Novel L-prolyl-L-leucylglycinamide (PLG) Tripeptidomimetics based on 2-azanorbornane Scaffold as Positive Allosteric Modulators of D_2R

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Supplementary Information

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1. General Notes

All chemicals were of reagent grade and were obtained from Aldrich Chemical Co., Bachem and Fluka and used without further purification. CPD was obtained by bidistillation of dicyclopentadiene, collected in a flask at 0ºC and used immediately. All air sensitive reactions were carried out under argon atmosphere. Flash chromatography was performed on silica gel (Merck 60, 230-240 mesh), and analytical TLC was carried out on pre-coated silica gel plates (Merck 60 F_{254}, 0.25 mm) using UV light and ethanolic solution of phosphomolybdic acid (followed by gentle heating) for visualization. Melting points were measured on a Reichert Kofler Thermopan apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded in different spectrometers at different institutions (CACTUS - Centro de Apoyo Científico Tecnológico de la Universidade de Santiago de Compostela and CEMUP - Centro de Materiais da Universidade do Porto) on Varian Mercury 300 (CACTUS), Bruker Avance III 400 (CEMUP) and Bruker DRX-500 (CACTUS) at 300, 400 and 500 MHz for ^{1}H and 75, 101, 126 MHz for ^{13}C, respectively. The NMR spetra were calibrated using the residual peak of protic solvent as an internal reference and/or TMS as internal standard. The nomenclature used for the assignment of protons and/or carbons for each α-amino acid residue in the peptide chains was made using a single letter system in subscript for the amino acid residue (L: L-leucine; V: L-valine, A: L-alanine; G: glycine) and indicating the proton (or group of protons) and/or the carbons in the structures by starting the numeration at the carbonyl carbon of the main chain of each α-amino acid residue. Mass spectra were recorded on Micromass Autospec or Bruker Microtof spectrometers (CACTUS).

2. Experimental protocols

2.1. General procedure for peptide coupling: synthesis of dipeptides 4a-c.

The appropriate carboxylic acid (**2a-c**) (1.0 mmol) and DIEA (4.0 mmol) were dissolved in dry CH_2Cl_2 under argon atmosphere. The solution was cooled to 0 °C (ice bath) and TBTU (1.0 mmol) was added and the resulting suspension was stirred for 30 min. After that the respective amino ester (**3a-c**) was added (1.0 mmol) and reaction was allowed to proceed at rt for 1 h. The solvent was removed to dryness and to the resulting residue was added 30 ml of ethyl acetate which was then washed successively with saturated aqueous $NaHCO_3$ and brine. The organic extracts were dried (anhydrous Na_2SO_4), filtered and the solvent of the filtrate was removed under reduced pressure to give an oily residue which was purified by flash chromatography on silica gel as specified below.

2.1.1. Methyl (tert-butoxycarbonyl)-L-*valyl*-L-*alaninate,* **4a**

The general procedure was applied to carboxylic acid **2a** (0.153 g, 0.704 mmol) dissolved in dry CH_2Cl_2 (20 ml) in presence of DIEA (0.48 ml, 2.82 mmol), TBTU (0.228 g, 0.71 mmol) and corresponding amine **3a** · HCl (0.099 g, 0.71 mmol). The work-up procedure yielded a crude oil, which was purified using CH_2Cl_2/CH_3OH (10:1) as eluent followed by crystallization from hexane cooled to 0°C. The crystals obtained were filtered under reduced pressure to afford dipeptide **4a** (0.187 g, 88%) as a white crystalline solid; **mp** = 139–140ºC. R_f: 0.79 in CH_2Cl_2/CH_3OH (10:1). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 6.63 (d, J = 7.1 Hz, 1H, D_2O exchange, CONH), 5.17 (d, J = 8.1 Hz, 1H, D_2O exchange, CONH), 4.58 (p, J = 7.2 Hz, 1H, H_A-2), 3.97 – 3.93 (m, 1H, H_V-2), 3.74 (s, 3H, OCH_3), 2.27 – 1.97 (m, 1H, H_V-3), 1.45 (s, 9H, ^{t}Bu), 1.41 (d, J = 7.3 Hz, 3H, H_A-3), [0.97 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.8 Hz, 3H), H_V-4]. ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: [174.07 (C), 172.16 (C), CONH + CO_2CH_3], 156.77 (C, $\underline{C}O_2{}^t$Bu), 80.72 (C, ^{t}Bu), 60.63 (CH_3, OCH_3), 53.29 (CH, C_V-2), 48.85 (CH, C_A-2), 31.97 (CH, C_V-3), 29.19 ($3CH_3$, ^{t}Bu), 20.04 (CH_3, C_V-4), 19.06 (CH_3, C_A-3), 18.65 (CH_3, C_V-4). ESI-TOF MS m/z: $[M+Na]^+$, ($C_{14}H_{26}N_2NaO_5^+$) 325.2, required 325.2.

2.1.2. Methyl (tert-butoxycarbonyl)-L-leucylglycinate, **4b**

The general procedure was applied to carboxylic acid **2b** · H_2O (2.00 g, 8.02 mmol) dissolved in dry CH_2Cl_2 (50 ml) in presence of DIEA (5.3 ml, 32.08 mmol), TBTU (2.58 g, 8.02 mmol) and corresponding amine **3b** · HCl (1.01 g, 8.02 mmol). The work-up procedure yielded a crude oil, which was purified using EtOAc as eluent affording dipeptide **4b** (2.33 g, 96%) as a colorless crystalline solid after recrystallization with Pentane/EtOAc; **mp** = 131–133ºC. R_f: 0.89 in EtOAc. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.83 (br s, 1H, D_2O exchange, CONH), 5.03 (br s, 1H, D_2O exchange, CONH), 4.17 (br s, 1H, H_L-2), 3.99 (d, J = 5.2 Hz, 2H, H_G-2) 3.72 (s, 3H, OCH_3), 1.78 – 1.60 (m, 2H, H_L-3), 1.51 – 1.36 (m, 10H, H_L-4 + ^{t}Bu), 0.98 – 0.86 (m, 6H, H_L-5). ^{13}C-NMR ($CDCl_3$, 101 MHz) δ: [173.02 (C), 170.16 (C), CONH + CO_2CH_3], 155.75 (C, $\underline{C}O_2{}^t$Bu), 80.10 (C, ^{t}Bu), 52.93 (CH, C_L-2), 52.28 (CH_3, OCH_3), 41.25 (CH_2, C_G-2), 41.11 (CH_2, C_L-3), 28.29 ($3CH_3$, ^{t}Bu), 24.70 (CH, H_L-4), [22.94 (CH_3), 21.89 (CH_3), C_L-5]. ESI-TOF HRMS m/z: $[M+Na]^+$, ($C_{14}H_{26}N_2NaO_5^+$) 325.1724, required 325.1734.

2.1.3. Methyl (tert-butoxycarbonyl)glycyl-L-leucinate, **4c**

The general procedure was applied to carboxylic acid **2c** (0.97 g, 5.5 mmol) dissolved in dry CH_2Cl_2 (40 ml) in presence of DIEA (3.60 ml, 22.0 mmol), TBTU (1.77 g, 5.5 mmol) and corresponding amine **3c** · HCl (1.00 g, 5.50 mmol). The work-up procedure yielded a crude oil, which was purified using CH_2Cl_2/CH_3OH (20:1) as eluent to afford dipeptide **4c** (1.64 g, 98%) as a yellow oil. R_f: 0.39 in CH_2Cl_2/CH_3OH (20:1). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 7.02 (br s, 1H, D_2O exchange, CONH), 5.64 (br s, 1H, D_2O exchange, CONH), 4.53 (br s, 1H, H_L-2), 3.89 – 3.68 (m, 2H, H_G-2), 3.63 (s, 3H, OCH_3), 1.67 – 1.53 (m, 3H, H_L-3 + H_L-4), 1.36 (s, 9H, ^{t}Bu), 0.97 – 0.73 (m, 6H, H_L-5).^{13}C-NMR ($CDCl_3$, 75 MHz) δ: [173.29 (C), 169.66 (C), CONH + CO_2CH_3], 156.12 (C, $\underline{C}O_2{}^t$Bu), 79.79 (C, ^{t}Bu), 52.13 (CH, C_L-2), 50.56 (CH_3, OCH_3), [43.99 (CH_2), 41.07 (CH_2), C_G-2 + C_L-3], 28.17 ($3CH_3$, ^{t}Bu), 24.64 (CH, C_L-4), [22.71 (CH_3), 21.65 (CH_3), C_L-5]. ESI-TOF HRMS m/z: $[M+Na]^+$, ($C_{14}H_{26}N_2NaO_5^+$) 325.1734, required 325.1734.

2.2. General procedure for acidolytic deprotection of dipeptides 4a-c

To a solution of the carbamate-ester **4a-c** (1 mmol) in dry CH_2Cl_2 under argon atmosphere and provided with stirring in a system cooled to 0°C (ice bath), was added dropwise TFA (30 mmol), which was allowed to stir for approximately 1 h. The solvent was removed in vacuo and excess of TFA was eliminated by co-evaporation with CH_2Cl_2 under reduced pressure. In some cases, the oils obtained were induced to crystallize with mixture of Et_2O/EtOAc to afford the desired dipeptides as trifluoracetate salts.

2.2.1. Methyl L*-valyl-*L*-alaninate trifluoracetate,* **5a**

The general procedure was applied to **4a** (1.08 g, 3.57 mmol) in dry CH_2Cl_2 (40 ml) and TFA (8.3 ml, 107 mmol). The work-up procedure followed by recrystallization in Et_2O/EtOAc (10:1) afforded dipeptide **5a** (1.19 g, 99%) as a white solid; **mp** = 140–141ºC. R_f: 0.26 in CH_2Cl_2/CH_3OH (10:1). ^{1}H-NMR (CD_3OD, 300 MHz) δ: 4.47 (q, J = 7.3 Hz, 1H, H_A-2), 3.72 (s, 3H, OCH_3), 3.68 (d, J = 5.8 Hz, 1H, H_V-2), 2.30 – 2.11 (m, 1H, H_V-3), 1.43 (d, J = 7.3 Hz, 3H, H_A-3), [1.11 (d, J = 6.8 Hz, 3H), 1.08 (d, J = 6.8 Hz, 3H), H_V-4]. ^{13}C-NMR (CD_3OD, 75 MHz) δ: [176.58 (C), 171.92 (C), CONH + CO_2CH_3], [62.09 (CH), 62.06 (CH), C_V-2 + C_A-2], 55.28 (CH_3, OCH_3), 34.09 (CH, C_V-3), [21.21 (CH_3), 20.39 (CH_3), C_V-4], 19.70 (CH, C_A-3). ESI-TOF MS m/z: $[M\text{-}TFA]^+$, ($C_9H_{19}N_2O_3^+$) 203.1, required 203.1.

2.2.2. Methyl L*-leucylglycinate trifluoracetate,* **5b**

The general procedure was applied to **4b** (0.500 g, 1.65 mmol) in dry CH_2Cl_2 (10 ml) and TFA (3.8 ml, 49 mmol). The work-up procedure afforded dipeptide **5b** (0.517 g, 99%) as a brownish dense oil. R_f: 0.26 in CH_2Cl_2/CH_3OH (10:1). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 4.02 – 3.93 (A-part of AB system, J = 17.6 Hz, 1H, H_G-2), 3.91 – 3.78 (m, 2H, B-part of AB system of H_G-2 + H_L-2], 3.64 (s, 3H, OCH_3), 1.75 – 1.52 (m, 3H, H_L-3 + H_L-4), 0.98 – 0.83 (m, 6H, H_L-5). ^{13}C-NMR (CD_3OD, 101 MHz) δ: [170.89 (C), 170.82 (C), CONH + CO_2CH_3], 52.44 (CH, C_L-2), 52.22 (CH_3, OCH_3), [41.31

(CH_2), 41.22 (CH_2), C_L-3 + C_G-2], 24.82 (CH, C_L-4), [22.42 (CH_3), 21.71 (CH_3), C_L-5]. ESI-TOF HRMS *m/z*: $[M\text{-}TFA]^+$, ($C_{11}H_{19}N_2O_3^+$) 203.1390, required 203.1390.

*2.2.3. Methyl glycyl-*L*-leucinate trifluoracetate,* **5c**

The general procedure was applied to **4c** (1.72 g, 5.69 mmol) in dry CH_2Cl_2 (50 ml) and TFA (13.2 ml, 171 mmol). The work-up procedure followed by recrystallization in Et_2O/EtOAc (10:1) afforded dipeptide **5c** (1.771 g, 98%) as a beige solid; **mp** = 114–115ºC. R_f: 0.25 in CH_2Cl_2/CH_3OH (10:1). ^{1}H-NMR (CD_3OD, 300 MHz) δ: 4.60 – 4.40 (m, 1H, H_L-2), 3.72 (s, 2H, H_G-2), 3.70 (s, 3H, OCH_3), 1.75 – 1.56 (m, 3H, H_L-3 + H_L-4), [0.95 (d, J = 6.0 Hz, 3H), 0.92 (d, J = 6.0 Hz, 3H), H_L-5]. ^{13}C-NMR (CD_3OD, 75 MHz) δ: 172.89 (C, CONH), 166.01 (C, CO_2CH_3), 51.38 (CH, C_L-2), 50.89 (CH_3, OCH_3), [40.05 (CH_2), 39.94 (CH_2), C_G-2 + C_L-3], 24.51 (CH, C_L-4), [21.76 (CH_3), 20.35 (CH_3), C_L-5]. ESI-TOF HRMS *m/z*: $[M\text{-}TFA]^+$, ($C_9H_{19}N_2O_3^+$) 203.1388, required 203.1390.

2.3. (±)-exo-2-benzyl-2-azabicyclo[2.2.1]hept-5-ene-3-carboxylic acid, (±)**-7**

To a stirred solution of (±)**-6**[1] (1.030 g, 4.23 mmol) in THF (10 ml) at 0ºC was added dropwise a solution of LiOH (1.270 g, 52.88 mmol) in THF/H_2O (1:1) (40 ml) and the reaction mixture was stirred for 0.5 h, warmed to rt and stirred for additional 48 h. The solvent was removed to dryness and the solid residue was dissolved in deionized H_2O (20 ml) and this solution was cooled at 0ºC (ice bath) and pH was adjusted to pH = 4-5 with H_2SO_4 1M. The solution was concentrated to give a white solid, which was triturated with warm (40ºC) mixture of Et_2O/EtOH (1:3) (150 ml) to afford, after concentration, (±)**-7** (0.882 g, 91%) as a white solid; **mp** = 111-112 ºC. ^{1}H-NMR (D_2O, 500 MHz) δ: 7.57 – 7.41 (m, 5H, ArH), 7.07 (dd, J = 5.9, 3.2 Hz, 1H, H-5), 6.56 (dd, J = 5.9, 3.2 Hz, 1H, H-6), 4.43 – 4.34 (A-part of AB system, J = 12.8 Hz, 1H, NCHHPh), 4.20 (s, 1H, H-1), 3.93 – 3.83 (B-part of AB system, J = 12.8 Hz, 1H, NCHHPh), 3.57 (d, 1H, J = 3.5 Hz, H-4), 3.45 (s, 1H, H-3),

1 Rodríguez-Borges, J. E.; García-Mera, X.; Fernández, F.; Lopes, V. H. C.; Magalhães, A. L.; Cordeiro, M. N. D. S. *Tetrahedron* 2005, **61**, 10951.

1.97 (s, 2H, H-7). ^{13}C-NMR (D_2O, 126 MHz) δ: 171.09 (C, CO_2H), 145.75 (CH, C-5), 130.48 (2CH, C-2' + C-6'), 130.05 (CH, C-6), 129.85 (C, C-1'), 129.82 (CH, C-4'), 129.37 (2CH, C-3' + C-5'), 71.56 (CH, C-3), 65.55 (CH, C-1), 51.17 (CH_2, NCH_2Ph), 48.12 (CH, C-4), 45.50 (CH_2, C-7). CI MS *m*/*z*: $[M]^+$, ($C_{14}H_{15}NO_2^+$) 229.9, required 229.3.

2.4. The general peptide coupling procedure for the synthesis of tripeptides 8a-c/8a'-c' is the same for dipeptides 4a-c

2.4.1. Methyl {(1SR,3SR,4RS)-2-benzyl-2-azabicyclo[2.2.1]hept-5-ene-3-carbonyl}-L-*valyl*-L-*alaninate,* **8a/8a'**

The general coupling procedure was applied to carboxylic acid (±)-**7** (0.630 g, 2.75 mmol) dissolved in dry CH_2Cl_2 (40 ml) in presence of DIEA (1.9 ml, 11 mmol), TBTU (0.883 g, 2.75 mmol) and corresponding amine **5a** (0.869 g, 2.75 mmol). The work-up procedure yielded a crude oil, which was purified using Hexane/EtOAc (1:4) as eluent to afford tripeptides **8a/8a'** (0.751 g, 66%) as a pale yellow oil. R_f: 0.53 in Hexane/EtOAc (1:4). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 8.28 (d, J = 8.5 Hz, 1H, D_2O exchange, CONH), 8.16 (d, J = 9.5 Hz, 1H, D_2O exchange, CONH), 7.39 – 7.22 (m, 10H, 2 x ArH), 6.72 – 6.60 (m, 2H, D_2O exchange, 2 x CONH), [6.59 – 6.53 (m, 2H), 6.23 (dd, J = 10.1, 4.0 Hz, 2H), 2 x (H-5 + H-6)], 4.59 – 4.47 (m, 2H, 2 x H_A-2), 4.22 (dd, J = 9.2, 6.5 Hz, 2H, 2 x H_V-2), 3.90 – 3.77 [3.85 (s), 3.80 (s), 2H, 2 x H-3], 3.77 – 3.70 [3.74 (s), 3.73 (s), 6H, 2 x OCH_3], 3.54 – 3.48 (A-part of AB system, J = 13.0 Hz, NCHHPh), 3.48 – 3.43 (AB system, J = 13.0 Hz, NCH_2Ph), 3.43 – 3.30 (B-part of AB system, J = 13.0 Hz, NCHHPh), 3.28 – 3.17 [3.24 (s), 3.22 (s), 2H, 2 x H-1], 2.48 – 2.30 [2.41 (s), 2.37 (s), 2H, 2 x H-4), [2.24 – 2.13 (m, 1H), 2.08 – 1.98 (m, 1H), 2H, 2 x H_V-3], 1.63 (t, J = 8.9 Hz, 2H, 2 x H-7$_{syn}$), 1.43 – 1.33 [1.39 (d, J = 7.0 Hz), 1.37 (d, J = 7.0 Hz), 8H, 2 x (H_A-3 + H-7$_{anti}$)], [0.97 (d, J = 7.0 Hz, 3H), 0.95 (d, J = 7.0 Hz, 3H), 0.88 (d, J = 7.0 Hz, 3H), 0.79 (d, J = 7.0 Hz, 3H), 12H, 2 x H_V-4]. ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: [173.14 (C), 173.13 (C),

173.09 (C), 172.97 (C), 170.73 (C), 170.37 (C), 4 x CONH + 2 x CO_2CH_3], [138.51 (CH), 138.42 (CH), 2 x C-5], [137.79 (C), 137.71 (C), 2 x C-1'], [133.14 (CH), 132.97 (CH), 2 x C-6], [129.19 (2CH), 128.91 (2CH), 2 x (C-2' + C-6')], [128.66 (2CH), 128.52 (2CH), 2 x (C-3' + C-5')], [127.37 (CH), 127.24 (CH), 2 x C-4'), [66.91 (CH), 66.82 (CH), 64.32 (CH), 63.56 (CH), 2 x (C-1 + C-3)], [58.55 (CH_2), 58.24 (CH_2), 2 x NCH_2Ph], [58.09 (CH), 57.95 (CH), 2 x C_V-2], 52.35 (CH_3, 2 x OCH_3), [49.15 (CH), 48.82 (CH), 47.98 (CH), 47.91 (CH), 2 x (C_A-2 + C_V-3)], [45.95 (CH_2), 45.91 (CH_2), 2 x C-7], [31.04 (CH), 30.91 (CH), 2 x C-4)], [19.29 (CH_3), 19.14 (CH_3), 18.13 (CH_3), 18.03 (CH_3), 17.97 (2CH_3, 2 x (C_A-3 + C_V-4)]. ESI-TOF HRMS *m/z*: $[M+H]^+$, ($C_{23}H_{32}N_3O_4^+$) 414.2388, required 414.2387.

2.4.2. Methyl {(1SR,3SR,4RS)-2-benzyl-2-azabicyclo[2.2.1]hept-5-ene-3-carbonyl}-L-*leucylglycinate*, **8b/8b'**

The general peptide coupling procedure was applied to carboxylic acid (±)**-7** (0.393 g, 1.71 mmol) dissolved in dry CH_2Cl_2 (40 ml) in presence of DIEA (1.1 ml, 6.8 mmol), TBTU (0.551 g, 1.71 mmol) and corresponding amine **5b** (0.541 g, 1.71 mmol). The work-up procedure yielded a crude oil, which was purified using Hexane/EtOAc (1:9) as eluent to afford tripeptides **8b/8b'** (0.495 g, 70%) as a pale yellow oil. R_f: 0.64 in Hexane/EtOAc (1:9). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.93 (d, J = 7.3 Hz, 2H, D_2O exchange, 2 x CONH), 7.53 – 7.18 (m, 10H, 2 x ArH), 6.91 (d, J = 4.3 Hz, 1H, D_2O exchange, CONH), 6.70 – 6.55 (m, 2H, H-5), 6.54 – 6.37 [m, 1H, D_2O exchange, CONH], 6.27 (dt, J = 6.1, 1.6 Hz, 2H, H-6), 4.50 – 4.26 (m, 2H, 2 x H_L-2), 4.07 – 3.80 [m, 6H, 2 x (H_G-2 + H-1)], 3.78 – 3.69 [3.74 (s), 3.73 (s), 6H, 2 x OCH_3)], 3.64 – 3.44 (m, 2H, NCH_2Ph), 3.43 – 3.32 (AB system, J = 12.8 Hz, 2H, NCH_2Ph), 3.32 – 3.16 (m, 2H, 2 x H-4), 2.42 (s, 2H, 2 x H-3), 1.84 – 1.19 [m, 10H, 2 x (H-7 + H_L-3 + H_L-4)], 1.03 – 0.80 [0.97 (d, J = 6.3 Hz, 3H), 0.91 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 2 x H_L-5]. ^{13}C-NMR ($CDCl_3$, 101 MHz) δ: [174.21 (C), 173.41 (C), 173.30 (C), 172.21 (C), 171.91 (C), 169.97 (C), 4 x CONH + 2 x CO_2CH_3], 138.55 (C, 2 x C-1'), [137.90 (CH), 137.87 (CH), 2 x C-5], [133.18 (CH), 133.04 (CH), 2 x C-6], [129.32,

(2CH), 129.02 (2CH), 2 x (C-2' + C-6')], [128.70 (2CH), 128.65 (2CH), 2 x (C-3' + C-5')], [127.46 (CH), 127.35 (CH), 2 x C-4'], [66.93 (CH), 66.60 (CH), 2 x C-3), [64.87 (CH), 64.72 (CH), 2 x C-1], [58.73 (CH_2), 58.55 (CH_2), 2 x NCH_2Ph], [52.24, 52.23, 51.37, 51.02, ($2CH_3$ + 2CH, 2 x OCH_3 + 2 x C_L-2)], [49.16 (CH), 48.87 (CH), 2 x C-4], [46.02 (CH_2), 45.94 (CH_2), 2 x C-7], [41.12 (CH_2), 41.09 (CH_2), 2 x C_G-2], [40.53 (CH_2), 40.40 (CH_2), 2 x C_L-3], [24.89 (CH), 24.57 (CH), 2 x C_L-4], [22.98 (CH_3), 22.85 (CH_3), 22.02 (CH_3), 21.85 (CH_3), 2 x C_L-5)]. ESI-TOF HRMS *m*/*z*: $[M+H]^+$, ($C_{23}H_{32}N_3O_4^+$) 414.2377, required 414.2387.

*2.4.3. Methyl {(1SR,3SR,4RS)-2-benzyl-2-azabicyclo[2.2.1]hept-5-ene-3-carbonyl}glycyl-*L*-leucinate*, **8c/8c'**

The general coupling procedure was applied to carboxylic acid (±)-**7** (0.590 g, 2.57 mmol) dissolved in dry CH_2Cl_2 (40 ml) in presence of DIEA (1.8 ml, 10.3 mmol), TBTU (0.825 g, 2.57 mmol) and corresponding amine **5c** (0.812 g, 2.57 mmol). The work-up procedure yielded a crude oil, which was purified using Hexane/EtOAc (1:4) as eluent to afford tripeptides **8c/8c'** (0.808 g, 76%) as a pale yellow oil. R_f: 0.43 in Hexane/EtOAc (1:4). ^{1}H-NMR ($CDCl_3$, 300 MHz, rotamers present) δ: 8.24 (br s, 2H, D_2O exchange, 2 x CONH), 7.57 – 7.14 (m, 10H, 2 x ArH), 6.95 – 6.70 (m, 2H, D_2O exchange, 2 x CONH), [6.64 (dd, J = 5.4, 2.7 Hz), 6.55 (dd, J = 4.7, 2.3 Hz), 6.37 (dd, J = 5.6, 2.5 Hz), 6.23 (dd, J = 5.5, 1.8 Hz), 4H, 2 x (H-5 + H-6)], [4.68 – 4.48 (m), 4.47 – 4.30 (m), 2 x H_L-2], 4.06 – 3.94 (AB system, J = 6.0 Hz, 2H, H_G-2), 3.83 (br s, 2H, H-1), 3.79 – 3.61 [3.71 (s), 3.72 (s), 8H, 2 x OCH_3 + H_G-2], 3.54 – 3.33 (m, 4H, 2 x NCH_2Ph), 3.31 – 3.16 (m, 2H, 2 x H-4), 2.37 (d, J = 3.6 Hz, 2H, 2 x H-3), 1.77 – 1.14 [m, 10H, 2 x (H-7 + H_L-3 + H_L-4)], 1.07 – 0.77 (m, 12H, 2 x H_L-5]. ^{13}C-NMR ($CDCl_3$, 75 MHz, rotamers present) δ: [173.82 (C), 173.06 (C), 168.77 (C), 168.72 (C), 4 x CONH + 2 x CO_2CH_3], 138.30 (2C, 2 x C-1'), [137.66 (CH), 137.63 (CH), 2 x C-5], [132.99 (CH), 132.98 (CH), 2 x C-6], 129.04 [4CH, 2 x (C-2' + C-6')], 128.46 [4CH, 2 x (C-3' + C-5')], 127.30 (2CH, 2 x C-4'), [66.62 (CH), 66.58 (CH), 65.75 (CH), 2 x C-3], [64.09 (CH), 64.00 (CH), 2 x C-1], [58.33 (CH_2), 58.29 (CH_2), 2 x NCH_2Ph], [52.22 (CH_3), 52.21 (CH_3), 2 x OCH_3], 50.70 (CH), 48.84

(CH), 48.81 (CH), 2 x (C_L-2 + C-4)], [45.94 (CH_2), 45.88 (CH_2), 2 x C-7], [43.03 (CH_2), 42.96 (CH_2), 41.20 (CH_2), 41.14 (CH_2), 2 x (C_G-2 + C_L-3)], [24.75 (CH), 24.71 (CH), 2 x C_L-4], [22.73 (CH_3), 22.71 (CH_3), 21.83 (CH_3), 21.72 (CH_3), 2 x C_L-5]. ESI-TOF HRMS *m*/*z*: $[M+H]^+$, ($C_{23}H_{32}N_3O_4^+$) 414.2371, required 414.2387.

2.5. General procedure for the synthesis of N-deprotected tripeptides 1a-c/1a'-c'

A solution of *N*-protected tripeptides **8a-c/8a'-c'** (1 mmol) in dry EtOAc was added to a suspension of 20% $Pd(OH)_2/C$ (0.5 mmol) in dry EtOAc. The reaction mixture was stirred at rt under a hydrogen atmosphere for 4-8 days (TLC). The catalyst was filtered off through a celite pad and washed with EtOAc. The solvent was removed under reduced pressure to give an oily residue which was purified by column chromatography on silica gel as specified below.

2.5.1. Methyl {(1SR,3RS,4RS)-2-azabicyclo[2.2.1]heptane-3-carbonyl}-L-*valyl*-L-*alaninate,* **1a/1a'**

The general procedure was applied to **8a/8a'** (0.150 g, 0.363 mmol) in dry EtOAc (10 ml) and presence of $Pd(OH)_2/C$ (0.026 g, 0.18 mmol). The reaction was stirred for 5 days. The work-up procedure yielded a crude oil, which was purified using EtOAc and then MeOH as eluents to afford tripeptides **1a/1a'** (86.2 mg, 73%) as a yellow solid with low meting point. R_f: 0.10 in EtOAc. ^{1}H-NMR ($CDCl_3$, 300 MHz, rotamers present) δ: [8.38 (d, J = 9.2 Hz), 8.28 (d, J = 9.0 Hz), 7.82 (d, J = 8.9 Hz), 2H, D_2O exchange, 2 x CONH], [7.34 (d, J = 7.2 Hz), 7.23 (d, J = 7.1 Hz), 7.04 (d, J = 7.8 Hz), 2H, D_2O exchange, 2 x CONH], 4.48 (p, J = 7.1 Hz, 2H, 2 x H_A-2), 4.31 – 4.15 (m, 2H, 2 x H_V-2), 3.69 (s, 6H, 2 x OCH_3), 3.63 (s, 2H, 2 x H-3), 3.49 (s, 2H, 2 x H-1), 2.72 (d, J = 1.8 Hz, 2H, 2 x H-4), 2.24 – 1.97 (m, 2H, 2 x H_V-3), 1.71 – 1.16 [1.35 (d, J = 7.0 Hz), 20H, two of them exchange with D_2O, 2 x (H-5 + H-6 + H-7 + H_A-3 + NH)], [0.93 (d, J = 6.6 Hz), 0.91 (d, J = 6.6 Hz), 0.89 (d, J = 6.6 Hz), 0.88 (d, J = 6.6 Hz), 12H, 2 x H_V-4]. ^{13}C-NMR ($CDCl_3$, 75 MHz, rotamers present) δ: [173.28 (C), 173.18 (C), 172.99 (C), 172.27 (C), 170.92 (C), 170.90 (C), 4 x CONH + 2 x CO_2CH_3],

[63.03 (CH), 62.83 (CH), 2 x C-3], [58.51 (CH), 58.37 (CH), 2 x C_V-2], [56.60 (CH), 56.25 (CH), 2 x C-1], [52.82 (CH_3), 52.27 (CH_3), 2 x OCH_3], [48.03 (CH), 47.98 (CH), 2 x C_A-2], [41.61 (CH), 41.33 (CH), 2 x C-4], [34.42 (CH_2), 34.35 (CH_2), 2 x C-7], 31.25 ($2CH_2$, 2 x C-6), [30.90 (CH), 30.72 (CH), 2 x C_V-3], [28.09 (CH_2), 27.89 (CH_2), 2 x C-5], [19.22 (CH_3), 18.18 (CH_3), 17.94 (CH_3), 17.76 (CH_3), 2 x C_V-4], 17.66 ($2CH_3$, 2 x C_A-3). ESI-TOF HRMS *m/z*: $[M+H]^+$, ($C_{16}H_{28}N_3O_4^+$) 326.2091, required 326.2074.

*2.5.2. Methyl {(1SR,3RS,4RS)-2-azabicyclo[2.2.1]heptane-3-carbonyl}-*L*-leucylglycinate,* **1b/1b'**

The general procedure was applied to **8b/8b'** (0.237 g, 0.573 mmol) in dry EtOAc (6 ml) and presence of $Pd(OH)_2/C$ (0.041 g, 0.29 mmol). The reaction was stirred for 8 days. The work-up procedure yielded a crude oil, which was purified using Hexane/EtOAc (1:9) as eluent to afford tripeptides **1b/1b'** (131.0 mg, 70%) as a yellow oil. R_f: 0.64 in Hexane/EtOAc (1:9). ^{1}H-NMR ($CDCl_3$, 400 MHz, rotamers present) δ: 8.59 (d, J = 7.9 Hz, 1H, D_2O exchange, CONH), 8.31 (d, J = 6.3 Hz, 1H, D_2O exchange, CONH), [8.05 (s), 7.97 (s), 7.89 (s), 2H, D_2O exchange, 2 x CONH), [4.72 – 4.49 (m), 4.48 – 4.25 (m), 4H, 2 x (H_L-2+ H-3)], 4.10 (s, 2H, 2 x H-1), 3.99 – 3.80 (m, 4H, 2 x H_G-2), 3.63 (s, 6H, 2 x OCH_3), [2.97 (s), 2.94 (s), 2H, 2 x H-4], 2.13 – 1.05 [m, 20H, two of them exchange with D_2O, 2 x (H-5, H-6, H-7, H_L-3, H_L-4, NH)], 0.93 – 0.63 (m, 12H, 2 x H_L-5). ^{13}C-NMR ($CDCl_3$, 101 MHz) δ: [173.50 (C), 173.42 (C), 171.26 (C), 170.11 (C), 167.72 (C), 167.63 (C), 4 x CONH + 2 x CO_2CH_3], [62.86 (CH), 62.28 (CH), 2 x C-3], [59.34 (CH), 59.18 (CH), 2 x C-1], [53.10 (CH), 52.53 (CH), 2 x C_L-2], [52.40 (CH_3), 52.20 (CH_3), 2 x OCH_3], [41.09 (CH_2), 40.98 (CH_2), 2 x C_G-2], 40.57 ($2CH_2$, 2 x C_L-3), 39.93 (2CH, 2 x C-4), [38.55 (CH_2), 38.47 (CH_2), 2 x C-7], [26.45 (CH_2), 26.31 (CH_2), 2 x C-6], [24.75 (CH), 24.55 (CH), 2 x C_L-4], [22.77 (CH_3), 22.51 (CH_2), 22.69 (CH_3), 22.48 (CH_2), 21.65 (CH_3), 21.22 (CH_3), 2 x (C-5 + C_L-5)]. ESI-TOF HRMS *m/z*: $[M+H]^+$, ($C_{16}H_{28}N_3O_4^+$) 326.2082, required 326.2074.

*2.5.3. Methyl {(1SR,3RS,4RS)-2-azabicyclo[2.2.1]heptane-3-carbonyl}glycyl-*L*-leucinate,* **1c/1c'**

The general procedure was applied to **8c/8c'** (0.285 g, 0.689 mmol) in dry EtOAc (10 ml) and presence of $Pd(OH)_2/C$ (0.049 g, 0.34 mmol). The reaction was stirred for 4 days. The work-up procedure yielded a crude oil, which was purified using Hexane/EtOAc (1:4) and then MeOH as eluents to afford tripeptides **1c/1c'** (186.1 mg, 83%) as a yellow solid with low melting point. R_f: 0.14 in EtOAc. ^{1}H-NMR ($CDCl_3$, 300 MHz, rotamers present) δ: [8.69 – 8.35 (m), 8.33 – 7.98 (m), 2H, D_2O exchange, 2 x CONH], 7.24 – 6.79 [7.18 (t, J = 8.5 Hz), 2H, D_2O exchange, 2 x CONH), 4.70 – 4.35 (m, 2H, 2 x H_L-2), 4.17 – 3.30 [3.75 (s), 3.69 (s), 14H, 2 x (H_G-2 + H-1 + H-3 + OCH_3)], 2.76 (s, 2H, 2 x H-4), 1.78 – 1.01 [m, 20H, two of them exchange with D_2O, 2 x (H-5 + H-6 + H-7 + H_L-3 + H_L-4 + NH)], 0.99 – 0.75 (m, 12H, 2 x H_L-5). ^{13}C-NMR ($CDCl_3$, 75 MHz, rotamers present) δ: [176.36 (C), 173.35 (C), 173.30 (C), 172.53 (C), 168.99 (C), 168.90 (C), 4 x CONH + 2 x CO_2CH_3], 62.64 (2CH, 2 x C-3), [56.61 (CH), 56.59 (CH), 2 x C-1], 52.21 ($2CH_3$, 2 x OCH_3), [50.75 (CH), 50.73 (CH), 50.45 (CH), 2 x C_L-2], [43.15 (CH_2), 43.04 (CH_2), 2 x C_G-2], [41.29 (CH), 41.28 (CH), 2 x C-4], 40.95 ($2CH_2$, 2 x C_L-3), 34.56 ($2CH_2$, 2 x C-7), [29.57 ($2CH_2$), 27.78 (CH_2), 27.76 (CH_2), 2 x (C-5 + C-6)], [24.92 (CH), 24.69 (CH), 2 x C_L-4], [22.70 (CH_3), 22.68 (CH_3), 22.07 (CH_3) 21.71 (CH_3), 21.66 (CH_3), 2 x C_L-5]. ESI-TOF HRMS *m/z*: $[M+H]^+$, ($C_{16}H_{28}N_3O_4^+$) 326.2066, required 326.2074.

2.6. Ethyl (±)-exo-2-(tert-butoxycarbonyl)-2-azabicyclo[2.2.1]heptane-3-carboxylate, (±)**-10**

To a round bottom flask was added a solution (±)**-9**[2,3] (1.051 g, 3.93 mmol) in MeOH p.a. (10 ml) and then Pd/C 10% (0.105 g) in MeOH p.a. (5 ml) in a system containing a pressure equilibrating addition funnel. The suspension was maintained with magnetic stirring at rt and added, dropwise, TES (5.96 ml, 37.4 mmol). After 10 min the solvent was eliminated under reduced pressure and the

2 Hursthouse, M.; Malik, K. M. A.; Hibbs, D. E.; Roberts, S. M.; Seago, A. J. H.; Sik, V.; Storer, R. *J. Chem. Soc. Perkin Trans. 1*, 1995, 2419.

3 Sousa, C. A. D.; Vale, M. L. C.; Rodríguez-Borges, J. E.; García-Mera, X. *New J. Chem.* 2010, **34**, 2546.

catalyst was filtered off by filtration over celite pad washing with CH_2Cl_2. The solvent of filtrate was then removed to dryness to obtain a light yellow oil which was purified by flash chromatography using as eluent Hexane/EtOAc (3:1) affording (±)-**10** (1.0585 g, quantitative) as clear light yellow oil. R_f: 0.45 in Hexane/EtOAc (3:1). ^{1}H-NMR ($CDCl_3$, 400 MHz, rotamers present) δ: 4.44 – 4.28 (m, 1H, H-1), 4.27 – 4.11 (m, 3H, H-3 + C<u>H</u>$_2$CH$_3$), 2.78 (s, 1H, H-4), 1.97 – 1.36 [1.46 (s), 1.40 (s), 15H, H-5 + H-6 + H-7 + ^{t}Bu], 1.36 – 1.21 [1.29 (t, J = 7.1 Hz), 1.28 (t, J = 7.1 Hz), 3H, CH$_2$C<u>H</u>$_3$]. ^{13}C-NMR ($CDCl_3$, 101 MHz, rotamers present) δ: [171.08 (C), 170.49 (C), CO_2Et], [153.68 (C), 153.03 (C), <u>C</u>O$_2^t$Bu], 79.44 (C, ^{t}Bu), [63.28 (CH), 62.60 (CH), C-3], 60.62 (CH$_2$, CH$_2$CH$_3$), [58.23 (CH), 57.13 (CH), C-1], [41.76 (CH), 41.08 (CH), C-4], [39.03 (CH$_2$), 38.86 (CH$_2$), C-7], [29.83 (CH$_2$), 29.74 (CH$_2$), C-6)], [28.47 (3CH$_3$), 28.29 (3CH$_3$), ^{t}Bu], 23.61 (CH$_2$, C-5), [14.33 (CH$_3$), 14.21 (CH$_3$), CH$_2$CH$_3$]. ESI-TOF HRMS m/z: $[M+H]^+$, ($C_{14}H_{24}NO_4^+$) 270.16931, required 270.16998.

2.7. Ethyl (1R,3S,4S)-2-(tert-butoxycarbonyl)-2-azabicyclo[2.2.1]heptane-3-carboxylate, **10**

To a round bottom flask was added a solution of enantiopure system **14**[4] (1.89 g, 9.18 mmol) in anhydrous CH_2Cl_2 (20 ml) followed by DIEA (3.2 ml, 18.4 mmol) and Boc_2O (3.01 g, 13.77 mmol). The reaction initially developed gas (CO_2) and was left stirring at rt for 4 h. The solvent was removed to dryness and to the resulting residue was added 30 ml of ethyl acetate which was then washed successively with saturated aqueous $NaHCO_3$ and brine. The organic extract was dried (anhydrous Na_2SO_4), filtered and the solvent of the filtrate was removed under reduced pressure to give a pale yellow oil which was purified by flash chromatography on silica gel using Hexane/EtOAc 3:1 as eluent affording **10** (2.40 g, 97%) as a pale yellow oil.

Spectral data for enantiopure **10** are in accordance with (±)-**10**.

4 Tararov, V. I.; Kadyrov, R.; Kadyrova, Z.; Dubrovina, N.; Börner, A. *Tetrahedron: Asymmetry* 2002, **13**, 25.

2.8. (±)-exo-2-(tert-butoxycarbonyl)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid, (±)**-11**

According to the alkaline hydrolysis procedure described above for (±)**-7**, to a stirred solution of (±)**-10** (1.0664 g, 3.96 mmol) in THF (10 ml) at 0ºC was added dropwise a solution of LiOH (1.1858 g, 49.5 mmol) in THF/H_2O (1:1) (40 ml) and the reaction mixture was stirred for 0.5 h, warmed to rt and stirred for a further stirred 48 h. After typical work-up it was obtained (±)**-11** (898.2 mg, 94%) as a beige solid; **mp** = 152-154 ºC. R_f: 0.11 in CH_2Cl_2/CH_3OH (20:1). ^{1}H-NMR ($CDCl_3$, 400 MHz, rotamers present) δ: 9.27 (br s, 1H, D_2O exchange, CO_2H), 4.42 – 4.22 (m, 1H, H-3), 4.15 (d, J = 2.8 Hz, 1H, H-1), 2.81 (br s, 1H, H-4), 1.91 – 1.30 [1.43 (s), 15H, H-5 + H-6 + H-7 + Boc]. ^{13}C-NMR (CD_3OD, 101 MHz, rotamers present) δ: [174.15 (C), 173.87 (C), CO_2H)], [154.47 (C), 154.17 (C), $\underline{C}O_2{}^t$Bu], [79.49 (C), 79.39 (C), ^{t}Bu], [64.28 (CH), 63.52 (CH), C-3], [58.69 (CH), 57.38 (CH), C-1], [41.43 (CH), 40.98 (CH), C-4], [38.46 (CH_2), 38.34 (CH_2), C-7], [29.04 (CH_2), 29.00 (CH_2), C-6], [27.47 (CH_3), 27.29 (CH_3), ^{t}Bu], [23.15 (CH_2), 23.10 (CH_2), C-5]. CI MS *m*/*z*: $[M]^+$, ($C_{12}H_{19}LiNO_4{}^+$) 248.14582, required 248.14686.

2.9. (1R,3S,4S)-2-(tert-butoxycarbonyl)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid, **11**

According to the alkaline hydrolysis procedure described above for (±)**-7**, to a stirred solution of **10** (2.02 g, 7.50 mmol) in THF (10 ml) at 0ºC was added dropwise a solution of LiOH (1.1858 g, 49.5 mmol) in THF/H_2O (1:1) (40 ml) and the reaction mixture was stirred for 0.5 h, warmed to rt and stirred for a further stirred 48 h. After typical work-up it was obtained **11** (1.61 g, 89%) as a beige solid; **mp** = 147–150 ºC.

Spectral data for enantiopure **11** are in accordance with (±)**-11**.

2.10. General procedure for the synthesis of N-Boc tripeptides 12b /12b', 12a and 12b is the same for coupling procedure described for tripeptides 8a-c/8a'-c'

2.10.1. Methyl {(1SR,3RS,4RS)-2-(tert-butoxycarbonyl)-2-azabicyclo[2.2.1]heptane-3-carbonyl}-L-leucylglycinate, **12b/12b'**

The general peptide coupling procedure was applied to carboxylic acid (±)-**11** (0.797 g, 3.30 mmol) dissolved in dry CH_2Cl_2 (40 ml) in presence of DIEA (2.18 ml, 13.2 mmol), TBTU (1.06 g, 3.30 mmol) and corresponding amine **5b** (1.252 g, 3.30 mmol). The work-up procedure yielded a crude oil, which was purified using EtOAc as the eluent to afford tripeptides **12b/12b'** (1.264 g, 90%) as a pale greenish oil. R_f: 0.63 in EtOAc. ^{1}H-NMR ($CDCl_3$, 400 MHz, rotamers present) δ: [7.57 (br s), 6.88 (br s), 6.60 (d, J = 7.2 Hz), 6.41 (br s), 4H, D_2O exchange, 4 x CONH], 4.70 – 4.49 (m, 2H, 2 x H_L-2), 4.44 (s, 2H, 2 x H-3), 4.14 – 3.89 [m, 6H, 2 x (H-1 + H_G-2)], 3.80 – 3.62 [3.73 (s), 3.73 (s), 3.70 (s), 3.70 (s), 6H, 2 x OCH_3], 3.00 – 2.78 [2.92 (s), 2.85 (s), 2H, 2 x H-4], 2.30 – 1.15 [1.43 (s), 1.42 (s), 36H, 2 x (H-5 + H-6 + H-7 + H_L-3 + H_L-4 + Boc)], 1.03 – 0.80 (m, 6H, H_L-5). ^{13}C-NMR ($CDCl_3$, 101 MHz, rotamers present) δ: [172.15 (C), 171.19 (C), 170.01 (C), 169.95 (C), 4 x CONH + 2 x CO_2CH_3], 155.48 (C, 2 x $\underline{C}O_2{}^t$Bu), [81.24 (C), 80.55 (C), 2 x ^{t}Bu], [66.19 (CH), 65.57 (CH), 2 x C-3], [60.36 (CH), 58.99 (CH), 2 x C-1], [52.25 (CH_3), 52.11 (CH_3), 2 x OCH_3], [51.17 (CH), 50.90 (CH), 2 x C_L-2], 41.69 (2CH, 2 x C-4), [41.15 (CH_2), 41.06 (CH_2), 2 x C_G-2], 40.50 ($2CH_2$, 2 x C_L-3), [39.23 (CH_2), 39.01 (CH_2), 2 x C-7], [30.30 (CH_2), 29.75 (CH_2), 2 x C-6], 28.24 ($6CH_3$, 2 x ^{t}Bu), [25.00 (CH), 24.42 (CH), 2 x C_L-4], [23.20 (CH_2), 23.16 (CH_3), 23.07 (CH_2), 23.00 (CH_3), 21.78 (CH_3), 21.44 (CH_3), 2 x (C-5 + C_L-5)]. ESI-TOF HRMS *m/z*: $[M+H]^+$, ($C_{21}H_{36}N_3O_6^+$) 426.25925, required 426.25986.

*2.10.2. Methyl {(1R,3S,4S)-2-(tert-butoxycarbonyl)-2-azabicyclo[2.2.1]heptane-3-carbonyl}-*L*-valyl-*L*-alaninate,* **12a**

The general peptide coupling procedure was applied to carboxylic acid (*1R,3S,4S*)-**11** (0.708 g, 2.93 mmol) dissolved in dry CH_2Cl_2 (40 ml) in presence of DIEA (2.04 ml, 11.7 mmol), TBTU (0.941 g, 2.93 mmol) and corresponding amine **5a** (0.927 g, 2.93 mmol). The work-up procedure yielded a crude oil, which was purified using EtOAc as the eluent to afford tripeptide **12a** (1.134 g, 91%) as a white solid. **mp**: 40–45ºC. R_f: 0.66 in EtOAc. ^{1}H-NMR ($CDCl_3$, 400 MHz, rotamers present) δ: [7.23 – 6.90 (m, 1H, CONH), 6.88 – 6.55 (m, 1H, CONH), 4.58 – 4.45 [4.52 (p, J = 7.3 Hz), 1H, H_V-2], 4.37 – 4.06 [4.29 (dd, J = 9.0, 5.9 Hz), 2H, H_A-2 + H-3], 3.85 – 3.52 [3.70 (s), 4H, H-1 + OCH_3], 2.94 – 2.65 (m, 1H, H-4), 2.42 – 2.03 (m, 1H, H_V-3), 1.83 – 1.26 (m, 18H, H-5 + H-6 + H-7+ H_A-3 + Boc), 0.98 – 0.77 (m, 6H, H_V-4). ^{13}C-NMR ($CDCl_3$, 101 MHz, rotamers present) δ: [173.08 (C), 171.39 (C), 171.03 (C), 170.73 (C), 2 x CONH + CO_2CH_3], 156.42 (C, $\underline{C}O_2{}^t$Bu), 80.93 (C, ^{t}Bu), 66.77 (CH, C-3), [58.22 (CH), 57.96 (CH), C-1 + C_A-2], 52.42 (CH_3, OCH_3), 48.17 (CH, C_V-2), [40.84 (CH), 40.31 (CH), C-4], [36.41 (CH_2), 36.08 (CH_2), C-7], [34.73 (CH), 30.00 (CH), C_V-3], [29.91 (CH_2), 29.77 (CH_2), C-6], 28.44 ($3CH_3$, ^{t}Bu), 27.01 (CH_2, C-5), [19.45 (CH_3), 18.22 (CH_3), 18.05 (CH_3), 17.31 (CH_3), 17.12 (CH_3), C_A-3 + C_V-4)]. ESI-TOF HRMS *m/z*: $[M+H]^+$, ($C_{21}H_{36}N_3O_6^+$) 426.25849, required 426.25986.

*2.10.3. Methyl {(1R,3S,4S)-2-(tert-butoxycarbonyl)-2-azabicyclo[2.2.1]heptane-3-carbonyl}-*L*-leucylglycinate,* **12b**

The general peptide coupling procedure was applied to carboxylic acid (*1R,3S,4S*)-**11** (0.615 g, 2.55 mmol) dissolved in dry CH_2Cl_2 (40 ml) in presence of DIEA (1.78 ml, 10.2 mmol), TBTU (0.819 g, 2.55 mmol) and corresponding amine **5b** (0.807 g, 2.55 mmol). The work-up procedure yielded a crude oil, which was purified using EtOAc as the eluent to afford tripeptide **12b** (1.063 g, 98%) as a white foam. **mp**: 42–45ºC. R_f: 0.62 in EtOAc. ^{1}H-NMR ($CDCl_3$, 400 MHz, rotamers present) δ: 7.52 – 6.44 [7.29 (br s), 7.03 (d, J = 8.7 Hz), 6.95 (d, J = 7.2 Hz), 6.67 (br s), 2H, D_2O exchange, 2 x

CONH], 4.62 – 4.44 (m, 1H, H_L-2), 4.30 – 3.83 [4.15 (s), 4.22 (s), 3H, H-3 + H_G-2), 3.83 – 3.58 [3.79 (s), 3.72 (s), 4H, H-1 + OCH_3], 3.00 – 2.71 [2.88 (s), 2.81 (s), 2H, H-4], 1.92 – 1.21 [1.47 (s), 18H, H-5 + H-6 + H-7 + H_L-3 + H_L-4 + Boc], 1.04 – 0.81 (m, 6H, H_L-5). ^{13}C-NMR ($CDCl_3$, 101 MHz, rotamers present) δ: [172.54 (C), 171.07 (C), 170.17 (C), 2 x CONH + CO_2CH_3], 156.61 (C, $\underline{C}O_2{}^t$Bu), 81.14 (C, ^{t}Bu), 66.78 (CH, C-3), 58.12 (CH, C-1), 52.21 (CH_3, OCH_3), 51.53 (CH, C_L-2), 41.14 (CH, C-4), 40.35 (CH_2, C_G-2), 36.33 (CH_2, C_L-3), 29.83 (CH_2, C-7), 28.37 (CH_2, C-6), 27.20 ($3CH_3$, ^{t}Bu), 25.01 (CH, C_L-4), 23.21 (CH_2, C-5), 21.51 (CH_3, C_L-5), 20.73 (CH_3, C_L-5)]. ESI-TOF HRMS *m*/*z*: $[M+H]^+$, ($C_{21}H_{36}N_3O_6^+$) 426.25829, required 426.25986.

2.11. General procedure for N-Boc deprotection of tripeptides 12a and 12b is the same for 4a-c

*2.11.1. Methyl {(1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carbonyl}-*L*-valyl-*L*-alaninate trifluoroacetate,* **1a** · **TFA**

The general procedure was applied to **12a** (0.520 g, 1.22 mmol) in dry CH_2Cl_2 (40 ml) and TFA (2.8 ml, 37 mmol). The work-up procedure followed by recrystallization in Et_2O/EtOAc (10:1) afforded tripeptide **1a** · **TFA** (0.525 g, 98%) as a pale greenish solid. R_f: 0.47 in CH_2Cl_2/MeOH 10:1. ^{1}H-NMR (DMSO-d_6, 400 MHz, rotamers present) δ: 9.20 (br s, 1Hm N$\underline{H}$H), 8.88 – 8.64 [8.82 (d, J = 9.0 Hz), 8.69 (d, J = 8.7 Hz), 1H, CONH], 8.63 – 8.42 [8.54 (d, J = 7.1 Hz), 8.49 (d, J = 6.6 Hz), 1H, CONH], 4.41 – 4.17 (m, 2H, H_V-2 + H_A-2), 4.08 – 4.01 (br s, H-3), 3.97 – 3.89 (br s, H-1), 3.67 – 3.54 [3.62 (s), 3.60 (s), 3H, OCH_3], 2.79 – 2.71 [2.73 (br s), 2.71 (br s), 1H, H-1), 2.09 – 1.97 (m, 1H, H_V-3), 1.79 – 1.43 (m, 6H, H-5 + H-6 + H-7), 1.33 – 1.23 [1.28 (d, J = 6.9 Hz), 1.27 (d, J = 7.2 Hz), 3H, H_A-3], 0.96 – 0.77 (m, 6H, H_V-4). ^{13}C-NMR (DMSO-d_6, 101 MHz, rotamers present) δ: [172.75 (C), 170.14 (C), 169.94 (C), 167.52 (C), 167.46 (C), 2 x CONH + CO_2CH_3], [61.95 (CH), 61.61 (CH), C-3], [58.06 (CH), 57.95 (CH), C_V-2], [57.62 (CH), C-1], [51.81 (CH_3), 51.71 (CH_3), OCH_3], [47.57 (CH), 47.45 (CH), C_A-2], [42.22 (CH), 41.87 (CH), C-4], [33.67 (CH_2), 33.49 (CH_2),

C-7], [31.17 (CH), 30.68 (CH), C_V-3], [26.69 (CH_2), 26.65 (CH_2), C-6], 24.71 (CH_2, C-5), [19.03 (CH_3), 19.00 (CH_3), 17.70 (CH_3), 17.54 (CH_3), C_V-4], [17.03 (CH_3), 16.69 (CH_3), C_A-3]. ESI-TOF HRMS *m*/*z*: $[M+H]^+$, ($C_{16}H_{28}N_3O_4^+$) 326.20694, required 326.20743.

*2.11.2. Methyl {(1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carbonyl}-*L*-leucylglicinate trifluoracetate,* **1b · TFA**

The general procedure was applied to **12b** (0.612 g, 1.44 mmol) in dry CH_2Cl_2 (40 ml) and TFA (3.3 ml, 43 mmol). The work-up procedure followed by recrystallization in Et_2O afforded tripeptide **1b · TFA** (0.633 g, 100%) as a pale greenish solid; **mp**: 126–129ºC. R_f: 0.1 in EtOAc. H-NMR (CD_3OD, 400 MHz, rotamers present) δ: 4.53 – 4.38 (m, 1H, H_L-2), 4.21 – 4.09 [4.16 (br s), 4.14 (br s), 1H, H-3], 4.07 – 3.79 [4.00 (d, J = 17.6 Hz), 3.94 (br s), 3.87 (d, J = 17.5 Hz), 3H, H-1 + H_G-2], 3.71 (s, 3H, OCH_3), 3.00 – 2.75 [2.96 (br s), 2.82 (br s), 1H, H-4], 1.95 – 1.53 (m, 9H, H-5 + H-6 + H-7 + H_L-3 + H_L-4), 1.05 – 0.86 [0.97 (d, J = 6.4 Hz), 0.94 (d, J = 6.4 Hz), 6H, H_L-5]. ^{13}C-NMR (CD_3OD, 101 MHz, rotamers present) δ: [174.80 (C), 174.76 (C), 171.52 (C), 171.46 (C), 168.86 (C), 2 x CONH + CO_2CH_3], [64.17 (CH), 63.89 (CH), C-3], 59.94 (CH, C-1), [53.73 (CH), 53.46 (CH), C_L-2], [52.58 (CH_3), 52.56 (CH_3), OCH_3], [43.41 (CH), 43.18 (CH), C-4], [41.88 (CH_2), 41.80 (CH_2), C_L-3], 41.77 (CH_2, C_G-2), [34.97 (CH_2), 34.84 (CH_2), C-7], [27.94 (CH_2), 27.88 (CH_2), C-6], [26.38 (CH_2), 26.27 (CH_2), C-5], [26.09 (CH), 25.85 (CH), C_L-4], [23.39 (CH_3), 23.27 (CH_3), 21.97 (CH_3), 21.68 (CH_3), C_L-5]. ESI-TOF HRMS *m*/*z*: $[M+H]^+$, ($C_{16}H_{28}N_3O_4^+$) 326.20744, required 326.20743.

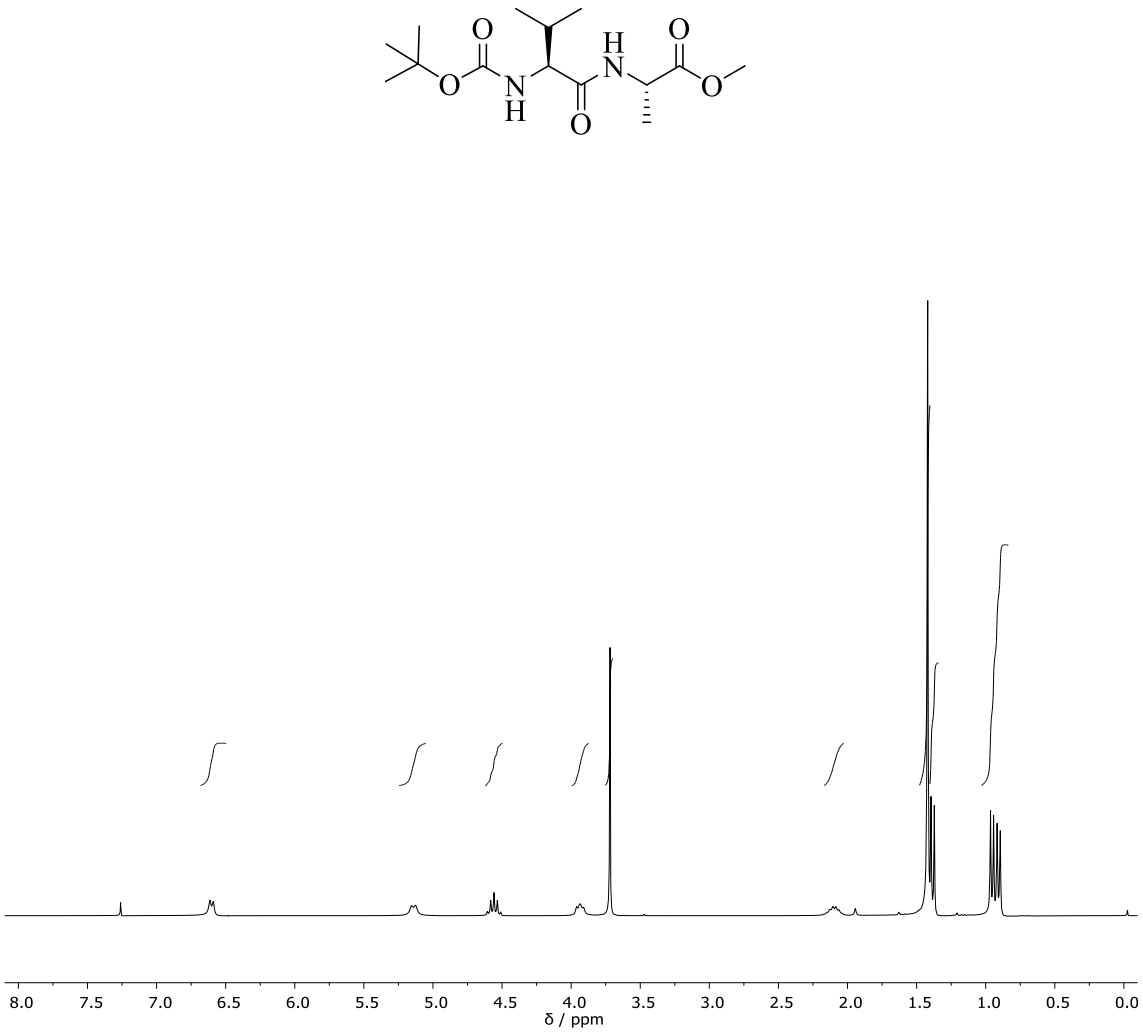

Fig. S1. ^{1}H-NMR espectrum ($CDCl_3$, 300 MHz) of compound **4a**.

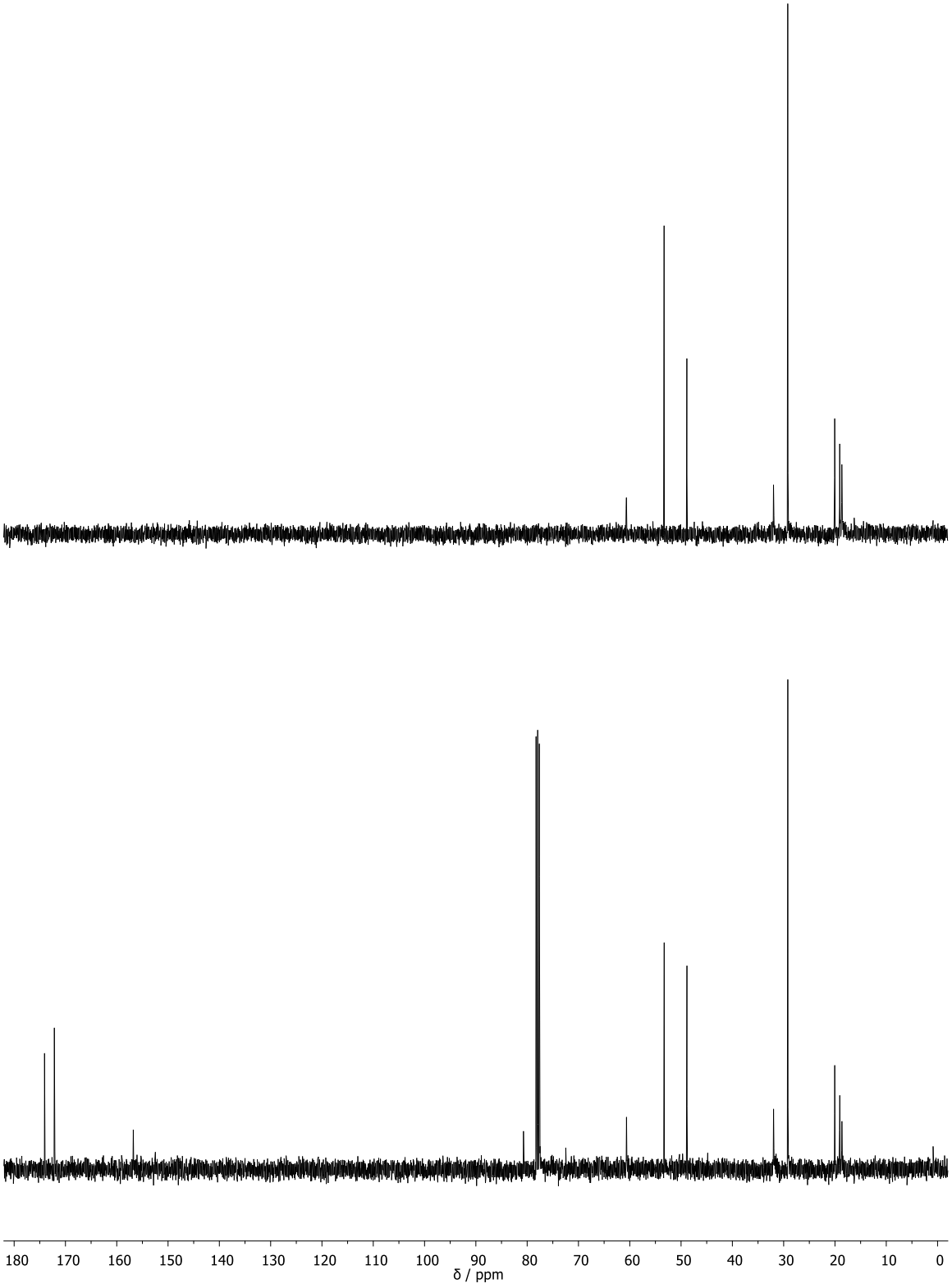

Fig. S2. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 75 MHz) of compound **4a**.

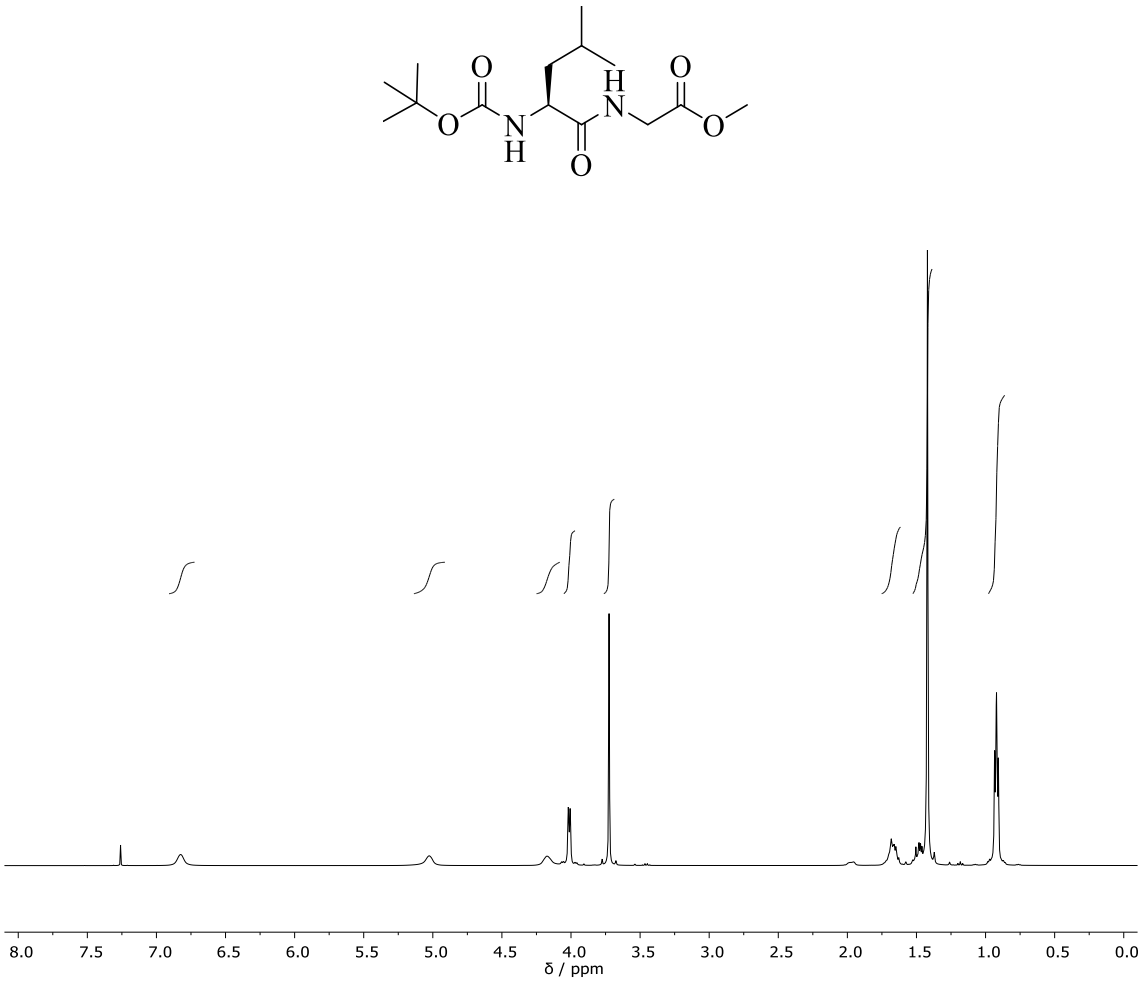

Fig. S3. ^{1}H-NMR espectrum ($CDCl_3$, 400 MHz) of compound **4b**.

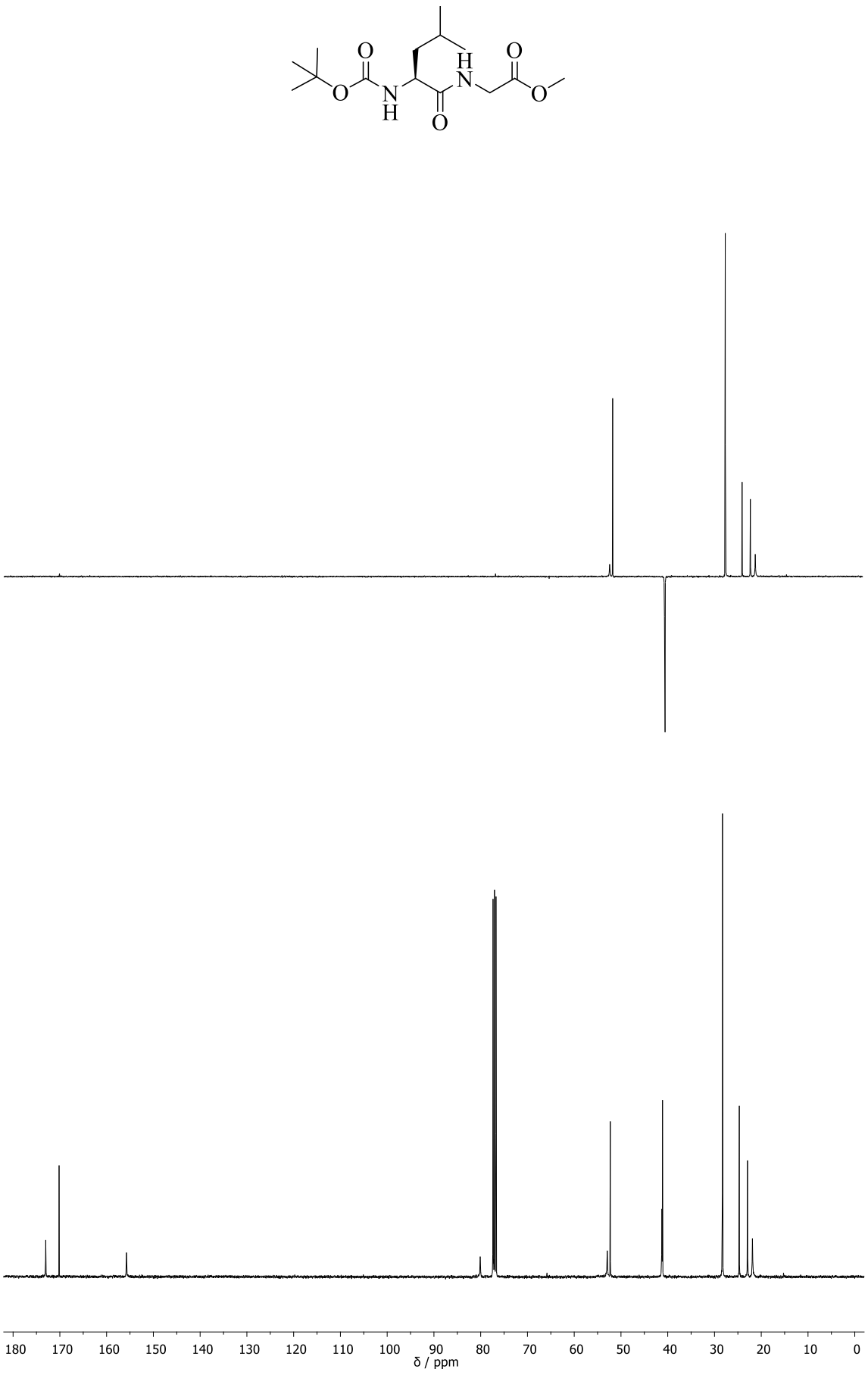

Fig. S4. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 101 MHz) of compound **4b**.

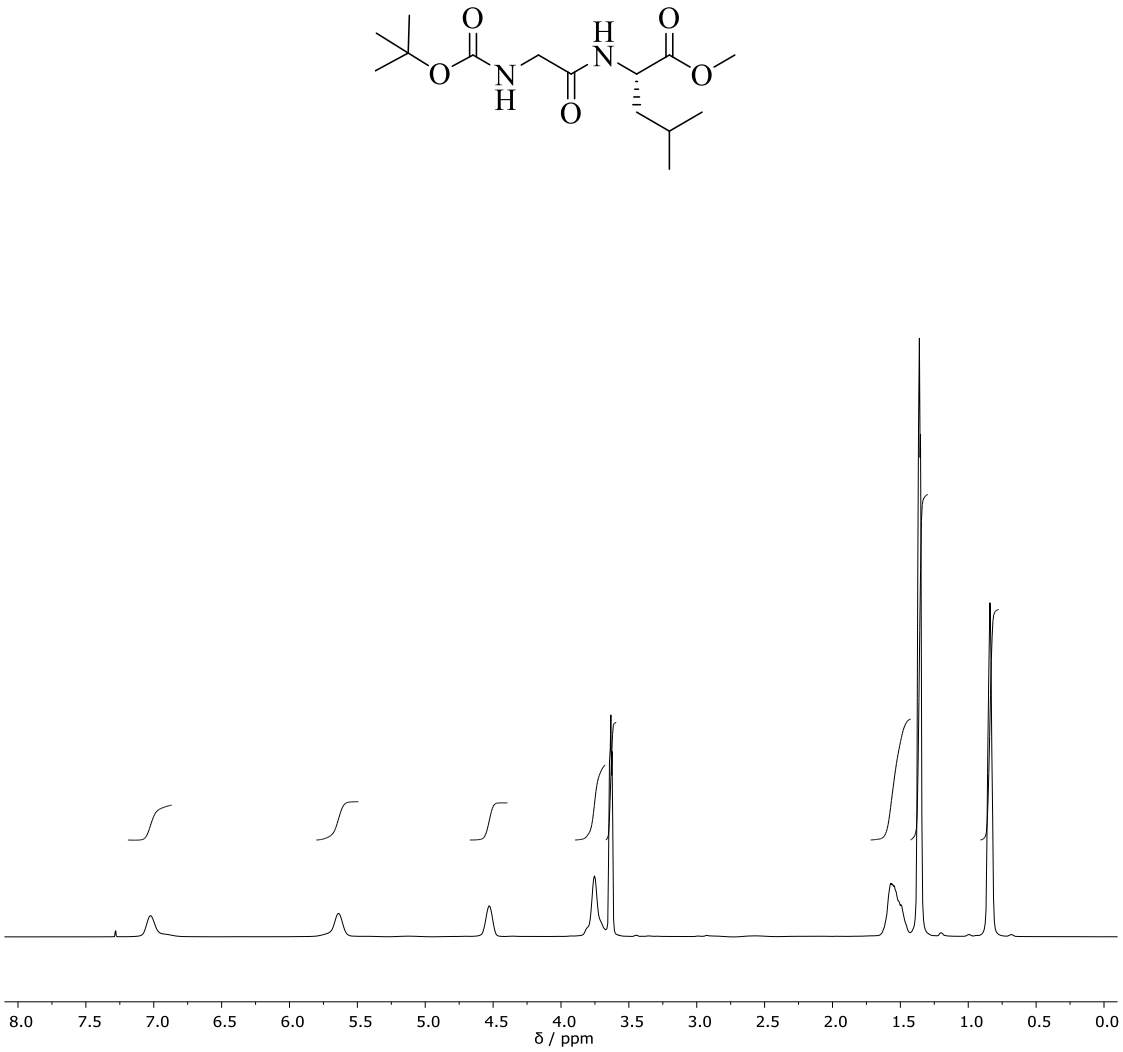

Fig. S5. ^{1}H-NMR espectrum ($CDCl_3$, 300 MHz) of compound **4c**.

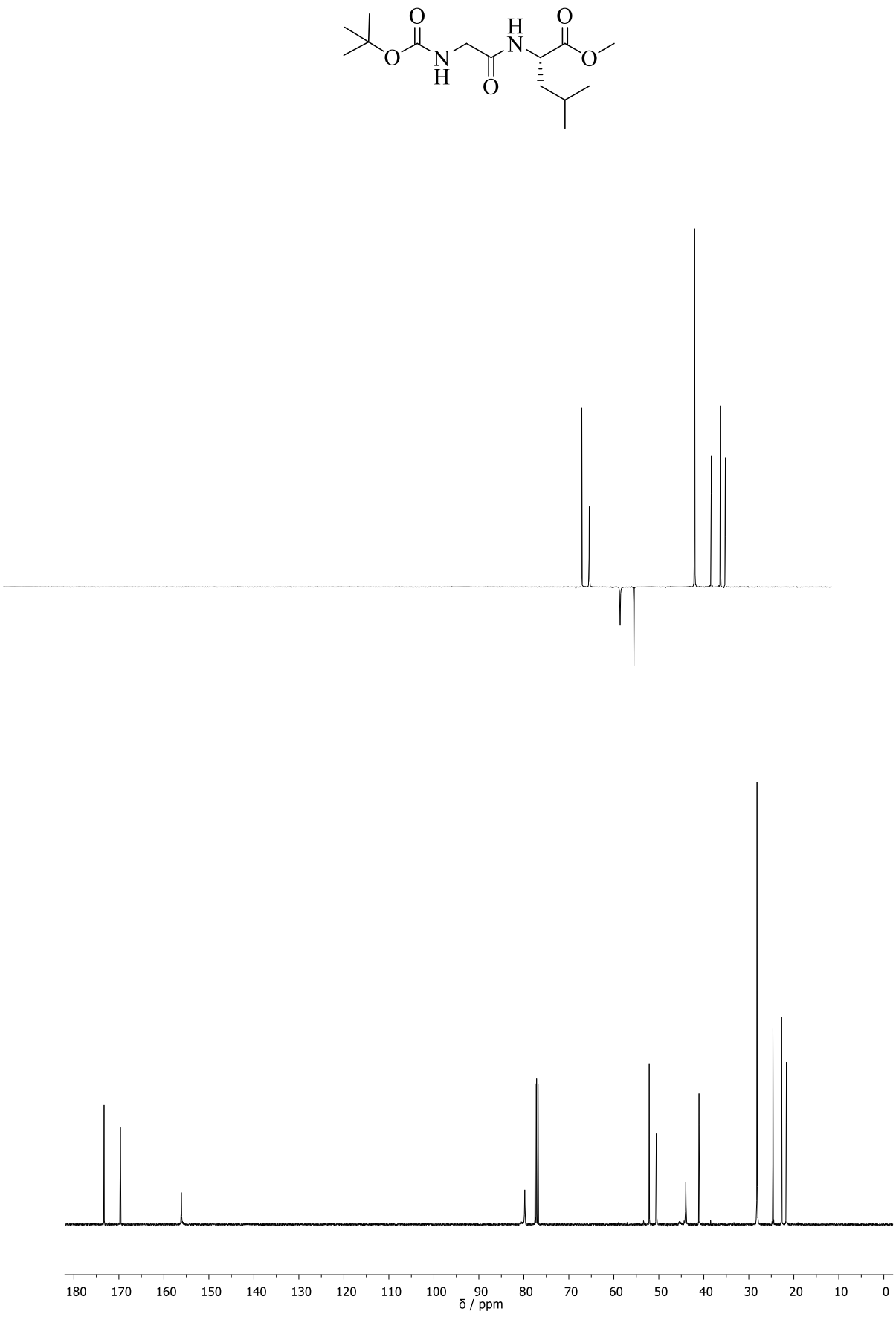

Fig. S6. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 75 MHz) of compound **4c**.

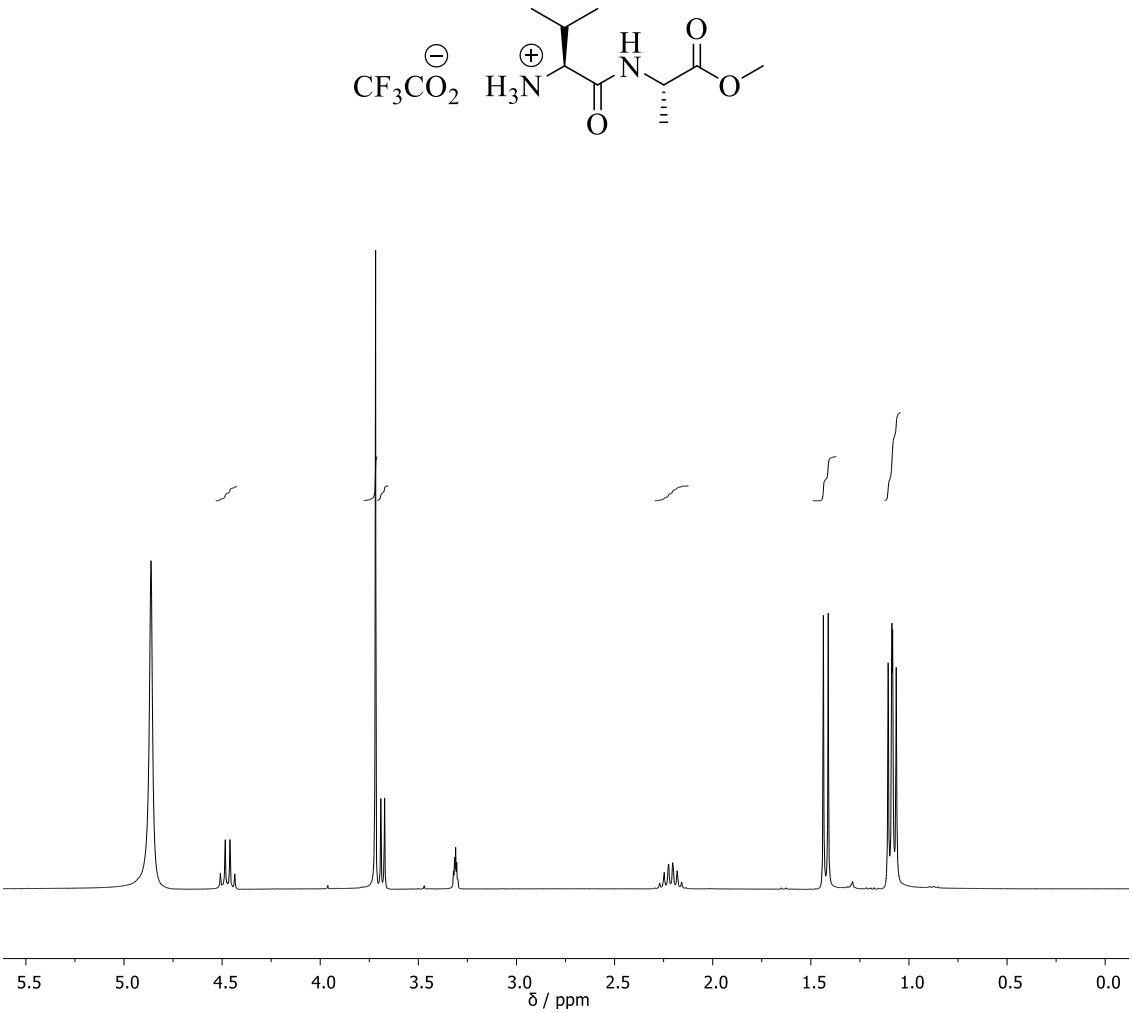

Fig. S7. ^{1}H-NMR espectrum (CD_3OD, 300 MHz) of compound **5a**.

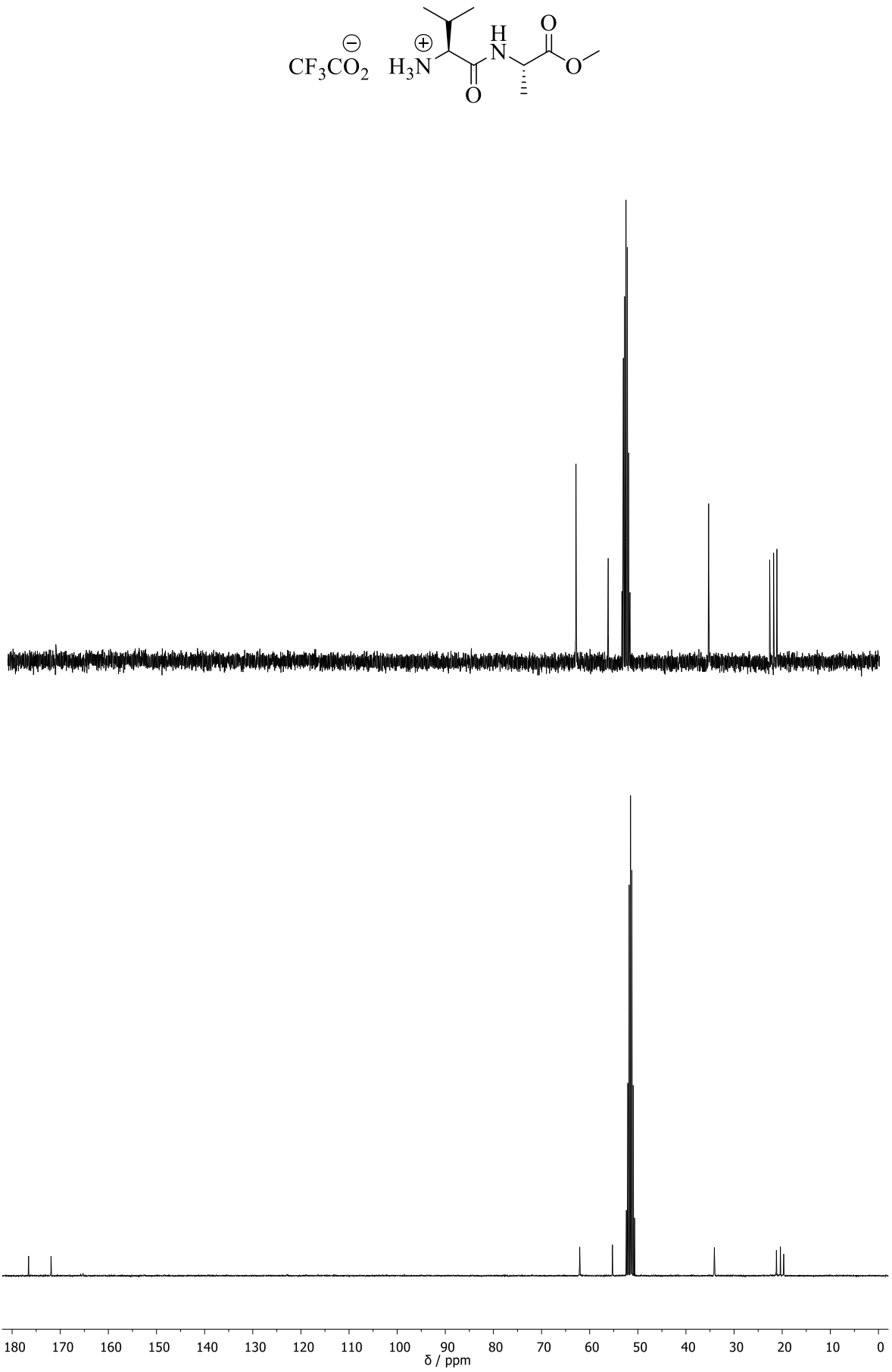

Fig. S8. DEPT (above) and ^{13}C-NMR (bottom) espectra (CD_3OD, 75 MHz) of compound **5a**.

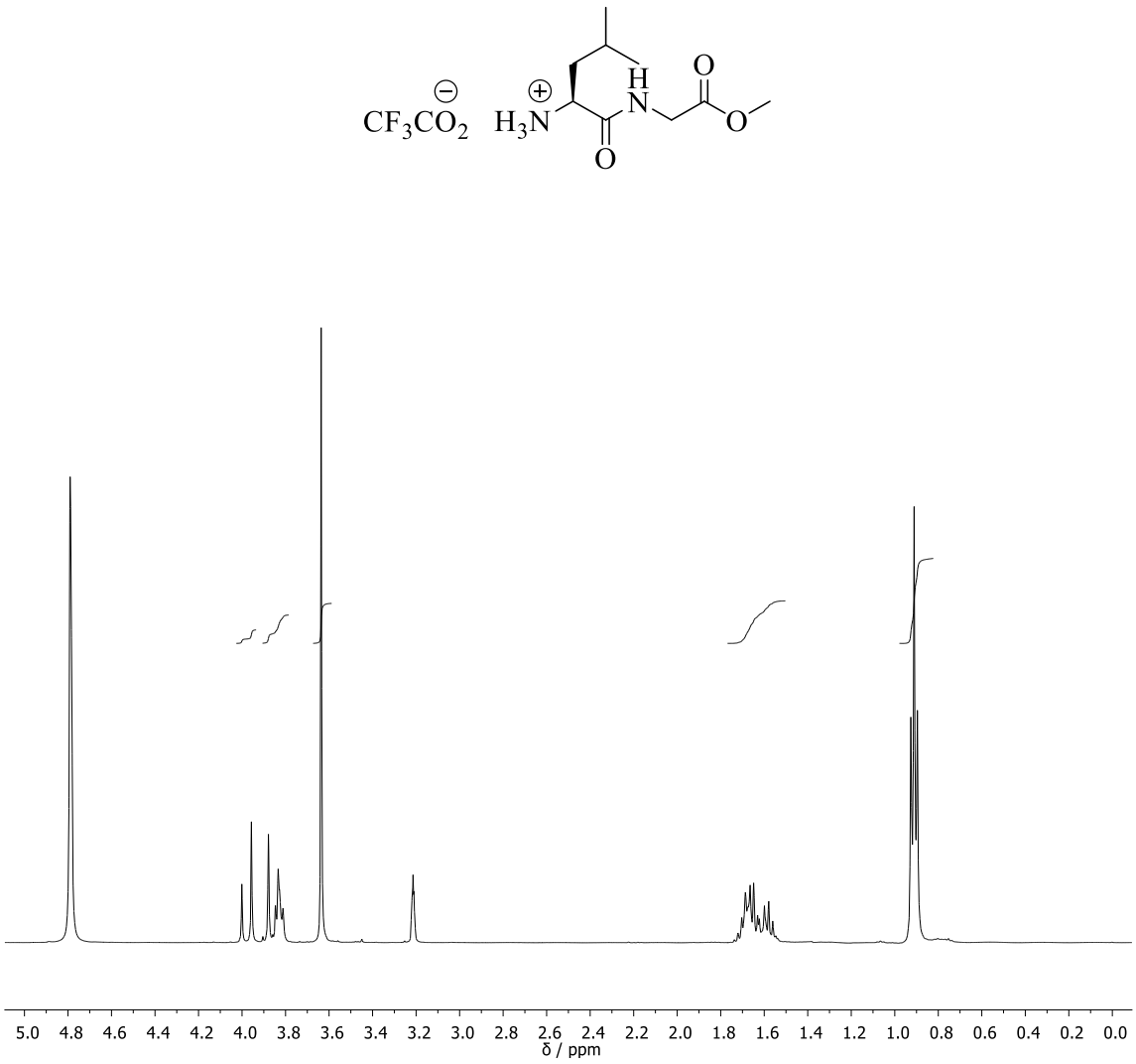

Fig. S9. ^{1}H-NMR espectrum (CD_3OD, 400 MHz) of compound **5b**.

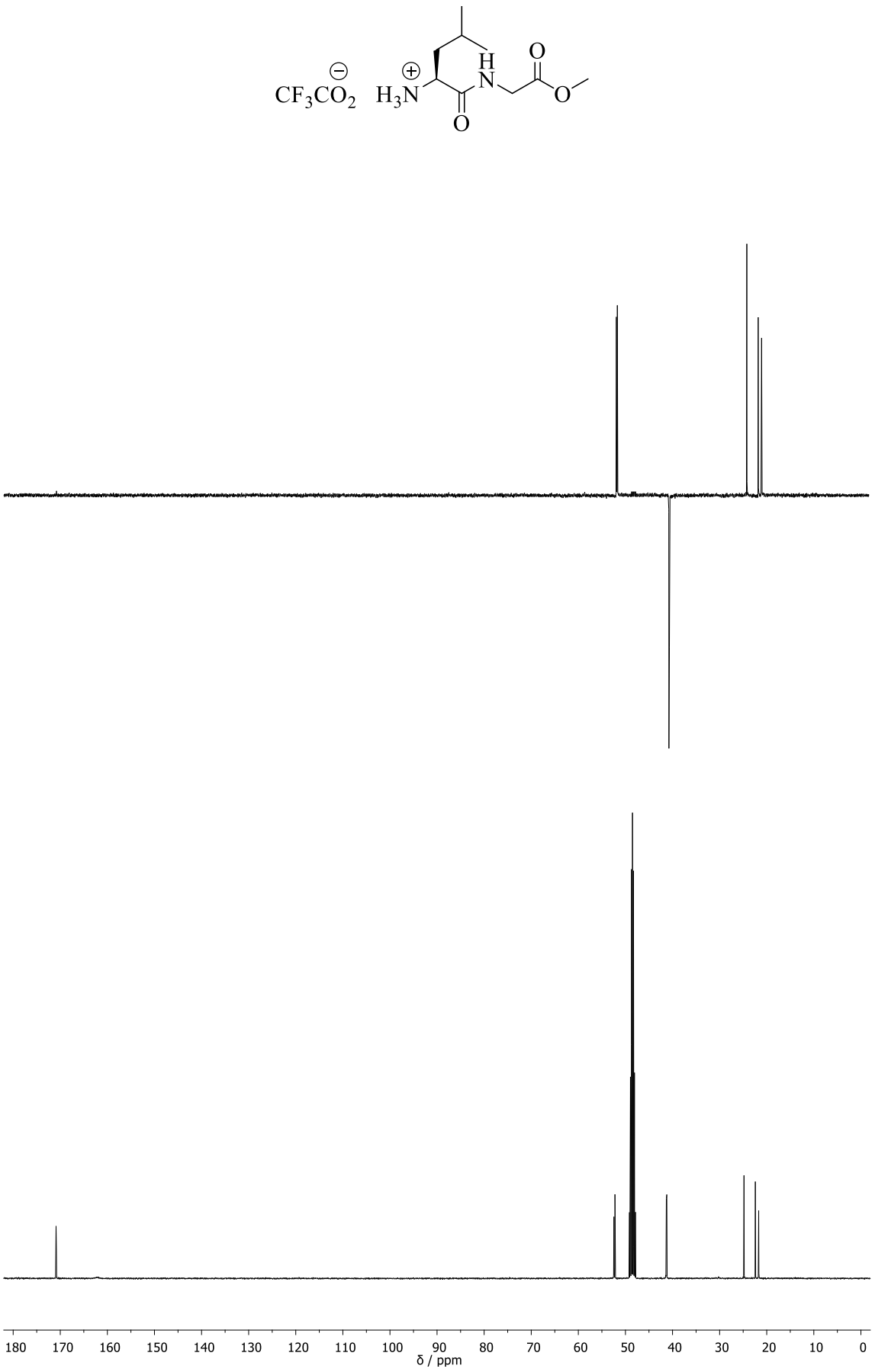

Fig. S10. DEPT (above) and ^{13}C-NMR (bottom) espectra (CD_3OD, 101 MHz) of compound **5b**.

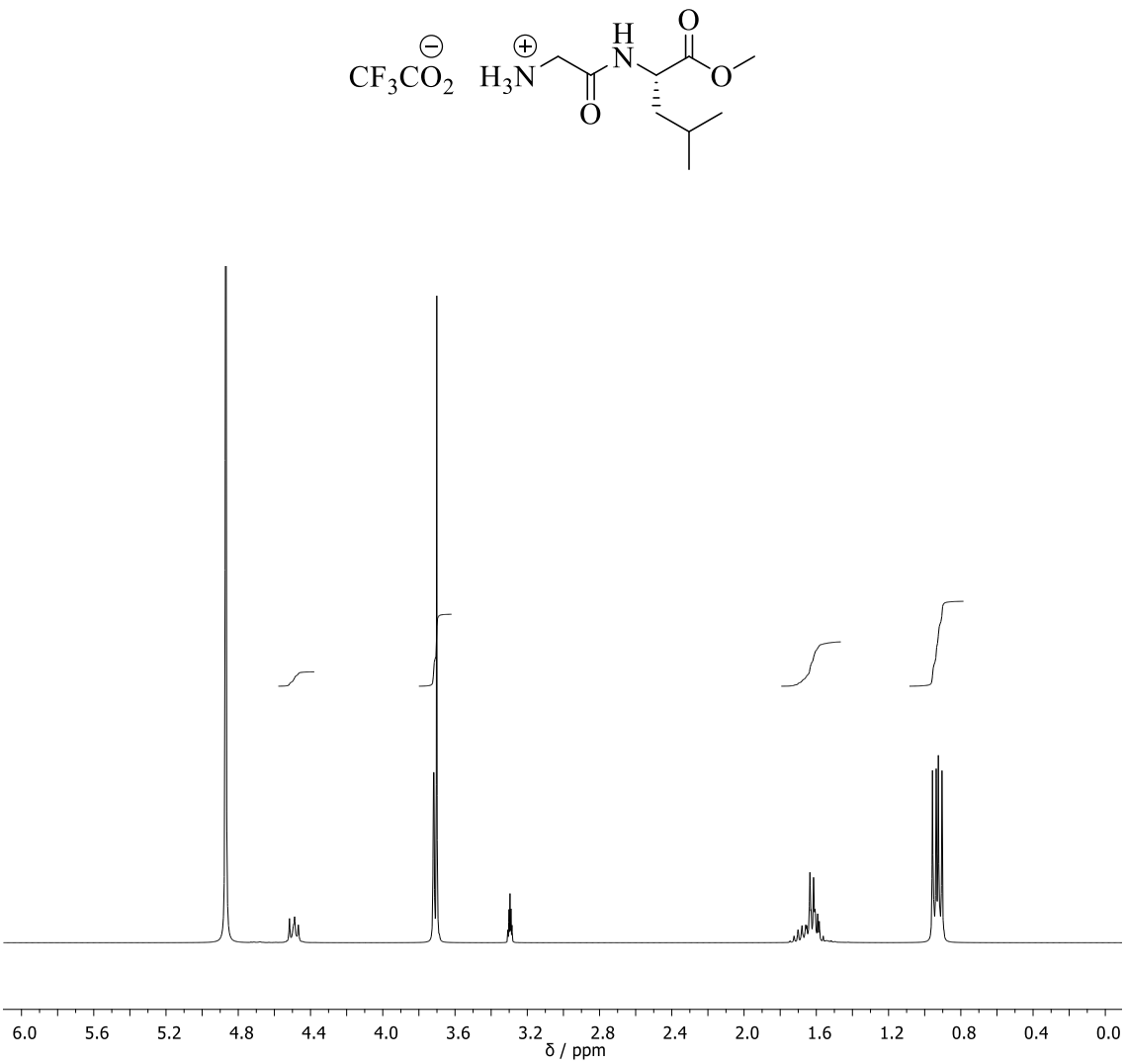

Fig. S11. ^{1}H-NMR espectrum (CD_3OD, 300 MHz) of compound **5c**.

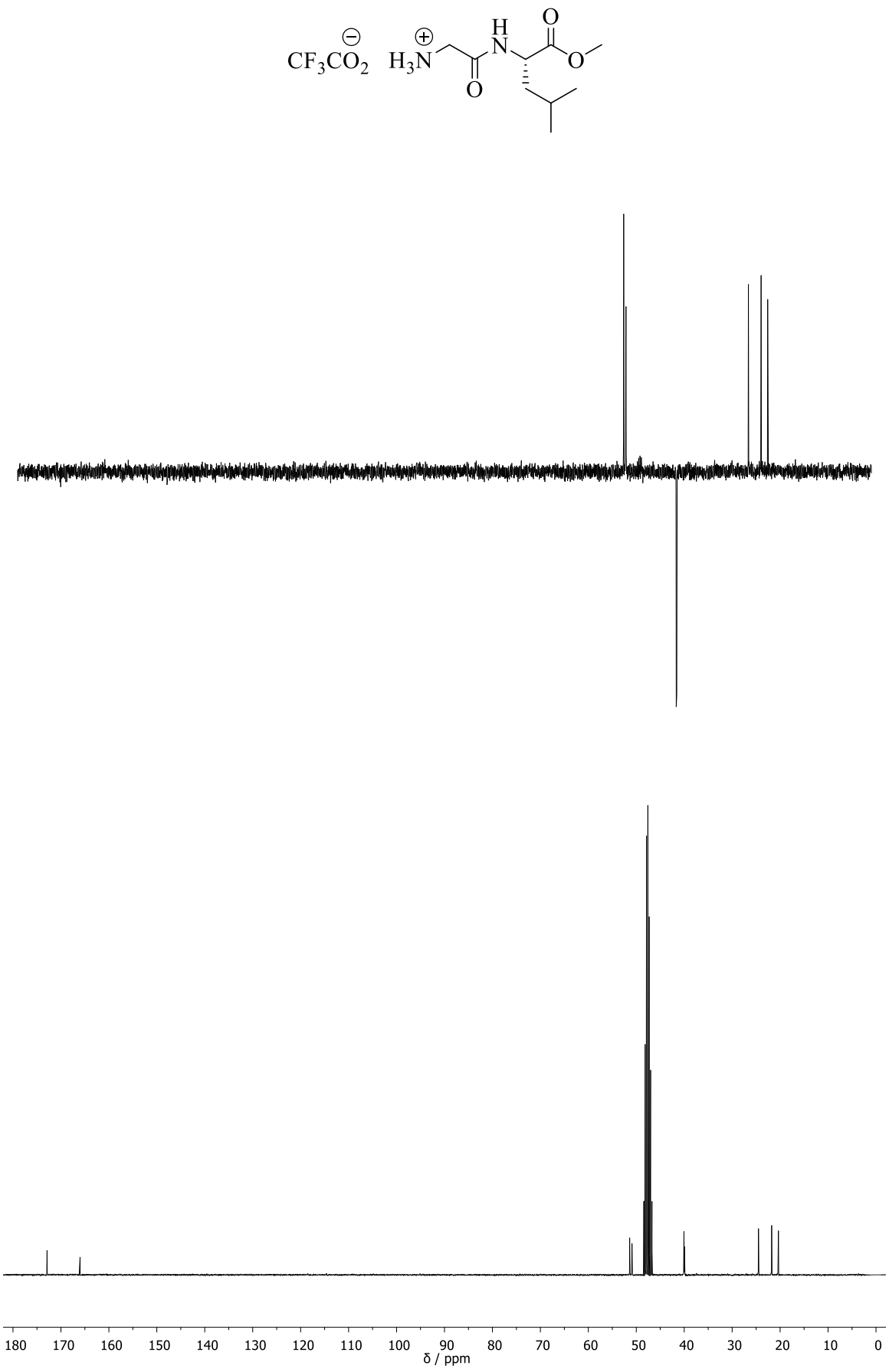

Fig. S12. DEPT (above) and ^{13}C-NMR (bottom) espectra (CD_3OD, 75 MHz) of compound **5c**.

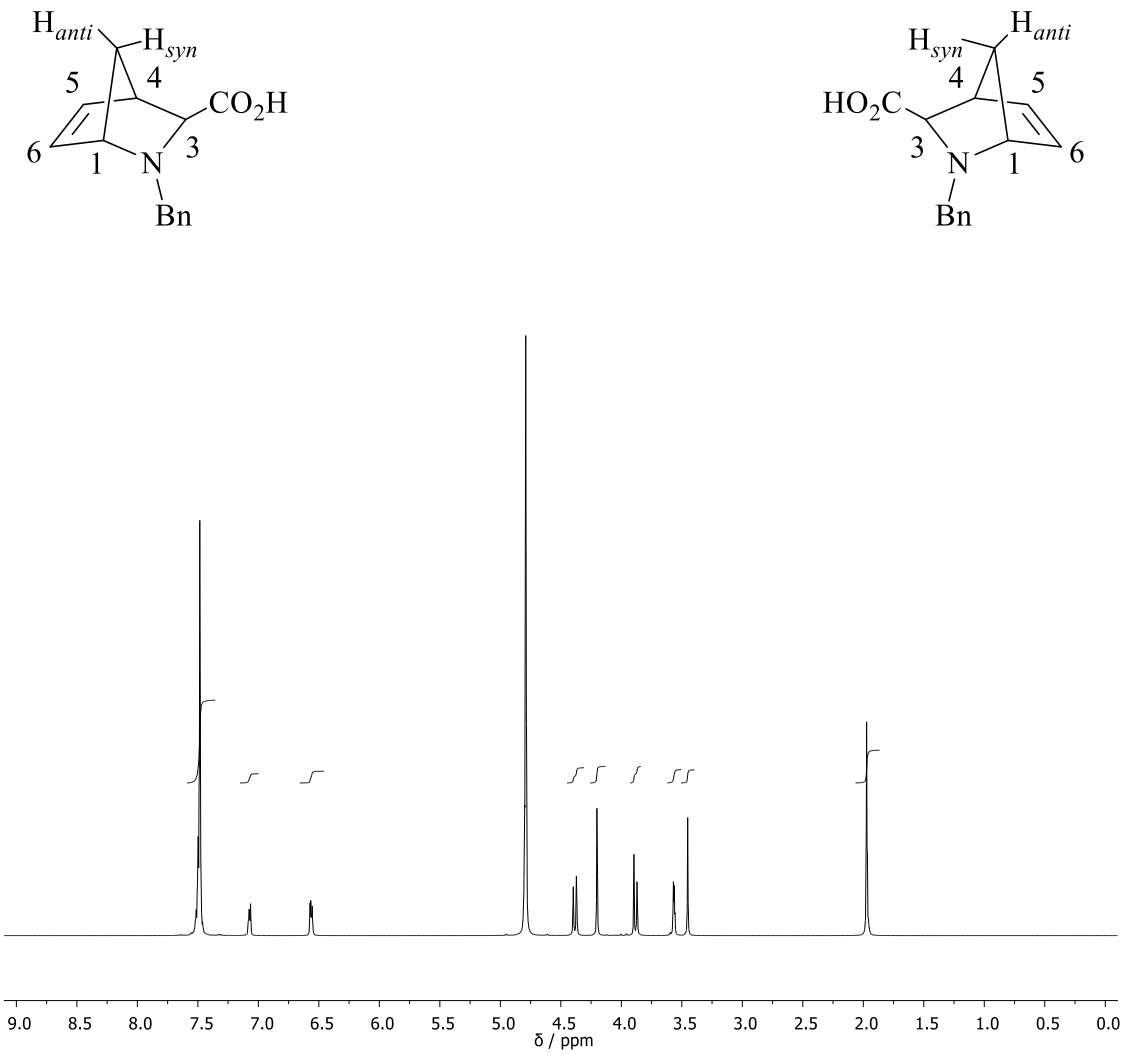

Fig. S13. ^{1}H-NMR espectrum (D_2O, 500 MHz) of compound (±)-**7**.

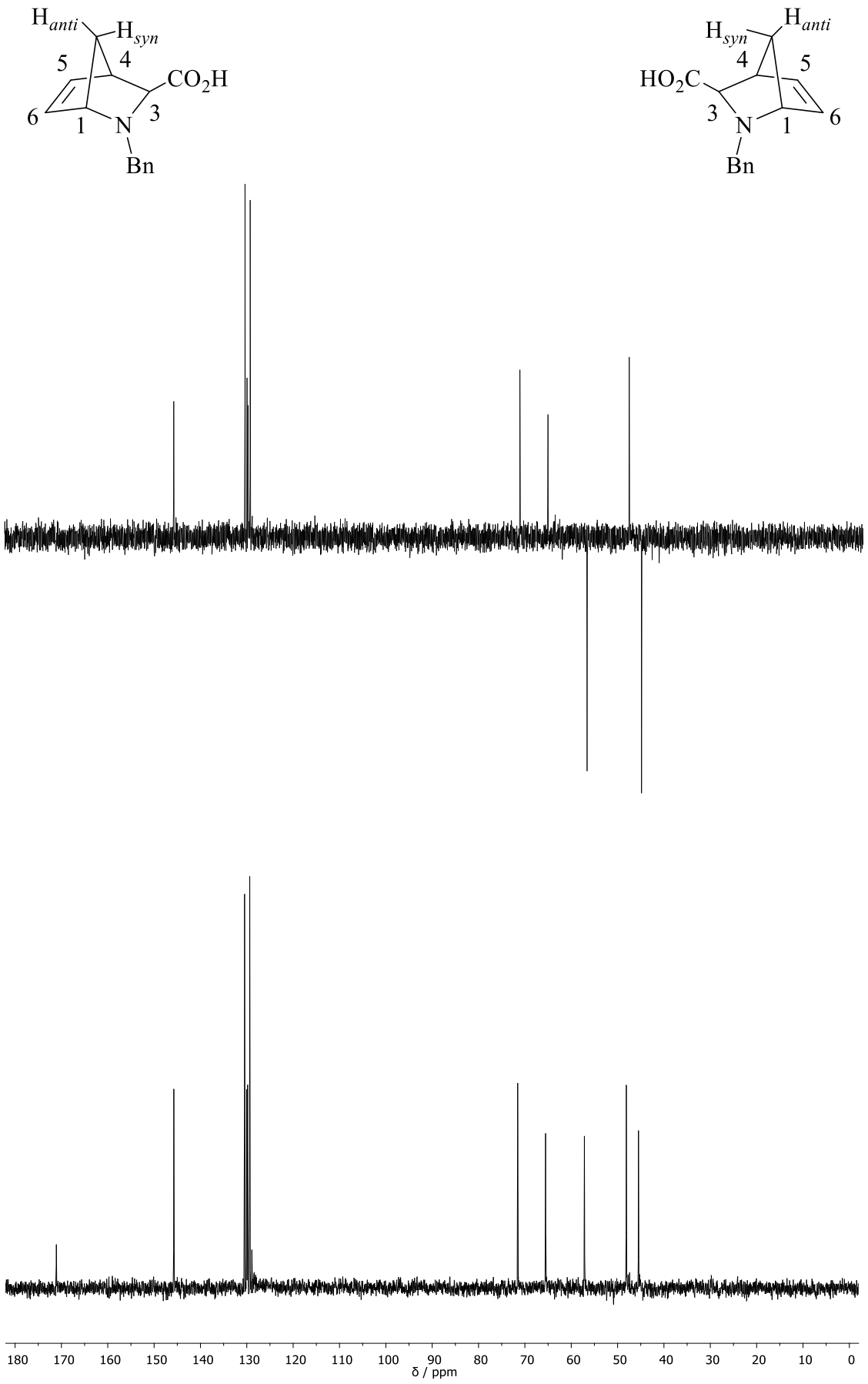

Fig. S14. DEPT (above) and ^{13}C-NMR (bottom) espectra (D_2O, 126 MHz) of compound (±)-**7**.

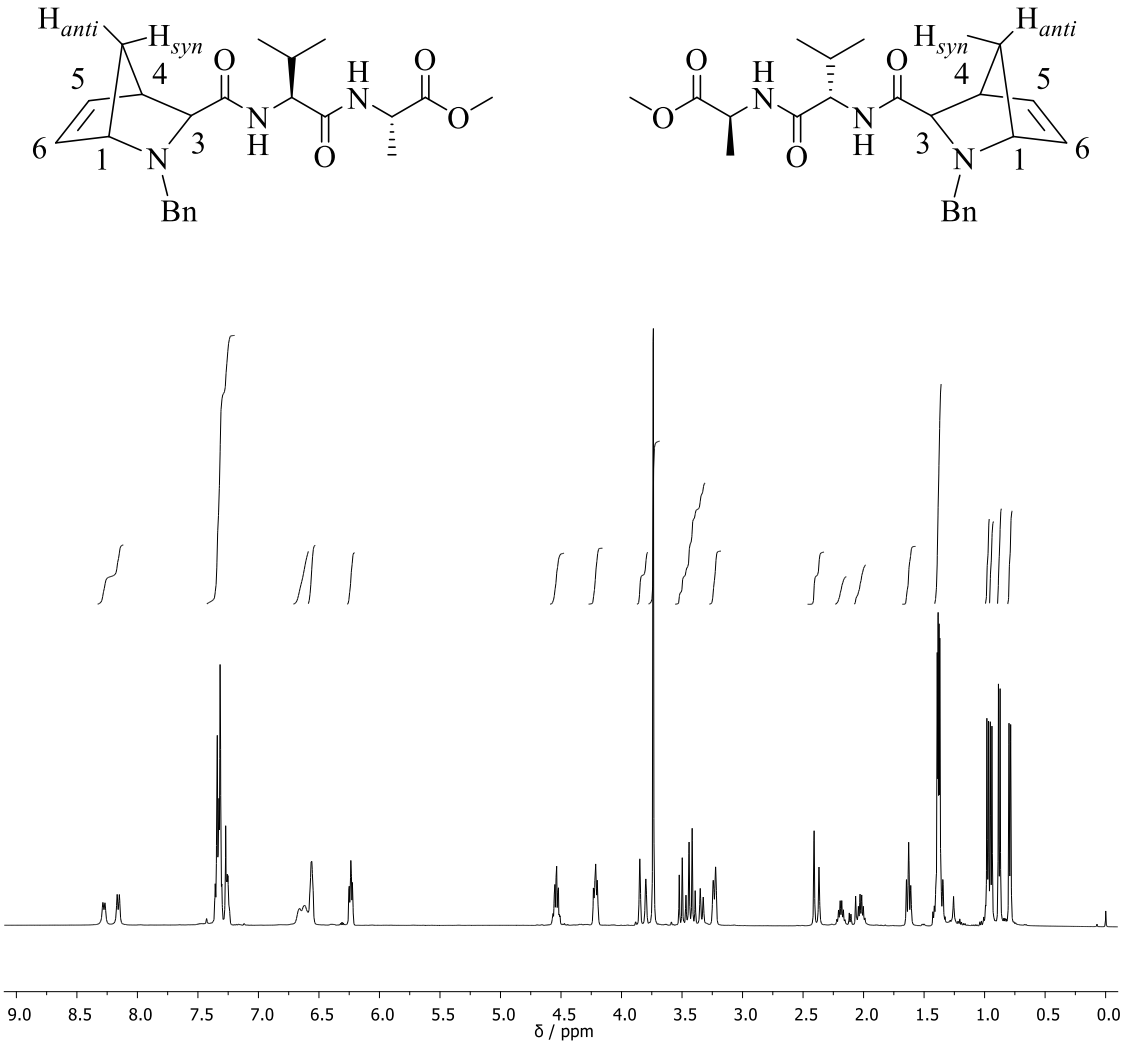

Fig. S15. ^{1}H-NMR espectrum ($CDCl_3$, 500 MHz) of compounds **8a/8a'**.

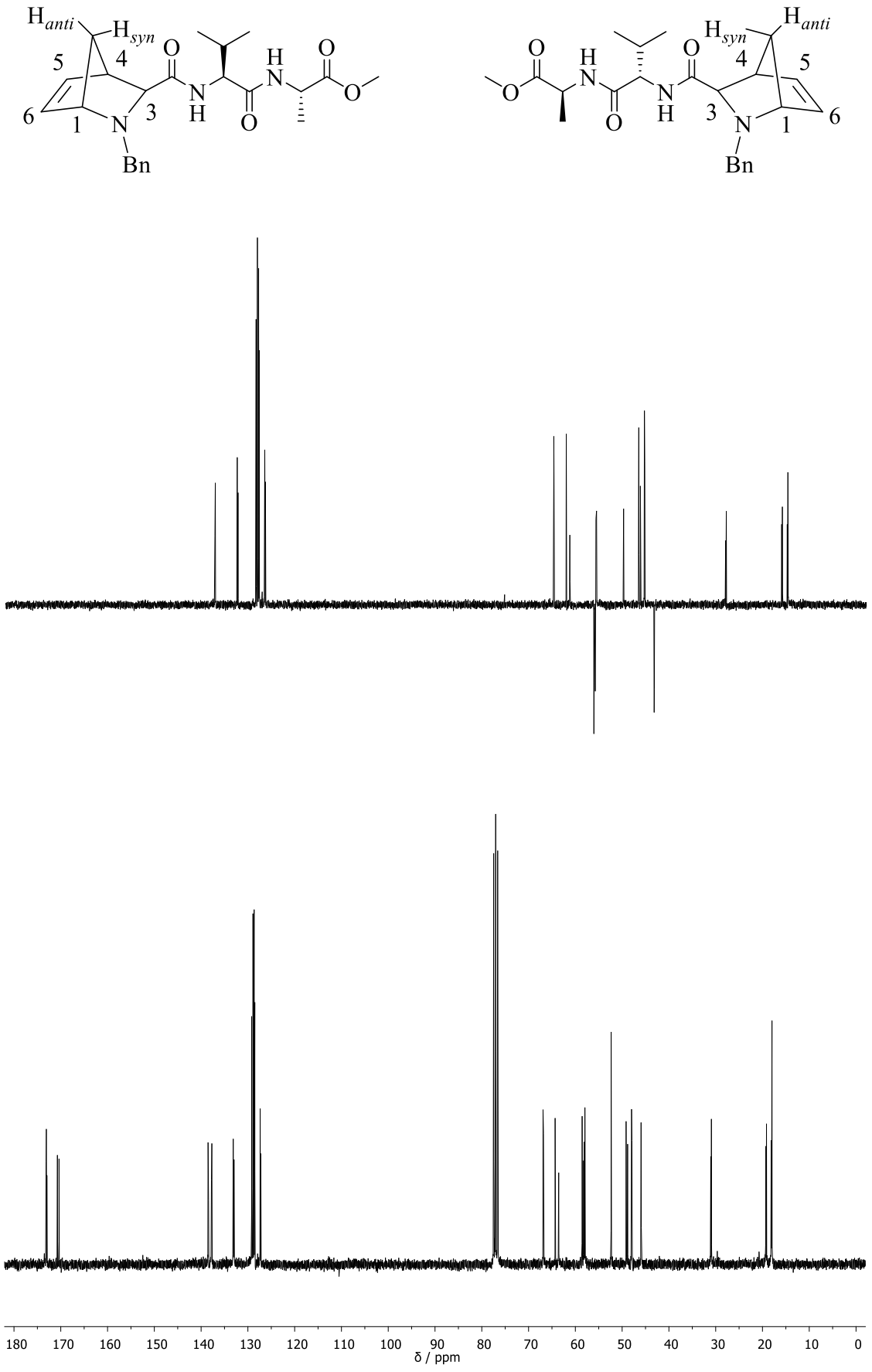

Fig. S16. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 126 MHz) of compounds **8a/8a'**.

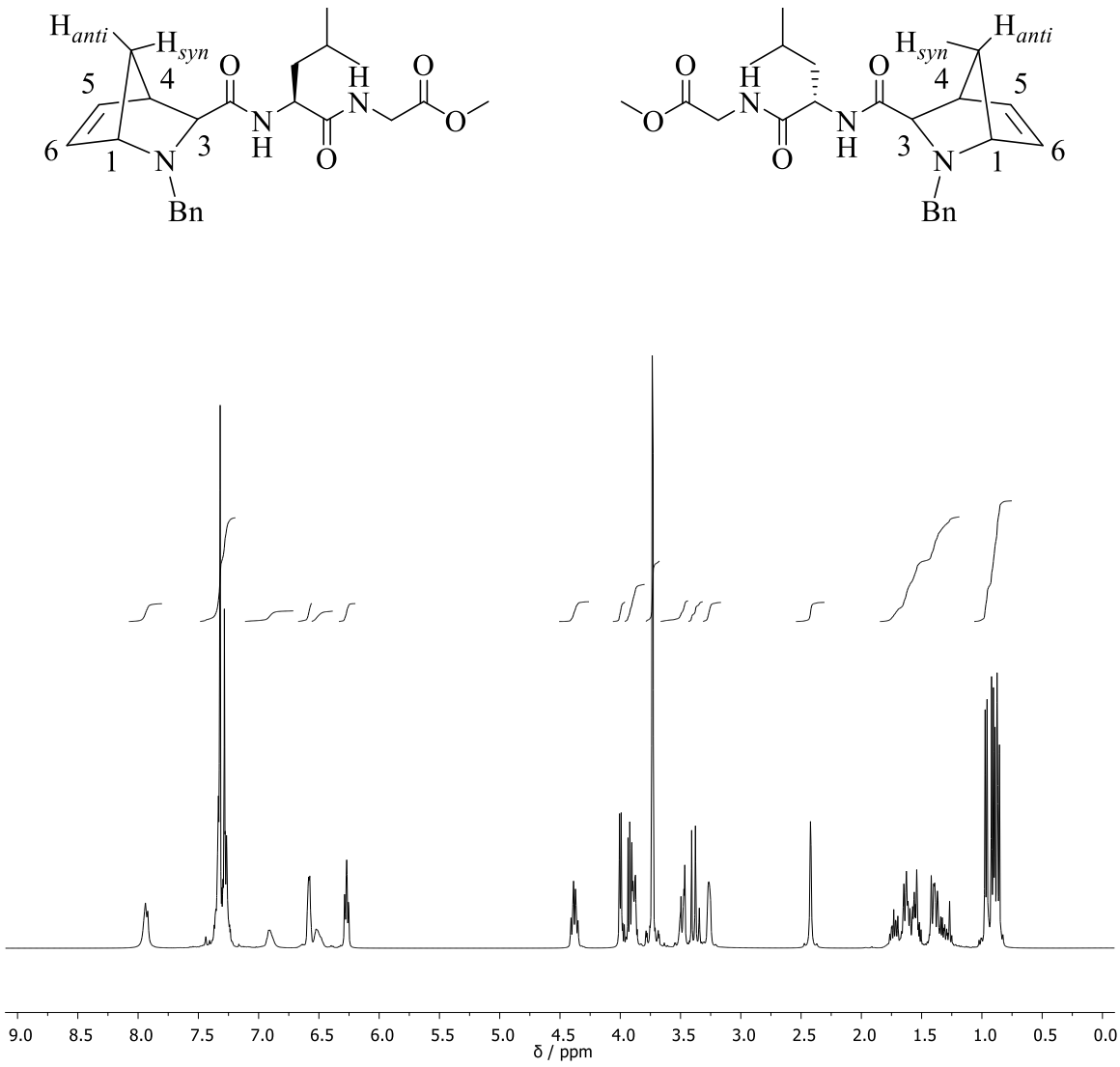

Fig. S17. ^{1}H-NMR espectrum ($CDCl_3$, 400 MHz) of compounds **8b/8b'**.

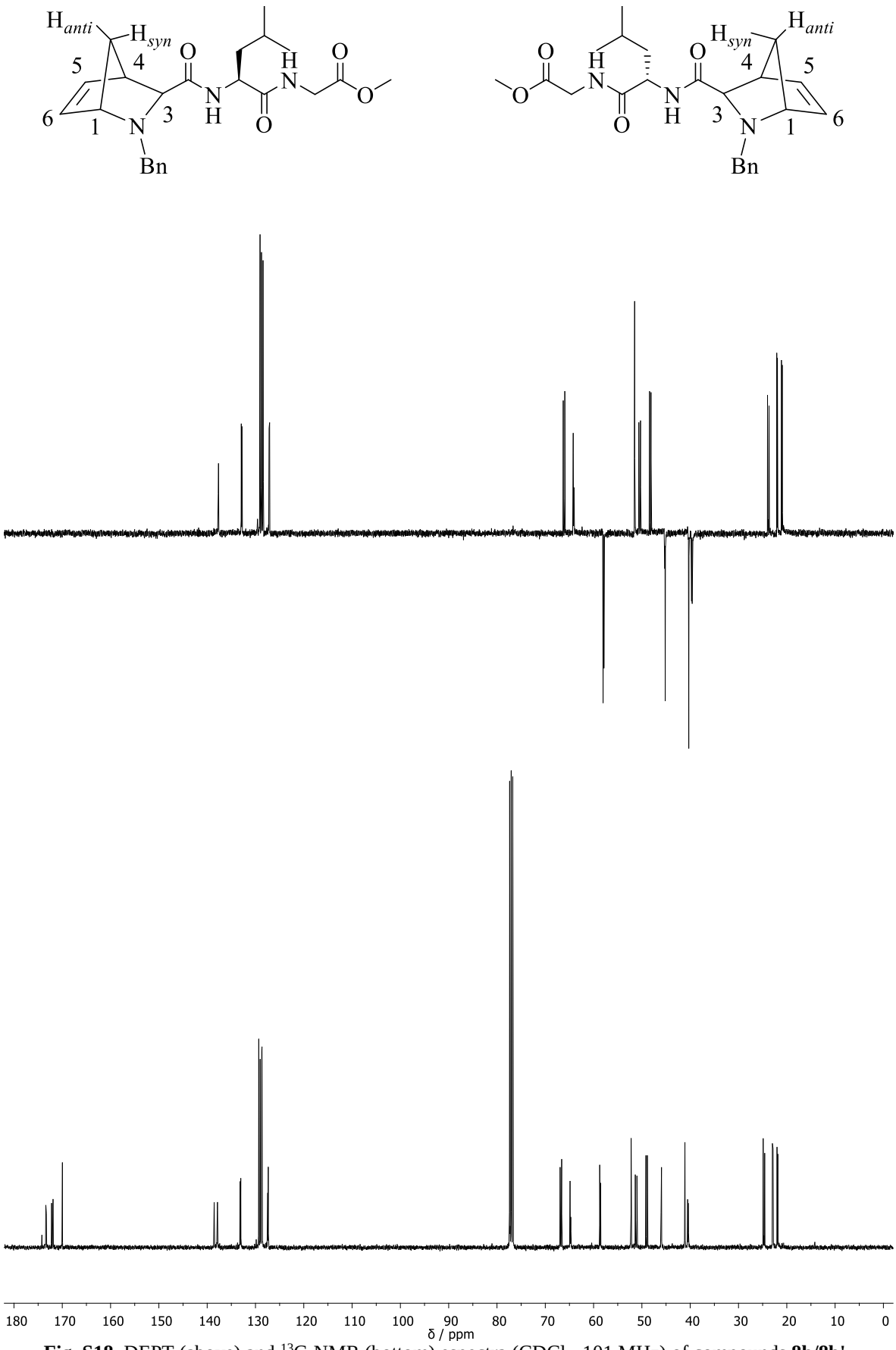

Fig. S18. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 101 MHz) of compounds **8b/8b'**.

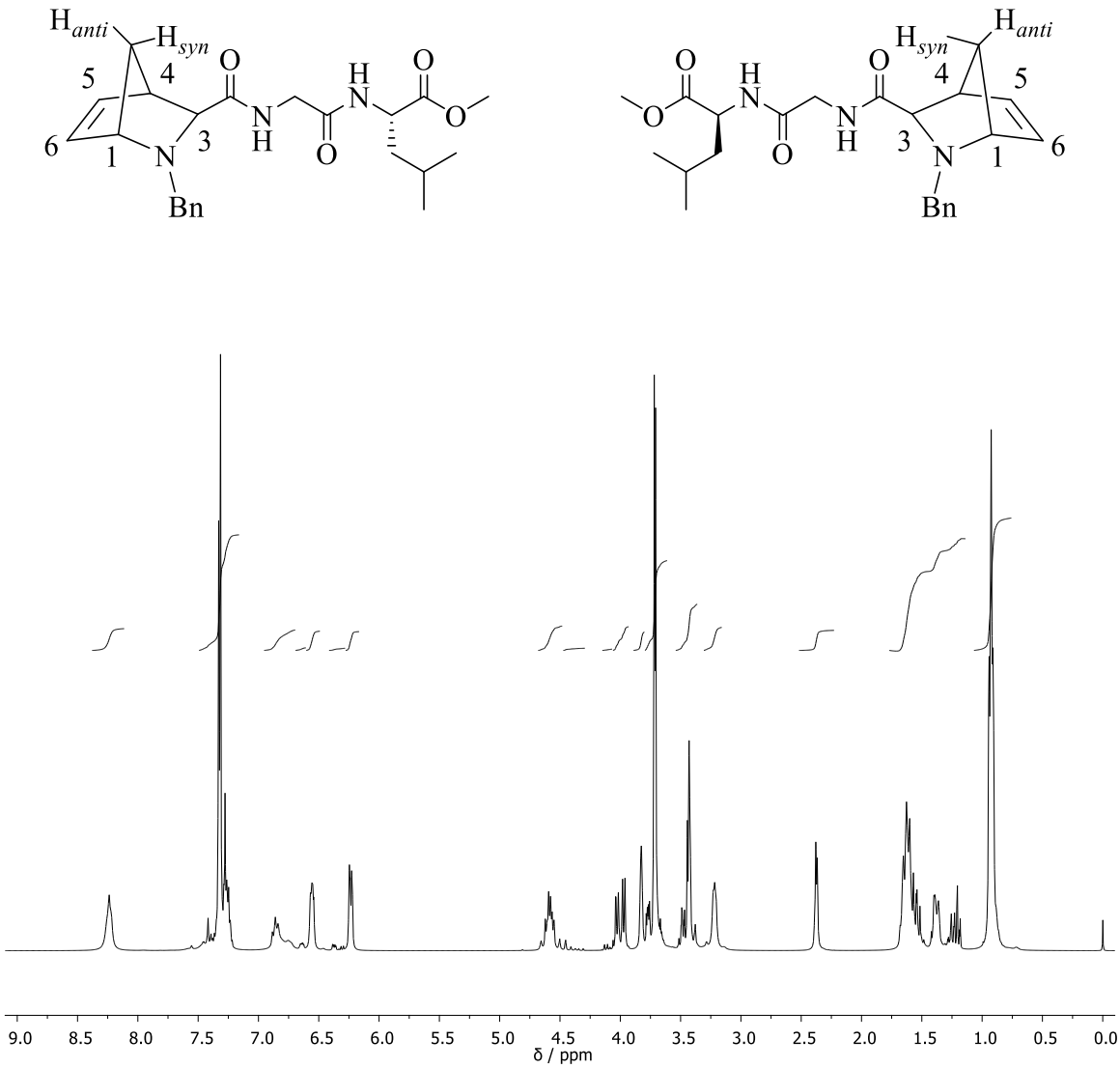

Fig. S19. ^{1}H-NMR espectrum ($CDCl_3$, 300 MHz) of compounds **8c/8c'**.

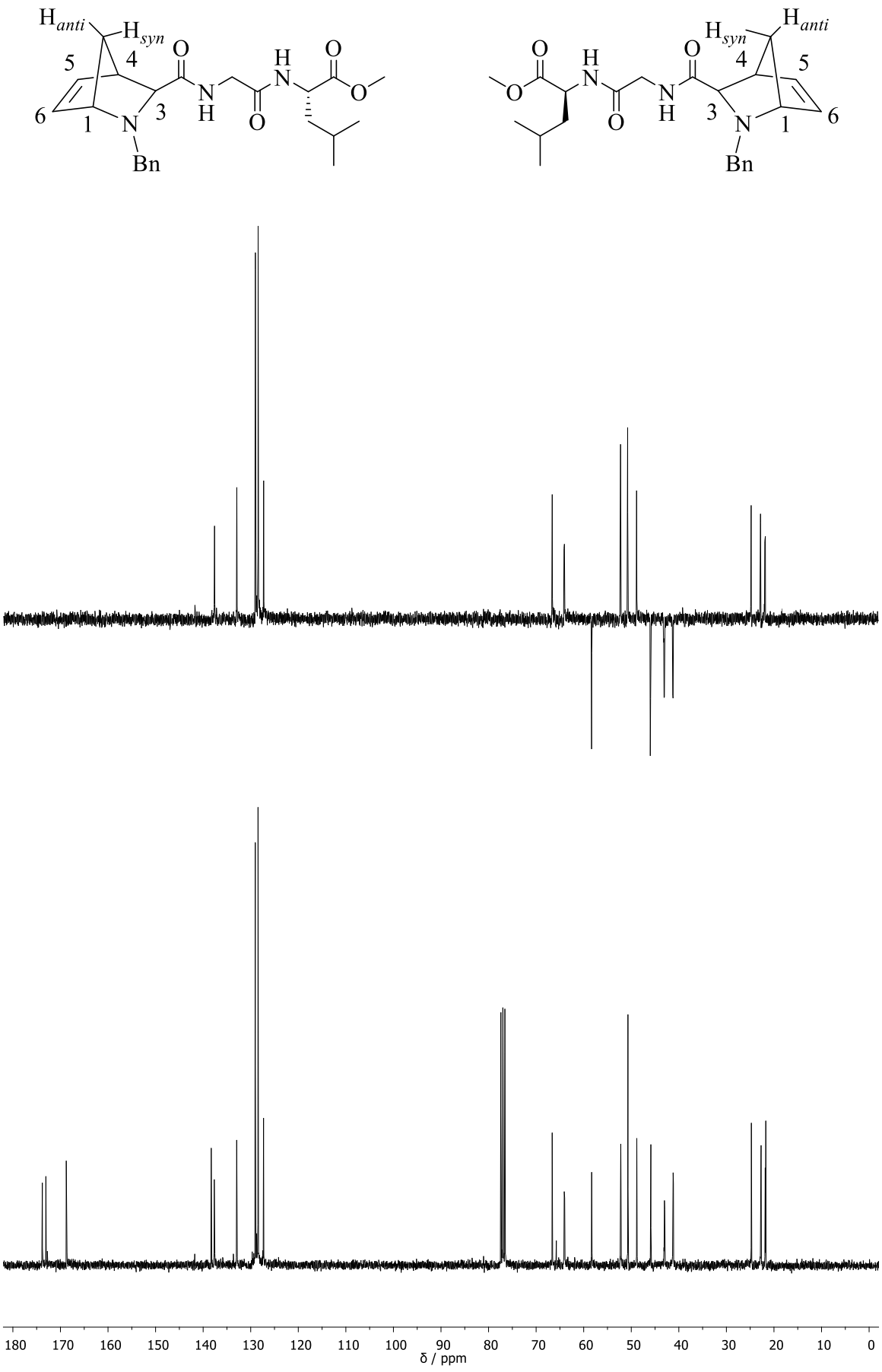

Fig. S20. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 75 MHz) of compounds **8c**/**8c'**.

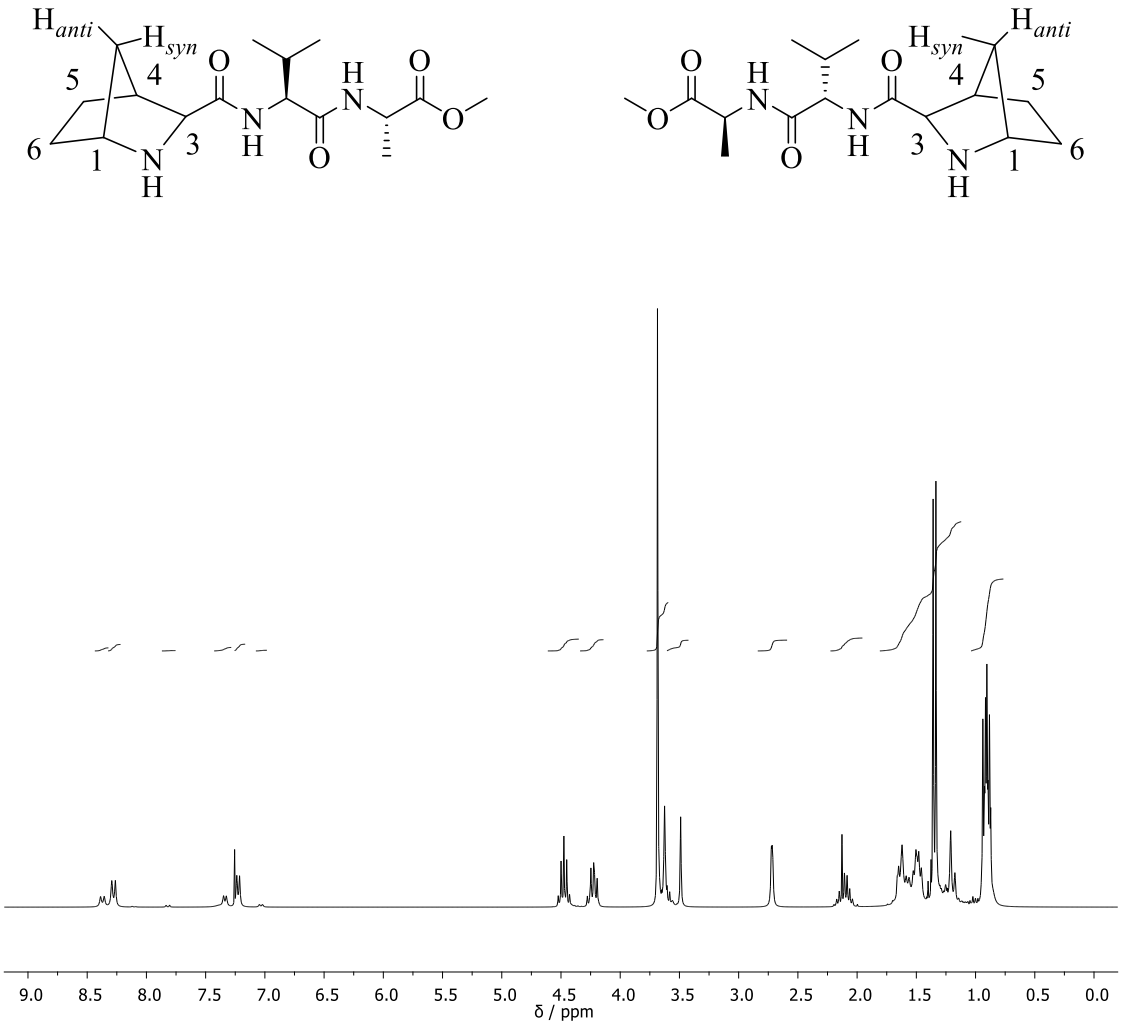

Fig. S21. ^{1}H-NMR espectrum ($CDCl_3$, 300 MHz) of compounds **1a/1a'**.

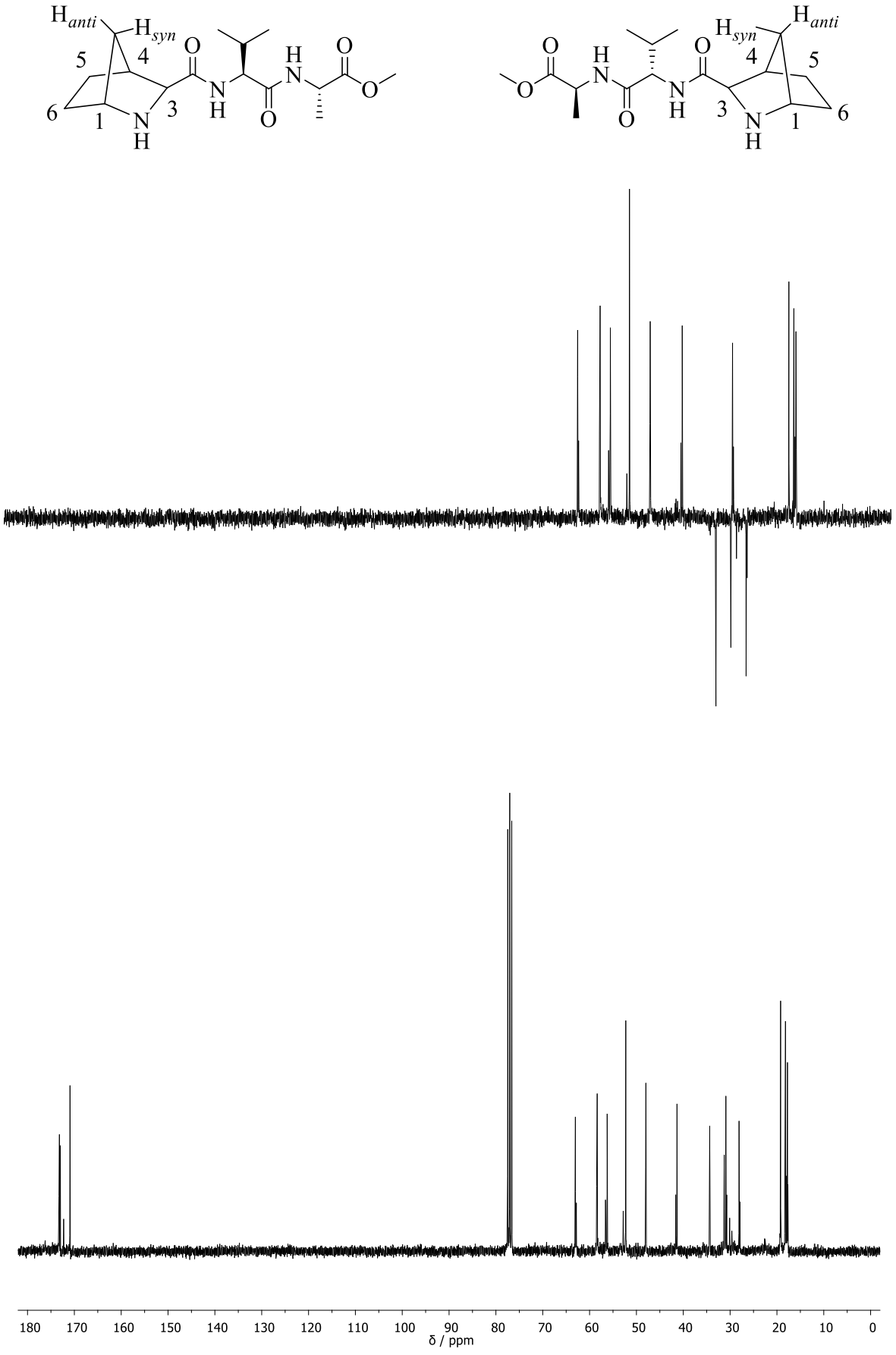

Fig. S22. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 75 MHz) of compounds **1a**/**1a'**.

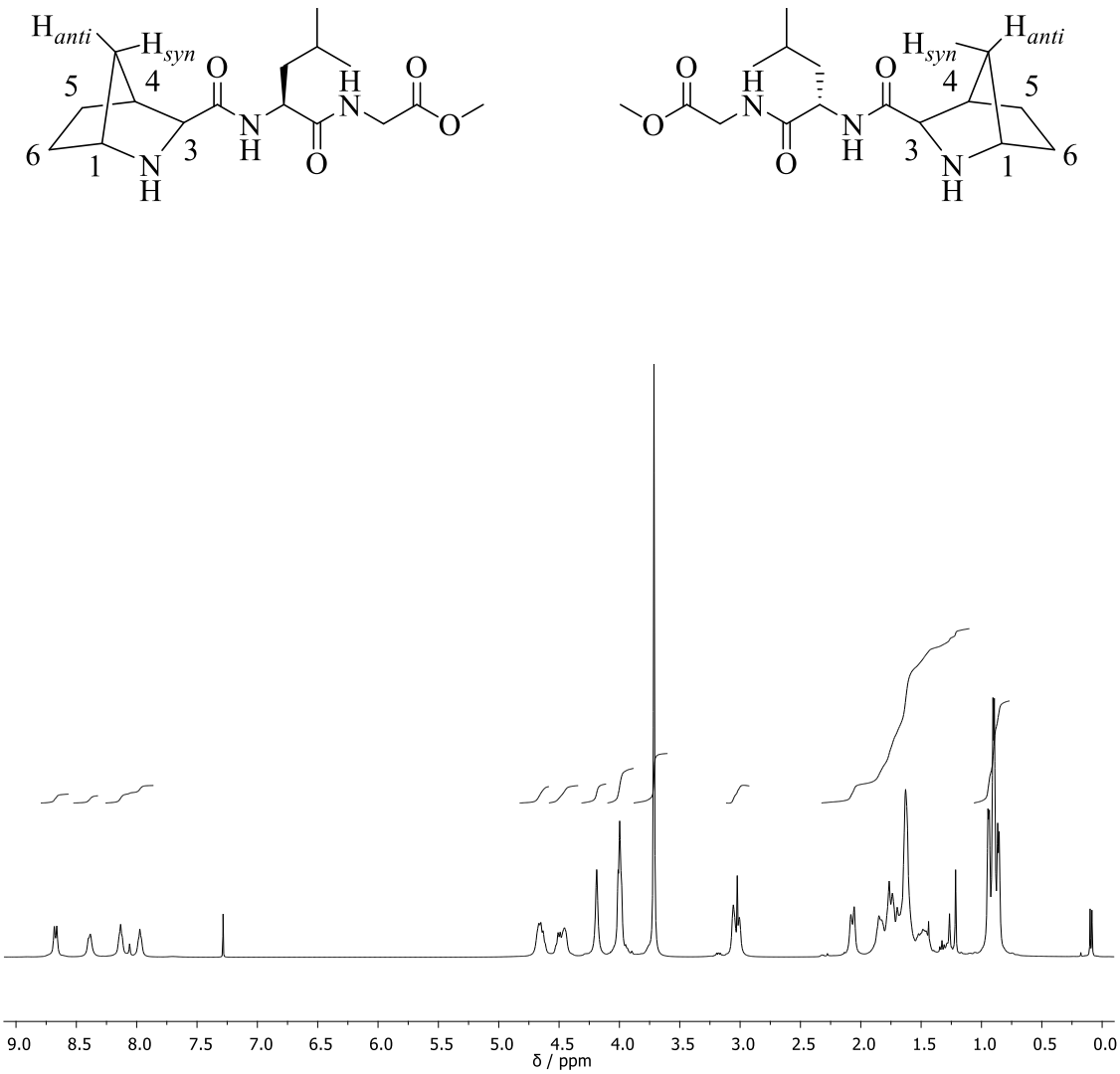

Fig. S23. ^{1}H-NMR espectrum ($CDCl_3$, 400 MHz) of compounds **1b/1b'**.

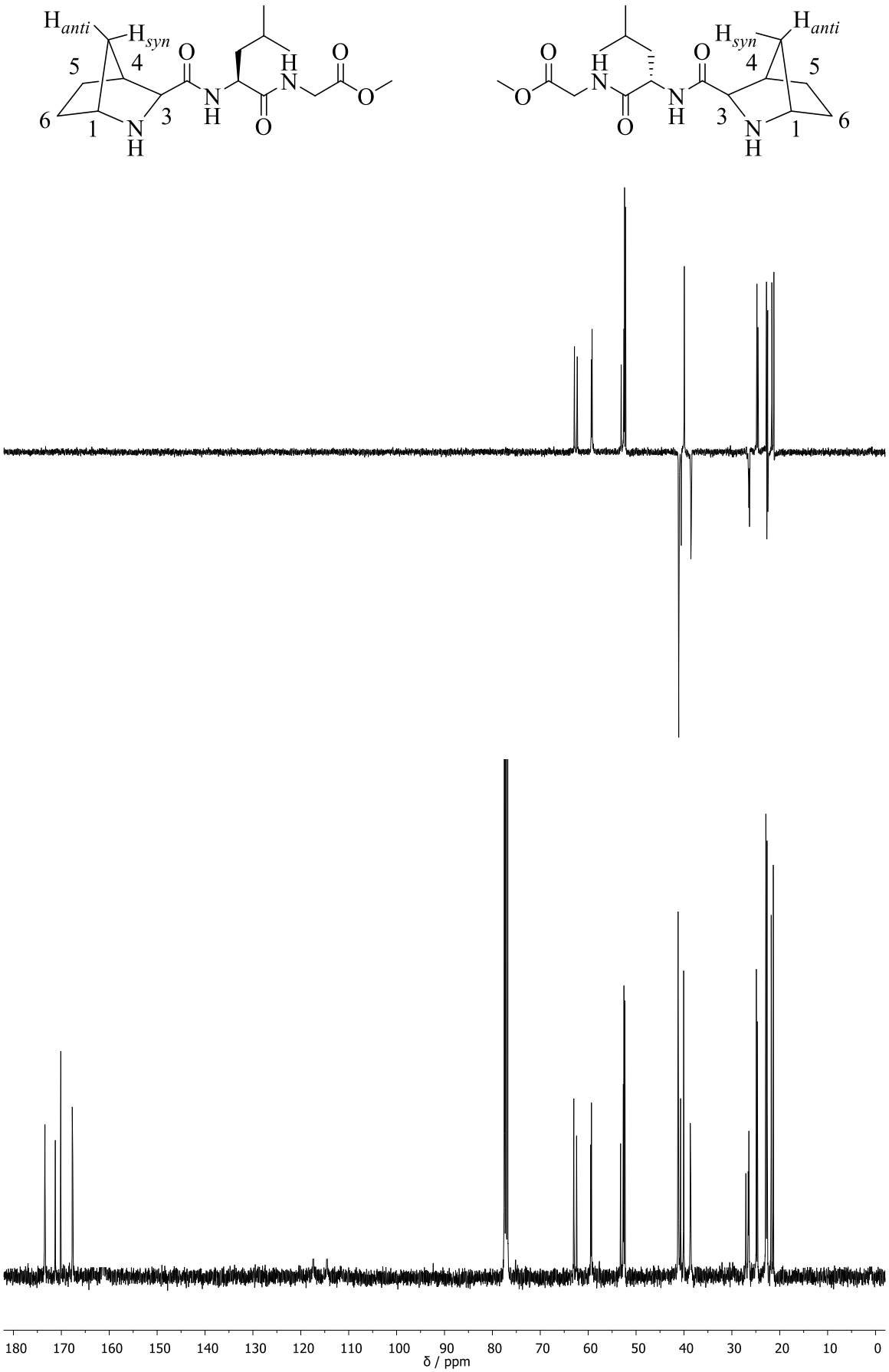

Fig. S24. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 101 MHz) of compounds **1b/1b'**.

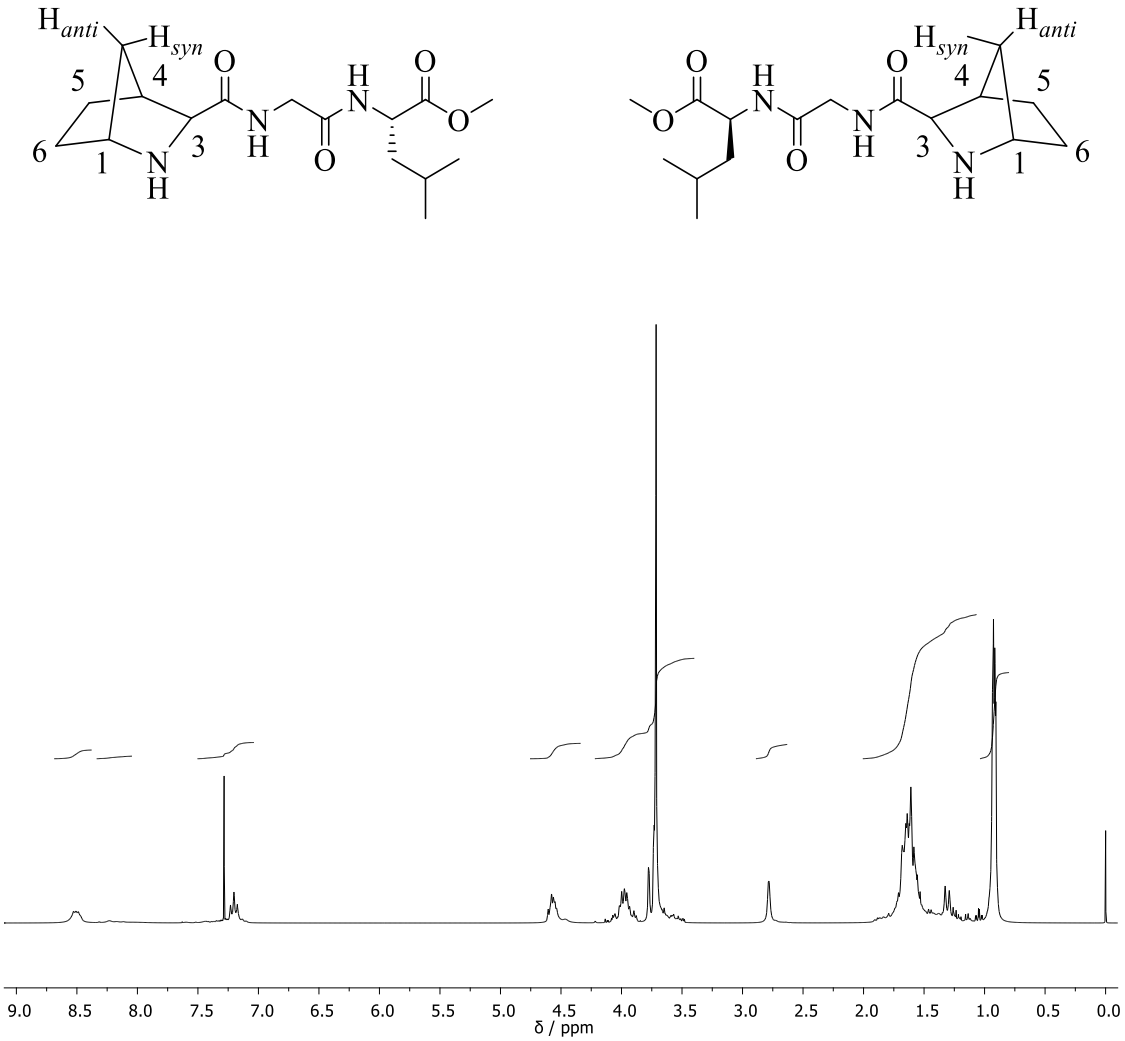

Fig. S25. ^{1}H-NMR espectrum ($CDCl_3$, 300 MHz) of compounds **1c/1c'**.

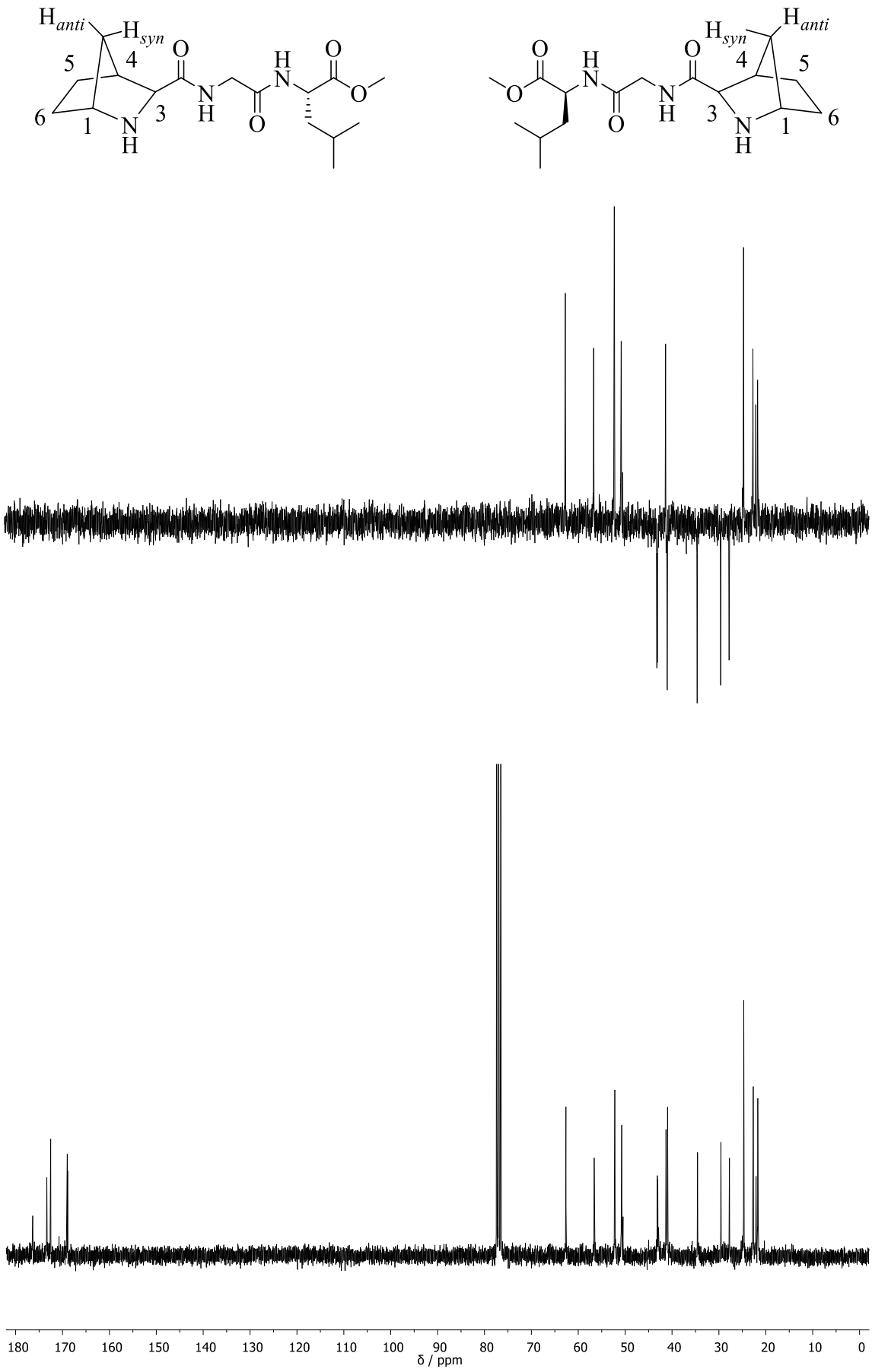

Fig. S26. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 75 MHz) of compounds **1c**/**1c'**.

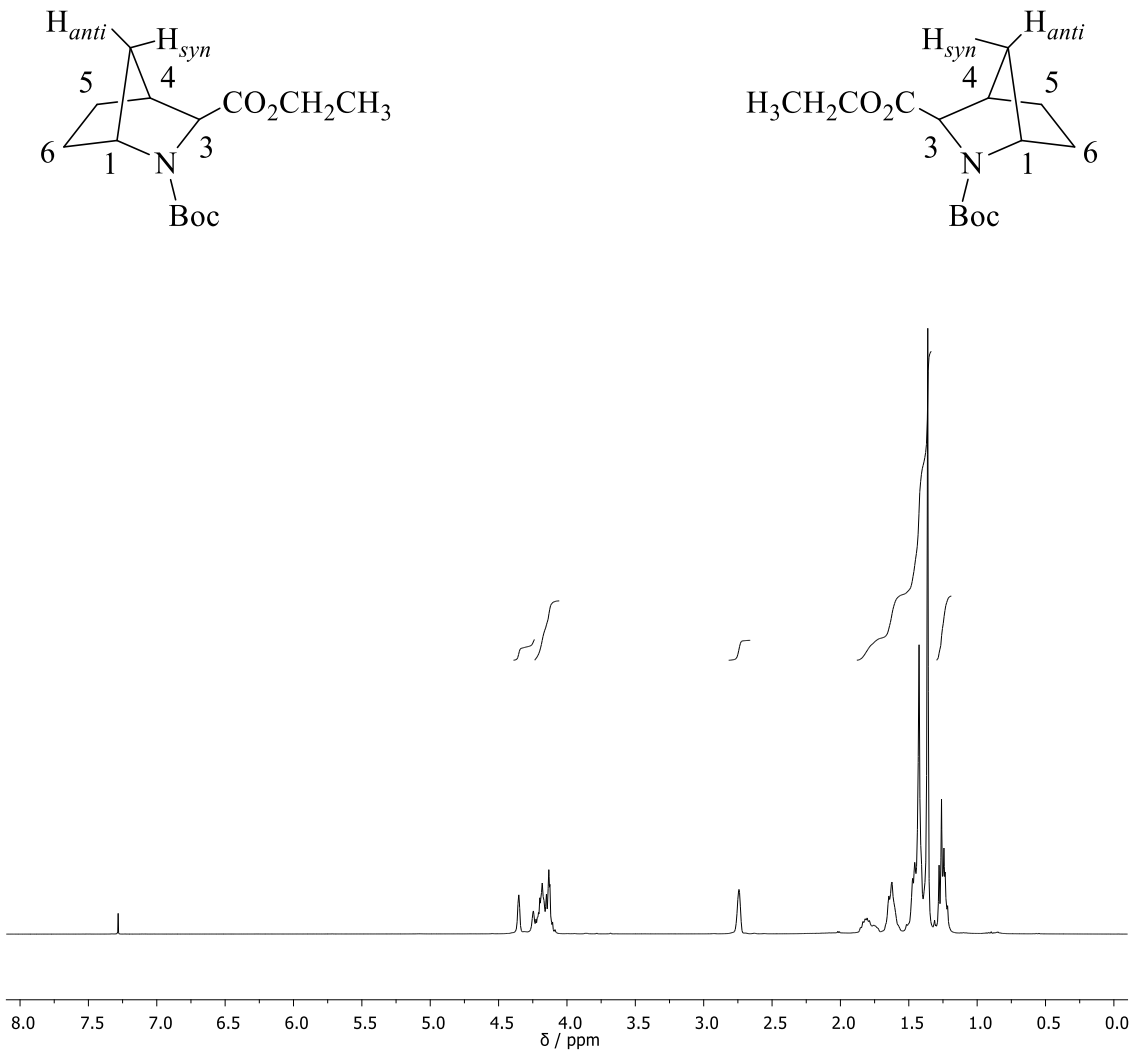

Fig. S27. ^{1}H-NMR espectrum ($CDCl_3$, 400 MHz) of compound (±)-**10**.

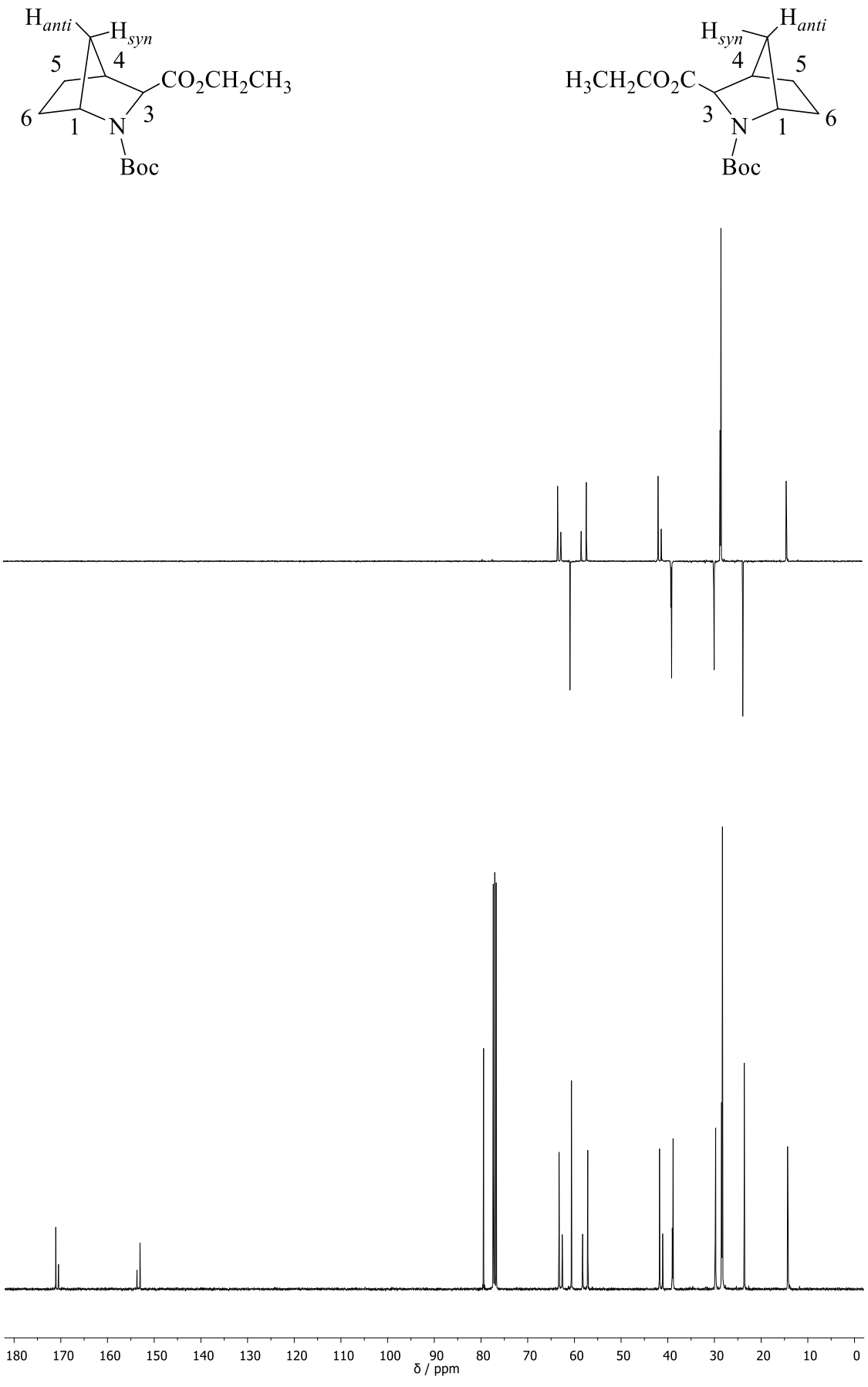

Fig. S28. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 101 MHz) of compound (±)-**10**.

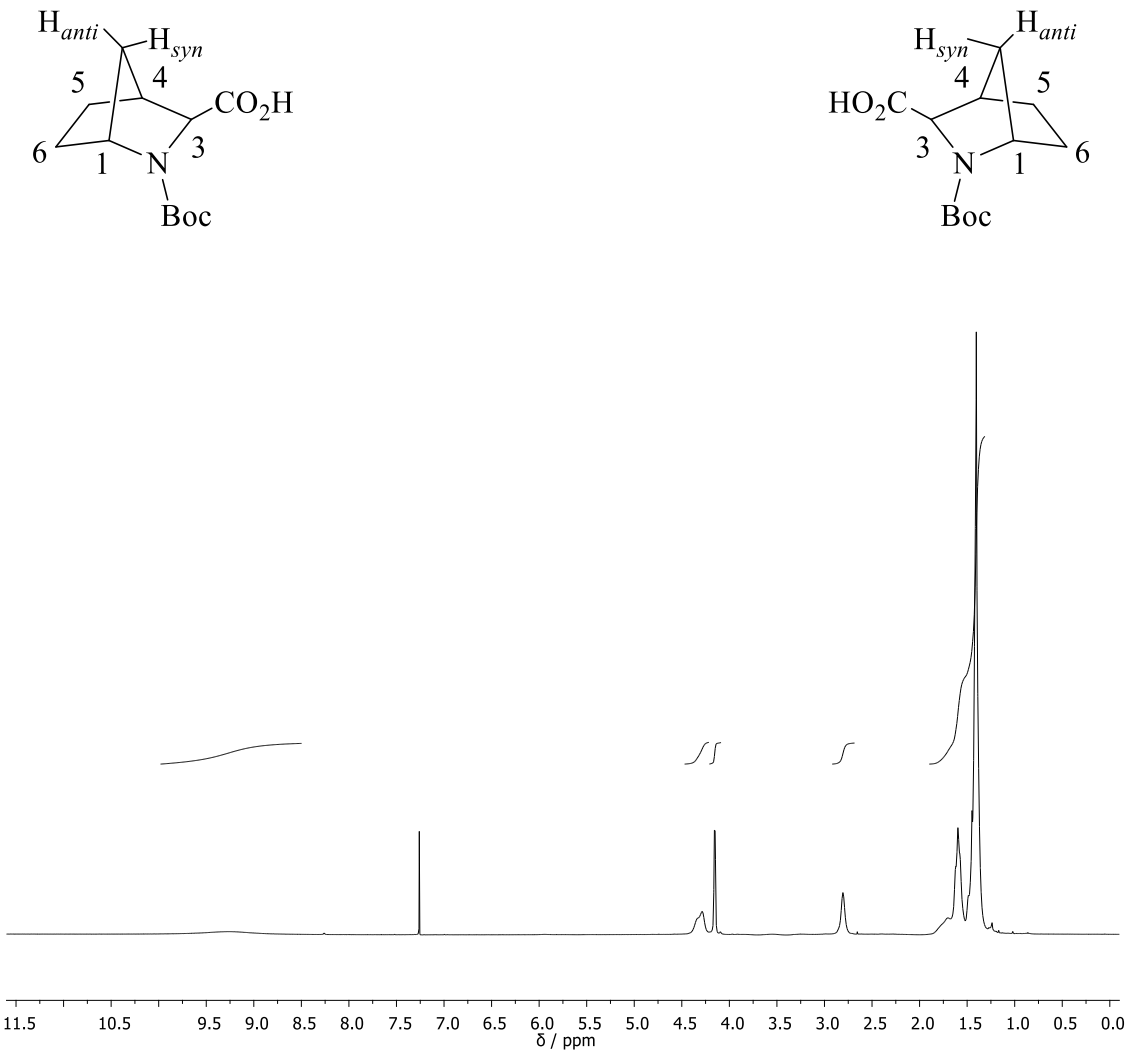

Fig. S29. ^{1}H-NMR espectrum ($CDCl_3$, 400 MHz) of compound (±)-**11**.

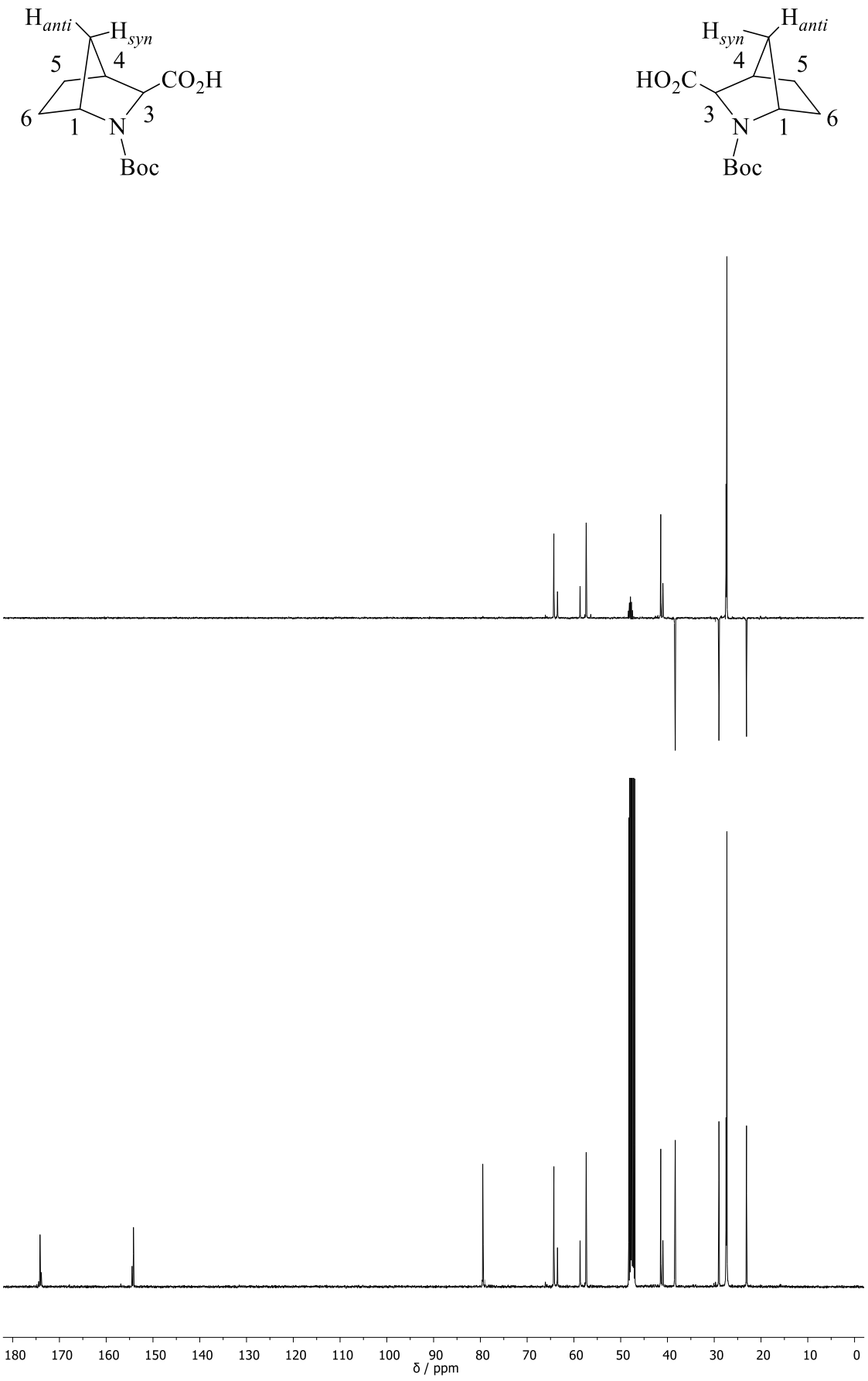

Fig. S30. DEPT (above) and ^{13}C-NMR (bottom) espectra (CD_3OD, 101 MHz) of compound (±)-**11**.

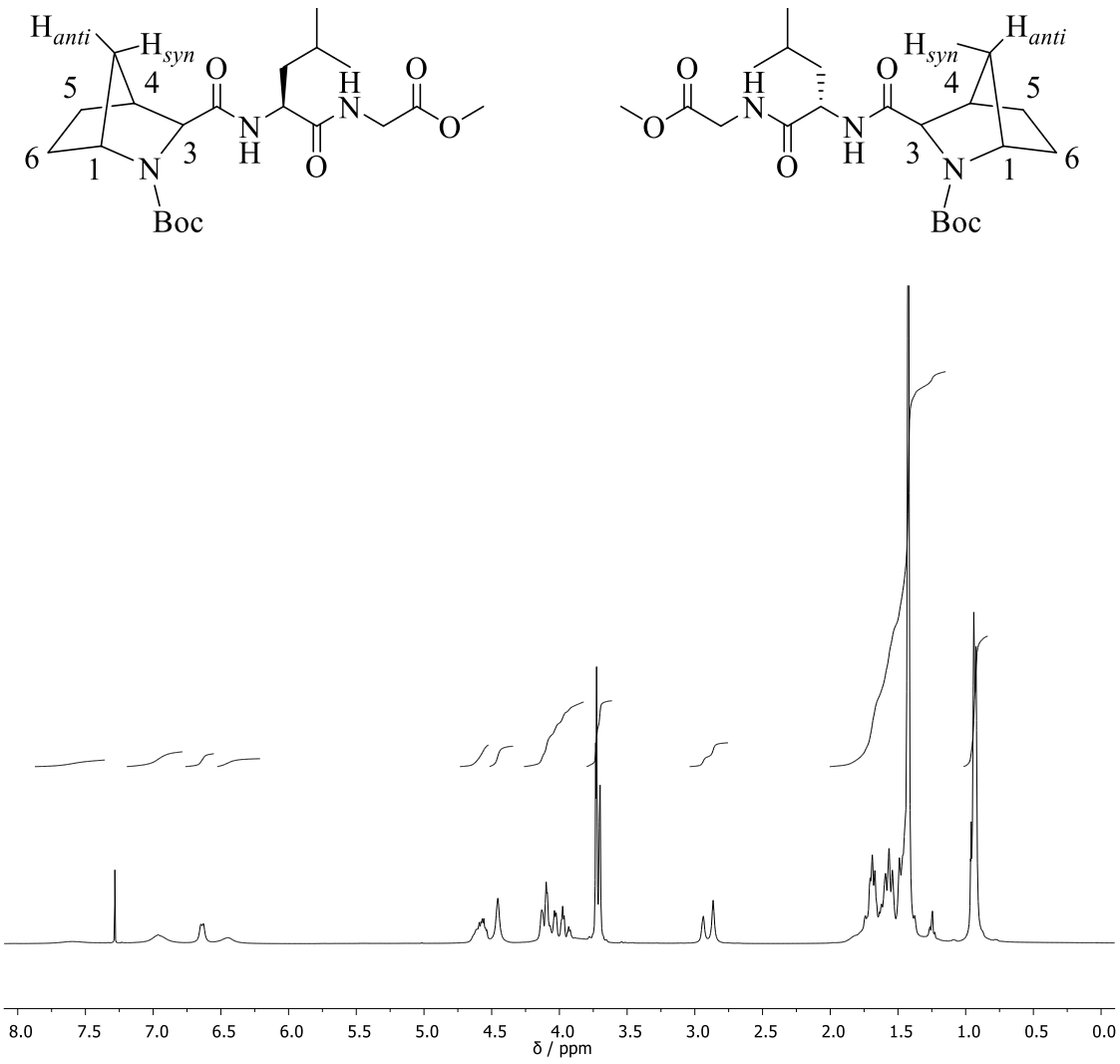

Fig. S31. ^{1}H-NMR espectrum ($CDCl_3$, 400 MHz) of compounds **12b/12b'**.

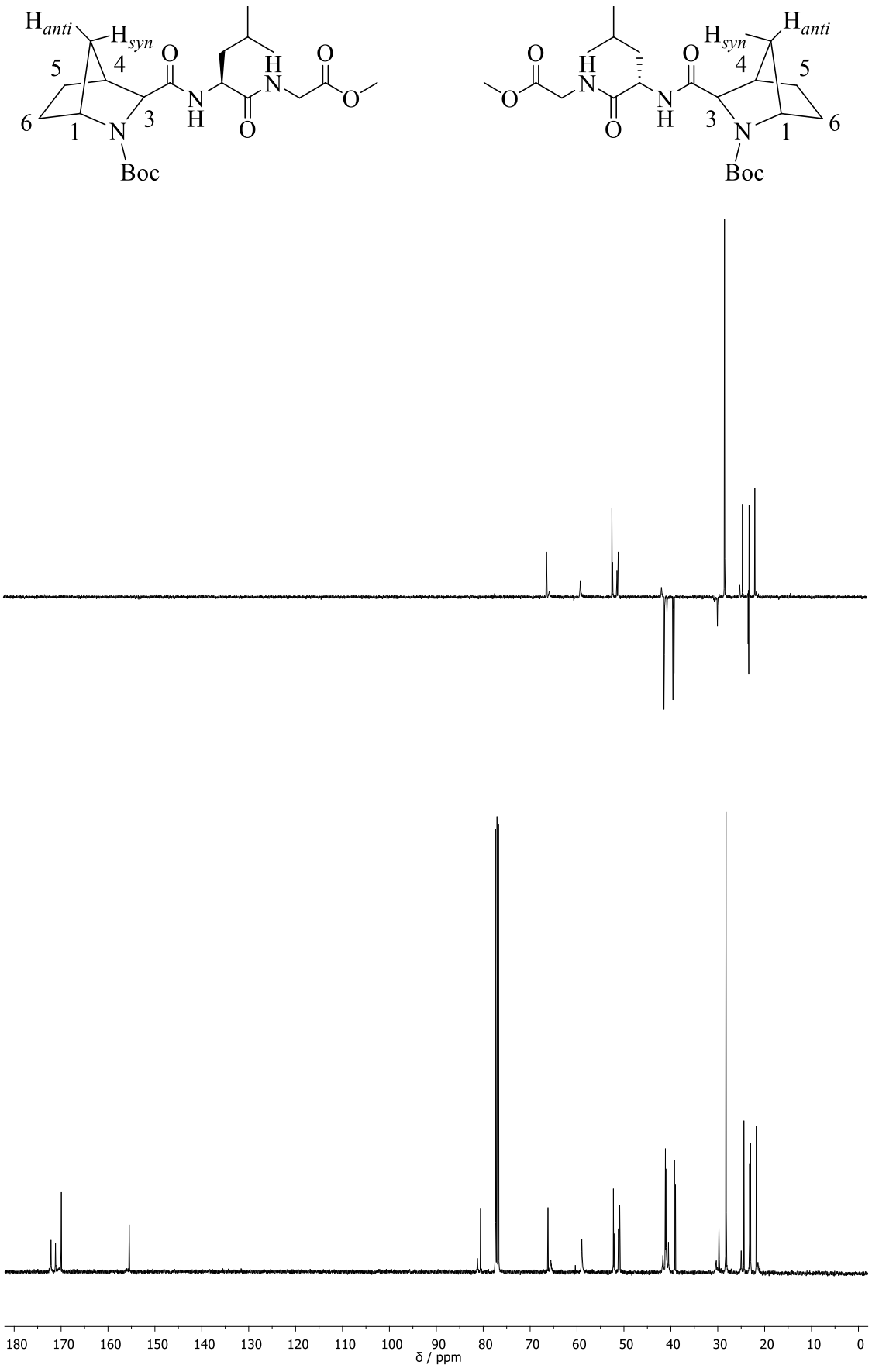

Fig. S32. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 101 MHz) of compounds **12b/12b'**.

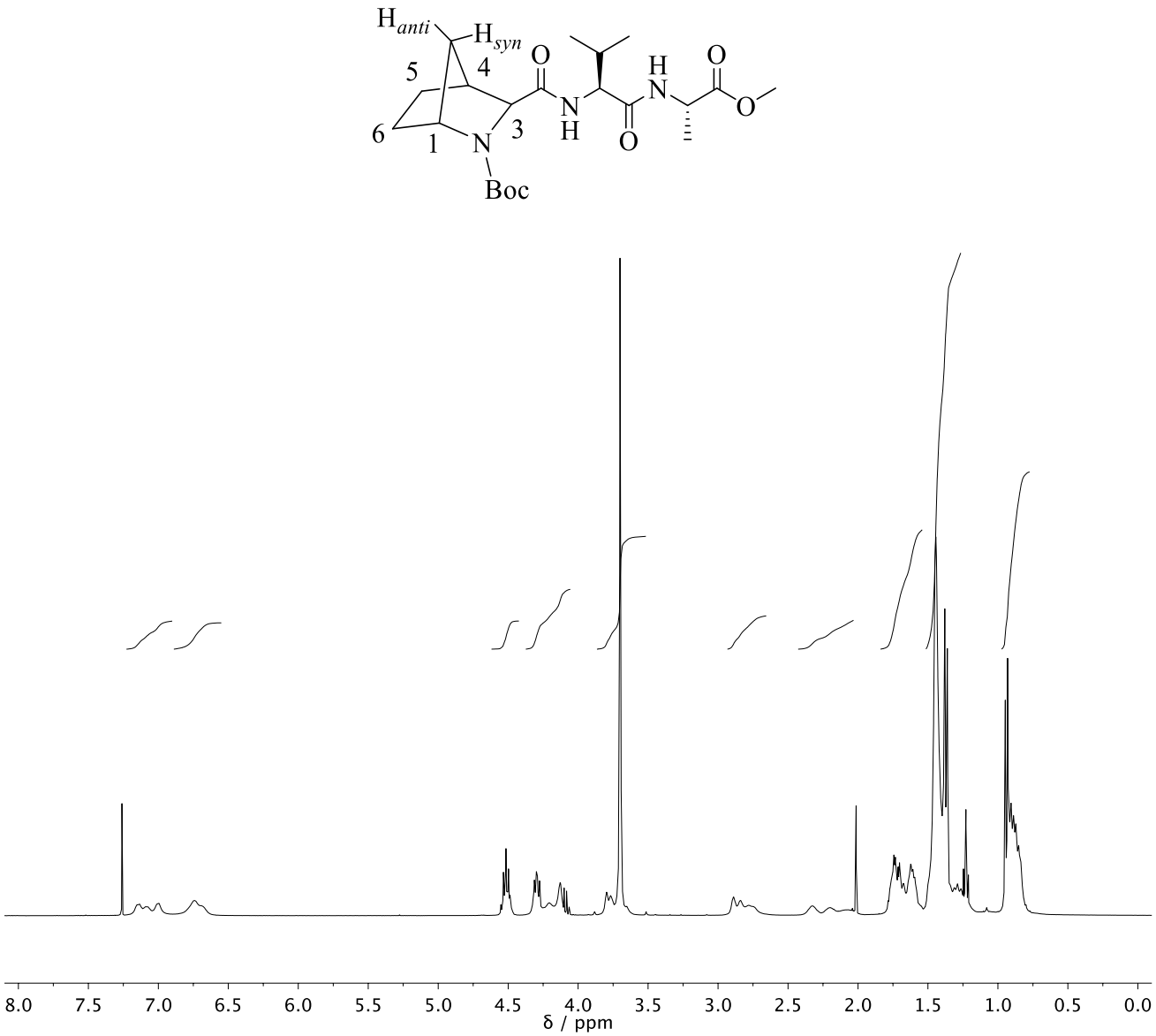

Fig. S33. ^{1}H-NMR espectrum ($CDCl_3$, 400 MHz) of compound **12a**.

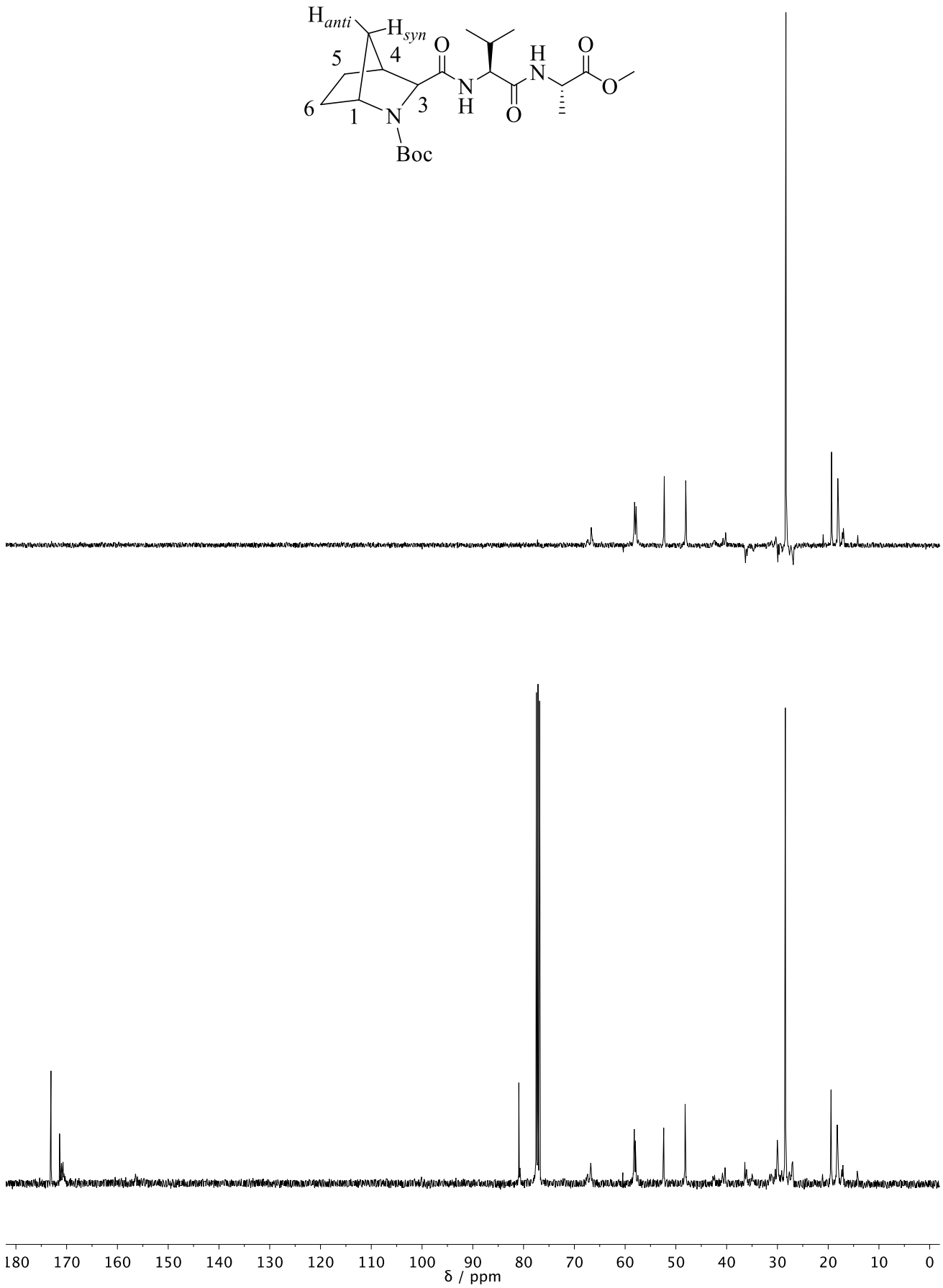

Fig. S34. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 101 MHz) of compound **12a**.

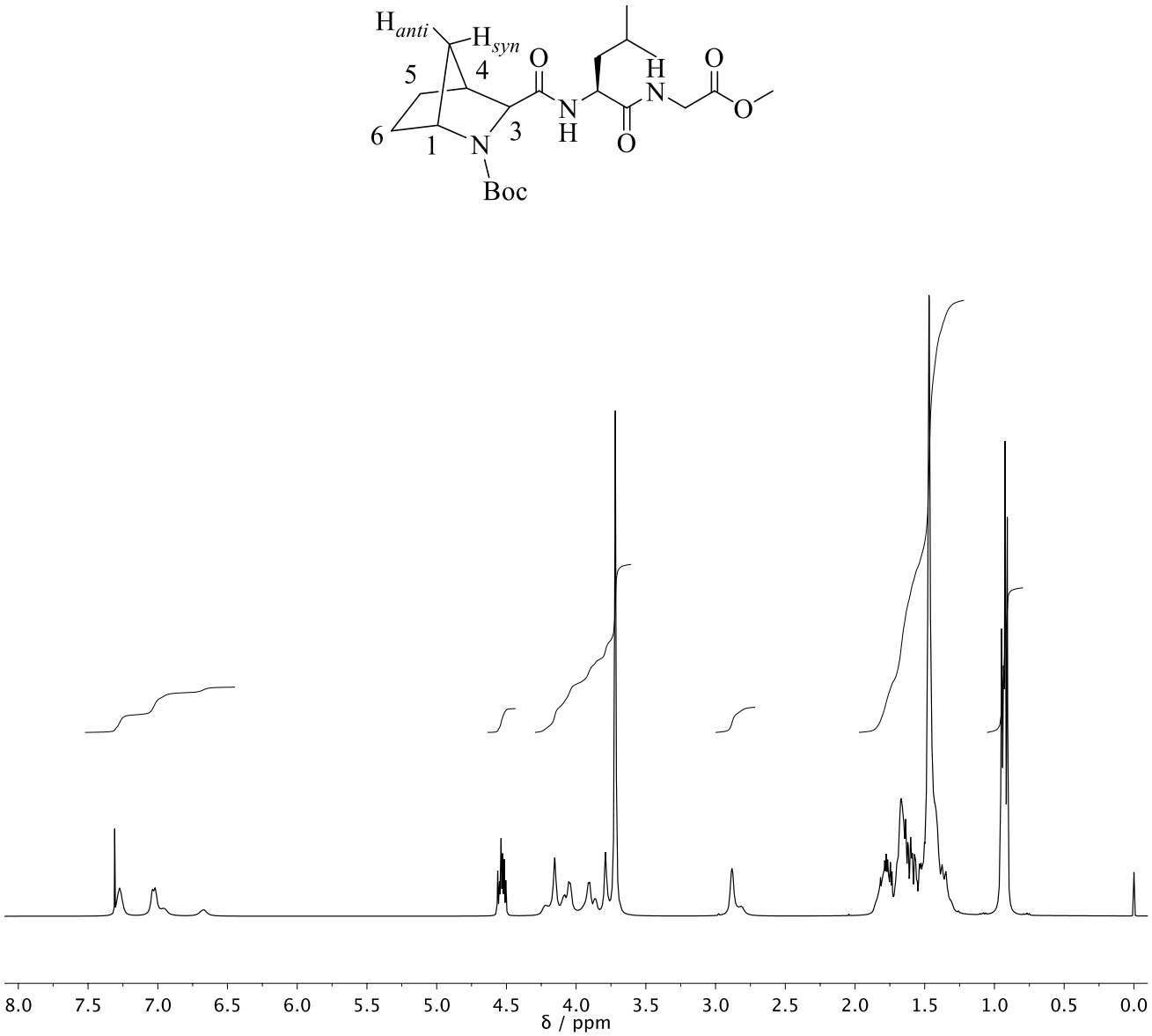

Fig. S35. ^{1}H-NMR espectrum ($CDCl_3$, 400 MHz) of compound **12b**.

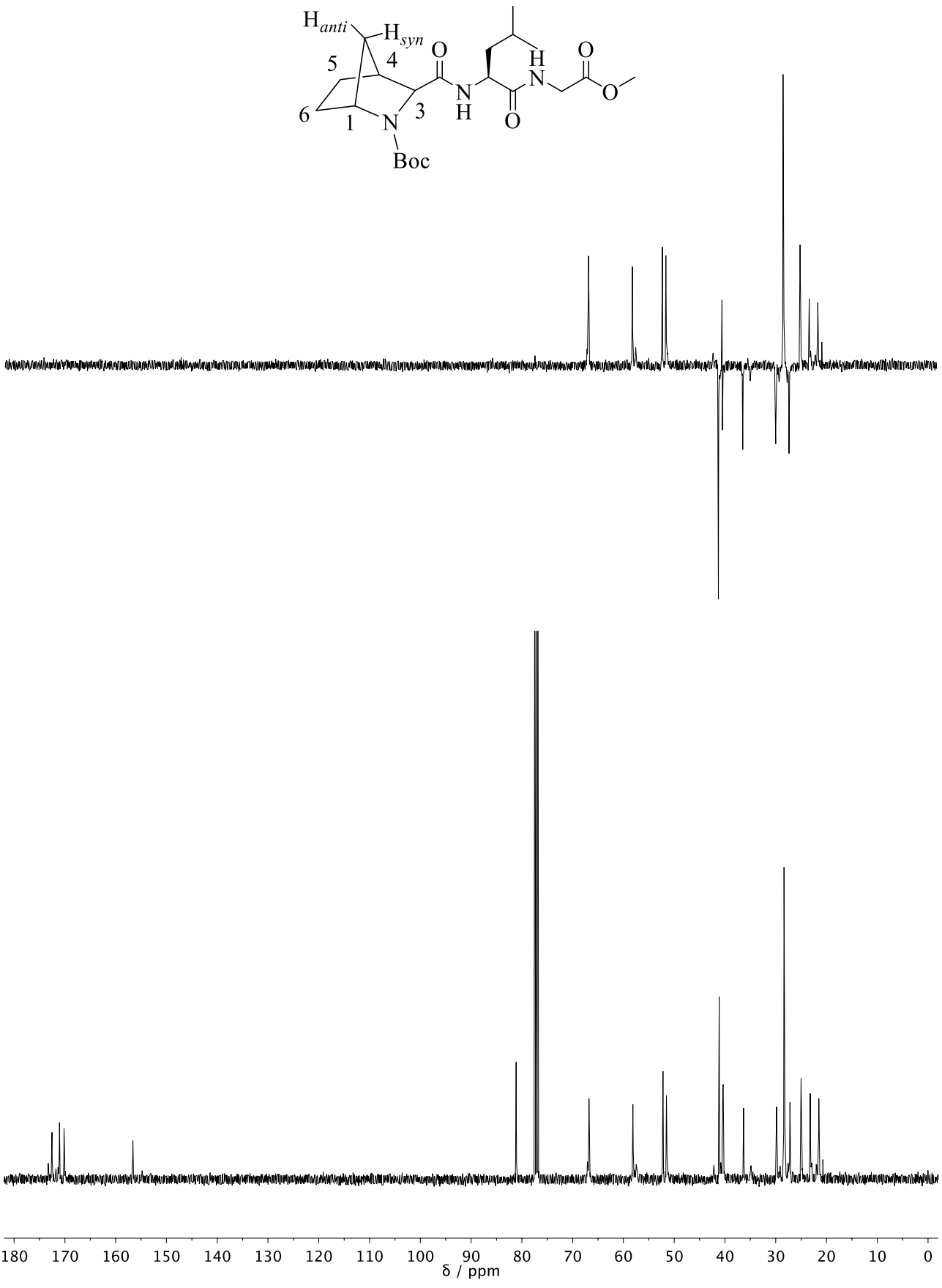

Fig. S36. DEPT (above) and ^{13}C-NMR (bottom) espectra ($CDCl_3$, 101 MHz) of compound **12b**.

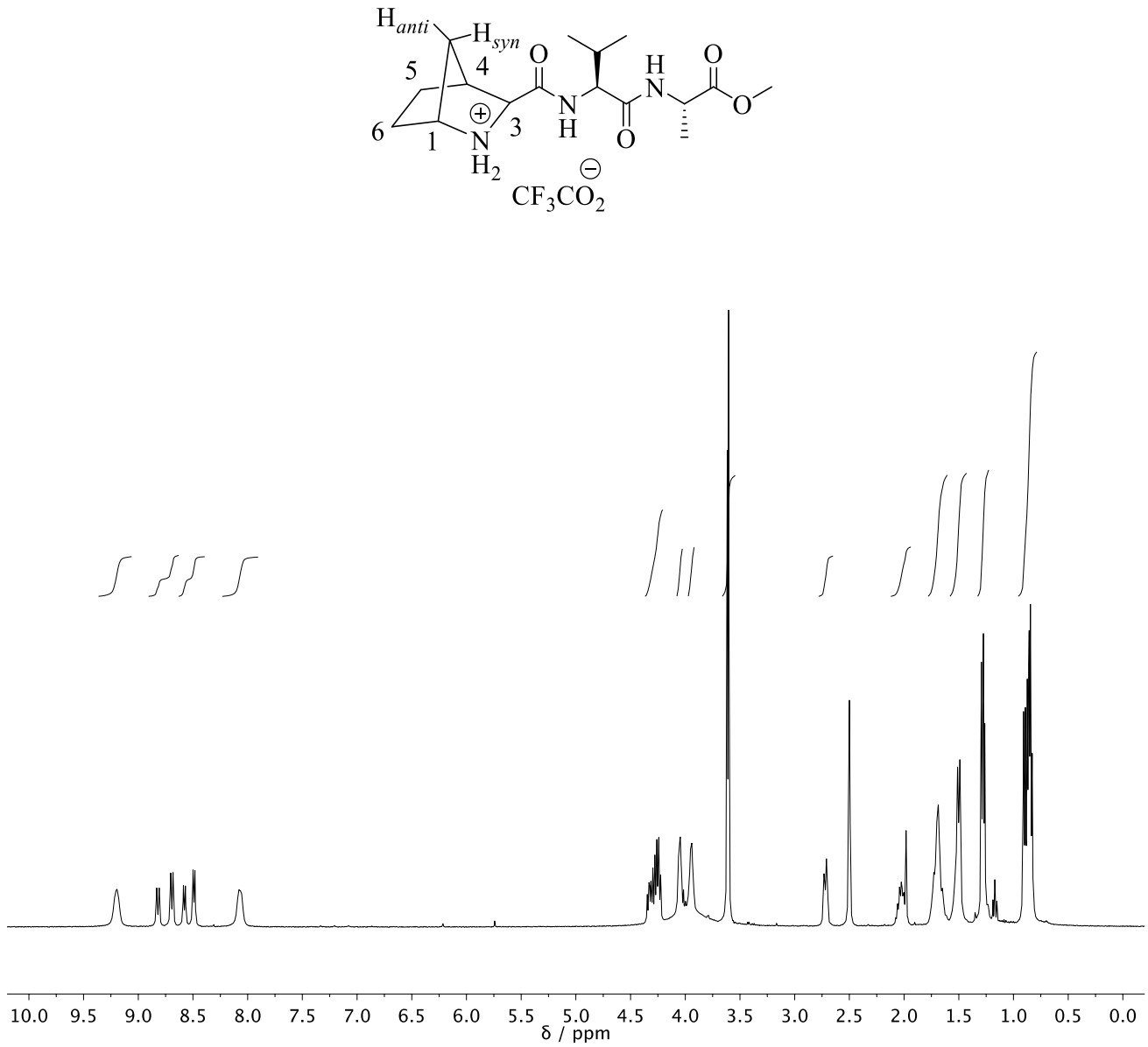

Fig. S37. ^{1}H-NMR espectrum (DMSO-d_6, 400 MHz) of compound **1a** · **TFA**.

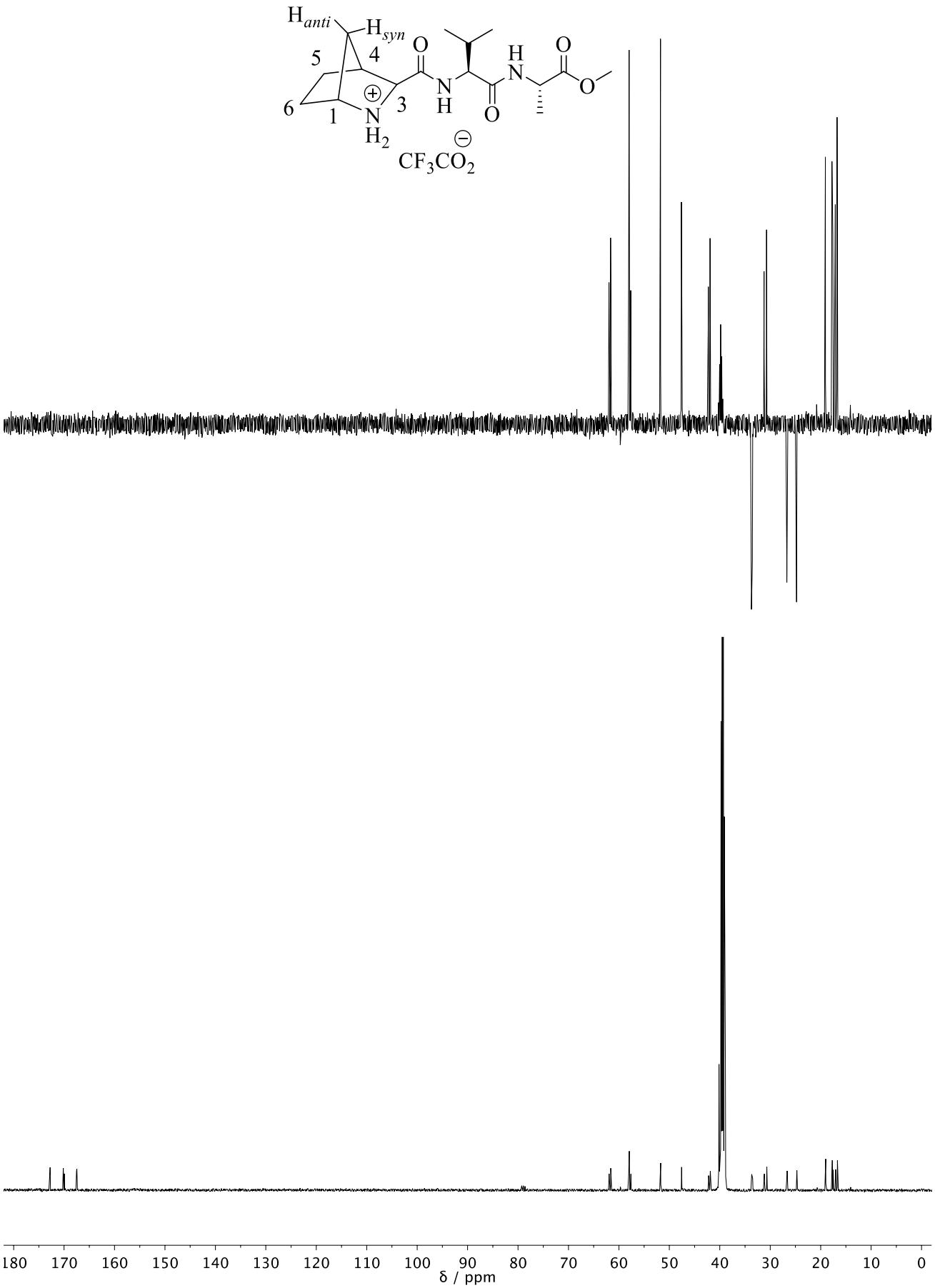

Fig. S38. DEPT (above) and ^{13}C-NMR (bottom) espectra (DMSO-d_6, 101 MHz) of compound **1a**· **TFA**.

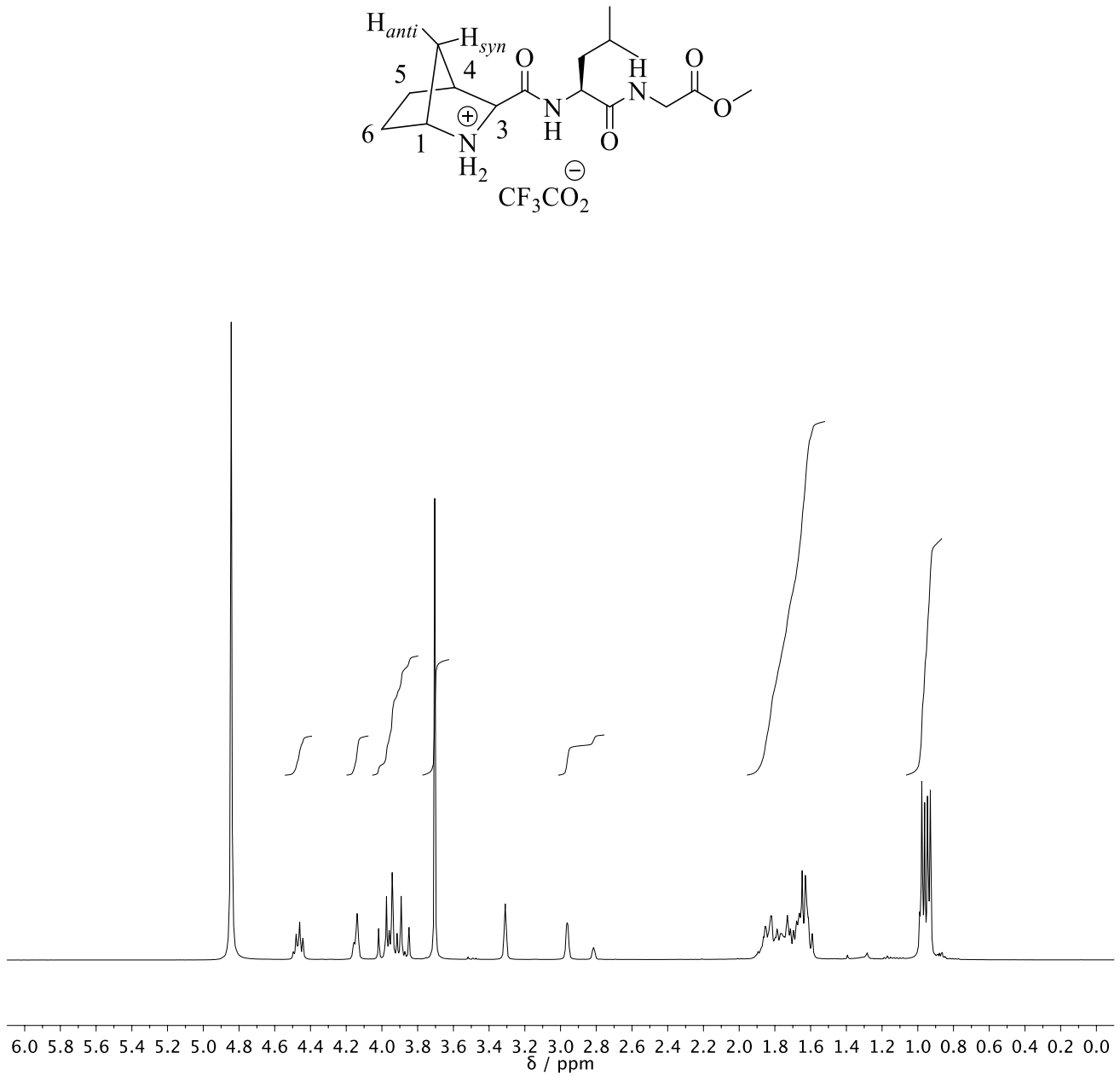

Fig. S39. ^{1}H-NMR espectrum (CD_3OD, 400 MHz) of compound **1b** · **TFA**.

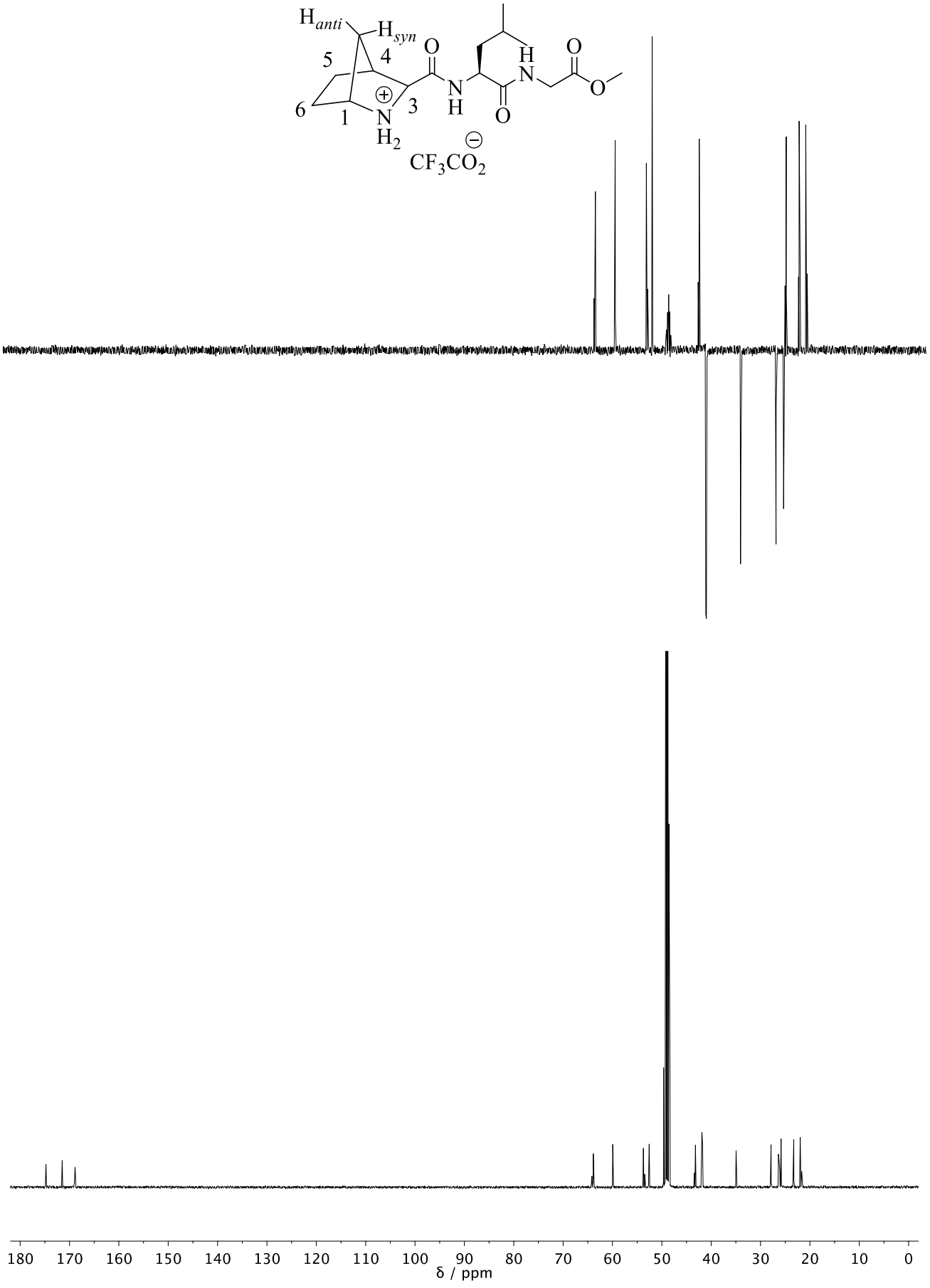

Fig. S40. DEPT (above) and ^{13}C-NMR (bottom) espectra (CD_3OD, 101 MHz) of compound **1b** · **TFA**.

Dopamine D_2 receptor binding assay

The ability of the peptidomimetics (1*R*,3*S*,4*S*)-**1a** and (1*R*,3*S*,4*S*)-**1b** to enhance the binding of [^{3}H]-NPA binding was analyzed using receptor binding studies. Chinese hamster ovary (CHO) cells expressing short isoform of human D_{2S} receptors were grown in 150 mm petri dishes in Dulbeco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 2 mM L-Glutamine. When cells were confluent, medium was removed and cells were washed twice with buffer A (5 mM Tris-HCl pH = 7.4, 2 mM EDTA). Cells were scrapped and homogenized twice in a Polytron. Cell suspension was centrifuged (300 g, 10 min, 4°C). Pellet was discarded and supernatant was centrifuged (48,400 g; 4°C; 60 min). Pellet was resuspended in buffer B (50 mM Tris-HCl; pH = 7.4) and protein quantity was measured by using Bradford method[5].

The binding of [^{3}H]-NPA to the membrane preparation was assayed in duplicate in 96-well plates. Membranes (30 μg/well) expressing human D_2 receptor were incubated with 0.25 nM [^{3}H]-NPA and test compounds for 60 min at 25°C in a 96-well polypropylene microplate with incubation buffer (50 mM Tris-HCl, pH = 7.4; 120 mM NaCl, 5 mM KCl, 4 mM $MgCl_2$, 1 mM EDTA) up to a total volume of 250 μL. Non-specific binding was defined in the presence of 1 μM (+)-butaclamol.

After incubation time 200 μL were transferred to a multiscreen FC microplate (Millipore) pre-treated with 0.5% polyethilenimine and samples were filtered and washed 4 times with 250 μL of wash buffer (50 mM Tris-HCl, pH = 7.4; 0.9% NaCl). Filters were dried and 35 μL of scintillation cocktail (Universol) were added to each well and radioactivity was detected in a microplate beta-scintillation counter (Microbeta Trilux).

Data are expressed as the increase of specific binding following the formula:

$$\%\ \text{Increase} = \left(\frac{(\text{X-NSB}) \times 100}{\text{BT-NSB}}\right) - 100$$

where X is the radioactivity detected in the test well; BT is the radioactivity detected when [^{3}H]-NPA was incubated in the absence of any compound and NSB is the radioactivity detected when [^{3}H]-NPA was co-incubated with 10 μM (+)-butaclamol.

ANOVA analysis was carried out to evaluate significant differences using SPSS software (V15.0). Statistical significance was set at $P < 0.05$.

[5] M.M. Bradford *Anal. Biochem*. 1976, **72**, 248.

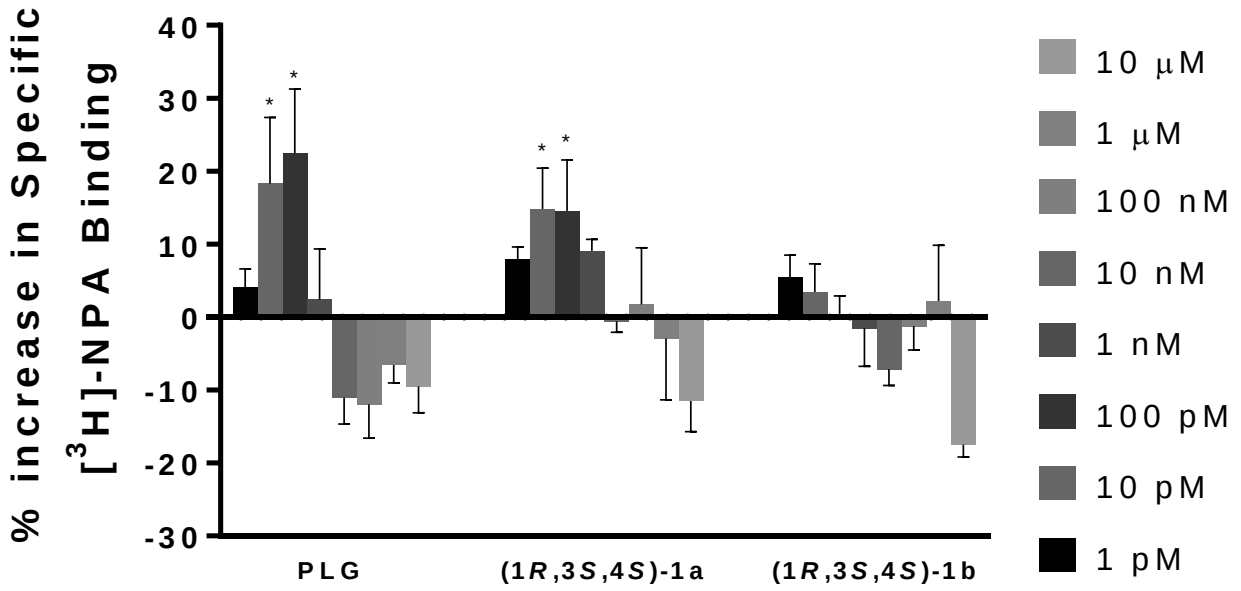

Fig. S41: Modulation of [^{3}H]-NPA binding of **PLG**, (1*R*,3*S*,4*S*)-**1a** and (1*R*,3*S*,4*S*)-**1b** exerted by the different compounds at eight different concentrations. Points represent the mean ± standard deviation (vertical bars) of three independent experiments carried out with duplicate points. **P* < 0.05 (ANOVA test; post-hoc Dunnet T3 test).