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Total Synthesis of Tubulysin U and N^{14}-Desacetoxytubulysin H

Bohua Long, ‡ Cheng Tao, ‡ Yinghong Li, Xiaobin Zeng,* Meiqun Cao,* Zhengzhi Wu*

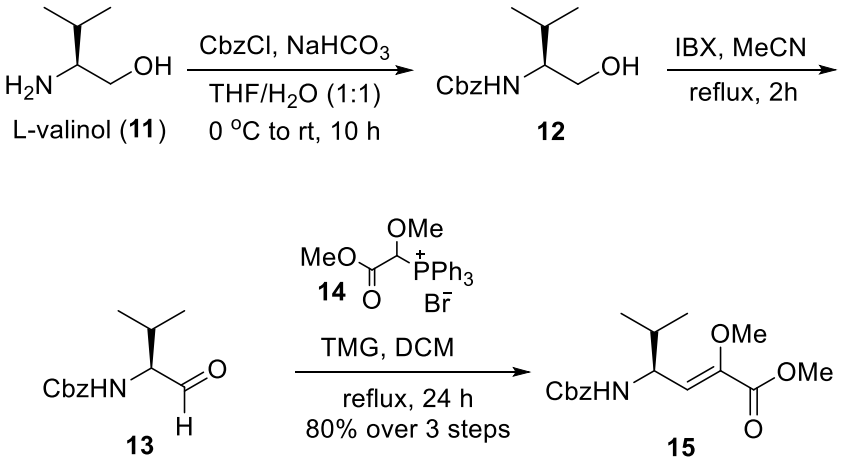
CONTENTS

General Information:

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 AV600 or 400 at 600 MHz (150 MHz) or 400 MHz (100 MHz), respectively. 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 and Analytical Data

(S,Z)-methyl 4-(benzyloxycarbonyl)-2-methoxy-5-methylhex-2-enoate (15)



To a solution of L-valinol **11** (10 g, 97 mmol) in THF/H_2O (1:1, 600 mL) was added $NaHCO_3$ (25.2 g, 300 mmol) and CbzCl (13.7 mL, 97 mmol) at 0 °C. 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 (2×300 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give corresponding compound **12** as an oil which was used for next step directly.

To a solution of the above **12** (2.5 g, 10.5 mmol) in dry MeCN (150 mL) was added IBX (5.9 g, 21 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 (100 mL). The total filtrate was concentrated in vacuo to afford the residue **13** as an oil which was used for next step directly.

To a solution of the above **13** in dry DCM (200 mL) was added phosphonium reagent **14** [1] (7.0 g, 15.8 mmol) and TMG (2 mL, 15.8 mmol) at room temperature. After 5 min, the resultant mixture was heated to reflux and stirred for 24h. The solution was concentrated in *vacuo,* then purified by silica gel column chromatography (EA/PE, 1:10) to afford **15** as an oil (2.7 g, 80% over 3 steps); $[\alpha]_D^{25}$ +8.50 (c 0.29, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.28 (m, 5H), 6.05 (d, J = 9.1 Hz, 1H), 5.15 – 5.04 (m, 2H), 5.00 (d, J = 7.9 Hz, 1H), 4.46 (dd, J = 15.6, 8.4 Hz, 1H), 3.78 (s, 3H), 3.72 (s, 3H), 1.82 (dd, J = 13.1, 6.5 Hz, 1H), 0.93 (dd, J = 11.3, 6.8 Hz, 6H); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 163.83, 155.73, 146.61, 136.38, 128.44, 128.05, 125.76, 66.67, 60.03, 52.92, 52.00, 32.59, 29.62, 18.51; HR-ESIMS m/z: calculated for $C_{17}H_{23}NO_5Na^+$ $[M+Na]^+$: 344.1474, found 344.1492.

[1] P. Seneci, I. Leger, M. Souchet, G. Nadler, *Tetrahedron*, **1997**, 53, 17097-17114

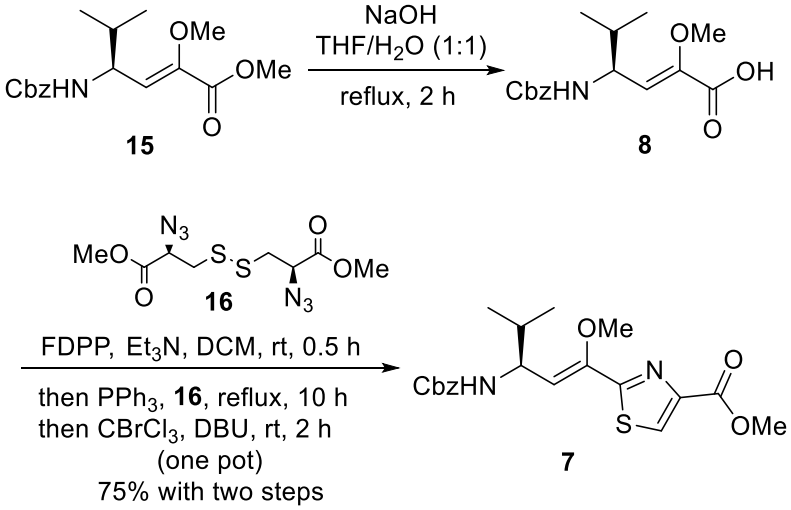
Another method for (S,Z)-methyl 4-(benzyloxycarbonyl)-2-methoxy-5-methylhex-2-enoate (15)

To a solution of L-valinol **11** (10 g, 97 mmol) in THF/H_2O (1:1, 600 mL) was added $NaHCO_3$ (25.2 g, 300 mmol) and CbzCl (13.7 mL, 97 mmol) at 0 °C. 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 (2×300 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give corresponding compound **12** as an oil which was used for next step directly.

To a solution of the above **12** (5.0 g, 21.1 mmol) in dry MeCN (200 mL) was added IBX (6.5 g, 23.2 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 residue **13** as an oil which was used for next step directly.

To a solution of the above **13** in isopropyl alcohol (200 mL) was added phosphonium reagent **14** [1] (11.1 g, 25.0 mmol) and K_2CO_3 (3.5 g, 25.0 mmol). The resultant mixture was stirred for 5h at room temperature. The solution was concentrated in *vacuo,* then purified by silica gel column chromatography (EA/PE, 1:10) to afford **15** as an oil (4.7 g, 70% over 3 steps)

(S,Z)-methyl 2-(3-(benzyloxycarbonyl)-1-methoxy-4-methylpent-1-enyl)thiazole-4- carboxylate (7)

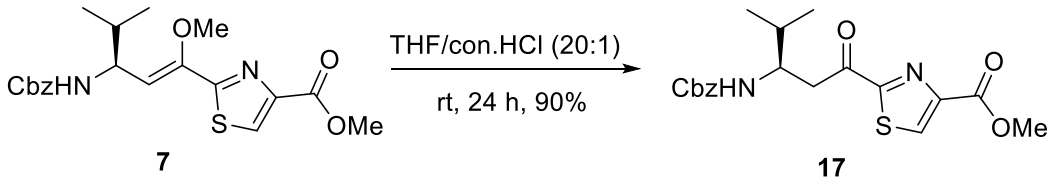


NaOH (3.4 g, 84 mmol) was added to a solution of compound **15** (2.7 g, 8.4 mmol) in THF/H_2O (1:1, 200 mL) at room temperature. After 10 min, the resultant mixture was heated to reflux and stirred for 2h. Then volatiles of the reaction mixture were removed in vacuo. The solution was diluted with water (100 mL) and adjusted to pH 2 by dropwise addition of $KHSO_4$ (1.0 M in water). The aqueous phase was extracted with ethyl acetate (2×300 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **8** as an oil which was used for next step directly.

To a solution of the above acid **8** in dry DCM (100 mL) was added pentafluorophenyl diphenylphosphinate (FDPP) (3.3 g, 8.4 mmol) and triethylamine (TEA) (2.4 mL, 16.8 mmol) at room temperature. After stirring for 30 min, compound **16** [2] (1.4 g, 4.2 mmol) and PPh_3 (11 g, 42 mmol) were added to the solution and heated to reflux for a further 10 h away from light. After cooling to 0 °C, 1,8-diazabicycloundec-7-ene (DBU) (3.8 mL, 25.2 mmol) and bromotrichloromethane ($CBrCl_3$) (1.7 mL, 16.8 mmol) were introduced via spyringe over 5 min and stirred for further 2h at room temperature. The solvent was quenched with saturated NH_4Cl solution and extracted with DCM (200 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; $[\alpha]_D^{25}$ -32.16 (c 1.61, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.38 – 7.27 (m, 5H), 6.06 (d, *J* = 9.8 Hz, 1H), 5.07 (q, *J* = 12.2 Hz, 2H), 4.92 (d, *J* = 9.1 Hz, 1H), 4.56 (q, *J* = 9.2 Hz, 1H), 3.94 (s, 3H), 3.84 (s, 3H), 1.81 (q, *J* = 6.7 Hz, 1H), 0.96 (dd, *J* = 12.1, 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.22, 161.69, 155.77, 148.97, 147.57, 136.38, 128.45, 128.05, 127.81, 117.24, 66.69, 61.05, 52.73, 52.45, 33.04, 18.69, 18.59. HR-ESIMS m/z: calculated for $C_{20}H_{24}N_2O_5SNa^+$ $[M+Na]^+$: 427.1304, found 427.1326.

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

(R) -methyl 2-(3-(benzyloxycarbonyl)-4-methylpentanoyl)thiazole-4-carboxylate (17)

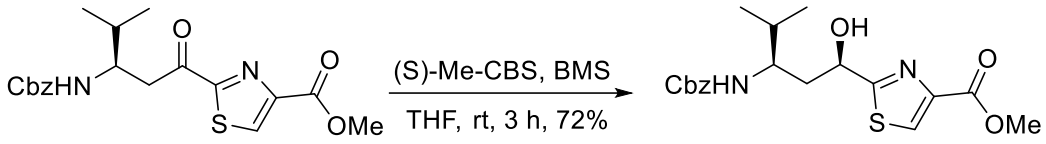


7 **17**

To a solution of compound **7** (2.55 g, 6.3 mmol) in THF (200 mL) was added concentrated hydrochloric acid (10 mL) at 0 °C. After being stirred at room temperature for 24 h, the reaction was quenched with saturated aqueous solution of $NaHCO_3$ (PH = 8). Volatiles of the reaction mixture were removed in vacuo. The residue was diluted with water (100 mL) and the aqueous phase was extracted with ethyl acetate (3×200 mL). The combined organic

phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (EA/PE, 1:4) to afford **17** (2.2 g, 90%) as an oil; $[\alpha]_D^{25}$ -12.45 (c 0.65, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.36 – 7.26 (m, 5H), 5.11 (d, *J* = 9.5 Hz, 1H), 5.02 (s, 2H), 4.13 – 4.06 (m, 1H), 3.96 (s, 3H), 3.47 – 3.28 (m, 2H), 1.94 (dq, *J* = 13.3, 6.6 Hz, 1H), 0.96 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.15, 167.21, 161.18, 155.95, 148.21, 136.49, 133.60, 128.43, 127.98, 66.58, 53.42, 52.63, 40.99, 32.05, 19.27, 18.28. HR-ESIMS m/z: calculated for $C_{19}H_{22}N_2O_5SNa^+$ $[M+Na]^+$: 413.1147, found 413.1175.

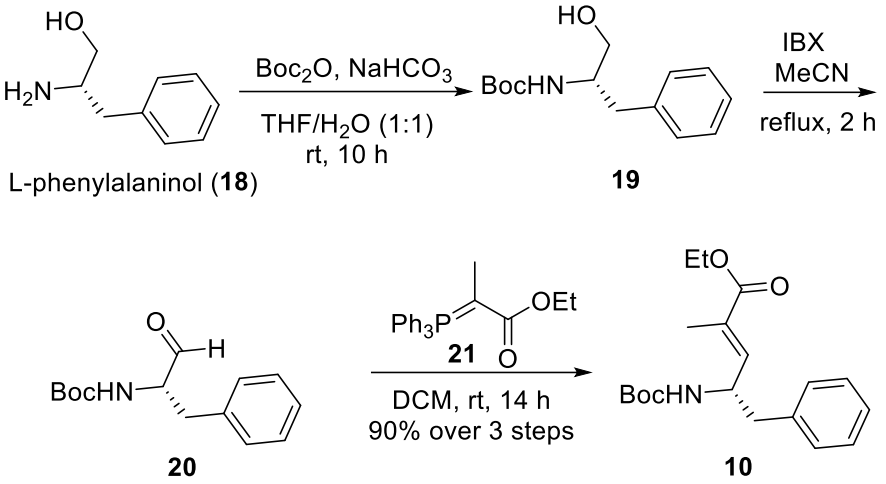
Methyl 2-((1R,3R)-3-(benzyloxycarbonyl)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (5)



17 Tuv fragment (**5**)

To a solution of compound **17** (2.2 g, 5.6 mmol) in dry THF (100 mL) was added (S)-Me-CBS (0.16 g, 0.56 mmol) and $BH_3{\cdot}SMe_2$ (10.0 M in DMS, 1.7 mL) at 0 °C. After 30 min at 0 °C, the solution was stirred for further 3h at room temperature. The reaction was quenched with MeOH (50 mL). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (EA/PE, 1:4) to afford **5** (1.6 g, 72%) as an oil; $[\alpha]_D^{25}$ +7.0 (c 0.66, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.33 – 7.27 (m, 5H), 5.11 (d, *J* = 1.8 Hz, 2H), 4.99 (d, *J* = 11.0 Hz, 1H), 4.78 (d, *J* = 9.5 Hz, 1H), 3.93 (s, 3H), 3.81 (dtt, *J* = 9.5, 6.5, 2.7 Hz, 1H), 2.19 – 2.08 (m, 1H), 1.85 – 1.73 (m, 2H), 0.98 – 0.92 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.39, 161.99, 158.29, 146.48, 135.91, 128.63, 128.37, 128.11, 127.58, 68.93, 67.47, 53.07, 52.38, 41.57, 32.18, 19.36, 18.21. HR-ESIMS m/z: calculated for $C_{19}H_{24}N_2O_5SNa^+$ $[M+Na]^+$: 415.1304, found 415.1321.

(S,E)-ethyl 4-(tert-butoxycarbonyl)-2-methyl-5-phenylpent-2-enoate (10)



To a solution of L-phenylalaninol **18** (10 g, 66.2 mmol) in THF/H_2O (1:1, 600 mL) was added $NaHCO_3$ (25.2 g, 300 mmol) and Boc_2O (15.2 mL, 66.2 mmol) at 0 °C. After being stirred at room temperature overnight, volatiles of the reaction mixture were removed in vacuo. The aqueous phase was extracted with ethyl acetate (2×300 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give corresponding compound **19** as an oil (16.6 g, 100%), which was used for next step directly.

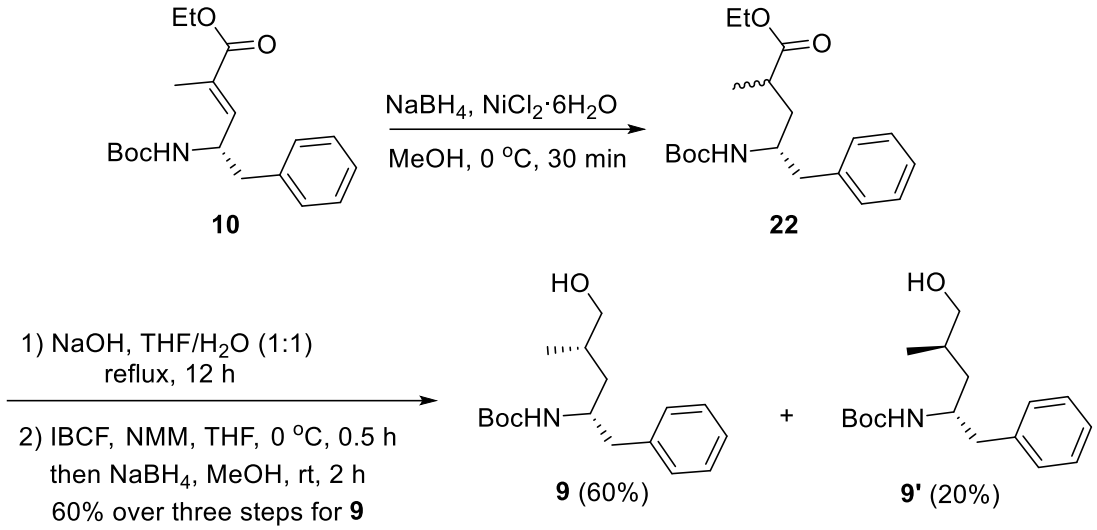
To a solution of **19** (5.0 g, 19.9 mmol) in dry MeCN (200 mL) was added IBX (11.2 g, 40 mmol) at room temperature. After 15 min, the resultant mixture was heated to reflux and stirred for 2h. The solution was cooled to room temperature and the solid was removed by filtration through a pad of celite and washed with MeCN (150 mL). The total filtrate was concentrated in vacuo to afford the residue **20** as an oil which was used for next step directly.

To a solution of the above **20** in dry DCM (300 mL) was added wittig reagent **21** [3] (8.7 g, 24 mmol) at room temperature. After 14h, the solution was concentrated in *vacuo,* then purified by silica gel column chromatography (EA/PE, 1:20) to afford **10** as an oil (4.86 g, 90% over three steps); The spectral data for synthetic **10** (^{1}H NMR and HMRS) were identical with those published by Wipf et al.[4]

[3] H. Hattori, E. Kaufmann, H. Miyatake-Ondozabal, R. Berg, K. Gademann, *J. Org. Chem.*, **2018**, 83, 7180-7205

[4] P. Wipf, T. Takada, and M. J. Rishel, *Org. Lett.*, **2004**, 6, 4057-4060.

***tert*-butyl (2R,4S)-5-hydroxy-4-methyl-1-phenylpentan-2-ylcarbamate (9)**



To a solution of compound **10** (4.86 g, 17.9 mmol) in MeOH (100 mL) was added $NiCl_2.6H_2O$ (0.86 g, 3.6 mmol) and $NaBH_4$ (2.04 g, 53.7 mmol) at 0 °C. After 30 min at 0 °C, the reaction was quenched with saturated aqueous solution of NH_4Cl (300 mL). The aqueous phase was extracted with ethyl acetate (3×300 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give corresponding compound **22** as an oil, which was used for next step directly.

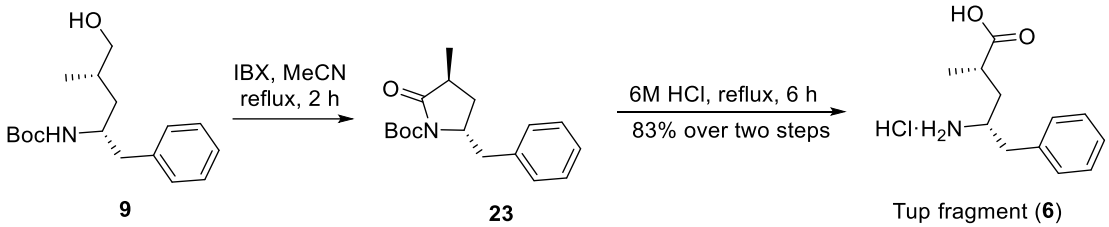
NaOH (7.2 g, 180 mmol) was added to a solution of the above compound **22** in THF/H_2O (1:1, 300 mL) at room temperature. After 10 min, the resultant mixture was heated to reflux and stirred for 12h. Then volatiles of the reaction mixture were removed in vacuo. The solution was diluted with water (100 mL) and adjusted to pH 2 by dropwise addition of $KHSO_4$ (1.0 M in water). The aqueous phase was extracted with ethyl acetate (2×300 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **22'** which was used for next step directly. To a solution of the above acid **22'** in dry THF (200 mL) was added NMM (3.3 mL, 30 mmol) and IBCF (2.3 mL, 18 mmol) at 0 °C. After 30 min at 0 °C, $NaBH_4$ (2.0 g, 53 mmol) and MeOH (50 mL) were added. After being stirred for 2h at room temperature, volatiles of the reaction mixture were removed in vacuo. The aqueous phase was extracted with ethyl acetate (2×300 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo.* The residue was purified by silica gel column chromatography (EA/PE, 1:4) to afford **9** as an oil (3.15 g, 60% over three steps) and **9'** as a by-product (1.05 g, 20% over three steps). The spectral data for compound **9** and **9'** were in good agreement with an authentic sample of this isomer prepared by a known method. [5]

Compound **9**: $[\alpha]_D^{25}$ -6.70 (c 0.50, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.16 (m, 5H), 4.55 (d, *J* = 7.9 Hz, 1H), 4.02 (s, 1H), 3.46 (d, *J* = 5.8 Hz, 2H), 2.86 – 2.59 (m, 3H), 1.84 – 1.70 (m, 1H), 1.54 (ddd, *J* = 13.1, 8.0, 4.8 Hz, 1H), 1.39 (s, 9H), 1.27 – 1.22 (m, 1H), 0.93 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.73, 137.95,

129.37, 128.20, 126.18, 79.13, 67.63, 49.49, 41.54, 39.03, 32.39, 28.26, 17.69. HR-ESIMS m/z: calculated for $C_{17}H_{27}NO_3Na^+$ $[M+Na]^+$: 316.1889, found 316.1910.

[5] P. Wipf, T. Takada, and M. J. Rishel, *Org. Lett.*, **2004**, 6, 4057-4060.

(2S,4R)-4-amino-2-methyl-5-phenylpentanoic acid (6)

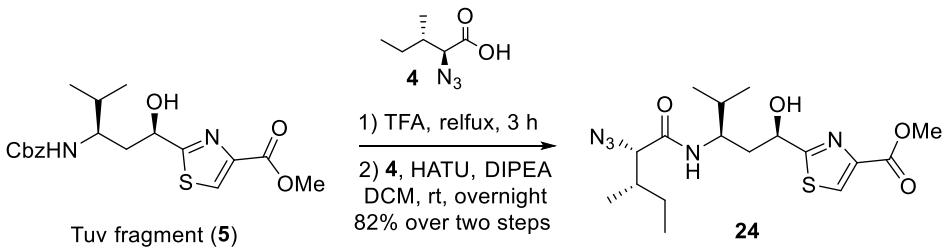


9 **23** Tup fragment (**6**)

To a solution of **9** (3.15 g, 10.7 mmol) in dry MeCN (200 mL) was added IBX (5.6 g, 20 mmol) at room temperature. After 5 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 (100 mL). The total filtrate was concentrated in vacuo to afford the residue **23** as an oil which was used for next step directly.

The above crude product **23** was dissolved in 6 M HCl (150 mL) and stirred for 6 h under reflux. After cooling to room temperature, the reaction mixture was then diluted with water (300 mL). The aqueous phase was extracted with ethyl acetate (600 mL) to remove the organic impurity. The aqueous phase was then evaporated under reduced pressure. The residue was then co-evaporated with MeCN (2×200 mL) to provide the desired product **6** (2.1 g, 83% over two steps) as an off-white solid. $[\alpha]_D^{25}$ -4.0 (c 1.0, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 7.47 – 7.32 (m, 5H), 3.69 – 3.58 (m, 1H), 3.06 (dd, *J* = 14.1, 6.6 Hz, 1H), 2.95 (dd, *J* = 14.2, 7.7 Hz, 1H), 2.72 (dq, *J* = 14.0, 7.0 Hz, 1H), 2.06 (ddd, *J* = 14.6, 8.6, 5.9 Hz, 1H), 1.76 (dt, *J* = 14.4, 6.6 Hz, 1H), 1.21 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, D_2O) δ 179.89, 135.44, 129.46, 129.10, 127.57, 51.26, 38.32, 35.78, 35.44, 16.63. HR-ESIMS m/z: calculated for $C_{12}H_{18}NO_2^+$ $[M+H]^+$: 208.1338, found 208.1328.

methyl 2-((1R,3R)-3-((2S,3S)-2-azido-3-methylpentanamido)-1-hydroxy- 4-methylpentyl) thiazole-4-carboxylate (24)

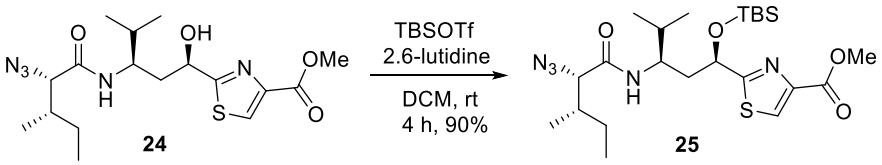


Tuv fragment (**5**) **24**

The compound **5** (2.0 g, 5.1 mmol) was dissolved in neat trifluoroacetic acid (TFA) (50 mL) and stirred for 3 h under reflux. The solvent was evaporated under reduced pressure. The residue was then co-evaporated with toluene to provide the desired amine **5'** as an oil which was used for next step directly. To a solution of the above amine **5'** in dry DCM (100 mL) was added compound **4** [6] (0.86g, 5.5 mmol) and HATU (3.8 g, 10 mmol) at room temperature. After DIPEA (3.3 mL, 20 mmol) was added, 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 (300 mL) and washed successively with saturated aqueous solution of NH_4Cl (100 mL) and brine (100 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 **24** (1.66 g, 82% over two steps) as an oil; $[\alpha]_D^{25}$ +13.10 (c 0.20, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 6.51 (d, *J* = 9.2 Hz, 1H), 5.25 (d, *J* = 4.1 Hz, 1H), 4.87 (d, *J* = 11.3 Hz, 1H), 4.01 (d, *J* = 3.6 Hz, 1H), 3.93 (s, 3H), 2.22 – 2.12 (m, 2H), 1.86 – 1.78 (m, 2H), 1.46 – 1.37 (m, 1H), 1.36 – 1.28 (m, 1H), 1.07 (d, *J* = 6.9 Hz, 3H), 0.98 – 0.90 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.17, 170.73, 161.95, 146.45, 127.62, 69.61, 68.74, 52.36, 51.55, 41.15, 38.56, 31.79, 24.14, 19.66, 18.25, 15.96, 11.64. HR-ESIMS m/z: calculated for $C_{17}H_{27}N_5O_4SNa^+$ $[M+Na]^+$: 420.1681, found 420.1713.

[6] J. T. Lundquist IV and J. C. Pelletier, *Org. Lett.*, **2001**, 3, 781-783

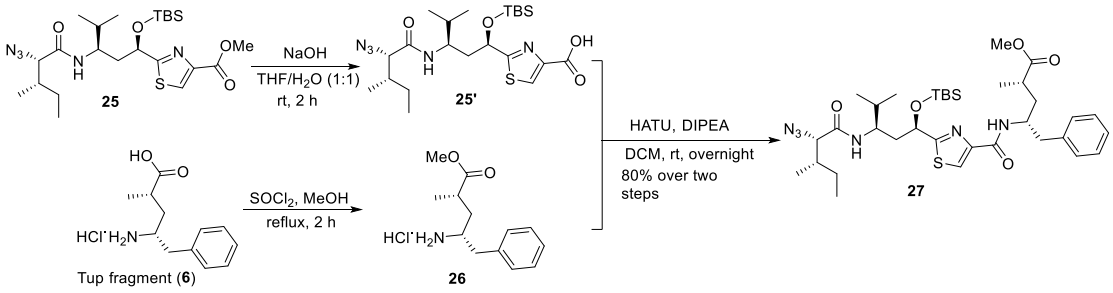
methyl2-((1R,3R)-3-((2S,3S)-2-azido-3-methylpentanamido)-1-(tert-butyldimethylsilyloxy)-4-methylpentyl)thiazole-4-carboxylate (25)



To a solution of **24** (1.66 g, 4.18 mmol) in dry DCM (100 mL) was added 2,6-lutidine (1.5 mL, 12.88 mmol) and TBSOTf (1.45 mL, 6.27 mmol) at 0 °C. After 15 min, the reaction mixture was allowed to warm to room temperature and stirred for 4h at N_2 atmosphere. Then the reaction was quenched with saturated aqueous solution of $NaHCO_3$ (150 mL). The aqueous phase was extracted with DCM (2×200 mL). The combined organic phase was washed by brine (150 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo.* The residue was purified by silica gel column chromatography (EA/PE, 1:8) to afford **25** (1.93 g, 90%) as an oil; $[\alpha]_D^{25}$ +41.0 (c 0.49, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 6.57 (d, *J* = 8.7 Hz, 1H), 5.15 – 5.06 (m, 1H), 4.07 – 3.98 (m, 1H), 3.94 (d, *J* = 1.7 Hz, 3H), 3.86 (d, *J* = 4.3 Hz, 1H), 2.11 (ddd, *J* = 9.5, 4.8, 2.8 Hz, 1H), 1.89 (dd, *J* = 7.5, 5.3 Hz, 3H), 1.44 (dtd, *J* = 15.0, 7.5, 4.2 Hz, 1H), 1.33 – 1.24 (m, 1H), 1.03 (d, *J* = 6.9 Hz, 3H), 0.93 (s, 9H), 0.91 – 0.79 (m, 9H), 0.11 (d, *J* = 7.0 Hz, 3H), -0.05 (d, *J* = 11.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.32, 168.53, 161.79,

146.28, 127.49, 70.39, 69.96, 52.42, 51.07, 40.11, 38.26, 31.64, 25.67, 24.22, 18.30, 17.24, 15.95, 11.49, -4.74, -5.17. HR-ESIMS m/z: calculated for $C_{23}H_{41}N_5O_4SSiNa^+$ $[M+Na]^+$: 534.2546, found 534.2588.

(2S,4R)-methyl4-(2-((1R,3R)-3-((2S,3S)-2-azido-3-methylpentanamido)-1-(tert-butyldimethylsilyloxy)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate (27)

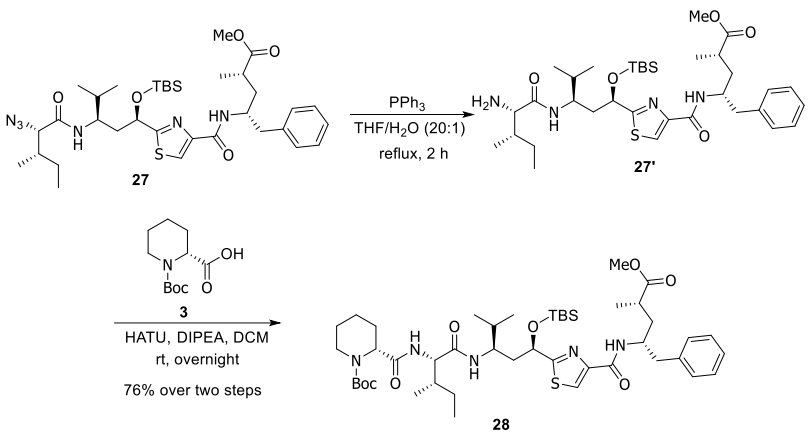


$SOCl_2$ (0.75 mL, 10 mmol) was added dropwise to a solution of Tup fragment (**6**) (0.97 g, 4 mmol) in MeOH (50 mL) at 0 °C. Then the resultant mixture was heated to reflux and stirred for 2 hours. The solution was concentrated in vacuo to give compound **26** which was used for next step directly.

NaOH (1.6 g, 40 mmol) was added to a solution of compound **25** (1.93 g, 3.77 mmol) in THF/H_2O (1:1, 200 mL) at 0 °C. After being stirred at room temperature for 2 h, volatiles of the reaction mixture were removed in vacuo. The solution was diluted with water (100 mL) and adjusted to pH 2 by dropwise addition of $KHSO_4$ (1.0 M in water). The aqueous phase was extracted with ethyl acetate (2×300 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **25'** as an oil which was used for next step directly. The acid **25'**, compound **26** and HATU (3.8 g, 10 mmol) were dissolved in dry DCM (100 mL) at 0 °C. After DIPEA (3.3 mL, 20 mmol) was added, 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 (300 mL) and washed successively with saturated aqueous solution of NH_4Cl (100 mL) and brine (100 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, 1:6) to afford **27** (2.11 g, 80% over two steps) as an oil; $[\alpha]_D^{25}$ +4.50 (c 0.28, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.29 – 7.15 (m, 5H), 6.42 (d, *J* = 9.0 Hz, 1H), 4.97 (dd, *J* = 7.7, 3.9 Hz, 1H), 4.39 (dd, *J* = 9.5, 6.0 Hz, 1H), 4.10 (tt, *J* = 9.2, 3.7 Hz, 1H), 3.85 (d, *J* = 4.2 Hz, 1H), 3.62 (s, 3H), 3.00 – 2.86 (m, 2H), 2.61 (ddd, *J* = 9.4, 7.0, 4.4 Hz, 1H), 2.15 – 1.95 (m, 4H), 1.93 – 1.81 (m, 2H), 1.61 (td, *J* = 9.9, 5.0 Hz, 1H), 1.46 (ddd, *J* = 11.8, 7.5, 4.4 Hz, 1H), 1.28 (s, 1H), 1.15 (d, *J* = 7.1 Hz, 3H), 1.04 (d, *J* = 6.9 Hz, 3H), 0.94 (s, 9H), 0.93 – 0.87 (m, 9H), 0.15 (s, 3H), -0.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.50,

176.38, 168.16, 160.63, 149.63, 137.63, 129.45, 128.26, 126.37, 122.98, 70.49, 70.34, 51.60, 51.09, 48.48, 41.11, 40.95, 38.23, 37.62, 36.41, 31.57, 25.63, 24.18, 17.94, 17.90, 17.71, 15.96, 11.51, -4.83, -5.00. HR-ESIMS m/z: calculated for $C_{35}H_{56}N_6O_5SSiNa^+$ $[M+Na]^+$: 723.3700, found 723.3732.

(R)-tert-butyl2-(((2S,3S)-1-((1R,3R)-1-(4-(((2R,4S)-5-methoxy-4-methyl-5-oxo-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-1-(tert-butyldimethylsilyloxy)-4-methylpentan-3-ylamino)-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (28)

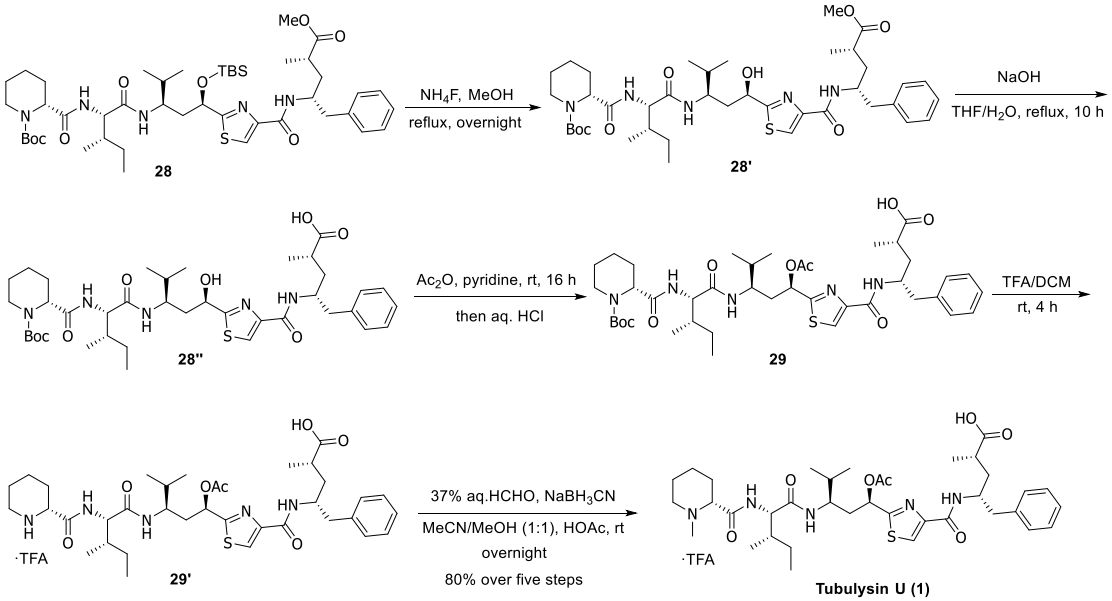


To a solution of **27** (2.11 g, 3.0 mmol) in THF (100 mL) was added H_2O (5 mL) and PPh_3 (7.9 g, 30 mmol) at room temperature. Then the resultant mixture was heated to reflux and stirred for 2 hours. The solution was concentrated in vacuo and the residue was then co-evaporated with toluene for 3 times to provide the desired amine **27'** which was used for next step directly. To a solution of the above amine **27'** in dry DCM (100 mL) was added compound **3** [7] (0.92 g, 4 mmol) and HATU (3.8 g, 10 mmol) at room temperature. After DIPEA (3.3 mL, 20 mmol) was added, 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 (400 mL) and washed successively with saturated aqueous solution of NH_4Cl (100 mL) and brine (100 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** (2.02 g, 76% over two steps) as an oil; $[\alpha]_D^{25}$ +6.20 (c 0.38, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) (*exists as rotamers*) δ 7.98 (d, *J* = 5.3 Hz, 1H), 7.27 – 7.20 (m, 5H), 6.60 (d, *J* = 8.8 Hz, 1H), 6.35 (d, *J* = 7.6 Hz, 1H), 4.91 (dd, *J* = 8.1, 2.8 Hz, 1H), 4.79 (d, *J* = 8.6 Hz, 1H), 4.39 (dd, *J* = 9.5, 6.1 Hz, 1H), 4.15 (dd, *J* = 10.5, 6.7 Hz, 1H), 4.10 – 4.06 (m, 1H), 4.00 – 3.85 (m, 1H), 3.61 (s, 3H), 3.02 – 2.96 (m, 1H), 2.90 – 2.85 (m, 1H), 2.77 (d, *J* = 14.0 Hz, 1H), 2.61 (dq, *J* = 5.1, 3.5, 2.8 Hz, 1H), 2.52 – 2.39 (m, 1H), 2.30 (d, *J* = 10.3 Hz, 1H), 2.04 – 1.78 (m, 6H), 1.63 (dd, *J* = 9.2, 4.9 Hz, 2H), 1.57 – 1.51 (m, 2H), 1.45 (s, 9H), 1.38 (s, 2H), 1.22 (s, 1H), 1.14 (d, *J* = 7.1 Hz, 3H), 0.93 – 0.84 (m, 21H), 0.16 (s, 3H), -0.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) (*exists as rotamers*)

δ 176.89, 176.61, 170.81, 160.72, 149.72, 137.77, 129.46, 129.31, 128.39, 128.33, 126.42, 126.38, 123.02, 122.91, 80.70, 70.45, 58.24, 51.67, 51.36, 48.73, 48.66, 45.19, 41.96, 41.10, 37.72, 37.66, 36.48, 35.73, 31.85, 29.63, 28.26, 25.72, 24.63, 21.39, 21.24, 20.46, 18.23, 17.98, 17.88, 17.72, 15.74, 10.86, -4.79. HR-ESIMS m/z: calculated for $C_{46}H_{75}N_5O_8SSiNa^+$ $[M+Na]^+$: 908.5003, found 908.5040.

[7] G. Xia, L. Liu, H. Liu, J. Yu, Z. Xu, Q. Chen, C. Ma, P. Li, B. Xiong, X. Liu, J. Shen, *ChemMedChem*, **2013**, 8, 577-581

(2S,4R)-4-(2-((1R,3R)-1-acetoxy-4-methyl-3-((2S,3S)-3-methyl-2-((R)-1-methylpiperidine-6-carboxamido)pentanamido)pentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (1)



To a solution of **28** (2.02 g, 2.28 mmol) in MeOH (100 mL) was added NH_4F (4.4 g, 120 mmol) at room temperature. Then the resultant mixture was stirred and heated to reflux overnight. The solution was concentrated in

vacuo and the residue was diluted with water (200 mL). The aqueous phase was extracted with ethyl acetate (2×400 mL). The combined organic phase was washed by brine (150 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the compound **28'** as an oil which was used for next step directly.

NaOH (1.2 g, 30 mmol) was added to a solution of the above compound **28'** in THF/H_2O (1:1, 200 mL) at room temperature. Then the resultant mixture was heated to reflux and stirred for 10 hours. After volatiles of the reaction mixture were removed in vacuo, the solution was diluted with water (100 mL) and adjusted to pH 2 by dropwise addition of $KHSO_4$ (1.0 M in water). The aqueous phase was extracted with ethyl acetate (2×400 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **28''** as an oil which was used for next step directly.

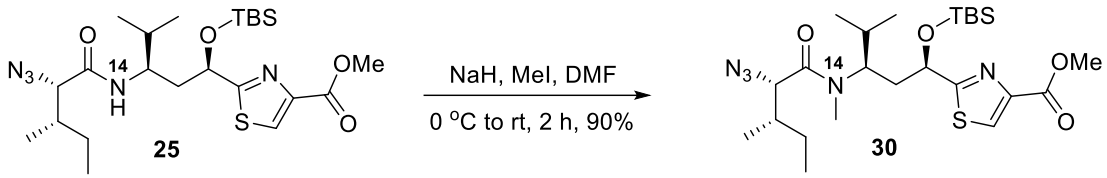
To a solution of the above acid **28''** in pyridine (40 mL) was added Ac_2O (10 mL) at room temperature. Then the resultant mixture was stirred for 16 h at room temperature under N_2 atmosphere. The reaction was quenched with H_2O (300 mL) and adjusted to pH 2 by dropwise addition of conc. HCl (45 mL) at 0 °C. The aqueous phase was extracted with ethyl acetate (3×200 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **29** as an oil which was used for next step directly.

The above acid **29** was dissolved in DCM (50 mL) and trifluoroacetic acid (TFA) (10 mL) was added. Then the resultant mixture was stirred for 4 h at room temperature under N_2 atmosphere. The solvent was evaporated under reduced pressure. The residue was then co-evaporated with toluene to provide the compound **29'** as an oil which was used for next step directly.

The above compound **29'** was suspended in MeCN/MeOH (1:1, 80 mL). A solution of 37% aqueous formaldehyde (4.0 mL) was added, and the reaction mixture was stirred until the peptide had completely dissolved (30 min). $NaBH_3CN$ (2.50 g, 40 mmol) was added followed by addition of glacial acetic acid (to reach pH = 5), and the reaction was stirred overnight at room temperature. The reaction mixture was subsequently concentrated in vacuo and the residue was purified by silica gel column chromatography (MeOH/DCM, 1:10), followed by trituration in i-Pr_2O to provide the desired product **1** as an oil (1.51g, 80% over five steps); $[\alpha]_D^{25}$ -10.5 (c 0.46, MeOH); ^{1}H NMR (400 MHz, CD_3OD) (*exists as rotamers*) δ 8.10 (s, 1H), 7.31 – 7.19 (m, 4H), 7.22 – 7.15 (m, 1H), 5.93 (dd, *J* = 10.9, 2.8 Hz, 1H), 4.39 – 4.32 (m, 1H), 4.24 (d, *J* = 8.2 Hz, 1H), 4.03 – 3.92 (m, 1H), 3.32 – 3.22 (m, 2H), 2.93 (d, *J* = 6.7 Hz, 2H), 2.67 – 2.61 (m, 1H), 2.58 – 2.50 (m, 1H), 2.53 (s, 3H), 2.29 – 2.21 (m, 1H), 2.17 (s, 3H), 2.16 – 2.09 (m, 1H), 2.02 – 1.98 (m, 1H), 1.94 – 1.85 (m, 2H), 1.84 – 1.78 (m, 2H), 1.79 – 1.52 (m, 5H), 1.49 – 1.37 (m, 1H), 1.27 – 1.22 (m, 1H), 1.17 (d, *J* = 7.1 Hz, 3H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.99 – 0.92 (m, 9H). ^{13}C NMR (150 MHz, CD_3OD)

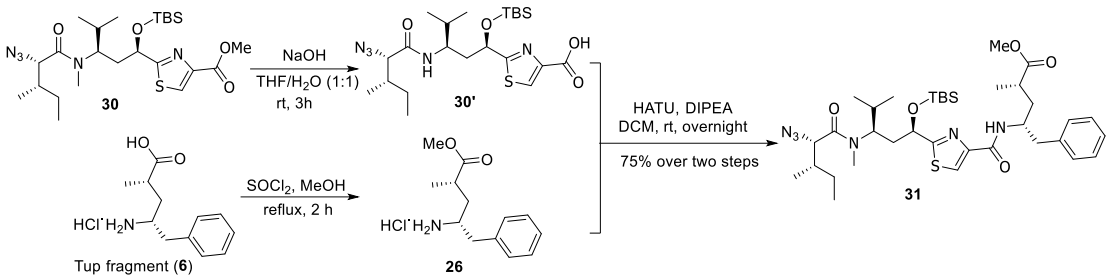
(*exists as rotamers*) δ 179.88, 173.45, 171.77, 169.19, 162.79, 150.79, 139.46, 130.45, 129.33, 127.43, 125.13, 71.19, 68.27, 60.13, 56.22, 52.11, 50.77, 42.96, 42.24, 39.17, 37.90, 37.82, 37.65, 33.80, 30.21, 25.69, 24.00, 22.36, 20.75, 19.57, 19.28, 18.54, 18.32, 16.22, 11.29, 9.17. HR-ESIMS m/z: calculated for $C_{37}H_{56}N_5O_7S^+$ $[M+H]^+$: 714.39004, found 714.38885.

Methyl 2-((1R,3R)-3-((2S,3S)-2-azido-N,3-dimethylpentanamido)-1-(tert-butyldimethylsilyloxy)-4-methylpentyl)thiazole-4-carboxylate (30)



To a solution of **25** (3.0 g, 5.86 mmol) in DMF (50 mL) was added 60% NaH (0.6 g, 15 mmol) at 0 °C. After 30 min, MeI (0.62 mL, 10 mmol) was added. The reaction mixture was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature and stirred for 2h under N_2 atmosphere. Then the reaction was quenched with saturated aqueous solution of NH_4Cl (500 mL) at 0 °C. The aqueous phase was extracted with ethyl acetate (3×300 mL). The combined organic phase was washed by brine (2×200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo.* The residue was purified by silica gel column chromatography (EA/PE, 1:10) to afford **30** (2.77 g, 90%) as an oil; $[\alpha]_D^{25}$ +46.5 (c 0.41, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) (*exists as rotamers*) δ 8.10 (s, 1H), 4.91 (dd, *J* = 6.6, 3.7 Hz, 1H), 4.47 (s, 1H), 3.93 (d, *J* = 5.3 Hz, 3H), 3.51 (d, *J* = 9.5 Hz, 1H), 2.94 (s, 3H), 2.18 (dq, *J* = 9.4, 3.2 Hz, 1H), 2.06 (dq, *J* = 6.5, 3.6 Hz, 2H), 1.79 – 1.74 (m, 1H), 1.25 (tt, *J* = 16.3, 7.3 Hz, 2H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.95 – 0.87 (m, 18H), 0.18 (s, 3H), -0.07 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) (*exists as rotamers*) δ 178.09, 169.57, 161.87, 146.28, 127.50, 70.98, 64.03, 57.23, 52.28, 40.46, 34.95, 30.37, 25.80, 25.06, 20.11, 19.12, 18.01, 16.01, 10.65, -4.67, -4.83. HR-ESIMS m/z: calculated for $C_{24}H_{43}N_5O_4SSiNa^+$ $[M+Na]^+$: 548.2703, found 548.2744.

(2S,4R)-methyl4-(2-((1R,3R)-3-((2S,3S)-2-azido-N,3-dimethylpentanamido)-1-(tert-butyldimethylsilyloxy)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate (31)

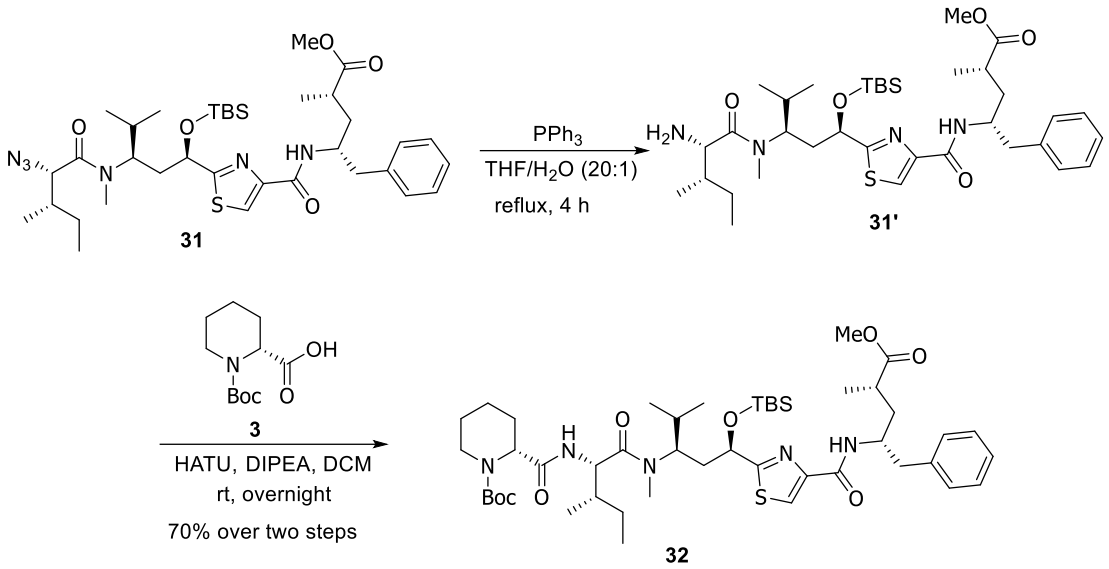


$SOCl_2$ (1.2 mL, 15 mmol) was added dropwise to a solution of Tup (**6**) (1.5 g, 6 mmol) in MeOH (100 mL) at 0 °C. Then the resultant mixture was heated to reflux and stirred for 2 hours. The solution was concentrated in vacuo to give compound **26** which was used for next step directly.

NaOH (2.4 g, 60 mmol) was added to a solution of compound **30** (2.77 g, 5.27 mmol) in THF/H_2O (1:1, 300 mL) at 0 °C. After being stirred at room temperature for 3 h, volatiles of the reaction mixture were removed in vacuo. The solution was diluted with water (100 mL) and adjusted to pH 2 by dropwise addition of $KHSO_4$ (1.0 M in water). The aqueous phase was extracted with ethyl acetate (2×300 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **30'** as an oil which was used for next step directly.

The acid **30'**, compound **26** and HATU (5.7 g, 15 mmol) were dissolved in dry DCM (200 mL) at 0 °C. After DIPEA (5.0 mL, 30 mmol) was added, 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 (300 mL) and washed successively with saturated aqueous solution of NH_4Cl (100 mL) and brine (100 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, 1:8) to afford **31** (2.83 g, 75% over two steps) as an oil; $[\alpha]_D^{25}$ +2.70 (c 0.35, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) (*exists as rotamers*) δ 7.97 (d, *J* = 6.5 Hz, 1H), 7.29 – 7.17 (m, 5H), 5.03 (ddd, *J* = 180.6, 6.8, 3.3 Hz, 1H), 4.57 – 4.31 (m, 2H), 3.61 (d, *J* = 9.1 Hz, 3H), 3.47 (dd, *J* = 31.5, 9.9 Hz, 1H), 3.07 – 2.93 (m, 1H), 2.95 – 2.83 (m, 3H), 2.70 – 2.57 (m, 1H), 2.44 (s, 1H), 2.18 – 1.96 (m, 4H), 1.76 (ddt, *J* = 12.0, 9.4, 6.3 Hz, 2H), 1.62 (ddd, *J* = 14.2, 9.9, 4.5 Hz, 1H), 1.29 (s, 1H), 1.23 (dd, *J* = 7.0, 2.0 Hz, 1H), 1.15 (dd, *J* = 10.1, 7.1 Hz, 3H), 1.04 (dd, *J* = 12.2, 6.5 Hz, 3H), 1.00 – 0.81 (m, 18H), 0.21 – -0.10 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) (*exists as rotamers*) δ 176.90, 176.43, 176.37, 176.13, 169.55, 169.42, 160.89, 160.73, 150.46, 149.64, 138.61, 137.62, 129.42, 129.29, 128.25, 128.02, 126.33, 125.94, 122.75, 72.02, 70.78, 63.93, 63.11, 61.15, 57.58, 51.58, 51.45, 48.87, 48.48, 41.47, 41.00, 40.70, 40.48, 38.03, 37.58, 36.43, 35.31, 35.01, 30.68, 30.36, 29.59, 25.71, 25.62, 25.08, 20.46, 20.04, 19.27, 18.18, 17.95, 17.65, 16.12, 15.62, 10.64, 10.58, -4.66, -4.92, -4.99, -5.06. HR-ESIMS m/z: calculated for $C_{36}H_{58}N_6O_5SSiNa^+$ $[M+Na]^+$: 737.3856, found 737.3893.

(R)-tert-butyl2-(((2S,3S)-1-(((1R,3R)-1-(4-(((2R,4S)-5-methoxy-4-methyl-5-oxo-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-1-(tert-butyldimethylsilyloxy)-4-methylpentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (32)



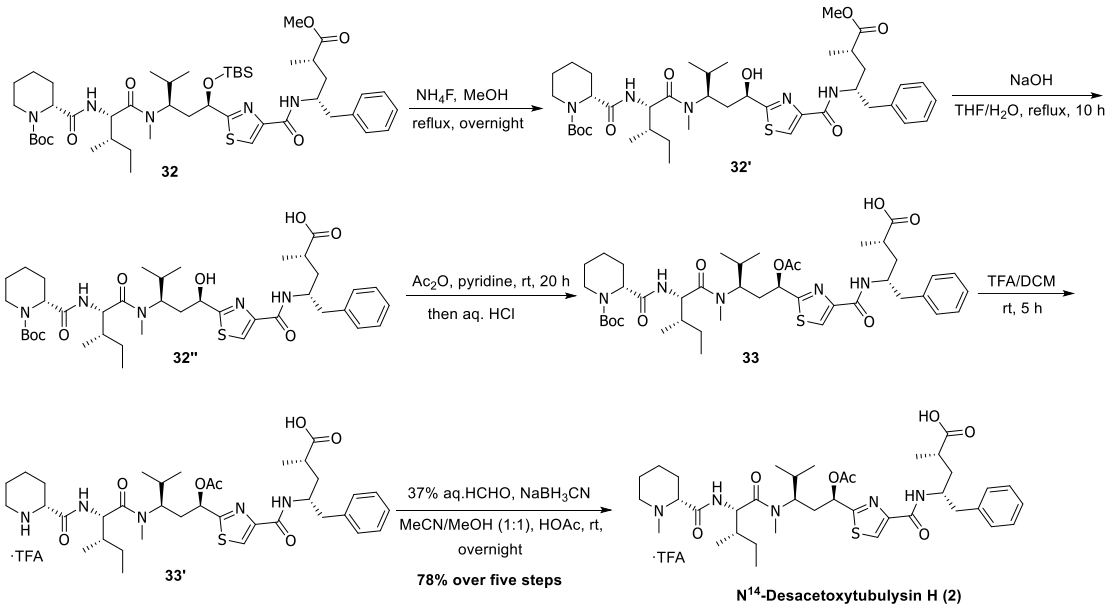
To a solution of **31** (2.83 g, 3.96 mmol) in THF (100 mL) was added H_2O (5 mL) and PPh_3 (10.5 g, 40 mmol) at room temperature. Then the resultant mixture was heated to reflux and stirred for 4 hours. The solution was concentrated in vacuo and the residue was then co-evaporated with toluene for 4 times to provide the desired amine **31'** which was used for next step directly.

To a solution of the above amine **31'** in dry DCM (150 mL) was added compound **3** [7] (0.92 g, 4 mmol) and HATU (5.7 g, 15 mmol) at room temperature. After DIPEA (5.0 mL, 30 mmol) was added, 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 (400 mL) and washed successively with saturated aqueous solution of NH_4Cl (100 mL) and brine (100 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 **32** (2.50 g, 70% over two steps) as an oil; $[\alpha]_D^{25}$ -0.90 (c 0.41, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) (*exists as rotamers*) δ 7.97 (d, *J* = 3.4 Hz, 1H), 7.27 – 7.15 (m, 5H), 6.99 (d, *J* = 8.8 Hz, 1H), 6.67 (d, *J* = 9.1 Hz, 1H), 4.85 – 4.68 (m, 3H), 4.48 (s, 1H), 4.44 – 4.34 (m, 1H), 4.13 – 3.90 (m, 1H), 3.59 (d, *J* = 3.3 Hz, 3H), 2.94 (s, 3H), 2.88 (d, *J* = 5.6 Hz, 2H), 2.74 (d, *J* = 20.9 Hz, 1H), 2.62 – 2.54 (m, 1H), 2.28 (d, *J* = 12.5 Hz, 1H), 2.05 – 1.98 (m, 2H), 1.93 (d, *J* = 10.3 Hz, 1H), 1.77 (s, 1H), 1.70 – 1.64 (m, 1H), 1.54 (dd, *J* = 18.6, 9.0 Hz, 4H), 1.44 (s, 9H), 1.34 (s, 2H), 1.16 – 1.11 (m, 3H), 1.00 – 0.96 (m, 3H), 0.92 – 0.76 (m, 18H), 0.16 (d, *J* = 3.2 Hz, 3H), -0.11 (d, *J* = 3.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$)

(*exists as rotamers*) δ 176.55, 176.28, 172.95, 170.76, 160.56, 149.40, 137.28, 129.38, 128.22, 126.38, 122.77, 80.34, 70.73, 57.12, 53.42, 51.57, 48.17, 40.83, 40.69, 37.80, 37.11, 36.36, 30.22, 30.00, 29.53, 28.18, 25.67, 25.56, 24.15, 20.43, 19.96, 19.32, 17.87, 17.68, 15.65, 10.91, -4.76, -5.01. HR-ESIMS m/z: calculated for $C_{47}H_{77}N_5O_8SSiNa^+$ $[M+Na]^+$: 922.5160, found 922.5199.

[7] G. Xia, L. Liu, H. Liu, J. Yu, Z. Xu, Q. Chen, C. Ma, P. Li, B. Xiong, X. Liu, J. Shen, *ChemMedChem*, **2013**, 8, 577-581

(2S,4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-6-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (2)



To a solution of **32** (2.50 g, 2.78 mmol) in MeOH (150 mL) was added NH_4F (5.6 g, 150 mmol) at room temperature. Then the resultant mixture was stirred and heated to reflux overnight. The solution was concentrated in vacuo and the residue was diluted with water (200 mL). The aqueous phase was extracted with ethyl acetate (2×400 mL). The combined organic phase was washed by brine (150 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the compound **32'** as an oil which was used for next step directly.

NaOH (1.6 g, 40 mmol) was added to a solution of the above compound **32'** in THF/H_2O (1:1, 200 mL) at room temperature. Then the resultant mixture was heated to reflux and stirred for 10 hours. volatiles of the reaction mixture were removed in vacuo, and the resulting solution was diluted with water (100 mL) and adjusted to pH 2 by dropwise addition of $KHSO_4$ (1.0 M in water). The aqueous phase was extracted with ethyl acetate (2×400 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **32''** as an oil which was used for next step directly.

To a solution of the above acid **32''** in pyridine (50 mL) was added Ac_2O (15 mL) at room temperature. Then the resultant mixture was stirred for 20 h at room temperature under N_2 atmosphere. The reaction was quenched with H_2O (300 mL) and adjusted to pH 2 by dropwise addition of conc. HCl (55 mL) at 0 °C. The aqueous phase was extracted with ethyl acetate (3×200 mL). The combined organic phase was washed by brine (200 mL), dried over sodium sulfate (anhydrous) and concentrated in *vacuo* to give the acid **33** as an oil which was used for next step directly.

The above acid **33** was dissolved in DCM (50 mL) and trifluoroacetic acid (TFA) (10 mL) was added. Then the resultant mixture was stirred for 5 h at room temperature under N_2 atmosphere. The solvent was evaporated under

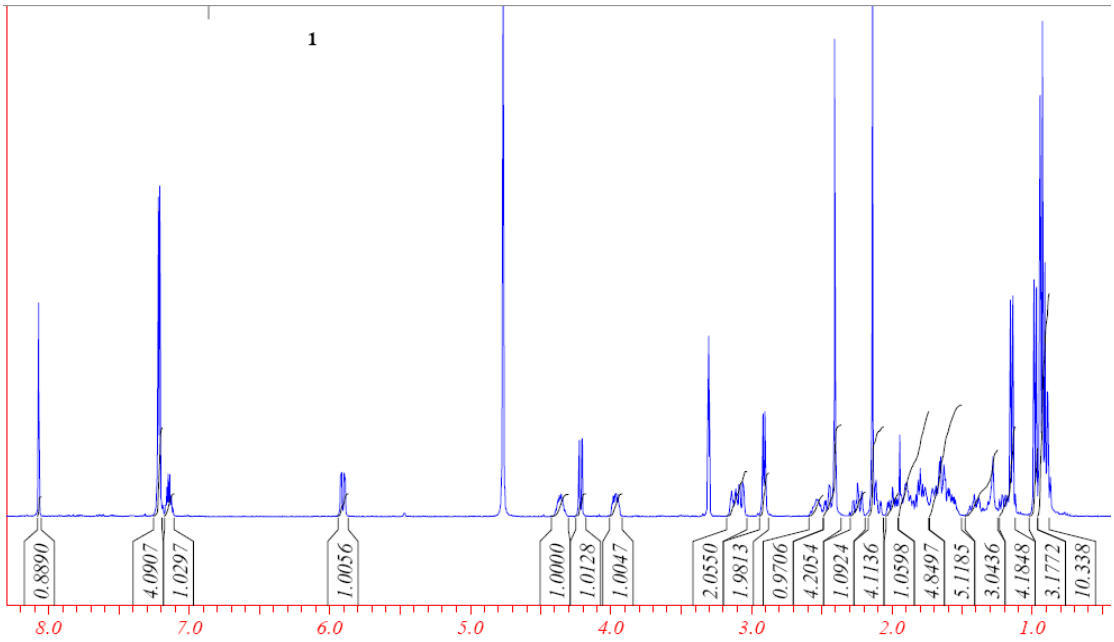
reduced pressure. The residue was then co-evaporated with toluene to provide the compound **33'** as an oil which was used for next step directly.

The above compound **33'** was suspended in MeCN/MeOH (1:1, 100 mL). A solution of 37% aqueous formaldehyde (5.0 mL) was added, and the reaction mixture was stirred until the peptide had completely dissolved (30 min). $NaBH_3CN$ (3.15 g, 50 mmol) was added followed by addition of glacial acetic acid (to reach pH = 5), and the reaction was stirred overnight at room temperature. The reaction mixture was subsequently concentrated in vacuo and the residue was purified by silica gel column chromatography (MeOH/DCM, 1:10), followed by trituration in i-Pr_2O to provide the desired product **2** as an oil (1.83 g, 78% over five steps); $[\alpha]_D^{25}$ -14.4 (c 0.26, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 8.09 (s, 1H), 7.27 – 7.21 (m, 4H), 7.19 – 7.13 (m, 1H), 5.71 (dd, *J* = 11.1, 2.3 Hz, 1H), 4.71 (d, *J* = 7.5 Hz, 1H), 4.51 – 4.26 (m, 2H), 3.76 (dd, *J* = 12.3, 2.8 Hz, 1H), 3.47 (d, *J* = 12.3 Hz, 1H), 3.12 (s, 3H), 3.09 – 3.04 (m, 1H), 2.90 (p, *J* = 7.2, 6.7 Hz, 2H), 2.74 (s, 3H), 2.54 (ddt, *J* = 10.0, 7.1, 4.1 Hz, 1H), 2.38 (td, *J* = 13.0, 11.3, 2.7 Hz, 1H), 2.33 – 2.23 (m, 1H), 2.20 – 2.16 (m, 1H), 2.15 (s, 3H), 2.02– 1.98 (m, 1H), 1.96 – 1.86 (m, 4H), 1.82 – 1.74 (m, 2H), 1.69 – 1.65 (m, 1H), 1.62 – 1.57 (m, 2H), 1.23 – 1.18 (m, 1H), 1.17 (d, *J* = 7.1 Hz, 3H), 1.03 (d, *J* = 6.5 Hz, 3H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H), 0.84 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 179.92, 174.61, 171.82, 171.62, 169.16, 162.80, 150.84, 139.50, 130.48, 129.33, 127.42, 125.16, 71.23, 68.18, 56.24, 56.02, 50.67, 42.93, 42.22, 39.09, 37.81, 37.42, 35.57, 30.91, 30.29, 25.26, 24.00, 22.39, 20.83, 20.52, 20.33, 18.52, 16.24, 11.32. HR-ESIMS m/z: calculated for $C_{38}H_{58}N_5O_7S^+$ $[M+H]^+$: 728.40569, found 728.40503.

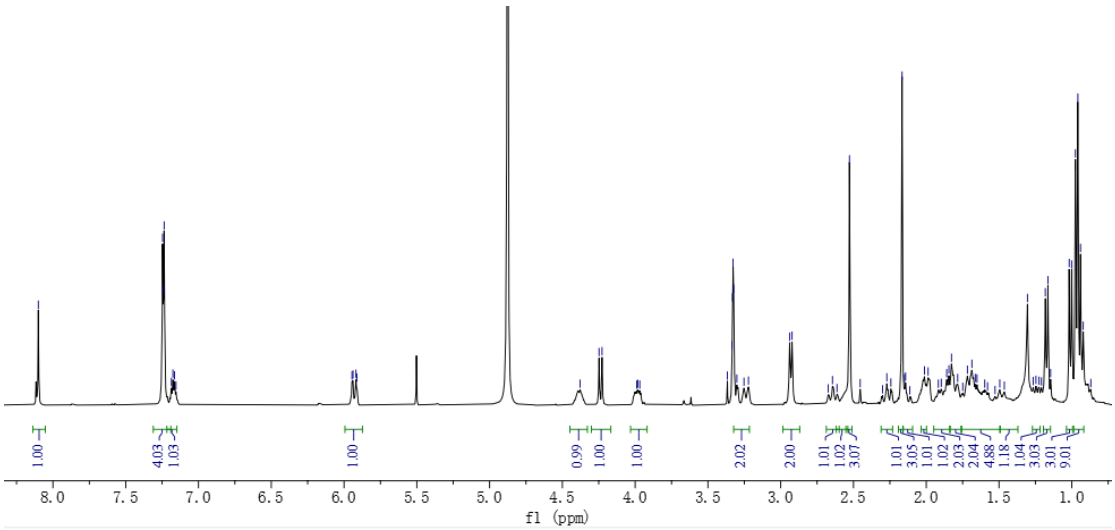
Comparison of Spectra of 1 and 2 with Previous Reports

^{1}H NMR of Tubulysin U (**1**)

Zanda's synthetic sample (Bruker AV 400, 400 MHz, CD_3OD)[8] (*Angew. Chem. Int. Ed.*, **2007**, 46, 3526-3529)

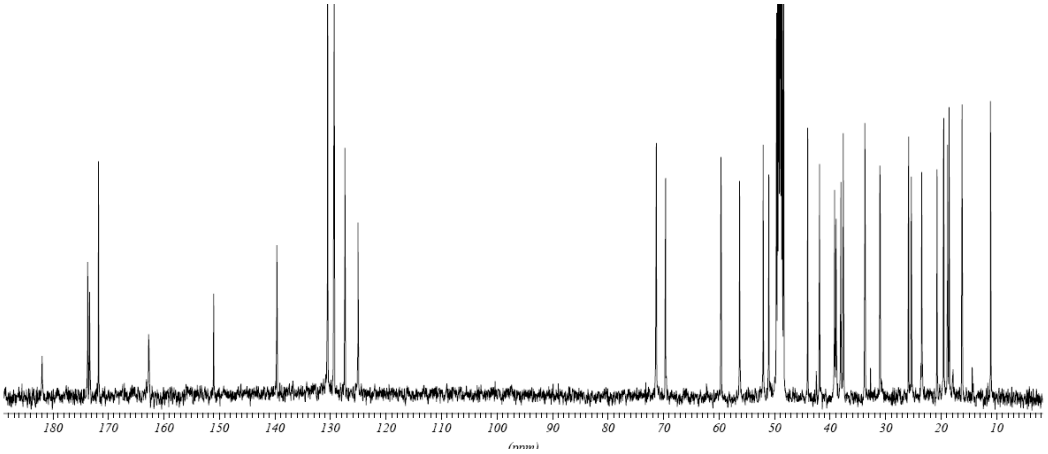


Our synthetic sample **1** (Bruker AV 400, 400 MHz, CD_3OD) (this work)

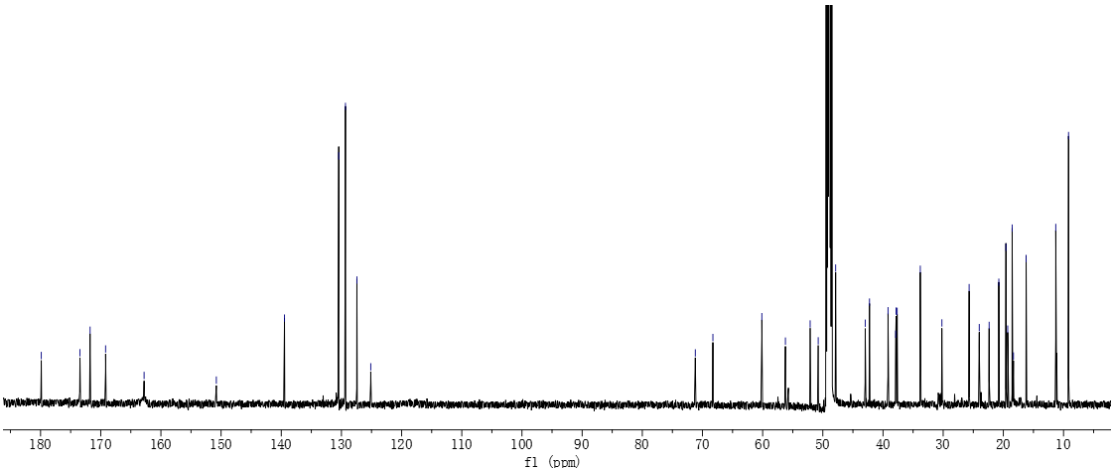


^{13}C NMR of Tubulysin U (**1**)

Zanda's synthetic sample (Bruker AV 400, 100 MHz, CD_3OD)[8] (*Angew. Chem. Int. Ed.*, **2007**, 46, 3526-3529)

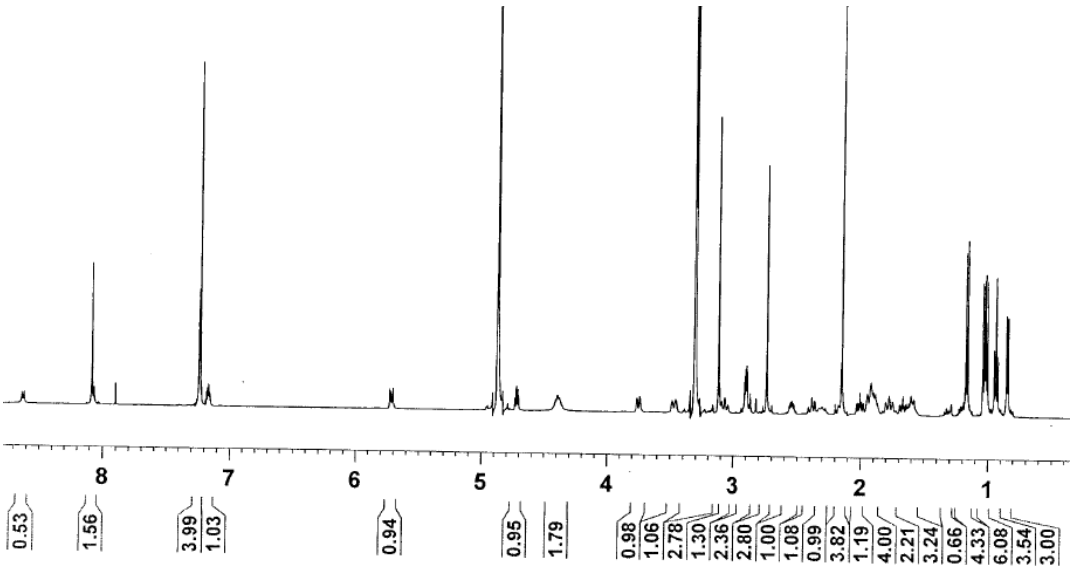


Our synthetic sample **1** (Bruker AV 600, 150 MHz, CD_3OD) (this work)

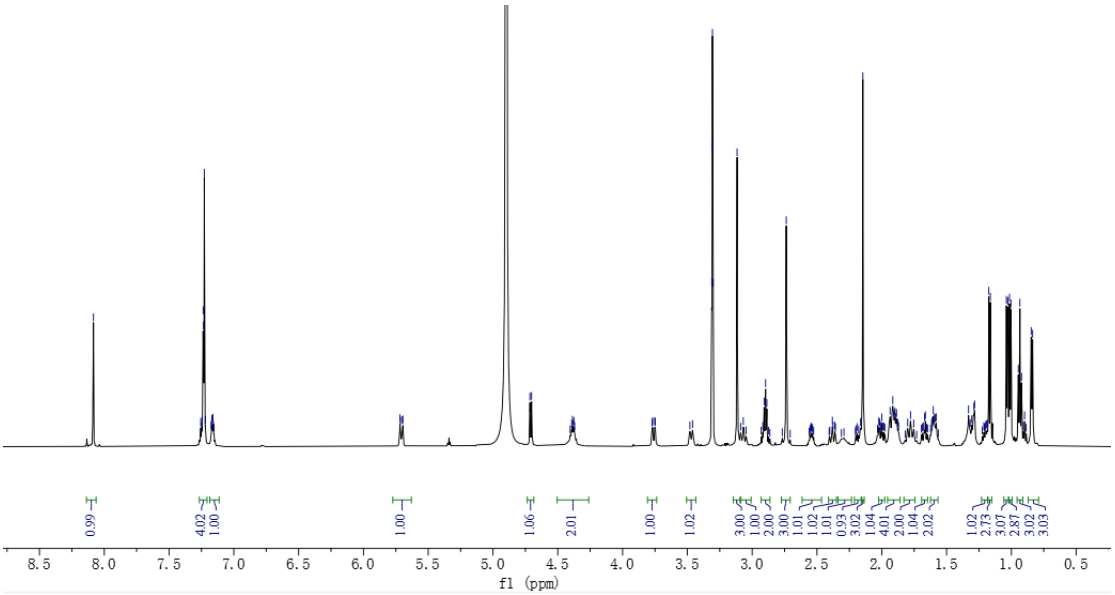


^{1}H NMR of N^{14}-Desacetoxytubulysin H (**2**)

Wipf 's synthetic sample (Bruker AV 500, 500 MHz, CD_3OD)[9] (*Org. Lett.*, **2007**, 9, 1605-1607)

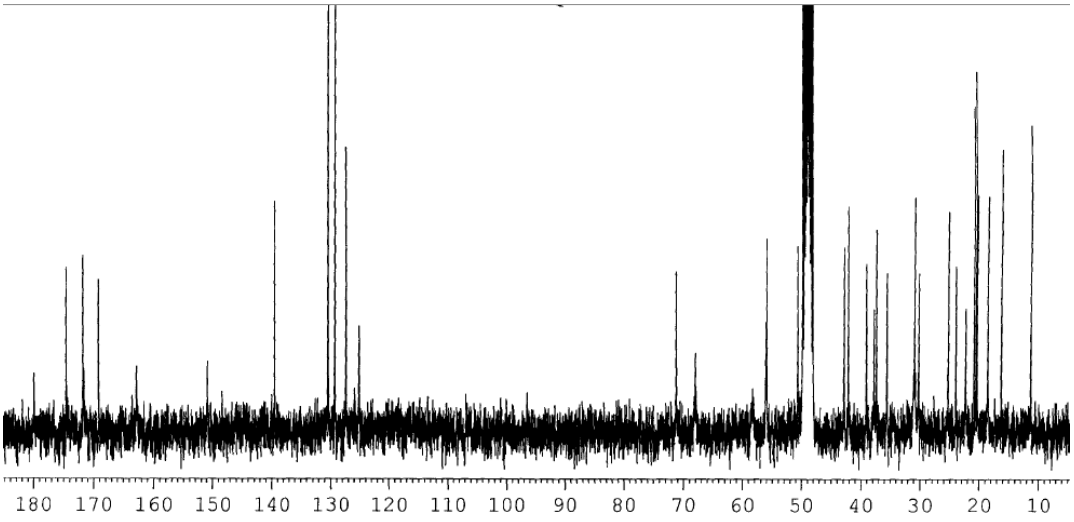


Our synthetic sample **2** (Bruker AV 600, 600 MHz, CD_3OD) (this work)



^{13}C NMR of N^{14}-Desacetoxytubulysin H (**2**)

Wipf 's synthetic sample (Bruker AV 300, 75 MHz, CD_3OD)[9] (*Org. Lett.*, **2007**, 9, 1605-1607)



Our synthetic sample **2** (Bruker AV 600, 150 MHz, CD_3OD) (this work)

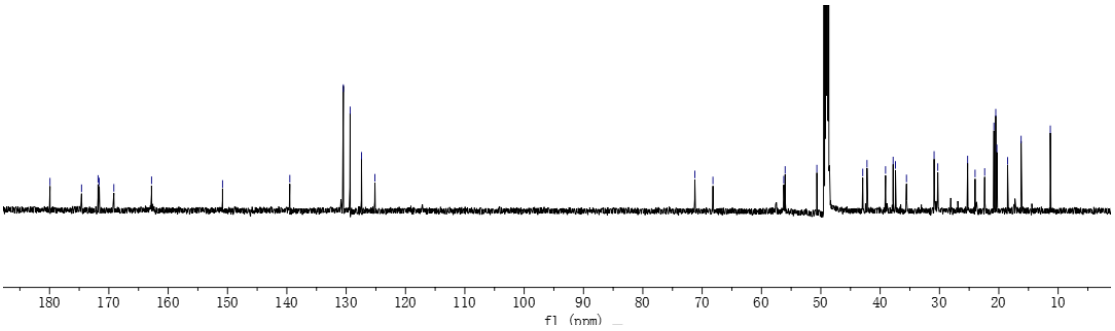


Table S1 ^{1}H NMR and ^{13}C NMR Spectroscopic Data of Our synthetic **1**, Zanda's synthetic **1** [8] in CD_3OD

[8] M. Sani, G. Fossati, F. Huguenot, and M. Zanda, *Angew. Chem. Int. Ed.*, **2007**, 46, 3526-3529

δH of Our synthetic **1** (400 MHz)	δH of Zanda's synthetic **1** (400 MHz)	δC of Our synthetic **1** (150 MHz)	δC of Zanda's synthetic **1** (100 MHz)
8.10 (s, 1H)	8.07 (s, 1H)	179.88	181.9
7.31 – 7.19 (m, 4H)	7.21 (d, *J* = 4.2 Hz, 4H)	173.45	173.7
7.22 – 7.15 (m, 1H)	7.17 – 7.11 (m, 1H)	171.77	173.3
5.93 (dd, *J* = 10.9, 2.8 Hz, 1H)	5.90 (dd, *J* = 10.8 and 3.0 Hz, 1H)	169.19	171.7
4.39 – 4.32 (m, 1H)	4.39 – 4.32 (m, 1H)	162.79	162.7
4.24 (d, *J* = 8.2 Hz, 1H)	4.21 (d, *J* = 8.2 Hz, 1H)	150.79	151.1
4.03 – 3.92 (m, 1H)	3.99 – 3.94 (m, 1H)	139.46	139.6
3.32 – 3.22 (m, 2H)	3.13 – 3.05 (m, 2H)	130.45	130.5
2.93 (d, *J* = 6.7 Hz, 2H)	2.91 (d, *J* = 6.8 Hz, 2H)	129.33	129.3
2.67 – 2.61 (m, 1H)	2.57 – 2.49 (m, 1H)	127.43	127.3
2.58 – 2.50 (m, 1H)	2.48 – 2.42 (m, 1H)	125.13	125.0
2.53 (s, 3H)	2.40 (s, 3H)	71.19	71.3
2.29 – 2.21 (m, 1H)	2.28 – 2.21 (m, 1H)	68.27	69.6
2.17 (s, 3H)	2.14 (s, 3H)	60.13	59.6
2.16 – 2.09 (m, 1H)	2.13 – 2.07 (m, 1H)	56.22	56.3
2.02 – 1.98 (m, 1H)	2.02 – 1.96 (m, 1H)	52.11	52.0
1.94 – 1.85 (m, 2H)	1.93 – 1.84 (m, 2H)	50.77	51.0
1.84 – 1.78 (m, 2H)	1.83 – 1.76 (m, 2H)	42.96	44.0
1.79 – 1.52 (m, 5H)	1.71 – 1.53 (m, 5H)	42.24	41.9
1.49 – 1.37 (m, 1H)	1.45 – 1.35 (m, 1H)	39.17	39.2

1.27 – 1.22 (m, 1H)	1.23 – 1.12 (m, 1H)	37.90	38.9
1.17 (d, *J* = 7.1 Hz, 3H)	1.14 (d, *J* = 7.0 Hz, 3H)	37.82	38.1
1.01 (d, *J* = 6.8 Hz, 3H)	0.97 (d, *J* = 6.8 Hz, 3H)	37.65	37.6
0.99 – 0.92 (m, 9H)	0.94 – 0.87 (m, 9H)	33.80	33.7
		30.21	31.0
		25.69	25.9
		24.00	25.4
		22.36	23.5
		20.75	20.7
		19.57	19.5
		19.28	18.8
		18.54	18.6
		16.22	16.2
		11.29	11.1

Table S2 ^{1}H NMR and ^{13}C NMR Spectroscopic Data of Our synthetic **2**, Ellman's synthetic **2** [9,10], Wipf 's synthetic **2** [11] in CD_3OD

[9] A. W. Patterson, H. M. Peltier, and J. A. Ellman, *J. Org. Chem.,* **2008***,* 73, 4362-4369

[10] A. W. Patterson, H. M. Peltier, F. Sasse, and J. A. Ellman, *Chem. Eur. J.*, **2007**, 13, 9534-9541

[11] P. Wipf and Z. Wang, *Org. Lett.*, **2007**, 9, 1605-1607.

δH of Our synthetic **2** (600 MHz)	δH of Ellman's synthetic **2** (500 MHz)	δH of Wipf 's synthetic **2** (500 MHz)	δC of Our synthetic **2** (150 MHz)	δC of Ellman's synthetic **2** (125 MHz)	δC of Wipf 's synthetic **2** (75 MHz)
		8.63 (d, *J* = 8.0 Hz, 1H)	179.92	182.5	179.9
8.09 (s, 1H)	8.08 (s, 1 H)	8.09 (s, 1H)	174.61	175.0	174.6
		8.07 (bs, 1H)	171.82	173.6	171.8
7.27 – 7.21 (m, 4H)	7.25 – 7.19 (m, 4 H)	7.24 – 7.23 (m, 4 H)	171.62	171.8	171.6
7.19 – 7.13 (m, 1H)	7.18 – 7.13 (m, 1 H)	7.19-7.16 (m, 1 H)	169.16	171.6	169.2
5.71 (dd, J = 11.1, 2.3 Hz, 1H)	5.71 (dd, *J* = 11.0, 2.5 Hz, 1H)	5.72 (dd, *J* = 11.0, 2.5 Hz, 1H)	162.80	162.7	162.8
4.71(d, *J* = 7.5 Hz, 1H)	4.73 (d, *J* = 8.0 Hz, 1H)	4.74-4.70 (m, 1 H)	150.84	151.1	150.8
4.51 – 4.26 (m, 2H)	4.50 – 4.30 (m, 2H)	4.42-4.36 (m, 2 H)	139.50	139.8	139.5
3.76 (dd, J = 12.3, 2.8 Hz, 1H),		3.75 (dd, *J* = 12.8, 3.8 Hz, 1H)	130.48	130.6	130.5

3.47 (d, *J* = 12.3, 1H)		3.49-3.45 (m, 1H)	129.33	129.3	129.3
3.12 (s, 3H)	3.10 (s, 3H)	3.12 (s, 3 H)	127.42	127.4	127.4
3.09 – 3.04 (m, 1H)	3.05 (d, *J* = 11.5 Hz, 1H)	3.11 – 3.04 (m, 1H)	125.16	125.1	125.1
2.90 (p, *J* = 7.2, *J* = 6.7, 2H)	2.92 (d, *J* = 6.5 Hz, 2H)	2.90 (dd, *J* = 6.8, 3.2 Hz, 2H)	71.23	71.2	71.2
2.74 (s, 3H)	2.85 (d, J = 10.5 Hz, 1H)	2.74 (s, 3 H)	68.18	69.7	68.0
2.54 (ddt, *J* = 10.0, 7.1, 4.1 Hz, 1H)	2.51 (br s, 1 H)	2.58-2.53 (m, 1H)	56.24	56.4	56.2
2.38 (td, *J* = 13.0, 11.3, 2.7 Hz, 1H)	2.41 – 2.23 (m, 3 H)	2.39 (ddd, *J* = 14.5, 11.5, 3.2 Hz, 1H)	56.02	55.2	56.0
2.33-2.23 (m, 1 H)	2.31 (s, 3 H)	2.33-2.28 (m, 1 H)	50.67	51.2	50.7
2.20-2.16 (m, 1H)		2.18-2.16 (m, 1H)	42.93	44.2	42.9
2.15 (s, 3H)	2.15 (s, 3 H)	2.15 (s, 3 H)	42.22	42.0	42.2
2.02 – 1.98 (m, 1H)	2.05 – 1.96 (m, 1H)	2.01 (ddd, *J* = 14.0, 10.0, 4.0 Hz, 1H)	39.09	39.6	39.1
1.96-1.86 (m, 4H)	1.92-1.75 (m, 4H)	1.95-1.89 (m, 4H)	37.81	39.5	37.8
1.82-1.74 (m, 2H)	1.74-1.56 (m, 5H)	1.81-1.74 (m, 2H)	37.42	37.6	37.4
1.69 – 1.65 (m, 1H)		1.67 (ddd, *J* = 14.4, 10.1, 4.5	35.57	35.6	35.6

		Hz, 1H)			
1.62 – 1.57 (m, 2H)	1.41-1.37 (m, 1H)	1.61-1.56 (m, 2H)	30.91	31.0	30.9
1.23-1.18 (m, 1H)	1.23-1.09 (m, 1H)	1.23-1.20 (m, 1H)	30.29	30.9	30.2
1.17 (d, J = 7.1 Hz, 3H)	1.16 (d, *J* = 7.0 Hz, 3H)	1.17 (d, *J* = 7.0 Hz, 3H)	25.26	25.5	25.2
1.03 (d, J = 6.5 Hz, 3H)	1.03 (d, *J* = 6.5 Hz, 3H)	1.04 (d, *J* = 6.5 Hz, 3H)	24.00	25.5	24.0
1.01 (d, J = 6.8 Hz, 3H)	0.98 (d, *J* = 6.5 Hz, 3H)	1.01 (d, *J* = 7.0 Hz, 3H)	22.39	23.7	22.3
0.93 (t, J = 7.4 Hz, 3H)	0.92 (t, *J* = 7.3 Hz, 3H)	0.94 (t, *J* = 7.2 Hz, 3H)	20.83	20.9	20.8
0.84 (d, J = 6.6 Hz, 3H)	0.81 (d, *J* = 6.5 Hz, 3H)	0.85 (d, *J* = 7.0 Hz, 3H)	20.52	20.6	20.5
			20.33	20.4	20.3
			18.52	19.1	18.5
			16.24	16.4	16.2
			11.32	11.3	11.3

NMR Spectra of New Compounds and Selected Known Compounds

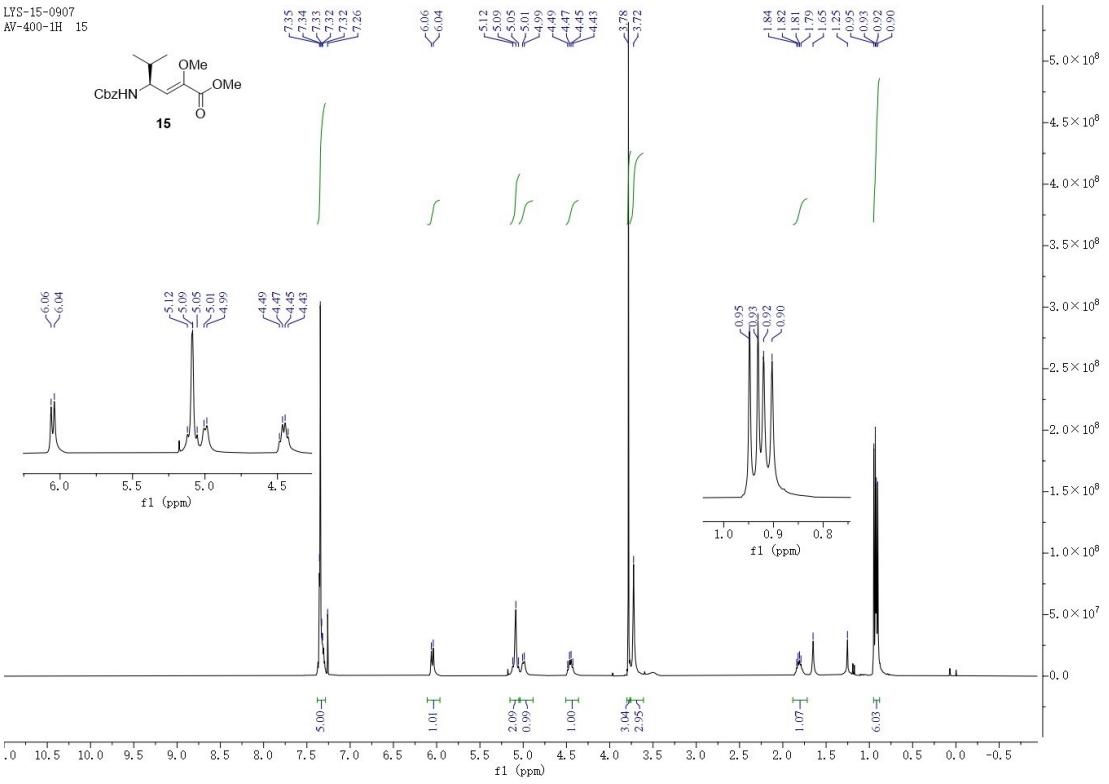


Figure S1. ^{1}H NMR of **15** ($CDCl_3$, 400 MHz)

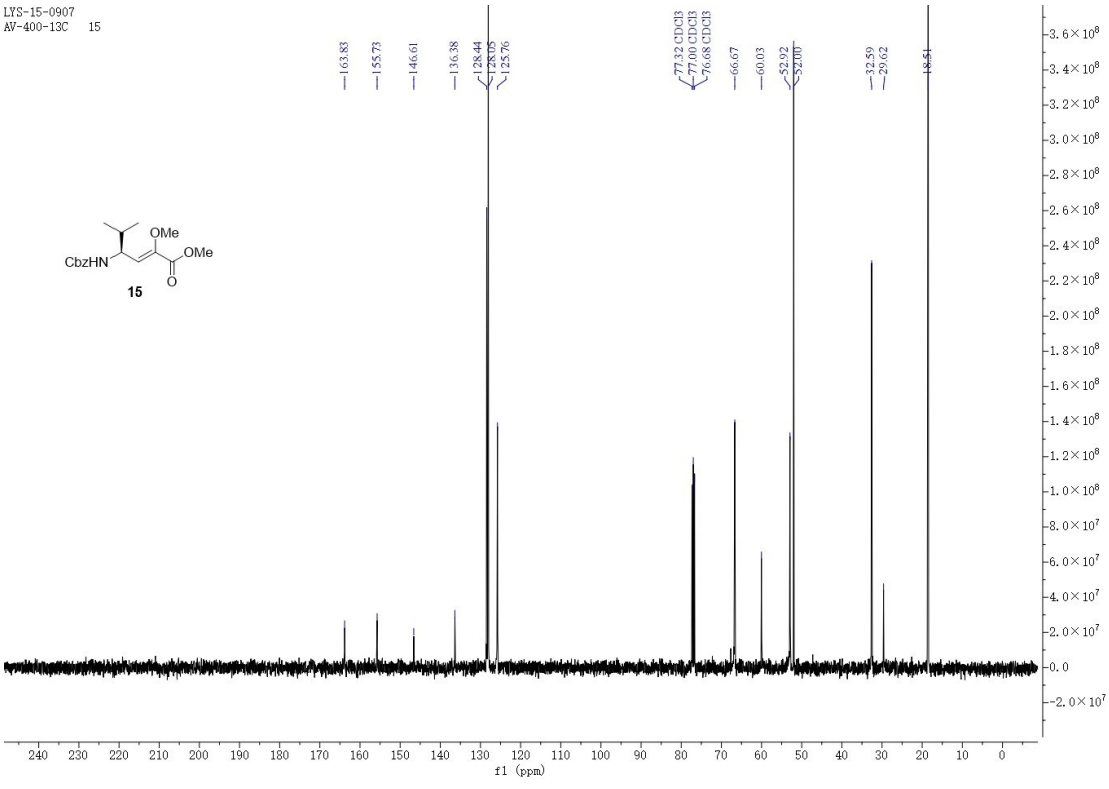


Figure S2. ^{13}C NMR of **15** ($CDCl_3$, 100 MHz)

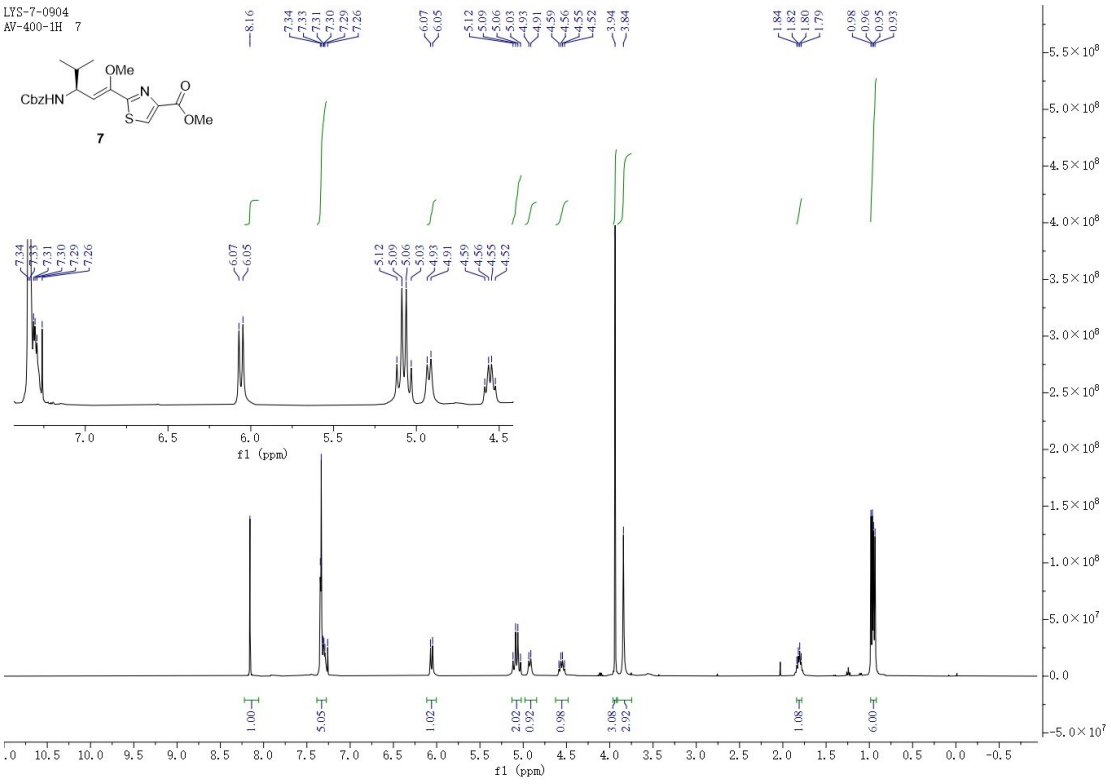


Figure S3. ^{1}H NMR of **7** ($CDCl_3$, 400 MHz)

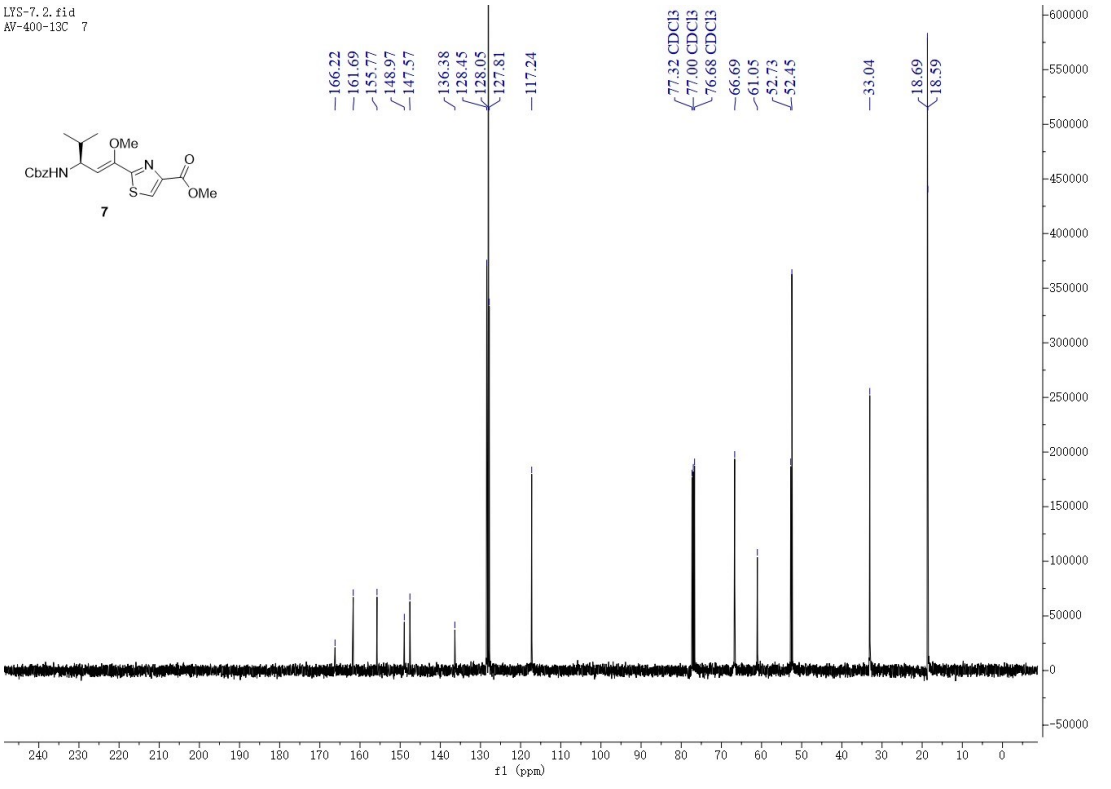


Figure S4. ^{13}C NMR of **7** ($CDCl_3$, 100 MHz)

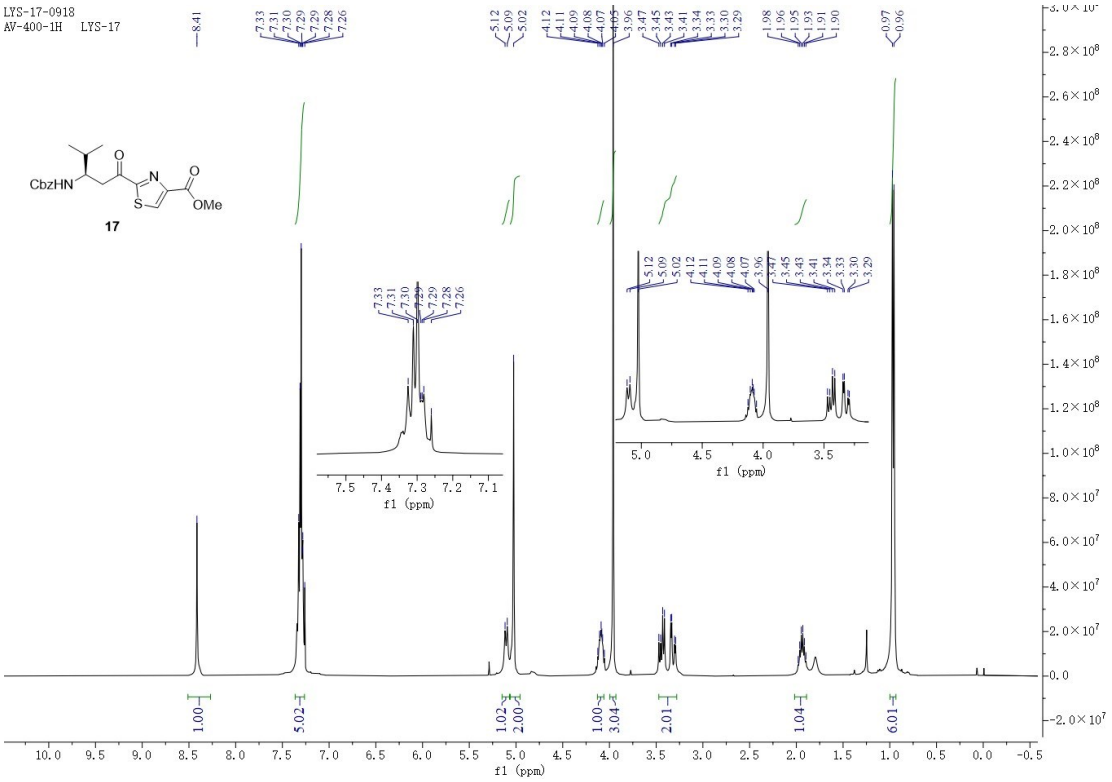


Figure S5. ^{1}H NMR of **17** ($CDCl_3$, 400 MHz)

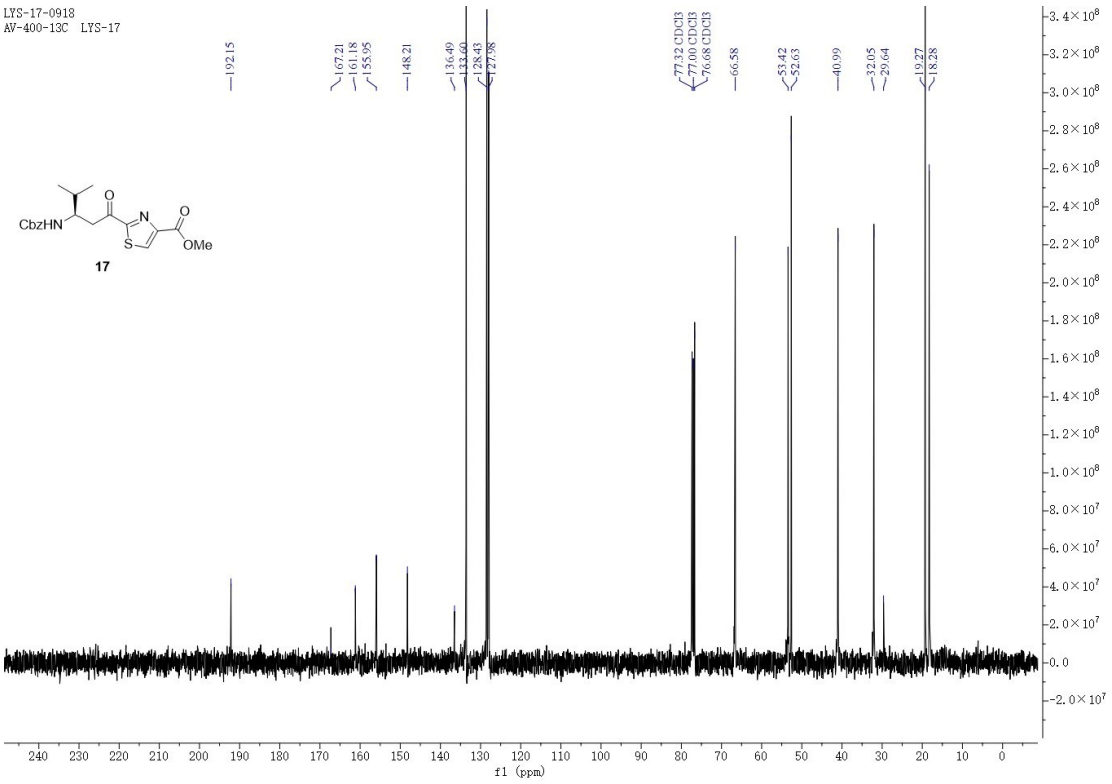


Figure S6. ^{13}C NMR of **17** ($CDCl_3$, 100 MHz)

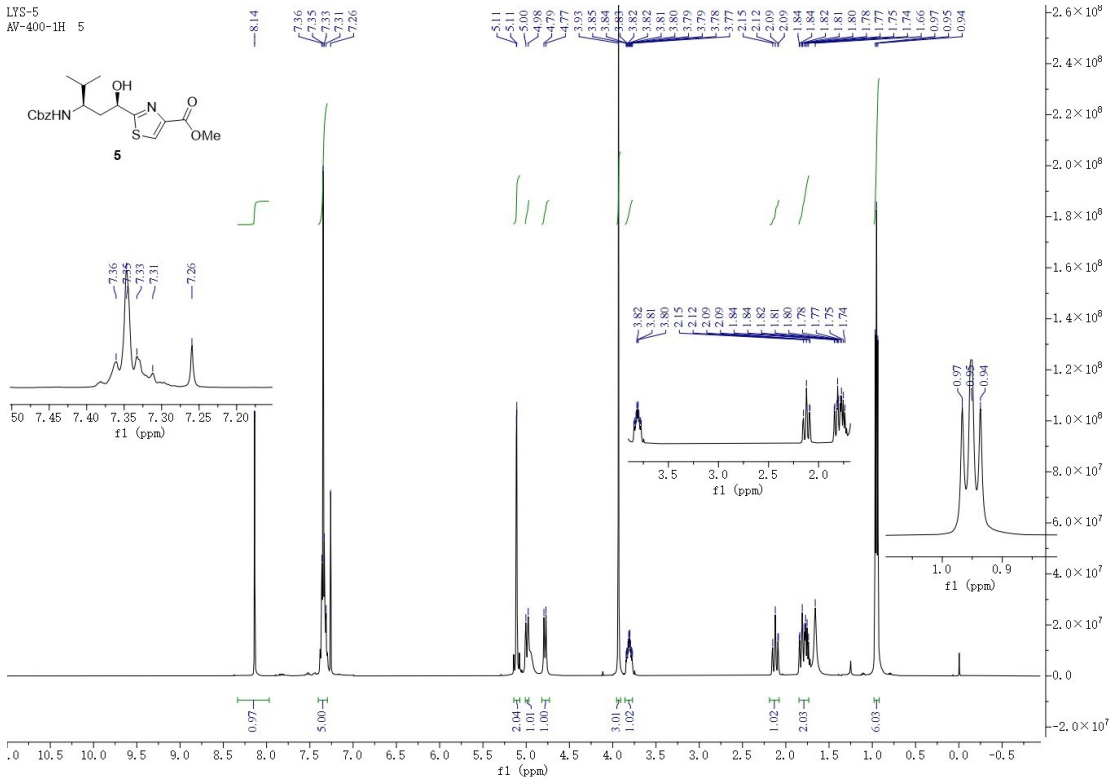


Figure S7. ^{1}H NMR of **5** ($CDCl_3$, 400 MHz)

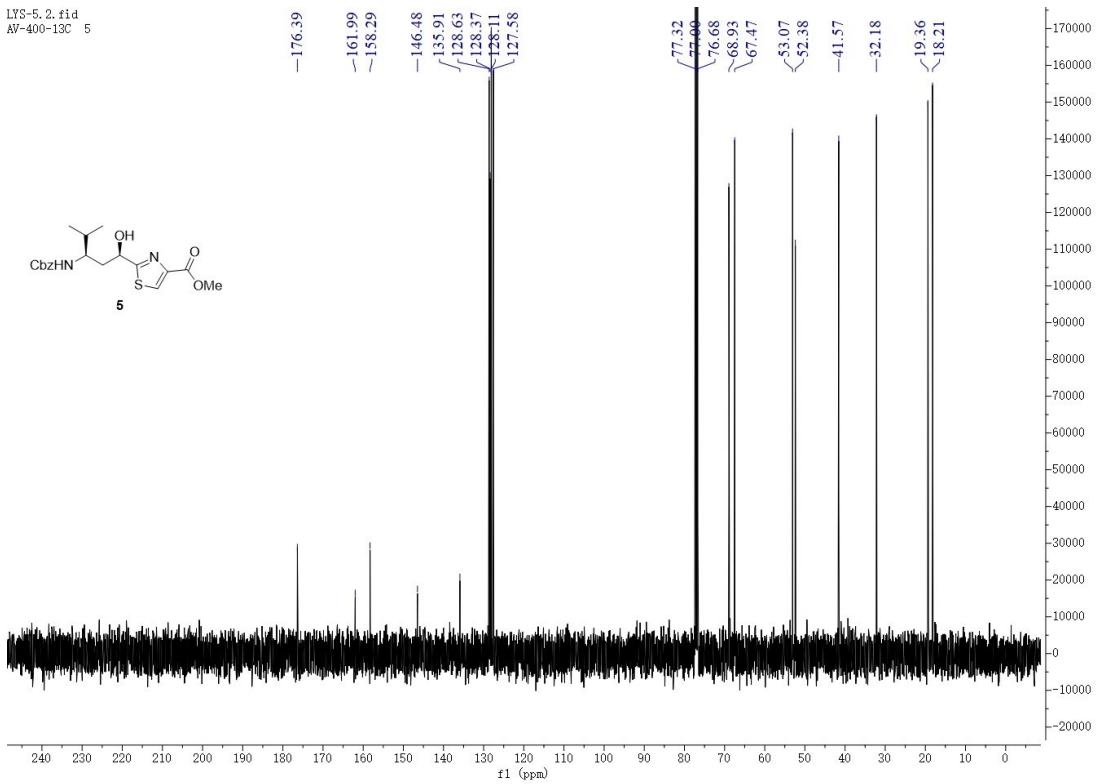


Figure S8. ^{13}C NMR of **5** ($CDCl_3$, 100 MHz)

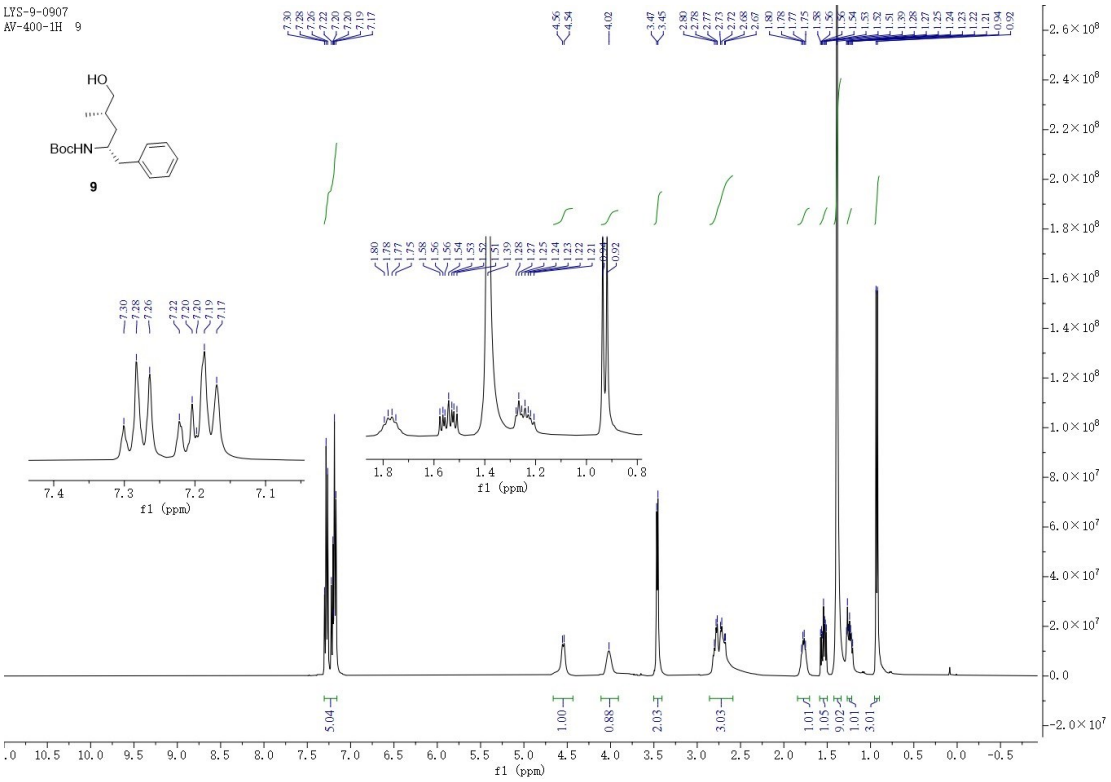


Figure S9. ^{1}H NMR of **9** ($CDCl_3$, 400 MHz)

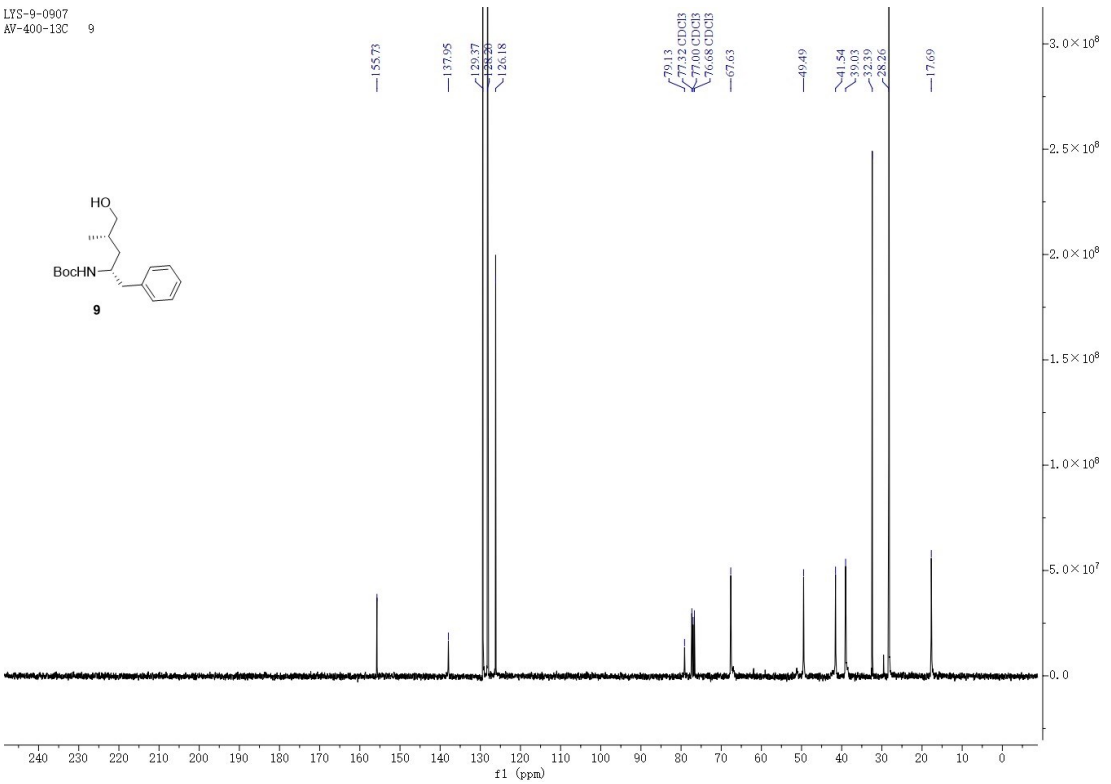


Figure S10. ^{13}C NMR of **9** ($CDCl_3$, 100 MHz)

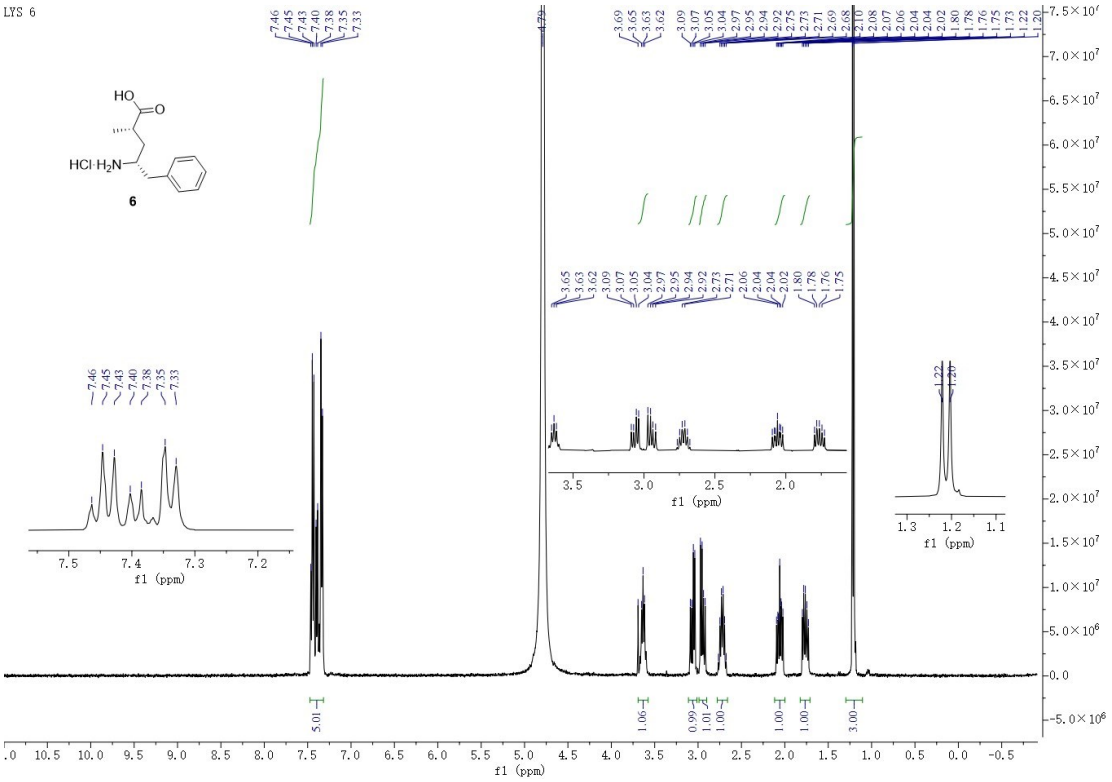


Figure S11. ^{1}H NMR of **6** (D_2O, 400 MHz)

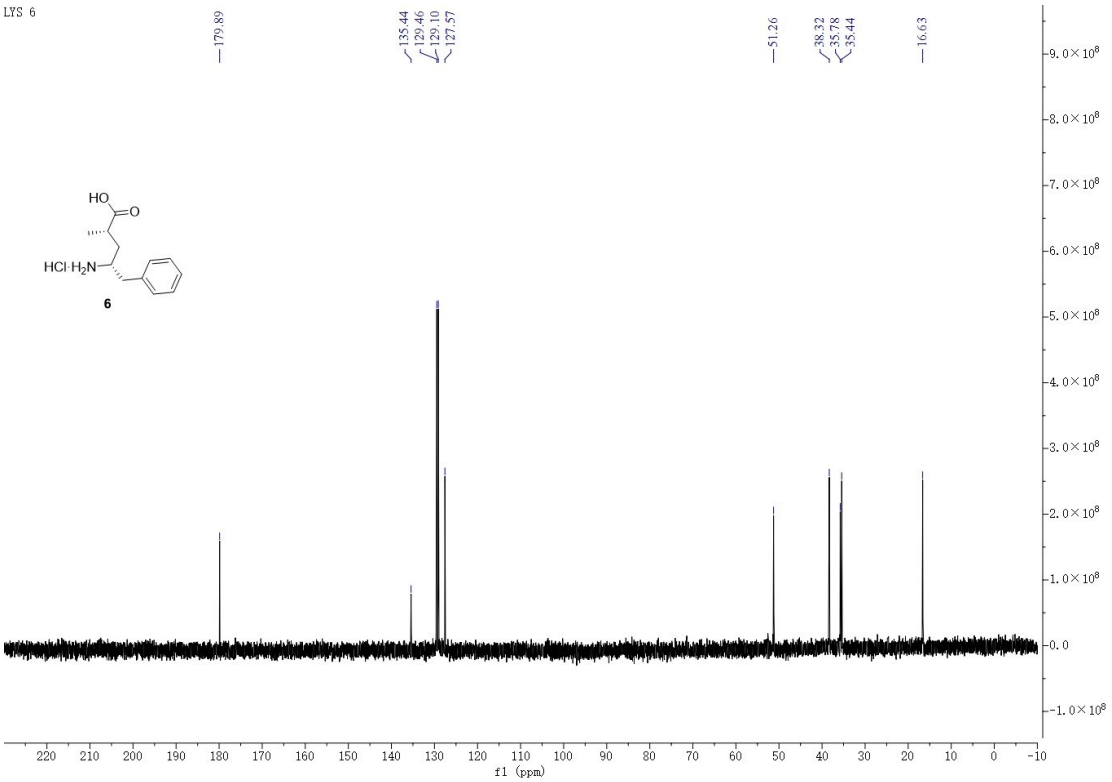


Figure S12. ^{13}C NMR of **6** (D_2O, 100 MHz)

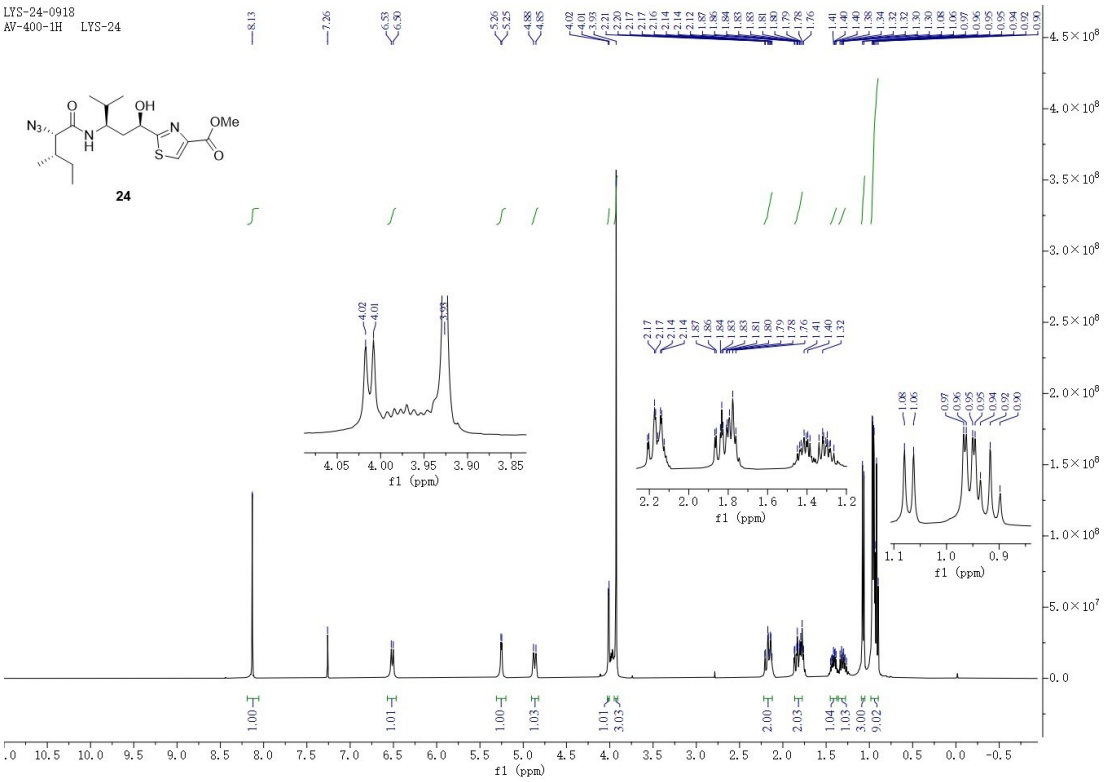


Figure S13. ^{1}H NMR of **24** ($CDCl_3$, 400 MHz)

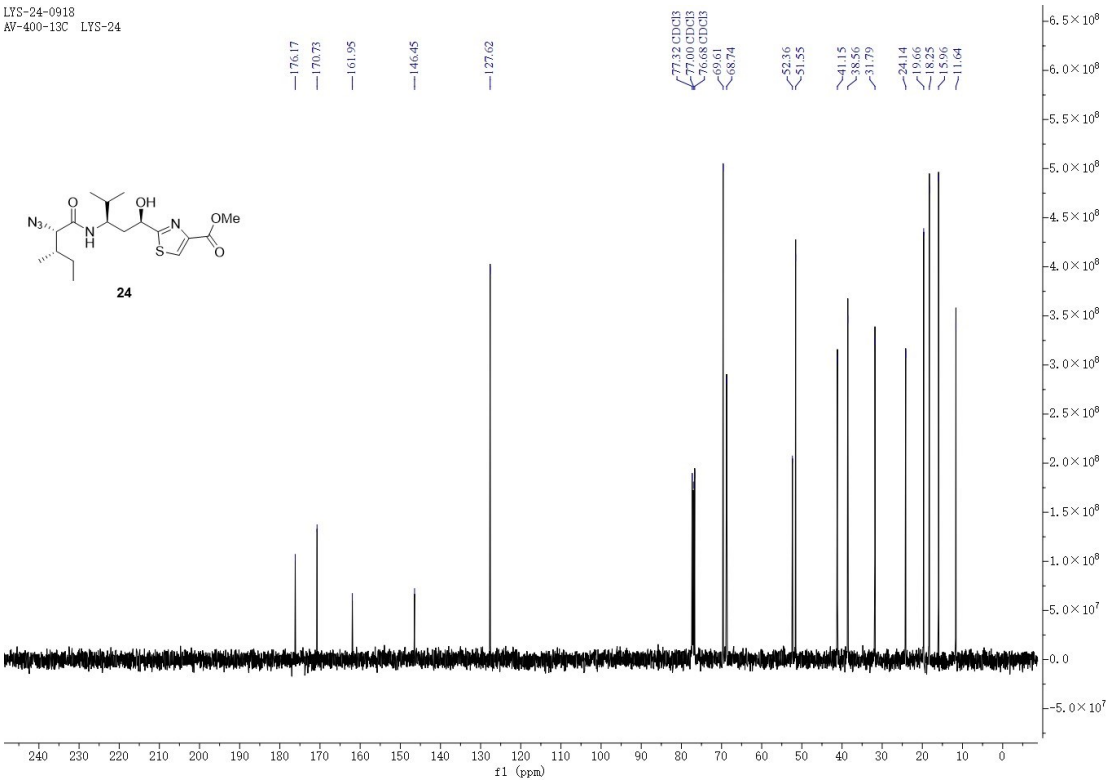


Figure S14. ^{13}C NMR of **24** ($CDCl_3$, 100 MHz)

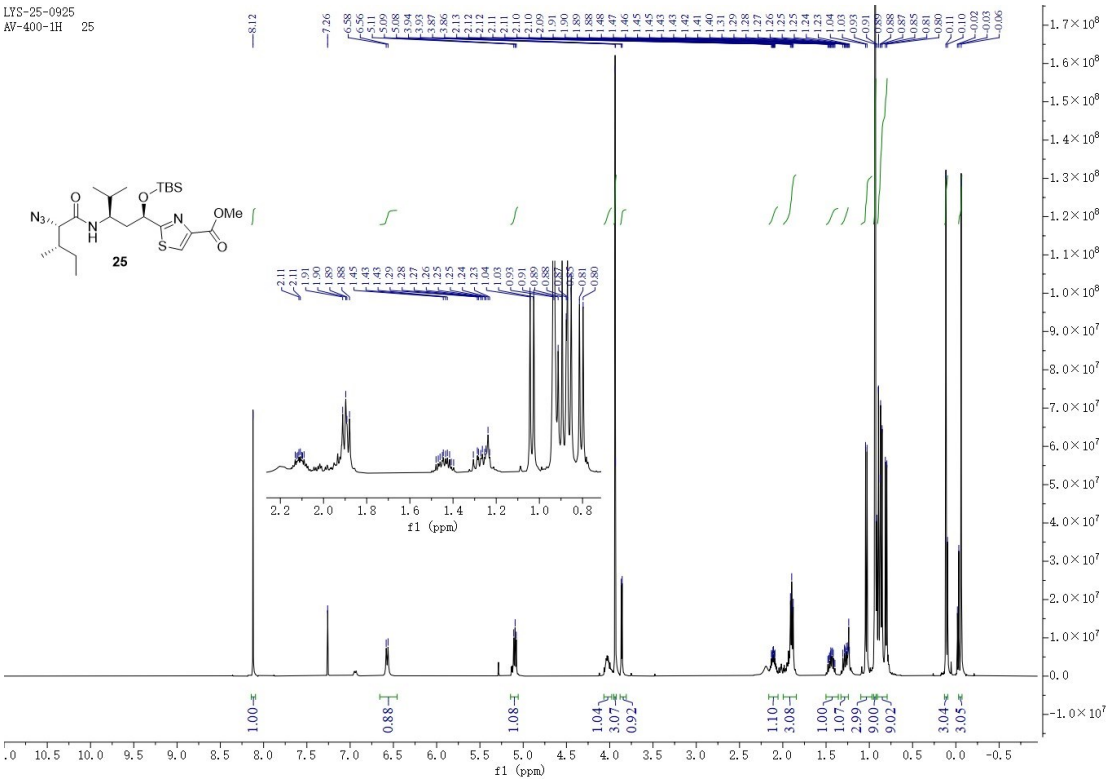


Figure S15. ^{1}H NMR of **25** ($CDCl_3$, 400 MHz)

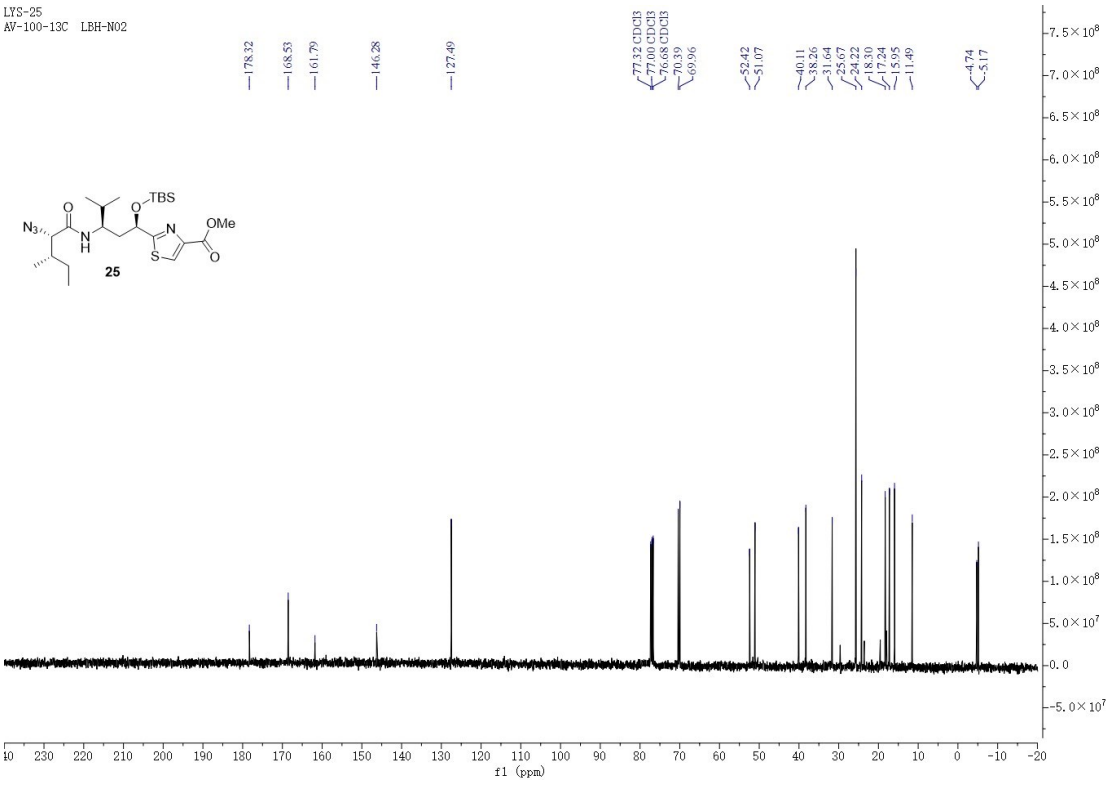


Figure S16. ^{13}C NMR of **25** ($CDCl_3$, 100 MHz)

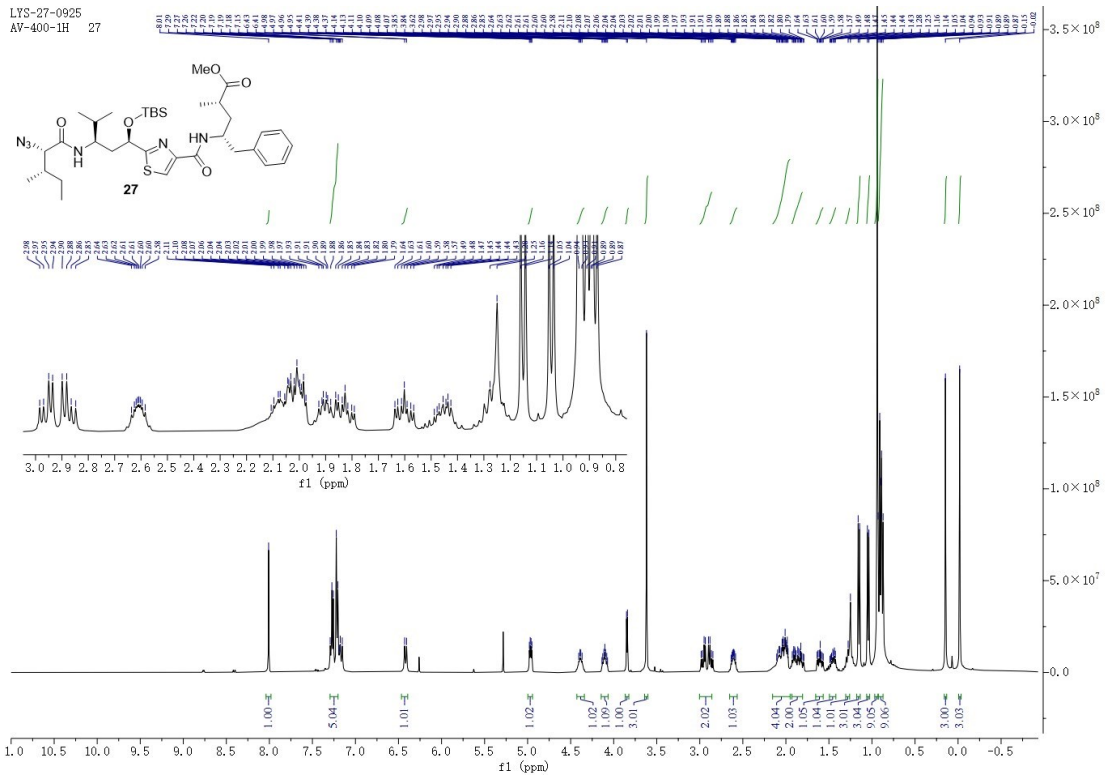


Figure S17. ^{1}H NMR of **27** ($CDCl_3$, 400 MHz)

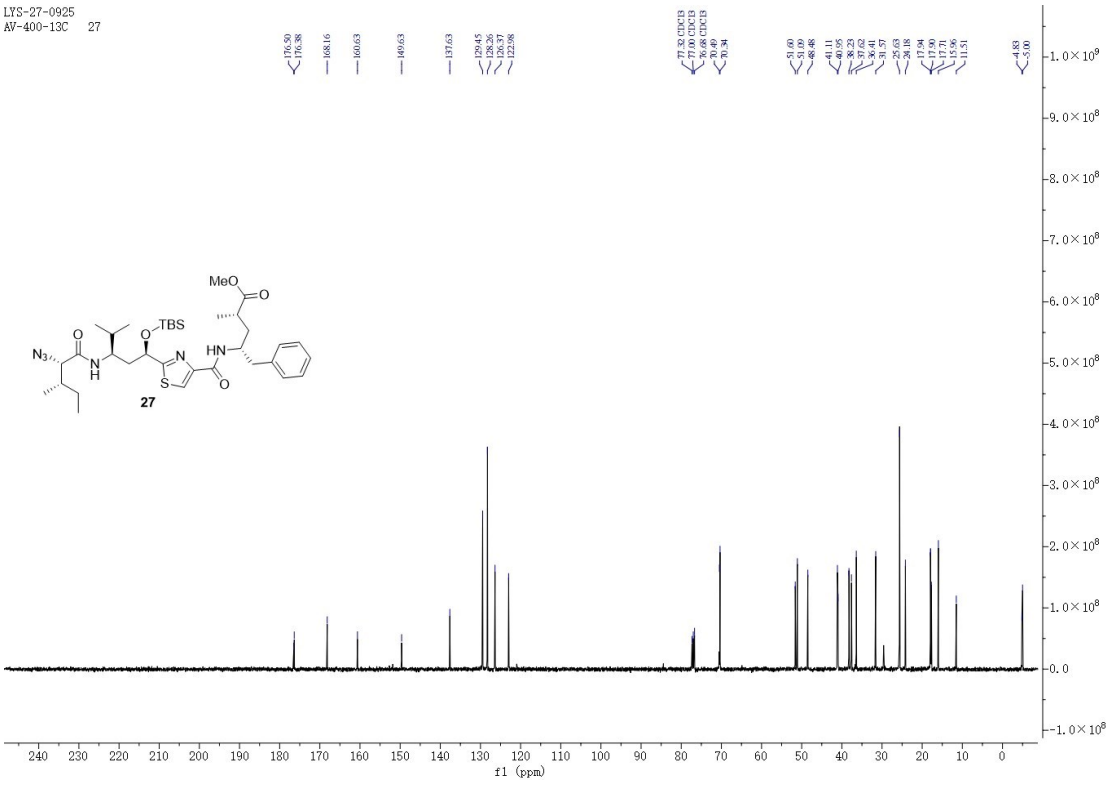


Figure S18. ^{13}C NMR of **27** ($CDCl_3$, 100 MHz)

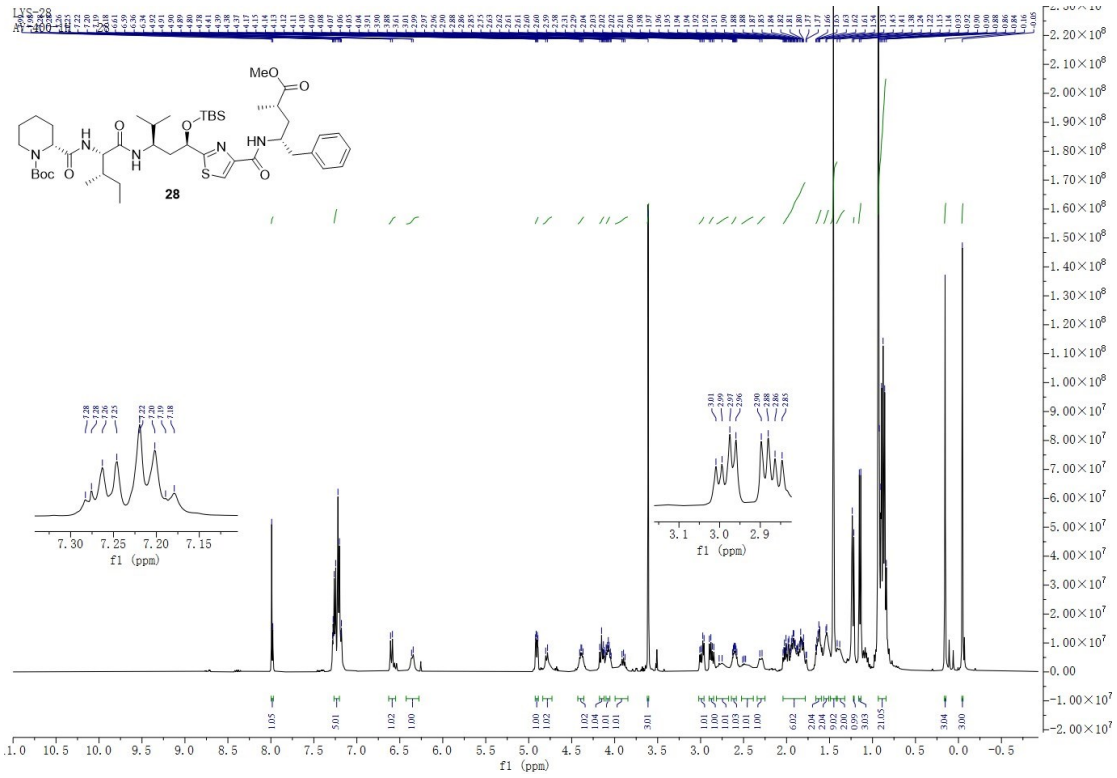


Figure S19. ^{1}H NMR of **28** ($CDCl_3$, 400 MHz)

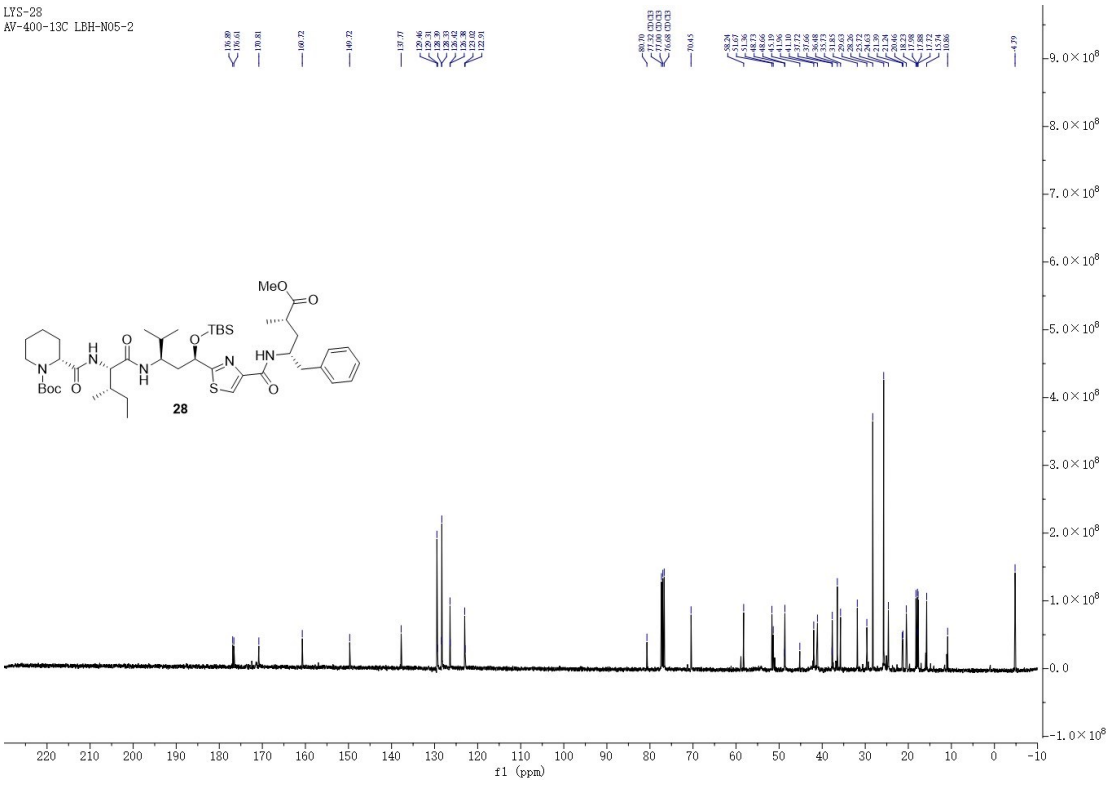


Figure S20. ^{13}C NMR of **28** ($CDCl_3$, 100 MHz)

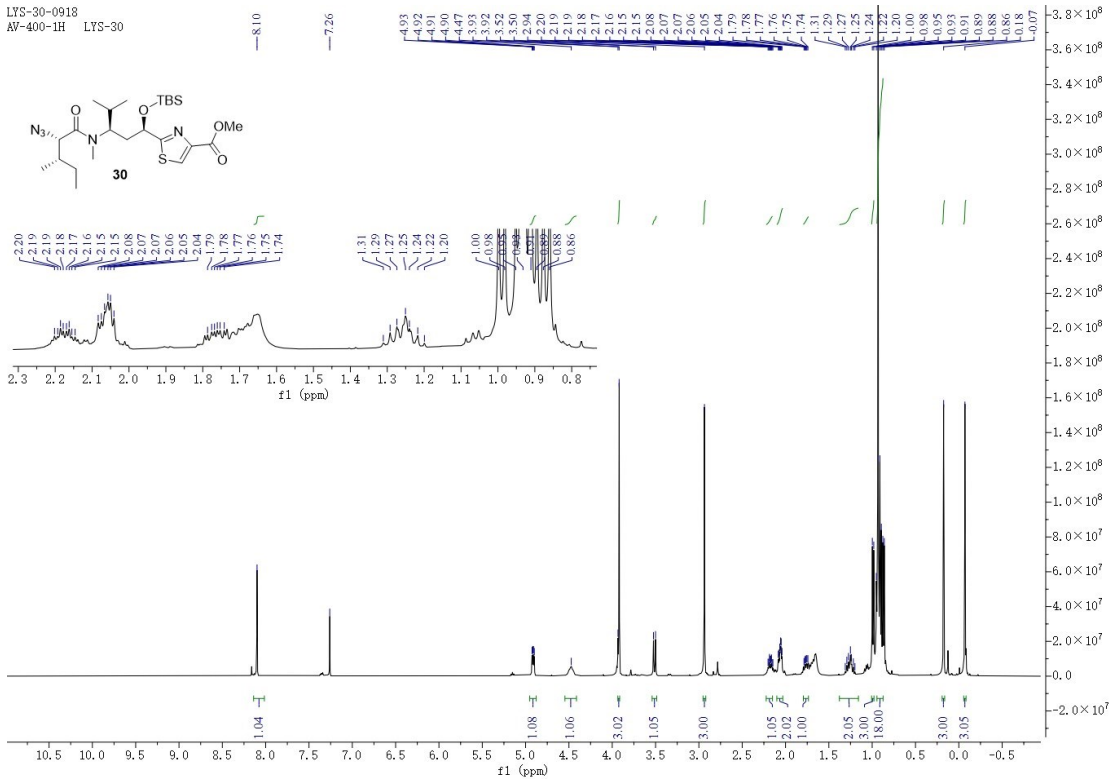


Figure S21. ^{1}H NMR of **30** ($CDCl_3$, 400 MHz)

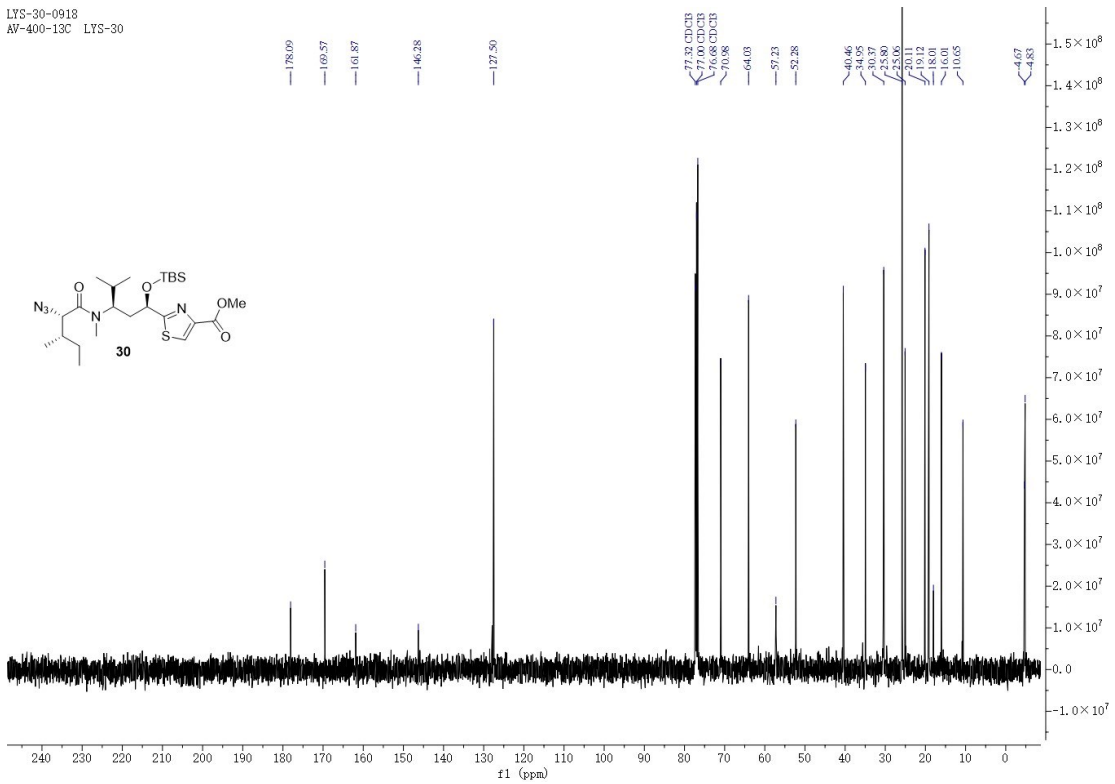


Figure S22. ^{13}C NMR of **30** ($CDCl_3$, 100 MHz)

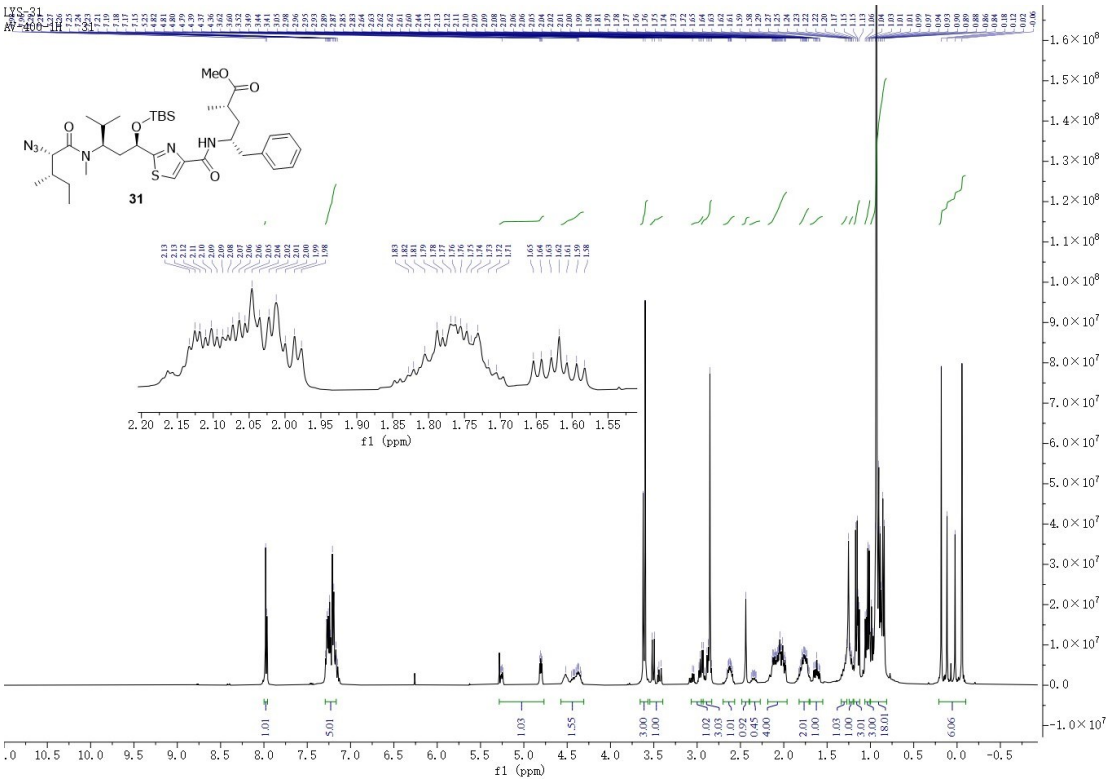


Figure S23. ^{1}H NMR of **31** ($CDCl_3$, 400 MHz)

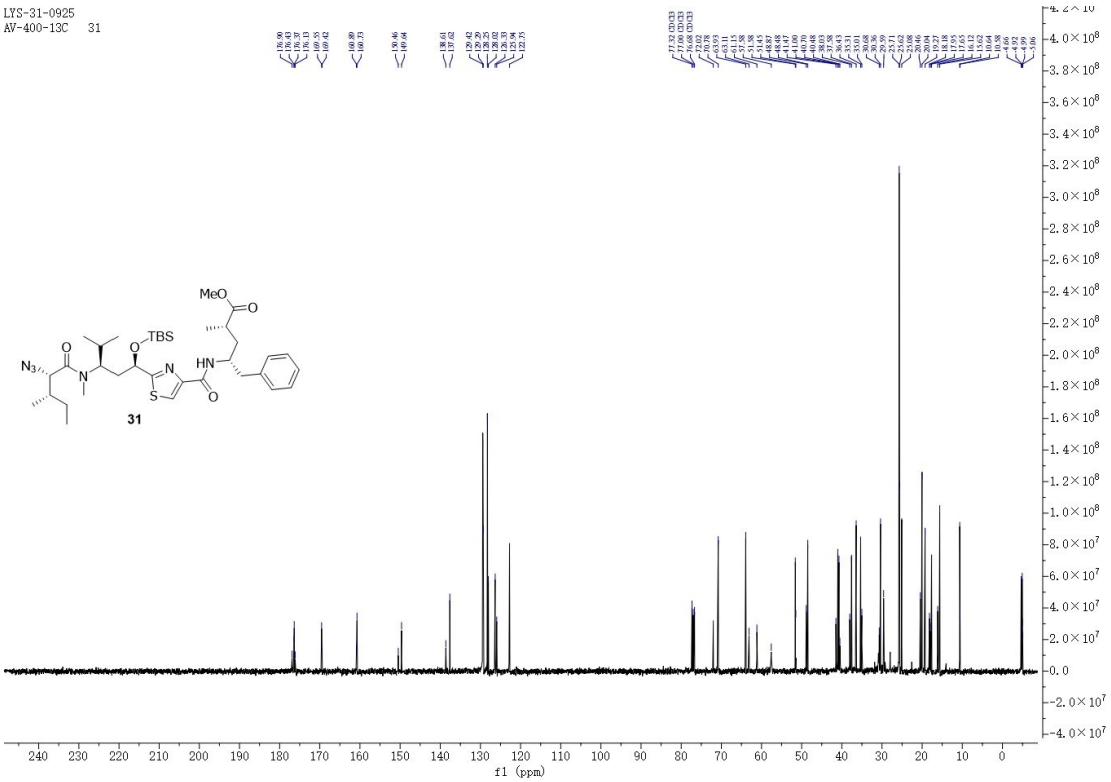


Figure S24. ^{13}C NMR of **31** ($CDCl_3$, 100 MHz)

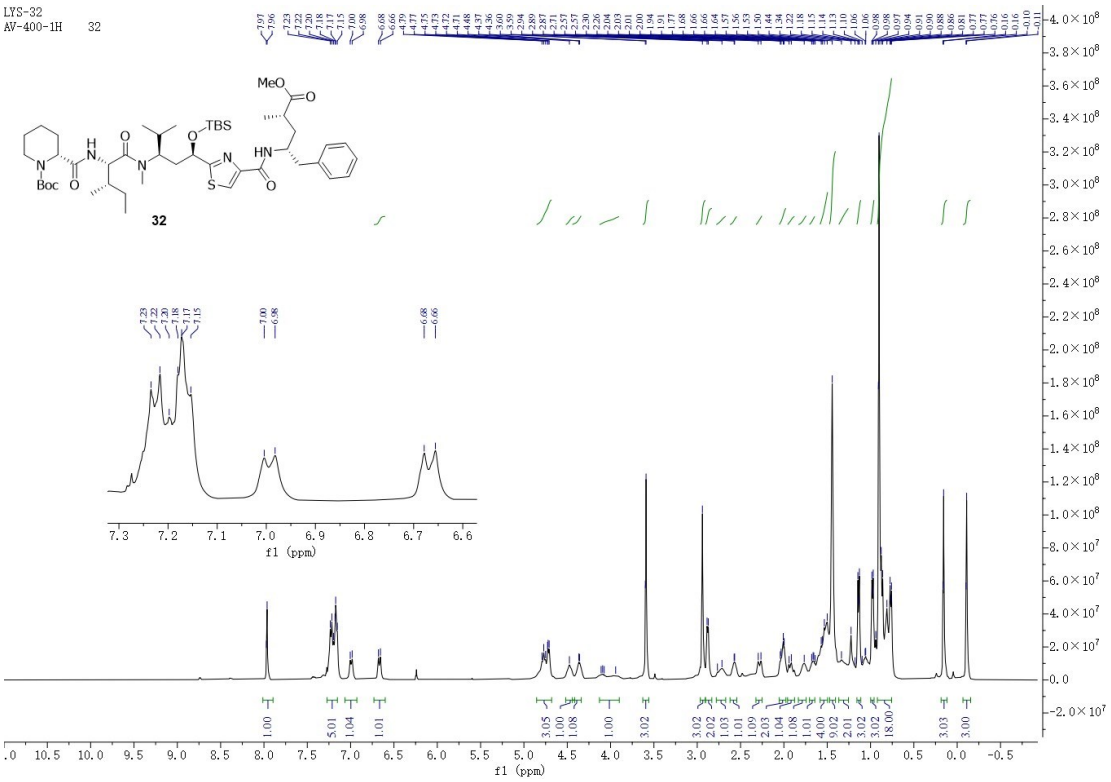


Figure S25. ^{1}H NMR of **32** ($CDCl_3$, 400 MHz)

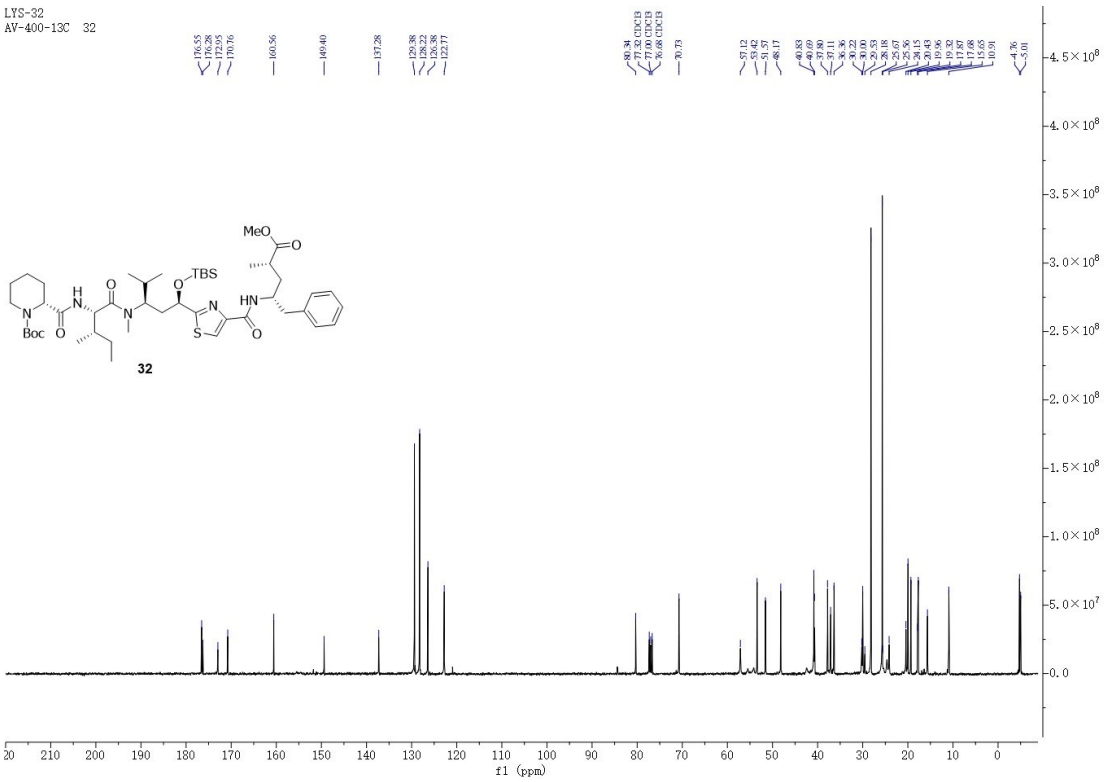


Figure S26. ^{13}C NMR of **32** ($CDCl_3$, 100 MHz)

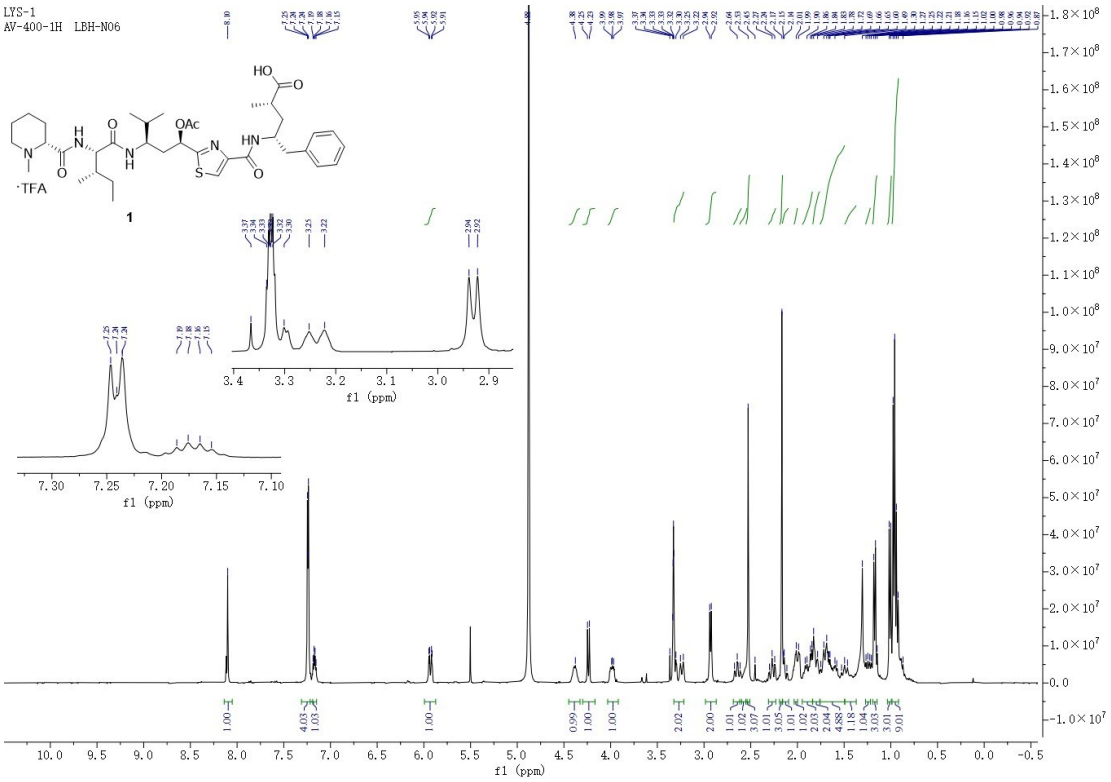


Figure S27. ^{1}H NMR of **1** (CD_3OD, 400 MHz)

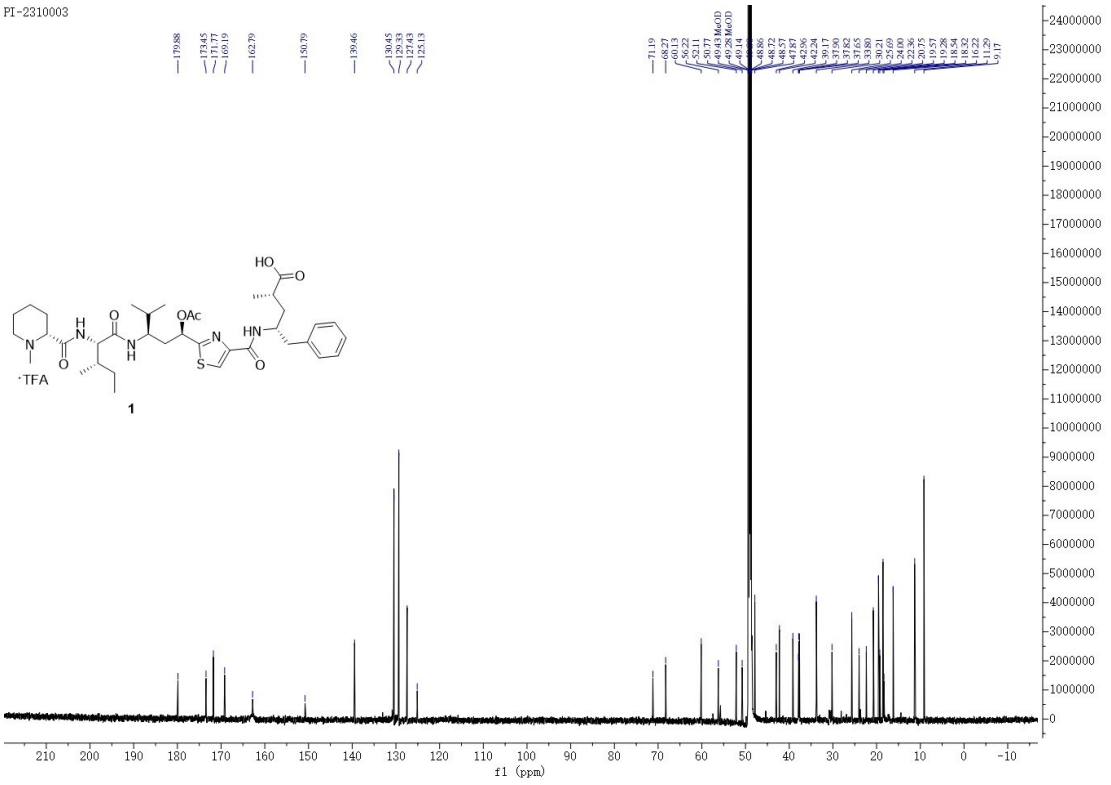


Figure S28. ^{13}C NMR of **1** (CD_3OD, 150 MHz)

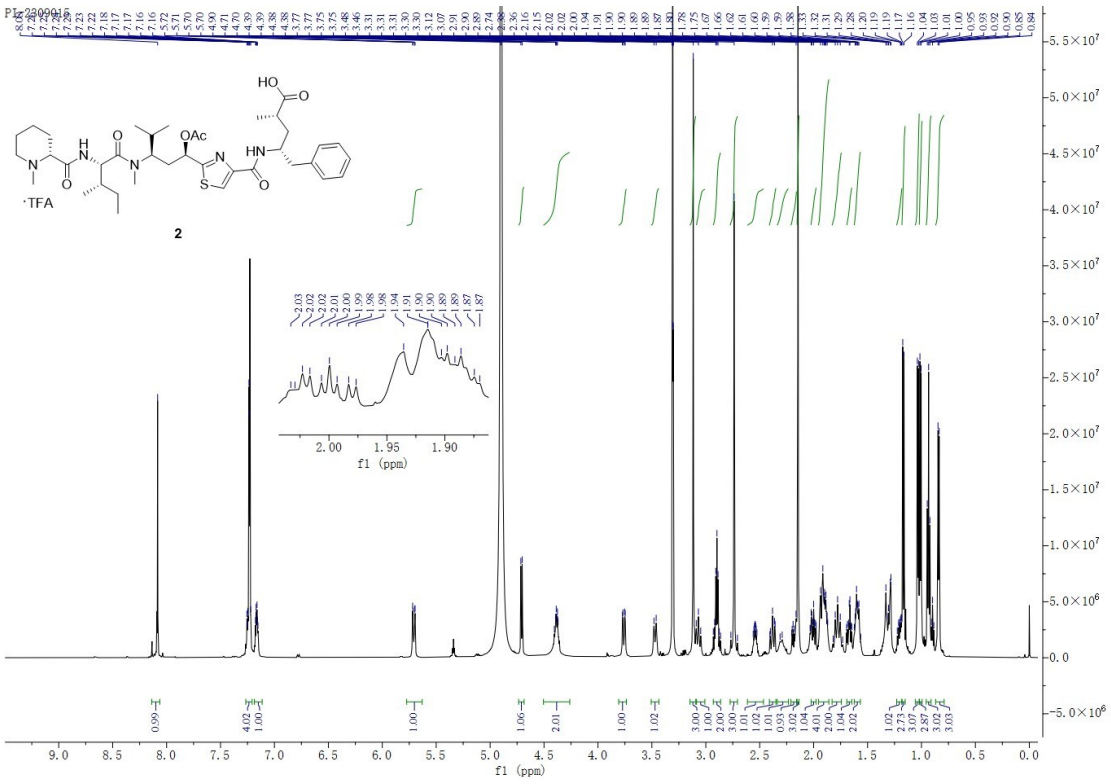


Figure S29. ^{1}H NMR of **2** (CD_3OD, 600 MHz)

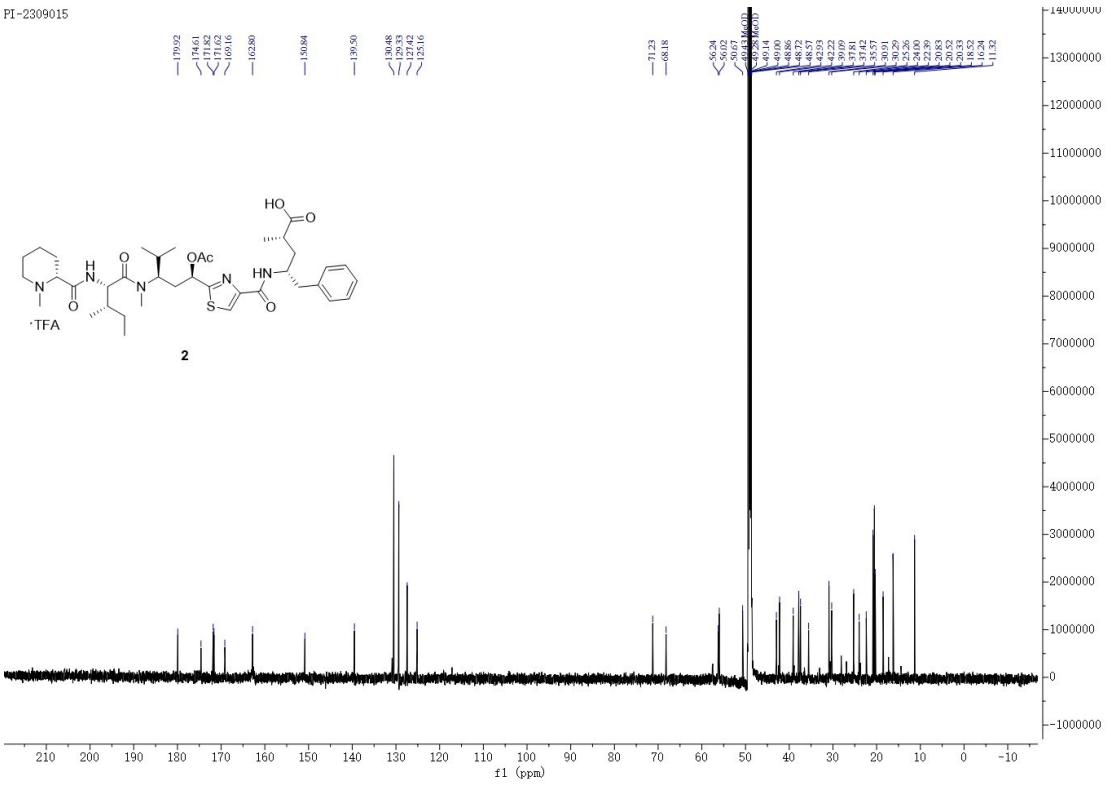


Figure S30. ^{13}C NMR of **2** (CD_3OD, 150 MHz)