Supporting Information

3-Aminopiperidine Based Peptide Analogues as the First Selective Noncovalent Inhibitors of the Bacterial Cysteine Protease IdeS

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Table of Contents

Synthesis of compounds shown in main text Schemes 3 - 6.

(-)-2-[(1-(L-Leucinyl-L-leucinyl)-(*R*)-piperidin-3-yl)(ethyl)amino]acetamide x 2 TFA ((*R*)-LLpipEtG) ((*R*)-11). A suspension of (*R*)-**9** (180 mg, 0.3 mmol) and Pd/C (10%) (*cat.*) in ethanol (2 mL) was stirred under a H_2 atmosphere at room temperature for 6 days. The catalyst was removed by filtration through a pad of Celite and the solvent was removed *in vacuo*. Purification by flash chromatography (ethyl acetate/methanol 20:1 followed by 20:3, R_f = 0.27) gave the ethylated intermediate (35 mg, 23%) and Cbz-deprotected material (106 mg, 73%) was collected. The ethylated intermediate (30 mg) was dissolved in dichloromethane (1.5 mL) and TFA (0.3 mL) was added. The reaction mixture was stirred at room temperature for 16 h. The solvent and excess of reagent were removed *in vacuo* to give the TFA salt of (*R*)-**LLpipEtG** ((*R*)-**11**) as a white solid (37 mg, 99%). Analytical reversed phase HPLC (the mobile phase was 10.8-37.2% CH_3CN in H_2O for 30 min, with 0.1% TFA throughout) t_R 7.1 min, 100% purity; ^{1}H NMR (CD_3OD) δ 4.92-4.83 (m, 1H,), 4.78 (app d, 1H), 4.47-4.32 (m, 1H), 4.16-3.88 (m, 3H), 3.44-3.26 (m, 3H), 3.15 (app t, 1H), 2.85 (app t, 1H), 2.71-2.58 (m, 1H) 2.29-2.14 (m, 1H), 2.05-1.88 (m, 1H), 1.87-1.56 (m, 6H), 1.55-1.40 (m, 1H), 1.39-1.25 (t, 3H), 1.06-0.85 (m, 12H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.2, 171.0, 168.5, 61.8, 52.9, 52.0, 50.9, 49.6, 46.8, 43.2, 41.8, 41.5, 25.9, 25.8, 25.3, 23.5, 23.2 (2C), 22.0, 21.7, 9.9; $[\alpha]_D^{20}$ -15.2 (*c* 1.0, CH_3OH); IR (KBr) *v* 3444, 2364, 1683, 1540, 1201 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{21}H_{41}N_5O_3$ $[M+H]^+$ 412.3280, found 412.3282.

(+)-2-[(1-(L-Leucinyl-L-leucinyl)-(*S*)-piperidin-3-yl)(ethyl)amino]acetamide x 2 TFA ((*S*)-LLpipEtG) ((*S*)-11). Compound (*S*)-**9** (91 mg, 0.15 mmol) was deprotected as described for (*R*)-**LLpipEtG** ((*R*)-**11**) to give both the ethylated intermediate (10 mg, 13%) and the Cbz-deprotected intermediate (41 mg, 58%). The ethylated intermediate (9 mg) was Boc-deprotected to give the TFA salt of (*S*)-**LLpipEtG** ((*S*)-**11**) as a white solid (11 mg, 98%). Analytical reversed phase HPLC (the mobile phase was 10.8-37.2% CH_3CN in H_2O for 30 min with 0.1% TFA throughout) t_R 9.0 min, 100% purity; ^{1}H NMR (CD_3OD) δ 4.68 (app d, 1H), 4.47 (app d, 1H), 4.37 (app d, 1H), 4.21-3.96 (m, 1H), 3.95-3.85 (m, 1H), 3.64-3.53 (m, 1H), 3.50-3.31 (m, 2H), 3.21 (app t, 1H), 2.99 (app t, 1H), 2.59 (app t, 1H), 2.28-2.16 (m, 1H), 2.07-1.97 (m, 1H), 1.96-1.87 (m, 1H), 1.86-1.55 (m, 6H), 1.54-1.40 (m, 1H), 1.39-1.25

(m, 3H), 1.04-0.86 (m, 12H); ^{13}C NMR (125 MHz, CD_3OD) δ 172.8, 170.8, 168.3, 62.1, 52.8, 52.2, 51.1, 46.4, 43.5, 41.8, 41.7, 26.0, 25.3, 25.1, 23.7, 23.6, 23.2 (2C), 22.0, 21.8, 9.9; $[\alpha]_D^{20}$ +9.8 (*c* 1.2, CH_3OH); IR (KBr) *v* 3446 br, 2368, 1683, 1540, 1201 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{21}H_{41}N_5O_3$ $[M+H]^+$ 412.3280, found 412.3285.

M**ethyl 2-[(1-*tert*-butoxycarbonylpiperid-2-en-3-yl)amino]acetate (12).** Compound **12** was synthesized from 1-Boc 3-piperidone (4.98 g, 25 mmol) and methyl glycinate (3.49 g, 27.8 mmol) as described for (rac)-**1** with $NaBH(OAc)_3$ (5.29 g, 25 mmol), acetic acid (2.25 g, 37.5 mmol) and molecular sieves (4.5 g) in dichloromethane (20 mL). Purification by flash chromatography (heptane/ethyl acetate 1:2 $R_f = 0.2$) gave recovered starting material (1.31 g) and the unsaturated by-product **12** as a yellow oil (0.83 g, 17% yield, based on recovered material). ^{1}H NMR δ 9.18 (s, 1H, ^{pip}H1), 6.33 (br s, 1H, ^{Gly}NH), 4.05 (d, *J* = 5.3 Hz, 2H, ^{Gly}H1), 3.76 (s, 3H, $^{methyl}CH_3$), 3.68 (t, *J* = 6.7 Hz 2H, ^{pip}H5), 2.24 (t, *J* = 7.3 Hz, 2H, ^{pip}H3), 1.90 (p, *J* = 7.2 Hz, 2H, ^{pip}H4), 1.54 (s, 9H, $^{Boc}CH_3$); ^{13}C NMR δ 172.3 (^{Gly}CO), 170.3 (^{pip}C2), 163.3 (^{pip}C1), 152.2 (^{Boc}CO), 84.1 (^{Boc}C), 52.1 (^{methyl}C), 41.1 (^{Gly}C1), 39.7 (^{pip}C5), 33.1 (^{pip}C3), 27.9 ($^{Boc}CH_3$), 24.2 (^{pip}C4); IR (neat) *v* 3317, 2956, 1739, 1686, 1345, 1152.

[(Piperid-2-en-3-yl)amino]acetamide x 2 TFA (pip(db)G) (13). A solution of NH_3 in methanol (7 *N*) (4.0 mL) was added to **12** (75 mg, 0.29 mmol) as described for (*S*)-**2** to give the intermediate amide as a white solid (66 mg, 93%). ^{1}H NMR δ 8.00 (s, 1H, ^{pip}H1), 6.86 (br s, 1H, N*H*), 6.02 (br s, 1H, N*H*), 5.02 (br s, 1H, N*H*), 3.94-3.60 (m, 2H, ^{Gly}H1), 3.06-2.78 (m, 2H, ^{pip}H5), 2.24-2.00 (m, 2H, ^{pip}H3), 1.75-1.52 (m, 2H, ^{pip}H4), 1.24 (s, 9H); ^{13}C NMR δ 174.3 (^{Gly}CO), 173.3 (^{pip}C2), 164.5 (^{pip}C1), 156.5 (^{Boc}CO), 79.1 (^{Boc}C), 42.7 (^{Gly}C1), 39.7 (^{pip}C5), 32.4 (^{pip}C3), 28.4 ($^{Boc}CH_3$), 25.9 (^{pip}C4); IR (KBr) *v* 3419, 1683, 1541, 1168 cm^{-1}. Deprotection of the intermediate (49 mg, 0.19 mmol) using TFA (0.5 mL) was performed as described for (*S*)-**pipG** ((*S*)-**3**) to give the TFA salt of **pip(db)G** (**13**) as a white solid (77 mg, 97%). ^{1}H NMR (CD_3OD) δ 8.08 (s, 1H, ^{pip}H1), 3.85 (s, 2H, ^{Gly}H1), 2.98 (t, *J* = 7.3 Hz, 2H, ^{pip}H5), 2.42 (t, *J* = 7.0 Hz, 2H, ^{pip}H3), 1.93 (p, *J* = 7.0 Hz, 2H, ^{pip}H4); ^{13}C NMR (CD_3OD) δ 175.1 (^{Gly}CO), 174.3 (^{pip}C2), 164.1 (^{pip}C1), 43.0 (^{Gly}C1), 40.3 (^{pip}C5), 33.4 (^{pip}C3), 24.2 (^{pip}C4); IR (KBr) *v* 3428, 1660, 1603, 1204 cm^{-1}.

2-[(Benzyloxycarbonyl)(1-(*tert*-butoxycarbonyl)-(*R*)-piperidin-3-yl)amino]acetic acid ((*R*)-14). Compound (*R*)-**14** was synthesized from (*R*)-**4** (0.88 g, 2.1 mmol) and LiOH (1M, aq.) (3.0 mL, 3.0 mmol) using the procedure described for (*S*)-**14** to give the product as a transparent oil (780 mg, 95%). ^{1}H NMR δ 7.42-7.25 (m, 5H, ^{Cbz}Ph), 5.19-5.08 (AB system, 2H, ^{Cbz}CH$_2$), 4.18-3.85 (m, 4H, ^{Pip}H1a, ^{Pip}H5a, ^{Gly}H1), 2.91-2.71 (m, 3H, ^{Pip}H1b, ^{Pip}H5b, ^{Pip}H2), 2.71-2.51 (m, 1H, ^{Pip}H3a), 1.97-1.85 (m, 1H, ^{Pip}H4a), 1.79-1.23 (m, 11H, ^{Pip}H4b, ^{Pip}H3b, ^{Boc}CH$_3$); ^{13}C NMR (CD$_3$OD) δ 173.8, (156.3, 155.3, 154.9 and 154.7, 2C, rot), 136.2 and 136.0 (rot), 128.4 and 128.3 (2C, rot), 128.0 and 127.8 (rot), 127.7 and 127.6 (2C, rot), 80.0, 67.9 and 67.4 (rot), 53.2, 47.0, 45.3, 43.3, 28.7, 28.3 (3C), 24.5; IR (KBr) *v* 3446, 2974, 2362, 1696, 1436, 1268, 1152 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{20}H_{28}N_2O_6$ [M+H]$^+$ 393.2020, found 393.2021.

2-[(Benzyloxycarbonyl)(1-(*tert*-butoxycarbonyl)-(*S*)-piperidin-3-yl)amino]acetic acid ((*S*)-14). Aqueous LiOH (1M) (3.0 mL, 3.0 mmol) was added to (*S*)-**4** (0.84 g, 2.0 mmol) in THF/MeOH/H$_2$O (3:1:1) (16 mL) at 0 °C and the reaction mixture was stirred for 30 min, and then for 3 h at room temperature. The reaction mixture was acidified with aqueous HCl (1M) and extracted with ethyl acetate (2 x 25 mL, 1 x 10 mL). The combined organic phases were washed with brine (1 x 25 mL), dried (Na$_2$SO$_4$) and filtered. The solvent was removed *in vacuo* to give (*S*)-**14** as a transparent oil (773 mg, 99%). ^{1}H NMR (CD$_3$OD) δ 7.42-7.25 (m, 5H, ^{Cbz}Ph), 5.20-5.07 (AB system, 2H, ^{Cbz}CH$_2$), 4.19-3.84 (m, 4H, ^{Pip}H1a, ^{Pip}H5a, ^{Gly}H1), 2.90-2.51 (m, 3H, ^{Pip}H1b, ^{Pip}H5b, ^{Pip}H2), 1.95-1.84 (m, 1H, ^{Pip}H3a), 1.79-1.56 (m, 2H, ^{Pip}H4), 1.44-1.23 (m, 10H, ^{Boc}CH$_3$, ^{Pip}H3b); ^{13}C NMR δ 173.8 (^{Gly}CO), 156.3, 155.3 and 155.0 and 154.7 (rot, 2C, ^{Boc}CO and ^{Cbz}CO), 136.2 and 136.0 (rot, ^{Cbz}C1), 128.5 and 128.4 (2C, rot, ^{Cbz}C3), 128.0 and 127.9 (rot, ^{Cbz}C4), 127.7 and 127.6 (2C, rot, ^{Cbz}C2), 80.1 and 80.0 (rot, ^{Boc}C), 67.9 and 67.4 (^{Cbz}CH$_2$), 53.2 (^{Pip}C2), 46.9 (^{Gly}C1), 45.4 (^{Pip}C1), 43.4 (^{Pip}C5), 28.7 (^{Pip}C3), 28.3 (3C, ^{Boc}CH$_3$), 24.6 (^{Pip}C4); IR (KBr) *v* 3446, 2974, 2362, 1696, 1436, 1268, 1152 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{20}H_{28}N_2O_6$ [M+H]$^+$ 393.2020, found 393.2021.

(-)-1-[((Benzyloxycarbonyl)(-1-*tert*-butoxycarbonyl-(*R*)-piperidin-3-yl)amino)acetyl]-L-proline amide ((*R*)-15). Compound (*R*)-**14** (615 mg, 1.57 mmol) and L-proline-NH$_2$ (195 mg, 1.72 mmol) were coupled as described in the procedure for (*S*)-**15** with EDC (330 mg, 1.72 mmol), HOBt (233 mg, 1.72 mmol), Et$_3$N (317 mg, 3.13 mmol) in dichloromethane (7 mL) to give (*R*)-**15** (630 mg, 82%) as a white solid. Analytical chiral HPLC (hexane/ethanol 70:30) t_R 10.9 min, 100% purity; ^{1}H NMR δ 7.41-7.21 (m, 5H), 5.24-4.92 (m, 3H), 4.66-4.47 (m, 1H), 4.25-3.37 (m, 6H), 3.31-3.21 (m, 1H), 3.09-2.50 (m, 2H), 2.46-2.28 (m, 1H), 2.14-1.79 (m, 3H), 1.79-1.63 (2H), 1.63-1.18 (10H); ^{13}C NMR δ 173.9 and 173.5 (rot), 168.2 and 168.0 (rot), (156.0, 155.1, 154.5 and 154.3 (rot, 2C), 136.0 and 135.9 (rot), 128.1 (2C), 127.6, 127.2 (2C), 79.3, 67.3 and 67.0 (rot), 59.9 and 59.6 (rot), 53.1, 46.8, 46.2, 45.7, 43.5, 28.8, 28.3, 28.0 (3C), 24.4, 24.0; $[\alpha]_D^{20}$ -84.5 (*c* 1.0, $CHCl_3$); IR (KBr) *v* 3421, 2972, 2362, 1684, 1422, 1264, 1151 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{25}H_{36}N_4O_6$ $[M+H]^+$ 489.2708, found 489.2705.

(-)-1-[((Benzyloxycarbonyl)(1-*tert*-butoxycarbonyl-(*S*)-piperidin-3-yl)amino)acetyl]-L-proline amide ((*S*)-15). EDC (309 mg, 1.61 mmol) and HOBt (218 mg, 1.61 mmol) were added to a solution of (*S*)-**14** (576 mg, 1.47 mmol) in dichloromethane (6.5 mL) at 0 °C and the reaction mixture was stirred for 30 min. Et_3N (297 mg, 2.94 mmol) and L-proline-NH_2 (183 mg, 1.61 mmol) were added and the mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with dichloromethane (25 mL) and washed with aqueous citric acid (10%) (2 x 10 mL), saturated aqueous $NaHCO_3$ (2 x 10 mL) and brine (1 x 10 mL). The organic phase was dried (Na_2SO_4) and filtered. The solvent was removed *in vacuo* to give (*S*)-**15** as a white solid (560 mg, 78%). Analytical chiral HPLC (hexane/ethanol 70:30) t_R 8.9 min, 100% purity. ^{1}H NMR δ 7.46-7.24 (m, 5H), 6.96-6.64 (m, 2H), 5.29-4.99 (m, 3H), 4.66-4.48 (m, 1H), 4.30-3.58 (m, 6H), 3.58-3.39 (m, 1H), 2.94-2.48 (m, 2H), 2.16-1.86 (m, 1H), 1.78-1.64 (m, 3H), 1.60-1.31 (m, 12H); ^{13}C NMR δ 174.1 and 173.8 and 173.5 (rot, ^{Pro}CO), 168.3 and 168.1 (rot, ^{Gly}CO), 156.1, 155.1, 154.5 and 154.4 (rot, 2C, ^{Cbz}CO and ^{Boc}CO), 136.1 and 136.0 (rot, ^{Cbz}C1), 128.2 (2C, ^{Cbz}C3), 127.7 (^{Cbz}C4), 127.3 and 127.2 (2C, rot, ^{Cbz}C2), 79.4 (^{Boc}C), 67.4, 67.3 and 67.1 ($^{Cbz}CH_2$), 59.8 and 59.5 (rot, ^{Pro}C1), 53.4 (^{Pip}C2), 46.9 (^{Pro}C4), 46.3 (^{Gly}C1), 46.2 (^{Pip}C1), 43.1 (^{Pip}C5), 28.7 (^{Pip}C3), 28.1 (3C, $^{Boc}CH_3$), 27.7 (^{Pro}C2), 24.5 (^{Pip}C4), 24.3 (^{Pro}C3). For further analyses, a portion of (*S*)-**15** was purified by flash chromatography (dichloromethane/methanol 95:5, R_f = 0.06). $[\alpha]_D^{20}$ -46.8 (*c* 1.0, $CHCl_3$); IR (KBr) *v* 3421, 2974, 2361, 1685, 1424, 1266, 1152 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{25}H_{36}N_4O_6$ $[M+H]^+$ 489.2708, found 489.2707.

(-)-1-[((*R*)-Piperidin-3-ylamino)acetyl]-L-proline amide x 2 TFA ((*R*)-pipGP) ((*R*)-16). Compound (*R*)-**15** (50 mg, 0.10 mmol) was deprotected as described for (*S*)-**LpipGP** ((*S*)-**18**) to give the TFA salt of (*R*)-**pipGP** ((*R*)-**16**) (52 mg, quant.) as a gray solid. ^{1}H NMR (CD_3OD) δ 4.45 (dd, *J* = 9.1, 4.1 Hz, 1H, ^{Pro}H1), 4.14 (s, 2H, ^{Gly}H1), 3.77 (app d, 1H, ^{Pip}H1a), 3.67-3.49 (m, 3H, ^{Pro}H4, ^{Pip}H2), 3.40 (app d, 1H, ^{Pip}H5a), 3.09 (app t, 1H, ^{Pip}H1b), 3.02-2.92 (m, 1H, ^{Pip}H5b), 2.41-2.16 (m, 2H, ^{Pip}H3a, ^{Pro}H2a), 2.15-1.93 (m, 4H, ^{Pip}H4b, ^{Pro}H2b, ^{Pro}H3), 1.88-1.65 (m, 2H, ^{Pip}H3b, ^{Pip}H4b); ^{13}C NMR (CD_3OD) δ 176.7, 165.2, 61.4, 52.9, 47.7, 46.9, 45.1, 44.7, 31.1, 26.4, 25.6, 21.6; $[\alpha]_D^{20}$ -30.8 (*c* 0.5, CH_3OH); IR (KBr) *v* 3405, 3224, 1683, 1653, 1458, 1202, 1131 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{12}H_{22}N_4O_2$ $[M+H]^+$ 255.1814, found 255.1812.

(-)-1-[2-((*S*)-Piperidin-3-ylamino)acetyl]-L-proline amide x 2 TFA ((*S*)-pipGP) ((*S*)-16). Compound (*S*)-**15** (40 mg, 0.8 mmol) was totally deprotected as described for (*S*)-**LpipGP** ((*S*)-**18**) to give the TFA salt of (*S*)-**pipGP** ((*S*)-**16**) (42 mg, quant.) as a gray solid. ^{1}H NMR (CD_3OD) δ 4.45 (dd, *J* = 9.0, 4.1 Hz, 1H, ^{Pro}H1), 4.21-4.05 (AB system, 2H, ^{Gly}H1), 3.80-3.67 (m, 1H, ^{Pip}H1eq), 3.66-3.49 (m, 3H, ^{Pip}H2ax, ^{Pro}H4), 3.44-3.33 (m, 1H, ^{Pip}H5eq), 3.16-3.03 (m, 1H, ^{Pip}H1ax), 3.02-2.89 (m, 1H, ^{Pip}H5ax), 2.41-2.16 (m, 2H, ^{Pip}H3eq, ^{Pro}H2a), 2.15-1.93 (m, 4H, ^{Pip}H4eq, ^{Pro}H2b, ^{Pro}H3), 1.88-1.65 (m, 2H, ^{Pip}H4ax, ^{Pip}H3ax); ^{13}C NMR (CD_3OD) δ 176.8 (^{Pro}CO), 165.8 (^{Gly}CO), 61.5 (^{Pro}C1), 53.0 (^{Pip}C2), 47.7 (^{Pro}C4), 47.3 (^{Gly}C1), 45.4 (^{Pip}C1), 44.7 (^{Pip}C5), 31.1 (^{Pro}C2), 26.5 (^{Pip}C3), 25.6 (^{Pro}C3), 21.5 (^{Pip}C4); $[\alpha]_D^{20}$ -27.0 (*c* 0.5, CH_3OH); IR (KBr) *v* 3420, 2993, 1674, 1457, 1429, 1203, 1131 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{12}H_{22}N_4O_2$ $[M+H]^+$ 255.1814, found 255.1812.

(-)-1-[((Benzyloxycarbonyl)(1-(*tert*-butoxycarbonyl-L-leucinyl)-(*R*)-piperidin-3-yl)amino)acetyl]-L-proline amide ((*R*)-17). Compound (*R*)-**17** was synthesized from (*R*)-**15** (400 mg, 0.82 mmol) using the procedure described for (*S*)-**17**. Purification by preparative HPLC (hexane/2-propanol 70:30, 8 mL/min) afforded the product as a white solid (304 mg, 64% yield over two steps). Analytical chiral HPLC (hexane/2-propanol 70:30) t_R 16.5 min, 99% purity, *d.e.* 100%; $[\alpha]_D^{20}$ -75.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 7.40-7.21 (m, 5H), 5.34-4.98 (m, 3H), 4.70-4.39 (m, 4H), 4.21-3.73 (m, 4H), 3.72-3.58 (m, 1H), 3.57-3.36 (m, 1H), 3.34-3.20 (m, 1H), 3.11-2.84 (m, 1H), 2.58-2.29 (m, 2H), 2.17-1.86 (m, 2H), 1.85-1.22 (m, 14H), 1.06-0.74 (m, 6H); ^{13}C NMR (CD_3OD, 50 °C) δ 177.1, 173.8 and 173.3 (rot), 170.1, 157.7, 157.4, 137.9, 129.5 (2C), 129.1, 128.9 (2C), 80.5, 68.6, 61.5 and 60.1 (rot), 55.1, 50.3, 47.7, 46.8 and 46.3 (rot), 43.2, 42.5 and 42.0 (rot), 30.5, 28.7 (3C), 26.5, 26.0 and 25.9 (rot) 25.7, 25.2, 23.6 and 23.5 (rot), 23.3, 22.1; IR (KBr) *v* 3420, 2959, 2364, 1702, 1653, 1456, 1261, 1167 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{31}H_{47}N_5O_7$ $[M+H]^+$ 602.3548, found 602.3545.

(-)-1-[((Benzyloxycarbonyl)(1-(*tert*-butoxycarbonyl-L-leucinyl)-(*S*)-piperidin-3-yl)amino)acetyl]-L-proline amide ((*S*)-17). Boc-deprotection of (*S*)-**15** (400 mg, 0.82 mmol) with TFA (560 mg, 4.91 mmol) in dichloromethane (5 mL) was performed as described for (*S*)-**5.** The crude intermediate were coupled to Boc-L-Leu x H_2O (225 mg, 0.90 mmol) using the procedure described for (*S*)-**5**, and EDC (173 mg, 0.90 mmol), HOBt (122 mg, 0.90 mmol), Et_3N (249 mg, 3.28 mmol) in dichloromethane (6 mL) with mol. sieves (160 mg). Purification by flash chromatography (ethyl acetate/methanol 20:1) gave (*S*)-**17** as a white solid (430 mg, 90% yield over two steps). Analytical chiral HPLC (hexane/2-propanol 70:30) t_R 26.3 min, 98% purity, *d.e.* 100%; $[\alpha]_D^{20}$ -8.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 7.48-7.20 (m, 5H), 5.32-4.93 (m, 3H), 4.70-3.13 (m, 11H), 3.09-2.91 (m, 1H), 2.82-2.61 (m, 1H), 2.58-2.24 (m, 1H), 2.23-1.60 (m, 6H), 1.59-1.14 (m, 10H), 1.03-0.68 (m, 6H); ^{13}C NMR (CD_3OD, 50 °C) δ 176.8, 173.4, 169.9, 157.5, 157.0, 137.7, 129.4 (2C), 129.0, 128.8 (2C), 80.3, 68.6, 61.4 and 61.0 (rot), 54.5, 50.2, 47.6, 46.8 and 46.3 (rot), 43.8, 42.7, 42.1, 33.2, 30.3, 28.8 (3C), 25.9, 25.6, 23.6, 23.3, 22.2; IR (KBr) *v* 3420, 2959, 2408, 1701, 1653, 1447, 1411, 1254, 1170 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{31}H_{47}N_5O_7$ $[M+H]^+$ 602.3548, found 602.3544.

1-[((1-L-Leucinyl-(*R*)-piperidin-3-yl)amino)acetyl]-L-proline amide × 2 TFA ((*R*)-LpipGP) ((*R*)-18). Compound (*R*)-**LpipGP** ((*R*)-**18**) was synthesized from (*R*)-**17** (240 mg, 0.40 mmol) with Pd/C using the procedure described for (*S*)-**LpipGP** ((*S*)-**18**), but by stirring for 48 h in the first deprotection step. Purification by flash chromatography (ethyl acetate:methanol 20:1, followed by methanol, R_f = 0.49) gave the Cbz-deprotected intermediate (111 mg) which was further deprotected as described for (*S*)-**LpipGP** ((*S*)-**18**) to give the TFA salt of (*R*)-**LpipGP** ((*R*)-**18**) as a white solid (171 mg, 72%). Analytical reversed phase HPLC (the mobile phase was 2.0-28.4% CH_3CN in H_2O for 30 min) t_R 14.0 min, 100% purity; $[\alpha]_D^{20}$ -40.1 (*c* 0.62, CH_3OH); ^{1}H NMR (CD_3OD) δ 4.48-4.40 (m, 1H, ^{Pro}H4a), 4.17-4.08 (m, 2H, ^{Pro}H1, ^{Leu}H1), 3.97 (s, 2H, ^{Gly}H1), 3.78-3.69 (m, 1H, ^{Pip}H1a), 3.67-3.52 (m, 2H, ^{Pip}H5), 3.27-3.10 (m, 2H, ^{Pip}H2, ^{Pip}H1b), 2.94-2.85 (m, 1H, ^{Pro}H4b), 2.35-2.18 (m, 2H, ^{Pro}H2a, ^{Pip}H3a), 2.13-1.90 (m, 4H, ^{Pro}H2b, ^{Pro}H3,

^{Pip}H4a), 1.79-1.56 (m, 5H, ^{Pip}H3b, ^{Pip}H4b, ^{Leu}H2, ^{Leu}H3), 1.06-0.99 (m, 6H, ^{Leu}H4, ^{Leu}H5); ^{13}C NMR (CD_3OD) δ 176.7 (^{Pro}CO), 170.0 (^{Leu}CO), 165.5 (^{Gly}CO), 61.6 (^{Pro}C1), 54.8 (^{Pip}C2), 50.6 (^{Leu}C1), 47.7 (^{Pip}C5), 46.8 (^{Gly}C1), 46.6 (^{Pip}C1), 44.6 (^{Pro}C4), 40.9 (^{Leu}C2), 31.0 (^{Pro}C2), 27.9 (^{Pip}C3), 25.5 (^{Pro}C3), 25.3 (^{Leu}C3), 24.9 (^{Pip}C4), 23.5 (^{Leu}C4 or ^{Leu}C5), 21.6 (^{Leu}C5 or ^{Leu}C4); IR (KBr) *v* 3421, 2964, 2366, 1682, 1457, 1204, 1133 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{18}H_{33}N_5O_3$ $[M+H]^+$ 368.2656, found 368.2651; pK_a1 = 4.41, pK_a2 = 10.11.

(-)-1-[((1-L-Leucinyl-(*S*)-piperidin-3-yl)amino)acetyl]-L-proline amide x 2 TFA (*S*)-LpipGP) ((*S*)-18). A suspension of (*S*)-**17** (250 mg, 0.41 mmol) and Pd/C (10%) (cat.) in ethanol (3 mL) was stirred under a H_2 atmosphere at room temperature for 24 h. The catalyst was removed by filtration through a pad of Celite and the solvents were removed *in vacuo*. The residue was dissolved in dichloromethane/TFA (1:1) (1 mL) and the reaction mixture was stirred at room temperature for 4 h. The solvent and excess of reagent were removed *in vacuo* to give the TFA salt of (*S*)-**LpipGP** ((*S*)-**18**) as a light yellow solid (180 mg, 73%). Analytical reversed phase HPLC (the mobile phase was 2.0-28.4% CH_3CN in H_2O for 30 min with 0.085% TFA throughout) t_R 15.2 min, 100% purity; $[\alpha]_D^{20}$ -15.9 (*c* 0.62, CH_3OH); ^{1}H NMR (CD_3OD) δ 4.48-4.40 (m, 2H, ^{Pro}H1, ^{Leu}H1), 4.21-4.03 (m, 2H, ^{Pip}H1a, ^{Pip}H5a), 3.97 (s, 2H, ^{Gly}H1), 3.69-3.48 (m, 3H, ^{Pip}H1b, ^{Pip}H5b, ^{Pip}H2), 3.36-3.31 (m, 2H, ^{Pro}H4), 2.32-2.17 (m, 2H, ^{Pip}H3a, ^{Pro}H2a), 2.11-1.49 (m, 9H, ^{Pro}H2b, ^{Leu}H3, ^{Pip}H4, ^{Pip}H3b, ^{Leu}H2, ^{Pro}H3), 1.09-0.95 (m, 6H, ^{Leu}H4, ^{Leu}H5); ^{13}C NMR (CD_3OD) δ 176.7 (^{Pro}CO), 170.3 (^{Leu}CO), 165.4 (^{Gly}CO), 61.6 (^{Pro}C1), 55.0 (^{Pip}C2), 50.5 (^{Leu}C1), 47.7 (^{Gly}C1), 47.0 (^{Pro}C4), 46.6 (^{Pip}C1), 44.3 (^{Pip}C5), 41.2 (^{Leu}C2), 31.0 (^{Pro}C2), 27.1 (^{Pip}C3), 25.5 (^{Leu}C3), 25.4 (^{Pro}C3), 23.5 (^{Leu}C4 or ^{Leu}C5), 23.2 (^{Pip}C4), 21.7 (^{Leu}C5 or ^{Leu}C4); IR (KBr) *v* 3422, 2966, 2363, 1676, 1457, 1202, 1130 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{18}H_{33}N_5O_3$ $[M+H]^+$ 368.2656, found 368.2655; pK_a1 = 4.35, pK_a2 = 10.06.

1-(1-*tert*-Butoxycarbonyl-(*R*)-piperidin-3-yl)-L-proline amide and
1-(-1-*tert*-Butoxycarbonyl-(*S*)-piperidin-3-yl)-L-proline amide ((*R*)- and (*S*)-19). The diastereomers of **19** were synthesized from 1-Boc 3-piperidone (1.8 g, 8.8 mmol) and L-proline-NH_2 (1.0 g, 8.8 mmol) as described for (rac)-**1** with $NaBH(OAc)_3$ (2.1 g, 9.7 mmol), acetic acid (0.8 g, 13.2 mmol) and molecular sieves (2.0 g) in dichloromethane (20 mL). Purification by flash chromatography (ethyl acetate/methanol 20:1 R_f = 0.19) gave the

product as a transparent oil (1.8 g, 68%). Analytical chiral HPLC (hexane/2-propanol 90:10) t_R 11.0 min 60% (*R*)-**19** and t_R 12.9 min 40% (*S*)-**19**; ^{1}H NMR δ 7.21 (br s, 1H), 5.75 (br s, 1H) 4.01-3.87 (m, 1H), 3.39-3.00 (m, 3H), 2.72-2.29 (m, 6H), 2.14-1.60 (m, 6H), 1.49-1.20 (m, 9H); ^{13}C NMR (rotamers or diastereomers) (CD_3OD) δ 182.1 and 181.8 (rot), 156.4, 81.0, 66.0 and 65.3 (rot), 61.3 and 60.6 (rot), 53.0, 52.0, 45.6 and 44.8 (rot), 32.2 and 32.1 (rot), 31.3 and 31.1 (rot), 30.0, 28.7 (3C), 25.3 and 25.3 (rot).

Separation of a portion of the diastereomeric mixture of **19** by preparative HPLC (Chiralpak AD) gave the two isolated diastereomers (*R*)- and (*S*)-**19**, which were used in further synthesis.

1-(1-(*tert*-Butoxycarbonylglycinyl)-(*R*)-piperidin-3-yl)-L-proline amide ((*R*)-20). Deprotection of (*R*)-**19** (878 mg, 3.0 mmol) with TFA (2.0 g, 17.7 mmol) in dichloromethane (20 mL) and subsequent coupling with Boc-glycine (570 mg, 3.3 mmol), EDC (625 mg, 3.3 mmol) and HOBt (441 mg, 3.3 mmol), Et_3N (2.5 g, 24.3 mmol) in dichloromethane (10 + 5 mL) were performed as described for (*S*)-**5** but without the acidic washing step. Purification by flash chromatography (ethyl acetate/methanol 20:1, $R_f = 0.09$) gave (*R*)-**20** as a white solid (475 mg, 45%). Analytical chiral HPLC (hexane/2-propanol 50:50, with a flow rate of 0.6 mL/min) t_R 5.5 min 98% and t_R 8.0 min 2%; ^{1}H NMR (CD_3OD) δ 4.48 (app d, 1H), 4.38 (app d, 1H), 4.00-3.86 (m, 2H), 3.69 (app d, 1H), 3.37-3.22 (m, 1H), 3.08-2.98 (m, 1H), 2.79-2.71 (m, 1H), 2.68-2.48 (m, 2H), 2.47-2.37 (m, 1H), 2.19-2.04 (m, 1H), 2.00-1.89 (m, 1H), 1.89-1.69 (m, 4H), 1.60-1.34 (m, 10H); ^{13}C NMR (CD_3OD) δ 182,0 169.4 and 169.3 (rot), 158.2, 80.4, 65.3 and 64.8 (rot), 61.4 and 60.9 (rot), 54.9 and 53.1 (rot), 52.8 and 50.0 (rot), 47.3 and 46.1 (rot), 43.7, 43.0 and 42.9 (rot), 32.1, 31.0 and 30.9 (rot), 28.7 (3C), 25.4 and 25.3 and 25.0 (rot); IR (neat) *v* 3420, 3303, 2976, 2866, 1711, 1654, 1444, 1382, 1350, 1123; HRMS (FT-ICR-MS) Calcd for $C_{17}H_{30}N_4O_4$ $[M+H]^+$ 355.2338 found 355.2330.

1-(1-(*tert*-Butoxycarbonylglycinyl)-(*S*)-piperidin-3-yl)-L-proline amide ((*S*)-20). Deprotection of (*S*)-**19** (726 mg, 2.4 mmol) with TFA (1.7 g, 14.6 mmol) in dichloromethane (15 mL) and subsequent coupling with Boc-glycine (471 mg, 2.7 mmol), EDC (518 mg, 2.7 mmol) and HOBt (366 mg, 2.7 mmol) in dichloromethane (10 mL) were performed as described for (*R*)-**20** to give (*S*)-**20** as a white solid (452 mg, 47%). Analytical chiral HPLC (hexane/2-propanol 50:50, with a flow rate of 0.6 mL/min) t_R 5.5 min 5% and t_R 8.0 min 95%; ^{1}H NMR (CD_3OD) δ 4.44-4.30 (m, 2H), 3.94-3.69 (m, 4H), 3.37-3.25 (m, 1H), 3.19-3.11 (m, 1H), 3.05-2.93 (m, 2H), 2.69-2.43 (m, 3H), 2.18-2.00 (m, 1H), 1.89-1.69 (m, 3H), 1.60-1.48 (m, 1H), 1.43 (s, 9H); ^{13}C NMR (CD_3OD) δ 181.5, 169.3, 158, 80.2, 65.3 and 65.2 (rot), 64.3 and 64.2 (rot), 54.9 and 51.9 (rot), 51.0 and 50.1 (rot), 47.6 and 46.0 (rot), 43.7, 42.9, 32.1, 29.6, 28.7 (3C), 25.4 and 25.2 and 24.9 (rot); IR (neat) *v* 3417, 2976, 2866, 1659, 1444, 1382, 1350, 1124; HRMS (FT-ICR-MS) Calcd for $C_{17}H_{30}N_4O_4$ $[M+H]^+$ 355.2338 found 355.2342.

1-(1-(glycinyl)-(*R*)-piperidin-3-yl)-L-proline amide x 2 TFA ((*R*)-GpipP) ((*R*)-21). Compound (*R*)-**20** (374 mg, 1.1 mmol) was deprotected by TFA/DCM (1:10) (6 mL) as described for (*S*)-**pipG** ((*S*)-**3**) to give (*R*)-**GpipP** ((*R*)-**21**) (268 mg, quant.). ^{1}H NMR (CD_3OD) δ 4.62-4.37 (app d, 2H), 4.11-3.9 (m, 2H), 3.85-3.77 (m, 1H), 3.67-3.59 (app d, 1H), 3.52-3.12 (m, 2H), 2.71-2.46 (m, 1H), 2.23-2.09 (m, 4H), 2.03-1.86 (m, 3H), 1.81-1.47 (m, 2H);^{13}C NMR (CD_3OD) δ 171.7, 166.4 and 166.0 (rot), 66.9 and 66.5 (rot), 62.0 and 61.9 (rot), 55.3 and 55.2 (rot), 47.0 and 45.5 (rot), 44.1 and 43.0 (rot), 41.1, 31.5 and 31.4 (rot), 27.6 and 27.5 (rot), 24.5, 24.2; IR *v* (neat) 3417 (v br), 1651-1633 (br), 1204, 1146; HRMS (FT-ICR-MS) Calcd for $C_{12}H_{22}N_4O_2$ $[M+H]^+$ 255.1814 found 255.1814.

1-(1-(glycinyl)-(*S*)-piperidin-3-yl)-L-proline amide x TFA ((*S*)-GpipP) ((*S*)-21). Compound (*S*)-**20** (311 mg, 0.9 mmol) was deprotected with TFA/DCM as described for (*S*)-**pipG** ((*S*)-**3**) to give (*S*)-**GpipP** ((*S*)-**21**) (223 mg, quant.). ^{1}H NMR (CD_3OD) δ 4.62 (app d, 1H), 4.46-4.35 (m, 1H), 4.12-3.82 (m, 2H), 3.82-3.60 (m, 2H), 3.54-3.06 (m, 3H), 3.02-2.86 (m, 1H), 2.76-2.48 (m, 1H), 2.34-2.09 (m, 3H), 2.06-1.75 (m, 3H), 1.71-1.58 (m, 1H); ^{13}C NMR (CD_3OD) δ 171.6, 166.4 and 166.1 (rot), 67.0 and 66.9 (rot), 61.6, 54.4 and 53.9 (rot), 46.9 and 45.4 (rot), 44.2 and 43.0 (rot), 41.0 31.3 and 31.0 (rot), 27.3 and 27.2 (rot), 24.6 and 24.3 (rot), 24.3 and 24.1 (rot); IR *v* (neat) 3417 (v br), 1643 (br), 1205, 1146; HRMS (FT-ICR-MS) Calcd for $C_{12}H_{22}N_4O_2$ $[M+H]^+$ 255.1814 found 255.1814

1-(1-[(*tert*-Butoxycarbonyl-L-leucinyl)-glycinyl]-(*R*)-piperidin-3-yl)-L-proline amide ((*R*)-22). Compound (*R*)-**20** (0.5 mmol) and Boc-L-leucine (150 mg, 0.7 mmol) were coupled as described for (*R*)-**19** mediated by EDC (93 mg, 0.5 mmol), HOBt (84 mg, 0.6 mmol) and Et_3N (361 mg, 3.6 mmol) in dichloromethane (7 mL) with molecular sieves (0.2 g).

Purification was performed by dissolving the crude product in ethyl acetate/methanol 20:3 (10 mL) and the solution was stirred with silica gel for 30 minutes and then filtrated. TLC analysis gave a single spot, R_f = 0.19. The solvents were removed *in vacuo* to give (*R*)-**22** as a white solid (230 mg, 93%). Analytical chiral HPLC (hexane/2-propanol 75:25 with a flow rate of 0.9 mL/min) t_R 14.0 min 98% and t_R 21.2 min 2%; ^{1}H NMR (CD_3OD) δ 4.50 (app d, 1H), 4.38 (app d, 1H), 4.16-3.98 (m, 3H), 3.98-3.94 (m, 1H), 3.70 (app d, 1H), 3.36-3.22 (m, 1H), 3.08-3.00 (m, 1H), 2.94 (app d, 1H), 2.73 (app d, 1H), 2.66-2.49 (m, 2H), 2.45-2.37 (m, 1H), 2.17-2.04 (m, 1H), 1.99-1.90 (m, 1H), 1.89-1.64 (m, 3H), 1.64-1.36 (m, 12H), 0.98-0.86 (m, 6H);^{13}C NMR (CD_3OD) δ 182.0, 175.7, 168.4 and 168.3 (rot), 157.8, 80.5, 65.3 and 64.8 (rot), 61.4 and 60.9 (rot), 54.5, 52.9 and 52.8 (rot), 50.1, 47.2 and 46.2 (rot), 43.7, 42.1 and 41.9 (rot), 32.2, 31.0 and 30.9 (rot), 28.7 (3C), 25.9, 25.5 and 25.4 (rot), 25.3 and 25.0 (rot), 23.7 and 23.5 (rot), 21.8; IR *v* (neat) 2443 (v br), 2074, 1651-1643, 1404, 1252, 1167; HRMS (FT-ICR-MS) Calcd for $C_{23}H_{41}N_5O_5$ $[M+H]^+$ 468.3178 found 468.3173.

1-(1-[(*tert*-Butoxycarbonyl-L-leucinyl)-glycinyl]-(*S*)-piperidin-3-yl)-L-proline amide ((*S*)-22). Compound (*S*)-**20** (0.5 mmol) was coupled to Boc-L-leucine (123 mg, 0.5 mmol) mediated by EDC (76 mg, 0.4 mmol) and HOBt (68 mg, 0.5 mmol) as described for (*R*)-**19**. Purification by short flash chromatography (ethyl acetate/methanol 20:3, R_f = 0.19) gave (*S*)-**22** as a white solid (160 mg, 78%). Analytical chiral HPLC (hexane/2-propanol 75:25, with a flow rate of 0.9 ml/min) t_R 14.7 min 4% and t_R 21.0 min, 96%; ^{1}H NMR (CD_3OD) δ 4.43-4.32 (m, 1H), 4.15-3.95 (m, 3H), 3.81 (app d, 1H), 3.71 (app d, 1H), 3.35-2.28 (m, 1H), 3.18-3.10 (m, 1H) 3.06-2.94 (m, 1H), 2.71-2.55 (m, 3H), 2.49-2.42 (m, 1H), 2.18-1.99 (m, 3H), 1.89-1.64 (m, 3H), 1.63-1.34 (m, 12H), 0.98-0.85 (m, 6H); ^{13}C NMR (CD_3OD) δ 181.7, 175.7, 168.4, 157.8, 80.5, 65.3 and 65.2 (rot), 60.3 and 60.2 (rot), 54.5, 51.9 and 50.9 (rot), 50.3, 47.6 and 46.2 (rot), 43.8, 42.2 and 42.1 (rot), 41.9 and 41.8 (rot), 32.2 and 32.1 (rot), 29.6, 27.7 (3C), 25.9, 25.3 and 25.0 (rot), 23.6 and 23.5 (rot), 21.8; IR *v* (neat) 3438 (v br), 2073, 1659-1633, 1404, 1252, 1166; HRMS (FT-ICR-MS) Calcd for $C_{23}H_{41}N_5O_5$ $[M+H]^+$ 468.3178 found 468.3181.

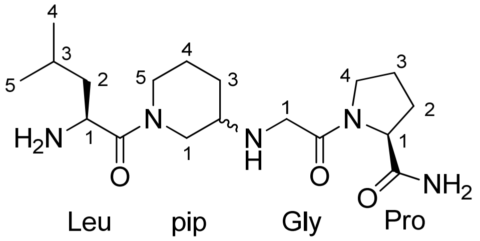
1-(1-[(L-Leucinyl)-glycinyl]-(*R*)-piperidin-3-yl)-L-proline amide x 2 TFA ((*R*)-LGpipP) ((*R*)-23). Deprotection of (*R*)-**22** (189 mg, 0.4 mmol) by TFA/dichloromethane (1:1) (6 mL) as described for (*S*)-**pipG** ((*S*)-**3**) gave the TFA salt of (*R*)-**LGpipP** ((*R*)-**23**) as a light brown solid (254 mg, quant.). ^{1}H NMR (CD_3OD) δ 4.48-4.35 (m, 2H), 4.21-4.09 (m, 2H), 3.97-3.91

(m, 1H), 3.85-3.77 (m, 1H), 3.72 (app d, 1H), 3.45-3.20 (m, 3H), 2.72-2.60 (m, 1H), 2.60-2.47 (m, 1H), 2.22-2.09 (m, 3H), 2.02-1.86 (m, 2H), 1.82-1.58 (m, 5H), 1.00 (app t, *J* = 6.2 Hz, 6H); ^{13}C NMR (CD_3OD) δ171.8 and 171.4 (rot), 169.1, 168.8, 67.1 and 66.6 (rot), 62.3 and 62.2 (rot), 55.5, 53.1, 47.2 and 45.6 (rot), 44.2 and 43.1 (rot), 42.1 and 42.0 (rot), 41.6, 31.5, 27.7 and 27.6 (rot), 25.6 and 25.3 (rot), 24.6, 24.3 and 24.2 (rot), 22.8 and 22.6 (rot), 22.3 and 22.2 (rot); IR *v* (neat) 3397 (v br), 2975, 2870, 2362, 1681, 1202, 1135; HRMS (FT-ICR-MS) Calcd for $C_{18}H_{33}N_5O_3$ $[M+H]^+$ 368.2654 found 368.2649.

1-(1-[(L-Leucinyl)-glycinyl]-(*S*)-piperidin-3-yl)-L-proline amide x 2 TFA ((*S*)-LGpipP) ((*S*)-23). Deprotection of (*S*)-**22** (132 mg, 0.3 mmol) by TFA/dichloromethane (1:1) (6 mL) as described for (*S*)-**pipG** ((*S*)-**3**) gave the TFA salt of (*S*)-**LGpipP** ((*S*)-**23**) as a light brown solid (163 mg, 98%). Analytical reversed phase HPLC (the mobile phase was 10.8-37.2% CH_3CN in H_2O for 30 min with 0.1% TFA throughout) t_R 25.7 min, 100% purity; ^{1}H NMR (CD_3OD) δ 4.66 (app d, 1H), 4.48-4.34 (m, 2H), 4.23-4.08 (m, 2H), 3.96-3.90 (m, 1H), 3.84 (app d, 1H), 3.79-3.71 (m, 1H), 3.41-3.24 (m, 2H), 3.13-3.04 (m, 1H), 2.88-2.78 (m, 1H), 2.58-2.49 (m, 1H), 2.32-2.23 (m, 1H), 2.20-2.08 (m, 2H), 2.03-1.88 (m, 2H), 1.86-1.60 (m, 4H), 1.00 (app t, *J* = 6.3 Hz, 6H);^{13}C NMR (CD_3OD) δ 171.5, 171.3, 169.0, 67.0, 61.9, 54.4, 53.1, 45.5, 44.4, 41.9, 41.6, 31.3 and 31.0 (rot), 27.5, 25.4, 25.0, 24.4, 22.9 and 22.6 (rot), 22.3; IR *v* (neat) 3418, 2976, 2871, 2360, 1681, 1203, 1140; HRMS (FT-ICR-MS) Calcd for $C_{18}H_{33}N_5O_3$ $[M+H]^+$ 368.2654 found 368.2649.

NMR spectral assignments of compounds **(*R*)-1**, **(*R*)-2,** **(*R*)-3**, **(*R*)-4**, **(*R*)-7** and **(*R*)-10**

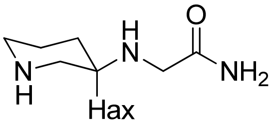
For the assignment of ^{1}H and ^{13}C NMR signals, the numbering used is shown in the structure below.



(-)-Ethyl 2-[(1-*tert*-butoxycarbonyl-(*R*)-piperidin-3-yl)amino]acetate ((*R*)-1). ^{1}H NMR δ 4.18 (q, *J* = 7.1 Hz, 2H, $^{Ethyl}CH_2$), 3.80-3.73 (m, 2H, ^{Pip}H5a, ^{Pip}H1a), 3.55-3.41 (AB system, 2H, ^{Gly}H1), 2.86-2.51 (m, 3H, ^{Pip}H5b, ^{Pip}H1b, ^{Pip}H2), 2.20-1.80 (m, 2H, ^{Gly}NH, ^{Pip}H3a), 1.73-1.64 (m, 1H, ^{Pip}H4a), 1.47-1.21 (m, 2H, ^{Pip}H3b, ^{Pip}H4b), 1.44 (s, 9H, $^{Boc}CH_3$), 1.26 (t, *J* = 7.1 Hz, 3H, $^{Ethyl}CH_3$); ^{13}C NMR δ 172.2 (^{Gly}CO), 154.6 (^{Boc}CO), 79.2 (^{Boc}C), 60.6 ($^{Ethyl}CH_2$), 53.3 (^{Pip}C2), 48.6 (^{Pip}C1), 48.1 (^{Gly}C1), 43.9 (^{Pip}C5), 31.1 (^{Pip}C3), 28.2 (3C, $^{Boc}CH_3$), 23.2 (^{Pip}C4), 14.0 ($^{Ethyl}CH_3$); $[\alpha]_D^{20}$ -8.5 (*c* 1.0, $CHCl_3$).

(-)-2-[(1-*tert*-Butoxycarbonyl-(*R*)-piperidin-3-yl)amino]acetamide ((*R*)-2). ^{1}H NMR δ 7.05 (br s, 2H, $^{amid}NH_2$), 5.53 (br s, 1H, ^{Gly}NH), 3.97-3.54 (m, 2H, ^{Pip}H5a, ^{Pip}H1a), 3.38-3.25 (AB system, 2H, ^{Gly}H1), 3.16-2.67 (m, 2H, ^{Pip}H5b, ^{Pip}H1b), 2.56 (app tt, *J* = 8.1, 8.1, 3.7, 3.7 Hz, 1H, ^{Pip}H2), 1.97-1.84 (m, 1H, ^{Pip}H3a), 1.73-1.63 (m, 1H, ^{Pip}H4a), 1.51-1.23 (m, 2H, ^{Pip}H4b, ^{Pip}H3b), 1.45 (s, 9H, $^{Boc}CH_3$); ^{13}C NMR δ 175.1 (^{Gly}CO), 154.6 (^{Boc}CO), 79.2 (^{Boc}C), 53.7 (^{Pip}C2), 49.5 (^{Gly}C1), 48.7 (^{Pip}C1), 43.8 (^{Pip}C5), 30.9 (^{Pip}C3), 28.1 (3C, $^{Boc}CH_3$), 22.9 (^{Pip}C4); $[\alpha]_D^{20}$ -4.6 (*c* 1.0, $CHCl_3$).

(-)-2-((*R*)-Piperidin-3-ylamino)acetamide x 2 TFA ((*R*)-pipG) ((*R*)-3). ^{1}H NMR (CD_3OD) δ 4.00-3.88 (AB system, 2H, ^{Gly}H1), 3.73 (dd, *J* = 12.1, 4.0 Hz, 1H, ^{Pip}H1eq), 3.63-3.51 (app tt, *J* = 11.4, 11.4, 4.1, 4.1 Hz, 1H, ^{Pip}H2ax), 3.40 (app dd, *J* = 12.9, 3.6 Hz, 1H, ^{Pip}H5eq), 3.08 (app t, *J* = 11.7 Hz, 1H, ^{Pip}H1ax), 2.96 (dt, *J* = 12.7, 12.7, 3.2 Hz, 1H, ^{Pip}H5ax), 2.32-2.24 (m, 1H, ^{Pip}H3eq), 2.14-2.04 (m, 1H, ^{Pip}H4eq), 1.87-1.78 (m, 1H, ^{Pip}H4ax), 1.71 (app ddt, *J* = 12.5, 12.5, 3.6 Hz, 1H, ^{Pip}H3ax); ^{13}C NMR (CD_3OD) δ 168.5 (^{Gly}CO), 53.0 (^{Pip}C2), 46.6 (^{Gly}C1), 45.0 (^{Pip}C1), 44.6 (^{Pip}C5), 26.2 (^{Pip}C3), 21.6 (^{Pip}C4). Suggested ring conformation of (*R*)-**pipG** ((*R*)-**3**):

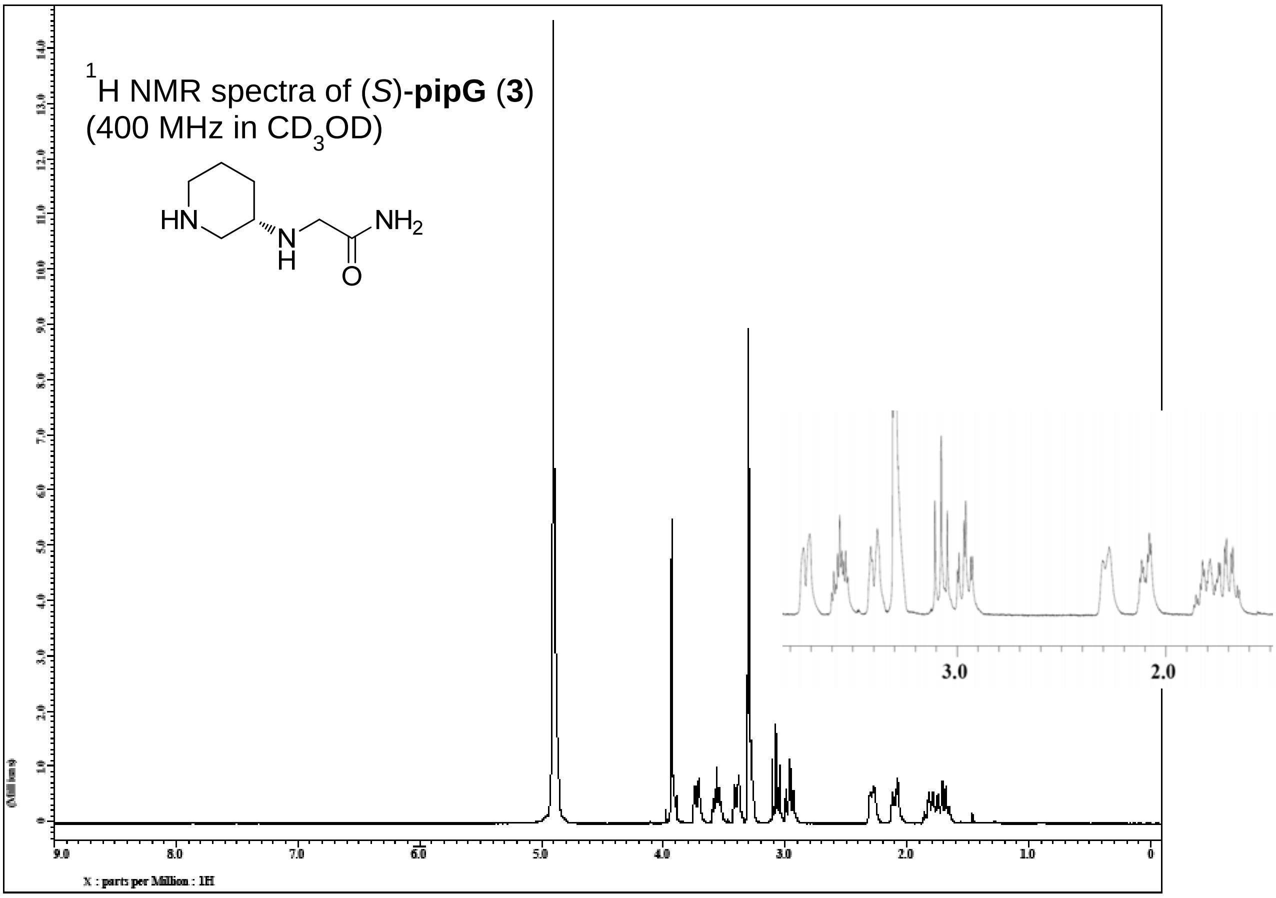


(-)-Ethyl 2-[(benzyloxycarbonyl)(1-*tert*-butoxycarbonyl-(*R*)-piperidin-3-yl)amino]acetate ((*R*)-4). ^{1}H NMR (rotamers) δ 7.59-7.20 (m, 5H, ^{Cbz}CH), 5.19 and 5.10 (rot, s, 2H, $^{Cbz}CH_2$), 4.34-3.72 (m, 6H, $^{Ethyl}CH_2$, ^{Pip}H1a, ^{Pip}H5a, ^{Gly}H1), 2.80-2.43 (m, 3H, ^{Pip}H1b, ^{Pip}H5b, ^{Pip}H2), 2.03-1.87 (m, 1H, ^{Pip}H3a), 1.80-0.98 (m, 1H, ^{Pip}H4a), 1.43-1.17 (m, 2H, ^{Pip}H3b, ^{Pip}H4b), 1.43 and 1.37 (rot, s, 9H, $^{Boc}CH_3$), 1.26 and 1.17 (rot, t, J = 7.1 Hz, 3H, $^{Ethyl}CH_3$); ^{13}C NMR δ 169.7 (^{Gly}CO), 155.8, 155.1, 154.5 and 154.3 (rot, 2C, ^{Boc}CO and ^{Cbz}CO), 136.1 (^{Cbz}C1), 128.3 and 128.2 (2C, rot, ^{Cbz}C3), 127.8 (^{Cbz}C4), 127.5 (2C, ^{Cbz}C2), 79.4 (^{Boc}C), 67.4 and 67.1 (rot, $^{Cbz}CH_2$), 60.9 ($^{Ethyl}CH_2$), 53.1 and 52.9 (rot, ^{Pip}C2), 47.0 and 46.5 (^{Pip}C1), 45.6 and 45.3 (^{Gly}C1), 43.2 (^{Pip}C5), 28.7 (^{Pip}C3), 28.1 (3C, $^{Boc}CH_3$), 24.4 (^{Pip}C4), 13.9 ($^{Ethyl}CH_3$).

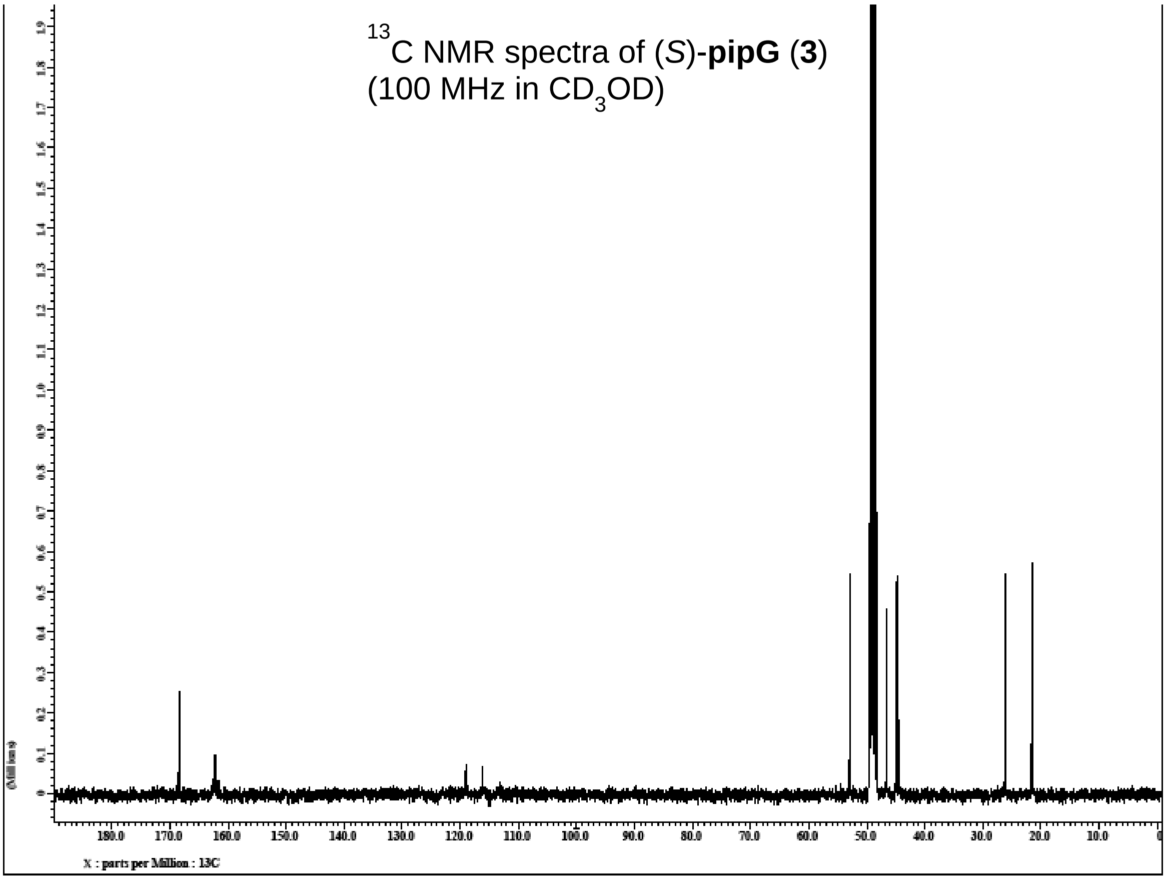
(-)-2-[(1-L-Leucinyl-(*R*)-piperidin-3-yl)amino]acetamide x 2 TFA ((*R*)-LpipG) ((*R*)-7). ^{1}H (CD_3OD) NMR δ 4.75 (app d, 1H, ^{pip}H1eq), 4.48-4.38 (m, 1H, ^{Leu}H1), 3.98-3.86 (m, 2H, ^{Gly}H1), 3.74 (app d, 1H, ^{pip}H5eq), 3.23-3.08 (m, 2H, ^{pip}H2, ^{pip}H5ax), 2.85 (app t, 1H, ^{pip}H1ax), 2.32-2.18 (m, 1H, ^{pip}H3a), 1.98-1.51 (m, 6H, ^{pip}H4, ^{pip}H3b, ^{Leu}H2, ^{Leu}H3), 1.05-0.95 (m, 6H, ^{Leu}H4, ^{Leu}H5).

(-)-2-[(L-Leucinyl-(1-L-leucinyl-(*R*)-piperidin-3-yl))amino]acetamide x 2 TFA ((*R*)-LLpipG) ((*R*)-10). ^{1}H NMR (CD_3OD) δ 4.93-4.82 (m, 1H, $^{C\text{-}Leu}$H1), 4.67 (app d, 1H, ^{pip}H1eq), 4.09 (app d, 1H, $^{N\text{-}Leu}$H1), 4.04-3.83 (m, 3H, ^{pip}H5eq, ^{Gly}H1), 3.82-3.64 (m, 2H, $^{N\text{-}Leu}$H2a, $^{N\text{-}Leu}$H3), 3.47-3.38 (m, 1H, $^{N\text{-}Leu}$H2b), 3.26-3.09 (m, 2H, ^{pip}H5ax, ^{pip}H2), 2.84 (app t, 1H, ^{pip}H1ax), 2.32-2.11 (m, 1H, ^{pip}H3a), 1.99-1.41 (m, 6H, ^{pip}H3b, ^{pip}H4, $^{C\text{-}Leu}$H3, $^{C\text{-}Leu}$H2), 1.04-0.76 (m, 12H, $^{N\text{-}Leu}$H4, $^{N\text{-}Leu}$H5, $^{C\text{-}Leu}$H4, $^{C\text{-}Leu}$H5).

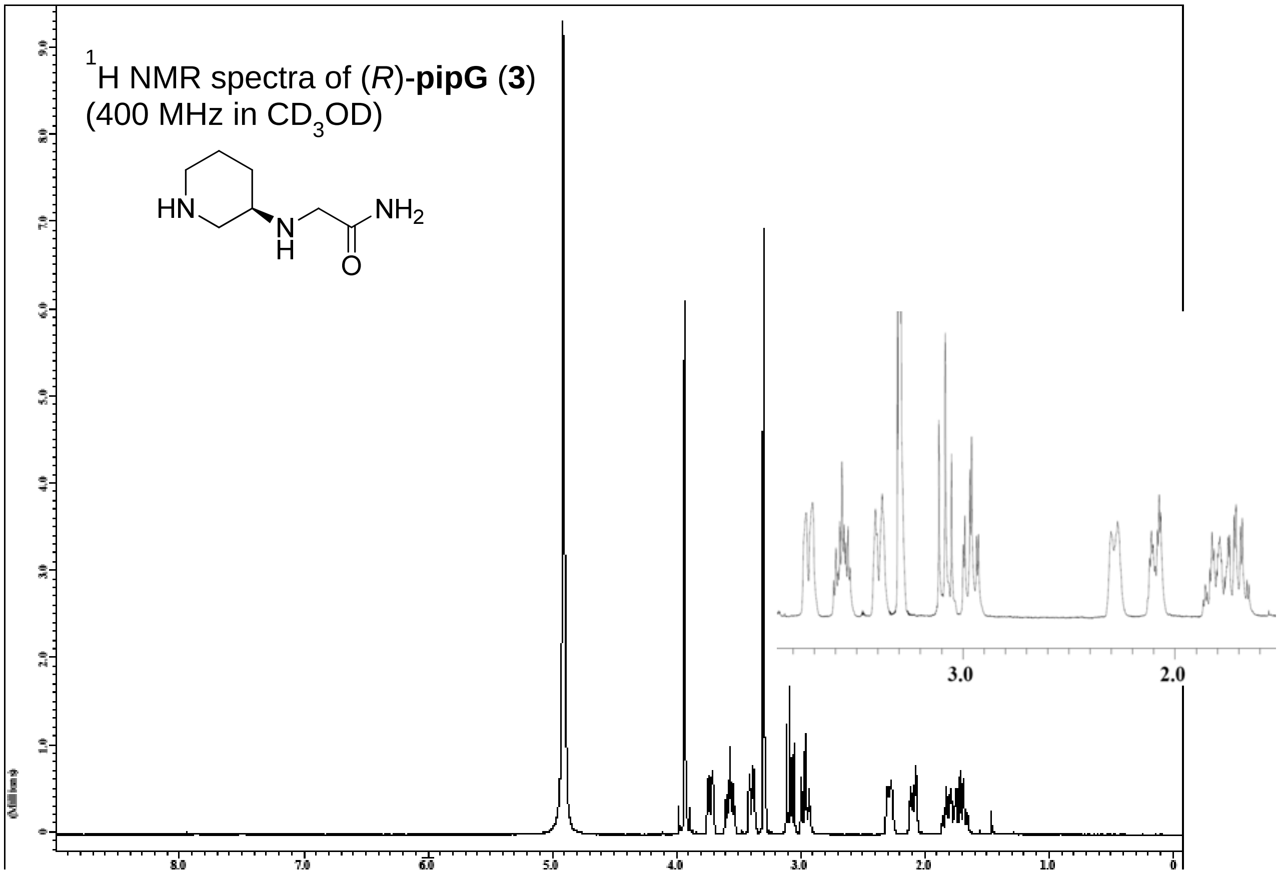
^{1}H and ^{13}C NMR spectra of tested compounds



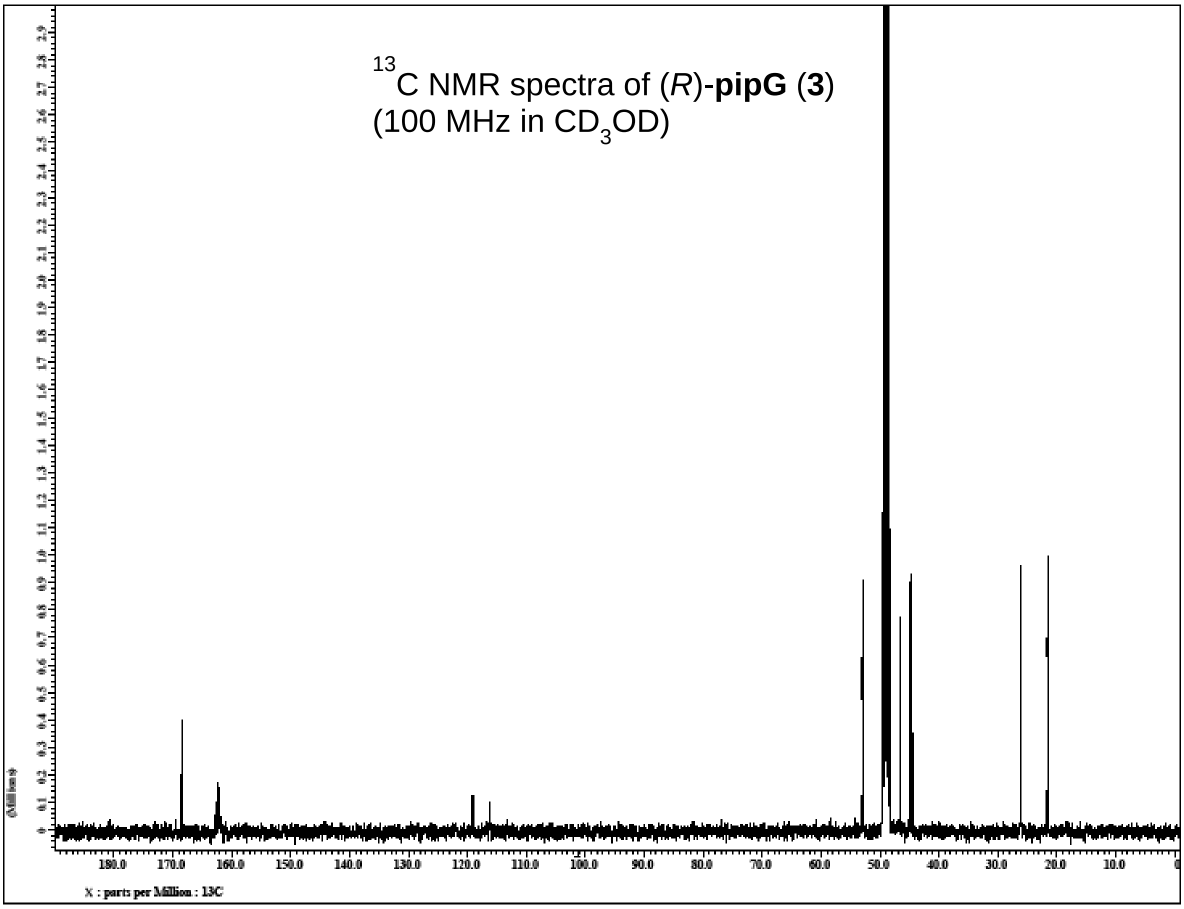
^{1}H NMR spectra of (*S*)-**pipG** (**3**) (400 MHz in CD_3OD)



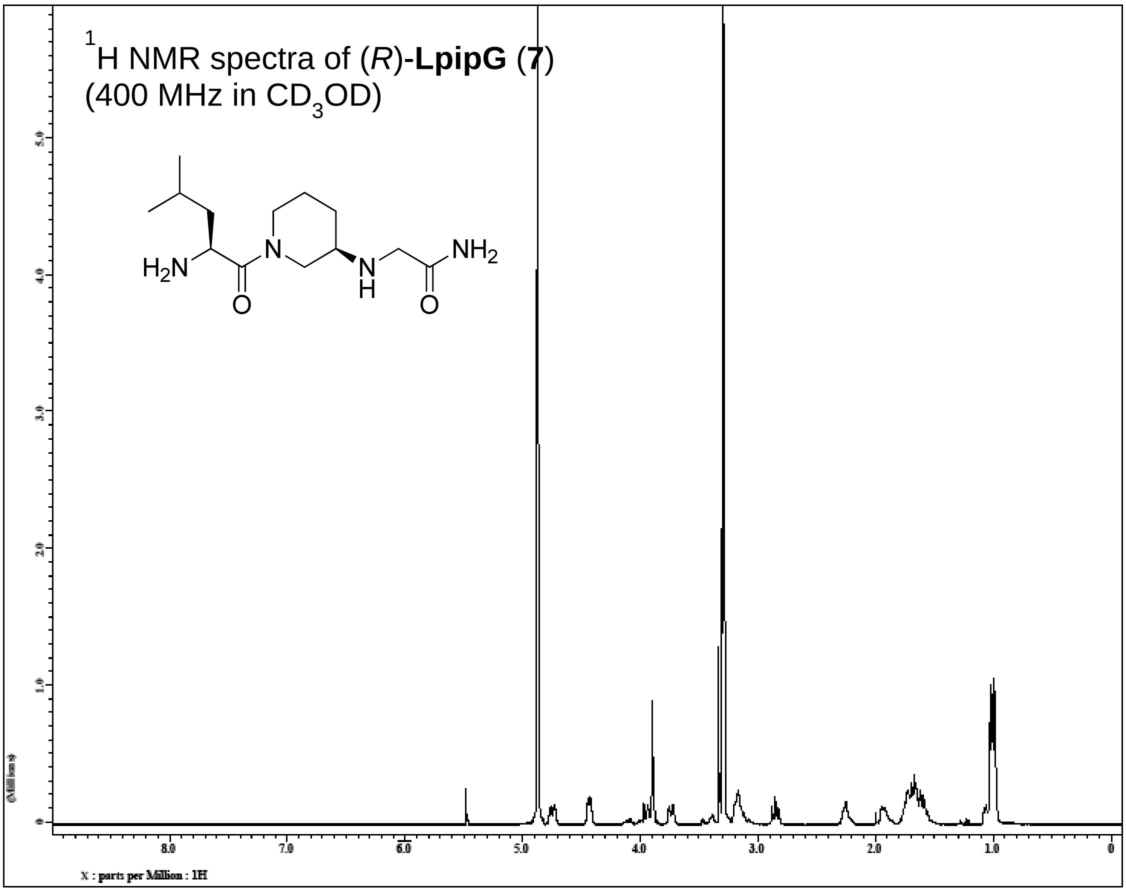
^{13}C NMR spectra of (*S*)-**pipG** (**3**) (100 MHz in CD_3OD)



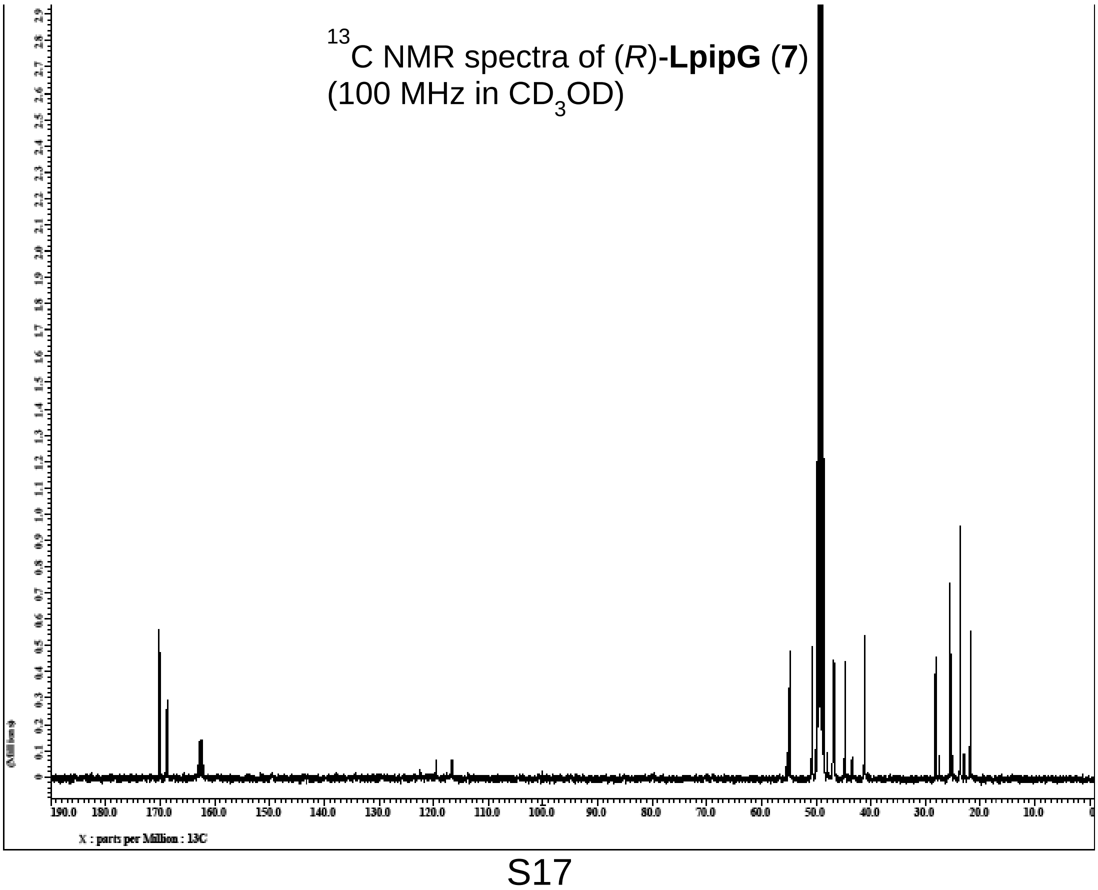
^{1}H NMR spectra of (*R*)-**pipG** (**3**) (400 MHz in CD_3OD)



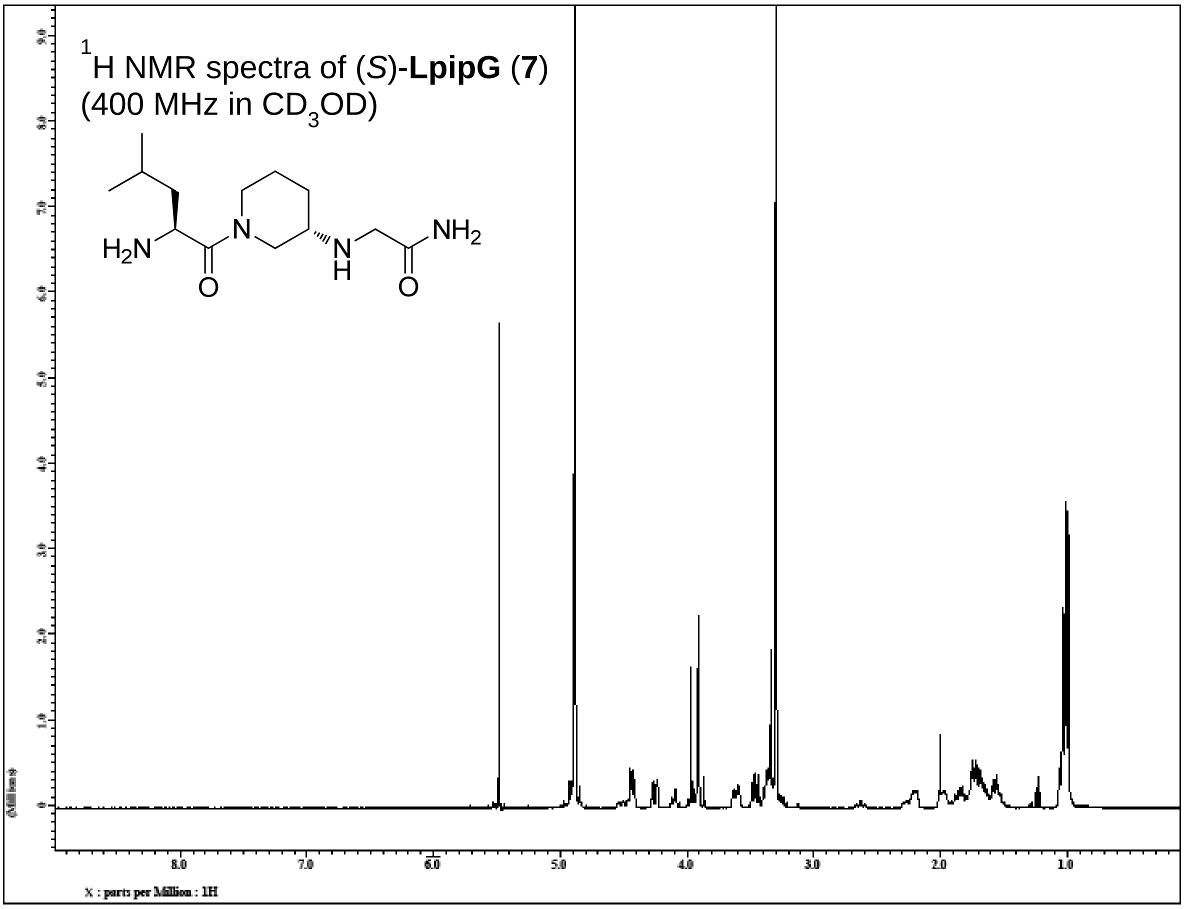
^{13}C NMR spectra of (*R*)-**pipG** (**3**) (100 MHz in CD_3OD)



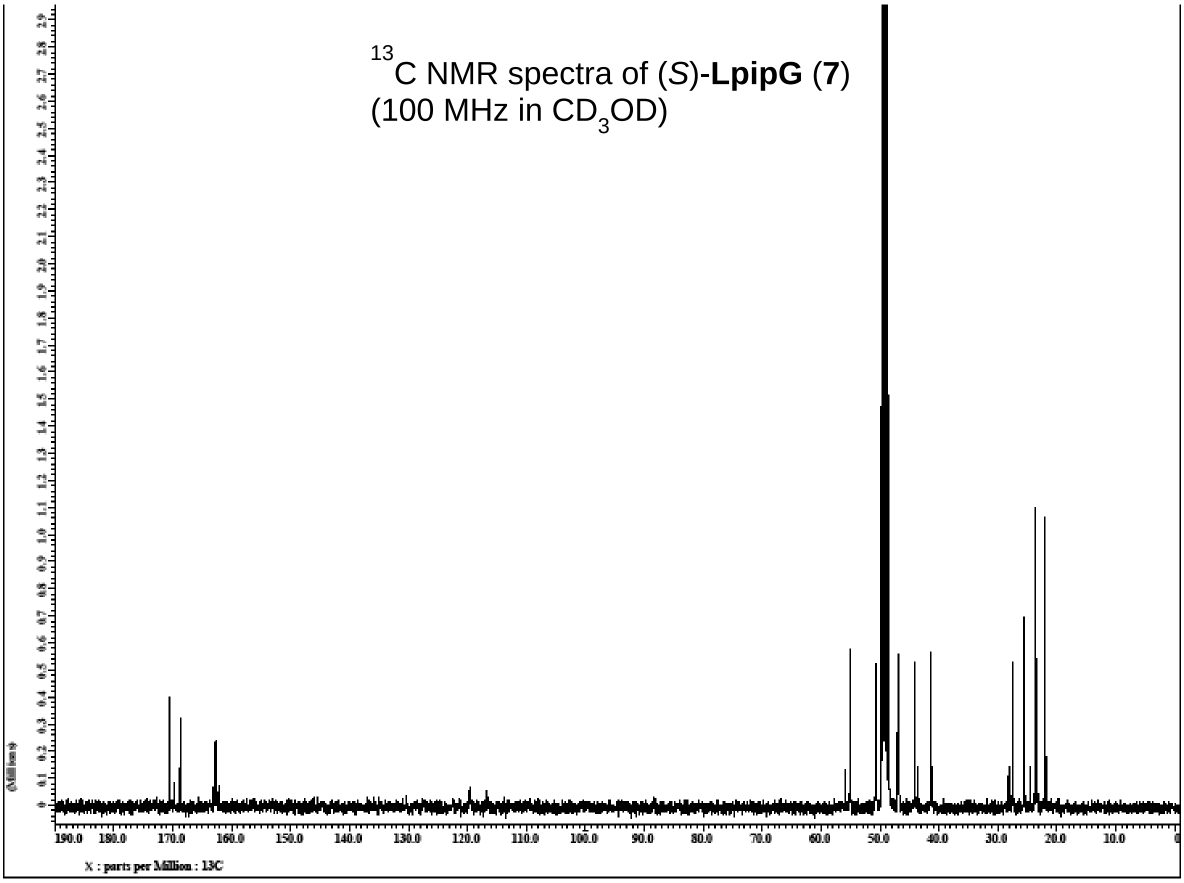
^{1}H NMR spectra of (*R*)-**LpipG** (**7**)
(400 MHz in CD_3OD)



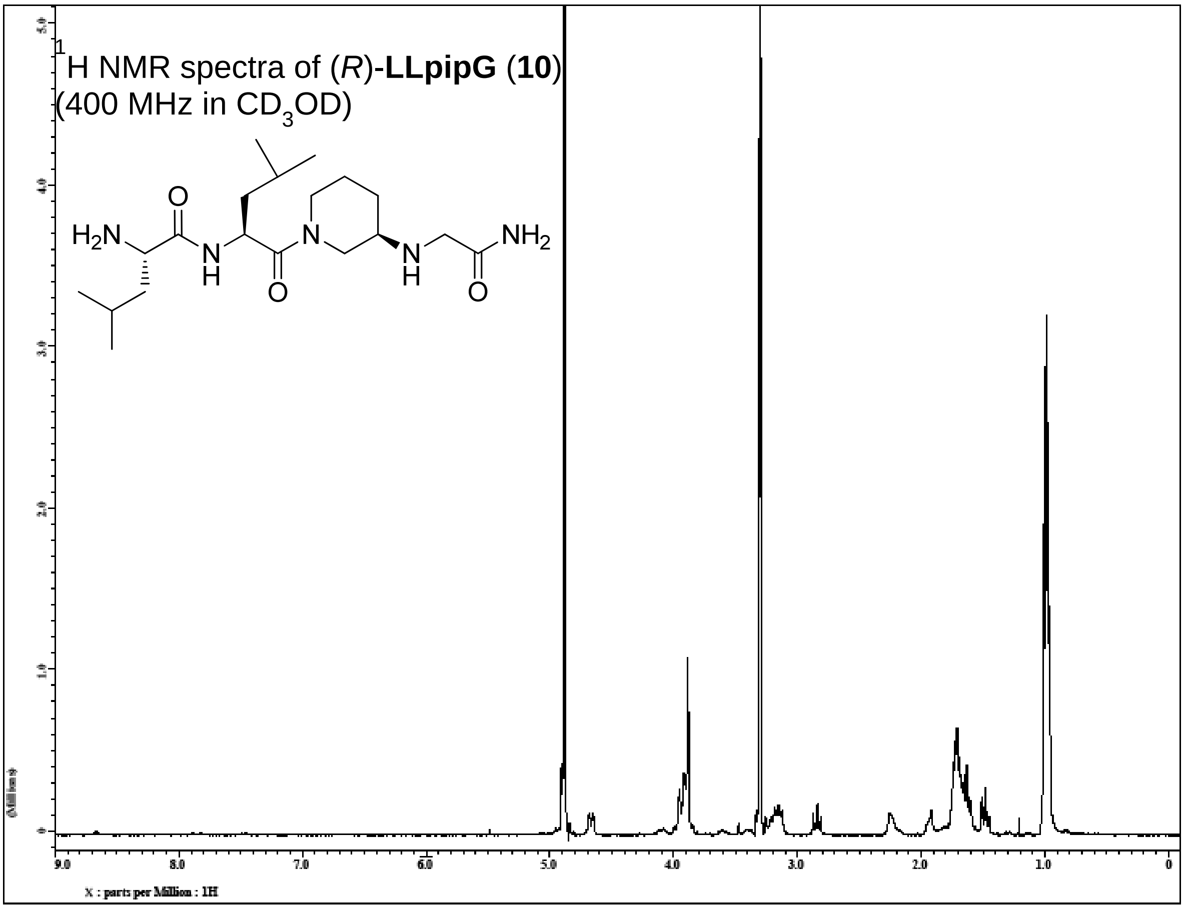
^{13}C NMR spectra of (*R*)-**LpipG** (**7**)
(100 MHz in CD_3OD)



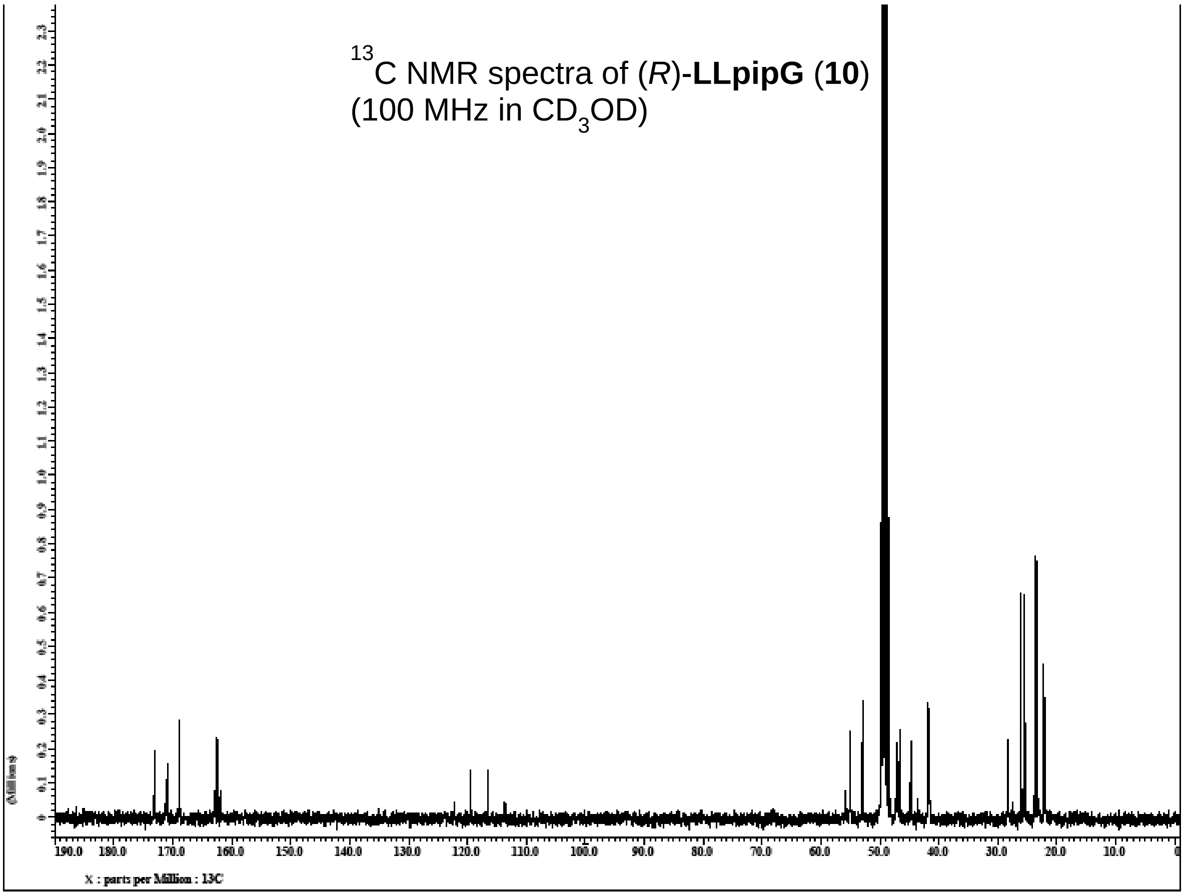
^{1}H NMR spectra of (*S*)-**LpipG** (**7**) (400 MHz in CD_3OD)



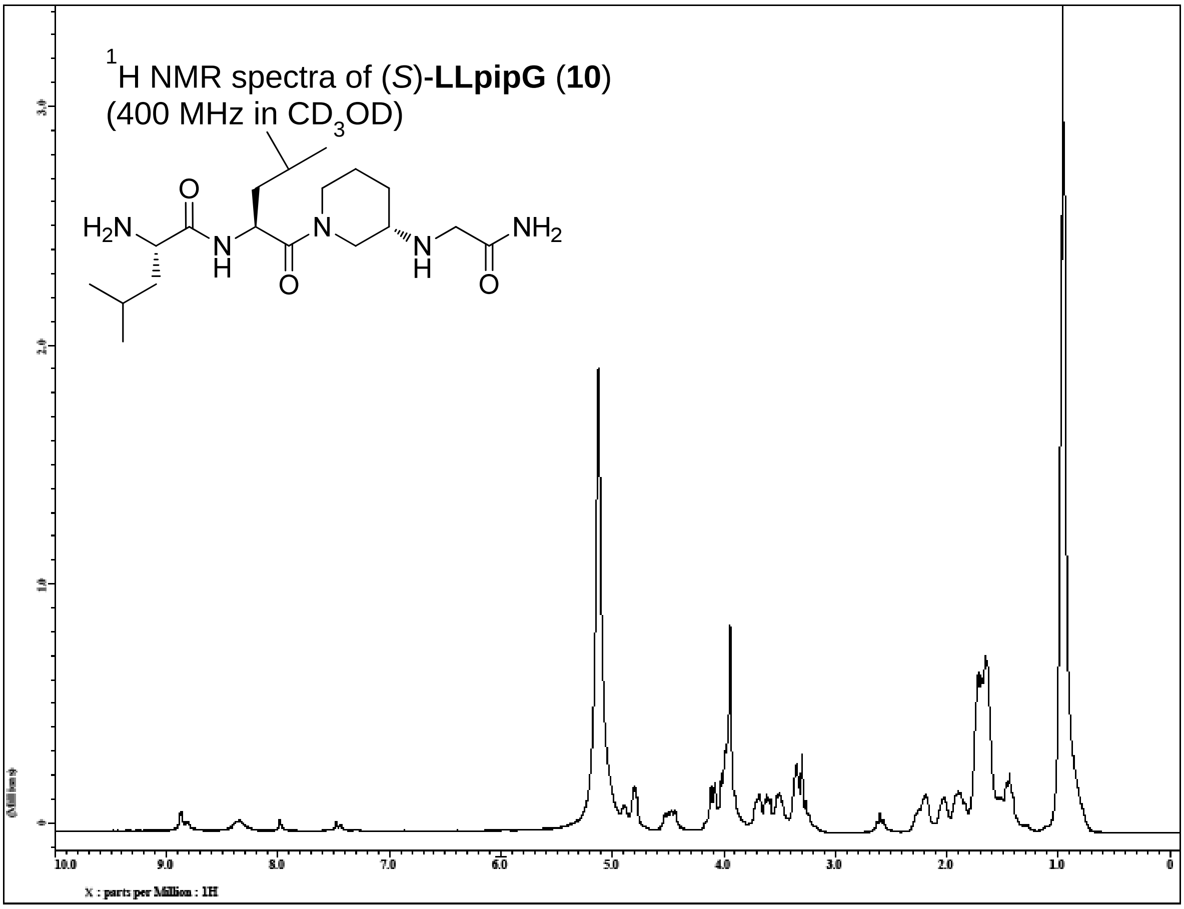
^{13}C NMR spectra of (*S*)-**LpipG** (**7**) (100 MHz in CD_3OD)



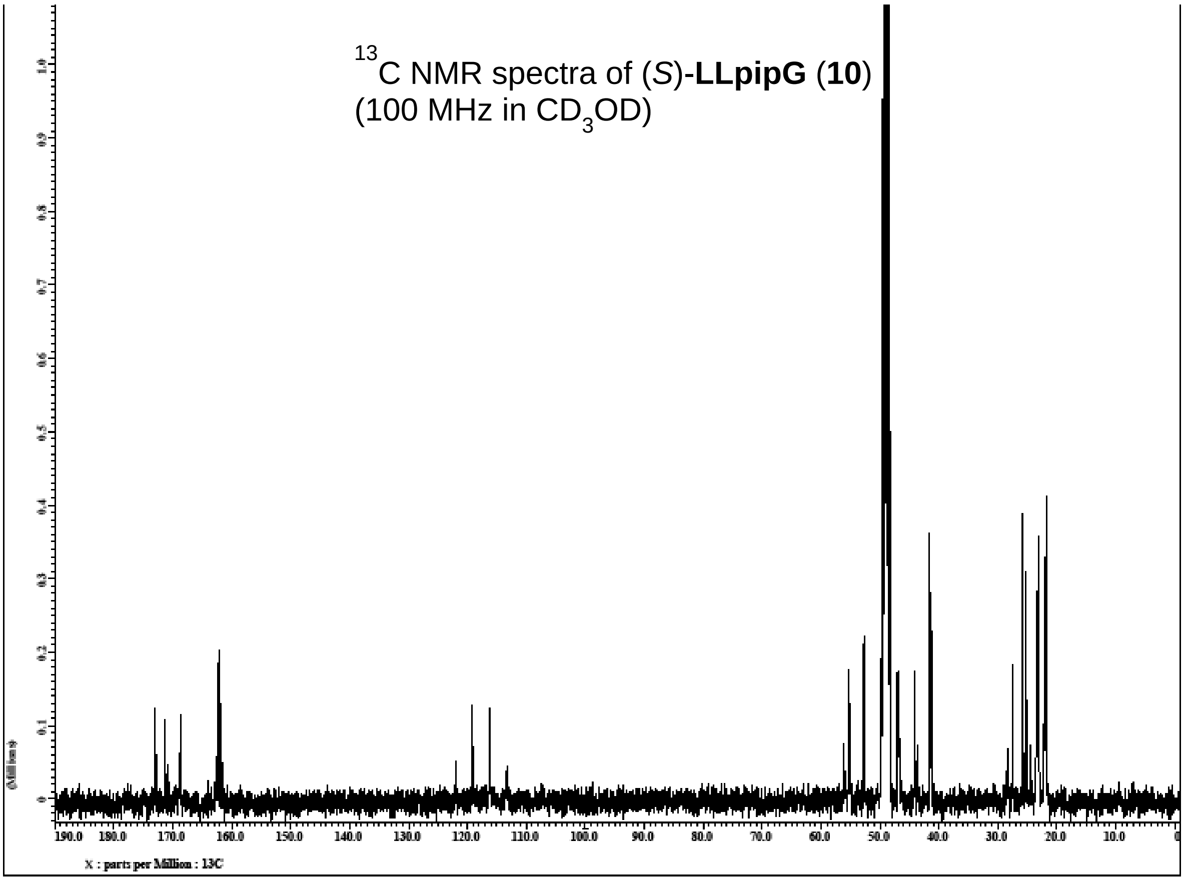
^{1}H NMR spectra of (*R*)-**LLpipG** (**10**)
(400 MHz in CD_3OD)



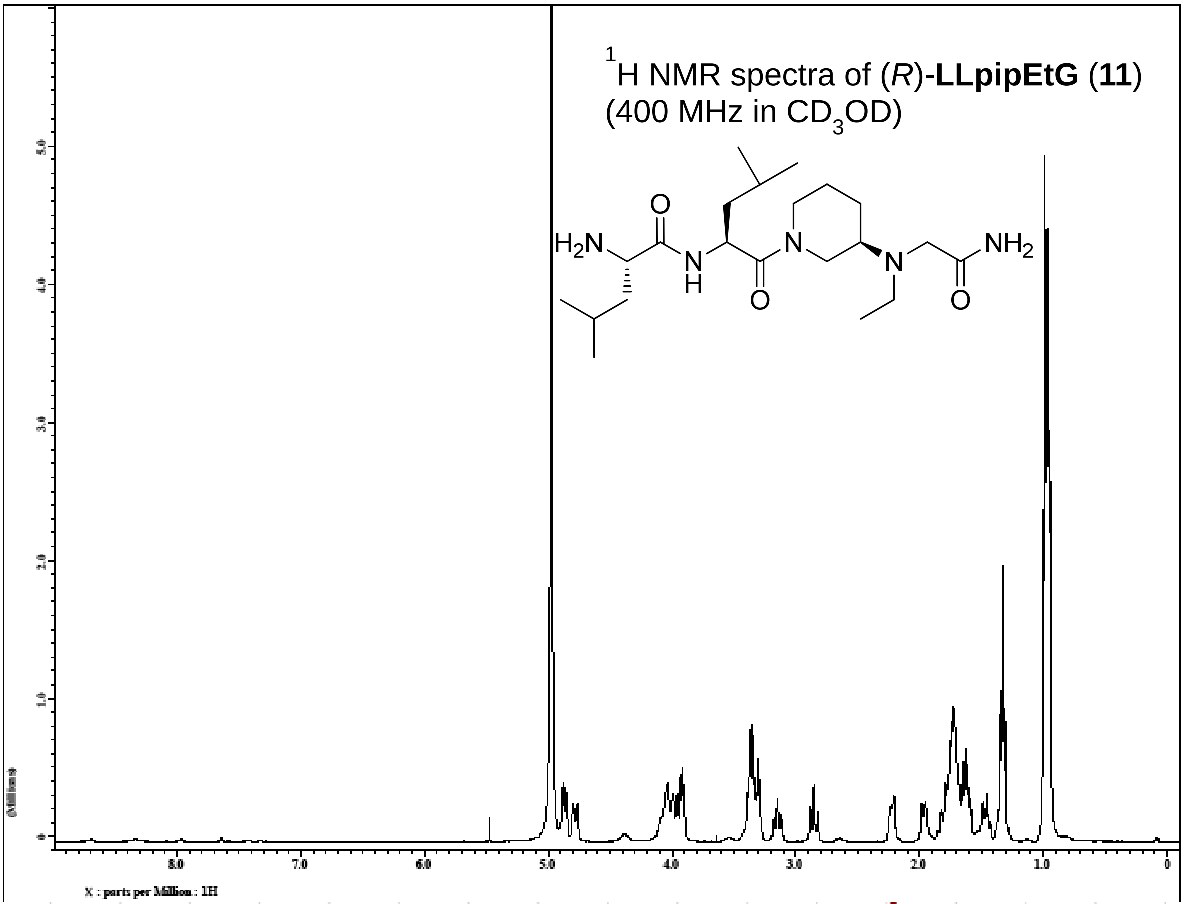
^{13}C NMR spectra of (*R*)-**LLpipG** (**10**)
(100 MHz in CD_3OD)



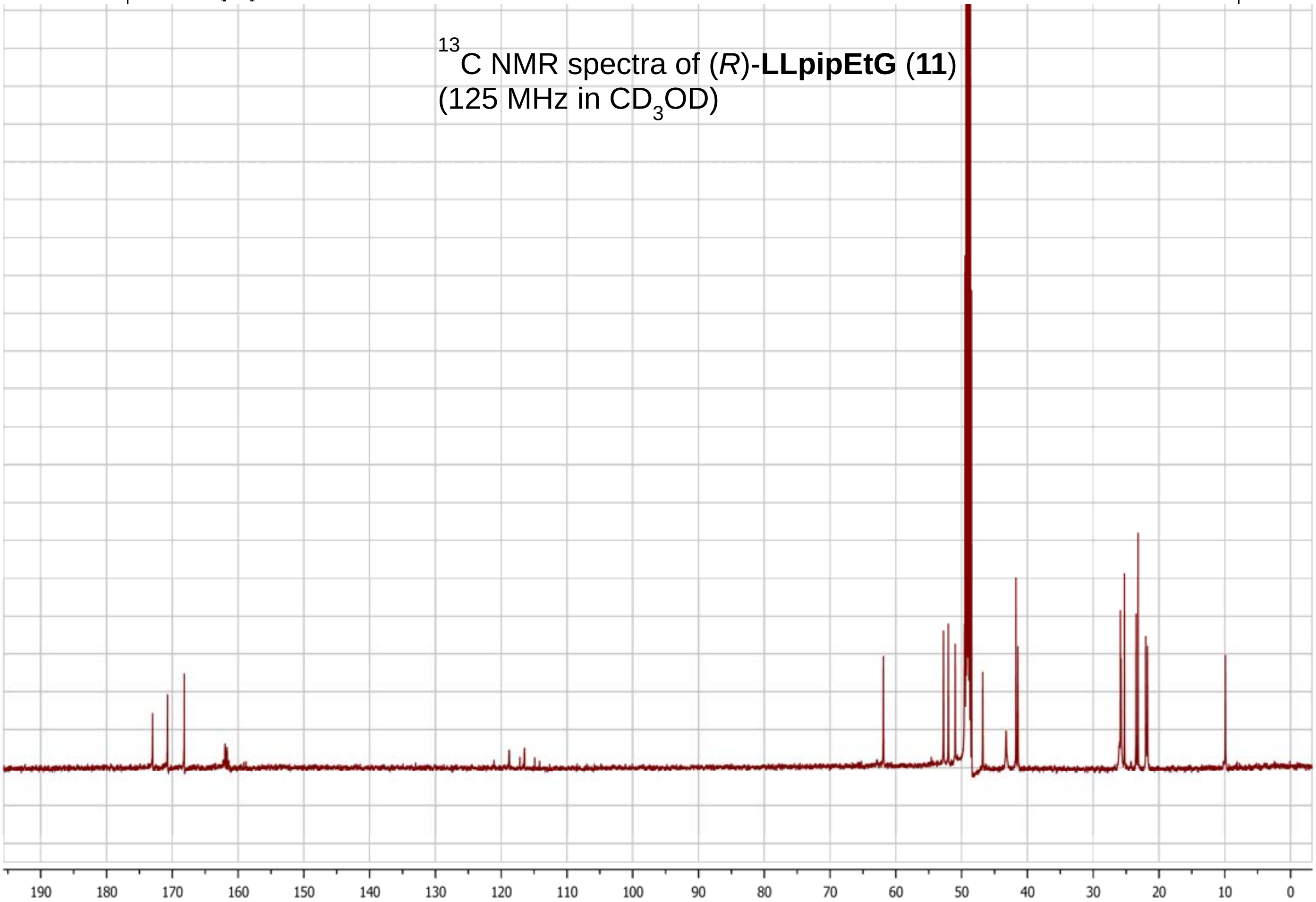
^{1}H NMR spectra of (*S*)-**LLpipG** (**10**)
(400 MHz in CD_3OD)



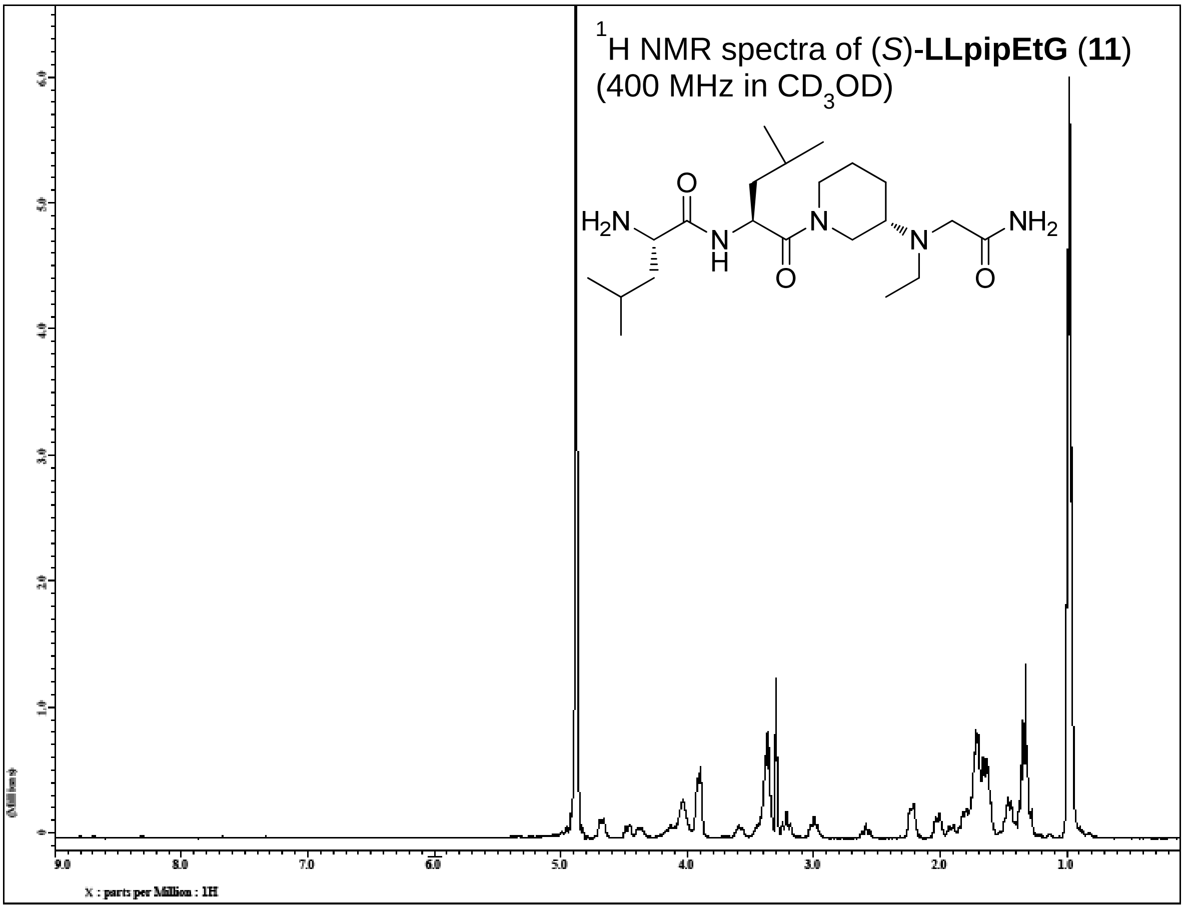
^{13}C NMR spectra of (*S*)-**LLpipG** (**10**)
(100 MHz in CD_3OD)



^{1}H NMR spectra of (*R*)-**LLpipEtG** (**11**)
(400 MHz in CD_3OD)

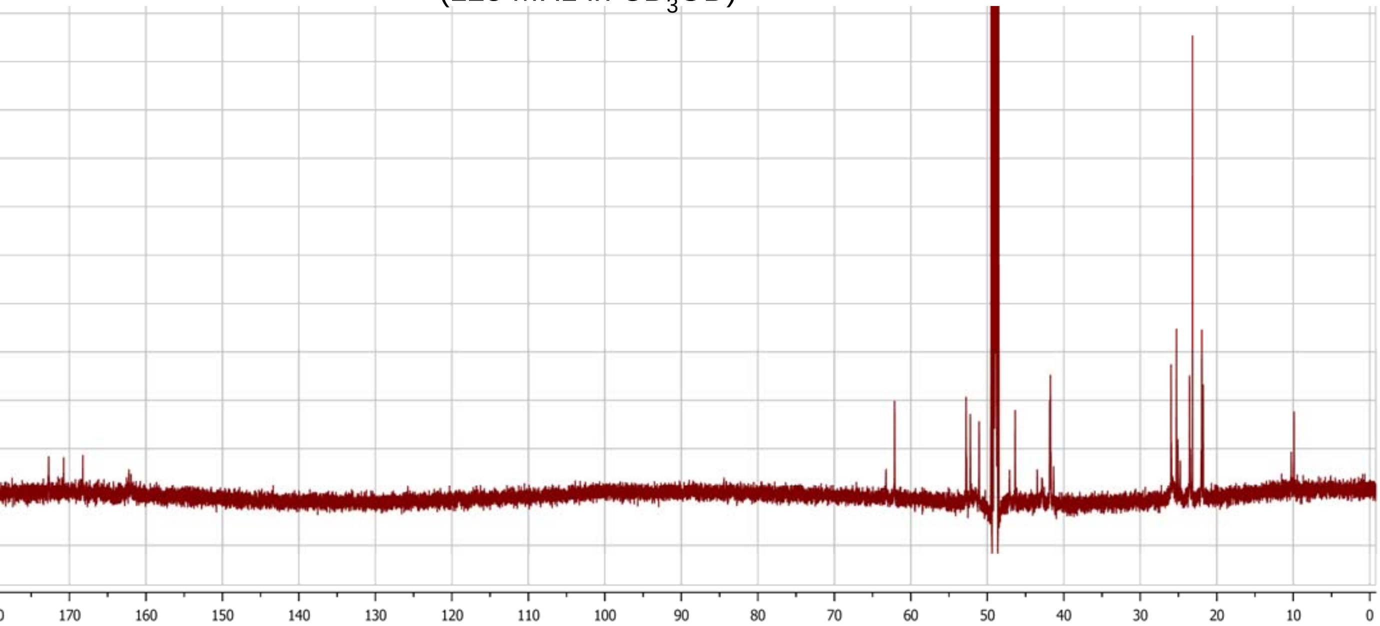


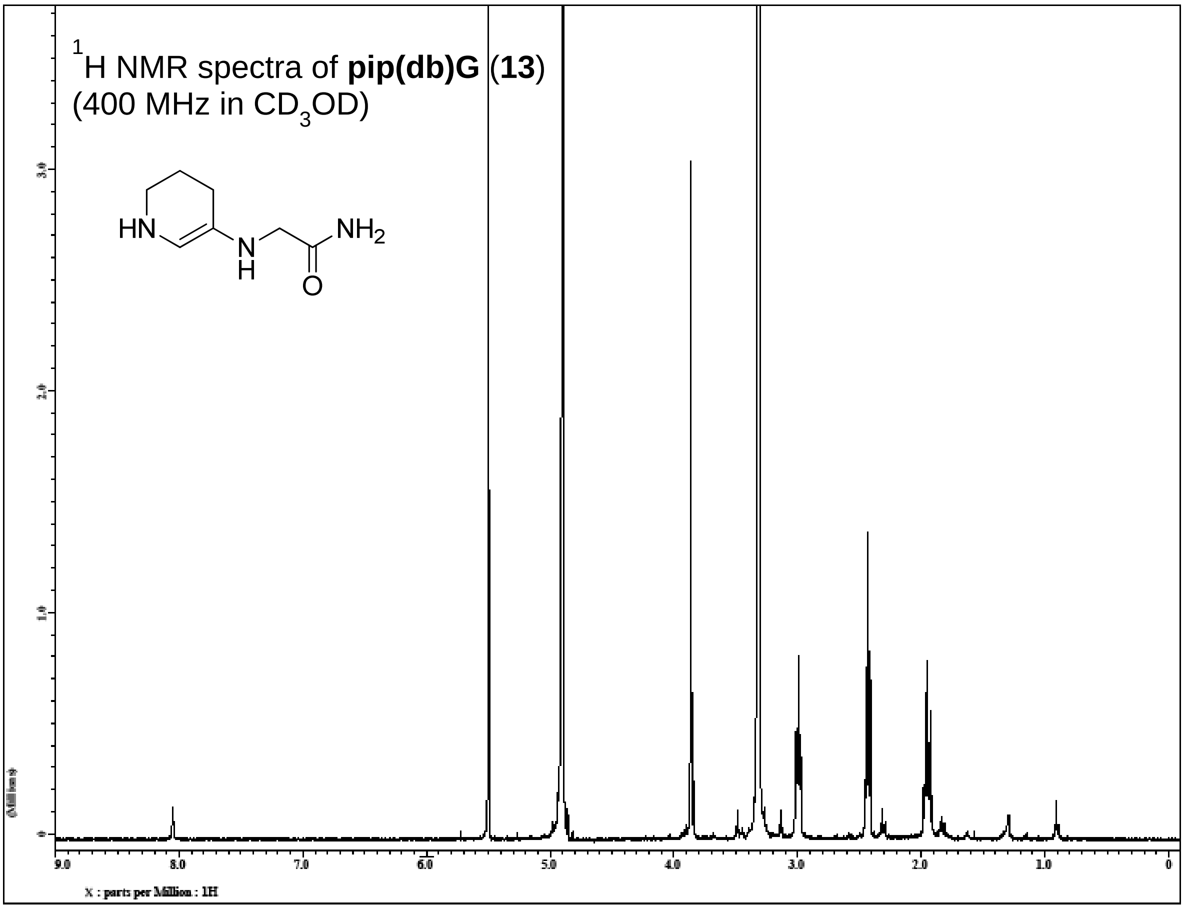
^{13}C NMR spectra of (*R*)-**LLpipEtG** (**11**)
(125 MHz in CD_3OD)



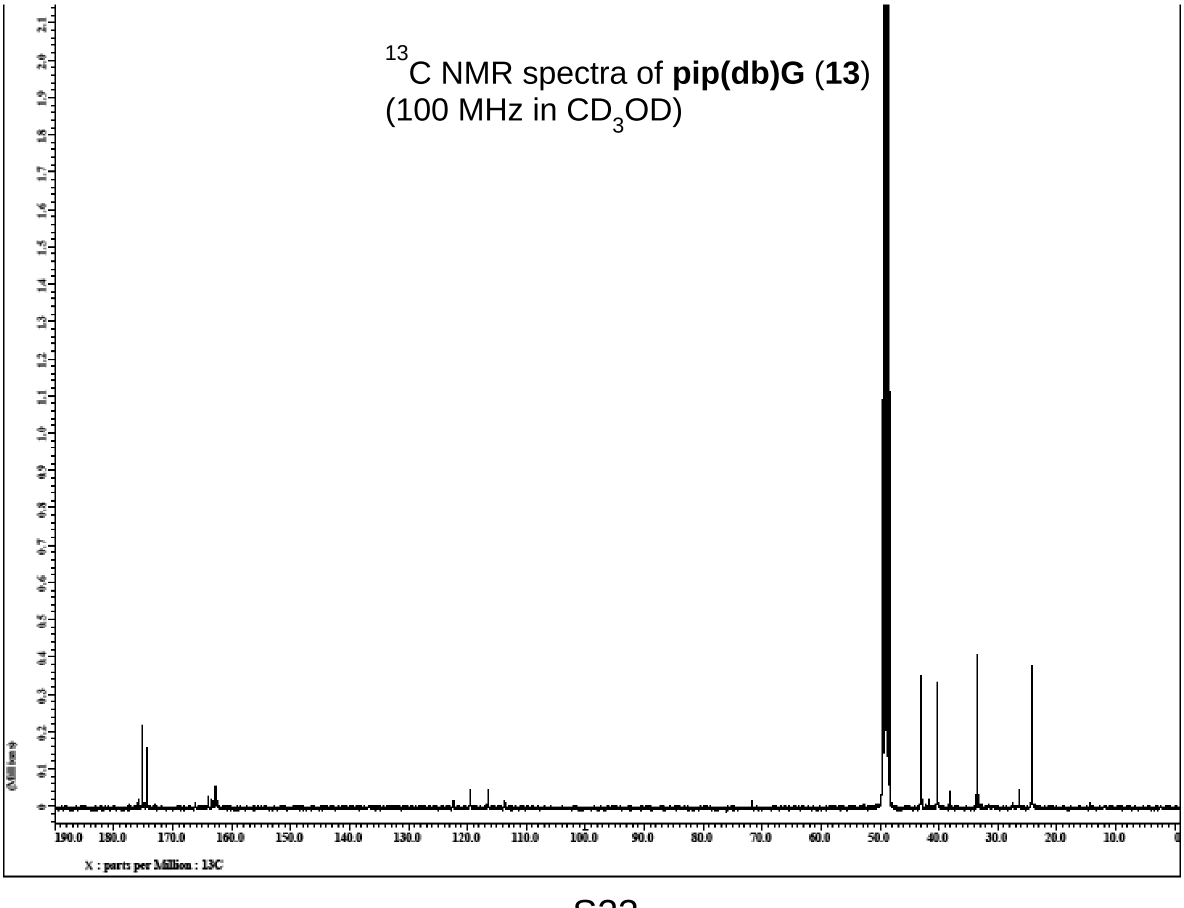
^{1}H NMR spectra of (*S*)-**LLpipEtG** (**11**) (400 MHz in CD_3OD)

^{13}C NMR spectra of (*S*)-**LLpipEtG** (125 MHz in CD_3OD)

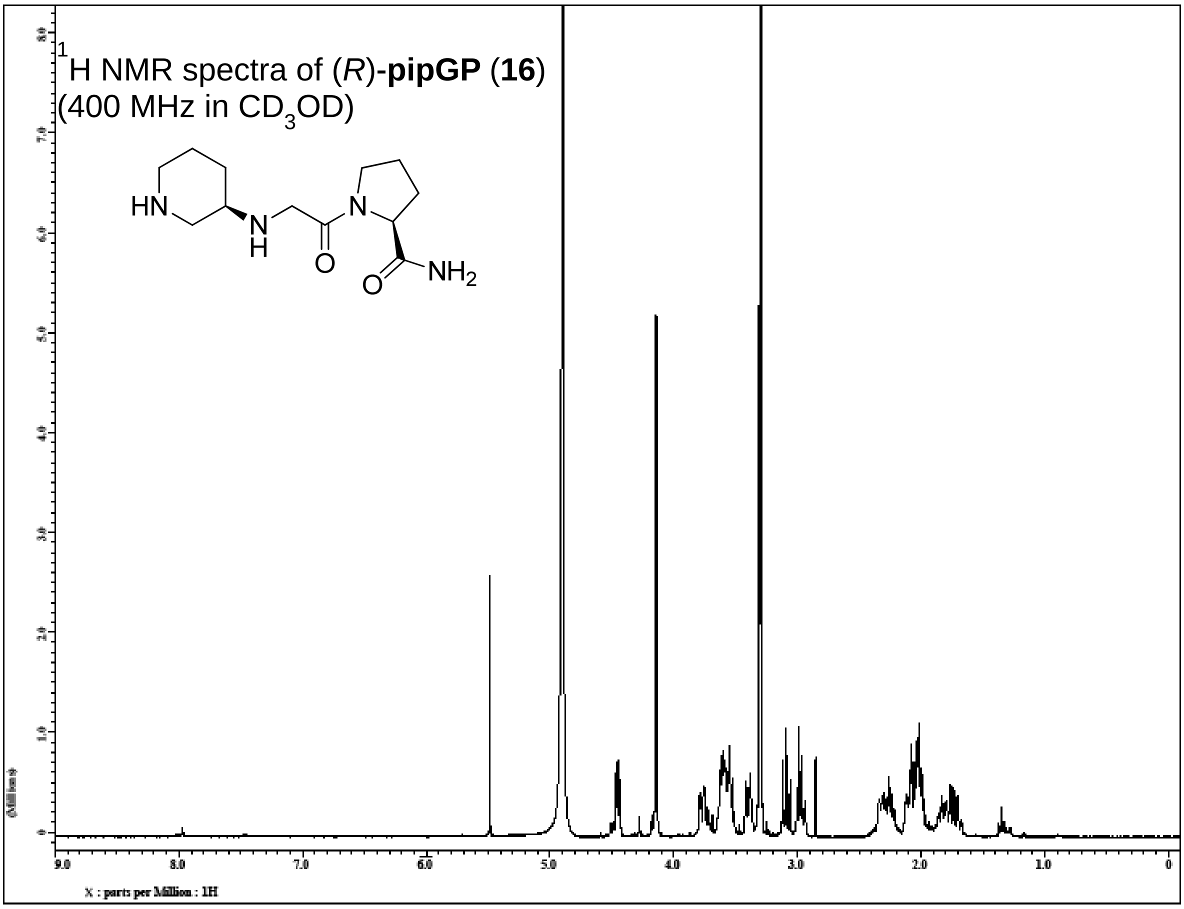




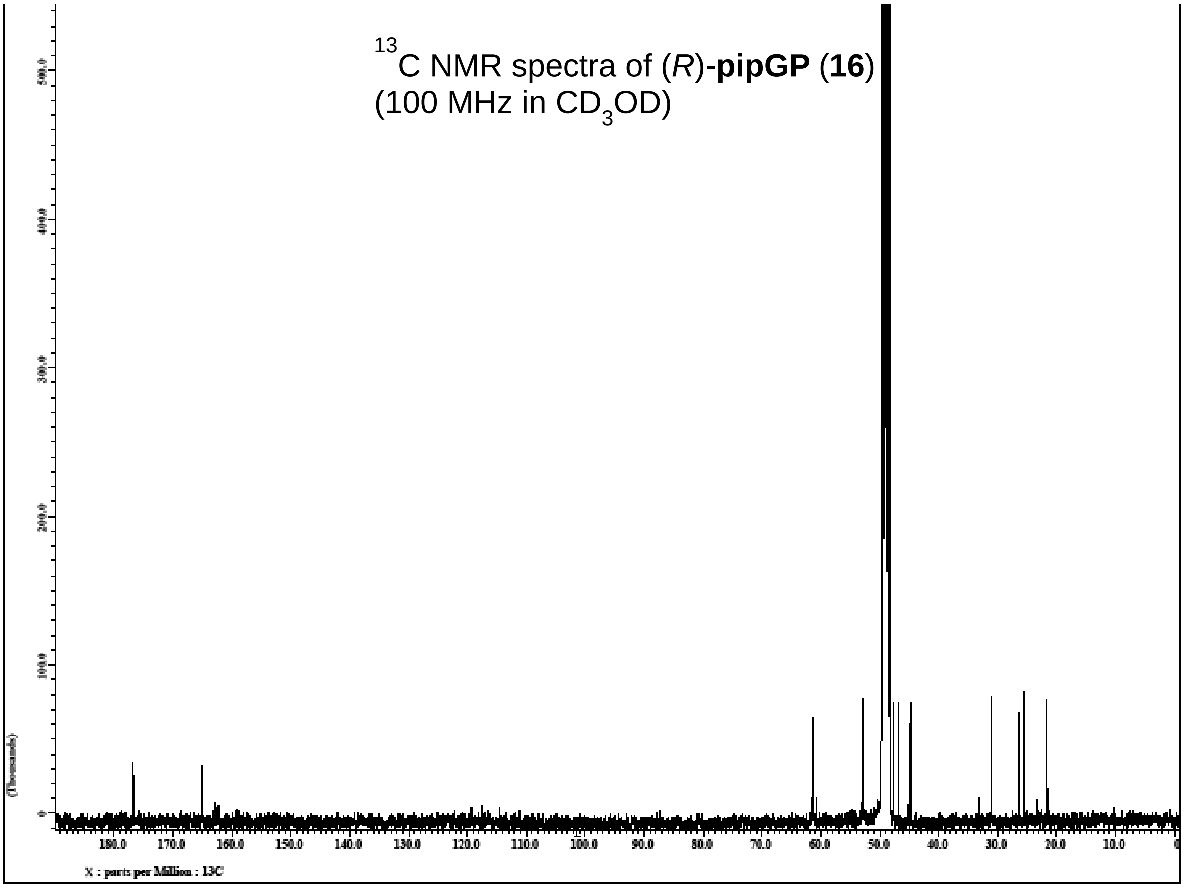
^{1}H NMR spectra of **pip(db)G** (**13**)
(400 MHz in CD_3OD)



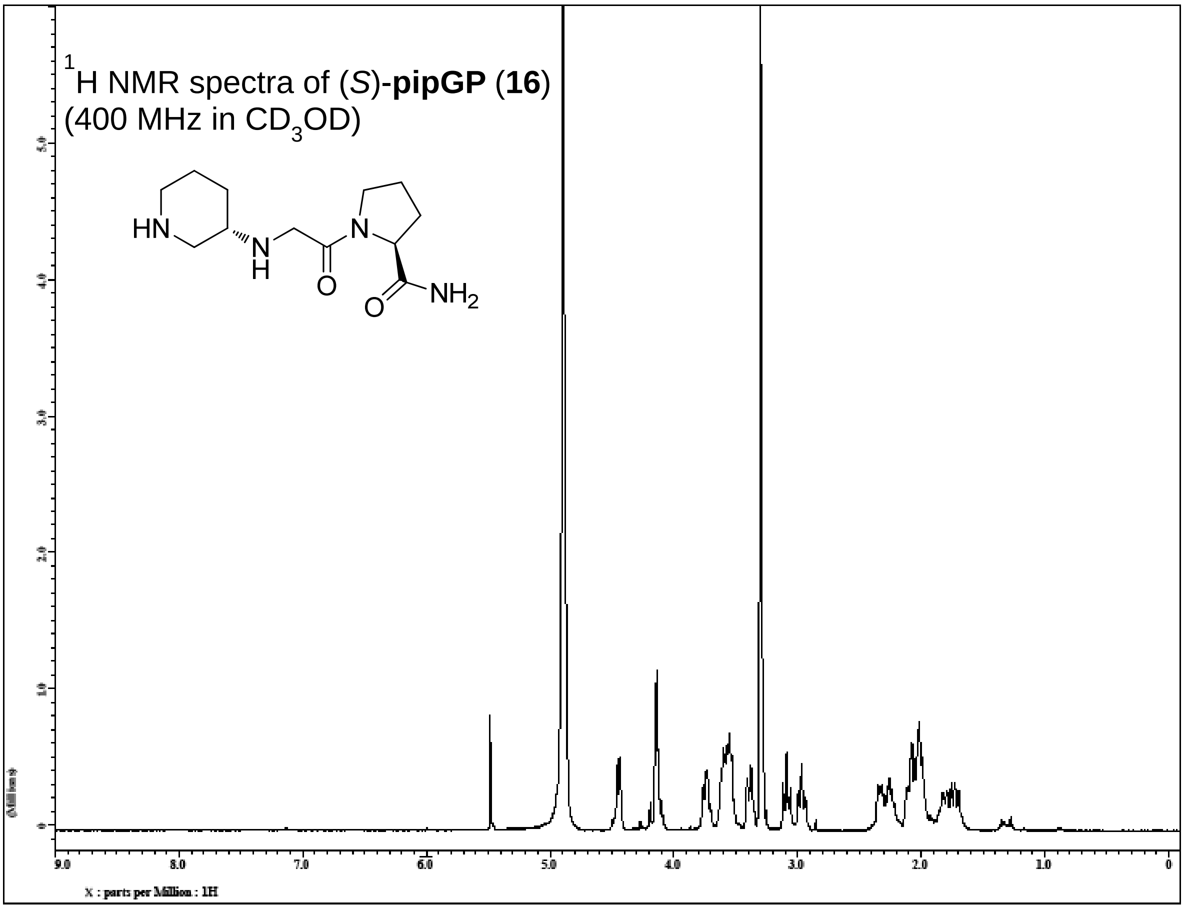
^{13}C NMR spectra of **pip(db)G** (**13**)
(100 MHz in CD_3OD)



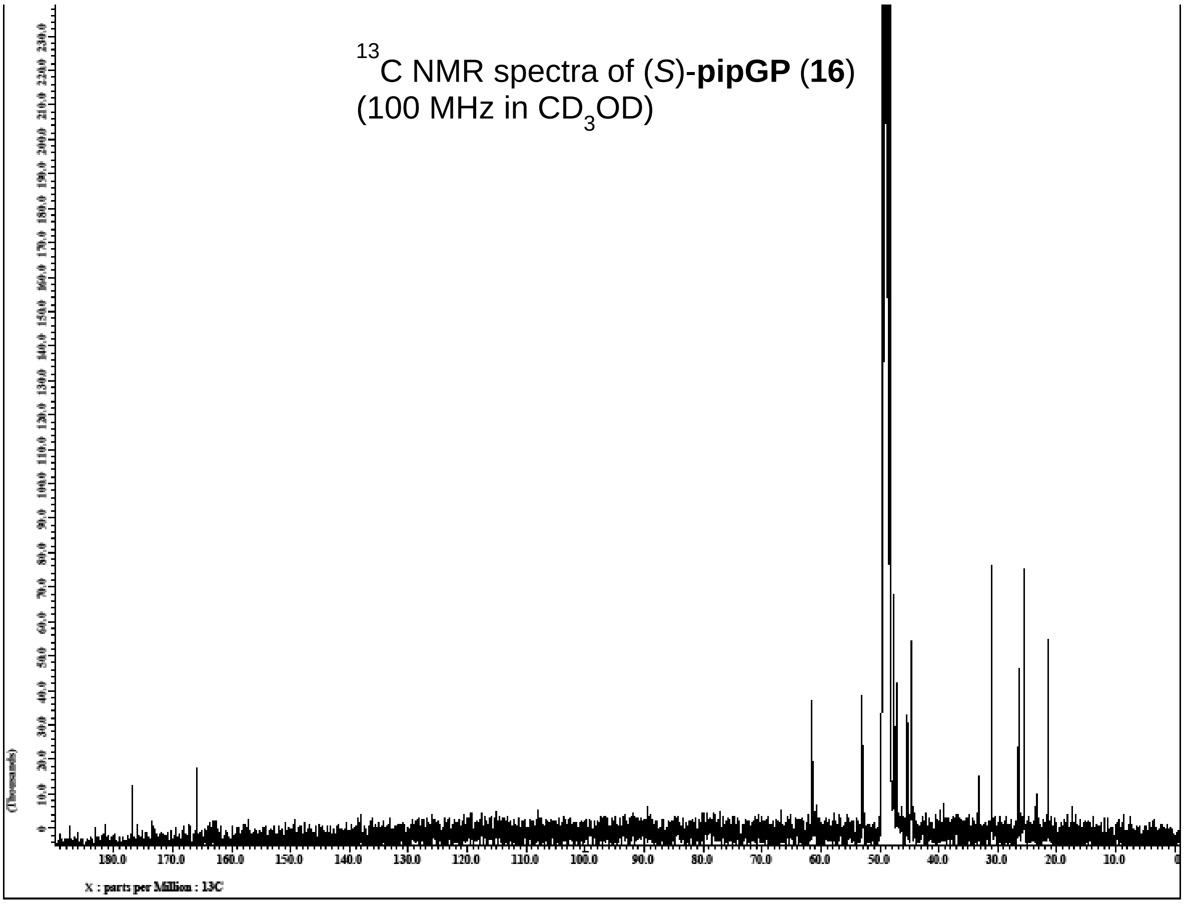
^{1}H NMR spectra of (*R*)-**pipGP** (**16**)
(400 MHz in CD_3OD)



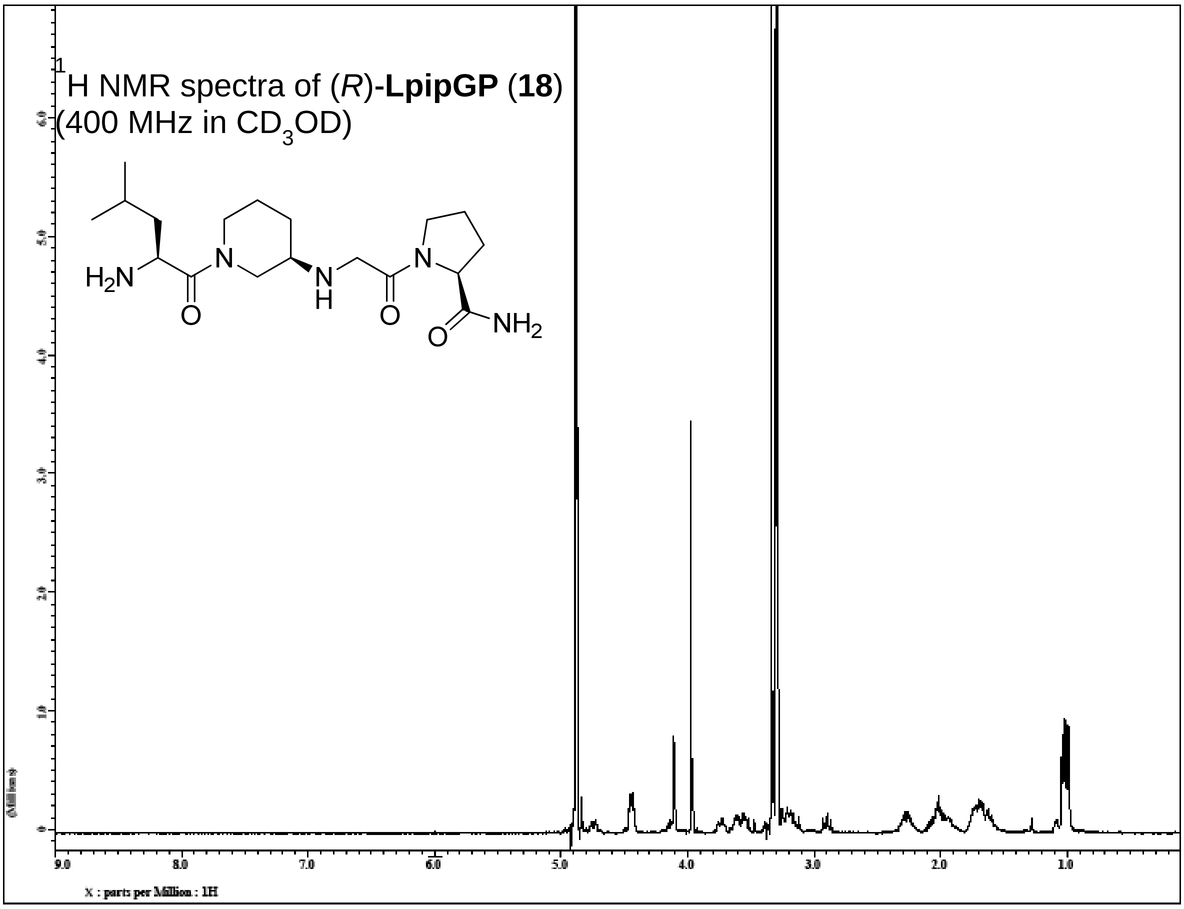
^{13}C NMR spectra of (*R*)-**pipGP** (**16**)
(100 MHz in CD_3OD)



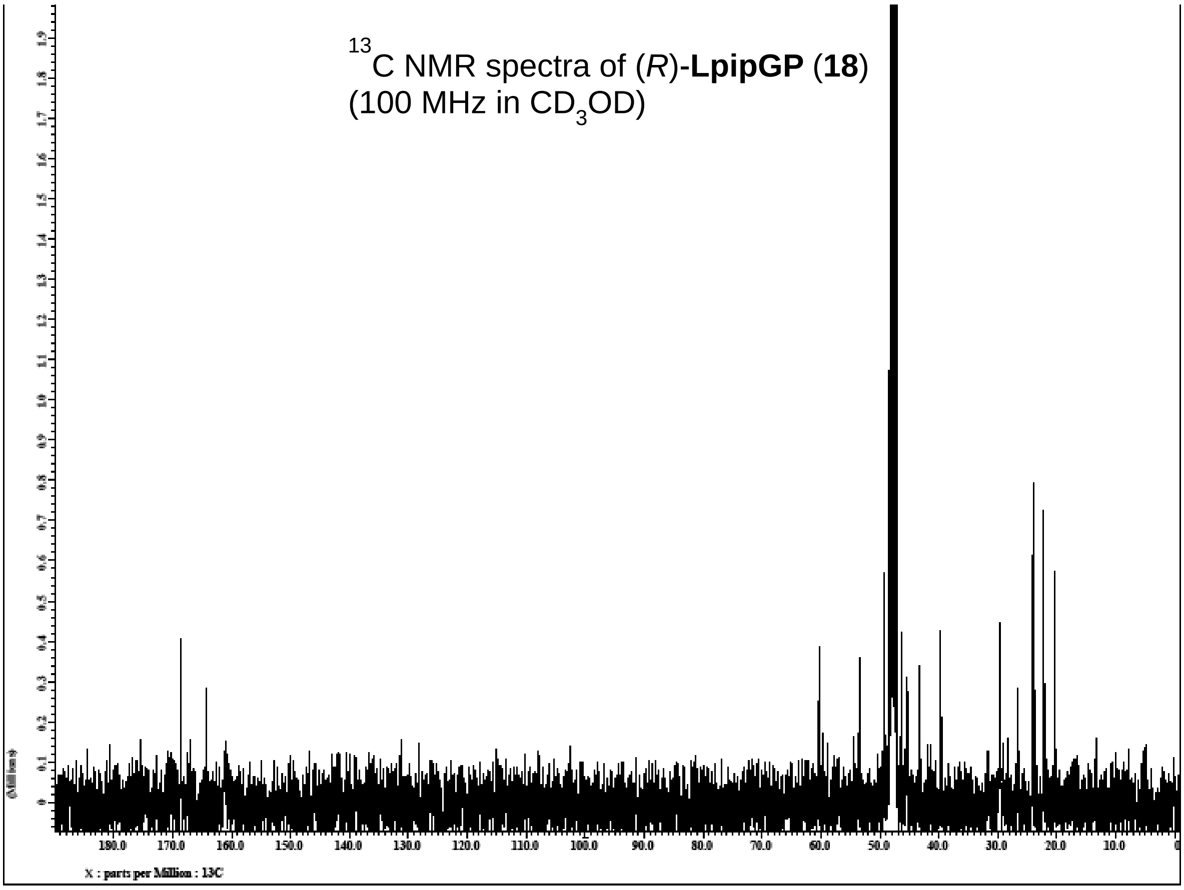
^{1}H NMR spectra of (*S*)-**pipGP** (**16**)
(400 MHz in CD_3OD)



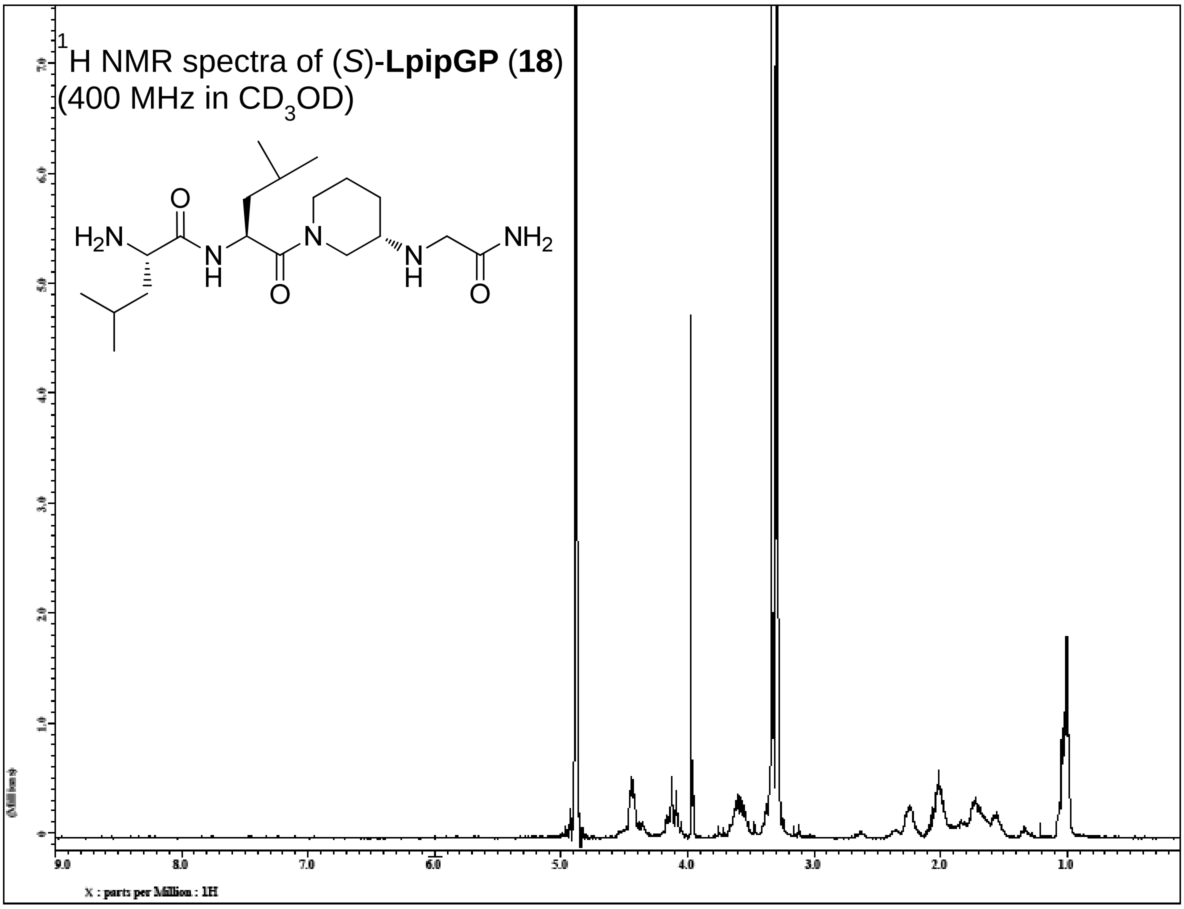
^{13}C NMR spectra of (*S*)-**pipGP** (**16**)
(100 MHz in CD_3OD)



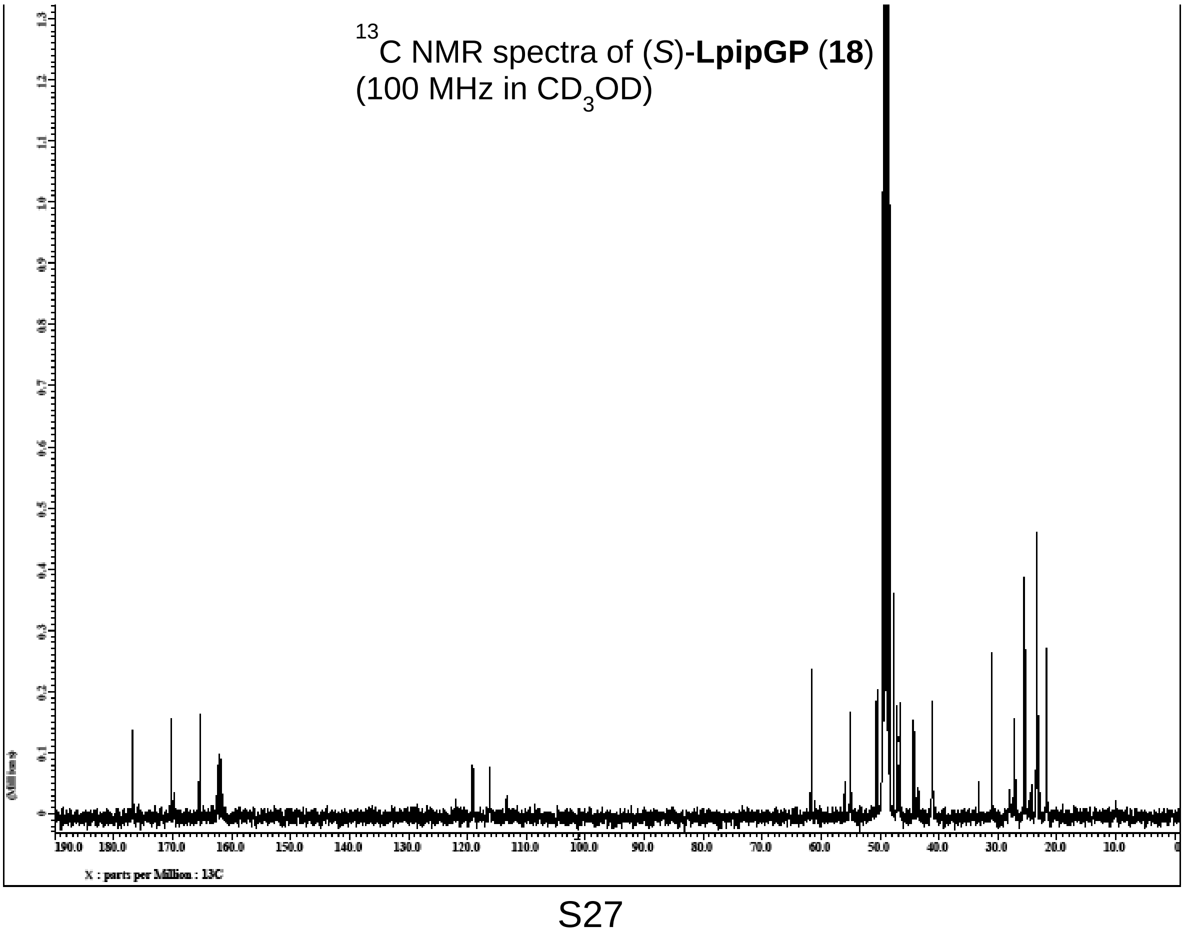
^{1}H NMR spectra of (*R*)-**LpipGP** (**18**)
(400 MHz in CD_3OD)



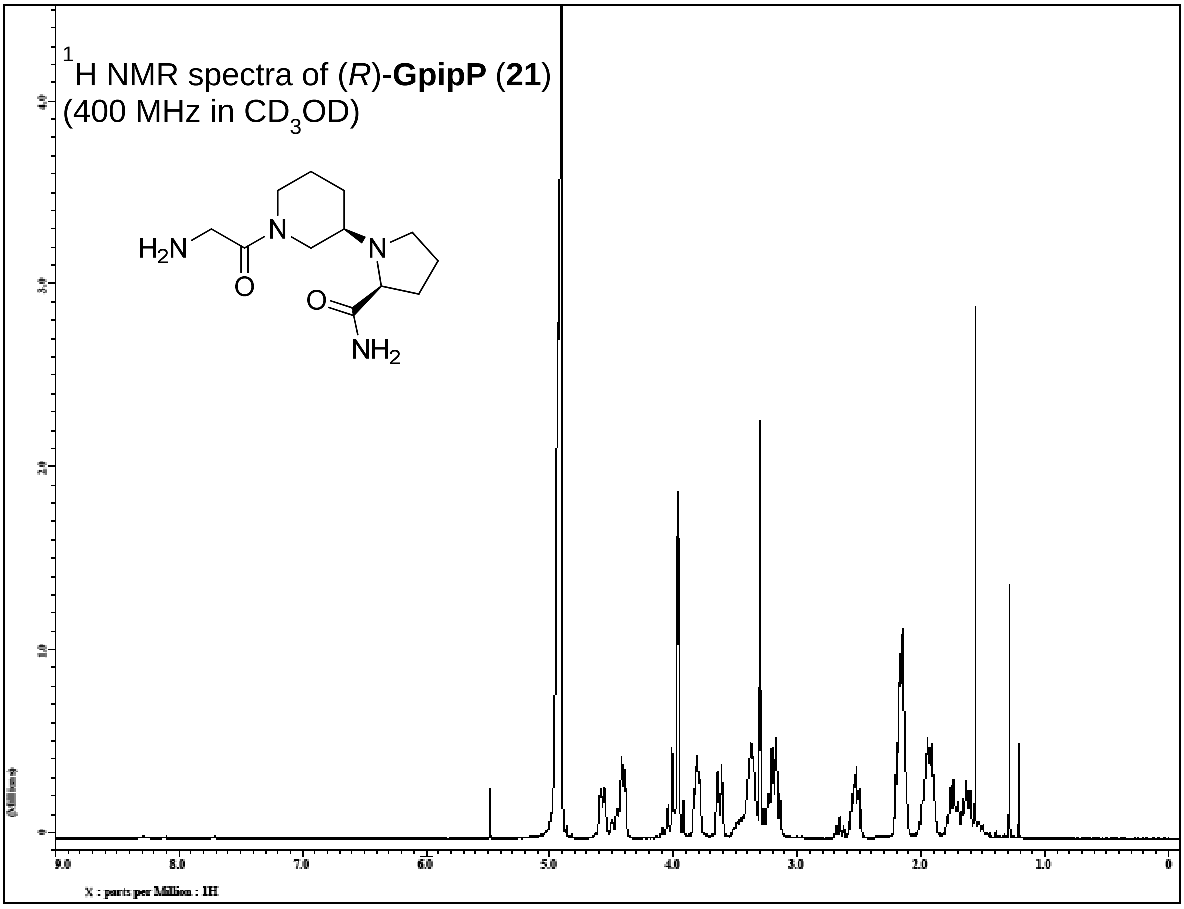
^{13}C NMR spectra of (*R*)-**LpipGP** (**18**)
(100 MHz in CD_3OD)



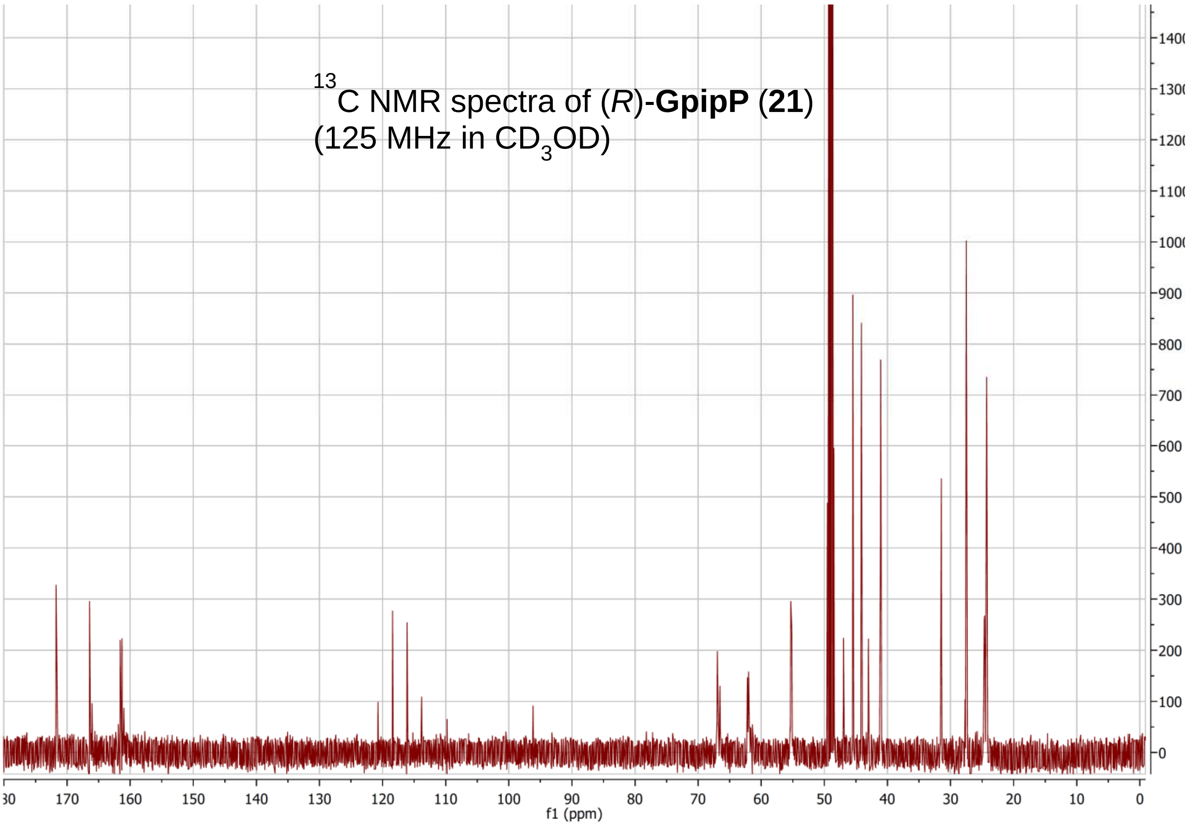
^{1}H NMR spectra of (*S*)-**LpipGP** (**18**) (400 MHz in CD_3OD)



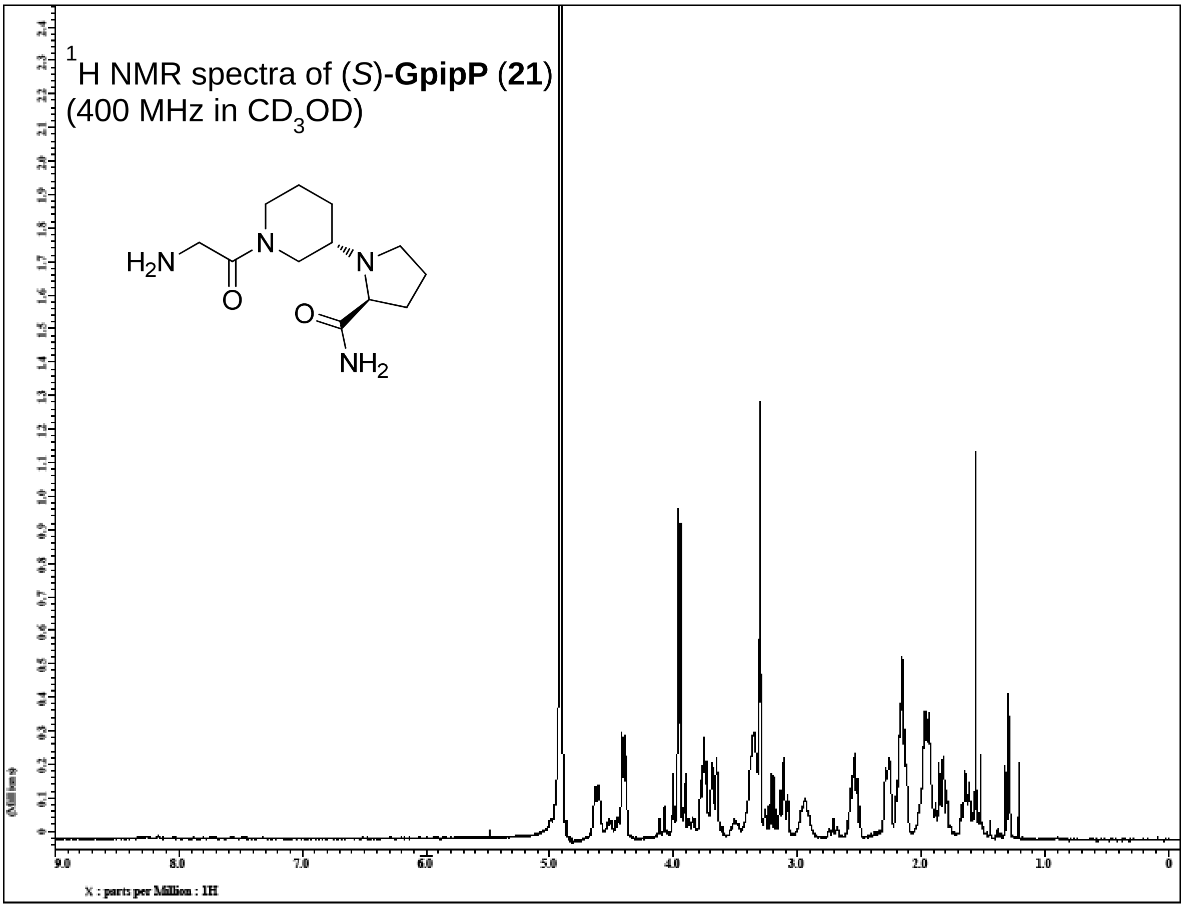
^{13}C NMR spectra of (*S*)-**LpipGP** (**18**) (100 MHz in CD_3OD)

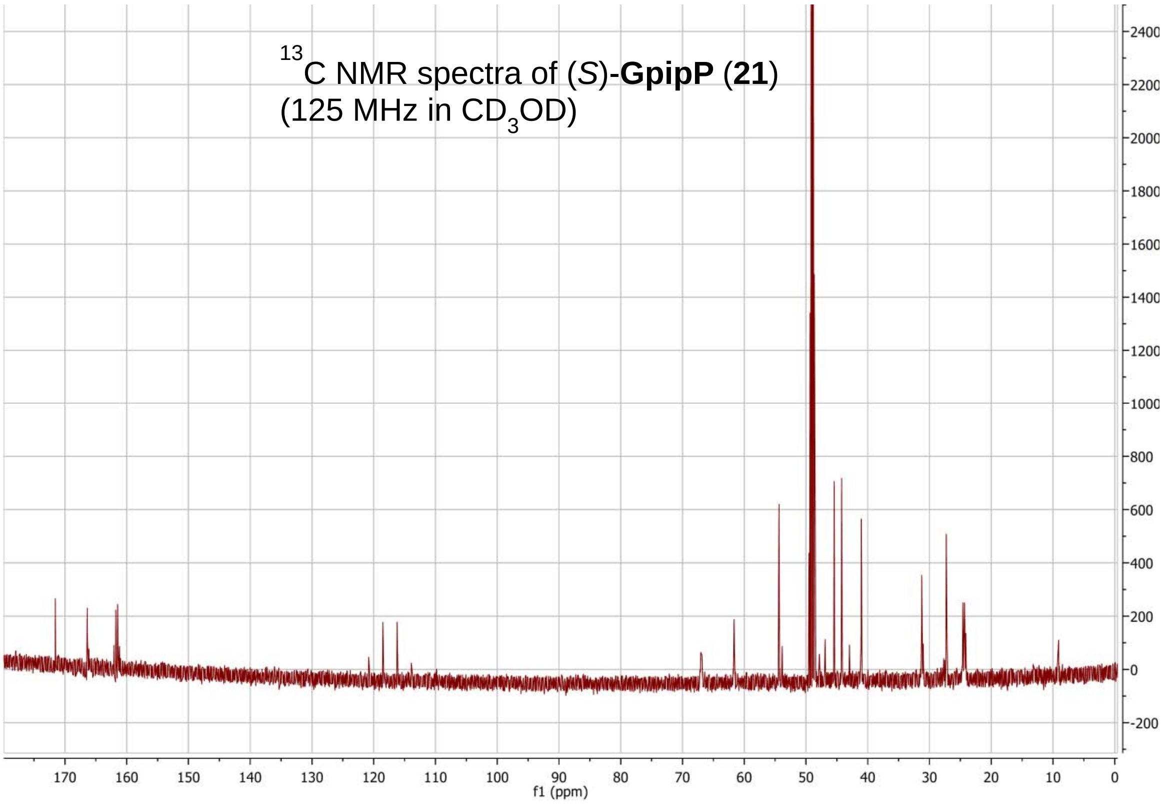


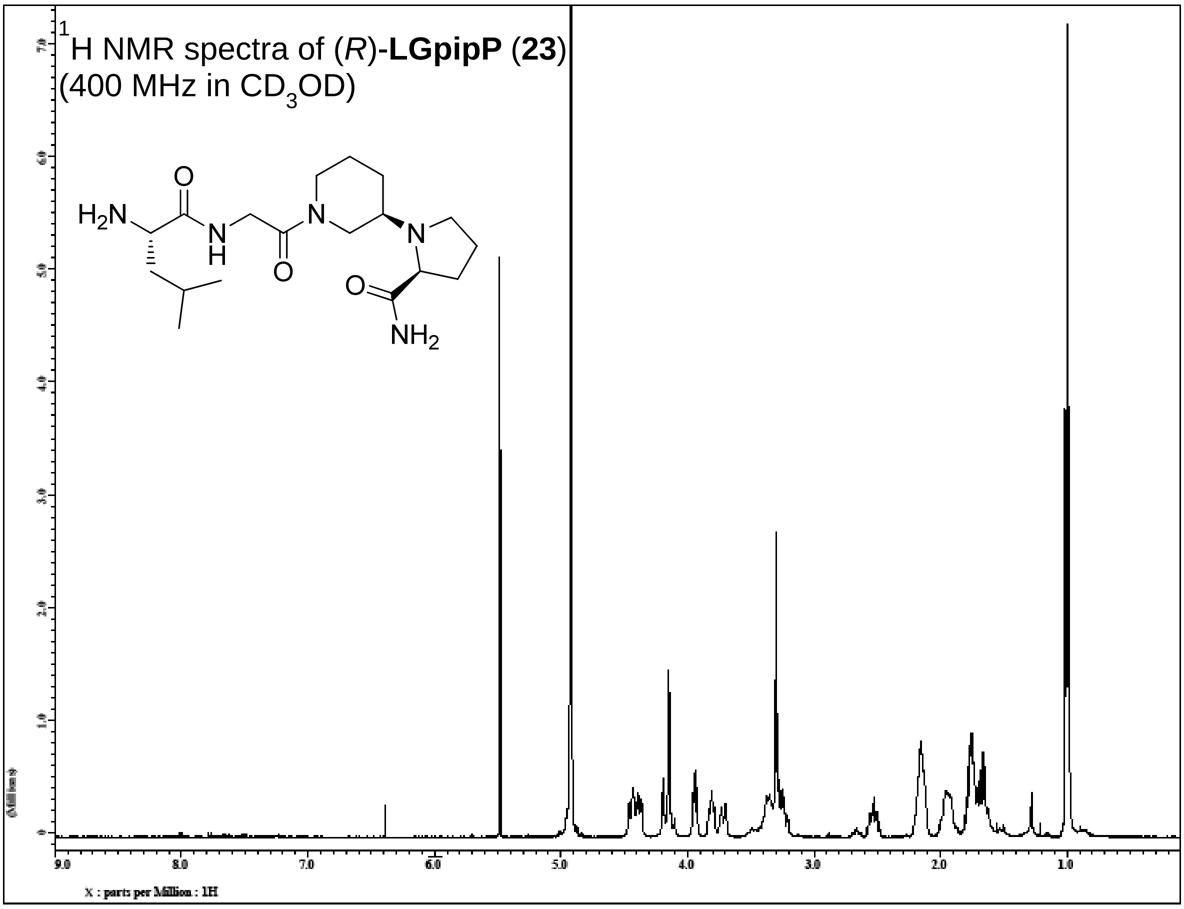
^{1}H NMR spectra of (*R*)-**GpipP** (**21**)
(400 MHz in CD_3OD)



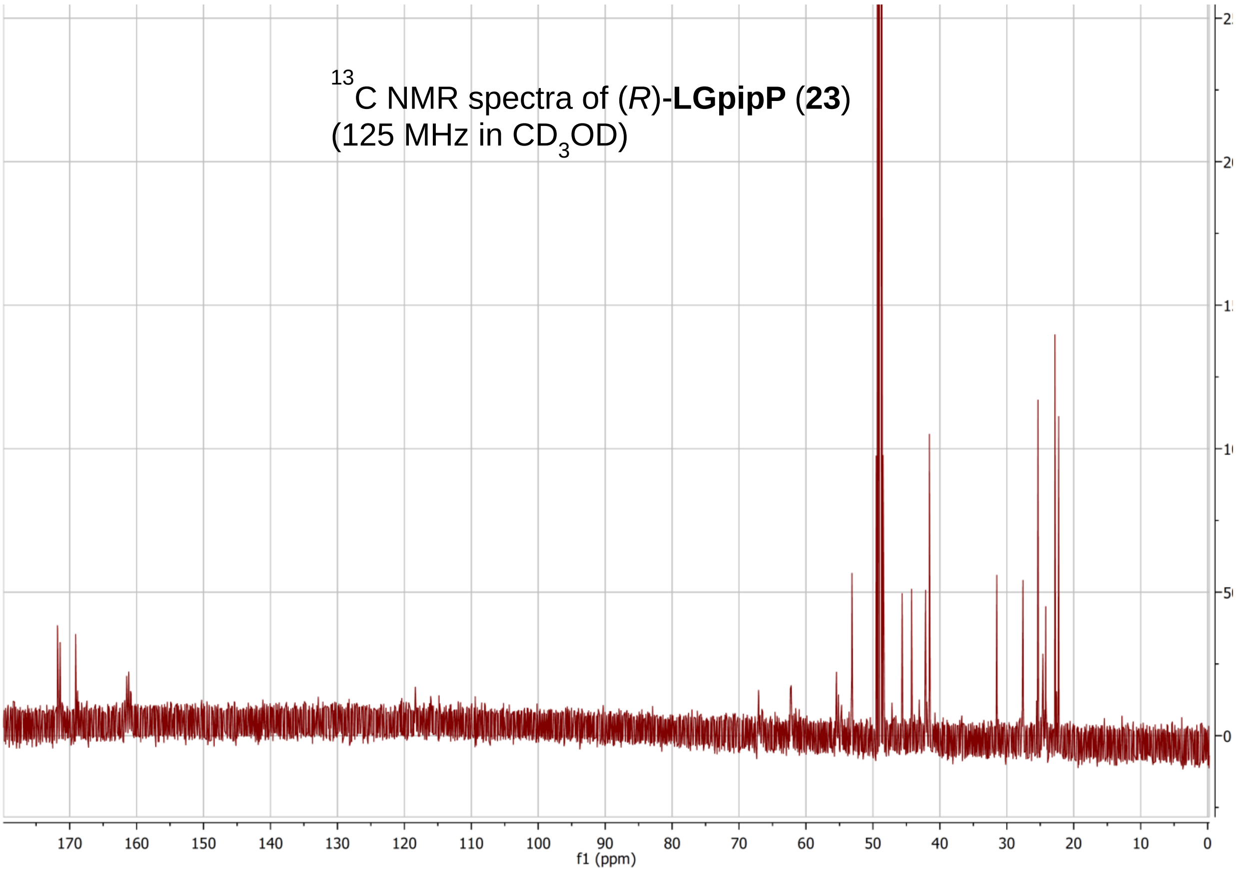
^{13}C NMR spectra of (*R*)-**GpipP** (**21**)
(125 MHz in CD_3OD)


^{1}H NMR spectra of (*S*)-**GpipP** (**21**)
(400 MHz in CD_3OD)

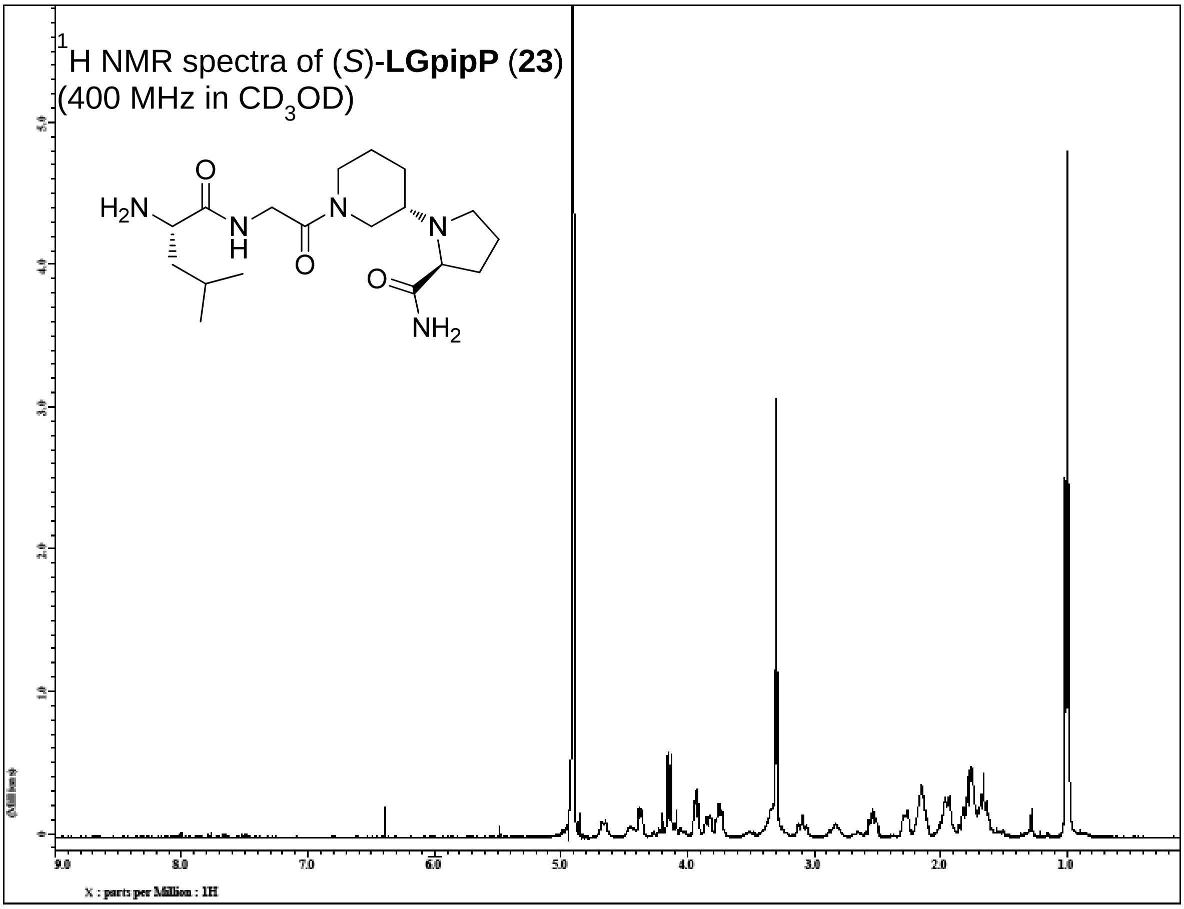

^{13}C NMR spectra of (*S*)-**GpipP** (**21**)
(125 MHz in CD_3OD)



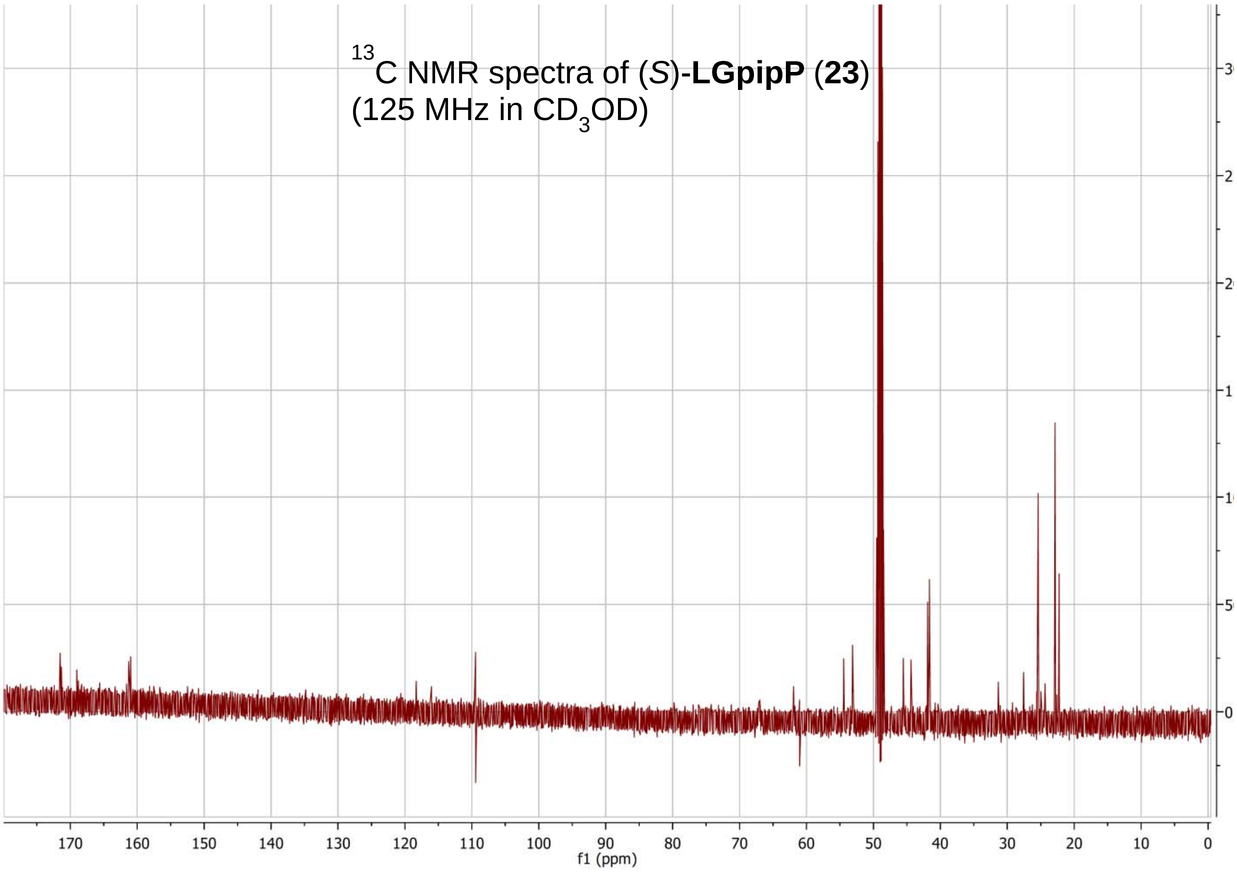
^{1}H NMR spectra of (*R*)-**LGpipP** (**23**)
(400 MHz in CD_3OD)



^{13}C NMR spectra of (*R*)-**LGpipP** (**23**)
(125 MHz in CD_3OD)



^{1}H NMR spectra of (*S*)-**LGpipP** (**23**)
(400 MHz in CD_3OD)



^{13}C NMR spectra of (*S*)-**LGpipP** (**23**)
(125 MHz in CD_3OD)