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Electronic Supplementary Information

Accessing the Disallowed Conformations of Peptides Employing Amide-to-Imidate Modification

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S1. Materials and Methods.

All the reactions were performed in oven dried apparatus and were stirred using magnetic stir-bars. Column chromatography was performed on silica gel (100-200 mess) (Acme's) purchased from Sd-fine chemicals. TLC was carried out on Merck DC Kieselgel 60 F_{254} aluminium sheets. Compounds were visualized by one of the (or all of the) following methods: (1) fluorescence quenching, (2) spray with a 0.2% (w/v) ninhydrin solution in absolute ethanol, (3) spray with 1% H_2SO_4 solution in EtOH/H_2O (1:5 v/v), (4) charring on hot plate. Ethylacetate and hexanes (or petroleum ether) were obtained from Sd-fine chemicals and were fractionally distilled at their respective boiling points, before use. Dichloromethane was dried by distillation over P_2O_5. NMM was distilled over CaH_2. NMR spectra were recorded on JEOL LA-300 (JEOL Ltd., Tokyo 196-8558, Japan) and BRUKER-AV400 spectrometer (Bruker Co., Faellanden, Switzerland) in $CDCl_3$. Chemical shifts are expressed in parts per million (ppm) from the residual non-deuterated chloroform in $CDCl_3$ (δ_H = 7.26 ppm, δ_C = 77.00 ppm). *J* values are in Hz. Multiplicities are indicated using the following abbreviations: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), quin (quintet), sep (septet), hept (heptet), m (multiplet), bs (broad singlet). Infrared (IR) spectra were recorded in thin-film (0.1 mm) made from solutions in $CHCl_3$ (10 mM) on sodium chloride plates or in neat (KBr pellets), using a JASCO FT/IR-410(Jasco Co., Hachioji City, Tokyo, Japan) spectrometer, and Perkin-Elmer FT/IR Spectrum BX, GX (Perkin-Elmer Co., Waltham, Massachusetts-02451, USA), with frequencies given in reciprocal centimetres (cm^{-1}). Mass spectra were obtained with Micromass Q-Tof (ESI-HRMS). Optical rotation ($[\alpha]_D^{20}$ deg cm^3 g^{-1} dm^{-1}) were recorded in JASCO-P-1020 polarimeter at 20 ^oC. All of the compounds were recorded with 1 cm cell path length quartz cell as solution in $CHCl_3$. Melting points were performed in VEEGO melting point apparatus (VEEGO Inst. Co., Mumbai, India).

S1.1. Crystal Structure Determination

Single crystals of the **1d** and corresponding peptide A→I analogue **2** were obtained by slow evaporation of dichloromethane : hexane mixture, in the Orthorhombic space group P212121, with four molecules in the asymmetric unit for **2** and in the Monoclinic space group P21, with two molecules in the asymmetric unit for **1d**. X-ray data were collected at 20 $^{\circ}C$ on a Brucker KAPPA APEX2 diffractometer using Mo K_α radiation. The data were collected using multi-scan

mode. The structure was obtained by using direct methods in SHELXD[1] and was refined against F_2 by the full matrix least squares method using SHELXL-97.[2] Hydrogen atoms were fixed geometrically in idealized positions and were refined as riding over the heavy atoms to which they are bonded. The final R-factor (R1) obtained for **1d** is 5.68% for 3005 observed reflections with |F| >4r (F) and for peptide A→I analogue **2** is 5.32% for 2436 observed reflections with |F| >4r (F). The crystal and diffraction parameters for peptides were provided in Table I. CCDC 797014 for **1d** and CCDC 797013 for **2** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam. ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

[1]Schneider, T. R.; Sheldrick, G. M. Substructure solution with SHELXD *Acta Crystallogr. Sect. D* **2002**, *58*, 1772.

[2]Sheldrick, G. M. SHELXL-97, A Program for Crystal Structure Refinement; University of Gottingen: Gottingen, 1997

S1.2. Circular Dichroism:

Far-UV CD spectra were recorded using a JASCO CD spectrometer equipped with a Peltier temperature-controlled cell holder using a 0.1 cm path length Suprasil quartz cell (Hellma, Forest Hills, NY). CD spectra were recorded from 190 to 300 nm at 20 °C with scan speed was set to 50 nm/min and spectra were averaged over 5 scans. Spectral baselines were obtained under analogous conditions as that for the samples. The blank solvent and each solution were recorded under the same condition. Solutions were prepared by weighing the peptide in a volumetric flask and adding the MeOH as a solvent by dilution followed by filtering throughout the 0.2 micron PVDF membrane filters (Pall India Pvt. Ltd. Mumbai). Most of the measurements were performed in the concentration range 1 x 10^{-4} to 1 x 10^{-3} M in dry MeOH.

S1.3. NMR experiments:

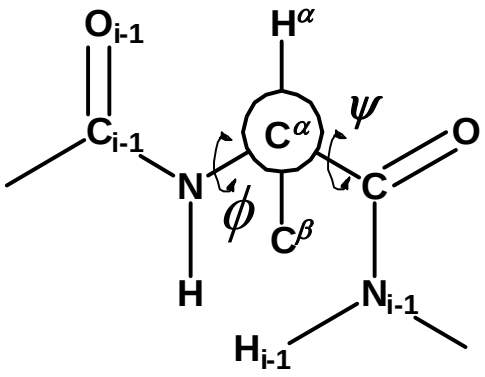
^{1}H & 2D NMR analysis of peptides were performed in deoxygenated 15 mM solution in $CDCl_3$. 1D and ^{1}H & 2D NMR spectra were recorded on a Bruker Avance (Bruker Co., Faellanden, Switzerland) 400 MHz spectrometer. 2D NMR spectra were recorded in phase sensitive mode using time-proportional phase incrementation for quadrature detection in the t_1 dimension.

S1.3.1. TOCSY Experiments: The TOCSY spectra were recorded at 298 K with a mixing time of 200 ms using the MLEVPH pulse sequence. A TOCSY continuous wave spin-lock of 1.5 KHz was used to collect 2k points in the *f2* domain and 512 points in the *f1* domain. The data were processed using Bruker TOPSPIN software.

S1.3.2. ROESY Experiments: The ROESY spectra were recorded at 298 K with a mixing time of 500 ms using the ROESYPH pulse sequence. A ROESY continuous wave spin-lock of 1.5 KHz was used to collect 2k points in the *f2* domain and 512 points in the *f1* domain. The data were processed using Bruker TOPSPIN software.

S2. Steric clashes of the type H···$X_{i\pm1}$ involving the backbone amide hydrogen (H) contributes to ~60% of the disallowed ϕ,ψ space in peptides.

A)



B)

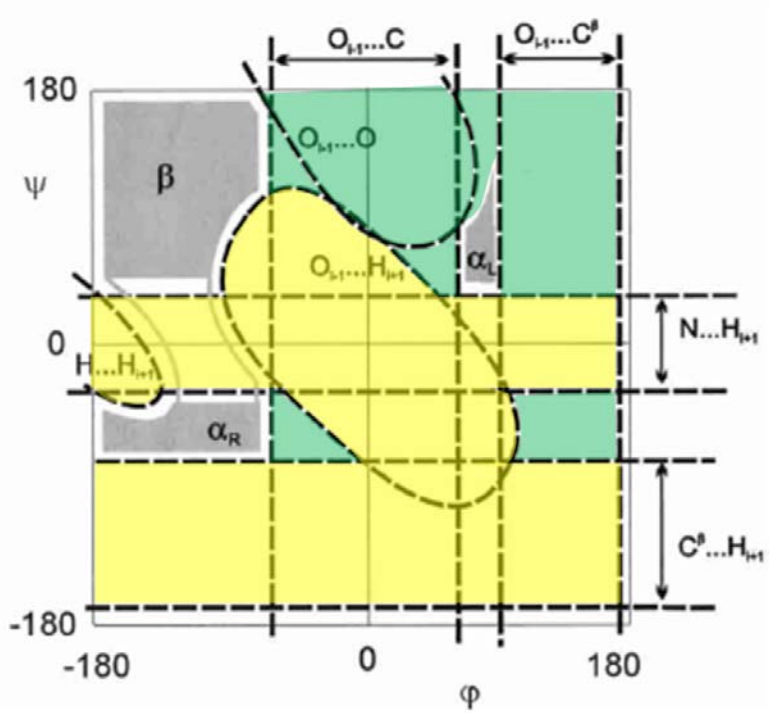
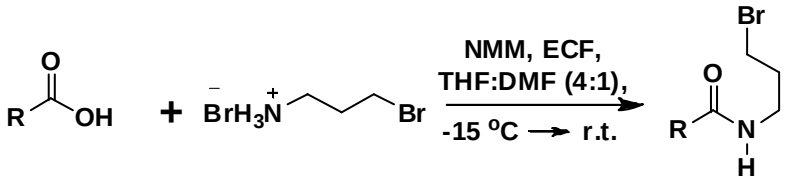
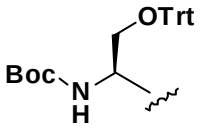
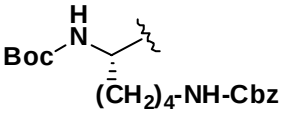
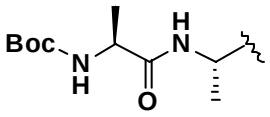
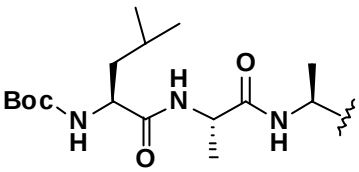
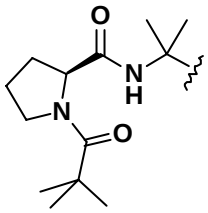


Figure S1: A) Schematic of the alanine dipeptide representing the protein backbone angles parameterized by the C_{i-1}-N-C^{α}-C (ϕ) and N-C^{α}-C-N_{i-1} (ψ) dihedral angles. B) The original Ramachandran steric map where the specific hard-sphere repulsions (dashed lines) identified by Mandel et al. (Mandel, N.; Mandel, G.; Trus, B. L.; Rosenberg, J.; Carlson, G.; Dickerson, R. E. *J. Biol. Chem.* **1977**, *252*, 4619.) define the allowed regions (grey) and the disallowed regions. The disallowed regions to which the H···$X_{i\pm1}$ clashes contribute to are colored in yellow and the rest of the disallowed region is colored in green.

S3. Synthesis of Peptides Containing C-terminal N-(3-Bromopropyl)amide:

Table 1. Synthesis of peptides containing C-terminal N-(3-bromopropyl)amide



S.No	R	Product	Yield
1		19	79
2		20	87
3		21	85
4		22	74
5		1c	81

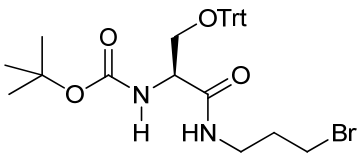
S3.2. General Procedure for Coupling of Carboxylicacids with Amines or Amine hydrohalides:

To a cold (-20 °C) solution of the carboxylic acid (1 mmol) and N-methyl morpholine (NMM) (1.5 mmol) in tetrahydrofuran (THF) (6 mL) was added ethylchloroformate (ECF) (1.03 mmol)

under N_2 atmosphere and vigorously stirred. After 2 min of stirring, a solution of aminehydrohalide (or) amine (1.05 mmol) in a mixture of THF : DMF (1 : 4 – v/v) was added to the mixture followed by NMM (2.5 mmol) and stirred. After 10 min the mixture was warmed to 25 ^{o}C and stirred for further 5 h. THF was removed under reduced pressure and the resulting viscous solution was diluted with water (5 mL) and thoroughly extracted with ethyl acetate (15 mL). The combined organic extracts were washed with 1 N HCl (5 mL), saturated aqueous sodium bicarbonate ($NaHCO_3$) (5 mL) and dried over anhydrous sodium sulphate (Na_2SO_4) and concentrated to give a residue, which was purified by silica gel (100-200 mesh) flash column chromatograph.

All peptides containing the C-terminal N-(3-Bromopropyl)amide functional group were synthesized following the reported procedure (Reddy, D. N.; Prabhakaran, E. N. *J. Org. Chem.* **2010**, *76*, 680), wherein the synthesis and structural data for the peptidyl-N-(3-bromopropyl)amides **12-16** and **20** are reported.

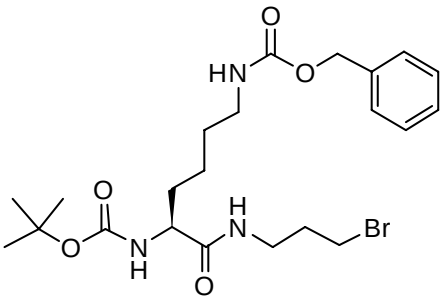
S3.2.1. N′-(3′-Bromopropyl)-(2-(S)-(N-*tert*-butyloxycarbonyl)amino-3-(O-trityl)hydroxy)-propanamide (19)



19

Amide **19** was synthesized by following the above general procedure for amide coupling and purified by silical gel column chromatography (EtOAc : Hexanes – 1 : 4) as a viscous oil (299 mg, 0.53 mmol, 79%) (TLC: EtOAc – R_f = 0.64). IR (NaCl, neat): 3320, 3034, 2978, 2933, 2876, 1661, 1490, 1450, 1367, 1260, 1166, 1095, 764, 747, 707 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.40 (d, *J* = 7.6 Hz, 6H), 7.32 (t, *J* = 7.7 Hz, 6H), 7.26-7.23 (m, 6H), 6.51 (bs, 1H), 5.14 (bs, 1H), 4.21-4.25 (m, 1H), 3.68 (dd, *J* = 9.1, 3.9 Hz, 1H), 3.46 (q, *J* = 6.1 Hz, 2H), 3.39 (t, *J* = 6.4 Hz, 2H), 3.19 (dd, *J* = 8.7, 5.2 Hz, 1H), 2.08 (quin, *J* = 5.6 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.5, 155.4, 143.3, 128.5, 128, 127.3, 87, 80.3, 63.6, 54.6, 38, 32, 30.7, 28.3; HRMS *m/z* for $C_{30}H_{35}N_2O_4Na$ $[M+Na]^+$ calcd 589.1678, Found 589.1673.

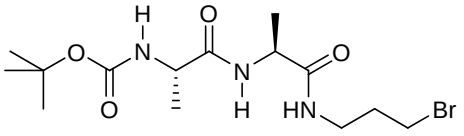
S3.2.2. N′-(3′-Bromopropyl)-2-((S)-(N-*tert*-butyloxycarbonyl)amino)-6-(N-benzyloxycarbonyl)amino-Hexanamide (20)



20

Amide **20** was synthesized by following the above general procedure for amide coupling and purified by silical gel column chromatography (EtOAc : Hexane – 3 : 7) as a viscousoil in good yields (297 mg, 0.6 mmol, 87%) (TLC: EtOAc – R_f = 0.64). IR (NaCl, 10 mM in $CHCl_3$): 3444, 3343, 3020, 2977, 2868, 1708, 1678, 1517, 1457, 1369, 1252, 1163, 1047 cm^{-1}; ^1H NMR (400 MHz , $CDCl_3$) δ ppm: 7.35–7.30 (m, 5H), 6.59 (bs, 1H), 5.26 (d, *J* = 7.2 Hz, 1H), 5.10 (s, 2H), 4.97 (bs, 1H), 4.04-3.99 (m, 1H), 3.40 (t, *J* = 6.4 Hz, 2H), 3.38 (q, *J* = 6.2 Hz, 2H), 3.19 (q, *J* = 6.1 Hz, 2H), 2.06 (quin, *J* = 6.8 Hz, 2H), 1.86-1.78 (m, 1H), 1.63-1.58 (m, 1H), 1.58-1.49 (m, 2H), 1.44 (s, 9H), 1.39-1.35 (m, 2H); ^{13}C NMR (100 MHz ,$CDCl_3$) δ ppm: 172.5, 156.7, 155.9, 136.5, 128.5, 128.09, 128.06, 80.2, 66.7, 54.4, 40.2, 37.9, 32, 31.5, 30.7, 29.4, 28.3, 22.4; HRMS *m/z* Calcd for $C_{22}H_{34}BrN_3O_5Na$ 522.1580 , Found 522.1584.

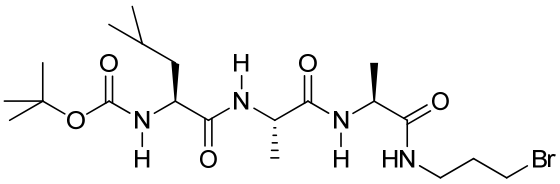
S3.2.3. N′-(3′-Bromopropyl)-2-((S)-(2-(S)-(N-*tert*-butyloxycarbonyl)-aminopropanoyl)-aminopropanoyl)-Propanamide (21)



21

Amide **21** was synthesized by following the above general procedure for amide coupling and purified by silical gel column chromatography (EtOAc : Hexane – 2 : 3) as a solid (m.p. 145-146 ^oC) in good yields (1.022 g, 2.73mmol, 85%) (TLC: EtOAc – R_f = 0.39). IR (NaCl, neat): 3304, 2978, 2927, 1697, 1640, 1538, 1447, 1365, 1252, 1167, 1050 cm^{-1}; ^1H NMR (300 MHz , $CDCl_3$) δ ppm: 6.8 (bs, 1H), 6.66 (bs, 1H), 4.98 (d, *J* = 5.4 Hz, 1H), 4.44 (quin, *J* = 7.2 Hz, 1H), 4.10 (quin, *J* = 6.9 Hz, 1H), 3.5-3.28 (m, 4H), 2.07 (quin, *J* = 6.6 Hz, 2H), 1.45 (s, 9H), 1.39 (d, *J* = 6.9 Hz, 3H), 1.37 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz ,$CDCl_3$) δ ppm: 172.8, 172.3, 155.8, 80.6, 50.8, 49, 38, 32.1, 30.6, 28.3, 18.1; HRMS *m/z* for $C_{14}H_{26}BrN_3O_4Na$ $[M+Na]^+$ calcd 402.1004, Found 402.1000.

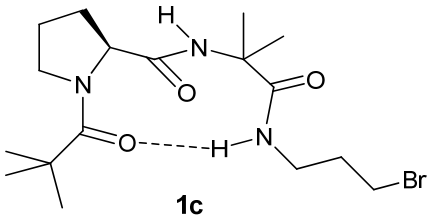
S3.2.4. N′-(3′-Bromopropyl)-2-((S)-(2-(S)-(2-(S)-(N-*tert*-butyloxycarbonyl)amino-4-(methyl) pentyl)-aminopropanoyl)-aminopropanoyl)-Propanamide (22)



22

Amide **22** was synthesized by following the above general procedure for amide coupling and purified by silical gel column chromatography (EtOAc : Hexane – 4 : 1) as a white solid (223 mg, 0.45 mmol, 74%) (m.p. = 166-167 °C) (TLC: EtOAc – R_f = 0.24). IR (NaCl, 10 mM in $CHCl_3$): 3428, 3346, 3011, 2938, 2875, 1698, 1676, 1672, 1665, 1523, 1499, 1160 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.28 (bs, 1H), 6.96 (bs, 1H), 6.57 (bs, 1H), 5.08 (bs, 1H), 4.48 (quin, *J* = 7.6 Hz, 1H), 4.26 (quin, *J* = 6.5 Hz, 1H), 4.01-3.96 (m, 1H), 3.45 (t, *J* = 6.9 Hz, 2H), 3.44-3.39 (m, 1H), 3.37-3.31 (m, 1H), 2.12 (quin, *J* = 6.7 Hz, 2H), 1.76-1.61 (m, 2H), 1.54-1.48 (m, 1H), 1.45 (s, 9H), 1.42 (d, *J* = 7.3 Hz, 6H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.8, 172.6, 171.8, 156.6, 81.3, 54.9, 50.4, 49.2, 40.6, 38.1, 32.3, 30.8, 28.2, 24.9, 22.9, 21.7, 17.5; HRMS *m/z* for $C_{20}H_{37}BrN_4O_5$ $[M+Na]^+$ calcd 515.1845, Found 515.1844.

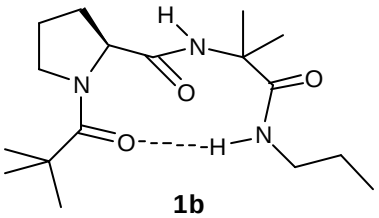
S3.2.5. N′-(3′-Bromopropyl)-2-Methyl-2-((S)-((N-Pivaloyl)-Pyrrolidine-2-Carbonyl)amino)-Propanamide (1c)



1c

Amide **1c** was synthesized by following the above general procedure for amide coupling and purified by silical gel column chromatography (EtOAc : Hexane – 4 : 1) as a white solid (459 mg, 1.14 mmol, 81% yield) (m.p. = 185-186 °C) (TLC- DCM : MeOH (20 : 1) – R_f = 0.51). IR (NaCl, 10 mM in $CHCl_3$): 3433, 3358, 3001, 2878, 1693, 1667, 1598, 1536, 1416, 1382, 1365, 1218 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.35 (bs, 1H), 6.07 (bs, 1H), 4.17 (t, *J* = 6.3 Hz, 1H), 3.75 (t, *J* = 6.5 Hz, 2H), 3.42 (t, *J* = 6.8 Hz, 2H), 3.32 (q, *J* = 6.3 Hz, 2H), 2.19-2.12 (m, 1H), 2.06 (quin, *J* = 6.8 Hz, 2H), 2.1-2.03 (m, 1H), 2.01-1.87 (m, 2H), 1.54 (s, 3H), 1.44 (s, 3H), 1.27 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 178.2, 174. 3, 172.1, 63.4, 57.4, 48.8, 38.9, 38.4, 32.5, 31.1, 27.7, 27.5, 27.3, 26.2, 24.3; HRMS *m/z* for $C_{17}H_{30}BrN_3O_3Na$ $[M+Na]^+$ calcd 426.1368, Found 426.1364; $[\alpha]_D^{20}$ = -1.9 (c = 1, $CHCl_3$).

S3.2.6. N′-Propyl-2-Methyl-2-((S)-((N-Pivaloyl)-Pyrrolidine-2-Carbonyl)amino)-Propanamide (1b)

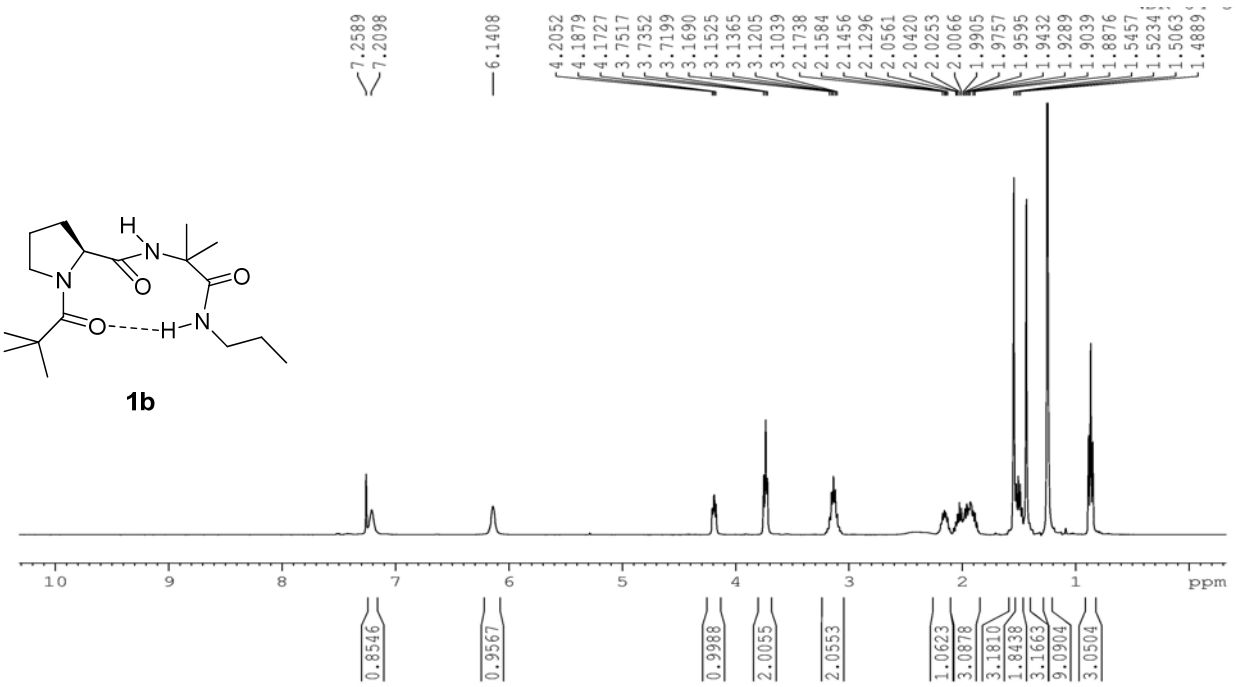


1b

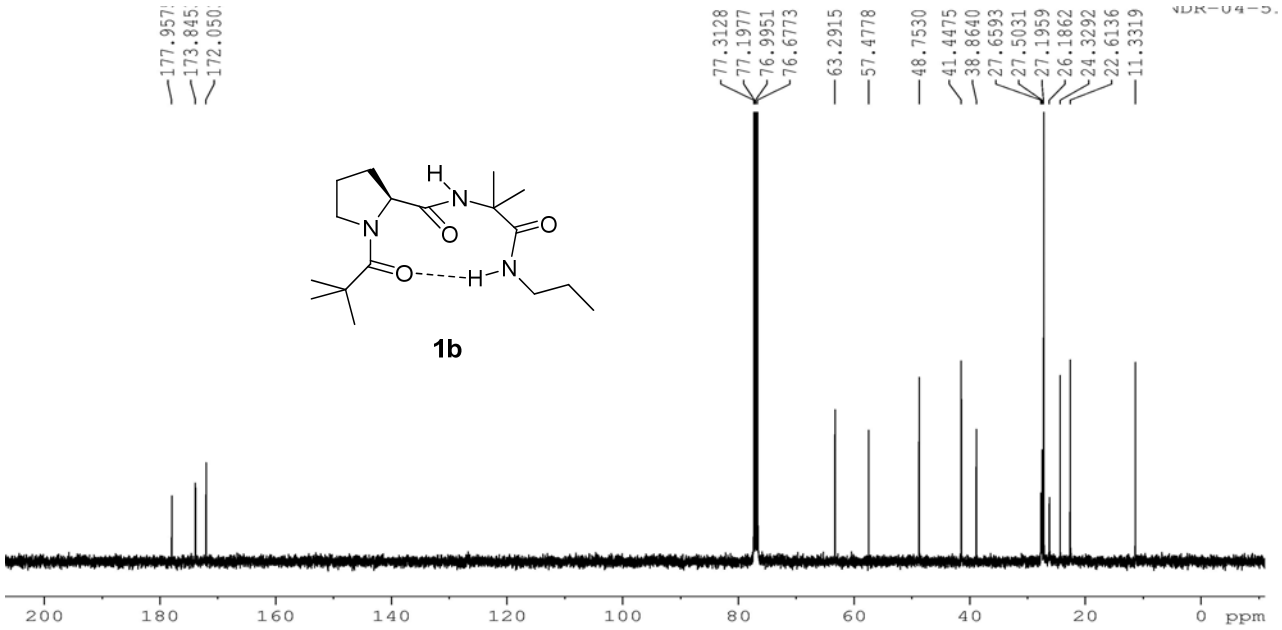
Amide **1b** was synthesized by following the above general procedure for amide coupling and purified by silical gel column chromatography (DCM : MeOH – 20 : 1) yielded the desired product as a white solid (49 mg, 0.15 mmol, 85% yield) (m.p. = 190-191 °C) (TLC- DCM : MeOH (10 : 1) – R_f = 0.53). IR (NaCl, 10 mM in $CHCl_3$): 3433, 3370, 3026, 3006, 1696, 1669, 1653, 1596, 1542, 1508, 1382, 1260, 1172 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.21 (bs, 1H), 6.14 (bs, 1H), 4.19 (t, J = 6.7 Hz, 1H), 3.74 (t, J = 6.6 Hz, 2H), 3.19-3.1 (m, 2H), 2.19-2.11 (m, 1H), 2.08-1.87 (m, 3H), 1.51 (sep, J = 6.8 Hz, 2H), 1.55 (s, 3H), 1.44 (s, 3H), 1.25 (s, 9H), 0.87 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 178, 173.9, 172.1, 63.3, 57.5, 48.8, 41.5, 38.9, 27.7, 27.5, 27.2, 26.2, 24.3, 22.6, 11.3; HRMS *m/z* for $C_{17}H_{31}N_3O_3Na$ $[M+Na]^+$ calcd 348.2263, Found 348.2266; $[\alpha]_D^{20}$ = -12.7 (c = 1, $CHCl_3$).

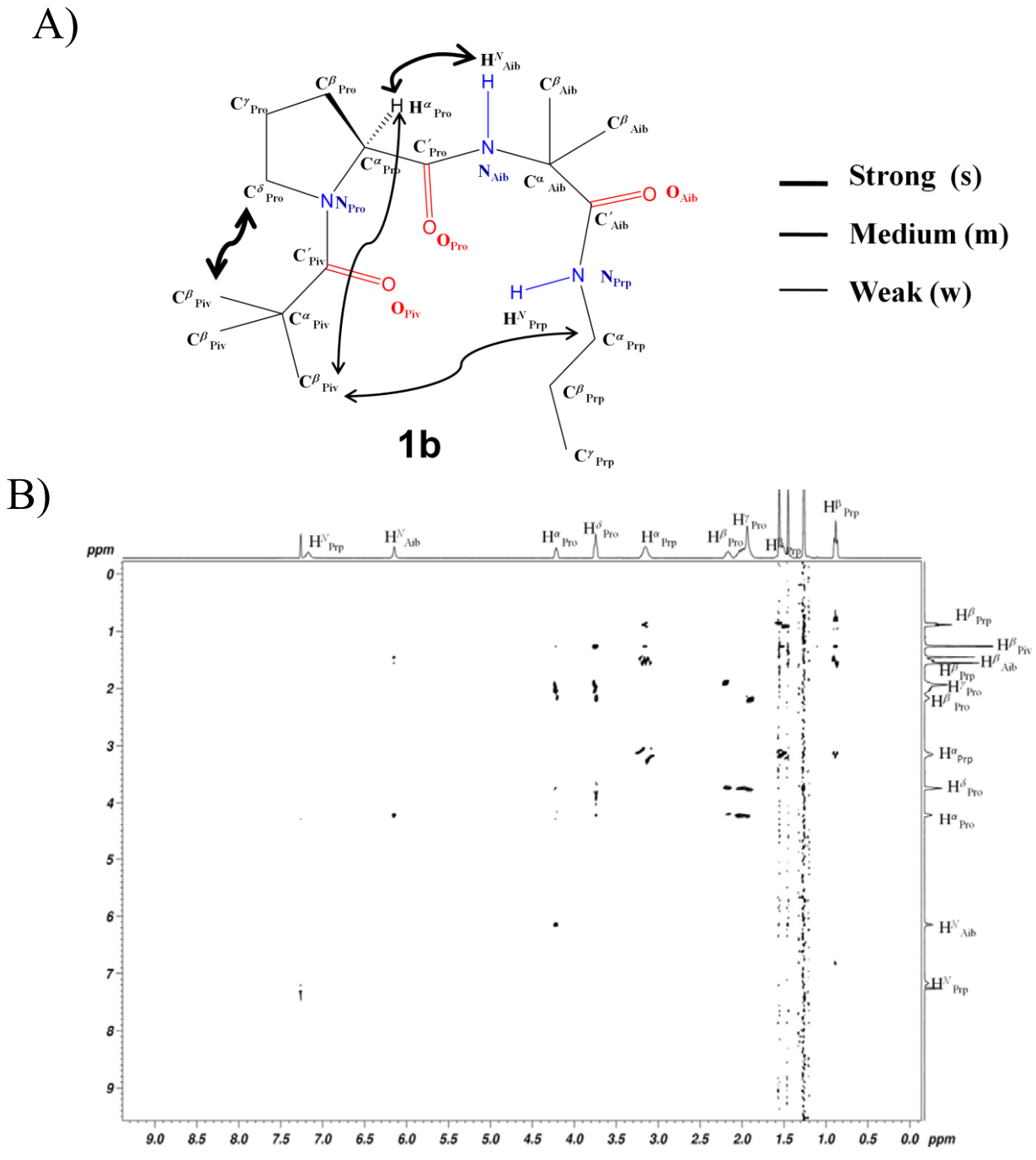
S4. Solution structure of peptide 1b.

S4.1. ^{1}H NMR Spectrum (400 MHz) of model peptide **1b** in $CDCl_3$ (10 mM)

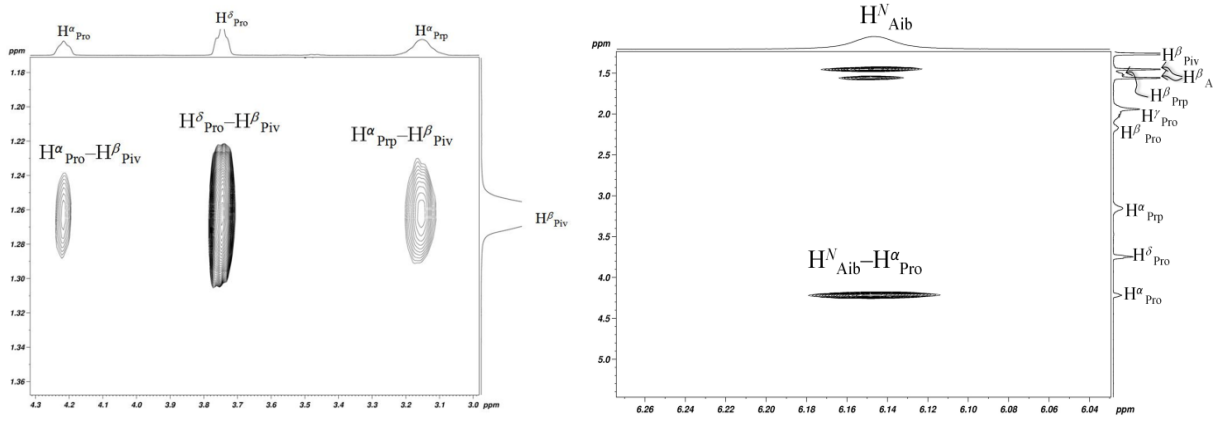


S4.2. ^{13}C NMR Spectrum (100 MHz) of model peptide **1b** in $CDCl_3$ (60 mM)





S4.3. Figure S2: A) Chemdraw diagram of the model peptide **1b** with labels for atoms. B) The complete 2D ROESY spectrum of model peptide **1b** (400 MHz NMR, $CDCl_3$, 10 mM, 20 °C).

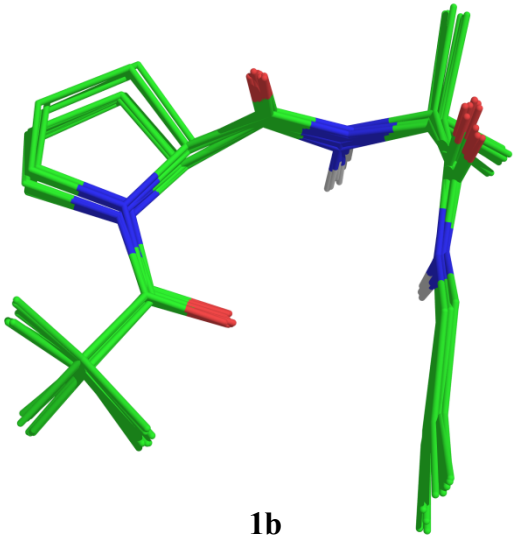


S4.4. Figure S3: Partial ROESY spectra of model peptide **1b** showing the relevant cross peaks.

S4.5. Method for calculation of peptide conformation from NOE distance constraints:

The solution structures were computed using Discover module (version 2000) of InsightII (Accelyrs, San Diego, CA) from ROESY cross-peaks. The NOE restraints were categorized into three groups: strong (2.5 Å upper limit), medium (3.5 Å upper limit), and weak (6 Å upper limit). These distances were employed using generic distance restraints with force constants of 1 kcal/mol/Å^2 and a maximum force value of 1000 kcal/mol/Å^2. The consistent valence force field (CVFF) was applied for all calculations. Prior to every restrained dynamics simulation the system was equilibrated for 1 ps. After that period, the structures were submitted to 100 ps of molecular dynamics at 1000 K with step size of 1 fs. At regular intervals of 1 ps, 100 conformers were extracted. The 10 lowest energy conformers with non violated restraints were taken and subjected to energy minimization by conjugate gradient method until the maximum derivative was less than 0.0001 kcal/mol. The resulting structures were analyzed with pymol and InsightII.

A)



1b

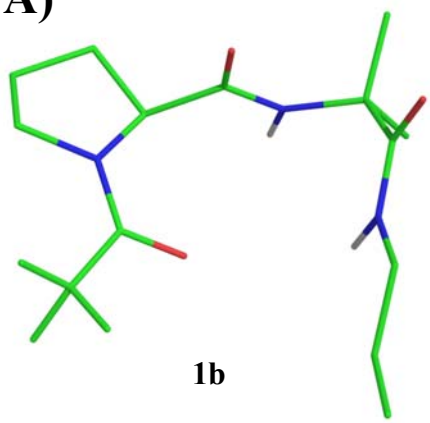
B)

Conformer	ϕ_{Pro}	ψ_{Pro}	ϕ_{Aib}	ψ_{Aib}
1	-75.5	115.2	56.1	52.6
2	-62.9	123.0	57.4	56.2
3	-62.9	123.0	57.5	56.3
4	-62.9	122.9	57.5	56.2
5	-75.4	115.2	56.0	52.6
6	-62.9	123.0	57.5	56.2
7	-65.7	113.7	53.1	45.7
8	-77.7	121.2	58.6	56.7
9	-62.9	123.0	57.4	56.2
10	-62.9	123.0	57.4	56.3
Average[a]	-67.2(6.3)	120.3(3.9)	56.8(1.5)	54.5(3.5)

[a] Values in parentheses are standard deviations

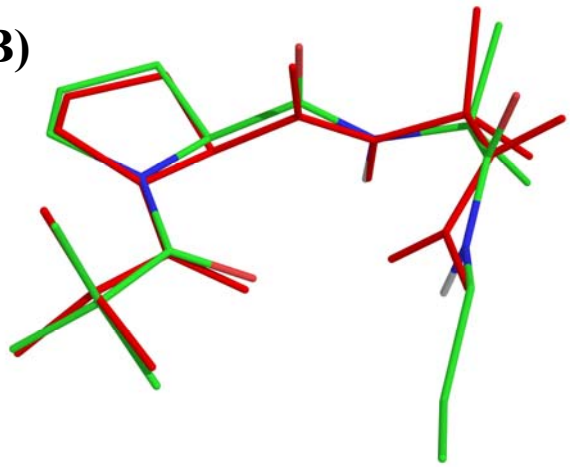
S4.6. Figure S4: A) Stick diagrams of the 10 lowest energy conformers of model peptide **1b**, obtained from molecular dynamic simulation with CVFF force field at 1000K using the constrains obtained from the 2D ROESY experiment, superimposed on one another. The amide hydrogens are selectively shown for clarity. B) The ϕ, ψ angles for Pro & Aib residues in the 10 least energy structures. Note: The 4→1 hydrogen bonding interaction was not used as a constraint.

A)



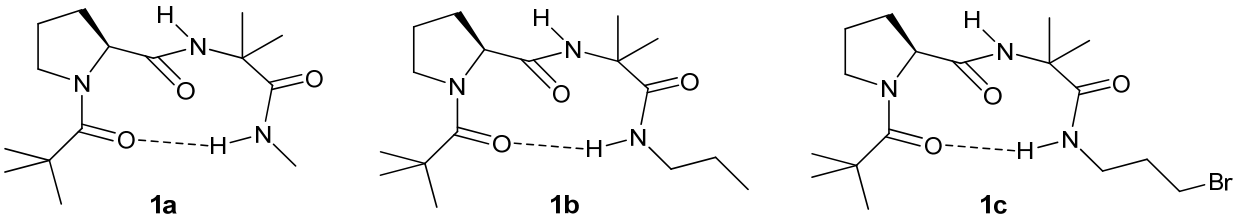
1b

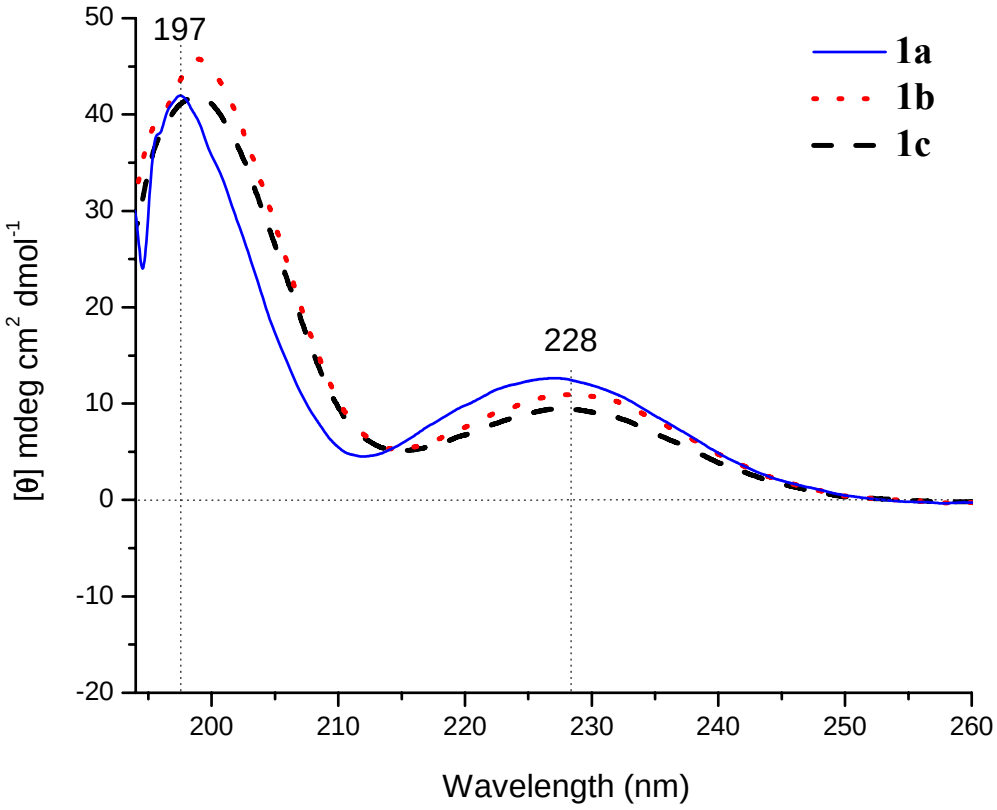
B)



1a & 1b

S4.7. Figure S5: A) Stick diagram representing the average of the 10 lowest energy conformers of the model peptide **1b** in $CDCl_3$ (10 mM). B) Overlap of the stick diagrams of the crystal structure of the reference peptide **1a** (red) (Prasad, B. V. V.; Balaram, H.; Balaram, P. *Biopolymers* **1982**, *21*, 1261.) and the average minimum energy structure of peptide **1b** (green) in $CDCl_3$ (10 mM) –RMSD of all relevant atoms (excluding hydrogens) = 0.085 Å.



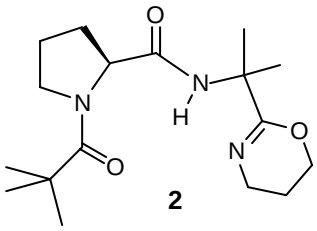


S4.8. Figure S6: CD spectra of the model compounds **1a [____]**, **1b[· · · ·]**, **1c[- - - -]** in MeOH (1 mM) at 20 °C.

S5. General Procedure for the Synthesis of the Oxazine Containing A→I Analogues from Peptidyl-N-(3-Bromopropyl)amides:

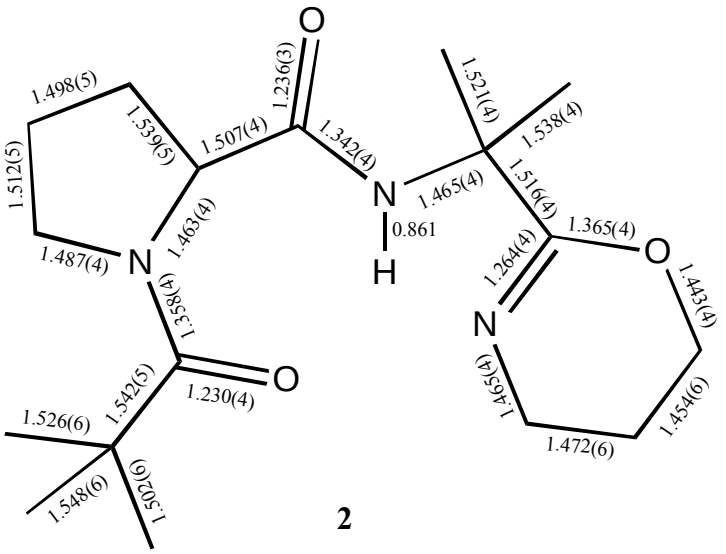
To a cold (0 °C) suspension of NaH (1.2 mmol) in THF (1 mL) was added a solution of the peptidyl-(3-bromopropyl)amide (1 mmol) in dry THF (33 mL). After 10 min the mixture was warmed to 25 °C and stirred further until TLC indicated complete consumption of the peptide. The mixture was filtered through celite and concentrated under vacuum to give the desired oxazine containing peptide products in high purity.

S5.1. 2-(1-Methyl-1-((S)-(N-Pivaloyl)-Pyrrolidine-2-Carbonyl)amino)-Ethyl)-5,6-Dihydro-4H-1,3-Oxazine (2)



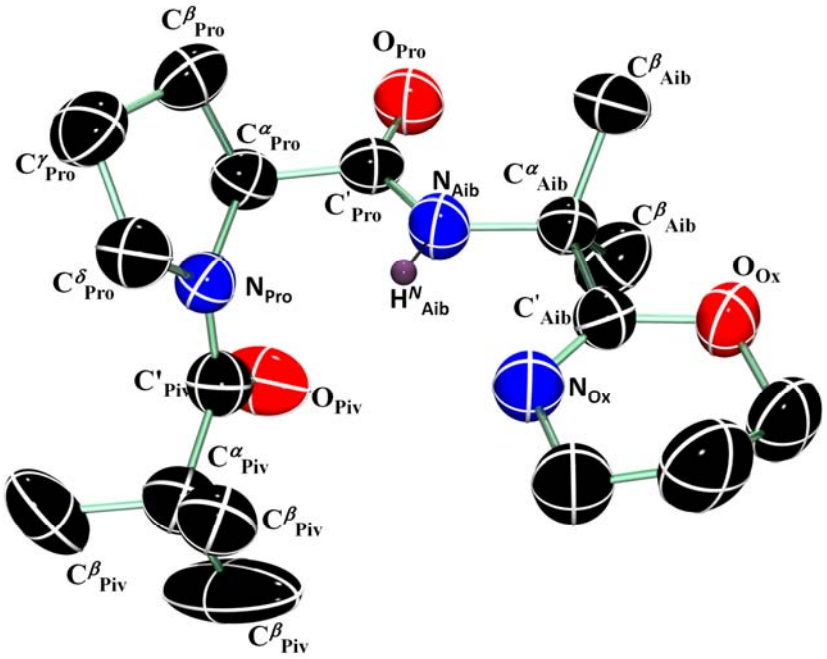
2

Oxazine containing peptide **2** was synthesized by following the above general procedure as a white solid (80 mg, 0.25 mmol, 100% yield) (m.p. = 137-138 °C) (TLC: DCM : MeOH (20 : 1) – R_f = 0.55). IR (NaCl, 10 mM in $CHCl_3$): 3335, 3024, 3002, 2989, 2973, 1681, 1663, 1602, 1516, 1457, 1406, 1364, 1259, 1158, 796 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.75 (bs, 1H), 4.52 (dd, J = 7.1, 2.9 Hz, 1H), 4.16 (t, J = 5.4 Hz, 2H), 3.73-3.67 (m, 2H), 3.35 (t, J = 6 Hz, 2H), 2.07-1.94 (m, 4H), 1.84 (quin, J = 5.6 Hz, 2H), 1.51 (s, 6H), 1.27 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 177.1, 170.8, 162.3, 65.2, 62.8, 55.4, 48.3, 41.7, 39.1, 27.6, 23.9, 23.8, 21.8; HRMS *m/z* for $C_{17}H_{30}N_3O_3$ $[M+H]^+$ calcd 324.2287, Found 324.2285; $[\alpha]_D^{20}$ = -104.9 (c = 1, $CHCl_3$).



2

S5.2. Figure S7: The bond lengths determined in the crystal structure of the A→I analogue **2** (values in parentheses are standard deviations).



2

S5.3. Figure S8: Illustration of an ORTEP-POV Ray rendered view of the A→I analogue **2**. The thermal ellipsoids are scaled to the 50% probability level.

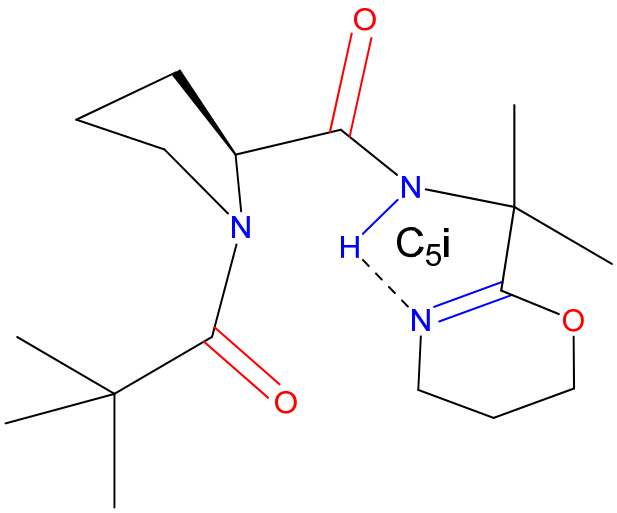
S5.4. Table 2: A list of selected dihedral angles obtained from the crystal structure of A→I analogue **2.**

Conformational Angles (deg)

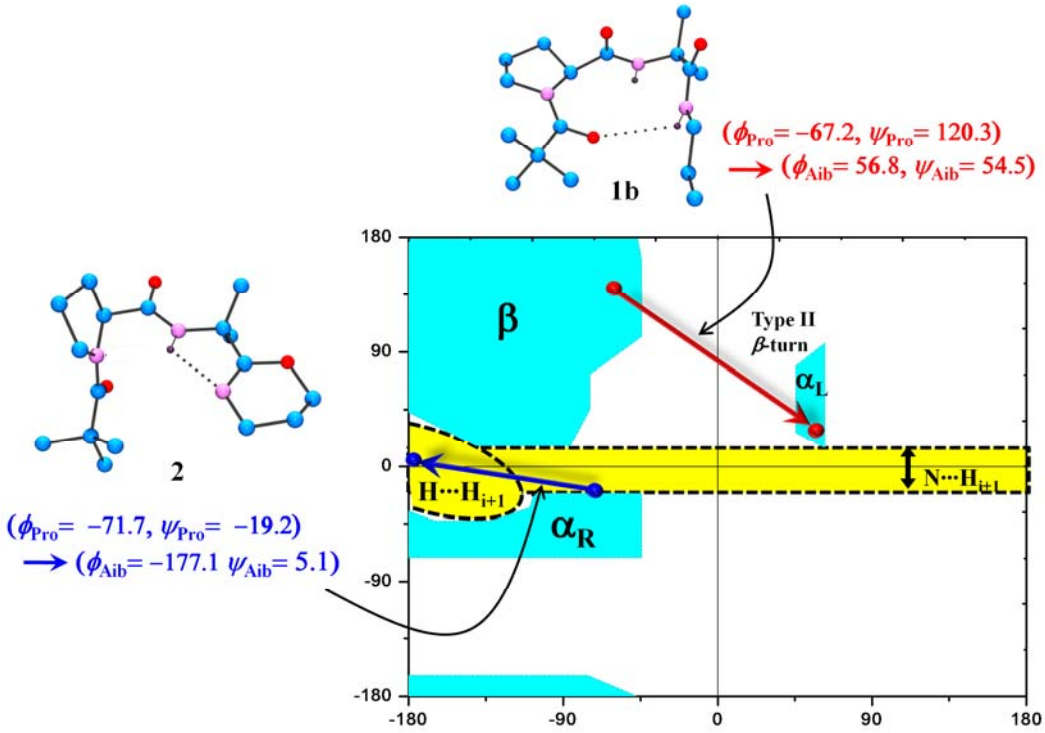
Peptide Backbone	**2**	Pyrrolidine Ring	**2**
$\omega_1(C^{\alpha}_{Piv}\text{-}C'_{Piv}\text{-}N_{Pro}\text{-}C^{\alpha}_{Pro})$	179.8(3)	$\theta(C^{\delta}_{Pro}\text{-}N_{Pro}\text{-}C^{\alpha}_{Pro}\text{-}C^{\beta}_{Pro})$	-3.1(3)
$\phi_1(C'_{Piv}\text{-}N_{Pro}\text{-}C^{\alpha}_{Pro}\text{-}C'_{Pro})$	-71.7(4)	$\chi^1_{Pro}(N_{Pro}\text{-}C^{\alpha}_{Pro}\text{-}C^{\beta}_{Pro}\text{-}C^{\gamma}_{Pro})$	-20.0(3)
$\psi_1(N_{Pro}\text{-}C^{\alpha}_{Pro}\text{-}C'_{Pro}\text{-}N_{Aib})$	-19.2(4)	$\chi^2_{Pro}(C^{\alpha}_{Pro}\text{-}C^{\beta}_{Pro}\text{-}C^{\gamma}_{Pro}\text{-}C^{\delta}_{Pro})$	35.2(4)
$\omega_2(C^{\alpha}_{Pro}\text{-}C'_{Pro}\text{-}N_{Aib}\text{-}C^{\alpha}_{Aib})$	-177.2(3)	$\chi^3_{Pro}(C^{\beta}_{Pro}\text{-}C^{\gamma}_{Pro}\text{-}C^{\delta}_{Pro}\text{-}N_{Pro})$	-36.2(3)
$\phi_2(C'_{Pro}\text{-}N_{Aib}\text{-}C^{\alpha}_{Aib}\text{-}C'_{Aib})$	-177.1(3)	$\chi^4_{Pro}(C^{\gamma}_{Pro}\text{-}C^{\delta}_{Pro}\text{-}N_{Pro}\text{-}C^{\alpha}_{Pro})$	24.5(3)
$\psi_2(N_{Aib}\text{-}C^{\alpha}_{Aib}\text{-}C'_{Aib}\text{-}N_{Ox})$	5.1(4)	$C^{\alpha}_{Pro}\text{-}N_{Pro}\cdots H_{Aib}\text{-}N_{Aib}$	-16.9
$\omega_3(C^{\alpha}_{Aib}\text{-}C^{2}_{Ox}\text{-}N_{Ox}\text{-}C^{6}_{Ox})$	178.8(3)	$N_{Aib}\text{-}H_{Aib}\cdots N_{Ox}\text{-}C'_{Aib}$	8.1

S5.5. Table 3. Data Collection and Refinement Parameters for Peptide **2**

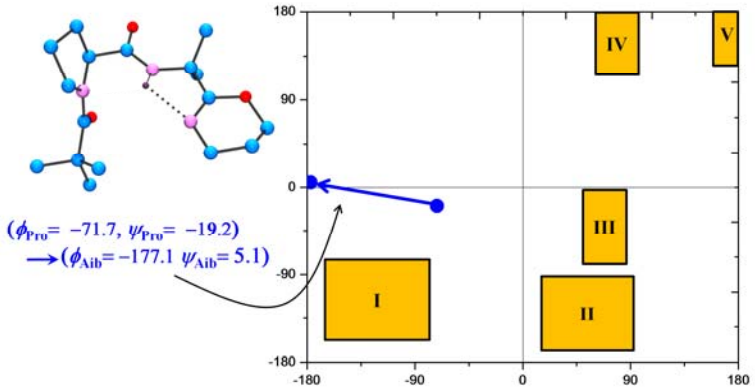
Empirical formula	$C_{17}H_{29}N_3O_3$
Crystal shape	colorless blocks
Crystal size (mm^3)	1862.4(14)
Crystallizing solvent	DCM/Hexane
Space group	P 21 21 21
Cell parameters	
a (Å)	10.100(5)
b (Å)	10.997(5)
c (Å)	16.768(5)
α (deg)	90.0
β (deg)	90.0
γ (deg)	90.0
Volume ($Å^3$)	1862.4(14)
Z	4
Molecular weight	1862.4(14)
Density (g/cm^3) (cal)	1.153
F (000)	704.0
Radiation (0.71073 Å)	Mo K_α
Temperature ($^\circ C$)	20
2θ max (deg)	52
Scan type	ω scan
Measured reflections	3668
Independent reflections	3668
Unique reflections	3668
Observed reflections [$\|F\| > 4\sigma(F)$]	2436
Final R (%)	5.32
Final wR2 (%)	15.41
Goodness-of-fit (*S*)	1.037
$\Delta\rho_{max}$ ($eÅ^{-3}$)	0.242
$\Delta\rho_{min}$ ($e Å^{-3}$)	-0.154
No. of restraints/ parameters	0/223
Data-param ratio	1.75 : 1.00



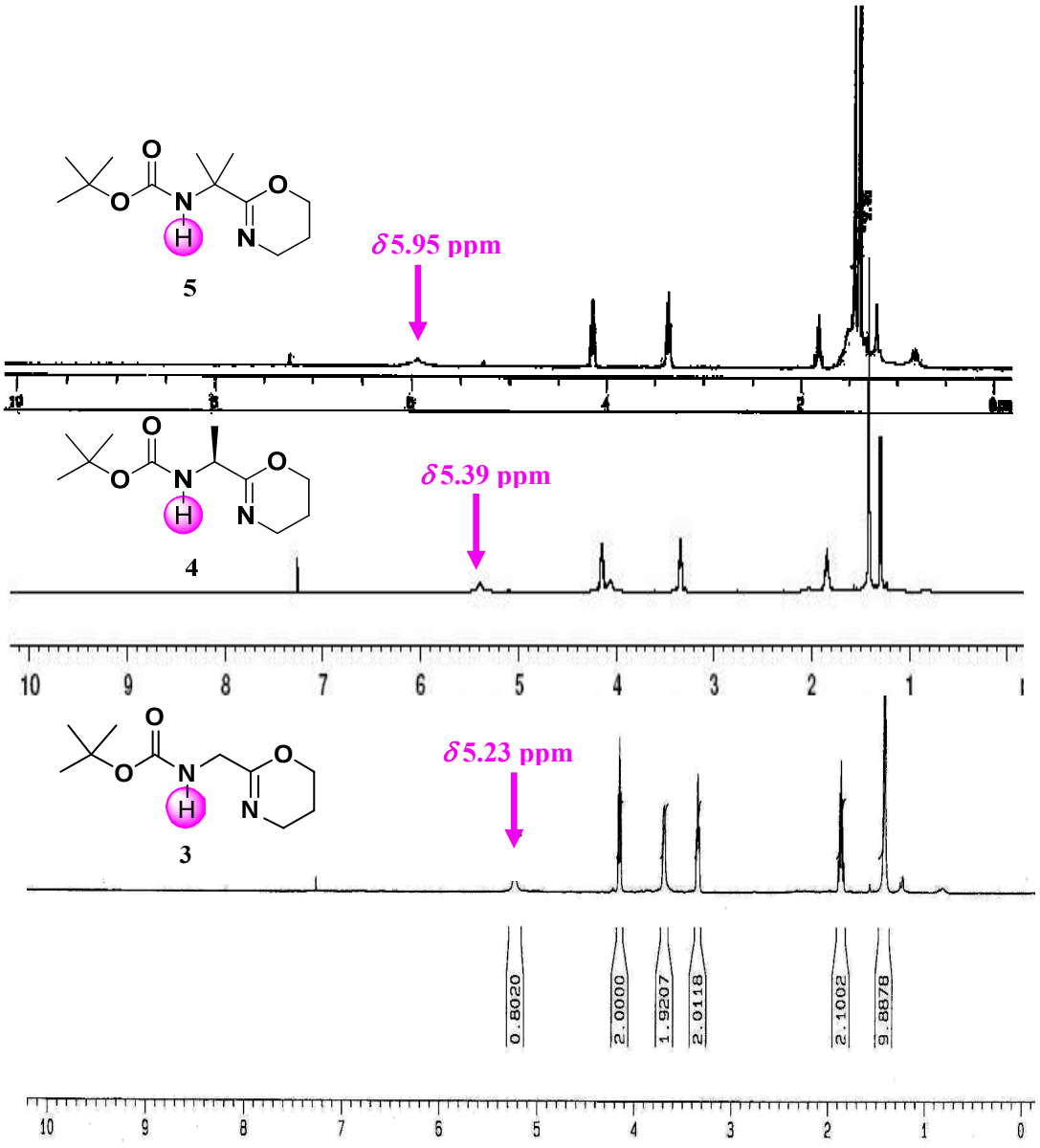
S5.6. Figure S9: Chemdraw diagram showing the C_5i hydrogen bonded 5-membered ring structure in the A→I analogue peptide **2**.



S6.1. Figure S10: Ramachandran map showing the allowed conformational space for peptide backbone (cyan); the arrow diagrams (Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425.) representing the type of turn present in the model peptide **1b** (red) and the A→I analogue peptide **2** (blue). The ϕ,ψ space that is disallowed for peptides due to the H···H_{i+1} and the H···N_{i+1} steric clashes are highlighted in yellow. Note: The $(\phi,\psi)_{Aib}$ falls in the disallowed space (ϕ=180±10, ψ= 0±10) in the A→I analogue peptide **2**.



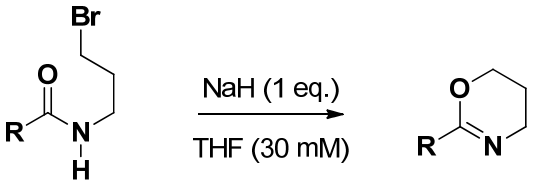
S6.2. Figure S11 : Ramachandran map showing the delineated clusters of disallowed ϕ,ψ angles (boxes highlighted in orange, identified by Roman numerals – Pal, D.; Chakrabarti, P. *Biopolymers* **2002**, *63*, 195.) that are observed in peptides; and the arrow diagrams representing the type of turn present in the model peptide **1b** (red) and the A→I analogue peptide **2** (blue).

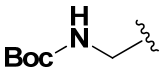
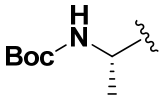
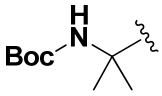
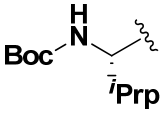
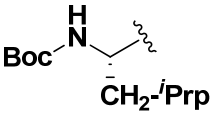
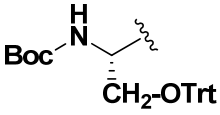
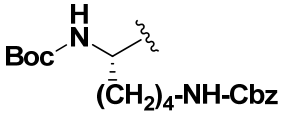
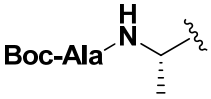
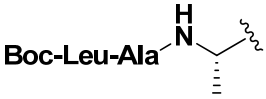
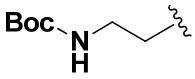


S7. Figure S12: The ^{1}H NMR spectra of the A→I analogue peptides **3**, **4** (400 MHz, $CDCl_3$, 10mM) and **5** (300 MHz, $CDCl_3$, 10mM). The chemical shift values of the carbamate NH are indicated with reference to the peak for $CHCl_3$ at δ 7.26 ppm.

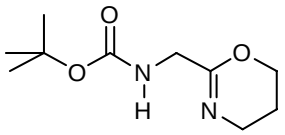
S8. Synthesis of N-(3-Hydroxypropyl)amides from Oxazines.

S8.1. Table 4. Synthesis of oxazine containing peptides (A ⟶ I mutants)



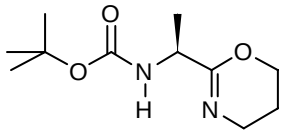
S.No	R	Reactant	Product	Yield
1		**14**	**3**	97
2		**15**	**4**	100
3		**16**	**5**	98
4		**17**	**6**	97
5		**18**	**7**	98
6		**19**	**8**	98
7		**20**	**9**	98
8		**21**	**10**	100
9		**22**	**11**	98
10		**23**	**12**	97

S8.2. 2-((N-*tert*-Butyloxycarbonyl)amino)-Methyl)-5,6-Dihydro-4H-1,3-Oxazine (3): The oxazine containing peptide **3** was synthesized by following the above general procedure, as a viscous oil (58 mg, 0.27 mmol, 97%) (TLC: EtOAc – R_f = 0.11). IR (NaCl, neat): 3392, 2976, 2931, 2870, 1704, 1694, 1518, 1368, 1168, 1062 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 5.23 (s, 1H), 4.15 (t, J = 5.7 Hz, 2H), 3.69 (d, J = 4.2 Hz, 2H), 3.34 (t, J = 5.7 Hz, 2H), 1.86 (quin, J = 5.7 Hz, 2 H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 156.3, 155.6, 79.3, 65, 42.3, 41.7, 28.3, 21.8; HRMS *m/z* for $C_{10}H_{18}N_2O_3Na$ $[M+Na]^+$ calcd 237.1215, Found 237.1219.



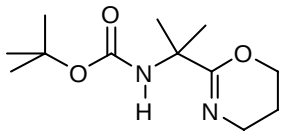
3

S8.3. 2-((S)-1-(N-*tert*-Butyloxycarbonyl)amino)-Ethyl)-5,6-Dihydro-4H-1,3-Oxazine (4): The oxazine containing peptide **4** was synthesized by following the above general procedure, as a viscous oil (21 mg, 0.09 mmol, 100%) (TLC: EtOAc – R_f = 0.15). IR (NaCl, neat): 3404, 2956, 2925, 2855, 1719, 1682, 1492, 1456, 1366, 1167, 1061 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 5.39 (bs, 1H), 4.19-4.06 (m, 3H), 3.35-3.32 (m, 2H), 1.88-1.81 (m, 2H), 1.41 (s, 9H), 1.36 (d, J = 7.2 Hz , 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 160, 155, 79.1, 65, 48.8, 41.7, 28.3, 21.8, 19.8; HRMS *m/z* for $C_{11}H_{20}N_2O_3Na$ $[M+Na]^+$ calcd 251.1372, Found 251.1374.



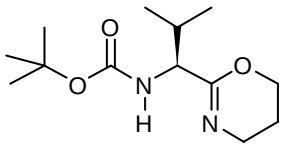
4

S.8.4. 2-{(1-(S)-(N-tert-Butyloxycarbonyl)amino)-1-Methyl)-Ethyl}-5,6-Dihydro-4H-1,3-Oxazine (5): The oxazine containing peptide **5** was synthesized by following the above general procedure, as a viscous oil (37 mg, 0.15 mmol, 98%) (TLC: EtOAc – R_f = 0.25). IR (NaCl, neat): 3375, 2978, 2930, 2869, 1715, 1674, 1495, 1454, 1294, 1160, 1067 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 5.95 (bs, 1H), 4.16 (t, J = 5.7 Hz, 2H), 3.38 (t, J = 5.7 Hz, 2H), 1.84 (quin, J = 5.7 Hz, 2H), 1.47 (s, 6H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 162.5, 154.3, 78.6, 65.2, 54.9, 41.8, 28.4, 28.3, 21.7; HRMS *m/z* for $C_{12}H_{23}N_2O_3[M+H]^+$ calcd 243.1709, Found 243.1706.

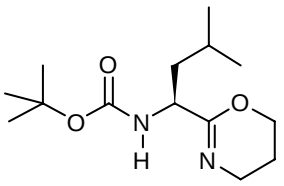


5

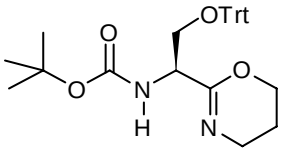
S8.5. 2-{(1-(*S*)-(N-*tert*-Butyloxycarbonyl)amino)-2-Methyl)-Propyl}-5,6-Dihydro-4*H*-1,3-Oxazine (6): The oxazine containing peptide **6** was synthesized by following the above general procedure, as a viscous oil (36 mg, 0.14 mmol, 97%) (TLC: EtOAc – R_f = 0.35). IR (NaCl, neat): 3447, 3017, 2964, 2919, 2851, 1700, 1684, 1507, 1498, 131368, 1216, 1158, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 5.20 (d, *J* = 7.9 Hz, 1H), 4.13 (td, *J* = 5.8, 4.8 Hz, 2H), 3.94 (dd, *J* = 7.7, 5.1 Hz, 1H), 3.34 (t, *J* = 5.6 Hz, 2H), 2.06-1.95 (m, 1H), 1.83 (quin, *J* = 5.7 Hz, 2H), 1.40 (s, 9H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 158.9, 155.6, 79, 64.8, 57.8, 41.7, 31.7, 28.3, 21.8, 19.1, 17.5; HRMS *m/z* for $C_{13}H_{24}N_2O_3Na$ $[M+Na]^+$ calcd 279.1685, Found 279.1691.


6

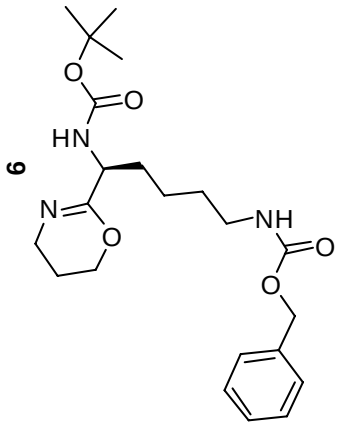
S8.6. 2-{(1-(*S*)-(N-*tert*-Butyloxycarbonyl)amino-3-Methyl)-Butyl}-5,6-Dihydro-4*H*-1,3-Oxazine (7): The oxazine containing peptide **7** was synthesized by following the above general procedure, as a viscous oil (53 mg, 0.2 mmol, 98%) (TLC: EtOAc – R_f = 0.41). IR (NaCl, neat): 3396, 2957, 2870, 1717, 1679, 1498, 1389, 1366, 1250, 1174, 1026 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 5.09 (d, *J* = 7 Hz, 1H), 4.17-4.07 (m, 3H), 3.32 (q, *J* = 5.5 Hz, 2H), 1.83 (quin, *J* = 5.6 Hz, 2H), 1.65 (sep, *J* = 6.7 Hz, 1H), 1.51-1.44 (m, 2H), 1.40 (s, 9H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 160.2, 155.2, 79, 64.9, 51.8, 43.3, 41.8, 28.3, 24.7, 23, 22.3, 21.8; HRMS *m/z* for $C_{14}H_{27}N_2O_3$ $[M+H]^+$ calcd 271.2022, Found 271.2020.


7

S8.7. 2-(1-(*S*)-(N-(*tert*-Butyloxycarbonyl)amino)-2-((O-Trityl)hydroxy)-Ethyl)-5,6-Dihydro-4H-1,3-Oxazine (8): The oxazine containing peptide **8** was synthesized by following the above general procedure, as a viscous oil (42 mg, 0.09 mmol, 98%) (TLC: EtOAc – R_f = 0.31). IR (NaCl, neat): 3410, 3034, 2976, 2890, 1714, 1683, 1492, 1450, 1365, 1230, 1168, 1080, 1066, 910, 734, 706 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.46 (d, *J* = 7.6 Hz, 6H), 7.33-7.23 (m, 10H), 5.75 (d, *J* = 7.6 Hz, 1H), 4.28-4.27 (m, 1H), 4.15-4.08 (m, 2H), 3.48 (t, *J* = 5.4 Hz, 2H), 3.44-3.42 (m, 1H), 3.29 (dd, *J* = 8.6, 3.3 Hz, 1H), 1.93-1.88 (m, 2H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 157.2, 155.1, 143.9, 128.6, 127.7, 126.9, 85.9, 79.2, 65, 63.8, 53.2, 41.9, 28.4, 21.8; HRMS *m/z* for $C_{30}H_{34}N_2O_4Na$ $[M+Na]^+$ calcd 509.2416, Found 509.2416.


8

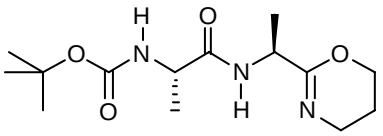
S8.8. 2-(1-(*S*)-(N-(*tert*-Butyloxycarbonyl)amino)-5-((N-benzyloxycarbonyl)amino)-Pentyl)-5,6-Dihydro-4H-1,3-Oxazine (9)



6

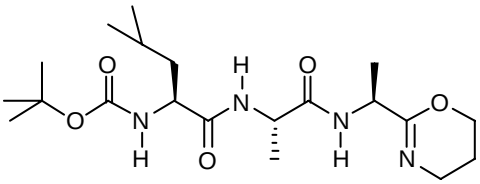
Oxazine containing peptide **9** was synthesized by following the above general procedure as a viscous oil (74 mg, 0.18 mmol, 98% yield) (TLC: EtOAc – R_f = 0.17). IR (NaCl, 10 mM in $CHCl_3$): 3450, 3014, 2933, 2867, 1710, 1678, 1513 (br), 1368, 1235, 1212, 1169, 1063 cm^{-1}; ^1H NMR (400 MHz , $CDCl_3$) δ ppm: 7.36–7.30 (m, 5H), 5.38 (d, *J* = 6.9 Hz, 1H), 5.14 (bs, 1H), 5.09 (s, 2H), 4.20-4.12 (m, 1H), 4.15 (q, *J* = 5.3 Hz, 1H), 4.10-4.05 (m, 1H), 3.36 (t, *J* = 5.6 Hz, 2H), 3.18 (q, *J* = 6.2 Hz, 2H), 1.85 (q, *J* = 5.3 Hz, 2H), 1.79-1.73 (m, 1H), 1.61-1.51 (m, 3H), 1.43 (s, 9H), 1.38-1.33 (m, 2H); ^{13}C NMR (100 MHz ,$CDCl_3$) δ ppm: 159.2, 156.4, 155.3, 136.6, 128.4, 128, 127.9, 79.2, 66.4, 64.9, 52.5, 41.6, 40.7, 33.2, 29.2, 28.3, 22, 21.7; HRMS *m/z* Calcd for $C_{22}H_{33}N_3O_5Na$ 442.2318 , Found 442.2316.

S8.9. 2-[{1-(*S*)-N-(2-(*S*)-(N-(*tert*-Butyloxycarbonyl)amino)-Propanoyl)amino}-Ethyl]-5,6-Dihydro-4H-1,3-Oxazine (10): The oxazine containing peptide **10** was synthesized by following the above general procedure, as a viscous oil (21 mg, 0.07 mmol, 100%) (TLC: DCM : MeOH (10 : 1) – R_f = 0.53). IR (NaCl, neat): 3301, 2978, 2929, 2857, 1714, 1677, 1668, 1517, 1454, 1368, 1248, 1168, 1069, 1020 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 6.97 (d, *J* = 5.2 Hz, 1H), 5.08 (bs, 1H), 4.22 (quin, *J* = 6.8 Hz, 1H), 4.16-4.07 (m, 3H), 3.28 (t, *J* = 5.7 Hz, 2H), 1.80 (quin, *J* = 5.4 Hz, 2H), 1.38 (s, 9H), 1.28 (d, *J* = 6.6 Hz, 3H), 1.26 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 171.4, 159.6, 155.2, 79.8, 65.1, 50.1, 47.6, 41.6, 28.3, 21.8, 19.3, 18.7; HRMS *m/z* for $C_{14}H_{25}N_3O_4Na$ $[M+Na]^+$ calcd 322.1743, Found 322.1733.



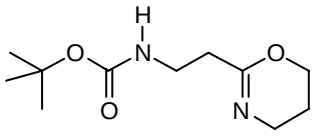
10

S8.10. 2-[{1-(*S*)-{2-(*S*)-N-(2-(*S*)-(N-(*tert*-Butyloxycarbonyl)amino)-4-Methyl-Pentanoyl)-Aminopropanoyl}-amino}-Ethyl]-5,6-Dihydro-4H-1,3-Oxazine (11): The oxazine containing peptide **11** was synthesized by following the above general procedure, as a viscous oil (24 mg, 0.06 mmol, 98%) (TLC:EtOAc– R_f = 0.17). IR (NaCl, neat): 3307, 3295, 2959, 2872, 1717, 1684, 1670, 1656, 1525, 1450, 1388, 1368, 1239, 1165, 1138, 1047 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 6.98 (d, J = 5.8 Hz, 1H), 6.73 (d, J = 7.3 Hz, 1H), 4.96 (d, J = 7.5 Hz, 1H), 4.43 (quin, J = 7.1 Hz, 1H), 4.26 (quin, J = 6.7 Hz, 1H), 4.2-4.13 (m, 3H), 3.34 (t, J = 5.7 Hz, 2H), 1.88-1.84 (m, 2H), 1.69-1.63 (m, 2H), 1.51-1.45 (m, 1H), 1.43 (s, 9H), 1.36 (d, J = 7.2 Hz, 3H), 1.31(d, J = 6.8 Hz, 3H), 0.93 (d, J = 6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 172.2, 170.7, 159.5, 155.7, 80, 65.2, 53, 48.9, 47.7, 41.7, 41.4, 28.3, 24.7, 23, 22, 21.8, 19.2, 18.5; HRMS *m/z* for $C_{20}H_{36}N_4O_5Na$ $[M+Na]^+$ calcd 435.2583, Found 435.2582.



11

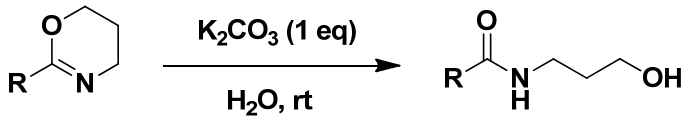
S8.11. 2-((*S*)-2-(N-*tert*-Butyloxycarbonyl)-amino)-Ethyl)-5,6-Dihydro-4H-1,3-Oxazine (12): The oxazine containing peptide **12** was synthesized by following the above general procedure, as a viscous oil (37 mg, 0.16 mmol, 97%) (TLC: EtOAc – R_f = 0.31). IR (NaCl, neat): 3348, 2955, 2926, 2859, 1710, 1674, 1511, 1366, 1276, 1245, 1170, 1077 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 5.24 (bs, 1H), 4.12 (t, J = 5.6 Hz, 2H), 3.39-3.27 (m, 4H), 2.25 (t, J = 6.4 Hz, 2H), 1.83 (quin, J = 6 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 158.6, 155.8, 78.9, 64.8, 41.9, 35, 29.6, 28.4, 21.7; HRMS *m/z* for $C_{11}H_{21}N_2O_3$ $[M+H]^+$ calcd 229.1552, Found 229.1554.

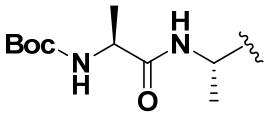
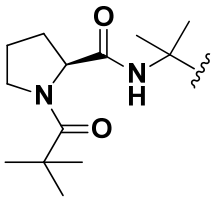


12

S9. Synthesis of N-(3-hydroxypropyl)amides.

S9.1. Table 5. Synthesis of N-(3-Hydroxypropyl)amides from Oxazines (I ⟶ A mutation)

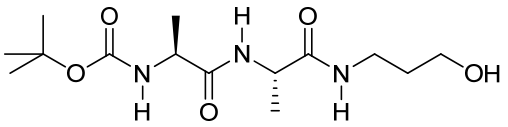


Entry	R	Product	Time (h)	Yield (%)
1		24	6.5	93
2		1d	33	100

S9.2. General procedure for synthesis of N-(3-hydroxypropyl)amides from Oxazines:

To a stirred solution of the oxazine (1 mmol) in distilled water (3 mL) was added potassium carbonate (K_2CO_3) (1 mmol) and stirred until TLC analysis indicated the complete consumption of the oxazine. The resulting mixture was extracted with EtOAc (2 x 5 mL) and the organic layer was dried (Na_2SO_4) and concentrated under high vacuum to yield a residue which was purified by silica gel flash column chromatography to get the desired product.

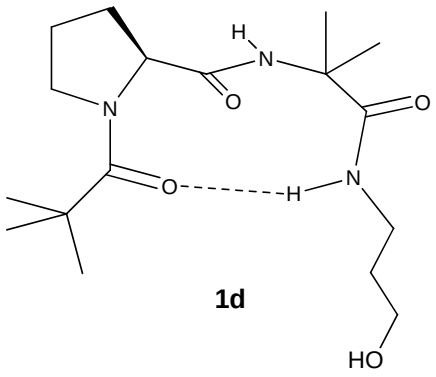
S9.2.1. N′-(3′-Hydroxypropyl)-2-((S)-(2-(S)-(N-*tert*-butyloxycarbonyl)-aminopropanoyl)-aminopropanoyl)-Propanamide (24): Amide **24** was synthesized by following the general procedure and purified by silica gel flash column chromatograph (EtOAc) to yield the desired product as a white solid (49 mg, 0.16 mmol, 93%) (m.p. = 135-136 °C) (TLC: DCM : MeOH (10 : 1) – R_f = 0.46). IR (NaCl, neat): 3307, 2937, 2886, 1688, 1648, 1533, 1454, 1367, 1252, 1166, 1071 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 6.87 (bs, 1H), 6.62 (d, J = 7.2 Hz, 1H), 4.97 (bs, 1H), 4.45 (quin, J = 7.2 Hz, 1H), 4.10 (quin, J = 6.4 Hz, 1H), 3.61 (t, J = 5.6 Hz, 2H), 3.44-3.36 (m, 2H), 3.29 (bs, 1H), 1.69 (quin, J = 5.6 Hz, 2H), 1.45 (s, 9H), 1.40 (d, J = 7.2 Hz, 3H), 1.38 (d, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.1, 172.8, 155.6, 80.7, 59.2, 50.8, 49, 36.2, 31.9, 28.3, 18; HRMS *m/z* for $C_{14}H_{27}N_3O_5Na$ $[M+Na]^+$ calcd 340.1848, Found 340.1848.



24

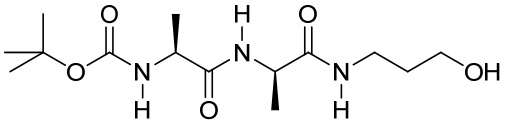
S9.2.2. N′-(3′-Hydroxypropyl)-2-Methyl-2-((S)-((N-Pivaloyl)-Pyrrolidine-2-Carbonyl)amino)-Propanamide (1d)

Amide **1d** was synthesized by following the general procedure and purified by silica gel flash column chromatograph (DCM : MeOH – 20 : 1) to yield the desired product as a white solid (48 mg, 0.14 mmol, 79% yield) (m.p. = 169-170 °C) (TLC: DCM : MeOH (10 : 1) – R_f = 0.41). IR (NaCl, 10 mM in $CHCl_3$): 3433, 3353, 3025, 3006, 1697, 1647, 1596, 1542, 1508, 1418, 1365, 1236, 1063, 912 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.49 (bs, 1H), 6.05 (bs, 1H), 4.19 (dd, J = 8.1, 5.6 Hz, 1H), 3.76 (t, J = 6.4 Hz, 2H), 3.62-3.58 (m, 2H), 3.44-3.33 (m, 2H), 2.23-2.13 (m, 1H), 2.11-2.02 (m, 1H), 2.06 (bs, 1H), 1.98-1.91 (m, 2H), 1.74-1.59 (m, 2H), 1.58 (s, 3H), 1.46 (s, 3H), 1.27 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 178.2, 175.2, 172.2, 63.4, 58.7, 57.6, 48.9, 38.9, 35.9, 32.3, 27.8, 27.2, 26.2, 24.2; HRMS *m/z* for $C_{17}H_{31}N_3O_4Na$ $[M+Na]^+$ calcd 364.2212, Found 364.2215; $[\alpha]_D^{20}$ = -1.3 (c = 1, $CHCl_3$).



1d

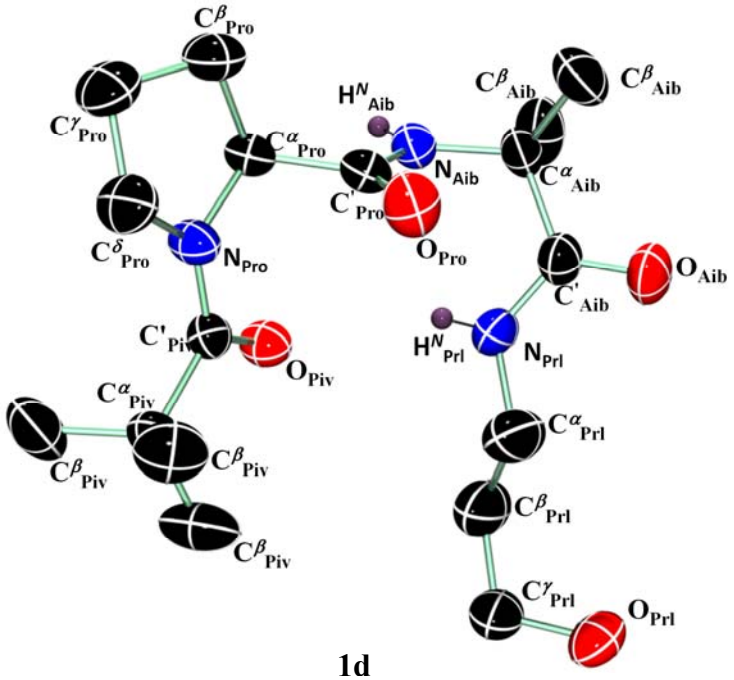
S9.2.3. N′-(3′-Hydroxypropyl)-2-((R)-(2-(S)-(N-*tert*-butyloxycarbonyl)-aminopropanoyl)-aminopropanoyl)-Propanamide (24LD): Amide **24LD** was synthesized by general procedure as described in section **S3.2** and purified by silica gel flash column chromatograph (EtOAc) to yield the desired product as a white solid (129 mg, 0.41 mmol, 71%) (m.p. = 164-165 °C) (TLC: DCM : MeOH (10 : 1) – R_f = 0.44). IR (NaCl, neat): 3338, 2979, 2880, 1664, 1536, 1364, 1251, 1166, 1072 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.19 (bs, 1H), 6.90 (d, *J* = 7.3 Hz, 1H), 5.23 (d, *J* = 4.9 Hz, 1H), 4.46 (quin, *J* = 7.4 Hz, 1H), 4.08 (quin, *J* = 7.0 Hz, 1H), 3.62 (t, *J* = 5.7 Hz, 2H), 3.45-3.32 (m, 2H), 3.07 (bs, 1H), 1.68 (quin, *J* = 5.6 Hz, 2H), 1.43 (s, 9H), 1.39 (d, *J* = 7.1 Hz, 3H), 1.35 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.03, 172.97, 155.9, 80.5, 59.5, 50.6, 49.1, 36.5, 31.9, 28.3, 17.9; HRMS *m/z* Calcd for $C_{14}H_{27}N_3O_5Na$ 340.1848, Found 340.1848.



24LD

S9.3. Table 6. Data Collection and Refinement Parameters for Peptide **1d**.

Empirical formula	$C_{17}H_{31}N_3O_4$
Crystal shape	Colorless blocks
Crystal size (mm^3)	974.3(10)
Crystallizing solvent	DCM/Hexane
Space group	P21
Cell parameters	
a (Å)	6.031(5)
b (Å)	8.634(5)
c (Å)	18.780(5)
α (deg)	90.0
β (deg)	94.9(5)
γ (deg)	90.0
Volume ($Å^3$)	974.3(10)
Z	2
Molecular weight	341.45
Density (g/cm^3) (cal)	1.164
F (000)	372.0
Radiation (0.71073 Å)	Mo K_α
Temperature ($^\circ$C)	20
2θ max (deg)	51.98
Scan type	ω scan
Measured reflections	3746
Independent reflections	3746
Unique reflections	3746
Observed reflections [$\|F\| > 4\sigma(F)$]	3005
Final R (%)	5.68
Final wR2 (%)	15.03
Goodness-of-fit (*S*)	1.066
$\Delta\rho_{max}$ ($eÅ^{-3}$)	0.571
$\Delta\rho_{min}$ ($e\ Å^{-3}$)	-0.288
No. of restraints/ parameters	1/223
Data-param ratio	1.83 : 0.98



1d

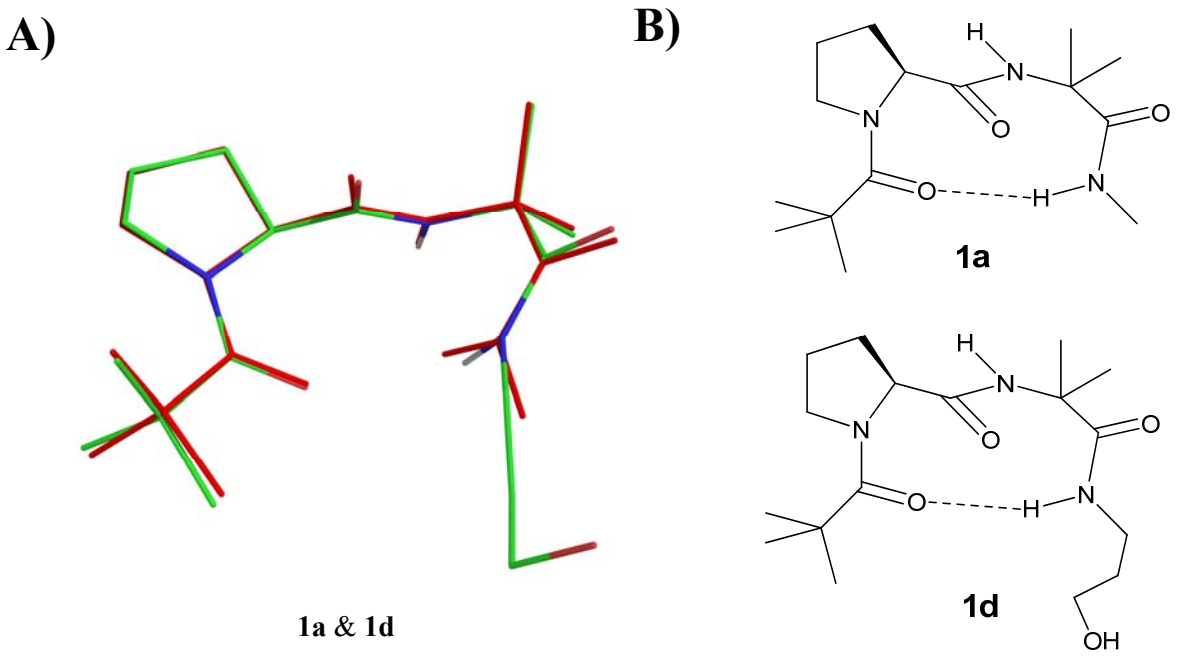
S9.4. Figure S13: Illustration of an ORTEP-POV Ray rendered view of the N-(3-hydroxypropyl)amide **1d**. The thermal ellipsoids are scaled to the 50% probability level.

S9.5. Table 7: Comparison of selected dihedral angles in the crystal structures of peptides **1a** and **1d**

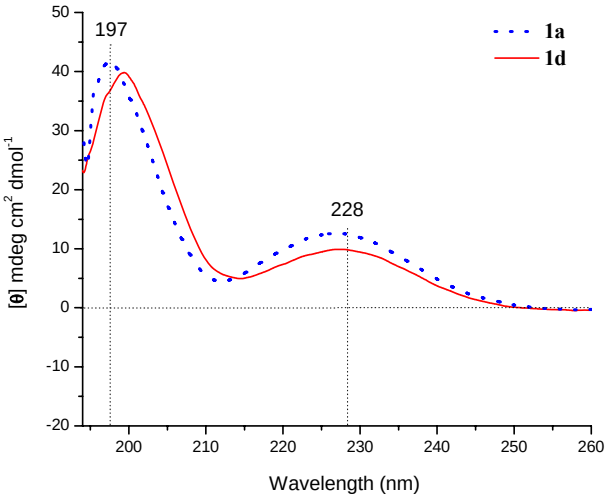
Conformational Angles (deg)

Peptide Backbone	**1a**[a]	**1d**	Pyrrolidine Ring	**1a**[a]	**1d**
$\omega_1(C^{\alpha}_{Piv}\text{-}C'_{Piv}\text{-}N_{Pro}\text{-}C^{\alpha}_{Pro})$	175.1(3)	179.5(2)	$\theta(C^{\delta}_{Pro}\text{-}N_{Pro}\text{-}C^{\alpha}_{Pro}\text{-}C^{\beta}_{Pro})$	0.8(6)	-2.3(3)
$\phi_1(C'_{Piv}\text{-}N_{Pro}\text{-}C^{\alpha}_{Pro}\text{-}C'_{Pro})$	-57.8(6)	-60.4(3)	$\chi^1_{Pro}(N_{Pro}\text{-}C^{\alpha}_{Pro}\text{-}C^{\beta}_{Pro}\text{-}C^{\gamma}_{Pro})$	-26.2(6)	-21.9(3)
$\psi_1(N_{Pro}\text{-}C^{\alpha}_{Pro}\text{-}C'_{Pro}\text{-}N_{Aib})$	139.2(5)	139.9(2)	$\chi^2_{Pro}(C^{\alpha}_{Pro}\text{-}C^{\beta}_{Pro}\text{-}C^{\gamma}_{Pro}\text{-}C^{\delta}_{Pro})$	40.5(7)	37.7(4)
$\omega_2(C^{\alpha}_{Pro}\text{-}C'_{Pro}\text{-}N_{Aib}\text{-}C^{\alpha}_{Aib})$	-179.3(5)	177.7(2)	$\chi^3_{Pro}(C^{\beta}_{Pro}\text{-}C^{\gamma}_{Pro}\text{-}C^{\delta}_{Pro}\text{-}N_{Pro})$	-39.7(7)	-38.5(4)
$\phi_2(C'_{Pro}\text{-}N_{Aib}\text{-}C^{\alpha}_{Aib}\text{-}C'_{Aib})$	61.4(7)	57.2(3)	$\chi^4_{Pro}(C^{\gamma}_{Pro}\text{-}C^{\delta}_{Pro}\text{-}N_{Pro}\text{-}C^{\alpha}_{Pro})$	24.8(7)	25.5(4)
$\psi_2(N_{Aib}\text{-}C^{\alpha}_{Aib}\text{-}C'_{Aib}\text{-}N_{Me})$	25.1(7)	28.0(4)			
$\omega_3(C^{\alpha}_{Aib}\text{-}C'_{Aib}\text{-}N_{Met}\text{-}C_{Met})$ (**1a**) $\omega_3(C^{\alpha}_{Aib}\text{-}C'_{Aib}\text{-}N_{Me}\text{-}C^{\alpha}_{Prl})$ (**1b**)	176.9(5)	-165.2(3)			

[a] Prasad, B. V. V.; Balaram, H.; Balaram, P. Biopolymers **1982**, *21*, 1261.



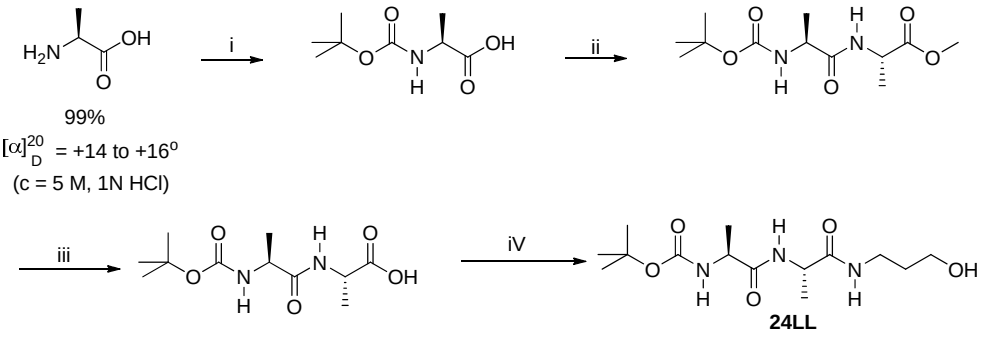
S9.7. Figure S14: Stick diagram representing the crystal structure of the peptide **1d** (red) and the reference peptide **1a** (green) superimposed on one another (RMSD of relevant atoms excluding hydrogens = 0.025 Å). B) & C) Chemdraw diagrams of the peptides **1a** & **1d** showing the 4→1 intramolecular hydrogen bonding interactions.



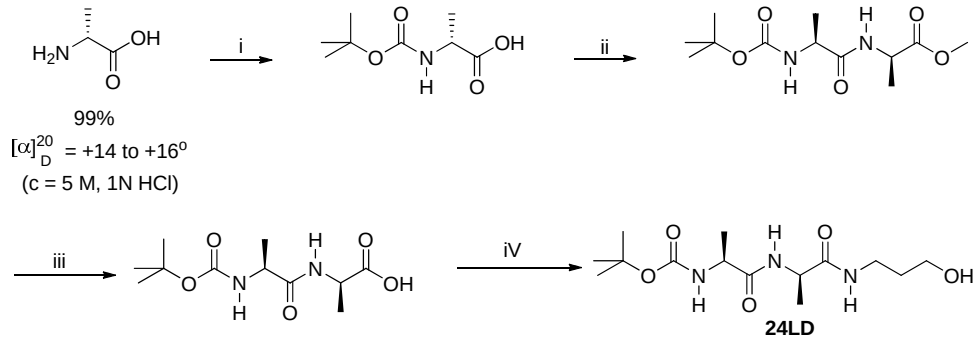
S9.8. Figure S15: CD spectra of the model compounds **1d [____]**, **1a [· · · ·]** in MeOH (1 mM) at 20 °C.

S10.1 Optical rotational studies of 24: The compounds **24LL**, **24LD** and **24** were synthesized as shown in the following schemes (1, 2) and their specific rotation values in different solvents were compared with those of the enantiomerically enriched N-(3-hydroxypropyl)amides **24LL** and **24LD**, synthesized as shown in scheme 1. The specific rotation values of **24**, **24LL** and **24LD** (Table 4) in different solvents are comparable (within the error range), indicating that there is no discernible epimerization at the C^{α} of the Alanine (the C-terminal aminoacid), under the conditions (1 equivalent NaH, THF) of cyclo-*O*-alkylation of the N-(3-bromopropyl) peptide **24**.

Scheme 1

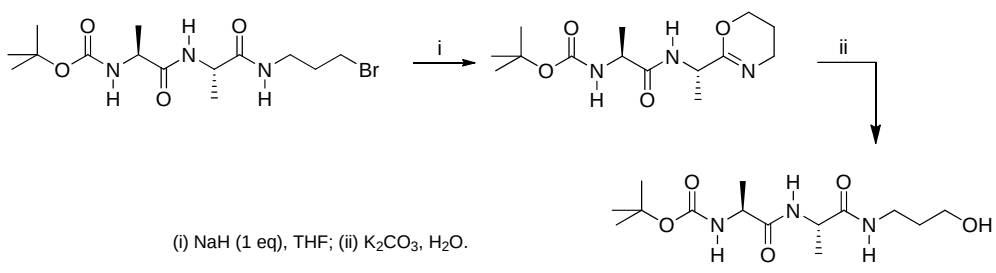


(i) $(Boc)_2O$, THF/H_2O, K_2CO_3; (ii) EtOCOCl, NMM, L-Methylalaninatehydrochloride, -20 to 0 °C; (iii) LiOH, $MeOH/H_2O$; (iV) EtOCOCl, NMM, $H_2N(CH_2)_3OH$, -20 to 0 °C.



(i) $(Boc)_2O$, THF/H_2O, K_2CO_3; (ii) EtOCOCl, NMM, D-Methylalaninatehydrochloride, -20 to 0 °C; (iii) LiOH, $MeOH/H_2O$; (iV) EtOCOCl, NMM, $H_2N(CH_2)_3OH$, -20 to 0 °C.

Scheme 2



(i) NaH (1 eq), THF; (ii) K_2CO_3, H_2O.

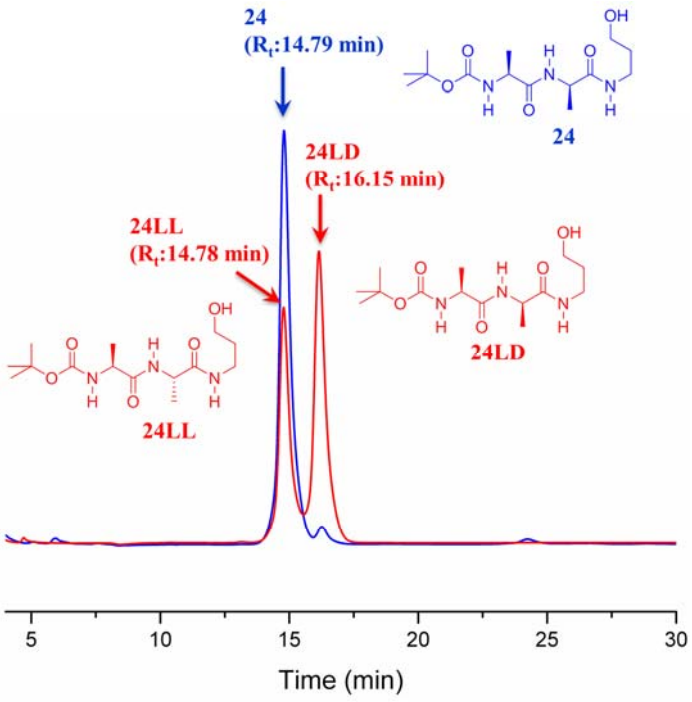
Table 4 : Specific rotation ($[\alpha]_D^{20}$) values for the compounds **24**, **24LL** and **24LD** in various solvents.

Solvent[a]	**24**	**24LL**	**24LD**
$CHCl_3$	-42.2 ± 1.0	-43.0±0.3	+21.7± 1.2
CH_2Cl_2	-40.6 ± 1.4	-38.4± 0.8	+13.5 ± 0.8
MeOH	-35.7 ± 0.4	-37.8±0.3	+14.0 ± 0.4

[a] (c = 1% by weight in solvent, at 20 °C); [b] all values are average of two measurements.

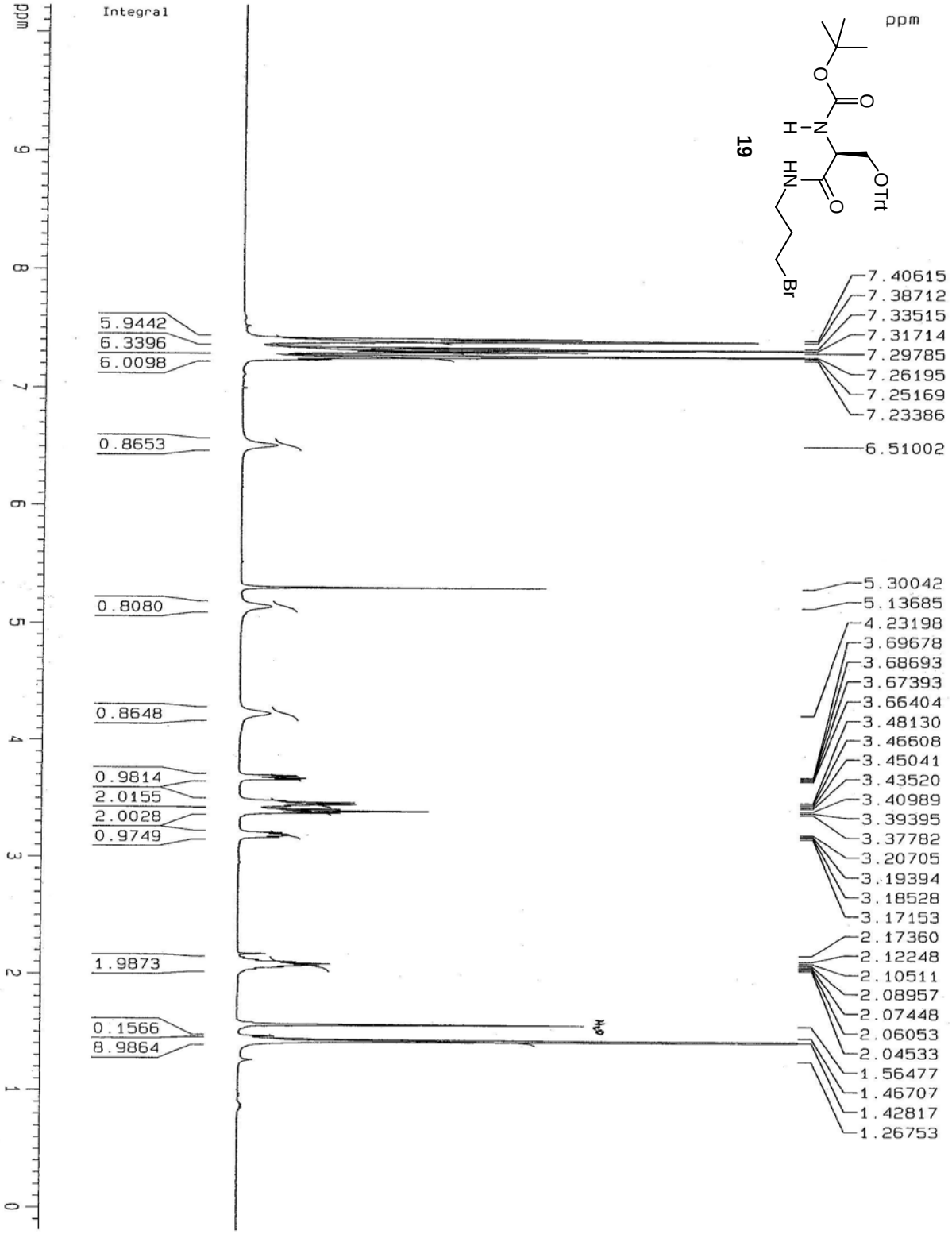
S10.2. HPLC studies of 24: High Performance Liquid Chromatography (HPLC) was performed on a Shimadzu System Eco chromatograph (Kyoto, Japan) equipped with a model LC-20AP variable wavelength UV detector and a injector fitted with a 20 µL sample loop. Ascentis C_{18} (5 µm, 4.6 x 250 mm) column was used. Isocratic RP-HPLC was done with a mixture of water and MeOH (13:7 v/v) at 1 mL/min flow rate, with the UV-vis detector to absorb at 214 nm.

Figure S16: RP-HPLC chromatogram of the dipeptide alcohols (**24LL, 24LD, 24**). The red chromatogram is for the elution of a pre-mixed solution of **24LL** (R_t =14.78 min) and **24LD** (R_t =16.15 min) in MeOH. The blue chromatogram is for the elution of a solution of **24** (R_t = 14.79 min) in MeOH. The relative intensity of the minor peak (R_t =16.15 min) in the blue chromatogram is 1.76 % compared to the intensity of the major peak (R_t = 14.79 min) (98.24%). The syntheses of **24LL** and **24LD** were accomplished by using 99% enantiopure L-Ala and D-Ala obtained from Spectrochem, India, Pvt Ltd.

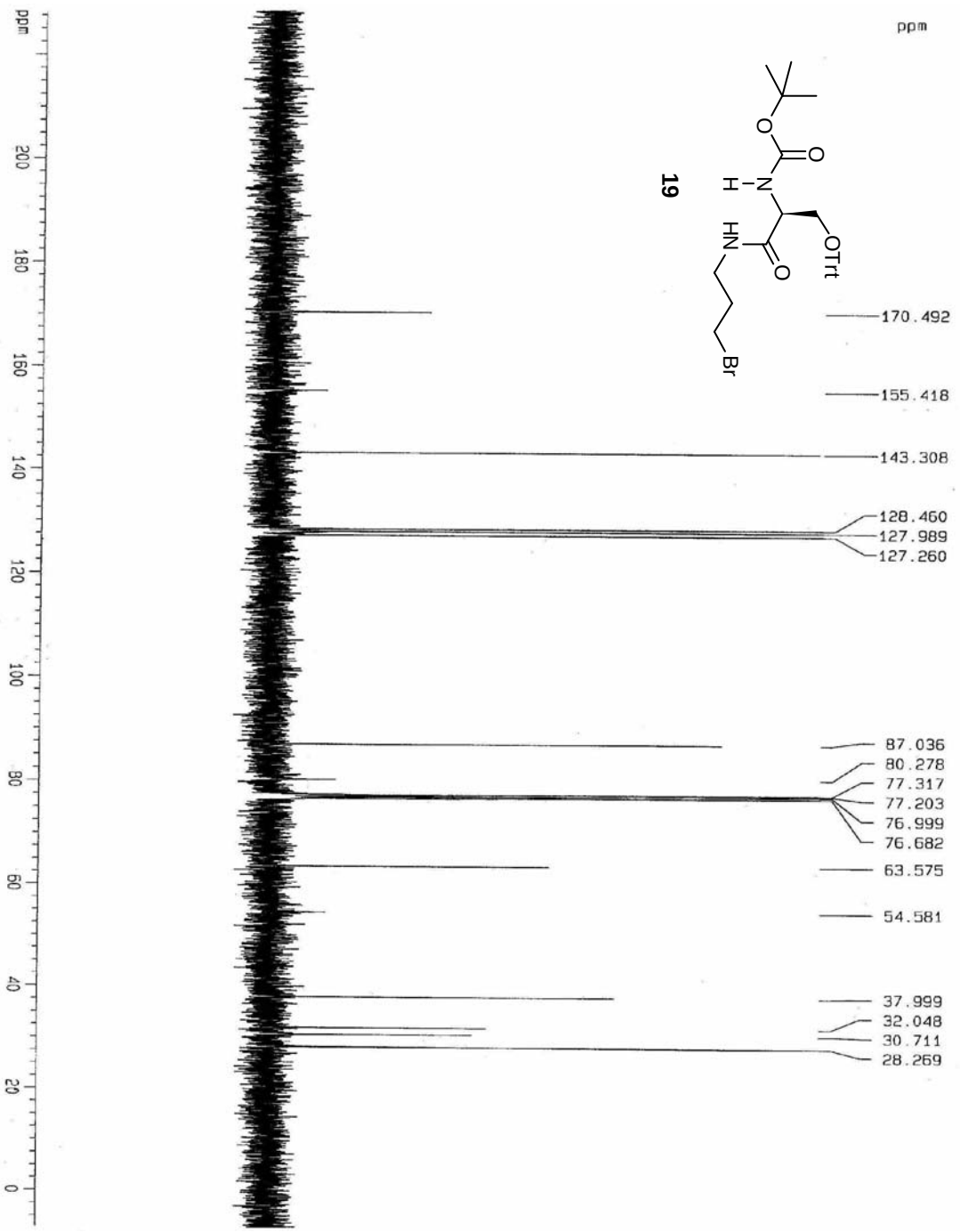


The results of the HPLC experiments concur with the results obtained from the specific rotation studies, indicating that there is no significant epimerization at the C-terminal residue under the conditions of base-mediated oxazinization.

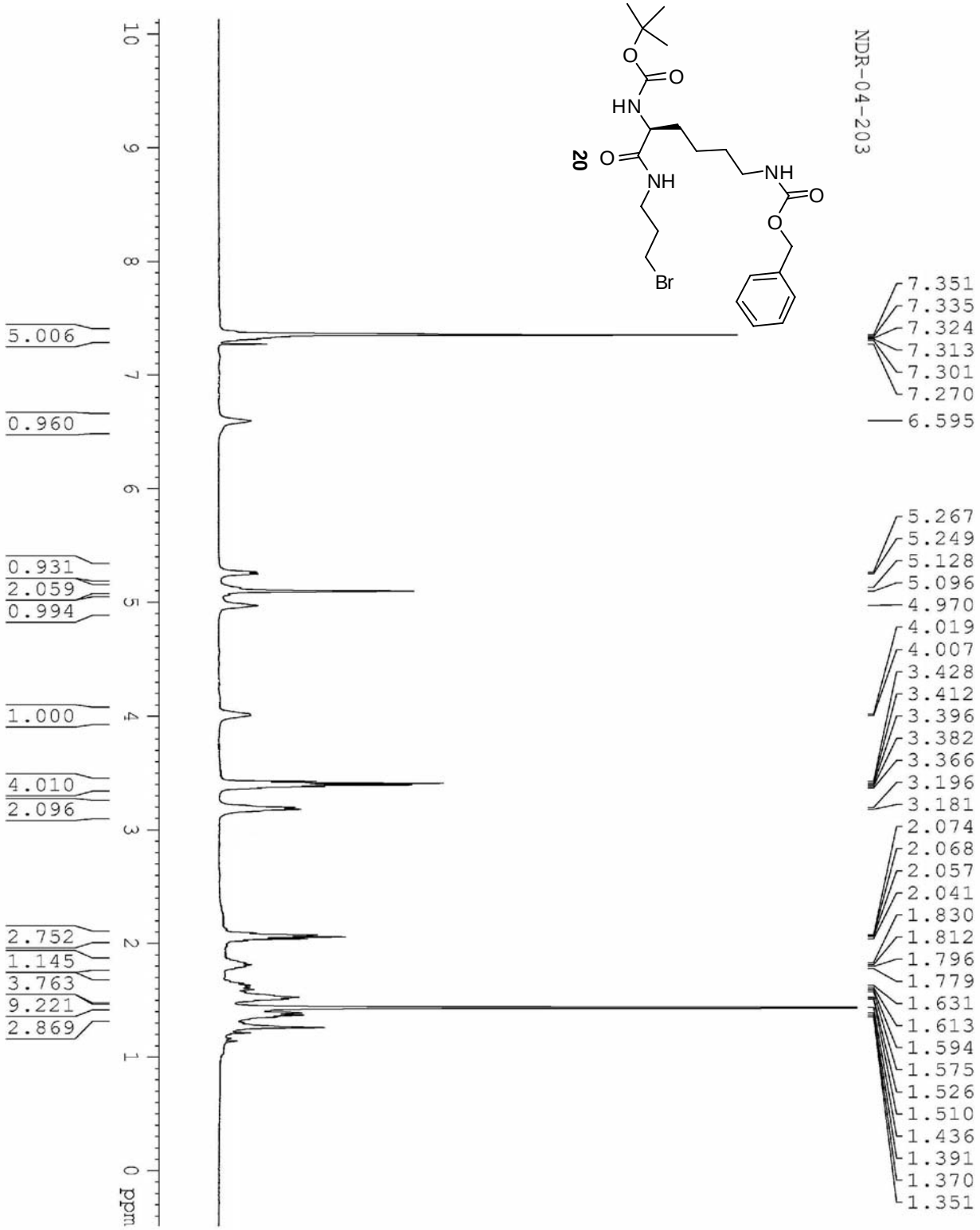
S11. ^{1}H NMR spectrum of peptide **19** in $CDCl_3$ (400 MHz, 60 mM).



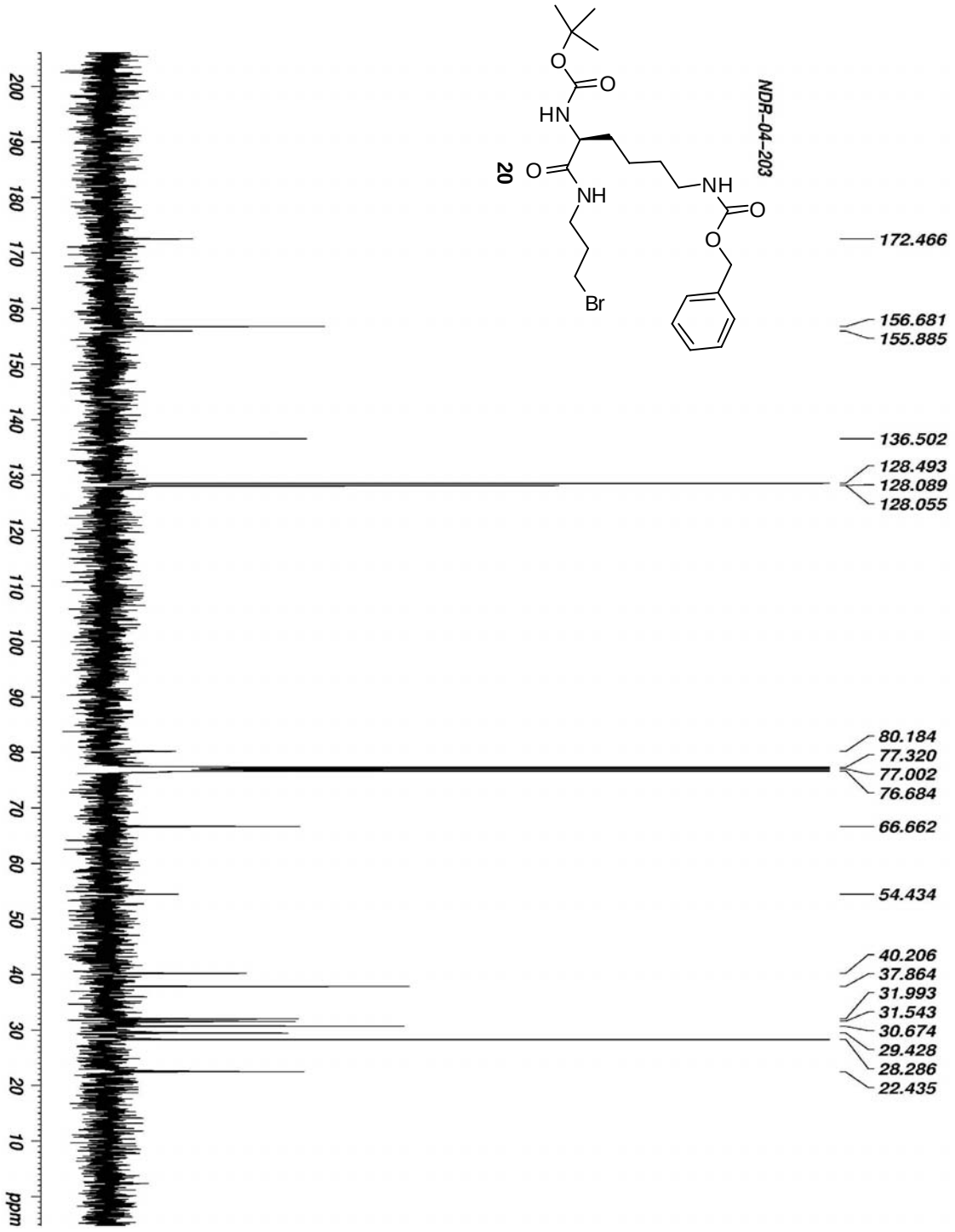
S12. ^{13}C NMR spectrum of peptide **19** in $CDCl_3$ (100 MHz, 60 mM)



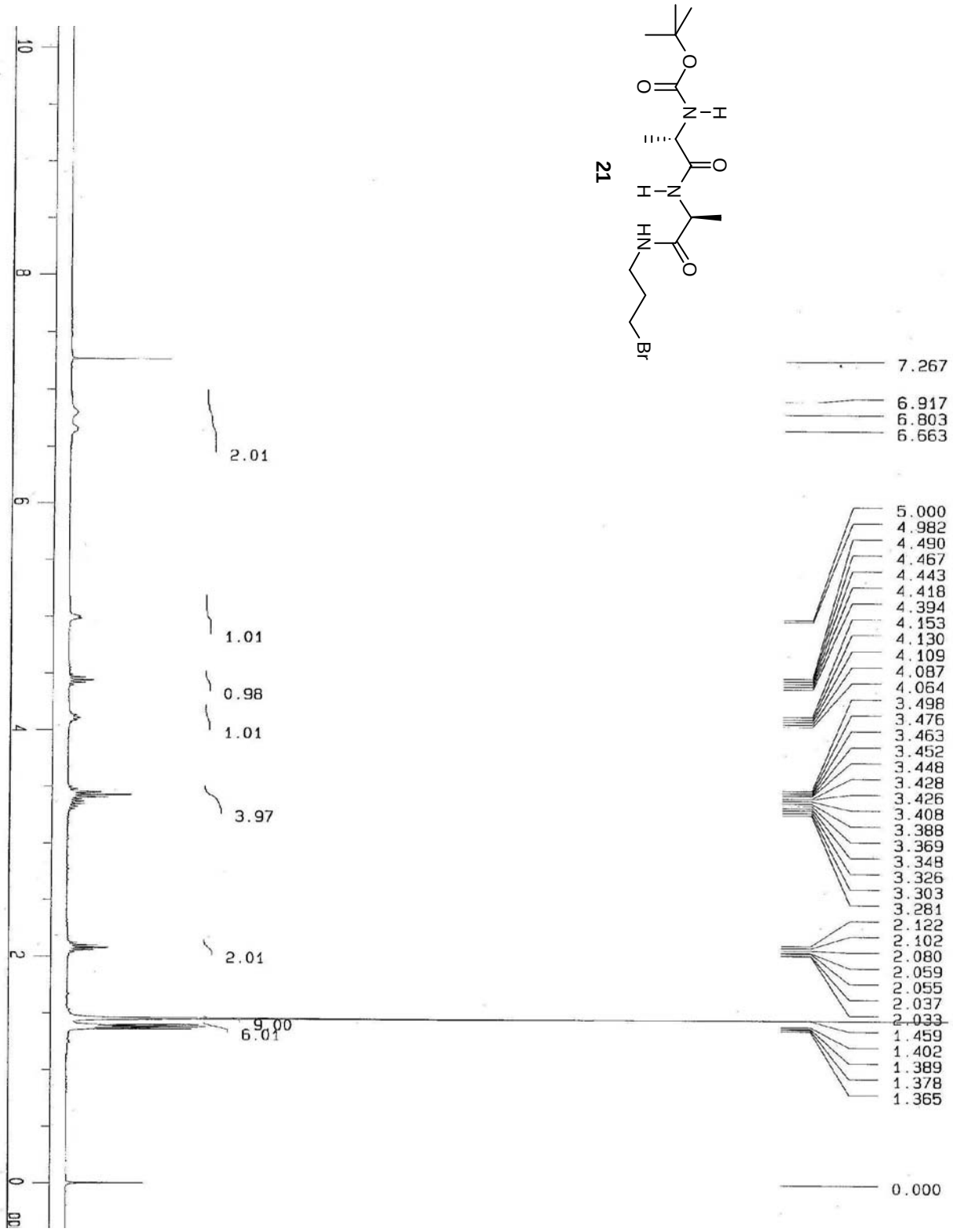
S13. ^{1}H NMR spectrum of peptide **20** in $CDCl_3$ (400 MHz, 60 mM)



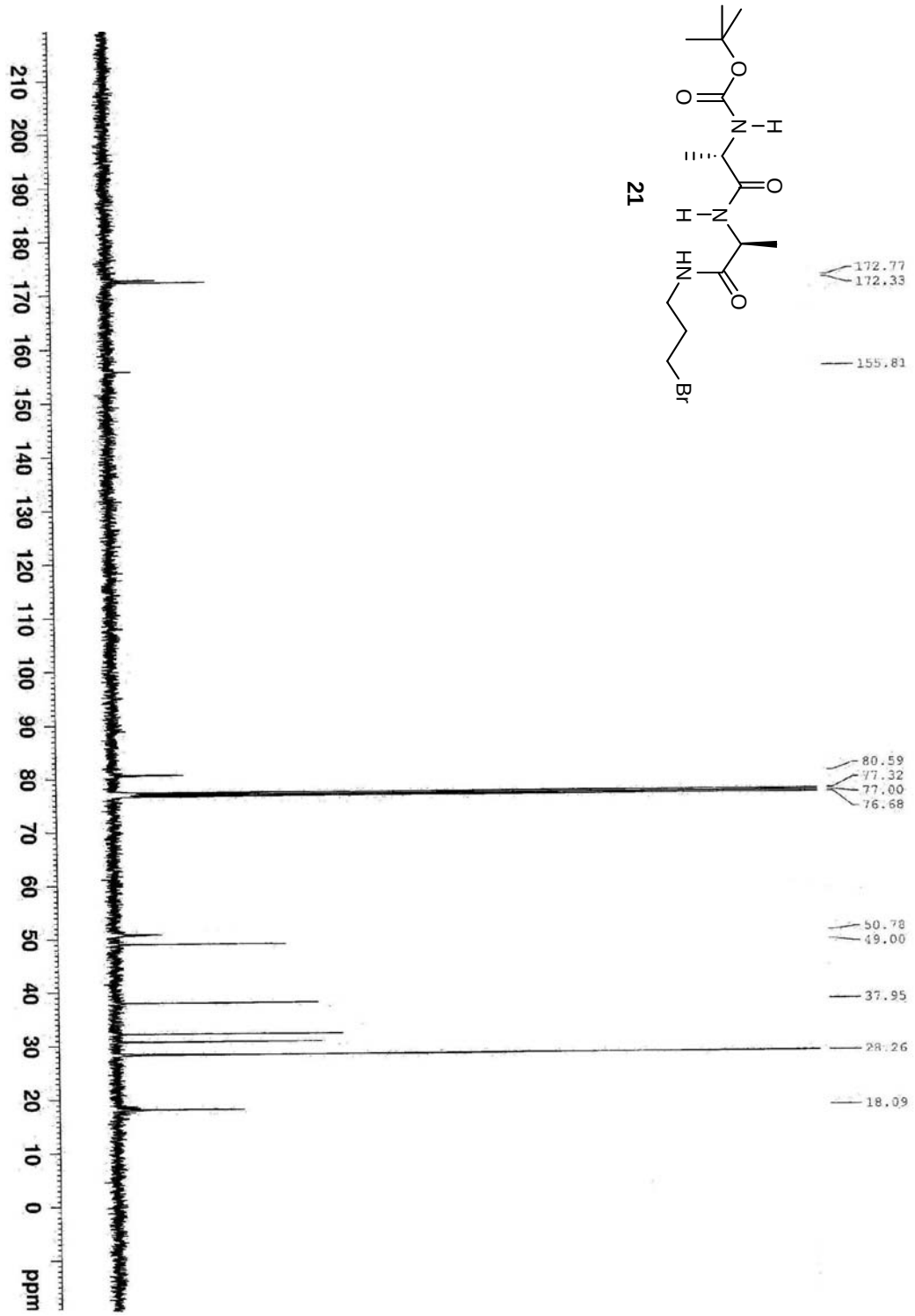
S14. ^{13}C NMR spectrum of peptide **20** in $CDCl_3$ (100 MHz, 60 mM)



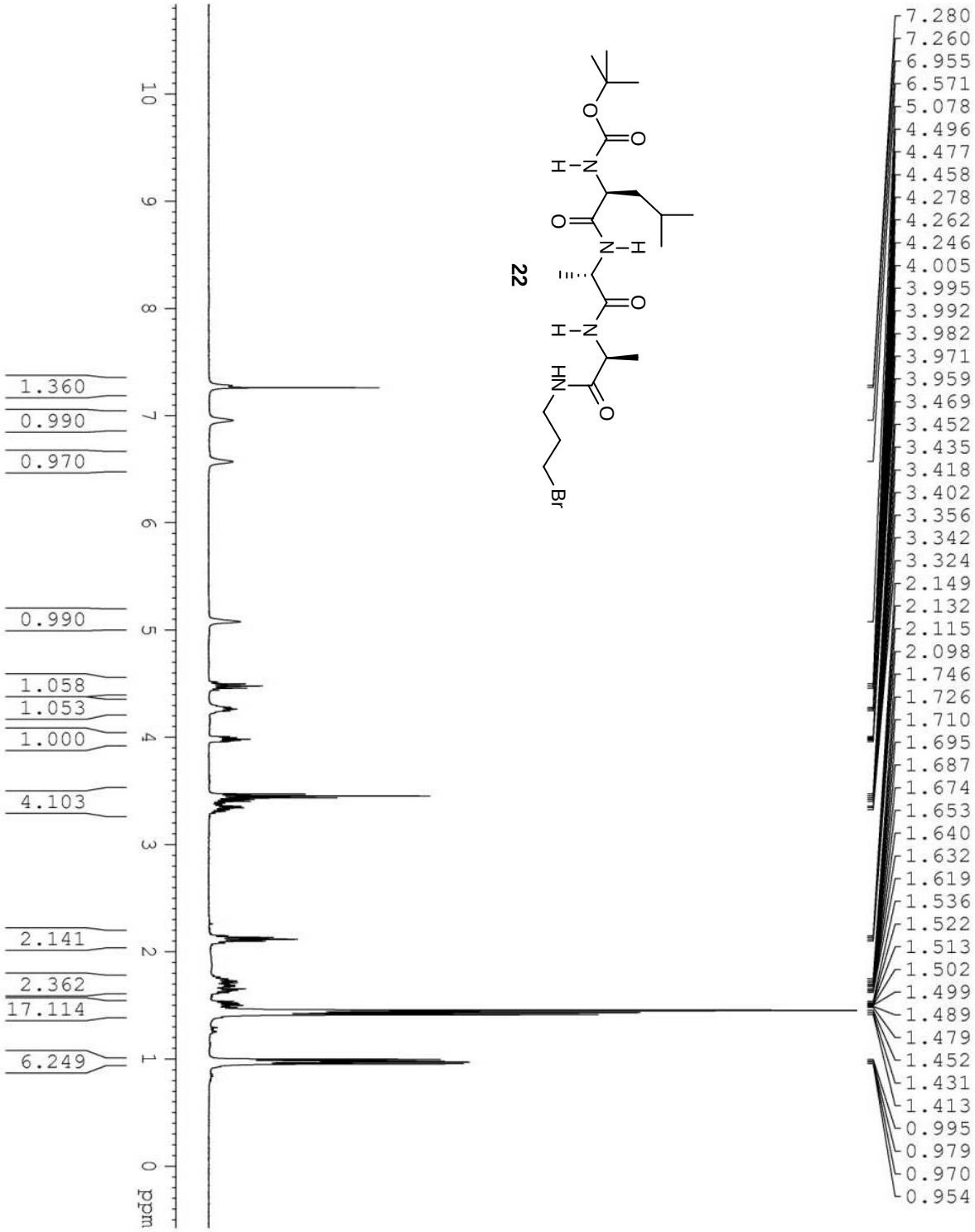
S15. ^{1}H NMR spectrum of peptide **21** in $CDCl_3$ (300 MHz, 60 mM)



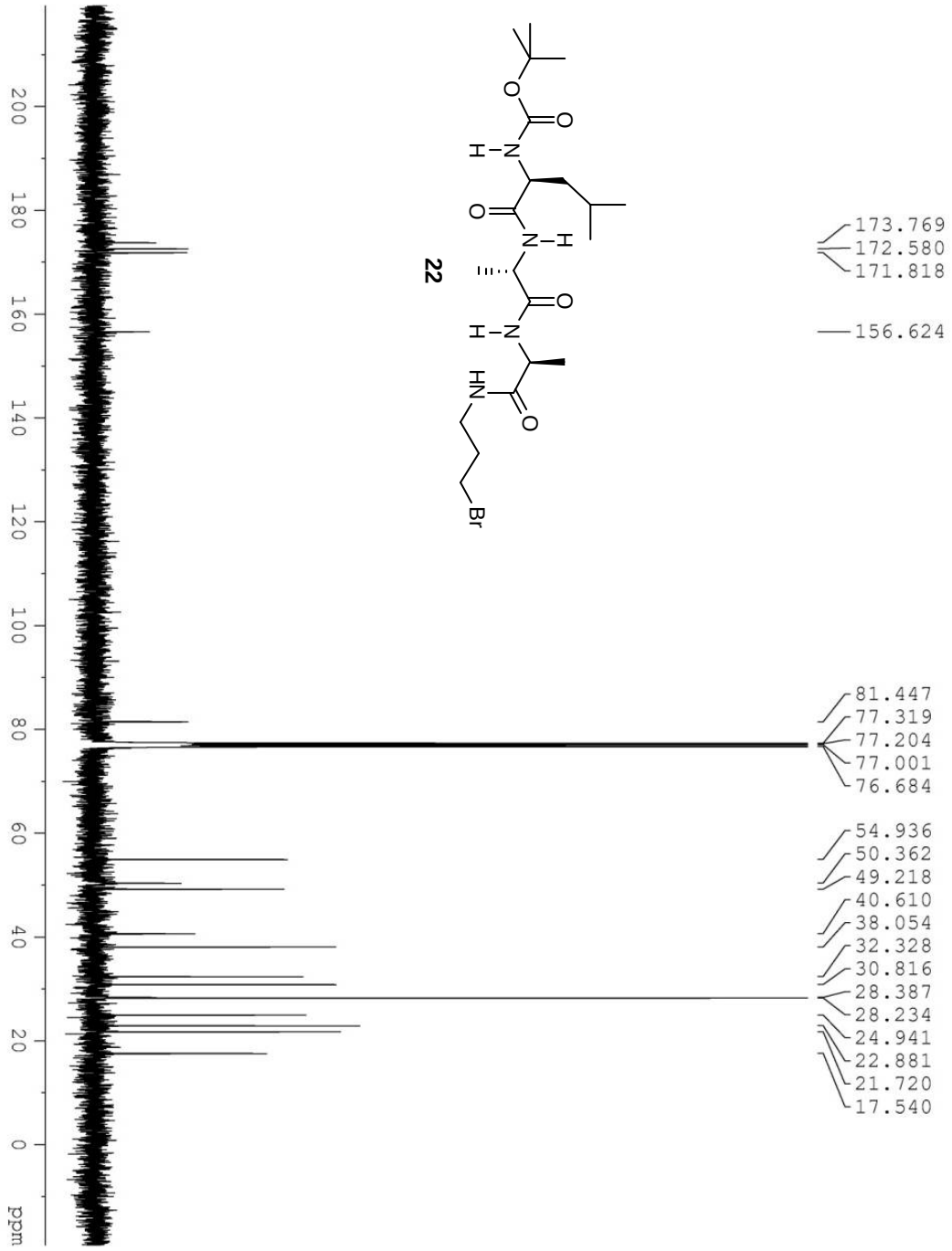
S16. ^{13}C NMR spectrum of peptide **21** in $CDCl_3$ (100 MHz, 60 mM)



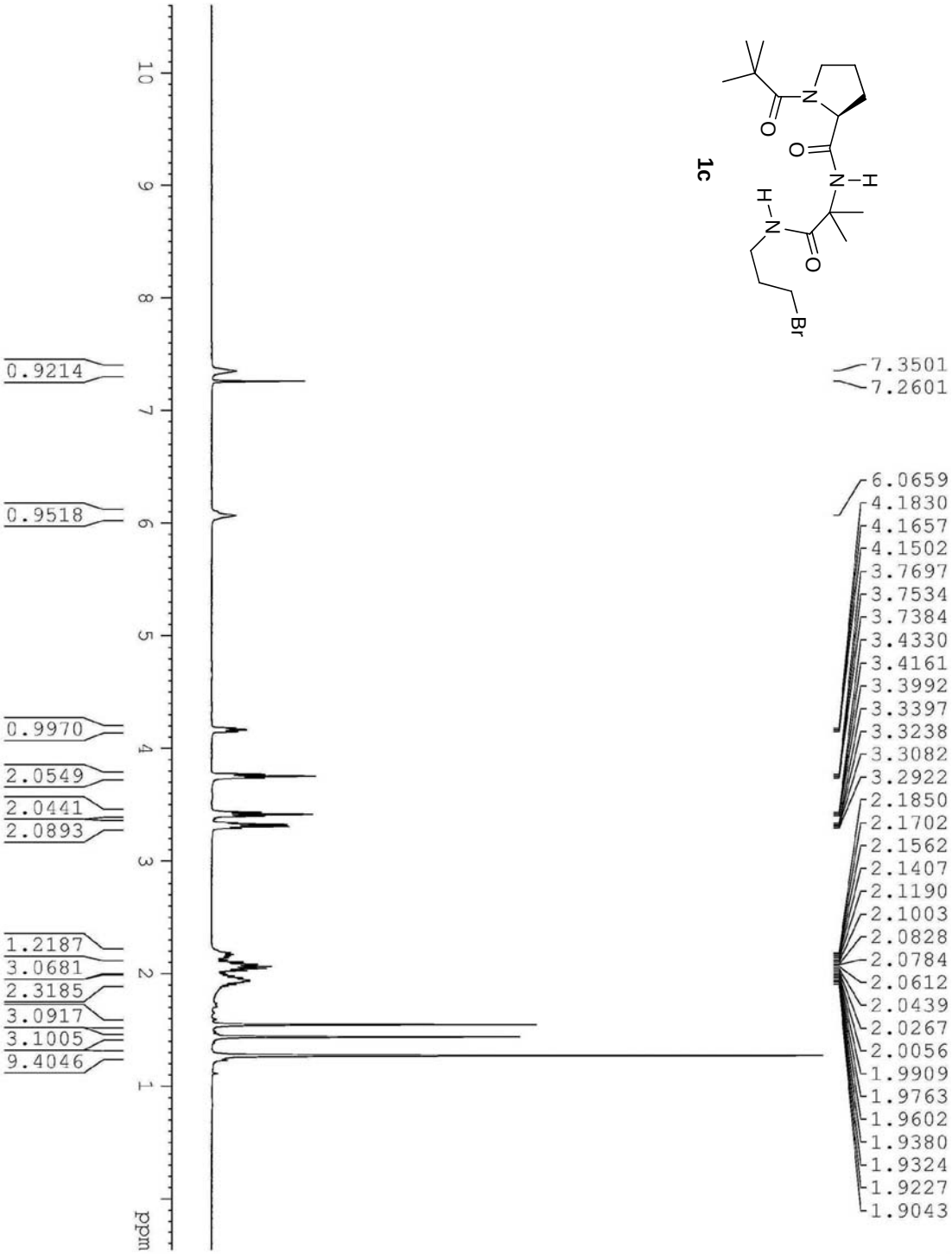
S17. ^{1}H NMR spectrum of peptide **22** in $CDCl_3$ (400 MHz, 60 mM)



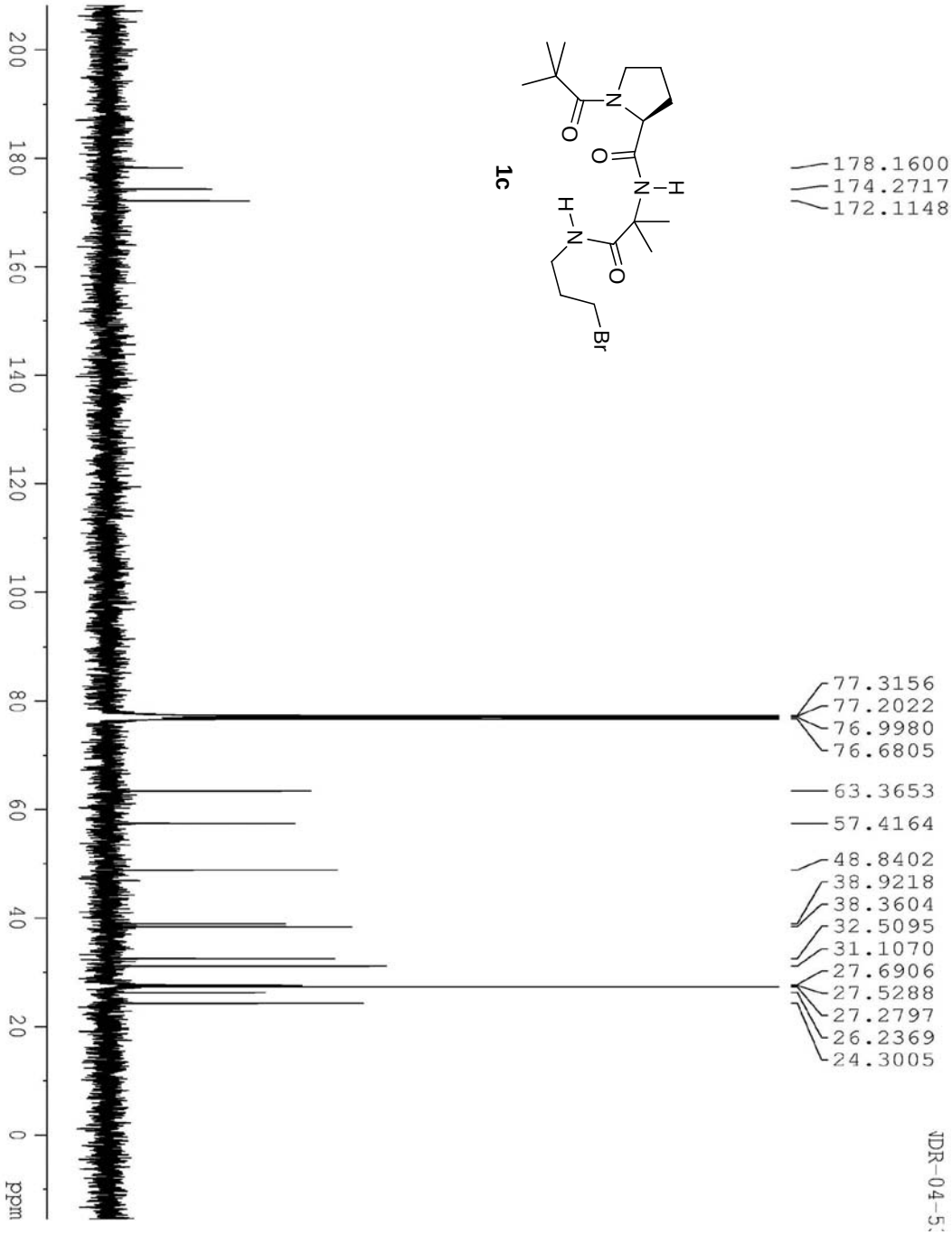
S18. ^{13}C NMR spectrum of peptide **22** in $CDCl_3$ (100 MHz, 60 mM)



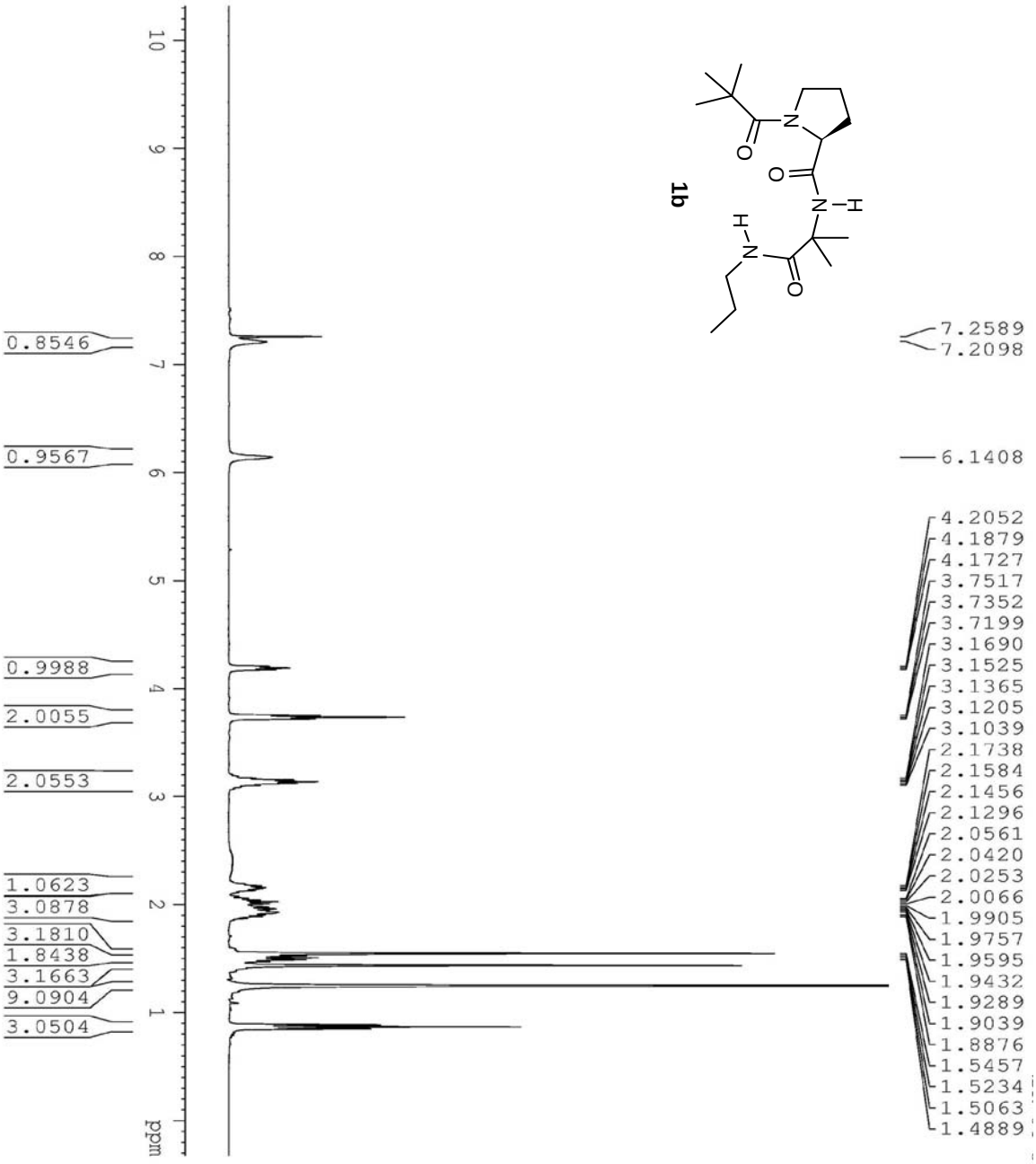
S19. ^{1}H NMR spectrum of peptide **1c** in $CDCl_3$ (400 MHz, 60 mM)



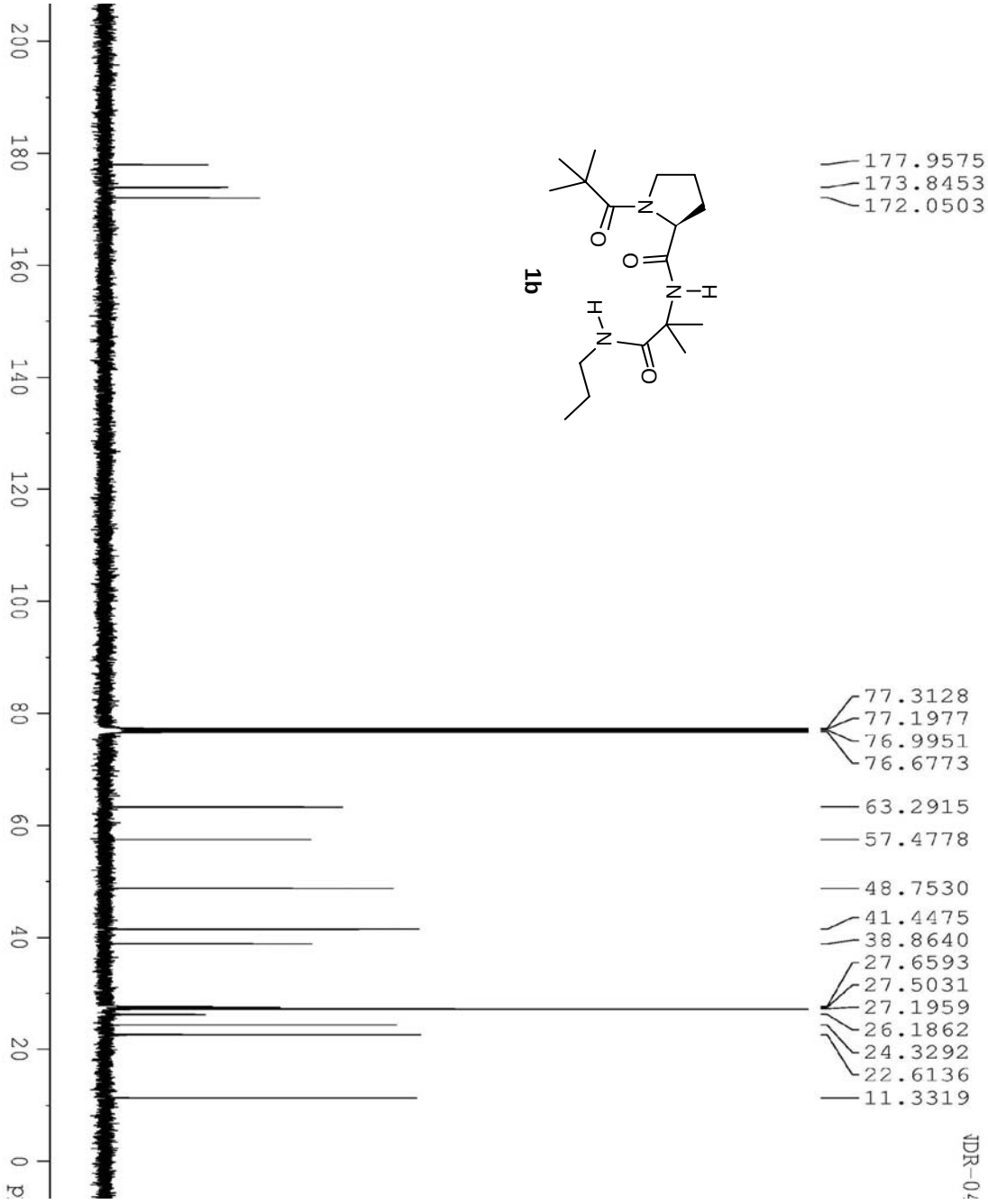
S20. ^{13}C NMR spectrum of peptide **1c** in $CDCl_3$ (100 MHz, 60 mM)



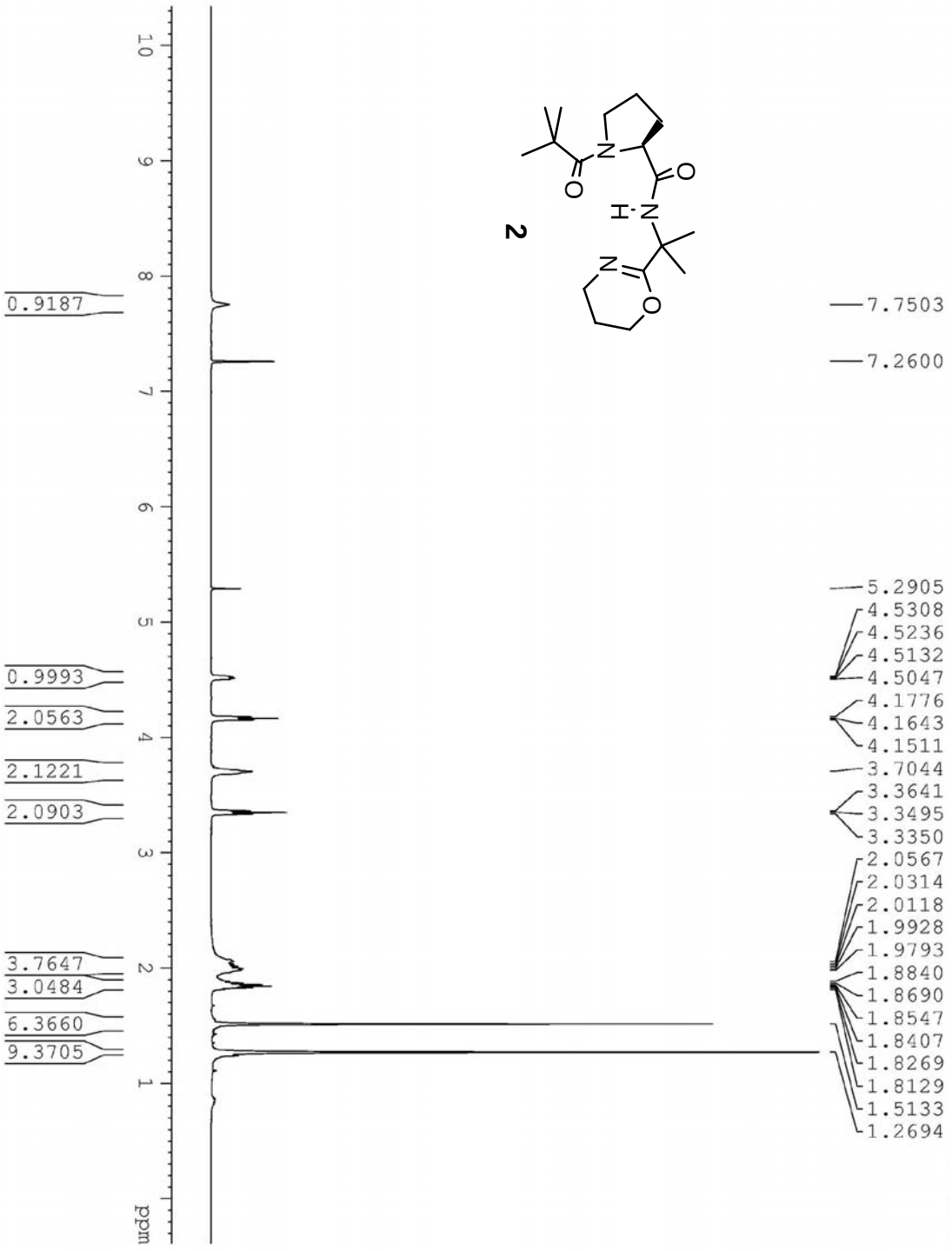
S21. ^{1}H NMR spectrum of peptide **1b** in $CDCl_3$ (400 MHz, 60 mM)



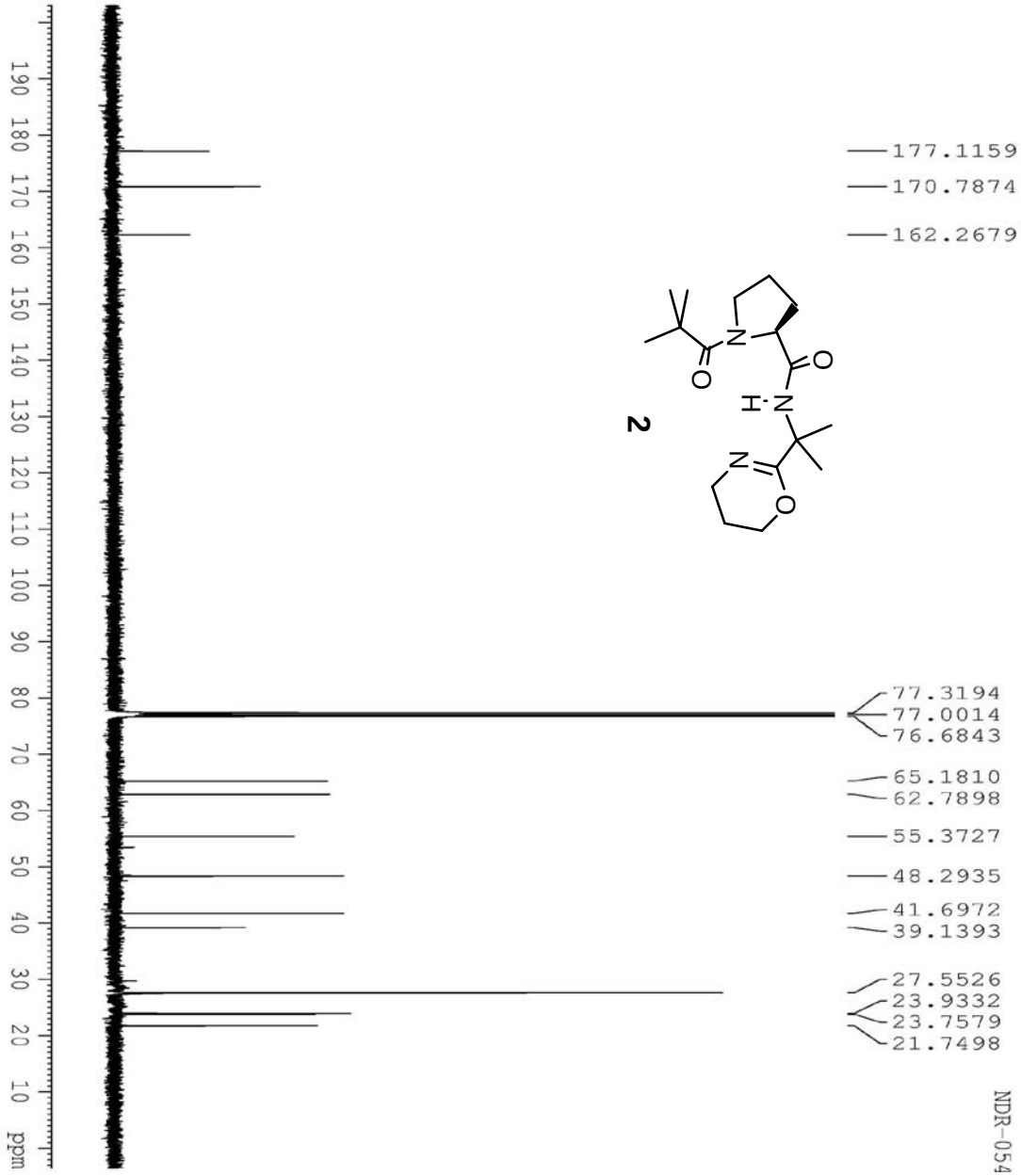
S22. ^{13}C NMR spectrum of peptide **1b** in $CDCl_3$ (100 MHz, 60 mM)



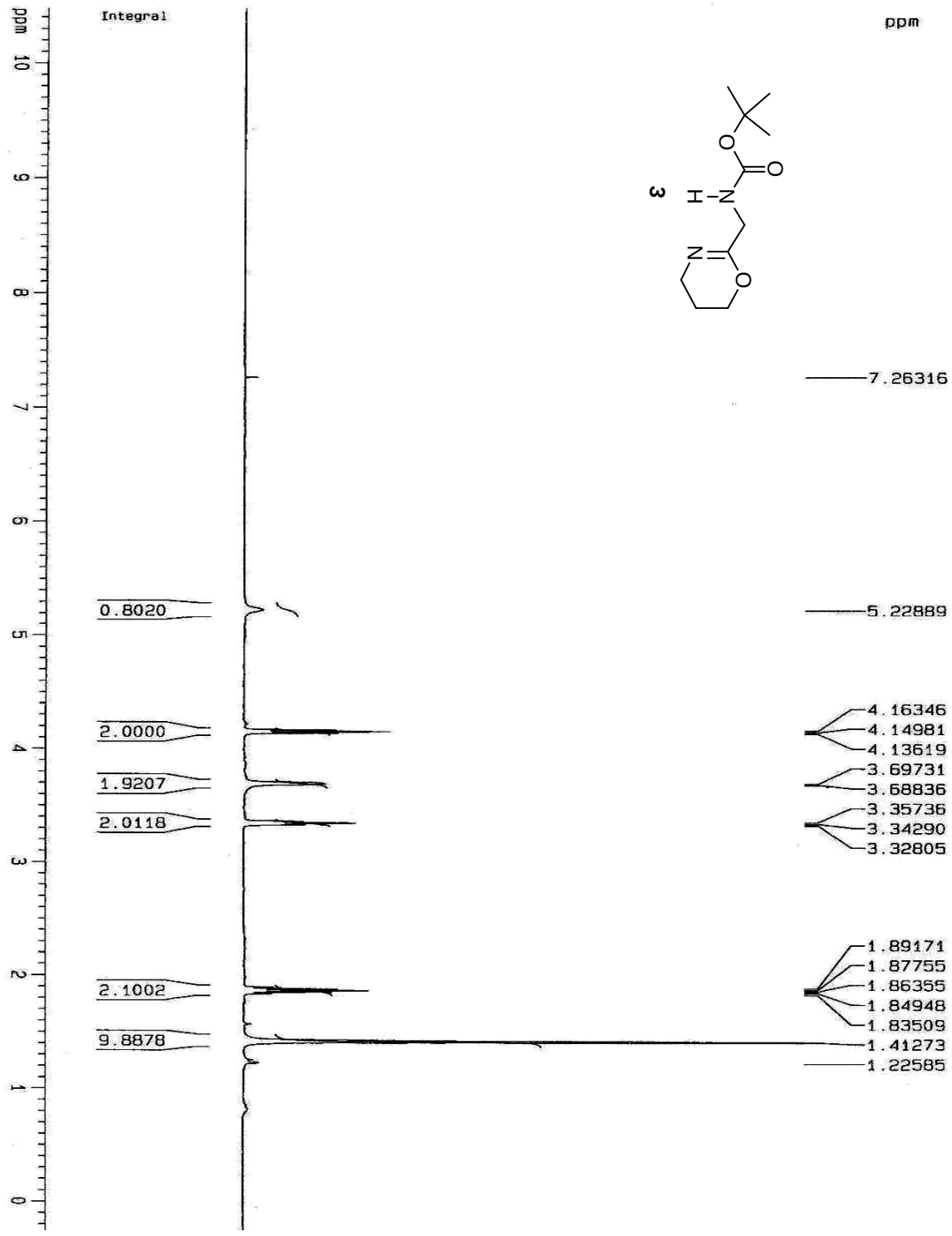
S23. ^{1}H NMR spectrum of the A→I analogue peptide **2** in $CDCl_3$ (400 MHz, 60 mM)



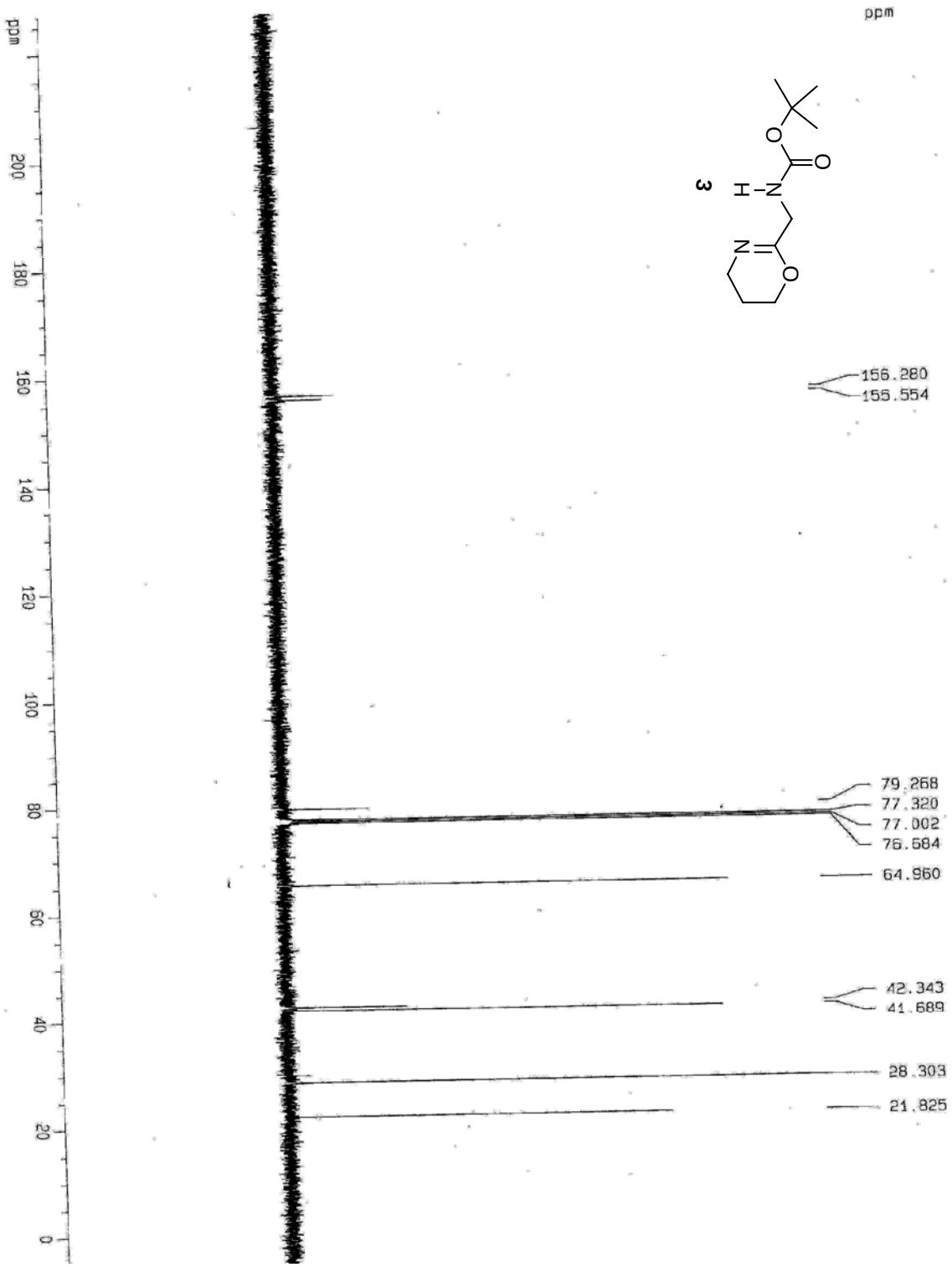
S24. ^{13}C NMR spectrum of the A→I analogue peptide **2** in $CDCl_3$ (100 MHz, 60 mM)



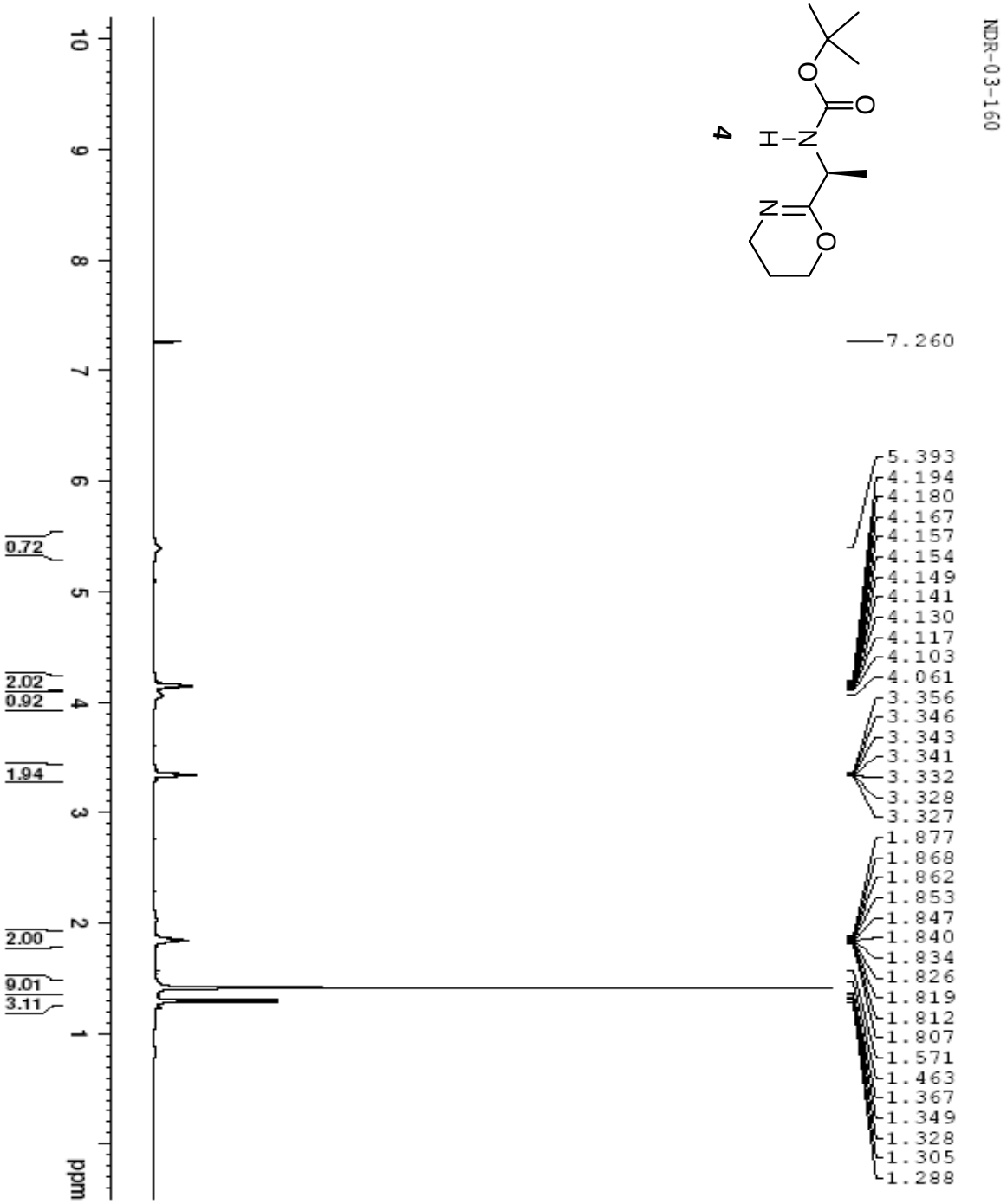
S25. ^{1}H NMR spectrum of the A→I analogue peptide **3** in $CDCl_3$ (400 MHz, 60 mM)



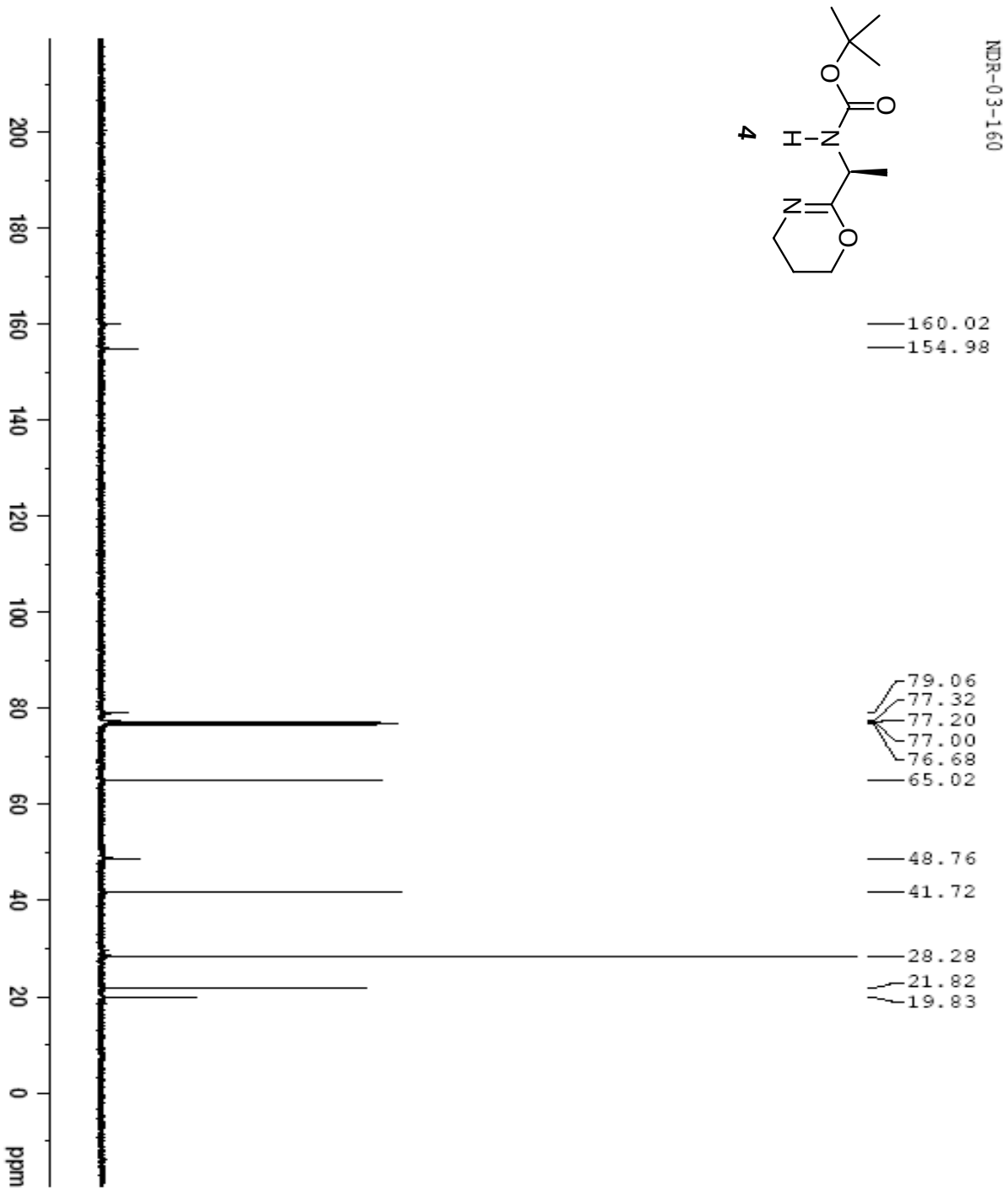
S26. ^{13}C NMR spectrum of the A→I analogue peptide **3** in $CDCl_3$ (100 MHz, 60 mM)



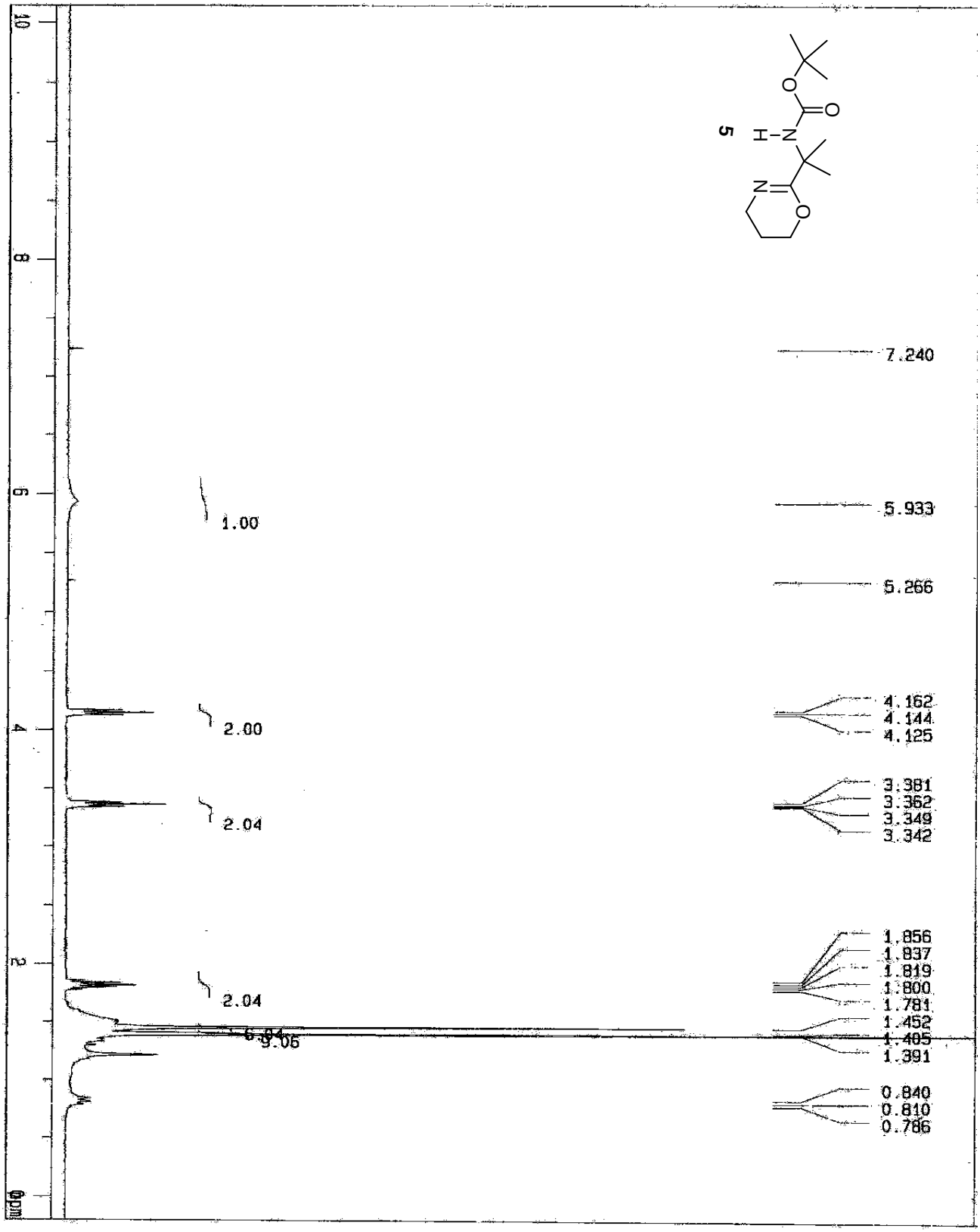
S27. ^{1}H NMR spectrum of the A→I analogue peptide **4** in $CDCl_3$ (400 MHz, 60 mM)



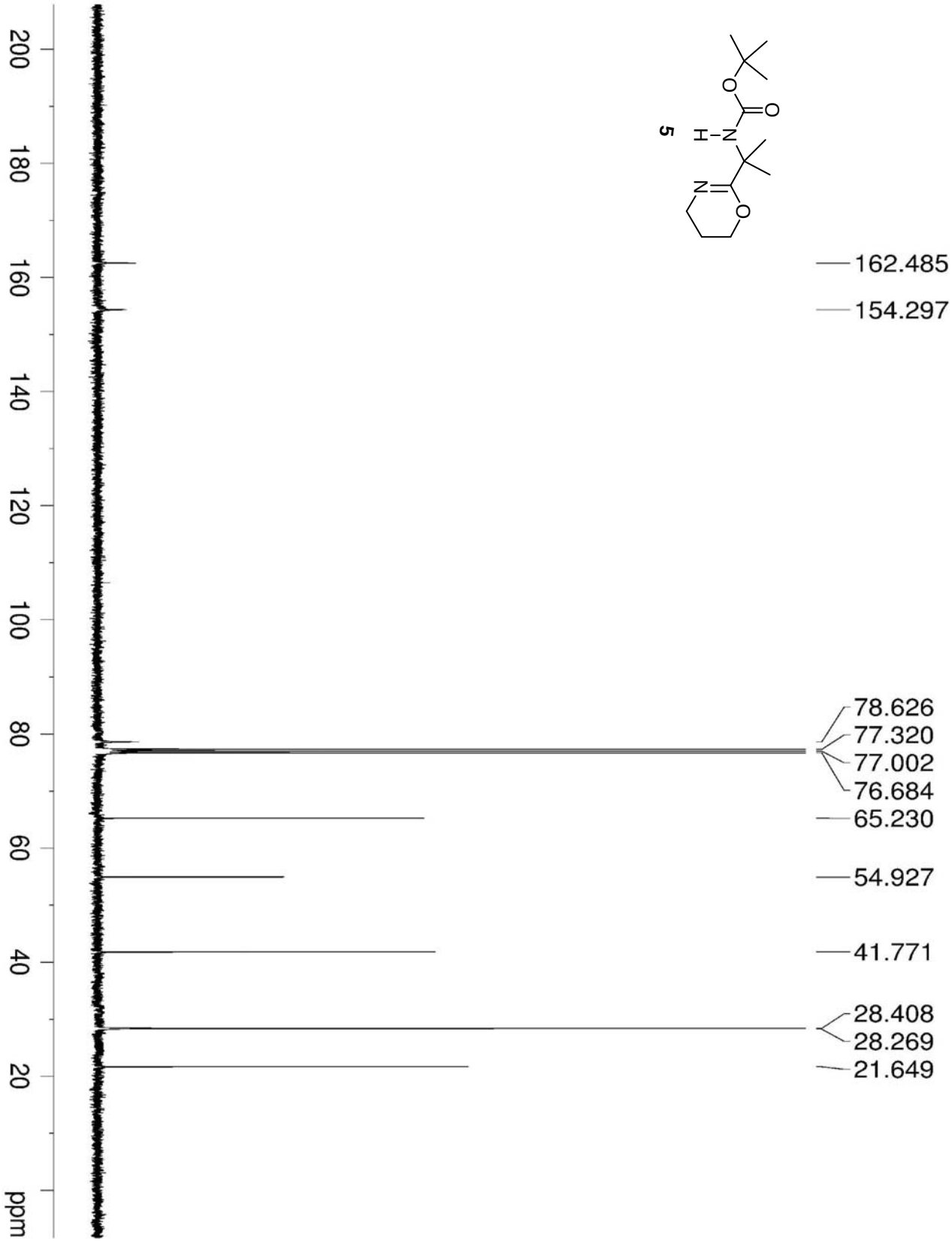
S28. ^{13}C NMR spectrum of the A→I analogue peptide **4** in $CDCl_3$ (100 MHz, 60 mM)



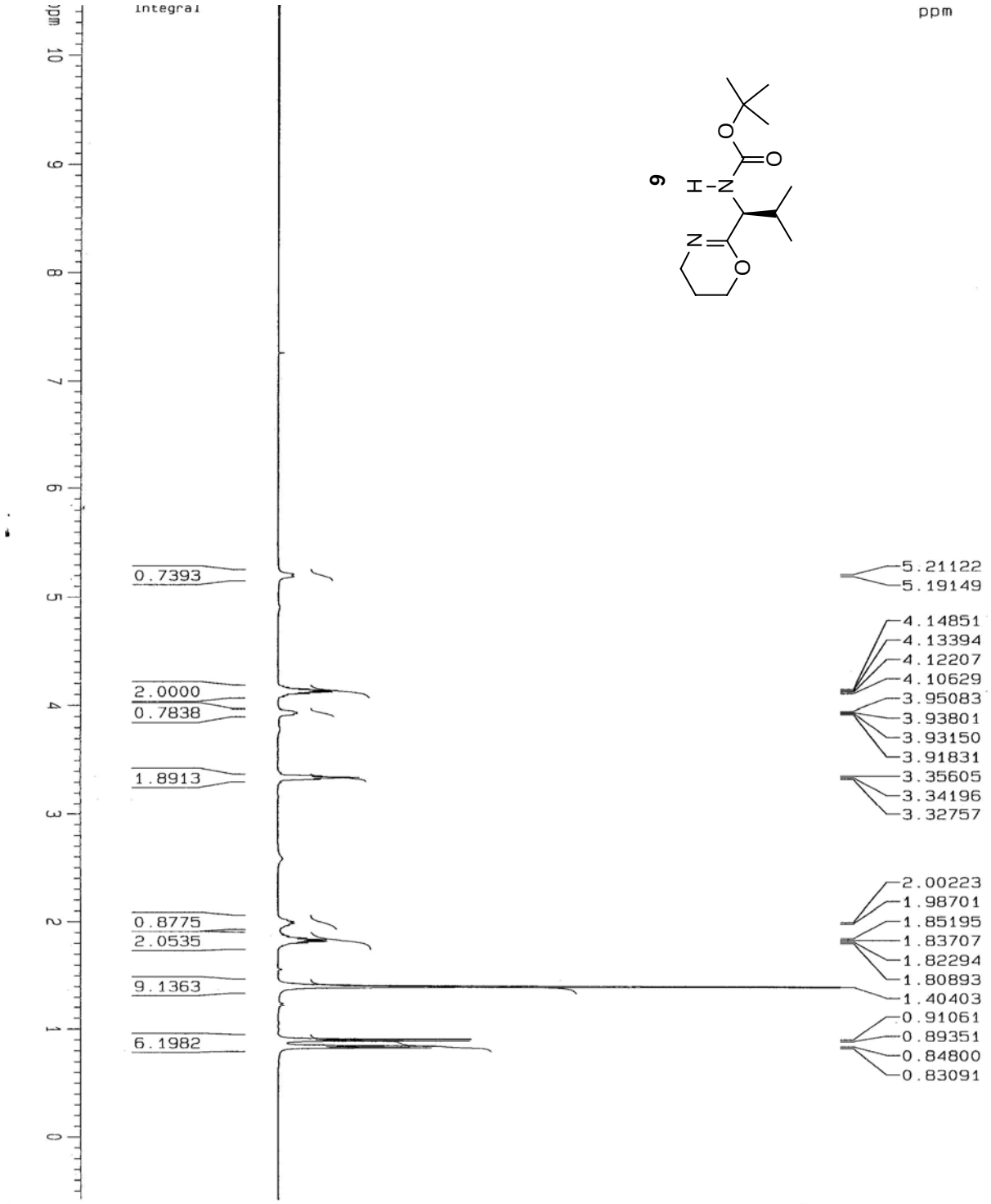
S29. ^{1}H NMR spectrum of the A→I analogue peptide **5** in $CDCl_3$ (300 MHz, 60 mM)



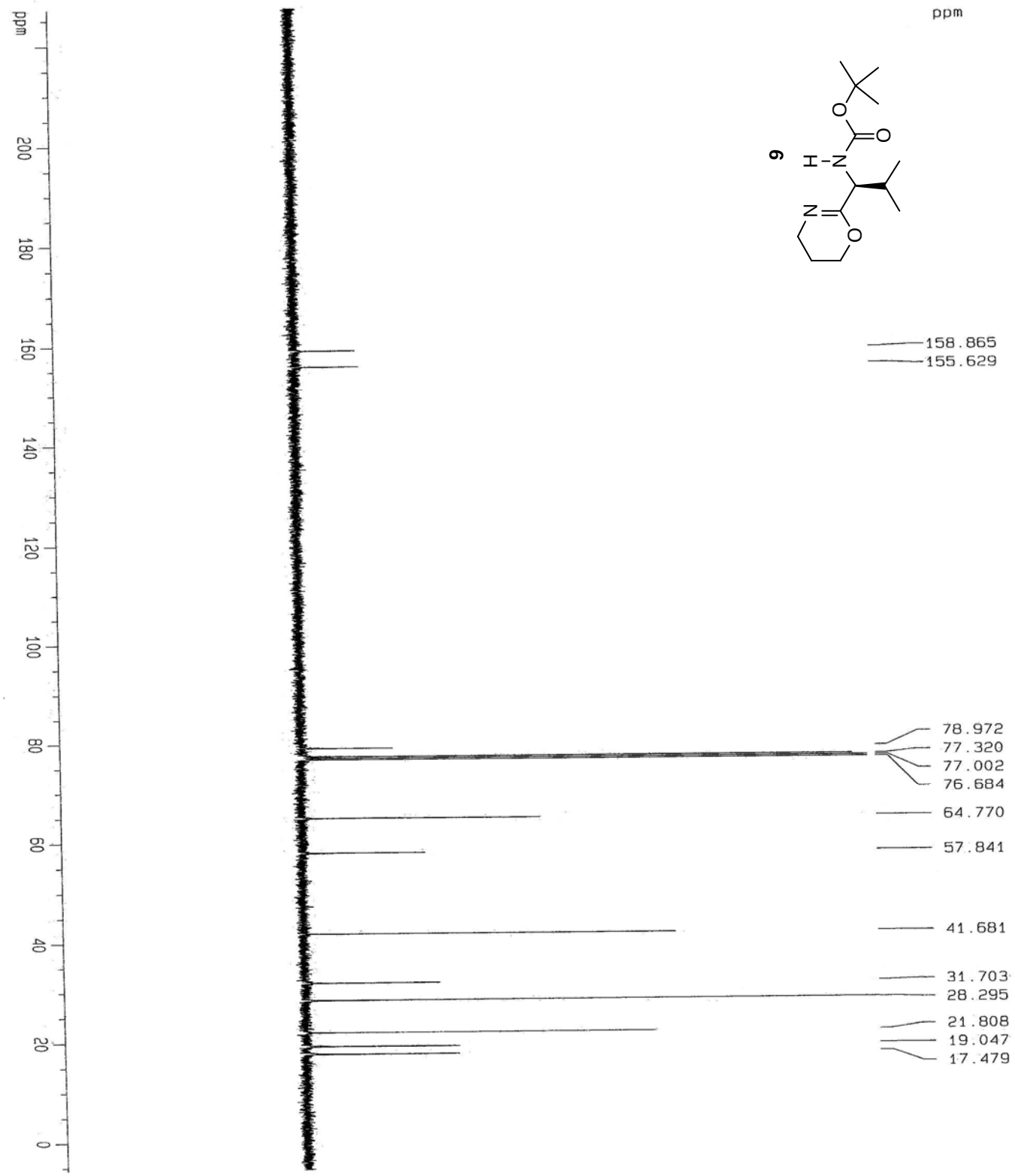
S30. ^{13}CNMR spectrum for the A→I analogue peptide **5** in $CDCl_3$ (100 MHz, 60 mM)



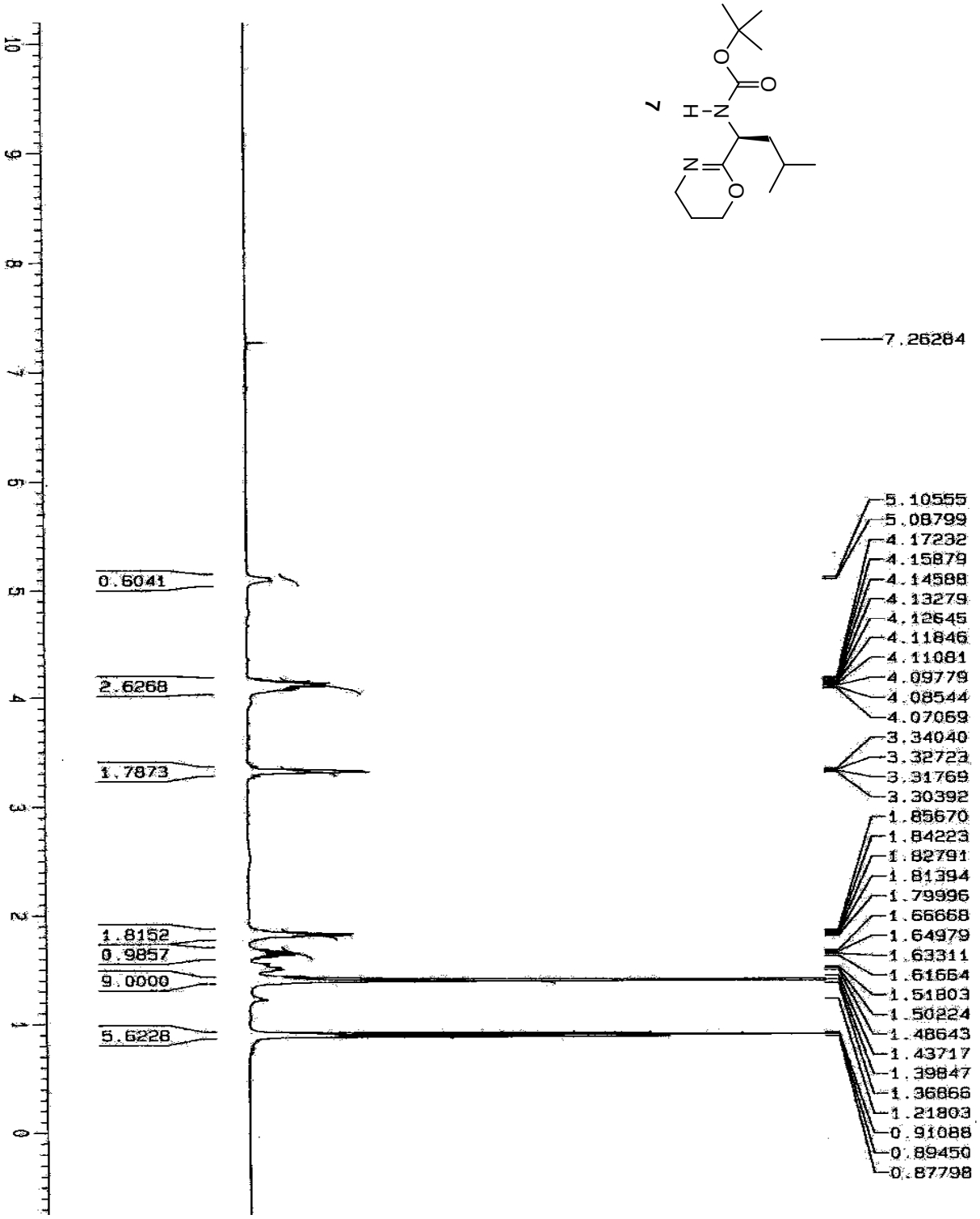
S31. ^{1}H NMR spectrum of the A→I analogue peptide **6** in $CDCl_3$ (400 MHz, 60 mM)



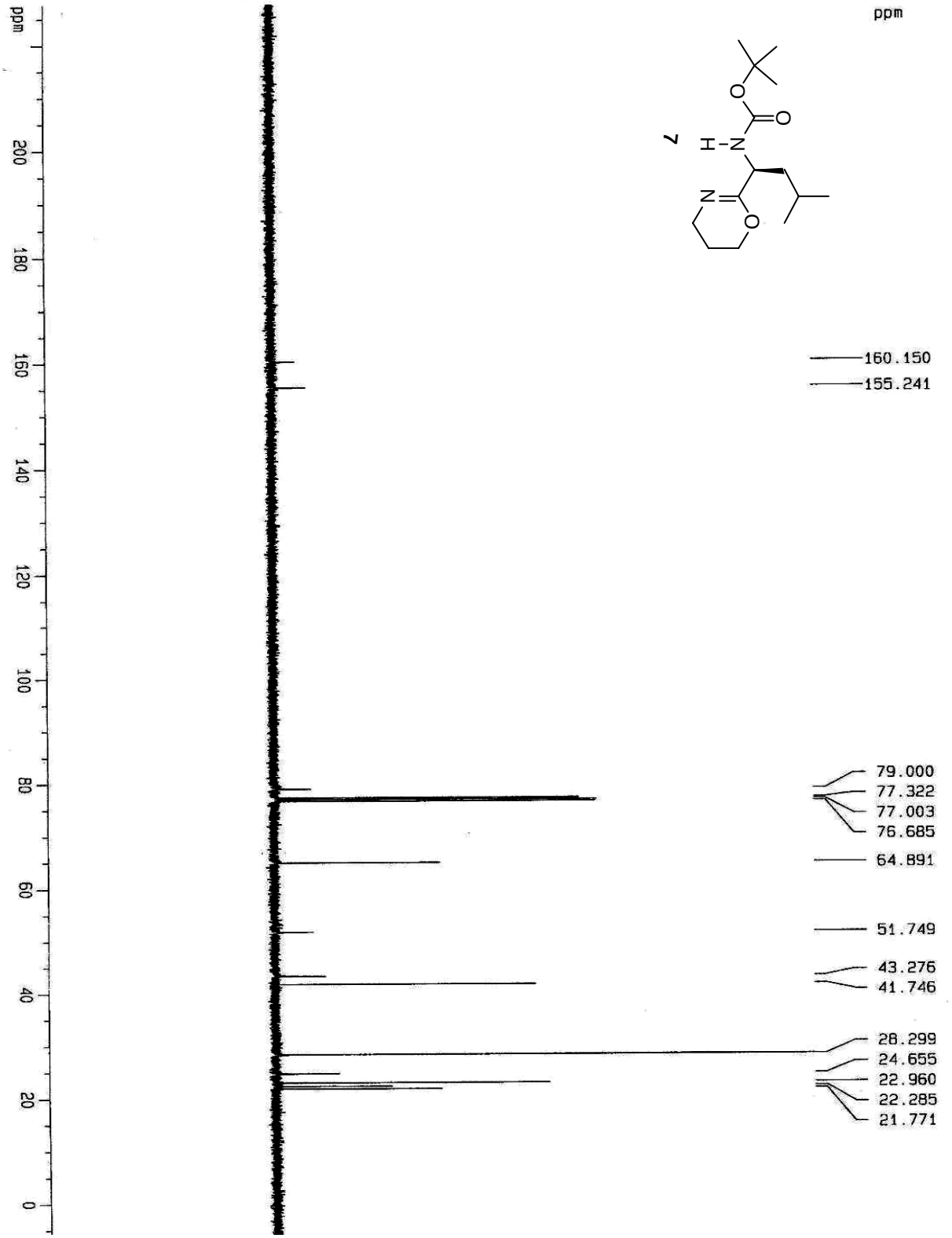
S32. ^{13}C NMR spectrum of the A→I analogue peptide **6** in $CDCl_3$ (100 MHz, 60 mM)



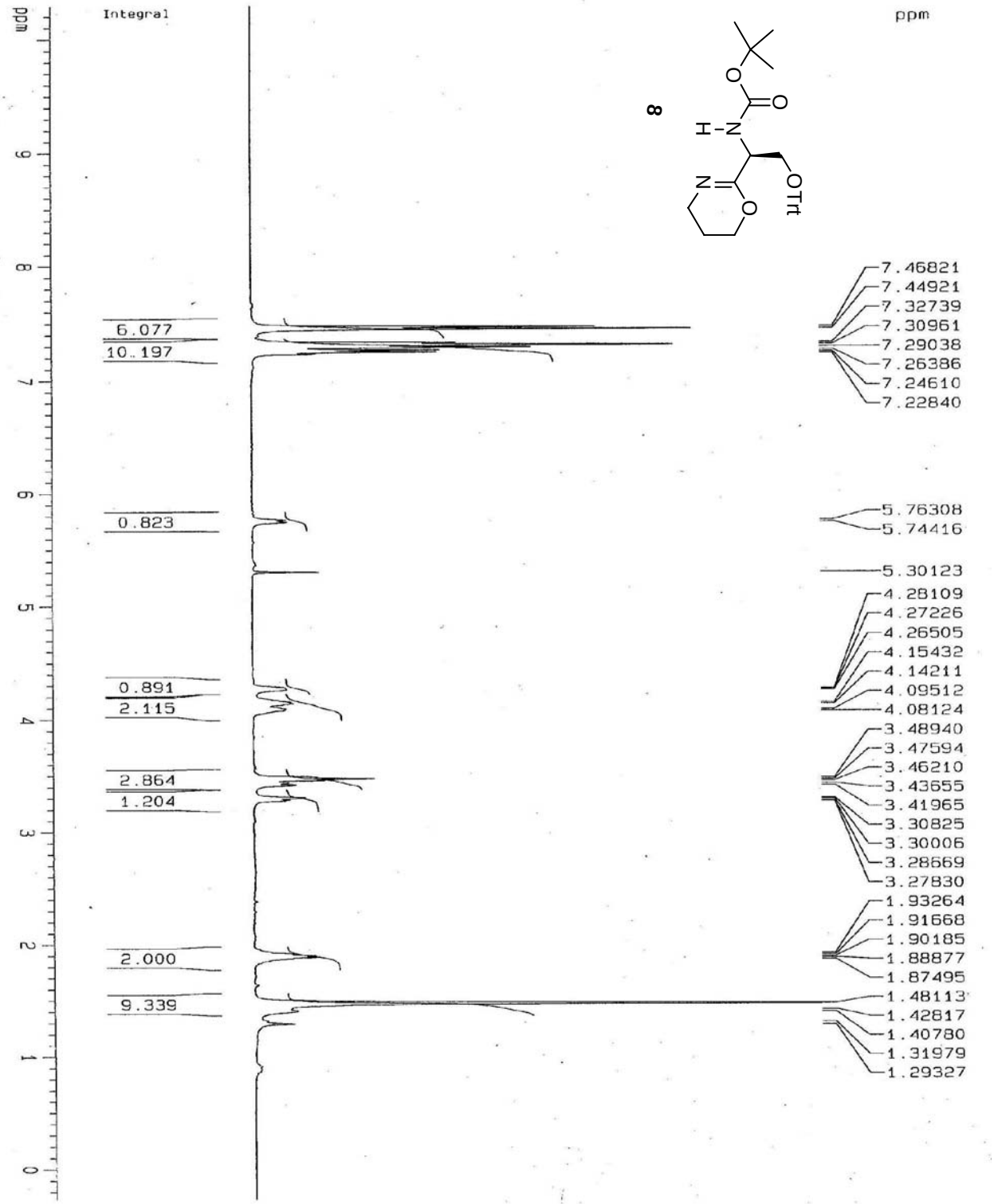
S33. ^{13}C NMR spectrum of the A→I analogue peptide **7** in $CDCl_3$ (400 MHz, 60 mM)



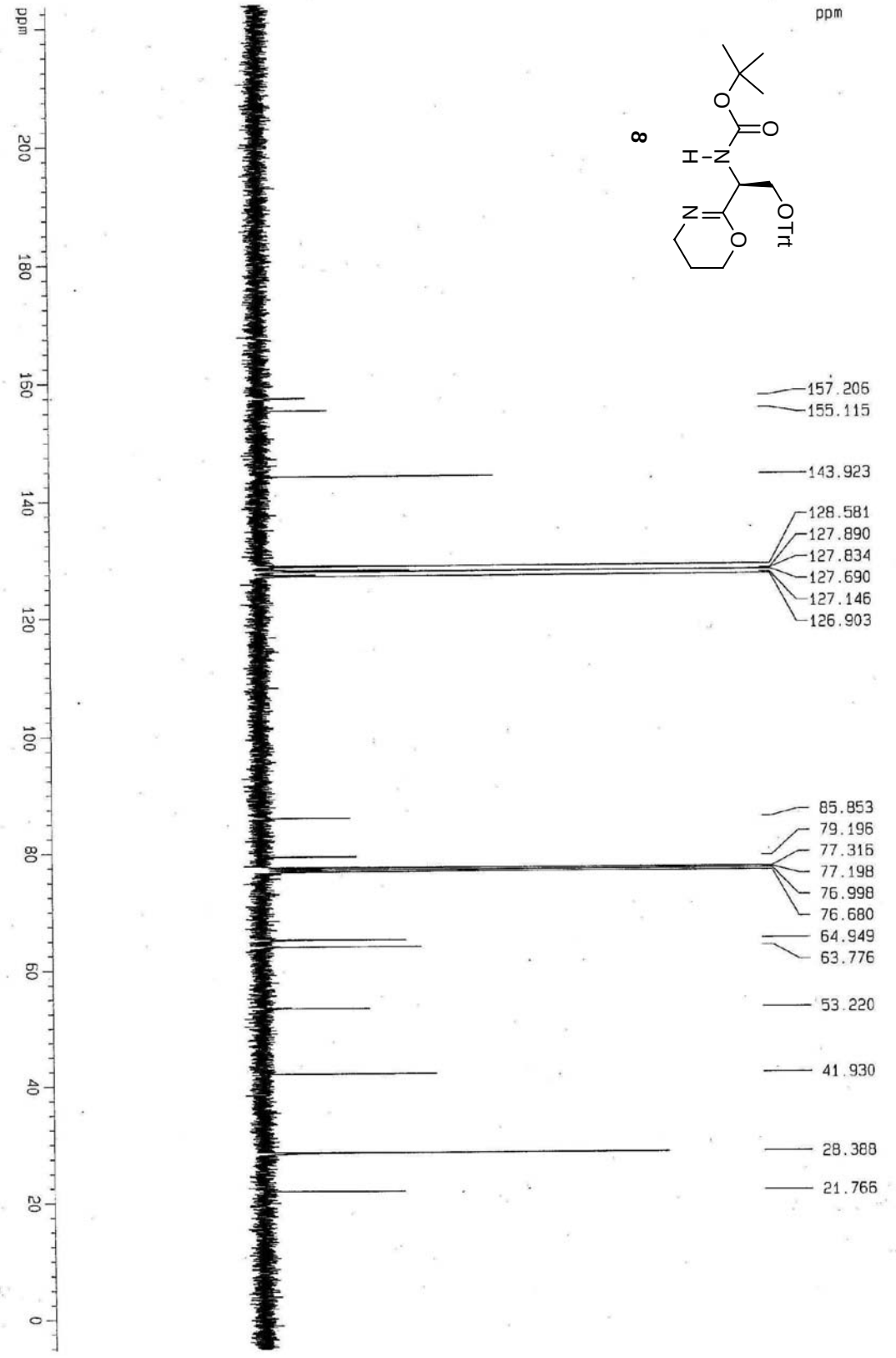
S34. ^{13}C NMR spectrum of the A→I analogue peptide **7** in $CDCl_3$ (100 MHz, 60 mM)



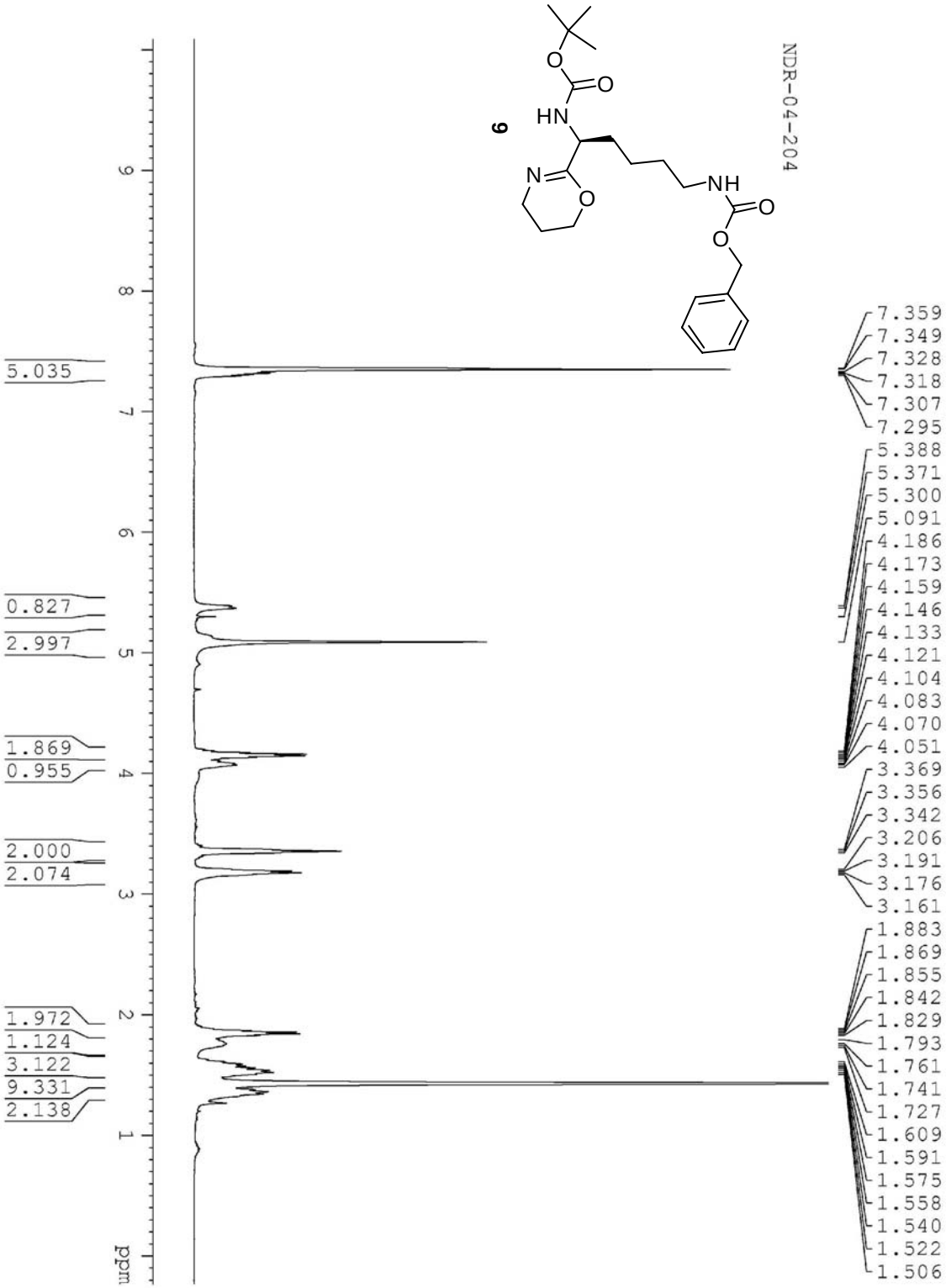
S35. ^{1}H NMR spectrum of the A→I analogue peptide **8** in $CDCl_3$ (400 MHz, 60 mM)



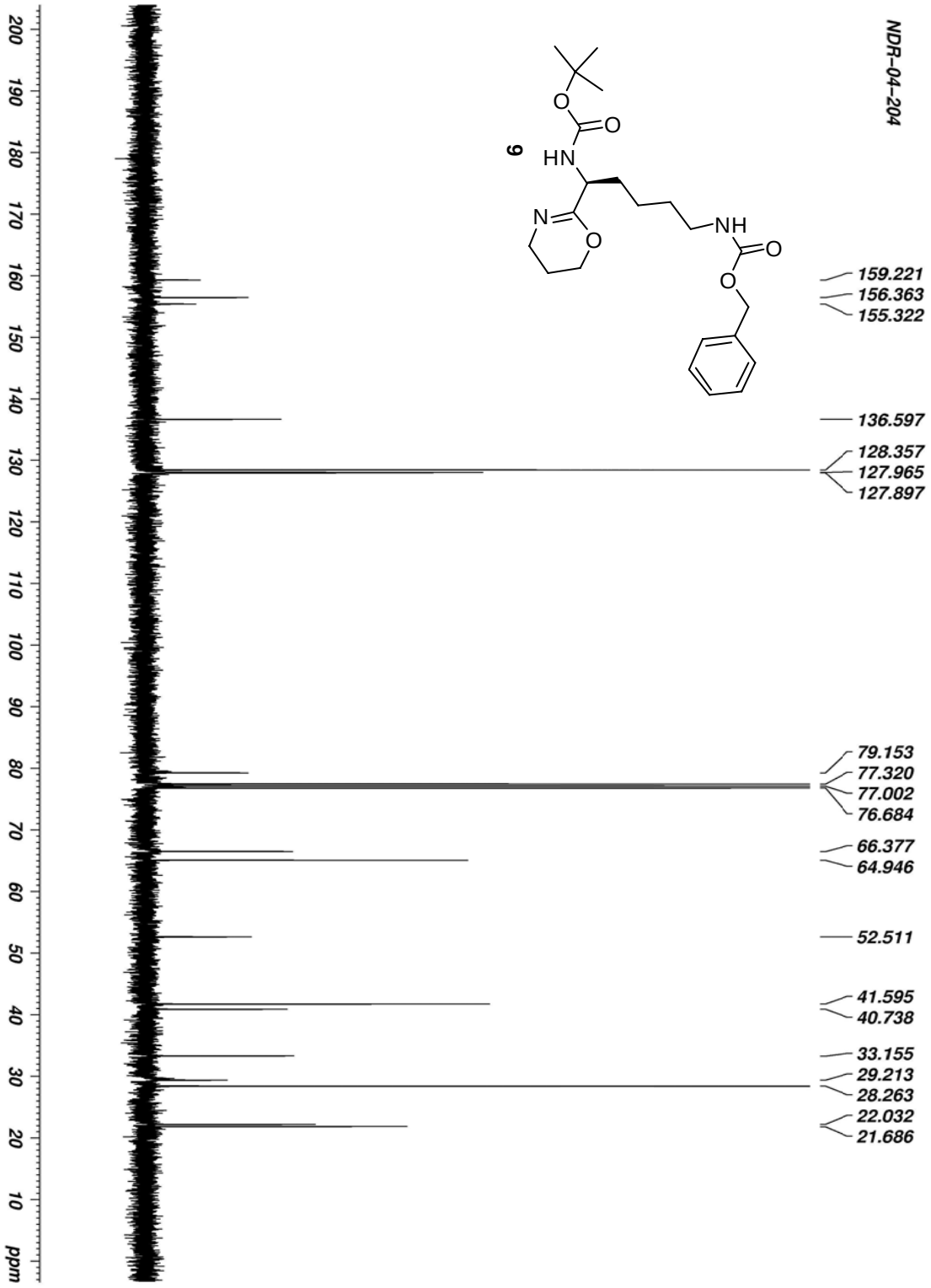
S36. ^{13}C NMR spectrum of the A→I analogue peptide **8** in $CDCl_3$ (100 MHz, 60 mM)



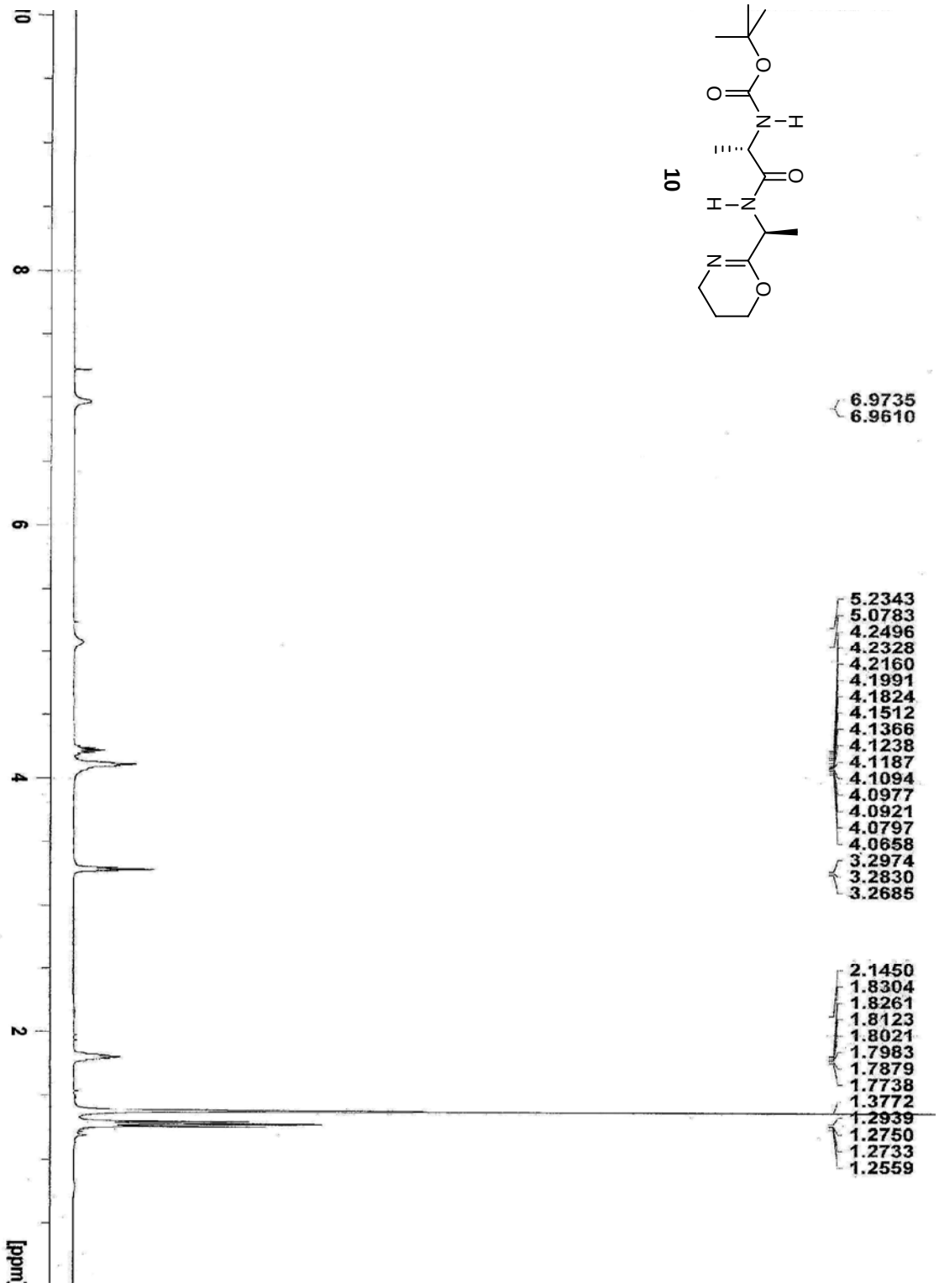
S37. ^{1}H NMR spectrum of the A→I analogue peptide **9** in $CDCl_3$ (400 MHz, 60 mM)



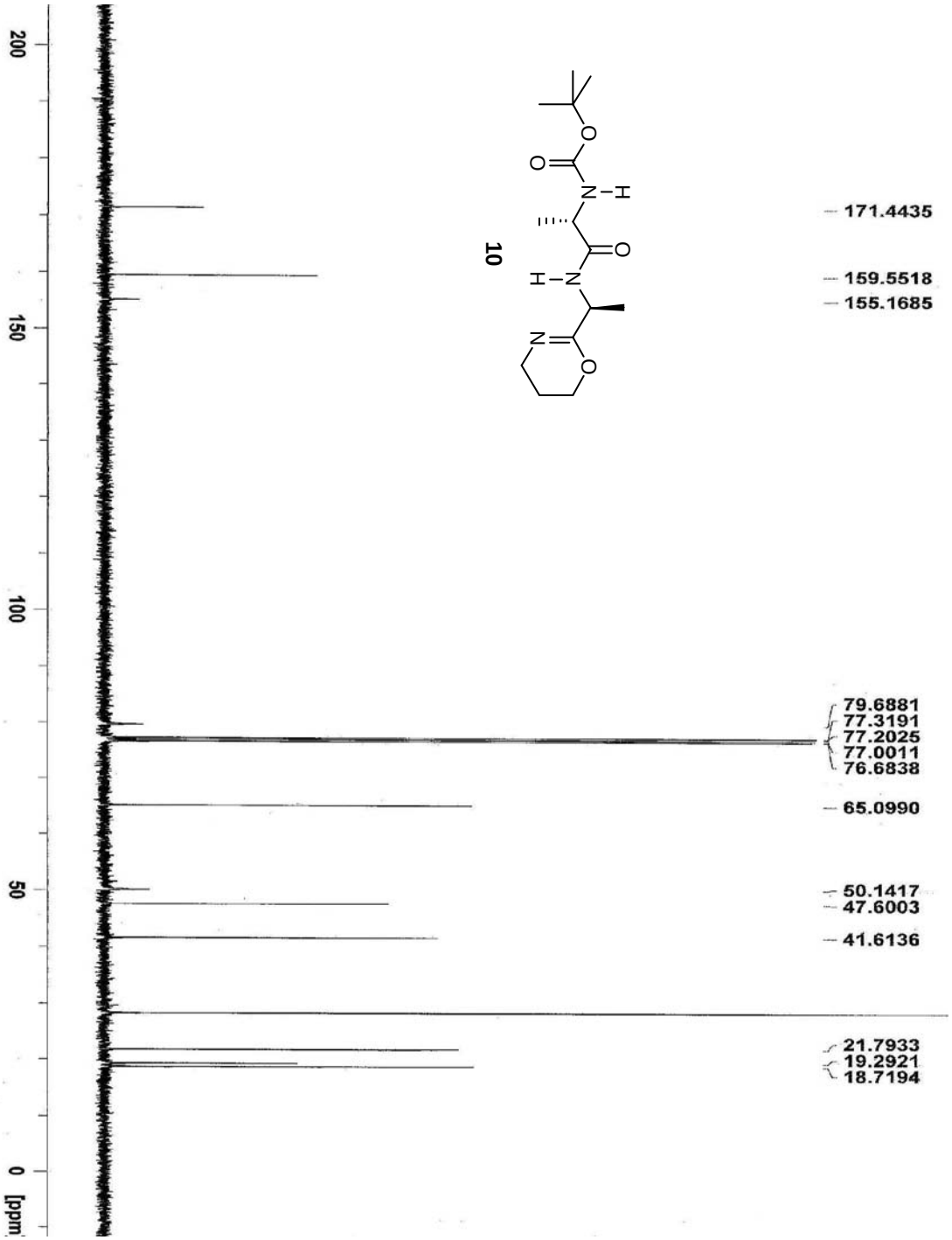
S38. ^{13}C NMR spectrum of the A→I analogue peptide **9** in $CDCl_3$ (100 MHz, 60 mM)



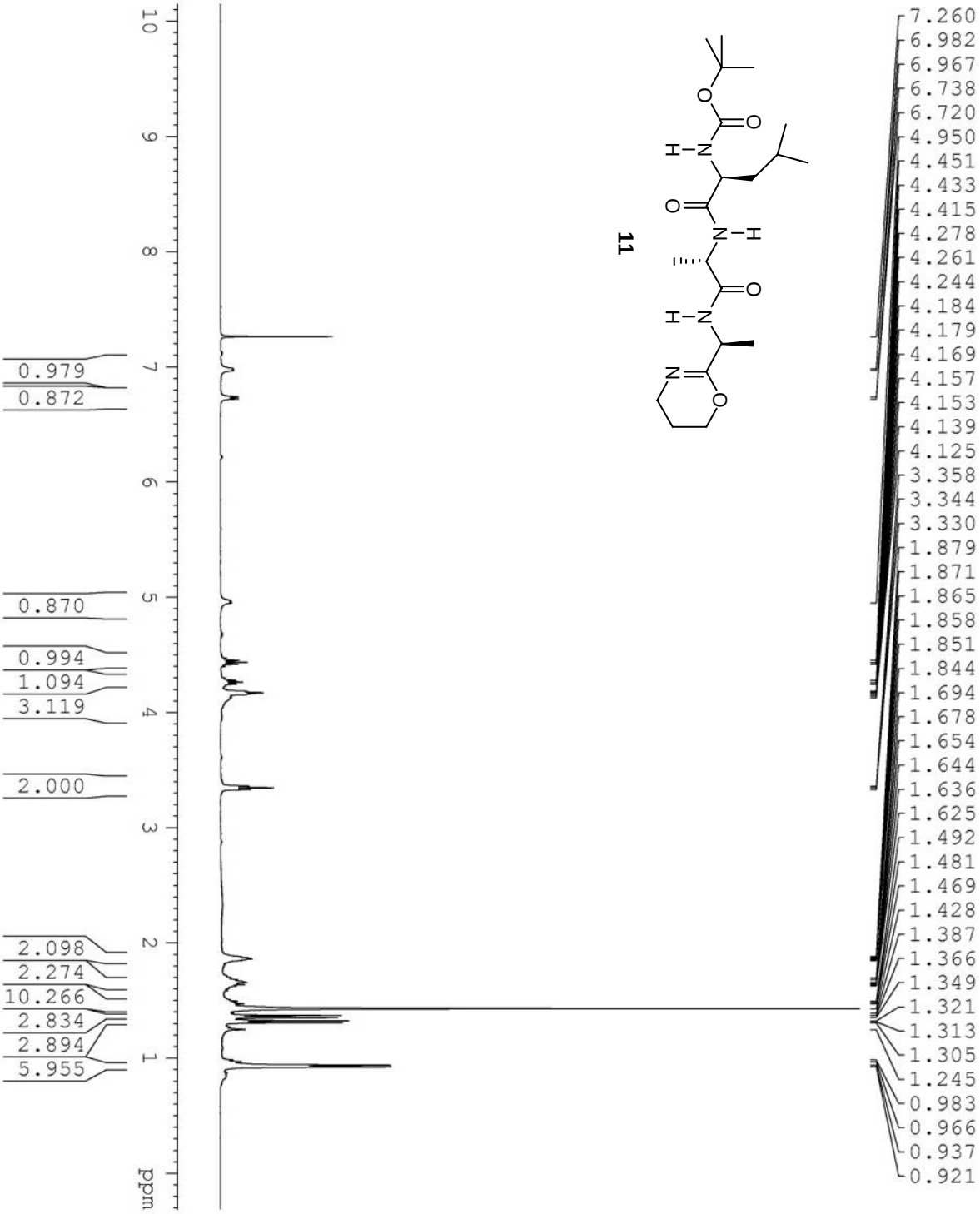
S39. 1H NMR spectrum of the A→I analogue peptide **10** in $CDCl_3$ (400 MHz, 60 mM)



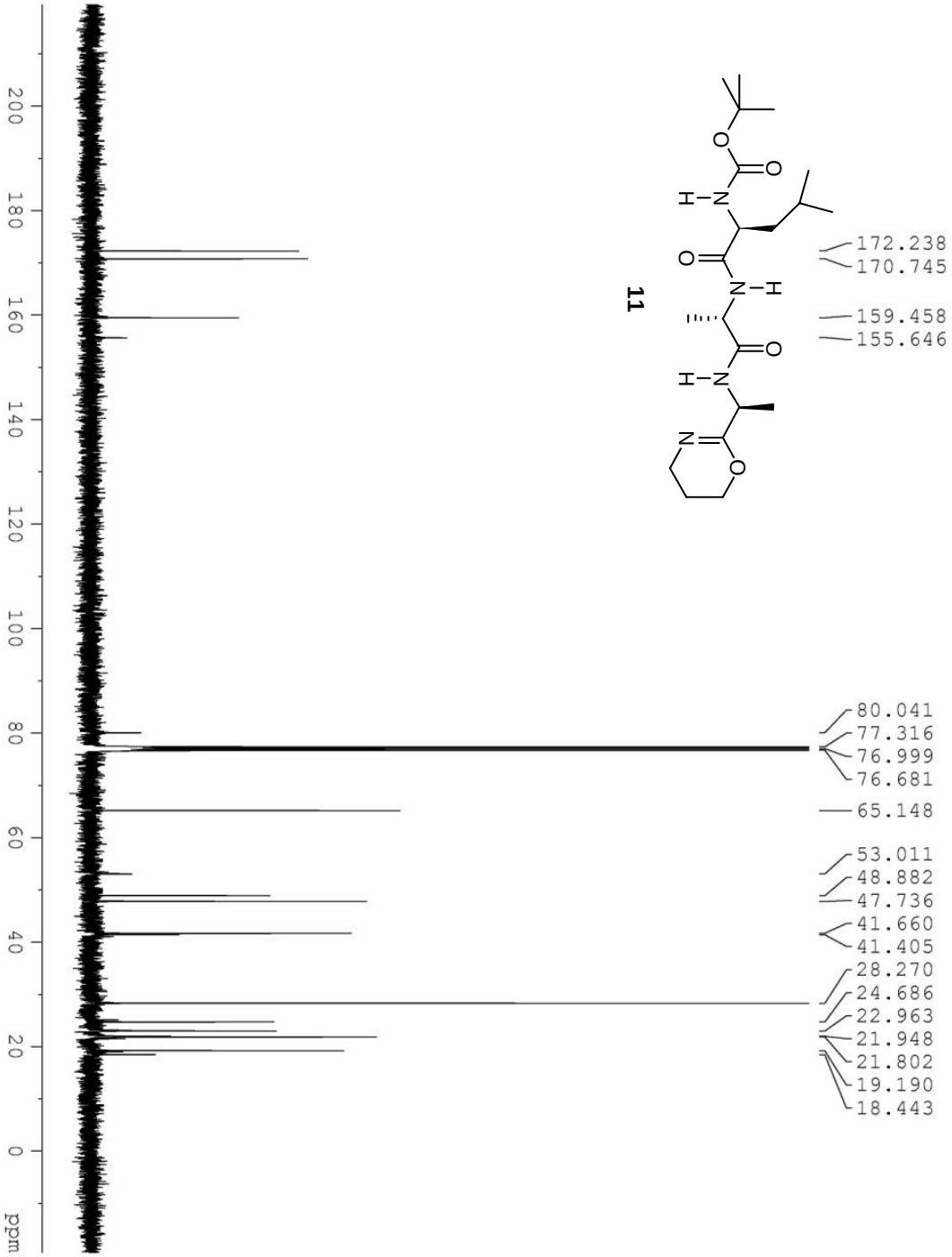
S40. ^{13}C NMR spectrum of the A→I analogue peptide **10** in $CDCl_3$ (100 MHz, 60 mM)



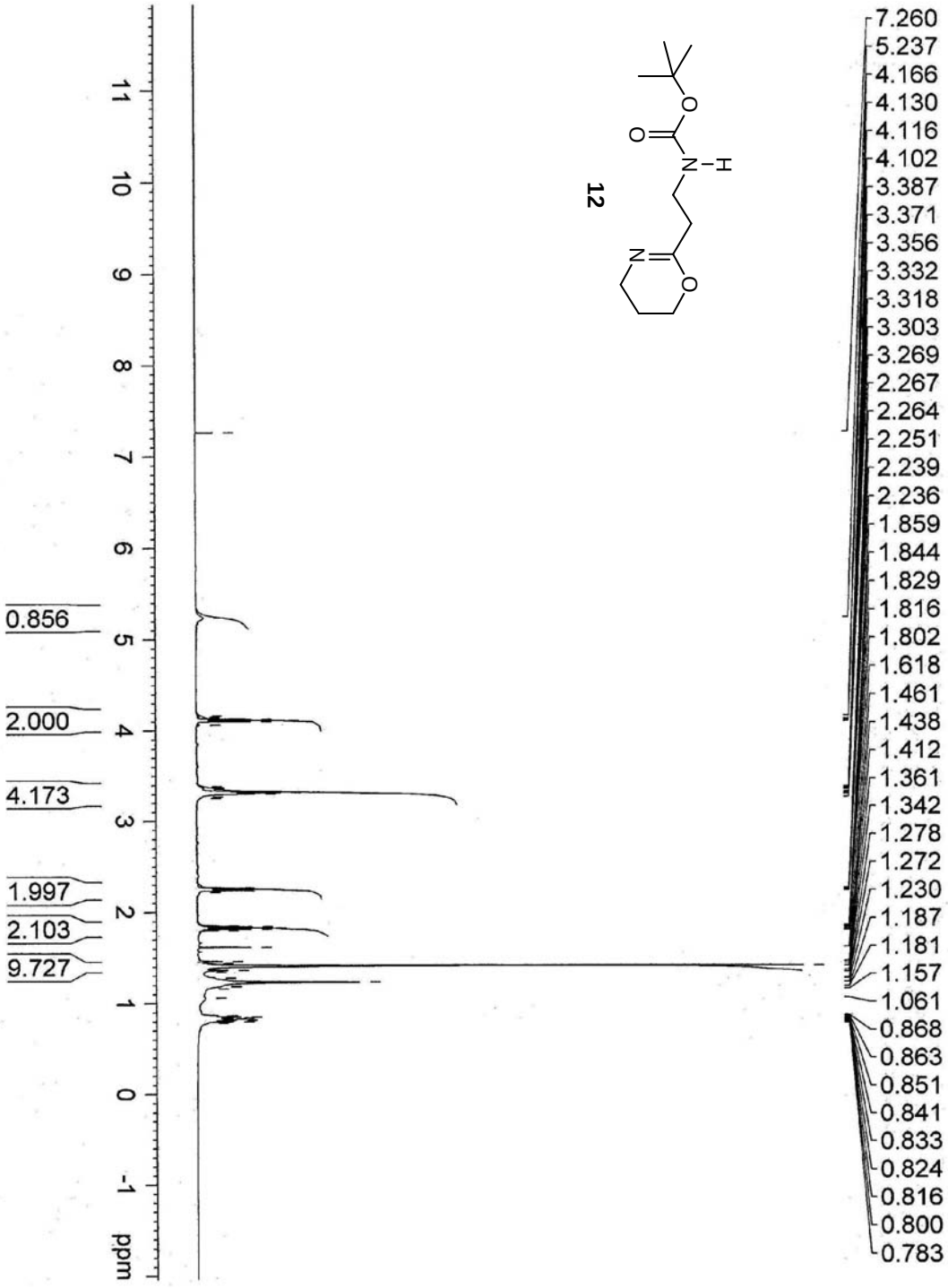
S41. ^{1}H NMR spectrum of the A→I analogue peptide **11** in $CDCl_3$ (400 MHz, 60 mM)



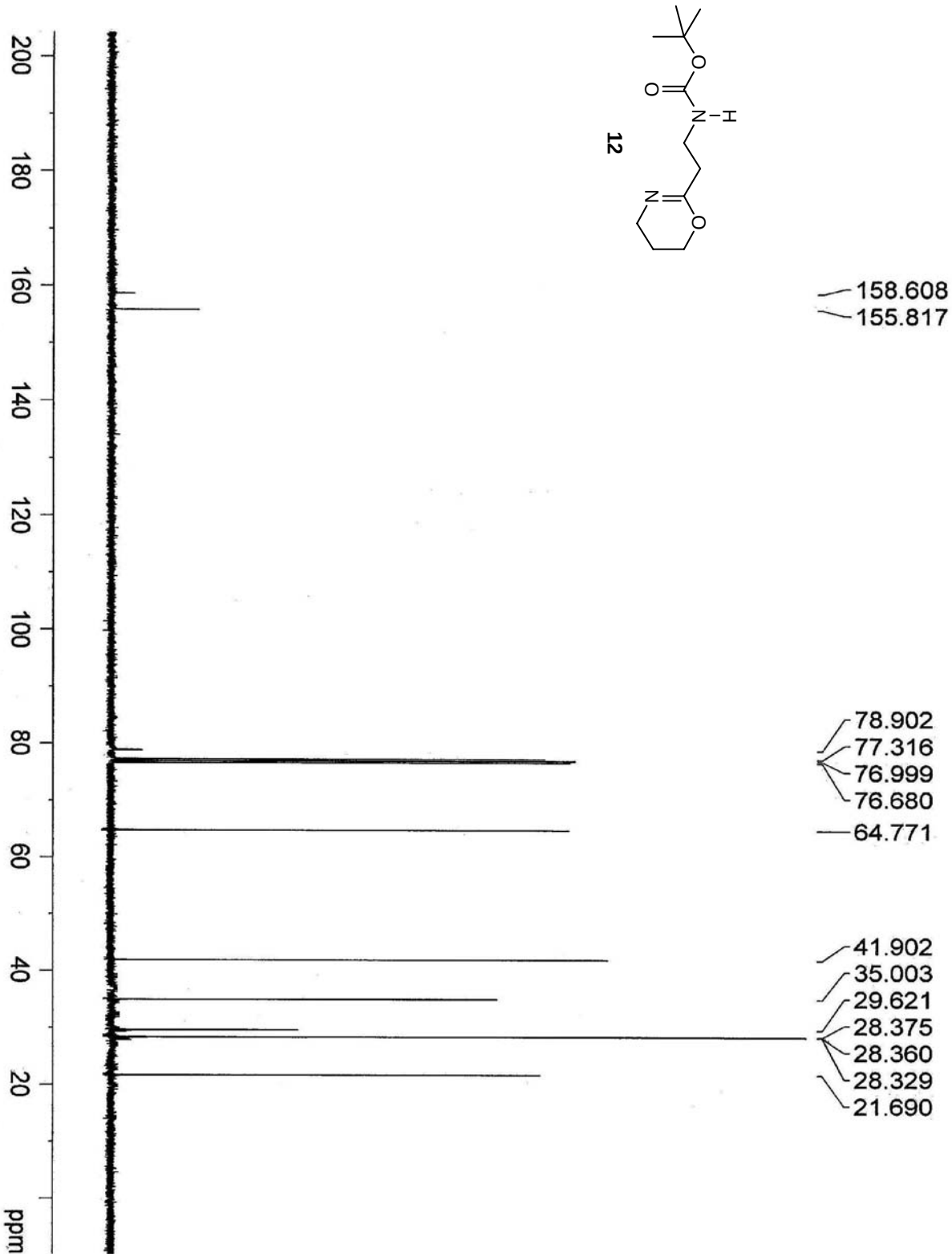
S42. ^{13}C NMR spectrum of the A→I analogue peptide **11** in $CDCl_3$ (100 MHz, 60 mM)



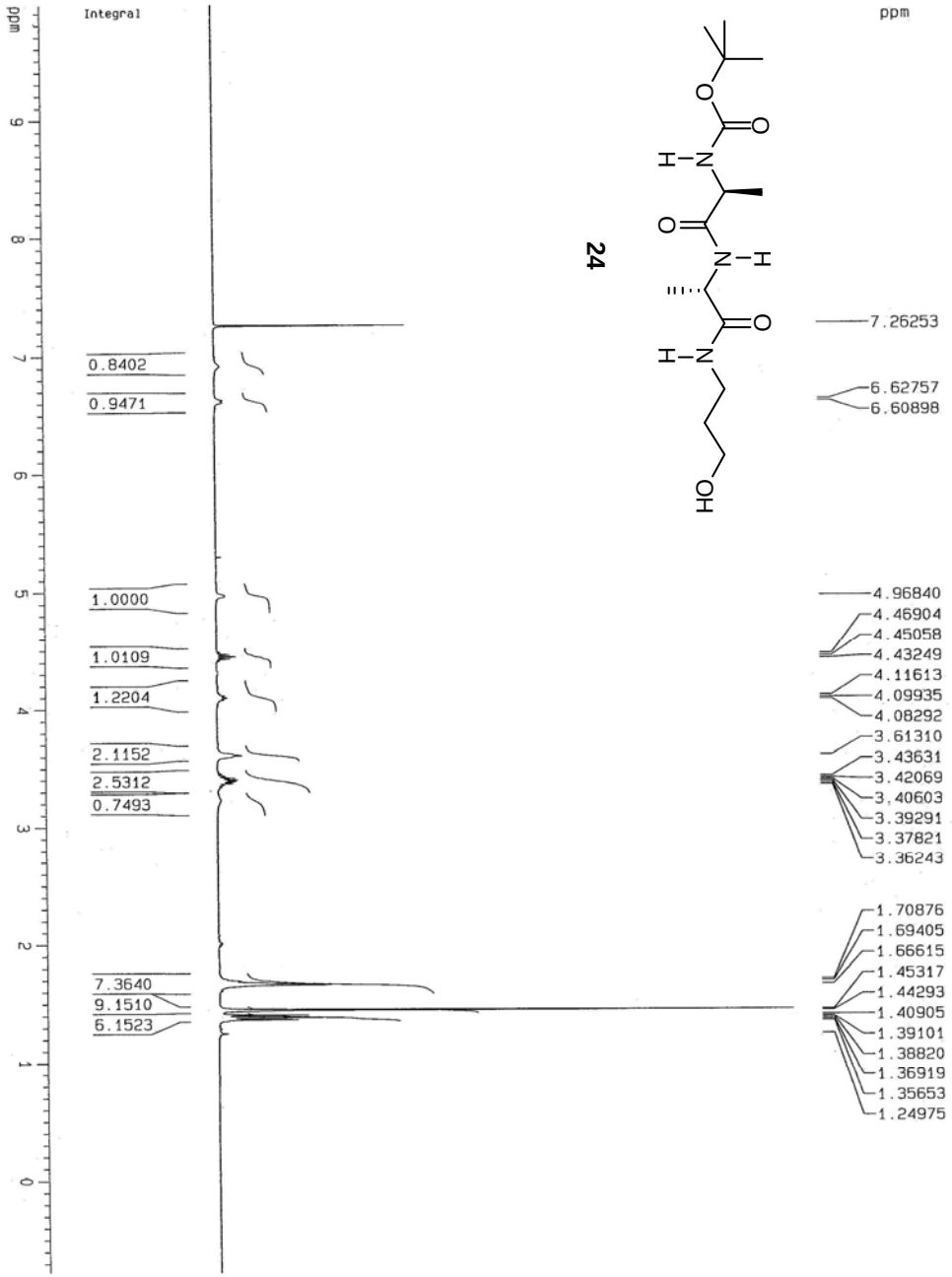
S43. ^{1}H NMR spectrum of the A→I analogue peptide **12** in $CDCl_3$ (400 MHz, 60 mM)



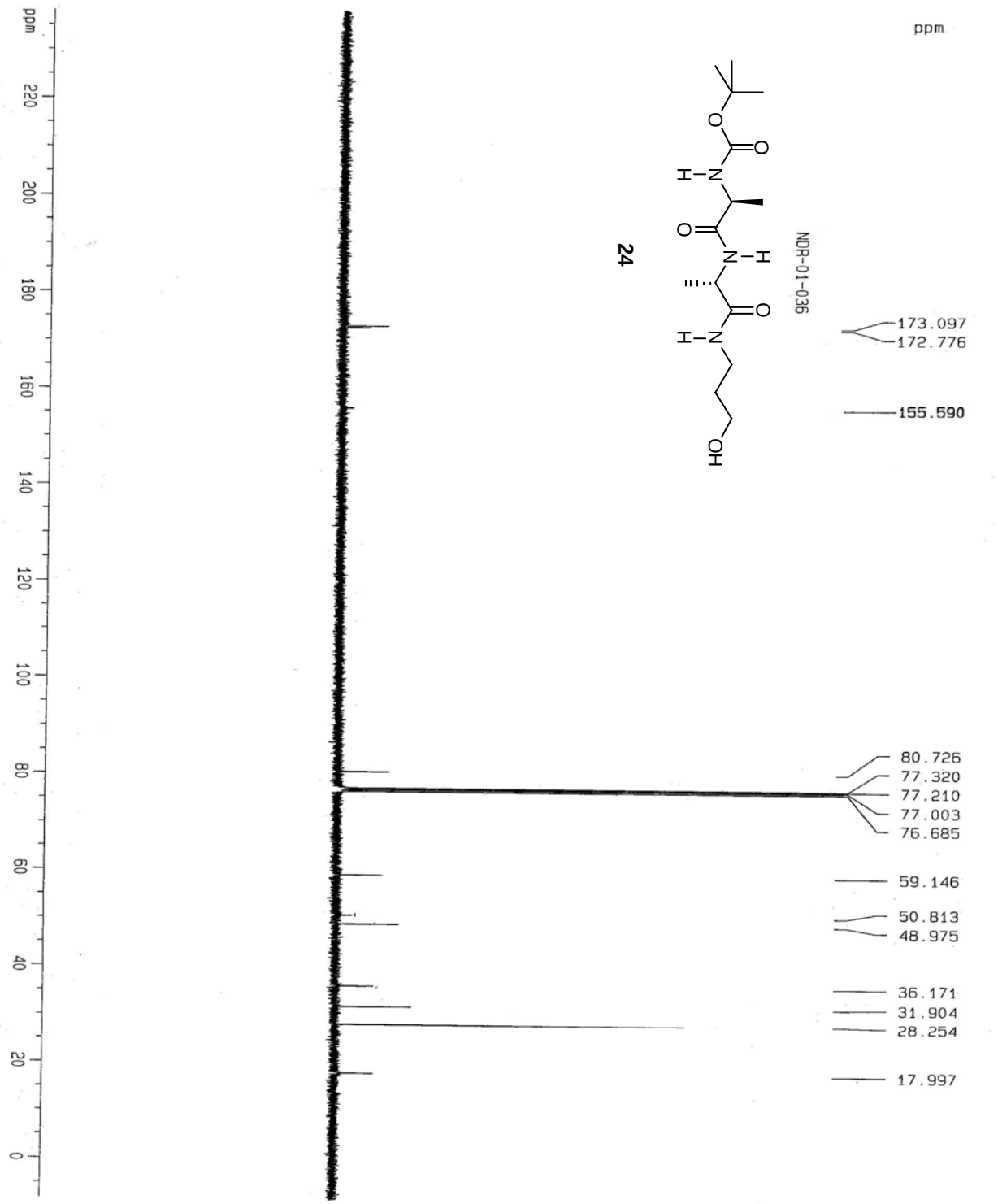
S44. ^{13}C NMR spectrum of the A→I analogue peptide **12** in $CDCl_3$ (100 MHz, 60 mM)



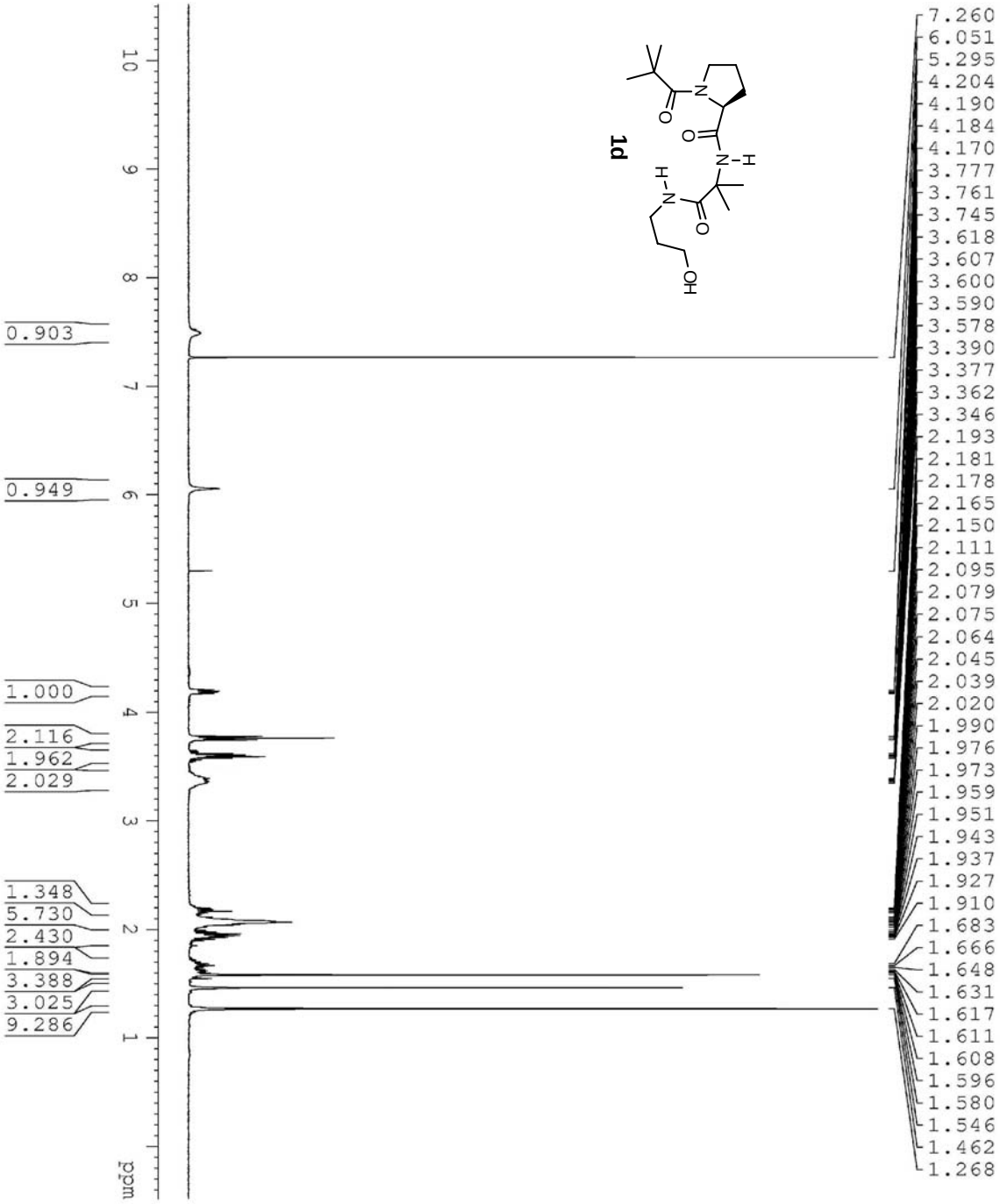
S45. ^{1}H NMR spectrum of the A→I analogue peptide **24** in $CDCl_3$ (400 MHz, 60 mM)



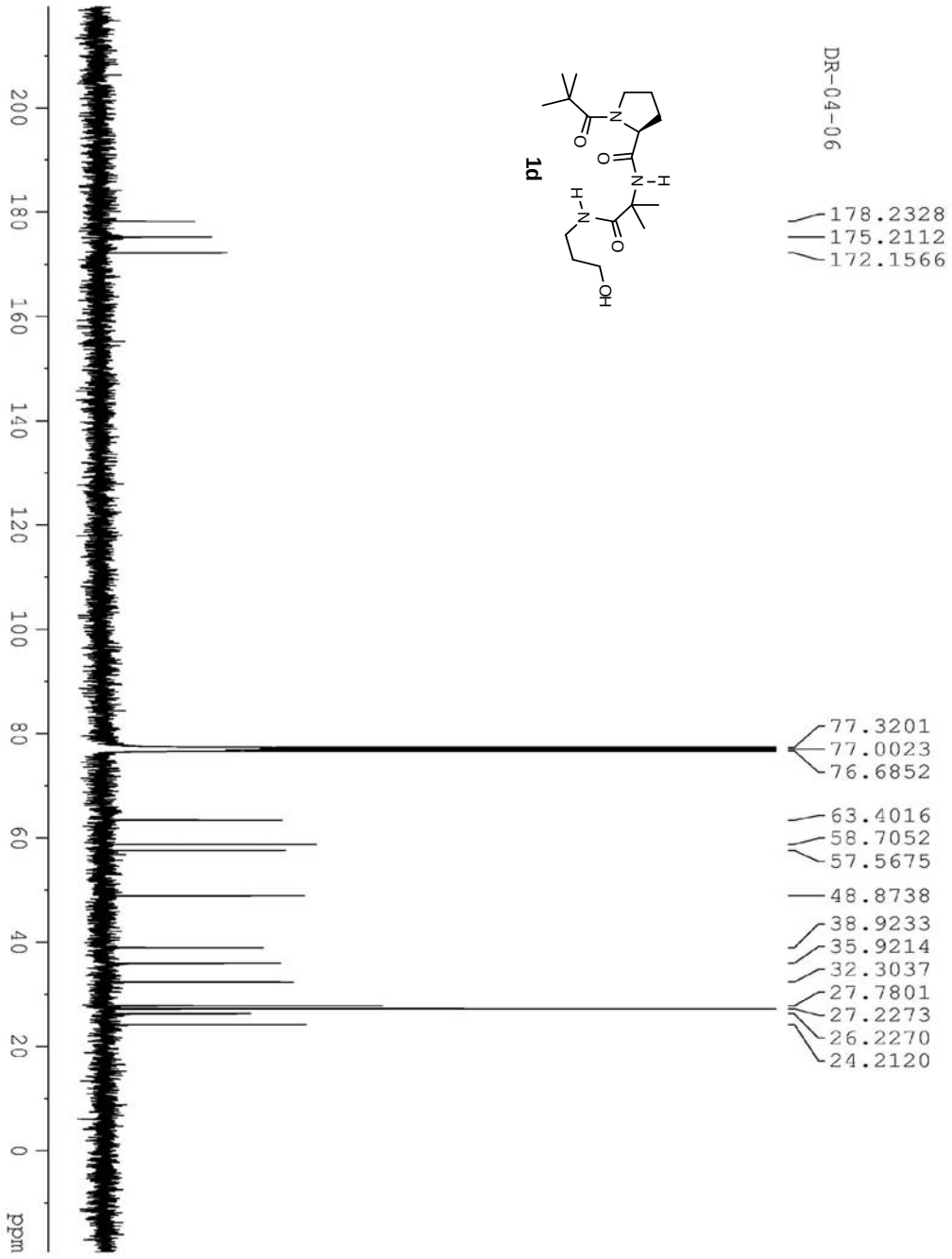
S46. ^{1}H NMR spectrum of the A→I analogue peptide **24** in $CDCl_3$ (100 MHz, 60 mM)



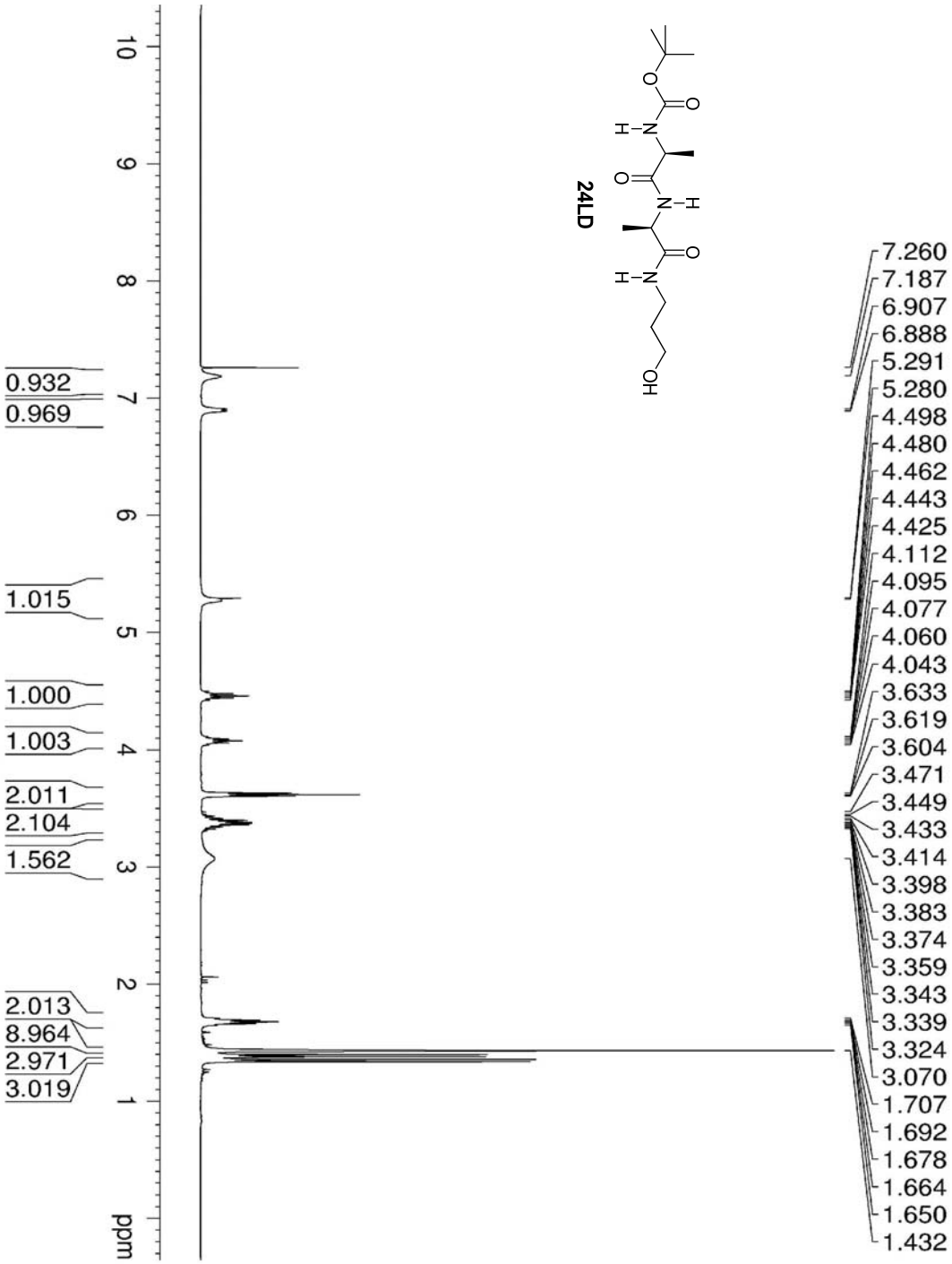
S47. ^{1}H NMR spectrum of the A→I analogue peptide **1d** in $CDCl_3$ (400 MHz, 60 mM)



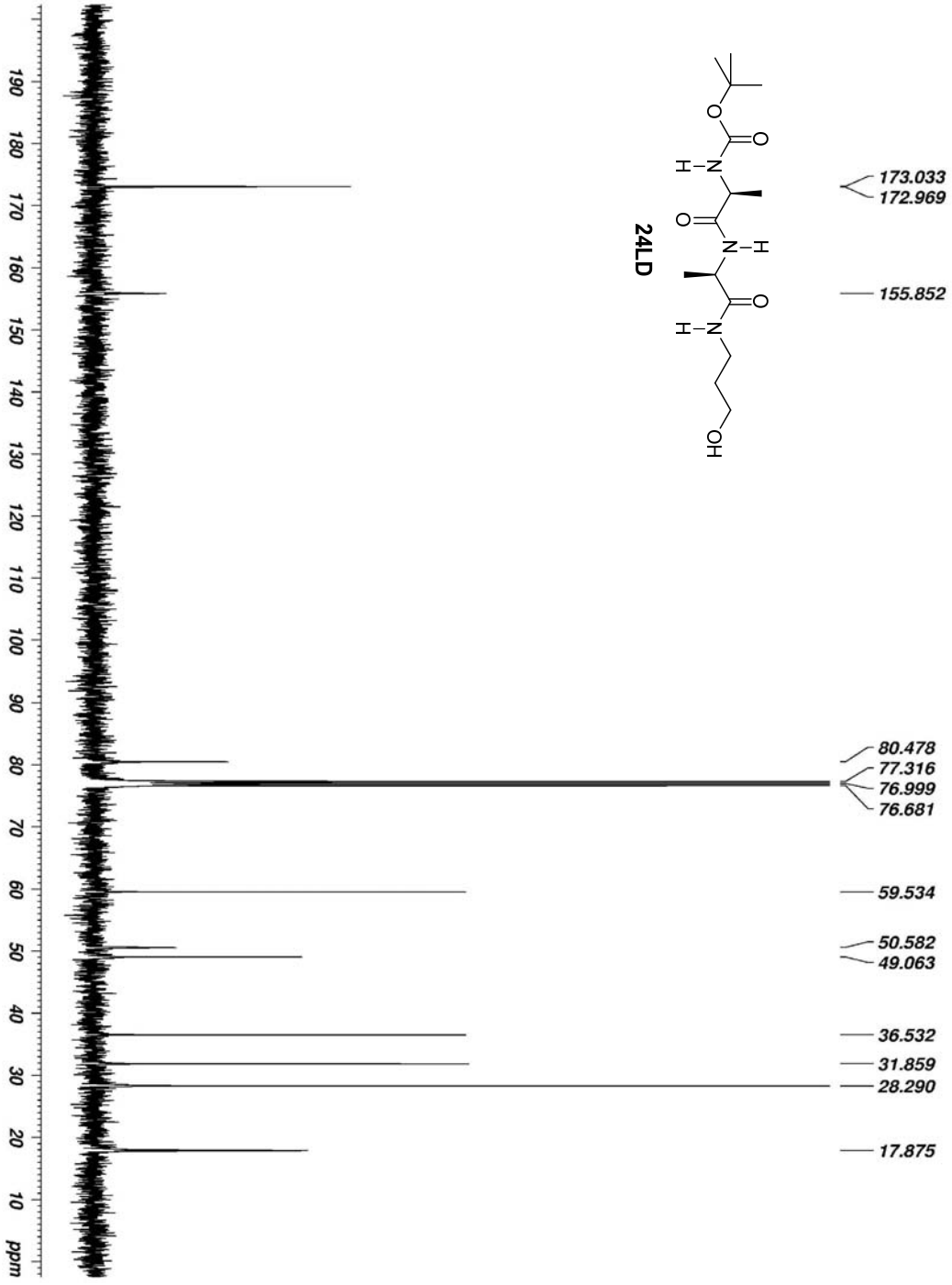
S48. ^{13}C NMR spectrum of the A→I analogue peptide **1d** in $CDCl_3$ (100 MHz, 60 mM)



S49. ^{1}H NMR spectrum of the A→I analogue peptide **24LD** in $CDCl_3$ (400 MHz, 60 mM)



S50. ^{13}C NMR spectrum of the A→I analogue peptide **24LD** in $CDCl_3$ (100 MHz, 60 mM)



S51. ^{1}H NMR spectrum of Boc-Aib-OMe in $CDCl_3$ (400 MHz, 60 mM)