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Supporting Information for

Target-Guided Screening of Fragments (TGSOF) in the Discovery of Inhibitors against EV-A71 3C Protease

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Content

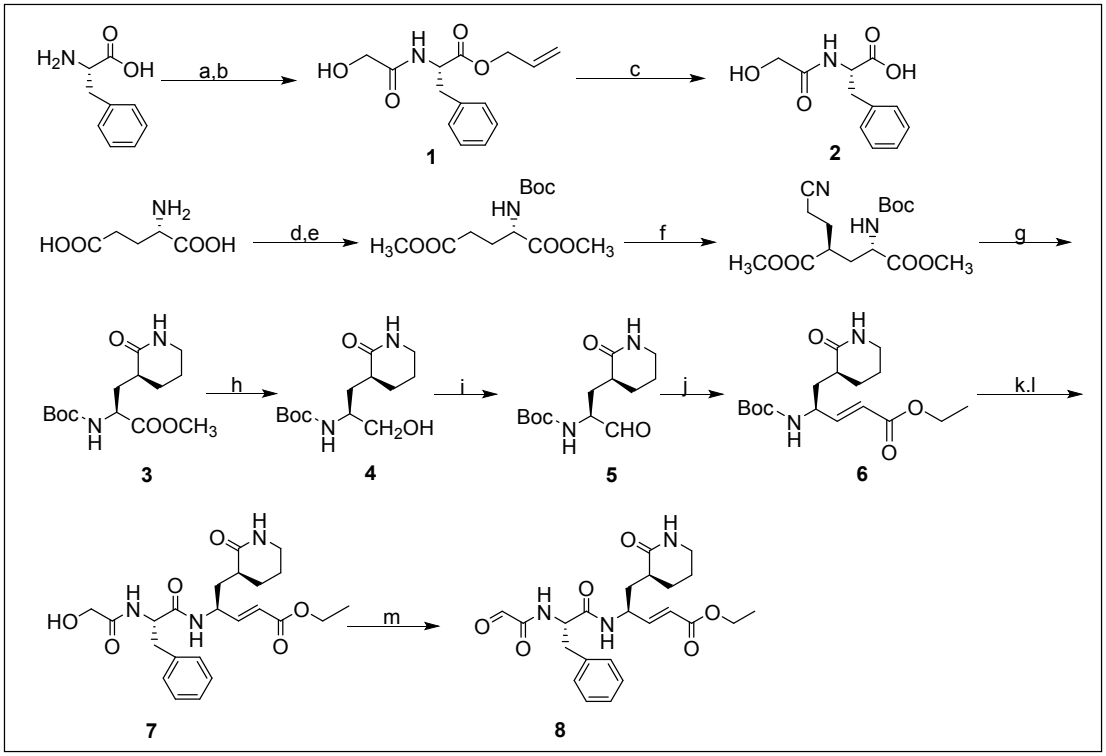
(87 pages)

Part A. Chemistry

Instrumentation and methods

All reagents were purchased from commercial suppliers and used as received. NMR spectra were recorded on a Bruker AVANCE-400 (400 MHz) (Bruker, Karlsruhe, Germany) NMR spectrometer. Mass analysis of synthetic products was conducted using an ESI mass spectrometry: Shimadzu LCMS-2020 (Shimadzu, Kyoto, Japan). Optical rotations were measured with an Insmark IP-120 automatic polarimeter (Insmark, Shanghai, China). Measurements were collected at 15 °C in DCM at 589 nm. $[\alpha]_D$ values are given in units of (deg × mL)/(g × dm). HRMS were recorded on a high-resolution ESI-FTICR mass spectrometer (Varian 7.0 T, Varian, USA). The purity of all tested compounds was determined to be >95% by means of analytical HPLC (Dionex UltiMate 3000, Germany). All protein sample analyses were performed on an LC-MS system that consisted of ultimate 3000 HPLC and an Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific). HPLC was performed at a constant flow rate of 800 μL/min using a binary solvent system. Mobile phase A consisted of 0.1% formic acid in HPLC grade H_2O and mobile phase B was 0.1% formic acid in acetonitrile. The Q- Exactive Orbitrap settings were as follows: sheath gas at 35, auxiliary gas at 10, sweep gas at 0, spray voltage at 3.5 kV, automatic gain control (AGC) at 3 x 106, max injection time at 200 ms. MS full scans were carried out using a mass range of 500 – 1500 *m/z*, and the resolution was 70,000. UV-VIS spectra were obtained on an Infinite Tecan spectrophotometer (Tecan, Mannedorf, Switzerland). Circular dichroism spectroscopic measurement was conducted in a MOS-450 spectropolarimeter (BioLogic, Grenoble, France). The data were processed using Graphpad Prism software.

Scheme S1. Synthesis of LBB (**8**)



Reagents and conditions: a. $SOCl_2$, allyl alcohol, reflux, 4 h; b. HOBt, EDC.HCl, TEA, DCM, glycolic acid, 65%; c. *N*-methylaniline, $Pd[P(C_6H_5)_3]_4$, THF, 75%; d. $SOCl_2$, MeOH, reflux, 4 h; e. $(Boc)_2O$, Et_3N, THF, RT, overnight, 87%; f. LHMDS, 3-bromopropionitrile, THF, -78 °C, 2 h, 51%; g. $CoCl_2{\cdot}6H_2O$, $NaBH_4$, MeOH, 25 °C, overnight, 55%; h. $NaBH_4$, MeOH, 25 °C, 5 h, 89%; i. DMP, DCM, 0°C, 1 h; j. $EtOOCCH_2P(O)(OEt)_2$, LHMDS, THF, 0.5 h, 79%; k. TFA, DCM, 25°C, 4 h; l. HOBt, EDC.HCl, **2**, TEA, DCM, 65%; m. DMP, DCM, 25 °C, 1 h, 79%.

Preparation of Alcohol 1. $SOCl_2$ (3.3 mL, 45.5 mmol) was added drop-wise to a solution of L-phenylalanine (10 g, 60.6 mmol) in allyl alcohol (200.0 mL) at 0 °C. After a further 20 min of stirring at 0 °C, the reaction was heated to reflux for 2.5 h and cooled to room temperature (RT), and then allyl alcohol was evaporated. A solution of the residue was obtained in anhydrous DCM (200 mL)，and then glycolic acid (8.1 g, 80.4 mmol), EDCI (23.06 g, 120.6 mmol) and HOBt (16.28 g, 120.6 mmol) were sequentially added. After 20 min, Et_3N (34.2 mL, 241.2 mmol) was added drop-wise. Then, the reaction mixture was stirred at RT for 3 h, followed by washing with saturated citric acid solution (400 mL×3), saturated $NaHCO_3$ solution (400 mL×3) and brine (400 mL×3). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (EtOAc: Petroleum ether, 1: 2 v/v) to afford the pure product **1** (11.95 g, 75%) as a yellow oil. $[\alpha]^{20}_D = 29.7$ (c = 0.38, CH_2Cl_2).^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.34 (d, *J* = 8.2 Hz, 1H), 7.29 – 7.17 (m, 3H), 7.13 (d, *J* = 6.7 Hz, 2H), 5.82 (ddt, *J* = 16.3, 10.6, 5.8 Hz, 1H), 5.24 (m, 2H), 4.87 (dd, *J* = 14.4, 6.4 Hz, 1H), 4.56 (d, *J* = 5.8 Hz, 2H),

4.45 (s, 1H), 3.96 (d, *J* = 2.1 Hz, 2H), 3.11 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ= 172.39, 171.01, 135.59, 131.22, 129.13, 128.49, 127.05, 118.93, 66.01, 61.77, 52.76, 37.83. HRMS (ESMS)：$C_{14}H_{18}NO_4$ $(M+H)^+$ calcd, 264.1230; found,264.1232.

Preparation of Acid 2. The alcohol **1** (1 g, 3.8 mmol) was dissolved in THF, and *N*-methylaniline (0.488 ml, 4.6 mmol) was added, following by $Pd(PPh_3)_4$ (0.433 g, 0.38 mmol). The resulting solution was stirred at RT for 1 h. The reaction was quenched by addition of HCl aqueous solution (1M, 10 mL), and then the THF was evaporated. The aqueous layer was extracted with DCM (30 mL×3). The combined organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by chromatography on silica gel (MeOH: DCM = 1: 30 v/v) to afford the pure product **2** (0.51 g, 60%) as a white solid. $[\alpha]^{20}_D$ = 42.3 (c = 0.3, CH_3OH).^{1}H NMR (400 MHz, MeOD): δ= 7.35 – 7.14 (m, 5H), 5.13 (s, 2H), 4.76 (dd, *J* = 7.4, 5.3 Hz, 1H), 4.01 – 3.88 (m, 2H), 3.23 (dd, *J* = 13.9, 5.1 Hz, 1H), 3.09 (dd, *J* = 13.9, 7.7 Hz, 1H). ^{13}C NMR (100 MHz, MeOD)：δ= 174.70, 174.17, 137.84, 130.28, 129.43, 127.87, 62.34, 54.12, 38.26. HRMS (ESMS)：$C_{11}H_{14}NO_4$ $(M+H)^+$ calcd, 224.0917; found, 224.0920.

Preparation of Intermediate 3. Compound 3 was prepared according to our previous report [1]**.** ^{1}H NMR (400 MHz, $CDCl_3$)：δ= 7.26 (s, 1H), 5.97 (d, *J* = 8.1 Hz, 1H), 4.39 – 4.17 (m, 1H), 3.68 (s, 3H), 3.36 – 3.17 (m, 2H), 2.38 – 2.20 (m, 2H), 2.13 – 2.02 (m, 1H), 1.89 – 1.63 (m, 3H), 1.57 – 1.47 (m, 1H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$)：δ= 174.58, 173.12, 155.81, 79.35, 51.98, 51.57, 41.92, 37.71, 33.86, 28.11, 26.29, 21.30. ESI-MS (*m/z*): $C_{14}H_{24}N_2NaO_5$ $(M+Na)^+$ 323.2.

Preparation of Alcohol 4. To a solution of **3** (1.0 g, 3.3 mmol) in MeOH (30.0 mL), $NaBH_4$ (1.3 g, 33.0 mmol) was added slowly at 0 °C. Then, the reaction mixture was stirred at RT for 4 h. Saturated NH_4Cl solution (30 mL) was added to quench the reaction. Subsequently, methanol was removed, and DCM (40 mL×3) was added to extract the aqueous residue. The combined organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by chromatography on silica gel (MeOH: DCM = 1: 30 v/v) to afford the pure product **4** (0.60 g, 60%) as white foam. $[\alpha]^{20}_D$ = – 24.6 (c = 0.2, CH_2Cl_2).^{1}H NMR (400 MHz, $CDCl_3$)： δ= 6.93 (s, 1H), 5.67 (d, *J* = 8.2 Hz, 1H), 4.06 (s, 1H), 3.72 (s, 1H), 3.56 (s, 2H), 3.30 (s, 2H), 2.36 (s, 1H), 2.07 (t, *J* = 9.9 Hz, 2H), 1.88 (dd, *J* = 9.0, 4.2 Hz, 1H), 1.79 – 1.51 (m, 3H), 1.43 (s, 9H). ^{13}C NMR (100

MHz, $CDCl_3$)：δ=175.98, 156.56, 79.20, 65.57, 50.28, 42.34, 37.97, 33.03, 28.43, 26.68, 21.50. HRMS (ESMS)：$C_{13}H_{25}N_2O_4$ $(M+H)^+$ calcd, 273.1809; found, 273.1804.

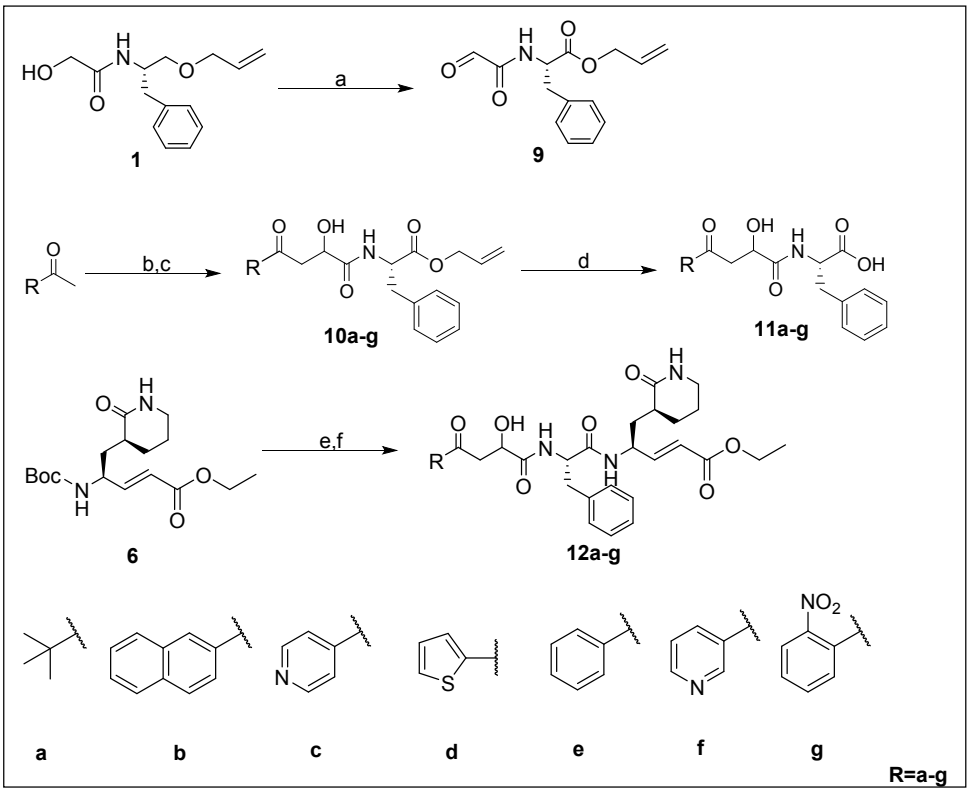
Preparation of Ester 6. To a solution of **4** (1.0 g, 4.4 mmol) in DCM (30.0 mL), DMP (2.8 g, 6.6 mmol) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 1.5h. Saturated $NaHCO_3$ solution (30 mL) and sodium thiosulfate (1.6 g, 2.2 mmol) were added to quench the reaction, and the solution was stirred for 30 min at RT. Subsequently, the aqueous phase was extracted with DCM (50 mL×3). The combined organic phase was dried over anhydrous sodium sulfate, and concentrated to provide **5** as a white foam. This product was used without further purification. Sodium bis(trimethylsilyl) amide (6.6 mL of 1.0 M solution in THF, 6.6 mmol) was added to a solution of triethyl phosphonoacetate (1.31 mL, 6.6 mmol) in THF (30 mL) at -78 °C, and the resulting mixture was stirred for 20 min at -78 °C. A solution of crude **5** (1.0 g, 4.4 mmol) in THF (13 mL) was added via cannula along the side of the reaction vessel, and the resulting mixture was stirred for 45 min at -78 °C, warmed to 0 °C, and then 1 M HCl (20 mL) was added to quench the reaction and stirred for 20 min. Subsequently, THF was removed, and DCM (40 mL×3) was added to extract the aqueous residue. The combined organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by chromatography on silica gel (MeOH: DCM = 1: 80 v/v) to afford the pure product **6** (0.75 g, 50%) as a white solid. $[\alpha]^{20}_D$ = – 13.4 (c = 0.36, CH_2Cl_2).^{1}H NMR (400 MHz, $CDCl_3$)：δ= 7.10 (s, 1H), 6.76 (dd, *J* = 15.6, 4.8 Hz, 1H), 5.85 (d, *J* = 15.6 Hz, 1H), 5.78 (d, *J* = 7.8 Hz, 1H), 4.31-4.17 (m, 1H), 4.08 (q, *J* =6.9 Hz, 2H), 3.29-3.10 (m, 2H), 2.34-2.20 (m, 1H), 2.13 – 1.95 (m, 2H), 1.83 – 1.70 (m, 1H), 1.69-1.54 (m, 1H), 1.52 – 1.39 (m, 2H), 1.33 (s, 9H), 1.17 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.94, 166.25, 155.65, 148.80, 120.36, 79.25, 60.24, 49.49, 42.07, 38.02, 36.01, 28.26, 26.70, 21.37, 14.13. HRMS (ESMS)：$C_{17}H_{29}N_2O_5$ $(M+H)^+$ calcd, 341.2071; found, 341.2067.

Preparation of Alcohol 7. To a solution of **6** (1.0 g, 2.9 mmol) in anhydrous DCM (30 mL), CF_3COOH (5.0 mL, 67 mmol) was added slowly at 0 °C. Then, the reaction mixture was stirred at RT for 2 h and concentrated. A solution of the residue was obtained in anhydrous DCM (40 mL) and Et_3N was added drop-wise to adjust the pH to 7.0 at 0 °C. Then**,** **2** (1.0 g, 4.4 mmol), EDC.HCl (0.84 g, 4.4 mmol) and HOBt (0.59 g, 4.4 mmol) were sequentially added. After 20 min, Et_3N (1.87 mL, 13.2 mmol) was added drop-wise. The reaction mixture was stirred at RT for 3 h,

followed by washing with saturated citric acid solution (50 mL×3), saturated $NaHCO_3$ solution (50 mL×3) and brine (50 mL×3). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (MeOH: DCM = 1: 30 v/v) to afford the pure product **7** (0.75 g, 60%) as a white oil. $[\alpha]^{20}_D$ = – 30.4 (c = 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ： 8.28 (d, *J* = 6.4 Hz, 1H), 7.53 (d, *J* = 8.7 Hz, 1H), 7.30-7.12 (m, 5H), 6.87 (s, 1H), 6.69 (dd, *J* = 15.7, 5.4 Hz, 1H), 5.72 (d, *J* = 15.7 Hz, 1H), 5.16 (s, 1H), 4.87-4.75 (m, 1H), 4.43-4.30 (m, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 4.11 – 4.01 (m, 1H), 3.94 (m, 1H), 3.23 (s, 2H), 3.13 (d, *J* = 6.5 Hz, 2H), 2.12-1.89 (m, 3H), 1.88-1.76 (m, 1H), 1.72-1.57 (m, 1H), 1.55-1.42 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ：175.51, 172.61, 171.33, 166.35, 147.22, 136.59, 129.33, 128.56, 126.93, 120.99, 62.37, 60.43, 53.78, 49.52, 42.17, 38.18, 37.52, 35.51, 27.66, 21.37, 14.24. HRMS (ESMS)：$C_{23}H_{32}N_3O_6$ $(M+H)^+$ calcd, 446.2286; found,446.2288.

Preparation of α-aldehyde 8. To a solution of **7** (0.5 g, 1.2 mmol) in DCM (30.0 mL), DMP (0.76 g, 1.8 mmol) was added slowly at 0 °C. The reaction mixture was stirred at RT for 1.5 h. Saturated $NaHCO_3$ solution (30 mL) and sodium thiosulfate (0.15 g, 0.6 mmol) were added to quench the reaction, and the solution was stirred for 30 min at RT. Subsequently, the aqueous phase was extracted with DCM (50 mL×3). The combined organic phases were dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (MeOH: DCM = 1: 30 v/v) to afford the pure product **8** (0.35 g, 70%) as a white oil. $[\alpha]^{20}_D$ = – 32.8 (c = 0.16, CH_2Cl_2). ^{1}H NMR (400 MHz, D_2O) δ：7.44 – 7.21 (m, 5H), 6.58 (dd, *J* = 15.7, 5.5 Hz, 1H), 5.45 (d, *J* = 15.4 Hz, 1H), 4.62 – 4.48 (m, 2H), 4.24 (q, *J* = 7.1 Hz, 2H), 3.31 – 3.11 (m, 3H), 3.10 – 3.00 (m, 1H), 2.36-2.25 (s, 1H), 1.98-1.76 (m, 3H), 1.74-1.59 (m, 2H), 1.57-1.44 (m, 1H), 1.33 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, D_2O) δ：175.94, 171.91, 171.78, 167.10, 148.77, 137.15, 129.96, 129.02, 127.41, 120.94, 87.87, 61.00, 55.10, 48.20, 42.21, 38.14, 37.78, 35.82, 26.22, 21.09, 14.25. HRMS (ESMS)：$C_{23}H_{30}N_3O_6$ $(M+H)^+$ calcd, 444.2129; found, 444.2131. HPLC purity: 99.64%. (Because the aldehyde **8** is a glyoxamide and can exist in a hydrated form, the aldehyde proton is missing in the spectrum.)

Scheme S2. Synthesis of **12a-g**



Reagents and conditions: a. DMP, DCM, 25°C, 1 h, 79%; b. ketone, TEA, TMS triflate; c. 9, $TiCl_4$, DCM, 45%; d. *N*-methylaniline, $Pd[P(C_6H_5)_3]_4$, THF, 75%; e. TFA, DCM, 25°C, 4 h; f. 11, HOBt, EDC.HCl, TEA, DCM, 65%.

General Procedure for the Synthesis of Esters (10a-g): Preparation of Ester 10a. To a solution of **1** (2.0 g, 7.6 mmol) in DCM (80 mL), DMP (4.8 g, 11.4 mmol) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 1.5h. Saturated $NaHCO_3$ solution (100 mL) and sodium thiosulfate (3.2 g, 4.4 mmol) were added to quench the reaction, and the solution was stirred for 30 min at RT. Subsequently, the aqueous phase was extracted with DCM (50 mL×3). The combined organic phases were dried over anhydrous sodium sulfate, and concentrated to provide crude **9** as white foam. This product was used without further purification. To a solution of pinacolone (2.3 g, 22.8 mmol) in anhydrous DCM (50 mL), triethylamine (4.69 mL, 34.2 mmol) and trimethylsilyl trifluoromethanesulfonate (4.2 mL, 22.8 mmol) were Subsequently added. The reaction mixture was stirred at 0 °C for 1 h. The resulting solution was directly used for the next step. To a solution of crude **9** in DCM (80 mL), $TiCl_4$ (15.2 mL, 15.2 mmol, 1 M in DCM) was

added drop-wise at – 78 °C. After stirred for 20 min, the above resulting solution was added slowly. The reaction was stirred at – 78 °C for 1 h. Saturated NH_4Cl solution (80 mL) was added to quench the reaction, and the solution was stirred for 30 min at RT. Subsequently, the aqueous phase was extracted with DCM (50 mL×3). The combined organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (EtOAc: Petroleum ether, 1: 1 v/v) to afford the product **10a** (1.1 g, 40%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ：7.23 (s, 1H), 7.08 – 6.89 (m, 5H), 5.67 – 5.55 (m, 1H), 5.02 (dd, *J* = 24.9, 13.9 Hz, 2H), 4.66 (s, 1H), 4.37 (s, 2H), 4.24 (s, 1H), 3.01 – 2.44 (m, 4H), 0.89 (d, *J* = 3.3 Hz, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ：215.85, 215.75, 172.71, 170.72, 170.68, 135.75, 135.69, 131.32, 129.21, 129.05, 128.32, 128.25, 126.85, 126.79, 118.57, 68.00, 65.71, 52.82, 52.64, 43.98, 40.52, 37.85, 37.65, 25.95. HRMS (ESMS)：$C_{20}H_{28}NO_5$ $(M+H)^+$ calcd, 362.1962; found, 362.1955.

Preparation of Ester 10b. 10b was prepared as a yellow oil by an analogous procedure to **10a** (40%, yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ= 8.66 (d, *J* = 8.5 Hz, 1H), 7.87 – 7.76 (m, 2H), 7.75 – 7.69 (m, 1H), 7.62 (t, *J* = 9.3 Hz, 1H), 7.51 – 7.36 (m, 2H), 7.34 – 7.23 (m, 1H), 7.15 (dt, *J* = 12.8, 7.1 Hz, 5H), 5.83 – 5.70 (m, 1H), 5.25 – 5.10 (m, 2H), 4.98 – 4.86 (m, 1H), 4.78 (d, *J* = 8.0 Hz, 2H), 4.57 – 4.45 (m, 2H), 3.62 – 3.22 (m, 2H), 3.20 – 3.01 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ= 202.53, 202.50, 172.87, 172.85, 170.77, 170.73, 135.69, 135.63, 134.22, 134.12, 133.55, 133.13, 131.21, 131.19, 129.79, 129.17, 129.01, 128.63, 128.58, 128.29, 128.24, 128.16, 127.86, 126.81, 126.76, 126.17, 125.54, 124.06, 118.55, 68.40, 65.70, 52.91, 52.66, 45.27, 37.76, 37.60. HRMS (ESMS)：$C_{26}H_{26}NO_5$ $(M+H)^+$ calcd, 432.1805; found, 432.1804.

Preparation of Ester 10c. 10c was prepared as a brown oil by an analogous procedure to **10a** (50%, yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ= 8.81 – 8.63 (m, 2H), 7.68 (dd, *J* = 5.8, 3.2 Hz, 2H), 7.63-7.56 (m, 1H), 7.28 – 7.12 (m, 5H), 5.90 – 5.80 (m, 1H), 5.34 – 5.19 (m, 2H), 4.96-4.85 (m, 1H), 4.76 – 4.68 (m, 1H), 4.65 – 4.49 (m, 2H), 3.58-3.43 (m,, 1H), 3.36 – 3.06 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=198.02, 197.86, 172.79, 172.72, 170.87, 170.82, 150.41, 142.45, 142.39, 135.73, 135.67, 131.29, 129.30, 129.10, 128.45, 128.42, 127.01, 126.98, 121.16, 118.87, 67.49, 65.96, 52.92, 52.72, 43.18, 37.97, 37.67. HRMS (ESMS)：$C_{21}H_{23}N_2O_5$ $(M+H)^+$ calcd, 383.1601; found, 383.1604.

Preparation of Ester 10d. 10d was prepared as a yellow oil by an analogous procedure to **10a**

(70%, yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.09 (d, *J* = 8.4 Hz, 1H), 7.86 – 7.72 (m, 2H), 7.34 – 7.14 (m, 6H), 5.99 – 5.79 (m, 1H), 5.37 – 5.17 (m, 2H), 4.84 – 4.75 (m, 1H), 4.70 – 4.52 (m, 3H), 3.27 – 3.02 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=191.93, 191.85, 175.87, 175.77, 172.20, 145.13, 137.67, 137.63, 135.63, 134.36, 134.29, 133.03, 133.00, 130.49, 130.29, 129.51, 129.49, 129.44, 127.96, 118.94, 69.13, 69.07, 66.93, 66.91, 54.70, 54.45, 44.65, 38.43, 38.28. HRMS (ESMS)：$C_{20}H_{22}NO_5S(M+H)^+$ calcd, 388.1213; found, 388.1210.

Preparation of Ester 10e. 10e was prepared as a yellow oil by an analogous procedure to **10a** (50% yield). **10e** was purified by column chromatography (EtOAc: Petroleum ether, 1: 2 v/v) to afford the two pure products. **(6S,9R)-10e** (30% yield): $[\alpha]^{20}_D$ = 10.4 (c = 0.3, CH_3OH). ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.93 (d, *J* = 8.0 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.27 – 7.18 (m, 3H), 7.14 (d, *J* = 6.9 Hz, 2H), 5.87 (ddt, *J* = 16.9, 11.4, 5.9 Hz, 1H), 5.31 (d, *J* = 17.2 Hz, 1H), 5.25 (d, *J* = 10.4 Hz, 1H), 4.91 (dd, *J* = 14.4, 6.4 Hz, 1H), 4.65 (d, *J* = 7.6 Hz, 1H), 4.62 (d, *J* = 5.9 Hz, 2H), 4.03 (s, 1H), 3.57 (dd, *J* = 18.3, 3.3 Hz, 1H), 3.29 (dd, *J* = 18.4, 8.4 Hz, 1H), 3.20 (dd, *J* = 13.9, 5.7 Hz, 1H), 3.11 (dd, *J* = 13.9, 6.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ= 200.20, 172.31, 170.95, 136.13, 135.84, 134.01, 131.54, 129.34, 128.82, 128.67, 128.33, 127.23, 119.08, 68.35, 66.15, 53.11, 42.39, 38.09. HRMS (ESMS)：$C_{22}H_{24}NO_5$ $(M+H)^+$ calcd, 382.1649; found, 382.1641. **(6S,9S)-10e** (20% yield): $[\alpha]^{20}_D$ = 18.3 (c = 0.1, CH_2Cl_2). ^{1}H NMR (400 MHz, MeOD): δ=7.96 – 7.87 (m, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 2H), 7.26 (m, 5H), 5.89 (ddt, *J* = 16.4, 11.0, 5.7 Hz, 1H), 5.36 – 5.17 (m, 2H), 4.82 (td, *J* = 5.5, 2.6 Hz, 1H), 4.66-4.55 (m, 3H), 3.28 – 3.06 (m, 4H). ^{13}C NMR (100 MHz, MeOD): δ=197.69, 174.76, 170.92, 136.81, 136.45, 133.11, 131.75, 129.22, 128.37, 128.19, 127.87, 126.65, 117.61, 67.71, 65.63, 53.10, 42.88, 37.17. HRMS (ESMS)：$C_{22}H_{24}NO_5$ $(M+H)^+$ calcd, 382.1649; found, 382.1646.

Preparation of Ester 10f. 10f was prepared as a yellow oil by an analogous procedure to **10a** (50% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.07 (d, *J* = 2.2 Hz, 1H), 8.69 (t, *J* = 5.4 Hz, 1H), 8.16 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.69 (dd, *J* = 8.3, 3.2 Hz, 1H), 7.42 – 7.30 (m, 1H), 7.26 – 7.12 (m, 5H), 5.84 (tt, *J* = 16.3, 5.7 Hz, 1H), 5.32 – 5.17 (m, 2H), 4.97-4.86 (m, 1H), 4.82-4.73 (m, 1H), 4.64 – 4.51 (m, 2H), 3.57 – 3.25 (m, 2H),3.23 – 3.07 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=197.08, 196.92, 172.88, 172.83, 170.71, 170.65, 152.97, 149.00, 135.61, 135.59, 131.60, 131.19, 129.14,

128.95, 128.23, 126.78, 123.53, 118.59, 118.57, 67.33, 65.71, 52.80, 52.56, 43.01, 37.76, 37.48. HRMS (ESMS)：$C_{21}H_{23}N_2O_5$ $(M+H)^+$ calcd, 383.1601; found, 383.1596.

Preparation of Ester 10g. 10g was prepared as a yellow oil by an analogous procedure to **10a** (50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.4 Hz, 1H), 7.67 (d, *J* = 4.9 Hz, 1H), 7.52 (dd, *J* = 29.9, 14.5 Hz, 2H), 7.36 – 6.99 (m, 5H), 5.83 (d, *J* = 5.7 Hz, 1H), 5.35 – 5.15 (m, 2H), 4.88 (s, 1H), 4.75 – 4.51 (m, 3H), 4.41 – 3.98 (m, 1H), 3.50 – 2.97 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.44, 201.18, 172.35, 172.17, 170.89, 170.83, 145.55, 145.51, 136.58, 135.75, 134.23, 131.35, 130.88, 129.30, 129.20, 128.51, 128.44, 127.61, 127.55, 127.06, 126.98, 124.27, 124.23, 118.86, 68.04, 65.97, 53.08, 52.97, 46.51, 46.37, 37.94, 37.75. HRMS (ESMS)：$C_{22}H_{23}N_2O_7$ $(M+H)^+$ calcd, 427.1500; found, 427.1506.

General Procedure for the Synthesis of Esters (12a-g): Preparation of Ester 12a. To a solution of **10a** (0.94 g, 2.6 mmol) in THF (35.0 mL), *N*-methylaniline (0.3 ml, 3.1 mmol) and $Pd(PPh_3)_4$ (0.03 g, 0.03 mmol) were sequentially added. The reaction mixture was stirred at RT for 1 h, and then was concentrated. The residue was purified by column chromatography to afford crude **11a**. To a solution of **6** (0.4 g, 1.16 mmol) in anhydrous DCM (20 mL), CF_3COOH (4.0 mL, 53.6 mmol) was added slowly at 0 °C. Then, the reaction mixture was stirred at RT for 2 h and concentrated. A solution of the residue was obtained in DCM (40 mL) and Et_3N was added drop-wise to adjust the pH to 7.0 at 0 °C. **11a** (0.35 g, 1.56 mmol), EDC.HCl (0.60 g, 3.12 mmol) and HOBt (0.42 g, 3.12 mmol) were sequentially added at 0 °C. After 20 min, Et_3N (0.66 mL, 4.68 mmol) was added drop-wise. The reaction mixture was stirred at RT for 12 h, followed by washing with saturated citric acid solution (50 mL×3), saturated $NaHCO_3$ solution (50 mL×3) and brine (50 mL×3). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (MeOH: DCM = 1: 30 v/v) to afford the pure product **12a** (0.39 g, 60%) as a yellow oil. ^{1}H NMR (400 MHz, MeOD) δ: 7.38 – 7.18 (m, 5H), 6.79 (dd, *J* = 15.9, 5.0 Hz, 1H), 5.73 (d, *J* = 15.8 Hz, 1H), 4.75 – 4.55 (m, 2H), 4.48 – 4.25 (m, 1H), 4.17 (dq, *J* = 14.3, 7.1 Hz, 2H), 3.29 – 3.09 (m, 4H), 3.08 – 2.94 (m, 2H), 2.34 – 2.17 (m, 2H), 2.10 – 1.78 (m, 2H), 1.77 – 1.43 (m, 3H), 1.28 (dt, *J* = 14.3, 7.1 Hz, 3H), 1.11 (d, *J* = 4.7 Hz, 9H). 13C NMR (100 MHz, MeOD) δ: 215.64, 214.73, 176.74, 176.57, 176.42, 176.36, 173.24, 173.17, 167.75, 149.06, 148.98, 138.03, 137.97, 130.54, 130.35, 129.70, 129.57, 128.06, 128.02,

121.80, 69.05, 68.79, 61.54, 61.47, 55.90, 55.81, 49.29, 49.14, 45.06, 42.98, 42.20, 41.89, 38.78, 38.73, 38.31, 38.28, 36.60, 27.28, 27.10, 26.48, 22.13, 14.58. HRMS (ESMS): $C_{29}H_{42}N_3O_7(M+H)^+$ calcd, 544.3017; found, 544.3009. HPLC purity: 99.15%.

Preparation of Ester 12b. 12b was prepared as a yellow oil by an analogous procedure to **12a** (50% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.64 (d, *J* = 8.2 Hz, 1H), 8.24 (dd, *J* = 24.8, 6.5 Hz, 1H), 8.02-7.79 (m, 3H), 7.48 (ddd, *J* = 14.9, 13.7, 8.2 Hz, 4H), 7.28 – 7.09 (m, 5H), 6.73 (ddd, *J* = 15.7, 10.5, 5.3 Hz, 1H), 6.66 – 6.30 (m, 1H), 5.82 (dd, *J* = 15.6, 5.8 Hz, 1H), 5.34-5.92 (m, 1H), 4.90-4.54 (m, 2H), 4.51-4.29(m, 1H), 4.19 – 4.01 (m, 2H), 3.67 – 3.04 (m, 6H), 2.13 – 1.83 (m, 3H), 1.73 (s, 1H), 1.64 – 1.35 (m, 3H), 1.28 – 1.15 (m, 3H). ^{13}C NMR (100MHz, $CDCl_3$): δ=202.58, 202.23, 175.59, 175.26, 173.67, 173.51, 171.09, 166.36, 166.32, 147.33, 147.26, 136.75, 136.70, 135.09, 134.63, 133.90, 133.85, 133.30, 133.13, 130.07, 129.53, 129.43, 128.78, 128.52, 128.46, 128.41, 128.10, 128.03, 126.81, 126.78, 126.50, 125.84, 125.76, 124.39, 121.08, 69.03, 68.92, 60.39, 60.28, 54.05, 53.69, 49.57, 49.29, 45.65, 45.55, 42.12, 38.23, 38.17, 37.66, 37.54, 35.65, 35.47, 27.58, 27.32, 21.26, 21.14, 14.24, 14.16. HRMS (ESMS): $C_{35}H_{40}N_3O_7$ $(M+H)^+$ calcd, 614.2861; found, 614.2858. HPLC purity: 95.57%.

Preparation of Ester 12c. 12c was prepared as a brown oil by an analogous procedure to **12a**. (50% yield). **12c** was purified by column chromatography (MeOH: DCM = 1: 30 v/v) to afford the two pure products. **12c-1** (20% yield): $[\alpha]^{20}_D$ = − 37.6 (c = 0.09, CH_3OH). ^{1}H NMR (400 MHz, MeOD): δ=8.76 (d, *J* = 5.2 Hz, 2H), 7.84 (dd, *J* = 4.5, 1.7 Hz, 2H), 7.34 – 7.19 (m, 5H), 6.73 (dd, *J* = 15.7, 5.4 Hz, 1H), 5.66 (dd, *J* = 15.8, 1.6 Hz, 1H), 4.67 – 4.54 (m, 3H), 4.17 (q, *J* = 7.0 Hz, 2H), 3.29 – 3.16 (m, 4H), 3.11 (dd, *J* = 18.1, 7.4 Hz, 2H), 2.29 (tt, *J* = 9.8, 4.9 Hz, 1H), 2.11 – 1.94 (m, 2H), 1.88 – 1.76 (m, 1H), 1.73 – 1.57 (m, 2H), 1.54 – 1.41 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, MeOD): δ=198.55, 176.84, 175.73, 173.09, 167.85, 151.44, 148.96, 144.95, 137.89, 130.57, 129.68, 128.15, 122.93, 121.82, 69.16, 61.61, 55.97, 49.17, 44.35, 42.94, 38.92, 38.79, 36.58, 27.26, 22.13, 14.56. HRMS (ESMS): $C_{30}H_{37}N_4O_7$ $(M+H)^+$ calcd, 565.2657; found, 565.2652. HPLC purity: 95.26%. **12c-2** (30% yield): $[\alpha]^{20}_D$ = − 5.9 (c = 0.10, CH_3OH). ^{1}H NMR (400 MHz, MeOD): δ=8.76 (d, *J* = 5.7 Hz, 2H), 7.95 – 7.82 (m, 2H), 7.40 – 7.20 (m, 5H), 6.78 (dd, *J* = 15.7, 5.1 Hz, 1H), 5.71 (dd, *J* = 15.7, 1.6 Hz, 1H), 4.66 (t, *J* = 7.1 Hz, 2H), 4.51 (t, *J* = 5.1 Hz, 1H), 4.09 (q, *J* = 7.1, 2H), 3.60 – 3.47 (m, 2H), 3.27 – 3.08 (m, 4H), 2.39 – 2.18 (m, 2H),

2.05 (ddt, *J* = 9.6, 6.3, 3.1 Hz, 1H), 1.84 (ddd, *J* = 9.6, 5.9, 3.0 Hz, 1H), 1.74 – 1.51 (m, 3H), 1.21 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, MeOD): δ=198.95, 176.80, 175.84, 173.13, 167.97, 151.43, 149.01, 144.71, 137.97, 130.57, 129.57, 128.14, 122.87, 121.71, 68.84, 61.60, 55.96, 49.15, 44.05, 42.88, 38.78, 38.57, 36.65, 27.12, 22.18, 14.50. HRMS (ESMS)：$C_{30}H_{37}N_4O_7$ $(M+H)^+$ calcd, 565.2657; found, 565.2648. HPLC purity: 97.01%.

Preparation of Ester 12d. 12d was prepared as a yellow oil by an analogous procedure to 12a (70% yield). ^{1}H NMR (400 MHz, MeOD): δ= 7.85 (dd, *J* = 12.4, 3.7 Hz, 2H), 7.26 (dd, *J* = 17.7, 9.7 Hz, 5H), 7.22 – 7.16 (m, 1H), 6.82 – 6.69 (m, 1H), 5.77 – 5.63 (m, 1H), 4.7-4.39 (m, 3H), 4.23 – 4.04 (m, 2H), 3.42 (d, *J* = 4.7 Hz, 2H), 3.28 – 3.21 (m, 2H), 3.20 – 3.09 (m, 2H), 2.38 – 2.19 (m, 2H), 2.05 (d, *J* = 11.9 Hz, 1H), 1.84 (s, 1H), 1.67 (dd, *J* = 26.4, 12.9 Hz, 2H), 1.58 – 1.45 (m, 1H), 1.24 (dt, *J* = 14.1, 6.5 Hz, 3H). ^{13}C NMR (100 MHz, MeOD): δ= 192.39, 192.12, 176.76, 176.66, 176.31, 176.24, 173.27, 173.19, 167.82, 149.15, 145.34, 145.19, 138.15, 138.04, 136.03, 135.84, 134.70, 134.53, 130.65, 130.45, 129.78, 129.71, 129.64, 128.16, 121.90, 121.82, 69.51, 69.20, 61.63, 61.53, 56.15, 55.96, 49.23, 49.20, 44.64, 44.27, 43.00, 38.95, 38.82, 38.55, 36.72, 36.62, 27.34, 27.21, 22.23, 14.71, 14.65. HRMS (ESMS)：$C_{29}H_{36}N_3O_7S$ $(M+H)^+$ calcd, 570.2268; found, 570.2262. HPLC purity: 95.08%.

Preparation of Ester (7S,2'S,10S,13R)-12e. (7S,2'S,10S,13R)-12e was prepared as a yellow oil by an analogous procedure to **12a** (50% yield). $[\alpha]^{20}_D = -9.9$ (c = 0.06, CH_3OH)^{1}H NMR (400 MHz, MeOD): δ= 7.86 (d, *J* = 7.4 Hz, 2H), 7.48 (d, *J* = 7.4 Hz, 1H), 7.37 (t, *J* = 7.7 Hz, 2H), 7.22 – 7.09 (m, 5H), 6.68 (dd, *J* = 15.7, 4.9 Hz, 1H), 5.61 (dd, *J* = 15.7, 1.5 Hz, 1H), 4.60 – 4.51 (m, 2H), 4.37 (t, *J* = 4.8 Hz, 1H), 3.98 – 3.88 (m, 2H), 3.39 (d, *J* = 4.9 Hz, 2H), 3.14 – 3.03 (m, 4H), 2.30-2.11 (m, 2H), 1.94 (ddd, *J* = 15.6, 7.5, 4.5 Hz, 1H), 1.77 – 1.67 (m, 1H), 1.64 – 1.52 (m, 2H), 1.42 (td, *J* = 12.9, 3.1 Hz, 1H), 1.05 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, MeOD): δ= 199.66, 176.70, 176.56, 173.25, 167.81, 148.99, 138.03, 137.98, 134.66, 130.36, 129.75, 129.73, 129.39, 128.11, 121.83, 69.00, 61.48, 56.04, 49.07, 43.61, 42.94, 38.76, 38.41, 36.63, 27.06, 22.15, 14.47. HRMS (ESMS)：$C_{31}H_{38}N_3O_7$ $(M+H)^+$ calcd, 564.2704; found, 564.2700. HPLC purity: 98.73%.

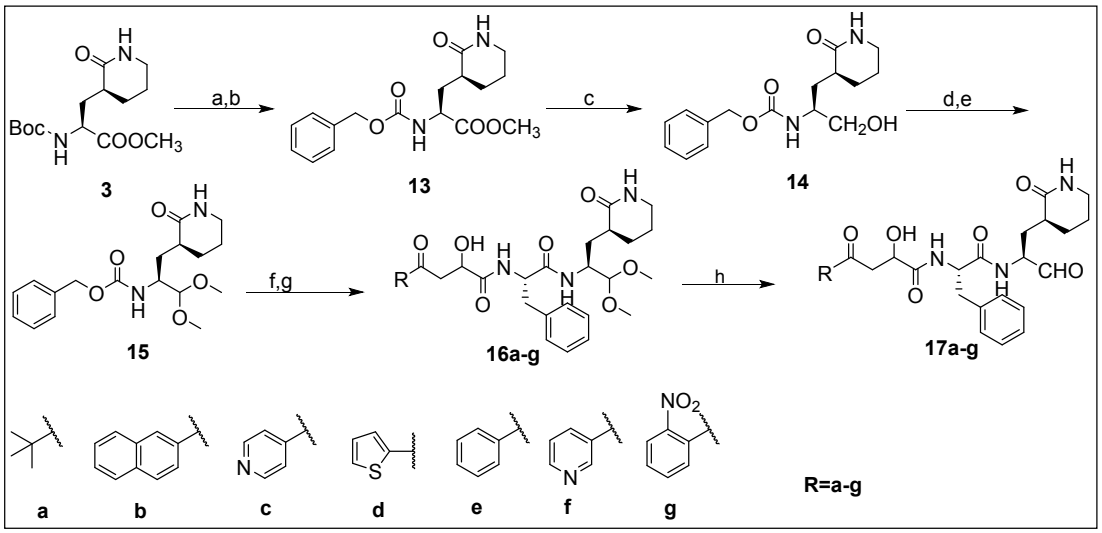
Preparation of Ester (7S,2'S,10S,13S)-12e. (7S,2'S,10S,13S)-12e was prepared as a yellow oil by an analogous procedure to **12a** (60% yield). $[\alpha]^{20}_D = -17.4$ (c = 0.12, CH_3OH) .^{1}H NMR (400 MHz, MeOD): δ= 7.99 – 7.93 (m, 2H), 7.67 – 7.57 (m, 1H), 7.51 (t, *J* = 7.7 Hz, 2H), 7.36 – 7.21

(m, 5H), 6.76 (dd, *J* = 15.7, 5.3 Hz, 1H), 5.69 (dd, *J* = 15.7, 1.5 Hz, 1H), 4.73 – 4.58 (m, 3H), 4.17 (q, *J* = 7.0 Hz, 2H), 3.36 – 3.04 (m, 6H), 2.36-2.28 (m, 1H), 2.19 – 1.95 (m, 2H), 1.88 – 1.78 (m, 1H), 1.73 – 1.58 (m, 2H), 1.55 – 1.44 (m, 1H), 1.29 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 199.29, 176.76, 176.06, 173.08, 167.80, 148.97, 138.12, 137.91, 134.49, 130.55, 129.72, 129.62, 129.26, 128.06, 121.77, 69.29, 61.58, 55.87, 49.15, 44.03, 42.90, 38.90, 38.76, 36.53, 27.22, 22.10, 14.56. HRMS (ESMS)：$C_{31}H_{37}N_3NaO_7$ $(M+Na)^+$ calcd, 586.2524; found, 586.2520. HPLC purity: 96.91%.

Preparation of Ester 12f. 12f was prepared as a yellow oil by an analogous procedure to **12a** (60% yield). ^{1}H NMR (400 MHz, MeOD): δ=9.07 (dd, *J* = 12.5, 2.1 Hz, 1H), 8.77 – 8.67 (m, 1H), 8.39 – 8.25 (m, 1H), 7.56 (dt, *J* = 8.0, 5.4 Hz, 1H), 7.37 – 7.16 (m, 5H), 6.76 (ddd, *J* = 17.4, 15.8, 5.1 Hz, 1H), 5.69 (td, *J* = 15.8, 1.6 Hz, 1H), 4.71 – 4.49 (m, 3H), 4.19 – 4.01 (m, 2H), 3.50 (d, *J* = 5.1 Hz, 1H), 3.27 – 3.01 (m, 5H), 2.37 – 1.94 (m, 3H), 1.86 – 1.61 (m, 3H), 1.55 – 1.42 (m, 1H), 1.29 – 1.13 (m, 3H). ^{13}C NMR (100 MHz, MeOD): δ=198.39, 197.95, 176.71, 176.60, 176.09, 175.72, 173.18, 173.02, 167.77, 167.72, 154.11, 153.95, 150.20, 150.08, 149.06, 149.00, 137.94, 137.83, 137.61, 137.53, 133.92, 133.77, 130.51, 130.19, 129.68, 129.61, 128.07, 125.36, 121.78, 69.15, 68.86, 61.57, 61.49, 55.94, 55.78, 49.09, 49.07, 44.14, 43.93, 42.89, 38.87, 38.71, 38.49, 36.61, 36.51, 27.17, 27.05, 22.07, 14.56. $C_{30}H_{37}N_4O_7$ $(M+H)^+$ calcd, 565.2657; found, 565.2651. HPLC purity: 95.82%.

Preparation of Ester 12g. 12g was prepared as a yellow oil by an analogous procedure to 12a (60% yield). ^{1}H NMR (400 MHz, MeOD) δ 8.08 (d, *J* = 7.9 Hz, 1H), 7.78 (t, *J* = 7.1 Hz, 1H), 7.68 (dd, *J* = 12.8, 7.3 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.31 – 7.15 (m, 5H), 6.72 (dd, *J* = 15.7, 4.8 Hz, 1H), 5.72 – 5.60 (m, 1H), 4.67 – 4.47 (m, 3H), 4.21 – 4.08 (m, 2H), 3.29 – 2.92 (m, 6H), 2.29 (d, *J* = 3.5 Hz, 1H), 2.14 – 1.92 (m, 2H), 1.84 – 1.72 (m, 1H), 1.60 (dt, *J* = 13.8, 11.2 Hz, 2H), 1.45 (dd, *J* = 19.3, 8.9 Hz, 1H), 1.34 – 1.15 (m, 3H). ^{13}C NMR (100 MHz, MeOD) δ 201.57, 201.28, 176.76, 176.64, 175.59, 175.38, 173.15, 173.07, 167.77, 148.98, 147.38, 147.27, 138.19, 138.15, 137.84, 137.79, 135.34, 135.28, 132.28, 130.51, 130.38, 129.66, 129.58, 129.28, 129.18, 128.09, 128.06, 125.31, 121.74, 69.24, 69.09, 61.52, 56.09, 55.88, 49.12, 49.10, 47.66, 42.88, 38.75, 38.72, 38.69, 36.59, 36.56, 27.22, 27.13, 22.11, 14.56. $C_{31}H_{37}N_4O_9$ $(M+H)^+$ calcd, 609.2555; found, 609.2559. HPLC purity: 96.72%.

Scheme S3. Synthesis of **17a-g**



Reagents and conditions: a. TFA, DCM, 25°C, 4 h; b. H_2O, THF, TEA, $NaHCO_3$, CbzCl, 12 h, 75%; c. $NaBH_4$, MeOH, 25 °C, 5 h, 89 %; d. DMP, DCM, 0°C, 1 h; e. MeOH, methylorthoformate, TsOH, reflux, 0.5 h, 70%; f. H_2, Pd-C, MeOH, 12 h; g. HOBt, EDC.HCl, TEA, DCM, **11**, 65%; h. TFA, H_2O, -20 °C, 24 h, 70%.

Preparation of Ester 13. To a solution of **3** (3.0 g, 10.0 mmol) in anhydrous DCM (60 mL), CF_3COOH (10.0 mL, 67 mmol) was added slowly at 0 °C. Then, the reaction mixture was stirred at RT for 2 h and concentrated. A solution of the residue was obtained in anhydrous THF (80 mL) and Et_3N was added drop-wise to adjust the pH to 7.0 at 0 °C. H_2O (20 ml), $NaHCO_3$ (4.2 g, 50.0 mmol), and benzyl carbonochloridate were sequentially added at 0 °C. The reaction mixture was stirred at RT for 12 h, and then the mixture was extracted with Et_2O (100 ml x 3). The combined organic layer was washed with saturated citric acid solution (100 mL×3), saturated $NaHCO_3$ solution (100 mL×3) and brine (100 mL×3). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (Petroleum ether/Ethyl acetate 1:1) to afford the pure product **13** (2.4 g, 70%) as a white oil. $[\alpha]^{20}_D = -21.9$ (c = 0.92, CH_2Cl_2).^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.43 – 7.22 (m, 5H), 6.85 (s, 1H), 6.51 (d, *J* = 7.1 Hz, 1H), 5.10 (s, 2H), 4.40 – 4.28 (m, 1H), 3.71 (s, 3H), 3.22 (s, 2H), 2.32 (d, *J* = 10.4 Hz, 2H), 2.02 (s, 1H), 1.90 – 1.76 (m, 2H), 1.67 (dqt, *J* = 10.4, 7.3, 3.2 Hz, 1H), 1.53 (q, *J* = 11.3, 10.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ= 174.46, 172.74, 156.32, 136.22, 128.08, 127.64, 127.60, 66.33, 52.00, 51.93, 41.60, 37.51, 33.31, 26.15, 21.07. HRMS (ESMS)：$C_{17}H_{23}N_2O_5$ $(M+H)^+$

calcd, 335.1601; found, 335.1609.

Preparation of Alcohol 14. 14 was prepared as a white oil by an analogous procedure to **4** (70% yield). $[\alpha]^{20}_{D}$ = – 41.4 (c = 0.42, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.36 – 7.23 (m, 5H), 6.84 (s, 1H), 6.33 (d, *J* = 8.3 Hz, 1H), 5.13 – 4.99 (m, 2H), 4.28 – 4.03 (m, 1H), 3.75 (dtt, *J* = 13.4, 9.1, 4.5 Hz, 1H), 3.64 – 3.48 (m, 2H), 3.22 – 3.10 (m, 2H), 2.29 (m, 1H), 2.17 – 1.96 (m, 2H), 1.84 – 1.72 (m, 1H), 1.60 (m, 2H), 1.51 – 1.43 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ= 175.96, 156.97, 136.69, 128.46, 127.97, 127.94, 66.53, 65.35, 50.81, 42.23, 37.83, 32.91, 26.57, 21.36. HRMS (ESMS)：$C_{16}H_{23}N_2O_4$ $(M+H)^+$ calcd, 307.1652; found, 307.1653.

Preparation of Acetal 15. To a solution of **14** (1.0 g, 3.4 mmol) in DCM (30.0 mL), DMP (2.2 g, 5.1 mmol) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 1.5h. Saturated $NaHCO_3$ solution (30 mL) and sodium thiosulfate (1.2 g, 1.7 mmol) were added to quench the reaction, and the solution was stirred for 30 min at RT. Subsequently, the aqueous phase was extracted with DCM (50 mL×3). The combined organic phase was dried over anhydrous sodium sulfate, and concentrated to provide crude aldehyde as white foam. This product was used without further purification. To a solution of the crude aldehyde in $HC(OMe)_3$/MeOH (1: 4, 48 mL), TsOH (58 mg, 0.34 mmol) was added at RT. Then, the reaction was heated to reflux for 0.5 h and cooled to RT. Saturated $NaHCO_3$ solution (20 mL) was added, and the organic solvent was evaporated. Subsequently, DCM (40 mL×3) was added to extract the aqueous residue. The combined organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (Petroleum ether/Ethyl acetate 1:2) to afford the pure product **15** (0.83 g, 70%) as a white oil. $[\alpha]^{20}_{D}$ = – 37.1 (c = 0.6, CH_2Cl_2).^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.32 (dd, *J* = 13.5, 5.8 Hz, 5H), 7.02 (s, 1H), 5.80 (d, *J* = 9.2 Hz, 1H), 5.09 (s, 2H), 4.24 (d, *J* = 4.0 Hz, 1H), 3.87 (td, *J* = 9.0, 3.7 Hz, 1H), 3.39 (d, *J* = 2.4 Hz, 6H), 3.24 – 3.15 (m, 2H), 2.30 – 2.02 (m, 3H), 1.79 (ddt, *J* = 9.6, 6.1, 3.5 Hz, 1H), 1.65 – 1.41 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ= 175.54, 156.73, 136.67, 128.38, 127.87, 127.76, 106.14, 66.47, 56.03, 55.30, 50.38, 42.13, 37.53, 31.08, 26.30, 21.25. HRMS (ESMS)：$C_{18}H_{26}N_2NaO_5$$(M+Na)^+$ calcd, 373.1734; found, 373.1730.

General Procedure for the Synthesis of Acetal (16a-g): Preparation of Acetal 16c. To a solution of **10c** (99 mg, 0.26 mmol) in THF (15.0 mL), *N*-methylaniline (0.03 ml, 0.31 mmol) and

$Pd(PPh_3)_4$ (0.03g, 0.03mmol) were sequentially added. The reaction mixture was stirred at RT for 1 h, and then was concentrated. The residue was purified by column chromatography to afford crude **11c**. To a solution of **15** (0.11 g, 0.31 mmol) in degassed MeOH (5 mL), 10% Pd/C (11 mg) was added. The resulting mixture was stirred for 6 h at RT under H_2, then filtered through a pad of celite. Subsequently, methanol was removed to afford the crude amine. To a solution of the crude amine and **11c** in DCM (15.0 mL), EDC.HCl (0.11 g, 0.6 mmol) and HOBt (0.08 g, 0.6 mmol) were sequentially added. After 20 min, Et_3N (0.13 mL, 0.9 mmol) was added drop-wise. The reaction mixture was stirred at RT for 3 h, followed by washing with saturated citric acid solution (10 mL×3), saturated $NaHCO_3$ solution (10 mL×3) and brine (10 mL×3). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (MeOH: DCM = 1: 40 v/v) to afford the pure product **16c** (56 mg, 50%) as a brown oil. ^{1}H NMR (400 MHz, MeOD) δ: 8.62 (d, *J* = 5.1 Hz, 2H), 7.92 (d, *J* = 8.7 Hz, 1H), 7.73-7.66 (m, 2H), 7.20 – 7.09 (m, 5H), 4.63 – 4.40 (m, 2H), 4.08 – 3.96 (m, 2H), 3.31 – 3.17 (m, 8H), 3.12-3.03(m, 3H), 2.93 (ddd, *J* = 13.7, 10.5, 8.1 Hz, 1H), 2.13 – 1.85 (m, 3H), 1.66 (tt, *J* = 9.4, 4.5 Hz, 1H), 1.56 – 1.43 (m, 2H), 1.36 – 1.27 (m, 1H). ^{13}C NMR (100 MHz, MeOD) δ: 198.68, 198.38, 177.07, 177.04, 175.62, 175.44, 173.37, 173.32, 151.41, 151.37, 144.70, 144.63, 138.10, 138.08, 130.61, 130.40, 129.52, 129.47, 127.84, 122.79, 122.76, 107.14, 69.01, 68.79, 56.44, 56.36, 55.62, 55.59, 55.49, 55.36, 49.63, 49.51, 44.40, 44.21, 42.90, 39.01, 38.72, 38.26, 38.23, 31.69, 26.84, 21.94, 21.86. HRMS (ESMS)：$C_{28}H_{36}N_4NaO_7$ $(M+Na)^+$ calcd, 563.2476; found, 563.2470.

Preparation of Acetal 16a. 16a was prepared as a yellow oil by an analogous procedure to **16c** (60% yield). ^{1}H NMR (400 MHz, MeOD) : δ= 7.35-7.30 (m, 5H), 4.74 (s, 1H), 4.38 (t, *J* = 13.1 Hz, 1H), 4.20-4.26 (m, 2H), 3.54 – 2.92 (m, 12H), 2.32 – 2.07 (m, 3H), 1.88 (s, 1H), 1.71 (dd, *J* = 25.4, 12.5 Hz, 2H), 1.54 (d, *J* = 10.3 Hz, 1H), 1.19 (s, 9H). ^{13}C NMR (100 MHz, MeOD) : δ= 215.03, 214.61, 177.22, 177.17, 176.35, 176.26, 173.52, 173.45, 138.19, 130.64, 130.43, 129.57, 129.46, 127.89, 107.25, 107.18, 69.01, 68.85, 56.44, 56.18, 55.55, 55.51, 55.31, 49.86, 49.71, 45.07, 45.01, 42.96, 42.31, 42.10, 39.07, 38.64, 38.38, 31.90, 31.65, 26.92, 26.49, 22.02, 21.95. HRMS (ESMS)：$C_{27}H_{42}N_3O_7$ $(M+H)^+$ calcd, 520.3017; found, 520.3012.

Preparation of Acetal 16b. 16b was prepared as a yellow oil by an analogous procedure to **16c**

(60% yield). ^{1}H NMR (400 MHz, MeOD) : δ=8.58 (dd, *J* = 19.5, 10.7 Hz, 1H), 8.12 – 7.99 (m, 2H), 7.99 – 7.85 (m, 2H), 7.62 – 7.49 (m, 3H), 7.36 – 7.15 (m, 5H), 4.81 – 4.56 (m, 2H), 4.14 (dd, *J* = 14.3, 5.9 Hz, 2H), 3.56 – 2.97 (m, 12H), 2.30 – 1.95 (m, 3H), 1.77-1.51 (m, 3H), 1.49 – 1.38 (m, 1H).^{13}C NMR (100 MHz, MeOD) : δ=203.32, 203.15, 177.21, 177.15, 176.16, 175.93, 173.53, 173.47, 138.16, 138.07, 135.33, 133.94, 133.84, 131.24, 131.21, 130.61, 130.43, 129.56, 129.48, 129.29, 129.21, 128.84, 128.73, 127.88, 127.86, 127.51, 126.87, 126.82, 125.57, 107.22, 107.13, 69.68, 69.48, 56.48, 56.19, 55.72, 55.54, 55.34, 49.67, 49.64, 47.51, 47.26, 42.89, 39.01, 38.78, 38.32, 31.98, 31.57, 26.91, 26.85, 21.85. HRMS (ESMS)：$C_{33}H_{40}N_3O_7$ $(M+H)^+$ calcd, 590.2861; found, 590.2865.

Preparation of Acetal 16d. 16d was prepared as a yellow oil by an analogous procedure to **16c** (60% yield). ^{1}H NMR (400 MHz, MeOD) : δ=8.08 – 7.80 (m, 2H), 7.44 – 7.20 (m, 6H), 4.74 (s, 1H), 4.61 (dd, *J* = 25.3, 21.8 Hz, 1H), 4.30 – 4.13 (m, 2H), 3.47 – 3.34 (m, 7H), 3.33 – 3.00 (m, 5H), 2.34 – 2.03 (m, 3H), 1.86 (s, 1H), 1.68 (dd, *J* = 27.3, 13.5 Hz, 2H), 1.53 (d, *J* = 10.2 Hz, 1H).^{13}C NMR (100 MHz, MeOD) : δ=192.24, 192.05, 177.27, 175.93, 175.70, 173.54, 173.46, 145.28, 145.23, 138.23, 138.18, 135.77, 135.64, 134.51, 134.35, 130.66, 130.43, 129.59, 129.53, 129.46, 127.91, 107.25, 69.44, 69.20, 56.45, 56.17, 55.73, 55.69, 55.56, 55.44, 49.80, 49.71, 44.68, 44.39, 42.99, 39.04, 38.70, 38.43, 32.00, 31.62, 26.96, 22.01. HRMS (ESMS)：$C_{27}H_{36}N_3O_7$ S$(M+H)^+$ calcd, 546.2268; found, 546.2273.

Preparation of 16e-1. 16e-1 was prepared as a yellow oil by an analogous procedure to **16c** (60% yield). $[\alpha]^{20}_D$ = − 6.5 (c = 0.08, CH_3OH). ^{1}H NMR (400 MHz, MeOD) : δ=7.97 (t, *J* = 12.4 Hz, 2H), 7.57 (s, 1H), 7.46 (s, 2H), 7.23 (d, *J* = 25.5 Hz, 5H), 4.67 (s, 1H), 4.48 (d, *J* = 3.0 Hz, 1H), 4.16 (t, *J* = 14.0 Hz, 2H), 3.38 (s, 2H), 3.29 (d, *J* = 10.8 Hz, 6H), 3.17 (d, *J* = 16.4 Hz, 3H), 3.10 – 3.00 (m,1H), 2.18 (dd, *J* = 27.4, 14.3 Hz, 2H), 2.02 (s, 1H), 1.77 (s, 1H), 1.72 – 1.57 (m, 2H), 1.44 (d, *J* = 10.3 Hz, 1H).^{13}C NMR (100 MHz, MeOD) : δ=199.45, 177.23, 176.20, 173.56, 138.21, 138.18, 134.55, 130.43, 129.73, 129.59, 129.31, 127.90, 107.28, 69.07, 56.27, 55.71, 55.44, 49.77, 43.85, 42.97, 38.67, 38.40, 31.81, 26.94, 22.01. HRMS (ESMS)：$C_{29}H_{38}N_3O_7(M+H)^+$ calcd, 540.2704; found, 540.2710.

Preparation of 16e-2. 16e-2 was prepared as a yellow oil by an analogous procedure to **16c** (60% yield). $[\alpha]^{20}_D$ = − 21.5 (c = 0.12, CH_3OH). ^{1}H NMR (400 MHz, MeOD) : δ=7.95 – 7.89 (m, 2H),

7.59 (t, *J* = 7.4 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.35 – 7.21 (m, 5H), 4.72 (dt, *J* = 8.5, 5.7 Hz, 1H), 4.61 (dd, *J* = 8.3, 3.4 Hz, 1H), 4.18 – 4.06 (m, 2H), 3.34 (d, *J* = 11.3 Hz, 6H), 3.26 – 2.95 (m, 6H), 2.28 – 1.94 (m, 3H), 1.83 – 1.72 (m, 1H), 1.70 – 1.55 (m, 2H), 1.42 (ddd, *J* = 24.6, 13.7, 7.3 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) : δ=199.13, 177.20, 175.97, 173.40, 134.44, 130.69, 130.67, 129.71, 129.51, 129.46, 129.22, 127.87, 107.23, 69.22, 56.44, 56.18, 55.51, 49.56, 44.14, 42.94, 39.07, 38.33, 31.57, 26.93, 21.92. HRMS (ESMS)：$C_{29}H_{38}N_3O_7(M+H)^+$ calcd, 540.2704; found, 540.2711.

Preparation of Acetal 16f. 16f was prepared as a brown oil by an analogous procedure to **16c** (60% yield). ^{1}H NMR (400 MHz, MeOD) : δ= 9.07 (d, *J* = 22.7 Hz, 1H), 8.73 (s, 1H), 8.33 (dd, *J* = 24.8, 7.4 Hz, 1H), 8.01 (s, 1H), 7.56 (s, 1H), 7.26 (t, *J* = 16.8 Hz, 5H), 4.76 – 4.52 (m, 2H), 4.21 – 4.05 (m, 2H), 3.78 – 3.39 (m, 2H), 3.35 (dd, *J* = 19.9, 9.9 Hz, 6H), 3.19 (dd, *J* = 24.0, 6.5 Hz, 3H), 3.06 (dt, *J* = 21.8, 10.3 Hz, 1H), 2.31 – 1.96 (m, 3H), 1.79 (s, 1H), 1.61 (dd, *J* = 24.3, 11.4 Hz, 2H), 1.52 – 1.41 (m, 1H). ^{13}C NMR (100 MHz, MeOD) : δ=198.20, 197.88, 177.17, 177.14, 175.83, 175.63, 173.48, 173.42, 154.06, 153.96, 150.18, 150.10, 138.20, 138.18, 137.55, 137.47, 133.99, 133.95, 130.66, 130.43, 129.56, 129.51, 127.88, 107.24, 69.14, 68.93, 56.45, 56.37, 55.78, 55.68, 55.53, 55.40, 49.64, 49.58, 44.37, 44.16, 42.95, 39.05, 38.72, 38.37, 31.76, 31.62, 26.95, 22.01, 21.94. HRMS (ESMS)：$C_{28}H_{37}N_4O_7(M+H)^+$ calcd, 541.2657; found, 541.2652.

Preparation of Acetal 16g. 16g was prepared as a brown oil by an analogous procedure to **16c** (60% yield). ^{1}H NMR (400 MHz, MeOD) δ 8.09 (d, *J* = 8.1 Hz, 1H), 7.83 – 7.74 (m, 1H), 7.68 (t, *J* = 7.8 Hz, 1H), 7.58 (dd, *J* = 13.8, 7.6 Hz, 1H), 7.35 – 7.13 (m, 5H), 4.65 (t, *J* = 6.0 Hz, 1H), 4.51 (dd, *J* = 22.4, 6.2 Hz, 1H), 4.11 (t, *J* = 10.2 Hz, 2H), 3.32 (dd, *J* = 17.2, 8.8 Hz, 6H), 3.19– 2.87 (m, 6H), 2.26 – 1.94 (m, 3H), 1.81 – 1.70 (m, 1H), 1.68 – 1.51 (m, 2H), 1.49 – 1.35 (m, 1H). ^{13}C NMR (100 MHz, MeOD) δ 201.51, 201.24, 177.25, 177.18, 175.53, 175.34, 173.43, 173.40, 147.41, 147.34, 138.29, 138.18, 138.12, 138.07, 135.33, 135.27, 132.28, 130.61, 130.44, 129.57, 129.48, 129.27, 129.20, 127.91, 127.87, 125.31, 107.22, 107.17, 69.21, 69.09, 56.46, 56.22, 55.77, 55.52, 55.45, 49.51, 49.46, 47.77, 42.93, 39.04, 38.83, 38.30, 31.81, 31.56, 26.92, 26.89, 21.96, 21.92. HRMS (ESMS)：$C_{29}H_{37}N_4O_9(M+H)^+$ calcd, 585.2555; found, 585.2557.

General Procedure for the Synthesis of Aldehyde (17a-g): Preparation of Aldehyde 17c. A solution of **16c** (30 mg, 0.056 mmol) in TFA/H_2O (2:1, 3 mL), was stirred at – 20 °C for 24 h. The

mixture was diluted with DCM (40ml) and saturated $NaHCO_3$ solution was added slowly, to adjust the pH to 7.0 at 0 °C. The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (MeOH: DCM = 1: 30 v/v) to afford the pure product **17c** (17 mg, 60%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.25 (d, *J* = 11.3 Hz, 1H), 8.68 (dd, *J* = 11.4, 5.4 Hz, 2H), 7.58 (dd, *J* = 5.6, 4.5 Hz, 2H), 7.38 – 7.48 (m, 1H),7.23 – 7.09 (m, 5H), 6.47 – 6.58 (m, 1H), 4.88 – 4.46 (m, 2H), 4.23 – 3.95 (m, 1H), 3.45 – 2.93 (m, 6H), 2.05 – 1.83 (m, 3H), 1.81 – 1.52 (m, 3H), 1.48 – 1.37 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 200.49, 198.39, 197.67, 175.59, 175.30, 173.42, 173.17, 171.97, 171.83, 150.95, 142.41, 142.24, 136.59, 136.54, 129.56, 129.41, 128.65, 128.62, 127.03, 126.99, 121.17, 68.30, 68.20, 58.20, 57.77, 53.89, 53.48, 43.05, 42.91, 42.28, 38.23, 37.91, 37.77, 37.53, 30.73, 30.68, 28.12, 27.78, 21.29, 21.26. HRMS (ESMS)：$C_{26}H_{31}N_4O_6$ $(M+H)^+$ calcd, 495.2238; found, 495.2232. HPLC purity: 99.43%.

Preparation of Aldehyde 17a. 17a was prepared as a yellow oil by an analogous procedure to **17c** (70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) : δ= 9.31 (d, *J* = 9.1 Hz, 1H), 8.40 (d, *J* = 6.8 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 7.32 – 7.16 (m, 5H), 6.49 (s, 1H), 4.84 (dt, *J* = 15.1, 6.7 Hz, 1H), 4.68 (s, 1H), 4.44 – 4.23 (m, 2H), 3.32 – 3.10 (m, 4H), 3.08 – 2.94 (m, 2H), 2.16 – 1.91 (m, 3H), 1.90 – 1.62 (m, 3H), 1.52 (dd, *J* = 19.8, 9.8 Hz, 1H), 1.10 (d, *J* = 7.4 Hz, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) : δ= 216.29, 215.20, 200.73, 186.78, 175.61, 175.35, 173.80, 173.65, 172.09, 171.95, 136.70, 129.59, 129.50, 128.67, 128.63, 127.02, 68.56, 68.45, 57.98, 57.57, 54.06, 53.65, 44.33, 44.29, 42.31, 40.75, 40.64, 38.06, 37.95, 37.71, 37.56, 30.78, 27.88, 27.52, 26.26, 26.17, 21.23, 21.17. HRMS (ESMS)：$C_{25}H_{36}N_3O_6$$(M+H)^+$ calcd, 474.2599; found, 474.2594. HPLC purity: 98.90%.

Preparation of Aldehyde 17b. 17b was prepared as a yellow oil by an analogous procedure to **17c** (70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) : δ=9.32 (d, *J* = 7.3 Hz, 1H), 8.74 – 8.52 (m, 2H), 7.92 (ddd, *J* = 24.0, 16.2, 8.2 Hz, 3H), 7.62 – 7.35 (m, 4H), 7.30 – 7.15 (m, 5H), 6.27 (d, *J* = 18.6 Hz, 1H), 5.00 – 4.56 (m, 2H), 4.11 (ddd, *J* = 14.5, 11.1, 4.9 Hz, 1H), 3.68 – 2.97 (m, 6H), 2.18 (s, 1H), 2.00 – 1.83 (m, 2H), 1.81 – 1.56 (m, 3H), 1.47 (dd, *J* = 21.8, 11.6 Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) : δ=203.15, 202.43, 201.30, 200.76, 175.69, 175.45, 173.93, 173.66, 172.10, 172.07, 136.64, 136.60, 135.02, 134.68, 133.98, 133.95, 133.46, 133.32, 130.13, 129.55, 129.45, 129.39,

128.85, 128.64, 128.61, 128.55, 128.23, 128.17, 126.96, 126.62, 125.83, 125.79, 124.47, 69.08, 68.98, 58.08, 57.91, 54.10, 53.72, 45.60, 45.56, 42.17, 38.04, 37.83, 37.70, 37.52, 30.81, 30.71, 27.86, 27.62, 21.10, 20.98. HRMS (ESMS)：$C_{31}H_{34}N_3O_6(M+H)^+$ calcd, 544.2442; found, 544.2447. HPLC purity: 98.64%.

Preparation of Aldehyde 17d. 17d was prepared as a yellow oil by an analogous procedure to **17c** (70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (d, *J* = 5.8 Hz, 1H), 8.58 – 8.46 (m, 1H), 7.66 – 7.50 (m, 2H), 7.26 – 7.07 (m, 5H), 7.02 (ddd, *J* = 25.9, 15.2, 10.7 Hz, 1H), 6.66 (d, *J* = 26.4 Hz, 1H), 4.86 – 4.42 (m, 2H), 4.20 – 4.02 (m, 1H), 3.27 – 2.88 (m, 6H), 2.08 – 1.78 (m, 3H), 1.77 – 1.48 (m, 3H), 1.37 (dd, *J* = 22.1, 11.1 Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 200.89, 200.78, 192.21, 191.44, 175.79, 175.55, 173.71, 173.53, 172.12, 172.07, 143.75, 143.51, 136.74, 134.76, 134.57, 133.45, 133.13, 129.61, 129.47, 128.66, 128.54, 128.48, 126.99, 68.59, 58.06, 57.71, 54.23, 53.83, 43.06, 42.25, 38.04, 37.76, 37.71, 37.44, 30.84, 30.73, 27.87, 27.53, 21.17, 21.10. HRMS (ESMS)：$C_{25}H_{30}N_3O_6S(M+H)^+$ calcd, 500.1850; found, 500.1846. HPLC purity: 98.65%.

Preparation of (2S,2'S,5S,8R)-17e. (2S,2'S,5S,8R)-17e was prepared as a yellow oil by an analogous procedure to **17c** (70% yield). $[\alpha]^{20}_D$ = − 5.3 (c = 0.12, CH_3Cl). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.33 (S, 1H), 8.54 (d, *J* = 6.5 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.61 – 7.50 (m, 2H), 7.42 (p, *J* = 7.4 Hz, 2H), 7.21 (tt, *J* = 14.3, 6.9 Hz, 5H), 6.53 (s, 1H), 4.86 (dd, *J* = 14.9, 6.6 Hz, 1H), 4.55 (dd, *J* = 6.8, 3.6 Hz, 1H), 4.23 (dq, *J* = 10.9, 6.5 Hz, 1H), 3.56 – 3.06 (m, 6H), 2.19 – 1.87 (m, 3H), 1.85-1.53 (m, 3H), 1.51 – 1.41 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.76, 199.38, 175.36, 173.57, 171.98, 136.70, 136.30, 133.81, 129.47, 128.78, 128.65, 128.35, 126.98, 68.62, 57.64, 54.05, 42.55, 42.28, 37.77, 37.53, 30.83, 27.56, 21.15. HRMS (ESMS)：$C_{27}H_{32}N_3O_6(M+H)^+$ calcd, 494.2286; found, 494.2283. HPLC purity: 97.95%.

Preparation of (2S,2'S,5S,8S)-17e. (2S,2'S,5S,8S)-17e was prepared as a yellow oil by an analogous procedure to **17c** (70% yield). $[\alpha]^{20}_D$ = − 27.2 (c = 0.15, CH_3Cl). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.62 (s, 1H), 7.80 (d, *J* = 7.0 Hz, 2H), 7.48 (d, *J* = 7.6 Hz, 2H), 7.42 – 7.31 (m, 2H), 7.17 (dd, *J* = 14.9, 7.1 Hz, 5H), 6.72 (s, 1H), 4.85 (d, *J* = 5.3 Hz, 1H), 4.65 (d, *J* = 8.2 Hz, 1H), 4.07 (s, 1H), 3.35 – 2.79 (m, 6H), 1.96-1.80 (m, 2H), 1.75 – 1.31 (m, 5H).^{13}C NMR (100 MHz, $CDCl_3$) δ 200.74, 198.52, 175.76, 173.85, 172.10, 136.78, 136.48, 133.66, 129.59, 128.74, 128.59, 128.27, 126.90, 68.39, 58.06, 53.66, 42.56, 42.30, 38.05, 37.76, 30.69, 27.93, 21.20.

HRMS (ESMS)：$C_{27}H_{32}N_3O_6(M+H)^+$ calcd, 494.2286; found, 494.2279. HPLC purity: 99.07%.

Preparation of Aldehyde 17f. 17f was prepared as a brown oil by an analogous procedure to **17c** (70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (d, *J* = 10.0 Hz, 1H), 8.97 (dd, *J* = 9.3, 7.5 Hz, 1H), 8.61 (ddd, *J* = 13.9, 6.9, 4.6 Hz, 1H), 8.11 – 7.99 (m, 1H), 7.49 (ddd, *J* = 20.0, 17.5, 8.8 Hz, 1H), 7.28 (td, *J* = 8.5, 5.0 Hz, 1H), 7.22 – 7.03 (m, 5H), 6.68 (d, *J* = 24.8 Hz, 1H), 4.85 – 4.46 (m, 2H), 4.22 – 3.98 (m, 1H), 3.40 – 3.20 (m, 2H), 3.17 – 3.00 (m, 4H), 2.18 – 1.79 (m, 3H), 1.73 – 1.48 (m, 3H), 1.45 – 1.35 (m, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 200.60, 197.97, 197.24, 175.58, 175.35, 173.78, 173.56, 172.08, 171.99, 153.65, 153.62, 149.75, 149.66, 136.65, 136.63, 135.76, 135.66, 131.99, 131.90, 129.56, 129.41, 128.62, 128.59, 126.96, 123.79, 123.73, 68.27, 68.16, 57.93, 57.54, 54.08, 53.73, 42.93, 42.89, 42.19, 41.96, 37.96, 37.75, 37.66, 37.45, 30.75, 30.69, 27.75, 27.42, 21.13, 21.09. HRMS (ESMS)：$C_{26}H_{31}N_4O_6(M+H)^+$ calcd, 495.2238; found, 495.2243. HPLC purity: 96.76%.

Preparation of Aldehyde 17g. 17g was prepared as a brown oil by an analogous procedure to **17c** (60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.38 – 9.31 (m, 1H)，9.15 – 8.90 (m, 1H), 8.15 – 8.05 (m, 1H), 7.74 (q, *J* = 7.8 Hz, 1H), 7.67 – 7.59 (m, 1H), 7.50 – 7.35 (m, 2H), 7.32 – 7.12 (m, 5H), 6.76 – 6.55 (m, 1H), 4.97 – 4.79 (m, 2H), 4.77 – 4.55 (m, 1H), 4.05 (ddd, *J* = 27.3, 12.0, 7.2 Hz, 1H), 3.52 – 3.38 (m, 1H), 3.36 – 3.15 (m, 5H), 2.12 – 1.78 (m, 4H), 1.76 – 1.47 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.15, 201.06, 200.44, 176.18, 175.77, 173.24, 173.10, 172.07, 172.04, 145.87, 145.31, 137.47, 137.01, 136.86, 136.57, 134.94, 134.31, 131.03, 130.86, 129.50, 129.42, 128.71, 128.58, 127.93, 127.77, 127.02, 126.85, 124.45, 68.39, 68.27, 58.83, 58.69, 53.58, 53.51, 46.72, 46.35, 42.42, 42.24, 38.69, 38.58, 37.42, 37.28, 31.06, 30.48, 28.78, 28.50, 21.41, 21.30. HRMS (ESMS)：$C_{27}H_{31}N_4O_8(M+H)^+$ calcd, 539.2136; found, 539.2133. HPLC purity: 95.67%.

Part B. Determination of the Absolute Configuration for (6S, 9R)-10e and (6S, 9S)-10e by Modified Mosher's Method

Scheme S4. Synthesis of *R*- and *S*-Mosher esters from compound **10e-2**

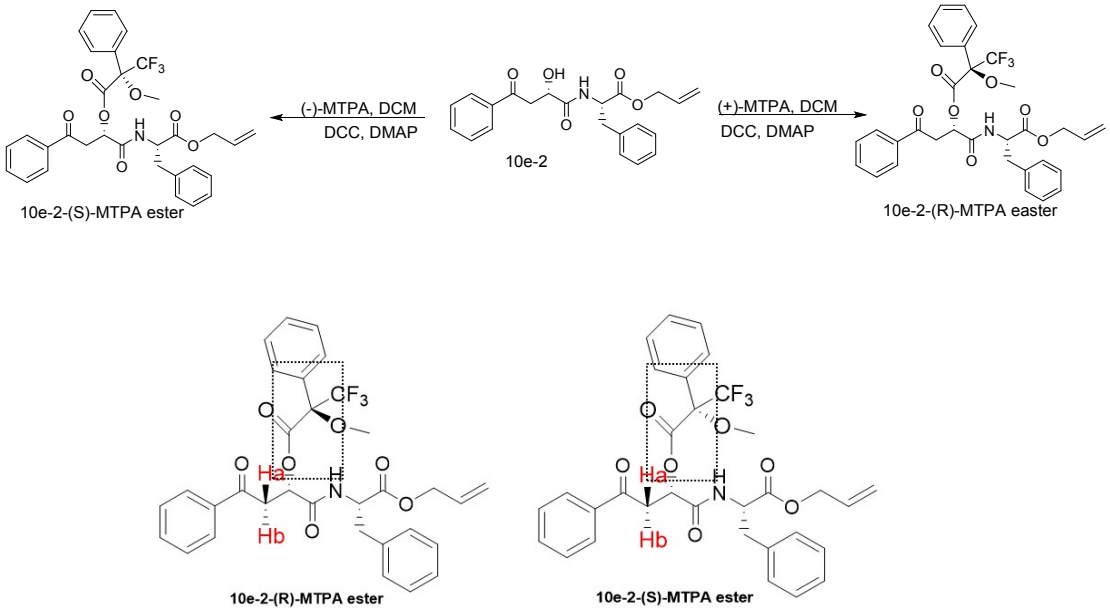


Figure S1. The MTPA plane of *S*- and *R*-MTPA esters derived from **10e-2**

Modified Mosher's method is effective in determination of the absolute configurations of secondary alcohols[2]. This method relies on the fact that the protons in diastereomeric MTPA-esters display different chemical shifts in their ^{1}H NMR spectra. The diastereomeric R- and S-MTPA esters of **10e-2** were prepared from (R)-MTPA-OH and (S)-MTPA-OH, respectively. The H_a and H_b proton signal of each diastereomeric Mosher ester were assigned by ^{1}H-^{1}H COSY experiments. The chemical shift of H_a in R-MTPA ester was δ_R =3.63, while the chemical shift of H_a in S-MTPA ester was δ_S =3.60. Hence, the $\delta_{SR} = \delta_S - \delta_R$ = 3.60-3.63 = -0.03 was negative (Figure S1). The chemical shift of H_b in R-MTPA ester was δ_R =3.39, while the chemical shift of H_b in S-MTPA ester as δ_S =3.35. The $\delta_{SR} = \delta_S - \delta_R$ = 3.35-3.39 = -0.04 was negative (Figure S1). According to the Mosher empirical rule, the absolute configuration of the Primary alcohol in **10e-2** was S-. At the same time, the absolute configuration of the primary alcohol in **10e-1** was R-.

Preparation of 10e-2-(R)-MTPA ester. To a solution of the **10e-2** (50.00 mg, 0.13 mmol) in DCM (10 mL), (R)-MTPA-OH (45.67 mg, 0.20 mmol), DMAP (1.60 mg, 0.013 mmol), and DCC (41.20 g, 0.20 mmol) was added. The reaction mixture was stirred at 25 °C for 1 h. The organic phase was concentrated, and the residue was purified by column chromatography (EtOAc: Petroleum ether, 1: 6 v/v) to afford the pure product **10e-2-(R)-MTPA ester** (62.00 mg, 80%) as a white oil. $[\alpha]^{20}_D$ = 28.5 (c = 0.38, CH_2Cl_2).^{1}H NMR (400 MHz, MeOD): δ= 7.94 (d, *J* = 7.5 Hz, 2H), 7.64 – 7.57 (m, 1H), 7.52 – 7.45 (m, 4H), 7.40 – 7.20 (m, 8H), 5.93 – 5.78 (m, 2H), 5.33 –

5.16 (m, 2H), 4.75 (dd, J = 8.2, 5.8 Hz, 1H), 4.59 (d, J = 5.7 Hz, 2H), 3.63 (dd, J = 18.1, 9.7 Hz, 1H), 3.46 (s, 3H), 3.39 (dd, J = 18.1, 2.6 Hz, 1H), 3.18 (dd, J = 13.9, 5.7 Hz, 1H), 3.03 (dd, J = 13.9, 8.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD): δ= 196.69, 172.14, 170.70, 166.88, 137.68, 137.50, 134.87, 133.44, 133.11, 130.74, 130.41, 129.87, 129.60, 129.34, 129.20, 128.71, 128.01, 124.60 (q, J_{C-F} = 286.0 Hz), 119.13, 85.87 (q, J_{C-F} = 27.7 Hz), 72.31, 67.04, 56.31, 55.28, 41.07, 38.27.HRMS (ESMS)：$C_{32}H_{31}F_3NO_7$ $(M+H)^+$ calcd, 598.2047; found, 598.2042.

Preparation of 10e-2-(S)-MTPA ester. 10e-2-(S)-MTPA ester was prepared from **10e-2** and (S)-MTPA-OH, by an analogous procedure to **10e-2-(R)-MTPA ester** (66% yield). $[\alpha]^{20}_D$ = 1.9 (c = 0.24, CH_2Cl_2). ^{1}H NMR (400 MHz, MeOD): δ= 7.86 (d, J = 7.7 Hz, 2H), 7.62 – 7.43 (m, 5H), 7.35 – 7.16 (m, 8H), 5.94 – 5.81 (m, 2H), 5.25 (dd, J = 35.9, 13.9 Hz, 2H), 4.78 (dd, J = 7.8, 6.1 Hz, 1H), 4.59 (d, J = 5.7 Hz, 2H), 3.60 (dd, J = 17.9, 9.4 Hz, 1H), 3.50 (s, 3H), 3.35 (dd, J = 17.8, 2.7 Hz, 1H), 3.19 (dd, J = 13.9, 5.7 Hz, 1H), 3.09 (dd, J = 13.9, 8.1 Hz, 1H). ^{13}C NMR (100 MHz, MeOD): δ= 196.30, 172.17, 170.70, 166.85, 137.59, 137.43, 134.69, 133.28, 133.04, 130.63, 130.37, 129.73, 129.56, 129.26, 129.10, 128.69, 127.97, 124.58 (q, J_{C-F} = 286.0 Hz), 119.07, 85.87 (q, J_{C-F} = 27.5 Hz), 72.19, 67.01, 56.23, 55.17, 41.00, 38.22. HRMS (ESMS)：$C_{32}H_{31}F_3NO_7$ $(M+H)^+$ calcd, 598.2047; found, 598.2038.

Part C. The Sequence of 3C Protease

MGSSHHHHHHSSGLVPRGSHMGPSLDFALSLLRRNVRQVQTDQGHFTMLGVRDRLAVL
PRHSQPGKTIWIEHKLVNVLDAVELVDEQGVNLELTLITLDTNEKFRDITKFIPENISTASD
ATLVINTEHMPSMFVPVGDVVQYGFLNLSGKPTHRTMMYNFPTKAGQCGGVVTSVGKII
GIHIGGNGRQGFCAGLKRSYFASEQ

MW [avg] = 22353.76 Da

Part D. General Procedure of Protein Reaction

LBB (**8**) was dissolved into DMSO to afford a solution with the concentration of 240 mM. This solution was added into the solution of protease in PB buffer (25 mM, pH= 7.0) to afford final solution with 3C protease (40 μM) and probe **8** (2.4 mM). The final solution was stood at room temperature for 12 hours. After that, the small molecules were removed by three times ultra-

filtration. The remained modified 3C protease was determined by LC-ESI-Orbitrap MS.

The solution of commercial available ketone was dissolved into DMSO to afford a solution with the ketone concentration of 2.0 M. The solution of ketone was injected into a solution of probe-modified 3C protease to afford final solution with 3C protease (40 μM) and ketone (80 mM). The mixture was stood at room temperature for 12 hours. After that, the small molecules and precipitate were removed by three times ultra-filtration. The remained protease was determined by LC-ESI-Orbitrap MS.

Part E. The LC−MS/MS Analysis of Modified Unique Peptide from Trypsin Digestion[3]

The gel bands of proteins were excised from the gel, reduced with DTT (5 mM) and alkylated with iodoacetamide (10 mM). Then, the gel digestion was carried out with sequencing grade modified trypsin (10 μL in 50 mM ammonium bicarbonate) at 37°C overnight. The residues were extracted twice with 0.1% trifluoroacetic acid in 50% acetonitrile aqueous solution for 30 min. Extracts were centrifuged to reduce the volume. For LC-MS/MS analysis, peptides were separated by the 120 min gradient elution at a flow rate 0.30 μL/min with a Thermo-Dionex Ultimate 3000 HPLC system, which was directly interfaced with a Thermo Scientific Q Exactive mass spectrometer (Bremen, Germany). The analytical column was a silica capillary column (75 μm ID, 150 mm length) packed with C-18 resin (100 Å, 2 μm). Mobile phase A consisted of 0.1% formic acid, and mobile phase B consisted of 80% acetonitrile and 0.08% formic acid. The Q Exactive mass spectrometer was operated in the data-dependent acquisition mode using Xcalibur 2.1.2 software and there was a single full-scan mass spectrum in the orbitrap (300-1800 *m/z*, 70,000 resolution) followed by 20 data-dependent MS/MS scans at 27% normalized collision energy (HCD). The MS/MS spectra from each LC-MS/MS run were searched against the fasta database using an in-house Proteome Discoverer (Version PD1.4, Thermo-Fisher Scientific, USA). The search criteria were as following: full tryptic specificity was required; one missed cleavage was allowed; carbamidomethylation (C) was set as the static modifications; Deamidated (N, Q), oxidation (M) and $C_4H_2O_3S$(N-terminus) were set as the dynamic modifications; precursor ion mass tolerances were set at 20 ppm for all MS acquired in an orbitrap mass analyzer; and the fragment ion mass tolerance was set at 20 mmu for all MS2 spectra acquired. The peptide false discovery rate (FDR) was calculated using Percolator provided by PD. When the q value was

smaller than 1%, the peptide spectrum match (PSM) was considered to be correct. FDR was determined based on PSMs when searched against the reverse, decoy database. Peptides only assigned to a given protein group were considered as unique. The false discovery rate (FDR) was also set to 0.01 for protein identifications.

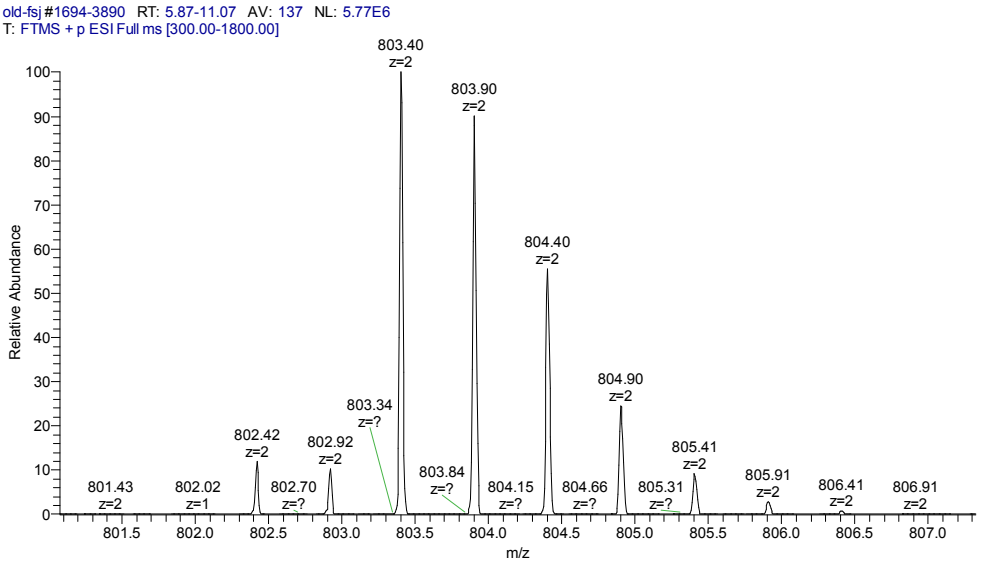


Figure S2. LC-MS / MS primary spectrum of modified $3C^{pro}$ peptides with LBB (**8**) from trypsin digestion

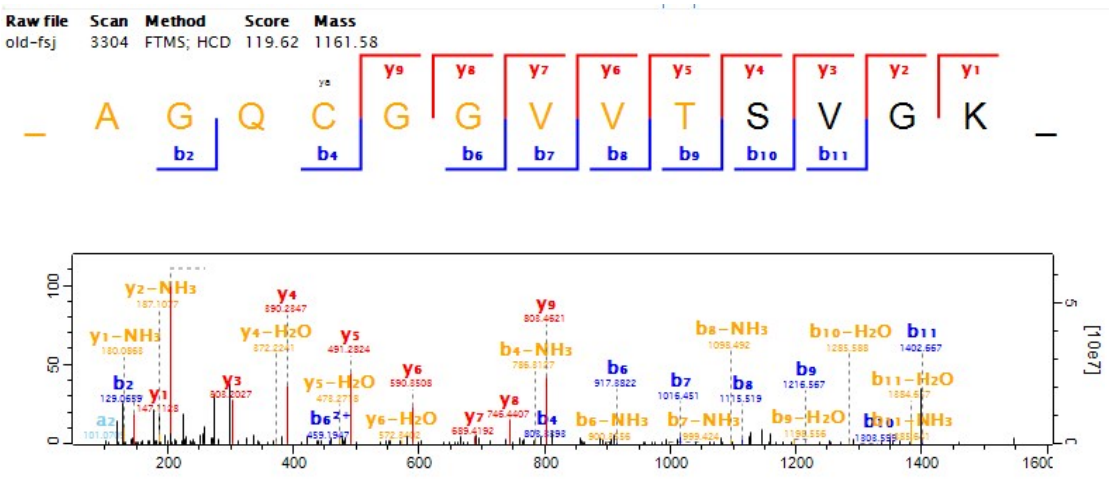


Figure S3. LC-MS / MS secondary spectrum of modified $3C^{pro}$ peptides with LBB (**8**) from trypsin digestion

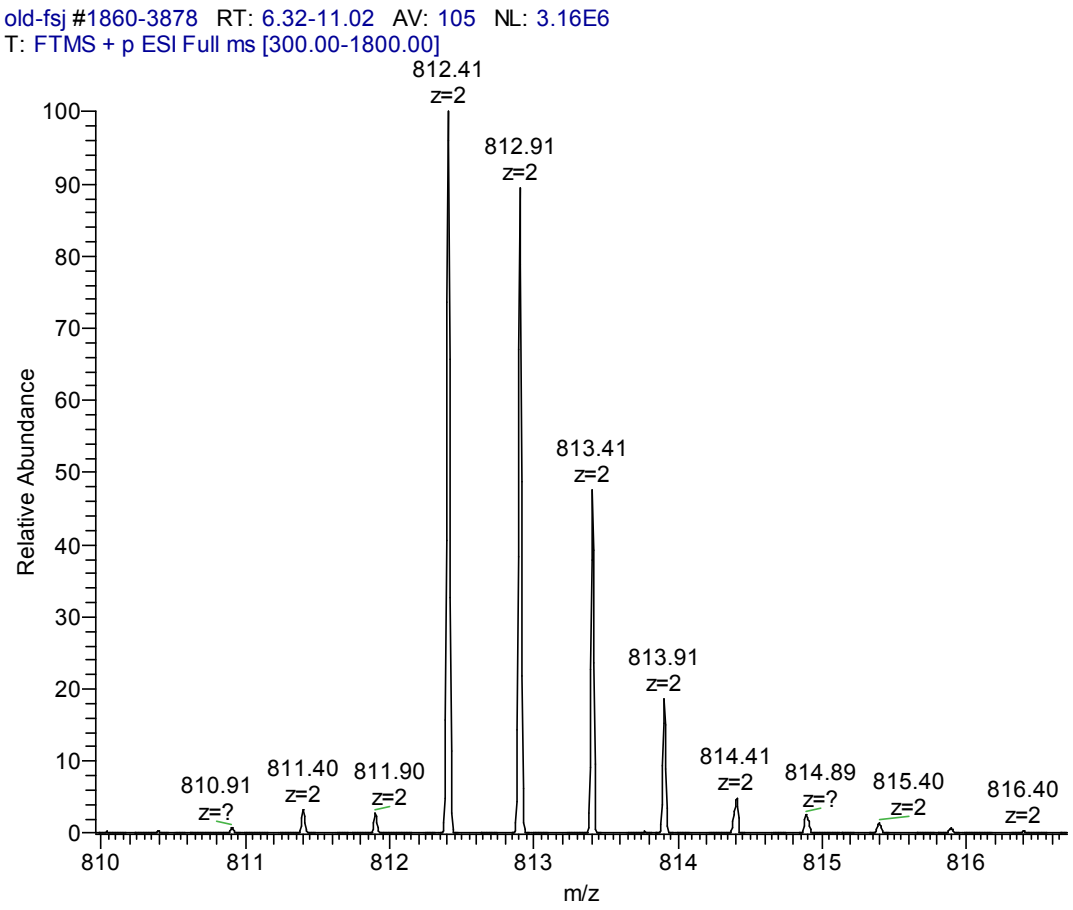


Figure S4. LC-MS / MS primary spectrum of modified $3C^{pro}$ peptides with LBB (**8**+H_2O) from trypsin digestion

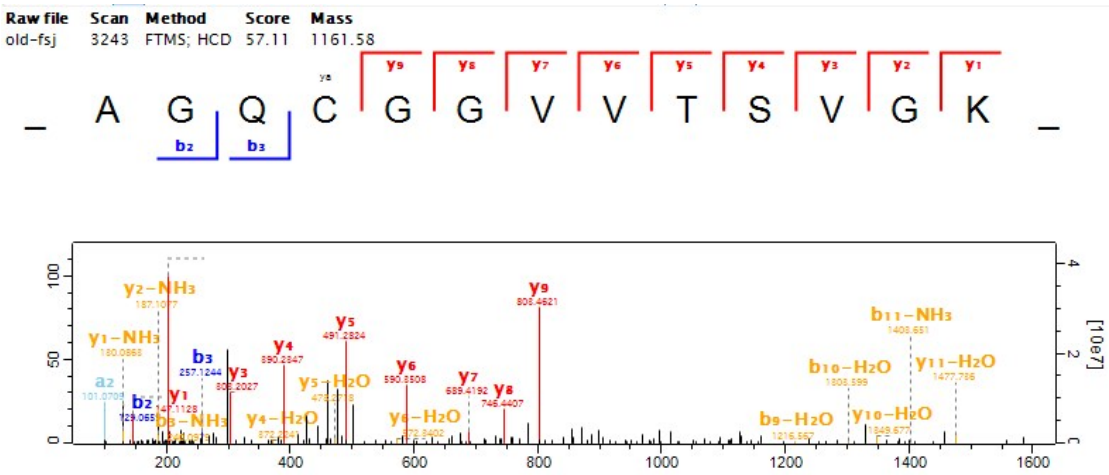


Figure S5. LC-MS / MS secondary spectrum of modified $3C^{pro}$ peptides with LBB (**8**+H_2O) from trypsin digestion

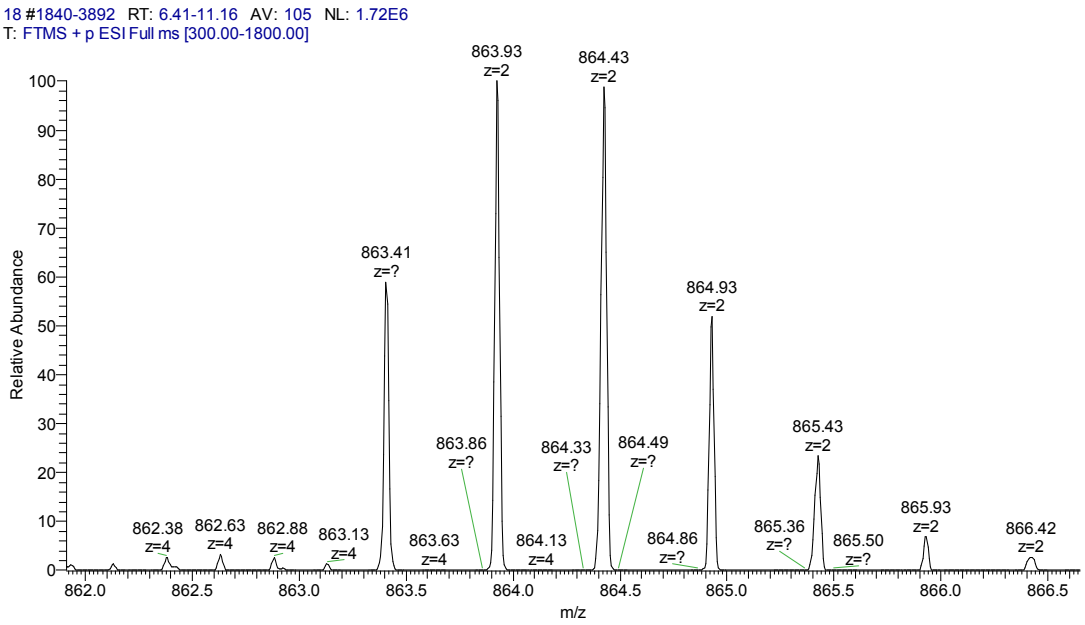


Figure S6. LC-MS / MS primary spectrum of aldol reaction peptide of modified protease with 4-acetylpyridine from trypsin digestion

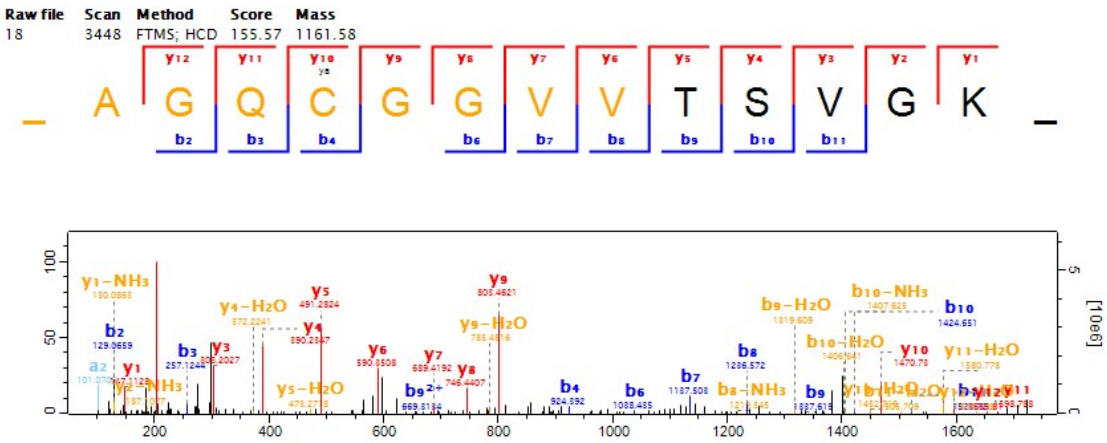


Figure S7. LC-MS / MS secondary spectrum of aldol reaction peptide of modified protease with 4-acetylpyridine from trypsin digestion

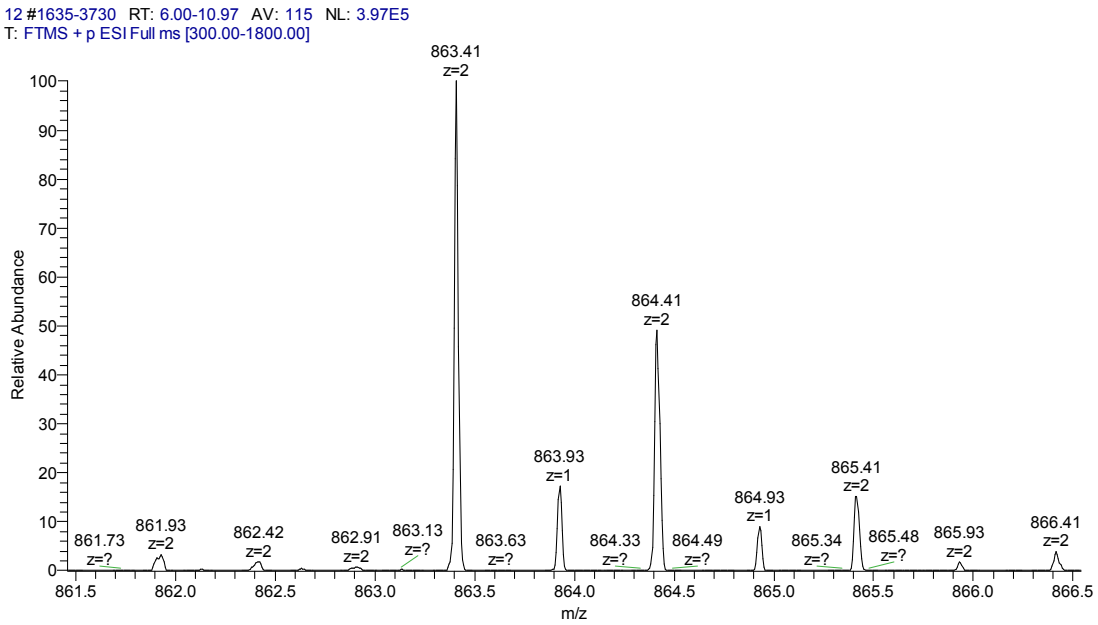


Figure S8. LC-MS / MS primary spectrum of aldol reaction peptide of modified protease with 3-acetylpyridine from trypsin digestion

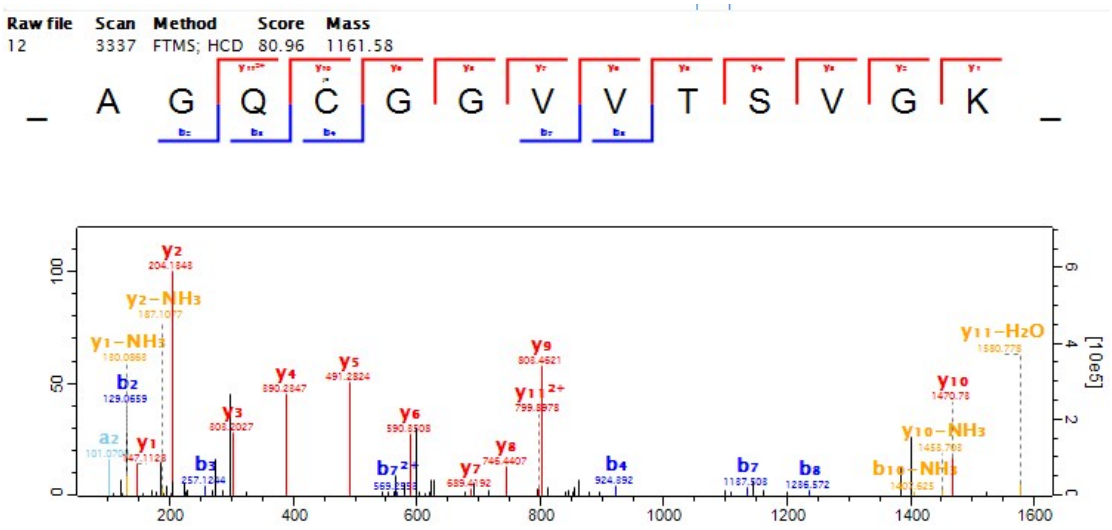


Figure S9. LC-MS / MS primary spectrum of aldol reaction peptide of modified protease with 3-acetylpyridine from trypsin digestion

Part F. The General Produces of the Protein Products Quantitatively Determined

The screening reaction quantitatively determined. The modified and aldol-reaction protein were quantified respectively by summing the top ten isotopic ions' peak areas of the most aboundant charge states $[M+27H]^{27+}$ or $[M+28H]^{28+}$ according to the published protocol[4]. The extracted ion chromatogram (EIC) window for quantitation was set at 5 ppm. Peak areas were calculated using Xcalibur software (Thermo Fisher Scientific, Waltham, MA).

The modified reaction quantitatively determined. The modified reaction was quantitatively

determined by an analogous procedure to the screening reaction.

Part G. Background Test

The aldol reaction of the 4-acetylpyridine or 3-acetylpyridine and LBB (8). 4-Acetylpyridine or 3-Acetylpyridine was injected into a solution of LBB (**8**) which offered a final mixture contained 5 mM **8** and 100 mM 4-Acetylpyridine or 3-Acetylpyridine in the PB buffer / DMSO-d_6 (20: 1). The PB buffer was prepared by the D_2O and DSS was added as internal standard. The mixture was cultivated at room temperature for 12 hours. After that, the final solution was characterized by H^1-NMR.

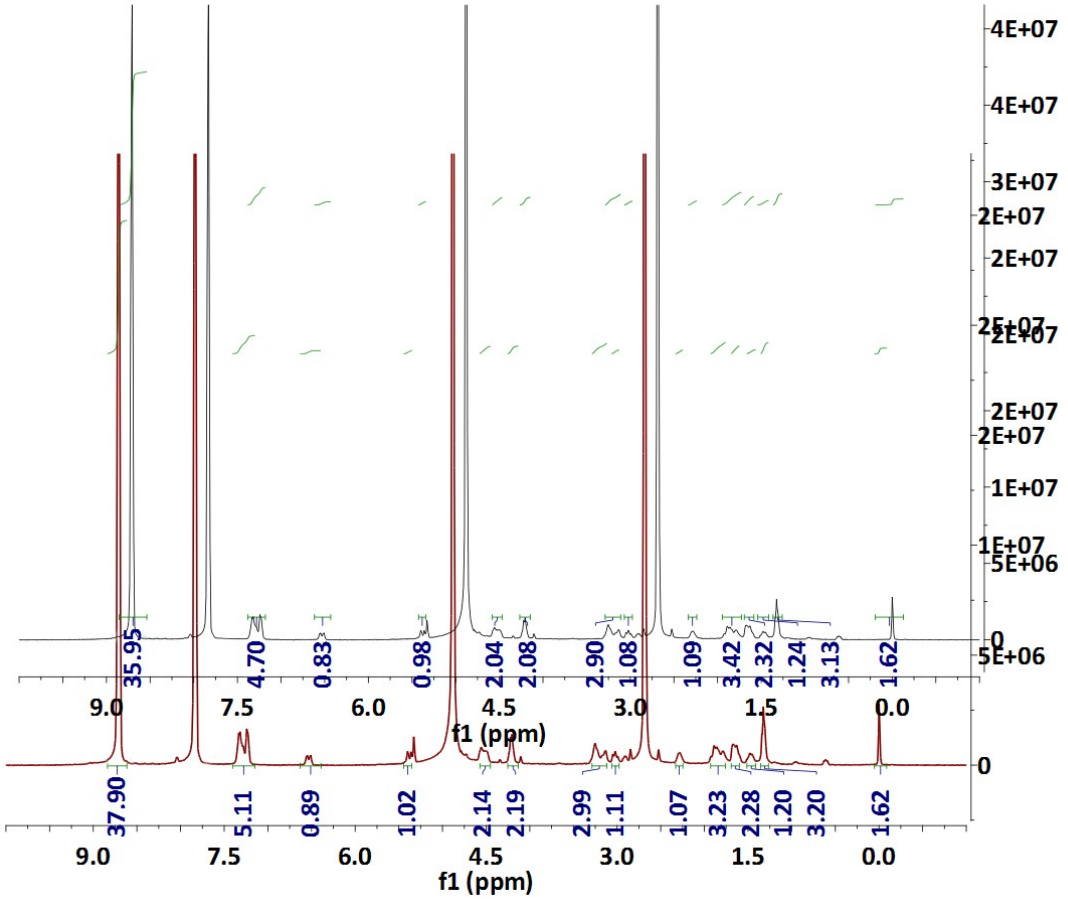


Figure S10. NMR spectrum of aldol reaction of the 4-Acetylpyridine and LBB in the absence of EV-A71 $3C^{pro}$. The aldol reaction rarely occurred, and no new compound was detected.

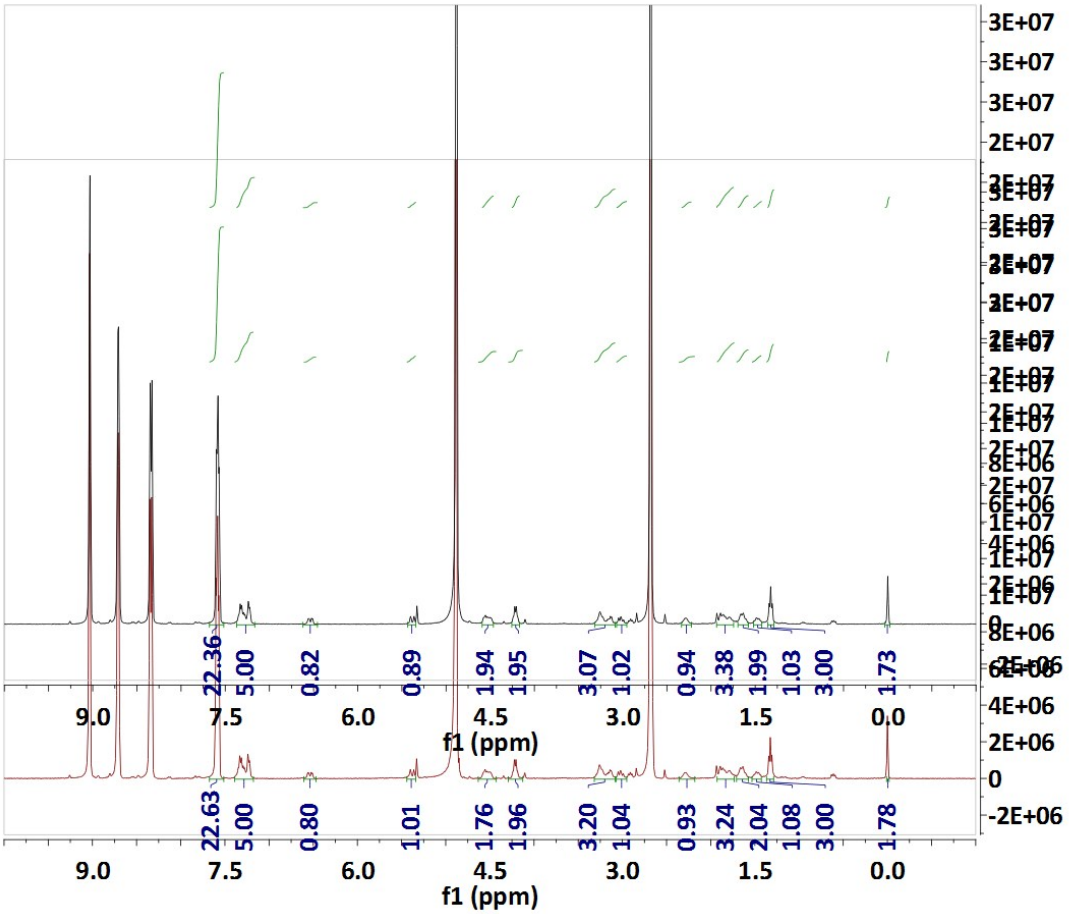
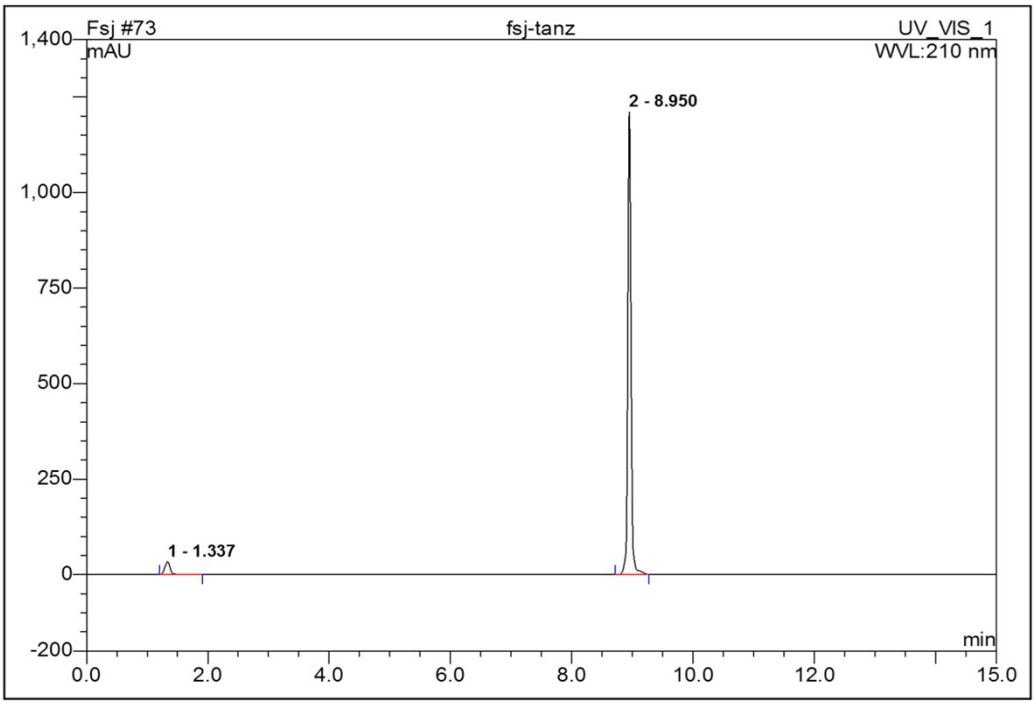


Figure S11. NMR spectrum of aldol reaction of the 3-Acetylpyridine and LBB in the absence of EV-A71 $3C^{pro}$. The aldol reaction rarely occurred, and no new compound was detected.

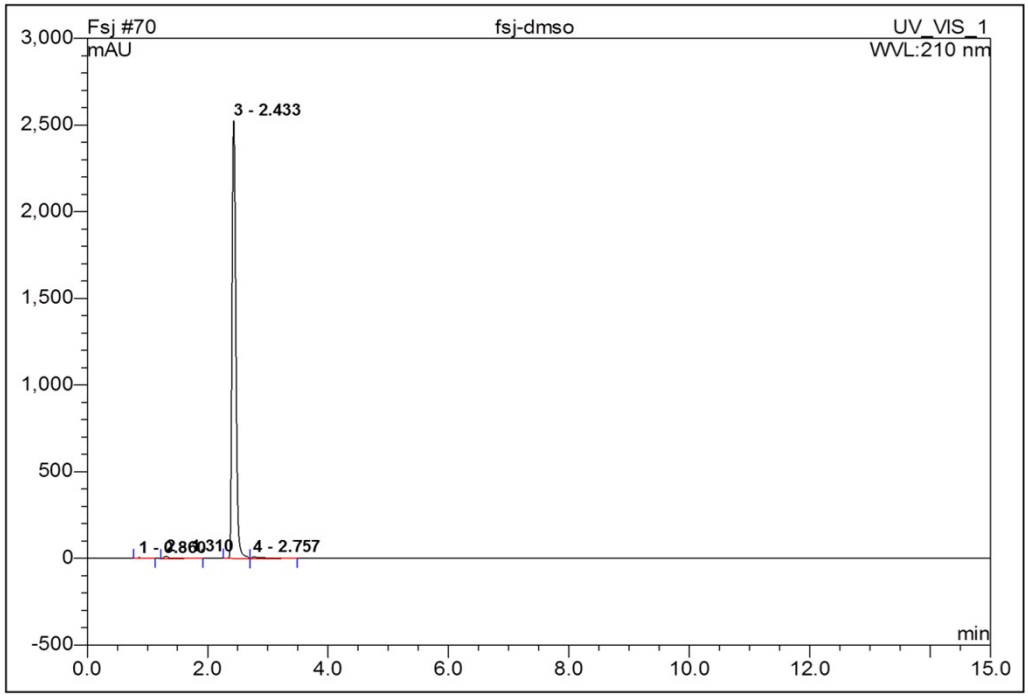
4-Acetylpyridine or 3-Acetylpyridine was injected into a solution of LBB (**8**) which offered a final mixture contained 100 mM **8** and 100 mM 4-Acetylpyridine or 3-Acetylpyridine in the PB buffer / DMSO(20: 1). The mixture was cultivated at room temperature for 12 hours.

The whole reaction was detected by HPLC. HPLC analysis was performed using the method: Guard column = Agela Technologies Venusil MP C18 (5.0 μm, 100 Å, 4.6 × 10 mm) and column = Agela Technologies Venusil MP C18 column (5.0 μm, 100 Å, 4.6 × 250 mm); temperature = 30 °C; solvent A = H_2O; solvent B = MeCN; flow rate = 1.0 mL/min; gradient: Solvent B was maintained at 5% (0.0–4.0 minutes), and linearly increased from 5%–70% (4.0–9.0 minute)，and then dropped from 70%–5% (9.0–10.0 minutes), and maintained at 5% (10.0–15.0 minutes).



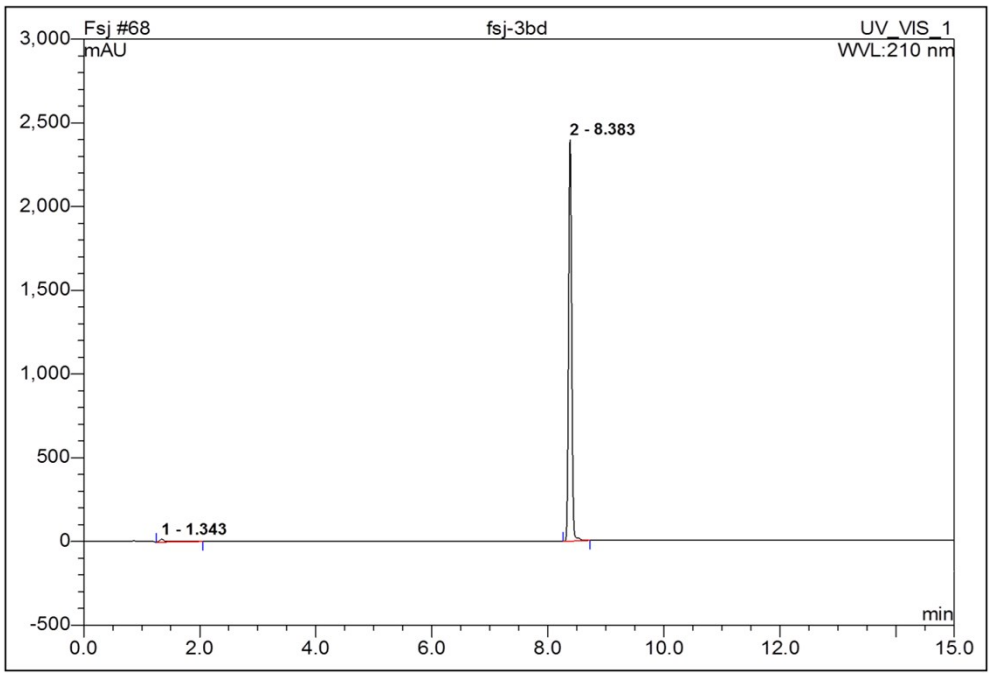
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.34	n.a.	33.910	3.526	4.90	n.a.	BMB
2	8.95	n.a.	1187.478	68.306	95.10	n.a.	BM
Total:			1221.388	71.832	100.00	0.000	

Figure S12. HPLC Chromatogram of **8**



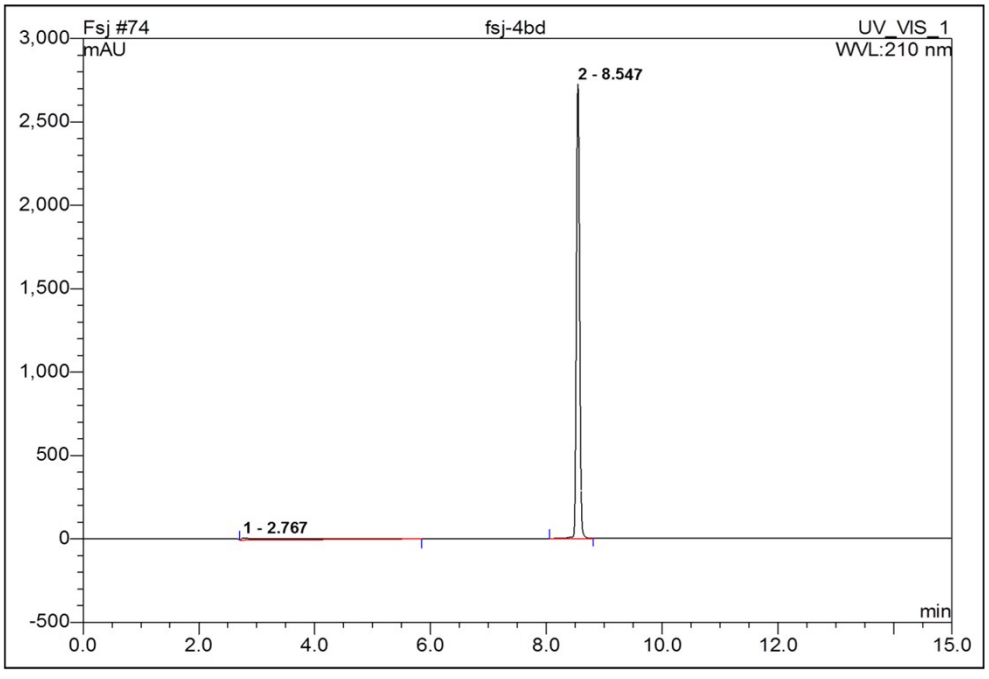
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	0.86	n.a.	3.050	0.243	0.12	n.a.	BMB
2	1.31	n.a.	12.462	1.550	0.80	n.a.	BMB
3	2.43	n.a.	2527.712	188.243	97.72	n.a.	BMb
4	2.76	n.a.	10.154	2.615	1.36	n.a.	bMB
Total:			2553.378	192.651	100.00	0.000	

Figure S13. HPLC Chromatogram of DMSO



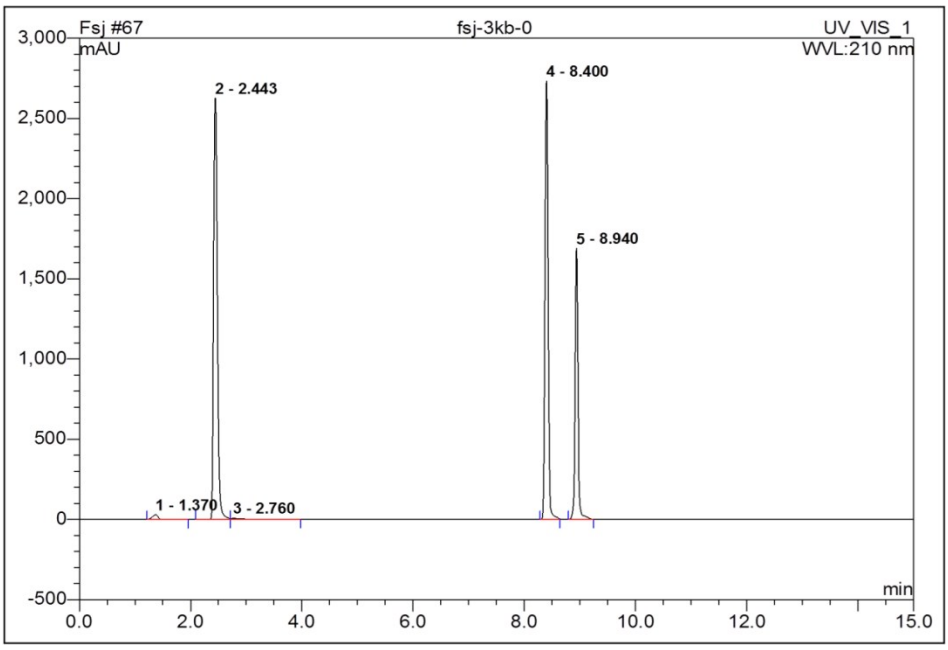
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.34	n.a.	17.199	2.802	1.85	n.a.	BMB
2	8.38	n.a.	2399.724	149.102	98.15	n.a.	BMB
Total:			2416.923	151.904	100.00	0.000	

Figure S14. HPLC Chromatogram of 3-acetylpyridine



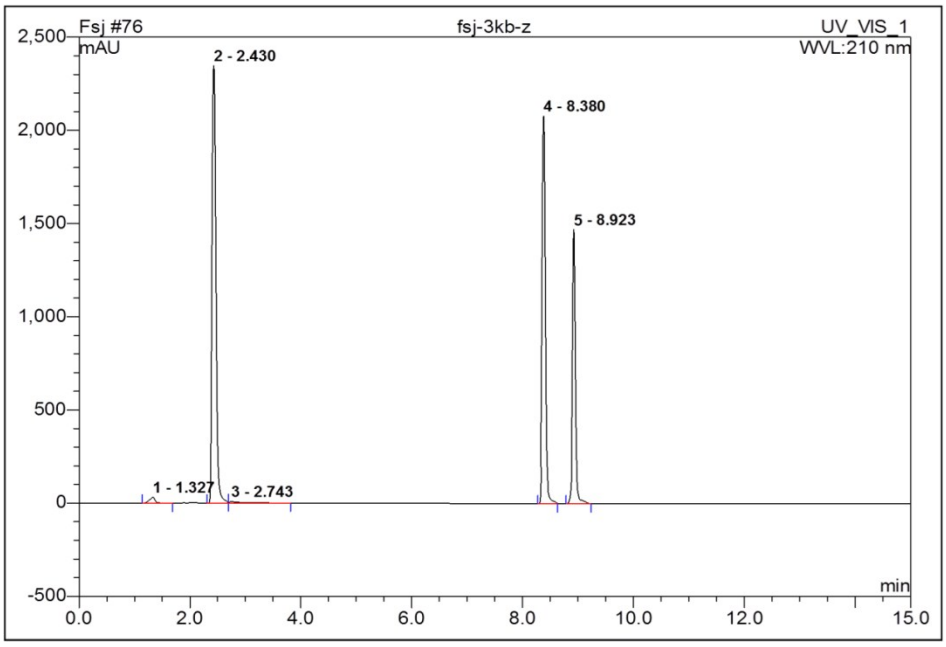
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	2.77	n.a.	12.217	12.525	6.46	n.a.	BMB
2	8.55	n.a.	2724.861	181.364	93.54	n.a.	BM
Total:			2737.078	193.889	100.00	0.000	

Figure S15. HPLC Chromatogram of 4-acetylpyridine



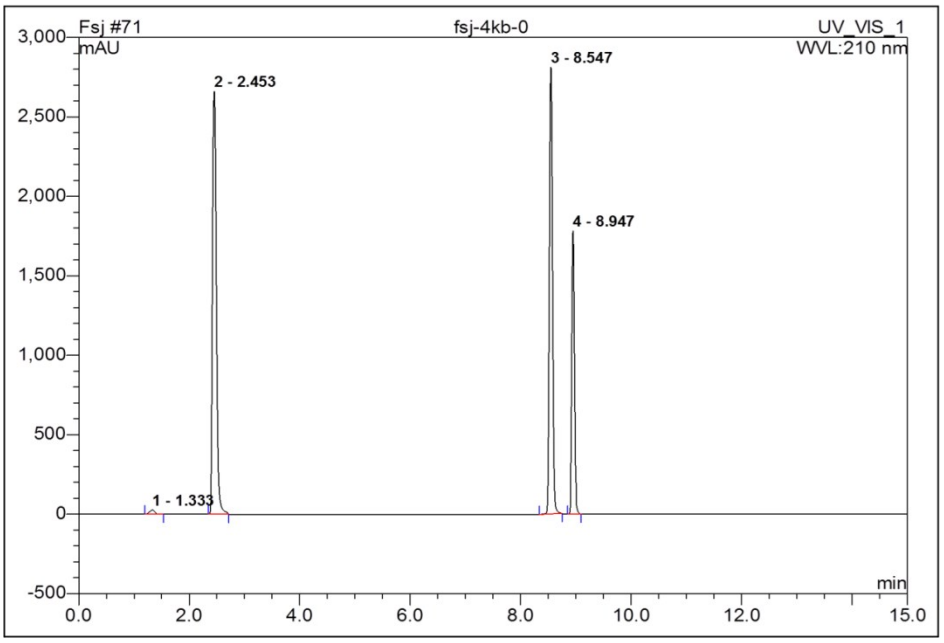
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.37	n.a.	29.879	3.757	0.77	n.a.	BMB
2	2.44	n.a.	2626.894	208.221	42.93	n.a.	BM
3	2.76	n.a.	7.712	1.896	0.39	n.a.	MB
4	8.40	n.a.	2735.478	175.791	36.21	n.a.	BM
5	8.94	n.a.	1672.260	96.111	19.70	n.a.	BM
Total:			7072.223	485.776	100.00	0.000	

Figure S16. HPLC Chromatogram of the pre-reaction of 3-acetylpyridine and **8**



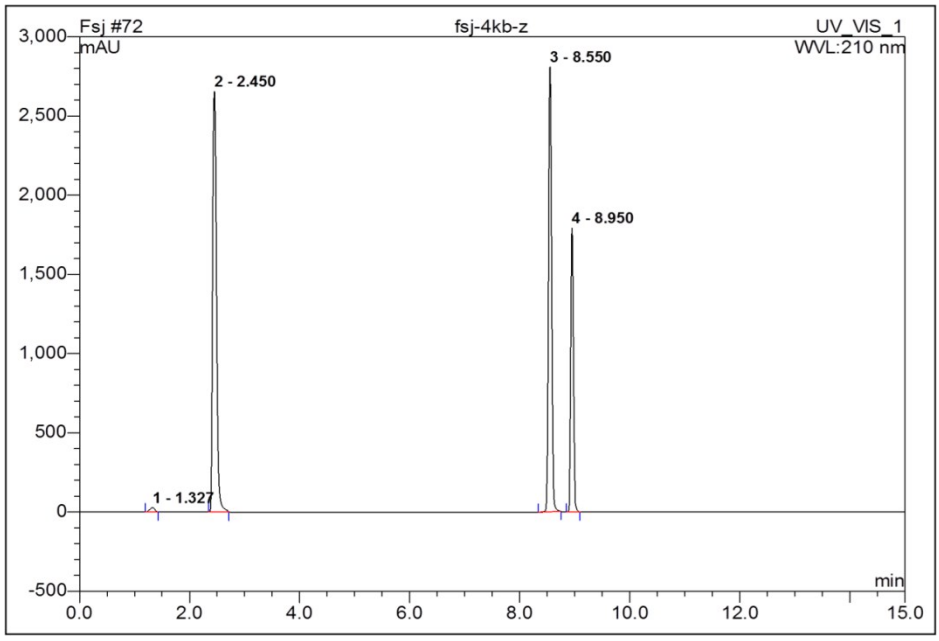
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.33	n.a.	30.824	3.628	0.84	n.a.	BMB
2	2.43	n.a.	2347.341	194.078	44.86	n.a.	BM
3	2.74	n.a.	7.891	1.779	0.41	n.a.	MB
4	8.38	n.a.	2078.748	147.330	34.06	n.a.	BM
5	8.92	n.a.	1445.259	85.002	19.83	n.a.	BM
Total:			5910.063	431.817	100.00	0.000	

Figure S17. HPLC Chromatogram of the post-reaction of 3-acetylpyridine and **8**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.33	n.a.	26.074	2.882	0.59	n.a.	BMB
2	2.45	n.a.	2660.260	219.528	42.89	n.a.	BMB
3	8.55	n.a.	2811.148	188.357	36.70	n.a.	BMB
4	8.95	n.a.	1760.356	101.697	19.82	n.a.	BM
Total:			7257.838	512.464	100.00	0.000	

Figure S18. HPLC Chromatogram of the pre-reaction of 4-acetylpyridine and **8**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.33	n.a.	26.501	2.764	0.54	n.a.	BMB
2	2.45	n.a.	2656.031	218.161	42.82	n.a.	BMB
3	8.55	n.a.	2806.491	188.152	36.77	n.a.	BMB
4	8.95	n.a.	1763.734	101.556	19.87	n.a.	BM
Total:			7252.757	510.633	100.00	0.000	

Figure S19. HPLC Chromatogram of the post-reaction of 4-acetylpyridine and **8**

The reaction of the native 3C^{pro} and 4-acetylpyridine or 3-acetylpyridine. The solution of 4-

acetylpyridine or 3-acetylpyridine was dissolved into DMSO to afford a solution with the concentration of 2 M. The solution of 4-acetylpyridine or 3-acetylpyridine was injected into a solution of native 3C protease to afford final solution with 3C protease concentration of 40 μM and 4-acetylpyridine or 3-acetylpyridine concentration of 80 mM. The mixture was stood at room temperature for 12 hours. After that, the small molecules and precipitate were removed by three times ultra-filtration, and the remained protease was determined by LC-ESI-Orbitrap MS.

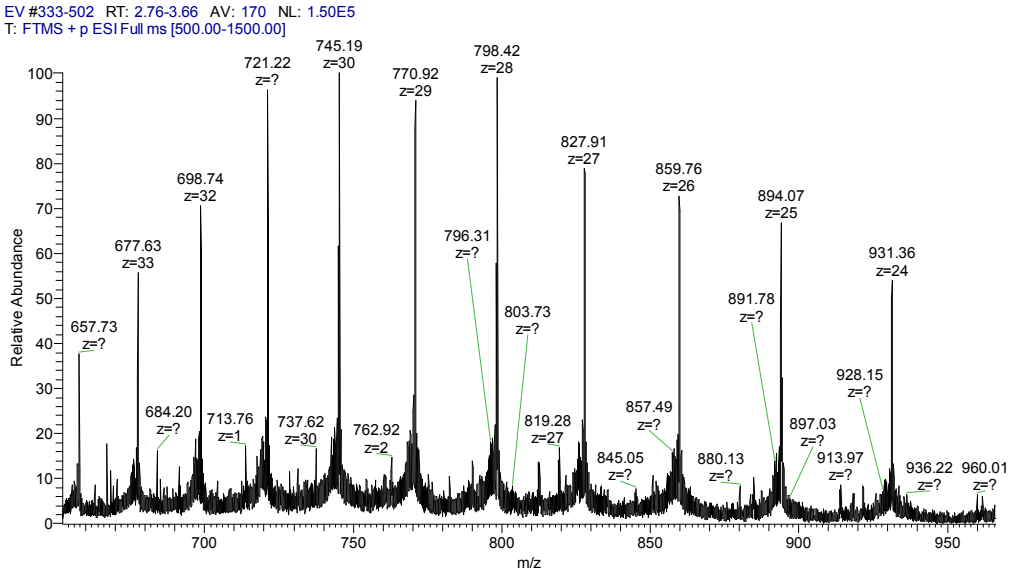


Figure S20. LC-ESI-Orbitrap analysis of the aldol reaction of native protease with 3-acetyl pyridine. Native $3C^{pro}$ that having no LBB functionality was employed as control and no product was observed.

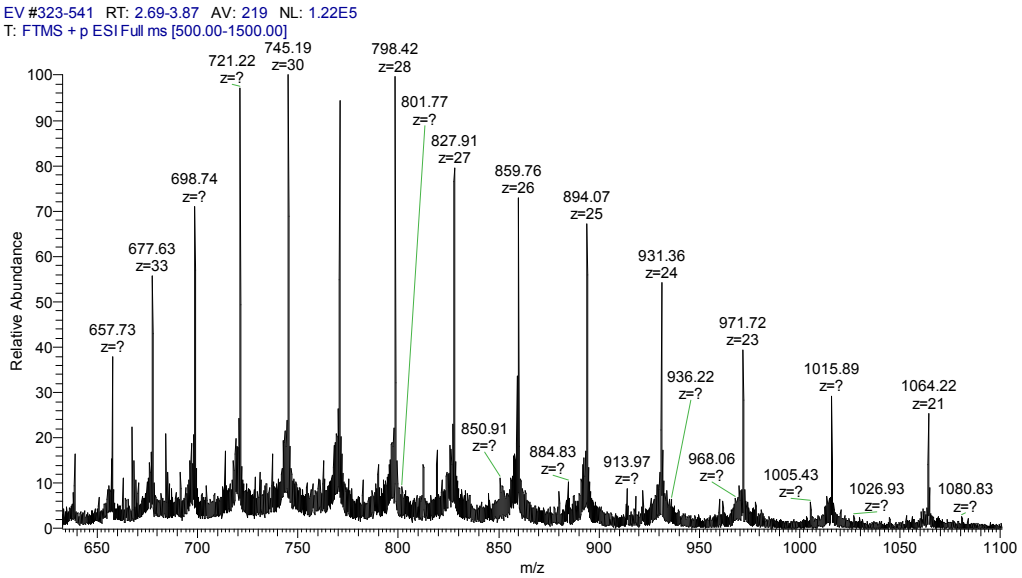


Figure S21. LC-ESI-Orbitrap analysis of the aldol reaction of native protease with 4-acetyl pyridine. Native $3C^{pro}$ that having no LBB functionality was employed as control and no product was observed.

Part H. Docking Models of 12a-g Bound to EV-A71 3C Protease

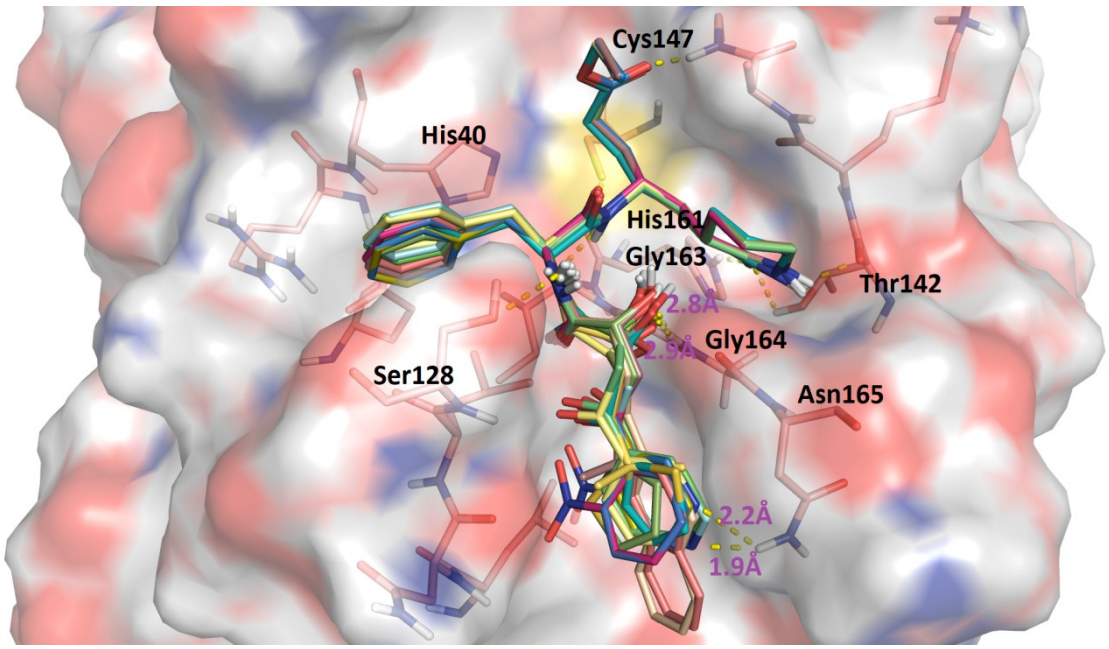


Figure S22. Docking models of **12a-g** bound to EV-A71 $3C^{pro}$ (PDB ID: 4GHT[5]).

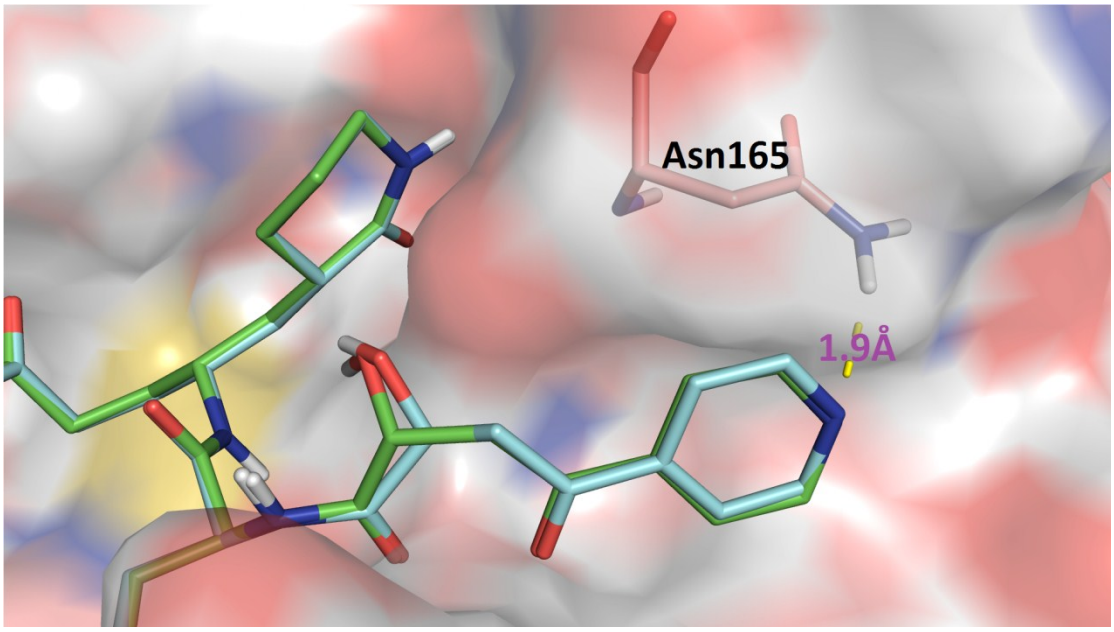


Figure S23. The partial amplification of docking models of **12c** bound to EV-A71 $3C^{pro}$ (PDB ID: 4GHT[5]).

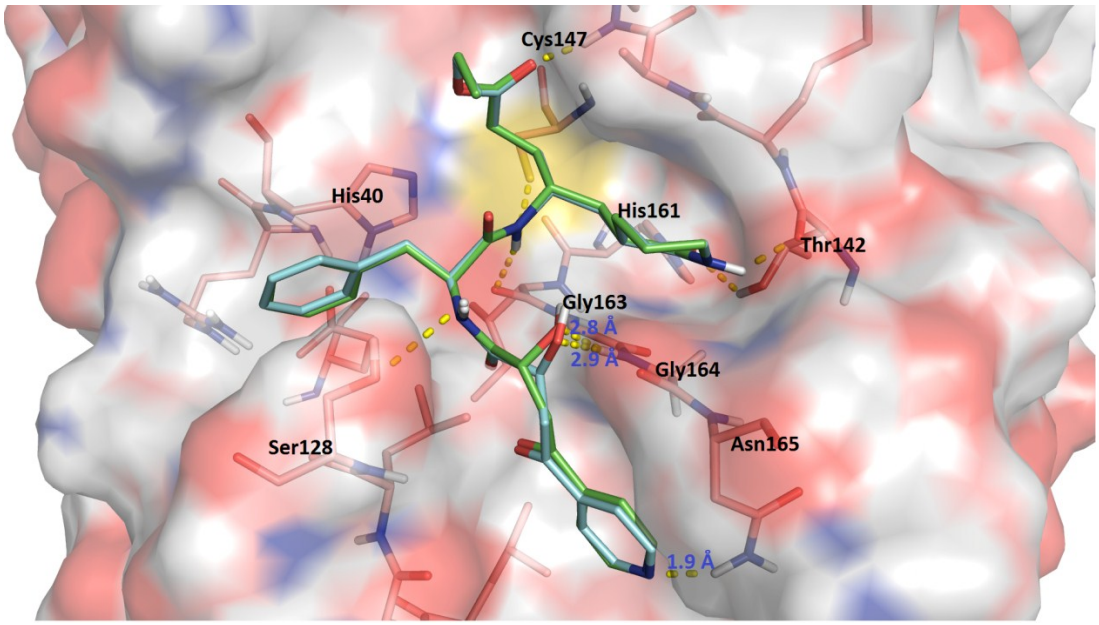


Figure S24. Docking models of **12c** bound to EV-A71 $3C^{pro}$ (PDB ID: 4GHT[5]).

Part I. LC-ESI-Orbitrap analysis of the aldol reaction

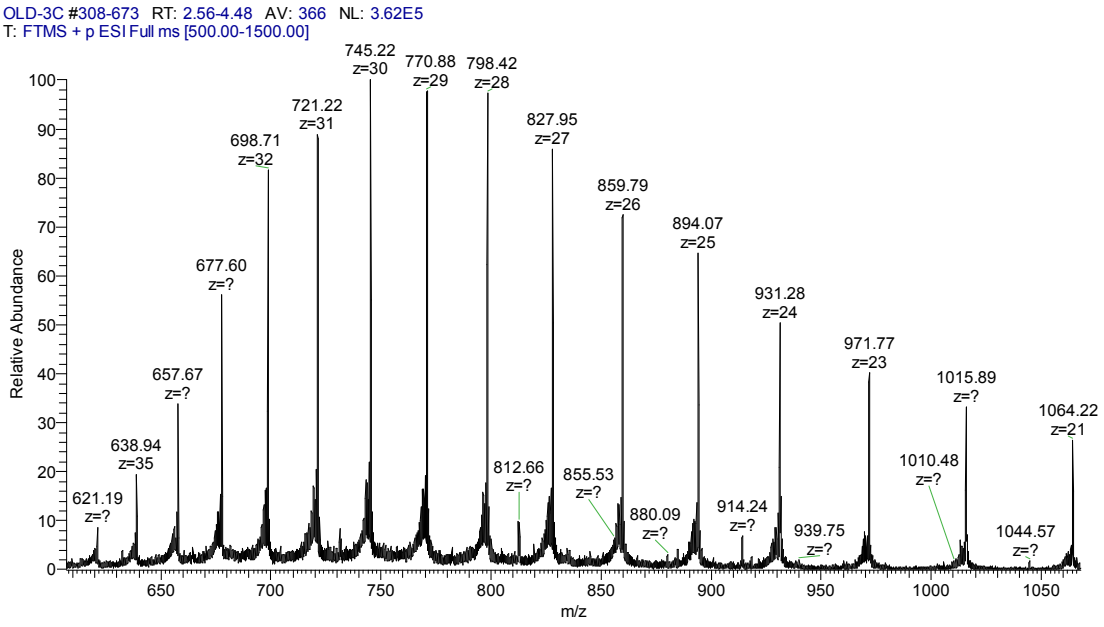


Figure S25. LC-ESI-Orbitrap analysis of native $3C^{pro}$.

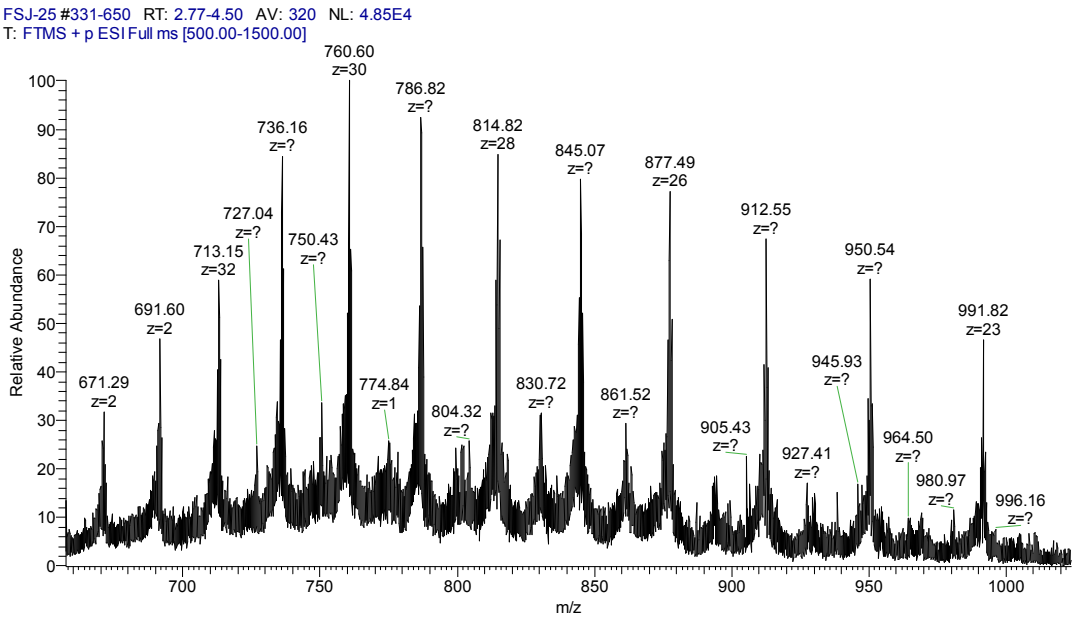


Figure S26. LC-ESI-Orbitrap analysis of modified $3C^{pro}$ with aldehyde

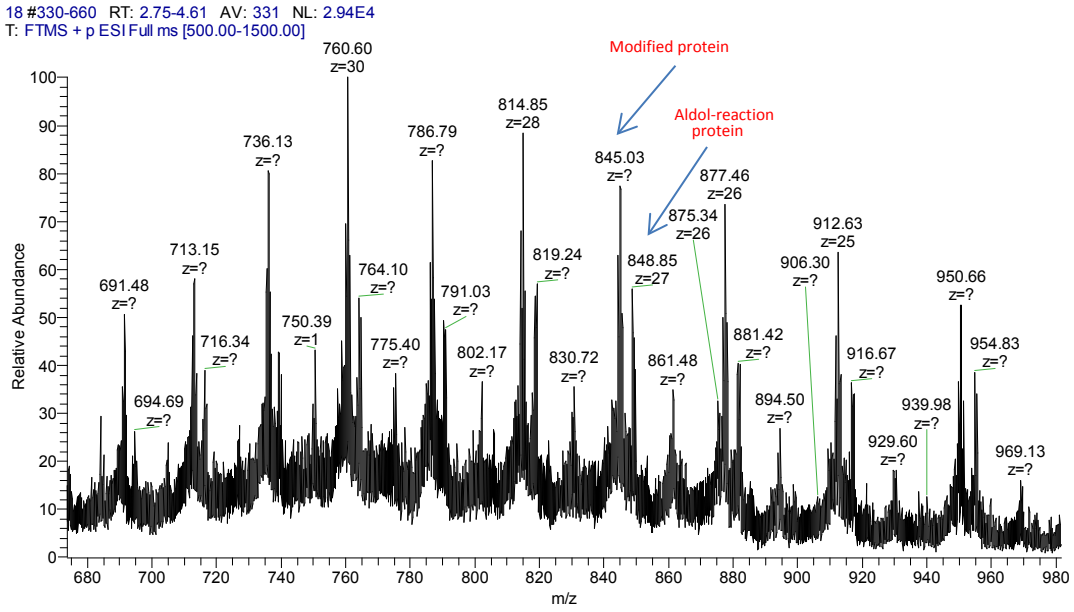


Figure S27. LC-ESI-Orbitrap analysis of the aldol reaction of modified protease with 4-acetylpyridine.

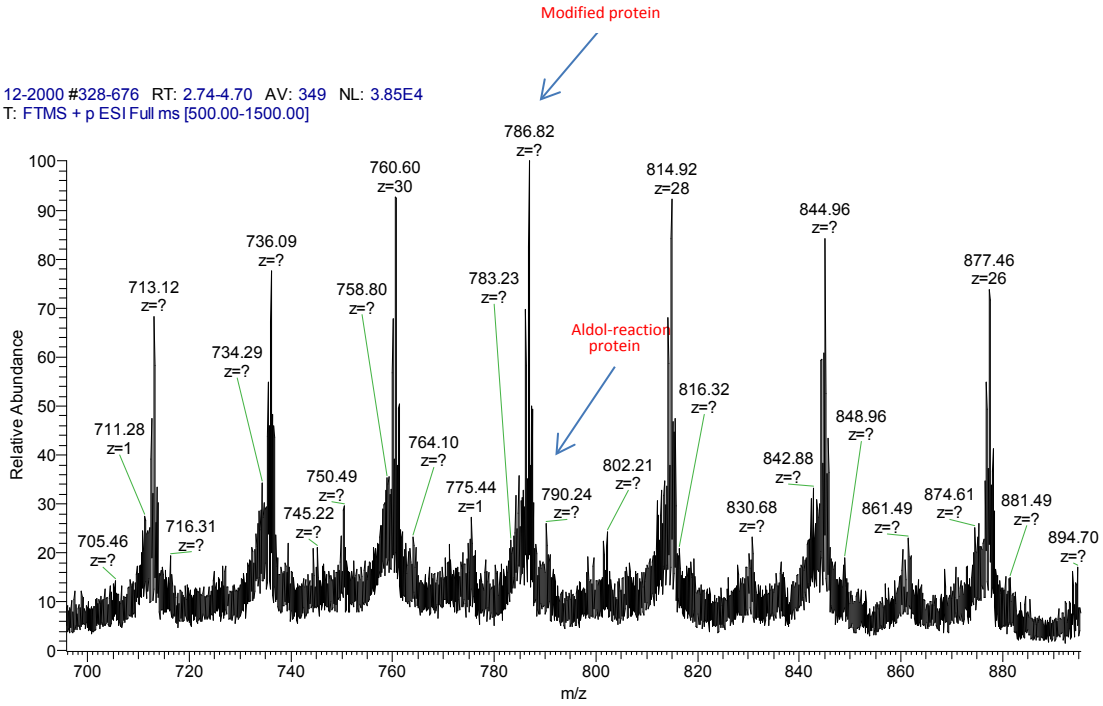
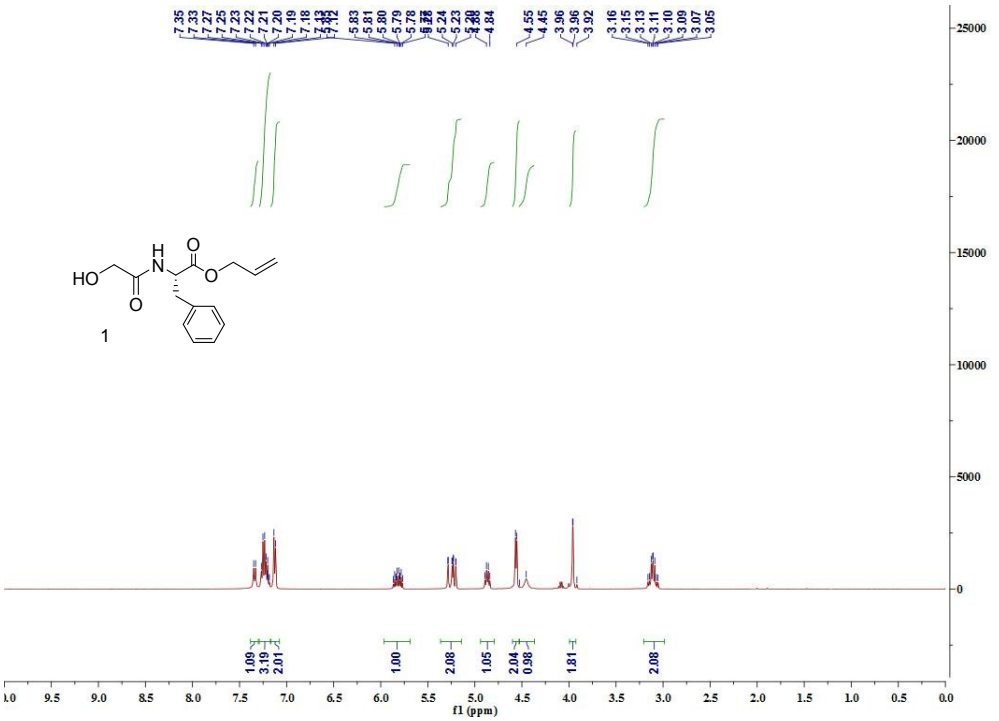


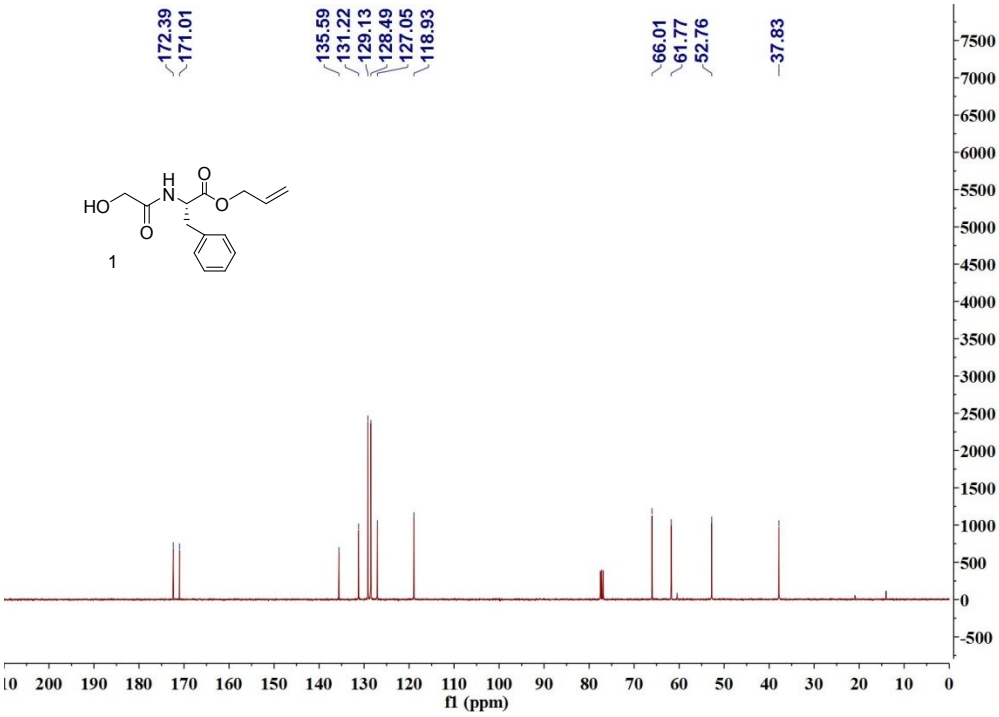
Figure S28. LC-ESI-Orbitrap analysis of the aldol reaction of modified protease with 3-acetylpyridine.

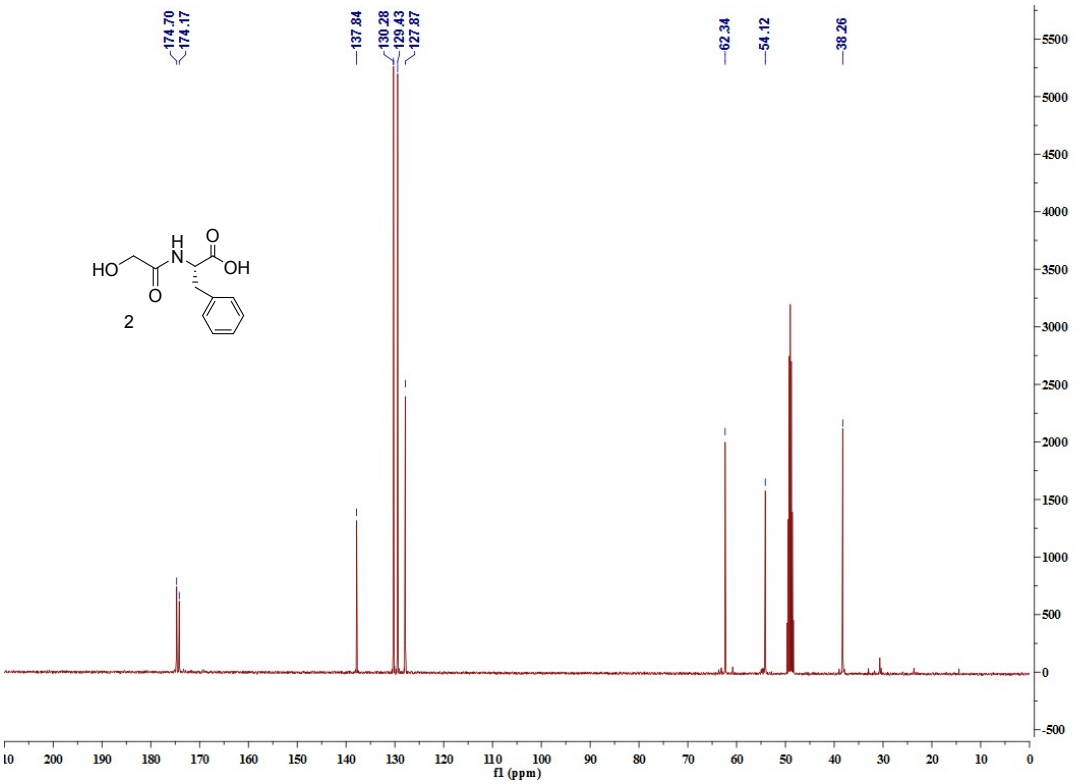
Part J. References

1. Y. Y. Zhai, X. S. Zhao, Z. J. Cui, M. Wang, Y. X. Wang, L. F. Li, Q. Sun, X. Yang, D. B. Zeng, Y. Liu, Y. N. Sun, Z. Y. Lou, L. Q. Shang and Z. Yin, *J. Med. Chem.*, 2015, **58**, 9414-9420.
2. F. Freire, J. M. Seco, E. Quinoa and R. Riguera, *J. Org. Chem.*, 2005, **70**, 3778-3790.
3. Y. J. Cheng, Y. L. Chen, X. L. Sun, Y. H. Li, C. B. Huang, H. T. Deng and Z. G. Li, *Inflammation*, 2014, **37**, 1459-1467.
4. Q. Ruan, Q. C. Ji, M. E. Arnold, W. G. Humphreys and M. S. Zhu, *Anal. Chem.*, 2011, **83**, 8937-8944.
5. C. M. Wu, Q. X. Cai, C. Chen, N. Li, X. J. Peng, Y. X. Cai, K. Yin, X. S. Chen, X. L. Wang, R. F. Zhang, L. J. Liu, S. H. Chen, J. Li and T. W. Lin, *Acta Crystallogr D*, 2013, **69**, 866-871.

Part K. Characterizations of compounds (NMR spectrum)



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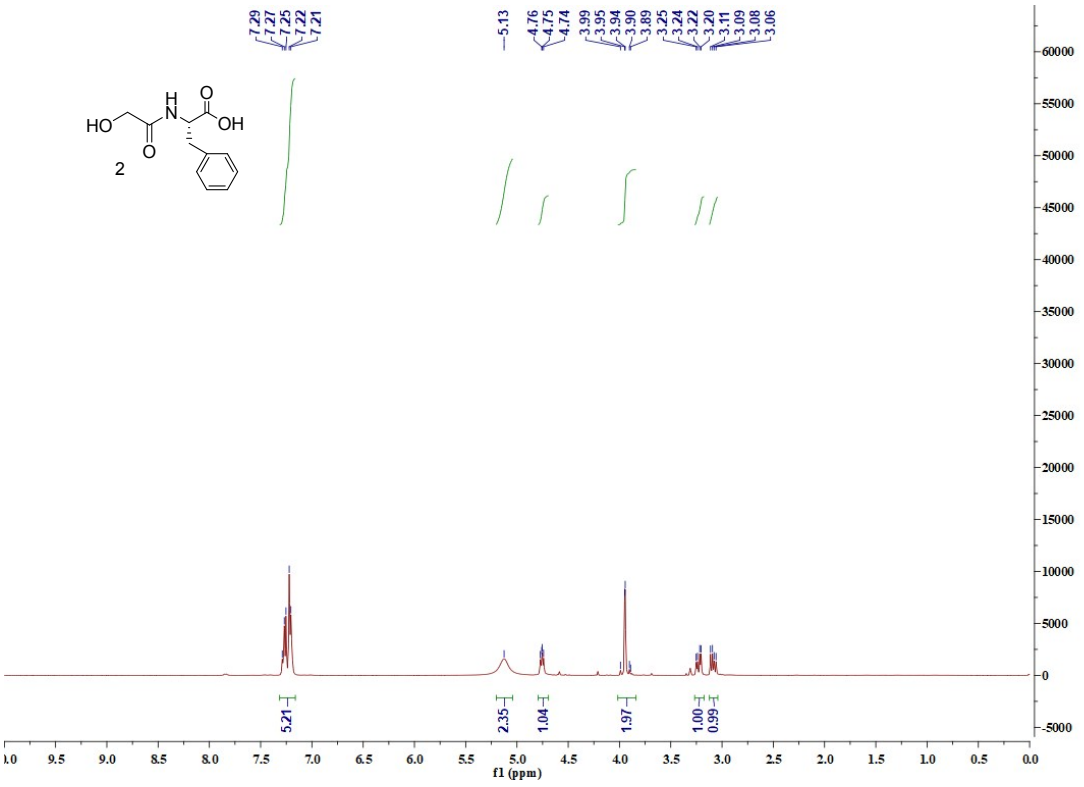
N
O
OH
O
2
f1 (ppm)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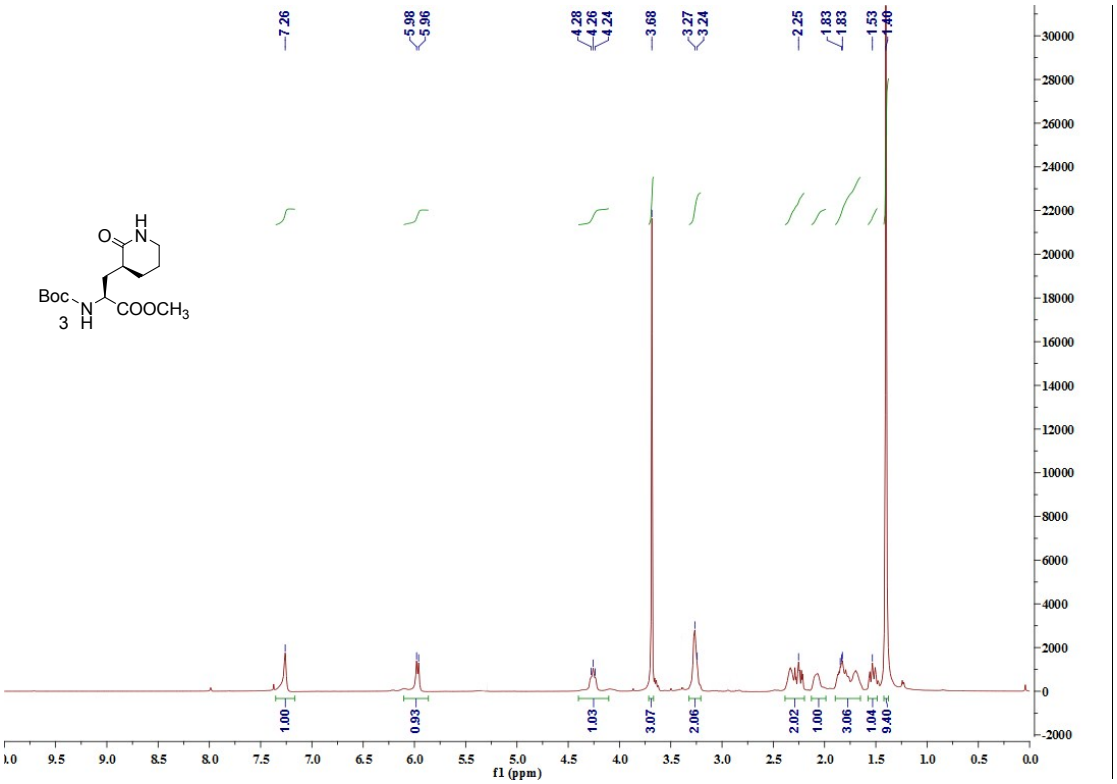
3.90
3.89
3.25
3.24
3.22
3.20
3.11
3.09
3.08
3.06
HO
H
N
O
OH
O
2
5.21
2.35
1.04
1.97
1.00
0.99
f1 (ppm)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1.83
1.83
1.53
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2.06
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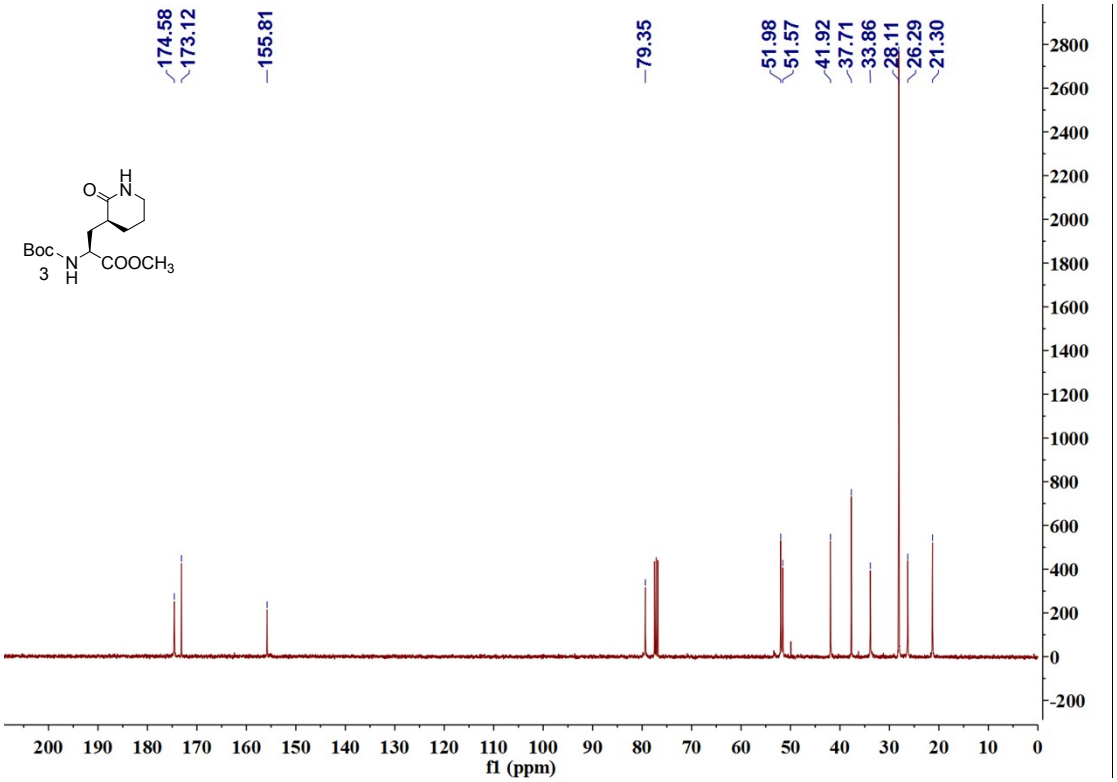
1.04
9.40
f1 (ppm)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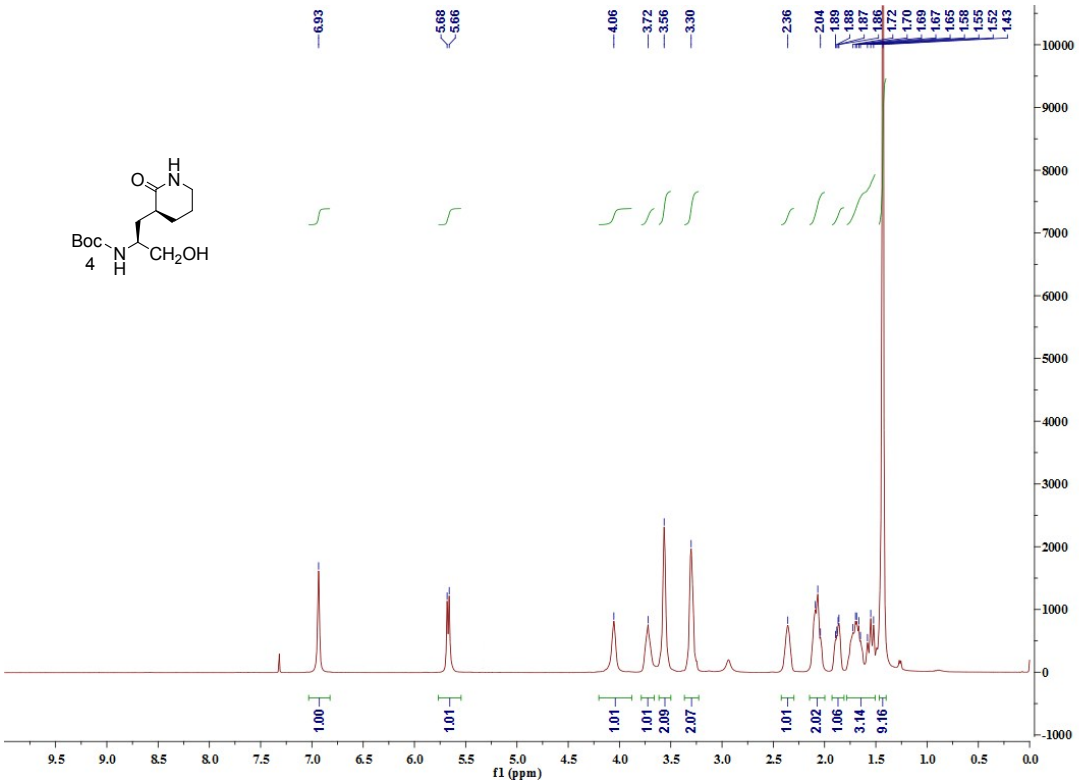
f1 (ppm)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1.69
1.67
1.65
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1.55
1.52
1.43
1.00
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1.01
1.01
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2.02
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f1 (ppm)
Boc
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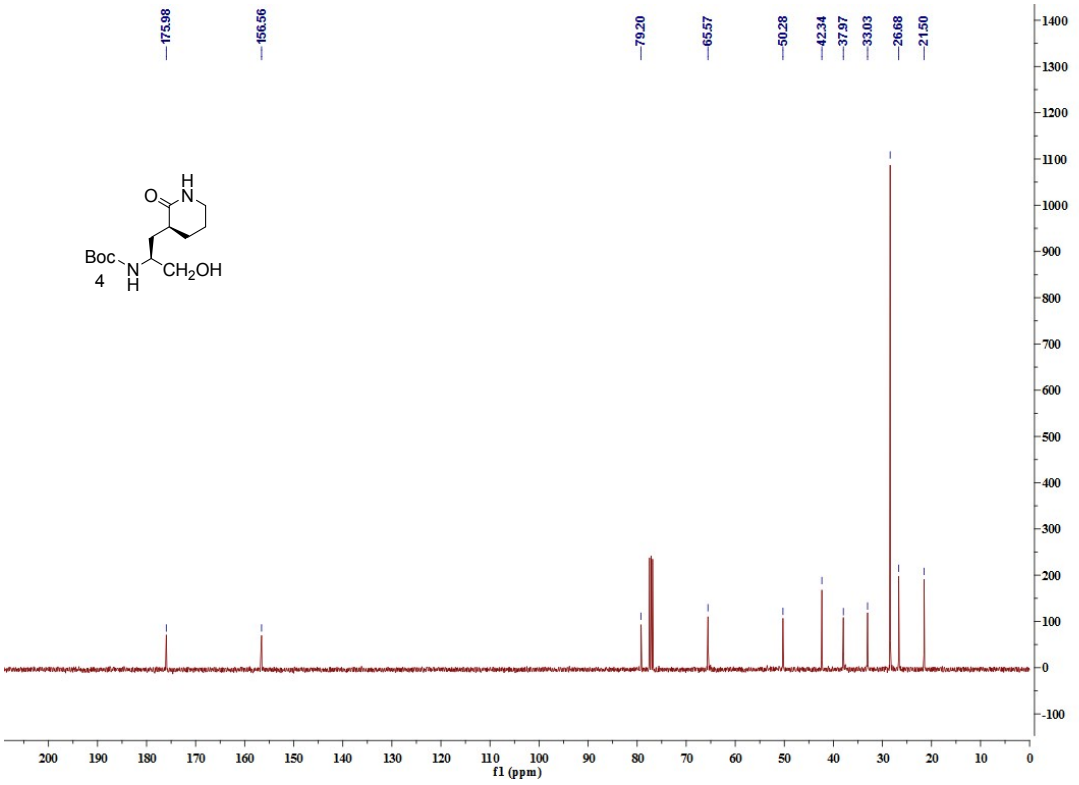
H
CH2OH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f1 (ppm)
Boc
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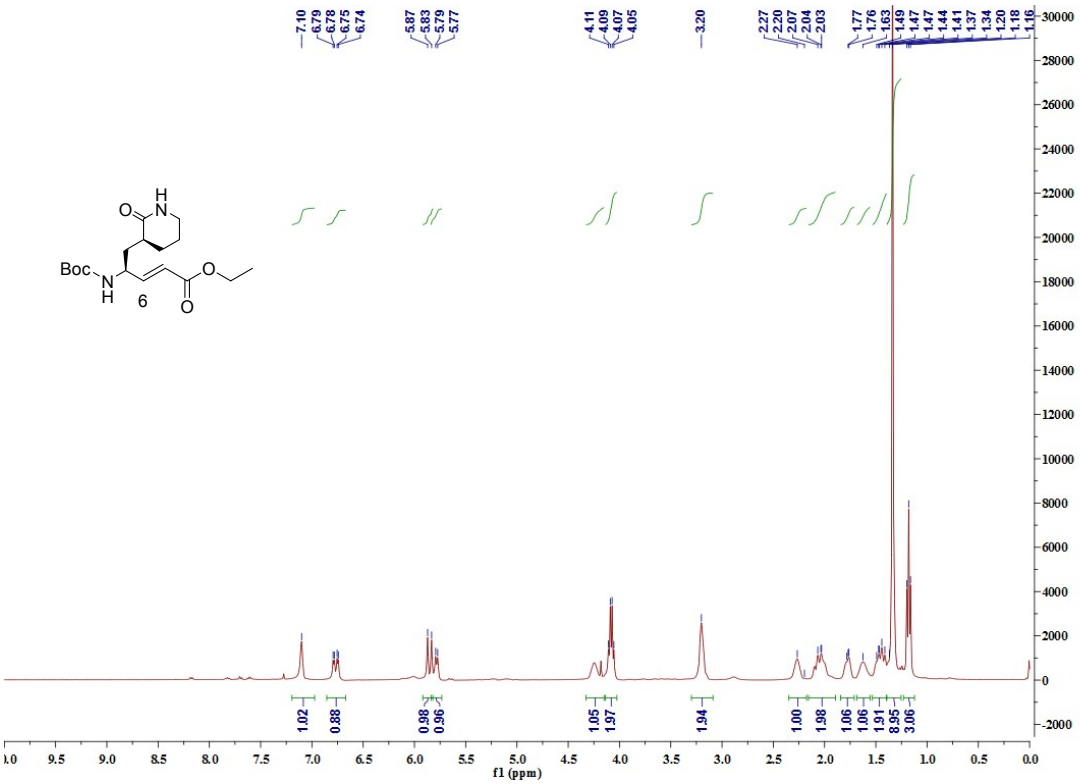
N
H
CH2OH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4.07
4.05
3.20
2.27
2.20
2.07
2.04
2.03
1.77
1.76
1.63
1.49
1.47
1.47
1.44
1.41
1.37
1.34
1.20
1.18
1.16
1.02
0.88
0.98
0.96
1.05
1.97
1.94
1.00
1.98
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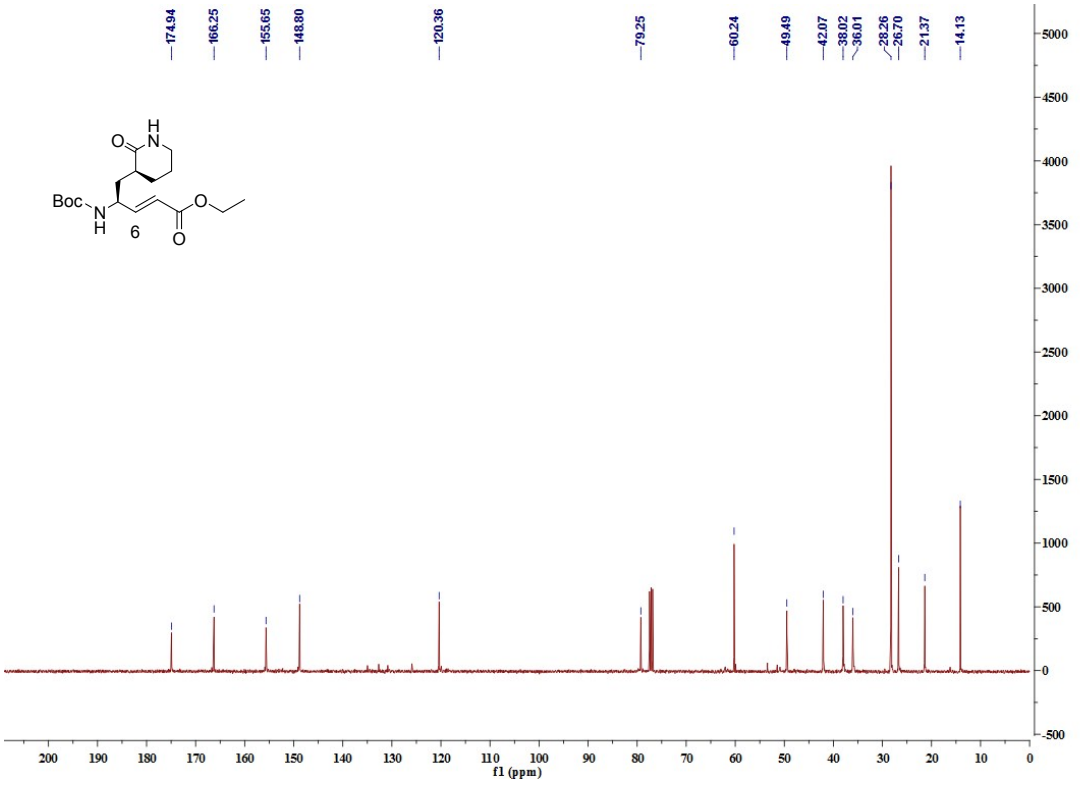
H
N
O
O
6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38.02
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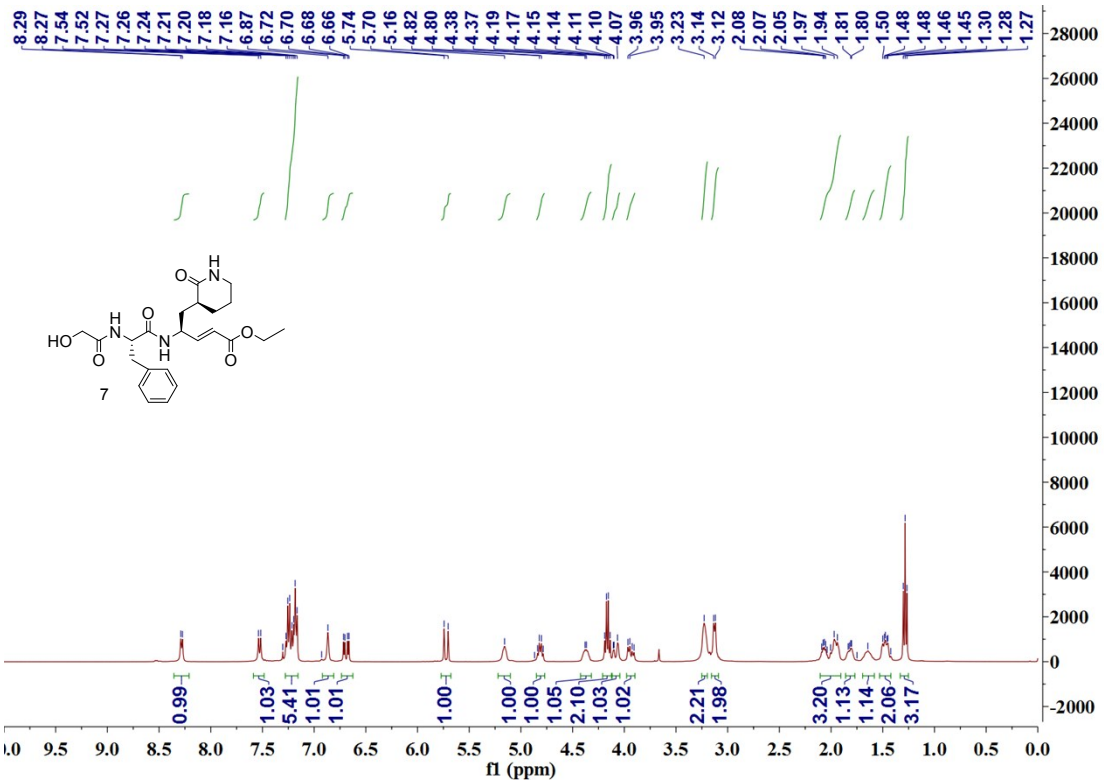
14.13
f1 (ppm)
Boc
N
H
O
H
N
O
O
6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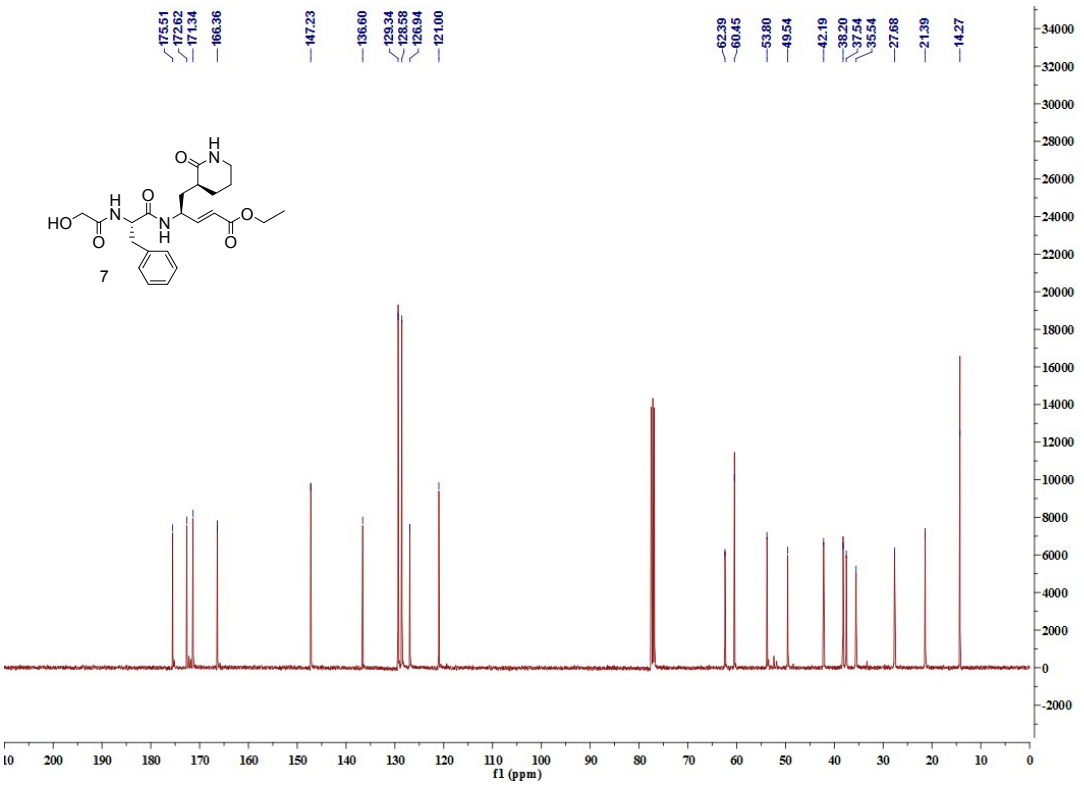
0.99 1.03 5.41 1.01 1.01 1.00 1.00 1.00 1.05 2.10 1.03 1.02 2.21 1.98 3.20 1.13 1.14 2.06 3.17
f1 (ppm)
7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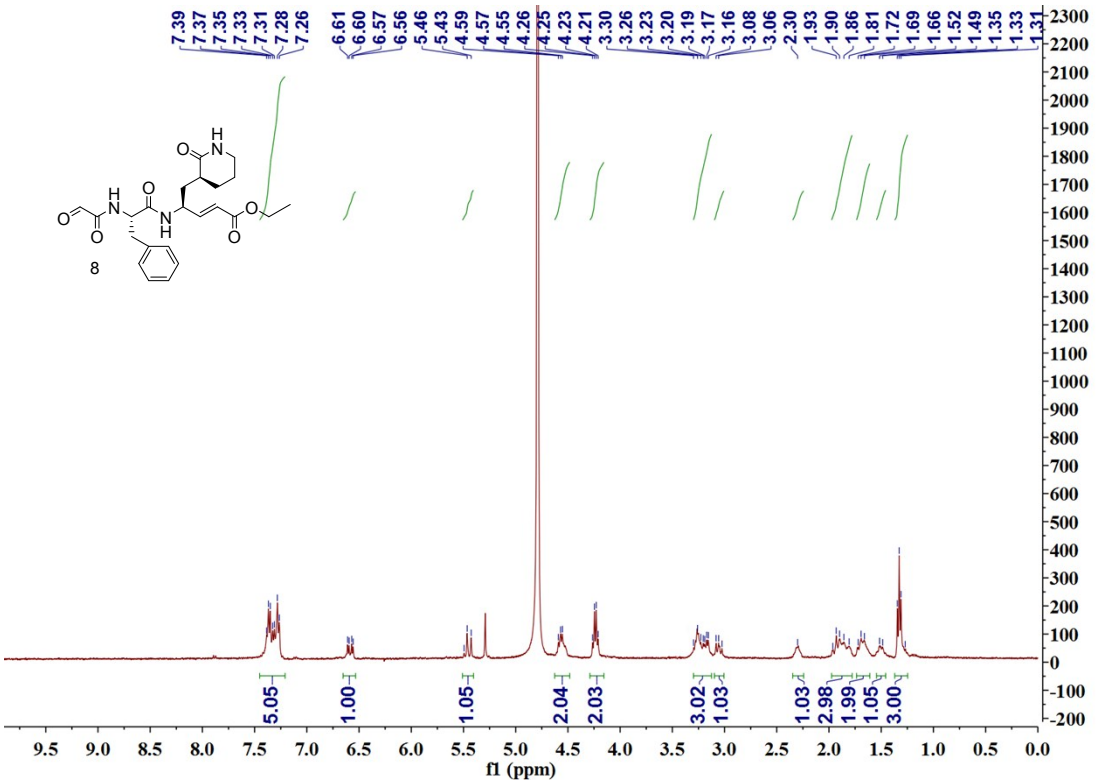
f1 (ppm)
7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1.00
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3.02
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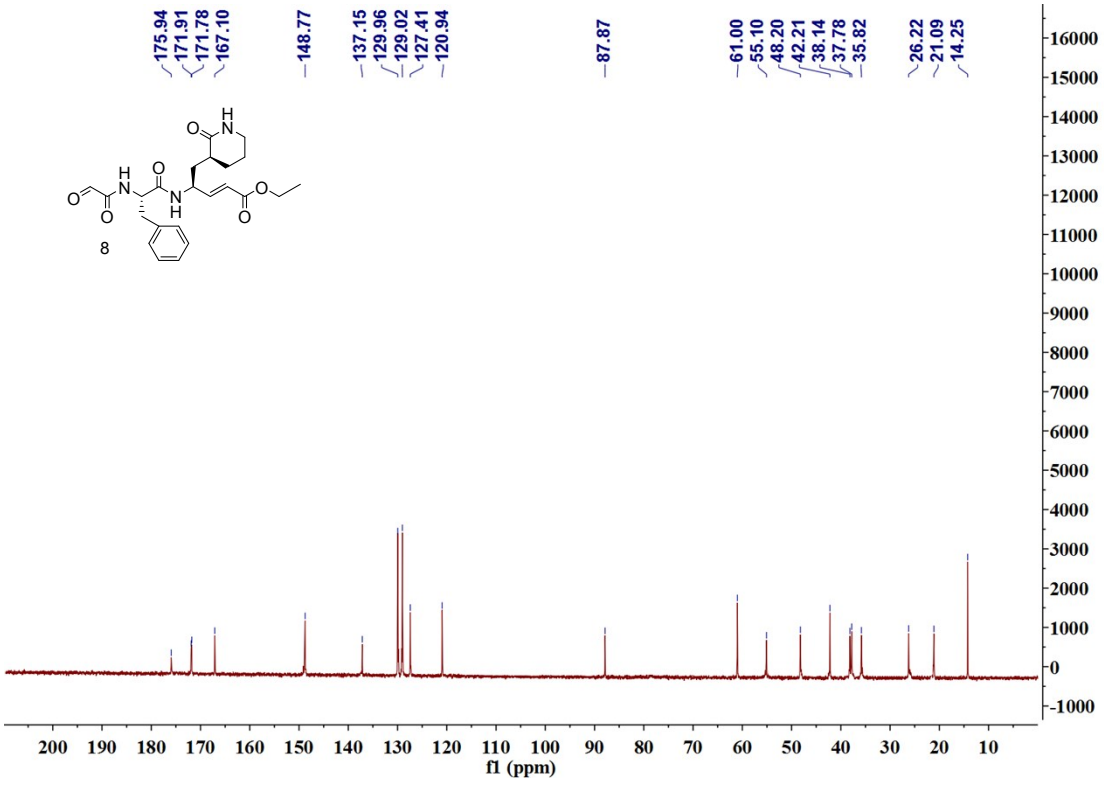
2.98
1.99
1.05
3.00
f1 (ppm)
8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42.21
38.14
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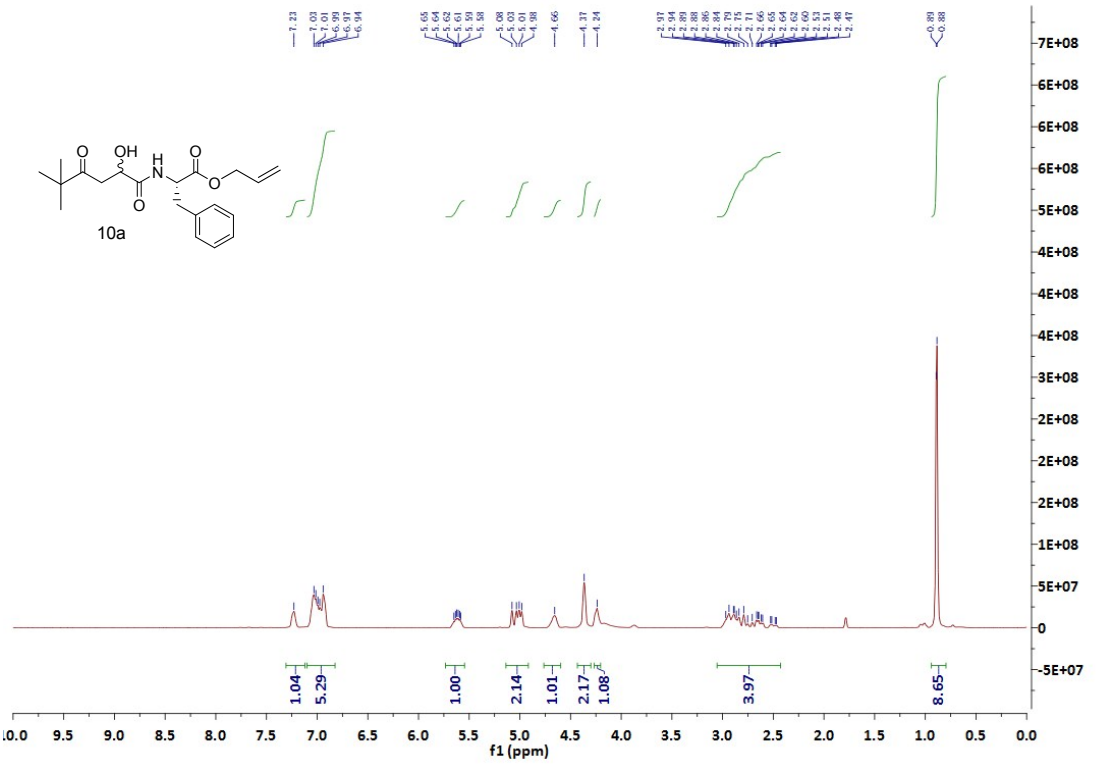
21.09
14.25
f1 (ppm)
8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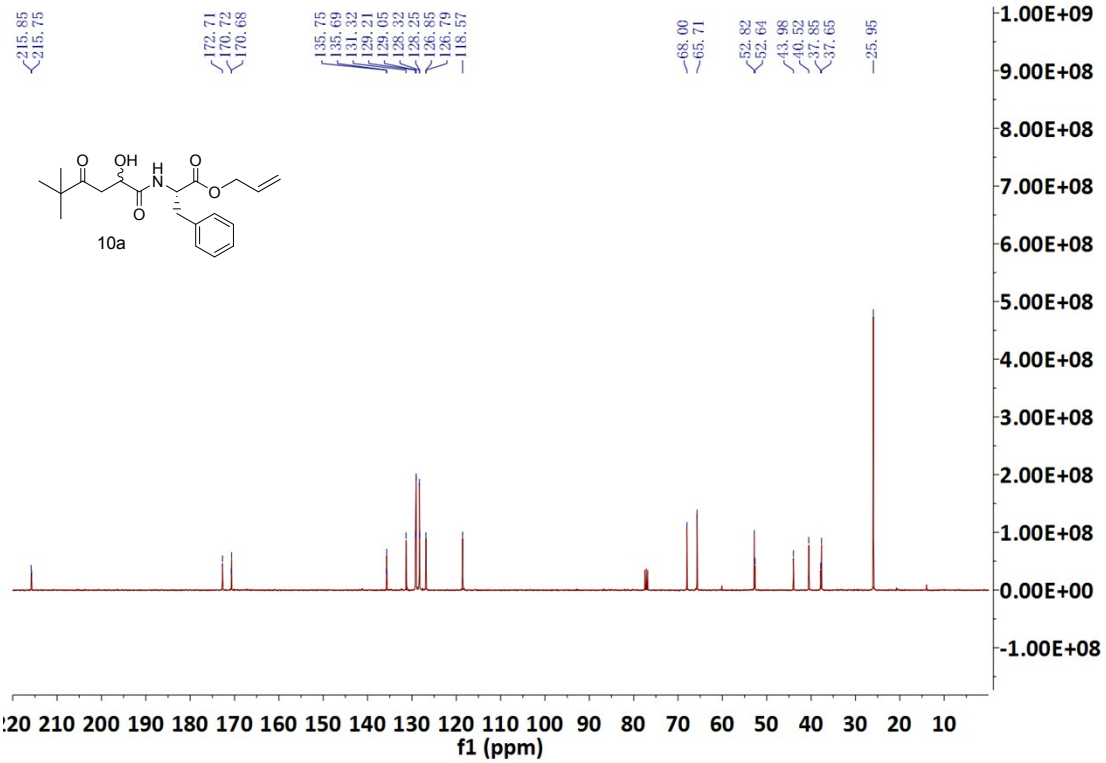
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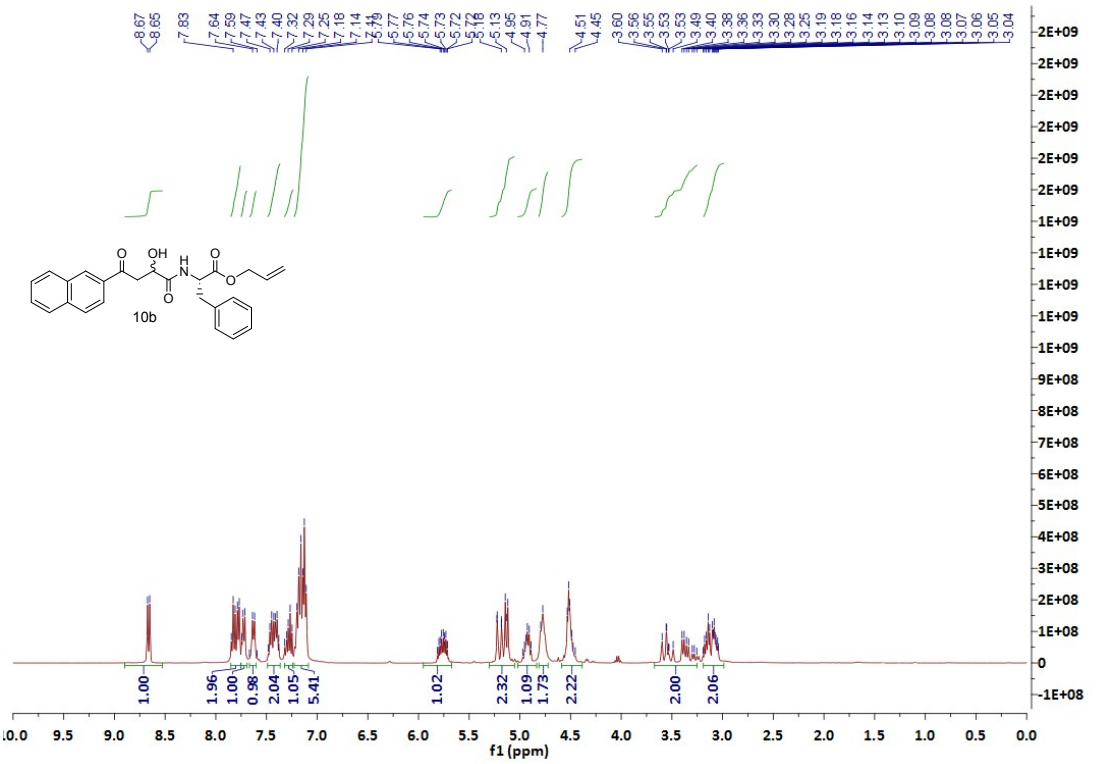
10b
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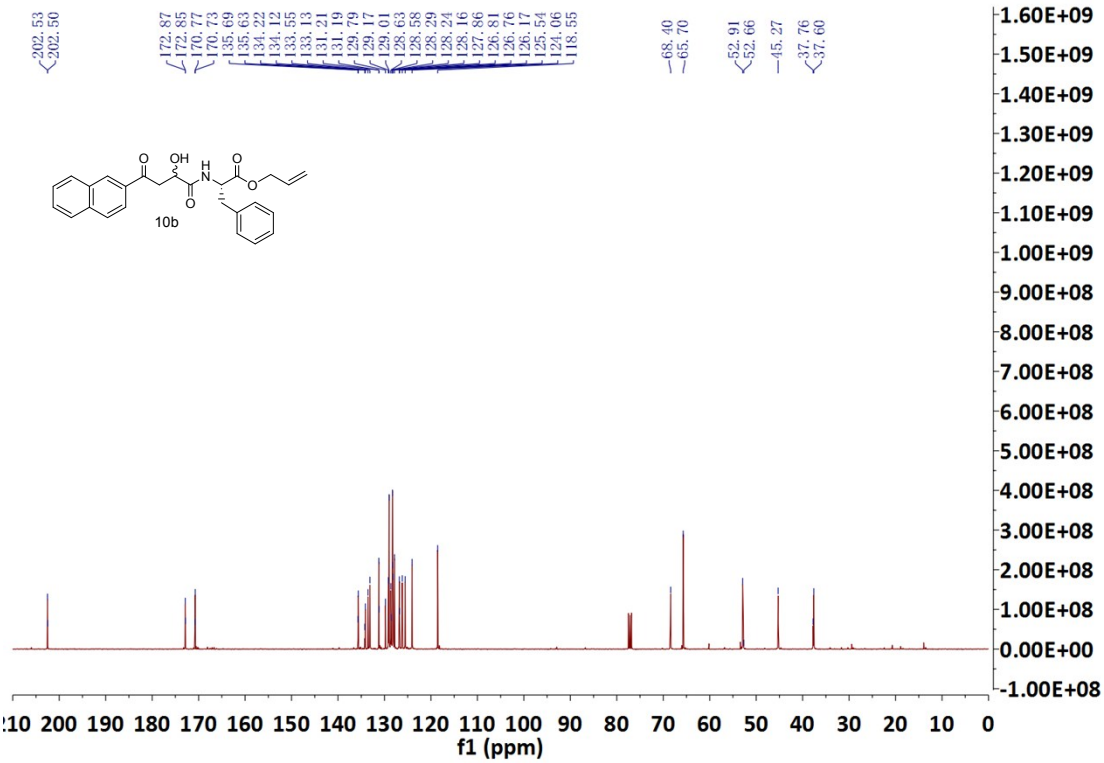
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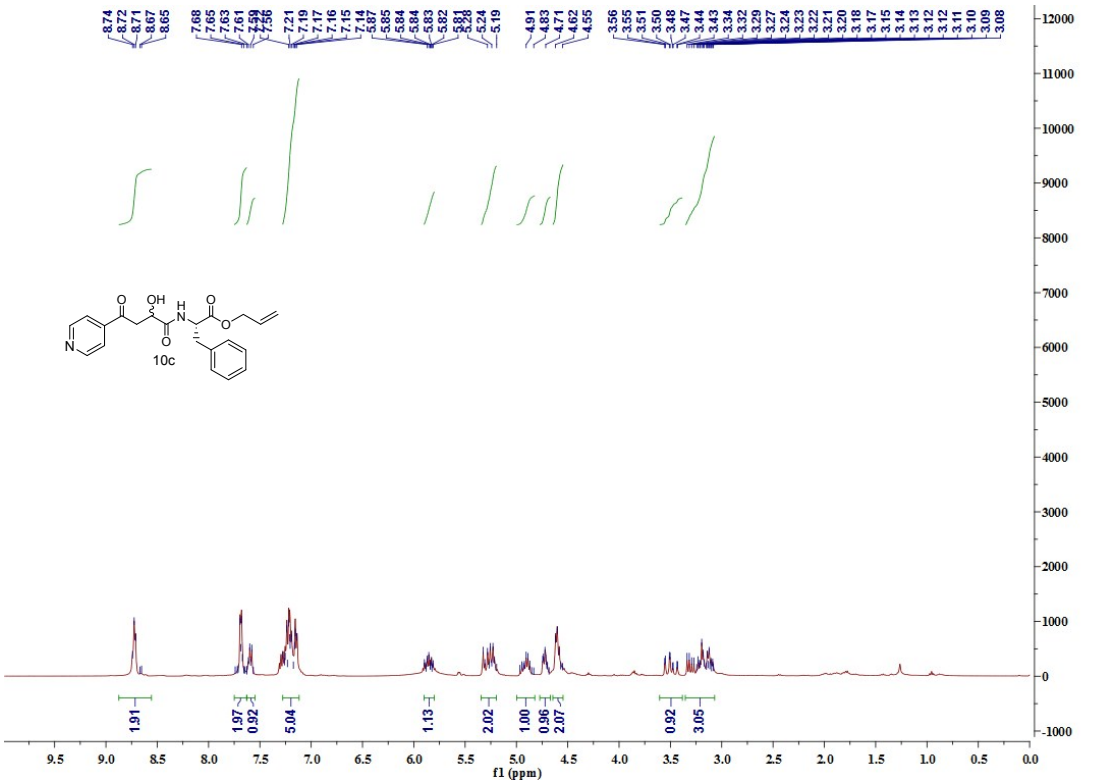
f1 (ppm)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7.14
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5.85
5.84
5.84
5.83
5.82
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4.83
4.71
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3.56
3.55
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3.47
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3.43
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3.29
3.27
3.24
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3.21
3.20
3.18
3.17
3.15
3.14
3.13
3.12
3.11
3.10
3.09
3.08
10c
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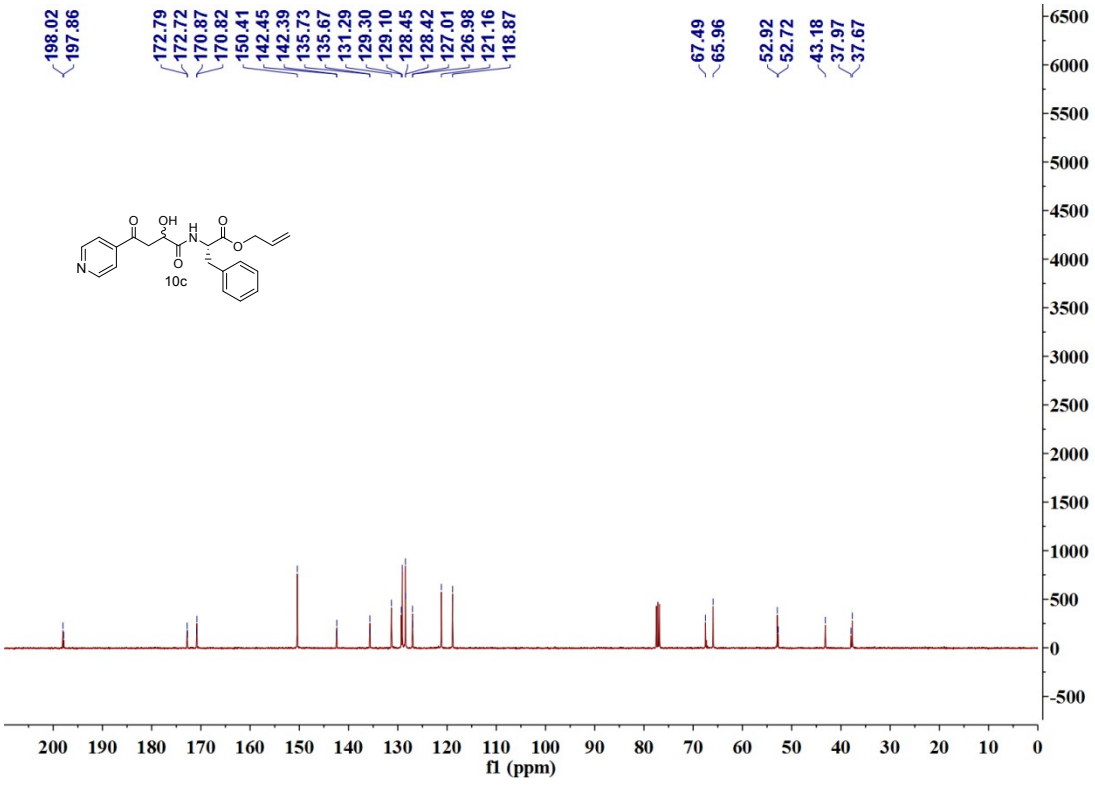
1.13
2.02
1.00
0.96
2.07
0.92
3.05
f1 (ppm)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128.45
128.42
127.01
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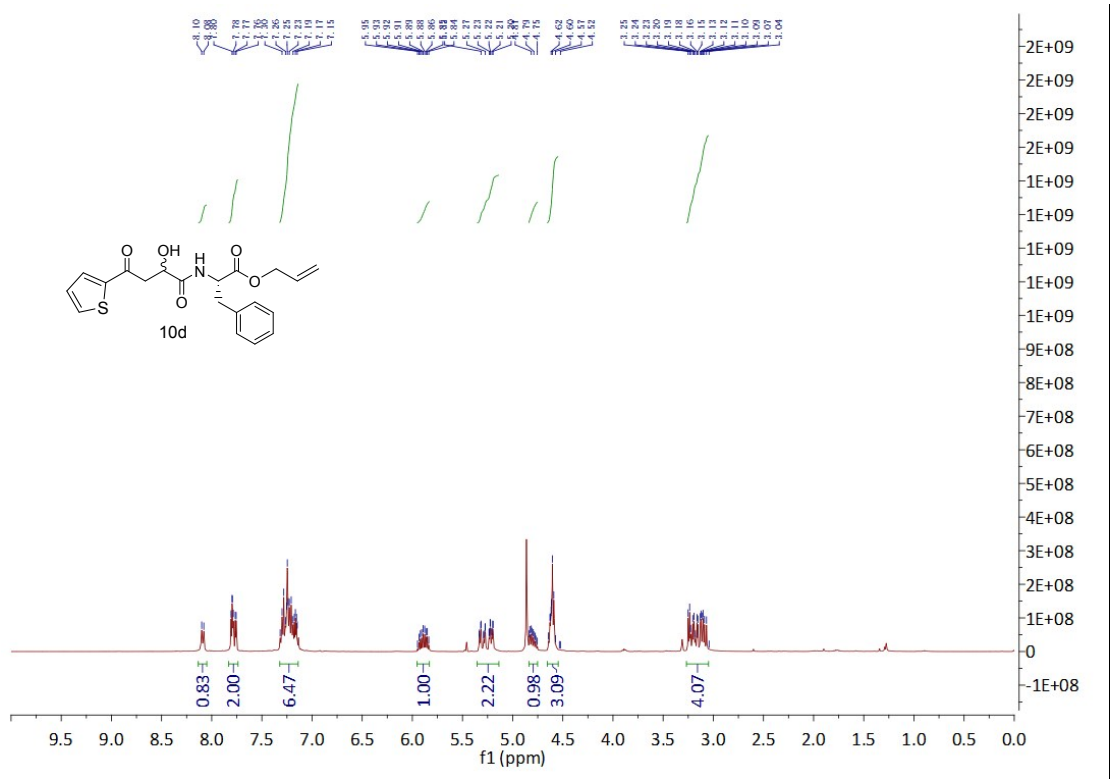
52.92
52.72
43.18
37.97
37.67
10c
f1 (ppm)


10d
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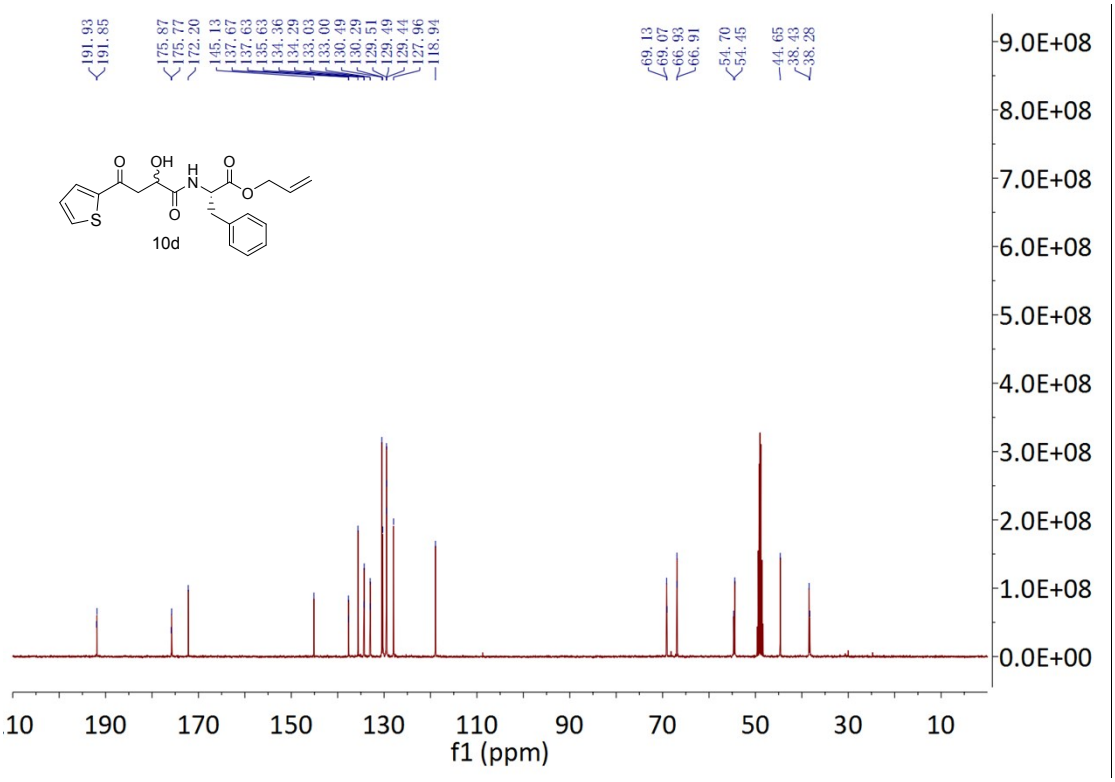
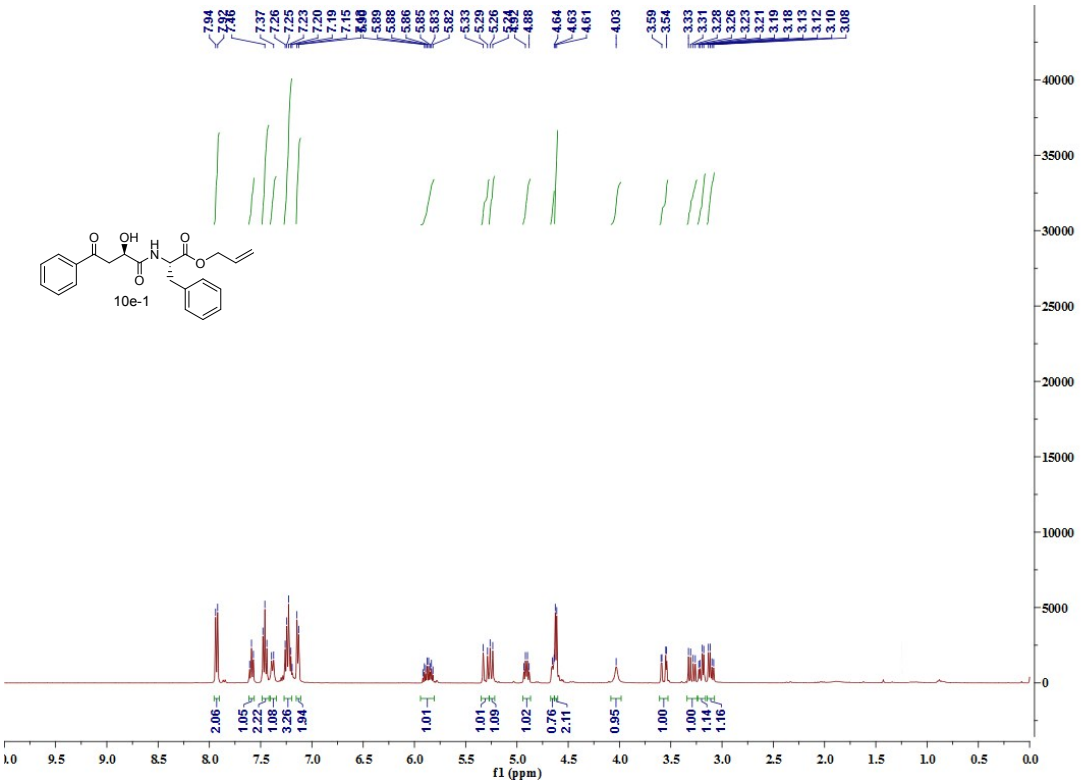
0.98
3.09
4.07
f1 (ppm)


10d
f1 (ppm)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7.23
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7.19
7.15
5.89
5.88
5.86
5.85
5.83
5.82
5.33
5.29
5.26
4.88
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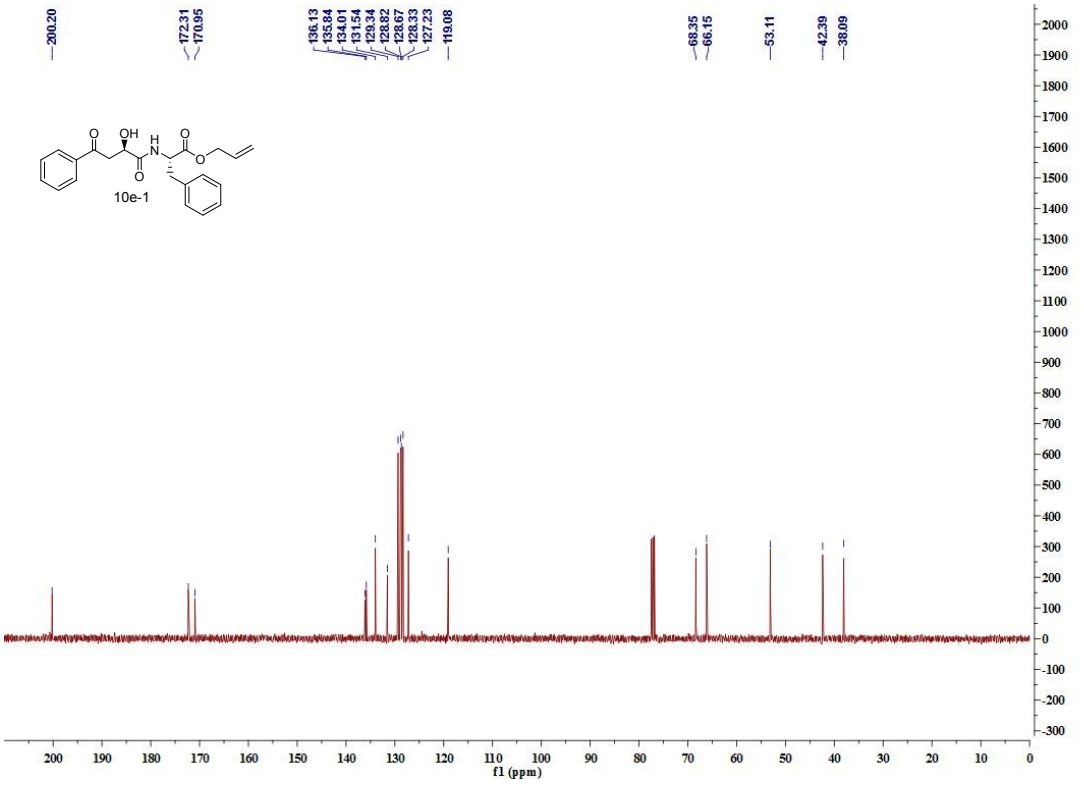
4.63
4.61
4.03
3.59
3.54
3.33
3.31
3.28
3.26
3.23
3.21
3.19
3.18
3.13
3.12
3.10
3.08
10e-1
2.06
1.05
2.22
1.08
3.26
1.94
1.01
1.01
1.09
1.02
0.76
2.11
0.95
1.00
1.00
1.14
1.16
f1 (ppm)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129.34
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127.23
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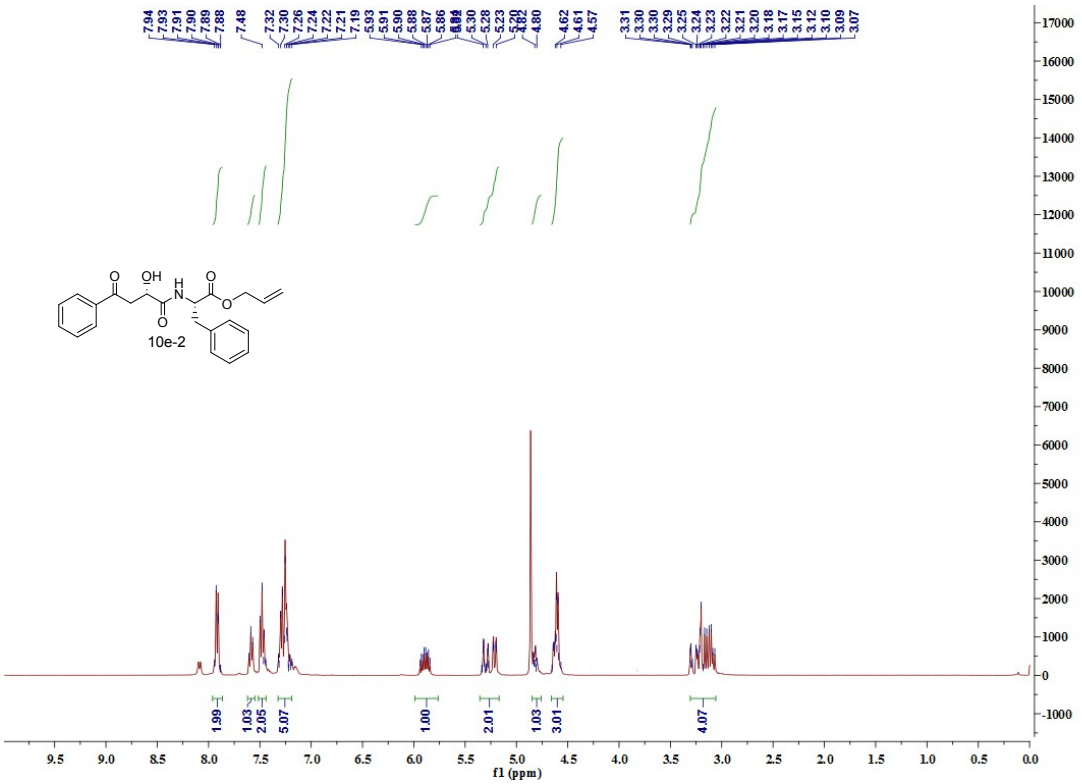
66.15
53.11
42.39
38.09
10e-1
f1 (ppm)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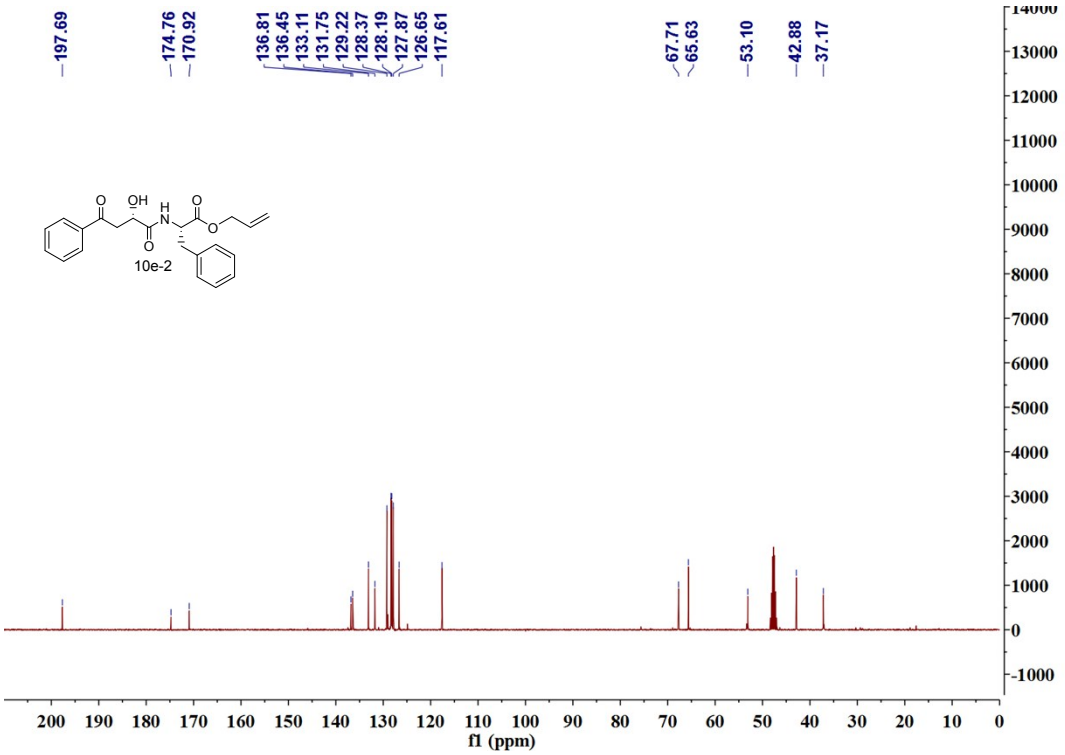
f1 (ppm)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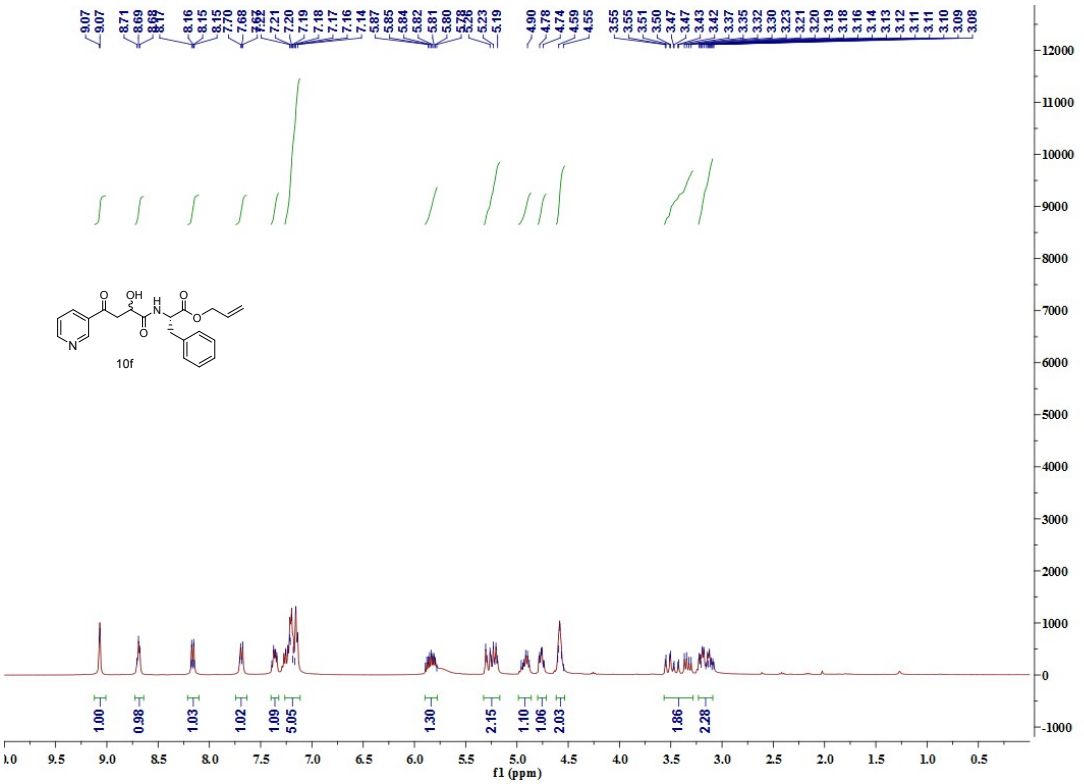
f1 (ppm)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9.07
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8.15
8.15
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7.68
7.22
7.21
7.20
7.19
7.18
7.17
7.16
7.14
5.87
5.85
5.84
5.82
5.81
5.80
5.28
5.23
5.19
4.90
4.78
4.74
4.59
4.55
3.55
3.55
3.51
3.50
3.47
3.47
3.43
3.42
3.37
3.35
3.32
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3.23
3.21
3.20
3.19
3.18
3.16
3.14
3.13
3.12
3.11
3.11
3.10
3.09
3.08
10f
1.00
0.98
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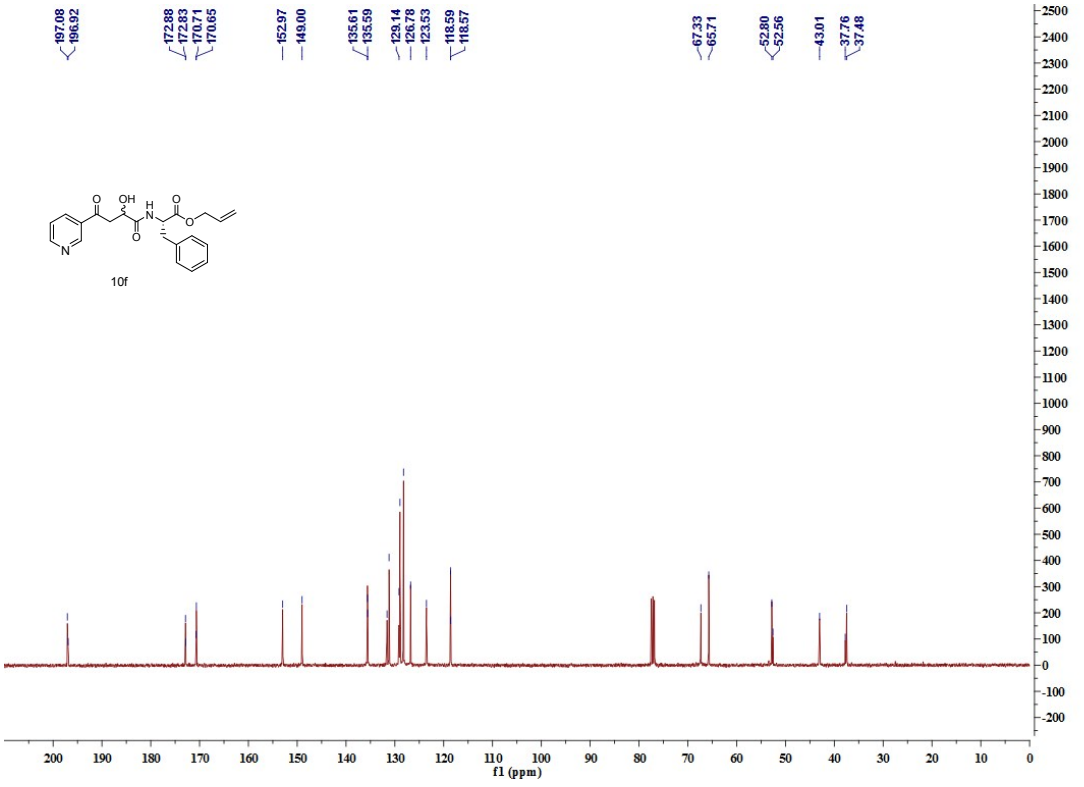
1.02
1.09
5.05
1.30
2.15
1.10
1.06
2.03
1.86
2.28
f1 (ppm)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135.59
129.14
126.78
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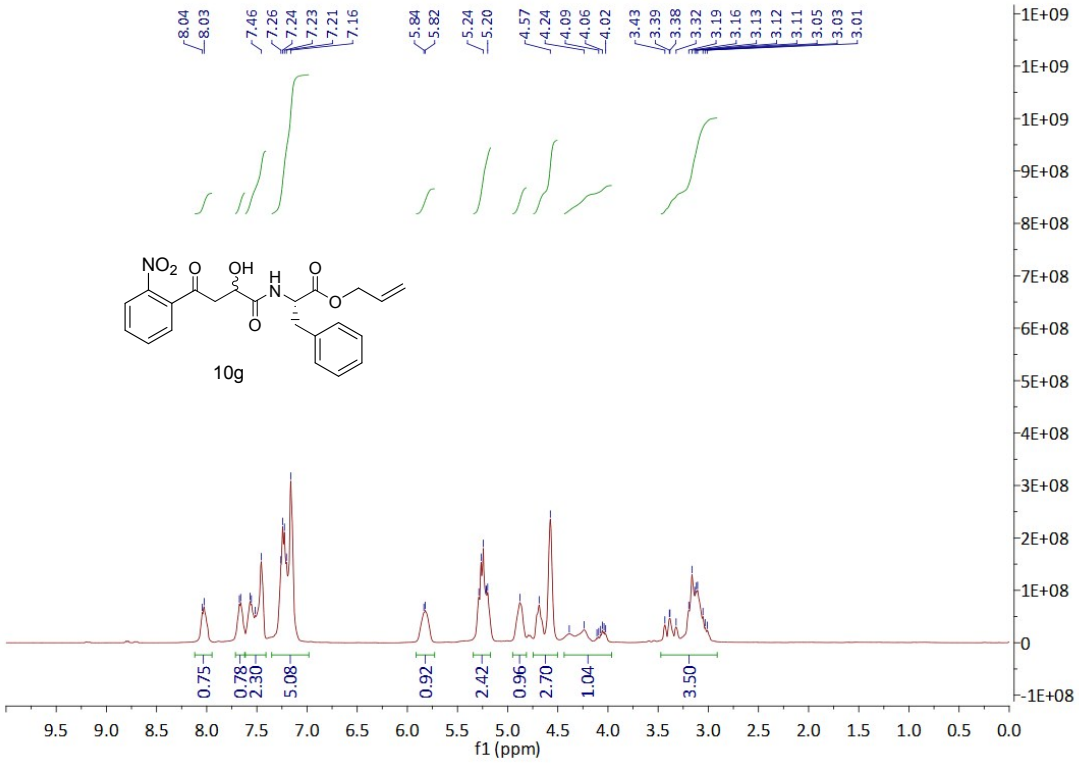
67.33
65.71
52.80
52.56
43.01
37.76
37.48
10f
f1 (ppm)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8.03
7.46
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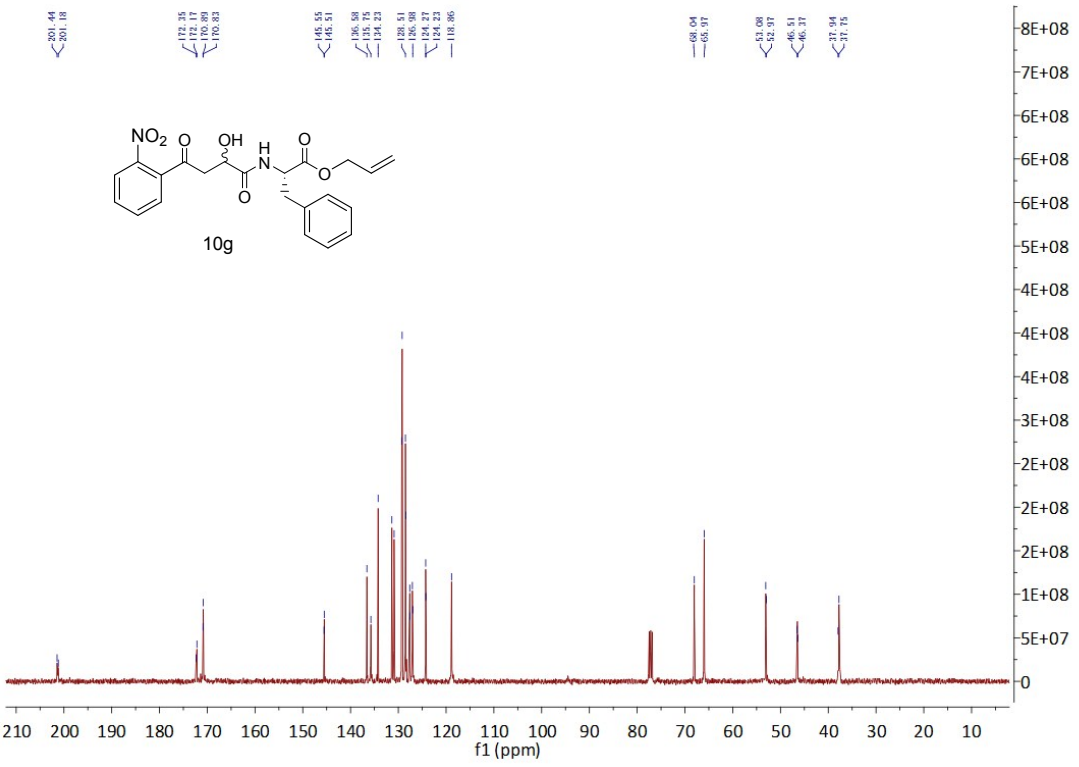
7.24
7.23
7.21
7.16
5.84
5.82
5.24
5.20
4.57
4.24
4.09
4.06
4.02
3.43
3.39
3.38
3.32
3.19
3.16
3.13
3.12
3.11
3.05
3.03
3.01
NO2
O
OH
H
N
O
O
O
10g
0.75
0.78
2.30
5.08
0.92
2.42
0.96
2.70
1.04
3.50
f1 (ppm)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128.98
124.27
124.23
118.86
68.04
65.97
53.08
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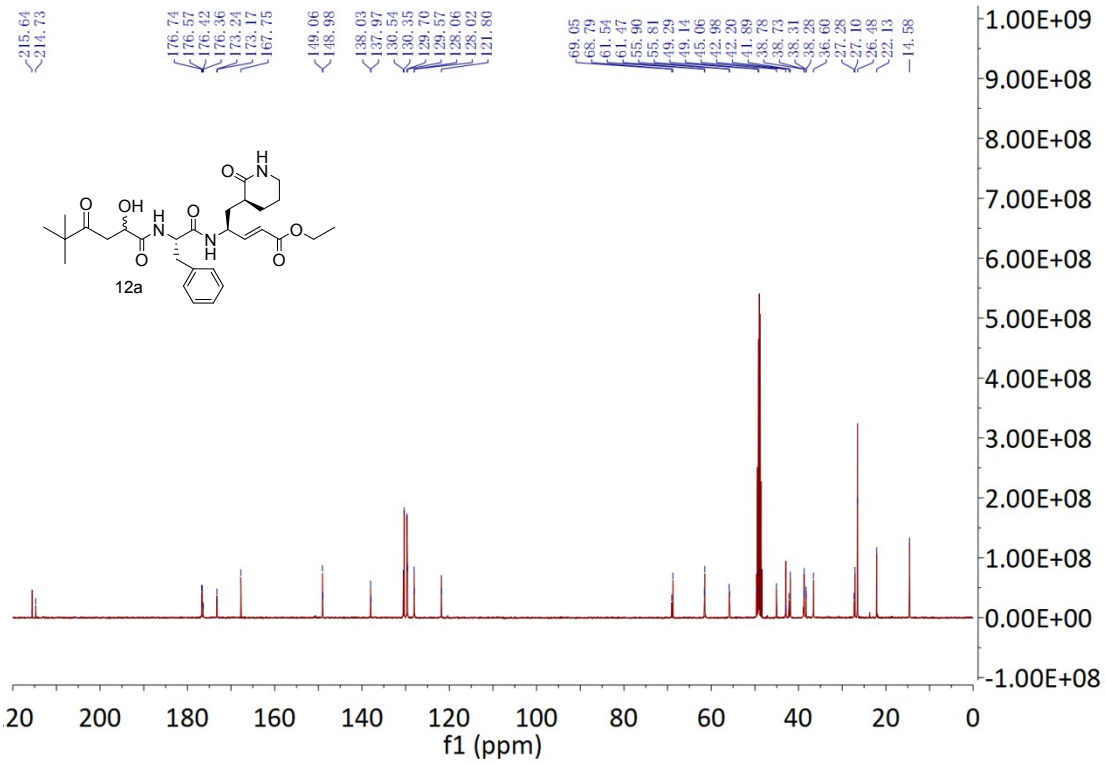
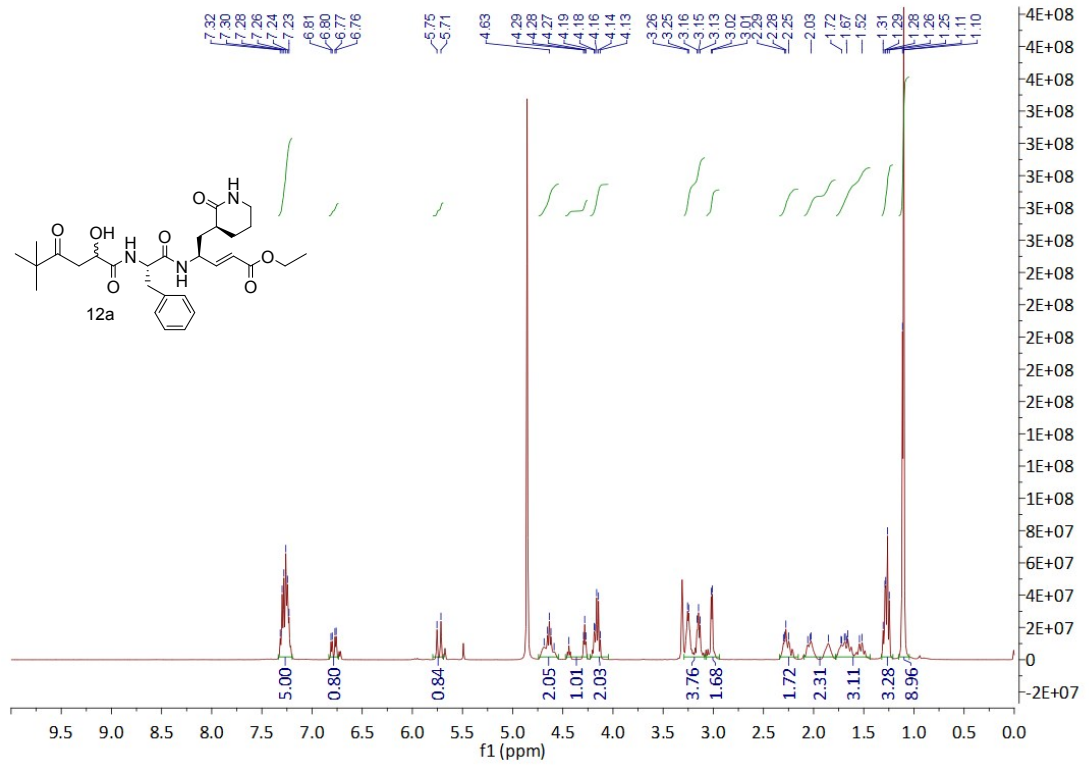
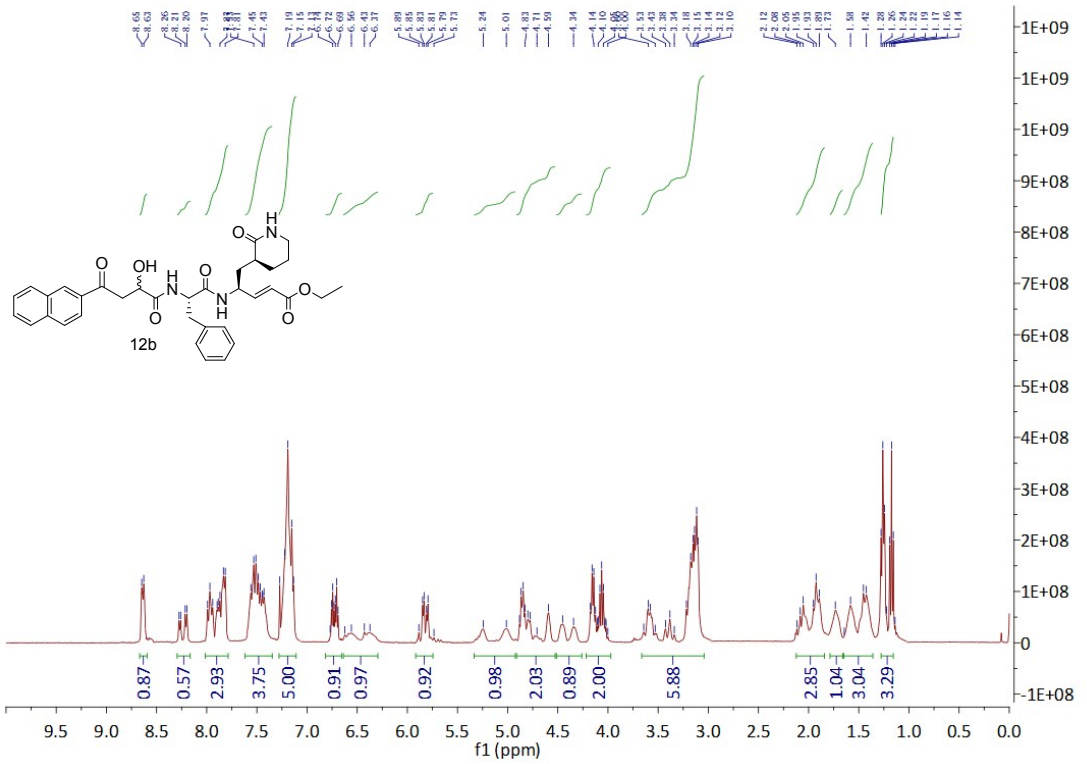
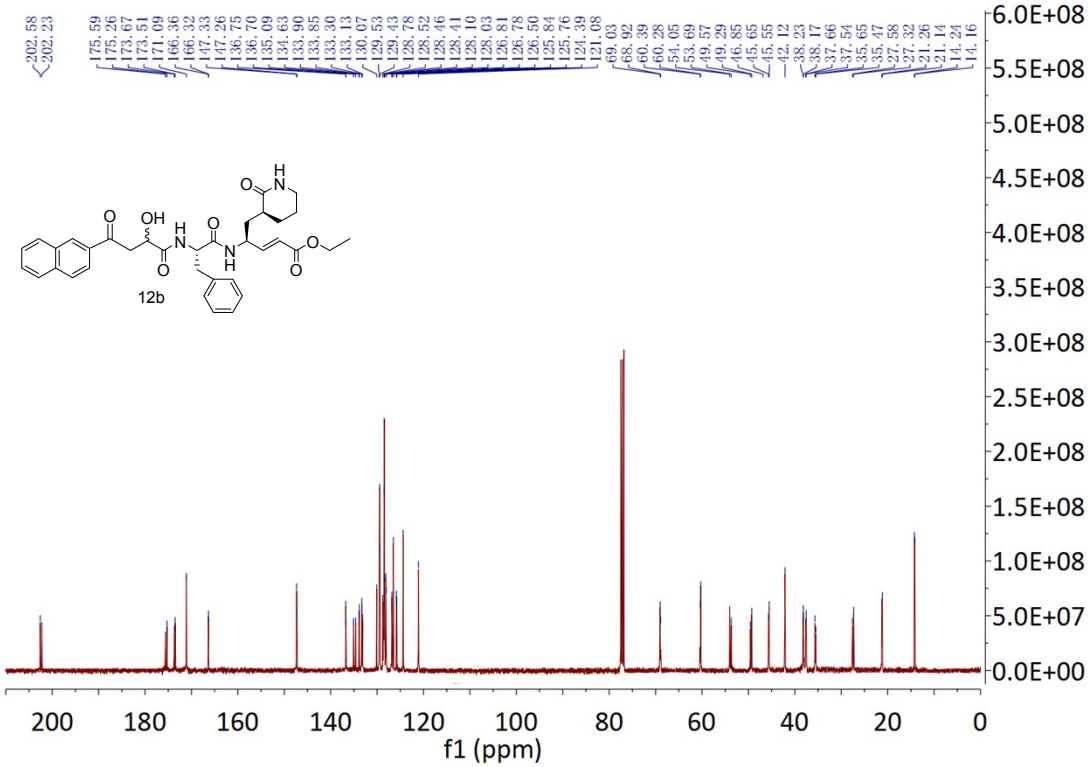
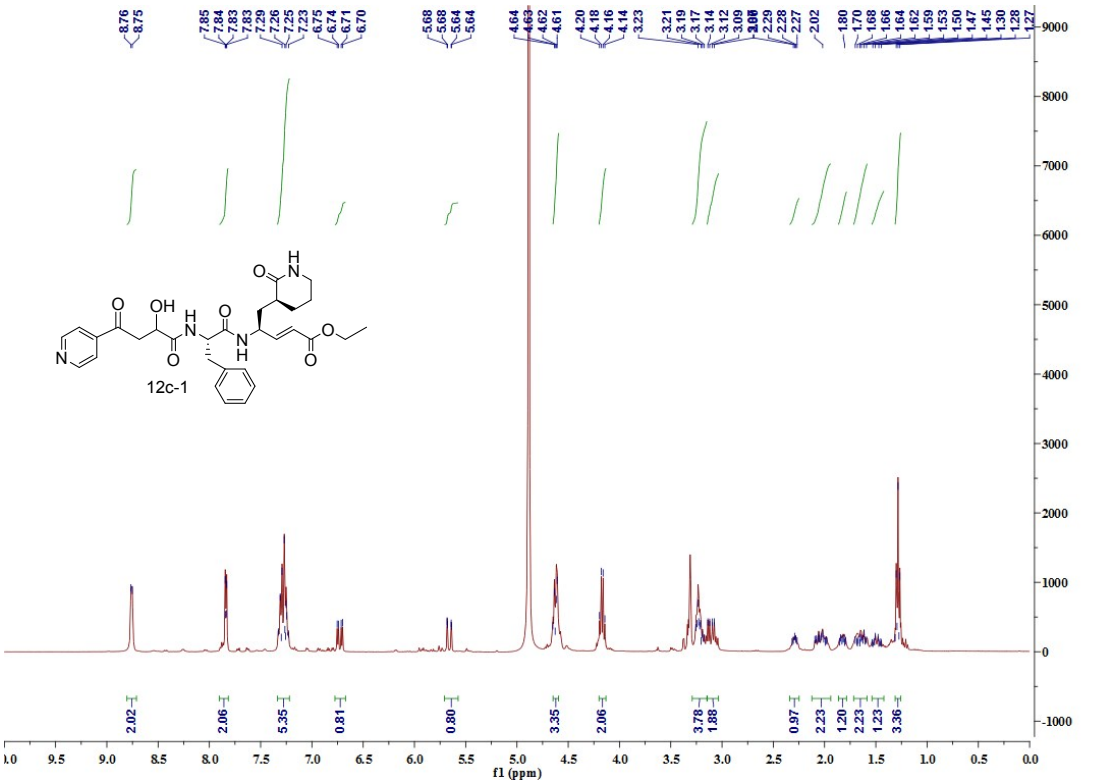
46.51
46.37
37.94
37.75
NO2
O
OH
H
N
O
O
O
10g
f1 (ppm)


12a
f1 (ppm)


12a
f1 (ppm)


12b
0.87
0.57
2.93
3.75
5.00
0.91
0.97
0.92
0.98
2.03
0.89
2.00
5.88
2.85
1.04
3.04
3.29
f1 (ppm)


12b
f1 (ppm)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7.23
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6.71
6.70
5.68
5.68
5.64
5.64
4.64
4.63
4.62
4.61
4.20
4.18
4.16
4.14
3.23
3.21
3.19
3.17
3.14
3.12
3.09
2.29
2.28
2.27
2.02
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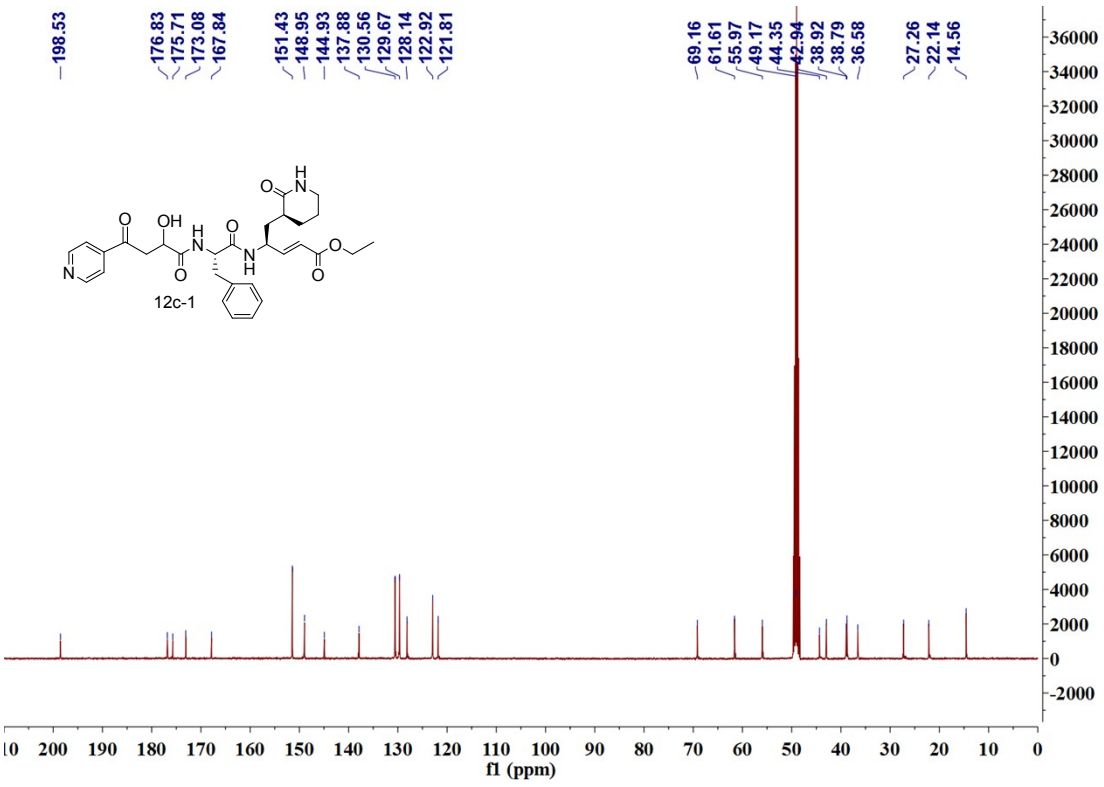
1.68
1.66
1.64
1.62
1.59
1.53
1.50
1.47
1.45
1.30
1.28
1.27
12c-1
2.02
2.06
5.35
0.81
0.80
3.35
2.06
3.78
1.88
0.97
2.23
1.20
2.23
1.23
3.36
f1 (ppm)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175.71
173.08
167.84
151.43
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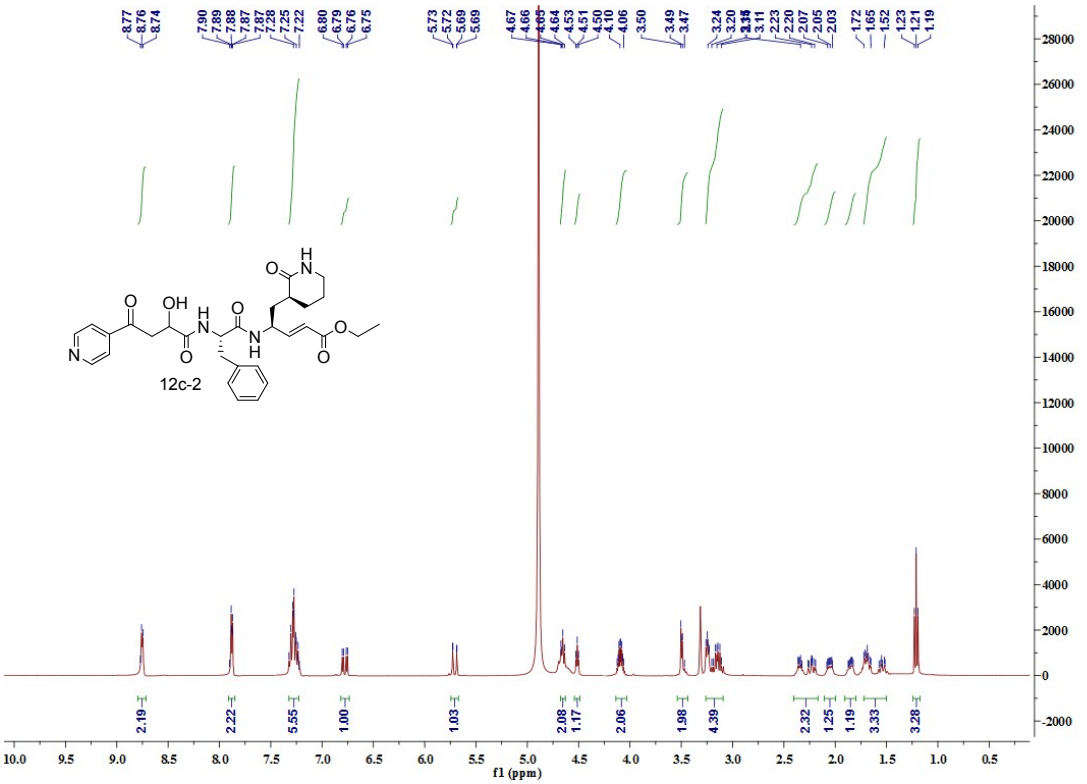
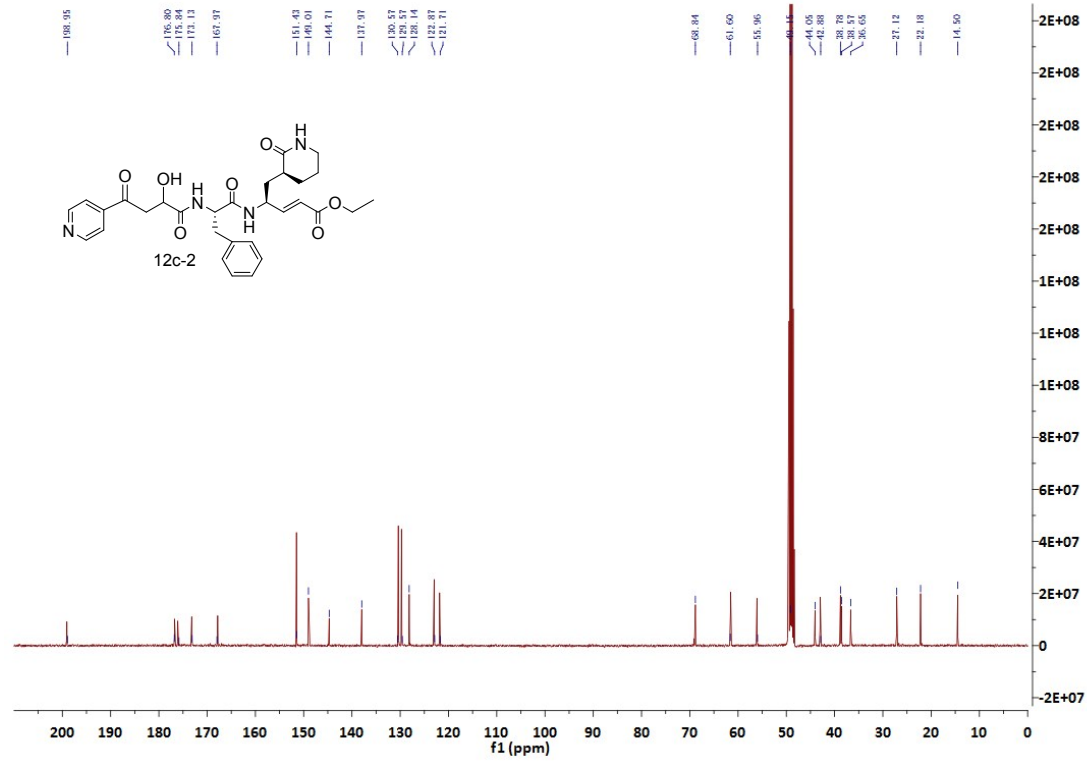
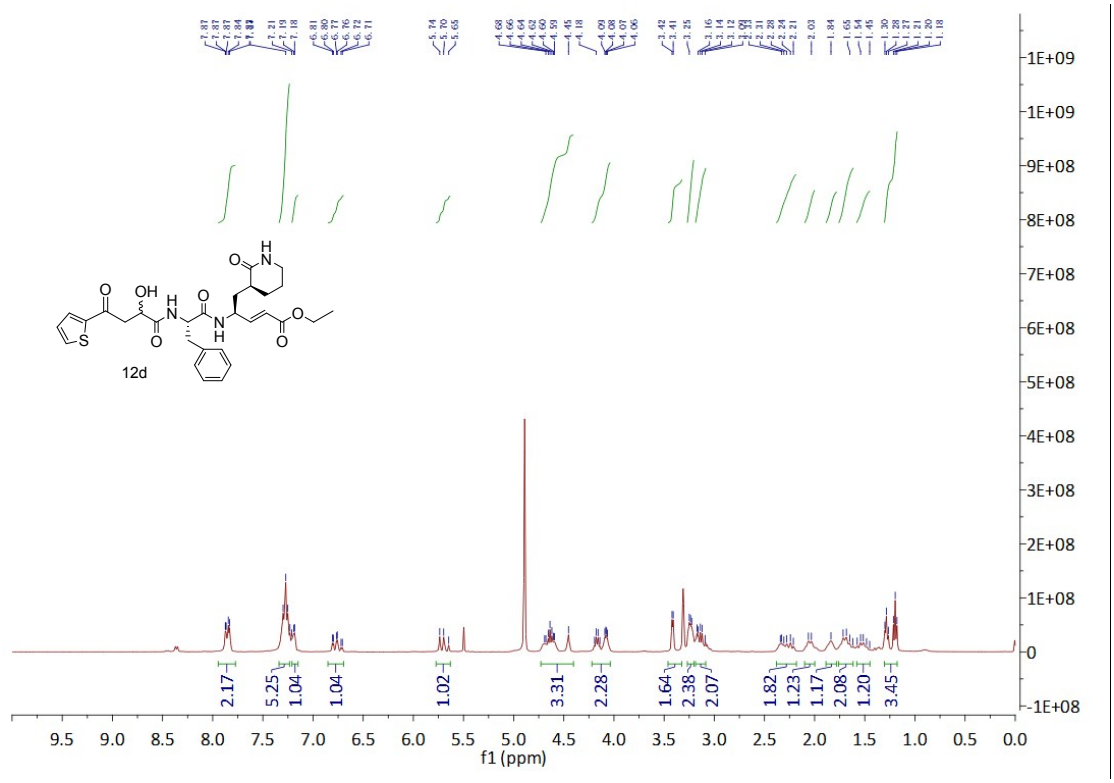
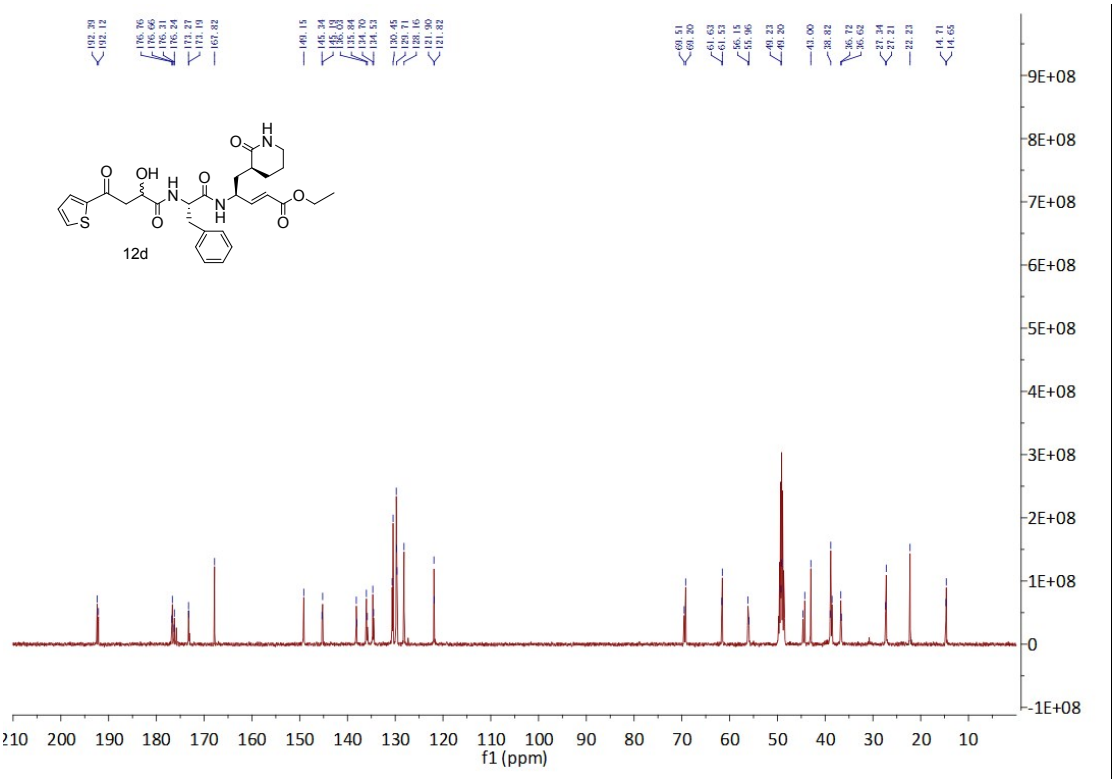
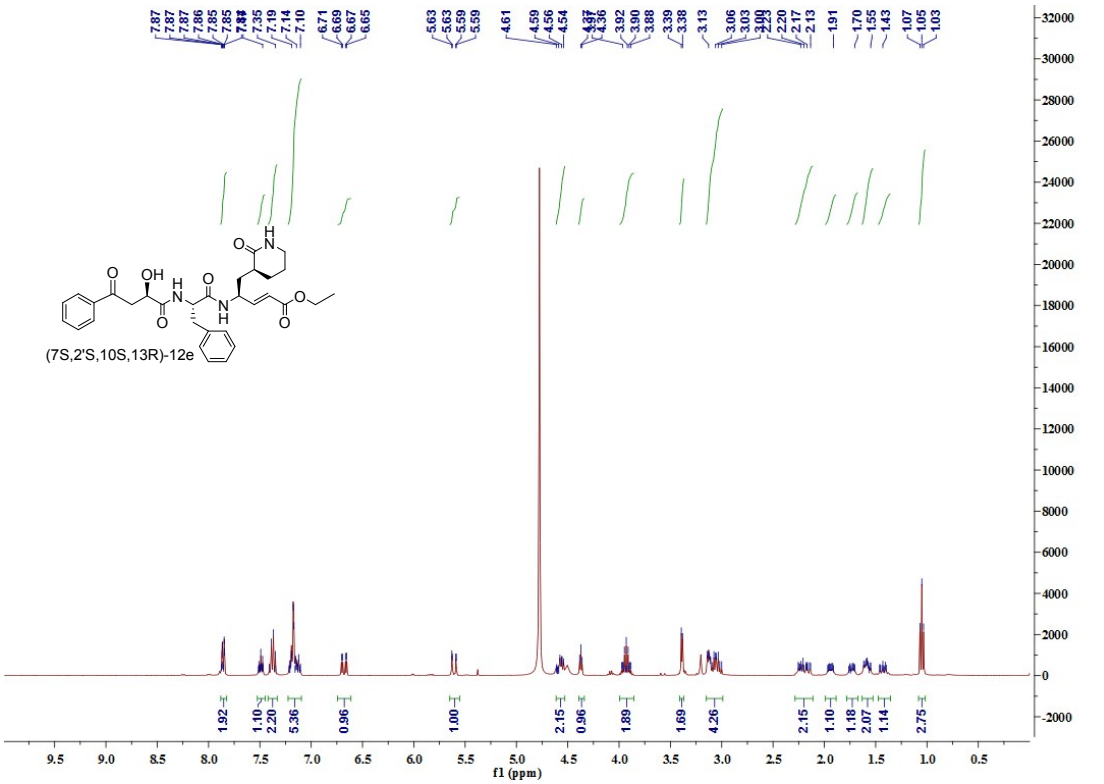
144.93
137.88
130.56
129.67
128.14
122.92
121.81
69.16
61.61
55.97
49.17
44.35
42.94
38.92
38.79
36.58
27.26
22.14
14.56
12c-1
f1 (ppm)

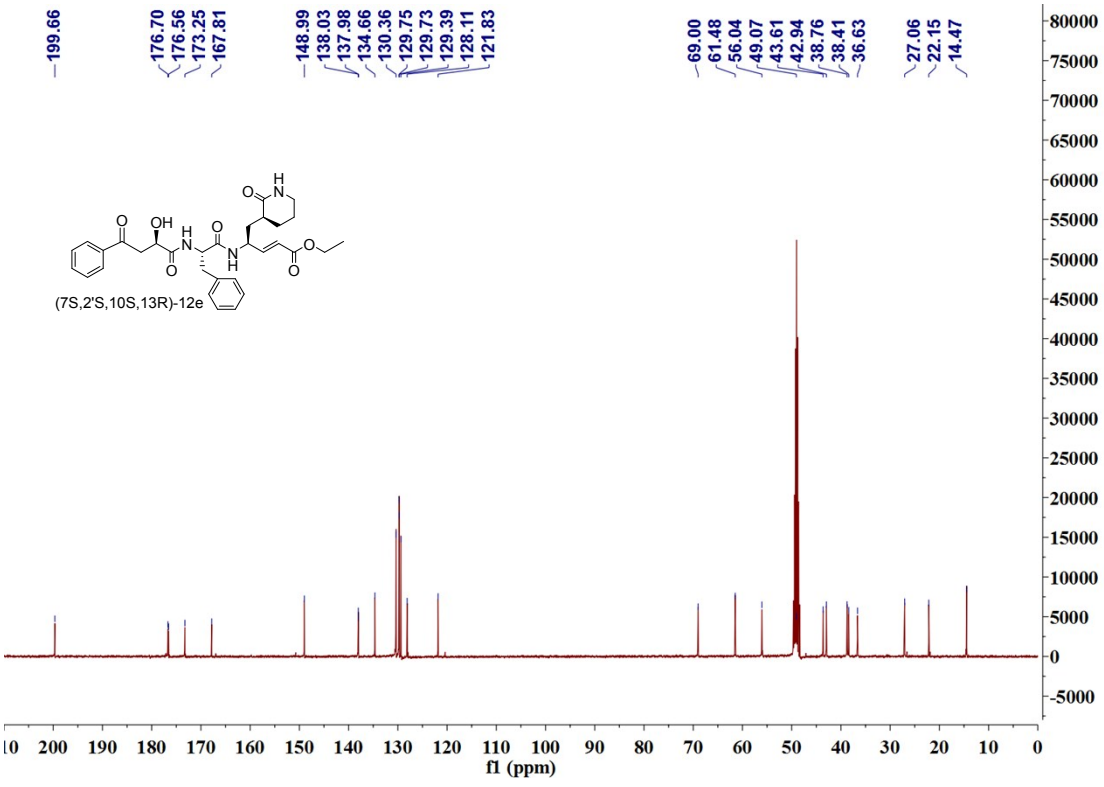

12c-2
f1 (ppm)

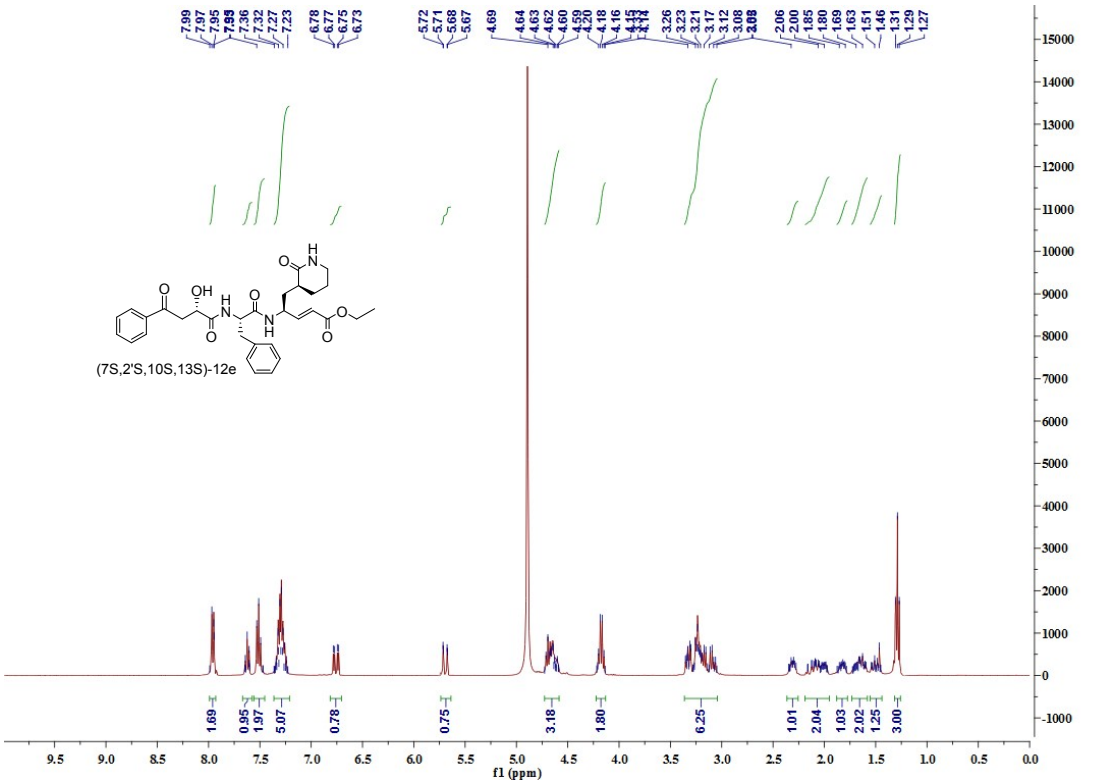

12c-2
f1 (ppm)

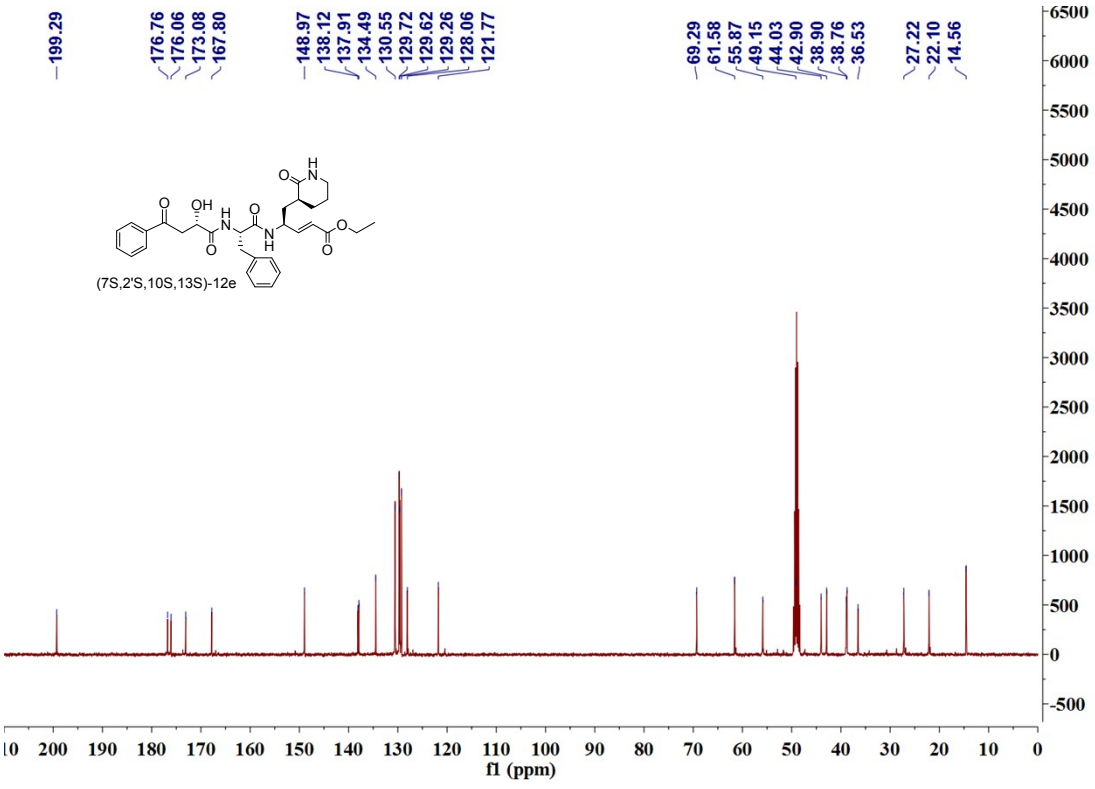

12d
2.17
5.25
1.04
1.04
1.02
3.31
2.28
1.64
2.38
2.07
1.82
1.23
1.17
2.08
1.20
3.45
f1 (ppm)

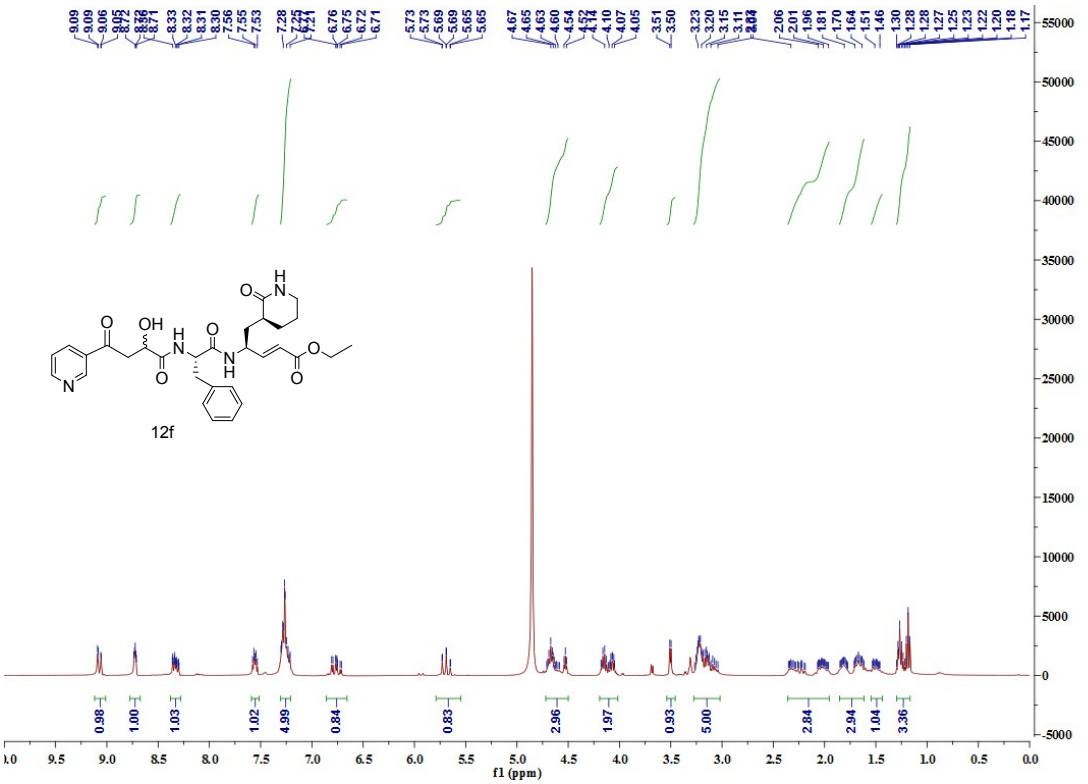

12d
f1 (ppm)

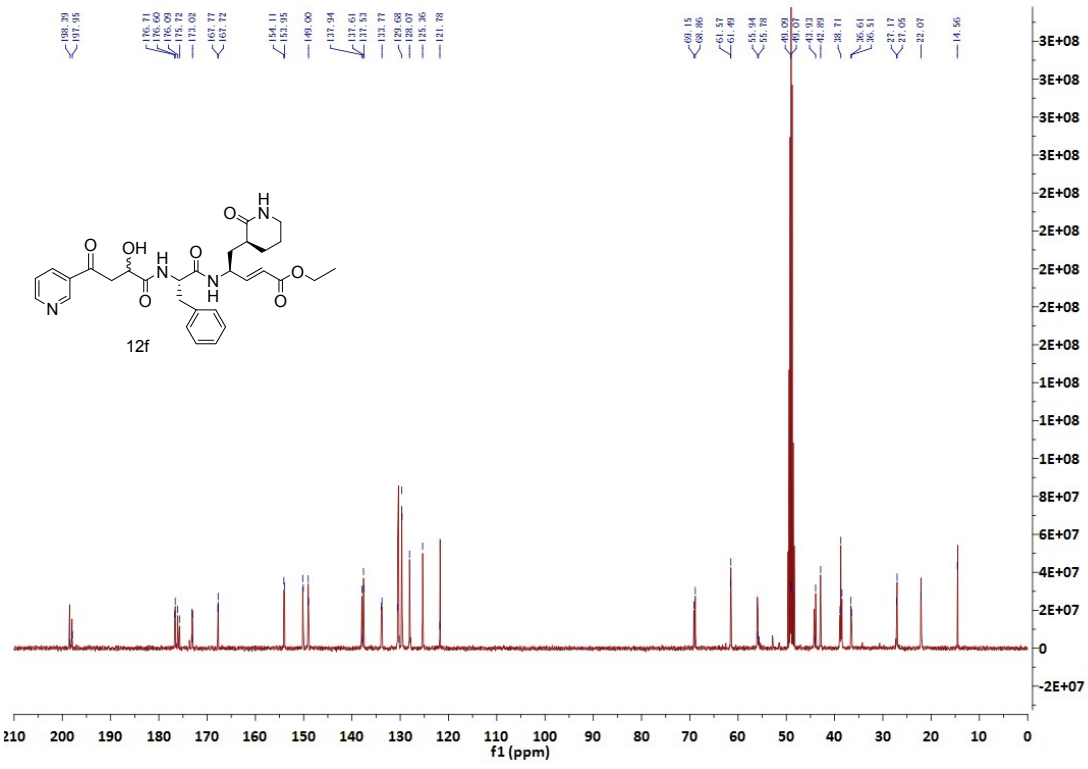

(7S,2'S,10S,13R)-12e
f1 (ppm)

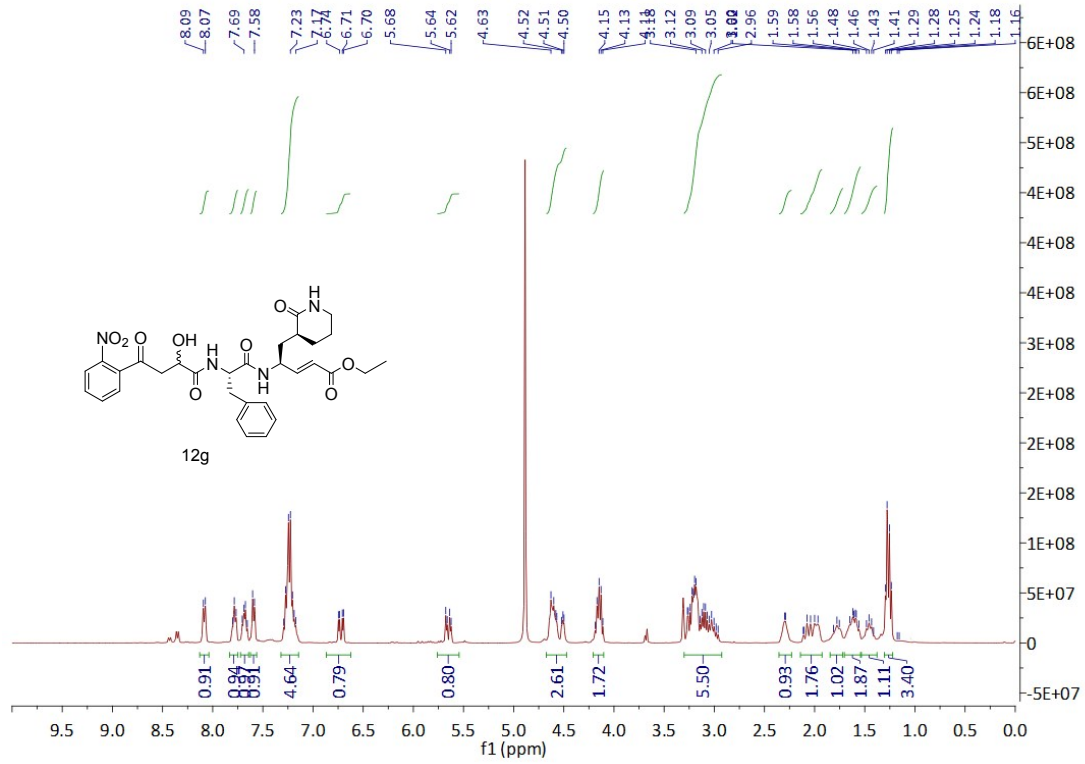

(7S,2'S,10S,13R)-12e
f1 (ppm)

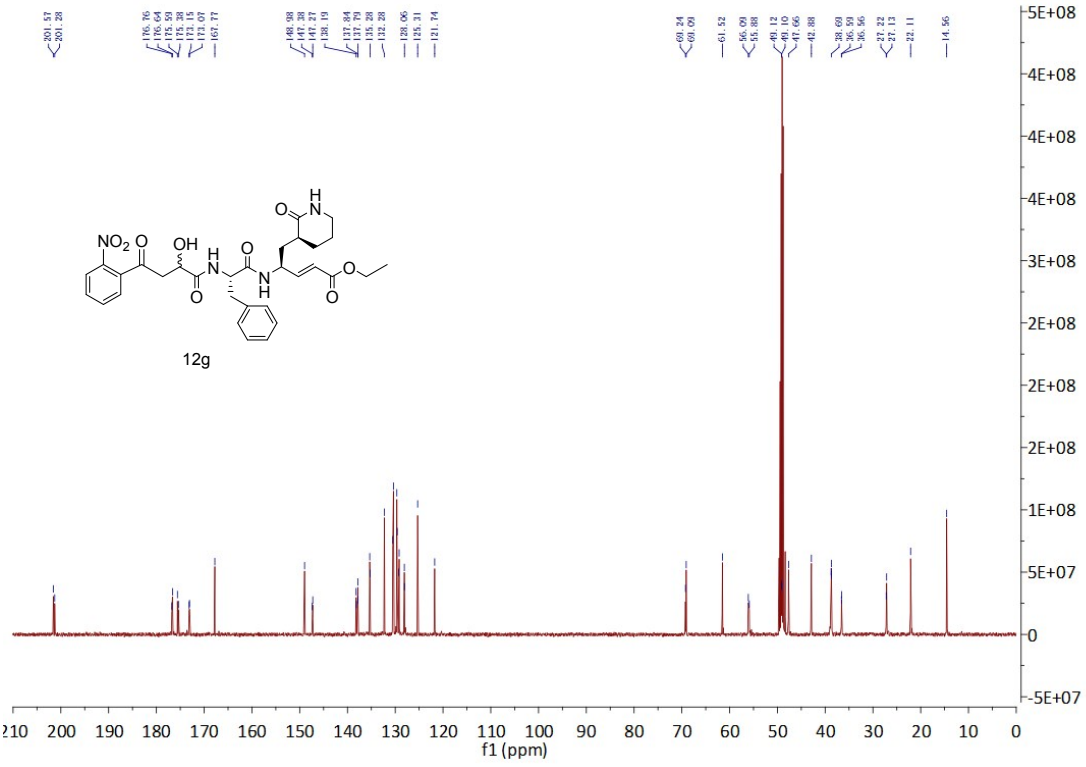

(7S,2'S,10S,13S)-12e
f1 (ppm)

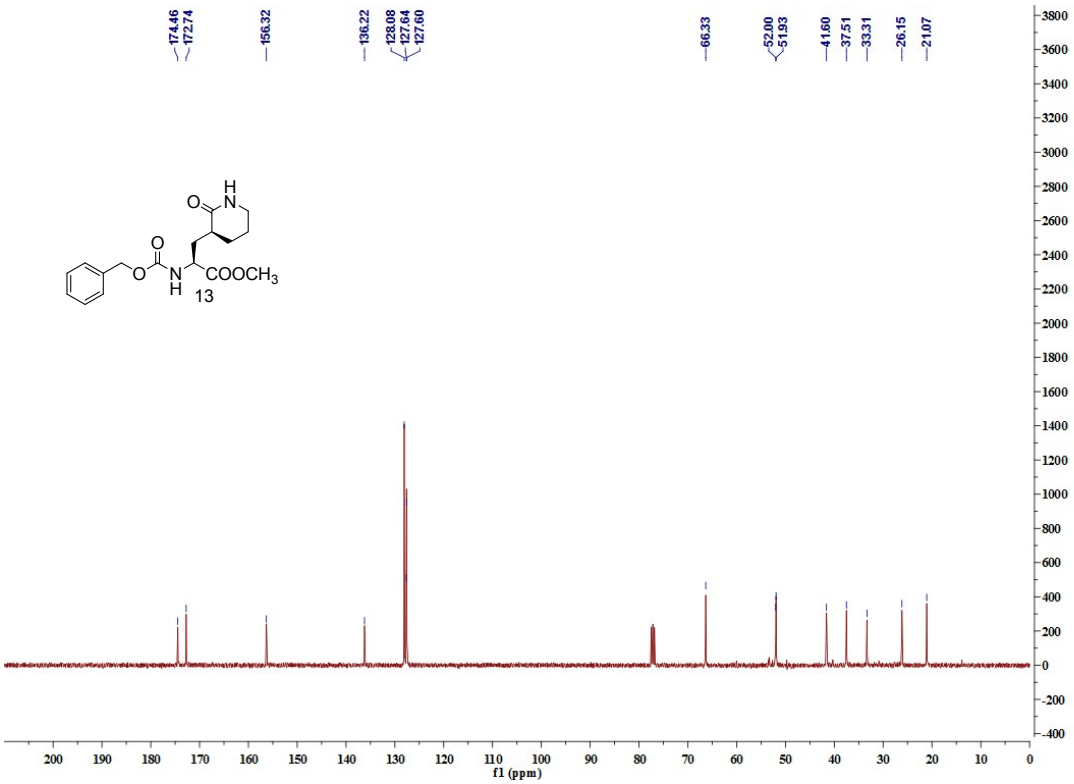

(7S,2'S,10S,13S)-12e
f1 (ppm)


12f
f1 (ppm)


12f
f1 (ppm)


12g
f1 (ppm)


12g
f1 (ppm)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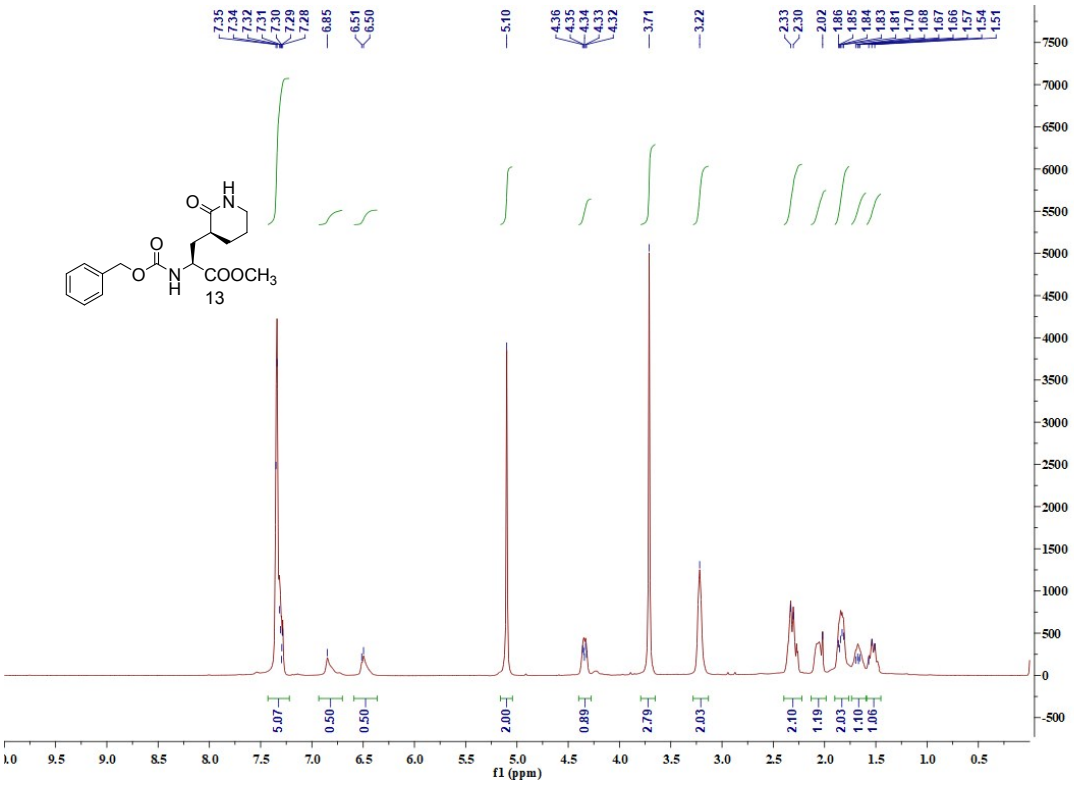
41.60
37.51
33.31
26.15
21.07
13
f1 (ppm)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4.34
4.33
4.32
3.71
3.22
2.33
2.30
2.02
1.86
1.85
1.84
1.83
1.81
1.70
1.68
1.67
1.66
1.57
1.54
1.51
5.07
0.50
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2.79
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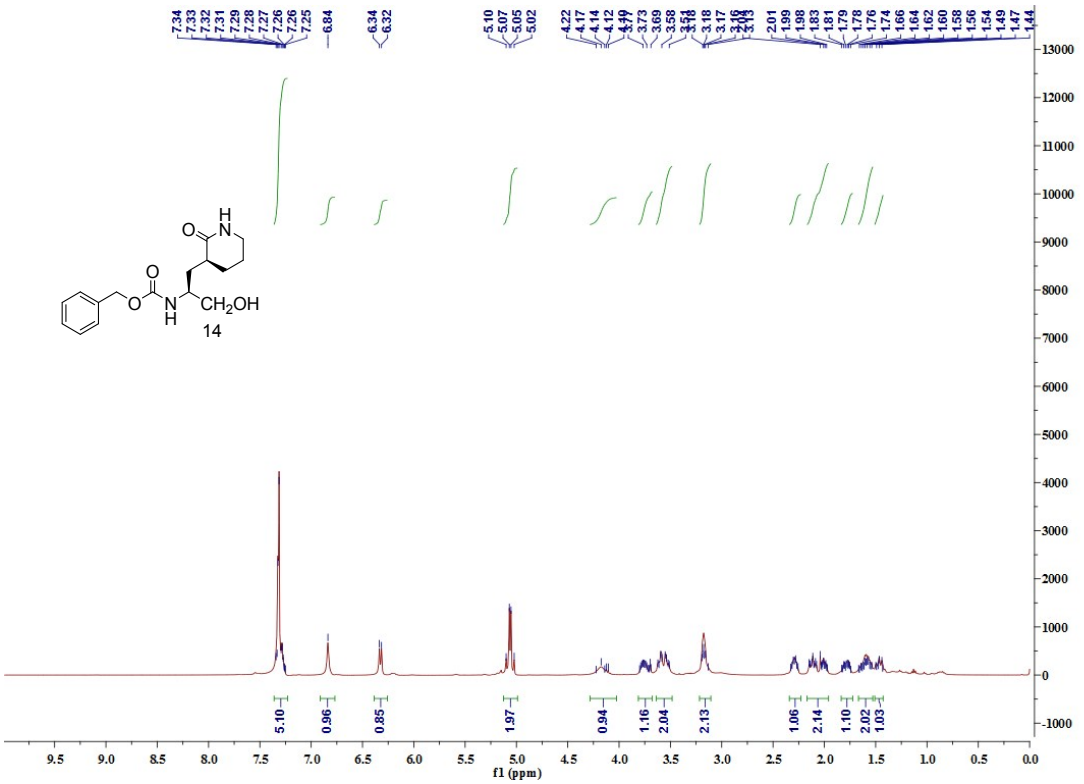
2.10
1.19
2.03
1.10
1.06
13
f1 (ppm)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6.32
5.10
5.07
5.05
5.02
4.22
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1.54
1.49
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1.44
5.10
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1.97
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1.16
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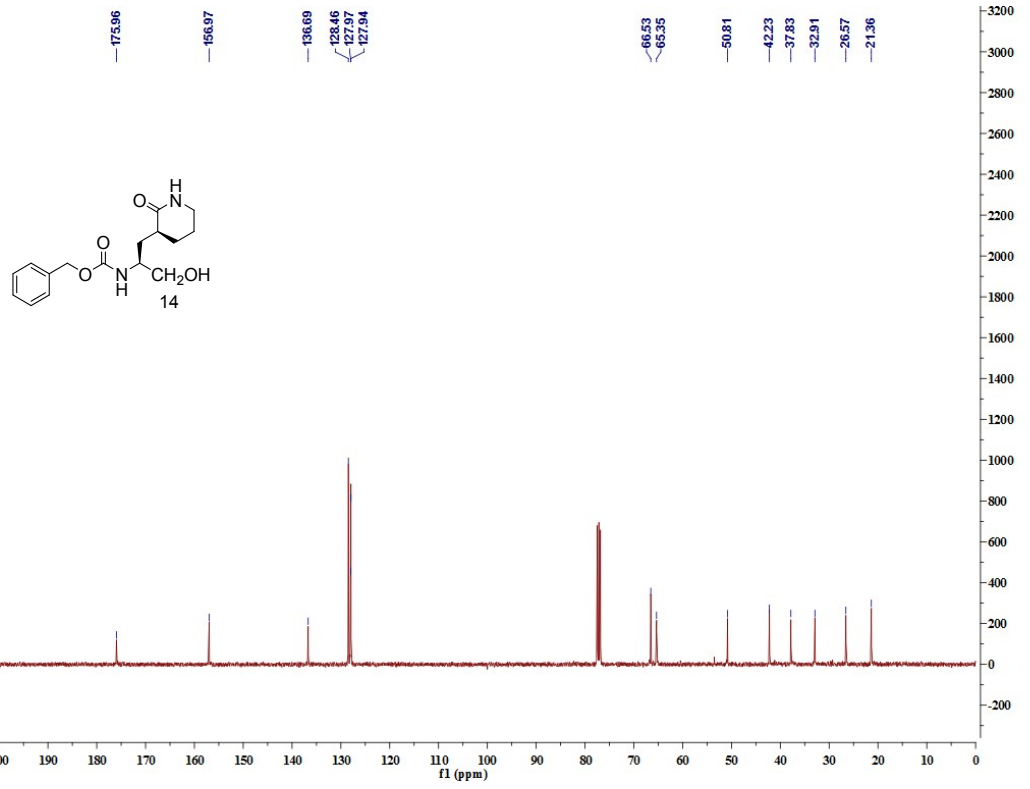
2.13
1.06
2.14
1.10
2.02
1.03
f1 (ppm)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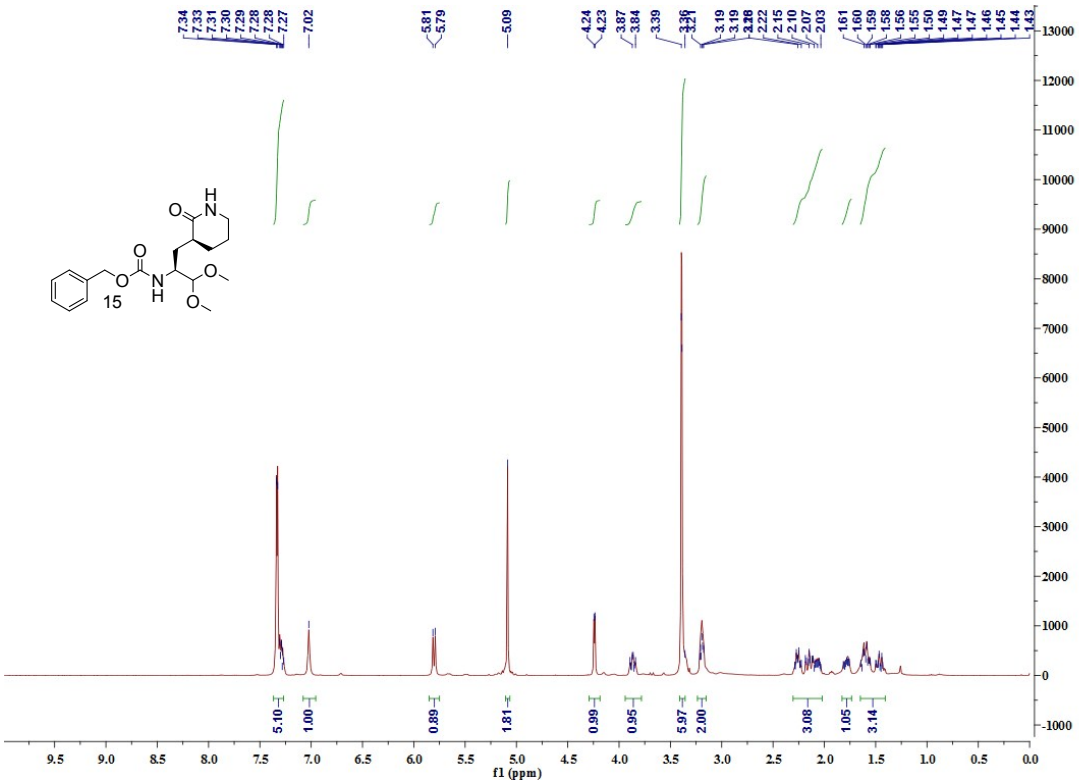
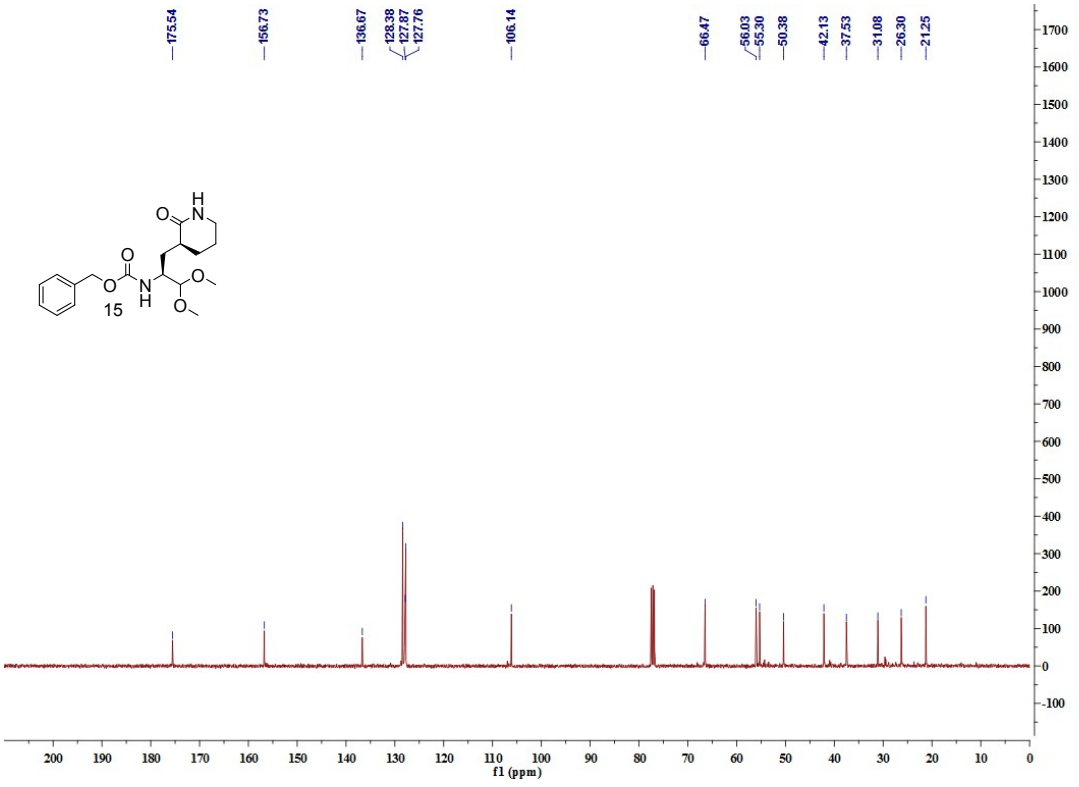
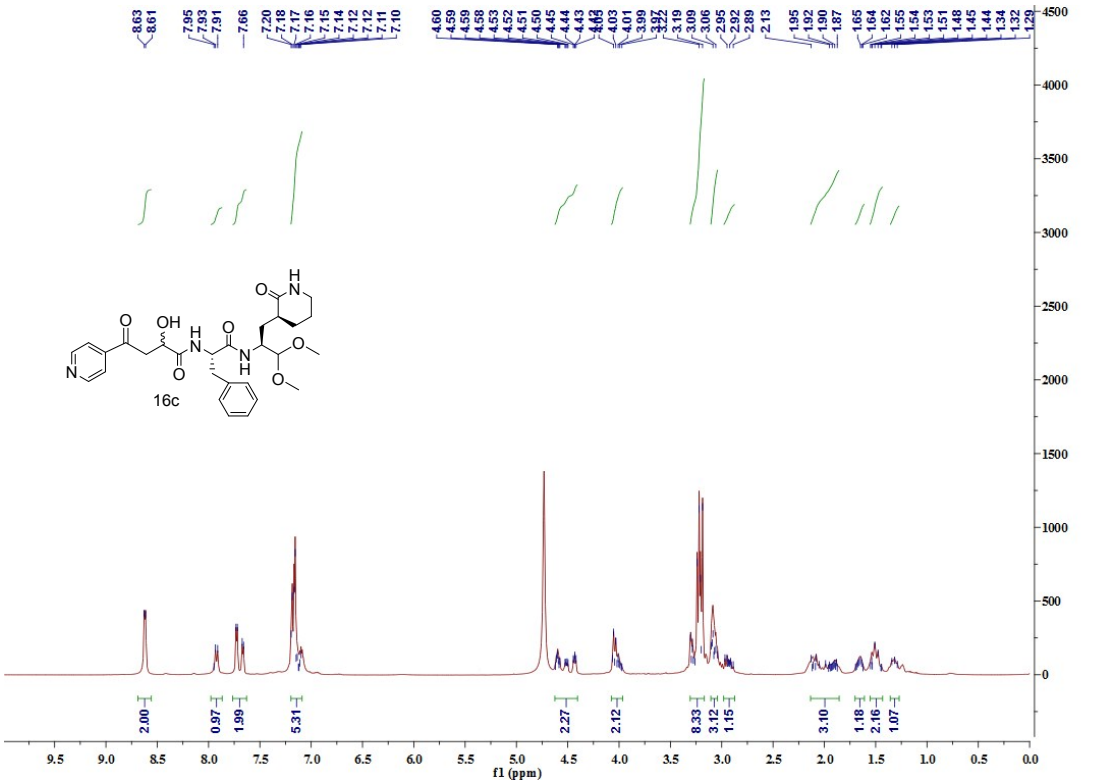
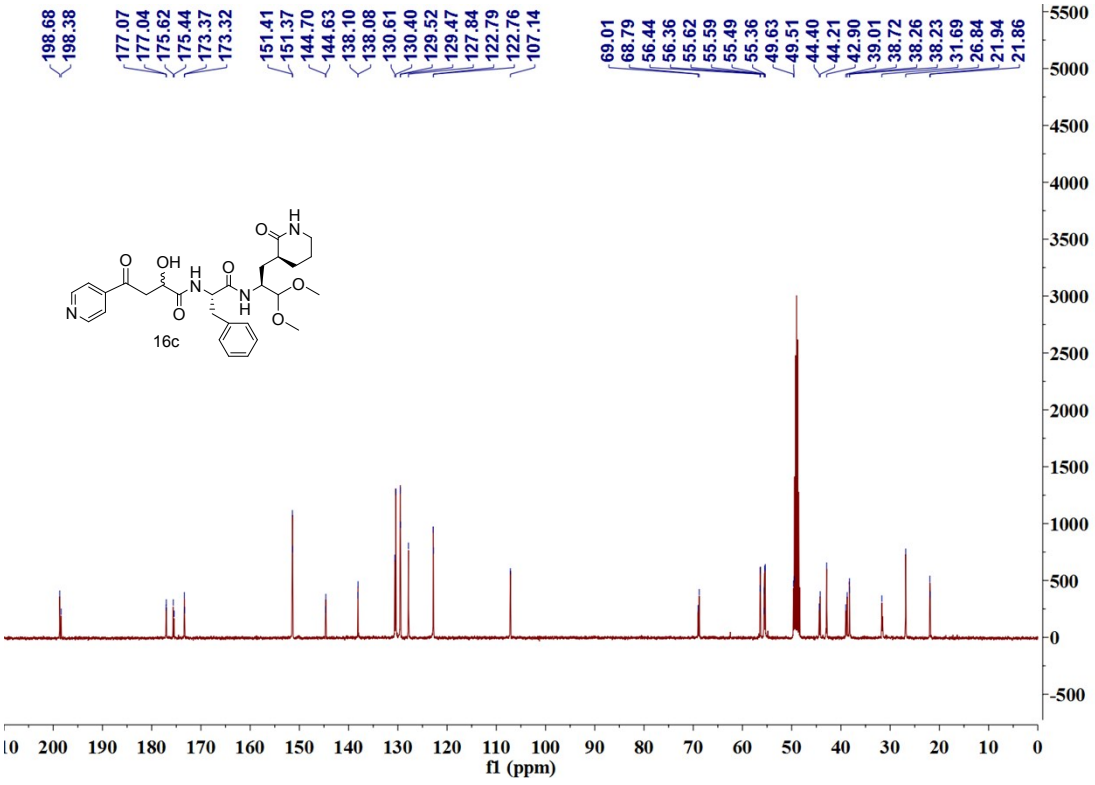
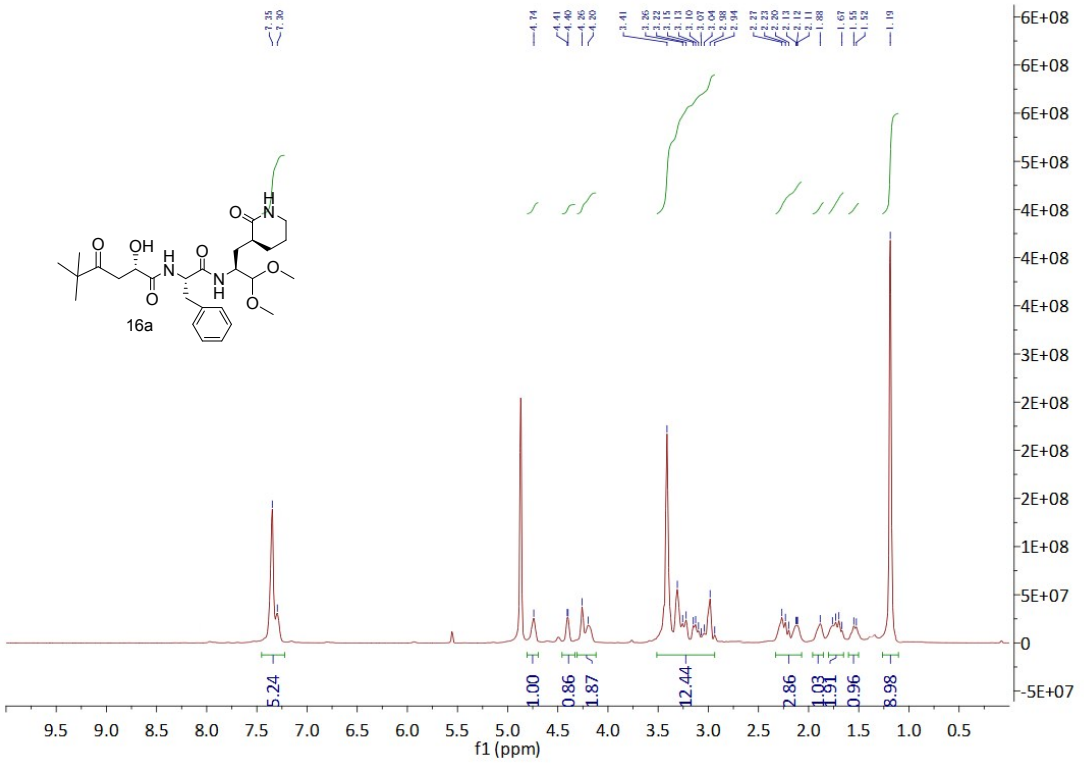
127.97
127.94
66.53
65.35
50.81
42.23
37.83
32.91
26.57
21.36
f1 (ppm)

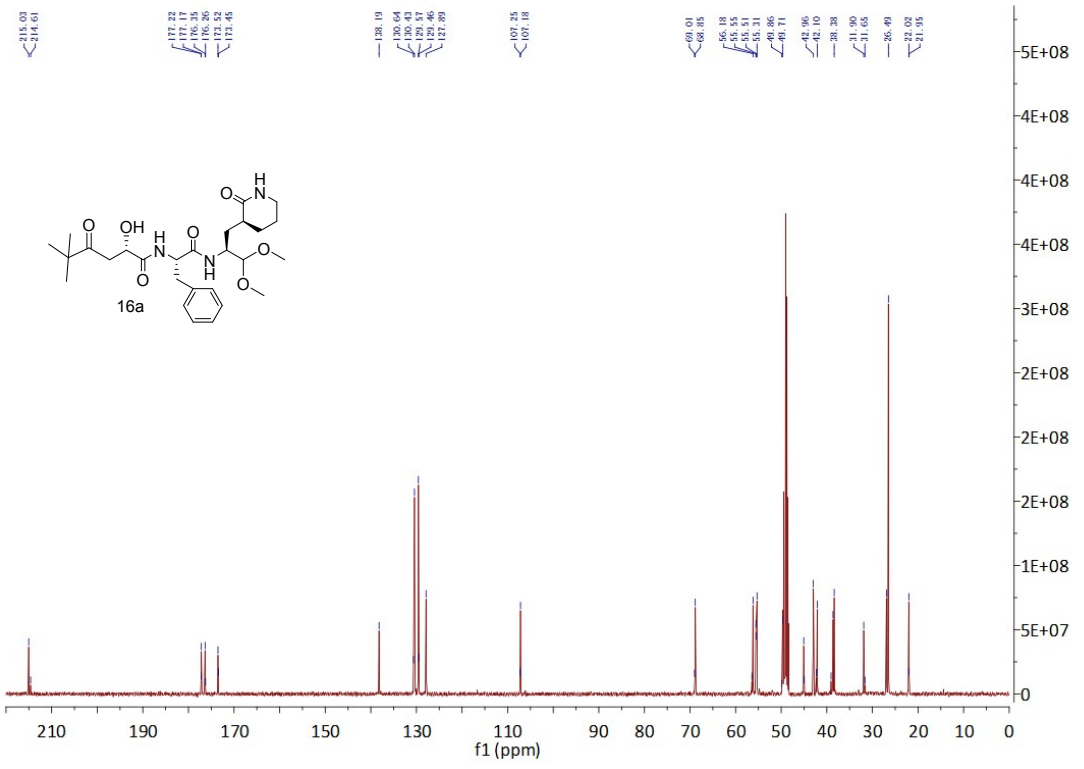

7.34
7.33
7.31
7.30
7.29
7.28
7.28
7.27
7.02
5.81
5.79
5.09
4.24
4.23
3.87
3.84
3.39
3.36
3.21
3.19
3.19
2.22
2.15
2.10
2.07
2.03
1.61
1.60
1.59
1.58
1.56
1.55
1.50
1.49
1.47
1.47
1.46
1.45
1.44
1.43
5.10
1.00
0.89
1.81
0.99
0.95
5.97
2.00
3.08
1.05
3.14
f1 (ppm)
15

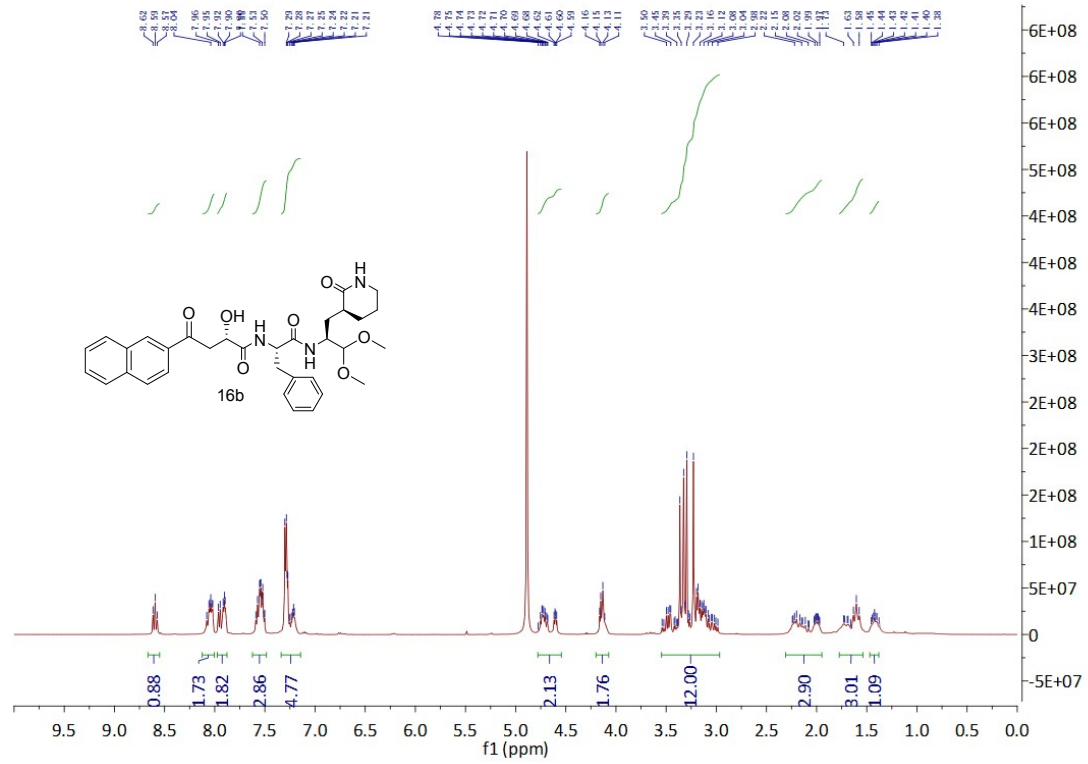

175.54
156.73
136.67
128.38
127.87
127.76
106.14
66.47
56.03
55.30
50.38
42.13
37.53
31.08
26.30
21.25
f1 (ppm)
15

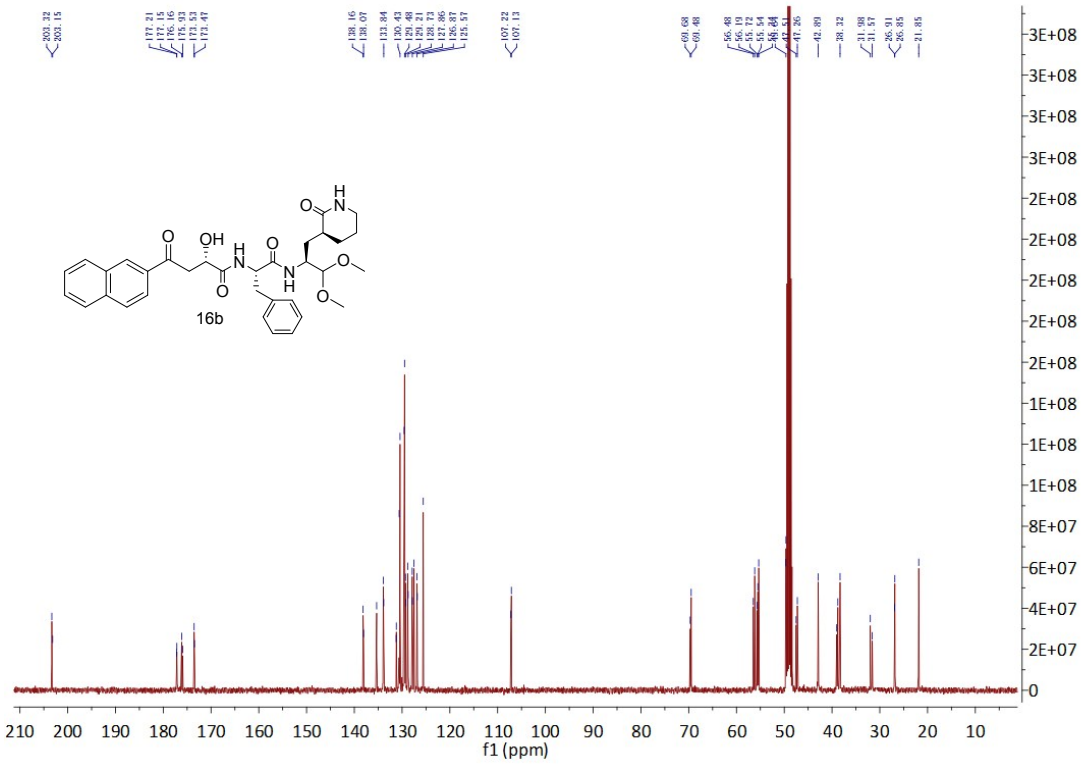

16c
f1 (ppm)

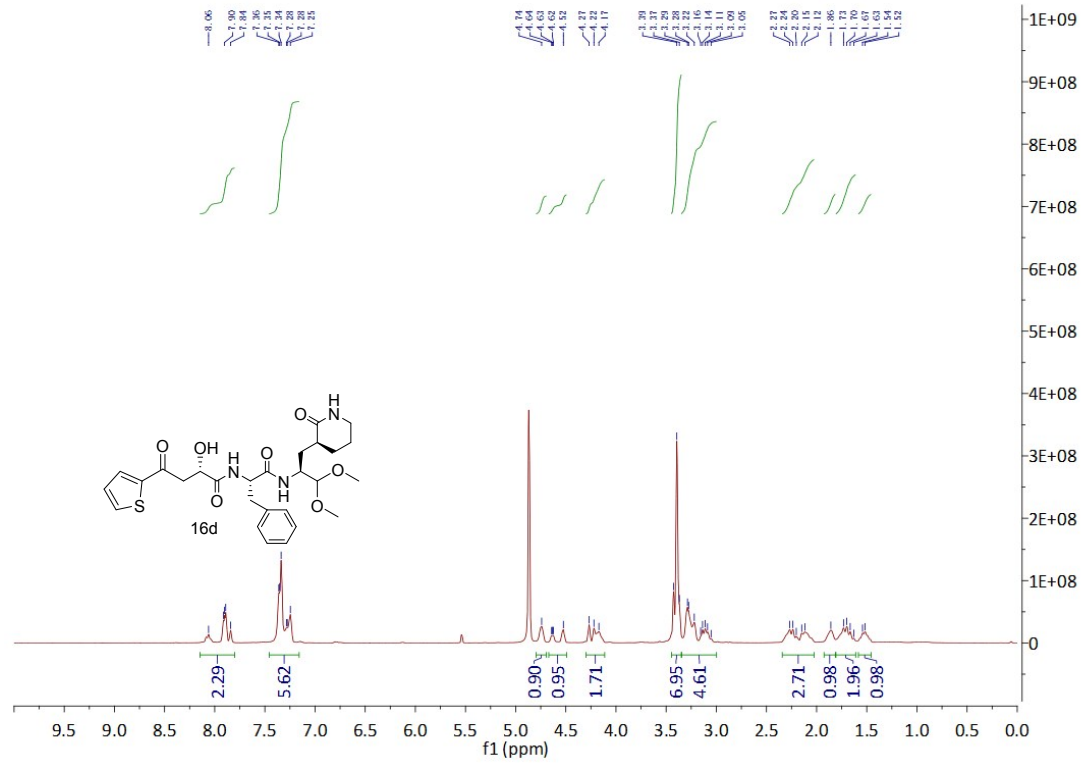

16c
f1 (ppm)

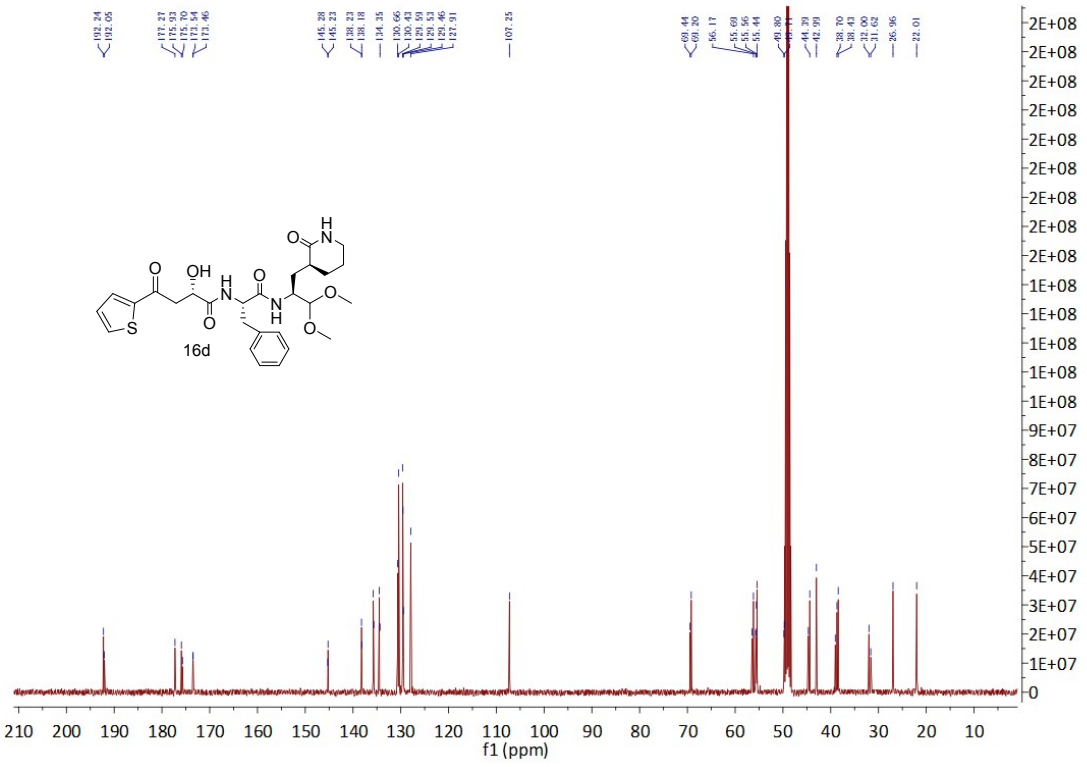

16a
f1 (ppm)

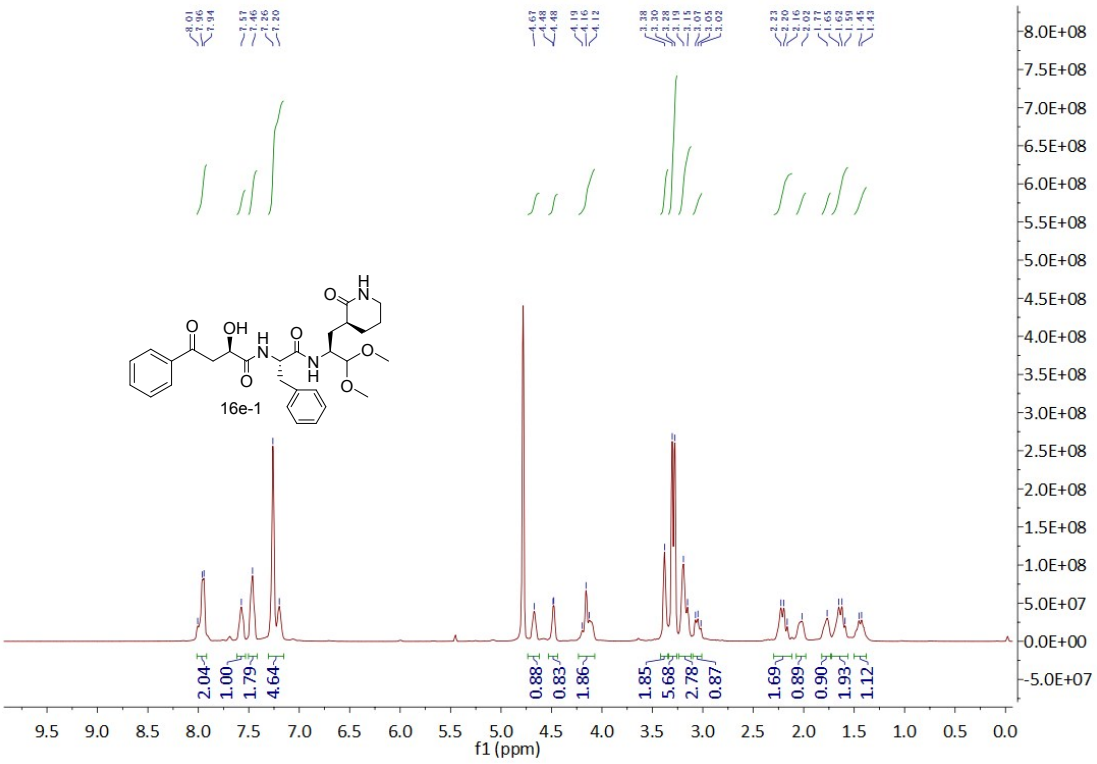

16a
f1 (ppm)

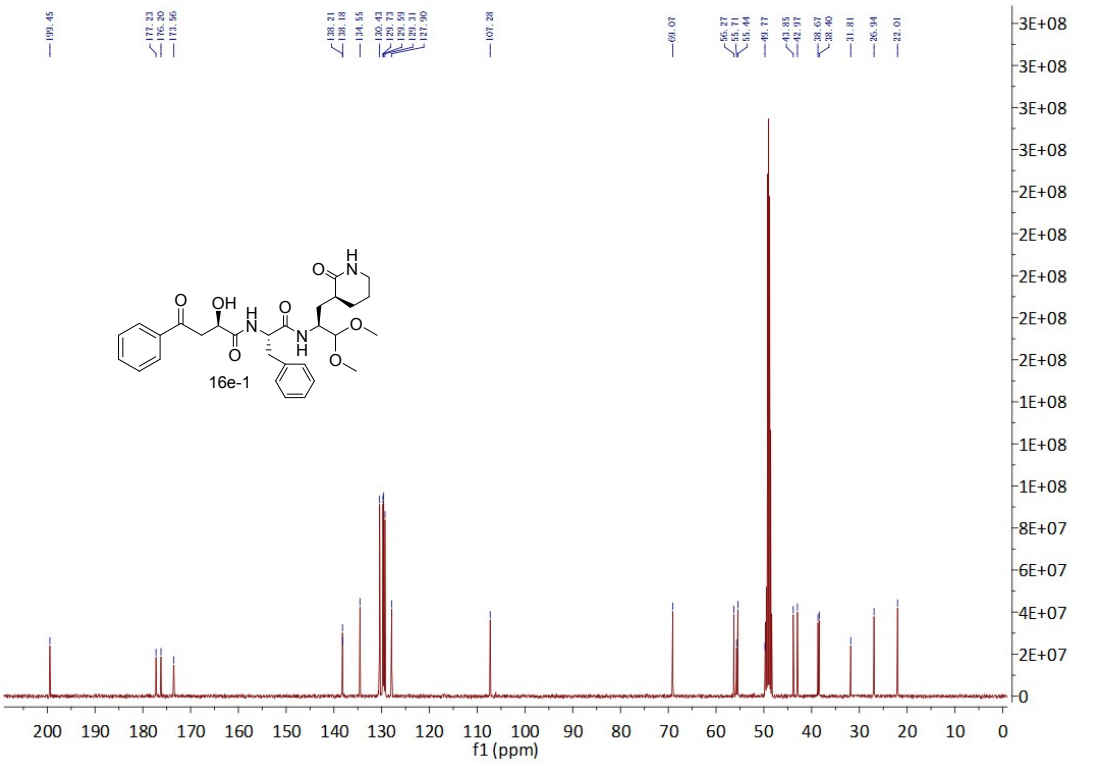

16b
0.88
1.73
1.82
2.86
4.77
2.13
1.76
12.00
2.90
3.01
1.09
f1 (ppm)

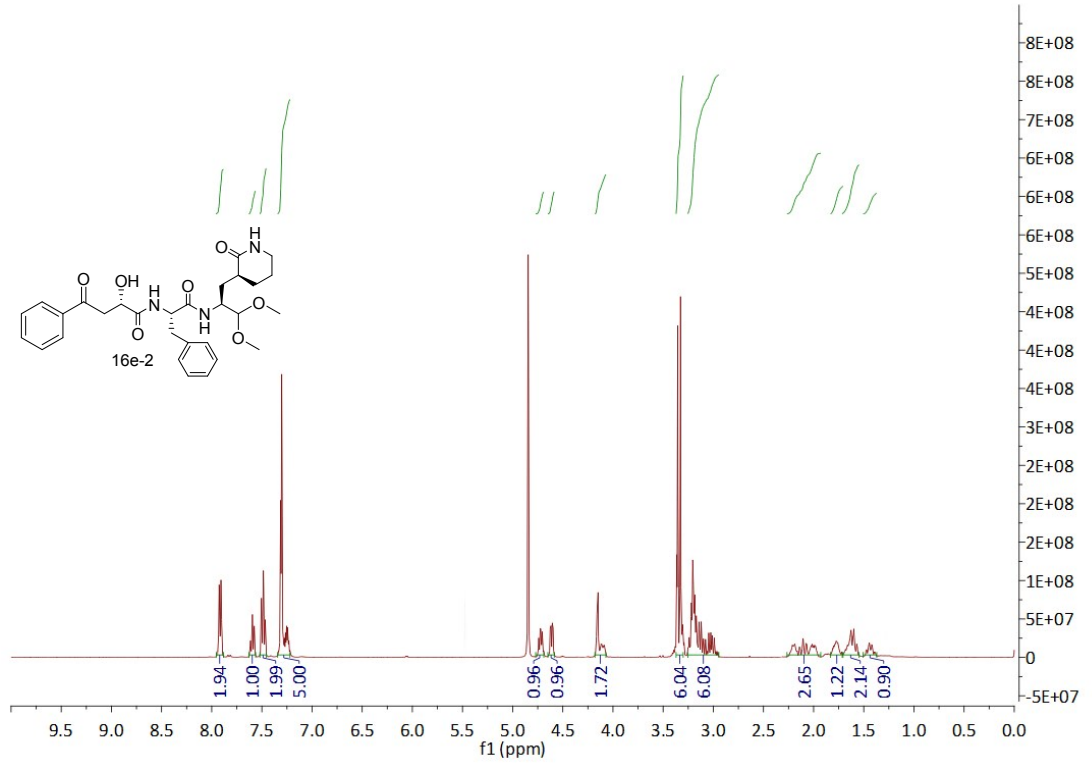

16b
f1 (ppm)

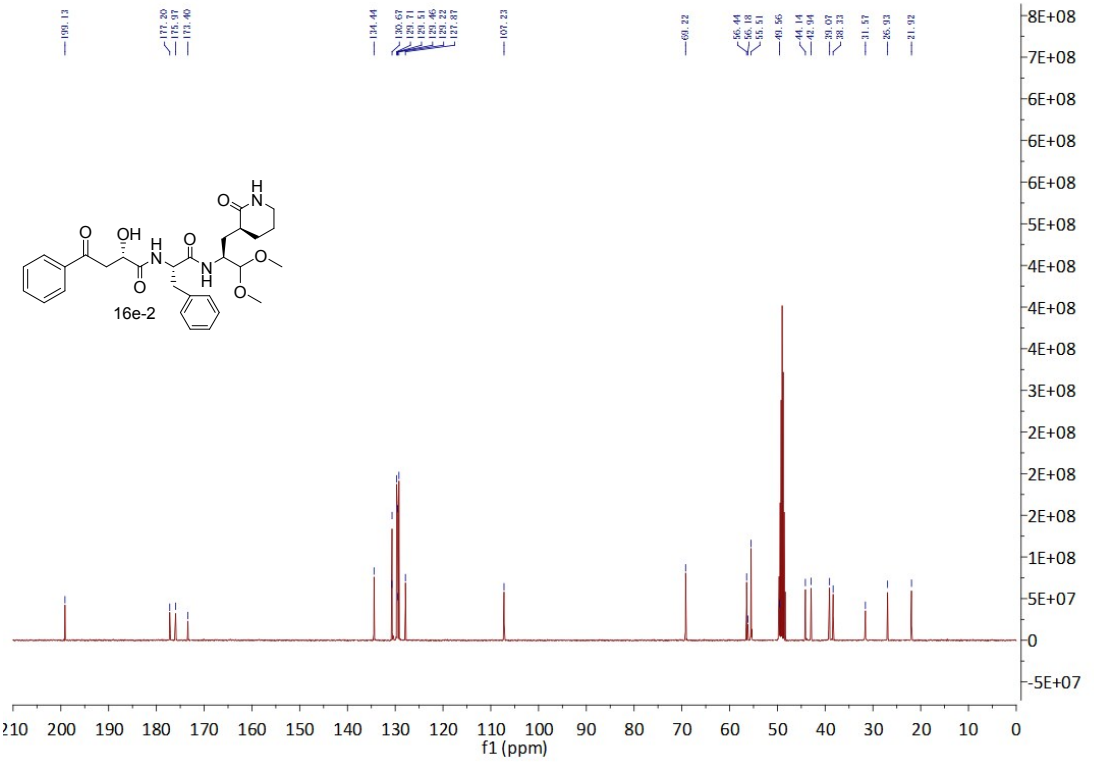

16d
2.29
5.62
0.90
0.95
1.71
6.95
4.61
2.71
0.98
1.96
0.98
f1 (ppm)

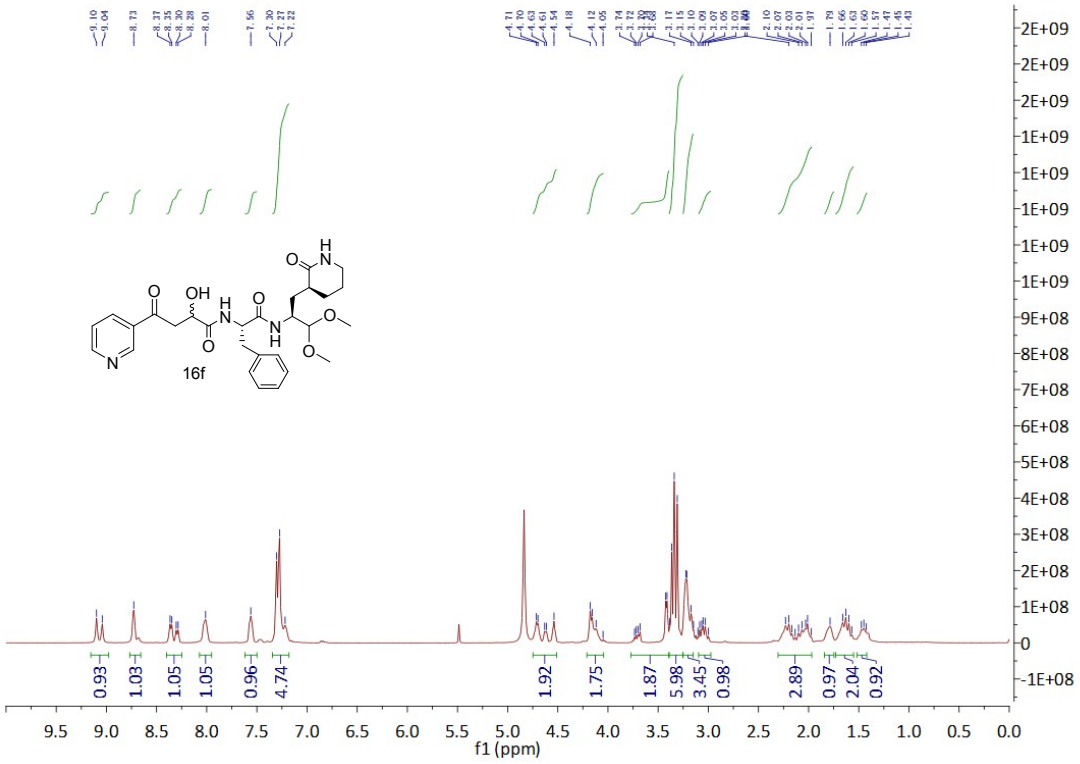

16d
f1 (ppm)

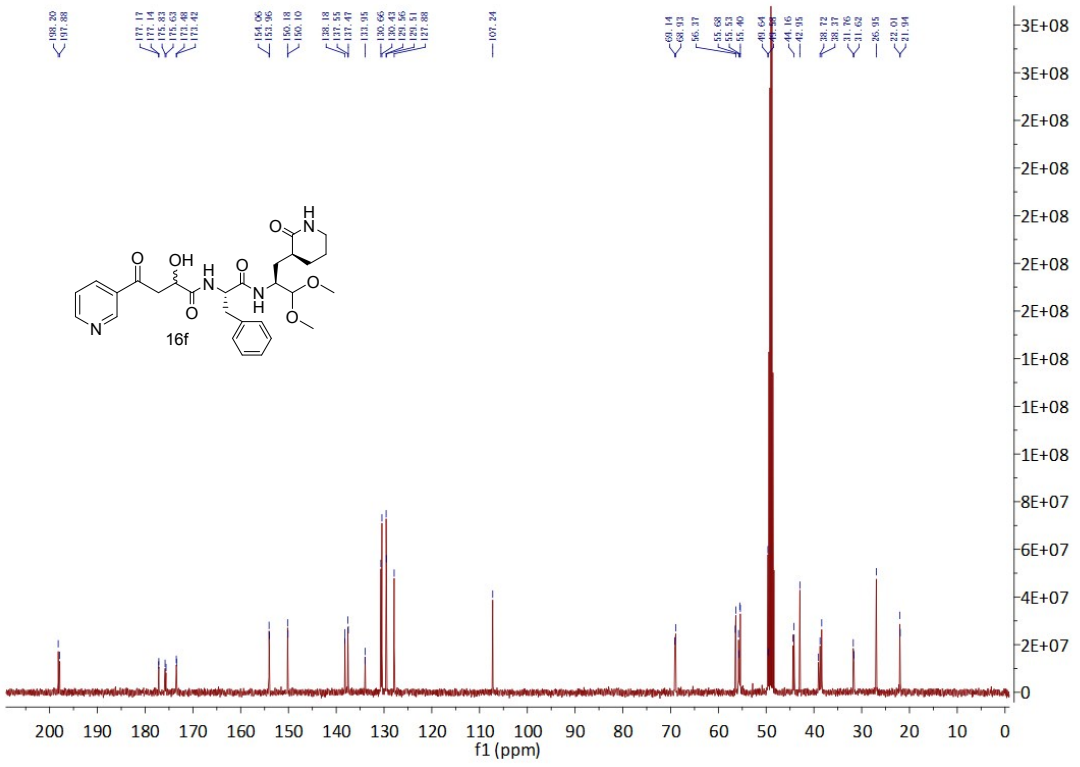

16e-1
f1 (ppm)

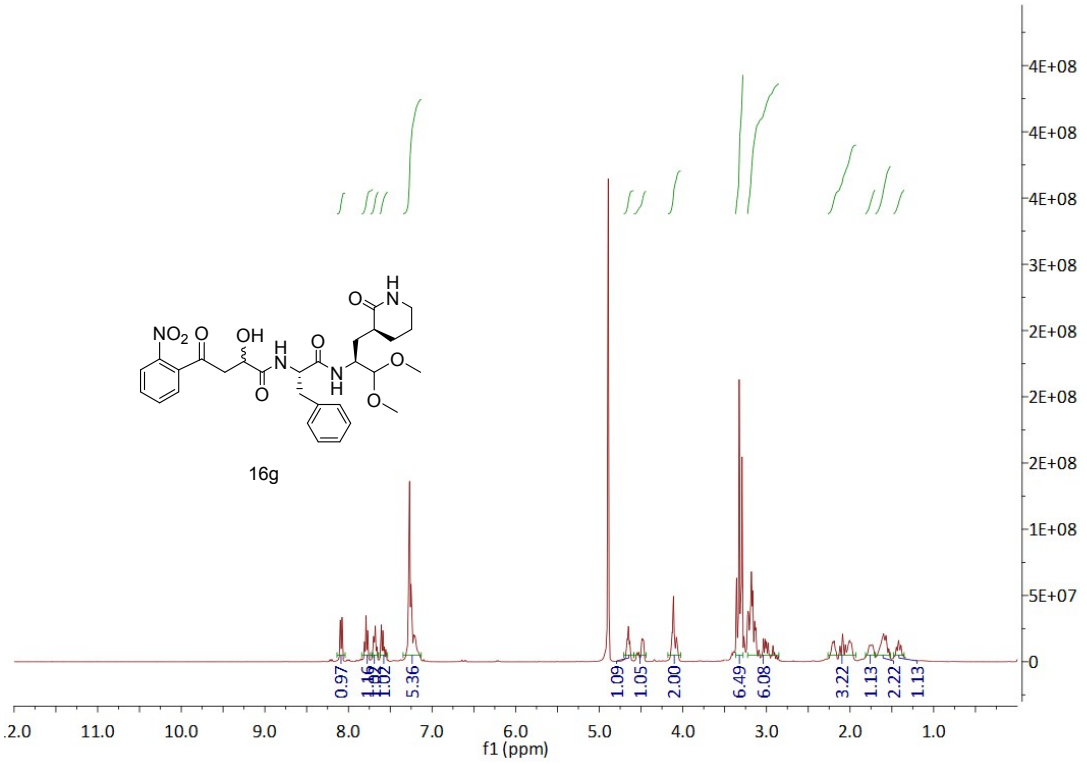

16e-1
f1 (ppm)

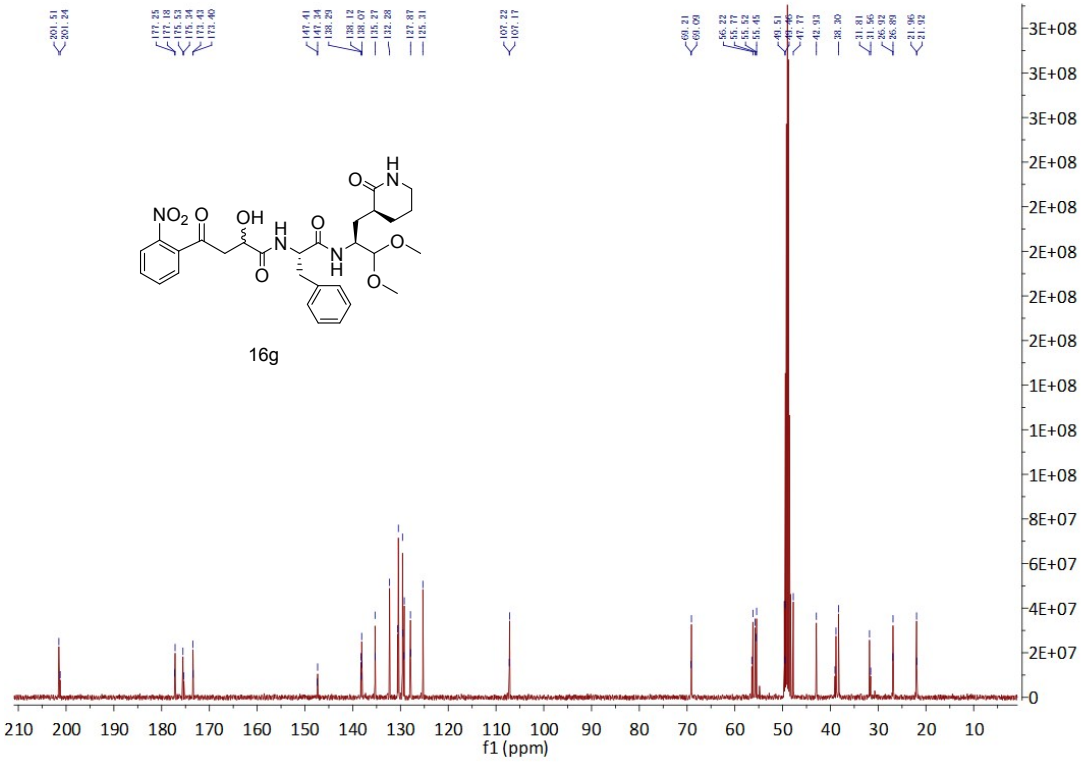

16e-2
1.94
1.00
1.99
5.00
0.96
0.96
1.72
6.04
6.08
2.65
1.22
2.14
0.90
f1 (ppm)

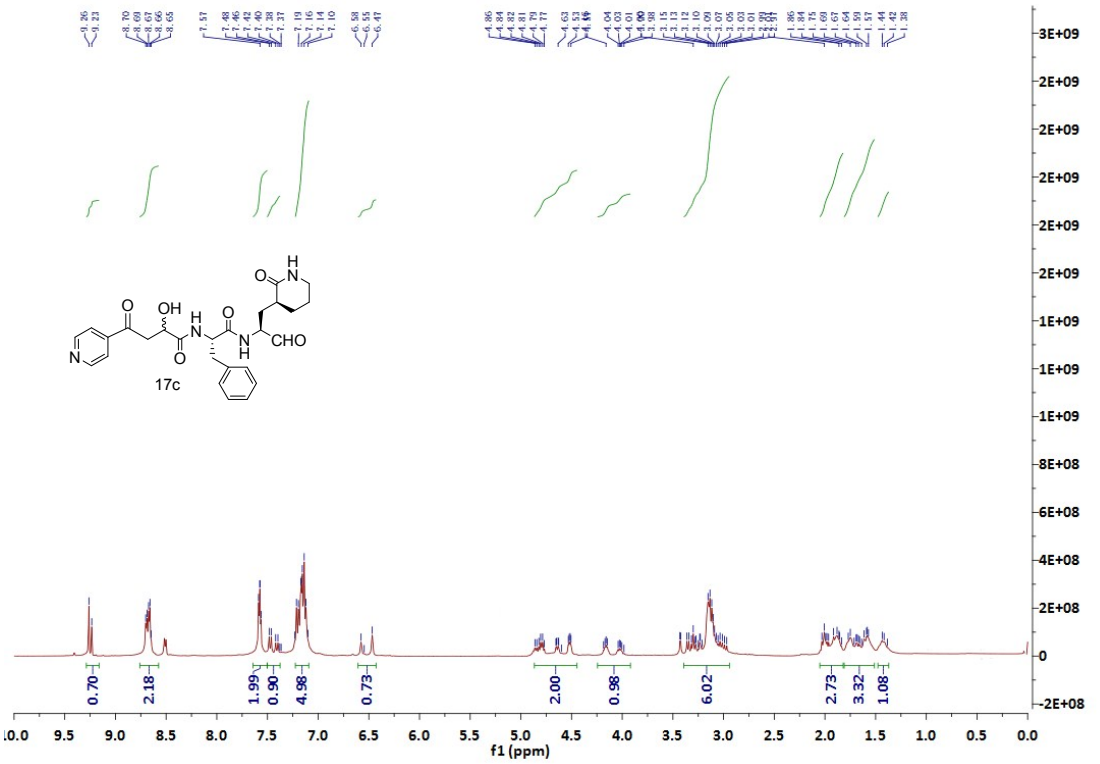

16e-2
f1 (ppm)

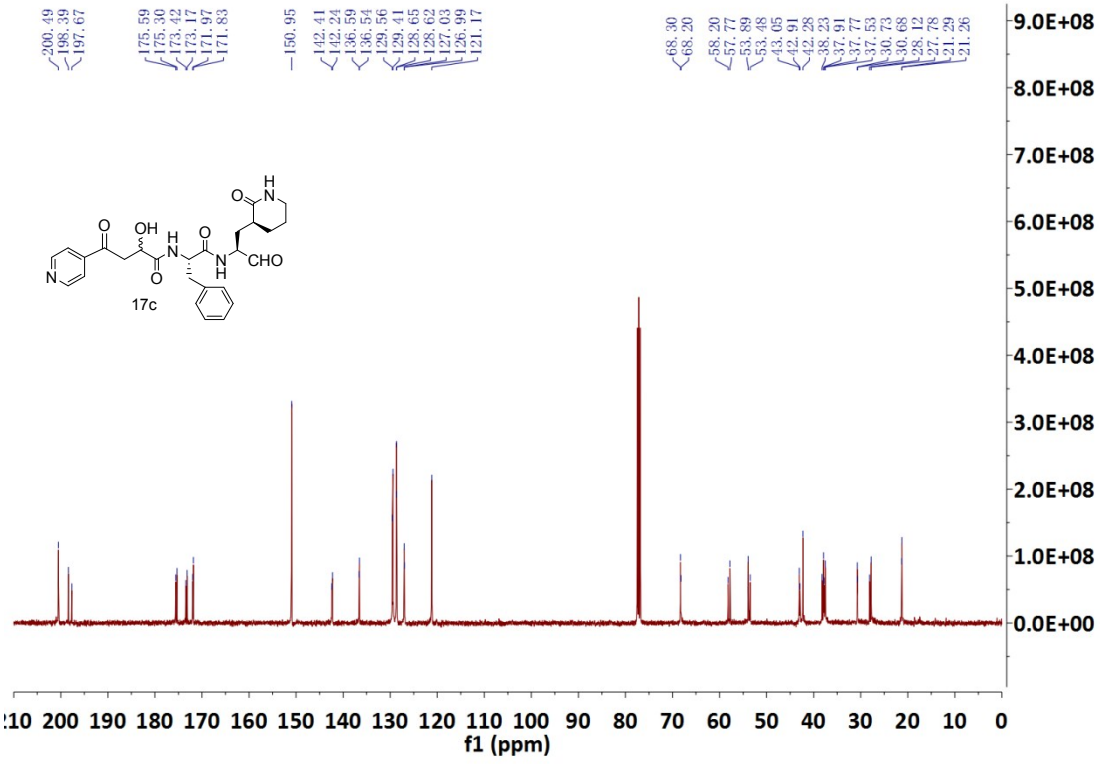

16f
0.93
1.03
1.05
1.05
0.96
4.74
1.92
1.75
1.87
5.98
3.45
0.98
2.89
0.97
2.04
0.92
f1 (ppm)

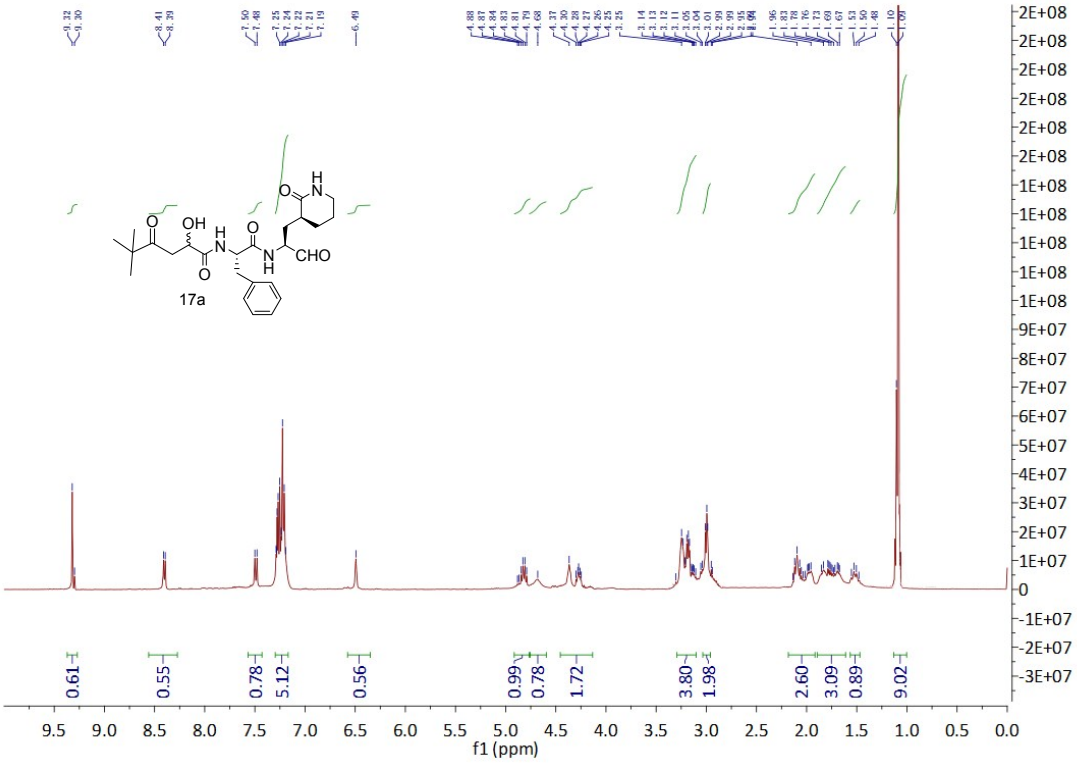

16f
f1 (ppm)

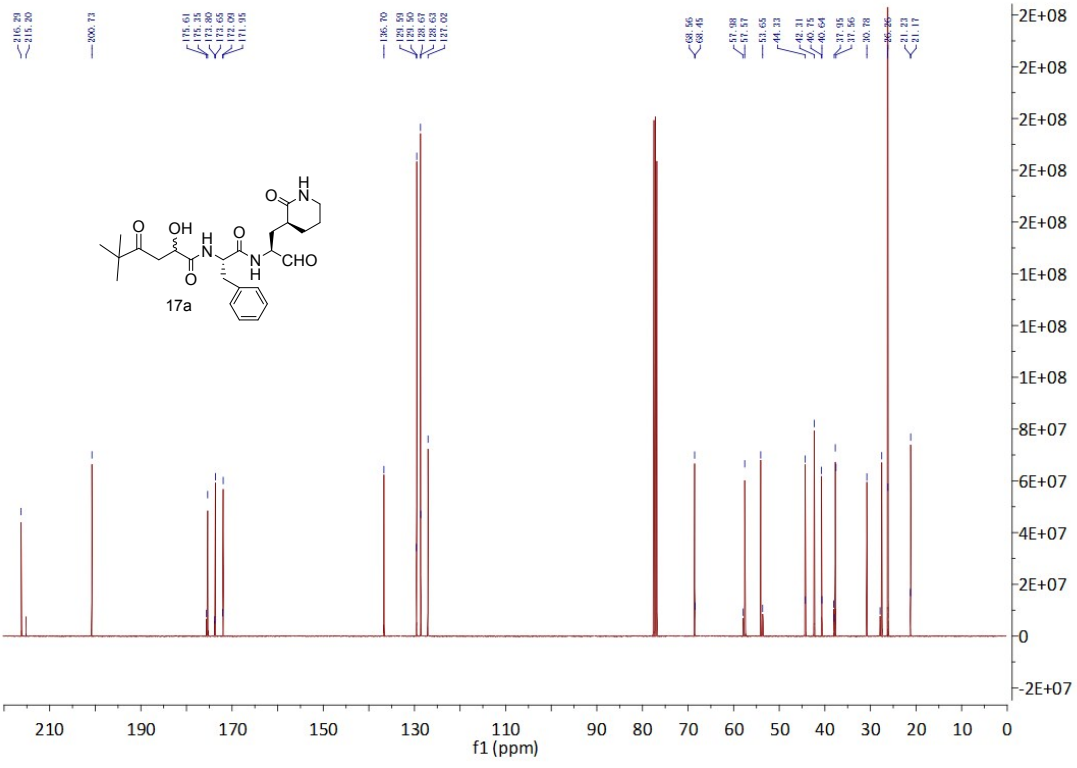

16g
0.97
1.16
1.02
5.36
1.09
1.05
2.00
6.49
6.08
3.22
1.13
2.22
1.13
f1 (ppm)

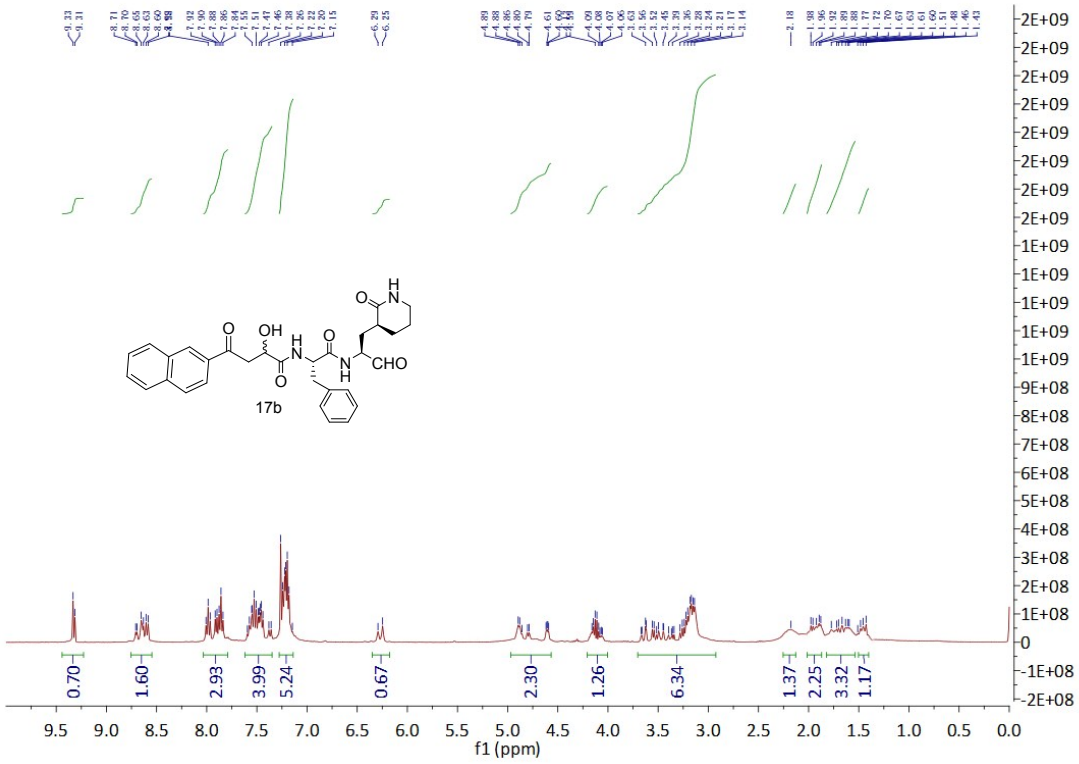

16g
f1 (ppm)

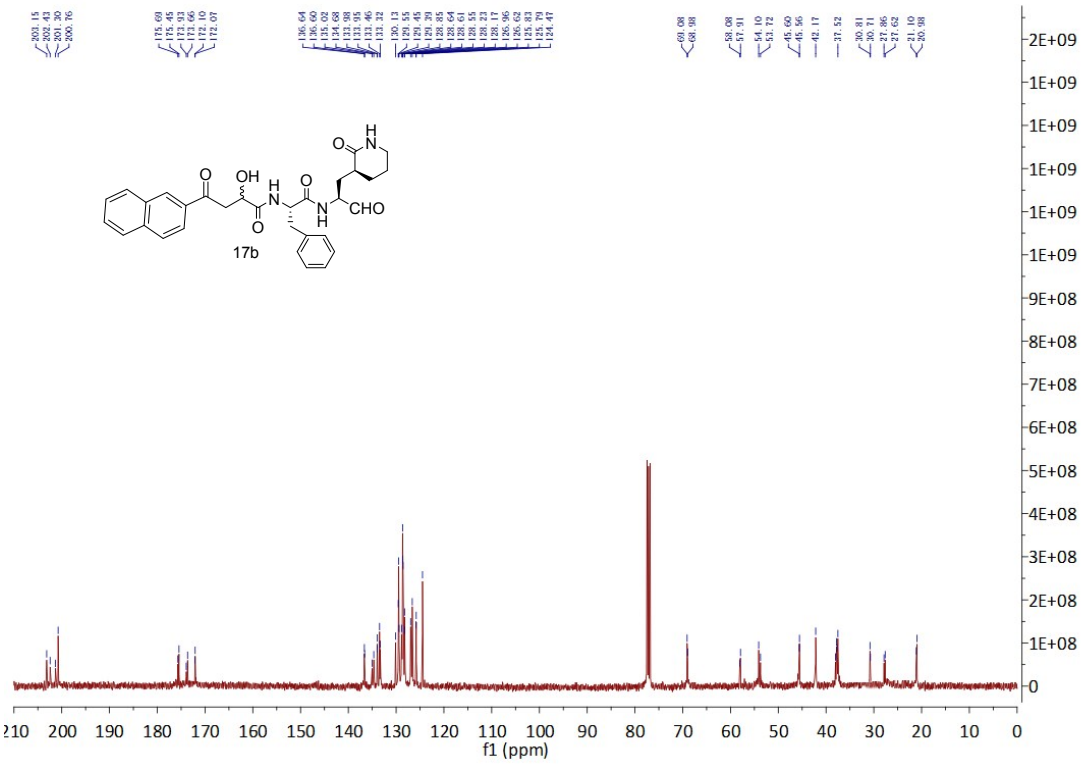

17c
f1 (ppm)

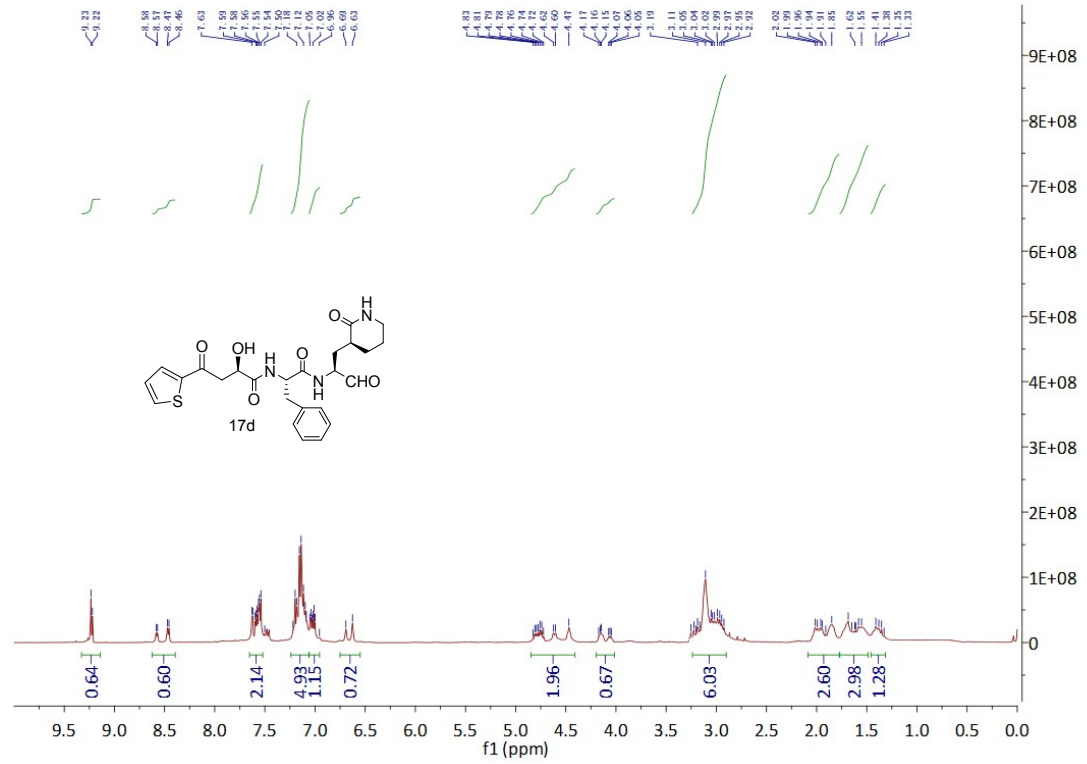

17c
f1 (ppm)

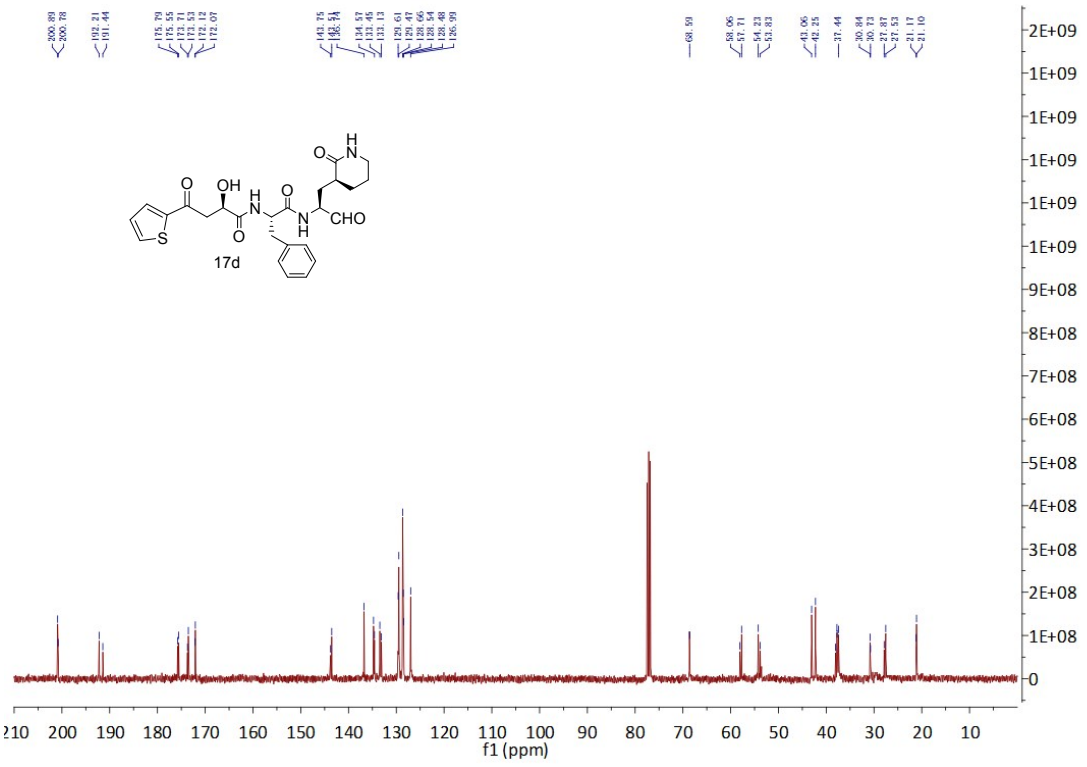

17a
f1 (ppm)

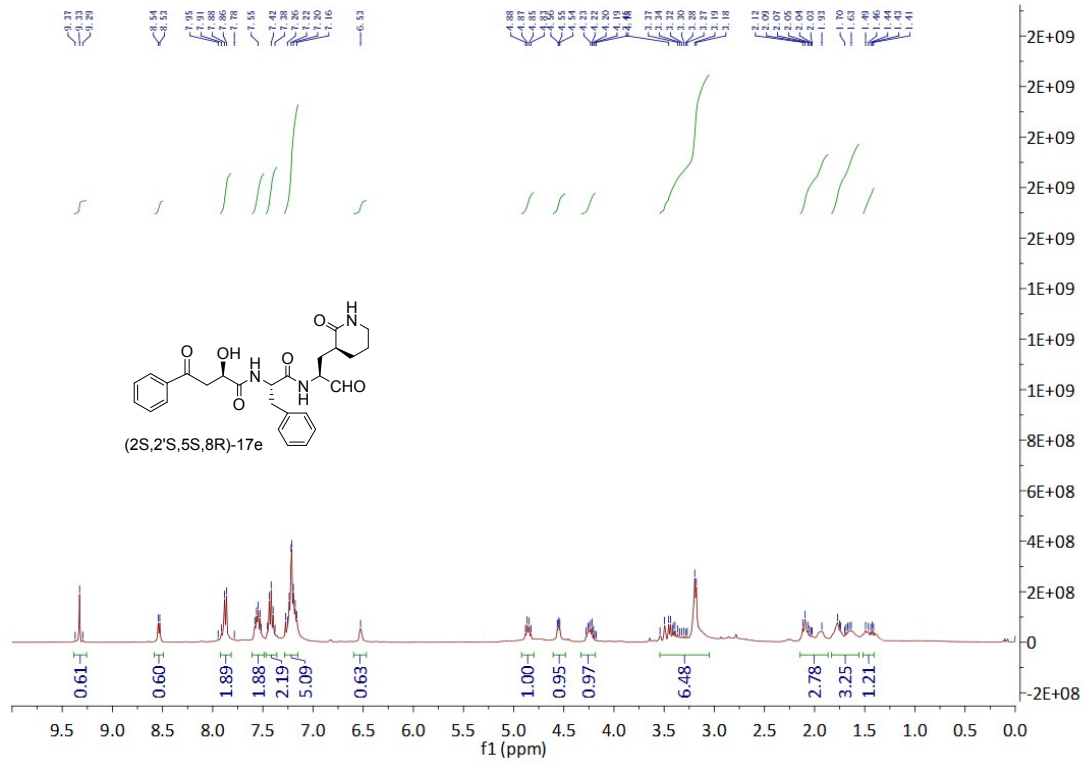

17a
f1 (ppm)

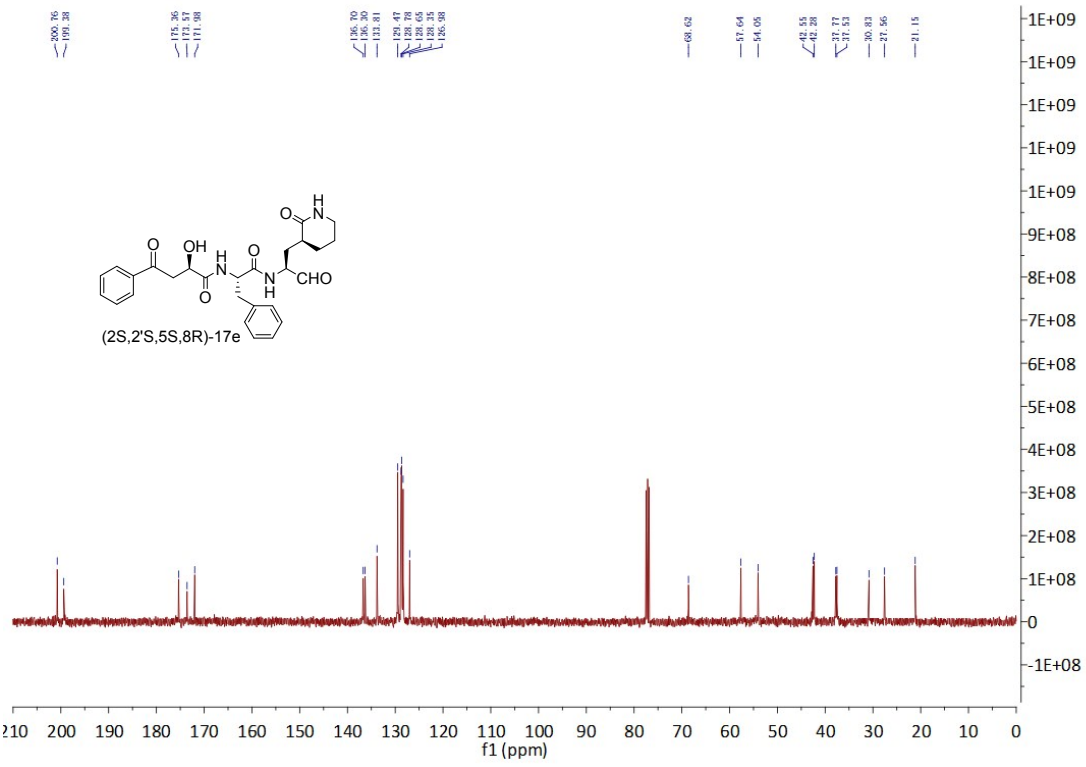

17b
0.70
1.60
2.93
3.99
5.24
0.67
2.30
1.26
6.34
1.37
2.25
3.32
1.17
f1 (ppm)

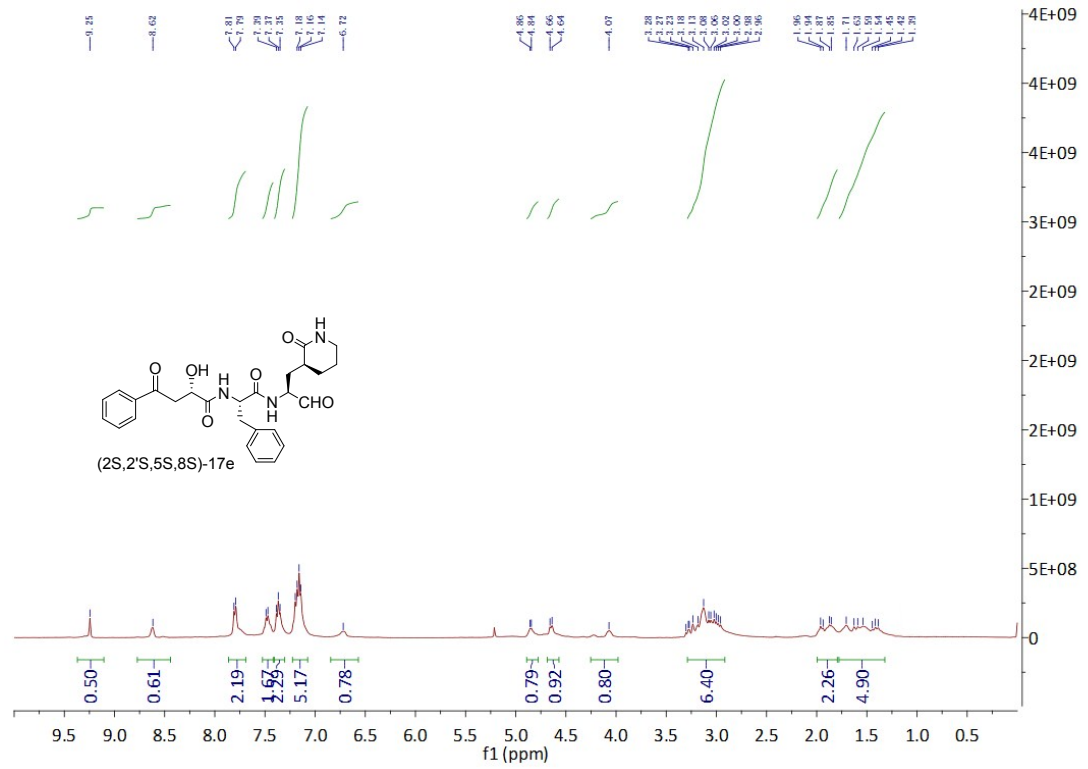

17b
f1 (ppm)

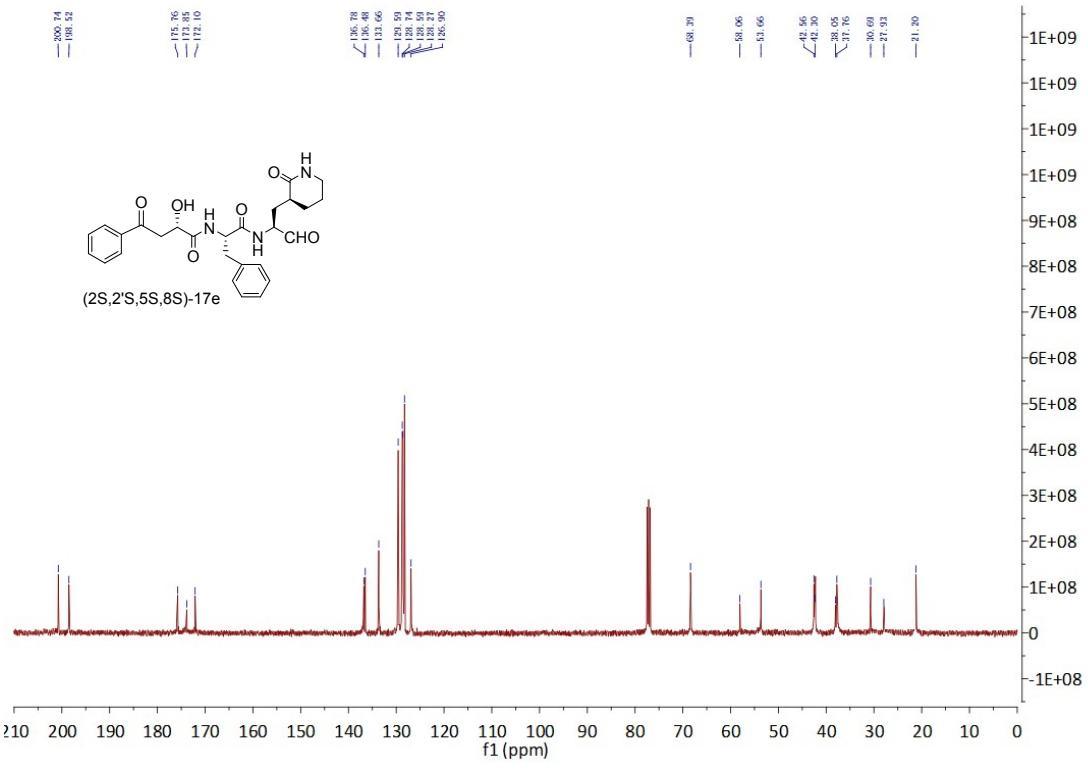

17d
0.64
0.60
2.14
4.93
1.15
0.72
1.96
0.67
6.03
2.60
2.98
1.28
f1 (ppm)

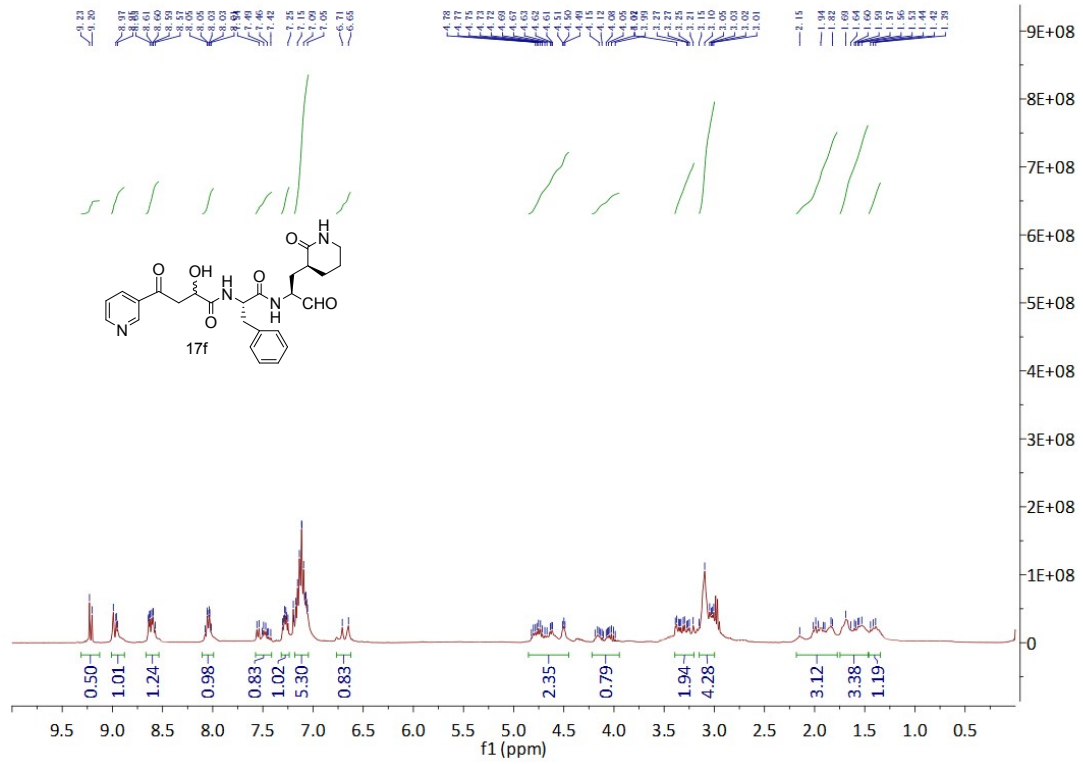

17d
f1 (ppm)

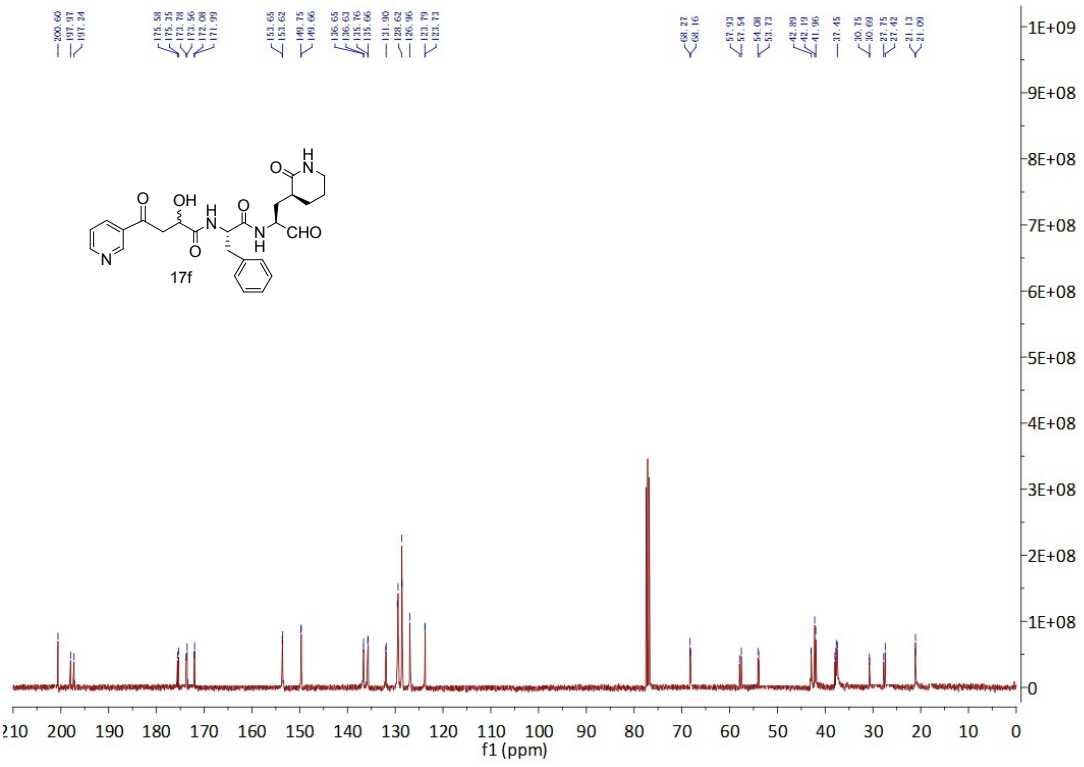

(2S,2'S,5S,8R)-17e
0.61
0.60
1.89
1.88
2.19
5.09
0.63
1.00
0.95
0.97
6.48
2.78
3.25
1.21
f1 (ppm)

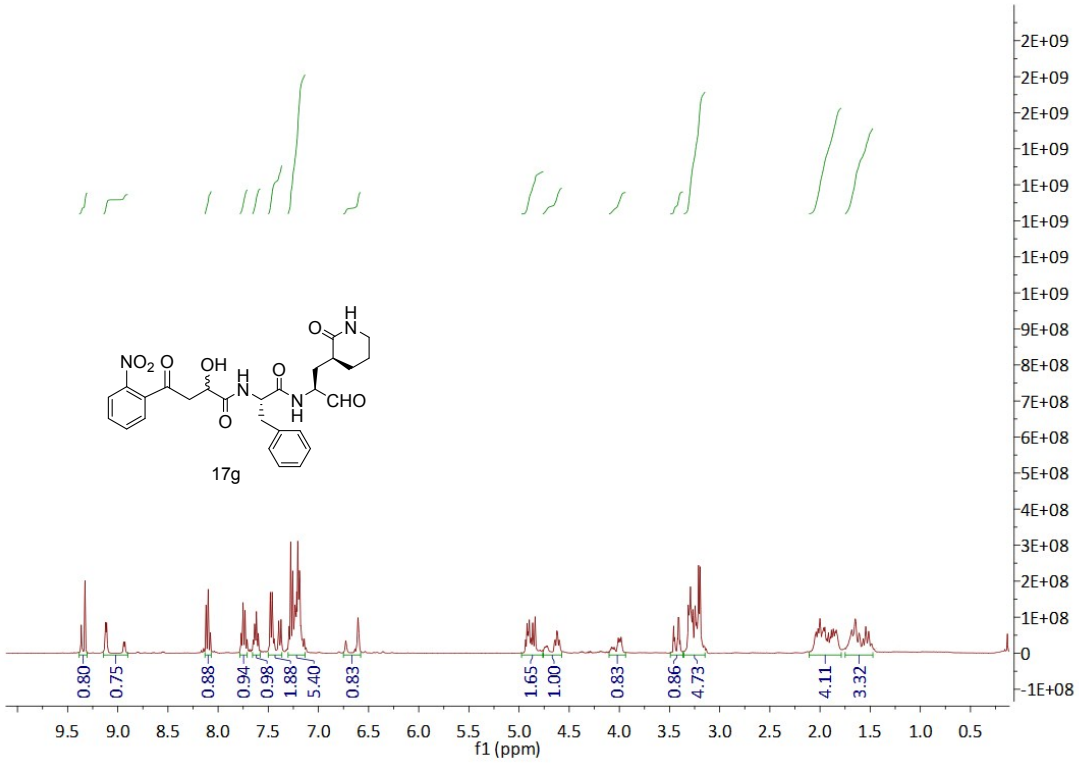

(2S,2'S,5S,8R)-17e
f1 (ppm)

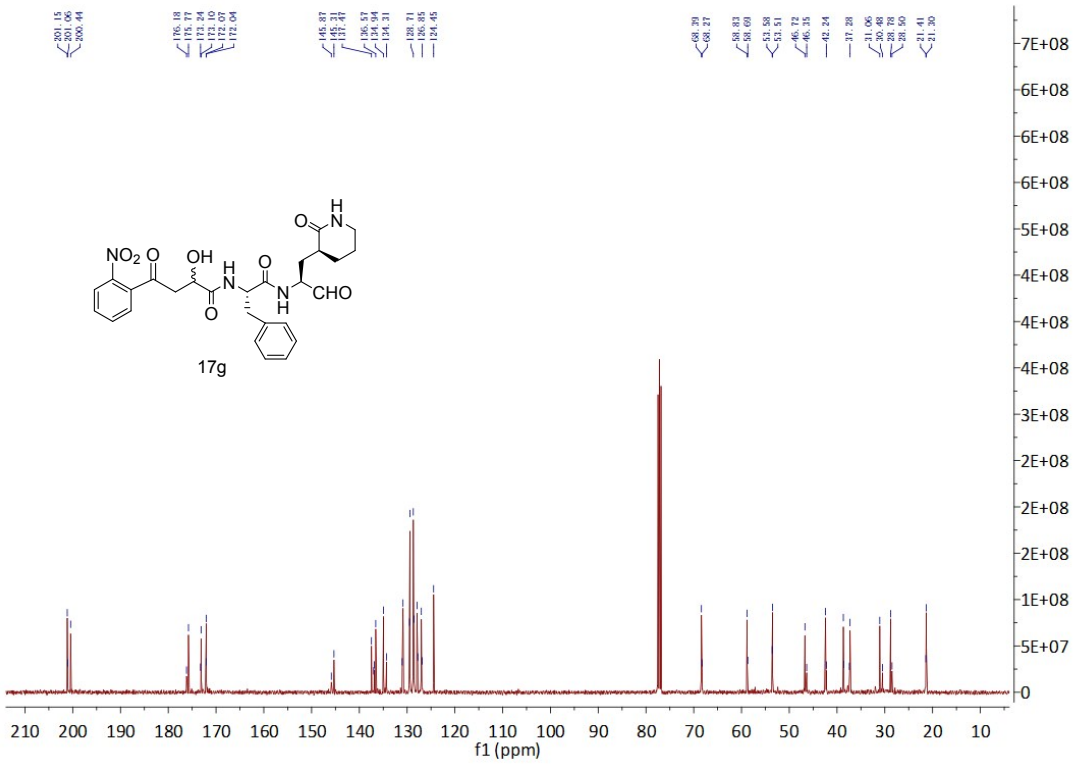

(2S,2'S,5S,8S)-17e
f1 (ppm)

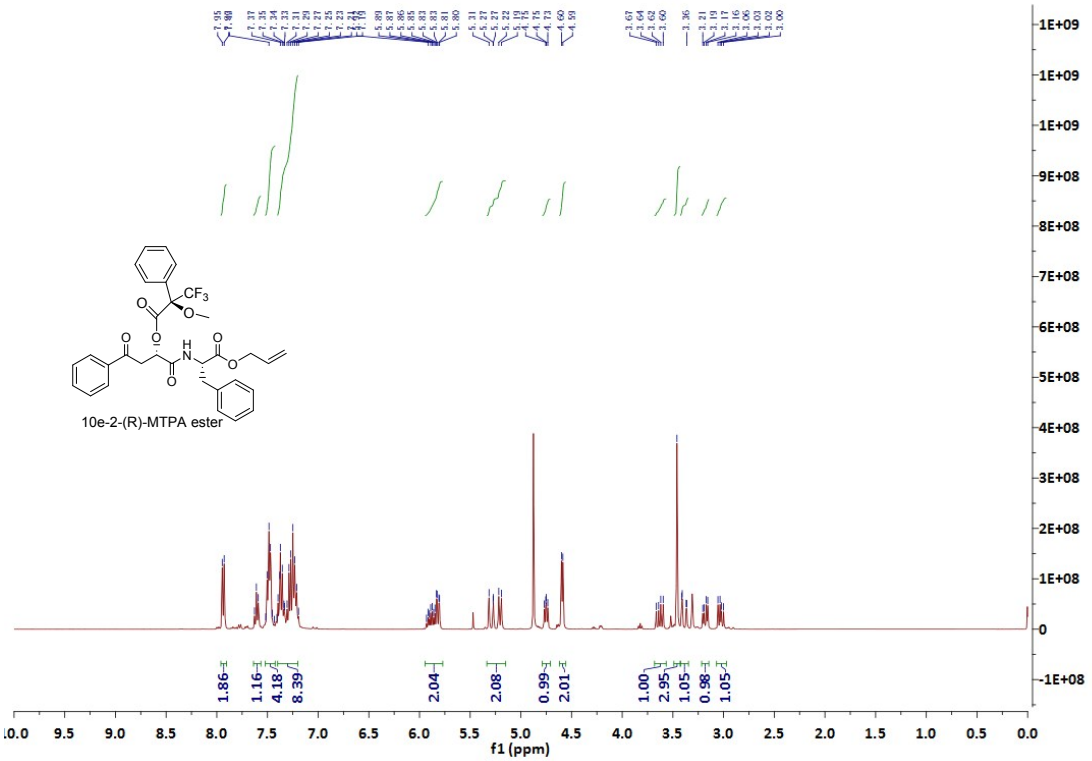

(2S,2'S,5S,8S)-17e
f1 (ppm)

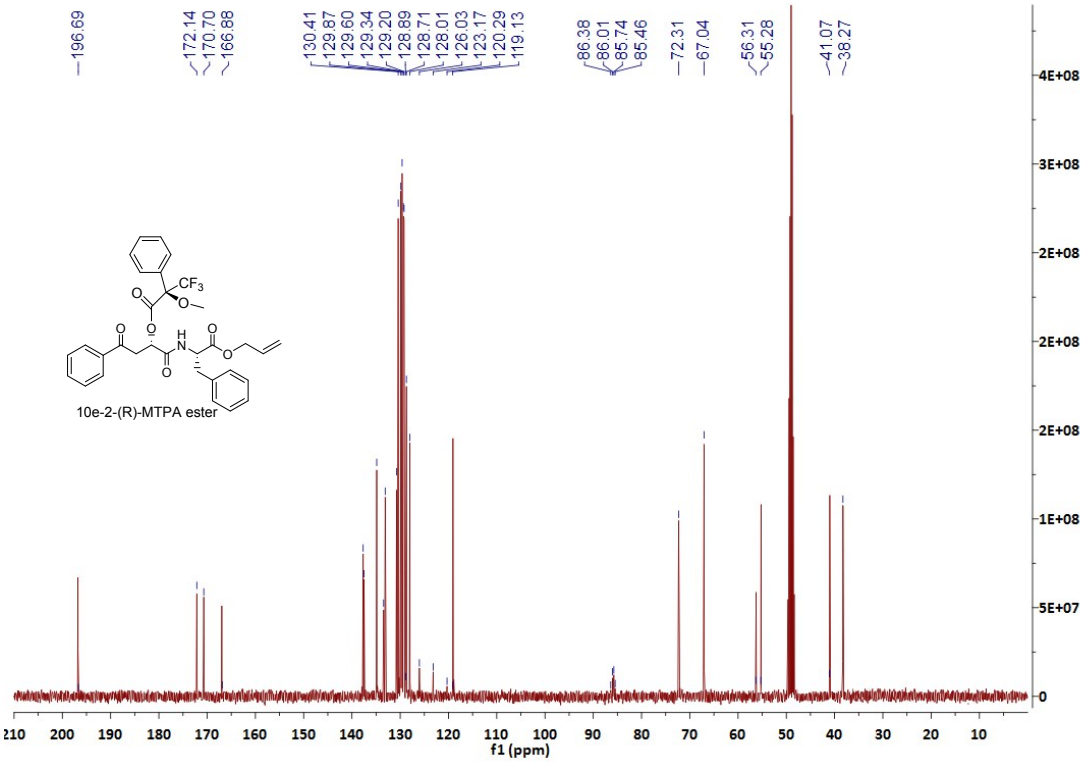

17f
0.50
1.01
1.24
0.98
0.83
1.02
5.30
0.83
2.35
0.79
1.94
4.28
3.12
3.38
1.19
f1 (ppm)

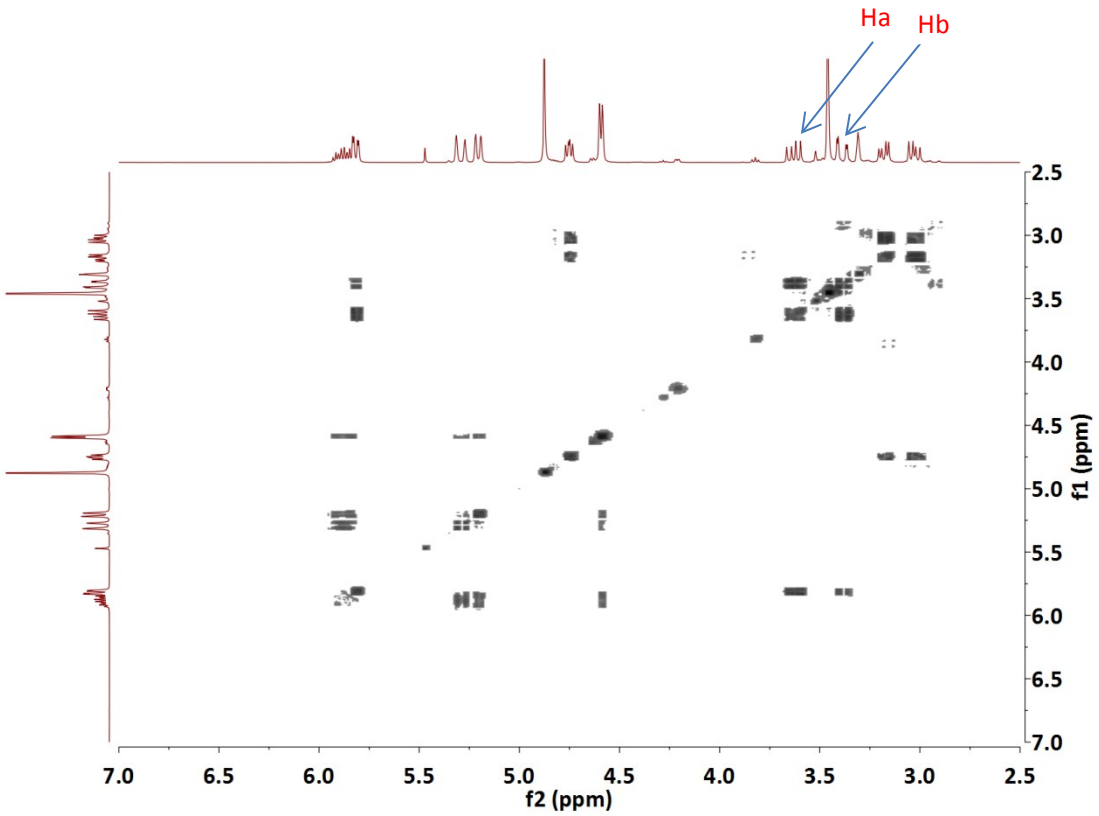

17f
f1 (ppm)

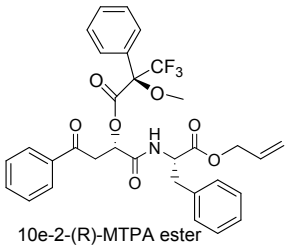

17g
0.80
0.75
0.88
0.94
0.98
1.88
5.40
0.83
1.65
1.00
0.83
0.86
4.73
4.11
3.32
f1 (ppm)


17g
f1 (ppm)

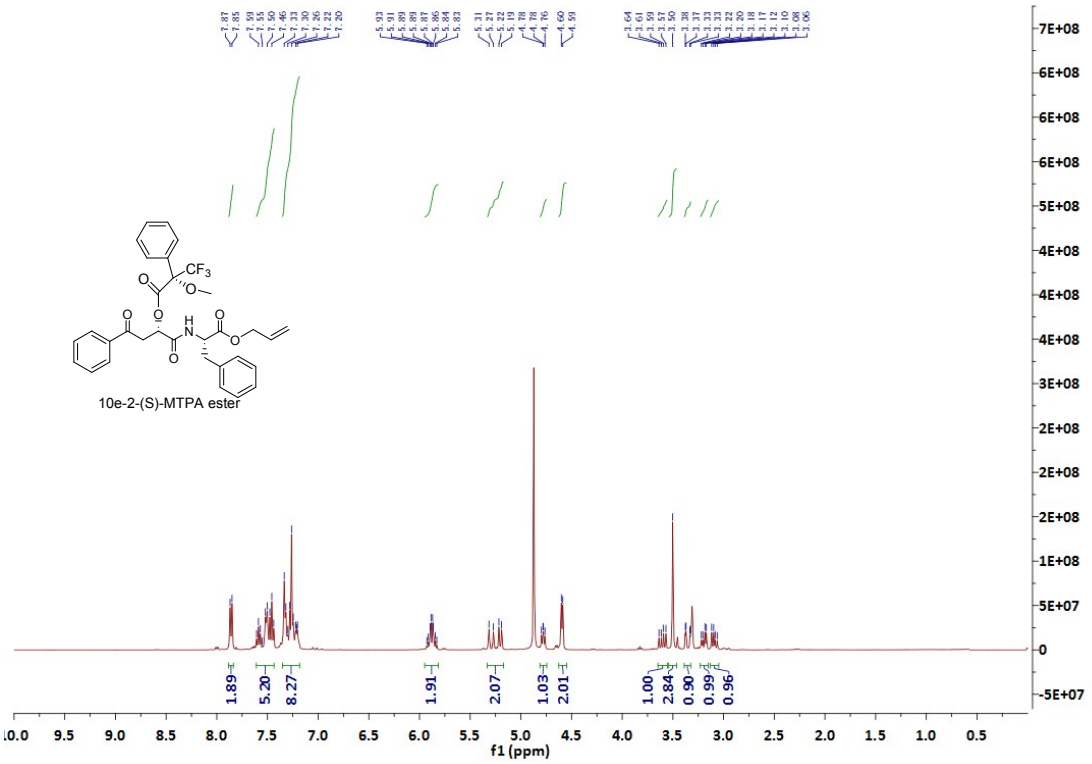

10e-2-(R)-MTPA ester
f1 (ppm)

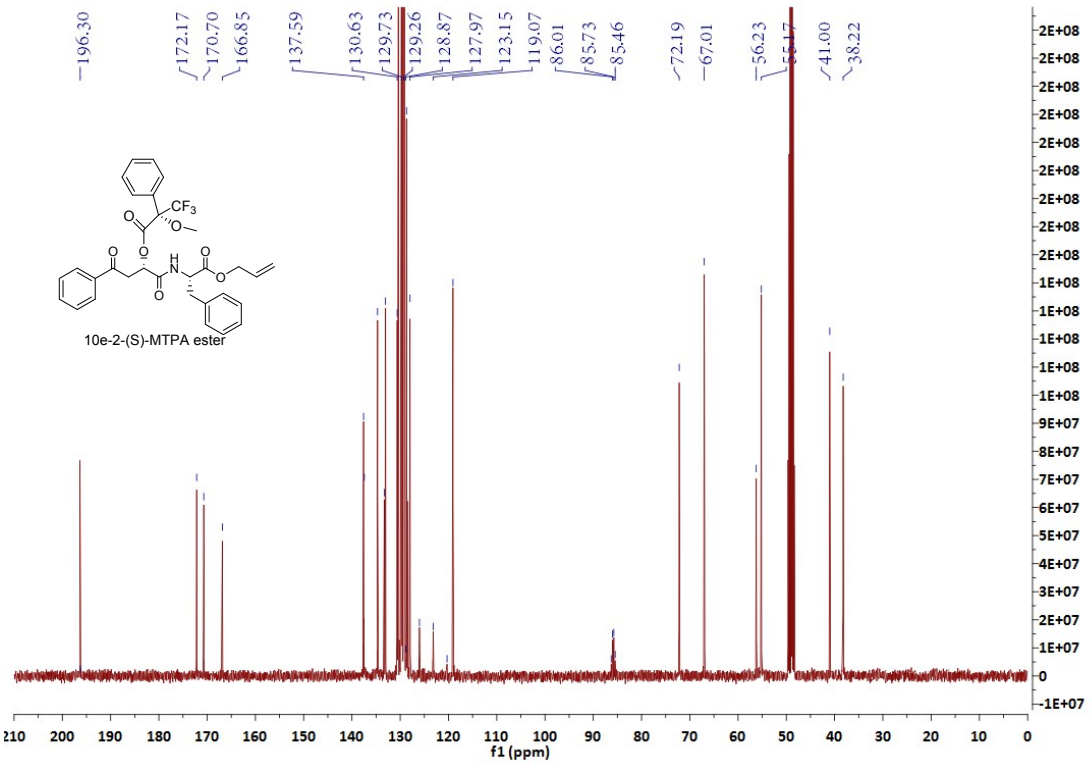

10e-2-(R)-MTPA ester
f1 (ppm)

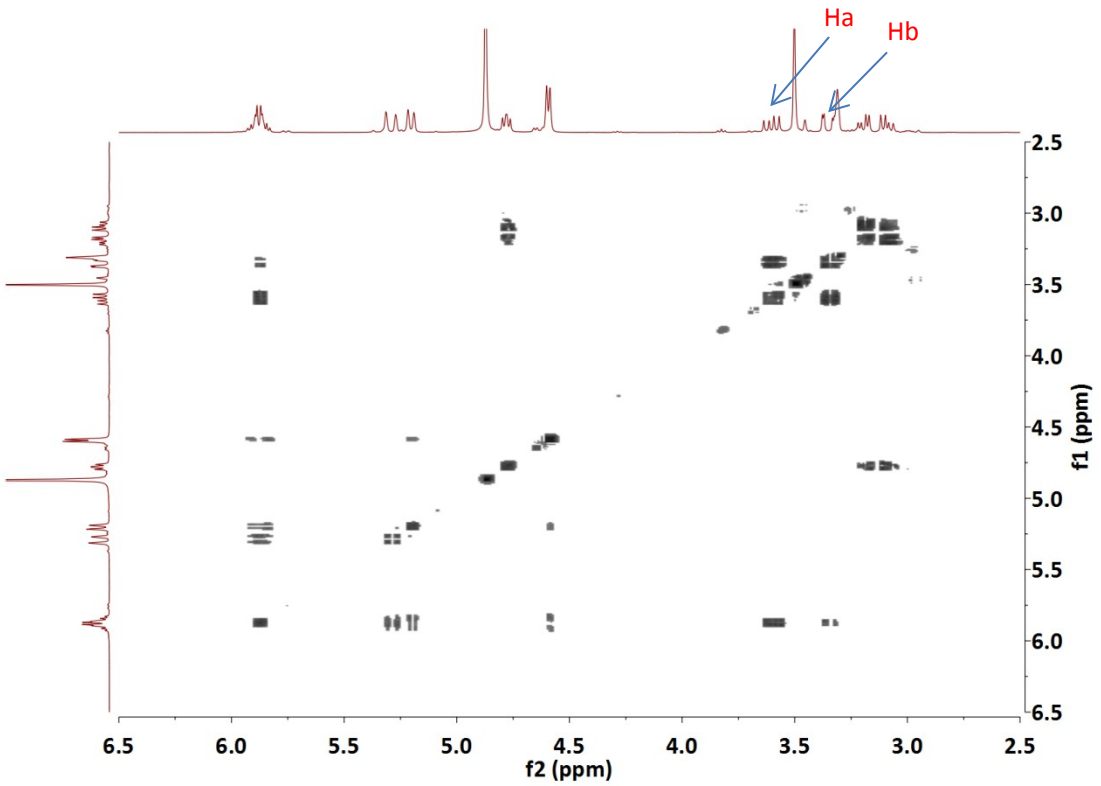


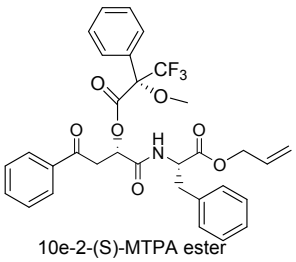


10e-2-(R)-MTPA ester


10e-2-(S)-MTPA ester
1.89
5.20
8.27
1.91
2.07
1.03
2.01
1.00
2.84
0.90
0.99
0.96
f1 (ppm)


196.30
172.17
170.70
166.85
137.59
130.63
129.73
129.26
128.87
127.97
123.15
119.07
86.01
85.73
85.46
72.19
67.01
56.23
55.17
41.00
38.22
10e-2-(S)-MTPA ester
f1 (ppm)





10e-2-(S)-MTPA ester