Electronic Supplementary Information

Overcoming synthetic challenges in targeting coenzyme A biosynthesis with the antimicrobial natural product CJ-15,801

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1. Materials and methods

All chemicals were purchased from Merck KGaA and were used without further purification unless otherwise noted. *S. aureus* PanK was obtained as previously described.[1-2] Pyruvate kinase was from Merck KGaA. Tetrahydrofuran (THF) was distilled under nitrogen from sodium wire using benzophenone as an indicator. Dichloromethane (CH_2Cl_2) was distilled under nitrogen from calcium hydride. *N*,*N*-Dimethylformamide (DMF) was dried and purified by shaking up over potassium hydroxide, followed by distillation under reduced pressure and a nitrogen atmosphere, and was stored over 3 Å molecular sieves. All column chromatography was performed using Merck silica gel 60 (particle size 0.040-0.063 mm) using combinations of hexane, ethyl acetate (EtOAc), CH_2Cl_2 and methanol (MeOH) as eluents. Thin layer chromatography (TLC) was carried out on aluminium-backed Merck silica gel 60 F254 plates. Visualization was performed with a UV lamp followed by spraying with a cerium ammonium molybdate or ninhydrin solution, followed by heating. All ^{1}H and ^{13}C NMR spectra were obtained using 300 MHz Varian VNMRS (75 MHz for ^{13}C), 400 MHz Varian Unity Inova (101 MHz for ^{13}C) or 600 MHz Varian Unity Inova (125 MHz for ^{13}C) instruments at the Central Analytical Facility (CAF) of Stellenbosch University. Chemical shifts (δ) were recorded using the residual solvent peak or an external reference. All chemical shifts are reported in ppm and all spectra were obtained at 25°C. Proton spectral data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad), coupling constant in Hz, and integration. All high resolution mass spectra (HRMS) were performed on a Waters API Q-TOF Ultima spectrometer at the Mass Spectrometry unit of CAF using either positive or negative ion mode ESI as appropriate.

Removal rate of the tetrabutylammonium salt was determined using the following equation:

$$Removal\ rate\ (\%) = \left(1 - \frac{y}{12x}\right) \times 100$$

where

x : total equivalent of TBAF

y : integration value of methyl group of residual tetrabutylammonium salt

2. Synthetic methods

2.1 Synthesis of pantoamides (3)

(*R*)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid amide (3a). The synthesis of **3a** was performed according to the literature procedure of Aquino *et al*.[3] To an oven-dried two-neck round bottom flask containing 2,4-dihydroxy-3,3-dimethyl-butyramide (1.58 g, 10.7 mmol) was added anhydrous acetone (27 mL) and CH_2Cl_2 (27 mL). To this solution was added isopropylmethyl ether (2.10 mL, 21.4 mmol) and toluenesulfonic acid (*p*-TsOH) (204 mg, 1.07 mmol). The resulting mixture was then stirred for 1 h at ambient temperature followed by filtration and neutralization by the addition of triethylamine (300 µL). The resulting mixture was then dried over Na_2SO_4. After filtration the solution was concentrated *in vacuo* and the amide purified using flash chromatography (hexane/EtOAc; 2:1) to afford **3a** as a yellow oil (1.24 g, 62%) which solidified on standing. The NMR data are in agreement with the literature spectra.[3] ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.10 (br s, 2H), 5.21 (d, J = 6.0 Hz, 1H), 4.47 (t, J = 5.5 Hz, 1H), 3.66 (d, J = 6.0 Hz, 1H), 3.30 (dd, J = 10.3, 5.4 Hz, 1H), 3.18 (dd, J = 10.3, 5.4 Hz, 1H), 0.82 (s, 3H), 0.81 (s, 3H).

(4*R*)-2-(4-Methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamide (3b). To a solution of 2,4-dihydroxy-3,3-dimethyl-butyramide[3] (500 mg, 3.40 mmol) and anisaldehyde dimethyl acetal (3.10 g, 17.0 mmol) in CH_2Cl_2 (30 mL) at ambient temperature, was added (+)-camphor-10-sulfonic acid (79.0 mg, 0.340 mmol). The reaction mixture was stirred at ambient temperature for 1 h before being quenched by the addition of $NaHCO_3$ (20 mL). The aqueous phase was extracted with CH_2Cl_2 (3×10 mL) and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc; 2:1) gave **3b** as a white solid (673 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (d, J = 8.6, 2H), 6.92 (d, J = 8.6 Hz, 2H), 6.44 (br s, 1H), 5.48 (s, 1H), 4.11 (s, 1H), 3.82 (s, 3H), 3.73 (d, J = 11.7 Hz, 1H), 3.67 (d, J = 11.7 Hz, 1H), 1.19 (s, 3H), 1.10 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 160.4, 130.4, 127.6, 113.9, 101.5, 84.2, 78.7, 55.5, 33.1, 21.9, 19.3. HRMS (ESI/Q-TOF) m/z: $[M + Na]^+$ Calcd for $C_{14}H_{19}NO_4Na$: 288.1206; Found 288.1212.

(*R*)-5,5-Dimethyl-2-oxo-1,3-dioxane-4-carboxamide (3c). To an ice-cooled stirred solution of 2,4-dihydroxy-3,3-dimethyl-butyramide[3] (300 mg, 2.31 mmol) in pyridine (15 mL) was added triphosgene (684 mg, 2.31 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction was warmed to ambient temperature and stirred for 12 h. After addition of saturated $NaHCO_3$ solution (20 mL), the reaction mixture was extracted with ethyl acetate (3×20 mL). Combined organic layers were washed with 2% HCl (25 mL), water (25 mL) and saturated NaCl solution (25 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (hexane/EtOAc; 4:1) to give **3c** as a colourless oil (56 mg, 14%). ^{1}H NMR (400

MHz, $CDCl_3$) δ 5.48 (s, 2H), 4.11 (s, 1H), 3.74 (d, J = 11.4 Hz, 1H), 3.67 (d, J = 11.4 Hz, 1H), 1.19 (s, 3H), 1.10 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 154.0, 89.2, 72.4, 37.8, 21.2. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_7H_{12}NO_4$: 174.0761; Found: 174.0771.

(*R*)-2,2-Di-tert-butyl-5,5-dimethyl-1,3,2-dioxasilinane-4-carboxamide (3d). To an oven dried two-neck round bottom flask containing 2,4-dihydroxy-3,3-dimethyl-butyramide[3] (300 mg, 2.31 mmol) dissolved in anhydrous DMF (14 mL), $AgNO_3$ (861 mg, 5.04 mmol) was added. The reaction mixture was cooled to 0 °C and di-*tert*-butyldichlorosilane (536 µL, 2.54 mmol) was added drop-wise over 5 minutes. The mixture was stirred on ice for 30 minutes and subsequently allowed to warm to ambient temperature. Et_3N (707 µL, 5.07 mmol) was added and stirred for a further 15 minutes at ambient temperature. Water (100 mL) was added and the reaction mixture was extracted with EtOAc (3×20 mL). The resulting mixture was dried over Na_2SO_4 and concentrated *in vacuo*, followed by purification of the residue using flash chromatography (hexane/EtOAc; 4:1) to give **3d** as a yellow oil (390 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (br s, 1H), 6.68 (br s, 1H), 4.37 (s, 1H), 3.99 (d, J = 11.6 Hz, 1H), 3.47 (d, J = 11.6 Hz, 1H), 1.07 (s, 3H), 1.03 (s, 18H), 0.97 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 81.5,75.5, 37.3, 28.7, 27.7, 27.4, 20.3, 20.0, 19.0. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{14}H_{30}NO_3Si$: 288.1990; Found: 288.1982.

2.2 Synthesis of 3-bromoacrylates (4)

General procedure for synthesis of (*E*)-3-bromoacrylates (4). With the exception of **4e**, the (*E*)-3-bromoacrylates **4** were prepared either by Fischer-esterification (*Method A*), by refluxing (*E*)-β-bromoacrylic acid[4-5] and the alcohol of interest in the presence of H_2SO_4, or by a carbodiimide-mediated coupling (*Method B*), in which 1 equiv. (*E*)-β-bromoacrylic acid, 1 equiv. dimethylaminopyridine (DMAP), 1 equiv. 3-hydroxyproprionitrile and 1.1 equiv. *N*,*N*′-diisopropylcarbodiimide (DIC; 339 µL, 2.19 mmol) is mixed in CH_2Cl_2 (0.5 M with respect to the bromoacrylic acid). Since the bromoacrylates were found to decompose upon storage, they were used immediately after purification for preparation of the protected CJ-15,801 precursors **5**.

Allyl (*E*)-3-bromoacrylate (4a). Synthesized using *Method A* by dissolving (*E*)-β-bromoacrylic acid[4-5] (2.00 g, 13.2 mmol) in allyl alcohol (49 mL), adding H_2SO_4 (217 µL, 3.91 mmol) and heating the solution was heated to reflux overnight. After cooling to ambient temperature and concentration *in vacuo* the desired ester was purified using flash chromatography (hexane/EtOAc; 19:1) yielding the product as a colourless liquid (1.54 g, 61%), which was used immediately in the subsequent step. The Z-isomer[3] and the iodo-homologue[6] have been synthesized previously. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 13.9 Hz, 1H), 6.55 (d, J = 13.9 Hz, 1H), 5.93 (ddt, J = 17.1, 10.4, 5.8 Hz, 1H), 5.30 (ddq, J = 25.1, 10.4, 1.4 Hz, 2H), 4.65 (dt, J = 5.8, 1.4 Hz, 2H). ^{13}C NMR (75MHz, $CDCl_3$): δ 163.9, 131.8, 128.7, 127.2, 118.9, 65.7.

Methyl (*E*)-3-bromoacrylate (4b). Methyl ester **4b** was prepared as for **4a** using *Method A* with (*E*)-β-bromoacrylic acid[4-5] (5.91 g, 39.2 mmol) and methanol. Purification using flash chromatography (hexane/EtOAc; 3:1) yielded the product as a yellow liquid (1.63 g, 25%), which was used immediately in the subsequent step. The NMR data are in agreement with the literature spectra.[4] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, J = 14.0 Hz, 1H), 6.53 (d, J = 14.0 Hz, 1H), 3.75 (s, 3H).

tert-Butyl (*E*)-3-bromoacrylate (4c). *tert*-Butyl ester **4c** was prepared using *Method A* with (*E*)-β-bromoacrylic acid[4-5] (2.0 g, 13 mmol) and *tert*-butyl alcohol. Purification using flash chromatography (hexane/EtOAc; 4:1) yielded the product as a colourless liquid after flash chromatography (0.51 g, 19%), which was used immediately in the subsequent step. Both the bromo[7] and iodo-versions[8] have been synthesized previously. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.47 (d, J = 13.9 Hz, 1H), 6.45 (d, J = 13.9 Hz, 1H), 1.49 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 163.5, 130.7, 125.5, 81.7, 28.2.

2-(Trimethylsilyl)ethyl (*E*)-3-bromoacrylate (4d). TMSE ester **4d** was prepared using *Method B*. (*E*)-β-bromoacrylic acid[4-5] (284 mg, 1.88 mmol) and DMAP (25 mg, 1.9 mmol) in an oven-dried two-neck flask were dissolved by addition of CH_2Cl_2 (5 mL) and 2-(trimethyl silyl)-ethanol (223 mg, 1.88 mmol) using oven-dried syringes. The solution was cooled to 0 °C and DIC (321 µL, 2.07 mmol) was added drop-wise using an oven-dried syringe. The reaction was allowed to warm to ambient temperature and stirred for 1 h. The reaction mixture was then filtered through Celite and concentrated *in vacuo*. The resulting syrup was taken up in EtOAc (50 mL) and washed with saturated $NaHCO_3$ (2×10 mL) and brine (1×10 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The desired product was obtained as a clear liquid (325 mg, 70%) after purification by flash chromatography (hexane/CH_2Cl_2; 5:1) and was used immediately in the subsequent step. The *Z*-chloro[9] and *Z*-iodo-homologs[10] have been synthesized previously. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 13.9 Hz, 1H), 6.45 (d, J = 13.9 Hz, 1H), 4.23–4.15 (m, 2H), 1.02–0.93 (m, 2H), 0.00 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.0, 129.1, 126.1, 77.2, 63.2, 17.3, -1.5.

4-Methoxybenzyl (E)-3-bromoacrylate (4e). K_2CO_3 (311 mg, 2.25 mmol) was added to a solution of (*E*)-β-bromoacrylic acid[4-5] (170 mg, 1.13 mmol) and *p*-methoxybenzyl bromide (162 µL, 1.13 mmol) in THF (5 mL) at ambient temperature. The reaction mixture was stirred at ambient temperature for 48 h before acetone (3 mL) and more *p*-methoxybenzyl bromide (162 µL, 1.13 mmol) were added. The reaction mixture was stirred for a further 20 h at ambient temperature, concentrated *in vacuo*, and purified by column chromatography (hexane/EtOAc 4:1). The ester product was obtained as an oil (349 mg, 54%), which was used immediately in the subsequent step.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, J = 14.1 Hz, 1H), 7.37 (d, J = 9.1 Hz, 2H), 6.91 (d, J = 9.1 Hz, 2H), 6.60 (d, J = 14.1 Hz, 1H), 4.53 (s, 2H), 3.81 (s, 3H).

2-Cyanoethyl (*E*)-3-bromoacrylate (4f). Cyanoethyl ester **4f** was prepared according to *Method B* (i.e. as for **4d**) using (*E*)-β-bromoacrylic acid[4-5] (300 mg, 1.99 mmol), DMAP (25 mg, 1.99 mmol), 3-hydroxyproprionitrile (150 mg, 1.99 mmol) and DIC (339 µL, 2.19 mmol) in CH_2Cl_2 (4 mL). The desired product was purified using flash chromatography (hexane/EtOAc; 9:1) to give the product as a clear liquid (388 mg, 96%), which was used immediately in the subsequent step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (d, *J* = 13.9 Hz, 1H), 6.50 (d, *J* = 13.9 Hz, 1H), 4.30 (t, *J* = 6.2 Hz, 2H), 2.70 (t, *J* = 6.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.2, 128.4, 127.5, 116.7, 77.2, 59.1, 17.8.

Benzyl (*E*)-3-bromoacrylate (4g). Benzyl ester **4g** was prepared according to *Method B* (i.e. as for **4d**) using (*E*)-β-bromoacrylic acid[4-5] (1.00 g, 6.62 mmol), DMAP (162 mg, 1.32 mmol), benzyl alcohol (3.44 mL, 33.10 mmol) and DIC (1.13 mL, 7.29 mmol) in CH_2Cl_2 (8 mL). The desired product was purified using flash chromatography (hexane/EtOAc 19:1) to give **4g** (1.0 g, 63%) as a pale yellow liquid, which was used immediately in the subsequent step. The NMR data are in agreement with the literature spectra.[5] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, *J* = 13.8 Hz, 1H), 7.39–7.33 (m, 5H), 6.58 (d, *J* = 13.8 Hz, 1H), 5.19 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 163.8, 135.4, 128.6, 128.5, 128.4, 128.3, 127.2, 66.7.

2.3 Synthesis of protected CJ-15,801 precursors (5)

General procedure for formation of protected CJ-15,801 precursors (5). The reactions were conducted according to an adapted procedure of Tanoury *et al*.[11] To an oven-dried Schlenk tube under a nitrogen atmosphere were added $Pd(OAc)_2$ (0.1 eq), Xantphos (0.15 eq), K_2CO_3 (2 eq), CTAB (0.2 eq), amide **3** (1 eq) bromide **4** (1.1 eq) and toluene (0.4 M with respect to amide). The suspension was then degassed under high vacuum until no further gas evolution was observed, after which it was warmed to 55 °C. After stirring for 1 h, 3 equiv. of water was added and the reaction stirred for a further 4 h at 55 °C. After cooling to ambient temperature the reaction was diluted with EtOAc, washed with water and the organic layer dried over Na_2SO_4. After filtration and concentration *in vacuo* the *E*- and *Z*-isomers of the product were separated and purified using flash chromatography. The stereochemistry of the products was assigned by comparing the coupling constants of the alkene protons.

Allyl (*R,E*)-3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)acrylate (5a). The synthesis of **5a** was performed according to the general procedure using amide **3a** (351 mg, 1.87 mmol) and bromoacrylate **4a** (393 mg, 2.06 mmol). The required *E*-isomer (306 mg, 55%) was obtained after purification by flash chromatography (hexane/EtOAc 4:1). The same reaction also yielded a small

amount of the *Z*-isomer (61 mg, 11%). The NMR data are in agreement with the literature spectra.[6] **(*E*)-5a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (br d, J = 11.6 Hz, 1H), 8.00 (dd, J = 14.0, 11.6 Hz, 1H), 5.95 (ddt, J = 17.0, 10.3, 5.5 Hz,), 5.63 (d, J = 14.0 Hz, 1H), 5.33 (d, J = 17.0 Hz, 1H), 5.23 (d, J = 10.3 Hz, 1H), 4.64 (d, J = 5.5 Hz, 2H), 4.20 (s, 1H), 3.71 (d, J = 11.8 Hz, 1H), 3.31 (d, J = 11.8 Hz, 1H), 1.51 (s, 3H), 1.45 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H). **(*Z*)-5a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.07 (br d, J = 10.0 Hz, 1H), 7.45 (dd, J = 11.7, 10.0 Hz, 1H), 6.00–5.87 (m, 1H), 5.33 (d, J = 17.2 Hz, 1H), 5.25 (d, J = 11.7 Hz, 1H), 5.20 (d, J = 9.0 Hz, 1H), 4.65 (d, J = 5.2 Hz, 2H), 4.21 (s, 1H), 3.73 (d, J = 11.7 Hz, 1H), 3.33 (d, J = 11.7 Hz, 1H), 1.58 (s, 3H), 1.46 (s, 3H), 1.04 (s, 3H), 1.03 (s, 3H).

Methyl (*R,E*)-3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)acrylate (5b). The synthesis of **5b** was performed according to the general procedure for Pd-catalyzed coupling reactions using amide **3a** (200 mg, 1.07 mmol) and bromoacrylate **4b** (194 mg, 1.18 mmol). The required *E*-isomer (206 mg, 71%) was obtained after purification by flash chromatography (hexane/EtOAc 3:1 to 2:1). The same reaction also yielded a small amount of the *Z*-isomer (12 mg, 4%). The NMR data are in agreement with the literature spectra.[12] **(*E*)-5b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (br d, J = 11.7 Hz, 1H), 7.98 (dd, J = 14.0, 11.7 Hz, 1H), 5.60 (d, J = 14.0 Hz, 1H), 4.19 (s, 1H), 3.73 (s, 3H), 3.71 (d, J = 12.0 Hz, 1H), 3.31 (d, J = 12.0 Hz, 1H), 1.51 (s, 3H), 1.45 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H). **(*Z*)-5b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.07 (br d, J = 11.1 Hz, 1H), 7.43 (dd, J = 11.1, 9.0 Hz, 1H), 5.17 (dd, J = 9.0 Hz, 1H), 4.21 (s, 1H), 3.74 (s, 3H), 3.73 (d, J = 12.7 Hz, 1H), 3.32 (d, J = 12.7 Hz, 1H), 1.59 (s, 3H), 1.47 (s, 3H), 1.05 (s, 3H), 1.03 (s, 3H).

***tert*-Butyl (*R,E*)-3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)acrylate (5c).** The synthesis of **5c** was performed according to the general procedure using amide **3a** (250 mg, 1.34 mmol) and bromoacrylate **4c** (304 mg, 1.47 mmol). The required *E*-isomer (248 mg, 59%) was obtained exclusively after purification by flash chromatography (hexane/EtOAc 4:1). The NMR data are in agreement with the literature spectra.[13] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (br d, J = 11.8 Hz, 1H), 7.85 (dd, J = 14.4, 11.8 Hz, 1H), 5.52 (d, J = 14.4 Hz, 1H), 4.18 (s, 1H), 3.71 (d, J = 12.0 Hz, 1H), 3.31 (d, J = 12.0 Hz, 1H), 1.50 (s, 3H), 1.48 (s, 9H), 1.45 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H).

2-(Trimethylsilyl)ethyl (*R,E*)-3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)acrylate (5d). The synthesis of **5d** was performed according to the general procedure using amide **3a** (125 mg, 0.667 mmol) and bromoacrylate **4d** (184 mg, 0.733 mmol). The required *E*-isomer (208 mg, 80%) was obtained after purification by flash chromatography (hexane/EtOAc 9:1). The same reaction also yielded a small amount of the *Z*-isomer (32 mg, 12%). The NMR data are in agreement with the literature spectra.[13] ^{1}H NMR (300 MHz, $CDCl_3$): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.39 (d, J = 12.0 Hz, 1H), 7.98 (dd, J = 14.2, 12.0 Hz, 1H), 5.60 (d, J = 14.2 Hz, 1H), 4.39 – 4.05 (m, 2H), 3.73 (d,

J = 11.8 Hz, 1H), 3.33 (d, *J* = 11.8 Hz, 1H), 1.53 (s, 3H), 1.47 (s, 3H), 1.07 (s, 3H), 1.02 (s, *J* = 3H), 0.06 (s, 6H).

4-Methoxybenzyl (*E*)-3-([(4*R*)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamido] acrylate (5e). The synthesis of **5e** was performed according to the general procedure using amide **3b** (355 mg, 1.34 mmol) and bromoacrylate **4e** (304 mg, 1.47 mmol). The required *E*-isomer (205 mg, 82%) was obtained exclusively after purification by flash chromatography (hexane/EtOAc 4:1) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (d, J = 12.2 Hz, 1H), 7.97 (dd, J = 14.4, 12.2 Hz, 1H), 7.42 (d, J = 8.7 Hz, 2H), 7.29 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 5.57 (d, J = 14.4 Hz, 1H), 5.49 (s, 1H), 5.10 (s, 2H), 4.20 (s, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.78 (d, J = 11.8 Hz, 1H), 3.72 (d, J = 11.8 Hz, 1H), 1.13 (s, 3H), 1.10 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.5, 167.1, 160.6, 136.3, 130.2, 127.7, 114.1, 114.0, 103.3, 101.8, 83.8, 78.5, 66.0, 55.5, 33.7, 21.9, 21.8, 19.3, 19.2. HRMS (ESI/QTOF) m/z: $[M–H]^-$ Calcd for $C_{25}H_{28}NO_7$: 454.1871; Found: 454.1870.

2-Cyanoethyl (*R,E*)-3-(5,5-dimethyl-2-oxo-1,3-dioxane-4-carboxamido)acrylate (5f). The synthesis of **5f** was performed according to the general procedure using amide **3c** (57 mg, 0.33 mmol) and bromoacrylate **4f** (66 mg, 0.36 mmol). The required *E*-isomer (34 mg, 35%) was obtained exclusively after purification by flash chromatography (hexane/EtOAc 3:1) as a pale oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, *J* = 11.7 Hz, 1H), 6.52 (d, *J* = 11.7 Hz, 1H), 4.31 (t, *J* = 6.2 Hz, 2H), 4.11 (s, 1H), 3.74 (d, *J* = 11.4 Hz, 1H), 3.67 (d, *J* = 11.4 Hz, 1H), 2.72 (t, *J* = 6.2 Hz, 2H), 1.19 (s, 3H), 1.10 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.2, 160.4, 128.4, 127.5, 113.9, 101.5, 84.2, 78.7, 59.1, 55.5, 33.1, 21.9, 19.3, 17.8. HRMS (ESI/QTOF): $[M+H]^+$ Calcd for $C_{13}H_{17}N_2O_6$: 297.1081; Found: 297.1080.

2-(Trimethylsilyl)ethyl (*R,E*)-3-(2,2-di-tert-butyl-5,5-dimethyl-1,3,2-dioxasilinane-4-carboxamido) acrylate (5g). The synthesis of **5g** was performed according to the general procedure using amide **3d** (206 mg, 0.72 mmol) and bromoacrylate **4d** (250 mg, 1.00 mmol). The required *E*-isomer (253 mg, 82%) was obtained exclusively after purification by flash chromatography (hexane/EtOAc 9:1) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, *J* = 12.1 Hz, 1H), 7.94 (dd, *J* = 14.1, 12.1 Hz, 1H), 5.50 (d, *J* = 14.1 Hz, 1H), 4.50 (s, 1H), 4.25–4.16 (m, 2H), 4.03 (d, *J* = 11.7 Hz, 1H), 3.51 (d, *J* = 11.7 Hz, 1H), 1.23–0.77 (m, 26H), 0.02 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 167.3, 136.1, 103.1, 81.5, 75.2, 62.5, 37.8, 28.6, 27.8, 20.4, 19.0, 17.5, -1.4. HRMS (ESI/QTOF): $[M+Na]^+$ Calcd for $C_{22}H_{43}NO_5Si_2Na$: 480.2572; Found: 480.2563.

2-Cyanoethyl (*R,E*)-3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)acrylate (5h). The synthesis of **5h** was performed according to the general procedure using amide **3a** (50 mg, 0.27 mmol) and bromoacrylate **4f** (60 mg, 0.29 mmol). The required *E*-isomer (60 mg, 71%) was obtained exclusively after purification by flash chromatography (hexane/EtOAc 2:1) as a yellow oil. ^{1}H

NMR (400 MHz, $CDCl_3$) δ 8.47 (d, J = 12.1 Hz, 1H), 8.01 (dd, J = 14.1, 12.1 Hz, 1H), 5.64 (d, J = 14.1 Hz, 1H), 4.34 (t, J = 6.3 Hz, 2H), 4.20 (s, 1H), 3.71 (d, J = 11.8 Hz, 1H), 3.31 (d, J = 11.8 Hz, 1H), 2.73 (t, J = 6.3 Hz, 2H), 1.51 (s, 3H), 1.45 (s, 3H), 1.04 (s, 3H), 1.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 166.4, 137.3, 116.8, 101.4, 99.5, 76.7, 71.2, 58.4, 33.4, 29.4, 21.8, 18.8, 18.6. HRMS (ESI/QTOF) m/z: $[M–H]^-$ Calcd for $C_{15}H_{21}N_2O_5$: 309.1450; Found: 309.1448.

Benzyl (*R,E*)-3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)acrylate (5i). The synthesis of **5i** was performed according to the general procedure using amide **3a** (530 mg, 2.83 mmol) and bromoacrylate **4g** (750 mg, 3.11 mmol). The required *E*-isomer (816 mg, 83%) was obtained exclusively after purification by flash chromatography (hexane/EtOAc 4:1) as a yellow solid. The NMR data are in agreement with the literature spectra.[13] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.40 (br d, J = 11.7 Hz, 1H), 8.01 (dd, J = 14.0, 11.7 Hz, 1H), 7.38- 7.35 (m, 4H), 7.34-7.29 (m, 1H), 5.60 (d, J = 14.5 Hz, 1H), 5.19 (s, 2H), 4.19 (s, 1H), 3.71 (d, J = 12.1 Hz, 1H), 3.32 (d, J = 11.7 Hz, 1H), 1.51 (s, 3H), 1.45 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.9, 166.9, 136.4, 136.2, 128.5, 128.1, 102.7, 99.5, 77.2, 71.2, 66.0, 33.4, 29.4, 21.8, 18.8, 18.7. HRMS (ESI/QTOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{26}NO_5$: 348.1805; Found: 348.1808.

2.4 Synthesis of CJ-15,801 (1) from precursor 5g

Small scale synthesis. TBAF (1 M in THF, 1.3 mL, 1.3 mmol) was added to **5g** (97 mg, 0.21 mmol) and the resulting mixture was stirred at ambient temperature overnight. To the reaction mixture were added $CaCO_3$ (408 mg), DOWEX 50W×8-400 (1.22 g, washed in MeOH) and MeOH (3 mL). The suspension was stirred at ambient temperature for 1 h. All insoluble materials were removed by filtration through a pad of Celite, and the filter cake was thoroughly washed with MeOH. Combined filtrates were evaporated under vacuum to give the crude product that was subsequently purified by flash column chromatography (EtOAc/MeOH/CH_3CN/H_2O 5:1:1:1). CJ-15,801 (**1**) was obtained as a yellow oil (62 mg, 93% when corrected for the presence of ~0.42 equiv. TBA as determined by ^{1}H NMR). The NMR data were in agreement with the literature spectra.[6] ^{1}H NMR (400 MHz, CD_3OD): δ 7.97 (d, J = 14.3 Hz, 1H), 5.72 (d, J = 14.3 Hz, 1H), 4.03 (s, 1H), 3.51 (d, J = 11.2 Hz, 1H), 3.41 (d, J = 11.2 Hz, 1H), 0.96 (s, 3H), 0.95 (s, 3H). HRMS (ESI/QTOF) m/z: $[M+H]^+$ Calcd for $C_9H_{16}NO_5$: 218.1023; Found: 218.1034.

Large scale synthesis. For the large scale synthesis the precursors **3d** and **4d** were prepared on a gram scale according to the methods outlined above for the respective small scale syntheses in comparable or improved yields (~50% for **3d** and 89% for **4d** respectively). The gram-scale Pd-catalyzed coupling was performed according to the outlined method for **5g** using amide **3d** (2.27 g, 7.89 mmol) and bromoacrylate **4d** (2.18 g, 8.68 mmol). **(*E*)-5g** (3.10 g, 86%) was obtained after purification by flash chromatography (hexane/EtOAc 9:1) as a yellow oil. Deprotection was

performed as described for the small scale synthesis, by combining **5g** (0.88 g, 1.9 mmol) and TBAF (1M in THF, 4.8 mL, 4.8 mmol). $CaCO_3$ (3.7 g), DOWEX 50W×8-400 (11.1 g, washed in MeOH) and MeOH (28 mL) were added. The crude product obtained in this manner was subsequently subjected to a further five rounds of the same CaF_2 precipitation/cation-exchange purification procedure. CJ-15,801 (**1**) was obtained as a yellow oil (400 mg, 70% when corrected for the presence of ~0.23 equiv. TBA as determined by ^{1}H NMR).

2.5 Synthesis of CJ-15,801 (1) from precursor 5h

Small scale synthesis. $BiCl_3$ (51 mg, 161 μmol) was added to **5h** (250 mg, 806 μmol) in CH_3CN (7 mL) and H_2O (290 μL) and the resulting mixture was stirred at ambient temperature overnight. The filtrate was evaporated under vacuum to give a crude product that was subsequently purified by flash column chromatography (DCM, then 95:5 DCM:MeOH) to give **6a** as a yellow oil (179 mg, 82%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.24 (d, *J* = 11.9 Hz, 1H), 8.03 (dd, *J* = 14.1, 11.9 Hz, 1H), 5.66 (d, *J* = 14.1 Hz, 1H), 4.78 (d, *J* = 4.5 Hz, 1H), 4.35 (t, *J* = 6.2 Hz, 2H), 4.16 (d, *J* = 4.5 Hz, 1H), 3.52 (s, 1H), 2.74 (t, *J* = 6.2 Hz, 2H), 0.98 (s, 3H), 0.95 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.1, 167.1, 138.1, 117.3, 101.5, 77.7, 71.1, 58.6, 39.6, 21.0, 20.4, 18.3. HRMS (ESI/QTOF) m/z: $[M+H]^+$ Calcd for $C_{12}H_{19}N_2O_5$: 271.1294; Found: 271.1286.

K_2CO_3 in MeOH (10% w/v; 200 mg in 2 mL) was added to **6a** (17 mg, 63 μmol) and the mixture was stirred at ambient temperature for 1 h. DOWEX 50W×8-40 was added to the reaction mixture until acidified to pH 5.5. The resin was washed with MeOH (20 mL) and the filtrate concentrated *in vacuo*. CJ-15,801 **1** was obtained after flash column chromatography (EtOAc/MeOH/CH_3CN/H_2O 5:1:1:1) as a light yellow oil (11 mg, 81%). The NMR data were in agreement with the literature spectra.[6]

Large Scale Synthesis. For the large scale synthesis the precursors **3d** and **4f** were prepared on a gram scale according to the methods outlined above for the respective small scale syntheses in comparable or improved yields (~50% for **3d** and 80% for **4f** respectively). The gram-scale Pd-catalyzed coupling was performed according to the general method using amide **3a** (2.88 g, 15.4 mmol) and bromoacrylate **4f** (3.73 g, 16.9 mmol). **(*E*)-5h** (3.35 g, 70%) was obtained as a yellow oil after purification by flash chromatography (Hexane/EtOAc 2:1). Deprotection was accomplished in two-steps by first combining **(*E*)-5h** (1.46 g, 4.70 mmol) in CH_3CN (30 mL) with $BiCl_3$ (148 mg, 0.47 mmol) and H_2O (1 mL) and stirring the resulting mixture at ambient temperature overnight. The filtrate was evaporated under vacuum to give the crude product that was subsequently purified by flash column chromatography (Hexane/EtOAc 1:4) to give **6a** as a yellow oil (966 mg, 76%). In the second deprotection step, K_2CO_3 in MeOH (10% w/v; 9 g in 90 mL) was added to **6a** (966 mg, 3.58 mmol) and the mixture was stirred at ambient temperature for

1 h. DOWEX 50W×8-40 (previously washed with deionised water) was added to the reaction mixture until the pH reached 5.5. The resin was washed with MeOH (100 mL) and the filtrate concentrated *in vacuo*. CJ-15,801 (**1**) was obtained after flash column chromatography (EtOAc/MeOH/CH_3CN/H_2O 5:1:1:1) as a light yellow oil (544 mg, 70%). The NMR data were in agreement with the literature spectra.[6]

2.6 Synthesis of protected precursors of 4'-phospho-CJ-15,801 (7)

2-Cyanoethyl (*R,E*)-3-(4-((bis(2-cyanoethoxy)phosphoryl)oxy)-2-hydroxy-3,3-dimethylbutanamido) acrylate (7a). To a solution of **6a** (90 mg, 0.333 mmol) and bis(2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite (130 µL, 0.499) in a mixture of dry CH_2Cl_2 (2 mL) and dry CH_3CN (2 mL) was added 1H-tetrazole solution (0.45 M in decane, 1.41 mL, 0.633 mmol). The reaction is kept at ambient temperature for 1 h, and then cooled to 0 °C. *tert*-Butylhydroperoxide (5-6 M solution in decane, 364 µL, 2.00 mmol) is added, and the stirring continued for 1 h while maintaining the temperature at 0 °C. The reaction mixture is diluted with EtOAc (15 mL) and the mixture is subsequently washed with water (3×3 mL). After drying (Na_2SO_4), filtration and removal of the solvent in vacuo, the product was purified by flash column chromatography (Hexane/EtOAc 4:1) to give **7a** as colourless oil (74.1 mg, 49%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.12 (d, *J* = 11.7 Hz, 1H), 8.03 (dd, *J* = 14.1, 11.7 Hz, 1H), 5.64 (d, *J* = 14.1 Hz, 1H), 4.36-4.32 (m, 6H), 4.20 (dd, *J* = 10.0, 7.0 Hz, 1H), 4.15 (s, 1H), 3.82 (dd, *J* = 10.0, 7.0 Hz, 1H), 2.82 (t, *J* = 5.9 Hz, 1H), 2.74 (t, *J* = 6.5 Hz, 1H), 1.16 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.7, 166.8, 137.9, 117.1, 116.6, 116.6, 101.5, 74.3 (d, $^2J_{C,P}$ = 6.1 Hz), 73.6, 62.9 (d, $^2J_{C,P}$ = 5.2 Hz), 62.8 (t, $^2J_{C,P}$ = 5.2 Hz), 58.6, 40.2, 40.1, 21.1, 20.0, 18.7, 18.3. ^{31}P (121 MHz, $CDCl_3$) δ -0.82. HRMS (ESI/QTOF): m/z $[M+H]^+$ calcd for $C_{18}H_{26}N_4O_8P$: 457.1483; found: 457.1492.

Benzyl (*R,E*)-3-(4-((bis(benzyloxy)phosphoryl)oxy)-2-hydroxy-3,3-dimethylbutanamido)acrylate (7b). $BiCl_3$ (148 mg, 0.470 mmol) and H_2O (846 µL) was added to **5i** (816 mg, 2.35 mmol) in CH_3CN (20 mL) and the resulting mixture was stirred at ambient temperature overnight. The reaction mixture was filtered through Celite and the filtrate was evaporated under vacuum. The resulting crude residue was re-dissolved in EtOAc (50 mL) and the organic layer was washed with sat. aqueous $NaHCO_3$ (2×20 mL). The aqueous layer was extracted with EtOAc (1×20 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo* before purification by flash column chromatography (Hexane/EtOAc 1:1 to 100% EtOAc) gave **6b** as a light yellow oil (614 mg, 85%). The analytical data were in agreement with those published previously.[13] 1H NMR (400 MHz, CDCl3) δ 9.12 (d, *J* = 11.9 Hz, 1H), 8.01 (dd, *J* = 14.2, 11.9 Hz, 1H), 7.35-7.30 (m, 5H), 5.65 (d, *J* = 14.2 Hz, 1H), 5.16 (s, 2H), 4.14 (s, 1H), 3.53 (d, *J* = 11.4 Hz, 1H), 3.49 (d, *J* = 11.4 Hz, 1H), 0.98 (s, 1H), 0.94 (s, 1H). Dibenzyl chlorophosphate was prepared

in situ by reacting *N*-chloro-succinimide (260 mg, 1.95 mmol) and dibenzylphosphite (511 mg, 1.95 mmol) in anhydrous toluene (4 mL) under an inert atmosphere for 2 h at rt. The reaction mixture was filtered to remove the succinimide. The dibenzyl chlorophosphate-containing solution was added dropwise with stirring to a solution of **6b** (200 mg, 0.651 mmol) in anhydrous pyridine (5 mL) at -40 °C under an inert atmosphere. The reaction mixture was stirred for an additional 2 h at -40 °C and the mixture was placed in the -20 °C freezer overnight. The reaction mixture was allowed to warm to rt and was subsequently quenched with H_2O (3 mL) and concentrated *in vacuo*. The resulting crude residue was re-dissolved in EtOAc (30 mL) and the organic layer was washed with 1% citric acid (2×10 mL), 1 M aqueous $NaHCO_3$ (2×10 mL) and sat. aqueous Na_2SO_4 (1×10 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo* before purification by flash column chromatography (Hexane/EtOAc 1:2) gave **7b** as a yellow oil (81.1 mg, 22%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.14 (d, *J* = 11.9 Hz, 1H), 8.02 (dd, *J* = 14.2, 11.9 Hz, 1H), 7.40-7.31 (m, 15H), 5.62 (d, *J* = 14.2 Hz, 1H), 5.18 (s, 2H), 5.08-4.97 (m, 4H), 4.06 (t, *J* = 10.0 Hz, 1H), 3.96 (s, 1H), 3.36 (t, *J* = 10.0 Hz, 1H), 1.08 (s, 3H), 0.75 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 167.3, 137.1, 136.4, 135.4, 135.4, 135.3, 135.2, 129.1, 129.0, 128.9, 128.7, 128.3, 128.2, 128.2, 128.2, 102.4, 73.6 (d, $^2J_{C,P}$ = 5.7 Hz), 73.3, 70.3 (d, $^2J_{C,P}$ = 5.9 Hz), 70.2 (d, $^2J_{C,P}$ = 6.0 Hz), 66.1, 40.2 (d, $^3J_{C,P}$ = 4.1 Hz), 21.1, 18.1. ^{31}P (162 MHz, $CDCl_3$) δ 1.98. HRMS (ESI/QTOF): m/z $[M+H]^+$ calcd for $C_{30}H_{35}NO_8P$: 568.2095; found: 568.2102.

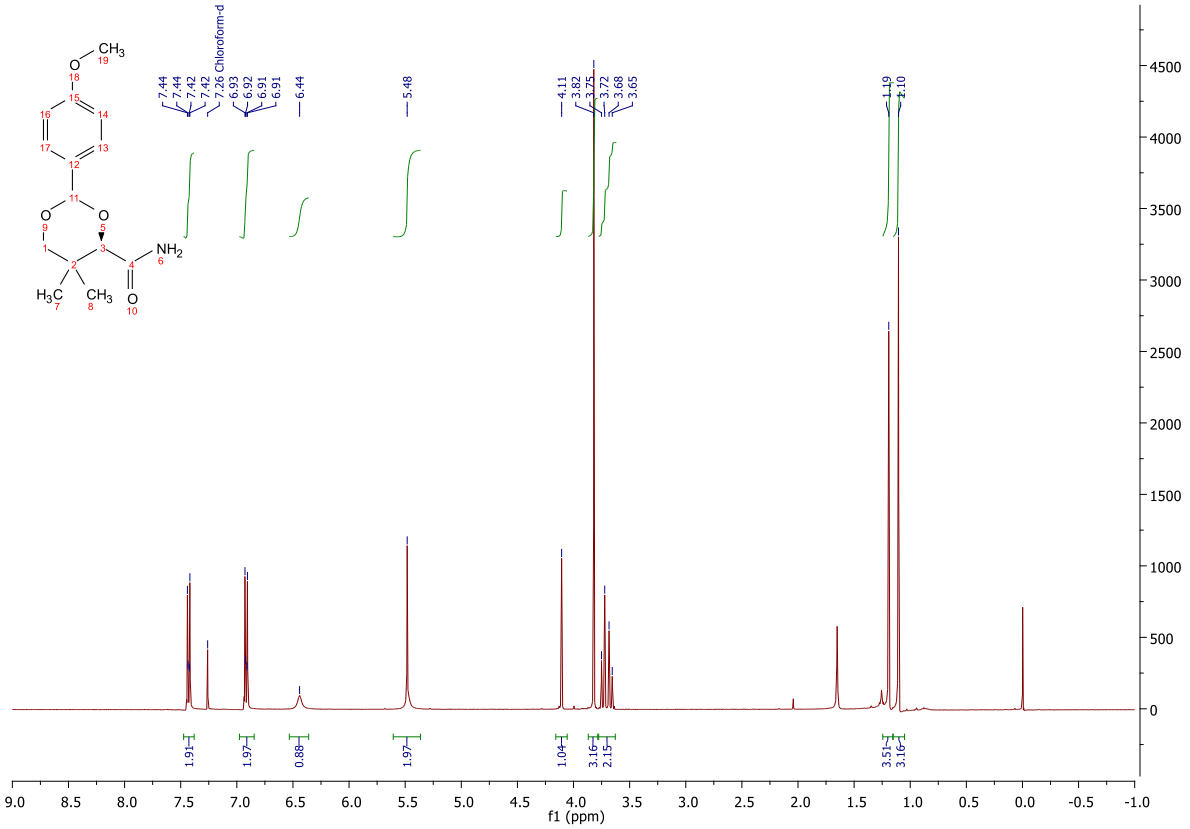
2.7 Purity determination of 4′-phospho-CJ-15,801 (8) by HPLC and UV-Vis absorbance analysis

Purified fractions from the chemoenzymatic synthesis of 4′-phospho-CJ-15,801 (**8**) was analysed by HPLC analysis on an Agilent 1100 instrument using a Luna® 5 µm C18 100 Å column (250 x 4.6 mm). The column was equilibrated with 95% 50 mM CH_3COONH_4 (pH 5.5) and 5% acetonitrile. Following injection of the sample (15 µL), the elution proceeded at a flow rate of 1 mL/min with 95% 50 mM CH_3COONH_4 (isocratic; 0–5 min), a linear gradient increase to 20% acetonitrile (5–6 min), a linear gradient increase to 40% acetonitrile (6–10 min), and isocratic elution at 40% acetonitrile (10–15 min). The elution was monitored at 254 nm.

The combined purified fractions gave 64.5 mg of a white solid upon lyophilization to constant weight. Using a pure sample of 4′-phospho-CJ-15,801 (**8**) prepared previously,[14] a standard curve was prepared by measuring the absorbance of a concentration series from 0.125–4 mM. The absorbance of the dissolved solid was compared to the standard curve, indicating a concentration equivalent to 34.4 mg pure 4′-phospho-CJ-15,801 (**8**) (60% purity, 70% yield).

3. NMR Spectra of Pantoamides (3)

Figure S1. ^{1}H and ^{13}C NMR Spectra of 3b



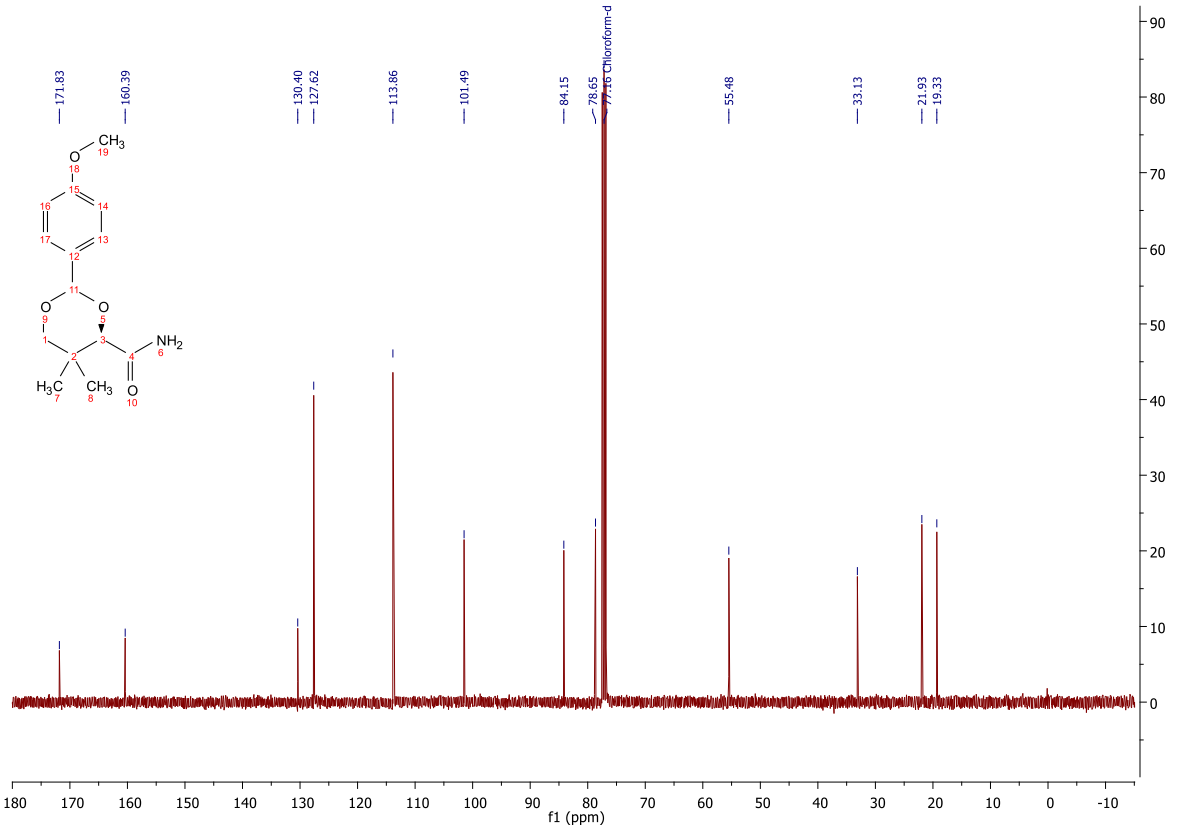
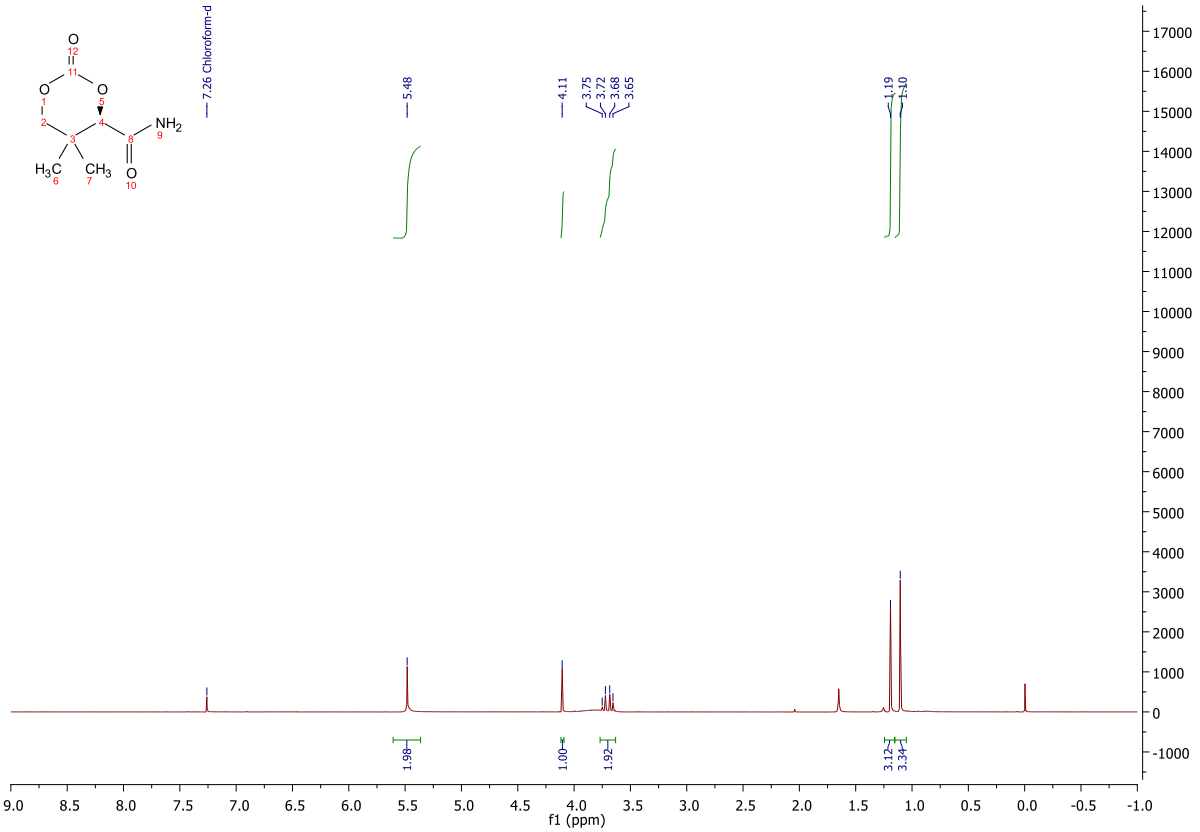


Figure S2. ^{1}H and ^{13}C NMR Spectra of 3c.



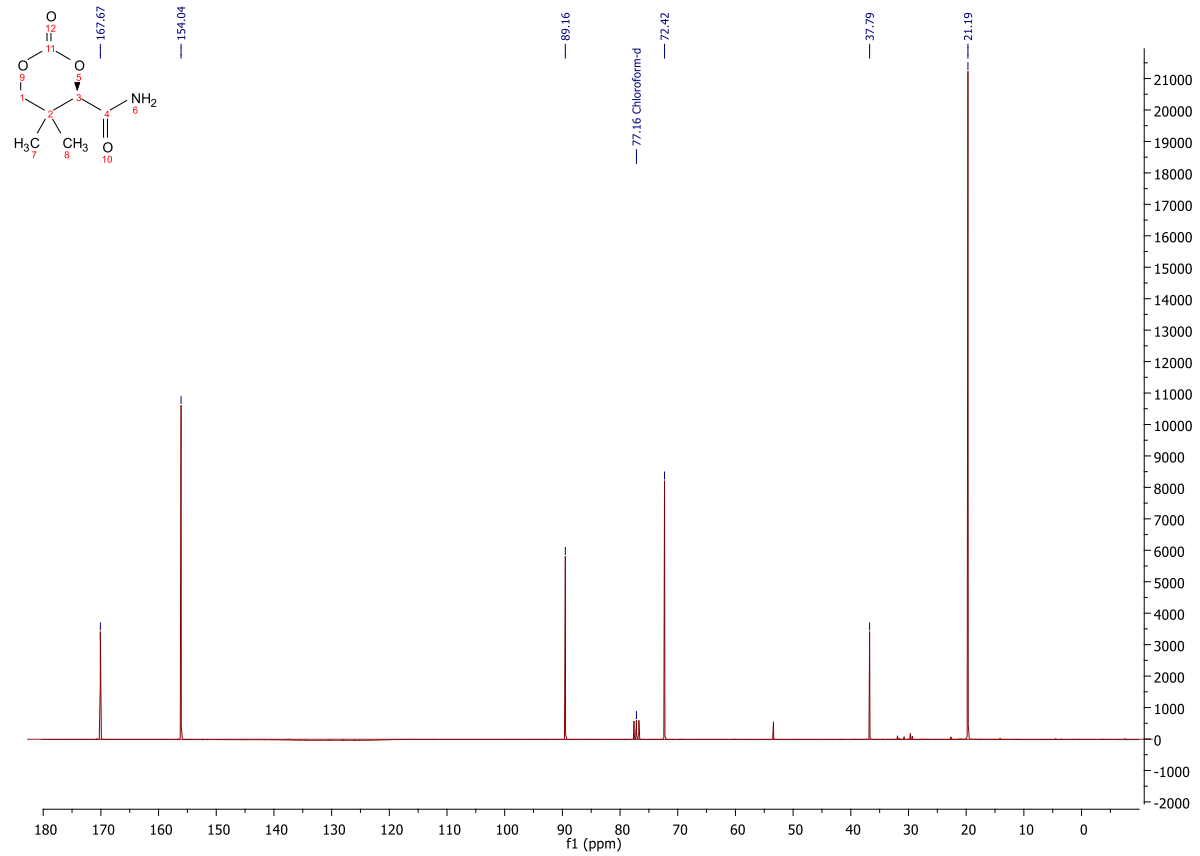
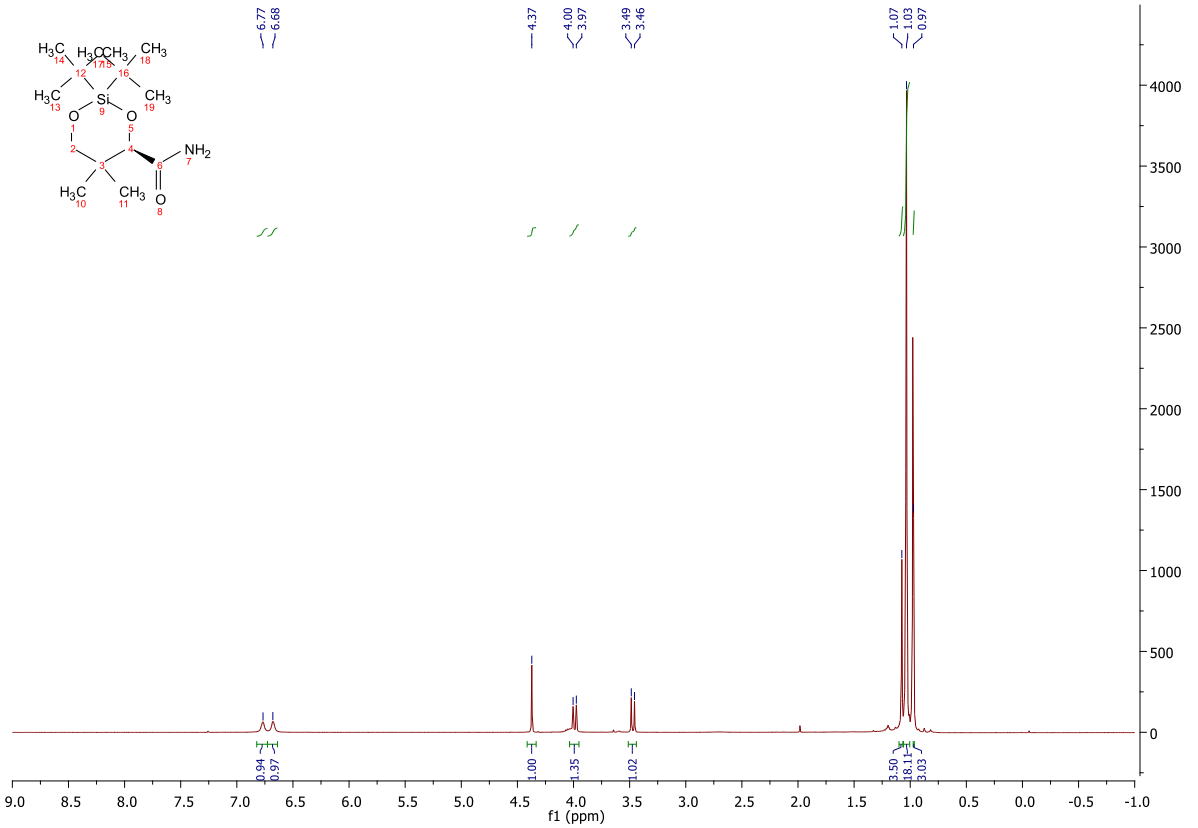


Figure S3. ^{1}H and ^{13}C NMR Spectra of 3d.



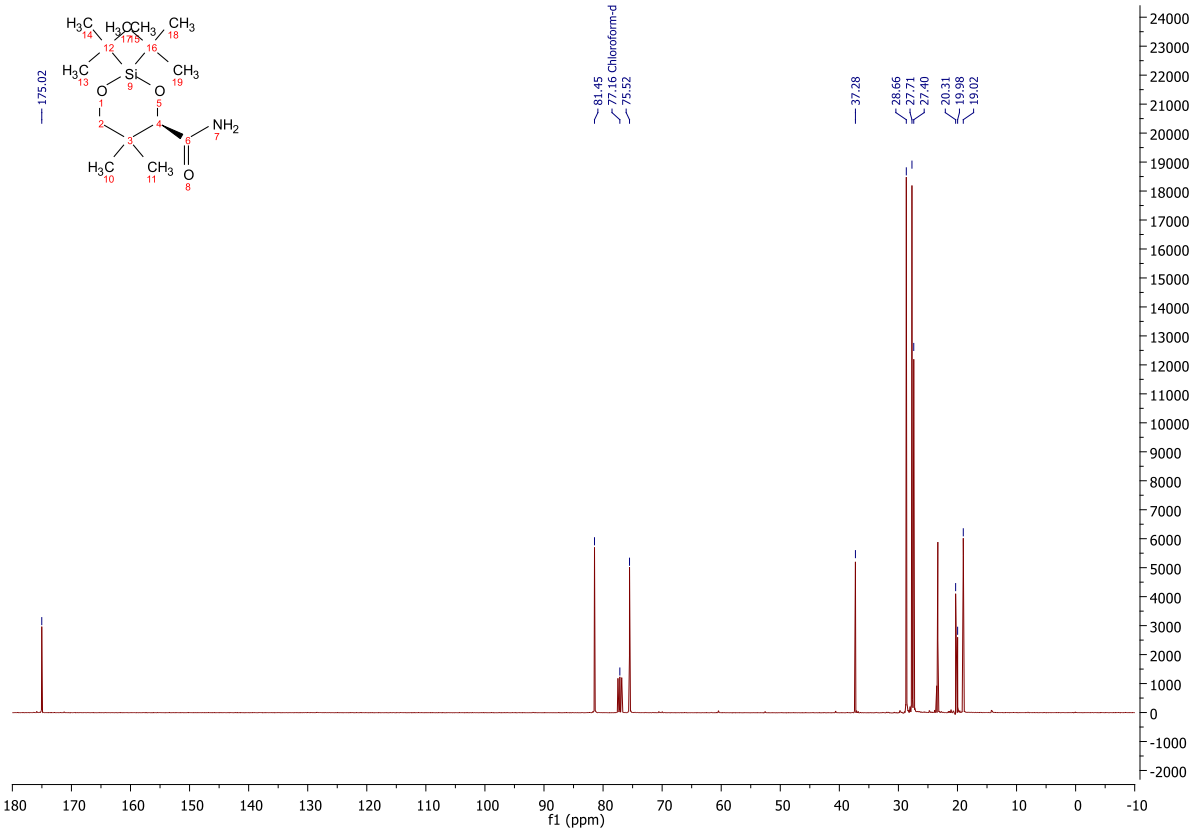
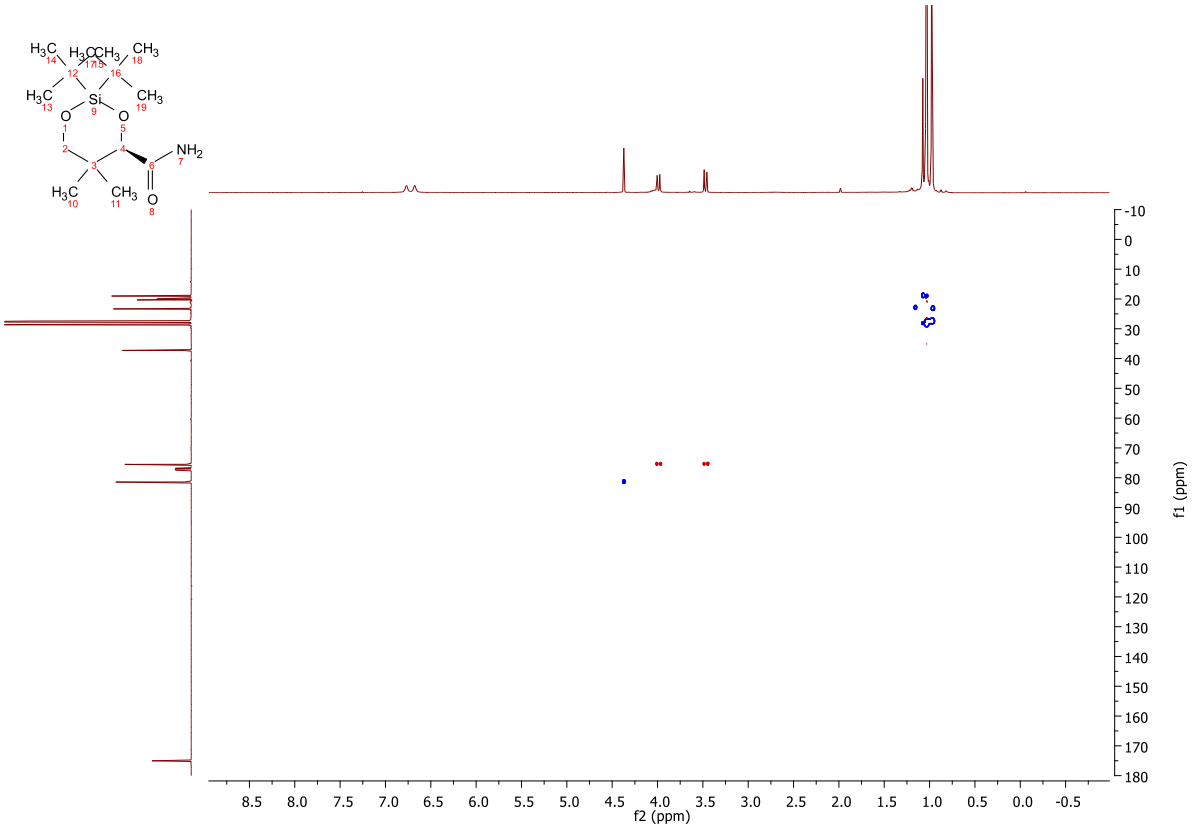
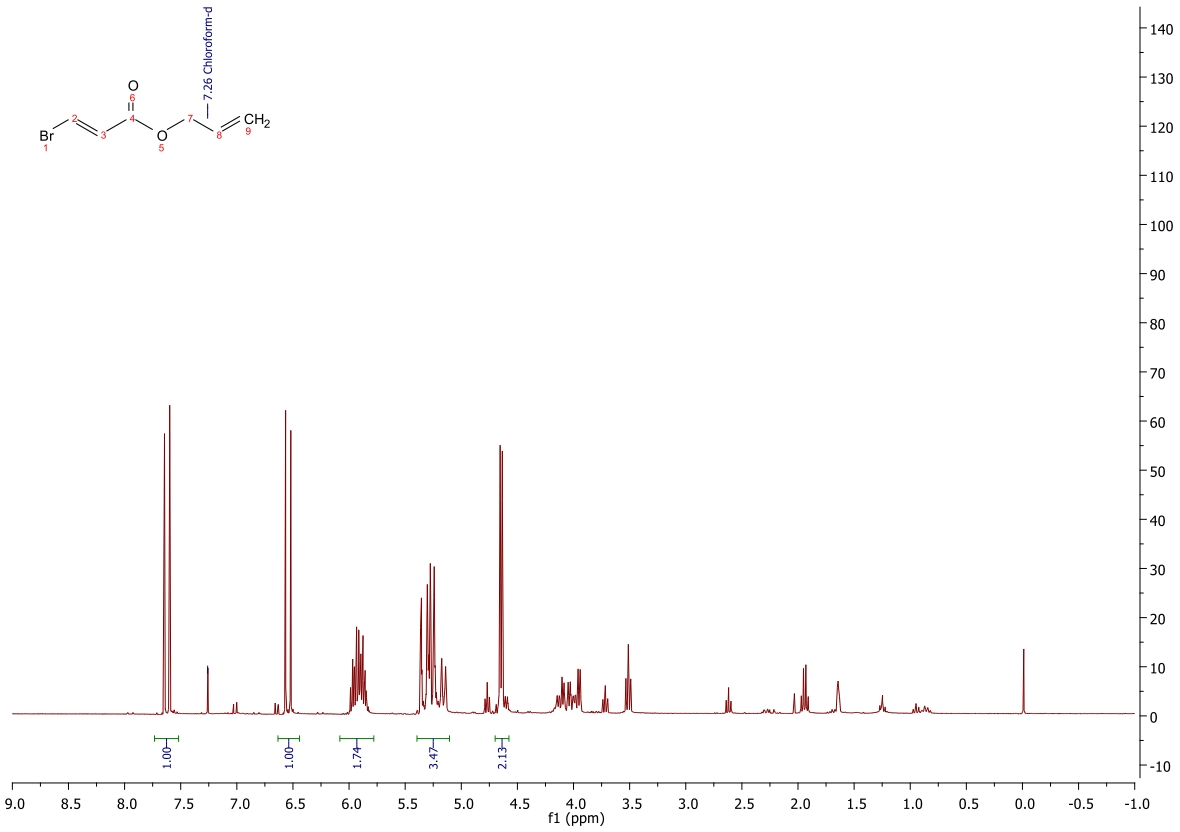


Figure S4. HSQC Spectrum of 3d.



4. NMR Spectra of 3-Bromoacrylates (4)

Figure S5. ^{1}H and ^{13}C NMR Spectra of 4a.



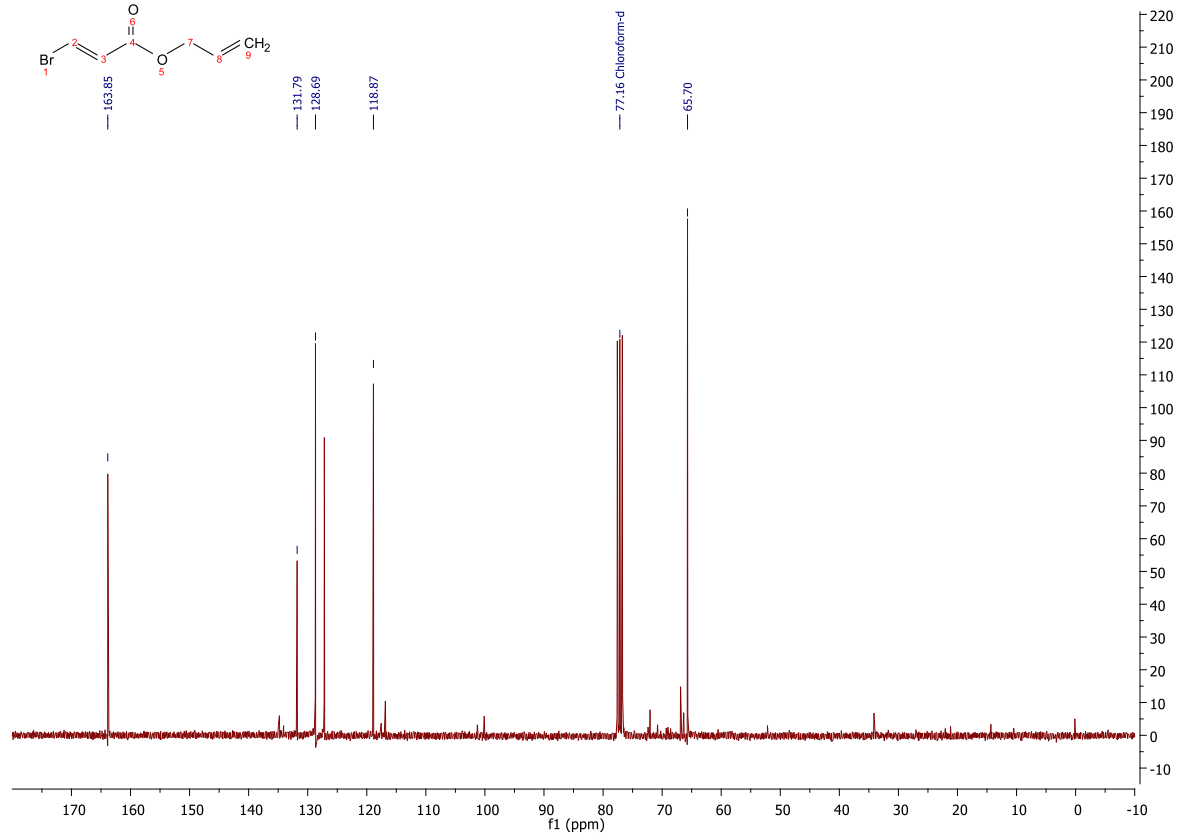
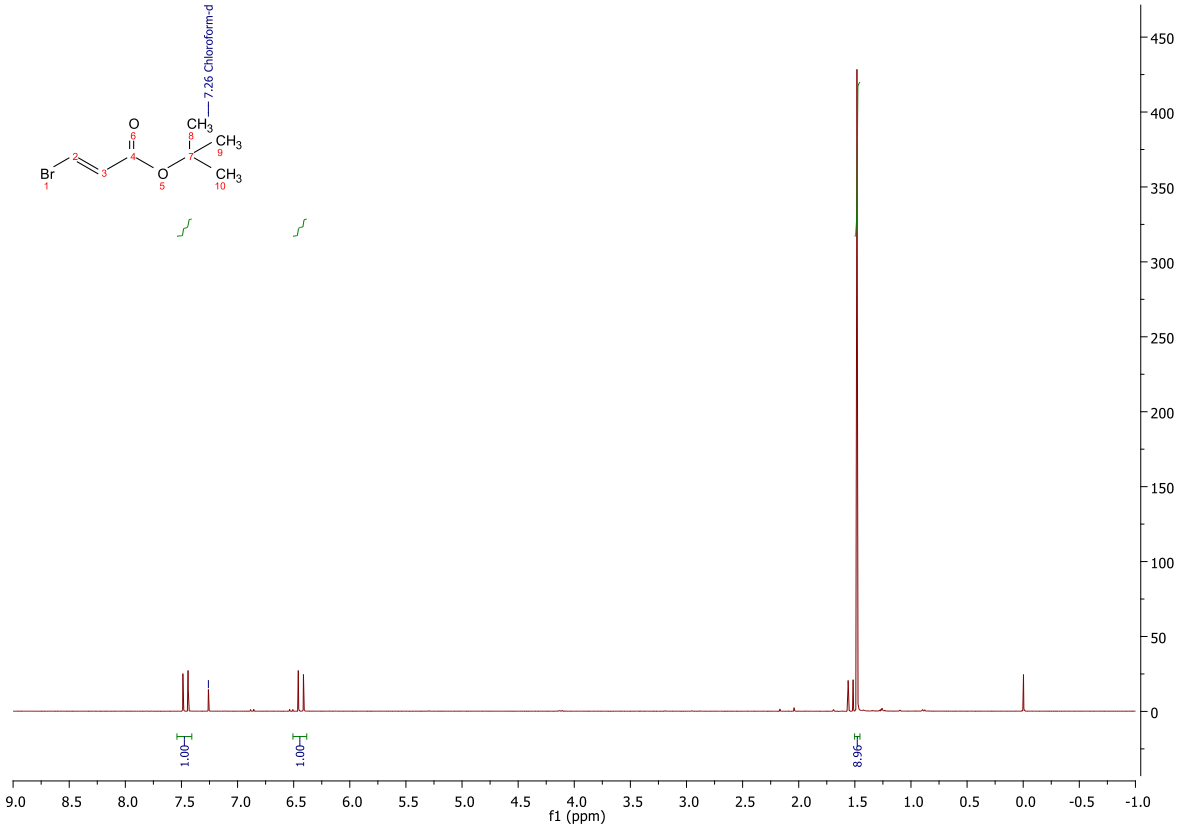


Figure S6. ^{1}H and ^{13}C NMR Spectra of 4c.



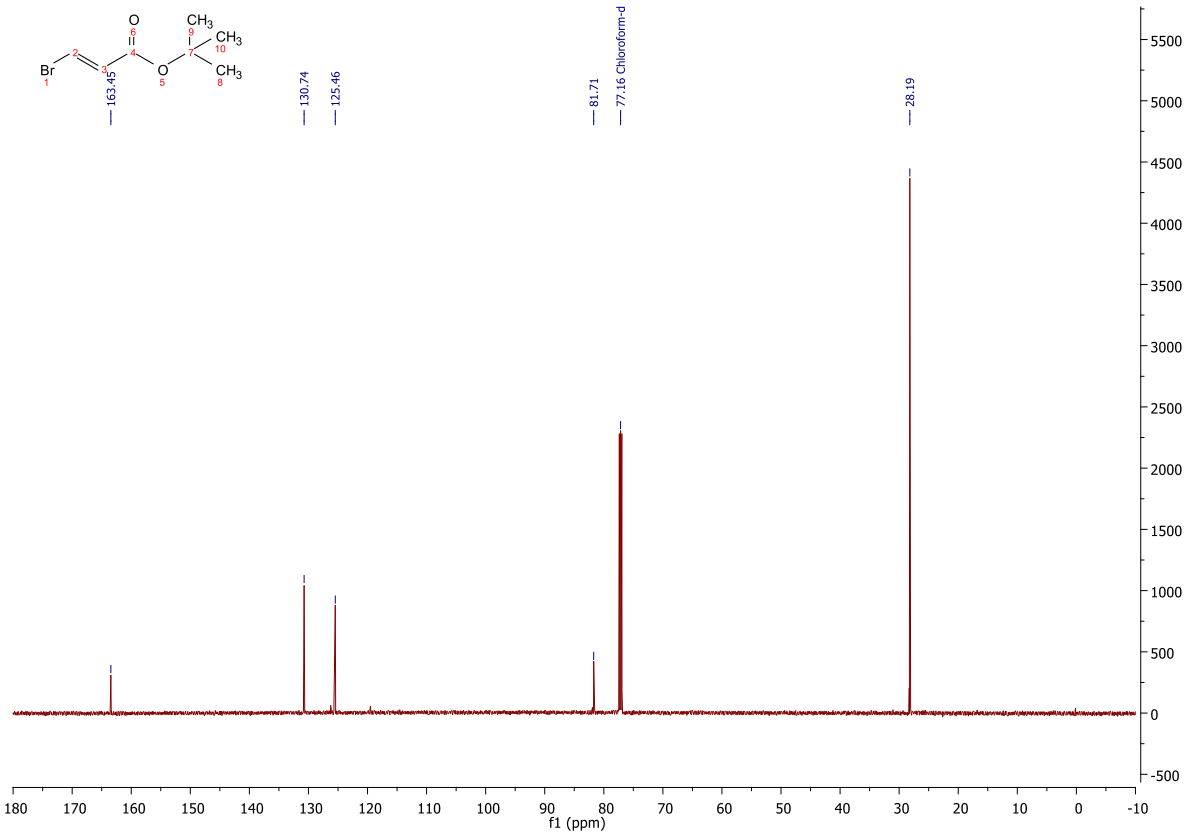
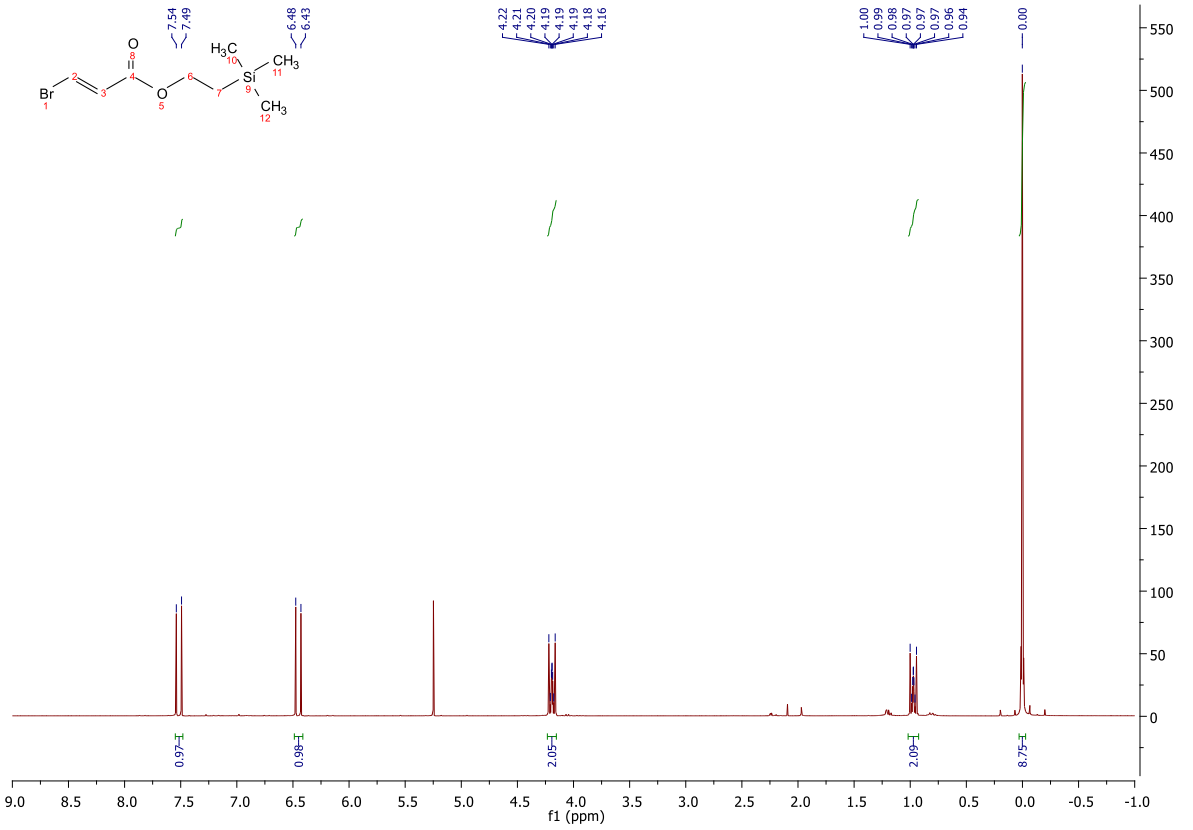


Figure S7. ^{1}H and ^{13}C NMR Spectra of 4d.



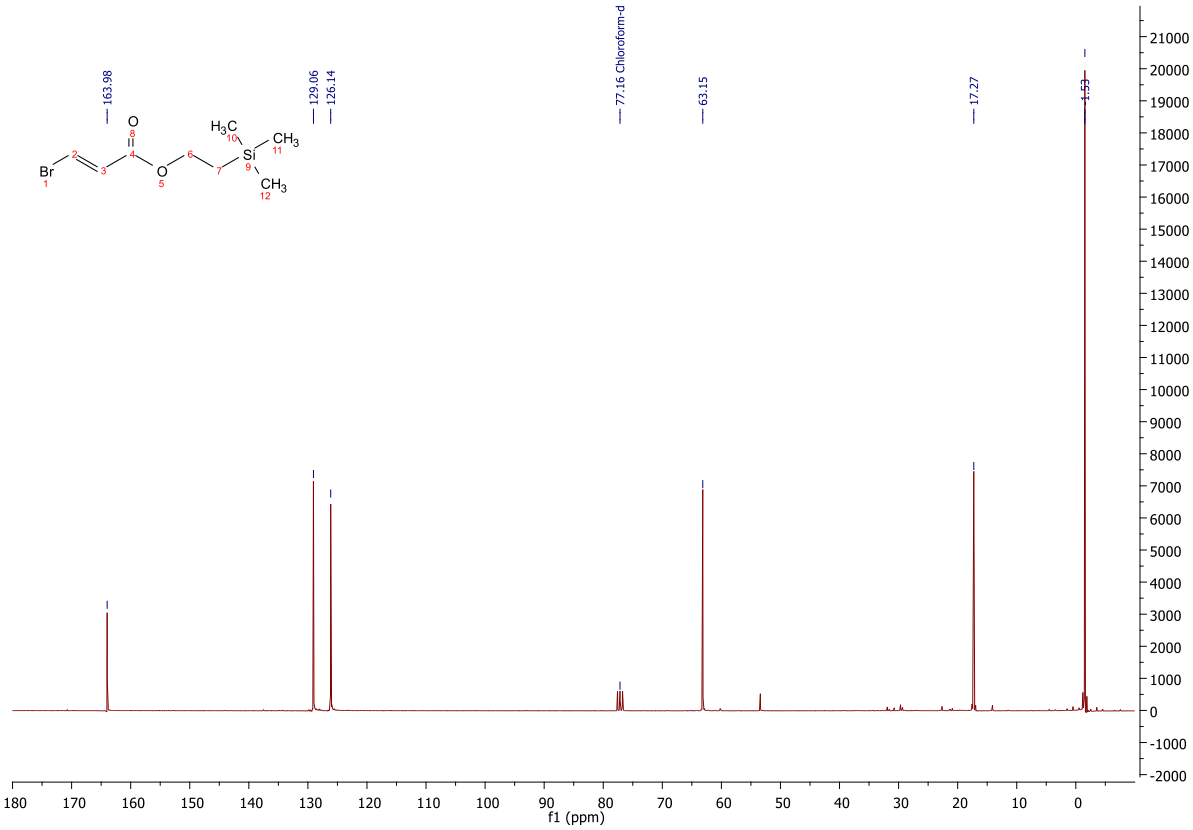


Figure S8. ^{1}H NMR spectrum of 4e.

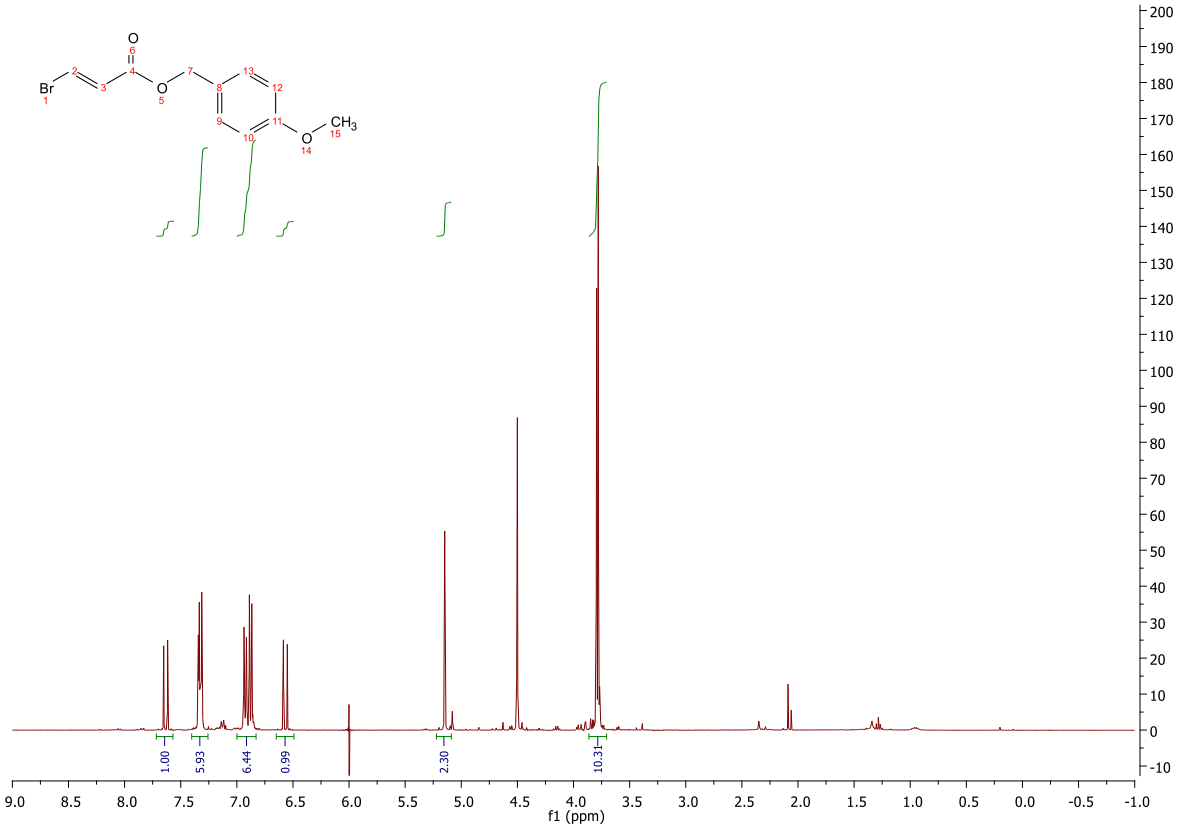
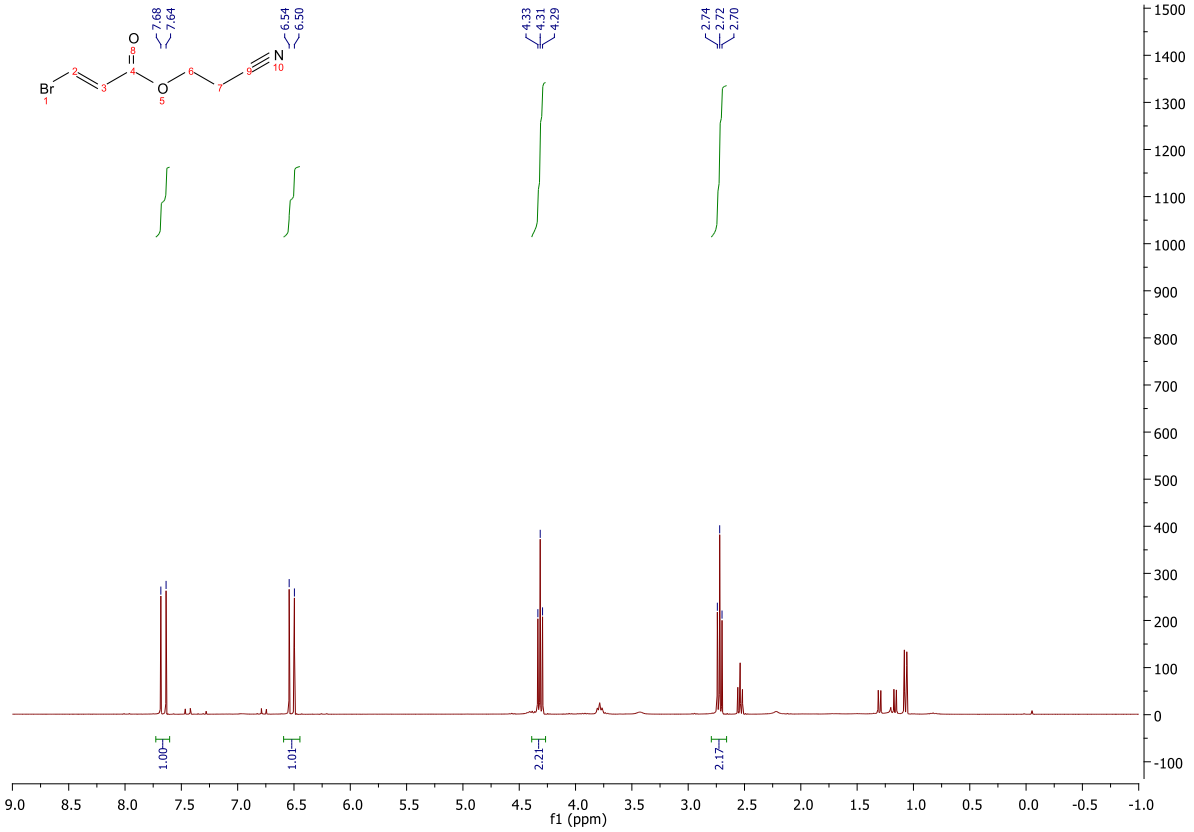
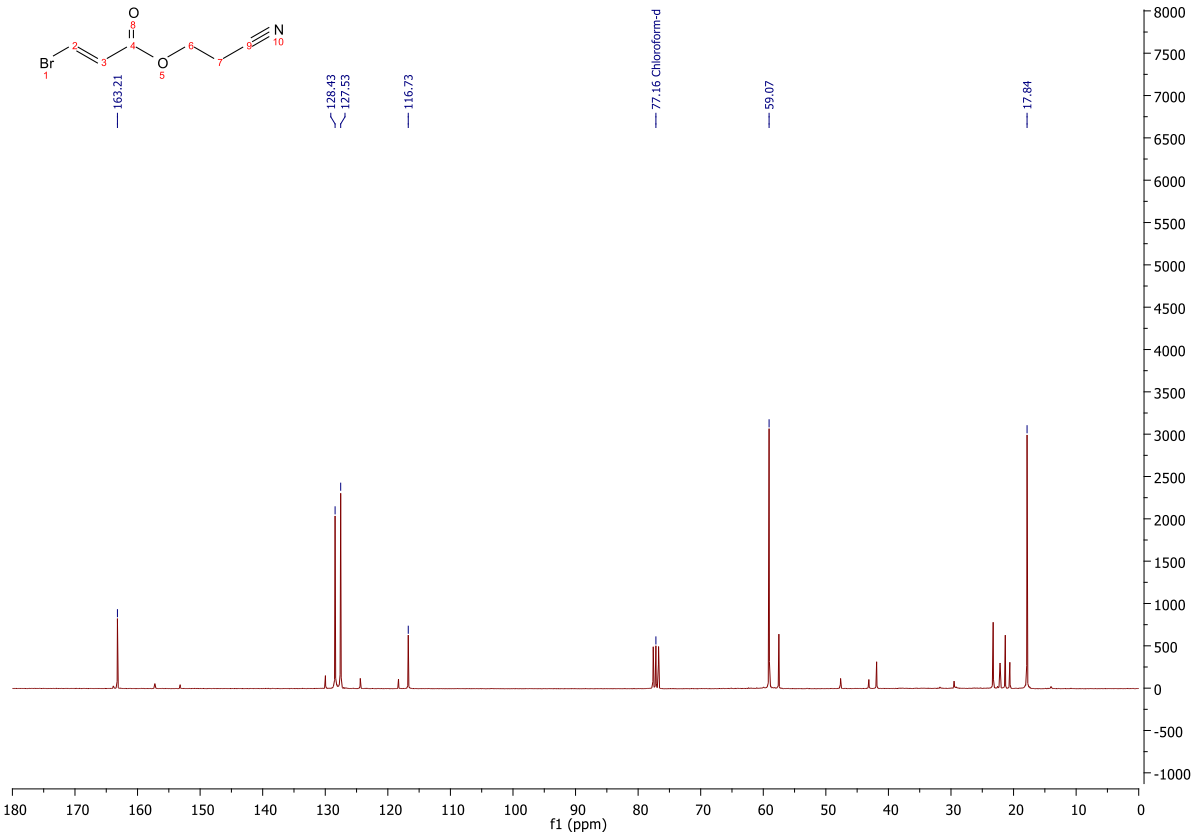


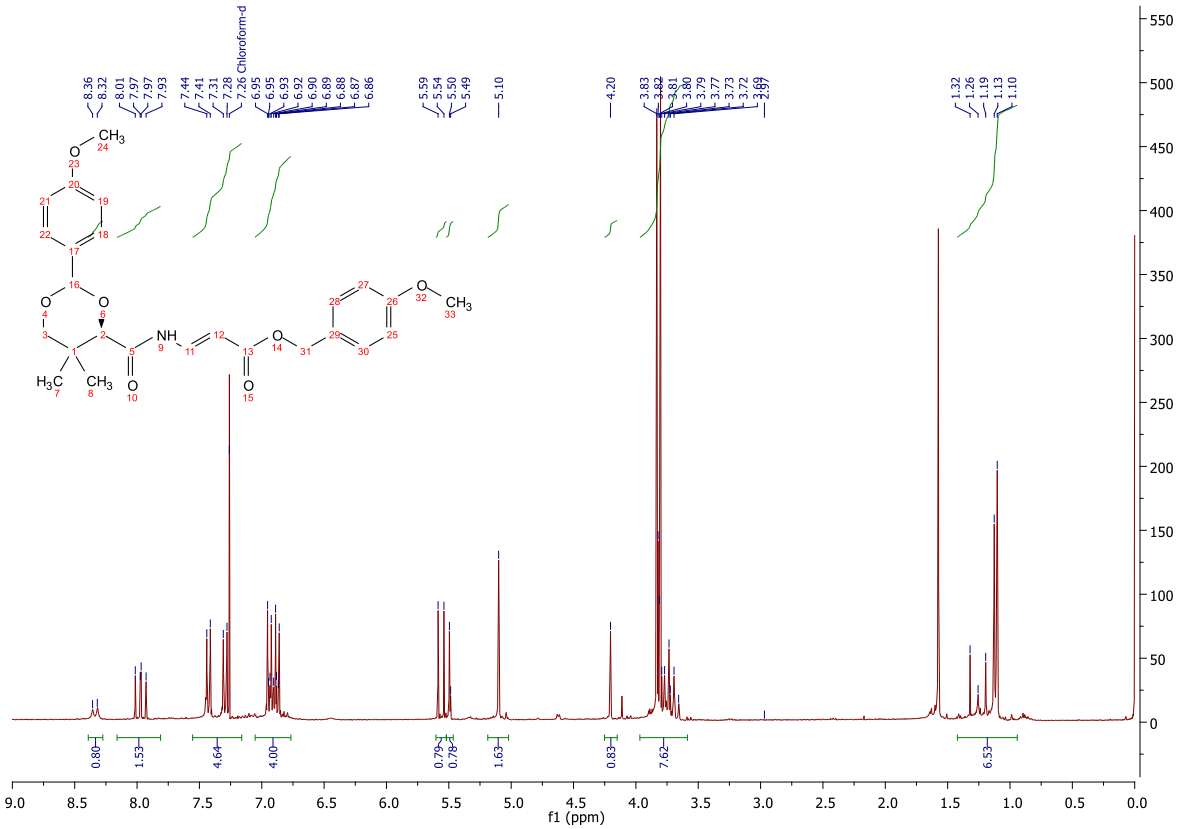
Figure S9. ^{1}H and ^{13}C NMR Spectra of 4f.





5. NMR Spectra of Protected Precursors (5)

Figure S10. ^{1}H and ^{13}C NMR Spectra of 5e.



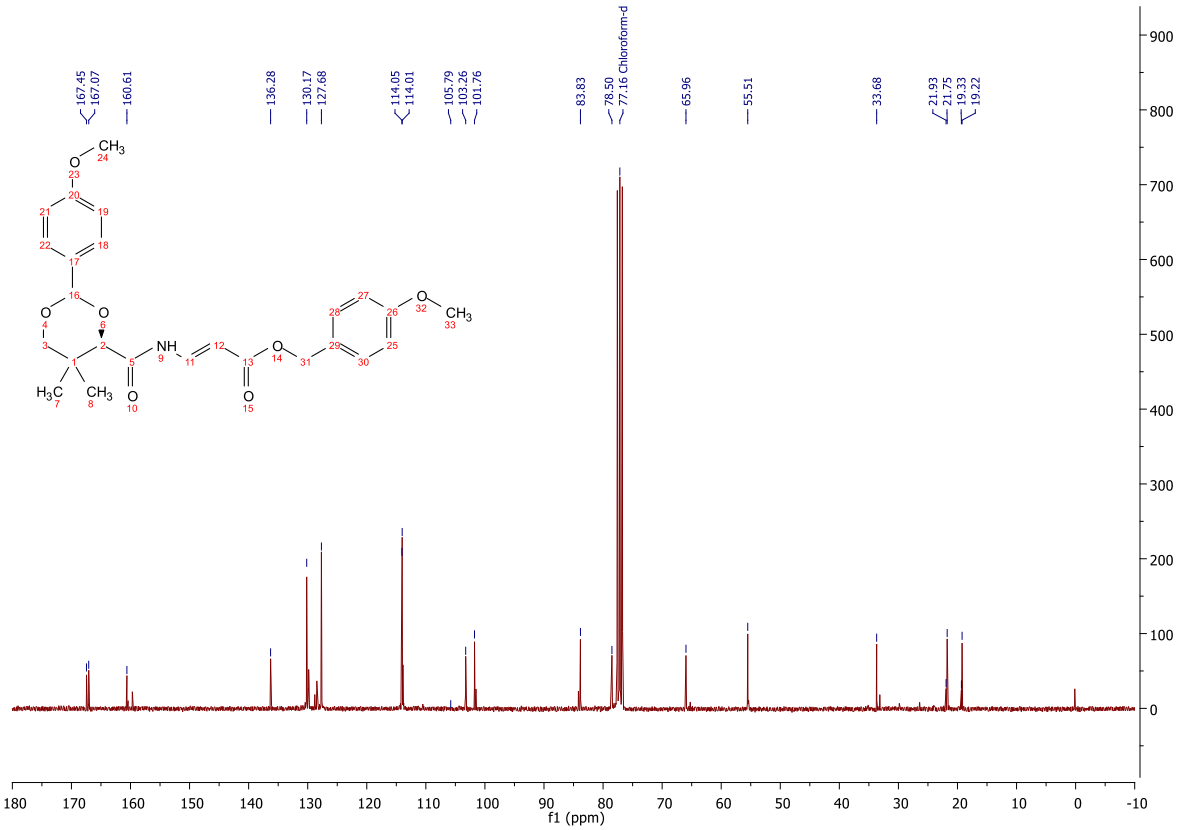
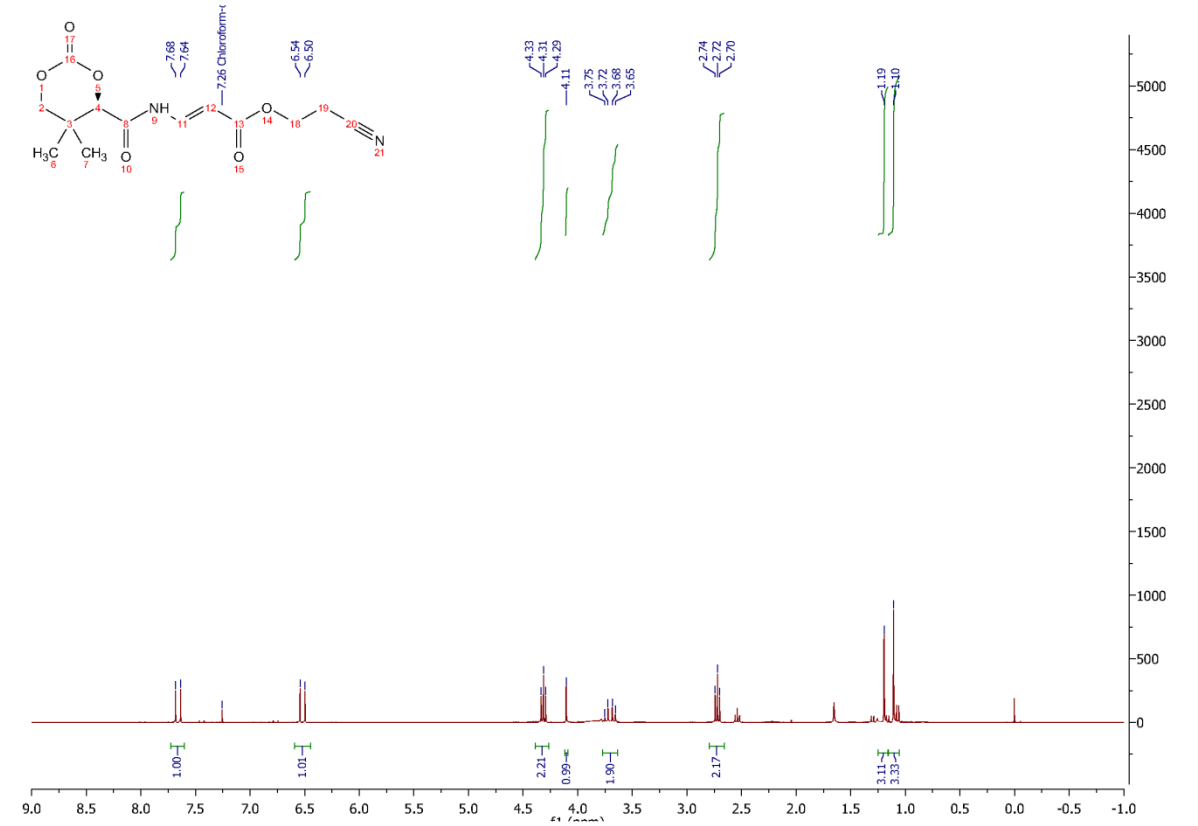


Figure S11. ^{1}H and ^{13}C NMR Spectra of 5f.



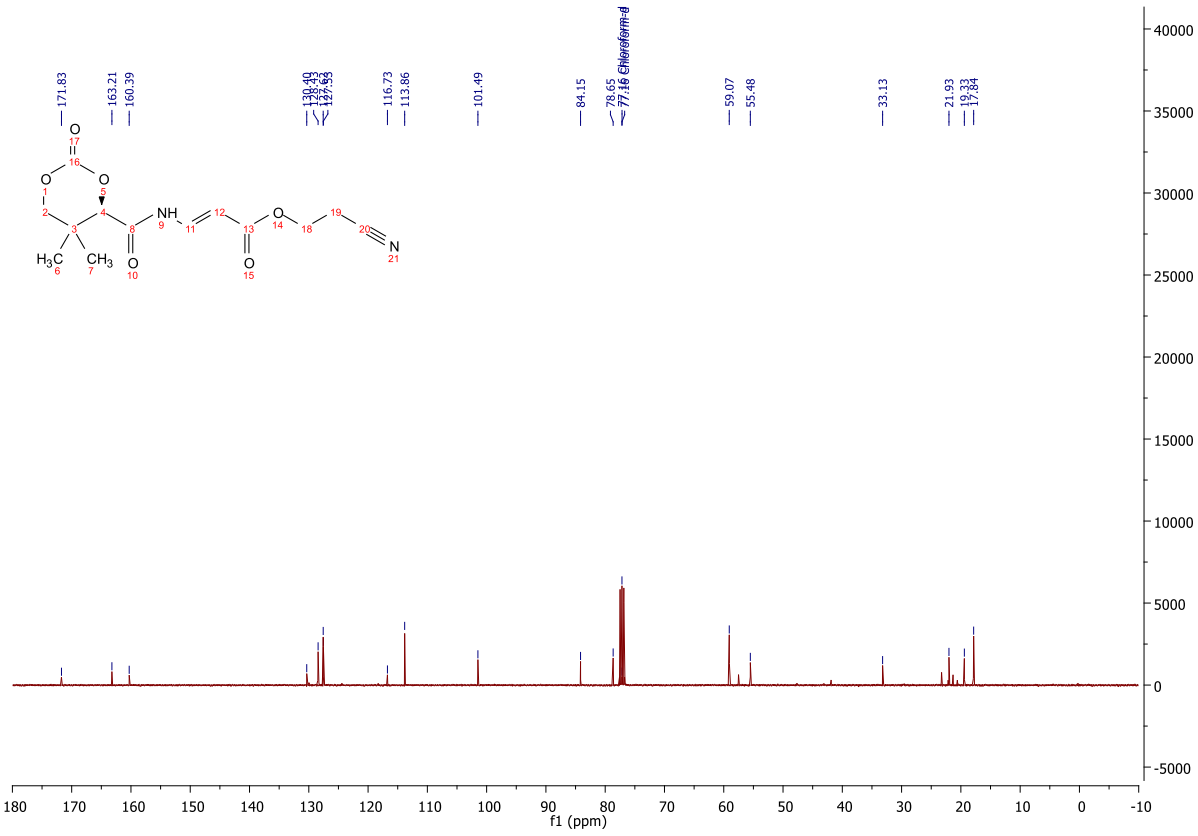
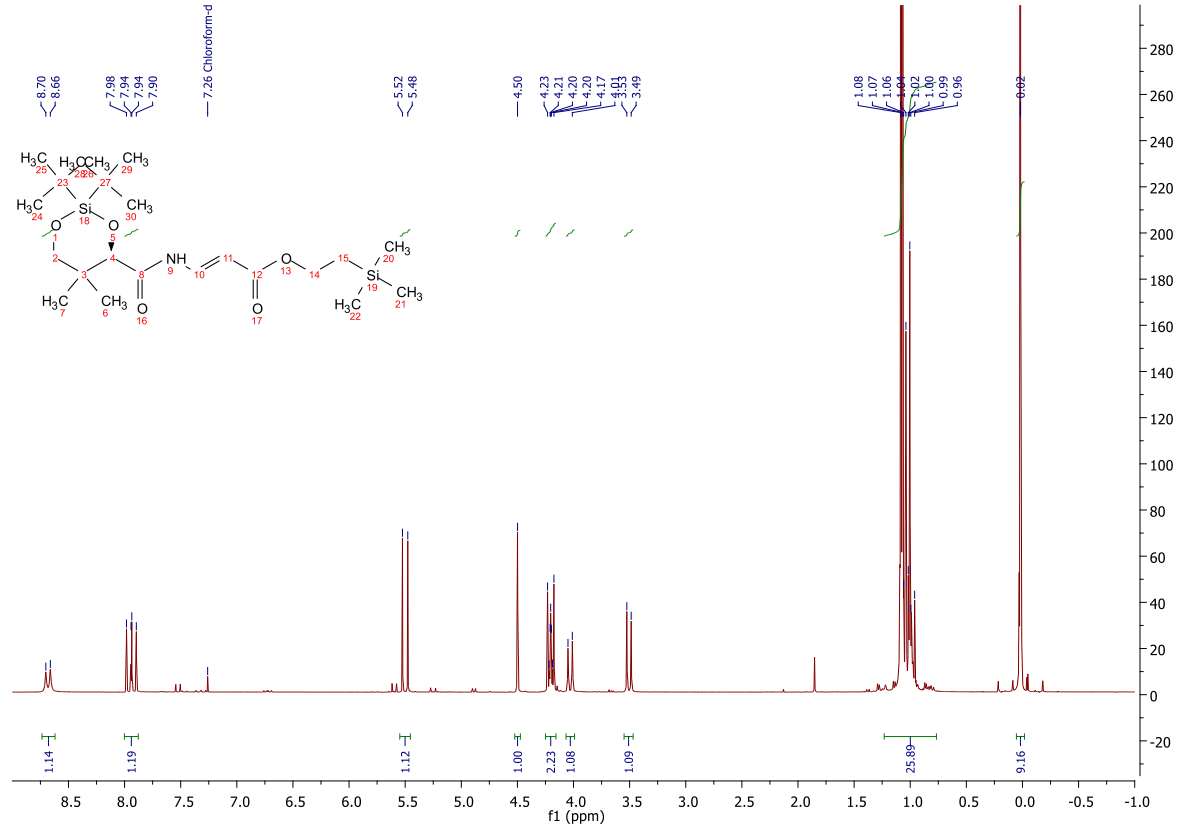


Figure S12. ^{1}H and ^{13}C NMR Spectra of 5g.



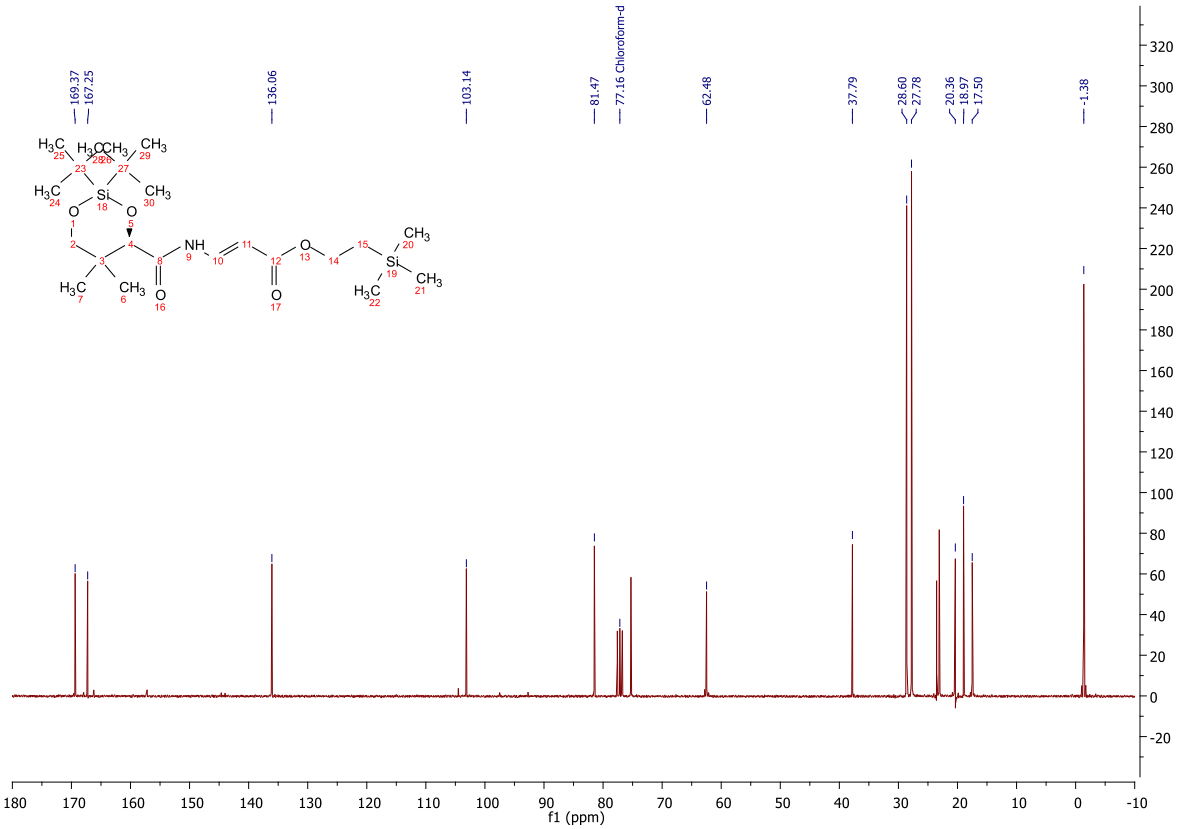


Figure S13. HSQC Spectrum of 5g.

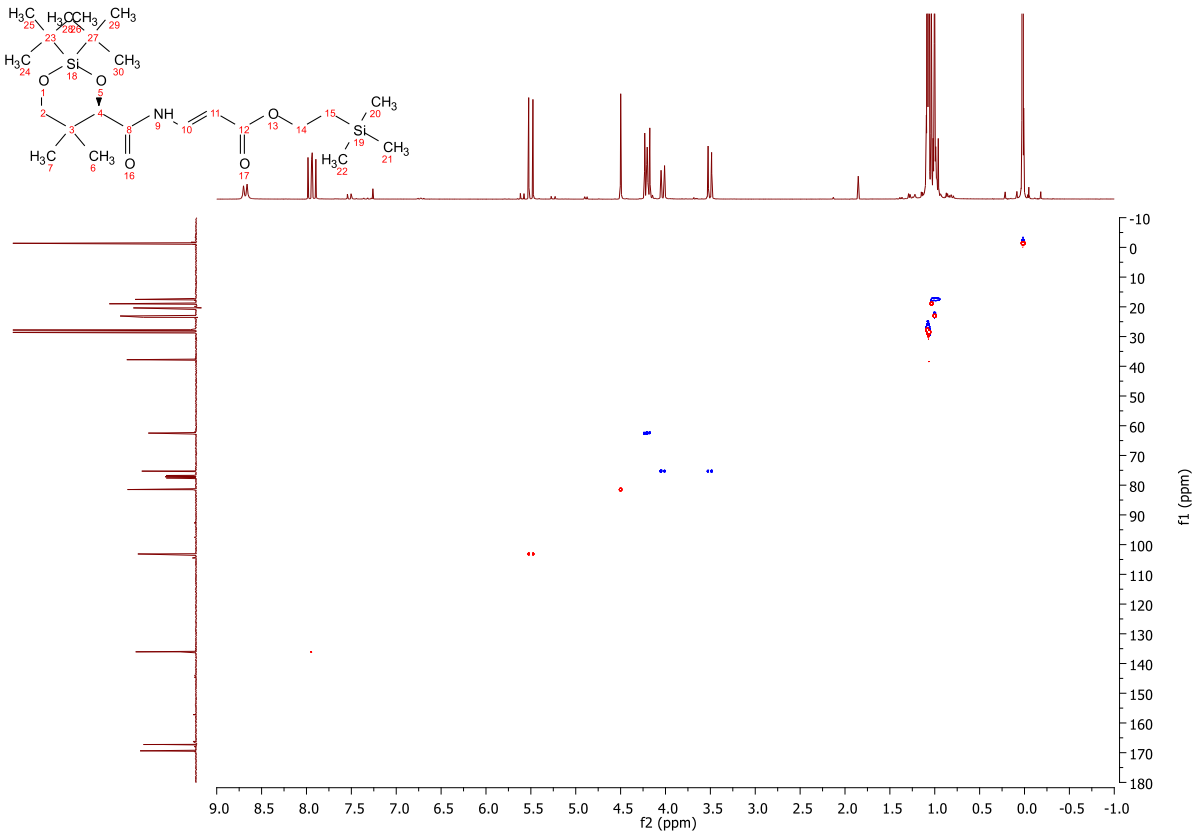
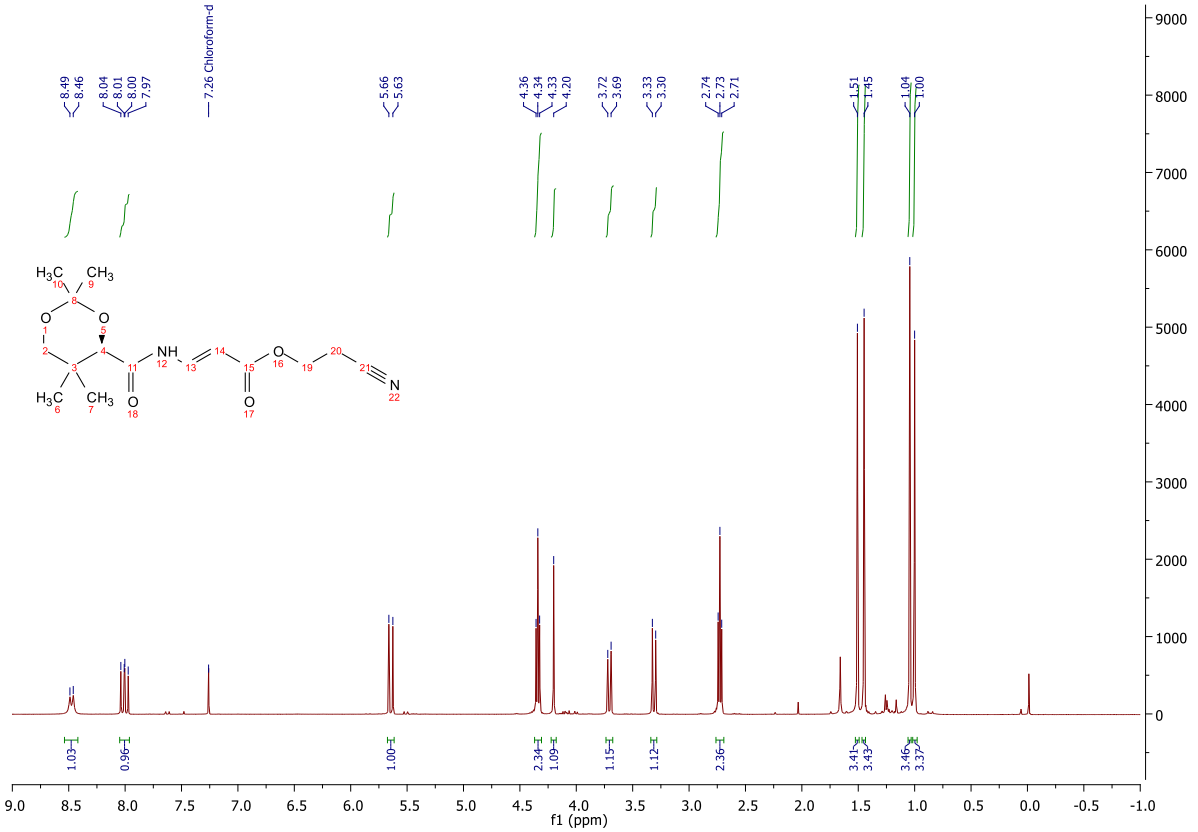
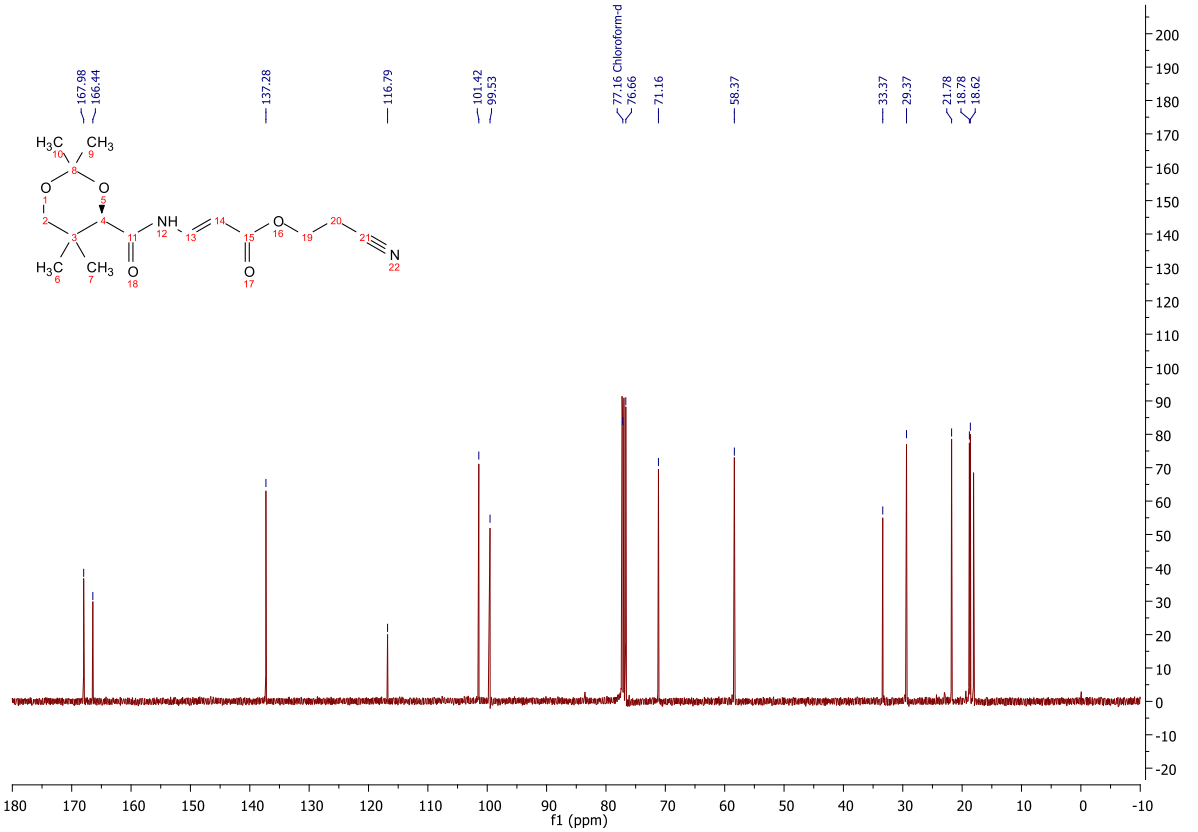


Figure S14. ^{1}H and ^{13}C NMR Spectra of 5h.





6. NMR Spectra of Other Synthesized Compounds

Figure S15. ^{1}H NMR Spectrum of CJ-15,801 (1).

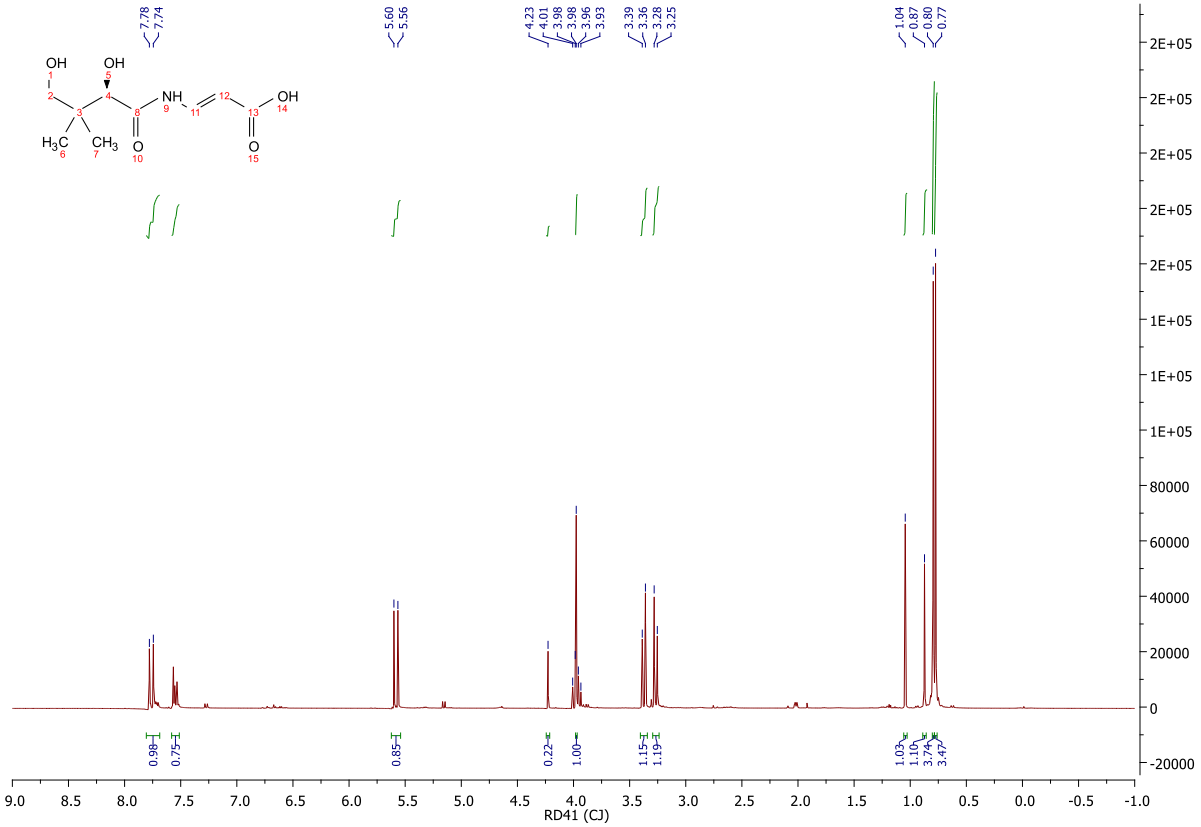
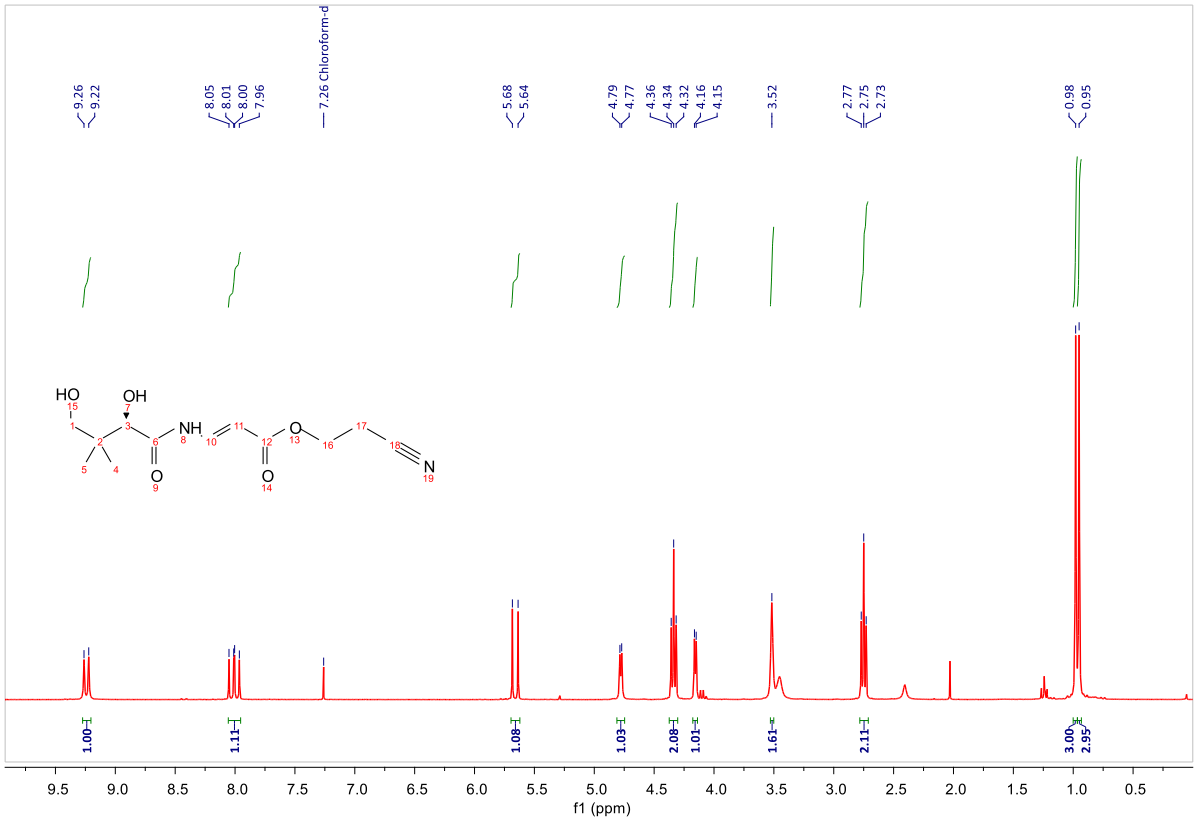


Figure S16. ^{1}H and ^{13}C NMR Spectra of 6a.



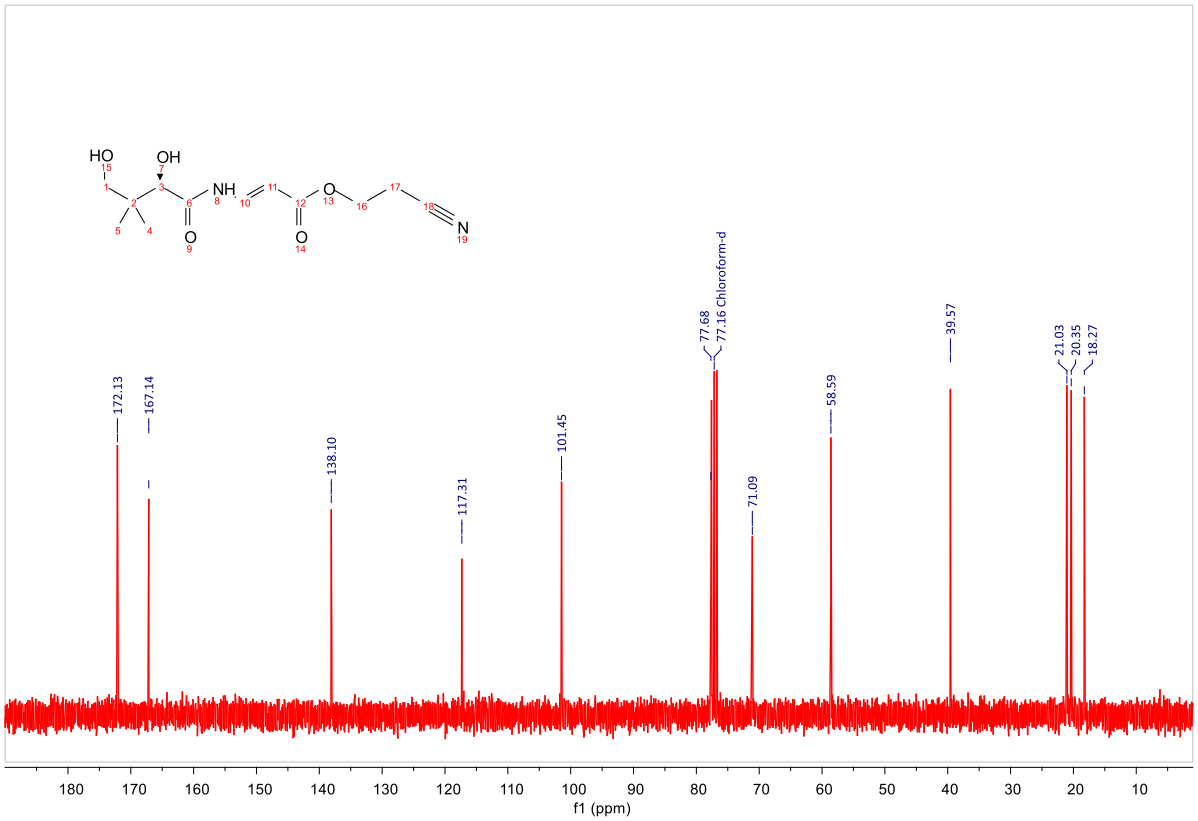
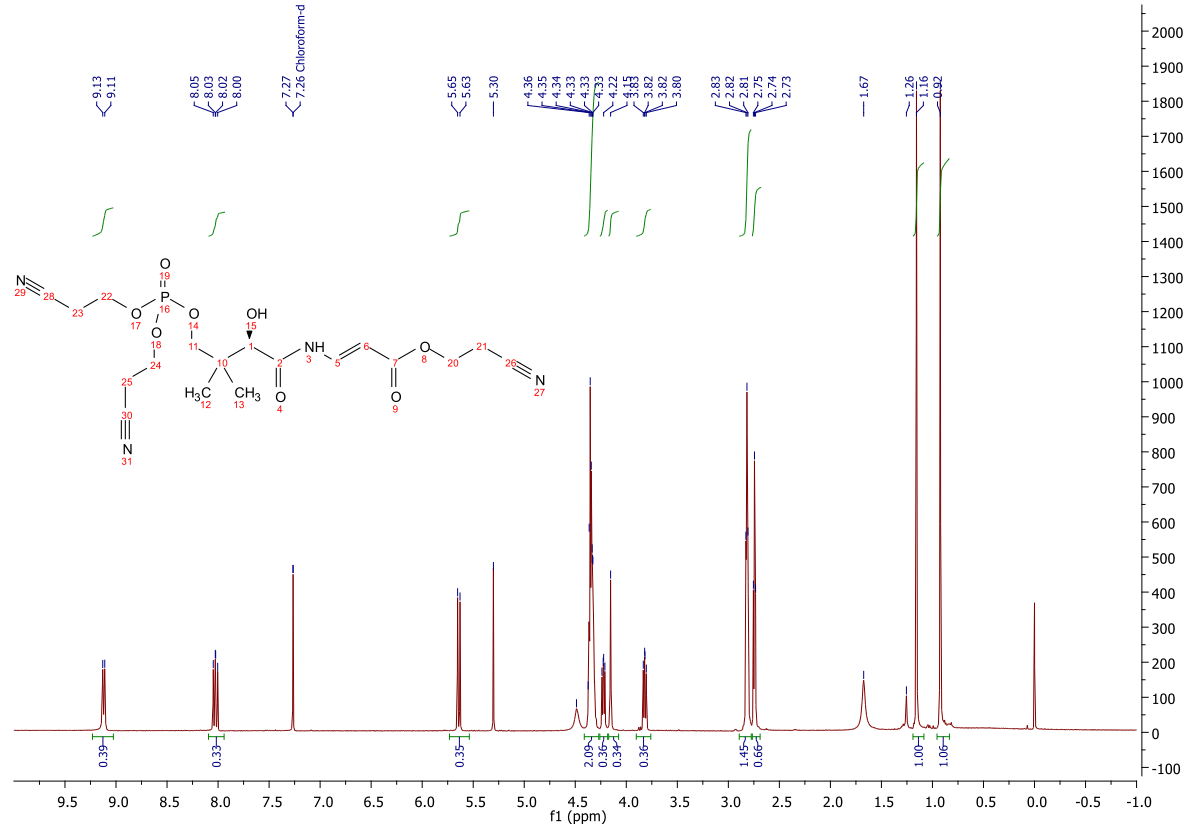


Figure S17. ^{1}H and ^{13}C NMR Spectra of 7a.



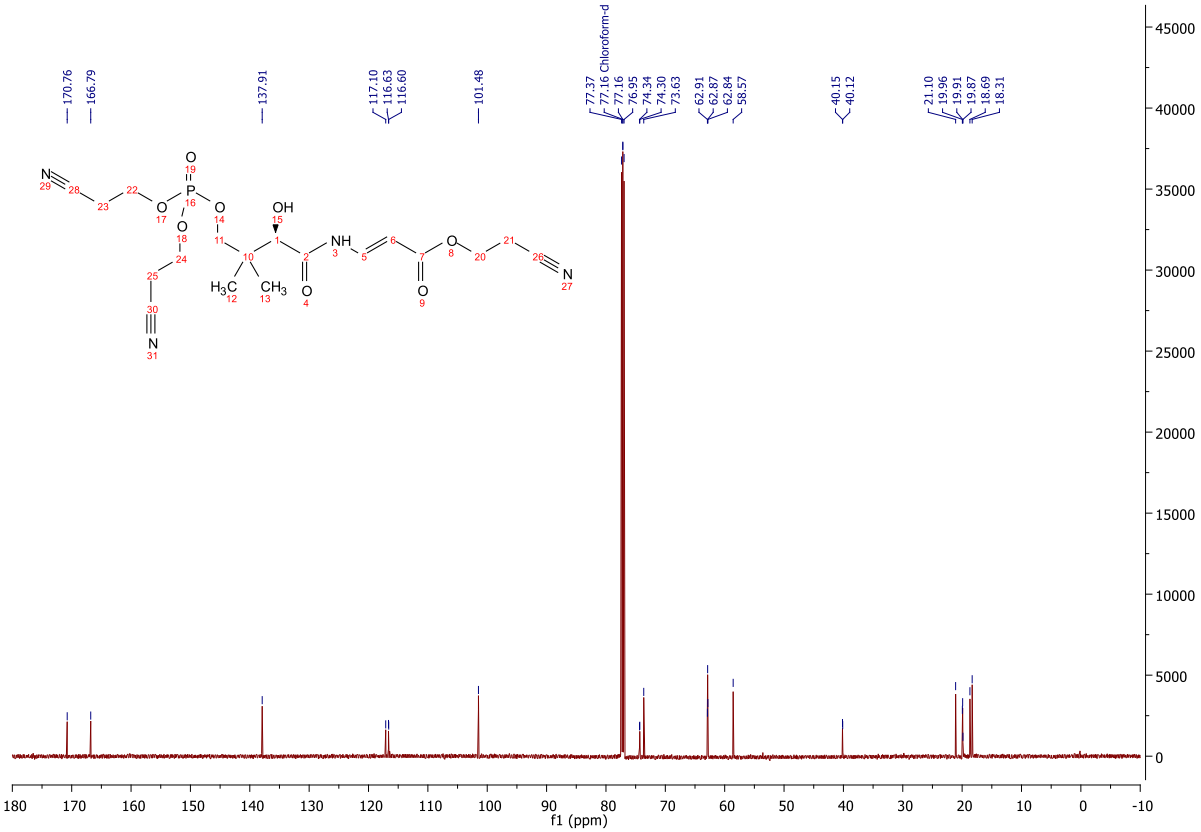
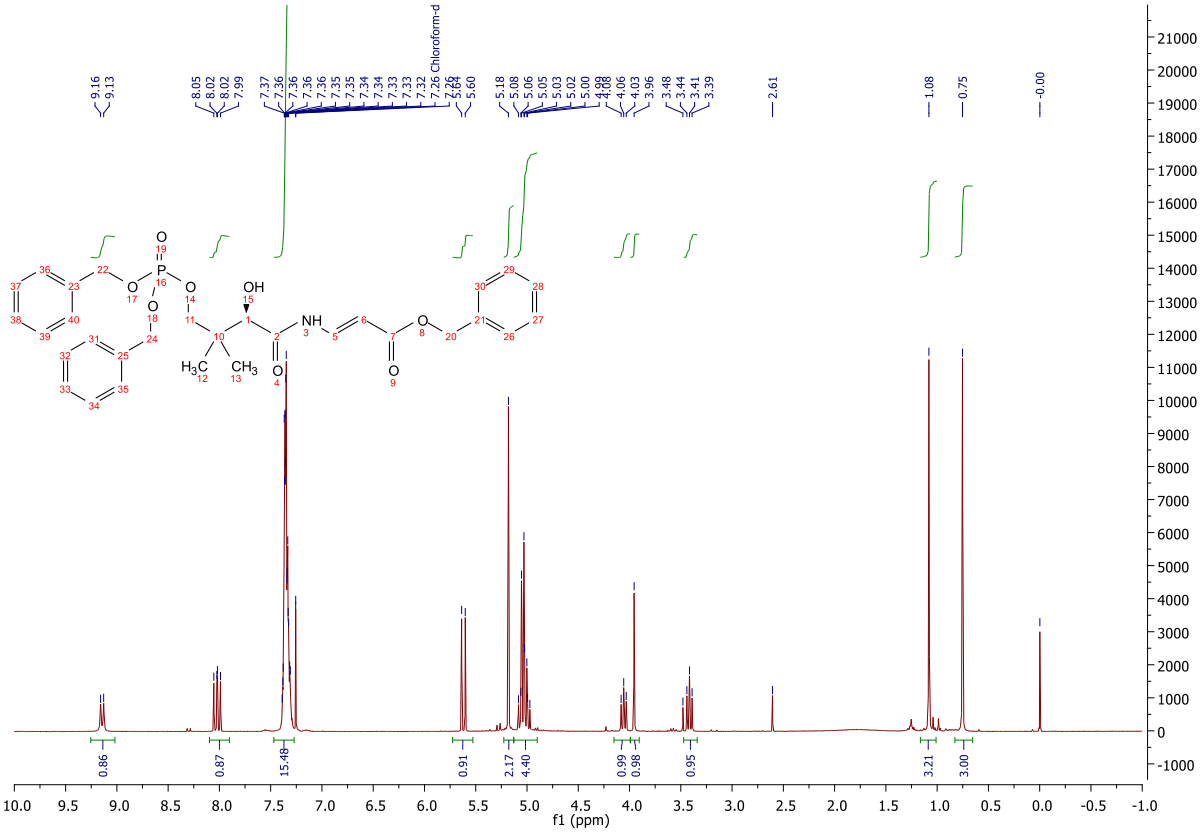


Figure S18. ^{1}H and ^{13}C NMR Spectra of 7b.



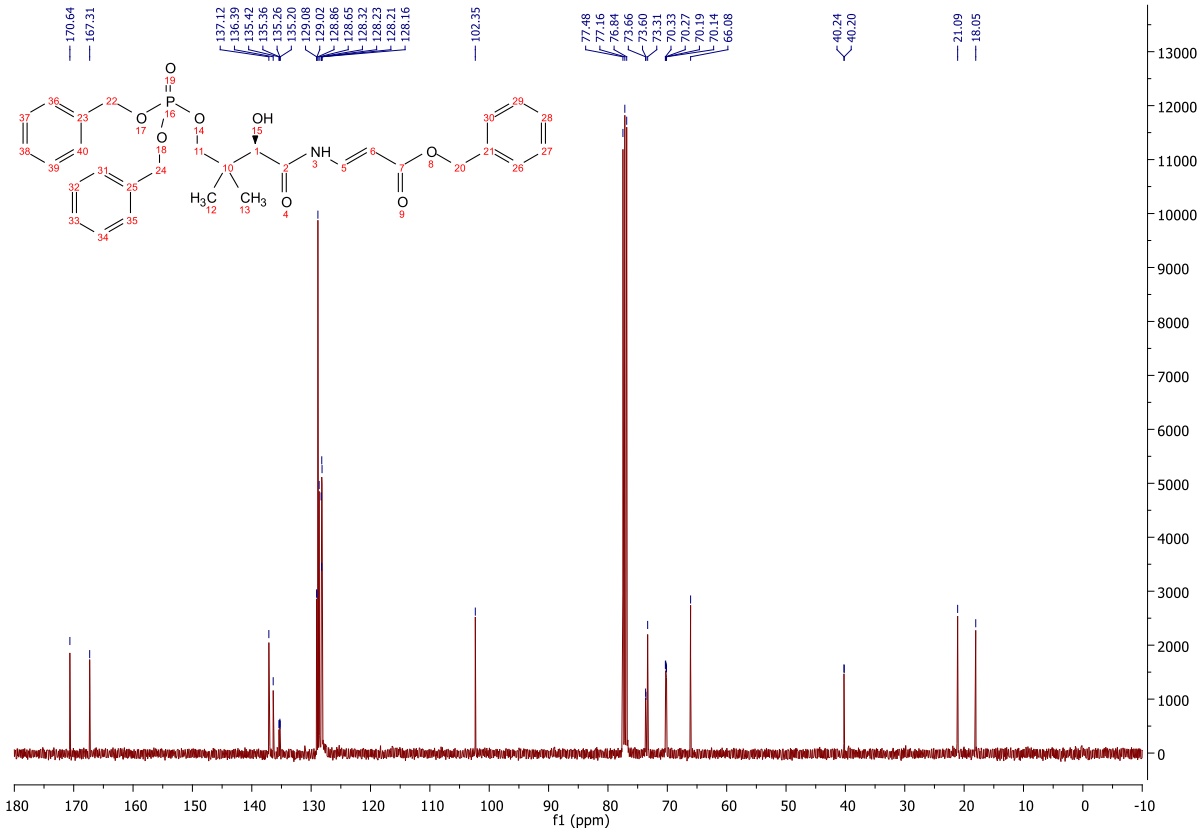
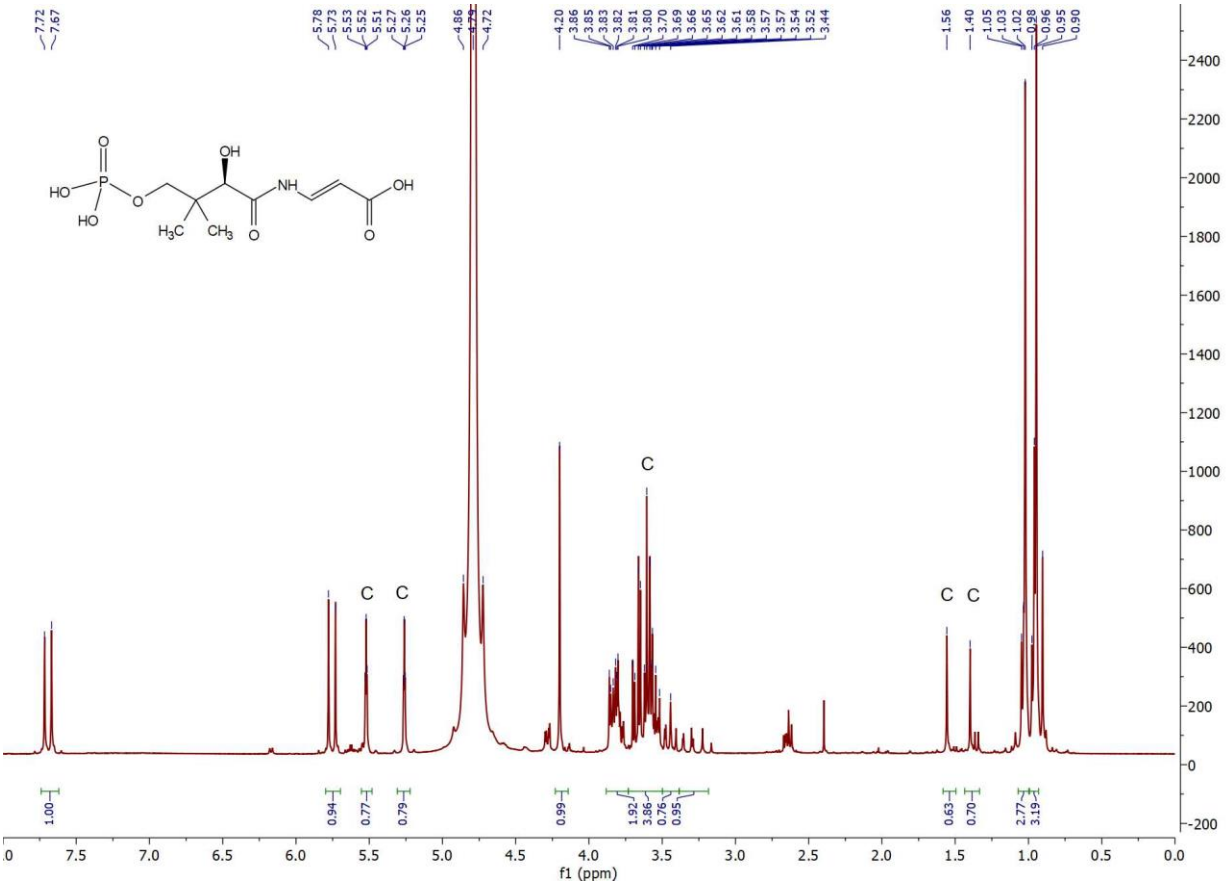
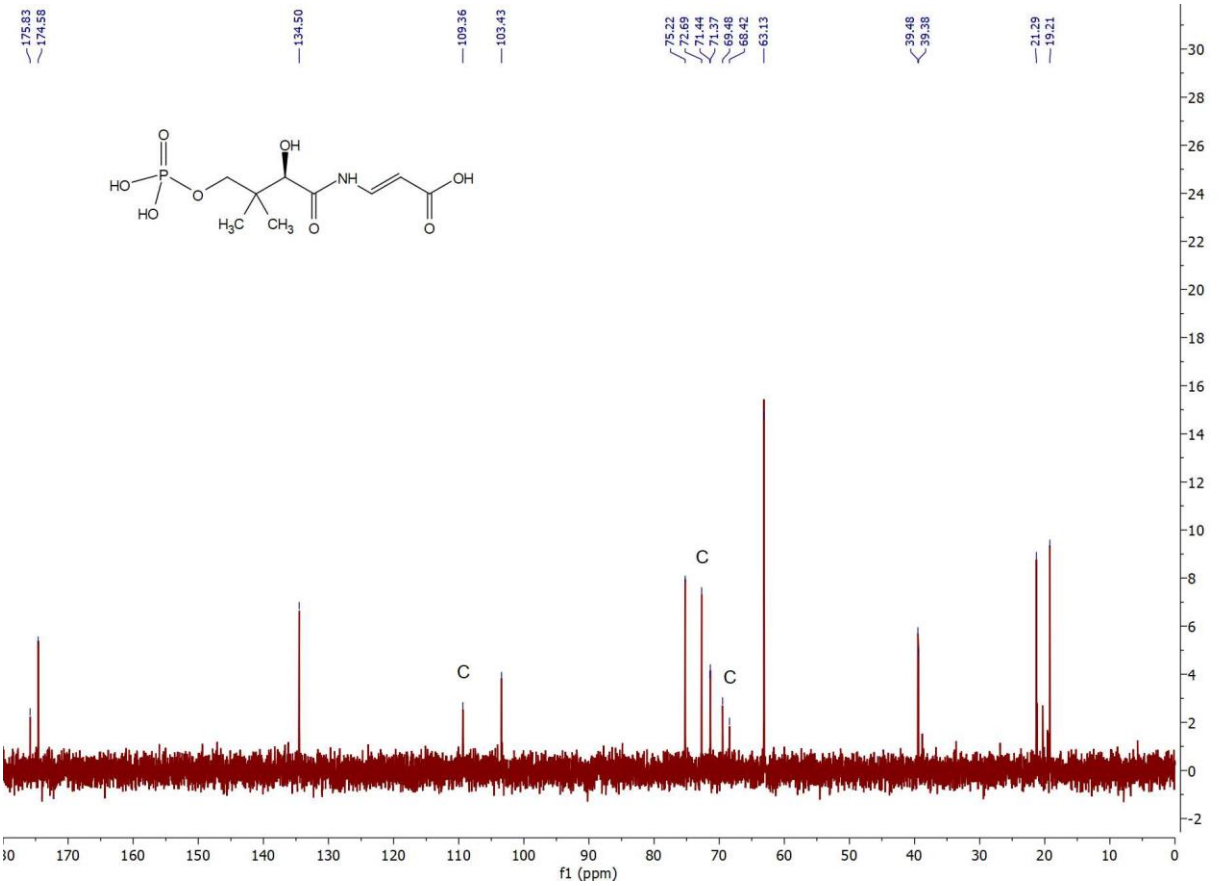


Figure S19. ^{1}H and ^{13}C NMR Spectra of 4′-phospho-CJ-15,801 (8) product mixture.





7. Additional Analytical Data on the Preparation of 4′-phospho-CJ-15,801 (8)

Figure S20. HRMS (ESI+) spectrum of 4′-phospho-CJ-15,801 (8) product mixture.

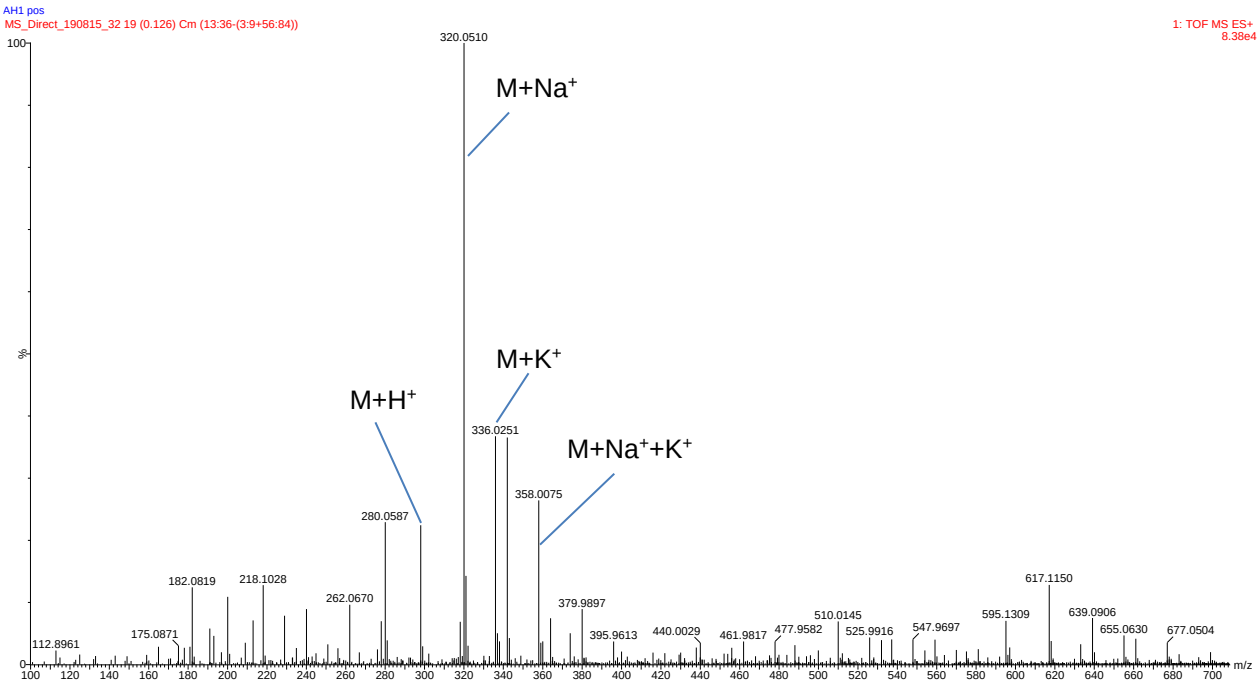
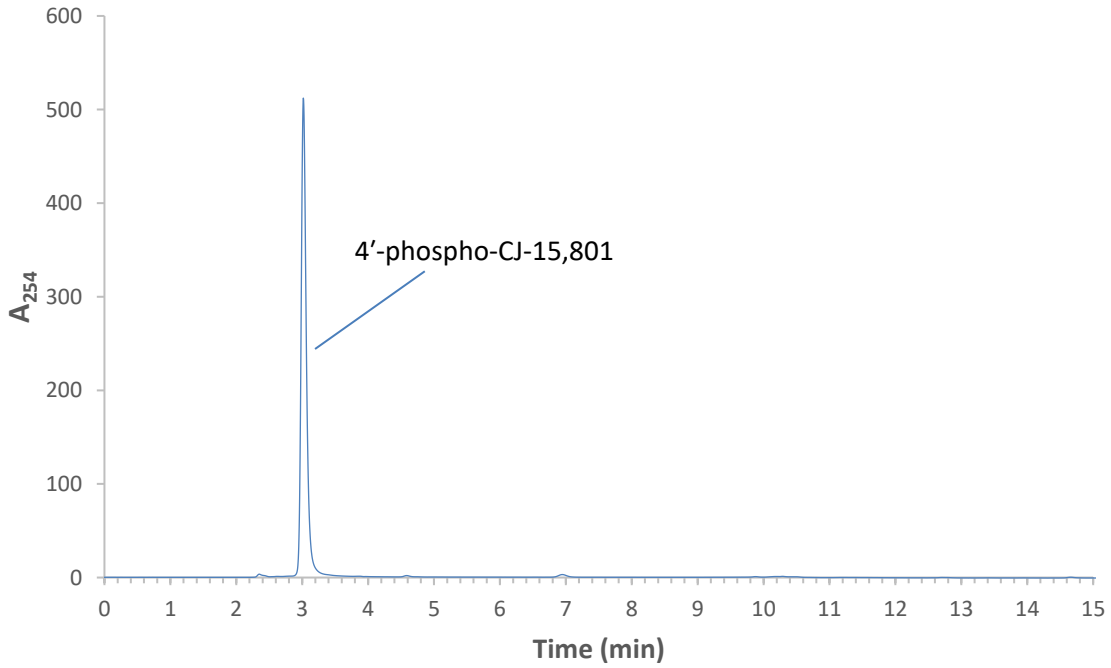


Figure S21. HPLC chromatogram of 4′-phospho-CJ-15,801 (8) product mixture.



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