Supporting Information

Synthesis of D- and L-2,3-*trans*-3,4-*cis*-4,5-*trans*-3,4-Dihydroxy-5-hydroxymethylproline and of Tripeptides Containing Them

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1. General Experimental Part.

The procedures described for racemic products were applied to the preparation of enantiomerically pure products. Apart from the [α] values and m.p., all other data were identical for enantiomerically pure products and the corresponding racemates. ^{1}H NMR and ^{13}C NMR signals assignments were confirmed by 2D COSY and HMQC. Optical rotations were measured at 25 °C. TLC was performed on silica gel HF_{254}, with detection by UV light and charring with Pancaldi, ninhydrin or $KMnO_4$. Silica gel (240-400 mesh) was used for preparative chromatography. Anh. solvents and reagents were freshly distilled under N_2 prior to use: THF from sodium and benzophenone, CH_2Cl_2 and *i*-Pr_2NEt from CaH_2.

2. Complete Spectral Characterization Data.

(±)-7-*tert*-Butoxycarbonyl-5,6-*exo*-isopropylidenedioxy-7-azabicyclo[2.2.1]heptane-2-*endo*-ol ((-)-9) and 7-*tert*-butoxycarbonyl-5,6-*exo*-isopropylidenedioxy-7-azabicyclo[2.2.1]-hept-2-ene (10). *Data for (-)-**9**:* $[\alpha]_D$ -8 (c 1.0, CH_2Cl_2), $[\alpha]_{577}$ -10.3, $[\alpha]_{546}$ -11.7, $[\alpha]_{435}$ -12.5, $[\alpha]_{405}$ -15.9. IR ν_{max} 3443, 2978, 1681, 1416, 1173, 1058 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 363 K, δ ppm, *J* Hz) δ 4.90 (bs, 1 H, OH), 4.71 (d, 1 H, $J_{5,6}$ = 5.6, H-5),* 4.24 (d, 1 H, H-6),* 4.07 (dt, 1 H, H-2), 3.99 (d, $J_{3a,4}$ = 5.9, H-4), 3.97 (d, $J_{1,2}$ = 4.8, H-1), 2.02 (ddd, 1 H, $J_{2,3a}$ = 9.8, $J_{3a,3b}$ = 13.0, H-3a), 0.86 (dd, 1 H, H-3b); ^{13}C NMR (100.5 MHz, DMSO-*d*$_6$, 363 K, δ ppm) δ 152.5 (CO), 108.3 (*C*$(CH_3)_2$), 80.1 (C-5),* 77.3 (*C*$(CH_3)_3$), 75.9 (C-6),* 66.2 (C-2), 61.0, 58.4 (C-1, C-4), 32.4 (C-3), 27.2 (C(*C*$H_3)_3$), 24.6, 23.4 (C(*C*$H_3)_2$); CIMS *m/z* 286 [4%, $(M+H)^+$], *m/z* 270 [8%, $(M-Me)^+$], *m/z* 230 [100%, $(M-Bu^t+2H)^+$], *m/z* 186 [25%, $(M-Boc+2H)^+$]; HRCIMS *m/z* found 286.1650, calcd for $C_{14}H_{23}NO_5$+H 286.1654; Analysis, calcd for $C_{14}H_{23}NO_5$: C, 58.95; H, 8.07; N, 4.91; Found: C, 58.76; H, 8.30; N, 4.70. *Data for **10**:* m. p. 77-79 °C; IR ν_{max} 2978, 2936, 1698, 1468, 1368, 1207, 1158, 1057, 882, 857 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 343 K, δ ppm, *J* Hz) δ 6.37 (t, 2 H, $J_{1,2} = J_{3,4} = J_{2,4} = J_{1,3}$ = 1.3, H-2 and H-3), 4.50 (t, 2 H, H-1 and H-4), 4.24 (s, 2 H, H-5 and H-6), 1.40 (s, 9H, C(C*H*$_3)_3$), 1.36, 1.25 (2 s, 3 H each, C(C*H*$_3)_2$); ^{13}C NMR (100.5 MHz, DMSO-*d*$_6$, 343 K, δ ppm) δ 152.6 (CO), 135.2 (C-2 and C-3), 113.9 (*C*$(CH_3)_2$), 78.5 (C-5

and C-6), 77.8 ($C(CH_3)_3$), 61.5 (C-1 and C-4), 27.2 ($C(CH_3)_3$), 25.3, 24.3 ($C(CH_3)_2$); CIMS *m/z* 252 [5%, $(M-Me)^+$], *m/z* 168 [10%, $(M-Boc+2H)^+$]; Analysis, calcd for $C_{14}H_{21}NO_4$: C, 62.90; H, 7.92; N, 5.24; Found: C, 62.60; H, 7.96; N, 5.16.

(+)-7-*tert*-Butoxycarbonyl-5,6-*exo*-isopropylidenedioxy-7-azabicyclo[2.2.1]heptane-2-*endo*-ol ((+)-9). This compound was prepared in the manner described for (-)-**9** except that pure (+)-**8** was used. Pure (+)-**9** was obtained in 73% yield.$[\alpha]_D$ +7.3 (*c* 1.0, CH_2Cl_2), $[\alpha]_{577}$ +9.2, $[\alpha]_{546}$ +10, $[\alpha]_{435}$ +12.1, $[\alpha]_{405}$ +14.5.

(1*R*,2*R*,3*S*,4*S*,4'*R*,5'*R*)- and (1*S*,2*S*,3*R*,4*R*,4'*R*,5'*R*)-4',5'-Diphenylspiro[2,3-*exo*-isopropylidenedioxy-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]hept-2,2'-imidazoline] (+)-12 and (-)-13. Data for (+)-**12**: $[\alpha]_D$ +73 (*c* 1.65, $CHCl_3$); ^{13}C NMR (75.4 MHz, $CDCl_3$-Et_3N, 298 K, δ ppm, mixture of rotamers 1.2:1) *Major rotamer*: δ 155.0 (CO), 141.3, 139.6 (2 C-1 of Ph), 128.1-126.9 (10 C-aromat.), 111.0 ($C(CH_3)_2$), 81.7 (C-6), 80.8, 78.6 (C-2, C-3), 79.6 ($C(CH_3)_3$), 70.2, 69.2 (C-4', C-5'), 66.3 (C-4), 59.2 (C-1), 43.0 (C-5), 28.3 ($C(CH_3)_3$), 25.5, 24.1 ($(CH_3)_2C$). *Minor rotamer*: δ 154.9 (CO), 141.3, 139.4 (2 C-1 of Ph), 128.1-126.9 (10 C-aromat.), 111.0 ($C(CH_3)_3$), 81.5 (C-6), 81.4, 78.7 (C-2, C-3), 79.7 ($C(CH_3)_3$), 70.2, 69.3 (C-4', C-5'), 67.5 (C-4), 58.3 (C-1), 42.7 (C-5), 28.3 ($C(CH_3)_3$), 25.5, 24.1 ($(CH_3)_2C$); CIMS *m/z* 478 [70%, $(M+H)^+$]; HRCIMS *m/z* found 477.2626, calcd for $C_{28}H_{35}N_3O_4$ 477.2628. Data for (-)-**13**: $[\alpha]_D$ -38 (*c* 1.65, $CHCl_3$); ^{13}C NMR (75.4 MHz, $CDCl_3$-Et_3N, 298 K, δ ppm, mixture of rotamers 3.5:1) *Major rotamer*: δ 154.7 (CO), 141.8, 138.8 (2 C-1 of Ph), 128.7-126.1 (10 C-aromat.), 110.8 ($C(CH_3)_2$), 83.4 (C-6), 81.3, 78.6 (C-2, C-3), 79.6 ($C(CH_3)_3$), 71.8, 69.2 (C-4', C-5'), 67.8 (C-4), 58.4 (C-1), 41.1 (C-5), 28.4 ($C(CH_3)_3$), 25.6, 24.3 ($(CH_3)_2C$). *Minor rotamer*: δ 154.7 (CO), 141.0, 140.9 (2 C-1 of Ph), 128.7-126.1 (10 C-aromat.), 110.8 ($C(CH_3)$), 82.9 (C-6), 81.6, 78.5 (C-2, C-3), 79.5 ($C(CH_3)_2$), 71.3, 69.3 (C-4', C-5'), 67.1 (C-4), 59.7 (C-1), 42.6 (C-5), 28.3 ($C(CH_3)_3$), 25.6, 24.3 ($(CH_3)_2C$); CIMS *m/z* 478 [100%, $(M+H)^+$]; HRCIMS *m/z* found 477.2620, calcd for $C_{28}H_{35}N_3O_4$ 477.2628.

(-)-7-*tert*-Butoxycarbonyl-5,6-*exo*-isopropylidenedioxy-7-azabicyclo[2.2.1]hept-2-one ((-)-3). Method a). To a stirred solution of oxalyl chloride (68 μl, 0.78 mmol) in anhydrous CH_2Cl_2 (2 ml) at –70 °C, was added a solution of DMSO (106 μl, 1.56 mmol) in anhydrous CH_2Cl_2 (0.5 ml) dropwise. After addition, the mixture was stirred at –70 °C for 10 min and then a solution of alcohol (-)-**9** (184 mg, 0.65 mmol) in anhydrous CH_2Cl_2 (2 ml) was added dropwise. The mixture was stirred for a further 20 min and then Et_3N (0.5 ml, 3.25 mmol) was added dropwise. The mixture was allowed to reach 20 ºC and then washed with H_2O. The organic layer was separated and the aqueous phase reextracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel (ether:petroleum ether, 3:1) to give (-)-**3** (162 mg, 91%) as a white solid. M.p. 85-87 °C; $[\alpha]_D$ -51.9 (*c* 1.11, CH_2Cl_2), $[\alpha]_{577}$ -54.7, $[\alpha]_{546}$ -65.5, $[\alpha]_{435}$ -181, $[\alpha]_{405}$ -282; IR ν_{max} 2978, 2936, 1771, 1704, 1403, 1368, 1210, 1171, 1104, 1058 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 313 K, δ ppm, *J* Hz) δ 4.67 (d, 1H, $J_{3a,4}$ = 5.4, H-4), 4.41 (d, 1 H, $J_{5,6}$ = 5.4, H-5),* 4.37 (d, 1 H, H-6),* 4.34 (s, 1 H, H-1), 2.40 (dd, 1 H, $J_{3a,3b}$ = 17.8, H-3a), 1.80 (d, 1 H, H-3b), 1.47 (s, 12 H, C(C*H*$_3$)$_3$, C(C*H*$_3$)$_2$), 1.2 (s, 3 H, C(C*H*$_3$)$_2$); ^{13}C NMR (100.5 MHz, $CDCl_3$, 313 K, δ ppm) δ 206.2 (CO of ketone), 154.1 (CO of carbamate), 113.4 (*C*(CH$_3$)$_2$), 81.8 (C-5),* 80.8 (*C*(CH$_3$)$_3$), 78.0 (C-6),* 68.0 (C-1), 59.2 (C-4), 39.3 (C-3), 28.2 (C(*C*H$_3$)$_3$), 25.6, 24.4 (C(*C*H$_3$)$_2$); CIMS *m/z* 284 [30%, $(M+H)^+$], *m/z* 268 [5%, $(M-Me)^+$], *m/z* 228 [90%, $(M-Bu^t+2H)^+$], *m/z* 184 [90%, $(M-Boc+2H)^+$]; HREIMS *m/z* found 283.1422, calcd for $C_{14}H_{21}NO_5$ 283.1420.

(+)-7-*tert*-Butoxycarbonyl-5,6-*exo*-isopropylidenedioxy-7-azabicyclo[2.2.1]hept-2-one ((+)-3). This compound was prepared in the way described for (-)-**3**, except that pure (+)-**9** (method a) and (+)-**12** (method b) were used. Pure (+)-**3** was obtained in 90% (method a) and 92% (method b) yield. Mp = 86-88 °C; $[\alpha]_D$ +50.0 (*c* 0.86, CH_2Cl_2), $[\alpha]_{577}$ +52.6, $[\alpha]_{546}$ +64.2, $[\alpha]_{435}$ +177, $[\alpha]_{405}$ +273.

(-)-7-*tert*-Butoxycarbonyl-2-{[(*tert*-butyl)dimethylsilyl]oxy}-5,6-*exo*-isopropylidenedioxy-7-azabicyclo[2.2.1]hept-2-ene ((-)-14). $[\alpha]_D$ -38.2 (*c* 1.1, $CHCl_3$), $[\alpha]_{577}$ -40.2, $[\alpha]_{546}$ -45.7, $[\alpha]_{435}$ -82.5,

$[\alpha]_{405}$ -102; IR ν_{max} 2933, 2860, 1709, 1619, 1368, 1310, 1257, 1177, 1161, 1099, 841 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 298 K, δ ppm, *J* Hz, mixture of rotamers) δ 4.86, 4.79 (2 bs, 1 H, H-3), 4.55, 4.46 (2 bs, 1 H, H-4), 4.46 (d, 1 H, $J_{5,6}$ = 5.4, H-5),* 4.38 (d, 1 H, H-6),* 4.26, 4.15 (2 bs, 1 H, H-1), 1.43, (s, 12 H, (CH_3)$_3$C, (CH_3)$_2$C), 1.30 (s, 3 H, (C(CH_3)$_2$), 0.89, 0.88 (2 bs, 9 H, (CH_3)$_3$C-Si), 0.15, 0.12 (2 s, 6 H, (CH_3)$_2$Si); ^{13}C NMR (100.5 MHz, $CDCl_3$, 298 K, δ ppm, mixture of rotamers) δ 162.9, 161.7 (1 C, C-2), 155.0, 154.9 (1 C, CO), 116.2, 116.1 (1C, *C*(CH_3)$_3$), 104.5, 103.1 (1 C, C-3), 83.0, 82.5 (1 C, C-5),* 80.3, 80.0 (1 C, C-6),* 80.2 (1 C, (CH_3)$_3$*C*), 65.4, 64.7 (1 C, C-1), 63.4, 62.8 (1 C, C-4), 28.7 (3 C, (*C*H_3)$_3$C), 26.7 (1 C, (*C*H_3)$_2$C), 25.9 (3 C, (*C*H_3)$_3$CSi), 25.7, 25.6 (1 C, (*C*H_3)$_2$C) , 18.5 (1 C, (*C*H_3)$_3$*C*Si), -4.4, -4.5 (1 C, (CH_3)$_2$Si), -4.6, -4.7 (1 C, (CH_3)$_2$Si); CIMS *m/z* 398 [20%, $(M+H)^+$], *m/z* 342 [19%, $(M-Bu^t+2H)^+$], *m/z* 297 [57%, $(M-Boc+H)^+$]; Analysis, calcd for $C_{20}H_{35}NSiO_5$: C, 60.42; H, 8.87; N, 3.52; Found: C, 60.22; H, 8.96; N, 3.46.

(+)-7-*tert*-Butoxycarbonyl-2-{[(*tert*-butyl)dimethylsilyl]oxy}-5,6-*exo*-isopropylidenedioxy-7-azabicyclo[2.2.1]hept-2-ene ((+)-14). This compound was prepared in the manner described for (-)-**14**, except that pure (+)-**3** was used. Pure (+)-**16** was obtained in 84% yield. $[\alpha]_D$ +36.6 (*c* 1.0, $CHCl_3$), $[\alpha]_{577}$ +38.3, $[\alpha]_{546}$ +44.7, $[\alpha]_{435}$ +82, $[\alpha]_{405}$ +99.

D-2,3-*trans*-3,4-*cis*-4,5-*trans*-*N*-(*tert*-Butoxycarbonyl)-5-hydroxymethyl-3,4-isopropylidenedioxy-proline (Boc-D-Thyp(CMe$_2$)-OH) ((-)-4). $[\alpha]_D$ -44 (*c* 0.8, $CHCl_3$), $[\alpha]_{577}$ -48, $[\alpha]_{546}$ -53.6, $[\alpha]_{435}$ -87.8, $[\alpha]_{405}$ -104; IR ν_{max} 3600-2400, 1676, 1587, 1408, 1382, 1257, 1216, 1171, 1136, 1063, 734 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, 373 K, δ ppm, *J* Hz) δ 4.67 (dd, 1 H, $J_{3,4}$ = 5.9, $J_{2,3}$ = 1.5, H-3), 4.64 (d, 1 H, H-4), 4.14 (bs, 1 H, H-2), 3.92 (t, 1 H, H-5), 3.72 (dd, 1 H, $^2J_{Ha,Hb}$ = 11.4, $J_{5,Ha}$ = 4.3, CH_aOH), 3.44 (dd, 1 H, $J_{5,Hb}$ = 3.2, CH_bOH), 1.40 (s, 9 H, (CH_3)$_3$C), 1.38, 1.29 (2 s, 3 H each, (C(CH_3)$_2$); ^{13}C NMR (100.5 MHz, DMSO-d_6, 373 K, δ ppm) δ 173.9 (COOH), 153.2 (CO), 110.2 (*C*(CH_3)$_2$), 83.0 (C-3), 81.7 (C-4), 77.6 (*C*(CH_3)$_3$), 68.4 (CH_2OH), 66.0 (C-2), 60.8 (C-5), 27.2 (C(*C*H_3)$_3$), 26.1, 24.3 (C(*C*H_3)$_2$); CIMS *m/z* 318 [5%, $(M+H)^+$], *m/z* 279 [5%, $(M-Bu^t+NH_4+H)^+$]; *m/z* 217 [20%, $(M-Boc+2H)^+$].

L-2,3-*trans*-3,4-*cis*-4,5-*trans*-*N*-(*tert*-Butoxycarbonyl)-5-hydroxymethyl-3,4-isopropylidenedioxy-proline (Boc-L-Thyp(CMe$_2$)-OH) ((+)-4). This compound was prepared in the manner described for (-)-**4** except that pure (+)-**14** was used. Pure (+)-**4** was obtained in 89% yield. $[\alpha]_D$ +45 (*c* 0.55, $CHCl_3$), $[\alpha]_{577}$ +50.7, $[\alpha]_{546}$ +54, $[\alpha]_{435}$ +86.3, $[\alpha]_{405}$ +98.

***meso*-(2*S*,3*S*,4*R*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-2,5-dihydroxymethyl-3,4-isopropylidenedioxy-pyrrolidine (15)**. To a solution of $Me_2S.BH_3$ complex (36 μl, 0.36 mmol, 95% in dimethyl sulfide) in anhydrous THF (1 ml), is added a solution of (-)-**4** (38 mg, 0.12 mmol) in anhydrous THF (1 ml). The mixture is heated under reflux for 2 h. Excess BH_3 is destroyed by dropwise addition of anhydrous MeOH. After removal of the solvent, the product is obtained as an oil that was purified by column chromatography on silica gel (CH_2Cl_2:MeOH, 50:1→20:1) to give **15** (21 mg, 59%) as a syrup. IR ν_{max} 3402, 2980, 2936, 1673, 1404, 1370, 1171, 1063 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, 363 K, δ ppm, *J* Hz) δ 4.65 (s, 2 H, H-3 and H-4), 4.57 (bs, 2 H, OH), 3.85 (dd, $J_{2,CHa} = J_{5,CHa} = 6.8$, $J_{2,CHb} = J_{5,CHb} = 3.9$, H-2 and H-5), 3.53 (dt, 1 H, $^2J_{CHa,CHb} = 10.8$, $J_{CHa,OH} = 4.7$, CH_aOH), 3.41 (m, 1H, CH_bOH), 1.42 (s, 9 H, (CH_3)$_3$C), 1.38, 1.26 (2 s, 3 H each, (C(CH_3)$_2$); ^{13}C NMR (100.5 MHz, DMSO-d_6, 363 K, δ ppm) δ 153.6 (CO), 110.1 (*C*(CH$_3$)$_2$), 81.4 (C-3 and C-4), 78.7 (*C*(CH$_3$)$_3$), 65.9 (C-2 and C-5), 61.1 (2 CH_2OH), 27.8 (C(*C*H$_3$)$_3$), 26.8, 24.9 (C(*C*H$_3$)$_2$); CIMS *m/z* 304 [46%, $(M+H)^+$], *m/z* 248 [100%, $(M\text{-}Bu^t+2H)^+$]; *m/z* 204 [55%, $(M\text{-}Boc+2H)^+$].

***meso*-(2*S*,3*S*,4*R*,5*R*)-2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine hydrochloride (16).**[19] Diol **15** (7 mg, 0.022 mmol) was dissolved in THF (0.5 ml)-HCl 1M (0.5 ml) and the mixture was stirred at 90 °C for 6 h. The solvent was evaporated and the excess HCl removed *in vacuo* to give **16** (4 mg, 100%) as a white foam.

H-L-Thyp(CMe$_2$)-Gly-OBn (17). To a solution of compound (+)-**4** (135 mg, 0.428 mmol) in dry DMF (3 ml), glycine benzyl ester *p*-toluenesulfonate (181 mg, 0.535 mmol), diisopropylethylamine (234 μl, 1.284 mmol) and PyBOP (274 mg, 0.535 mmol) were added. The solution was stirred for 1 h.,

then evaporated to dryness. The crude was dissolved in CH_2Cl_2, washed with saturated aqueous solution of citric acid and brine, and the organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The corresponding resdiue was purified by flash chromatography using ether as eluent to give Boc-L-Thyp(CMe_2)-Gly-OBn (159 mg, 0.34 mmol, 80% yield) as a white solid. Dipeptide Boc-L-Thyp(CMe_2)-Gly-OBn was dissolved in TFA (20%)-DCM (4 ml) and the mixture was stirred for 30 min. Then, the solution was concentrated to dryness and the crude co-evaporated with Et_3N. The corresponding residue was purified by flash chromatography (CH_2Cl_2:MeOH, 30:1) to give **17** (97 mg, 0.267 mmol, 78% yield) as a colorless oil. $[\alpha]_D$ +6.6 (*c* 1.25, CH_3OH), $[\alpha]_{577}$ +5.8, $[\alpha]_{546}$ +7.5, $[\alpha]_{435}$ +12.6, $[\alpha]_{405}$ +13.7; IR ν_{max} 3337, 2987, 2935, 1748, 1654, 1522, 1382, 1211 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, 298 K, δ ppm, *J* Hz) δ 7.41-7.35 (m, 5 H, H-aromat.), 5.21 (d, 1 H, $^2J_{H,H}$ = 12.2, C*H*$_2$Ph), 5.18 (d, 1 H, C*H*$_2$Ph), 4.84 (dd, 1 H, $J_{3,4}$ = 5.8, $J_{2,3}$ = 2.4, H-3), 4.50 (dd, 1 H, $J_{4,5}$ = 2.0, H-4), 4.10 (d, 1 H, $^2J_{H,H}$ = 17.7, H-1'a), 3.97 (d, 1 H, H-1'b), 3.80 (d, 1 H, H-2), 3.50 (d, 2 H, $J_{5,6}$ = 6.0, H-6a and H-6b), 3.37 (td, 1 H, H-5), 1.51, 1.33 (2 s, 3 H each, (C(C*H*$_3$)$_2$); ^{13}C NMR (100.5 MHz, CD_3OD, 298 K, δ ppm) δ 176.8 (COOBn), 172.1 (CONHR), 138.0, 130.5, 130.3, 130.2 (6 C, C-aromat.), 114.3 (*C*(CH_3)$_2$), 87.0 (C-3), 85.4 (C-4), 70.1 (C-2), 68.9 (*C*H$_2$Ph), 68.3 (C-5), 64.6 (C-6), 42.8 (C-1'), 28.2, 25.9 ((*C*H$_3$)$_2$C); CIMS *m/z* 365 [100%, $(M+H)^+$]; HRCIMS *m/z* found 365.1719, calcd for $C_{18}H_{24}N_2O_6$ +H 365.1726.

H-L-Phe-L-Thyp-Gly-OBn (19). $[\alpha]_D$ +36.2 (*c* 0.65, CH_3OH), $[\alpha]_{577}$ +34.9, $[\alpha]_{546}$ +41.5, $[\alpha]_{435}$ +72.0, $[\alpha]_{405}$ +84.5; IR ν_{max} 3298, 2937, 1748, 1652, 1558, 1456, 1213, 1055 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, 298 K, δ ppm, *J* Hz, mixture of rotamers) *Major rotamer*: δ 7.41-7.20 (m, H-aromat.), 5.21 (s, 2 H, C*H*$_2$Ph), 4.81 (dd, 1 H, $J_{3,4}$ = 5.8, $J_{2,3}$ = 2.2, H-3), 4.70 (br. d, 1 H, H-4), 4.32 (t, 1 H, $J_{1'',2''}$ = 3.9, H-1''), 4.25 (d, 1 H, H-2), 4.10 (d, 1 H, $^2J_{H,H}$ = 17.5, H-1'a), 4.05 (d, 1 H, H-1'b), 3.88 (dd, 1 H, $^2J_{H,H}$ = 11.5, $J_{2a'',1''}$ = 4.8, H-2''a), 3.70 (dd, 1 H, $J_{2''b,1''}$ = 3.1, H-2''b), 3.67 (dd, 1 H, $J_{5,6a}$ = 8.2, $J_{5,6b}$ = 5.8, H-5), 2.99 (dd, 1 H, $J_{6a,6b}$ = 13.2, H-6a), 2.85 (dd, 1 H, H-6b), 1.28, 1.26 (2 s, 3 H each, (C(C*H*$_3$)$_2$). *Minor rotamer*: δ 7.41-7.20 (m, H-aromat.), 5.21 (s, 2 H, C*H*$_2$Ph), 4.85 (dd, 1 H, $J_{3,4}$ = 5.7, $J_{2,3}$ = 2.7, H-3), 4.68

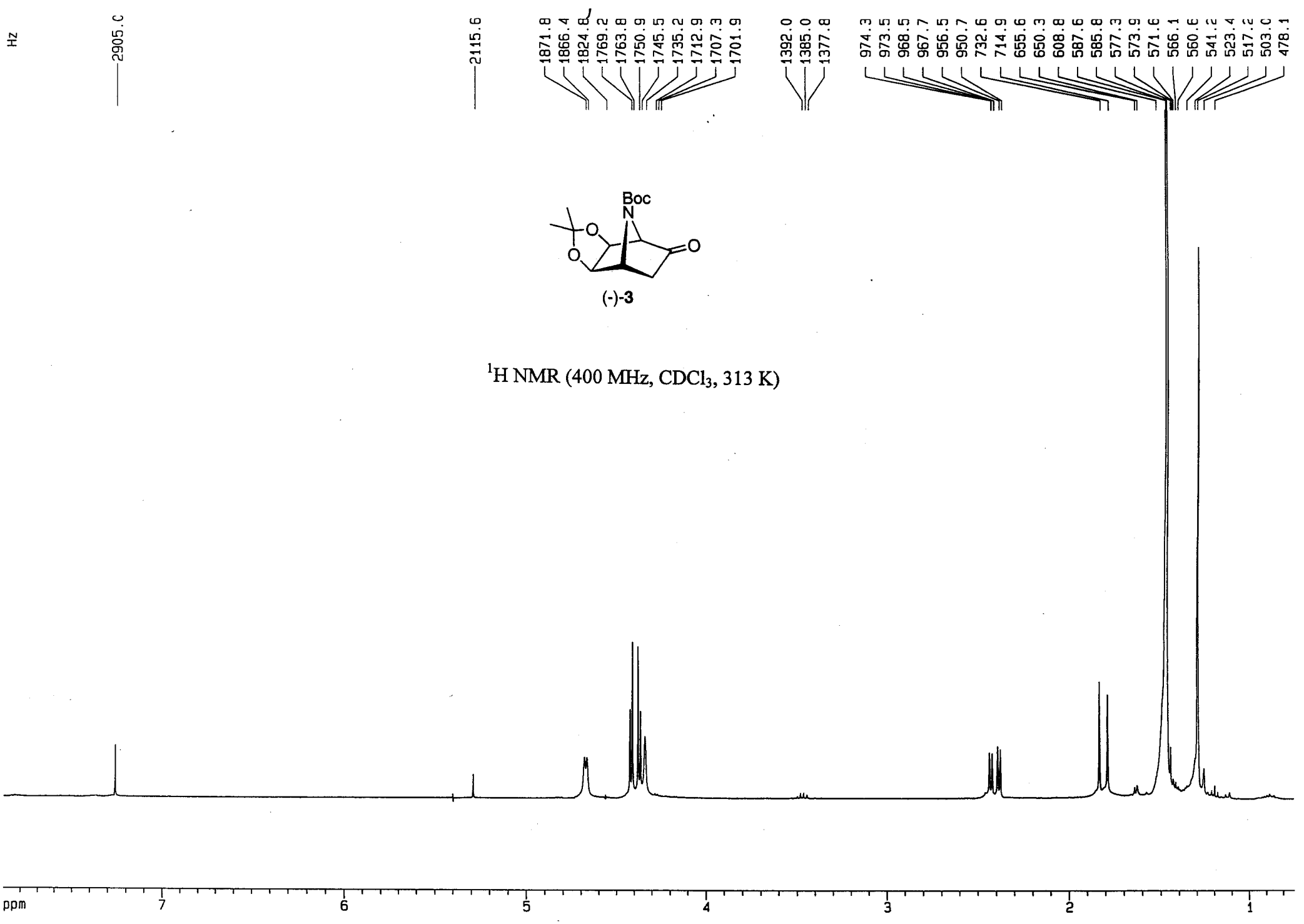
(d, 1 H, H-4), 4.66 (d, 1 H, H-2), 4.42 (t, 1 H, $J_{1'',2''}$ = 4.9, H-1''), 4.06 (d, 1 H, $^{2}J_{H,H}$ = 17.6, H-1'a), 3.99 (d, 1 H, H-1'b), 3.91-3.89 (m, 1 H, H-5), 3.32 (dd, 1 H, H-2''a), 3.24 (dd, 1 H, $^{2}J_{H,H}$ = 11.9, $J_{2b'',1''}$ = 5.3, H-2''b), 3.14 (dd, 1 H, $J_{6a,6b}$ = 12.8, $J_{5,6a}$ = 7.4, H-6a), 2.81 (dd, 1 H, $J_{5,6b}$ = 6.5, H-6b), 1.47, 1.33 (2 s, 3 H each, (C(C*H*$_3$)$_2$).^{13}C NMR (100.5 MHz, CD_3OD, 298 K, δ ppm, mixture of rotamers) *Major rotamer*: δ 177.3 (COOBn), 174.6 (CONR'R''), 171.8 (CONHR), 139.7-128.7 (12 C, C-aromat.), 114.2 (*C*(CH$_3$)$_2$), 85.8 (C-3), 83.3 (C-4), 70.9 (C-2), 68.9 (COO*C*H$_2$Ph), 68.3 (C-1''), 62.3 (C-2''), 56.3 (C-5), 43.1 (C-1'), 42.6 (C-6), 28.5, 26.2 ((*C*H$_3$)$_2$C). *Minor rotamer*: δ 177.6 (COOBn), 174.3 (CONR'R''), 171.6 (CONHR), 139.0-128.7 (12 C, C-aromat.), 114.2 (*C*(CH$_3$)$_2$), 84.9 (C-4), 83.8 (C-3), 70.4 (C-2), 68.9 (COO*C*H$_2$Ph), 67.9 (C-1''), 64.3 (C-2''), 55.9 (C-5), 43.7 (C-1'), 43.1 (C-6), 28.4, 26.2 ((*C*H$_3$)$_2$C); CIMS *m/z* 512 [100%, $(M+H)^+$]; HRCIMS *m/z* found 512.2401, calcd for $C_{27}H_{33}N_3O_7$ +H 512.2397.

H-D-Thyp-L-Val-OBn (20). To a solution of compound (-)-**4** (55 mg, 0.173 mmol) in dry DMF (1.5 ml), L-valine benzyl ester *p*-toluenesulfonate (84 mg, 0.223 mmol), diisopropylethylamine (93 μl, 0.51 mmol) and PyAOP (117 mg, 0.225 mmol) were added. The solution was stirred for 1 h., then evaporated to dryness. The crude was dissolved in CH_2Cl_2, washed with saturated aqueous solution of citric acid and brine, and the organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography using ether as eluent to give Boc-D-Thyp(CMe$_2$)-L-Val-OBn (65 mg, 0.128 mmol, 74% yield) as a white solid. Dipeptide Boc-D-Thyp-L-Val-OBn was dissolved in TFA (80%)-H_2O (3 ml) and the mixture was stirred for 2 h. Then, the solution was concentrated to dryness and the corresponding residue was purified by flash chromatography (CH_2Cl_2:MeOH, 30:1) to give **20** (55 mg, 89% yield) as a colorless oil. $[\alpha]_D$ -7 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD, 298 K, δ ppm, *J* Hz) δ 7.38-7.29 (m, 5 H, H-aromat.), 5.21 (d, 1 H, $^{2}J_{H,H}$ = 12.2, C*H*$_2$Ph), 5.15 (d, 1 H, C*H*$_2$Ph), 4.46 (d, 1 H, $J_{1',2'}$ = 5.61, H-1'), 4.26 (d, 1 H, $J_{2,3}$ = 4.4, H-2), 4.23 (t, 1 H, $J_{3,4}$ = 4.2, H-3), 4.01 (dd, 1 H, $J_{4,5}$ = 6.4, H-4), 3.87 (dd, 1 H, $J_{6a,6b}$ = 11.9, $J_{5,6a}$ = 4.0, H-6a), 3.81 (dd, 1 H, $J_{5,6b}$ = 5.9, H-6b), 2.22 (m, 1 H, H-2'), 0.92 (d, 1 H, $^{3}J_{H,H}$ = 5.64, CH_3), 0.89 (d, 1 H, $^{3}J_{H,H}$ = 5.64, CH_3); ^{13}C NMR (75.4 MHz,

CD_3OD, 298 K, δ ppm) δ 172.4 (COOBn), 168.7 (CONHR), 137.0, 129.6, 129.5 (6 C, C-aromat.), 76.0 (C-3), 72.7 (C-4), 68.1 (*C*H_2Ph), 65.6 (C-2), 64.9 (C-5), 59.7 (C-1'), 59.5 (C-6), 31.9 (C-2'), 19.5, 18.1 (2 CH_3) ; FABMS *m/z* 367 [40%, $(M+H)^+$]; HRFABMS *m/z* 367.1879, calcd for $C_{18}H_{26}N_2O_6$ +H 367.1869.

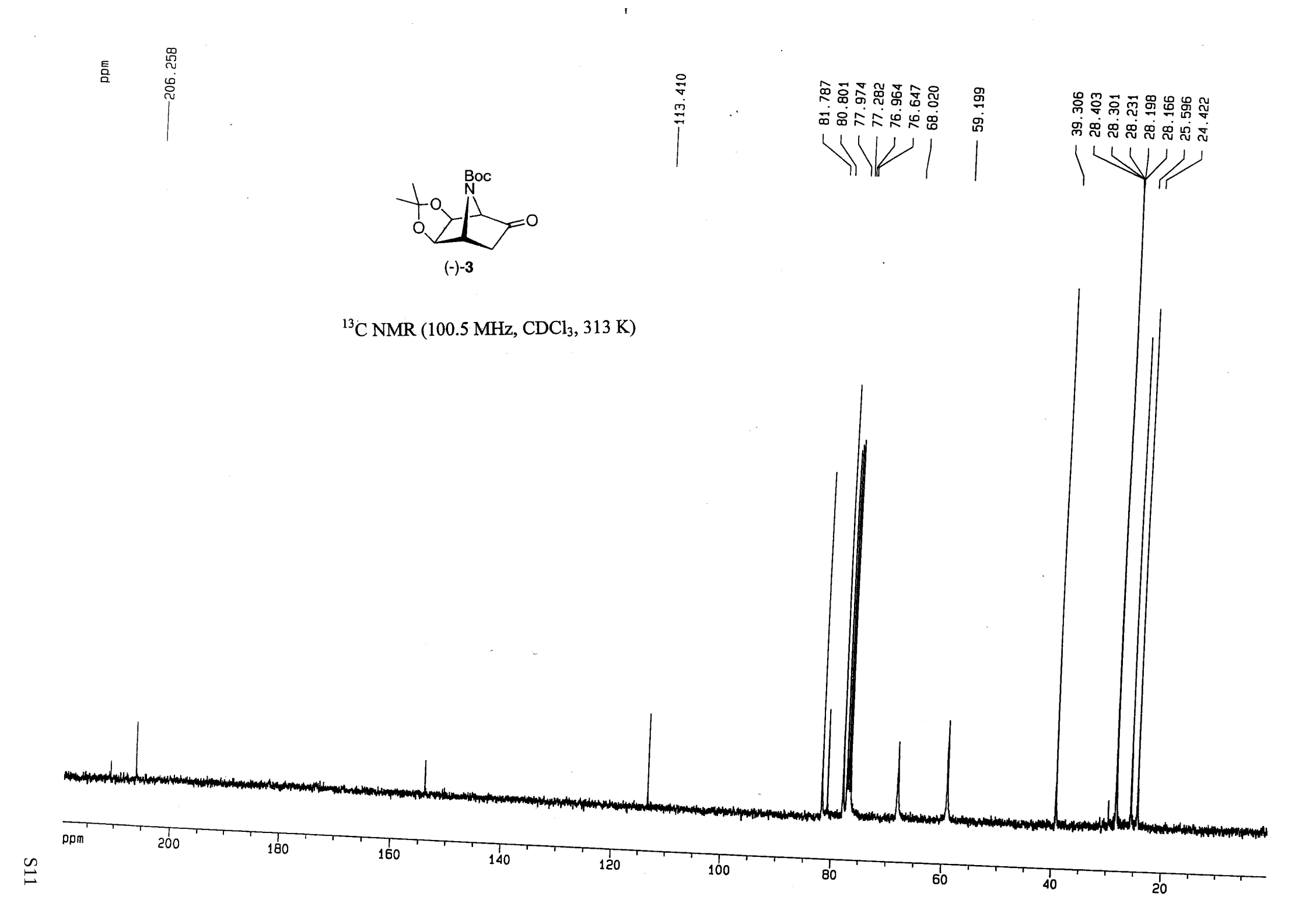
Fmoc-L-Ala-D-Thyp-L-Val-OBn (21). To a solution of compound **20** (33 mg, 0.069 mmol) in dry DMF (1.5 ml), Fmoc-L-alanine (25 mg, 0.082 mmol), diisopropylethylamine (37 μl, 0.21 mmol) and PyAOP (44 mg, 0.082 mmol) were added. The solution was stirred for 1 h., then evaporated to dryness. The crude was dissolved in CH_2Cl_2, washed with saturated aqueous solution of citric acid and brine, and the organic layer was dried over $MgSO_4$ and concentrated. The corresponding residue was purified by flash chromatography (CH_2Cl_2:MeOH, 30:1) to give **21** (33 mg, 70% yield) as a white solid. Compound **21** was characterized as the unprotected tripeptide **22**.

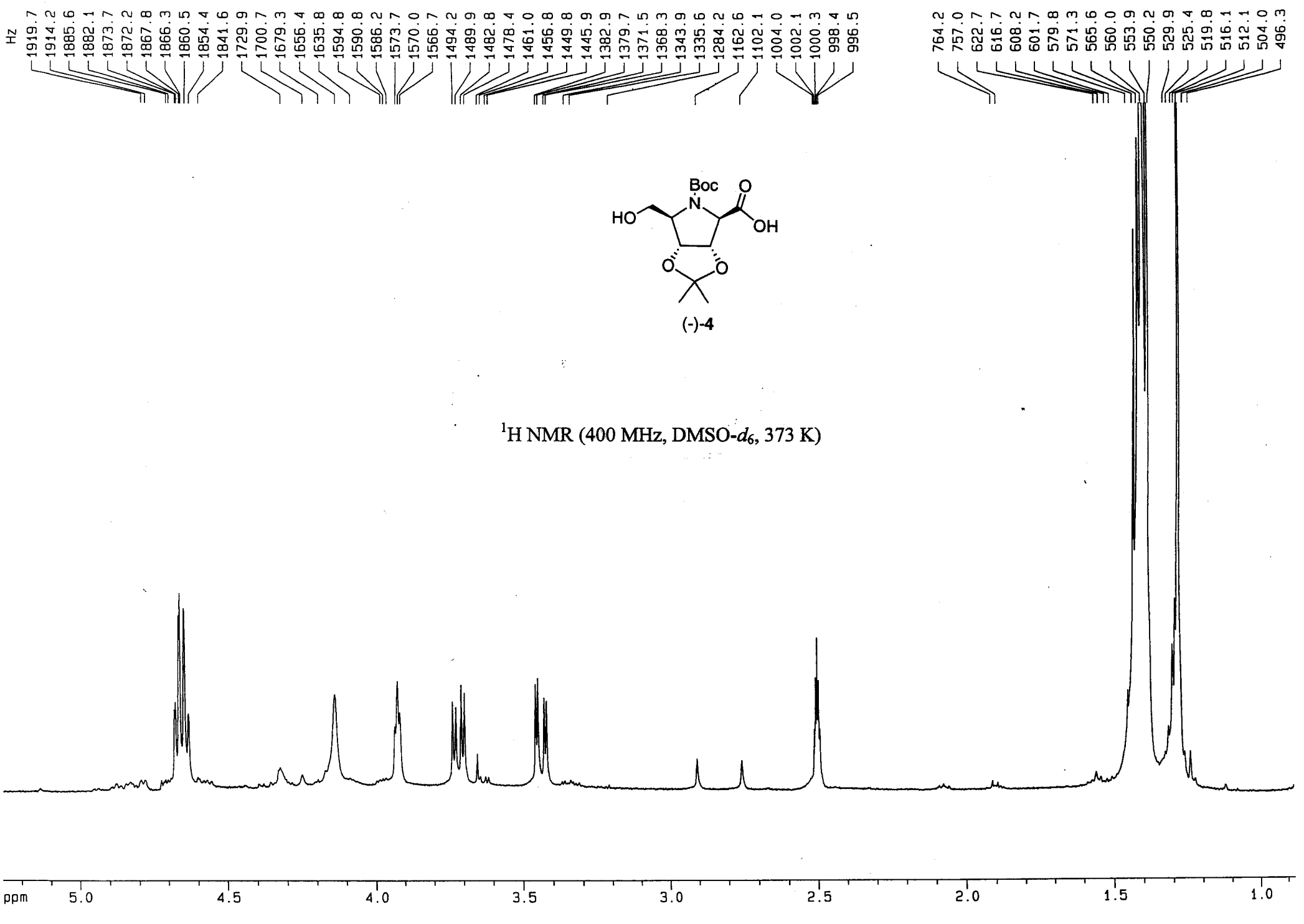
H-L-Phe-D-Thyp-L-Val-OBn (22). Tripeptide **21** (33 mg, 0.048 mmol) was dissolved in CH_2Cl_2 (3 ml) and Et_2NH (0.3 ml) was added. The mixture was stirred for 30 min, then the solution was concentrated to dryness and the crude was purified by flash chromatography (CH_2Cl_2:MeOH, 5:1) to give **22** (19 mg, 90%) as a colorless oil. $[\alpha]_D$ -11 (*c* 0.37, CH_3OH); ^{13}C NMR (100.5 MHz, CD_3OD, 298 K, δ ppm, mixture of rotamers 1:1) δ 173.8, 173.5, 172.6 (COOBn, CONHR, CONHR'R''), 137.2, 130.0, 129.6, 129.5, 129.4, 129.3 (C-aromat.), 76.6, 75.3, 74.5, 72.3 (C-3, C-4), 68.9, 67.3 (C-2), 67.9, 67.8 (*C*H_2Ph), 67.2, 66.3 (C-5), 62.5, 59.9 (C-6), 60.0, 59.3 (NH*C*H of Val), 49.6-48.3 (CH of Ala, under MeOD), 32.0, 31.2 ($CH(CH_3)_2$ of Val), 20.3, 19.2 (CH_3 of Ala), 19.6, 19.5 (CH_3 of Val), 18.7, 18.4 (CH_3 of Val); FABMS *m/z* 460 [30%, $(M+Na)^+$]; HRFABMS *m/z* 460.2037, calcd for $C_{21}H_{31}N_3O_7$ +Na 460.2060.



(-)-**3**

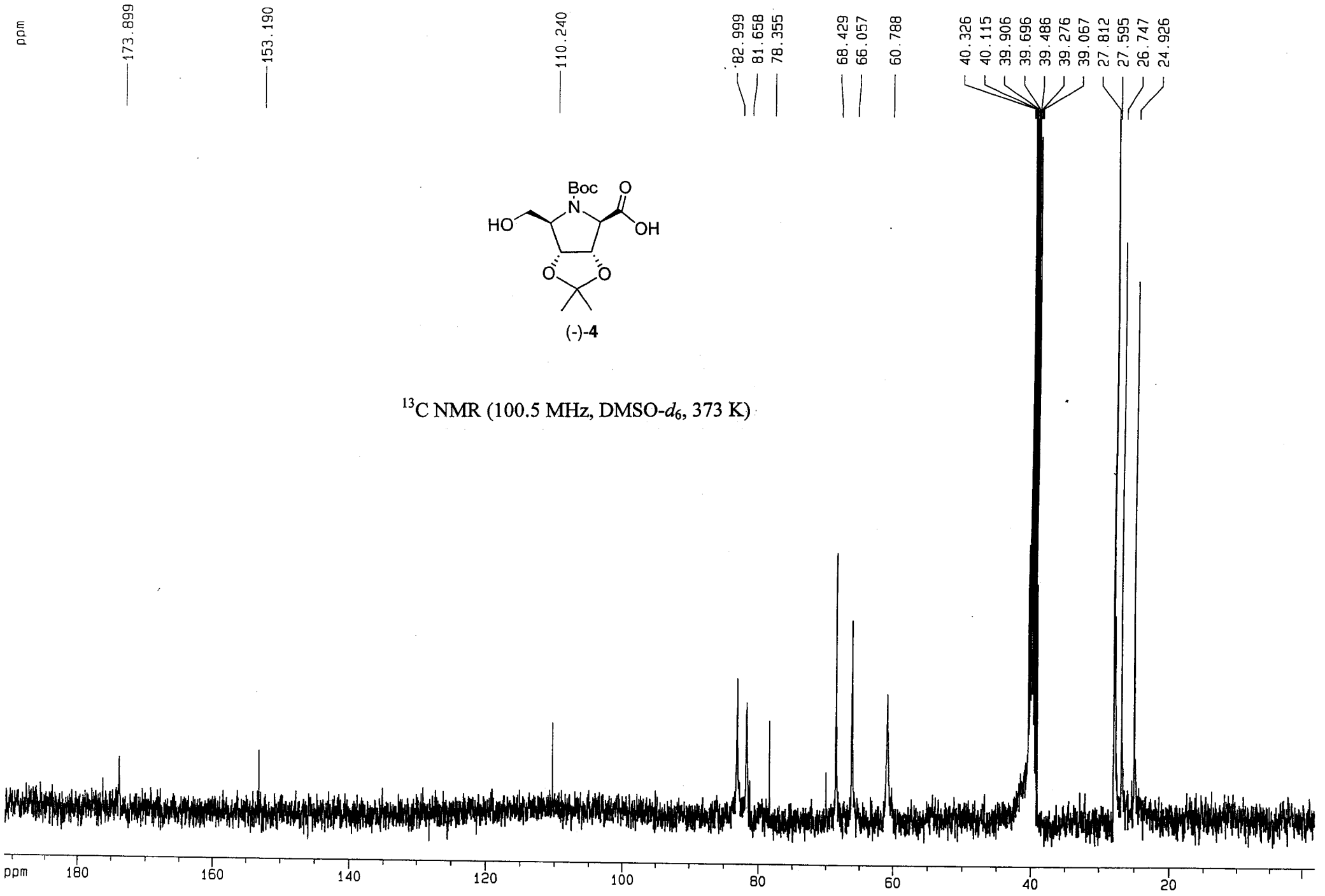
^{1}H NMR (400 MHz, $CDCl_3$, 313 K)


ppm
206.258
113.410
81.787
80.801
77.974
77.282
76.964
76.647
68.020
59.199
39.306
28.403
28.301
28.231
28.198
28.166
25.596
24.422
Boc
N
O
O
O
(-)-3
13C NMR (100.5 MHz, CDCl3, 313 K)
ppm
200
180
160
140
120
100
80
60
40
20



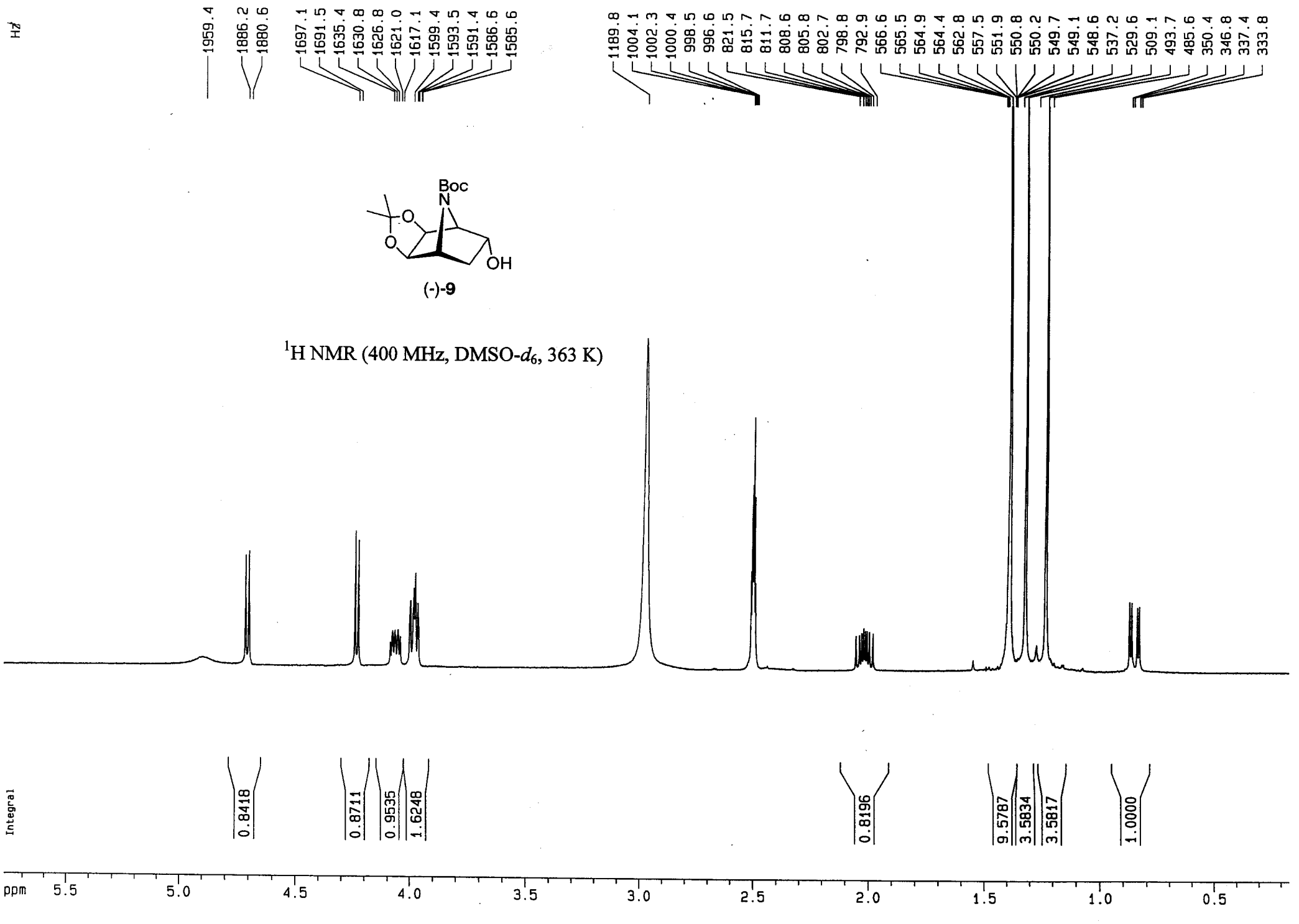
(-)-**4**

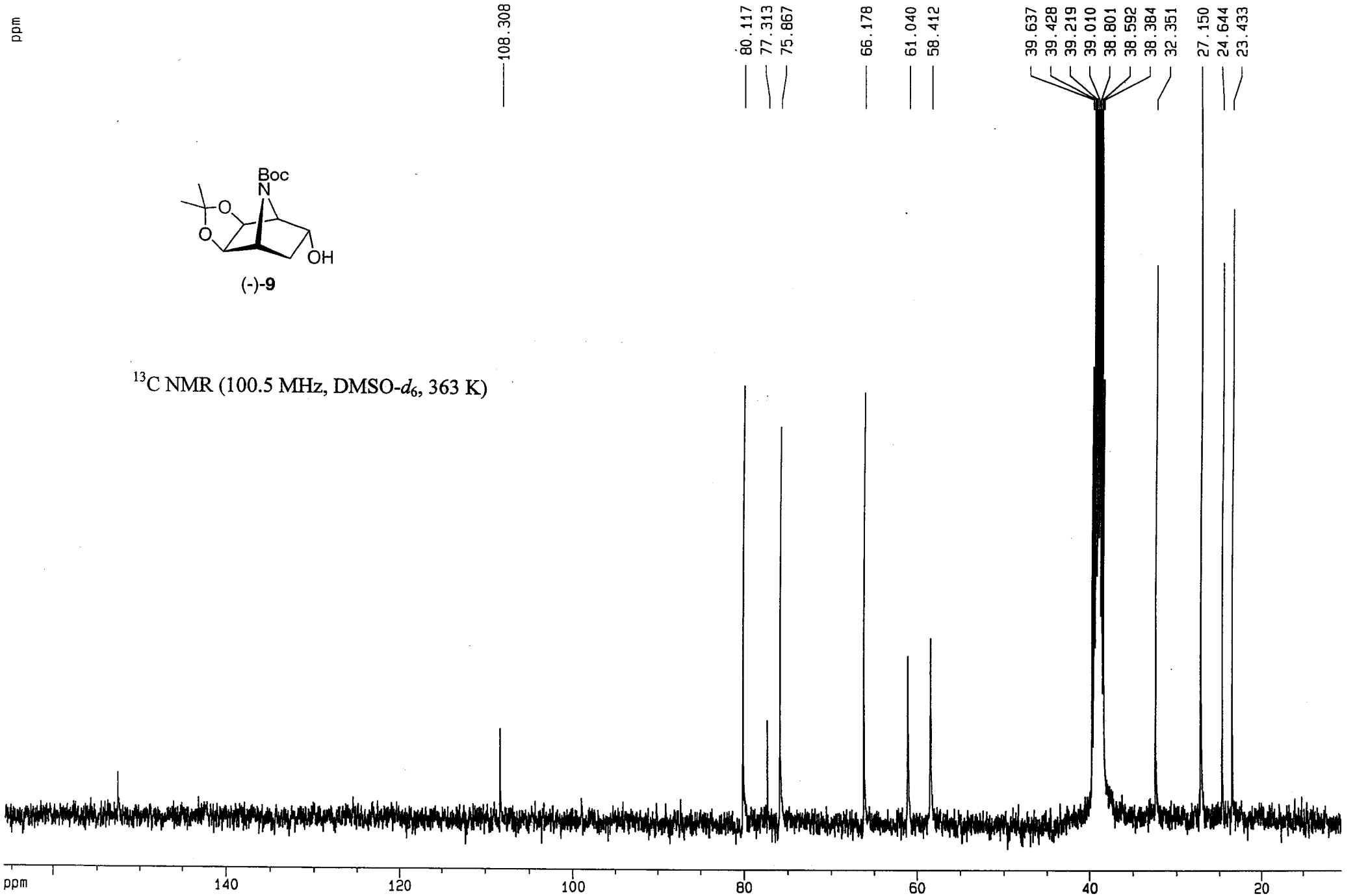
^{1}H NMR (400 MHz, DMSO-d_6, 373 K)

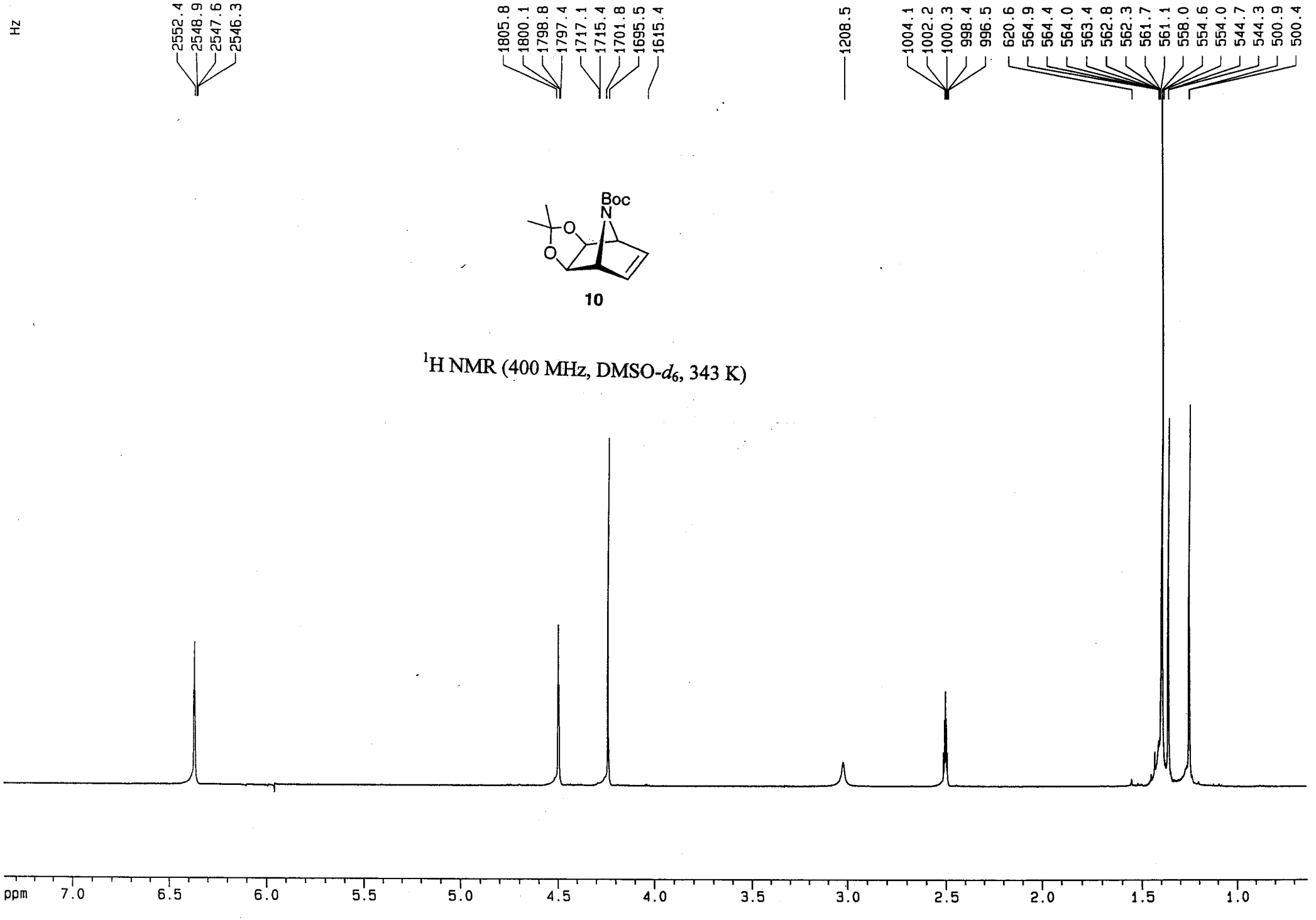


(-)-**4**

^{13}C NMR (100.5 MHz, DMSO-d_6, 373 K)

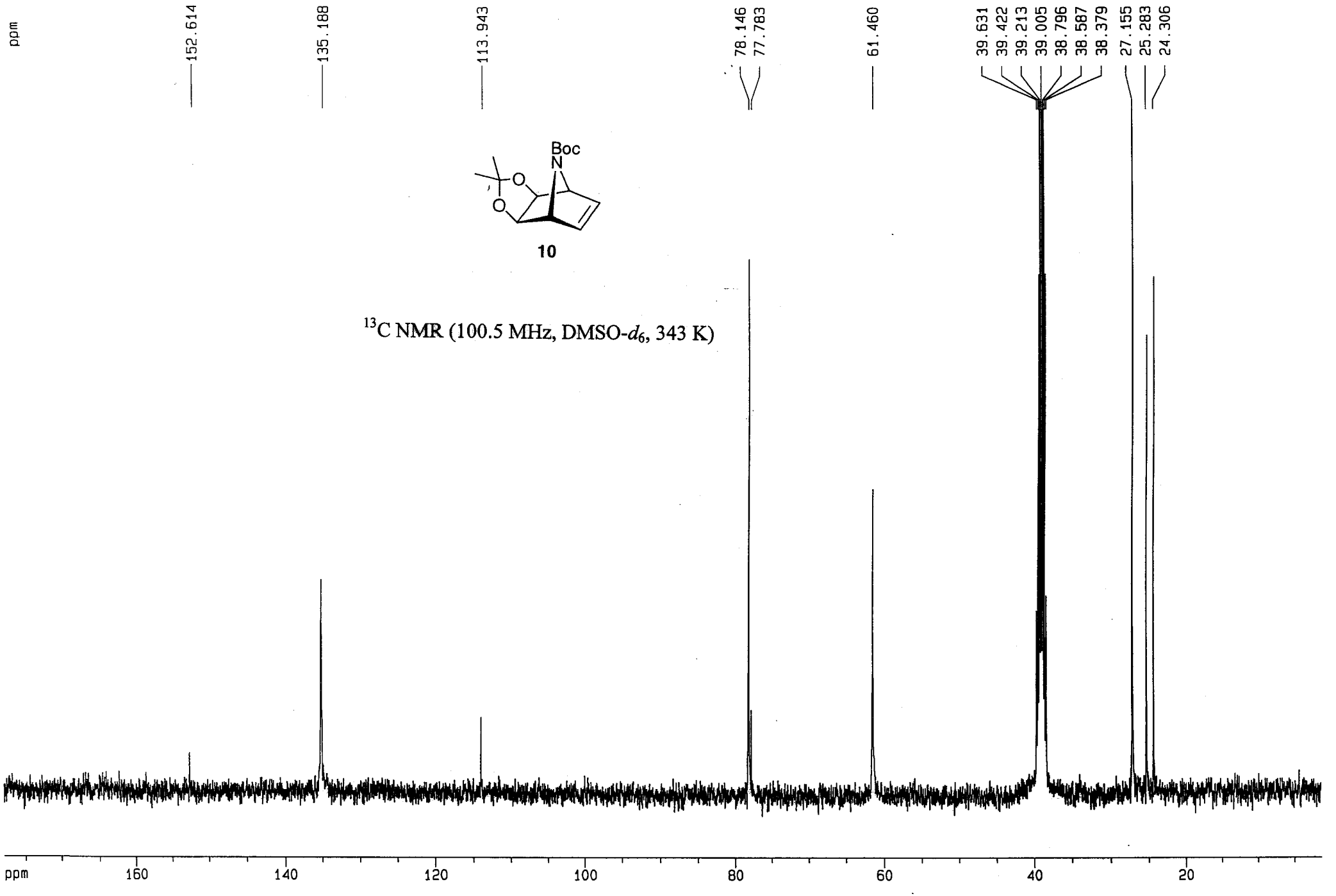

Hz
1959.4
1886.2
1880.6
1697.1
1691.5
1635.4
1630.8
1626.8
1621.0
1617.1
1599.4
1593.5
1591.4
1586.6
1585.6
1189.8
1004.1
1002.3
1000.4
998.5
996.6
821.5
815.7
811.7
808.6
805.8
802.7
798.8
792.9
566.6
565.5
564.9
564.4
562.8
557.5
551.9
550.8
550.2
549.7
549.1
548.6
537.2
529.6
509.1
493.7
485.6
350.4
346.8
337.4
333.8
Boc
N
O
O
OH
(-)-9
^{1}H NMR (400 MHz, DMSO-d_6, 363 K)
Integral
0.8418
0.8711
0.9535
1.6248
0.8196
9.5787
3.5834
3.5817
1.0000
ppm
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

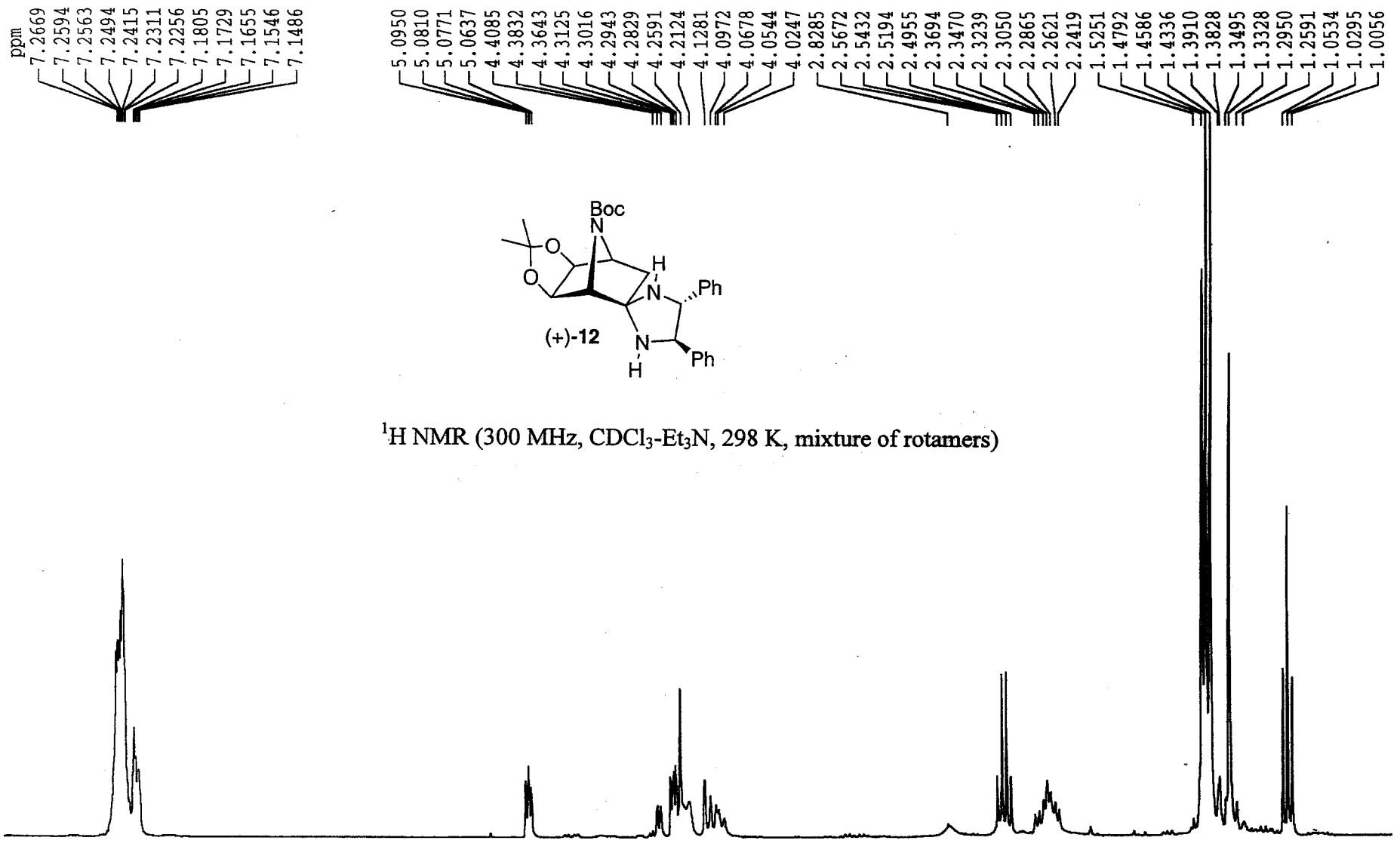

ppm
108.308
80.117
77.313
75.867
66.178
61.040
58.412
39.637
39.428
39.219
39.010
38.801
38.592
38.384
32.351
27.150
24.644
23.433
Boc
N
O
O
OH
(-)-9
^{13}C NMR (100.5 MHz, DMSO-d_6, 363 K)
ppm
140
120
100
80
60
40
20



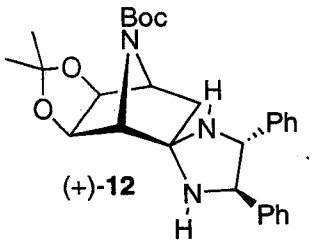
10

^{1}H NMR (400 MHz, DMSO-d_6, 343 K)


ppm
152.614
135.188
113.943
78.146
77.783
61.460
39.631
39.422
39.213
39.005
38.796
38.587
38.379
27.155
25.283
24.306
Boc
N
O
O
10
^{13}C NMR (100.5 MHz, DMSO-d_6, 343 K)
ppm
160
140
120
100
80
60
40
20

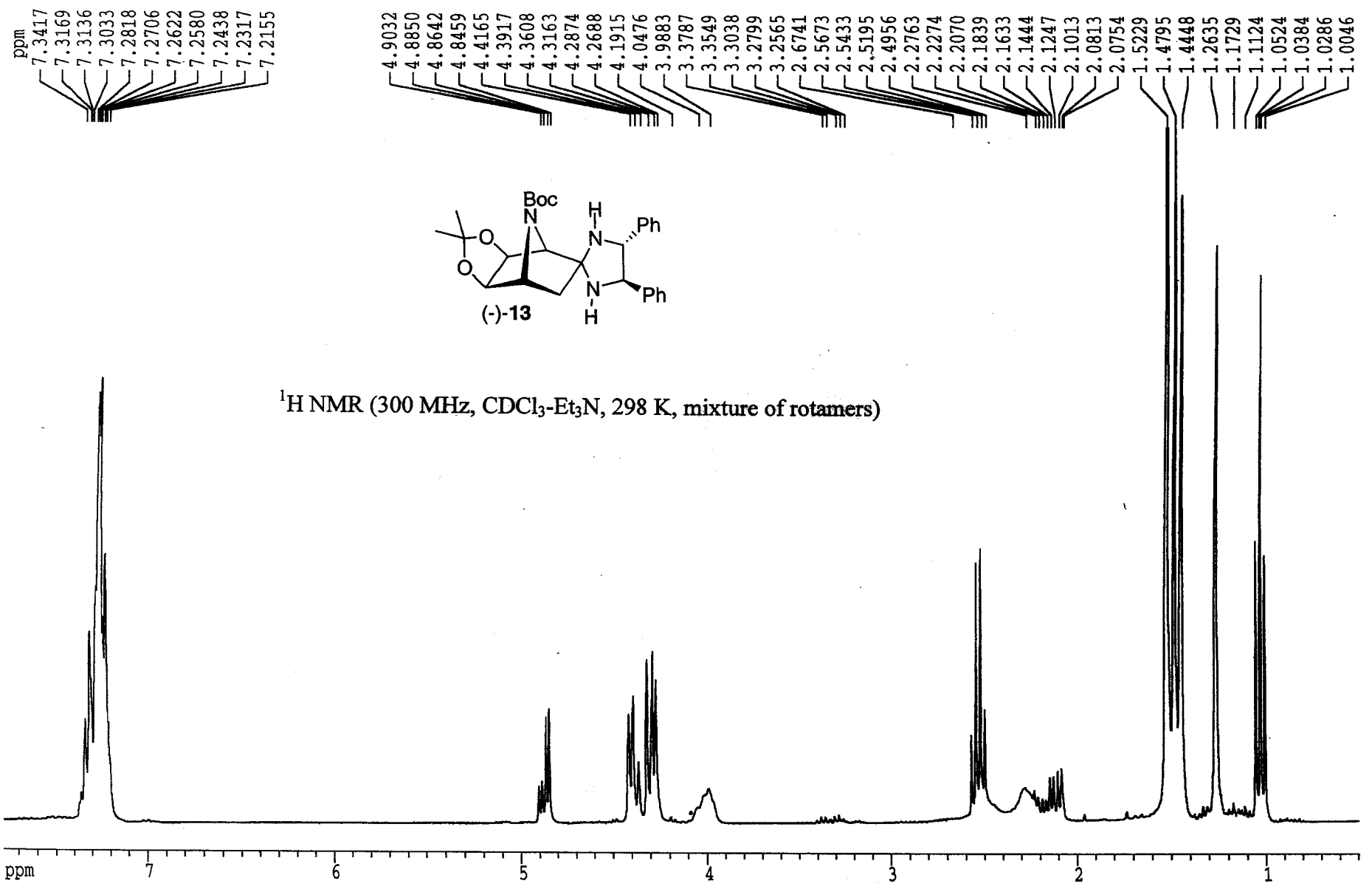


^{1}H NMR (300 MHz, $CDCl_3$-Et_3N, 298 K, mixture of rotamers)

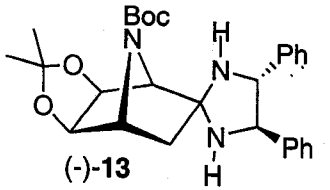


(+)-**12**

^{13}C NMR (75.4 MHz, $CDCl_3$-Et_3N, 298 K, mixture of rotamers)

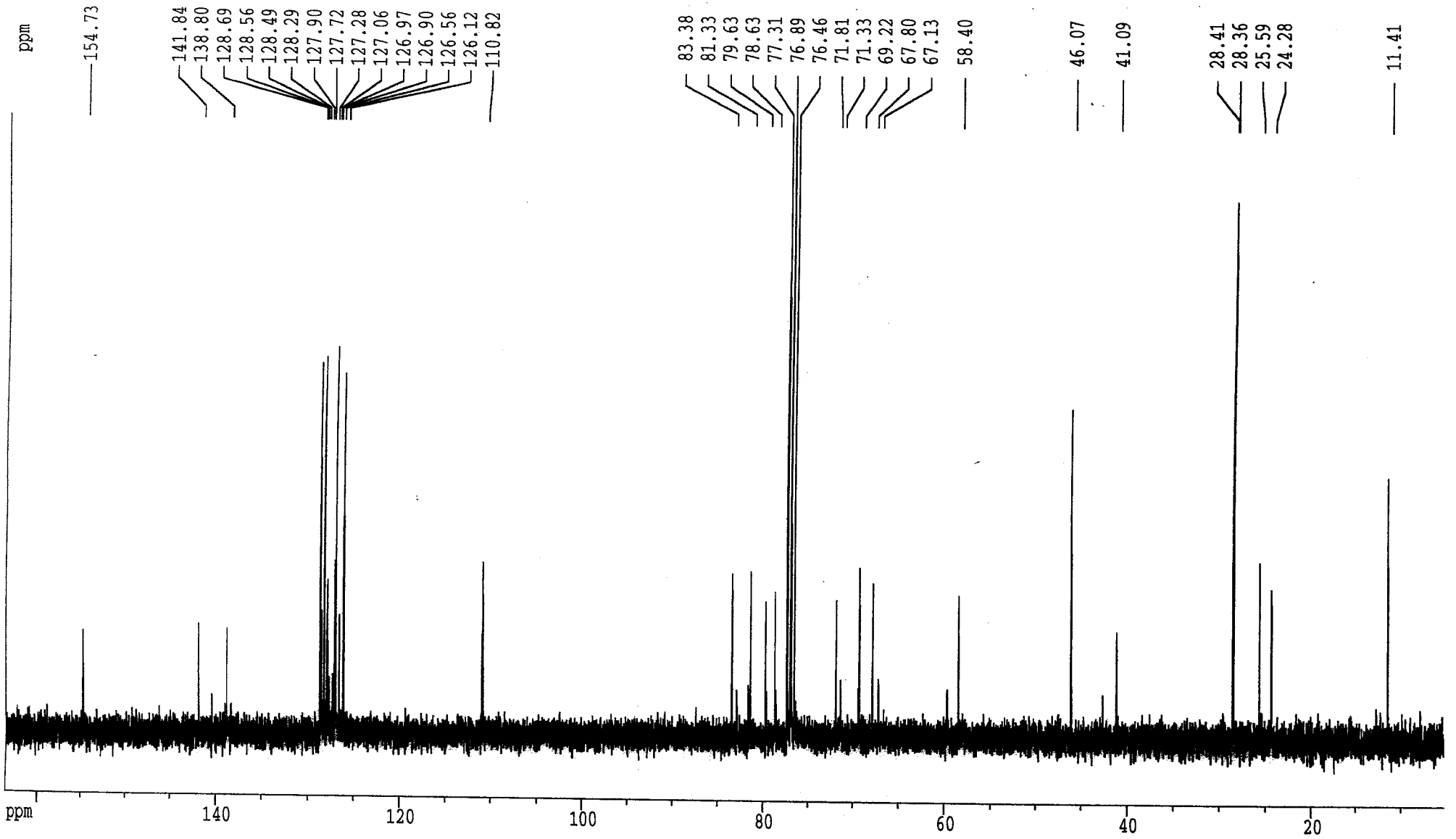


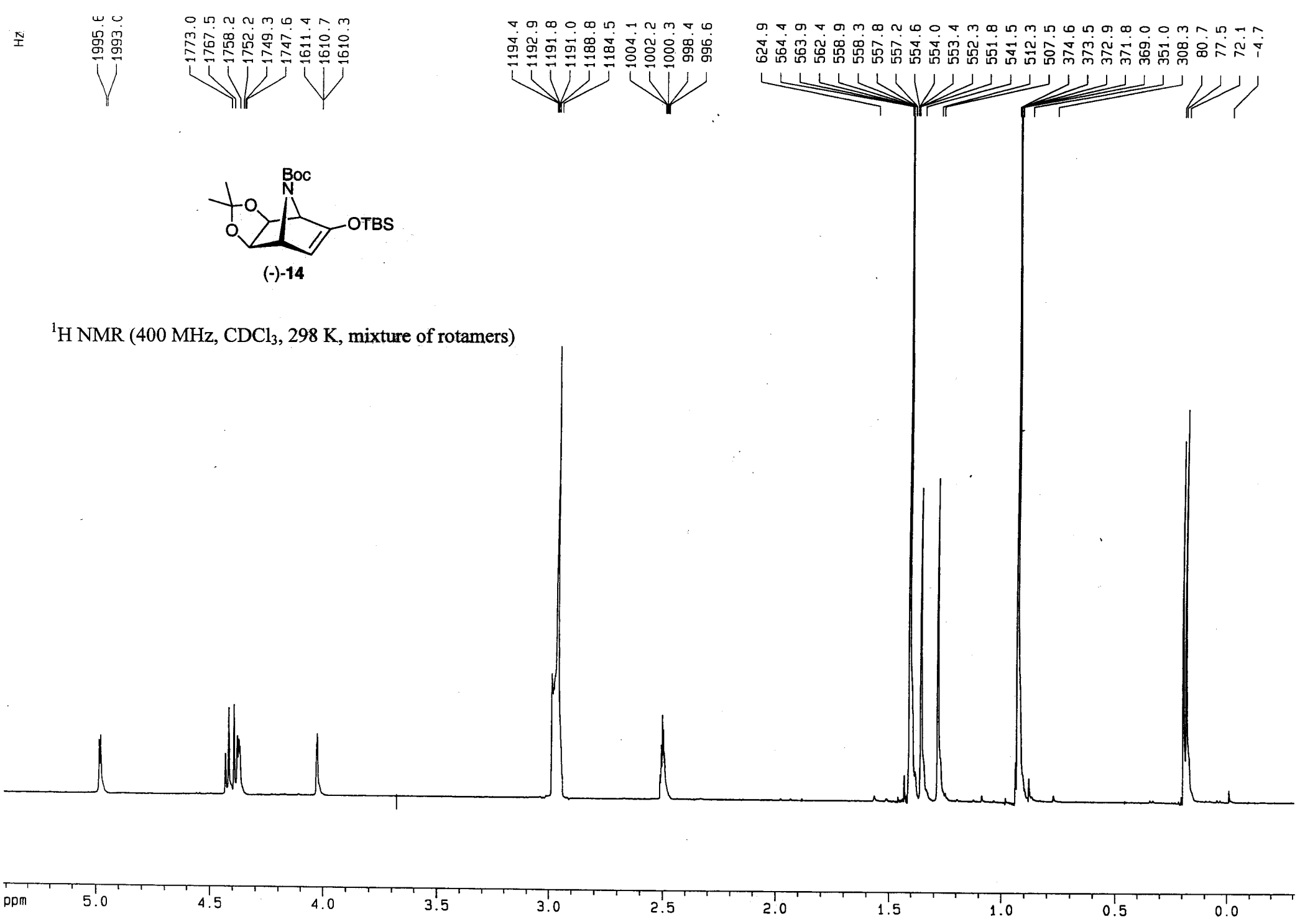
^{1}H NMR (300 MHz, $CDCl_3$-Et_3N, 298 K, mixture of rotamers)



(-)-**13**

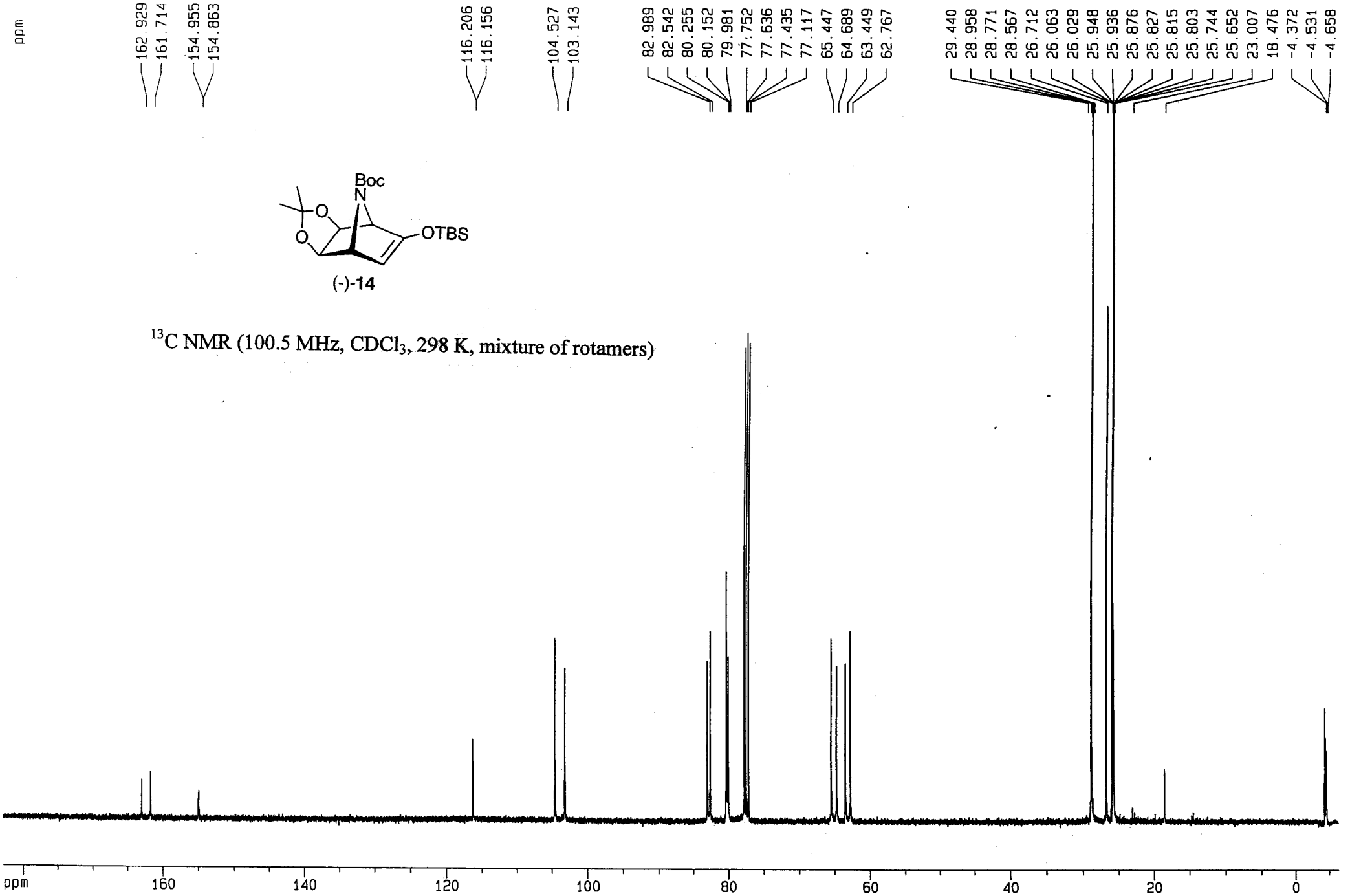
^{13}C NMR (75.4 MHz, $CDCl_3$-Et_3N, 298 K, mixture of rotamers)





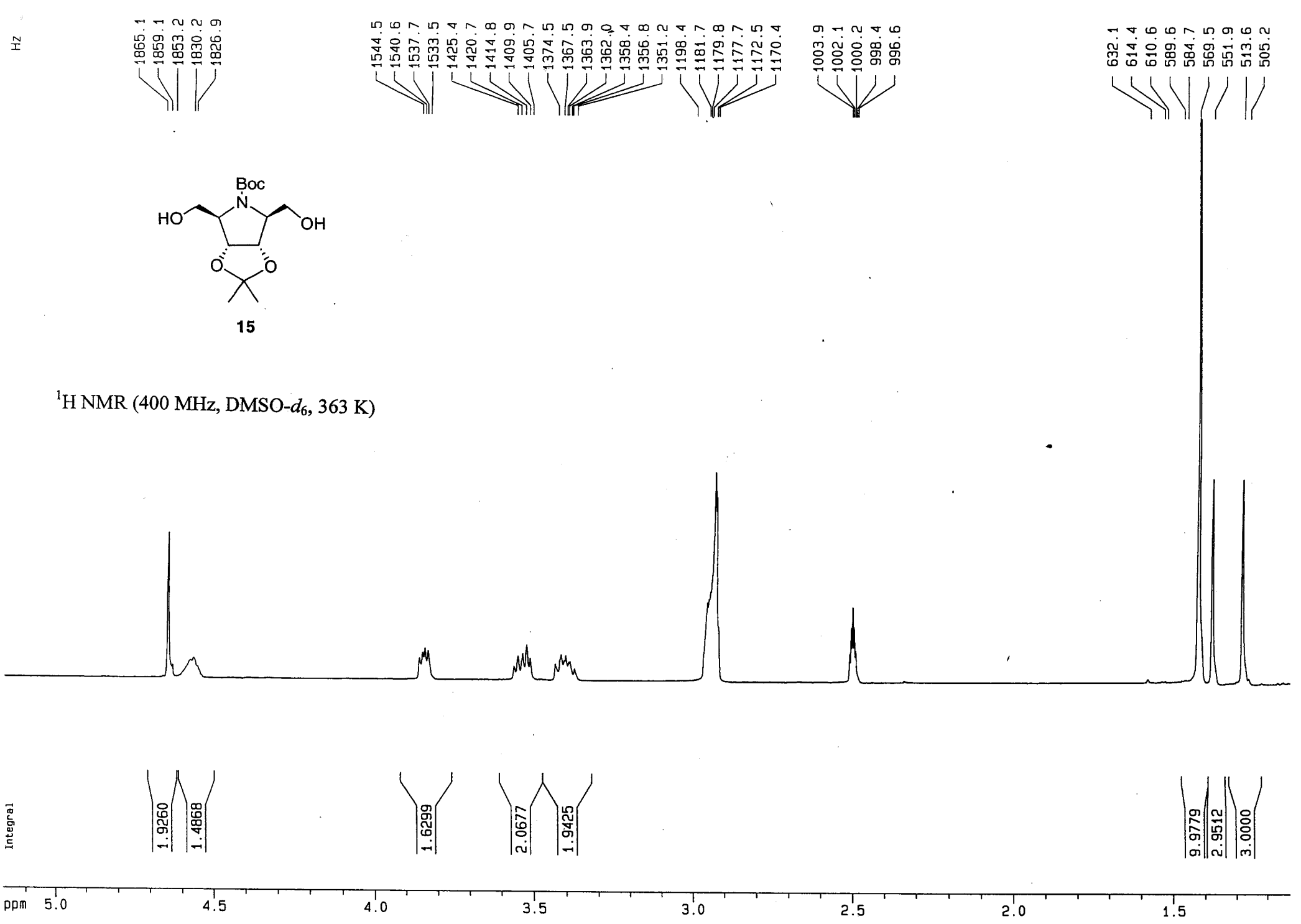
(-)-**14**

^{1}H NMR (400 MHz, $CDCl_3$, 298 K, **mixture of rotamers**)

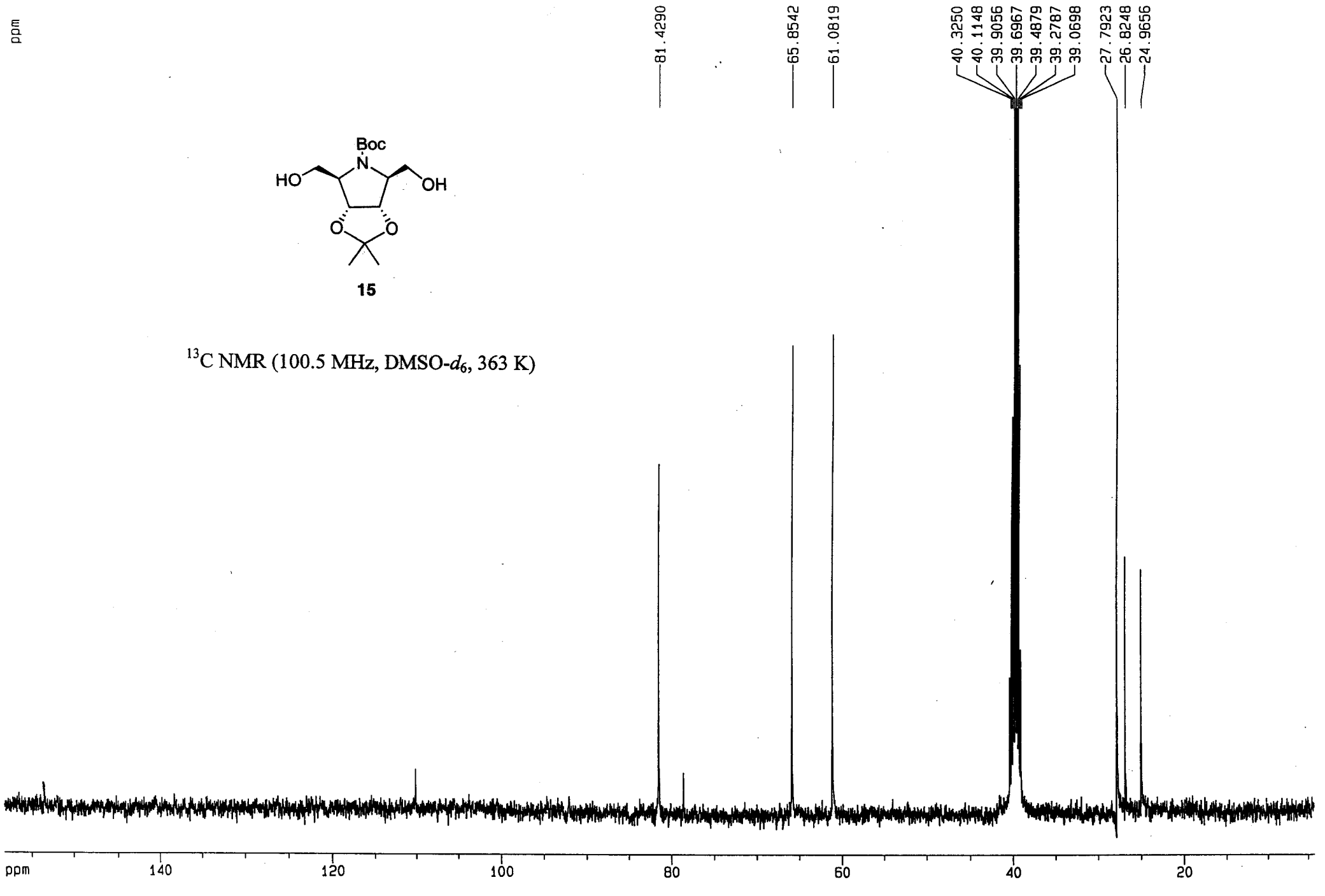


(-)-**14**

^{13}C NMR (100.5 MHz, $CDCl_3$, 298 K, mixture of rotamers)

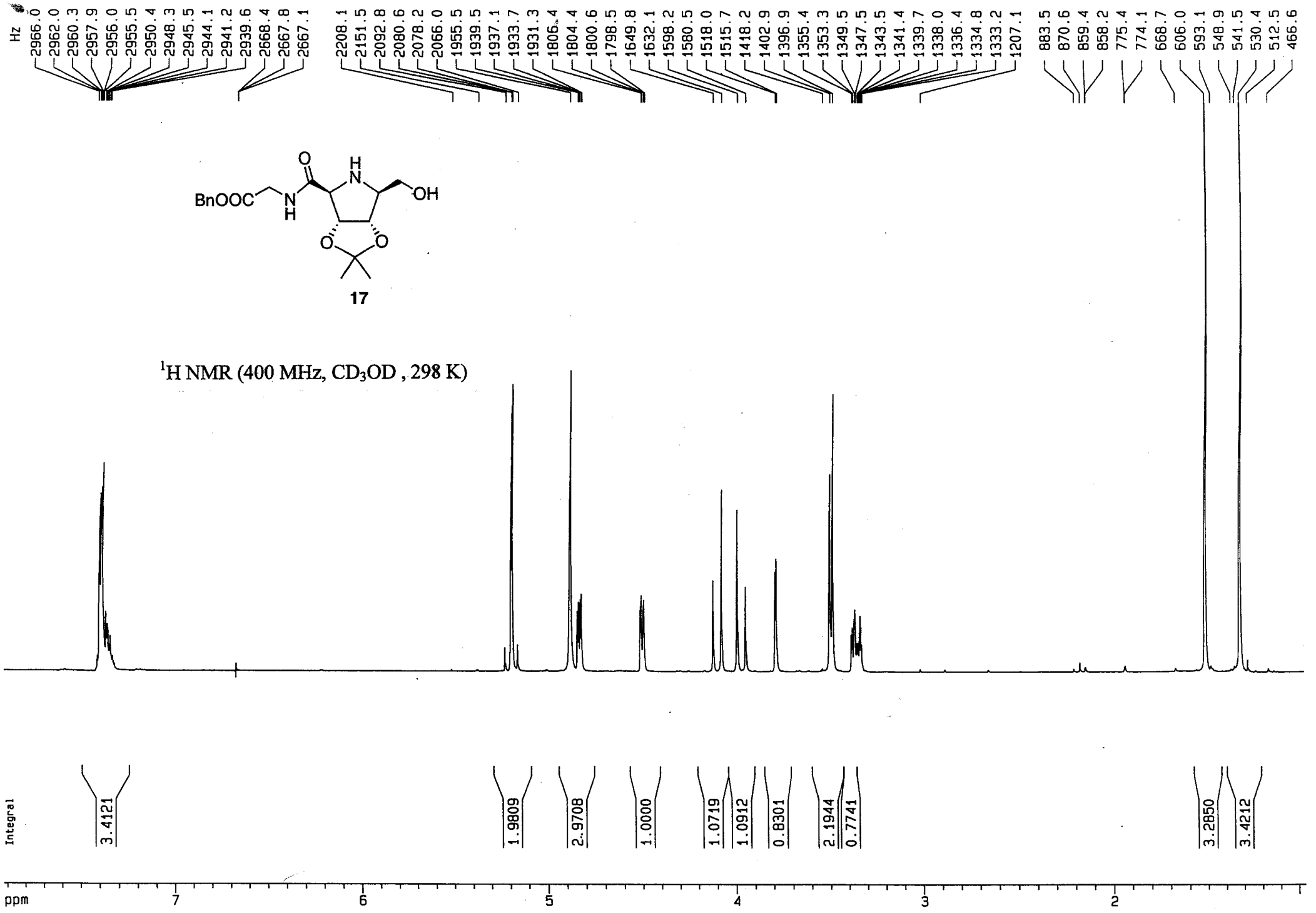


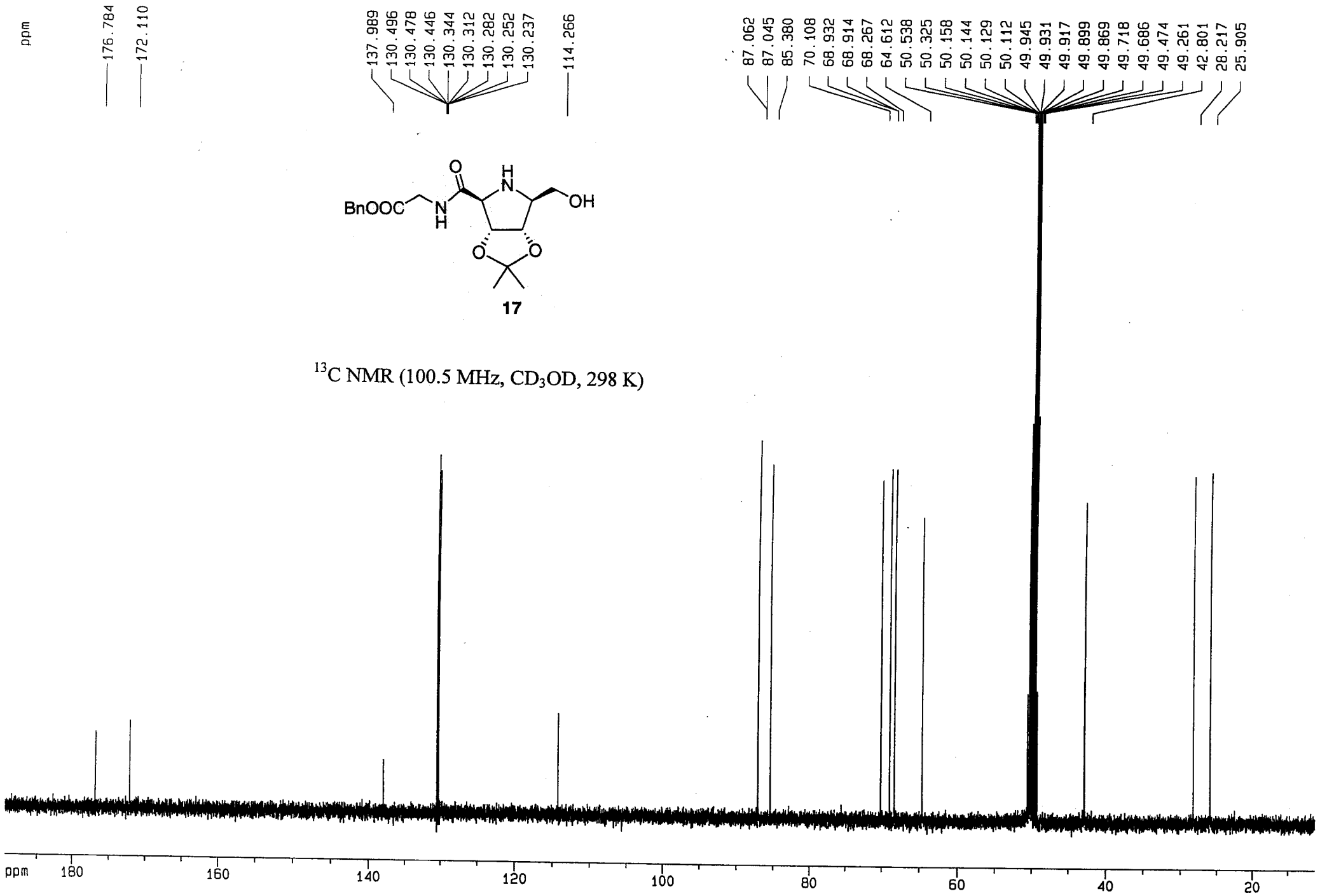
^{1}H NMR (400 MHz, DMSO-d_6, 363 K)



15

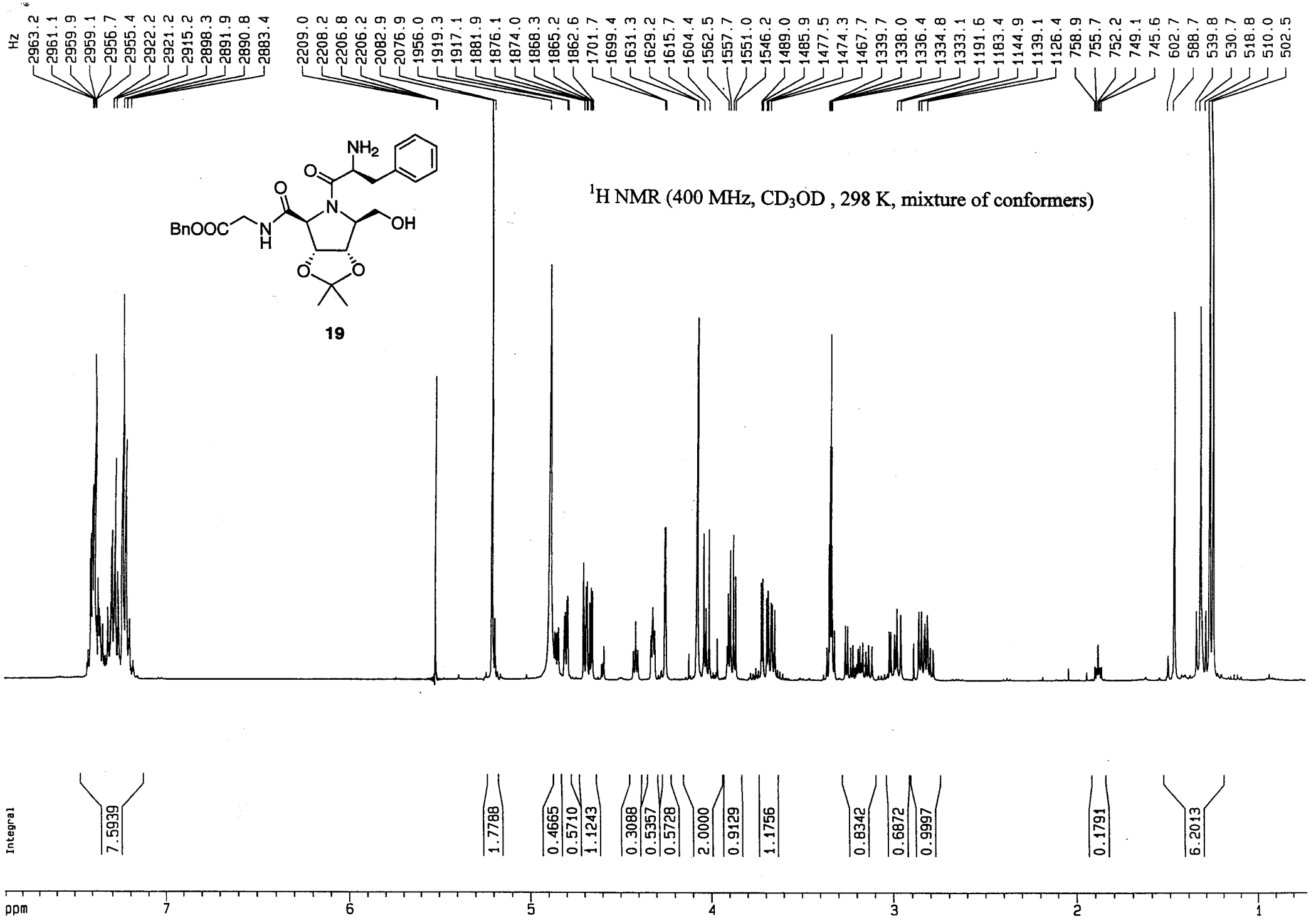
^{13}C NMR (100.5 MHz, DMSO-d_6, 363 K)

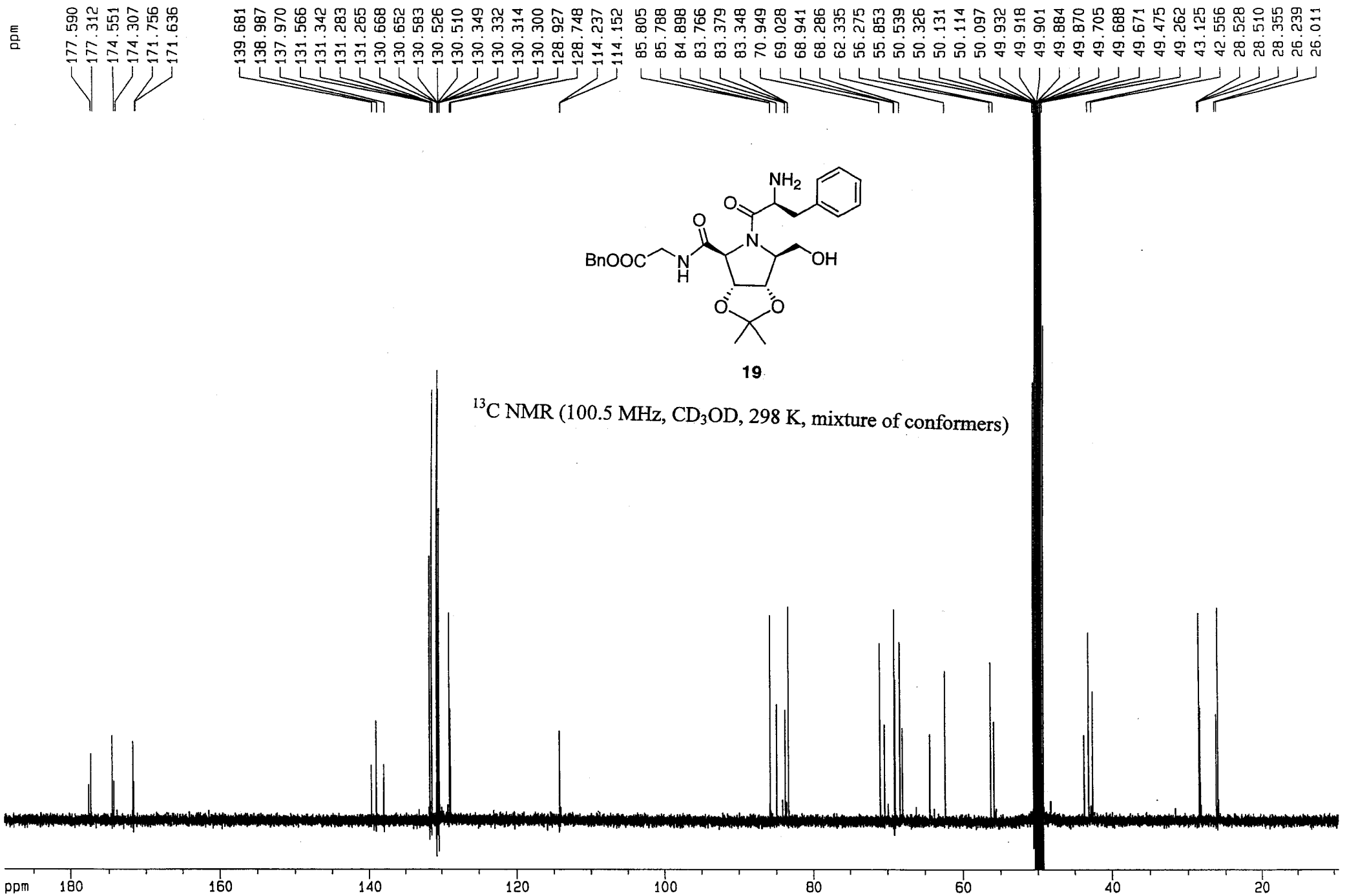

Hz
2966.0
2962.0
2960.3
2957.9
2956.0
2955.5
2950.4
2948.3
2945.5
2944.1
2941.2
2939.6
2668.4
2667.8
2667.1
2208.1
2151.5
2092.8
2080.6
2078.2
2066.0
1955.5
1939.5
1937.1
1933.7
1931.3
1806.4
1804.4
1800.6
1798.5
1649.8
1632.1
1598.2
1580.5
1518.0
1515.7
1418.2
1402.9
1396.9
1355.4
1353.3
1349.5
1347.5
1343.5
1341.4
1339.7
1338.0
1336.4
1334.8
1333.2
1207.1
883.5
870.6
859.4
858.2
775.4
774.1
668.7
606.0
593.1
548.9
541.5
530.4
512.5
466.6
BnOOC
N
H
O
H
N
OH
O
O
17
^{1}H NMR (400 MHz, CD_3OD , 298 K)
Integral
3.4121
1.9809
2.9708
1.0000
1.0719
1.0912
0.8301
2.1944
0.7741
3.2850
3.4212
ppm
7
6
5
4
3
2



17

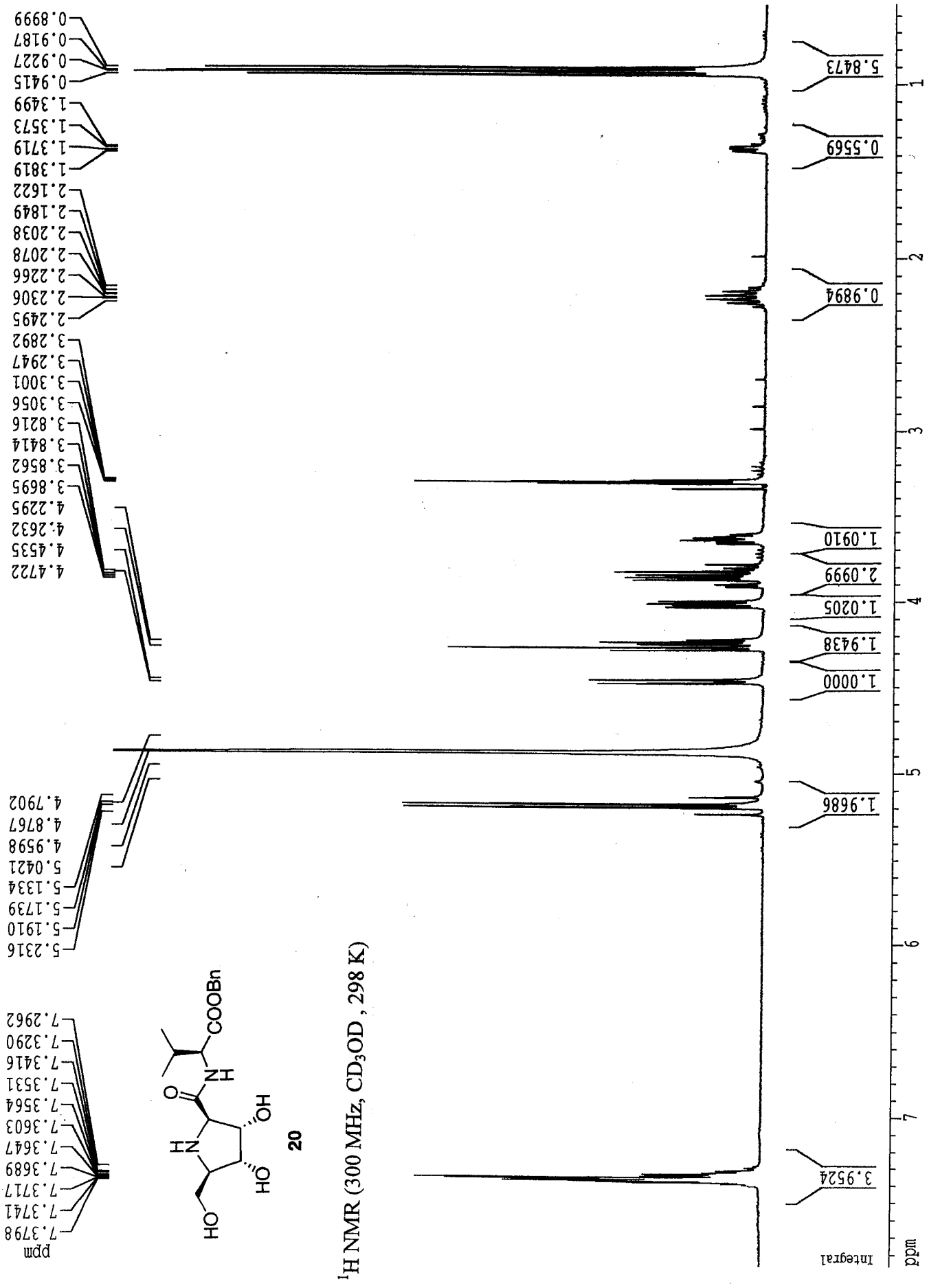
^{13}C NMR (100.5 MHz, CD_3OD, 298 K)

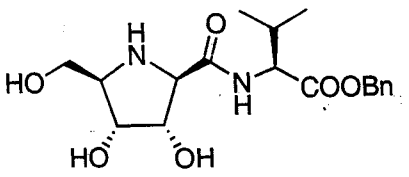

Hz
2963.2
2961.1
2959.9
2959.1
2956.7
2955.4
2922.2
2921.2
2915.2
2898.3
2891.9
2890.8
2883.4
2209.0
2208.2
2206.8
2206.2
2082.9
2076.9
1956.0
1919.3
1917.1
1881.9
1876.1
1874.0
1868.3
1865.2
1862.6
1701.7
1699.4
1631.3
1629.2
1615.7
1604.4
1562.5
1557.7
1551.0
1546.2
1489.0
1485.9
1477.5
1474.3
1467.7
1339.7
1338.0
1336.4
1334.8
1333.1
1191.6
1183.4
1144.9
1139.1
1126.4
758.9
755.7
752.2
749.1
745.6
602.7
588.7
539.8
530.7
518.8
510.0
502.5
NH2
O
O
N
OH
BnOOC
N
H
O
O
19
1H NMR (400 MHz, CD3OD , 298 K, mixture of conformers)
Integral
7.5939
1.7788
0.4665
0.5710
1.1243
0.3088
0.5357
0.5728
2.0000
0.9129
1.1756
0.8342
0.6872
0.9997
0.1791
6.2013
ppm
7
6
5
4
3
2
1



19

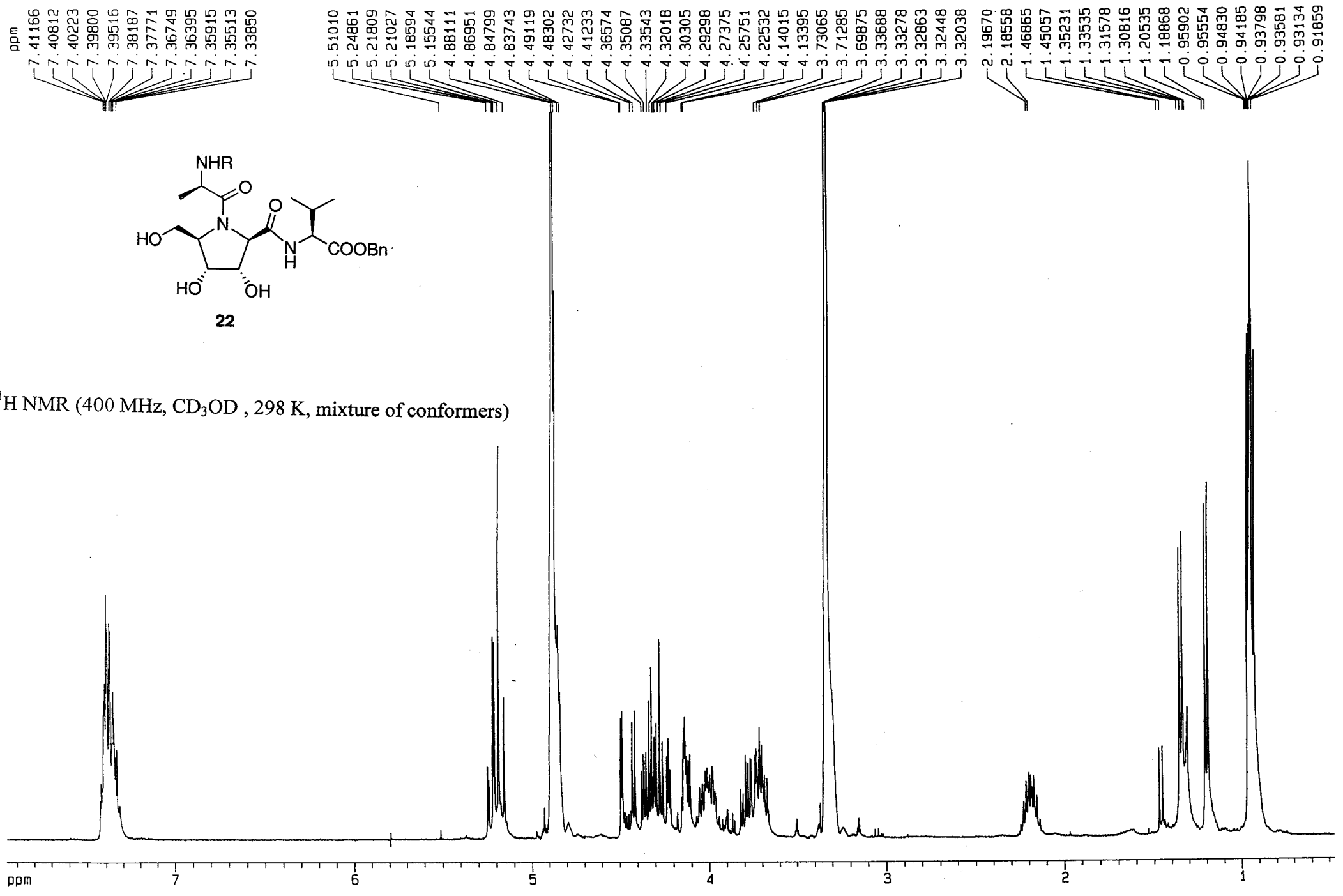
^{13}C NMR (100.5 MHz, CD_3OD, 298 K, mixture of conformers)


0.8999
0.9187
0.9227
0.9415
1.3499
1.3573
1.3719
1.3819
2.1622
2.1849
2.2038
2.2078
2.2266
2.2306
2.2495
3.2892
3.2947
3.3001
3.3056
3.8216
3.8414
3.8562
3.8695
4.2295
4.2632
4.4535
4.4722
4.7902
4.8767
4.9598
5.0421
5.1334
5.1739
5.1910
5.2316
7.2962
7.3290
7.3416
7.3531
7.3564
7.3603
7.3647
7.3689
7.3717
7.3741
7.3798
ppm
5.8473
0.5569
0.9894
1.9686
1.0910
2.0999
1.0205
1.9438
1.0000
3.9524
Integral
1
2
3
4
5
6
7
ppm
COOBn
NH
O
N
OH
HO
HO
20
^{1}H NMR (300 MHz, CD_3OD , 298 K)

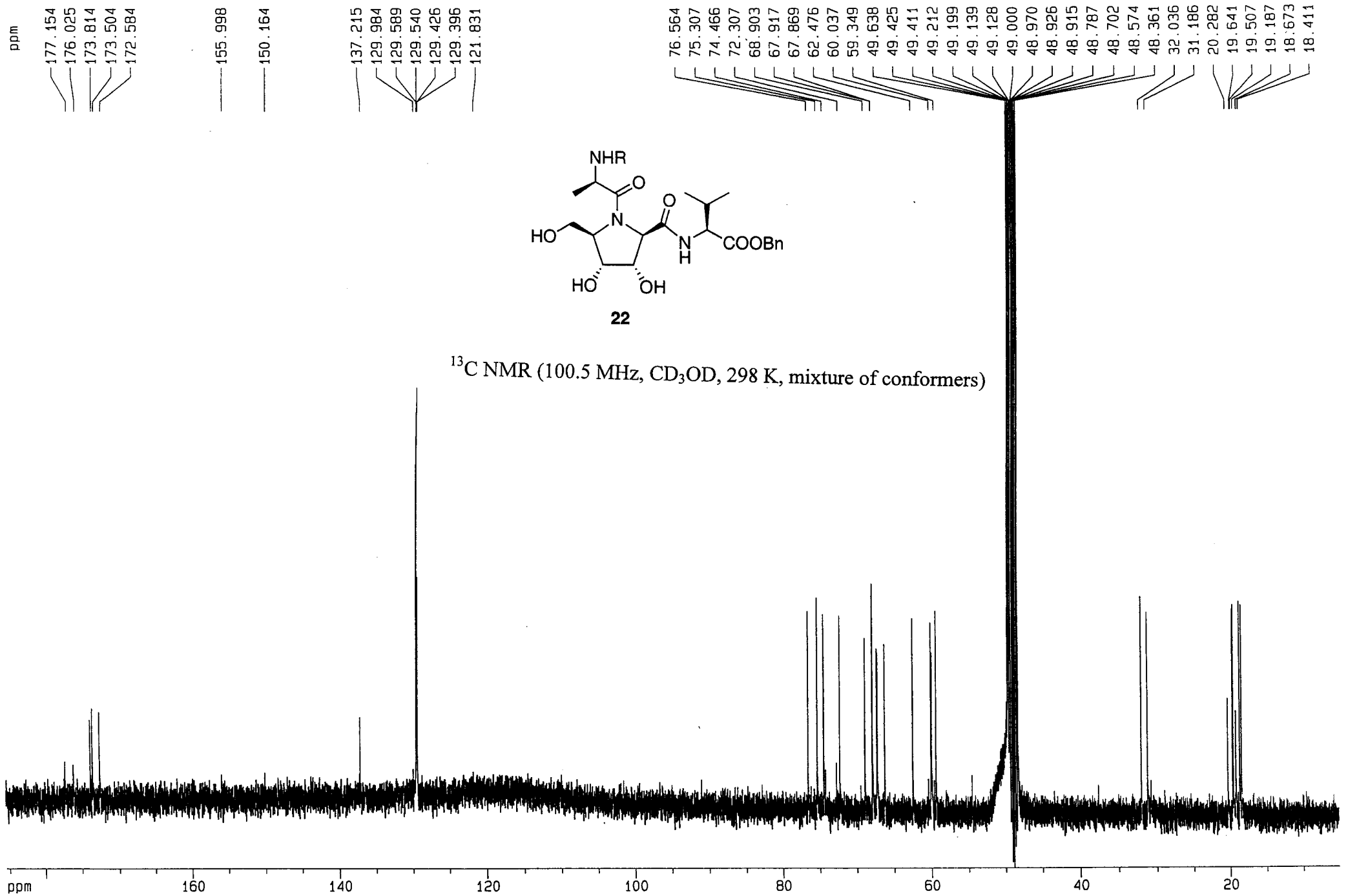


20

^{13}C NMR (75.4 MHz, CD_3OD, 298 K)



^{1}H NMR (400 MHz, CD_3OD , 298 K, mixture of conformers)



22

^{13}C NMR (100.5 MHz, CD_3OD, 298 K, mixture of conformers)