Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Total Synthesis and Absolute Stereochemistry of the Proteasome Inhibitors Cystargolides A and B

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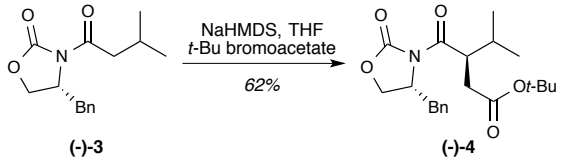
SUPPLEMENTARY INFORMATION

GENERAL CONSIDERATIONS

All moisture sensitive reactions were conducted in oven-dried glassware under an atmosphere of dry nitrogen. Reaction solvents (Et_2O, CH_2Cl_2, THF) were dried and degassed by passing through a column of activated alumina in a solvent purification system. Dimethylformamide was dried over activated 4Å molecular sieves and distilled under high vacuum. *N*-Methyl morpholine was distilled from CaH_2. All other solvents and reagents were purchased from commercial suppliers and used as received, unless otherwise specified. Thin layer chromatography (TLC) was performed using glass plates precoated with silica 60 Å F-254 (250 μm) and visualized by UV light, $KMnO_4$, phosphomolybdic acid, anisaldehyde or Cerium Ammonium Molybdate stains, followed by heating. Silica gel (particle size 40–63 μm) was used for flash column chromatography. ^1H and ^{13}C NMR spectra were recorded using a Bruker instrument working at a frequency of 400 MHz for ^1H, and at 100 MHz for ^{13}C. Chemical shifts are reported in ppm using residual solvent resonances as internal reference (δ 7.26 and δ 77.0 for ^1H and ^{13}C in $CDCl_3$, δ 2.50 and δ 39.51 for ^1H and ^{13}C in DMSO-d6). Data for ^1HNMR are reported as follows: b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Coupling constants are given in Hertz. IR measurements were performed in a Nicolet FT IR as thin films. Optical rotations were measured on a Rudolph Autopol III polarimeter. High-resolution mass spectrometry analyses were conducted at the University of New Mexico Mass Spectrometry facility.

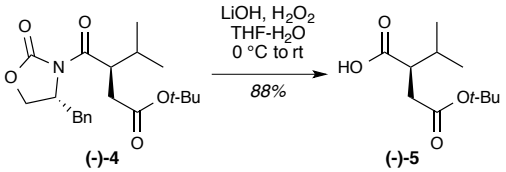
EXPERIMENTAL PROCEDURES AND CHARACTERIZATION DATA



(-)-3 (-)-4

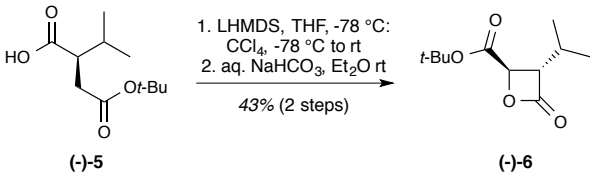
(*R*)-tert-butyl 3-((*R*)-4-benzyl-2-oxooxazolidine-3-carbonyl)-4-methylpentanoate (-)-4. A solution of imide (-)-**3**[1] (1.05 g, 4.05 mmol) in 37 mL of dry THF was cooled to –78 °C and treated with NaHMDS (3.03 mL, 2M soln. in THF, 6.07 mmol) dropwise. Enolate formation was allowed at the same temperature for 1 h before *t*-Butyl bromoacetate (1.2 mL, 8.1 mmol) was added neat. Reaction mixture was stirred and allowed to gradually reach room temperature for 12 h before quenching with 0.5 mL of acetic acid. The mixture was concentrated in rotavapor and the residue triturated with hexanes, filtered and dried under high vacuum to give 954.1 mg of the title product in 62% yield.

^1H, ^{13}C NMR and optical rotation data was in agreement with literature values.[1]



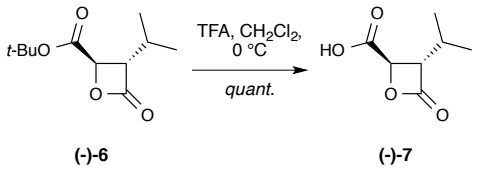
(*R*)-4-(*tert*-butoxy)-2-isopropyl-4-oxobutanoic acid (-)-5. A solution of imide (-)-**4** (673.4 mg, 1.79 mmol) in 8.5 mL of THF and 1.5 mL of water was cooled to 0 °C and treated with 1.25 mL of a 30% wt. H_2O_2 solution. The mixture was stirred for 5 min. before a freshly prepared solution of LiOH (173.1 mg, 4.12 mmol) in 3 mL of water was added dropwise. The reaction mixture was stirred and allowed to gradually reach room temperature for 12 h before cooling back to 0 °C and quenching with 2 mL of a 2M $Na_2S_2O_3$ solution. Stirring continued for 10 min. before the THF was removed in rotavapor. The aqueous residue was diluted with 2N NaOH (pH 11) and washed with CH_2Cl_2. The organic washings containing the auxiliary were set aside. The aqueous layer containing the carboxylate product was acidified with 10% HCl solution (pH 2) and extracted with CH_2Cl_2. This second organic layer containing the acid product was dried over $MgSO_4$, filtered and concentrated to give 344.9 mg of the title compound in 88% yield.

^{1}H, ^{13}C NMR and optical rotation data was in agreement with literature values.[1]



(2*R*,3*S*)-*tert*-butyl 3-isopropyl-4-oxooxetane-2-carboxylate (-)-6. Hexamethyldisilazane (1.14 mL, 5.50 mmol) was dissolved in 8 mL of dry THF and cooled to 0 °C. *n*-BuLi (3.46 mL of a 1.59 M solution in hexanes, 5.50 mmol) was slowly added. The mixture was stirred at 0 °C for about 20 min. The resulting LHMDS solution was cooled to –78 °C and treated with a solution of acid (-)-**5** (476.5 mg, 2.2 mmol) in 8 mL of dry THF. Enolate formation was allowed at the same temperature for 1 h before CCl_4, freshly distilled from CaH_2 (0.25 mL, 2.64 mmol) was added neat. Reaction mixture was stirred and allowed to gradually reach room temperature for 12 h before carefully concentrating in rotavapor. The gummy solid residue was suspended in 40 mL of Et_2O and treated with 20 mL of 5% $NaHCO_3$ solution. The mixture was vigorously stirred at room temperature for 24 h and then diluted with Et_2O, washed with $NaHCO_3$ saturated solution and brine, dried over $MgSO_4$, filtered and concentrated. The residue was loaded into a flash chromatography column and purified using 5:1 hexanes/Et_2O to give 205.5 mg of the title compound in 43% yield over 2 steps.

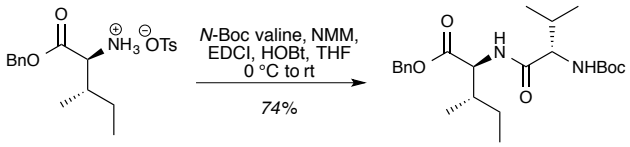
$[\alpha]_D^{22}$ = –7.0 (*c* = 1.0, $CHCl_3$); **IR** (ν, cm^{-1}) 3675, 2988, 2900, 1821, 1744; **^{1}HNMR** (400 MHz, $CDCl_3$) δ 4.51 (d, *J* = 4.4 Hz, 1H), 3.43 (dd, *J* = 8.4, 4.4 Hz, 1H), 2.06-2.21 (m, 1 H), 1.49 (s, 9H), 1.10 (d, *J* = 6.7 Hz, 3H), 1.05 (d, *J* = 6.7 Hz, 3H); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 168.6, 167.3, 83.3, 70.0, 63.8, 27.7, 27.5, 19.8, 19.5; **HRMS** (ESI+) *m/z*: $[M + Na]^+$ calc'd for $C_{11}H_{18}O_4Na$ 237.1103; found 237.1106.



(2*R*,3*S*)-3-isopropyl-4-oxooxetane-2-carboxylic acid (-)-7. A solution of lactone (-)-**6** (205.0 mg, 0.95 mmol) in 1 mL of dry CH_2Cl_2 was cooled to 0 °C and treated with 1 mL of TFA. The mixture was stirred at

the same temperature for 12 h before careful removal of the solvent and excess reagent (at 0 °C) under high vacuum. The residue was loaded into a flash chromatography column and purified using 95:5:0.1 $CHCl_3$-MeOH-AcOH to give 150.2 mg of the title compound in quantitative yield.

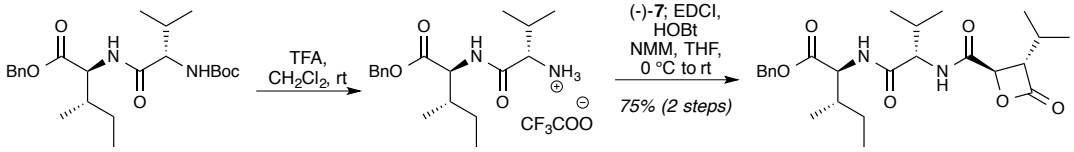
$[\alpha]_D^{20}$ = –0.8 (*c* = 1.0, $CHCl_3$); **IR** (ν, cm^{-1}) 2967, 1832, 1735; **^{1}HNMR** (400 MHz, $CDCl_3$) δ 10.00 (bs, 1H), 4.69 (d, *J* = 4.2 Hz, 1H), 3.63 (dd, *J* = 8.3, 4.2 Hz, 1H), 2.14-2.30 (m, 1H), 1.12 (d, *J* = 6.7 Hz, 3H), 1.08 (d, *J* = 6.7 Hz, 3H); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 173.4, 168.3, 69.3, 64.4, 27.7, 19.8, 19.4; **HRMS** (ESI-) *m/z*: [M-H]$^-$ calc'd for $C_7H_9O_4$ 157.0501, found 157.0499.



(-)-8 (-)-9

(2*S*,3*S*)-benzyl 2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-3-methylpentanoate (-)-9. A mixture of L-Isoleucine benzyl ester (-)-**8**[2] (100 mg, 0.25 mmol) and *N*-Boc L-Valine[3] (57.3 mg, 0.26 mmol) was dissolved in 1.4 mL of dry THF and cooled to 0 °C. *N*-methyl morpholine (0.14 mL, 1.31 mmol) was added, followed by HOBt (40.9 mg, 0.3 mmol) and EDCI (58.1 mg, 0.3 mmol). The mixture was stirred and allowed to gradually reach room temperature for 12 h before concentrating in rotavapor. The residue was taken in ethyl acetate and washed with 1N HCl solution, $NaHCO_3$ saturated solution, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The product was purified by flash column chromatography using 8:2 hexanes-ethyl acetate to obtain 79.2 mg of the title compound in 74% yield.

$[\alpha]_D^{22}$ = –16.0 (*c* = 1.0, $CHCl_3$); **IR** (ν, cm^{-1}) 3310, 2969, 1743, 1682, 1647, 1527; **^{1}HNMR** (400 MHz, $CDCl_3$) δ 7.28-7.37 (m, 5H), 6.39 (bs, 1H), 5.19 (d, *J* = 12.2 Hz, 1H), 5.11 (d, *J* = 12.2 Hz, 1H), 5.07 (bs, 1H), 4.62 (dd, *J* = 8.5, 4.8 Hz, 1H), 3.85-3.93 (m, 1H), 2.02-2.16 (m, 1H), 1.85-1.97 (m, 1H), 1.43 (s, 9H), 1.32-1.44 (m, 1H), 1.07-1.20 (m, 1H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H) 0.87 (d, *J* = 7.0 Hz, 3H), 0.84 (d, *J* = 7.0 Hz, 3H); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 171.5, 171.4, 155.8, 135.2, 128.5, 128.41, 128.35, 79.8, 67.0, 60.1, 56.4, 37.9, 30.7, 28.3, 24.9, 19.2, 17.8, 15.4, 11.5; **HRMS** (ESI+) *m/z*: $[M+Na]^+$ calc'd for $C_{23}H_{36}N_2O_5Na$ 443.2522; found: 443.2523.

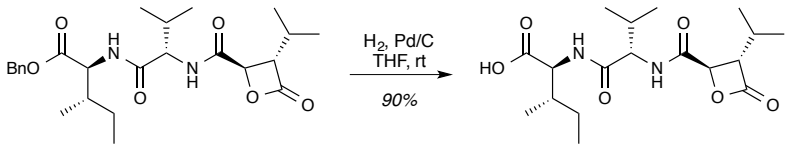


(-)-9 S2 (-)-10

(2*S*,3*S*)-benzyl-2-((*S*)-2-((2*R*,3*S*)-3-isopropyl-4-oxooxetane-2-carboxamido)-3-methylbutanamido)-3-methylpentanoate (-)-10. A solution of *N*-Boc peptide (-)-**9** (100 mg, 0.23 mmol) in 0.5 mL of dry CH_2Cl_2 was treated with 0.5 mL of neat TFA at room temperature. The mixture was stirred for about 30 min before concentrating in rotavapor/high vacuum to obtain the trifluoroacetate salt intermediate **S2** as a white solid that was used in the coupling reaction without extensive characterization: A solution of acid (-)-**7** (72.7 mg, 0.46 mmol) and *N*-methyl morpholine (0.15 mL, 1.38 mmol) in 2.3 mL of dry THF was added to a flask containing a stirring bar and trifluoroacetate salt **S2**, held at 0 °C. The mixture was stirred for 5 min. before HOBt (66.8 mg, 0.49 mmol) and EDCI (94.8 mg, 0.49 mmol) were quickly added in one portion. The flask was flushed with nitrogen and the mixture stirred and allowed to gradually reach room temperature for 12 h before concentrating in rotavapor. The residue was taken in 30 mL of ethyl acetate and washed with 1N HCl solution, $NaHCO_3$ saturated solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was loaded

into a flash chromatography column and purified using 75:25 to 7:3 hexanes-ethyl acetate to give 80.0 mg of the title product in 75% yield over 2 steps.

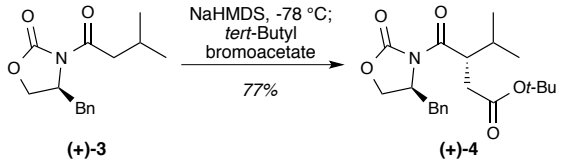
$[\alpha]_D^{20}$ = –9.3 (*c* = 1.0, $CHCl_3$); **IR** (ν, cm^{-1}) 3296, 2964, 1839, 1739, 1644, 1544; **^{1}HNMR** (400 MHz, $CDCl_3$) δ 7.29-7.39 (m, 5H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.35 (d, *J* = 8.2 Hz, 1H), 5.2 (d, *J* = 12.1 Hz, 1H), 5.11 (d, *J* = 12.1 Hz, 1H), 4.62 (d, *J* = 4.6 Hz, 1H), 4.60 (d, *J* = 4.7 Hz, 1H), 4.26 (dd, *J* = 8.0, 7.2 Hz, 1H), 3.49 (dd, *J* = 8.1, 4.5 Hz, 1H), 2.06-2.25 (m, 2H), 1.82-1.96 (m, 1H), 1.28-1.40 (m, 1H), 1.11 (m, overlapped, 1H), 1.10 (d, *J* = 6.7 Hz, 3H), 1.07 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 171.4, 169.9, 168.4, 168.0, 135.1, 128.6, 128. 5, 128.4, 71.0, 67.1, 64.3, 58.5, 56.5, 37.7, 30.9, 27.8, 25.0, 20.0, 19.4, 19.1, 18.1, 15.5, 11.5; **HRMS** (ESI+) *m/z*: $[M+Na]^+$ calc'd for $C_{25}H_{36}N_2O_6Na$ 483.2471; found 483.2478.



(-)-10 Cystargolide A [(-)-**1**]

Cystargolide A (**1**). A solution of benzyl ester (-)-**10** (80.0 mg, 0.17 mmol) in 14 mL of dry THF was treated with Pd/C (80.0 mg, 10% wt.). The flask was flushed with hydrogen gas and the mixture stirred under a hydrogen atmosphere (balloon) at room temperature for 12 h. The mixture was then diluted with THF and filtered through a short plug of celite, rinsing with THF. The collected filtrate was concentrated and the residual white solid triturated and washed with hexanes until the product was clean of all non-polar impurities, as judged by TLC. Product dried under high vacuum to give 58.3 mg of the title compound in 90% yield.

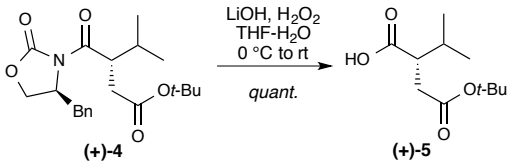
$[\alpha]_D^{20}$ = –49.0 (*c* = 0.5, MeOH); **IR** (ν, cm^{-1}) 3284, 2965, 1840, 1647, 1544; **^{1}HNMR** (400 MHz, DMSO-d6) δ 12.57 (bs, 1H), 8.56 (d, *J* = 9.0 Hz, 1H), 8.17 (d, *J* = 7.9 Hz, 1H), 5.01 (d, *J* = 4.0 Hz, 1H), 4.37 (dd, *J* = 8.5, 7.2 Hz, 1H), 4.15 (dd, *J* = 7.2, 7.2 Hz, 1H), 3.50 (dd, *J* = 8.2, 4.0 Hz, 1H), 2.06-2.16 (m, 1H), 1.94-2.04 (m, 1H), 1.73-1.93 (m, 1H), 1.35-1.47 (m, 1H), 1.44-1.22 (m, 1H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.80-0.87 (m, 12H); **^{13}CNMR** (100 MHz, DMSO-d6) δ 172.7, 170.6, 170.1, 167.3, 70.1, 62.7, 57.2, 56.4, 36.1, 30.9, 26.7, 24.8, 19.4, 19.3, 19.1, 17.9, 15.5, 11.3; **HRMS** (ESI-) *m/z*: $[M-H]^-$ calc'd for $C_{18}H_{29}N_2O_6$ 369.2026; found 369.2026.



(+)-3 **(+)-4**

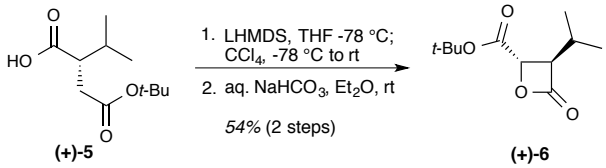
(*S*)-*tert*-butyl 3-((*S*)-4-benzyl-2-oxooxazolidine-3-carbonyl)-4-methylpentanoate (+)-4. A solution of imide (+)-**3**[4] (1.6 g, 6.12 mmol) in 57 mL of dry THF was cooled to –78 °C and treated with NaHMDS (4.59 mL, 2M soln. in THF, 9.18 mmol) dropwise. Enolate formation was allowed at the same temperature for 1 h before *t*-Butyl bromoacetate (1.8 mL, 12.24 mmol) was added neat. Reaction mixture was stirred and allowed to gradually reach room temperature for 12 h before quenching with 0.5 mL of acetic acid. The mixture was concentrated in rotavapor and the residue was triturated with hexanes, filtered and dried under high vacuum to give 1.77 g of the title product in 77% yield.

^{1}H, ^{13}C NMR and optical rotation data was in agreement with literature values.[4]



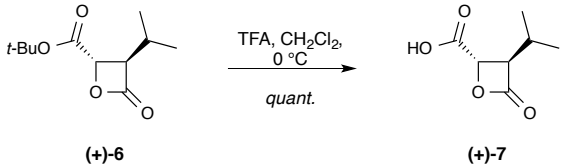
(*S*)-4-(*tert*-butoxy)-2-isopropyl-4-oxobutanoic acid (+)-5. A solution of imide (+)-**4** (1.45 g, 3.86 mmol) in 18 mL of THF and 3 mL of water was cooled to 0 °C and treated with 2.7 mL of a 30% wt. H_2O_2 solution. The mixture was stirred for 5 min. before a freshly prepared solution of LiOH (372.7 mg, 8.88 mmol) in 6 mL of water was added dropwise. Reaction mixture was stirred and allowed to gradually reach room temperature for 12 h before cooling back to 0 °C and quenching with 4 mL of a 2M $Na_2S_2O_3$ solution. Stirring continued for 10 min. before the THF was removed in rotavapor. Aqueous residue was diluted with 2N NaOH (pH 11) and washed with CH_2Cl_2. The organic washings containing the auxiliary were set aside. The aqueous layer containing the carboxylate product was acidified with 10% HCl solution (pH 2) and extracted with CH_2Cl_2. This second organic layer containing the acid product was dried over $MgSO_4$, filtered and concentrated to give 839.4 mg of the title compound in quantitative yield.

^{1}H, ^{13}C NMR and optical rotation data was in agreement with literature values.[4]



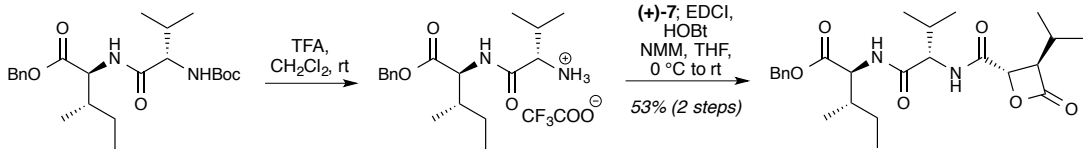
(2*S*,3*R*)-tert-butyl 3-isopropyl-4-oxooxetane-2-carboxylate (+)-6. Hexamethyldisilazane (0.48 mL, 2.31 mmol) was dissolved in 4 mL of dry THF and cooled to 0 °C. *n*-BuLi (1.45 mL of a 1.59 M solution in hexanes, 2.31 mmol) was slowly added. The mixture was stirred at 0 °C for about 20 min. The resulting LHMDS solution was cooled to −78 °C and treated with a solution of acid (+)-**5** (200 mg, 0.92 mmol) in 4 mL of dry THF. Enolate formation was allowed at the same temperature for 1 h before CCl_4, freshly distilled from CaH_2 (0.1 mL, 1.10 mmol) was added neat. The reaction mixture was stirred and allowed to gradually reach room temperature for 12 h before carefully concentrating in rotavapor. The gummy solid residue was suspended in 20 mL of Et_2O and treated with 10 mL of 5% $NaHCO_3$ solution. The mixture was vigorously stirred for 24 h and then diluted with Et_2O, washed with $NaHCO_3$ saturated solution and brine, dried over $MgSO_4$, filtered and concentrated. Residue was loaded into a flash chromatography column and purified using 5:1 hexanes-Et_2O to give 108.3 mg of the title compound in 54% yield over 2 steps.

$[\alpha]_D^{20}$ = +8.0 (*c* = 1.0, $CHCl_3$); IR, ^{1}H and ^{13}C NMR data for this compound are identical to that described for (-)-**6**.



(2*S*,3*R*)-3-isopropyl-4-oxooxetane-2-carboxylic acid (+)-7. A solution of lactone (+)-**6** (100.0 mg, 0.46 mmol) in 1.5 mL of dry CH_2Cl_2 was cooled to 0 °C and treated with 1.5 mL of TFA. The mixture was stirred at the same temperature for 12 h before careful removal of the solvent and excess reagent (at 0 °C) under high vacuum. Residual product loaded into a flash chromatography column and purified using 95:5:0.1 $CHCl_3$-MeOH-AcOH to give 76.9 mg of the title compound in quantitative yield.

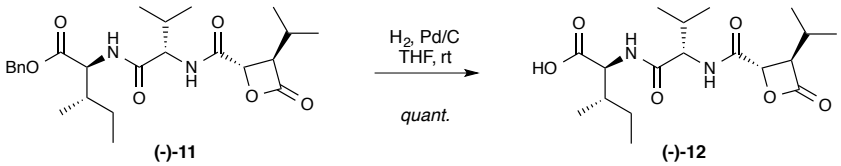
$[\alpha]_D^{22}$ = +0.9 (*c* = 1.0, $CHCl_3$); IR, ^{1}H and ^{13}C NMR data for this compound are identical to that described for (-)-**7**.



(-)-9 S2 (-)-11

(2*S*,3*S*)-benzyl 2-((*S*)-2-((2*S*,3*R*)-3-isopropyl-4-oxooxetane-2-carboxamido)-3-methylbutanamido)-3-methylpentanoate (-)-11. A solution of peptide (-)-**9** (100 mg, 0.23 mmol) in 0.5 mL of CH_2Cl_2 was treated with 0.5 mL of TFA. The mixture was stirred at room temperature for about 30 min. before concentrating in rotavapor and high-vacuum. The residual trifluoroacetate salt **S2** as a white solid that was used in the coupling reaction without extensive characterization: A solution of acid (+)-**7** (72.7 mg, 0.46 mmol) and *N*-methyl morpholine (0.15 mL, 1.38 mmol) in 2.3 mL of THF was added to a flask containing a stirring bar and trifluoroacetate salt **S2**, held at 0 °C. HOBt (66.8 mg, 0.49 mmol) and EDCI (94.8 mg, 0.49 mmol) were quickly added in one portion. Reaction mixture was stirred and allowed to reach room temperature for 12 h before concentrating in rotavapor. The residue was taken in ethyl acetate and washed with 1N HCl solution, $NaHCO_3$ saturated solution, and brine, dried over $MgSO_4$, filtered and concentrated. The residue was loaded into a flash chromatography column and purified using 7:3 hexanes-ethyl acetate to give 56.6 mg of the title compound in 53% yield.

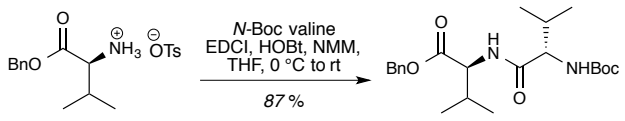
$[\alpha]_D^{23}$ = –17.0 (*c* = 0.5, $CHCl_3$); **IR** (ν, cm^{-1}) 3287, 2963, 1840, 1733, 1643, 1553; **^{1}HNMR** (400 MHz, $CDCl_3$) δ 7.29-7.36 (m, 5H), 7.04 (bd, *J* = 8.7 Hz, 1H), 6.57 (bs, 1H), 5.21 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 4.61 (dd, *J* = 8.5, 4.6 Hz, 1H), 4.60 (d, *J* = 4.4 Hz, 1H), 4.33 (dd, *J* = 8.6, 7.3 Hz, 1H), 3.46 (dd, *J* = 8.1, 4.5 Hz, 1H), 2.15-2.23 (m, 1H), 2.05-2.14 (m, 1H), 1.83-1.94 (m, 1H), 1.25-1.38 (m, 1H), 1.13 (m, overlapped, 1H), 1.11 (d, *J* = 6.7 Hz, 3H), 1.08 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 7.1 Hz, 3H), 0.90 (d, *J* = 7.1 Hz, 3H), 0.82-0.86 (m, 6H); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 171.4, 170.1, 178.6, 168.1, 135.1, 128.54, 128.45, 128.4, 71.1, 67.1, 64.6, 58.3, 56.6, 37.7, 31.3, 27.8, 25.0, 20.0, 19.3, 19.1, 18.2, 15.4, 11.5; **HRMS** (ESI+) *m/z*: $[M+Na]^+$ calc'd for $C_{25}H_{36}N_2O_6Na$ 483.2471; found 483.2466.



(-)-11 (-)-12

(2*S*,3*S*)-2-((*S*)-2-((2*S*,3*R*)-3-isopropyl-4-oxooxetane-2-carboxamido)-3-methylbutanamido)-3-methylpentanoic acid (-)-12. A solution of benzyl ester (-)-**11** (56.0 mg, 0.12 mmol) in 10 mL of THF was treated with Pd/C (56 mg, 10% wt). The flask was flushed with hydrogen, and the mixture stirred under a hydrogen atmosphere (balloon) for 12 h before diluting with THF and filtering through a short plug of celite. Solids were rinsed with THF and the combined filtrate concentrated to a white solid that was triturated and washed with hexanes until all non-polar impurities were removed, as judged by TLC. Drying under high vacuum yielded 44.7 mg of the title compound in quantitative yield.

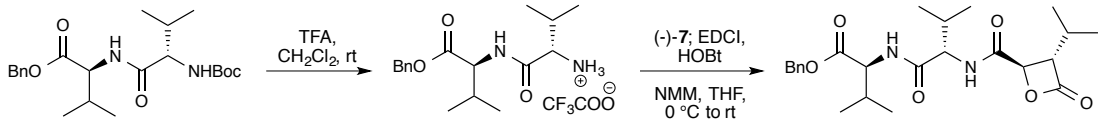
$[\alpha]_D^{20}$ = –2.2 (*c* = 0.45, MeOH); **IR** (ν, cm^{-1}) 3296, 2966, 1838, 1648, 1542; **^{1}HNMR** (400 MHz, DMSO-d6) δ 12.65 (bs, 1H), 8.43 (d, *J* = 8.9 Hz, 1H), 8.12 (d, *J* = 7.8 Hz, 1H), 4.98 (d, *J* = 4.0 Hz, 1H), 4.36 (dd, *J* = 7.8, 7.8 Hz, 1H), 4.14 (dd, *J* = 6.8, 6.8 Hz, 1H), 3.50 (dd, *J* = 8.0, 4.1 Hz, 1H), 2.06-2.15 (m, 1H), 1.96-2.05 (m, 1H), 1.71-1.82 (m, 1H), 1.32-1.45 (m, 1H), 1.12-1.23 (m, 1H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.79-0.90 (m, 12H); **^{13}CNMR** (100 MHz, DMSO-d6) δ 172.8, 170.4, 170.1, 167.2, 70.4, 62.4, 57.4, 56.4, 36.1, 31.0, 26.6, 24.7, 19.6, 19.13, 19.10, 18.0, 15.5, 11.3; **HRMS** (ESI-) *m/z*: $[M-H]^-$ calc'd for $C_{18}H_{29}N_2O_6$ 369.2026; found 369.2019.



(-)-13 (-)-14

(*S*)-benzyl 2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-3-methylbutanoate (-)-14. A mixture of valine benzyl ester (-)-**13**[5] (100 mg, 0.26 mmol) and *N*-Boc valine (57.1 mg, 0.26 mmol) was dissolved in 1.8 mL of THF and cooled to 0 °C. *N*-methyl morpholine (0.14 mL, 1.31 mmol) was added, followed by HOBt (40.0 mg, 0.3 mmol) and EDCI (57.5 mg, 0.3 mmol). The mixture was stirred allowed to gradually reach room temperature for 12 h before concentrating in rotavapor. Residue was taken in ethyl acetate and washed with 1N HCl solution, $NaHCO_3$ saturated solution and brine, dried over $MgSO_4$, filtered and concentrated. The residue was loaded into a flash chromatography column and purified using 8:2 hexanes-ethyl acetate to give 92.5 mg of the title compound in 87% yield.

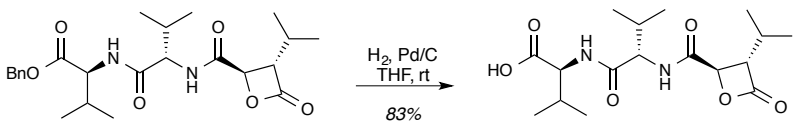
^{1}H and ^{13}C NMR data were in agreement with literature values.[5]



(-)-14 S3 (-)-15

(*S*)-benzyl 2-((*S*)-2-((2*R*,3*S*)-3-isopropyl-4-oxooxetane-2-carboxamido)-3-methylbutanamido)-3-methylbutanoate (-)-15. A solution of peptide (-)-**14** (103.5 mg, 0.25 mmol) in 0.5 mL of CH_2Cl_2 was treated with 0.5 mL of TFA. The mixture was stirred at room temperature for about 30 min. before concentrating in rotavapor and high-vacuum. The residual trifluoroacetate salt **S3** as a white solid that was used in the coupling reaction without extensive characterization. A solution of acid (-)-**7** (79.8 mg, 0.50 mmol) and *N*-methyl morpholine (0.17 mL, 1.38 mmol) in 2.3 mL of THF was added to a flask containing a stirring bar and trifluoroacetate salt **S3**, held at 0 °C. HOBt (74.0 mg, 0.54 mmol) and EDCI (105.0 mg, 0.54 mmol) were quickly added in one portion. The mixture was stirred and allowed to gradually reach room temperature for 12 h before concentrating in rotavapor. The residue was taken in ethyl acetate and washed with 1N HCl solution, $NaHCO_3$ saturated solution, and brine, dried over $MgSO_4$, filtered and concentrated. The residue was loaded into a flash chromatography column and purified using 7:3 hexanes-ethyl acetate to give 55.2 mg of the title compound in 48% yield over two steps.

$[\alpha]_D^{18}$ = –13.2 (*c* = 0.5, $CHCl_3$); **IR** (ν, cm^{-1}) 3277, 2964, 1838, 1741, 1645, 1549; **^{1}HNMR** (400 MHz, $CDCl_3$) δ 7.30-7.36 (m, 5H), 7.04 (d, *J* = 8.4 Hz, 1H), 6.53 (d, *J* = 8.6 Hz, 1H), 5.20 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 4.63 (d, *J* = 4.5 Hz, 1H), 4.57 (dd, *J* = 8.6, 4.8 Hz, 1H), 4.31 (dd, *J* = 7.7, 7.7 Hz, 1H), 3.49 (dd, *J* = 8.1, 4.5 Hz, 1H), 2.06-2.24 (m, 3H), 1.09 (d, *J* = 6.7 Hz, 3H), 1.06 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 171.4, 170.2, 168.5, 168.0, 135.1, 128.54, 128.45, 128.4, 71.0, 67.1, 64.3, 58.6, 57.1, 31.0, 30.8, 27.7, 20.0, 19.4, 19.1, 18.9, 18.1, 17.6; **HRMS** (ESI+) *m/z*: $[M+Na]^+$ calc'd for $C_{24}H_{34}O_4Na$ 469.2315; found 469.2311.

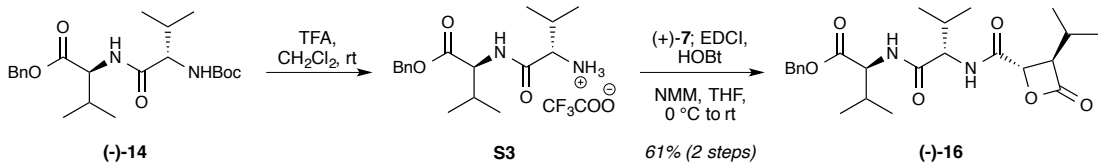


(-)-15 Cystargolide B (**2**)

Cystargolide B (2). A solution of benzyl ester (-)-**15** (55.2 mg, 0.12 mmol) in 10 mL of THF was treated with Pd/C (55 mg, 10% wt.). The flask was flushed with hydrogen and the mixture stirred under an atmosphere of hydrogen (balloon) at room temperature for 12 h before diluting with THF and filtering through a short plug of celite. Solids were rinsed with THF and the combined filtrate concentrated in

rotavapor. Crude product was triturated and washed with hexanes until all non-polar impurities were removed, as judged by TLC. Drying under high vacuum yielded 35.4 mg of the title compound in 83% yield.

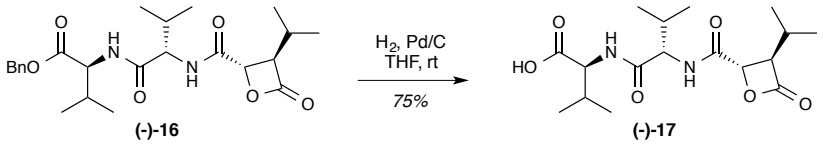
$[\alpha]_D^{19}$ = –54.6 (*c* = 0. 5, MeOH); **IR** (ν, cm^{-1}) 3304, 2966, 1839, 1720, 1647, 1546; **^{1}HNMR** (400 MHz, DMSO-d6) δ 12.57 (bs, 1H), 8.56 (d, *J* = 9.0 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 5.02 (d, *J* = 4.2 Hz, 1H), 4.39 (dd, *J* = 8.8, 7.0 Hz, 1H), 4.11 (dd, *J* = 7.8, 6.0 Hz, 1H), 3.51 (dd, *J* = 8.3, 4.0 Hz, 1H), 1.94-2.19 (m, 3H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.87 (d, *J* = 6.7 Hz, 3H); **^{13}CNMR** (100 MHz, DMSO-d6) δ 172.8, 170.7, 170.1, 167.3, 70.1, 62.7, 57.4, 57.2, 30.9, 29.6, 26.7, 19.4, 19.3, 19.1, 19.1, 18.1, 17.9; **HRMS** (ESI-) *m/z*: $[M-H]^-$ calc'd for $C_{17}H_{27}N_2O_6$ 181.0477; found 355.1872.



(-)-14 S3 61% (2 steps) (-)-16

(*S*)-benzyl 2-((*S*)-2-((2*S*,3*R*)-3-isopropyl-4-oxooxetane-2-carboxamido)-3-methylbutanamido)-3-methylbutanoate (-)-16. A solution of peptide (-)-**14** (82.5 mg, 0.20 mmol) in 0.4 mL of CH_2Cl_2 was treated with 0.4 mL of TFA. The mixture was stirred at room temperature for about 30 min. before concentrating in rotavapor and high-vacuum. The residual trifluoroacetate salt **S3** was used in the coupling reaction without extensive characterization: A solution of acid (+)-**7** (48.1 mg, 0.30 mmol) and *N*-methyl morpholine (0.13 mL, 1.21 mmol) in 2.0 mL of THF was added to a flask containing a stirring bar and trifluoroacetate salt **S3**, held at 0 °C. HOBt (58.9 mg, 0.43 mmol) and EDCI (82.5 mg, 0.43 mmol) were quickly added in one portion. Reaction mixture was stirred and allowed to reach room temperature for 12 h before concentrating in rotavapor. The residue was taken in ethyl acetate and washed with 1N HCl solution, $NaHCO_3$ saturated solution, and brine, dried over $MgSO_4$, filtered and concentrated. The residue was loaded into a flash chromatography column and purified using 7:3 hexanes-ethyl acetate to give 55.4 mg of the title compound in 61% yield over two steps.

$[\alpha]_D^{26}$ = –21.6 (*c* = 0.5, $CHCl_3$); **IR** (ν, cm^{-1}) 3309, 2964, 1836, 1739, 1647, 1538; **^{1}HNMR** (400 MHz, $CDCl_3$) δ 7.28-7.37 (m, 5H), 7.09 (d, *J* = 8.8 Hz, 1H), 6.66 (d, *J* = 8.5 Hz, 1H), 5.20 (d, *J* = 12.2 Hz, 1H), 5.11 (d, *J* = 12.2 Hz, 1H), 4.60 (d, *J* = 4.5 Hz, 1H), 4.57 (dd, *J* = 8.7, 4.8 Hz, 1H), 4.37 (dd, *J* = 8.8, 7.2 Hz, 1H), 3.46 (dd, *J* = 8.1, 4.5 Hz, 1H), 2.06-2.24 (m, 3H), 1.11 (d, *J* = 6.7 Hz, 3H), 1.08 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 171.4, 170.3, 168.6, 168.2, 135.1, 128.54, 128.45, 128.4, 71.1, 67.1, 64.6, 58.3, 57.2, 31.2, 31.0, 27.8, 20.0, 19.3, 19.1, 18.9, 18.2, 17.6; **HRMS** (ESI+) *m/z*: $[M+Na]^+$ calc'd for $C_{24}H_{34}N_2O_6Na$ 469.2315; found 469.2325.



(-)-16 (-)-17

(*S*)-2-((*S*)-((2*S*,3*R*)-3-isopropyl-4-oxooxetane-2-carboxamido)-3-methylbutanamido)-3-methylbutanoic acid (-)-17. A solution of benzyl ester (-)-**16** (50.2 mg, 0.11 mmol) in 10 mL of THF was treated with Pd/C (50 mg, 10% wt.). The flask was flushed with hydrogen and the mixture stirred under an atmosphere of hydrogen (balloon) at room temperature for 12 h before diluting with THF and filtering through a short plug of celite. Solids were rinsed with THF and the combined filtrate concentrated in rotavapor. Crude product was triturated and washed with hexanes and 5:1 hexanes-Et_2O until all non-

polar impurities were removed, as judged by TLC. Drying under high vacuum yielded 30.1 mg of the title compound in 75% yield.
$[\alpha]_D^{27}$ = –0.47 (*c* = 0. 21, MeOH); **IR** (ν, cm^{-1}) 3296, 2965, 1837, 1718, 1653, 1540; **^{1}HNMR** (400 MHz, DMSO-d6) δ 12.58 (bs, 1H), 8.41 (d, *J* = 8.9 Hz, 1H), 8.08 (d, *J* = 8.2 Hz, 1H), 4.99 (d, *J* = 4.1 Hz, 1H), 4.38 (dd, *J* = 8.8, 6.9 Hz, 1H), 4.11 (dd, *J* = 8.0, 6.0 Hz, 1H), 3.51 (dd, *J* = 8.0, 4.1 Hz, 1H), 1.96-2.17 (m, 3H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 3H), 0.82-0.89 (m, 12H); **^{13}CNMR** (100 MHz, DMSO-d6) δ 172.7, 170.5, 170.0, 167.2, 70.3, 62.4, 57.4, 57.2, 30.9, 29.6, 26.5, 19.6, 19.09, 19.08, 19.0, 18.0, 18.0; **HRMS** (ESI-) *m/z*: $[M-H]^-$ calc'd for $C_{17}H_{27}N_2O_6$ 355.1869; found 355.1864.

Biological Assays.

Jurkat Lysate Preparation. Human T-cell leukemia cell line Jurkat (ATCC TIB-152, E6-1 clone) was cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (Invitrogen), 100mg/L penicillin G and 100 mg/L streptomycin (PenStrep; Invitrogen) until the cell culture reached a density of approximately $2x10^6$ cells/mL. The cells were pelleted, washed with HEPES saline solution (pH 7.4), and lysed by resuspending the cells in Lysis Buffer (150 mM NaCl, 50 mM HEPES pH 7.6, 5 mM EDTA, and 1% Triton X-100) to a cell density of $2x10^6$ cells/mL. The lysate was immediately put on ice and agitated for one hour. Using the BioRad protein assay, the final protein concentration of the lysed cells was ~0.5 mg/mL. The lysate was stored at -84 °C.

Proteasome Assay. The 20S proteasomal activity assay was based on a commercial kit (Cayman Chemical) which measured the degree of peptide cleavage in Jurkat cell lysate. Precipitated proteins were removed from thawed cell lysate using a 3 minute centrifugation at ~10,000G, and 90 μL of Jurkat lysate was added per well on a 96 well plate. Separately, 100 mM stock solutions of the compounds were prepared in DMSO. Each stock solution was diluted further in Lysis Buffer, and 10 μL of the drug, at ten times the final concentration, was added to each lysate-containing well. The well plate was incubated at 37 °C for 30 minutes to allow for the binding of the drug to the proteasomes. Thereafter, 10 μL of the fluorescent substrate, 100 μM SUC-LLVY-AMC (ENZO Life Sciences) in Lysate Buffer, was added to each well. The plate was incubated for 60 minutes, and the proteasomal activity calculated in terms of the fluorescence of the cleaved substrate. Fluorescence of each well was measured with an excitation wavelength of 360 nM and emission wavelength of 480 nm using the WALLAC Victor^2 1420 multilabel counter. The IC_{50} values were calculated, using GrapPad Prism 6.0 non-linear regression dose-response, variable slope model, normalizing the values to the untreated and internal controls [(-)-**15**, 100 μM] as maximal and minimal responses to adjust the values of fluorescence to relative activity between 0 and 100 percent. The IC_{50} values were measured in technical triplicate for compounds (-)-**1**, (-)-**2**, (-)-**10** and (-)-**15** as confirmation, and were estimated from technical duplicate for compounds (-)-**11**, (-)-**12**, (-)-**16** and (-)-**17.**

Cell Culture. Human mammary carcinoma MCF7 (ATCC® HTB-22™)) cells were cultured in DMEM supplemented with 10% FBS and 100 mg/L penicillin G and 100 mg/L streptomycin. Non-tumorigenic mammary gland epithelial MCF 10A (ATCC: CRL-10317) cell line was cultured in RPMI (Invitrogen) supplemented with 5% horse serum (Invitrogen), epidermal growth factor EGF (20 ng/mL, QED Bioscience Inc.), hydrocortisone (0.5 ug/mL, Sigma), cholera toxin (100 ng/mL, Sigma), insulin (10 ug/mL, Sigma), and PenStrep (Invitrogen). The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

MTT Assay[6]. 100 μL of MCF-7 or MCF10A cells were transferred to each well of a 96-well microtiter plate at a concentration of $4x10^4$ cells/mL and incubated for 24 h to allow proper adhesion. Cells were

treated with the panel of test compounds at a series of concentrations and 0.1% vol/vol DMSO as solvent control. After 48h incubation, 20 μL of MTT reagent ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Alfa Aesar) in serum- free medium (5 mg/mL) was added to each well of the plates. The plate was incubated for 2 h at 37 °C. Media was removed from each well and the resulting formazan crystals dissolved in 100 μL of DMSO. Optical density (OD) at 490 nm was measured using a ThermoMAX microplate reader. The experiments were performed in four replicates and repeated at least twice for each compound per cell line unless otherwise stated. Cells treated with 1 μM phenylarsine oxide (PAO) served as a positive killing control. IC_{50} values were calculated using GrapPad Prism 6.0 using a non-linear regression, dose-response, variable slope model. Data were normalized using untreated and internal controls to calculate maximal and minimal responses.

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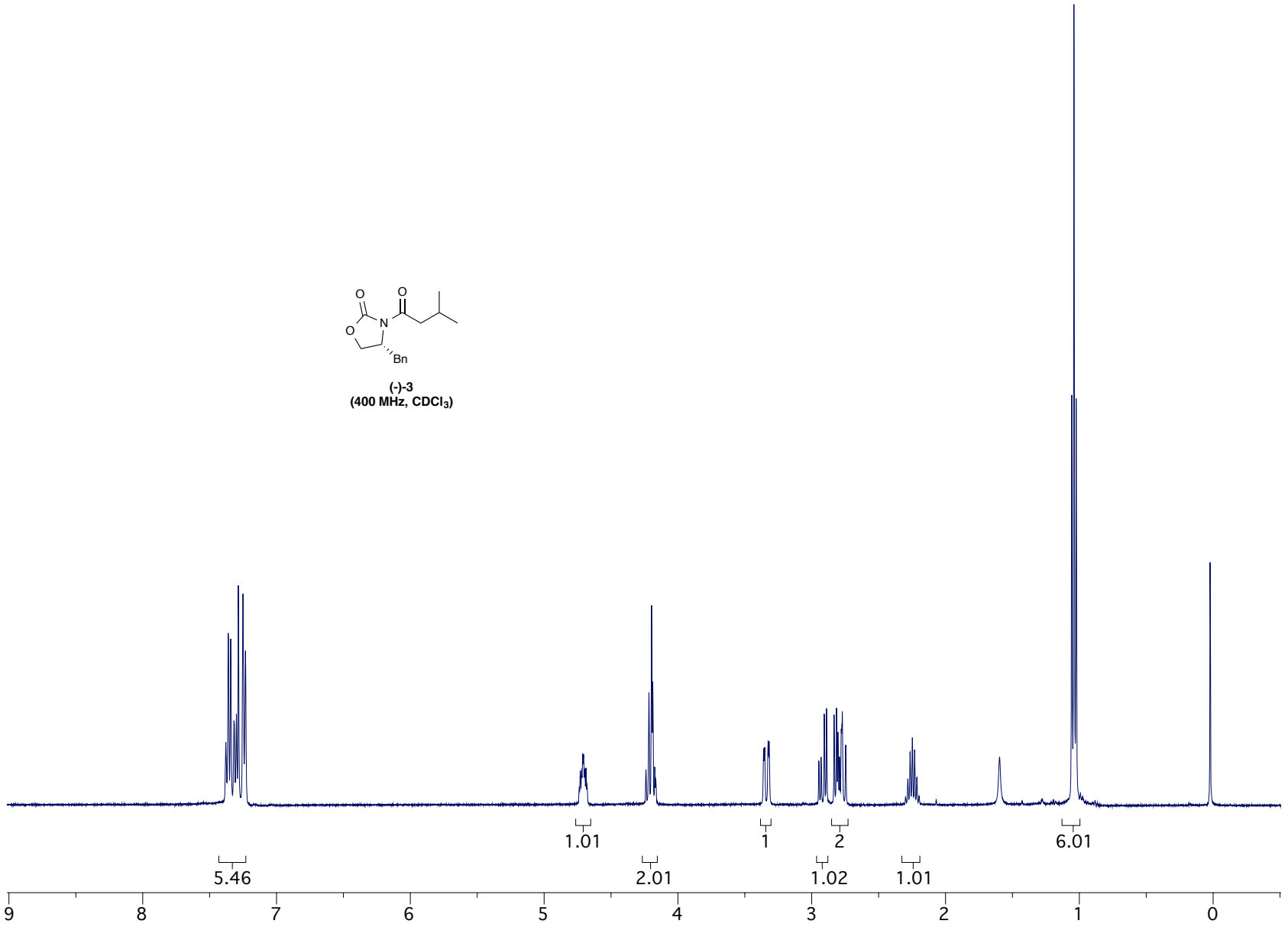
Copies of ^{1}H and ^{13}C NMR Spectra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O
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Bn
(-)-3
(400 MHz, CDCl3)
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8
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6
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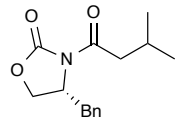

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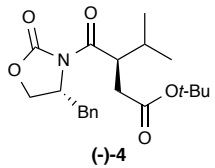

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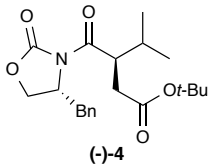

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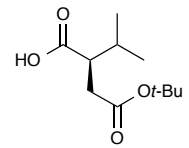

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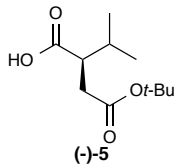

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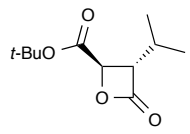
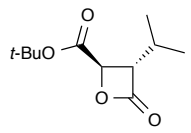
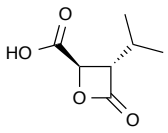


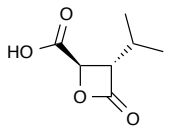
(-)-6
(400 MHz, $CDCl_3$)



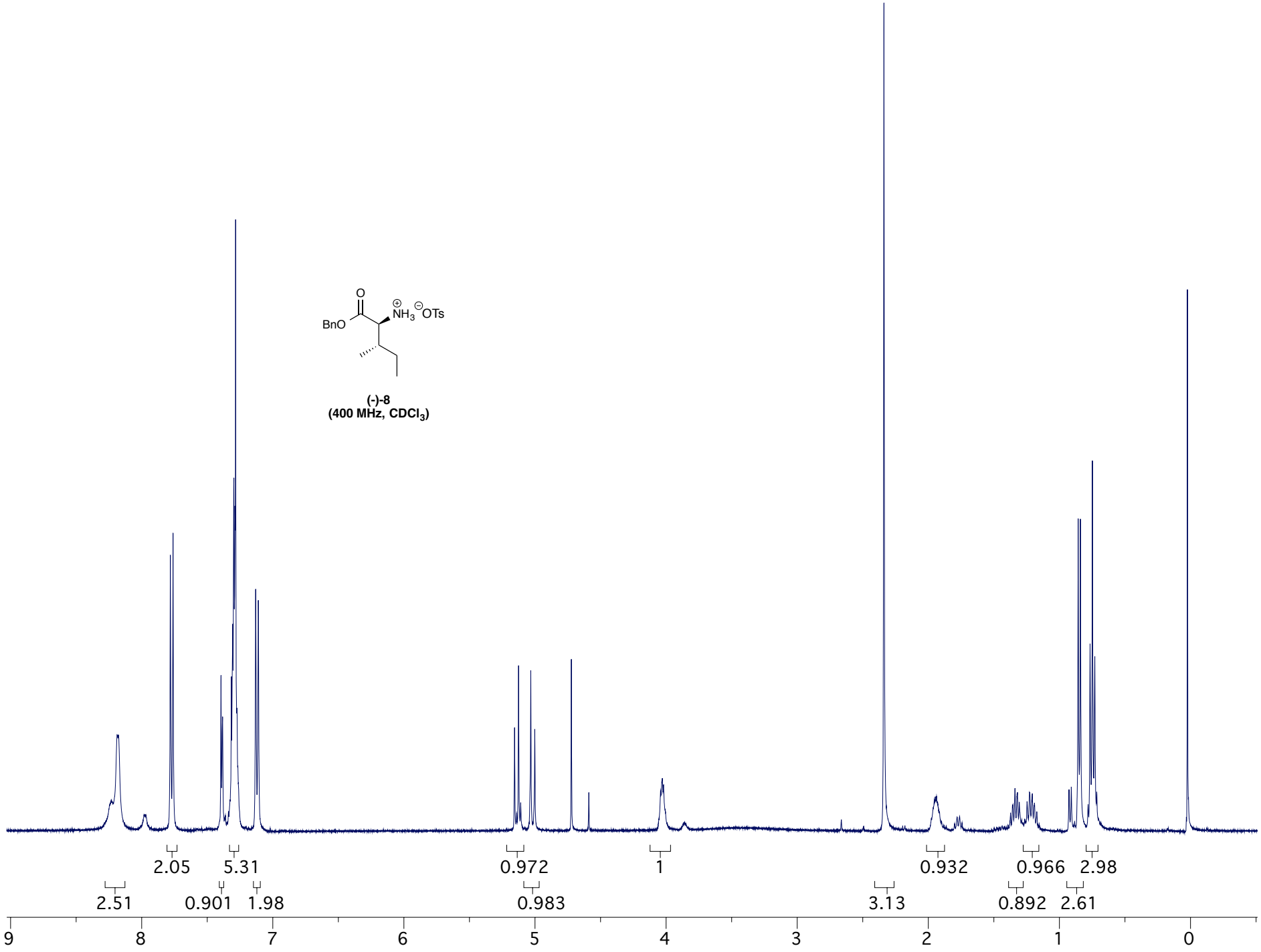
(-)-6
(100 MHz, $CDCl_3$)

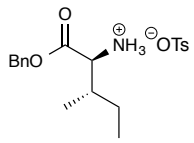


(-)-7
(400 MHz, $CDCl_3$)

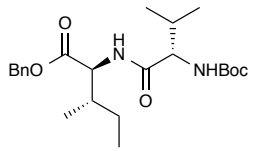


(-)-7
(100 MHz, $CDCl_3$)

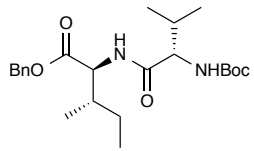

BnO
NH3
OTs
(-)-8
(400 MHz, CDCl3)
2.05
5.31
0.972
1
0.932
0.966
2.98
2.51
0.901
1.98
0.983
3.13
0.892
2.61
9
8
7
6
5
4
3
2
1
0



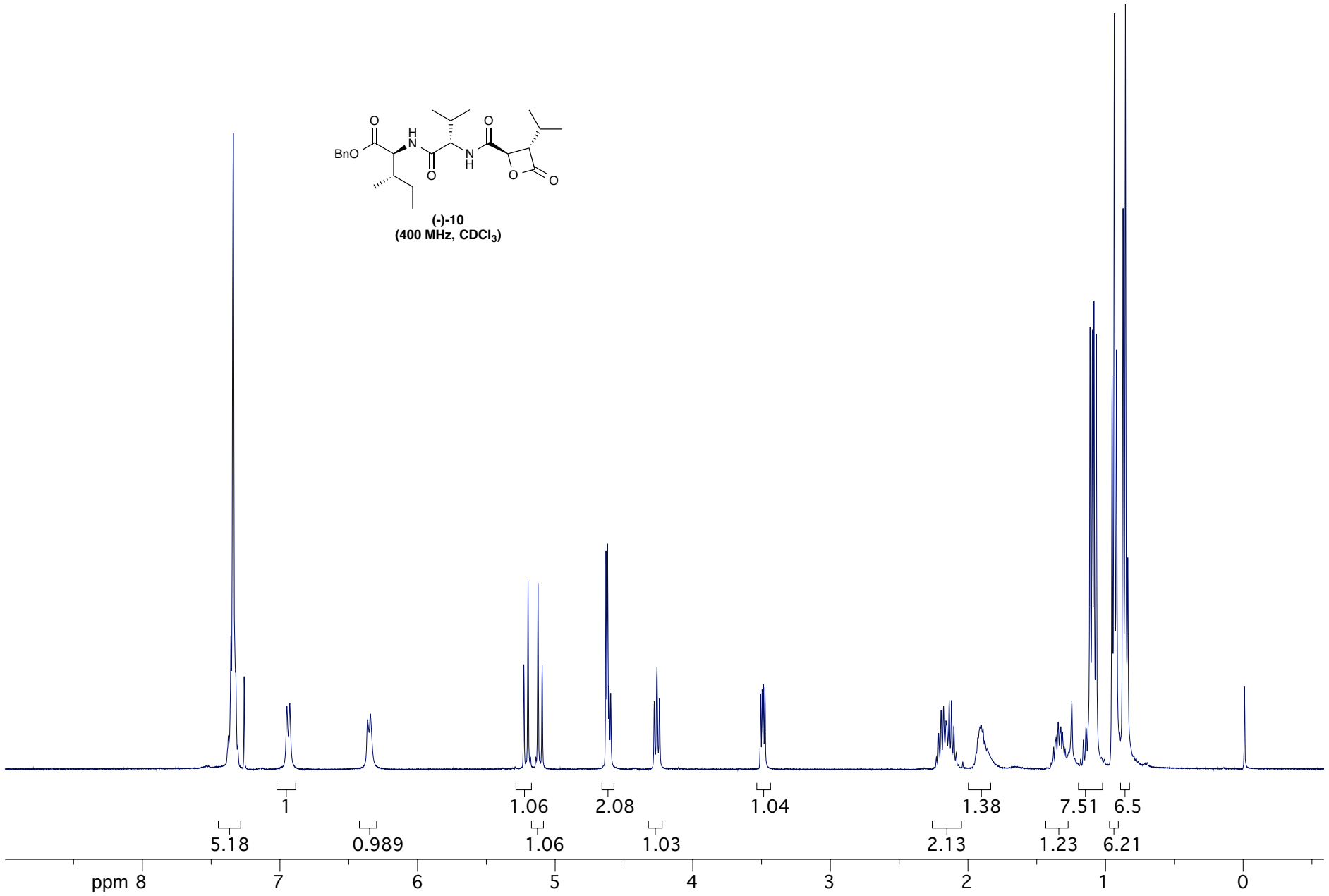
(-)-8
(100 MHz, $CDCl_3$)

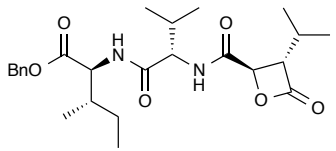


(-)-9
(400 MHz, $CDCl_3$)

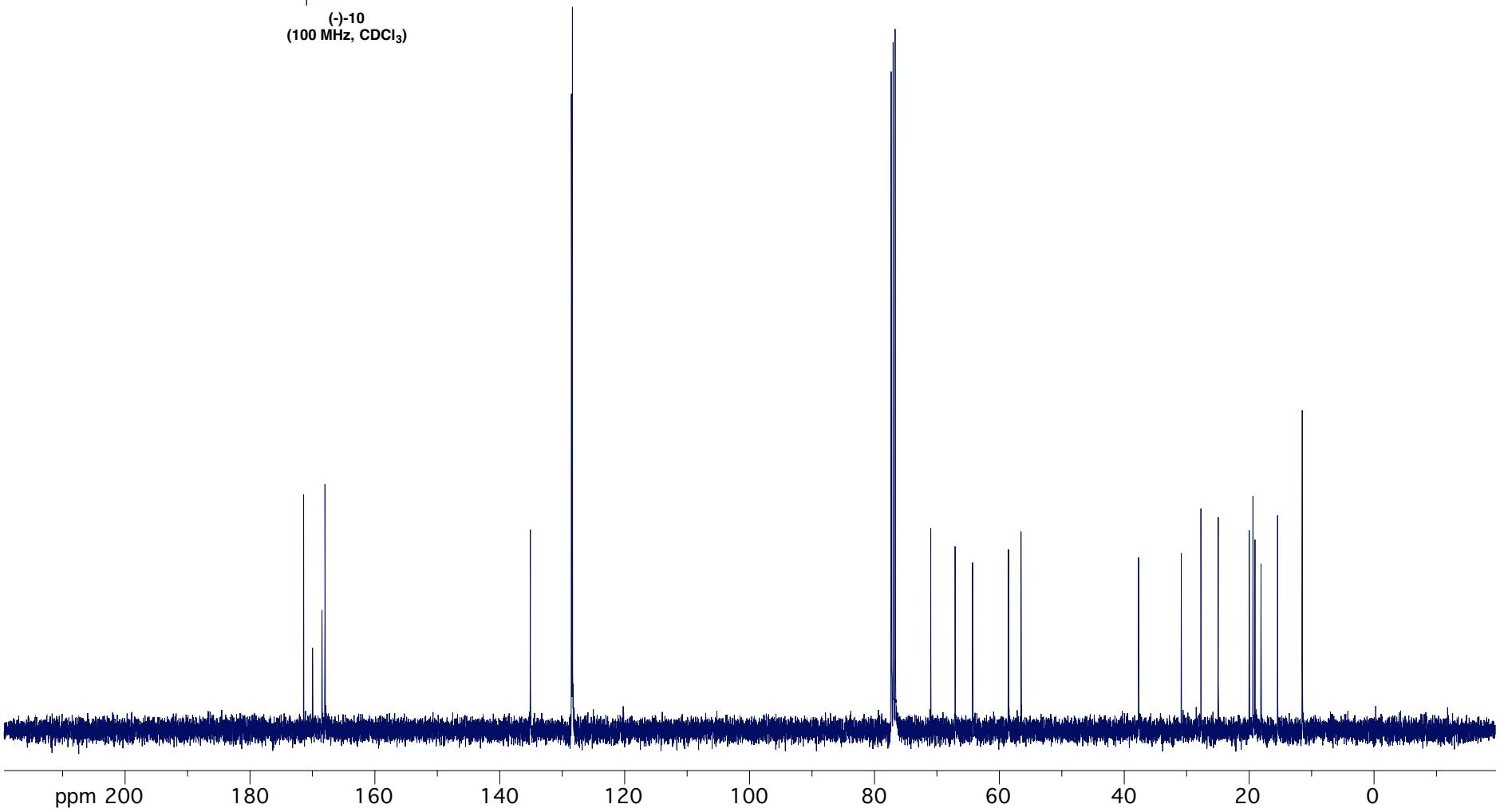


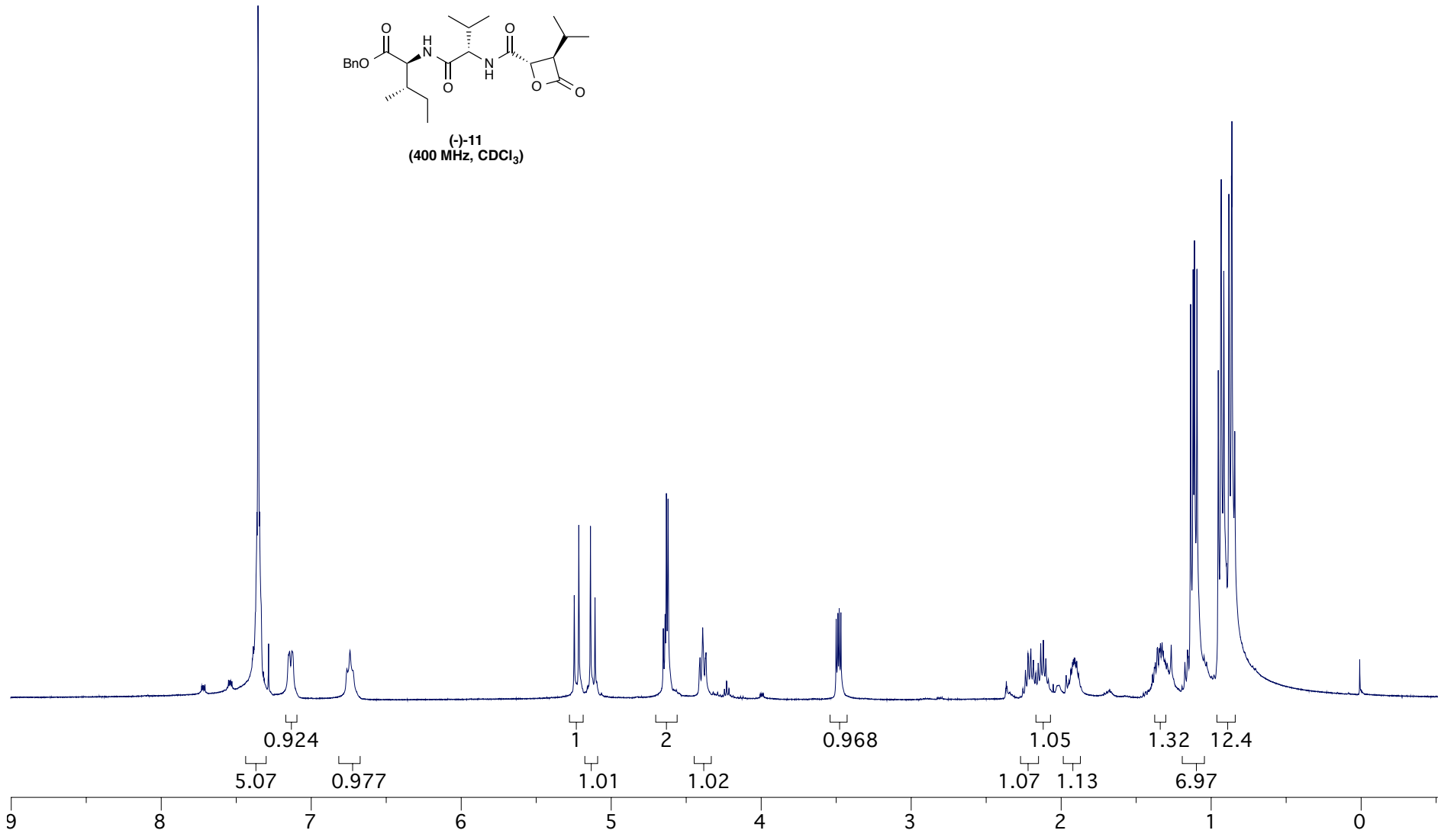
(-)-9
100 MHz, $CDCl_3$)

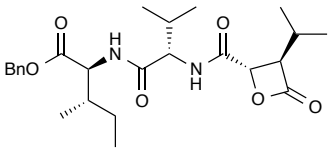

BnO
O
H
N
O
N
H
O
O
O
O
(-)-10
(400 MHz, CDCl3)
1
1.06
2.08
1.04
1.38
7.51
6.5
5.18
0.989
1.06
1.03
2.13
1.23
6.21
ppm 8
7
6
5
4
3
2
1
0



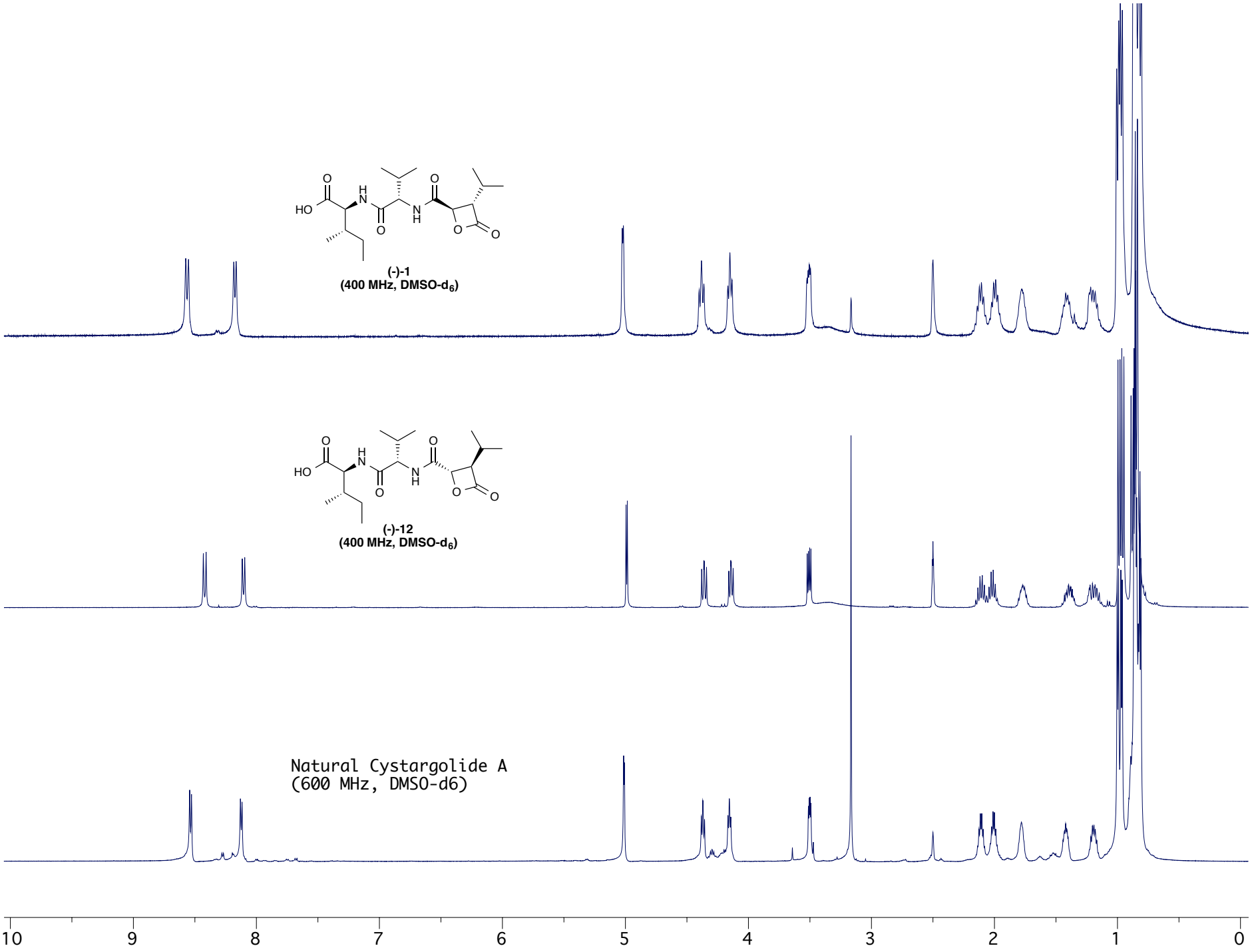
(-)-10
(100 MHz, $CDCl_3$)

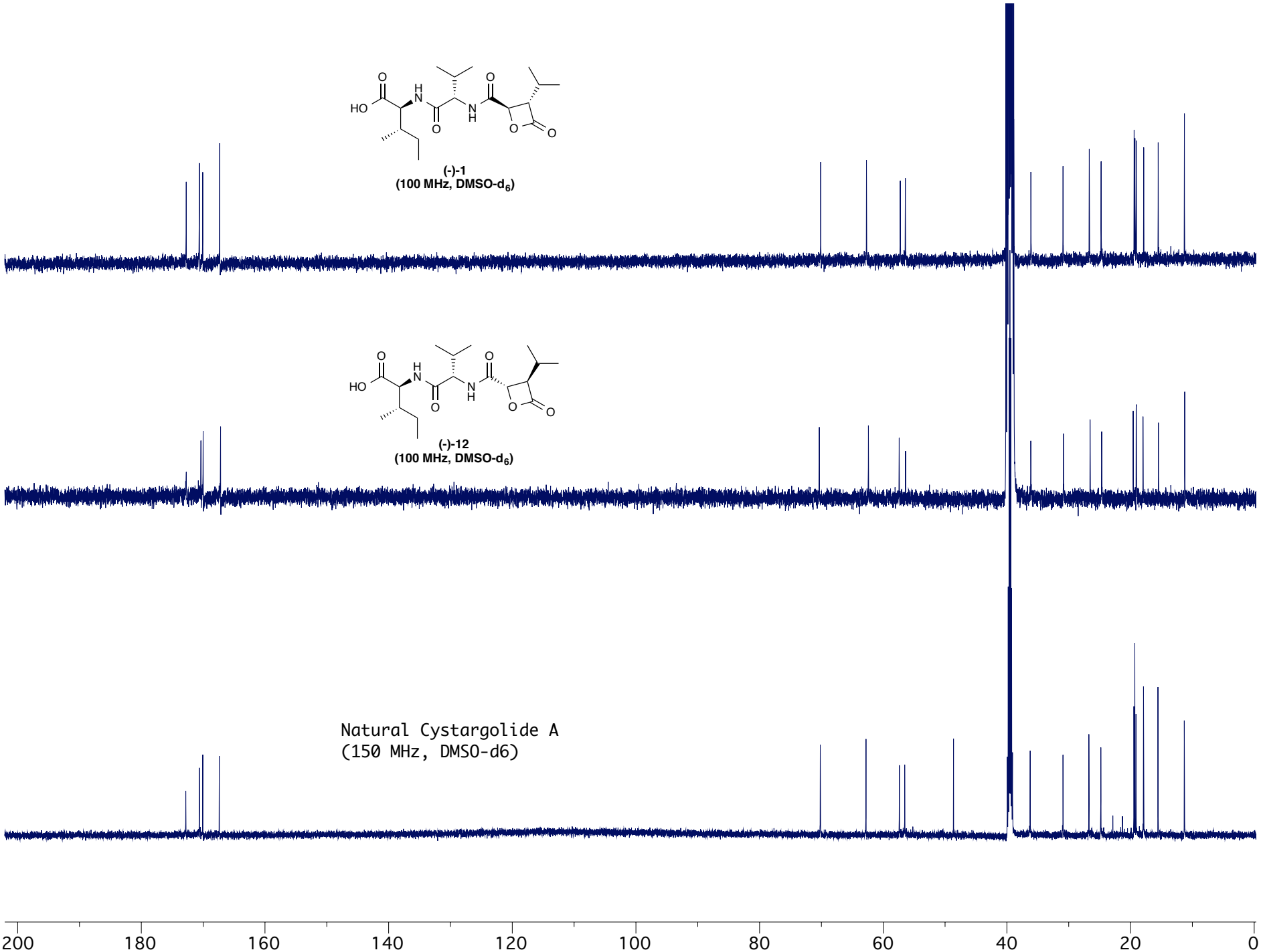


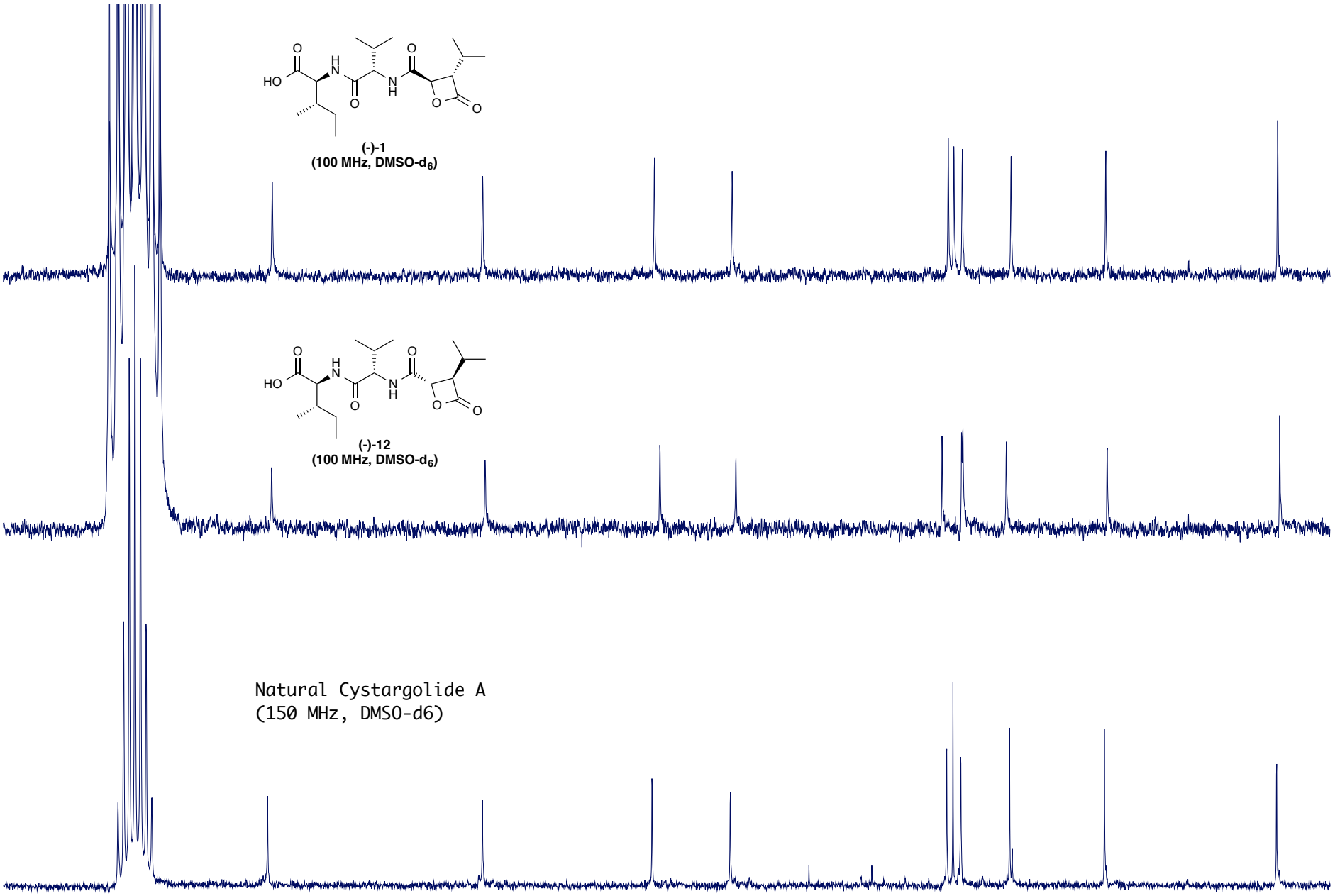

BnO
O
H
N
O
N
H
O
O
O
O
(-)-11
(400 MHz, CDCl3)
0.924
5.07
0.977
1
1.01
2
1.02
0.968
1.05
1.07
1.13
1.32
6.97
12.4
9
8
7
6
5
4
3
2
1
0

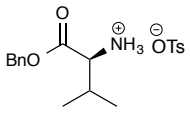


(-)-11
(100 MHz, $CDCl_3$)

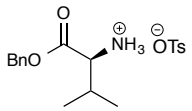

(-)-1
(400 MHz, DMSO-d6)
(-)-12
(400 MHz, DMSO-d6)
Natural Cystargolide A
(600 MHz, DMSO-d6)
10
9
8
7
6
5
4
3
2
1
0


(-)-1
(100 MHz, DMSO-d6)
(-)-12
(100 MHz, DMSO-d6)
Natural Cystargolide A
(150 MHz, DMSO-d6)
200
180
160
140
120
100
80
60
40
20
0

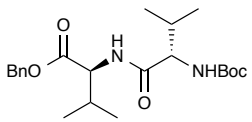

(-)-1
(100 MHz, DMSO-d6)
(-)-12
(100 MHz, DMSO-d6)
Natural Cystargolide A
(150 MHz, DMSO-d6)
ppm 40
35
30
25
20
15



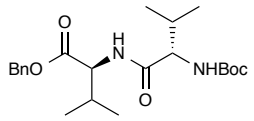
(-)-13
(400 MHZ, $CDCl_3$)



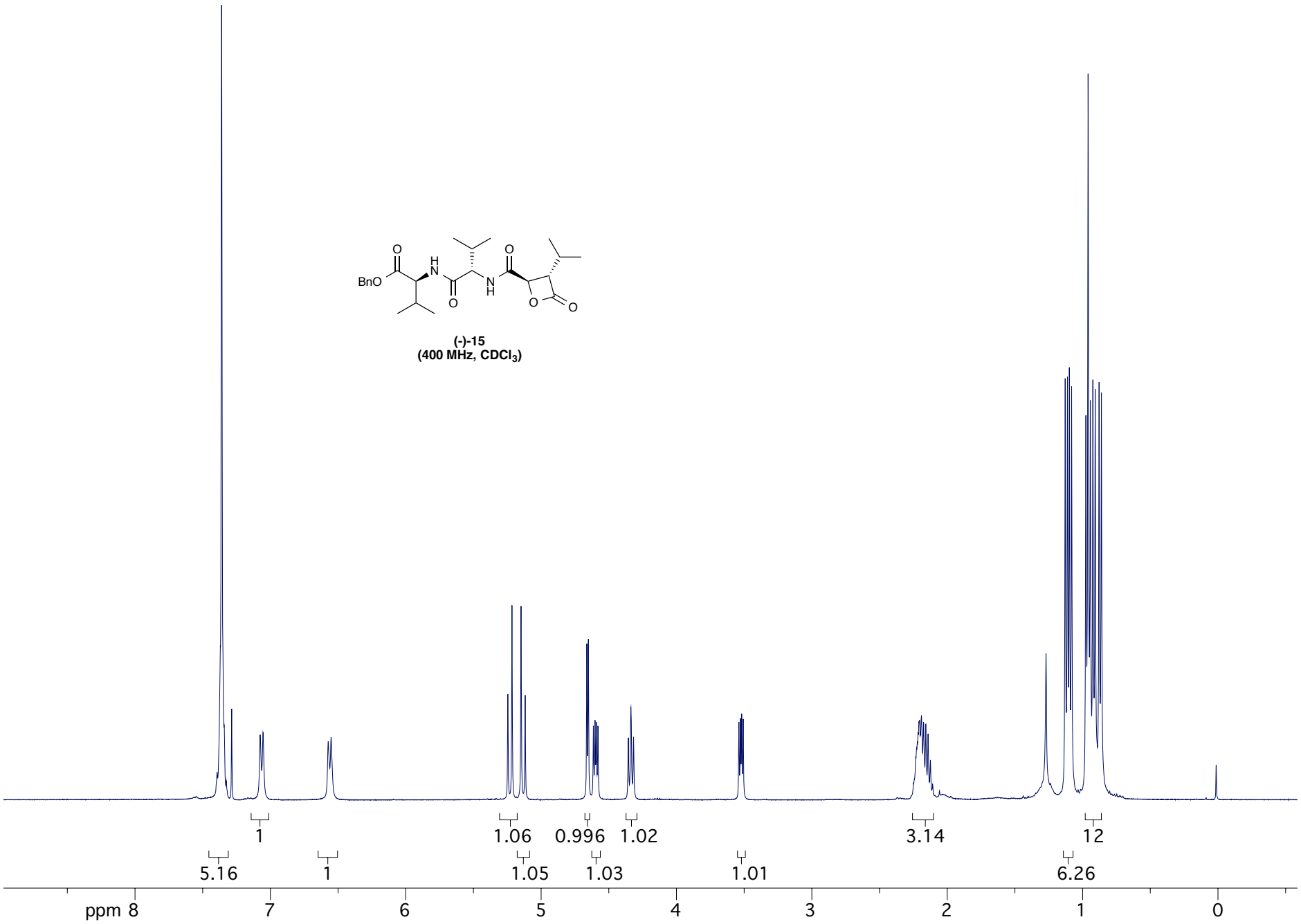
(-)-13
100 MHz, $CDCl_3$)

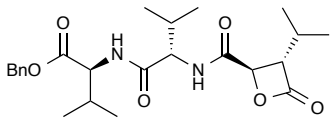


(-)-14
(400 MHz, $CDCl_3$)

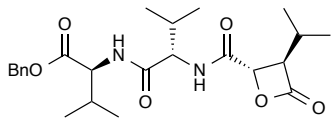


(-)-14
(100 MHz, $CDCl_3$)

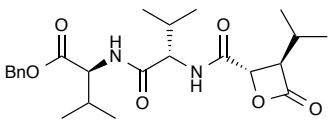

BnO
O
H
N
O
N
H
O
O
O
O
(-)-15
(400 MHz, CDCl3)
1
1.06
0.996
1.02
3.14
12
5.16
1
1.05
1.03
1.01
6.26
ppm 8
7
6
5
4
3
2
1
0



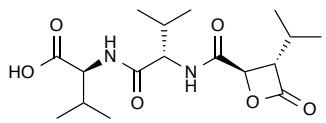
(-)-15
(100 MHz, $CDCl_3$)



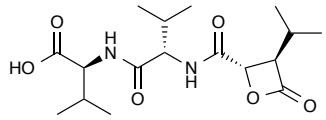
(-)-16
(400 MHz, $CDCl_3$)



(-)-16
(100 MHz, $CDCl_3$)



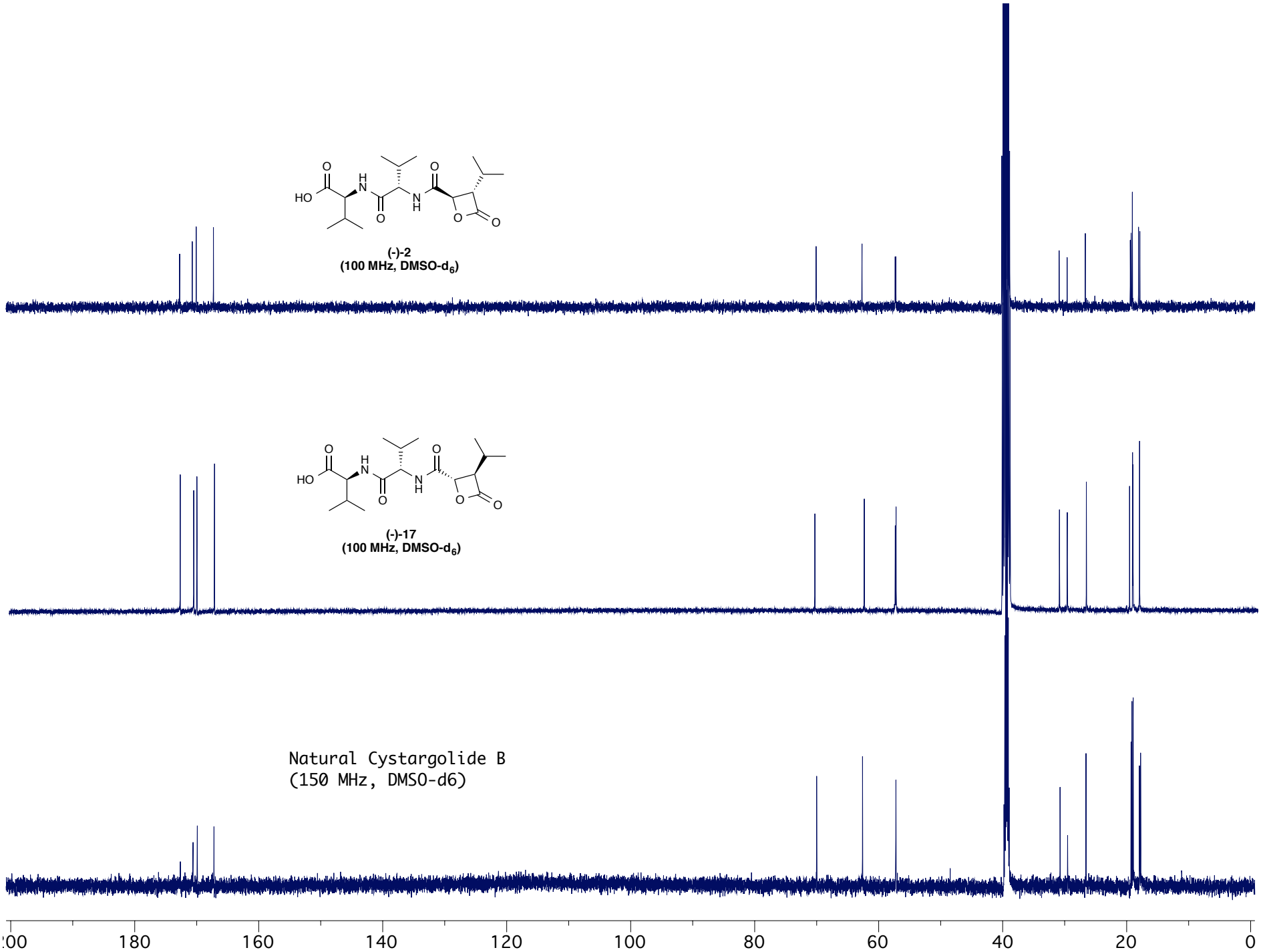
(-)-2
(400 MHz, DMSO-d_6)

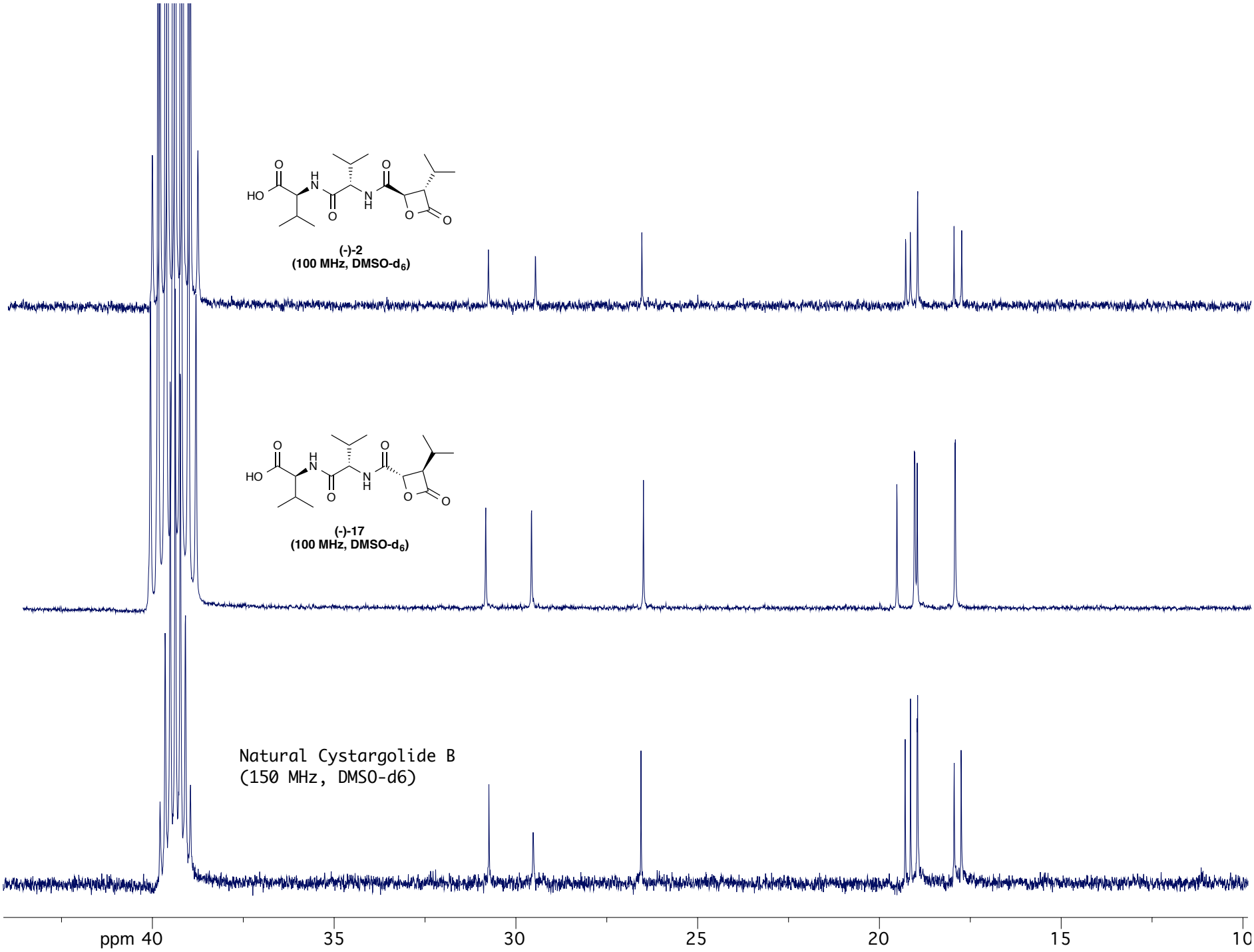


(-)-17
(400 MHz, DMSO-d_6)

Natural Cystargolide B
(600 MHz, DMSO-d6)

10 9 8 7 6 5 4 3 2 1 0


(-)-2
(100 MHz, DMSO-d6)
(-)-17
(100 MHz, DMSO-d6)
Natural Cystargolide B
(150 MHz, DMSO-d6)
180
160
140
120
100
80
60
40
20
0


(-)-2
(100 MHz, DMSO-d6)
(-)-17
(100 MHz, DMSO-d6)
Natural Cystargolide B
(150 MHz, DMSO-d6)
ppm 40
35
30
25
20
15
10