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Rapid and Column-Chromatography-Free Peptide Chain Elongation via a One-Flow, Three-Component Coupling Approach

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General techniques

NMR spectra were recorded on a JEOL-ECS400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) or JEOL-ECZ400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) instrument in the indicated solvent. Chemical shifts were reported in units of parts per million (ppm) relative to tetramethylsilane (0.00 ppm) in $CDCl_3$ or DMSO-d_5 (2.50 ppm) or HDO (4.79 ppm) for ^{1}H NMR and $CDCl_3$ (77.16 ppm) or DMSO-d_6 (39.52 ppm) for ^{13}C NMR. Multiplicities were reported by using the following abbreviations: s; singlet, d; doublet, t; triplet, q; quartet, m; multiplet, br; broad, *J*; coupling constants in Hertz (Hz). IR spectra were recorded on a JASCO FT/IR-4100 Fourier Transform Infrared Spectrophotometer. Only the strongest and/or structurally important peaks were reported as the IR data given in cm^{-1}. Optical rotations were measured with a JASCO P-2200. High resolution mass spectra (HRMS) were obtained on a Bruker Daltonics Compact in electrospray ionization (ESI) method. Column chromatography was performed on Silica Gel PSQ 60B purchased from Fuji Silysia Chemical LTD. Automated column chromatography was performed using a Biotage-Isolera Flash Purification System with Biotage SNAP Cartridge. Analytical HPLC was carried out using a JASCO PU-4580 HPLC pump system with a JASCO MD-2018 PDA Detector, a Shimadzu CTO-20A Column Oven or a JASCO C0-4060 Column Oven, a JASCO LG-4580 Quaternary Gradient Unit, a JASCO DG-4580 Degassing Unit, a JASCO AS-4550 Autosampler, and a JASCO LC-NetII/ADC Interface Box. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) with UV light, visualized by 5% ethanolic *p*-anisaldehyde solution or 10% ethanolic phosphomolybdic acid.

Micro-flow reactor set-up

Stainless steel V-shaped and T-shaped mixers (**Figures S1-6**) were purchased from Sanko Seiki Co. Ltd. (inner diameter: 0.25 mm). Teflon® tubes (inner diameter: 0.80 or 0.25 mm) were purchased from Senshu Scientific Co., Ltd. PEEK fittings, PEEK unions, stainless steel tubes, stainless steel fittings, stainless steel unions (inner diameter: 0.80 mm) and back pressure regulator (40 psi) were purchased from GL Science Inc. Solutions were injected into a micro-flow system with syringe pumps (Harvard PHD ULTRA) equipped gastight syringes (SGE 10 mL). The gastight syringes and the Teflon tubes were connected with joints purchased from Flon Industry Co., Ltd.

The employed micro-flow system for synthesis of α-amino acid *N*-carboxy anhydrides **1** was shown in **Figure S1**. The gastight syringes and V-shaped and T-shaped mixers were connected with the Teflon tubes. The V-shaped mixer was connected with the reaction tube 1 (Teflon tube). The T-shaped mixer was connected with the reaction tube 2 (Teflon tube). The mixers and reaction tubes were immersed in a water bath.

The employed micro-flow system for synthesis of amino acid derivatives **2** and **S1** was shown in **Figure S2**. The gastight syringes and T-shaped mixer were connected with the Teflon tubes and stainless steel tubes. The T-shaped mixers were connected with the reaction tubes 1 and 2 (Teflon tube). The mixers and reaction tubes were immersed in water bath.

The employed micro-flow system for synthesis of tripeptides **7** was shown in **Figure S3**. The gastight syringes and T-shaped mixer were connected with the Teflon tubes and/or stainless steel tubes. The T-shaped

mixers were connected with the reaction tube 1-4 (Teflon tube). The mixers and reaction tubes were immersed in water bath.

The employed micro-flow system for synthesis of Cbz-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (**7r**), Fmoc-L-Phe-L-Phe-L-MePhe-OMe (**7q**), protected penta-, hepta-peptide **8a** and **9a** was shown in **Figure S4**. The gastight syringes and T-shaped mixer were connected with the Teflon tubes. The T-shaped mixers were connected with the reaction tube 1-3 (Teflon tube). The mixers and reaction tubes were immersed in water bath.

The employed micro-flow system for synthesis of protected beefy meaty peptide (**10a**) and H-L-Phe-NCA (**1a**) was shown in **Figure S5**. The gastight syringes and T-shaped mixer were connected with the Teflon tubes and stainless steel tubes. The T-shaped mixer was connected with the reaction tube 1 (Teflon tube). The mixers and reaction tubes were immersed in water bath.

The employed micro-flow system for one-flow synthesis of Fmoc-L-Phe-L-Phe-L-Ala-O*t*-Bu (**7a**) was shown in **Figure S6**. The gastight syringes and T-shaped mixer were connected with the Teflon tubes and/or stainless steel tubes. The T-shaped mixers were connected with the reaction tubes 1-5 (Teflon tube). The mixers and reaction tubes were immersed in water bath.

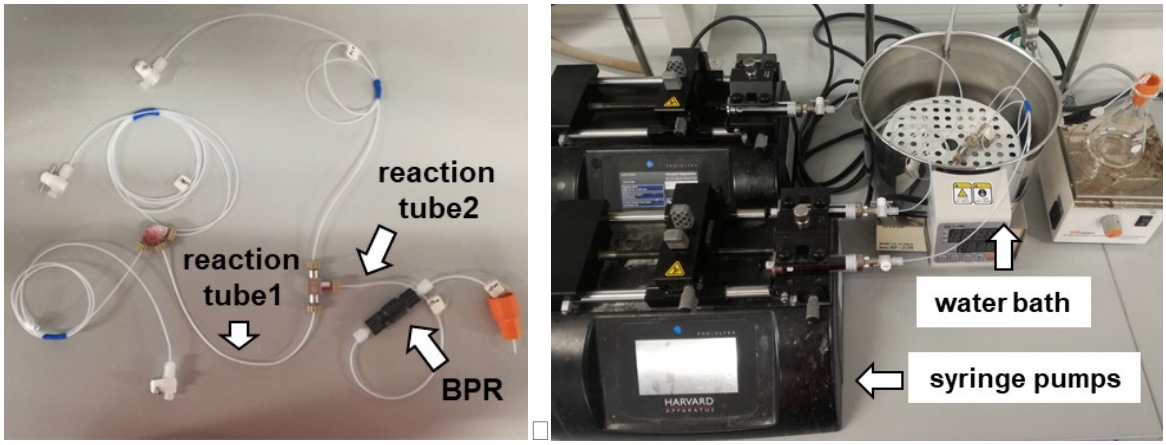


Figure S1. Micro-flow reactor set-up for synthesis of α-NCAs **1**

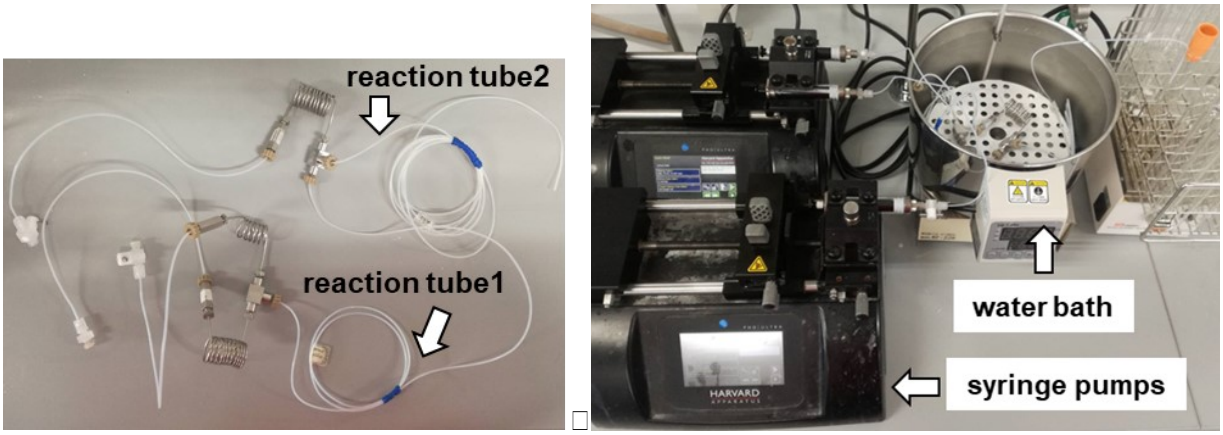


Figure S2. Micro-flow reactor set-up for synthesis of amino acid derivatives **2** and **S1**

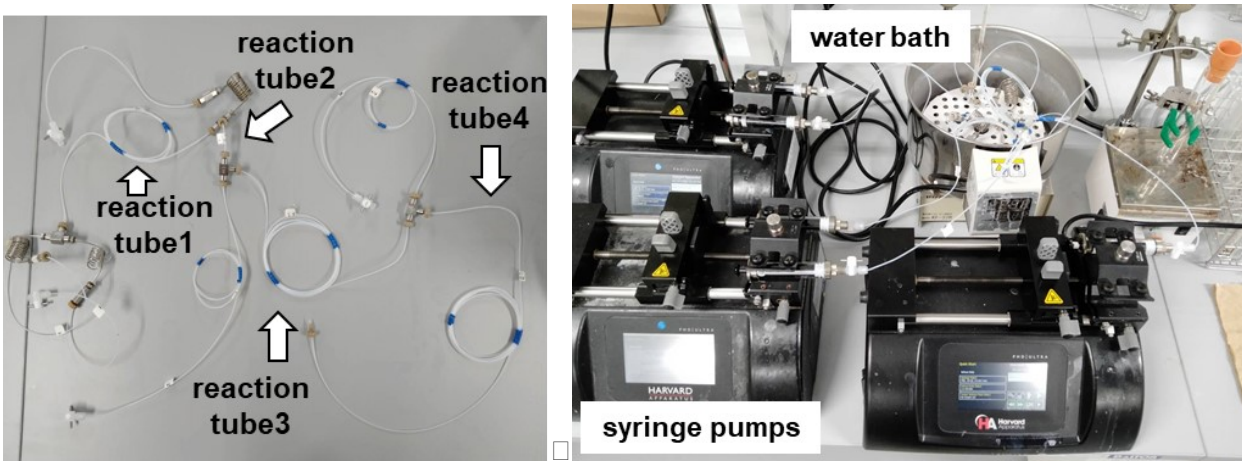


Figure S3. Micro-flow reactor set-up for synthesis of tripeptides **7**

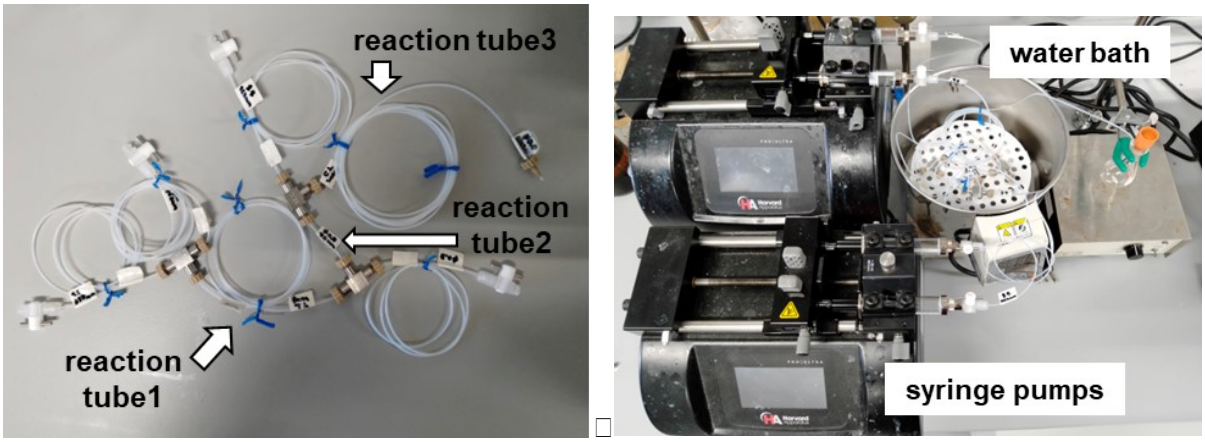


Figure S4. Micro-flow reactor set-up for synthesis of **7r**, **7q**, **8a** and **9a**

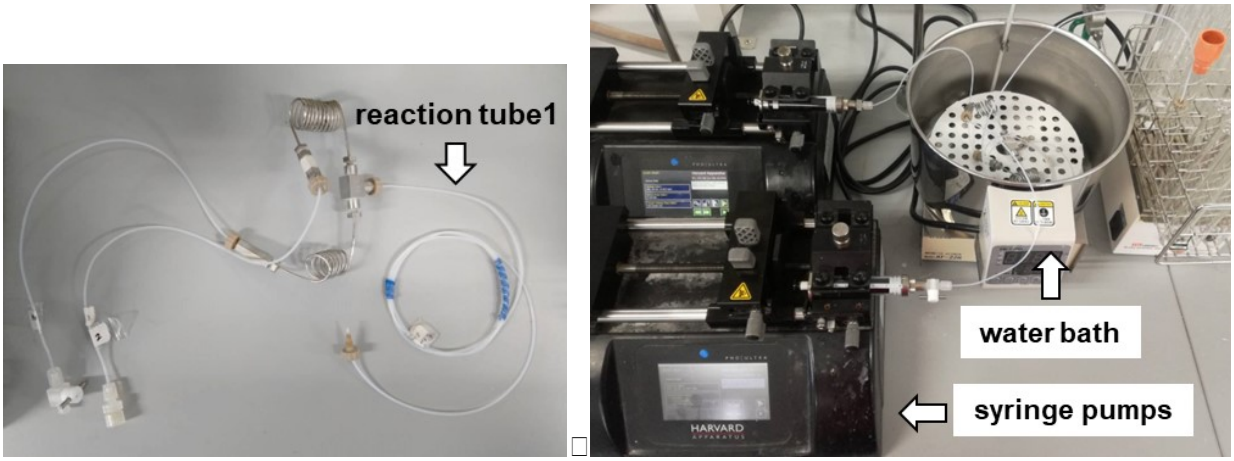


Figure S5. Micro-flow reactor set-up for synthesis of **10a** and **1a**

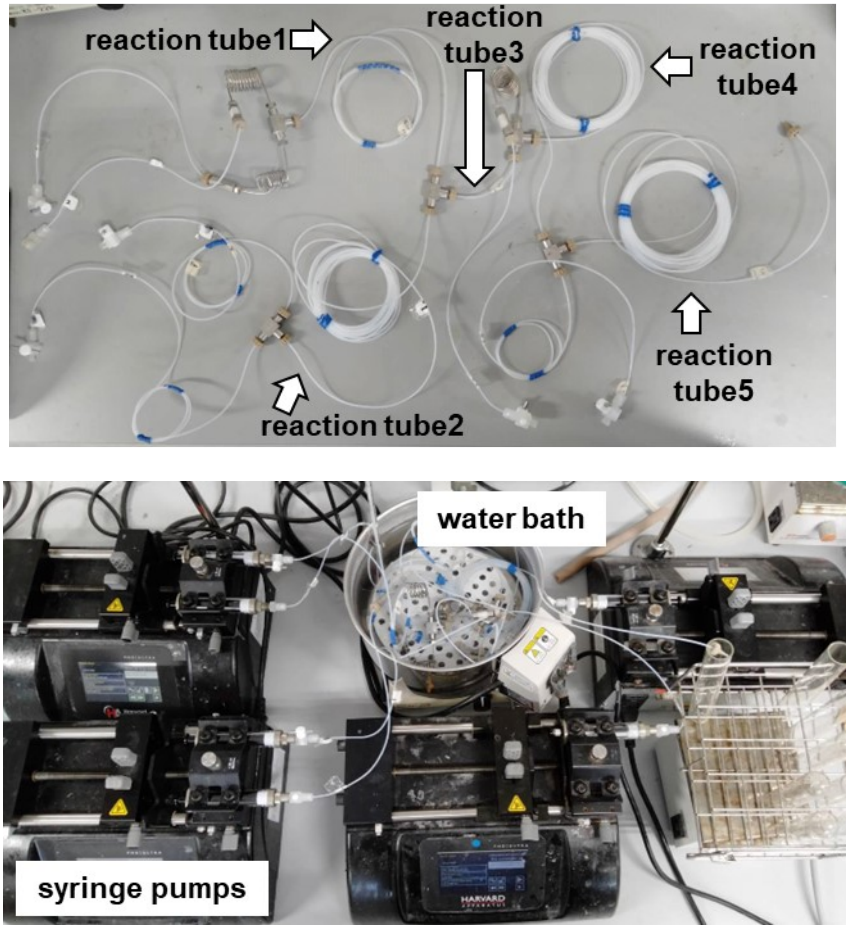
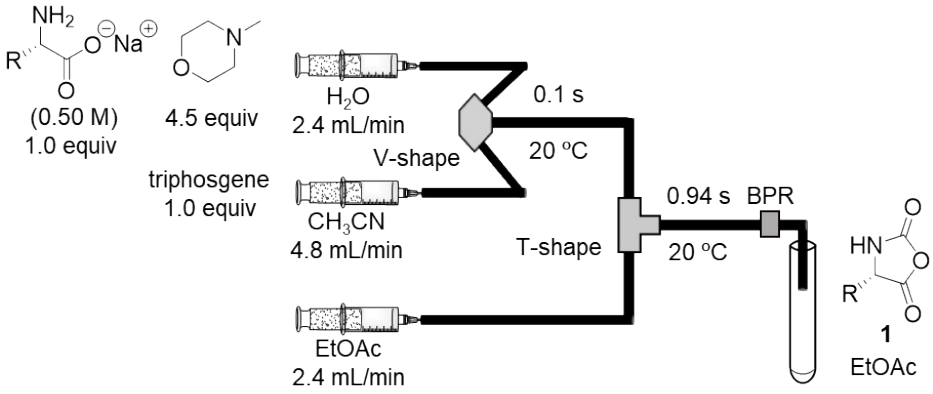


Figure S6. Micro-flow reactor set-up for one-flow synthesis of **7a**

General procedure for synthesis of α-NCAs 1[1)]



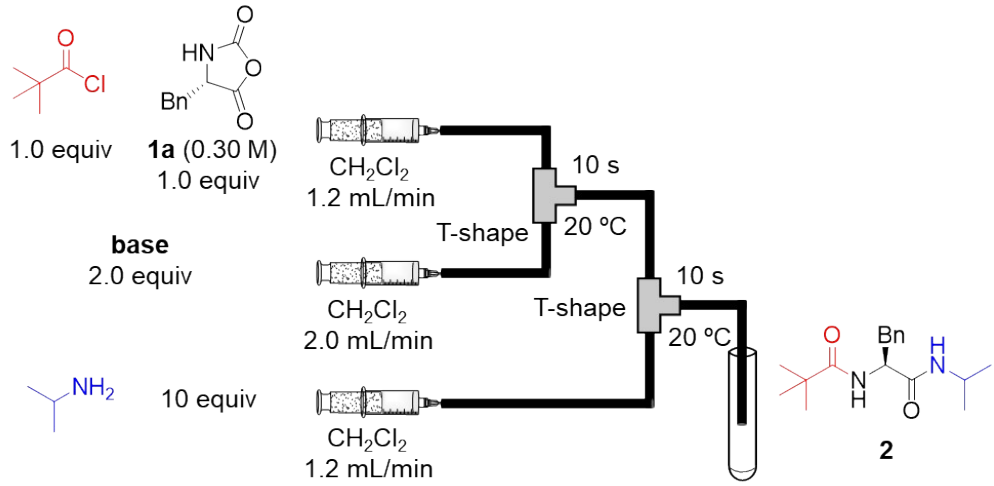
The employed micro-flow system was shown in **Figure S1**.

A solution of **α-amino acid sodium salt** (0.500 M, 1.00 equiv), *N*-methyl morpholine (2.25 M, 4.50 equiv) in H_2O (flow rate: 2.40 mL/min) and a solution of triphosgene (0.250 M, 1.00 equiv) in CH_3CN (flow rate: 4.80 mL/min) were injected into the V-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.250 mm, length: 244 mm, volume: 12.0 μL, reaction time: 0.10 s) at the same temperature. Then, the resultant mixture and EtOAc (flow rate: 2.40 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through

the reaction tube 2 (inner diameter: 0.800 mm, length: 298 mm, volume: 150 μL, reaction time: 0.94 s) at the same temperature. After being eluted for 20 s to reach a steady state, the reaction mixture was poured into EtOAc (40 mL) for appropriate time at room temperature. The aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. The appropriate purification afforded α-NCA **1**.

Examination of synthesis of 2

Table S1. Examination of bases for synthesis of 2



entry	**base** (p*K*aH)[a]		HPLC yield (%)
1	*N*-methylimidazole (NMI) (7.0)[2)]		35
2	*N*-methylmorpholine (NMM) (7.4)[3)]		2
3	dimethylbenzylamine (Me_2NBn) (8.9)[3)]		<1
4	triethylamine (Et_3N) (10.7)[3)]		8
5	*N*,*N*-diisopropylethylamine (*i*-Pr_2NEt) (11.4)[3)]		<1
6[b]	*i*-Pr_2NEt	pyridine (5.2)[2)]	10
7[b]	*i*-Pr_2NEt	DMAP (9.7)[4)]	99
8[b]	Me_2NBn	NMI	89
9[b]	*i*-Pr_2NEt	NMI	>99 (>99)[c]

[a]p*K*a of conjugated acid. [b]The combined use of bases. [c]Isolated yield.

The employed micro-flow system was shown in **Figure S2**.

A solution of **H-L-Phe-NCA (1a)** (0.300 M, 1.00 equiv), pivaloyl chloride (0.300 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) and a solution of **base** (0.360 M, 2.00 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. Then, the resultant mixture and a solution of **isopropylamine** (3.00 M, 10.0 equiv) in

CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 1459 mm, volume: 733 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 60 s to reach a steady state, the resultant mixture was poured into 1 M HCl aq. (1.5 mL) and CH_2Cl_2 (6 mL) for 25 s at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. Yields were determined by HPLC-UV analysis (conditions: COSMOSIL 5C_{18}-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min: 30 to 100%, 20-25 min: 100%, 25-27 min: 100 to 30%, 27-30 min: 30%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 °C, retention time: 8.7 min) using a calibration curve shown in **Figure S7** in order to allow rapid analysis of reaction results without silica gel column chromatography.

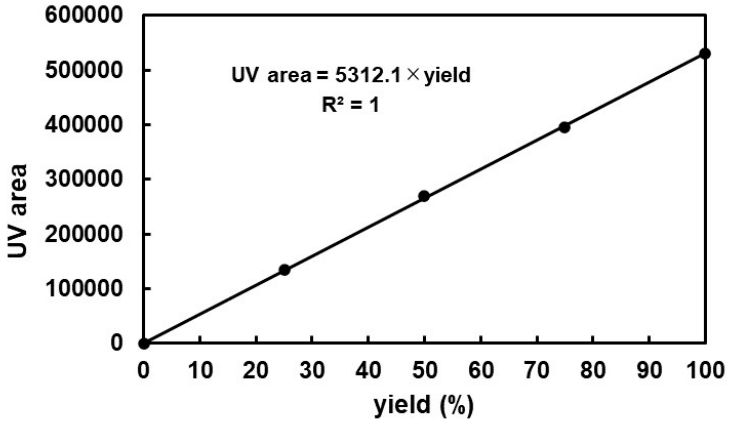
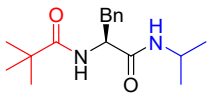


Figure S7. Calibration curve of **2**

(*S*)-*N*-Isopropyl-3-phenyl-2-pivalamidopropanamide (2)



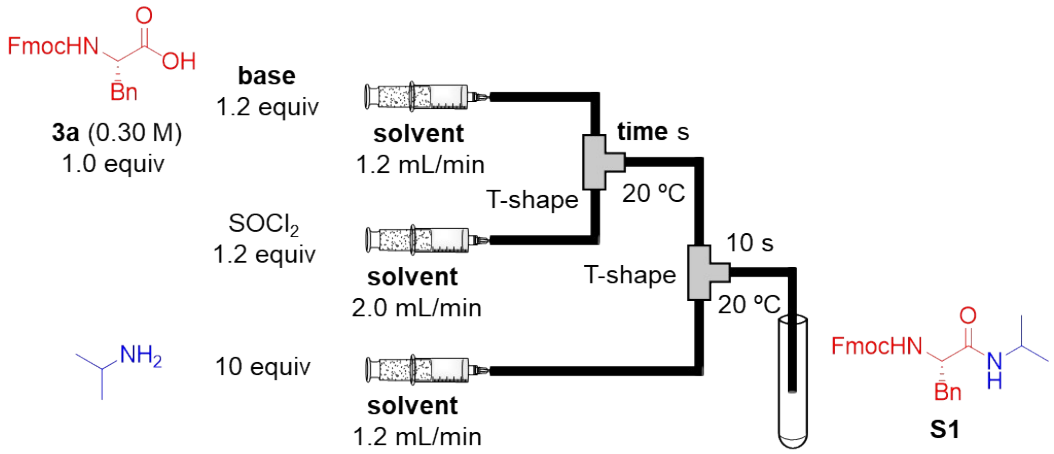
Purification method: The resultant mixture in CH_2Cl_2 was washed with 1 M HCl aq, *sat.* $NaHCO_3$ aq. and brine.

43.6 mg, 0.15 mmol, >99%.

White solid, mp 168-171 °C, IR (neat): 3285, 2969, 1633, 1556, 1216 cm^{-1}; $[\alpha]^{23}_D$ = -17.3 (c 2.05, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.30-7.20 (m, 5H), 6.45 (d, *J* = 6.4 Hz, 1H), 5.82 (d, *J* = 7.2 Hz, 1H), 4.57 (ddd, *J* = 6.4, 6.4, 6.4 Hz, 1H), 4.00-3.91 (m, 1H), 3.10 (dd, *J* = 6.4, 13.2 Hz, 1H), 2.97 (dd, *J* = 6.4, 13.2 Hz, 1H), 1.15 (s, 9H), 1.04 (d, *J* = 6.4 Hz, 3H), 0.98 (d, *J* = 6.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.4, 170.1, 137.0, 129.5, 128.6, 127.0, 54.5, 41.5, 38.9, 38.8, 27.5, 22.6, 22.5 ppm; HRMS (ESI): calcd for $[C_{17}H_{26}N_2O_2+H]^+$ 291.2067, found 291.2066 and $[C_{17}H_{26}N_2O_2+Na]^+$ 313.1886, found 313.1885.

Examination of acid chloride formation

Table S2. Examination of bases, solvents, and reaction times for acid chloride formation



entry	**base**	**solvent**	**time** (s)	HPLC yield (%)
1	pyridine	CH_2Cl_2	10	-[a]
2	NMI	CH_2Cl_2	10	98
3	NMM	CH_2Cl_2	10	>99
4	Et_3N	CH_2Cl_2	10	>99
5	*i*-Pr_2NEt	CH_2Cl_2	10	>99 (98)[b]
6	*i*-Pr_2NEt	CH_3CN	10	97
7	*i*-Pr_2NEt	1,4-dioxane	10	-[c]
8	*i*-Pr_2NEt	EtOAc	10	-[c]
9	*i*-Pr_2NEt	CH_2Cl_2	5	>99
10	*i*-Pr_2NEt	CH_2Cl_2	1	96

[a]Fmoc-L-Phe-OH was not soluble in CH_2Cl_2. [b]Isolated yield. [c]The reactor was clogged.

The employed micro-flow system was shown in **Figure S2**.

A solution of **Fmoc-L-Phe-OH** (**3a**) (0.300 M, 1.00 equiv), **base** (0.360 M, 1.2 equiv) in **solvent** (flow rate: 1.20 mL/min) and a solution of thionyl chloride (0.216 M, 1.20 equiv) in **solvent** (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, reaction time: 1 or 5 or 10 s) at the same temperature. Then, the resultant mixture and a solution of isopropylamine (3.00 M, 10.0 equiv) in **solvent** (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 1459 mm, volume: 733 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 60 s to reach a steady state, the resultant mixture was poured into 1 M HCl aq. (1.5 mL) and CH_2Cl_2 (6 mL) for 25 s at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. Yields were determined by HPLC-UV analysis (conditions: COSMOSIL 5C_{18}-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min:

30 to 100%, 20-25 min: 100%, 25-27 min: 100 to 30%, 27-30 min: 30%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 ℃, retention time: 14.3 min) using a calibration curve shown in **Figure S8** in order to allow rapid analysis of reaction results without silica gel column chromatography.

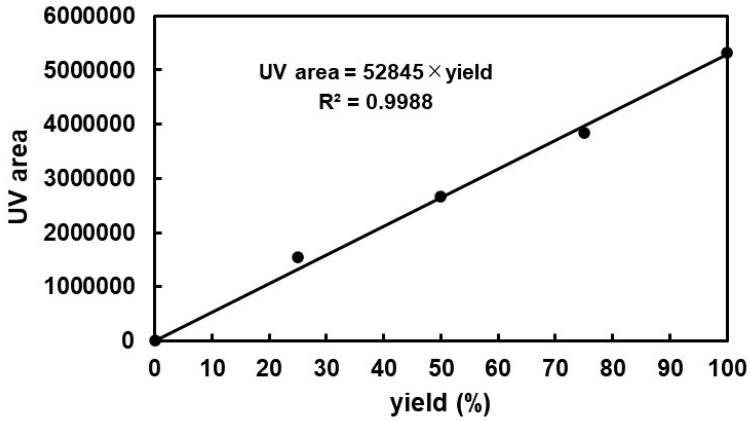


Figure S8. Calibration curve of **S1**

(9H-fluoren-9-yl)methyl (*S*)-(1-(isopropylamino)-1-oxo-3-phenylpropan-2-yl)carbamate (S1)

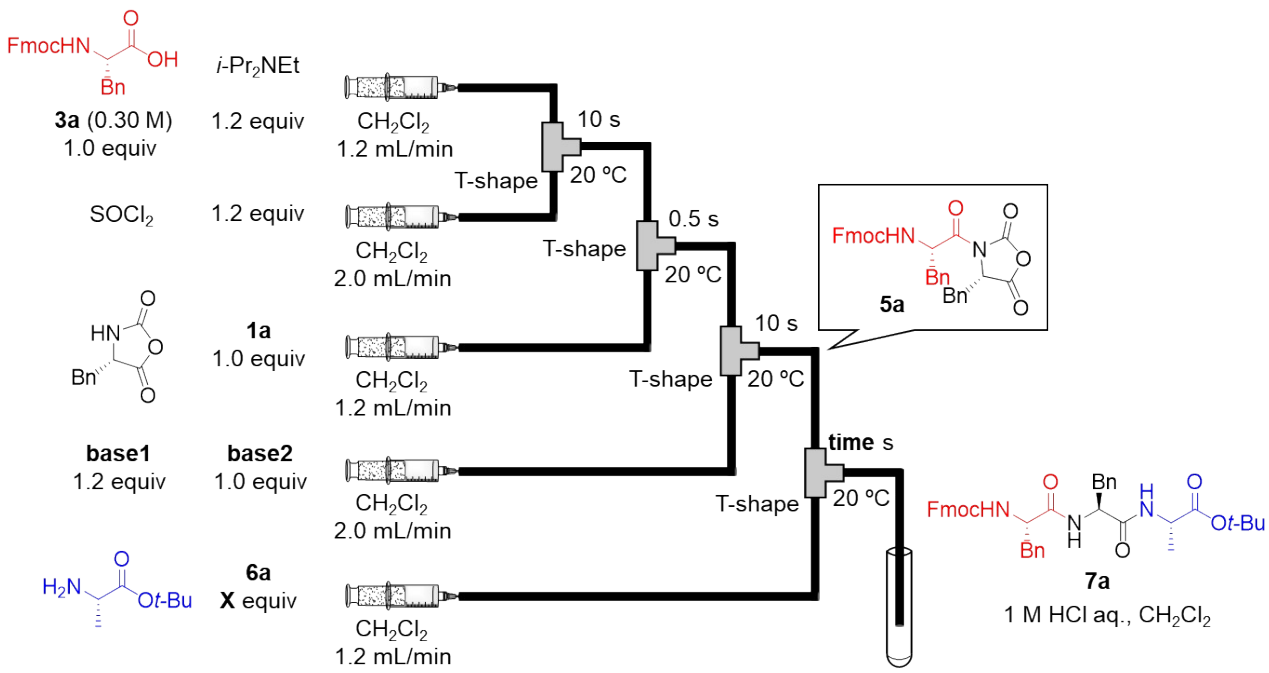
Chromatographic conditions: CH_2Cl_2 : CH_3OH = 98 : 2 to 80 : 20.

63.3 mg, 0.15 mmol, 98%.

White solid, mp 188-191 ℃, IR (neat): 3295, 1697, 1651, 1541, 1261, 757, 740 cm^{-1}; $[\alpha]^{24}_D$ = -0.95 (c 2.05, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.56 (dd, *J* = 7.6 Hz, 2H), 7.40 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.33-7.21 (m, 7H), 5.05 (d, *J* = 6.0 Hz, 1H), 5.25 (brs, 1H), 4.45-4.28 (m, 3H), 4.20 (t, *J* = 6.8 Hz, 1H), 4.00-3.91 (m, 1H), 3.16-3.14 (m, 1H), 2.97-2.92 (m, 1H), 1.03 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 156.0, 143.9, 141.4, 136.8, 129.5, 128.8, 127.9, 127.21, 127.17, 125.1, 120.1, 67.1, 56.7, 47.3, 41.6, 39.4, 22.6, 22.5 ppm; HRMS (ESI): calcd for $[C_{27}H_{28}N_2O_3+H]^+$ 429.2173, found 429.2171 and $[C_{27}H_{28}N_2O_3+Na]^+$ 451.1992, found 451.1992.

Examination of coupling reaction between 5a and 6a

Table S3. Examination of bases, amounts of 6a, and reaction times in the coupling reaction between 5a and 6a.



entry	**base1**	**base2**	**X** (equiv)	**time** (s)	HPLC yield (%)
1	*i*-Pr_2NEt	NMI	1.0	10	73
2	*i*-Pr_2NEt	NMI	1.2	10	86, 88, 88
3	*i*-Pr_2NEt	DMAP	1.2	10	76
4	*i*-Pr_2NEt	NMI	1.2	15	88
5	*i*-Pr_2NEt	NMI	1.5	10	89

The employed micro-flow system was shown in **Figure S3**.

A solution of **Fmoc-L-Phe-OH** (**3a**) (0.300 M, 1.00 equiv), *N*,*N*-diisopropylethylamine (0.360 M, 1.2 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of thionyl chloride (0.216 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. The resultant mixture and a solution of **H-L-Phe-NCA** (**1a**) (0.300 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 72.9 mm, volume: 37 μL, reaction time: 0.50 s) at the same temperature. The resultant mixture and a solution of **base1** (0.216 M, 1.2 equiv) and **base2** (0.180 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 3 (inner diameter: 0.800 mm, length: 2122 mm, volume: 1067 μL, reaction time: 10 s) at the same temperature. The resultant mixture and a solution of **H-L-Ala-O*t*-Bu** (**6a**) (**X** equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shape mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 4 (inner diameter: 0.800 mm, reaction time: **Y** s) at the same temperature. After being eluted for *ca.* 60 s to reach a steady state, the resultant mixture was poured into 1 M HCl aq. (1.5

mL) and CH_2Cl_2 (8 mL) for 25 s at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. Yields were determined by HPLC-UV analysis (conditions: COSMOSIL $5C_{18}$-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min: 30 to 100%, 20-25 min: 100%, 25-27 min: 100 to 30%, 27-30 min: 30%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 ℃, retention time: 17.3 min) using a calibration curve shown in **Figure S9** in order to allow rapid analysis of reaction results without silica gel column chromatography.

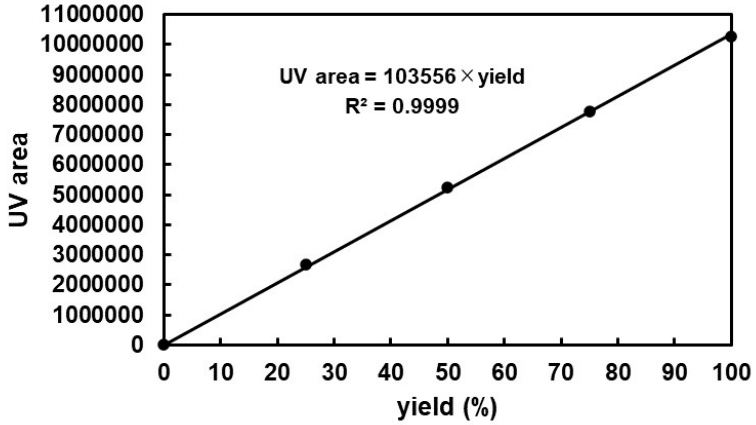
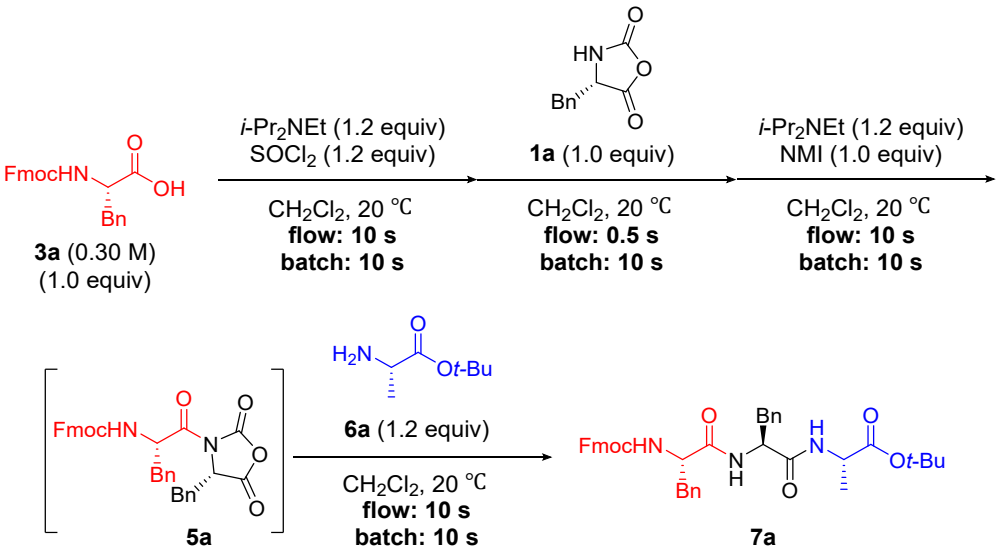


Figure S9. Calibration curve of **7a**

Comparison between micro-flow conditions and batch conditions

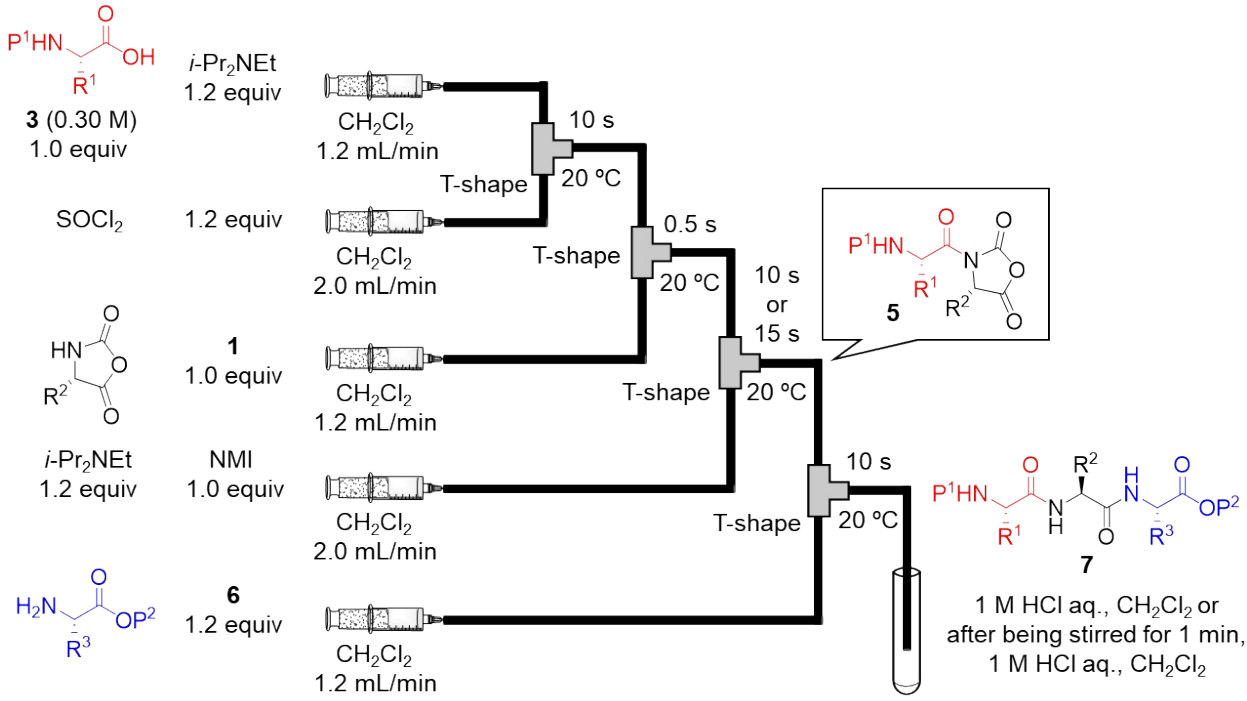
Table S4. Procedure for synthesis of 7a using a batch reactor and comparison of yields of 7a between micro-flow conditions and batch conditions



entry	HPLC yields (%)	
	micro-flow conditions	batch conditions
1	86	69
2	88	73
3	88	75

Comparison between micro-flow and batch conditions for synthesis of **7a**. Quantities of compounds, solvents and temperature were identical to those of flow condition. To a vigorously stirred (magnetic stirrer, 1,000 rpm) solution of **Fmoc-L-Phe-OH** (**3a**) (0.300 M, 1.00 equiv), *N*,*N*-diisopropylethylamine (0.360 M, 1.2 equiv) in CH_2Cl_2 (0.50 mL), a solution of thionyl chloride (0.216 M, 1.20 equiv) in CH_2Cl_2 (0.83 mL) was added in one portion of 20 °C under argon atmosphere. After being stirred for 10 s at the same temperature, a solution of **H-L-Phe-NCA** (**1a**) (0.300 M, 1.0 equiv) in CH_2Cl_2 (0.50 mL) was added in one portion of 20 °C under argon atmosphere. After being stirred for 10 s at the same temperature, a solution of *N*,*N*-diisopropylethylamine (0.216 M, 1.2 equiv), *N*-methylimidazole (0.180 M, 1.0 equiv) in CH_2Cl_2 (0.83 mL) was added in one portion at 20 °C under argon atmosphere. After being stirred for 10 s at the same temperature, a solution of **H-L-Ala-O*t*-Bu** (**6a**) (0.360 M, 1.20 equiv) in CH_2Cl_2 (0.50 mL) was added in one portion at 20 °C under argon atmosphere. After being stirred for 10 s at the same temperature, 1 M HCl aq. (1.5 mL) and CH_2Cl_2 (8 mL) were added. The aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. Yields were determined by HPLC-UV analysis (conditions: COSMOSIL $5C_{18}$-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min: 30 to 100%, 20-25 min: 100%, 25-27 min: 100 to 30%, 27-30 min: 30%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 °C, retention time: 17.3 min) using a calibration curve shown in **Figure S9** in order to allow rapid analysis of reaction results without silica gel column chromatography.)

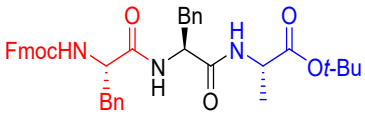
General procedure for examination of substrate scope of tripeptide 7



The employed micro-flow system was shown in **Figure S3**.

A solution of ***N*-protected amino acid 3** (0.300 M, 1.00 equiv), *N*,*N*-diisopropylethylamine (0.360 M, 1.2 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of thionyl chloride (0.216 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. The resultant mixture and a solution of **α-amino acid *N*-carboxy anhydride 1** (0.300 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 72.9 mm, volume: 37 μL, reaction time: 0.50 s) at the same temperature. The resultant mixture and a solution of *N*,*N*-diisopropylethylamine (0.216 M, 1.20 equiv) and *N*-methylimidazole (0.180 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 3 (inner diameter: 0.800 mm, reaction time: 10 s or 15 s) at the same temperature. The resultant mixture and a solution of **amino acid alkyl ester 6** (0.360 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 4 (inner diameter: 0.800 mm, length: 2,519 mm, volume: 1,267 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 90 s to reach a steady state, the resultant mixture was poured into 1 M HCl aq. (1.5 mL) and CH_2Cl_2 (8 mL) for 25 s at room temperature, or the resultant mixture was poured into test tube for 25 s and stirred for 1 min at room temperature, then, 1 M HCl aq. (1.5 mL) and CH_2Cl_2 (8 mL) were added. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with *sat.* $NaHCO_3$ aq., brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. The residue was purified by recrystallization.

Fmoc-L-Phe-L-Phe-L-Ala-O*t*-Bu (7a)



Purification method: The crude product was recrystallized from CH_2Cl_2/hexane.

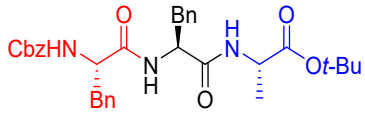
The reaction mixture was collected for 870 s (14 min 30 s).

HPLC conditions; DAICEL CHIRALPAK IH 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 8.8 min.

2.98 g, 4.5 mmol, 86%.

White solid, mp 162-164 °C, IR (neat): 3286, 1732, 1704, 1644, 1539, 1451, 1254, 1146, 741 cm^{-1}; $[\alpha]^{25}_D$ = -21.7 (c 0.88, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.50 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.39 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.31-7.07 (m, 12H), 6.67 (brs, 1H), 6.54 (brs, 1H), 5.44 (d, *J* = 6.0 Hz, 1H), 4.68 (d, *J* = 6.4 Hz, 1H), 4.46 (brs, 1H), 4.42-4.38 (m, 1H), 4.34-4.31 (m, 1H), 4.24 (brs, 1H), 4.14 (t, *J* = 6.8 Hz, 1H), 3.01-2.99 (m, 4H), 1.43 (s, 9H), 1.28 (d, *J* = 6.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 170.8, 169.8, 156.1, 143.8, 141.4, 136.3, 129.4, 128.8, 128.6, 127.9, 127.2, 127.1, 125.2, 125.1, 120.1, 82.1, 67.2, 56.2, 54.4, 48.9, 47.2, 38.5, 38.4, 28.0, 18.6 ppm; HRMS (ESI): calcd for $[C_{40}H_{43}N_3O_6+Na]^+$ 684.3044, found 684.3041.

Cbz-L-Phe-L-Phe-L-Ala-O*t*-Bu (7b)

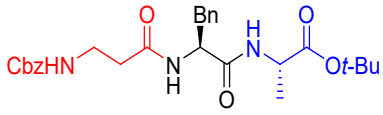


Purification method: The crude product was recrystallized from CH_2Cl_2/hexane.

76.3 mg, 0.13 mmol, 89%.

White solid, mp 112-115 °C, IR (neat): 3290, 1734, 1703, 1644, 1538, 1148 cm^{-1}; $[\alpha]^{26}_D$ = -17.7 (c 1.41, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.36-7.07 (m, 15H), 6.55 (d, *J* = 7.2 Hz, 1H), 6.50 (d, *J* = 5.2 Hz, 1H), 5.35 (d, *J* = 6.4 Hz, 1H), 5.06 (d, *J* = 12.4 Hz, 1H), 5.01 (d, *J* = 12.4 Hz, 1H), 4.66 (ddd, *J* = 7.2, 7.2, 7.2 Hz, 1H), 4.44-4.42 (m, 1H), 4.35-4.28 (m, 1H), 3.06-2.94 (m, 4H), 1.44 (s, 9H), 1.28 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 170.9, 170.0, 156.1, 136.4, 136.3, 129.4, 128.7, 128.6, 128.2, 128.1, 127.1, 127.0, 82.1, 67.1, 56.2, 54.3, 48.9, 38.6, 38.5, 28.0, 18.5 ppm; HRMS (ESI): calcd for $[C_{33}H_{39}N_3O_6+Na]^+$ 596.2731, found 596.2732.

Cbz-β-Ala-L-Phe-L-Ala-O*t*-Bu (7c)

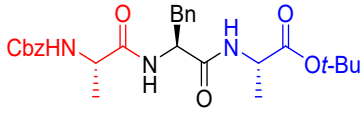


Purification method: The crude product was recrystallized from CH_2Cl_2/hexane.

70.6 mg, 0.14 mmol, 95%.

White solid, mp 97-99 °C, IR (neat): 3279, 1726, 1641, 1550, 1455, 1369, 1251, 1149 cm^{-1}; $[\alpha]^{26}_D$ = -0.77 (c 1.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.32-7.09 (m, 10H), 6.71 (d, *J* = 6.8 Hz, 1H), 6.57 (d, *J* = 7.2 Hz, 1H), 5.56 (brs, 1H), 5.10-5.03 (m, 2H), 4.72 (ddd, *J* = 6.8, 6.8, 6.8 Hz, 1H), 4.39-4.32 (m, 1H), 3.41-3.40 (m, 2H), 3.07 (dd, *J* = 6.8, 13.6 Hz, 1H), 3.00 (dd, *J* = 6.8, 13.6 Hz, 1H), 2.40-2.35 (m, 2H), 1.43 (s, 9H), 1.29 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 171.4, 170.4, 156.6, 136.6, 136.4, 129.4, 128.7, 128.6, 128.1, 127.1, 82.2, 66.7, 54.4, 48.9, 38.6, 37.2, 36.1, 28.0, 18.5 ppm; HRMS (ESI): calcd for $[C_{27}H_{35}N_3O_6+Na]^+$ 520.2418, found 520.2419.

Cbz-L-Ala-L-Phe-L-Ala-O*t*-Bu (7d)



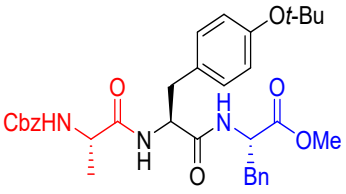
Purification method: The crude product was recrystallized from CH_2Cl_2/hexane

60.9 mg, 0.13 mmol, 82%.

White solid, mp 129-133 °C, IR (neat): 3310, 3272, 1733, 1698, 1645, 1539, 1239, 1150 cm^{-1}; $[\alpha]^{26}_D$ = -31.0 (c 1.02, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.30 (m, 5H), 7.29-7.09 (m, 5H), 6.82 (d, *J* = 6.8 Hz, 1H), 6.67 (d, *J* = 5.6 Hz, 1H), 5.46 (d, *J* = 6.8 Hz, 1H), 5.11 (d, *J* = 12.0 Hz, 1H), 5.05 (d, *J* = 12.0 Hz, 1H), 4.73 (ddd, *J* = 6.8, 6.8, 6.8 Hz, 1H), 4.38-4.31 (m, 1H), 4.27-4.24 (m, 1H), 3.06 (d, *J* = 6.8 Hz, 2H), 1.44 (s, 9H), 1.30 (d, *J* = 6.8 Hz, 3H), 1.30 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 171.7, 170.0, 156.1, 136.4, 136.3, 129.5, 128.7, 128.4, 128.2, 127.1, 82.1, 67.2, 54.3, 50.8, 48.9, 38.5, 28.0, 18.7, 18.5 ppm; HRMS (ESI): calcd for $[C_{27}H_{35}N_3O_6+Na]^+$ 520.2418, found 520.2419.

Spectral data of ^1H and ^{13}C NMR were identical to those previously reported[5].

Cbz-L-Ala-L-Tyr(*t*-Bu)-L-Phe-OMe (7e)

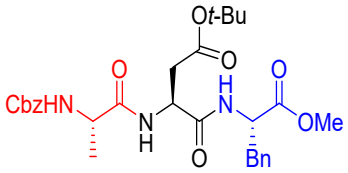


Purification method: The crude product was recrystallized from CH_2Cl_2/hexane

75.0 mg, 0.12 mmol, 83%.

White solid, mp 163-166 ℃, IR (neat): 3296, 1742, 1698, 1646, 1538, 1507, 1236, 1163 cm^{-1}; $[\alpha]^{24}_D$ = -3.3 (c 1.85, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.36-7.18 (m, 8H), 7.06-7.00 (m, 4H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.68 (d, *J* = 6.8 Hz, 1H), 6.41 (d, *J* = 5.6 Hz, 1H), 5.30 (d, *J* = 6.8 Hz, 1H), 5.11 (d, *J* = 12.4 Hz, 1H), 5.03 (d, *J* = 12.4 Hz, 1H), 4.75 (ddd, *J* = 5.6 Hz, 1H), 4.60 (ddd, *J* = 6.8, 6.8, 6.8 Hz, 1H), 4.21-4.17 (m, *J* = 6.4 Hz, 1H), 3.66 (s, 3H), 3.07 (dd, *J* = 5.6, 13.2 Hz, 1H), 3.00-2.95 (m, 3H), 1.30 (s, 9H), 1.26 (d, *J* = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 171.4, 170.3, 154.6, 136.2, 135.8, 131.1, 129.9, 129.3, 128.7, 128.4, 128.3, 127.2, 124.4, 78.5, 67.3, 54.4, 53.5, 52.4, 50.7, 37.9, 37.5, 28.9, 18.5 ppm; HRMS (ESI): calcd for $[C_{34}H_{41}N_3O_7+H]^+$ 604.3017, found 604.3016, $[C_{34}H_{41}N_3O_7+Na]^+$ 626.2837, found 626.2837.

Cbz-L-Ala-L-Asp(O*t*-Bu)-L-Phe-OMe (7f)

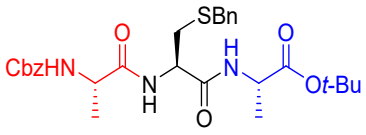


Purification method: The crude product was recrystallized from CH_2Cl_2/CH_3OH/hexane

66.3 mg, 0.12 mmol, 80%.

White solid, mp 139-142 ℃, IR (neat): 3299, 1731, 1653, 1523, 1217, 1155 cm^{-1}; $[\alpha]^{23}_D$ = +19.1 (c 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.13 (m, 11H), 7.04 (d, *J* = 7.2 Hz, 1H), 5.26 (brs, 1H), 5.11 (d, *J* = 12.4 Hz, 1H), 5.07 (d, *J* = 12.4 Hz, 1H), 4.79-4.74 (m, 2H), 4.21-4.18 (m, 1H), 3.69 (s, 3H), 3.13 (dd, *J* = 6.0, 13.6 Hz, 1H), 3.05 (dd, *J* = 6.0, 13.6 Hz, 1H), 2.91-2.88 (m, 1H), 2.53 (dd, *J* = 6.4, 16.4 Hz, 1H), 1.43 (s, 9H), 1.31 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 171.5, 170.1, 156.1, 136.3, 135.9, 129.4, 128.71, 128.68, 128.3, 128.2, 127.2, 82.1, 67.2, 53.8, 52.4, 50.8, 49.2, 37.7, 36.8, 28.1, 18.7 ppm; HRMS (ESI): calcd for $[C_{29}H_{37}N_3O_8+Na]^+$ 578.2473, found 578.2473.

Cbz-L-Ala-L-Cys(Bn)-L-Ala-O*t*-Bu (7g)



Purification method: The crude product was recrystallized from CH_2Cl_2/hexane

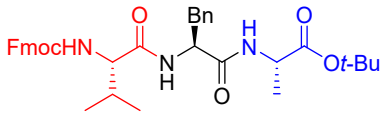
HPLC conditions; DAICEL CHIRALPAK IB 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 13.9 min.

66.6 mg, 0.12 mmol, 82%.

Yellow amorphous solid, IR (neat): 3301, 1731, 1692, 1641, 1538, 1239, 1146, 698 cm^{-1}; $[\alpha]^{23}_D$ = -23.4 (c 1.08, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.34-7.21 (m, 10H), 6.97 (d, *J* = 5.6 Hz, 1H), 6.90 (d, *J* = 6.4 Hz, 1H), 5.42 (d, *J* = 6.4 Hz, 1H), 5.11 (s, 2H), 4.56-4.54 (m, 1H), 4.43-4.38 (m, 1H), 4.28-4.26 (m, 1H), 3.74 (s, 2H), 2.91 (dd, *J* = 5.2, 13.6 Hz, 1H), 2.73 (dd, *J* = 6.4, 13.6 Hz, 1H), 1.45 (s, 9H), 1.37 (d, *J* = 7.6 Hz, 3H),

1.35 (d, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 171.6, 169.4, 156.1, 138.2, 136.2, 129.2, 128.8, 128.7, 128.4, 128.2, 127.4, 82.2, 67.2, 52.3, 50.9, 49.2, 36.6, 33.7, 28.1, 18.8, 18.4 ppm; HRMS (ESI): calcd for $[C_{28}H_{37}N_3O_6S_1+Na]^+$ 566.2295, found 556.2297.

Fmoc-L-Val-L-Phe-L-Ala-O*t*-Bu (7h)

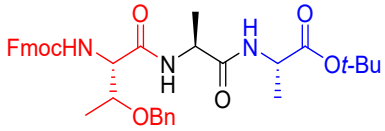


Purification method: The crude product was recrystallized from CH_2Cl_2/CH_3OH/hexane

79.4 mg, 0.13 mmol, 86%.

White solid, mp 192-196 °C, IR (neat): 3289, 1734, 1696, 1644, 1541, 1247, 1147, 741 cm^{-1}; $[\alpha]^{24}_D$ = -26.2 (c 1.41, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J = 7.6 Hz, 2H), 7.59 (d, J = 7.6 Hz, 2H), 7.41-7.38 (m, 2H), 7.33-7.26 (m, 2H), 7.23-7.05 (m, 5H), 6.64 (d, J = 7.2 Hz, 1H), 6.55 (d, J = 6.8 Hz, 1H), 5.43 (d, J = 8.0 Hz, 1H), 4.74-4.70 (m, 1H), 4.45 (dd, J = 6.8 Hz, 10.0 Hz, 1H), 4.35-4.31 (m, 2H), 4.20 (t, J = 6.8 Hz, 1H), 4.04-4.01 (m, 1H), 3.07-3.01 (m, 2H), 2.10-2.07 (m, 1H), 1.43 (s, 9H), 1.29 (d, J = 6.4 Hz, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 171.2, 170.0, 156.5, 144.0, 141.5, 136.4, 129.4, 128.7, 127.9, 127.22, 127.17, 125.2, 120.1, 82.1, 67.2, 60.5, 54.3, 48.9, 47.3, 38.5, 31.1, 28.1, 19.3, 18.6, 17.8 ppm; HRMS (ESI): calcd for $[C_{36}H_{43}N_3O_6+Na]^+$ 636.3044, found 636.3044.

Fmoc-L-Thr(Bn)-L-Ala-L-Ala-O*t*-Bu (7i)

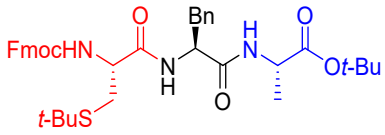


Purification method: The crude product was recrystallized from CH_2Cl_2/CH_3OH/hexane

82.2 mg, 0.13 mmol, 87%.

White solid, mp 182-185 °C, IR (neat): 3294, 1732, 1705, 1641, 1537, 1451, 1245, 1154, 739 cm^{-1}; $[\alpha]^{24}_D$ = -9.6 (c 1.28, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J = 7.6 Hz, 2H), 7.60 (d, J = 7.2 Hz, 2H), 7.41-7.29 (m, 9H), 7.02 (d, J = 7.2 Hz, 1H), 6.69 (d, J = 6.8 Hz, 1H), 5.82 (d, J = 6.4 Hz, 1H), 4.68 (d, J = 11.6 Hz, 1H), 4.57-4.36 (m, 6H), 4.22 (t, J = 6.8 Hz, 1H), 4.16-4.15 (m, 1H), 1.46 (s, 9H), 1.34 (d, J = 7.6 Hz, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.17 (d, J = 6.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9, 171.1, 169.4, 156.4, 144.0, 143.8, 141.4, 137.9, 128.6, 128.1, 127.9, 127.2, 125.2, 120.1, 82.1, 74.8, 71.7, 67.3, 58.0, 49.1, 48.9, 47.3, 28.1, 18.5, 18.2, 15.2 ppm; HRMS (ESI): calcd for $[C_{36}H_{43}N_3O_7+Na]^+$ 652.2993, found 652.2990.

Fmoc-L-Cys(*t*-Bu)-L-Phe-L-Ala-O*t*-Bu (7j)



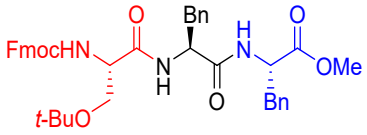
Purification method: The crude product was recrystallized from CH_2Cl_2/CH_3OH/hexane

HPLC conditions; DAICEL CHIRALPAK IH 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 10.2 min.

84.2 mg, 0.125 mmol, 83%.

White solid, mp 126-128 °C, IR (neat): 3290, 1732, 1708, 1645, 1538, 1452, 1245, 1150, 737 cm^{-1}; $[\alpha]^{24}_D$ = -17.0 (c 1.81, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.40 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.31 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.26-7.15 (m, 5H), 6.80 (d, *J* = 7.2 Hz, 1H), 6.53 (d, *J* = 5.2 Hz, 1H), 5.67 (brs, 1H), 4.69 (ddd, *J* = 7.2, 7.2, 7.2 Hz, 1H), 4.43-4.28 (m, 4H), 4.21 (t, *J* = 7.2 Hz, 1H), 3.15-3.06 (m, 2H), 2.93 (dd, *J* = 5.6, 13.2 Hz, 1H), 2.80 (dd, *J* = 6.8, 13.2 Hz, 1H), 1.44 (s, 9H), 1.30 (s, 12H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 170.0, 169.7, 156.1, 143.8, 143.8, 141.4, 136.3, 129.5, 128.8, 127.9, 127.2, 125.2, 120.1, 82.0, 67.4, 55.0, 54.5, 48.9, 47.2, 43.4, 38.0, 31.0, 30.8, 28.0, 18.5 ppm; HRMS (ESI): calcd for $[C_{38}H_{47}N_3O_6S_1+Na]^+$ 696.3078, found 696.3078.

Fmoc-L-Ser(*t*-Bu)-L-Phe-L-Phe-OMe (7k)

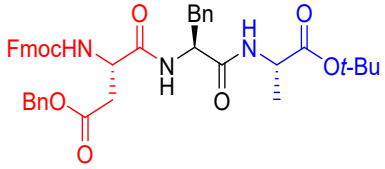


Purification method: The crude product was recrystallized from CH_2Cl_2/hexane

97.1 mg, 0.14 mmol, 94%.

White solid, mp 131-135 °C, IR (neat): 3294, 2974, 1745, 1698, 1647, 1541, 1241, 1195, 738 cm^{-1}; $[\alpha]^{25}_D$ = +11.4 (c 1.75, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.39 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.31 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.29-7.15 (m, 10H), 7.01 (d, *J* = 6.0 Hz, 2H), 6.40 (d, *J* = 5.2 Hz, 1H), 5.63 (d, *J* = 5.2 Hz, 1H), 4.75-4.71 (m, 1H), 4.63 (ddd, *J* = 6.8, 6.8, 6.8 Hz, 1H), 4.39-4.33 (m, 2H), 4.21 (t, *J* = 7.6 Hz, 1H), 4.14 (brs, 1H), 3.74-3.62 (m, 4H), 3.26 (dd, *J* = 8.0 Hz, 1H), 3.09-2.95 (m, 4H), 1.12 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 170.3, 156.2, 143.9, 143.8, 141.4, 136.4, 135.9, 129.4, 129.3, 128.8, 128.7, 127.9, 127.2, 125.2, 120.1, 74.6, 67.3, 61.6, 54.5, 53.6, 52.4, 47.2, 37.9, 27.4 ppm; HRMS (ESI): calcd for $[C_{41}H_{45}N_3O_7+Na]^+$ 714.3150, found 714.3150.

Fmoc-L-Asp(OBn)-L-Phe-L-Ala-O*t*-Bu (7l)

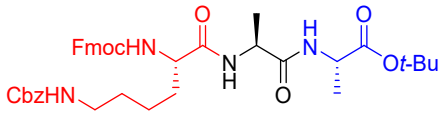


Purification method: The crude product was recrystallized from CH_2Cl_2/CH_3OH/hexane

89.3 mg, 0.12 mmol, 83%.

White solid, mp 112-115 °C, IR (neat): 3294, 1736, 1697, 1646, 1541, 1227, 1148, 739 cm^{-1}; $[\alpha]^{24}_D$ = -7.4 (c 1.82, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.56 (d, *J* = 7.6 Hz, 2H), 7.42-7.10 (m, 14H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.41 (d, *J* = 6.0 Hz, 1H), 5.78 (d, *J* = 8.4 Hz, 1H), 5.12 (s, 2H), 4.63-4.54 (m, 2H), 4.43-4.32 (m, 3H), 4.19 (t, *J* = 6.8 Hz, 1H), 3.08-2.98 (m, 3H), 2.76 (dd, *J* = 6.0, 16.4 Hz, 1H), 1.43 (s, 9H), 1.29 (d, *J* = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 170.2, 169.7, 156.1, 143.8, 141.4, 136.4, 135.4, 129.4, 128.8, 128.6, 128.4, 127.9, 127.3, 127.2, 125.2, 120.2, 82.1, 67.5, 67.1, 54.7, 51.3, 48.9, 47.2, 38.0, 36.2, 28.0, 18.5 ppm; HRMS (ESI): calcd for $[C_{42}H_{45}N_3O_8+Na]^+$ 742.3099, found 742.3098.

Fmoc-L-Lys(Cbz)-L-Ala-L-Ala-O*t*-Bu (7m)

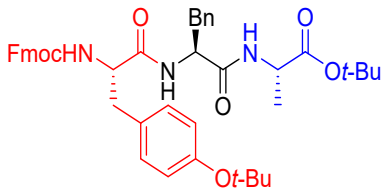


Purification method: The crude product was recrystallized from CH_2Cl_2/CH_3OH/hexane

96.8 mg, 0.14 mmol, 92%.

White solid, mp 156-159 °C, IR (neat): 3287, 1732, 1716, 1685, 1638, 1541, 1261, 1151, 739 cm^{-1}; $[\alpha]^{24}_D$ = -8.1 (c 1.73, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.2 Hz, 2H), 7.57 (d, *J* = 7.2 Hz, 2H), 7.40-7.26 (m, 9H), 6.71 (d, *J* = 7.2 Hz, 1H), 6.66 (d, *J* = 6.8 Hz, 1H), 5.68 (d, *J* = 6.0 Hz, 1H), 5.10-5.07 (m, 3H), 4.51-4.37 (m, 4H), 4.19 (t, *J* = 6.8 Hz, 1H), 3.20-3.15 (m, 1H), 1.84 (brs, 1H), 1.68-1.67 (m, 1H), 1.51-1.31 (m, 19H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 171.63, 171.56, 156.1, 156.0, 143.9, 143.8, 140.7, 137.3, 128.3, 127.7, 127.6, 127.1, 125.3, 120.1, 80.3, 65.6, 65.1, 54.5, 48.3, 47.6, 46.7, 31.6, 29.1, 27.6, 22.8, 18.4, 16.8 ppm; HRMS (ESI): calcd for $[C_{39}H_{48}N_4O_8+H]^+$ 701.3545, found 701.3544, $[C_{39}H_{48}N_4O_8+Na]^+$ 723.3364, found 723.3363.

Fmoc-L-Tyr(*t*-Bu)-L-Phe-L-Ala-O*t*-Bu (7n)



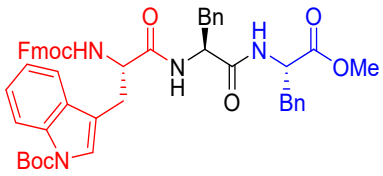
Purification method: The crude product was recrystallized from CH_2Cl_2/hexane

97.2 mg, 0.13 mmol, 88%.

White solid, mp 122-125 °C, IR (neat): 3290, 1733, 1703, 1644, 1540, 1507, 1236, 1160, 739 cm^{-1}; $[\alpha]^{24}_D$ = -11.7 (c 1.70, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.53-7.51 (m, 2H), 7.40 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.33-7.29 (m, 2H), 7.22-7.00 (m, 7H), 6.88 (d, *J* = 8.0 Hz, 2H), 6.49 (brs, 1H), 6.37 (brs, 1H), 5.23 (brs, 1H), 4.62 (ddd, *J* = 6.8, 6.8, 6.8 Hz, 1H), 4.41-4.30 (m, 4H), 4.15 (t, *J* = 6.8 Hz, 1H), 3.01-2.94

(m, 4H), 1.44 (s, 9H), 1.30 (s, 9H), 1.29 (d, J = 10.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 170.7, 169.7, 154.7, 143.8, 141.4, 136.3, 130.9, 129.9, 129.4, 128.7, 127.9, 127.24, 127.18, 125.2, 125.1, 124.4, 120.1, 82.1, 78.5, 67.3, 56.3, 54.4, 48.9, 47.2, 38.4, 37.6, 29.0, 28.1, 18.5 ppm; HRMS (ESI): calcd for $[C_{44}H_{51}N_3O_7+Na]^+$ 756.3619, found 756.3622.

Fmoc-L-Trp(Boc)-L-Phe-L-Phe-OMe (7o)

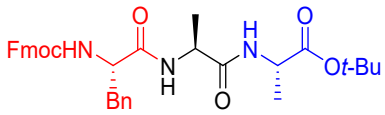


Purification method: The crude product was recrystallized from CH_2Cl_2/hexane.

108.8 mg, 0.13 mmol, 87%.

White solid, mp 184-188 °C, IR (neat): 3304, 1732, 1706, 1672, 1643, 1539, 1453, 1369, 1270, 1160, 739 cm^{-1}; $[\alpha]^{24}_D$ = -10.0 (c 1.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 7.2 Hz, 2H), 7.57 (d, J = 7.6 Hz, 1H), 7.51-7.46 (m, 3H), 7.39-7.35 (m, 2H), 7.32-7.15 (m, 7H), 7.13-7.07 (m, 3H), 6.99-6.96 (m, 4H), 6.68 (d, J = 4.8 Hz, 1H), 6.37 (brs, 1H), 5.52 (brs, 1H), 4.73-4.67 (m, 1H), 4.60-4.51 (m, 2H), 4.34 (dd, J = 7.6, 10.4 Hz, 1H), 4.25-4.23 (m, 1H), 4.14 (t, J = 7.2 Hz, 1H), 3.61 (s, 3H), 3.13-2.89 (m, 6H), 1.61 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 171.0, 170.0, 156.2, 149.6, 143.8, 141.4, 136.2, 135.8, 135.6, 130.4, 129.3, 129.2, 128.7, 128.6, 127.8, 127.2, 127.1, 125.2, 124.8, 124.6, 122.9, 120.1, 119.1, 115.5, 115.3, 83.9, 67.4, 55.0, 54.4, 53.6, 52.3, 47.1, 38.2, 38.0, 28.3, 27.9 ppm; HRMS (ESI): calcd for $[C_{50}H_{50}N_4O_8+Na]^+$ 857.3521, found 857.3523.

Fmoc-L-Phe-L-Ala-L-Ala-O*t*-Bu (7p)



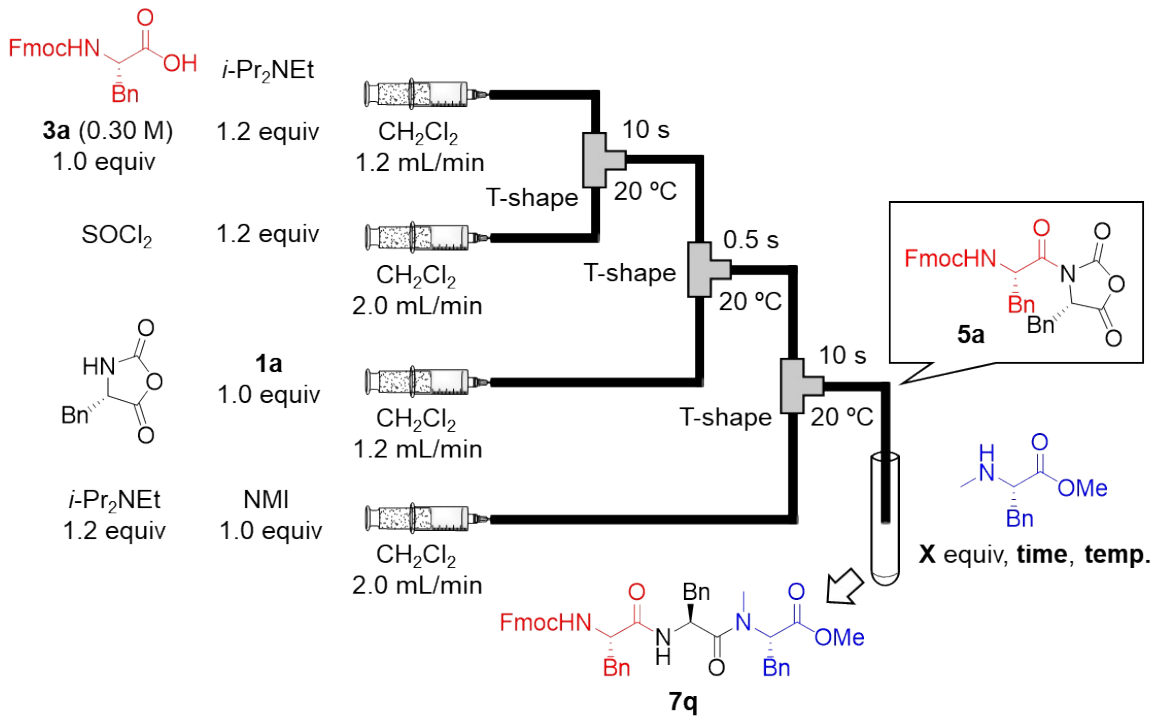
Purification method: The crude product was recrystallized from CH_2Cl_2/hexane

76.8 mg, 0.13 mmol, 87%.

White solid, mp 153-156 °C, IR (neat): 3296, 1731, 1707, 1643, 1539, 1451, 1254, 1154, 739 cm^{-1}; $[\alpha]^{25}_D$ = -16.5 (c 1.53, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, J = 7.6 Hz, 2H), 7.51 (dd, J = 7.6, 7.6 Hz, 2H), 7.38 (dd, J = 7.6, 7.6 Hz, 2H), 7.29-7.18 (m, 7H), 6.92 (brs, 2H), 5.73 (d, J = 7.2 Hz, 1H), 4.59-4.52 (m, 2H), 4.43-4.37 (m, 2H), 4.27-4.23 (m, 1H), 4.15 (t, J = 6.8 Hz, 1H), 3.07 (d, J = 5.6 Hz, 2H), 1.44 (s, 9H), 1.34 (d, J = 6.8 Hz, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.0, 171.4, 170.9, 156.1, 143.9, 141.4, 136.4, 129.5, 128.7, 127.8, 127.2, 125.3, 125.2, 120.1, 82.2, 67.2, 56.1, 49.0, 48.9, 47.2, 38.8, 28.1, 18.9, 18.6 ppm; HRMS (ESI): calcd for $[C_{34}H_{39}N_3O_6+Na]^+$ 608.2731, found 608.2729.

Examination of synthesis of *N*-methylated peptide 7q

Table S5. Examination of amounts of H-MePhe-OMe, reaction times, and temperatures for the synthesis of *N*-methylated peptide 7q



entry	**X** equiv	**time** (h)	**temp.** (°C)	HPLC yield (%)
1	2.0	3	r.t.	58
2	2.0	1	r.t.	52
3	1.5	3	r.t.	54 (51)[a]
4	1.2	3	r.t.	51
5	1.2	3	40	44

[a]Isolated yield.

The employed micro-flow system was shown in **Figure S4**.

A solution of **Fmoc-L-Phe-OH** (**3a**) (0.300 M, 1.00 equiv), *N*,*N*-diisopropylethylamine (0.360 M, 1.2 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of thionyl chloride (0.216 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. The resultant mixture and a solution of **H-L-Phe-NCA** (**1a**) (0.300 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 72.9

mm, volume: 37 μL, reaction time: 0.50 s) at the same temperature. The resultant mixture and a solution of *N*,*N*-diisopropylethylamine (0.216 M, 1.2 equiv) and *N*-methylimidazole (0.180 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 3 (inner diameter: 0.800 mm, length: 2122 mm, volume: 1067 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 90 s to reach a steady state, the resultant mixture was poured into flask including **H-L-MePhe-OMe** (**X** equiv) in CH_2Cl_2 for 25 s at room temperature. After being stirred for **time** (h) at **temp.** (°C), the reaction was quenched with 1 M HCl aq. The aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. Yields were determined by HPLC-UV analysis (conditions: COSMOSIL $5C_{18}$-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min: 30 to 100%, 20-25 min: 100%, 25-27 min: 100 to 30%, 27-30 min: 30%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 °C, retention time: 18.0 min) using a calibration curve shown in **Figure S10** in order to allow rapid analysis of reaction results without silica gel column chromatography.

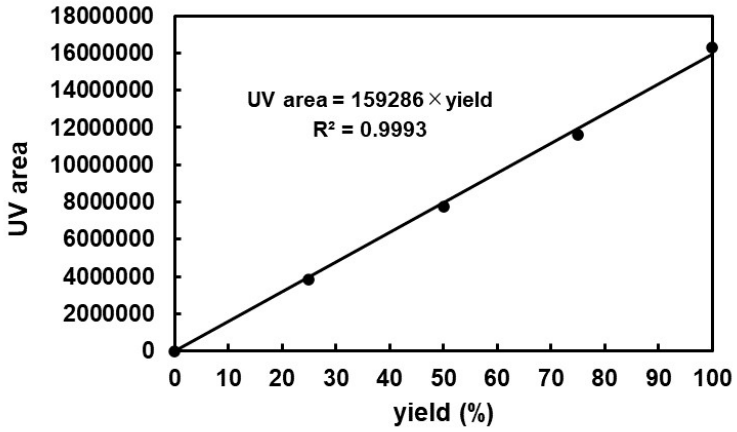
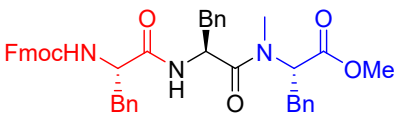


Figure S10. Calibration curve of **7q**

Fmoc-L-Phe-L-Phe-L-MePhe-OMe (7q)



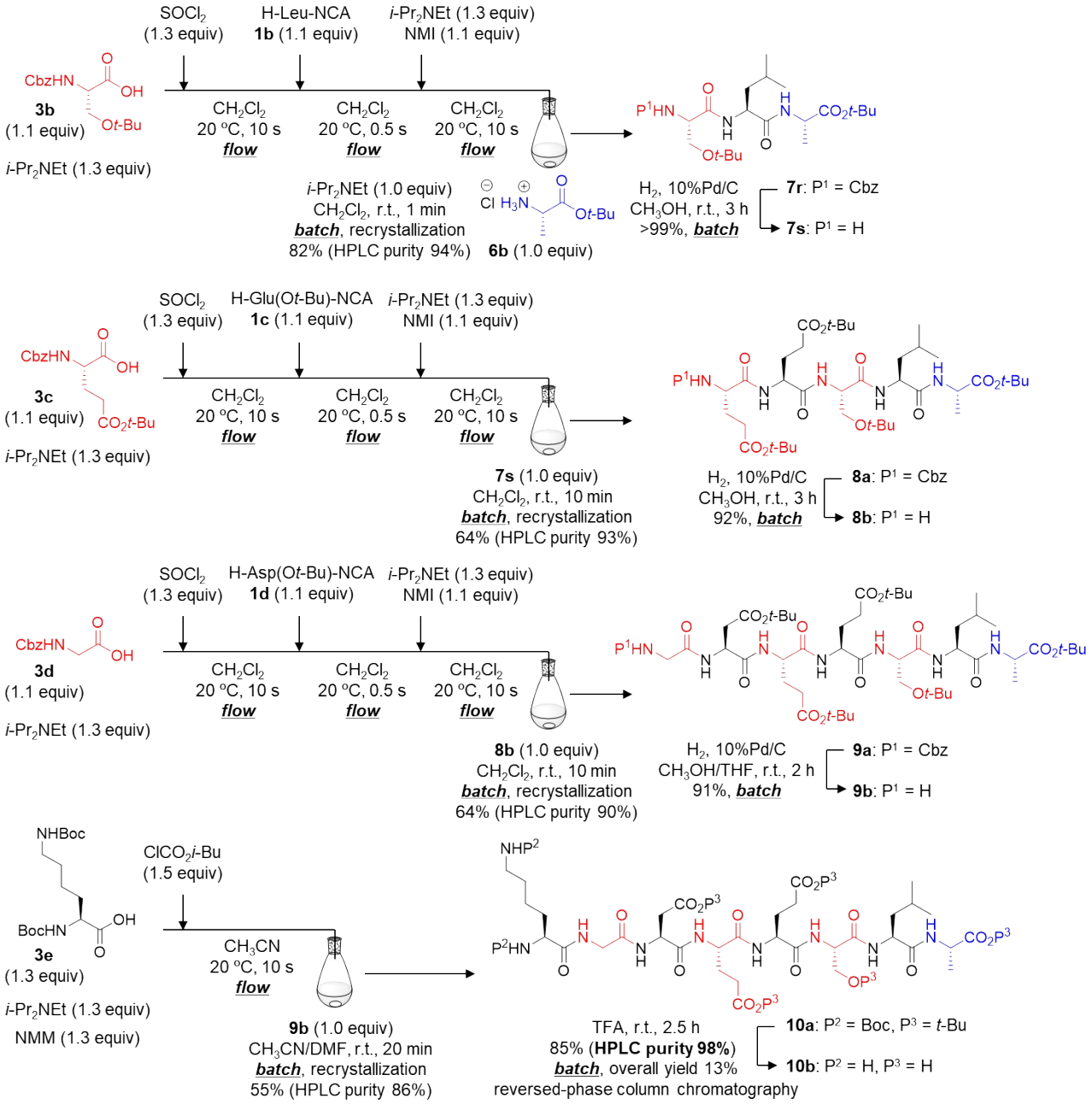
Chromatographic conditions: hexane : EtOAc = 90 : 10 to 20 : 80.

54.7 mg, 0.077 mmol, 51%.

White solid, mp 80-84 °C, IR (neat): 3296, 1739, 1724, 1637, 1538, 1496, 1452, 1256, 741 cm^{-1}; $[\alpha]^{21}_D$ = -55.9 (c 0.77, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): (^1H NMR spectrum was observed as conformational isomers. Only NMR signals of major isomer were shown) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.55-7.50 (m, 2H), 7.40 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.31 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.25-7.10 (m, 15H), 6.51 (d, *J* = 7.6 Hz, 1H), 5.26-5.24 (m, 1H), 5.13 (d, *J* = 7.6 Hz, 1H), 5.01-4.95 (m, 1H), 4.46-4.42 (m, 1H), 4.40-4.35 (m, 1H), 4.30-4.26

(m, 1H), 4.19-4.12 (m, 1H), 3.68 (s, 3H), 3.32 (dd, J = 4.8, 14.0 Hz, 1H), 3.02-2.80 (m, 5H), 2.68 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 170.8, 169.9, 155.8, 143.9, 141.4, 136.7, 136.3, 135.9, 129.7, 129.5, 129.0, 128.8, 128.6, 128.5, 127.9, 127.2, 127.1, 127.0, 125.3, 125.2, 120.1, 67.1, 58.7, 55.8, 52.4, 50.5, 47.2, 38.9, 38.3, 34.8, 32.7 ppm; HRMS (ESI): calcd for $[C_{44}H_{43}N_3O_6+Na]^+$ 732.3044, found 732.3046.

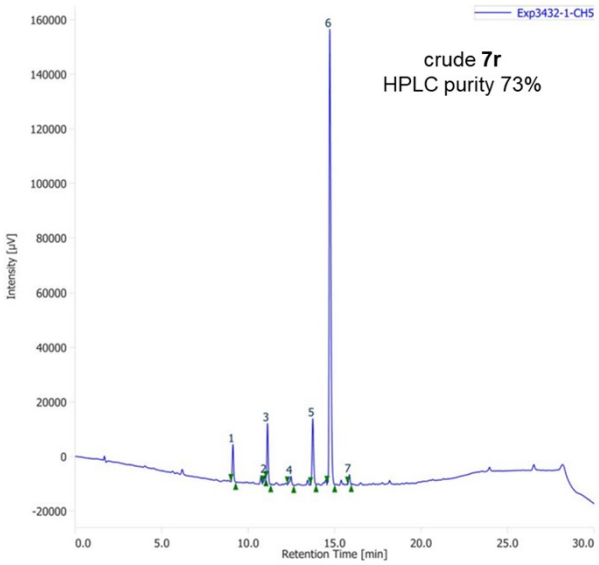
Procedure for synthesis of beefy meaty peptide (10b)



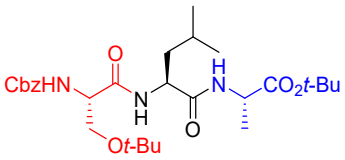
The employed micro-flow system was shown in **Figure S4**.

A solution of **Cbz-L-Ser(*t*-Bu)-OH** (**3b**) (0.324 M, 1.10 equiv), *N*,*N*-diisopropylethylamine (0.389 M, 1.32 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of thionyl chloride (0.233 M, 1.32 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1,061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. The resultant mixture and a solution of **H-L-Leu-NCA** (**1b**) (0.324 M, 1.10 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 72.9 mm, volume: 37 μL, reaction time: 0.50 s) at the same temperature. The resultant mixture and a solution of *N*,*N*-diisopropylethylamine (0.233 M, 1.32 equiv) and *N*-methylimidazole (0.194 M, 1.10 equiv)

in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 3 (inner diameter: 0.800 mm, length: 2,122 mm, volume: 1,067 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 90 s to reach a steady state, the resultant mixture was poured into flask including **H-L-Ala-O*t*-Bu·HCl** (**6b**) (1.00 equiv) and *N*,*N*-diisopropylethylamine (1.00 equiv) in CH_2Cl_2 for 265 s at room temperature. After being stirred for 1 min at room temperature, the reaction was quenched with 1 M HCl aq. (5 mL). Water was added to the mixture, which was extracted with CH_2Cl_2. The organic layer was washed with *sat.* $NaHCO_3$ aq., water, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* at room temperature. The crude tripeptide **7r** was obtained (HPLC purity: 73%).



Cbz-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (7r)

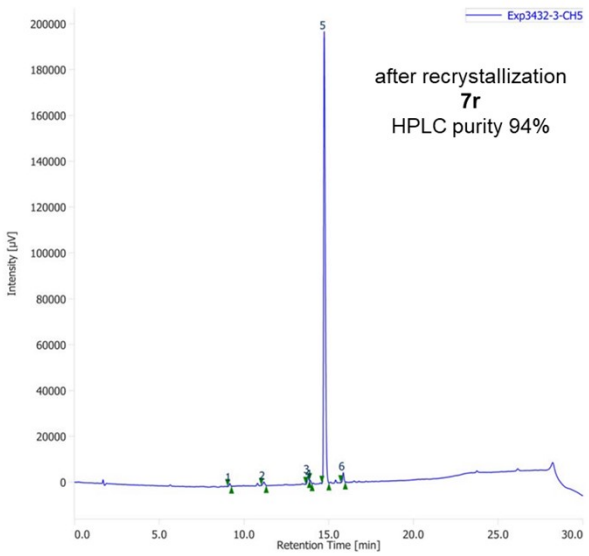


Purification method: The crude product was recrystallized from n-hexane.

HPLC conditions; COSMOSIL 5C_{18}-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min: 30 to 100%, 20-25 min: 100%, 25-27 min: 100 to 30%, 27-30 min: 30%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 °C, retention time: 14.7 min.

685.3 mg, 1.28 mmol, 82%, HPLC purity: 94%.

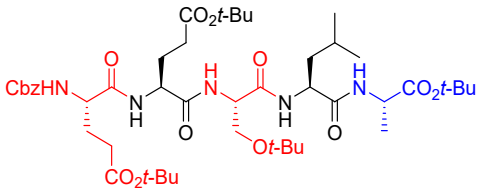
Colorless amorphous solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.33 (m, 5H), 6.93 (d, *J* = 7.8 Hz, 1H), 6.55 (d, *J* = 6.4 Hz, 1H), 5.96 (d, *J* = 4.8 Hz, 1H), 5.12 (s, 2H), 4.49-4.39 (m, 2H), 4.25 (brs, 1H), 3.85-3.83 (m, 1H), 3.43 (dd, *J* = 7.2, 8.8 Hz, 1H), 1.70-1.51 (m, 3H), 1.46 (s. 9H), 1.35 (d, *J* = 7.2 Hz, 3H), 1.18 (s, 9H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.0 Hz, 3H) ppm; HRMS (ESI): calcd for $[C_{28}H_{45}N_3O_7+H]^+$ 536.3330, found 536.3330, $[C_{28}H_{45}N_3O_7+Na]^+$ 558.3150, found 558.3152.



To a solution including semi-pure **Cbz-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu** (**7r**) (1.0 equiv) in CH_3OH, 10 wt% Pd/C (25 wt%) was added at room temperature. The reaction mixture was stirred under hydrogen atmosphere at room temperature. After being stirred for 3 h, the reaction mixture was filtered through a pad of charcoal and concentrated *in vacuo* at room temperature. The residue was dissolved in EtOAc, which was washed with 10% K_2CO_3 aq, water, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* at room temperature.

A solution of **Cbz-L-Glu(O*t*-Bu)-OH** (**3c**) (0.324 M, 1.10 equiv), *N*,*N*-diisopropylethylamine (0.389 M, 1.32 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of thionyl chloride (0.233 M, 1.32 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1,061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. The resultant mixture and a solution of **H-L-Glu(O*t*-Bu)-NCA** (**1c**) (0.324 M, 1.10 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 72.9 mm, volume: 37 μL, reaction time: 0.50 s) at the same temperature. The resultant mixture and a solution of *N*,*N*-diisopropylethylamine (0.233 M, 1.32 equiv) and *N*-methylimidazole (0.194 M, 1.10 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 3 (inner diameter: 0.800 mm, length: 2,122 mm, volume: 1,067 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 90 s to reach a steady state, the resultant mixture was poured into flask including semi-pure **H-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu** (**7s**) (1.00 equiv) in CH_2Cl_2 for 106 s at room temperature. After being stirred for 10 min at room temperature, the reaction was quenched with 1 M HCl aq. (3.5 mL). Water was added to the mixture, which was extracted with CH_2Cl_2. The organic layer was washed with water, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* at room temperature.

Cbz-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (8a)



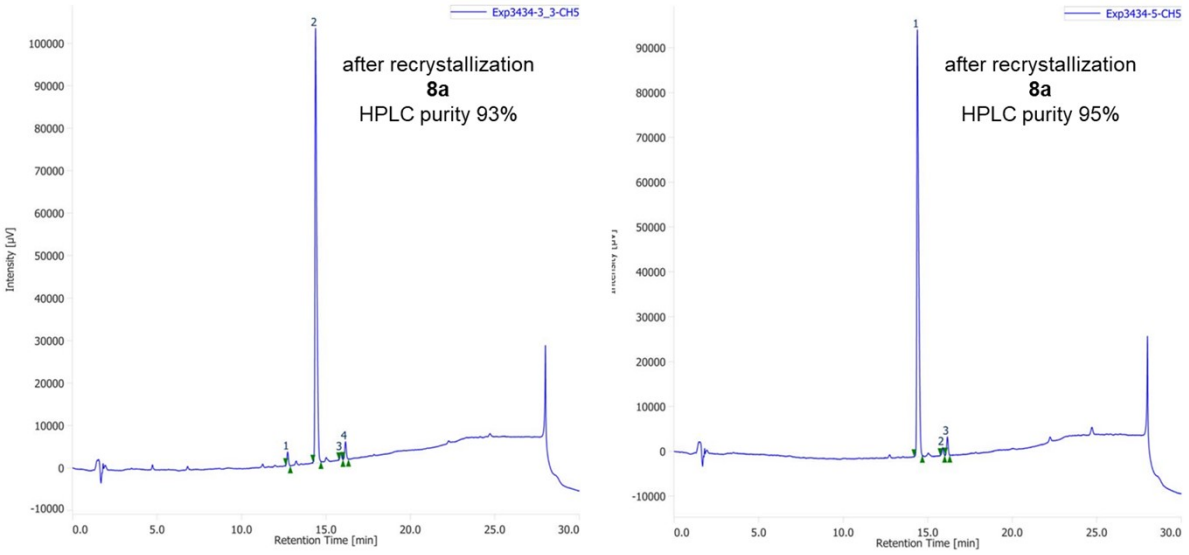
Purification method: The crude product was recrystallized from EtOAc.

HPLC conditions; COSMOSIL 5C_{18}-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min: 50 to 100%, 20-25 min: 100%, 25-30 min: 50%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 ℃, retention time: 14.5 min.

295.1 mg, 0.326 mmol, 52%, HPLC purity: 93%,

69.5 mg, 0.076 mmol, 12%, HPLC purity: 95%.

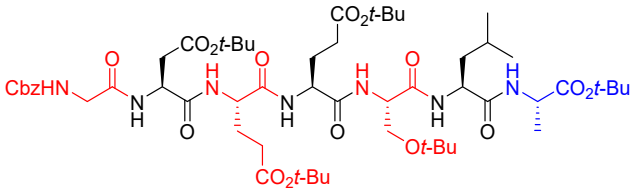
Colorless amorphous solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, *J* = 4.4 Hz, 1H), 7.36-7.31 (m, 5H), 7.11 (d, *J* = 8.0 Hz, 2H), 6.81 (d, *J* = 7.6 Hz, 1H), 5.79 (d, *J* = 5.6 Hz, 1H), 5.16 (d, *J* = 12.4 Hz, 1H), 5.08 (d, *J* = 12.4 Hz, 1H), 4.53-4.47 (m, 1H), 4.43-4.34 (m, 2H), 4.21-4.13 (m, 2H), 3.85 (dd, *J* = 3.7, 8.8 Hz, 1H), 3.52 (dd, *J* = 4.8, 8.8 Hz, 1H), 2.53-2.29 (m, 4H), 2.19-1.91 (m, 4H), 1.78-1.61 (m, 3H), 1.45 (s, 9H), 1.44 (s, 9H), 1.43 (s, 9H), 1.35 (d, *J* = 7.3 Hz, 3H), 1.17 (s, 9H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.90 (d, *J* = 6.4 Hz, 3H) ppm; HRMS (ESI): calcd for $[C_{46}H_{75}N_5O_{13}+H]^+$ 906.5434, found 906.5432, $[C_{46}H_{75}N_5O_{13}+Na]^+$ 928.5254, found 928.5254.



To a solution including semi-pure **Cbz-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (8a)** (1.0 equiv) in CH_3OH, 10 wt% Pd/C (25 wt%) was added at room temperature. The reaction mixture was stirred under hydrogen atmosphere at room temperature. After being stirred for 3 h, the reaction mixture was filtered through a pad of charcoal and concentrated *in vacuo* at room temperature. The residue was dissolved in EtOAc, which was washed with 1M NaOH aq., water, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* at room temperature.

A solution of **Cbz-Gly-OH** (**3d**) (0.324 M, 1.10 equiv), *N*,*N*-diisopropylethylamine (0.389 M, 1.32 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of thionyl chloride (0.233 M, 1.32 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. The resultant mixture and a solution of **H-L-Asp(O*t*-Bu)-NCA** (**1d**) (0.324 M, 1.10 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 72.9 mm, volume: 37 μL, reaction time: 0.50 s) at the same temperature. The resultant mixture and a solution of *N*,*N*-diisopropylethylamine (0.233 M, 1.32 equiv) and *N*-methylimidazole (0.194 M, 1.10 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 3 (inner diameter: 0.800 mm, length: 2122 mm, volume: 1067 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 90 s to reach a steady state, the resultant mixture was poured into flask including semi-pure **H-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu** (**8b**) (1.00 equiv) in CH_2Cl_2 for 33 s at room temperature. After being stirred for 10 min at room temperature, the reaction was quenched with 1 M HCl aq. (2 mL). Water was added to the mixture, which was extracted with CH_2Cl_2. The organic layer was washed with water, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* at room temperature.

Cbz-Gly-L-Asp(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (9a)

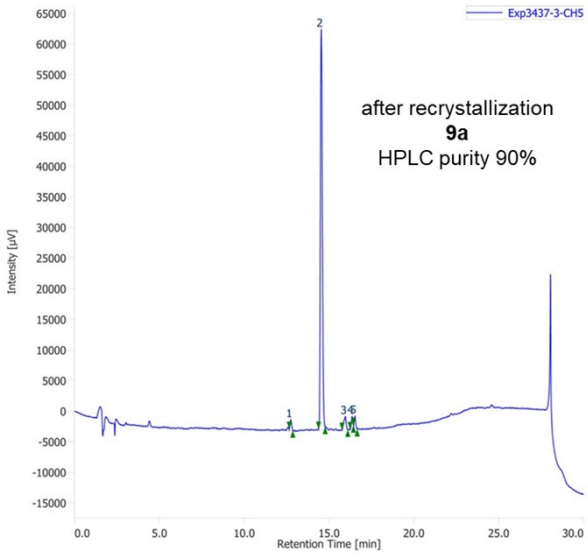


Purification method: The crude product was recrystallized from EtOAc-CH_3OH.

HPLC conditions; COSMOSIL 5C_{18}-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min: 50 to 100%, 20-25 min: 100%, 25-30 min: 50%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 °C, retention time: 14.5 min.

135.6 mg, 0.120 mmol, 64%, HPLC purity: 90%.

Colorless amorphous solid, ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.18 (d, J = 7.6 Hz, 1H), 8.11 (d, J = 6.8 Hz, 1H), 7.97-7.94 (m, 2H), 7.87 (d, J = 7.6 Hz, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.48 (brt, J = 6.0 Hz, 1H), 7.35-7.32 (m, 5H), 5.02 (s, 2H), 4.63-4.58 (m, 1H), 4.40-4.21 (m, 4H), 4.11-4.08 (m, 1H), 3.64 (d, J = 6.0 Hz, 2H), 3.46-3.41 (m, 1H), 3.33 (overlapped with H_2O, 1H), 2.64 (dd, J = 4.8, 16.0 Hz, 1H), 2.50 (overlapped with DMSO, 1H), 2.28-2.14 (m, 4H), 1.87 (brs, 2H), 1.78-1.70 (m, 2H), 1.66-1.59 (m, 1H), 1.47-1.34 (m, 38H), 1.23 (d, J = 7.2 Hz, 3H), 1.09 (s, 9H), 0.87 (d, J = 6.8 Hz, 3H), 0.84 (d, J = 6.4 Hz, 3H) ppm; HRMS (ESI): calcd for $[C_{56}H_{91}N_7O_{17}+H]^+$ 1134.6544, found 1134.6542, $[C_{56}H_{91}N_7O_{17}+Na]^+$ 1156.6364, found 1156.6363.

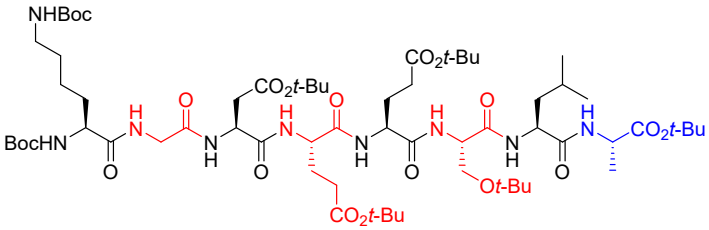


To a solution including semi-pure **Cbz-Gly-L-Asp(*t*-Bu)-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu** (**9a**) (1.0 equiv) in CH_3OH-THF, 10 wt% Pd/C (25 wt%) was added at room temperature. The reaction mixture was stirred under hydrogen atmosphere at room temperature. After being stirred for 2 h, the reaction mixture was filtered through a pad of charcoal and concentrated *in vacuo* at room temperature. The residue was dissolved in EtOAc-CH_3OH, which was washed with 1 M NaOH aq., water, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* at room temperature.

The employed micro-flow system was shown in **Figure S5**.

A solution of **Boc-Lys(Boc)-OH** (**3e**) (0.333 M, 1.27 equiv), *N*-methylmorpholine (0.333 M, 1.27 equiv) *N*,*N*-diisopropylethylamine (0.333 M, 1.27 equiv) in CH_3CN (flow rate: 1.20 mL/min), a solution of isobutyl chloroformate (0.240 M, 1.52 equiv) in CH_3CN (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1,061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 60 s to reach a steady state, the resultant mixture was poured into flask including semi-pure **H-Gly-L-Asp(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu** (**9b**) (1.00 equiv) in CH_3CN-DMF for 20 s at room temperature. After being stirred for 20 min at room temperature, the reaction was quenched with 1 M HCl aq. (2 mL). Water was added to the mixture, which was extracted with EtOAc. The organic layer was washed with water, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* at room temperature.

Boc-L-Lys(Boc)-Gly-L-Asp(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (10a)

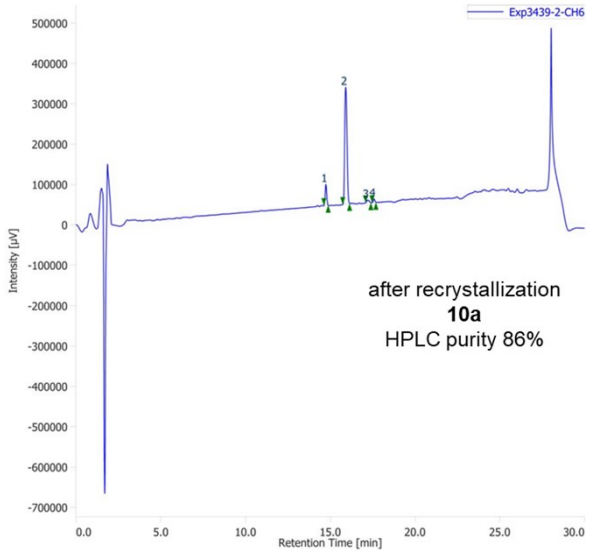


Purification method: The crude product was recrystallized from CH_3OH.

HPLC conditions; COSMOSIL $5C_{18}$-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min: 50 to 100%, 20-25 min: 100%, 25-30 min: 50%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 °C, retention time: 15.9 min.

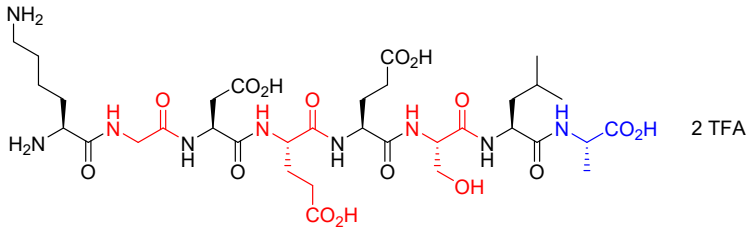
76.3 mg, 0.058 mmol, 55%, HPLC purity: 86%.

Colorless amorphous solid, ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11 (d, J = 6.8 Hz, 1H), 8.10 (d, J = 8.4 Hz, 1H), 8.04 (brt, J = 5.2 Hz, 1H), 7.95 (d, J = 6.8 Hz, 1H), 7.94 (d, J = 6.8 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 8.8 Hz, 1H), 6.91 (d, J = 7.2 Hz, 1H), 6.75 (brt, J = 4.8 Hz, 1H), 4.65-4.59 (m, 1H), 4.38-4.21 (m, 4H), 4.11-4.07 (m, 1H), 3.88-3.83 (m, 1H), 3.77-3.65 (m, 2H), 3.51-3.40 (m, 2H), 2.90-2.85 (m, 2H), 2.66 (dd, J = 5.2, 16.0 Hz, 1H), 2.43 (dd, J = 8.2, 16.0 Hz, overlapped with DMSO, 1H), 2.28-2.13 (m, 4H), 1.92-1.83 (m, 2H), 1.77-1.69 (m, 2H), 1.66-1.58 (m, 1H), 1.47-1.33 (m, 62H), 1.24 (d, J = 7.2 Hz, 3H), 1.09 (s, 9H), 0.87 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H) ppm; HRMS (ESI): calcd for $[C_{64}H_{113}N_9O_{20}+Na]^+$ 1350.7994, found 1350.7993.



A solution of semi-pure **Boc-L-Lys(Boc)-Gly-L-Asp(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (10a)** (53.1 mg, 0.040 mmol) in TFA was stirred at room temperature for 2.5 h. The mixture was concentrated *in vacuo* at room temperature and the residue was co-evaporated with water.

Beefy meaty peptide·2TFA (H-L-Lys-Gly-L-Asp-L-Glu-L-Glu-L-Ser-L-Leu-L-Ala-OH·2TFA) (10b)

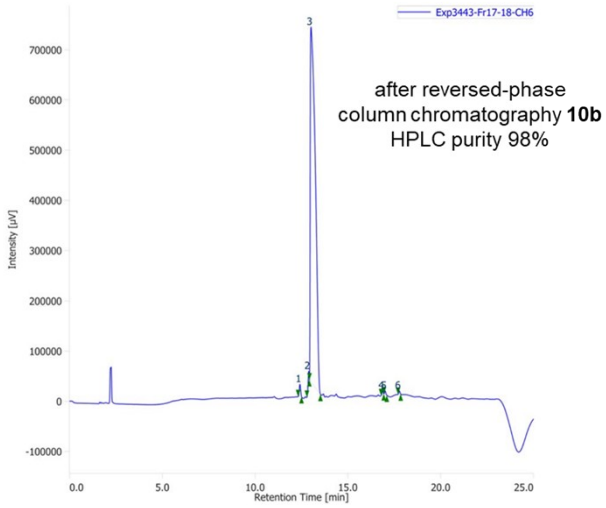


Chromatographic conditions (reverse phase): Biotage Sfär Bio C18 Duo 25 g, 0.1% TFA in H_2O : 0.1% TFA in CH_3CN = 100 : 0 to 70 : 30, flow rate 5 mL/min, detection wavelength 215 nm and Biotage Sfär Bio C18 Duo 10 g, 0.1% TFA in H_2O : 0.1% TFA in CH_3CN = 100 : 0 to 70 : 30, flow rate 2 mL/min, detection wavelength 215 nm.

HPLC conditions; COSMOSIL 5C_{18}-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% TFA/H_2O+0.1% TFA, 0-15 min: 0 to 20%, 15-20 min: 20%, 20-25 min: 0%, flow rate 1.0 mL/min, detection wavelength 215 nm, temperature 40 ℃, retention time: 13.1 min.

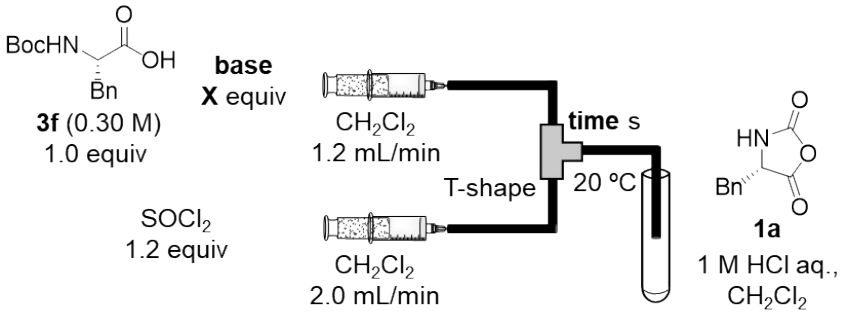
36.4 mg, 0.034 mmol, 85% (total yield 13%), HPLC purity: 98%.

Colorless amorphous solid, ^{1}H NMR (400 MHz, D_2O): δ 4.79 (overlapped with H_2O, 1H), 4.44 (t, J = 6.0 Hz, 1H), 4.41-4.33 (m, 4H), 4.08 (t, J = 6.8 Hz, 1H), 4.04 (s, 2H), 3.85 (d, J = 6.0 Hz, 2H), 3.01 (t, J = 8.0 Hz, 2H), 2.98-2.94 (m, 1H), 2.89 (dd, J = 6.8, 17.2 Hz, 1H), 2.51-2.48 (m, 4H), 2.18-2.11 (m, 2H), 2.06-1.98 (m, 2H), 1.96-1.92 (m, 2H), 1.76-1.63 (m, 5H), 1.53-1.47 (m, 2H), 1.42 (d, J = 7.2 Hz, 3H), 0.93 (d, J = 5.6 Hz, 3H), 0.88 (d, J = 6.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, D_2O, CH_3CN was used as internal standard): δ 176.1, 176.0, 175.2, 173.2, 173.1, 172.32, 172.27, 171.5, 170.5, 169.8, 169.4, 161.9 (q, $J_{C\text{-}F}$ = 35.2 Hz), 115.5 (q, $J_{C\text{-}F}$ = 290.8 Hz), 60.0, 54.6, 52.5, 52.3, 52.1, 51.3, 49.1, 47.7, 41.5, 38.8, 38.1, 34.4, 29.4, 29.0, 25.4, 25.0, 24.9, 23.3, 21.3, 20.3, 19.7, 15.2 ppm; HRMS (ESI): calcd for $[C_{34}H_{57}N_9O_{16}+H]^+$ 848.3996, found 848.3998, $[C_{34}H_{57}N_9O_{16}+Na]^+$ 870.3815, found 870.3815.



Examination of synthesis of NCA 1a

Table S6. Examination of bases, amounts of base, and reaction time for synthesis of 1a *via* Leuchs method



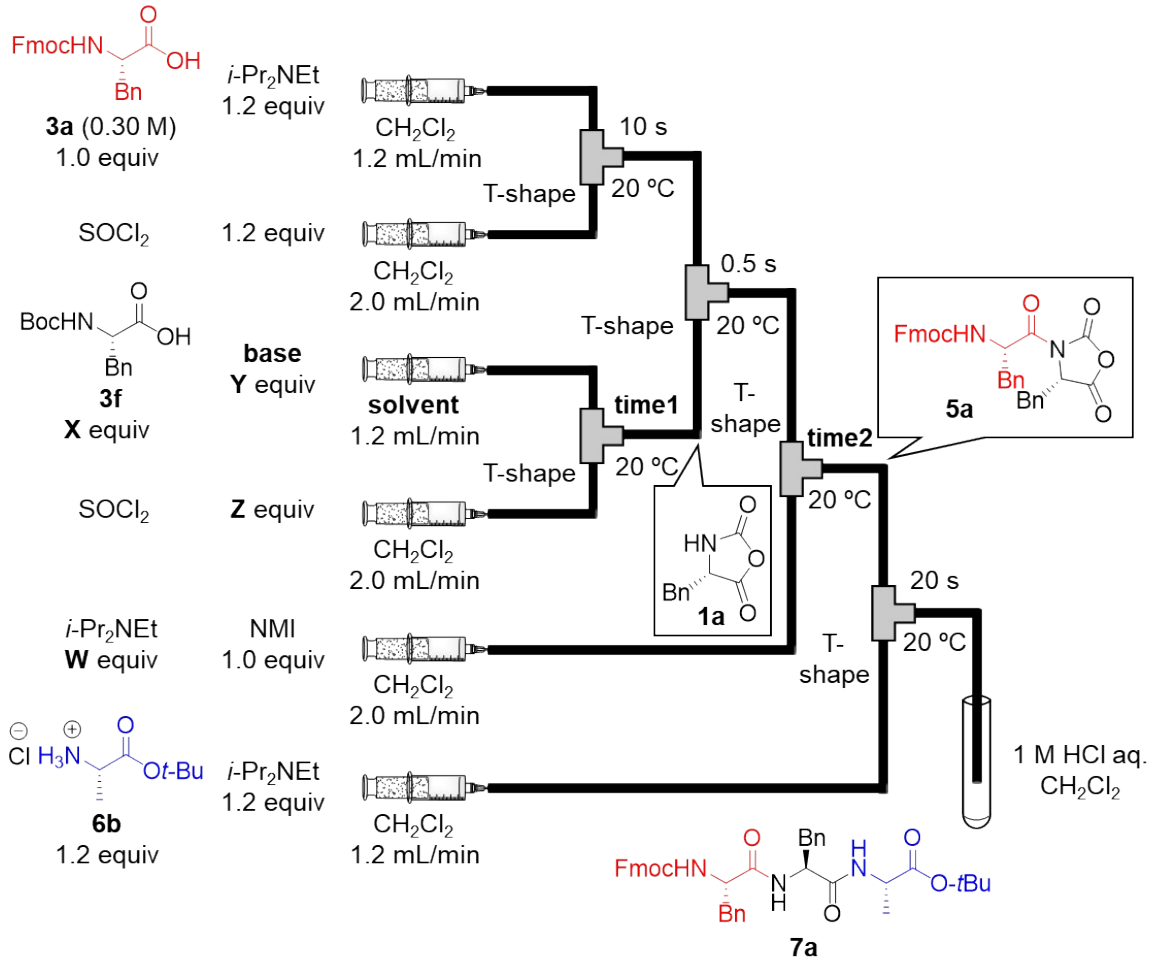
entry	**base**	**X equiv**	**time** (s)	^{1}H NMR yield (%)
1	NMM	2.4	30	>99
2	Me_2NBn	2.4	30	90
3	*i*-Pr_2NEt	2.4	30	21
4	none	0	10	0
5	NMM	2.4	10	>99
6	Me_2NBn	2.4	10	89
7	*i*-Pr_2NEt	2.4	10	39
8	*i*-Pr_2NEt	2.0	10	>99
9	*i*-Pr_2NEt	1.2	10	>99

The employed micro-flow system was shown in **Figure S5**.

A solution of **Boc-L-Phe-OH (3f)** (0.300 M, 1.00 equiv), **base** (**X equiv**) in CH_2Cl_2 (flow rate: 1.20 mL/min) and a solution of thionyl chloride (0.216 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, length: 1,061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 60 s to reach a steady state, the resultant mixture was poured into 1 M HCl aq. (1.5 mL) and CH_2Cl_2 (6 mL) for 25 s at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. Yields were determined by ^{1}H NMR using 1,1,2-trichloroethane as internal standard.

Examination of one-flow synthesis of 7a

Table S7. Examination of bases, solvents, amounts of substrates and base, and reaction times for one-flow synthesis of 7a



entry	base	solvent	X equiv	Y equiv	Z equiv	W equiv	time1 (s)	time2 (s)	HPLC yield (%)
1	NMM	CH_2Cl_2	1.0	2.4	1.2	1.2	30	10	<1
2	NMM	CH_2Cl_2	1.0	2.4	1.2	3.6	30	10	5
3	*i*-Pr_2NEt	CH_2Cl_2	1.0	1.2	1.2	2.4	10	10	38
4	*i*-Pr_2NEt	CH_2Cl_2	1.0	1.2	1.2	2.4	10	20	43
5	*i*-Pr_2NEt	CH_2Cl_2	1.5	1.8	1.8	3.6	10	20	62
6	*i*-Pr_2NEt	CH_2Cl_2	2.0	2.4	2.4	4.8	10	20	63
7	*i*-Pr_2NEt	CH_2Cl_2	1.5	1.8	1.8	3.6	20	20	68
8	*i*-Pr_2NEt	CH_2Cl_2	1.5	1.8	1.8	3.6	50	20	77 (80)[a]
9	*i*-Pr_2NEt	CH_2Cl_2	1.5	1.8	1.8	3.6	90	20	78
10	*i*-Pr_2NEt	THF	1.5	1.8	1.8	3.6	50	20	79

11	*i*-Pr_2NEt	CH_3CN	1.5	1.8	1.8	3.6	50	20	78

[a]Isolated yiled.

The employed micro-flow system was shown in **Figure S6**.

A solution of **Fmoc-L-Phe-OH** (**3a**) (0.300 M, 1.00 equiv), *N*,*N*-diisopropylethylamine (0.360 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of thionyl chloride (0.216 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1061 mm, volume: 533 μL, reaction time: 10 s) at the same temperature. A solution of **Boc-L-Phe-OH** (**3f**) (**X** equiv), *N*,*N*-diisopropylethylamine (**Y** equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of thionyl chloride (**Z** equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, **reaction time1**) at the same temperature. The two resultant mixtures were introduced to T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 3 (inner diameter: 0.800 mm, length: 106 mm, volume: 53 μL, reaction time: 0.50 s) at the same temperature. The resultant mixture and a solution of a solution of *N*,*N*-diisopropylethylamine (**W** equiv) and *N*-methylimidazole (0.180 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 4 (inner diameter: 0.800 mm, **reaction time2**) at the same temperature. The resultant mixture and a solution of **H-L-Ala-O*t*-Bu·HCl** (**6b**) (0.360 M, 1.20 equiv) and *N*,*N*-diisopropylethylamine (0.360 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 5 (inner diameter: 0.800 mm, length: 6,366 mm, volume: 3,200 μL, reaction time: 20 s) at the same temperature. After being eluted for 120-190 s to reach a steady state, the resultant mixture was poured into 1 M HCl aq. (1.5 mL) and CH_2Cl_2 (8 mL) for 25 s at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. In case of examination of reaction conditions, yields were determined by HPLC-UV analysis (conditions: COSMOSIL 5C_{18}-AR-II 4.6 mm I.D. × 150 mm, CH_3CN+0.1% formic acid/H_2O+0.1% formic acid, 0-20 min: 30 to 100%, 20-25 min: 100%, 25-27 min: 100 to 30%, 27-30 min: 30%, flow rate 1.0 mL/min, detection wavelength 254 nm, temperature 40 °C, retention time: 17.3 min) using a calibration curve shown in **Figure S9** in order to allow rapid analysis of reaction results without silica gel column chromatography. The residue was purified by silica gel column chromatography (CH_2Cl_2 : CH_3OH = 98 : 2 to 87 : 13) and recrystallization from CH_2Cl_2/hexane (**7a**: 79.6 mg, 0.12 mmol, 80%, HPLC purity: 95%). Racemization was not detected by HPLC-UV analysis (DAICEL CHIRALPAK IH 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 8.8 min.).

The comparison of cost and time between the developed approach and previously reported approach

The comparison between our developed approach for synthesis of Cbz-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (**7r**) and Wassenaar's approach[6] for synthesis of the similar Fmoc-L-Ser(*t*-Bu)-L-Leu-L-Ala-HMP (**7t**) resin was conducted. Employed conditions for removal of protecting groups at the *N*-terminus of peptides were different between our developed approach (Pd/C, H_2) and Wassenaar's approach (piperidine), therefore, we only compared syntheses of *C*,*N*-protected tripeptides **7r** and **7t**.

Postulate: Wassenaar's approach synthesized the beefy meaty peptide (BMP) in 86% total yield based on the starting resin. Wassenaar *et al*. used *N*-Fmoc-protected amino acids and HMP resin for peptide chain elongation. On the other hand, we used *N*-Cbz-protected amino acids, *C,N*-unprotected amino acids (for preparation of NCAs), and *C*-*t*-Bu-protected amino acids as components for one-flow 3CC coupling. Although Wassenaar's approach required the excess amounts of piperidine for removal of Fmoc group, the detailed amount used was not reported, therefore, we did not include the cost of piperidine in the comparison shown below.

Table S8. The cost of coupling substrates, reagents, and bases

reagents	manufacturer	Mw.	volume	density	cost (JPY)	cost/mmol
Fmoc-Ser(*t*-Bu)-OH	Watanabe	383.45	100 g	–	48,000	184
Cbz-Ser(t-Bu)-OH	Watanabe	295.34	100 g	–	68,000	201
Fmoc-Leu-OH	Watanabe	353.42	100 g	–	15,000	53.0
H-Leu-OH	Watanabe	131.17	1 kg	–	24,000	3.15
Fmoc-Phe-OH	Watanabe	387.44	100 g	–	15,000	58.1
Boc-Phe-OH	Watanabe	265.31	100 g	–	18,000	47.8
Fmoc-Ala-HMP resin	Watanabe	0.3-0.9 mmol/g[a]	5 g	–	13,000	4,333
H-Ala-O*t*-Bu·HCl	Watanabe	181.66	25 g	–	46,000	334
HBTU	TCI	379.25	100 g	–	21,600	81.9
HOBt monohydrate	TCI	153.15	200 g	–	21,800	16.7
i-Pr_2NEt	TCI	129.25	500 mL	0.76	12,900	4.39

triphosgene	TCI	296.73	250 g	–	43,500	51.6
NMM	TCI	101.15	500 mL	0.92	4,000	0.880
NaOH	Wako	40.0	5 kg	–	13,140	0.105
$SOCl_2$	TCI	118.96	500 mL	1.64	3,100	0.450
NMI	TCI	82.11	500 g	1.04	12,900	2.04

[a]Loading amount of resin. Average loading amount (= 0.6 mmol/g) was used for calculate cost/mmol. Watanabe = Watanabe Chemical Industries, LTD. TCI = Tokyo Chemical Industry Co., Ltd. Wako = FUJIFILM Wako Pure Chemical Corporation. The last access date (3rd, Feb, 2023).

Wassenaar's approach for synthesis of 7t from Fmoc-Ala-HMP resin (0.54 mmol/g, 2.25 g, 1.215 mmol)
Time: 4 min (NMP wash) + 2 min (30%(v/v) piperidine/NMP) + 20 min (30%(v/v) piperidine/NMP) + 10 min (NMP wash) + 60 min (coupling step) + 4 min (NMP wash) + 2 min (30%(v/v) piperidine/NMP) + 20 min (30%(v/v) piperidine/NMP) + 10 min (NMP wash) + 60 min (coupling step) = 192 min.
We assumed that the yield of **7t** was 100% for the calculation of productivity. Productivity: 0.00632 mmol/min.
Time for synthesis of 1 mmol of **7t**: 158 min/mmol.
Cost: (4,333 JPY/mmol × 1 equiv of Fmoc-Ala-HMP resin) + (53.0 JPY/mmol × 3.29 equiv of Fmoc-Leu-OH) + (184 JPY/mmol × 3.29 equiv of Fmoc-Ser(*t*-Bu)-OH) + (81.9 JPY/mmol × 3.29 equiv of HBTU × 2) + (16.7 JPY/mmol × 3.29 equiv of HOBt × 2) + (4.39 JPY/mmol × 6.58 equiv of *i*-Pr_2NEt × 2) = 5,819 JPY/mmol.

Our developed approach for synthesis of 7r

The preparation of H-L-Leu-NCA (1b).[1)]

Time: 1335 s (collection time) = 1335 s.
24.2 mmol, Yield 91%. Productivity: 1.09 mmol/min.
Time for synthesis of 1 mmol of **1b**: 0.917 min/mmol.
Cost: (3.15 JPY/mmol × 1.0 equiv of H-Leu-OH) + (51.6 JPY/mmol × 1.0 equiv of triphosgene) + (0.880 JPY/mmol × 4.5 equiv of NMM) + (0.105 JPY/mmol × 1.0 equiv of NaOH) = 58.8 JPY/mmol.
The cost for synthesis of 1 mmol of **1b**: 58.8/0.91 = 64.7 JPY/mmol.

The synthesis of 7r (HPLC purity: 94%) from 1b

Time: 265 s (collection time) + 60 s = 325 s.
1.28 mmol, Yield: 82% (HPLC purity: 94%). Productivity: 0.236 mmol/min (HPLC purity: 94%).
Time for synthesis of 1 mmol of **7r** (HPLC purity: 94%): 4.23 min/mmol.
Cost: (201 JPY/mmol × 1.10 equiv of Cbz-Ser(*t*-Bu)-OH (**3b**)) + (64.7 JPY/mmol × 1.10 equiv of **1b**) + (0.450 JPY/mmol × 1.32 equiv of $SOCl_2$) + (4.39 JPY/mmol × 3.64 equiv of *i*-Pr_2NEt) + (2.04 JPY/mmol × 1.1 equiv of NMI) + (334 JPY/mmol × 1.0 equiv of H-Ala-O*t*-Bu·HCl) = 645 JPY/mmol.
The total time for synthesis of **7r** (HPLC purity: 94%) from H-L-Leu-OH: 5.15 min/mmol.

The total cost for synthesis of 1 mmol of **7r** (HPLC purity: 94%): 645/0.82 = 787 (JPY/mmol).

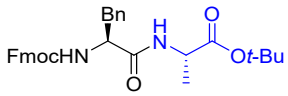
The procedure for synthesis of *epi*-7a-1 and *epi*-7a-2

To a solution of **Fmoc-L-Phe-OH** or **Fmoc-D-Phe-OH** (1.0 equiv) and *N*-methylmorpholine (1.1 equiv) in CH_2Cl_2, isobutyl chloroformate (1.0 equiv) was added at –20 °C. After stirred at –20 °C for 1 min, **H-L-Ala-O*t*-Bu·HCl** (1.1 equiv) and *N*,*N*-diisopropylethylamine (1.1 equiv) was added at –20 °C. After stirred at room temperature for 15 min, the reaction mixture was poured into a solution of CH_2Cl_2 and 1 M HCl aq. at the same temperature. The aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography.

To a solution of **Fmoc-L-Phe-L-Ala-O*t*-Bu** or **Fmoc-D-Phe-L-Ala-O*t*-Bu** (1.0 equiv) in CH_2Cl_2, DBU (1.0 equiv) was added at 0 °C. After reaction was monitored by thin-layer chromatography. After the reaction was completion, the resultant solution was purified by silica gel column chromatography.

To a solution of **Fmoc-L-Phe-OH** or **Fmoc-D-Phe-OH** (1.0 equiv) and *N*-methylmorpholine (1.1 equiv) in CH_2Cl_2, isobutyl chloroformate (1.1 equiv) was added at –20 °C. After stirred at –20 °C for 1 min, **H-L-Phe-L-Ala-O*t*-Bu** or **H-D-Phe-L-Ala-O*t*-Bu** (1.1 equiv) was added at –20 °C. After stirred at room temperature for 15 min, the reaction mixture was poured into a solution of CH_2Cl_2 and 1 M HCl aq. at the same temperature. The aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with *sat*. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography.

Fmoc-L-Phe-L-Ala-O*t*-Bu (S2)



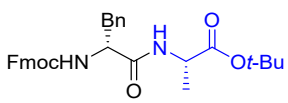
Chromatographic conditions: hexane : EtOAc = 90 : 10 to 20 : 80.

557.4 mg, 1.08 mmol, >99%.

White solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, *J* = 7.2 Hz, 2H), 7.51-7.46 (m, 2H), 7.35-7.31 (m, 2H), 7.24-7.12 (m, 7H), 7.02 (d, *J* = 6.8 Hz, 1H), 5.96 (d, *J* = 8.0 Hz, 1H), 4.64-4.60 (m, 1H), 4.43-4.34 (m, 2H), 4.20-4.15 (m, 1H), 4.10 (t, *J* = 6.8 Hz, 1H), 3.12-3.02 (m, 2H), 1.42 (s, 9H), 1.29 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 170.8, 156.0, 143.8, 143.7, 141.2, 136.5, 129.4, 128.5, 127.6, 127.0, 126.9, 125.15, 125.07, 119.9, 81.8, 67.1, 56.0, 48.7, 47.0, 38.8, 27.9, 18.3 ppm.

Spectral data of ^{1}H NMR and ^{13}C NMR were identical to those previously reported[7].

Fmoc-D-Phe-L-Ala-O*t*-Bu (S3)

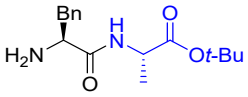


Chromatographic conditions: hexane : EtOAc = 90 : 10 to 20 : 80.

541.1 mg, 1.05 mmol, >99%.

White solid, mp 133-135 °C, IR (neat): 3299, 1732, 1716, 1655, 1540, 1450, 1253, 1147, 757, 741 cm^{-1}; $[\alpha]^{24}_{D}$ = +18.5 (c 2.38, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.53 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.38 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.31-7.20 (m, 7H), 6.32 (d, *J* = 4.8 Hz, 1H), 5.55 (d, *J* = 7.2 Hz, 1H), 4.47-4.31 (m, 4H), 4.18 (t, *J* = 7.2 Hz, 1H), 3.15-3.01 (m, 2H), 1.41 (s, 9H), 1.20 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 170.1, 155.9, 143.9, 143.8, 141.4, 136.5, 129.5, 128.8, 127.8, 127.2, 125.2, 120.1, 82.2, 67.2, 56.3, 48.7, 47.2, 39.1, 28.0, 18.4 ppm; HRMS (ESI): calcd for $[C_{31}H_{34}N_2O_5+Na]^+$ 537.2360, found 537.2363.

H-L-Phe-L-Ala-O*t*-Bu (S4)



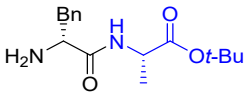
Chromatographic conditions: CH_2Cl_2 : CH_3OH = 98 : 2 to 80 : 20.

212.7 mg, 0.73 mmol, 93%.

Colorless oil, ^1H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 7.6 Hz, 1H), 7.33-7.29 (m, 2H), 7.25-7.21 (m, 3H), 4.50-4.42 (m, 1H), 3.62 (dd, *J* = 3.6, 9.6 Hz, 1H), 3.24 (dd, *J* = 3.6, 14.0 Hz, 1H), 2.72 (dd, *J* = 9.2, 14.0 Hz, 1H), 1.46 (s, 11H), 1.36 (d, *J* = 7.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 172.2, 137.8, 129.4, 128.7, 126.8, 81.8, 56.3, 48.3, 41.0, 28.0, 18.6 ppm.

Spectral data of ^1H NMR were identical to those previously reported[8].

H-D-Phe-L-Ala-O*t*-Bu (S5)



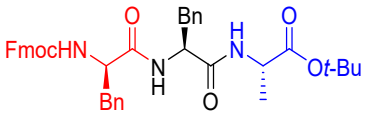
Chromatographic conditions: CH_2Cl_2 : CH_3OH = 98 : 2 to 80 : 20.

229.2 mg, 0.78 mmol, 85%.

Colorless oil, ^1H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, *J* = 7.6 Hz, 1H), 7.34-7.30 (m, 2H), 7.26-7.22 (m, 3H), 4.50-4.43 (m, 1H), 3.59 (dd, *J* = 4.4, 10.0 Hz, 1H), 3.30 (dd, *J* = 4.0, 13.6 Hz, 1H), 2.66 (dd, *J* = 9.6, 13.6 Hz, 1H), 1.47 (s, 11H), 1.36 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.9, 172.3, 138.3, 129.4, 128.8, 126.9, 81.9, 56.8, 48.4, 41.2, 28.1, 18.7 ppm.

Spectral data of ^1H NMR were identical to those previously reported[8].

Fmoc-D-Phe-L-Phe-L-Ala-O*t*-Bu (*epi*-7a-1)



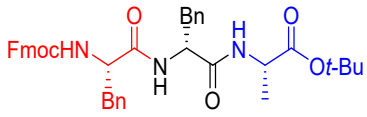
Chromatographic conditions: CH_2Cl_2 : CH_3OH = 99 : 1 to 90 : 10, and the crude product was recrystallized from CH_2Cl_2/hexane.

HPLC conditions; DAICEL CHIRALPAK IH 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 13.1 min.

377.3 mg, 0.57 mmol, 83%.

White solid, mp 97-101 ℃, IR (neat): 3283, 1732, 1644, 1541, 1245, 1224, 1148, 757, 741 cm^{-1}; $[\alpha]^{24}_D$ = +5.20 (c 2.92, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.72-7.70 (m, 2H), 7.48 (dd, *J* = 8.0, 8.0 Hz, 2H), 7.34 (dd, *J* = 7.2, 8.0 Hz, 2H), 7.25-6.96 (m, 14H), 5.94 (d, *J* = 8.4 Hz, 1H), 4.84-4.81 (m, 1H), 4.52 (d, *J* = 7.2 Hz, 1H), 4.38-4.31 (m, 2H), 4.19-4.08 (m, 2H), 3.05-2.87 (m, 4H), 1.39 (s, 9H), 1.26 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 171.2, 170.3, 156.0, 143.9, 143.8, 141.3, 136.5, 136.4, 129.5, 128.63, 128.56, 127.7, 127.1, 127.0, 125.2, 125.1, 120.0, 81.9, 67.2, 56.4, 54.2, 48.9, 47.1, 38.8, 38.3, 28.0, 18.3 ppm; HRMS (ESI): calcd for $[C_{40}H_{43}N_3O_6+Na]^+$ 684.3044, found 684.3047.

Fmoc-L-Phe-D-Phe-L-Ala-O*t*-Bu (*epi*-7a-2)



Chromatographic conditions: hexane : EtOAc = 90 : 10 to 20 : 80.

HPLC conditions; DAICEL CHIRALPAK IH 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 10.9 min.

453.8 mg, 0.69 mmol, 97%.

Colorless solid, mp 138-142 ℃, IR (neat): 3285, 1732, 1705, 1640, 1533, 1450, 1226, 1147, 757, 741 cm^{-1}; $[\alpha]^{24}_D$ = +1.85 (c 3.19, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, *J* = 7.6 Hz, 2H), 7.51 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.38 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.29-7.13 (m, 10H), 7.24 (d, *J* = 6.8 Hz, 2H), 6.65-6.60 (m, 2H), 5.67 (d, *J* = 7.6 Hz, 1H), 4.71 (d, *J* = 6.4 Hz, 1H), 4.40-4.30 (m, 3H), 4.24-4.12 (m, 2H), 3.04-3.02 (m, 3H), 2.80 (dd, *J* = 8.0, 13.2 Hz, 1H), 1.38 (s, 9H), 1.15 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 170.9, 169.7, 156.1, 143.8, 141.4, 136.4, 129.5, 128.8, 128.7, 127.8, 127.2, 127.1, 125.3, 125.2, 120.1, 82.0, 67.3, 56.7, 54.2, 48.7, 47.2, 38.6, 38.2, 28.0, 18.2 ppm; HRMS (ESI): calcd for $[C_{40}H_{43}N_3O_6+Na]^+$ 684.3044, found 684.3046.

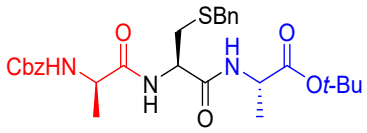
The procedure for synthesis of *epi*-7g-1, *epi*-7g-2, *epi*-7j-1 and *epi*-7j-2

The employed micro-flow system was shown in **Figure S3**.

A solution of **Fmoc-Cys(*t*-Bu)-OH** or **Cbz-Ala-OH** (0.300 M, 1.00 equiv), *N*,*N*-diisopropylethylamine (0.360 M, 1.2 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of thionyl chloride (0.216 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, 1061 mm, volume:

533 μL, reaction time: 10 s) at the same temperature. The resultant mixture and a solution of **H-L-Phe-NCA** or **H-L-Cys(Bn)-NCA** (0.300 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 72.9 mm, volume: 37 μL, reaction time: 0.50 s) at the same temperature. The resultant mixture and a solution of *N*,*N*-diisopropylethylamine (0.216 M, 1.20 equiv) and *N*-methylimidazole (0.180 M, 1.00 equiv) in CH_2Cl_2 (flow rate: 2.00 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 3 (inner diameter: 0.800 mm, reaction time: 10 s or 15 s) at the same temperature. The resultant mixture and a solution of **H-L-Ala-O*t*-Bu** (**6a**) (0.360 M, 1.20 equiv) in CH_2Cl_2 (flow rate: 1.20 mL/min) were injected into the T-shaped mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 4 (inner diameter: 0.800 mm, length: 2,519 mm, volume: 1,267 μL, reaction time: 10 s) at the same temperature. After being eluted for *ca.* 90 s to reach a steady state, the resultant mixture was poured into 1 M HCl aq. (1.5 mL) and CH_2Cl_2 (8 mL) for 25 s at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with *sat.* $NaHCO_3$ aq., brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. The residue was purified by recrystallization.

Cbz-D-Ala-L-Cys(Bn)-L-Ala-O*t*-Bu (*epi*-7g-1)



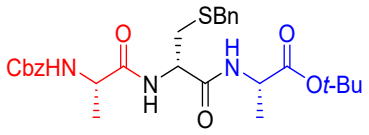
Purification method: The crude product was washed with hexane.

HPLC conditions; DAICEL CHIRALPAK IB 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 15.6 min.

73.0 mg, 0.13 mmol, 90%.

Yellow oil, IR (neat): 3287, 1732, 1645, 1541, 1508, 1234, 1148, 698 cm^{-1}; $[\alpha]^{24}_D$ = +11.1 (c 1.16, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.32-7.22 (m, 10H), 7.05 (d, *J* = 6.4 Hz, 1H), 6.95 (d, *J* = 7.6 Hz, 1H), 5.52 (d, *J* = 7.6 Hz, 1H), 5.13 (d, *J* = 12.4 Hz, 1H), 5.05 (d, *J* = 12.4 Hz, 1H) 4.57 (d, *J* = 6.0 Hz, 1H), 4.42-4.36 (m, 1H), 4.25-4.22 (m, 1H), 3.74 (s, 2H), 2.89 (dd, *J* = 4.0, 13.2 Hz, 1H), 2.73 (dd, *J* = 6.4, 13.2 Hz, 1H), 1.44 (s, 9H), 1.37 (d, *J* = 7.2 Hz, 3H), 1.35 (d, *J* = 7.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.5, 171.6, 169.5, 156.1, 138.1, 136.2, 129.2, 128.7, 128.6, 128.3, 128.2, 127.3, 82.1, 67.2, 52.2, 50.9, 49.1, 36.6, 33.7, 28.0, 18.7, 18.3 ppm; HRMS (ESI): calcd for $[C_{28}H_{37}N_3O_6S_1+Na]^+$ 566.2295, found 566.2295.

Cbz-L-Ala-D-Cys(Bn)-L-Ala-O*t*-Bu (*epi*-7g-2)



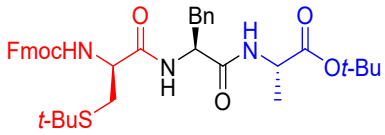
Purification method: The crude product was washed with hexane.

HPLC conditions; DAICEL CHIRALPAK IB 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 12.1 min.

70.3 mg, 0.13 mmol, 86%.

Yellow oil, IR (neat): 3296, 1732, 1717, 1647, 1540, 1523, 1241, 1150, 698 cm^{-1}; $[\alpha]^{24}_{D}$ = 1.25 (c 1.41, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.33-7.22 (m, 10H), 7.06 (d, *J* = 6.4 Hz, 1H), 6.90 (d, *J* = 7.6 Hz, 1H), 5.46 (d, *J* = 6.4 Hz, 1H), 5.43 (d, *J* = 12.4 Hz, 1H), 5.04 (d, *J* = 12.4 Hz, 1H), 4.60 (d, *J* = 6.4 Hz, 1H), 4.44-4.37 (m, 1H), 4.21-4.14 (m, 1H), 3.73 (s, 2H), 2.95 (dd, *J* = 5.2, 14.0 Hz, 1H), 2.74 (dd, *J* = 6.4, 14.0 Hz, 1H), 1.44 (s, 9H), 1.38 (d, *J* = 6.8 Hz, 3H), 1.35 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6, 171.9, 169.5, 156.2, 138.2, 136.2, 129.2, 128.74, 128.67, 128.4, 128.2, 127.3, 82.1, 67.2, 52.1, 51.1, 49.1, 36.5, 33.3, 28.1, 18.3, 18.2 ppm; HRMS (ESI): calcd for $[C_{28}H_{37}N_3O_6S_1+Na]^+$ 566.2295, found 566.2292.

Fmoc-D-Cys(*t*-Bu)-L-Phe-L-Ala-O*t*-Bu (*epi*-7j-1)



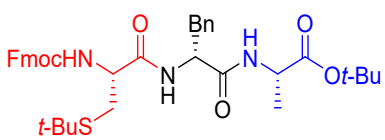
Purification method: The crude product was recrystallized from CH_2Cl_2/hexane

HPLC conditions; DAICEL CHIRALPAK IB 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 11.7 min.

89.7 mg, 0.13 mmol, 89%.

White solid, mp 152-156 °C, IR (neat): 3283, 1732, 1644, 1550, 1226, 1149, 758, 741 cm^{-1}; $[\alpha]^{24}_{D}$ = -0.061 (c 0.95, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.39 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.30 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.26-7.13 (m, 5H), 6.86 (d, *J* = 6.4 Hz, 1H), 6.47 (d, *J* = 7.2 Hz, 1H), 5.72 (brs, 1H), 4.72-4.67 (m, 1H), 4.40-4.28 (m, 4H), 4.20 (t, *J* = 6.8 Hz, 1H), 3.15 (dd, *J* = 6.4, 13.6 Hz, 1H), 3.07 (dd, *J* = 6.8, 13.6 Hz, 1H), 2.94 (dd, *J* = 6.0, 12.8 Hz, 1H), 2.76 (dd, *J* = 6.8, 12.8 Hz, 1H), 1.42 (s, 9H), 1.31 (d, *J* = 8.4 Hz, 12H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 170.2, 169.8, 156.1, 143.9, 143.8, 141.4, 136.3, 129.5, 128.7, 127.8, 127.2, 125.3, 120.1, 82.1, 67.5, 55.2, 54.5, 49.0, 47.2, 43.3, 38.2, 31.0, 30.9, 28.0, 18.5 ppm; HRMS (ESI): calcd for $[C_{38}H_{47}N_3O_6S_1+Na]^+$ 696.3078, found 696.3080.

Fmoc-L-Cys(*t*-Bu)-D-Phe-L-Ala-O*t*-Bu (*epi*-7j-2)



Purification method: The crude product was recrystallized from CH_2Cl_2/hexane

HPLC conditions; DAICEL CHIRALPAK IB 4.6 mm × 25 cm, 10% IPA in hexane, flow rate 1 mL/min, detection wavelength 254 nm, retention time 11.9 min.

75.5 mg, 0.11 mmol, 74%.

White solid, mp 125-127 ℃, IR (neat): 3352, 1731, 1703, 1657, 1524, 1451, 1235, 1151, 758, 741 cm^{-1}; $[\alpha]^{24}_D$ = -7.5 (c 0.84, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.40 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.31-7.19 (m, 7H), 6.86 (d, *J* = 6.8 Hz, 1H), 6.39 (d, *J* = 6.4 Hz, 1H), 5.73 (brs, 1H), 4.72-4.69 (m, 1H), 4.42-4.19 (m, 5H), 3.15 (dd, *J* = 6.4, 13.2 Hz, 1H), 3.07 (dd, *J* = 7.6, 13.2 Hz, 1H), 2.97 (dd, *J* = 6.0, 12.8 Hz, 1H), 2.78 (dd, *J* = 7.2, 12.8 Hz, 1H), 1.41 (s, 9H), 1.32 (s, 9H), 1.21 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9, 170.2, 169.6, 156.1, 143.9, 143.8, 141.4, 136.5, 129.5, 128.8, 127.9, 127.2, 125.3, 120.1, 82.1, 67.5, 55.3, 54.6, 48.8, 47.2, 43.3, 38.3, 31.0, 30.8, 28.0, 18.3 ppm; HRMS (ESI): calcd for $[C_{38}H_{47}N_3O_6S_1+Na]^+$ 696.3078, found 696.3077.

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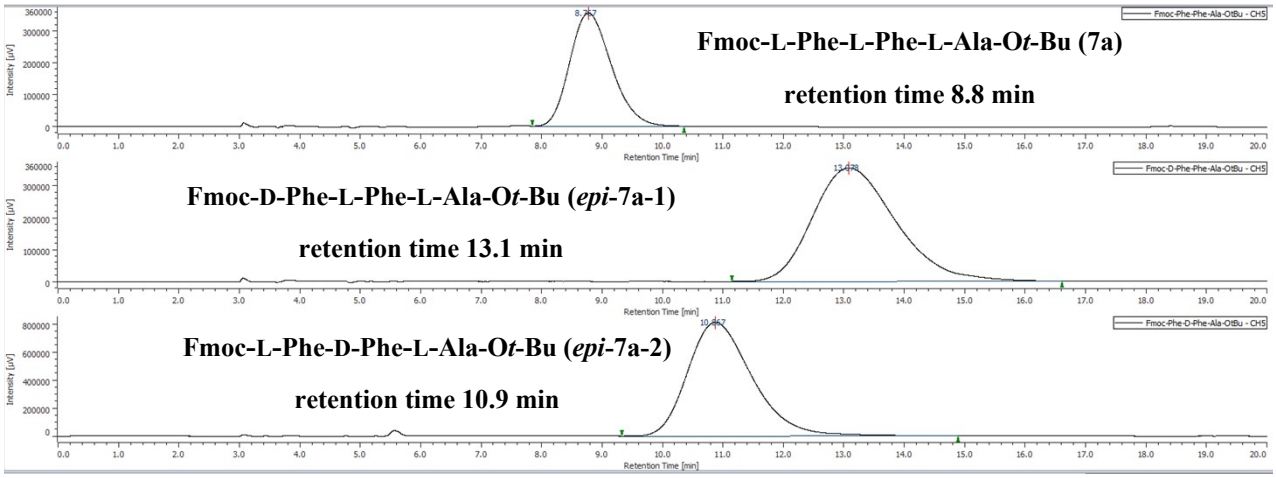
HPLC analysis for detection of racemization of tripeptide 7a, 7g and 7j

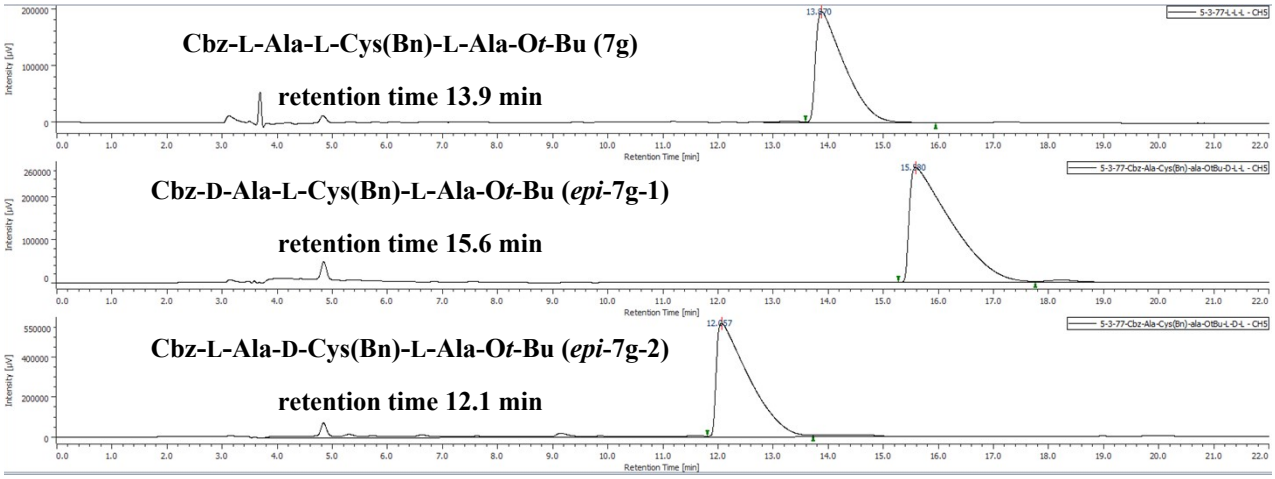
Fmoc-Phe-Phe-Ala-O*t*-Bu (7a, *epi*-7a-1, *epi*-7a-2)

(DAICEL CHIRALPAK IH, 4.6 mm × 25 cm, 10% IPA in hexane)



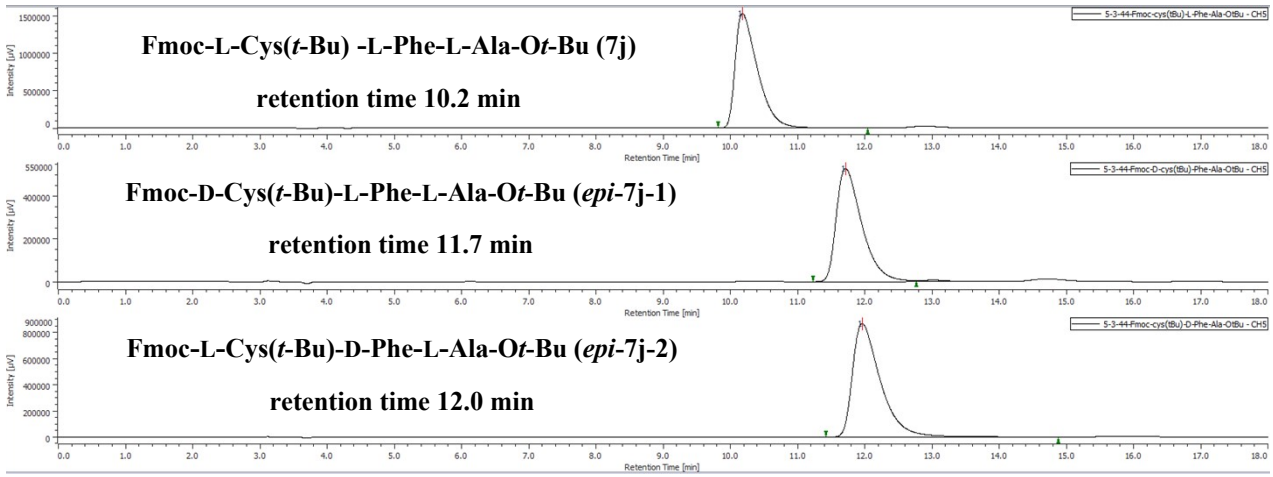
Cbz-Ala-Cys(Bn)-Ala-O*t*-Bu (7g, *epi*-7g-1, *epi*-7g-2)

(DAICEL CHIRALPAK IB, 4.6 mm × 25 cm, 10% IPA in hexane)



Fmoc-Cys(*t*-Bu)-Phe-Ala-O*t*-Bu (7j, *epi*-7j-1, *epi*-7j-2)

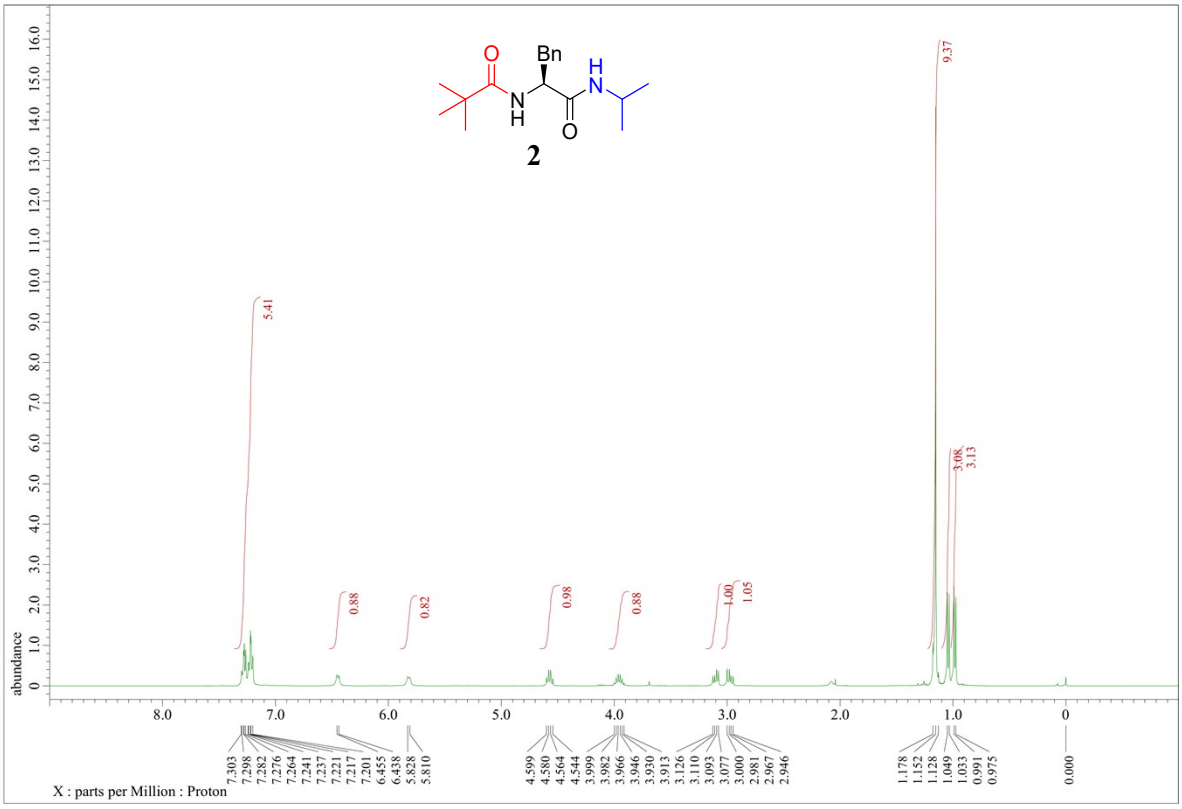
(DAICEL CHIRALPAK IB, 4.6 mm × 25 cm, 10% IPA in hexane)



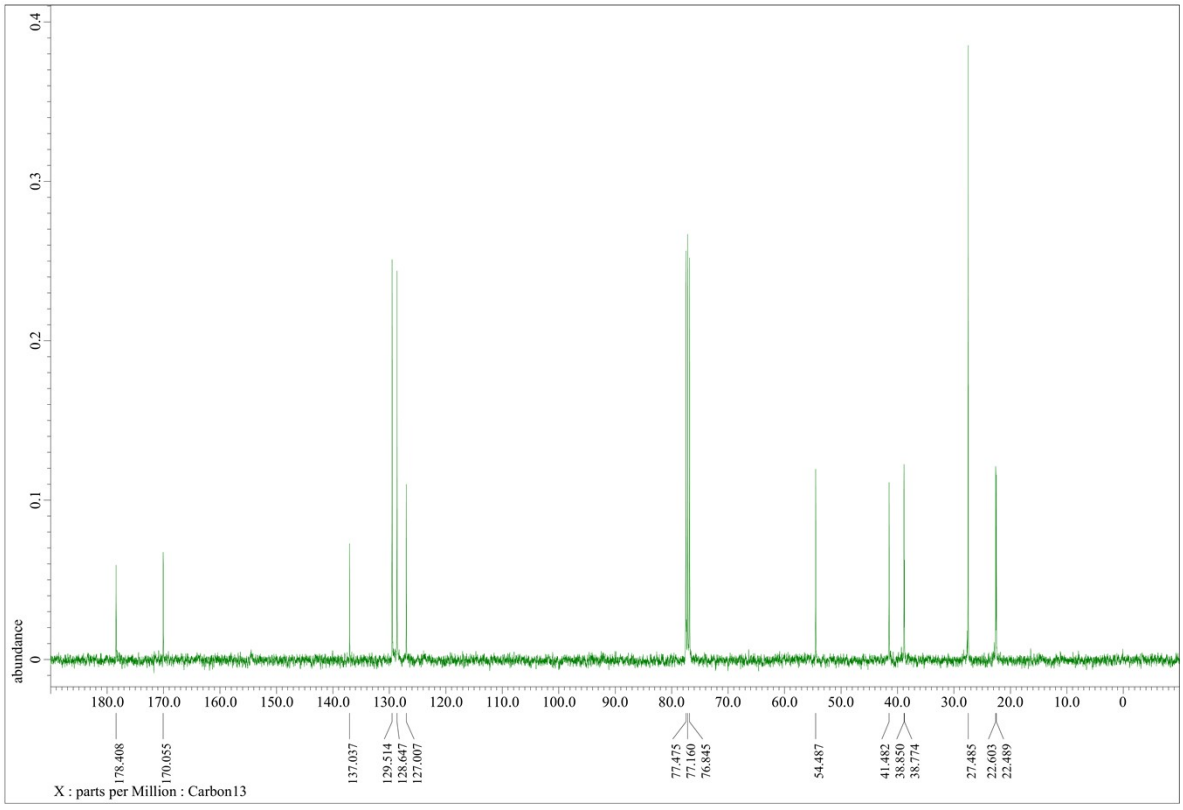
NMR spectra

(*S*)-*N*-Isopropyl-3-phenyl-2-pivalamidopropanamide (2)

(^{1}H NMR, 400 MHz, $CDCl_3$)

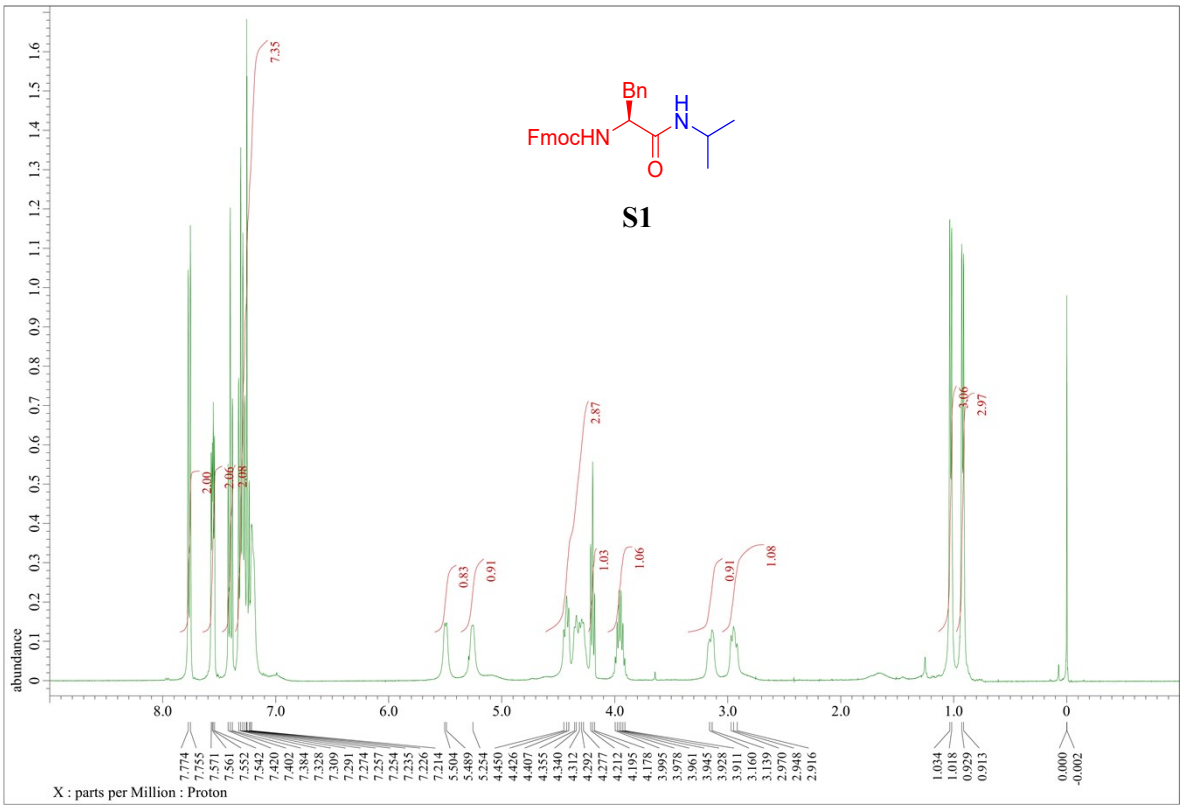


(^{13}C NMR, 100 MHz, $CDCl_3$)

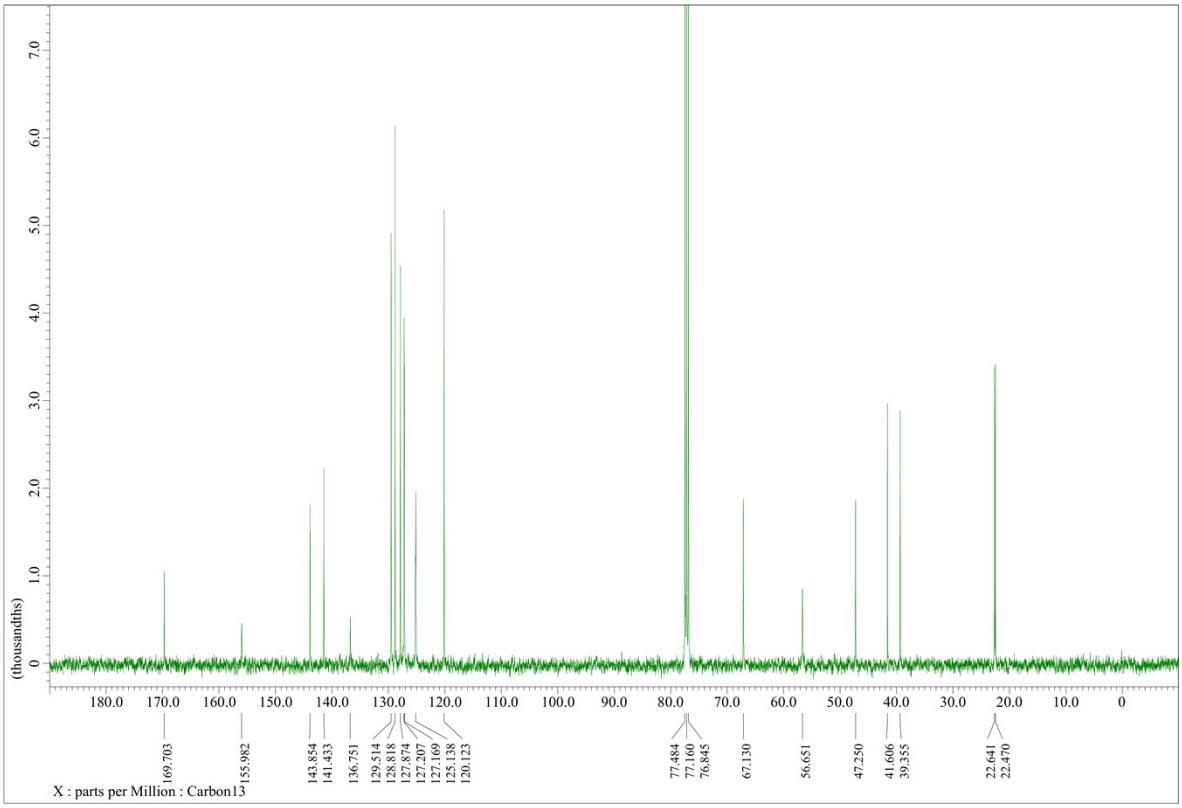


(9H-fluoren-9-yl)methyl (*S*)-(1-(isopropylamino)-1-oxo-3-phenylpropan-2-yl)carbamate (S1)

(^{1}H NMR, 400 MHz, $CDCl_3$)

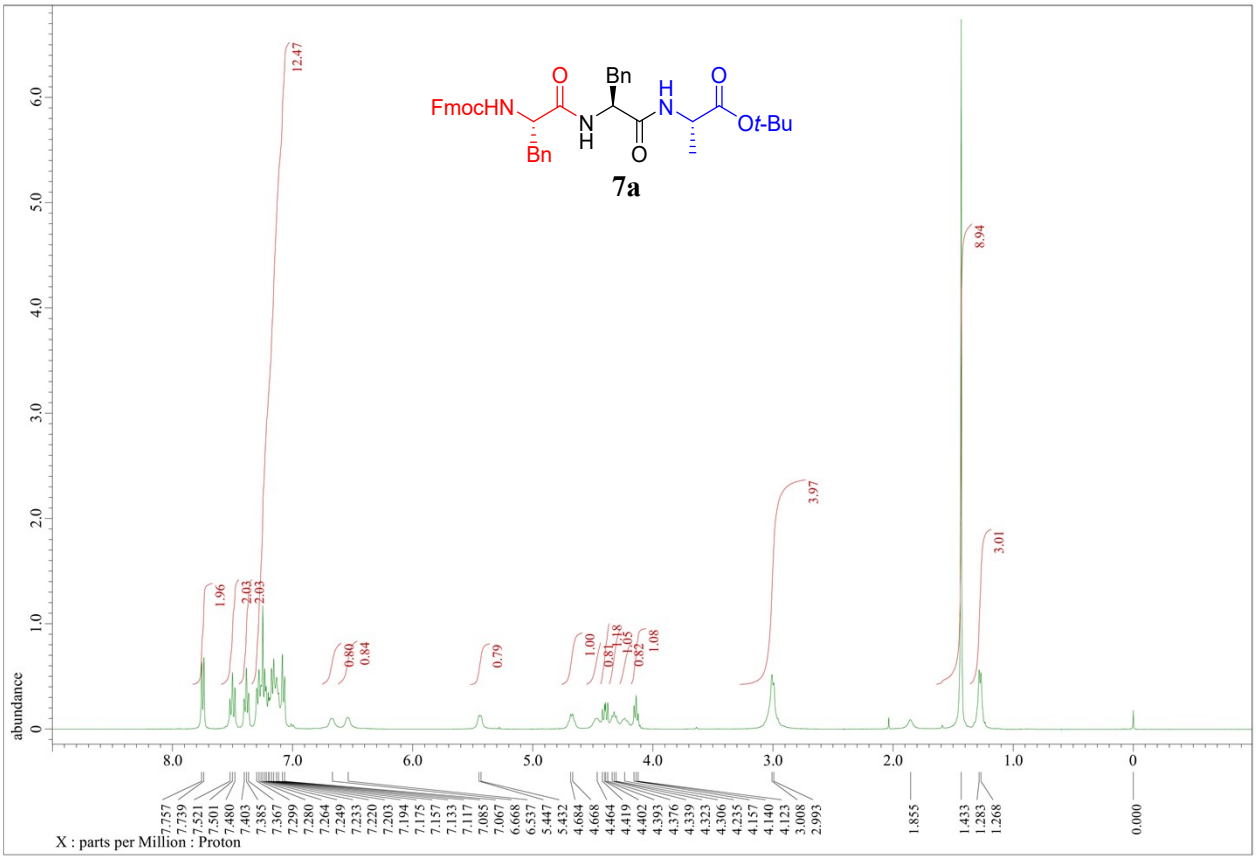


(^{13}C NMR, 100 MHz, $CDCl_3$)

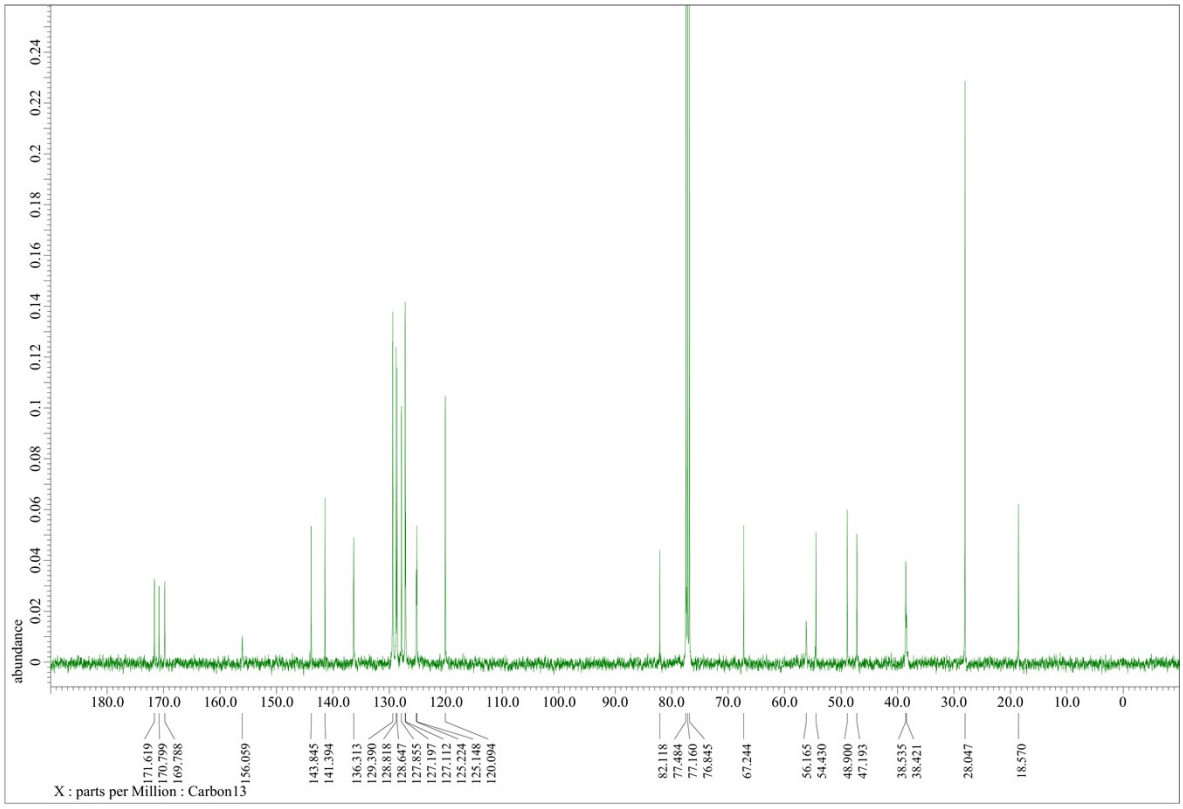


Fmoc-L-Phe-L-Phe-L-Ala-O*t*-Bu (7a)

(^{1}H NMR, 400 MHz, $CDCl_3$)

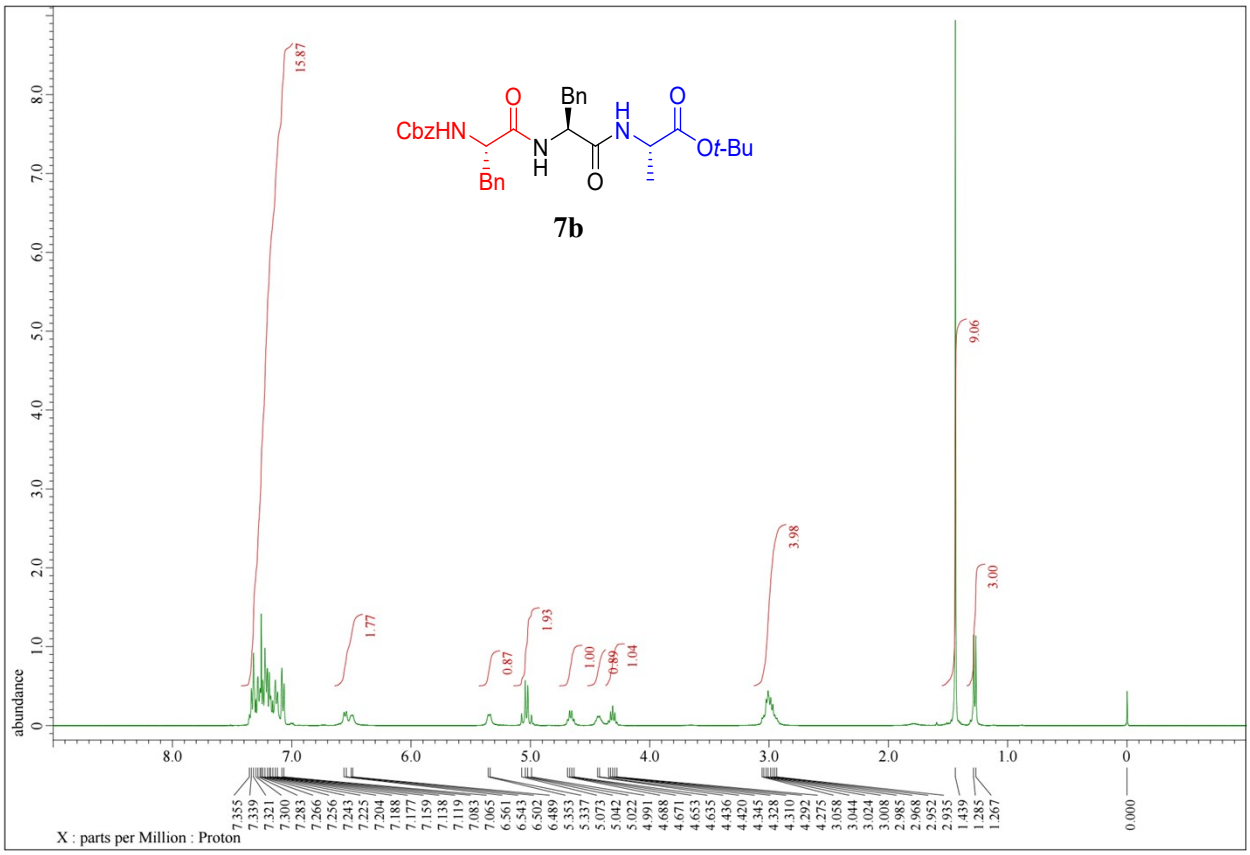


(^{13}C NMR, 100 MHz, $CDCl_3$)

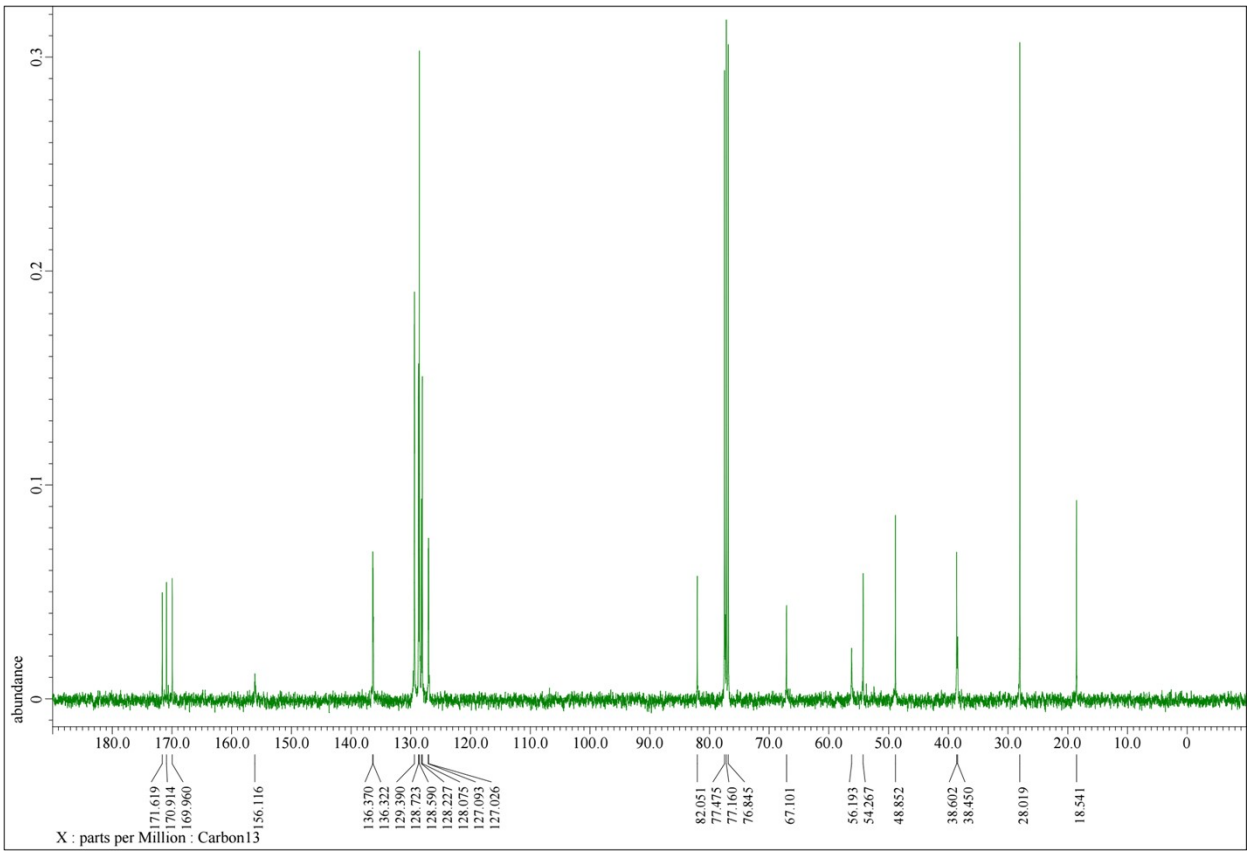


Cbz-L-Phe-L-Phe-L-Ala-O*t*-Bu (7b)

(^{1}H NMR, 400 MHz, $CDCl_3$)

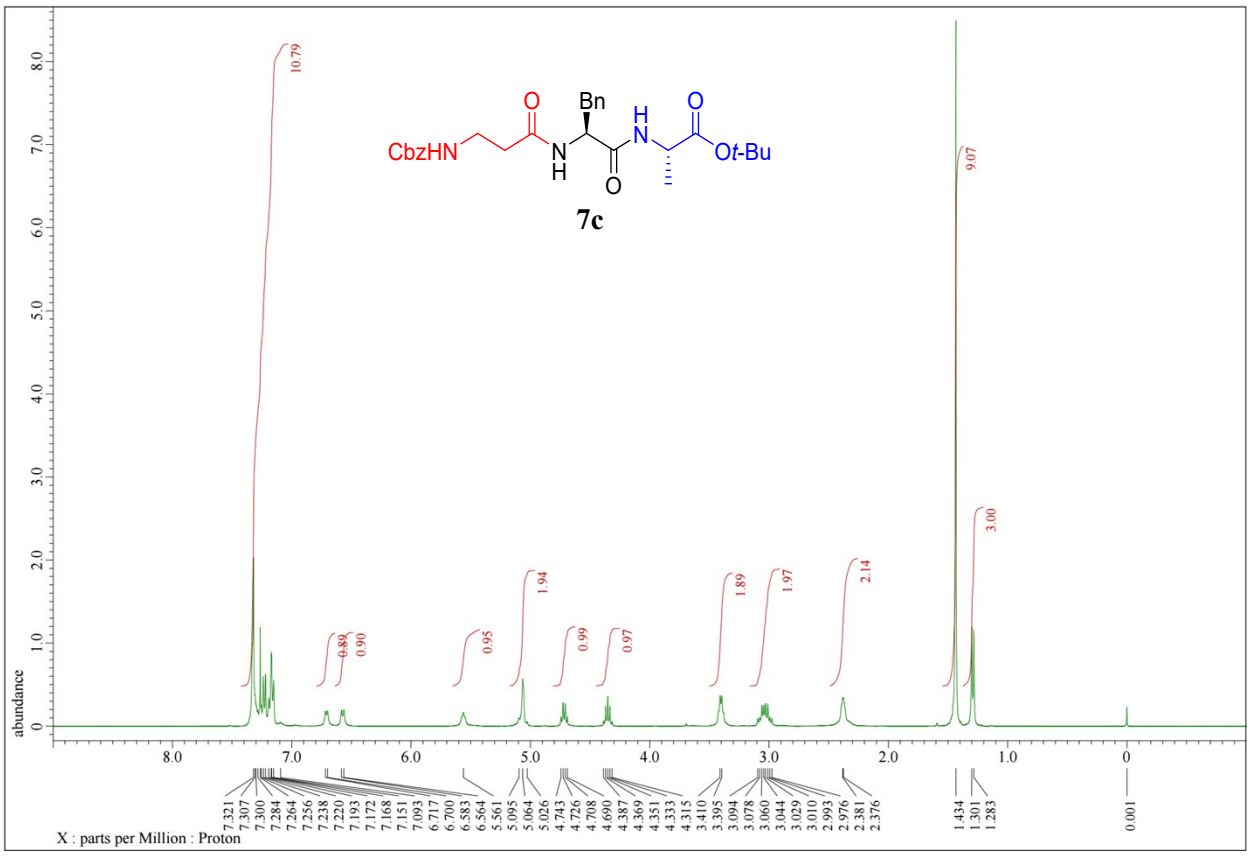


(^{13}C NMR, 100 MHz, $CDCl_3$)

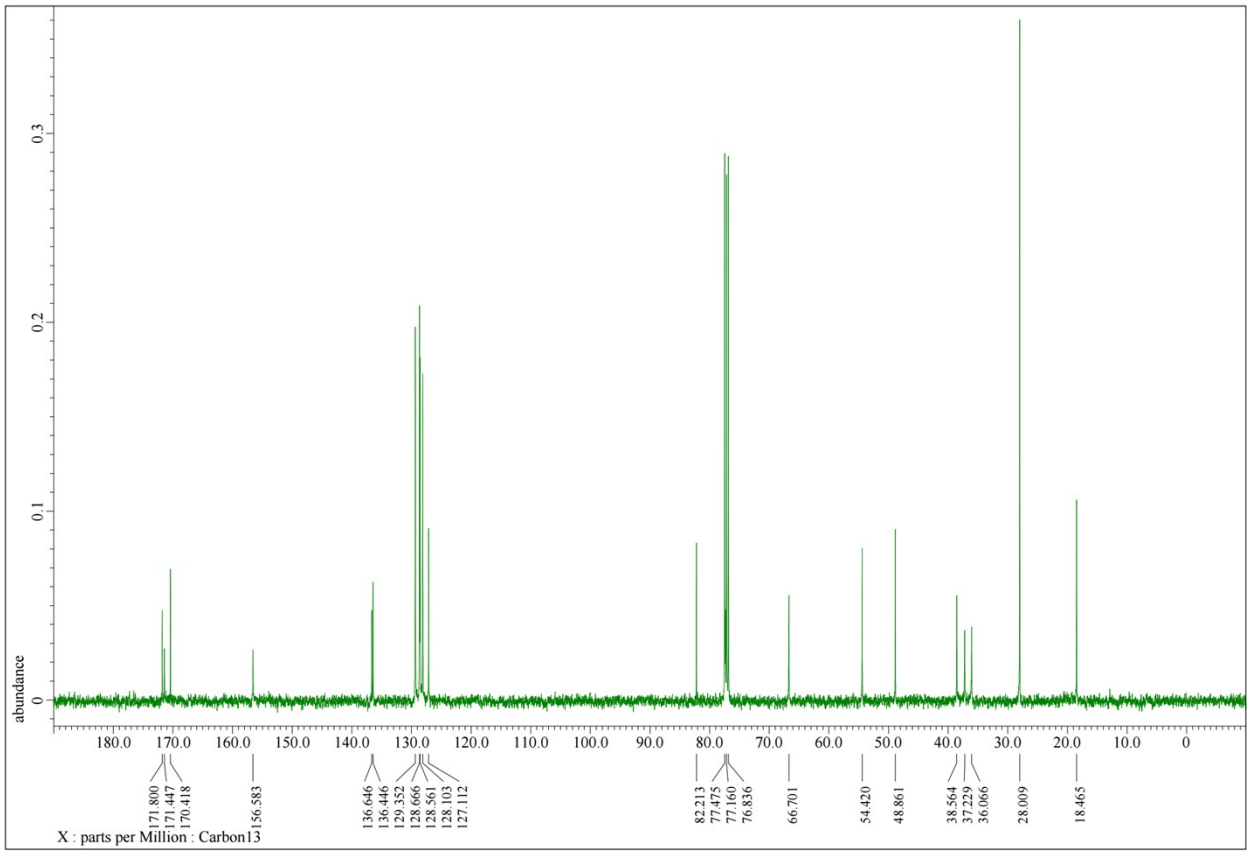


Cbz-β-Ala-L-Phe-L-Ala-O*t*-Bu (7c)

(^{1}H NMR, 400 MHz, $CDCl_3$)



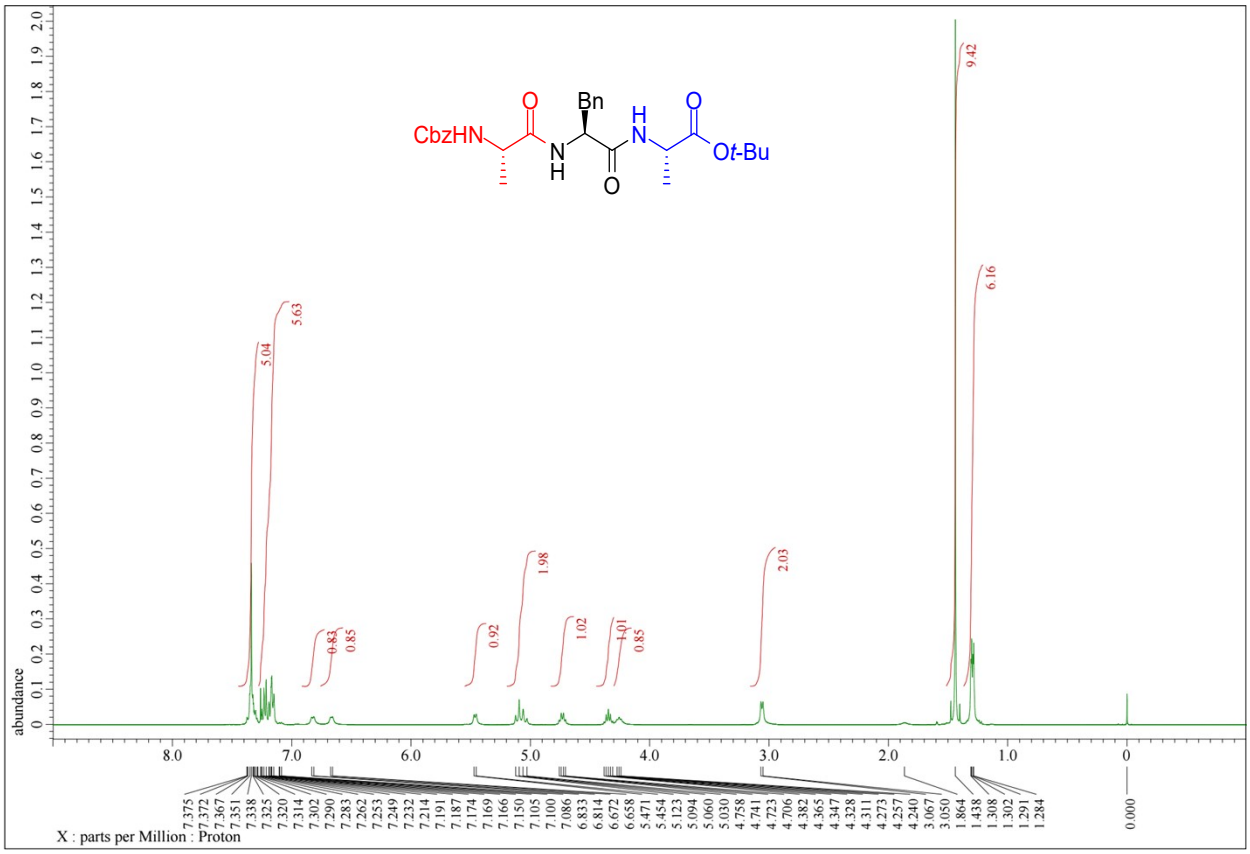
(^{13}C NMR, 100 MHz, $CDCl_3$)



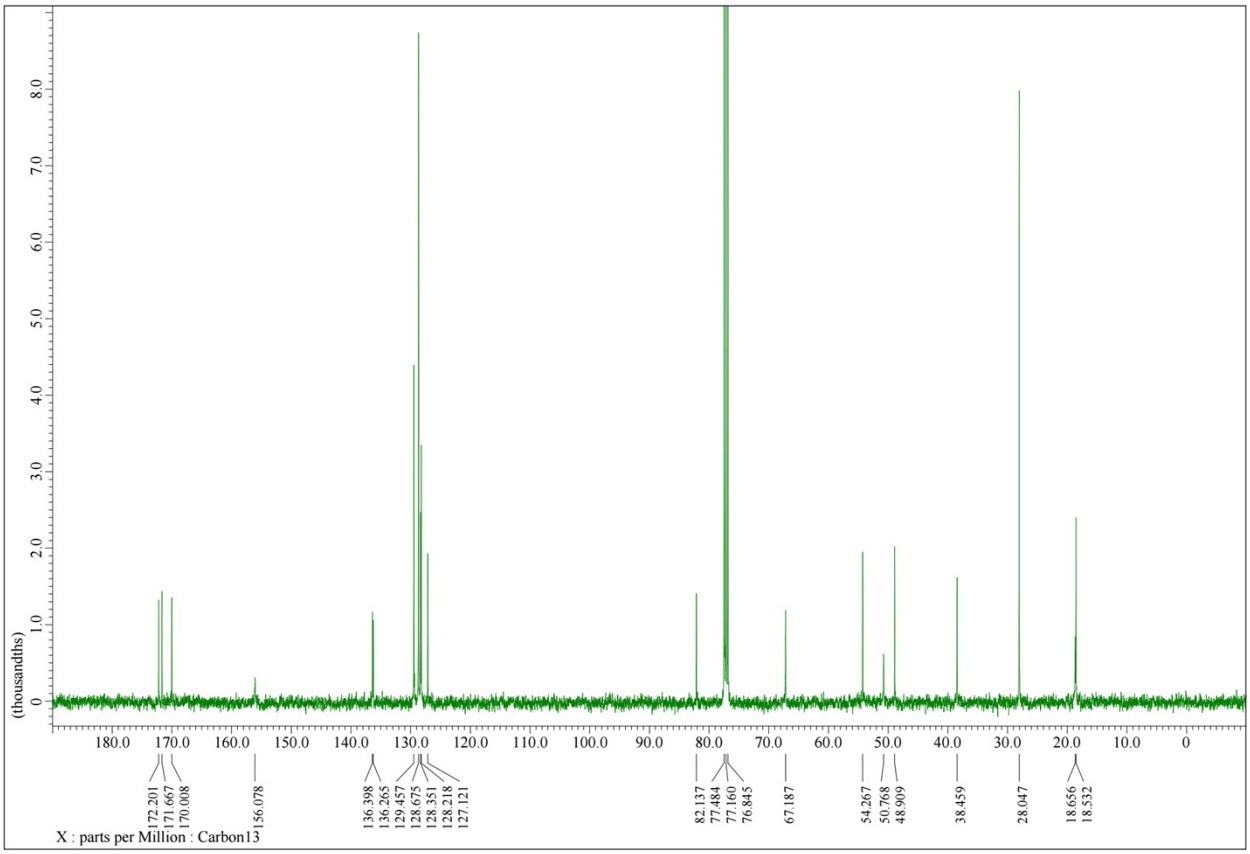
Cbz-L-Ala-L-Phe-L-Ala-O*t*-Bu (7d)

(^{1}H NMR, 400 MHz, $CDCl_3$)

7d

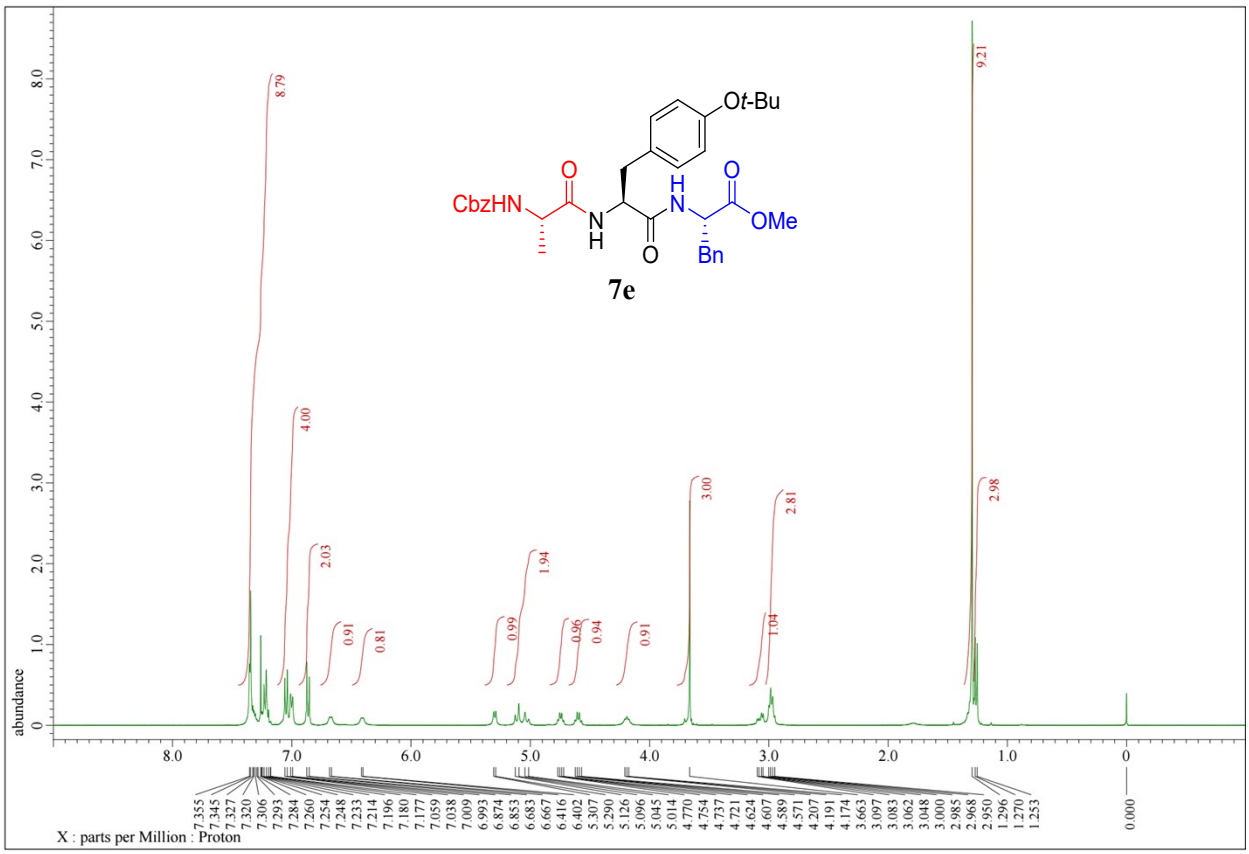


(^{13}C NMR, 100 MHz, $CDCl_3$)

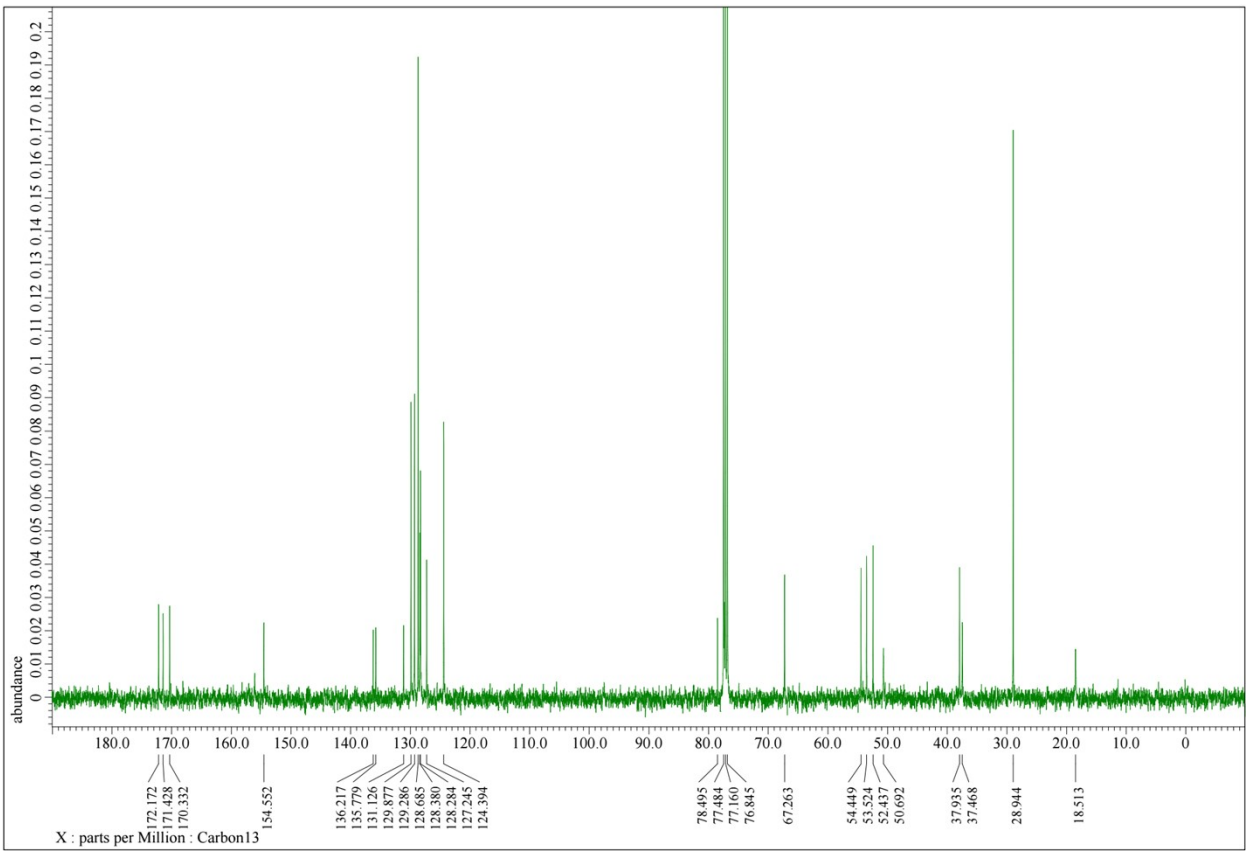


Cbz-L-Ala-L-Tyr(*t*-Bu)-L-Phe-OMe (7e)

(^{1}H NMR, 400 MHz, $CDCl_3$)

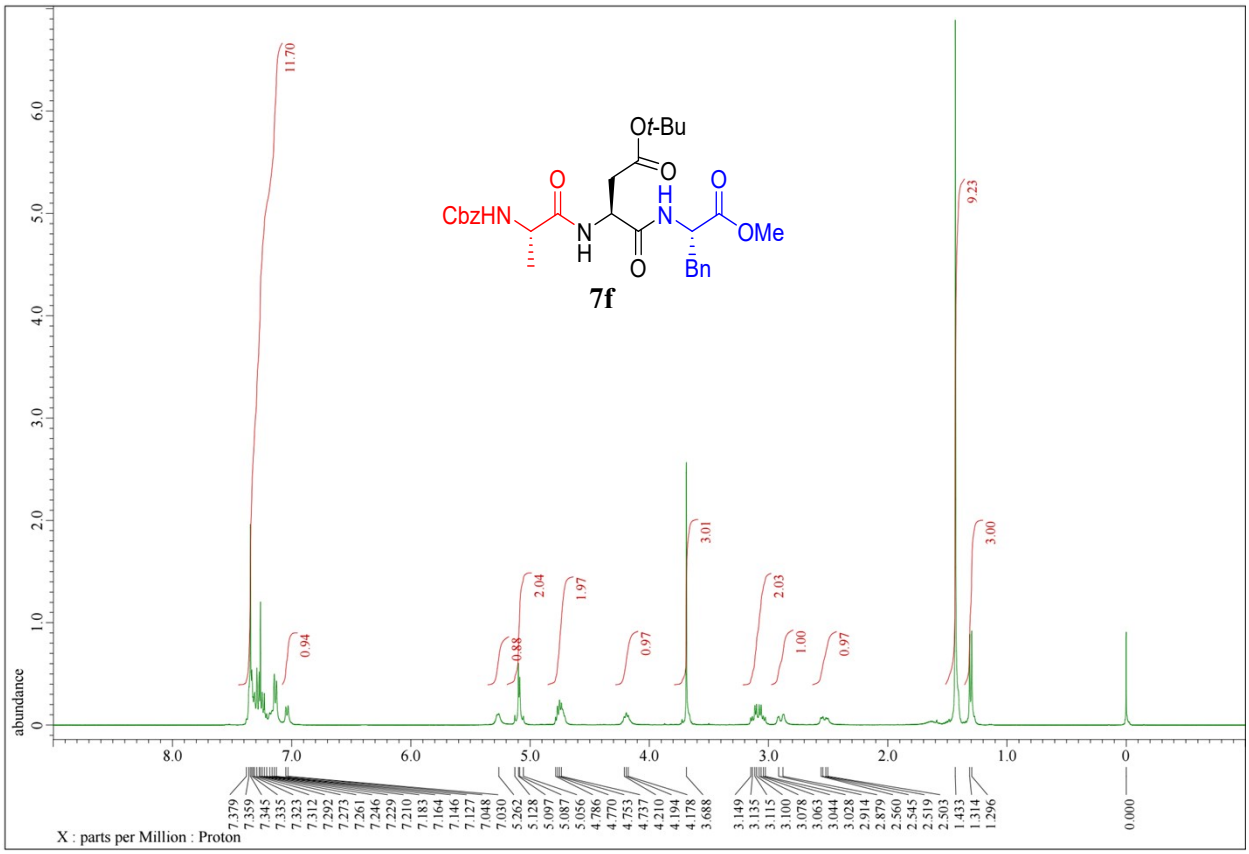


(^{13}C NMR, 100 MHz, $CDCl_3$)

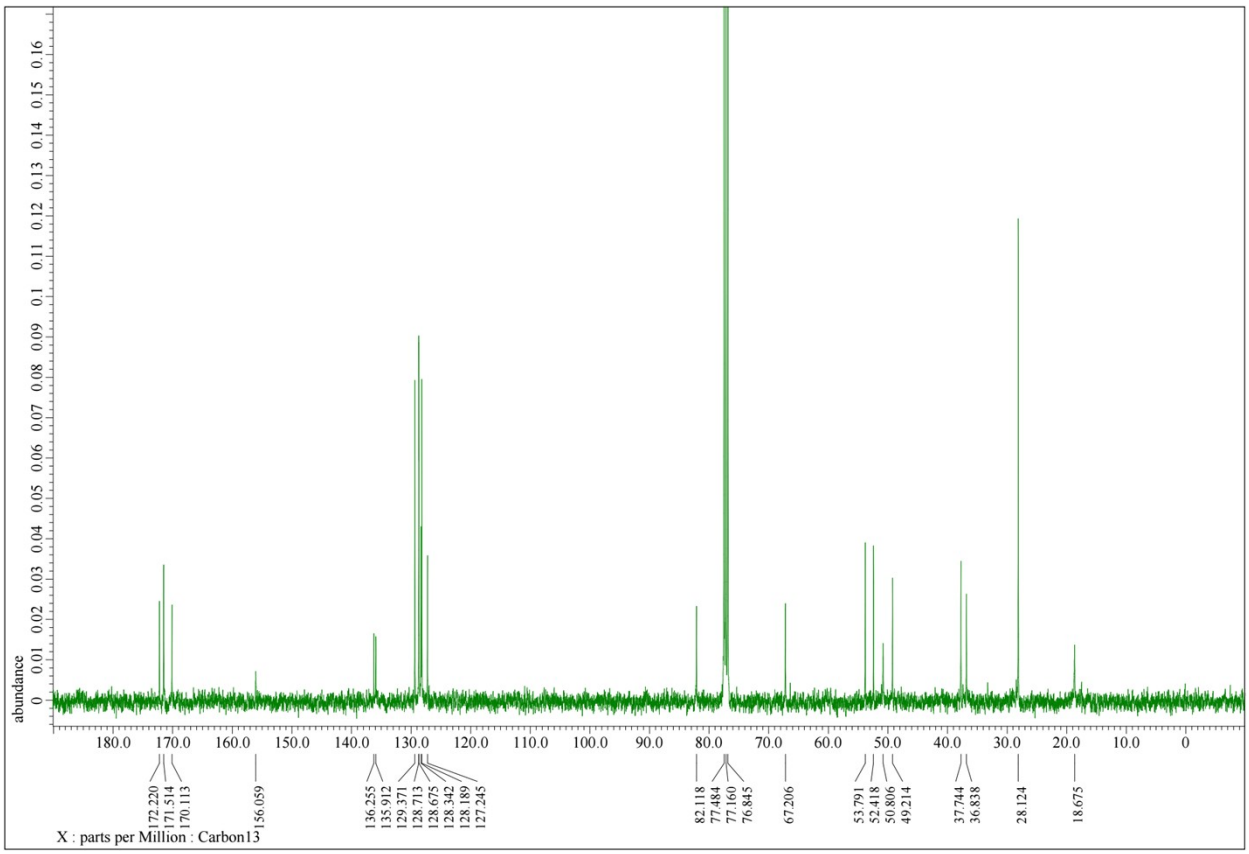


Cbz-L-Ala-L-Asp(O*t*-Bu)-L-Phe-OMe (7f)

(^{1}H NMR, 400 MHz, $CDCl_3$)

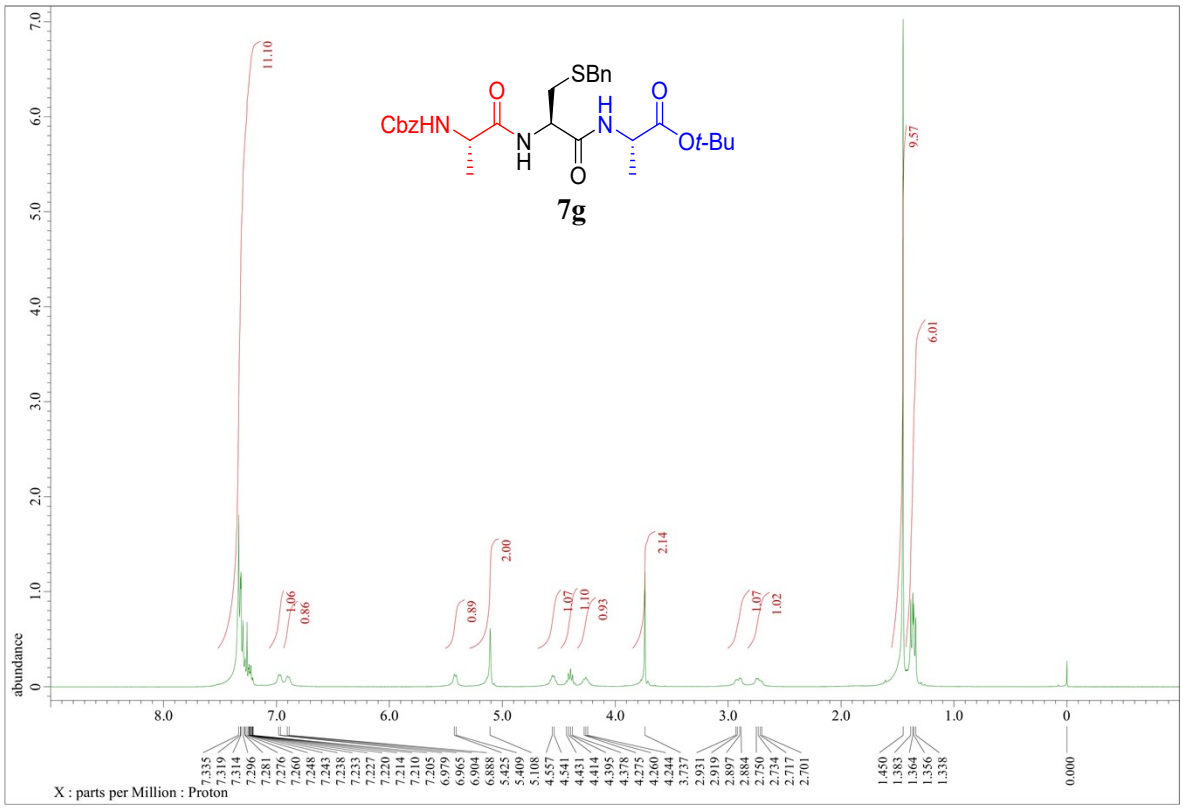


(^{13}C NMR, 100 MHz, $CDCl_3$)

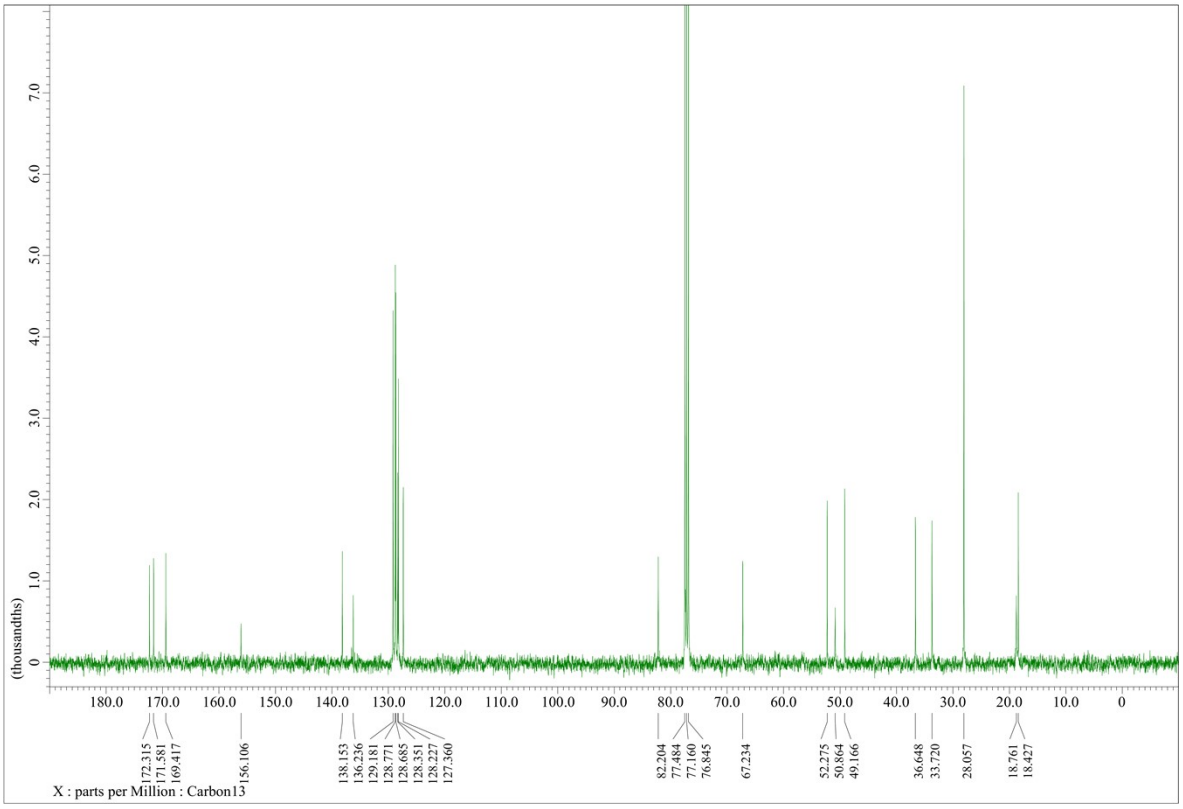


Cbz-L-Ala-L-Cys(Bn)-L-Ala-O*t*-Bu (7g)

(^{1}H NMR, 400 MHz, $CDCl_3$)

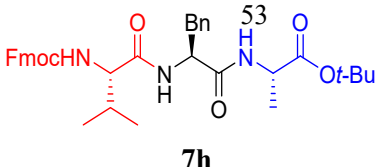


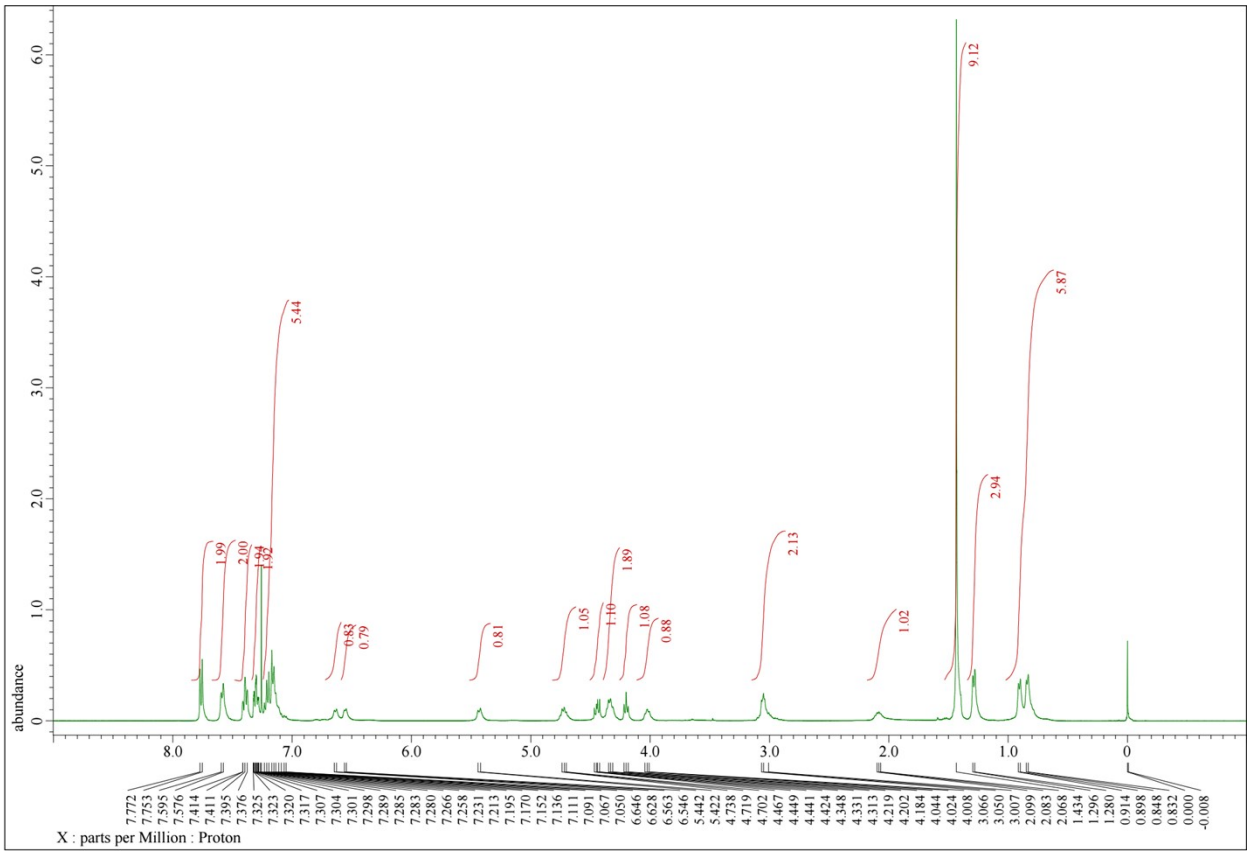
(^{13}C NMR, 100 MHz, $CDCl_3$)



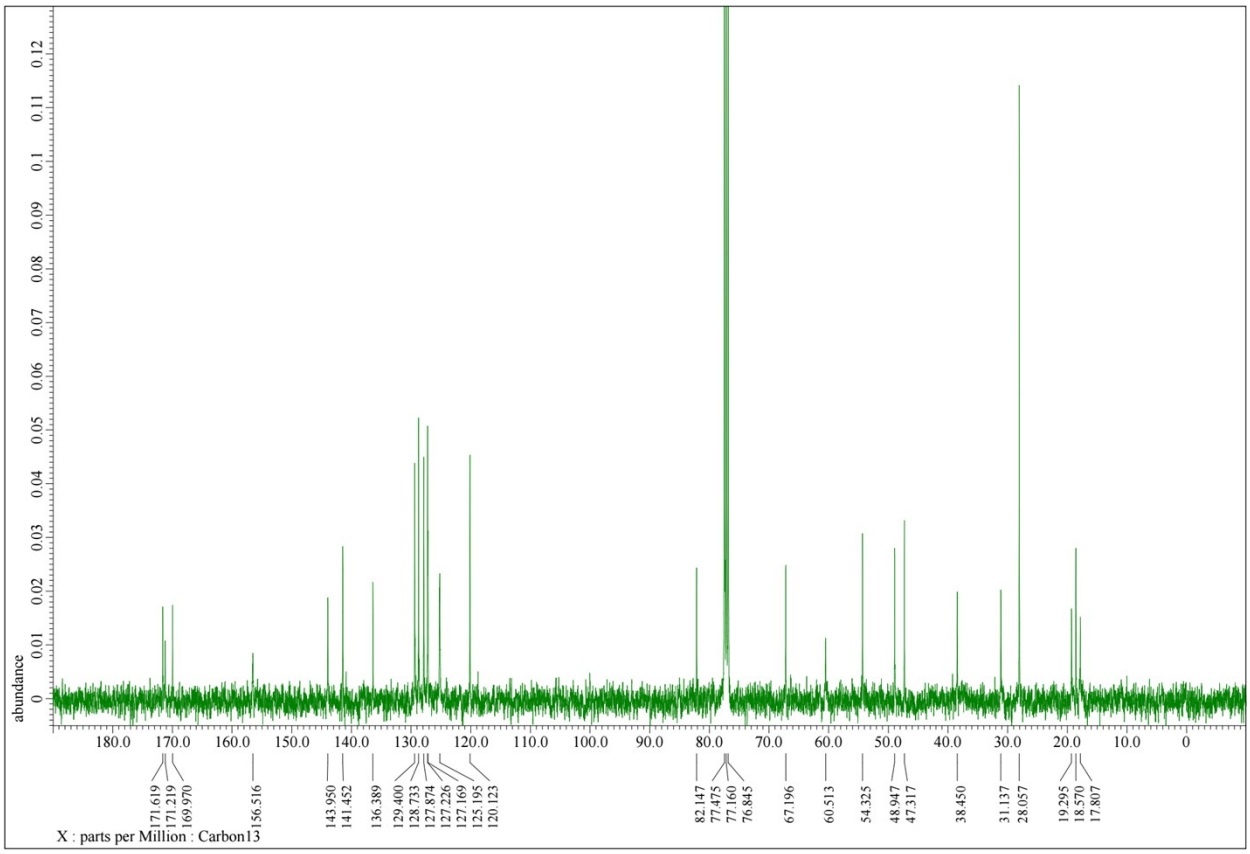
Fmoc-L-Val-L-Phe-L-Ala-O*t*-Bu (7h)

(^{1}H NMR, 400 MHz, $CDCl_3$)



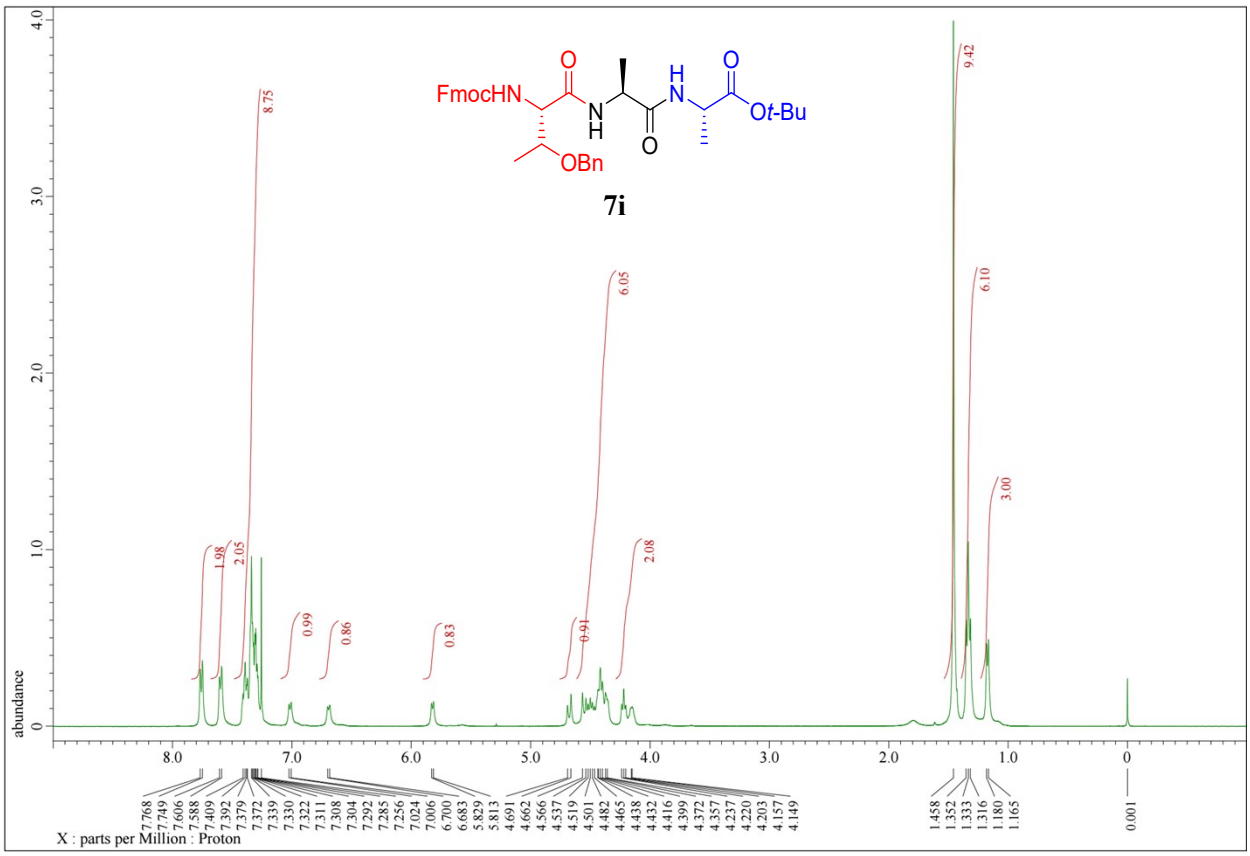


(^{13}C NMR, 100 MHz, $CDCl_3$)

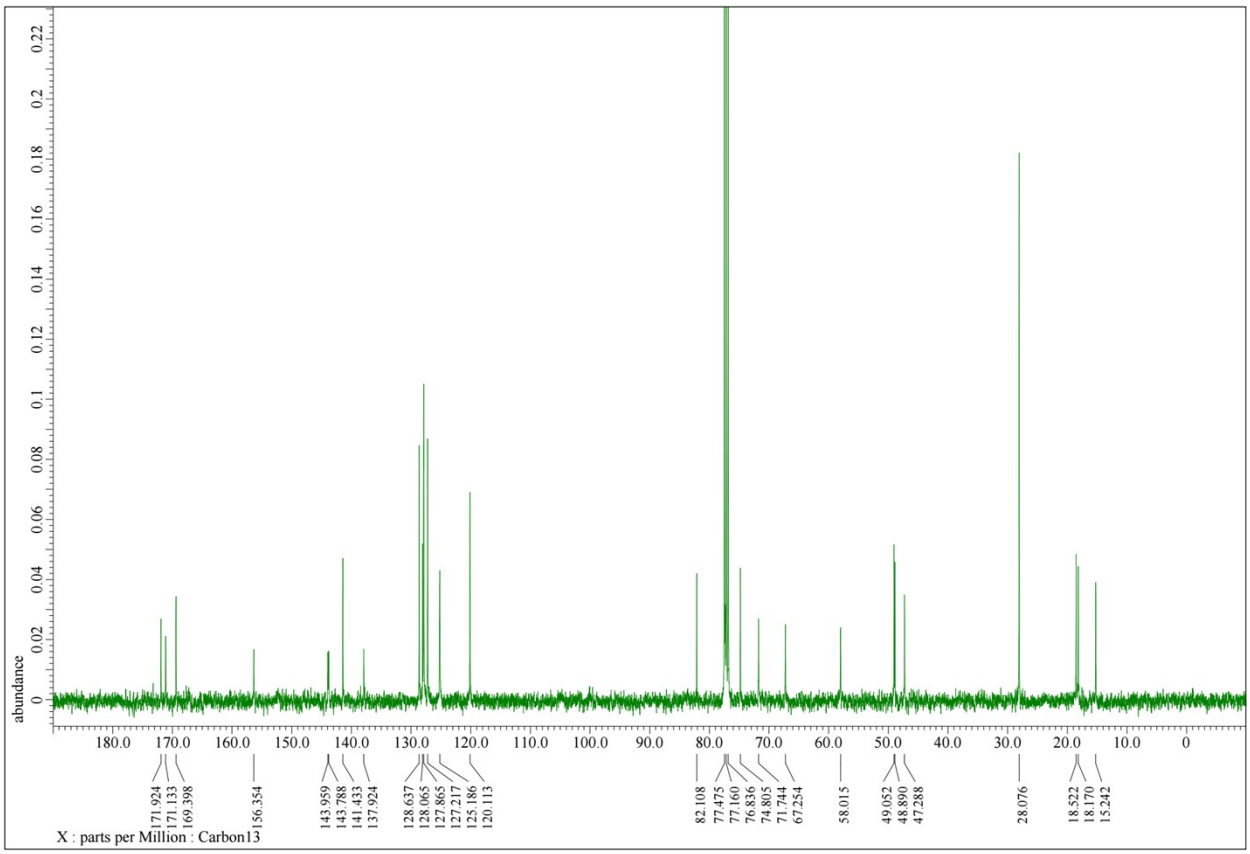


Fmoc-L-Thr(Bn)-L-Ala-L-Ala-O*t*-Bu (7i)

(^{1}H NMR, 400 MHz, $CDCl_3$)

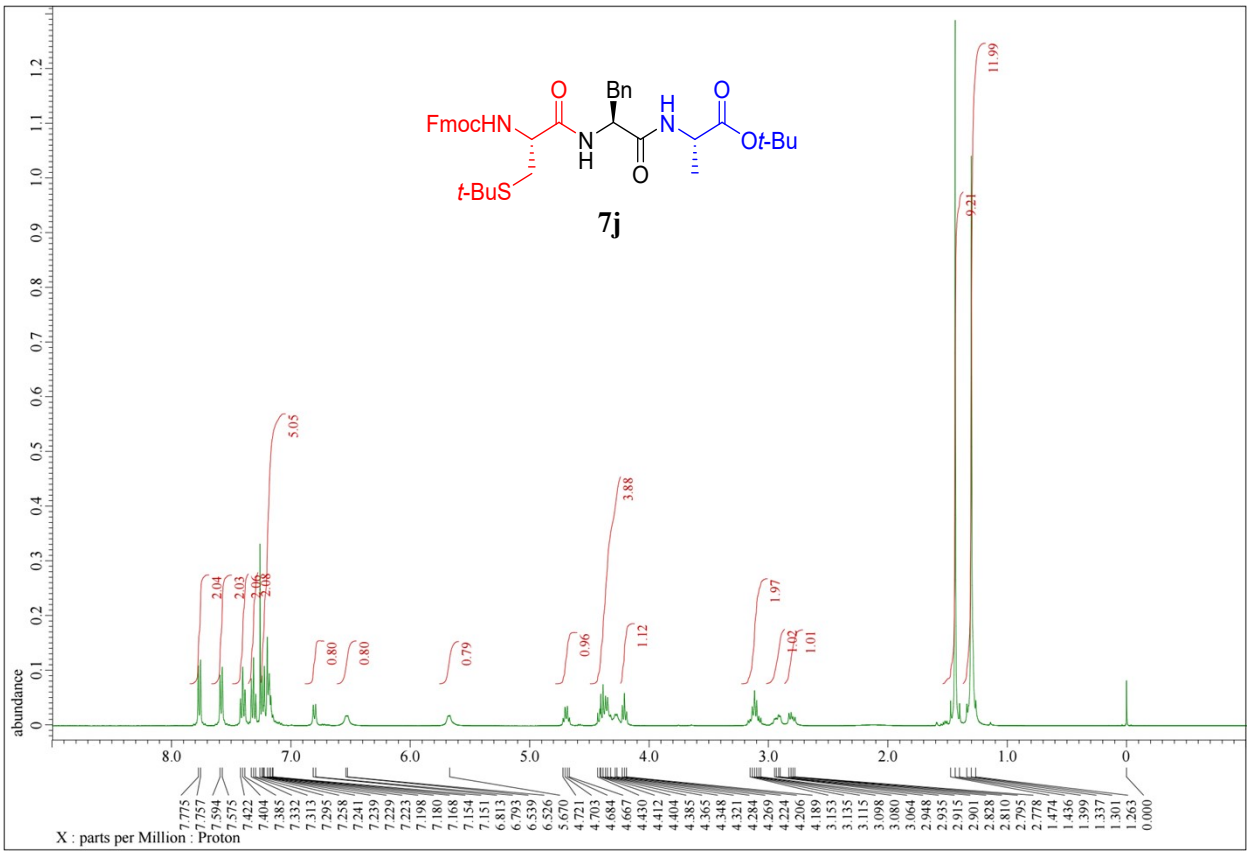


(^{13}C NMR, 100 MHz, $CDCl_3$)

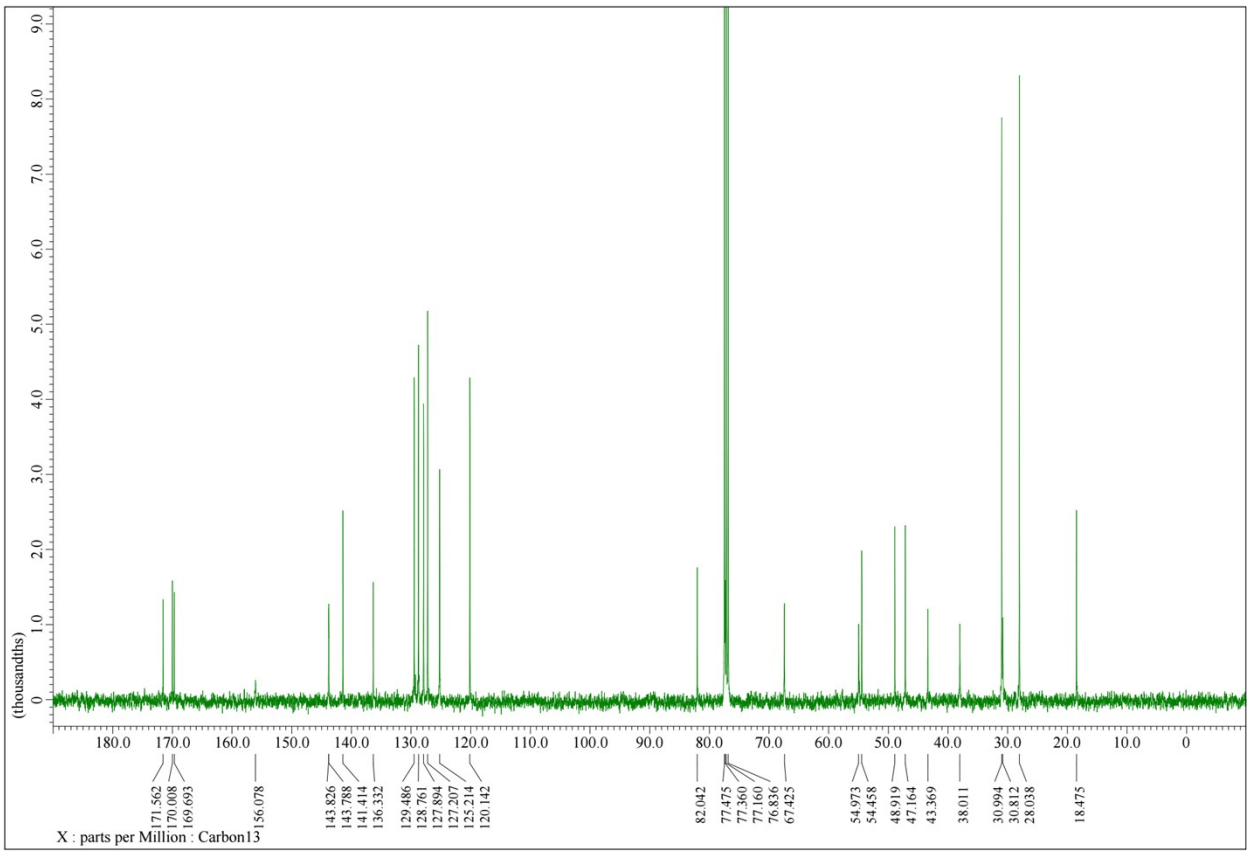


Fmoc-L-Cys(*t*-Bu)-L-Phe-L-Ala-O*t*-Bu (7j)

(^{1}H NMR, 400 MHz, $CDCl_3$)

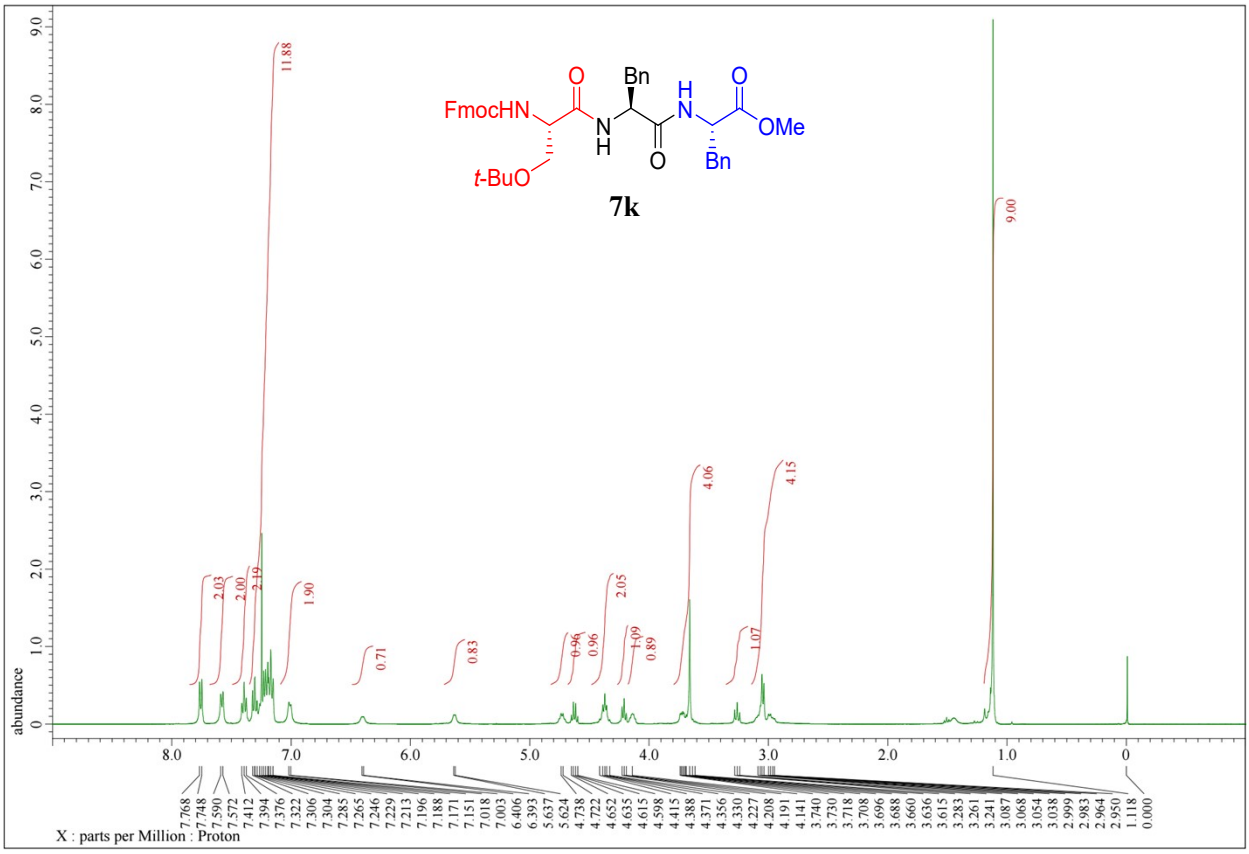


(^{13}C NMR, 100 MHz, $CDCl_3$)

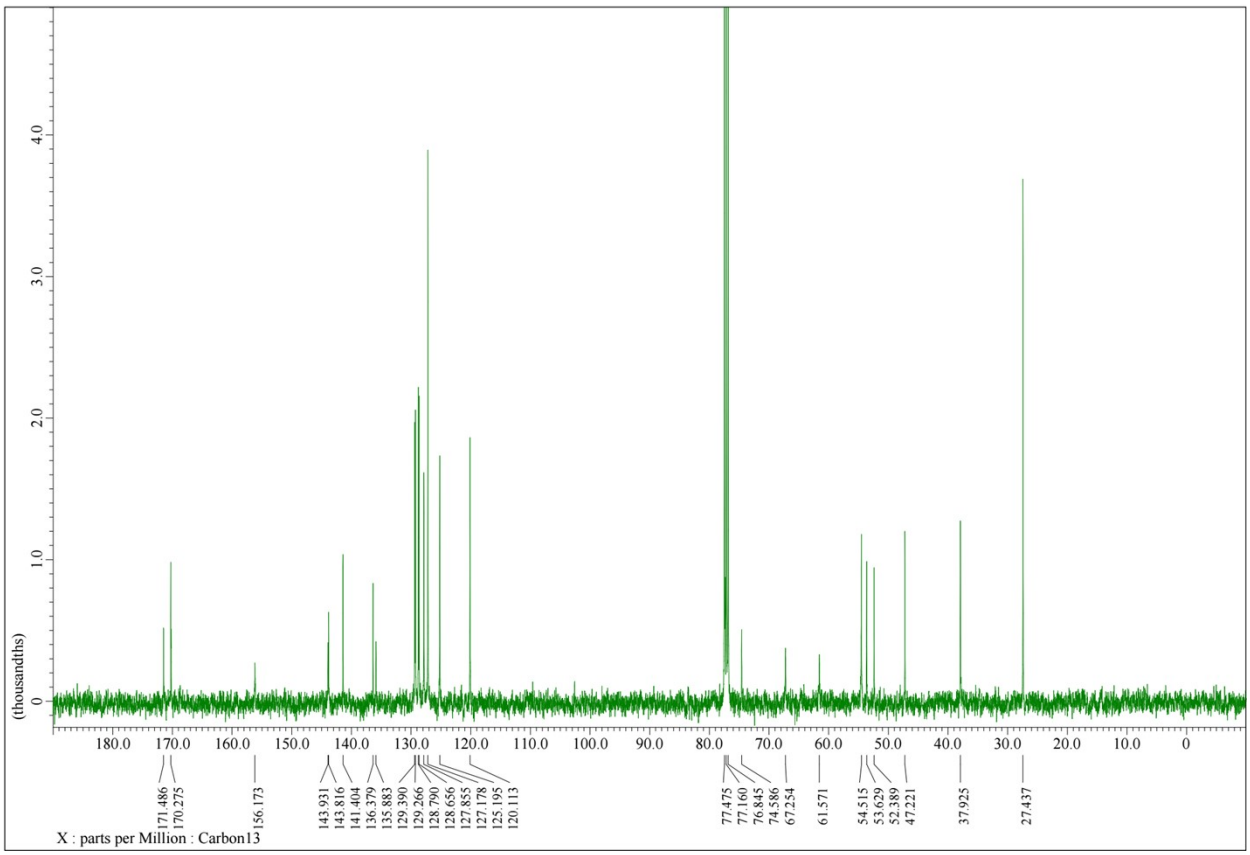


Fmoc-L-Ser(*t*-Bu)-L-Phe-L-Phe-OMe (7k)

(^{1}H NMR, 400 MHz, $CDCl_3$)

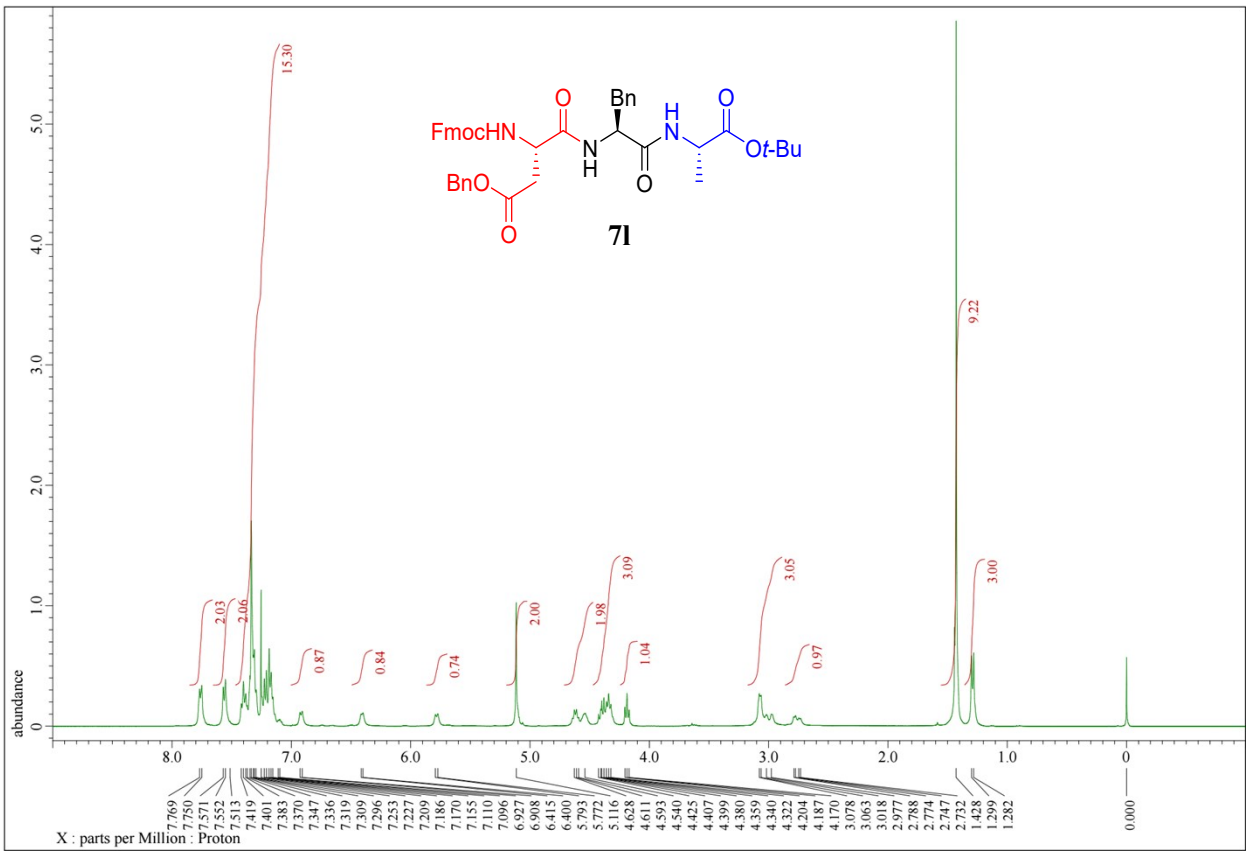


(^{13}C NMR, 100 MHz, $CDCl_3$)

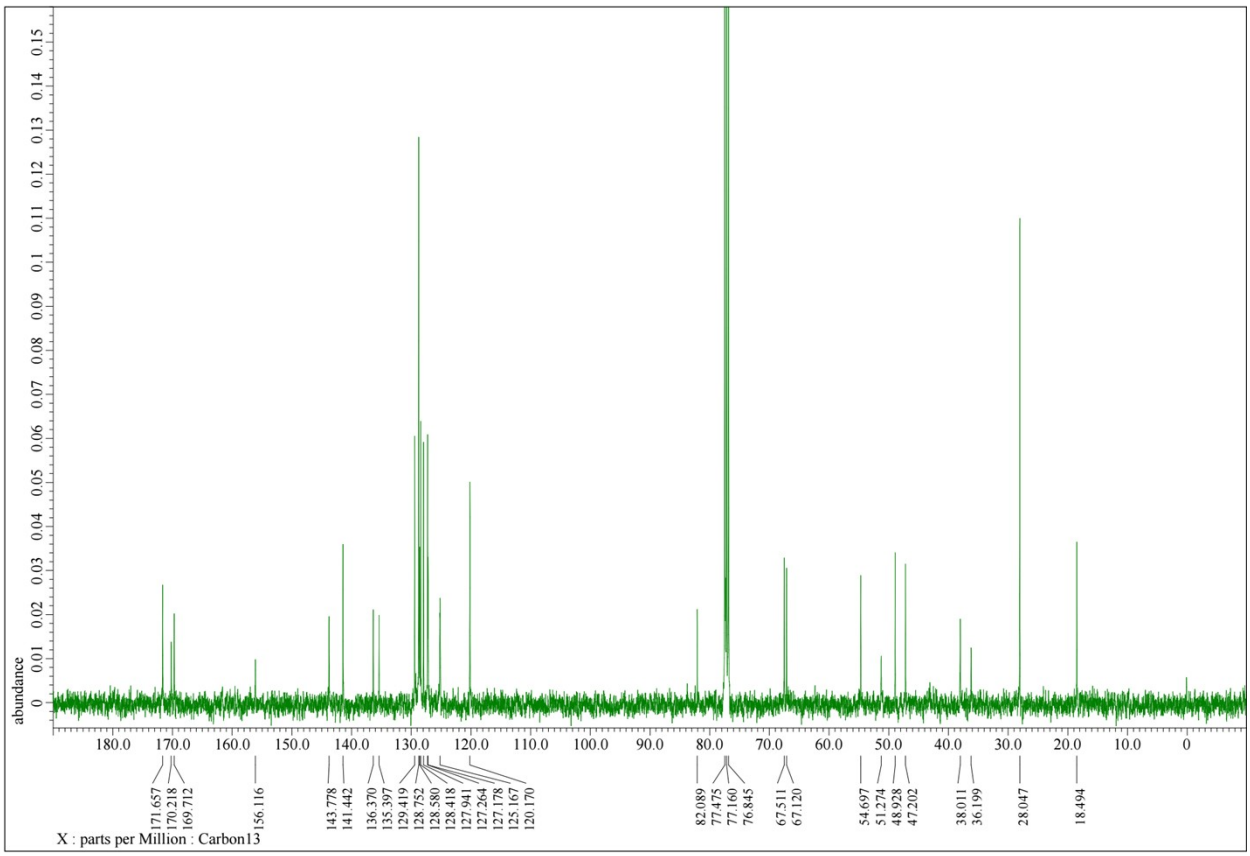


Fmoc-L-Asp(OBn)-L-Phe-L-Ala-O*t*-Bu (7l)

(^{1}H NMR, 400 MHz, $CDCl_3$)

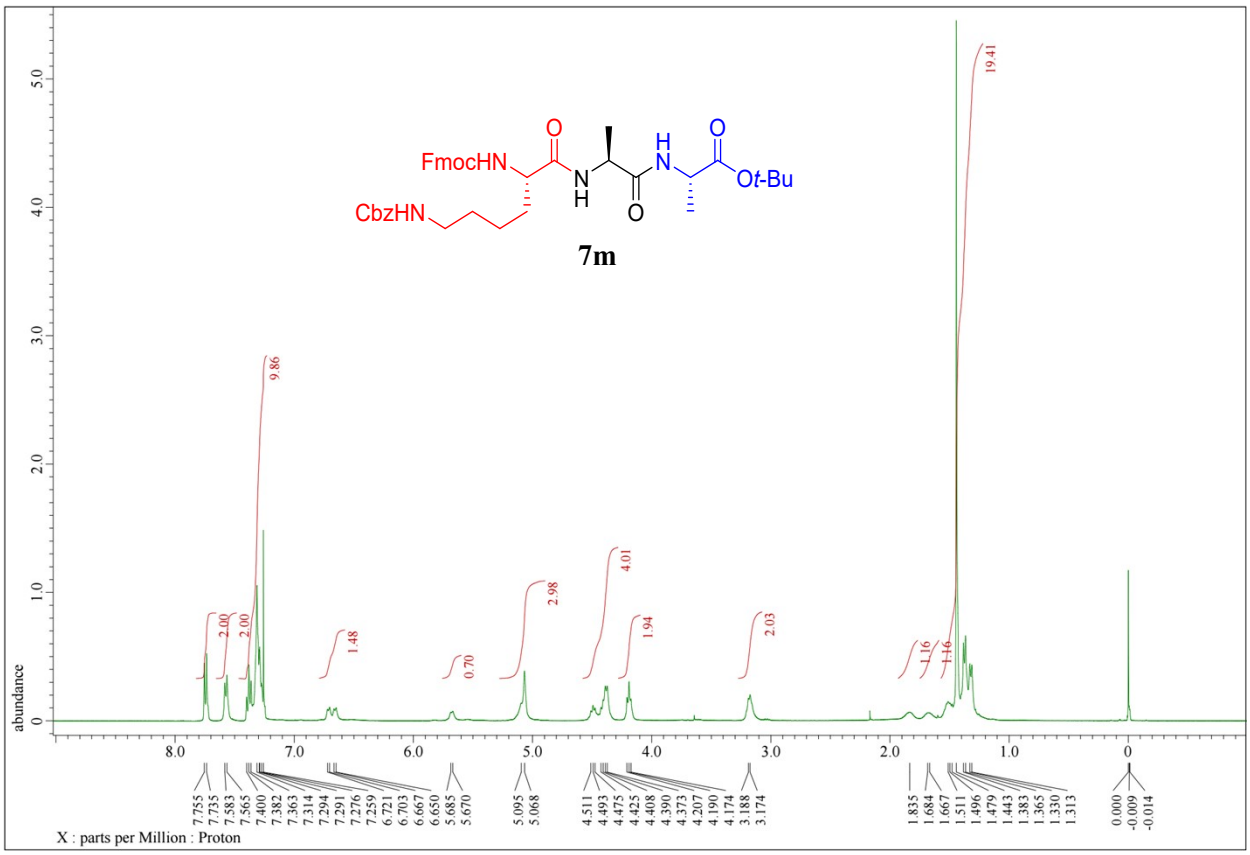


(^{13}C NMR, 100 MHz, $CDCl_3$)

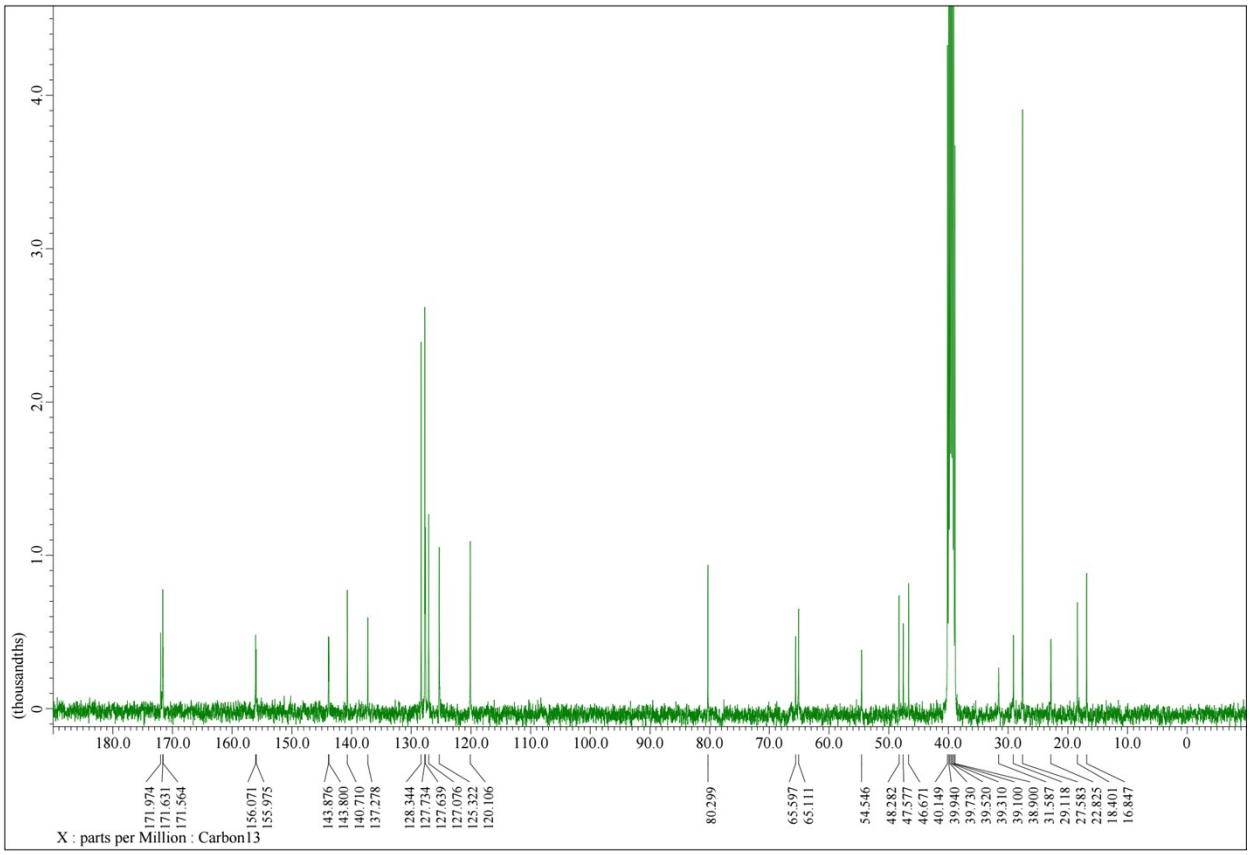


Fmoc-L-Lys(Cbz)-L-Ala-L-Ala-O*t*-Bu (7m)

(^{1}H NMR, 400 MHz, $CDCl_3$)

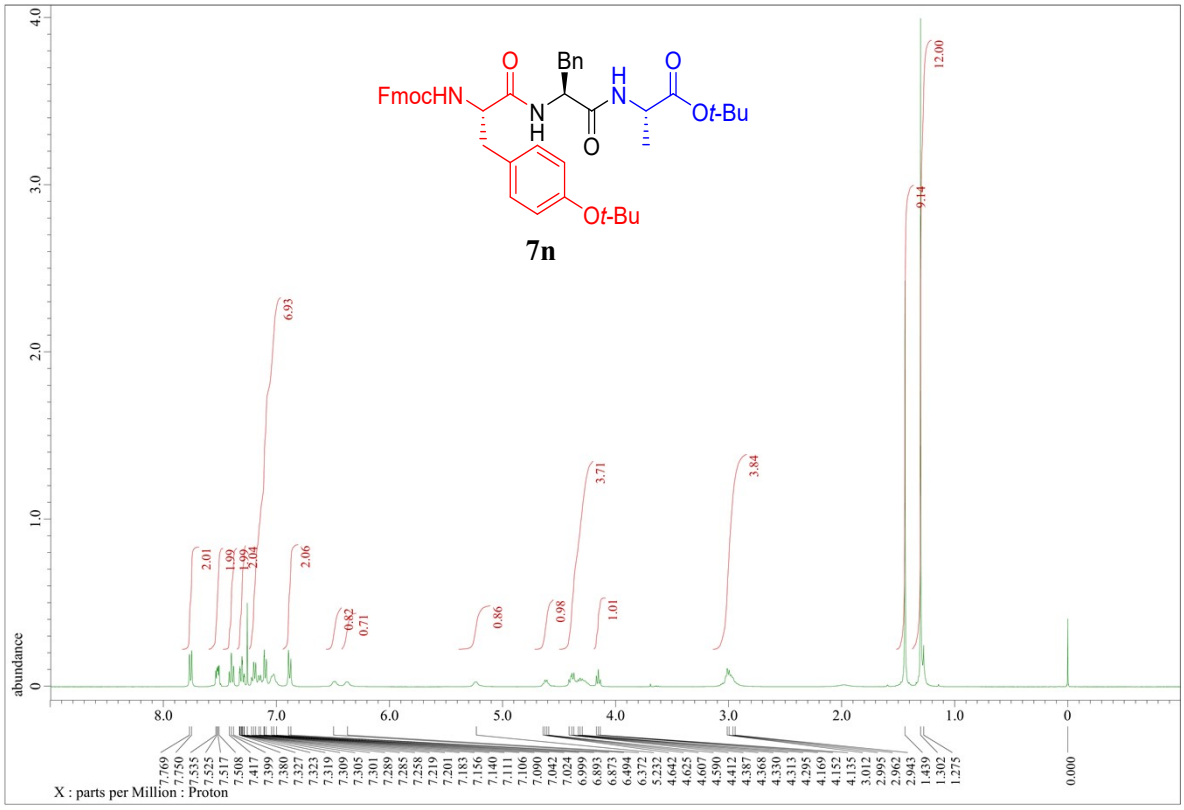


(^{13}C NMR, 100 MHz, DMSO-d_6)

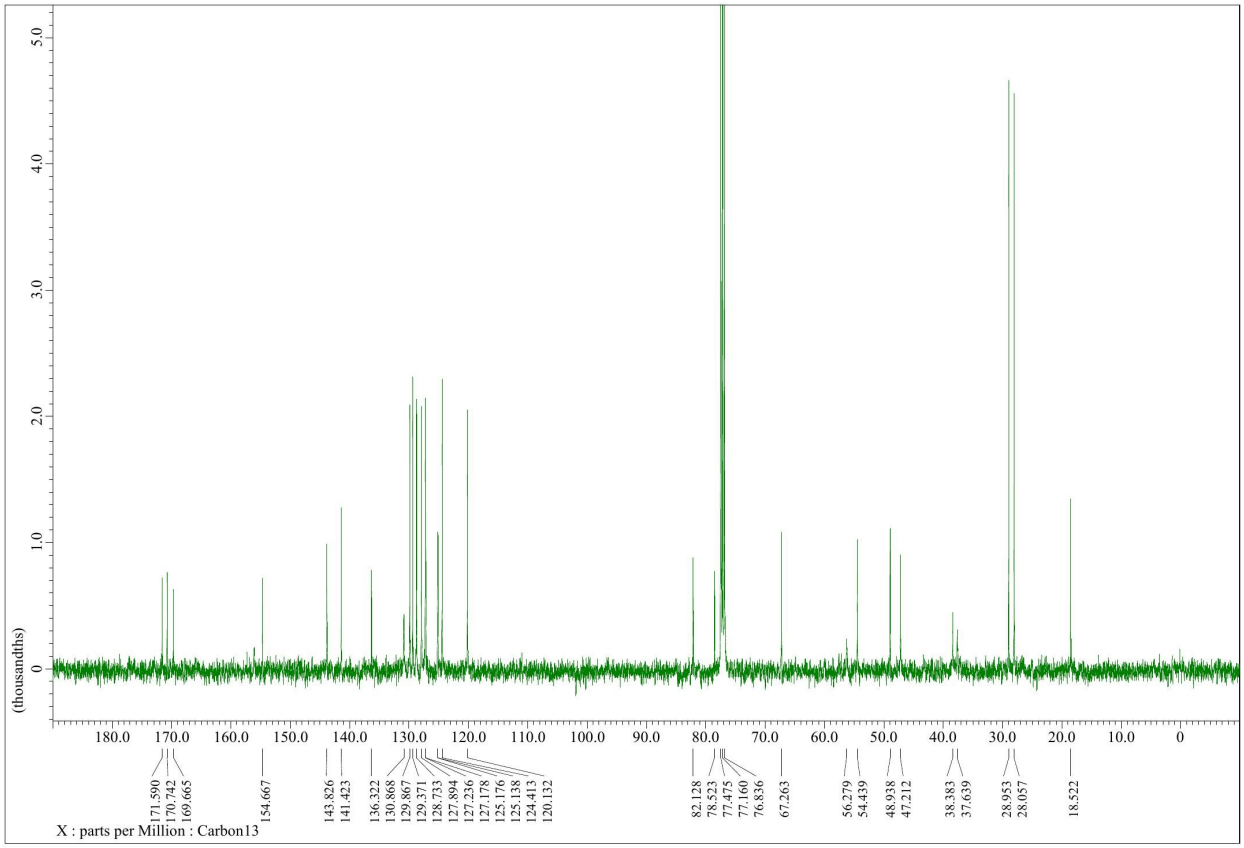


Fmoc-L-Tyr(*t*-Bu)-L-Phe-L-Ala-O*t*-Bu (7n)

(^{1}H NMR, 400 MHz, $CDCl_3$)

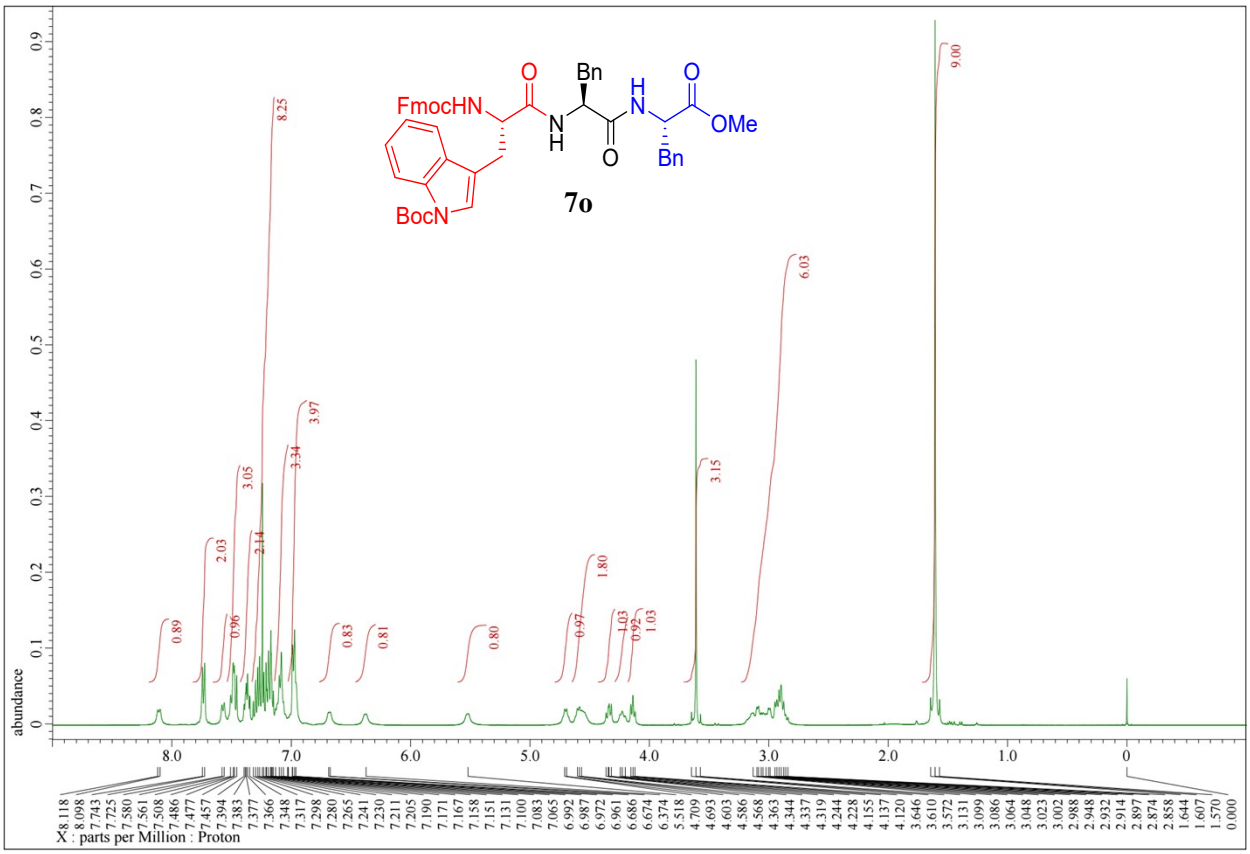


(^{13}C NMR, 100 MHz, $CDCl_3$)

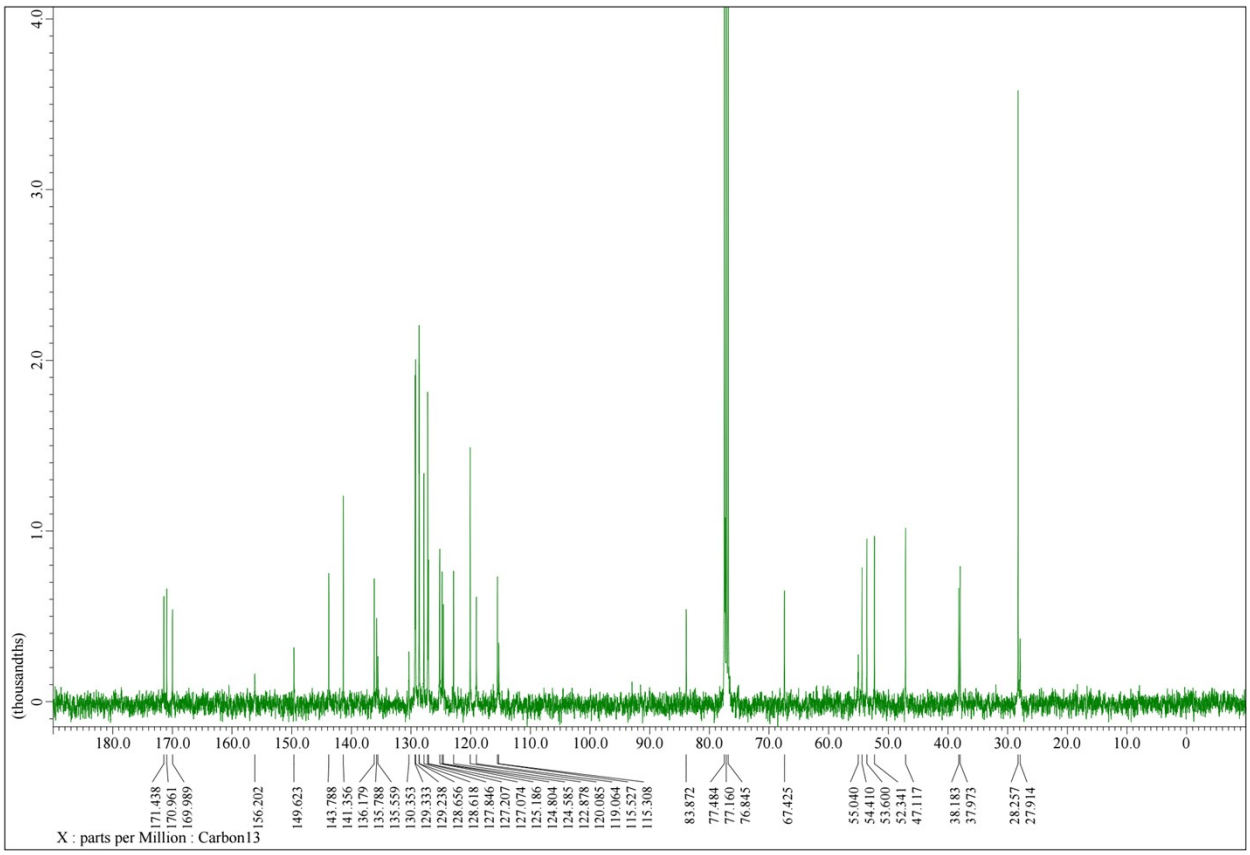


Fmoc-L-Trp(Boc)-L-Phe-L-Phe-OMe (7o)

(^{1}H NMR, 400 MHz, $CDCl_3$)

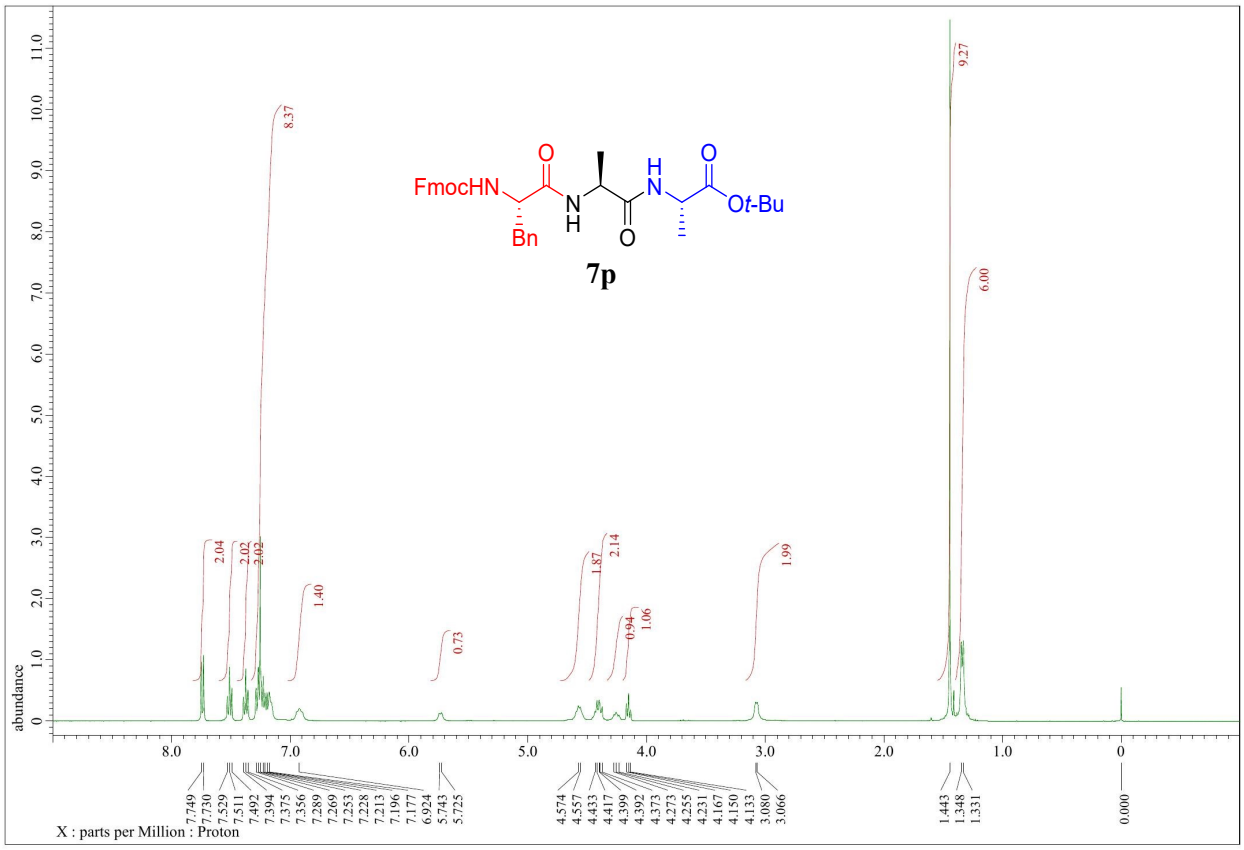


(^{13}C NMR, 100 MHz, $CDCl_3$)

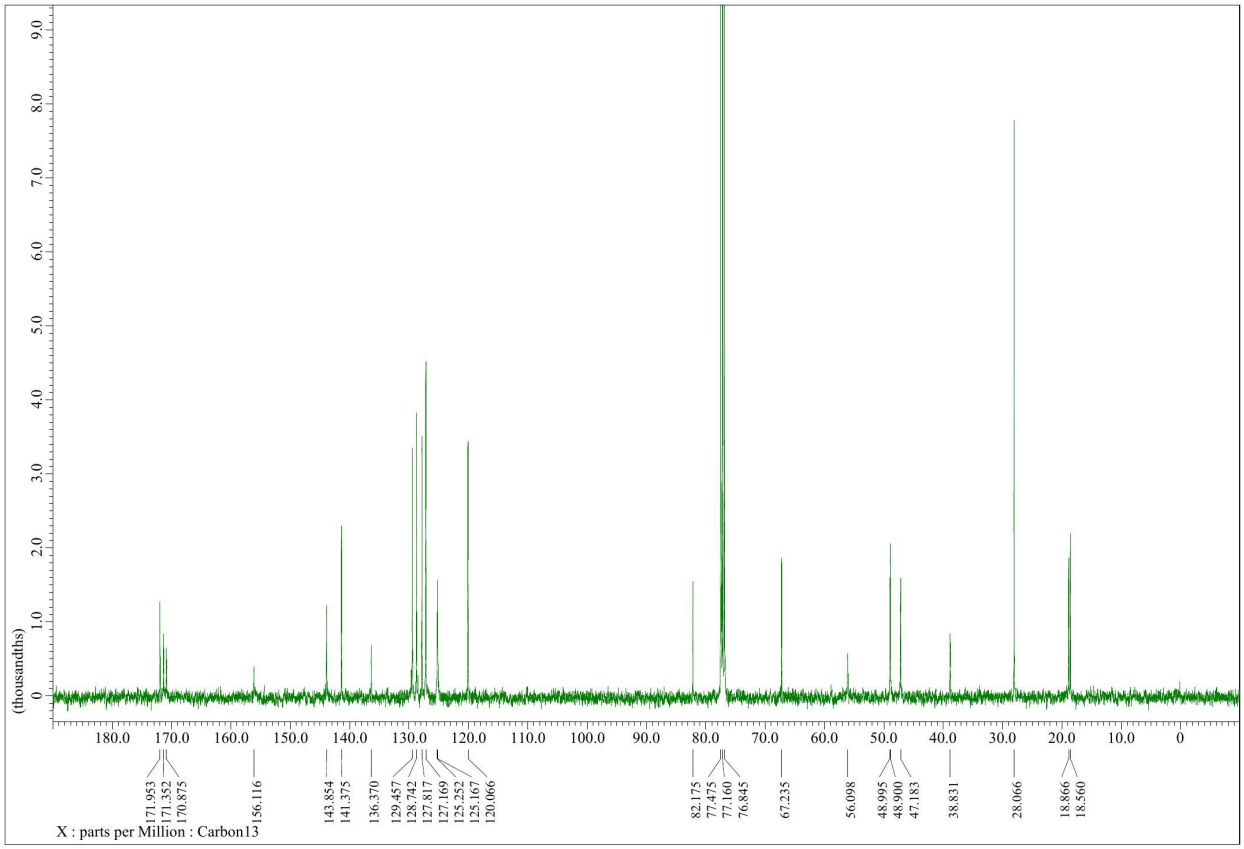


Fmoc-L-Phe-L-Ala-L-Ala-O*t*-Bu (7p)

(^{1}H NMR, 400 MHz, $CDCl_3$)

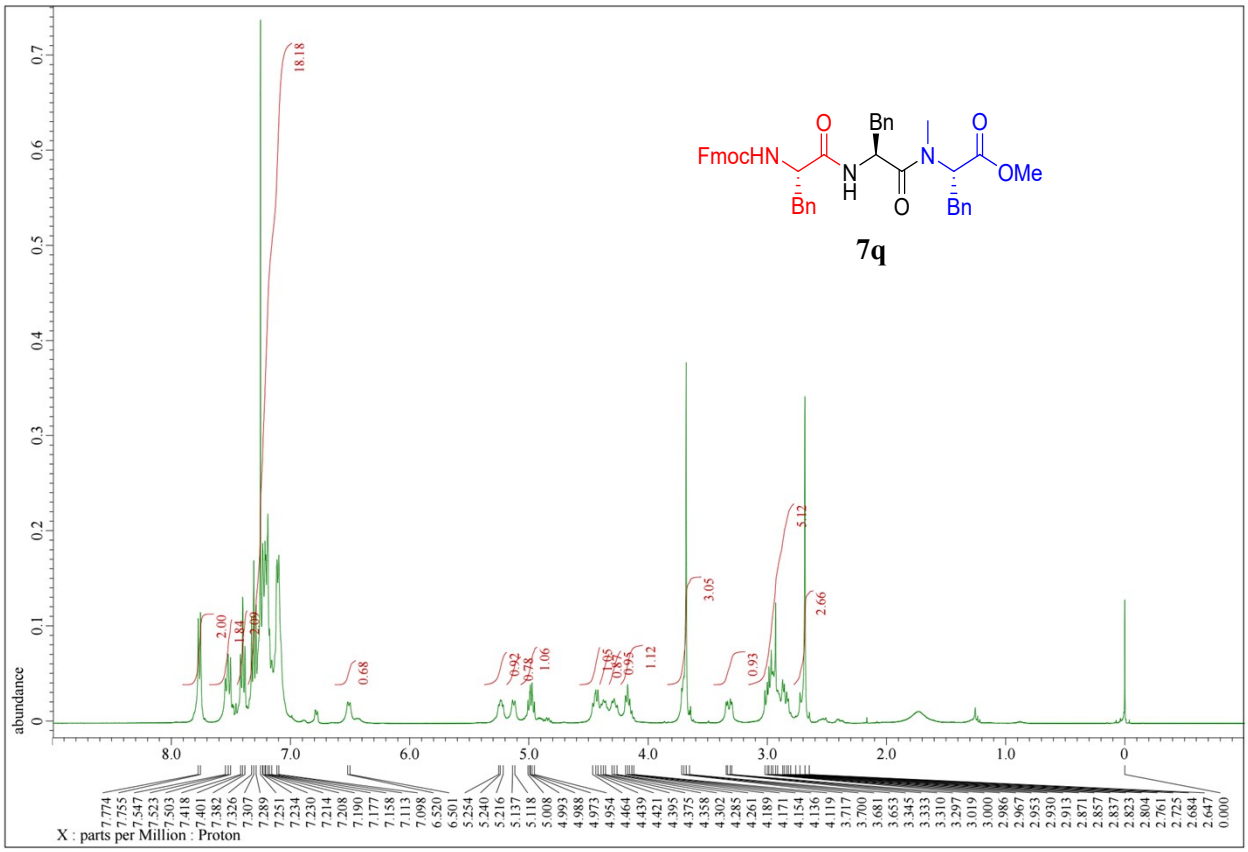


(^{13}C NMR, 100 MHz, $CDCl_3$)

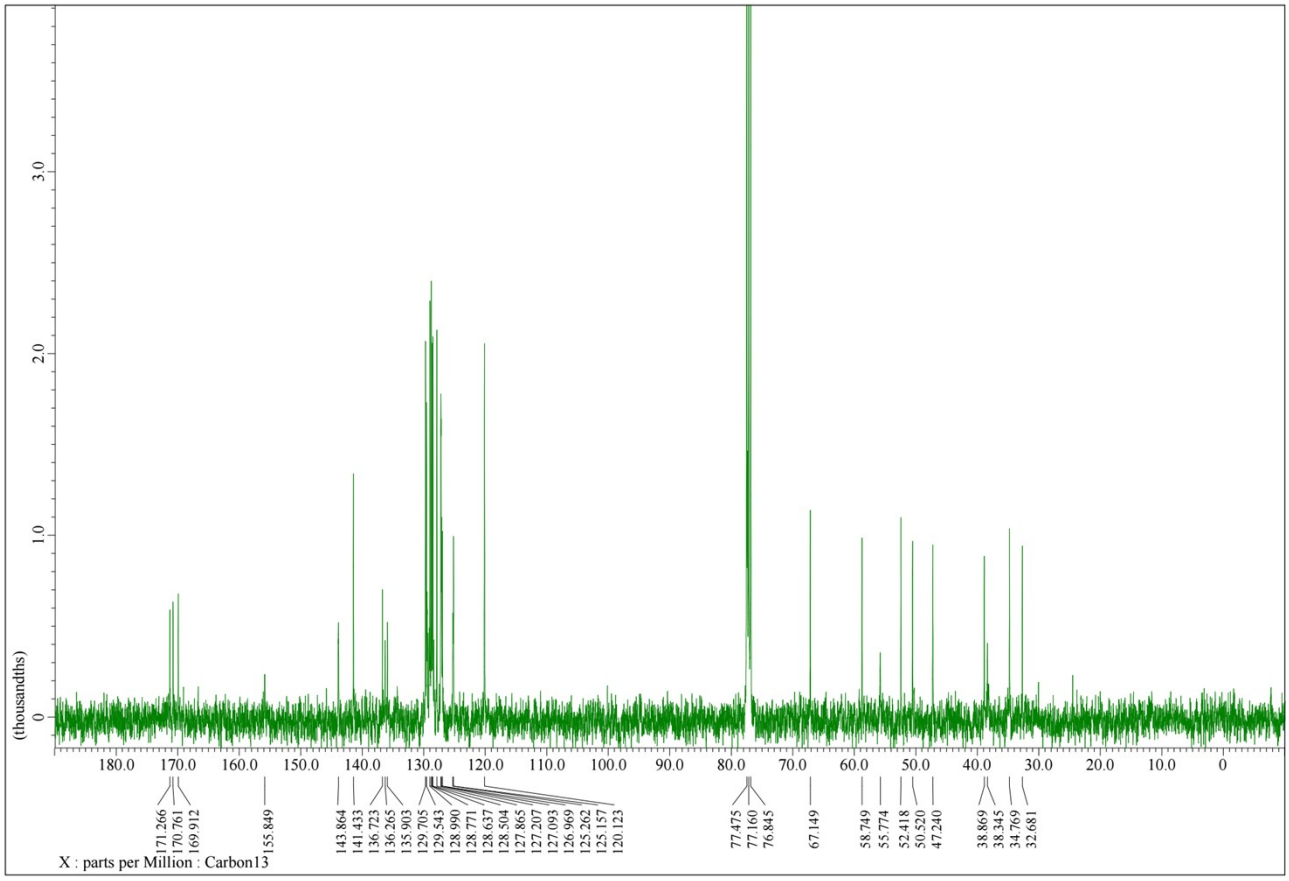


Fmoc-L-Phe-L-Phe-L-MePhe-OMe (7q)

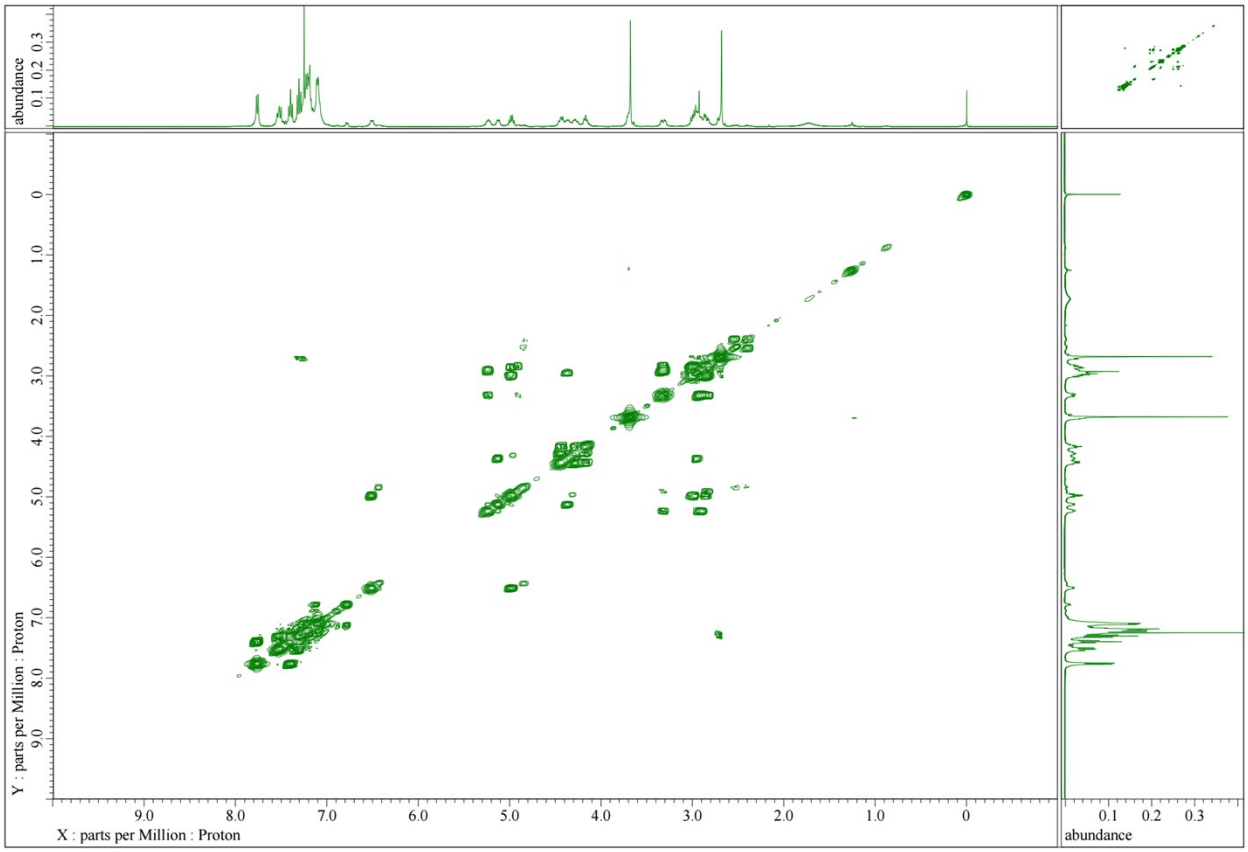
(^{1}H NMR, 400 MHz, $CDCl_3$)



(^{13}C NMR, 100 MHz, $CDCl_3$)

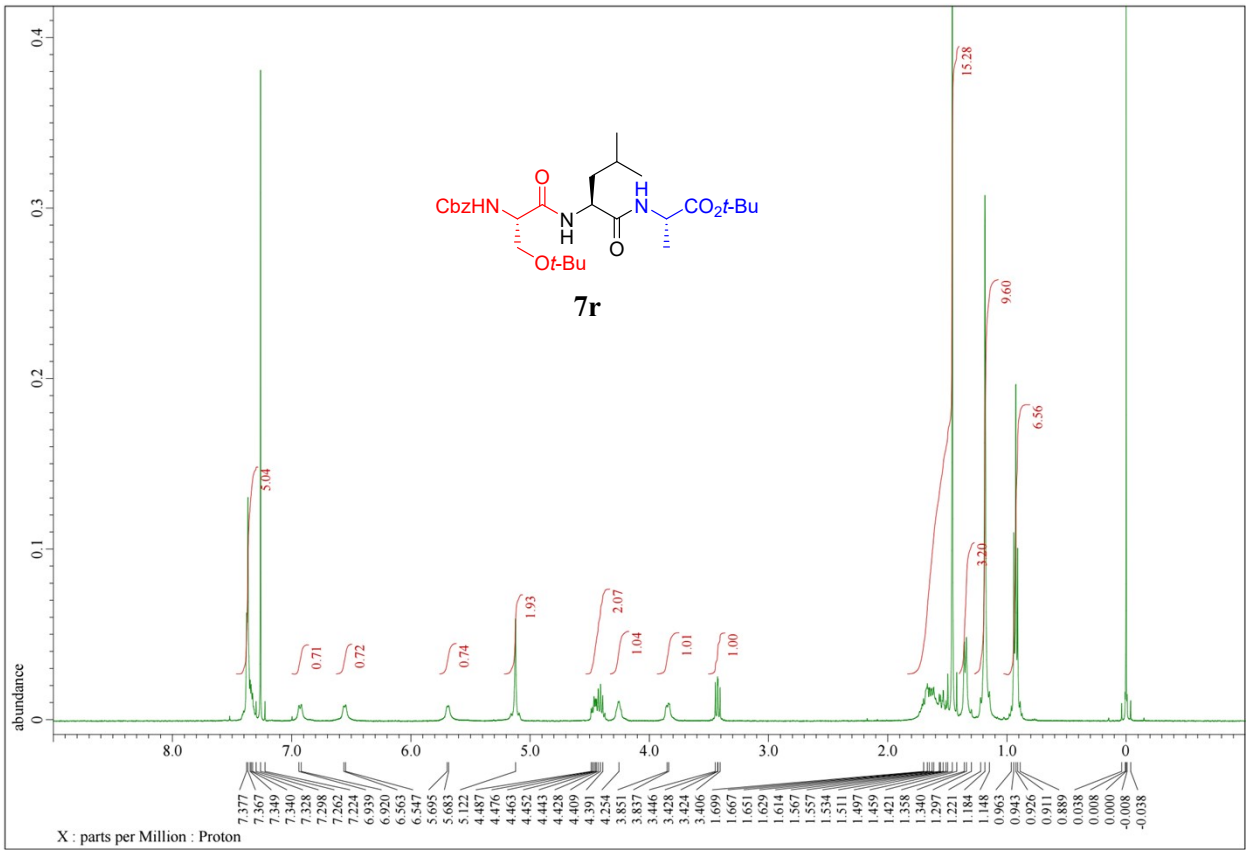


(COSY, $CDCl_3$)

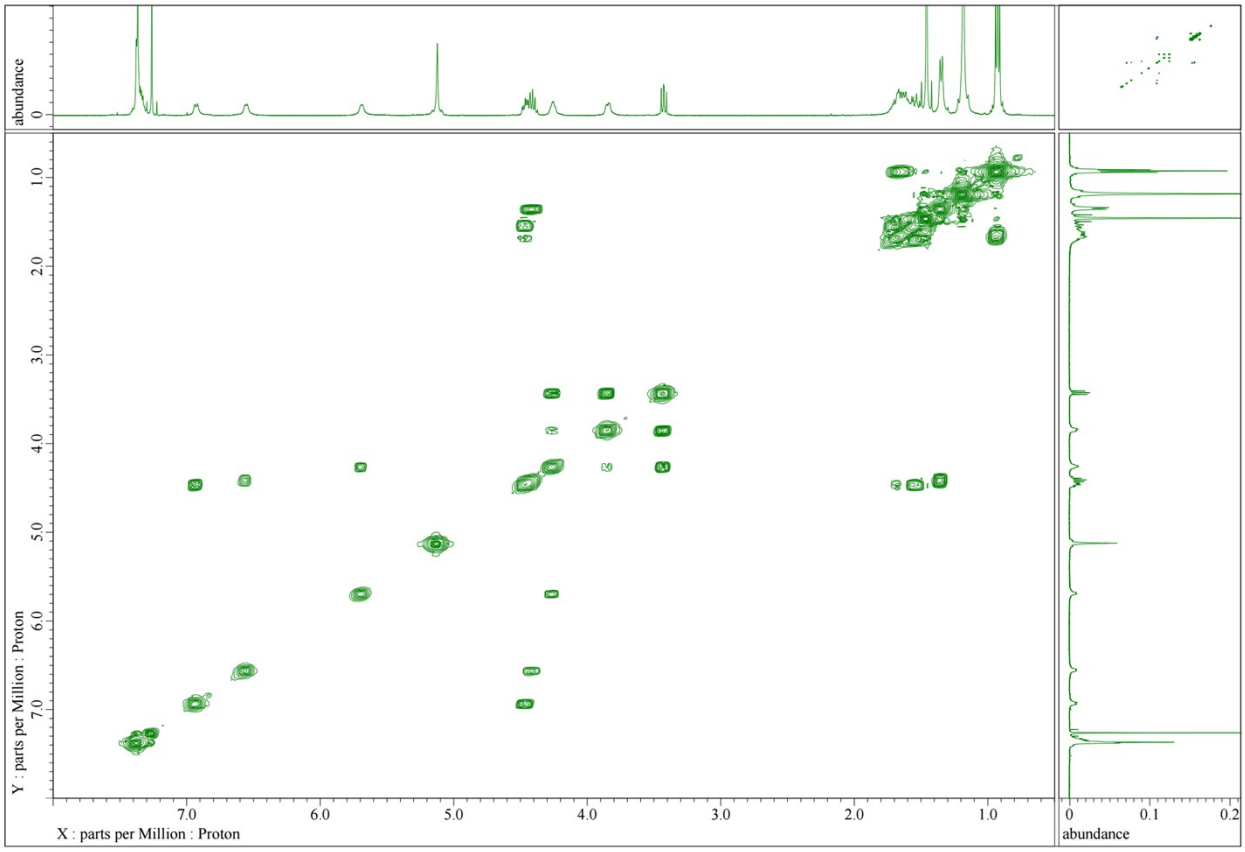


Cbz-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (7r)

(^{1}H NMR, 400 MHz, $CDCl_3$)

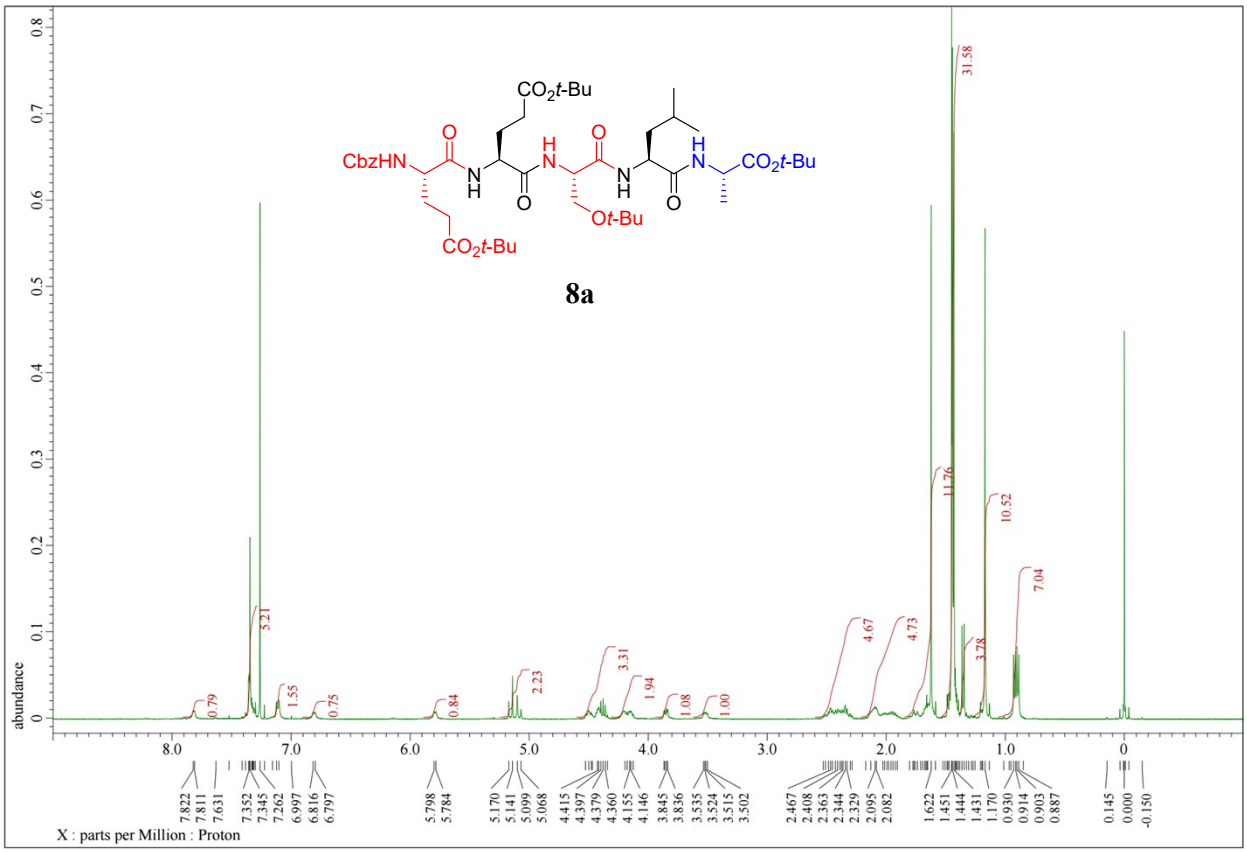


(COSY, 400 MHz, $CDCl_3$)

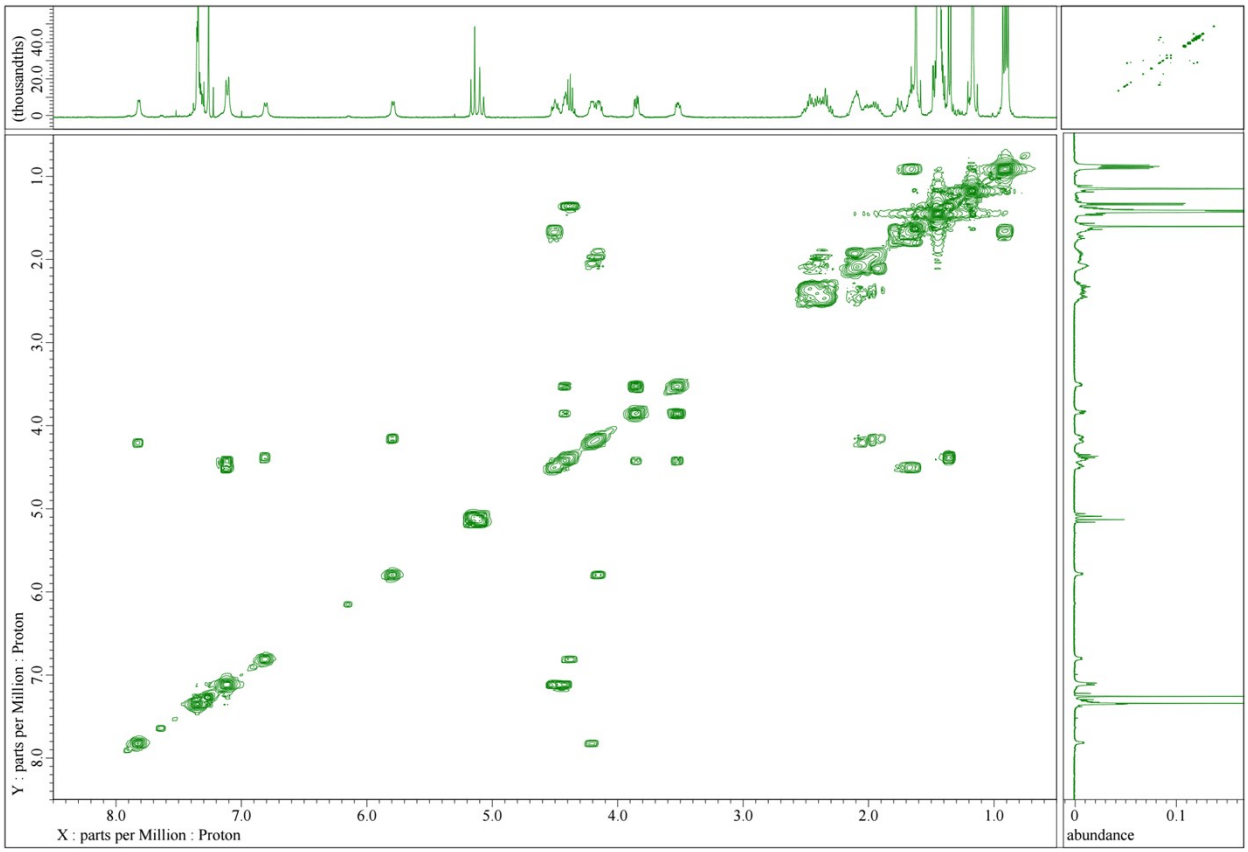


Cbz-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (8a)

(^{1}H NMR, 400 MHz, $CDCl_3$)

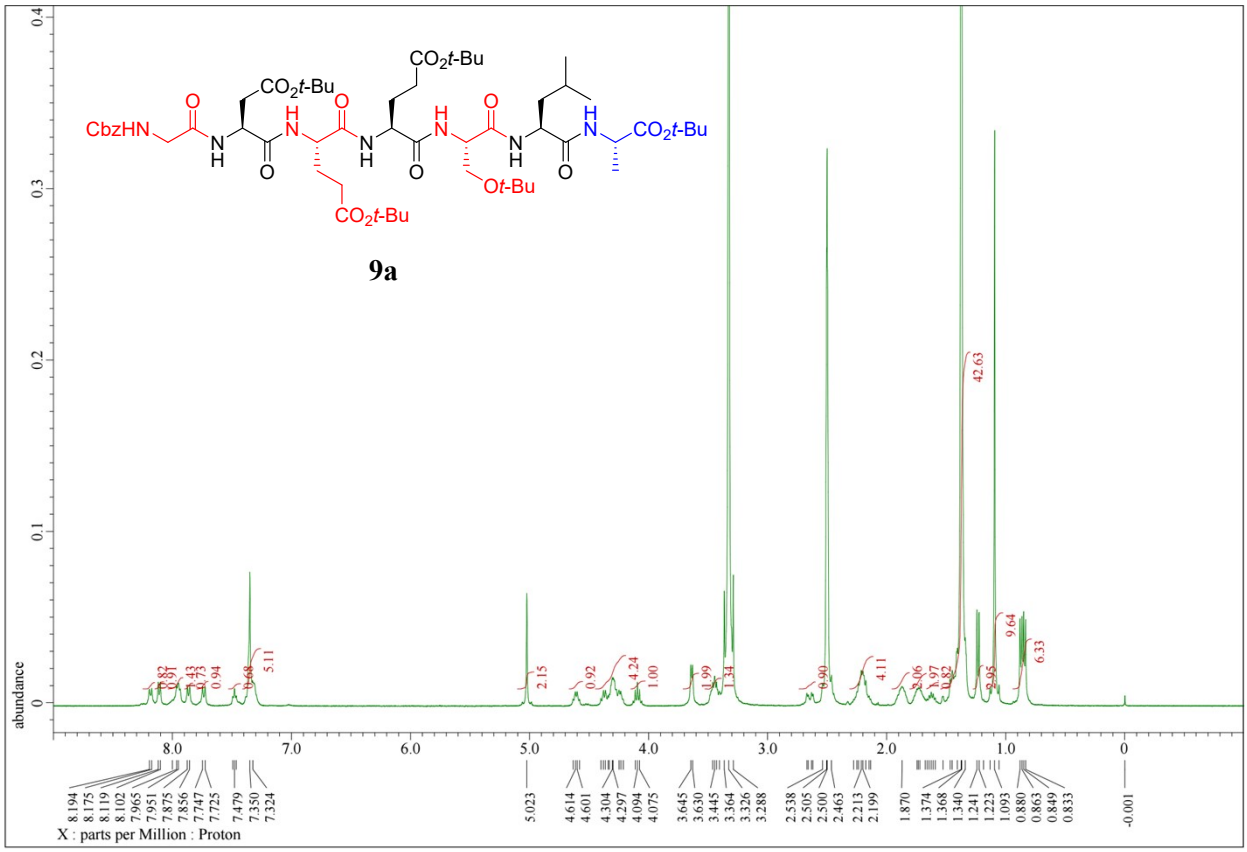


(COSY, 400 MHz, $CDCl_3$)

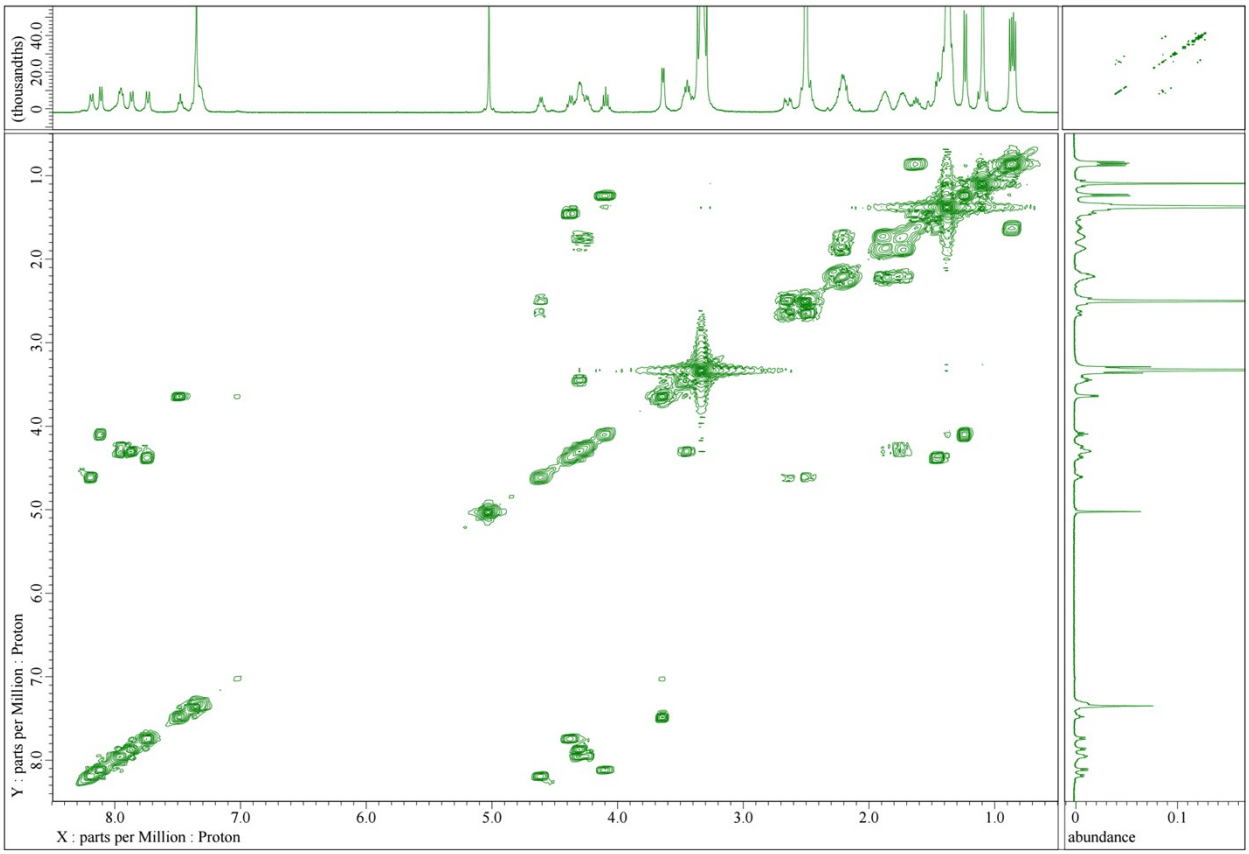


Cbz-Gly-L-Asp(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (9a)

(^{1}H NMR, 400 MHz, DMSO-d_6)

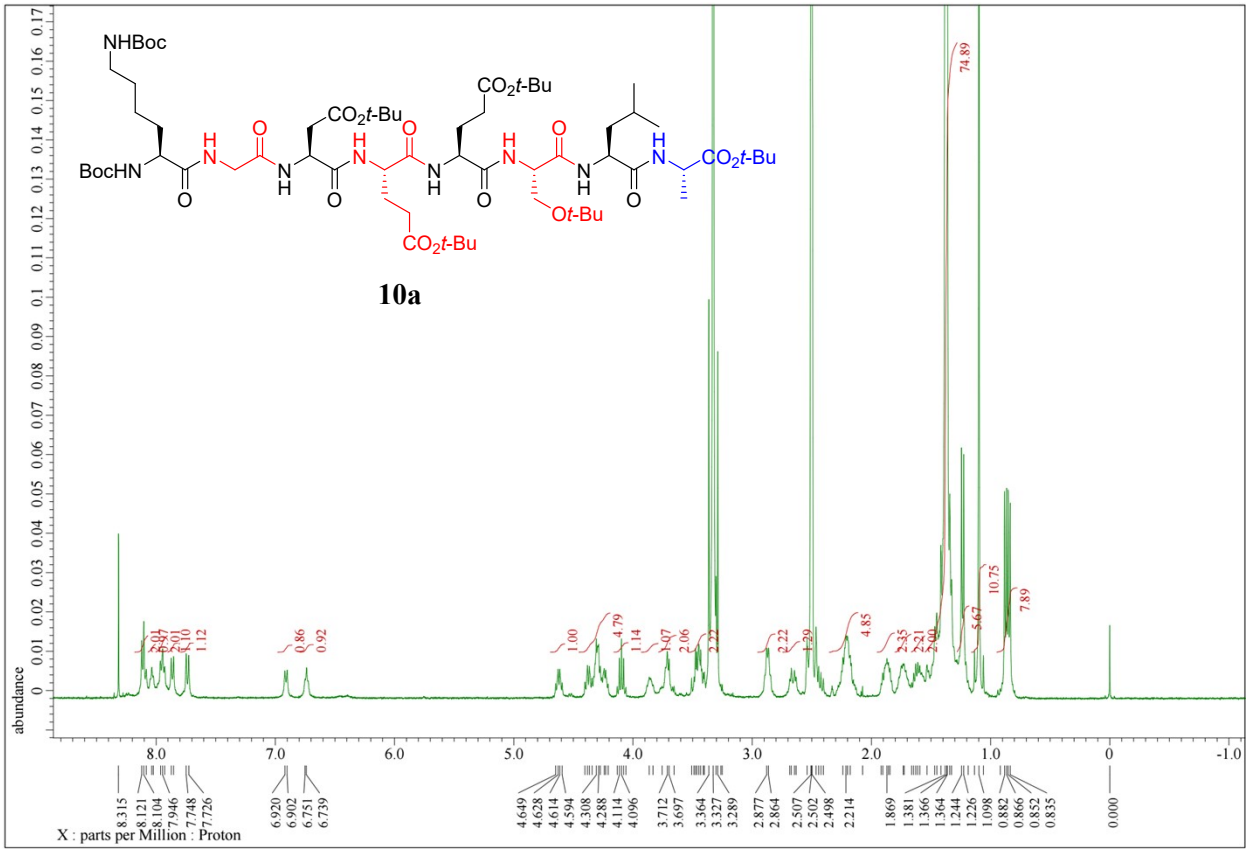


(COSY, 400 MHz, DMSO-d_6)

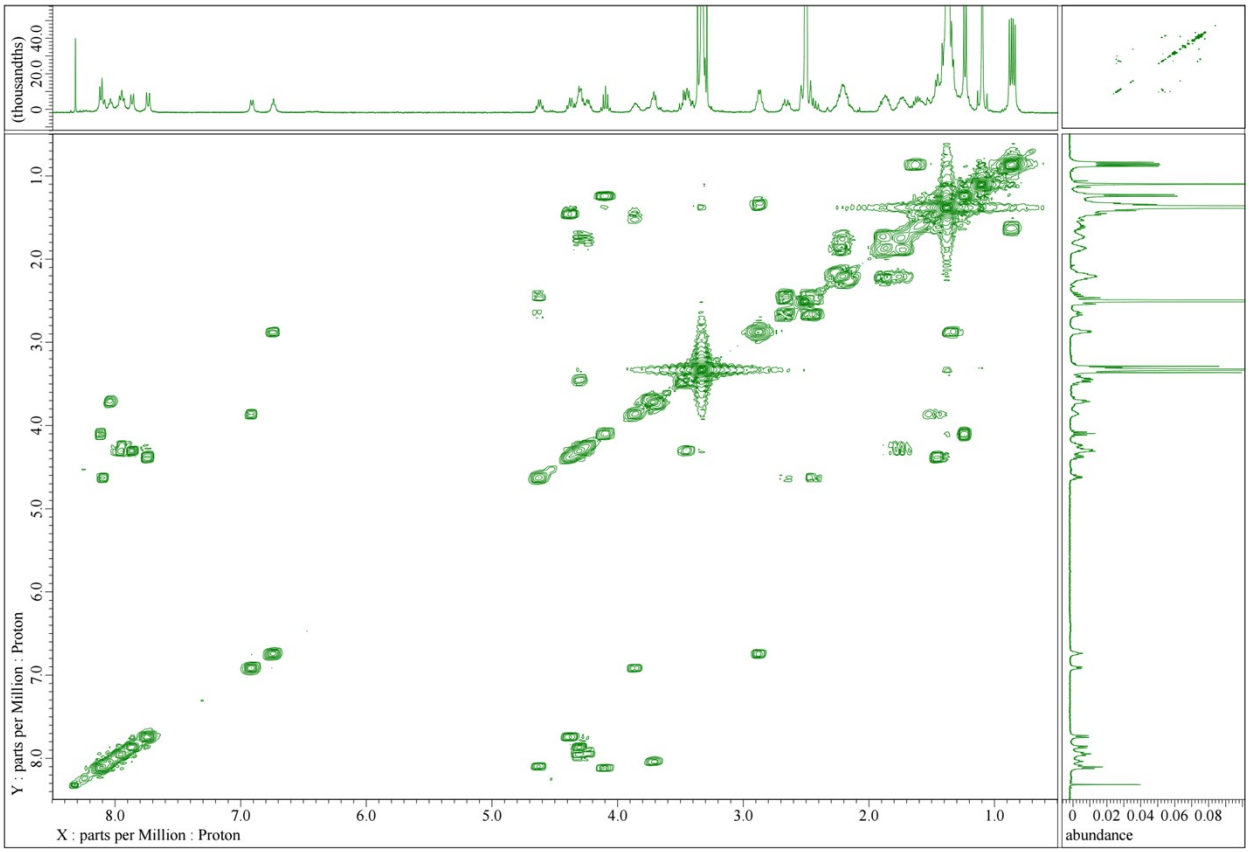


Boc-L-Lys(Boc)-Gly-L-Asp(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Glu(O*t*-Bu)-L-Ser(*t*-Bu)-L-Leu-L-Ala-O*t*-Bu (10a)

(^{1}H NMR, 400 MHz, DMSO-d_6)

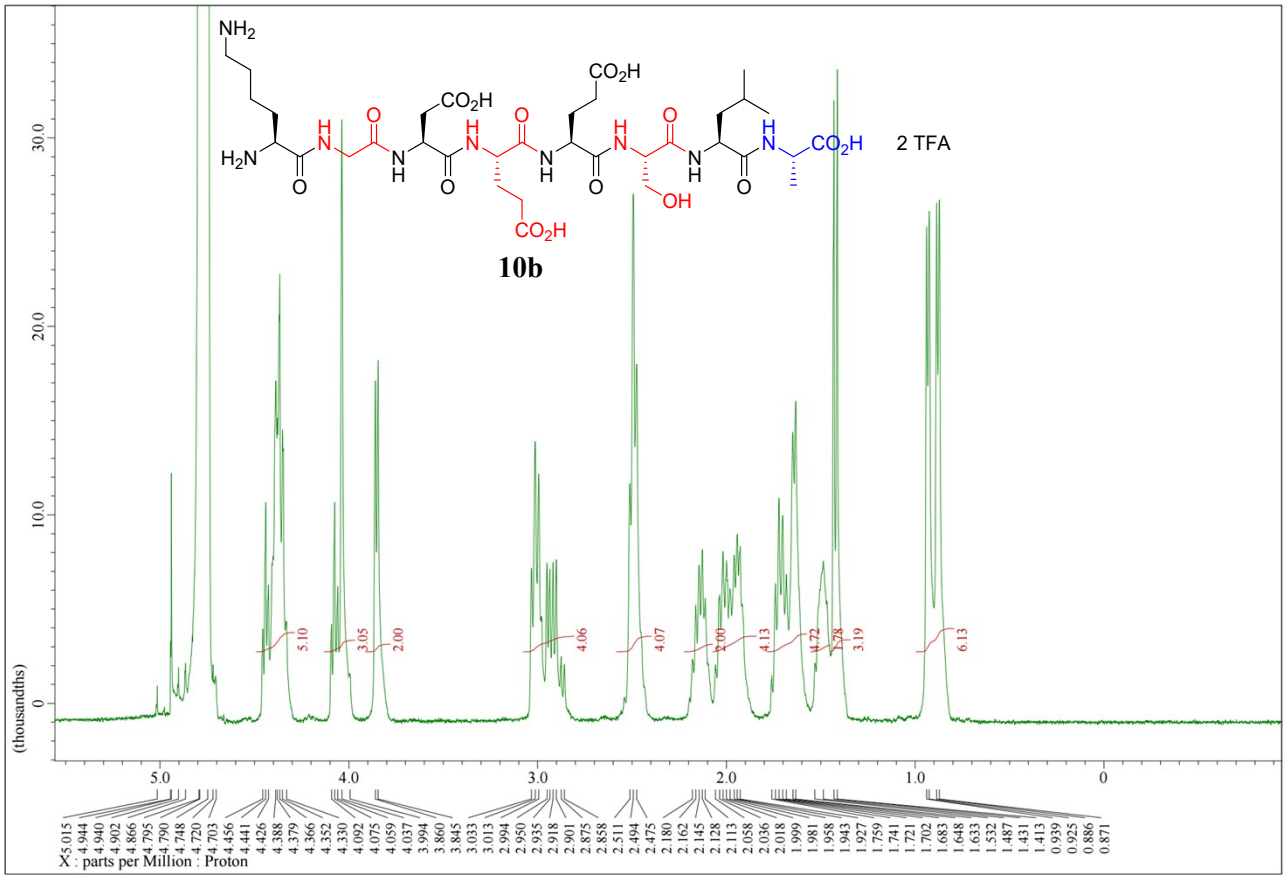


(COSY, 400 MHz, DMSO-d_6)

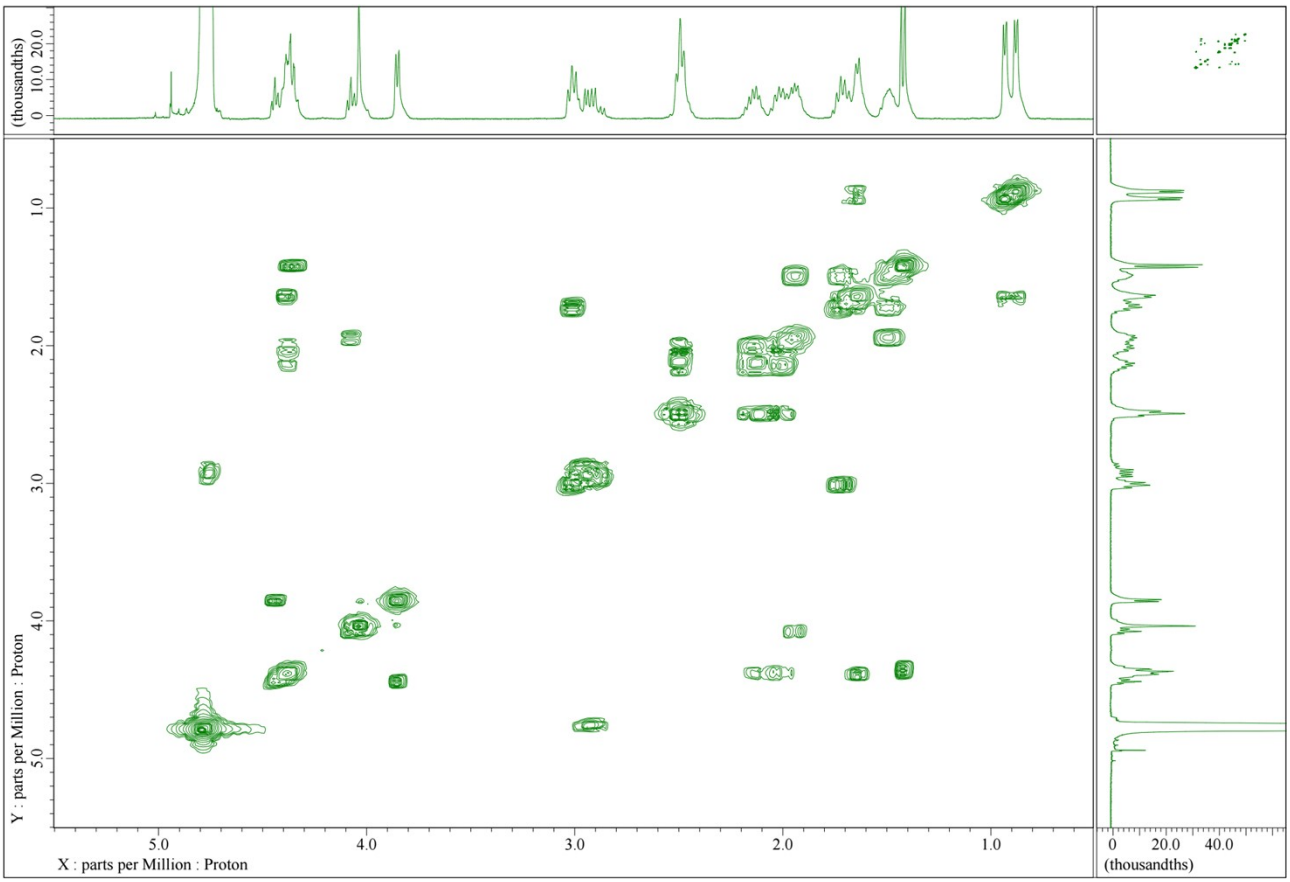


Beefy meaty peptide·2TFA (H-L-Lys-Gly-L-Asp-L-Glu-L-Glu-L-Ser-L-Leu-L-Ala-OH·2TFA) (10b)

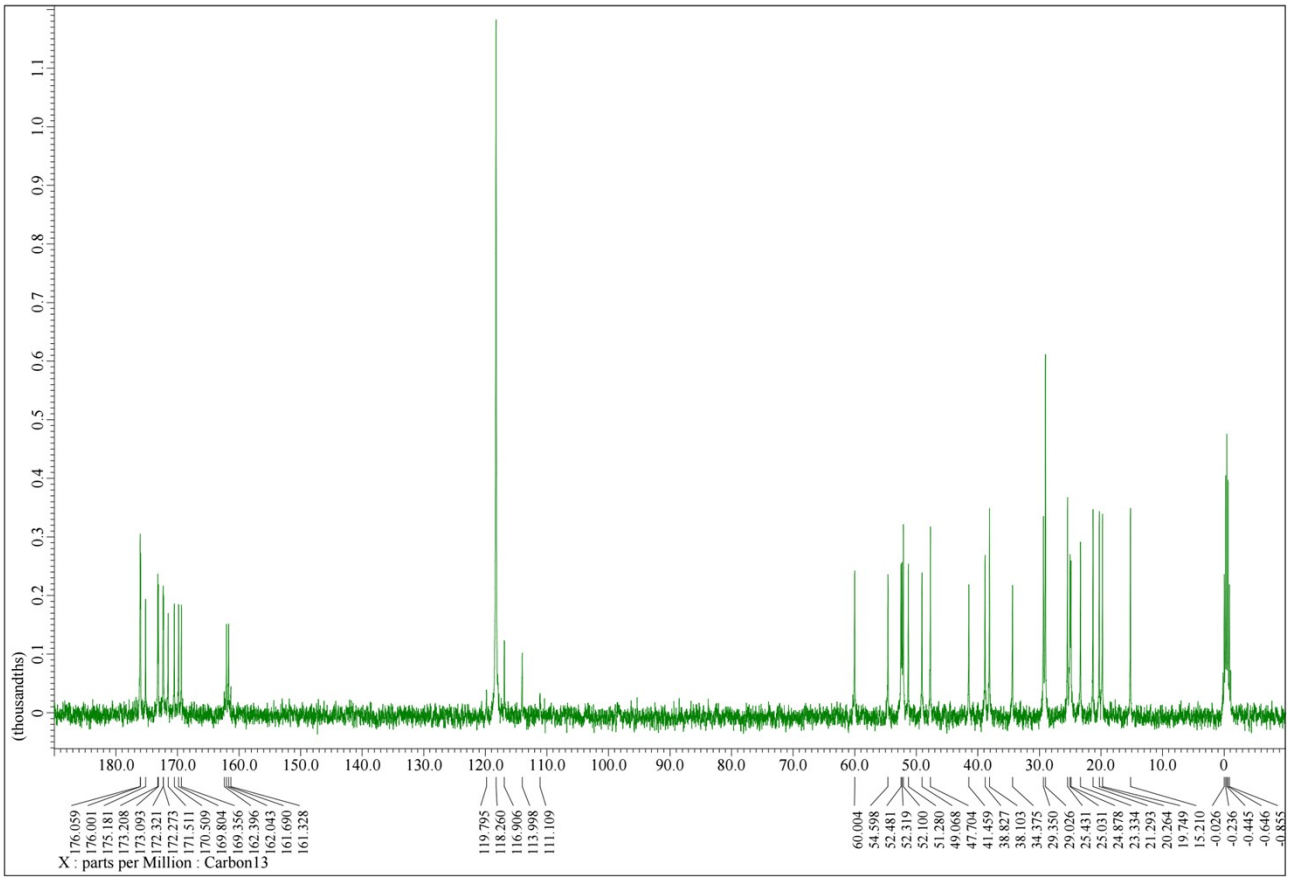
(^{1}H NMR, 400 MHz, D_2O)



(COSY, 400 MHz, D_2O)

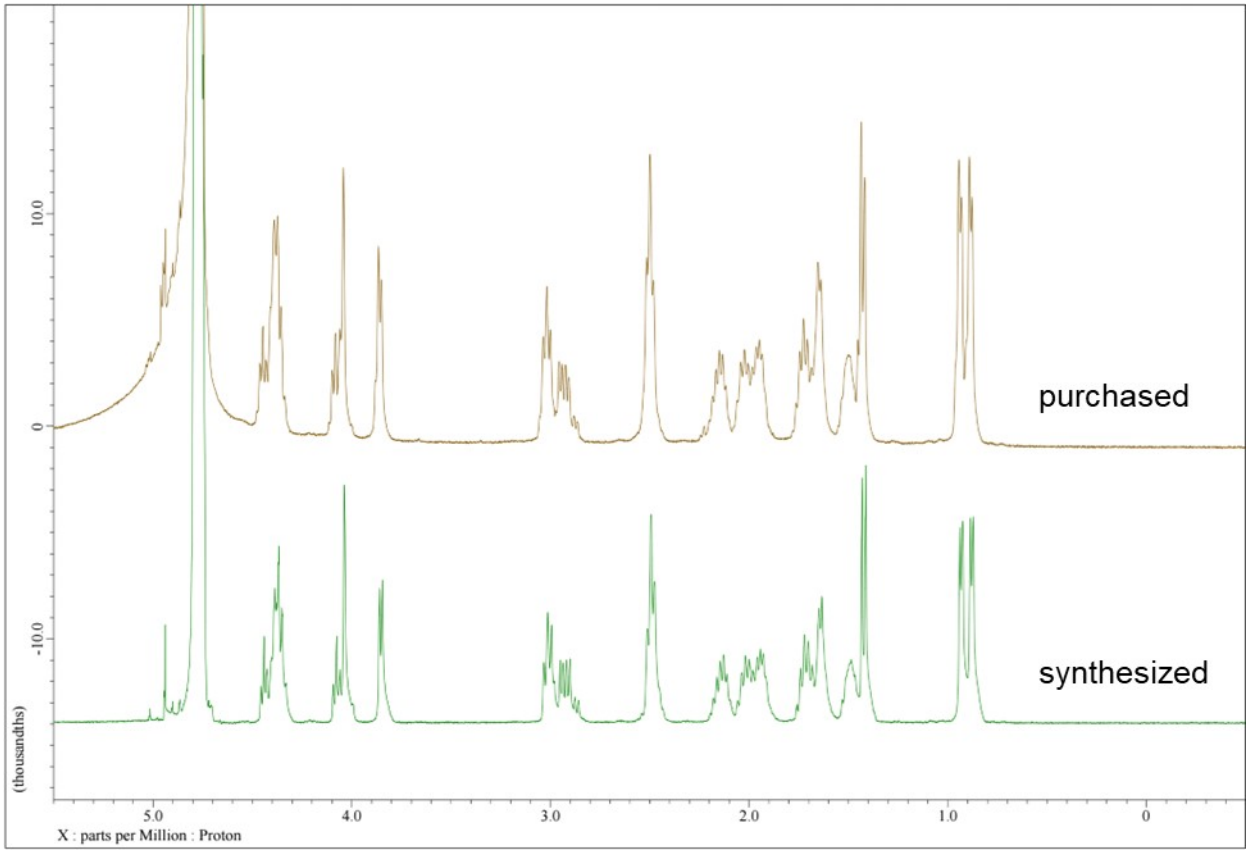


(^{13}C NMR, 100 MHz, D_2O)

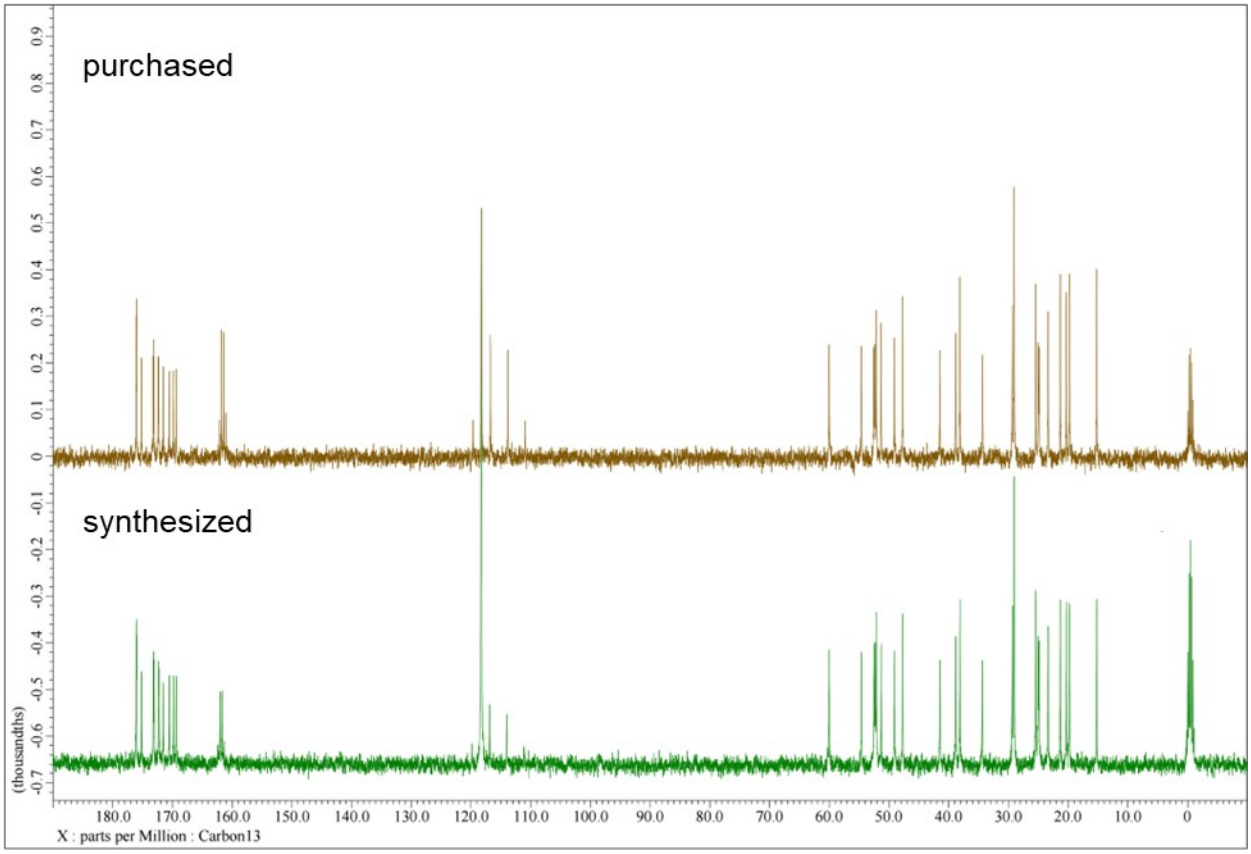


Comparison of NMR spectra between purchased and synthesized beefy meaty peptide · 2TFA

(^{1}H NMR, 400 MHz, D_2O)

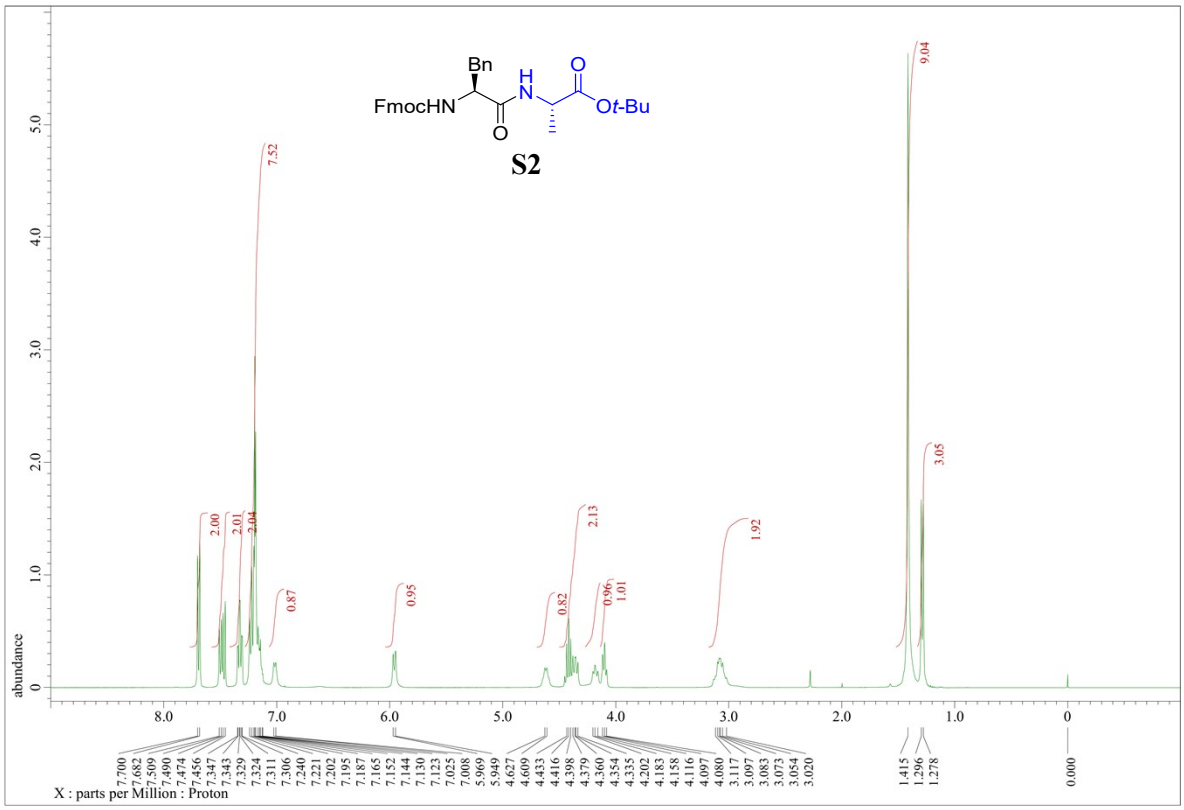


(^{13}C NMR, 100 MHz, D_2O)

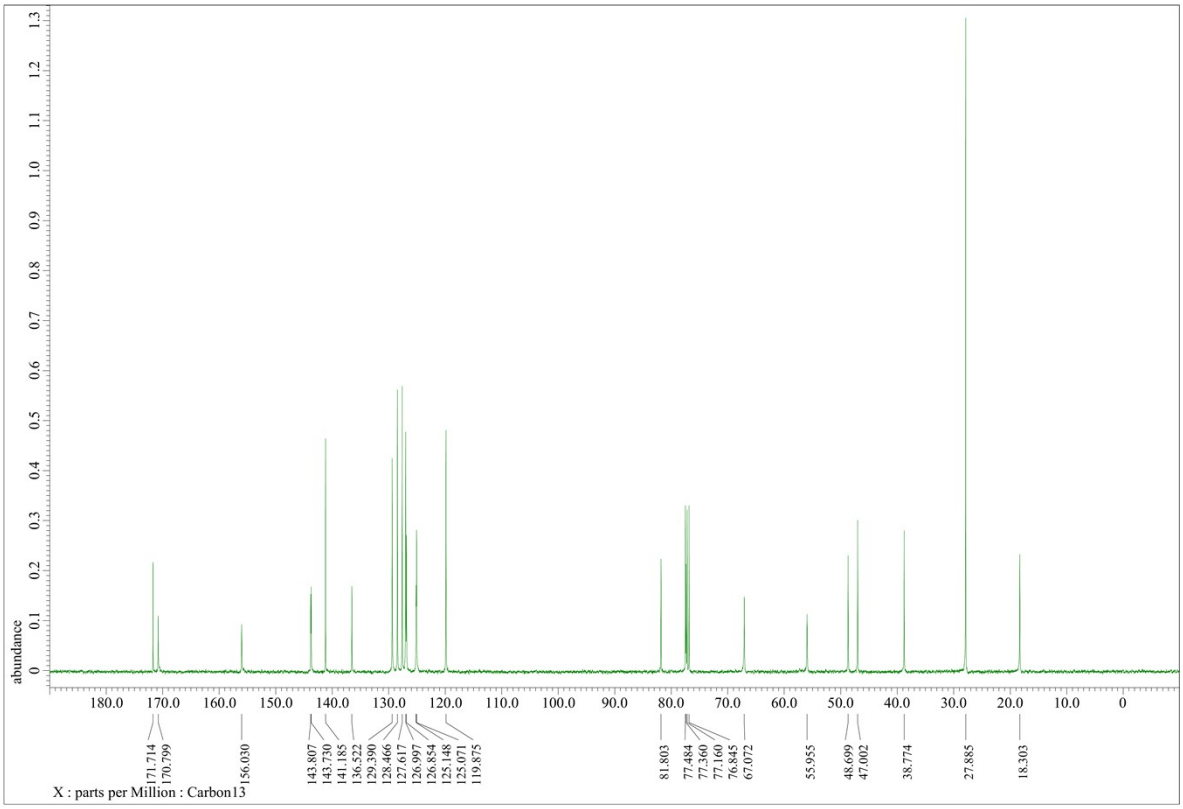


Fmoc-L-Phe-L-Ala-O*t*-Bu (S2)

(^{1}H NMR, 400 MHz, $CDCl_3$)

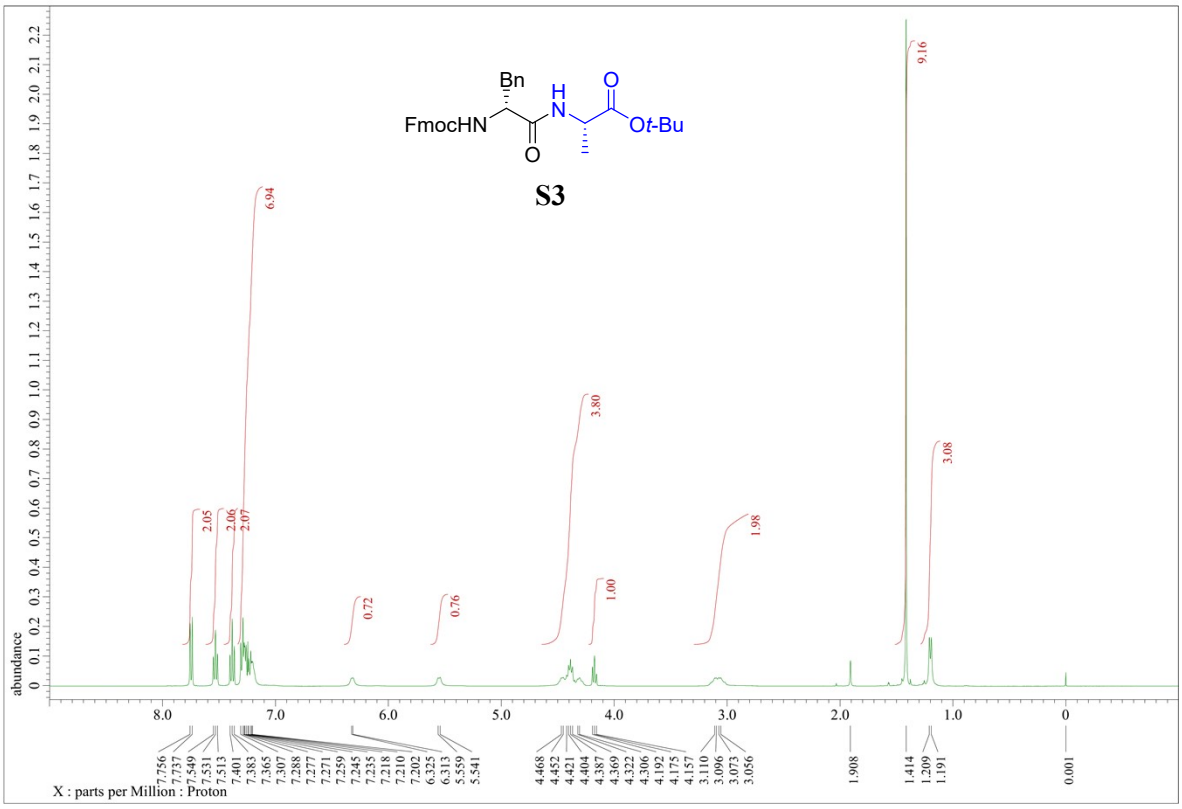


(^{13}C NMR, 100 MHz, $CDCl_3$)

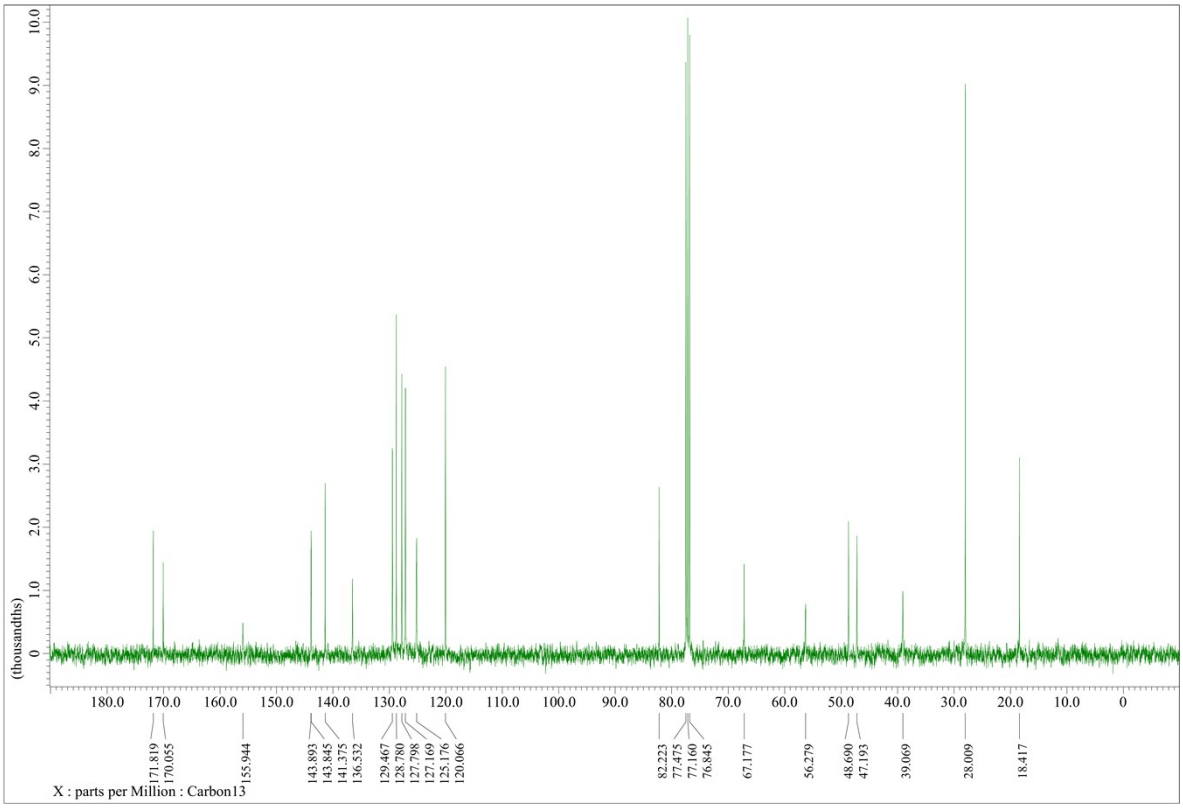


Fmoc-D-Phe-L-Ala-O*t*-Bu (S3)

(^{1}H NMR, 400 MHz, $CDCl_3$)

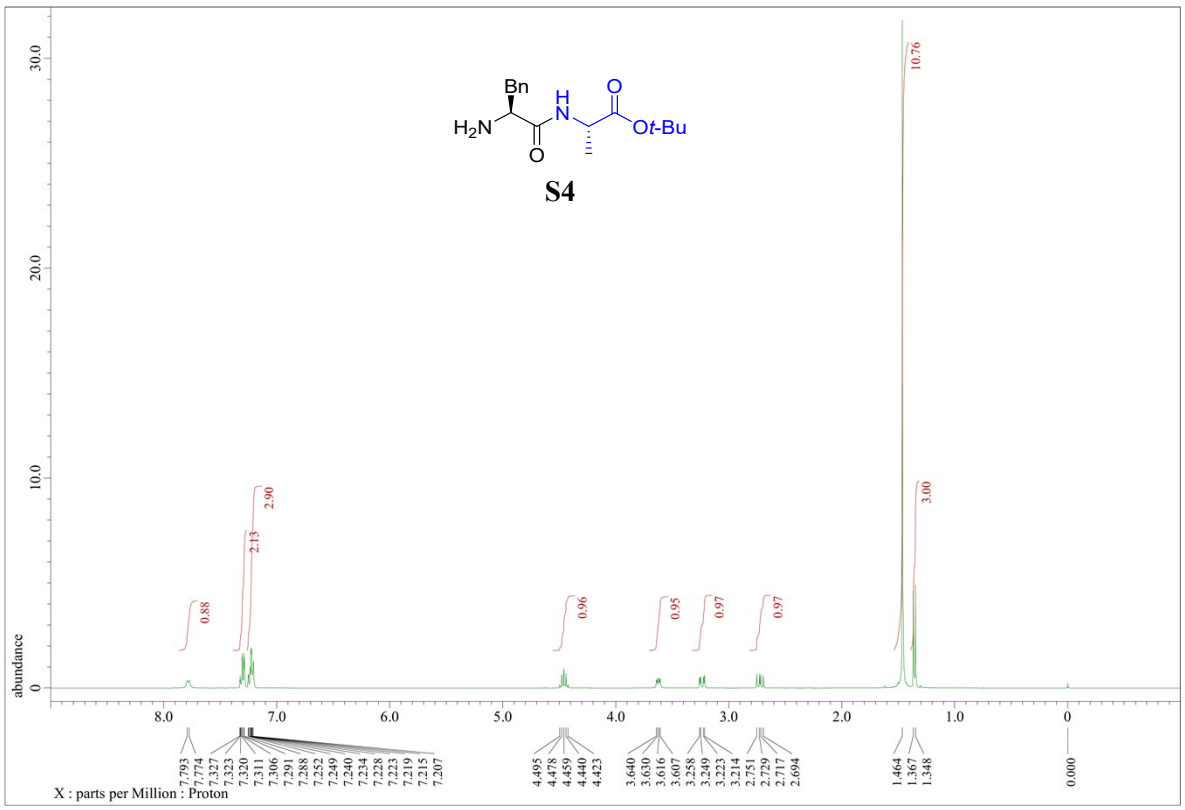


(^{13}C NMR, 100 MHz, $CDCl_3$)

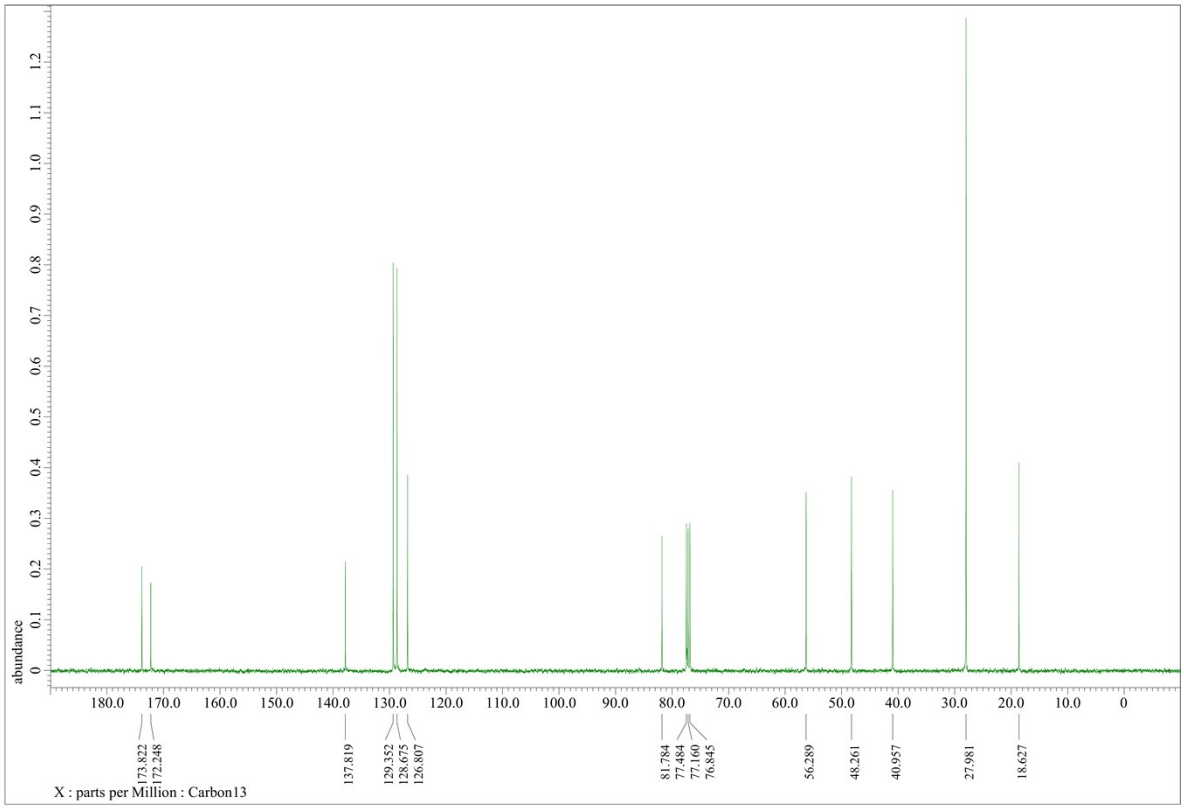


H-L-Phe-L-Ala-O*t*-Bu (S4)

(^{1}H NMR, 400 MHz, $CDCl_3$)

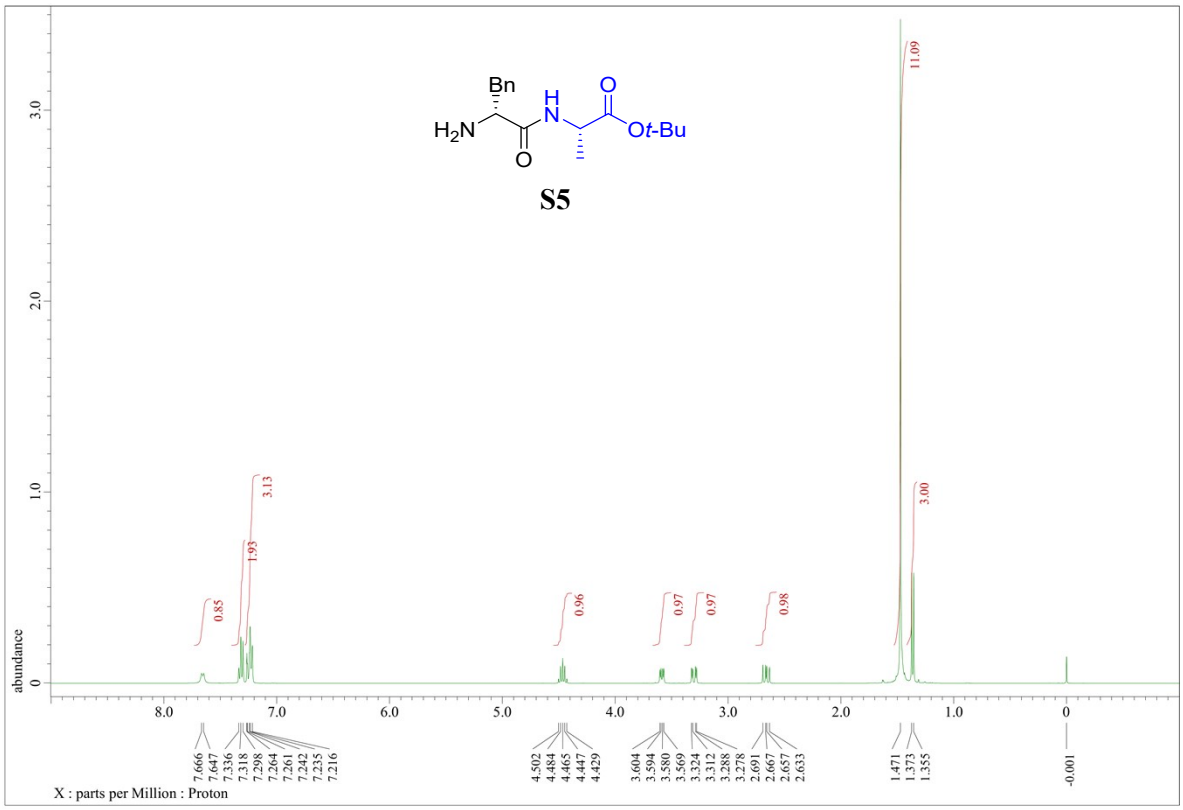


(^{13}C NMR, 100 MHz, $CDCl_3$)

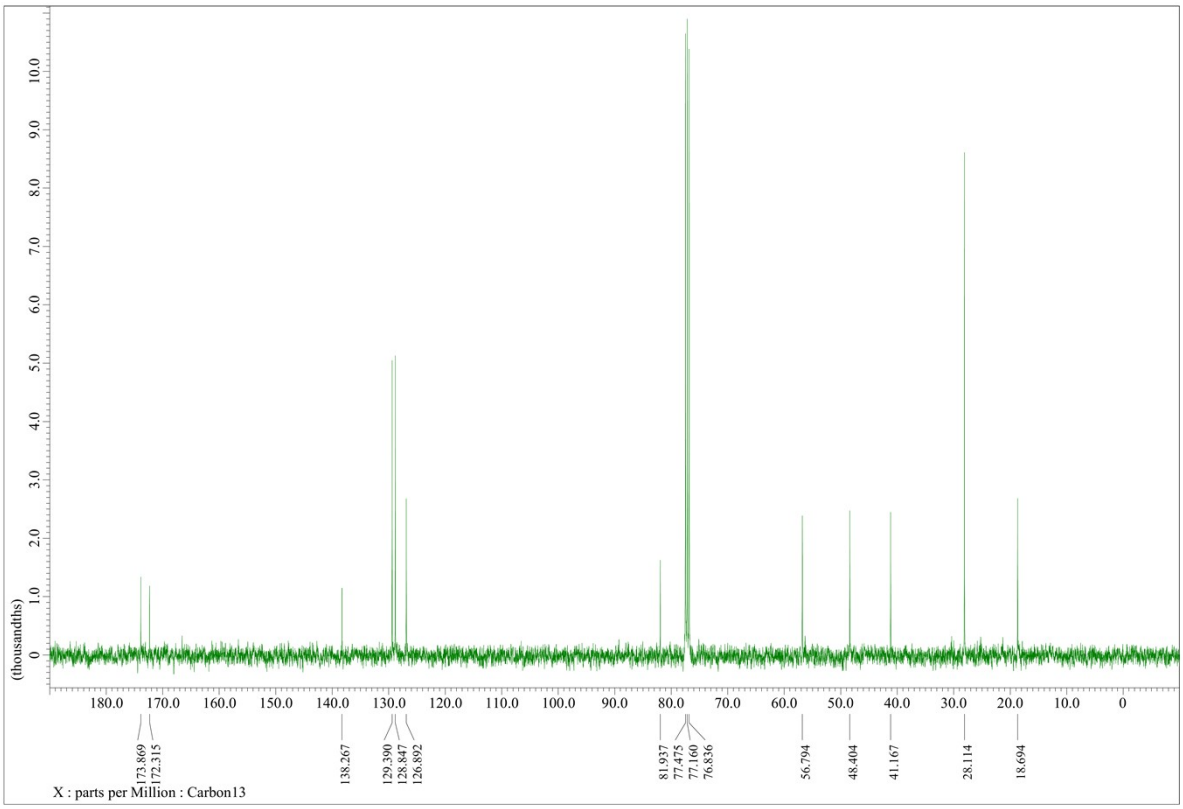


H-D-Phe-L-Ala-O*t*-Bu (S5)

(^{1}H NMR, 400 MHz, $CDCl_3$)

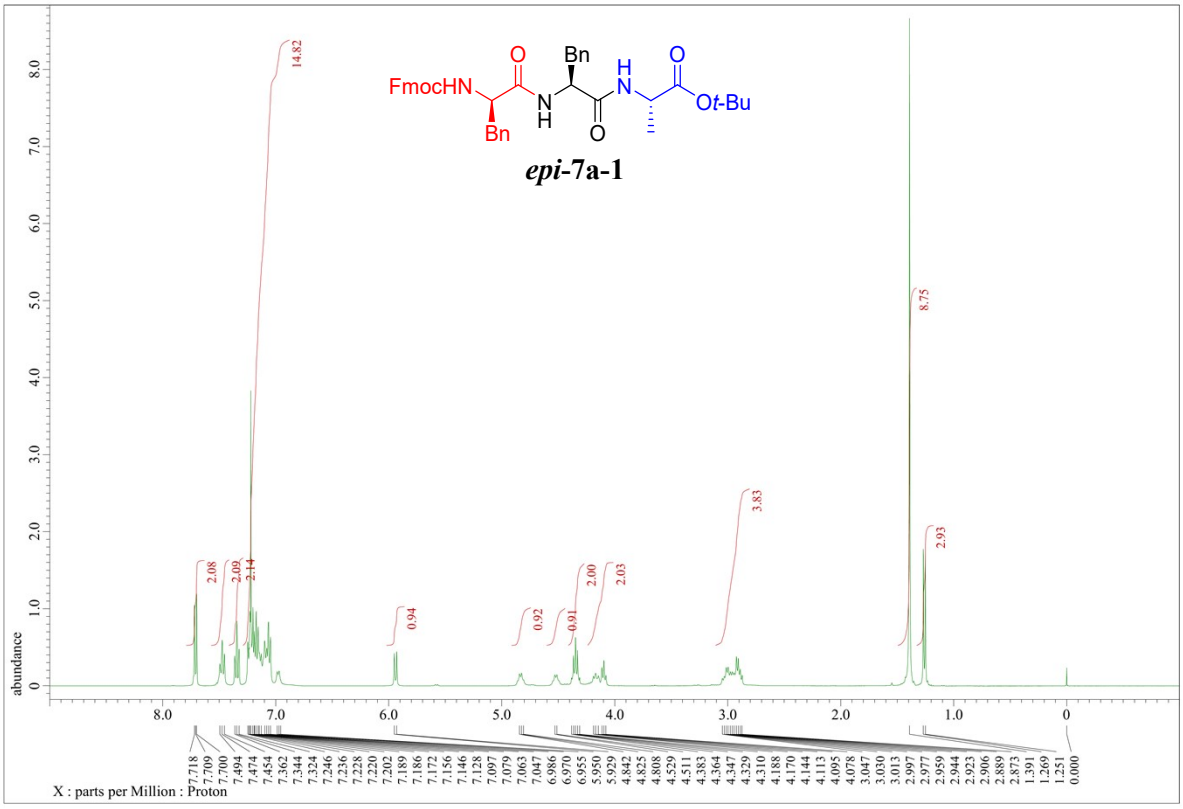


(^{13}C NMR, 100 MHz, $CDCl_3$)

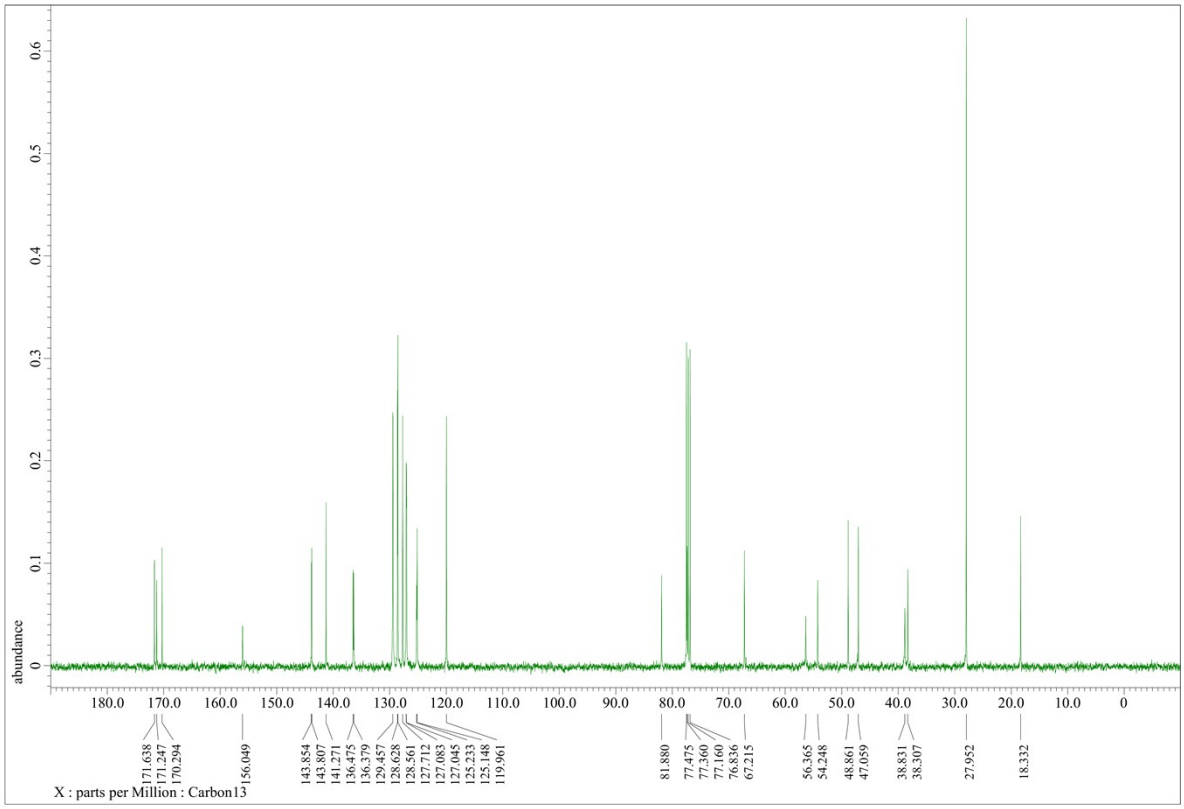


Fmoc-D-Phe-L-Phe-L-Ala-O*t*-Bu (*epi*-7a-1)

(^{1}H NMR, 400 MHz, $CDCl_3$)

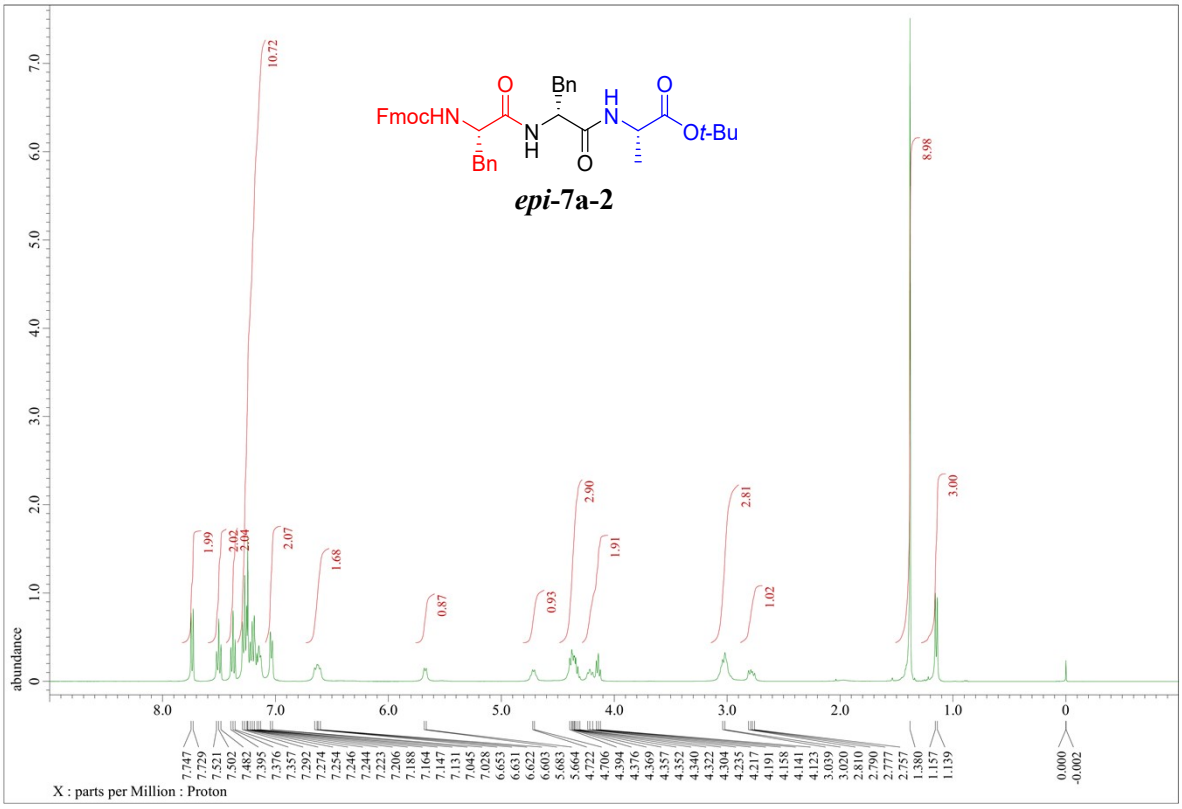


(^{13}C NMR, 100 MHz, $CDCl_3$)

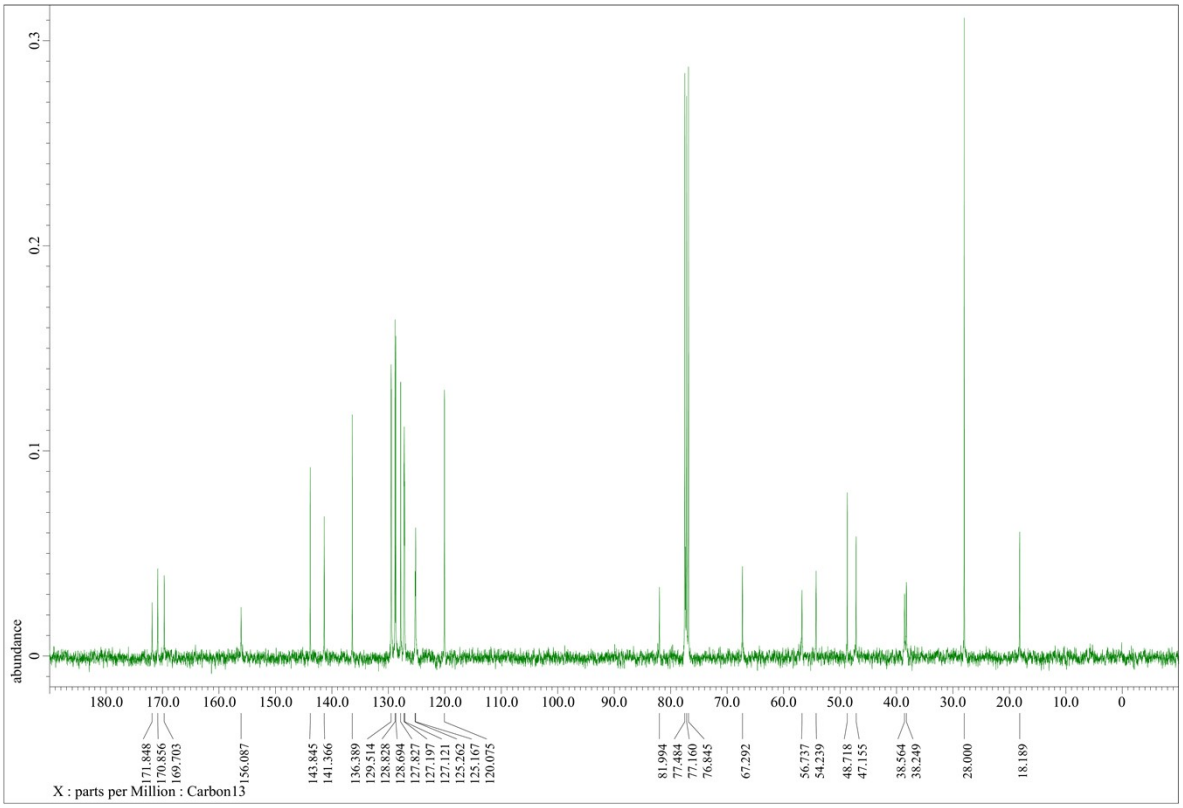


Fmoc-L-Phe-D-Phe-L-Ala-O*t*-Bu (*epi*-7a-2)

(^{1}H NMR, 400 MHz, $CDCl_3$)

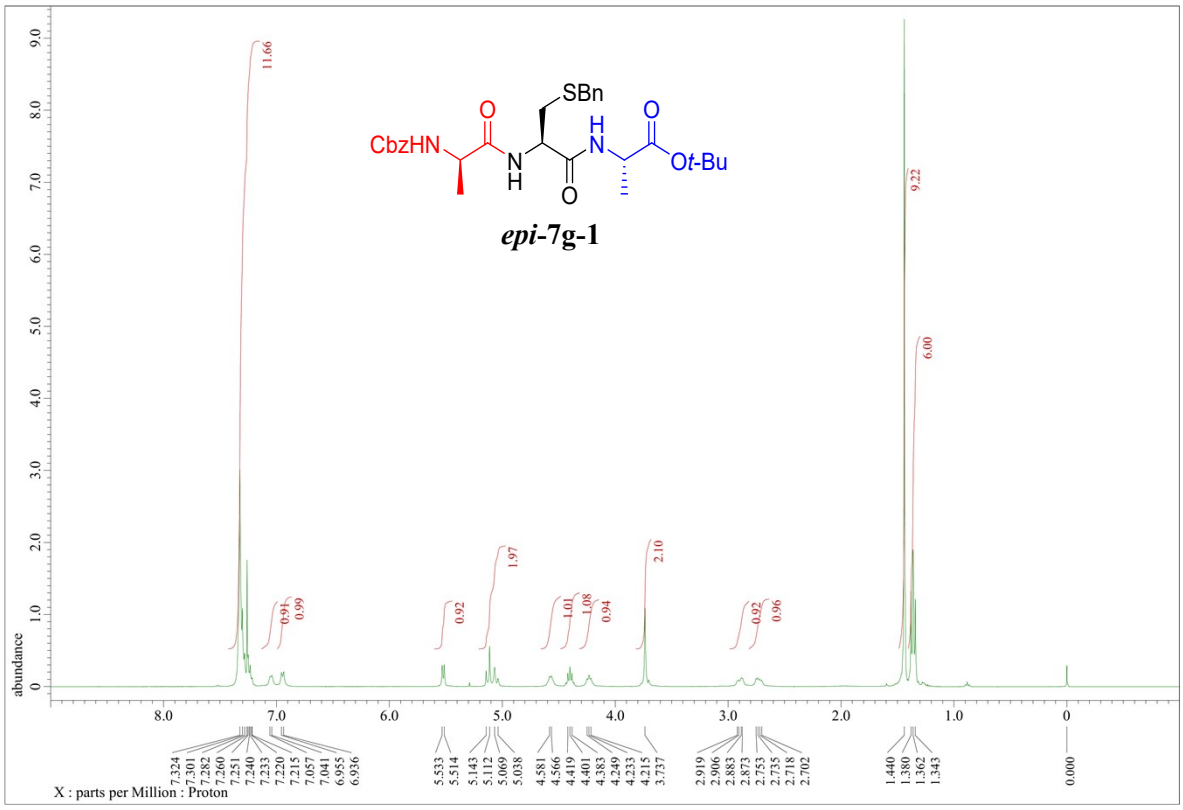


(^{13}C NMR, 100 MHz, $CDCl_3$)

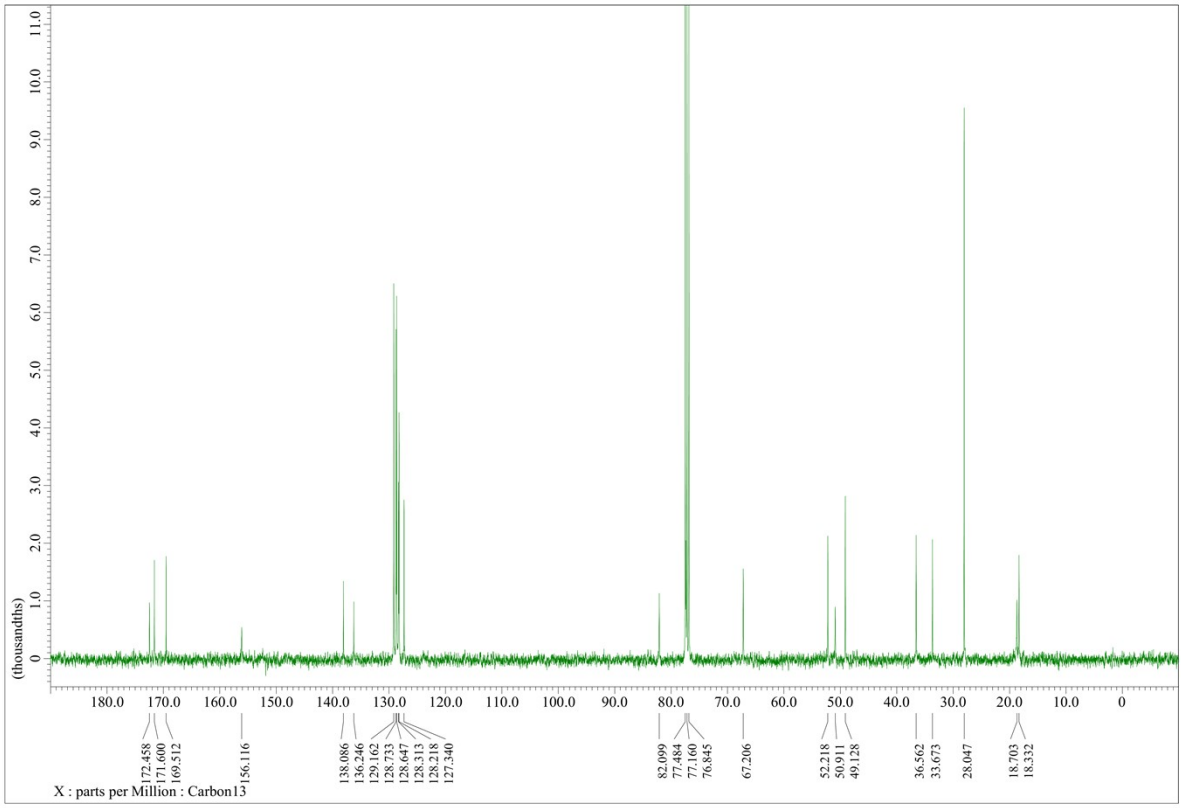


Cbz-D-Ala-L-Cys(Bn)-L-Ala-O*t*-Bu (*epi*-7g-1)

(^{1}H NMR, 400 MHz, $CDCl_3$)

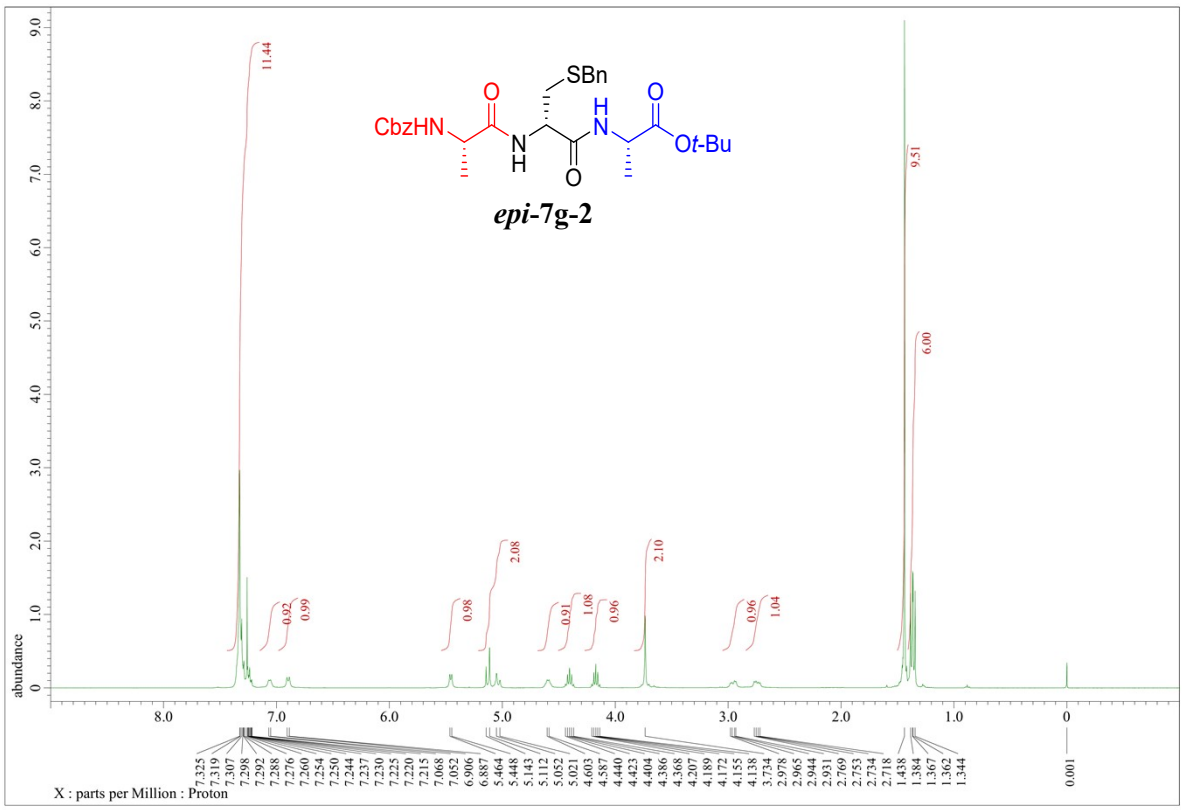


(^{13}C NMR, 100 MHz, $CDCl_3$)

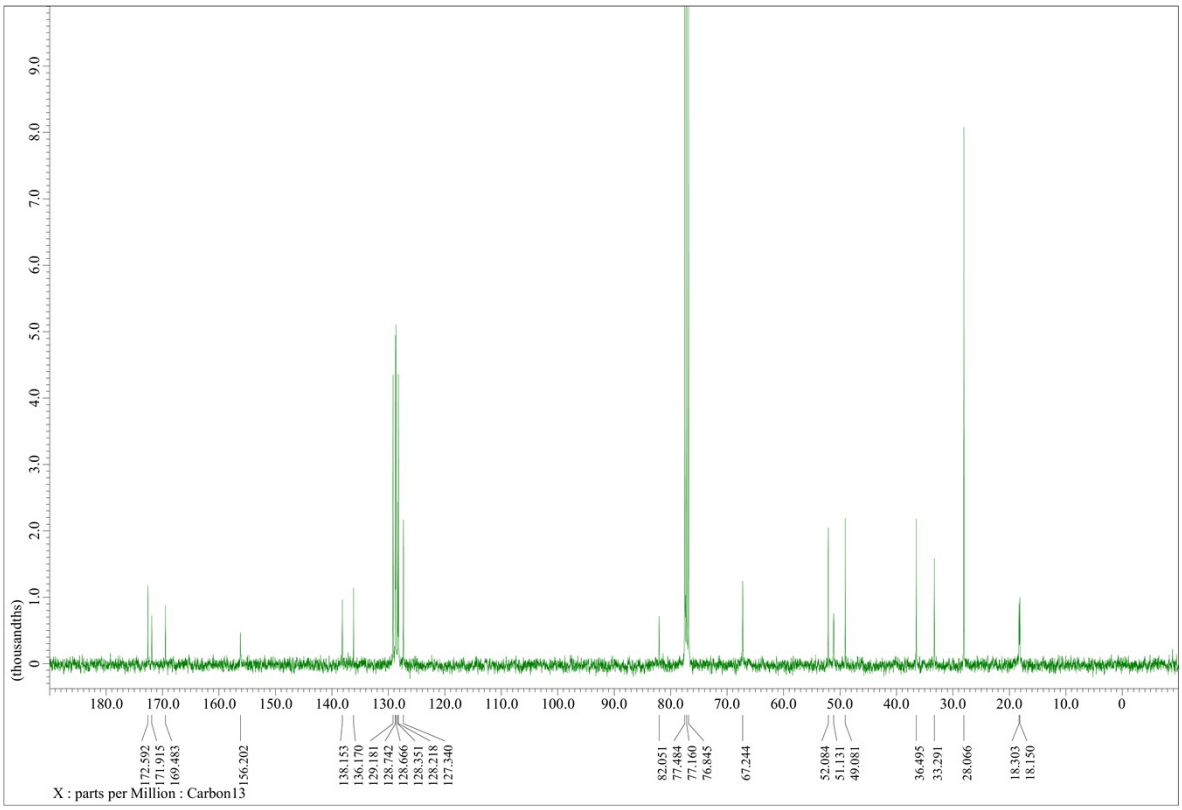


Cbz-L-Ala-D-Cys(Bn)-L-Ala-O*t*-Bu (*epi*-7g-2)

(^{1}H NMR, 400 MHz, $CDCl_3$)

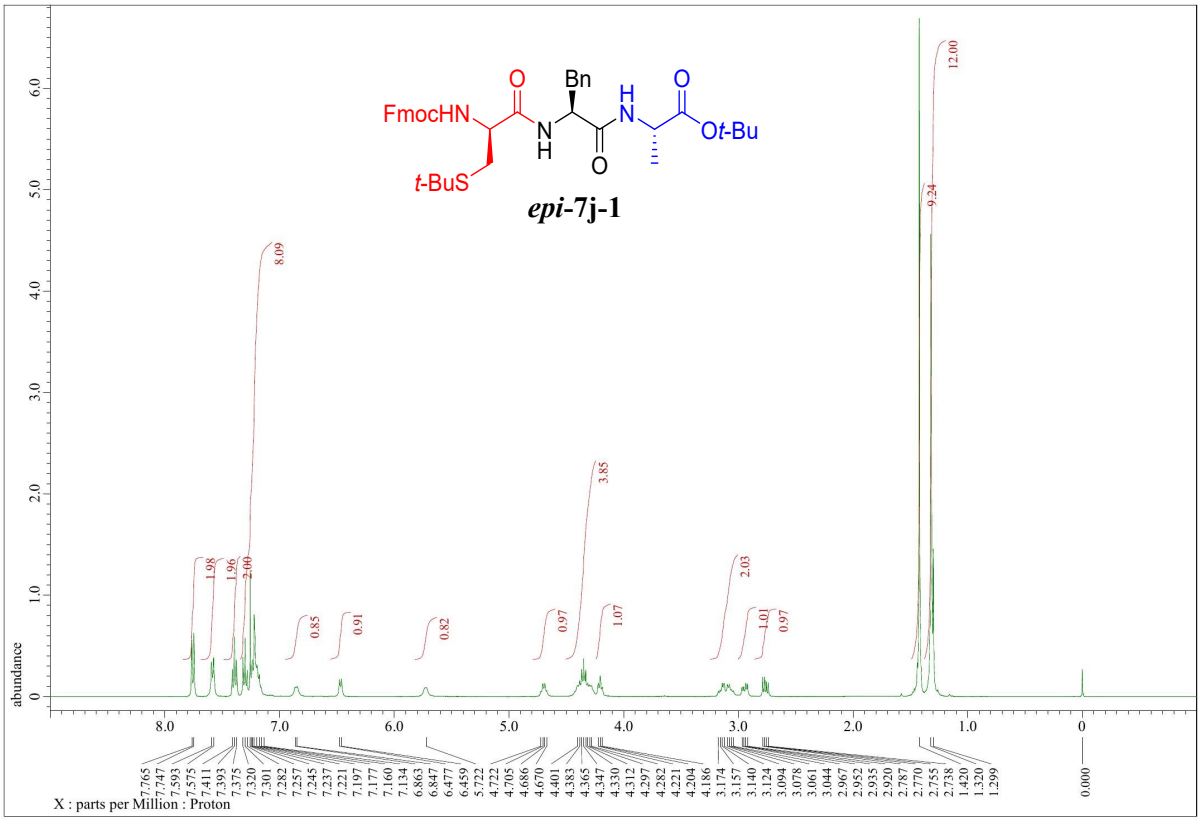


(^{13}C NMR, 100 MHz, $CDCl_3$)

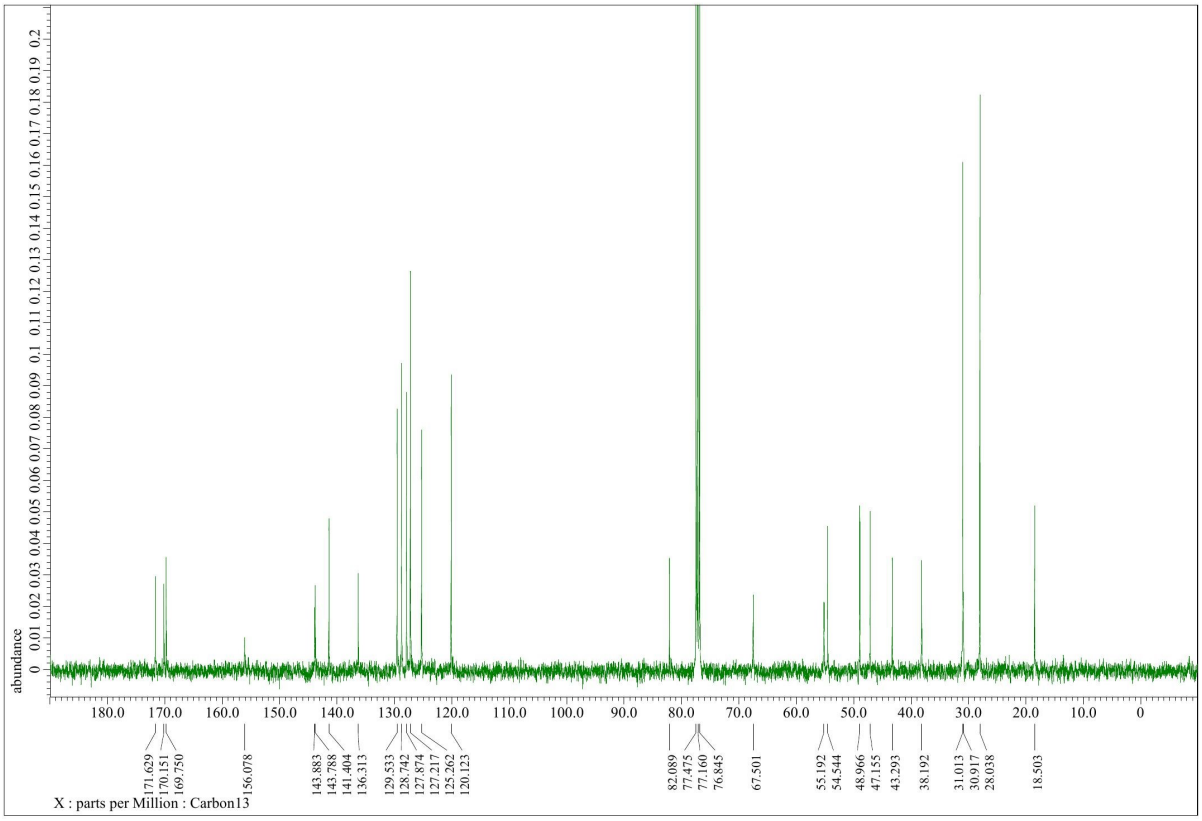


Fmoc-D-Cys(*t*-Bu)-L-Phe-L-Ala-O*t*-Bu (*epi*-7j-1)

(^{1}H NMR, 400 MHz, $CDCl_3$)

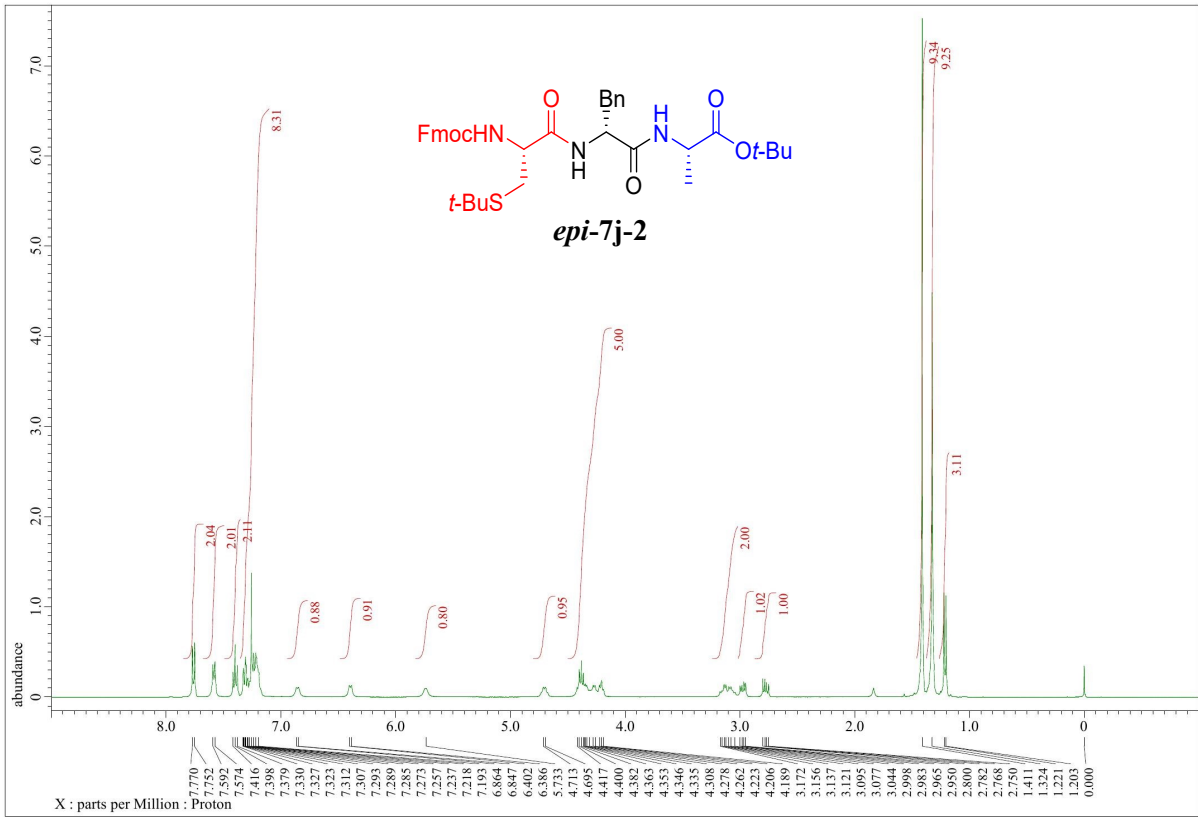


(^{13}C NMR, 100 MHz, $CDCl_3$)



Fmoc-L-Cys(*t*-Bu)-D-Phe-L-Ala-O*t*-Bu (*epi*-7j-2)

(^{1}H NMR, 400 MHz, $CDCl_3$)



(^{13}C NMR, 100 MHz, $CDCl_3$)