Formal Synthesis of Cyclotheonellazole A

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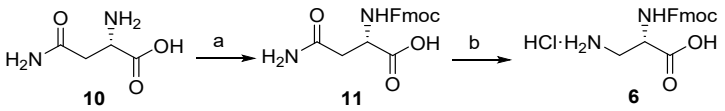
General Experimental

Commercially available reagents were used without further purification unless otherwise stated. All solvents were distilled prior to use: toluene, benzene, diethyl ether and tetrahydrofuran were distilled from Na/benzophenone; while dichloromethane, dimethylformamide, acetonitrile, triethylamine and diisopropylethylamine were distilled from CaH_2. Methanol was distilled under a N_2 atmosphere from Mg/I_2. All reactions were conducted in oven-dried (120 °C) or flame-dried glasswares under a N_2 atmosphere, and at ambient temperature (20 to 25 °C) unless otherwise stated. All non-aqueous reactions were performed by standard syringe in septa techniques. Evaporation and concentration under reduced pressure was performed at 50-500 mbar. ^{1}H NMR spectra were recorded in $CDCl_3$ (unless stated otherwise) on a Bruker Avance AV 400 at 400 MHz (100 MHz). Chemical shifts are reported as δ values (ppm) referenced to either a tetramethylsilane (TMS) internal standard or the signals due to the solvent residual. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz), integration. Some peptide intermediates exist as rotational conformers, the chemical shift for the minor isomers were indicated using parentheses next to the peak for their major isomers. Mass spectra were measured on ABI Q-star Elite. Optical rotations were measured on a Perkin-Elmer 351 polarimeter at 589 nm with a 100 mm path length cell at 20 °C (reported as follows: concentration (c in g/100 mL), solvent). The reaction progresses were checked on pre-coated thin layer chromatography (TLC) plates. TLC was carried out using pre-coated sheets (Qingdao silica gel 60-F250, 0.2 mm) which, after development, were visualized under UV light at 254nm. Flash column chromatography was performed using the indicated solvents on E. Qingdao silica gel 60 (230-400 mesh ASTM). Yields refer to chromatographically purified compounds, unless otherwise stated.

Experimental procedures

3-Amino-N-[(9H-fluoren-9-ylmethoxy)carbonyl]-L-alanine (6)

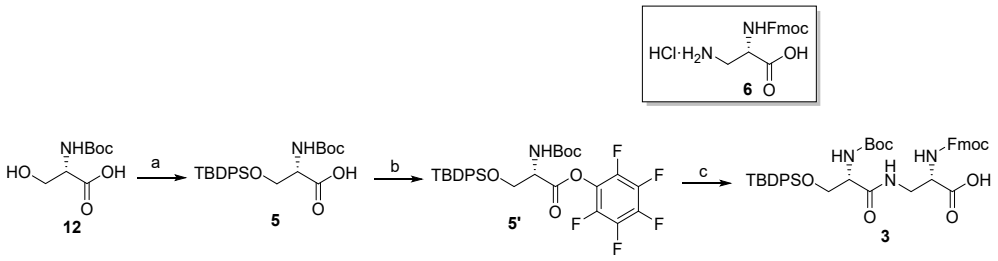


(a) Fmoc-OSu, $NaHCO_3$, THF/H_2O, rt; (b) $PhI(OAc)_2$, MeCN/EA/H_2O, rt

To a solution of L-asparagine **10** (50 g, 378.5 mmol) in THF/H_2O (1:1, 1000 mL) was added $NaHCO_3$ (100 g, 1.2 mol) and Fmoc-Osu (127.7 g, 378.5 mmol) at 0 °C. After being stirred at room temperature for 16 h, volatiles of the reaction mixture were removed in vacuo. The solution was then diluted with water (500 mL) and adjusted to pH 1 by addition of $KHSO_4$. The aqueous phase was extracted with ethyl acetate (3×1000 mL). The combined organic phase was washed by brine (500 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **11** as a white solid, which was used for next step directly.

To a solution of the above acid **11** in MeCN/EA/H_2O (2:2:1) (1000 mL) was added $PhI(OAc)_2$ (146.6 g, 455 mmol). After being stirred for 14 h at room temperature, the reaction mixture was concentrated under reduced pressure. The concentrated reaction mixture was then diluted with water (1000 mL) and adjusted to pH 1 by addition of conc. HCl. The aqueous phase was extracted with ethyl acetate (2×1000 mL) to remove the organic impurity. **The aqueous phase** was then evaporated under reduced pressure. The residue was then co-evaporated with MeCN (2×500 mL) to provide the desired product **6** (123.6 g, 90% over two steps) as a yellow solid. mp 148-152 °C; $[\alpha]_D^{25}$ -15.0 (c 1.0, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (s, 3H), 7.85 (t, J = 9.3 Hz, 3H), 7.71 (d, J = 6.2 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 4.38 – 4.12 (m, 4H), 3.31 – 3.13 (m, 1H), 3.06 (dd, J = 9.0, 5.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.18, 156.58, 144.03, 143.98, 141.03, 128.10, 127.54, 125.67, 120.49, 66.40, 52.08, 46.85; HR-ESIMS m/z: calculated for $C_{18}H_{19}N_2O_4^+$ $[M+H]^+$: 327.1339, found 327.1340.

(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((S)-2-((tert-butoxycarbonyl)amino)-3-((tert-butyldiphenylsilyl)oxy)propanamido)propanoic acid (3)



(a) TBDPSCl, Imidazole, DCM, reflux; (b) C_6F_5OH, EDCI, DMAP, DCM; (c) **6**, $NaHCO_3$, THF/H_2O, rt

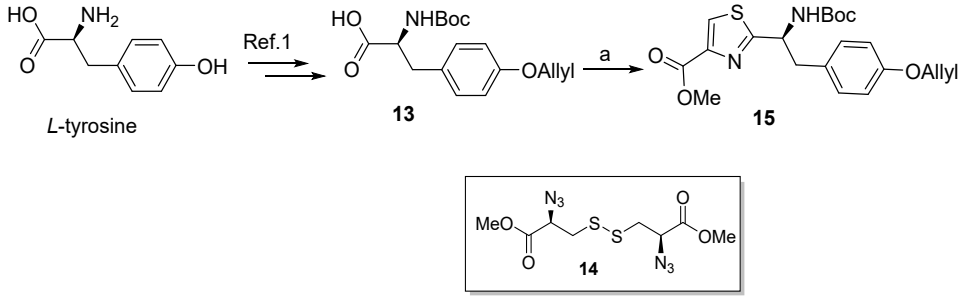
To a solution of Boc-L-serine **12** (50 g, 243.7 mmol) in dry DCM (800 mL) was added imidazole (50 g, 730 mmol) and TBDPSCl (64.0 mL, 245 mmol) at 0 °C. After 30 min, the resultant mixture was heated to reflux and stirred for 15h. The solution was diluted with water (1000 mL) and adjusted to pH 2 by addition of $KHSO_4$. The aqueous phase was extracted with DCM (3×600 mL). The combined organic phase was washed by brine (500 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **5** as a colourless oil, which was used for next step directly.

To a solution of the above acid **5** in dry DCM (500 mL) was added pentafluorophenol (47.0 g, 255 mmol), EDCI (61.3 g, 320 mmol) and DMAP (3.0 g, 24 mmol). After being stirred for 15 h at room temperature under N_2 atmosphere, the reaction was quenched with water (600 mL) and adjusted to pH 2 by addition of $KHSO_4$. The aqueous phase was extracted with DCM (3×500 mL). The combined organic phase was washed by brine (500 mL), dried over sodium sulfate (anhydrous) and concentrated in vacuo to give the pentafluorophenyl ester **5'**, which was used for next step directly.

To a solution of the above pentafluorophenyl ester **5'** in THF/H_2O (1:1, 1400 mL) was added compound **6** (95.0 g, 260 mmol) and $NaHCO_3$ (67.2 g, 800 mmol) at 0 °C. After being stirred at room temperature for 10 h, volatiles of the reaction mixture were removed in vacuo. The concentrated reaction mixture was then diluted with water (500 mL) and adjusted to pH 1 by slow addition of $KHSO_4$. The aqueous phase was extracted with ethyl acetate (3×800 mL). The combined organic phase was washed by brine (500 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo.* The residue was purified by silica gel column chromatography (EA/PE = 1:10; then MeOH/EA = 1:10) to afford **3** as an oil (146.6 g, 80% over three steps). $[\alpha]_D^{25}$ +6.3 (c 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.60 (dd, *J* = 7.6, 6.6 Hz, 6H), 7.43 – 7.33 (m, 9H), 7.29 (d, *J* = 7.4 Hz, 2H), 6.19 (s, 1H), 5.34 (s, 1H), 4.41 (m, 1H), 4.38 – 4.24 (m, 3H), 4.19 (d, *J* = 7.0 Hz, 1H), 3.96 (m, 1H), 3.83 (dd, *J* = 10.0, 5.0 Hz, 1H), 3.71 (m, 1H), 1.42 (s, 9H), 1.03 (s, 9H); ^{13}C NMR (100 MHz, MeOD) δ 173.39, 173.14, 158.47, 157.63, 145.18, 145.14, 142.51, 136.65, 136.62, 135.93, 134.19, 134.11, 130.99, 130.40, 128.88, 128.77, 128.55, 128.17, 126.32, 120.89, 80.95, 68.21, 65.30, 57.94, 55.31, 48.24, 41.69, 38.87, 28.73, 27.28, 20.04; HR-

ESIMS m/z: calculated for $C_{42}H_{48}N_3O_8Si^-$ [M-H]$^-$: 750.3216, found 750.3218.

Methyl (S)-2-(2-(4-(allyloxy)phenyl)-1-((tert-butoxycarbonyl)amino)ethyl)thiazole-4-carboxylate (15)



(a) FDPP, Et_3N, DCM, rt, 0.5 h; then PPh_3, **14**, reflux, 10h; then $CBrCl_3$, DBU, rt, 2h, 75% (one pot)

To a solution of the acid **13** (25.0 g, 77.8 mmol) in dry DCM (300 mL) was added pentafluorophenyl diphenylphosphinate (FDPP) (30.7 g, 80 mmol) and triethylamine (TEA) (22.5 mL, 160 mmol) at room temperature. After stirring for 30 min, compound **14** [2] (12.5 g, 39.0 mmol) and PPh_3 (102 g, 389 mmol) were added to the solution and heated to reflux for a further 14 h away from light. After cooling to 0 °C, 1,8-diazabicycloundec-7-ene (DBU) (23.3 mL, 156 mmol) and bromotrichloromethane ($CBrCl_3$) (15.4 mL, 156 mmol) were introduced via spyringe over 5 min and stirred for further 2h at room temperature. The solvent was quenched with saturated aqueous $KHSO_4$ solution (800 mL) and extracted with DCM (600 mL×3). The combined organic layer was washed with brine (200 mL) and dried over anhydrous Na_2SO_4, filtered, concentrated in vacuo, and purified by FC (silica gel, EA/PE, 1:6) to give compound **7** (2.55 g, 75% over two steps) as an oil;

The combined dichloromethane extracts were washed with a 10% solution of hydrogen peroxide (2×400 mL) to oxidize any remaining phosphorus species to TPPO. The organic layer was then washed with a saturated solution of Na_2SO_3 (2×500 mL), brine (500 mL), and dried over sodium sulfate. The resulting solution was filtered and concentrated on a rotary evaporator to a colourless residue.

The residue was dissolved in 1000 mL of absolute toluene, then magnesium chloride (325 mesh powder, 74.0 g, 778 mmol) was added. The reaction mixture was vigorously stirred at 60 °C under N_2 atmosphere. After 3h the mixture was cooled to ambient temperature and filtered to remove the solids (TPPO complex and excess magnesium chloride).

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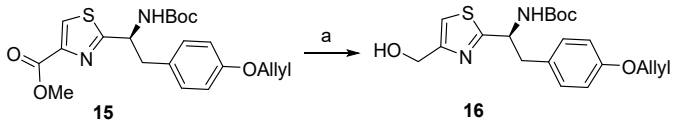
[1] S. Kappler, L. Karmann, C. Prudel, J. Herrmann, G. Caddeu, R. Müller, A. M. Vollmar, S. Zahler, U. Kazmaier, *Eur. J. Org. Chem.*, **2018**, 6952-6965.

[2] Y. Liu, J. Liu, X. Qi, and Y. Du, *J. Org. Chem.*, **2012**, 77, 7108-7113.

The filter cake was washed with toluene (600 mL) and the combined filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (EA/PE, 1:4) to afford **15** as an oil (24.4 g, 75%). $[\alpha]_D^{25}$ -19.0 (c 2.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 6.98 (d, *J* = 8.6 Hz, 2H), 6.80 (d, *J* = 8.6 Hz, 2H), 6.03 (ddd, *J* = 22.6, 10.5, 5.3 Hz, 1H), 5.39 (dd, *J* = 17.3, 1.5 Hz, 1H), 5.27 (dd, *J* = 10.5, 1.3 Hz, 2H), 4.56 – 4.43 (m, 2H), 3.96 (s, 3H), 3.25 (m, 2H), 3.07 (s, 1H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.95, 161.78, 157.62, 154.93, 146.82, 133.19, 130.34, 128.19, 127.38, 117.56, 114.83, 80.05, 68.73, 53.99, 52.38, 40.63, 28.19; HR-ESIMS m/z: calculated for $C_{21}H_{26}N_2O_5SNa^+$ $[M+Na]^+$: 441.1455, found 441.1458.

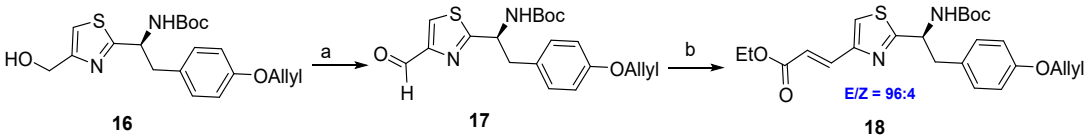
Tert-butyl (S)-(2-(4-(allyloxy)phenyl)-1-(4-(hydroxymethyl)thiazol-2-yl)ethyl)carbamate (16)



(a) $NaBH_4$, LiCl, EtOH, reflux

To a solution of **13** (24.4 g, 58.3 mmol) in dry EtOH (300 mL) was added $NaBH_4$ (6.65 g, 175 mmol) and LiCl (7.4 g, 175 mmol) at 0 °C. After 30 min, the solution was heated to reflux for a further 15h. Volatiles of the reaction mixture were removed in vacuo. The concentrated reaction mixture was then diluted with water (500 mL) and adjusted to pH 3 by slow addition of $KHSO_4$. The aqueous phase was extracted with ethyl acetate (3×500 mL). The combined organic phase was washed by brine (400 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo.* The residue was purified by silica gel column chromatography (EA/PE, 1:2) to afford **16** as an oil (21.6 g, 95%). $[\alpha]_D^{25}$ -23.3 (c 1.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (s, 1H), 6.96 (d, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 8.5 Hz, 2H), 6.07 – 5.95 (m, 1H), 5.39 (d, *J* = 1.5 Hz, 1H), 5.35 (d, *J* = 1.5 Hz, 1H), 5.25 (dd, *J* = 10.5, 1.4 Hz, 1H), 5.16 (s, 1H), 4.72 (s, 2H), 4.46 (d, *J* = 5.3 Hz, 2H), 3.18 (dd, *J* = 13.9, 6.3 Hz, 2H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.46, 157.47, 156.32, 154.95, 133.21, 132.03, 130.33, 128.50, 117.48, 114.67, 114.39, 79.99, 68.69, 60.59, 53.80, 40.90, 28.20; HR-ESIMS m/z: calculated for $C_{20}H_{26}N_2O_4SNa^+$ $[M+Na]^+$: 413.1505, found 413.1507.

Ethyl (S,E)-3-(2-(2-(4-(allyloxy)phenyl)-1-((tert-butoxycarbonyl)amino)ethyl)thiazol-4-yl)acrylate (18)



16 17 18

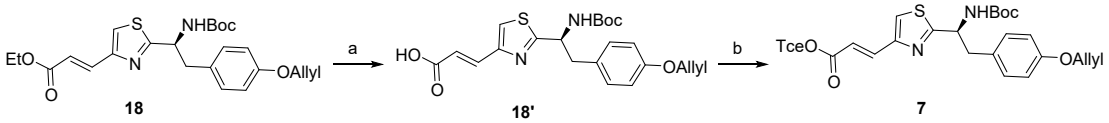
(a) IBX, DMSO, rt; (b) $Ph_3P{=}CHCO_2Et$, DCM, rt

To a solution of the above **16** (21.6 g, 55.3 mmol) in DMSO (100 mL) was added IBX (20.2 g, 72 mmol) at room temperature. After 2h, the reaction was quenched with saturated aqueous solution of $NaHCO_3$ (800 mL). The aqueous phase was extracted with EtOAc (3×500 mL). The combined organic phase was washed with H_2O (500 mL), brine (500 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to afford the aldehyde **17** as an oil which was used for next step directly.

To a solution of the above aldehyde **17** in CH_2Cl_2 (300 mL) was added $Ph_3P{=}CHCO_2Et$ (24.4 g, 70 mmol). The mixture was stirred for an additional 2h at ambient temperature. After concentrated in *vacuo,* the residue was purified by silica gel column chromatography (EA/PE, 1:4) to afford a small amount of the corresponding (Z)-isomer (0.9 g, 3.5 % over two steps) and the corresponding (E)-isomer **18** (21.6 g, 85% over two steps) as an oil. The (Z)-isomer move a little faster than the corresponding (E)-isomer on the TLC plates.

(E)-isomer compound **18**: $[\alpha]_D^{25}$ -21.4 (c 1.4, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 15.5 Hz, 1H), 7.29 (s, 1H), 7.01 (d, *J* = 6.8 Hz, 2H), 6.83 – 6.79 (m, 2H), 6.77 (d, *J* = 15.6 Hz, 1H), 6.03 (ddd, *J* = 22.5, 10.5, 5.3 Hz, 1H), 5.39 (dd, *J* = 17.3, 1.5 Hz, 1H), 5.32 – 5.21 (m, 2H), 4.49 (d, *J* = 5.3 Hz, 2H), 4.27 (q, *J* = 7.1 Hz, 2H), 3.24 (s, 2H), 1.39 (d, *J* = 27.9 Hz, 9H), 1.33 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.12, 157.55, 154.93, 151.61, 141.58, 136.17, 133.23, 131.54, 130.45, 128.41, 127.85, 121.06, 120.78, 117.51, 114.73, 80.2, 68.73, 60.46, 53.96, 40.69, 28.23, 14.24; HR-ESIMS m/z: calculated for $C_{24}H_{30}N_2O_5SNa^+$ $[M+Na]^+$: 481.1768, found 481.1770.

2,2,2-Trichloroethyl (S,E)-3-(2-(2-(4-(allyloxy)phenyl)-1-((tert-butoxycarbonyl)amino)ethyl)thiazol-4-yl)acrylate (7)



18 18' 7

(a) NaOH, THF/MeOH/H_2O, reflux; (b) TceOH, EDCI, DMAP, DCM, rt

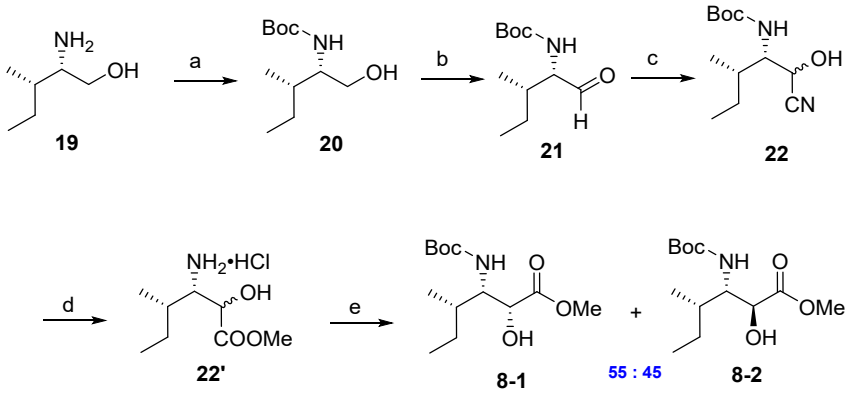
NaOH (20.0 g, 500 mmol) was added to a solution of compound **18** (21.6 g, 47.1 mmol) in THF/MeOH/H_2O

(1:1:1, 300 mL) at room temperature. After 10 min, the resultant mixture was heated to reflux and stirred for 2h. Then volatiles of the reaction mixture were removed in vacuo. The solution was diluted with water (400 mL) and adjusted to pH 2 by addition of $KHSO_4$. The aqueous phase was extracted with ethyl acetate (3×500 mL). The combined organic phase was washed by brine (400 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **18'** as an oil in quantitative yield which was used for next step directly.

The above acid **18'**, EDCI (13.4 g, 70 mmol) and DMAP (1.2 g, 10 mmol) were dissolved in dry DCM (200 mL). After 2,2,2-trichloroethanol (TceOH) (6.7 mL, 70 mmol) was added, the reaction mixture was stirred overnight at room temperature. Then the reaction was quenched with water (500 mL) and adjusted to pH 2 by addition of $KHSO_4$. The aqueous phase was extracted with DCM (3×500 mL). The combined organic phase was washed by brine (500 mL), dried over sodium sulfate (anhydrous) and concentrated in vacuo to give **7** (26.5 g, 100% yield), which was pure enough and could be used for next step directly. $[\alpha]_D^{25}$ -20.0 (c 2.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 15.5 Hz, 1H), 7.38 (s, 1H), 7.26 (s, 1H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.84 (dd, *J* = 25.5, 11.9 Hz, 3H), 6.03 (ddd, *J* = 15.8, 10.5, 5.3 Hz, 1H), 5.39 (dd, *J* = 17.3, 1.5 Hz, 1H), 5.33 – 5.10 (m, 3H), 4.88 (s, 2H), 4.74 (s, 1H), 4.50 (d, *J* = 5.3 Hz, 2H), 3.24 (s, 2H), 1.42 (d, *J* = 4.0 Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.38, 157.63, 154.84, 151.26, 150.60, 138.35, 133.23, 130.46, 130.38, 128.28, 122.51, 118.82, 117.61, 114.80, 98.30, 95.10, 80.26, 74.11, 74.02, 68.77, 54.06, 40.74, 28.27; HRMS (*m/z*): calcd for $C_{24}H_{27}Cl_3N_2NaO_5S^+$ ([M + Na]$^+$) 583.0598, found 583.0599.

Methyl (2R,3S,4S)-3-((tert-butoxycarbonyl)amino)-2-hydroxy-4-methylhexanoate (8-1)

Methyl (2S,3S,4S)-3-((tert-butoxycarbonyl)amino)-2-hydroxy-4-methylhexanoate (8-2)



(a) Boc_2O, $NaHCO_3$, THF/H_2O, rt; (b) IBX, MeCN, reflux; (c) $Me_2C(OH)CN$, Et_3N, DCM; (d) $SOCl_2$, MeOH, reflux; (e) Boc_2O, $NaHCO_3$, THF/H_2O, rt.

To a solution of the L-Isoleucinol (**19**) (30 g, 256 mmol) in THF/H_2O (1:1, 1000 mL) was added $NaHCO_3$

(33.6 g, 400 mmol) and Boc_2O (58.8 mL, 256 mmol). After being stirred at room temperature for 12h, volatiles of the reaction mixture were removed in vacuo. The aqueous phase was extracted with ethyl acetate (3×500 mL). The combined organic phase was washed by brine (500 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the corresponding compound **20** in quantitative yield, which was used for next step directly.

To a solution of the above compound **20** in dry MeCN (500 mL) was added IBX (78.4 g, 280 mmol) at room temperature. After 15 min, the resultant mixture was heated to reflux and stirred for 2h. The solution was cooled to room temperature and the solid was removed by filtration through a pad of celite and washed with MeCN (200 mL). The total filtrate was concentrated in vacuo to afford the aldehyde **21** as an oil which was used for next step directly.

To a solution of the above aldehyde **21** in dry DCM (400 mL) was added Et_3N (42.0 mL, 300 mmol) and acetone cyanohydrin (27.5 mL, 300 mmol) at room temperature. After being stirred at room temperature for 2h, volatiles of the reaction mixture were removed in vacuo. The compound **22** was obtained in quantitative yield, which was used for next step directly.

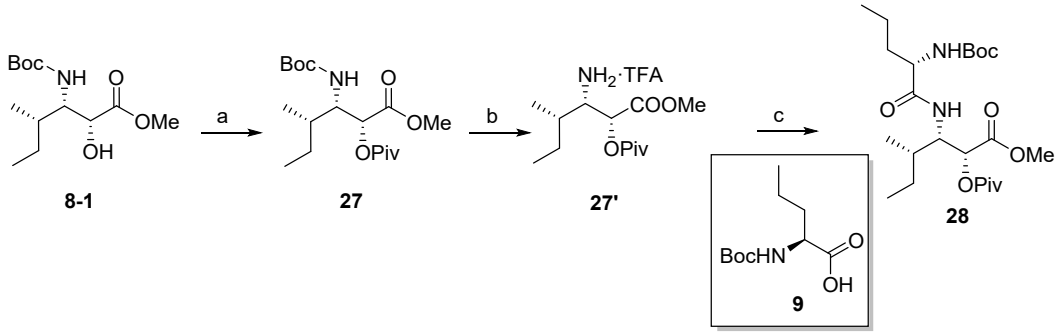
To a solution of the above compound **22** in MeOH (500 mL) was dropwise added $SOCl_2$ (90 mL) at 0 °C. After 30 min, the resultant mixture was heated to reflux and stirred for 10h. The solution was cooled to room temperature and volatiles of the reaction mixture were removed in vacuo to obtain the compound **22'**, which was used for next step directly.

To a solution of the above **22'** in THF/H_2O (1:1, 800 mL) was added $NaHCO_3$ (33.6 g, 400 mmol) and Boc_2O (58.8 mL, 256 mmol). After being stirred at room temperature for 10 h, volatiles of the reaction mixture were removed in vacuo. The aqueous phase was extracted with ethyl acetate (3×500 mL). The combined organic layer was washed with brine (500 mL) and dried over anhydrous Na_2SO_4, filtered, concentrated in vacuo, and purified by flash column chromatography (silica gel, EA/PE, 1:10 then 1:5) to give compound **8-1** (35.2 g, 50 % over 5 steps) and **8-2** (28.5g, 40.5% over 5 steps). The two diastereomers could be easily separated by silica gel column chromatography. The threo compound (**8-1**) move a little faster than the corresponding erythro (**8-2**) on the TLC plates.

The threo compound (**8-1**): $[\alpha]_D^{25}$ -48.6 (c 1.41, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.83 (d, J = 9.0 Hz, 1H), 4.36 (s, 1H), 3.77 (s, 3H), 3.32 (s, 1H), 1.62 (dd, J = 15.3, 7.1 Hz, 2H), 1.40 (s, 9H), 1.34 – 1.03 (m, 1H), 0.99 (d, J = 6.5 Hz, 3H), 0.90 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.87, 155.46, 79.25, 70.28, 56.96, 52.66, 36.18, 28.19, 25.52, 15.66, 10.92; HR-ESIMS m/z: calculated for $C_{13}H_{25}NO_5Na^+$ $[M+Na]^+$: 298.1625, found 298.1627.

The erythro compound (**8-2**): $[\alpha]_D^{25}$ -8.1 (c 0.62, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.79 (d, *J* = 8.7 Hz, 1H), 4.31 (m, 1H), 3.79 (s, 3H), 3.37 (s, 1H), 1.64 – 1.60 (m, 2H), 1.45 (d, *J* = 3.9 Hz, 9H), 1.21 – 1.05 (m, 1H), 0.93 – 0.87 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.85, 156.18, 79.68, 72.59, 58.01, 52.49, 35.37, 28.30, 28.19, 24.84, 15.94, 11.13; HR-ESIMS m/z: calculated for $C_{13}H_{25}NO_5Na^+$ $[M+Na]^+$: 298.1625, found 298.1627.

Methyl (2R,3S,4S)-3-((S)-2-((tert-butoxycarbonyl)amino)pentanamido)-4-methyl-2-(pivaloyloxy)hexanoate (28)



(a) PivCl, Et_3N, DMAP, DCM, reflux, 2h; (b) TFA, DCM; (c) Boc-L-norvaline **9**, HATU, DIPEA, DCM, rt, overnight.

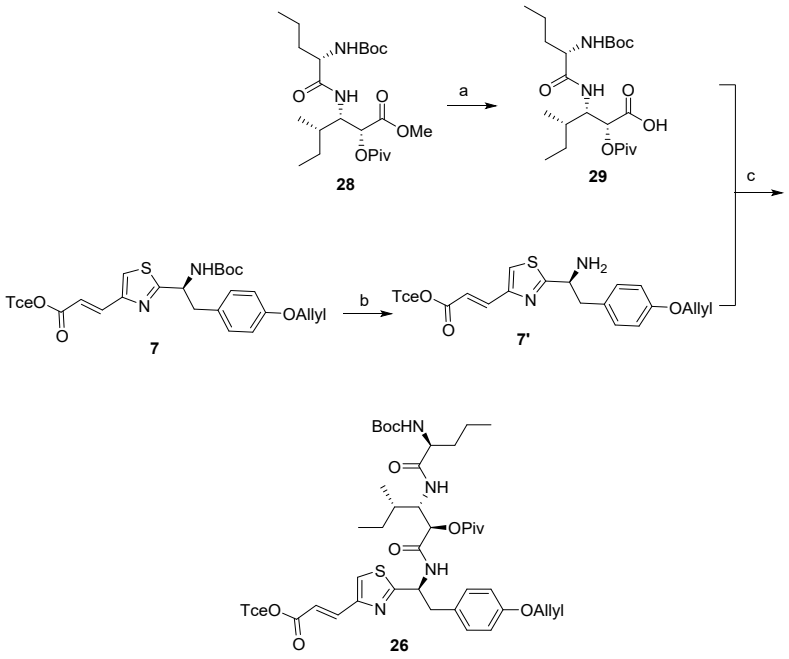
To a solution of compound **8-1** (35.2 g, 127.8 mmol) in dry DCM (500 mL) was added Et_3N (26.4 mL, 190 mmol), DMAP (1.6 g, 13 mmol) and PivCl (16.0 mL, 130 mmol) at 0 °C. After 30 min, the resultant mixture was heated to reflux and stirred for 2h. The reaction was then quenched with saturated aqueous $KHSO_4$ solution (600 mL) and extracted with DCM (3×500 mL). The combined organic layer was washed with saturated aqueous solution of $NaHCO_3$ (500 mL), brine (500 mL) and dried over anhydrous Na_2SO_4, filtered, concentrated in vacuo to give the compound **27** (46.0 g, 100%) as an oil, which was pure enough and could be used for next step directly.

$[\alpha]_D^{25}$ -46.9 (c 0.67, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.15 (d, *J* = 2.3 Hz, 1H), 4.64 (d, *J* = 10.4 Hz, 1H), 4.07 – 3.95 (m, 1H), 3.70 (s, 3H), 1.60 – 1.55 (m, 1H), 1.43 (s, 1H), 1.41 (d, *J* = 5.0 Hz, 9H), 1.26 (d, *J* = 3.0 Hz, 9H), 1.23 (s, 1H), 0.89 (dd, *J* = 5.3, 4.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.38, 169.12, 155.28, 79.60, 71.88, 55.23, 52.24, 38.85, 36.84, 28.22, 27.02, 26.47, 25.54, 15.44, 10.94; HR-ESIMS m/z: calculated for $C_{18}H_{33}NO_6Na^+$ $[M+Na]^+$: 382.2200, found 382.2203.

To a solution of the above compound **27** in dry DCM (200 mL) was added neat trifluoroacetic acid (TFA) (50 mL). After being stirred at room temperature for 3h, volatiles of the reaction mixture were removed in vacuo. The residue was then co-evaporated with toluene (2×300 mL) to provide the desired amine **27'** (TFA salt) as an oil, which was used for next step directly.

To a solution of the above amine **27'** in dry DCM (400 mL) was added compound **9** (28.2 g, 130 mmol) and HATU (59.3 g, 156 mmol) at room temperature. After DIPEA (64.5 mL, 390 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature and stirred overnight at N_2 atmosphere. The solution was diluted with DCM (1500 mL) and washed successively with saturated aqueous $KHSO_4$ solution (400 mL), saturated aqueous solution of $NaHCO_3$ (400 mL), brine (500 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (EA/PE, 1:4) to afford **28** (50 g, 85% over 3 steps) as an oil. $[\alpha]_D^{25}$ -50.7 (c 0.67, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (d, *J* = 9.7 Hz, 1H), 5.15 (d, *J* = 2.0 Hz, 1H), 5.07 (dd, *J* = 15.5, 6.3 Hz, 1H), 4.35 – 4.29 (m, 1H), 4.02 – 3.96 (m, 1H), 3.67 (s, 3H), 1.74 (d, *J* = 7.1 Hz, 1H), 1.52 – 1.46 (m, 4H), 1.41 (s, 9H), 1.32 (d, *J* = 7.4 Hz, 4H), 1.26 (s, 9H), 1.16 (s, 2H), 0.92 – 0.87 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.27, 171.94, 168.80, 155.86, 79.98, 71.37, 54.27, 53.48, 52.30, 38.83, 36.53, 28.21, 27.09, 26.96, 25.35, 18.71, 15.30, 13.63, 10.80; HR-ESIMS m/z: calculated for $C_{23}H_{42}N_2O_7Na^+$ [M+Na]$^+$: 481.2884, found 481.2886.

2,2,2-Trichloroethyl (E)-3-(2-((6S,9S,10R,13S)-14-(4-(allyloxy)phenyl)-9-((S)-sec-butyl)-2,2-dimethyl-4,7,11-trioxo-10-(pivaloyloxy)-6-propyl-3-oxa-5,8,12-triazatetradecan-13-yl)thiazol-4-yl)acrylate (26)



(a) LiI, pyridine, relux; (b) TFA, DCM; (c) HATU, DIPEA, DCM, rt, overnight.

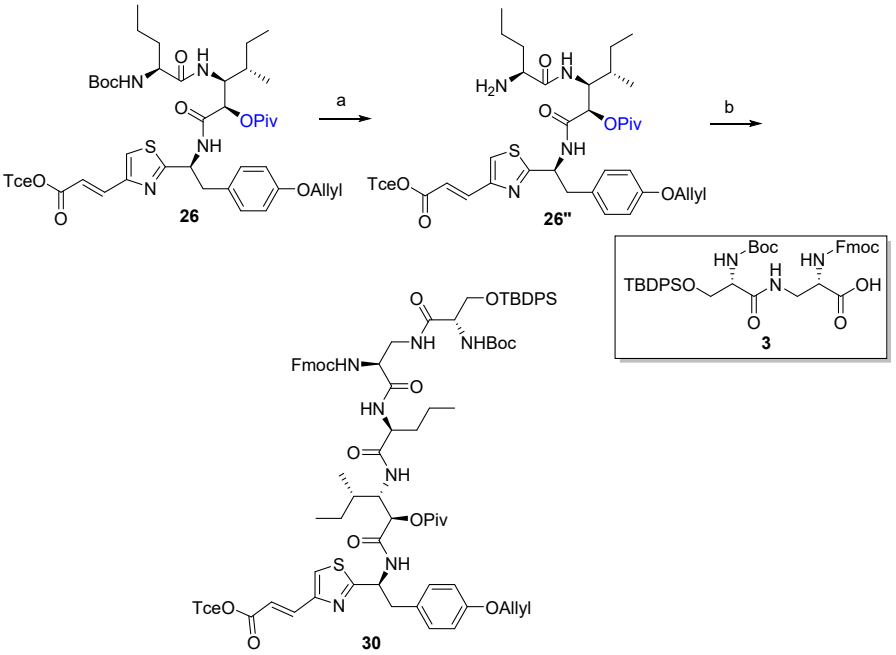
To a solution of the above compound **28** (50 g, 109.0 mmol) in dry pyridine (300 mL) was added LiI (29.2 g, 218 mmol). The reaction mixture was heated to reflux and stirred for 6h. Then volatiles of the mixture were removed in vacuo. The residue was diluted with water (500 mL) and adjusted to pH 2 by addition of $KHSO_4$. The

aqueous phase was extracted with EtOAc (3×500 mL). The combined organic phase was washed by brine (500 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **29** (46.0 g, 95%) as a yellow oil, which was used for next step directly.

To a solution of compound **7 (**26.5 g, 47.2 mmol) in dry DCM (200 mL) was added neat trifluoroacetic acid (TFA) (50 mL). After being stirred at room temperature for 3h, volatiles of the reaction mixture were removed in vacuo and the residue was quenched with saturated aqueous solution of $NaHCO_3$ (500 mL). The aqueous phase was extracted with DCM (3×500 mL). The combined organic phase was washed with brine (400 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to afford the free amine **7'** as an oil which was used for next step directly.

To a solution of the above amine **7'** in dry DCM (400 mL) was added the above acid **29** (22.2 g, 50 mmol) and HATU (28.5 g, 75 mmol) at room temperature. After DIPEA (16.5 mL, 100 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature and stirred overnight at N_2 atmosphere. The solution was diluted with DCM (1000 mL) and washed successively with saturated aqueous $KHSO_4$ solution (300 mL), saturated aqueous solution of $NaHCO_3$ (300 mL), brine (300 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (EA/PE, 1:2) to afford **26** (34.4 g, 82% over 2 steps) as an oil. $[\alpha]_D^{25}$ -4.8 (c 0.58, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 15.5 Hz, 1H), 7.39 (s, 1H), 7.10 (d, *J* = 7.3 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 6.74 (t, *J* = 11.7 Hz, 3H), 6.51 (d, *J* = 10.0 Hz, 1H), 6.00 (ddd, *J* = 22.4, 10.5, 5.3 Hz, 1H), 5.47 (dd, *J* = 8.6, 6.1 Hz, 2H), 5.36 (dd, *J* = 17.3, 1.4 Hz, 1H), 5.24 (dd, *J* = 10.5, 1.2 Hz, 1H), 4.90 (d, *J* = 10.0 Hz, 1H), 4.83 (t, *J* = 9.5 Hz, 2H), 4.48 – 4.44 (m, 2H), 4.35 (t, *J* = 8.3 Hz, 1H), 3.82 (dd, *J* = 14.3, 8.0 Hz, 1H), 3.24 (dd, *J* = 13.7, 4.5 Hz, 1H), 3.10 (dd, *J* = 13.8, 7.5 Hz, 1H), 1.52 – 1.42 (m, 3H), 1.39 (s, 9H), 1.25 (d, *J* = 11.9 Hz, 9H), 1.11 (dd, *J* = 6.3, 3.5 Hz, 4H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.82 (t, *J* = 7.2 Hz, 3H), 0.71 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.26, 171.60, 170.19, 167.93, 165.15, 157.59, 155.97, 150.51, 137.82, 133.14, 130.60, 127.40, 122.80, 118.97, 117.53, 114.63, 94.99, 80.00, 74.11, 72.65, 68.65, 54.13, 52.54, 40.74, 38.95, 36.36, 33.35, 28.18, 27.01, 25.06, 18.70, 15.58, 13.59, 10.89; HR-ESIMS m/z: calculated for $C_{41}H_{57}Cl_3N_4O_9SNa^+$[M+Na]$^+$: 909.2804, found 909.2806.

2,2,2-Trichloroethyl (E)-3-(2-((6S,10S,13S,16S,17R,20S)-10-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-21-(4-(allyloxy)phenyl)-6-((tert-butoxycarbonyl)amino)-16-((S)-sec-butyl)-2,2-dimethyl-7,11,14,18-tetraoxo-3,3-diphenyl-17-(pivaloyloxy)-13-propyl-4-oxa-8,12,15,19-tetraaza-3-silahenicosan-20-yl)thiazol-4-yl)acrylate (30)



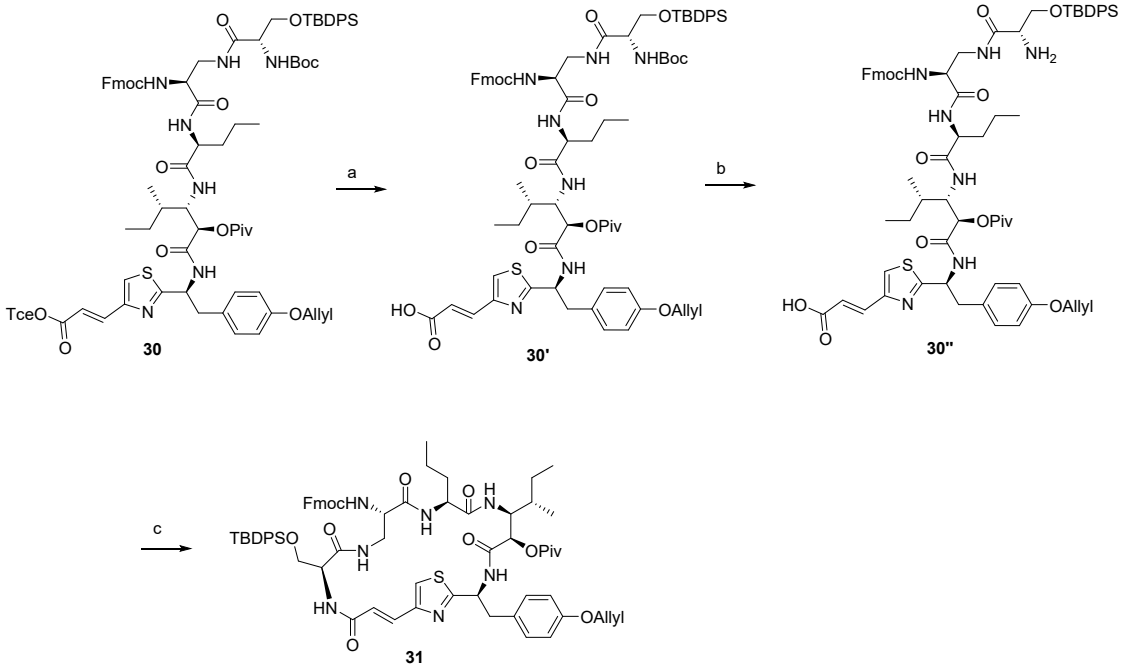
(a) TFA, DCM; (b) **3**, HATU, DIPEA, DCM, rt, overnight.

To a solution of compound **26 (**34.4 g g, 38.8 mmol) in dry DCM (200 mL) was added neat trifluoroacetic acid (TFA) (50 mL). After being stirred at room temperature for 3h, volatiles of the reaction mixture were removed in vacuo and the residue was quenched with saturated aqueous solution of $NaHCO_3$ (400 mL). The aqueous phase was extracted with DCM (3×500 mL). The combined organic phase was washed with brine (500 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to afford the free amine **26''** as an oil which was used for next step directly.

To a solution of the above amine **26''** in dry DCM (400 mL) was added the acid **3** (30.1 g, 40 mmol) and HATU (22.8 g, 60 mmol) at room temperature. After DIPEA (13.2 mL, 80 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature and stirred overnight at N_2 atmosphere. The solution was diluted with DCM (1000 mL) and washed successively with saturated aqueous $KHSO_4$ solution (300 mL), saturated aqueous solution of $NaHCO_3$ (300 mL), brine (300 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (EA/PE, 1:2) to afford **30** (50.2 g, 85% over 2 steps) as an oil. $[\alpha]_D^{25}$ -4.4 (c 0.70, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.5 Hz, 3H), 7.65 – 7.42 (m, 8H), 7.43 – 7.28 (m, 10H), 7.25 (td, *J* = 7.2, 2.7 Hz, 3H), 6.83 (d, *J* = 8.3 Hz, 2H), 6.81 – 6.53 (m, 4H), 6.21 (s, 1H), 6.04 – 5.89 (m, 1H), 5.60 – 5.27 (m, 4H), 5.21 (dd, *J* = 10.5, 1.1 Hz, 1H), 4.81 (s, 2H), 4.41 (d, *J* = 5.2 Hz, 2H), 4.31 (dd, *J* = 11.8, 4.7 Hz, 3H), 4.15 (d, *J* = 6.0 Hz, 4H), 3.89 (s, 1H), 3.83 (dd, *J* = 10.1, 5.1 Hz, 1H), 3.71 – 3.53 (m, 1H), 3.40 (s, 1H), 3.30 – 3.20 (m, 1H), 3.16 (dd, *J* = 13.7, 7.3 Hz, 1H), 1.56 (d, *J* = 14.7 Hz, 3H), 1.36 (d, *J* = 12.3 Hz, 9H), 1.27 – 1.13 (m, 12H), 1.01 (s, 9H), 0.87

(d, J = 6.7 Hz, 3H), 0.72 (dt, J = 14.2, 7.3 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.32, 171.44, 170.47, 170.10, 167.94, 164.99, 157.28, 155.86, 150.08, 143.51, 141.00, 14096, 138.02, 135.29, 135.21, 133.01, 132.53, 132.36, 131.75, 130.36, 129.70, 127.62, 127.48, 126.87, 124.88, 123.09, 119.71, 119.47, 118.33, 117.25, 114.39, 94.87, 80.16, 73.84, 72.88, 68.42, 66.98, 63.34, 56.91, 55.91, 54.21, 53.94, 52.30, 46.76, 41.33, 39.96, 38.70, 35.22, 32.95, 28.50, 28.02, 26.77, 26.53, 24.88, 18.97, 15.39, 13.31, 10.42; HR-ESIMS m/z: calculated for $C_{78}H_{96}Cl_3N_7O_{14}SSiNa^+$ $[M+Na]^+$: 1542.5463, found 1542.5466.

(12Z,2S,5R,6S,9S,12S,16S,19E)-12-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-(allyloxy)benzyl)-6-((S)-sec-butyl)-16-(((tert-butyldiphenylsilyl)oxy)methyl)-4,8,11,15,18-pentaoxo-9-propyl-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-5-yl pivalate (31)



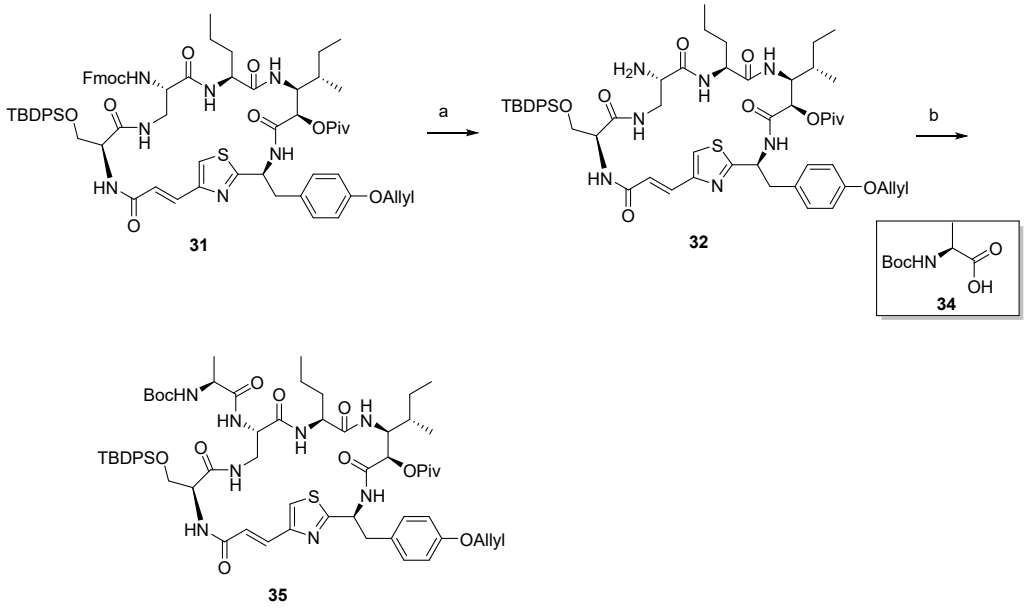
(a) Zn dust, 90% HOAc, THF; (b) TFA, DCM; (c) HATU, DIPEA, dilute DCM, rt, 2d.

To a solution of the above compound **30** (50.2 g, 33.0 mmol) in THF (500 mL) was added 90% aqueous HOAc (100 mL) and Zn dust (43 g, 660 mmol) at room temperature. After being stirred at room temperature for 2h, volatiles of the reaction mixture were removed in vacuo. The residue was diluted with EtOAc (2000 mL) and quenched with saturated aqueous solution of $NaHCO_3$ (1500 mL). The mixture was filtered through a Celite pad. The aqueous phase was then extracted with EtOAc (3×1000 mL). The combined organic phase was washed with brine (800 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to afford the acid **30'** as an oil which was used for next step directly.

To a solution of the above compound **30'** in dry DCM (300 mL) was added neat trifluoroacetic acid (TFA) (60 mL). After being stirred at room temperature for 3h, volatiles of the reaction mixture were removed in vacuo and the residue was quenched with saturated aqueous solution of $NaHCO_3$ (500 mL). The aqueous phase was extracted with DCM (3×1000 mL). The combined organic phase was washed with brine (500 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to afford the desired free amino acid **30''** as an oil which was used for next step directly.

To a solution of the above amino acid **30''** in dry DCM (10 L) was added HATU (19.0 g, 50 mmol) at room temperature. After DIPEA (16.5 mL, 100 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature and stirred for 2 days at N_2 atmosphere. The solution was washed successively with saturated aqueous $KHSO_4$ solution (1000 mL), saturated aqueous solution of $NaHCO_3$ (1000 mL), brine (1000 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (EA/PE, 1:1) to afford **31** (33.6 g, 80% over 3 steps) as an oil. $[\alpha]_D^{25}$ +32.3 (c 1.13, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.53 (m, 9H), 7.52 – 7.28 (m, 11H), 6.99 (s, 2H), 6.81 (d, *J* = 8.3 Hz, 2H), 6.03 (ddd, *J* = 22.4, 10.5, 5.2 Hz, 1H), 5.65 (s, 1H), 5.42 – 5.27 (m, 2H), 4.57 – 4.26 (m, 6H), 4.20 (t, *J* = 6.5 Hz, 1H), 4.06 (d, *J* = 8.2 Hz, 1H), 3.88 (s, 1H), 3.18 (dt, *J* = 18.5, 9.3 Hz, 2H), 1.51 (s, 2H), 1.43 (d, *J* = 3.2 Hz, 1H), 1.25 (d, *J* = 17.1 Hz, 4H), 1.17 (d, *J* = 11.2 Hz, 9H), 1.12 – 1.04 (m, 9H), 0.95 – 0.60 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.16, 170.16, 168.11, 166.42, 157.79, 150.28, 143.68, 143.55, 141.21, 135.49, 135.39, 135.33, 133.26, 133.09, 130.43, 129.93, 127.85, 127.71, 127.66, 127.29, 127.07, 127.01, 125.18, 125.01, 123.40, 121.99, 119.91, 117.62, 117.53, 114.81, 68.70, 67.38, 53.10, 52.01, 46.93, 41.62, 38.75, 29.63, 28.23, 26.98, 26.92, 26.71, 19.45, 19.17, 18.92, 15.71, 13.45, 11.50; HR-ESIMS m/z: calculated for $C_{71}H_{85}N_7O_{11}SSiNa^+$[M+Na]$^+$: 1294.5689, found 1294.5692.

(12Z,2S,5R,6S,9S,12S,16S,19E)-2-(4-(allyloxy)benzyl)-12-((S)-2-((tert-butoxycarbonyl)amino)propanamido)-6-((S)-sec-butyl)-16-(((tert-butyldiphenylsilyl)oxy)methyl)-4,8,11,15,18-pentaoxo-9-propyl-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-5-yl pivalate (35)



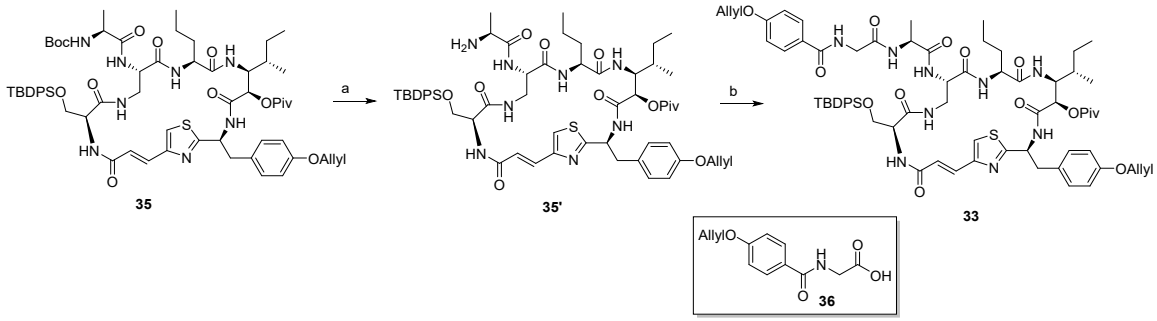
(a) DEA, MeCN, rt, 1h; (b) **34**, HATU, DIPEA, DCM, rt, overnight

To a solution of the above compound **31** (33.6 g, 26.4 mmol) in dry MeCN (200 mL) was added diethylamine (DEA) (50 mL). After being stirred at room temperature for 2h, volatiles of the reaction mixture were removed in vacuo. The residue was then co-evaporated with toluene (2×300 mL) to provide the desired free amine **32** as an oil, which was used for next step directly.

To a solution of the above amine **32** in dry DCM (200 mL) was added Boc-L-alanine **34** (5.7 g, 30 mmol) and HATU (15.2 g, 40 mmol) at room temperature. After DIPEA (9 mL, 55 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature and stirred overnight at N_2 atmosphere. The solution was diluted with DCM (1500 mL) and washed successively with saturated aqueous $KHSO_4$ solution (400 mL), saturated aqueous solution of $NaHCO_3$ (400 mL), brine (400 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (EA/PE, 70:30) to afford **35** (25.8 g, 80% over 2 steps) as an oil. $[\alpha]_D^{25}$ +40.5 (c 0.84, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 3.2 Hz, 2H), 7.64 (dd, *J* = 6.6, 2.7 Hz, 2H), 7.54 (d, *J* = 15.0 Hz, 1H), 7.38 (dd, *J* = 6.3, 4.4 Hz, 6H), 7.33 (s, 1H), 6.99 (d, *J* = 6.7 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.02 (ddt, *J* = 17.2, 10.6, 5.3 Hz, 1H), 5.65 (s, 1H), 5.43 (d, *J* = 7.4 Hz, 1H), 5.42 – 5.35 (m, 1H), 5.26 (dd, *J* = 10.5, 1.3 Hz, 1H), 5.07 (s, 1H), 4.73 (s, 1H), 4.55 (s, 1H), 4.47 (dd, *J* = 4.1, 1.2 Hz, 2H), 4.28 (dd, *J* = 9.9, 5.3 Hz, 1H), 4.10 (s, 1H), 3.81 (s, 1H), 3.22 – 3.02 (m, 2H), 1.56 (s, 1H), 1.41 (d, *J* = 8.3 Hz, 9H), 1.27 (d, *J* = 11.7 Hz, 3H), 1.15 (s, 9H), 1.06 (s, 9H), 0.84

– 0.61 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.37, 171.88, 170.32, 168.42, 166.30, 157.73, 155.69, 150.19, 135.33, 133.12, 130.35, 129.88, 129.79, 127.84, 127.81, 127.47, 123.51, 121.83, 117.58, 114.78, 80.44, 68.67, 55.06, 51.79, 41.64, 38.53, 35.75, 29.61, 28.29, 26.95, 19.47, 19.13, 15.79, 13.38; HR-ESIMS m/z: calculated for $C_{64}H_{88}N_8O_{12}SSNa^+$[M+Na]$^+$: 1243.5904, found 1243.5907.

(12Z,2S,5R,6S,9S,12S,16S,19E)-12-((S)-2-(2-(4-(allyloxy)benzamido)acetamido)propanamido)-2-(4-(allyloxy)benzyl)-6-((S)-sec-butyl)-16-(((tert-butyldiphenylsilyl)oxy)methyl)-4,8,11,15,18-pentaoxo-9-propyl-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-5-yl pivalate (33)



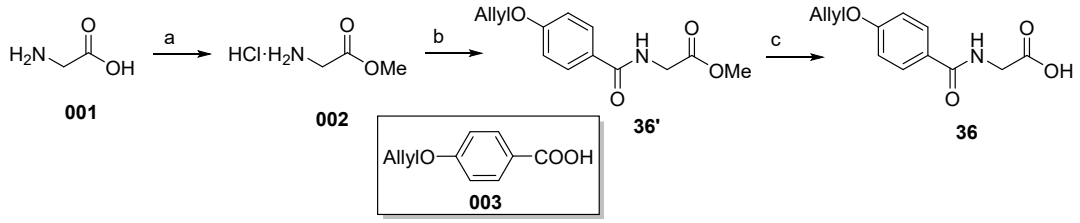
(a) TFA, DCM; (b) **36**, EDCI, HOBt, DIPEA, DCM, rt, overnight.

To a solution of the above compound **35** (25.8 g, 21.0 mmol) in dry DCM (200 mL) was added neat trifluoroacetic acid (TFA) (40 mL). After being stirred at room temperature for 4h, volatiles of the reaction mixture were removed in vacuo and the residue was quenched with saturated aqueous solution of $NaHCO_3$ (400 mL). The aqueous phase was extracted with DCM (3×500 mL). The combined organic phase was washed with brine (500 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to afford the free amine **35'** as an oil which was used for next step directly.

To a solution of the above amine **35'** in dry DCM (400 mL) was added the acid **36** (5.9 g, 25 mmol), EDCI (14.4 g, 75 mmol) and HOBt (1.4 g, 10 mmol) at room temperature. After DIPEA (16.5 mL, 100 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature and stirred overnight at N_2 atmosphere. The solution was diluted with DCM (1500 mL) and washed successively with saturated aqueous $KHSO_4$ solution (500 mL), saturated aqueous solution of $NaHCO_3$ (500 mL), brine (500 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (MeOH/EA, 1:20) to afford **33** (19.7 g, 70% over 2 steps) as an oil.

$[\alpha]_D^{25}$ +56.4 (c 0.78, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.64 – 7.33 (m, 11H), 6.89 (dd, *J* = 43.6, 35.6 Hz, 6H), 5.99 (dd, *J* = 14.7, 7.8 Hz, 2H), 5.29 (ddd, *J* = 55.6, 40.5, 27.3 Hz, 6H), 5.06 (d, *J* = 62.5 Hz, 2H), 4.70 – 4.22 (m, 9H), 4.23 – 3.83 (m, 4H), 3.13 (d, *J* = 5.4 Hz, 2H), 2.35 (s, 2H), 1.50 – 1.33 (m, 6H), 1.24 (dd, *J* = 20.0, 7.6 Hz, 6H), 1.12 (s, 9H), 1.05 – 0.83 (m, 11H), 0.64 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.89, 173.88, 172.80, 171.73, 170.54, 169.73, 169.04, 168.57, 167.58, 166.20, 164.45, 161.45, 157.77, 156.21, 150.34, 147.06, 135.51, 135.38, 133.10, 132.62, 132.07, 132.00, 131.97, 130.25, 129.81, 129.37, 128.57, 128.45, 127.81, 127.40, 125.60, 123.92, 121.86, 117.98, 117.63, 114.81, 114.50, 74.37, 68.76, 68.69, 65.10, 60.40, 55.51, 51.75, 48.31, 43.35, 42.30, 41.53, 38.96, 38.72, 35.87, 33.34, 31.87, 31.45, 31.38, 30.08, 29.46, 29.31, 28.32, 27.00, 26.79, 26.53, 25.22, 22.64, 20.26, 19.31, 19.04, 16.21, 15.24, 15.19, 14.08, 13.67, 13.31, 11.89, 11.59; HR-ESIMS m/z: calculated for $C_{71}H_{91}N_9O_{13}SSiNa^+$[M+Na]$^+$: 1360.6119, found 1360.6120.

Methyl (4-(allyloxy)benzoyl)glycinate (36')



(a) $SOCl_2$, MeOH, reflux; (b) **003**, EDCI, HOBt, DIPEA, DCM, rt, overnight. (c) NaOH, THF/MeOH/H_2O, rt

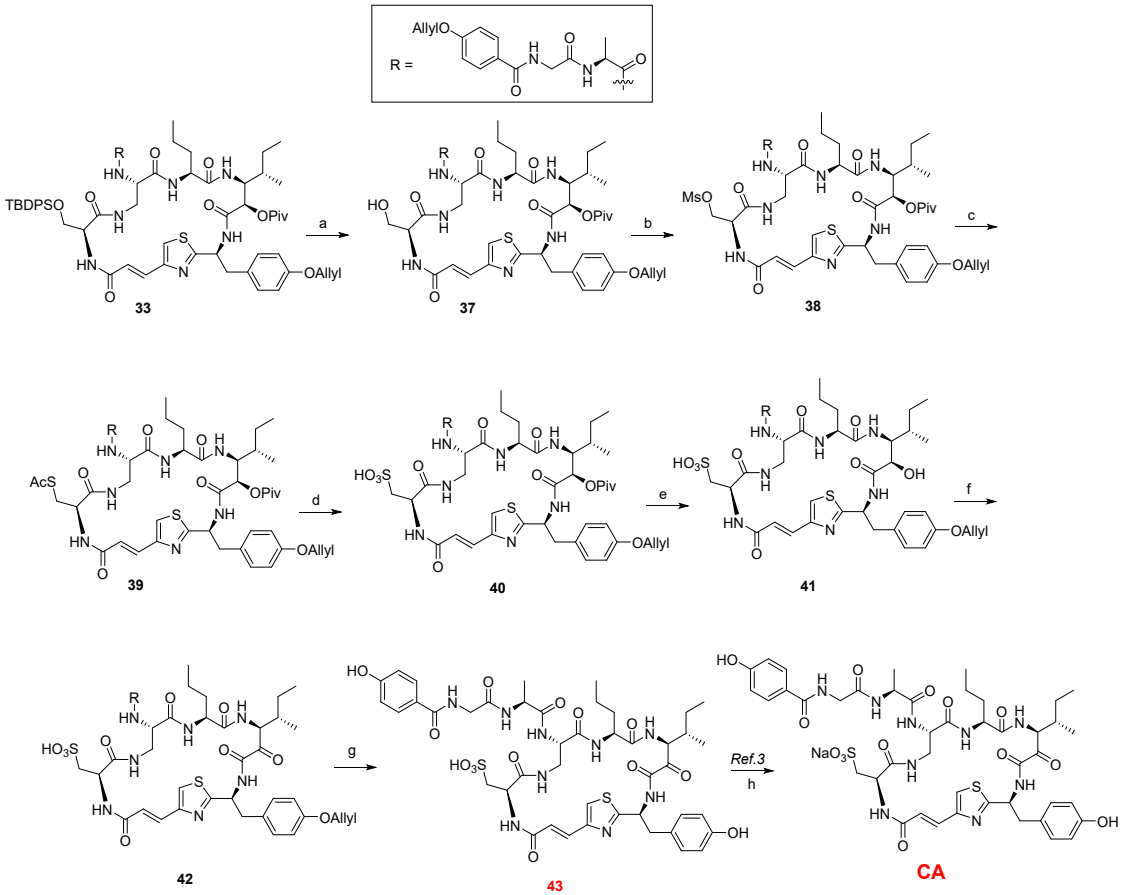
$SOCl_2$ (29 mL, 400 mmol) was added dropwise to a solution of glycine **001** (15 g, 200 mmol) in MeOH (400 mL) at 0 °C. Then the resultant mixture was heated to reflux and stirred for 2 hours. The solution was concentrated in vacuo to give **002** in quantitative yield which was used for next step directly.

To a solution of the above compound **002** in dry DCM (600 mL) was added 4-(allyloxy)benzoic acid **003** (35.7 g, 200 mmol), EDCI (48 g, 250 mmol) and HOBt (2.7 g, 20 mmol) at room temperature. After DIPEA (83 mL, 500 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature and stirred overnight at N_2 atmosphere. The solution was diluted with DCM (2000 mL) and washed successively with saturated aqueous $KHSO_4$ solution (800 mL), saturated aqueous solution of $NaHCO_3$ (800 mL), brine (500 mL). The organic phase was then dried over sodium sulfate (anhydrous) and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (EA/PE, 1:1) to afford **36'** (37.4 g, 75% over 2 steps) as an oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.69 (m, 2H), 6.99 – 6.84 (m, 2H), 6.65 (s, 1H), 6.14 – 5.94 (m, 1H), 5.46 – 5.36 (m, 1H), 5.34 – 5.26 (m, 1H), 4.57 (dd, *J* = 5.2, 1.2 Hz, 2H), 4.31 – 4.11 (m, 2H), 3.78

(s 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.67, 166.93, 161.35, 132.57, 128.88, 126.00, 118.02, 114.45 , 68.79, 52.35, 41.64; HR-ESIMS m/z: calculated for $C_{13}H_{15}NO_4Na^+$[M+Na]$^+$: 272.0893, found 272.0895.

NaOH (30.0 g, 750 mmol) was added to a solution of the above compound **36'** (37.4 g, 150 mmol) in THF/MeOH/H_2O (1:1:1, 450 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature and stirred for 3h. Volatiles of the reaction mixture were removed in vacuo. The solution was diluted with water (600 mL) and adjusted to pH 2 by addition of $KHSO_4$. The aqueous phase was extracted with ethyl acetate (3×800 mL). The combined organic phase was washed by brine (500 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **36** as a white solid in quantitative yield, which was used for next step directly.

Sodium ((12Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-butyl)-12-((S)-2-(2-(4-hydroxybenzamido)acetamido)propanamido)-2-(4-hydroxybenzyl)-4,5,8,11,15,18-hexaoxo-9-propyl-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-16-yl)methanesulfonate (CA)



(a) NH_4F, MeOH, reflux, 2h; (b) MsCl, Et_3N, DCM, rt, 2h; (c) KSAc, DMF, rt, 2h; (d) Oxone, AcOH, AcOK, 60 °C, 14h, 60% yield over 4 steps; (e) Et_3N/H_2O/MeOH (1:1:10), reflux, 2d; (f) IBX, MeCN, reflux, 2h; (g) SeO_2, HOAc, dioxane, reflux, 2h, 55% yield over 3 steps. (h) NaCl, MeOH/H_2O, rt.

To a solution of **33** (19.7 g, 14.7 mmol) in MeOH (400 mL) was added NH_4F (16.3 g, 441 mmol) at room temperature. Then the resultant mixture was stirred and heated to reflux for **6h**. The solution was concentrated in vacuo and the residue was diluted with water (500 mL). The aqueous phase was extracted with ethyl acetate (3×600 mL). The combined organic phase was washed by saturated aqueous solution of $NaHCO_3$ (400 mL), brine (400 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the compound **37** as an oil, which was used for next step directly.

To a solution of the above compound **37** in dry DCM (400 mL) was added Et_3N (8.4 mL, 60 mmol) and MsCl (2.4 mL, 30 mmol) at 0 °C. After 30 min, the resultant mixture was allowed to warm to room temperature and stirred for 2h at N_2 atmosphere. The reaction was then quenched with saturated aqueous $NaHCO_3$ solution (500 mL) and extracted with DCM (3×600 mL). The combined organic layer was washed successively with saturated aqueous $KHSO_4$ solution (500 mL), saturated aqueous solution of $NaHCO_3$ (500 mL), brine (500 mL), and dried over anhydrous Na_2SO_4, filtered, concentrated in vacuo to give compound **38** as an oil, which was pure enough and could be used for next step directly.

To a solution of the above compound **38** in dry DMF (60 mL) was added KSAc (8.4 g, 73.5 mmol) at room temperature. The resultant mixture was stirred for 2h, then quenched with saturated aqueous $NaHCO_3$ solution (800 mL) and extracted with EtOAc (3×600 mL). The combined organic layer was washed with brine (2×500 mL), and dried over anhydrous Na_2SO_4, filtered, concentrated in vacuo to give thioacetate **39** as an oil, which was used for next step directly.

To a solution of the above thioacetate **39** in AcOH (400 mL) was added KOAc (180 g) and Oxone (185 g, 300 mmol). The reaction mixture was heated to 60 °C and stirred for 14h at N_2 atmosphere. Then the reaction was quenched with water (1500 mL) and adjusted to pH 8 by addition of NaOH and $NaHCO_3$ at 0 °C. The aqueous phase was extracted with EtOAc (3×1000 mL). The combined organic phase was washed by brine (500 mL), dried over sodium sulfate (anhydrous) and concentrated in vacuo. The residue was purified by silica gel column chromatography (MeOH/EA, 1:5) to afford the sulfonic acid **40** (10.3 g, 60% yield over 4 steps).

$[\alpha]_D^{25}$ +62.5 (c 0.4, MeOH); ^{1}H NMR (400 MHz, MeOD) δ 8.62 (s, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 7.62 – 7.50 (m, 2H), 7.35 (d, *J* = 15.1 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 6.90 (s, 3H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.11 – 5.87 (m, 2H), 5.59 (t, *J* = 7.2 Hz, 1H), 5.37 (ddd, *J* = 17.3, 9.3, 1.6 Hz, 2H),

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[3] Y. Cui, M. Zhang, H. Xu, T. Zhang, S. Zhang, X. Zhao, P. Jiang, J. Li, B. Ye, Y. Sun, M. Wang, Y. Deng, Q. Meng, Y. Liu, Q. Fu, J. Lin, L. Wang, and Y. Chen, *J. Med. Chem.*, **2022**, 65, 2971-2987.

5.27 – 5.11 (m, 3H), 5.00 (d, *J* = 11.2 Hz, 1H), 4.57 (d, *J* = 5.1 Hz, 3H), 4.47 (d, *J* = 5.1 Hz, 2H), 4.42 (dd, *J* = 10.6, 3.7 Hz, 2H), 4.04 (s, 2H), 4.00 – 3.93 (m, 1H), 3.72 (d, *J* = 12.1 Hz, 1H), 3.10 (t, *J* = 10.1 Hz, 3H), 2.63 (s, 1H), 2.41 (s, 1H), 1.67 (s, 1H), 1.54 (s, 2H), 1.32 (d, *J* = 6.9 Hz, 3H), 1.25 (d, *J* = 5.5 Hz, 2H), 1.22 (s, 9H), 0.92 (s, 3H), 0.72 (t, *J* = 7.0 Hz, 3H), 0.60 (d, *J* = 5.8 Hz, 3H); ^{13}C NMR (100 MHz, MeOD) δ 178.91, 176.14, 174.06, 173.09, 172.81, 172.36, 171.67, 171.53, 169.86, 167.89, 162.92, 159.07, 152.75, 151.52, 139.00, 134.83, 134.34, 134.28, 131.31, 130.40, 129.43, 126.96, 126.06, 124.66, 123.88, 117.87, 117.45, 115.82, 115.44, 75.52, 69.77, 69.68, 56.26, 55.89, 53.46, 52.70, 51.76, 51.09, 50.03, 44.25, 42.79, 42.09, 39.81, 37.62, 35.30, 33.79, 30.68, 27.68, 23.49, 21.37, 20.65, 18.49, 17.27, 13.91, 12.30; HR-ESIMS m/z: calculated for $C_{55}H_{73}N_9O_{15}S_2^-$ [M-H]$^-$: 1162.4595, found 1162.4598.

The above sulfonic acid **40** was dissolved in MeOH (400 mL), then Et_3N (50 mL) and H_2O (50 mL) were added. Then the resultant mixture was stirred and heated to reflux for 2 days. The solution was concentrated in vacuo and the residue was co-evaporated with MeCN (2×500 mL) to provide the desired compound **41** as an oil, which was used for next step directly.

To a solution of the above compound **41** in dry MeCN (400 mL) was added IBX (12.3 g, 44 mmol) at room temperature. After 15 min, the resultant mixture was heated to reflux and stirred for 2h. The solution was cooled to room temperature and the solid was removed by filtration through a pad of celite and washed with MeCN (300 mL). The total filtrate was concentrated in vacuo to afford the oxo compound **42**, which was used for next step directly.

To a solution of the above oxo compound **42** in 1, 4-dioxane (400 mL) was added HOAc (8.4 mL, 147 mmol) and SeO_2 (8.2 g, 73.5 mmol) at room temperature. After 15 min, the resultant mixture was heated to reflux and stirred for 2h. The solution was concentrated in vacuo and the residue was purified by silica gel column chromatography (MeOH/EA, 1:5) to afford the sulfonic acid **43** (4.8 g, 55% yield over 3 steps).

$[\alpha]_D^{25}$ -21.0 (c 0.1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (d, *J* = 7.9 Hz, 1H), 8.62 (d, *J* = 7.0 Hz, 1H), 8.47 (t, *J* = 5.7 Hz, 1H), 8.33 (d, *J* = 9.4 Hz, 1H), 8.17 (d, *J* = 9.3 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.87 (s, 2H), 7.74 (d, *J* = 8.7 Hz, 2H), 7.41 (d, *J* = 15.1 Hz, 1H), 7.19 (s, 2H), 6.78 (t, *J* = 11.5 Hz, 3H), 6.70 (d, *J* = 8.4 Hz, 2H), 5.22 (dd, *J* = 15.5, 8.2 Hz, 1H), 5.05 (dd, *J* = 9.4, 3.4 Hz, 1H), 4.75 (m, 1H), 4.34 (m, 2H), 4.03 (m, 1H), 3.92 – 3.86 (m, 1H), 3.85 – 3.76 (m, 2H), 3.27 (m, 1H), 3.14 – 3.01 (m, 2H), 2.76 – 2.68 (m, 1H), 2.41 (m, 1H), 2.31 (m, 1H), 1.65 (m, 1H), 1.56 (m, 1H), 1.34 (m, 1H), 1.17 (d, *J* = 7.0 Hz, 3H), 1.15 (m, 1H), 1.07 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H), 0.78 (d, *J* = 6.9 Hz, 3H), 0.73 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.96,

174.74, 171.31, 170.58, 168.72, 166.18, 164.81, 163.71, 160.22, 156.15, 149.52, 132.48, 130.42, 129.28, 127.40, 124.74, 123.38, 123.12, 115.12, 114.80, 60.05, 54.43, 50.92, 49.32, 47.74, 40.15, 39.10, 36.92, 32.12, 23.25, 19.68, 18.40, 16.12, 13.79, 11.78; HR-ESIMS m/z: calculated for $C_{44}H_{55}N_9O_{14}S_2^-$ $[M-H]^-$: 996.3237, found 996.3239.

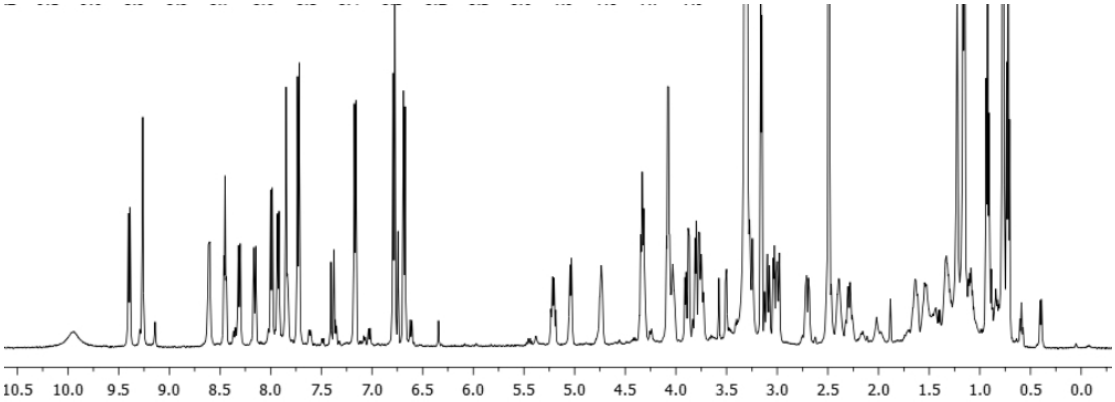
To a stirred solution of the above sulfonic acid **43** in MeOH/H_2O (1:1) (60 mL) at 0 °C was added NaCl (0.3 g, 5 mmol). After 1h, The solvent was evaporated carefully under reduced pressure. The crude residue was then purified by HPLC on a preparative RP-8 column with a mixture of 45:55 MeCN/H_2O to afford the pure product (**CA**) (4.5 g).

$[\alpha]_D^{25}$ -21.0 (c 0.1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (d, *J* = 8.0 Hz, 1H), 8.62 (d, *J* = 7.1 Hz, 1H), 8.44 (s, 1H), 8.33 (d, *J* = 9.4 Hz, 1H), 8.17 (d, *J* = 9.3 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.94 (d, *J* = 7.5 Hz, 1H), 7.86 (s, 2H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 15.1 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 2H), 6.78 (*J* = 8.0 Hz, 3H), 6.69 (d, *J* = 8.4 Hz, 2H), 5.22 (dd, *J* = 15.6, 8.2 Hz, 1H), 5.05 (dd, *J* = 9.4, 3.5 Hz, 1H), 4.74 (d, *J* = 8.9 Hz, 1H), 4.42 – 4.30 (m, 2H), 4.05 (s, 1H), 3.89 (dd, *J* = 14.0, 5.0 Hz, 1H), 3.84 – 3.72 (m, 2H), 3.16 – 2.99 (m, 3H), 2.76 – 2.67 (m, 1H), 2.39 (s, 1H), 2.30 (d, *J* = 12.8 Hz, 1H), 1.66 (s, 1H), 1.56 (s, 1H), 1.35 (s, 1H), 1.17 (d, *J* = 7.0 Hz, 3H), 1.15 – 1.03 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H), 0.83 – 0.70 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ197.92, 174.70, 171.28, 171.07, 170.56, 170.53, 168.71, 166.17, 164.78, 163.66, 160.55, 156.15, 149.48, 132.45, 131.42, 130.38, 129.23, 127.33, 124.95, 123.34, 123.07, 116.42, 115.09, 114.83, 62.46, 60.01, 55.65, 54.39, 54.29, 54.05, 50.88, 50.62, 50.41, 49.90, 49.27, 48.92, 47.70, 42.83, 42.48, 41.12, 40.75, 40.48, 36.89, 32.08, 23.21, 19.64, 18.36, 16.07, 13.74, 11.74; HR-ESIMS m/z: calculated for $C_{44}H_{54}N_9Na_2O_{14}S_2^+$ $[M+Na]^+$: 1042.3022, found 1042.3024.

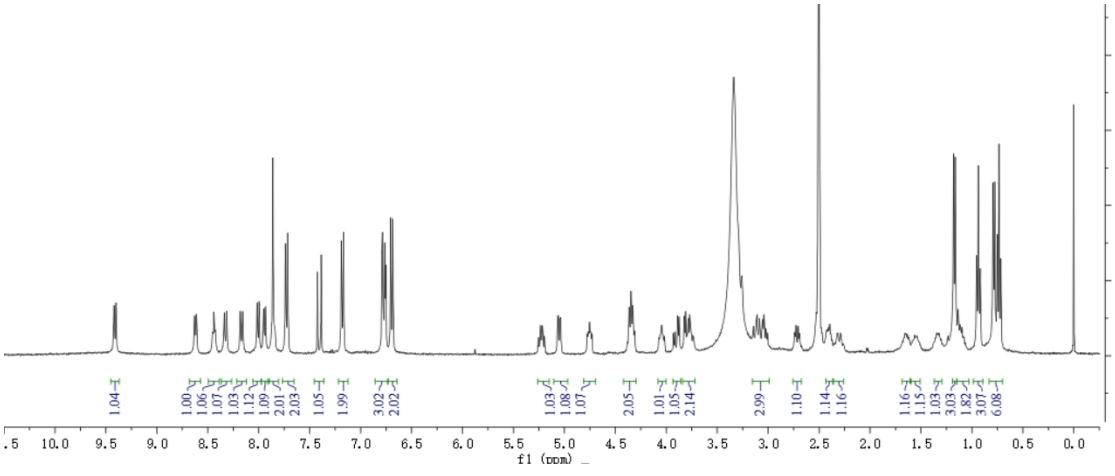
Comparison of Spectra of Our synthetic CA with Natural product CA

^{1}H NMR of CA

Carmeli's natural sample **CA** (Bruker AV 500, 500 MHz, DMSO-*d6*) (*J. Nat. Prod.*, **2017**, 80, 1110-1116)

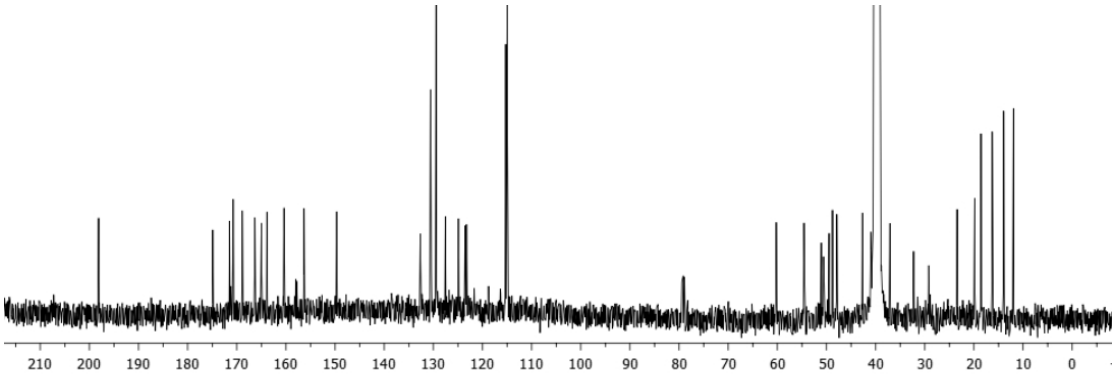


Our synthetic sample **CA** (Bruker AV 400, 400 MHz, DMSO-d_6) (this work)



^{13}C NMR of CA

Carmeli's natural sample **CA** (Bruker AV 400, 100 MHz, DMSO-*d6*) (*J. Nat. Prod.*, **2017**, 80, 1110-1116)



Our synthetic sample **CA** (Bruker AV 400, 100 MHz, DMSO-d_6) (this work)

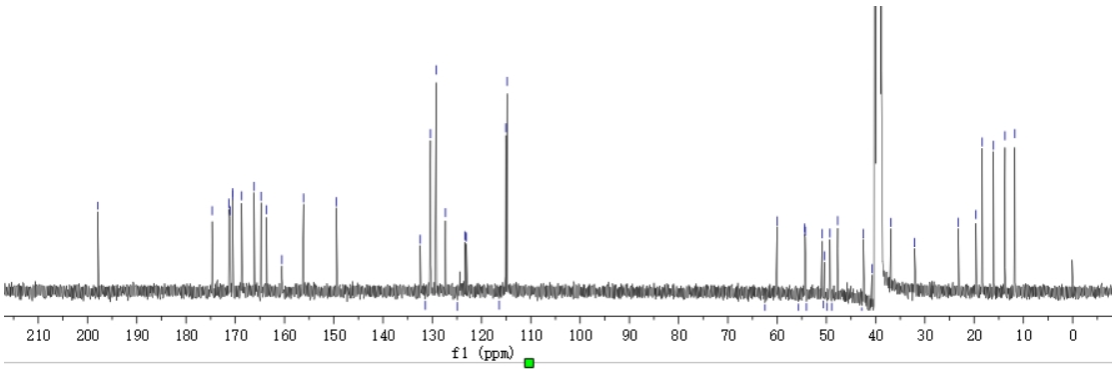
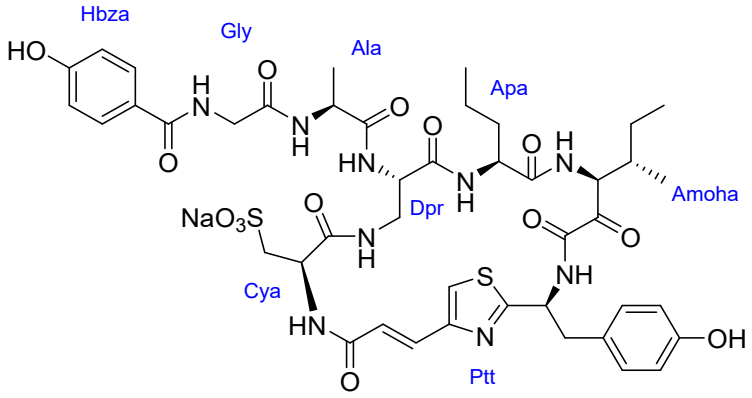


Table S1 ^{1}H NMR and ^{13}C NMR Spectroscopic Data of Our synthetic **CA** and Natural product **CA** [3] in DMSO-d_6

[3] M. Issac, M. Aknin, A. Gauvin-Bialecki, N. D. Voogd, A. Ledoux, M. Frederich, Y. Kashman, and S. Carmeli, *J. Nat. Prod.*, **2017**, 80, 1110-1116.



position	δH of Our synthetic **CA** (400 MHz)	δH of natural product **CA** (500 MHz)	δC of Our synthetic **CA** (100 MHz)	δC of natural product **CA** (100 MHz)
Hbza 1			166.17	166.3
2			124.95	124.9
3, 3'	7.73, d (*J* =8.0)	7.73, d (*J* =9.0)	129.23	129.4
4, 4'	6.78, d (*J* =8.0)	6.78, d (*J* =9.0)	115.09	115.0
5			160.55	160.4
5-OH	Our DMSO-d_6 contained an appropriate amount of H_2O, HDO and D_2O, which leaded to vanishing OH signals due to hydrogen–deuterium exchange. This H atom was very acidic and active.	9.96 brs		
Gly 1			168.71	168.9
2	3.79, dd (*J* =14.0, 5.0) 3.89, dd (*J* =14.0, 5.0)	3.79, dd (*J* =16.5, 5.5) 3.89, dd (*J* =16.5, 5.5)	42.83	42.7
NH	8.44, t (*J* =4.0)	8.46, t (*J* =5.5)		
Ala 1			171.28	171.5
2	4.35, m	4.33, m	47.70	47.9

3	1.17, d (*J* =8.0)	1.16, d (*J* =7.0)	18.36	18.6
NH	8.01, d (*J* =8.0)	8.00, d (*J* =7.5)		
Dpr 1			171.28	171.3
2	4.05, m	4.03, m	50.88	51.1
3	2.48, m 3.75, m	2.49, m 3.75, m	40.75	40.9
α-NH	7.94, d (*J* =8.0)	7.93, d (*J* =7.5)		
β-NH	7.86, brs	7.85, brs		
Apa 1			174.70	174.9
2	4.35, m	4.32, m	54.39	54.5
3	1.63, m 2.29, m	1.63, m 2.29, m	32.08	32.3
4	1.35, m 1.56, m	1.31, m 1.53, m	19.64	19.8
5	0.94, t (*J* =8.0)	0.92, t (*J* =7.0)	13.74	13.9
NH	8.62, d (*J* =8.0)	8.61, d (*J* =7.0)		
Amoha 1			163.66	163.9
2			197.92	198.1
3	5.05, dd (*J* =9.0, 3.6)	5.04, dd (*J* =9.5, 3.5)	60.01	60.2
4	2.39, m	2.39, m	36.89	37.1
5	0.78, d (*J* =6.8)	0.77, d (*J* =7.0)	16.07	16.3
6	1.10, m 1.15, m	1.08, m 1.14, m	23.21	23.4
7	0.73, t (*J* =8.0)	0.72, t (*J* =7.5)	11.74	11.9
NH	8.32, d (*J* =12.0)	8.31, d (*J* =9.5)		
Ptt 1			164.78	164.9
2	6.78, d (*J* =14.0)	6.76, d (*J* =15.0)	123.34	123.6
3	7.40, d (*J* =14.0)	7.39, d (*J* =15.0)	132.45	132.6
4			149.48	149.7
5	7.86, s	7.85, s	123.34	123.3

6			170.56	170.7
7	5.23, m	5.21, m	54.39	54.6
8	3.03, dd (*J* =13.5, 5.0) 3.12, dd (*J* =13.5, 5.0)	3.02, dd (*J* =14.0, 5.5) 3.10, dd (*J* =14.0, 4.0)	39.28	39.2
9			127.33	127.6
10， 10’	7.18, d (*J* =8.0)	7.17, d (*J* =8.5)	130.38	130.6
11， 11’	6.69, d (*J* =8.0)	6.68, d (*J* =8.5)	115.09	115.3
12			156.15	156.3
12-OH	Our DMSO-d_6 contained an appropriate amount of H_2O, HDO and D_2O, which leaded to vanishing OH signals due to hydrogen–deuterium exchange. This H atom was very acidic and active.	9.27, s		
NH	9.41, d (*J* =8.0)	9.40, d (*J* =8.0)		
Cya 1			170.56	170.7
2	4.74, m	4.74, m	49.27	49.5,
3	2.71, dd (*J* =13.5, 5.5) 3.25, m	2.70, dd (*J* =14.0, 6.0) 3.25, m	50.62	50.6
NH	8.17, d (*J* =8.0)	8.16, d (*J* =9.0)		

Enzyme Inhibition Assays

Methods

(1) TMPRSS2 activity assay

According to the instructions provided with TMPRSS2 Fluorogenic assay kit (BPS Bioscience, 78083), in each well of black 96-well plates, 10 μL TMPRSS2 enzyme (150 ng/reaction) was mixed with 30 μL tested compounds (**CA** or positive control camostat) at room temperature for 30 min, 10 μL substrate (final concentration: 10 μM) was then added. Kinetic fluorescence measurements (excitation: 383 nm； emission: 455 nm) were monitored for up to 30 min.

(2) Neutrophil Elastase activity assay

According to the instructions provided with Fluorometric Neutrophil Elastase Inhibitor Screening Kit (Abcam, ab118971). 50 μL assay buffer containing neutrophil elastase was mixed with 25 μL tested compounds (**CA** or positive control SPCK) in black 96-well plates and incubated at 37℃ for 5 min, 25 μL substrate was then pipetted into each well. Fluorescence changes were monitored kinetically at 37 ℃ for 30 min (excitation: 400 nm； emission: 505 nm).

Results

As shown in Fig.1, **CA** effectively inhibited the enzyme activity of TMPRSS2 (IC_{50}, 19.17 μM). More encouragingly, **CA** fully inhibited neutrophil elastase activity with an IC_{50} value of 1.00 nM (Fig. 2).

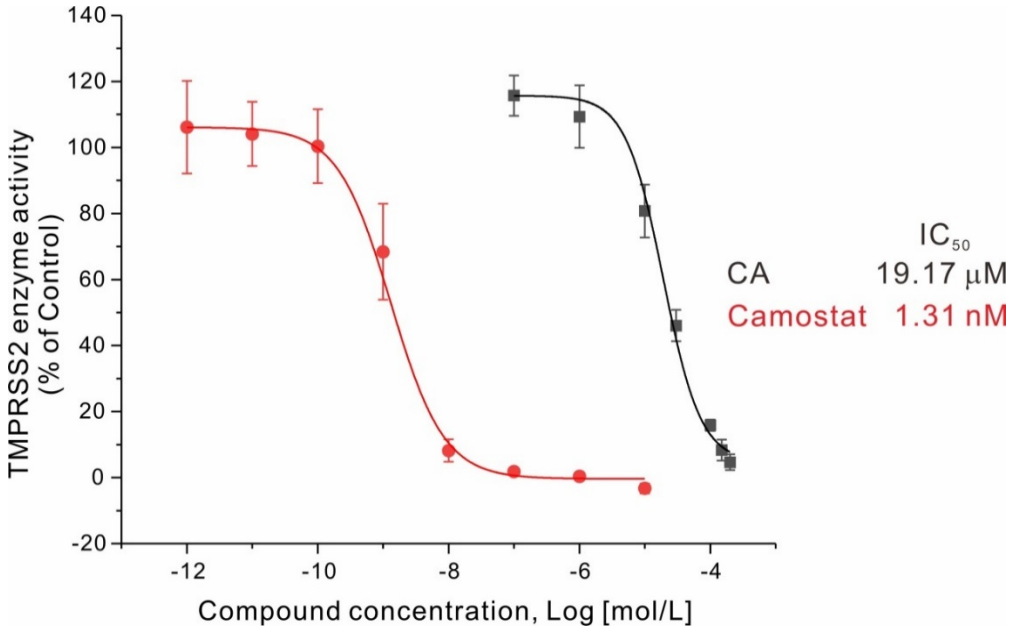


Fig. 1 CA effectively inhibited TMPRSS2 enzymatic activity.

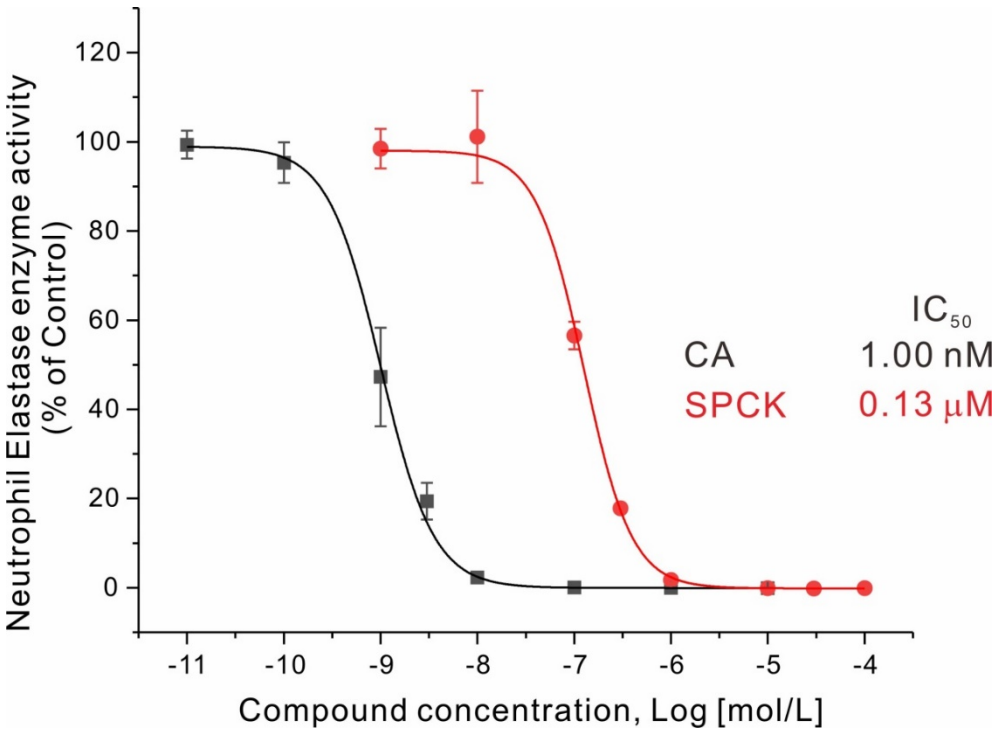


Fig. 2 CA fully inhibited neutrophil elastase enzymatic activity.

NMR Spectra of New Compounds and Selected Known Compounds

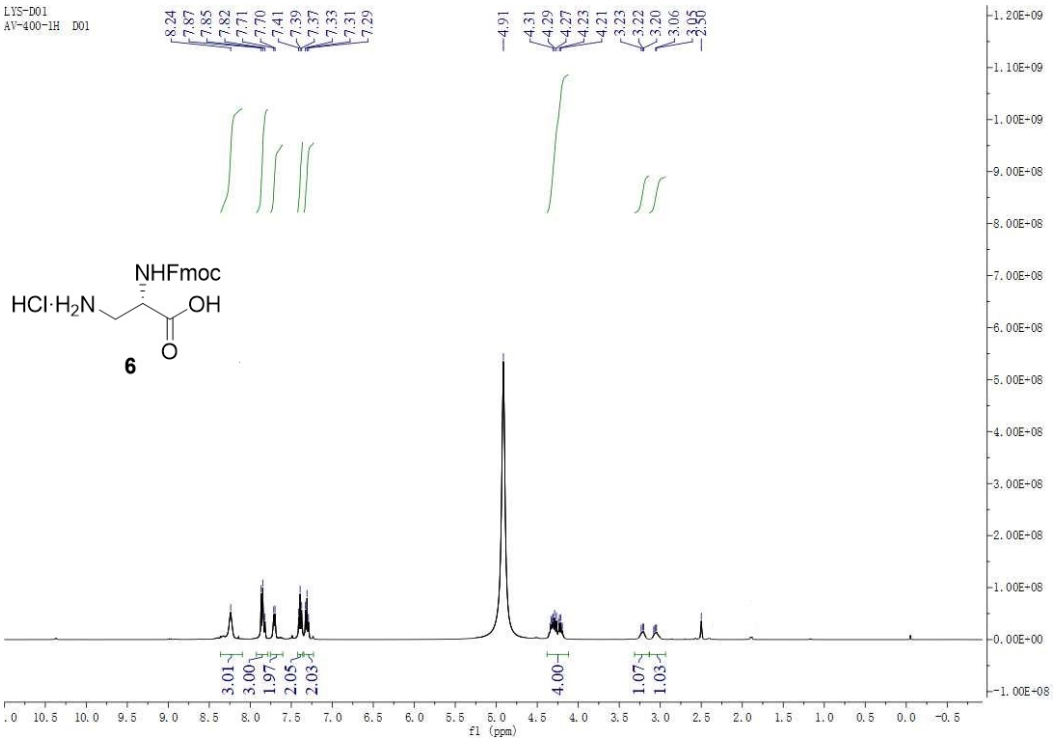


Figure S1. ^{1}H NMR of **6** (DMSO-d_6, 400 MHz)

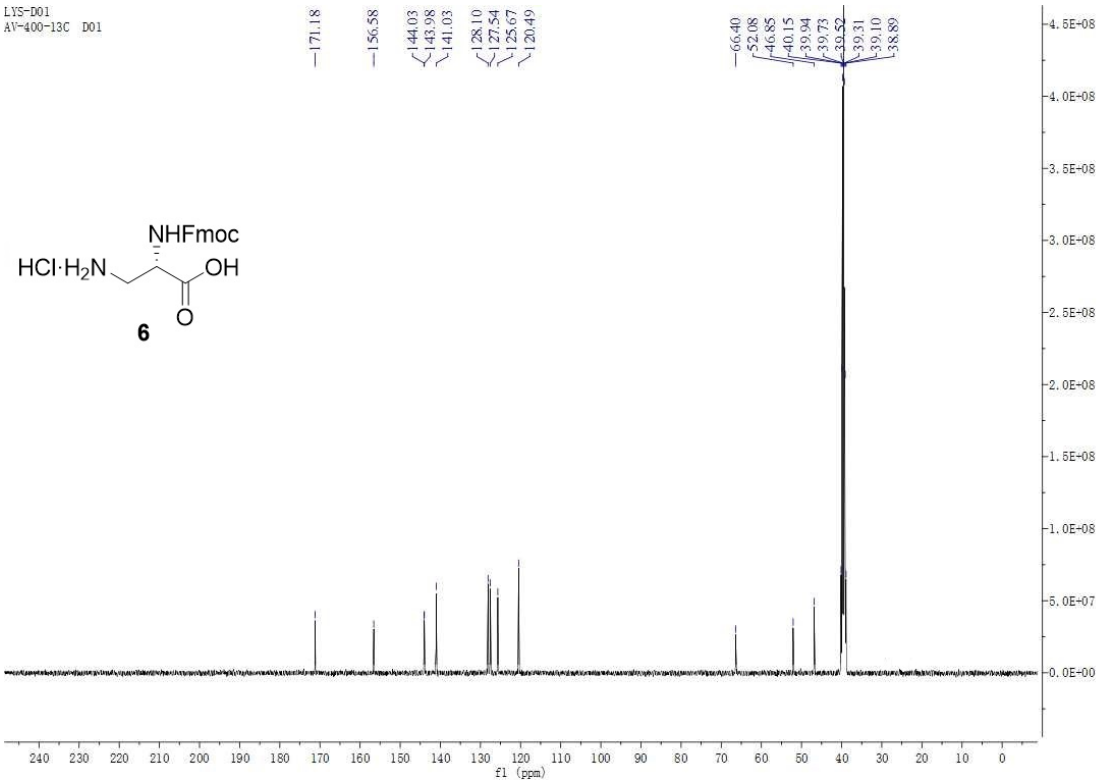


Figure S2. ^{13}C NMR of **6** (DMSO-d_6, 100 MHz)

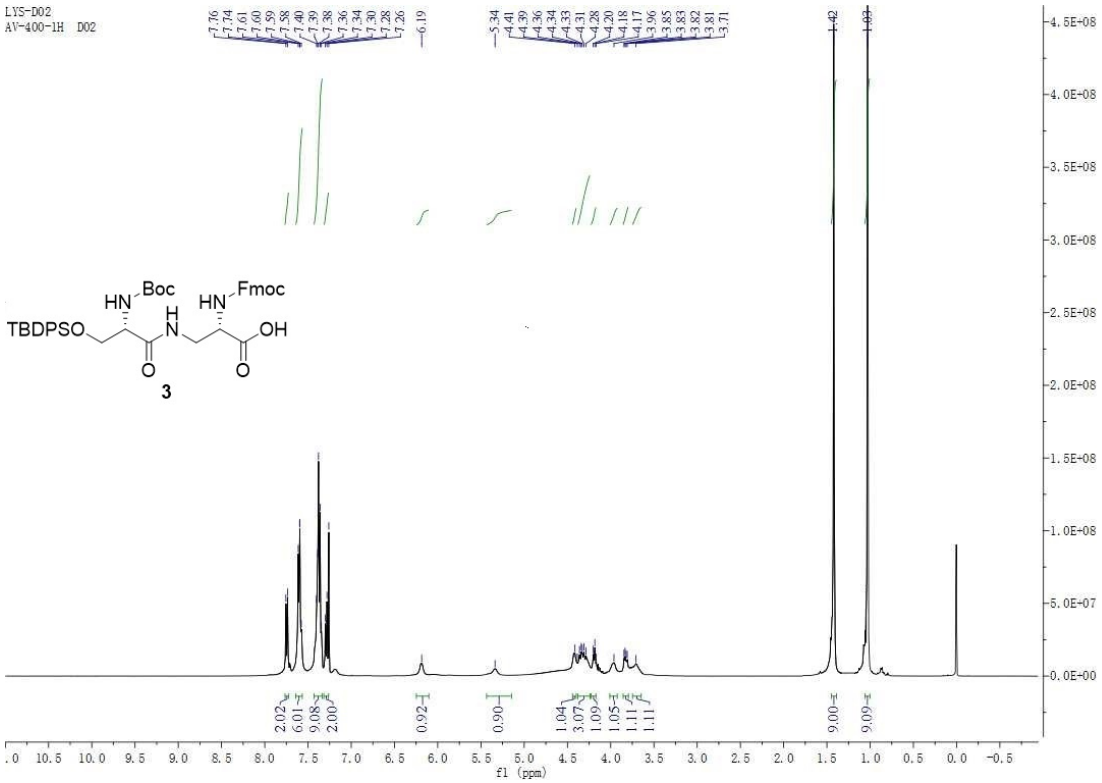


Figure S3. ^{1}H NMR of **3** ($CDCl_3$, 400 MHz)

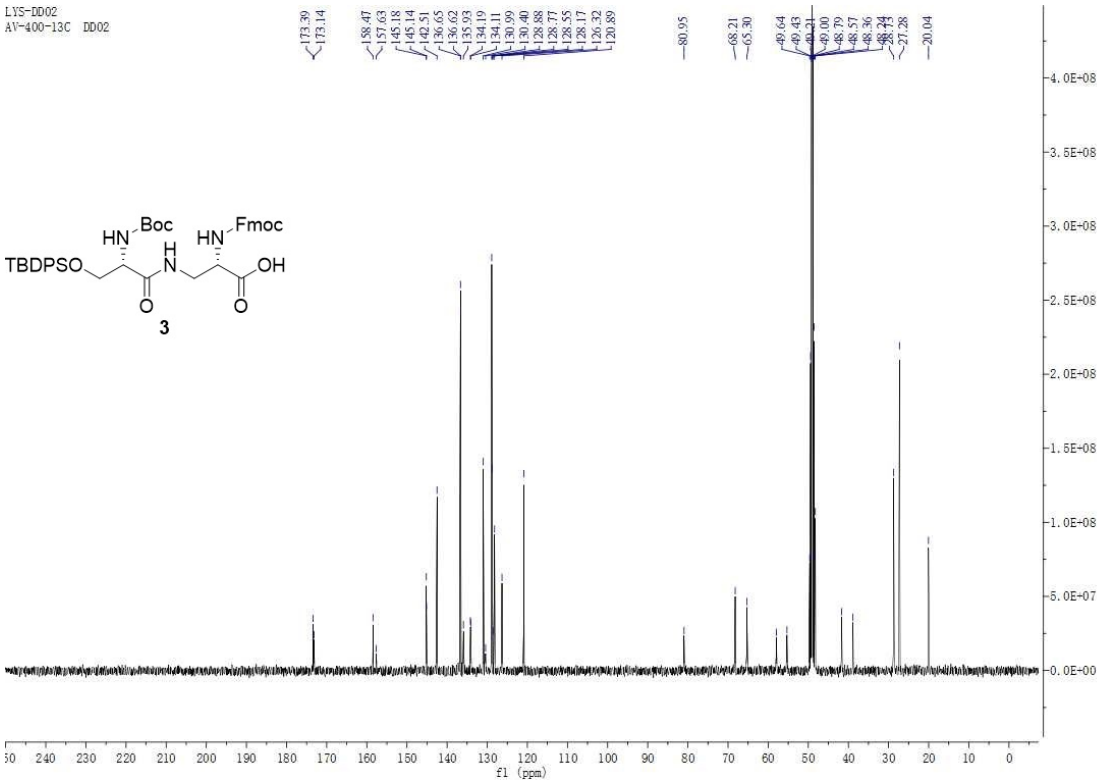


Figure S4. ^{13}C NMR of **3** (MeOD, 100 MHz)

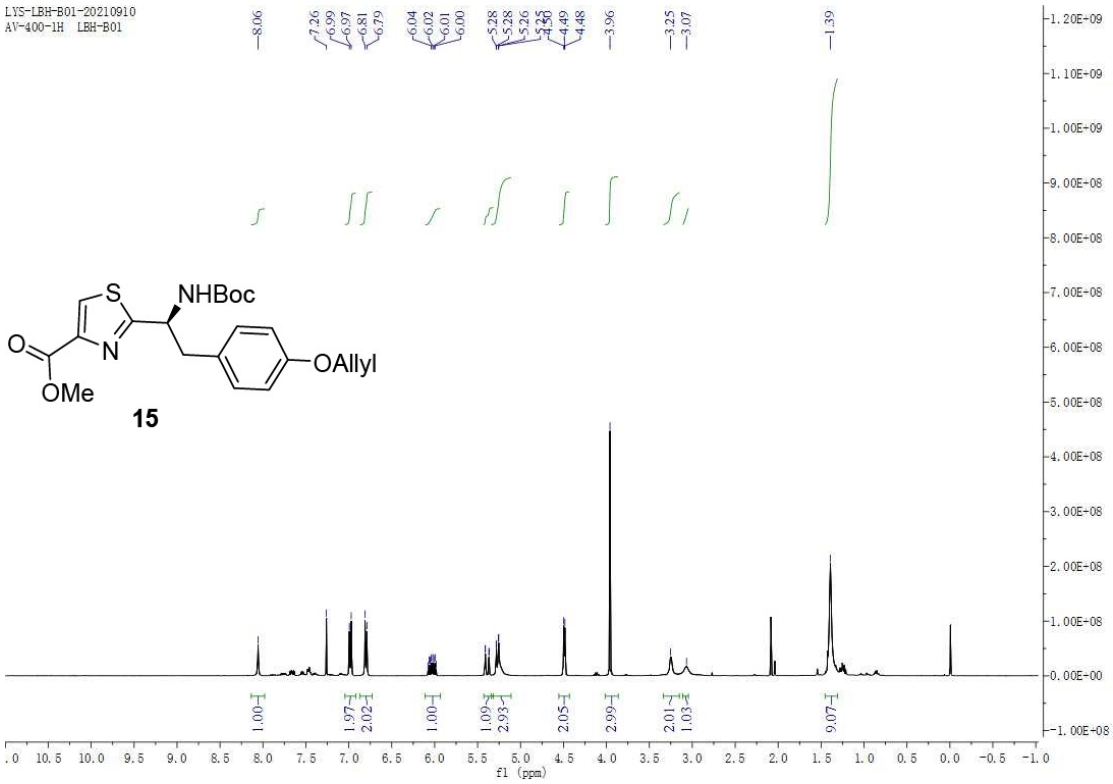


Figure S5. ^{1}H NMR of **15** ($CDCl_3$, 400 MHz)

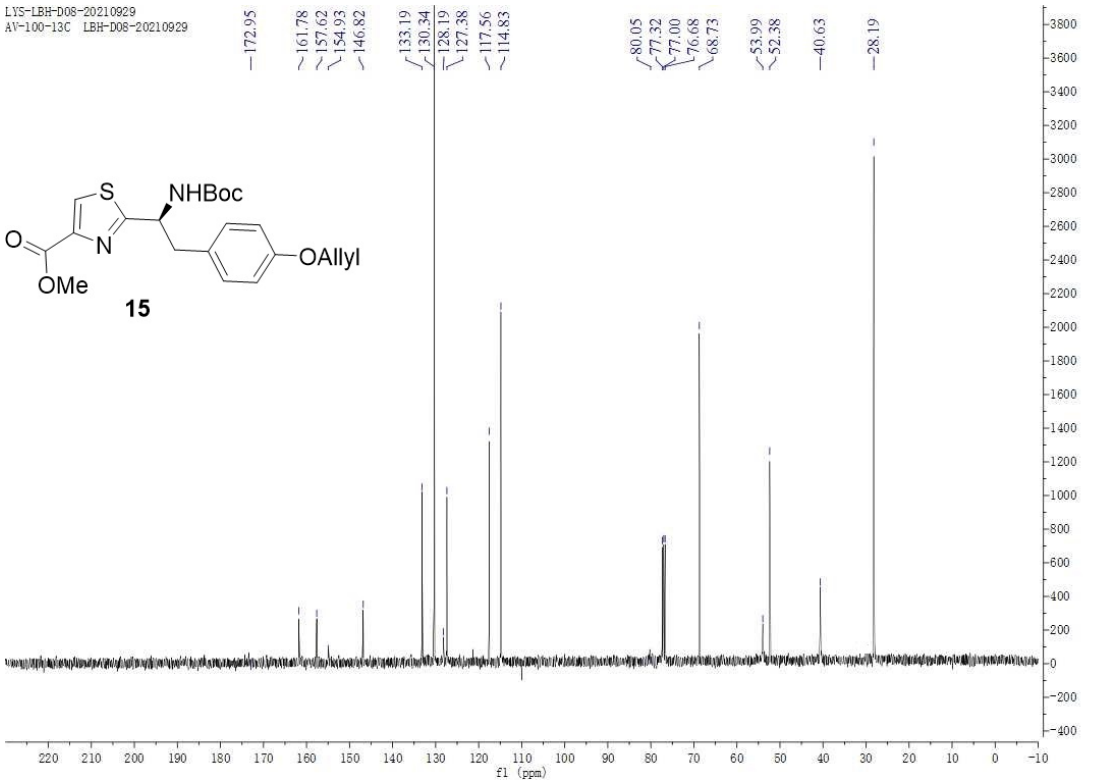


Figure S6. ^{13}C NMR of **15** ($CDCl_3$, 100 MHz)

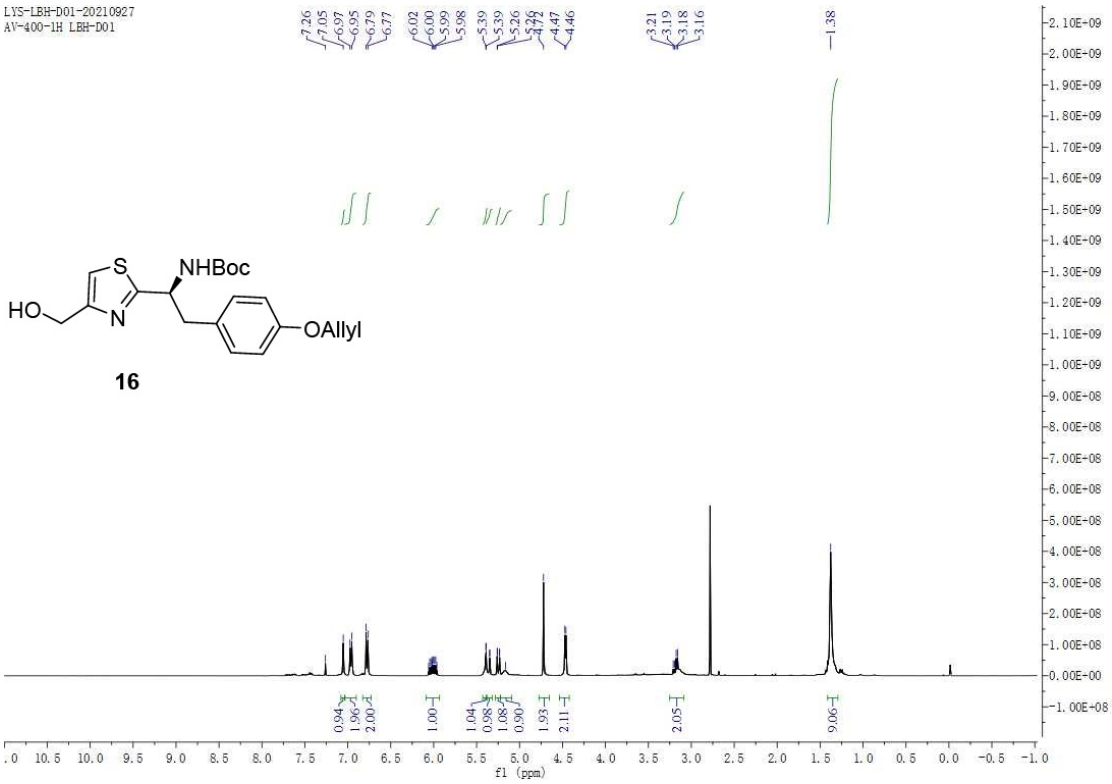


Figure S7. ^{1}H NMR of **16** ($CDCl_3$, 400 MHz)

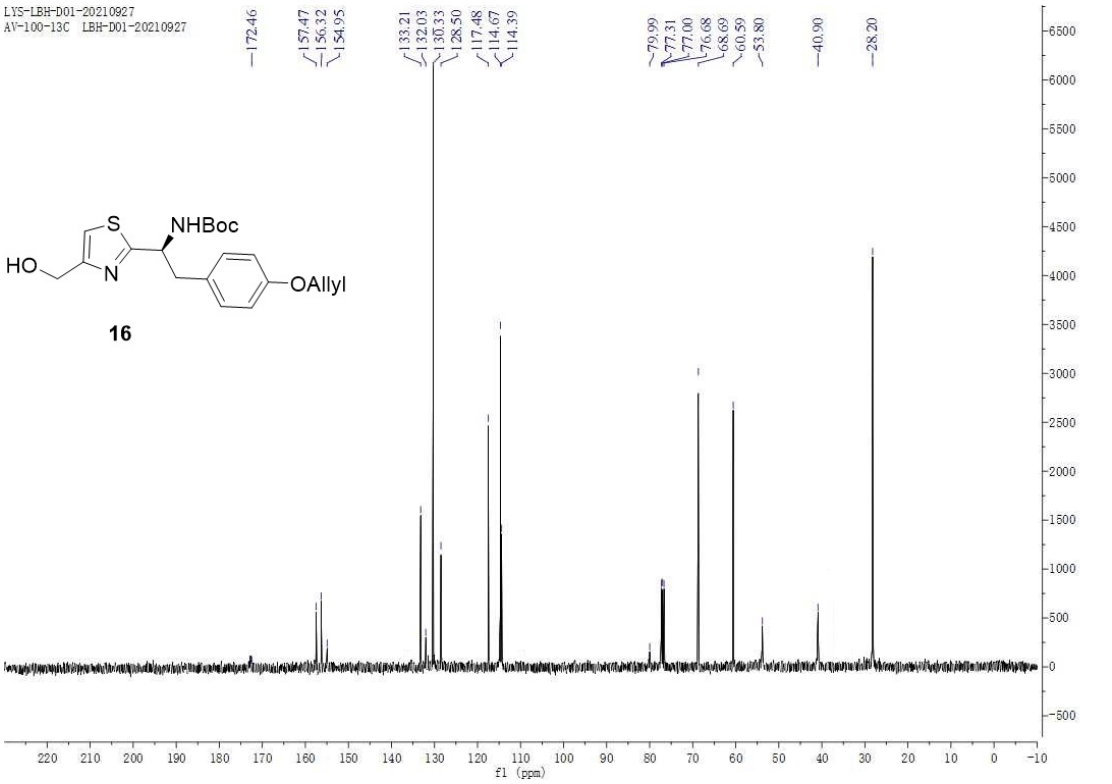


Figure S8. ^{13}C NMR of **16** ($CDCl_3$, 100 MHz)

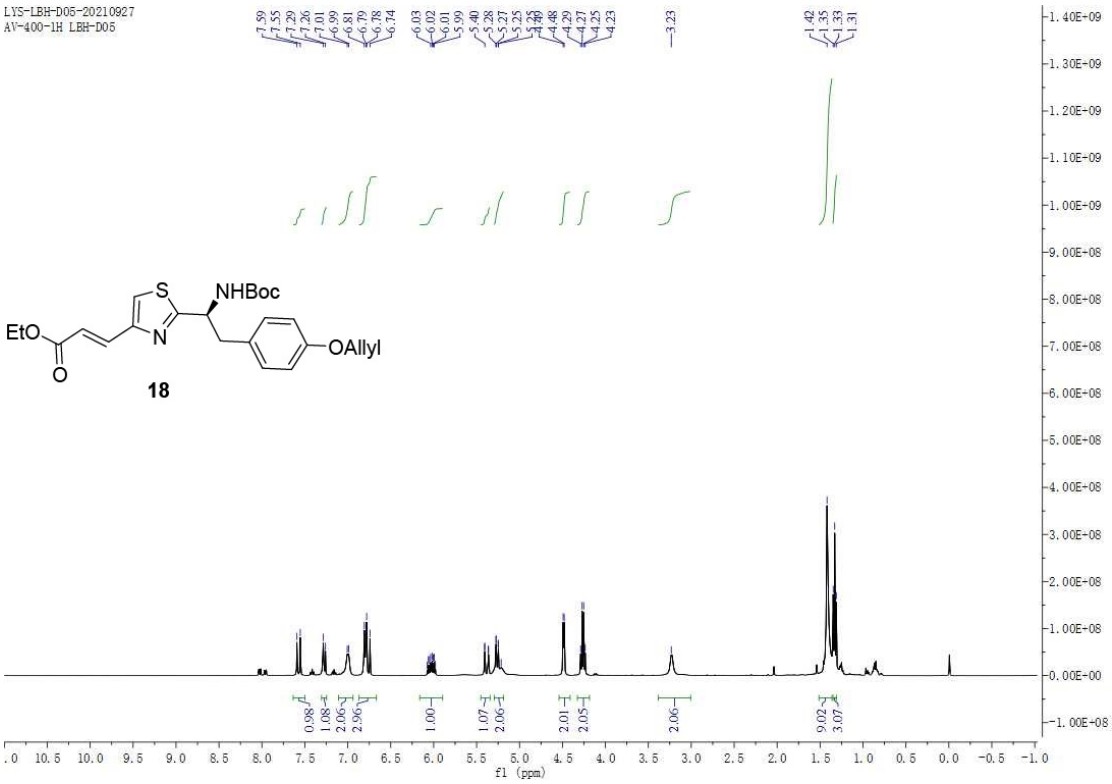


Figure S9. ^{1}H NMR of **18** ($CDCl_3$, 400 MHz)

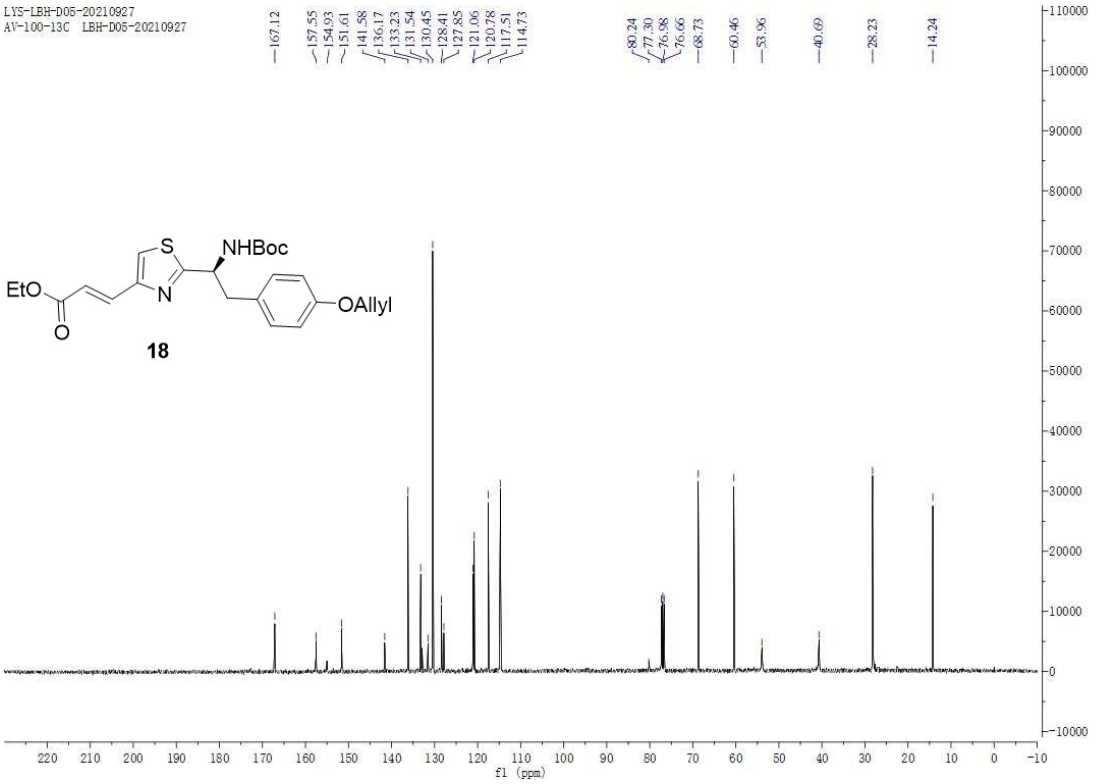


Figure S10. ^{13}C NMR of **18** ($CDCl_3$, 100 MHz)

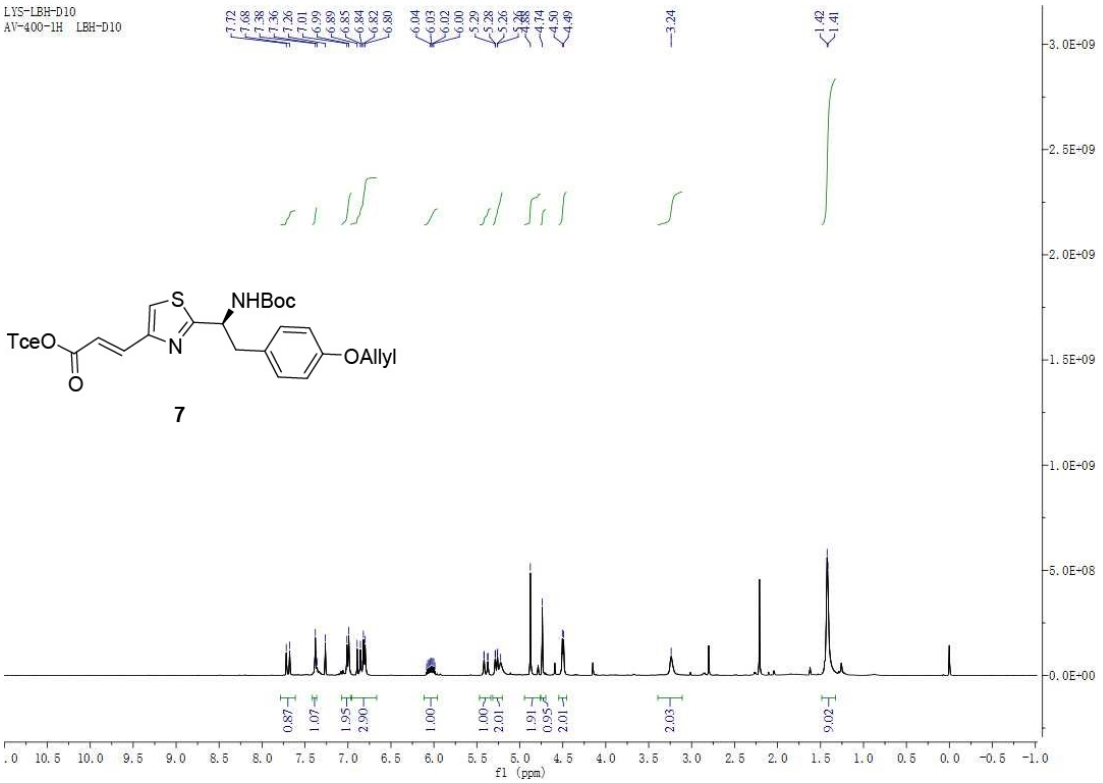


Figure S11. ^{1}H NMR of **7** ($CDCl_3$, 400 MHz)

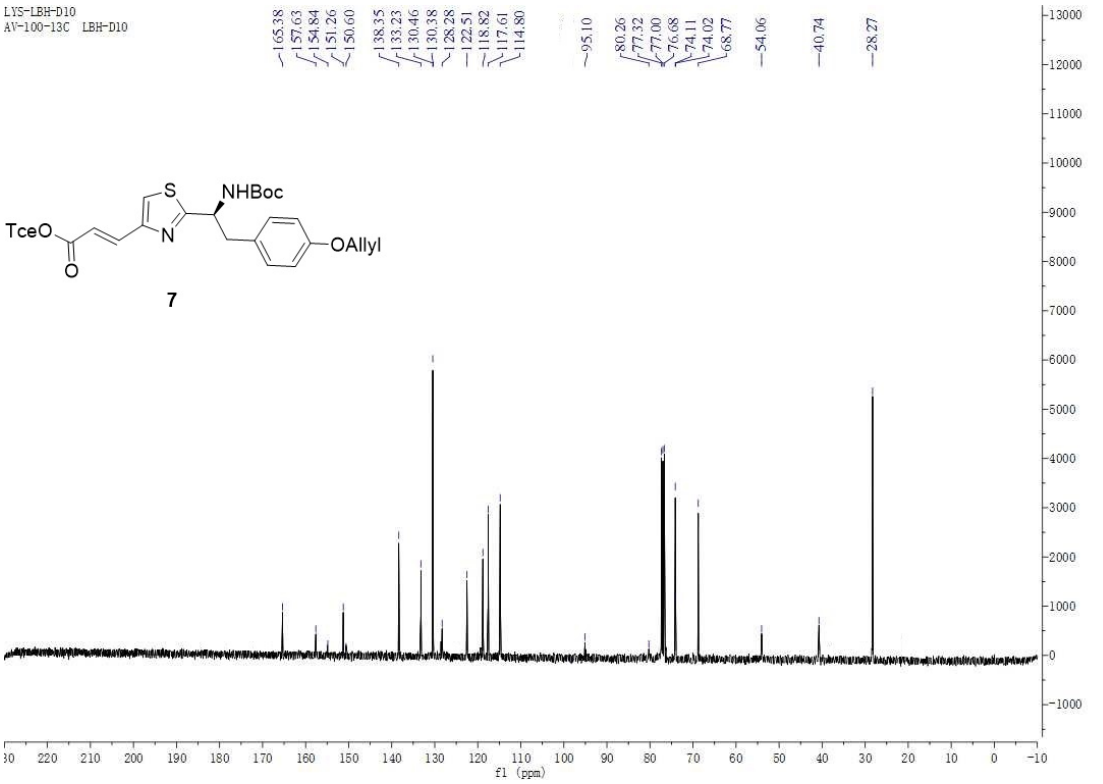


Figure S12. ^{13}C NMR of **7** ($CDCl_3$, 100 MHz)

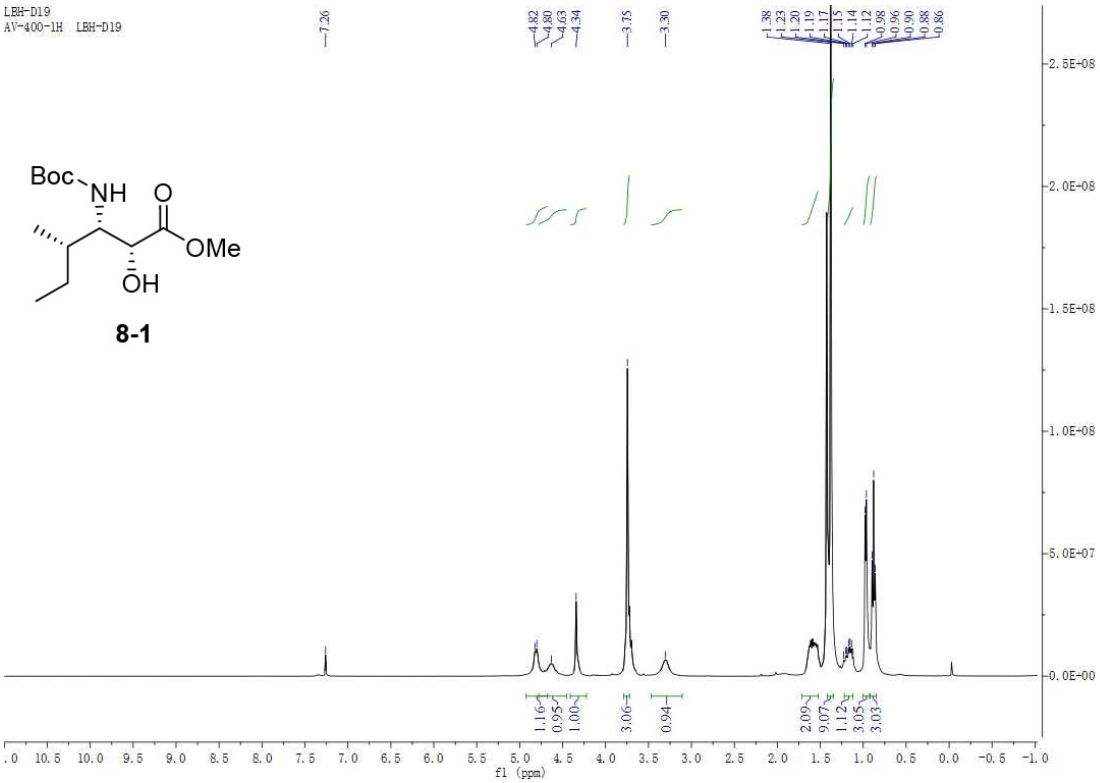


Figure S13. ^{1}H NMR of **8-1** ($CDCl_3$, 400 MHz)

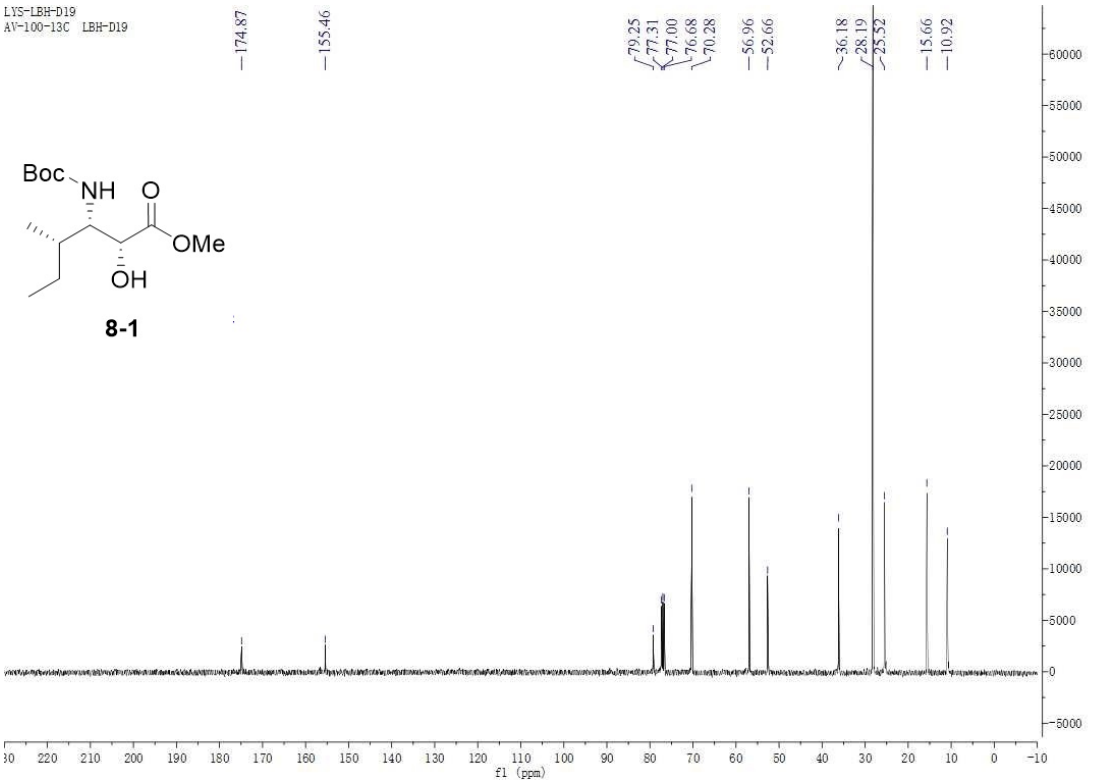


Figure S14. ^{13}C NMR of **8-1** ($CDCl_3$, 100 MHz)

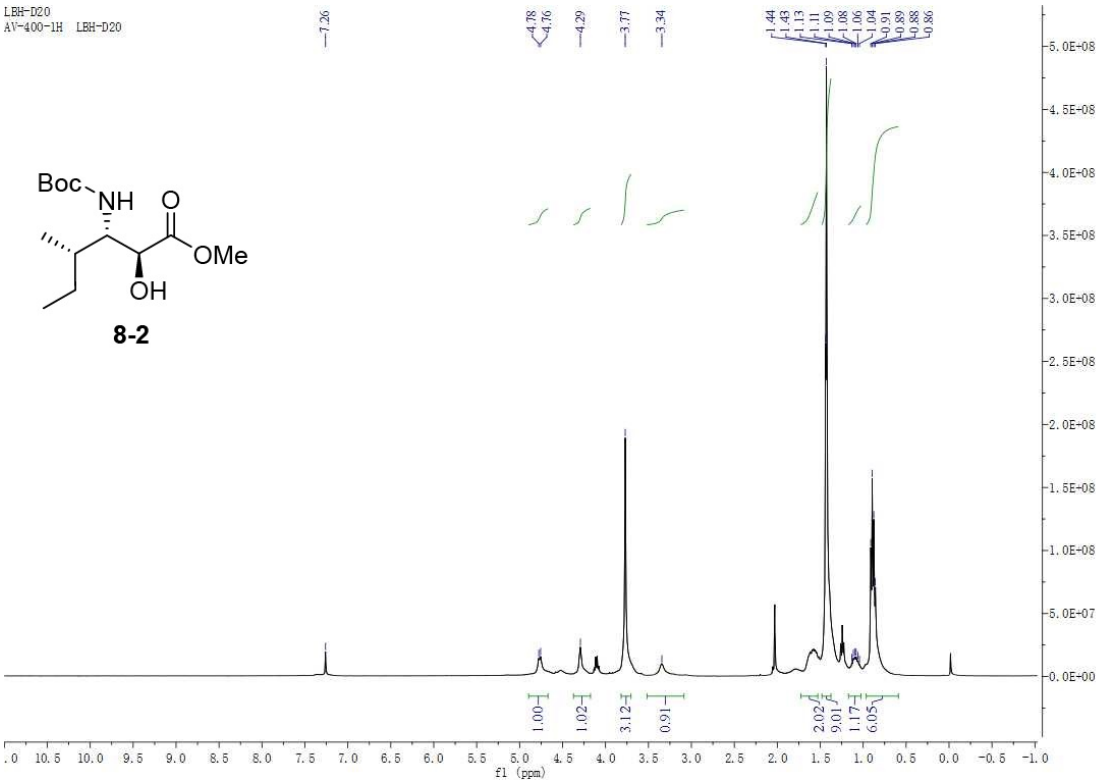


Figure S15. ^{1}H NMR of **8-2** ($CDCl_3$, 400 MHz)

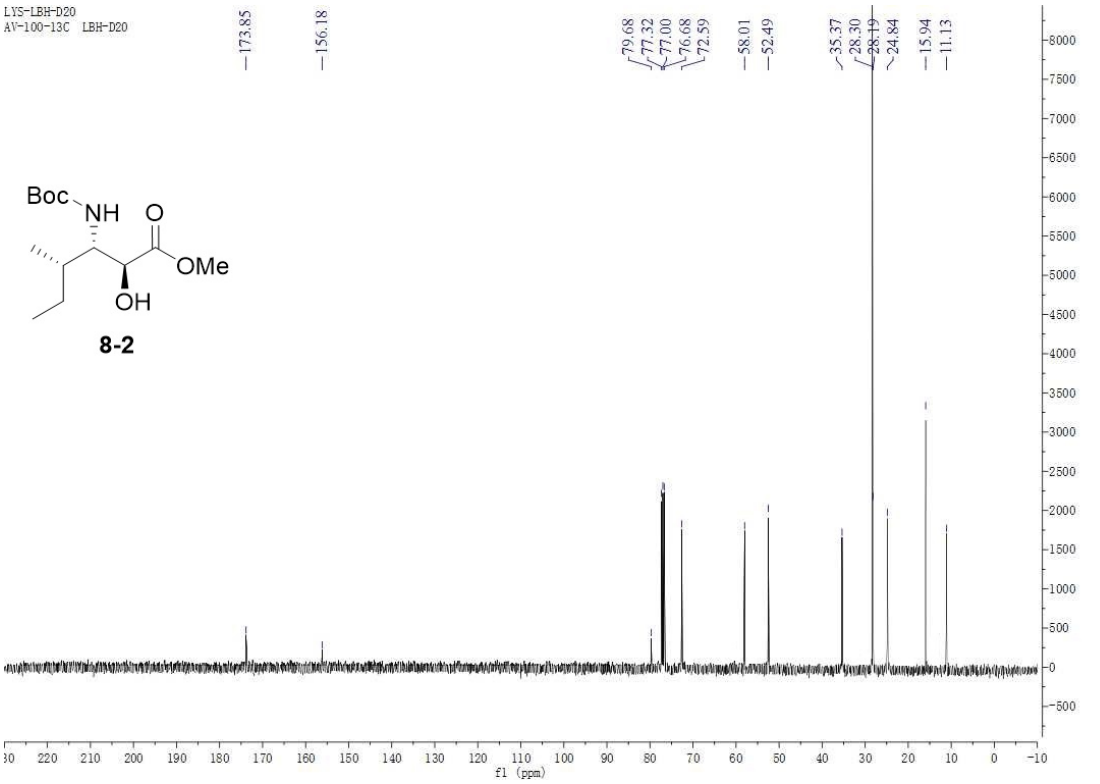


Figure S16. ^{13}C NMR of **8-2** ($CDCl_3$, 100 MHz)

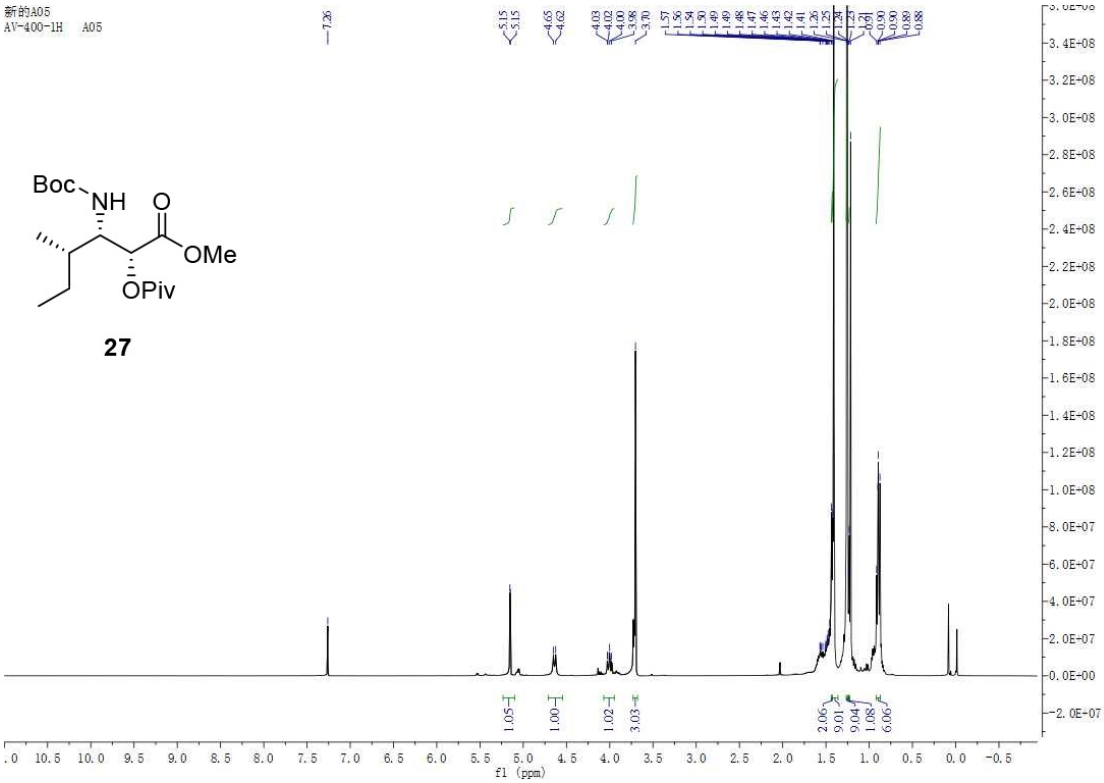


Figure S17. ^{1}H NMR of **27** ($CDCl_3$, 400 MHz)

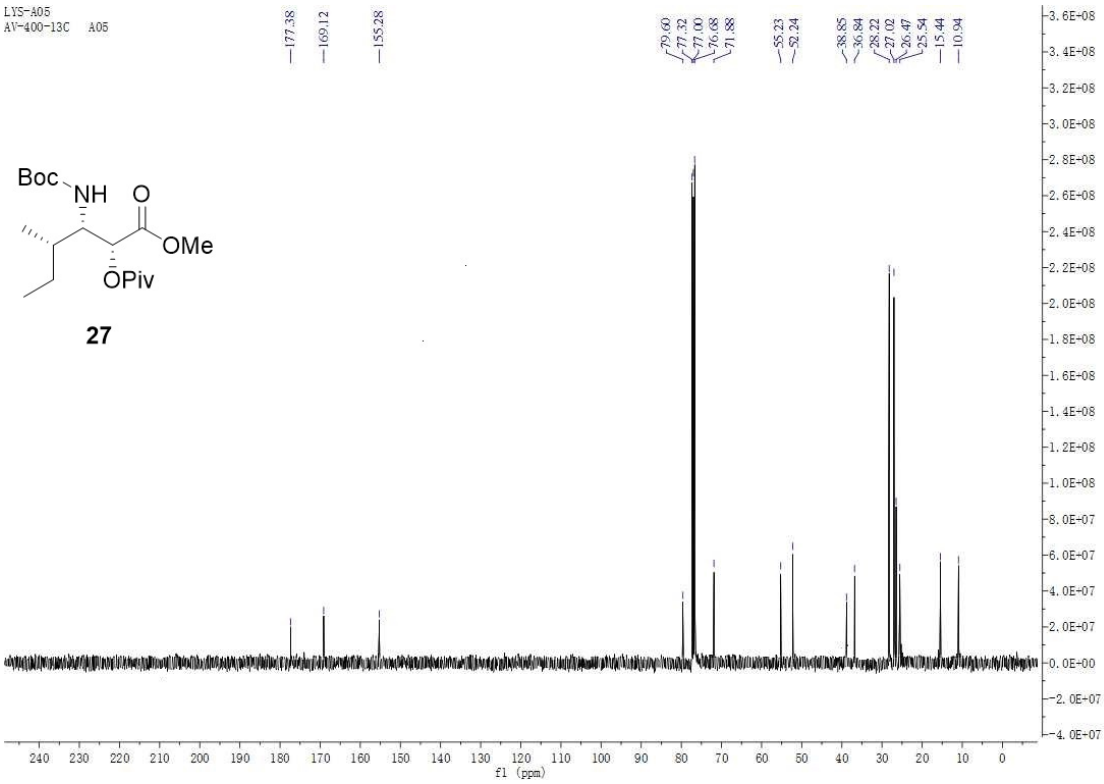


Figure S18. ^{13}C NMR of **27** ($CDCl_3$, 100 MHz)

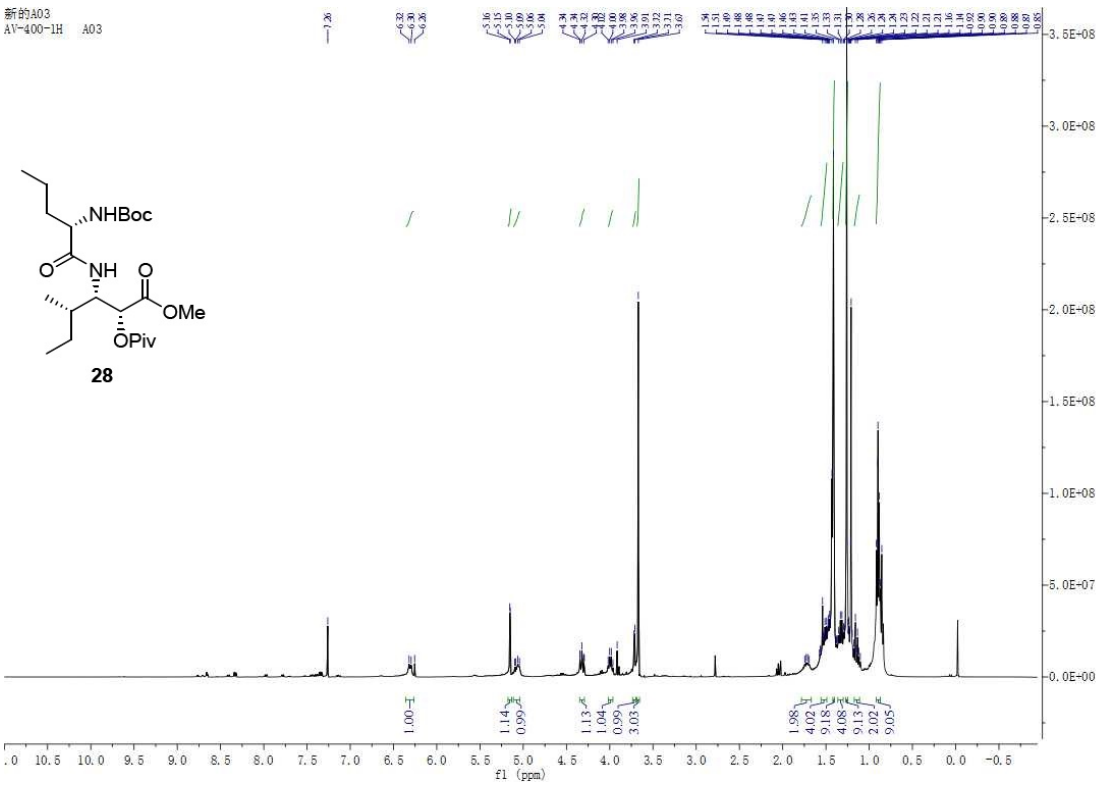


Figure S19. ^{1}H NMR of **28** ($CDCl_3$, 400 MHz)

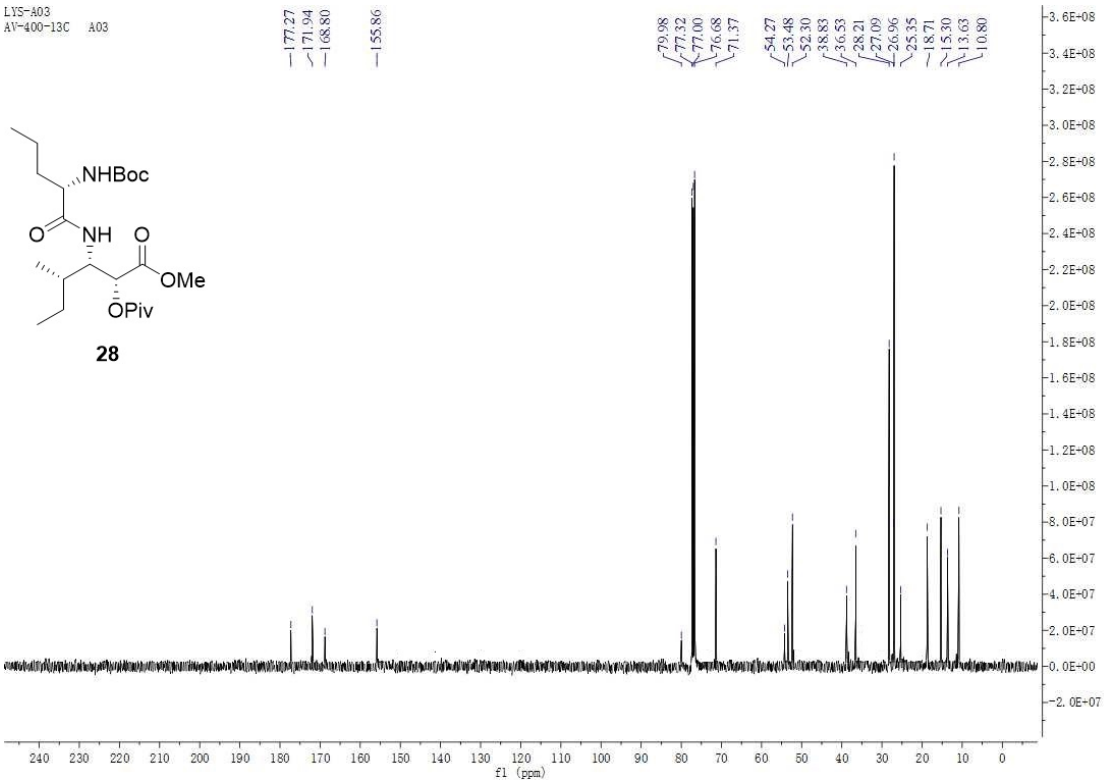


Figure S20. ^{13}C NMR of **28** ($CDCl_3$, 100 MHz)

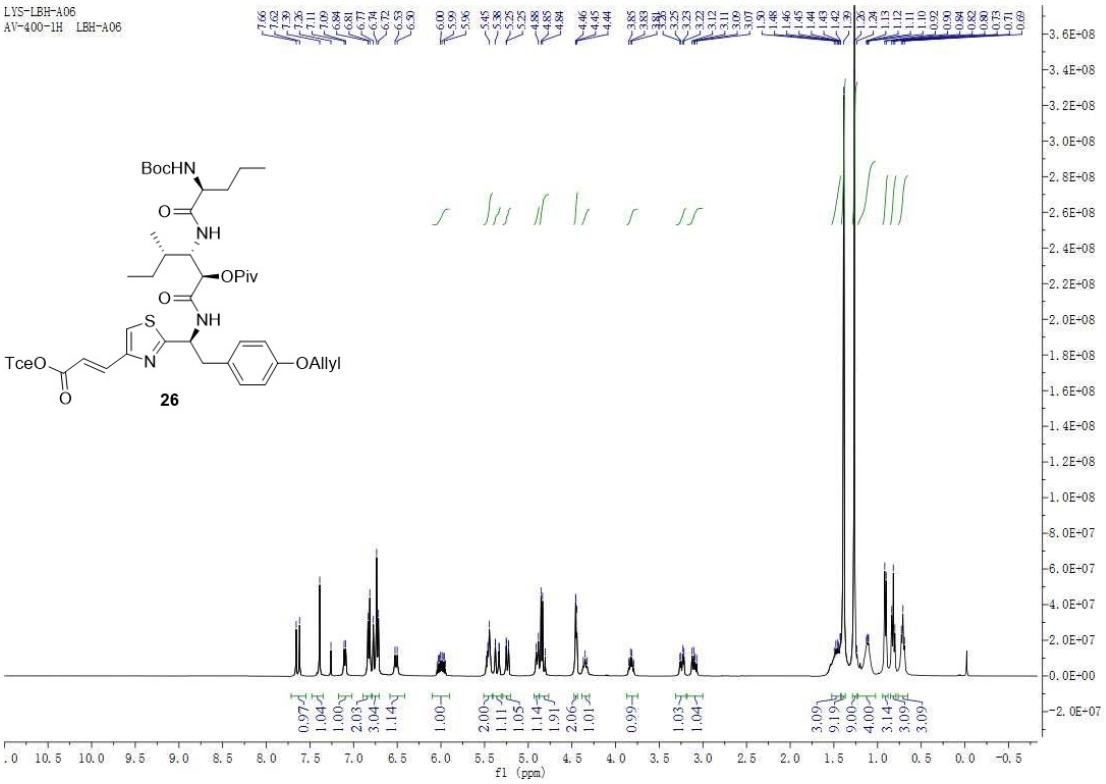


Figure S21. ^{1}H NMR of **26** ($CDCl_3$, 400 MHz)

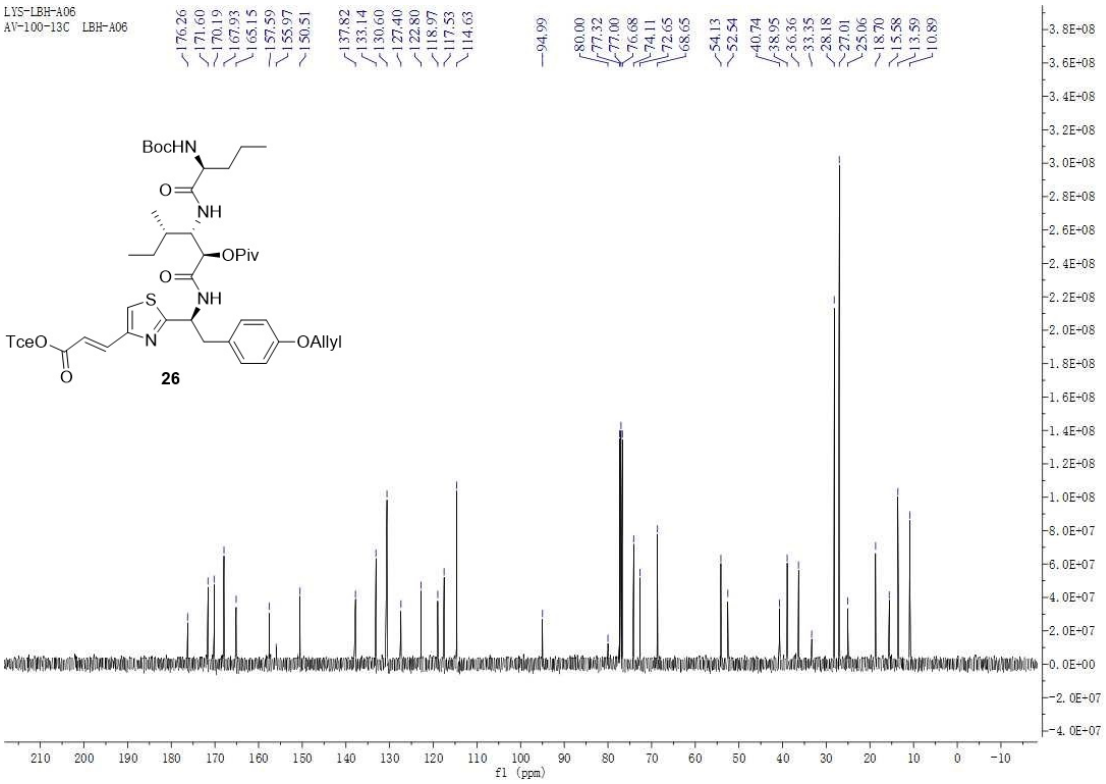


Figure S22. ^{13}C NMR of **26** ($CDCl_3$, 100 MHz)

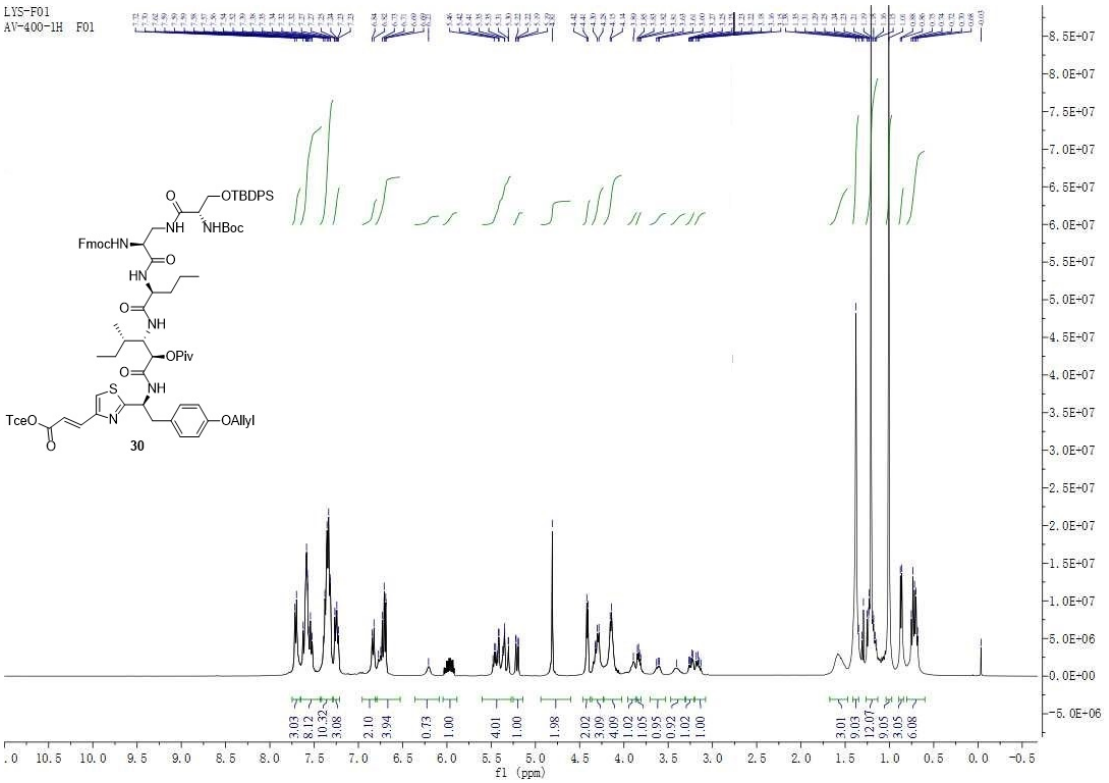


Figure S23. ^{1}H NMR of **30** ($CDCl_3$, 400 MHz)

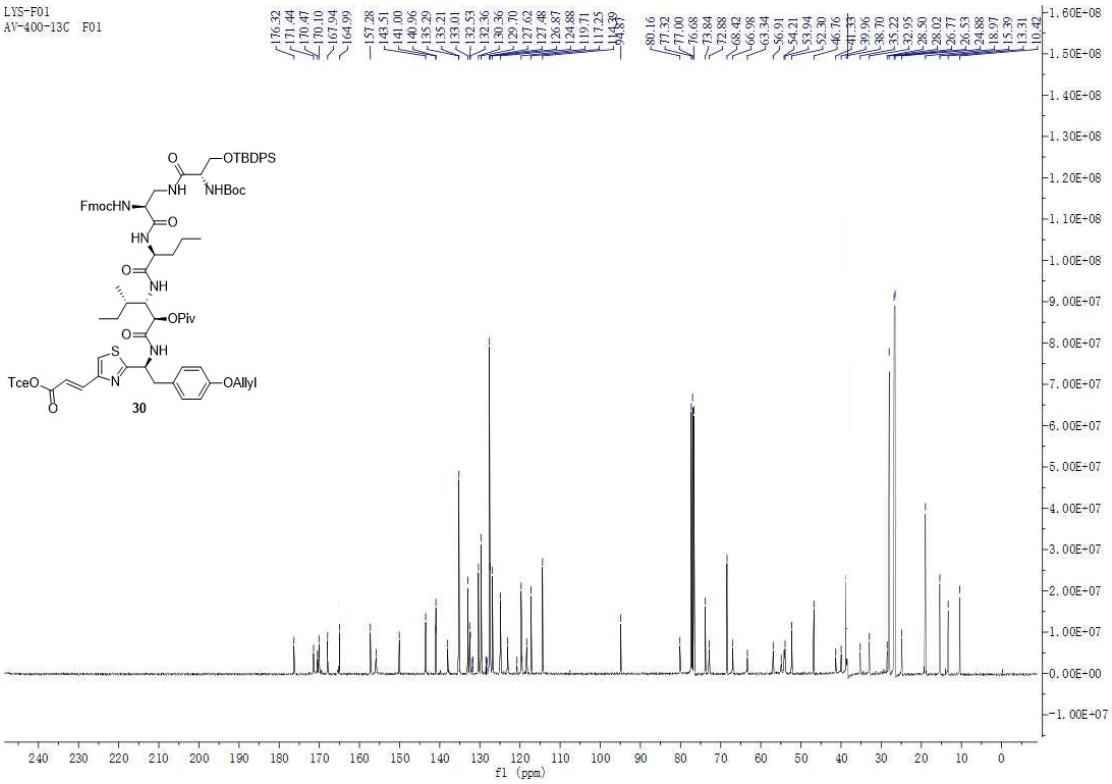


Figure S24. ^{13}C NMR of **30** ($CDCl_3$, 100 MHz)

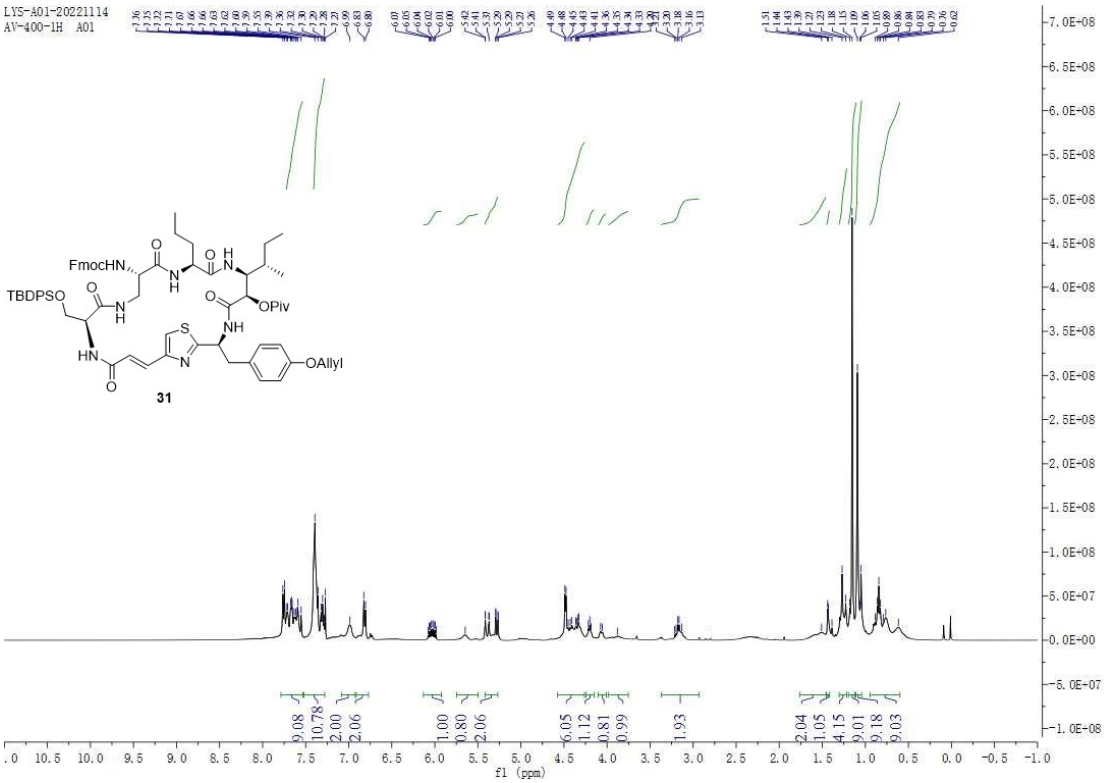


Figure S25. ^{1}H NMR of **31** ($CDCl_3$, 400 MHz)

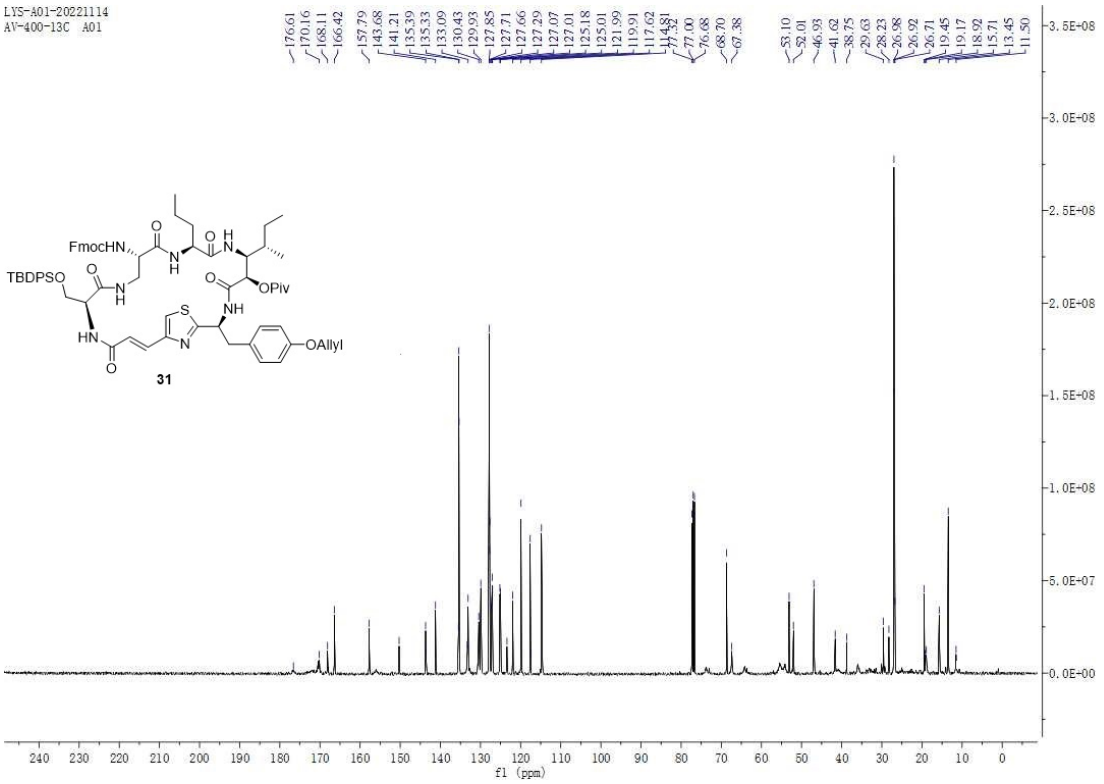


Figure S26. ^{13}C NMR of **31** ($CDCl_3$, 100 MHz)

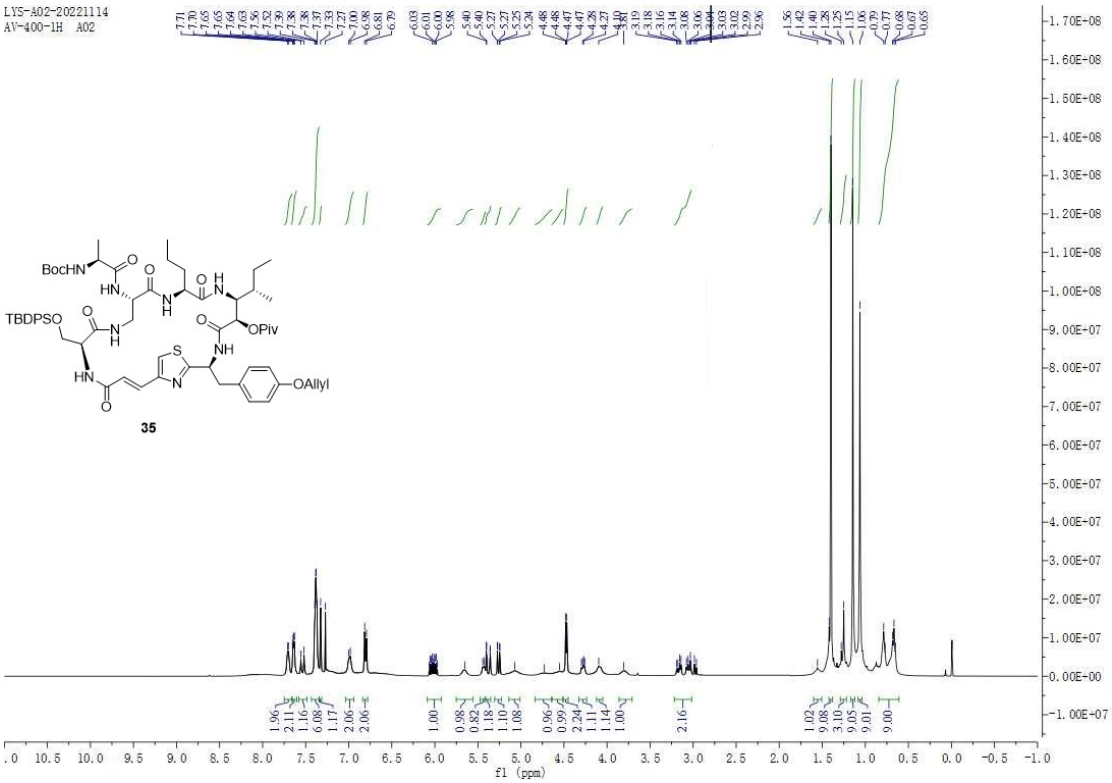


Figure S27. ^{1}H NMR of **35** ($CDCl_3$, 400 MHz)

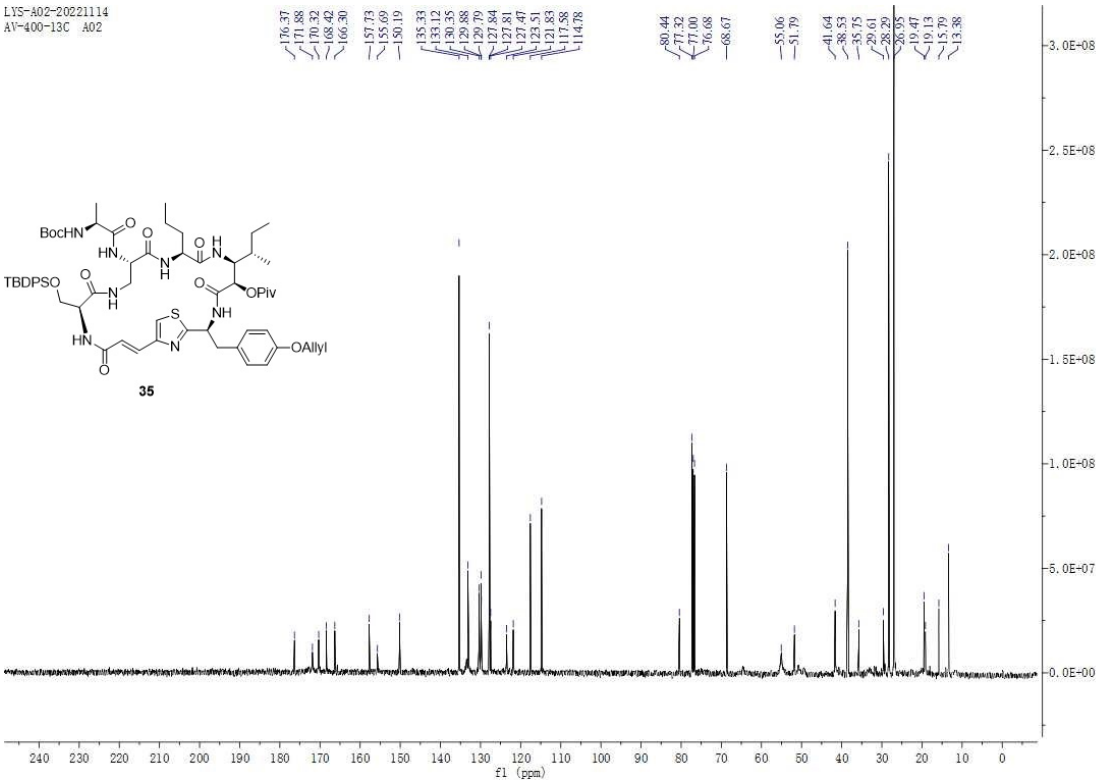


Figure S28. ^{13}C NMR of **35** ($CDCl_3$, 100 MHz)

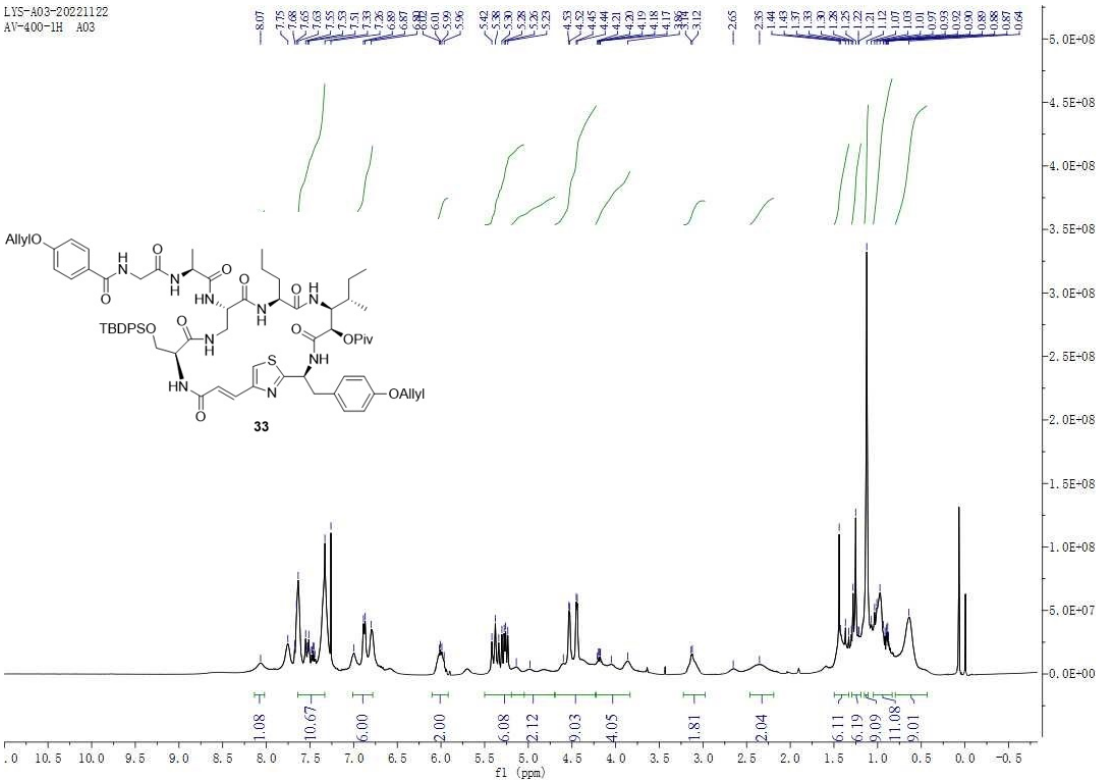


Figure S29. ^{1}H NMR of **33** ($CDCl_3$, 400 MHz)

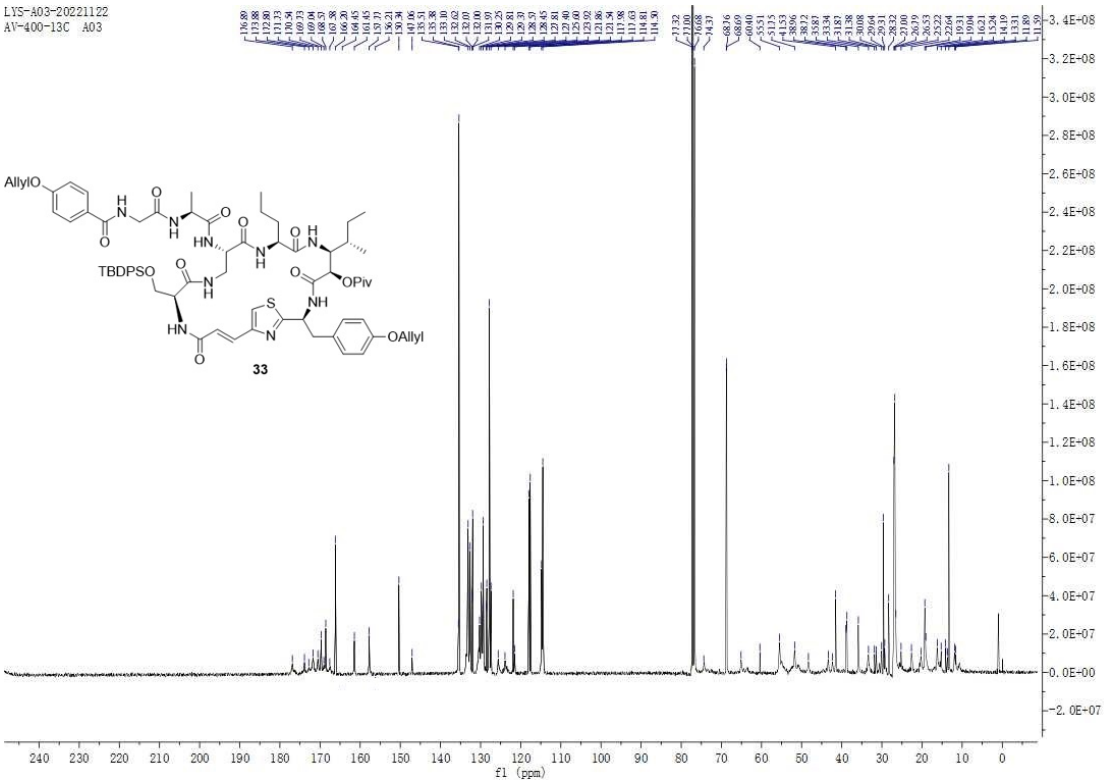


Figure S30. ^{13}C NMR of **33** ($CDCl_3$, 100 MHz)

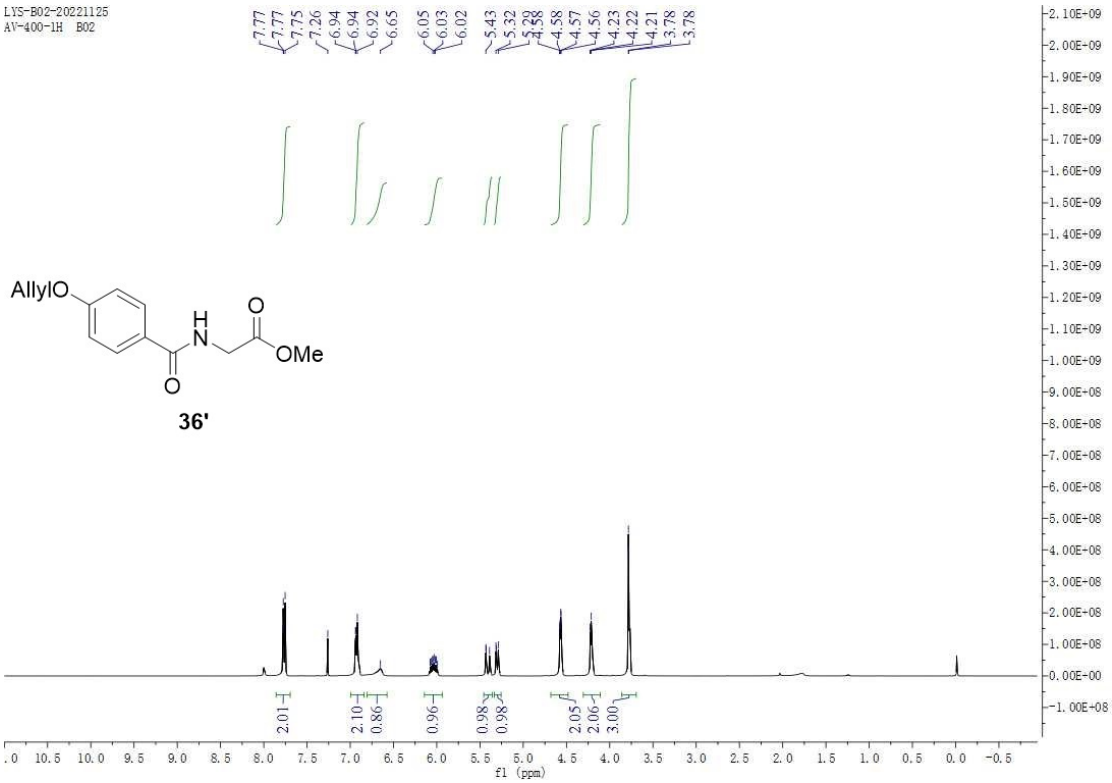


Figure S31. ^{1}H NMR of **36'** ($CDCl_3$, 400 MHz)

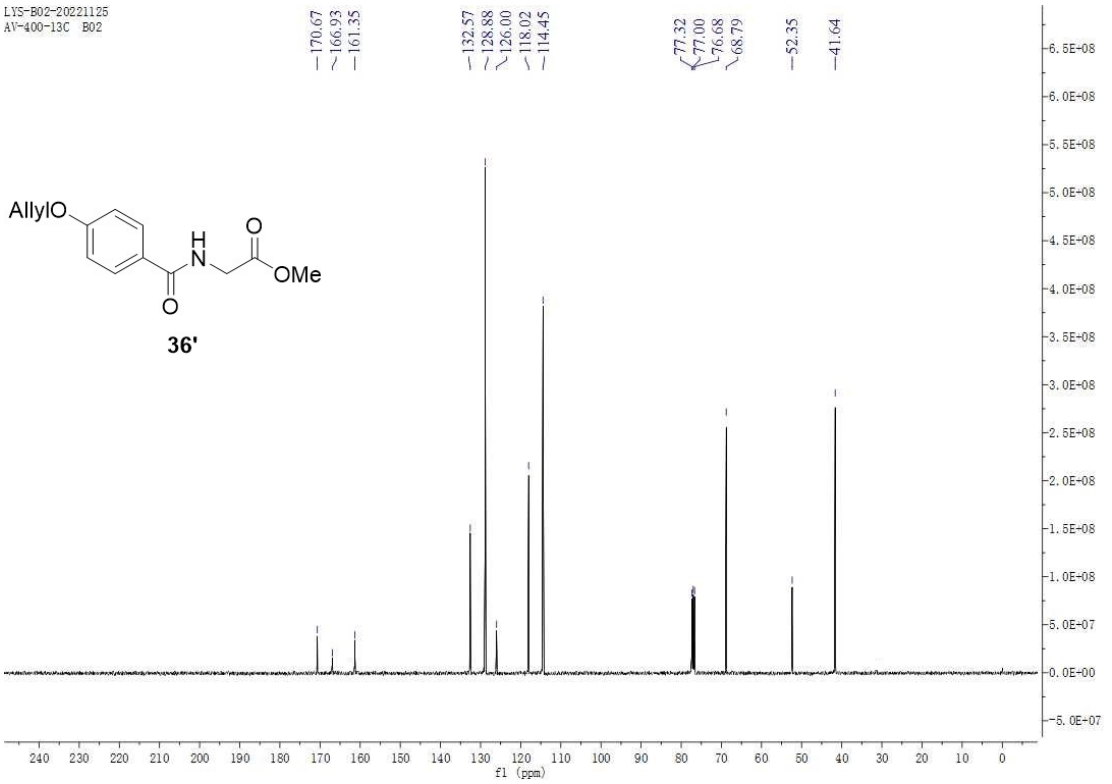


Figure S32. ^{13}C NMR of **36'** ($CDCl_3$, 100 MHz)

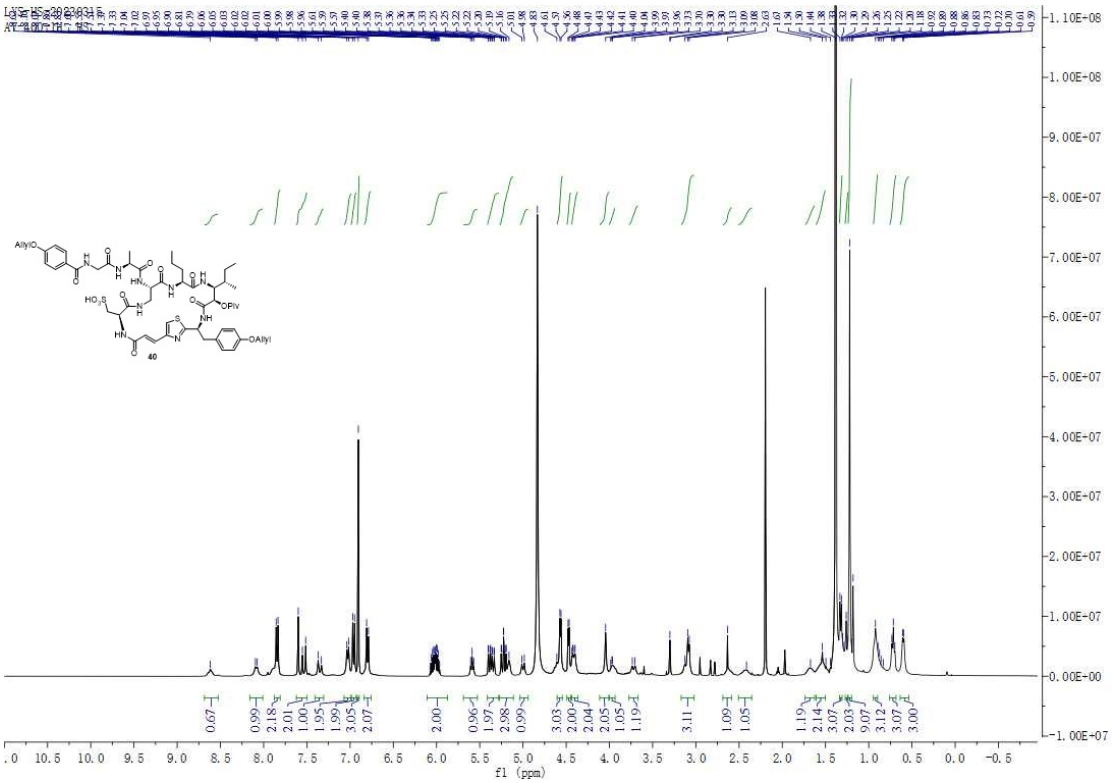


Figure S33. ^{1}H NMR of **40** (MeOD, 400 MHz)

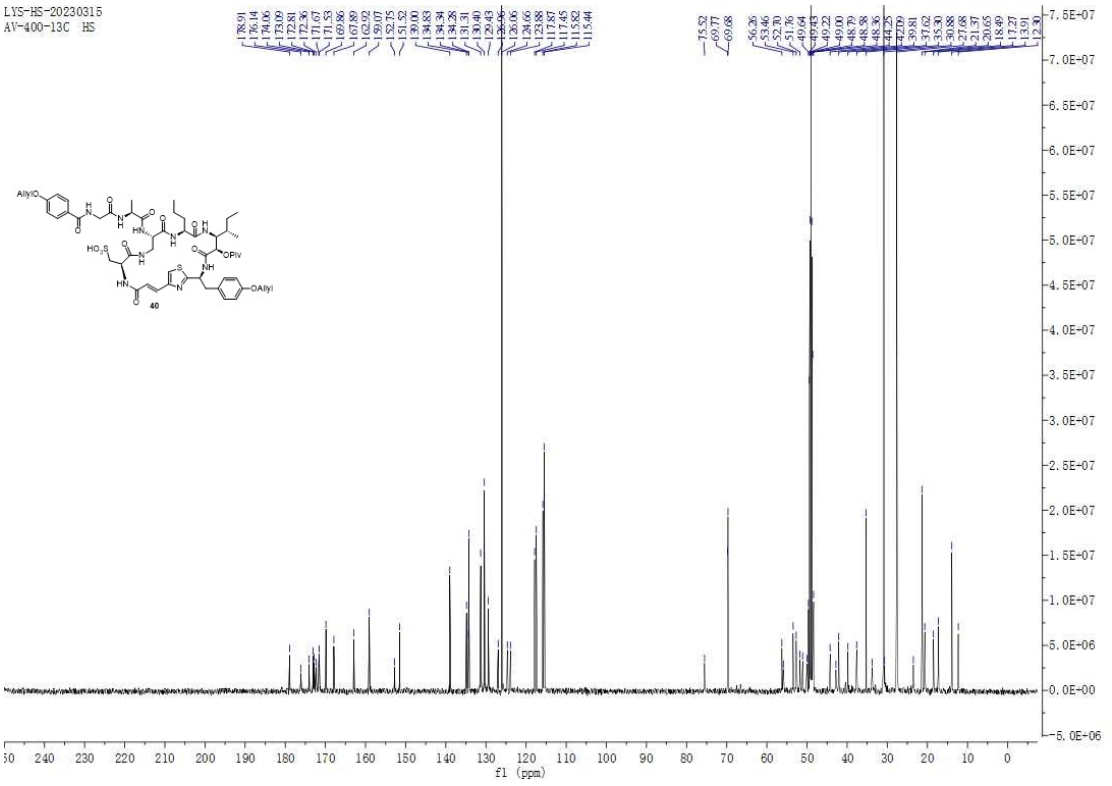


Figure S34. ^{13}C NMR of **40** (MeOD, 100 MHz)

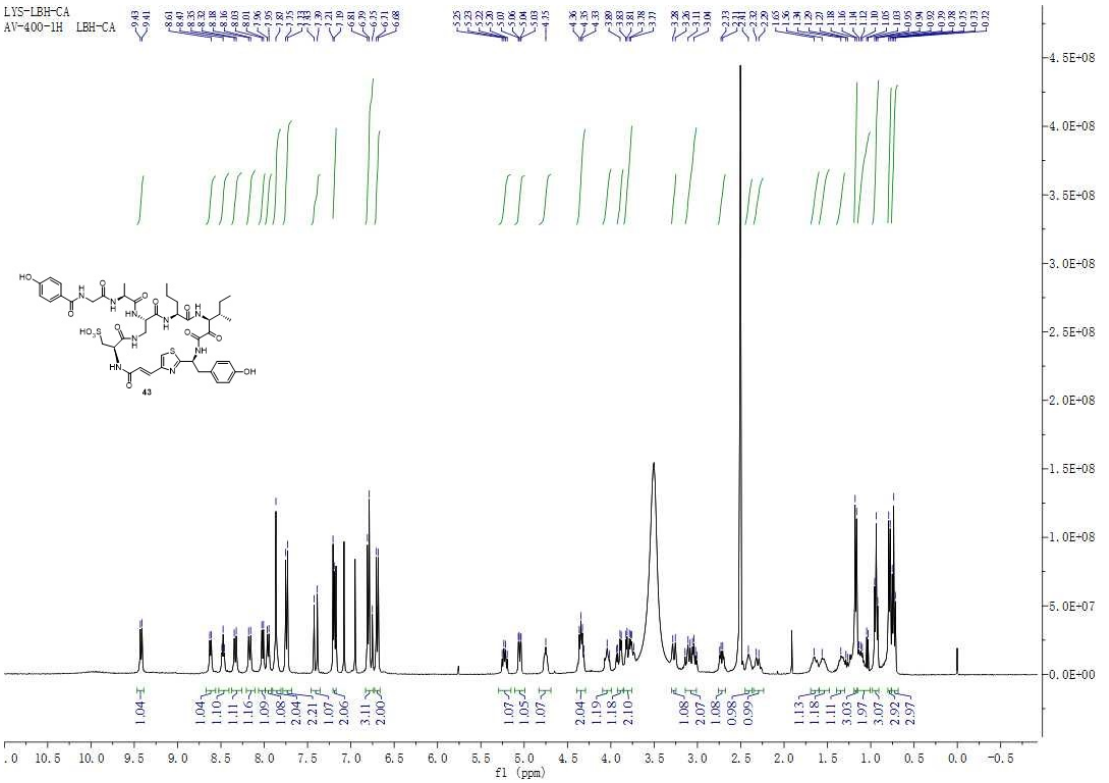


Figure S35. ^{1}H NMR of **43** (DMSO-d_6, 400 MHz)

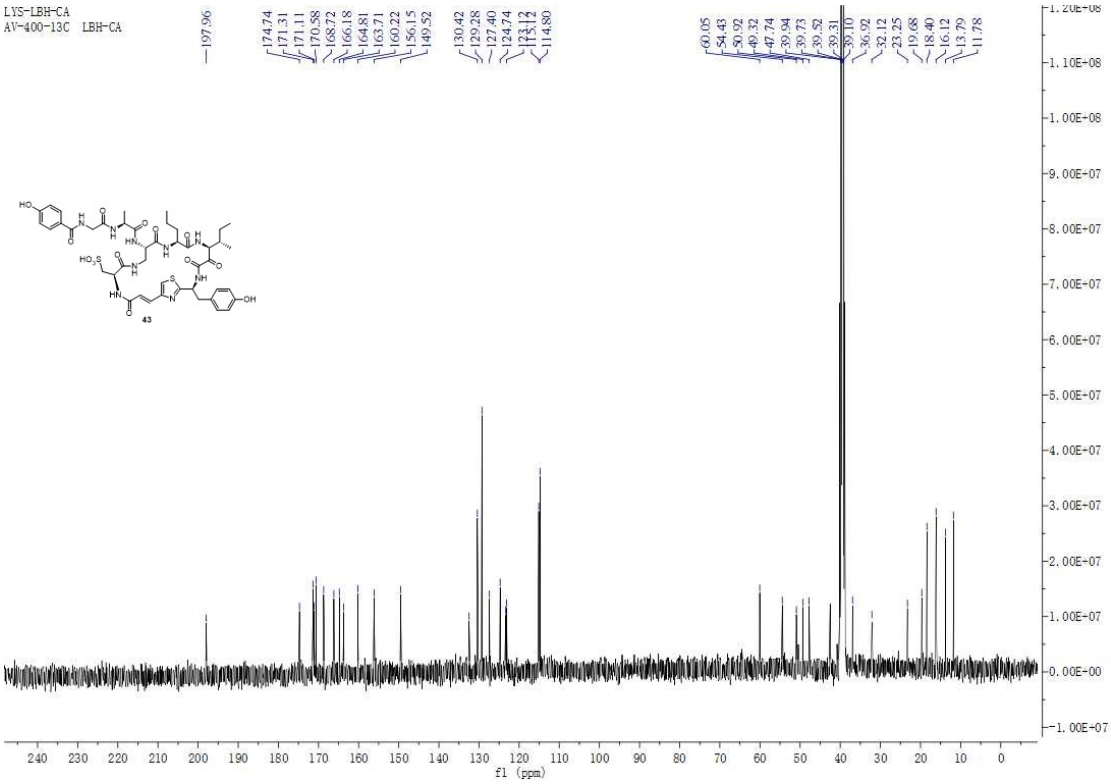


Figure S36. ^{13}C NMR of **43** (DMSO-d_6, 100 MHz)

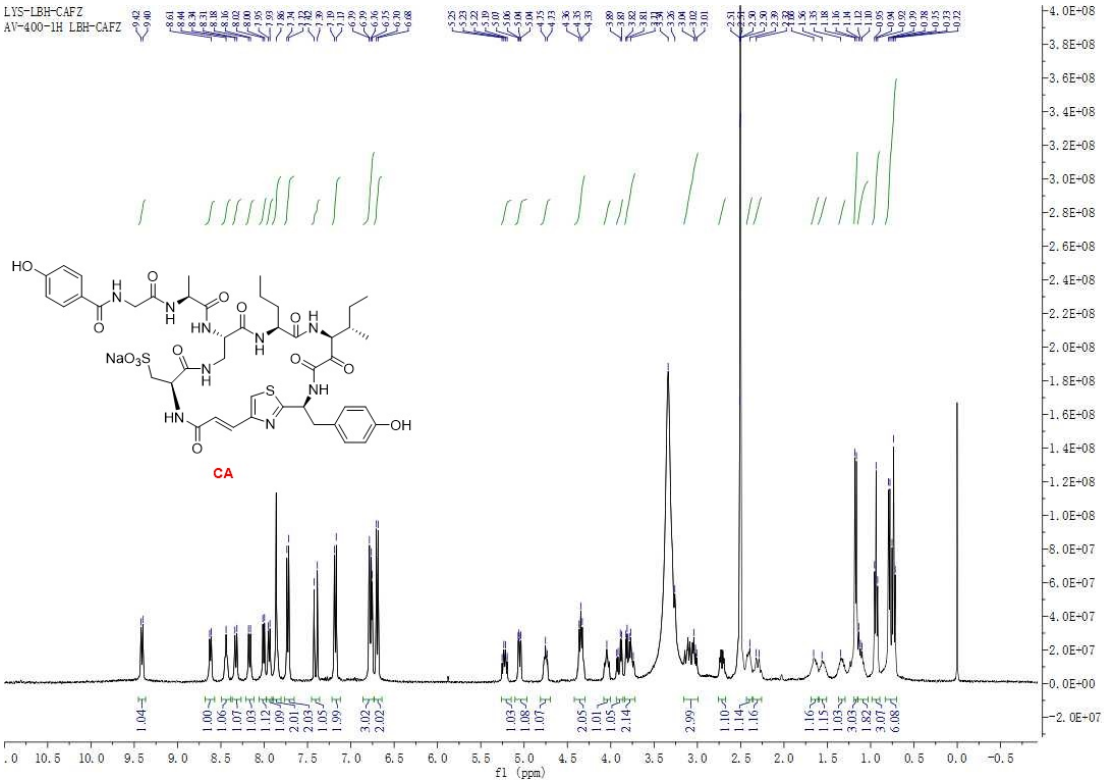


Figure S37. ^{1}H NMR of **CA** (DMSO-d_6, 400 MHz)

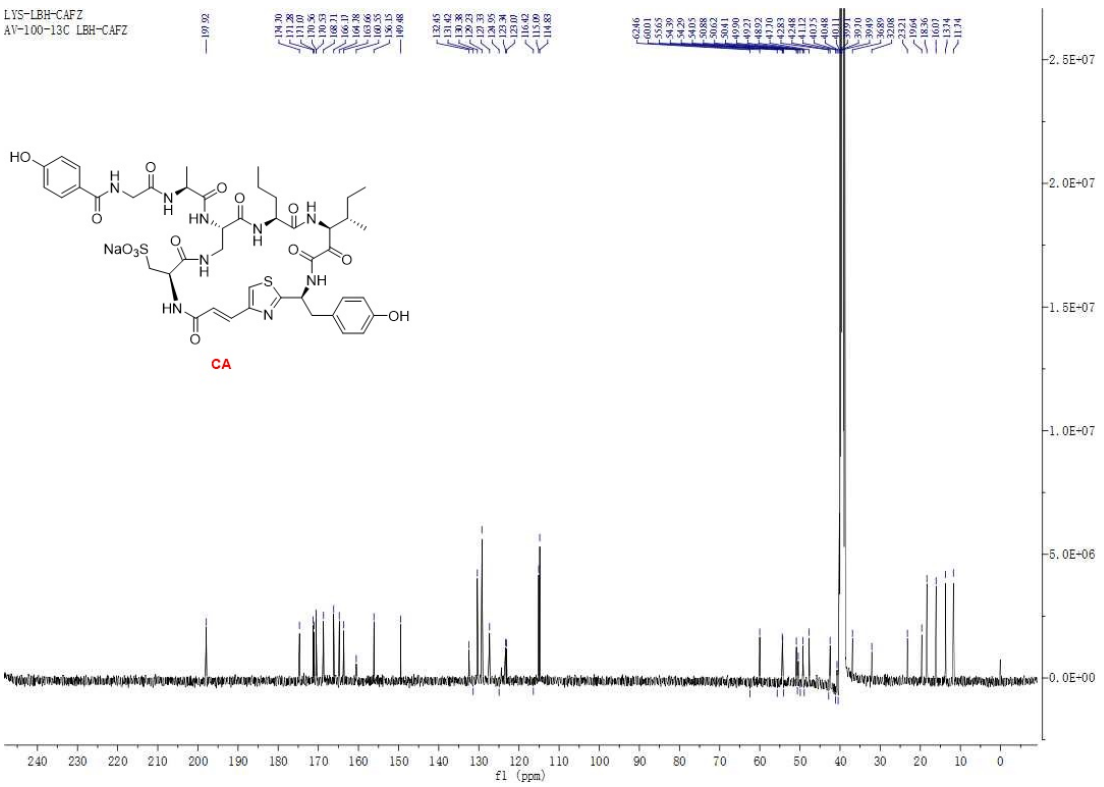


Figure S38. ^{13}C NMR of **CA** (DMSO-d_6, 100 MHz)