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Manuscript title: A kinetic method for detecting intramolecular peptide H-bonds

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Supporting Information.

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 mesh) (Acme's) purchased from S D Fine Chem. Ltd, India. Thin Layer Chromatography (TLC) was carried out on Merck DC Kieselgel 60 F254 aluminium sheets. Compounds were visualized by one (or all of the) following methods: (1) fluorescence quenching, (2) spraying 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. Ethyl acetate and hexanes (or low boiling fractions of petroleum ether) were obtained from S D Fine Chem. Ltd, India and were fractionally distilled at their respective boiling points, before use. Dichloromethane (DCM) was dried by distillation over phosphorus pentoxide (P_2O_5). N-methyl morpholine (NMM) was distilled over calcium hydride (CaH_2). Nuclear Magnetic Resonance (NMR) spectra were recorded on BRUKER-AV400 spectrometer (Bruker Co., Faellanden, Switzerland). Chemical shifts are expressed as δ values in parts per million (ppm) from the residual non-deuterated chloroform in $CDCl_3$ (δ_H = 7.26 ppm, δ_C = 77.00 ppm). $^{1,3}J_{H,H}$ coupling constant values are expressed in hertz (Hz). Multiplicities are indicated using the following abbreviations: s (singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplets), t (triplet), q (quartet), quin (quintet), sext (sextet), hept (heptet), m (multiplet), bs (broad singlet). Infrared (IR) spectra were recorded in a FT/IR spectrometer, for thin-films (0.1 mmol) made from solutions in $CHCl_3$ (10 mmol) on sodium chloride plates or in neat (KBr pellets), with frequencies given in reciprocal centimetres (cm^{-1}). Mass spectra were obtained with Micromass Q-Tof (ESI-HRMS). Melting points (m.p.) analyses were performed in VEEGO melting point apparatus (VEEGO Inst. Co., Mumbai, India). Far-UV CD spectra were recorded using a JASCO CD spectrometer (model No - J-815) equipped with a peltier temperature-controlled cell holder using a 0.1 cm path length Suprasil quartz cell (Hellma, Forest Hills, NY, USA).

S2. Experimental Procedures.

S2.1. General procedure for coupling of carboxylic acids with amines or amine hydrobromides.

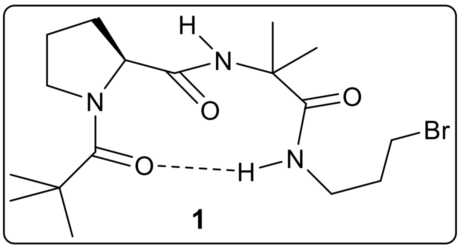
To a cold (-20 °C) solution of the carboxylic acid (1 mmol) and *N*-methyl morpholine (NMM) (1.5 mmol) in tetrahydrofuran (THF), 6 mL ethylchloroformate (ECF) (1.03 mmol) was added under N_2 atmosphere and vigorously stirred. After 2 min of stirring, a solution of amine hydrobromide or amine (1.05 mmol) 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 °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.

S2.2. General procedure for the synthesis and isolation of 2-substituted-5,6-Dihydro-4H-1,3-Oxazine hydrobromides.

Typically the reaction conditions involved the shaking of the amidopropylbromide precursors in $CHCl_3$ (60 mM) in a shaker, set at an internal temperature of 32 °C. 1 equivalent of DIEA was added to the reaction mixture to act as an acid scavenger, and accelerate the autocyclization reaction to appreciable and observable rates. The salts were easily isolated in high purity by trituration of the mixtures with cold dry diethylether (25 mL). The ether wash, containing the soluble amides, was removed by decantation. The resulting insoluble residue was dried under vacuum and directly characterized without any further purification.

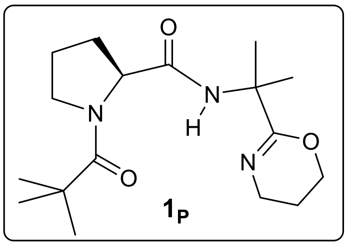
S3. Spectral details.

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



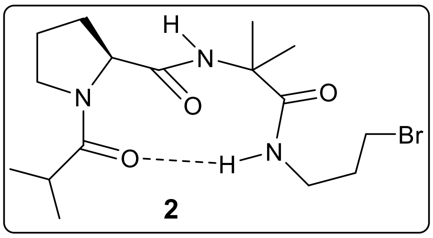
Amide **1** was synthesized by following the general procedure for peptide coupling (**S2.1.**) and purified by silica gel column chromatography (EtOAc : Hexane – 4 : 1) as a white solid (459 mg, 1.14 mmol, 81% yield); (mp = 185-186 °C); (TLC- DCM : MeOH (20 : 1) – R_f = 0.51). IR (NaCl, 10 mM in $CHCl_3$): 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 (p, 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* Calcd for $C_{17}H_{30}BrN_3O_3Na$ 426.1368, Found 426.1364; $[\alpha]_D^{20}$ = -1.9 (c 1, $CHCl_3$).

S3.2. 2-(1-Methyl-1-((S)-(N-Pivaloyl)-Pyrrolidine-2-Carbonyl)amino)-Ethyl)-5,6-Dihydro-4H-1,3-Oxazine (1_P)



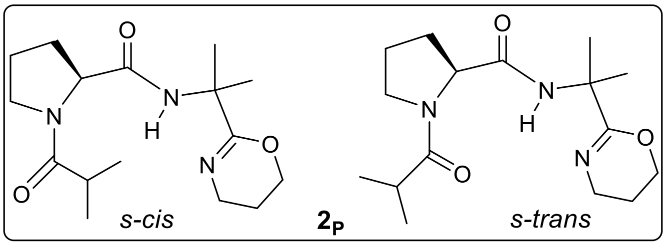
Oxazine **1_P** was synthesized as a white solid (80 mg, 0.25 mmol, 100% yield); (mp = 137-138 °C); (TLC: DCM : MeOH (20 : 1) – R_f = 0.55); IR (NaCl, 10 mM in $CHCl_3$): 3335, 3024, 3002, 2989, 2973, 1681, 1663, 1602, 1516, 1457, 1406, 1364, 1259, 1158, 796 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.75 (bs, 1H), 4.52 (dd, J = 7.1, 7.6 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* Calcd for $C_{17}H_{30}N_3O_3$ 324.2287, Found 324.2285; $[\alpha]_D^{20}$ = -104.9 (c 1, $CHCl_3$).

S3.3. N′-(3′-Bromopropyl)-2-Methyl-2-((S)-((N-Isobutyryl)-Pyrrolidine-2-Carbonyl)amino)-Propanamide (2)



Amide **2** was synthesized by following the general procedure for peptide coupling (**S2.1.**) and purified by silica gel column chromatography (EtOAc : Hexane – 4 : 1) as a white solid (597 mg, 1.53 mmol, 81% yield); (mp = 85-86 °C); (TLC- DCM : MeOH (20 : 1) – R_f = 0.31). IR (NaCl, 10 mM in $CHCl_3$): 3433, 3353, 3006, 2878, 1694, 1669, 1616, 1540, 1472, 1438, 1276, 1179, 1089 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.29 (t, *J* = 5.7 Hz, 1H), 6.43 (bs, 1H), 4.18 (dd, *J* = 7.6, 5 Hz, 1H), 3.69-3.63 (m, 1H), 3.60-3.54 (m, 1H), 3.42 (t, *J* = 6.6 Hz, 2H), 3.36-3.27 (m, 2H), 2.70 (hep, *J* = 6.7 Hz, 1H), 2.21-2.13 (m, 1H), 2.12-2.01 (m, 3H), 1.98-1.93 (m, 2H), 1.53 (s, 3H), 1.43 (s, 3H), 1.14 (d, *J* = 6.6 Hz, 3H), 1.12 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 177.4, 174.2, 171.5, 61.1, 57.4, 47.5, 38.3, 32.4, 32.3, 31.2, 28.5,27.2, 25.3, 24.4, 19.1, 18.7; HRMS *m/z* Calcd for $C_{16}H_{28}BrN_3O_3Na$ 412.1212, Found 412.1212.

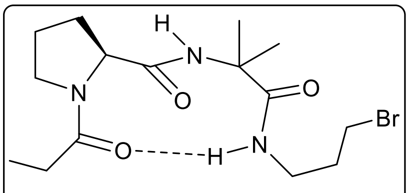
S3.4. 2-(1-Methyl-1-((S)-(N-Isobutyryl)-Pyrrolidine-2-Carbonyl)amino)-Ethyl)-5,6-Dihydro-4H-1,3-Oxazine (2_P)



Oxazine **2P** was synthesized as a crystaline solid (156 mg, 0.51 mmol, 100% yield); (m.p. = 133-135 °C); (TLC: DCM : MeOH (10 : 1) – R_f = 0.31). IR (NaCl, 10 mM in $CHCl_3$): 3331, 3002, 2874, 1679, 1658, 1652, 1603, 1515, 1457, 1426, 1158 cm^{-1}; ***trans* isomer**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.84 (bs, 1H), 4.52 (dd, *J* = 7.8, 2.8 Hz, 1H), 4.19-4.16 (m, 2H), 3.67-3.57 (m, 1H), 3.46-3.38 (m, 1H), 3.36 (t, *J* = 5.9 Hz, 2H), 2.70 (hep, *J* = 6.8 Hz, 1H), 2.26-2.17 (m, 1H), 2.07-1.99 (m, 1H), 1.97-1.89 (m, 2H), 1.84 (p, *J* = 5.6 Hz, 2H), 1.51 (s, 6H), 1.18 (d, *J* = 6.8 Hz, 3H), 1.12 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 176.6, 170.3, 161.8, 65.4, 60.6, 55.5, 47.1, 41.6, 32.3, 28.5, 24.8, 24.2, 24, 21.7, 19.4, 18.5; ***cis* isomer**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.91 (bs, 1H), 4.23 (t, *J* = 5.8 Hz, 1H), 4.19-4.16 (m, 2H), 3.67-3.57 (m, 2H), 3.36 (t, *J* = 5.9 Hz, 2H), 2.48 (hep, *J* = 6.6 Hz, 1H), 2.23-2.14 (m, 2H), 1.91-1.83 (m, 2H), 1.84 (p, *J* = 5.6 Hz, 2H), 1.53 (s, 6H), 1.09 (d, *J* = 6.5 Hz, 3H), 1.08

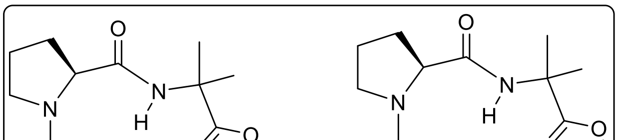
(d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 177.1, 170.7, 161.8, 65.3, 62.1, 55.4, 46.5, 41.8, 32.6, 31.9, 23.7, 23.6, 22.6, 21.6, 19.5, 19.2; HRMS *m/z* Calcd for $C_{16}H_{28}N_3O_3$ 310.2131, Found 310.2133.

S3.5. N′-(3′-Bromopropyl)-2-Methyl-2-((S)-((N-Propionyl)-Pyrrolidine-2-Carbonyl)amino)-Propanamide (3)


3

Amide **3** was synthesized by following the general procedure for peptide coupling (**S2.1.**) and purified by silica gel column chromatography (EtOAc : Hexane – 9 : 1) as a white solid (107 mg, 0.28 mmol, 73% yield); (mp = 100-102 °C); (TLC- DCM : MeOH (10 : 1) – R_f = 0.43). IR (NaCl, 10 mM in $CHCl_3$): 3433, 3355, 3006, 2880, 1693, 1667, 1620, 1537, 1510, 1440, 1382, 1219 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.28 (t, J = 5.4 Hz, 1H), 6.48 (bs, 1H), 4.17 (dd, J = 7.4, 4.7 Hz, 1H), 3.63-3.58 (m, 1H), 3.52-3.46 (m, 1H), 3.43-3.39 (m, 2H), 3.37-3.25 (m, 2H), 2.36 (q, J = 7.4 Hz, 2H), 2.21-2.11 (m, 2H), 2.05 (p, J = 6.3 Hz, 2H), 2.03-1.99 (m, 1H), 1.95-1.91 (m, 1H), 1.55 (s, 3H), 1.43 (s, 3H), 1.34 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.2, 174, 171.6, 61.1, 57.5, 47.6, 38.2, 32.2, 31.2, 28.6, 27.8, 27.4, 25.2, 24.2, 8.8; HRMS *m/z* Calcd for $C_{15}H_{26}BrN_3O_3Na$ 398.1055, Found 398.1053.

S3.6. 2-(1-Methyl-1-((S)-(N-Propionyl)-Pyrrolidine-2-Carbonyl)amino)-Ethyl)-5,6-Dihydro-4H-1,3-Oxazine (3_P)

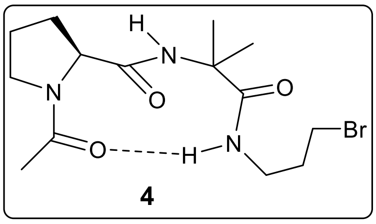


3_P

Oxazine $\mathbf{3_P}$ was synthesized as a viscous oil (79 mg, 0.28 mmol, 98% yield); (TLC: DCM : MeOH (10 : 1) – R_f = 0.29). IR (NaCl, 10 mM in $CHCl_3$): 3327, 3002, 2884, 1682, 1661, 1654, 1638, 1602, 1516, 1458, 1421, 1348, 1218 cm^{-1}; ***trans* isomer**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.78 (bs, 1H), 4.47 (dd, J = 8.2, 2.5 Hz, 1H), 4.15 (t, J = 5.6 Hz, 2H), 3.64-3.56 (m, 1H), 3.46-3.38 (m, 1H), 3.37 (t, J = 5.8 Hz, 2H), 2.39-2.28 (m, 2H), 2.23-2.16 (m, 1H), 2.09-2.02 (m, 1H), 1.98-1.91 (m, 1H), 1.86-1.81 (m, 3H), 1.52 (s, 3H), 1.51 (s, 3H), 1.16 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.2, 170.2, 162.1, 65.1, 60.5, 55.4, 47.1, 41.7, 28.7, 27.8, 24.6, 24.1, 23.8, 21.6, 8.9; ***cis* isomer**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.81 (bs, 1H), 4.19-4.15 (m, 3H), 3.64-3.56 (m, 2H), 3.36 (t, J = 5.8 Hz, 2H), 2.25-2.20 (m, 2H), 2.23-2.16 (m, 1H), 1.98-

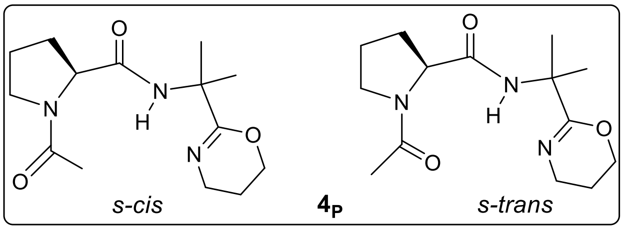
1.92 (m, 1H), 1.87-1.78 (m, 3H), 1.53 (s, 3H), 1.52 (s, 3H), 1.12 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.5, 170.4, 161.7, 65.3, 62.2, 55.3, 46.5, 41.7, 31.9, 27.5, 23.7, 23.5, 22.6, 21.6, 9.1; HRMS *m/z* Calcd for $C_{15}H_{26}N_3O_3$ 296.1974, Found 296.1971.

S3.7. N′-(3′-Bromopropyl)-2-Methyl-2-((S)-((N-Acetyl)-Pyrrolidine-2-Carbonyl)amino)-Propanamide (4)



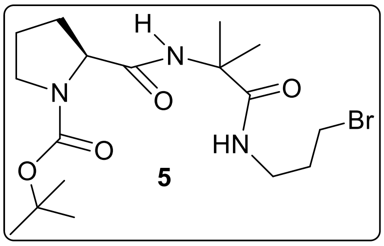
Amide **4** was synthesized by following the general procedure for peptide coupling (**S2.1.**) and purified by silica gel column chromatography (EtOAc : Hexane – 9 : 1) as a white solid (191 mg, 0.49 mmol, 79% yield); (mp = 133-134°C); (TLC- DCM : MeOH (10 : 1) – R_f = 0.41). IR (NaCl, 10 mM in $CHCl_3$): 3432, 3357, 3012, 2280, 1693, 1668, 1625, 1538, 1509, 1446, 1383, 1363, 1216, 1037 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.20 (bs, 1H), 6.51 (bs, 1H), 4.19 (dd, *J* = 7.2, 4.2 Hz, 1H), 3.66-3.61 (m, 1H), 3.53-3.48 (m, 1H), 3.44-3.38 (m, 3H), 3.30-3.22 (m, 1H), 2.20-2.11 (m, 2H), 2.12 (s, 3H), 2.09-2.01 (m, 3H), 1.98-1.94 (m, 1H), 1.55 (s, 3H), 1.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.2, 171.4, 170.9, 61, 57.5, 48.5, 38.1, 32.2, 31.3, 28.7, 27.3, 25.2, 24.3, 22.6 ; HRMS *m/z* Calcd for $C_{14}H_{24}BrN_3O_3Na$ 384.0899, Found 384.0894.

S3.8. 2-(1-Methyl-1-((S)-(N-Acetyl)-Pyrrolidine-2-Carbonyl)amino)-Ethyl)-5,6-Dihydro-4H-1,3-Oxazine (4$_{P}$)



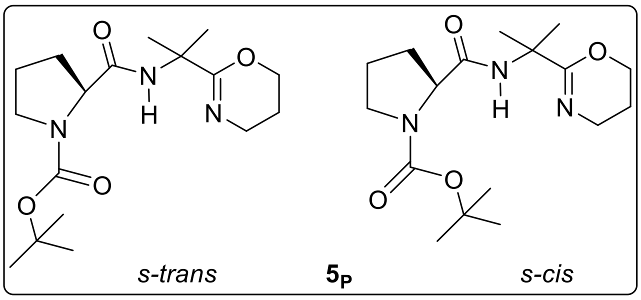
Oxazine **4**$_{P}$ was synthesized as a viscous oil (79 mg, 0.28 mmol, 98% yield); (TLC: DCM : MeOH (10 : 1) – R_f = 0.29). IR (NaCl, 10 mM in $CHCl_3$): 3432, 3322, 3007, 2880, 1678, 1662, 1634, 1540, 1515, 1456, 1420, 1359, 1277, 1251, 1157, 1079 cm^{-1}; ***trans* isomer**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.79 (bs, 1H), 4.43 (dd, *J* = 8.1, 2.3 Hz, 1H), 4.18-4.14 (m, 2H), 3.64-3.55 (m, 1H), 3.47-3.41 (m, 1H), 3.39-3.34 (m, 2H), 2.24-2.15 (m, 1H), 2.09 (s, 3H), 2.11-1.99 (m, 1H), 1.98-1.91 (m, 1H), 1.87-1.81 (m, 2H), 1.50 (s, 6H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.2, 170, 162.2, 65.2, 60.4, 54.4, 48, 41.8, 28.9, 24.7, 24.1, 23.8, 22.4, 21.6; ; ***cis* isomer**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.84 (bs, 1H), 4.18-4.14 (m, 3H), 3.64-3.55 (m, 2H), 3.39-3.34 (m, 2H), 2.22-2.15 (m, 1H), 2.01 (s, 3H), 2.11-1.99 (m, 1H), 1.99-1.91 (m, 1H), 1.87-1.81 (m, 2H), 1.53 (s, 6H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.3, 169.9, 161.8, 65.3, 62.9, 55.3, 46.5, 41.7, 31.9, 23.7, 23.5, 22.8, 22.3, 21.6; HRMS *m/z* Calcd for $C_{14}H_{23}N_3O_3Na$ 304.1637, Found 304.1635.

S3.9. N′-(3′-Bromopropyl)-2-Methyl-2-((S)-((N-*tert*-Butyloxycarbonyl)-Pyrrolidine-2-Carbonyl)amino)-Propanamide (5)



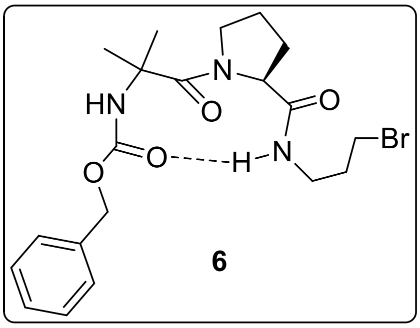
Amide **5** was synthesized by following the general procedure for peptide coupling (**S2.1.**) and purified by silica gel column chromatography (EtOAc : Hexane – 2 : 3) as a white solid (243 mg, 0.58 mmol, 79% yield); (mp = 138-139 °C); (TLC-EtOAc – R_f = 0.23). IR (NaCl, KBr): 3366, 3280, 2976, 2875, 1702, 1668, 1548, 1416, 1285, 1191, 1175, 1131, 1098 cm^{-1}; IR (NaCl, 10 mM in $CHCl_3$): 3430, 3358, 3010, 2882, 1693, 1677, 1665, 1534, 1507, 1395, 1368, 1265, 1178, 1158, 917, 830 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.24 (bs, 1H), 6.46 (bs, 1H), 4.06 (t, *J* = 6.3 Hz, 1H), 3.50-3.35 (m 1H), 3.32-3.23 (m, 1H), 2.08 (p, *J* = 6.6 Hz, 2H), 2.06-2.03 (m, 2H), 1.99-1.91 (m, 1H), 1.91-1.84 (m, 1H), 1.50 (s, 6H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 174.3, 171.7, 155.8, 80.8, 61.1, 157.3, 47.3, 38.1, 32.3, 31.1, 29.2, 28.4, 25.8, 24.7; HRMS *m/z* Calcd for $C_{17}H_{30}BrN_3O_4Na$ 442.1317, Found 442.1321.

S3.10. 2-(1-Methyl-1-((S)-(N-*tert*-Butyloxycarbonyl)-Pyrrolidine-2-Carbonyl)amino)-Ethyl)-5,6-Dihydro-4H-1,3-Oxazine (5_P)



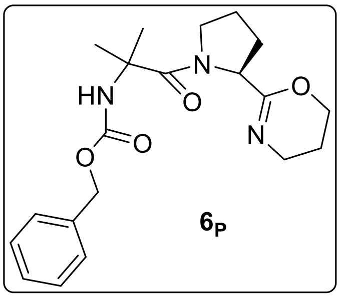
Oxazine **5_P** was synthesized as a viscous oil (80 mg, 0.24 mmol, 99% yield); (TLC: DCM : MeOH (20 : 1) – R_f = 0.55). IR (NaCl, 10 mM in $CHCl_3$): 3329, 2984, 2932, 1700, 1672, 1668, 1515, 1457, 1391, 1367, 1161, 1117 cm^{-1}; ***trans* isomer**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.87 (bs, 1H), 4.25-4.21(m, 1H), 4.17 (t, *J* = 5.1 Hz, 2H), 3.54-3.43 (m, 2H), 3.36 (t, *J* = 5.8 Hz, 2H), 2.26-2.18 (m, 1H), 2.15-2.06 (m, 1H), 1.91-1.79 (m, 4H), 1.55 (s, 6H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.8, 161.9, 154.9, 179.6, 65.2, 60.9, 55.3, 46.8, 41.7, 29.6, 28.3, 23.5, 21.7, 23.7; ***cis* isomer**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.79 (bs, 1H), 4.17 (t, *J* = 5.1 Hz, 2H), 4.11-4.01 (m, 1H), 3.54-3.43 (m, 2H), 3.36 (t, *J* = 5.8 Hz, 2H), 2.15-2.06 (m, 2H), 1.91-1.79 (m, 4H), 1.54 (s, 6H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 171.1, 161.8, 154.4, 79.9, 65.2, 61.6, 55.1, 46.7, 41.7, 31.1, 28.3, 23.7, 21.7, 22.6; HRMS *m/z* Calcd for $C_{17}H_{30}N_3O_4$ 340.2236, Found 340.2240.

S3.11. Benzyloxycarbonyl-α-Aminoisobutyryl-L-Prolyl-N-(3-bromopropyl)amide (6)



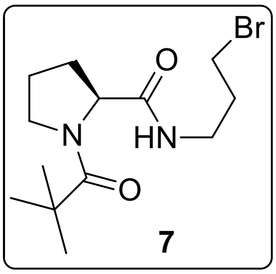
Amide **6** was synthesized by following the general procedure for peptide coupling (**S2.1.**) and purified by silica gel flash column chromatography (EtOAc : Hexane – 3 : 2) as a white solid (532 mg, 1.78 mmol, 78% yield); (mp = 133-134 °C); (TLC: EtOAc – R_f = 0.37). IR (NaCl, 10 mM in $CHCl_3$): 3435, 3353, 3027, 3008, 2878, 1714, 1652, 1649, 1602, 1541, 1505, 1402, 1367, 1265, 1086, 887 814 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.32-7.29 (m, 6H), 5.66 (bs, 1H), 5.17 (d, *J* = 12 Hz, 1H), 5 (d, *J* = 12 Hz, 1H), 4.5 (dd, *J* = 7.2 Hz, 1H), 3.65-3.6 (m, 1H), 3.5-3.35 (m, 3H), 3.21-3.12(m, 2H), 2.12-2.03 (m, 3H), 1.8-1.66 (m, 3H), 1.54 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 172.03, 171.98, 155.4, 136.1, 128.5, 128.4, 128.3, 67.1, 62.7, 57, 48.1, 37.6, 32.2, 31.2, 28.5, 26.5, 25.6, 24.5; HRMS *m/z* Calcd for $C_{20}H_{28}BrN_3O_4Na$ 476.1161, Found 476.1168; $[\alpha]_D^{20}$ = (*c* 1, $CHCl_3$).

S3.12. 2-(2-(*S*)-((N-Benzyloxycarbonyl)-1-Methyl-Ethylcarbonyl)amino)-Pyrrolidine)-5,6-Dihydro-4H-1,3-Oxazine (6_P)



Oxazine **6_P** was synthesized as a viscous oil (40 mg, 0.11 mmol, 96% yield); (TLC: DCM : MeOH (20 : 1) – R_f = 0.43); IR (NaCl, 10 mM in $CHCl_3$): 3375, 3105, 3030, 3013, 2929, 2856, 1716, 1681, 1676, 1634, 1628, 1497, 1456, 1410, 1265, 1222, 1210, 1164, 1087, 1075 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.35-7.29 (m, 5H), 5.06 (bs, 3H), 4.46-4.44 (m, 1H), 4.15-4.08 (m, 2H), 3.65-3.56 (m, 2H), 3.34-3.31 (m, 2H), 2.03-2.01 (m, 1H), 1.84-1.79 (m, 3H), 1.61-1.58 (m, 2H), 1.24 (s, 6H); HRMS *m/z* Calcd for $C_{20}H_{28}N_3O_4$ 374.208, Found 374.2082; $[\alpha]_D^{20}$ = -39.1 (*c* 0.1, $CHCl_3$).

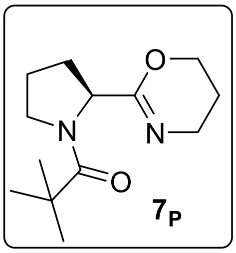
S3.13. N-Pivaloyl-N'-(3-bromopropyl)-L-Prolinamide (7)



7

Amide **7** was synthesized by following the general procedure for peptide coupling (**S2.1.**) and purified by silica gel flash column chromatography (EtOAc : Hexane – 3 : 2) as a viscous oil (404 mg, 1.27 mmol, 79% yield); (TLC - DCM : MeOH (20 : 1)– R_f = 0.44); IR (NaCl, 10 mM in $CHCl_3$): 3444, 3313, 2968, 2877, 1674, 1603, 1538, 1522, 1409, 1366, 1237 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 6.89 (bs, 1H), 4.58 (dd, J = 8.1, 3.3Hz, 1H), 3.74-3.62 (m, 2H), 3.40 (t, J = 6.6Hz, 2H), 3.35 (q, J = 6.4Hz, 2H), 2.25-2.19 (m, 1H), 2.13-2.00 (m, 3H), 1.95-1.81 (m, 2H), 1.26 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 178, 172.2, 61.7, 48.3, 39.2, 37.7, 32, 30.8, 27.5, 27.3, 25.9; HRMS m/z Calcd for $C_{13}H_{23}BrN_2O_2Na$ 341.0841, Found 341.0844; $[\alpha]_D^{20}$ = -86.5 (c 1, $CHCl_3$).

S3.14. 2-((*S*)-((S)-((N-Pivaloyl)amino)-Pyrrolidine)-5,6-Dihydro-4*H*-1,3-Oxazine (7_P)

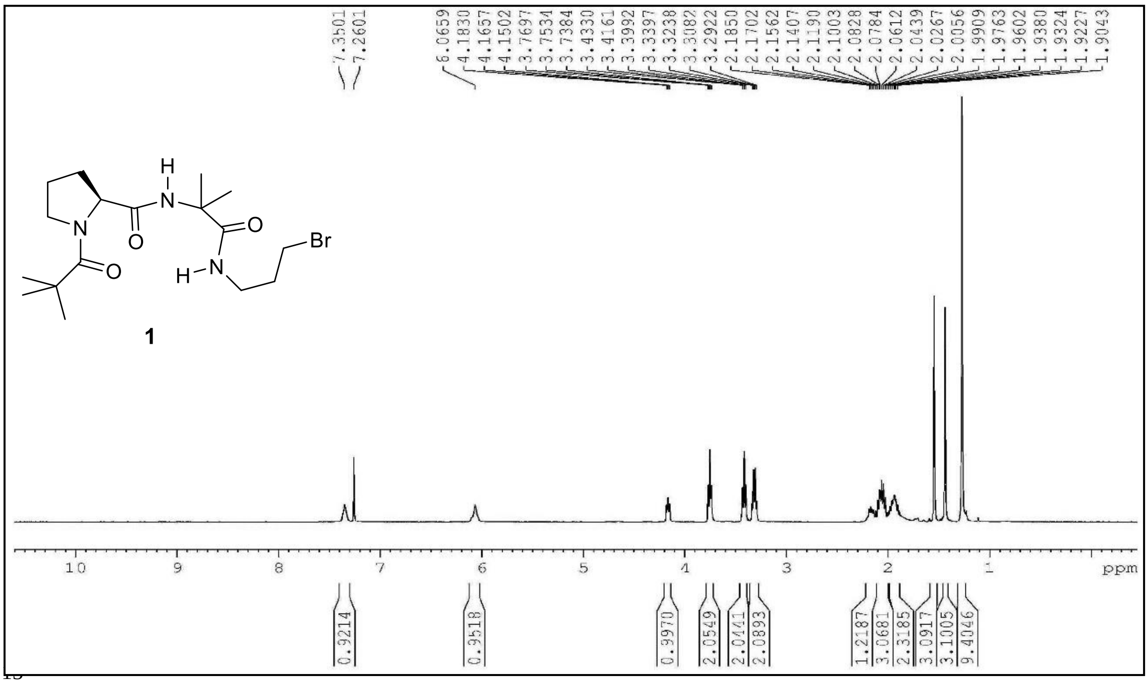


7_P

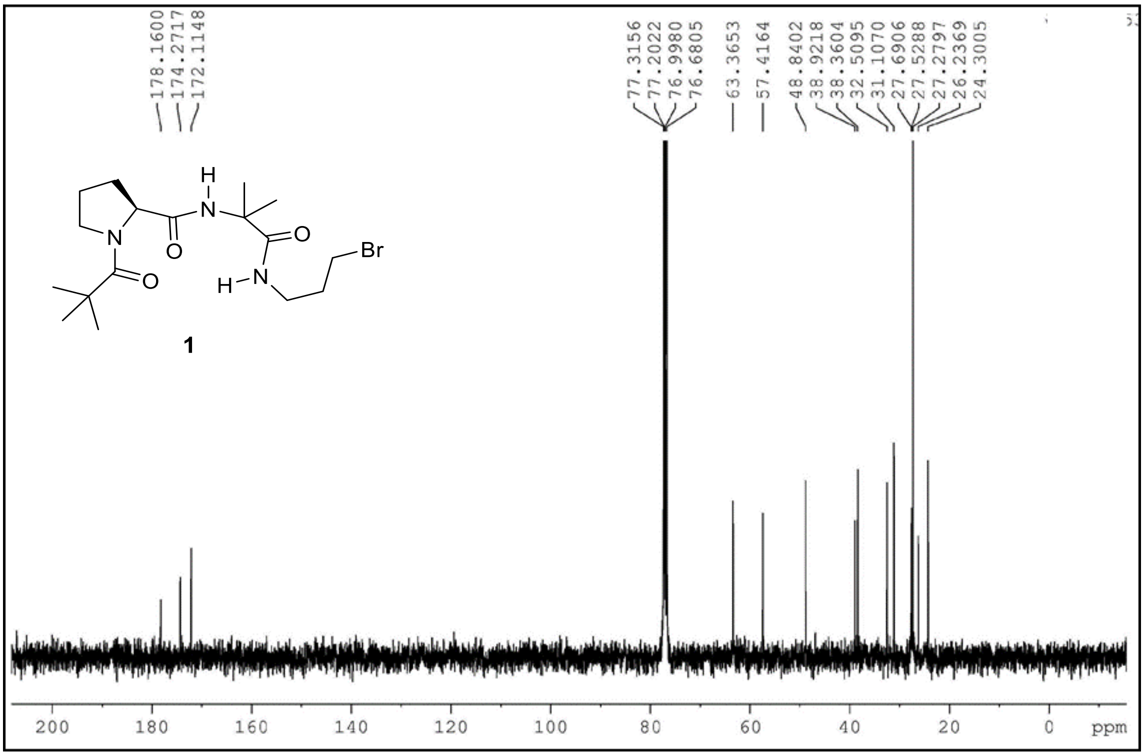
Oxazine **7_P** was synthesized as a viscous oil (36 mg, 0.15 mmol, 99% yield); (TLC- DCM : MeOH (20 : 1) – R_f = 0.27). FT-IR (NaCl, 10 mM in $CHCl_3$): 2991, 2878, 1676, 1607, 1411, 1385, 1365, 1189, 1089 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 4.48-4.38 (m, 1H), 4.15-4.05 (m, 2H), 3..70-3.63 (m, 2H), 3.37-3.29 (m, 2H), 2.11-1.98 (m, 2H), 1.87-1.78 (m, 4H), 1.23 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 176.3, 160.2, 64.9, 61.7, 48.2, 41.8, 38.9, 29, 27.5, 25.4, 21.8; HRMS m/z Calcd for $C_{13}H_{22}N_2O_2Na$ 261.1579, Found 261.1576; $[\alpha]_D^{20}$ = -45.7 (c 1, $CHCl_3$).

S4. ¹H NMR and ¹³C NMR Spectra

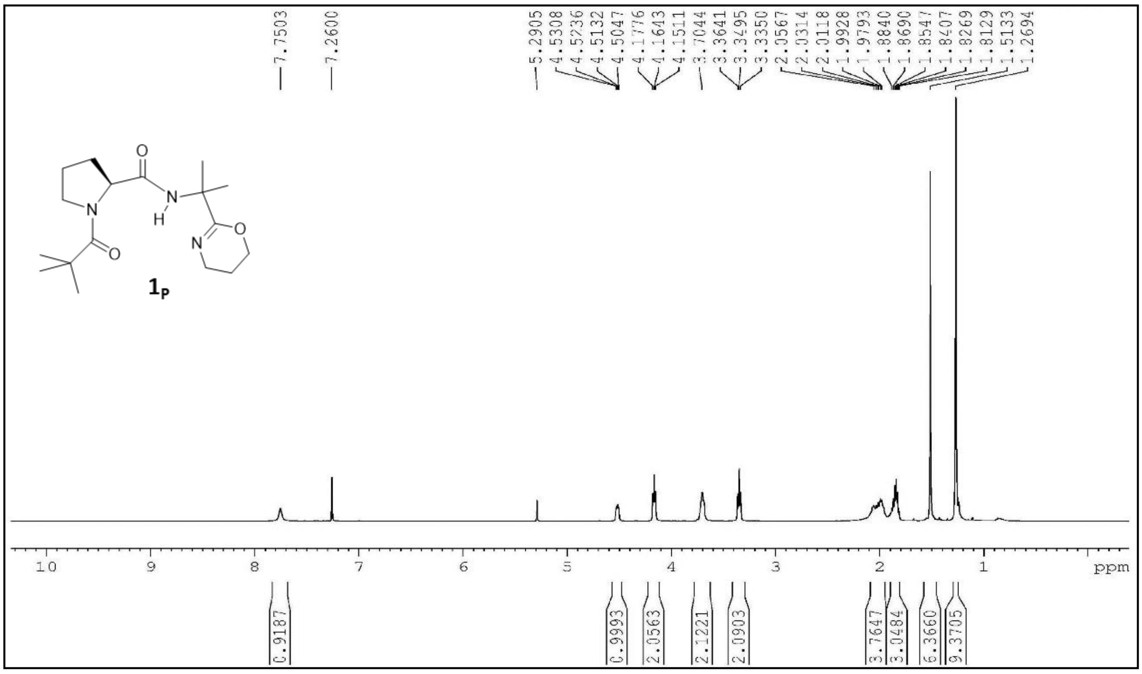
S4.1. Figure S1: ¹H NMR of 1 in $CDCl_3$ (400 MHz, 60 mM)



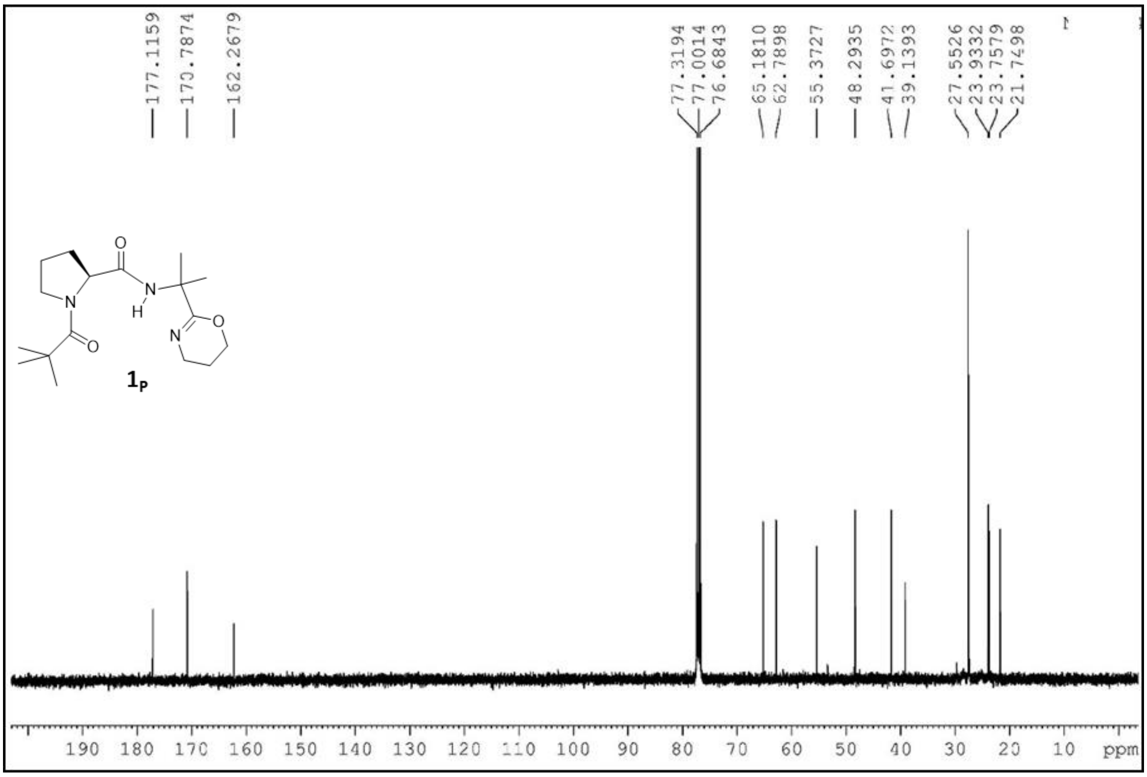
S4.2. Figure S2: ¹³C NMR of 1 in $CDCl_3$ (100 MHz, 60 mM)



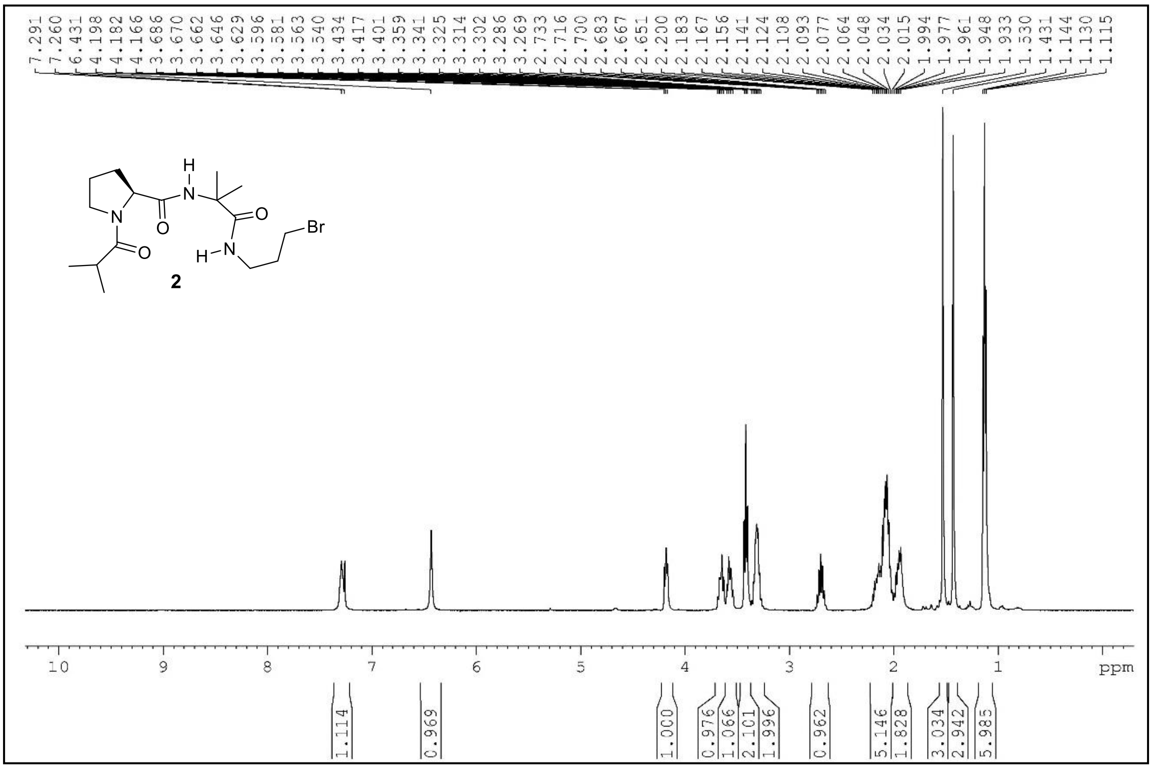
S4.3. Figure S3: ^{1}H NMR of **1$_{P}$** in $CDCl_3$ (400 MHz, 60 mM)



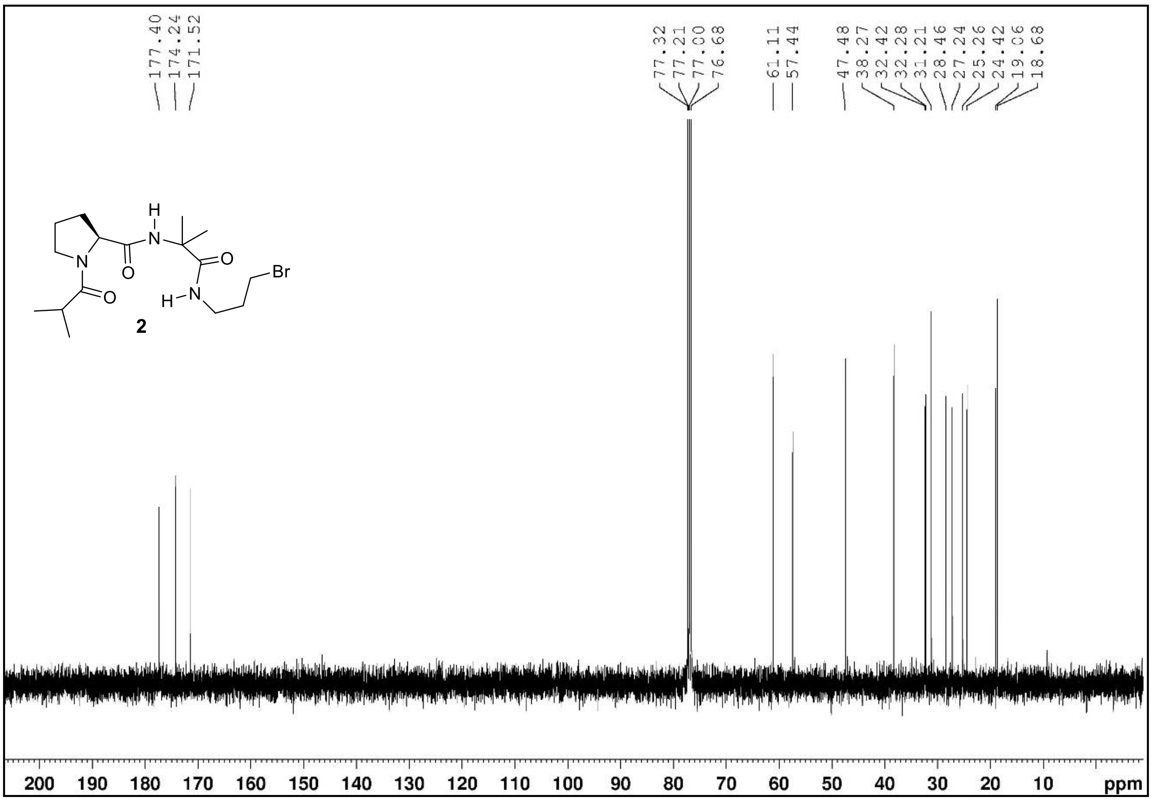
S4.4. Figure S4: ^{13}C NMR of **1$_{P}$** in $CDCl_3$ (100 MHz, 60 mM)



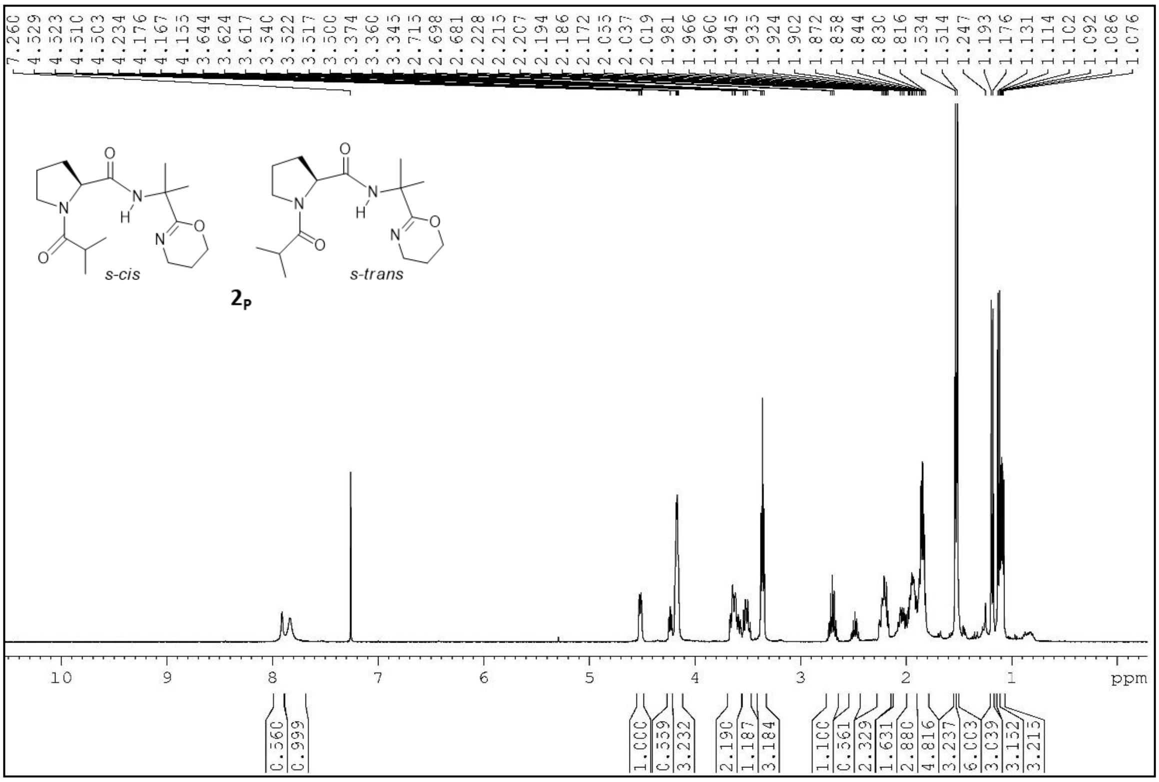
S4.5. Figure S5: ^{1}H NMR of **2** in $CDCl_3$ (400 MHz, 60 mM)



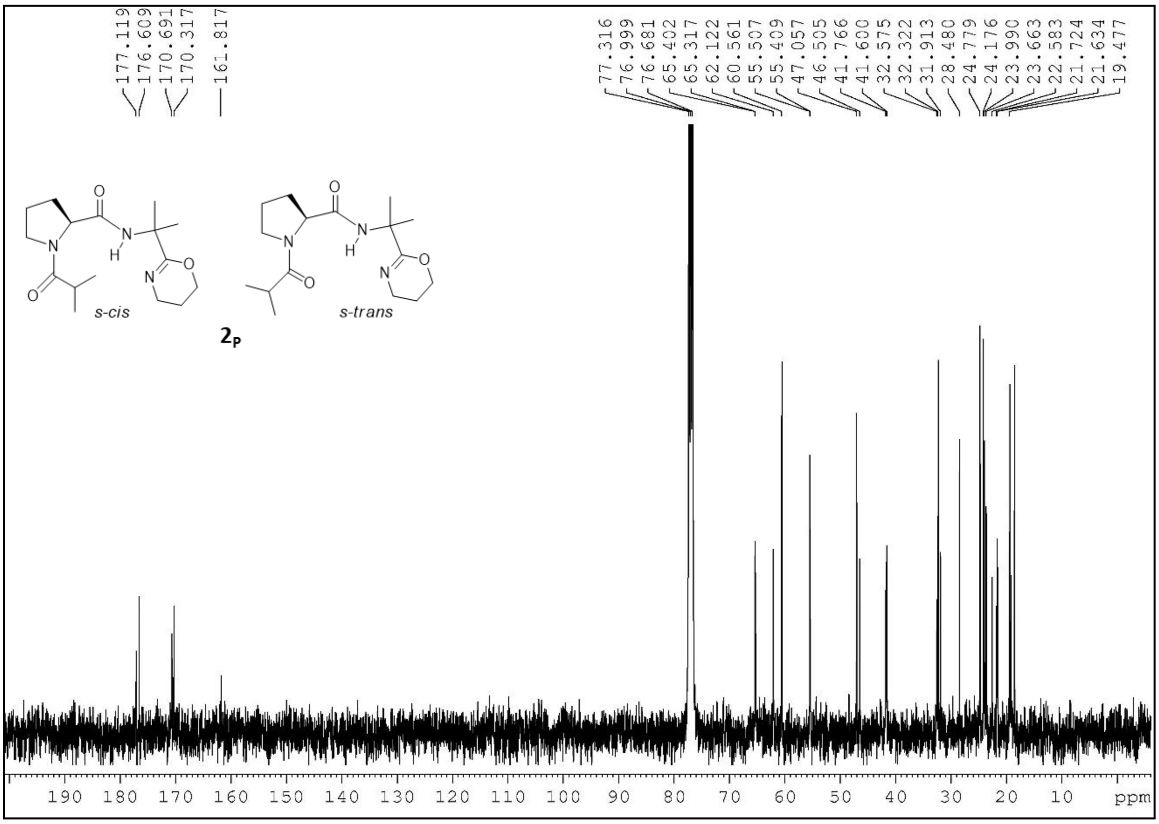
S4.6. Figure S6: ^{13}C NMR of **2** in $CDCl_3$ (100 MHz, 60 mM)



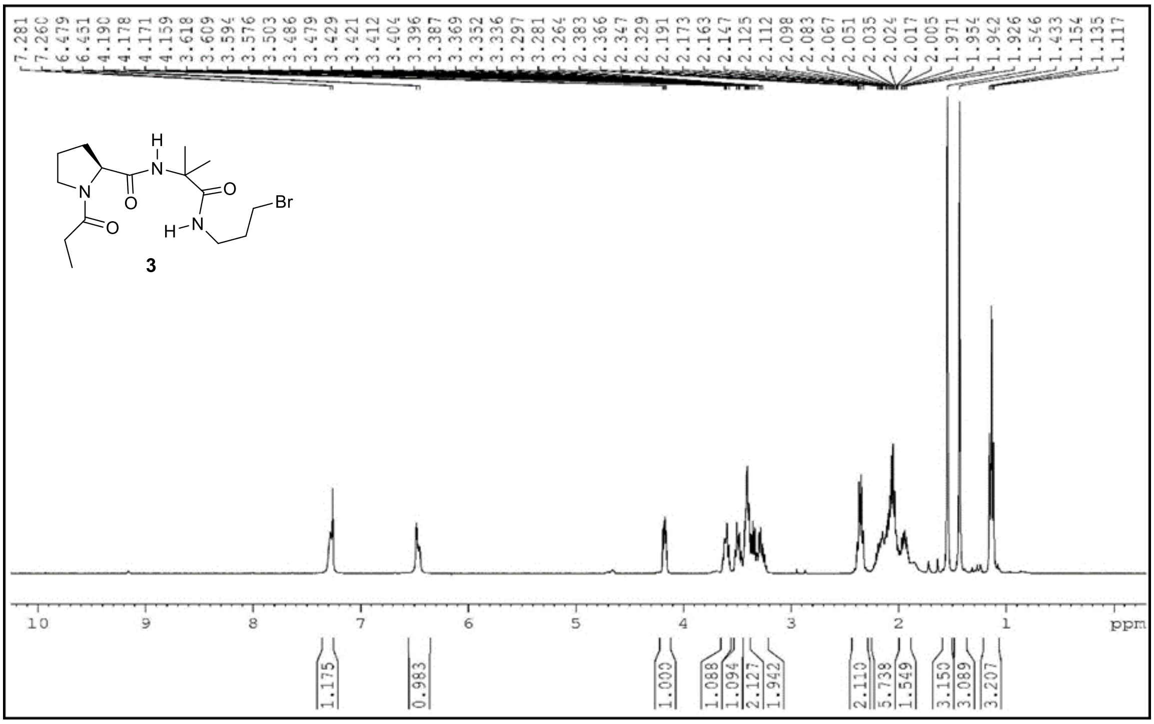
S4.7. Figure S7: ^{1}H NMR of **2$_{P}$** in $CDCl_3$ (400 MHz, 60 mM)



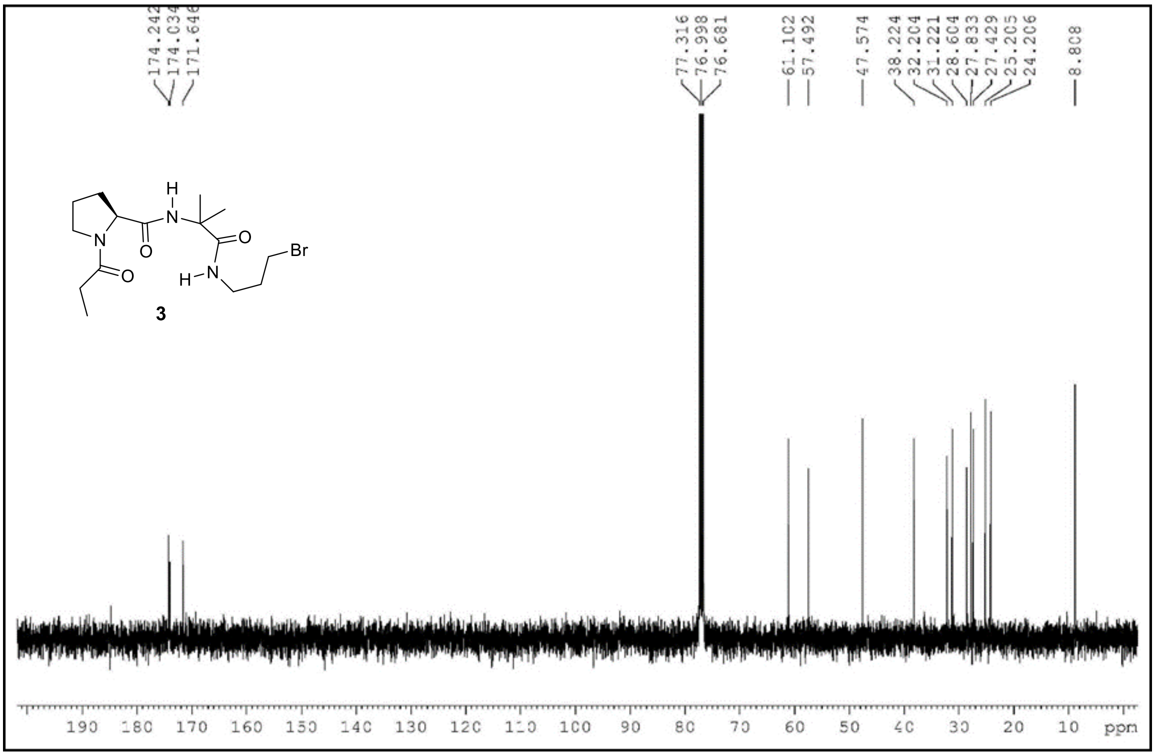
S4.8. Figure S8: ^{13}C NMR of **2$_{P}$** in $CDCl_3$ (100 MHz, 60 mM)



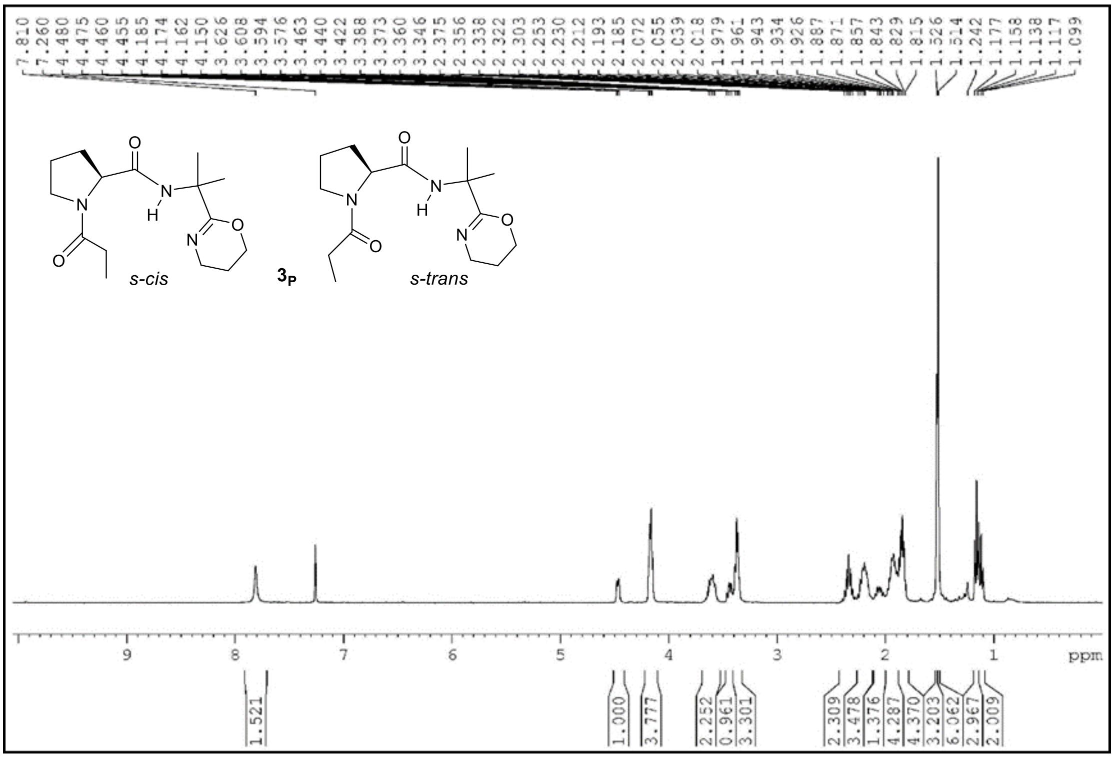
S4.9. Figure S9: ^{1}H NMR of **3** in $CDCl_3$ (400 MHz, 60 mM)



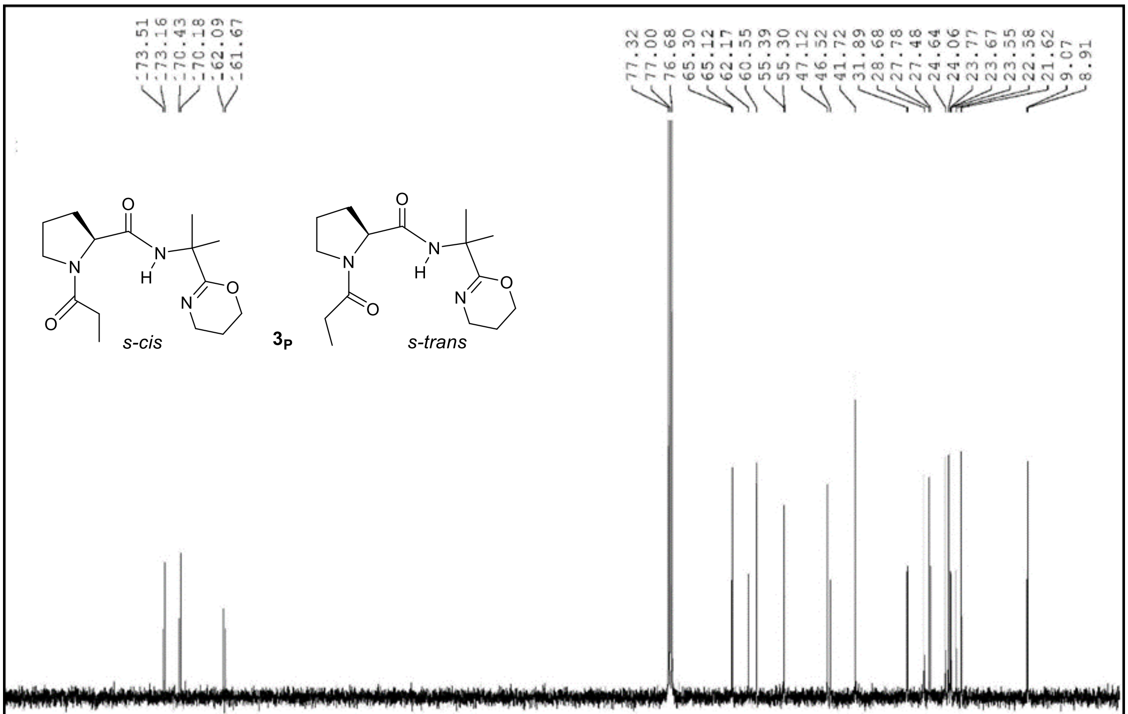
S4.10. Figure S10: ^{13}C NMR of **3** in $CDCl_3$ (100 MHz, 60 mM)



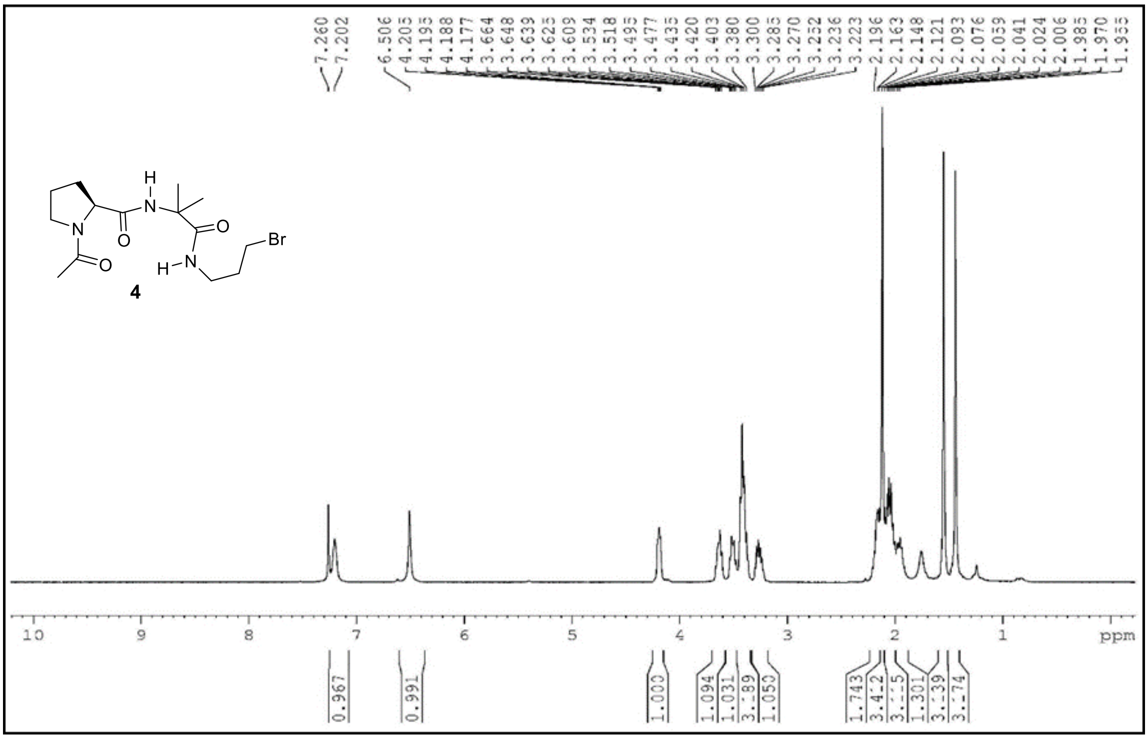
S4.11. Figure S11: ^{1}H NMR of $\mathbf{3_P}$ in $CDCl_3$ (400 MHz, 60 mM)



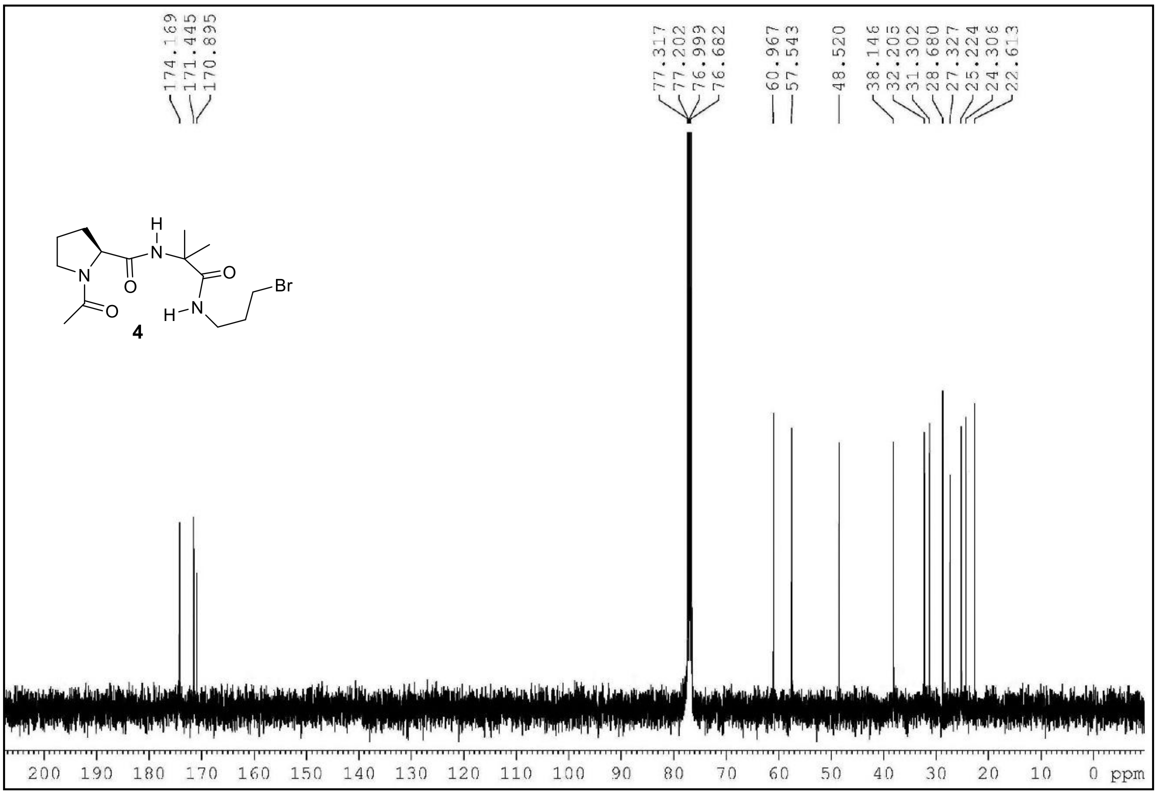
S4.12. Figure S12: ^{13}C NMR of $\mathbf{3_P}$ in $CDCl_3$ (100 MHz, 60 mM)



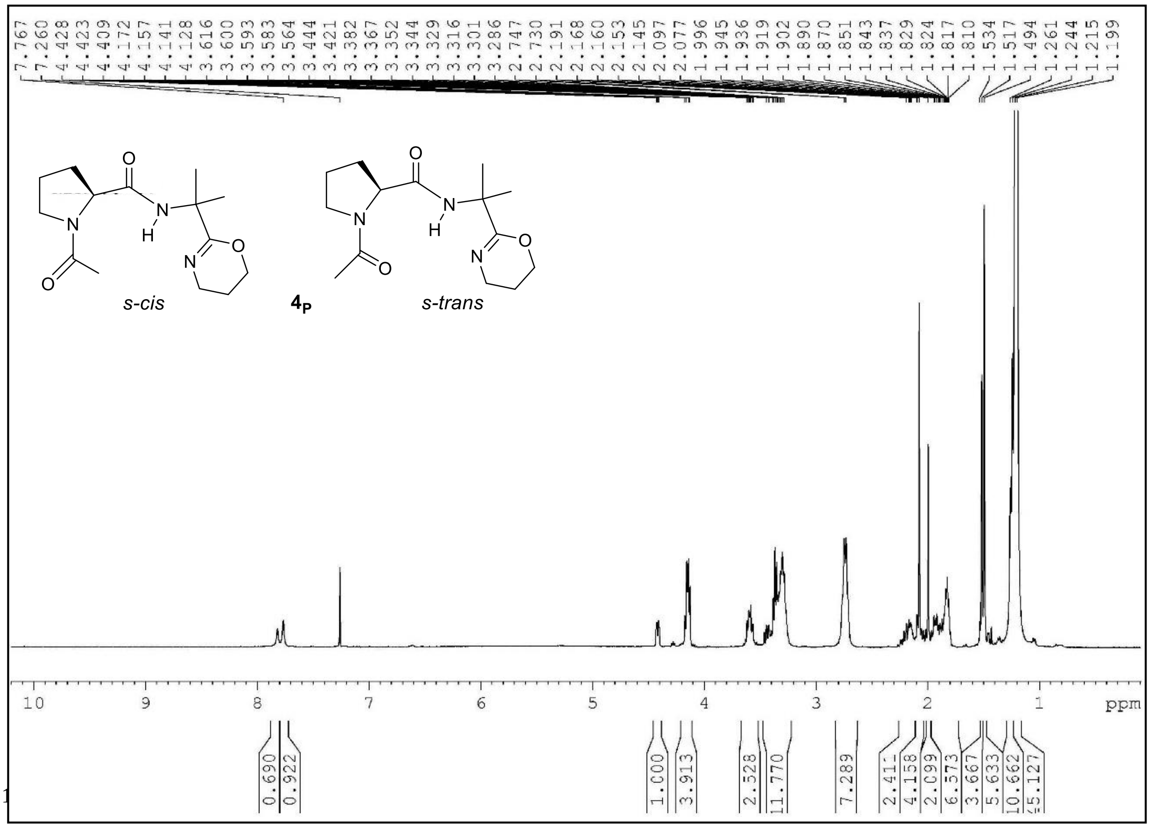
S4.13. Figure S13: ^{1}H NMR of **4** in $CDCl_3$ (400 MHz, 60 mM)



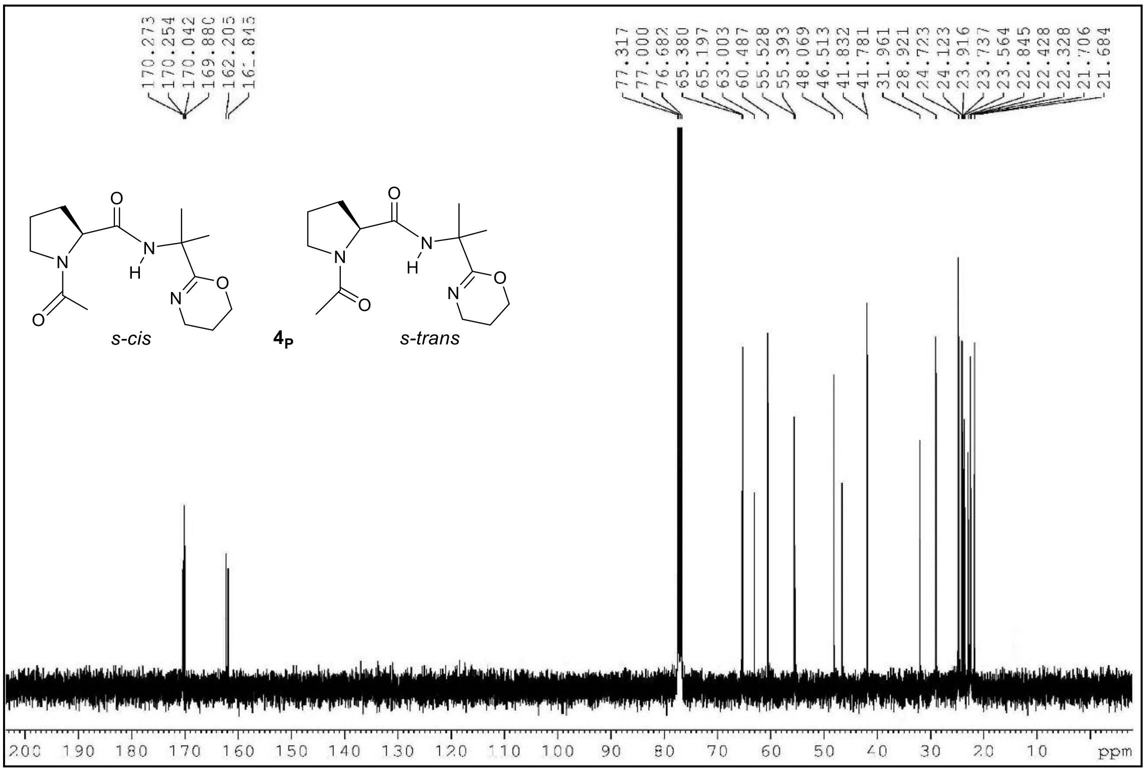
S4.14. Figure S14: ^{13}C NMR of **4** in $CDCl_3$ (100 MHz, 60 mM)



S4.15. Figure S15: ^{1}H NMR of $\mathbf{4_P}$ in $CDCl_3$ (400 MHz, 60 mM)



S4.16. Figure S16: ^{13}C NMR of $\mathbf{4_P}$ in $CDCl_3$ (100 MHz, 60 mM)



S5. Procedure for monitoring the progress of auto-cyclization reaction by ^{1}H NMR spectroscopy

The ^{1}H NMR methylene signals of $-CH_2-Br$ in the N-(3-bromopropyl)amides and $-CH_2-O-$ in the corresponding 5,6-dihydro-4H-1,3-oxazines are well-separated. Hence determining the kinetics of the reaction involves the calculation of the ratios of the intensities of the ^{1}H NMR signals corresponding to the two methylene protons, with time. The mole fraction of starting material $[a_0/(a_0+p)]$ was calculated with the progress of the reaction in time periods. The logarithmic mole fraction of starting material ($\ln[a_0/(a_0+p)]$) was plotted as a function of reaction time (h). The data for all substrates fit to a straight line, whose slope was equal to k^{-1} (sec), where k is the rate constant of the reaction. Thus all the reactions followed first-order kinetics as expected for an intramolecular nucleophilic cyclization reaction. The reaction rate constant was calculated using the following equation.

$$k = (1/t)\ln[a_0/(a_0+p)] \text{ sec}^{-1}$$

Where,

k = Reaction rate constant in (sec^{-1})

a_0 = ^{1}H NMR signal integral value of $-CH_2-Br$ in the N-(3-bromopropyl)amides at a given time

p = ^{1}H NMR signal integral value of $-CH_2-O-$ in the corresponding 5,6-dihydro-4H-1,3-oxazines at a given time

t = Reaction time (sec)

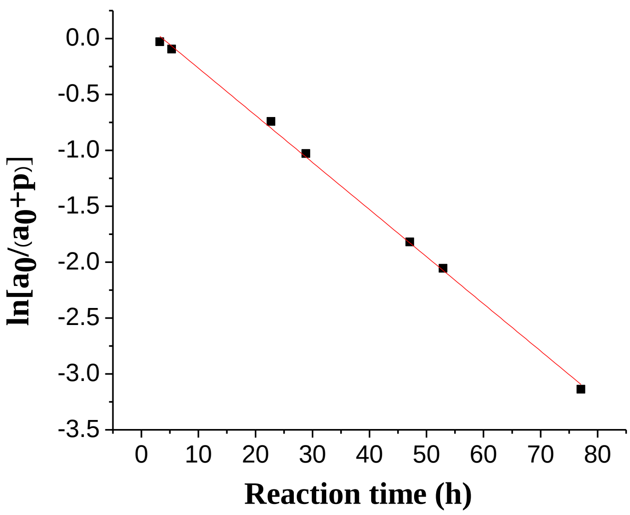
The half-life ($t_{1/2}$) of the reaction was calculated using the following equation:

$$t_{1/2} = 0.693/k \text{ (sec)}$$

S5.1. Conversion of amide **1** to 1,3-oxazine hydrobromide $\mathbf{1_P}$ ($CDCl_3$,60 mM, 32 ^oC) in DIEA (1eq.).

Table 1.

S. No.	**Reaction time (h)**	**Percent conversion (%)**	**ln[$a_0/(a_0$+p)]**
1	3.25	3	-0.0286
2	5.33	9	-0.0935
3	22.75	52	-0.741
4	28.83	64	-1.0296
5	47.08	84	-1.8197
6	52.92	87	-2.055
7	77.08	96	-3.138
8	86	100	



k = 11.70 × 10^{-6} sec^{-1}

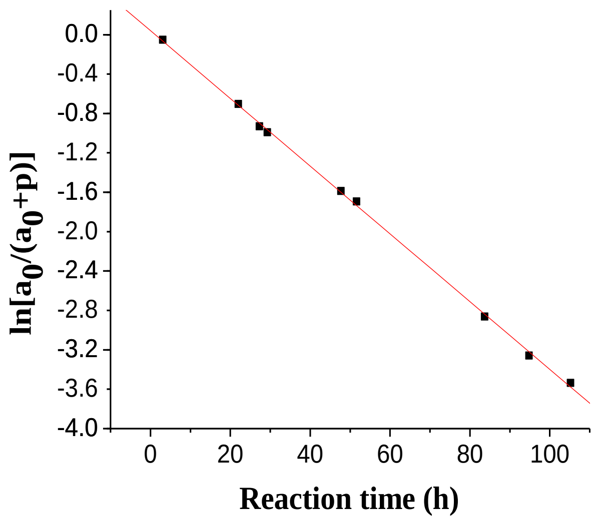
Half life ($t_{1/2}$) =16.4 h

The k and $t_{1/2}$ for the autocyclization reactions of **1** in 2, 3, 4 equivalents of DIEA were similarly calculated and found to be ~ equal to the values in 1 eq. DIEA.

S5.2. Conversion of amide **2** to 1,3-oxazine hydrobromide **2$_P$** ($CDCl_3$,60 mM, 32 ^oC) in DIEA (1 eq.).

Table 2.

S. No.	**Reaction time (h)**	**Percent conversion (%)**	**ln[a$_0$/(a$_0$+p)]**
1	3.1	5	-0.053
2	22	51	-0.703
3	27.3	61	-0.932
4	29.3	63	-0.99
5	47.7	80	-1.587
6	51.6	82	-1.694
7	83.7	94	-2.862
8	94.8	96	-3.258
9	105.2	97	-3.536



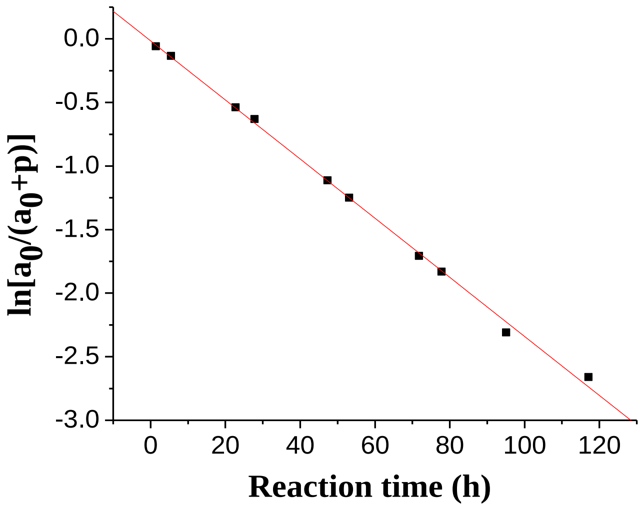
$k = 9.56 \times 10^{-6}$ sec^{-1}

Half life ($t_{1/2}$) =20.4 h

S5.3. Conversion of amide **3** to 1,3-oxazine hydrobromide $\mathbf{3_P}$ ($CDCl_3$,60 mM, 32 °C) in DIEA (1 eq.).

Table 3.

S. No.	Reaction time (h)	Percent conversion (%)	ln[a_0/(a_0+p)]
1	1.43	6	-0.059
2	5.45	13	-0.135
3	22.75	42	-0.539
4	27.83	47	-0.631
5	47.3	67	-1.112
6	53.13	71	-1.25
7	71.75	82	-1.708
8	77.8	84	-1.831
9	95.1	90	-2.31
10	117.1	93	-2.66



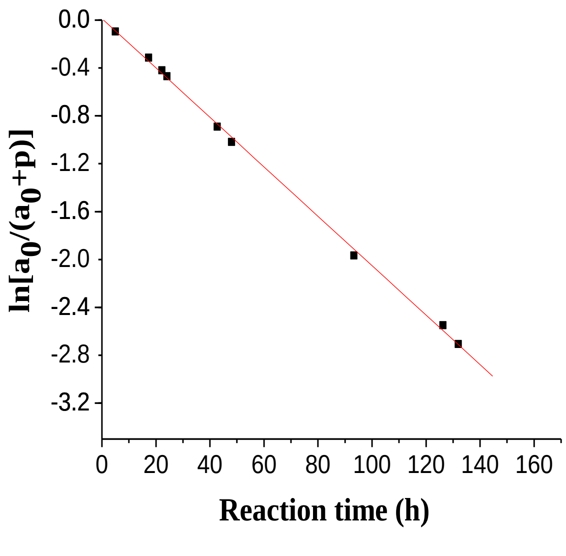
$k = 6.46 \times 10^{-6}\ sec^{-1}$

Half life ($t_{1/2}$) =30.1 h

S5.4. Conversion of amide **4** to 1,3-oxazine hydrobromide **4_P** ($CDCl_3$,60 mM, 32 °C) in DIEA (1 eq.).

Table 4.

S. No.	Reaction time (h)	Percent conversion (%)	ln[a_0/(a_0+p)]
1	5	9	-0.095
2	17.3	27	-0.315
3	22.25	34	-0.419
4	24.1	38	-0.47
5	42.75	59	-0.89
6	48	64	-1.019
7	93.3	86	-1.967
8	126.25	92	-2.549
9	132	93	-2.707



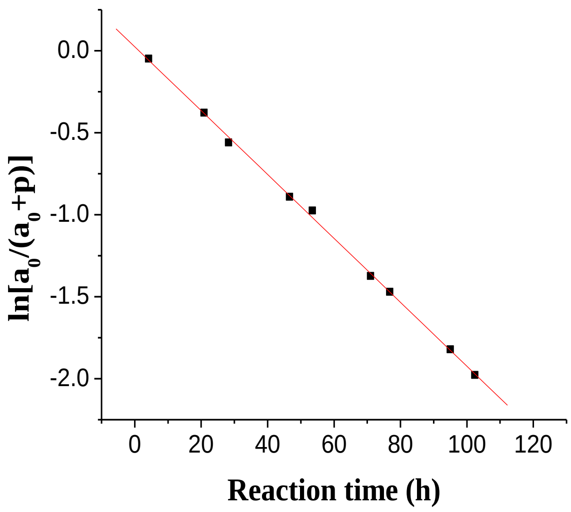
$k = 5.74 \times 10^{-6}\ sec^{-1}$

Half life ($t_{1/2}$) =33.6 h

S5.5. Conversion of amide **5** to 1,3-oxazine hydrobromide **5P** ($CDCl_3$,60 mM, 32 °C) in DIEA (1 eq.).

Table 5.

S. No.	Reaction time (h)	Percent conversion (%)	ln[a_0/(a_0+p)]
1	4.2	5	-0.049
2	20.9	31	-0.378
3	28.25	43	-0.56
4	46.6	59	-0.89
5	53.5	62	-0.975
6	71	75	-1.374
7	76.8	77	-1.47
8	95	84	-1.821
9	102.4	86	-1.977



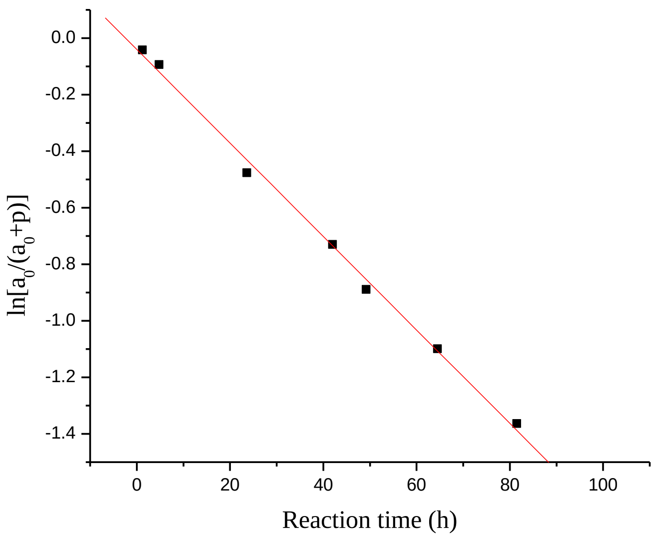
k = 5.42 × 10^{-6} sec^{-1}

Half life ($t_{1/2}$) =35.6 h

S5.6. Conversion of amide **6** to 1,3-oxazine hydrobromide **6_P** ($CDCl_3$,60 mM, 32 ^oC) in DIEA (1 eq.).

Table 6.

S. No.	Reaction time (h)	Percent conversion (%)	$\ln[a_0/(a_0+p)]$
1	1.25	4.1	-0.042
2	4.78	9	-0.094
3	23.62	37.9	-0.476
4	42	51.8	-0.73
5	49.2	58.9	-0.889
6	64.5	66.7	-1.099
7	81.51	74.4	-1.364



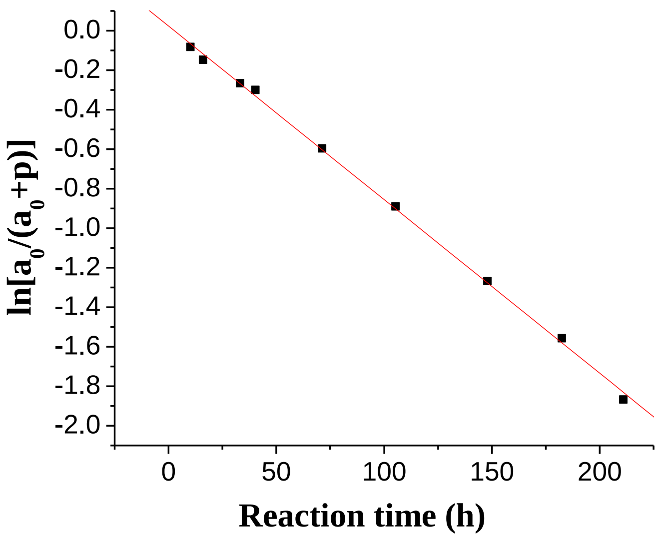
$k = 4.59\times 10^{-6}\ sec^{-1}$

Half life ($t_{1/2}$) =41.9 h.

S5.7. Conversion of amide **7** to 1,3-oxazine hydrobromide **7P** ($CDCl_3$,60 mM, 32 °C) in DIEA (1 eq.).

Table 7.

S. No.	**Reaction time (h)**	**Percent conversion (%)**	**ln[a_0/(a_0+p)]**
1	10.2	8	-0.082
2	16	14	-0.148
3	33.2	23	-0.266
4	40.3	26	-0.3
5	71.3	45	-0.596
6	105.3	59	-0.89
7	148	72	-1.268
8	182.5	79	-1.558
9	211	85	-1.868



k = 2.44 × 10^{-6} sec^{-1}

Half life ($t_{1/2}$) =78.8 h

S.6. The Taft equation (R. W. Taft Jr, *J. Am. Chem. Soc*, 1952, **74**, 3120-3128)

The Taft Equation is a linear free energy relationship (LFER) developed as a modification to the Hammett equation.

For aliphatic compounds, the Taft equation is described as:

$\log(k_s/k_{CH_3}) = \rho^*\sigma^* + \delta E_s$; where:

$\log(k_s/k_{CH3})$ → the ratio of the rate of the substituted reaction compared to the reference reaction,

ρ^* → polar reaction constant: sensitivity factor for the reaction to polar effects,

σ^* → the polar substituent constant that describes the field and inductive effects of the substituent,

δ → steric reaction constant: sensitivity factor for the reaction to steric effects,

E_s → the steric substituent constant that describes the steric effects of the substituent.

Case 1: when only the electronic sensitivity factor of the homologous substituents influence the kinetics.

Simplified Taft Equation: $\log(k_s/k_{CH_3}) = \rho^*\sigma^*$

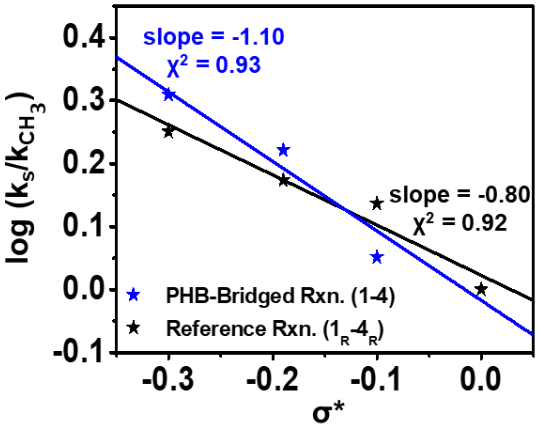


Figure S17. Linear Taft correlation of the PHB-bridged autocyclization of **1-4** and the corresponding reference reaction of **1_R-4_R**; $\chi^2 > 0.9$ for both series.

Case 2: when only the steric sensitivity factor of the homologous substituents influence the kinetics.

Simplified Taft Equation: $\log(k_s/k_{CH_3}) = \delta E_s$

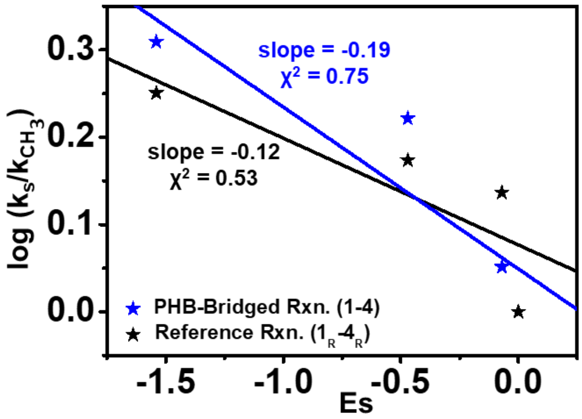


Figure S18. Taft correlation of the PHB-bridged autocyclization of **1-4** and the corresponding reference reaction of **1_R-4_R**; χ^2 significantly diminishes for both series.

S7. Stacked FT-IR Spectra of 1-4

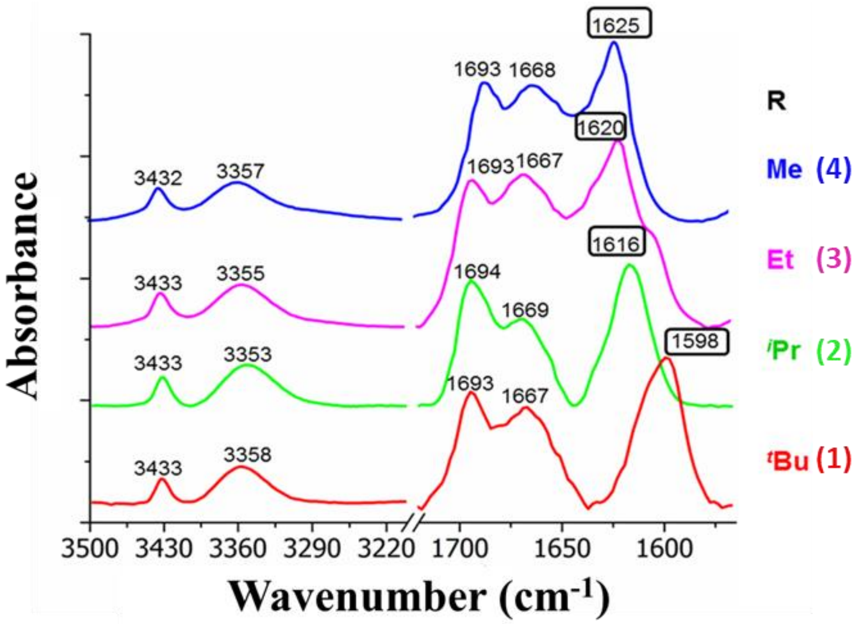


Figure S19. Stacked FT-IR spectra of **1-4** in 10 mM solution in $CHCl_3$.

S8. CD Spectral Data of the different turn conformations

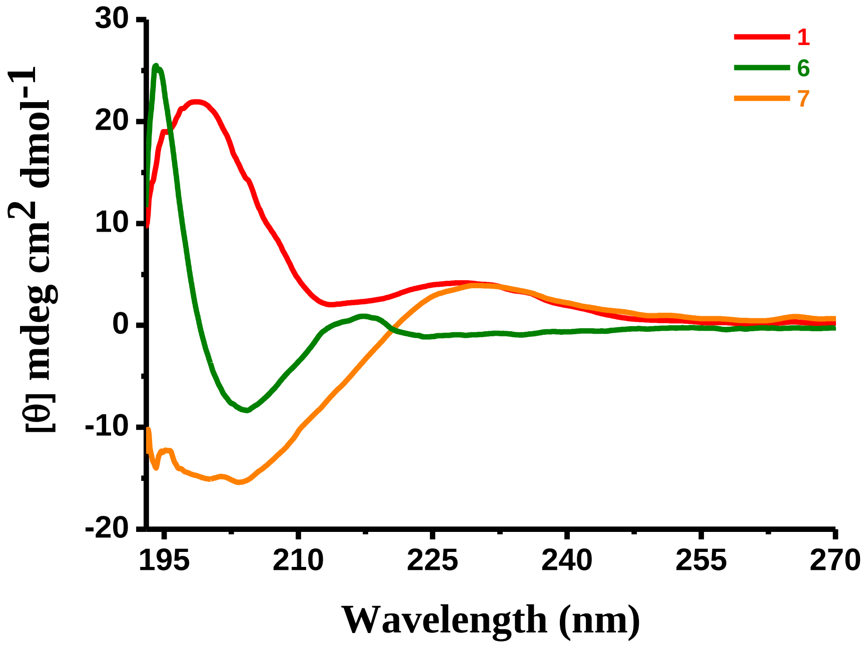


Figure S20. CD spectra of **1,6,7** in 0.5 mM solution in MeOH at 25 °C.

Table 8. Assignment of Secondary structures of **1,6,7** by comparison with reference CD signatures.

Model	Observed Maxima (nm)		Reference Maxima (nm)		Secondary Structure	Ref.
	-ve	+ve	-ve	+ve		
1	--	~198, ~228	--	~202, ~230	Type-II β-Turn	**a**
6	204.2, 224.1	194.3	~206, ~220	~190	Type-III β-Turn	**b**
7	203.3	--	~202	--	γ-Turn	**c**

(a) M. Crisma, G. Fasman, H. Balaram and P. Balaram, *Int. J. Pept. Protein Res*. 1984, **23**, 411-419.
(b) J. Bandekar, D. Evans, S. Krimm, S. Leach, S. Lee, J. McQuie, E. Minasian, G. Nemethy, M. Pottle and H. Scheraga, *Int. J. Pept. Protein Res.*, 1982, **19**, 187-205.
(c) J. R. Cann and R. O. Coombs, *Biochemistry*, 1972, **11**, 2654-2659.

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S9. Crystal Structure of the isostructural derivative of Piv-Pro-NH-$(CH_2)_3$-OH (CCDC 797014)

(D. N. Reddy, R. Thirupathi and E. N. Prabhakaran, *Chem. Commun.*, 2011, **47**, 9417-9419.)

The crystal structure shows the clear presence of an intramolecular 4→1 PHB and the Type-II *β*-turn conformation along the Pro-Aib sequence.

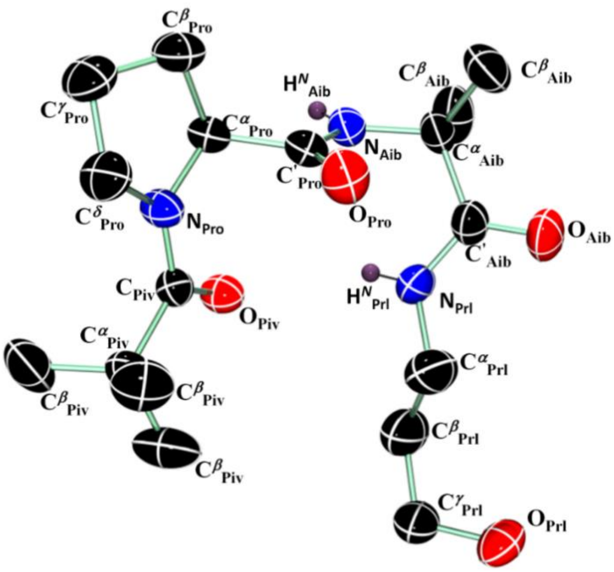

Figure S21. Illustration of an ORTEP-POV Ray rendered view of the N-(3-hydroxypropyl)amide derivative of **1**. The thermal ellipsoids are scaled to the 50% probability level.

Table 9. List of selected dihedral angles (in degrees) obtained from the above crystal structure and parameters of the 4→1 intramolecular hydrogen bond.

Amino Acid	ϕ	ψ
Pro_2	-60.45	139.85
Aib_3	57.16	28.05

	N---O (Å)	∠H-N---O
4→1 PHB	3.10	42.98

S10. Crystal Structure of the isostructural derivative of 5 (CCDC 1114882)

(P. van Roey, G. D. Smith, T. Balasubramanian, E. Czerwinski, G. R. Marshall and F. S. Mathews, *Int. J. Pept. Protein Res.*, 1983, **22**, 404-409).

The crystal structure shows the clear presence of an intramolecular 4→1 PHB and a Type-III *β*-turn conformation along the Pro-Aib sequence.

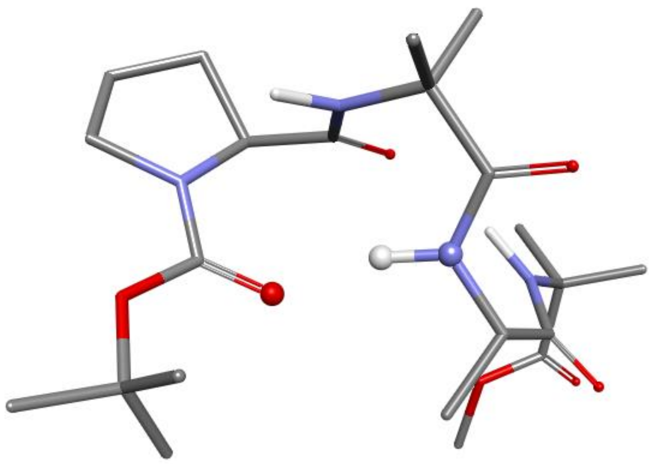

Figure S22. PDB structure of Boc-Pro-Aib-Ala-Aib-OMe

Table 10. List of selected dihedral angles (in degrees) obtained from the above crystal structure and parameters of the 4→1 intramolecular hydrogen bond.

Amino Acid	ϕ	ψ
Pro_2	-48.54	-45.62
Aib_3	-64.53	-11.34
Ala_4	-74.98	-11.38
Aib_5	56.90	32.87
	N---O (Å)	∠H-N---O
4→1 PHB	2.95	11.14

S11. Crystal Structure of the isostructural derivative of 6 (CCDC 1117967)

(B. V. Prasad, N. Shamala, R. Nagaraj, R. Chandrasekaran and P. Balaram, *Biopolymers*, 1979, **18**, 1635-1646).

The crystal structure shows the clear presence of an intramolecular 4→1 PHB and a Type-III *β*-turn conformation along the Aib-Pro sequence.

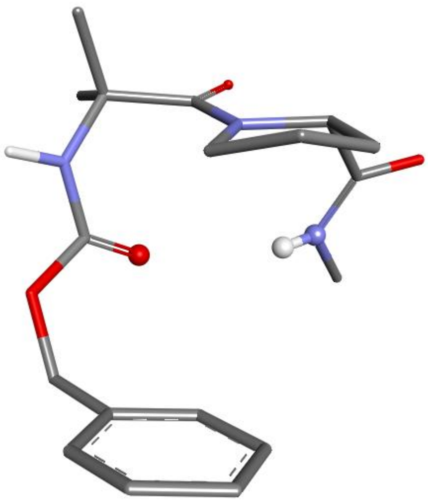

Figure S23. PDB structure of Cbz-Aib-Pro-NHMe.

Table 11. List of selected dihedral angles (in degrees) obtained from the above crystal structure and parameters of the 4→1 intramolecular hydrogen bond.

Amino Acid	ϕ	ψ
Aib_2	-50.95	-39.71
Pro_3	-64.87	-25.50
	N---O (Å)	∠H-N---O
4→1 PHB	3.12	29.33

S12. Crystal Structure of the isostructural derivative of 7 (CCDC 1165866)

(G. Valle, M. Crisma and C. Toniolo, *Acta Crystallogr., Sect. C*, 1988, **44**, 850-853.)

The N-methylamide derivative of **7** has been reported to crystallize in a conformation that is not conducive for intramolecular hydrogen-bonding. However, it does exhibit some characteristics of a 3→1 intramolecular hydrogen bonded *γ*-turn conformation from IR absorption spectra (solution as well as solid-state)[1] and ^{1}H and ^{13}C NMR studies[2].

1. E. Benedetti, A. Bavoso, B. D. Blasio, V. Pavone, C. Pedone, C. Toniolo and G. M. Bonora, *Int. J. Pept. Protein Res.*, 1982, **20**, 312-319.
2. R. Nagaraj, Y. Venkatachalapathi and P. Balaram, *Int. J. Pept. Protein Res.*, 1980, **16**, 291-298.

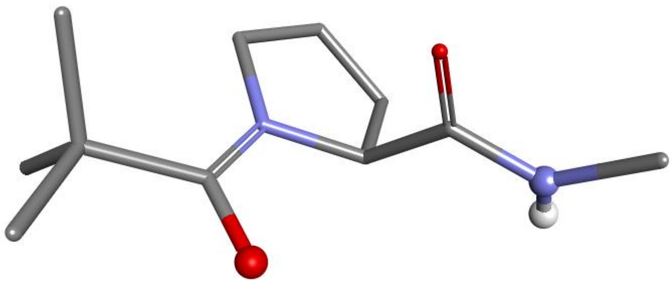

Figure S24. PDB structures of Piv-Pro-NHMe.

Table 12. List of selected dihedral angles (in degrees) obtained from the above crystal structure.

Amino Acid	ϕ	ψ
Pro_2	-70.49	163.25