiments respectively. Although their structures have not been determined, it is clear that a selective C-H functionalization/palladation reaction had occurred at the central carbon atom (denoted as *) of the pentamethine group.



**Synthesis of *N*-Ethyl-6-Vinylquinoline-2-carboxamide 10 and its Heck Reaction with Arylpalladium(II) Reagent 2.**

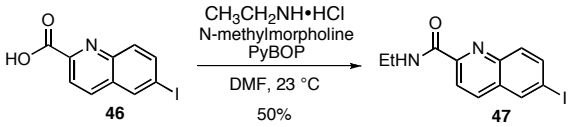



6-Iodo-2-methylquinoline (**45**). Concentrated hydrochloric acid (4.6 mL) was added to a solution of paraldehyde (6.03 g, 45.7 mmol, 2.0 equiv) and 4-iodoaniline (5.00 g, 22.8 mmol, 1.0 equiv) at 23 °C. The reaction flask was fitted with a reflux condenser and the reaction assembly was placed in an oil bath (bath temperature 100 °C). After 4 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The reaction mixture was diluted with water (50 mL) and the solution was brought to pH 10 with 1.0 N sodium hydroxide. The aqueous solution was extracted with dichloromethane (5 x 100 mL). The combined organic layers were dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (20% ethyl acetate-hexanes initially, grading to 30% ethyl acetate-hexanes). The product purified by flash-column chromatography was crystallized from warm ether (cooling to –20 °C overnight) to provide 1.40 g of 6-iodo-2-methylquinoline (**45**) as a brown crystalline solid (23 %). TLC: (20% ethyl acetate–hexanes) $R_f$ = 0.17 ($KMnO_4$). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.16 (d, 1H, J = 2.0 Hz), 7.94-7.90 (m, 2H), 7.75 (d, 1H, J = 9.0 Hz), 7.30 (d, 1H, J = 8.5 Hz), 2.74 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 160.0, 147.1, 138.3, 136.4, 135.1, 130.7, 128.5, 122.9, 91.1, 25.7. FTIR (neat), cm$^{-1}$: 1593 (w), 1483 (w), 1300 (w), 907 (s), 827 (m), 727 (s). HRMS (ESI): calcd for $(C_{10}H_8IN + H)^+$ 269.9774, found 269.9779.

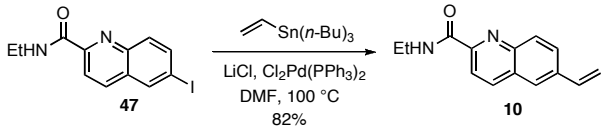



6-Iodoquinoline-2-carboxylic acid (**46**). 6-Iodo-2-methylquinoline (**45**, 1.40 g, 5.20 mmol, 1.0 equiv) was added to a suspension of selenium dioxide (1.15 g, 10.4 mmol, 2.0 equiv) in pyridine (26 mL) at 23 °C. The reaction flask was fitted with a reflux condenser and the reaction assembly was placed in an oil bath (bath temperature 80 °C). After 4 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The mixture was filtered through a pad of Celite, rinsing with 25% methanol-dichloromethane (100 mL) and the filtrate was concentrated to provide 1.46 g of 6-iodoquinoline-2-carboxylic acid (**46**) as a tan solid (94%). This material was used in the next transformation without purification. TLC: (10% methanol–dichloromethane) $R_f$ = 0.14 ($KMnO_4$). $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 13.35 (br s, 1H), 8.59 (d, 1H, J = 1.5 Hz), 8.49 (d, 1H, J = 8.5 Hz), 8.12 (d, 1H, J = 8.5 Hz), 8.11 (dd, 1H, J = 8.5, 2.0 Hz), 7.92 (d, 1H, J = 9.0 Hz). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 166.9, 149.9, 146.3, 139.6, 137.2, 132.1, 131.1, 127.0, 122.2, 96.2. FTIR (neat), cm$^{-1}$: 3435 (w),

2920 (w), 2500 (w), 1709 (m), 1053 (s), 1024 (s), 1007 (s). HRMS (ESI): calcd for $(C_{11}H_6INO_2 + H)^+$ 299.9516, found 299.9515.

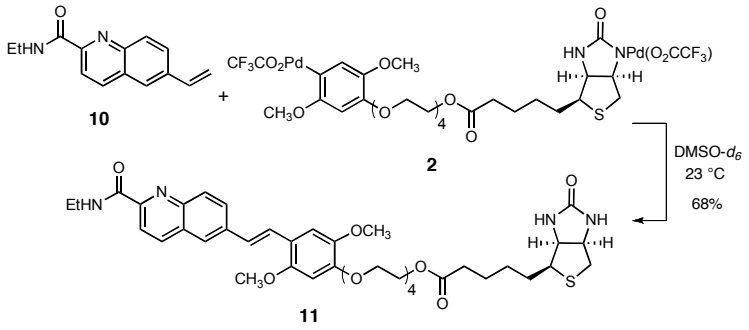



*N*-Ethyl-6-iodoquinoline-2-carboxamide (**47**). *N*-Methylmorpholine (138 µL, 1.26 mmol, 0.7 equiv) was added to a suspension of ethylamine hydrochloride (306 mg, 3.75 mmol, 2.1 equiv) and 6-iodoquinoline-2-carboxylic acid **46** (534 mg, 1.78 mmol, 1.0 equiv) in *N,N*-dimethylformamide (8.9 mL) at 23 °C. A solution of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (1.02 g, 1.96 mmol, 1.1 equiv) and *N*-methylmorpholine (138 µL, 1.26 mmol, 0.7 equiv) in *N,N*-dimethylformamide (8.9 mL) was then added via cannula at 23 °C. After 45 h, the reaction mixture was poured into a separatory funnel containing water (40 mL). The aqueous solution was extracted with dichloromethane (2 x 40 mL). The combined organic layers were dried over magnesium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate-hexanes) to provide 428 mg of *N*-ethyl-6-iodoquinoline-2-carboxamide (**47**) as a yellow solid (74%). TLC: (50% methanol–dichloromethane) $R_f$ = 0.46 ($KMnO_4$). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.27 (d, 1H, J = 8.5 Hz), 8.19 (s, 1H), 8.16 (br s, 1H), 8.11 (d, 1H, J = 8.5 Hz), 7.91 (dd, 1H, J = 8.5, 1.5 Hz), 7.74 (d, 1H, J = 9.0 Hz), 3.55 (app quint, 2H, J = 6.9 Hz), 1.30 (t, 3H, J = 7.2 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 164.1, 150.6, 145.5, 139.0, 136.7, 136.4, 131.3, 130.9, 119.8, 94.1, 34.7, 15.2. FTIR (neat), cm$^{-1}$: 3391 (m), 1667 (s), 1524 (s), 1483 (s), 1184 (m), 826 (s). HRMS (ESI): calcd for $(C_{12}H_{11}IN_2O + Na)^+$ 348.9808, found 348.9791.



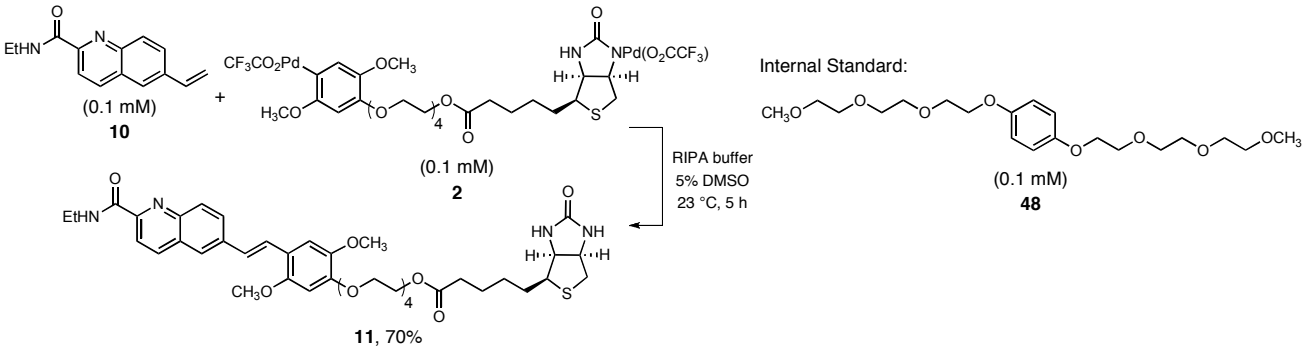

*N*-ethyl-6-vinylquinoline-2-carboxamide (**10**). To a solid mixture of lithium chloride (24.9 mg, 0.766 mmol, 5.0 equiv), dichlorobis(triphenylphosphine)palladium(II) (10.8 mg, 0.0153 mmol, 0.1 equiv), and *N*-ethyl-6-iodoquinoline-2-carboxamide **47** (50.0 mg, 0.153 mmol, 1.0 equiv) at 23 °C in a pressure tube were added *N,N*-dimethylformamide (1.5 mL) and tributyl(vinyl)stannane (67.0 µL, 0.230 mmol, 1.5 equiv) in sequence. The pressure tube was sealed and heated in an oil bath at 100 °C. After 4.5 h, the heating bath was removed and the reaction mixture was allowed to cool to 23 °C. The reaction mixture was poured into aqueous potassium fluoride (1.0 N, 15 mL) and the aqueous solution was extracted with ethyl

acetate (3 x 15 mL). The combined organic layers were washed with water (30 mL) and the washed solution was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash column chromatography (5% ethyl acetate-hexanes initially, grading to 10% ethyl acetate-hexanes) on a column that had been deactivated with 10% triethylamine-ethyl acetate to provide 39.6 mg of *N*-ethyl-6-vinylquinoline-2-carboxamide (**10**) as a tan solid (82%). TLC: (30% ethyl acetate–hexanes) $R_f$ = 0.24 ($KMnO_4$). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.30 (d, 1H, J = 8.5 Hz), 8.23 (d, 2H, J = 8.5 Hz), 8.05 (d, 1H, J = 8.5 Hz), 7.90 (d, 1H, J = 9.0 Hz), 7.75 (s, 1H), 6.90 (dd, 1H, J = 18.0, 11.0 Hz), 5.94 (d, 1H, J = 17.5 Hz), 5.44 (d, 1H, J = 11.0 Hz), 3.59 (app quint, 2H, J = 6.8 Hz), 1.34 (t, 3H, J = 7.2 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 164.5, 150.0, 146.6, 137.6, 137.2, 136.2, 130.0, 129.7, 127.7, 125.9, 119.5, 116.4, 34.6, 15.2. FTIR (neat), $cm^{-1}$: 2974 (w), 1666 (s), 1522 (s), 1497 (s). HRMS (ESI): calcd for $(C_{14}H_{14}N_2O + H)^+$ 227.1179, found 227.1213.




Heck product **11**. *N*-Ethyl-6-vinylquinoline-2-carboxamide **10** (4.1 mg, 18.1 µmol, 1.0 equiv) was added to a solution of the arylpalladium(II) reagent **2** (1.43 mL of a 18.1 mM solution in DMSO-$d_6$, 25.9 µmol, 1.4 equiv) in a 20-mL scintillation vial at 23 °C open to the air. After 20 h, DL-dithiothreitol (20.0 mg, 130 µmol, 5.0 equiv with respect to **2**) was added. After 5 min, the reaction mixture was filtered through a pad of Celite, rinsing with dichloromethane (35 mL) and the filtrate was concentrated. The residue was resuspended in ethyl acetate (20 mL) and the suspension was poured into a separatory funnel containing 1.0 N hydrochloric acid (20 mL). The layers were separated. The aqueous layer was extracted with ethyl acetate (2 x 20 mL). The combined organic layers were washed with water (2 x 20 mL) and the washed solution was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by semi-preparative reverse-phase HPLC to provide 9.7 mg of the Heck product **11** as a yellow oil (68%). TLC: (10% methanol–dichloromethane) $R_f$ = 0.13 (CAM). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.30 (d, 1H, J = 8.5 Hz), 8.23 (d, 2H, J = 8.5 Hz), 8.05 (d, 1H, J = 8.5 Hz), 7.90 (d, 1H, J = 9.0 Hz), 7.75 (s, 1H), 6.90 (dd, 1H, J = 18.0, 11.0 Hz), 5.94 (d, 1H, J = 17.5 Hz), 5.44 (d, 1H, J = 11.0 Hz), 3.59 (app quint, 2H, J = 6.8 Hz), 1.34 (t, 3H, J = 7.2 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ:

164.5, 150.0, 146.6, 137.6, 137.2, 136.2, 130.0, 129.7, 127.7, 125.9, 119.5, 116.4, 34.6, 15.2. FTIR (neat), $cm^{-1}$: 2974 (w), 1666 (s), 1522 (s), 1497 (s). HRMS (ESI): calcd for $(C_{40}H_{52}N_4O_{10}S + H)^+$ 781.3477, found 781.3474.




Representative Procedure for Heck-type Coupling of Arylpalladium(II) Reagent **2** and *N*-Ethyl-6-Vinylquinoline-2-Carboxamide **10** in Aqueous Media. *N*-Ethyl-6-vinylquinoline-2-carboxamide **10** (10.0 µL of a 18.1 mM solution in DMSO, 0.181 µmol, 1.0 equiv), 1,4-bis(1,4,7,10-tetraoxaundecyl)benzene **48** (18.1 µL of a 10 mM aqueous solution, 0.181 µmol, 1.0 equiv), and DMSO (70.0 µL) were added in sequence to RIPA buffer, pH 8.0 (1.70 mL) at 23 °C in a 1.5–mL microcentrifuge tube open to the air. A solution of arlypalladium(II) reagent **2** in DMSO-$d_6$ (10.0 µL of an 18.1 mM solution, 0.181 µmol, 1.0 equiv) was added at 23 °C. The microcentrifuge tube was then capped and placed on an end-over-end rotator. After 4 h of mixing, the reaction mixture was analyzed by HPLC (ODS column, 35 to 70% acetonitrile in 0.1% trifluoroacetic acid–water over 15 min) after filtering a 900 µL-aliquot through a 0.2-µm Teflon syringe filter and rinsing with 100 µL of methanol. The product conversion and yield were calculated using compound **48** as an inert internal standard. A representative HPLC trace of a mixture of the internal standard **48**, the *N*-ethyl-6-vinylquinoline-2-carboxamide **10**, and the Heck product **11** is shown below to illustrate their relative retention times. When the amount of DMSO was lowered to 1%, the coupling product **11** was formed in 60% yield after 4 h at 23 ºC. Also, see Table S1 for the coupling yields obtained from other buffer solutions.

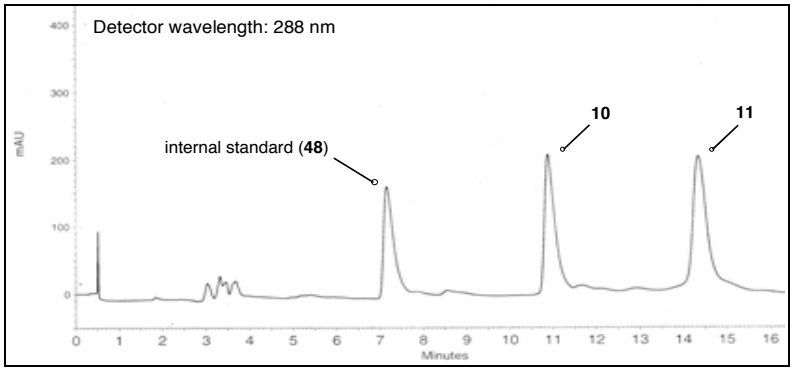
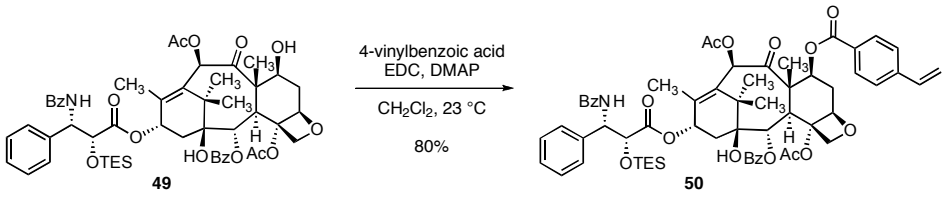



**Table S1.**

| Entry | Buffer Composition[a] | Yield[b] |
|---|---|---|
| 1 | 100 mM Tris, pH 8.0 | 49% |
| 2 | 100 mM Tricine, pH 8.0 | 70% |
| 3 | 100 mM Hepes•KOH<br>140 mM NaCl, 1 mMEDTA<br>1% sodium deoxycholate<br>1% Triton X-100, pH 8.0 | 73% |
| 4 | 100 mM sodium phosphate<br>pH 8.0 | < 5% |

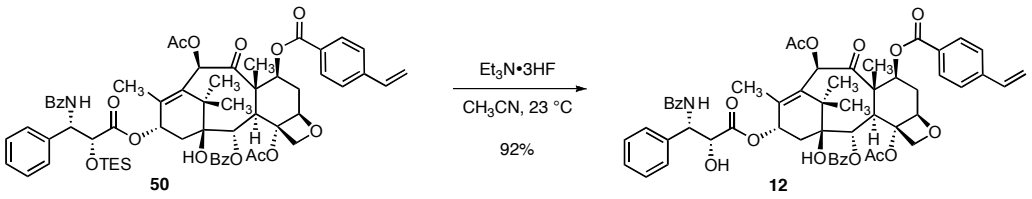
[a] with 5% DMSO. [b] measured against phenol as an internal standard.

**Synthesis of 7-(4-Vinylbenzoyl)taxol 12 and its Heck Reaction with Arylpalladium(II) Reagent 7**

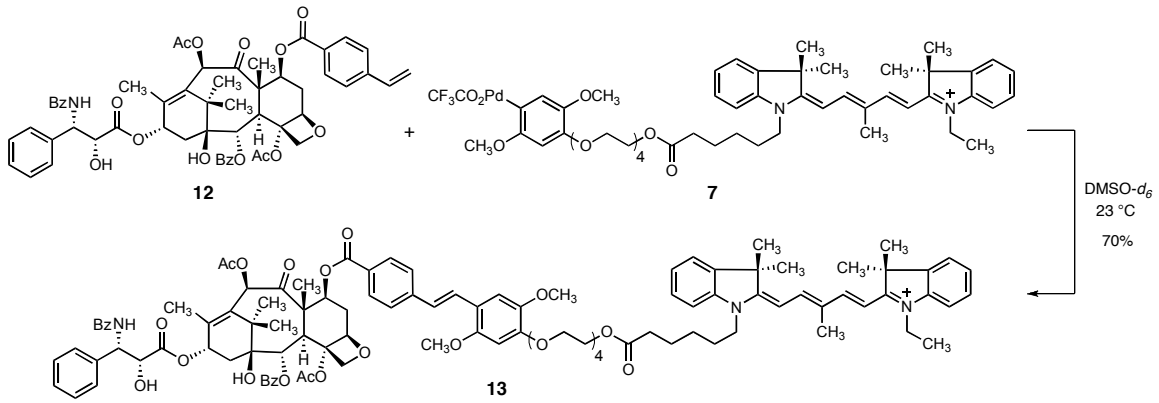



2′-Triethylsiloxy-7-(4-vinylbenzoyl)taxol (**50**). *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (42.1 mg, 0.22 mmol, 2.5 equiv) and 4-dimethylaminopyridine (11.0 mg, 0.088 mmol, 1.0 equiv) were added to a solution of 2′-triethylsiloxy taxol **49**[9] (85.0 mg, 0.088 mmol, 1.0 equiv) and 4-vinylbenzoic acid (32.5 mg, 0.22 mmol, 2.5 equiv) in dichloromethane (3.0 mL) at 23 °C. After 36 h, the product solution was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–hexanes) to provide 77.4 mg of 2′-triethylsiloxy-7-(4-vinylbenzoyl)taxol (**50**) as a white solid (80%). TLC: (30% ethyl acetate–hexanes) $R_f$ = 0.36 ($KMnO_4$). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.16 (d, 2H, *J* = 8.3 Hz), 7.89 (d, 2H, *J* = 8.3 Hz), 7.76 (d, 2H, *J* = 7.8 Hz), 7.63 (t, 1H, *J* = 7.8 Hz), 7.55 (t, 1H, *J* = 7.8 Hz), 7.50 (t, 1H, *J* = 7.8 Hz), 7.45–7.31 (m, 8H), 7.13 (d, 1H, *J* = 8.8 Hz), 6.75 (dd, 1H, *J* = 10.7, 17.1 Hz), 6.43 (s, 1H), 6.26 (t, 1H, *J* = 9.3 Hz), 5.86 (d, 1H, *J* = 17.5 Hz), 5.80–5.76 (m, 2H), 5.71 (d, 1H, *J* = 8.8 Hz), 5.37 (d, 1H, *J* = 10.8 Hz), 5.02 (d, 1H, *J* = 9.3 Hz), 4.72 (s, 1H), 4.38 (d, 1H, *J* = 8.3 Hz), 4.27 (d, 1H, *J* = 8.8 Hz), 4.06 (d, 1H, *J* = 6.8 Hz), 2.82–2.75 (m, 1H), 2.57 (s, 3H), 2.46 (dd, 1H, *J* = 9.8, 15.6 Hz), 2.19 (dd, 1H, *J* = 8.8, 15.1 Hz), 2.03 (s, 3H), 1.99–1.95 (m, 1H), 1.98 (s, 3H), 1.96 (s, 3H), 1.22 (s, 3H), 1.19 (s, 3H), 0.83 (t, 9H, *J* = 8.3 Hz), 0.53–0.38 (m, 6H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 202.7, 171.9, 170.1, 168.6, 167.3, 167.3, 165.4, 142.0, 141.3, 138.7, 136.5, 134.4, 134.0, 133.2, 132.0, 130.5, 130.3, 129.6, 129.4, 129.1, 128.9, 128.2, 127.3, 126.7, 126.2, 116.5, 84.3, 81.2, 79.0, 75.0, 75.0, 74.9, 72.3, 71.6, 56.5, 56.0, 46.9, 43.6, 35.9, 33.7, 26.6, 23.2, 21.7, 20.7, 14.8, 11.4, 6.8, 4.6. FTIR (neat), $cm^{-1}$: 3437 (w), 2955 (w), 1749 (m), 1722 (s), 1667 (m), 1607 (w), 1514 (m). HRMS (ESI): calcd for $(C_{62}H_{71}NO_{15}Si + HCOO)^-$ 1142.4575, found 1142.3540.

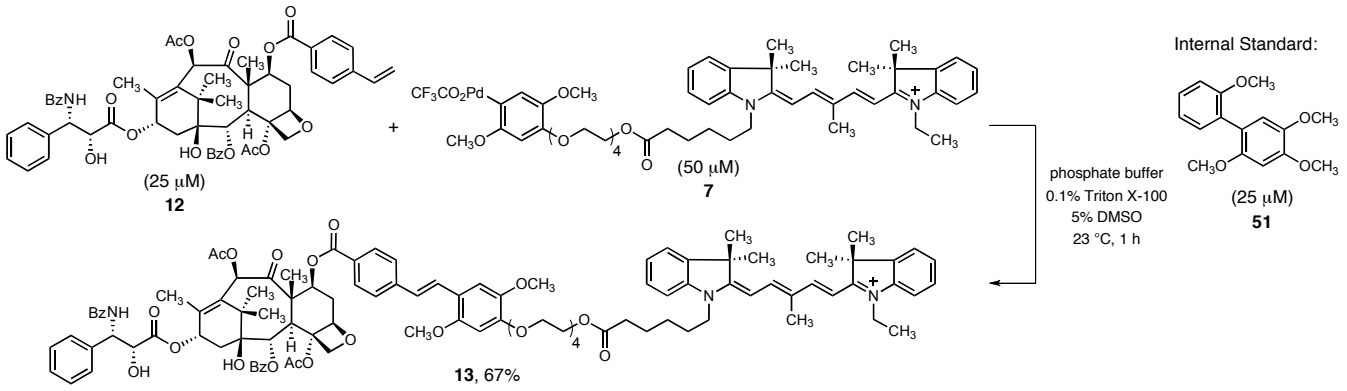
[8] Altstadt, T. J. *et al. J. Med. Chem*. **2001**, *44*, 4577–4583.



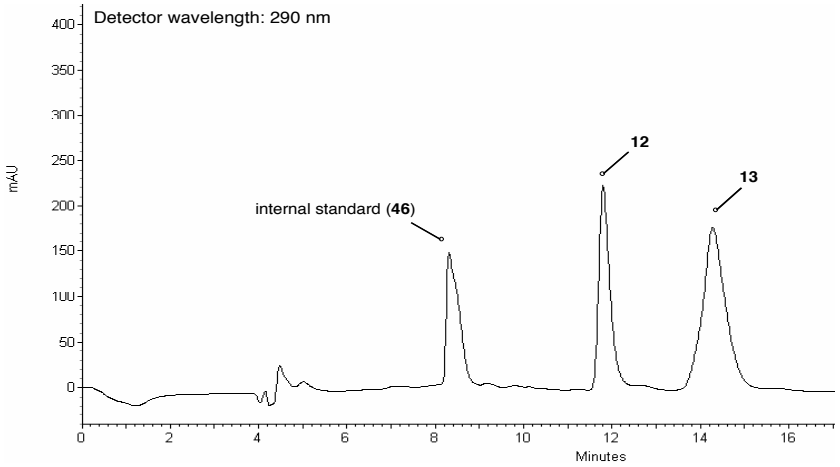

7-(4-Vinylbenzoyl)taxol (**12**). Triethylamine-trihydrofluoride (82 μL, 0.50 mmol, 10 equiv) was added to a solution of 2′-triethylsiloxy-7-(4-vinylbenzoyl)taxol **50** (55.0 mg, 0.050 mmol, 1.0 equiv) in acetonitrile (2.0 mL) at 23 °C. After 90 min, the product solution was diluted with ethyl acetate (30 mL). The diluted solution was washed sequentially with saturated aqueous sodium bicarbonate solution (25 mL), water (25 mL), and then saturated aqueous sodium chloride solution. The washed solution was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (30% ethyl acetate–hexanes initially, grading to 50% ethyl acetate–hexanes) to provide 45.5 mg of 7-(4-vinylbenzoyl)taxol (**12**) as a white solid (92%). TLC: (50% ethyl acetate–hexanes) $R_f$ = 0.44 ($KMnO_4$). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.14 (d, 2H, *J* = 7.3 Hz), 7.87 (d, 2H, *J* = 8.3 Hz), 7.76 (d, 2H, *J* = 7.3 Hz), 7.63 (t, 1H, *J* = 7.3 Hz), 7.54–7.49 (m, 4H), 7.45–7.39 (m, 5H), 7.35 (t, 1H, *J* = 7.1 Hz), 7.10 (d, 1H, *J* = 7.3 Hz), 6.75 (dd, 1H, *J* = 10.7, 17.6 Hz), 6.37 (s, 1H), 6.19 (br t, 1H, *J* = 9.1 Hz), 5.87–5.81 (m, 2H), 5.75–5.70 (m, 2H), 5.37 (d, 1H, *J* = 10.8 Hz), 4.99 (d, 1H, *J* = 9.3 Hz), 4.82 (br s, 1H), 4.35 (d, 1H, *J* = 8.3 Hz), 4.24 (d, 1H, *J* = 8.8 Hz), 4.01 (d, 1H, *J* = 6.8 Hz), 2.81–2.74 (m, 1H), 2.41 (s, 3H), 2.36 (app d, 2H, *J* = 8.3 Hz), 1.98 (s, 3H), 1.96 (s, 3H), 1.95–1.92 (m, 1H), 1.88 (s, 3H), 1.20 (s, 3H), 1.19 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 202.6, 172.7, 170.6, 168.6, 167.2, 165.3, 142.1, 140.6, 138.3, 136.5, 134.0, 133.5, 132.2, 130.4, 130.3, 129.5, 129.3, 129.3, 129.0, 129.0, 128.6, 127.3, 127.3, 126.2, 116.5, 84.2, 81.3, 78.9, 75.1, 74.7, 73.5, 72.4, 72.3, 56.7, 55.1, 47.1, 43.5, 35.9, 33.7, 26.8, 22.8, 21.1, 20.7, 14.9, 11.3. FTIR (neat), $cm^{-1}$: 2976 (w), 1724 (s), 1647 (m), 1606 (w), 1487 (m). HRMS (ESI): calcd for $(C_{62}H_{71}NO_{15} + H)^+$ 984.3801, found 984.3805.




Heck-type Coupling of **12** with Arylpalladium(II) Reagent **7** in DMSO. 7-(4-Vinylbenzoyl)taxol **12** (20.5 mg, 20.9 μmol, 1.5 equiv) was added to a solution of the arylpalladium(II) reagent **7** in DMSO-$d_6$ (1.00 mL of 13.9 mM solution, 13.9 μmol, 1 equiv) in a 20-mL scintillation vial at 23 °C. After 2 h, the reaction solution was partitioned between water (15 mL) and dichloromethane (20 mL). The organic layer was washed with saturated aqueous sodium chloride solution (15 mL) and the washed solution was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (2% methanol–dichloromethane initially, grading to 5% methanol–dichloromethane, then grading to 11% methanol–dichloromethane) to provide 18.2 mg of the Heck product **13** as a blue solid (70%). TLC: (1:1:7 methanol–hexanes–dichloromethane) $R_f$ = 0.42 (visible without staining, blue). $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.33 (d, 1H, *J* = 7.8 Hz), 8.11 (d, 2H, *J* = 7.8 Hz), 8.04 (d, 2H, *J* = 7.3 Hz), 7.95 (t, 2H, *J* = 12.7 Hz), 7.84 (d, 2H, *J* = 8.3 Hz), 7.63 (t, 1H, *J* = 7.3 Hz), 7.55–7.48 (m, 7H), 7.45–7.32 (m, 8H), 7.25–7.19 (m, 2H), 7.15 (d, 1H, *J* = 7.8 Hz), 7.11 (s, 1H), 7.06 (d, 1H, *J* = 7.8 Hz), 6.97 (d, 1H, *J* = 16.6 Hz), 6.63 (s, 1H), 6.35 (s, 1H), 6.14–6.04 (m, 3H), 5.72–5.65 (m, 3H), 4.96–4.92 (m, 2H), 4.31 (d, 1H, *J* = 8.3 Hz), 4.25–4.16 (m, 7H), 4.30–4.05 (m, 2H), 3.97 (d, 1H, *J* = 6.1 Hz), 3.89–3.87 (m, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.72–3.61 (m, 10H), 2.77–2.71 (m, 1H), 2.41 (s, 3H), 2.34 (t, 2H, *J* = 7.3 Hz), 2.20 (dd, 1H, *J* = 8.8, 15.1 Hz), 2.09 (s, 3H), 2.03 (dd, 1H, *J* = 9.3, 15.1 Hz), 1.95 (s, 3H), 1.93 (s, 3H), 1.90–1.88 (m, 1H), 1.89 (s, 3H), 1.85–1.79 (m, 2H), 1.76 (app s, 12H), 1.72–1.66 (m, 2H), 1.50–1.42 (m, 5H), 1.15 (app s, 6H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 202.9, 173.5, 173.2, 172.9, 172.7, 170.7, 168.5, 167.5, 167.2, 165.3, 154.8, 154.1, 152.3, 149.8, 144.2, 142.7, 142.2, 141.8, 141.5, 141.3, 139.3, 134.1, 134.0, 132.9, 131.7, 130.4, 130.4, 129.4, 129.0, 128.8, 128.7, 128.6, 128.0, 127.9, 126.3, 126.1, 125.7, 125.6, 125.4, 122.7, 118.6, 110.9, 110.6, 100.3, 100.1, 99.8, 84.3, 80.8, 78.9, 75.2, 74.8, 73.8, 72.3, 71.3, 71.1, 70.9, 70.8, 70.8, 70.1, 69.3, 69.3, 63.8, 57.1, 56.8, 56.5, 49.8, 49.6, 46.9, 44.4, 43.4, 39.8, 35.7, 34.0, 33.7, 28.3, 28.2, 27.2, 26.7, 26.7, 24.7, 22.9, 21.4, 20.7, 14.8, 12.5, 11.9, 11.3. FTIR (neat), cm$^{-1}$: 2928 (m), 1722 (m), 1651 (w), 1601 (w), 1491 (s), 1479 (s). HRMS (ESI): calcd for $(C_{106}H_{122}N_3O_{23} + Na)^{2+}$ 913.9178, found 913.9239.



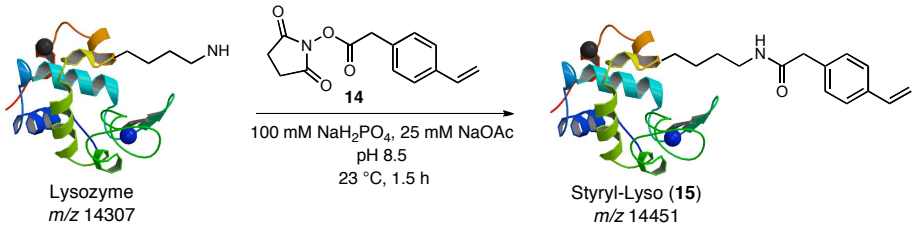

Representative Procedure for Heck-type Coupling of Arylpalladium(II) Reagent **7** and 7-(4-Vinylbenzoyl)taxol **12** in Aqueous Media. 7-(4-Vinylbenzoyl)taxol **12** (5.0 μL of a 5.0 mM solution in DMSO, 0.025 μmol, 1.0 equiv), 2,2',4,5-tetramethoxy-1,1'-biphenyl **51** (5.0 μL of a 5.0 mM solution in DMSO, 0.025 μmol, 1.0 equiv), and DMSO (36.7 μL) were added in sequence to 50 mM potassium phosphate buffer solutions containing 0.1% Triton X-100 (950 μL) at pHs of 5.8, 6.5, 7.4, and 8.0 (four separate experiments) at 23 °C in 1.5–mL microcentrifuge tubes open to the air. A solution of arylpalladium(II) reagent **7** in DMSO-$d_6$ (3.3 μL of an 15.2 mM solution, 0.050 μmol, 2.0 equiv) was added at 23 °C. Each microcentrifuge tube was then capped and placed on an end-over-end rotator. After 1 h of mixing, DL-dithiothreitol (5.0 μL of a 100 mM aqueous solution, 0.50 μmol, 10 equiv with respect to **7**) was added at 23 °C. After 10 min, the solution was filtered through a 0.2-μm Teflon syringe filter and rinsing with methanol (2 x 200 μL) and the eluent was analyzed by HPLC (ODS column, 65 to 90% methanol in 0.1% trifluoroacetic acid–water over 3 min followed by 90% methanol in 0.1% trifluoroacetic acid–water for 15 min). The product conversion and yields were determined using compound **51** as an inert internal standard (yields of product **13** were comparable in all four experiments at various pHs). A representative HPLC trace of a mixture of the internal standard **51**, 7-(4-Vinylbenzoyl)taxol **12**, and the Heck product **13** is shown below to illustrate their relative retention times.



**Heck-type Coupling of Cy5 Reagent 7 with Styryl-modified Lysozyme.**

**Instrumentation.** Electrospray LC/MS analysis was performed using an Agilent 6210 Time-of-Flight LC/MS system equipped with an Agilent 1200 series LC pump. Protein liquid chromatography was performed using an Agilent Zorbax Eclipse Plus C18 reversed phase column (2.1 mm x 100 mm). Flow rate was 500 µL/min. A linear gradient of 5-90% B in A over 7 min followed by 90% B for 2 min was applied for all lysozyme runs in which solvent A was 0.1% aqueous formic acid and solent B was 100% acetonitrile containing 0.1% formic acid.

**Gel Analyses.** For protein analysis on lysozyme and lysozyme derivatives, sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed using NuPAGE® 10% bis–tris gels (Invitrogen). Noves® sharp pre-stained protein standard (Invitrogen) was applied to at least one lane of each gel for calculation of apparent molecular weight. Electrophoresis equipment was purchased from Owl Separation Systems (model P8DS). Fluorescent gel imaging was accomplished on a Typhoon TRIO variable mode imager (Amersham Bioscience) using a red laser (633 nm) and a Cy5 (670 nm) band-pass filter. Fluorescent images were processed and quantified using ImageQuant TL software. Visualization of protein bands was accomplished by staining with Noves® Colloidal Blue staining kit (Invitrogen).




Preparation of Styryl-modified Lysozyme (**15**). 2-(4-Vinylphenyl)acetic acid *N*-hydroxysuccinimide ester **14**[10] (5.0 µL of a 50 mM solution in DMF, 0.25 µmol, 1.0 equiv) was added to a buffered solution of chicken egg white lysozyme (1.0 mL of a 250 µM aqueous solution containing 100 mM $NaH_2PO_4$ and 25 mM NaOAc, pH 8.5, 0.25 µmol) at 23 °C in a 1.5 mL–microcentrifuge tube open to air. The microcentrifuge tube was then capped and placed on an end-over-end rotator at 23 °C. After 90 min, the protein products were separated from small molecules by gel-filtration chromatography using a NAP-10 column (GE Healthcare) with 50 mM Tris buffer (pH 8.0) as the eluent. The protein mixture was found to contain approximately 34% of unreacted lysozyme, 40% of singly modified lysozyme, Styryl-Lyso (**15**), and 20% of doubly modified lysozyme, $(Styryl)_2$-Lyso (**52**), and 6% of triply modified lysozyme, $(Styryl)_3$-Lyso (**53**). The ratio was estimated by LC/MS analysis comparing extracted ion mass areas (Figure S1): Lysozyme, calcd[11] 14307 Da, found 14305.4±0.1; Styryl-Lyso (**15**), calcd 14451 Da, found 14449.6±0.03; $(Styryl)_2$-Lyso (**52**), calcd 14595 Da, found 14593.6±0.1; $(Styryl)_3$-Lyso (**53**), calcd 14739 Da, found 14737.7±0.2.

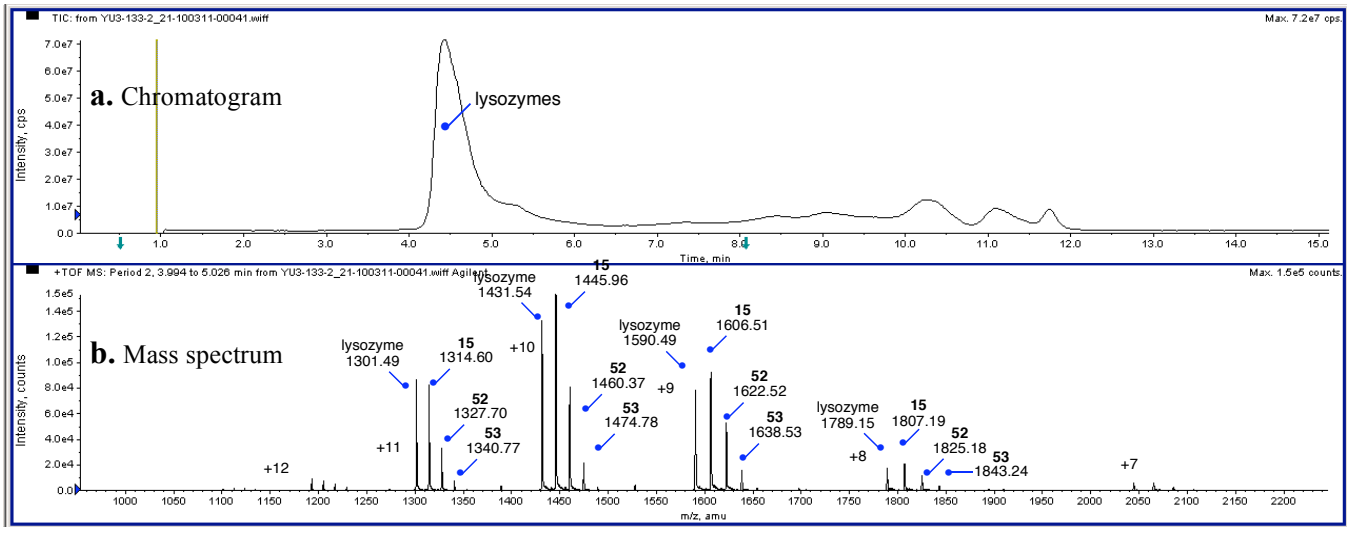
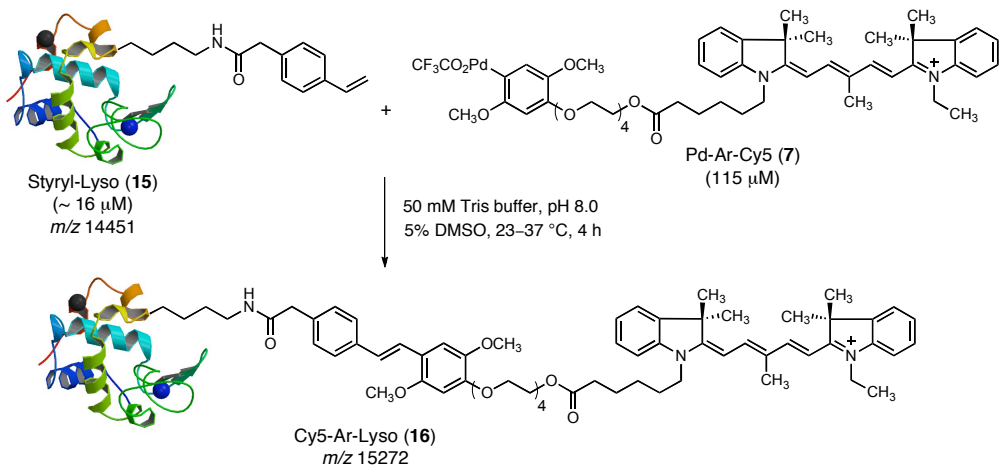



**Figure S1.** (a) Chromatogram of the lysozyme mixture. (b) The mass spectrum of the protein peak eluting from 4–5 min.

[10] Ho, C.-M.; Zhang, J.-L.; Zhou, C.-Y.; Chan, O.-Y.; Yan, J. J.; Zhang, F.-Y.; Huang, J.-S.; Che, C.-M. *J. Am. Chem. Soc.* **2010**, *132*, 1886–1894.
[11] Canfield, R. E. *J. Biol. Chem.* **1963**, *238*, 2698–2706.




Heck-type Coupling of Cy5 Reagent **7** with Styryl-modified Lysozyme. Dimethyl sulfoxide (4 μL) was added to a buffered solution of a mixture of styryl-modified lysozymes (125 μL of a 39 μM protein solution in 50 mM Tris buffer, pH 8.0), comprising approximately 34% of unmodified lysozyme, 40% of singly modified lysozyme, Styryl-Lyso (**15**), and 20% of doubly modified lysozyme, $(Styryl)_2$-Lyso (**52**), and 6% of triply modified lysozyme, $(Styryl)_3$-Lyso (**53**), at 23 °C in a 1.5 mL–microcentrifuge tube open to air. The solution was mixed briefly by vortexing at 23 °C. A solution of arylpalladium(II) reagent **7** in DMSO-$d_6$ (2 μL of a 7.5 mM solution, 0.015 μmol) was added at 23 °C. The solution was mixed briefly by vortexing at 23 °C. The microcentrifuge tube was then capped and placed on an end-over-end rotator at 37 °C (Final concentration: Styryl-Lyso (**15**) = 16 μM, Pd-Ar-Cy5 (**7**) = 115 μM, DMSO = 4.6%). After 4 h, a solution of DL-dithiothreitol (5.8 μL of a 25 mM solution in 50 mM Tris buffer, containing 2.5% Triton X-100, pH 8.0) was added at 23 °C to convert the remaining Pd-Ar-Cy5 (**7**) to the protiodepalladation product, H-Ar-Cy5 (**17**). After 30 min, a 35-μL aliquot of the reaction solution was extracted and analyzed by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The gel was visualized by fluorescence imaging and Coomassie staining (Figure 4b). The remaining reaction solution was immediately passed through a MicroSpin G-25 column (GE Healthcare) with 50 mM Tris buffer containing 0.1% Triton X-100 (pH 8.0) as the eluent (by re-hydrating the column with 250 μL of 50 mM Tris buffer containing 0.1% Triton X-100, pH 8.0, prior to the sample application). Analysis by LC/MS (Figure S2) confirmed the formation of Heck product Cy5-Ar-Lyso (**16**): calcd 15272 Da, found 15273.1±0.1. The conversion of Styryl-Lyso (**15**) to Cy5-Ar-Lyso (**16**) was estimated to be ~75% based on the respective ion extracted mass area (MA) in the LC/MS according to the equation, $MA_{Cy5\text{-}Ar\text{-}Lyso}/(MA_{Cy5\text{-}Ar\text{-}Lyso} + MA_{Styryl\text{-}Lyso})$. When unmodified lysozyme was exposed to analogous reaction conditions, no modification was observed (Figure S3).

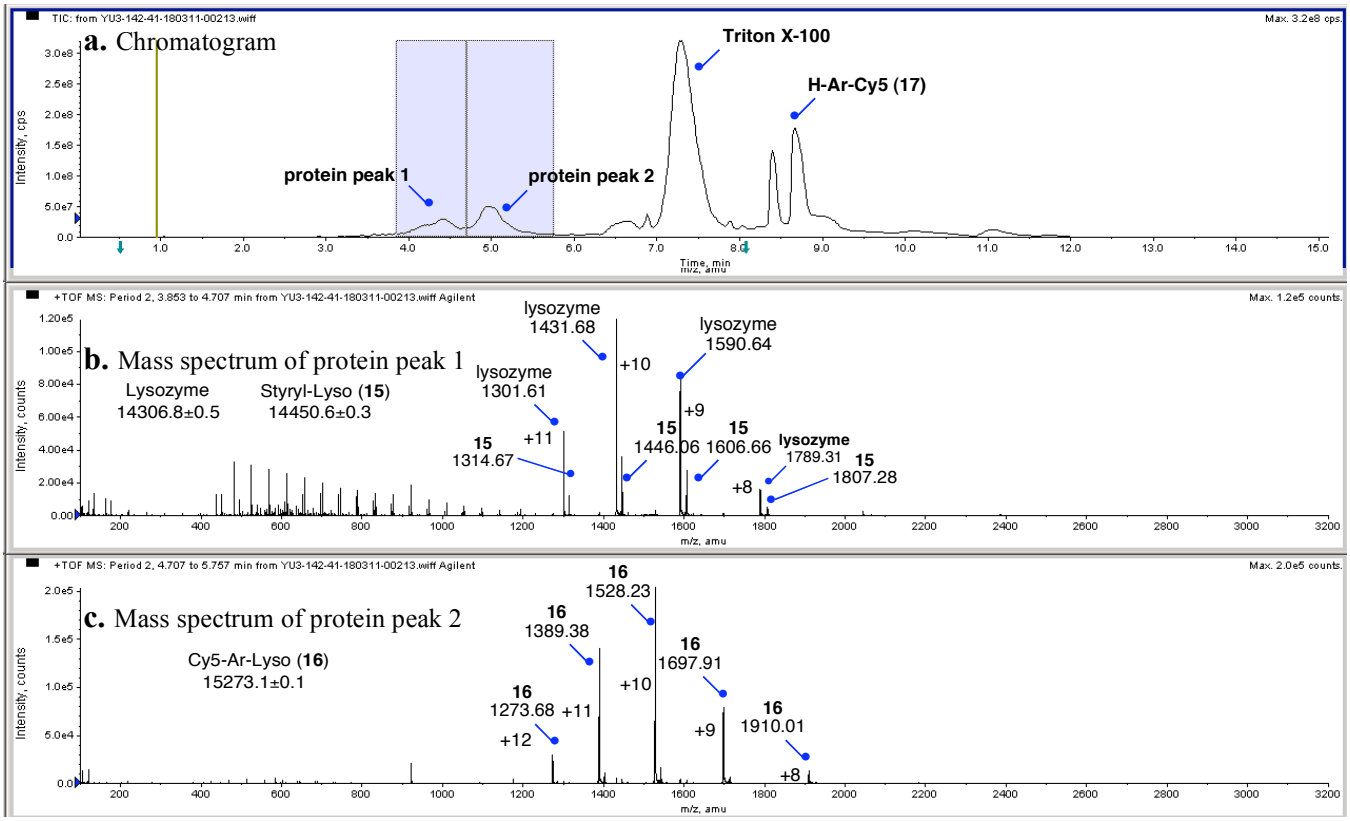



**Figure S2.** (a) Chromatogram obtained from the Heck-type coupling reaction between Styryl-Lyso (**15**) and Pd-Ar-Cy5 (**7**). (b) The mass spectrum of the protein peak 1, eluted from 3.9 to 4.7 min, consists of mainly the unmodified lysozyme and some unreacted Styryl-Lyso (**15**). (c) The mass spectrum of the protein peak 2, eluted from 4.7 to 5.8 min, consists primarily of the Heck product Cy5-Ar-Lyso (**16**).

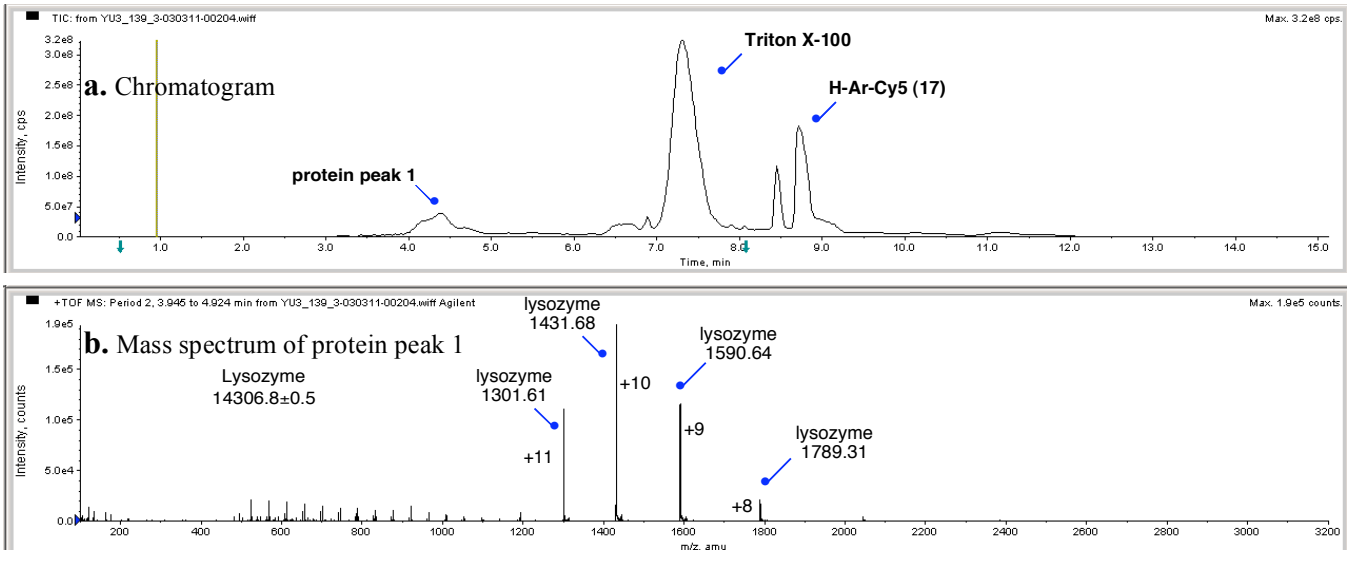



**Figure S3.** (a) Chromatogram after mixing un modified lysozyme with Pd-Ar-Cy5 (**7**). (b) The mass spectrum of the protein peak, eluted from 3.9 to 4.9 min, consists of only the unmodified lysozyme. No protein modification was observed.

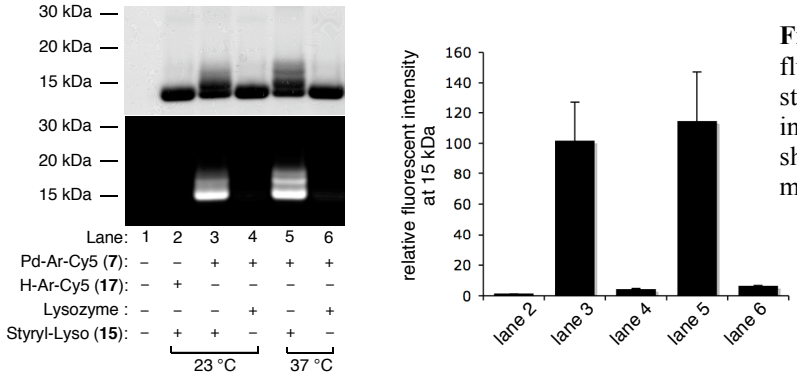



**Figure 4b.** SDS-PAGE with visualization by fluorescence imaging (bottom) and Coomassie staining (top) and, at right, the relative fluorescence intensities of lane2 2 to 6 at 15 kDa. Error bars show the standard deviation for three independent measurements.

**Affinity-enrichment Experiments involving Heck-type Coupling of Biotin Reagent 2 with Styryl-modified FK-506.**

**General Experimental Procedures.** Cell culture work was conducted in a class II biological safety cabinet. Buffers were filter-sterilized (0.2 μm) prior to use. Reagent **2** was prepared as a 15.2 mM stock solution in DMSO-$d_6$, and was stored frozen at –20 ºC as described previously. FK506 and compounds **18–19** were also stored as frozen 10 mM stocks in DMSO at –20 ºC.

**Materials.** Jurkat cells were a gift from the Verdine laboratory and were cultured in RPMI-1640 (ATCC or Mediatech) containing 10% fetal bovine serum (FBS). Chinese hamster ovary (CHO) cells were a gift from the Sames laboratory and were cultured in DMEM (Mediatech) containing 10% fetal bovine serum. Cells were grown in BD Falcon tissue culture flasks with vented caps. Bradford Reagent was purchased from Sigma Aldrich. The nuclear factor of activated T-cells (NFAT) reporter gene assay was conducted in pre-sterilized, 96-well, flat-bottomed plates from Nunc. Solutions of luciferin were purchased from Promega as the Bright-Glo Luciferase Assay System, and were used according to the manufacturer's instructions. The pGL3-NFAT luciferase plasmid was purchased from Addgene. The pGL3-control vector was purchased from Promega. Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed using pre-cast Novex tris–glycine mini gels (10–20% gradient, Invitrogen). Electrophoresis and semi-dry electroblotting equipment was purchased from Owl Separation Systems. Benchmark pre-stained protein ladders were purchased from Invitrogen. Nitrocellulose membranes were purchased from Amersham Biosciences. A mouse monoclonal antibody to FKBP12 was purchased from Abcam (ab58072). A rabbit monoclonal antibody to calcineurin A was purchased from Abcam (ab52761). A rabbit polyclonal antibody to calcineurin B was purchased from Santa Cruz Biotechnology (sc-33166). A mouse monoclonal antibody to calmodulin was purchased from Millipore (05-173). FK506 was purchased from LC Laboratories.

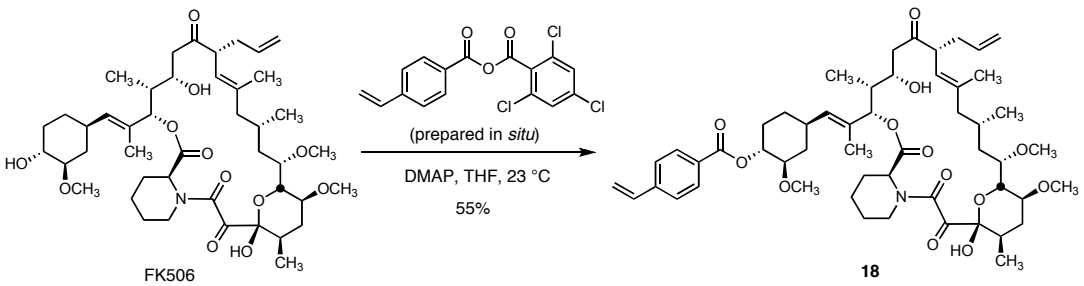
Western-blot detection was performed using the SuperSignal West Pico Chemiluminescence kit (including goat anti-rabbit-HRP and goat anti-mouse-HRP conjugates) from Pierce. Western blots were visualized using CL-XPosure X-ray film from Pierce, or were imaged on an AlphaImager. Streptavidin–agarose and protease inhibitor cocktail were purchased from Sigma Aldrich. Lipofectamine RNAiMax was purchased from Invitrogen (P/N56531).

**Preparation of Solutions.**

| Lysis Buffer | TBS-TX Buffer | Tris Buffer |
|---|---|---|
| 50 mM Tris (pH 7.5) | 50 mM Tris (pH 8.0) | 50 mM Tris (pH 7.5) |
| 100 mM potassium chloride | 100 mM potassium chloride | |
| 1 mM β-mercaptoethanol | 0.1% Triton X-100 | TBS-T Buffer |
| 0.1% Triton X-100 | 1% protease inhibitor cocktail | 0.1% TWEEN 20 |
| 1% protease inhibitor cocktail | | in TBS |

**Preparation of Resins.** A 2-mL aliquot of streptavidin–agarose suspension was transferred to a 15-mL centrifuge tube. TBS-TX (1 mL) was added, a cap was affixed and the tube was centrifuged at 1643 x g (5 min, 23 ºC). The supernatant was removed and discarded. TBS-TX (1 mL) was added, a cap was affixed and the tube was rotated end-over-end at 23 ºC for 5 min. The resin was isolated by centrifugation (1643 x g, 5 min, 23 ºC), and the supernatant was discarded. The resin was washed twice more with 1 mL of TBS-TX, then was suspended in 1 mL TBS-TX and mixed thoroughly prior to use.

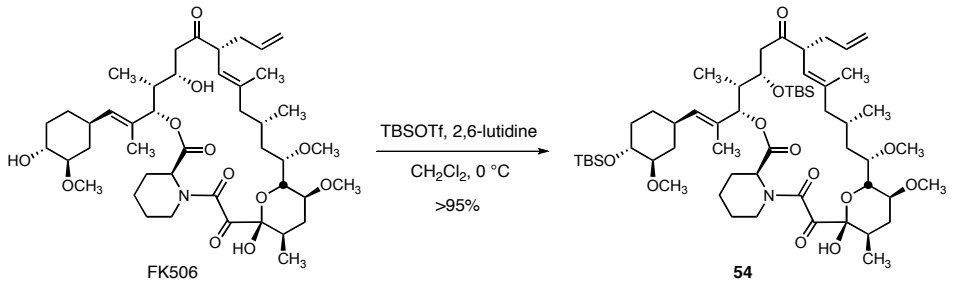
**Synthesis of FK506-based Affinity Probes.**




FK506 **18**

Styryl–FK506 (**18**). To an ice-cooled solution of 4-vinylbenzoic acid (2.0 mg, 13.7 µmol, 2.2 equiv) in tetrahydrofuran (622 µL) was added triethylamine (3 µL, 18.7 µmol, 3.0 equiv) followed by 2,4,6-trichlorobenzoyl chloride (2 µL, 13.7 µmol, 2.2 equiv). The reaction solution was stirred at 0 °C for 70 minutes, providing a stock supply of the mixed anhydride depicted in the equation above. An aliquot of the mixed anhydride solution (311 µL) was added by syringe to a second, 25-mL round-

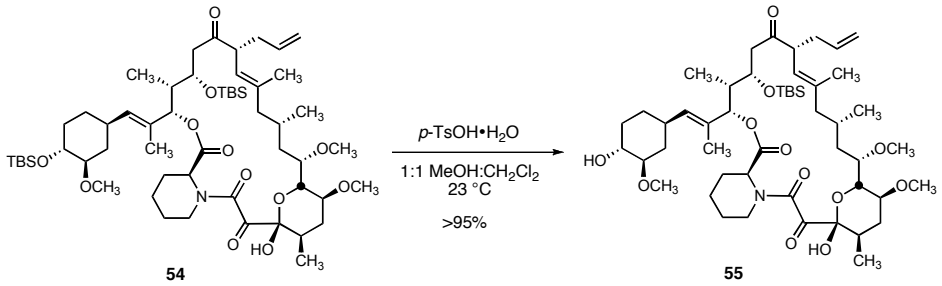
bottomed flask containing FK506 (5.0 mg, 6.22 μmol, 1 equiv) and 4-dimethylaminopyridine (0.80 mg, 6.22 μmol, 1 equiv) in tetrahydrofuran (311 μL) at 0 °C. The resulting pale yellow solution was stirred at 0 °C for 30 min, then was allowed to warm to 23 °C. After 1.5 h, the remainder of the mixed anhydride solution (311 μL) was added to the reaction solution by syringe. After 2 h, the product suspension was poured into saturated aqueous sodium bicarbonate solution (15 mL). The aqueous layer was separated and washed with ethyl acetate (2 x 10 mL). The combined organic phases were washed with brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (20% ethyl acetate–hexanes grading to 40% ethyl acetate–hexanes) to afford the Styryl–FK506 (**18**) as a colorless oil (3.1 mg, 55%). TLC: (50% ethyl acetate–hexanes) $R_f$ = 0.72 (CAM). $^1$H NMR (500 MHz, $CDCl_3$, 1.8:1 mixture of rotamers, asterisk denotes peaks associated with the minor rotamer) δ: 8.02 (d, 2H, *J* = 8.0 Hz), 7.48 (d, 2H, *J* = 8.5 Hz), 6.77 (dd, 1H, *J* = 17.5, 10.8 Hz), 5.88 (d, 1H, *J* = 17.5 Hz), 5.78–5.69 (m, 1H), 5.40 (d, 1H, *J* = 11.0 Hz), 5.36 (br s, 1H), 5.22* (br s, 1H), 5.12–4.93 (m, 5H), 4.89* (s, 1H), 4.64 (d, 1H, *J* = 4.5 Hz), 4.45 (d, 1H, *J* = 13.5 Hz), 4.23 (s, 1H), 3.97–3.89 (m, 2H), 3.78* (d, 1H, *J* = 12.0 Hz), 3.69 (d, 1H, *J* = 9.5 Hz), 3.61–3.58 (m, 1H), 3.51–3.32 (m, 12H), 3.16* (d, 1H, *J* = 2.5 Hz), 3.07–3.02 (m, 1H), 2.81 (dd, 1H, *J* = 16.0, 2.5 Hz), 2.75* (d, 1H, *J* = 17.0 Hz), 2.54–0.84 (m, 38H). $^{13}$C NMR (125 MHz, $CDCl_3$, peaks associated with the minor rotameric form are included) δ: 213.2, 213.1, 196.4, 192.6, 169.2, 168.9, 166.1, 164.9, 142.1, 140.1, 139.3, 136.3, 135.8, 135.6, 133.0, 132.3, 130.2, 130.0, 129.2, 129.0, 126.3, 122.8, 122.6, 116.9, 116.7, 98.9, 97.2, 81.2, 76.8, 76.7, 75.4, 74.0, 73.9, 73.1, 72.4, 70.4, 69.4, 58.3, 58.2, 57.8, 57.2, 56.9, 56.6, 56.4, 53.2, 53.0, 48.7, 48.6, 44.1, 43.8, 43.0, 40.4, 39.9, 39.5, 37.0, 36.9, 36.0, 35.8, 35.3, 35.0, 34.9, 34.8, 33.8, 33.0, 32.8, 30.8, 30.1, 28.0, 26.5, 26.4, 26.2, 24.9, 24.8, 23.6, 21.5, 21.1, 20.8, 19.6, 16.5, 16.3, 16.0, 14.7, 14.6, 10.0, 9.6. FTIR (neat), cm$^{-1}$: 2936 (m), 1742 (m), 1713 (s), 1651 (s), 1450 (m), 1273 (s), 1101 (s). HRMS (ESI): calcd for $(C_{53}H_{75}NO_{13} + NH_4)^+$ 951.5577, found 951.5578.



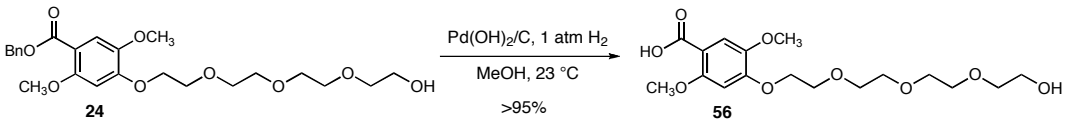

Bis-(*tert*-butyldimethylsilyl) ether **56**. The following is a modification of the procedure of Tamura et al.[12] *tert*-Butyldimethylsilyl trifluoromethanesulfonate (33.0 μL, 144 μmol, 3.5 equiv) was added by syringe to an ice-cooled solution of FK506 (33.0 mg, 41.0 μmol, 1 equiv) and 2,6-lutidine (24.0 μL, 205 μmol, 5.0 equiv) in dichloromethane (684 μL). The resulting clear yellow solution was stirred at 0 °C for 45 min, then a second portion of *tert*-butyldimethylsilyl trifluoromethanesulfonate (10.0 μL, 43.5 μmol, 1 equiv) was added. The reaction solution was stirred at 0 °C for 15 min. Methanol (250 μL) was added and the product solution was allowed to warm to 23 °C. Ethyl acetate (20 mL) was added and the resulting solution was washed sequentially with saturated aqueous sodium bicarbonate solution (15 mL), water (15 mL) and brine (10 mL). The organic layer was dried over anhydrous sodium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (25% ethyl acetate–hexanes) to afford the bis-(*tert*-butyldimethylsilyl) ether **13** as a colorless oil (43.1 mg, >95%). TLC: (25% ethyl acetate–hexanes) $R_f$ = 0.42 (CAM). $^1$H NMR (500 MHz, $CDCl_3$, 1.8:1.0 mixture of rotamers, asterisk denotes peaks associated with the minor rotamer) δ: 5.76–5.64 (m, 1H), 5.50* (br s, 1H), 5.27* (d, 1H, *J* = 10.0 Hz), 5.26 (br s, 1H), 5.21 (d, 1H, *J* = 7.0 Hz), 5.14* (d, 1H, *J* = 10.0 Hz), 5.04–4.96 (m, 2H), 4.83 (d, 1H, *J* = 10.0 Hz), 4.43–4.40 (m, 1H), 4.25* (p, 1H, *J* = 3.8 Hz), 4.20 (br s, 1H), 4.09–4.06 (m, 1H), 3.95* (dd, 1H, *J* = 9.8, 2.2 Hz), 3.91* (br d, 1H, *J* = 13.5 Hz), 3.81 (dd, 1H, *J* = 9.5, 1.0 Hz), 3.63–3.57 (m, 1H), 3.51–3.46 (m, 2H), 3.43–3.25 (m, 12H), 3.12 (br t, 1H, *J* = 12.8 Hz), 2.99–2.93 (m, 1H), 2.81–2.77 (m, 1H), 2.56–0.74 (m, 56H), 0.09 (s, 3H), 0.09* (s, 3H), 0.08 (s, 3H), 0.07* (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$, peaks associated with the minor rotameric form are included) δ: 210.4, 209.6, 196.8, 191.3, 169.2, 168.4, 166.0, 164.8, 139.7, 138.6, 136.4, 135.8, 132.7, 131.7, 130.0, 123.4, 122.3, 116.7, 116.3, 99.0, 97.8, 84.4, 76.6, 75.6, 75.4, 73.9, 73.8, 73.0, 71.8, 71.2, 69.9, 58.3, 58.2, 57.7, 57.4, 56.6, 56.5, 54.0, 53.9, 52.9, 49.5, 48.5, 44.7, 43.8, 41.4, 40.8, 39.3, 36.9, 36.6, 36.3, 35.8, 35.3, 35.1, 35.0, 34.8, 34.1, 34.0, 33.5, 33.0, 32.8, 31.1, 30.9, 27.9, 26.7, 26.4, 26.1, 26.0, 25.6, 24.9, 24.8, 24.5, 23.6, 21.1, 20.8, 19.7, 19.0, 18.4, 18.2, 18.1, 16.8, 16.4, 16.3, 15.7, 14.5, 10.5, –4.0, –4.1, –4.2, –4.3, –4.5, –4.7. FTIR

[5] Tamura, T.; Terada, T.; Tanaka, A. *Bioconjugate Chem*. **2003**, *14*, 1222–1230.

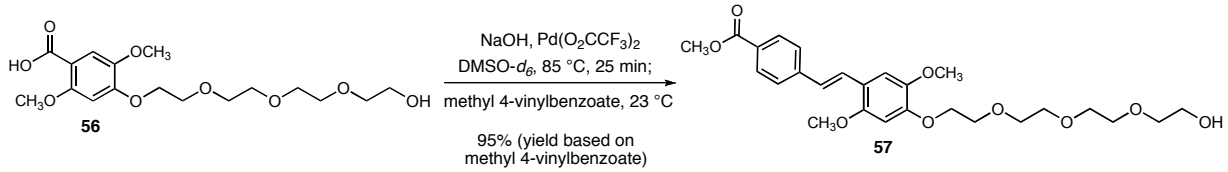
(neat), $cm^{-1}$: 2930 (s), 2859 (m), 1748 (w), 1717 (w), 1653 (s), 1460 (m), 1450 (m), 1103 (s). HRMS (ESI): calcd for $(C_{56}H_{97}NO_{12}Si_2 + Na)^+$ 1054.6442, found 1054.6437.




<u>*tert*-Butyldimethylsilyl ether **55**</u>. *p*-Toluenesulfonic acid monohydrate (2.0 mg, 10.4 μmol, 0.25 equiv) was added to a solution of the bis-(*tert*-butyldimethylsilyl) ether **54** (43.1 mg, 41.7 μmol, 1 equiv) in dichloromethane–methanol (1:1, 1.04 mL), forming a pale yellow solution. After 90 min, the reaction solution was diluted with ethyl acetate (30 mL). The diluted product solution was washed sequentially with saturated aqueous sodium bicarbonate solution (25 mL), water (25 mL), and brine (20 mL). The washed solution was dried over anhydrous sodium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (50% ethyl acetate–hexanes grading to 80% ethyl acetate–hexanes) to afford the *tert*-butyldimethylsilyl ether **55** as a colorless oil (37.6 mg, >95%). TLC: (80% ethyl acetate–hexanes) $R_f$ = 0.27 (CAM). $^1$H NMR (500 MHz, $CDCl_3$, 1.6:1.0 mixture of rotamers, asterisk denotes peaks associated with the minor rotamer) δ: 5.75–5.65 (m, 1H), 5.48* (br s, 1H), 5.29 (d, 1H, *J* = 9.0 Hz), 5.27* (br s, 1H), 5.22 (d, 1H, *J* = 7.0 Hz), 5.14* (d, 1H, *J* = 9.0 Hz), 5.06–4.96 (m, 2H), 4.82 (d, 1H, *J* = 10.0 Hz), 4.43–4.42 (m, 1H), 4.26–4.25* (m, 1H), 4.21* (br s, 1H), 4.07 (br s, 1H), 3.96–3.90 (m, 1H), 3.82 (d, 1H, *J* = 9.5 Hz), 3.63–3.58 (m, 1H), 3.51–3.46 (m, 2H), 3.43–3.25 (m, 12H), 3.16–3.11 (m, 1H), 3.04–3.00 (m, 1H), 2.81–2.77 (m, 1H), 2.56–0.74 (m, 47H), 0.05 (s, 3H), 0.04 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$, peaks associated with the minor rotameric form are included) δ: 210.4, 209.6, 196.7, 191.4, 169.2, 168.5, 166.0, 164.8, 139.7, 138.7, 136.4, 135.8, 133.0, 132.0, 129.8, 123.4, 122.3, 116.8, 116.4, 98.9, 97.8, 84.3, 76.5, 75.6, 73.9, 73.8, 73.7, 73.0, 71.9, 71.2, 69.9, 64.6, 57.7, 57.4, 56.8, 56.6, 56.5(8), 56.5, 54.0, 53.9, 52.9, 49.5, 48.5, 44.8, 43.9, 41.5, 40.8, 39.3, 36.2, 35.8, 35.2, 35.1, 35.0(9), 35.0, 34.8, 33.6, 33.0, 32.8, 31.5, 31.4, 30.9, 30.6, 27.9, 26.7, 26.4, 26.1, 26.0, 25.6, 24.9, 24.5, 21.1, 20.8, 19.7, 19.4, 19.0, 18.2, 18.1, 16.8, 16.4, 16.3, 15.7, 14.5, 10.6, –4.0, –4.1, –4.2, –4.6. FTIR (neat), $cm^{-1}$: 2932 (s), 1746 (m), 1717 (m), 1651 (s), 1450 (m), 1092 (s). HRMS (ESI): calcd for $(C_{50}H_{83}NO_{12}Si + NH_4)^+$ 935.6023, found 935.6025.

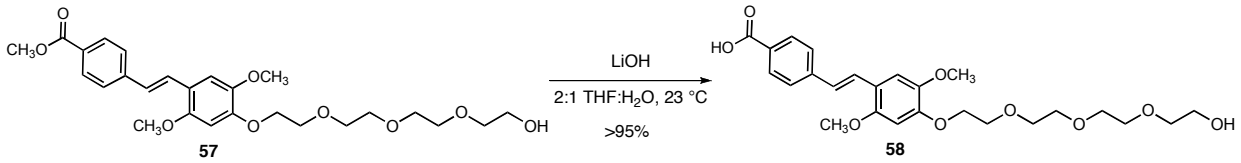



Benzoic acid derivative **56**. Palladium(II) hydroxide on carbon (20% w/w, 121.2 mg, 0.173 mmol, 0.2 equiv) was added to a stirring solution of the benzyl ester **24**[Error! Bookmark not defined.] (402.0 mg, 0.865 mmol, 1 equiv) in methanol (28.8 mL) in a 50-mL round-bottomed flask. The flask was evacuated, then filled with hydrogen (repeated twice more). A hydrogen balloon (1 atm) was affixed and the reaction mixture was stirred for 2 h 15 min at 23 °C. The black product suspension was filtered through a pad of Celite, rinsing with ethyl acetate (30 mL). The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography (5% methanol–dichloromethane) to provide the benzoic acid derivative **56** as a colorless oil (322.5 mg, >95%). TLC: (5% methanol–dichloromethane) $R_f$ = 0.19 (CAM); $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.63 (s, 1H), 6.71 (s, 1H), 4.30 (t, 2H, *J* = 5.0 Hz), 4.05 (s, 3H), 3.93 (t, 2H, *J* = 5.0 Hz), 3.88 (s, 3H), 3.76–3.65 (m, 10H), 3.61 (t, 2H, *J* = 4.8 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 165.5, 154.0, 153.6, 144.7, 115.2, 109.6, 98.7, 72.7, 71.1, 70.8, 70.7(9), 70.5, 69.8, 69.2, 61.9, 57.6, 56.6. FTIR (neat), cm$^{-1}$: 3460 (w), 3271 (w), 2872 (m), 1717 (s), 1611 (s). HRMS (ESI): calcd for $(C_{17}H_{26}O_9 + H)^+$ 397.1469, found, 397.1489.



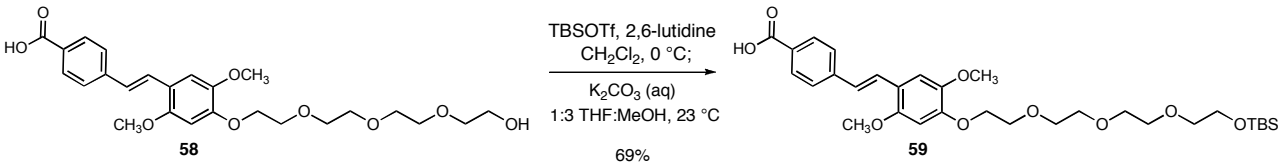

Stilbene derivative **57**. The benzoic acid derivative **56** (209 mg, 0.558 mmol, 1.2 equiv) and solid palladium(II) trifluoroacetate (556 mg, 1.67 mmol, 3.0 equiv) were added in sequence to a 0.22 M solution of sodium hydroxide in DMSO-$d_6$ (5.10 mL, 1.12 mmol, 2.0 equiv) in a 20-mL scintillation vial. The resulting brown solution was heated in an oil bath at 80 °C for 25 min open to the air. The oil bath was removed and the opaque, brown solution was allowed to cool to 23 °C. Solid methyl 4-vinylbenzoate (72.4 mg, 0.446 mmol, 1 equiv) was added and the mixture was stirred at 23 °C for 21 h. Solid DL-dithiothreitol (258.1 mg, 1.673 mmol, 3.0 equiv) was added to the product mixture. After 5 min, the resulting orange suspension was filtered through a pad of Celite, rinsing with ethyl acetate (40 mL). The filtrate was poured directly into 1.0 N aqueous hydrochloric acid solution (50 mL) and the layers were separated. The aqueous layer was washed with ethyl acetate (30 mL). The organic layers were combined, and the combined solution was washed with water (50 mL). The washed solution was dried over anhydrous sodium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (2.5% methanol–dichloromethane), affording the stilbene

derivative **57** as a neon yellow solid (150.3 mg, 95%). TLC: (5% methanol–dichloromethane) $R_f$ = 0.44 (CAM); $^1$H NMR (500 MHz, $CDCl_3$. 19:1 *E:Z* olefin isomers, asterisk denotes peaks associated with the *Z* stereoisomer) δ: 8.01 (d, 2H, *J* = 8.5 Hz), 7.90* (d, 2H, *J* = 8.5 Hz), 7.57 (d, 2H, *J* = 8.5 Hz), 7.54 (d, 1H, *J* = 16.5 Hz), 7.35* (d, 2H, *J* = 8.5 Hz), 7.14 (s, 1H), 7.01 (d, 1H, *J* = 16.5 Hz), 6.77* (d, 1H, *J* = 12.0 Hz), 6.67* (s, 1H), 6.63 (s, 1H), 6.60* (s, 1H), 6.57* (d, 1H, *J* = 12.5 Hz), 4.26 (t, 2H, *J* = 5.2 Hz), 3.93 (s, 3H), 3.92 (t, 2H, *J* = 5.0 Hz), 3.90 (s, 3H), 3.88 (s, 3H), 3.78–3.69 (m, 10H), 3.62 (t, 2H, *J* = 4.5 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 167.2, 152.4, 149.8, 144.2, 143.0, 130.2, 128.5, 126.3, 126.0, 125.9, 118.5, 110.6, 100.1, 72.7, 71.0, 70.9, 70.8, 70.6, 70.0, 69.1, 62.0, 57.0, 56.7, 52.2. FTIR (neat), cm$^{-1}$: 3460 (w), 2931 (m), 2868 (m), 1715 (s), 1599 (s). HRMS (ESI): calcd for $(C_{26}H_{34}O_9 + H)^+$ 491.2276, found, 491.2284.

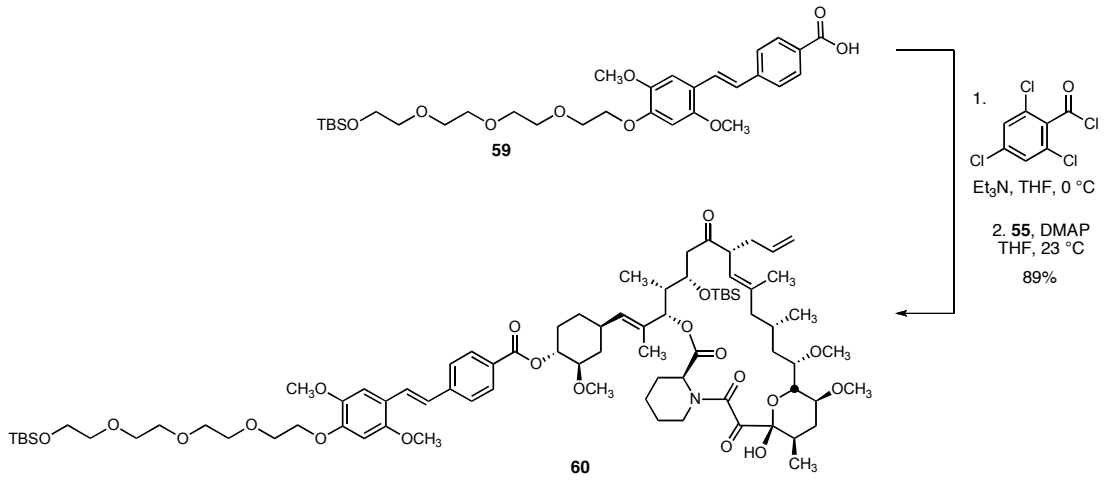



**57** **58**

Hydroxy carboxylic acid **58**. Solid lithium hydroxide (18.4 mg, 0.766 mmol, 2.5 equiv) was added to a stirring solution of the stilbene derivative **17** (150.3 mg, 0.306 mmol, 1 equiv) in a 2:1 mixture of tetrahydrofuran–water (6.13 mL) open to the air, forming a neon yellow suspension. After stirring in the dark at 23 °C for 16 h, a second portion of lithium hydroxide (35.0 mg, 1.458 mmol, 4.8 equiv) was added and the reaction mixture was stirred for an additional 30 h at 23 °C. The product mixture was poured into 1.0 N aqueous sodium hydroxide solution (25 mL) and dichloromethane (25 mL) was added. The layers were separated. The aqueous layer was acidified to pH 2 with 6 N aqueous sulfuric acid solution and the acidified solution was extracted with dichloromethane (8 x 40 mL). The organic phases were combined and the combined solution was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo to afford the hydroxy carboxylic acid **58** as a neon yellow solid (146.0 mg, >95%). TLC: (5% methanol–dichloromethane) $R_f$ = 0.13 (CAM); $^1$H NMR (500 MHz, $CDCl_3$, 12:1 *E:Z* olefin isomers, asterisk denotes peaks associated with the *Z* stereoisomer) δ: 8.06 (d, 2H, *J* = 8.5 Hz), 7.96* (d, 2H, *J* = 7.5 Hz), 7.59 (d, 2H, *J* = 9.0 Hz), 7.56 (d, 1H, *J* = 16.5 Hz), 7.38* (d, 2H, 8.5 Hz), 7.14 (s, 1H), 7.01 (d, 1H, *J* = 16.5 Hz), 6.80* (d, 1H, *J* = 12.5 Hz), 6.67* (s, 1H), 6.62 (s, 1H), 6.60* (s, 1H), 6.59* (d, 1H, *J* = 12.5 Hz), 4.26 (t, 2H, *J* = 5.2 Hz), 3.93 (t, 2H, *J* = 5.2 Hz), 3.90 (s, 3H), 3.87 (s, 3H), 3.78–3.70 (m, 10H), 3.64 (t, 2H, *J* = 4.5 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 171.1, 152.5, 150.0, 144.2, 143.7, 130.8, 127.6, 126.4, 126.3, 125.9, 118.4, 110.8, 100.1, 72.7, 71.1, 70.9, 70.8, 70.6, 70.0, 69.2, 62.0, 57.1, 56.7. FTIR (neat), cm$^{-1}$: 2926 (m), 2866 (m), 1713 (s), 1688 (s), 1599 (s), 1211 (s). HRMS (ESI): calcd for $(C_{25}H_{32}O_9 + H)^+$ 477.2119, found, 477.2106.



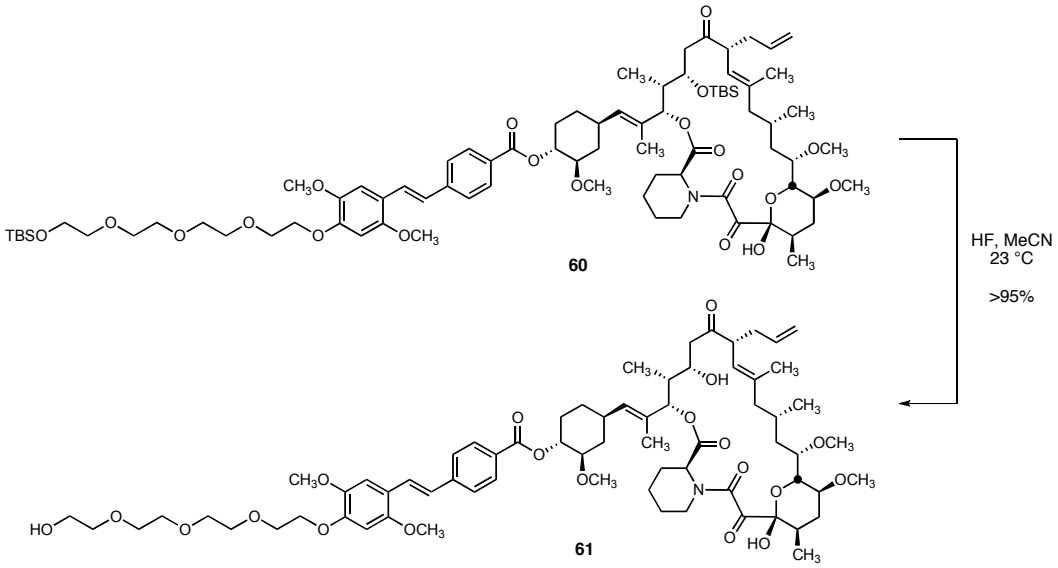

*tert*-Butyldimethylsilyloxy carboxylic acid **59**. To an ice-cooled solution of the hydroxy carboxylic acid **58** (146.0 mg, 0.306 mmol, 1 equiv) in dichloromethane (6.00 mL) was added 2,6-lutidine (210 µL, 1.80 mmol, 6.0 equiv) followed by *tert*-butyldimethylsilyl trifluoromethanesulfonate (248 µL, 1.08 mmol, 3.5 equiv), producing a neon yellow solution. After stirring in the dark at 0 °C for 60 min, additional portions of 2,6-lutidine (105 µL, 0.900 mmol, 3.0 equiv) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (125 µL, 0.544 mmol, 1.8 equiv) were added. After 30 min methanol (1.00 mL) was added. The product solution was allowed to warm to 23 °C, then was poured into saturated aqueous ammonium chloride solution (25 mL). The layers were separated. The aqueous phase was washed with dichloromethane (3 x 25 mL). The organic phases were combined and dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo. The residue was dissolved in a 1:3 mixture of tetrahydrofuran–methanol (3.60 mL) and the resulting solution was treated with 0.72 M aqueous potassium carbonate solution (1.00 mL, 0.720 mmol, 2.4 equiv). The neon yellow solution was stirred in the dark at 23 °C for 30 min. The product mixture was poured into a separatory funnel containing brine (30 mL) and sufficient 2 M aqueous sodium bisulfate solution was added to achieve pH 2. The acidified solution was washed with dichloromethane (7 x 25 mL). The organic phases were combined and dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (50% ethyl acetate–hexanes grading to 75% ethyl acetate–hexanes) to afford the *tert*-butyldimethylsilyloxy carboxylic acid **59** as a neon yellow solid (125.3 mg, 69%). TLC: (100% ethyl acetate) $R_f$ = 0.35 (CAM); $^1$H NMR (500 MHz, $CDCl_3$, 19:1 *E:Z* olefin isomers, asterisk denotes peaks associated with the *Z* stereoisomer) δ: 8.08 (d, 2H, *J* = 8.5 Hz), 7.97* (d, 2H, *J* = 8.5 Hz), 7.60 (d, 2H, *J* = 8.5 Hz), 7.57 (d, 1H, *J* = 17.0 Hz), 7.39* (d, 2H, *J* = 8.5 Hz), 7.14 (s, 1H), 7.02 (d, 1H, *J* = 16.5 Hz), 6.80* (d, 1H, *J* = 14.5 Hz), 6.67* (s, 1H), 6.63 (s, 1H), 6.59* (d, 1H, *J* = 13.5 Hz), 4.26 (t, 2H, *J* = 5.0 Hz), 3.92 (t, 2H, *J* = 5.2 Hz), 3.90 (s, 3H), 3.88 (s, 3H), 3.79–3.67 (m, 10H), 3.57 (t, 2H, *J* = 5.5 Hz), 0.90 (s, 9H), 0.08 (s, 6H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 171.4, 152.5, 150.0, 144.2, 143.8, 130.8, 127.6, 126.4, 126.3(5), 125.9, 118.4, 110.8, 100.1, 72.9, 71.1, 71.0, 70.9, 70.0, 69.2, 63.0, 57.1, 56.7, 26.2, 18.6, –5.0. FTIR (neat), cm$^{-1}$: 2928 (m), 2859 (m), 1715 (m), 1686 (m), 1599 (m), 1211 (s), 1101 (s). HRMS (ESI): calcd for $(C_{31}H_{46}O_9Si + H)^+$ 591.2984, found, 591.2980.




Bis-(*tert*-butyldimethylsilyl) ether **60**. The following is a modification of the procedure of Banaszynski et al.[13] A solution of the *tert*-butyldimethylsilyloxy carboxylic acid **59** (113 mg, 191 μmol, 5.0 equiv) in tetrahydrofuran (1.91 mL) was added in 7 273-μL aliquots every ten minutes for 1 h to an ice-cooled solution of 2,4,6-trichlorobenzoyl chloride (29.8 μL, 191 μmol, 5.0 equiv) and triethylamine (53.1 μL, 381 μmol, 10.0 equiv) in tetrahydrofuran (1.91 mL) in a 25-mL round-bottomed flask. After the final addition, the neon yellow solution was stirred in the dark at 0 °C for 30 min. In a separate 25-mL round-bottomed flask, tetrahydrofuran (1.90 mL), 4-dimethylaminopyridine (4.6 mg, 38.1 μmol, 1 equiv), and the mono-(*tert*-butyldimethylsilyl) ether **14** (35.0 mg, 38.1 μmol, 1 equiv) were combined. The resulting solution was transferred to the flask containing the mixed anhydride by cannula and the transfer was quantitated with a tetrahydrofuran rinse (950 μL). The neon yellow suspension was heated at 55 °C for 3 h, then the mixture was allowed to cool to 23 °C. The product solution was diluted with ethyl acetate (35 mL) and the diluted mixture was washed sequentially with saturated aqueous sodium bicarbonate (30 mL), water (30 mL), and brine (20 mL). The organic layer was collected and dried over anhydrous sodium sulfate. The dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (40% ethyl acetate–hexanes grading to 60% ethyl acetate–hexanes) to afford the bis-(*tert*-butyldimethylsilyloxy) ether **60** as a neon yellow oil (50.7 mg, 89%). TLC: (80% ethyl acetate–hexanes) $R_f$ = 0.46 (CAM). $^1$H NMR (500 MHz, $CDCl_3$, 12:1 *E:Z* olefin isomers, 1.7:1:0 mixture of rotamers, asterisk denotes peaks associated with the *Z* stereoisomer, double asterisk denotes peaks associated with the minor rotamer) δ: 8.01 (d, 2H, *J* = 8.0 Hz), 8.00** (d, 2H, *J* = 9.0 Hz), 7.89* (d, 2H, *J* = 8.0 Hz), 7.56 (d, 2H, *J* = 8.0 Hz), 7.53 (d, 1H, *J* = 16.5 Hz), 7.36* (d, 2H, *J* = 8.0 Hz), 7.13 (s, 1H), 7.03** (d, 1H, *J* = 16.0 Hz), 7.00 (d, 1H, *J* = 16.5 Hz), 6.76* (d, 1H, *J* = 12.5 Hz), 6.67* (s, 1H), 6.62 (s, 1H), 6.59* (s,

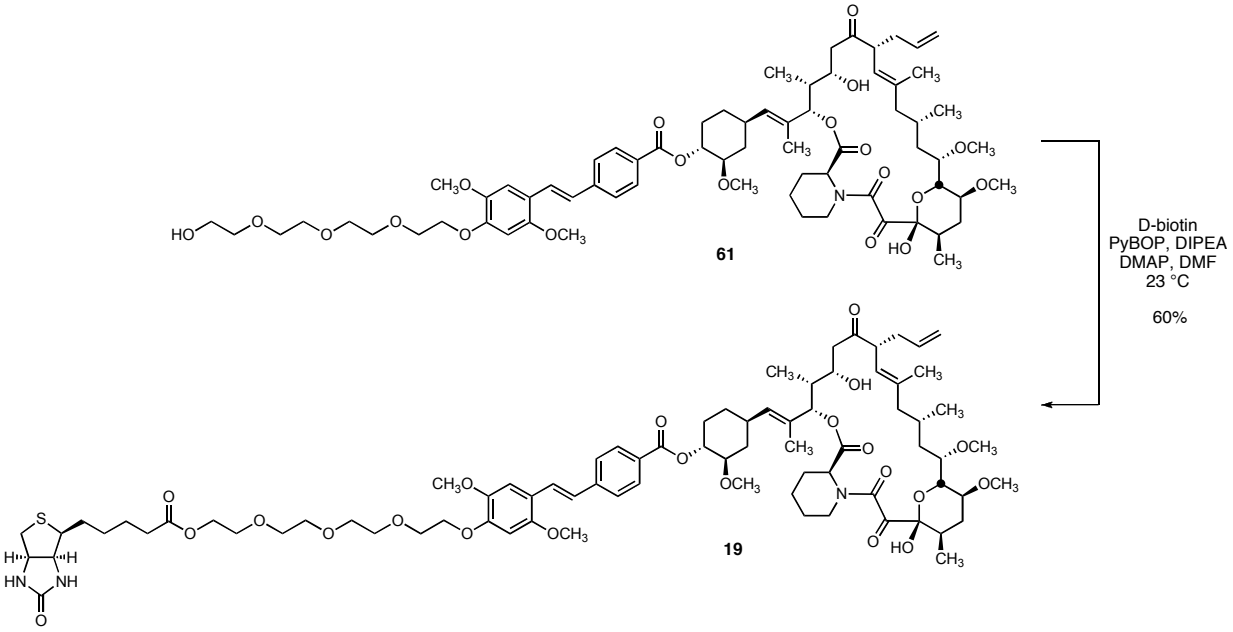
[13] Banaszynski, L. A.; Liu, C. W.; Wandless, T. J. *J. Am. Chem. Soc.* **2005**, *127*, 4715–4721.

1H), 6.56* (d, 1H, *J* = 13.0 Hz), 5.75–5.63 (m, 1H), 5.55** (br s, 1H), 5.28 (d, 1H, *J* = 9.0 Hz), 5.25 (br s, 1H), 5.23** (d, 1H, *J* = 6.5 Hz), 5.15** (d, 1H, *J* = 10.5 Hz), 5.05–4.91 (m, 2H), 4.83 (d, 1H, *J* = 10.5 Hz), 4.43–4.40 (m, 1H), 4.27–4.21** (m, 1H), 4.24 (t, 2H, *J* = 5.0 Hz), 4.19** (br s, 1H), 4.09 (br s, 1H), 3.96–3.54 (m, 23H), 3.50–3.24 (m, 12H), 3.15–3.08 (m, 1H), 2.79–2.75 (m, 1H), 2.56–1.14 (m, 29H), 1.02** (d, 3H, *J* = 6.5 Hz), 0.96 (d, 3H, *J* = 7.0 Hz), 0.90–0.84 (m, 27H), 0.82** (d, 3H, *J* = 5.5 Hz), 0.06–0.03 (m, 12H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 210.5, 209.7, 196.7, 191.2, 169.2, 168.4, 166.2, 166.1, 164.8, 152.4, 149.9, 144.3, 142.9, 139.8, 138.7, 136.4, 135.8, 133.2, 132.4, 130.2, 129.0, 126.3, 126.1, 125.8, 123.3, 122.3, 118.5, 116.8, 116.4, 110.7, 100.2, 99.0, 97.8, 81.1, 76.7, 76.6, 76.5, 75.6, 73.9, 73.8, 73.1, 72.9, 71.8, 71.1, 71.0, 70.9, 70.0, 69.2, 63.0, 58.2, 58.0, 57.7, 57.4, 57.1, 56.7, 56.5, 54.0, 53.8, 52.9, 49.4, 48.5, 44.8, 43.9, 41.3, 40.8, 39.3, 37.0, 36.9. 36.7, 36.3, 35.8, 35.1, 35.0, 34.8, 33.6, 33.0, 32.8, 30.8, 30.6, 30.2, 30.1, 29.9, 27.8, 26.8, 26.4, 26.2, 26.1, 26.0(7), 25.7, 24.9, 24.5, 21.1, 21.0, 19.8, 19.0, 18.6, 18.2, 18.1, 16.8, 16.4, 16.3, 15.8, 14.6, 12.8, 10.5, –4.0, –4.0(4), –4.2, –4.6, –5.0. FTIR (neat), cm$^{-1}$: 2930 (m), 2859 (w), 1746 (w), 1713 (m), 1651 (w), 1601 (w), 1450 (m), 1271 (s), 1099 (s), 835 (s). HRMS (ESI): calcd for $(C_{81}H_{127}NO_{20}Si_2 + NH_4)^+$ 1507.8828, found 1507.8840.




Alcohol **61**. A 48% solution of aqueous hydrofluoric acid (111 μL) was added to a Teflon reaction vessel containing a neon yellow solution of the bis-(*tert*-butyldimethylsilyloxy) ether **60** (39.8 mg, 26.7 μmol, 1 equiv) in acetonitrile (2.56 mL) open to the air. The reaction solution was stirred in the dark for 2.5 h at 23 °C. Ethyl acetate (20 mL) was added. The organic layer was washed sequentially with saturated aqueous sodium bicarbonate (20 mL), water (20 mL), and brine (20 mL). The organic phase was then dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo to afford the alcohol **61** as a neon yellow solid (34.0 mg, >95%). This material was used in the next

reaction without further purification. TLC: (5% methanol–dichloromethane) $R_f$ = 0.43 (CAM). $^1$H NMR (500 MHz, $CDCl_3$, 2.1:1.0 mixture of rotamers, asterisk denotes peaks associated with the minor rotamer) δ: 8.03 (d, 2H, *J* = 8.5 Hz), 8.02* (d, 2H, *J* = 8.5 Hz), 7.58 (d, 2H, *J* = 8.5 Hz), 7.57* (d, 2H, *J* = 8.5 Hz), 7.54 (d, 1H, *J* = 16.5 Hz), 7.14 (s, 1H), 7.02 (d, 1H, *J* = 16.0 Hz), 6.64 (s, 1H), 5.78–5.68 (m, 1H), 5.36 (s, 1H), 5.22* (s, 1H), 5.12–4.94 (m, 4H), 4.90* (s, 1H), 4.64 (d, 1H, *J* = 4.5 Hz), 4.46 (d, 1H, *J* = 12.5 Hz), 4.26 (t, 2H, *J* = 5.2 Hz), 4.22 (s, 1H), 3.94–3.90 (m, 6H), 3.88 (s, 3H), 3.77–3.67 (m, 12H), 3.63–3.56 (m, 3H), 3.49–3.30 (m, 12H), 3.16 (d, 1H, *J* = 2.5 Hz), 3.05 (td, 1H, *J* = 13.5, 3.0 Hz), 2.81 (dd, 1H, *J* = 16.0, 2.5 Hz), 2.75* (d, 1H, *J* = 17.0 Hz), 2.54–1.16 (m, 27H), 1.09–1.05* (m, 2H), 1.02 (d, 3H, *J* = 6.0 Hz), 0.99* (d, 3H, *J* = 7.0 Hz), 0.95 (d, 3H, *J* = 6.5 Hz), 0.94* (d, 3H, *J* = 7.0 Hz), 0.88 (d, 3H, *J* = 7.5 Hz), 0.84* (d, 3H, *J* = 7.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 213.2, 213.1, 196.5, 192.6, 169.2, 168.9, 166.2, 164.9, 152.4, 149.9, 144.2, 142.9, 140.1, 139.3, 135.8, 135.6, 133.0, 132.3, 130.2, 129.2, 129.0, 126.3, 126.1, 125.9, 122.8, 122.6, 118.6, 116.9, 110.7, 100.2, 98.9, 97.2, 81.2, 77.7., 76.7(4), 76.7(2), 75.4, 73.9, 73.1, 72.7, 72.3, 71.1, 70.9, 70.8, 70.6, 70.4, 70.0, 69.2, 62.0, 58.3, 58.2, 57.8, 57.2, 57.1, 56.9, 56.7, 56.6, 56.4, 53.2, 52.9, 48.7, 44.1, 43.8, 42.9, 40.4, 39.8, 39.5, 37.1, 36.9, 35.9, 35.8, 35.3, 34.9, 34.8, 33.8, 33.0, 32.8, 30.8, 30.1, 29.9, 29.6, 28.0, 26.5, 26.2, 24.8, 21.5, 21.1, 20.8, 19.6, 16.5, 16.3, 16.0, 14.6, 10.0, 9.6. FTIR (neat), $cm^{-1}$: 3491 (br), 2936 (s), 2870 (m), 1742 (m), 1711 (s), 1649 (m), 1601 (m), 1271 (s), 1101 (s). HRMS (ESI): calcd for $(C_{69}H_{99}NO_{20} + H)^+$ 1262.6833, found 1262.6831.




Biotin–Ar–FK506 (**19**). To a mixture of alcohol **61** (34.0 mg, 26.9 μmol, 1 equiv), D-biotin (32.9 mg, 135 μmol, 5.0 equiv), and 4-dimethylaminopyridine (3.3 mg, 26.9 μmol, 1 equiv) in *N,N*-dimethylformamide (673 μL), was added diisopropylethylamine (23.5 μL, 135 μmol, 5.0 equiv) followed by a solution of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (70.0 mg, 135 μmol, 5.0 equiv) and diisopropylethylamine (23.5 μL, 135 μmol, 5.0 equiv) in *N,N*-dimethylformamide (673 μL). The resulting yellow mixture was stirred in the dark at 23 °C. After 24 h, solid (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (10.0 mg, 39.9 μmol, 1.5 equiv) and solid D-biotin (5.0 mg, 20.5 μmol, 0.8 equiv) were added. After 3 h, ethyl acetate (15 mL) was added and the diluted product solution was washed sequentially with saturated aqueous ammonium chloride (15 mL), water (15 mL), and brine (15 mL). The organic layer was dried over anhydrous sodium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by reverse-phase, semi-preparatory HPLC to afford Biotin–Ar–FK506 (**19**) as a neon yellow oil (24.3 mg, 60%). TLC: (10% methanol–dichloromethane) $R_f$ = 0.49 (CAM). $^1$H NMR (500 MHz, $CDCl_3$, 19:1 *E:Z* olefin isomers, 2.2:1.0 mixture of rotamers, asterisk denotes peaks associated with the *Z* stereoisomer, double asterisk denotes peaks associated with the minor rotamer) δ: 8.00 (d, 2H, *J* = 8.0 Hz), 7.88* (d, 2H, *J* = 8.5 Hz), 7.56 (d, 2H, *J* = 8.5 Hz), 7.53 (d, 1H, *J* = 16.5 Hz), 7.33* (d, 2H, *J* = 8.0 Hz), 7.13 (s, 1H), 7.00 (d, 1H, *J* = 16.5 Hz), 6.76* (d, 1H, *J* = 11.5 Hz), 6.65* (s, 1H), 6.61 (s, 1H), 6.58* (s, 1H), 6.56* (d, 1H, *J* = 11.5 Hz), 5.77–5.66 (m, 1H), 5.34** (s, 1H), 5.30 (s, 1H), 5.20** (s, 1H), 5.10–4.92 (m, 4H), 4.90** (s, 1H), 4.86 (s, 1H), 4.62 (d, 1H, *J* = 4.5 Hz), 4.50–4.47 (m, 1H), 4.44 (d, 1H, *J* = 14.0 Hz), 4.30–4.28 (m, 1H), 4.24–4.18 (m, 5H), 3.93–3.88 (m, 6H), 3.86 (s, 3H), 3.75–3.65 (m, 10H), 3.58 (dd, 2H, *J* = 11.0, 3.0

Hz), 3.49–3.26 (m, 12H), 3.20 (d, 1H, *J* = 2.5 Hz), 3.15–3.11 (m, 1H), 3.05–3.00 (m, 1H), 2.90 (dd, 1H, *J* = 12.5, 5.0 Hz), 2.79 (dd, 1H, *J* = 16.5, 2.0 Hz), 2.73** (dd, 1H, *J* = 18.0, 2.0 Hz), 2.72 (d, 1H, *J* = 13.0 Hz), 2.52–1.15 (m, 37H), 1.07–1.01** (m, 1H), 1.01 (d, 3H, *J* = 6.0 Hz), 0.97** (d, 3H, *J* = 6.5 Hz), 0.94 (d, 3H, *J* = 6.5 Hz), 0.92** (d, 3H, *J* = 6.5 Hz), 0.87 (d, 3H, *J* = 7.5 Hz), 0.82** (d, 3H, *J* = 6.5 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 213.2, 213.1, 196.5, 192.7, 173.8, 169.2, 168.9, 166.2, 164.9, 163.2, 152.4, 149.9, 144.2, 142.9, 140.1, 139.3, 135.8, 135.6, 133.0, 132.3, 130.2, 129.2, 129.1, 126.3, 126.1, 125.8, 122.8, 122.6, 118.5, 116.9, 110.7, 100.2, 98.9, 97.2, 81.2, 76.8, 75.4, 73.9, 73.8(6), 73.1, 72.3, 71.1, 70.9, 70.8, 70.7(6), 70.4, 70.0, 69.4, 69.2, 63.7, 62.1, 60.3, 58.3, 58.2, 57.8, 57.2, 57.1, 56.9, 56.8, 56.6., 56.4, 55.5, 53.2, 53.0, 48.7, 48.6, 44.1, 43.8, 43.0, 40.8, 40.4, 39.9, 39.5, 37.0, 36.9, 35.9, 35.8, 35.4, 34.9, 34.8, 34.0, 33.8, 33.0, 32.8, 30.8, 30.1, 28.5, 28.0, 26.5, 26.2, 24.9, 24.8, 21.5, 21.1, 20.8, 19.6, 16.5, 16.3, 16.0, 14.7, 14.6, 10.0, 9.6. FTIR (neat), $cm^{-1}$: 3391 (w), 2936 (m), 1705 (s), 1647 (m), 1601 (w), 1450 (m), 1271 (s), 1101 (s). HRMS (ESI): calcd for $(C_{79}H_{113}N_3O_{22}S + H)^+$ 1488.7609, found 1488.7604.

**Nuclear Factor of Activated T-cells (NFAT) Reporter Gene Assay.** Jurkat cells grown to approximately 90% confluence were diluted 10-fold in fresh Opti-MEM I reduced-serum medium (Invitrogen) and the cells were counted by trypan blue exclusion using a hemacytometer. The cells were pelleted by centrifugation at 183 x g (10 min, 4 ºC), the pellet was washed with 10 mL Opti-MEM I reduced-serum medium, and the washed pellet was suspended in sufficient Opti-MEM I reduced-serum medium to produce a concentration of 1 x $10^7$ cells/mL.

A cell culture flask (75 $cm^2$) was charged with Opti-MEM I reduced-serum medium (3.9 mL), DMRIE-C reagent (62.4 μL, Invitrogen), and pGL3-NFAT luciferase plasmid (39.0 μg). The resulting solution was mixed by gently swirling. After incubation at 23 °C for 45 min, the cell suspension (1.56 mL) was added and the flask was incubated at 37 ºC under an atmosphere of 5% $CO_2$. After 4 h, RPMI-1640 containing 15% fetal bovine serum, 160 nM ionomycin, 1 μg/mL phytohemagglutinin (PHA-M), and 50 ng/mL phorbol 12-myristate 13-acetate (PMA, 15.6 mL) was added and the flask was incubated at 37 ºC under an atmosphere of 5% $CO_2$ for an additional 5 h.

A 4.0-mL aliquot of a solution of FK506, at 1 mM in DMSO, was diluted in 596 mL of medium to achieve a working concentration of 6.7 nM. A 4.0-mL aliquot of a solution of analog **18** (30 mM in DMSO) was diluted in 596 mL of medium to achieve a working concentration of 200 nM. A 4.0-mL aliquot of a solution of analog **19** (50 mM in DMSO) was diluted in 596 mL of medium to achieve a working concentration of 333 nM. Solutions with a range of different concentrations of drug were obtained by serial dilution. 25-μL aliquots of the diluted drug solutions were added to each of

the wells of a 96-well, flat-bottomed plate, resulting in final assay concentrations of up to 1.7 nM for FK506, 50.0 nM for analog **18**, 83.2 nM for analog **19**.

A 10-μL aliquot of cells was stained with trypan blue solution (10 μL) and the cells were counted using a hemacytometer. The cell suspension was diluted to a concentration of 3.0 x $10^5$ cells/mL with RPMI-1640 containing 15% fetal bovine serum, 160 nM ionomycin, 1 μg/mL PHA-M (Sigma L8902), and 50 ng/mL PMA. A multi-channel pipette was used to charge each of the wells of the 96-well plate containing the drug solutions with 75 μL of the diluted cell suspension per well.

The treated cells were incubated for 13 h at 37 ºC under an atmosphere of 5% $CO_2$, then were removed from the incubator and allowed to cool to 23 ºC. To each well was added 100 μL of Bright-Glo Luciferase Assay System, and the luminescence was recorded on a 96-well luminescence counter following a 5-min incubation period at 23 ºC.

Maximal NFAT activity was calculated for each well based upon the following formula: Percent maximal NFAT activity = 100 x $(S - B_o)/(B_t - B_0)$, where S is the sample reading, $B_o$ is the average reading of wells containing medium at the completion of the assay, and $B_t$ is the average reading for an untreated population of cells at the completion of the assay.

Each analog was assayed a minimum of six times over a time period of 3 weeks. For each compound, seven separate concentrations were used in the assay. The average NFAT activity at each concentration was plotted against concentration and a curve fit was generated. Final $IC_{50}$ values reflect the concentrations at which the resulting curves pass through fifty percent inhibition of NFAT.

**Affinity-Isolation of the FK506–Immunosuppressive Complex.**

Preparation of CHO Whole-Cell Lysate.

CHO cells were grown to approximately 95% confluence in 24 T-150 tissue culture flasks. The medium was discarded, and the cells were washed with PBS (10 mL per flask). The cells were harvested by trypsinization (10 min, 37 ºC , 8 mL per flask, 0.05% trypsin, 0.53 mM EDTA). Fresh cell culture medium (16 mL) was added to each flask, and the suspensions were transferred to 50-mL centrifuge tubes. The cells were pelletted by centrifugation (183 x g, 10 min, 4 ºC). The supernatant was discarded, and the cell pellets were resuspended in 1 mL PBS and transferred to two 15-mL centrifuge tubes, along with a 1-mL PBS rinse. The cells were pelletted once again by centrifugation (2000 x g, 10 min, 4 ºC) and were washed twice more with 1 mL PBS.

Packed cells (1.5 mL) were suspended in ice-cold lysis buffer (6.0 mL, see above for formulation) and the suspension was transferred to a 7-mL Dounce homogenizer. The sample was homogenized on ice (25 strokes). The resulting lysate was transferred to fresh 1.7-mL centrifuge tubes, along with two 1-mL lysis buffer rinses. The lysate was centrifuged (12,000 x g, 20 min, 4 ºC) and the supernatant transferred to a clean, 15-mL centrifuge tube. A 750-µL aliquot of washed, well-suspended streptavidin–agarose resin (see above for resin preparation) was added, and the resulting slurry mixed at 4 ºC for 5 h. The mixture was centrifuged (549 x g, 5 min, 4 ºC) and the supernatant carefully removed and partitioned into nine 1.0-mL aliquots, which were flash-frozen in liquid $N_2$ and stored at –80 ºC prior to use. The lysate contained 7.66 mg/mL total protein (Bradford method[14]).

Affinity-Isolation of the FK506–Immunosuppressive Complex.

A 1.0-mL aliquot of CHO whole-cell lysate was thawed at 4 ºC and diluted with 6.66 mL TBS-TX to afford a working lysate of 1.0 mg/mL total protein. This was partitioned into 1.7-mL centrifuge tubes, and treated (on ice) with DMSO and solutions of FK506, **18**, or **19** (from 10 mM stocks in DMSO):

| Sample | lysate | DMSO | 10 mM FK506 | 10 mM **18** | 10 mM **19** | 18.1 mM **2** | 10 mM DTT | QuadraPure EDA | Total Volume | DMSO (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 495 µL | 5.0 µL | X | X | X | X | 12.5 µL (250 µM) | 2.5 µg | 500 µL | 2 |
| 2 | 495 µL | 2.6 µL | 1.0 µL (20 µM) | X | X | 1.4 µL (50 µM) | 12.5 µL (250 µM) | 2.5 µg | 500 µL | 2 |
| 3 | 495 µL | 2.6 µL | X | 1.0 µL (20 µM) | X | 1.4 µL (50 µM) | 12.5 µL (250 µM) | 2.5 µg | 500 µL | 2 |
| 4 | 495 µL | 4.0 µL | X | X | 1.0 µL (20 µM) | X | 12.5 µL (250 µM) | 2.5 µg | 500 µL | 2 |

The samples were mixed end-over-end at 4 ºC for 4 h, then were allowed to warm to 23 ºC and treated with a freshly mixed solution of **2** containing 0.1% by volume aqueous $EtNH_2$. The reactions were rotated end-over-end at 23 ºC. After 14 h, a 10 mM aqueous solution of DTT (12.5 µL) and solid QuadraPure EDA (2.5 mg) were added in sequence to the reaction solutions. The samples were rotated end-over-end at 23 ºC for an additional 2 h, then centrifuged (12,000 x g, 10 min, 4 ºC). The supernatants (450 µL) were transferred to clean, 1.7-mL centrifuge tubes and each sample was treated with a 150-µL

[14] Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.

aliquot of washed, well-suspended, streptavidin–agarose resin (see above for resin preparation) at 23 ºC. After 2 h, the resins were collected by centrifugation (12,000 x g, 10 min, 4 ºC), and the supernatants were removed and discarded.

The collected resins were washed with 500 μL lysis buffer at 4 ºC, then three times with 500 μL Tris buffer at 4 ºC. Each wash consisted of 10 min mixing, followed by 10 min centrifugation (12,000 x g at 4 ºC).

The washed resins were suspended in Laemmli loading buffer (Sigma, 2x concentration, 25 μL per sample) and the samples were heat denatured at 95 ºC for 6 min. Two tris-glycine mini gels (10–20%, 15-well) were loaded with denatured protein mixture (gel 1: 2.5 μL of each sample; gel 2: 17.5 μL of each sample). One lane was loaded with 7 μL of Benchmark Pre-stained Protein Ladder (Invitrogen). The samples were electroluted (150 V, 23 ºC, gel 1: 40 min, gel 2: 65 min), then transferred under semi-dry conditions to a nitrocellulose membrane (100 mA, 15 h, 23 ºC).

Membrane 1 was blocked for 1 h (40 mL 5% low-fat milk in TBS-T), then rinsed (three five min washes with 40 mL TBS-T) and treated 2.5 h with primary antibody solution (20 mL 1% low-fat milk in TBS-T containing 7.5 μg anti-FKBP12 antibody). The membrane was rinsed again (three five min washes with 40 mL TBS-T) and treated 1 h with secondary antibody solution (20 mL 1% low-fat milk in TBS-T containing 20 μg goat anti-mouse-HRP conjugate). The membrane was rinsed once more (three ten min washes with 40 mL TBS-T) and treated with 6 mL of a 1:1 mixture stabilized peroxide solution:enhanced luminol solution (Pierce; WestPico Chemiluminescent Substrate kit) for 3 min. Finally, the membrane was sealed in plastic wrap and exposed to X-ray film to provide the Western blot of **Fig. 5b** to identify FKBP12.

Membrane 2 was blocked for 1 h (40 mL 5% low-fat milk in TBS-T), then rinsed (three five min washes with 40 mL TBS-T) and treated 2.5 h with primary antibody solution (20 mL 1% low-fat milk in TBS-T containing 5 μg anti-calcineurin B antibody). The membrane was rinsed again (three five min washes with 40 mL TBS-T) and treated with secondary antibody solution (20 mL 1% low-fat milk in TBS-T containing 20 μg goat anti-rabbit-HRP conjugate). The membrane was rinsed once more (three ten min washes with 40 mL TBS-T) and treated with 6 mL of a 1:1 mixture stabilized peroxide solution:enhanced luminol solution (Pierce; WestPico Chemiluminescent Substrate kit) for 3 min. Finally, the membrane was sealed in plastic wrap and exposed to X-ray film to provide the Western blot of **Fig. 5b** to identify calcineurin B.

Membrane 2 was then treated 17 h with primary antibody solution (20 mL 1% low-fat milk in TBS-T containing 10 μg anti-calmodulin antibody) at 4 ºC. The membrane was rinsed (three five min washes with 40 mL TBS-T) and treated with secondary antibody solution (20 mL 1% low-fat milk in TBS-T containing 20 μg goat anti-mouse-HRP conjugate). The membrane was rinsed once more (three ten min washes with 40 mL TBS-T) and treated with 6 mL of a 1:1 mixture stabilized peroxide solution:enhanced luminol solution (Pierce; WestPico Chemiluminescent Substrate kit) for 3 min. Finally, the

membrane was sealed in plastic wrap and exposed to X-ray film to provide the Western blot of **Fig. 5b** to identify calmodulin.

Finally, membrane 2 was treated 2 h with primary antibody solution (20 mL 5% bovine serum albumin in TBS-T containing 10 μg anti-calcineurin A antibody). The membrane was rinsed (three five min washes with 40 mL TBS-T) and treated with secondary antibody solution (20 mL 1% low-fat milk in TBS-T containing 20 μg goat anti-rabbit-HRP conjugate). The membrane was rinsed once more (three ten min washes with 40 mL TBS-T) and treated with 6 mL of a 1:1 mixture stabilized peroxide solution:enhanced luminol solution (Pierce; WestPico Chemiluminescent Substrate kit) for 3 min. Finally, the membrane was sealed in plastic wrap and exposed to X-ray film to provide the Western blot of **Fig. 5b** to identify calcineurin A.

Using these conditions, we also explored the arylpalladium(II) reagent (**2**)-, time-, and drug-dependency of the Heck-based affinity-enrichment of FKBP12 from CHO lysate (Figure S4).

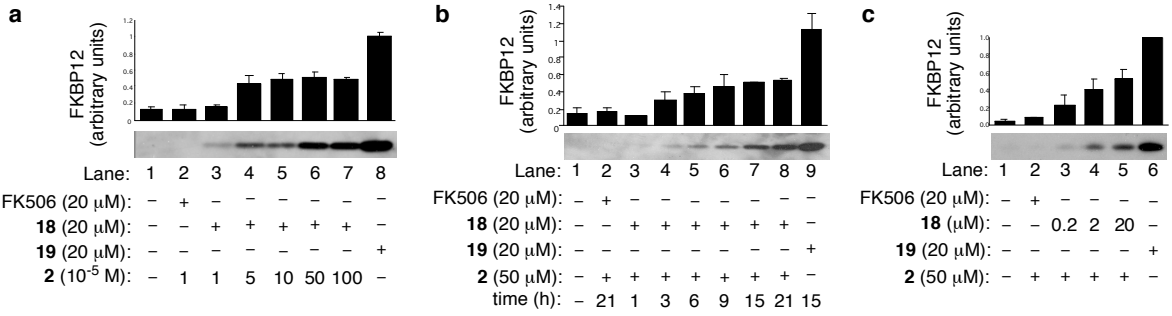
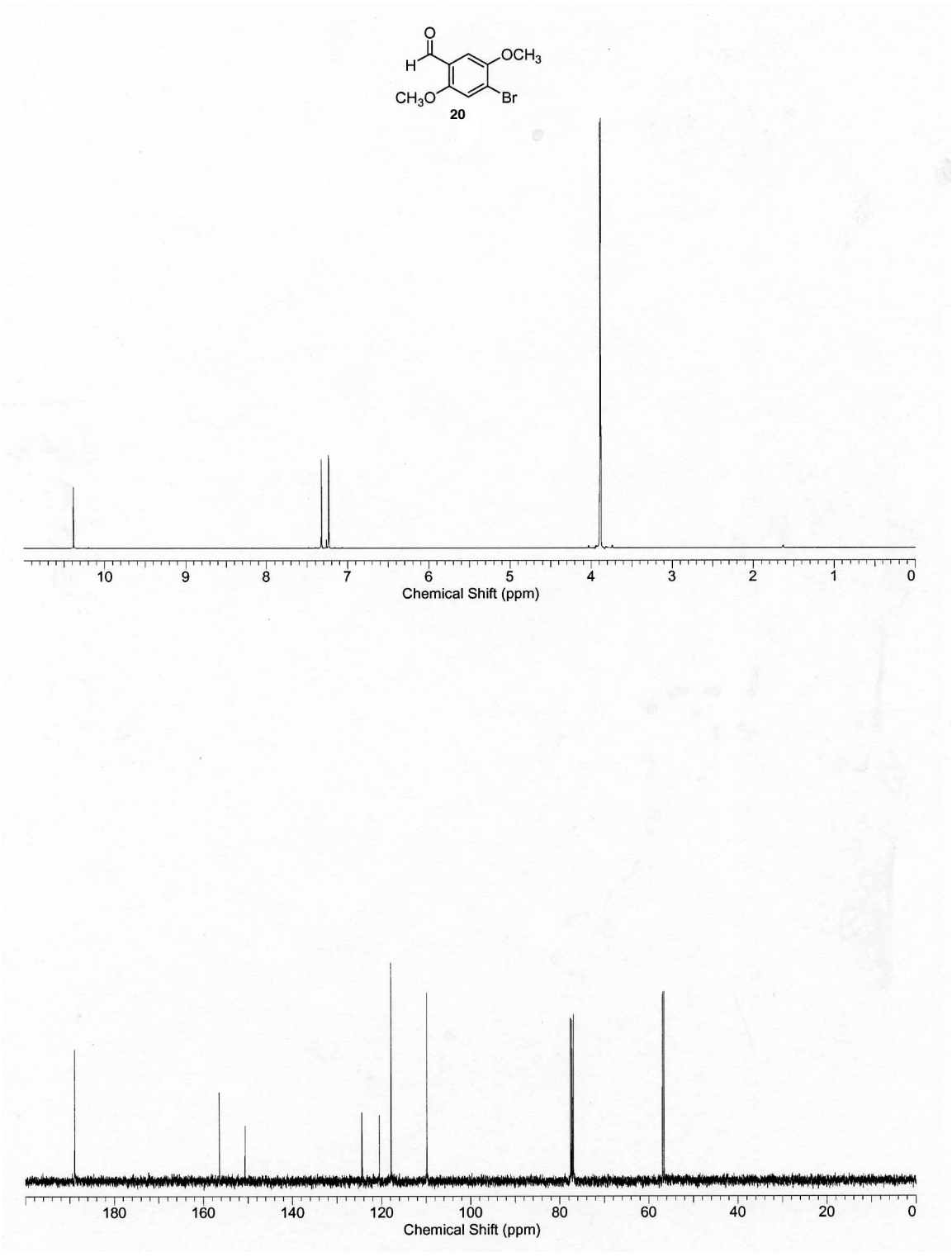



**Figure S4** Affinity-isolation of FKBP12. (a) Affinity-isolation experiments conducted with varying concentrations of reagent Pd-Ar-Biotin (**2**). (b) Affinity-isolation experiments conducted by varying the duraction of the Heck reaction from 0–21h. (c) Affinity-isolation experiments conducted with varying concentrations of probe Styryl-FK506 (**18**). The amount of FKBP12 was quantified by densitometry scanning of each lane and is presented as means±s.e.m. of at least three independent experiments.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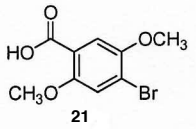
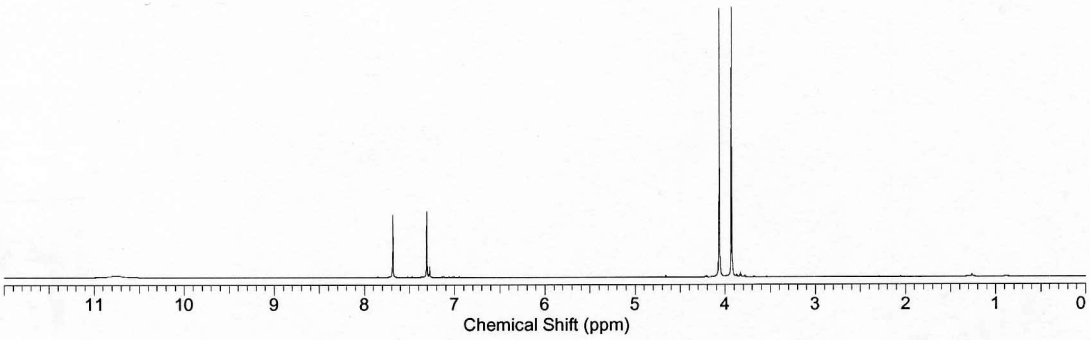
H
OCH3
CH3O
Br
20
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


O
HO
OCH3
CH3O
Br

**21**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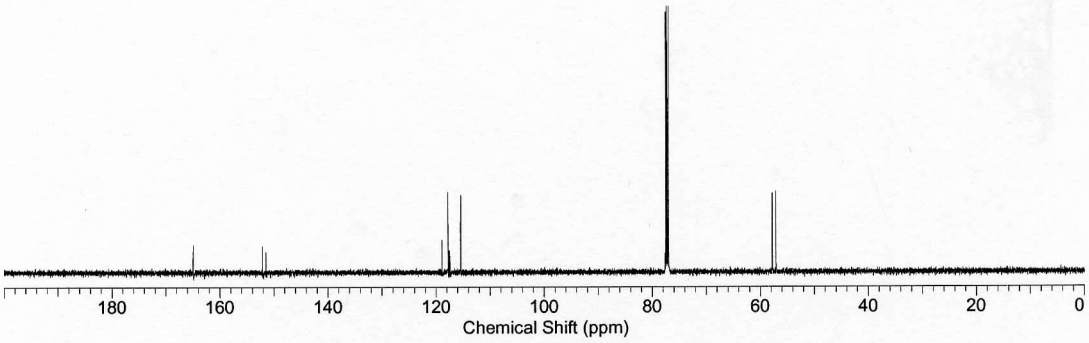
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)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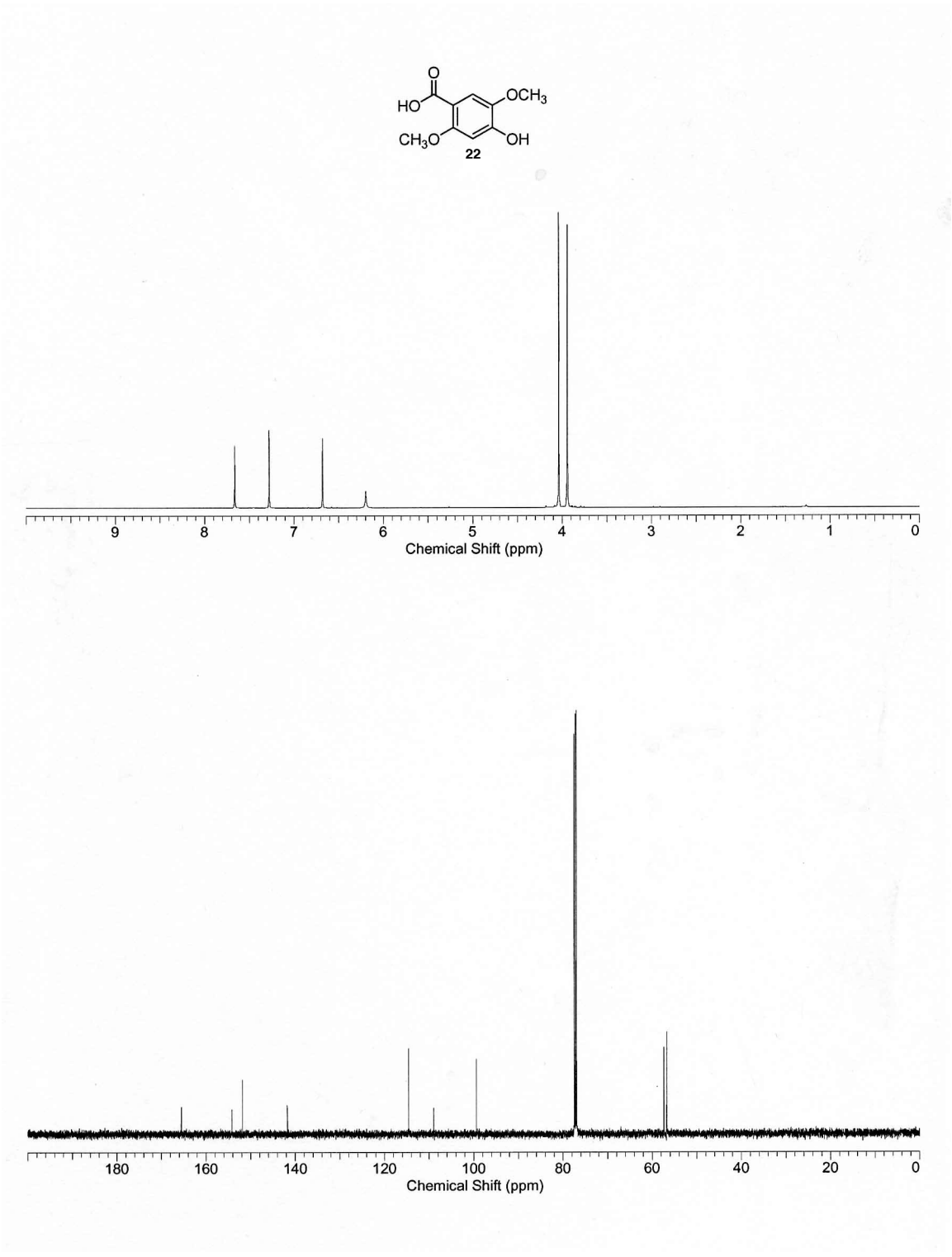
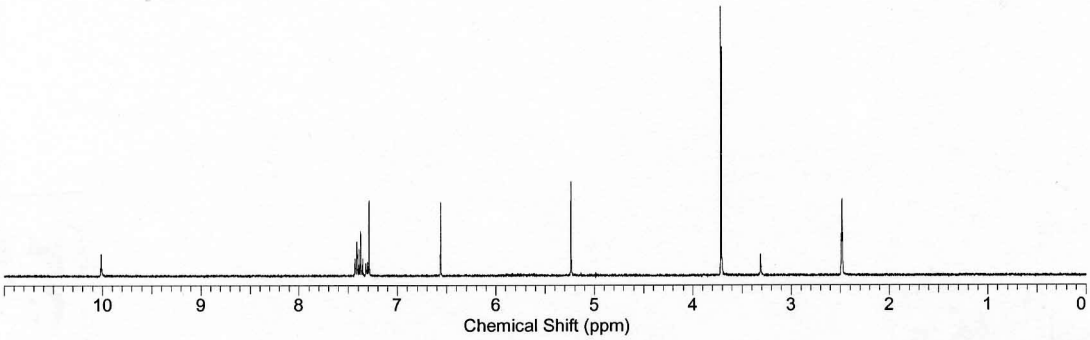
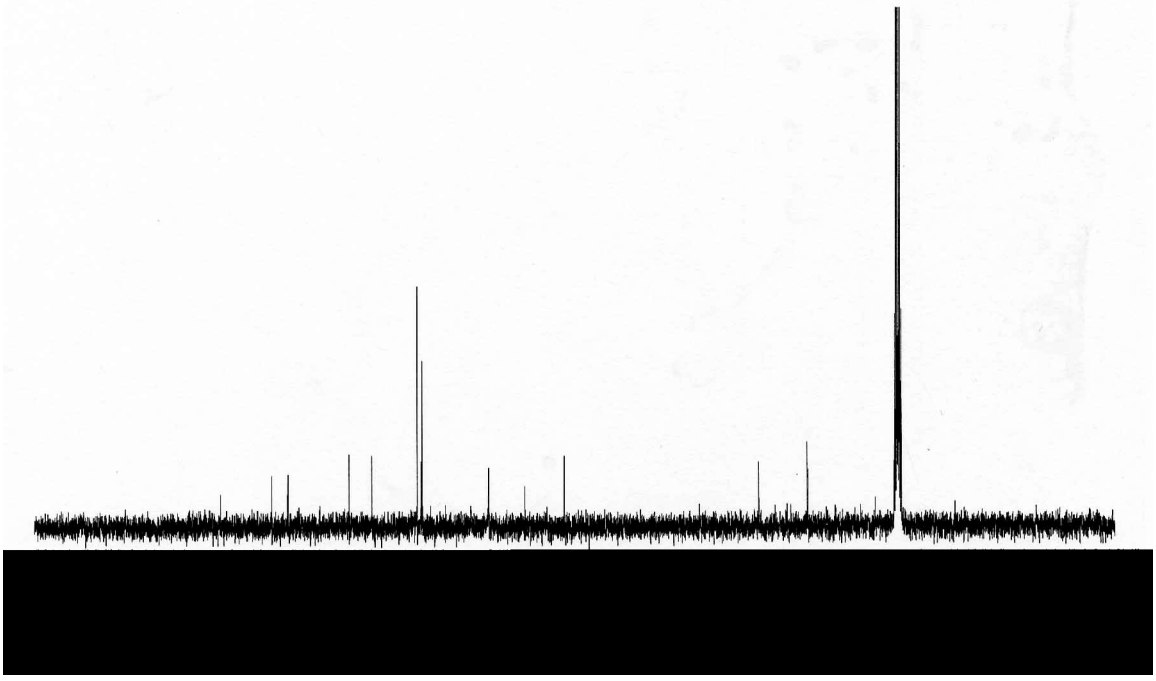
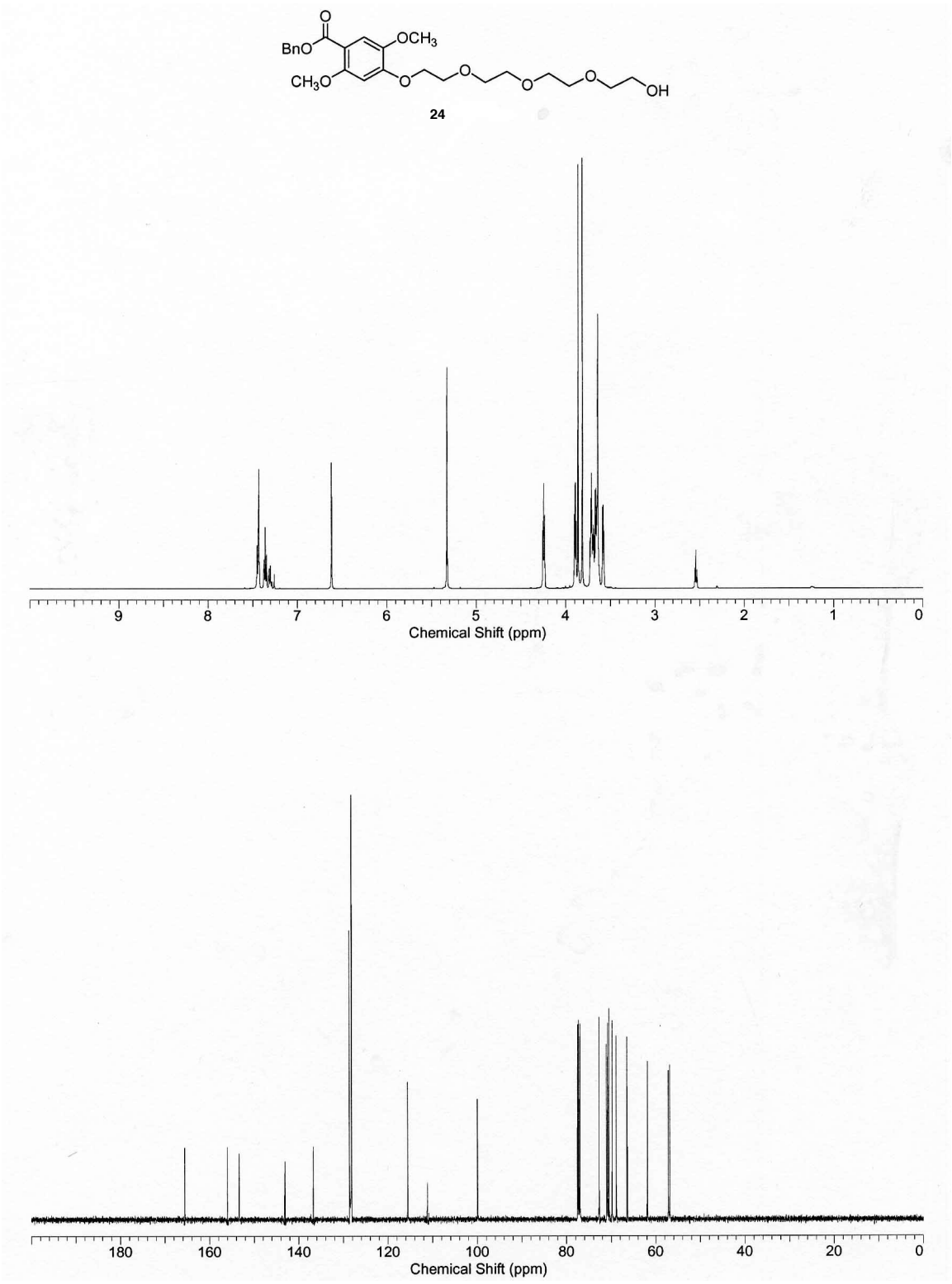
60
40
20
0
Chemical Shift (ppm)


HO
O
OCH3
CH3O
OH
22
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


**23**

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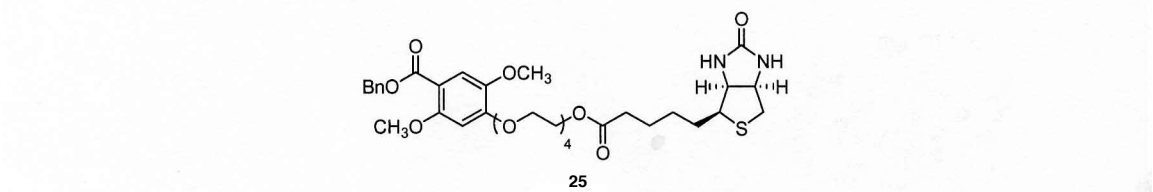
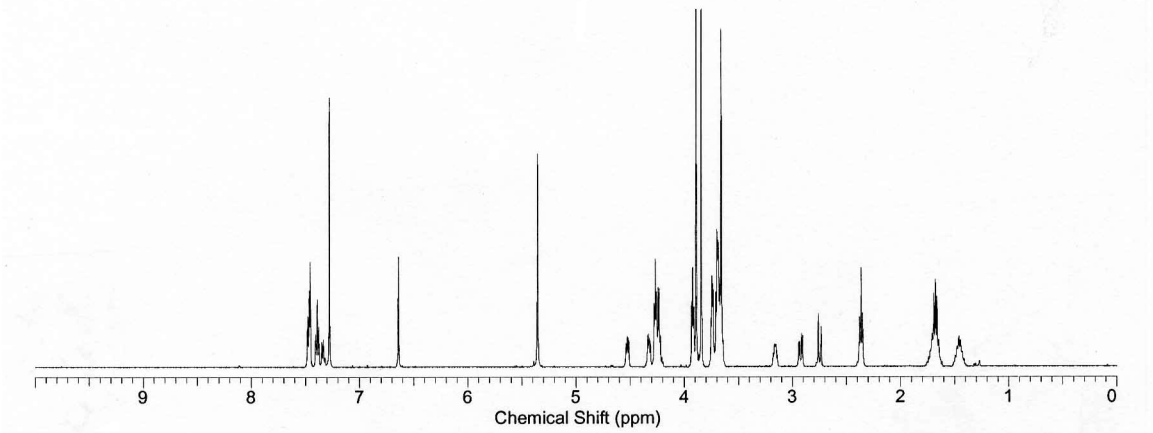
OCH3
CH3O
O
O
O
O
O
OH
24
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


BnO
OCH3
CH3O
O
4
HN
NH
H
H
S

**25**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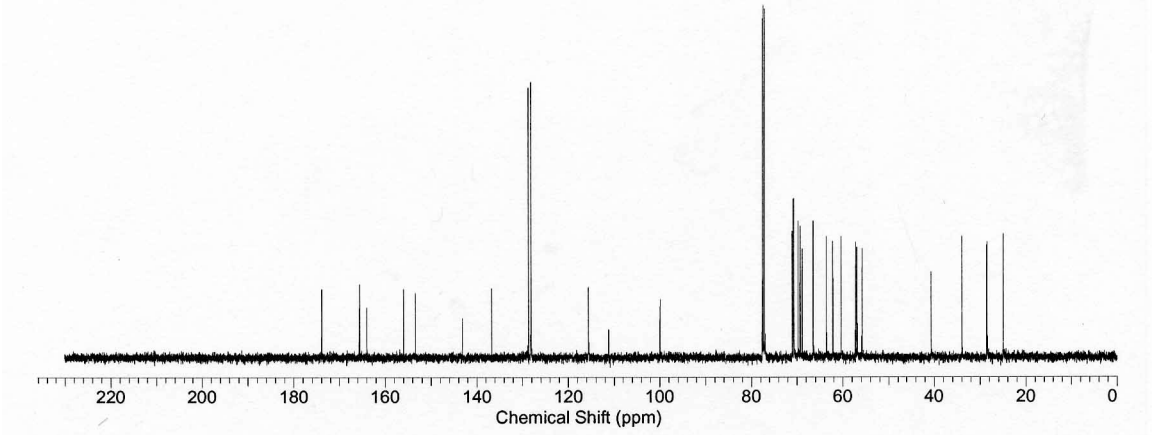
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)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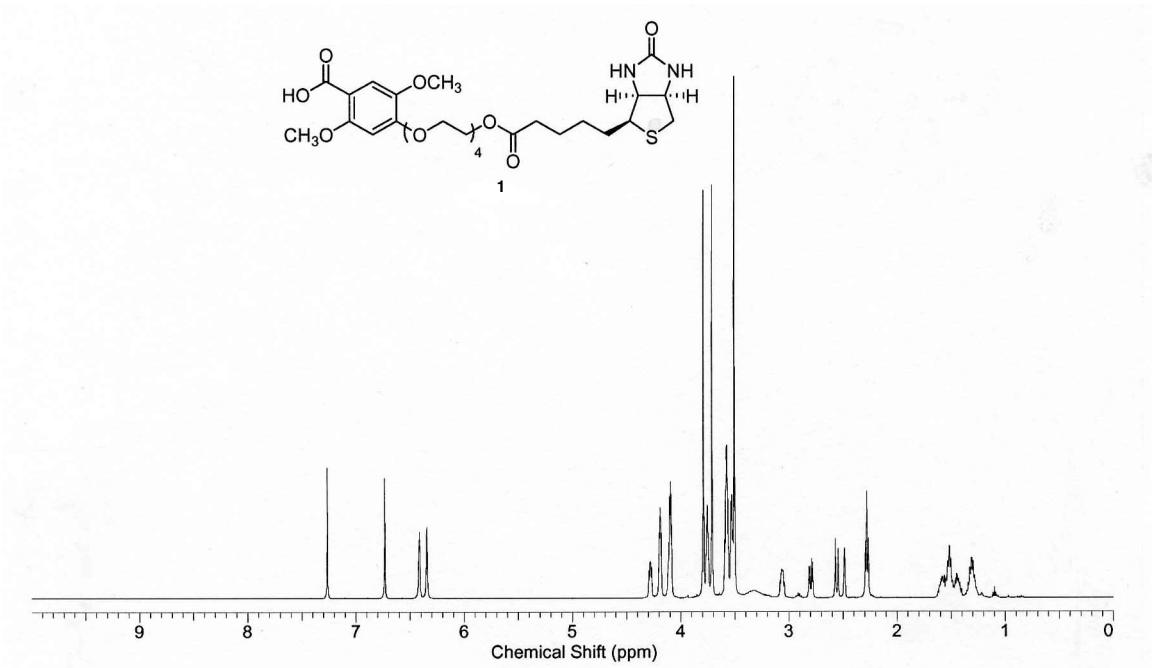
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O
OCH3
CH3O
O
4
O
O
HN
NH
H
H
S
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9
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7
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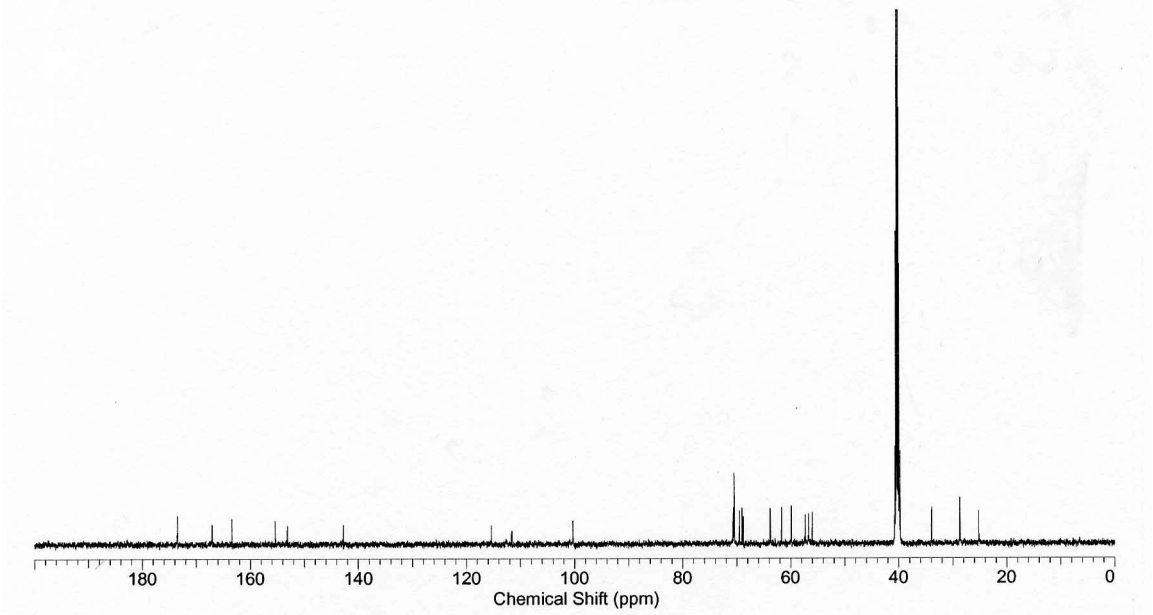
5
4
3
2
1
0
Chemical Shift (ppm)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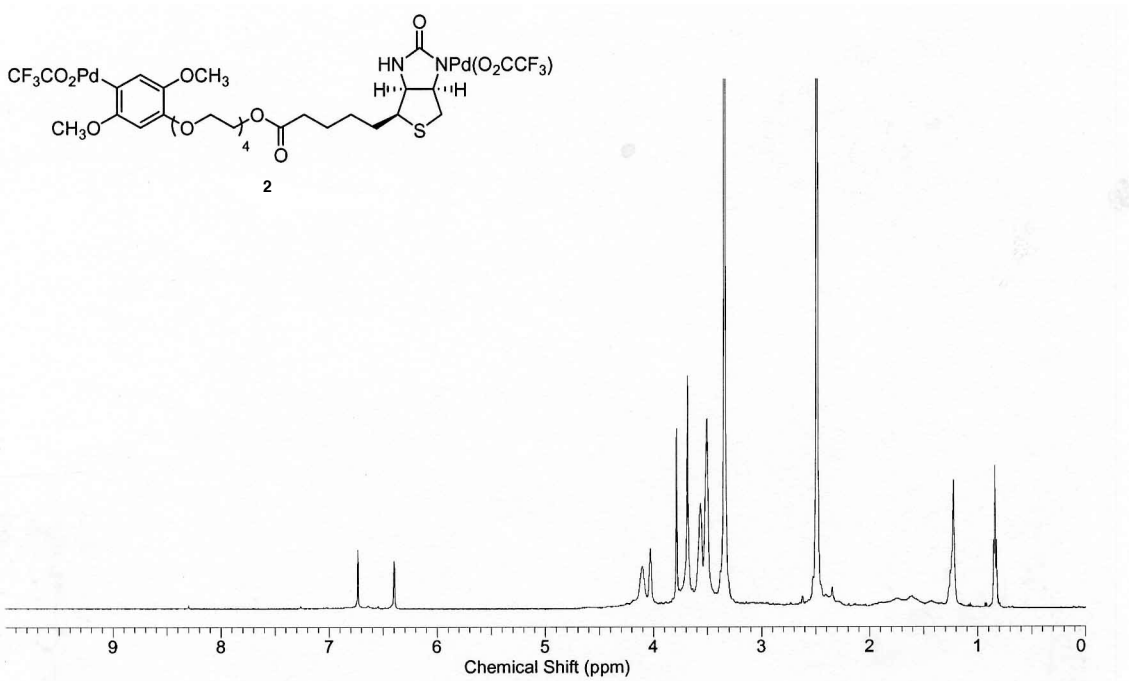
Chemical Shift (ppm)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CH3O
HN
NPd(O2CCF3)
H
H
S
O
4
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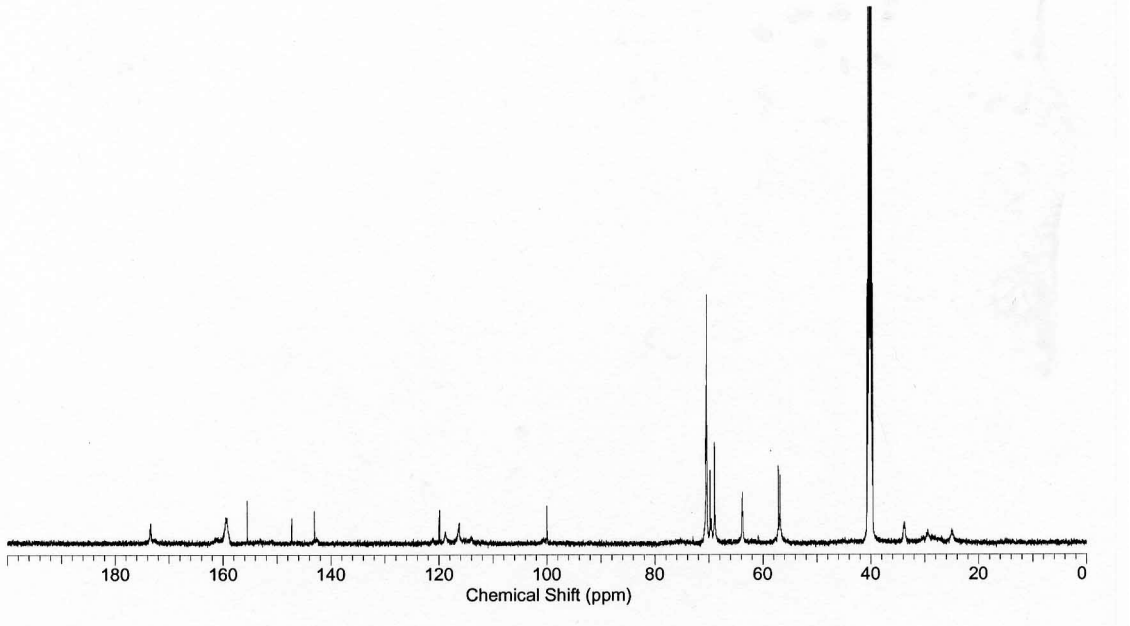
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

2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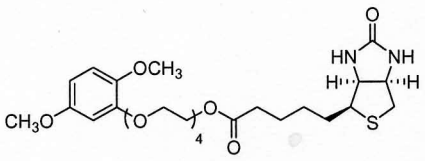
60
40
20
0
Chemical Shift (ppm)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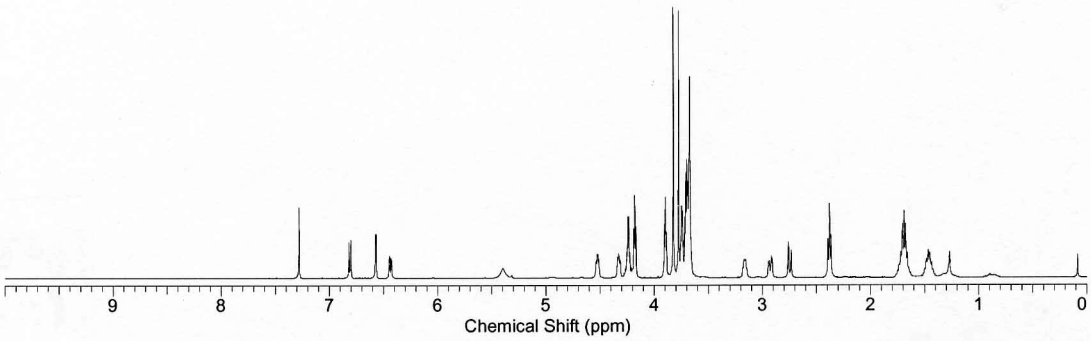
CH3O
O
4
O
O
HN
NH
H
H
S

**26**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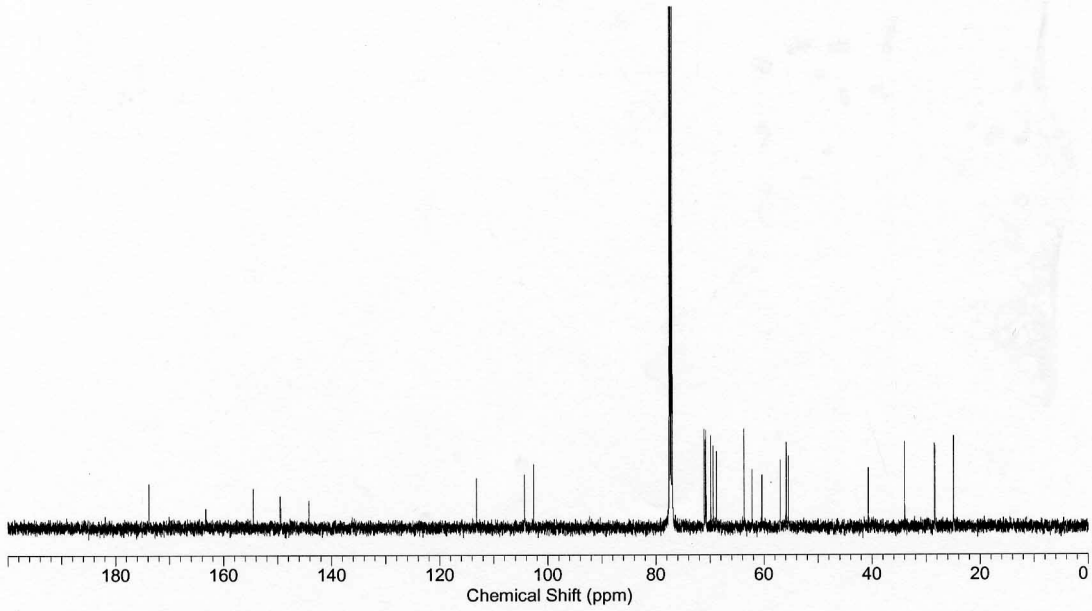
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)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40
20
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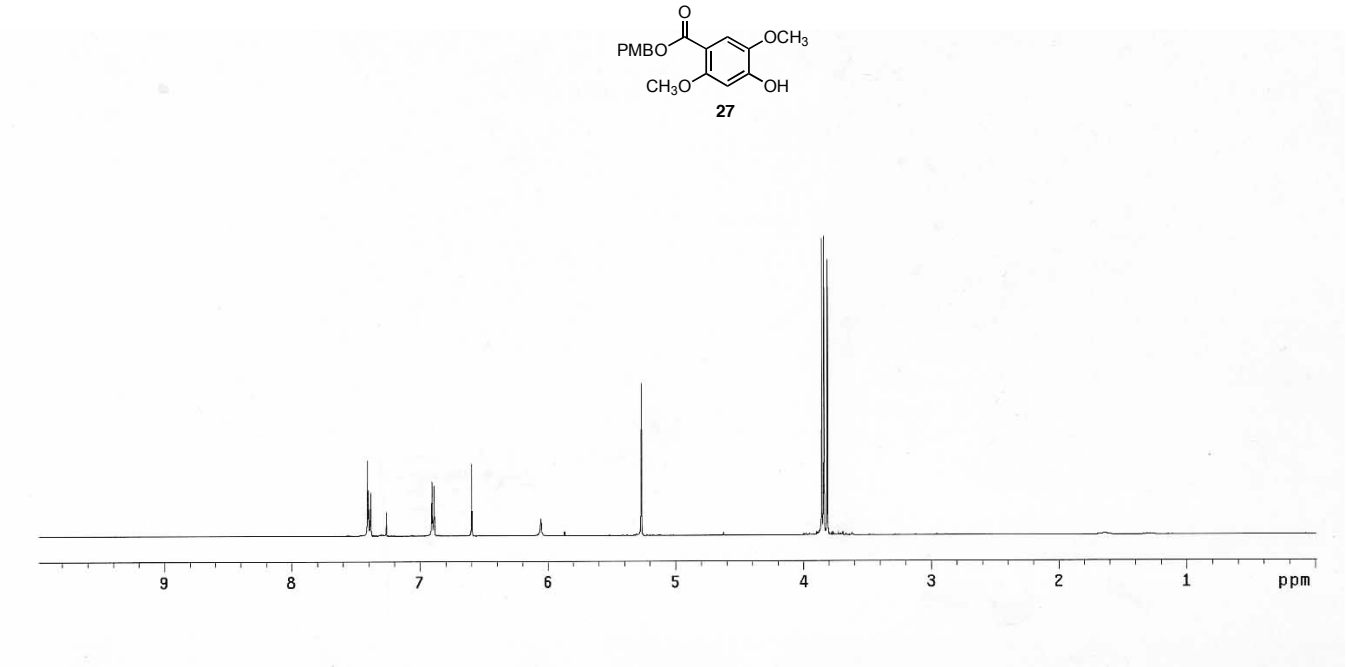
Chemical Shift (ppm)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CH3O
OH
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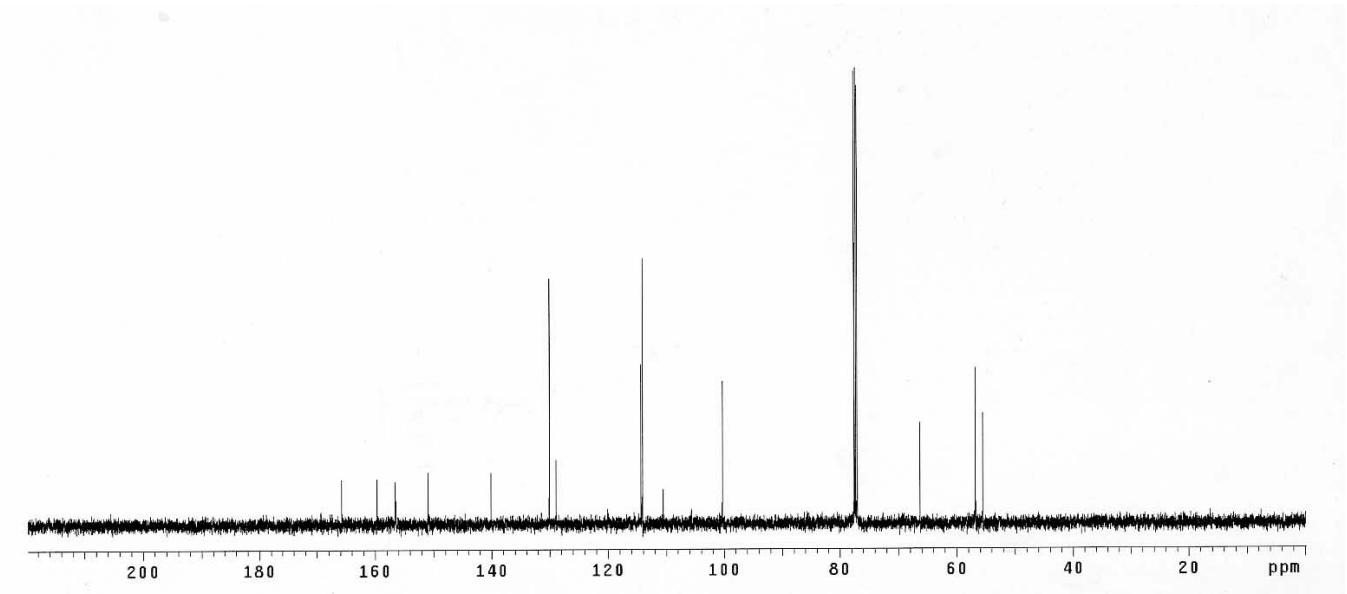
9
8
7
6
5
4
3
2
1
ppm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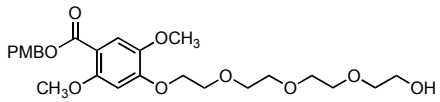
ppm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PMBO
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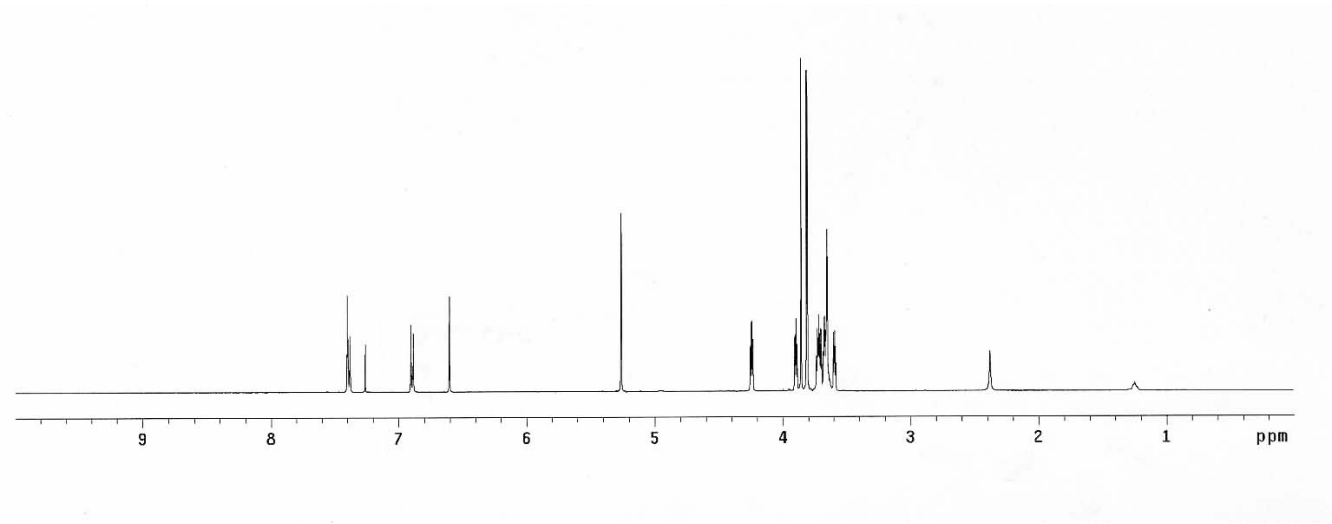
CH3O
O
O
O
O
OH

**28**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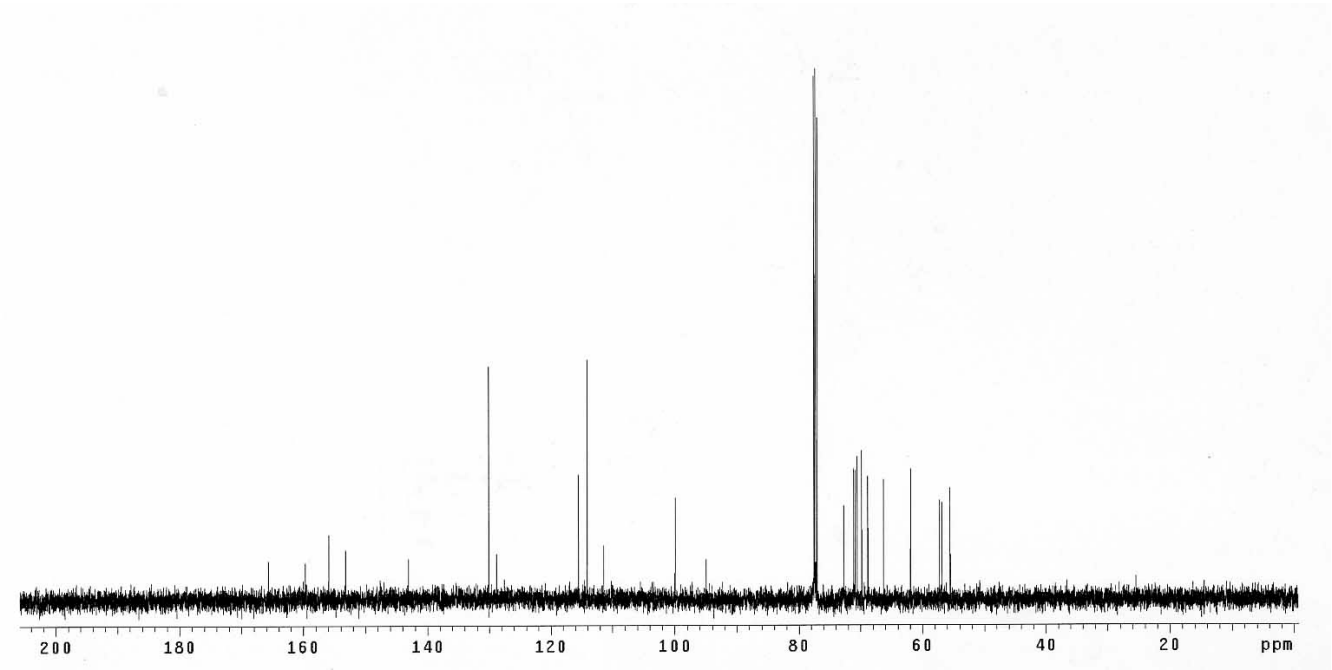
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80
60
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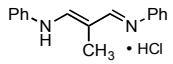
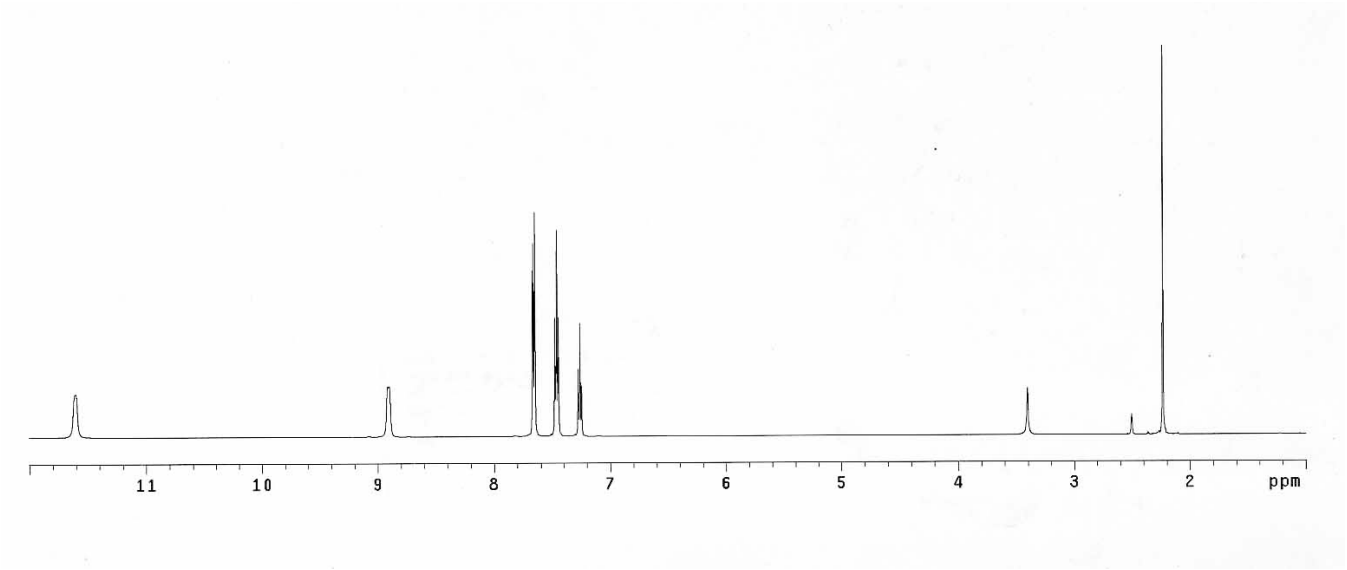
20
ppm


Ph
N
H
N
Ph
CH3
• HCl

**30**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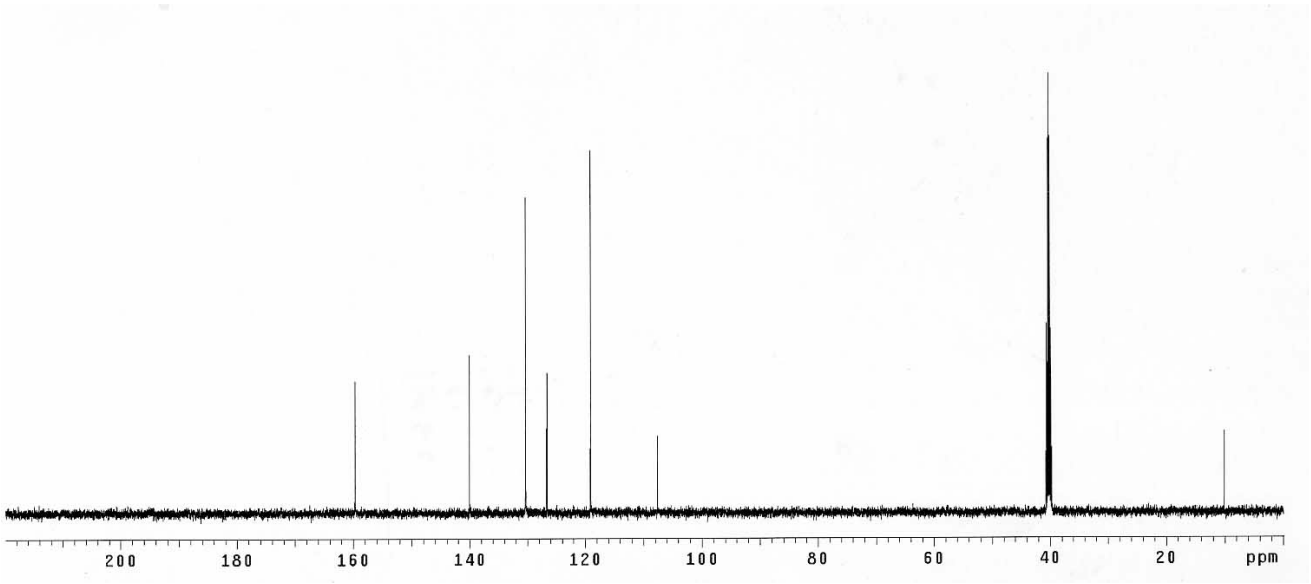
9
8
7
6
5
4
3
2
ppm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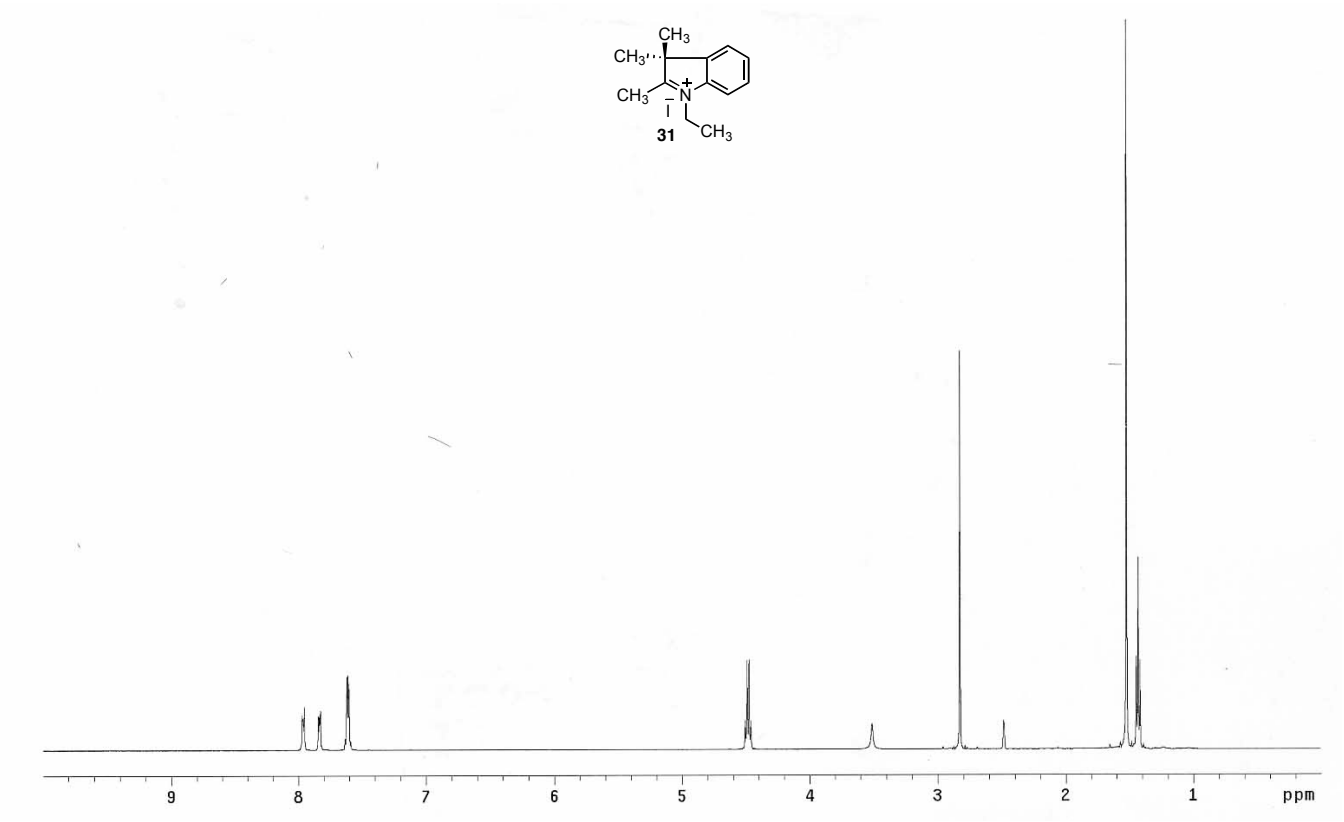
80
60
40
20
ppm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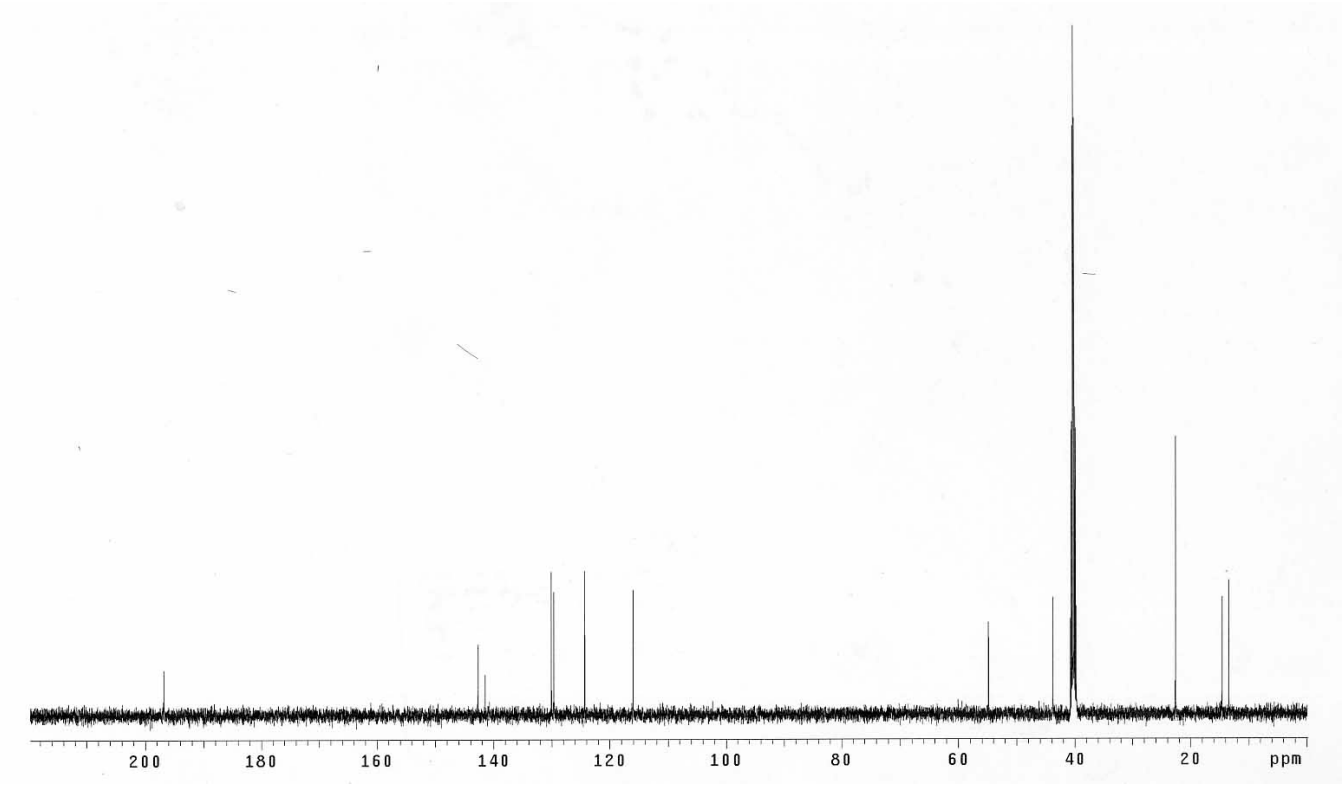
CH3
CH3
N
I
CH3
31
9
8
7
6
5
4
3
2
1
ppm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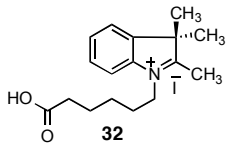
60
40
20
ppm

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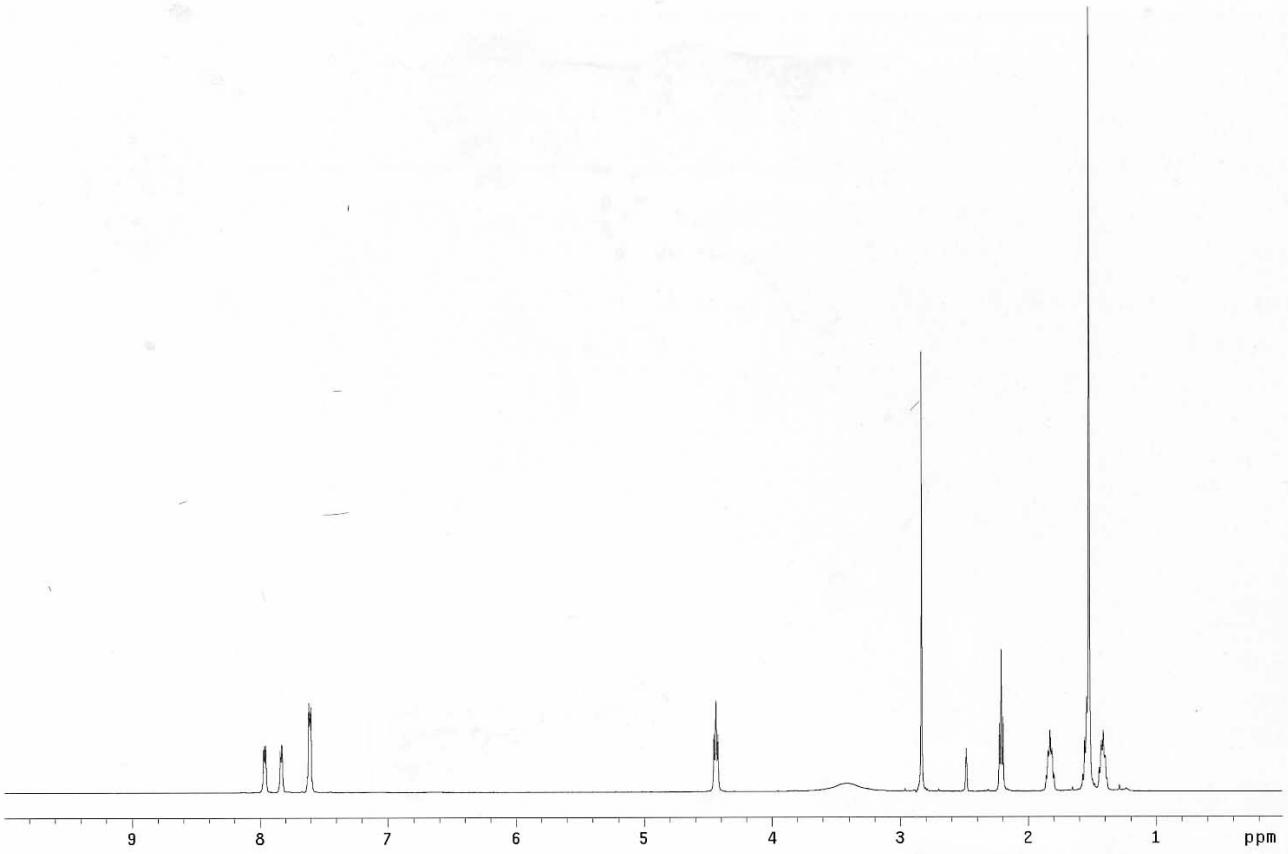
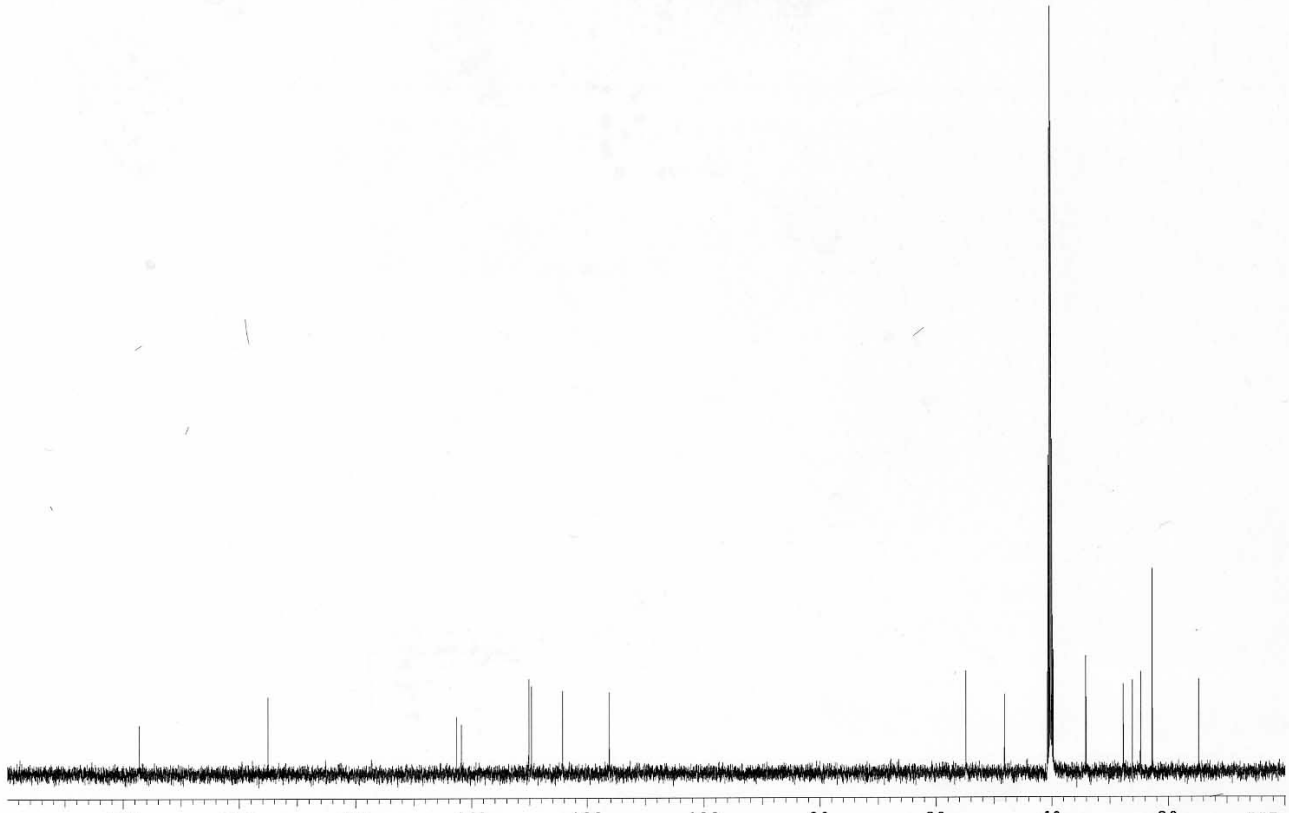
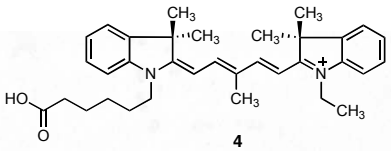
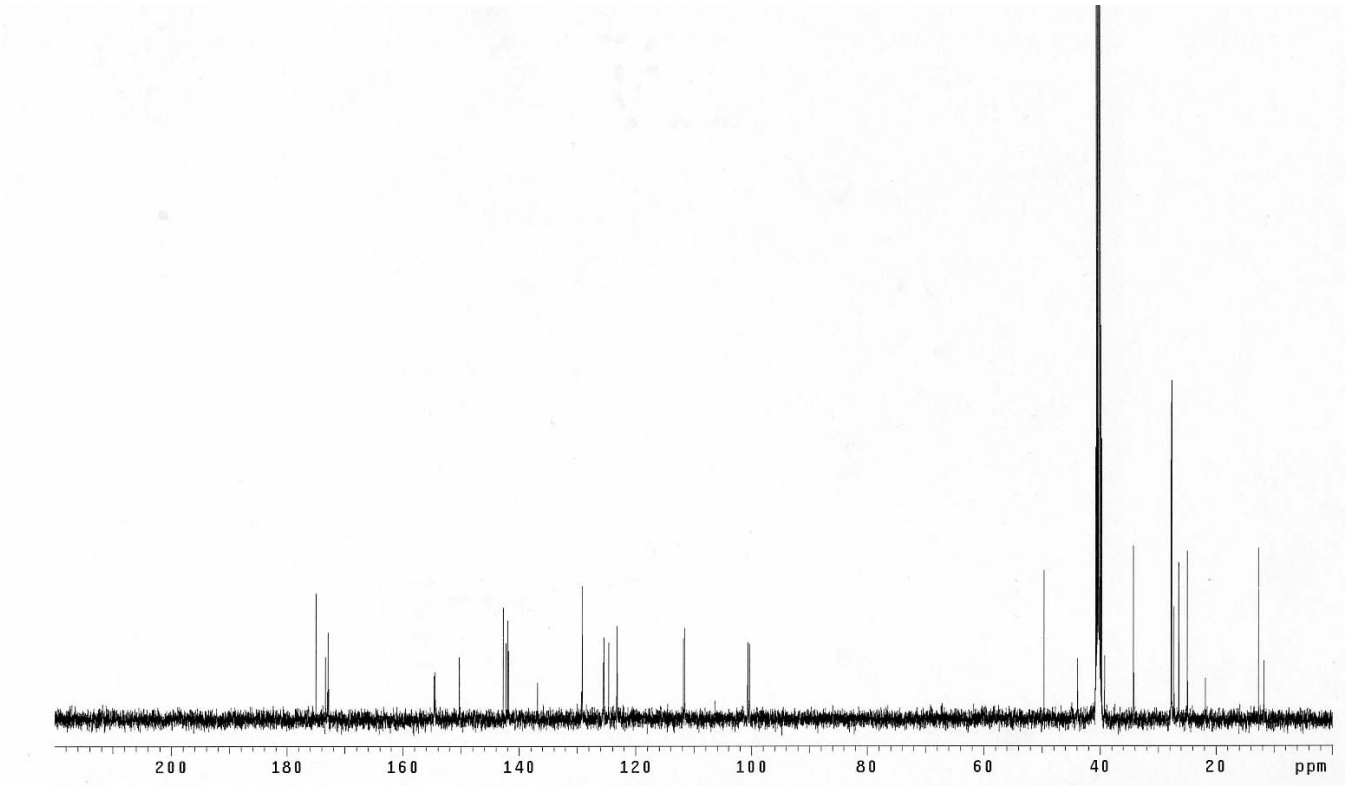



CH3
CH3
CH3
CH3
N
N
HO
O
CH3
CH3

**4**


9
8
7
6
5
4
3
2
1
ppm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60
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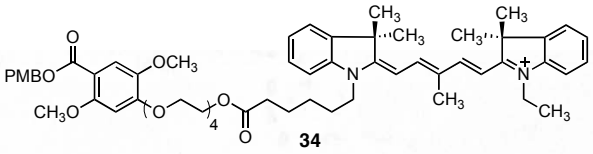
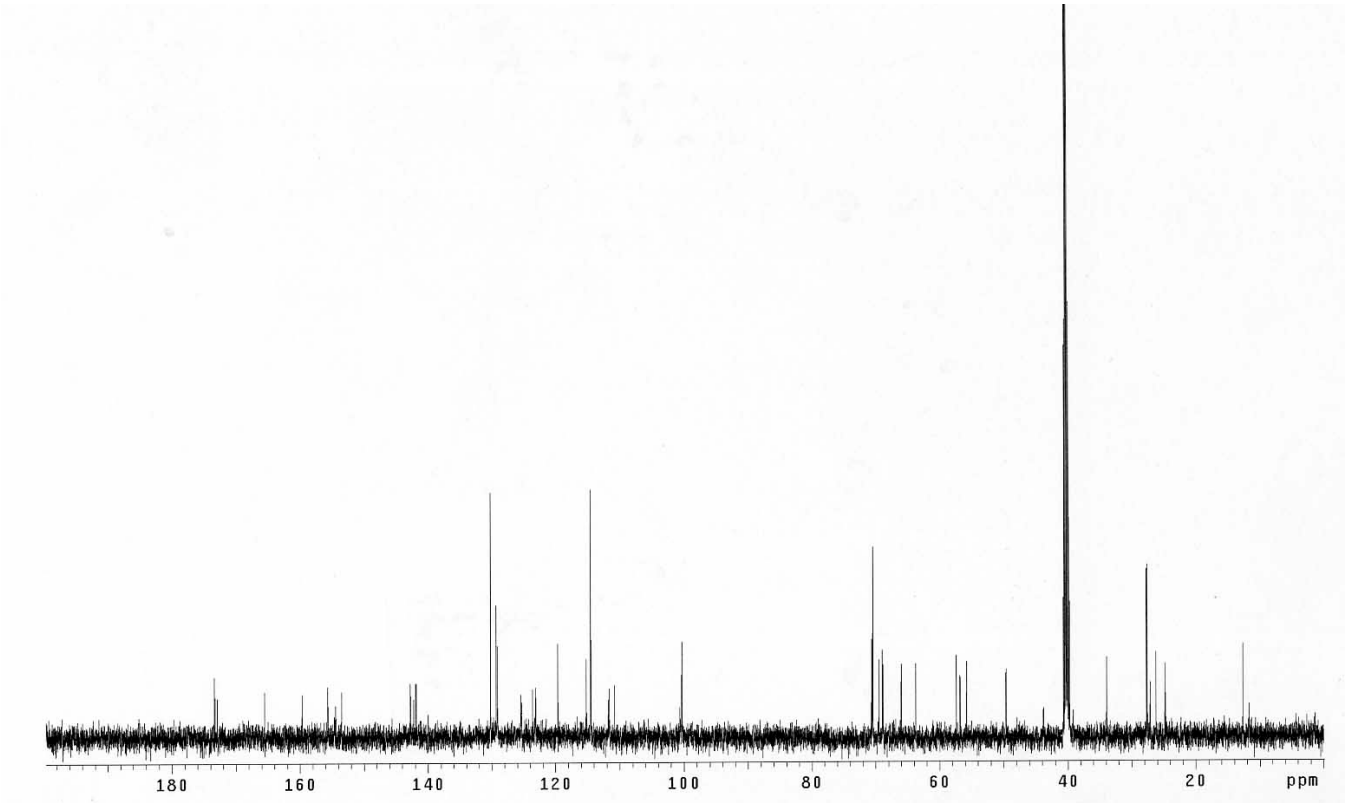
20
ppm


CH3
CH3
CH3
CH3
O
PMBO
OCH3
CH3O
O
4
O
O
N
CH3
N
CH3

**34**


9
8
7
6
5
4
3
2
1
ppm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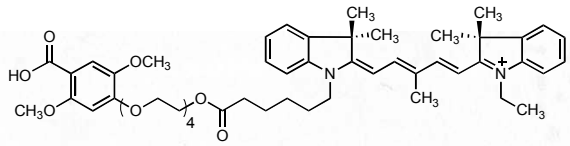
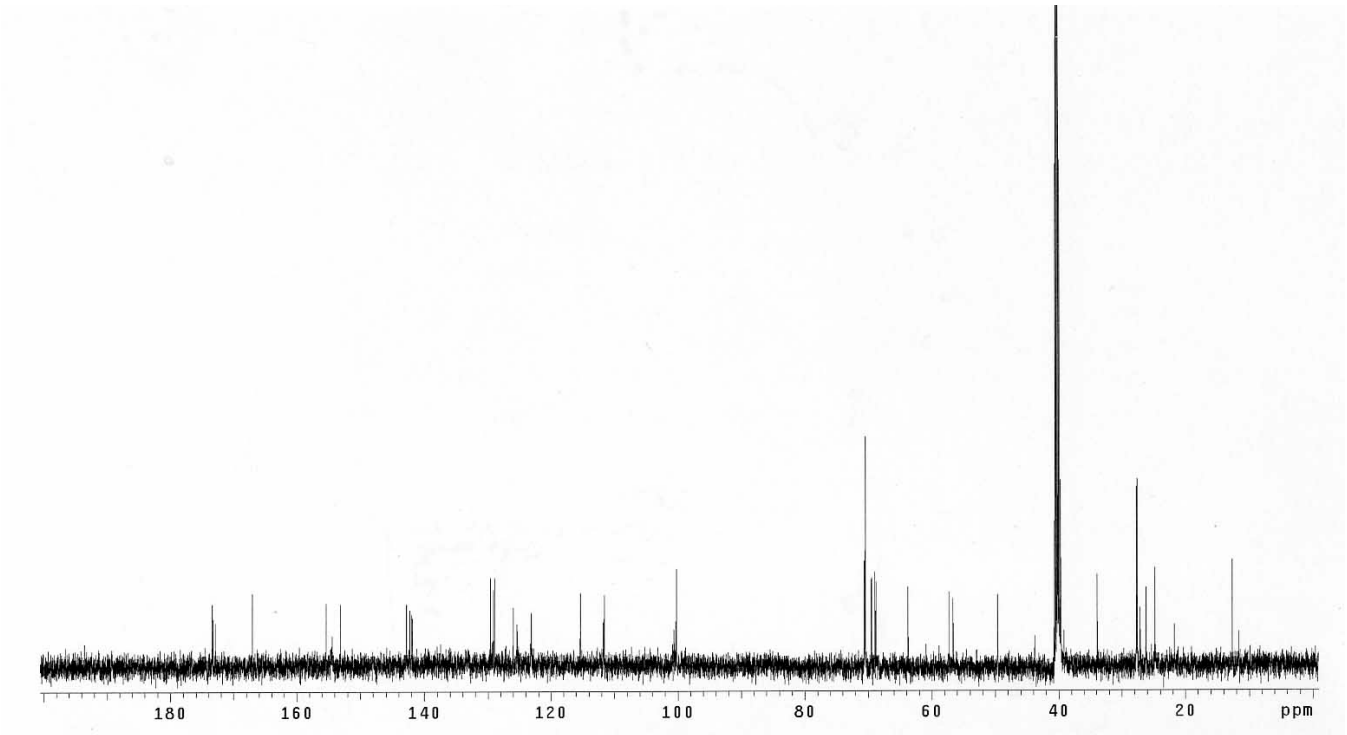
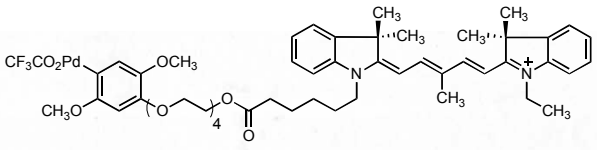
160
140
120
100
80
60
40
20
ppm



**6**




CF3CO2Pd
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CH3O
O
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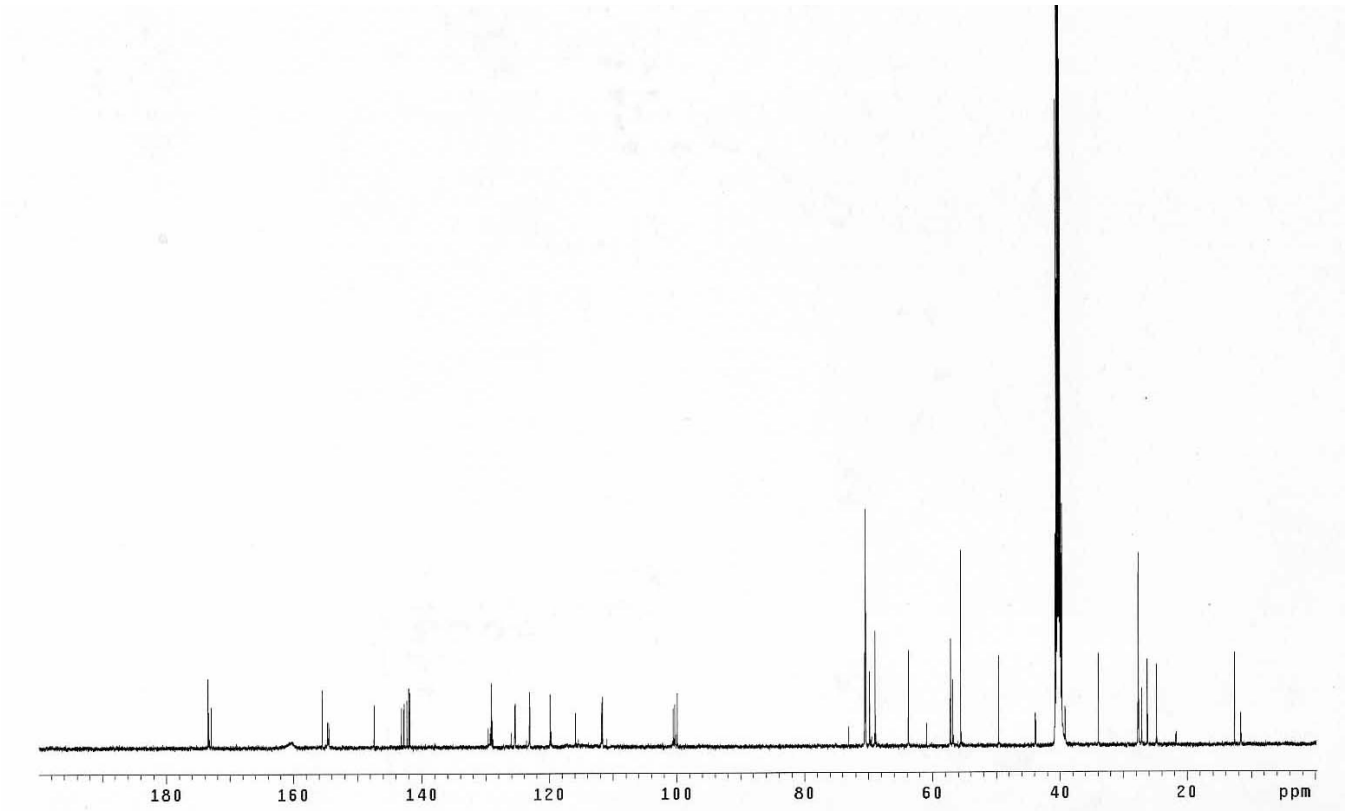
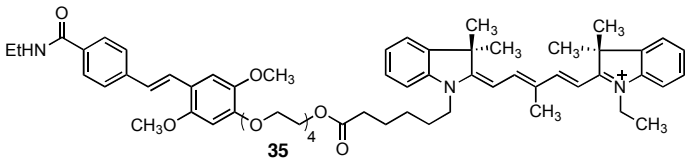
O
O
CH3
CH3
CH3
CH3
CH3
N
N
+
CH3

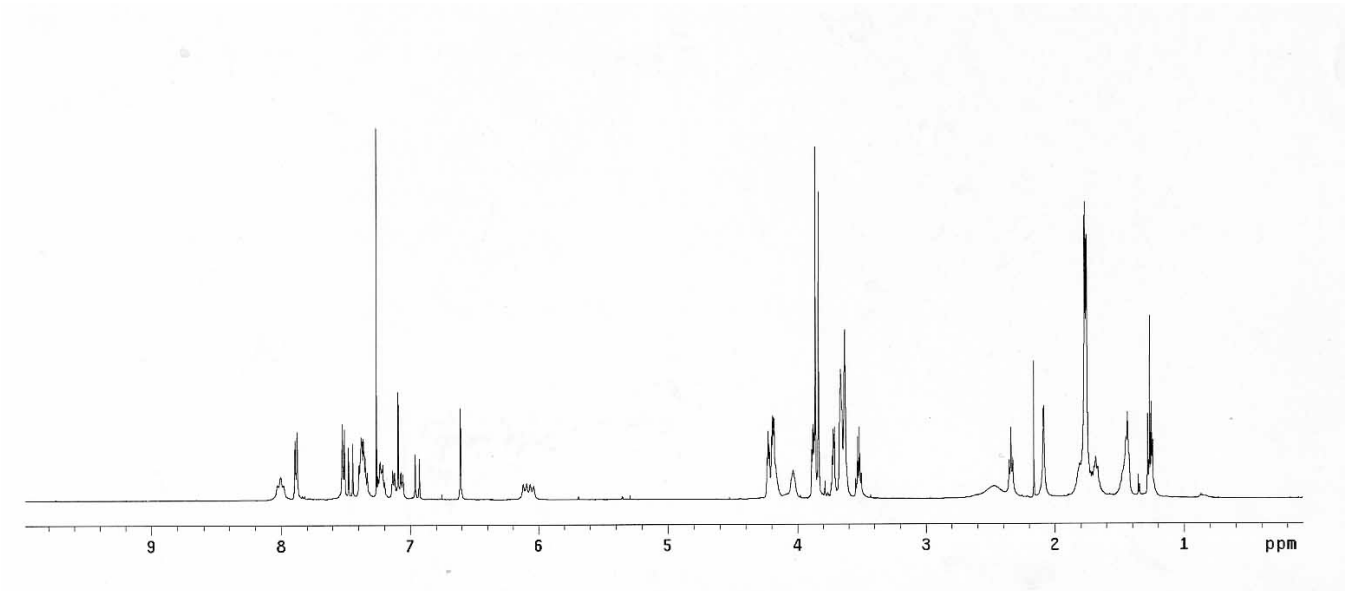
**7**

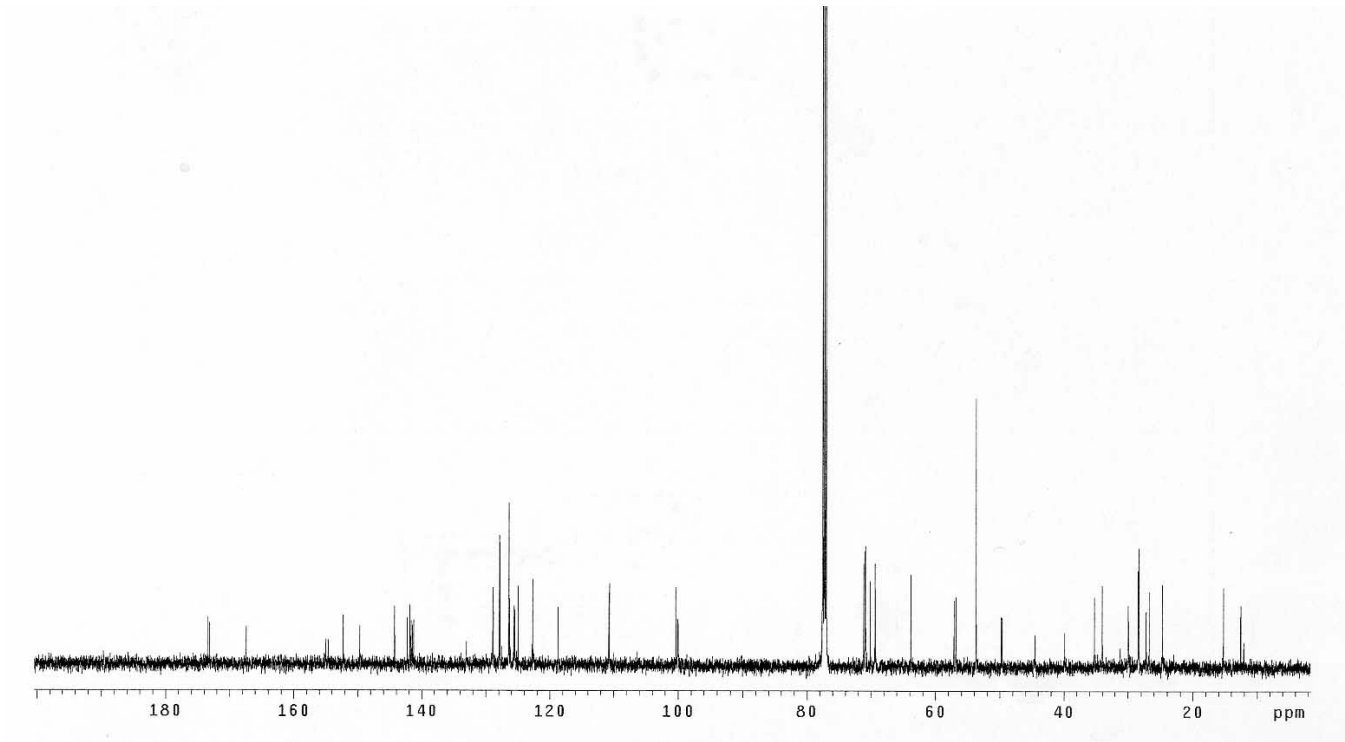

9
8
7
6
5
4
3
2
1
ppm

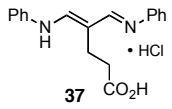

180
160
140
120
100
80
60
40
20
ppm


EtHN
O
OCH3
CH3O
O
4
O
O
CH3
CH3
CH3
CH3
N
CH3
N+
CH3

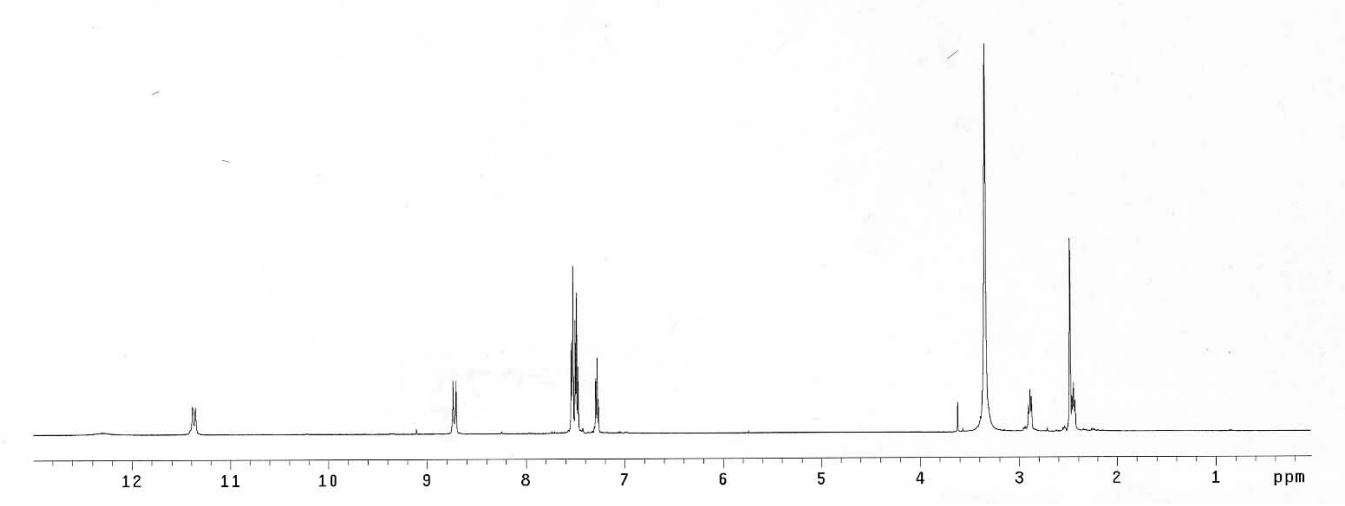
**35**

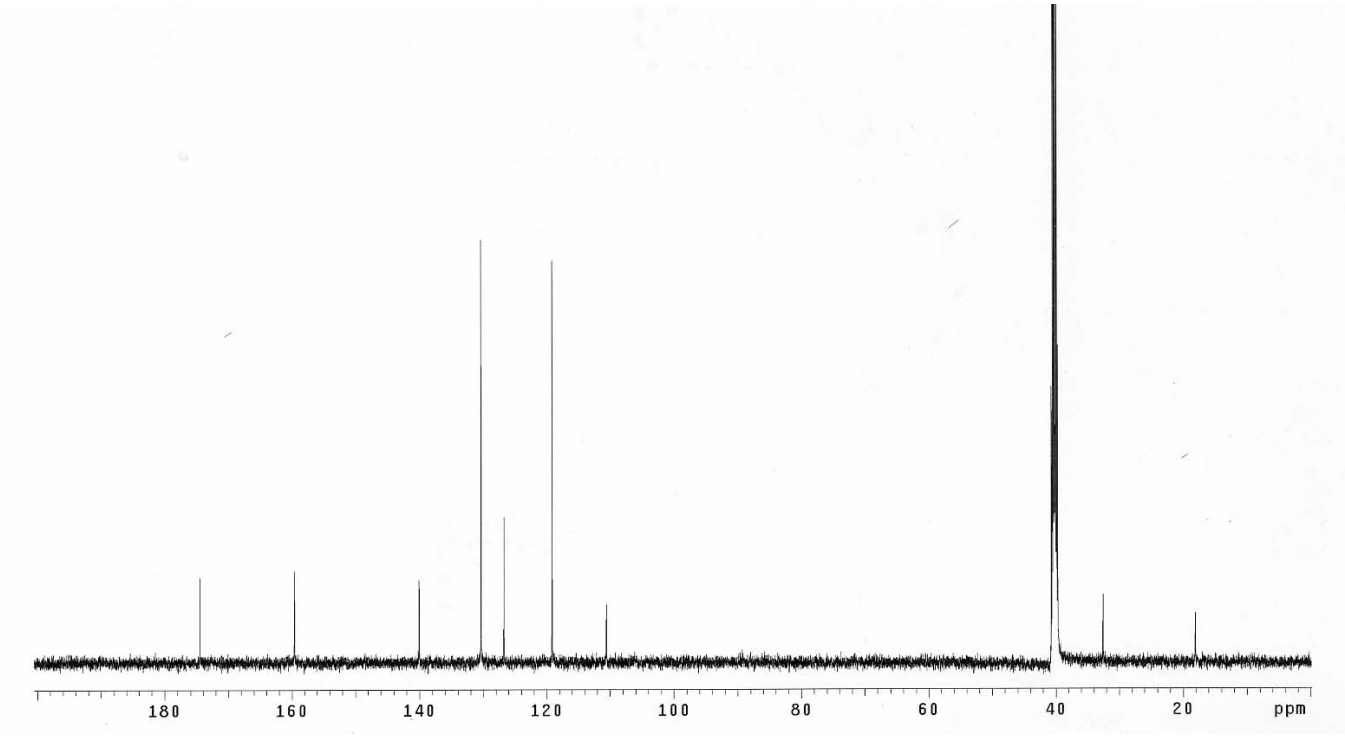

9
8
7
6
5
4
3
2
1
ppm

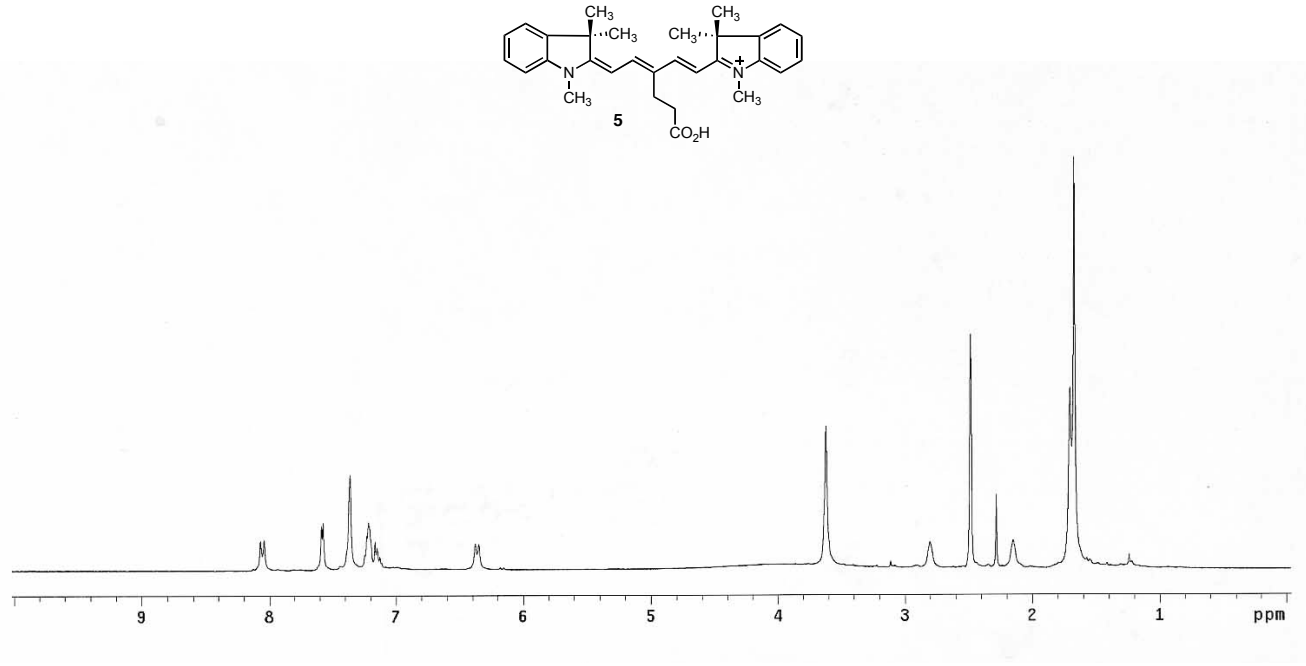

180
160
140
120
100
80
60
40
20
ppm


Ph
N
H
N
Ph
• HCl
CO2H

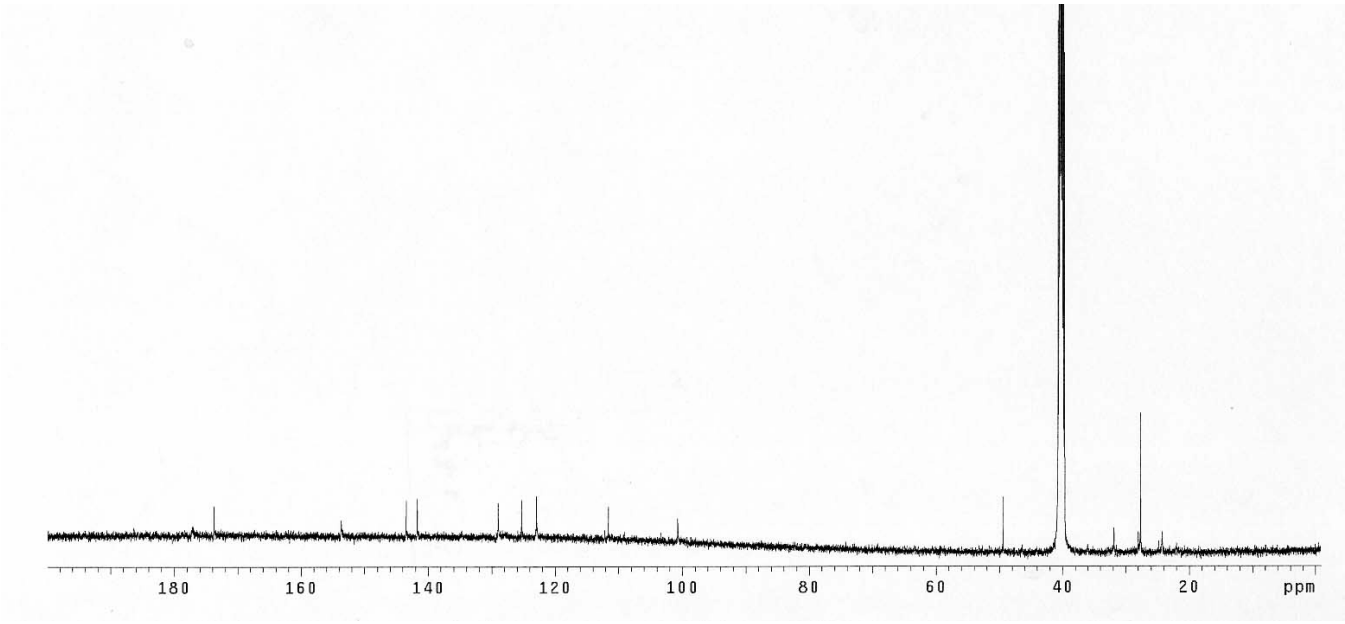
**37**

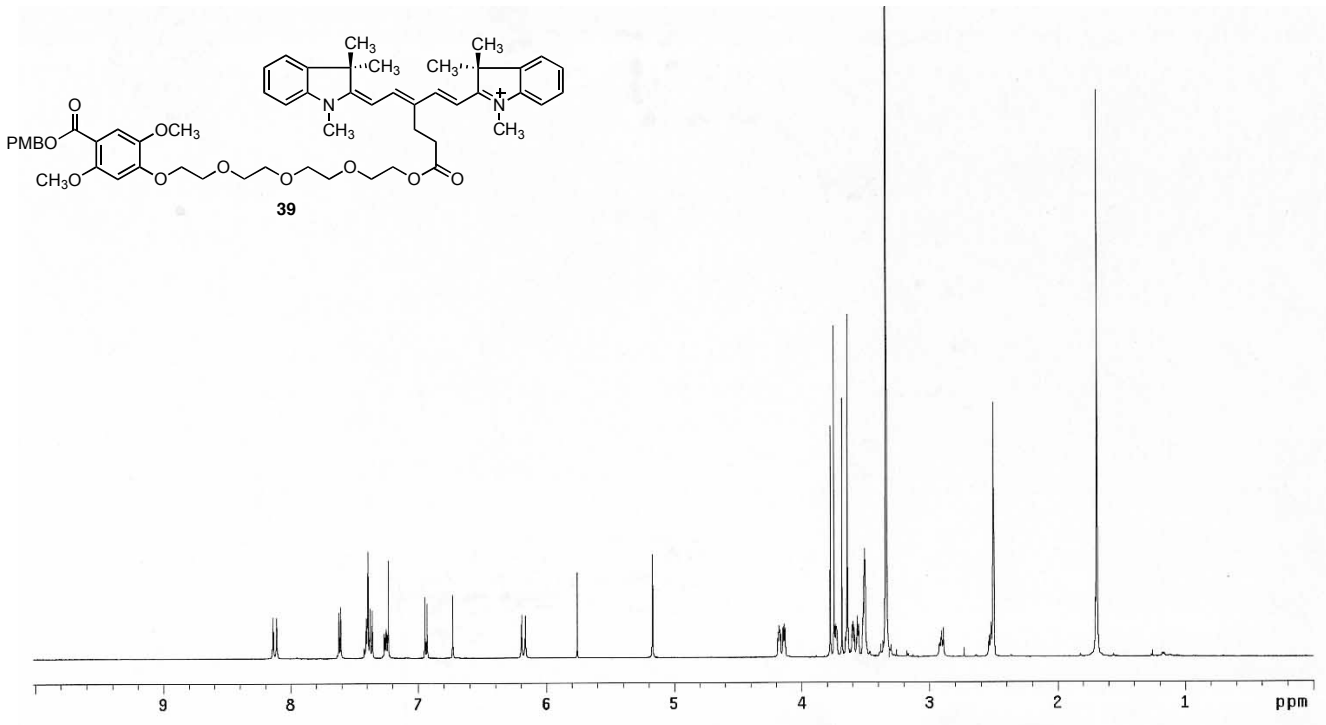

12
11
10
9
8
7
6
5
4
3
2
1
ppm

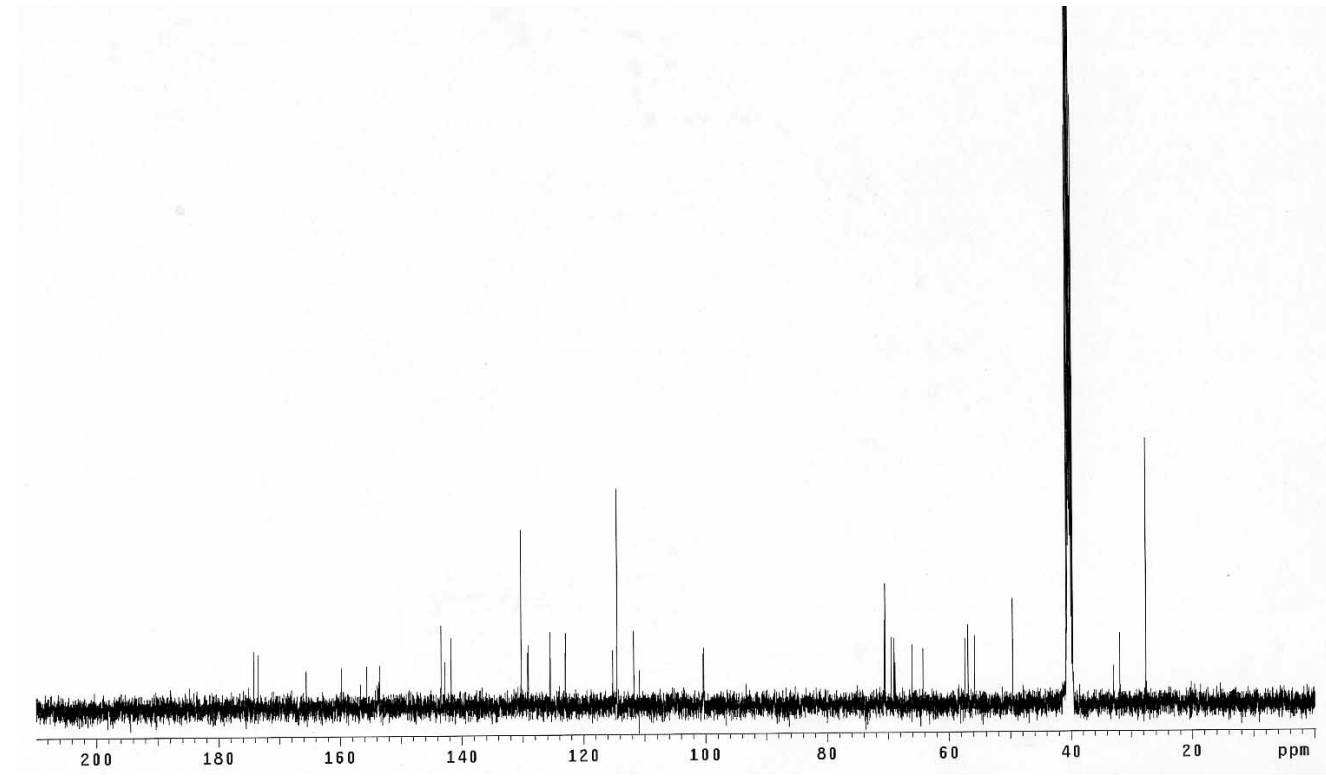

180
160
140
120
100
80
60
40
20
ppm

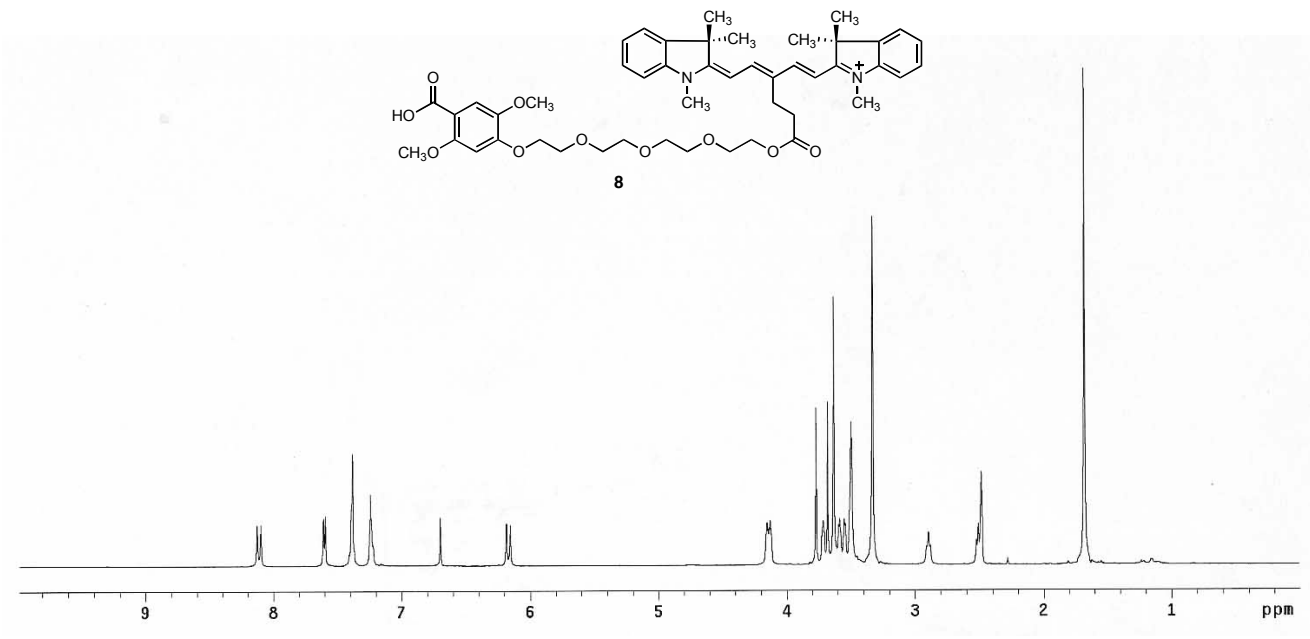

CH3
CH3
CH3
CH3
N
CH3
N+
CH3
CO2H
9
8
7
6
5
4
3
2
1
ppm

**5**

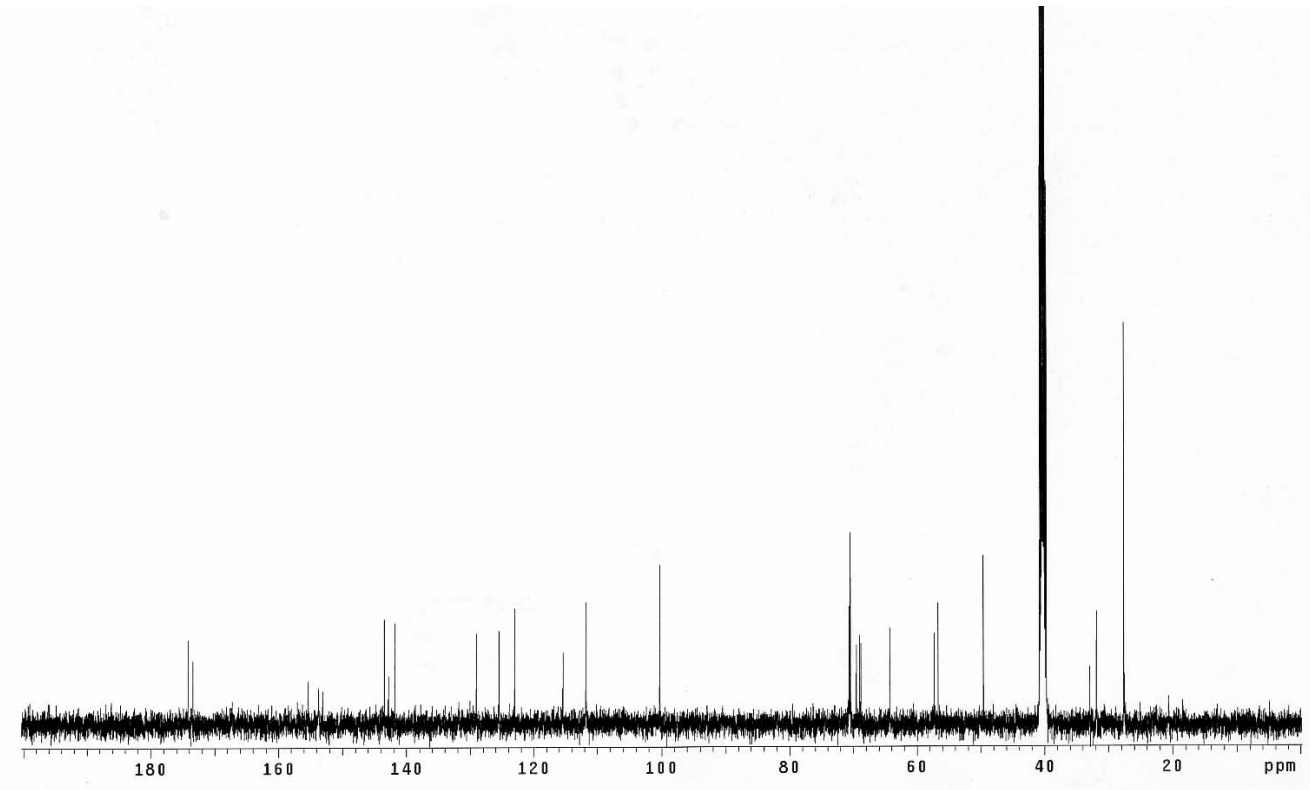

180
160
140
120
100
80
60
40
20
ppm

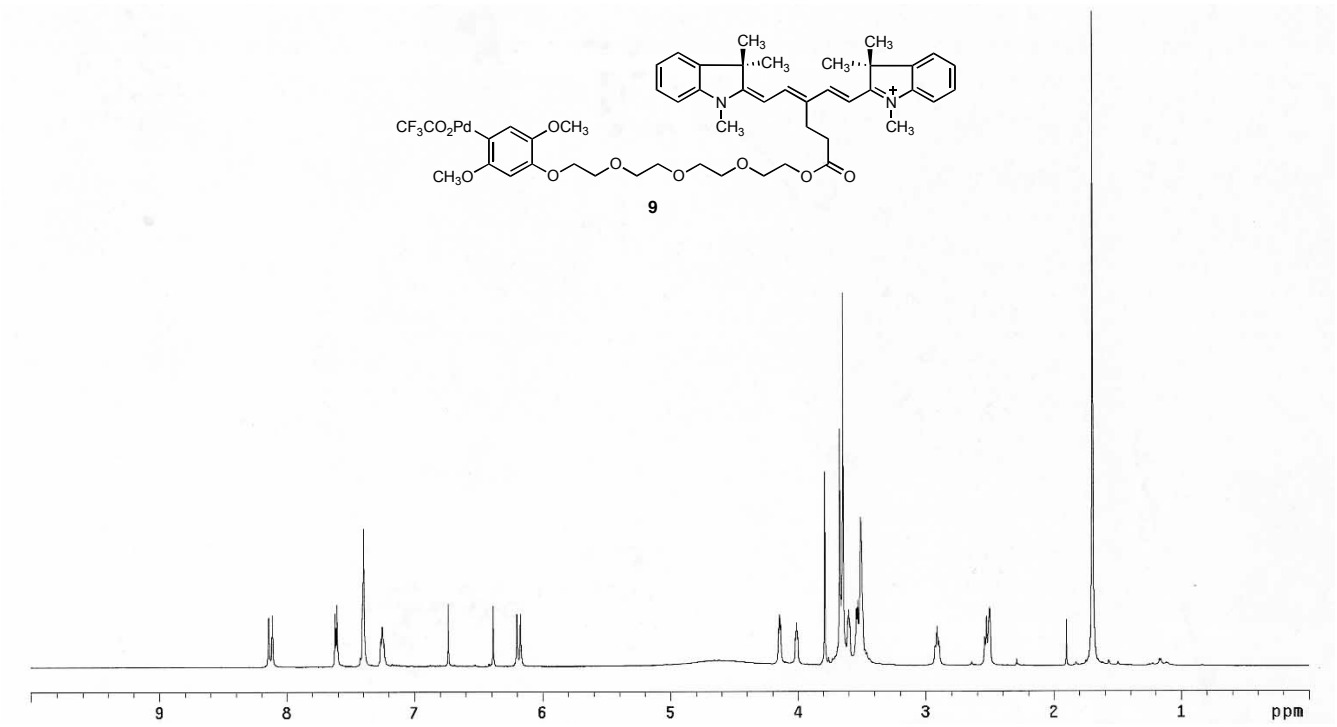

CH3
CH3
CH3
CH3
N
CH3
N
CH3
O
PMBO
OCH3
CH3O
O
O
O
O
O
O
39
9
8
7
6
5
4
3
2
1
ppm

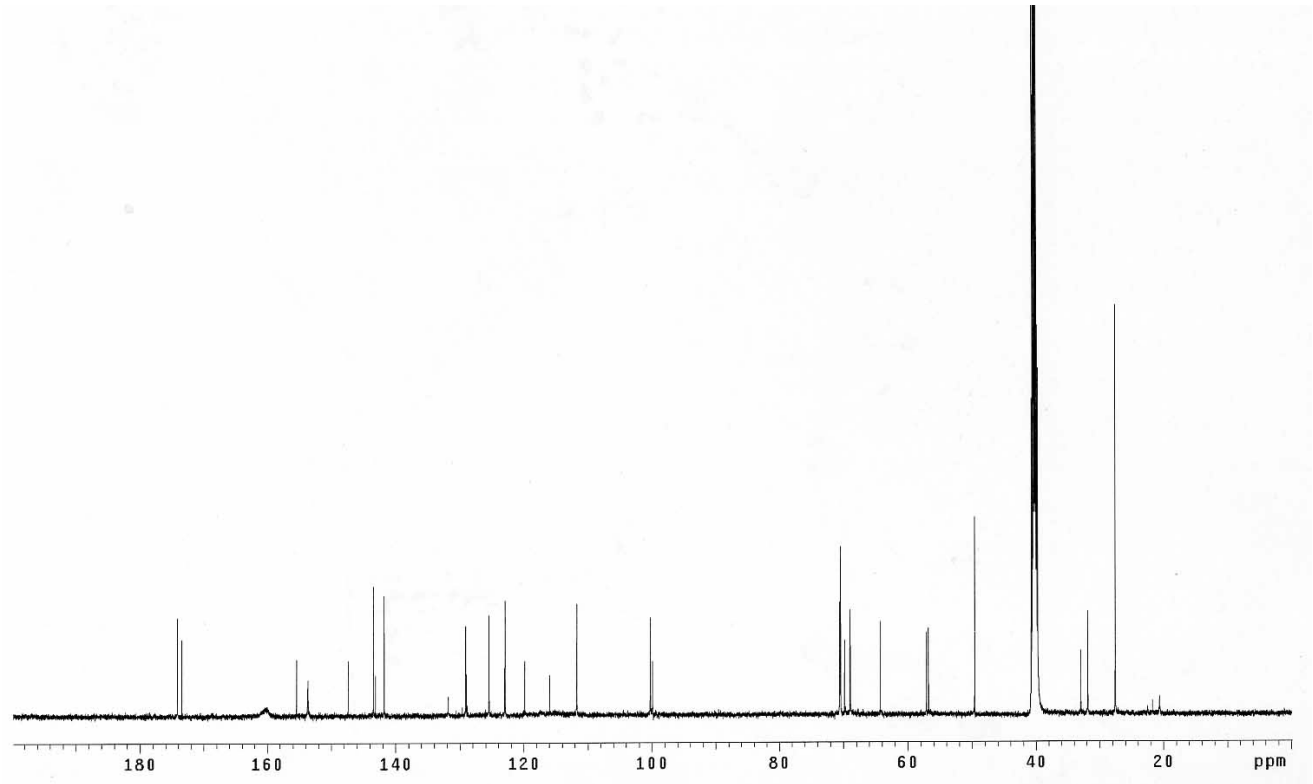

200
180
160
140
120
100
80
60
40
20
ppm

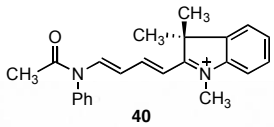

HO
O
OCH3
CH3O
O
O
O
O
O
O
N
CH3
CH3
CH3
CH3
CH3
N
CH3
9
8
7
6
5
4
3
2
1
ppm

8

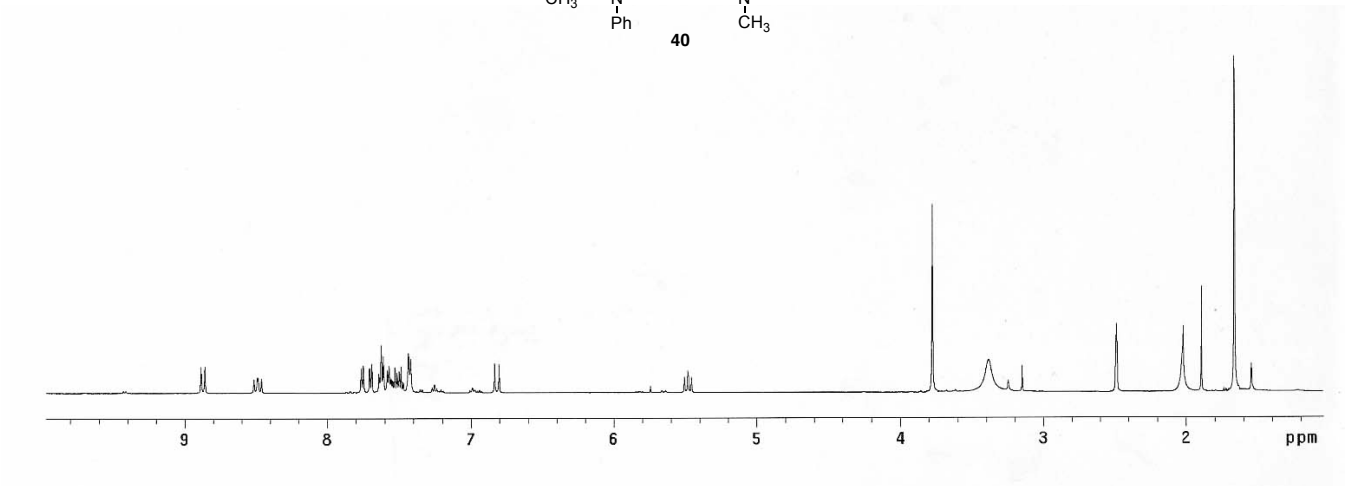

180
160
140
120
100
80
60
40
20
ppm

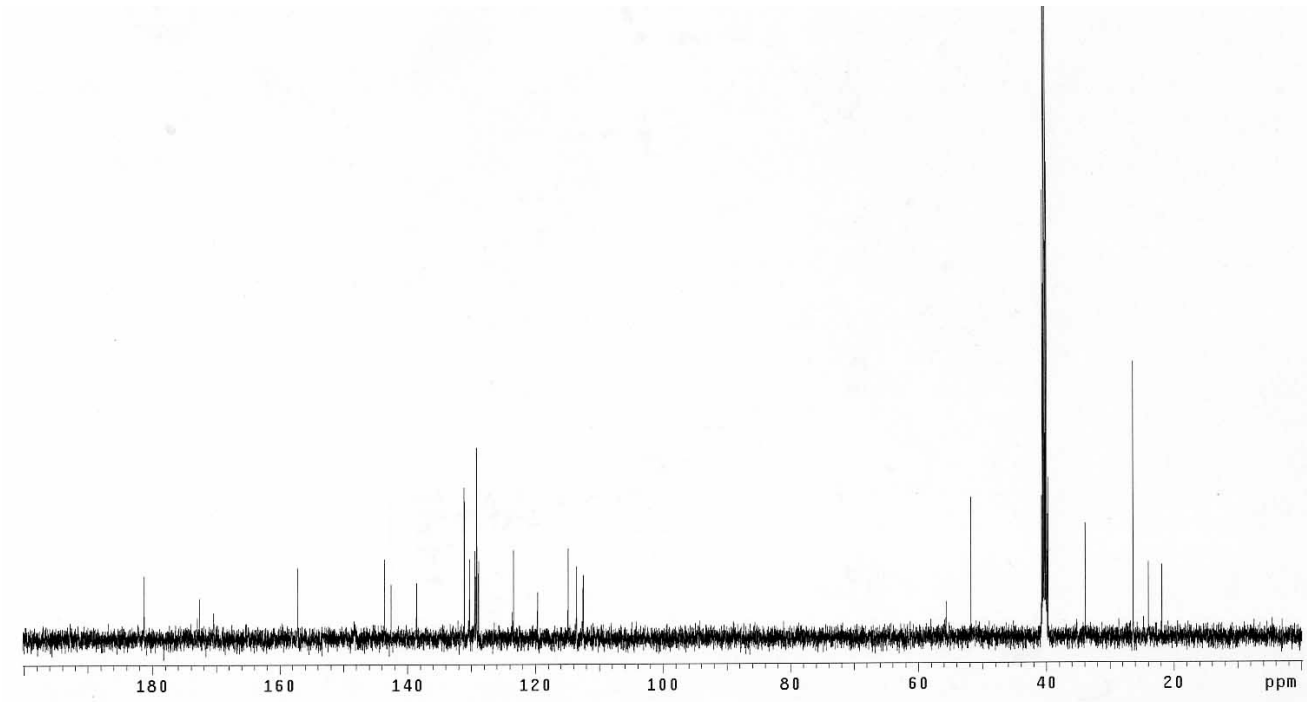

CF3CO2Pd
OCH3
CH3O
CH3
CH3
CH3
CH3
CH3
N
CH3
N
CH3
O
O
O
O
O
O
9
9
8
7
6
5
4
3
2
1
ppm

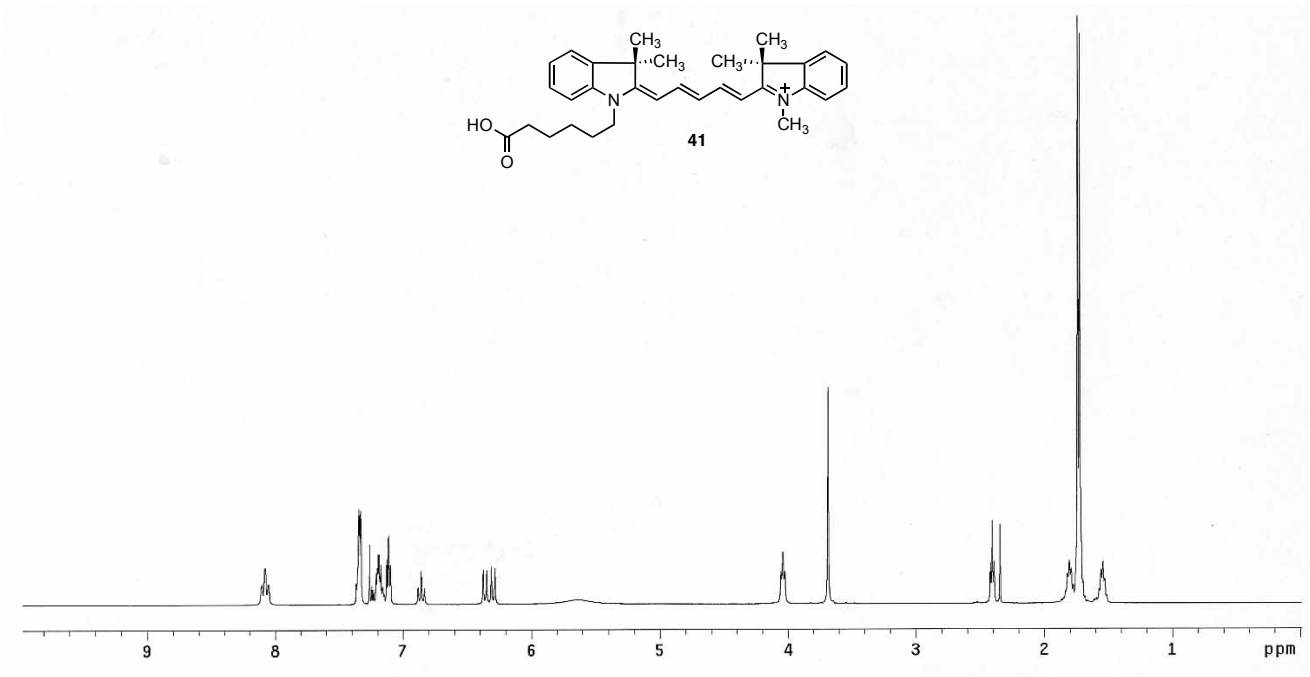

180
160
140
120
100
80
60
40
20
ppm

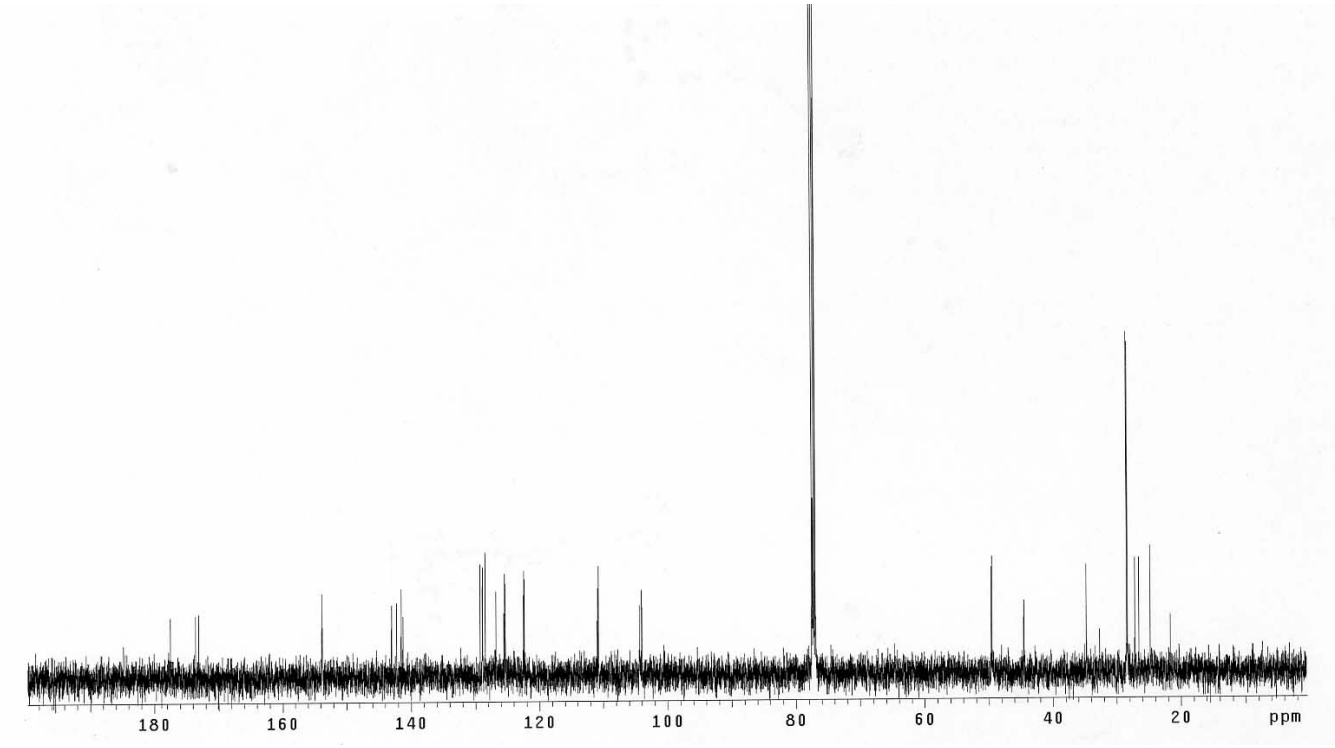

CH3
CH3
O
CH3
N
Ph
N+
CH3

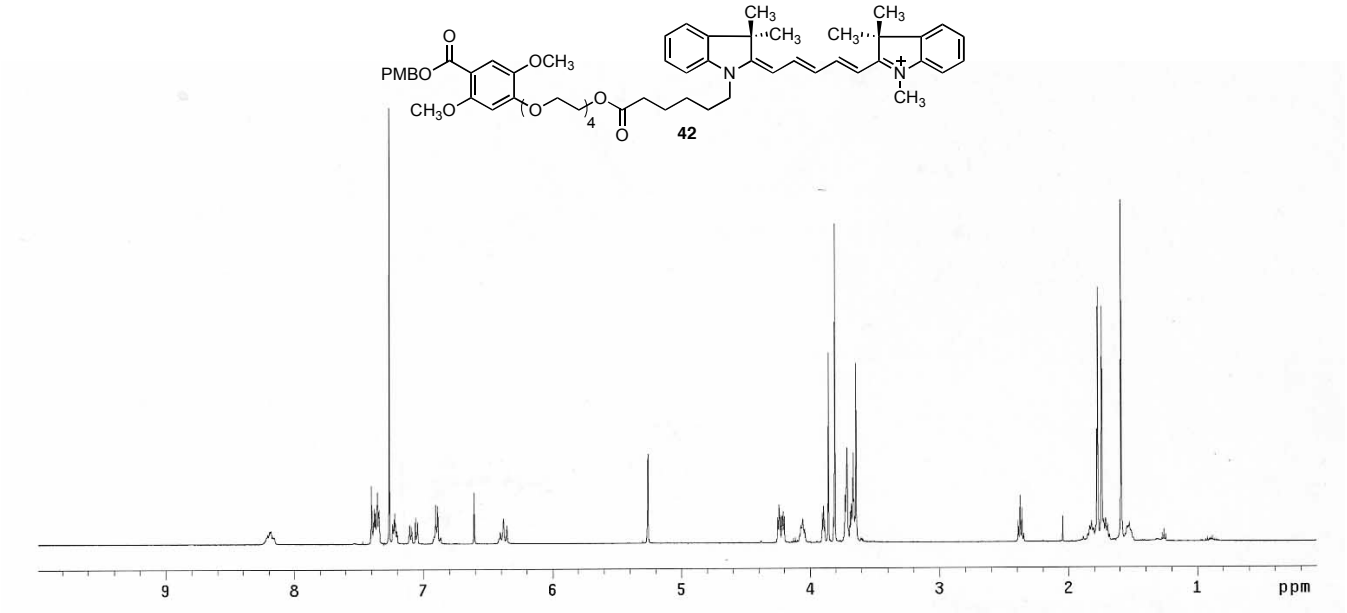
**40**

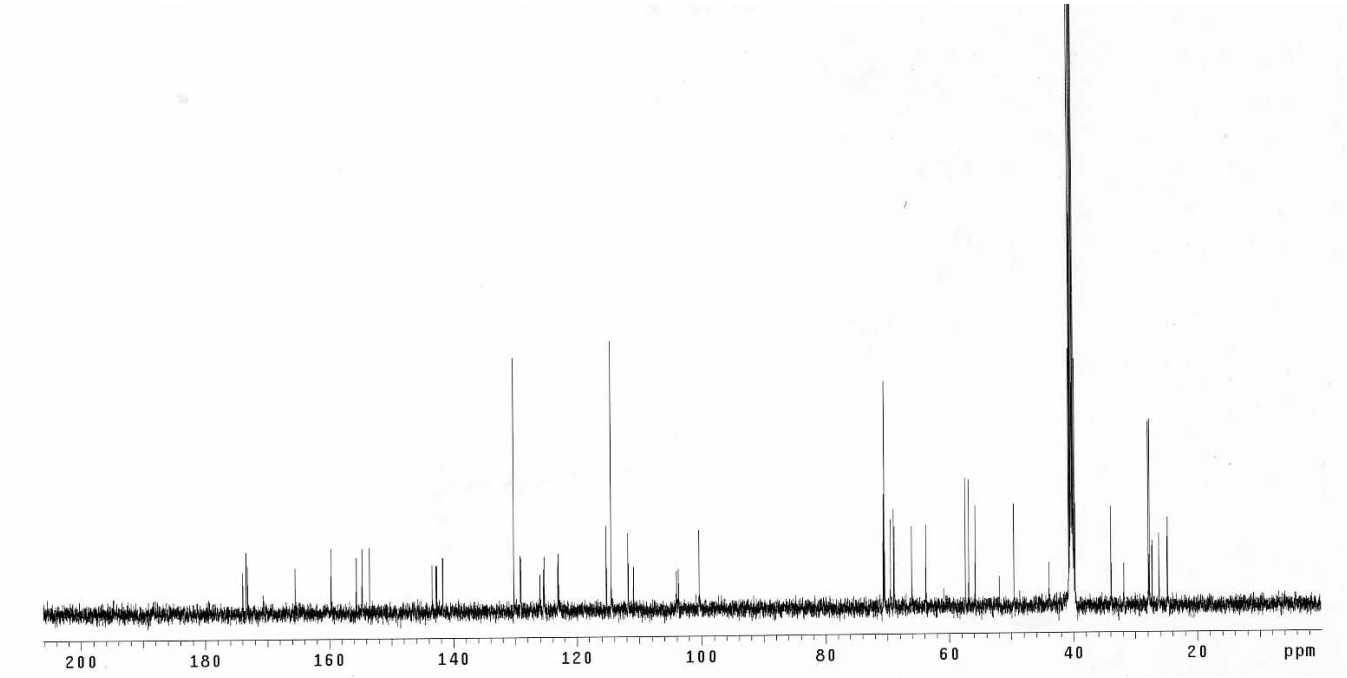

9
8
7
6
5
4
3
2
ppm

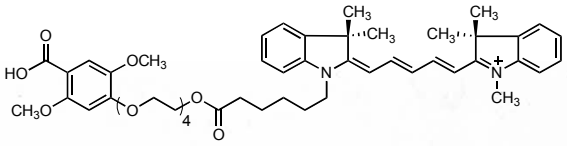

180
160
140
120
100
80
60
40
20
ppm


CH3
CH3
CH3
CH3
N
N
CH3
HO
O
41
9
8
7
6
5
4
3
2
1
ppm


180
160
140
120
100
80
60
40
20
ppm

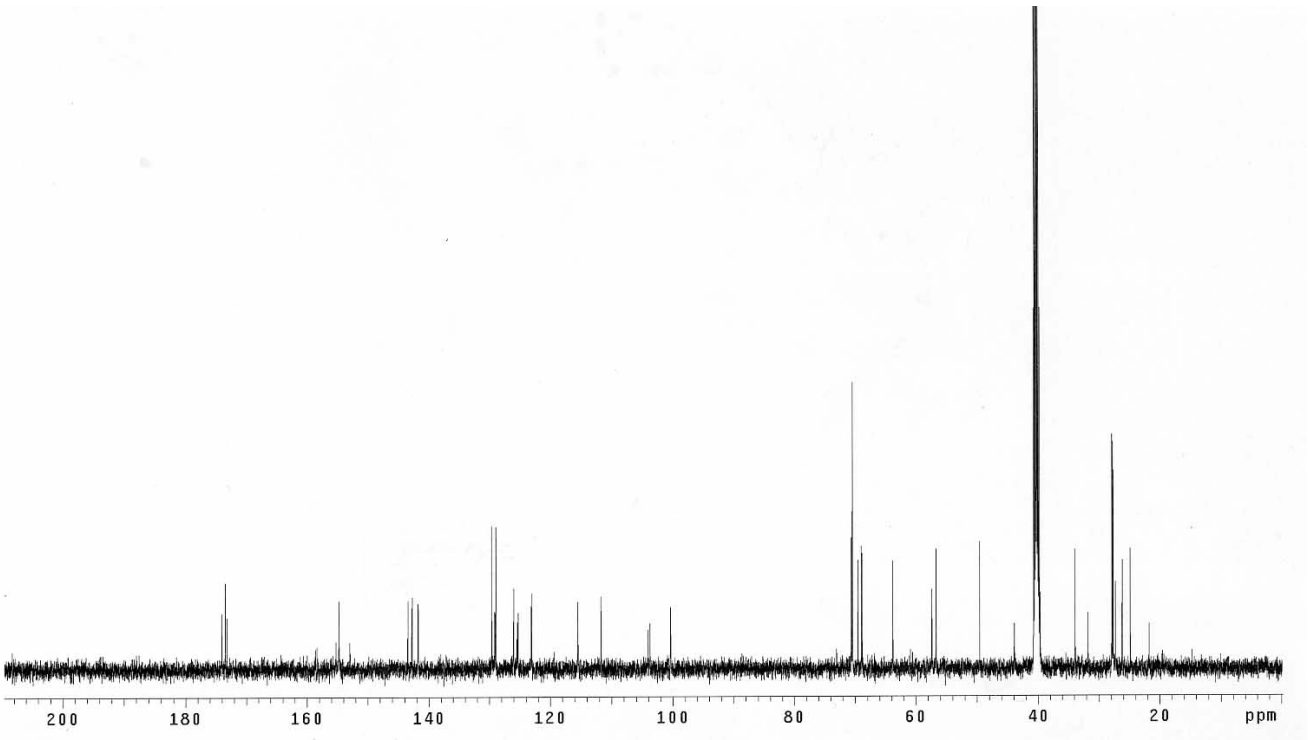

PMBO
OCH3
CH3O
O
4
CH3
CH3
CH3
CH3
N
N
CH3
42
9
8
7
6
5
4
3
2
1
ppm

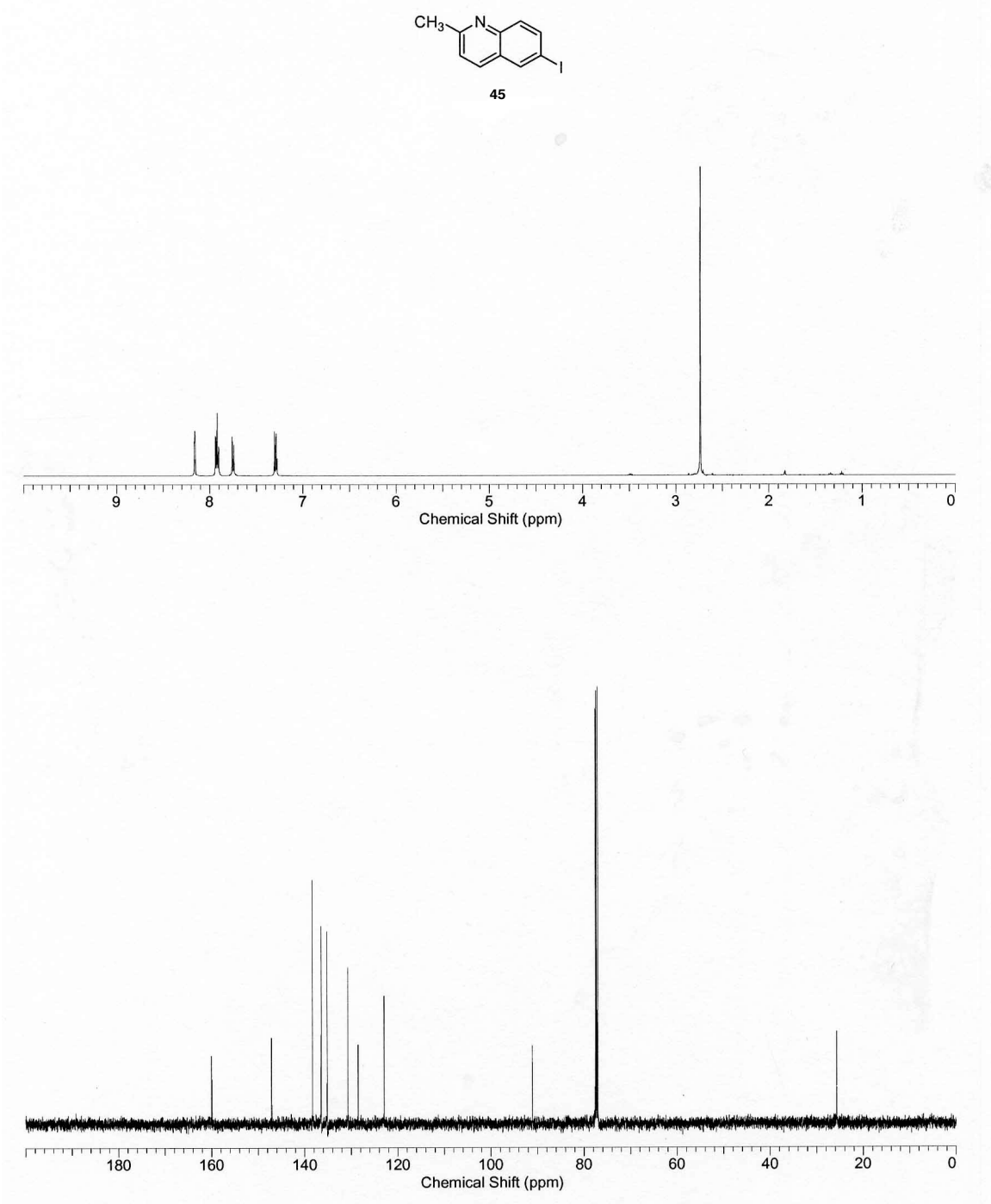

200
180
160
140
120
100
80
60
40
20
ppm

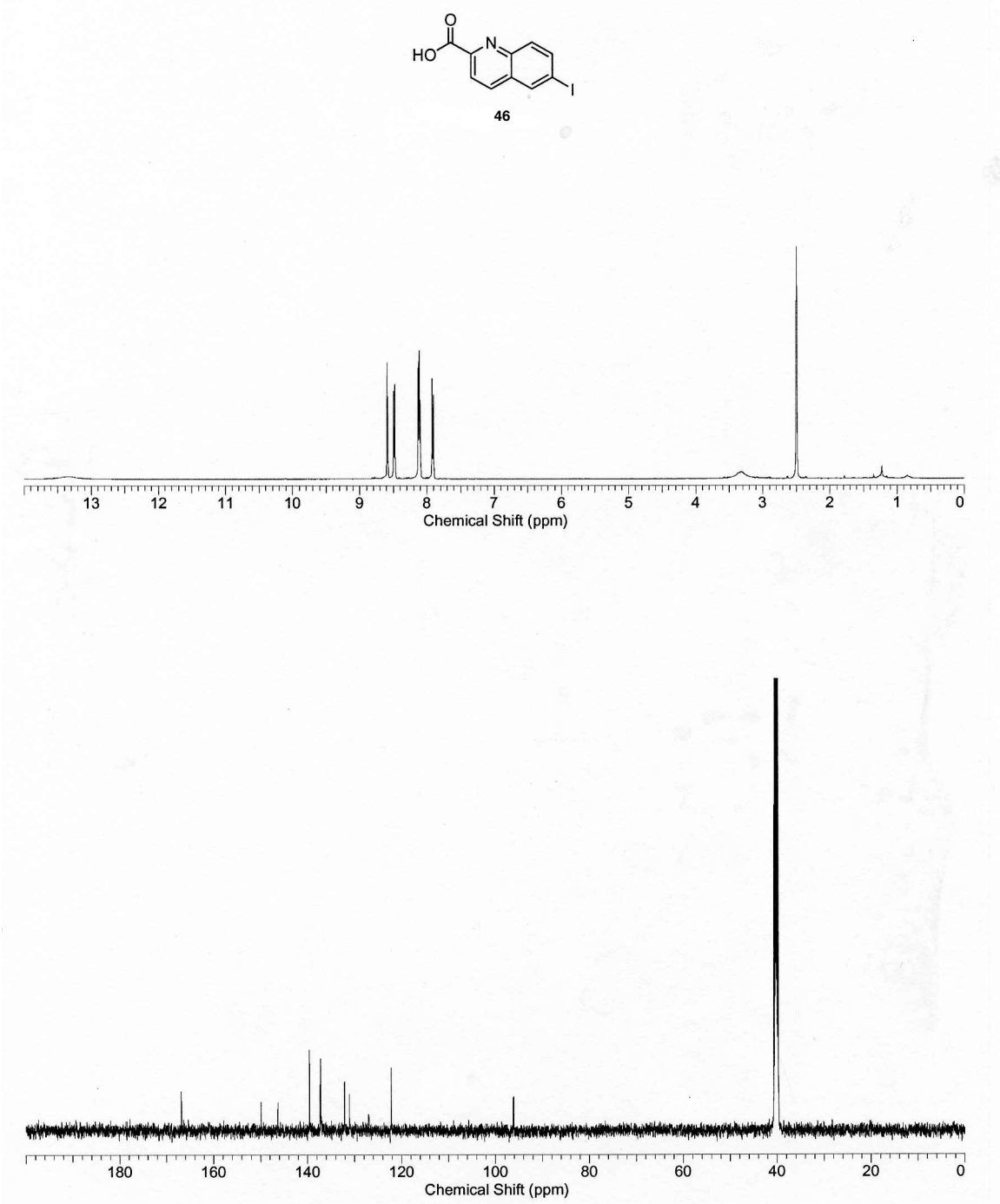


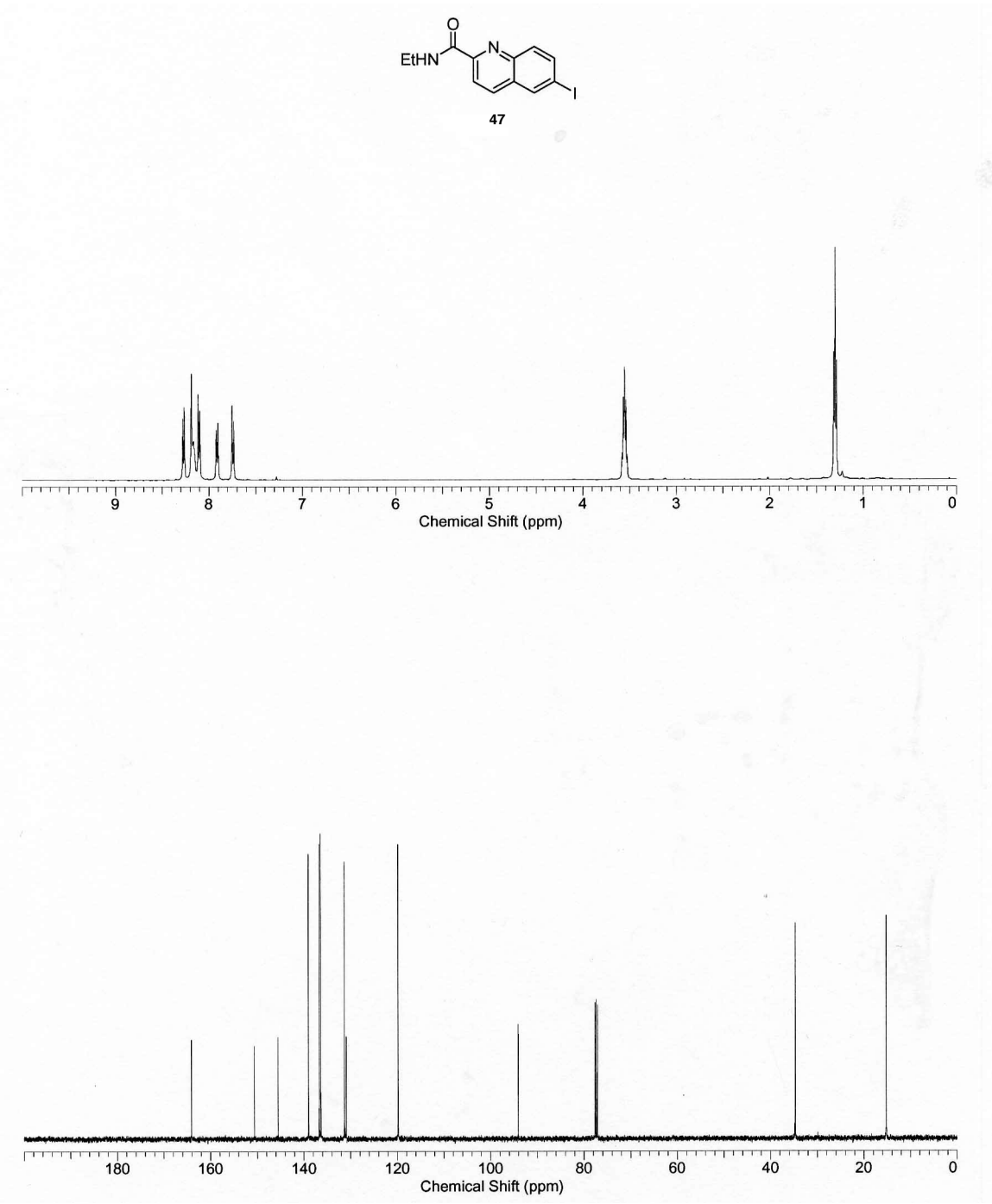
**3**

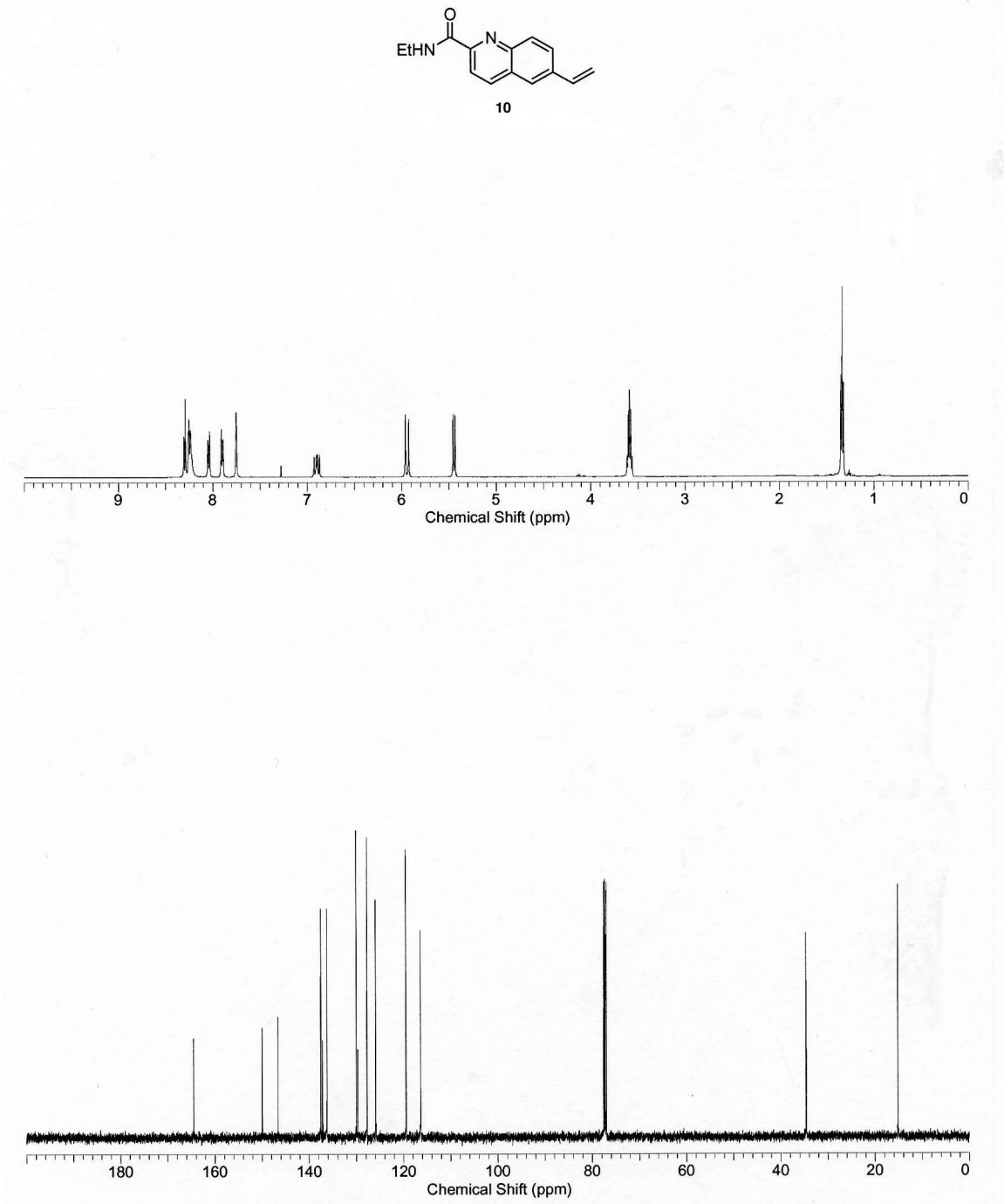


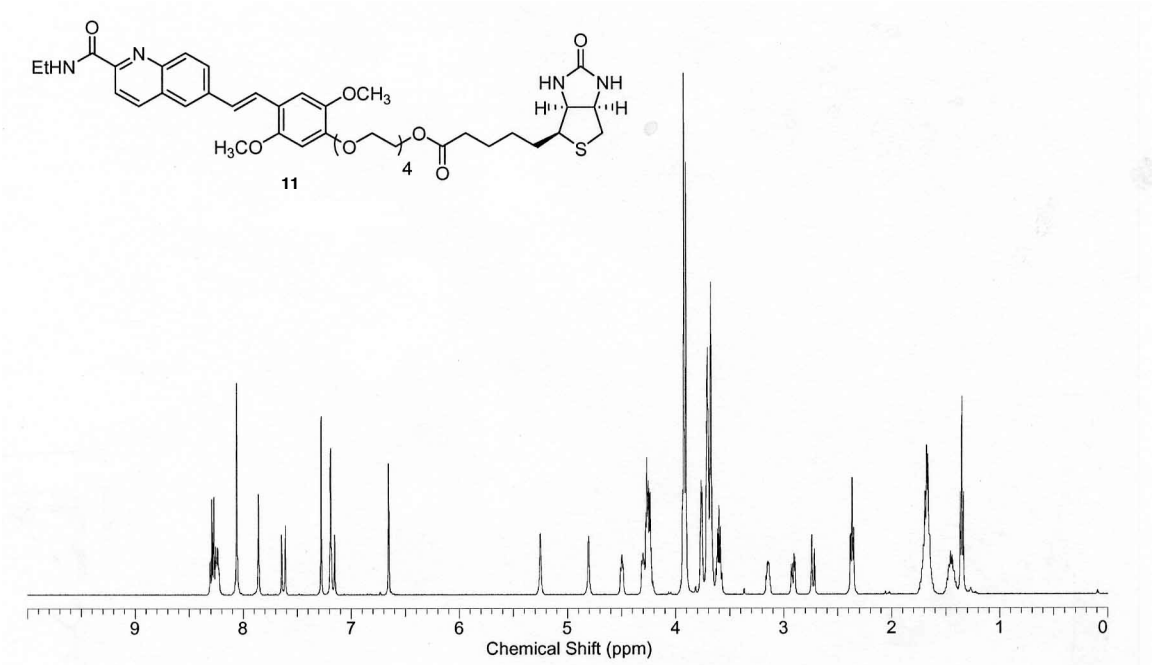




CH3
N
I
45
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

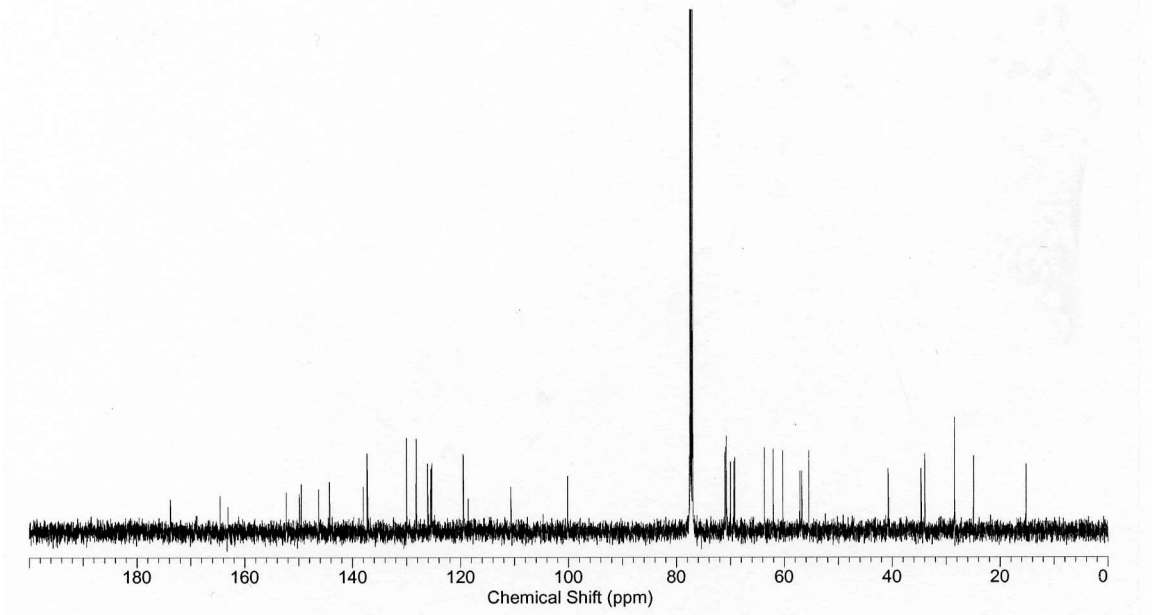

O
HO
N
I
46
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

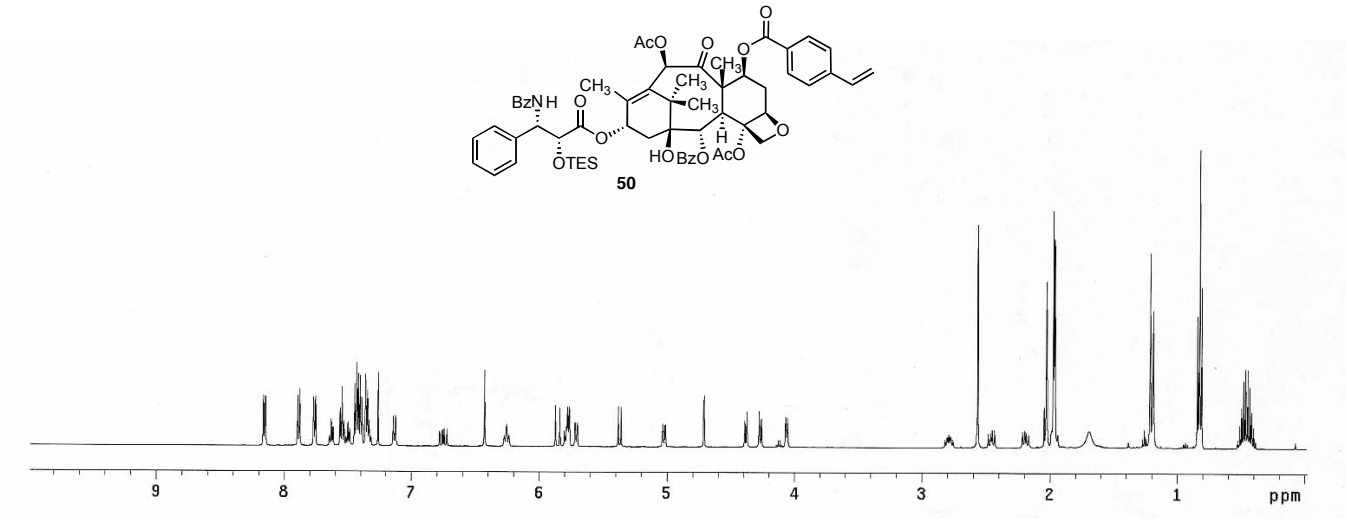

O
EtHN
N
I
47
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


EtHN
O
N
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

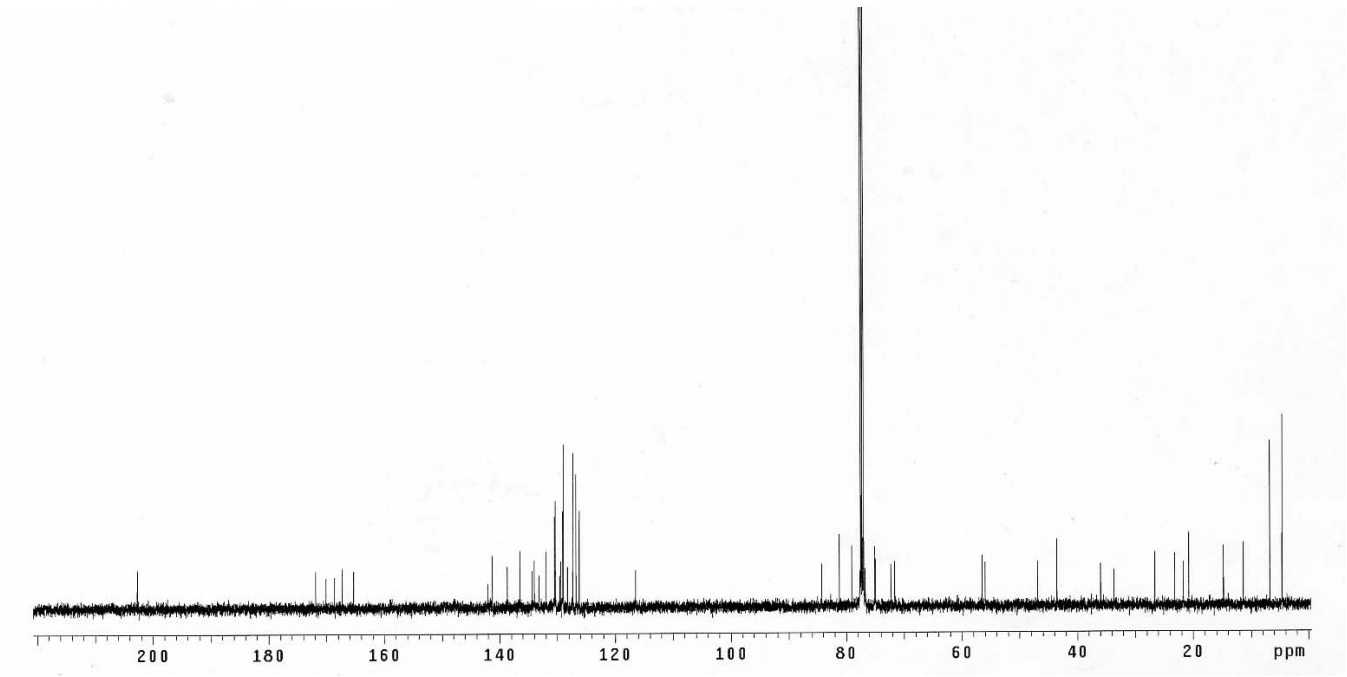

EtHN
O
N
OCH3
H3CO
O
4
O
HN
NH
H
H
S
O
Chemical Shift (ppm)

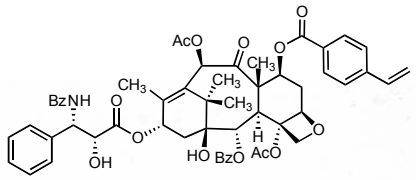
11


Chemical Shift (ppm)

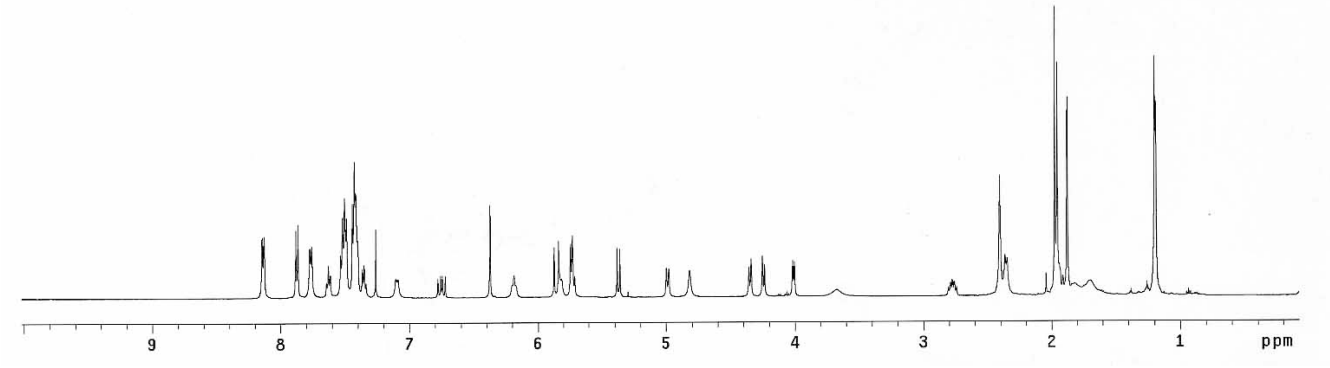

AcO
O
O
O
CH3
CH3
CH3
CH3
BzNH
O
O
H
HO
BzO
AcO
OTES
9
8
7
6
5
4
3
2
1
ppm

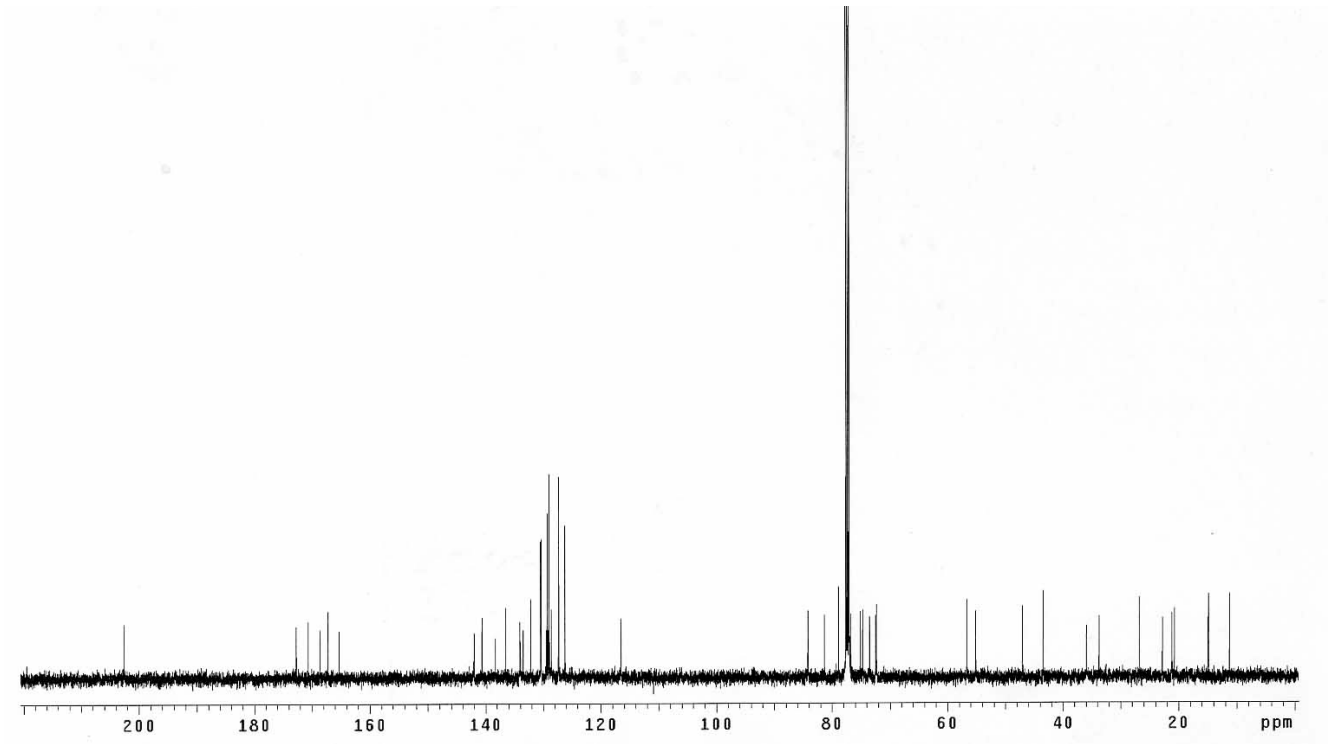
**50**

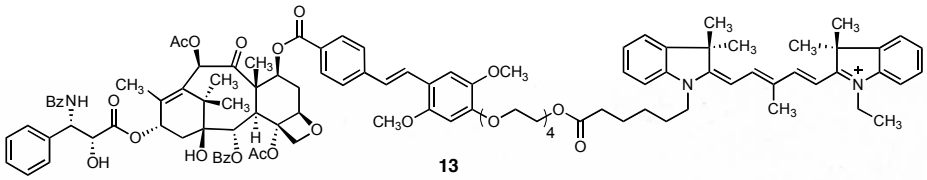

200
180
160
140
120
100
80
60
40
20
ppm


AcO
O
O
O
CH3
CH3
CH3
CH3
BzNH
O
O
OH
H
O
HO
BzO
AcO

**12**

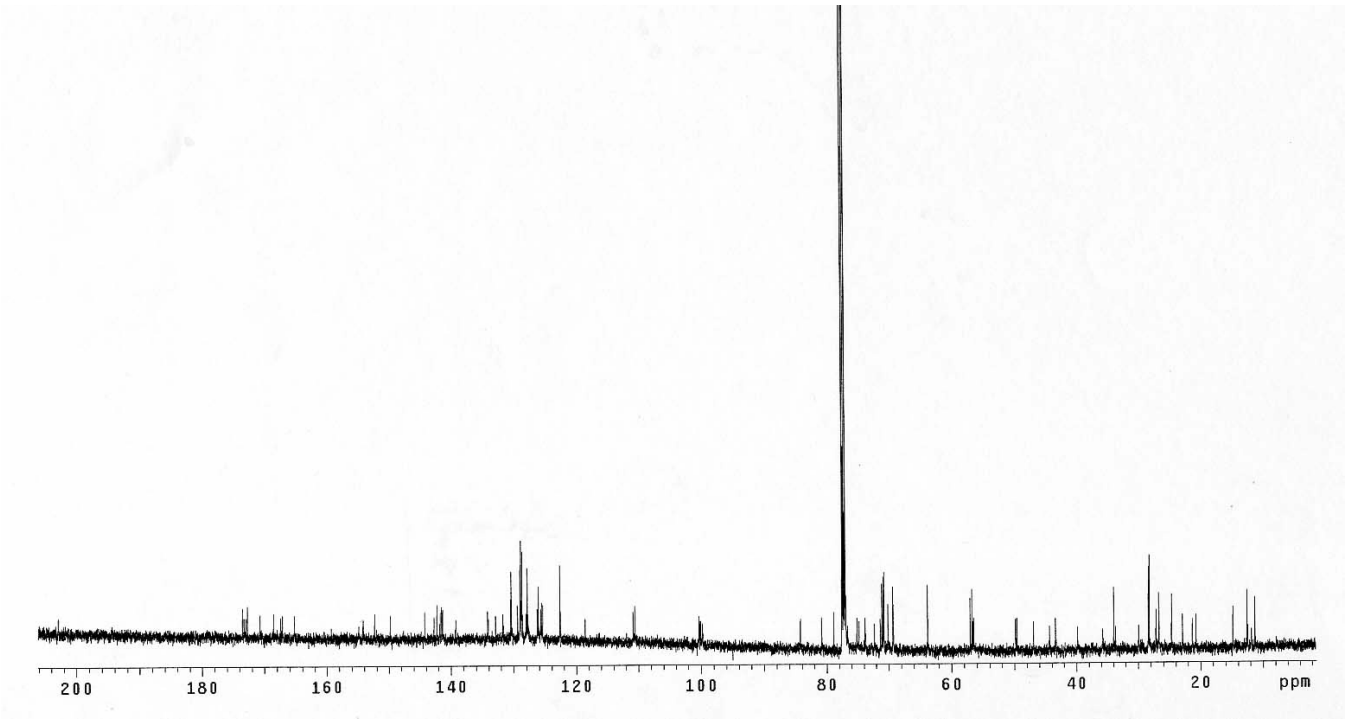

9
8
7
6
5
4
3
2
1
ppm

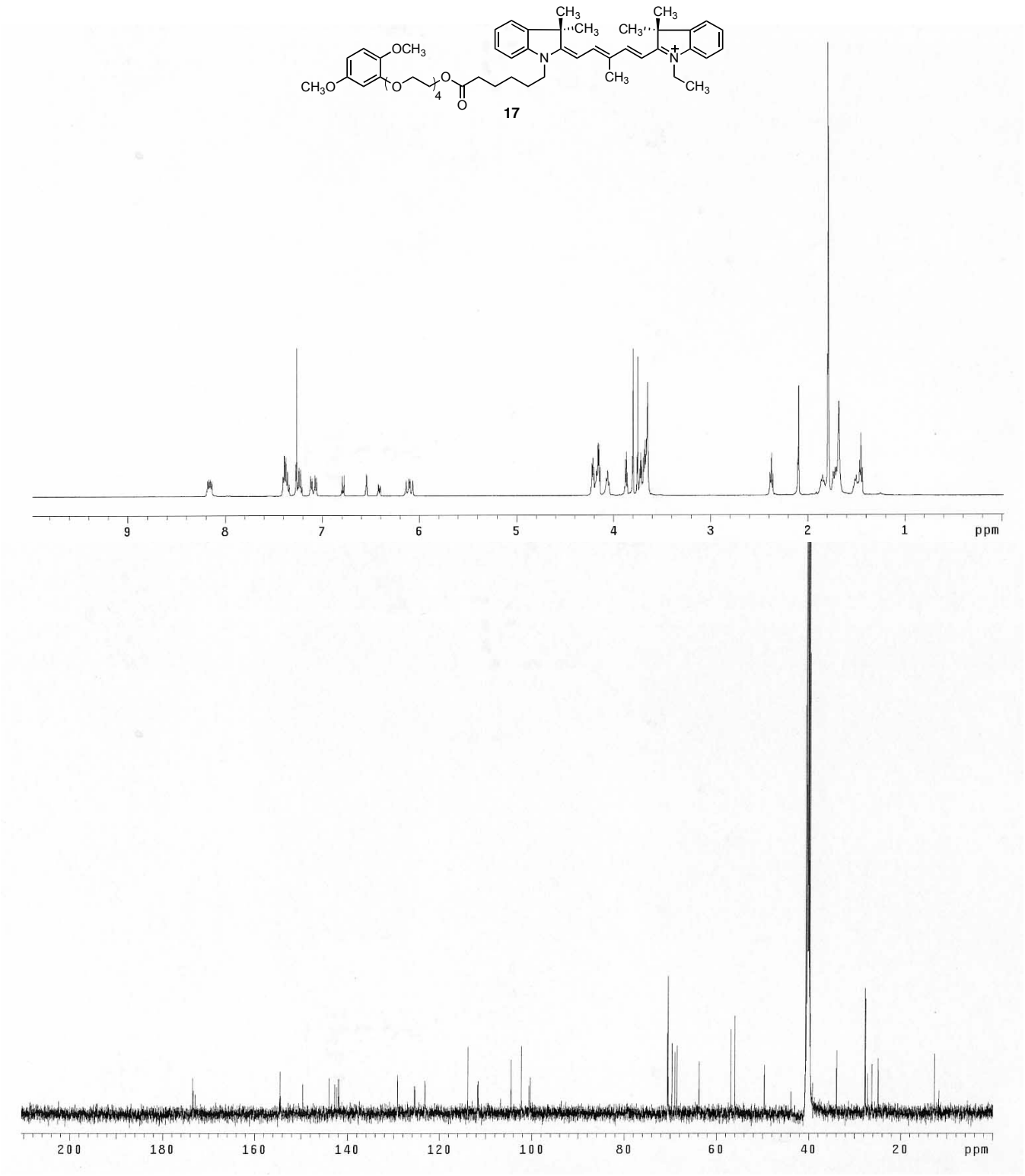

200
180
160
140
120
100
80
60
40
20
ppm

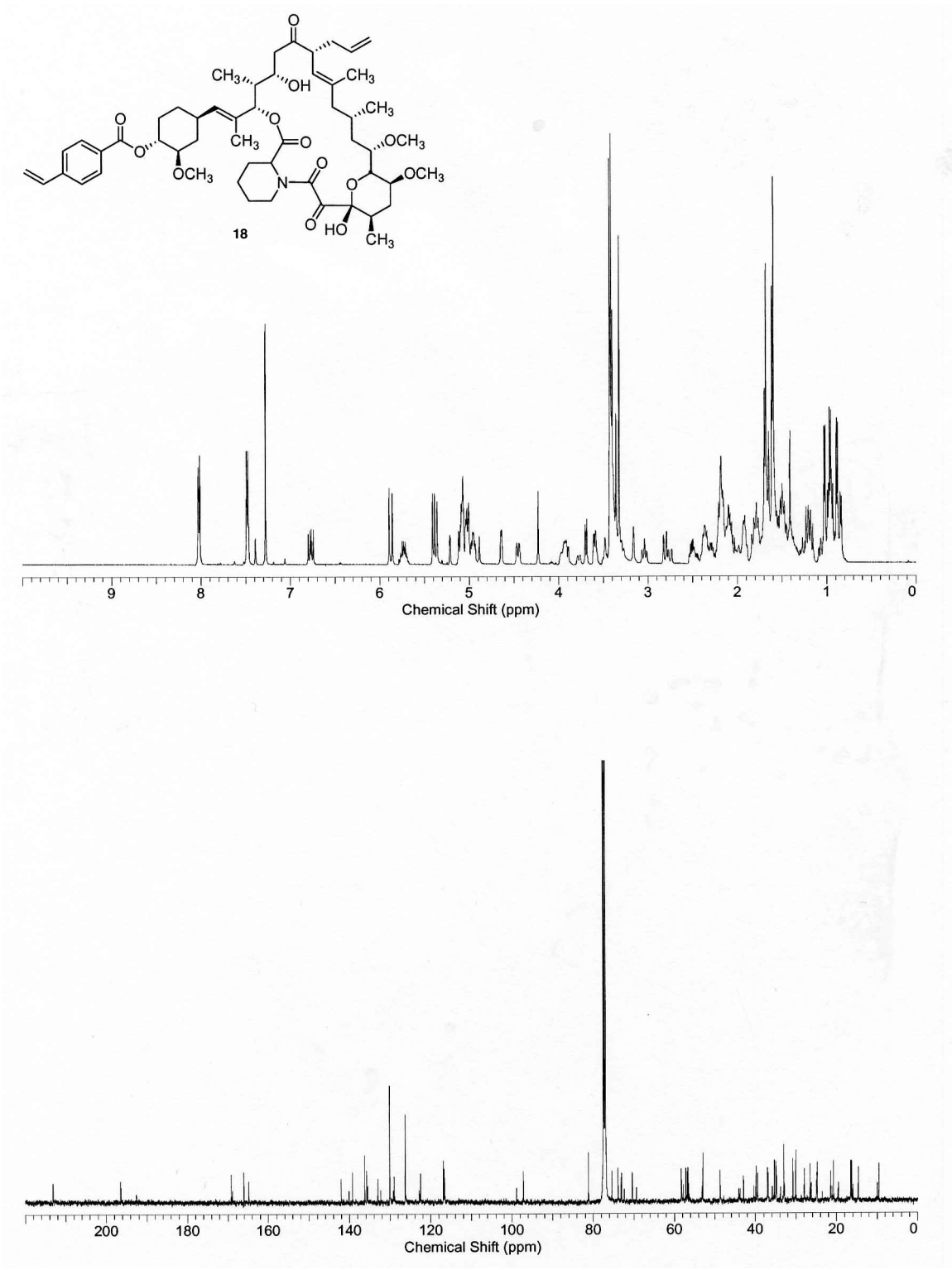


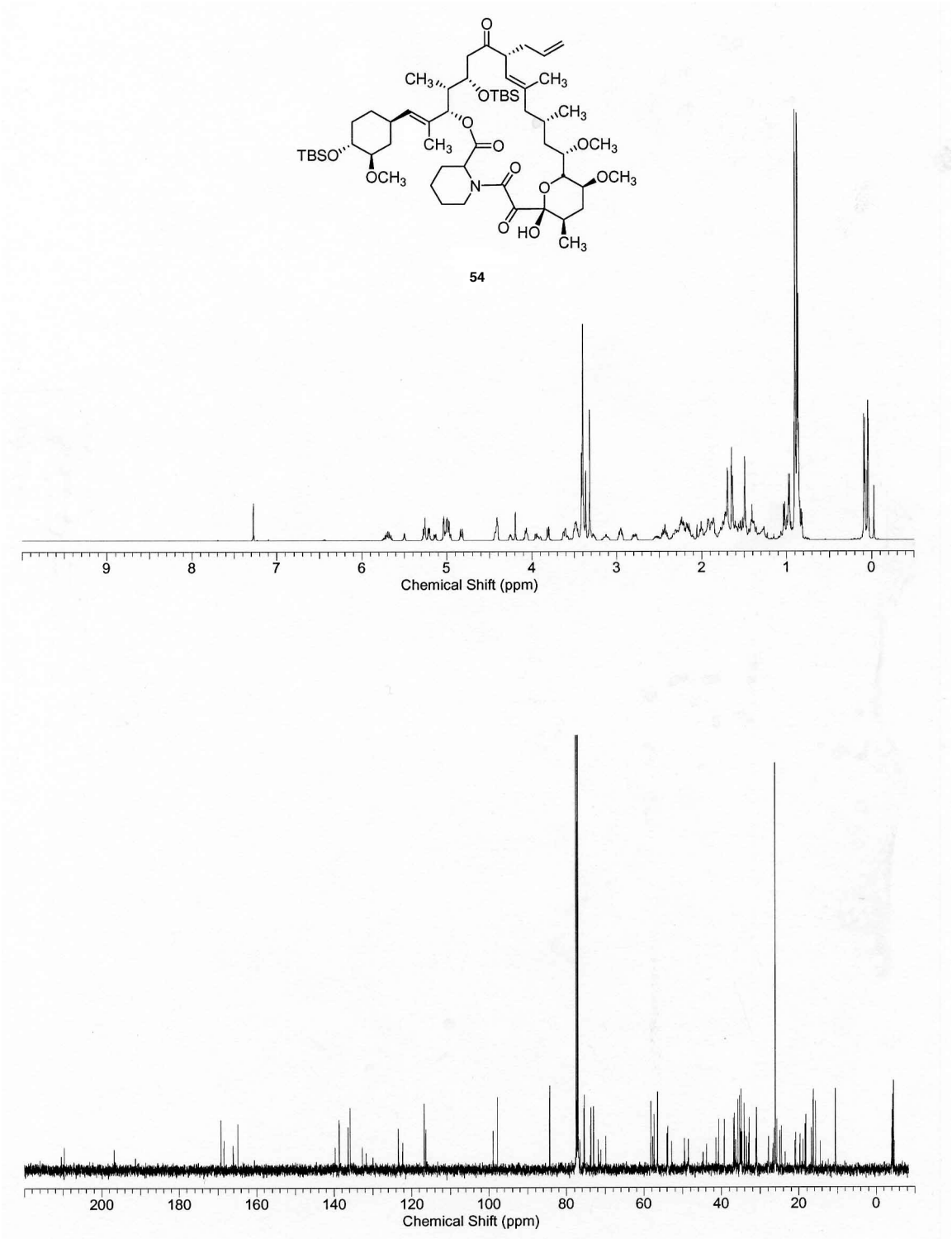
**13**

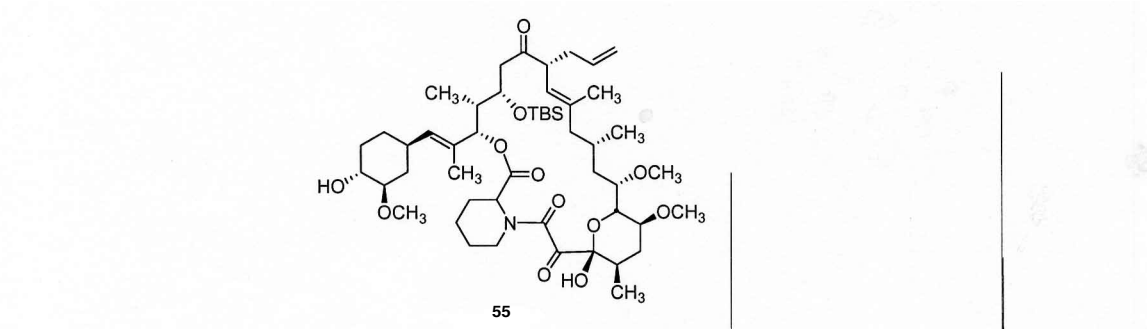



CH3
CH3
CH3
CH3
OCH3
CH3O
O
4
O
O
N
N
CH3
CH3
17
9
8
7
6
5
4
3
2
1
ppm
200
180
160
140
120
100
80
60
40
20
ppm

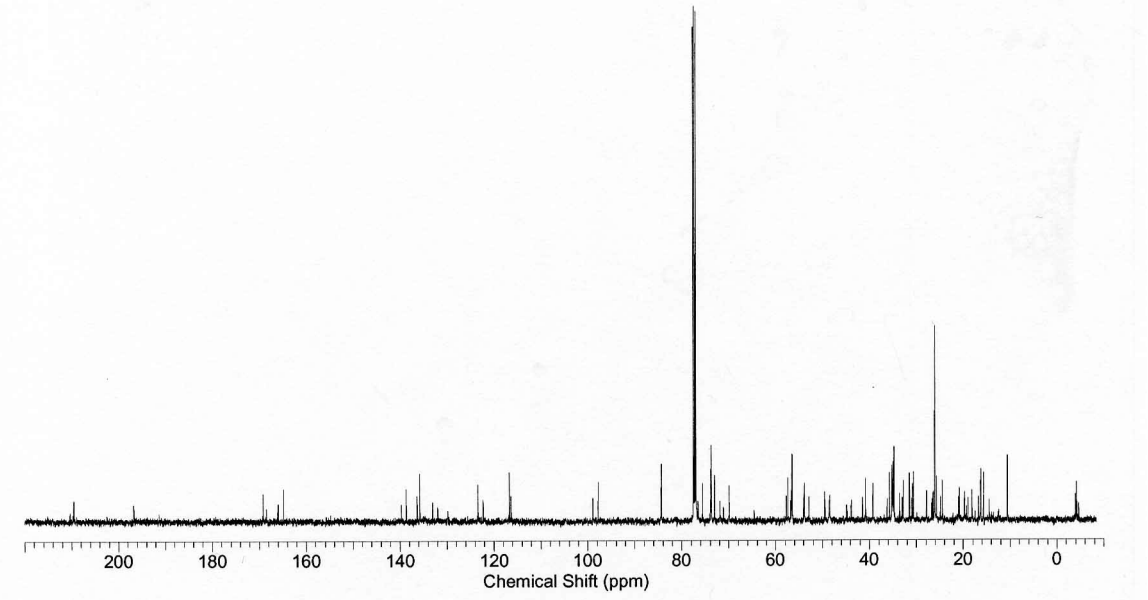

18
Chemical Shift (ppm)
Chemical Shift (ppm)

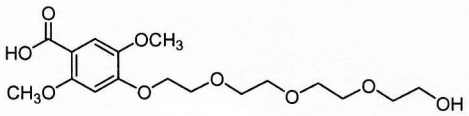

O
CH3
OTBS
CH3
CH3
O
CH3
O
OCH3
TBSO
OCH3
N
O
O
OCH3
O
HO
CH3
54
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)



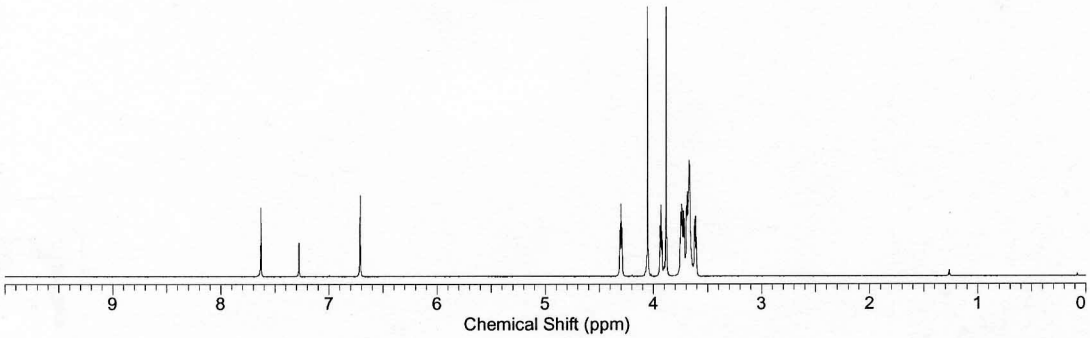
55

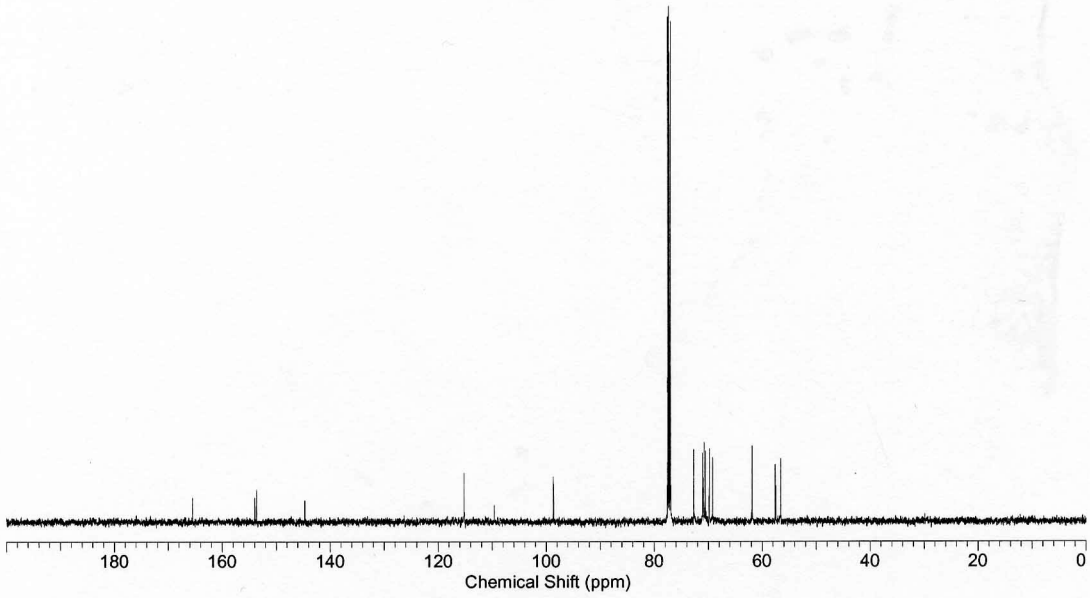

9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

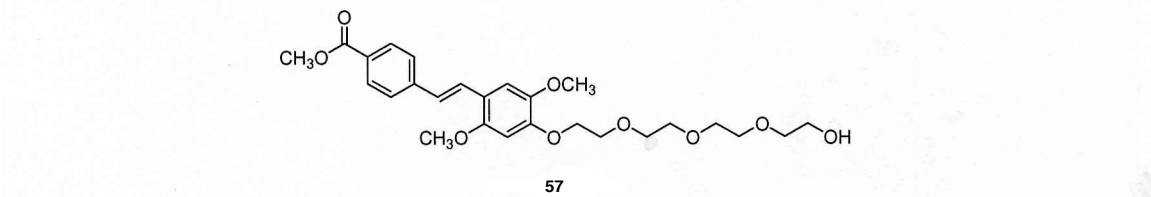

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


O
HO
OCH3
CH3O
O
O
O
O
OH

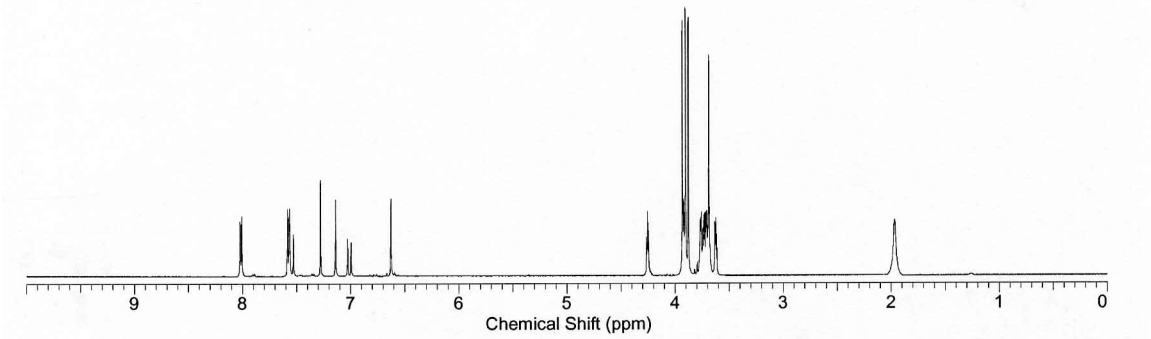
**56**

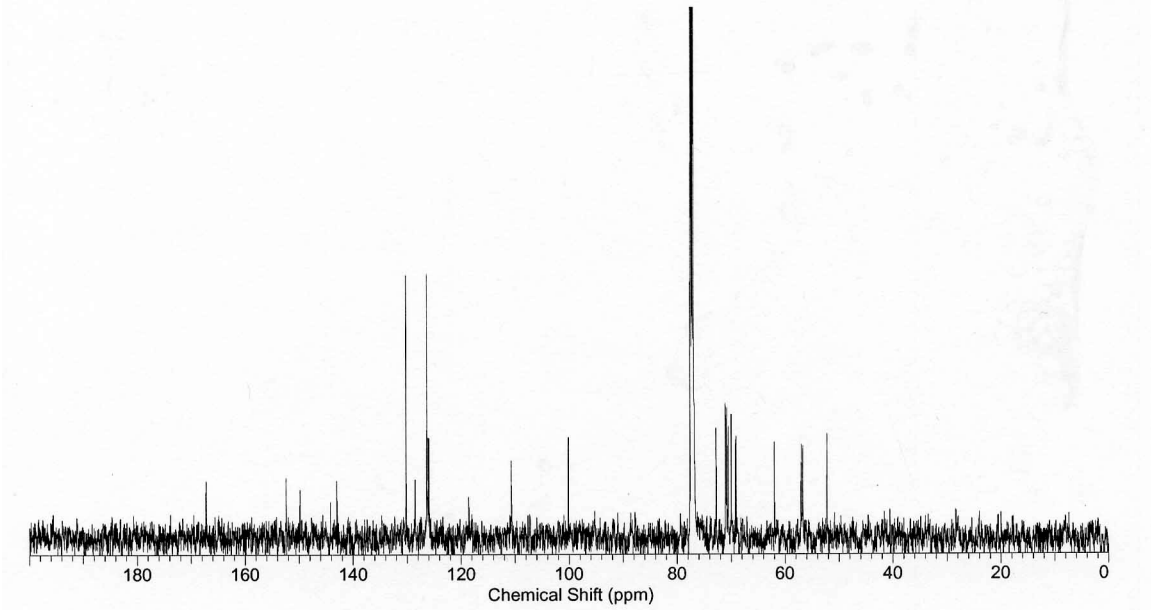

9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

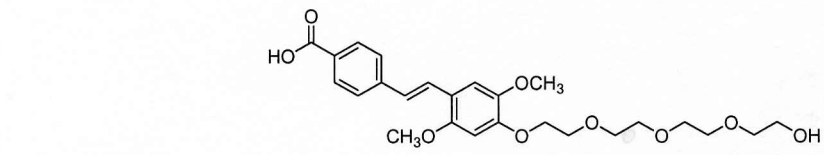

180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


O
CH3O
OCH3
CH3O
O
O
O
O
OH

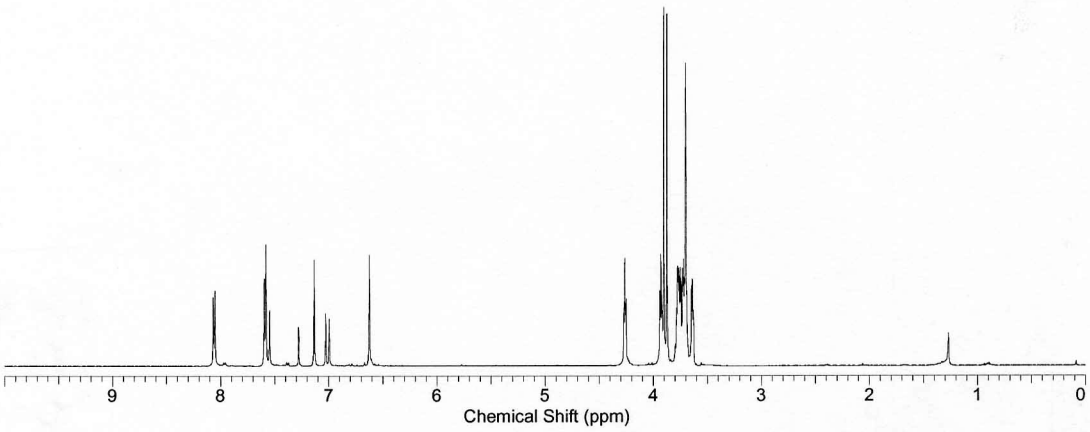
**57**

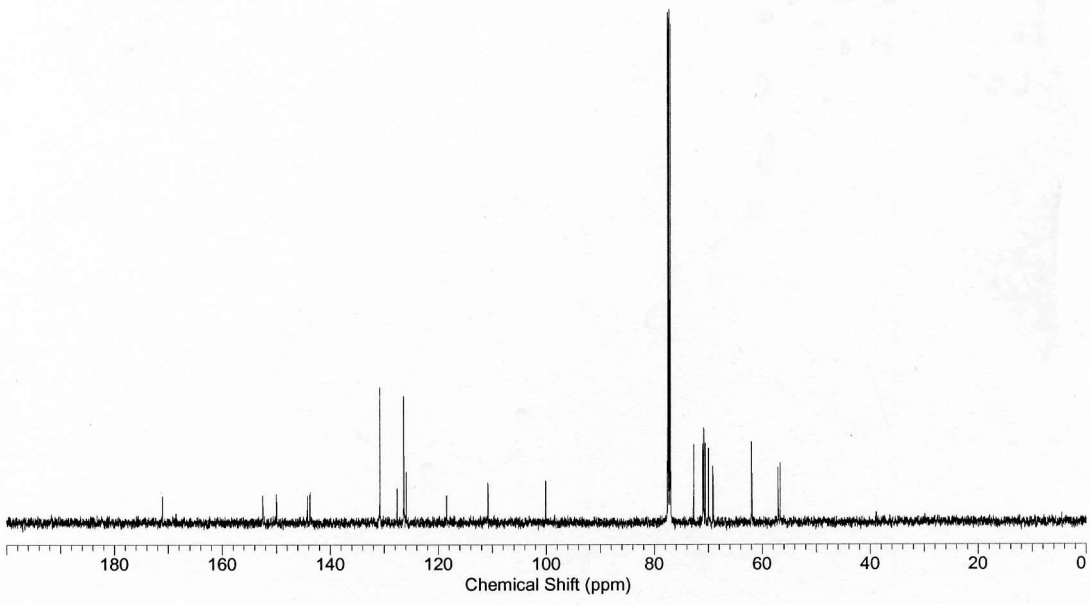

9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

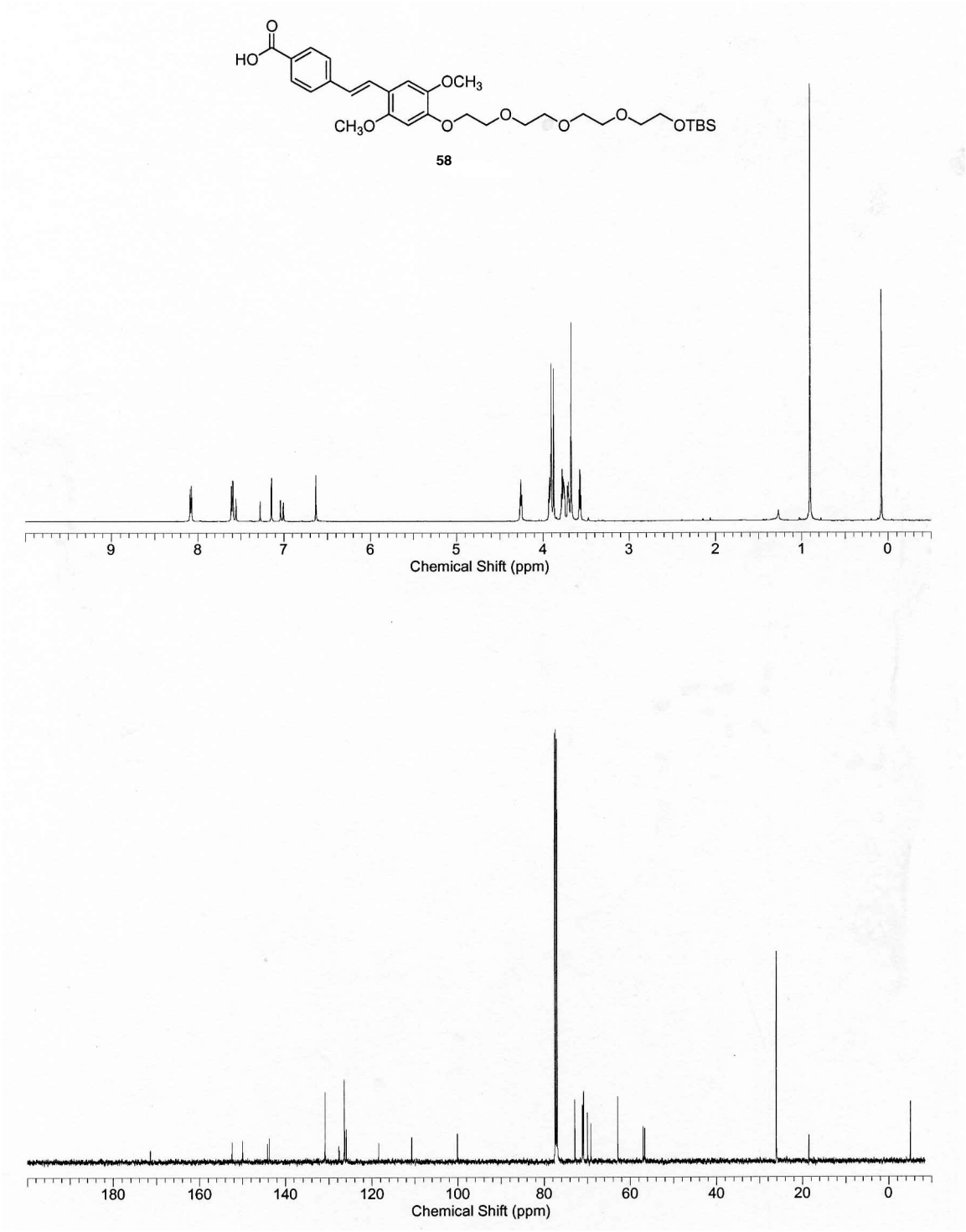

180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

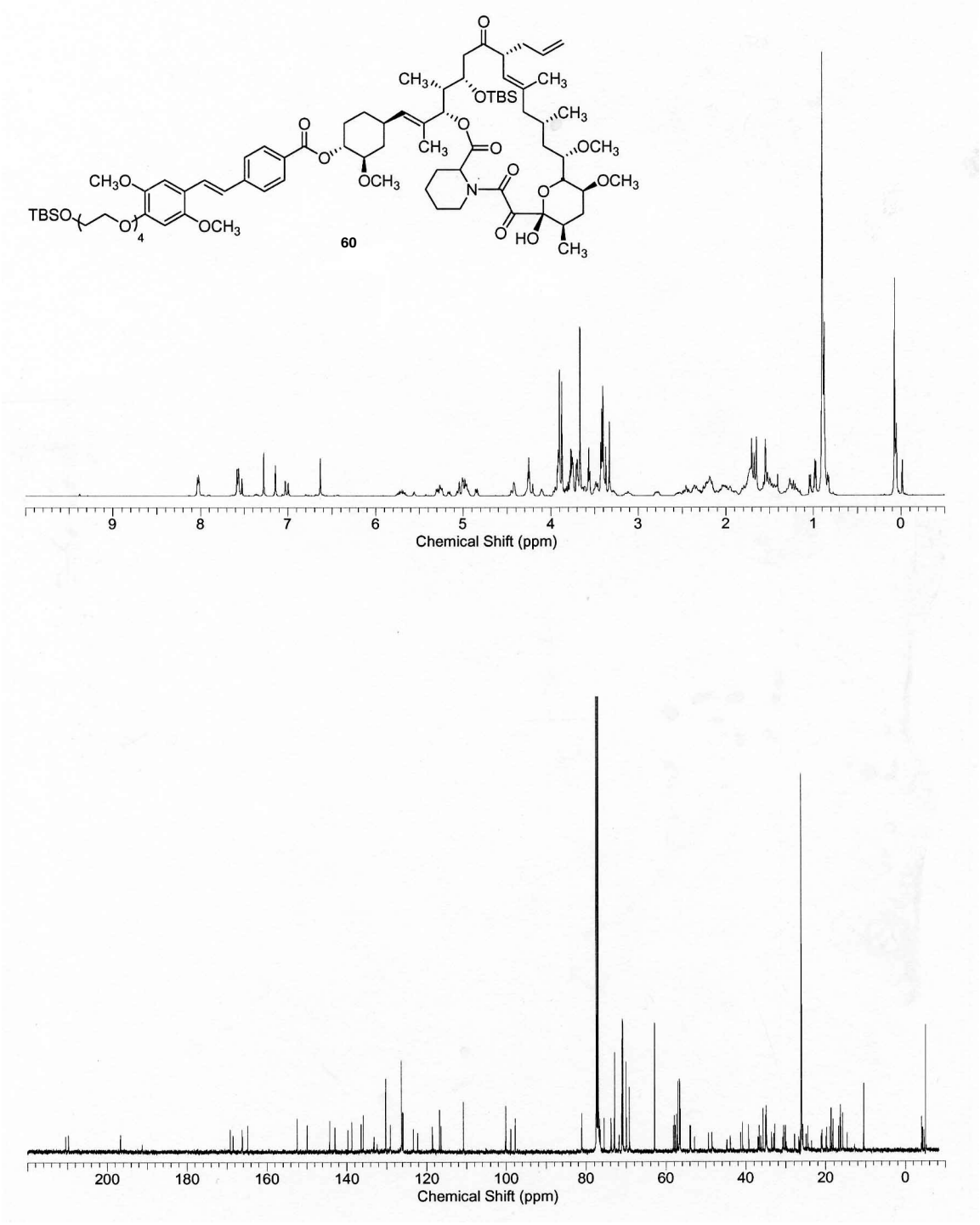

HO
O
OCH3
CH3O
O
O
O
O
O
OH

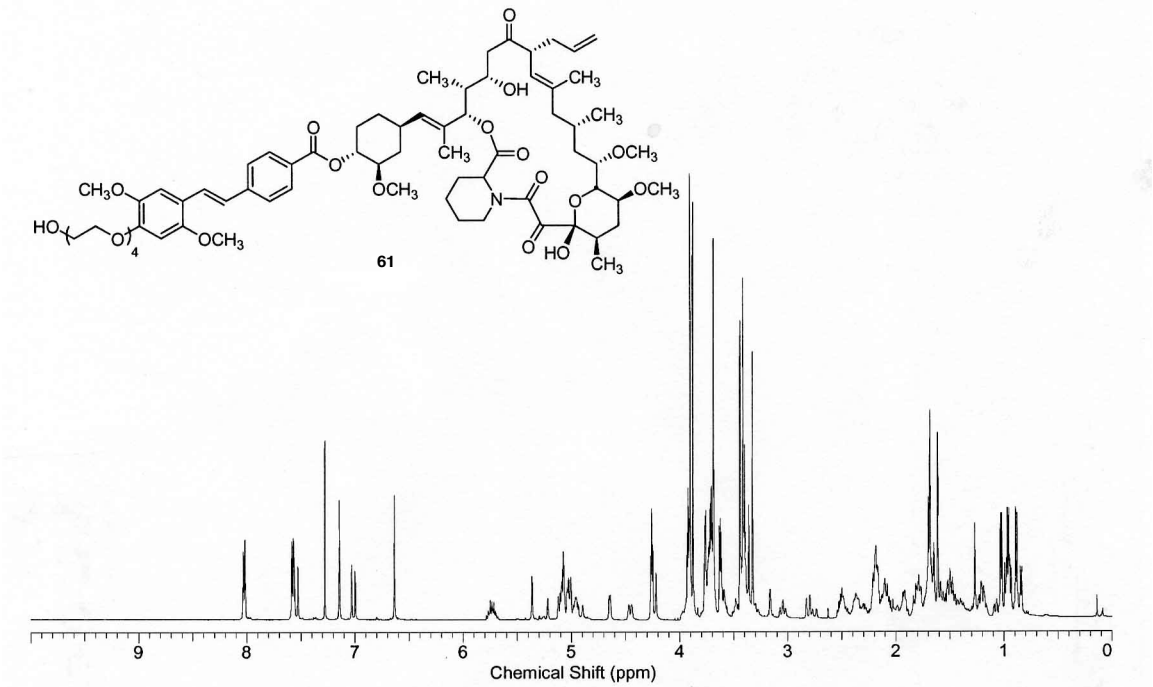
58

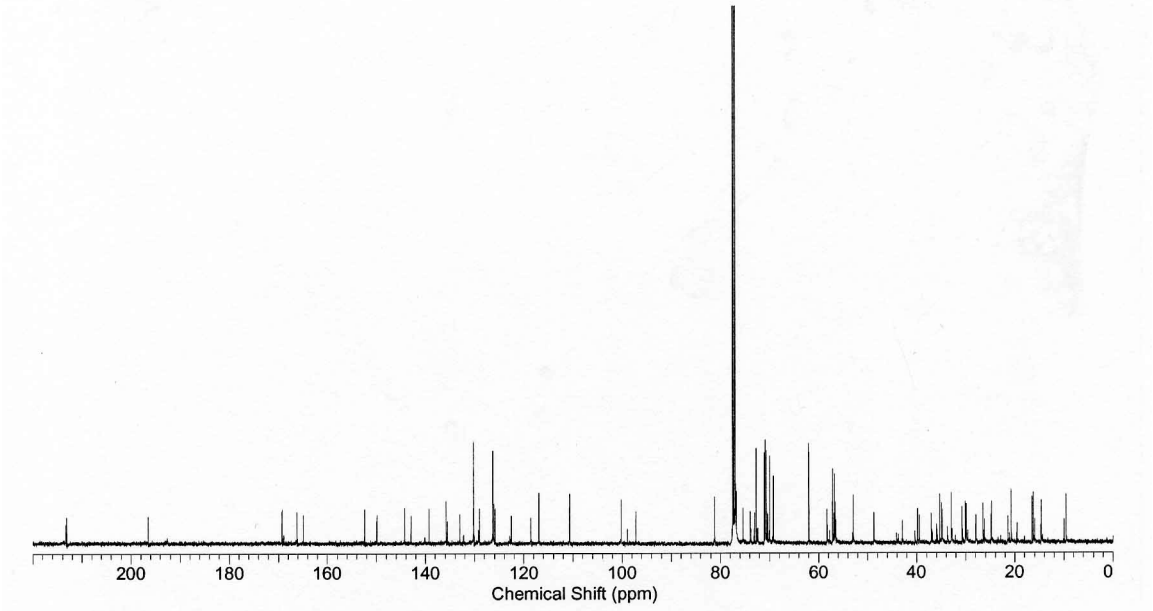

9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

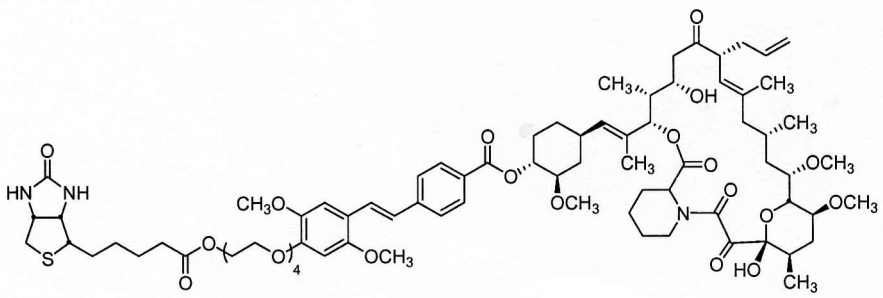

180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


HO
O
OCH3
CH3O
O
O
O
O
OTBS
58
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

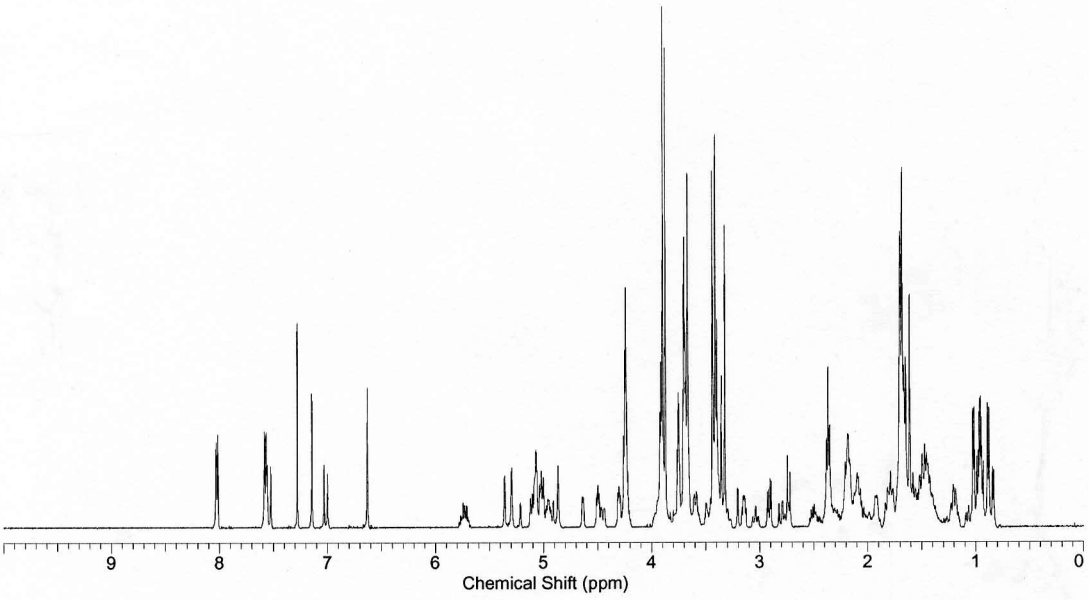

60
Chemical Shift (ppm)
Chemical Shift (ppm)

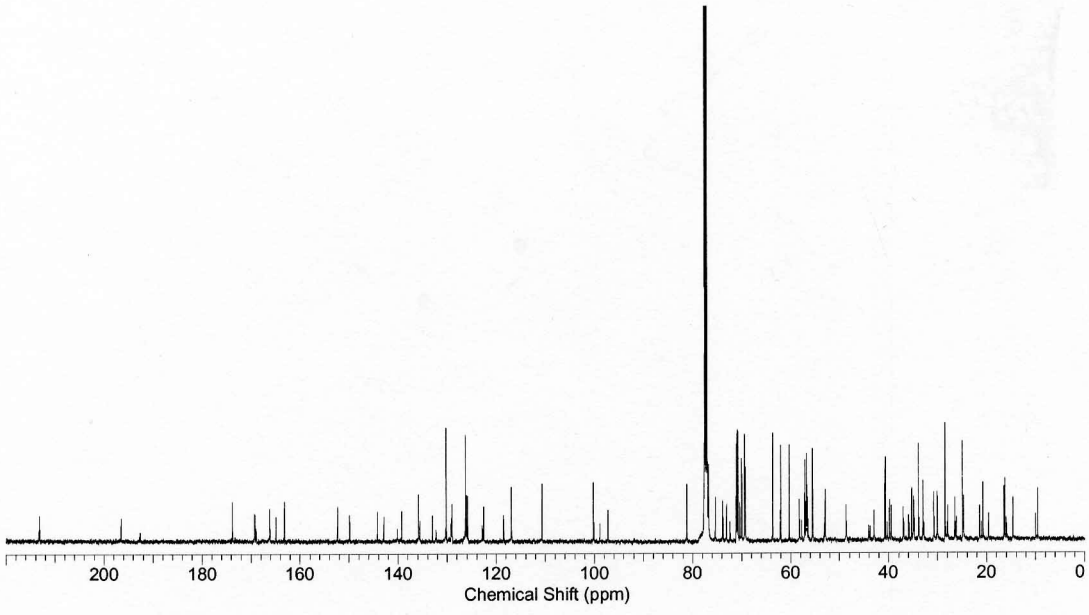

61
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)



19


9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)