Tubulysin synthesis featuring stereoselective catalysis and highly convergent multi-component assembly

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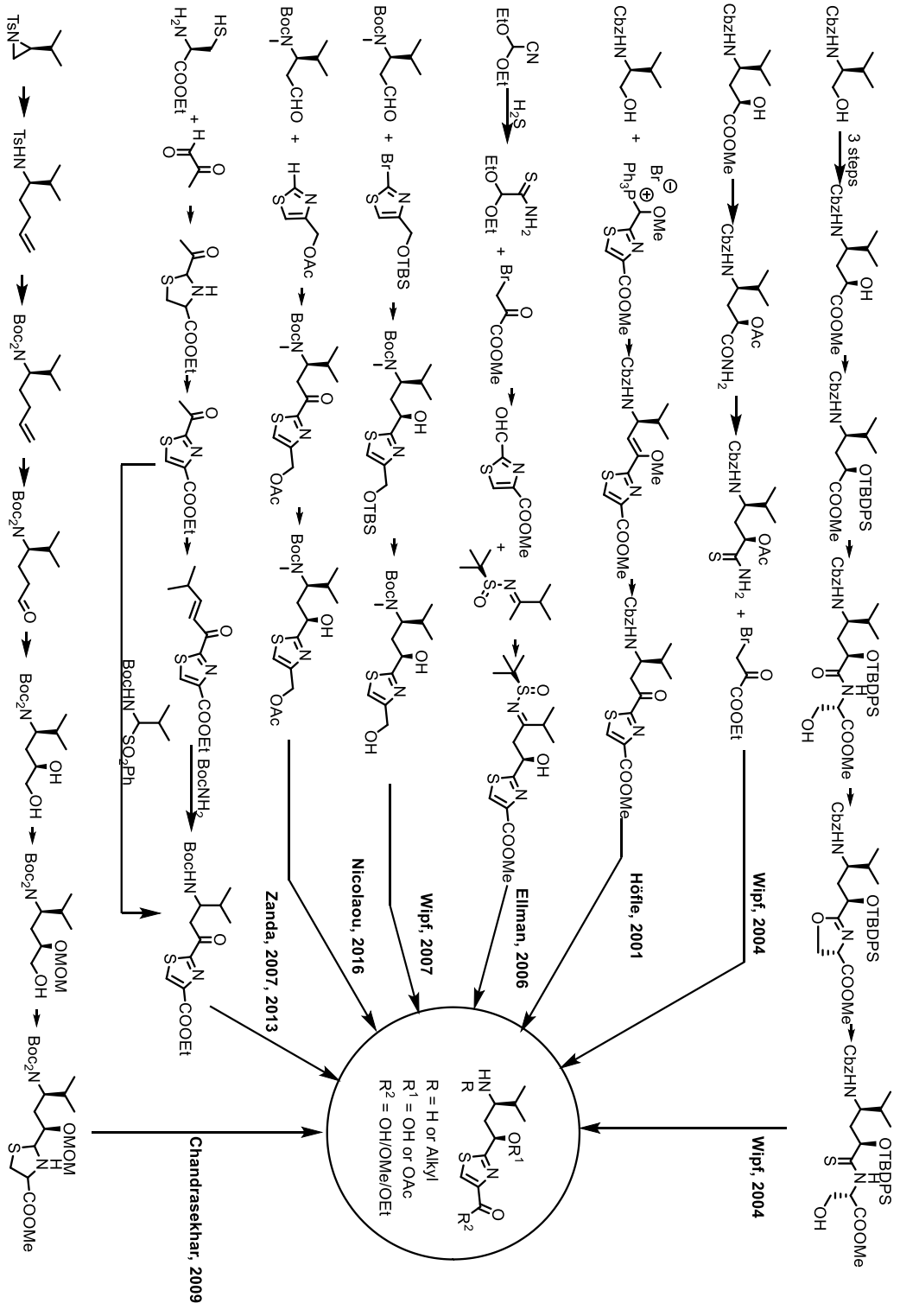
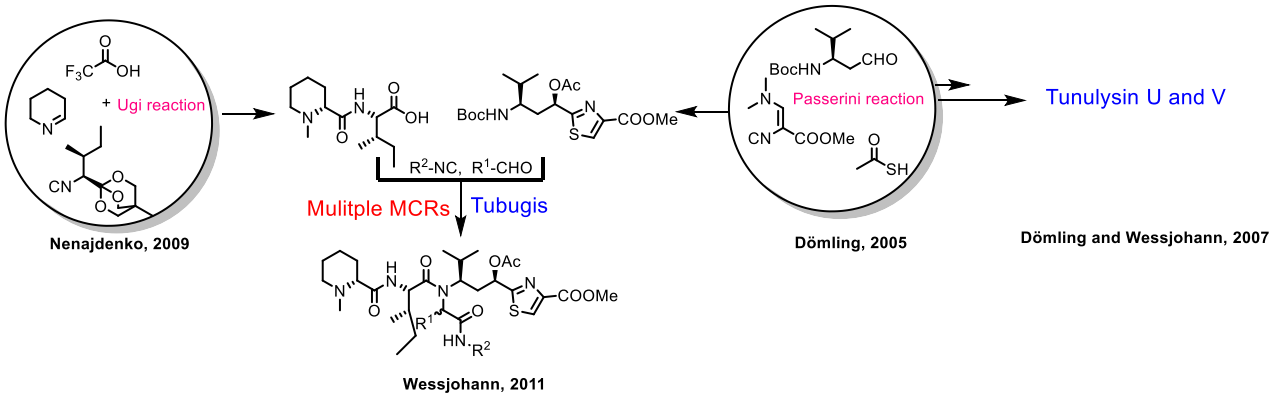
General Information

All reactions involving moisture-sensitive reagents were conducted in oven-dried glassware under a nitrogen or argon atmosphere. All chemicals and catalysts were purchased in Sigma Aldrich and used as it is. Analytical thin-layer chromatography (TLC) was performed on SiO_2 60 F-254 plates. Visualization was accomplished by UV irradiation at 254 nm or by staining with any one of the following reagents: iodine, 5% phosphomolybdic acid hydrate in ethanol, ninhydrin (0.3% w/v in glacial acetic acid/n-butyl alcohol 3:97), Vaughn's reagent (4.8 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, and 0.2 g of $Ce(SO_4)_2\cdot 4H_2O$ in 10 mL of conc. H_2SO_4 and 90 mL of H_2O), or anisaldehyde (7.5 mL of *p*-anisaldehyde, 25 mL of conc. H_2SO_4, and 7.5 mL of acetic acid in 675 mL of 95% ethanol). Column chromatography was performed using SiO_2 60 (particle size 0.040−0.055 mm, 230−400 mesh). Flash chromatography was conducted on a silica gel (230-400 mesh) using a Teledyne ISCO CombiFlash® Rf or Grace Reveleris X2. Hydrogenation reactions were performed on an H-cube. Melting points were obtained on a capillary melting point apparatus fitted with a digital thermometer and are not corrected. Optical rotation was obtained on Jasco P-1020 instrument at the designated concentration and temperature using a 1 dm cell. Proton and carbon NMR spectra were obtained on 400, 500, and 600 MHz NMR spectrometers. Chemical shifts are reported as δ values in parts per million (ppm) as referenced to residual solvent. ^{1}H NMR spectra are tabulated as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), number of protons, and coupling constant(s). HRMS was obtained on Shimadzu LCMS-IT-TOF. RP-HPLC analyses were carried out on a Chromaster system equipped with a Hitachi 5160 pump, a Hitachi 5260 auto sampler and a Hitachi 5430 diode array detector. SFC-HPLC chromatograms were Supercritical Fluid Chromatograph (SFC) with a 3100 MS Detector (ESI) using a solvent system of methanol or isopropanol and CO_2 on a Viridis silica gel column (4.6 × 250 mm, 5 µm particle size) or Ethyl pyridine column and reported as (m/z). Conditions and columns used for RP-HPLC or SFC-HPLC analysis are given in the individual compounds.

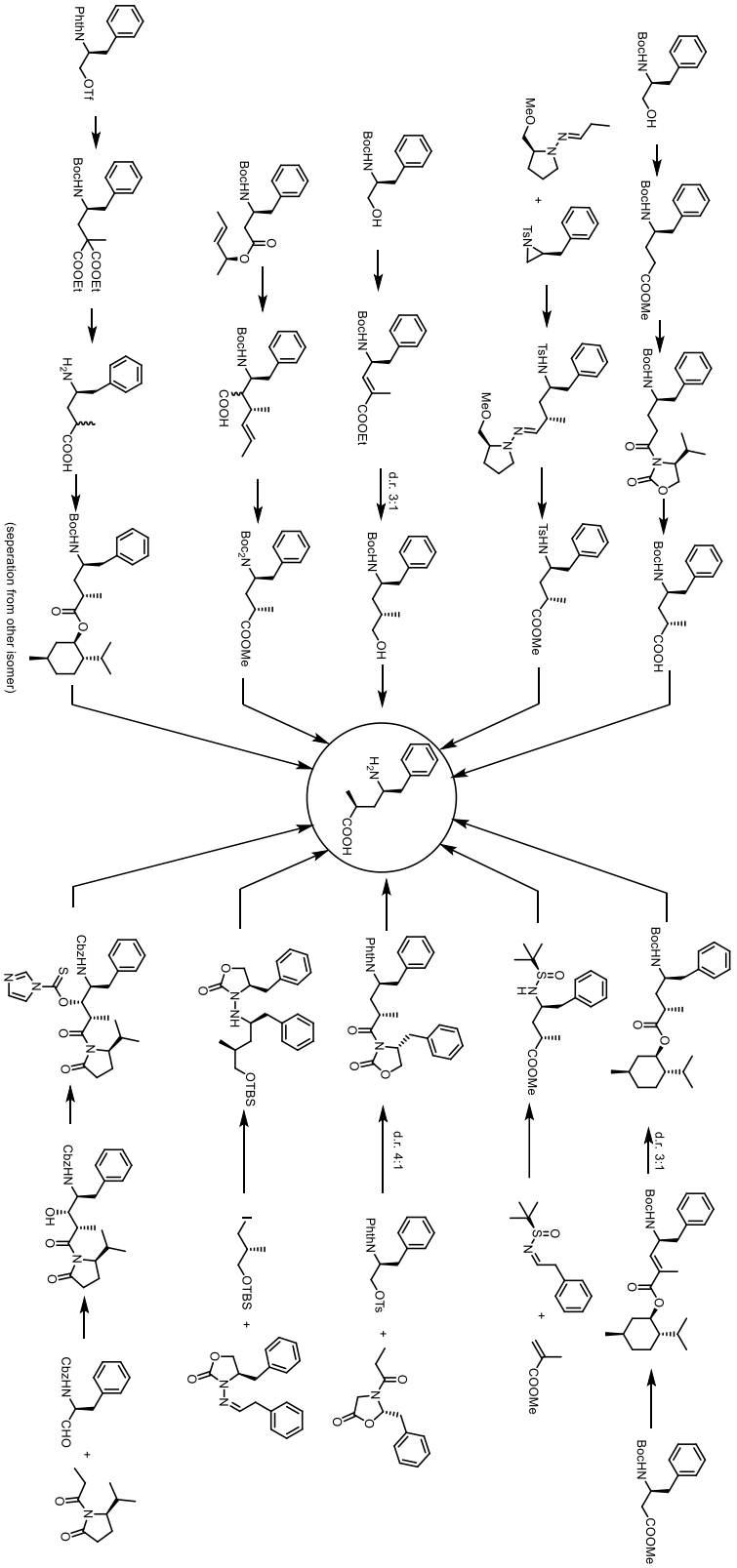
Previous methods for the synthesis of Tubuvaline



Previous MCR approaches



Previous methods for the synthesis of Tubuphenylalanine

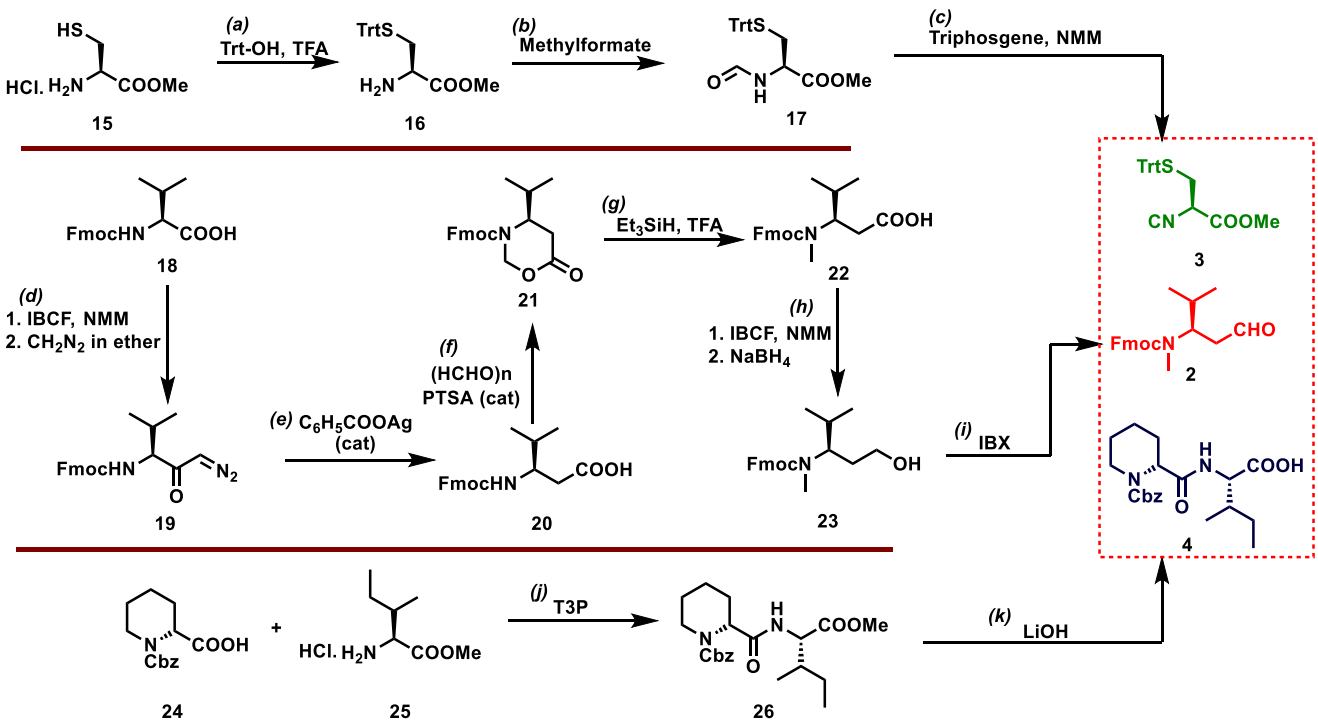


For Review See: U. Kazmaier, A. Ullrich, J. Hoffmann, *The Open Natural Products Journal* **2013**, *6*, 12. And reference cited therein.

Recent works by Nicolaou see:
K. C. Nicolaou, R. D. Erande, J. Yin, D. Vourloumis, M. Aujay, J. Sandoval, S. Munneke, J. Gavrilyuk, *J. Am. Chem. Soc*. **2018**, *140*, 3690.

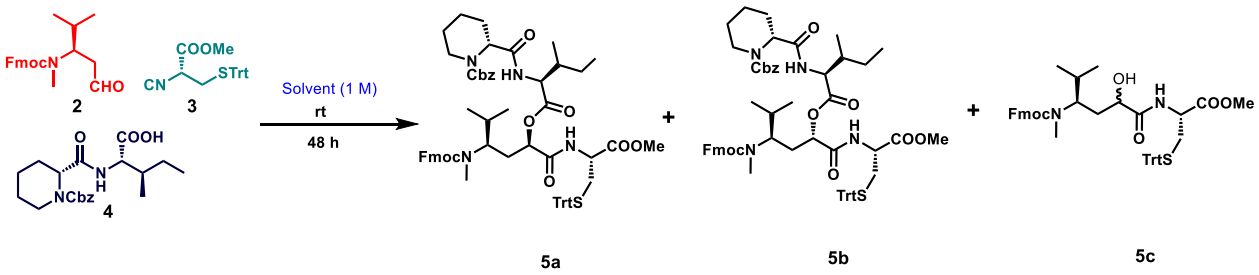
Recent work by Wipf see:
R. Colombo, Z. Wang, J. Han, R. Balachandran, H. N. Daghestani, D. P. Camarco, A. Vogt, B. W. Day, D. Mendel, P. Wipf, *J. Org. Chem.* **2016**, *81*, 10302

Synthesis of building blocks for the present studies



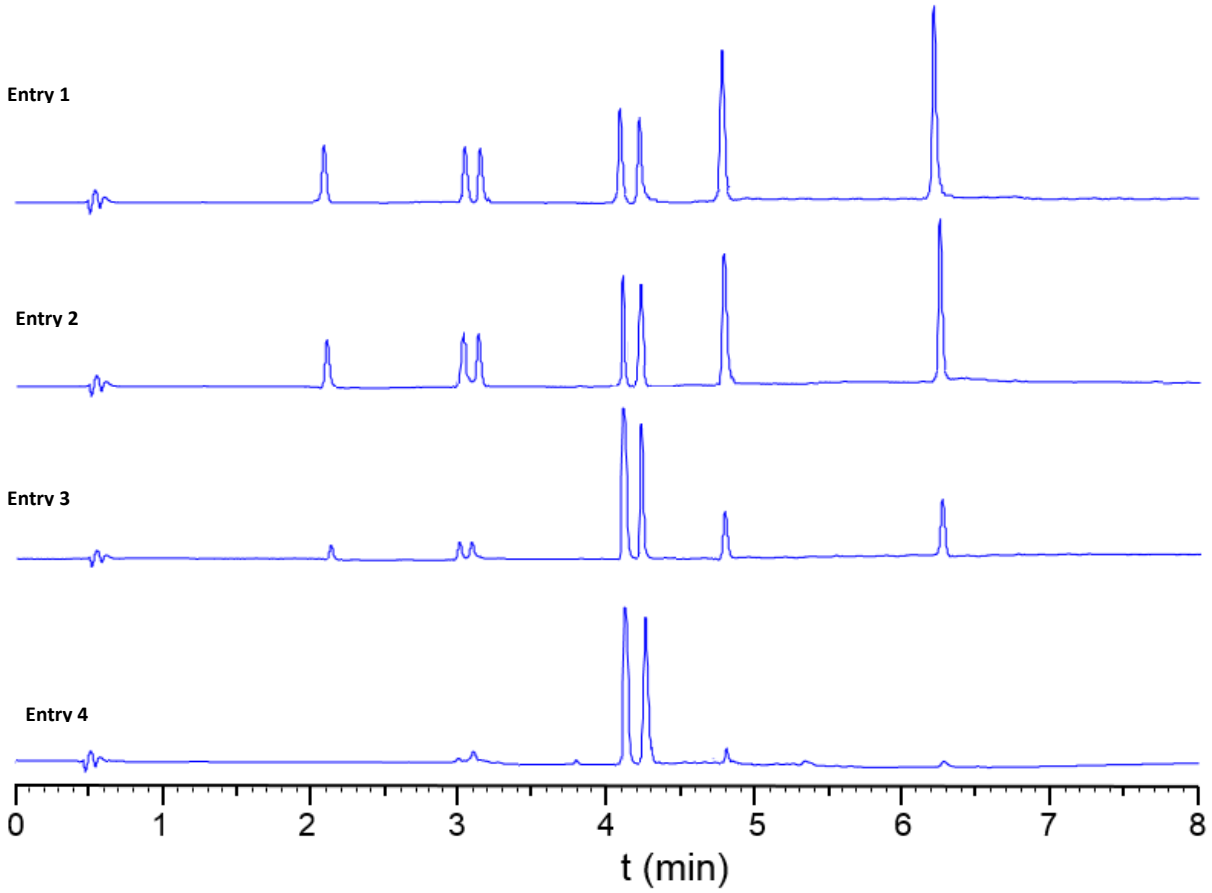
Diastereoselective synthesis of 5a

Solvent studies



Entry	Solvent	Yield (%)[a] (mixture of **5a** and **5b**)	dr[b] (**5a**:**5b**)	Yield (%)[a] **5c**
1	CH_3CN	52	1:1	15
2	THF	57	1:1	20
3	$CHCl_3$	82	1:1	08
4	CH_2Cl_2	92	1:1	02
[a]Isolated yields are given; [b]dr ratios are measured by integrating the area percentage at 4.1 and 4.3 min				

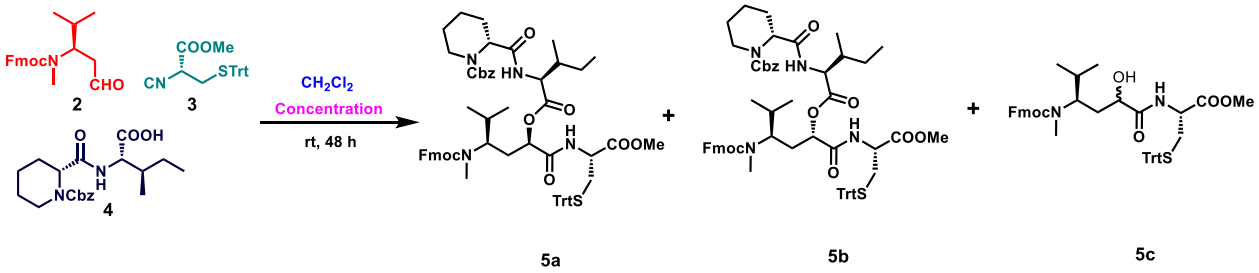
Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous $MgSO_4$ (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), and dry solvents (0.1 mL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature for 48 h. The solvent was evaporated and diluted with EtOAc (5 mL) and washed with water. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was directly analysed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min)



RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 80 - 90% CH_3CN/H_2O +0.1%TFA over 8 min.
Absorbance: 214 nm

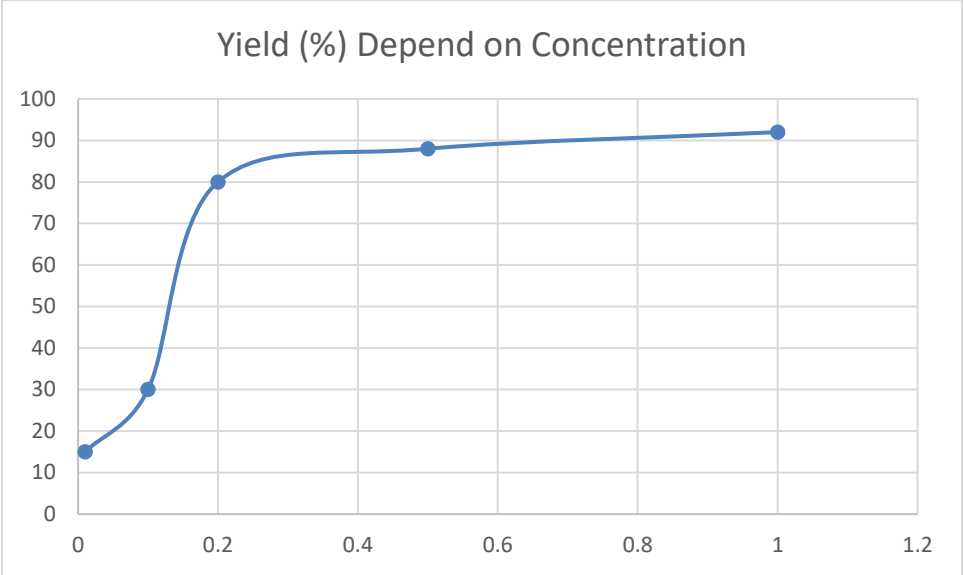
Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

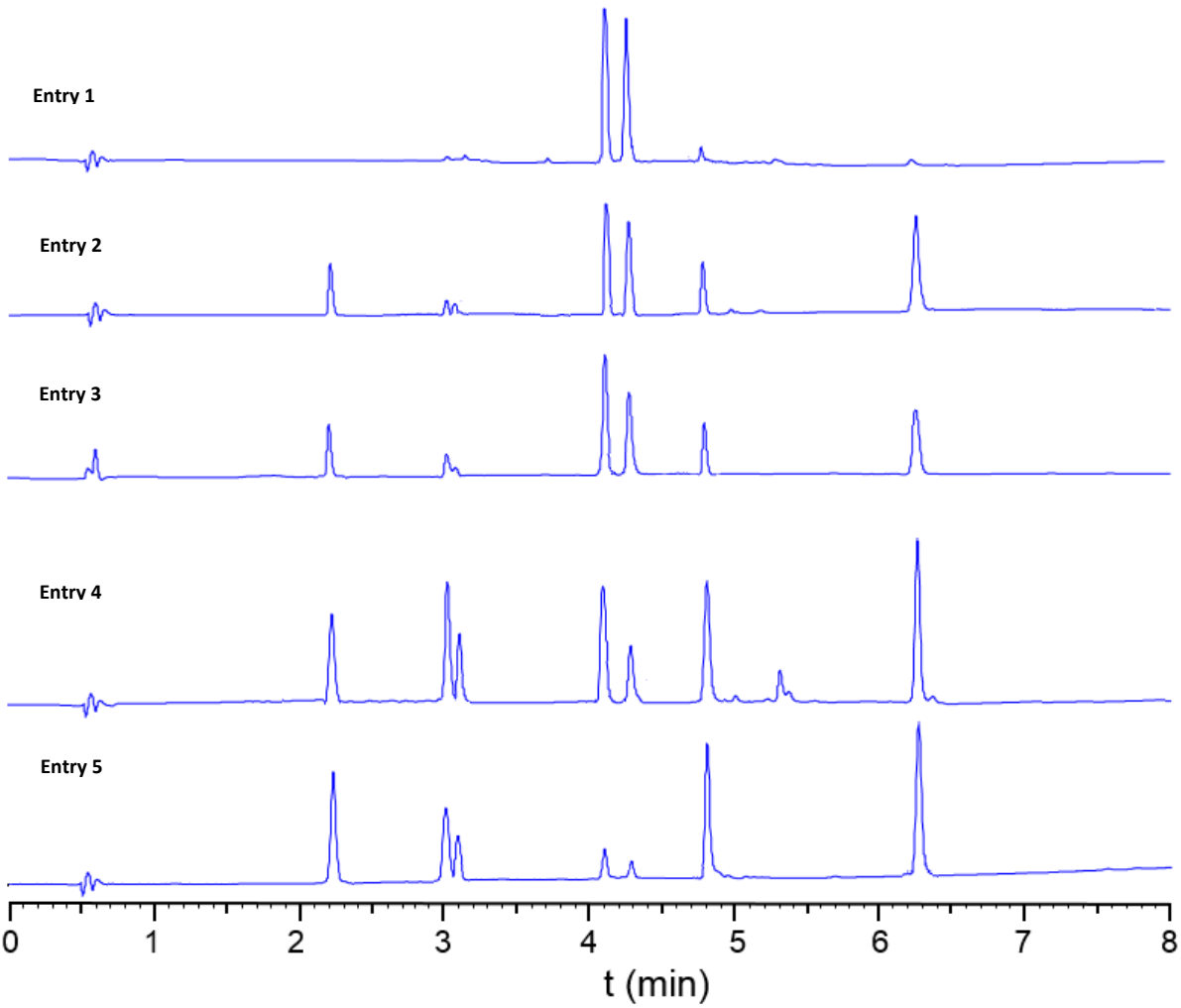
Solvent concentration studies



Entry	Concentration	Yield (%)[a] (mixture of **5a** and **5b**)	dr[b] (**5a**:**5b**)	Yield (%)[a] **5c**
1	1 M	92	1:1	02
2	0.5 M	88	58:42	05
3	0.2 M	80	65:35	08
4	0.1 M	30	68:32	32
5	0.01 M	15	70:30	50
[a]Isolated yields are given; [b] dr ratios are measured by integrating the area percentage at 4.1 and 4.3 min				

Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous $MgSO_4$ (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), and dry CH_2Cl_2 with indicated concentrations in the table. The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature for 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was directly analyzed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min)

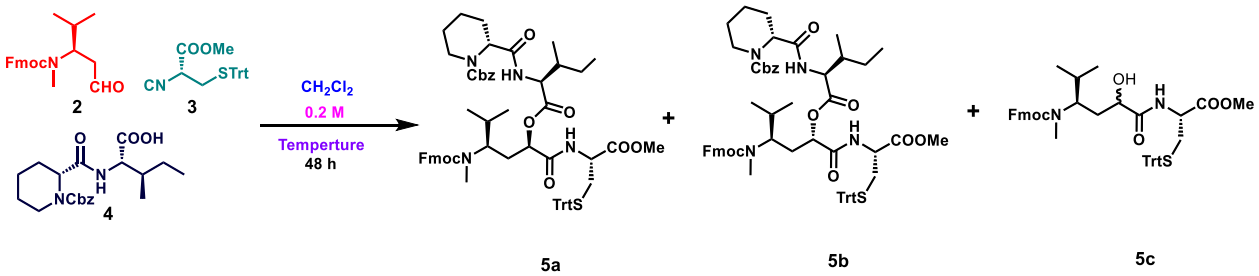

Yield (%) Depend on Concentration
100
90
80
70
60
50
40
30
20
10
0
0
0.2
0.4
0.6
0.8
1
1.2


Entry 1
Entry 2
Entry 3
Entry 4
Entry 5
0
1
2
3
4
5
6
7
8
t (min)

RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 80 - 90% CH_3CN/H_2O + 0.1%TFA over 8 min.
Absorbance: 214 nm

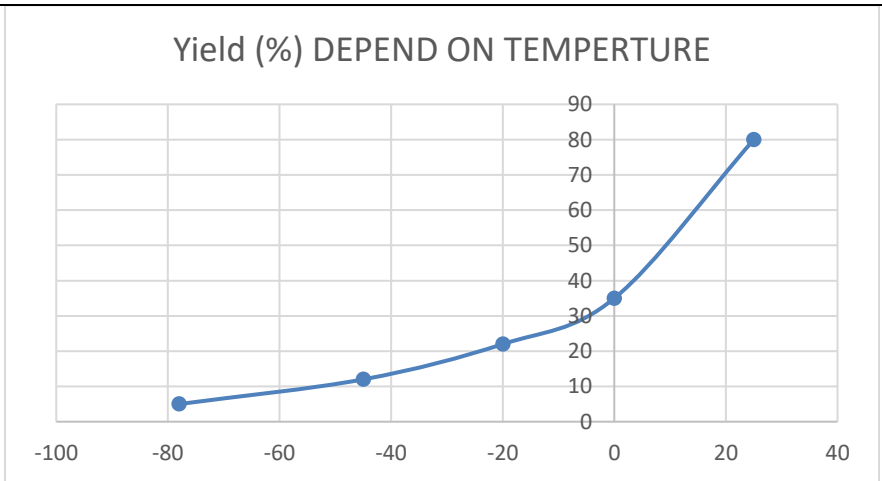
Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

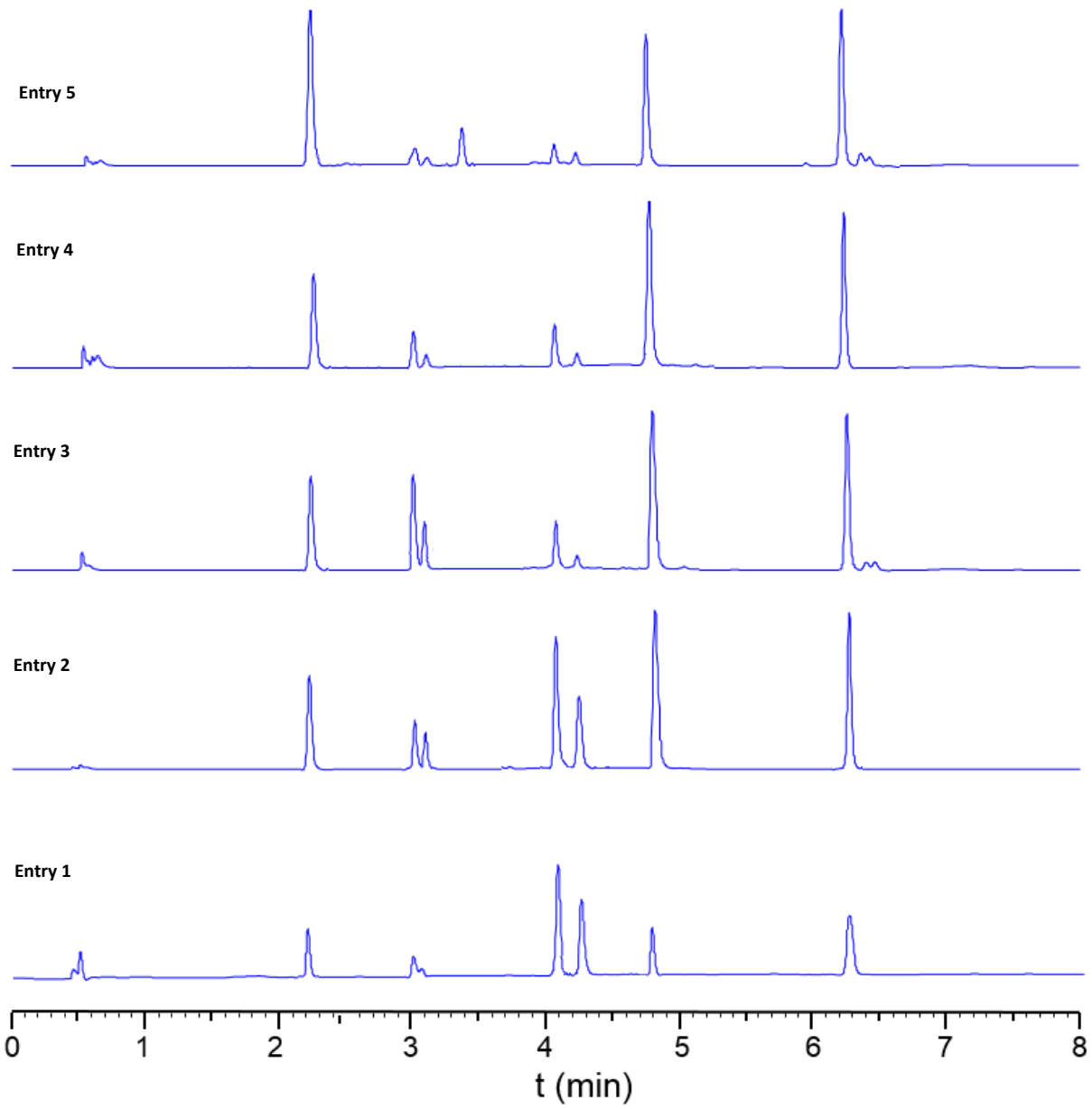
Screening of temperature



Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous $MgSO_4$ (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), and dry CH_2Cl_2 (0.8 mL) were cooled to indicated temperature in the table. The resulting solution was stirred for 10 min at indicated temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at same temperature for 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was directly analyzed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min)

Entry	Temperature	Yield (%)[a] (mixture of **5a** and **5b**)	dr[b] (**5a**:**5b**)	Yield (%)[a] **5c**
1	25 °C	80	65:35	08
2	0 °C	35	65:35	40
3	-20 °C	22	63:37	30
4	-45 °C	12	68:32	15
5	-78 °C	5	62:48	8
[a]Isolated yields are given; [b] dr ratios are measured by integrating the area percentage at 4.1 and 4.3 min				

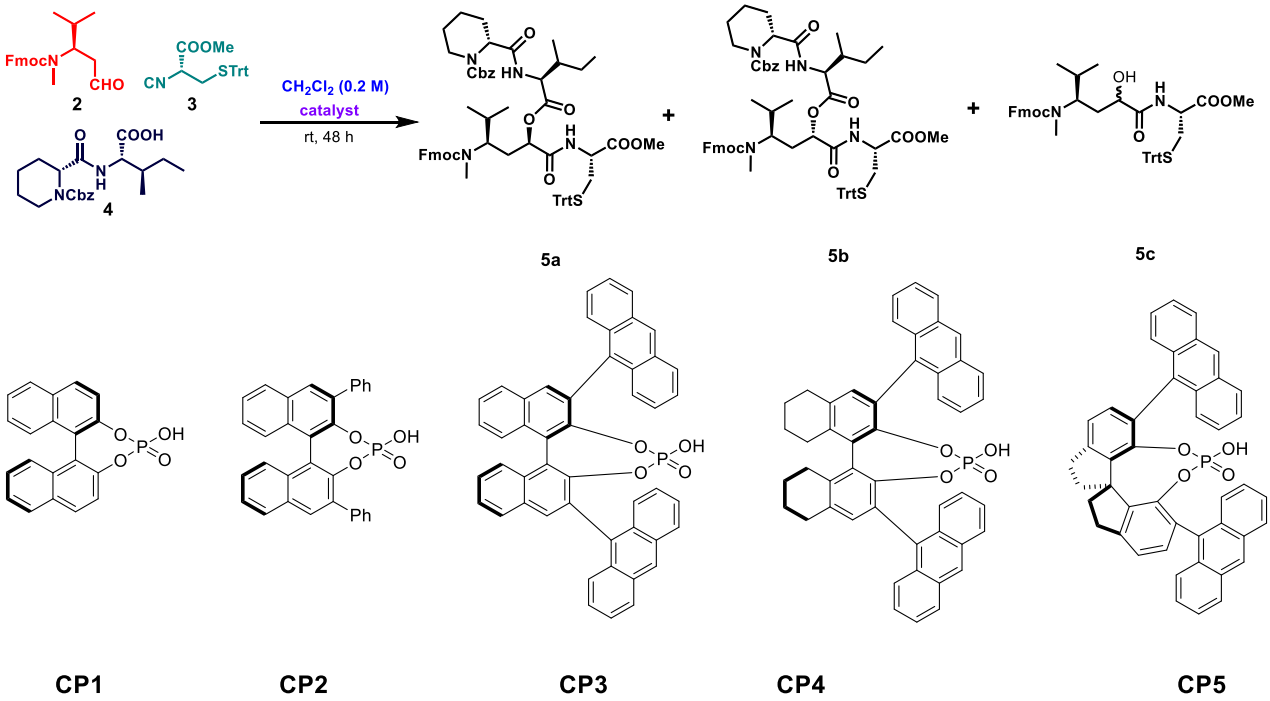




RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 80 - 90% CH_3CN/H_2O +0.1%TFA over 8 min.
Absorbance: 214 nm

Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

Screening of chiral phosphoric acid catalysts

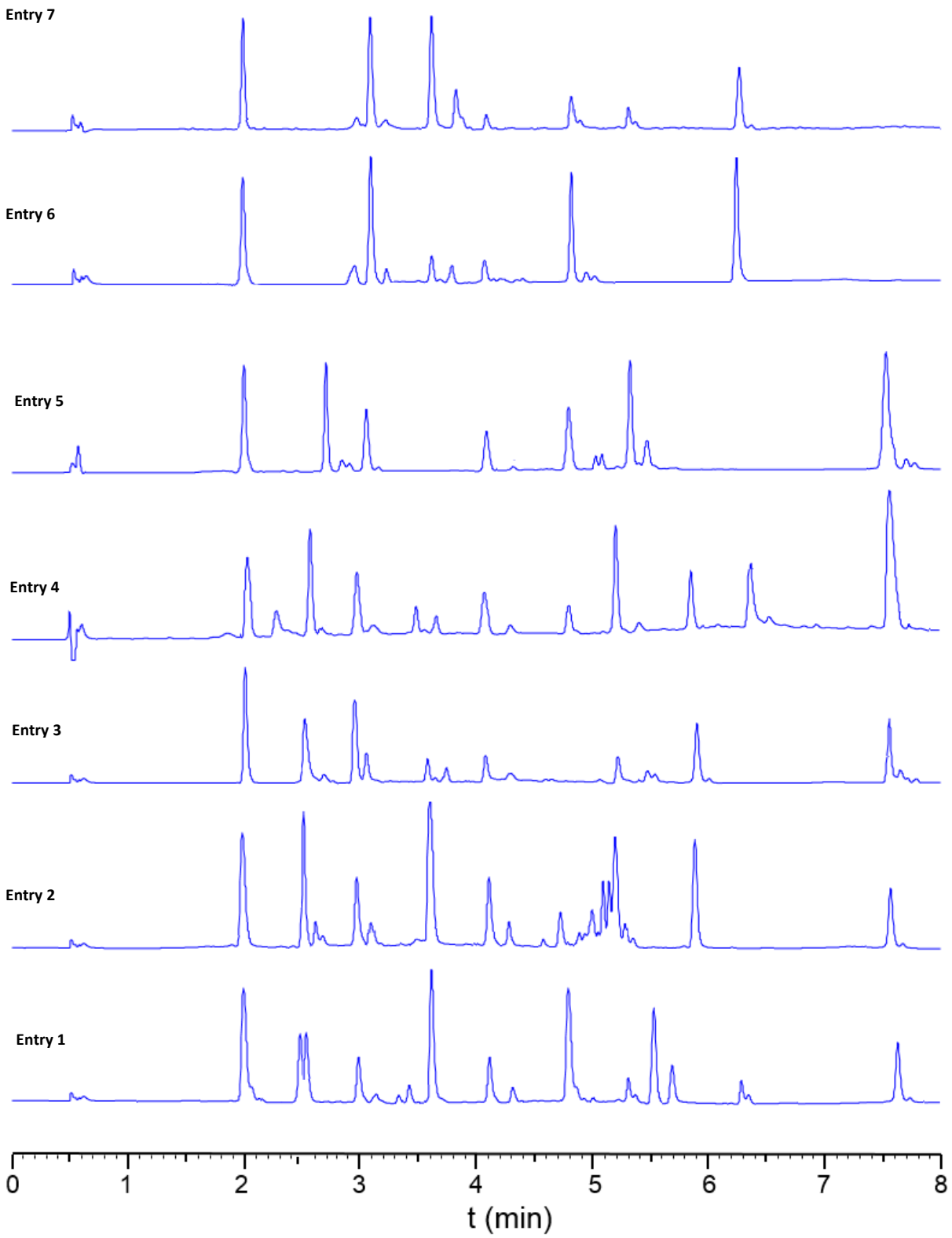


Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous $MgSO_4$ (100 mg),

Entry	Catalyst (10 mol%)	Additive (20 mol%)	Yield (%)[a,b] (mixture of **5a** and **5b**)	dr[c] (**5a:5b**)	Yield (%)[d] **5c**
1	CP1	-	20 (8)	80:20	20
2	CP2	-	18 (12)	85:15	22
3	CP3	-	15 (10)	88:12	26
4	CP4	-	22 (18)	92:8	18
5	CP5	-	38 (31)	95:5	15
6	CP5	Py (20)	minor[e]	-	60
7	CP4	Py (20)	minor[e]	-	62

[a]Isolated yields of **5a** and **5b** is are given; [b] Isolated yields of **5a** is given in the parenthesis; [c]dr ratios are measured by integrating the area percentage at 4.1 and 4.3 min ; [d] isolated yields of **5c** is given; [e]not isolated

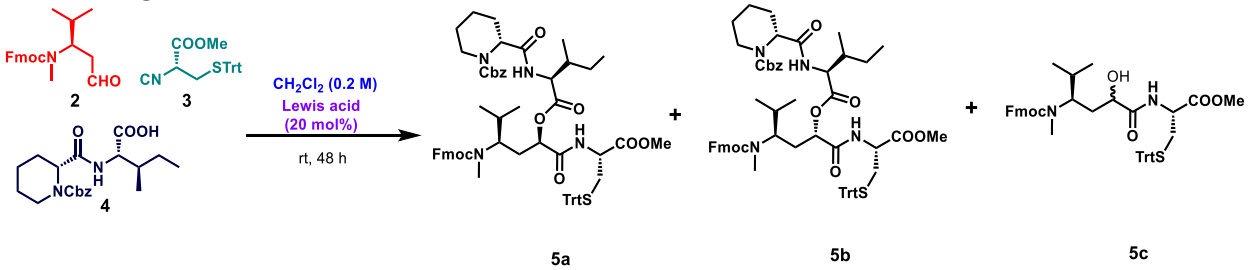
aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), chiral phosphoric acid (0.01 mmol) were dissolved in dry CH_2Cl_2 (800 µL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was directly analyzed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min). The combined fractions of **5a** and **5b** were again purified to obtain pure **5a**.



RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 80 - 90% CH_3CN/H_2O +0.1%TFA over 8 min.
Absorbance: 214 nm

Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1
Triphenyl methyl cation (trityl)	7.6

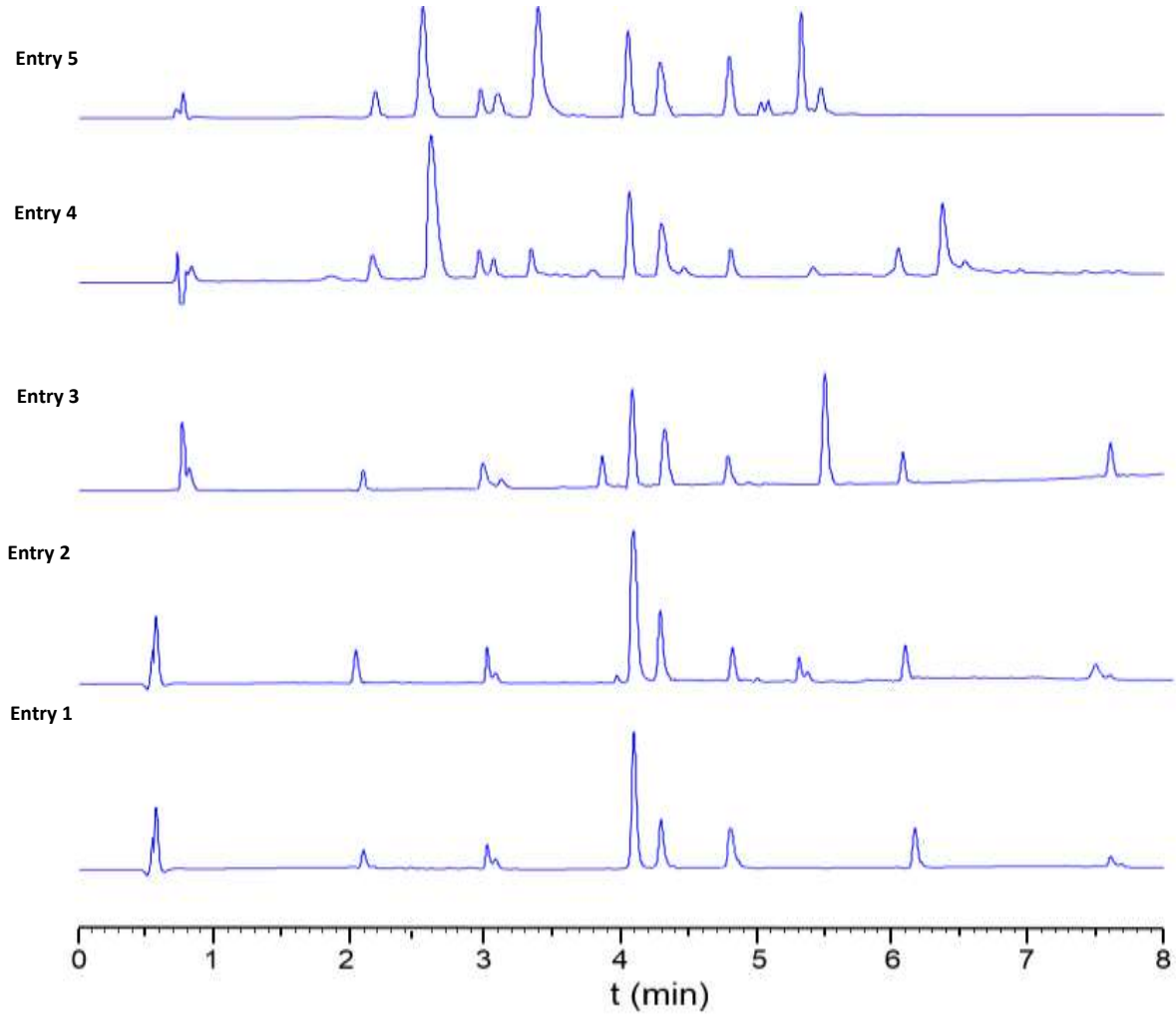
Screening of Lewis acids



Entry	Lewis acid (20 mol%)	Yield (%)[a,b] (mixture of **5a** and **5b**)	dr[c] (**5a:5b**)	Yield (%)[d] **5c**
1	$ZnBr_2$	86 (61)	80:20	5
2	AgOTf	65 (35)	70:30	10
3	CuOTf	63 (28)	66:34	5
4	$Zn(OTf)_2$	50 (20)	75:25	12
5	$Ti(OiPr)_4$	55 (22)	73:27	18

[a]Isolated yields of **5a and 5b** is are given; [b] Isolated yields of **5a** is given in the parenthesis; [c]dr ratios are measured by integrating the area percentage at 4.1 and 4.3 min; [d] isolated yields of **5c** is given.

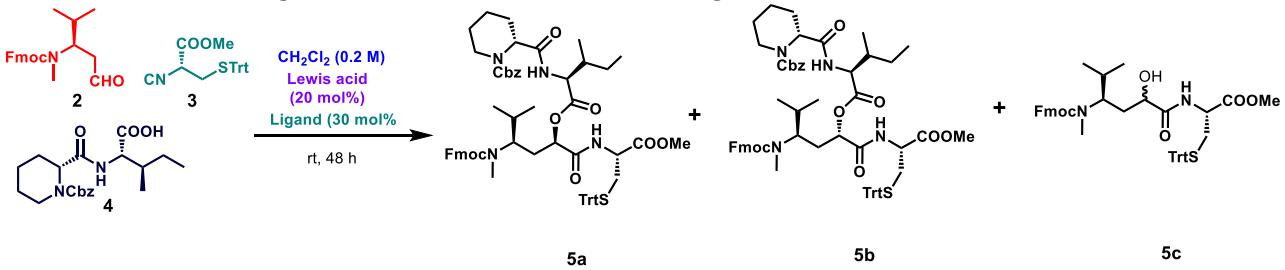
Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous $MgSO_4$ (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), Lewis acid (0.02 mmol) were dissolved in dry CH_2Cl_2 (800 µL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was directly analysed through HPLC. The crude residue was directly analysed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min). The combined fractions of **5a** and **5b** were again purified to obtain pure **5a**.



RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 80 - 90% CH_3CN/H_2O + 0.1%TFA over 8 min.
Absorbance: 214 nm

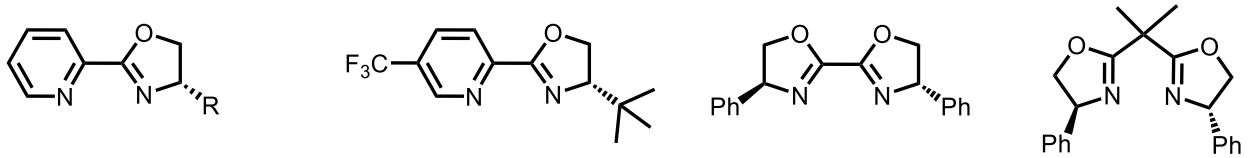
Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

Table 7. Screening of Lewis acids and chiral ligands



Entry	Ligand (30 mol%)	Yield (%)[a,b] (mixture of **5a** and **5b**)	dr[c] **5a**:**5b**	Yield (%)[d] **5c**
1	**L1**	**76 (71)**	**92:8**	**5**
2	L2	68 (51)	70:30	8
3	L3	60 (49)	85:15	10
4	L4	56 (33)	76:24	28
5	L5	70 (45)	72:28	15
6	L6	55 (28)	69:39	10

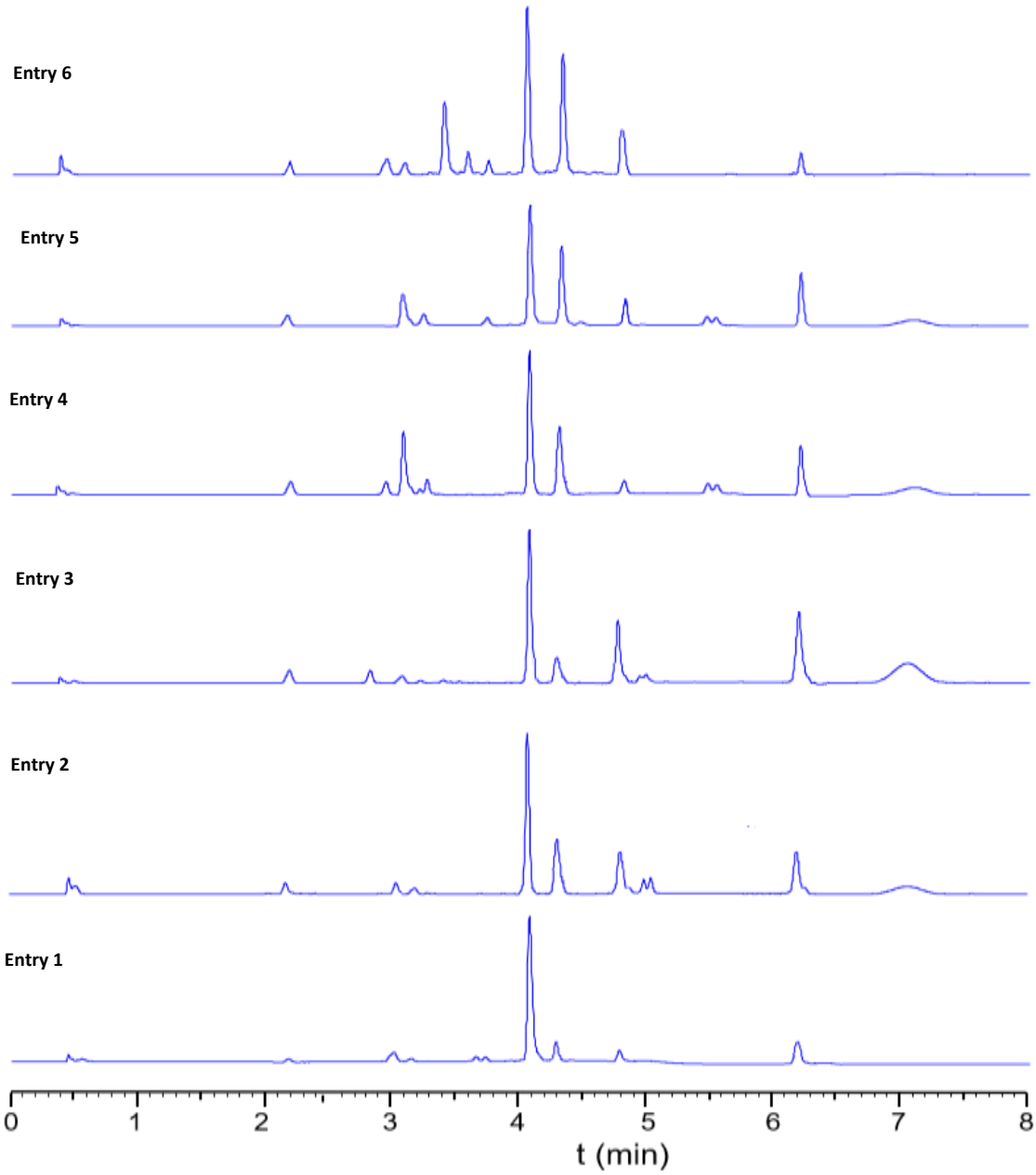
[a]Isolated yields of **5a** and **5b** is are given; [b] Isolated yields of **5a** is given in the parenthesis; [b] dr ratios are measured by integrating the area percentage at 4.1 and 4.3 min; [d] isolated yields of **5c** is given



L1, **R = ^{t}Bu;**
L2, R = Ph;
L3, R = ^{i}Pr;

L4 **L5** **L6**

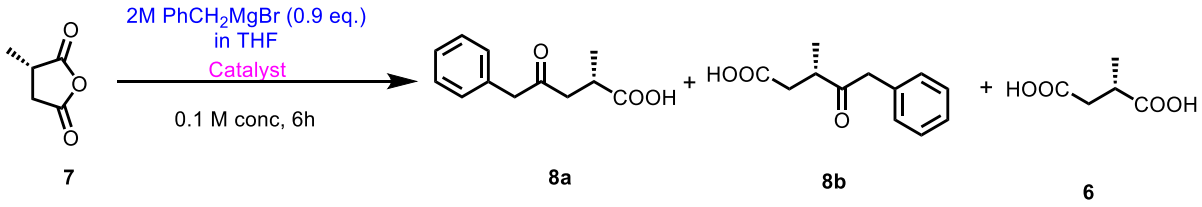
Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous $MgSO_4$ (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), Lewis acid (0.02 mmol) and Ligand (0.03 mmol) were dissolved in dry CH_2Cl_2 (800 µL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was directly analyzed through HPLC. The crude residue was directly analyzed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min). The combined fractions of **5a** and **5b** were again purified to obtain pure **5a**.

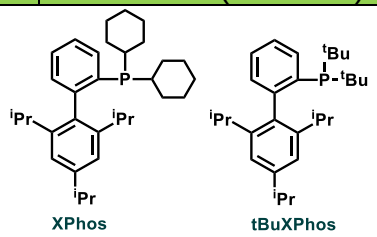


RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 80 - 90% CH_3CN/H_2O + 0.1%TFA over 8 min.
Absorbance: 214 nm

Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

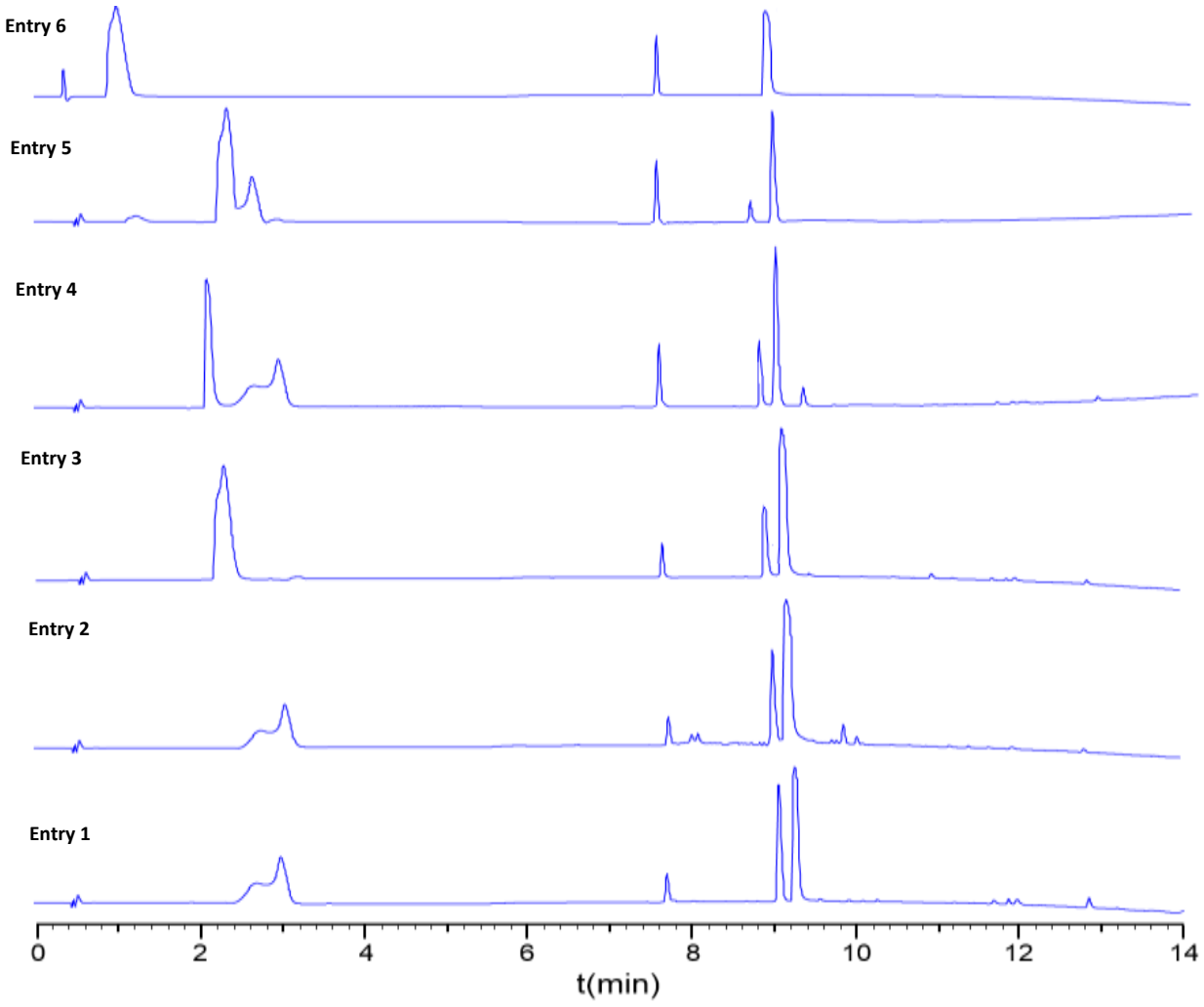
Regioselective ring opening of 7



Entry	Temperature	Catalyst	Yield (%)[a] **6/ 8a/ 8b**
1	0 °C	-	8/ 45/ 43
2	-78 °C	-	3/ 55/ 40
3	-78 °C	CuI (10 mol%)	10/ 58/ 33
4	-78 °C	CuI (10mol%) / PPh_3 (20 mol%)	20/ 65/ 20
5	-78 °C	CuI (10 mol%) / Xphos (20 mol%)	17/ 72/10
6	-78 °C	CuI (10 mol%)+ ^{t}BuXPhos (20 mol%)	20/ 70/0
 XPhos tBuXPhos			

[a] HPLC yields are given by integrating the retention times at 7.7, 9.1 and 9.3 min

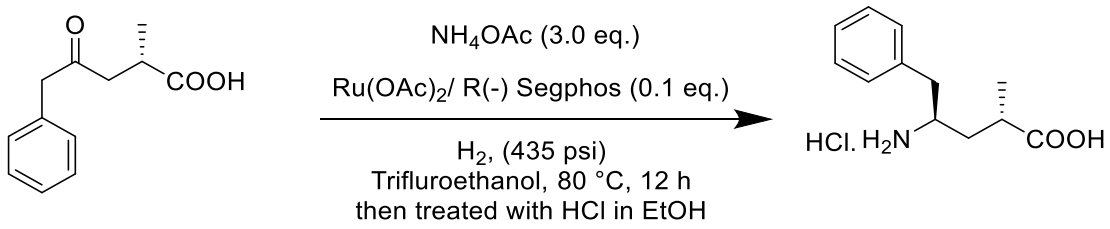
To a solution of anhydride **7** (114 mg, 1.0 mmol) in anhydrous THF (10 mL), the respective catalyst was added. The round bottomed flask was degassed and filled with Nitrogen. The solution was then cooled to desired temperature as shown in table. Benzyl magnesium bromide (2.0 M in THF, 0.9 mmol) was slowly added to the reaction mixture in 30 mins *via* syringe pump (a purple colour forms, but it disappears after the addition is finished). The reaction mixture was stirred at same temperature for additional 6h and then warm to room temperature for 1h. H_2O (20 mL) and 1N HCl (20 mL) were added and stirred for 5 mins. The reaction mixture diluted with was EtOAc (2 X 20 mL) and filtered through pad of Cellite. The organic layer was separated, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was analysed through RP-HPLC analysis. The major desired regioisomer was purified through flash column chromatography on silica (n-hexane/EtOAc, 100:0 to 90:10) confirmed through ^{1}H NMR analysis**.**



RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 10 - 90% CH_3CN/H_2O + 0.1%TFA over 14 min.
Absorbance: 211 nm

Compound	Retention time (min)
8a	9.3
8b	9.1
6 (methyl succinic acid)	7.7
Benzyl alcohol and phenolic by-products	1-4 broad peaks

Diastereoselective synthesis of Tubuphenylalanine (9)

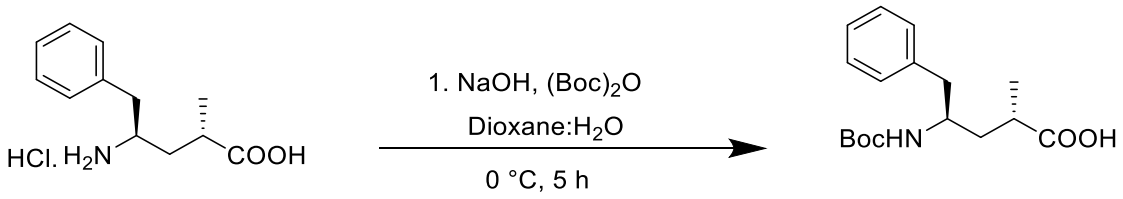


18 **9**

To a 100-mL stainless steel autoclave equipped with a glass inner lining and a Teflon coated stirrer bar was placed $Ru(OAc)_2$((*R*)-dm-segphos) (4.7 mg, 0.005 mmol), **18** (103.0 mg, 0.5 mmol) and ammonium acetate (192.5 mg, 2.5 mmol). The atmosphere was replaced with nitrogen gas, followed by addition of Trifluoroethanol (2 mL). Hydrogen was initially introduced into the autoclave at a pressure of 145 psi, before being reduced to 14.5 psi by carefully releasing the stop valve. After this procedure was repeated three times, hydrogen was introduced at 435 psi. The autoclave was placed in a pre-warmed oil-bath set at 80 °C, and the mixture was stirred for 12 h. The solution was cooled to 20 °C then hydrogen gas was carefully released. Hydrochloric acid (1 mL, 1M) was added to the reaction mixture and the aqueous phase was washed with diethyl ether for three times and basified with sodium hydroxide(0.5 mL, 5 M), followed by extraction with ethyl ether (2 mL x 3). The combined organic phase was dried over sodium sulfate, filtered and evaporated under reduced pressure. The resulting free amine was then dissolved in acetonitrile (1 mL) was cooled to 0 °C then HCl in EtOH (1M, 1mL) was added dropwise with stirring. The resulting hydrochloride slat of **9** was isolated by filtration and washing with cold acetonitrile (3 X 1 mL) and dried under vacuum desiccator. The white powder obtained was directly subjected to NMR analysis, measurement of optical rotation and HRMS.

To facilitate the measurement of chiral SFC, the **9** obtained was derived into its Boc-protected **9a**.

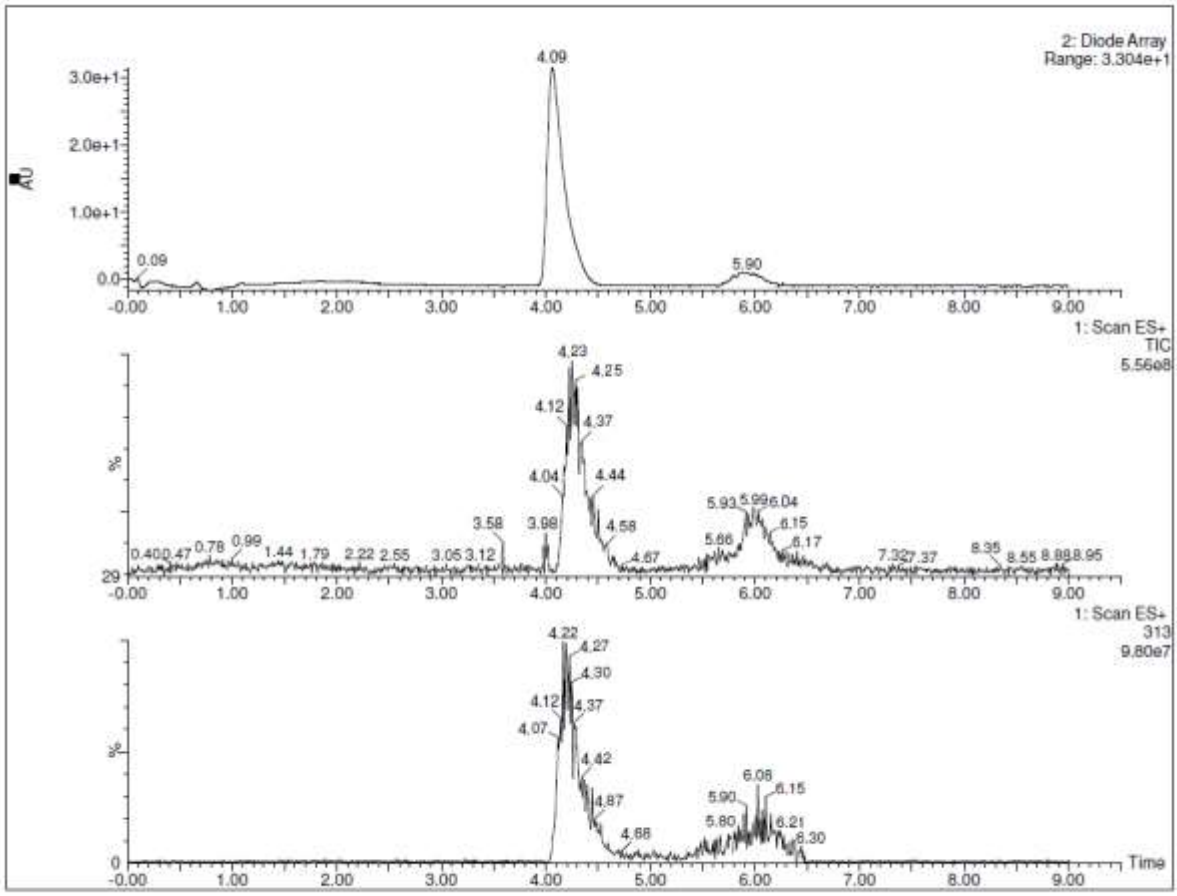
Chiral SFC analysis of 9 as its derivative 9a



9 **9a**

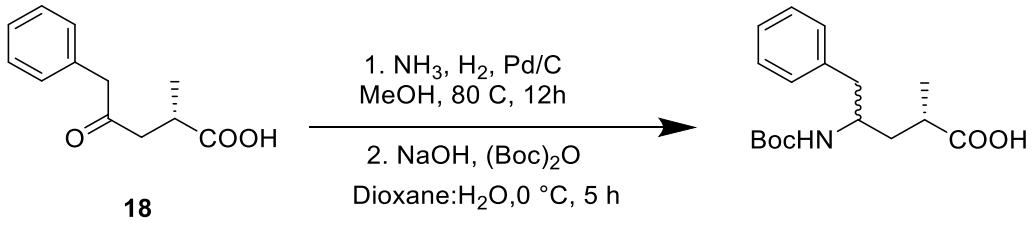
9 (0.1 mg, 0.0004 mmol) was dissolved in Dioxane:H_2O (1:1, 0.5 mL) followed by addition of NaOH (0.032 mg, 0.0008 mmol) at 0 °C. After stirring for 1min $(Boc)_2O$ (0.113 mg, 0.00052 mmol) was added. The reaction mixture was stirred at same temperate for 5 h. The solvent was evaporated and dilute with H_2O (2 mL) and washed with diethyl ether (2 X 2 mL) and the aqueous layer was acidified to pH 2 using citric acid solution and extract with EtOAc (2 X 2 mL). The organic phase was separated, dried and evaporated under reduced pressure. The corresponding **9a** obtained were used for chiral SFC-HPLC test to evaluate the de value.

Chiral SFC HPLC analysis:
Method: Reprosil Chiral-AM column (4.6 × 250 mm, 5µm) with 5 - 30% *i*-PrOH in CO_2 for 9 min; γ = 214 nm.
Indicated 98:2 er: t_R (major) = 4.09 min, t_R (minor) = 5.90 min.



Chiral SFC HPLC chromatogram of **9a**

Chiral SFC analysis of diastereomeric 9 as derivative of 9b

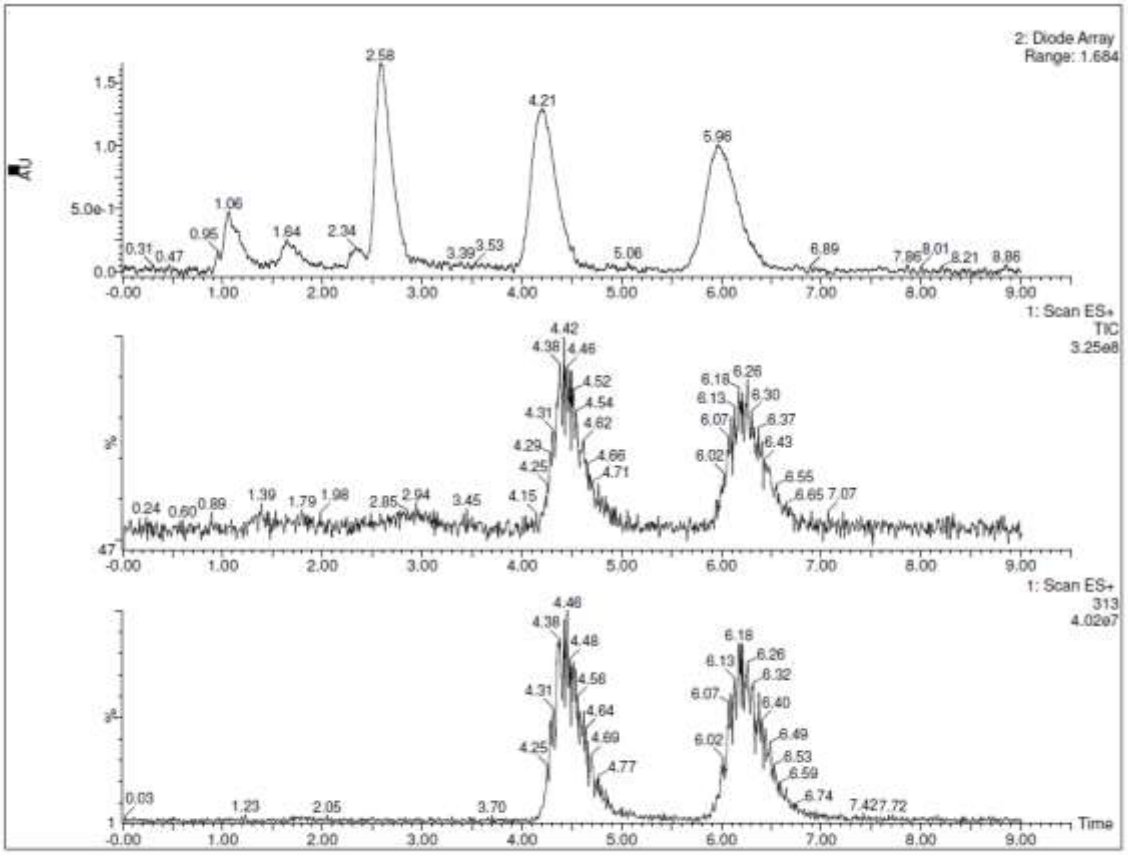


18 **9b**

Step1: To a 50-mL stainless steel autoclave equipped with a glass inner lining and a Teflon coated stirrer bar was placed **18** (50.0 mg, 0.25 mmol), MeOH (0.5 mL) and ammonia solution (7N in MeOH, 0.2 mL, 1.25 mmol). Hydrogen was initially introduced into the autoclave at a pressure of 145 psi, before being reduced to 14.5 psi by carefully releasing the stop valve. After this procedure was repeated three times, hydrogen was introduced at 500 psi. The autoclave was placed in a pre-warmed oil-bath set at 80 °C, and the mixture was stirred for 12 h. The solution was cooled to 20 °C then hydrogen gas was carefully released. Hydrochloric acid (1 mL, 1M) was added to the reaction mixture and the aqueous phase was washed with diethyl ether for three times and basified with sodium hydroxide (0.5 mL, 5 M), followed by extraction with ethyl ether (2 mL x 3). The combined organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure.

Step 2: The resulting free amine was then protected with Boc by using the protocol as described for the synthesis of **9a**. The resulting racemic **9b** were used for chiral SFC-HPLC test.

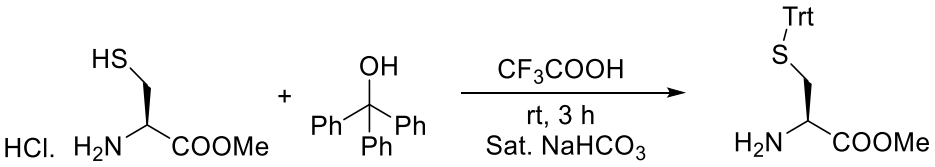
Chiral SFC HPLC analysis:
Method: Reprosil Chiral-AM column (4.6 × 250 mm, 5µm) with 5 - 30% *i*-PrOH in CO_2 for 9 min; γ = 214 nm.
t_R = 4.21 min and 5.96 min.



Chiral SFC HPLC chromatogram of racemic **9b**

Experimental procedure and spectroscopic data of all compounds

Synthesis of methyl S-trityl-L-cysteine (16)



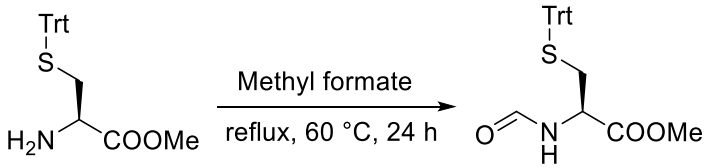
15 **16**

To a solution of triphenyl methanol (95.98 g, 369 mmol, 1.0 eq.) in 400 mL of trifluoroacetic acid L-cysteine methyl ester hydrochloride **15** (50 g, 369.0 mmol). The resulting red-orange solution was left to stir at room temperature for 3 h before it was concentrated to orange oil, which was partitioned between 1000 mL of CH_2Cl_2 and 1000 mL of H_2O, resulting in bleaching of the orange color. Portions of K_2CO_3 were then slowly added to the aqueous layer until a basic pH was sustained for up to 1 h. The organic layer was then separated, washed with saturated $NaHCO_3$ (3 X 200 mL) and brine, dried over $MgSO_4$ and concentrated to a pale oil which solidified after overnight stirring in hexanes. The white solids thus obtained were isolated *via* vacuum filtration.

Yield: 97% (135 g)
TLC: 0.41 (Petroleum ether/EtOAc, 50:50)
White solid
Mp: 55 – 57 °C
$[\alpha]_D^{25}$ = +40.2 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{23}H_{24}NO_2S$ 378.1527; found 378.1521

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 – 7.45 (m, 6H), 7.37 – 7.30 (m, 6H), 7.30 – 7.18 (m, 3H), 3.69 (s, 3H), 3.24 (dd, *J* = 7.8, 4.8 Hz, 1H), 2.64 (dd, *J* = 12.5, 4.9 Hz, 1H), 2.52 (dd, *J* = 12.5, 7.8 Hz, 1H).
^{13}C NMR (126 MHz, $CDCl_3$): δ 174.1, 144.5, 130.0, 129.5, 128.3, 127.9, 126.8, 66.8, 53.7, 52.2, 36.9.

Synthesis of methyl N-formyl-S-trityl-L-cysteine (17)



16 **17**

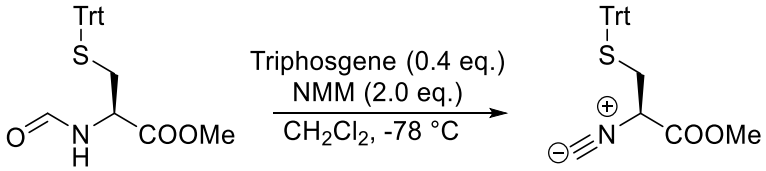
Amine **16** (40.0 g, 106 mmol) was dissolved in methyl formate (700 mL, solvent) and the assembly was allowed to reflux in an oil bath at 60 °C until TLC showed complete

consumption of starting material (usually 24 h). The solvent was evaporated under reduced pressure. Diethyl ether was added to the crude reaction mixture and stirred at 0 ˚C until the product gets precipitated (1 h). The solid product was collected by filtration and washed thoroughly with cold ether (3 X 200 mL). The filtrate is stored at 0 ˚C overnight and the remaining solid were filtered off. The white solid **17** was used without further purification.

Yield: 95% (40.8 g)
TLC: 0.50 (Petroleum ether/EtOAc, 50:50)
White solid
Mp = 130 –132 ˚C
$[\alpha]_D^{25}$ = +28.2 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{20}H_{29}N_2O_5$: 406.1476; found 406.1471

^1H NMR (600 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.38 – 7.31 (m, 5H), 7.27 – 7.22 (m, 5H), 7.22 – 7.20 (m, 3H), 7.19 – 7.16 (m, 2H), 5.78 (d, *J* = 8.1 Hz, 1H), 4.63 – 4.57 (m, 1H), 3.66 (s, 3H), 2.72 (dd, *J* = 12.7, 5.6 Hz, 1H), 2.55 (dd, *J* = 14.3, 7.8 Hz, 1H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 170.3, 160.2, 144.2, 128.1, 128.0, 127.0, 126.9, 67.1, 52.8, 33.6.

28g Synthesis of methyl (*R*)-2-isocyano-3-(tritylthio)propanoate (3)



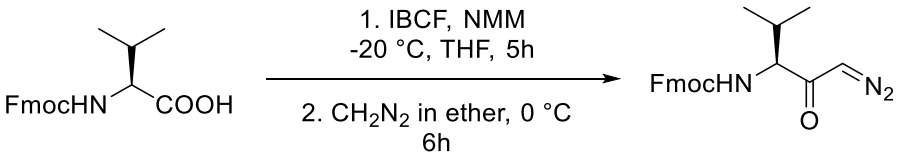
17 **3**

A solution of *N*-formyl Cys(Trt)-methyl ester **17** (35 g, 86.3 mmol) in CH_2Cl_2 (450.0 mL), was cooled to -78 °C. N-methylmorpholine (19.0 mL, 172.6 mmol, 2 eq.) was added. After 5 min triphosgene (10.2 g, 34.52 mmol, 0.40 eq.) in CH_2Cl_2 (150 mL) was added drop wise over 30 min. After addition was complete the reaction mixture was stirred for 7h at -78 ° C (TLC analysis). Saturated $NaHCO_3$ solution (200 mL) was added at same temperature and then allowed to warm to room temperature. The organic layer was separated and the aqueous phase was washed CH_2Cl_2 (3 X 100 mL). The combined organic extracts were separated, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was dissolved in diethyl ether (300 mL) and stored -15 °C for 5 h resulted in pure solid of isocyanide **3** which was collected by filtration. The isocyanide **3** was used further without purification.

TLC: 0.42 (Petroleum ether/EtOAc, 9:1)
Yield: 86% (28.7 g)
White solid
Mp = 97 – 99 ˚C
$[\alpha]_D^{25}$ = +38.4 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{24}H_{22}NO_2S$ 388.1365; found 388.1366

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.13 (m, 15H), 3.65 (s, 3H), 3.27 (dd, *J* = 8.0, 5.8 Hz, 1H), 2.75 – 2.70 (m, 1H), 2.68 (dd, *J* = 13.7, 8.0 Hz, 1H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 165.6, 160.9, 146.8, 143.9, 129.4, 128.2, 127.9, 127.2, 127.1, 67.5, 53.4, 34.2.

40g Synthesis of (*S*)-(9*H*-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (19)



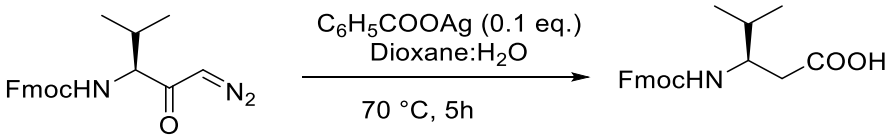
18 **19**

Under an argon atmosphere *N*-methyl morpholine (20.28 mL, 184.5 mmol, 1.5 eq,) and isobutylchloroformate (20.7 mL, 159.9 mmol, 1.30 eq) were carefully added at -20 °C to the Fmoc-Val-OH **18** (43.0 g, 123.0 mmol) dissolved in dry THF (550 mL). The reaction mixture was stirred at -20 °C for 5 hr and then under exclusion of light diazomethane in diethyl ether (3.0 eq. generated from the *N*-nitroso *N*-methyl urea using Diazard kit), was added and it was stirred at rt for 12 h. Afterwards, the excess of diazomethane was quenched by addition of glacial acetic acid (10 mL) and the mixture was taken up in 10% $NaHCO_3$ (3 X 200 mL) and extracted with EtOAc (3 X 300 mL). The combined organic phases were washed with saturated NH_4Cl (3 X 200 mL)), saturated NaCl, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product (yellow solid) was recrystalized using CH_2Cl_2: n-pentane (70:135 mL) and pure **19** collected by filtration and dried.

Yield: 88% (39.3 g)
TLC: 0.52 (Petroleum ether/EtOAc, 7:3)
Pale yellow solid
mp 122 − 124 ˚C
$[\alpha]_D^{25}$ = +16.3 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{21}H_{22}N_3O_3$ 364.1661; found 364.1655

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.6 Hz, 2H), 7.66 – 7.55 (m, 2H), 7.46 – 7.30 (m, 4H), 5.51 (d, *J* = 9.0 Hz, 1H), 5.36 (br, s, 1H), 4.46 (d, *J* = 7.2 Hz, 2H), 4.24 (t, *J* = 6.7 Hz, 1H), 4.18 – 4.12 (m, 1H), 2.18 – 2.08 (m, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H).
^{13}C NMR (121 MHz, $CDCl_3$) δ 193.3, 156.3, 143.8, 141.3, 127.7, 127.7, 127.1, 127.0, 125.1, 125.0, 120.0, 66.8, 47.3, 31.0, 25.5, 19.4, 17.4.

26g Synthesis of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (20)



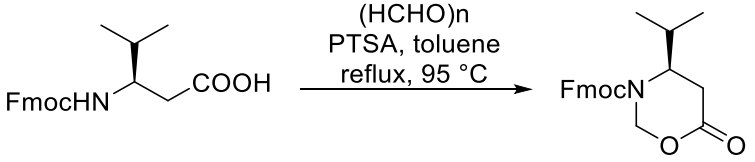
19 **20**

The diazo ketone **19** (30 g, 82.6 mmol) was dissolved in Dioxane/H_2O (9:1, 495 mL) and under exclusion of light silver benzoate (1.8 g, 8.26 mmol, 0.1 eq.) was added at 0 ˚C and the reaction mixture was stirred at same temperature for 30 min. Then the reaction mixture was refluxed in an oil bath at 70 ˚C for 5 h. After completion of the reaction (TLC analysis), the mixture was cooled down to room temperature and diluted with H_2O (330 mL), adjusted to pH = 2-3 by the addition of 1.0 M HCl and extracted with EtOAc (3 X 300 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was taken up in DCM (128 mL) and was cooled to -22 °C, cold pentane (78 mL) was added dropwise. The obtained precipitate was filtered off, washed with cold pentane (2 X 50 mL) and dried overnight under reduced pressure to yield **20**.

Yield: 95% (26.3 g)
TLC: 0.42 ($CHCl_3$/ MeOH/ AcOH, 40:2:1)
White solid
mp 153 − 155 ˚C
$[\alpha]_D^{25}$ = - 37.5 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{21}H_{24}NO_4$ 354.1705; found 354.1700

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.85 (s, 1H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.33 (td, *J* = 7.5, 1.2 Hz, 2H), 5.17 (d, *J* = 3.8 Hz, 1H), 4.43 (d, *J* = 7.0 Hz, 2H), 4.24 (t, *J* = 6.8 Hz, 1H), 3.84 (td, *J* = 12.2, 9.8, 6.0 Hz, 1H), 2.66 – 2.53 (m, 2H), 1.89 (dt, *J* = 13.7, 6.9 Hz, 1H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 7.2 Hz, 3H).
^{13}C NMR (121 MHz, $CDCl_3$) δ 176.9, 156.1, 143.8, 141.3, 127.6, 127.1, 127.0, 125.1, 125.0, 119.9, 66.6, 54.0, 47.3, 36.8, 31.6, 19.3, 18.6.

20g Synthesis of (*R*)-(9*H*-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (21)



20 **21**

The Fmoc-β-Val-OH **20** (22 g, 66 mmol), paraformaldehyde (12.4 g) and *p*-toluene sulfonic acid (1.2 g, 0.1 eq.)) were suspended in toluene (800 mL). The mixture was

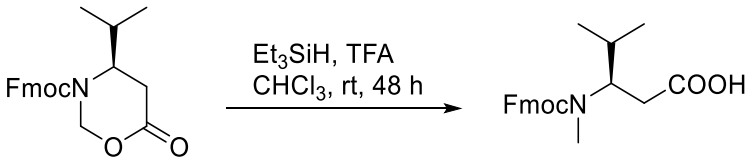
refluxed in an oil bath at 95 °C in a Dean–Stark setup until no more starting material could be detected by TLC analysis (6 h). The solution was cooled, washed with saturated $NaHCO_3$ (3 X 200 mL) and the aqueous phase was extracted with EtOAc (3 X 200 mL). The combined organic layer was then washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* gave the crude product, which was purified by silica gel column chromatography with ethyl acetate–*n*-hexane (3:7).

Yield: 82% (19.7 g)
TLC: 0.45 (Petroleum ether/EtOAc, 7:3)
Gummy solid
$[\alpha]_D^{25}$ = + 93.1 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{22}H_{24}NO_4$ 366.1705; found 366.1695

^1H NMR (600 MHz, $CDCl_3$) (major rotamer) δ 7.64 – 7.18 (m, 8H), 5.65 (d, *J* = 22.4 Hz, 1H), 5.62 (d, *J* = 20.6 Hz, 1H), 4.43 (d, *J* = 10.6 Hz, 2H), 4.11 (t, *J* = 16.6 Hz, 1H), 4.09 – 4.03 (m, 1H), 2.65 (s, 3H), 2.56 (dd, *J* = 15.1, 4.3 Hz, 1H), 2.69 – 2.57 (m, 1H), 2.18 – 2.10 (m, 2H), 0.84 (d, *J* = 6.5 Hz, 3H), 0.75 (d, *J* = 6.4 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) (major rotamer) δ 170.1, 154.9, 143.2, 141.1, 127.7, 127.1, 124.4, 119.8, 72.8, 67.0, 46.9, 31.6, 25.6, 18.1.

^1H NMR (600 MHz, $CDCl_3$) (minor rotamer) δ 7.64 – 7.18 (m, 5H), 5.34 (d, *J* = 20.4 Hz, 0.55 H), 5.27 (d, *J* = 18.6 Hz, 0.55H), 4.60 (d, *J* = 10.6 Hz, 1.4H), 4. 09 (t, *J* = 14.4 Hz, 0.5H), 4.04 – 4.00 (m, 0.7H), 2.62 – 2.03 (m, 1.5 H), 0.52 (d, *J* = 6.6 Hz, 2H), 0.39 (d, *J* = 6.4 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) (minor rotamer) δ 170.9, 154.7, 143.3, 141.2, 127.6, 127.0, 124.3, 119.8, 71.6, 67.8, 49.2, 31.0, 23.7, 20.7.

9g Synthesis of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (22)



21 **22**

The oxazolidinone **21** (10 g, 27.3 mmol) was dissolved in 300 mL of $CHC1_3$, and 300 mL of trifluoroacetic acid and triethylsilane (13.0 mL, 82.0 mmol) were added. The solution was stirred at room temperature for 48 h followed by concentration in vacuo to an oil. The oil was dissolved in CH_2C1_2 and reconcentrated several times with CH_2Cl_2. The resultant oil was purified on silica gel flash column chromatography (PE/ EA 100:0 to 20:80 then to 0:100).

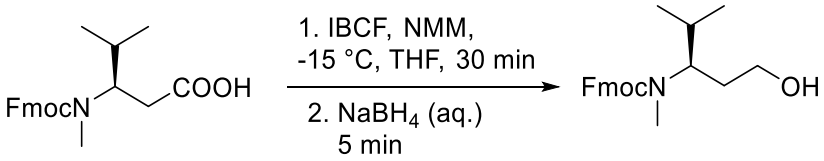
Yield: 90% (9.01 g)

TLC: 0.34 (Petroleum ether/ EtOAc/ AcOH, 5:5:0.2)
$[\alpha]_D^{25}$ = -23.6 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + Na]^+$ calculated for $C_{22}H_{25}NO_4Na$ 390.1681; 390.1680

^1H NMR (600 MHz, $CDCl_3$) (major rotamer) δ 8.86 (s, 1H), 7.65 (dt, *J* = 15.9, 7.4 Hz, 2H), 7.57 – 7.46 (m, 2H), 7.28 (q, *J* = 7.5 Hz, 2H), 7.20 (q, *J* = 7.4, 6.4 Hz, 2H), 4.37 (d, *J* = 22.4, 2H), 4.29 (t, *J* = 10.6, 1H), 4.03 – 3.93 (m, 1H), 2.65 (s, 3H), 2.56 (dd, *J* = 15.1, 4.3 Hz, 1H), 2.46 (dd, *J* = 15.2, 10.5 Hz, 1H), 2.21 (d, *J* = 14.6 Hz, 1H), 2.05 (s, 1H), 1.80 – 1.70 (m, 1H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.75 (d, *J* = 6.6 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) (major rotamer) δ 176.9, 156.7, 144.2, 141.3, 127.6, 127.5, 127.0, 125.1, 124.7, 119.9, 67.3, 60.2, 47.3, 37.6, 35.8, 30.5, 19.8, 19.5.

^1H NMR (600 MHz, $CDCl_3$) (minor rotamer) δ 7.65 – 7.20 (m, 5H), 4.52 (d, *J* = 10.9, 0.7H), 4.13 (t, *J* = 6.9 Hz, 0.5H), 4.03 – 3.93 (m, 0.3H), 2.68 (s, 1.7H), 2.56 (dd, *J* = 15.1, 4.3 Hz, 2H), 2.46 (dd, *J* = 15.2, 10.5 Hz, 2H), 2.21 (dd, *J* = 14.6, 7.4 Hz, 0.5H), 2.05 (dd, *J* = 12.6, 8.2 Hz, 0.5H), 1.80 – 1.70 (m, 2H), 0.62 (d, *J* = 6.6 Hz, 1.7H), 0.49 (d, *J* = 6.6 Hz, 1.8H).
^{13}C NMR (151 MHz, $CDCl_3$) (minor rotamer) δ 176.5, 156.3, 144.1, 141.3, 127.6, 127.1, 127.0, 125.0, 119.8, 67.1, 47.3, 37.6, 35.6, 30.5, 19.8, 19.2.

Synthesis of (*R*)-(9*H*-fluoren-9-yl)methyl (1-hydroxy-4-methylpentan-3-yl)(methyl)carbamate (23)



22 **23**

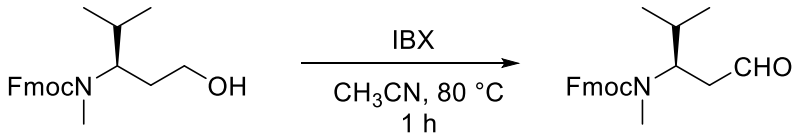
A solution of **23** (3.67 g, 10 mmol) in THF (50 mL) was cooled to -15 °C (ice/ salt bath) under a nitrogen atmosphere. NMM (1.62 mL, 15 mmol, 1.5 eq.) and isobutyl chlorformate (IBCF, 1.55 mL, 12 mmol, 1.2 eq.) were added successively in a dropwise manner. After 3 h, the reaction mixture was filtered. The filtrate was cooled to -15 °C (ice/ salt bath), and a solution of $NaBH_4$ (1.6 g, 40 mmol, 4.0 eq.) in H_2O (2.5 mL) was added in one portion. After complete reduction (TLC analysis), the suspension was diluted with EtOAc and H_2O (1:1). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 X 50 mL) and the combined organic layer was dried over $MgSO_4$ and evaporated under vacuum. The crude product was purified on silica gel flash column chromatography (Pet ether / EtOAc, 100:0 to 20:80).

Yield: 96% (3.39 g)
TLC: 0.25 (Petroleum ether/EtOAc, 5:5)
$[\alpha]_D^{25}$ = -10.4 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{22}H_{28}NO_3$ 354.2069; found 354.2060

^{1}H NMR (600 MHz, $CDCl_3$) (major rotamer) δ 7.82 – 7.34 (m, 8H), 4.53 – 4.50 (m, 2H), 4.26 (t, *J* = 4.3 Hz, 1H), 3.86 (ddd, *J* = 12.1, 10.4, 3.2 Hz, 1H), 3.54 (ddd, *J* = 11.7, 5.6, 2.9 Hz, 1H), 3.34 – 3.26 (m, 1H), 2.61 (s, 3H), 1.95 (dddd, *J* = 14.1, 10.7, 5.5, 3.2 Hz, 1H), 1.70 (dp, *J* = 10.4, 6.6 Hz, 1H), 1.47 – 1.35 (m, 1H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) (major rotamer) δ 157.2, 143.3, 140.7, 126.9, 126.8, 126.3, 126.2, 124.1, 123.6, 119.1, 66.3, 58.1, 52.6, 46.7, 31.1, 29.3, 26.8, 19.3, 18.5.

^{1}H NMR (600 MHz, $CDCl_3$) (minor rotamer) (selected signals) δ 7.82 – 7.34 (m, 3H), 4.68 (dd, *J* = 10.7, 4.5 Hz, 0.6H), 4.22 (t, *J* = 4.7 Hz, 0.3H), 3.86 (ddd, *J* = 12.1, 10.4, 3.2 Hz, 3H), 3.54 (ddd, *J* = 11.7, 5.6, 2.9 Hz, 3H), 3.34 – 3.26 (m, 0.3H), 2.63 (s, 0.7H), 1.47 – 1.35 (m, 0.3H), 0.73 (d, *J* = 6.8 Hz, 1H), 0.49 (d, *J* = 6.6 Hz, 1H).
^{13}C NMR (151 MHz, $CDCl_3$) (minor rotamer) δ 155.9, 143.0, 140.5, 126.8, 126.3, 126.2, 124.0, 123.6, 119.1, 65.6, 57.6, 52.2, 46.57, 30.8, 29.1, 19.2, 19.1.

Synthesis of (*R*)-(9*H*-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2)



23 **2**

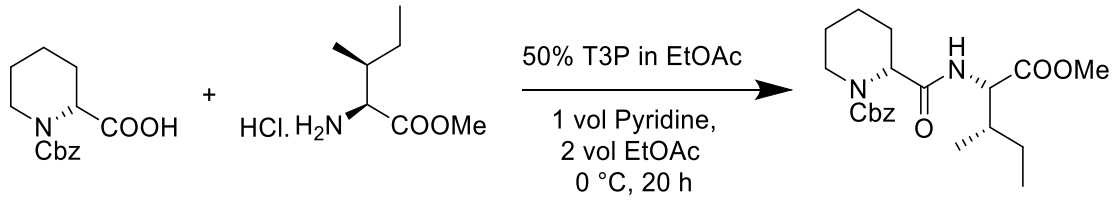
Alcohol **23** (3.0 g, 8.4 mmol) was dissolved in CH_3CN (50 mL) and freshly prepared IBX (4.7 g, 16.8 mmol, 2.0 eq.) was added. The resulting suspension was immersed in an oil bath set to 80 °C and stirred vigorously in an open atmosphere. After 2 h (TLC monitoring), the reaction was cooled to 5 °C and filtered through a medium glass frit. The filter cake was washed with cold ethyl acetate (3 X 10 mL), and the combined filtrates were concentrated. The crude aldehyde **2** was pure enough for further reactions. For NMR and SFC analysis small amount was purified by flash column chromatography (PE/EA 100:0 to 70:30) to yield aldehyde **2** as a yellow gum.

Yield: 96% (2.4 g)
TLC: 0.51 (Petroleum ether/EtOAc, 7:3)
$[\alpha]_D^{25}$ = -92.6 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{22}H_{26}NO_3$ 352.1912; found 352.1906

^{1}H NMR (500 MHz, $CDCl_3$) (major rotamer) δ 9.66 (s, 1H), 7.79 – 7.30 (m, 8H), 4.72 – 4.63 (m, 1H), 4.47 (d, *J* = 6.8 Hz, 2H), 4.34 – 4.22 (m, 1H), 2.73 (s, 3H), 2.70 (dd, *J* = 4.3, 1.2 Hz, 1H), 2.56 (dd, *J* = 10.8, 4.2 Hz, 1H), 1.86 – 1.81 (m, 1H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) (major rotamer) δ 200.9, 156.4, 144.1, 143.8, 141.4, 127.5, 127.1, 126.9, 124.9, 124.8, 124.3, 119.8, 67.0, 57.6, 47.3, 44.7, 31.8, 29.6, 19.8, 19.2, 18.8.

^{1}H NMR (500 MHz, $CDCl_3$) (minor rotamer) (selected signals) δ 9.04 (s, 0.7H), 7.79 – 7.30 (m, 5.6H), 4.72 – 4.63 (m, 1H), 4.34 – 4.22 (m, 2H), 2.66 (s, 2H), 2.25 (dd, *J* = 16.4, 4.5 Hz, 1H), 2.15 (dd, *J* = 9.9, 3.5 Hz, 1H), 1.36 – 1.34 (m, 0.8H), 0.66 (d, *J* = 6.6 Hz, 2H), 0.50 (d, *J* = 6.6 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) (minor rotamer) δ 200.5, 155.9, 143.9, 141.2, 127.1, 126.9, 124.8, 124.3, 119.7, 66.4, 57.8, 47.2, 44.6, 30.2, 29.2, 19.7, 18.8.

Synthesis of (*R*)-benzyl 2-(((2*S*,3*R*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26)



24 **25** **26**

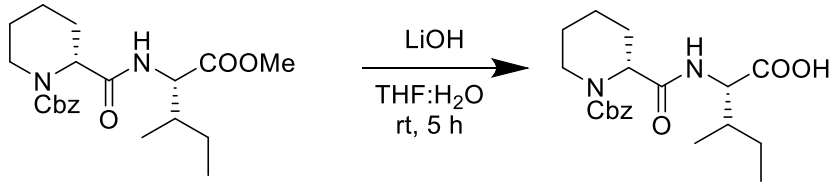
Acid **24** (20.0 g, 76.0 mmol), amine **25** (12.1 g, 83.6 mmol, 1.1 eq.), pyridine (20.7 mL, 266 mmol, 1 volume), and EtOAc (42 mL, 2 volumes) were charged in an 500 mL flask with constant stirring. The heterogeneous mixture was cooled between -20 °C and -10 °C as 91.4 mL of T3P solution (50 wt% in EtOAc, 152 mmol, 2.0 eq.) were added *via* addition funnel at a rate to maintain an internal temperature below 0 °C. The resulting homogeneous, golden-yellow solution was stirred at 0 °C for 20 h, at which time the coupling was complete and amide **26** was formed. The reaction mixture was again cooled between -20 °C and -10 °C as 60 mL (3 volumes) of 0.5 M aq HCl solution were added (exothermic) *via* addition funnel at a rate to maintain an internal temperature below 5 °C. The mixture was diluted with 300 mL of EtOAc and the phases were separated. The organic layer was washed with 100 mL of 0.5 M aq HCl, 100 mL of sat. NaCl solution, and concentrated. The pure product was isolated by flash column chromatography (Pet Ether / EtOAc, 100:0 to 70:30).

Yield: 89% (26.4 g)
TLC: 0.35 (Petroleum ether/EtOAc, 8:2)
Colour less gum
$[\alpha]_D^{25}$ = + 35.5 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + Na]^+$ calculated for $C_{21}H_{30}N_2O_5Na$ 413.2052; found 413.2051

^{1}H NMR (600 MHz, MeOD) δ 7.41 – 7.27 (m, 5H), 5.16 (s, 2H), 4.87 (dd, *J* = 6.2, 2.3 Hz, 1H), 4.44 (d, *J* = 6.2 Hz, 1H), 4.09 – 4.03 (m, 1H), 3.69 (s, 3H), 3.26 (dt, *J* = 13.2, 6.5 Hz, 1H), 2.19 – 2.12 (m, 1H), 1.91 (dtd, *J* = 8.8, 6.5, 4.2 Hz, 1H), 1.75 – 1.71 (m, 1H), 1.64 (td, *J* = 7.1, 3.5 Hz, 1H), 1.48 – 1.41 (m, 4H), 1.24 – 1.14 (m, 1H), 0.94 – 0.88 (m, 6H).

^{13}C NMR (151 MHz, MeOD) δ 172.6, 172.0, 156.8, 136.6, 128.2, 128.0, 127.7, 127.4, 126.8, 67.1, 63.8, 56.7, 51.1, 41.9, 36.9, 26.8, 24.9, 24.3, 19.7, 14.8, 10.3.

18g Synthesis of (2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)



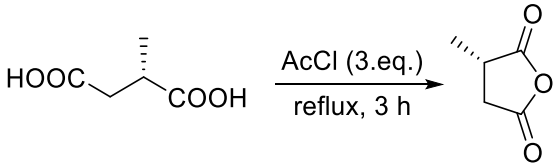
26 **4**

To a solution of the **26** (20 g, 51.2 mmol) in THF: H_2O: MeOH (1:0.5:1, 100 mL), lithium hydroxide (2.10 g, 76.8 mmol) was added at 0 °C. The mixture was stirred for 3 h at R.T. and then acidified to pH 2.0 with 1N HCl. The aqueous layer was extracted with ethyl acetate (3 X 150 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated in vacuum to yield **4** and used without further purification.

Yield: 95% (18.3 g)
TLC: 0.35 ($CHCl_3$/ MeOH/ AcOH, 40:2:1)
Colour less gum
$[\alpha]_D^{25}$ = +42.3 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{20}H_{29}N_2O_5$ 377.2076; found 377.2069

^{1}H NMR (600 MHz, MeOD) δ 7.36 (d, *J* = 5.8 Hz, 3H), 7.30 (d, *J* = 8.6 Hz, 2H), 5.16 (s, 2H), 4.87 (dd, *J* = 6.2, 2.3 Hz, 1H), 4.46 – 4.40 (m, 1H), 4.09 – 4.05 (m, 2H), 3.24 (td, *J* = 12.9, 3.1 Hz, 1H), 2.16 (t, *J* = 18.2 Hz, 1H), 1.93 (tdd, *J* = 12.4, 8.4, 5.3 Hz, 1H), 1.71 – 1.64 (m, 2H), 1.48 (tdd, *J* = 15.1, 11.9, 7.7 Hz, 3H), 1.18 (ddt, *J* = 14.2, 8.8, 7.2 Hz, 1H), 0.96 – 0.90 (m, 6H).
^{13}C NMR (151 MHz, MeOD) δ 173.1, 171.5, 156.2, 136.5, 128.1, 127.6, 127.4, 67.1, 60.1, 56.5, 41.9, 36.9, 24.7, 19.4, 19.3, 14.7, 13.0, 10.3.

Synthesis of (*S*)-3-methyldihydrofuran-2,5-dione (7)



6 **7**

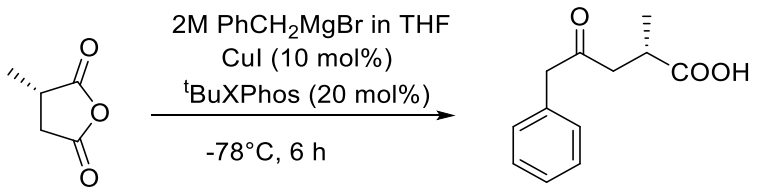
(*S*)-3-methyldihydrofuran-2,5-dione (1.32 g, 10.0 mmol) and acetyl chloride (2.83 mL, 30.0 mmol) were placed in a single neck round bottomed flask and the assembly was refluxed in an oil bath (75 °C) on the steam bath for 3 h. The solution is allowed to cool undisturbed and is finally chilled in an ice bath. The succinic anhydride **7**, which separates in beautiful crystals, and is collected on a Büchner funnel, washed with cold ether (2 X 75 mL), and dried in a vacuum desiccator.

Yield: 98% (1.11 g)
TLC: 0.54 (Petroleum ether/EtOAc, 6:4)

$[\alpha]_D^{25}$ = -11.0 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + Na]^+$ calculated for $C_5H_6O_3Na$ 137.0214; found 137.0204

^1H NMR (500 MHz, $CDCl_3$) δ 3.27 – 3.14 (m, 2H), 2.70 – 2.59 (m, 1H), 1.45 (d, *J* = 6.1 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 174.4, 170.0, 36.0, 35.0, 16.1.

Synthesis of (*S*)-2-methyl-4-oxo-5-phenylpentanoic acid (8a)



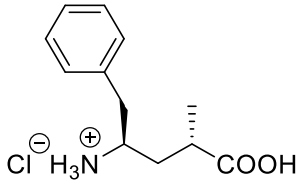
7 **8a**

To a solution of anhydride **17** (1.14 g, 10 mmol), CuI (190 mg, 1 mmol, 0.1 eq.) and ^{t}BuXPhos (849.2 mg, 0.2 mmol, 0.2 eq.) in dry THF (100 mL) was cooled to -78 °C. benzyl magnesium bromide (2.0 M in THF, 0.9 mmol) slowly added to the reaction mixture over 30 min (A purple color forms, but it disappear after the addition is finished). The reaction mixture was stirred at same temperature for additional 6h and then room temperature for 1h. H_2O (50 mL) and 1N HCl (50 mL) were added and stirred for 5 mins. The reaction mixture diluted with was EtOAc (2 X 200 mL) and filtered through pad of Cellite. The organic layer was separated, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified though column chromatography. No other regioisomer was observed through HPLC analysis.

Yield: 70 % (798 mg)
TLC: 0.51 (Petroleum ether/EtOAc/TFA, 7:3:0.1)
$[\alpha]_D^{25}$ = -25.8 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + Na]^+$ calculated for $C_{12}H_{14}O_3Na$ 229.0840; found 229.0837

^1H NMR (500 MHz, $CDCl_3$) δ 10.54 (s, 1H), 7.40 – 7.34 (m, 5H), 5.17 (s, 2H), 3.07 – 2.96 (m, 1H), 2.53 (dd, *J* = 6.9, 3.0 Hz, 1H), 2.50 (dd, *J* = 12.5, 6.0 Hz, 1H), 1.29 (d, *J* = 7.2 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 201.8, 175.0, 135.7, 128.0, 128.2, 128.0, 125.7, 66.5, 44.5, 37.3, 16.8.

Synthesis of (2*S*,4*R*)-4-(l4-azaneyl)-2-methyl-5-phenylpentanoic acid (Tup, 9)

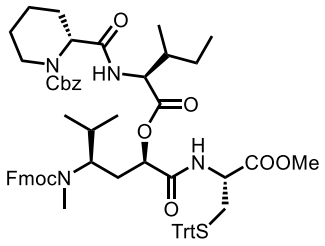


To a 100-mL stainless steel autoclave equipped with a glass inner lining and a Teflon coated stirrer bar was placed $Ru(OAc)_2$((*R*)-dm-segphos) (4.7 mg, 0.005 mmol), **18** (103.0 mg, 0.5 mmol) and ammonium acetate (192.5 mg, 2.5 mmol). The atmosphere was replaced with nitrogen gas, followed by addition of Trifluoroethanol (2 mL). Hydrogen was initially introduced into the autoclave at a pressure of 145 psi, before being reduced to 14.5 psi by carefully releasing the stop valve. After this procedure was repeated three times, hydrogen was introduced at 435 psi. The autoclave was placed in a pre-warmed oil-bath set at 80 °C, and the mixture was stirred for 12 h. The solution was cooled to 20 °C then hydrogen gas was carefully released. Hydrochloric acid (1 mL, 1M) was added to the reaction mixture and the aqueous phase was washed with diethyl ether for three times and basified with sodium hydroxide (0.5 mL, 5 M), followed by extraction with ethyl ether (2 mL x 3). The combined organic phase was dried over sodium sulfate, filtered and evaporated under reduced pressure. The resulting free amine was then dissolved in acetonitrile (1 mL) was cooled to 0 °C then HCl in EtOH (1M, 1mL) was added dropwise with stirring. The resulting hydrochloride slat of **9** was isolated by filtration and washing with cold acetonitrile (3 X 1 mL) and dried under vacuum desiccator. The white powder obtained was directly subjected to NMR analysis, measurement of optical rotation and HRMS.

Yield: 81% (103.2 mg)
TLC: 0.31 (CH_2Cl_2/ MeOH/ NH_4OH, 9:1:0.2)
$[\alpha]_D^{25}$ = +11.2 (*c* 1, MeOH)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{12}H_{18}NO_2$ 208.1332; found 208.1330

^1H NMR (600 MHz, D_2O) δ 7.30 (d, *J* = 7.5 Hz, 2H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.22 – 7.14 (m, 2H), 3.54 – 3.42 (m, 1H), 2.88 (dd, *J* = 14.1, 6.8 Hz, 1H), 2.83 – 2.78 (m, 1H), 2.64 – 2.54 (m, 1H), 1.94 – 1.86 (m, 1H), 1.67 – 1.56 (m, 1H), 1.05 (d, *J* = 7.2 Hz, 3H).
^{13}C NMR (151 MHz, D_2O) δ 179.7, 135.4, 129.4, 129.2, 129.1, 127.5, 126.1, 52.5, 38.3, 35.8, 35.7, 16.7.

Synthesis of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (5a)



Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous $MgSO_4$ (100 mg), aldehyde **2** (351.2 mg, 1.0 mmol), acid **4** (376.2 mg, 1.0 mmol), $ZnBr_2$ (45.04 mg, 0.2 mmol, 0.2 eq.) and Ligand **L1** (61.2 mg, 0.3 mmol, 0.3 eq.) were dissolved in dry CH_2Cl_2 (4.5 mL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 1.0 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature 48 h, the solvent was evaporated and diluted with EtOAc (15 mL) and washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was directly analysed through HPLC. The crude residue was directly analysed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min). The combined fractions of **5a** and **5b** were again purified to obtain pure **5a**.

Major Diasteroemer (**5a**): TLC: 0.31 (Petroleum ether/EtOAc, 7.5:2.5)
Minor Diasteroemer(**5b**): TLC: 0.34 (Petroleum ether/EtOAc, 7.5:2.5)

Yield: 71% (790 mg)
TLC: 0.34 (Petroleum ether/EtOAc, 7:3)
$[\alpha]_D^{25}$ = -96.5 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{66}H_{75}N_4O_{10}S$ 1115.5198; found 1115.5182.

^{1}H NMR (600 MHz, $CDCl_3$) (major rotamer) δ 7.77 (br, s, 1H), 7.66 (t, *J* = 7.7 Hz, 2H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 7.5 Hz, 1H), 7.35 – 6.96 (m, 22H), 6.55 (br, s, 1H), 4.90 – 4.69 (m, 2H), 4.55 (d, *J* = 8.1 Hz, 2H), 4.28 (t, *J* = 6.5 Hz, 1H), 3.50 (s, 3H), 2.67 – 2.66 (m, 1H), 2.63 (s, 3H), 2.62 – 2.58 (m, 2H), 2.58 – 2.53 (m, 2H), 2.50 (d, *J* = 11.1 Hz, 1H), 2.16 – 2.01 (m, 2H), 2.00 – 1.88 (m, 4H), 1.85 (dd, *J* = 5.5, 1.9 Hz, 2H), 1.60 (dt, *J* = 9.8, 6.4 Hz, 2H), 1.56 – 1.43 (m, 2H), 1.43 – 1.23 (m, 3H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.84 (t, *J* = 6.6 Hz, 3H), 0.80 (d, *J* = 11.7, 6.7 Hz, 3H), 0.72 (d, *J* = 6.7, 2.5 Hz, 3H).

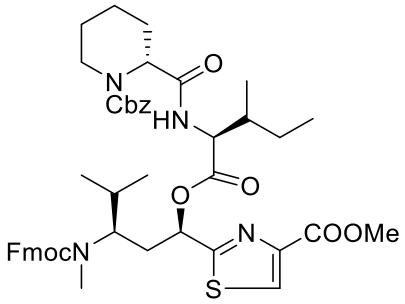
^{13}C NMR (151 MHz, $CDCl_3$) (major rotamer) δ 169.9, 168.8, 168.6, 167.9, 156.1, 154.8, 142.6, 142.3, 142.1, 139.5, 134.5, 127.8, 127.6, 126.7, 126.2, 125.9, 125.7, 125.6, 125.2

125.1, 124.9, 123.3, 123.1, 118.1, 118.0, 117.9, 114.6, 70.0, 65.8, 65.8, 65.3, 65.1, 56.1, 54.9, 50.7, 50.4, 49.2, 45.6, 45.5, 35.5, 34.2, 31.7, 31.1, 29.9, 29.8, 28.5, 27.8, 23.1, 22.8, 18.5, 18.33, 18.0, 17.9, 17.4, 13.8, 13.7, 9.8.

^{1}H NMR (600 MHz, $CDCl_3$) (minor rotamer) (selected signals) δ 7.48 (d, *J* = 7.5 Hz, 1H), 7.47 – 6.96 (m, 12), 6.25 (br, s, 0.5H), 5.05 (s, 0.8H), 4.65 – 4.59 (m, 1H), 4.38 (dd, *J* = 10.6, 6.9 Hz, 1H), 4.20 – 4.13 (m, 2H), 4.11 (q, *J* = 7.5, 6.3 Hz, 2H), 3.98 (s, 1H), 3.54 (s, 1.1H), 3.51 (s, 1H), 2.75 (s, 1.2H), 2.29 – 2.16 (m, 2H), 1.73 – 2.16 (s, 2H), 1.03 (d, *J* = 6.5 Hz, 1.2H), 1.00 – 0.64 (m, 4H).

^{13}C NMR (151 MHz, $CDCl_3$) (minor rotamer) δ 168.8, 168.82, 168.7, 168.4, 167.0, 155.1, 142.6, 142.2, 142.1, 139.4, 127.7, 127.6, 126.7, 126.3, 126.1, 125.8, 125.6, 125.2, 124.9, 123.2, 123.1, 118.1, 117.9, 69.4, 65.8, 65.4, 65.3, 56.5, 56.0, 54.6, 50.6, 50.1, 45.6, 45.5, 35.5, 33.93, 31.3, 30.0, 28.7, 28.6, 28.1, 27.8, 23.1, 23.0, 18.5, 18.2, 17.9, 13.8, 13.7, 13.6, 9.7.

Synthesis of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9H-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxa-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10)



A solution of **5a** (1.0 mmol) in dry CH_2Cl_2 (10 mL) was treated with $TiCl_4$ (1.0 M solution in CH_2Cl_2, 3.0 mmol) and stirred at 0 °C for 18 h. The reaction mixture was stirred vigorously with cold saturated aqueous $NaHCO_3$ (20 mL) for 30 min. The aqueous layer was extracted with CH_2Cl_2 (2 X 10 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The resultant crude product was pass with pad of silica and washed with EtOAc and evaporated to dryness. The crude thiazoline was dissolved in $CHCl_3$ (5 mL), activated MnO_2 (< 10 micron, 10 mmol) was added. The reaction mixture was refluxed in an oil bath at 70 °C for 5 h, then filtered through a short silica gel and celite column and washed with $CHCl_3$. The organic solution was concentrated. The resulting crude product was purified by flash chromatography (EtOAc/hexane, 0:100 to 30:70) to afford compound **10** as a white foam.

Yield: 81% (901 mg)
TLC: 0.30 (Petroleum ether/EtOAc, 7:3)
$[\alpha]_D^{25}$ = +12.5 (*c* 1, $CHCl_3$)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{47}H_{56}N_4O_9S$ 853.3840; found 853.3837

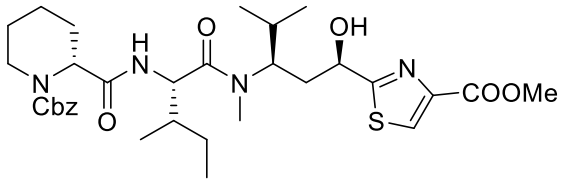
^{1}H NMR (600 MHz, $CDCl_3$) (major rotamer) δ 8.00 (s, 1H), 7.66 (d, *J* = 7.6, 2H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.33 – 7.16 (m, 9H), 6.43 (br, s, 1H), 5.91 d, *J* = 6.5 Hz, 1H), 5.11 (s, 2H), 4.79 (d, *J* = 6.5 Hz, 1H), 4.59 (dd, *J* = 8.9, 4.6 Hz, 1H), 4.32 – 4.30 (m, 2H), 4.13 (t, *J* = 23.8, 7.0 Hz, 1H), 3.84 (s, 3H), 3.77 – 3.67 (m, 1H), 3.66 – 3.52 (m, 1H), 2.83 (td, *J* = 13.0, 3.0 Hz, 1H), 2.64 (s, 3H), 2.47 – 2.43 (m, 1H), 2.25 – 2.11 (m, 5H), 1.98 – 1.91 (m, 2H), 1.91 – 1.88 (m, 2H), 1.57 –1.50 (m, 2H), 1.47 – 1.42 (m, 2H), 1.32 (dddd, *J* = 16.3, 12.2, 6.2, 2.8 Hz, 2H, 0.90 (d, *J* = 6.6 Hz, 3H), 0.87 – 0.83 (m, 6H), 0.75 (d, *J* = 6.6 Hz, 3H).

^{1}H NMR (600 MHz, $CDCl_3$) (minor rotamer) (selected signals) δ 7.68 (s, 0.5 H), 7.63 (d, *J* = 7.4 Hz, 0.5H), 7.45 (d, *J* = 7.2 Hz, 0.5H), 7.33 – 7.16 (m, 8H), 6.03 (br, d, 0.5H), 5.10 (s, 1H), 4.73 (br, s, 0.5H), 4.53 (dd, *J* = 8.6, 4.4 Hz, 0.5H), 4.28 – 4.25 (m, 1H), 4.10 (t, *J* = 25.8, 7.0 Hz, 0.5H), 3.83 (s, 1.5H), 3.77 – 3.67 (m, 0.5H), 3.66 – 3.52 (m, 0.5H), 2.81 (td, *J* = 13.0, 3.0 Hz, 0.5H), 2.61 (s, 1.5H), 2.54 – 2.53 (m, 0.5H), 2.32 – 2.30 (m, 1H), *J* = 16.4, 4.6 Hz, 1H), 2.33 – 2.30 (m, 1H), 2.26 – 2.16 (m, 4H), 1.69 – 1.66 (m, 1H), 1.57 – 1.50 (m, 1H), 1.47 – 1.42 (m, 1H),), 1.33 – 1.26 (m, 1H), 0.87 (d, *J* = 6.4 Hz, 1.5H), 0.77 – 0.79 (m, 3H), 0.67 (d, *J* = 6.6 Hz, 1.5H).

^{13}C NMR (151 MHz, $CDCl_3$) (major rotamer) δ 171.4, 170.7, 170.1, 161.6, 157.0, 155.5, 146.6, 144.3, 144.0, 141.3, 141.3, 136.3, 128.5, 128.4, 128.0, 127.7, 127.6, 127.5, 127.1, 127.0, 126.9, 125.1,124.7, 119.9, 119.8, 71.1, 67.6, 67.2, 61.4, 56.4, 52.3, 47.3, 42.1, 37.1, 31.9, 30.3, 29.7, 24.8, 24.6, 22.7, 20.3, 20.1, 19.4, 15.6, 11.6.

^{13}C NMR (151 MHz, $CDCl_3$) (minor rotamer) δ 169.6, 161.3, 156.8, 156.7, 146.6, 144.20, 144.1, 141.3, 141.3, 136.6, 128.5, 128.2, 128.1, 127.8, 127.6, 127.5, 127.0, 126.9, 125.1, 124.7, 119.9, 119.9, 71.5, 67.6, 67.3, 61.7, 56.5, 52.4, 47.4, 42.2, 37.2, 34.9, 30.3, 29.6, 24.7, 24.5, 22.7, 20.4, 20.0, 19.7, 15.7, 11.5.

Synthesis of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11)



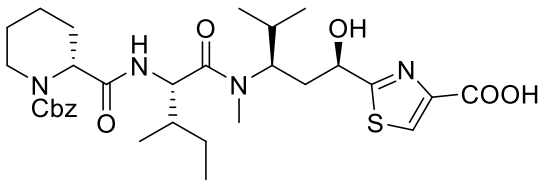
To a solution of **10** (852 mg, 1.0 mmol) in CH_3CN (12 mL) at 0 °C under Ar was added Et_2NH (8 mL). The solution was stirred at room temperature for 1 h. After complete deprotection of Fmoc group (TLC analysis) the mixture was evaporated and co-evaporated with CH_2Cl_2 to remove DEA. The crude product which was dissolved in CH_2Cl_2 (10 mL) and DIPEA (0.52 mL) was added and the resulting mixture was stirred at 50 °C for 24 h. The crude hydroxyl derivative **11** was purified by column chromatography.

The non-acylated amine was isolated and again stirred in presence of CH_2Cl_2/DIPEA for overnight.

Yield: 93% (585 mg)
TLC: 0.32 (Petroleum ether/ EtOAc/ AcOH, 3:7)
$[\alpha]_D^{25}$ = +68.2 (*c* 1, MeOH)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{32}H_{46}N_4O_7S$ 631.3160, found 631.3158

^{1}H NMR (600 MHz, MeOD) δ 8.50 (s, 1H), 7.64 (d, *J* = 13.2 Hz, 1H), 7.33 (dt, *J* = 21.4, 5.5 Hz, 5H), 5.13 (s, 2H), 4.79 (d, *J* = 5.7 Hz, 1H), 4.73 (t, *J* = 8.8 Hz, 1H), 4.33 (br, s, 1H), 4.06 (d, *J* = 12.1 Hz, 1H), 3.94 (s, 3H), 3.13 (s, 3H), 3.12 (dt, *J* = 12.6, 5.6, 3.9 Hz, 1H), 3.05 – 3.02 (m, 2H), 2.37 (d, *J* = 14.1 Hz, 1H), 2.19 (d, *J* = 13.8 Hz, 1H), 2.06 – 1.94 (m, 1H), 1.94 – 1.83 (m, 1H), 1.70 – 1.59 (m, 4H), 1.56 – 1.52 (m, 1H), 1.49 – 1.39 (m, 2H), 1.38 – 1.30 (m, 1H), 1.10 (d, *J* = 6.5 Hz, 3H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.72 (d, *J* = 6.9 Hz, 3H).
^{13}C NMR (151 MHz, MeOD) δ 174.6, 173.1, 172.0, 158.2, 157.6, 138.1, 129.7, 129.2, 129.2, 128.9, 72.8, 68.7, 62.8, 62.5, 55.3, 52.8, 43.5, 38.5, 37.7, 31.9, 25.7, 25.6, 21.4, 20.6, 20.1, 16.3, 11.2.

Synthesis of 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylic acid (12)



LiOH (35.95 mg, 1.5 mmol, 3.0 eq.) was added to a solution of **11** (315 mg, 0.5 mmol) in a mixture of THF: MeOH: H_2O (1:1:0.5, 0.5 M), and the reaction mixture was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure. The crude product was purified through silica get column chromatography using CH_2Cl_2/MeOH.

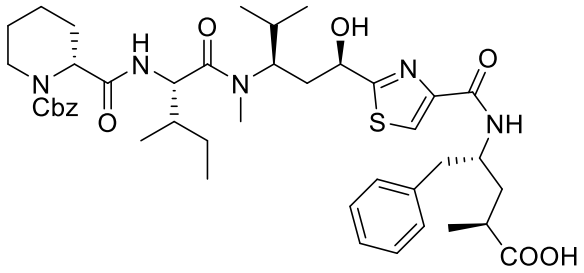
Yield: 98% (302 mg)
TLC: 0.25 (CH_2Cl_2/ MeOH/ AcOH, 9:1:0.2)
$[\alpha]_D^{25}$ = +38.4 (*c* 1, MeOH)
HRMS (ESI-TOF) m/z: $[M + Na]^+$ calculated for $C_{31}H_{44}N_4O_7SNa$ 639.2828; found 639.2820

^{1}H NMR (600 MHz, MeOD) δ 8.31 (s, 1H), 7.68 (d, *J* = 26.3 Hz, 1H), 7.36 (t, *J* = 6.6 Hz, 4H), 7.31 (dt, *J* = 17.0, 5.6 Hz, 1H), 5.15 (s, 2H), 4.85 (t, *J* = 6.5 Hz, 1H), 4.82 – 4.79 (m, 1H), 4.75 (t, *J* = 8.7 Hz, 1H), 4.36 (br, m, 1H), 4.07 (d, *J* = 13.5 Hz, 1H), 3.13 (dd, *J* = 13.1, 2.9 Hz, 1H), 3.09 (s, 3H), 3.06 – 3.04 (m, 2H), 2.33 (dd, *J* = 6.1, 3.6 Hz, 1H), 2.31 (dd, *J* = 6.4, 3.8 Hz, 1H), 2.27 – 2.11 (m, 1H), 2.07 – 1.99 (m, 1H), 1.90 (ddt, *J* = 12.0,

6.9, 2.7 Hz, 1H), 1.66 (d, *J* = 13.9 Hz, 2H), 1.56 – 1.53 (m, 1H), 1.48 – 1.41 (m, 2H), 1.39 – 1.34 (m, 1H), 1.13 (d, *J* = 6.5 Hz, 3H), 0.96 (d, *J* = 7.0 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.75 (d, *J* = 4.5 Hz, 3H).

^{13}C NMR (151 MHz, MeOD) δ 177.6, 173.2, 171.6, 162.8, 155.9, 146.8, 136.5, 128.1, 127.7, 127.7, 127.6, 127.4, 69.8, 67.1, 54.7, 53.8, 48.0, 47.8, 47.7, 47.6, 47.4, 47.3, 47.1, 41.9, 37.4, 36.1, 30.4, 24.3, 24.2, 24.1, 19.8, 19.1, 18.6, 14.7, 9.6.

Synthesis of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13)



Acid **12** (50.0 mg, 0.0811 mmol) was added to a 0.2 M solution of pentafluorophenol (23.0 mg, 0.125 mmol) and DIC (14.4 µL, 0.0919 mmol) in CH_2Cl_2 at 0 °C. The reaction mixture was warmed to rt, stirred for 24 h, and concentrated under reduced pressure. EtOAc (10 mL) was added, and the crude product was filtered, with rinsing of the reaction vessel with EtOAc. The filtrate was concentrated under reduced pressure, and the crude material was used without further purification. DMF (0.335 mL, 0.25 M) was added to the crude product, followed by the hydrochloride salt of tubuphenylalanine (**9**) (61.0 mg, 0.250 mmol) and diisopropylethylamine (73.0 µL, 0.419 mmol). The reaction mixture was stirred for 24 h at rt, and DMF was removed under vacuum. Column purification (100:0 to 90:10 CH_2Cl_2:MeOH) afforded **13** as white foamy solid.

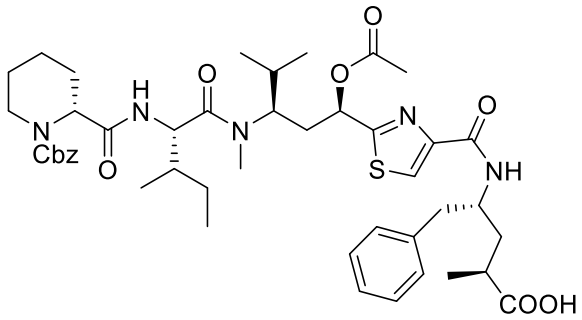
Yield: 88% (56.5 mg)
TLC: 0.28 (CH_2Cl_2/MeOH/AcOH, 9:1:0.1)
$[\alpha]_D^{25}$ = +11.5 (*c* 1, MeOH)
HRMS (ESI-TOF) m/z: $[M + Na]^+$ calculated for $C_{43}H_{59}N_5O_8SNa$ 828.3982, found 828.3975

^{1}H NMR (600 MHz, MeOD) δ 8.59 (d, *J* = 9.3 Hz, 1H), 8.01 (s, 1H), 7.35 – 7.29 (m, 5H), 7.26 – 7.20 (m, 5H), 7.17 (qd, *J* = 6.5, 5.5, 3.8 Hz, 1H), 5.15 (s, 2H), 4.84 – 4.81 (m, 1H), 4.72 (d, *J* = 3.7 Hz, 1H), 4.70 (d, *J* = 3.8 Hz, 1H), 4.42 – 4.29 (m, 1H), 4.13 (dt, *J* = 15.5, 9.5 Hz, 1H), 3.70 (dd, *J* = 18.1, 12.3 Hz, 1H), 3.59 (dd, *J* = 18.0, 12.2 Hz, 1H), 3.38 – 3.34 (m, 1H), 3.14 (s, 3H), 2.96 (dd, *J* = 13.7, 7.7 Hz, 2H), 2.88 – 2.85 (m, 1H), 2.61 (ddd, *J* = 10.5, 7.2, 4.3 Hz, 1H), 2.27 (d, *J* = 8.8 Hz, 1H), 2.26 (d, *J* = 8.5 Hz, 1H), 2.26 – 2.23 (m, 1H), 2.12 – 2.10 (m, 1H), 2.07 (d, *J* = 3.3 Hz, 1H), 2.03 (d, *J* = 3.4 Hz, 1H), 1.90 (ddt, *J* =

11.9, 6.6, 2.8 Hz, 2H), 1.68 (d, *J* = 12.8 Hz, 3H), 1.56 – 1.52 (m, 1H), 1.51 – 1.42 (m, 2H), 1.41 – 1.27 (m, 2H), 1.16 (d, *J* = 7.1 Hz, 3H), 0.95 – 0.91 (m, 3H), 0.87 (d, *J* = 6.5 Hz, 3H), 0.83 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (151 MHz, MeOD) δ 178.4, 176.0, 173.1, 161.8, 161.0, 149.2, 138.4, 136.4, 128.9, 128.2, 127.8, 127.8, 127.4, 125.9, 122.3, 72.0, 68.6, 67.3, 60.8, 53.8, 53.7, 42.1, 41.5, 38.0, 37.8, 37.0, 36.6, 30.6, 29.2, 24.7, 24.2, 19.9, 19.1, 18.8, 17.5, 14.3, 9.7.

Synthesis of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14)



A 0.1 M solution of **13** (40 mg, 0.049 mmol) in pyridine (0.50 mL) was cooled to 0 °C, and acetic anhydride (34.0 µL, 0.366 mmol) was added. The reaction mixture was allowed to warm to rt over 2 h and was stirred at rt for 24 h. The reaction mixture was then cooled to 0 °C, and a 1:1 mixture of dioxane/water (1.80 mL) was added. The mixture was allowed to warm to rt, and was stirred for 6 h at rt. The solvent was removed under reduced pressure. Column chromatography (100% CH_2Cl_2 to 10% MeOH/CH_2Cl_2) afforded 14 as an amorphous solid.

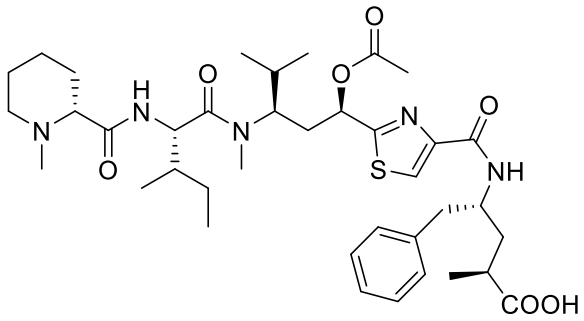
Yield: 88% (36.4 mg)
TLC: 0.42 (CH_2Cl_2/MeOH/ AcOH, 9:1:0.1)
$[\alpha]_D^{25}$ = +26.5 (*c* 1, MeOH)
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{45}H_{62}N_5O_9S$ 848.4268; found 848.4261

^{1}H NMR (600 MHz, MeOD) δ 8.47 (d, *J* = 9.3 Hz, 1H), 8.09 (s, 1H), 7.46 (br, s, 1H), 7.33 (s, 4H), 7.28 – 7.20 (m, 4H), 7.16 (h, *J* = 4.4 Hz, 1H), 5.86 (dd, *J* = 10.9, 3.6 Hz, 1H), 5.13 (s, 2H), 4.84 (d, *J* = 9.4 Hz, 2H), 4.68 – 4.61 (m, 1H), 4.36 (d, *J* = 4.8 Hz, 1H), 4.13 (d, *J* = 8.5 Hz, 1H), 3.38 – 3.33 (m, 2H), 3.16 (s, 3H), 3.11 – 3.09 (m, 2H), 2.95 (dd, *J* = 13.7, 7.7 Hz, 1H), 2.89 – 2.86 (m, 1H), 2.61 (t, *J* = 9.8 Hz, 1H), 2.26 – 2.24 (m, 1H), 2.22 (d, *J* = 26.6 Hz, 2H), 2.18 (s, 3H), 2.05 (d, *J* = 7.2 Hz, 2H), 1.93 (d, *J* = 7.4 Hz, 1H), 1.85 (s, 1H), 1.67 (d, *J* = 13.1 Hz, 3H), 1.60 – 1.51 (m, 1H), 1.50 – 1.44 (m, 1H), 1.41 – 1.33 (m, 1H), 1.16 (d, *J* = 7.1 Hz, 3H), 0.98 – 0.95 (m, 3H), 0.89 (d, *J* = 6.4 Hz, 3H), 0.80 (d, *J* = 52.4 Hz, 3H).

^{13}C NMR (151 MHz, MeOD) δ 179.9, 174.8, 173.1, 171.3, 170.3, 162.9, 151.0, 146.0, 139.8, 138.0, 130.5, 129.7, 129.4, 129.3, 129.0, 127.5, 124.7, 71.9, 68.8, 57.1, 55.4, 55.3,

49.7, 43.0, 39.4, 38.4, 38.1, 36.3, 31.8, 30.9, 30.6, 25.8, 25.7, 21.4, 20.9, 20.6, 20.2, 18.9, 16.0, 11.2.

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



The compound **14** (20 mg, 0.023 mmol) was dissolved in a mixture of MeOH (3 mL). 37% aqueous formaldehyde (8.6 mg, 5.0 eq.) and 20% Pd/C (0.09 mg, 0.0023 mmol) were added. The reaction mixture was stirred under hydrogen atmosphere for 24 h and afterwards filtered through Cellite. The solvent was then removed under reduced pressure. The product was purified through column chromatography (100% CH_2Cl_2 to 10% MeOH/CH_2Cl_2) to afford **1** as a pale-yellow film that was further purified by RP chromatography (12-g C18 cartridge, 0.1% TFA/H_2O/CH_3CN 90:10 − 0:60). Lyophilization afforded **1** as a colorless fluffy solid.

Yield: 90% (15.0 mg)
TLC: 0.25 (CH_2Cl_2/ MeOH/ AcOH; 9:1:0.1).
$[\alpha]_D^{25}$ = + 19.2 (*c* 1.0, MeOH).
HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{38}H_{58}N_5O_7S$ 728.4056; found 728.4050

^{1}H NMR (600 MHz, MeOD) δ 8.36 (d, *J* = 9.2 Hz, 1H), 8.12 (s, 1H), 7.28 – 7.23 (m, 4H), 7.19 – 7.17 (m, 1H), 5.89 (d, *J* = 3.5 Hz, 1H), 4.73 (d, *J* = 8.2 Hz, 1H), 4.37 (ddd, *J* = 12.0, 5.7, 3.7 Hz, 2H), 3.77 (dd, *J* = 10.4, 1.8 Hz, 1H), 3.52 (d, *J* = 11.6 Hz, 1H), 3.22 (td, *J* = 13.6, 3.9 Hz, 1H), 3.14 (s, 3H), 3.00 (dd, *J* = 13.7, 7.8 Hz, 1H), 2.93 (dd, *J* = 13.7, 6.1 Hz, 1H), 2.77 (s, 3H), 2.63 (ddd, *J* = 9.5, 7.0, 4.4 Hz, 1H), 2.37 (ddd, *J* = 13.9, 9.5, 3.3 Hz, 1H), 2.33 – 2.30 (m, 1H), 2.26 – 2.19 (m, 4H), 2.06 – 2.04 (m, 1H), 1.91 – 1.84 (m, 4H), 1.84 – 1.77 (m, 2H), 1.66 (ddd, *J* = 13.8, 10.7, 3.1 Hz, 1H), 1.65 – 1.59 (m, 2H), 1.30 – 1.22 (m, 4H), 1.20 (d, *J* = 7.1 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.94 (t, *J* = 7.0 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (151 MHz, MeOD) δ 178.9, 173.3, 170.3, 170.0, 166.8, 161.5, 149.9, 138.0, 129.0, 127.5, 126.4, 124.7, 69.9, 66.6, 57.1, 54.8, 54.4, 48.6, 41.7, 40.3, 37.2, 36.3, 35.9, 34.0, 29.7, 28.1, 24.2, 23.0, 21.4, 20.2, 19.5, 18.9, 17.5, 14.3, 10.0.

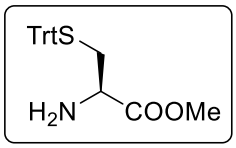
Comparison of Spectral data for 1

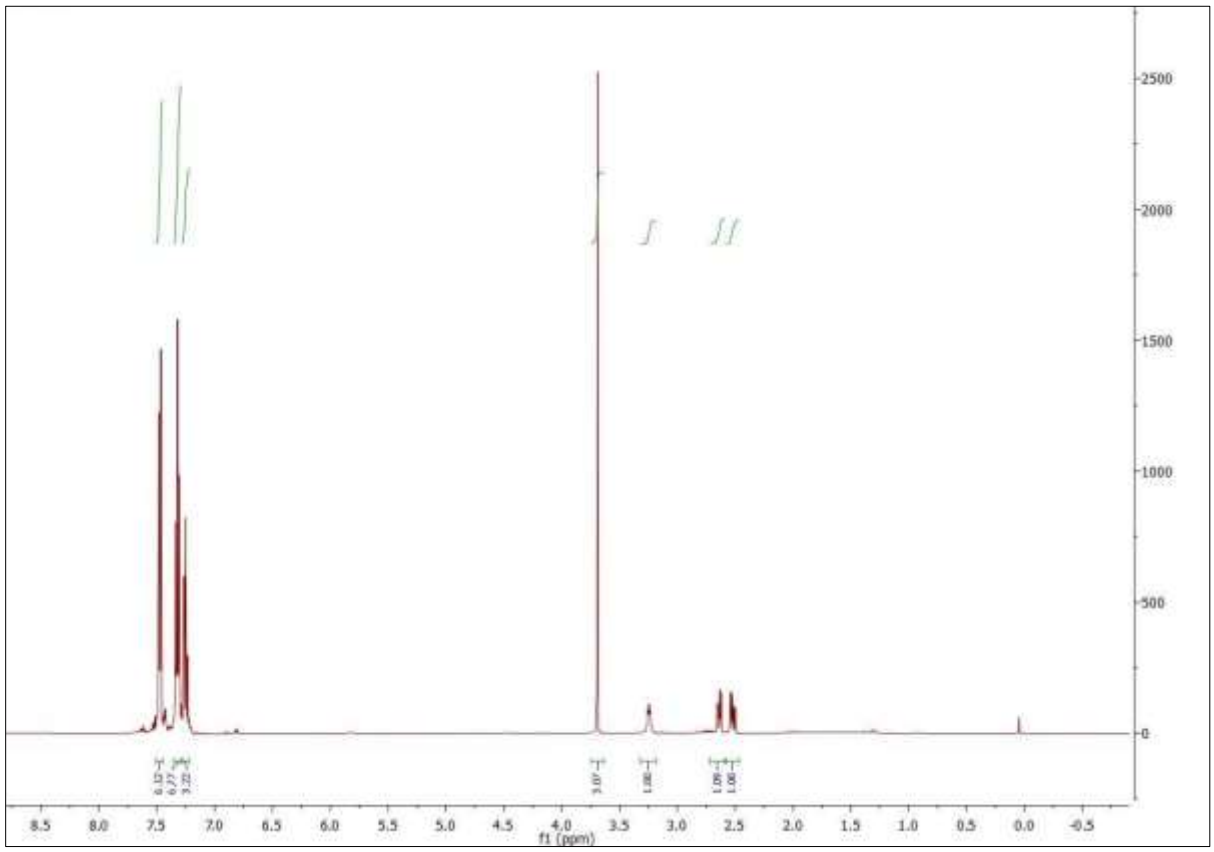
C-atoms	Reported[1] (101 MHz, MeOD)	Our synthesis (151 MHz, MeOD)
Tup CO_2H	178.5	178.9
Ile CON	173.2	173.3
Tuv thiazole C_2	170.34	170.3
Tuv $COCH_3$	170.2	170
Mep CO-NH	167.7	166.8
Tuv CO-NH)	161.4	161.5
Tuv thiazole C_4	149.4	149.9
Tup phenyl C_1	138.1	138
Tup m,m′-phenyl CHs	129.1	129
Tup o,o′-phenyl CHs	127.9	127.5
Tup p-phenyl CH	126	126.4
Tuv thiazole CH	123.7	124.7
Tuv CH-OAc	69.8	69.9
Mep CH-N	66.8	66.6
Tuv CH-N	56.9	57.1
Mep CH_2-N	54.8	54.8
Ile CH-NH	54.6	54.4
Tup CH-NH	49.3	48.6
Mep CH_3	41.5	41.7
Tup CH_2-Ph	40.8	40.3
Tup β CH_2	37.7	37.2
Tup α CH	36.4	36.3
Ile $CHCH_3$	36	35.9
Tuv CH_2	34.2	34
Tuv CH-CH_3, Tuv N-CH_3	29.5	29.7
Mep β CH_2	28.9	28.1
Ile CH_2	23.8	24.2
Mep δ CH_2	22.6	23
Mep γ CH_2	21	21.4
Tuv $COCH_3$	19.4	20.2

[1] P. Wipf et al. J. Org. Chem. **2016**, *81*, 10302−10320.

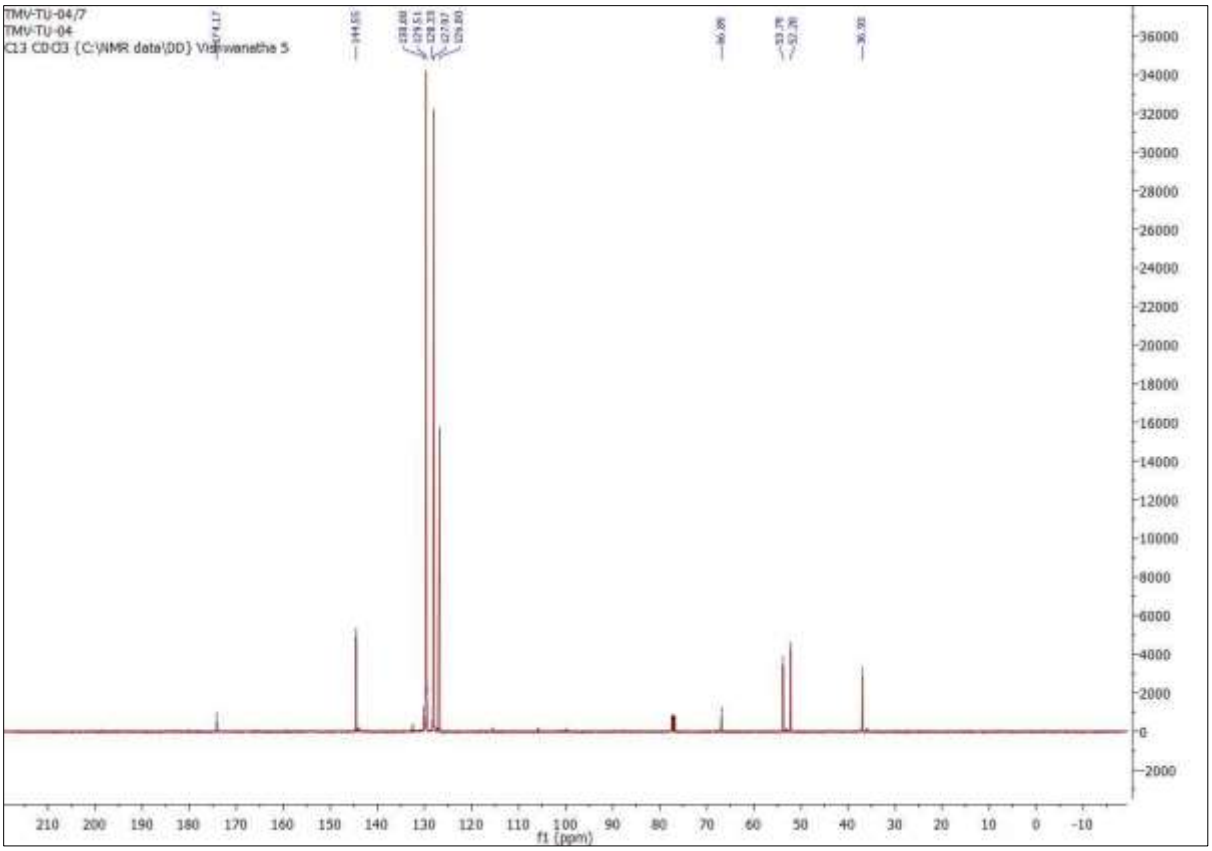
Tuv CH-CH_3 a	19.1	19.5
Tuv CH-CH_3 b	18.9	18.9
Tup CH_3	17.1	17.5
Ile CH-CH_3	14.8	14.3
Ile CH_2-CH_3	9.9	10

Protons	Reported δ (m, *J* in Hz) (500 MHz)	Our synthesis δ (m, *J* in Hz) (600 MHz)
Tup amide NH		8.36 (d, *J* = 9.2 Hz, 1H)
Tuv thiazole CH	8.1 (s, 1H)	8.12 (s, 1H)
Tup o-, o′-, m-, and m′-phenyl CHs	7.27-7.24 (m, 4H)	7.28-7.23 (m, 4H)
Tup p-phenyl CH	7.20-7.17 (m, 1H)	7.19-7.17 (m, 1H)
Tuv CHOAc	5.74 (d, J = 11.0, 1 H)	5.89 (d, J = 3.5 Hz, 1H)
Ile CH-NH	4.74 (d, J = 7.5, 1 H)	4.73 (d, J = 8.2 Hz, 1H)
Tuv CH-N, Tup CH-NH	4.42-4.38 (m, 2H)	4.37 (ddd, 12.0, 5.7, 3.7Hz, 2H)
Mep CH-N	3.77 (dd, J = 12.2, 2.1 Hz, 1 H)	3.77 (dd, J = 10.4, 1.8 Hz, 1H)
Mep CH_2-N a	3.49 (d, J = 12.7 Hz, 1H)	3.52 (d, J = 11.6 Hz, 1H)
Tuv N-CH_3	3.14 (s, 3H)	3.14 (s, 3H)
Mep CH_2-N b	3.09 (td, J = 12.9, 2.1Hz 1H)	3.22 (td, J = 13.6, 3.9 Hz, 1H)
Tup CH_2-Ph	2.95-2.89 (m, 2H)	3.00 (dd, J = 13.7, 7.8 Hz, 1H), 2.93 (dd, J = 13.7, 6.1 Hz, 1H)
Mep CH_3	2.76 (s, 3H)	2.77 (s, 3H)
Tup α CH	2.59-2.54 (m, 1 H)	2.63 (ddd, J = 9.5, 7.0, 4.4 Hz, 1H)
Tuv CH_2	2.41 (ddd, J = 14.7, 11.6, 2.9 Hz, 1H)	2.37 (ddd, J = 13.9, 9.5, 3.3 Hz, 1H)
Tuv CH_2 b	2.35-2.30 (m, 1 H)	2.33 – 2.30 (m, 1H)
Tuv $COCH_3$, Mep β CH_2 a	2.19-2.17 (m, 4 H)	2.26 – 2.19 (m, 4H
Tup β CH_2 a	2.02 (ddd, J = 13.69, 9.9, 4.0 Hz, 1H)	2.06 – 2.04 (m, 1H)
Tuv $CHCH_3$, Ile CH-CH_3, Mep γ CH_2 a, Mep δ CH_2 a	1.97 -1.87 (m, 4 H)	1.91 – 1.84 (m, 4H)
Mep β CH_2 b, Mep δ CH_2 b	1.84-1.75 (m, 2 H)	1.84 – 1.77 (m, 2H)
Tup β CH_2 b	1.69 (ddd, J = 14.2, 10.2, 4.1 Hz, 1H)	1.66 (ddd, J = 13.8, 10.7, 3.1 Hz, 1H)
Mep γ CH_2 b, Ile CH_2 a	1.65-1.58 (m, 2H)	1.65 – 1.59 (m, 2H)
Ile CH_2 b, Tup CH_3	1.24-1.19 (m, 4H)	1.30 – 1.22 (m, 4H)
Tuv CH-CH_3 a	1.06 (d, 6.6 Hz, 3H)	1.20 (d, J = 7.1 Hz, 3H)
Ile CH-CH_3	1.04 (J = 6.8 Hz, 3 H)	1.06 (d, J = 6.8 Hz, 3H)
Ile CH2-CH_3	0.96 (t, J = 7.4 Hz, 3H)	0.94 (t, J = 7.0 Hz, 3H)
Tuv CH-CH_3	0.87 (d, J = 6.6 Hz, 3 H)	0.82 (d, J = 6.6 Hz, 3H)

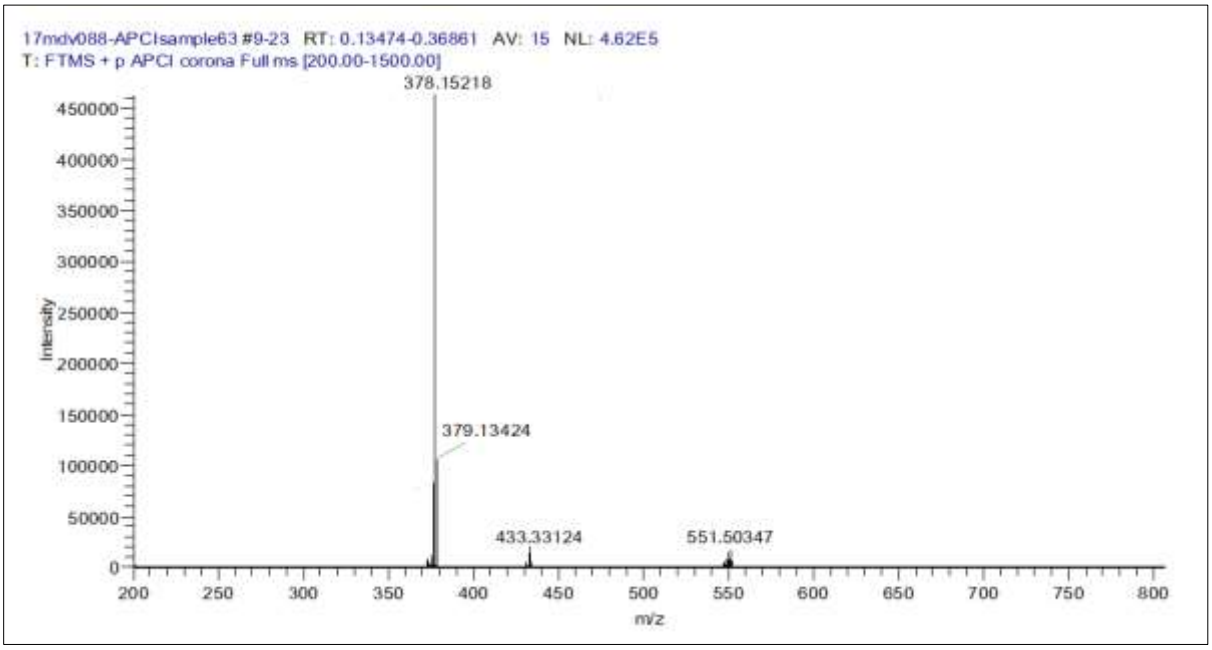




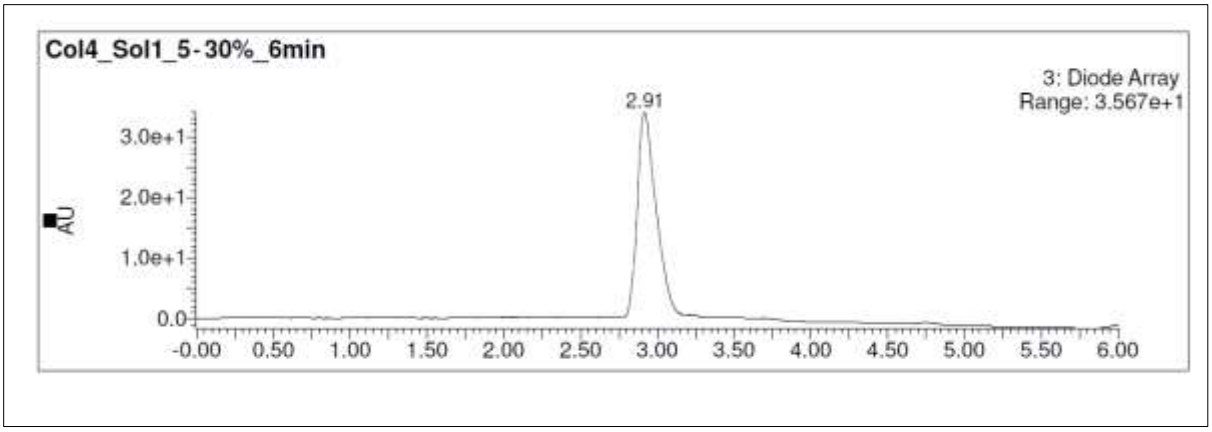
^{1}H NMR of methyl S-trityl-L-cysteine (16)
(500 MHz, $CDCl_3$)



^{13}C NMR of methyl S-trityl-L-cysteine (16)
(126 MHz, $CDCl_3$)

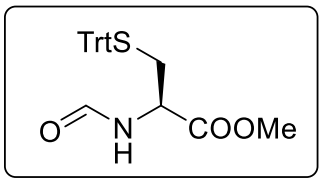


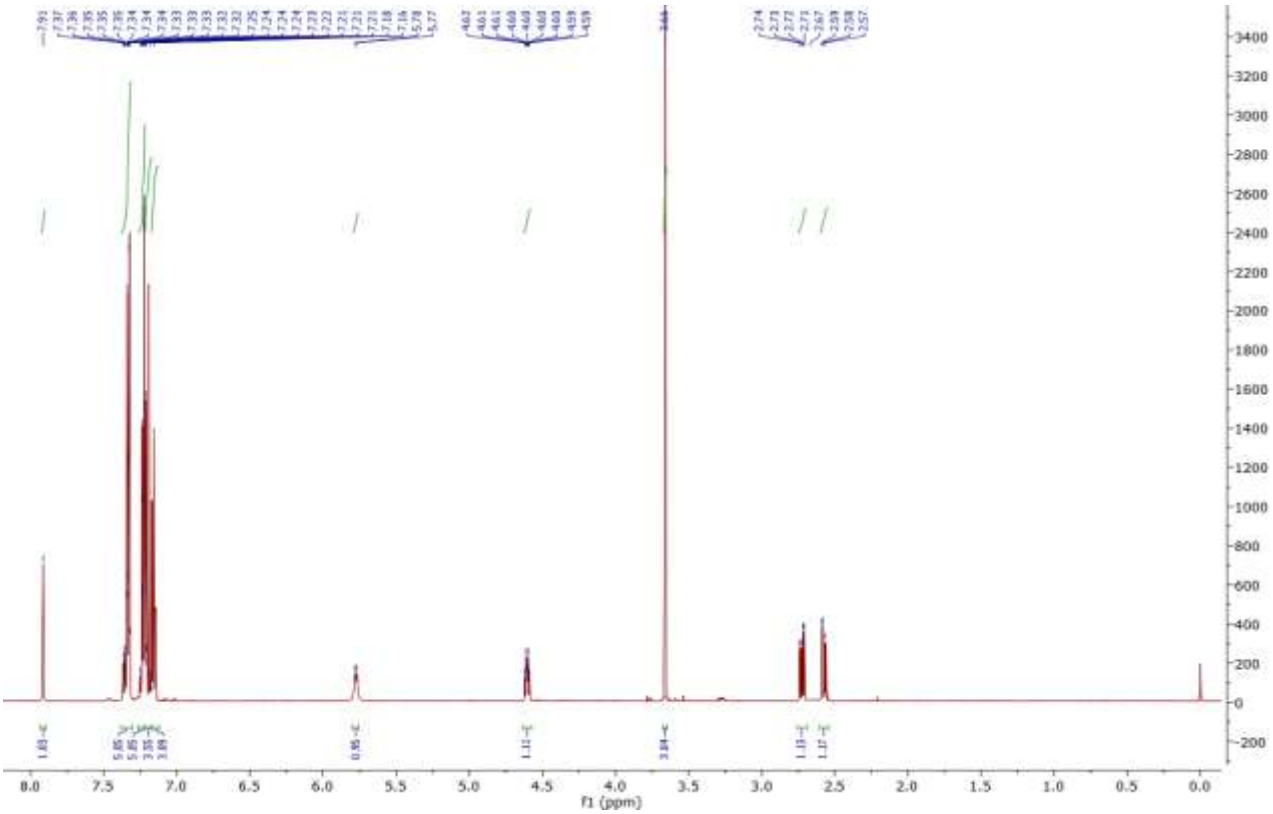
HRMS of methyl S-trityl-L-cysteine (16)



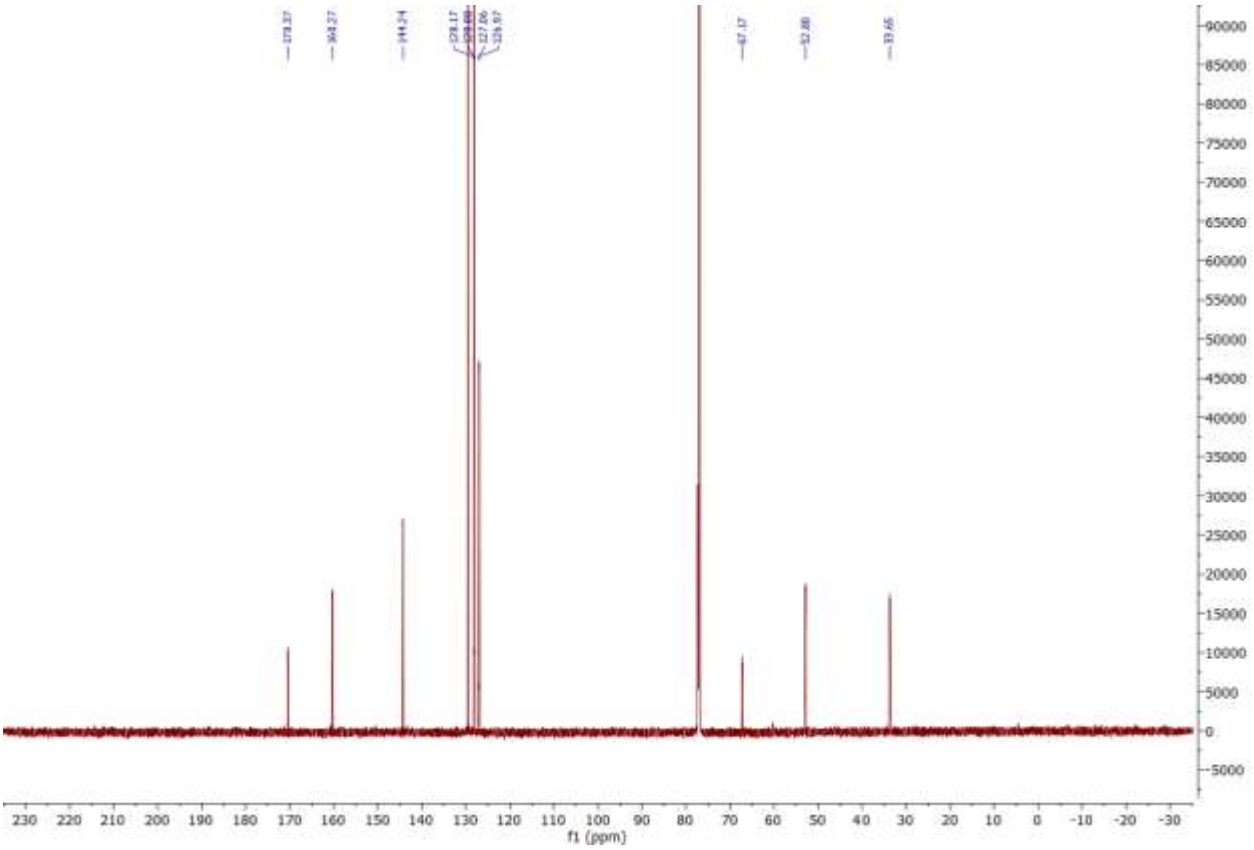
SFC-HPLC of methyl S-trityl-L-cysteine (16)

SFC-HPLC: (Viridis silica gel column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO_2 over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm,
t_R = 2.91 min

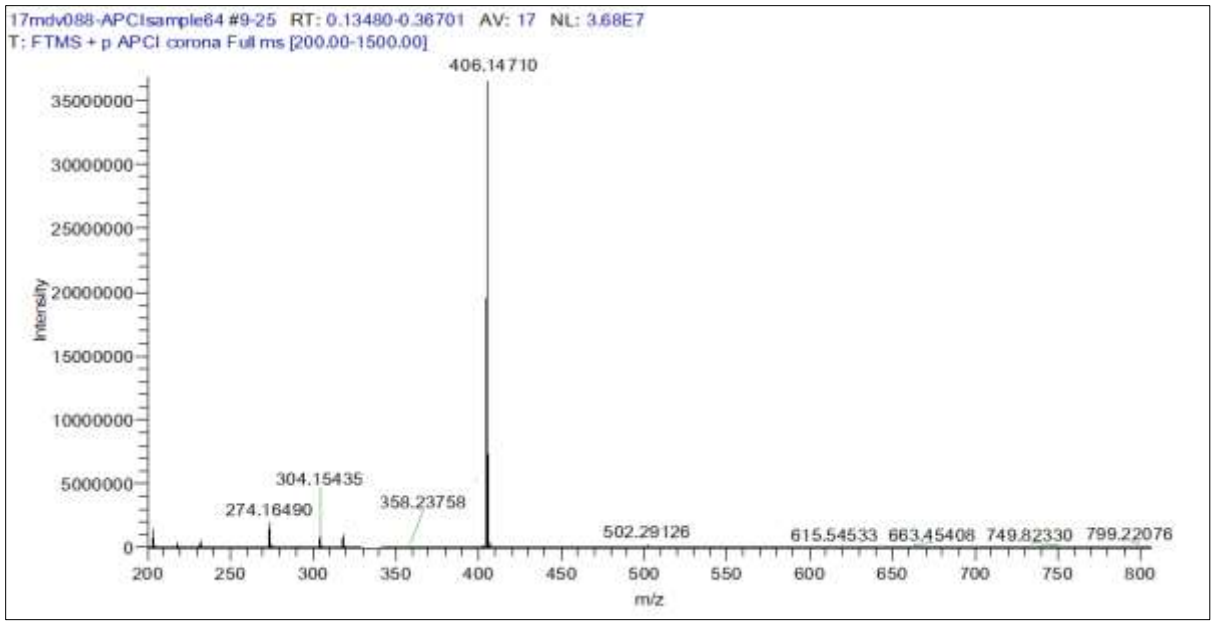




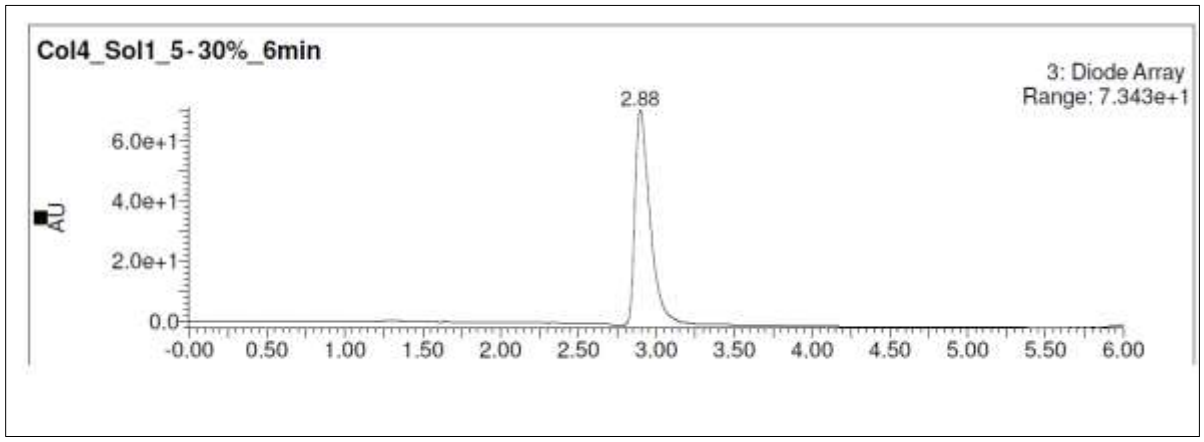
^{1}H NMR of methyl *N*-formyl-*S*-trityl-L-cysteine (17)
(600 MHz, $CDCl_3$)



^{13}C NMR of methyl *N*-formyl-*S*-trityl-L-cysteine (17)
(151 MHz, $CDCl_3$)

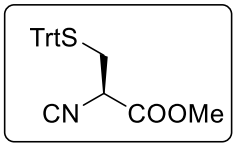


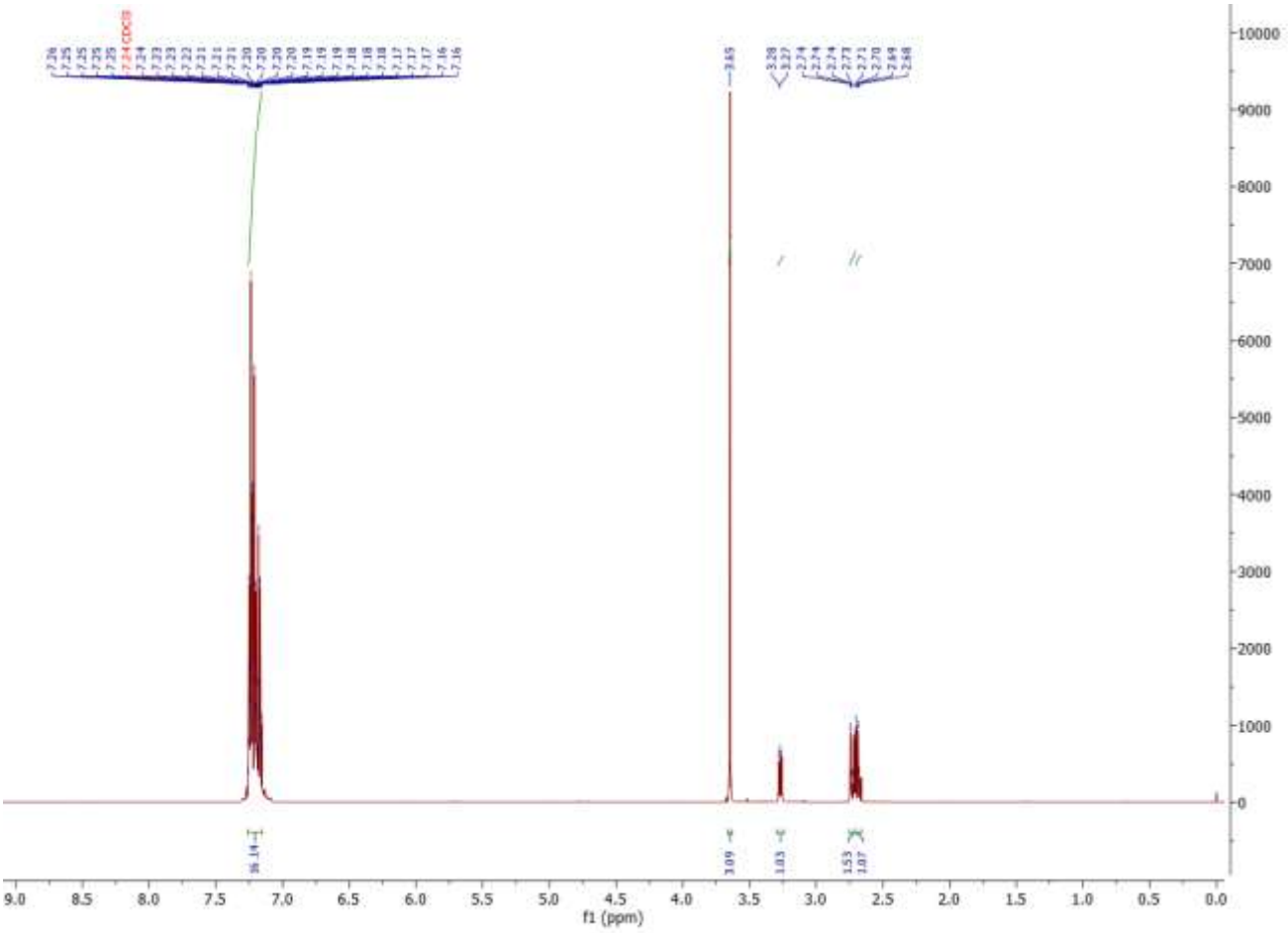
HRMS of methyl *N*-formyl-*S*-trityl-L-cysteine (17)



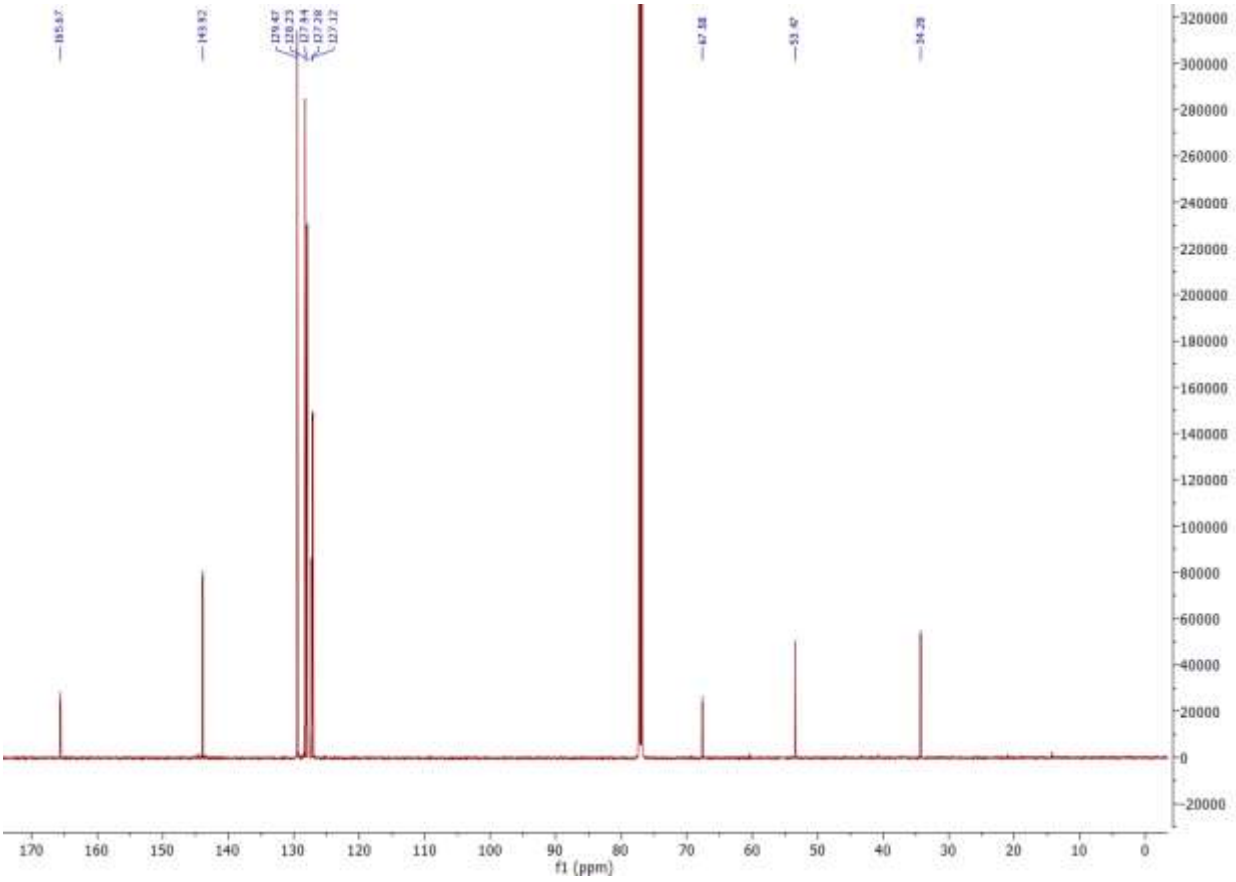
SFC-HPLC of methyl *N*-formyl-*S*-trityl-L-cysteine (17)

SFC-HPLC: (Viridis silica gel column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO_2 over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm,
t_R = 2.88 min

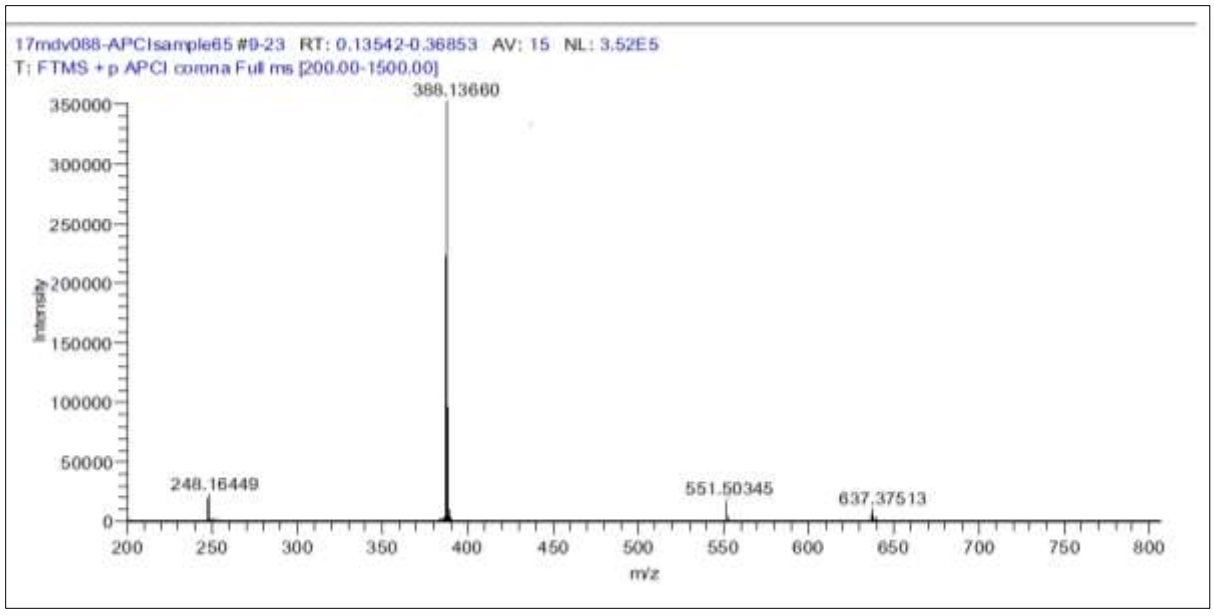




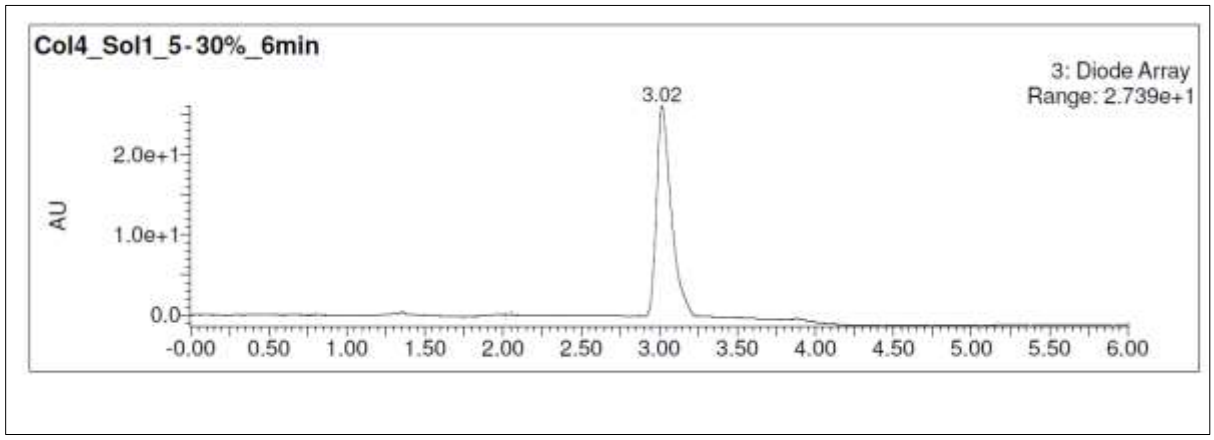
^{1}H NMR of methyl (*R*)–2–isocyano–3–(tritylthio)propanoate (3) (600 MHz, $CDCl_3$)



^{13}C NMR of methyl (*R*)–2–isocyano–3–(tritylthio)propanoate (3) (151 MHz, $CDCl_3$)

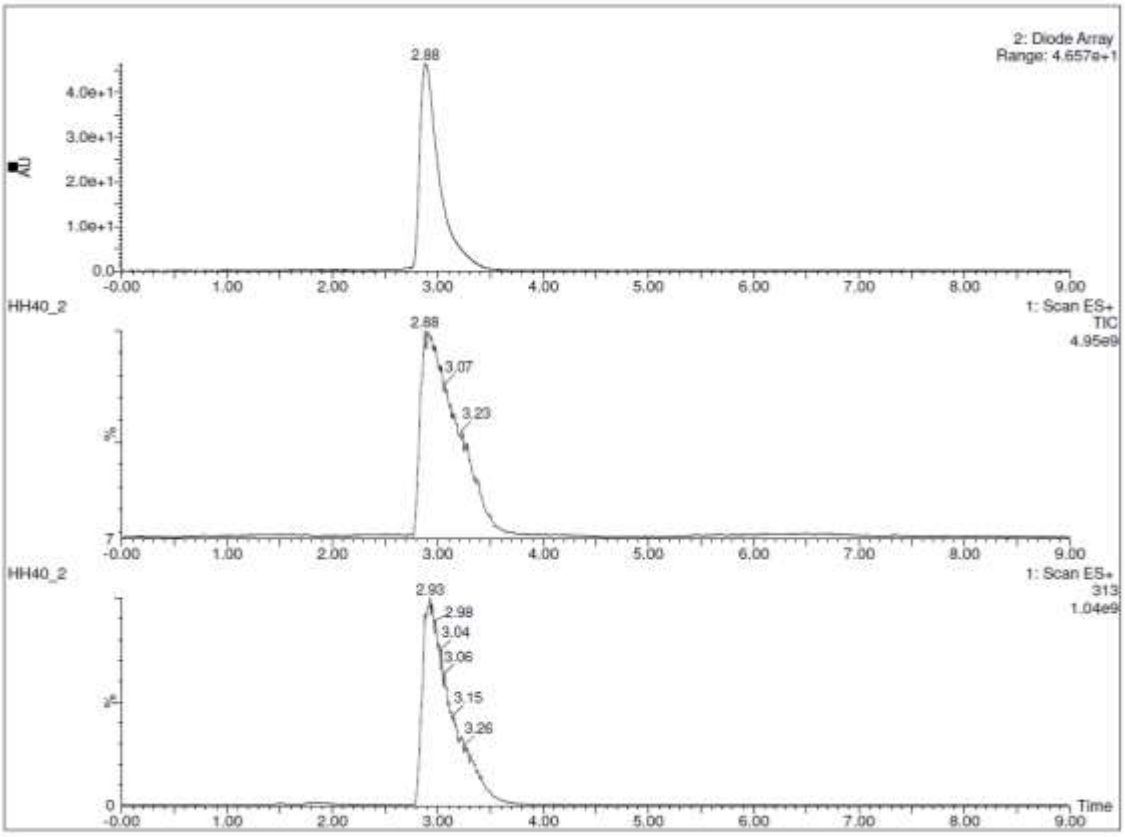


HRMS of methyl (*R*)-2-isocyano-3-(tritylthio)propanoate (3)



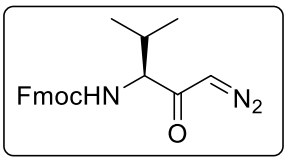
SFC-HPLC of methyl (*R*)-2-isocyano-3-(tritylthio)propanoate (3)

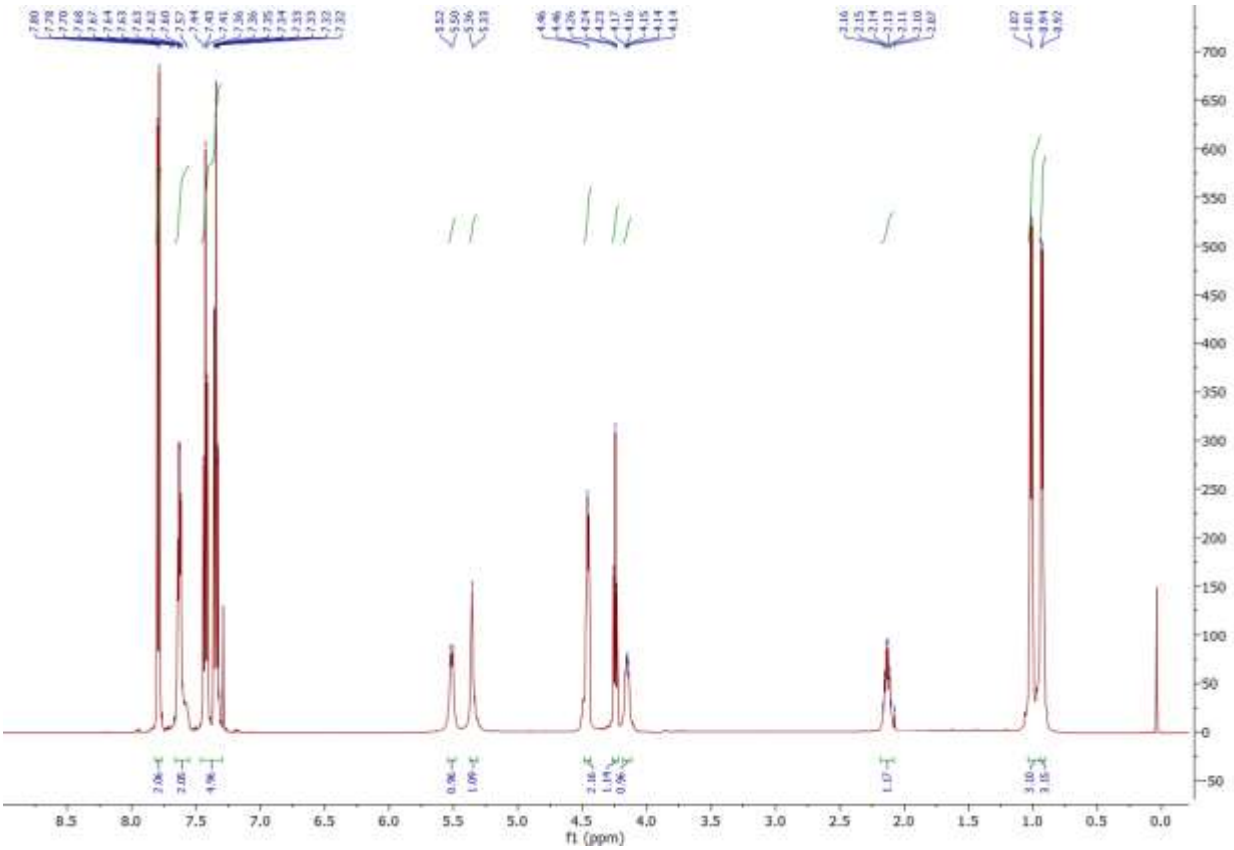
SFC-HPLC: (Viridis silica gel column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO_2 over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 3.02 min



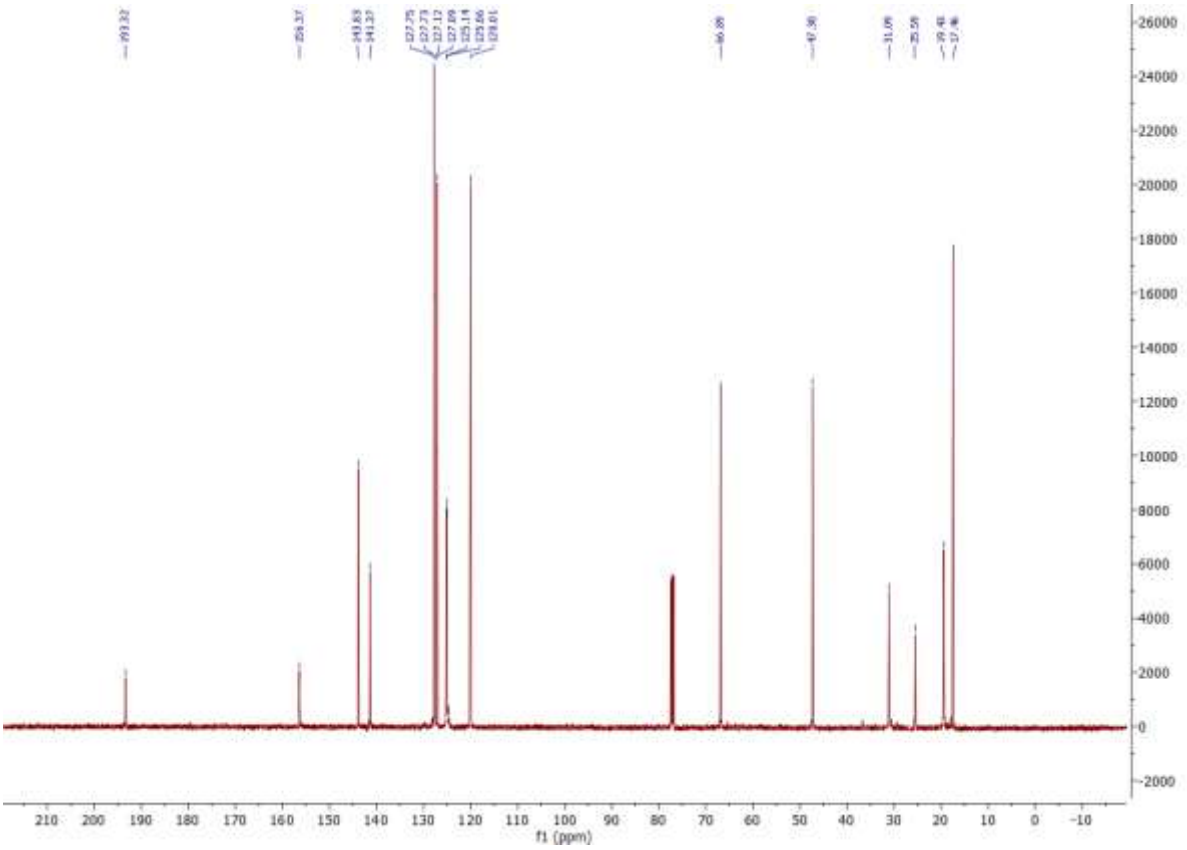
Chiral SFC–HPLC chromatogram of enantiopure methyl (*R*)–2–isocyano–3–(tritylthio)propanoate (3)

Method**:** Reprosil Chiral-AM column (4.6 X 250 mm, 5µm) with 5 - 30% *i*PrOH in CO_2 for 9 min; Absorbance = 230 nm, flow rate: 0.8 mL/min, t_R = 2.88 min ee: >98%.

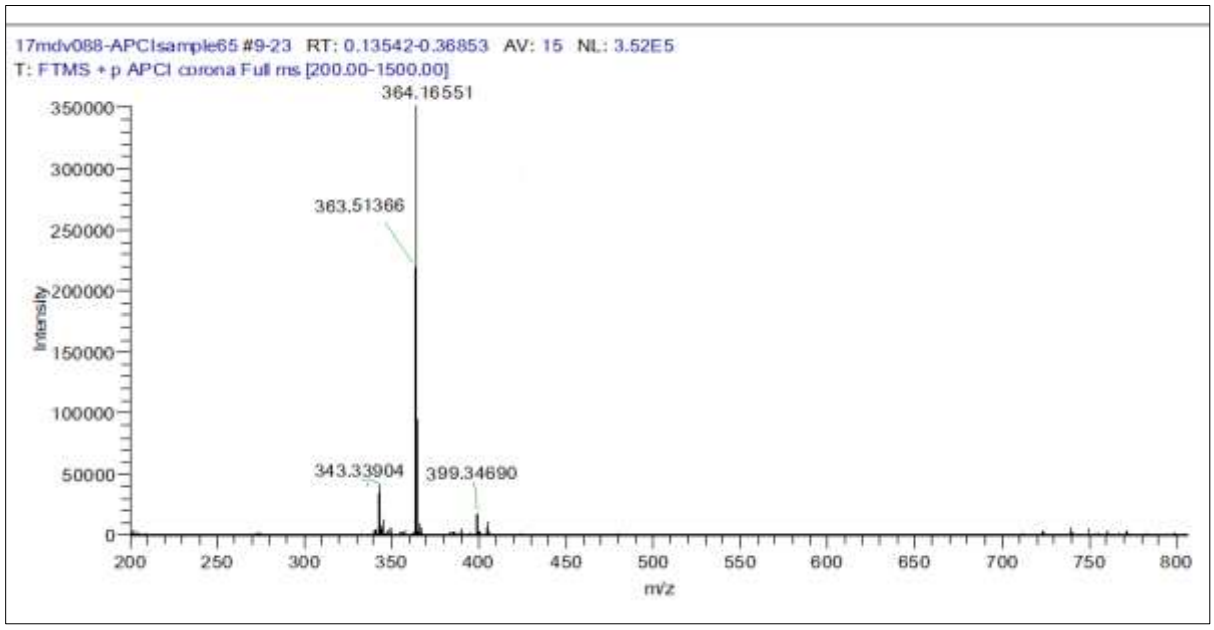




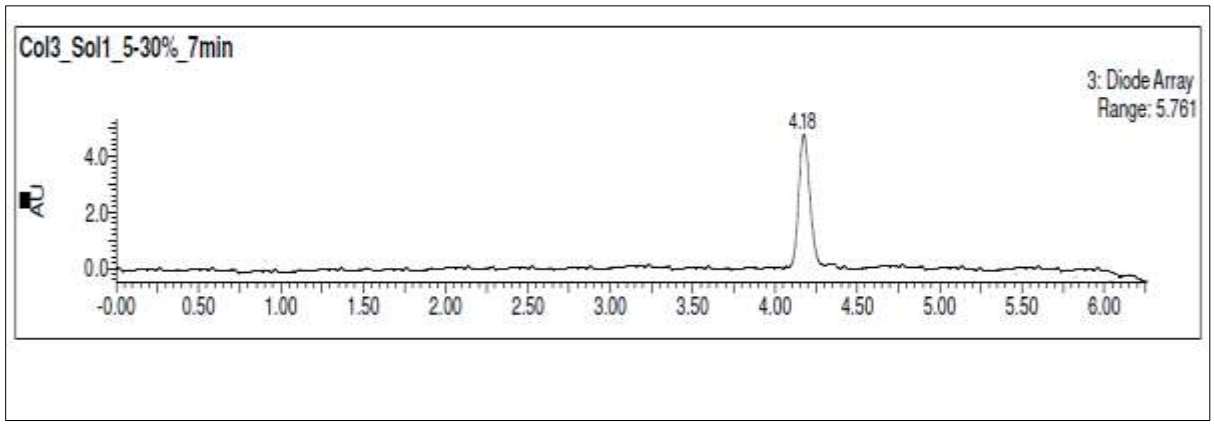
^{1}H NMR of (*S*)-(9*H*-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (19)
(500 MHz, $CDCl_3$)



^{13}C NMR of (*S*)-(9*H*-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (19)
(126 MHz, $CDCl_3$)

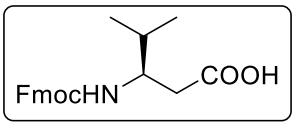


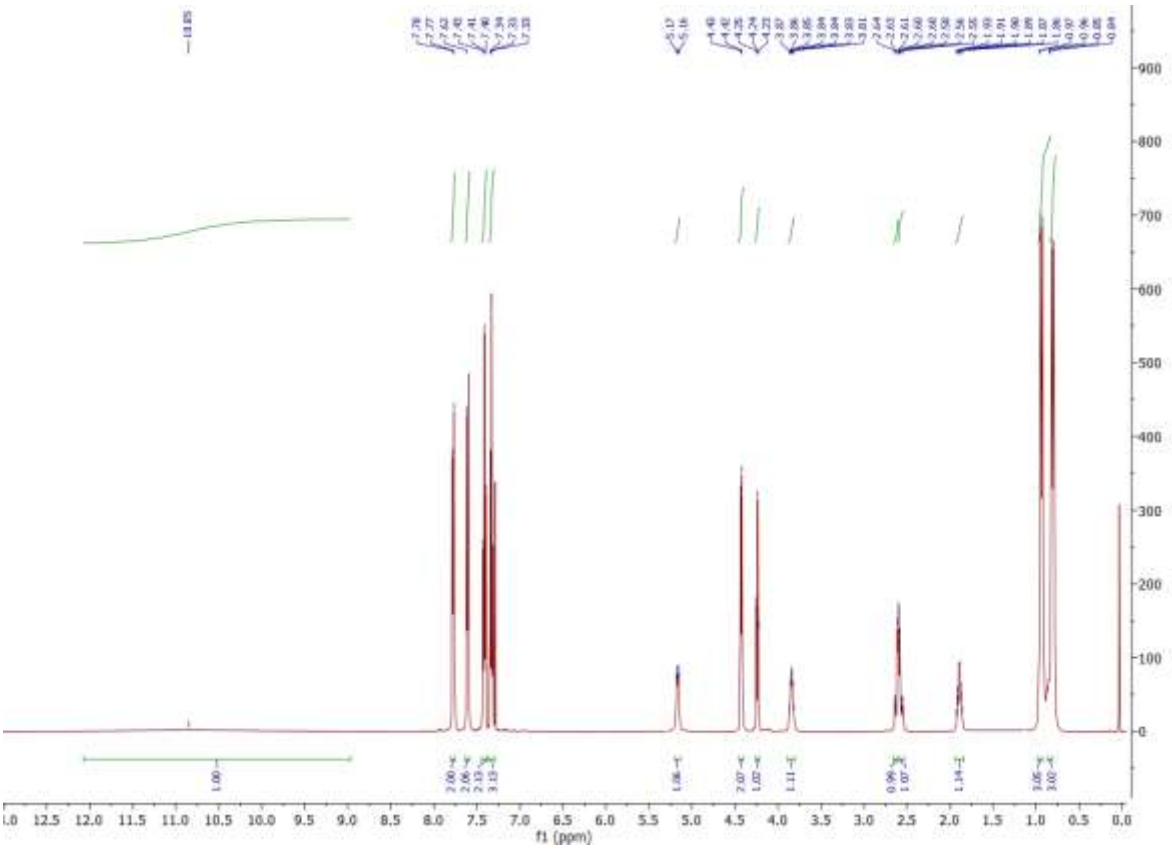
HRMS of (*S*)-(9*H*-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (19)



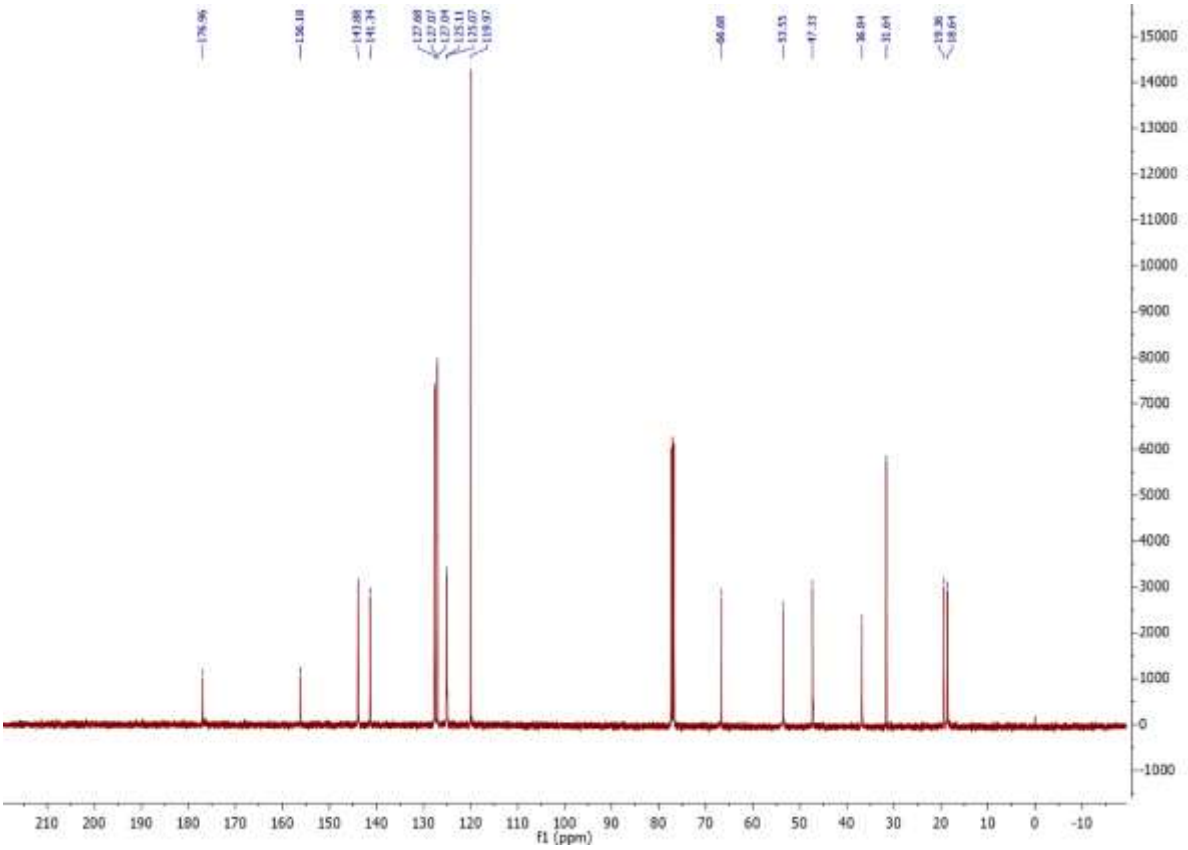
SFC-HPLC of (*S*)-(9*H*-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (19)

SFC-HPLC: (Ethyl pyridine column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO_2 over 7 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 4.18 min

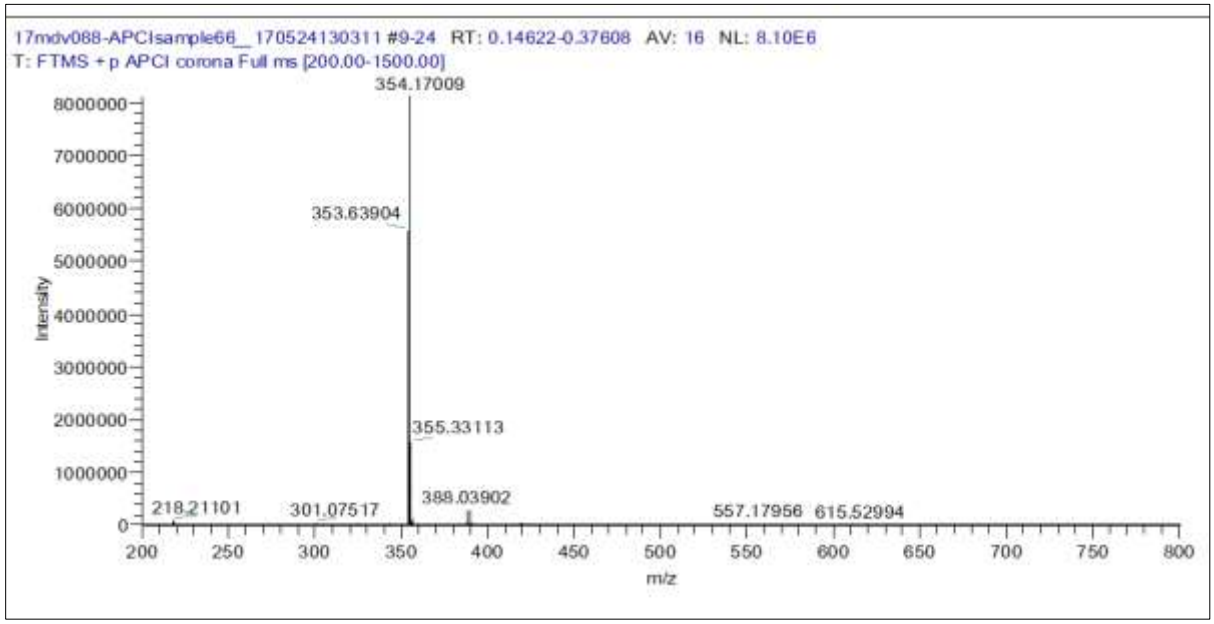




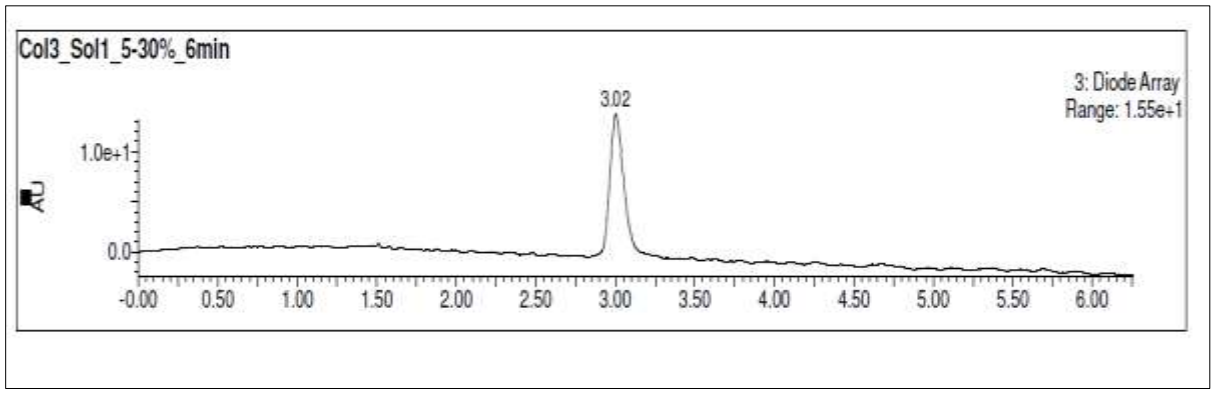
^{1}H NMR of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (20)
(400 MHz, $CDCl_3$)



^{13}C NMR of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (20)
(121 MHz, $CDCl_3$)

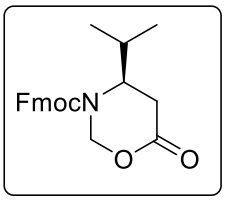


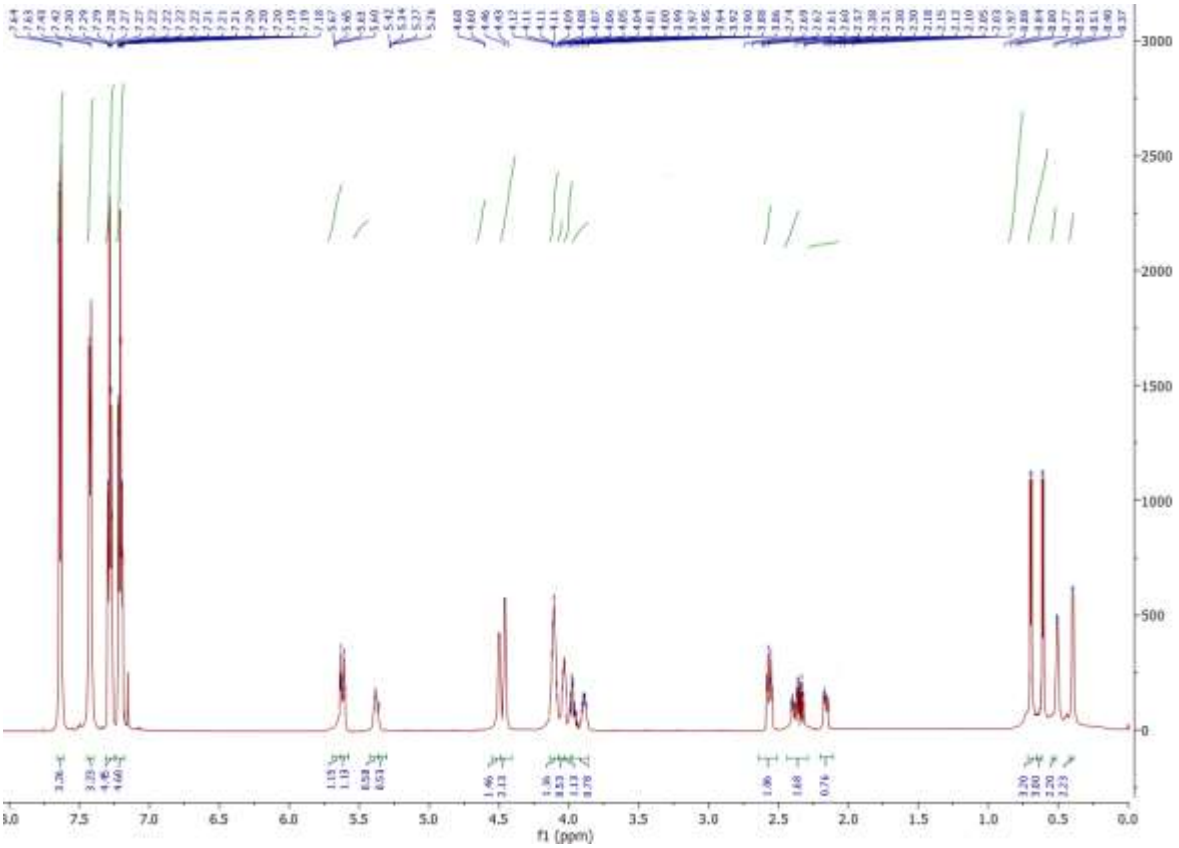
HRMS (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (20)



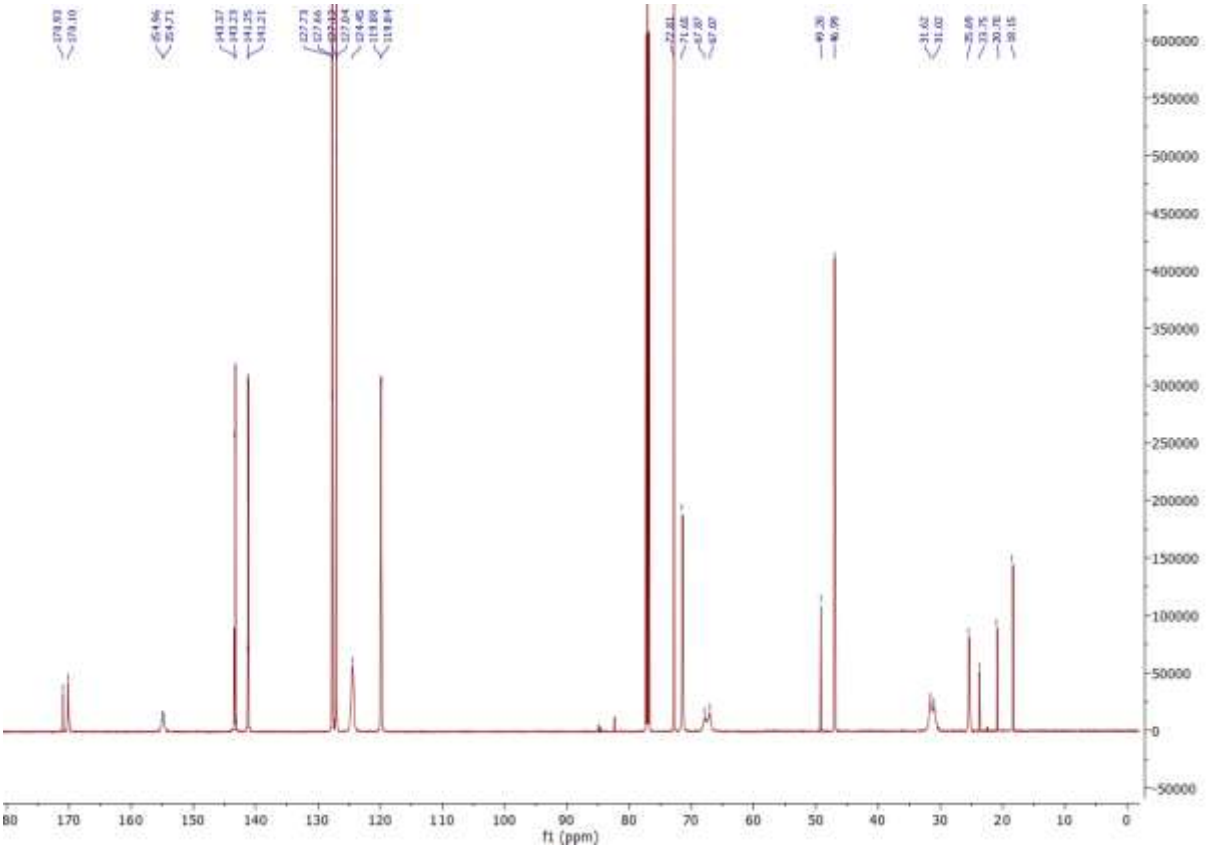
SFC-HPLC of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (20)

SFC-HPLC: (Ethyl pyridine column (4.6 × 250 mm, 5 µm, gradient: 5 – 40% MeOH in CO_2 over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 3.02 min

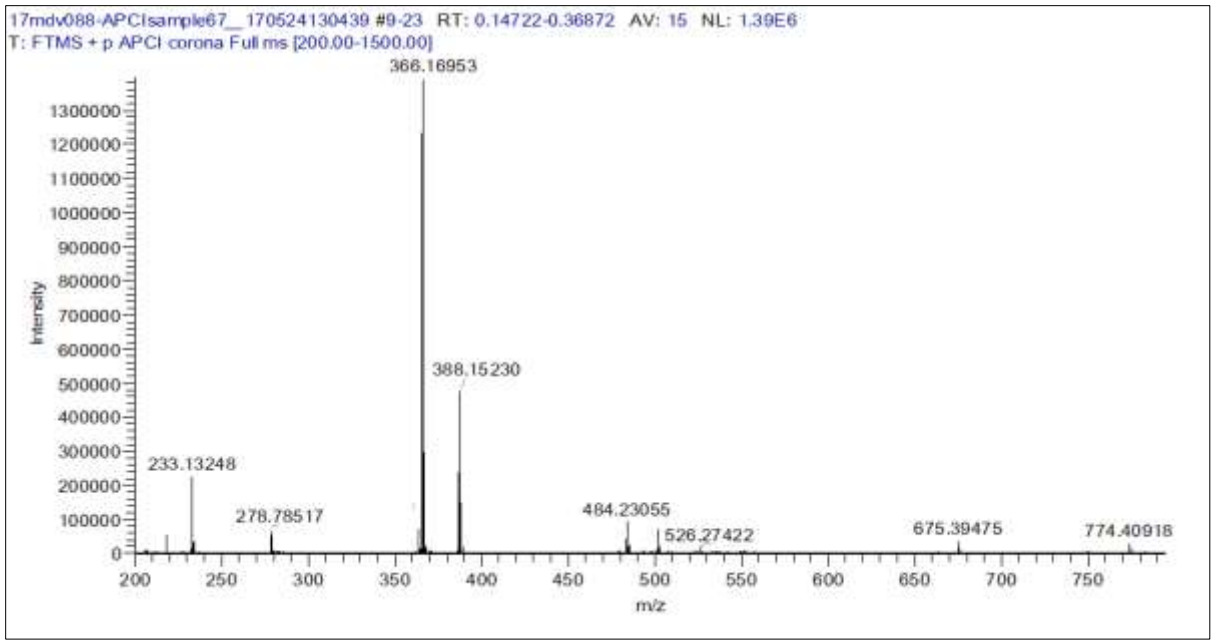




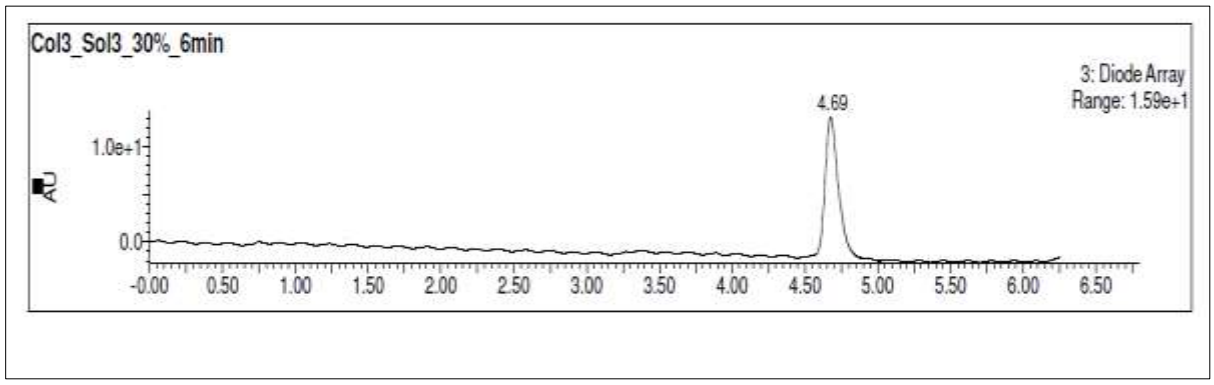
^{1}H NMR of (*R*)-(9*H*-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (21)
(600 MHz, $CDCl_3$)



^{13}C NMR of (*R*)-(9*H*-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (21)
(151 MHz, $CDCl_3$)

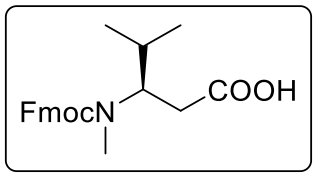


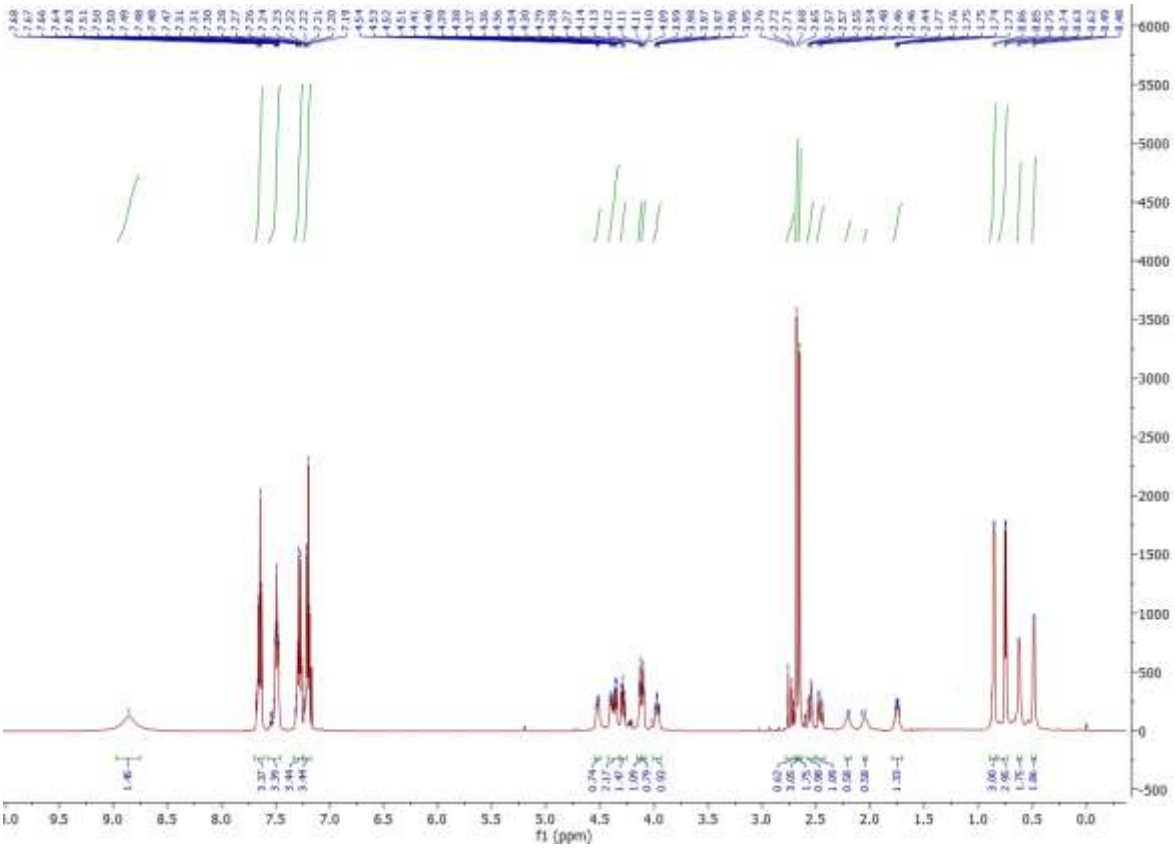
HRMS of (*R*)-(9*H*-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (21)



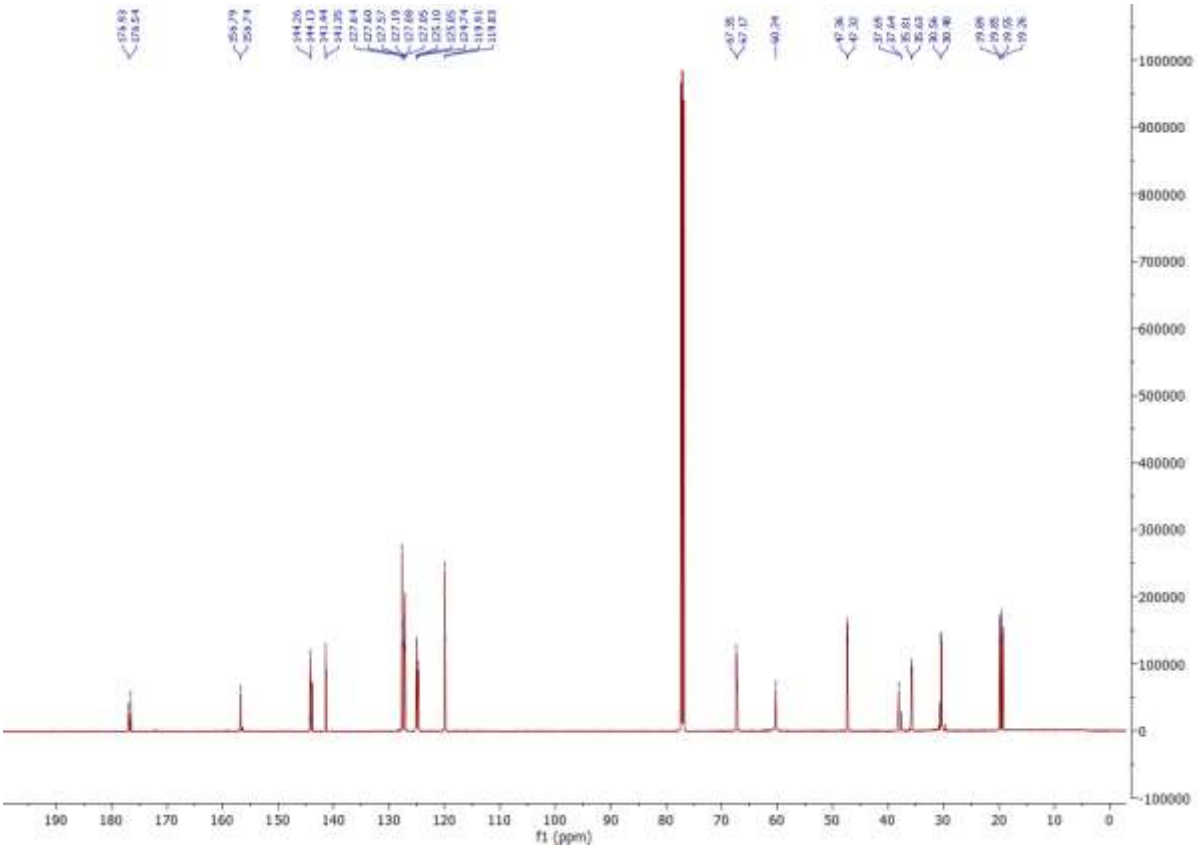
SFC-HPLC of (*R*)-(9*H*-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (21)

SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 µm, gradient: 10 – 30% MeOH in CO_2 over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 4.69 min

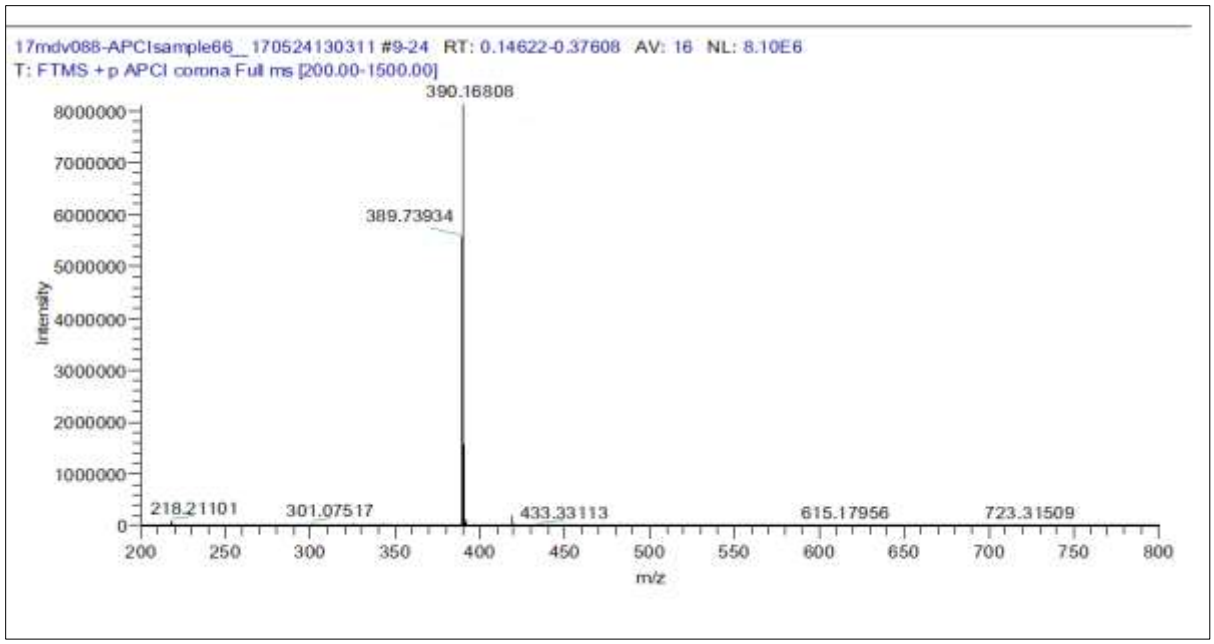




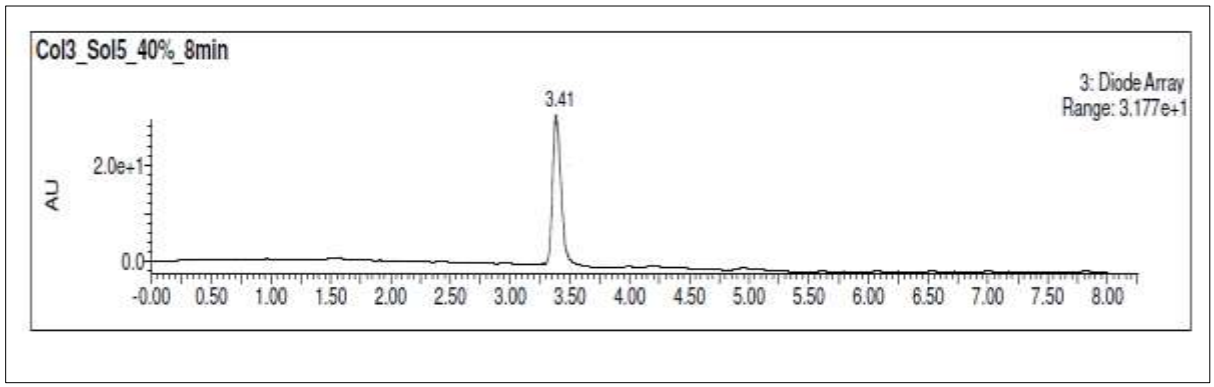
^{1}H NMR of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (22)
(600 MHz, $CDCl_3$)



^{13}C NMR of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (22)
(151 MHz, $CDCl_3$)

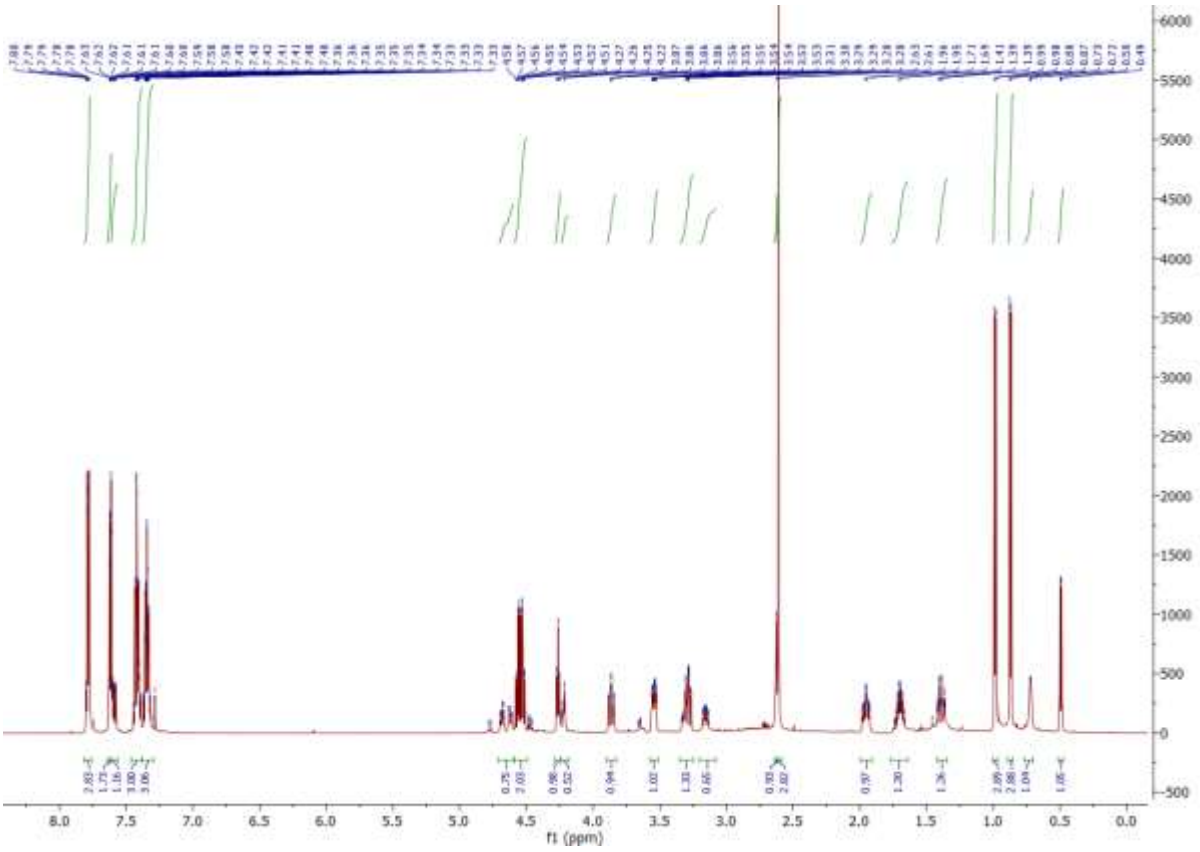


HRMS of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (22)

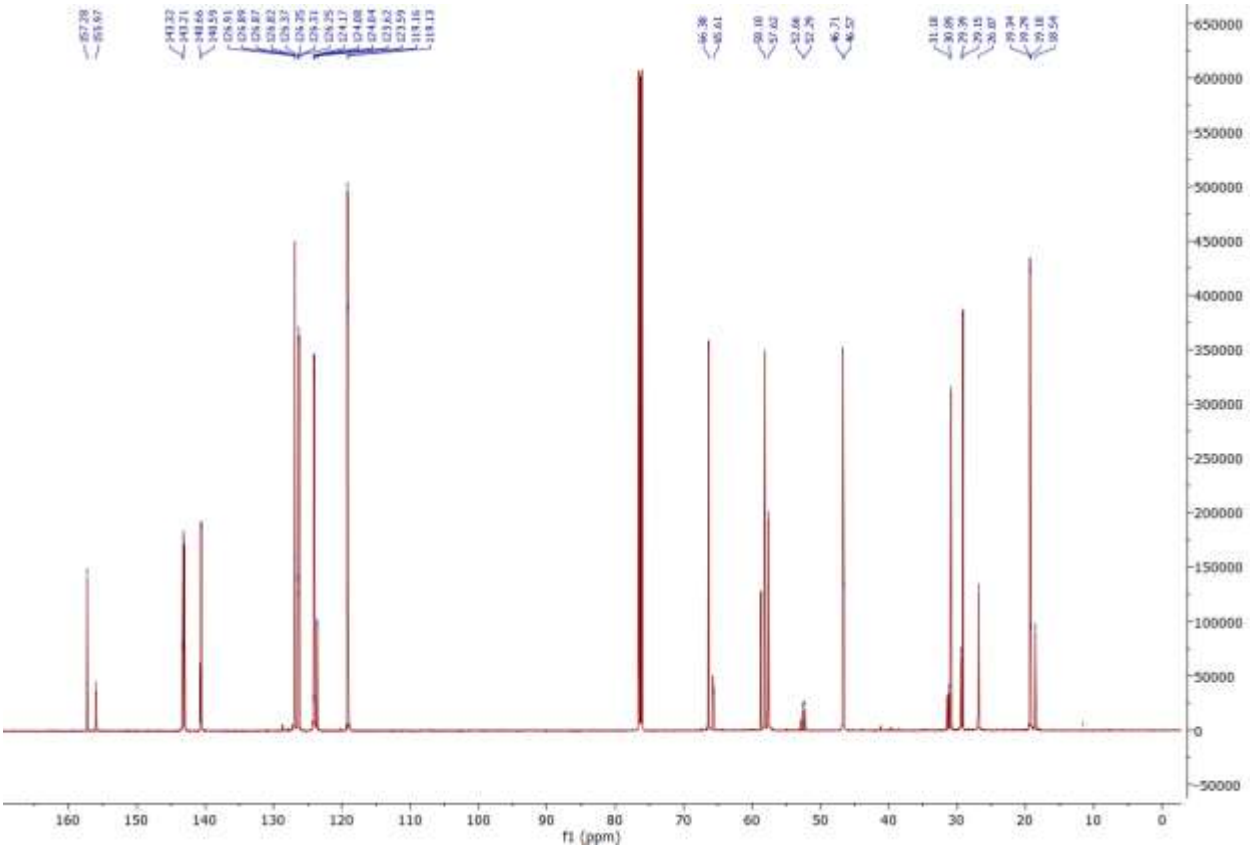


SFC-HPLC of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (22)

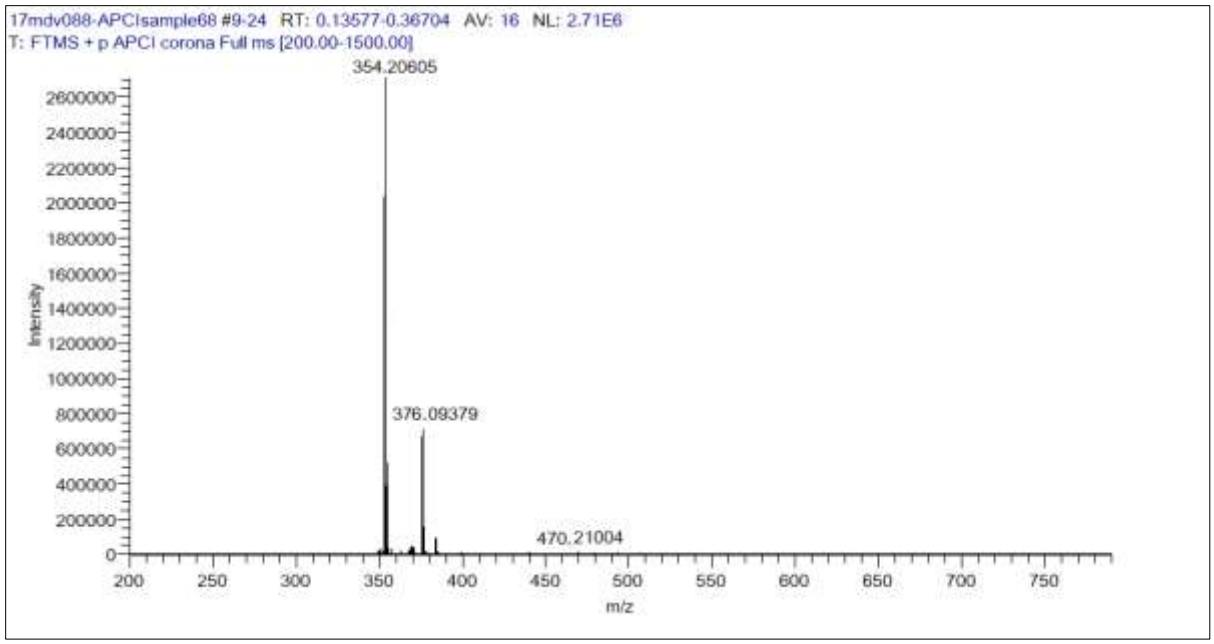
SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 µm, gradient: 5 – 40% MeOH in CO_2 over 8 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 3.41 min



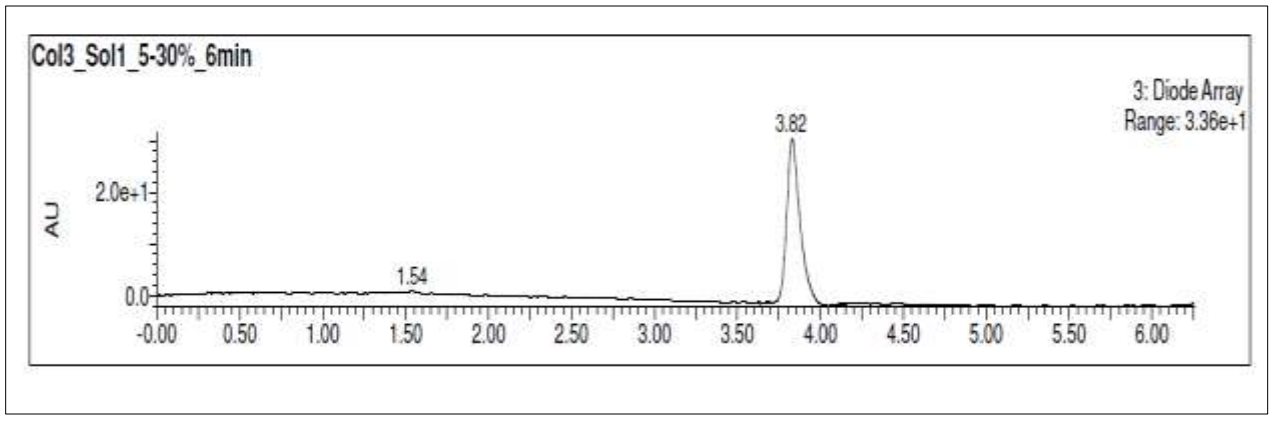
^{1}H NMR of (*R*)-(9*H*-fluoren-9-yl)methyl (1-hydroxy-4-methylpentan-3-yl)(methyl)carbamate (23)
(600 MHz, $CDCl_3$)



^{13}C NMR of HRMS of (*R*)-(9*H*-fluoren-9-yl)methyl (1-hydroxy-4-methylpentan-3-yl)(methyl)carbamate (23)
(151 MHz, $CDCl_3$)

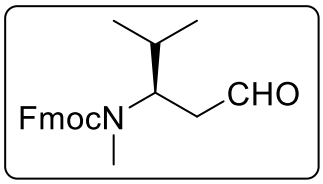


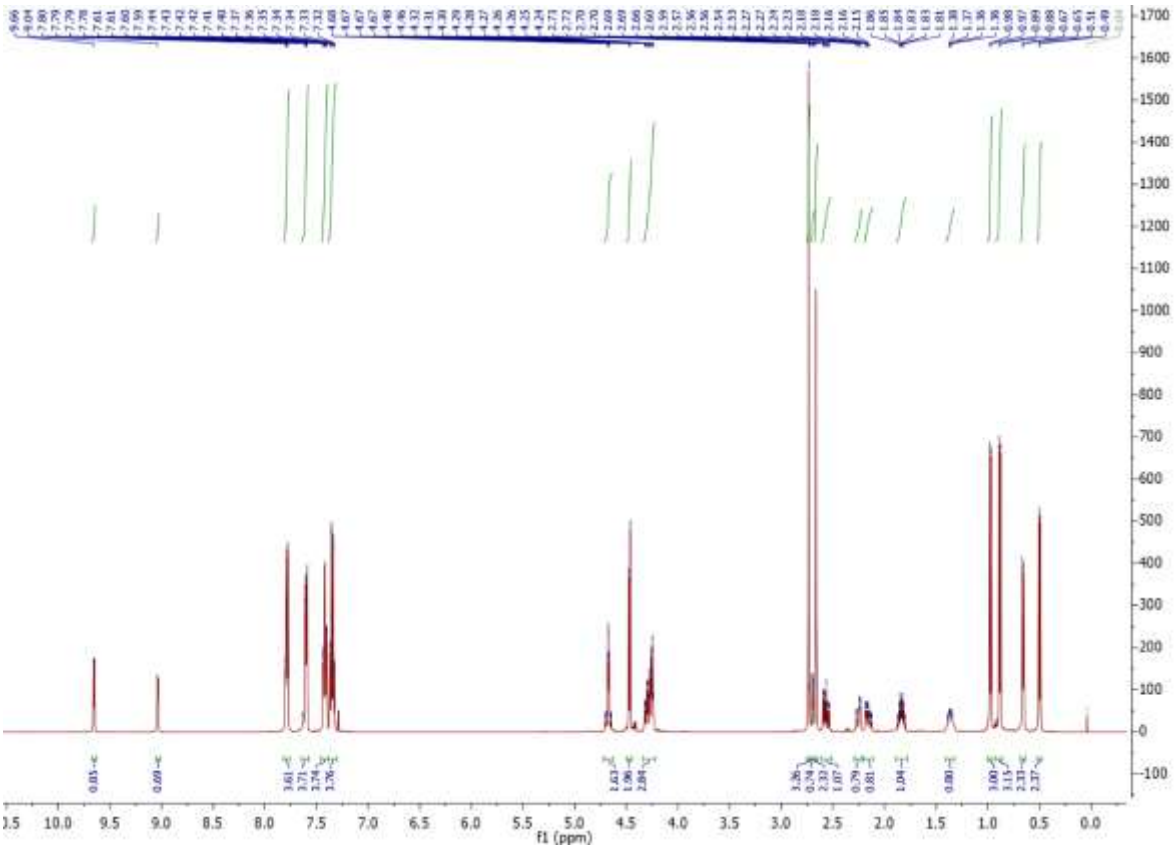
HRMS of (*R*)-(9*H*-fluoren-9-yl)methyl (1-hydroxy-4-methylpentan-3-yl)(methyl)carbamate (23)



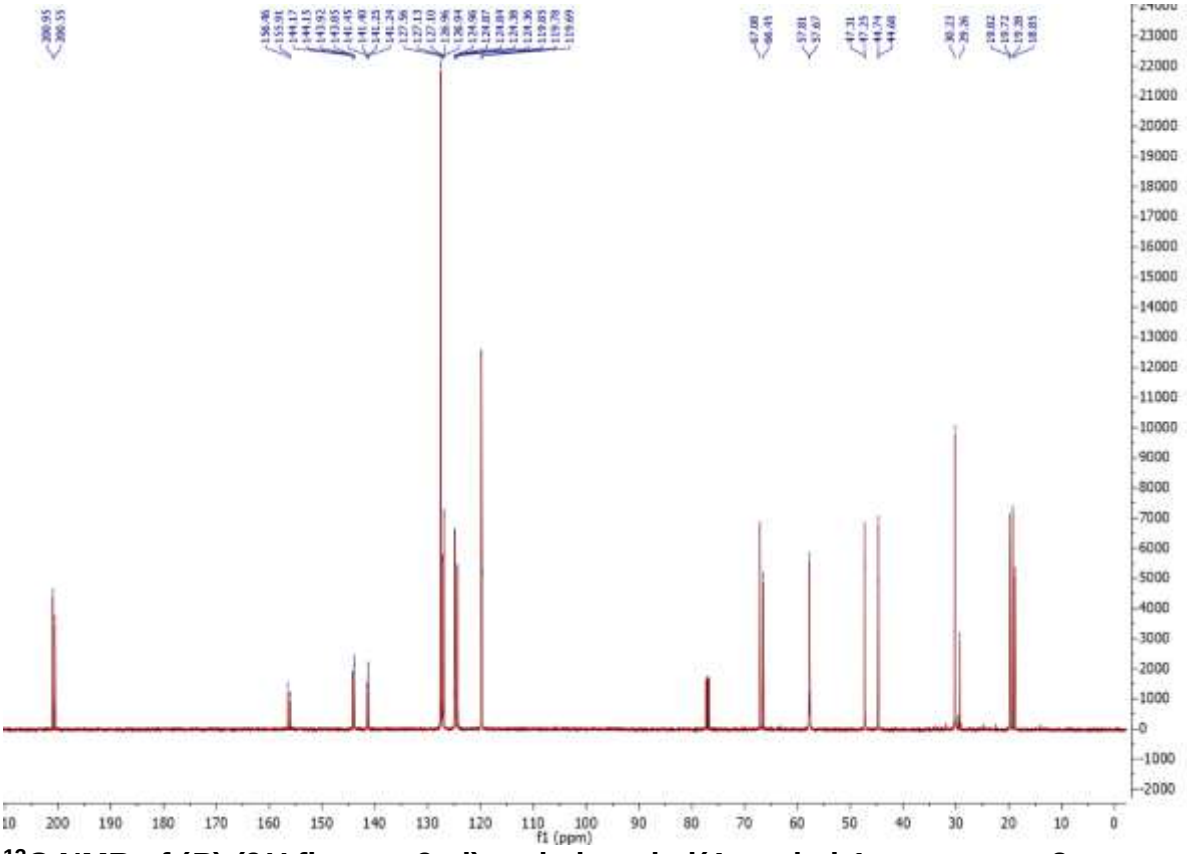
SFC-HPLC of (*R*)-(9*H*-fluoren-9-yl)methyl (1-hydroxy-4-methylpentan-3-yl)(methyl)carbamate (23)

SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO_2 over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 3.82 min

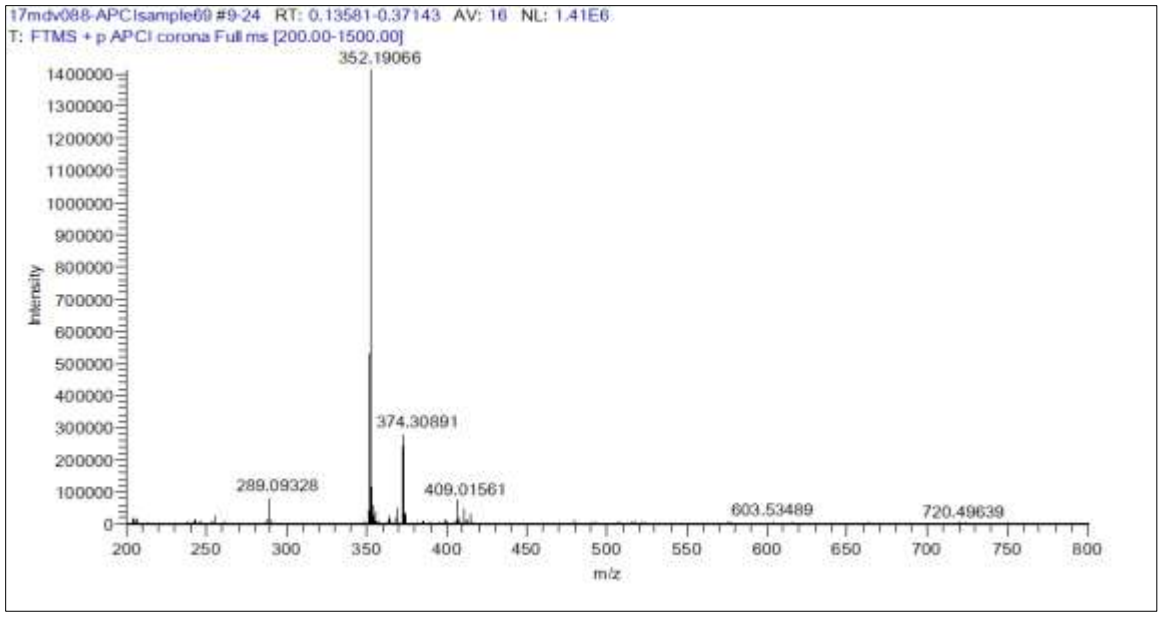




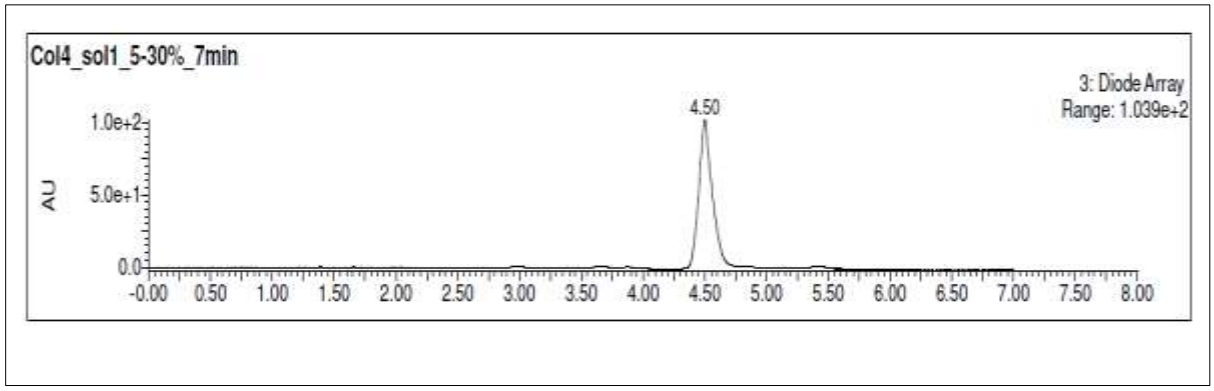
^{1}H NMR of (*R*)-(9*H*-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2)
(500 MHz, $CDCl_3$)



^{13}C NMR of (*R*)-(9*H*-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2)
(126 MHz, $CDCl_3$)

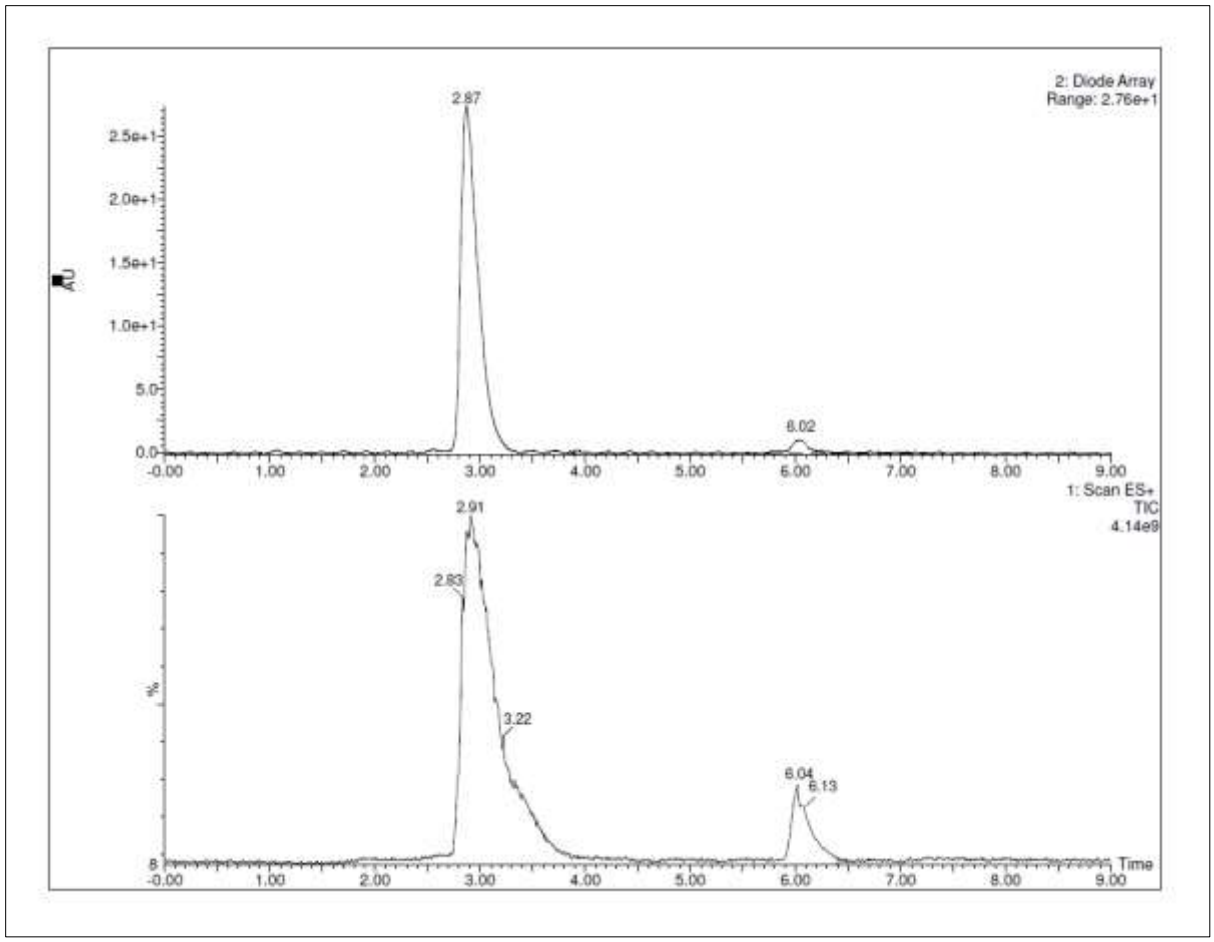


HRMS of (*R*)-(9*H*-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2)



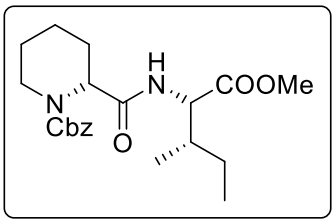
SFC-HPLC of (*R*)-(9*H*-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2)

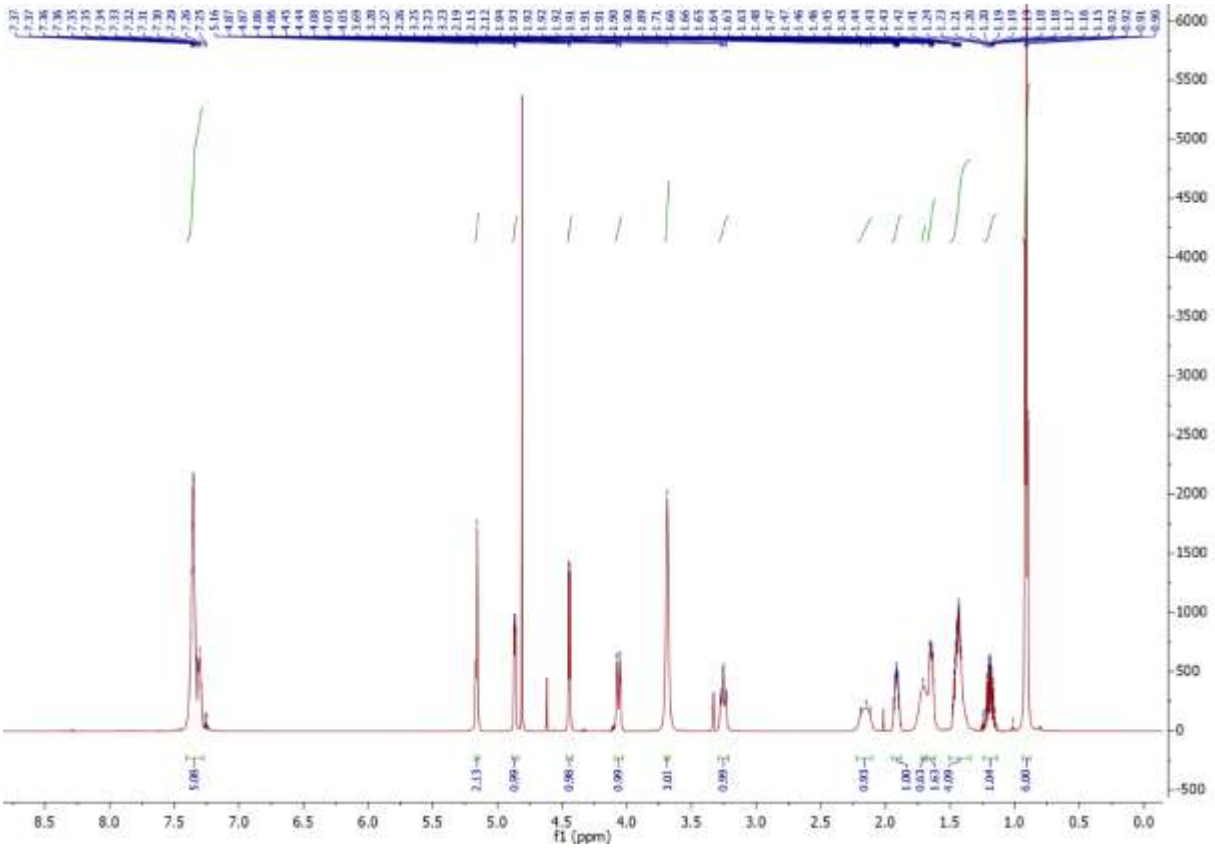
SFC-HPLC: Viridis silica gel column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO_2 over 7 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 4.50 min



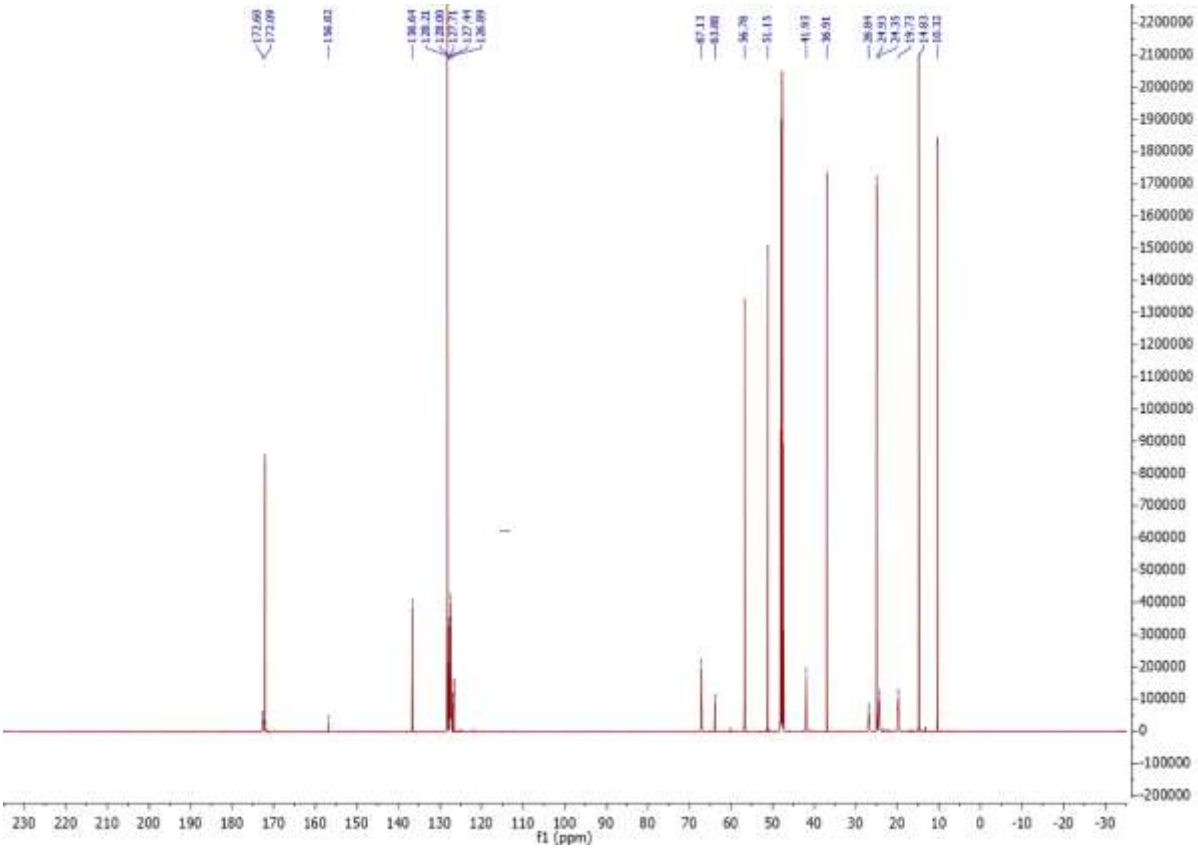
Chiral SFC–HPLC chromatogram of (*R*)-(9*H*-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2)

Method: Reprosil Chiral-AM column (4.6 × 250 mm, 5µm, with 2 -40% *i*PrOH in CO_2 for 9 min; γ = 254 nm, flow rate: 0.8 mL/ min). t_R = 2.87 min; ee >98%

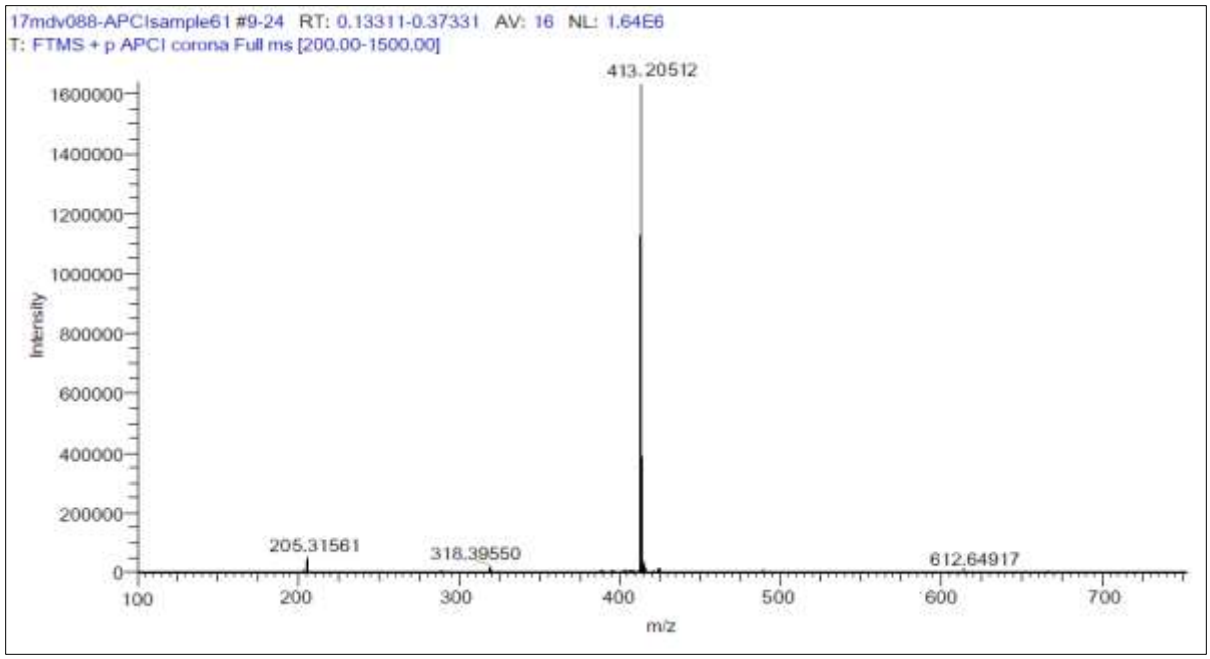




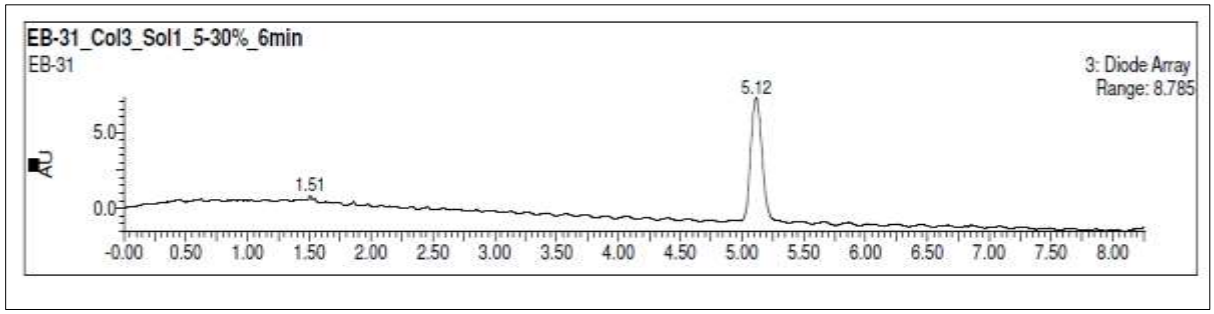
^{1}H NMR of (*R*)-benzyl 2-(((2*S*,3*R*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26)
(600 MHz, d_4-MeOD)



^{13}C NMR of (*R*)-benzyl 2-(((2*S*,3*R*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26)
(151 MHz, *d*$_4$-MeOD)

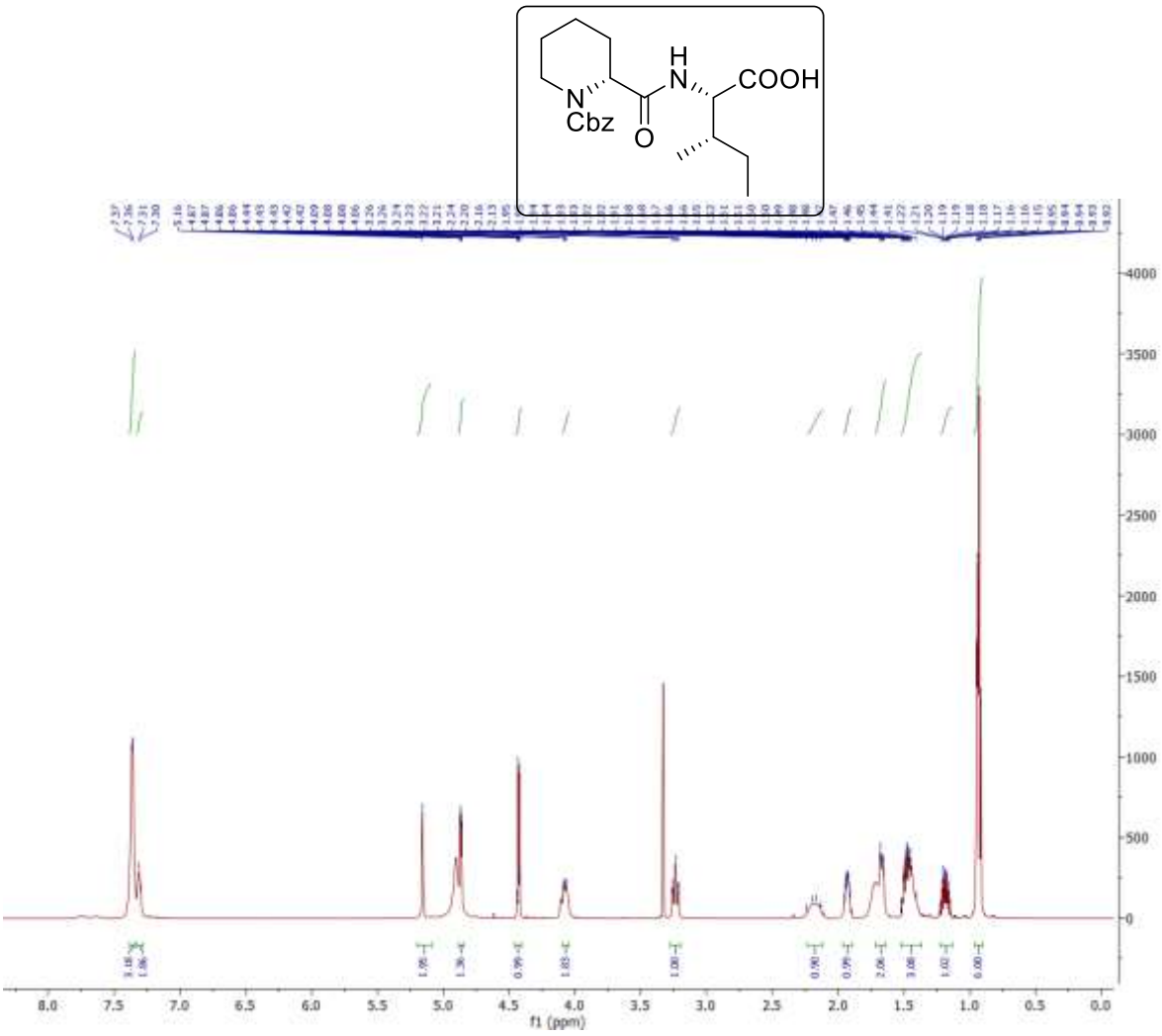


HRMS of (*R*)-benzyl 2-(((2*S*,3*R*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26)

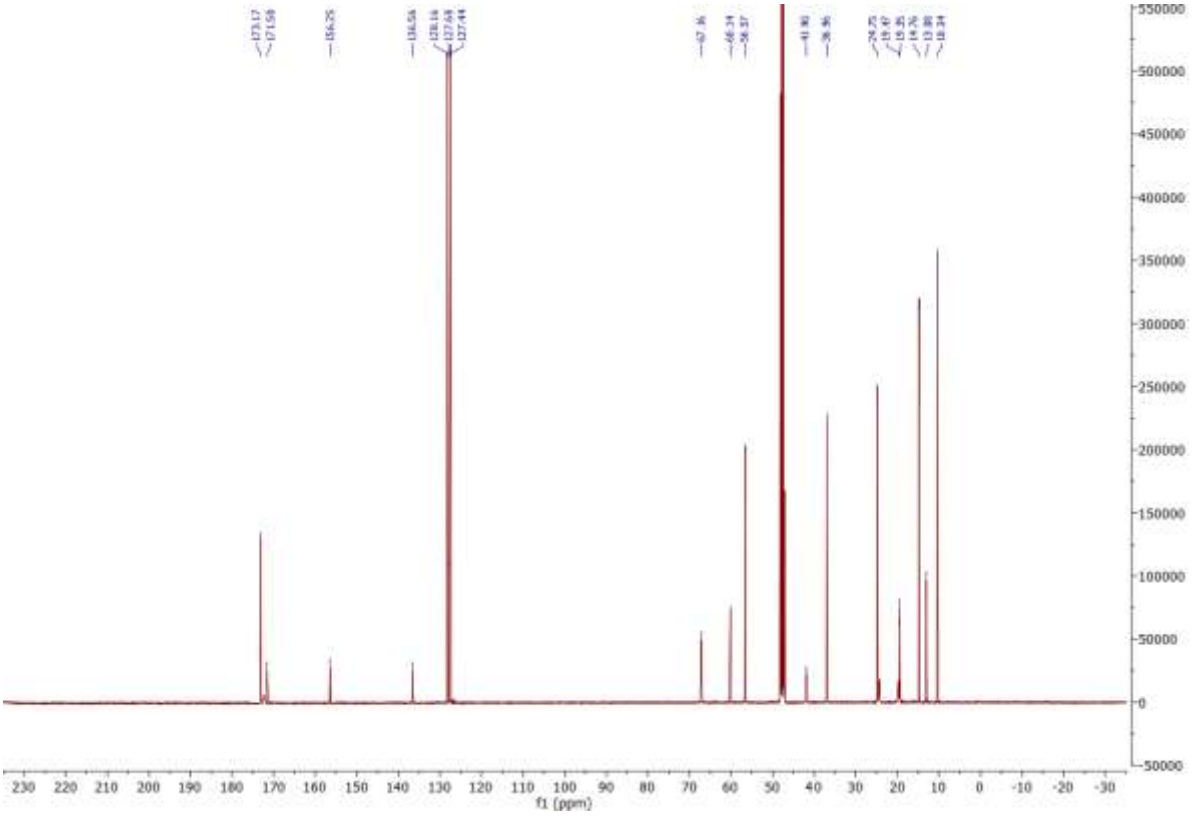


SFC-HPLC of (*R*)-benzyl 2-(((2*S*,3*R*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26)

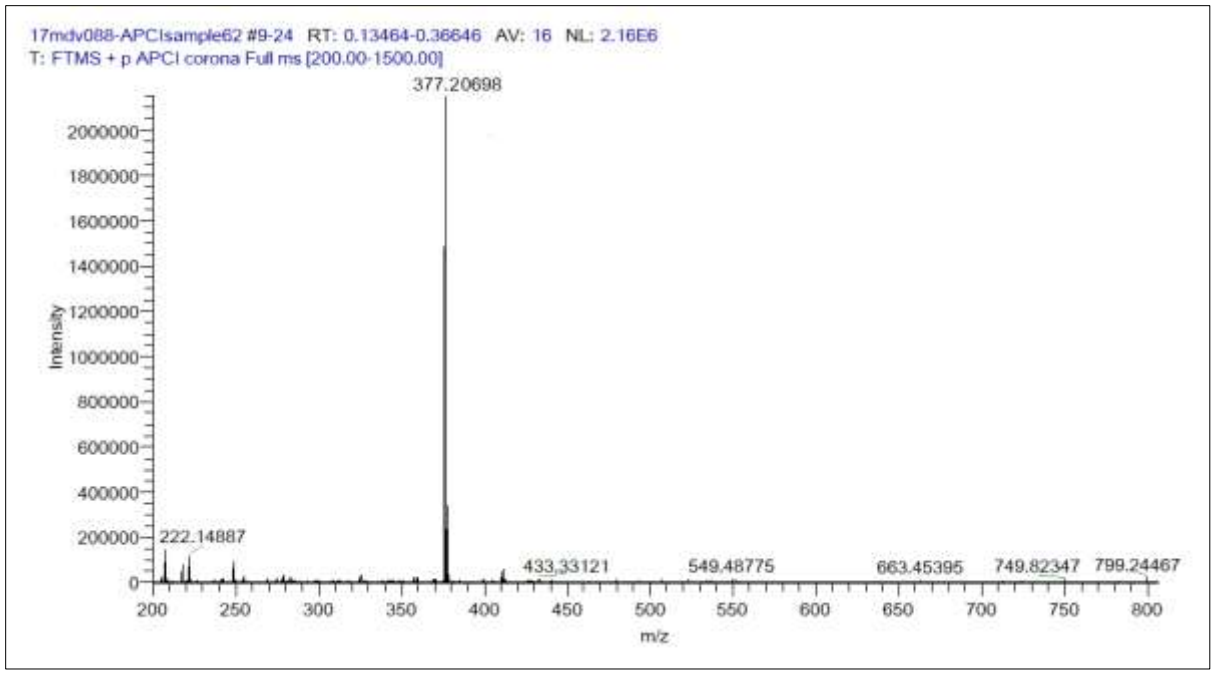
SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO_2 over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 5.12 min



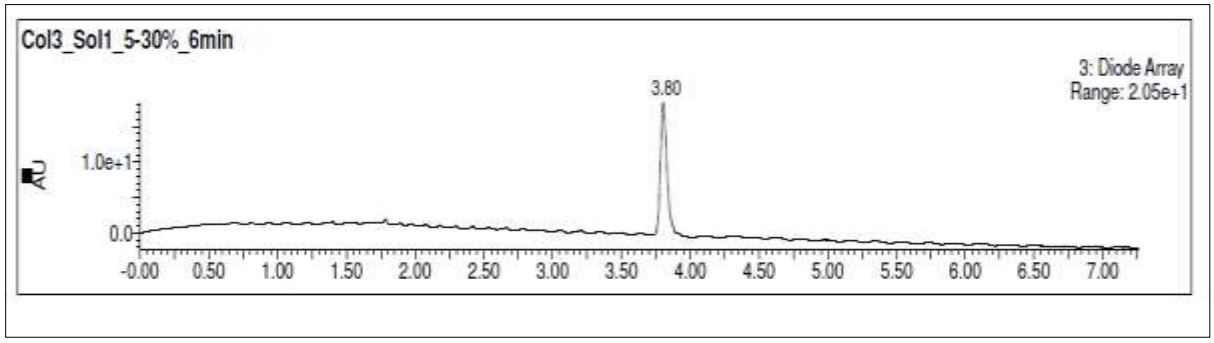
^{1}H NMR of (2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)
(600 MHz, d_4-MeOD)



^{13}C NMR of (2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)
(151 MHz, d_4-MeOD)

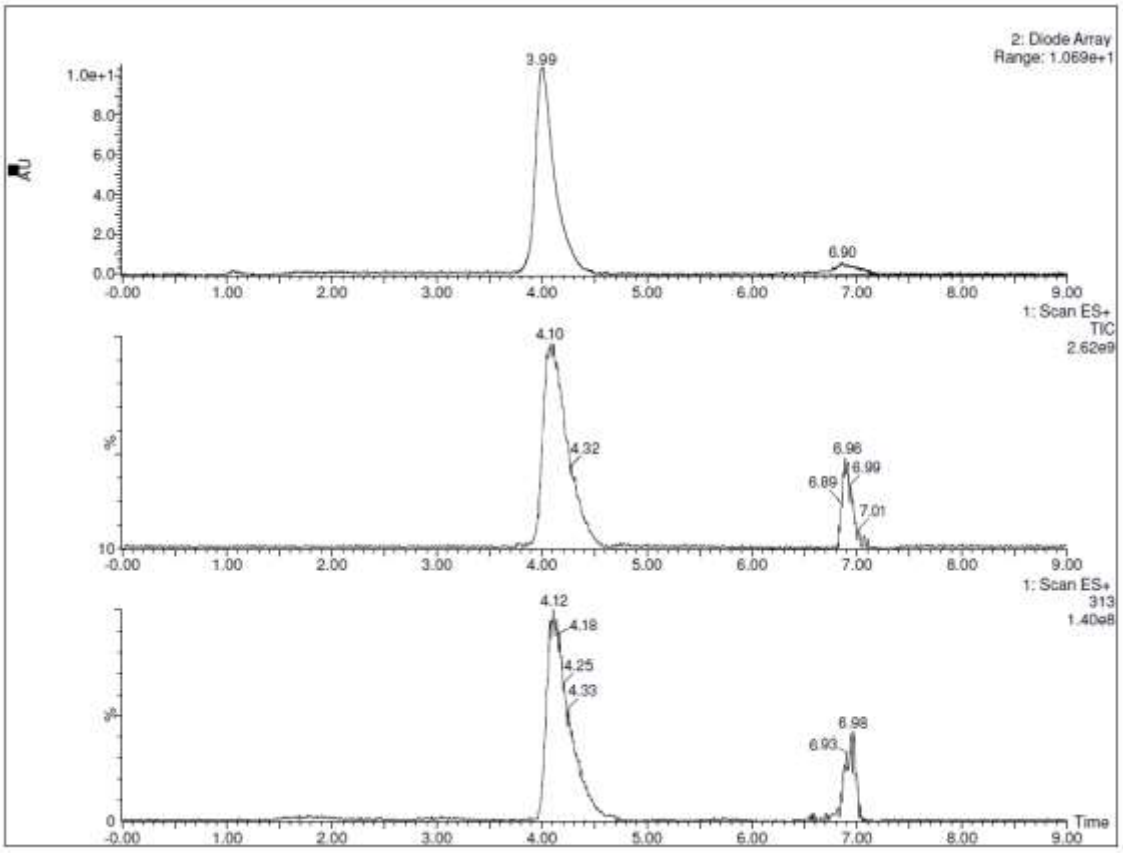


HRMS of (2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)



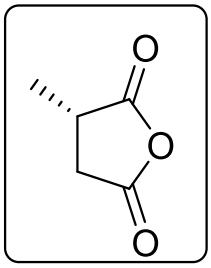
SFC-HPLC of (2S,3R)-2-((R)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)

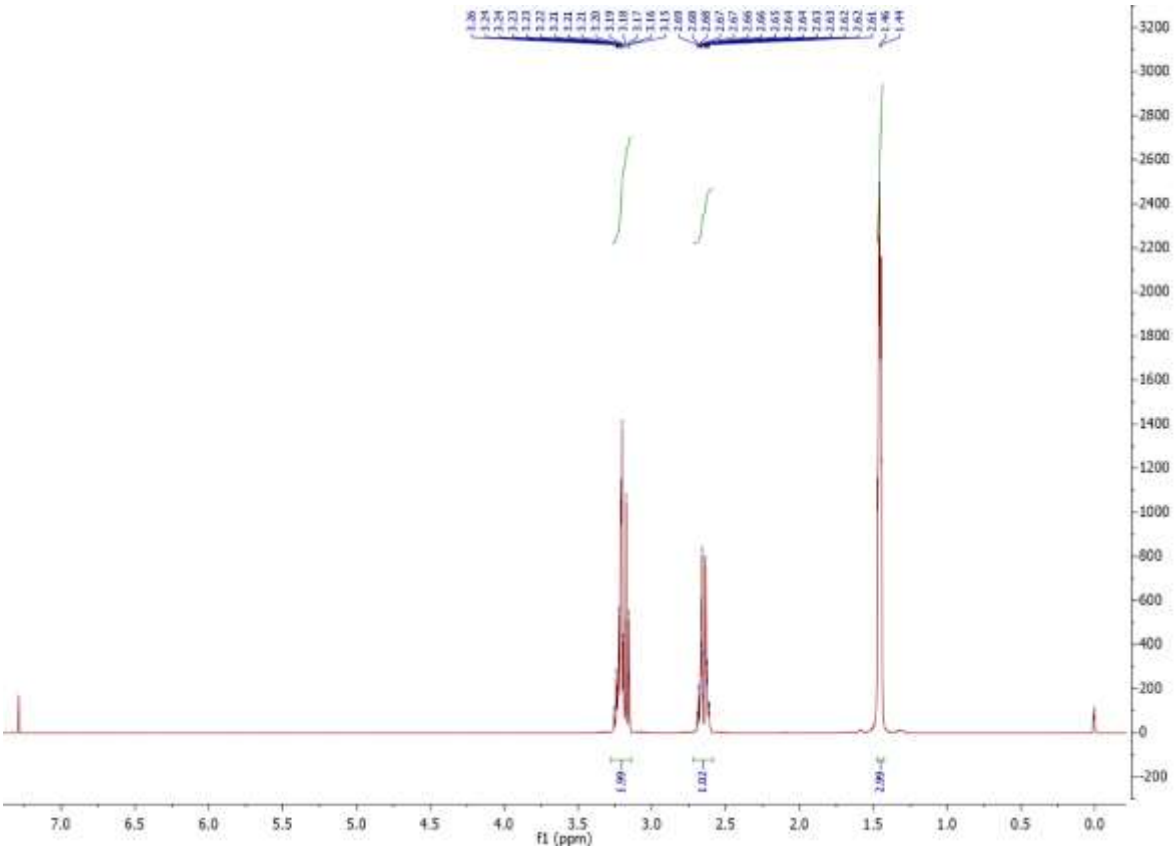
SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO_2 over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 3.30 min



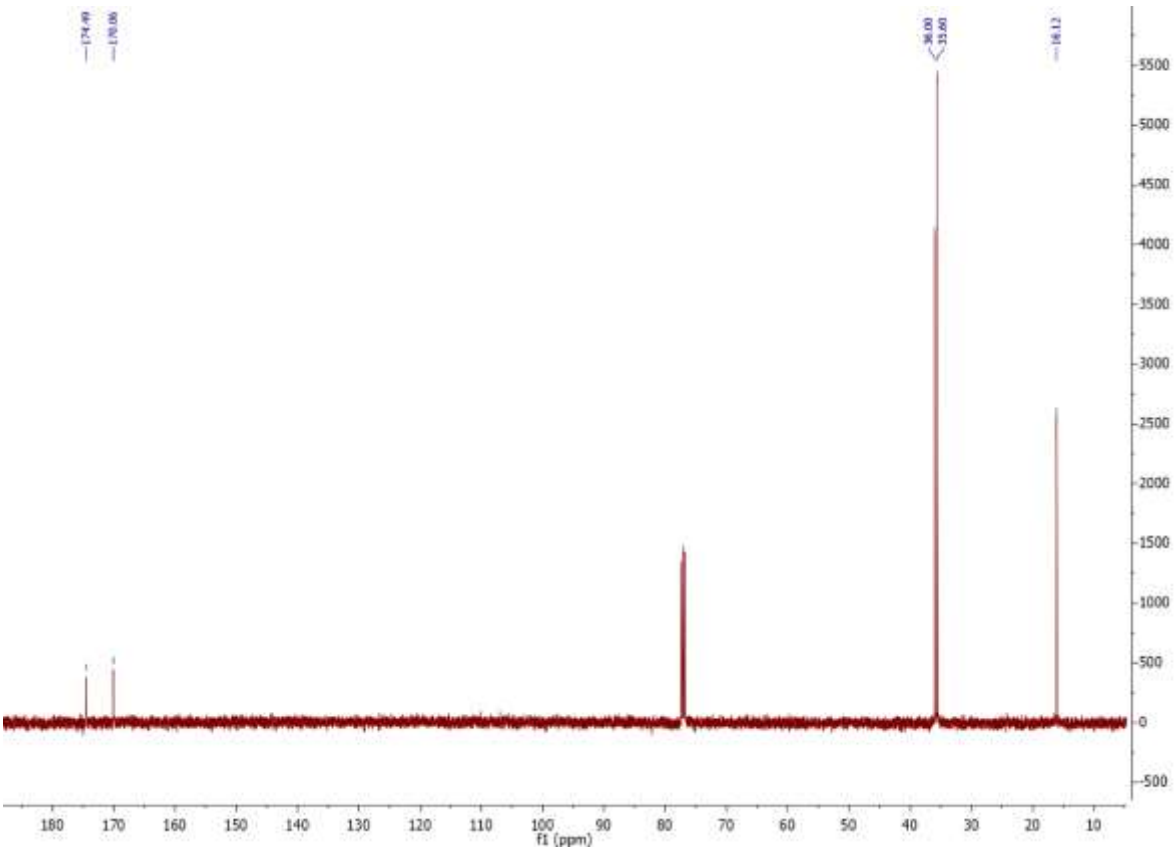
Chiral SFC–HPLC chromatogram of (2S,3R)-2-((R)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)

Method**:** Reprosil Chiral-AM column (4.6 × 250 mm, 5µm, with 3 - 30% *i*PrOH in CO_2 for 9 min; γ = 214 nm, flow rate: 0.6 mL/ min). t_R = 3.99 min; ee >98%

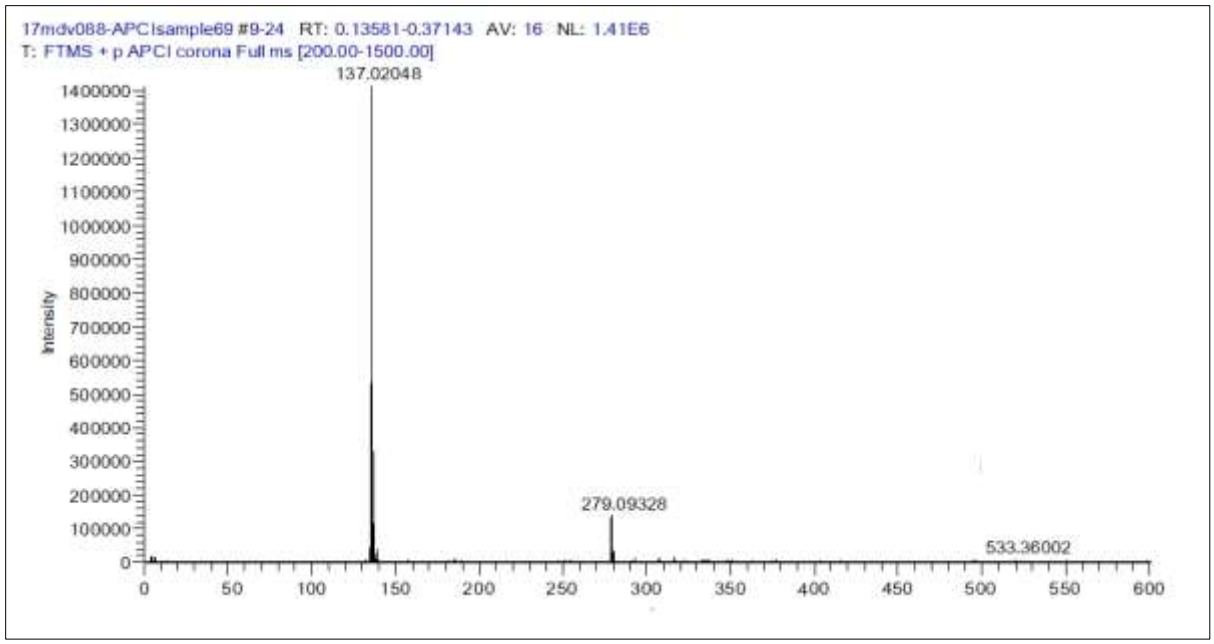




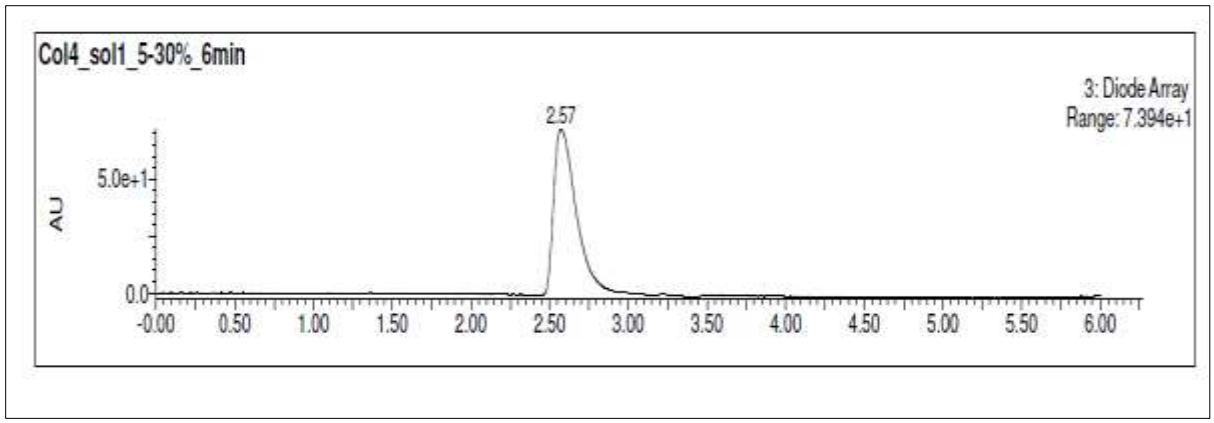
^{1}H NMR of (*S*)-3-methyldihydrofuran-2,5-dione (7)
(500 MHz, $CDCl_3$)



^{13}C NMR of (*S*)-3-methyldihydrofuran-2,5-dione (7)
(126 MHz, $CDCl_3$)

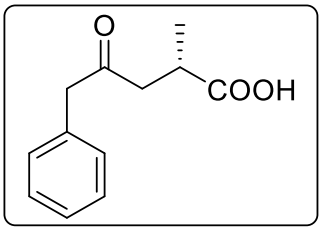


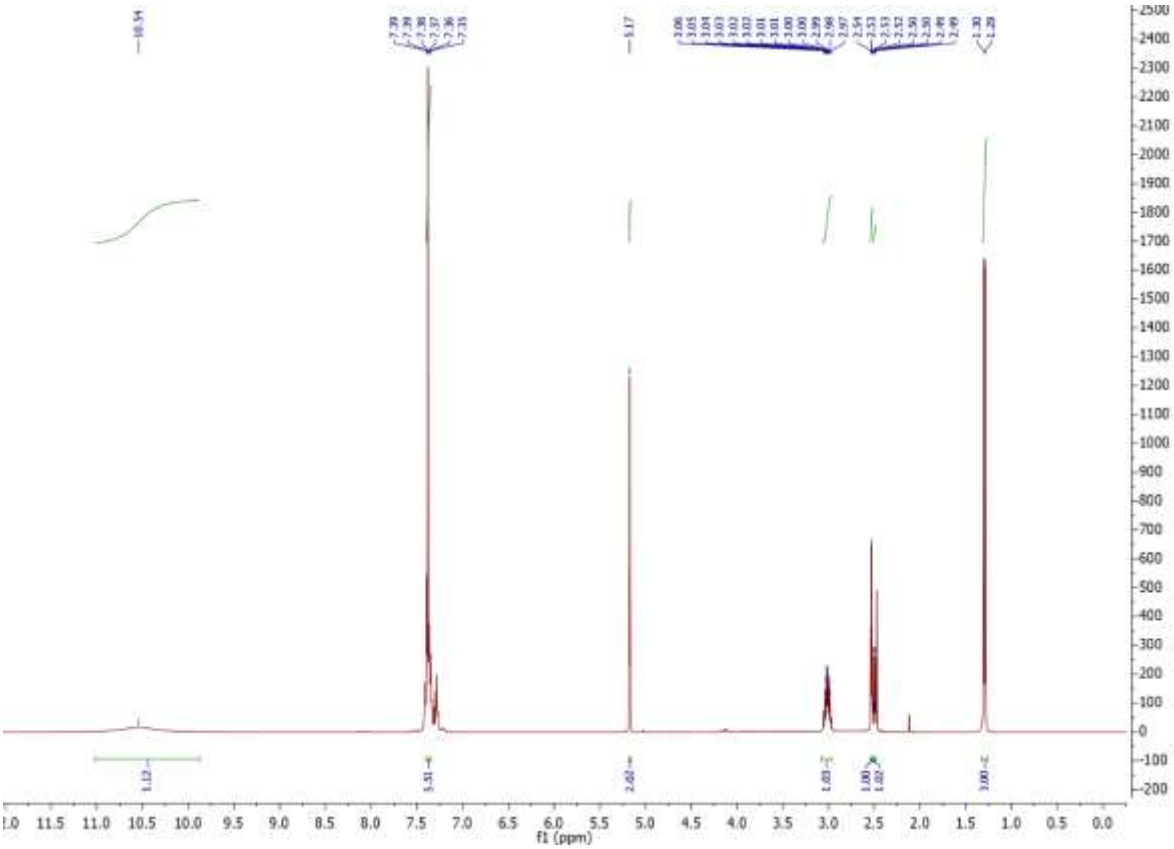
HRMS of (*S*)-3-methyldihydrofuran-2,5-dione (7)



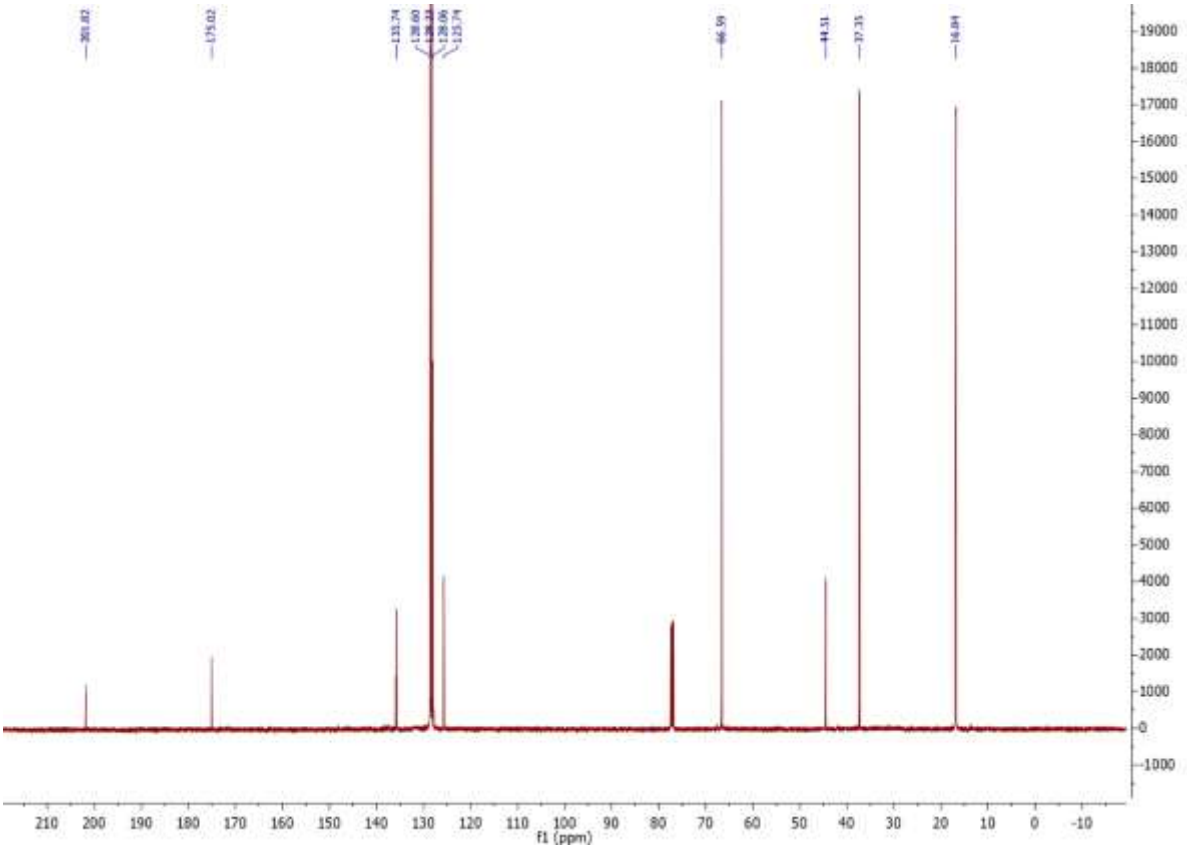
SFC-HPLC of (*S*)-3-methyldihydrofuran-2,5-dione (7)

SFC-HPLC: Viridis silica gel column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO_2 over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 2.57 min

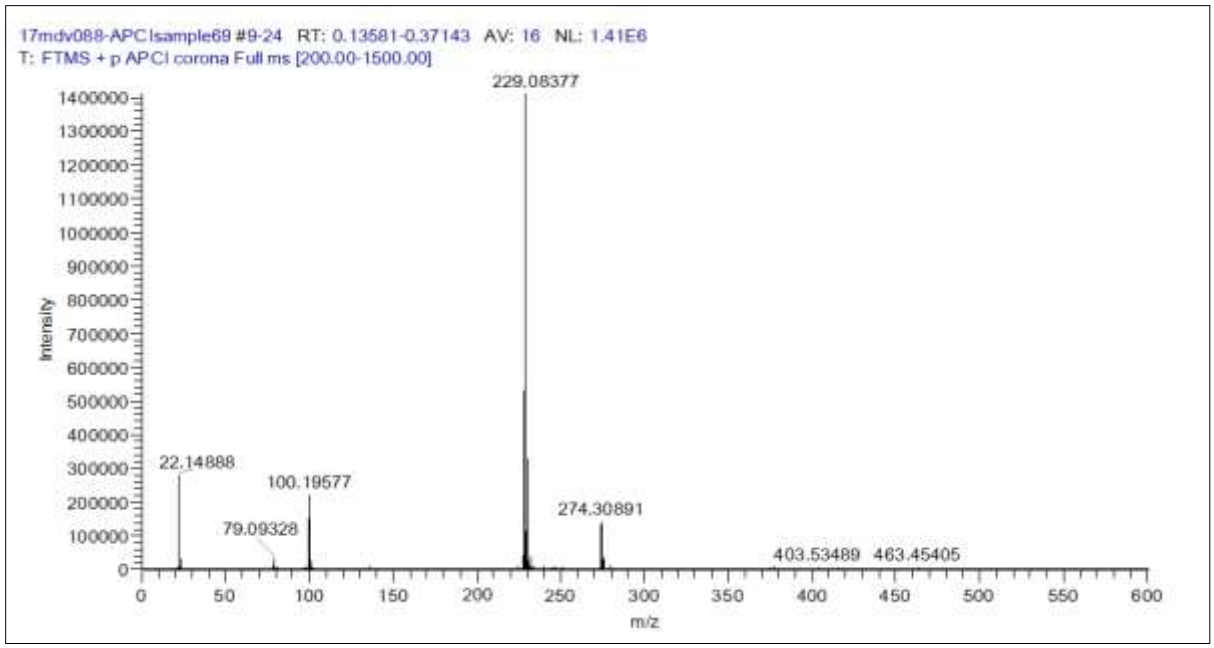




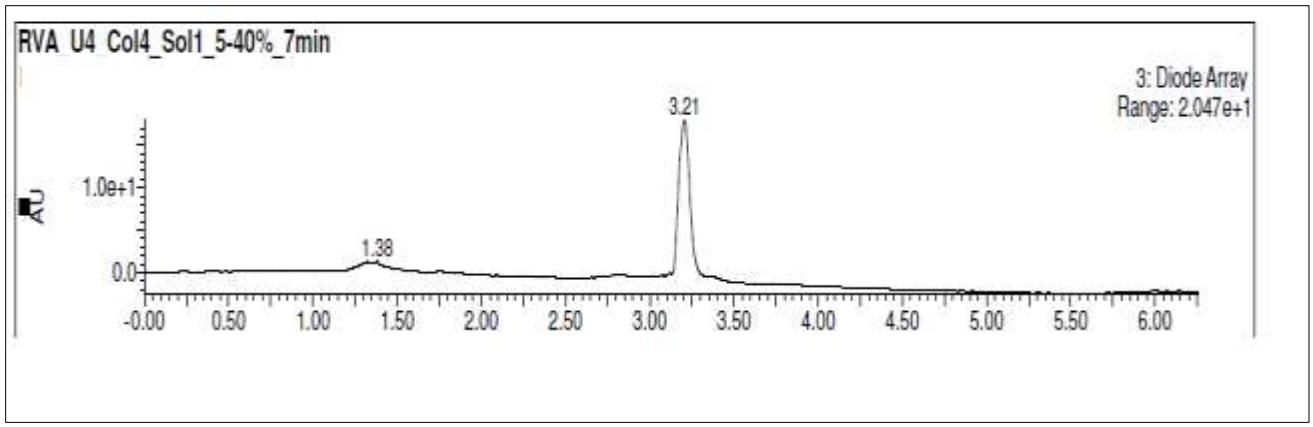
^{1}H NMR of (*S*)-2-methyl-4-oxo-5-phenylpentanoic acid (8a) (500 MHz, $CDCl_3$)



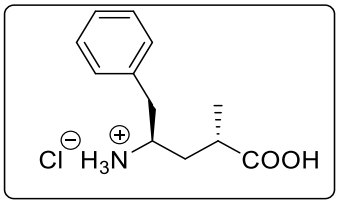
^{13}C NMR of (*S*)-2-methyl-4-oxo-5-phenylpentanoic acid (8a) (126 MHz, $CDCl_3$)

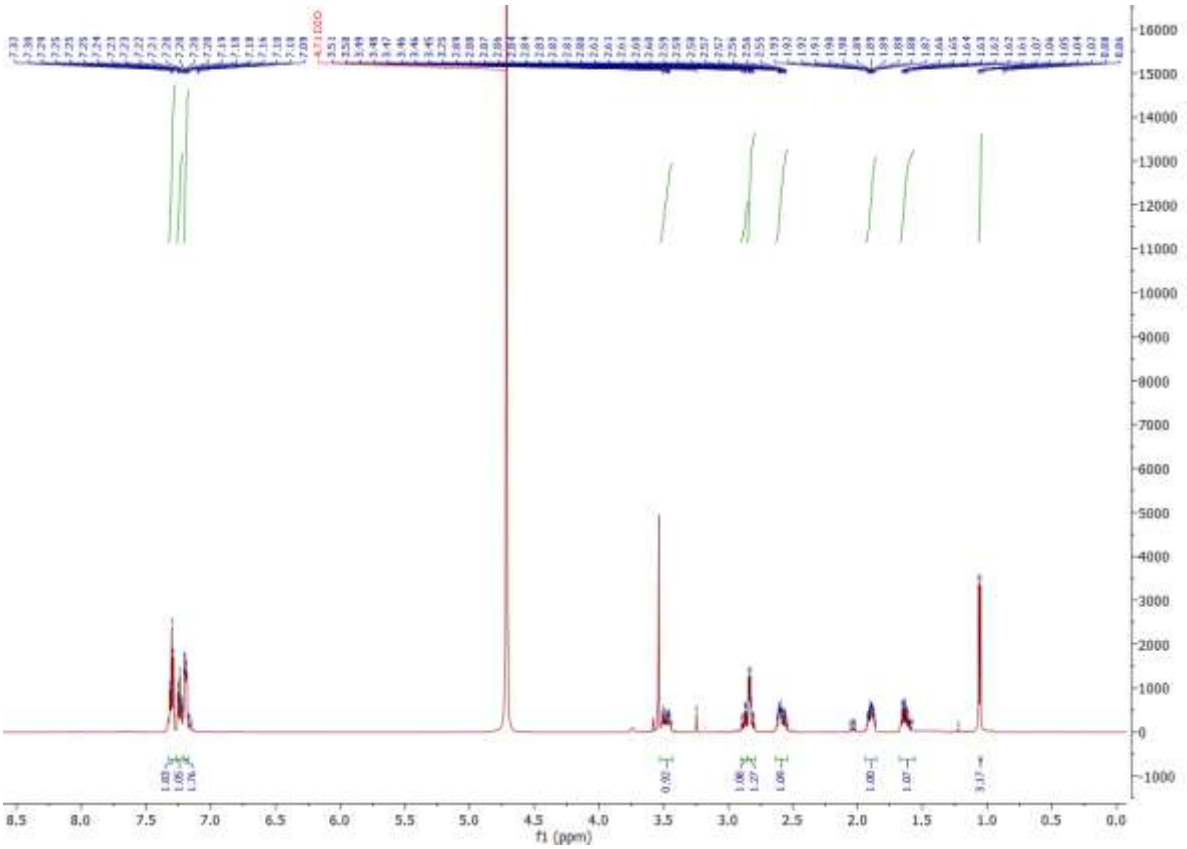


HRMS of (*S*)-2-methyl-4-oxo-5-phenylpentanoic acid (8a)

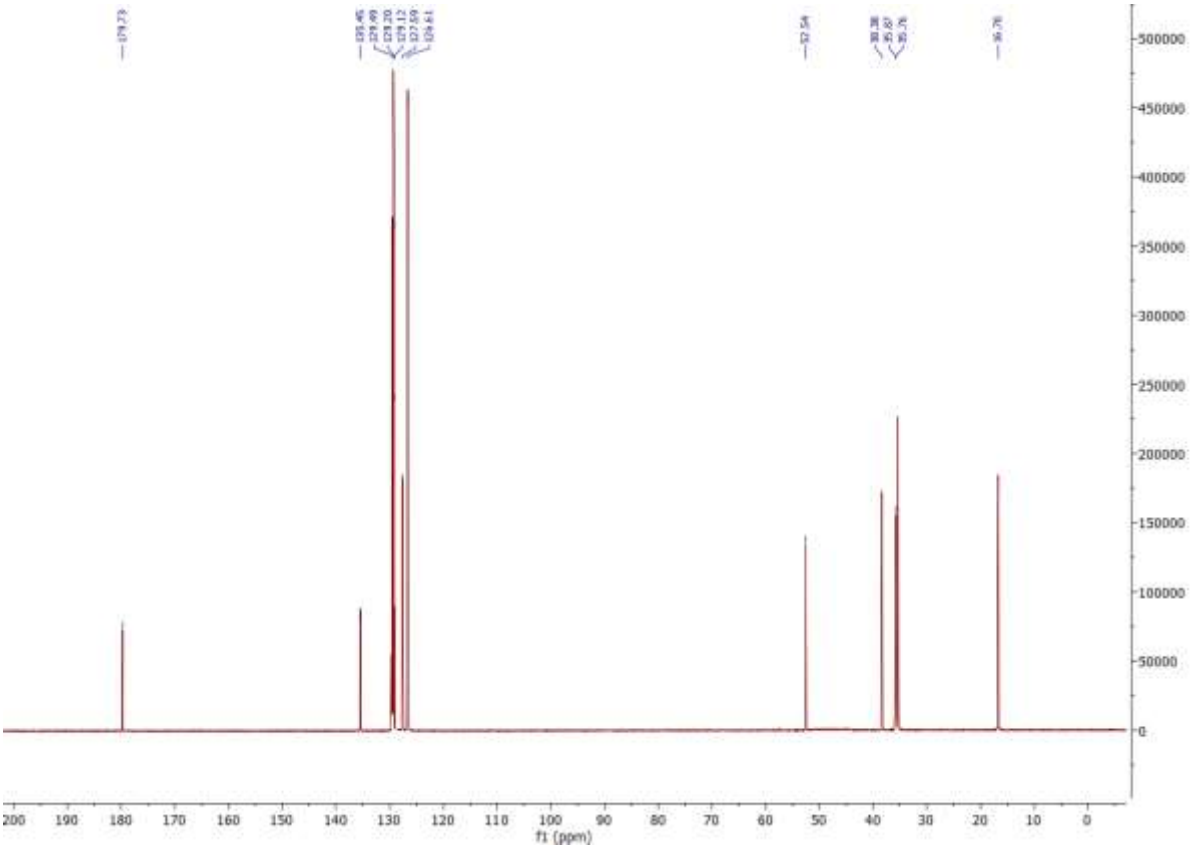


SFC-HPLC: Viridis silica gel column (4.6 × 250 mm, 5 µm, gradient: 5 – 40% MeOH in CO_2 over 7 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 3.21 min



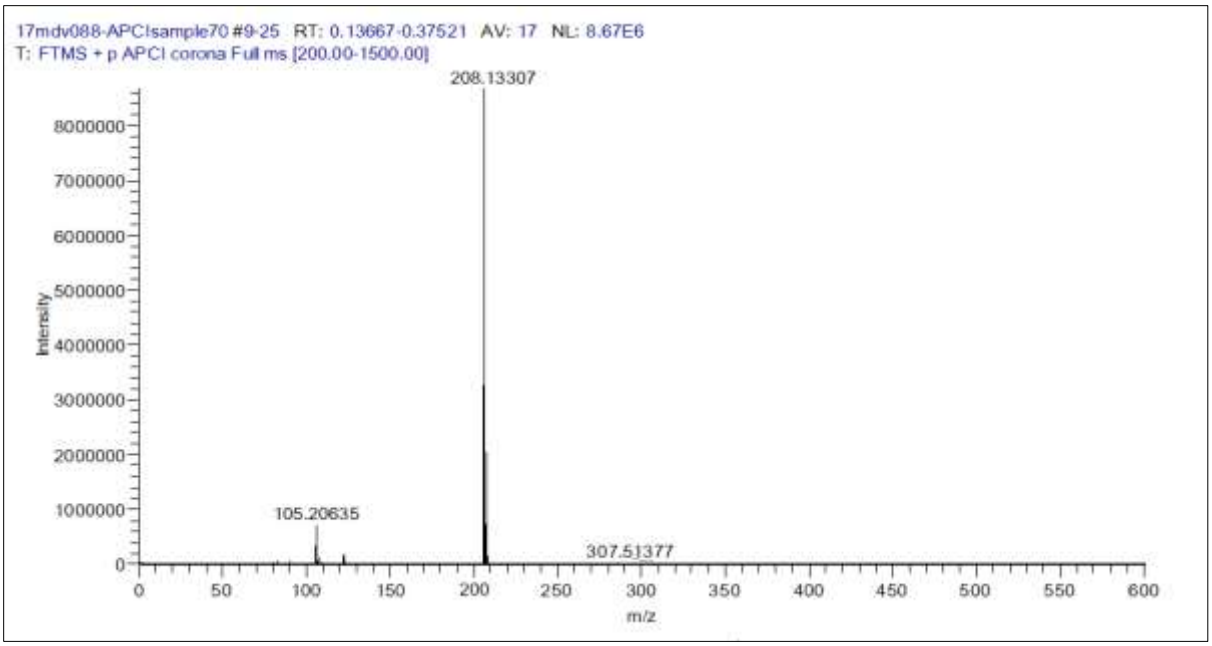


^{1}H NMR of (2*S*,4*R*)-4-(l4-azaneyl)-2-methyl-5-phenylpentanoic acid (Tup,9) (600 MHz, D_2O)

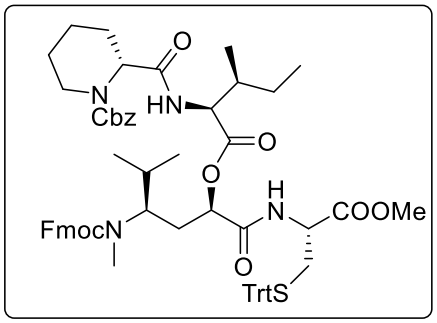


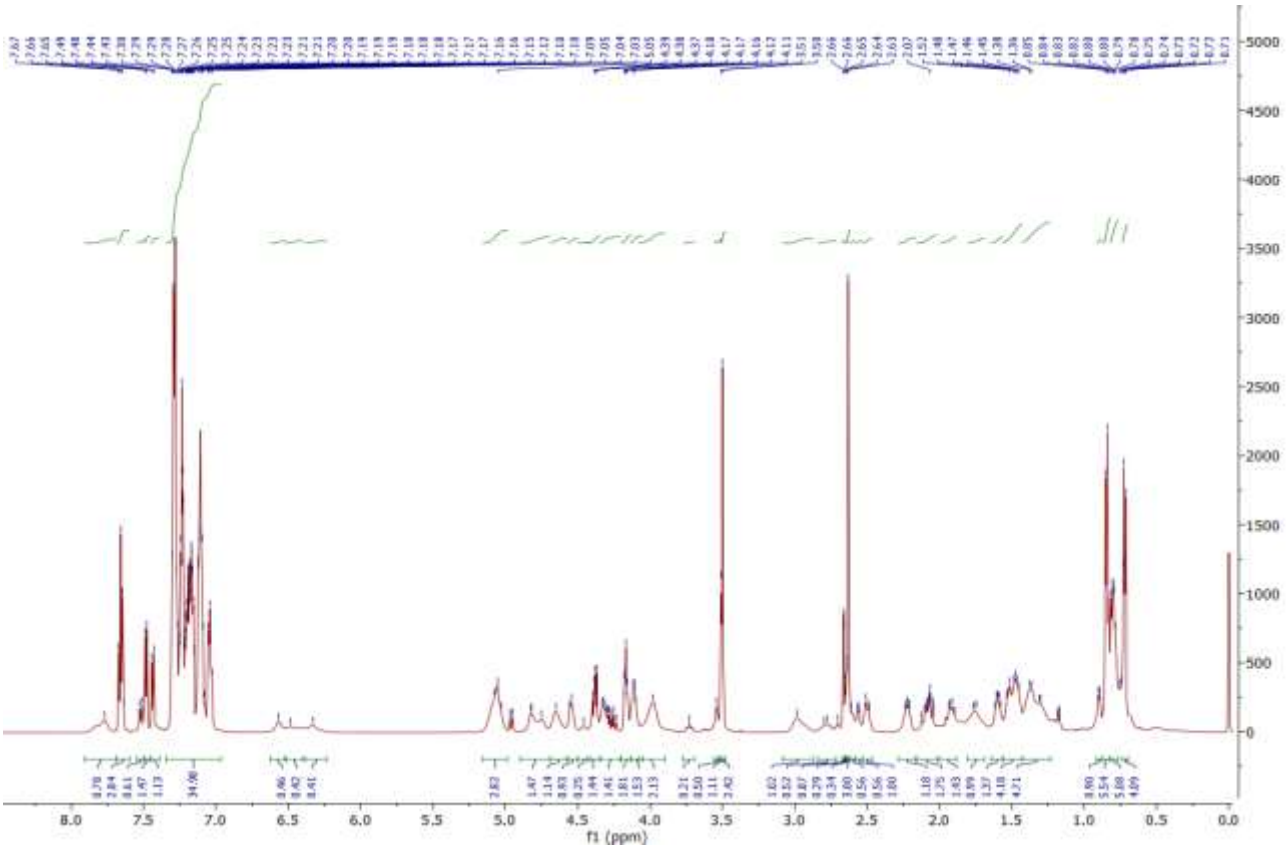
^{13}C NMR of (2*S*,4*R*)-4-(l4-azaneyl)-2-methyl-5-phenylpentanoic acid (Tup,9)

(151 MHz, D_2O)

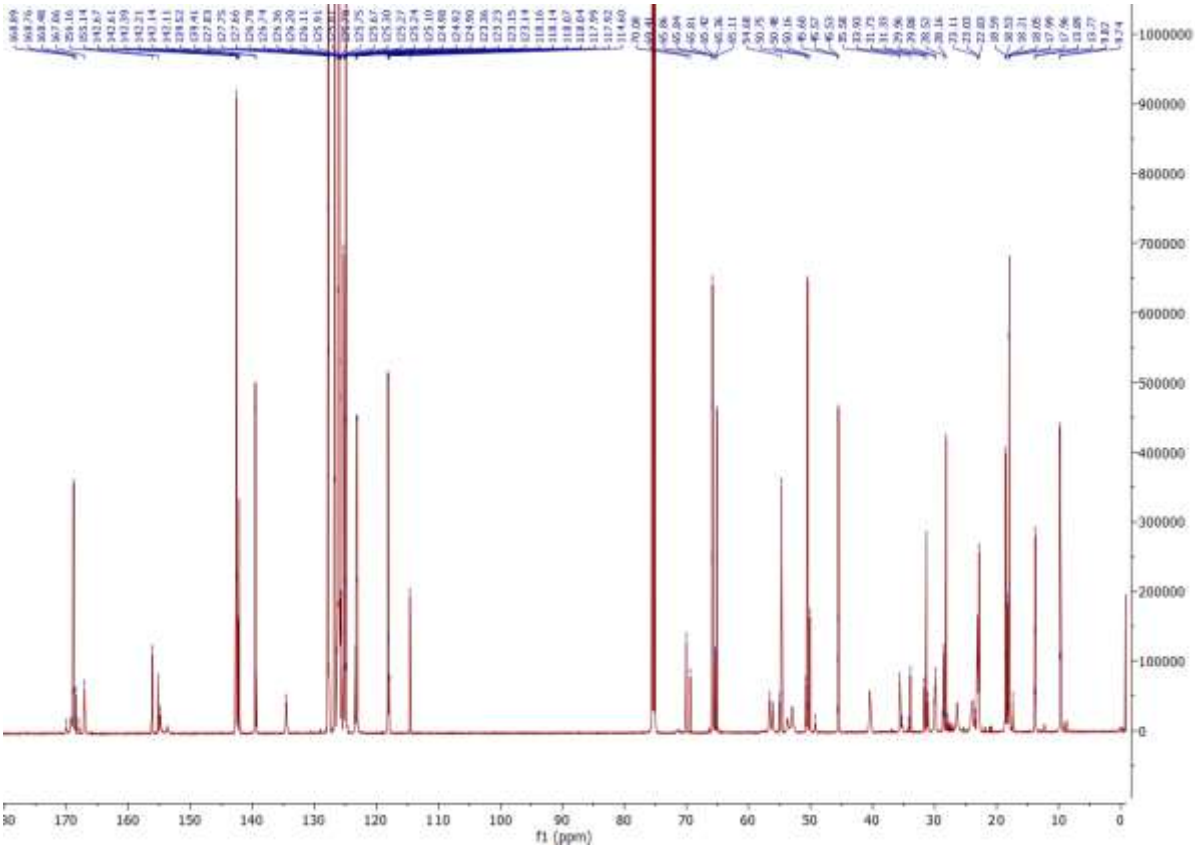


HRMS of (2*S*,4*R*)-4-(l4-azaneyl)-2-methyl-5-phenylpentanoic acid (Tup,9)

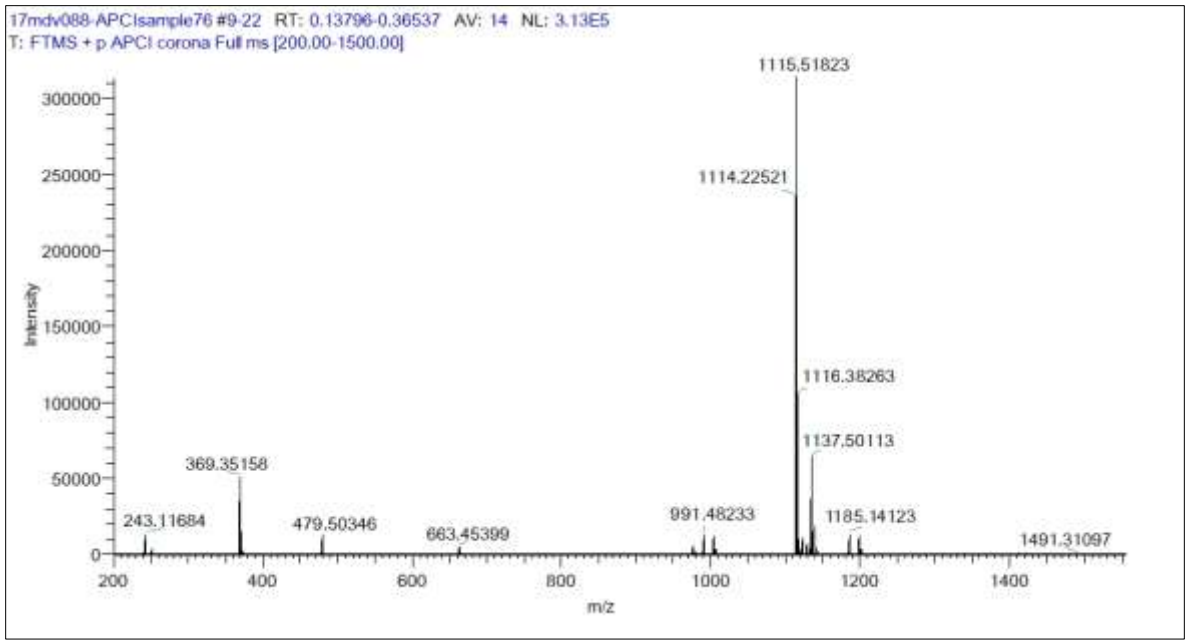




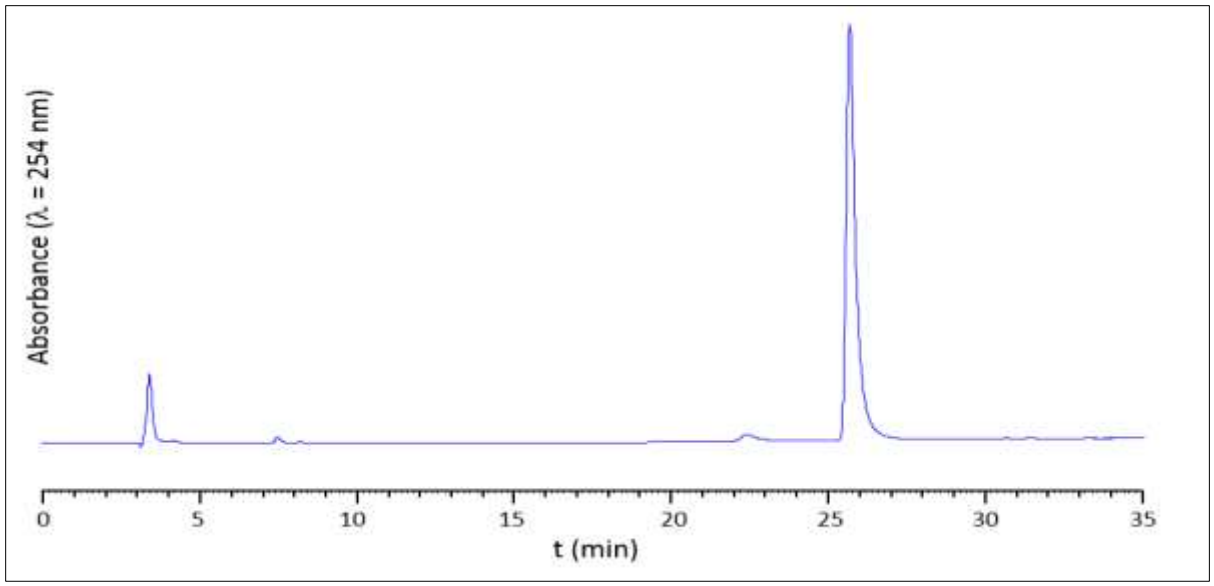
^{1}H NMR of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (5a)
(600 MHz, $CDCl_3$)



^{13}C NMR of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (5a)
(151 MHz, $CDCl_3$)

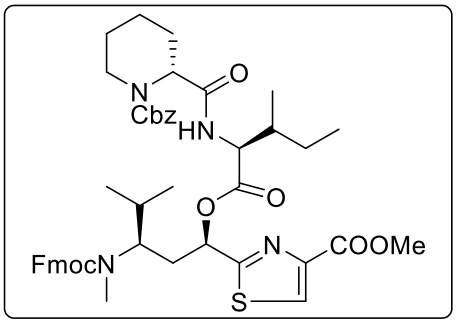


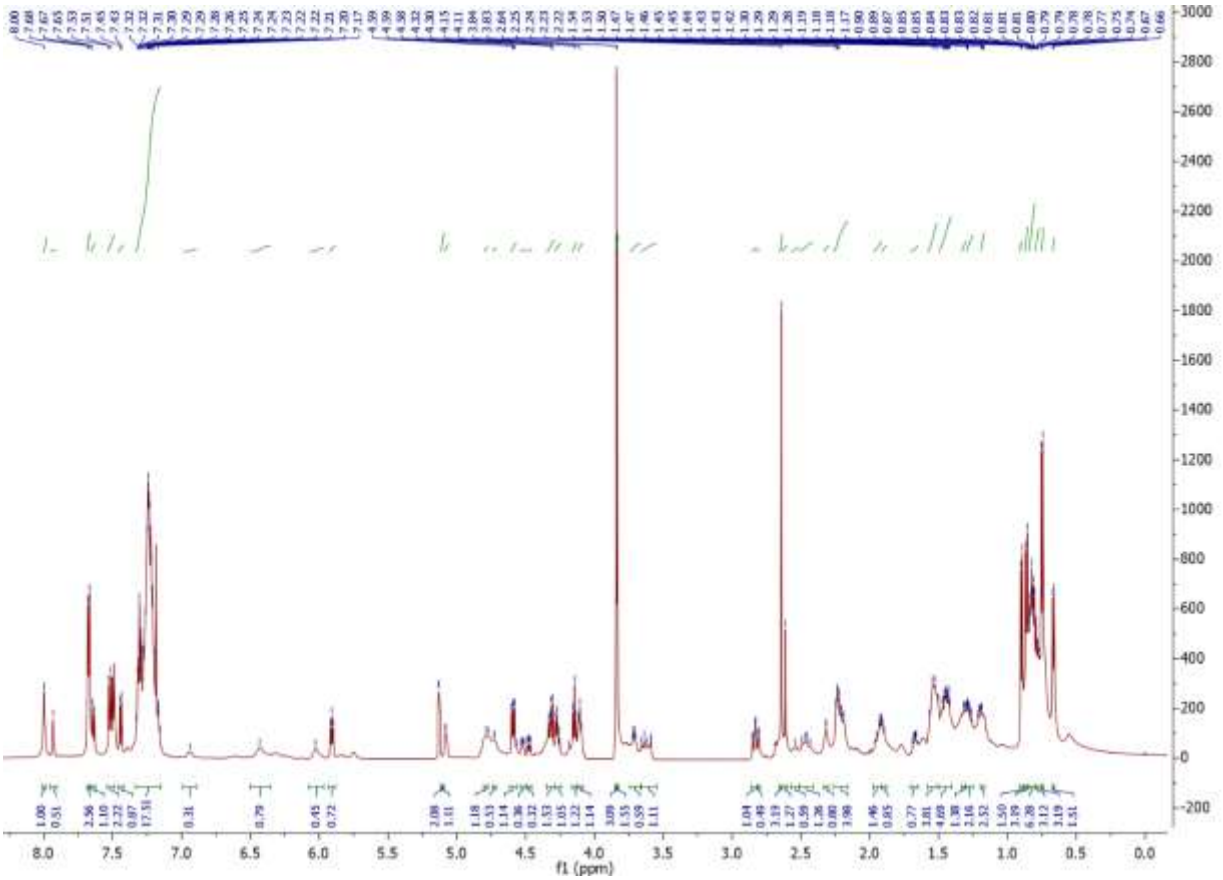
HRMS of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (5a)



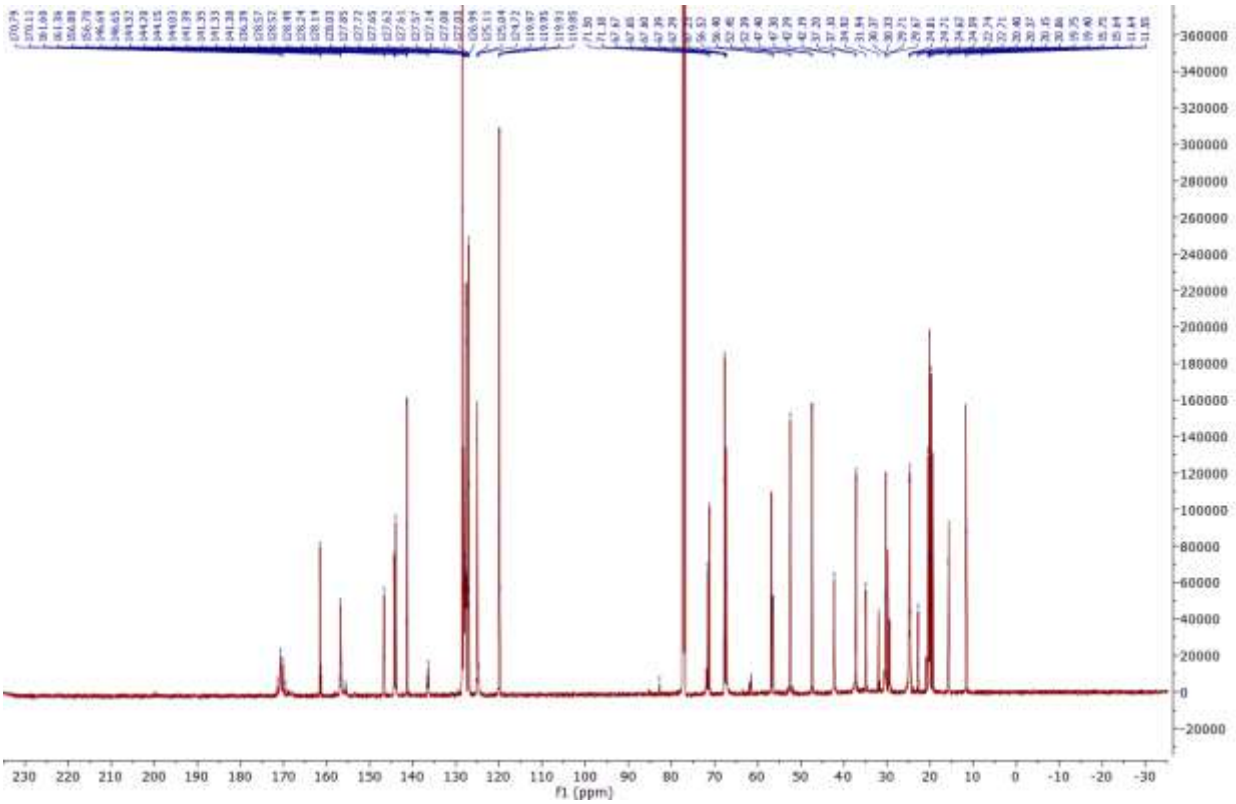
RP-HPLC of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (5a)

Analytical RP-HPLC: (Agilent Zorbax SB C_{18} column: 2.1 x 150 mm, 5 µm, gradient: 50 - 90% CH_3CN/H_2O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), Absorbance: 254 nm, t_R = 25.7 min

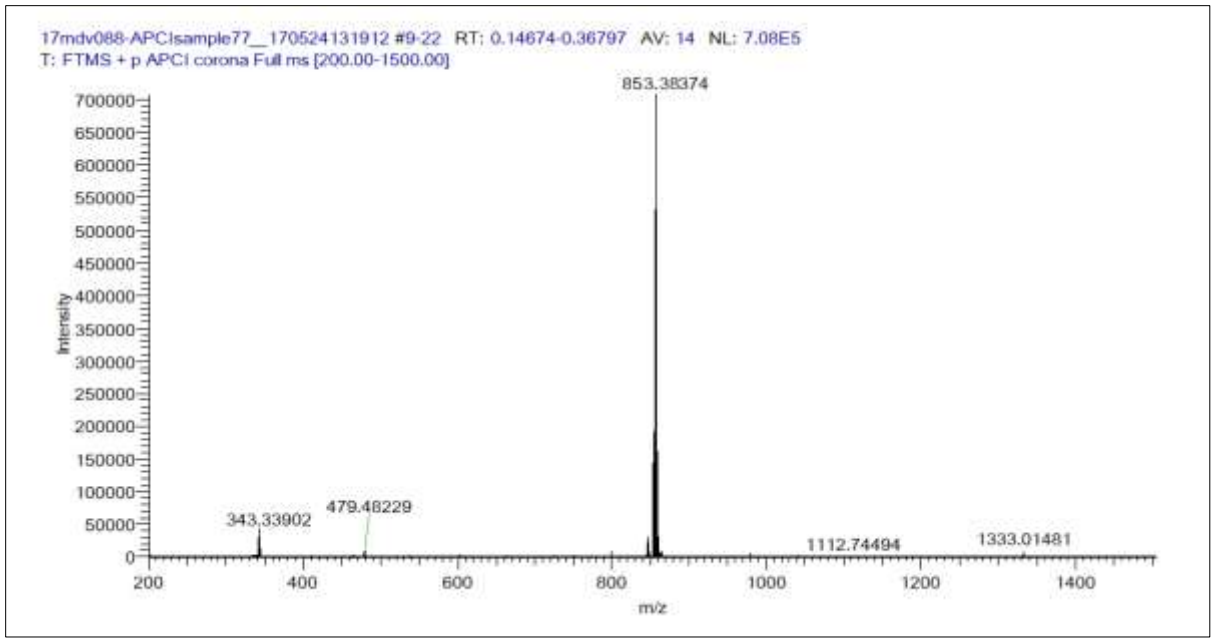




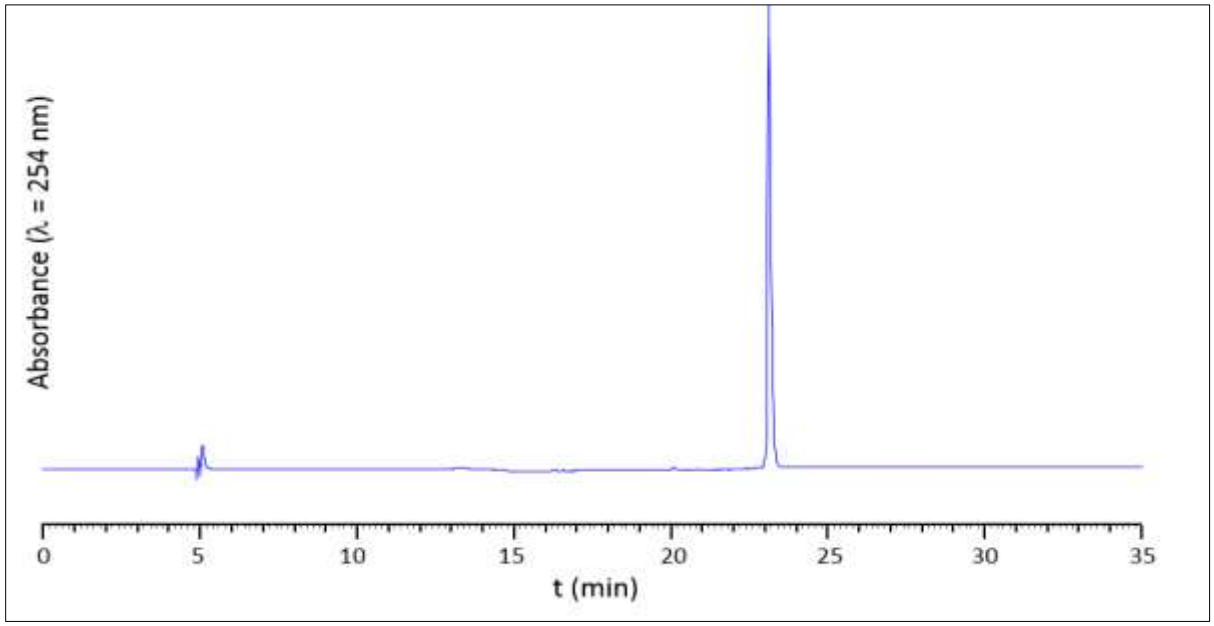
^{1}H NMR of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9H-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxa-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10)
(600 MHz, $CDCl_3$)



^{13}C NMR of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9H-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxa-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10)
(151 MHz, $CDCl_3$)

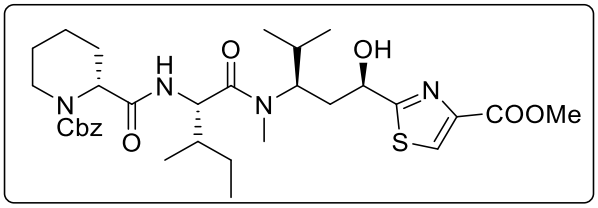


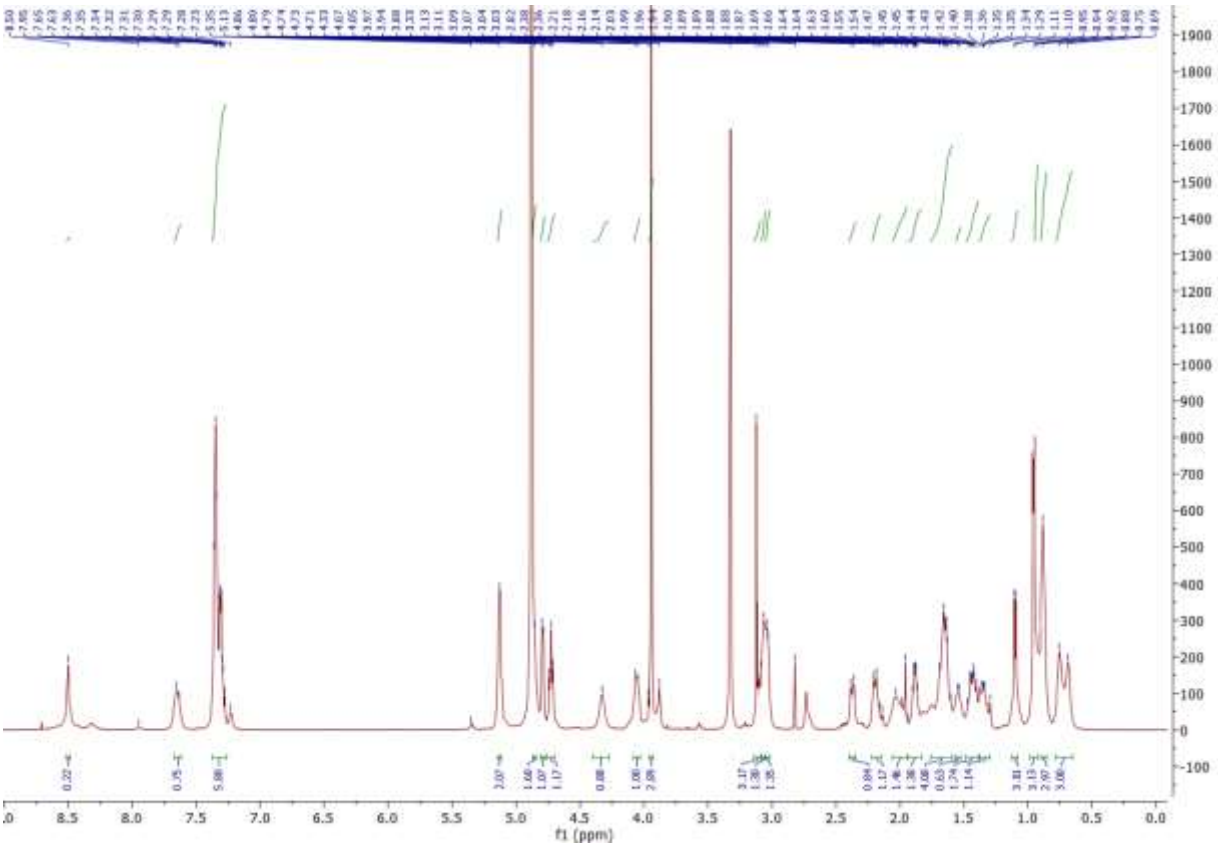
HRMS of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9H-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxa-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10)



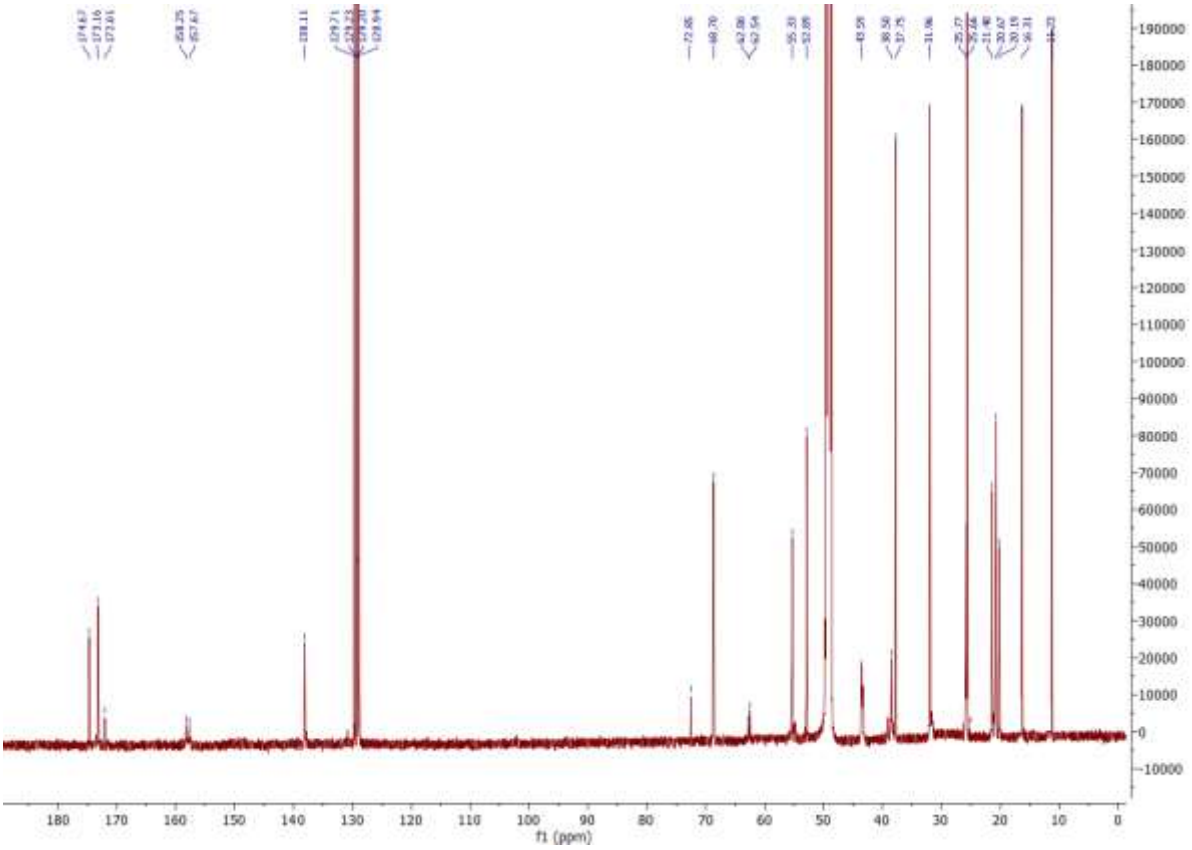
RP-HPLC of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9H-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxa-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10)

Analytical RP-HPLC: (Agilent Zorbax SB C_{18} column: 2.1 x 150 mm, 5 µm, gradient: 50 - 90% CH_3CN/H_2O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), **Absorbance**: 254 nm, t_R = 23.2 min

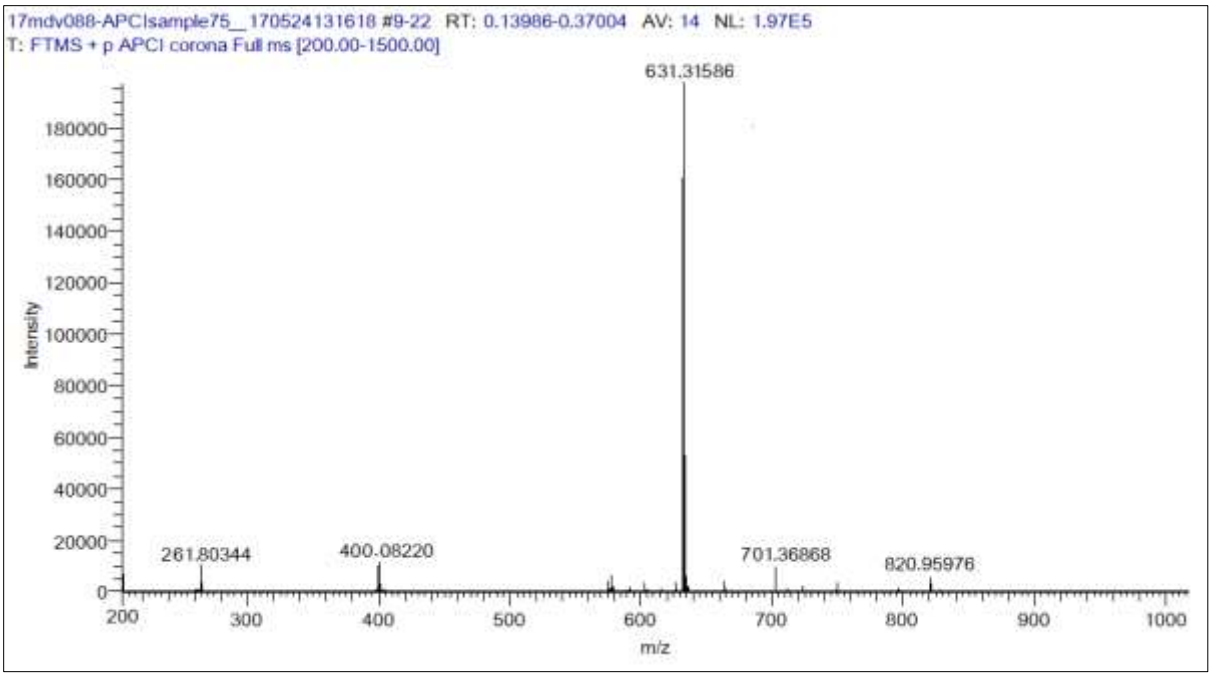




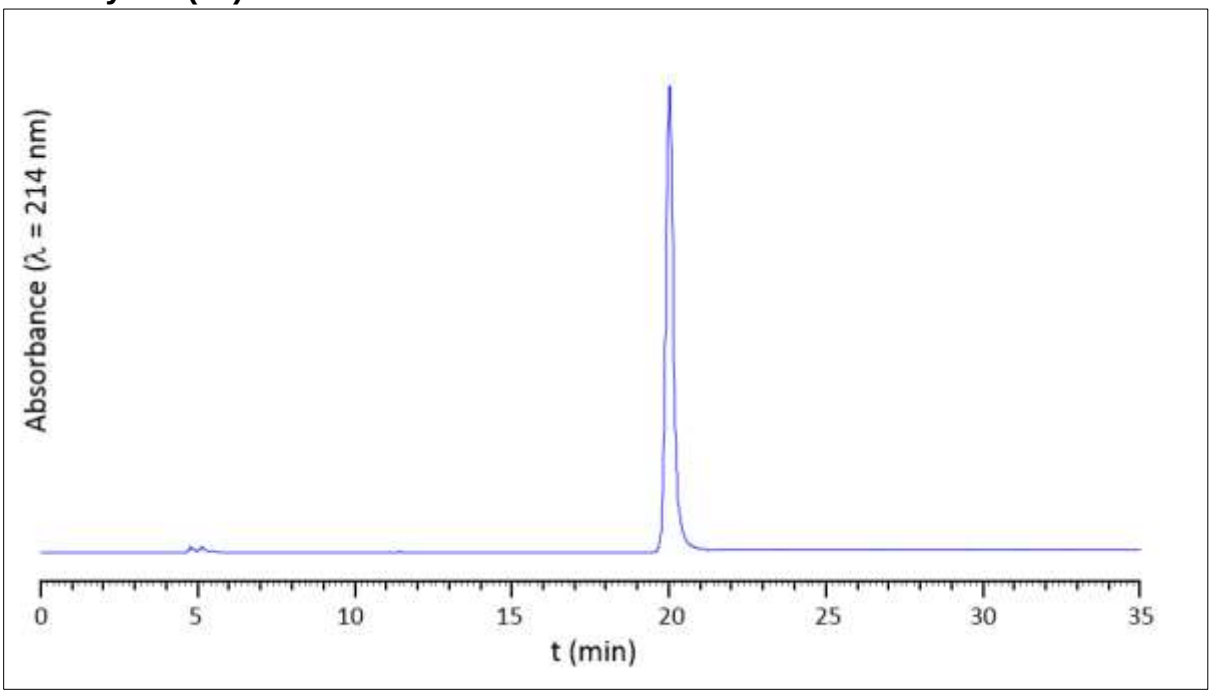
^{1}H NMR of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11)
(600 MHz, d_4-MeOD)



^{13}C NMR of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11)
(151 MHz, d_4-MeOD)



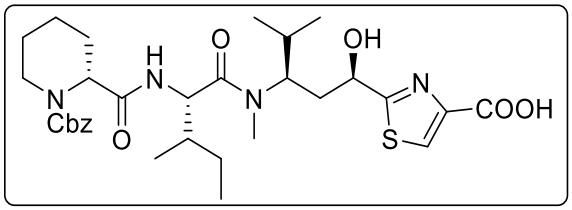
HRMS of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11)

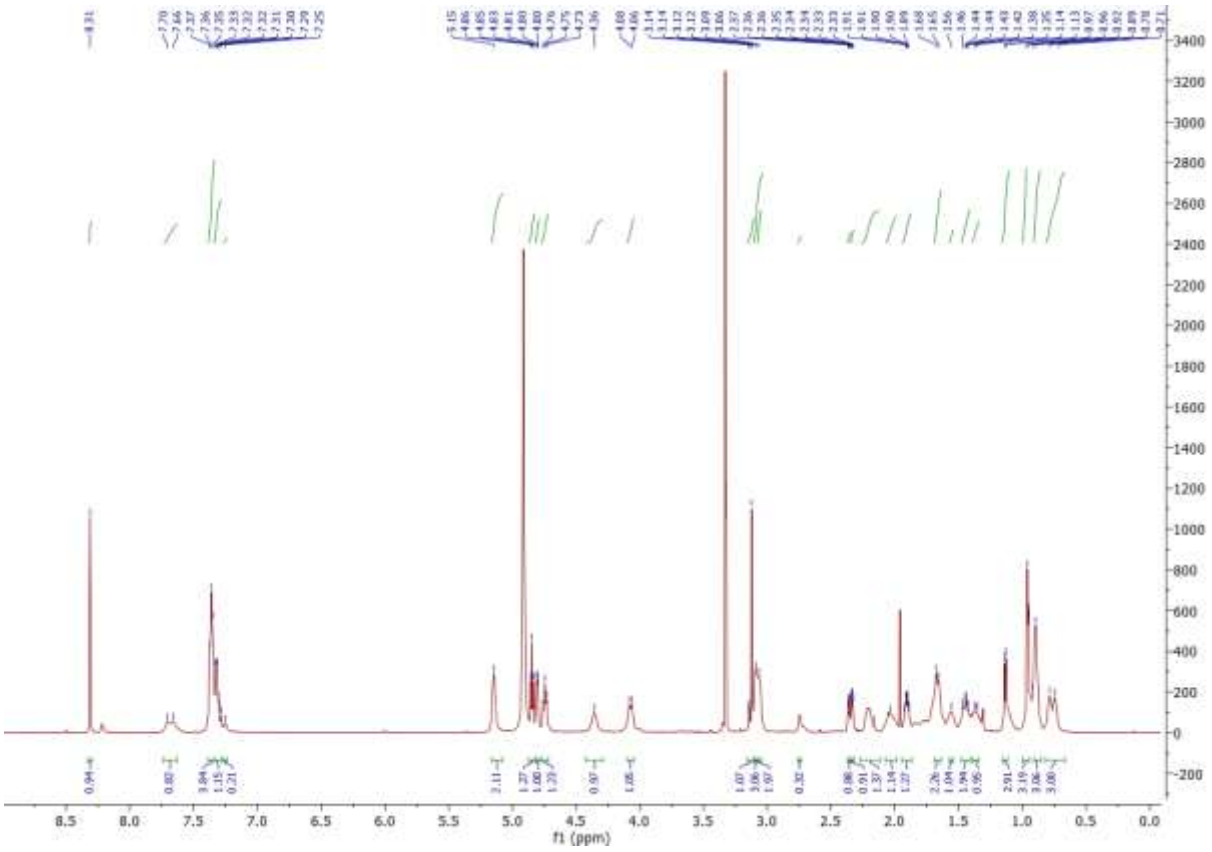


RP-HPLC of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11)

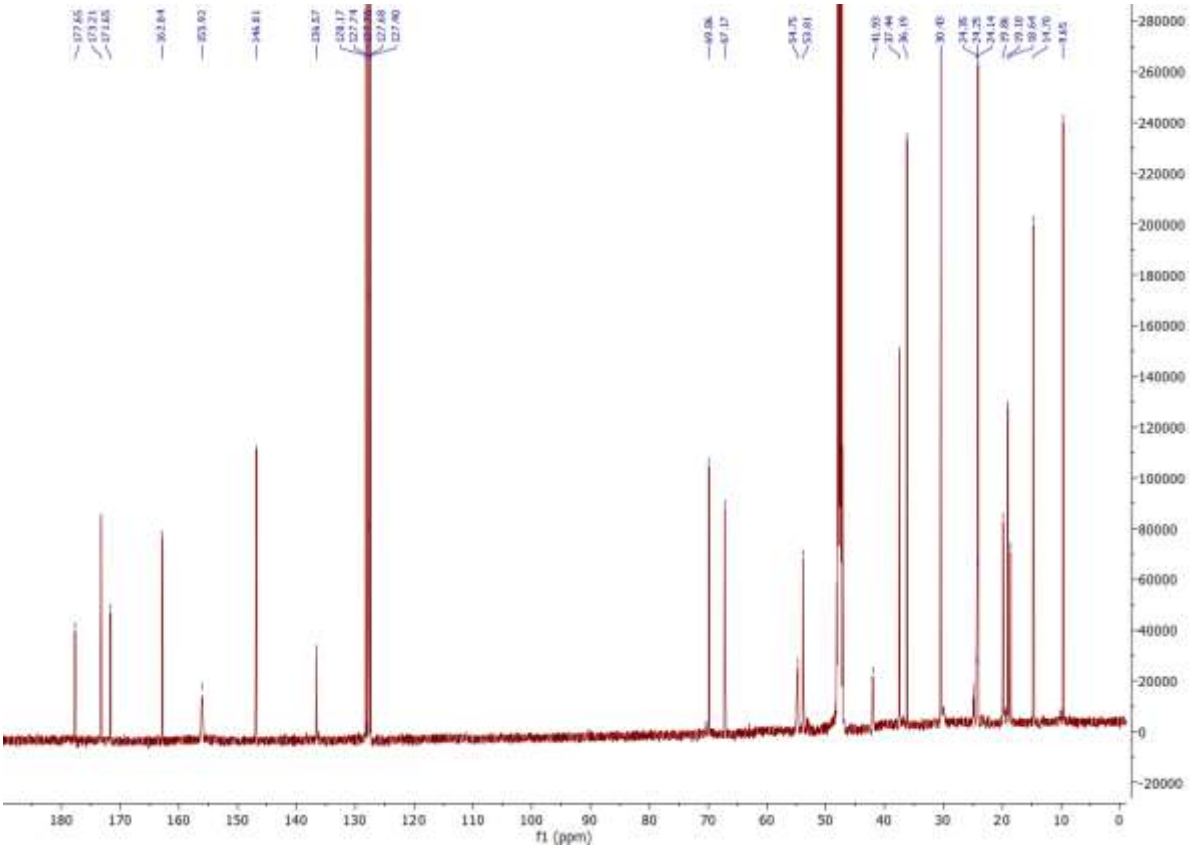
Analytical RP-HPLC: (Agilent Zorbax SB C_{18} column: 2.1 x 150 mm, 5 µm, gradient: 50 – 70 % CH_3CN/H_2O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), **Absorbance**: 214 nm,

t_R = 20.1 min

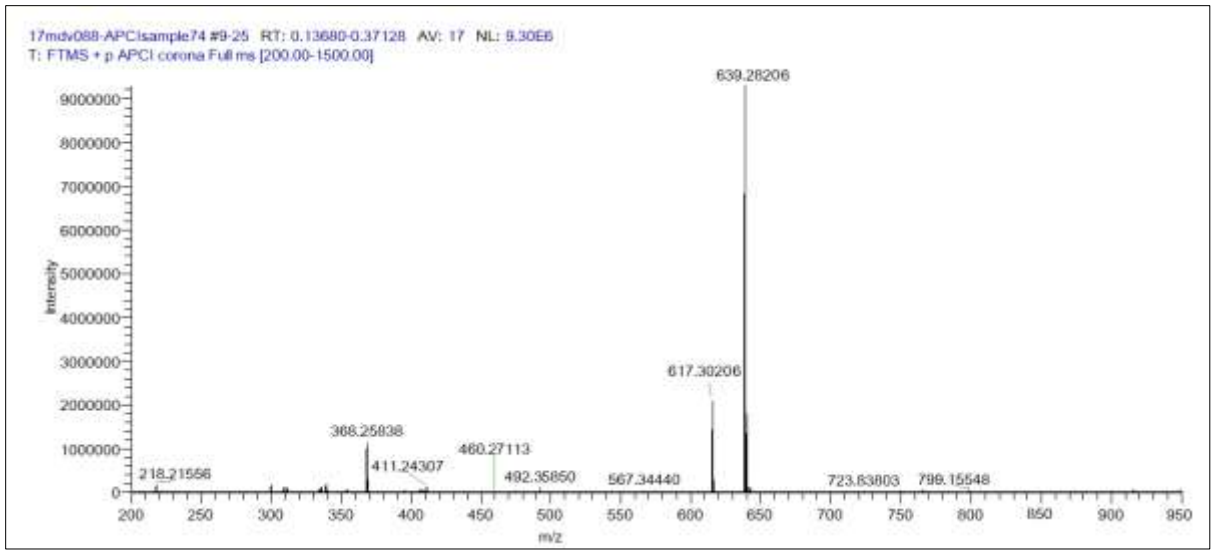




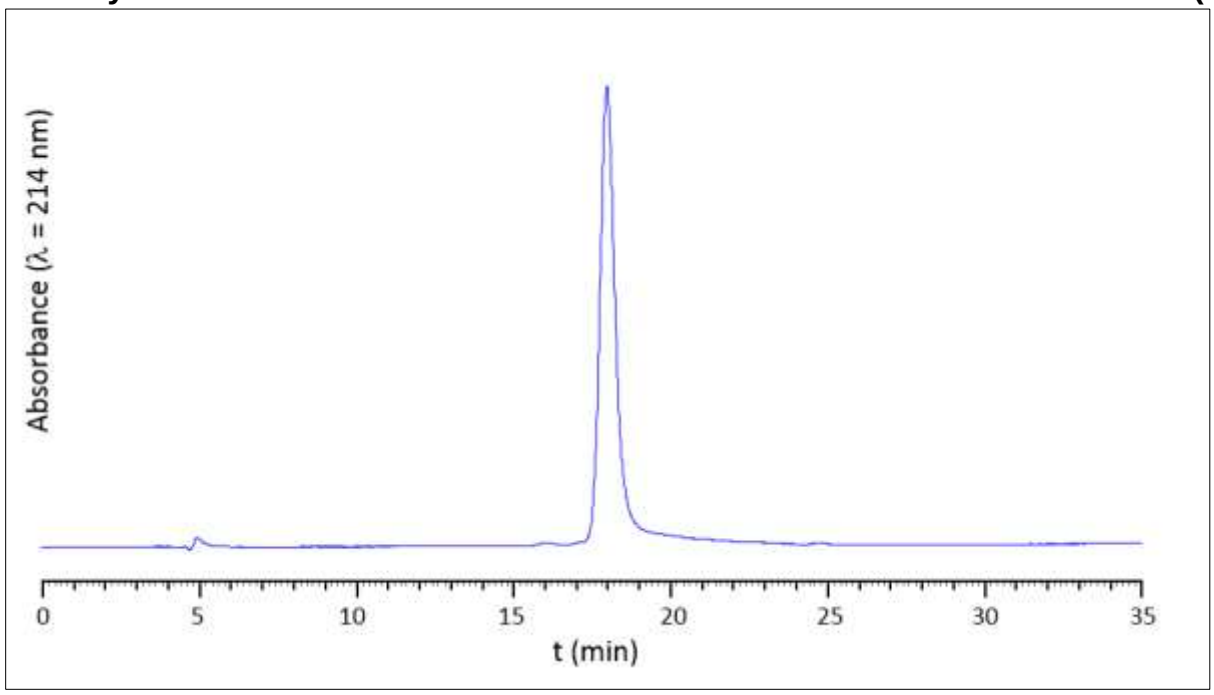
^{1}H NMR of 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylic acid (12)
(600 MHz, *d*$_4$-MeOD)



^{13}C NMR of 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylic acid (12)
(151 MHz, d_4-MeOD)

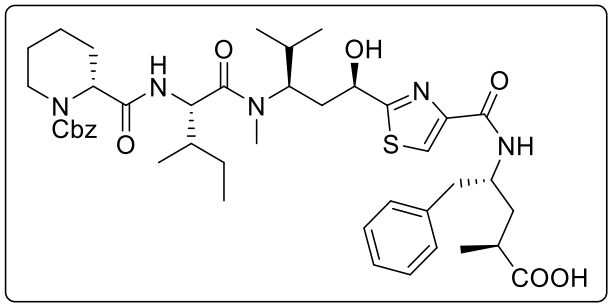


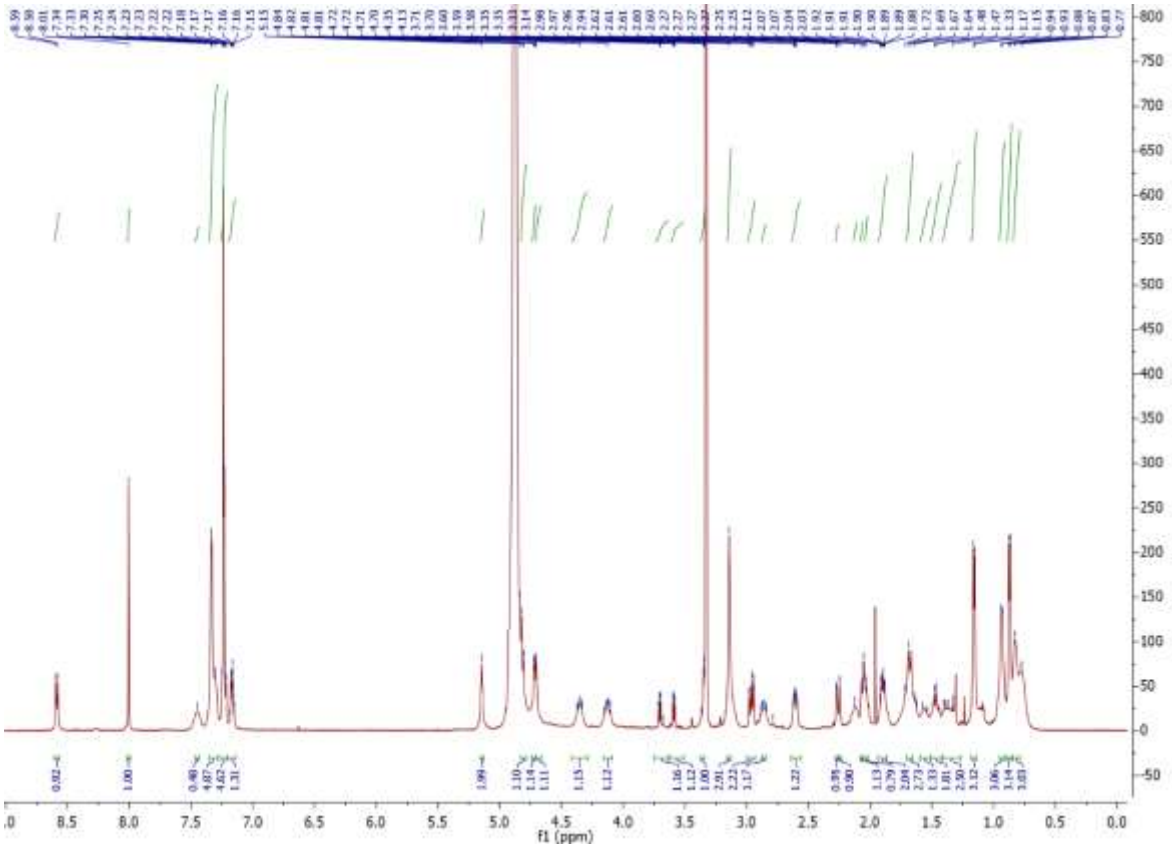
HRMS of 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylic acid (12)



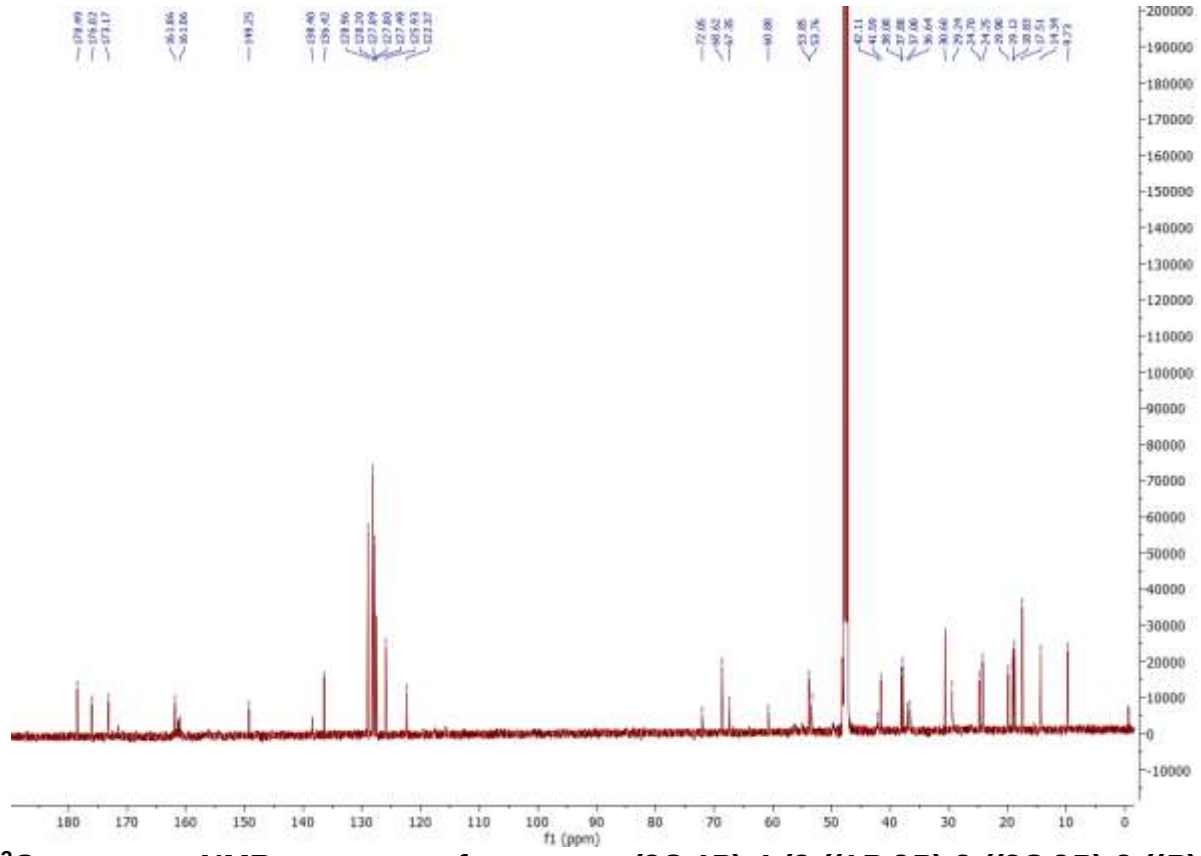
RP-HPLC of 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylic acid (12)

Analytical RP-HPLC: (Agilent Zorbax SB C_{18} column: 2.1 x 150 mm, 5 µm, gradient: 50 - 70% CH_3CN/H_2O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), **Absorbance**: 214 nm,
t_R = 18.0 min

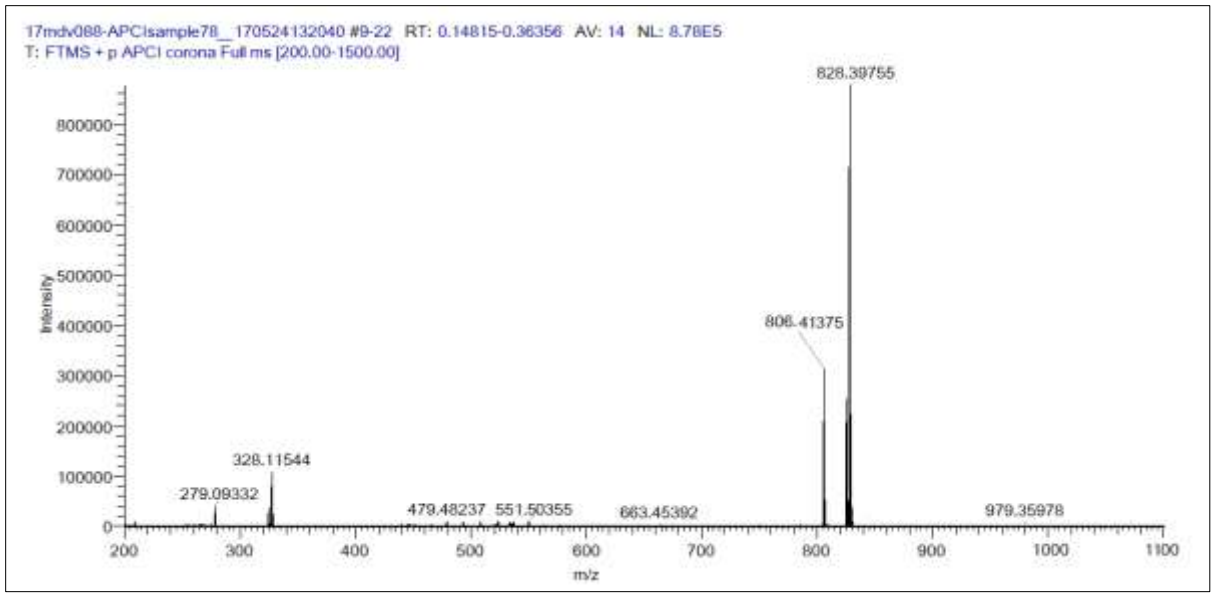




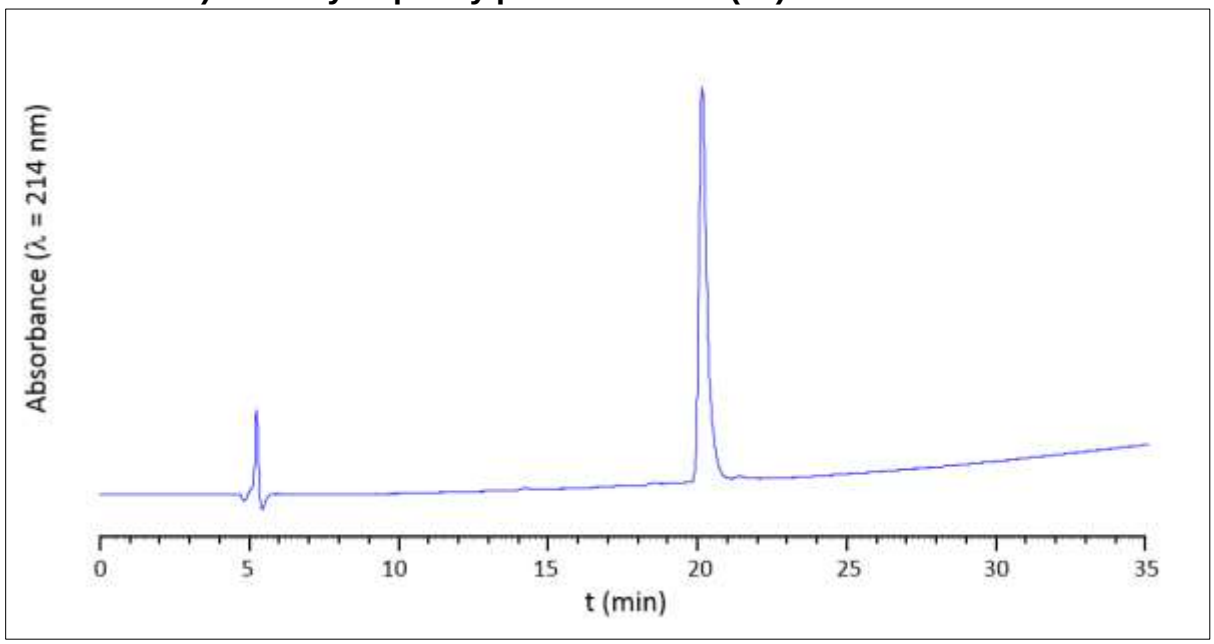
^{1}H NMR of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13)
(600 MHz, d_4-MeOD)



^{13}C NMR of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13)
(151 MHz, *d*$_4$-MeOD)

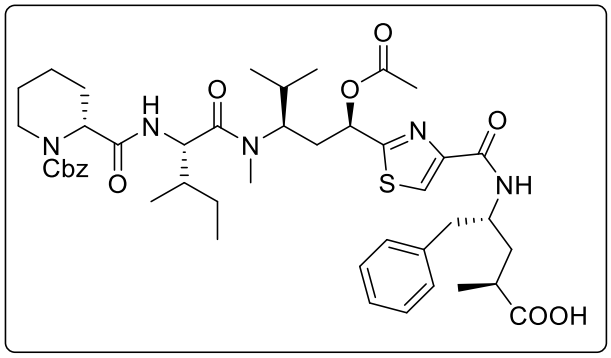


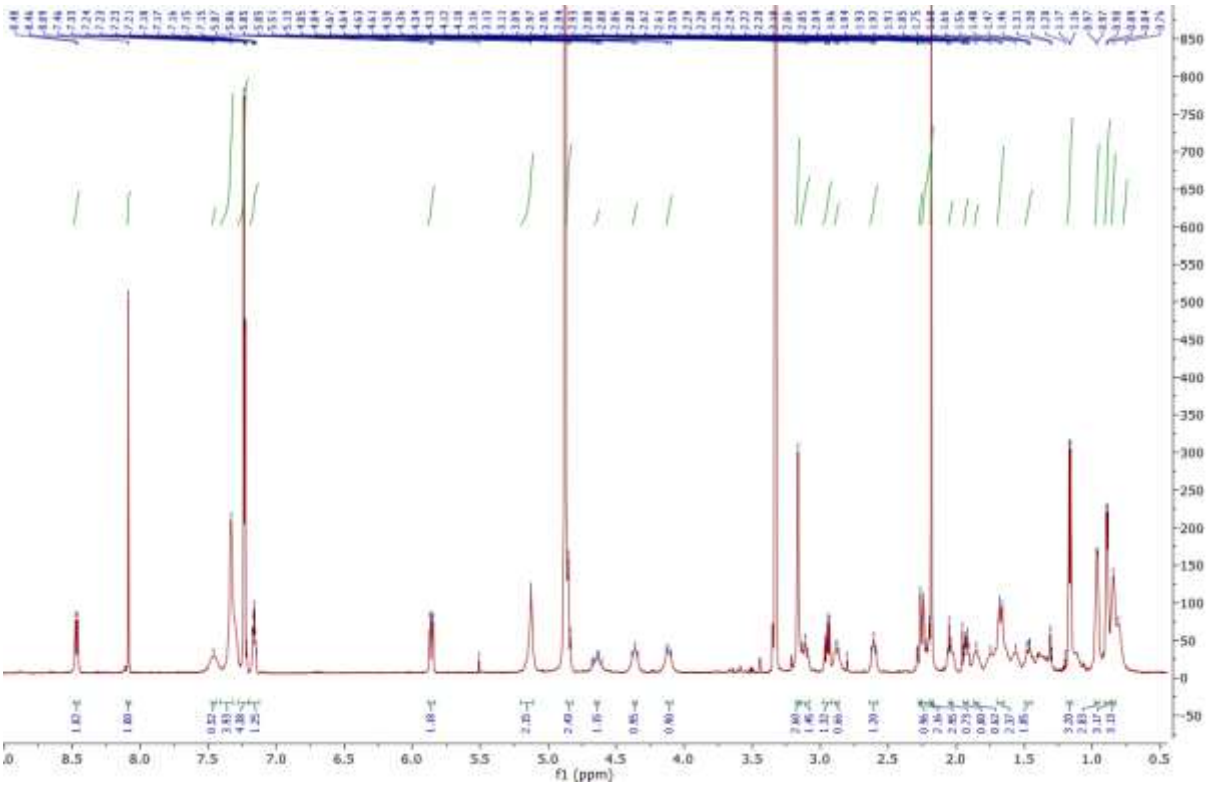
HRMS of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13)



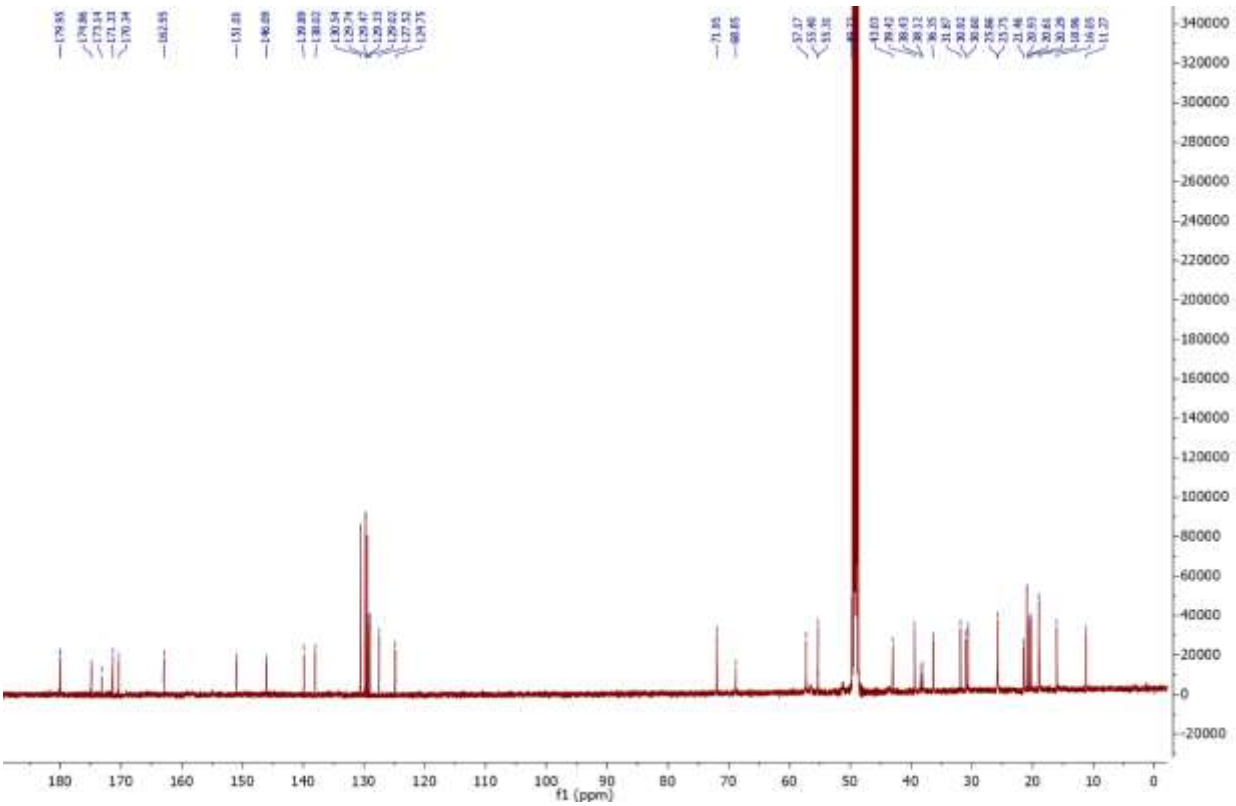
RP-HPLC of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13)

Analytical RP-HPLC: (Agilent Zorbax SB C_{18} column: 2.1 x 150 mm, 5 µm, gradient: 30 – 60 % CH_3CN/H_2O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), Absorbance: 214 nm,
t_R = 20.0 min

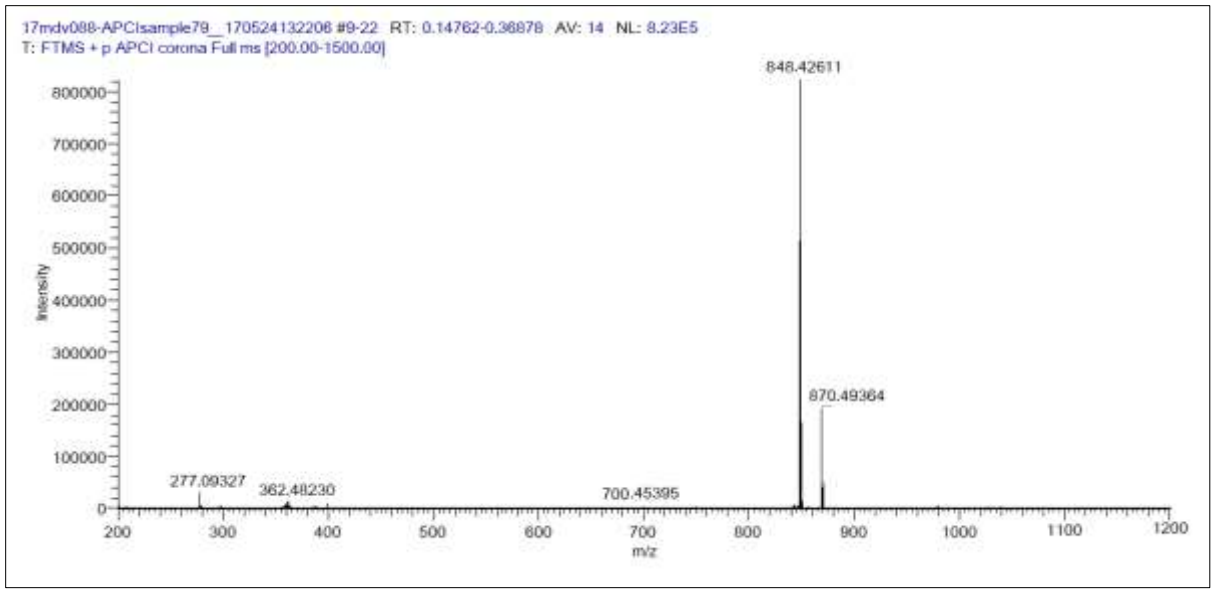




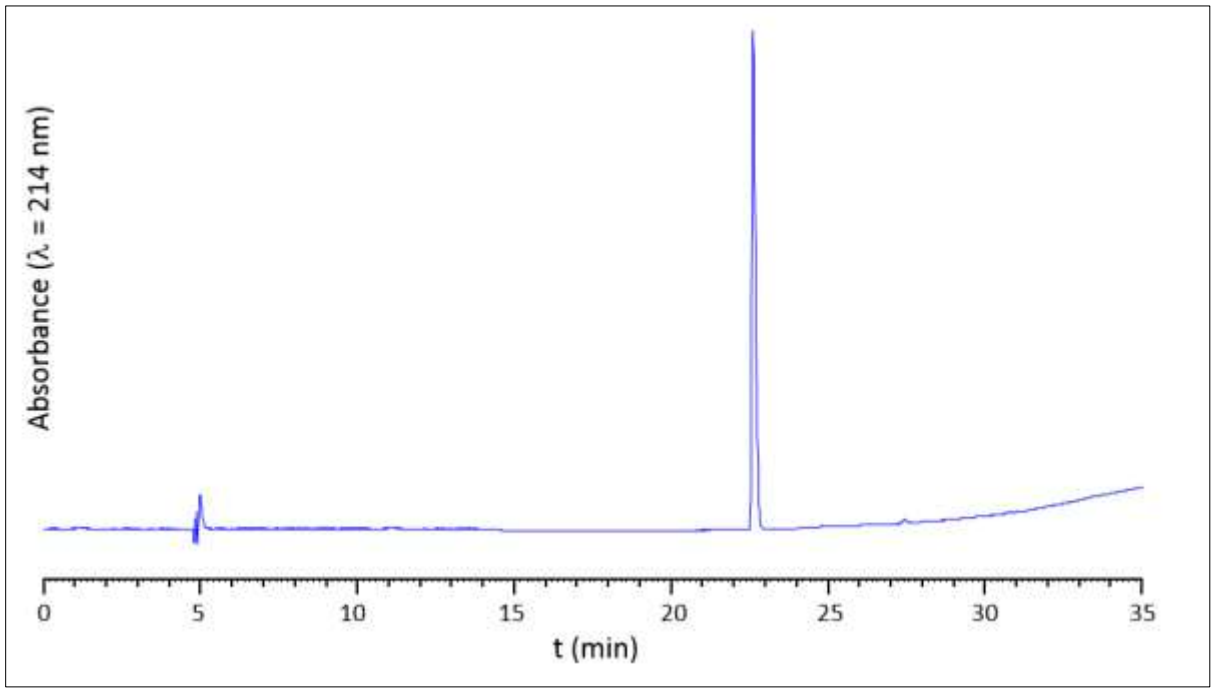
^{1}H NMR of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14) (600 MHz, *d*$_4$-MeOD)



^{13}C NMR of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14) (151 MHz, *d*$_4$-MeOD)

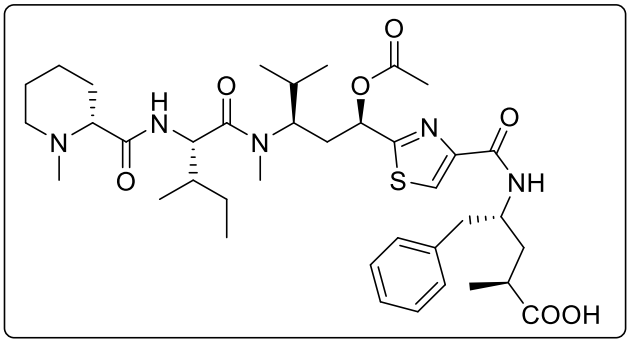


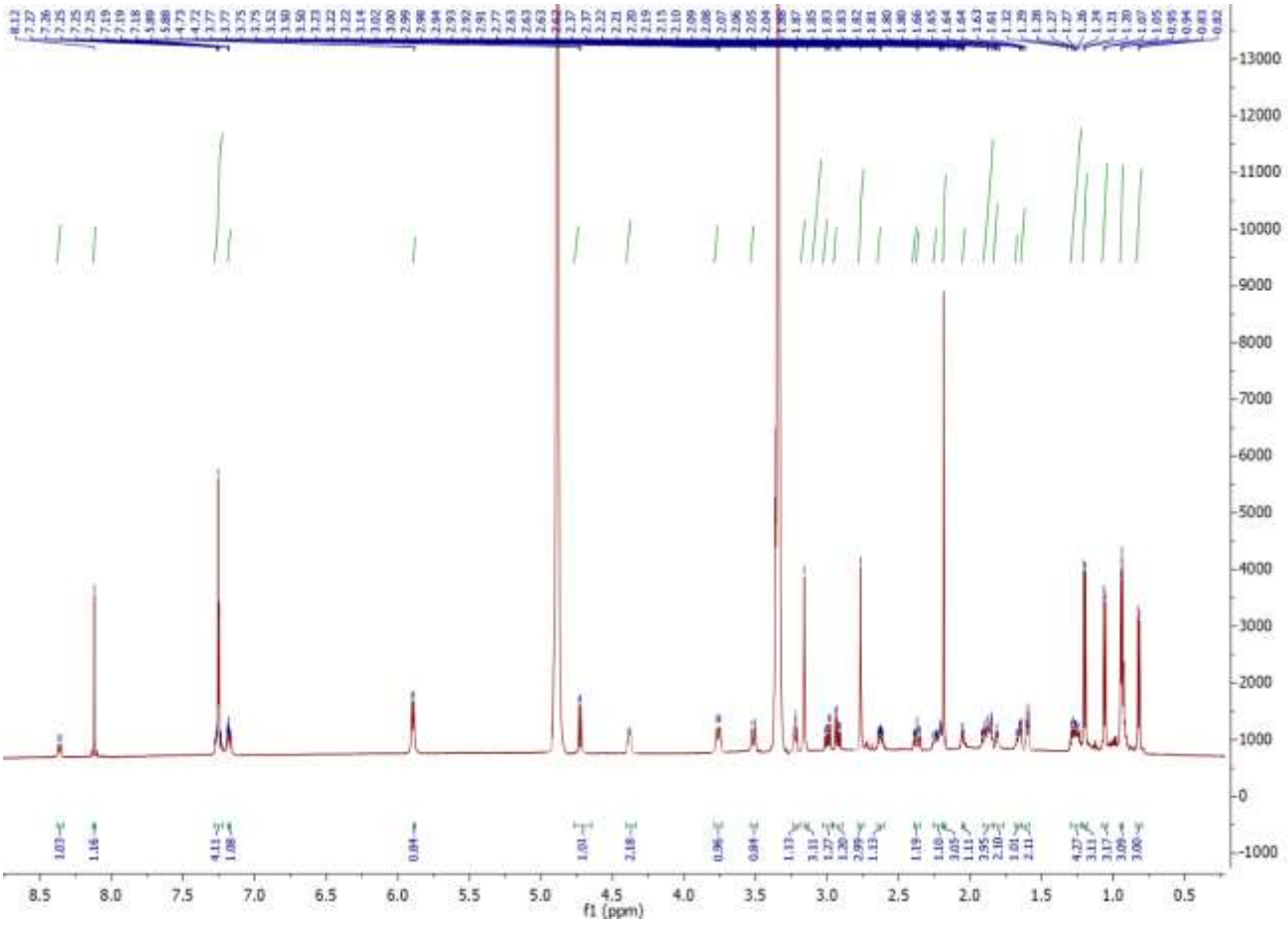
HRMS of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14)



RP-HPLC of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14)

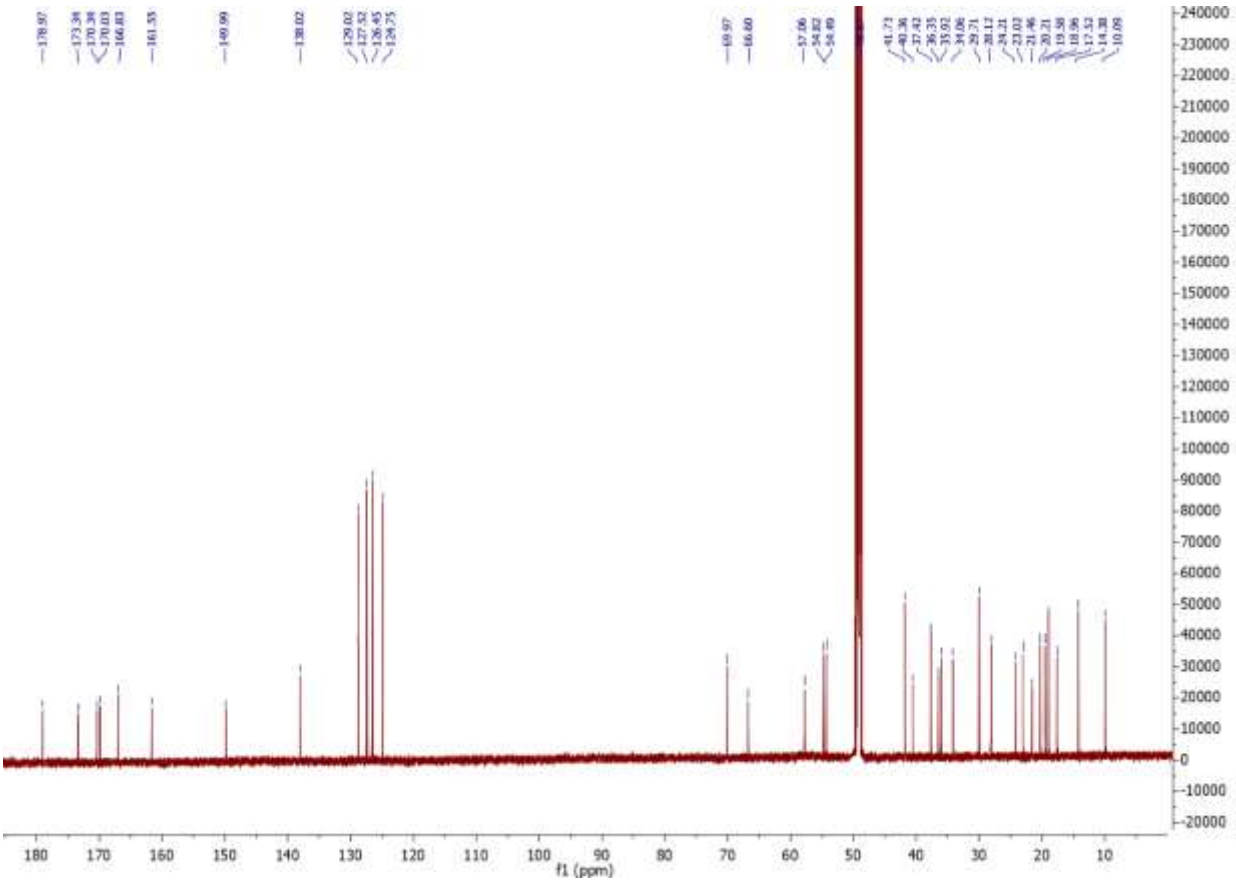
Analytical RP-HPLC: (Agilent Zorbax SB C_{18} column: 2.1 x 150 mm, 5 µm, gradient: 20 – 90 % CH_3CN/H_2O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), **Absorbance**: 214 nm,
t_R = 22.8 min



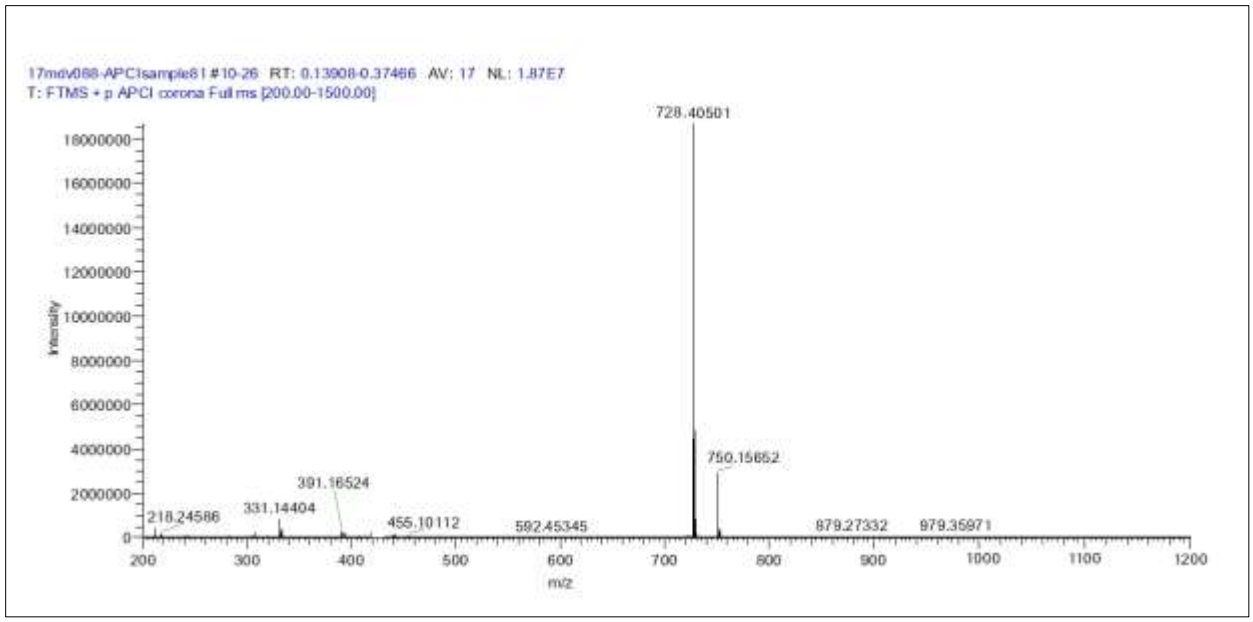


^{1}H NMR of (2S,4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3R)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (1)

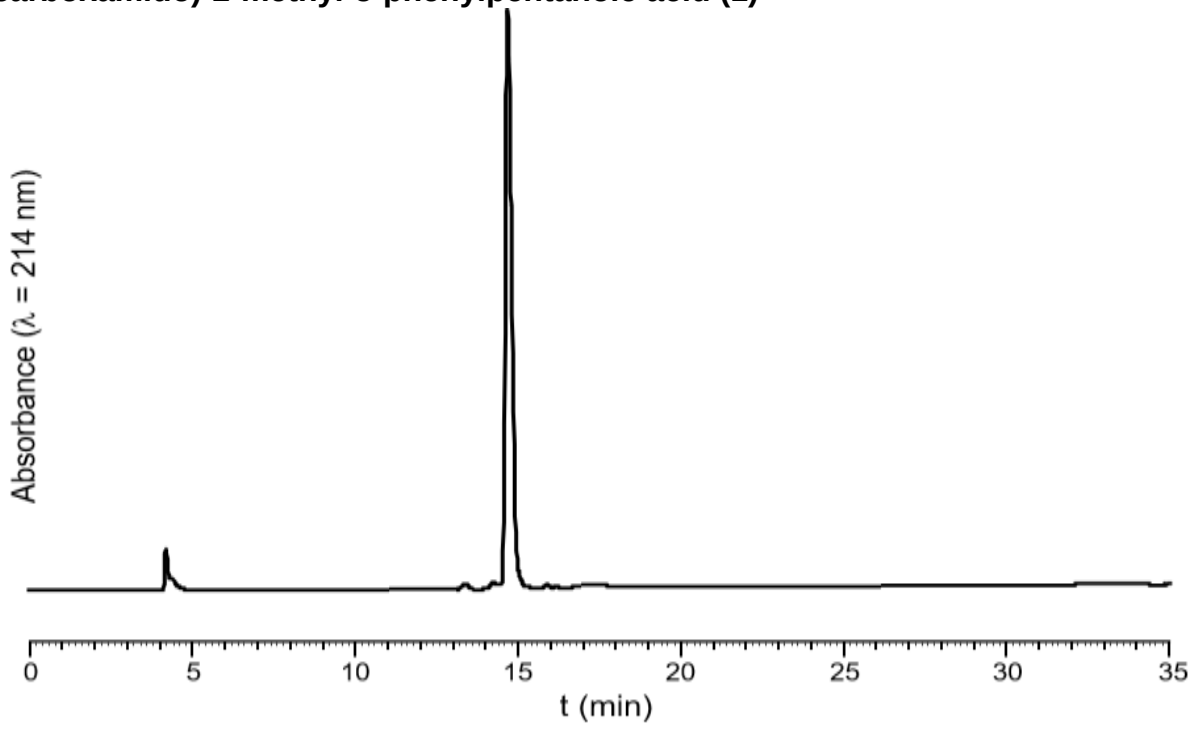
(600 MHz, *d*$_4$-MeOD)



^{13}C NMR of (2S,4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3R)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (1)
(151 MHz, *d*$_4$-MeOD)



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



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

Analytical RP-HPLC: (Agilent Zorbax SB C_{18} column: 2.1 x 150 mm, 5 µm, gradient: 20 - 80% CH_3CN/H_2O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), **Absorbance**: 214 nm, t_R = 14.8 min.

Cell based screening

Dose-dependent inhibition of cell growth by tubulysin in HCT-116 cells - The human colon cancer cell line HCT-116 were maintained at 37'C in a humidified atmosphere with 5% CO2 and after trypsinization plated 1:10 in polystyrene 24well plates (costar). The cells were then transferred to the Incucyte S3 live cell imaging system inside a Panasonic MCO-230AIC incubator at 37'C in a humidified atmosphere with 5% CO2and imaged using a phase contrast with a 20x objective over a period of 4 days. From each well, four areas were analyzed for confluency and the average confluency is plotted below.

Note that TMV inhibition was most prominent in HCT-116 cells, the effect was only seen at sub uM concentrations in HeLa cells and HEK293T cells.