Supplementary Methods

Modular Nanoparticulate Prodrug Design Enables Efficient Treatment of Solid Tumors Using Bioorthogonal Activation

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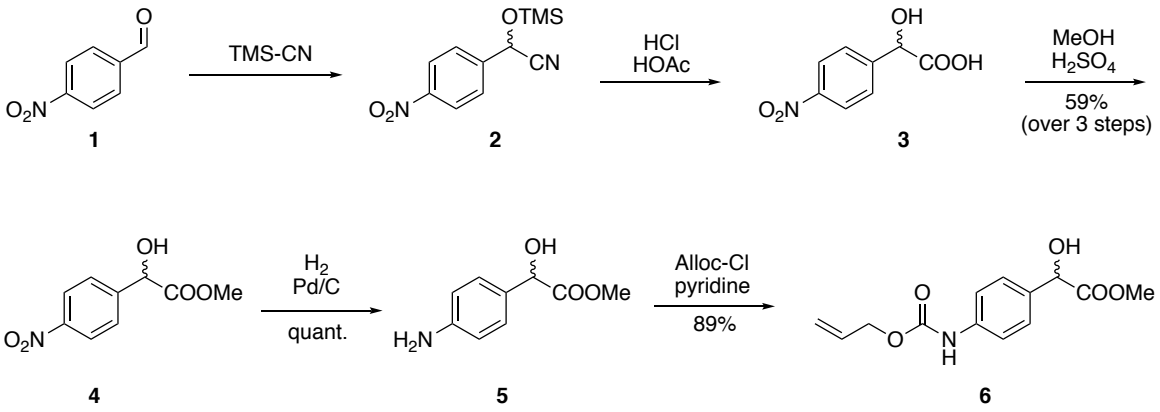
Content

1. Synthesis

1.1. General

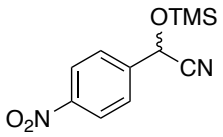
Unless otherwise noted, reactions were carried out under an atmosphere of nitrogen or argon in air-dried glassware with magnetic stirring. Air- and/or moisture-sensitive liquids were transferred via syringe. All reagents were obtained from commercial sources and used without further purification. $dPEG_4$-NH_2 (Amino-dPEG®4-OH) was obtained from Quanta BioDesign (OH, USA). Doxorubicin was obtained from LC Laboratories (MA, USA) and MMAE was obtained from AK Scientific (CA, USA). Dry solvents were obtained from Sigma Aldrich. Analytical thin layer chromatography (TLC) was performed using plates cut from glass sheets (silica gel 60 F-254, Silicycle). Visualization was achieved under a 254 nm or 365 nm UV light and by immersion in a solution of cerium sulfate in ethanol followed by heating with a heat gun. Column chromatography was carried out using silica gel G-25 (40-63 μM) or C18 flash cartridges (SNAP C18 and SNAP C18 Ultra, Biotage). NMR spectra were recorded on a Bruker Avance IIIHD 600 MHz spectrometer equipped with a Prodigy BBO cryo probe, or on a Bruker Avance UltraShield 400 MHz spectrometer. Chemical shifts are reported in parts per million (δ) and calibrated using residual undeuterated solvent. Data are represented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, b = broad), coupling constant (J, Hz) and integration. High performance liquid chromatography-mass spectrometry analysis (HPLC-MS, LCMS) was performed on a Waters instrument equipped with a Waters 2424 ELS Detector, Waters 2998 UV-Vis Diode array Detector, Waters 2475 Multi-wavelength Fluorescence Detector, and a Waters 3100 Mass Detector. Separations employed an HPLC-grade water/acetonitrile solvent gradient. Columns: XTerra MS C18 Column, 125., 5 μm, 4.6 mm X 50 mm column; Waters XBridge Protein BEH C4 Column, 300., 3.5 μm, 2.1 mm X 50 mm. Routine analysis were conducted with 0.1 % formic acid added added to both solvents.

1.2. Alloc-protected self-immolative linker (Alloc-SIL)



The synthesis of intermediate **5** was conducted according to Venkatesan *et al*.[1]

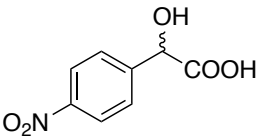
(±)-2-(4-Nitrophenyl)-2-((trimethylsilyl)oxy)acetonitrile (2)



2

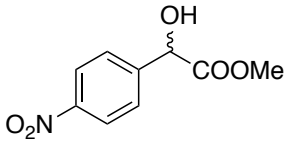
To a solution of 4-nitrobenzaldehyde (**1**) (5 g, 33 mmol) in dichloromethane (50 mL) was added zinc iodide (1.05 g, 3.3 mmol) and trimethylsilyl cyanide (TMS-CN) (4.56 g, 46 mmol). The mixture was heated to reflux for 4 h. After addition of 1M HCl (30 mL) heating was continued at 60 °C for 4 h. The reaction mixture was cooled to room temperature, diluted with water (50 mL) and extracted with dichloromethane (3 x 100 mL). The combined organic layer was washed with water (200 mL) and brine (200 mL), dried over Na_2SO_4 and concentrated. The crude product was used without further purification.

(±)-2-Hydroxy-2-(4-nitrophenyl)acetic acid (3)



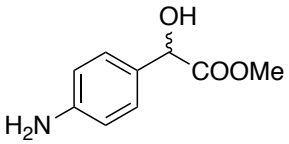
To a solution of crude **2** in acetic acid (40 mL) was added 10M HCl (40 mL) and the mixture was heated to 100 °C for 6 h. After cooling to room temperature, the mixture was concentrated and dried at reduced pressure. The obtained crude product was used without further purification.

(±)-Methyl 2-hydroxy-2-(4-nitrophenyl)acetate (4)



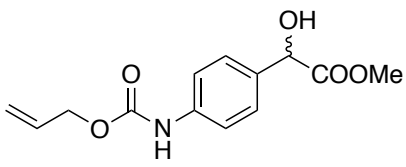
Crude **3** was dissolved in methanol (50 mL) and H_2SO_4 (4 mL) was added. The solution was refluxed for 8 h, then cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in EtOAc (100 mL), washed with water (100 mL) and brine (100 mL), and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (200 g SiO_2, EtOAc in hexanes, gradient elution) to obtain **4** (4.11 g, 59% over 3 steps). Analytical data matched those reported in the literature.[1]

(±)-Methyl 2-(4-aminophenyl)-2-hydroxyacetate (5)



Palladium on activated charcoal (10% Pd/C, 1 g) was added to a solution of **4** (4 g, 19 mmol) in methanol (50 mL). The flask was flushed with H_2 and the mixture was stirred under a positive pressure of H_2 (balloon) for 18 h. The mixture was filtrated over Celite® and concentrated under reduced pressure to obtain compound **5** (3.42 g, quant.). Analytical data matched those reported in the literature.[1]

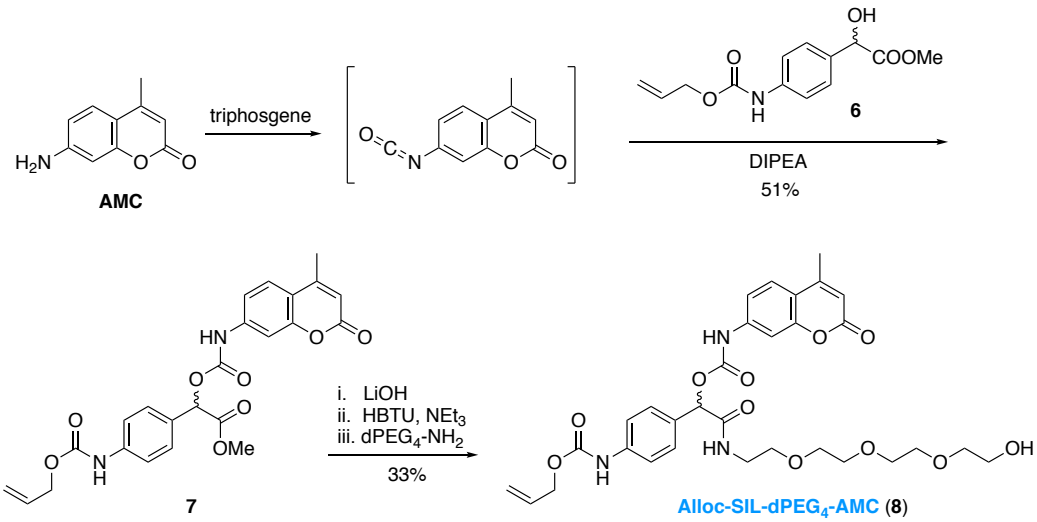
Alloc-SIL (6); (±)-methyl 2-(4-(((allyloxy)carbonyl)amino)phenyl)-2-hydroxyacetate



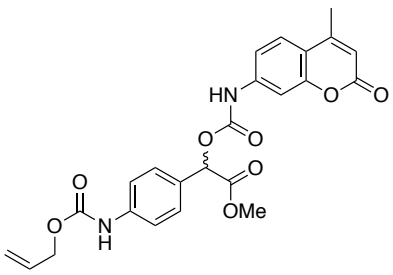
To a solution of **5** (362.4 mg, 2 mmol) and pyridine (322 µL, 4 mmol) in dichloromethane (10 mL) was slowly added a solution of Alloc-Cl (301.3 mg, 2.5 mmol) in dichloromethane (3 mL). The mixture was stirred at room temperature for 12 h, then diluted with dichloromethane (20 mL), washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (40 g SiO_2, EtOAc in hexanes, gradient elution) to obtain Alloc-SIL (**6**) as a white powder (472 mg, 89%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.32 (m, 4H), 6.72 (bs, 1H), 5.96 (ddt, J = 17.1, 10.6, 5.7 Hz, 1H), 5.36 (dq, J = 17.1, 1.4 Hz, 1H), 5.26 (dq, J =

10.6, 1.3 Hz, 1H), 5.14 (s, 1H), 4.66 (dt, J = 5.7, 1.3 Hz, 2H), 3.75 (s, 3H), 3.42 (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 153.1, 138.1, 133.3, 132.3, 127.4, 118.8, 118.4, 72.4, 66.0, 53.1; HRMS $[M+H]^+$ calcd. 266.1023 for $C_{13}H_{16}NO_5^+$, found 266.1020.

1.3. Synthesis of fluorogenic Alloc-SIL-dPEG$_4$-AMC

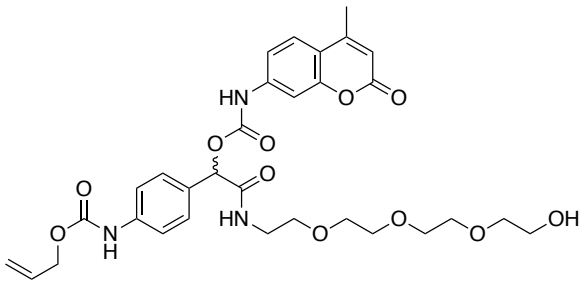


Alloc-SIL-AMC (7)



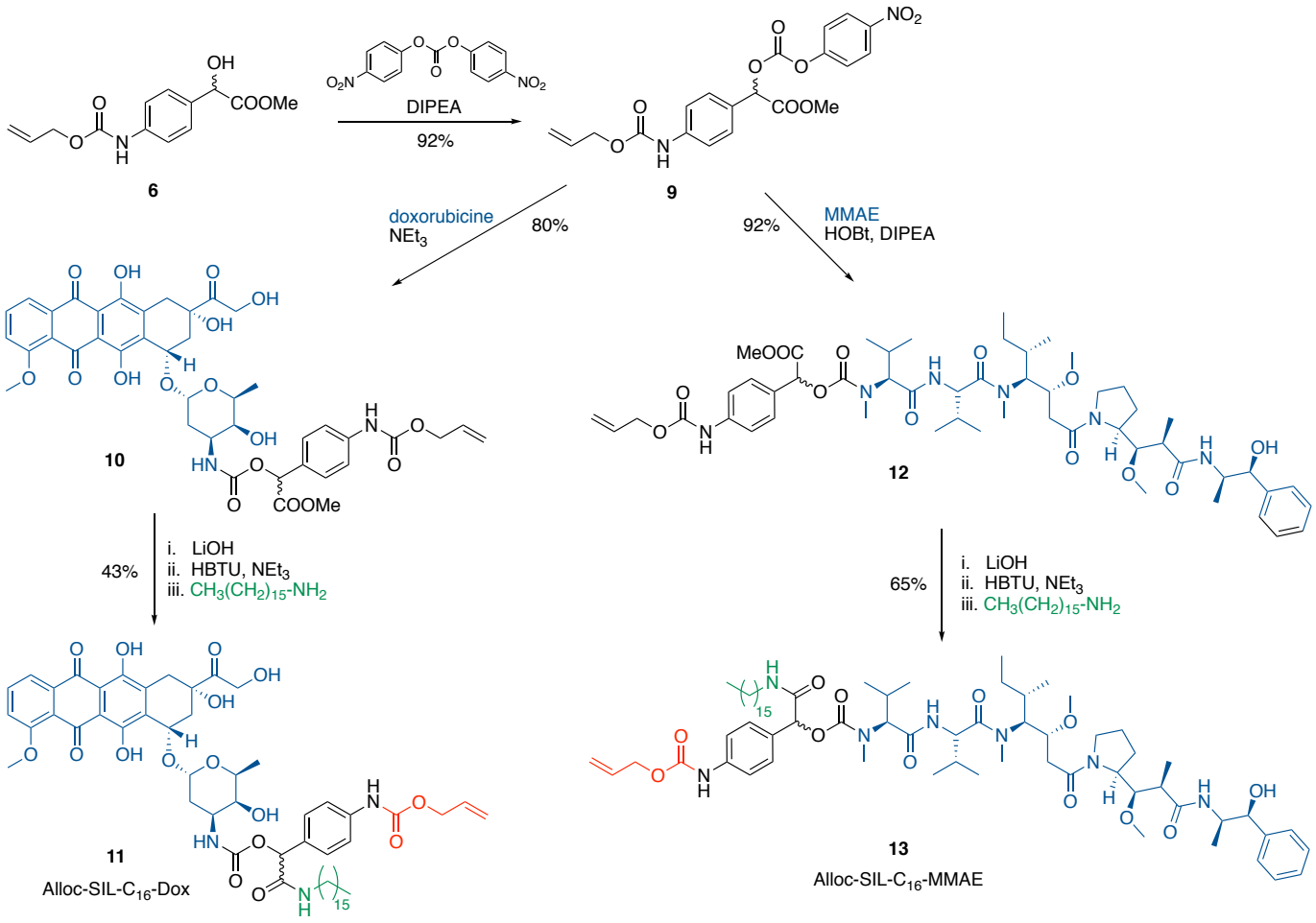
7-Amino-4-methylcoumarin (**AMC**) (105.1 mg, 0.6 mmol) and triphosgene (89 mg, 0.3 mmol) were suspended in 10 mL dry toluene (10 mL) and the mixture was refluxed for 4 h. Argon was passed through the mixture for 10 min. After concentration a white solid was obtained that was reacted with Alloc-SIL (**6**) (155 mg, 0.58 mmol) and DIPEA (522 µL, 3 mmol) in dry THF (10 mL) at room temperature for 18 h. The mixture was concentrated and the residue was purified by column chromatography (Biotage SNAP Ultra C18 column, 30 g, H_2O/acetonitrile gradient elution, 0.1% formic acid) to obtain Alloc-SIL-AMC (**7**) (143 mg, 51%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 9.90 (s, 1H), 7.71 (d, J = 8.9 Hz, 1H), 7.57-7.51 (m, 3H), 7.47-7.41 (m, 3H), 6.25 (d, J = 1.1 Hz, 1H), 5.99 (ddt, J = 17.2, 10.6, 5.3 Hz, 1H), 5.98 (s, 1H), 5.37 (dq, J = 17.2, 1.5 Hz, 1H), 5.25 (dq, J = 10.6, 1.3 Hz, 1H), 4.62 (dt, J = 5.4, 1.4 Hz, 2H), 3.71 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 160.0, 153.8, 153.2, 153.1, 152.4, 142.2, 140.2, 133.2, 128.7, 127.4, 126.1, 118.3, 117.7, 114.7, 114.3, 112.1, 104.6, 74.1, 64.8, 52.4, 18.0; HRMS $[M+H]^+$ calcd. 467.1449 for $C_{24}H_{23}N_2O_8^+$, found 467.1442.

Alloc-SIL-dPEG$_4$-AMC (8)

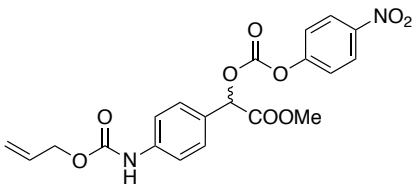


LiOH monohydrate (8.8 mg, 0.21 mmol) was added to a solution of Alloc-SIL-AMC (**7**) (32.7 mg, 0.07 mmol) in THF/H_2O/MeOH (6.75 mL, v/v/v = 20/2/5). The solution was stirred until LCMS indicated full saponification to the respective acid (~30 min). Acidic cation exchange resin (Dowex 500X8-H, 0.5 g) was added and stirring was continued for 1 min. The resin was removed by filtration and washed with methanol. The filtrate was concentrated under reduced pressure and the residue was redissolved in dry DMF (2 mL). To the solution was added NEt_3 (29.3 µL, 0.21 mmol), HBTU (39.8 mg, 0.105 mmol) and dPEG$_4$-NH_2 (Amino-dPEG®$_4$-OH, 27.1 mg, 0.14 mmol) and the mixture was stirred until LCMS indicated full conversion. The mixture was directly loaded onto a Biotage SNAP Ultra C18 column (30 g) and reversed phase chromatography (H_2O/acetonitrile gradient elution, 0.1% formic acid) afforded Alloc-SIL-AMC-dPEG$_4$ (**8**) (14.4 mg, 33%); ^{1}H NMR (600 MHz, DMSO-d_6, two rotamers, ~65/35) δ 10.44 (bs, 0.65H), 10.38 (s, 0.35H), 9.84 (s, 0.65H), 9.75 (s, 0.35H), 8.39 (t, J = 5.7 Hz, 1H), 7.86 (d, J = 1.9 Hz, 0.35H), 7.72 (dd, J = 8.7, 1.9 Hz, 0.35H), 7.70 (d, J = 8.7 Hz, 1H), 7.54 (d, J = 1.9 Hz, 0.65H), 7.50-7.45 (m, 1.3H), 7.45-7.40 (m, 3.35H), 6.26 (q, J = 1.3 Hz, 0.35H), 6.24 (q, J = 1.3 Hz, 0.65H), 6.01-5.93 (m, 1H), 5.83 (s, 0.65H), 5.36 (dq, J = 17.2, 1.7 Hz, 0.65H), 5.35 (dq, J = 17.2 Hz, 1.7 Hz, 0.35H), 5.25-5.21 (m, 1H), 5.08 (s, 0.35H), 4.61-4.58 (m, 2H), 3.49-3.45 (m, 7H), 3.44-3.33 (m, 7H, underneath water peak), 3.24 (q, J = 5.7 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6, two rotamers) δ 172.3, 168.4, 160.1, 153.9, 153.54, 153.28, 153.25, 153.22, 153.19, 152.4, 142.6, 142.1, 139.6, 138.7, 134.5, 133.33, 133.27, 129.7, 128.5, 127.2, 126.1, 125.8, 118.0, 117.7, 117.6, 115.8, 115.3, 114.5, 114.3, 112.4, 112.0, 106.2, 104.5, 75.0, 73.8, 72.4, 69.83, 69.77, 69.74, 69.6, 68.8, 64.8, 64.7, 60.2, 18.1, 18.0; HRMS $[M+H]^+$ calcd. 628.2501 for $C_{31}H_{38}N_3O_{11}^+$, found 628.2493.

1.3. Alloc-SIL-C_{16}-drug conjugates

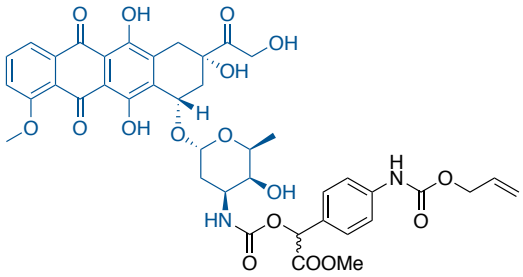


Alloc-SIL-PNP (9)



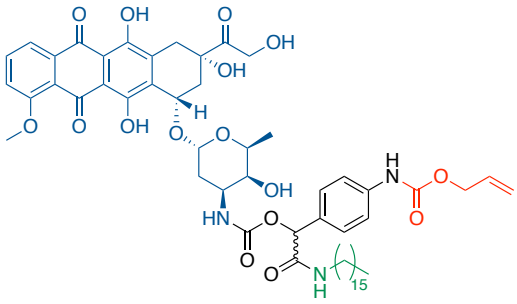
Alloc-SIL (**6**) (132.6 mg, 0.5 mmol) and DIPEA (130.6 µL, 0.75 mmol) were dissolved in dry DMF (2 mL). The solution was cooled to 0 °C and bis(4-nitrophenyl) carbonate (304.2 mg, 1 mmol) was added in one portion. The mixture was stirred at room temperature for 4 h, stored at -20 °C overnight, and then poured into water/EtOAc (100 mL, v/v = 1/1). The layers were separated and the aqueous phase was extracted with EtOAc (2 x 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (40 g SiO_2, Et_2O in hexanes, gradient elution) to afford compound **9** as a white solid (197 mg, 92%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 8.37-8.32 (m, 2H), 7.61-7.56 (m, 2H), 7.55 (d, J = 8.6 Hz, 2H), 7.44 (d, J = 8.6 Hz, 2H), 6.08 (s, 1H), 5.99 (ddt, J = 17.2, 10.6, 5.4 Hz, 1H), 5.37 (dq, J = 17.2, 1.5 Hz, 1H), 5.25 (dq, J = 10.6, 1.5 Hz, 1H), 4.63 (dt, J = 5.4, 1.4 Hz, 2H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 154.9, 153.1, 151.3, 145.4, 140.5, 133.2, 128.7, 126.2, 125.6, 122.4, 118.3, 117.7, 77.3, 64.8, 52.8; HRMS $[M+H]^+$ calcd. 431.1085 for $C_{20}H_{19}N_2O_9^+$, found 431.1086.

Alloc-SIL-Dox (10)



Doxorubicin hydrochloride (174 mg, 0.3 mmol) was added to a solution of Alloc-SIL-PNP (**9**) (142.1 mg, 0.33 mmol) and NEt_3 (46 µL, 0.33 mmol) in dry DMSO (3 mL). The mixture was stirred at room temperature for 2 h (LCMS showed full conversion), then directly loaded onto a Biotage SNAP C18 column (30 g). Reversed phase chromatography (H_2O/acetonitrile gradient elution, 0.1% formic acid) afforded Alloc-SIL-C_{16}-Dox (**10**) (199.3 mg, 80%); ^1H NMR (400 MHz, DMSO-d_6, mixture of two diastereomers, ~1/1) δ 14.01-13.93 (m, 1H), 13.24-13.18 (m, 1H), 9.87-9.76 (m, 1H), 7.88-7.80 (m, 2H), 7.61-7.55 (m, 1H), 7.50-7.41 (m, 2H), 7.37-7.29 (m, 2H),. 7.26-7.14 (m, 1H), 6.03-5.91 (m, 1H), 5.65 (s, 1H), 5.40 (d, J = 8.3 Hz, 1H), 5.38-5.31 (m, 1H), 5.27-5.19 (m, 2H), 4.93-4.84 (m, 2H), 4.76-4.68 (m, 1H), 4.63-4.56 (m, 4H), 4.21-4.13 (m, 1H), 3.99-3.92 (m, 3H), 3.79-3.67 (m, 1H), 3.59 (s, 1.4H, COOMe diastereomer 1), 3.54 (s, 1.6H, COOMe diastereomer 2), 3.48-3.42 (m, 1H), 3.03-2.81 (m, 2H), 2.25-2.17 (m, 1H), 2.13-2.05 (m, 1H), 1.95-1.80 (m, 1H), 1.54-1.43 (m, 1H), 1.18-1.09 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, mixture of diastereomers) δ 212.2, 184.7, 184.6, 168.2, 159.1, 154.4, 152.9, 152.8, 151.5, 138.2, 134.5, 133.7, 132.9, 132.3, 131.5, 126.7, 126.6, 126.4, 126.3, 118.2, 118.0, 117.3, 116.5, 116.0, 109.1, 108.9, 98.6, 73.3, 71.8, 68.1, 66.6, 66.2, 65.1, 65.0, 63.1, 62.1, 54.9, 50.4, 45.7, 34.8, 30.4, 28.2, 28.0, 15.4; HRMS $[M+H]^+$ calcd. 835.2556 for $C_{41}H_{43}N_2O_{17}^+$, found 835.2569.

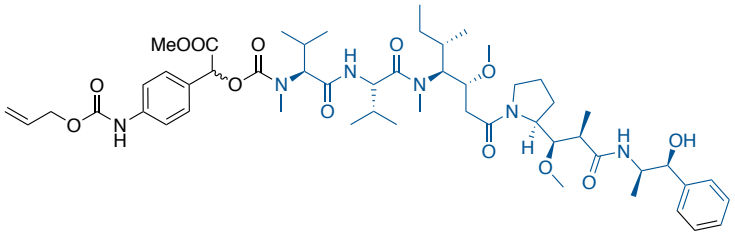
Alloc-SIL-C_{16}-Dox (11)



To a solution of Alloc-SIL-Dox (**10**) (83.5 mg, 0.1 mmol) in MeOH (3 mL) and THF (5 mL) was added an aqueous solution of LiOH monohydrate (420 mM, 0.714 mL, 0.3 mmol). The solution was stirred for 25 min. Acidic cation exchange resin (Dowex 500X8-H, 0.5 g) was added and stirring was continued for 1 min. The resin was removed by filtration and washed with methanol. The filtrate was concentrated under reduced pressure and the residue was redissolved in dry DMF (0.4 mL) and dry methanol (0.8 mL). To the solution was added NEt_3 (69.7 µL, 0.5 mmol), HBTU (41.7 mg, 0.11 mmol) and a solution of hexadecylamine (50 mM in dry THF, 3 mL, 0.15 mmol). The mixture was stirred until LCMS indicated full conversion and then directly loaded onto a Biotage SNAP C18 column (30 g). Reversed phase chromatography (H_2O/acetonitrile gradient elution, 0.1% formic acid) afforded Alloc-SIL-C_{16}-Dox (**11**) (45 mg, 43%); ^1H NMR (600 MHz, DMF-d_7, mixture of diastereomers) δ 14.31-14.16 (m, 1H), 13.45-13.29 (m, 1H), 9.77-9.66 (m, 1H), 8.13-8.08 (m, 1H), 8.03 (s, 1H), 7.98-7.91 (m, 2H), 7.74-7.69 (m, 1H), 7.58-7.50 (m, 2H), 7.42-7.35 (m, 2H), 7.02-6.87 (m, 1H), 6.05-5.95 (m, 1H), 5.73 (s, 1H), 5.42-5.32 (m, 2H), 5.24-5.19 (m, 1H), 5.12-5.04 (m, 1H), 4.84-4.72 (m, 2H), 4.68-4.56 (m, 3H), 4.35-4.28 (m, 1H), 4.11-4.04 (m, 4H), 3.91-3.84 (m, 2H), 3.18-3.01 (m, 5H), 2.50-2.38 (m, 1H), 2.29-2.21 (m, 1H), 2.05-1.95 (m, 1H), 1.80-1.63 (m, 1H), 1.45-1.37 (m, 2H), 1.29-1.12 (m, 33H), 0.90-0.84 (m, 3H); ^{13}C NMR (150 MHz, DMF-d_7, mixture of diastereomers) δ 214.4, 187.3, 187.2, 169.30, 169.28, 163.2, 161.64, 161.61, 156.92, 156.88, 155.5, 155.4, 154.9, 154.8, 153.83, 153.80, 139.9, 136.5, 135.9, 135.5, 134.6, 133.8, 131.8, 128.2, 128.1, 120.8, 120.7,

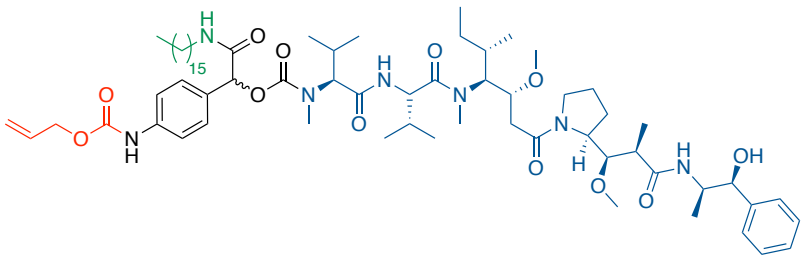
119.91, 119.88, 119.4, 118.2, 117.13, 117.11, 111.4, 111.3, 101.3, 101.2, 76.0, 75.5, 75.4, 70.5, 69.1, 67.6, 67.5, 65.2, 65.1, 64.9, 56.67, 56.65, 47.92, 47.88, 39.0, 38.9, 37.0, 32.9, 32.0, 29.8, 26.9, 25.6, 22.8, 17.0, 13.9; HRMS $[M-H]^-$ calcd. 1042.4918 for $C_{56}H_{72}N_3O_{16}^-$, found 1042.4930.

Alloc-SIL-MMAE (12)



MMAE (108 mg, 0.15 mmol) was added to a solution of Alloc-SIL-PNP (**9**) (94.7 mg, 0.22 mmol), DIPEA (40.1 µL, 0.23 mmol), and HOBt (20.3 mg, 0.15 mmol) in dry DMSO (1.5 mL). The mixture was stirred at room temperature for 2 h (LCMS showed full conversion). Excess Alloc-SIL-PNP was quenched by addition of 2-(2-aminoethoxy)ethanol (10 µL, 0.1 mmol) and stirring was continued for 30 min. The mixture was directly loaded onto a Biotage SNAP Ultra C18 column (30 g). Reversed phase chromatography (H_2O/acetonitrile gradient elution, 0.1% formic acid) afforded MMAE-SIL-C_{16}-Dox (**10**) (140 mg, 92%); ^{1}H NMR (400 MHz, DMSO-d_6, mixture of diastereomers and rotamers) δ 9.87-9.79 (m, 1H), 8.33-8.04 (m, 1H), 7.92-7.85 (m, 0.6H), 7.65-7.59 (m, 0.4H), 7.54-7.45 (m, 3H), 7.43-7.37 (m, 1H), 7.34-7.24 (m, 4H), 7.22-7.15 (m, 1H), 5.99 (ddt, J = 17.1, 10.6, 5.4 Hz, 1H), 5.87-5.76 (m, 1H), 5.43-5.32 (m, 2H), 5.24 (dq, J = 10.6, 1.3 Hz, 1H), 4.81-4.66 (m, 1H), 4.64-4.59 (m, 2H), 4.53-4.20 (m, 3H), 4.07-3.91 (m, 2H), 3.82-3.76 (m, 0.5H), 3.64-3.54 (m, 4H), 3.53-3.42 (m, 0.5H), 3.33 (s, 3H), 3.27-3.10 (m, 9H), 3.06-2.86 (m, 5H), 2.47-2.37 (m, 1H), 2.34-2.21 (m, 1H), 2.19-2.09 (m, 2H), 2.08-2.06 (m, 4H), 1.86-1.69 (m, 3H), 1.60-1.44 (m, 2H), 1.38-1.24 (m, 1H), 1.08-0.96 (m, 7H), 0.96-0.71 (m, 19H), 0.65-0.55 (m, 1H), ; ^{13}C NMR (100 MHz, DMSO-d_6, mixture of diastereomers and rotamers) δ 170.7, 170.6, 168.0, 167.9, 167.1, 161.4, 153.6, 153.3, 151.5, 142.0, 138.2, 138.1, 138.0, 131. 6, 126.6, 126.5, 126.4, 126.3, 126.11, 126.06, 125.1, 125.0, 124.8, 124.7, 116.7, 116.4, 116.0, 83.8, 80.0, 76.0, 75.3, 73.1, 72.9, 63.1, 61.8, 61.6, 59.3, 58.6, 57.0, 56.5, 55.5, 55.4, 53.3, 52.6, 52.5, 50.5, 48.1, 47.5, 45.5, 44.6, 42.1, 41.6, 35.5, 33.5, 30.1, 29.9, 28.6, 28.4, 28.2, 25.7, 25.2, 25.1, 23.7, 23.6, 22.7, 21.5, 17.6, 17.4, 17.3, 17.2, 17.13, 17.06, 16.9, 16.7, 14.2, 14.0, 13.8, 13.7, 13.6, 13.3, 8.7, 8.6; HRMS $[M+H]^+$ calcd. 1009.5856 for $C_{53}H_{81}N_6O_{13}^+$, found 1009.5843.

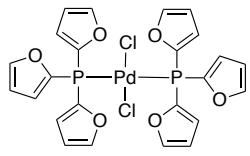
Alloc-SIL-C_{16}-MMAE (13)



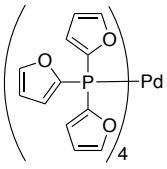
To a solution of Alloc-SIL-MMAE (**12**) (124.2 mg, 0.123 mmol) in MeOH (9 mL) was added an aqueous solution of LiOH monohydrate (420 mM, 0.878 mL, 0.369 mmol). The solution was stirred for 30 min. Acidic cation exchange resin (Dowex 500X8-H, 0.5 g) was added and stirring was continued for 1 min. The resin was removed by filtration and washed with methanol. The filtrate was concentrated under reduced pressure and the residue was redissolved in dry DMF (1 mL). To the solution was added NEt_3 (51.4 µL, 0.37 mmol), a solution of HBTU (166 mM in THF/DMF, v/v = 2/1, 1.04 mL, 0.17 mmol) and a solution of hexadecylamine (50 mM in dry THF, 3.44 mL, 0.17 mmol). The mixture was

stirred until LCMS indicated full conversion and then directly loaded onto a Biotage SNAP C18 column (30 g). Reversed phase chromatography (H_2O/acetonitrile gradient elution, 0.1% formic acid) afforded Alloc-SIL-C_{16}-MMAE (**13**) (98.1 mg, 65%); ^{1}H NMR (600 MHz, DMF-d_7, mixture of diastereomers and rotamers) δ 9.81-9.72 (m, 1H), 8.36-8.30 (m, 0.5H), 8.24-8.18 (m, 0.4H), 8.10-8.05 (m, 0.5H), 8.03 (s, 0.5H), 7.98-7.91 (m, 0.6H), 7.89-7.79 (m, 0.5H), 7.71-7.67 (m, 0.5H), 7.65-7.58 (m, 2H), 7.55-7.48 (m, 2H), 7.46-7.40 (m, 2H), 7.38-7.30 (m, 2H), 7.26-7.18 (m, 1H), 6.05-5.96 (m, 1.6H), 5.93-5.86 (m, 0.4H), 5.39-5.34 (m, 1H), 5.25-5.20 (m, 1H), 4.96-4.85 (m, 0.5H), 4.82-4.67 (m, 1.5H), 4.66-4.53 (m, 3H), 4.39-4.25 (0.4H), 4.25-4.09 (m, 2H), 3.98-3.93 (m, 0.4H), 3.92-3.87 (m, 0.4), 3.69-3.57 (m, 1H), 3.55-3.46 (m, 1H), 3.40-3.24 (m, 8H), 3.21-3.09 (m, 4H), 3.08-2.99 (m, 1H), 2.65-2.41 (m, 1.3H), 2.34-2.18 (m, 1.5H), 2.15 (s, 1.5H), 2.10-1.81 (m, 2.4H), 1.75-1.53 (m, 1H), 1.50-1.38 (m, 2.5H), 1.31-1.19 (m, 27H), 1.17-1.01 (m, 7H), 1.01-0.74 (m, 22H); ^{13}C NMR (150 MHz, DMF-d_7, mixture of diastereomers and rotamers) δ 173.4, 173.3, 173.2, 170.8, 170.7, 170.3, 170.3, 170.2, 170.2, 169.7, 169.6, 169.5, 169.5, 169.4, 169.4, 169.1, 169.1, 169.0, 169.0, 163.2, 156.2, 156.1, 155.2, 155.1, 155.0, 153.8, 153.8, 144.2, 144.2, 144.1, 140.2, 140.1, 133.8, 131.6, 131.6, 131.5, 131.5, 131.3, 131.1, 131.1, 128.4, 128.3, 128.2, 128.1, 128.1, 127.2, 127.2, 127.1, 127.0, 127.0, 126.8, 118.3, 117.9, 117.1, 86.1, 82.5, 78.4, 77.8, 77.0, 76.9, 76.7, 76.7, 75.8, 75.8, 75.6, 65.2, 64.7, 64.6, 64.5, 64.4, 64.3, 63.1, 61.1, 60.6, 59.5, 59.0, 57.4, 56.6, 55.7, 55.2, 55.1, 54.9, 54.8, 54.7, 52.7, 50.8, 50.2, 50.1, 47.8, 46.8, 46.7, 44.7, 44.2, 39.3, 39.1, 39.0, 37.7, 37.7, 35.9, 32.0, 27.3, 27.2, 27.2, 27.0, 26.9, 26.8, 26.0, 25.9, 25.0, 24.9, 23.6, 22.8, 19.3, 19.2, 19.1, 19.0, 18.9, 18.8, 18.7, 18.6, 18.4, 18.3, 18.2, 18.1, 16.3, 16.1, 15.8, 15.6, 15.4, 14.9, 14.8, 14.7, 14.1, 13.9, 13.8, 12.0, 11.9, 11.9, 10.5, 10.4, 10.3, 10.2, 7.3; HRMS $[M-H]^-$ calcd. 1218.8363 for $C_{68}H_{112}N_7O_{12}^+$, found 1218.8377.

1.4. Palladium catalysts



Pd-1



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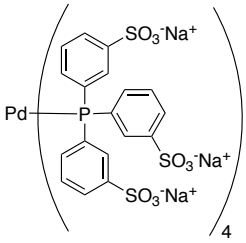
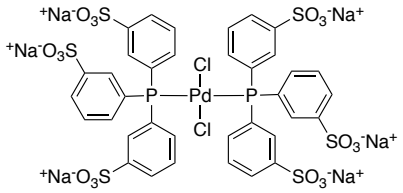


Pd-3



Pd-4

Palladium catalysts **Pd-1**, **Pd-2**, **Pd-3**, and **Pd-4** were prepared as described in the literature.[2-5]

2. Fluorescence measurements

2.1. Reaction kinetics and catalyst performance

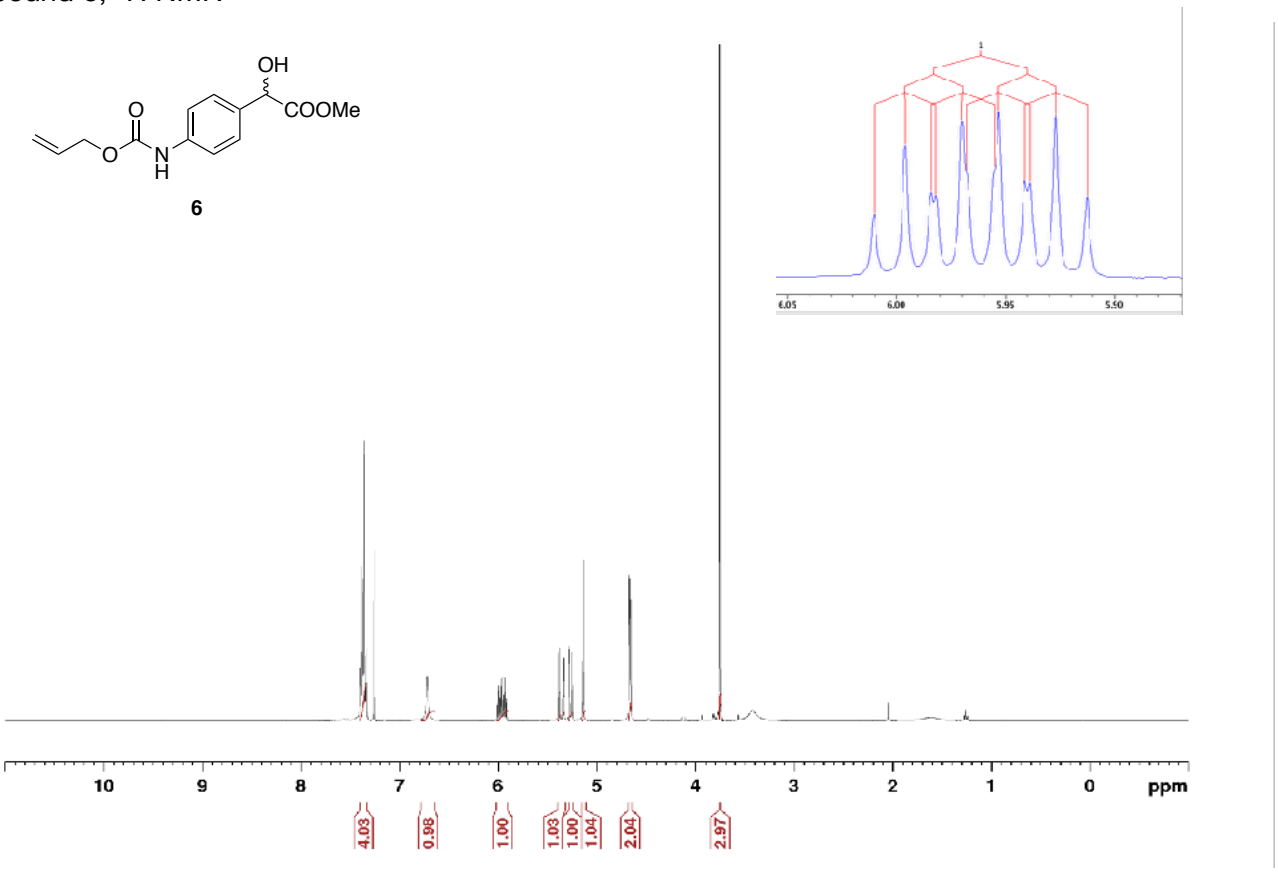
Alloc-SIL-dPEG4-AMC (**8**) was reacted at a concentration of 5 µM with palladium catalyst (**Pd-1**, **Pd-2**, **Pd-3**, **Pd-4**) at a concentration of 10 µM in HBSS and MEM (see Fig. S1). $Alloc_2R110$ was reacted at a concentration of 5 µM with **Pd-1** at a concentration of 10 µM in HBSS as described previously.[2] Fluorescence was monitored for 10 h at room temperature using 96 well plates and a Tecan Safire 2 fluorescence plate reader.

2.2. Fluorescence spectra

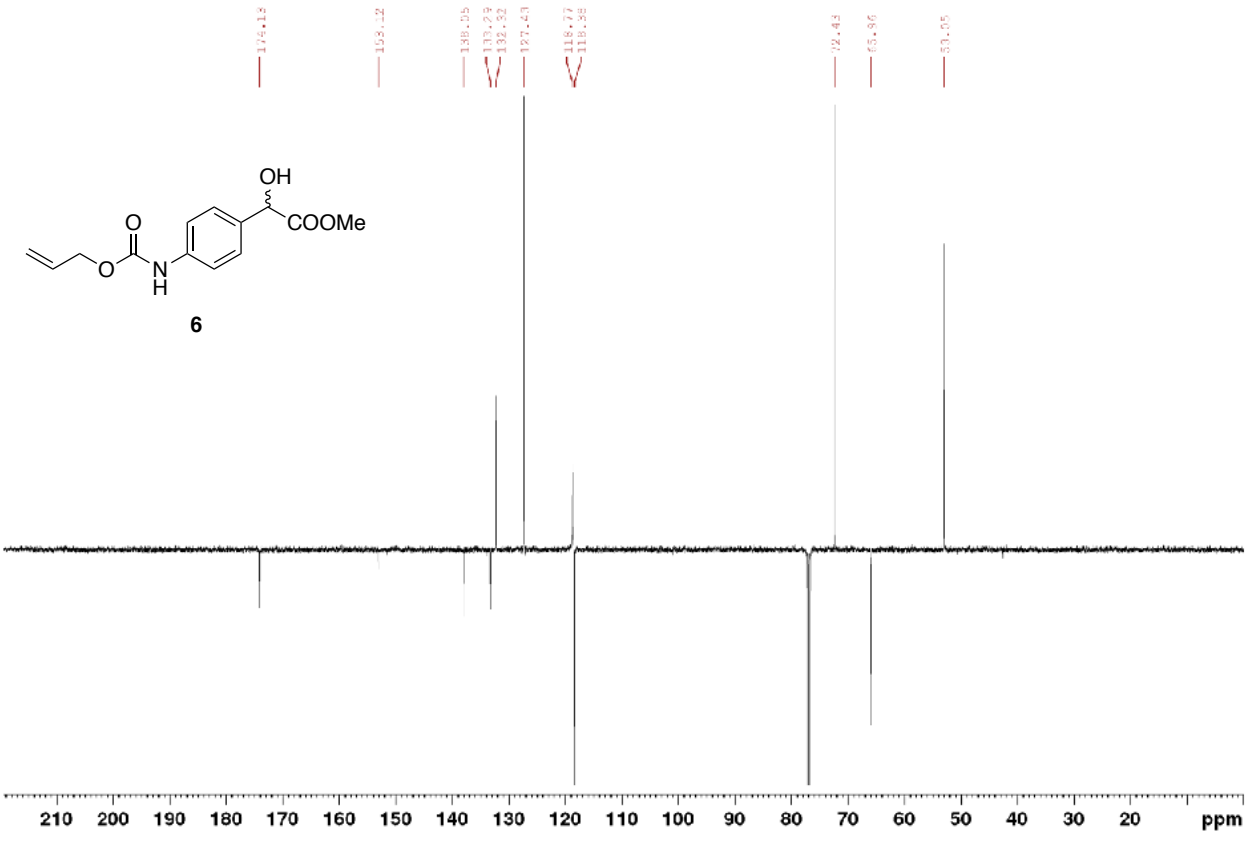
Fluorescence spectra (see Fig. S1) were recorded on an LS55 fluorimeter (PerkinElmer Inc., MA, USA) at a concentration of 1 µM 7-amino-4-methylcoumarin (AMC) or Alloc-SIL-dPEG4-AMC (**8**) in PBS, and after reacting Alloc-SIL-dPEG4-AMC (**8**) (1 µM in PBS) with **Pd-1** (2 µM in PBS).

3. NMR Spectra

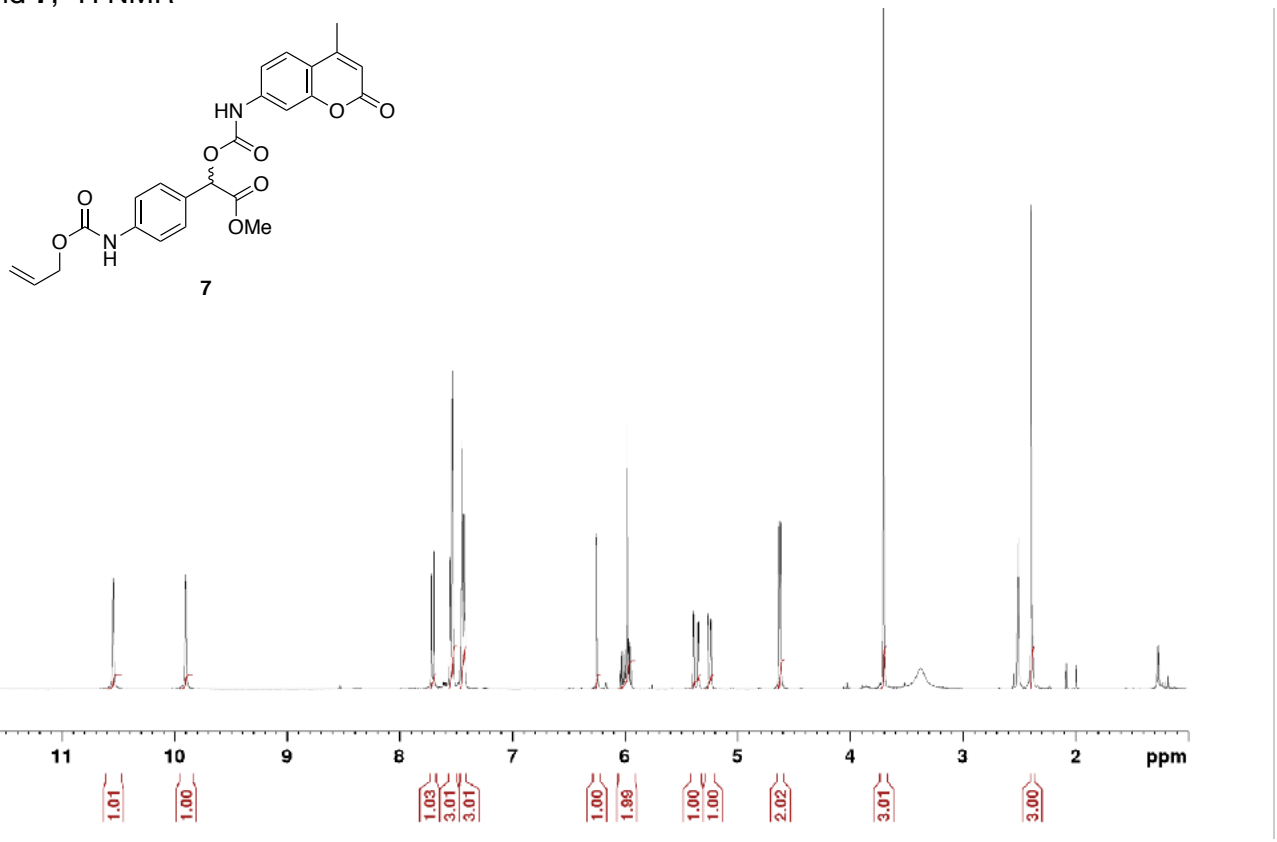
Compound **6**, ^{1}H NMR



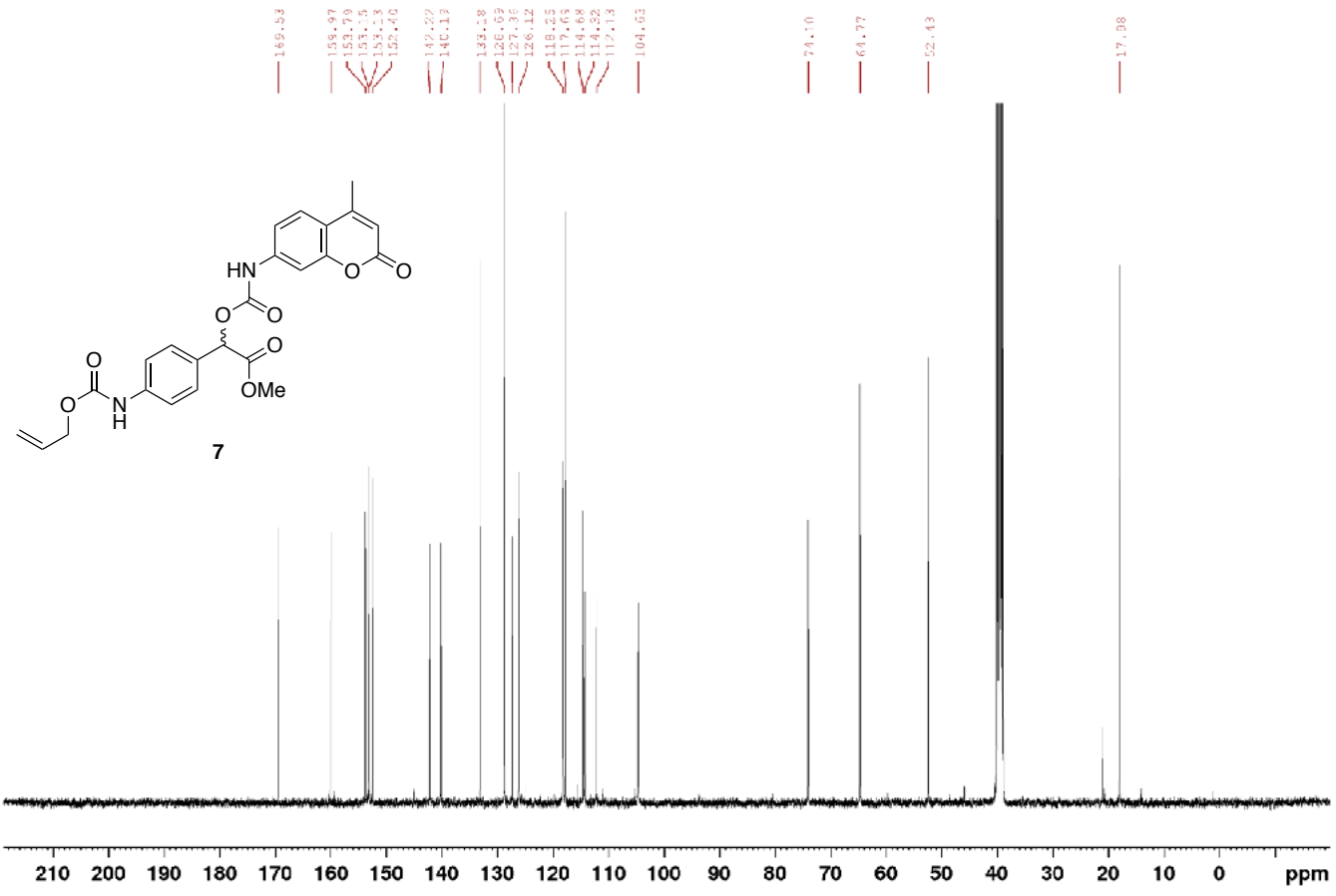
Compound **6**, ^{13}C NMR



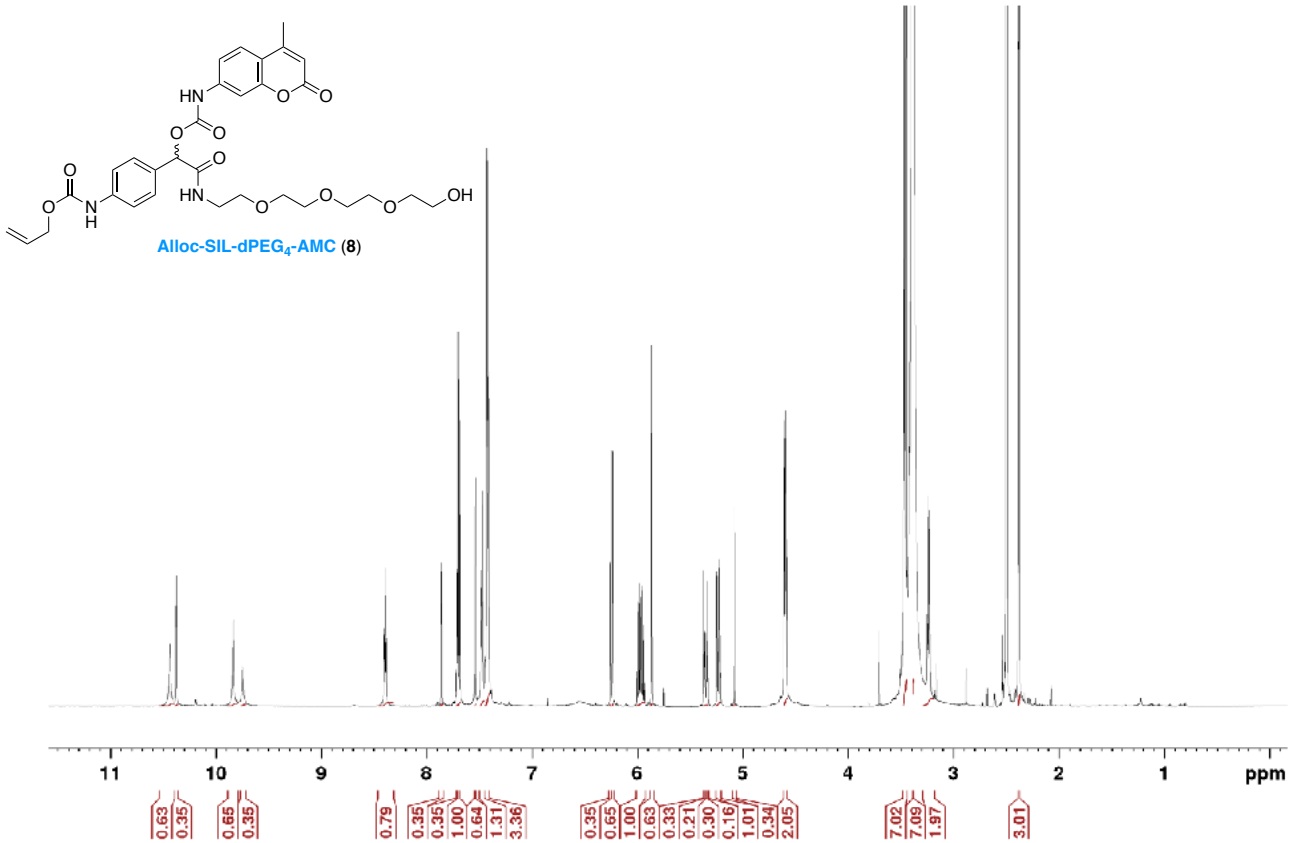
Compound **7**, ^{1}H NMR



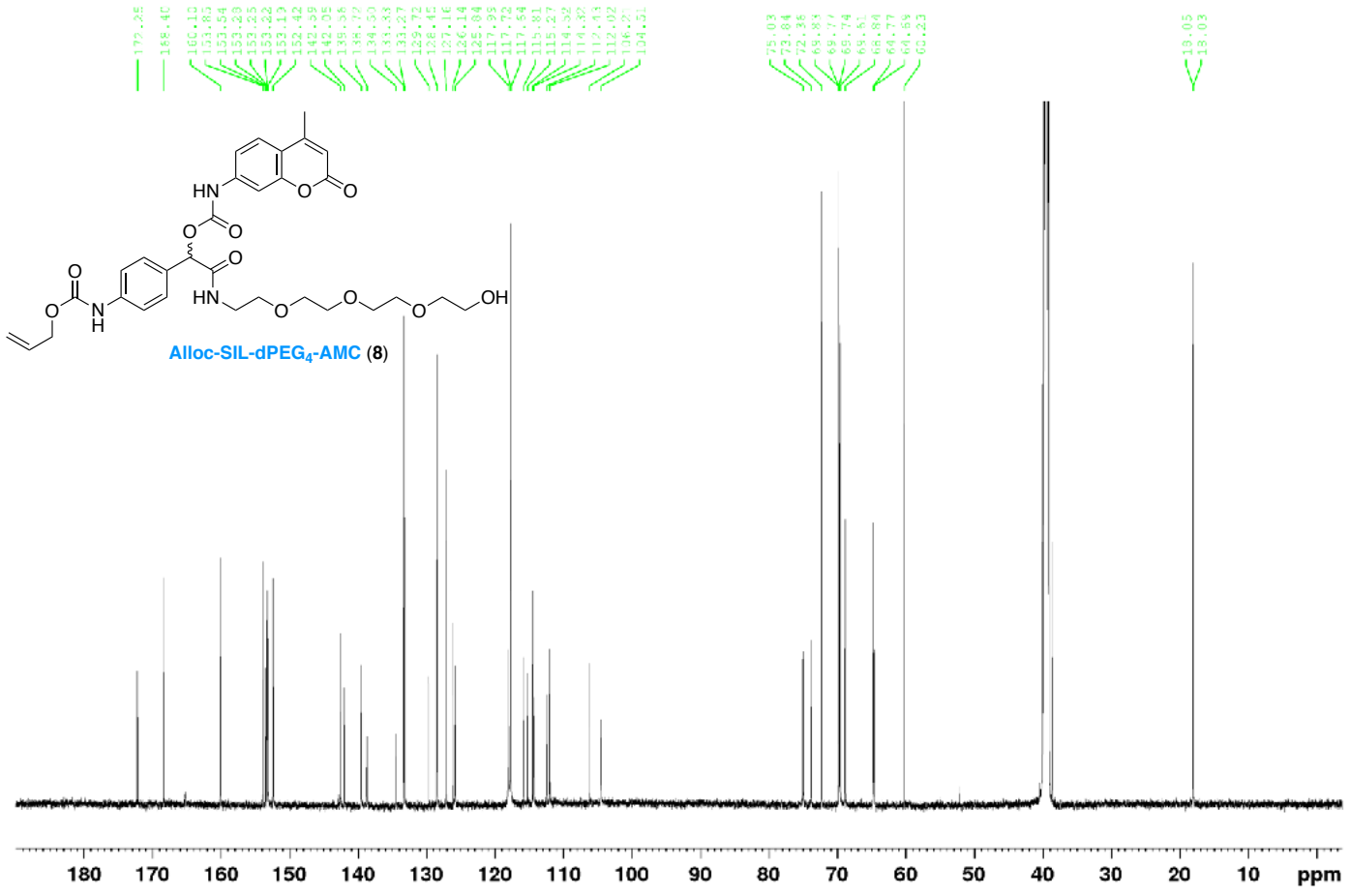
Compound **7**, ^{13}C NMR



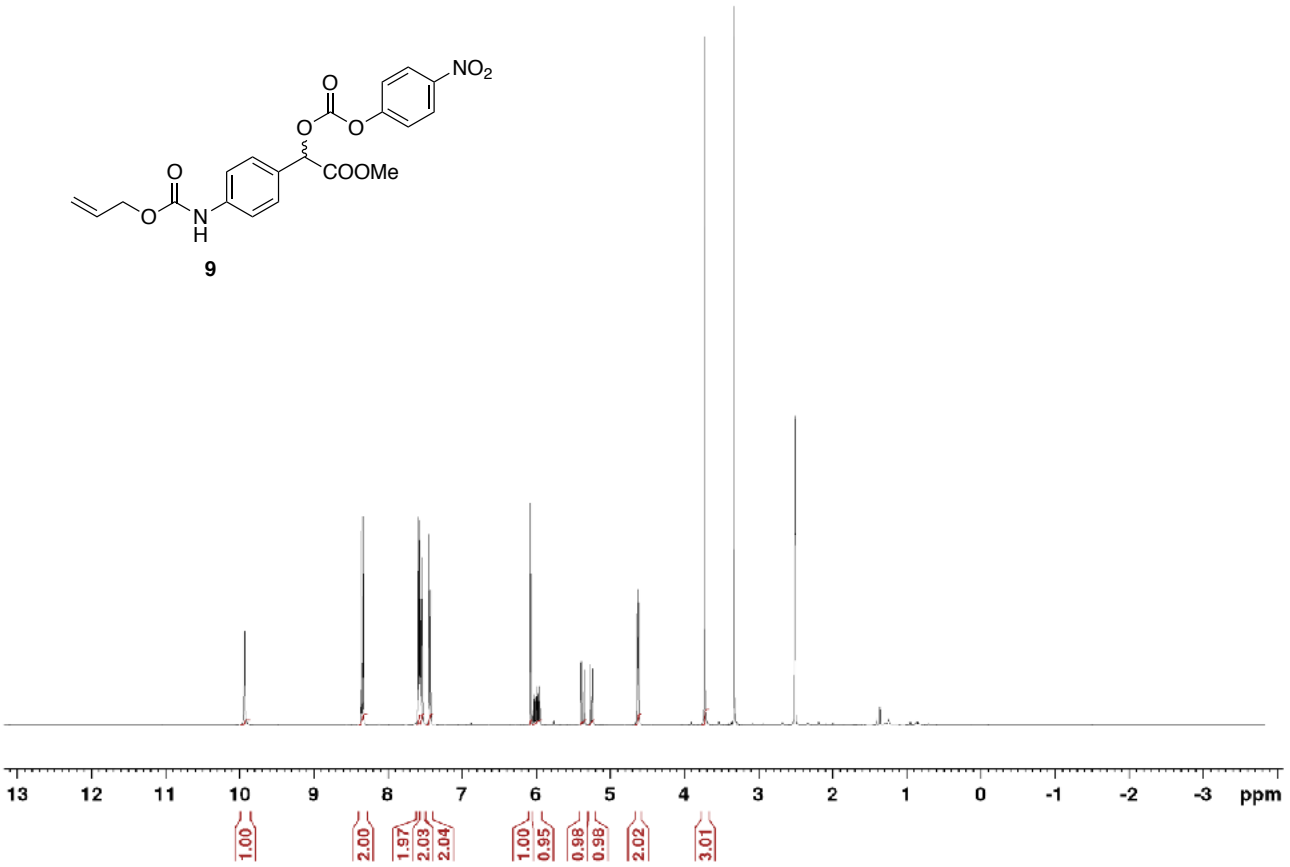
Compound **8**, ^{1}H NMR



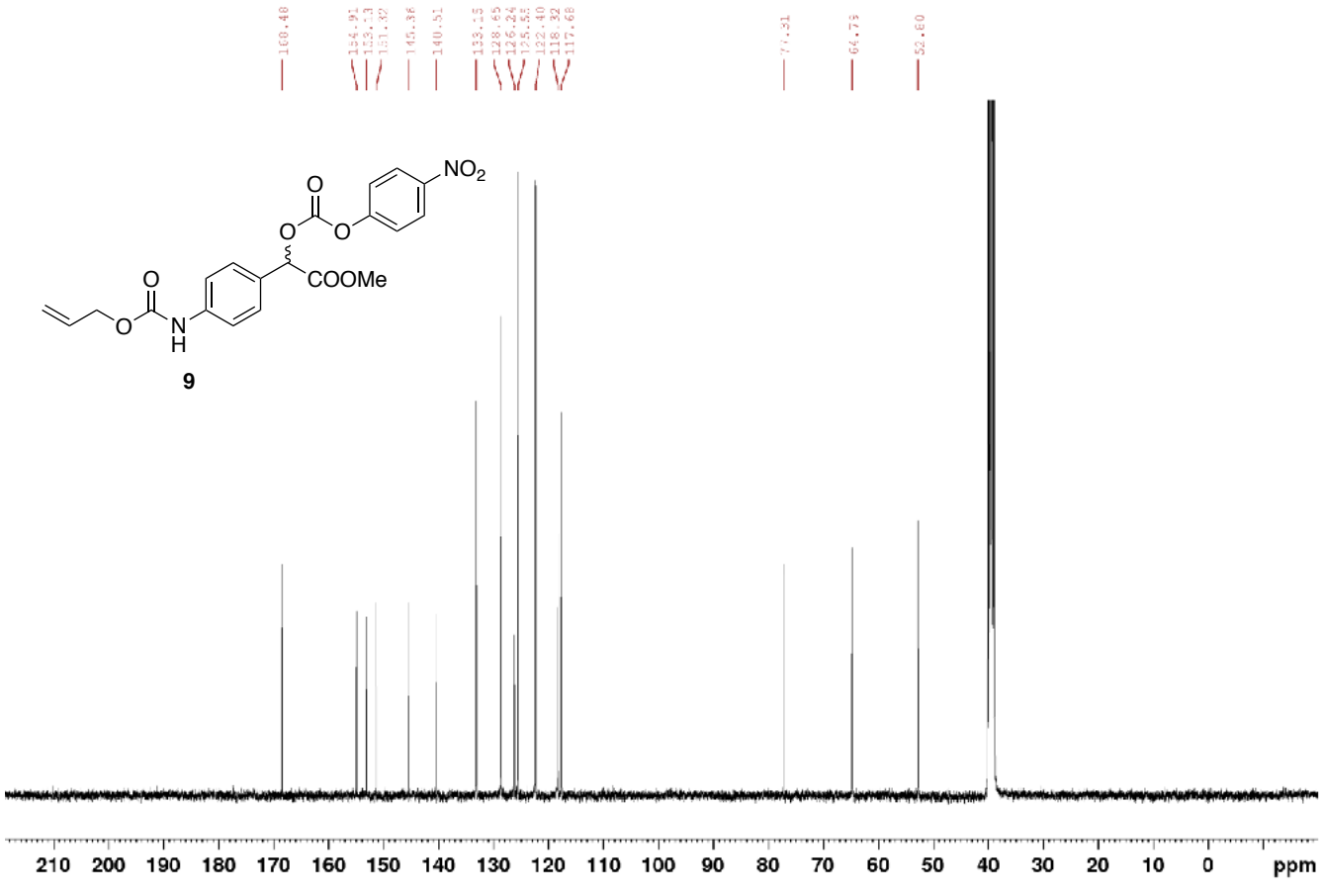
Compound **8**, ^{13}C NMR



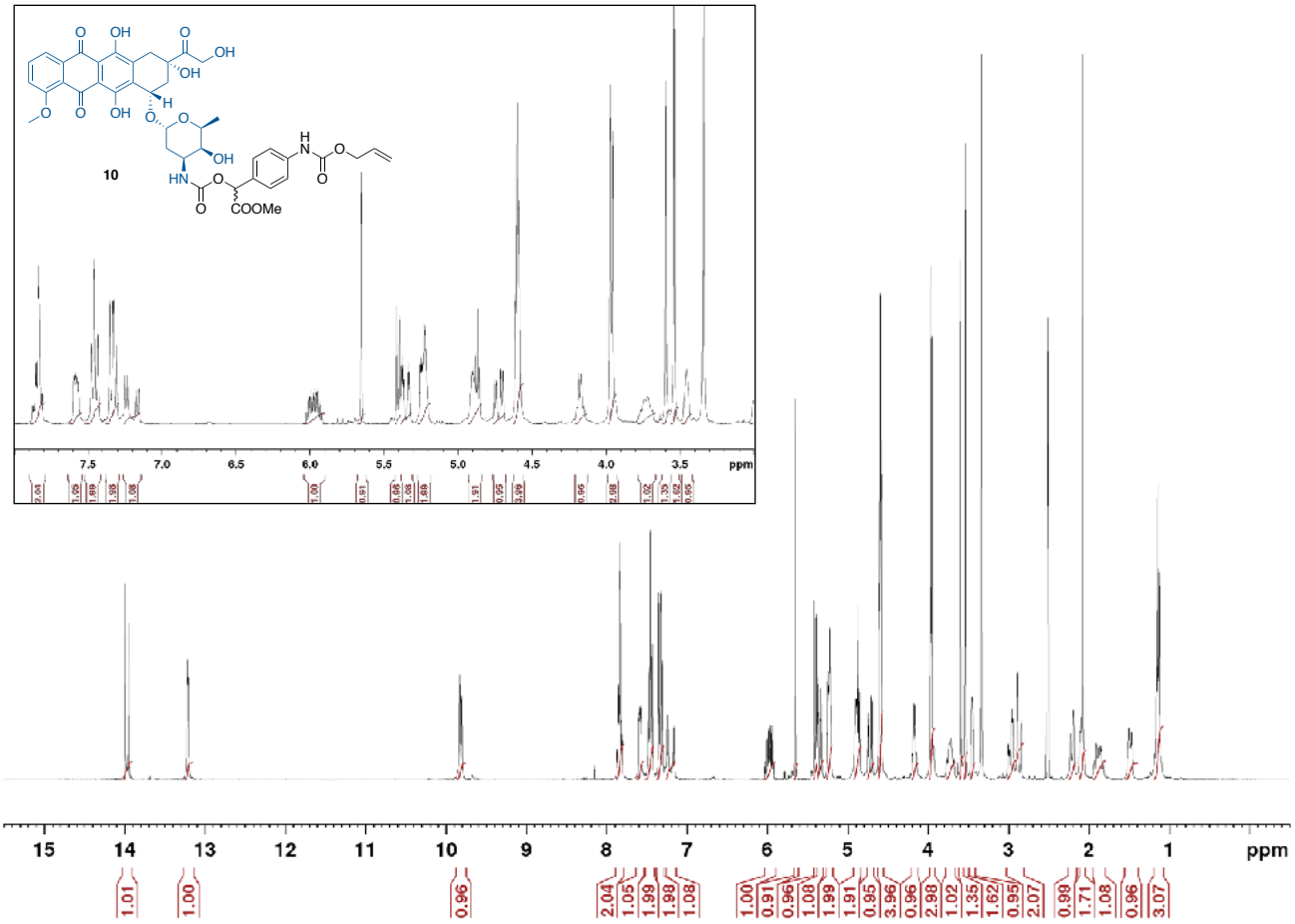
Compound **9**, ^{1}H NMR



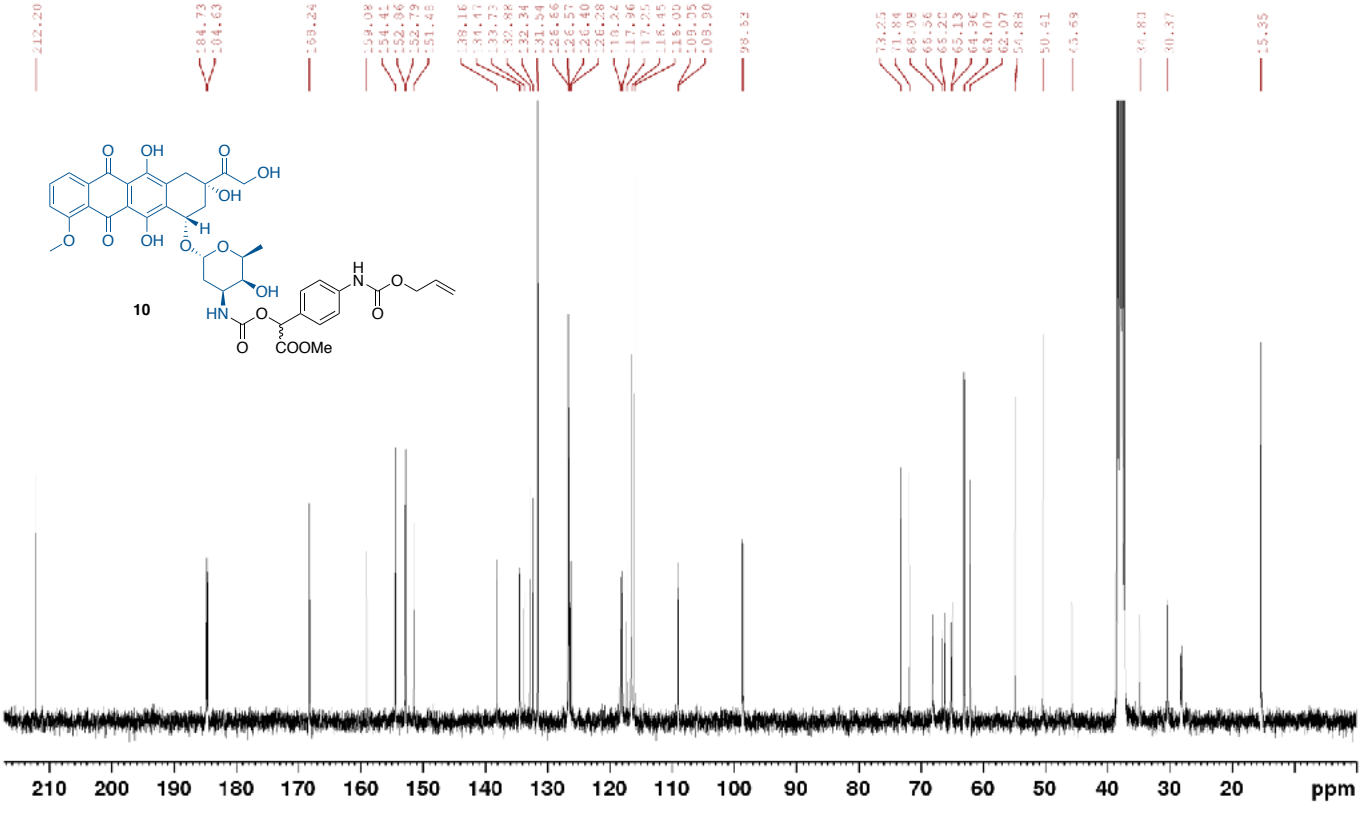
Compound **9**, ^{13}C NMR



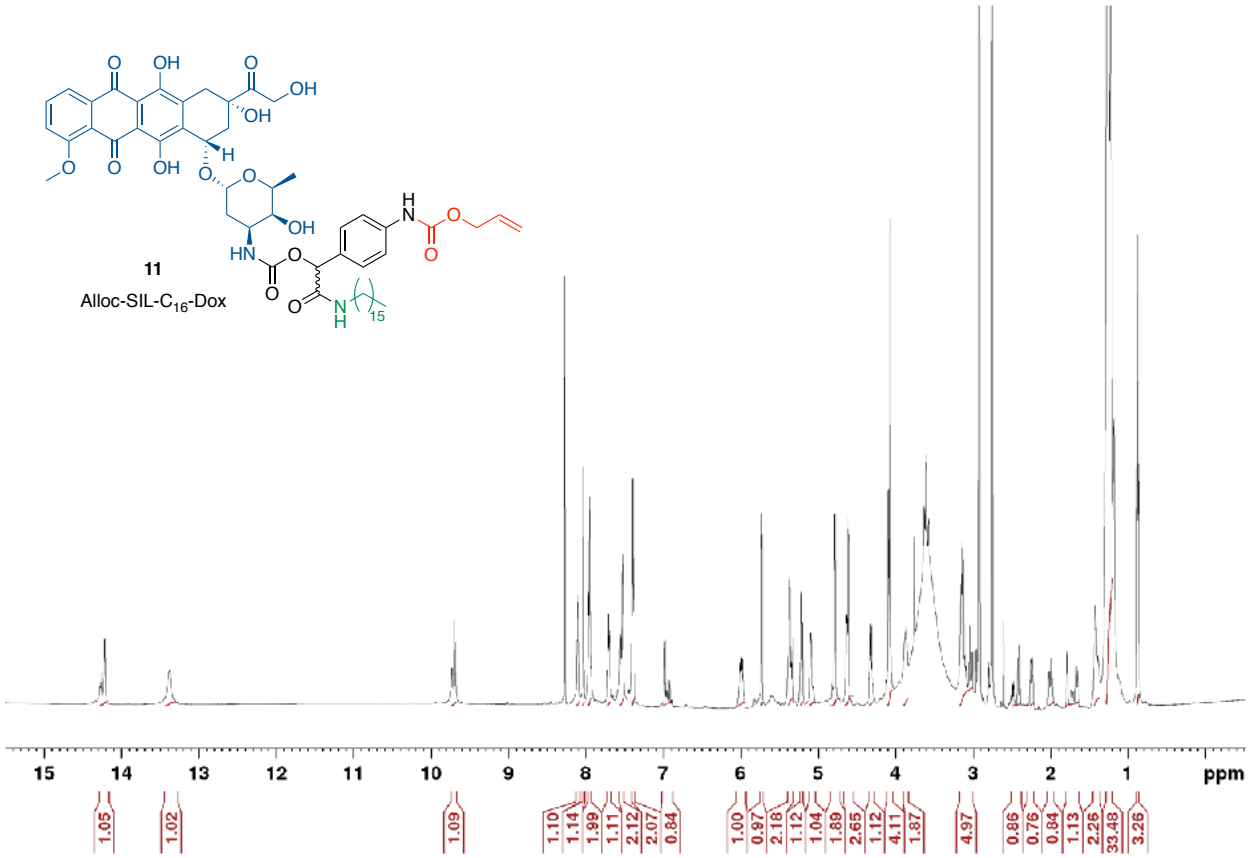
Compound **10**, ^{1}H NMR



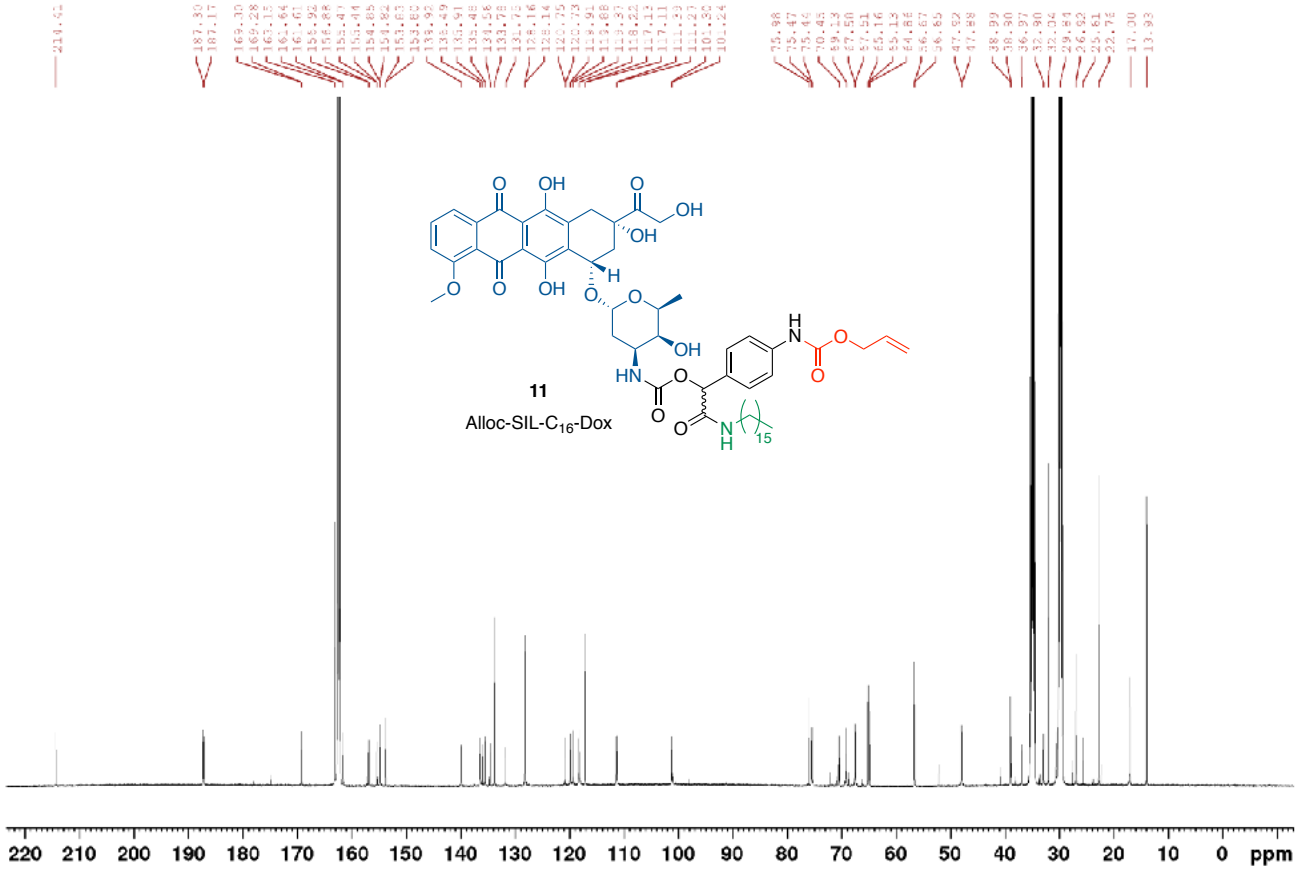
Compound **10**, ^{13}C NMR



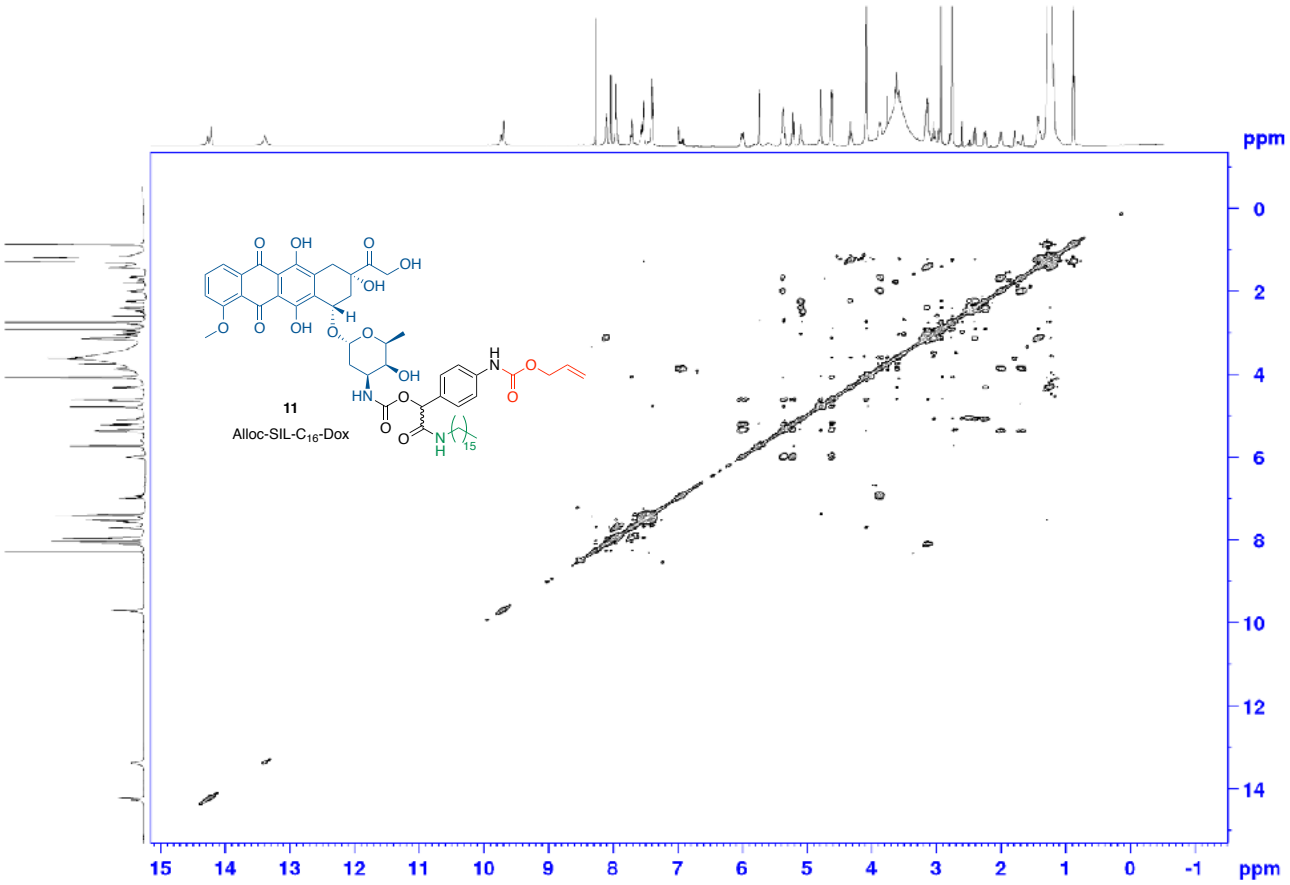
Compound **11**, ^{1}H NMR



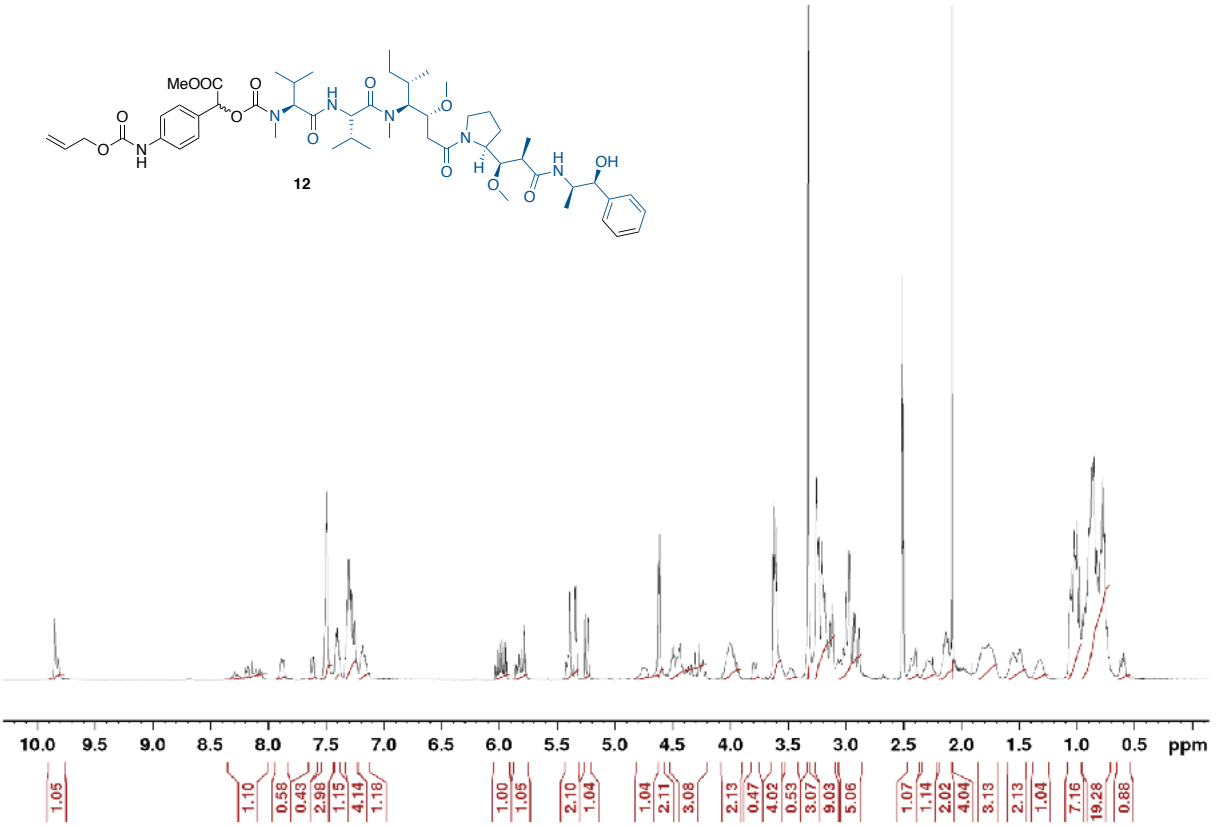
Compound **11**, ^{13}C NMR



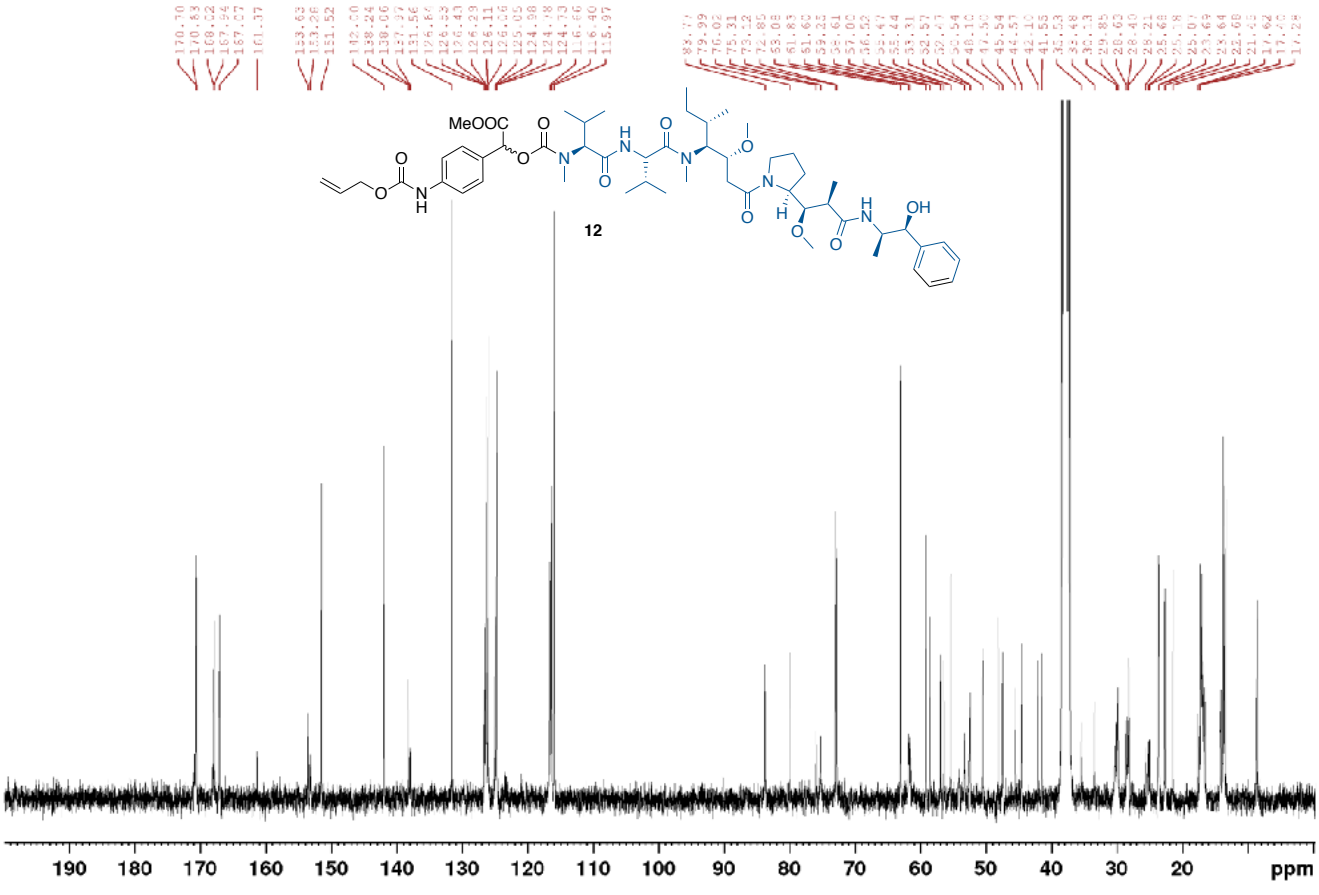
Compound **11**, COSY



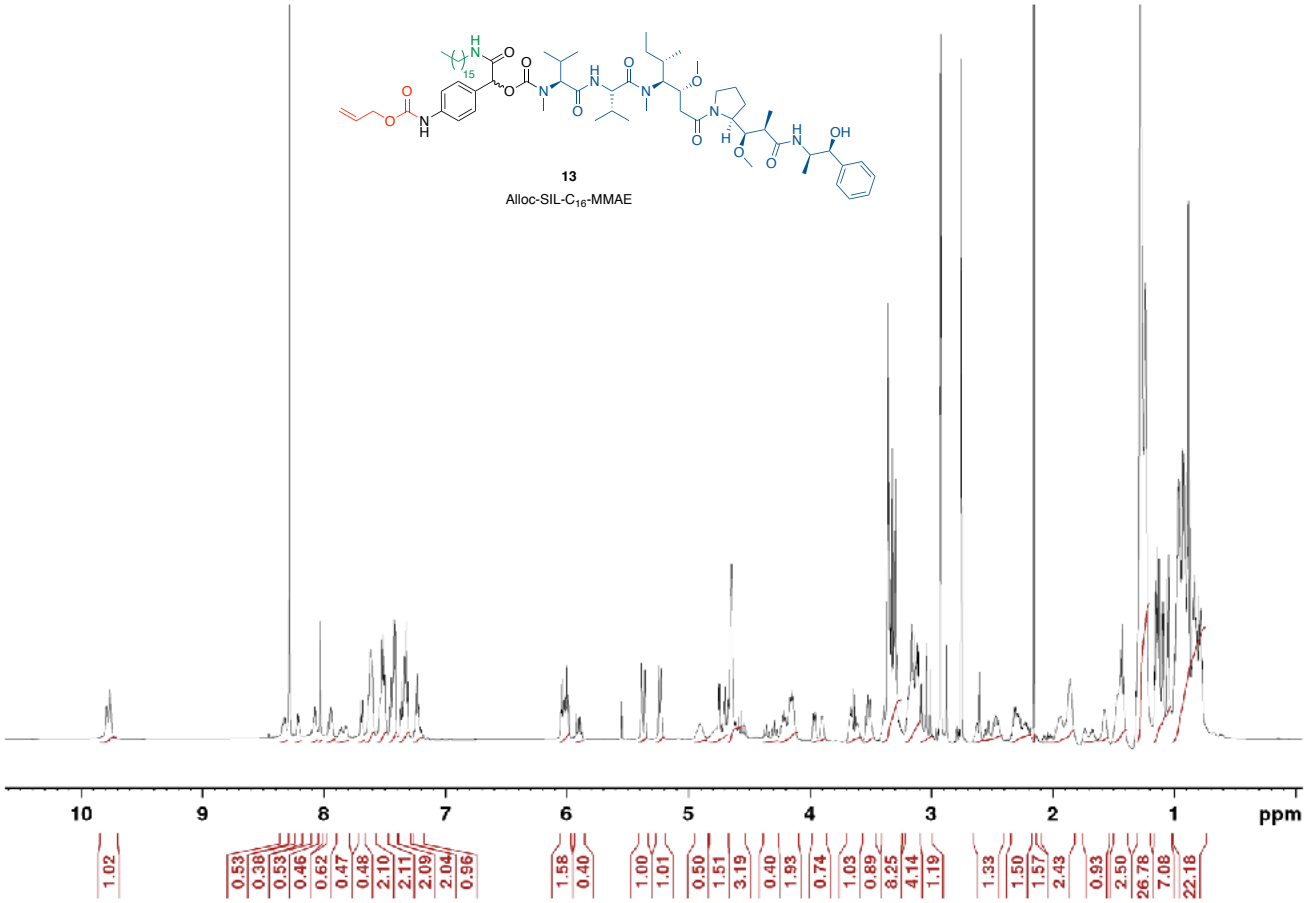
Compound **12**, ^{1}H NMR



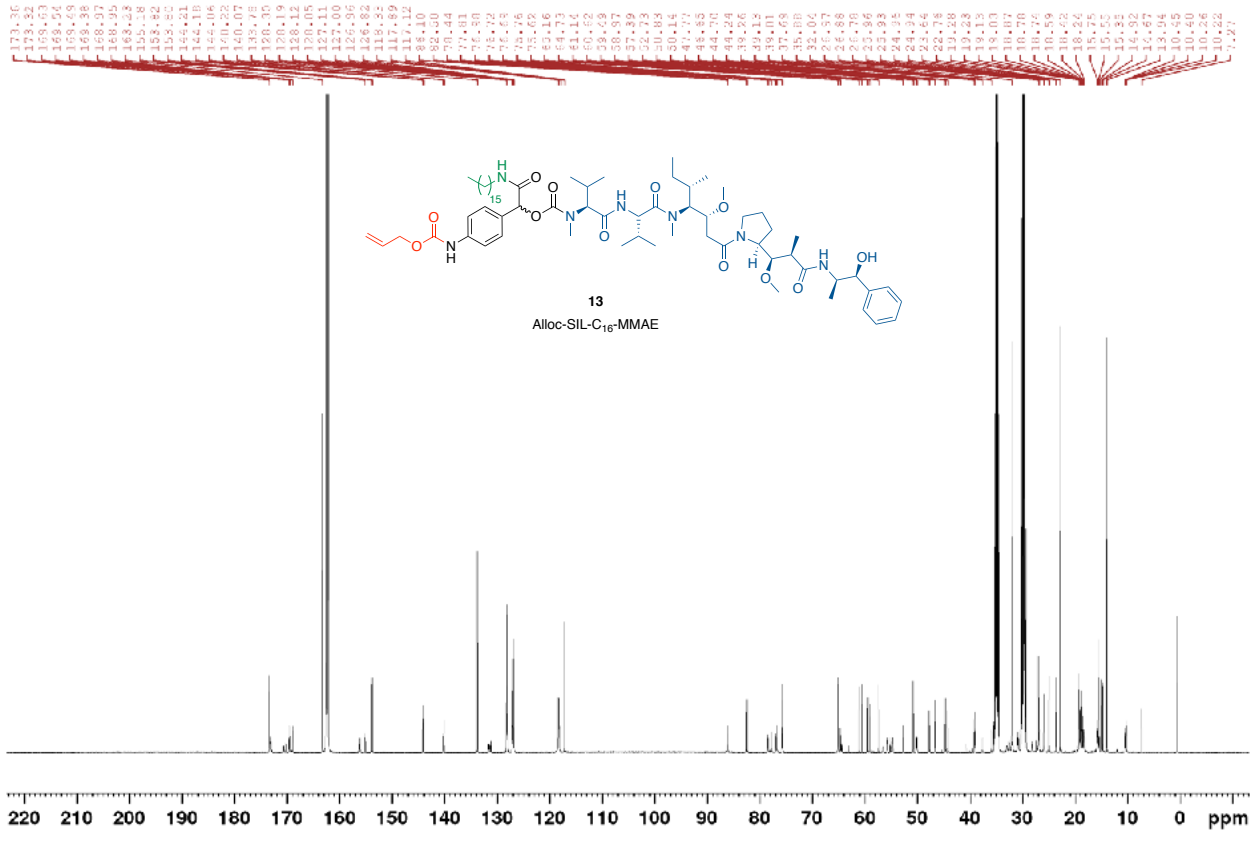
Compound **12**, ^{13}C NMR



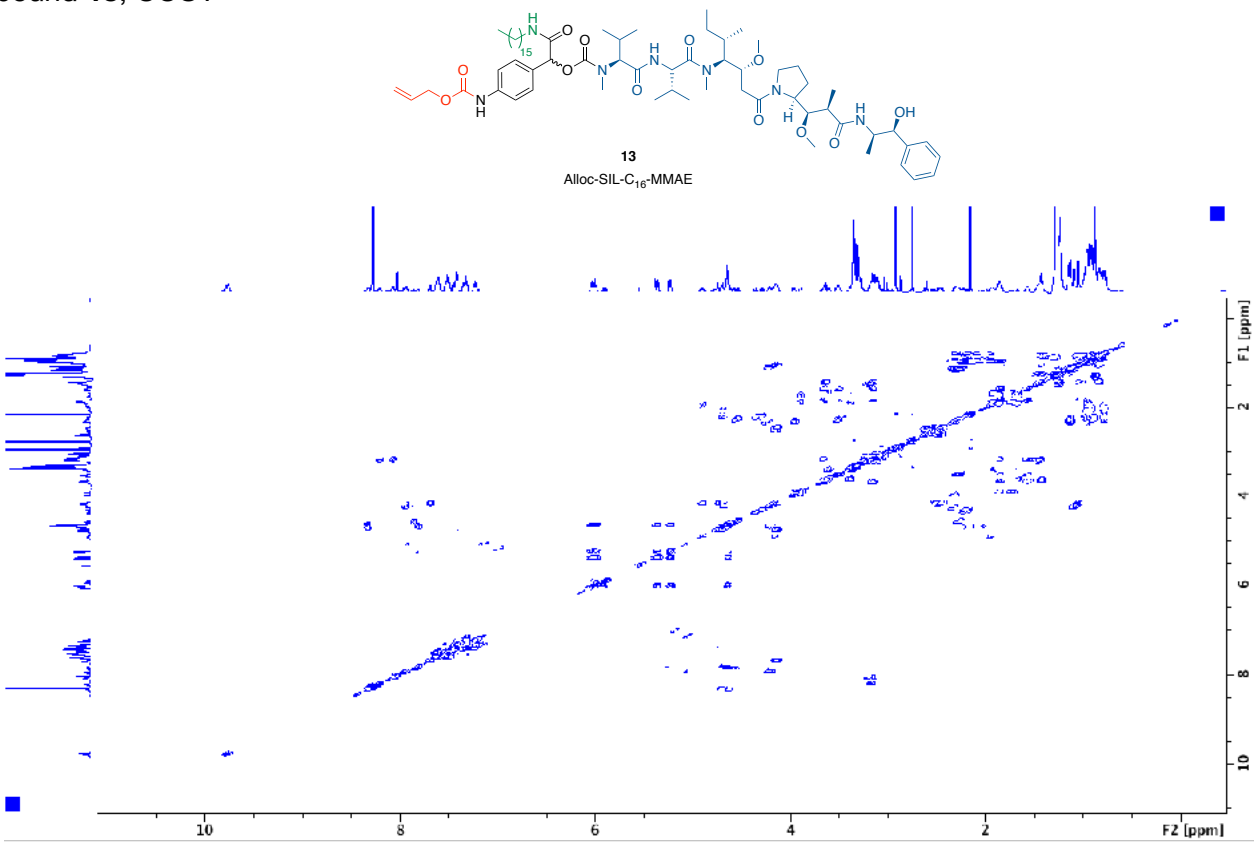
Compound **13**, ^{1}H NMR



Compound **13**, ^{13}C NMR



Compound **13**, COSY



4. References

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