Electronic Supplementary Information

Design, Synthesis, and Complementary Recognition of β-Hairpin Peptides Stabilized by Artificial DNA Base-Pairing Amino Acids

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Contents

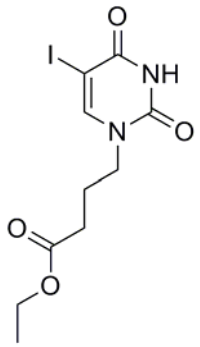
Experimental Section

General. Column chromatography was carried out on silica gel (Kanto Kagaku, silica gel 60N). ^{1}H, ^{13}C, and COSY spectra were recorded on JEOL JNM-AL300 spectrometers using $CDCl_3$ or DMSO-d_6 as a solvent, and tetramethylsilane was used as an internal standard. Chemical shifts (δ) were expressed in parts per million downfield from tetramethylsilane. ROESY, ^{1}H, and COSY spectra for peptide conformation analyses were recorded on Bluker BioSpin DRX-600 spectrometer using $CDCl_3$ as a solvent. High resolution electrospray ionization (HR-ESI) mass spectra were obtained on a mass spectrometer equipped in the LCMS-IT-TOF system (Shimazu, Co.). CD spectra were measured on a JASCO J-820 spectropolarimeter. IR spectra were measured by on a Nicolet 6700 FT-IR spectrometer. X-ray crystallographic analysis was made on a Bruker SMART APEX CCDarea-detecter with graphite monochromated Mo-Kα radiation.

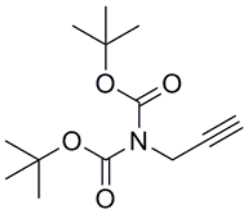
Material. All solvents and reagents were commercially available (from Nakarai Tesque, Sigma-Aldrich Co. or Wako Pure Chemical Industries, Ltd.) and used without further purification.

Titration experiments for analysing the equilibrium constant (*K*a) between T-derivatives and DAPs. Titration samples were prepared with the dilution of $CDCl_3$ solution of T-derivatives (compound **7**: 5.00×10^{-4} M or **9**: 3.00×10^{-3} M , 750 μL) with **DAP** solution (3.00×10^{-3} M , 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 μL). ^{1}H NMR spectra were measured for the several samples at RT. Downfield shifts of imide proton of T-derivatives were observed with increasing of **DAP** contents (Figure S3). This downfield shift shows the molecular complex formation between T-derivaties and **DAP** based on complementary hydrogen bonds. Both of the titration curves were fit to a 1:1 binding isotherm with GAsFit program,[1,2] which gave binding constants. The titration and fitting curves of compound **7** and **9** with **DAP** were shown in Figure S4 and S5.

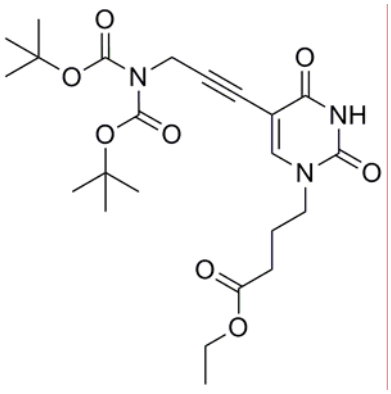
Computational methods. Conformational search (semi-empirical, PM_3) and structural optimization (DFT, B3LYP/6-31G*) was carried out using the Spartan 08[3] program.



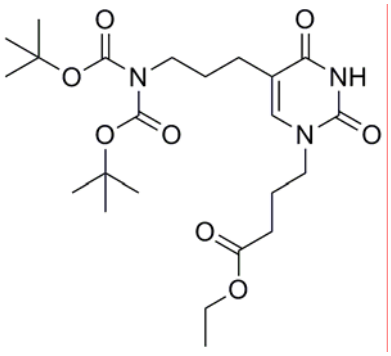
Ethyl 4-(5-iodo-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)butanoate (1). To a suspension of NaOH (0.960 g, 24.0 mmol) in DMF (200 mL) was added 5-iodouracil (4.76 g, 20.0 mmol) and stirred at 80 °C for 24 h. The redish solution was evaporated and filtered to afford brown oil. The residue was subjected to SiO_2 column chromatography, where major fraction was collected to afford yellow solid of **1** (3.67 g, 52%). ^{1}H NMR (300.4 MHz, DMSO-d_6): δ 1.17 (t, *J* = 7.1 Hz, 3H, -CH_2CH_3), 1.83 (tt, *J* = 6.8, 6.8 Hz, 2H, -$CH_2CH_2CH_2$-), 2.32 (t, *J* = 7.2 Hz, 2H, -CH_2CH_2CO-), 3.70 (t, *J* = 6.8 Hz, 2H, -NCH_2CH_2-), 4.01 (q, *J* = 7.1 Hz, 2H, -OCH_2CH_3), 8.16 (s, 1H, -NC*H*=C-), 11.6 (s, 1H, -CON*H*CO-). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 14.1 (-CH_2CH_3), 23.7 (-$CH_2CH_2CH_2$-), 30.4 (-CH_2CH_2CO-), 47.2 (-NCH_2CH_2-), 59.9 (-OCH_2CH_3), 68.1 (-CO*C*I=CH-), 149.9 (-C=C*H*N-), 150.7 (-NH*C*ON-), 161.1 (-CI*C*ONH-), 172.2 (-CH_2*C*OO-). ESI-MS (HR) *m/z* calcd for $C_{10}H_{13}IN_2O_4$ 374.9818 $(M+Na)^+$, found 374.9763.



***N*,*N*-Bis(*t*-butoxycarbonyl)-2-propynamine (2).** To a suspension of di-*t*-butylaimidodicarboxylate (8.69 g, 40.0 mmol) and cesium carbonate (15.6 g, 48.0 mmol) in DMF (200 mL) was added propargylbromide solution in toluene (80 wt%, 4.90 mL) under nitrogen atmosphere. The heterogeneous solution was stirred at RT for 12 h. The mixture solution was evaporated, and filtered to remove solid precipitates. The residue was subjected to SiO_2 column chromatography, where major fraction was collected to afford pale yellow liquid of **2** (9.83 g, 96%). ^{1}H NMR (300.4 MHz, $CDCl_3$): δ 1.53 (s, 18H, -C(CH_3)$_3$), 2.24 (s, 1H, -C≡C*H*), 4.35 (s, 2H, -NCH_2C-). ^{13}C NMR (75.45 MHz, $CDCl_3$): δ 27.6, 35.3, 70.3, 79.2, 82.5, 151.1. ESI-MS (HR) *m/z* calcd for $C_{26}H_{42}N_2O_8$ 533.2839 $(2M+Na)^+$, found 533.2780.

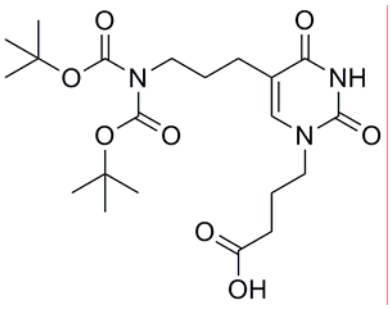


Ethyl 4-(5-(3-(bis(*t*-butoxycarbonyl)amino)prop-1-ynyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)butanoate (5-I-U3-OEt, 3). To a solution of **1** (1.06 g, 3.00 mmol), **2** (1.53 g, 6.00 mmol), copper iodide (0.457 g, 2.40 mmol), and triethylamine (0.911 g, 9.00 mmol) in THF (30.0 mL) was added tetrakis(triphenylphosphine)palladium (0) (0.347 g, 0.300 mmol). The mixture was stirred at RT for 12 h. The resulting mixture was evaporated to remove solvent. The residue was subjected to SiO_2 column chromatography, where major fraction was collected to afford **3** (1.18 g, 82%). ^{1}H NMR (300.4 MHz, $CDCl_3$): δ 1.27 (t, *J* = 7.2 Hz, 3H, -CH_2CH_3), 1.54 (s, 18H, -$C(CH_3)_3$), 2.01 (t, *J* = 7.2 Hz, 2H, -$CH_2CH_2CH_2$-), 2.37 (t, *J* = 7.1 Hz, 2H, -CH_2CH_2CO-), 3.80 (t, *J* = 7.1 Hz, 2H, -NCH_2CH_2-), 4.15 (q, *J* = 7.2 Hz, 2H, -OCH_2CH_3), 4.57 (s, 2H, -NCH_2C-), 7.44 (s, 1H, -C=CHN-), 8.32 (br, 1H, -CONHCO-). ^{13}C NMR (75.45 MHz, $CDCl_3$): δ 14.2 (-CH_2CH_3), 24.1 (-$CH_2CH_2CH_2$-), 28.0 (-$C(CH_3)_3$), 30.6 (-CH_2CH_2CO-), 36.6 (-NCH_2C-), 48.3 (-NCH_2CH_2-), 60.8 (-OCH_2CH_3), 72.8 (-$CH_2C{\equiv}C$-), 83.2 (-$OC(CH_3)_3$), 90.3 (-C≡*C*C-), 99.8 (-CO*C*(C≡)=CH-), 147.1 (-C=*C*HN-), 149.5 (-NH*C*ON-), 151.6 (-O*C*ON-), 161.2 (-C(C≡)*C*ONH-), 172.3 (-CH_2COO-). ESI-MS (HR) *m/z* calcd for $C_{23}H_{33}N_3O_8$ 502.2165 $(M+Na)^+$, found 502.2088.



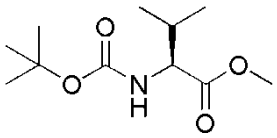
Ethyl 4-{5-(3-(di-*t*-butoxycarbonylamino)propyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl}butanoate ($(Boc)_2$-Taa3-OEt, 4). A suspension of **3** (1.10 g, 2.30 mmol), NBSH (2.00 g, 9.20 mmol), and triethylamine (1.86 g, 18.4 mmol) in CH_2Cl_2 (23.0

mL) was stirred at RT for 2 d. The solution was washed with saturated aqueous $NaHCO_3$ solution (50.0 mL × 2), H_2O (50.0 mL), Brine (50.0 mL), and dried over Na_2SO_4. The obtained residue was subjected to SiO_2 column chromatography, where major fraction was collected to afford yellow oil of **4** (0.523 g, 47%). ^{1}H NMR (300.4 MHz, $CDCl_3$): δ 1.26 (t, *J* = 7.2 Hz, 3H, -CH_2CH_3), 1.51 (s, 18H, -$C(CH_3)_3$), 1.81 (tt, *J* = 7.3, 7.3 Hz, 2H, -$CH_2CH_2CH_2$-), 2.02 (tt, *J* = 7.0, 7.0 Hz, -$CH_2CH_2CH_2$-), 2.25-2.45 (m, 4H, -CH_2CH_2CO-, -CH_2CH_2C-), 3.59 (t, *J* = 7.3 Hz, 2H, $(Boc)_2NCH_2CH_2$-), 3.78 (t, *J* = 7.2 Hz, 2H, -NCH_2CH_2-), 4.14 (q, *J* = 7.1 Hz, 2H, -OCH_2CH_3), 7.13 (s, 1H, -C=CHN-), 8.40 (br, 1H, -CONHCO-). ^{13}C NMR (75.45 MHz, $CDCl_3$): δ 14.2 (-CH_2CH_3), 23.8 (-$CH_2CH_2CH_2$-), 24.2 (-$CH_2CH_2CH_2$-), 27.2 (-CH_2CH_2C-), 28.1 (-$C(CH_3)_3$), 30.8 (-CH_2CH_2CO-), 45.5 ($(Boc)_2NCH_2CH_2$-), 47.7 (-NCH_2CH_2-), 60.8 (-OCH_2CH_3), 82.3 (-$OC(CH_3)_3$), 113.9 (-$CO C^5(CH_2$-)=CH-), 140.7 (-C=C^6HN-), 150.6 (-NCONH-), 152.8 (-OCON-), 163.4 (-CCONH-), 172.5 (-CH_2COO-). ESI-MS (HR): *m/z* calcd. for $C_{23}H_{37}N_3O_8$ $[M+Na]^+$ 506.2478, found 506.2394.



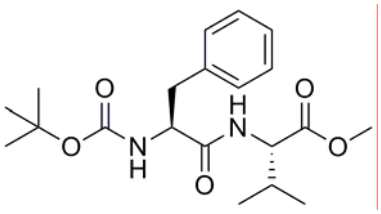
4-(5-(3-(Bis(*t*-butoxycarbonyl)amino)propyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)butanoic acid ($(Boc)_2$-Taa_3-OH, 5). A solution of **4** (2.13 g, 4.44 mmol) in MeOH (25.0 mL) was added aqueous 1 M NaOH solution (25.0 mL), and stirred at RT for 2 h. The solution was neutralized with DOWEX® 50WX4-50 to pH 7.0. After filtering ion-exchange resins, the solution was extracted with CH_2Cl_2 (30.0 mL × 3). The combined organic layer was washed with Brine (50.0 mL), and dried over Na_2SO_4. The solution was filtered, and evaporated to afford white solid of **5** (1.39 g, 70%). ^{1}H NMR (300.4 MHz, $CDCl_3$): δ 1.50 (s, 18H, -$C(CH_3)_3$), 1.81 (tt, *J* = 7.3, 7.3 Hz, 2H, -$CH_2CH_2CH_2$-), 2.08 (t, *J* = 6.2 Hz, -$CH_2CH_2CH_2$-), 2.32 (t, *J* = 7.0 Hz, 2H, -$CH_2CH_2C^5$-), 2.45 (br, 2H, -CH_2CH_2CO-), 3.58 (t, *J* = 7.6 Hz, 2H, $(Boc)_2NCH_2CH_2$-), 3.83 (t, *J* = 6.3 Hz, 2H, -NCH_2CH_2-), 7.13 (s, 1H, -C=CHN-), 8.87 (br, 1H, -CONHCO-). ^{13}C NMR (75.45 MHz, $CDCl_3$): δ 23.6 (-$CH_2CH_2CH_2$-), 23.8 (-$CH_2CH_2CH_2$-), 27.1 (-CH_2CH_2C-), 28.1 (-$C(CH_3)_3$), 30.7 (-CH_2CH_2CO-), 45.4 ($(Boc)_2NCH_2CH_2$-), 48.0 (-NCH_2CH_2-), 82.5 (-$OC(CH_3)_3$), 113.9 (-$CO C^5(CH_2$-)=CH-), 141.0 (-C=C^6HN-), 151.0 (-NCONH-), 152.9 (-OCON-), 163.9 (-CCONH-), 175.5 (-CH_2COOH). ESI-MS (HR): *m/z* calcd. for

$C_{21}H_{33}N_3O_8$ $[M+Na]^+$ 478.2165, found 478.2000.



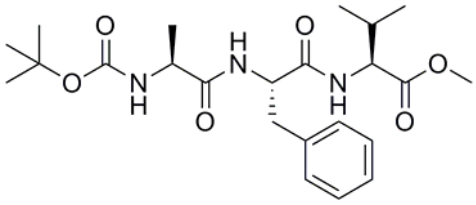
Boc-Val-OMe. Into a solution of Boc-Val-OH (4.78 g, 22.0 mmol), DCC (4.95 g, 20.0 mmol), DMAP (0.269 g, 2.20 mmol) in CH_2Cl_2 (200 mL) was added MeOH (0.641 g, 20.0 mmol) dropwise at 0 ºC and stirred for 1 h. After warming to RT, the solution was stirred further 3 h. The solution was filtered to remove DCUrea, and evaporated. The colorless residue was subjected to SiO_2 flash column chromatography, where major fraction was collected to afford colorless liquid of **Boc-Val-OMe** (4.62 g, 99%). ^{1}H NMR ($CDCl_3$, 300.4 MHz): δ 0.82 (d, *J* = 7.0 Hz, 3H, -CH(C*H*$_3$)$_2$), 0.89 (d, *J* = 6.9 Hz, 3H, -CH(C*H*$_3$)$_2$), 1.38 (s, 9H, -C(C*H*$_3$)$_3$), 2.05 (m, 1H, -CHC*H*(CH$_3$)$_2$), 3.67 (s, 3H, -OC*H*$_3$), 4.15 (dd, *J* = 8.9, 4.9 Hz, 1H, -NHCHCO-), 4.95 (d, *J* = 7.9 Hz, 1H, -CON*H*CH-). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 17.6, 18.9, 28.3, 31.3, 52.0, 58.5, 79.7, 155.7, 172.9. ESI-MS (HR) *m/z* calcd for $C_{22}H_{42}N_2O_8$ 485.2839 $(2M+Na)^+$, found 485.2855.

Peptide synthetic procedures. A solution of Boc-X-OMe (1.00 mmol) in CH_2Cl_2 (8.00 mL) was added TFA (2.00 mL), and stirred at RT for 30 min. The solution was neutralized with aqueous 1 M NaOH solution to pH 7.0. The organic layer was washed with aqueous sat. $NaHCO_3$ solution (50.0 mL), water (50.0 mL), Brine (50.0 mL), and dried over Na_2SO_4. After evaporation, the deprotected amine was dissolved into CH_2Cl_2 (10.0 mL), followed by triethylamine (1.20 mmol), Boc-Y-OH (1.10 mmol), DCC (1.20 mmol), and stirred at 0 ºC for 1h. The solution was warmed to RT, and stirred for further 12 h. The mixture solution was washed with aqueous 1 M HCl solution (50.0 mL), aqueous sat. $NaHCO_3$ solution (50.0 mL), Brine (50.0 mL), and dried over Na_2SO_4. The residue was purified by SiO_2 column chromatography to afford Boc-YX-OEt.

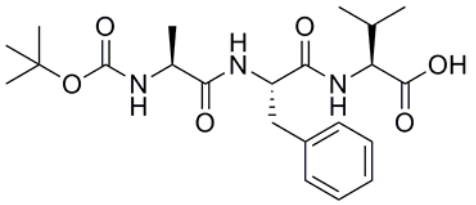


Boc-PheVal-OMe. Yield in 44%. ^{1}H NMR ($CDCl_3$, 300.4 MHz): δ 0.84 (d, *J* = 7.0 Hz, 3H, -CH(C*H*$_3$)$_2$), 0.87 (d, *J* = 7.0 Hz, 3H, -CH(C*H*$_3$)$_2$), 1.42 (s, 9H, (C*H*$_3$)$_3$C-), 3.08 (d, *J* = 6.9 Hz, 2H, -CHC*H*$_2$C$_6$H$_5$), 3.69 (s, 3H, -OC*H*$_3$), 4.34 (d, *J* = 7.1 Hz, 1H, -NHC*H*^{Phe}CO-), 4.46 (dd, *J* = 8.5, 5.1 Hz, 1H, -NHC*H*^{Val}CO-), 5.09 (br, 1H, -CON*H*CH^{Phe}-), 6.32 (d, *J* = 8.5 Hz, 1H, -CON*H*CH^{Val}-), 7.18-7.34 (m, 5H, -CH$_2$C$_6$H$_5$). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 17.7, 18.8, 28.2, 31.3, 52.0, 52.1, 57.2, 57.3, 126.9,

128.7, 129.2, 129.3, 153.1, 171.1, 171.7. ESI-MS (HR) *m/z* calcd for $C_{20}H_{30}N_2O_5$ 401.2052 $[M+Na]^+$, found 401.2012.

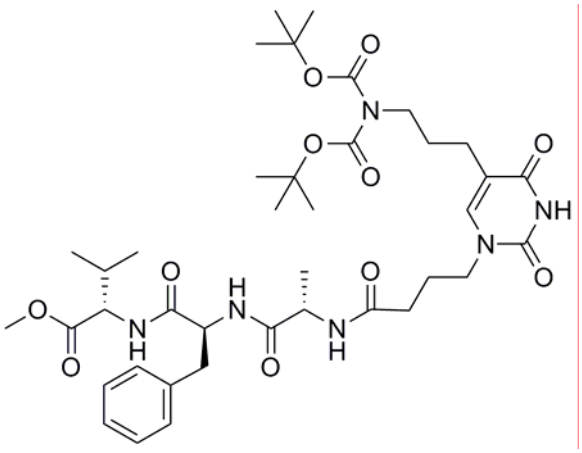


Boc-AlaPheVal-OMe (8). Yield in 42%. ^{1}H NMR ($CDCl_3$, 300.4 MHz): δ 0.82 (d, *J* = 7.0 Hz, 3H, -CH(CH_3)$_2$), 0.85 (d, *J* = 6.9 Hz, 3H, -CH(CH_3)$_2$), 1.30 (d, *J* = 7.1 Hz, 3H, -CHCH_3), 1.42 (s, 9H, -C(CH_3)$_3$), 2.00-2.18 (m, 1H, -CHC*H*(CH$_3$)$_2$), 3.09 (t, *J* = 7.0 Hz, 2H, -CHCH_2C$_6$H$_5$), 3.69 (s, 3H, -OCH_3), 4.12 (q, *J* = 7.1 Hz, 1H, -NHC*H*^{Ala}CO-), 4.42 (dd, *J* = 8.6, 5.2 Hz, 1H, -NHC*H*^{Val}CO-), 4.67 (td, *J* = 7.2, 7.2 Hz, 1H, -NHC*H*^{Phe}CO-), 4.90 (br, 1H, BocN*H*CH^{Ala}-), 6.38 (d, *J* = 6.9 Hz, -CON*H*CH^{Val}-), 6.71 (d, *J* = 7.7 Hz, 1H, -CON*H*CH^{Phe}-), 7.16-7.36 (m, 5H, -CH$_2$C$_6$*H*$_5$). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 17.8 (-CH(*C*H$_3$)$_2$) , 18.3 (-CH*C*H$_3$), 18.8 (-CH(*C*H$_3$)$_2$), 28.2 (-C(*C*H$_3$)$_3$), 31.1 (-CH*C*H(CH$_3$)$_2$), 37.8 (-CH*C*H$_2$C$_6$H$_5$), 50.3 (-NH*C*H^{Ala}CO-), 52.1 (-O*C*H$_3$), 54.3 (-NH*C*H^{Phe}CO-), 57.4 (-NH*C*H^{Val}CO-), 80.3 (-O*C*(CH$_3$)$_3$), 127.0 (-CH$_2$*C*$_6$H$_5$), 128.6 (-CH$_2$*C*$_6$H$_5$), 129.3 (-CH$_2$*C*$_6$H$_5$), 136.3 (-CH$_2$*C*$_6$H$_5$), 155.3 (-O*C*ONH-), 170.4 (-CH*C*ONH-), 171.6 (-CH*C*ONH-), 172.5 (-CH*C*OO-). ESI-MS (HR) *m/z* calcd for $C_{23}H_{35}N_3O_6$ 472.2424 $[M+Na]^+$, found 472.2176.

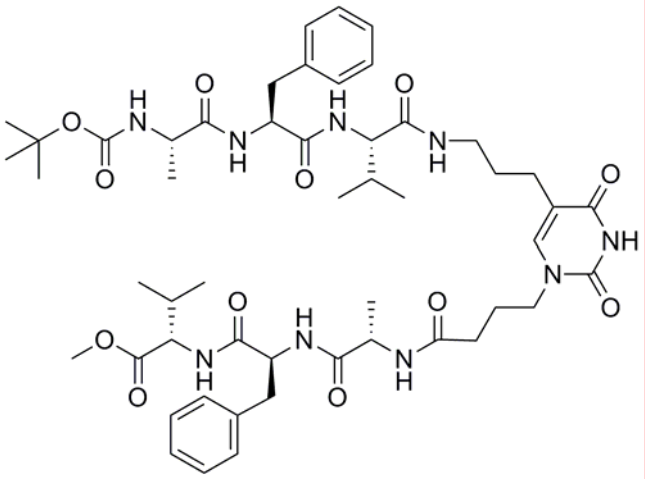


Boc-AlaPheVal-OH. This compound was deprotected with the same procedure of **(Boc)$_2$-Taa$_3$-OH** (**6**). Yield in 97%. ^{1}H NMR ($CDCl_3$, 300.4 MHz): δ 0.89 (d, *J* = 6.9 Hz, 3H, -CH(CH_3)$_2$), 0.92 (d, *J* = 6.9 Hz, 3H, -CH(CH_3)$_2$), 1.30 (d, *J* = 6.9 Hz, 3H, -CHCH_3), 1.42 (s, 9H, -C(CH_3)$_3$), 2.06-2.32 (m, 1H, -CHC*H*(CH$_3$)$_2$), 3.00-3.22 (m, 2H, -CHCH_2C$_6$H$_5$), 4.16 (br, 1H, -NHC*H*^{Ala}CO-), 4.37 (br, 1H, -NHC*H*^{Val}CO-), 4.76 (td, *J* = 6.9, 7.6 Hz, 1H, -NHC*H*^{Phe}CO-), 5.02 (br, 1H, BocN*H*CH^{Ala}-), 6.76 (d, *J* = 7.5 Hz, -CON*H*CH^{Val}-), 7.05 (d, *J* = 7.7 Hz, 1H, -CON*H*CH^{Phe}-), 7.15-7.35 (m, 5H, -CH$_2$C$_6$*H*$_5$). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 17.9 (-CH*C*H$_3$), 18.4 (-CH(*C*H$_3$)$_2$), 18.9 (-CH(*C*H$_3$)$_2$), 28.3 (-C(*C*H$_3$)$_3$), 30.5 (-CH*C*H(CH$_3$)$_2$), 37.9 (-CH*C*H$_2$C$_6$H$_5$), 54.4 (-NH*C*H^{Ala}CO-), 54.5 (-NH*C*H^{Phe}CO-), 58.2 (-NH*C*H^{Val}CO-), 127.1 (-CH$_2$*C*$_6$H$_5$), 128.7 (-CH$_2$*C*$_6$H$_5$), 129.3 (-CH$_2$*C*$_6$H$_5$), 136.2 (-CH$_2$*C*$_6$H$_5$), 155.6 (-O*C*ONH-), 171.2 (-CH*C*ONH-), 172.9

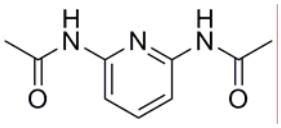
(-CH*C*ONH-), 173.1 (-CH*C*OO-). ESI-MS (HR) *m/z* calcd for $C_{22}H_{33}N_3O_6$ 458.2267 $[M+Na]^+$, found 458.2214.



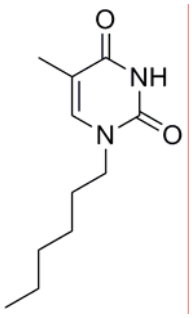
$(Boc)_2$-Taa$_3$AlaPheVal-OMe (6). Yield in 52%. ^{1}H NMR ($CDCl_3$, 300.4 MHz): δ 0.85 (d, *J* = 7.0 Hz, 3H, -CH(C*H*$_3$)$_2$), 0.88 (d, *J* = 7.0 Hz, 3H, -CH(C*H*$_3$)$_2$), 1.28 (d, *J* = 7.1 Hz, 3H, -CHC*H*$_3$), 1.50 (s, 18H, -C(C*H*$_3$)$_3$), 1.82 (tt, *J* = 7.1 Hz, -CH$_2$C*H*$_2$CH$_2$-), 1.88-2.02 (m, 1H, -CH$_2$C*H*$_2$CH$_2$-), 2.02-2.24 (m, 3H, -CH$_2$C*H*$_2$CH$_2$-, -CHC*H*(CH$_3$)$_2$), 2.24-2.42 (m, 4H, -CH$_2$C*H*$_2$CO-, -CH$_2$C*H*$_2$C-), 2.94-3.16 (m, 2H, -CHC*H*$_2$C$_6$H$_5$), 3.60 (t,. *J* = 7.4 Hz, 2H, (Boc)$_2$NC*H*$_2$CH$_2$-), 3.69 (s, 3H, -OCH$_3$), 3.76 (t, *J* = 6.2 Hz, 2H, -NC*H*$_2$CH$_2$-), 4.48 (dd, *J* = 8.6, 5.2 Hz, 1H, -NHC*H*^{Val}CO-), 4.52 (q, *J* = 7.2 Hz, 1H, -NHC*H*^{Ala}CO-), 4.76 (td, *J* = 7.4, 7.4 Hz, 1H, -NHC*H*^{Phe}CO-), 6.99 (d, *J* = 7.7 Hz, 1H, -CON*H*CH^{Ala}-), 7.04 (d, *J* = 8.7 Hz, 1H, -CON*H*CH^{Val}-), 7.10-7.27 (m, 6H, -CH$_2$C$_6$*H*$_5$, -C=C*H*N-), 8.01 (s, 1H, -CON*H*CO-). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 17.7, 17.9, 18.8, 23.8, 24.6, 27.1, 28.1, 31.1, 32.8, 37.3, 45.5, 47.7, 49.0, 52.1, 54.8, 57.4, 82.4, 114.6, 126.8, 128.5, 129.3, 136.8, 140.5, 151.7, 152.8, 163.3, 171.0, 171.4, 171.8, 172.4. ESI-MS (HR) *m/z* calcd for $C_{39}H_{58}N_6O_{11}$ 809.4061 $[M+Na]^+$, found 809.3634.



Boc-AlaPheValTaa$_3$AlaPheVal-OMe (7). The Boc group of peptide **6** (226 mg, 0.287 mmol) was deprotected as shown above to afford yellow residue. The residue, Boc-AlaPheVal-OH (126 mg, 0.290 mmol), EDCl (61.2 mg, 0.319 mmol), and HOSu (36.7 mg, 0.319 mmol) were dissolved into DMF (3.00 mL). The mixture solution was stirred at 0 ºC for 1 h, and at RT for further 12 h. The solvent was removed under vacuum to afford yellow oil. The oil was dissolved into CH_2Cl_2 (10.0 mL), washed with water (10.0 mL), Brine (10.0 mL), and dried over Na_2SO_4. The residue was purified by SiO_2 flash column chromatography. Further purification was carried out using recycling GPC to afford colorless solid of **7** (82.5 mg, 29%). ^{1}H NMR (MeOH-d_4, 300.4 MHz): δ 1.16 (d, J = 6.7 Hz, 12H, -CH(CH_3)$_2$), 1.34-1.62 (m, 6H, -CHCH_3), 1.66 (s, 9H, -C(CH_3)$_3$), 1.80-2.05 (m, 2H, -CH$_2$CH_2CH$_2$-), 2.14-2.43 (m, 4H, -CH$_2$CH_2CH$_2$-, -CHCH(CH$_3$)$_2$), 2.50 (t, J = 7.0 Hz, 2H, -CH$_2$CH_2CO-), 2.58 (t, J = 7.3 Hz, 2H, -CH$_2$CH_2C-), 3.10-3.50 (m, 4H, -CHCH_2C$_6$H$_5$), 3.93 (s, 3H, -OCH$_3$), 3.99 (t, J = 6.3 Hz, 2H, -NCH_2CH$_2$-), 4.17-4.42 (m, 2H, -NHCH^{Val}CO-), 4.44-4.64 (m, 2H, -NHCH^{Ala}CO-), 4.86-5.05 (m, 2H, -NHCH^{Phe}CO-), 7.30-7.57 (m, 10H, -CH$_2$C$_6$$H_5$), 7.64 (s, 1H, -C=C$H$N-). ^{13}C NMR (MeOH-$d_4$, 75.45 MHz): δ 18.0, 18.9, 19.0, 19.6, 24.9, 25.0, 25.9, 26.0, 28.3, 28.9, 31.8, 32.0, 32.6, 33.3, 36.1, 38.8, 40.5, 50.7, 52.7, 55.9, 59.5, 80.8, 114.7, 127.9, 129.8, 130.6, 138.5, 143.8, 144.1, 153.2, 166.5, 172.5, 173.4, 173.6, 175.1, 175.4. ESI-MS (HR) *m/z* calcd for $C_{51}H_{73}N_9O_{12}Na$ 1026.5276 $(M+Na)^+$, found 1026.4770.



***N*,*N'*-(pyridine-2,6-diyl)diacetamide.** This compound was synthesized based on literature procedure.[4]



1-Hexyl-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9). To a suspension of thymine (1.26 g, 10.0 mmol), NaOH (0.440 g, 11.0 mmol) in DMF (100 mL) was added 1-bromohexane (1.65 g, 10.0 mmol) and stirred at 80 °C for 24 h. The mixture solution was evaporated and filtered to afford colorless oil. The residue was subjected to SiO_2 column chromatography, where major fraction was collected to afford colorless solid of **9**

(1.43 g, 68%). ^{1}H NMR (300.4 MHz, $CDCl_3$): δ 0.89 (t, *J* = 6.3 Hz, 3H, -CH_2CH_3), 1.20-1.44 (m, 6H, -$CH_2CH_2CH_2$-), 1.55-1.78 (m, 2H, -$CH_2CH_2CH_2$-), 1.92 (s, 3H, -CCH_3), 3.68 (t, *J* = 7.4 Hz, 2H, -NCH_2CH_2-), 8.97 (s, 1H, -C=CHN-), 8.52 (br, 1H, -CONHCO-). ^{13}C NMR (75.45 MHz, $CDCl_3$): δ 12.3 (-CCH_3), 13.9 (-CH_2CH_3), 22.4 (-$CH_2CH_2CH_2$-), 26.1 (-$CH_2CH_2CH_2$-), 29.0 (-$CH_2CH_2CH_2$-), 31.3 (-$CH_2CH_2CH_2$-), 48.5 (-NCH_2CH_2-), 110.5 (-$COC(CH_3)$=CH-), 140.4 (-C=CHN-), 150.7 (-NHCON-), 164.0 (-CCONH-). ESI-MS (HR) *m/z* calcd for $C_{11}H_{18}N_2O_2$ 233.1266 $[M+Na]^+$, found 233.1246.

References

1. Blight, B. A.; Camara-Campos, A.; Djurdjevic, S.; Kaller, M.; Leign, D. A.; McMillan, F. M.; McNab, H.; Slawin, A. M. Z. *J. Am. Chem. Soc.* **2009**, *131*, 14116.
2. GAs-Fit: http://gasfit.djurdjevic.org.uk
3. Spartan version 08.1.0.0, Wavefunction, Inc., Irvine, CA, 2008.
4. Huang, M.-Y.; Yeh, C.-Y.; Lee, G.-H.; Peng, S.-M. *Dalton Trans.* **2006**, 5683-5690.

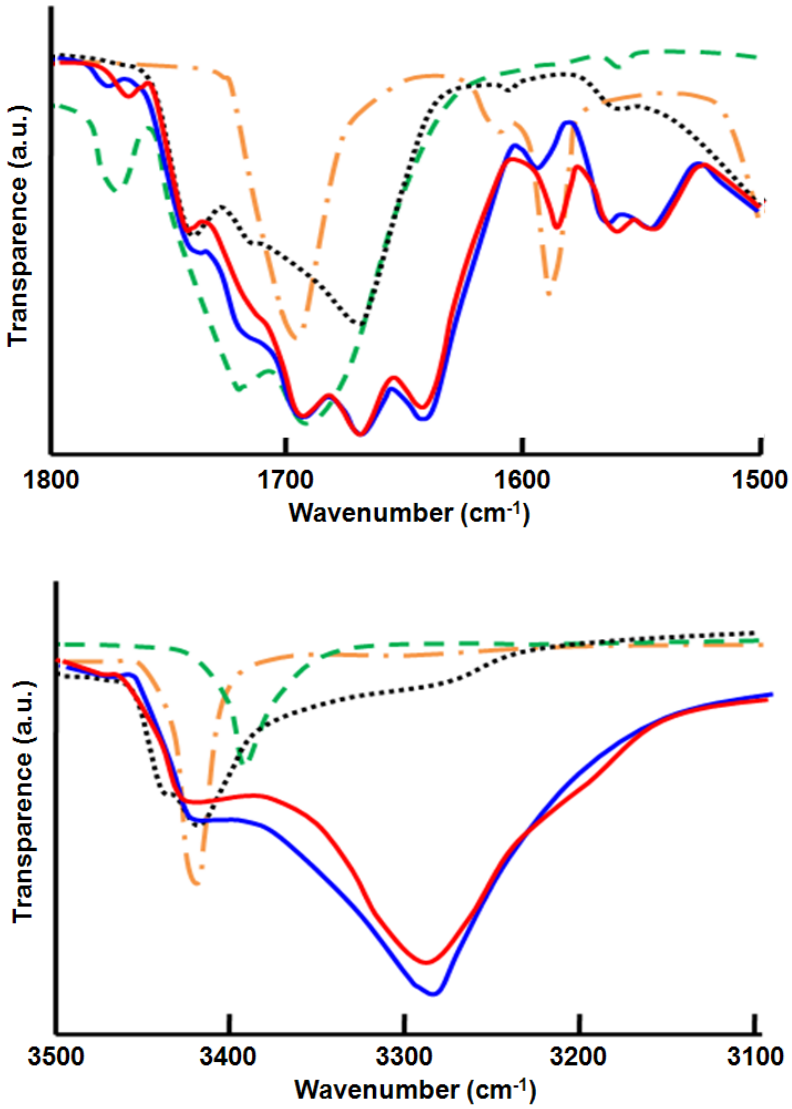


Figure S1. IR spectra of **4** (5.0×10^{-2} M, dashed green), **8** (1.0×10^{-1} M, dotted black), **DAP** (5.0×10^{-2} M, dash-dotted orange), **7** (5.0×10^{-2} M, blue), and **7**:**DAP** = 1:1 (2.5×10^{-2} M, red) in $CHCl_3$. (a) 1500-1800 cm^{-1} and (b) 3100-3500 cm^{-1} region.

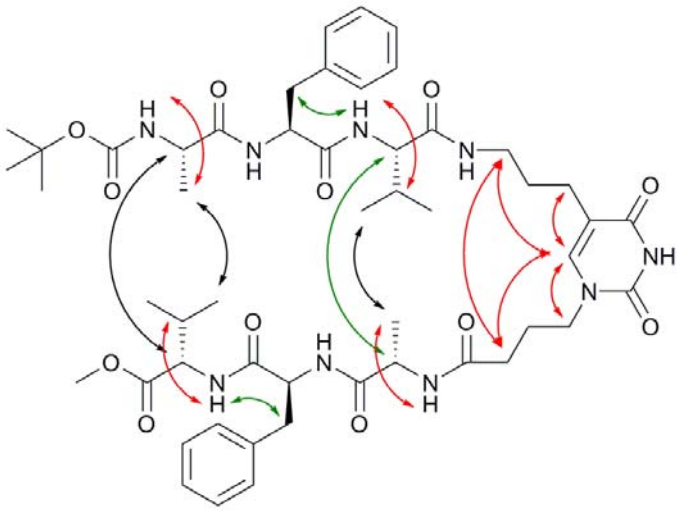


Figure S2. Observed NOEs (600 MHz) of peptide **7** in $CDCl_3$ (1.00×10^{-3} M). Red: strong NOEs, greens: medium NOEs, black: weak NOEs.

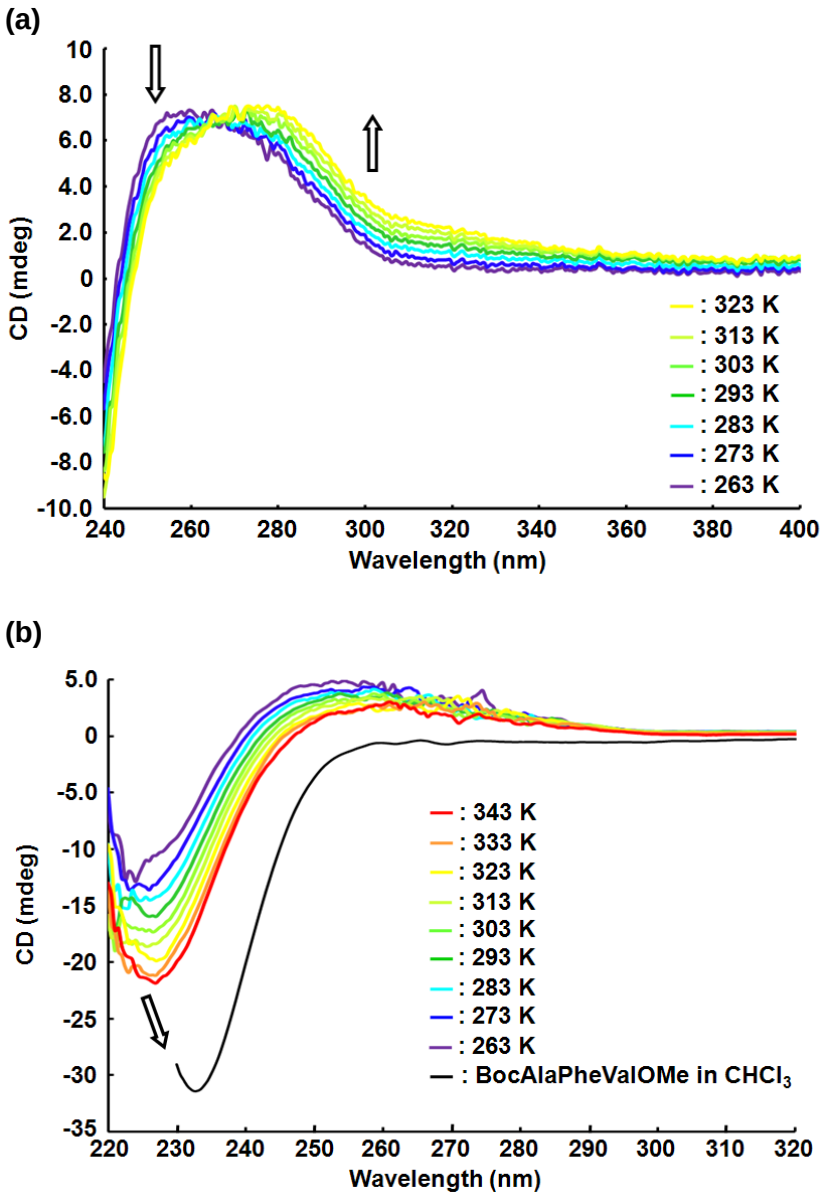


Figure S3. VT-CD spectra of peptide **7** (2.0×10^{-3} M) in $CHCl_3$ (a) and trifluoroethanol (b). CD spectrum of **8** (4.0×10^{-3} M) in $CHCl_3$ is shown for comparison.

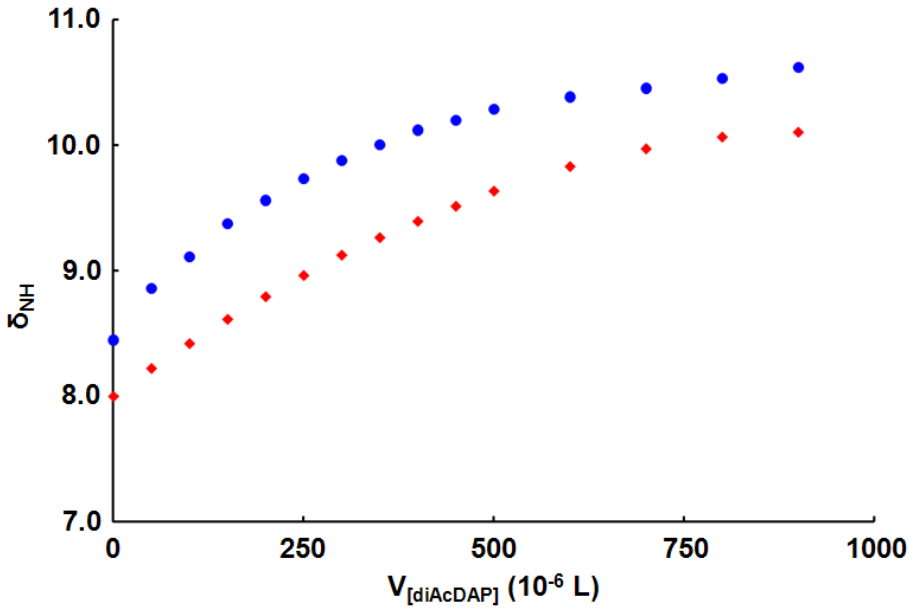
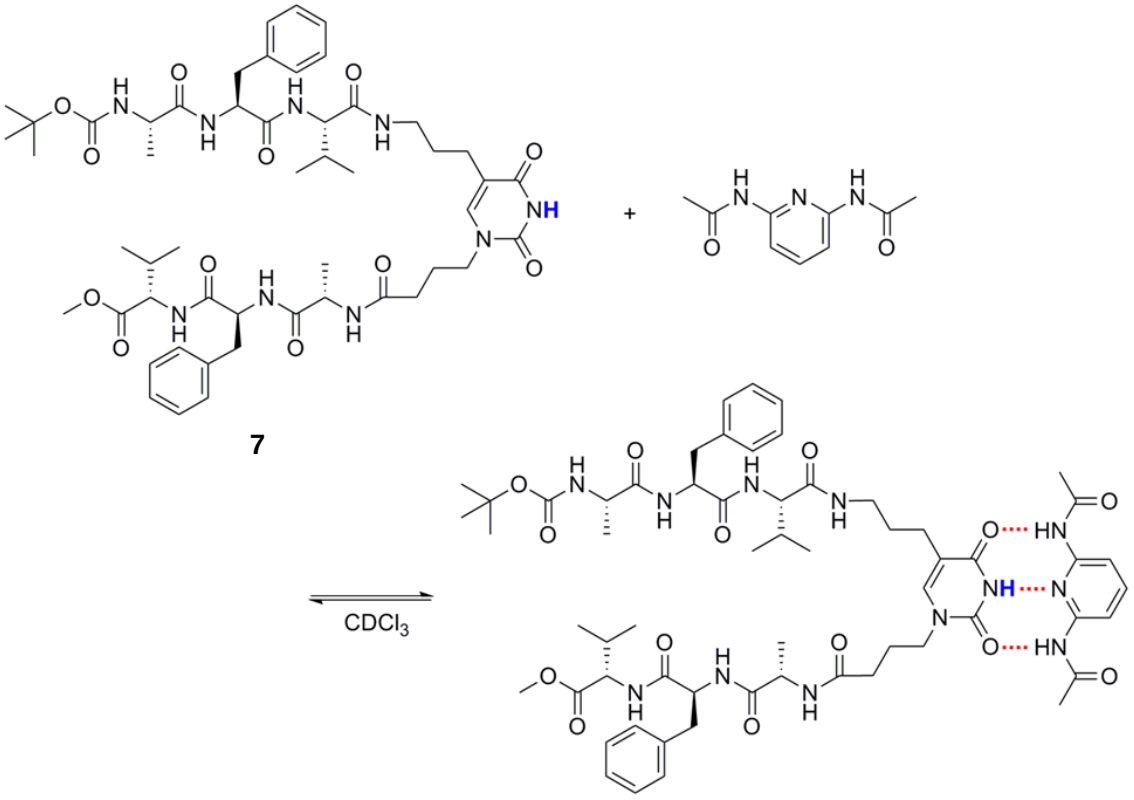


Figure S4. ^{1}H NMR (300 MHz) chemical shifts of imide protons of T-derivatives (**7**: blue, 5.00×10^{-4} M, **9**: red, 3.00×10^{-3} M) with the various contents of **DAP** (3.00×10^{-3} M) in $CDCl_3$.



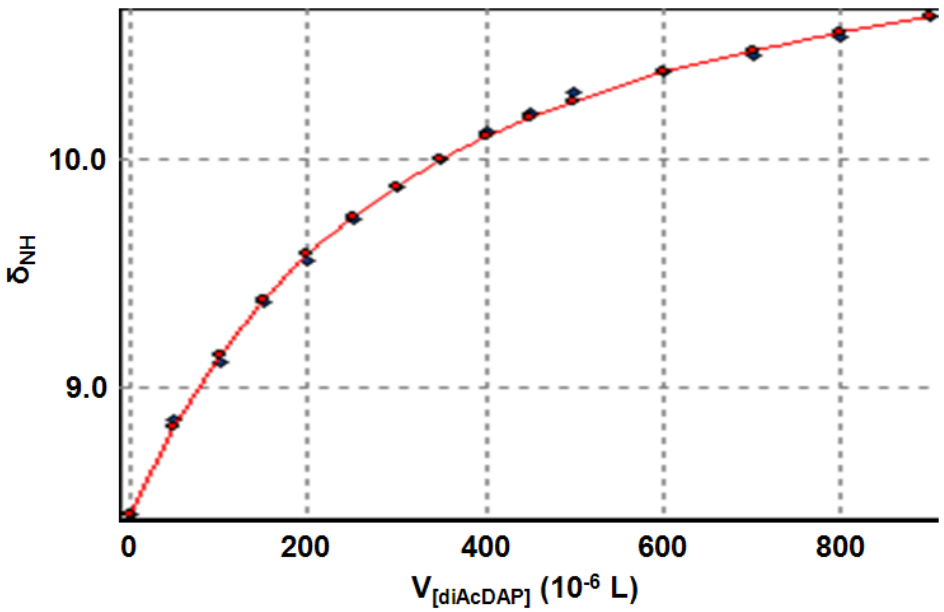
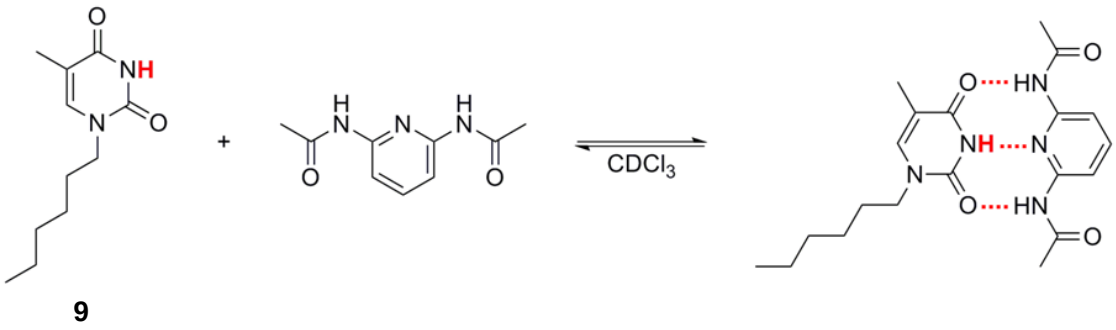


Figure S5. Curve-fitting profile of **7**-**DAP** association with the GAsFit program. Diamonds show experimental results and circles show theoretical values.



9

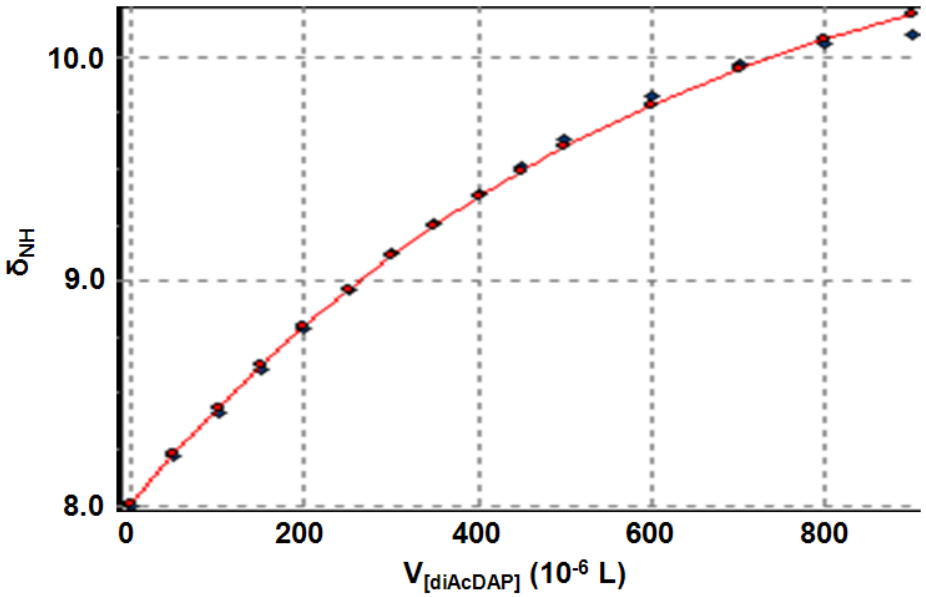


Figure S6. Curve-fitting profile of **9-DAP** association with the GAsFit program. Diamonds show experimental results and circles show theoretical values.

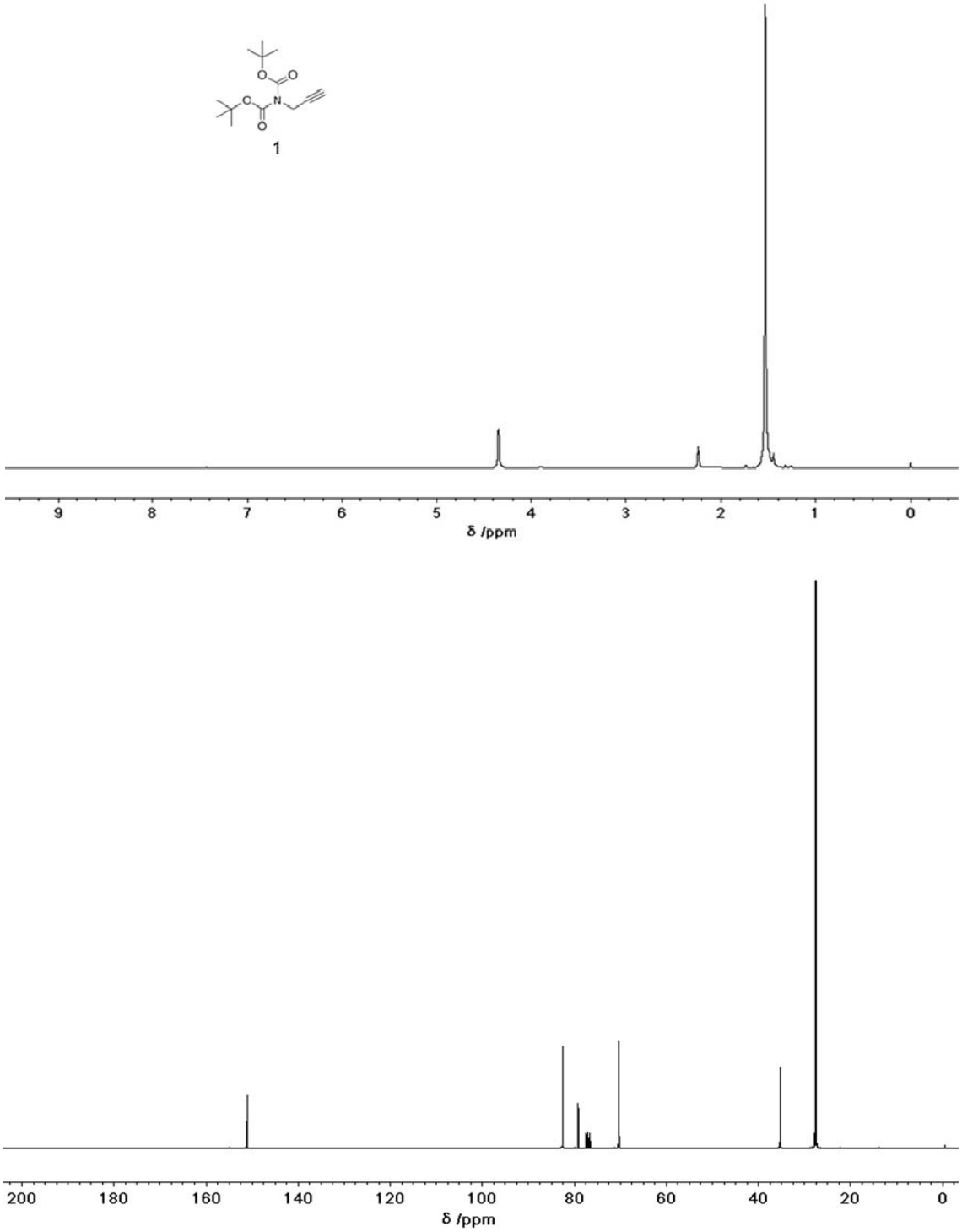


Figure S7. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR spectra of **1**.

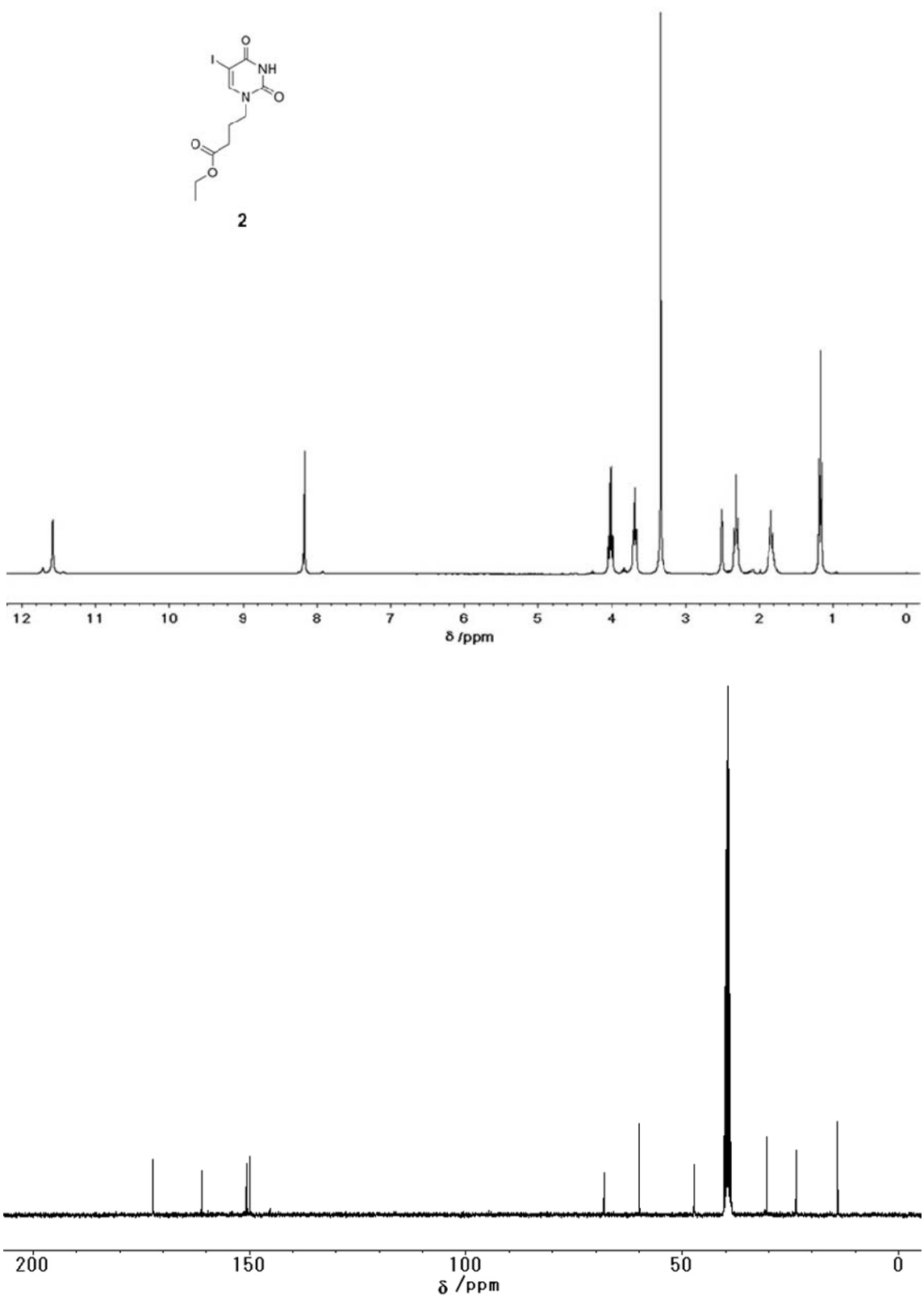


Figure S8. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR spectra of **2**.

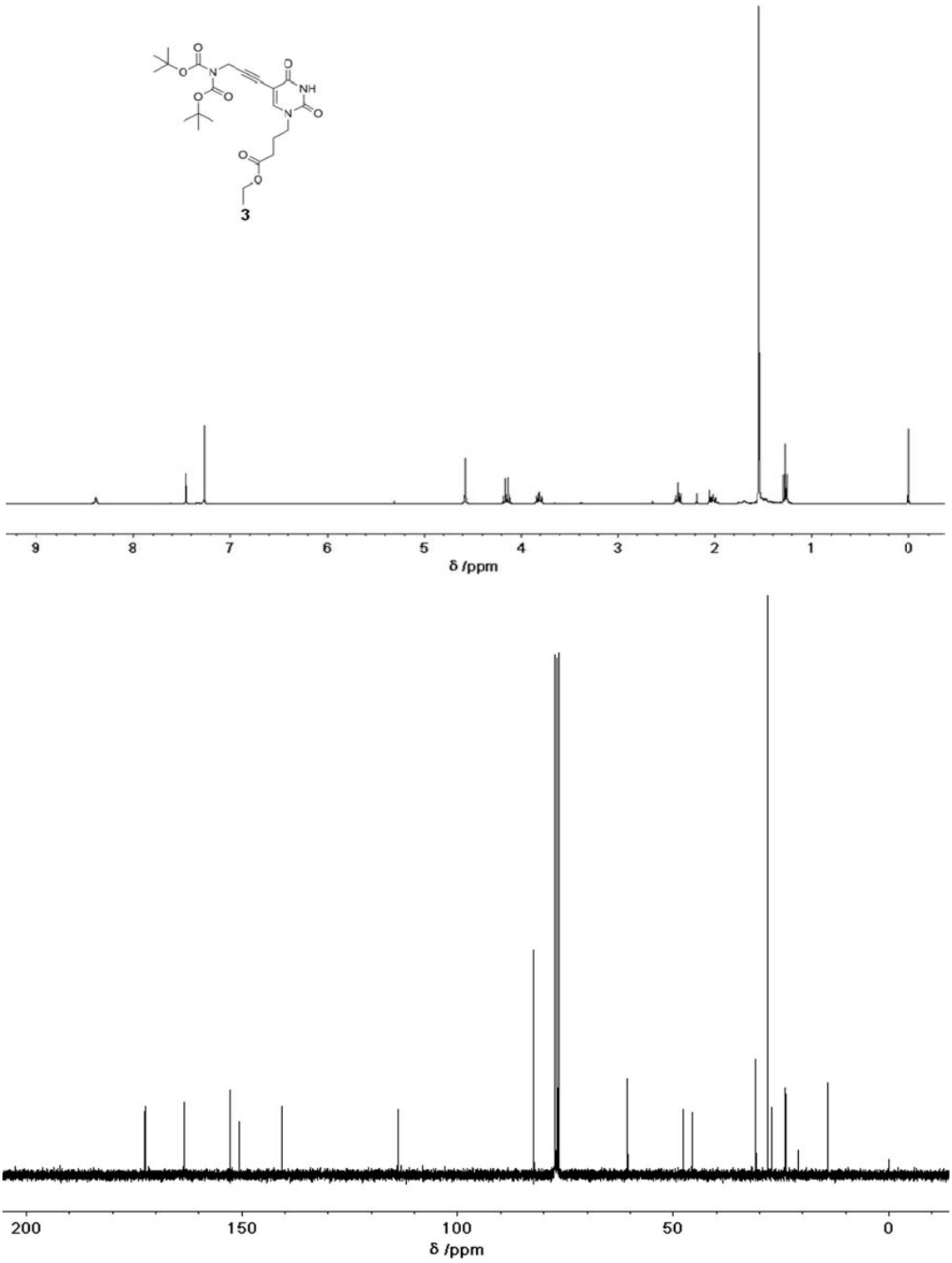


Figure S9. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR spectra of **3**.

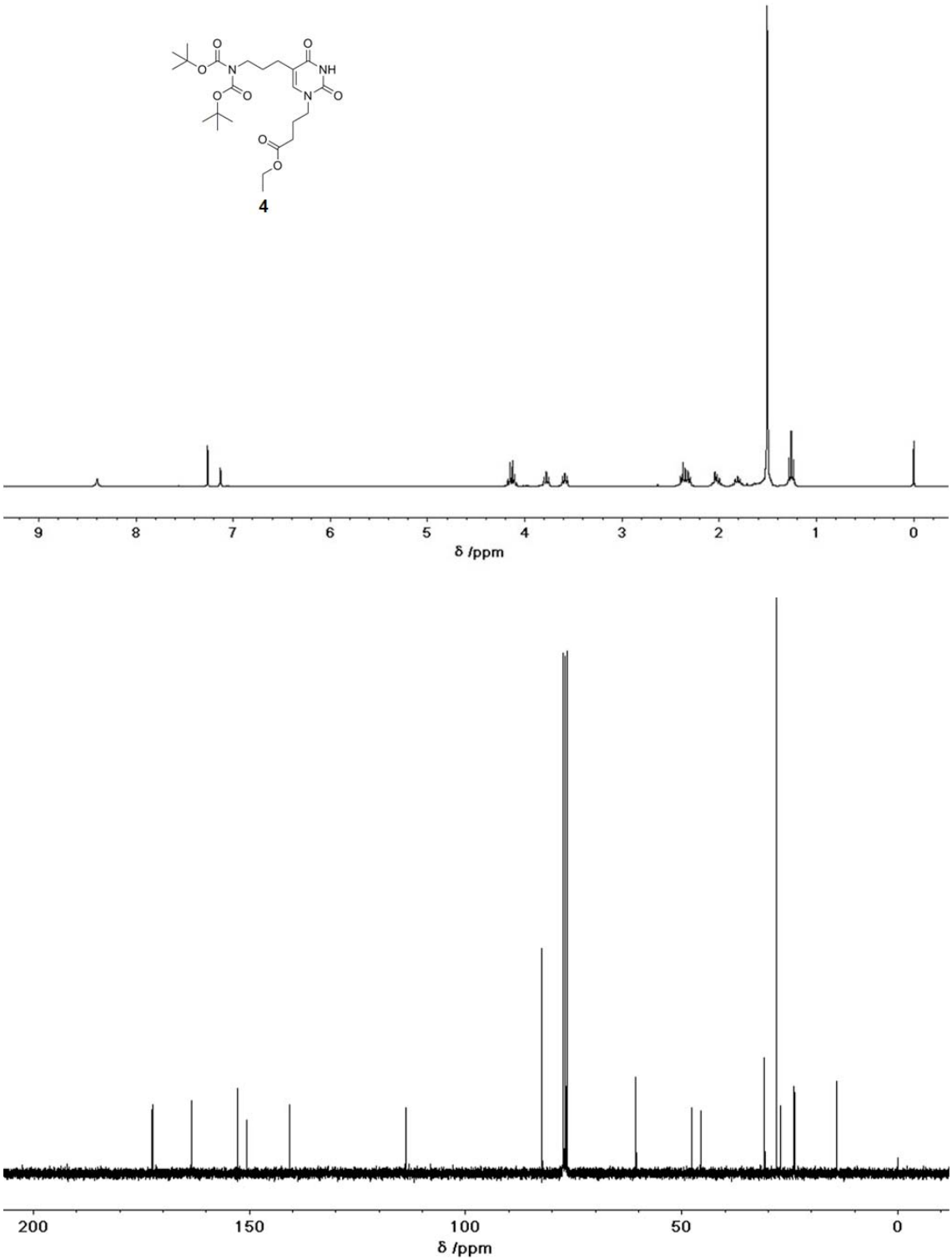


Figure S10. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR spectra of **4**.

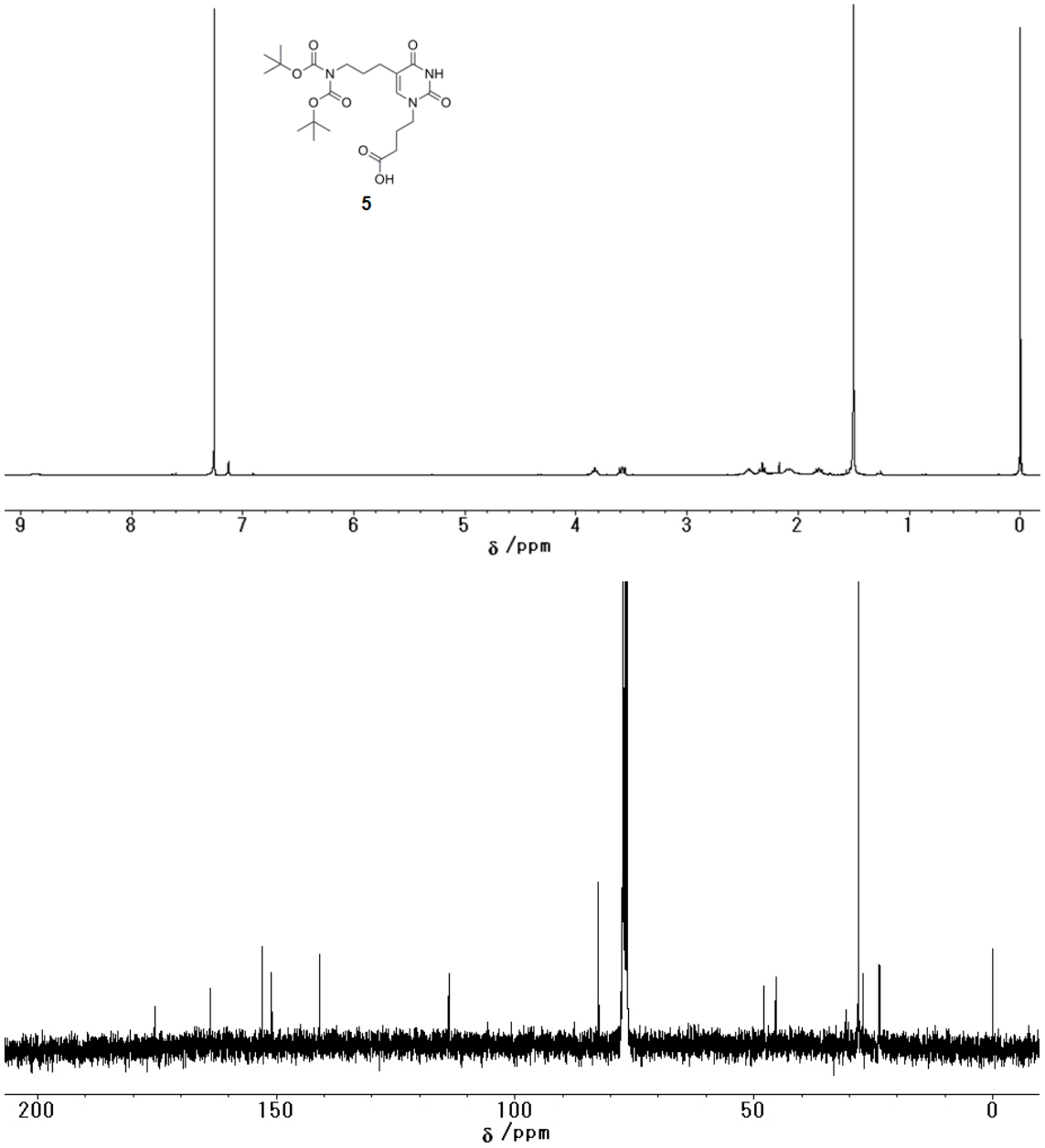


Figure S11. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR spectra of **5**.

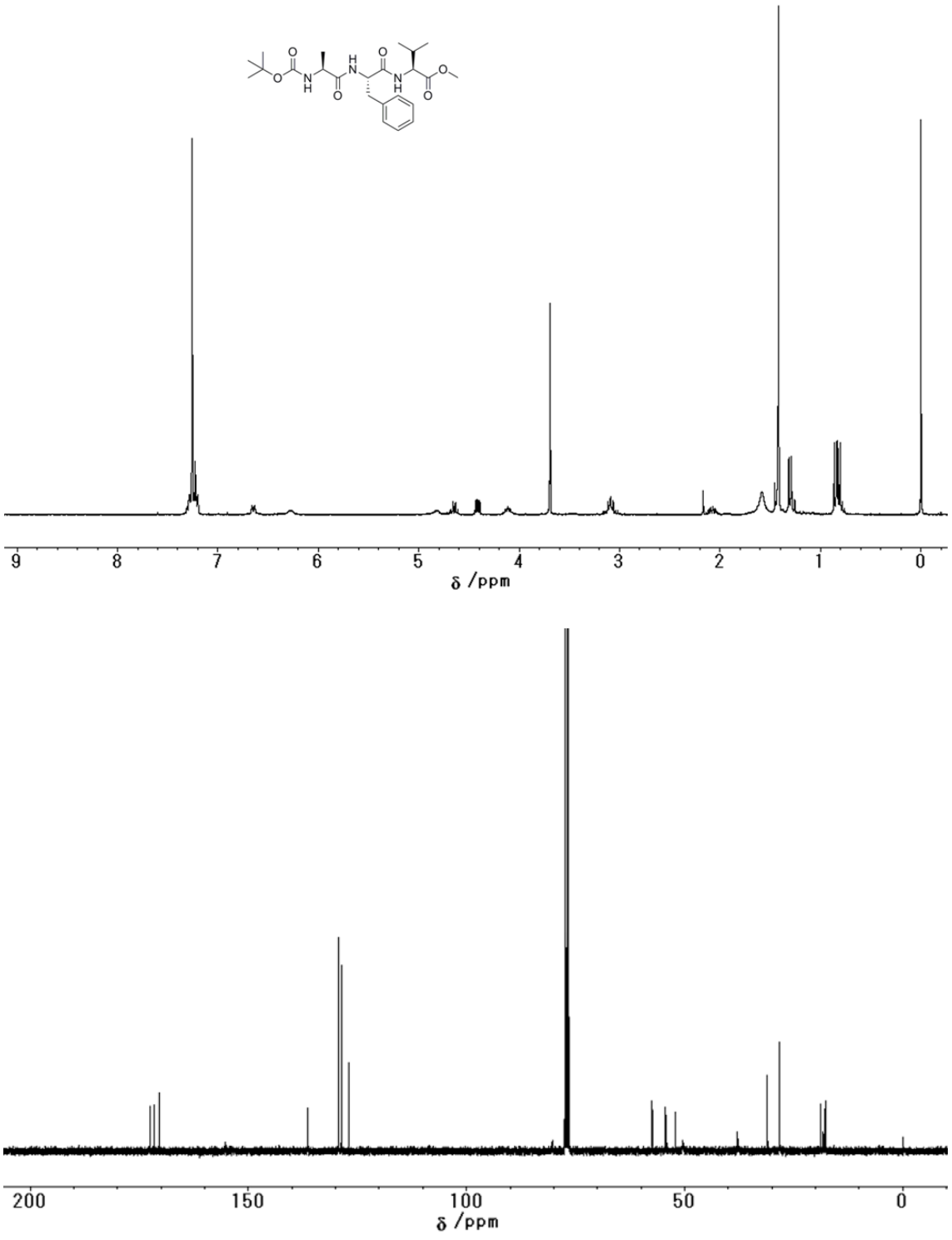


Figure S12. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR spectra of **8**.

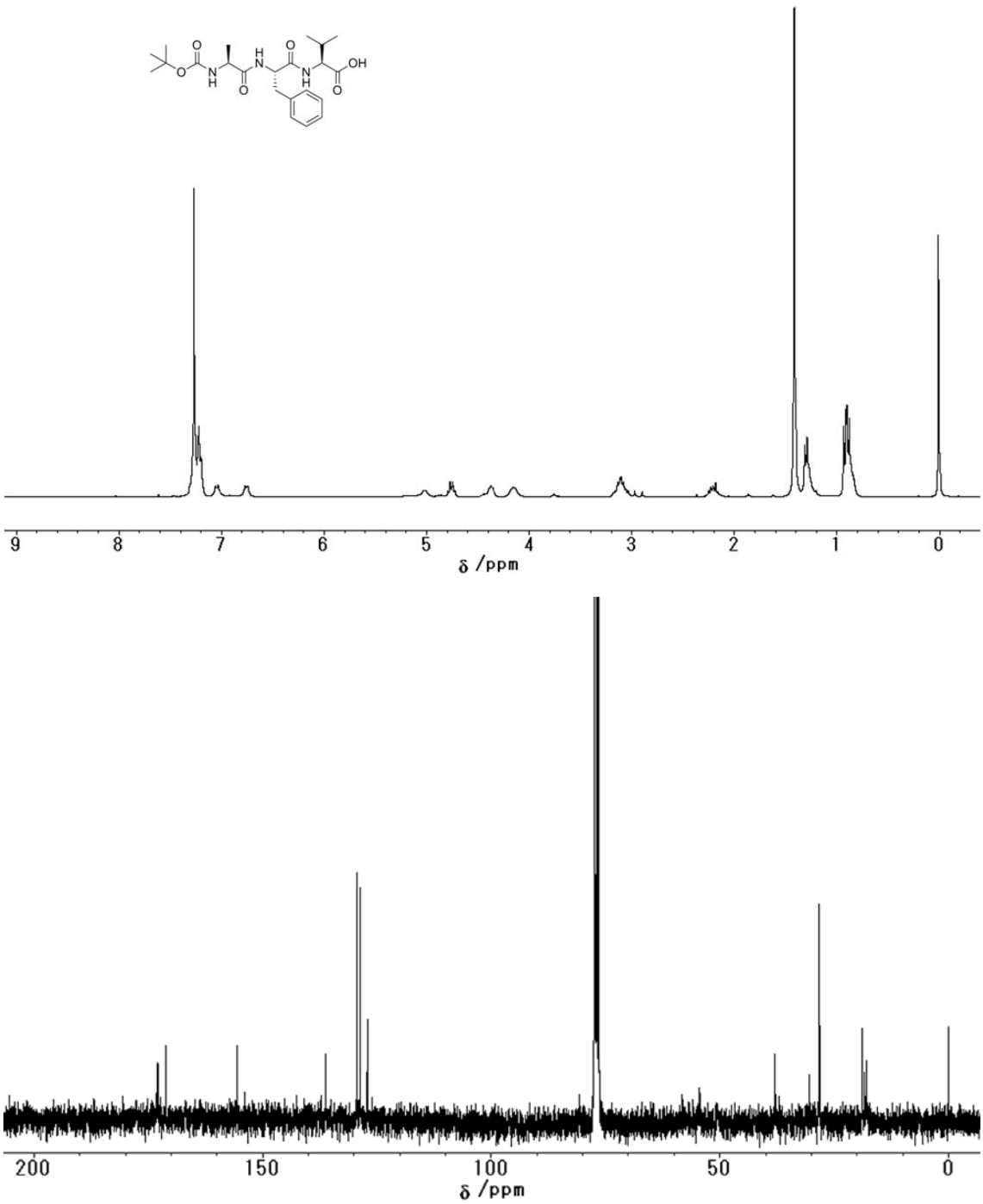


Figure S13. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR spectra of **BocAFVOH**.

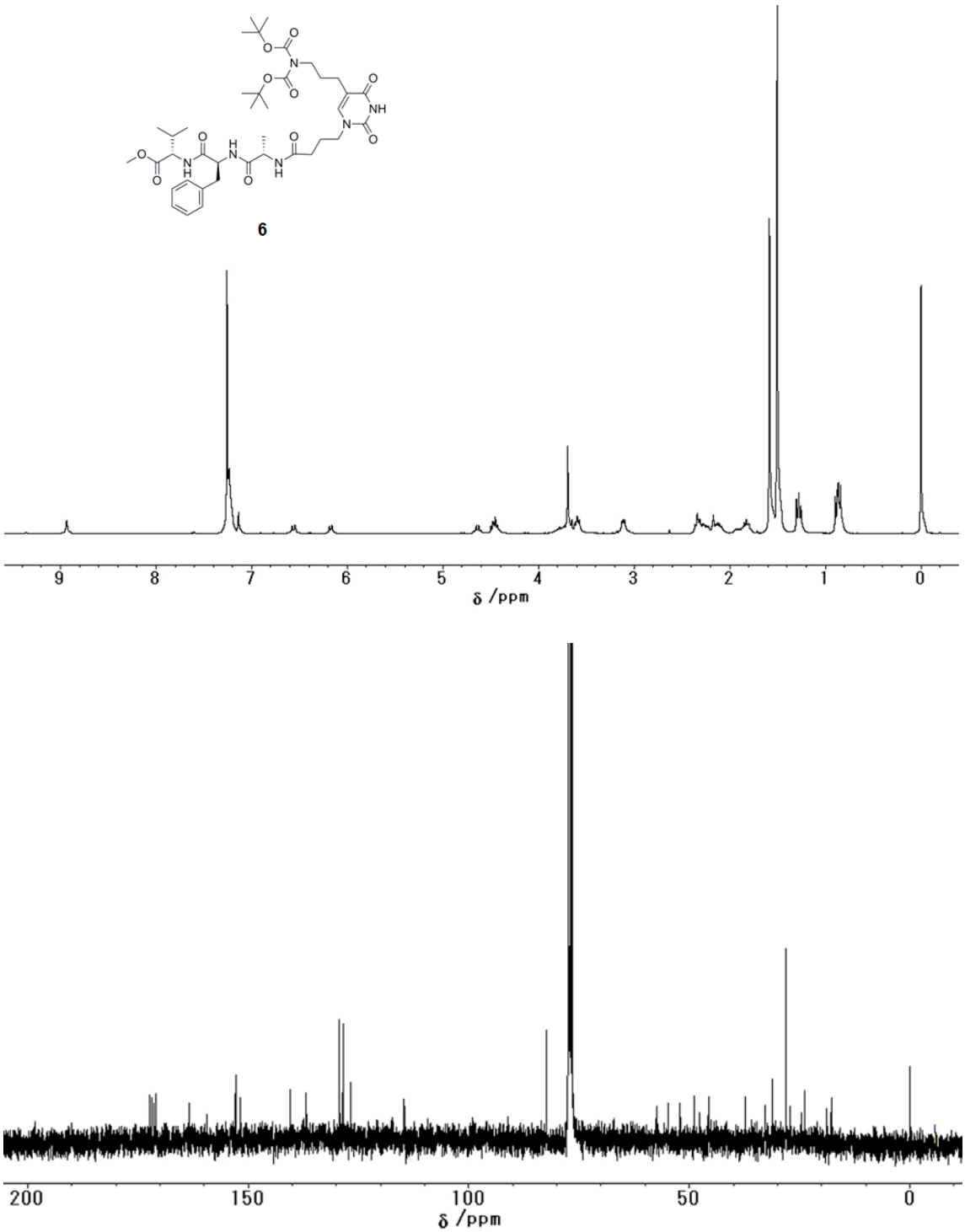


Figure S14. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR spectra of **6**.

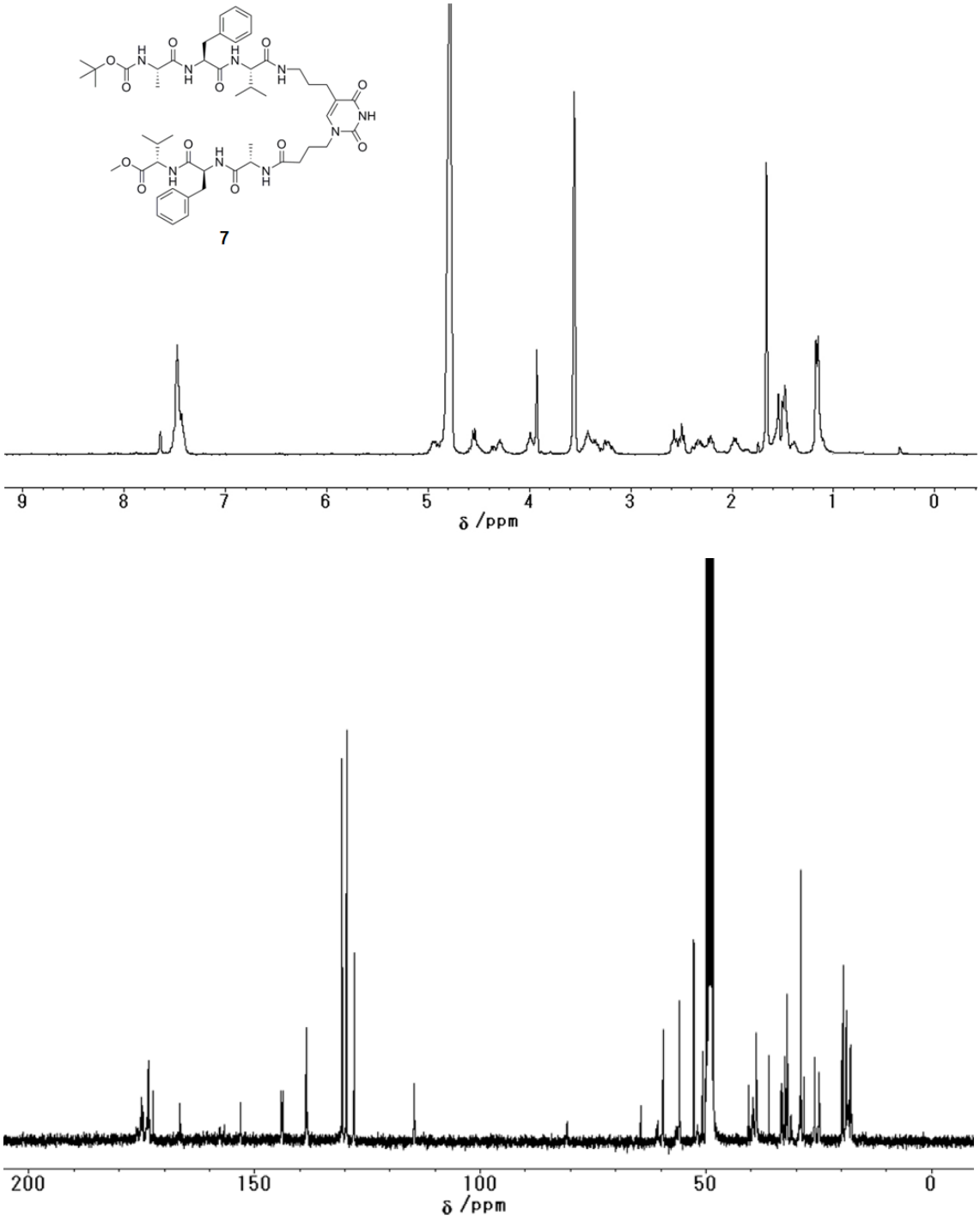


Figure S15. ^{1}H NMR (300 MHz, MeOH-d_4) and ^{13}C NMR spectra of **7**.

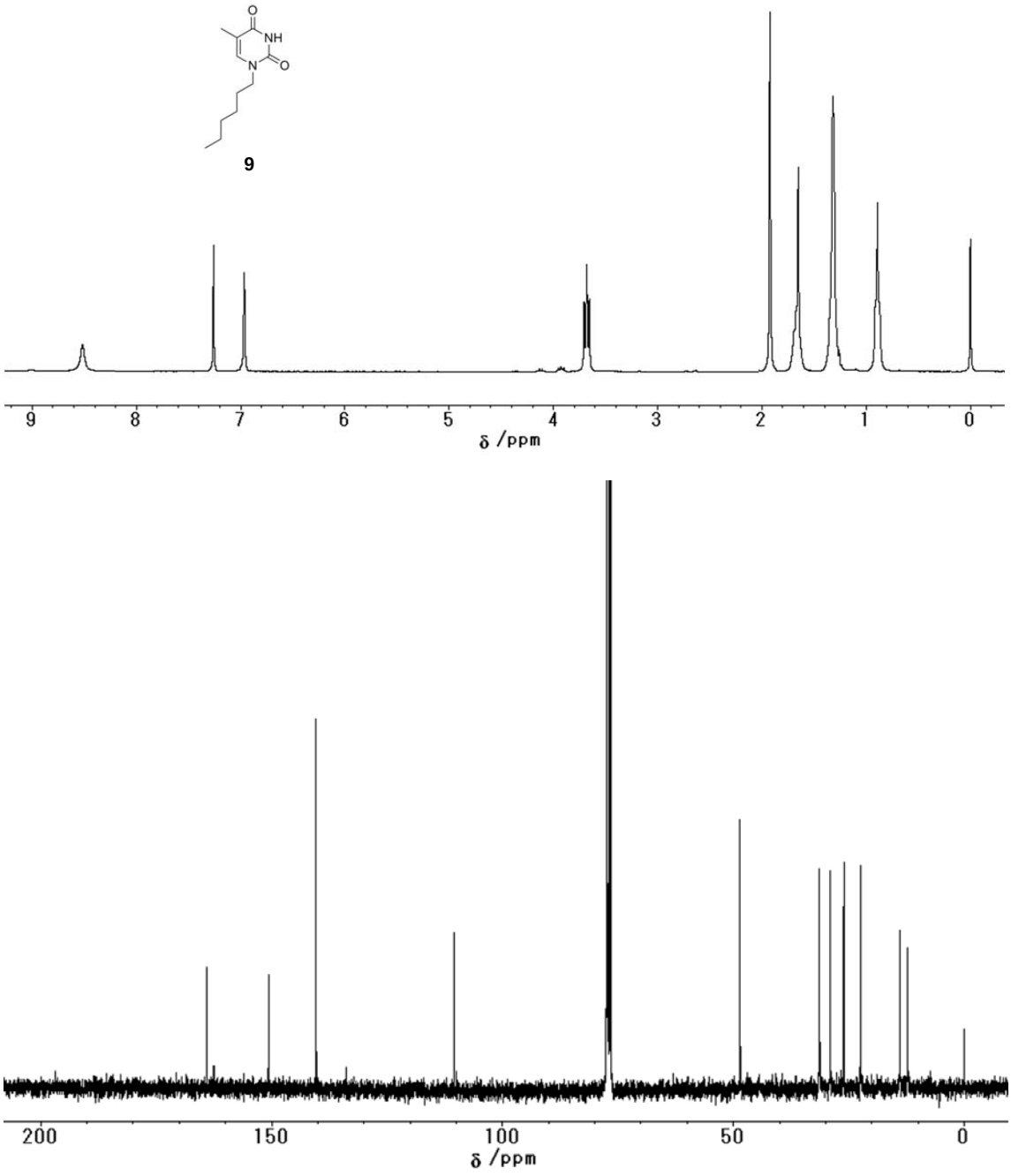


Figure S16. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR spectra of **9**.