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Supporting Information

Micro-Flow Synthesis of β-Amino Acid Derivatives via a Rapid Dual Activation Approach

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General techniques

NMR spectra were recorded on a Bruker biospin AVANCE II 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) and Bruker biospin AVANCE III HD 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) instrument in the indicated solvent. Chemical shifts are reported in units of parts per million (ppm) relative to tetramethylsilane (0.00 ppm) or $CDCl_3$ (7.26 ppm) or $(CD_3)_2CO$ (2.50 ppm) for ^{1}H NMR and $CDCl_3$ (77.16 ppm) or $(CD_3)_2CO$ (39.52 ppm) for ^{13}C NMR. Multiplicities were reported by using the following abbreviations: s; singlet, d; doublet, t; triplet, m; multiplet, br; broad, *J*; coupling constants in Hertz (Hz). IR spectra were recorded on a JASCO Corporation FT/IR-4100 FT-IR Spectrometer. ATR PRO ONE was attached to the FT/IR-4100 in measuring solid IR spectroscopy by single reflection attenuated total reflection. Only the strongest and/or structurally important peaks were reported as the IR data given in cm^{-1}. Optical rotations were measured with a Rudolph Research Analytical Autopol IV. HRMS (ESI-TOF) were measured with a Bruker micrOTOF II.

Column chromatography was performed on Silica Gel PSQ 60B purchased from Fuji Silysia Chemical LTD. Gel permeation chromatography (GPC) for purification was performed using a Japan Analytical Industry Model LC-9225 NEXT (recycling preparative HPLC) and a Japan Analytical Industry Model UV-600 NEXT ultra violet detector with a polystyrene gel column (JAIGEL-1H, 20 mm × 600 mm), using chloroform as a solvent (3.5 mL/min). Analytical HPLC was carried out using a JASCO PU-4580 HPLC pump system with a JASCO MD-2018 PDA Detector, a JASCO CO-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 ethanol solution *p*-anisaldehyde contains acetic acid and H_2SO_4. EtOAc and CH_2Cl_2, $CHCl_3$ were dried over molecular sieves 4A. MeCN was dried over molecular sieves 3A.

Micro-flow reactor set-up

Stainless steel V-shape and T-shape mixer (**Figure S-1-3**) were purchased from Sanko Seiki Co. Ltd. (inner diameter: 0.25 mm). Teflon® tubes (inner diameter: 0.8 or 0.25 mm) and PEEK tube (inner diameter: 0.5 mm) were purchased from Senshu Scientific Co., Ltd. PEEK fittings, PEEK unions, stainless steel tubes, stainless steel fittings, stainless steel unions (inner diameter: 0.8 mm) and back pressure regulator (40 psi) were purchased from GL Science Inc. Solutions were introduced to a micro-flow system with syringe pumps (Harvard PHD ULTRA and Harvard PHD 2000) 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 β-NCA **1** was shown in **Figure S-1**. The gastight syringes and V-shape and T-shape mixers were connected with the Teflon tubes and stainless steel tubes (for controlling the temperature of solutions). The V-shape mixer was connected with the reaction tube 1 (Teflon tube). The T-shape mixer and the back pressure regulator (BPR) were connected with the reaction tube 2 (Teflon tube). The mixers and reaction tubes were immersed in water bath.

The employed micro-flow system for synthesis of **2** and **3** was shown in **Figure S-2**. The gastight syringes and T-shape mixer were connected with the Teflon tubes and stainless steel tubes (for controlling the temperature of solutions). The T-shape 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 synthesis of **4** and **5** was shown in **Figure S-3**. The gastight syringes and T-shape mixer were connected with the Teflon tubes and stainless steel tubes (for controlling the temperature of solutions). The T-shape mixer was connected with the reaction tube 1 (Teflon tube). The T-shape mixer was connected with the reaction tube 2 (Teflon tube). The mixers and reaction tubes were immersed in water bath.

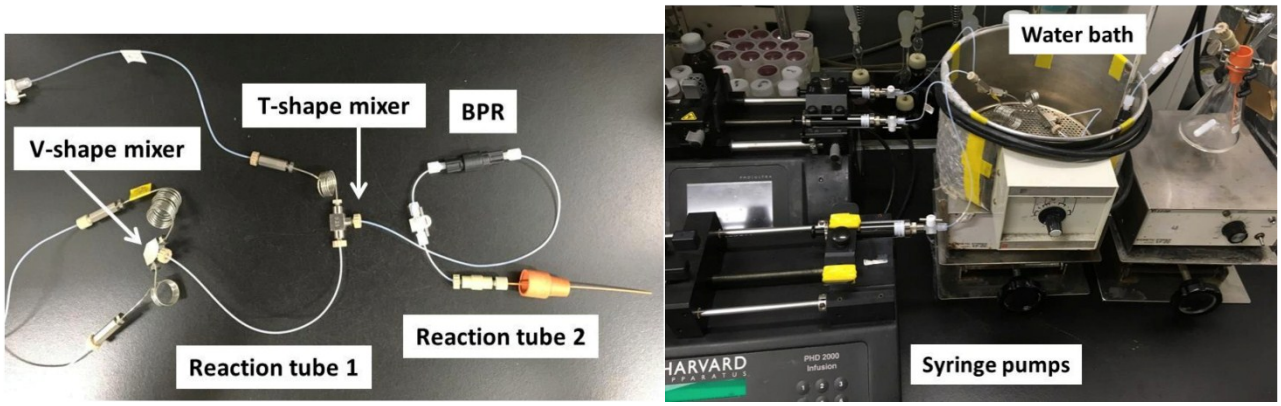


Figure S-1

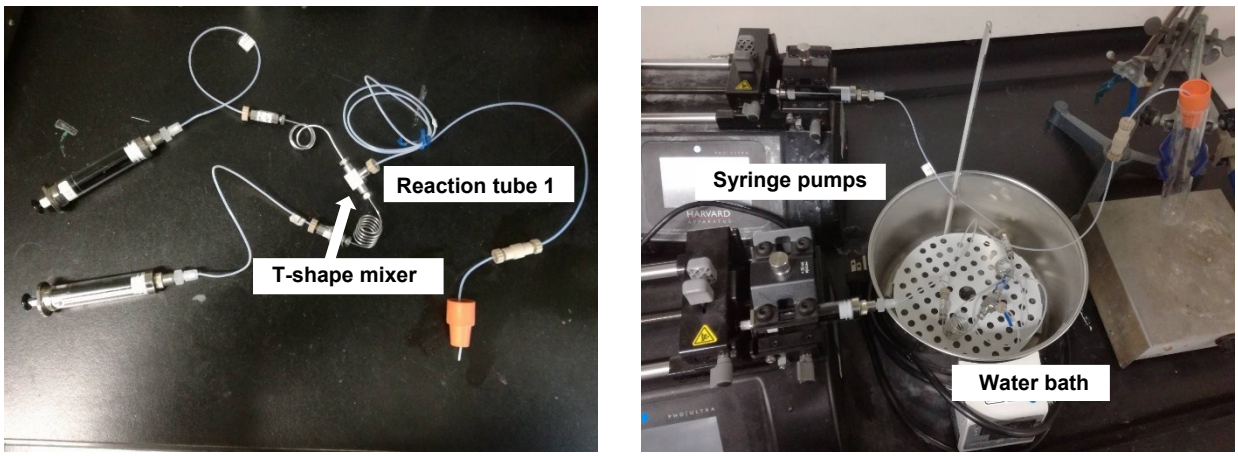


Figure S-2

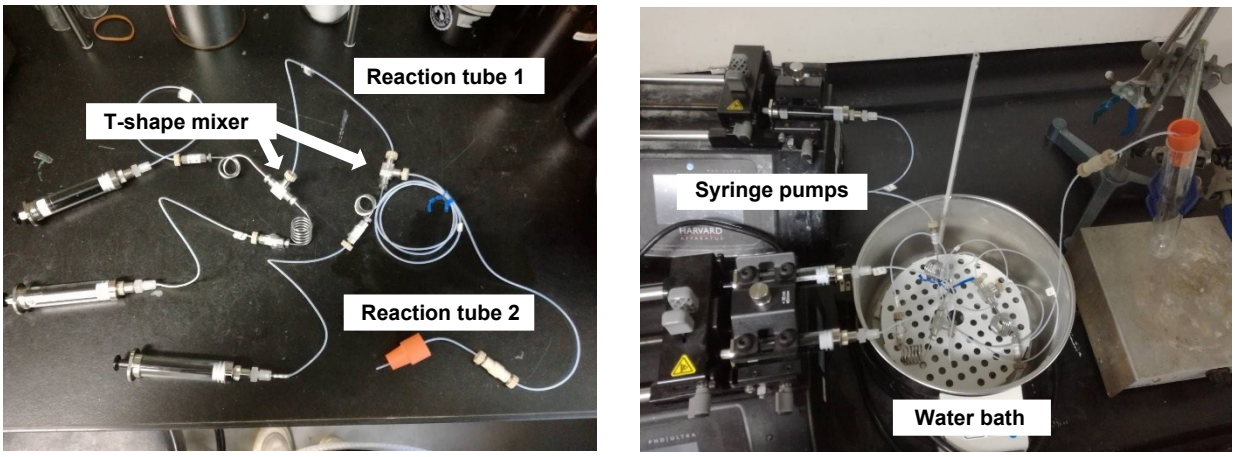
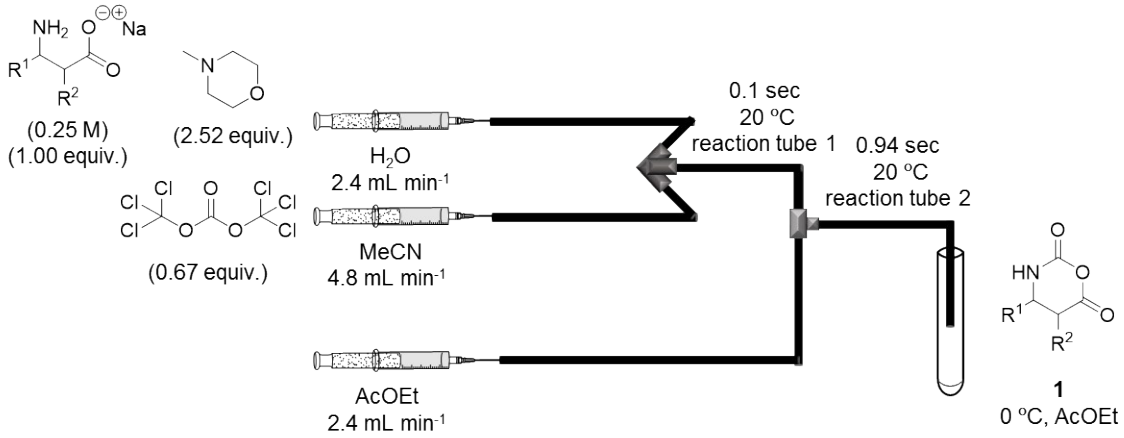


Figure S-3

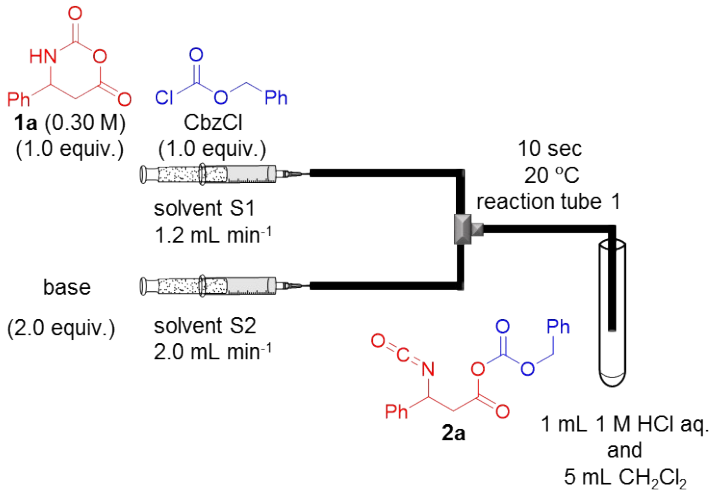
General procedure for synthesis of β-NCA 1[1)]



The employed micro-flow system was shown in **Figure S-1**.

A solution of amino acid sodium salt (0.250 M, 1.00 eq.), *N*-methyl morpholine (0.630 M, 2.52 equiv.) in H_2O (flow rate: 2.40 mL/min) and a solution of triphosgene (0.250 M, 0.670 equiv.) in MeCN (flow rate: 4.80 mL/min) were introduced to the V-shape 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: 0.100 s) at the same temperature. Then, the resultant mixture and EtOAc (flow rate: 2.40 mL/min) were introduced to the T-shape 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: 0.940 s) at the same temperature. After being eluted for 20 s to reach a steady state, the resultant mixture was poured into EtOAc (40 mL) for 100 s at 0 °C. The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at 25 °C (***caution*: *β-NCAs gradually decomposed***).

Optimization of reaction conditions for synthesis of 2a



The employed micro-flow system was shown in **Figure S-2**.

A solution of β-phenylalanine-NCA **1a** (0.300 M, 1.00 eq.), benzyl chloroformate (0.300 M, 1.00 eq.) in solvent **S1** (flow rate: 1.20 mL/min), a solution of base (0.360 M, 2.00 eq.) in solvent **S2** (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, length: 1062 mm, volume: 533 µL, reaction time: 10 or 3.3 s) at the same temperature. After being eluted for 40 s to reach a steady state, the resultant mixture was poured into CH_2Cl_2 (5 mL) and 1 M HCl aq. (1 mL) for 25 s at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo at 25 °C (***caution*: 2a *gradually decomposed***). Yields were determined by ^{1}H NMR analysis with 1,1,2-trichloroethane as an internal standard.

Examination of reaction time in synthesis of 2a

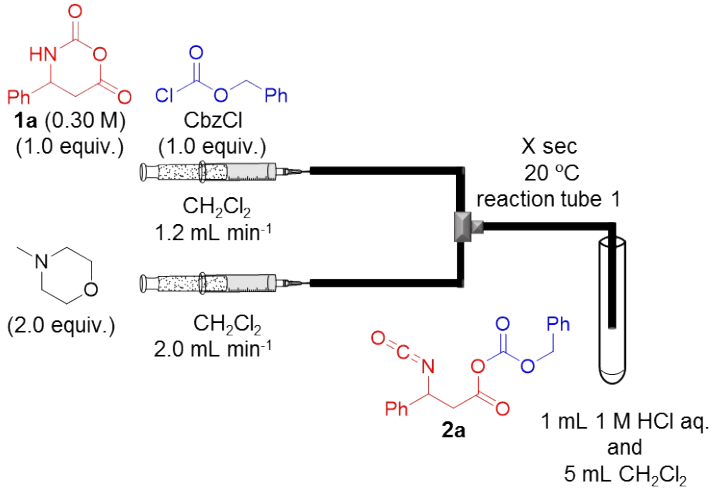


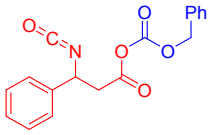
Table S-1

entry	X (sec)	yield (%)
1	0.3	96
2	3.3	>99
3	10	>99

The employed micro-flow system was shown in **Figure S-2**.

A solution of β-phenylalanine-NCA **1a** (0.300 M, 1.00 eq.), benzyl chloroformate (0.300 M, 1.00 eq.) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of *N*-methyl morpholine (0.360 M, 2.00 eq.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape 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: X s) at the same temperature. After being eluted for 40 s to reach a steady state, the resultant mixture was poured into CH_2Cl_2 (5 mL) and 1 M HCl aq. (1 mL) for 25 s at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo at 25 °C (***caution:* 2a *gradually decomposed***). Yields were determined by ^{1}H NMR analysis with 1,1,2-trichloroethane as an internal standard.

(Benzyl carbonic) 3-isocyanato-3-phenylpropanoic anhydride (2a)

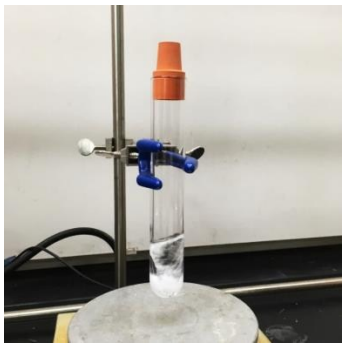


Purification method: **2a** was obtained without further purification.

48.9 mg, 1.00 mmol, >99%

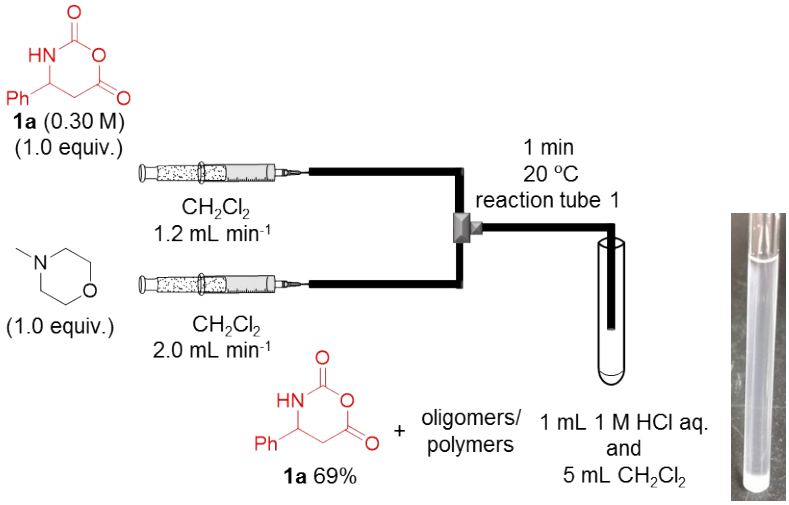
Colorless oil; IR (neat): 3066, 3031, 2267, 1822, 1761, 1496, 1455, 1227, 1095 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (m, 10H), 5.27 (s, 2H), 5.14 (m, 1H), 2.96 (dd, J = 9.6, 17.2 Hz, 1H), 2.87 (dd, J = 4.8, 16.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.6, 148.5, 139.4, 133.9, 129.3, 128.9, 128.8, 125.8, 124.5, 71.6, 54.8, 43.8; HRMS (ESI-TOF): calcd for $[C_{18}H_{15}NO_5+Na]^+$ 348.0842, found 348.0841.

Procedure for synthesis of 2a using a batch reactor



Comparison between micro-flow and batch conditions for synthesis of **2a**. Quantities of compounds, solvents and temperature were identical to those of flow condition. To a vigorously stirred (Magnetic stirrer, 1000 rpm) solution of β-phenylalanine NCA **1a** (0.300 M, 1.00 eq.), Cbz-Cl (0.300 M, 1.00 eq.) in CH_2Cl_2 (0.500 mL), a solution of *N*-methylmorpholine (0.360 M, 2.00 eq.) in CH_2Cl_2 (0.833 mL) was added in one portion at 20 ºC under argon atmosphere. After being stirred for 10 s at the same temperature, solution of CH_2Cl_2 (5 mL) and 1 M HCl aq. (1 mL) was added in one portion at 20 ºC. (Under the flow conditions, formation of **2a** was performed in 3.3 sec. However, under the batch conditions, it was impossible to operate the reaction within 3.3 sec. Thus, the reaction time was extended to 10 s). 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 25 °C (***caution*: 2a *gradually decomposed***). Yields were determined by ^{1}H NMR analysis with 1,1,2-trichloroethane as an internal standard.

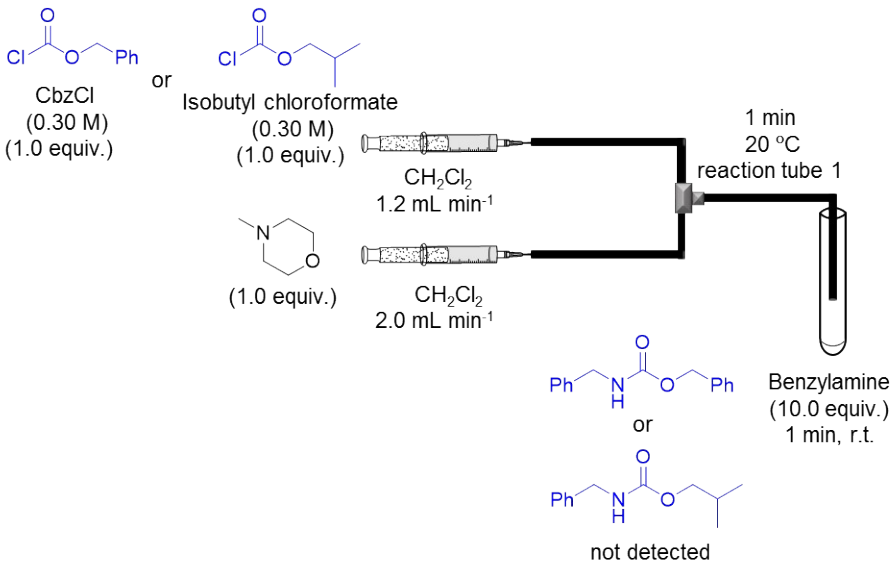
Consideration of lifetime of β-phenylalanine NCA 2a in the presence of *N*-methylmorpholine



The employed micro-flow system was shown in **Figure S-2**.

A solution of β-phenylalanine-NCA **1a** (0.300 M, 1.00 eq.) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of *N*-methylmorpholine (0.180 M, 1.00 eq.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, length: 6372 mm, volume: 3201 μL, reaction time: 60 s) at the same temperature. After being eluted for 90 s to reach a steady state, the resultant mixture was poured into CH_2Cl_2 (5 mL) and 1 M HCl aq. (1 mL) for 25 s at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo at 25 °C. Recovery yield of **1a** was determined by ^{1}H NMR analysis with 1,1,2-trichloroethane as an internal standard. In the above figure, generation of undesired, insoluble oligomers/polymers from **1a** in NMR tube was shown.

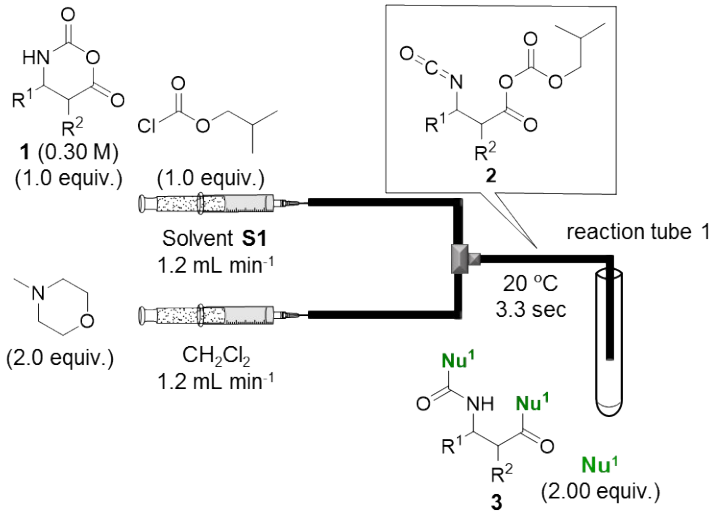
Consideration of lifetime of alkyl chloroformate in the presence of *N*-methylmorpholine



The employed micro-flow system was shown in **Figure S-2**.

A solution of benzyl chloroformate or isobutyl chloroformate (0.300 M, 1.00 eq.) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of *N*-methylmorpholine (0.180 M, 1.00 eq.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, length: 6372 mm, volume: 3201 μL, reaction time: 60 s) at the same temperature. After being eluted for 90 s to reach a steady state, the resultant mixture was poured into benzyl amine (10.0 equiv.) for 25 s at room temperature. After being stirred for 1 min at the room temperature, the organic layer was washed with 1 M HCl aq. and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Yields were determined by ^{1}H NMR analysis with 1,1,2-trichloroethane as an internal standard.

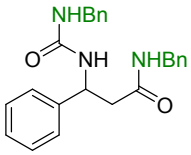
General procedure for examination of substrate scope of 3



The employed micro-flow system was shown in **Figure S-2**.

A solution of β-NCA **1** (0.300 M, 1.00 eq.), isobutyl chloroformate (0.300 M, 1.00 eq.) in solvent **S1** (flow rate: 1.20 mL/min), a solution of *N*-methylmorpholine (0.360 M, 2.00 eq.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, length: 355 mm, volume: 178 μL, reaction time: 3.3 s) at the same temperature. After being eluted for 40 s to reach a steady state, the resultant mixture was poured into a solution of **nucleophile**[1] (2.00 equiv.) in CH_2Cl_2 (0.200 mL) for 25 s at room temperature. The reaction was carried out for 1 h at room temperature. The reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel or preparative TLC.

_N_-Benzyl-3-(3-benzylureido)-3-phenylpropanamide (3a)

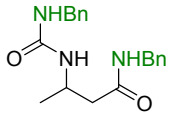


Purification method: **3a** was obtained by silica gel column chromatography (CH_2Cl_2/MeOH = 9/1).

52.2 mg, 0.13 mmol, 90%.

White solid; mp 195-197 ºC, IR (ATR): 3262, 3062, 3029, 1631, 1557, 1496, 1453, 1375, 1247 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$): δ 8.35 (t, *J* = 6.0 Hz 1H), 7.24 (m, 13H), 7.00 (d, *J* = 6.4 Hz, 2H), 6.74 (d, *J* = 8.4 Hz, 1H), 6.60 (t, *J* = 5.6 Hz, 1H), 5.13 (m, 1H), 4.20 (m, 4H), 2.62 (m, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ 169.6, 157.2, 143.7, 140.8, 139.2, 128.12, 128.07, 128.0, 126.91, 126.88, 126.54, 126.49, 126.47, 126.3, 50.8, 42.8, 42.7, 41.8; HRMS (ESI-TOF): calcd for $[C_{24}H_{25}N_3O_2+Na]^+$ 410.1839, found 410.1838.

_N_-Benzyl-3-(3-benzylureido)butanamide (3b)



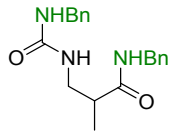
*THF was used as Solvent **S1**

Purification method: **3b** was obtained by silica gel column chromatography (CH_2Cl_2/MeOH = 9/1).

44.1 mg, 0.14 mmol, 90%.

White solid; mp 206-209 ºC, IR (ATR): 3263, 3031, 2973, 1626, 1542, 1454, 1377, 1296, 1201 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$): δ 8.39 (t, *J* = 5.2 Hz 1H), 7.26 (m, 10H), 6.40 (t, *J* = 5.6 Hz, 1H), 5.97 (d, *J* = 8.0 Hz, 1H), 4.27 (d, *J* = 6.0 Hz, 2H), 4.27 (d, *J* = 6.0 Hz, 2H), 4.20 (d, *J* = 5.6 Hz, 2H), 3.99 (m, 1H), 2.37 (dd, *J* = 5.6, 14.0 Hz, 1H), 2.21 (dd, *J* = 8.0, 14.0 Hz, 1H), 1.06 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ 170.3, 157.3, 140.9, 139.5, 128.2, 128.1, 127.2, 127.0, 126.7, 126.5, 43.0, 42.8, 42.5, 42.0, 20.9; HRMS (ESI-TOF): calcd for $[C_{19}H_{23}N_3O_2+Na]^+$ 348.1682, found 348.1681.

***N*-Benzyl-3-(3-benzylureido)-2-methylpropanamide (3c)**



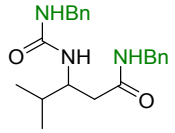
*CH_2Cl_2/MeCN (*v*/*v* =1/1) was used as Solvent **S1**

Purification method: **3c** was obtained by silica gel column chromatography (CH_2Cl_2/MeOH = 9/1).

40.5 mg, 0.12 mmol, 83%.

White solid; mp 195-197 ºC, IR (ATR): 3336, 3269, 1626, 1572, 1541, 1453, 1256, 1221, 1066 cm^{-1}; ^1H NMR (500 MHz, $(CD_3)_2CO$): δ 8.39 (t, *J* = 5.5 Hz 1H), 7.30 (m, 4H), 7.24 (m, 6H), 6.42 (t, *J* = 5.5 Hz, 1H), 6.00 (t, *J* = 5.5 Hz, 1H), 4.28 (d, *J* = 6.0 Hz, 2H), 4.20 (d, *J* = 6.0 Hz, 2H), 3.12 (t, *J* = 6.5 Hz, 2H), 2.54 (m, 1H), 1.01 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ 174.6, 158.1, 140.9, 139.6, 128.3, 128.2, 127.02, 126.96, 126.6, 126.5, 42.9, 42.6, 41.9, 40.5, 15.7; HRMS (ESI-TOF): calcd for $[C_{19}H_{23}N_3O_2+Na]^+$ 348.1682, found 348.1684.

***N*-Benzyl-3-(3-benzylureido)-4-methylpentanamide (3d)**

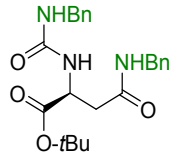


Purification method: **3d** was obtained by preparative TLC (CH_2Cl_2/MeOH = 9/1).

47.1 mg, 0.13 mmol, 89%.

White solid; mp 171-174 ºC, IR (ATR): 3304, 3258, 2870, 1638, 1577, 1558, 1261, 1239, 1030 cm^{-1}; ^1H NMR (500 MHz, $(CD_3)_2CO$): δ 8.35 (t, *J* = 6.0 Hz 1H), 7.26 (m, 10H), 6.38 (t, *J* = 5.5 Hz, 1H), 5.88 (d, *J* = 9.5 Hz, 1H), 4.26 (d, *J* = 6.0 Hz, 2H), 4.21 (d, *J* = 6.0 Hz, 2H), 3.89 (m, 1H), 2.27 (m, 2H), 1.73 (m, 1H), 0.830 (d, *J* = 6.5 Hz, 6H); ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ 170.6, 157.8, 141.0, 139.5, 128.21, 128.19, 127.1, 126.9, 126.6, 126.5, 51.7, 42.9, 42.0, 38.7, 31.3, 19.3, 17.6; HRMS (ESI-TOF): calcd for $[C_{21}H_{27}N_3O_2+Na]^+$ 376.1995, found 376.1995.

***tert*-Butyl N^4-benzyl-N^2-(benzylcarbamoyl)-L-asparaginate (3e)**



*THF was used as Solvent **S1**

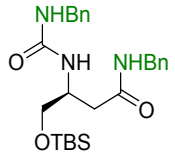
Purification method: **3e** was obtained by silica gel column chromatography (CH_2Cl_2/MeOH = 9/1).

59.4 mg, 0.14 mmol, 96%.

Colorless oil; IR (neat): 3064, 3030, 2978, 2930, 1732, 1643, 1558, 1250, 1157 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$): δ 8.45 (t, *J* = 7.0 Hz 1H), 7.23 (m, 10H), 6.80 (t, *J* = 5.6 Hz, 1H), 6.31 (d, *J* = 8.8 Hz, 1H), 4.41 (m, 1H), 4.27 (t, *J* = 6.0 Hz, 1H), 4.21 (d, *J* = 6.0 Hz, 1H), 2.65 (dd, *J* = 6.0, 15.6 Hz, 1H), 2.56 (dd, *J* = 5.2, 15.6 Hz,

1H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ 171.3, 169.4, 157.6, 140.7, 139.3, 128.2, 128.1, 127.2, 126.9, 126.7, 126.5, 80.2, 50.2, 42.8, 42.1, 37.9, 27.6; HRMS (ESI-TOF): calcd for $[C_{23}H_{29}N_3O_4+Na]^+$ 434.2050, found 434.2052. $[\alpha]^{24}_D$ = +18.94 (c 1.00, CH_2Cl_2).

(*S*)-*N*-Benzyl-3-(3-benzylureido)-4-((*tert*-butyldimethylsilyl)oxy)butanamide (3f)



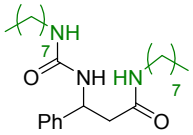
*THF was used as Solvent **S1**

Purification method: **3f** was obtained by preparative TLC (CH_2Cl_2/MeOH = 9/1).

57.9 mg, 0.13 mmol, 85%.

White solid; mp 149-151 ºC, IR (ATR): 3260, 3089, 2927, 2855, 1641, 1563, 1249, 1075, 834 cm^{-1}; ^{1}H NMR (500 MHz, ($CDCl_3$, CH_2Cl_2 was used as internal standard): δ 7.25 (m, 10H), 6.61 (brs, 1H), 5.50 (brs, 1H), 4.33 (d, *J* = 5.5 Hz, 2H), 4.27 (s, 2H), 4.10 (brs, 1H), 3.68 (dd, *J* = 4.0, 9.5 Hz, 1H), 3.51 (dd, *J* = 6.5, 9.5 Hz, 1H), 2.48 (m, 2H), 0.85 (s, 9H), 0.03 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.6, 158.2, 139.4, 138.2, 128.7, 128.6, 127.7, 127.50, 127.48, 127.2, 65.1, 49.4, 44.4, 43.6, 38.7, 26.0, 18.3; HRMS (ESI-TOF): calcd for $[C_{25}H_{37}N_3O_3Si+Na]^+$ 478.2499, found 478.2496. $[\alpha]^{24}_D$ = -14.00 (c 1.00, CH_2Cl_2).

***N*-Octyl-3-(3-octylureido)-3-phenylpropanamide (3g)**

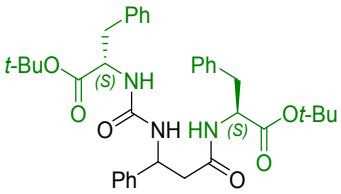


Purification method: **3g** was obtained by preparative TLC (CH_2Cl_2/MeOH = 9/1).

69.0 mg, 0.16 mmol, >99%.

White amorphous solid; IR (neat): 3286, 2954, 2923, 2853, 1637, 1577, 1572, 1467, 1265 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37 (brs, 1H), 7.24 (m, 5H), 6.70 (brs, 1H), 5.59 (brs, 1H), 5.31 (m, 1H), 3.07 (m, 4H), 2.74 (m, 2H), 1.38 (s, 4H), 1.24 (m, 20H), 0.86 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$)**:** δ 171.3, 158.6, 143.1, 128.6, 127.1, 126.3, 51.6, 44.2, 40.6, 39.9, 32.01, 31.99, 30.5, 29.6, 29.5, 29.4, 27.2, 22.81, 22.79, 14.2; HRMS (ESI-TOF): calcd for $[C_{26}H_{45}N_3O_2+Na]^+$ 457.3404, found 454.3407.

***tert*-Butyl ((3-(((*S*)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylpropyl)carbamoyl)-L-phenylalaninate (3h)**



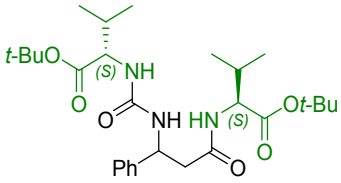
*L-Phenylalanine *tert*-butyl ester hydrochloride was used as **Nu[1]**. DIEA (2.0 equiv.) was used to trap hydrogen chloride.

Purification method: **3h** was obtained by preparative TLC (CH_2Cl_2/MeOH = 9/1).

94.5 mg, 0.15 mmol, >99%.

White solid; mp 164-167 ºC, IR (neat): 3294, 3062, 3030, 2978, 2932, 1734, 1638, 1559, 1367, 1154 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, diastereomeric mixture): δ 7.17 (m, 12H), 7.08 (d, *J* = 6.4 Hz, 1H), 6.86 (brs, 1H), 6.33 (dd, *J* = 8.4, 63.6 Hz, 1H), 6.10 (dd, *J* = 7.2, 61.6 Hz, 1H), 5.15 (m, 2H), 4.62 (m, 2H), 2.92 (m, 4H), 2.64 (m, 2H), 1.37 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$, diastereomeric mixture): δ 172.0, 171.6, 171.1, 170.7, 170.4, 170.1, 156.83, 156.75, 142.0, 141.7, 136.8, 136.7, 136.3, 136.2, 129.69, 129.66, 129.54, 129.48, 128.7, 128.6, 128.44, 128.38, 128.3, 127.4, 127.3, 127.0, 126.9, 126.8, 126.7, 126.4, 82.6, 82.5, 82.0, 81.9, 54.7, 53.81, 53.75, 52.0, 51.4, 43.0, 42.3, 39.0, 38.7, 38.0, 37.9, 28.1, 28.0; HRMS (ESI-TOF): calcd for $[C_{36}H_{45}N_3O_6+Na]^+$ 638.3201, found 638.3200.

***tert*-Butyl ((3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)amino)-3-oxo-1-phenylpropyl)carbamoyl)-L-valinate (3i)**



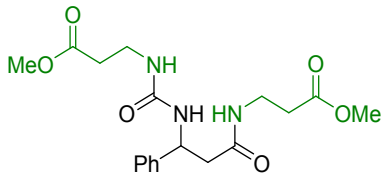
*L-Valine *tert*-butyl ester hydrochloride was used as **Nu[1]**. DIEA (2.0 equiv.) was used to trap hydrogen chloride.

Purification method: **3i** was obtained by preparative TLC (CH_2Cl_2/MeOH = 9/1).

65.2 mg, 0.13 mmol, 84%.

White solid; mp 193-196 ºC, IR (neat): 3297, 3064, 2968, 2933, 1735, 1638, 1564, 1368, 1158 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, diastereomeric mixture): δ 7.25 (m, 5H), 6.56 (t, *J* = 8.4 Hz, 1H), 6.25 (dd, *J* = 8.8, 28.0 Hz, 1H), 5.50 (d, *J* = 7.6 Hz, 0.5H), 5.22 (m, 1H), 5.14 (m, 0.5H), 4.31 (m, 2H), 2.73 (m, 2H), 2.04 (m, 2H), 1.44 (m, 18H), 0.80 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, diastereomeric mixture): δ 172.6, 172.1, 172.0, 171.2, 170.7, 170.5, 157.6, 157.3, 142.1, 141.8, 128.7, 128.4, 127.4, 127.0, 126.3, 126.2, 82.3, 82.1, 81.54, 81.48, 58.6, 58.4, 57.6, 57.5, 52.1, 51.3, 43.5, 42.3, 31.6, 31.4, 31.3, 31.2, 28.1, 19.1, 18.95, 18.86, 18.8, 17.8, 17.6; HRMS (ESI-TOF): calcd for $[C_{28}H_{45}N_3O_6+Na]^+$ 542.3201, found 542.3203.

Methyl 3-(3-(3-((3-methoxy-3-oxopropyl)amino)-3-oxo-1-phenylpropyl)ureido)propanoate (3j)



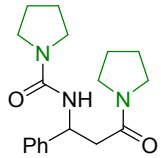
*β-Alanine methyl ester hydrochloride was used as **Nu[1]**. DIEA (2.0 equiv.) was used as trap a of hydrochloride.

Purification method: **3j** was obtained by preparative TLC (CH_2Cl_2/MeOH = 9/1).

56.4 mg, 0.15 mmol, 99%.

White amorphous solid, IR (neat): 3287, 3091, 2952, 1734, 1637, 1567, 1438, 1257, 1200; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.26 (m, 4H), 7.19 (m, 2H), 6.56 (d, *J* = 8.0 Hz, 1H), 5.71 (brs, 1H), 5.22 (m, 1H), 3.63 (s, 3H), 3.61 (s, 3H), 3.40 (m, 4H), 2.67 (m, 2H), 2.46 (t, *J* = 6.0 Hz, 2H), 2.40 (dd, *J* = 6.5, 13.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.1, 172.7, 171.3, 158.0, 142.5, 128.6, 127.3, 126.2, 51.8, 51.7, 51.6, 43.8, 35.9, 35.1, 34.9, 33.8; HRMS (ESI-TOF): calcd for $[C_{18}H_{25}N_3O_6+Na]^+$ 402.1636, found 402.1636.

***N*-(3-Oxo-1-phenyl-3-(pyrrolidin-1-yl)propyl)pyrrolidine-1-carboxamide (3k)**



Purification method: **3k** was obtained by preparative TLC (CH_2Cl_2/MeOH = 9/1).

50.7 mg, 0.16 mmol, >99%.

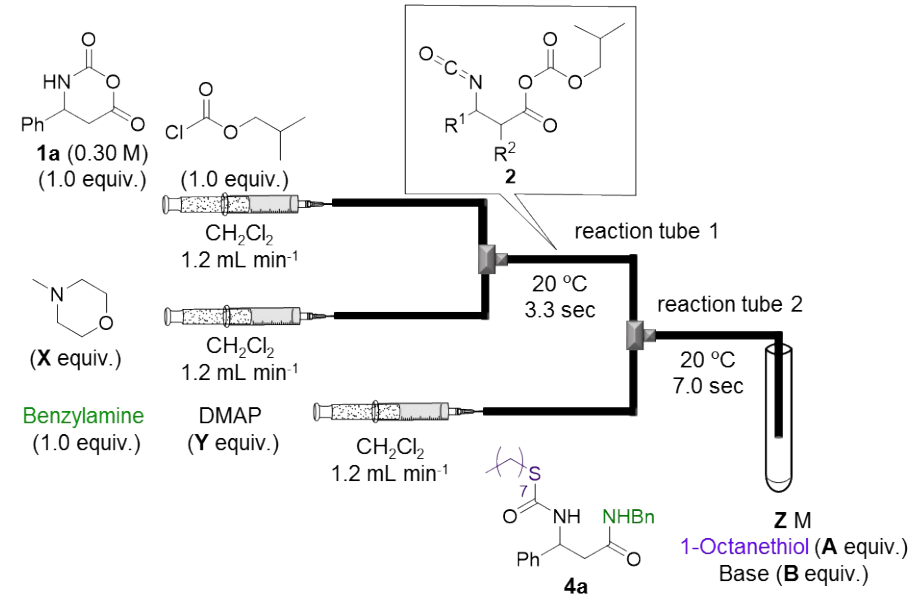
Colorless oil; IR (neat): 2970, 2950, 2871, 1629, 1525, 1451, 1398, 1254, 1192; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (m, 2H), 7.28 (m, 2H), 7.19 (m, 1H), 6.83 (d, *J* = 7.2 Hz, 1H), 5.23 (m, 1H), 3.37 (m, 6H), 3.26 (m, 1H), 2.82 (m, 2H), 2.73 (dd, *J* = 4.4, 14.4 Hz, 1H) 1.89 (s, 4H), 1.74 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$)**:** δ 170.1, 156.3, 143.1, 128.4, 127.0, 126.2 51.3, 46.9, 45.6, 45.5, 40.2, 26.0, 25.6, 24.3; HRMS (ESI-TOF): calcd for $[C_{18}H_{25}N_3O_2+Na]^+$ 338.1839, found 338.1837.

Optimization of reaction conditions for synthesis of 4a

The employed micro-flow system was shown in **Figure S-3**.

A solution of β-phenylalanine-NCA (0.300 M, 1.00 eq.), isobutyl chloroformate (0.300 M, 1.00 eq.) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of *N*-methylmorpholine (**X** equiv.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, length: 355 mm, volume: 178 μL, reaction time: 3.3 s) at the same temperature. The resultant mixture and a solution of benzylamine (0.180 M, 1.00 eq.) and catalyst (**+cat**, **Y** equiv.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 1200 mm, volume: 603 μL, reaction time: 7.0 s) at the same temperature. After being eluted for 40 s to reach a steady state, the resultant

mixture was poured into a solution of 1-octanethiol (**A** equiv.) and base (**B** equiv.) in CH_2Cl_2 (0.200 mL) for 25 s at room temperature. The reaction was carried out overnight at room temperature. The reaction was concentrated in vacuo. Yields of **4a** was determined by HPLC-UV (conditions: Inertsil^{TM}, ODS-3 5 μm, 4.6 x 75 mm, MeCN+0.1% formic acid/H_2O+0.1% formic acid, 0-10 min: 0 to 100%, 10-15 min: 100%, 15-17 min: 100 to 0%, flow rate 1.0 mL/min, 40 °C, detection wavelength 254 nm) using a calibration curve shown in **Figure S-4** in order to allow rapid analysis of reaction results without silica gel column chromatography.



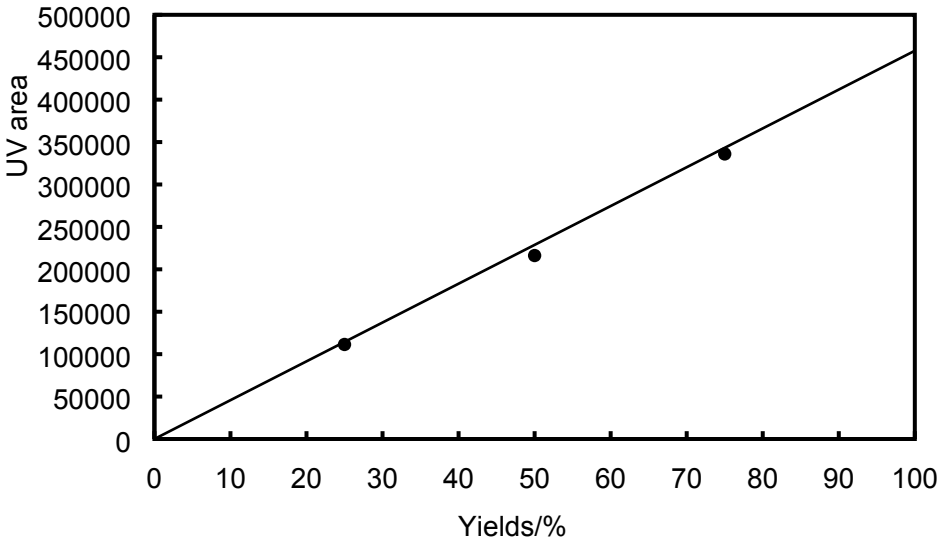


Figure S-4 Calibration curve of **4a**

Table S-2. Optimization of reaction conditions

entry	**X equiv.**	**+cat**	**Y** equiv.	**Z** M	**A** equiv.	Base	**B** equiv.	yield (%)
1	2.0	-	-	0.04	1.0	-	-	37
2	2.0	DMAP	0.1	0.04	1.0	-	-	50
3	2.0	DMAP	0.1	0.06	1.0	-	-	53
4	**2.0**	**DMAP**	**0.1**	**0.06**	**1.0**	**DIEA**	**1.0**	**68±1 (72** [b])
5	2.0	DMAP	0.1	0.06	1.5	DIEA	1.0	69
6	2.0	-	-	0.06	1.0	DMAP + DIEA	0.1+1.0	49
7	2.0	DMAP	0.1	0.06	1.0	DIEA	3.0	62
8	1.0	DMAP	0.1	0.06	1.0	DIEA	1.0	41
9	1.2	DMAP	0.1	0.06	1.0	DIEA	1.0	47
10	1.5	DMAP	0.1	0.06	1.0	DIEA	1.0	57
11	3.0	DMAP	0.1	0.06	1.0	DIEA	1.0	68
12	2.0	DMAP	0.05	0.06	1.0	DIEA	1.0	62
13	2.0	DMAP	0.2	0.06	1.0	DIEA	1.0	68
14	2.0	DMAP	0.3	0.06	1.0	DIEA	1.0	65

Procedure for synthesis of 4a using a batch reactor

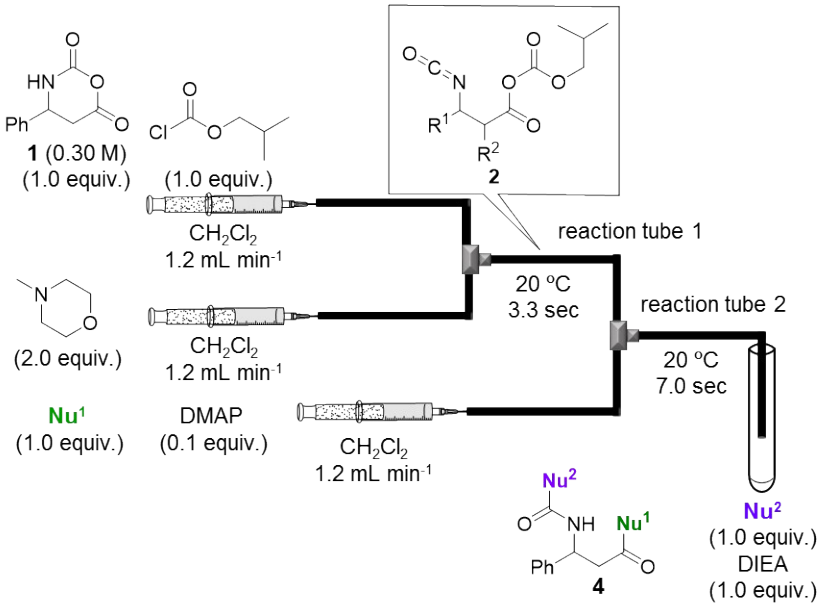
Quantities of compounds, solvents and temperature were identical to those of flow condition. To a vigorously stirred (Magnetic stirrer, 1000 rpm) solution of β-phenylalanine NCA (**1a**) (0.300 M, 1.00 eq.), isobutyl chloroformate (0.300 M, 1.00 eq.) in CH_2Cl_2 (0.500 mL), a solution of *N*-methylmorpholine (0.360 M, 2.00 eq.) in CH_2Cl_2 (0.833 mL) was added in one portion at 20 ºC under argon atmosphere. After being stirred for 10 s at 20 ºC (Under the flow conditions, formation of **3** was performed in 3.3 sec. However, under the batch conditions, it was impossible to operate the reaction within 3.3 sec. Thus, the reaction time was extended to 10 s), a solution of benzyl amine (0.180 M, 1.00 equiv.) and DMAP (0.018 M, 0.10 equiv.) and CH_2Cl_2 was added in one portion at 20 ºC. After being stirred for 10 s (Under the flow conditions, reaction was performed in 7.0 sec.). A solution of 1-octanethiol (1.00 equiv.), DIEA (1.00 equiv.) and CH_2Cl_2 (0.2 mL) was added in one portion at 20 ºC. The reaction was carried out overnight at room temperature. The reaction was concentrated in vacuo. Yields of **4a** was determined

by HPLC-UV (conditions: Inertsil™, ODS-3 5 μm, 4.6 x 75 mm, MeCN+0.1% formic acid/H_2O+0.1% formic acid, 0-10 min: 0 to 100%, 10-15 min: 100%, 15-17 min: 100 to 0%, flow rate 1.0 mL/min, 40 °C, detection wavelength 254 nm) using a calibration curve shown in **Figure S-4**

Table S-3

entry	yield (%)
1	53
2	48
3	54

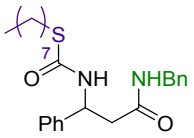
General procedure for examination of substrate scope 4



The employed micro-flow system was shown in **Figure S-3**.

A solution of β-phenylalanine-NCA (0.300 M, 1.00 eq.), isobutyl chloroformate (0.300 M, 1.00 eq.) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of *N*-methylmorpholine (0.360 M, 2.00 eq.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, length: 355 mm, volume: 178 μL, reaction time: 3.3 s) at the same temperature. The resultant mixture and a solution of **nucleophile[1]** (0.180 M, 1.00 eq.) DMAP (0.0180 M, 0.10 equiv.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 1200 mm, volume: 603 μL, reaction time: 7.0 s) at the same temperature. After being eluted for 40 s to reach a steady state, the resultant mixture was poured into a solution of **nucleophile[2]** (1.00 equiv.) and DIEA (1.00 equiv.) in CH_2Cl_2 (0.200 mL) for 25 s at room temperature. The reaction was carried out overnight at room temperature. The reaction mixture was concentrated in vacuo. The residue was purified by preparative TLC.

***S*-Octyl (3-(benzylamino)-3-oxo-1-phenylpropyl)carbamothioate (4a)**

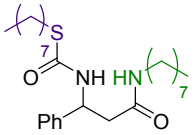


Purification method: **4a** was obtained by preparative TLC (CH_2Cl_2).

45.8 mg, 0.11 mmol, 72%.

White solid; mp 107-109 ºC, IR (ATR): 3264, 3028, 2924, 2868, 1635, 1557, 1453, 1246, 1028 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.41 (d, *J* = 7.6 Hz, 1H), 7.26 (m, 8H), 6.97 (dd, *J* = 1.6, 6.4 Hz, 2H), 6.00 (t, *J* = 5.6 Hz, 1H), 5.23 (s, 1H), 4.34 (dd, *J* = 6.0, 14.8 Hz, 1H), 4.20 (dd, *J* = 5.2, 14.8 Hz, 1H), 2.84 (m, 2H), 2.75 (dd, *J* = 5.2, 14.4 Hz, 1H), 2.62 (dd, *J* = 6.0, 14.4 Hz, 1H), 1.56 (m, 2H), 1.30 (m, 10H), 0.87 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 167.5, 140.8, 137.7, 128.8, 128.7, 127.64, 127.61, 127.5, 126.2, 52.5, 43.5, 42.1, 31.9, 30.5, 30.1, 29.3, 29.2, 28.9, 22.7, 14.2; HRMS (ESI-TOF): calcd for $[C_{25}H_{34}N_2O_2S+Na]^+$ 449.2233, found 449.2233.

_S_-Octyl (3-(octylamino)-3-oxo-1-phenylpropyl)carbamothioate (4b)

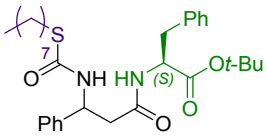


Purification method: **4b** was obtained by preparative TLC (CH_2Cl_2).

50.5 mg, 0.11 mmol, 75%,

White solid; mp 120-123 ºC; IR (ATR): 3303, 2954, 2919, 2849, 1646, 1551, 1523, 1222, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.47 (d, *J* = 6.8 Hz, 1H), 7.27 (m, 5H), 5.63 (brs, 1H), 5.27 (brs, 1H), 3.09 (m, 2H), 2.84 (m, 2H), 2.72 (dd, *J* = 5.2, 14.4 Hz, 1H), 2.56 (dd, *J* = 5.6, 14.4 Hz, 1H), 1.59 (m, 22H), 0.87 (m, 6H) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 167.5, 141.0, 128.7, 127.6, 126.2, 52.6, 42.2, 39.7, 31.9, 30.5, 30.1, 29.5, 29.32, 29.28, 29.23, 28.9, 26.9, 22.7, 14.2; HRMS (ESI-TOF): calcd for $[C_{26}H_{44}N_2O_2S+Na]^+$ 471.3016, found 471.3013.

***tert*-Butyl (3-(((octylthio)carbonyl)amino)-3-phenylpropanoyl)-L-phenylalaninate (4c)**



*L-Phenylalanine *tert*-butyl ester hydrochloride was used as **Nu[1]**. DIEA (1.0 equiv.) was used used to trap hydrogen chloride.

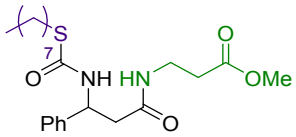
Purification method: **4c** was obtained by preparative TLC (CH_2Cl_2)

53.0 mg, 0.098 mmol, 65%.

Colorless oil; IR (neat): 3317, 2926, 2854, 1733, 1649, 1523, 1221, 1156, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, diastereomeric mixture): δ 7.17 (m, 10H), 6.73 (m, 1H), 5.96 (d, *J* = 7.6 Hz, 1H), 5.31 (m, 1H), 4.63 (m, 1H), 2.80 (m, 6H), 1.59 (m, 2H), 1.36 (m, 10H), 1.24 (brs, 9H), 0.87 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$,

diastereomeric mixture): δ 170.4, 170.3, 169.9, 169.6, 167.4, 140.9, 140.8, 136.1, 135.8, 129.6, 128.8, 128.5, 128.4, 127.7, 127.1, 127.0, 126.29, 126.26, 82.8, 82.7, 53.7, 53.5, 52.4, 52.2, 42.0, 41.7, 38.0, 31.9, 30.5, 30.1, 29.3, 29.2, 28.9, 28.1, 28.0, 22.7, 14.2; HRMS (ESI-TOF): calcd for $[C_{31}H_{44}N_2O_4S+Na]^+$ 563.2914, found 563.2916.

Methyl 3-(3-(((octylthio)carbonyl)amino)-3-phenylpropanamido)propanoate (4d)



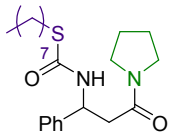
*β-Alanine methyl ester hydrochloride was used as **Nu[1]**. DIEA (1.0 equiv.) was used used to trap hydrogen chloride.

Purification method: **4d** was obtained by preparative TLC (CH_2Cl_2).

39.5 mg, 0.094 mmol, 62%.

White solid; mp 118-119 ºC; IR (ATR): 3315, 2953, 2924, 2850, 1736, 1639, 1519, 1221, 1194 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.27 (m, 6H), 6.14 (brs, 1H), 5.28 (brs, 1H), 3.63 (s, 3H), 3.42 (m, 1H), 3.32 (m, 1H), 2.86 (m, 1H), 2.71 (dd, *J* = 5.2, 14.4 Hz, 1H), 2.57 (dd, *J* = 5.6, 14.4 Hz, 1H), 2.34 (m, 2H), 1.58 (m, 2H), 1.29 (m, 10H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0, 170.4, 167.4, 140.8, 128.7, 127.6, 126.1, 52.4, 51.9, 42.1, 34.8, 33.6, 31.9, 30.5, 30.1, 29.3, 29.2, 28.9, 22.7, 14.2; HRMS (ESI-TOF): calcd for $[C_{22}H_{34}N_2O_4S+Na]^+$ 445.2131, found 445.2131.

***S*-Octyl (3-oxo-1-phenyl-3-(pyrrolidin-1-yl)propyl)carbamothioate (4e)**

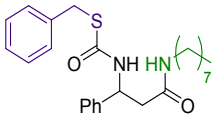


Purification method: **4e** was obtained by preparative TLC (CH_2Cl_2).

37.8 mg, 0.10 mmol, 65%.

Colorless oil; IR (neat): 2954, 2925, 2854, 1675, 1621, 1453, 1199, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.74 (brs, 1H), 7.30 (m, 4H), 7.25 (m, 1H), 5.31 (brs, 1H), 3.36 (m, 2H), 3.23 (m, 1H), 2.85 (m, 4H), 2.73 (dd, *J* = 5.0, 15.0 Hz, 1H), 1.75 (m, 4H), 1.58 (m, 2H), 1.30 (m, 10H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.2, 167.3, 141.5, 128.6, 127.4, 126.2, 52.1, 46.9, 45.6, 39.5, 31.9, 30.6, 30.0, 29.3, 29.2, 19.2, 14.2; HRMS (ESI-TOF): calcd for $[C_{24}H_{25}N_3O_2+Na]^+$ 413.2233, found 413.2233.

***S*-Benzyl (3-(octylamino)-3-oxo-1-phenylpropyl)carbamothioate (4f)**

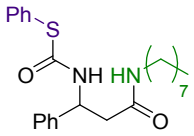


Purification method: **4f** was obtained by preparative TLC (CH_2Cl_2).

45.5 mg, 0.11 mmol, 71%.

White solid; mp 119-120 ºC; IR (ATR): 3296, 3197, 3028, 2924, 2851, 1670, 1638, 1557, 1230 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, *J* = 7.2 Hz, 1H), 7.25 (m, 10H), 5.53 (brs, 1H), 5.28 (brs, 1H), 4.11 (q, *J* = 13.6 Hz, 2H), 3.06 (m, 2H), 2.71 (dd, *J* = 5.2, 14.4 Hz, 1H), 2.53 (dd, *J* = 5.2, 14.4 Hz, 1H), 1.29 (m, 12H), 0.88 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 166.8, 140.8, 138.4, 129.0, 128.8, 128.6, 127.7, 127.2, 126.2, 52.7, 42.1, 39.6, 34.3, 31.9, 29.4, 29.30, 29.26, 26.9, 22.7, 14.2; HRMS (ESI-TOF): calcd for $[C_{25}H_{34}N_2O_2S+Na]^+$ 449.2233, found 449.2230.

_S_-Phenyl (3-(octylamino)-3-oxo-1-phenylpropyl)carbamothioate (4g)

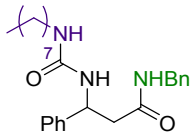


Purification method: **4g** was obtained by preparative TLC (CH_2Cl_2).

42.7 mg, 0.10 mmol, 69%

White solid; mp 135-137 ºC; IR (ATR): 3303, 2921, 2852, 1644, 1544, 1523, 1438, 1353, 1214 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.52 (s, 2.5 H), 7.38 (s, 2.5H), 7.26 (m, 5H), 5.54 (brs, 1H), 5.22 (brs, 1H), 3.06 (m, 2H), 2.67 (d, *J* = 14.4 Hz, 1H), 2.51 (m, 1H), 1.20 (m, 12H), 0.88 (t, *J* = 6.8 Hz, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 166.0, 140.7, 135.5, 129.5, 129.3, 128.8, 128.5, 127.7, 126.2, 52.8, 41.9, 39.6, 31.9, 29.4, 29.31, 29.28, 26.9, 22.8, 14.2; HRMS (ESI-TOF): calcd for $[C_{24}H_{32}N_2O_2S+Na]^+$ 435.2077, found 435.2076.

_N_-Benzyl-3-(3-octylureido)-3-phenylpropanamide (4h)



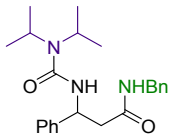
*The reaction was carried out for 1 h. DIEA was not used.

Purification method: **4h** was obtained by preparative TLC (CH_2Cl_2/MeOH = 9/1) and GPC.

36.8 mg, 0.090 mmol, 60%.

White solid; mp 151-153 ºC; IR (ATR): 3315, 3256, 3027, 2920, 2851, 1637, 1557, 1452, 1255 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.14 (brs, 1H), 7.18 (m, 8H), 7.03 (d, *J* = 6.0 Hz, 2H), 6.83 (brs, 1H), 5.70 (brs, 1H), 5.34 (m, 1H), 4.22 (brs, 2H), 2.99 (m, 3H), 2.71 (d, *J* = 8.8, 1H), 1.21 (m, 12H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$)**:** δ 171.6, 158.6, 143.1, 138.3, 128.6, 128.5, 127.3, 127.0, 126.3, 51.6, 44.1, 43.3, 40.5, 32.0, 30.4, 29.5, 29.4, 27.1, 22.8, 14.2; HRMS (ESI-TOF): calcd for $[C_{23}H_{31}N_3O_2+Na]^+$ 432.2621, found 432.2620.

_N_-Benzyl-3-(3,3-diisopropylureido)-3-phenylpropanamide (4i)



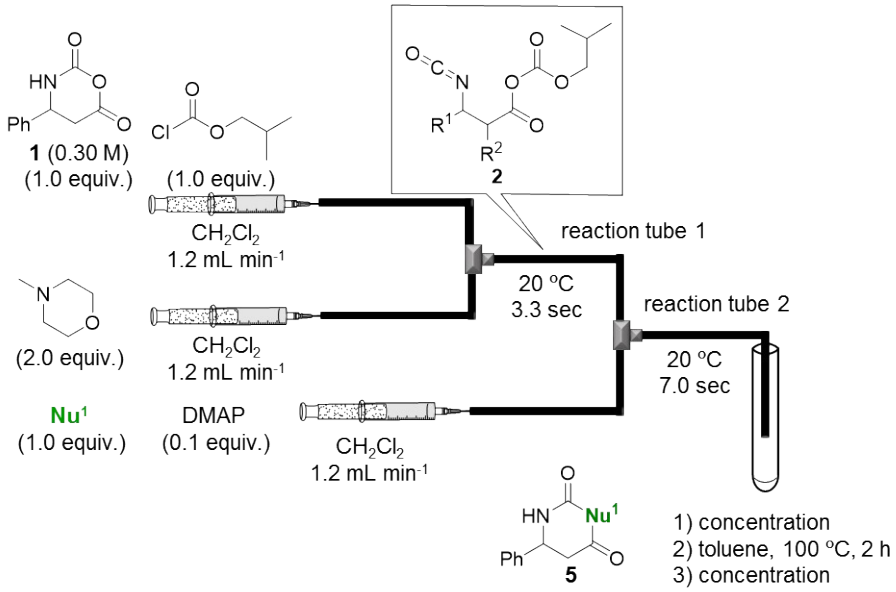
*2.00 equiv. of **Nu²** was used. DIEA was not used.

Purification method: **4i** was obtained by preparative TLC (CH_2Cl_2).

51.3 mg, 0.13 mmol, 90%.

White solid; mp 156-159 ºC; IR (ATR): 3370, 3249, 3060, 2964, 2920, 1645, 1621, 1530, 1327 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 8H), 6.94 (m, 2H), 6.50 (d, *J* = 7.6 Hz, 1H), 6.30 (brs, 1H), 5.28 (m, 1H), 4.37 (dd, *J* = 6.0, 14.8 Hz, 1H), 4.14 (dd, *J* = 5.2, 14.8 Hz, 1H), 3.94 (m, 2H), 2.78 (dd, *J* = 4.4, 14.4 Hz, 1H), 2.59 (dd, *J* = 4.4, 14.4 Hz, 1H), 1.27 (s, 6H), 1.25 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 156.6, 142.7, 137.9, 128.63, 128.61, 127.6, 127.4, 127.0, 126.2, 51.6, 45.2, 43.3, 42.9, 21.5, 21.4; HRMS (ESI-TOF): calcd for $[C_{25}H_{35}N_3O_2+Na]^+$ 404.2308, found 404.2308.

General procedure for examination of substrate scope 5

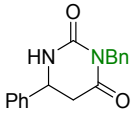


The employed micro-flow system was shown in **Figure S-3**.

A solution of β-phenylalanine-NCA (0.300 M, 1.00 eq.), isobutyl chloroformate (0.300 M, 1.00 eq.) in CH_2Cl_2 (flow rate: 1.20 mL/min), a solution of *N*-methylmorpholine (0.360 M, 2.00 eq.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 1 (inner diameter: 0.800 mm, length: 355 mm, volume: 178 μL, reaction time: 3.3 s) at the same temperature. The resultant mixture and a solution of **nucleophile¹** (0.180 M, 1.00 eq.) DMAP (0.0180 M, 0.10 equiv.) in CH_2Cl_2 (flow rate: 2.00 mL/min) were introduced to the T-shape mixer at 20 °C with the syringe pumps. the resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 1200 mm, volume: 603 μL, reaction time: 7.0 s) at the same temperature. After being eluted for 40 s to reach a steady state, the resultant

mixture was poured into flask for 25 s at room temperature. The reaction mixture was concentrated in vacuo. Then, toluene (2.00 mL) was added. The reaction was carried out for 2 h at 100 ºC. The reaction mixture was concentrated in vacuo. The residue was purified by preparative TLC.

3-Benzyl-6-phenyldihydropyrimidine-2,4(1*H*,3*H*)-dione (5a)

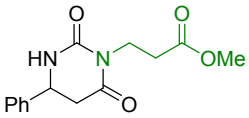


Purification method: **5a** was obtained by preparative TLC (CH_2Cl_2).

32.9 mg, 0.12 mmol, 78%.

White solid; mp 140-143 ºC; IR (ATR): 3209, 3062, 1722, 1665, 1436, 1352, 1310, 762, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.39-7.23 (m, 10H), 5.91 (s, 1H), 4.96 (dd, *J* = 7.6, 14 Hz, 2H), 4.67-4.63 (m, 1H), 2.94 (ddd, *J* = 1.6, 4.8, 6.4, 1H), 2.82 (dd, *J* = 10.4, 16.4, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.5, 154.6, 139.1, 137.6, 129.4, 129.0, 128.9, 128.5, 127.6, 126.1, 51.2, 43.6, 40.1; HRMS (ESI-TOF): calcd for $[C_{17}H_{16}N_2O_2+Na]^+$ 303.1104, found 303.1104.

Methyl 3-(2,6-dioxo-4-phenyltetrahydropyrimidin-1(2*H*)-yl)propanoate (5b)



*β-Alanine methyl ester hydrochloride was used as **Nu[1]**. DIEA (1.0 equiv.) was used to trap hydrogen chloride.

Purification method: **5b** was obtained by preparative TLC (CH_2Cl_2).

27.4 mg, 0.099 mmol, 66%.

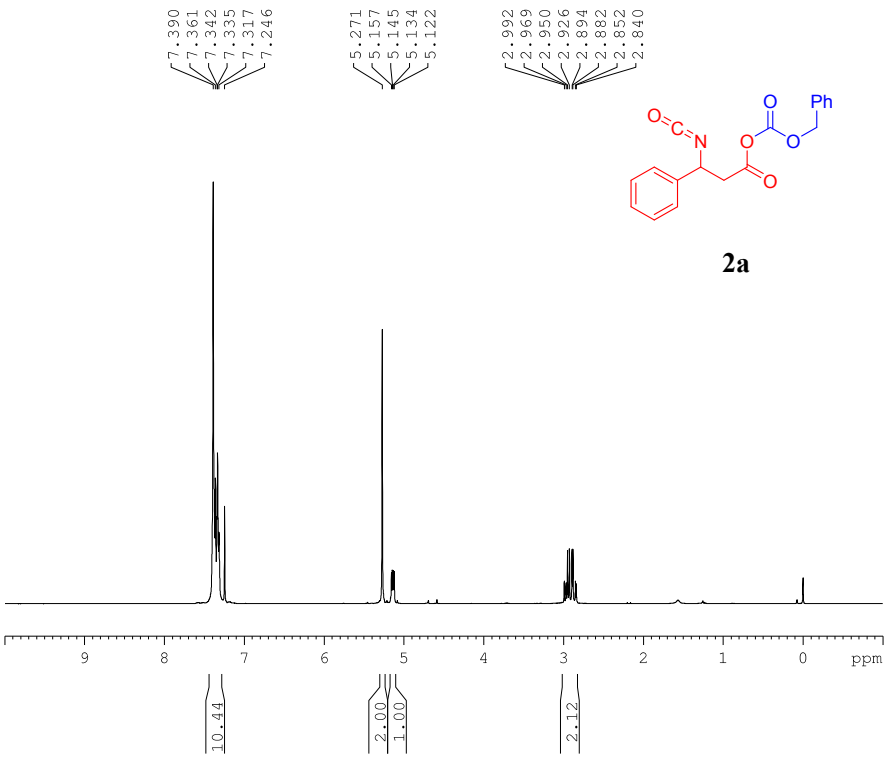
White solid; mp 141-144 ºC; IR (ATR): 3313, 2952, 1719, 1666, 1457, 1429, 1360, 1202, 1153 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.36 (m, 5H), 5.87 (s, 1H), 4.69 (dd, *J* = 4.4, 10.4 Hz, 1H), 4.10 (m, 1H), 3.66 (s, 3H), 2.95 (dd, *J* = 1.2, 4.8 Hz, 1H), 2.93 (ddd, *J* = 1.2, 4.8, 16.8 Hz, 1H), 2.61 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 168.5, 154.2, 139.0, 129.4, 129.0, 126.1, 51.9, 51.2, 40.0, 36.3, 32.9; HRMS (ESI-TOF): calcd for $[C_{14}H_{16}N_2O_4+Na]^+$ 299.1002, found 299.1002.

References

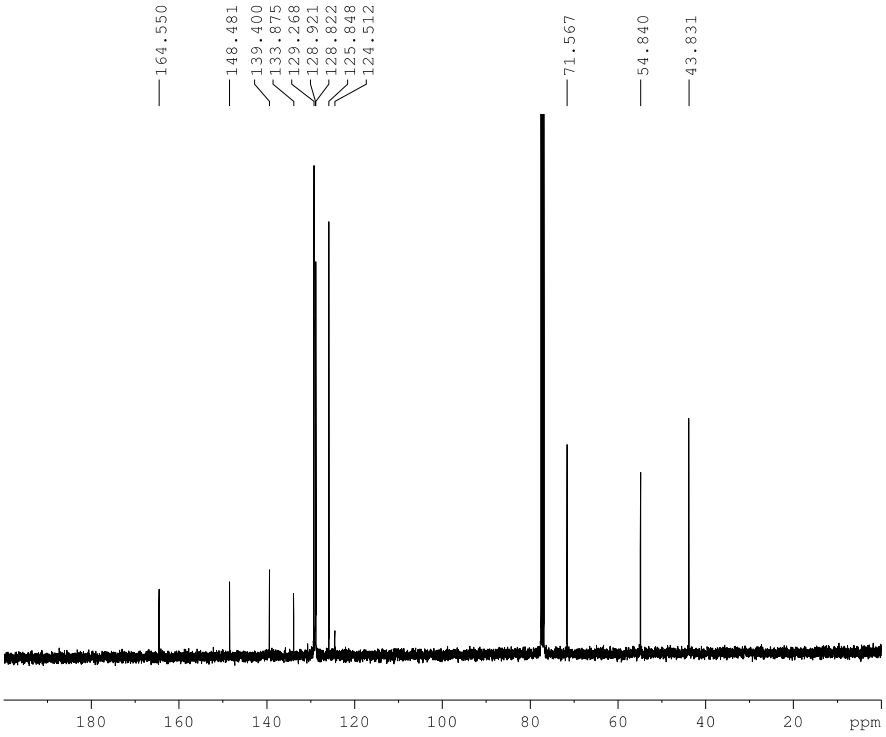
1) N. Sugisawa, Y. Otake, S. Fuse, H. Nakamura, *Chem. Asian J.* 2020, **15**, 79-84.

(Benzyl carbonic) 3-isocyanato-3-phenylpropanoic anhydride (2a)

(^{1}H NMR)

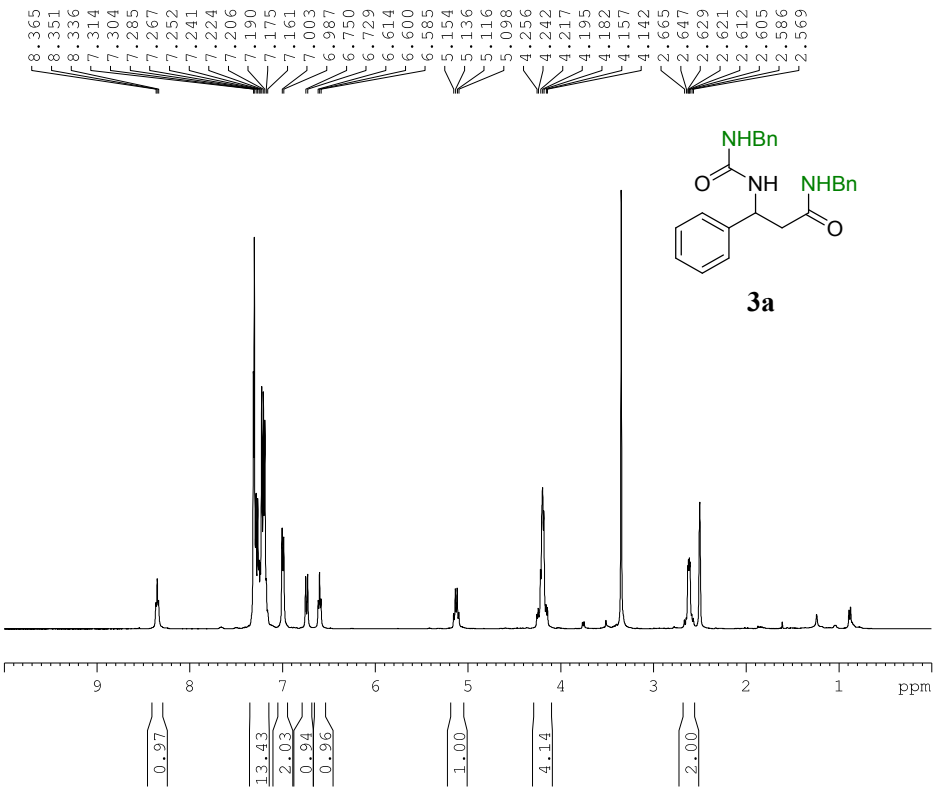


(^{13}C NMR)

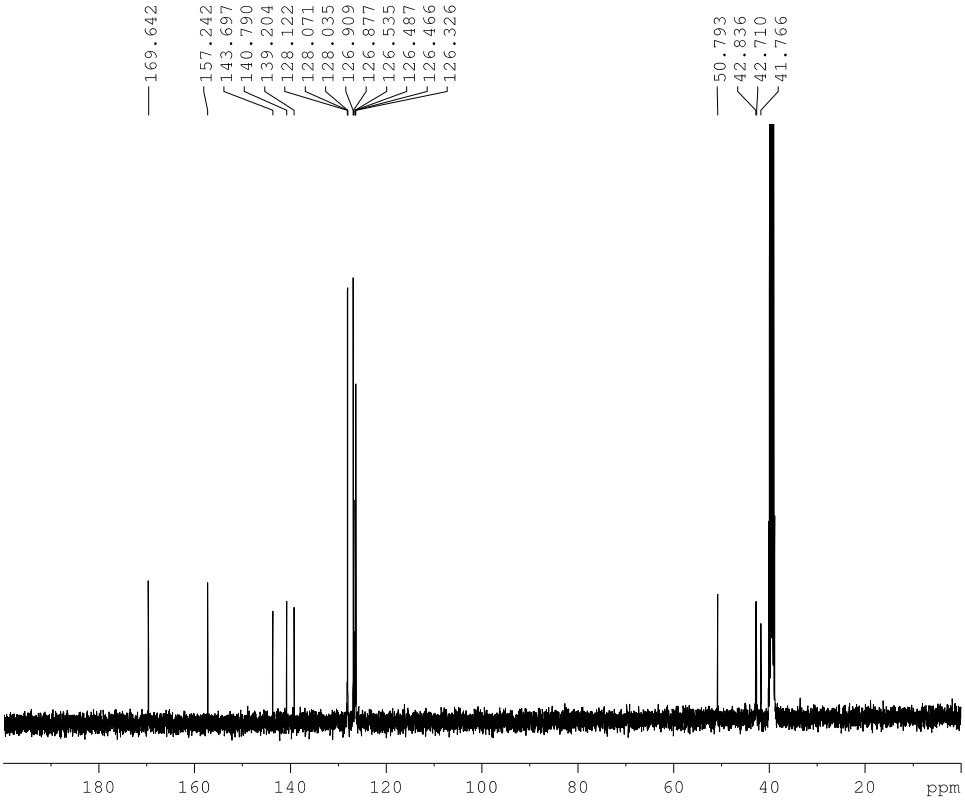


***N*-Benzyl-3-(3-benzylureido)-3-phenylpropanamide (3a)**

(^{1}H NMR)

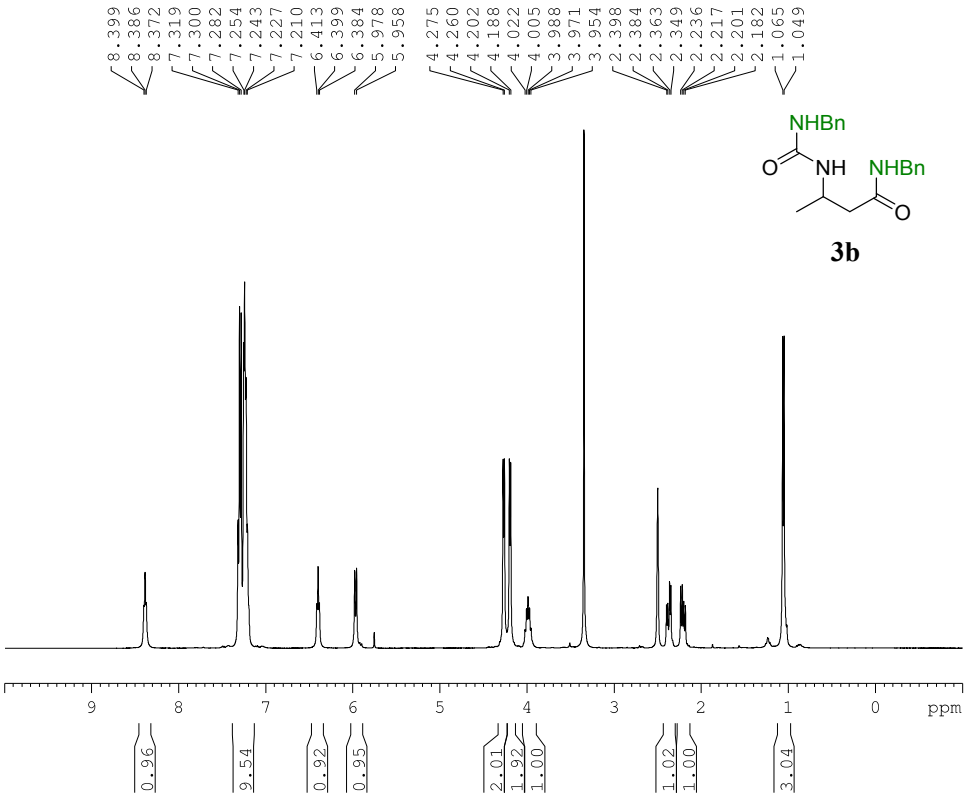


(^{13}C NMR)

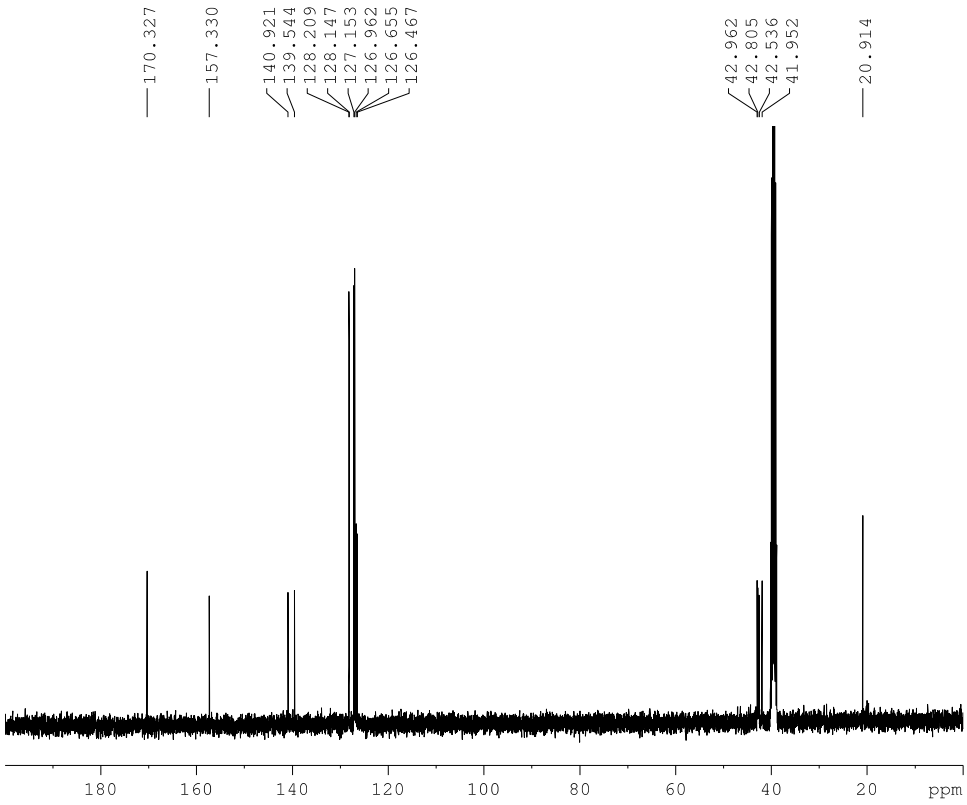


***N*-Benzyl-3-(3-benzylureido)butanamide (3b)**

(^{1}H NMR)

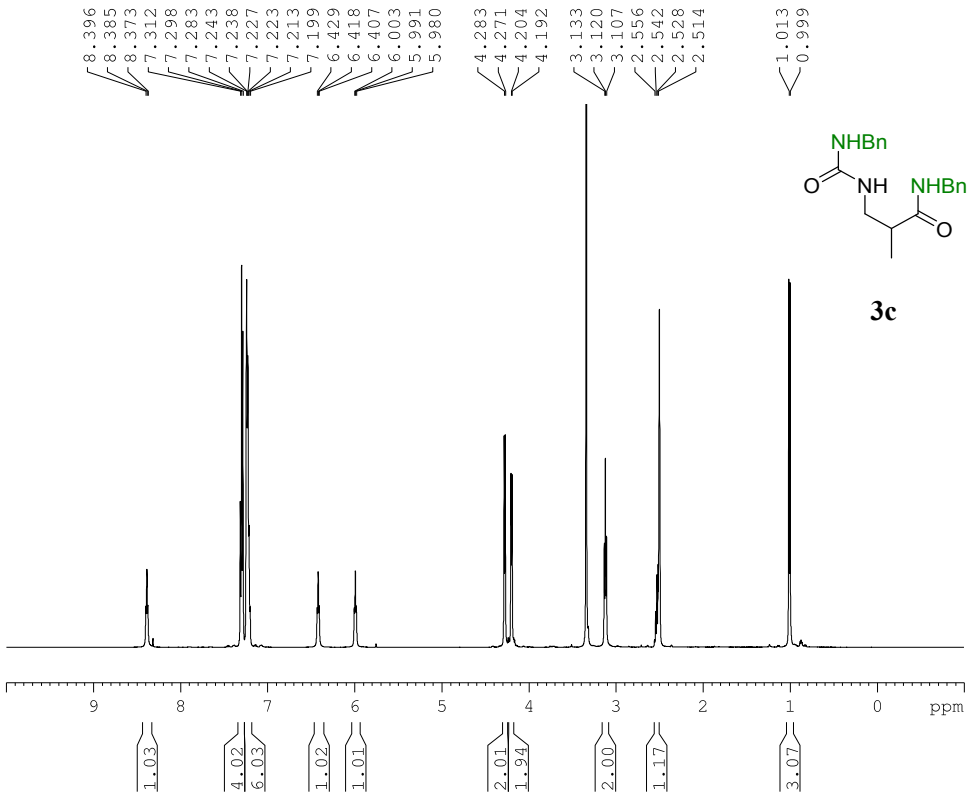


(^{13}C NMR)

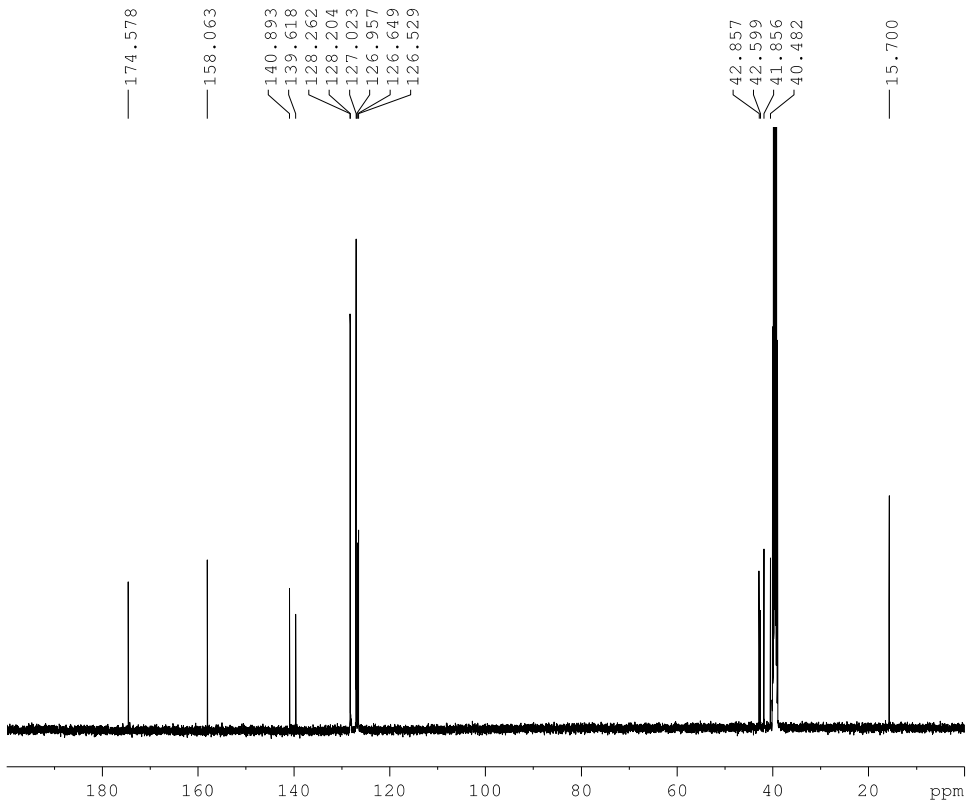


***N*-Benzyl-3-(3-benzylureido)-2-methylpropanamide (3c)**

(^{1}H NMR)

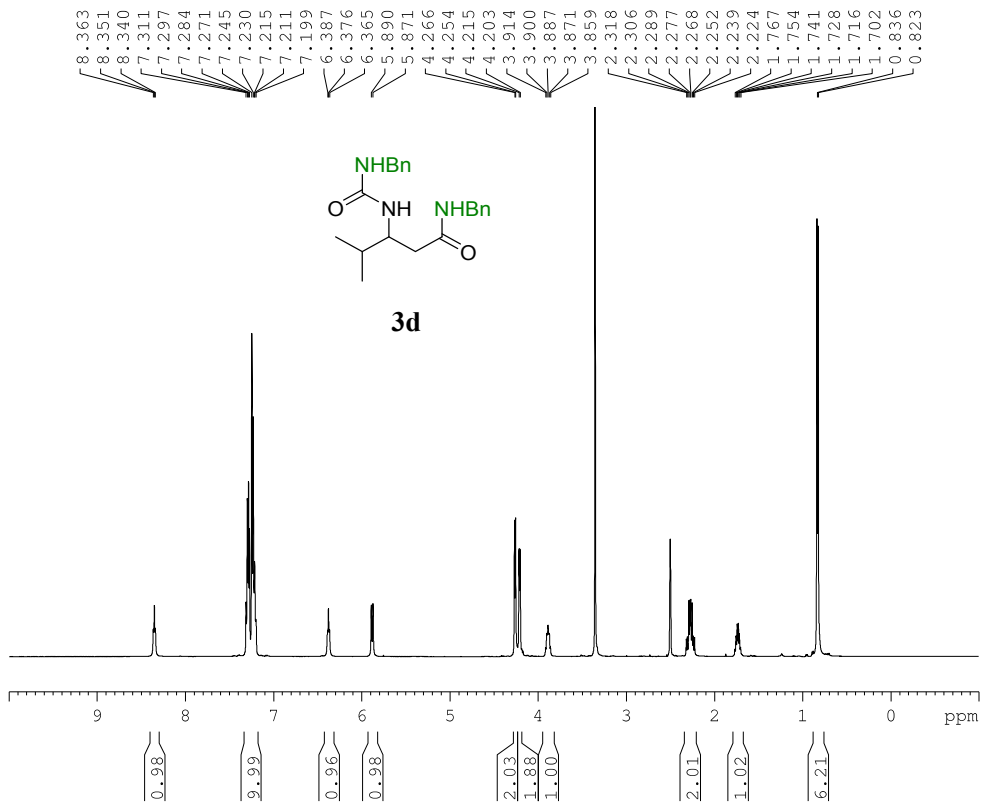


(^{13}C NMR)

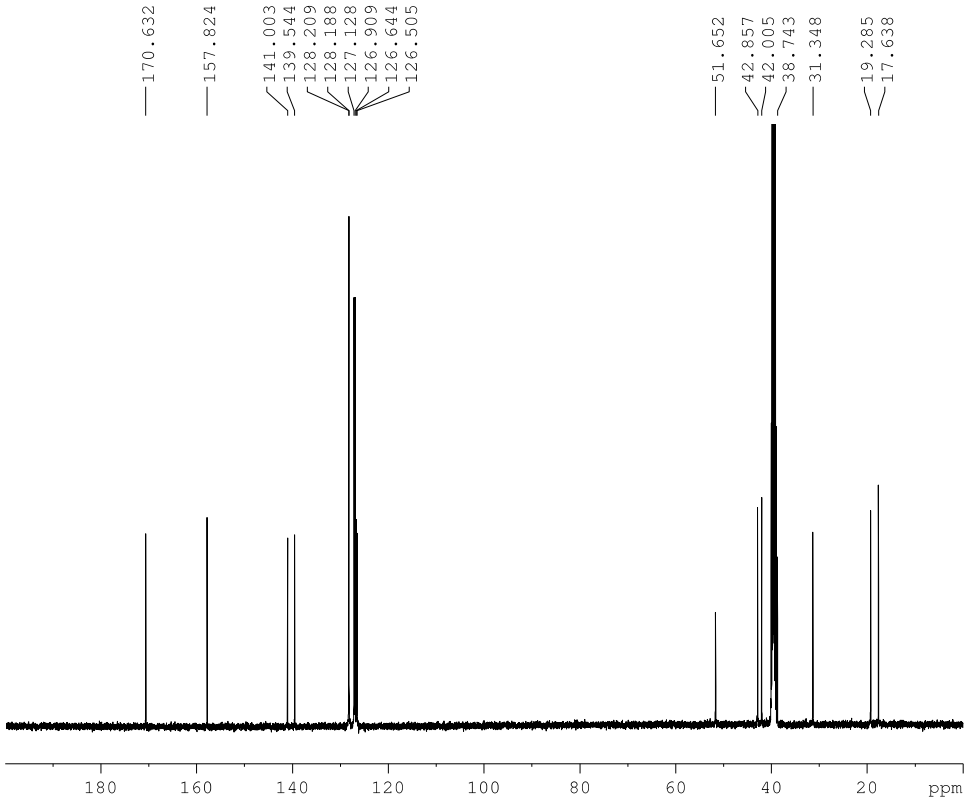


***N*-Benzyl-3-(3-benzylureido)-4-methylpentanamide (3d)**

(^{1}H NMR)

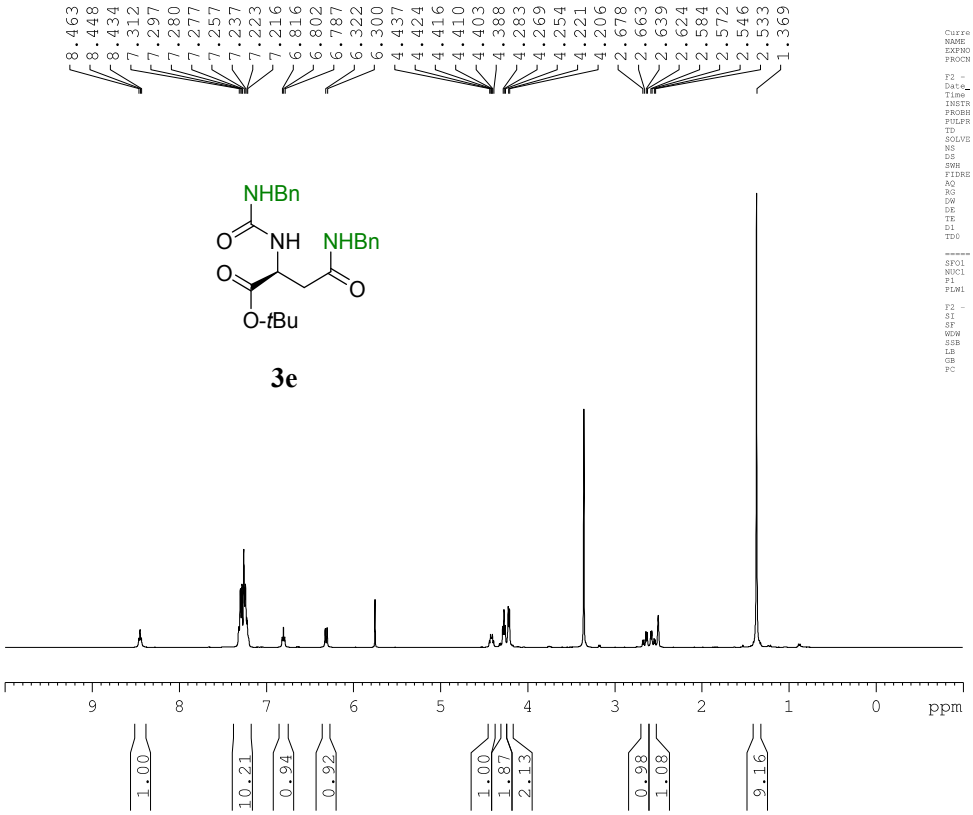


(^{13}C NMR)

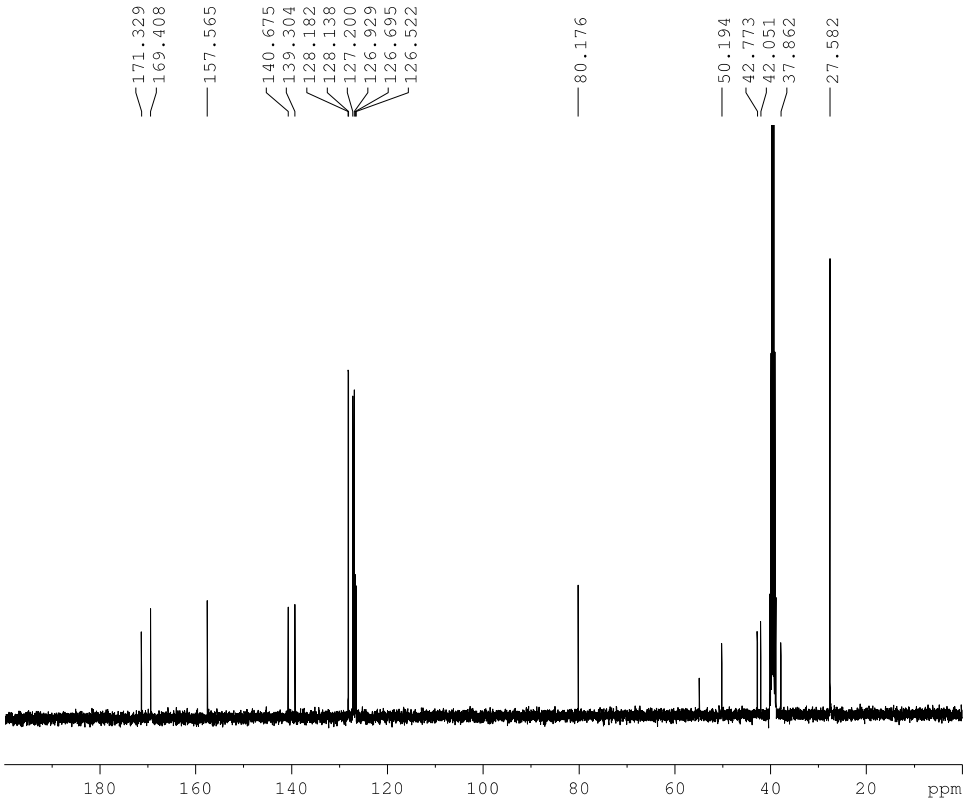


***tert*-Butyl N^4-benzyl-N^2-(benzylcarbamoyl)-L-asparaginate (3e)**

(^{1}H NMR)

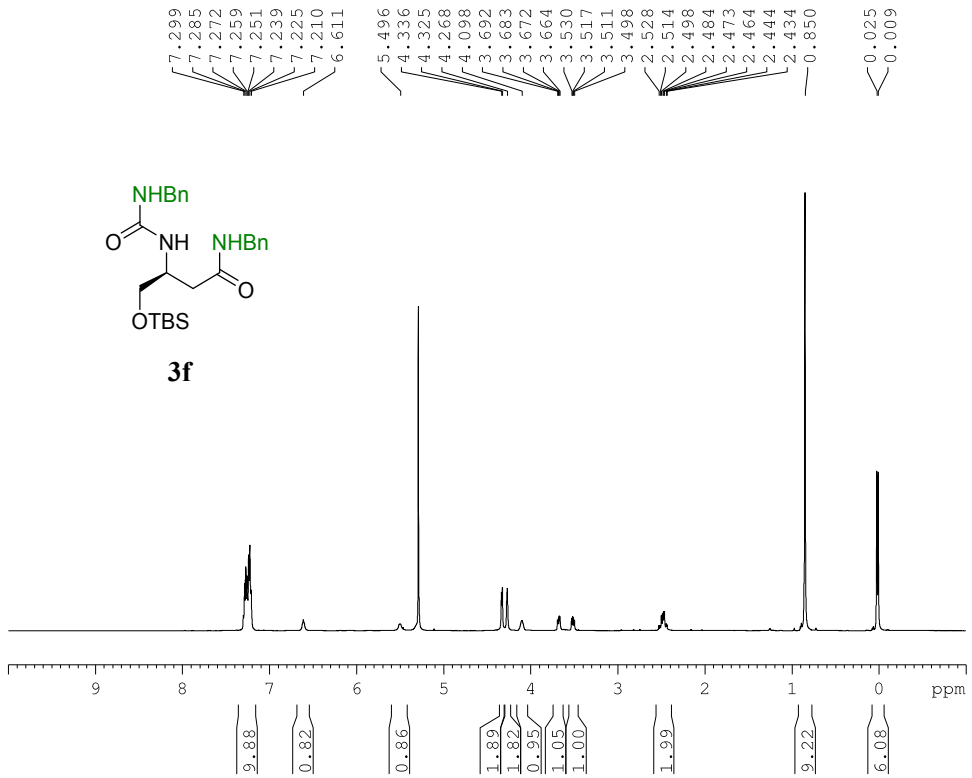


(^{13}C NMR)

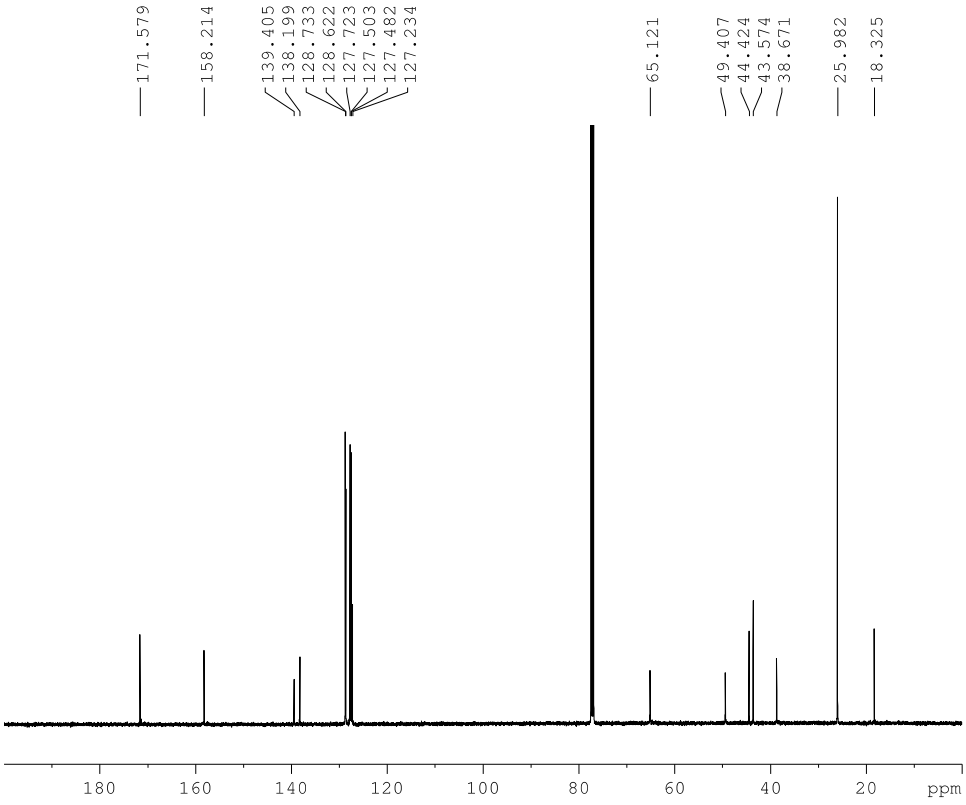


(*S*)-*N*-Benzyl-3-(3-benzylureido)-4-((*tert*-butyldimethylsilyl)oxy)butanamide (3f)

(^{1}H NMR)

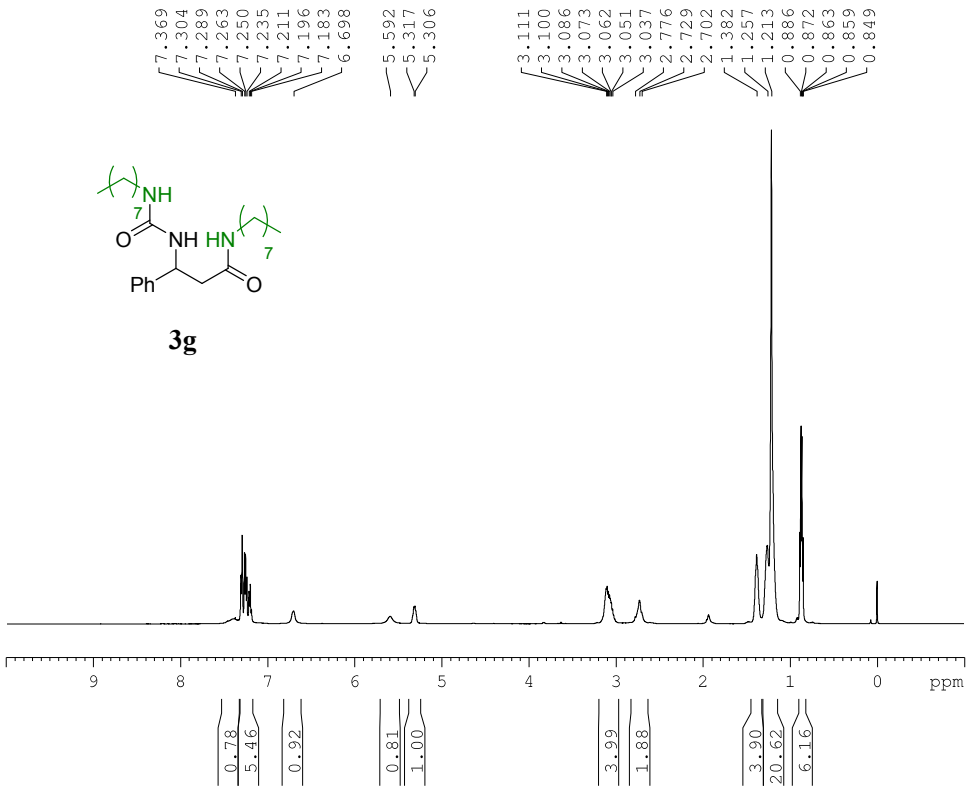


(^{13}C NMR)

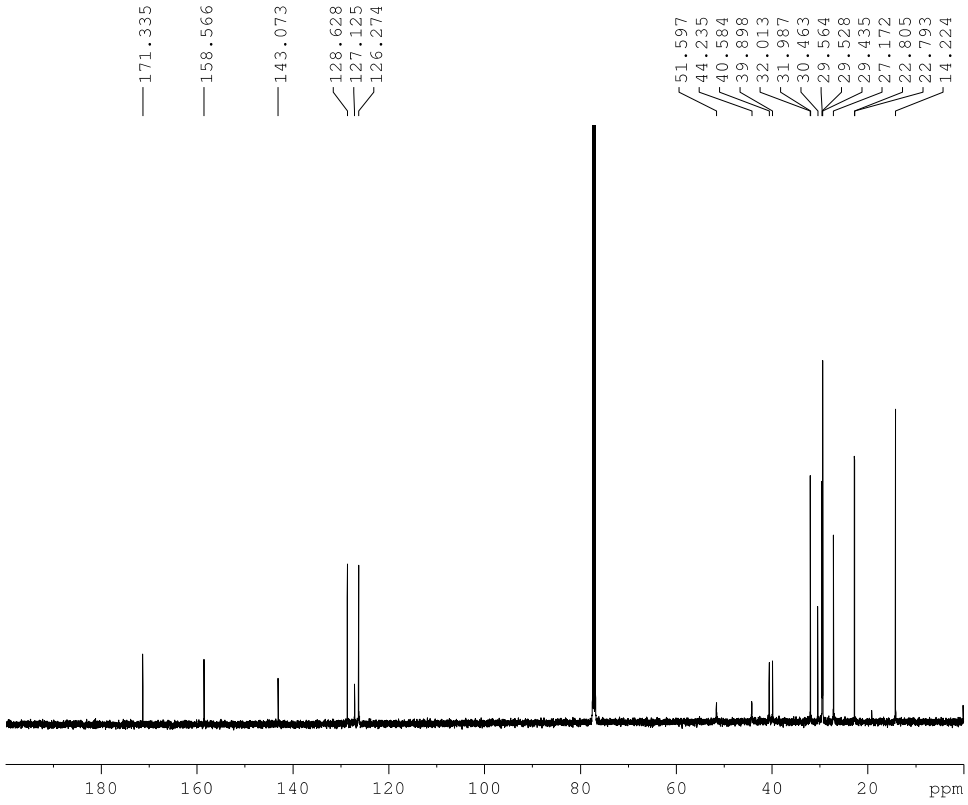


_N_-Octyl-3-(3-octylureido)-3-phenylpropanamide (3g)

(^{1}H NMR)

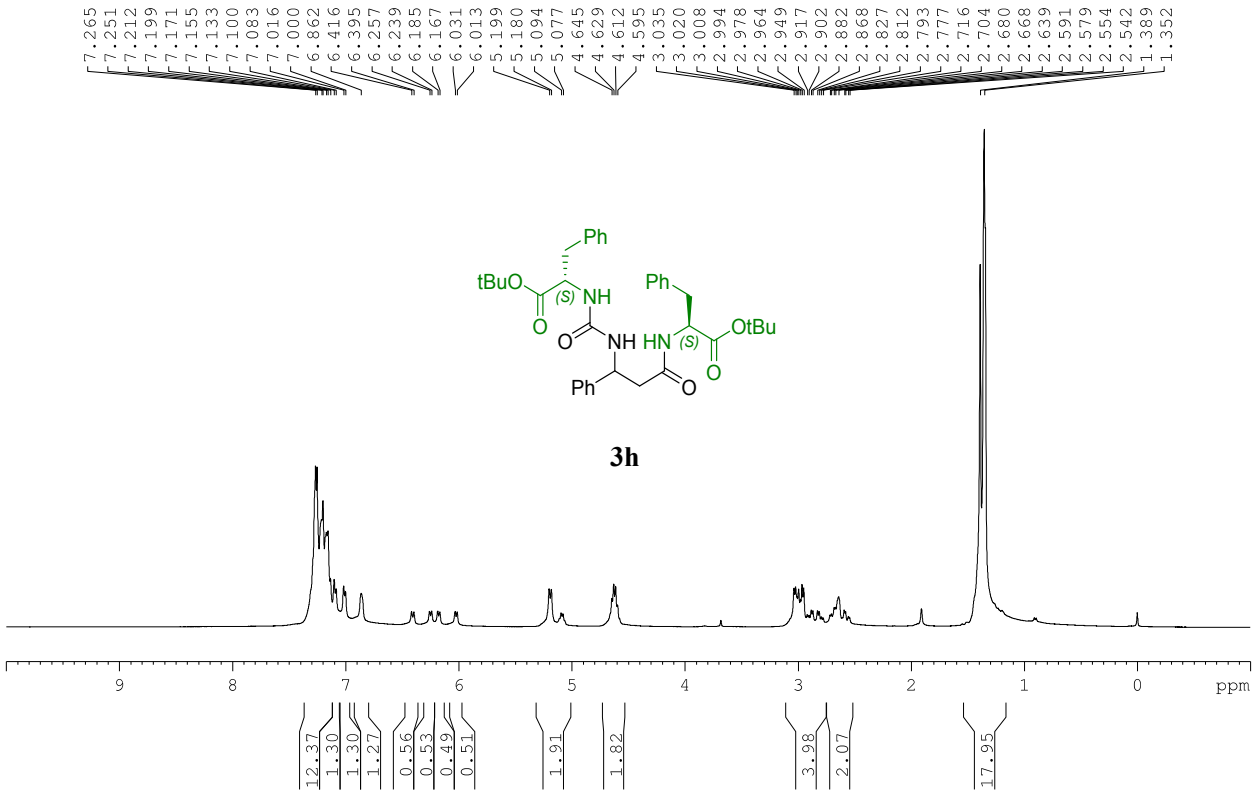


(^{13}C NMR)



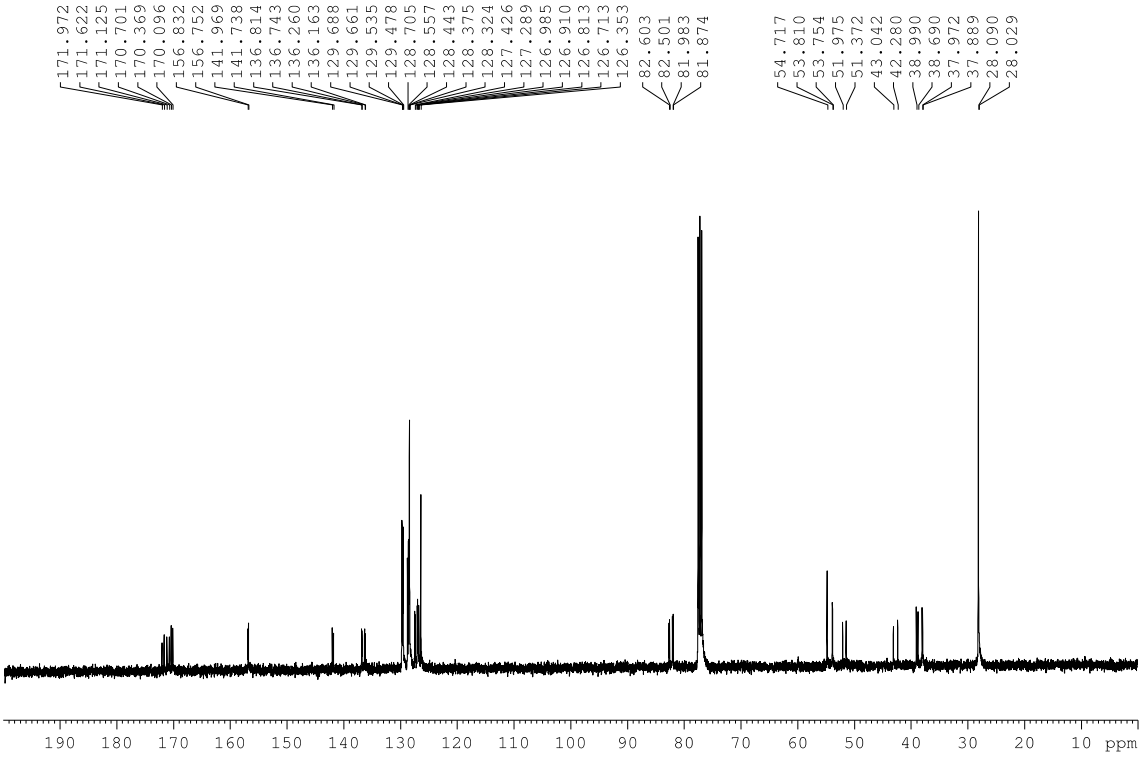
***tert*-Butyl ((3-(((S)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylpropyl)carbamoyl)-L-phenylalaninate (3h)**

(^{1}H NMR)



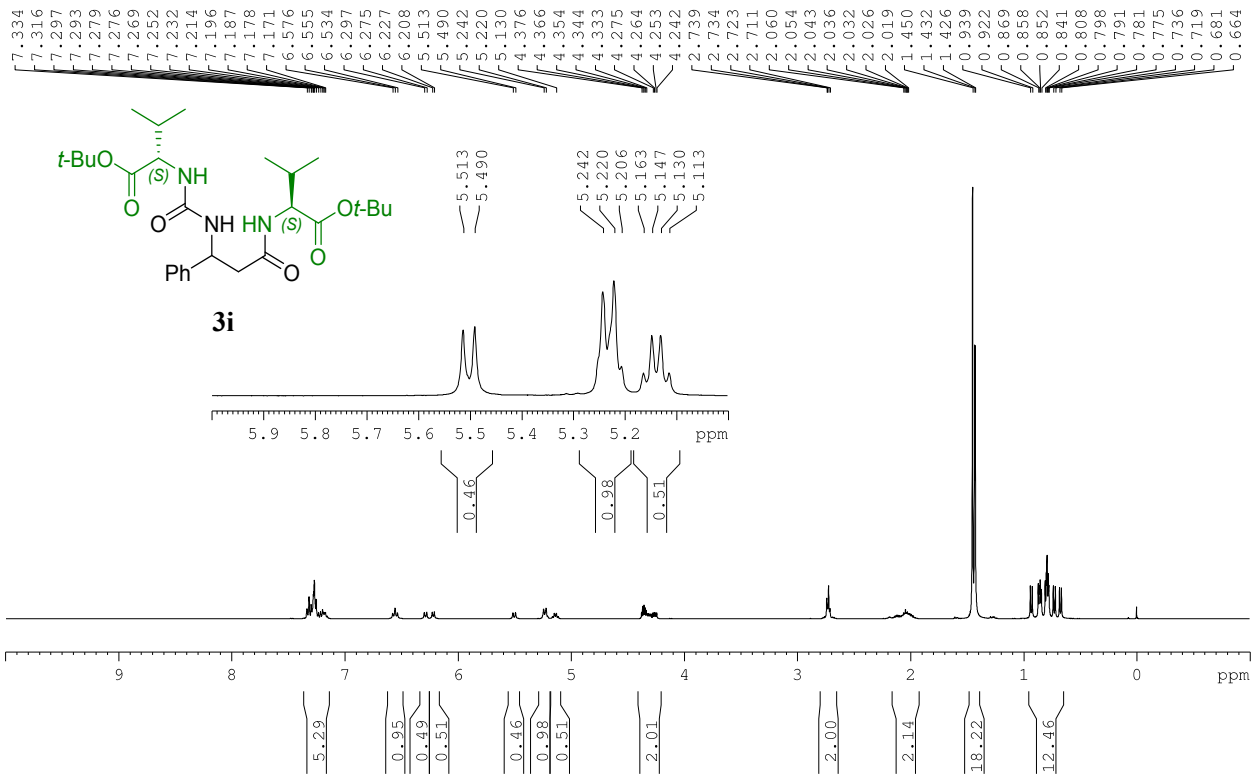
3h

(^{13}C NMR)

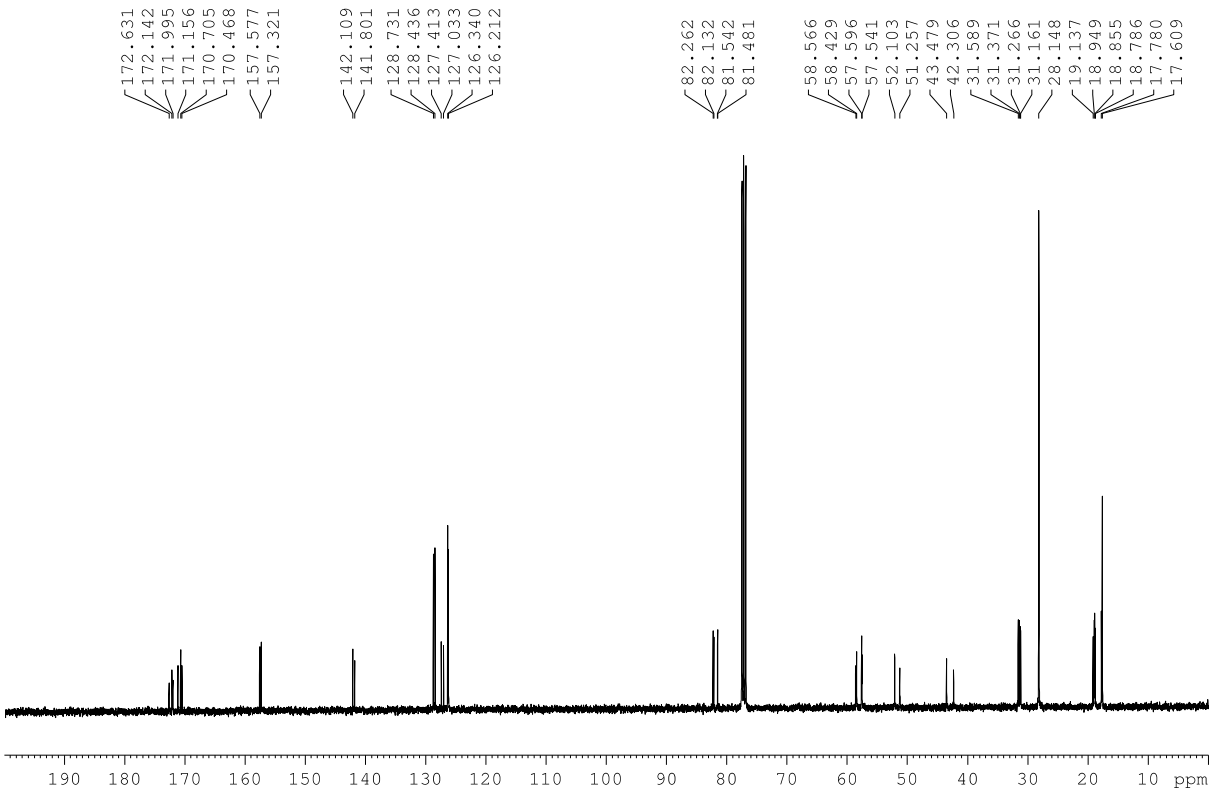


***tert*-Butyl ((3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)amino)-3-oxo-1-phenylpropyl)carbamoyl)-L-valinate (3i)**

(^{1}H NMR)

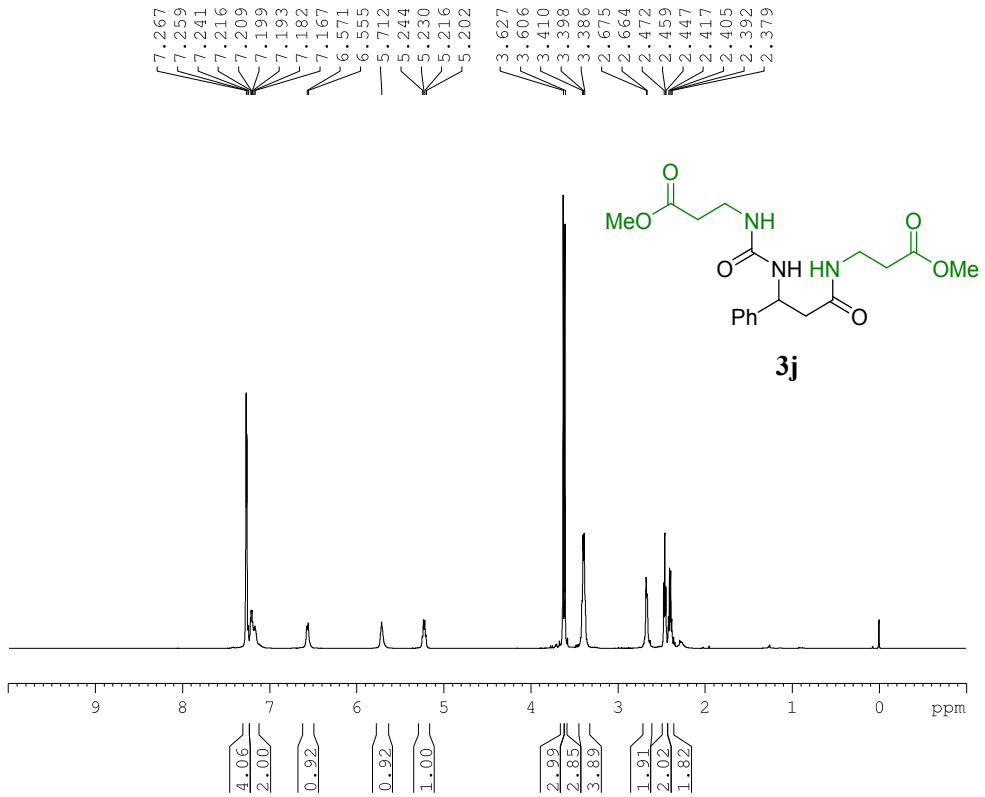


(^{13}C NMR)

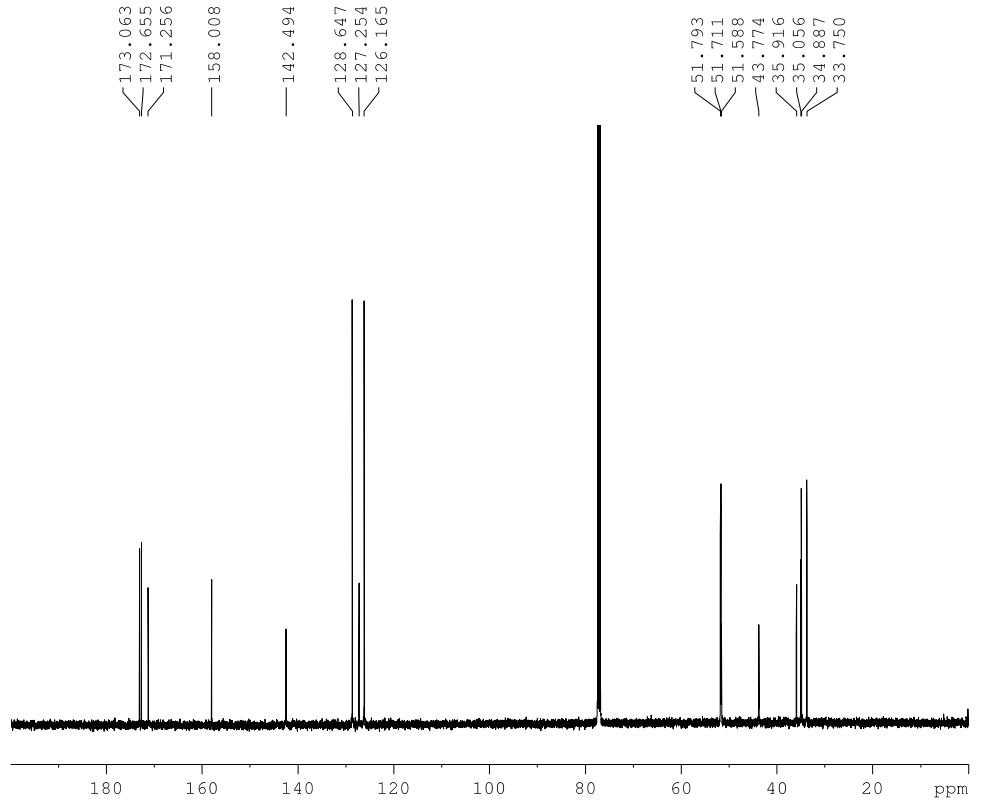


Methyl 3-(3-(3-((3-methoxy-3-oxopropyl)amino)-3-oxo-1-phenylpropyl)ureido)propanoate (3j)

(^{1}H NMR)

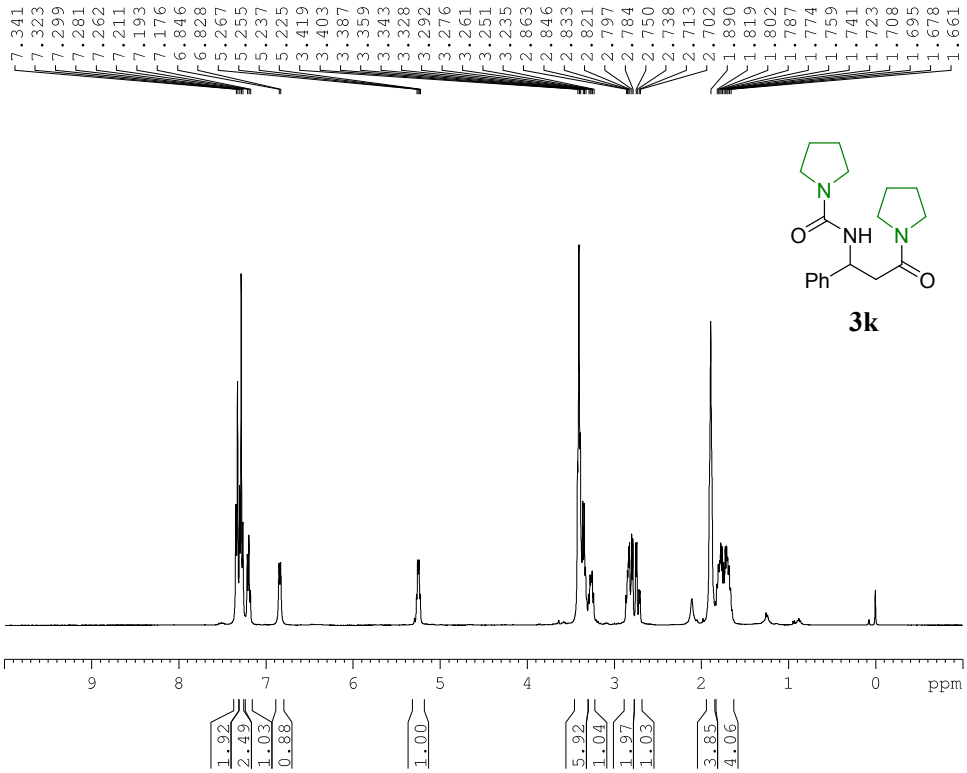


(^{13}C NMR)

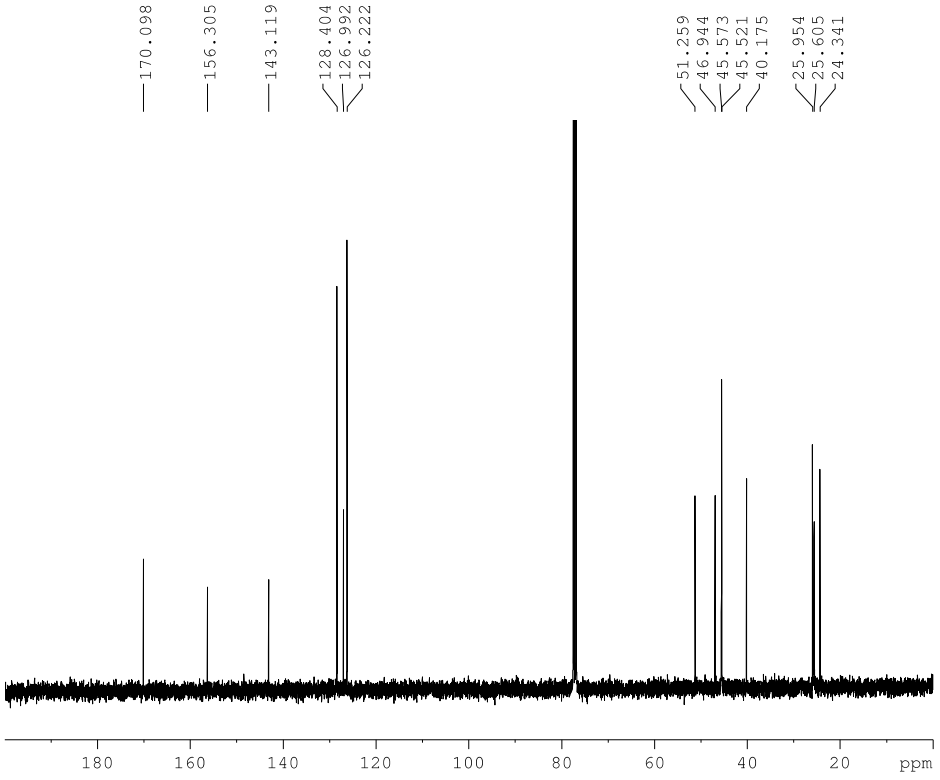


***N*-(3-Oxo-1-phenyl-3-(pyrrolidin-1-yl)propyl)pyrrolidine-1-carboxamide (3k)**

(^{1}H NMR)

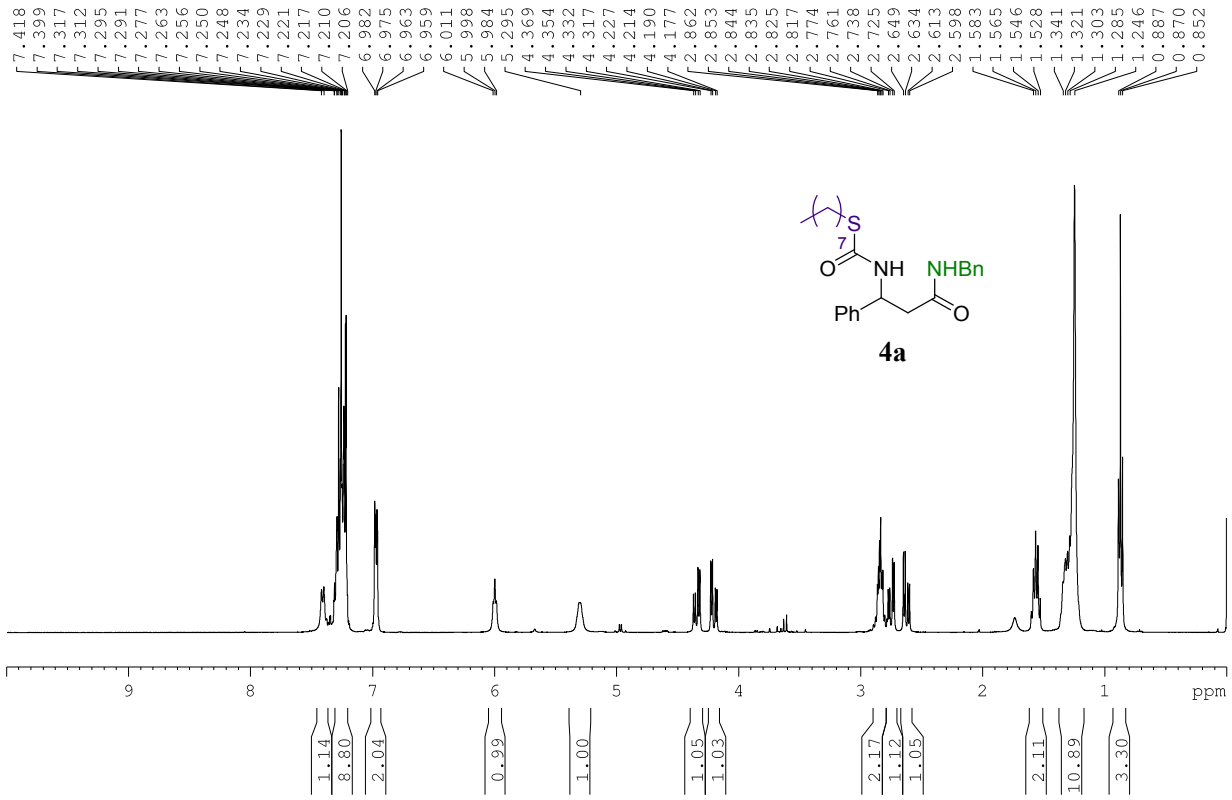


(^{13}C NMR)

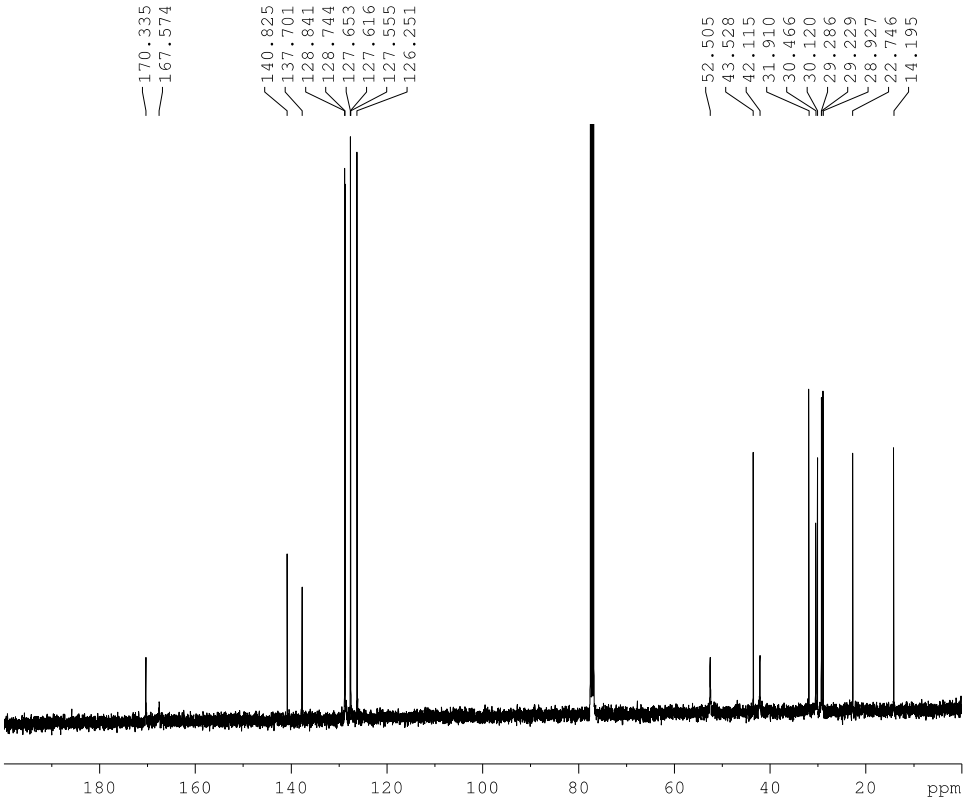


***S*-Octyl (3-(benzylamino)-3-oxo-1-phenylpropyl)carbamothioate (4a)**

(^{1}H NMR)

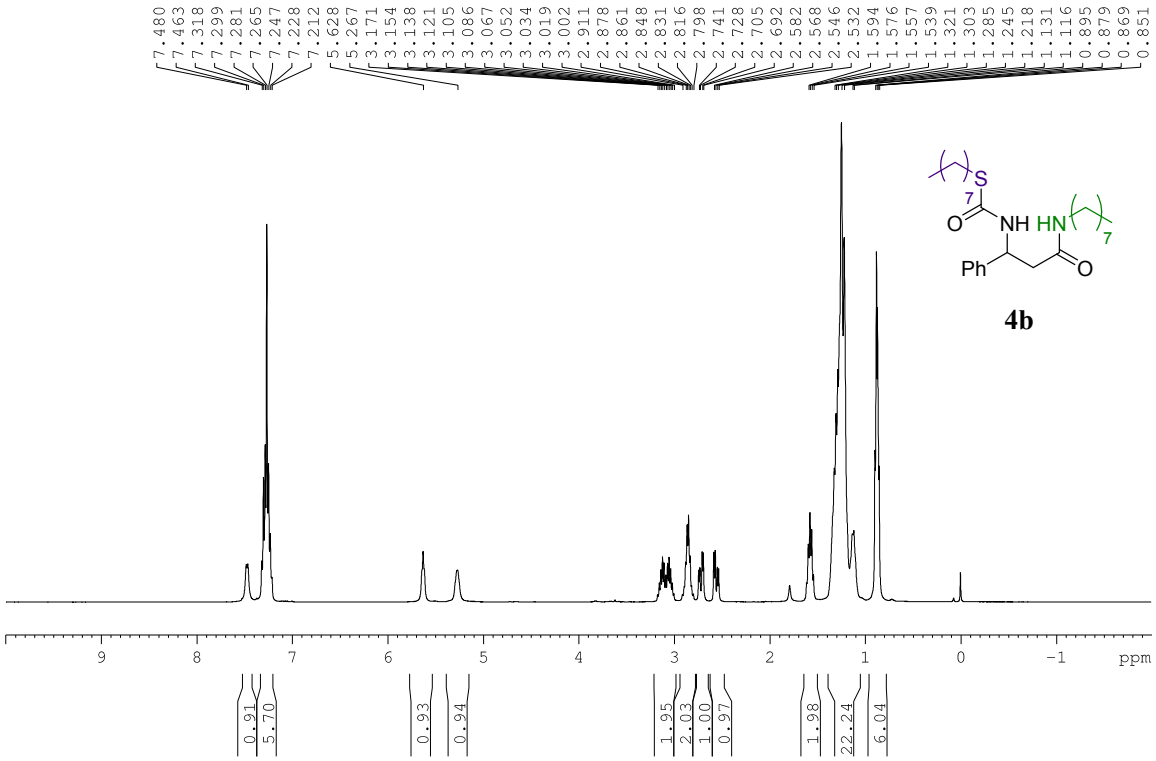


(^{13}C NMR)

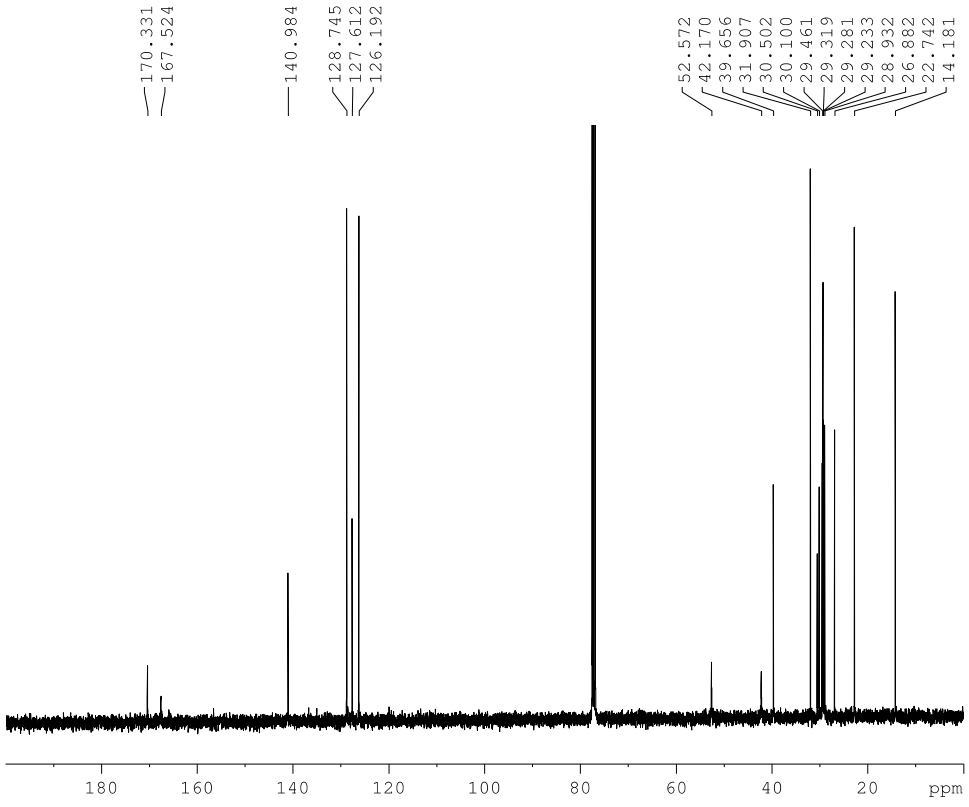


***S*-Octyl (3-(octylamino)-3-oxo-1-phenylpropyl)carbamothioate (4b)**

(^{1}H NMR)

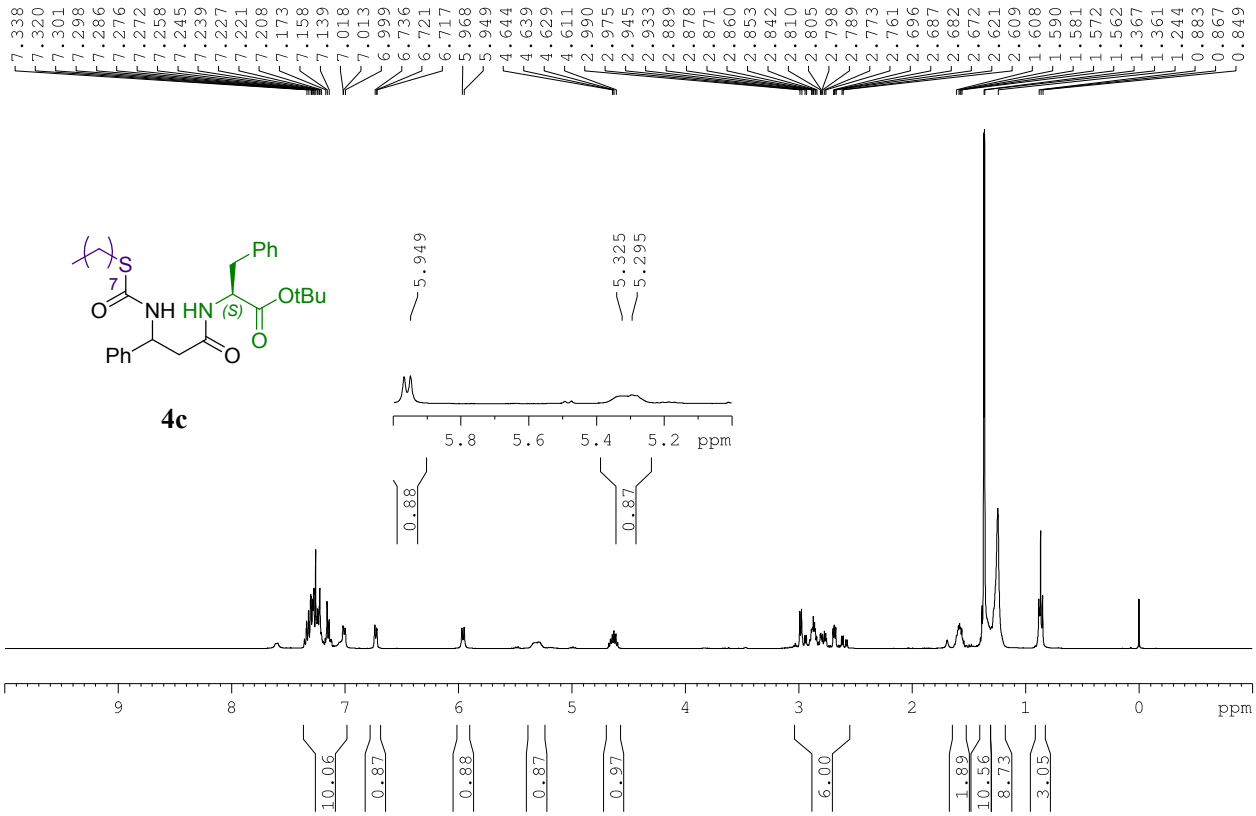


(^{13}C NMR)



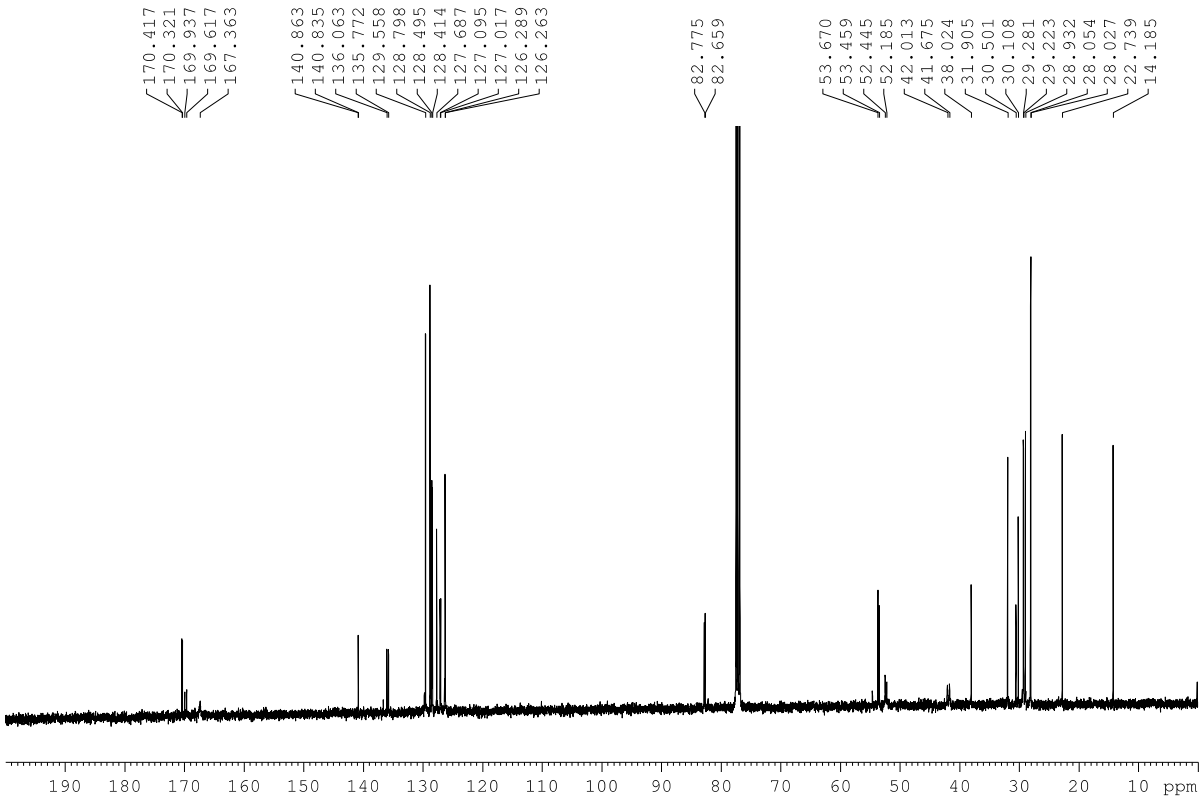
***tert*-Butyl (3-(((octylthio)carbonyl)amino)-3-phenylpropanoyl)-L-phenylalaninate (4c)**

(^{1}H NMR)



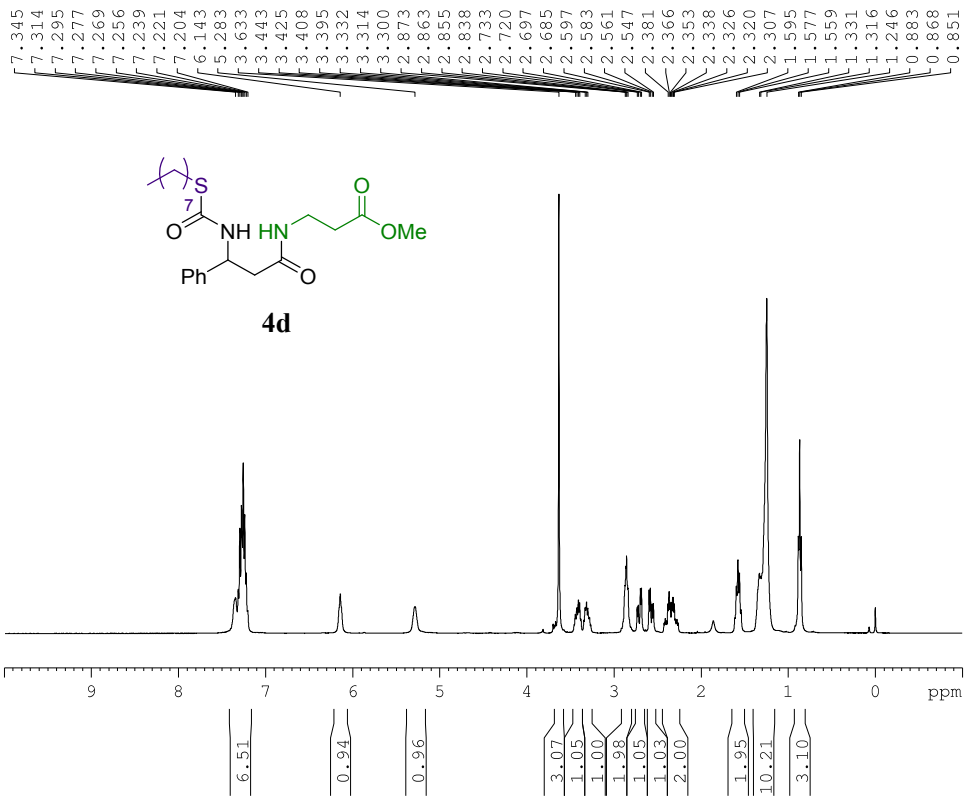
4c

(^{13}C NMR)

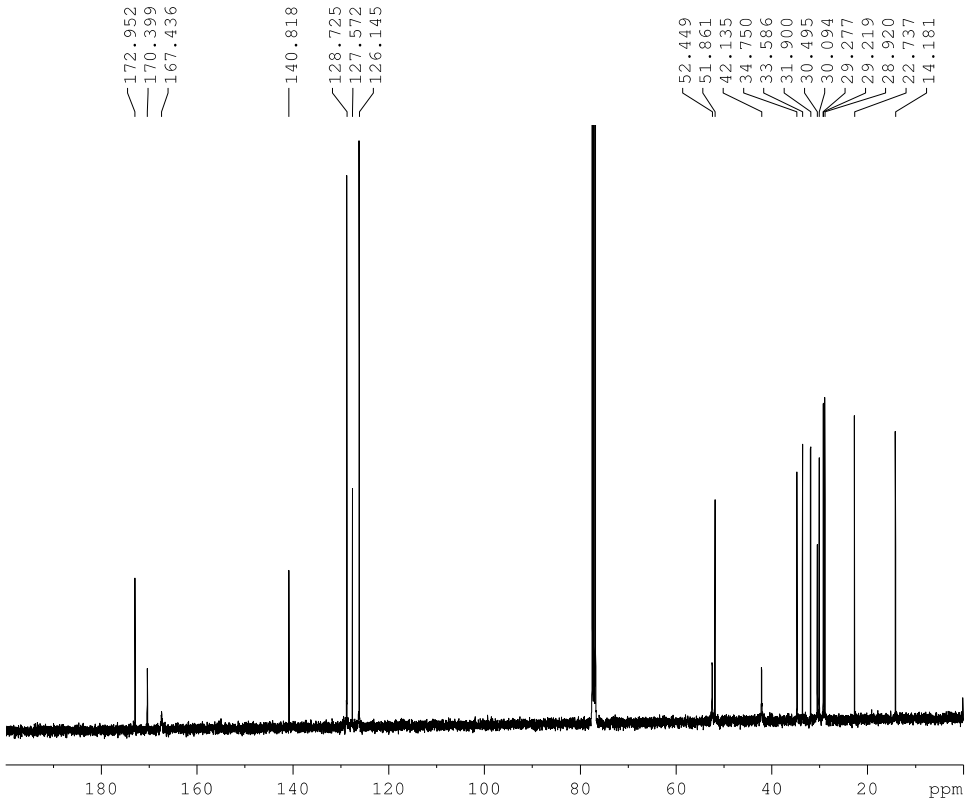


Methyl 3-(3-(((octylthio)carbonyl)amino)-3-phenylpropanamido)propanoate (4d)

(^{1}H NMR)

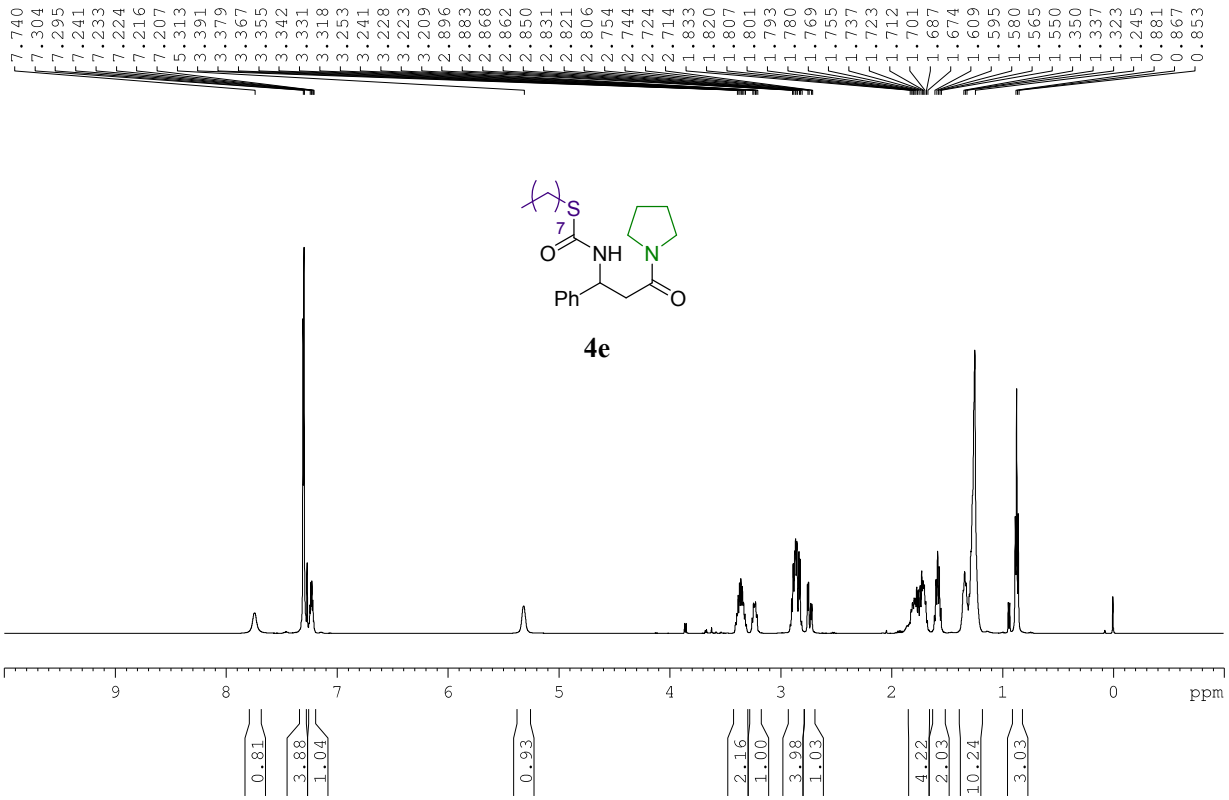


(^{13}C NMR)

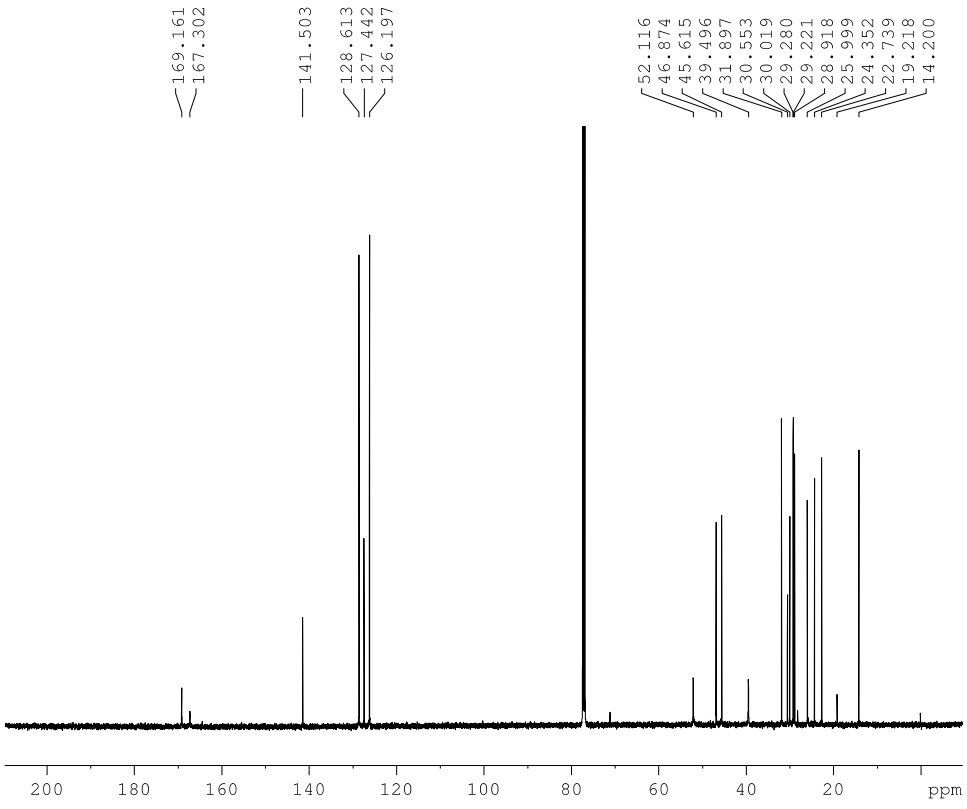


***S*-Octyl (3-oxo-1-phenyl-3-(pyrrolidin-1-yl)propyl)carbamothioate (4e)**

(^{1}H NMR)

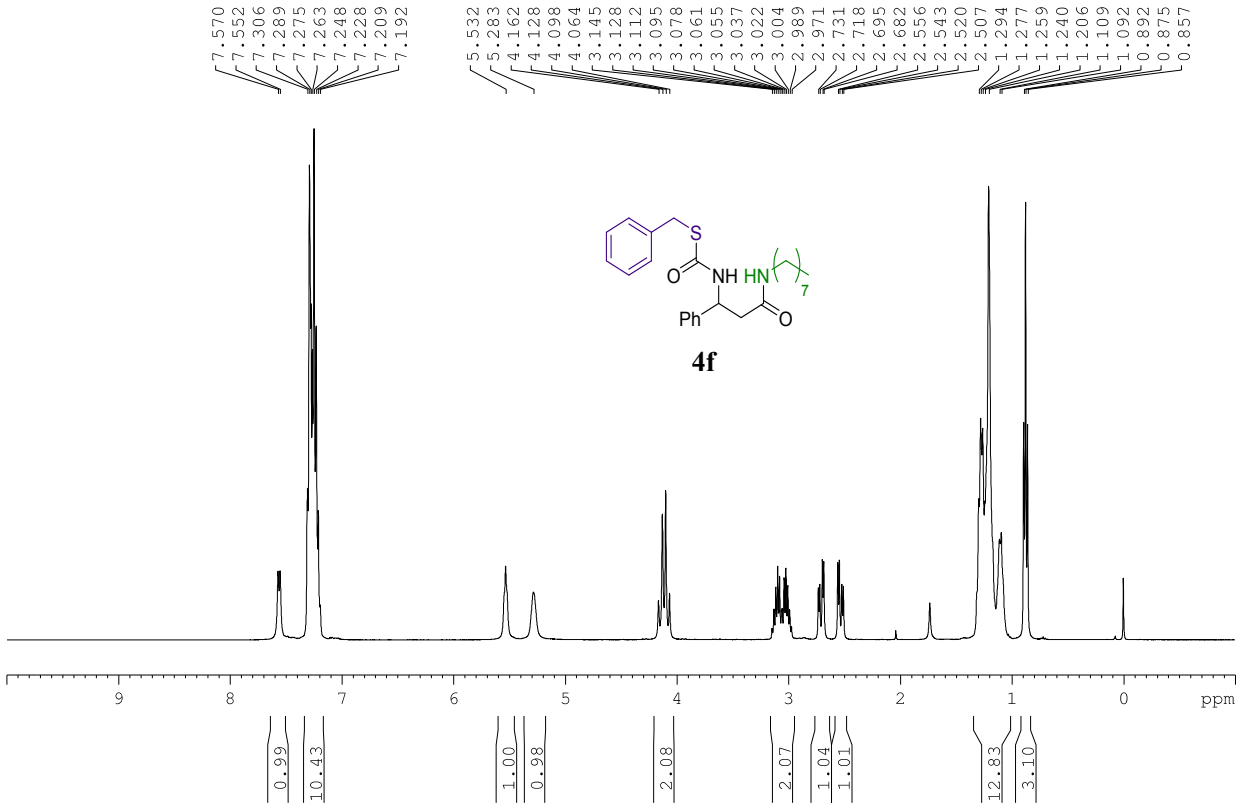


(^{13}C NMR)

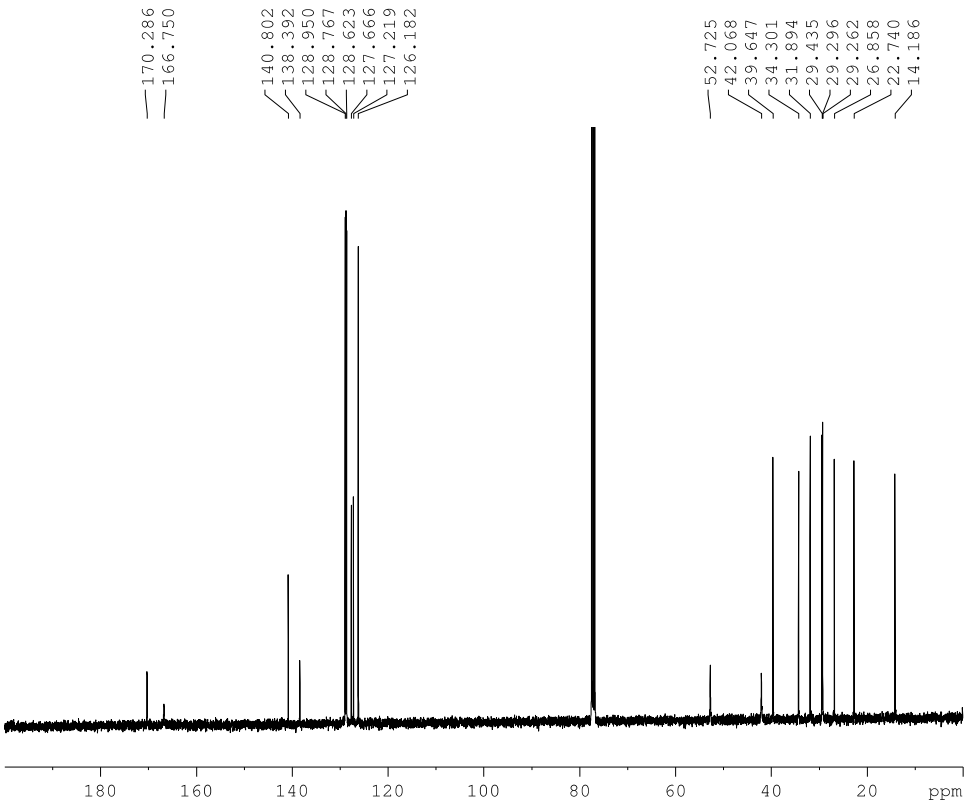


***S*-Benzyl (3-(octylamino)-3-oxo-1-phenylpropyl)carbamothioate (4f)**

(^{1}H NMR)

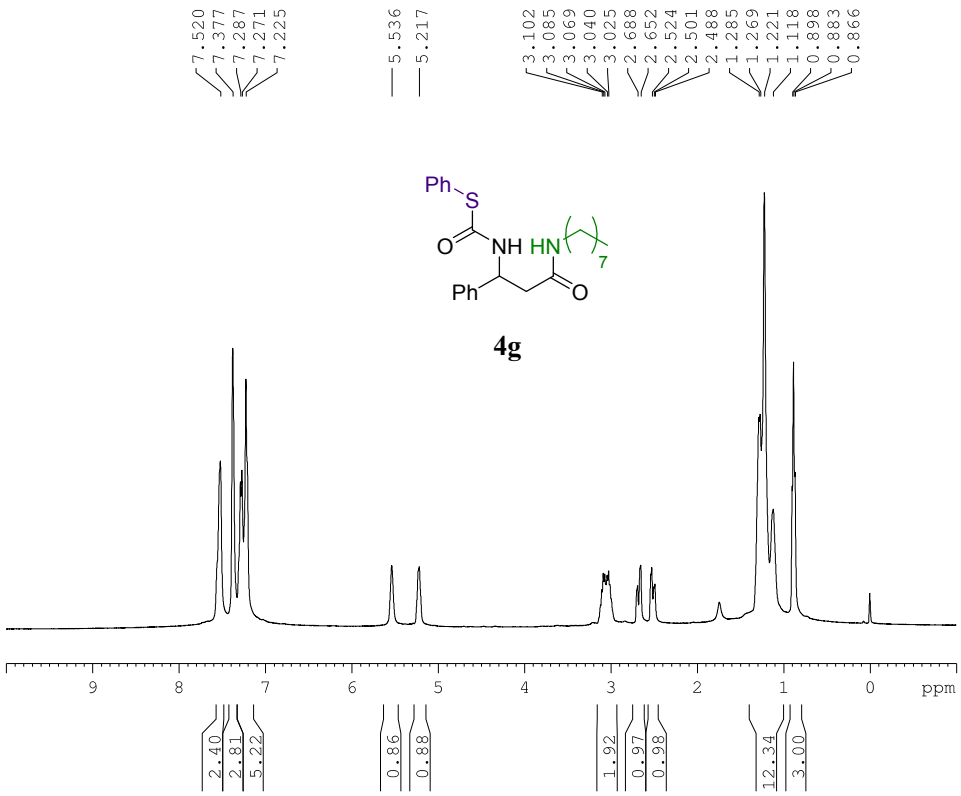


(^{13}C NMR)

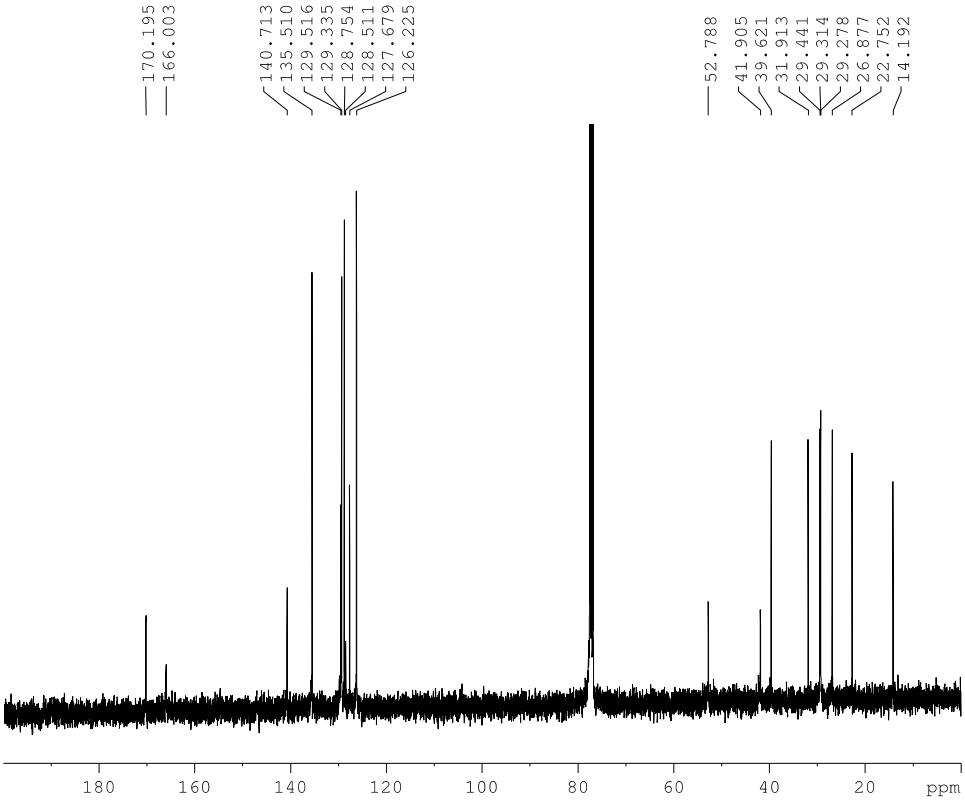


***S*-Phenyl (3-(octylamino)-3-oxo-1-phenylpropyl)carbamothioate (4g)**

(^{1}H NMR)

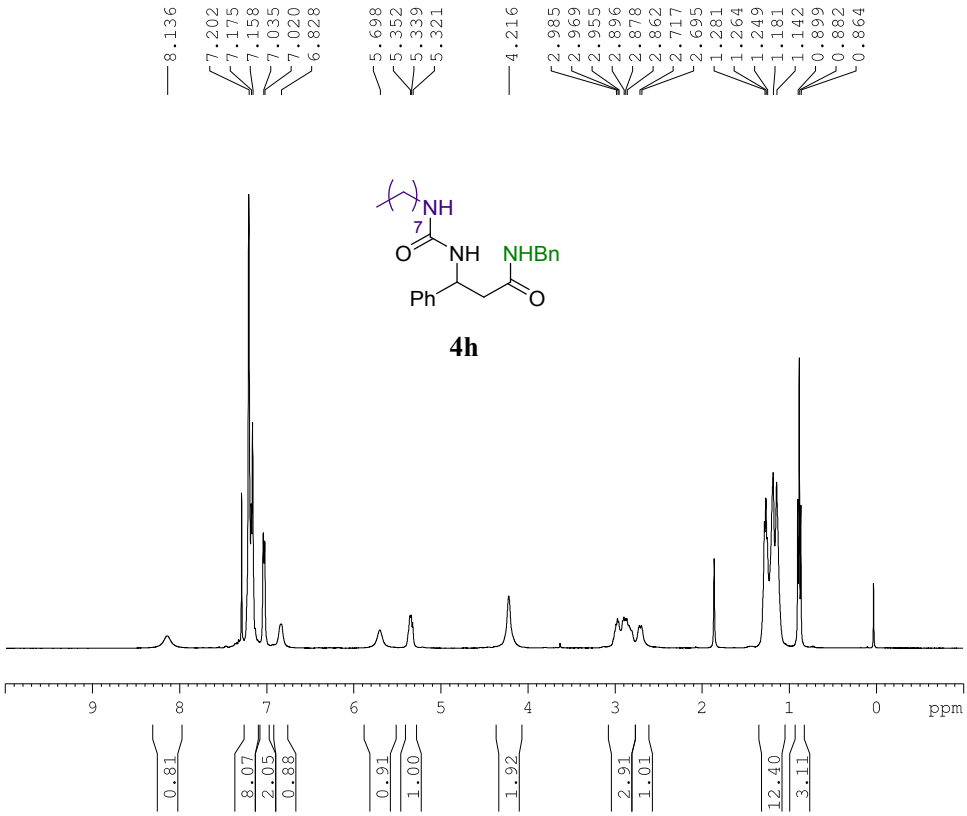


(^{13}C NMR)



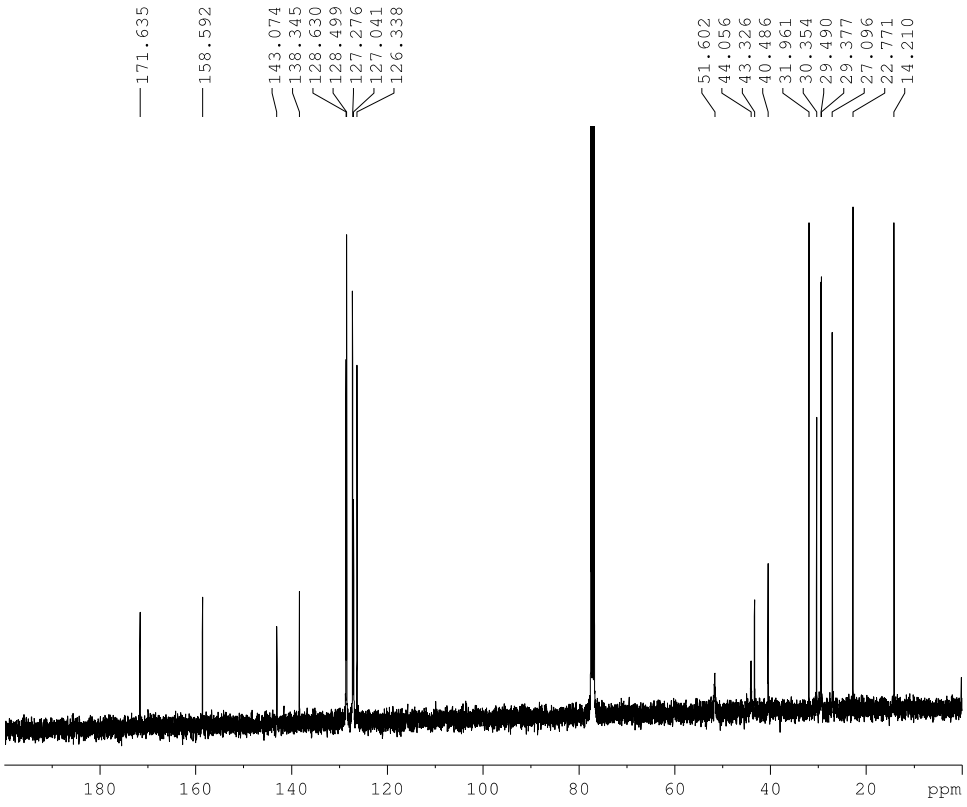
***N*-Benzyl-3-(3-octylureido)-3-phenylpropanamide (4h)**

(^{1}H NMR)



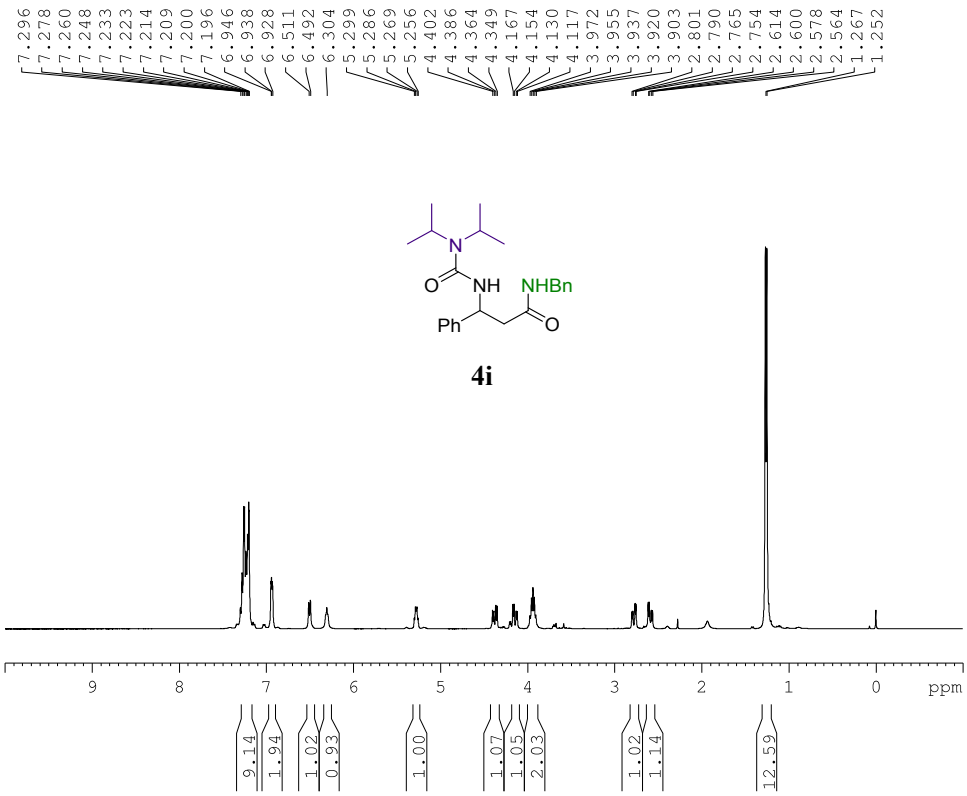
4h

(^{13}C NMR)

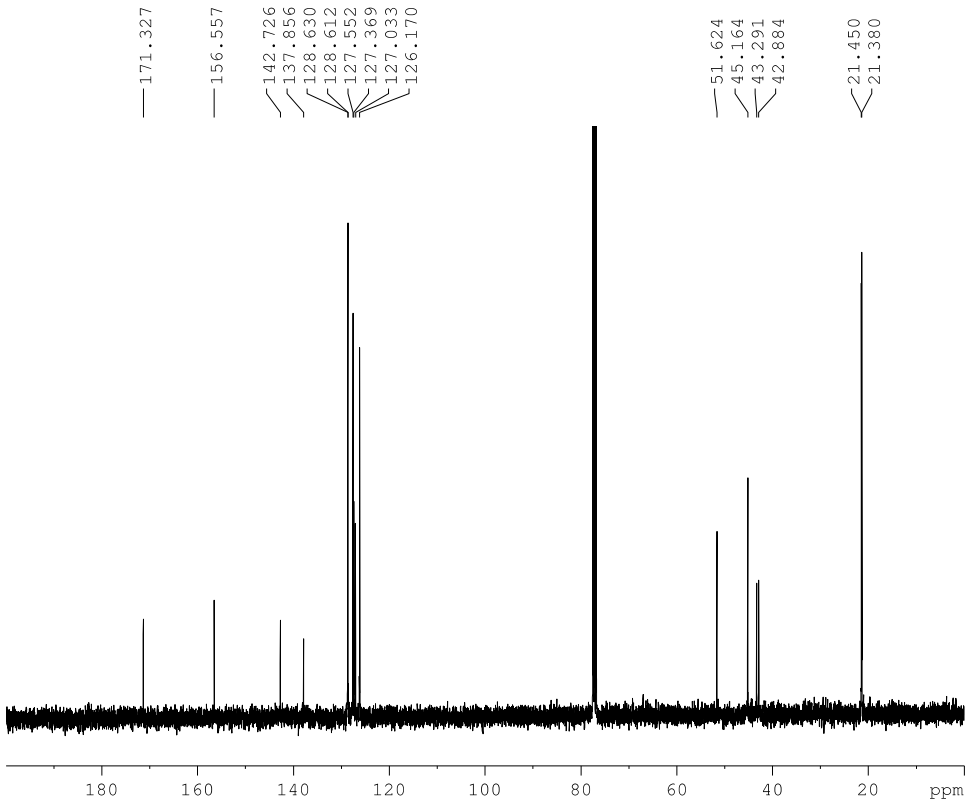


***N*-Benzyl-3-(3,3-diisopropylureido)-3-phenylpropanamide (4i)**

(^{1}H NMR)

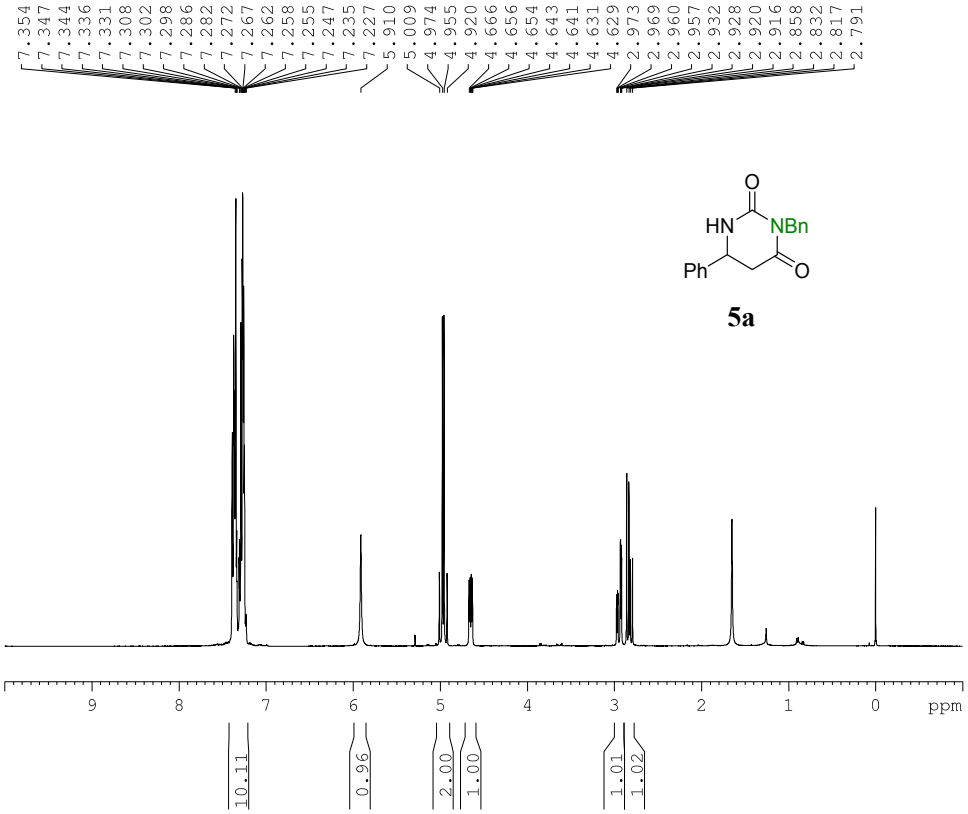


(^{13}C NMR)

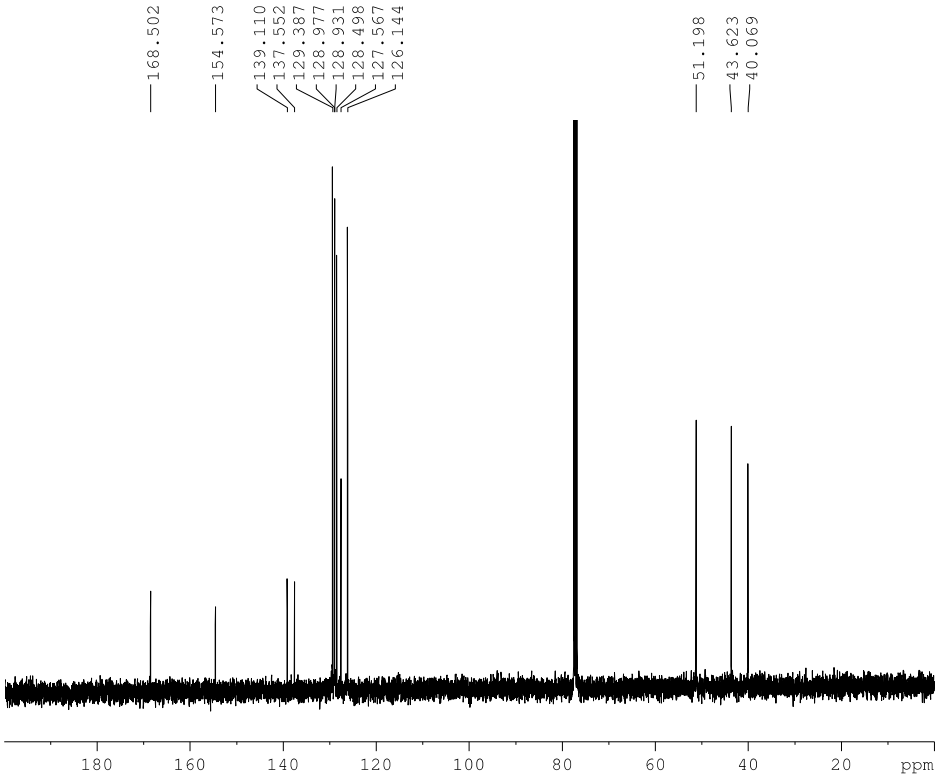


3-Benzyl-6-phenyldihydropyrimidine-2,4(1*H*,3*H*)-dione (5a)

(^{1}H NMR)

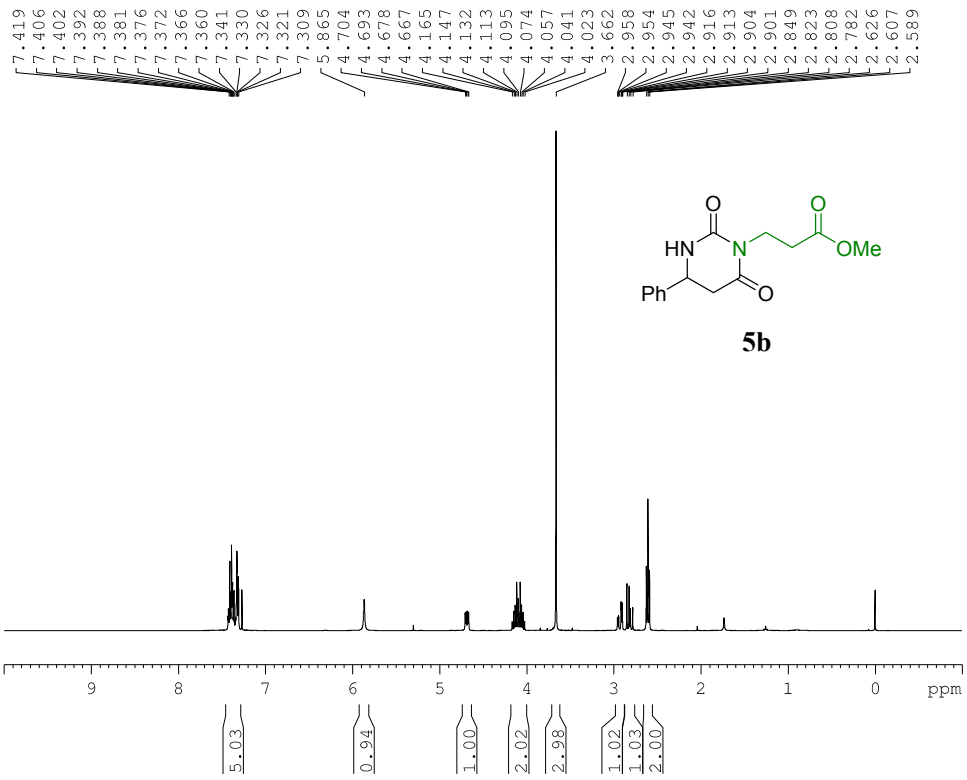


(^{13}C NMR)



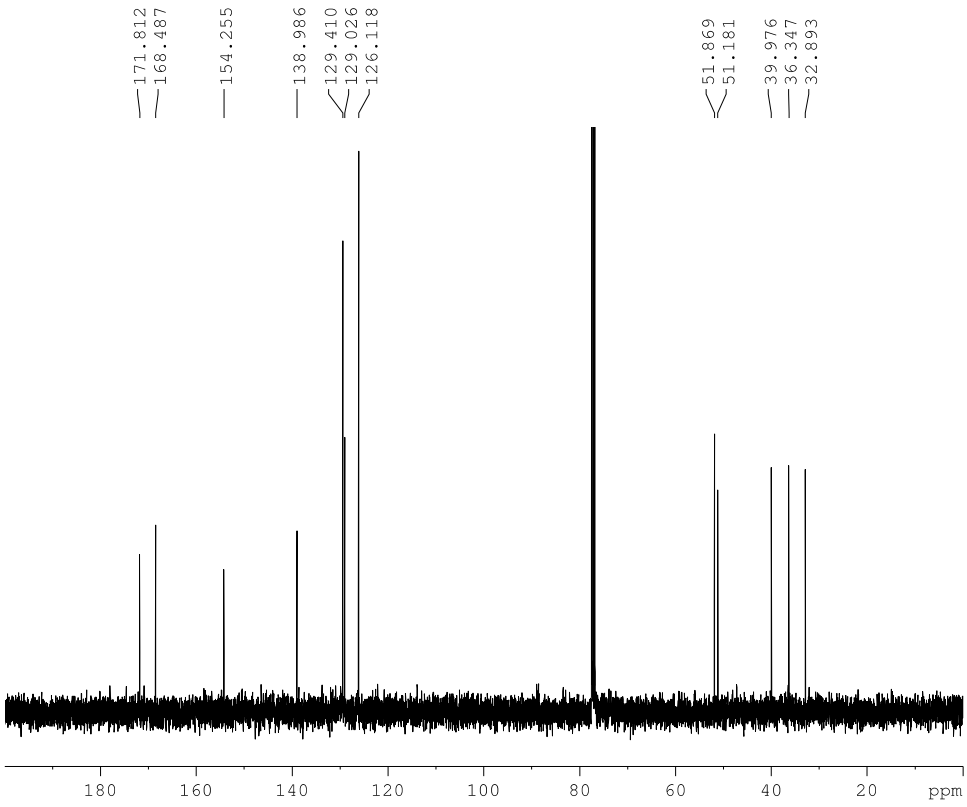
Methyl 3-(2,6-dioxo-4-phenyltetrahydropyrimidin-1(2*H*)-yl)propanoate (5b)

(^{1}H NMR)



5b

(^{13}C NMR)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154.255
138.986
129.410
129.026
126.118
51.869
51.181
39.976
36.347
32.893
180
160
140
120
100
80
60
40
20
ppm