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

Supplementary Information

Rapid Chemoenzymatic Route to Glutamate Transporter Inhibitor L-TFB-TBOA and Related Amino Acids

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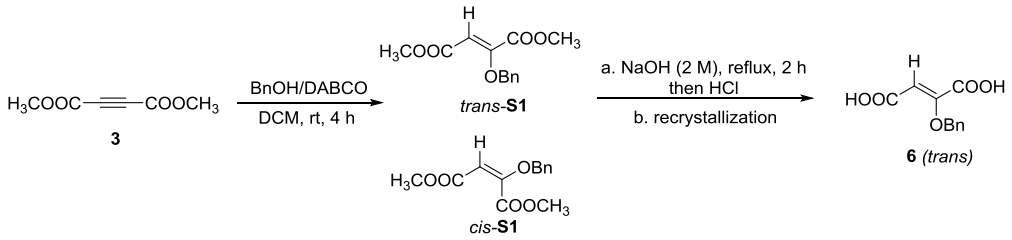
I) General information

All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) or Thermo Fisher Scientific Co. unless stated otherwise. Solvents were purchased from Biosolve (Valkenswaard, The Netherlands) or Sigma-Aldrich Chemical Co. The boiling point of the petroleum ether used for chemical purification was 40-60 °C. Authentic sample of L-TFB-TBOA was purchased from Tocris Bioscience. Ingredients for buffers and media were obtained from Duchefa Biochemie (Haarlem, The Netherlands) or Merck (Darmstadt, Germany). A previously engineered variant of methylaspartate ammonia lyase (MAL-L384A) was overproduced in *Escherichia coli* and purified as described previously[1]. Ni-Sepharose 6 fast flow resin and prepacked PD-10 Sephadex G-25 columns for protein purification were purchased from GE Healthcare Bio-Sciences (Little Chalfont, UK). Proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions on gels containing 10% polyacrylamide. The gels were stained with Coomassie brilliant blue. High performance liquid chromatography (HPLC) was performed with a Shimadzu LC-10AT HPLC with a Shimadzu SP-M10A ELSD detector. NMR analyses were performed on a Varian Inova 400 MHz machine at the NMR Center of the University of Groningen, or on a Brucker 500 MHz machine at the Drug Design laboratory of the University of Groningen. Chemical shifts (δ) are reported in parts per million (ppm). Optical rotations were measured on a Schmidt+Haensch Polartronic MH8 polarimeter with a 10 cm cell (*c* given in g/100 mL). Electrospray ionization orbitrap high resolution mass spectrometry (HRMS) was performed by the Mass Spectrometry core facility of the University of Groningen.

II) Detailed experimental procedures

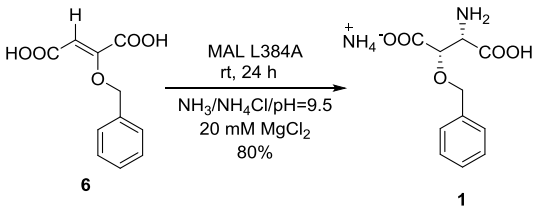
1 Chemoenzymatic synthesis of L-TBOA (**1**) at multigram scale.

1.1 *trans*-2-benzyloxy fumaric acid (6)



To a stirred solution of dimethyl acetylenedicarboxylate (**3**, 2.84 g, 20.0 mmol) in DCM (150 mL) was added DABCO (0.22 g, 2.0 mmol) and benzyl alcohol (2.16 g, 20.0 mmol) at room temperature. After completion of the reaction (TLC monitoring), the solvent was removed under vacuum to provide crude products as a dark oil, which contained *trans*-**S1** and *cis*-**S1** isomers (*trans*/*cis* = 6/4). The crude product was dissolved in ethanol (50 mL) and subjected to basic hydrolysis using 2 M NaOH (50 mL) at reflux for 2 h. After complete hydrolysis, the reaction mixture was cooled to room temperature and extracted using EtOAc (2 x 50 mL). The aqueous layer was acidified with HCl (con.) until pH = 1 (ice-bath) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over anhydrous Na_2SO_4 and evaporated to provide a dark yellow solid. Recrystallization was performed using hexane/Et_2O (v/v=1/3) to provide pure *trans*-2-benzyloxy-fumaric acid **6** (1.64 g, two steps yield 37%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.43 – 7.32 (m, 5H), 6.08 (s, 1H), 5.10 (s, 2H). The NMR data are in agreement with published data.[1] Following the same procedures, we prepared **6** at multigram-scale (35 g) from dimethyl acetylenedicarboxylate (**3**, 60 g) and benzyl alcohol (44.3 g).

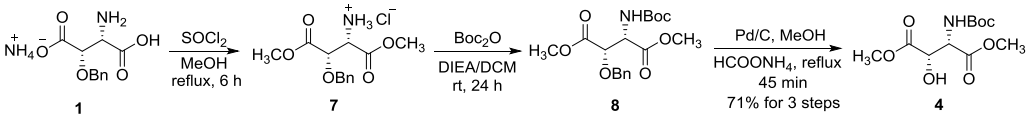
1.2 (L-*threo*)-3-benzyloxyaspartate (**1**, L-TBOA)



To a slowly stirred solution of *trans*-2-benzyloxy-fumaric acid **6** (2.44 g, 11 mmol) in 220 mL of buffer (5 M NH_3/NH_4Cl, 20 mM $MgCl_2$, pH = 9.5) was added MAL L384A[1] (0.01 mol%, 5 mL, 12.3 mg/mL), and the reaction mixture was incubated for 24 hours at room temperature. After completion of the enzymatic reaction (^{1}HNMR monitoring, >98% conversion), the reaction mixture was warmed up to 60 °C for 10 min until the

enzyme precipitated, followed by filtration through cotton to remove the white precipitates. Most of the water in the reaction mixture was evaporated under vacuum, then the resulting concentrated mixture was acidified with HCl (conc.) to pH = 1 (in an ice-bath). The acidified solution was loaded onto a column packed with cation-exchange resin (1000 g of Dowex 50W X8, 50-100 mesh), which was pre-treated with 2 M aqueous ammonia (4 column volumes, C.Vs.), 1 M HCl (2 C.Vs.) and distilled water (4 C.Vs.). The column was washed with water (2 C.Vs.) and the product was eluted with 2 M aqueous ammonia (2 C.Vs.). The ninhydrin-positive fractions were collected and lyophilized to yield the product L-TBOA (**1**) ammonium salt (white powder, 2.26 g, 80%). ^{1}H NMR (500 MHz, D_2O) δ 7.43 – 7.34 (m, 5H), 4.71 (d, J = 11.6 Hz, 1H), 4.47 (d, J = 11.6 Hz, 1H), 4.32 (d, J = 2.3 Hz, 1H), 3.99 (d, J = 2.3 Hz, 1H). NMR data are in agreement with published data.[1] Following the same procedures, we prepared L-TBOA at multigram-scale (**1**, 24.8 g) from **6** (25 g) in several batches.

2 Synthesis of chiral building block (**4**)



2.1 (L-*threo*)-dimethyl 2-amino-3-(benzyloxy)succinate hydrochloride (7)

To a stirred suspension of L-TBOA (**1**, 512 mg, 2 mmol) in dry MeOH (15 mL) at was added $SOCl_2$ (1.45 mL, 20 mmol) dropwise (in an ice-bath). After 20 minutes, the cooling system was removed and the reaction mixture was heated to reflux for 6 h. After completion of the reaction (TLC monitoring, MeOH/DCM 4:1, Rf = 0.8 ninhydrin), the reaction mixture was cooled to room temperature, and solvent was removed to provide crude product **7** as a white solid (580 mg, 96%). No purification was needed, the crude product **7** was directly used for the next step. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (s, 3H), 7.39 – 7.32 (m, 5H), 4.77 (d, J = 11.8 Hz, 1H), 4.64 (d, J = 3.6 Hz, 1H), 4.55 (d, J = 11.8 Hz, 1H), 4.46 (d, J = 3.6 Hz, 1H), 3.75 (s, 3H), 3.64 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.36, 167.07, 136.64, 128.29 (2), 128.22 (2), 128.05, 75.19, 72.60, 54.07, 53.16, 52.83. HRMS: calcd. for $C_{13}H_{18}NO_5$ $[M+H]^+$: 268.1180, found 268.1174.

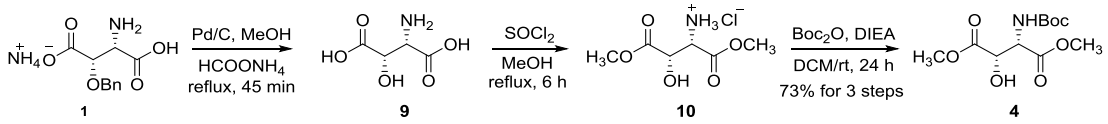
2.2 (L-*threo*)-dimethyl 2-(benzyloxy)-3-[(*tert*-butoxycarbonyl)amino]succinate (8)

To a stirred solution of **7** (580 mg, 1.92 mmol) in dry DCM (20 mL) was added DIEA (495μL, 3 mmol) and Boc_2O (436 mg, 2 mmol) under cooling in an ice-bath. After 10 minutes, the cooling was removed and the reaction mixture was stirred at room temperature for further 24 h. After completion of the reaction, the reaction mixture was diluted with DCM (20 mL), and washed with 0.5 M HCl (50 mL), saturated $NaHCO_3$ solution (50

mL) and brine (50 mL). The organic layer was dried over Na_2SO_4 (s) and concentrated under vacuum to give crude product **8** as a clear oil (640 mg, 91%). No purification was needed, analytically pure product **8** was directly used for the next step. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 – 7.28 (m, 5H), 7.05 (d, J = 9.5 Hz, 1H), 4.67 (d, J = 11.9 Hz, 1H), 4.62 (dd, J = 9.5, 4.3 Hz, 1H), 4.47 (d, J = 4.3 Hz, 1H), 4.42 (d, J = 11.9 Hz, 1H), 3.67 (s, 3H), 3.60 (s, 3H), 1.36 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.72, 169.66, 155.44, 136.55, 128.43, 128.29 (2), 128.20 (2), 80.22, 76.75, 72.75, 56.01, 52.58, 52.45, 28.18 (3). HRMS: calcd. for $C_{18}H_{25}NO_7Na$ $[M+Na]^+$: 390.1523, found 390.1523.

2.3 (L-*threo*)-dimethyl 2-[(*tert*-butoxycarbonyl)amino]-3-hydroxy succinate (4)

To a stirred solution of **8** (640 mg, 1.75 mmol) in dry MeOH (15 mL) was added Pd/C (10%, 0.6 g) and $HCOONH_4$ (0.7 g). The mixture was heated to reflux for 45 min. After completion of the reaction, the reaction mixture was filtered through Celite and evaporated under vacuum to provide crude product **4**. Purification was conducted *via* flash chromatography (EtOAc/Petroleum ether, 15%, v/v) to provide compound **4** as clear oil (392 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.29 (d, J = 9.5 Hz, 1H), 4.78 (dd, J = 9.3, 2.0 Hz, 1H), 4.69 (dd, J = 5.8, 2.0 Hz, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 3.22 (d, J = 5.7 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.41, 169.85, 155.27, 80.41, 71.07, 56.12, 53.24, 52.94, 28.16 (3). HRMS: calcd. for $C_{11}H_{19}NO_7Na$ $[M+Na]^+$: 300.1054, found 300.1053. We prepared precursor **4** at multigram-scale (6.9 g) from **7** (9.8 g) following the same procedures.



2.4 (L-*threo*)-dimethyl 2-amino-3-hydroxy succinate (10)

To a stirred solution of **1** (256 mg, 1 mmol) in dry MeOH (10 mL) was added Pd/C (10%, 0.25 g) and $HCOONH_4$ (0.35 g). The mixture was heated to reflux for 45 min. Subsequently, the reaction mixture was filtered through Celite and washed with MeOH (10 mL). The filtrate was collected and evaporated under vacuum to provide product (L-*threo*)-3-hydroxy aspartate **9**. ^{1}H NMR (500 MHz, D_2O) δ 4.54 (d, J = 2.1 Hz, 1H), 4.06 (d, J = 2.1 Hz, 1H); ^{13}C NMR (126 MHz, D_2O) δ 176.57, 172.39, 70.67 (d, J = 12.6 Hz), 56.94 (t, J = 32.8 Hz). HRMS: calcd. for $C_4H_8NO_5$ $[M+H]^+$: 150.0397, found 150.0397.

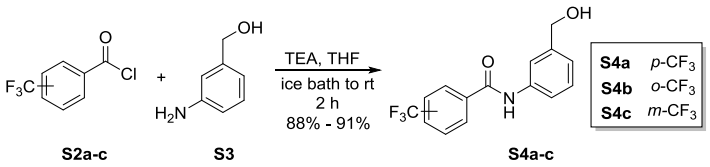
In the subsequent step, compound **9** was dissolved in dry MeOH (10 mL), followed by dropwise addition of $SOCl_2$ (0.73 mL, 10 mmol) in an ice-bath. After 20 minutes, the cooling system was removed and the reaction

mixture was heated to reflux for 6 h. After completion of the reaction, the reaction mixture was cooled to room temperature and solvent was removed under vacuum to provide product **10** as white solid (192 mg, two-step yield 90%). No purification was needed, analytically pure product **10** was directly used for the next step. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.65 (d, J = 2.4 Hz, 1H), 3.92 (d, J = 2.4 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 2.48 (brs, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.99 (2), 72.13, 56.73, 53.17, 52.85. HRMS: calcd. for $C_6H_{12}NO_5$ $[M+H]^+$: 178.0710, found 178.0712.

2.5 (L-*threo*)-dimethyl 2-[(*tert*-butoxycarbonyl)amino]-3-hydroxy succinate (4)

To a stirred solution of **10** (192 mg, 0.9 mmol) in dry DCM (10 mL) was added DIEA (330 μL, 2 mmol) and Boc_2O (218 mg, 1 mmol) under cooling using an ice-bath. After 10 minutes, the cooling system was removed and the reaction mixture was stirred at room temperature for further 24 h. After completion of the reaction, the reaction mixture was diluted with DCM (10 mL), and washed with 0.5 M HCl (20 mL), saturated $NaHCO_3$ solution (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 (s). The solvent was evaporated under vacuum to give crude product **4**, which was purified *via* flash chromatography (EtOAc/Petroleum ether, 15%, v/v) to provide pure compound **4** as a clear oil (202 mg, 81%).

3. Synthesis of substituted benzyl bromide (**5**)



3.1 *N*-[3-(hydroxymethyl)phenyl]-4-(trifluoromethyl)benzamide (S4a)

To a stirred solution of 3-aminobenzyl alcohol (**S3**, 861 mg, 7.0 mmol) and 4-trifluoromethylbenzoyl chloride (**S2a**, 1.04 g, 5.0 mmol) in THF (30 mL) was added triethylamine (707 mg, 7.0 mmol) under cooling using an ice-bath . After 10 minutes, the cooling bath was removed and reaction was run at room temperature for further 2 h. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (50 mL) and washed with 1 M aqueous HCl (3 x 50 mL), saturated aqueous $NaHCO_3$ (2 x 50 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and evaporated under vacuum to give the crude product **S4a**. The product was precipitated from ethyl acetate/pentane to give pure **S4a** as a white powder (1.30 g, 88%). ^{1}H NMR (400 MHz, Methanol-d_4): δ 8.09 (d, J = 8.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H), 7.71 (s, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.17 (d, J = 7.6 Hz, 1H), 4.62 (s, 2H); ^{19}F NMR (376 MHz, Methanol-d_4) δ -64.5 (s). NMR data

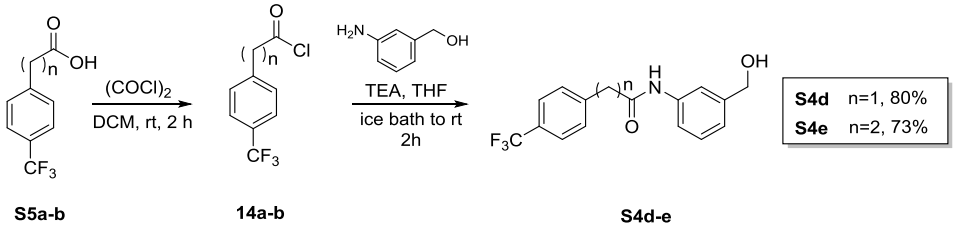
are in agreement with published data.[2, 3]

3.2 ***N*-[3-(hydroxymethyl)phenyl]-2-(trifluoromethyl)benzamide (S4b).**

Compound **S4b** was prepared from 3-aminobenzyl alcohol (**S3,** 861 mg, 7.0 mmol), 2-trifluoromethylbenzoyl chloride (**S2b**, 1.04 g, 5.0 mmol) and triethylamine (707 mg, 7.0 mmol) following a procedure similar to that used for **S4a**. The compound **S4b** was obtained as a white solid (1.32 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H), 8.32 (s, 1H), 8.28 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.80 – 7.76 (m, 2H), 7.69 (d, J = 8.1 Hz, 1H), 7.32 (t, J = 7.8 Hz, 1H), 7.08 (d, J = 7.5 Hz, 1H), 5.26 (t, J = 5.7 Hz, 1H), 4.53 (d, J = 5.6 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.94, 143.22, 138.71, 135.81, 131.87, 129.72, 129.19 (q, J = 32.8 Hz), 128.35, 128.12 (q, J = 3.8 Hz), 124.02 (q, J = 273.4 Hz), 124.26 (q, J = 3.8 Hz), 122.06, 118.89, 118.61, 62.87; ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.13 (s). HRMS: calcd. for $C_{15}H_{13}F_3NO_2$ $[M+H]^+$: 296.0893, found 296.0885.

3.3 ***N*-[3-(hydroxymethyl)phenyl]-3-(trifluoromethyl)benzamide (S4c).**

Compound **S4c** was prepared from 3-aminobenzyl alcohol (**S3**, 861 mg, 7.0 mmol), 3-trifluoromethylbenzoyl chloride (**S2c**, 1.04 g, 5.0 mmol) and triethylamine (707 mg, 7.0 mmol) by following a procedure similar to that used for **S4a**. The title compound was obtained as a white solid (1.35 g, 91%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.72 – 7.68 (m, 3H), 7.53 (d, J = 8.3 Hz, 1H), 7.29 (t, J = 7.8 Hz, 1H), 7.06 (d, J = 7.6 Hz, 1H), 5.24 (t, J = 5.7 Hz, 1H), 4.50 (d, J = 5.7 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.56, 143.33, 138.81, 136.33, 132.62, 129.99, 128.52, 128.44, 126.32 (q, J = 6.3 Hz), 125.80 (q, J = 31.5 Hz), 123.82 (q, J = 273.4 Hz), 121.95, 118.05, 117.74, 62.88; ^{19}F NMR (376 MHz, Methanol-d_4) δ -60.55 (s). HRMS: calcd. for $C_{15}H_{13}F_3NO_2$ $[M+H]^+$: 296.0893, found 296.0886.



3.4 ***N*-[3-(hydroxymethyl)phenyl]-2-[4-(trifluoromethyl)phenyl]acetamide (S4d).**

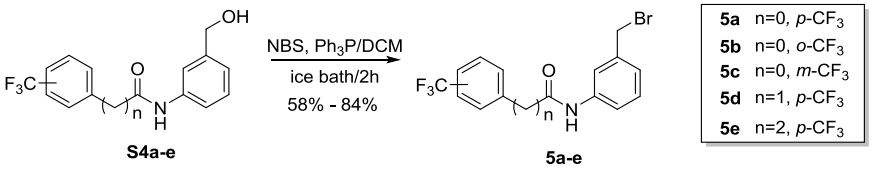
To a stirred solution of 2-[4-(trifluoromethyl)phenyl]acetic acid (**S5a**, 0.50 g, 2.45 mmol) in dry DCM (20 mL) at room temperature was added oxalyl chloride solution (10 mL, 2 M in DCM) and one drop of dry DMF as

catalyst. The reaction was stirred at same temperature for further 2 h. Subsequently, solvents and access oxalyl chloride were removed under reduced pressure, and the resulting acid chloride **14a** was directly used for the next step.

The freshly prepared acid chloride **14a** was diluted with dry THF (30 mL) under ice-bath condition, after which 3-aminobenzyl alcohol (361 mg, 2.94 mmol) and triethylamine (297 mg, 2.94 mmol) were added. After 10 minutes, the cooling system was removed, and the reaction mixture was stirred for another 2 hours at room temperature. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (100 mL) and washed with 1 M HCl (3 x 100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic phase was dried over Na_2SO_4 and the solvent was partially evaporated. The product **S4d** was precipitated from EtOAc/Petroleum ether to give white powder (0.61 g, 80%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.22 (s, 1H), 7.70 (d, J = 8.0 Hz, 2H), 7.58 – 7.54 (m, 3H), 7.48 (d, J = 8.4 Hz, 1H), 7.23 (t, J = 7.8 Hz, 1H), 6.98 (d, J = 7.6 Hz, 1H), 5.19 (t, J = 5.7 Hz, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.76 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.27, 143.25, 140.88, 138.92, 130.00 (2), 128.39, 127.29 (q, J = 32.8 Hz), 125.12 (2, q, J = 3.8 Hz), 124.39 (q, J = 272.2 Hz), 121.34, 117.45, 117.17, 62.79, 42.95; ^{19}F NMR (376 MHz, DMSO-d_6) δ -60.86 (s). HRMS: calcd. for $C_{16}H_{15}F_3NO_2$ $[M+H]^+$: 310.1049, found 310.1049.

3.5 *N*-[3-(hydroxymethyl)phenyl]-3-[4-(trifluoromethyl)phenyl]propanamide (S4e)

Compound **S4e** was prepared from 3-[4-(trifluoromethyl)phenyl]propanoic acid (**S5b**, 0.50 g, 2.29 mmol) following a procedure similar to that used for **S4d**. The title compound was obtained as a white solid (0.62 g, 73%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.91 (s, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.54 (s, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.7 Hz, 1H), 7.22 (t, J = 7.8 Hz, 1H), 6.96 (d, J = 7.6 Hz, 1H), 5.18 (t, J = 5.7 Hz, 1H), 4.45 (d, J = 5.6 Hz, 2H), 3.00 (t, J = 7.6 Hz, 2H), 2.66 (t, J = 7.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.96, 146.24, 143.16, 139.01, 129.13 (2), 128.33, 126.74 (q, J = 31.5 Hz), 125.12 (2, q, J = 3.8 Hz), 124.43 (q, J = 272.2 Hz), 121.10, 117.39, 117.12, 62.83, 37.30, 30.51; ^{19}F NMR (376 MHz, DMSO-d_6) δ -60.74 (s). HRMS: calcd. for $[M+H]^+$: $C_{17}H_{17}F_3NO_2$ 324.1206, found 324.1199.



3.6 ***N*-[3-(bromomethyl)phenyl]-4-(trifluoromethyl)benzamide (5a)**

To a stirred solution of compound **S4a** (295 mg, 1.0 mmol) in DCM (20 mL), in an ice-bath, was added *N*-bromosuccinimide (213 mg, 1.2 mmol) and triphenylphosphine (314mg, 1.2 mmol). The reaction was allowed to proceed at the same temperature for 2 h. After completion of the reaction, the solvent was evaporated and product **5a** was purified by flash column chromatography (EtOAc/Petroleum ether, 10%, v/v) to give a white powder (299 mg, 84%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.51 (s, 1H), 8.13 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 8.0 Hz, 2H), 7.89 (s, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.19 (d, J = 7.6 Hz, 1H), 4.70 (s, 2H); ^{19}F NMR (376 MHz, DMSO-d_6): -61.3 (s). HRMS: calcd. for $C_{15}H_{12}BrF_3NO$ $[M+H]^+$: 358.0049, found: 358.0047. NMR data are in agreement with published data.[3]

3.7 ***N*-[3-(bromomethyl)phenyl]-2-(trifluoromethyl)benzamide (5b).**

Compound **5b** was prepared from **S4b** (1.20 g, 4.07 mmol), NBS (866 mg, 4.88 mmol) and Ph_3P (1.28 g, 4.88 mmol) following a procedure similar to that used for **5a**. The title compound was obtained as a white solid (1.10 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 7.89 – 7.83 (m, 2H), 7.80 (t, J = 7.2 Hz, 1H), 7.72 (t, J = 7.4 Hz, 2H), 7.57 – 7.55 (m, 1H), 7.34 (t, J = 7.9 Hz, 1H), 7.20 (d, J = 7.7 Hz, 1H), 4.71 (s, 2H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 168.73, 140.52, 139.99, 137.31, 133.45, 131.21, 130.29, 129.47, 128.24 (q, J = 32.8 Hz), 127.51(q, J = 5.0 Hz), 126.38, 125.22 (q, J = 273.4 Hz), 122.01, 121.29, 33.73; ^{19}F NMR (376 MHz, Methanol-d_4) δ -60.54 (s). HRMS: calcd. for $C_{15}H_{12}BrF_3NO$ $[M+H]^+$: 358.0049, found 358.0049.

3.8 ***N*-[3-(bromomethyl)phenyl]-3-(trifluoromethyl)benzamide (5c).**

Compound **5c** was prepared from **S4c** (1.10 g, 3.73 mmol), *N*-bromosuccinimide (794 mg, 4.47 mmol) and triphenylphosphine (1.17 g, 4.47 mmol) following a procedure similar to that used for **5a**. The title compound was obtained as a white solid (960 mg, 72%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.55 (s, 1H), 8.31 (s, 1H), 8.28 (d, J = 7.9 Hz, 1H), 7.97 (d, J = 10.0 Hz, 1H), 7.91 (t, J = 1.7 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.72 (d, J = 9.1 Hz, 1H), 7.36 (t, J = 7.9 Hz, 1H), 7.21 (d, J = 7.6 Hz, 1H), 4.72 (s, 2H); ^{13}C NMR (126 MHz, Methanol-d_4) δ 167.04, 140.40, 139.99, 137.16, 132.31, 131.97 (q, J = 32.8 Hz), 130.62, 130.20, 129.34 (q, J = 3.8 Hz), 126.40, 125.57 (q, J = 5.0 Hz), 122.79, 122.04, 33.81; ^{19}F NMR (376 MHz, Methanol-d_4) δ -64.16 (s). HRMS: calcd. for $C_{15}H_{12}BrF_3NO$ $[M+H]^+$: 358.0049, found 358.0049.

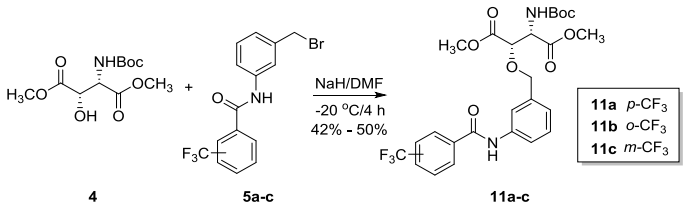
3.9 ***N*-[3-(bromomethyl)phenyl]-2-[4-(trifluoromethyl)phenyl]acetamide (5d)**

Compound **5d** was prepared from **S4d** (0.61 g, 1.97 mmol), NBS (419 mg, 2.36 mmol) and Ph_3P (618 mg, 2.36 mmol) following a procedure similar to that used for **5a**. The title compound was obtained as a white solid (0.47 g, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, J = 8.0 Hz, 2H), 7.55 (t, J = 2.0 Hz, 1H), 7.46 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.2 Hz, 1H), 7.27 (t, J = 7.8 Hz, 1H), 7.19 (s, 1H), 7.14 (d, J = 7.6 Hz, 1H), 4.43 (s, 2H), 3.78 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.27, 143.25, 140.88, 138.92, 130.00 (2), 128.40, 127.29 (q, J = 32.8 Hz), 125.12 (2, q, J = 3.8 Hz), 124.39 (q, J = 272.2 Hz), 121.34, 117.45, 117.17, 62.78, 42.94; ^{19}F NMR (376 MHz, Methanol-d_4) δ -63.94 (s). HRMS: calcd. for $C_{16}H_{14}BrF_3NO$ $[M+H]^+$: 372.0205, found 372.0205.

3.10 ***N*-[3-(bromomethyl)phenyl]-3-[4-(trifluoromethyl)phenyl]propanamide (5e)**

Compound **5e** was prepared from **S4e** (0.60 g, 1.86 mmol), NBS (396 mg, 2.23 mmol) and Ph_3P (584mg, 2.23 mmol) following a procedure similar to that used for **5a**. The title compound was obtained as a white solid (0.42 g, 58%). ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.61 (t, J = 1.7 Hz, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.45 – 7.39 (m, 3H), 7.25 (t, J = 7.9 Hz, 1H), 7.12 (d, J = 7.7 Hz, 1H), 4.50 (s, 2H), 3.07 (t, J = 7.7 Hz, 2H), 2.69 (t, J = 7.7 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.33, 146.33, 139.54, 138.61, 129.26 (2), 129.16, 126.92 (q, J = 31.5 Hz), 125.28 (2, q, J = 3.8 Hz), 124.57 (q, J = 272.2 Hz), 124.05, 119.91, 119.15, 37.42, 34.77, 30.59; ^{19}F NMR (376 MHz, Methanol-d_4) δ -63.90 (s). HRMS: calcd. for $C_{17}H_{16}BrF_3NO$ $[M+H]^+$: 386.0362, found 386.0362.

4. Synthesis of L-TFB-TBOA (**2a**) and its derivatives (**2b-c**).



4.1 **(L-*threo*)-dimethyl 2-[(*tert*-butoxycarbonyl)amino]-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy] succinate (11a)**

To a stirred solution of compound **4** (50 mg, 0.18 mmol) in dry DMF (3 mL) was added **5a** (129 mg, 0.36 mmol) at -20 °C. After 10 min, NaH (60% in mineral oil, 7.2 mg, 0.18 mmol) was added to the reaction mixture and the mixture was stirred at -20 °C for further 4 h. After completion of the reaction, the reaction mixture was quenched with cold water, extracted with EtOAc (3 x 20 mL), washed with brine (3 x 50 mL), and dried over Na_2SO_4. The solvent was evaporated to provide crude product **11a**, which was purified *via* flash chromatography (EtOAc/Petroleum ether, 15%, v/v) to give **11a** as a clear oil (45 mg, 45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25

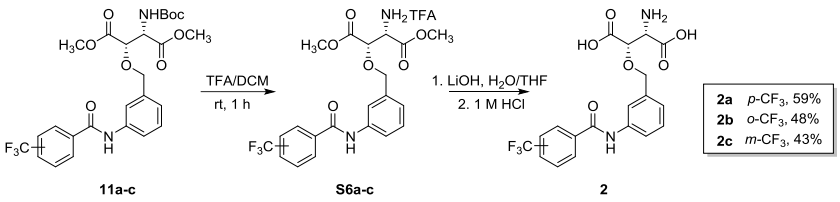
(s, 1H), 7.96 (d, *J* = 8.1 Hz, 2H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.53 (s, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 7.6 Hz, 1H), 5.36 (d, *J* = 9.9 Hz, 1H), 4.82 – 4.77 (m, 2H), 4.50 (d, *J* = 2.4 Hz, 1H), 4.36 (d, *J* = 11.9 Hz, 1H), 3.76 (s, 3H), 3.63 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.89, 169.76, 164.69, 155.55, 138.17, 137.93, 137.89, 133.59 (q, *J* = 32.8 Hz), 129.32, 127.75 (2), 125.86 (2, q, *J* = 3.8 Hz), 123.70 (q, *J* = 272.2 Hz), 124.56, 120.32, 120.08, 80.47, 77.29, 72.66, 56.13, 52.81, 52.62, 28.26 (3). HRMS: calcd. for $C_{26}H_{30}F_3N_2O_8$ $[M+H]^+$: 555.1949, found 555.1954.

4.2 **(L-*threo*)-dimethyl 2-[(*tert*-butoxycarbonyl)amino]-3-[3-[2-(trifluoromethyl)benzoylamino]benzyloxy] succinate (11b)**

Compound **11b** was prepared from **4** (50 mg, 0.08 mmol), **5b** (129 mg, 0.36 mmol) and NaH (60% in mineral oil, 7.2 mg, 0.18 mmol) following a procedure similar to that used for **11a**. The title compound was obtained as clear oil (50 mg, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.8 Hz, 1H), 7.65 – 7.58 (m, 5H), 7.48 (s, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 5.35 (d, *J* = 10.0 Hz, 1H), 4.83 – 4.80 (m, 2H), 4.51 (d, *J* = 2.4 Hz, 1H), 4.38 (d, *J* = 12.0 Hz, 1H), 3.77 (s, 3H), 3.65 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.85, 169.77, 165.80, 155.55, 137.98, 137.81, 135.79, 132.38, 130.41, 129.40, 128.66, 127.40 (q, *J* = 31.5 Hz), 126.70 (q, *J* = 5.0 Hz), 124.73, 123.72 (q, *J* = 273.4 Hz), 120.02, 119.78, 80.39, 77.30, 72.63, 56.13, 52.83, 52.63, 28.29 (3). HRMS: calcd. for $C_{26}H_{30}F_3N_2O_8$ $[M+H]^+$: 555.1949, found 555.1942.

4.3 **(L-*threo*)-dimethyl 2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(trifluoromethyl) benzoylamino]benzyloxy] succinate (11c)**

Compound **11c** was prepared from **4** (50 mg, 0.08 mmol), **5c** (129 mg, 0.36 mmol) and NaH (60% in mineral oil, 7.2 mg, 0.18 mmol) following a procedure similar to that used for **11a**. The title compound was obtained as clear oil (42 mg, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.99 (s, 1H), 7.83 – 7.80 (m, 1H), 7.65 (dt, *J* = 15.6, 7.9 Hz, 2H), 7.52 (s, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 5.36 (d, *J* = 10.0 Hz, 1H), 4.85 – 4.80 (m, 2H), 4.51 (d, *J* = 2.3 Hz, 1H), 4.39 (d, *J* = 11.9 Hz, 1H), 3.78 (s, 3H), 3.66 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.79, 169.67, 164.27, 155.44, 137.84, 137.72, 135.64, 131.38 (q, *J* = 32.8 Hz), 130.37, 129.50, 129.30, 128.52 (q, *J* = 2.5 Hz), 124.50, 124.11 (q, *J* = 5.0 Hz), 123.63 (q, *J* = 273.4 Hz), 120.13, 119.89, 80.34, 77.13, 72.52, 56.02, 52.73, 52.54, 28.17 (3). HRMS: calcd. for $C_{26}H_{30}F_3N_2O_8$ $[M+H]^+$: 555.1949, found 555.1950.



4.4 **(L-*threo*)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (2a, L-TFB-TBOA)**

To a stirred solution of **11a** (45 mg, 0.08 mmol) in dry DCM (2 mL)，in an ice-bath，was added trifluoroacetic acid (0.8 mL) dropwise. After the complete addition of trifluoroacetic acid, the ice-bath was removed and the reaction was allowed to proceed at room temperature for further 1.5 h. After completion of the starting material, solvent was removed in vacuo to provide **S6a** quantitatively. Compound **S6a** was directly used for the next step without purification.

To a stirred solution of **S6a** in THF/H_2O (1:1, each 1 mL) was added LiOH (11.6 mg, 0.5 mmol) and the reaction mixture was stirred at room temperature for 2 h. After completion of the starting material, THF was removed in vacuo and the resulting aqueous solution was reverse extracted with EtOAc (1 mL). The aqueous layer was acidified with 1 M HCl (pH=1) until white precipitates appeared. The white precipitates were filtered through a Büchner funnel and washed with 5 mL of cold water. The obtained white solid was dried under vacuum over night to provide the final product **2a** (L-TFB-TBOA) (22 mg, two-step yield 59%, *de* >98%, *ee* >99%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.49 (s, 1H), 8.16 (d, J = 8.1 Hz, 2H), 7.92 (d, J = 8.2 Hz, 2H), 7.76 (s, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.28 (d, J = 7.7 Hz, 1H), 4.83 (d, J = 10.6 Hz, 1H), 4.48 (d, J = 10.6 Hz, 1H), 4.13 (d, J = 9.5 Hz, 1H), 3.83 (d, J = 9.5 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.74, 168.42, 164.29, 138.68, 138.47, 138.40, 131.34 (q, J = 31.5 Hz), 128.62 (2), 128.36, 125.38 (2, q, J = 5.0 Hz), 124.17, 123.94 (q, J = 272.2 Hz), 120.42, 119.83, 75.09, 72.50, 53.35; ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.32 (s). HRMS: calcd. for $C_{19}H_{18}F_3N_2O_6$ $[M+H]^+$: 427.1112, found 427.1108. $[\alpha]_D^{25}$ = -55.0 (*c* 0.60, DMSO). Comparison of the ^{1}H NMR data of **2a** with the ^{1}H NMR data of chemically prepared racemic DL-*erythro*-**S12a** showed that the *de* of product **2a** is >98%. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (L-*threo*-**2a**) = 2.8 min, *ee* >99%. Following the same procedures, we prepared **2a** at multigram-scale (2.3 g) from **11a** (4.15 g).

4.5 **(L-*threo*)-3-[3-[2-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (2b)**

Compound **2b** was prepared from **11b** (45 mg, 0.08 mmol) following a procedure similar to that used for **2a**. The title compound was obtained as a white solid (16 mg, two-step yield 43%, *de* >98%, *ee* >99%). ^{1}H NMR (400

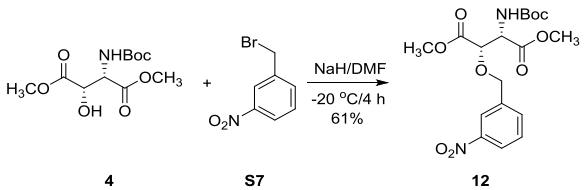
MHz, DMSO-d_6) δ 10.56 (s, 1H), 7.85 (d, J = 7.7 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.73 – 7.66 (m, 3H), 7.62 (d, J = 7.8 Hz, 1H), 7.34 – 7.26 (m, 2H), 4.80 (d, J = 10.5 Hz, 1H), 4.46 (d, J = 10.5 Hz, 1H), 4.11 (d, J = 9.4 Hz, 1H), 3.81 (d, J = 9.3 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.58, 168.41, 165.56, 138.56, 138.46, 136.22, 132.60, 130.00, 128.50 (2), 126.32 (q, J = 5.0 Hz), 125.78 (q, J = 31.5 Hz), 124.14, 123.79 (q, J = 274.7 Hz), 119.52, 118.95, 74.84, 72.69, 53.13; ^{19}F NMR (376 MHz, DMSO-d_6) δ -57.91 (s). HRMS: calcd. for $C_{19}H_{18}F_3N_2O_6$ $[M+H]^+$: 427.1112, found 427.1106. $[\alpha]_D^{25}$ = -53.1 (c 0.43, DMSO). Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (L-*threo*-**2b**) = 2.6 min, *ee* >99%.

4.6 **(L-*threo*)-3-[3-[3-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (2c)**

Compound **2c** was prepared from **11c** (42 mg, 0.076 mmol) following a procedure similar to that used for **2a**. The title compound was obtained as a white solid (18 mg, two-step yield 48%, *de* >98%, *ee* >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 8.31 (s, 1H), 8.28 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.73 (d, J = 7.7 Hz, 2H), 7.35 (t, J = 7.8 Hz, 1H), 7.27 (d, J = 7.6 Hz, 1H), 4.81 (d, J = 10.7 Hz, 1H), 4.47 (d, J = 10.7 Hz, 1H), 4.11 (d, J = 8.9 Hz, 1H), 3.77 (d, J = 9.0 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.14, 168.88, 164.38, 138.90, 138.83, 136.16, 132.33, 130.20, 129.61 (q, J = 32.8 Hz), 128.83, 128.62 (q, J = 3.8 Hz), 124.72 (q, J = 3.8 Hz), 124.67, 124.46 (q, J = 273.4 Hz), 120.99, 120.39, 75.44, 73.01, 53.73; ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.08 (s). HRMS: calcd. for $C_{19}H_{18}F_3N_2O_6$ $[M+H]^+$: 427.1112, found 427.1112. $[\alpha]_D^{25}$ = -50.7 (c 0.43, DMSO). Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (L-*threo*-**2c**) = 2.7 min, *ee* >99%.

5. Synthesis of L-TFB-TBOA derivatives from protected *meta*-NH_2-TBOA (**13**)

5.1 **(L-*threo*)-dimethyl 2-(3-nitrobenzyloxy)-3-[(*tert*-butoxycarbonyl)amino] succinate (12)**

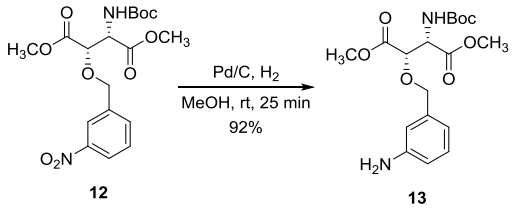


4 **S7** **12**

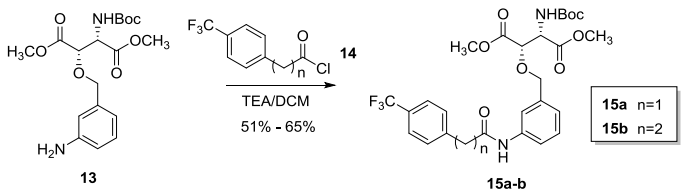
To a stirred solution of compound **4** (277 mg, 1.08 mmol) in dry DMF (5 mL) was added bromomethyl-3-nitrobenzene (**S7**, 466 mg, 2.16 mmol) at -20 °C. After 10 min, NaH (60% in mineral oil, 43 mg, 1.08 mmol) was added to the reaction mixture and the mixture was stirred at -20 °C for further 4 h. After completion of the

reaction, the reaction mixture was quenched with cold water and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over Na_2SO_4 and evaporated to provide crude product **12**, which was purified *via* flash chromatography (EtOAc/Petroleum ether, 17%, v/v) to give pure **12** as a clear oil (275 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, J = 8.1 Hz, 1H), 8.12 (s, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.52 (t, J = 7.9 Hz, 1H), 5.31 (d, J = 10.0 Hz, 1H), 4.92 – 4.87 (m, 2H), 4.56 (d, J = 2.4 Hz, 1H), 4.48 (d, J = 12.1 Hz, 1H), 3.79 (s, 3H), 3.74 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.72, 169.39, 155.44, 148.41, 139.10, 133.77, 129.56, 123.18, 122.66, 80.55, 78.15, 71.74, 56.12, 53.04, 52.72, 28.28 (3). HRMS: calcd. for $C_{18}H_{24}N_2O_9Li$ $[M+Li]^+$: 419.1636, found 419.1638.

5.2 **(L-*threo*)-dimethyl 2-(3-aminobenzyloxy)-3-[(*tert*-butoxycarbonyl)amino] succinate (13)**



To a stirred solution of **12** (275 mg, 0.67 mmol) in dry MeOH (10 mL) was added Pd/C 10% (50.0 mg) under nitrogen atmosphere. The reaction was stirred under H_2 atmosphere (balloon) for 25 min at room temperature. After completion of the reaction, the reaction mixture was filtered through Celite and washed with MeOH (5 mL). The filtrate was concentrated under vacuum to provide product **13** as a colorless oil (235 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (t, J = 7.7 Hz, 1H), 6.62 – 6.60 (m, 2H), 6.57 (d, J = 2.0 Hz, 1H), 5.37 (d, J = 9.9 Hz, 1H), 4.79 (dd, J = 10.0, 2.3 Hz, 1H), 4.72 (d, J = 11.9 Hz, 1H), 4.47 (d, J = 2.3 Hz, 1H), 4.26 (d, J = 12.0 Hz, 1H), 3.77 (s, 3H), 3.68 (s, 2H), 3.64 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.93, 169.85, 155.59, 146.72, 137.83, 129.46, 118.50, 114.98, 114.89, 80.31, 76.64, 72.82, 56.13, 52.73, 52.57, 28.31 (3). HRMS: calcd. for $C_{18}H_{26}F_3N_2O_7Li$ $[M+Li]^+$:389.1895, found 389.1895.



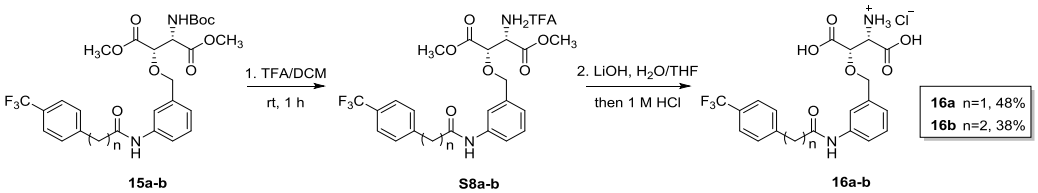
5.3 **(L-*threo*)-dimethyl 2-[(*tert*-butoxycarbonyl)amino]-3-[3-[2-[4-(trifluoromethyl)phenyl]acetamido] benzyloxy] succinate (15a)**

To a stirred solution of **13** (80 mg, 0.21 mmol) in dry DCM (2 mL), in an ice-bath, was added TEA (64 mg, 0.63

mmol), followed by dropwise addition of acid chloride solution (**14a**, 100 mg in 1 mL of DCM, 0.42 mmol). After 10 min, the ice-bath was removed and the reaction was kept at room temperature for further 2 h. After completion of the reaction, the solvent was evaporated under vacuum and the resulting residue was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with 1 M aqueous HCl (30 mL), saturated aqueous $NaHCO_3$ solution (50 mL), brine (50 mL), and dried over Na_2SO_4. The solvent was evaporated to provide crude product, which was purified *via* flash chromatography (EtOAc/Petroleum ether, 30%, v/v) to give pure **15a** as a clear oil (77 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, J = 8.0 Hz, 2H), 7.48 (d, J = 8.0 Hz, 3H), 7.30 – 7.27 (m, 2H), 7.21 (s, 1H), 7.00 (d, J = 7.6 Hz, 1H), 5.32 (d, J = 10.0 Hz, 1H), 4.81 (dd, J = 9.9, 2.4 Hz, 1H), 4.76 (d, J = 11.9 Hz, 1H), 4.47 (d, J = 2.3 Hz, 1H), 4.33 (d, J = 11.8 Hz, 1H), 3.77 (s, 2H), 3.77 (s, 3H), 3.60 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.83, 169.75, 168.38, 155.55, 138.63, 137.91, 137.70, 129.83 (2), 129.18, 125.92 (2, q, J = 3.8 Hz), 124.14 (q, J = 272.2 Hz), 124.25, 119.81, 119.58, 80.46, 77.23, 72.65, 56.10, 52.74, 52.60, 44.26, 28.25 (3). HRMS: calcd. for $C_{27}H_{31}F_3N_2O_8Li$ $[M+Li]^+$: 575.2187, found 575.2192.

5.4 (L-*threo*)-dimethyl 2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-[4-(trifluoromethyl)phenyl] propanamido]benzyloxy] succinate (15b)

Compound **15b** was prepared from **13** (80 mg, 0.21 mmol), TEA (64 mg, 0.63 mmol) and acid chloride solution (**14b**, 100 mg in 1 mL of DCM, 0.45 mmol) following a procedure similar to that used for **16a**. The title product was obtained as a clear oil (75 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, J = 8.0 Hz, 2H), 7.47 (d, J = 7.8 Hz, 1H), 7.36 – 7.33 (m, 3H), 7.27 (t, J = 8.0 Hz, 1H), 7.22 (s, 1H), 6.99 (d, J = 7.4 Hz, 1H), 5.33 (d, J = 9.9 Hz, 1H), 4.81 (dd, J = 9.9, 2.3 Hz, 1H), 4.76 (d, J = 11.9 Hz, 1H), 4.48 (d, J = 2.4 Hz, 1H), 4.34 (d, J = 11.8 Hz, 1H), 3.77 (s, 3H), 3.62 (s, 3H), 3.10 (t, J = 7.5 Hz, 2H), 2.67 (t, J = 7.5 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.85 (2), 169.77, 155.56, 144.91, 137.95, 137.78, 129.26, 128.89 (2), 125.64 (2, q, J = 3.8 Hz), 124.35 (q, J = 272.2 Hz), 124.11, 119.72, 119.49, 80.44, 77.24, 72.69, 56.13, 52.78, 52.63, 38.87, 31.17, 28.30 (3). HRMS: calcd. for $C_{28}H_{34}F_3N_2O_8$ $[M+H]^+$: 583.2268 found 583.2265.



5.5 (L-*threo*)-3-[3-[2-[4-(trifluoromethyl)phenyl]acetamido]benzyloxy]aspartate (16a)

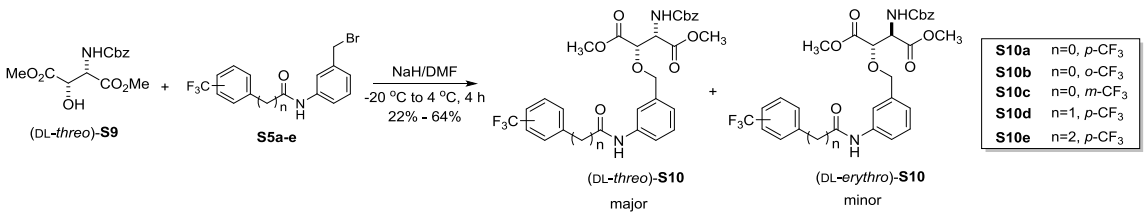
Compound **16a** was prepared from **15a** (70 mg, 0.12 mmol) following a procedure similar to that used for **2a**. The title compound was obtained as a white solid (26 mg, two-step yield 48%, *de* >98%, *ee* >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.59 (s, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.61 – 7.56 (m, 4H), 7.23 (t, J = 7.7 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 4.73 (d, J = 10.9 Hz, 1H), 4.43 (d, J = 10.9 Hz, 1H), 4.17 (d, J = 7.9 Hz, 1H), 3.80 (s, 2H), 3.79 (d, J = 8.3 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.95, 168.45 (2), 141.03, 138.91, 138.41, 130.07 (2), 128., 127.26 (q, J = 31.5 Hz), 125.08 (2, q, J = 3.8 Hz), 124.42 (q, J = 272.2 Hz), 123.24, 118.92, 118.41, 75.42, 72.40, 53.63, 42.85; ^{19}F NMR (376 MHz, DMSO-d_6) δ -60.84 (s). HRMS: calcd. for $C_{20}H_{20}F_3N_2O_6$ $[M+H]^+$: 441.1268, found 441.1268. $[\alpha]_D^{25}$ = -46.7 (*c* 0.45, DMSO). Comparison of the ^{1}H NMR data of **16a** with the ^{1}H NMR data of chemically prepared racemic DL-*erythro*-**S12d** showed that *de* of product **16a** is >98%. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (L-*threo*-**16a**) = 2.6 min, *ee* >99%.

5.6 **(L-*threo*)-3-[3-[3-[4-(trifluoromethyl)phenyl]propanamido]benzyloxy]aspartate (16b)**

Compound **16b** was prepared from **15b** (75 mg, 0.13 mmol) following a procedure similar to that used for **2a**. The title compound was obtained as a white solid (22 mg, two-step yield 38%, *de* >98%, *ee* >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.58 (d, J = 8.1 Hz, 1H), 7.54 (s, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.21 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 4.69 (d, J = 11.0 Hz, 1H), 4.40 (d, J = 11.0 Hz, 1H), 4.16 (d, J = 7.0 Hz, 1H), 3.72 (d, J = 7.0 Hz, 1H), 2.99 (t, J = 7.6 Hz, 2H), 2.69 (t, J = 7.6 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.31, 170.12, 168.46, 146.29, 138.97, 138.46, 129.16 (2), 128.29, 126.72 (q, J = 31.5 Hz), 125.12 (2, q, J = 3.8 Hz), 124.45 (q, J = 272.2 Hz), 122.91, 118.73, 118.29, 75.94, 72.13, 53.94, 37.27, 30.57; ^{19}F NMR (376 MHz, DMSO-d_6) δ -60.72 (s). HRMS: calcd. for $C_{21}H_{22}F_3N_2O_6$ $[M+H]^+$: 455.1424 found 455.1425. $[\alpha]_D^{25}$ = -34.3 (*c* 0.42, DMSO). Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (L-*threo*-**16b**) = 2.6 min, *ee* >99%.

6. Synthesis of DL-TFB-TBOA and its derivatives as chiral reference compounds

The chemical synthesis of compound (DL-*threo*)-**S9** has been described elsewhere.[4]



6.1 **(DL-*threo*)-dimethyl 2-[(benzyloxycarbonyl)amino]-3-[3-[4-(trifluoromethyl)benzoylamino] benzyloxy] succinate. (DL-*threo*-S10a)**

To a stirred solution of compound (DL-*threo*)-**S9** (156 mg, 0.5 mmol) in dry DMF (3 mL) was added **5a** (358 mg, 1.0 mmol) at -20 °C. After 10 min, NaH (60% in mineral oil, 20 mg, 0.5 mmol) was added to the reaction mixture and the mixture was stirred at -20 °C for further 2 h. Subsequently, the reaction mixture was warmed up to 4 °C and stirred for another 2 h. After completion of the reaction, the reaction mixture was quenched with cold water and extracted with EtOAc (3 x 20 mL), washed with brine (3 x 50 mL), and dried over Na_2SO_4. The solvent was evaporated under vacuum to provide crude product, which was purified *via* flash chromatography (EtOAc/Petroleum ether, 25%, v/v) to give (DL-*threo*)-**S10a** as a clear oil (190 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.97 (d, J = 8.1 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.1 Hz, 1H), 7.52 (s, 1H), 7.35 – 7.29 (m, 6H), 7.05 (d, J = 7.6 Hz, 1H), 5.62 (d, J = 9.8 Hz, 1H), 5.08 (d, J = 1.9 Hz, 2H), 4.89 (dd, J = 9.9, 2.4 Hz, 1H), 4.80 (d, J = 11.9 Hz, 1H), 4.54 (d, J = 2.4 Hz, 1H), 4.39 (d, J = 11.8 Hz, 1H), 3.74 (s, 3H), 3.66 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.62, 164.60, 156.26, 138.18, 137.90, 137.87, 136.14, 133.66 (q, J = 34.0 Hz), 129.38, 128.61 (2), 128.29, 128.05 (2), 127.72 (2), 125.92 (2, q, J = 3.8 Hz), 124.55, 123.70 (q, J = 273.4 Hz), 120.28, 120.00, 77.12, 72.75, 71.04, 67.35, 56.55, 52.95, 52.68. HRMS: calcd. for $C_{29}H_{28}F_3N_2O_8$ $[M+H]^+$: 589.1792, found 589.1794. In addition, a mixture of (DL-*threo*)-**S10a** and (DL-*erythro*)-**S10a** (50 mg, 16%, *threo/erythro* = 1:2) was obtained.

6.2 **(DL-*threo*)-dimethyl 2-[(benzyloxycarbonyl)amino]-3-[3-[2-(trifluoromethyl)benzoylamino] benzyloxy]succinate. (DL-*threo*-S10b)**

Compound (DL-*threo*)-**S10b** was prepared from (DL-*threo*)-**S9** (156 mg, 0.5 mmol), **5b** (359 mg, 1.0 mmol) and NaH (60% in mineral oil, 20 mg, 0.5 mmol) following a procedure similar to that used for (DL-*threo*)-**S10a**. The title product (DL-*threo*)-**S10b** was obtained as a clear oil (130 mg, 44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.67 (d, J = 7.5 Hz, 1H), 7.55 – 7.50 (m, 5H), 7.33 – 7.27 (m, 6H), 7.03 (d, J = 7.5 Hz, 1H), 5.62 (d, J = 9.8 Hz, 1H), 5.03 (s, 2H), 4.84 (dd, J = 9.9, 2.4 Hz, 1H), 4.76 (d, J = 11.9 Hz, 1H), 4.51 (d, J = 2.4 Hz, 1H), 4.35 (d, J = 11.9 Hz, 1H), 3.70 (s, 3H), 3.62 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.51, 169.42, 165.91, 156.14,

137.91, 137.63, 136.07, 135.66, 132.12, 130.14, 129.17, 128.48 (2), 128.39, 128.13, 127.90 (2), 127.21 (q, *J* = 32.8 Hz), 126.49 (q, *J* = 5.0 Hz), 124.50, 123.60 (q, *J* = 273.4 Hz), 120.00, 119.80, 76.93, 72.63, 67.14, 56.41, 52.80, 52.50. HRMS: calcd. for $C_{29}H_{28}F_3N_2O_8$ $[M+H]^+$: 589.1792, found 589.1797.

6.3 **(DL-*threo*)-dimethyl 2-[(benzyloxycarbonyl)amino]-3-[3-[3-(trifluoromethyl)benzoylamino]benzyloxy] succinate. (DL-*threo*-S10c)**

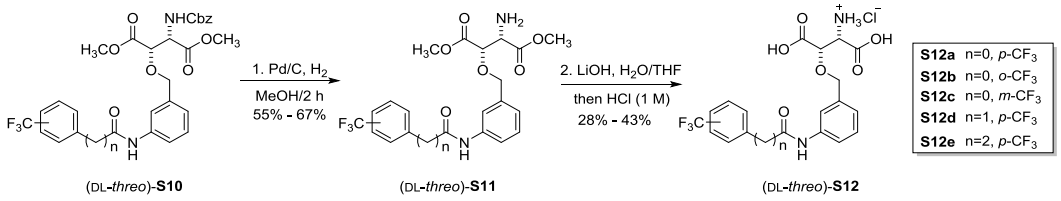
Compound (DL-*threo*)-**S10c** was prepared from (DL-*threo*)-**S9** (161 mg, 0.52 mmol), **5c** (370 mg, 1.04 mmol) and NaH (60% in mineral oil, 24 mg, 0.52 mmol) following a procedure similar to that used for (DL-*threo*)-**S10a**. The title product (DL-*threo*)-**S10c** was obtained as a clear oil (144 mg, 47%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.97 (s, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.52 (s, 1H), 7.37 – 7.31 (m, 6H), 7.06 (d, *J* = 7.6 Hz, 1H), 5.60 (d, *J* = 9.9 Hz, 1H), 5.09 (d, *J* = 2.4 Hz, 2H), 4.89 (dd, *J* = 9.8, 2.3 Hz, 1H), 4.82 (d, *J* = 11.9 Hz, 1H), 4.54 (d, *J* = 2.3 Hz, 1H), 4.41 (d, *J* = 11.9 Hz, 1H), 3.75 (s, 3H), 3.67 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.52, 169.51, 164.26, 156.13, 137.74, 136.04, 135.63, 131.37 (q, *J* = 32.8 Hz), 130.36, 129.49, 129.30, 128.53, 128.50 (2), 128.17, 128.00, 127.96, 124.48, 124.12 (q, *J* = 3.8 Hz), 120.18, 119.90, 76.96, 72.62, 67.24, 56.43, 52.85, 52.58. HRMS: calcd. for $C_{29}H_{28}F_3N_2O_8$ $[M+H]^+$: 589.1792, found 589.1794.

6.4 **(DL-*threo*)-dimethyl 2-[(benzyloxycarbonyl)amino]-3-[3-[2-[4-(trifluoromethyl)phenyl]acetamido] benzyloxy] succinate. (DL-*threo*-S10d)**

Compound (DL-*threo*)-**S10d** was prepared from (DL-*threo*)-**S9** (100 mg, 0.32 mmol), **5d** (239 mg, 0.64 mmol) and NaH (60% in mineral oil, 13 mg, 0.32 mmol) following a procedure similar to that used for (DL-*threo*)-**S10a**. The title product (DL-*threo*)-**S10d** was obtained as a clear oil (60 mg, 43%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (d, *J* = 8.0 Hz, 2H), 7.47 – 7.44 (m, 4H), 7.34 – 7.30 (m, 6H), 7.24 (t, *J* = 7.8 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 5.59 (d, *J* = 9.9 Hz, 1H), 5.08 (d, *J* = 2.5 Hz, 2H), 4.87 (dd, *J* = 9.9, 2.4 Hz, 1H), 4.74 (d, *J* = 11.8 Hz, 1H), 4.50 (d, *J* = 2.4 Hz, 1H), 4.33 (d, *J* = 11.8 Hz, 1H), 3.73 (s, 5H), 3.60 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.61, 169.55, 168.25, 156.25, 138.54, 137.84, 137.67, 136.14, 129.86 (2), 129.25, 128.61(2), 128.29, 128.06 (2), 126.00 (2, q, *J* = 3.8 Hz), 125.98, 124.30, 119.81, 119.55, 77.04, 72.73, 67.34, 56.52, 52.89, 52.67, 44.35. HRMS: calcd. for $C_{30}H_{30}F_3N_2O_8$ $[M+H]^+$: 603.1949, found 603.1950. In addition, a mixture of (DL-*threo*)-**S10d** and (DL-*erythro*)-**S10d** (20 mg, 14%, *threo/erythro* = 1:2) was obtained.

6.5 **(DL-*threo*)-dimethyl 2-[(benzyloxycarbonyl)amino]-3-[3-[3-[4-(trifluoromethyl)phenyl]propanamido] benzyloxy] succinate. (DL-*threo*-S10e)**

Compound (DL-*threo*)-**S10e** was prepared from (DL-*threo*)-**S9** (125 mg, 0.40 mmol), **5e** (313 mg, 0.81 mmol) and NaH (60% in mineral oil, 16 mg, 0.40 mmol) following a procedure similar to that used for (DL-*threo*)-**S10a**. The title product (DL-*threo*)-**S10e** was obtained as a clear oil (54 mg, 22%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.35 – 7.29 (m, 9H), 7.23 (d, J = 7.9 Hz, 1H), 6.97 (d, J = 7.6 Hz, 1H), 5.62 (d, J = 9.8 Hz, 1H), 5.07 (s, 2H), 4.87 (dd, J = 9.8, 2.4 Hz, 1H), 4.73 (d, J = 11.8 Hz, 1H), 4.51 (d, J = 2.3 Hz, 1H), 4.33 (d, J = 11.7 Hz, 1H), 3.73 (s, 3H), 3.60 (s, 3H), 3.06 (t, J = 7.6 Hz, 2H), 2.64 (t, J = 7.6 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.05, 169.61, 169.53, 156.25, 144.94, 138.07, 137.58, 136.10, 129.17, 128.82 (2), 128.57 (2), 128.48 (q, J = 34.0 Hz), 128.25 (2), 128.01 (2), 125.54 (q, J = 3.8 Hz), 124.32 (q, J = 272.2 Hz), 123.98, , 119.76, 119.53, 77.00, 72.78, 67.30, 56.50, 52.85, 52.61, 38.63, 31.09. HRMS: calcd. for $C_{31}H_{32}F_3N_2O_8$ $[M+H]^+$: 617.2105, found 617.2108.



(DL-*threo*)-**S10** (DL-*threo*)-**S11** (DL-*threo*)-**S12**

6.6 **(DL-*threo*)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (DL-*threo*-S12a, DL-TFB-TBOA)**

To a stirred solution of (DL-*threo*)-**S10a** (190 mg, 0.32 mmol) in dry MeOH (10 mL) was added Pd/C 10% (10.0 mg) under nitrogen atmosphere. The reaction mixture was stirred under H_2 atmosphere (balloon) for 2 h at room temperature. After completion of the reaction, the reaction mixture was filtered through Celite and washed with MeOH (5 mL). The filtrate was concentrated under vacuum to provide product (DL-*threo*)-**S11a** as a clear oil (88 mg, 61%). No purification was needed, the product (DL-*threo*)-**S11a** was directly used for the next step.

To a stirred solution of (DL-*threo*)-**S11a** (88 mg, 0.19 mmol) in THF/H_2O (1:1, each 1.5 mL) was added LiOH (24 mg, 1.0 mmol) and the reaction mixture was stirred at room temperature for 2 h. After completion of the starting material, THF was removed in vacuo and the resulting aqueous solution was reverse extracted with EtOAc (1 mL). The aqueous layer was acidified with 1 M HCl (pH=1) until white precipitates appeared. The white precipitates were filtered through a Büchner funnel and washed with 5 mL of cold water. The white solid was dried under vacuum overnight to provide the product (DL-*threo*)-**S12a** (DL-TFB-TBOA) (35 mg, two steps yield 43%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.16 (d, J = 8.1 Hz, 2H), 7.92 (d, J = 8.2 Hz, 2H),

7.75 (s, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.28 (d, J = 7.6 Hz, 1H), 4.83 (d, J = 10.6 Hz, 1H), 4.48 (d, J = 10.6 Hz, 1H), 4.12 (d, J = 9.5 Hz, 1H), 3.82 (d, J = 9.5 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.54, 168.44, 164.29, 138.69, 138.46, 138.31, 131.34 (q, J = 32.8 Hz), 128.59 (2), 128.39, 125.39 (2, q, J = 5.0 Hz), 124.28, 123.94 (q, J = 273.4 Hz), 120.50, 119.87, 74.72, 72.71, 53.11; ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.33 (s). HRMS: calcd. for $C_{19}H_{18}F_3N_2O_6$ $[M+H]^+$: 427,1112, found 427,1111. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (D-*threo*-**S12a)** = 2.4 min, t_R (L-*threo*-**S12a**) = 2.8 min.

6.7 **(DL-*erythro*)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (DL-*erythro*-S12a)**

Compound (DL-*erythro*)-**S12a** was prepared from the mixture of (DL-*threo*)-**S10a** and (DL-*erythro*)-**S10a** (50 mg, 16%, *threo/erythro* = 1:2), following a procedure similar to that used for (DL-*threo*)-**S12a**. The title product (DL-*erythro*)-**S12a** was obtained as a white solid (7 mg, two steps yield 44%, *erythro/threo* = 93:7). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.15 (d, J = 8.1 Hz, 2H), 7.92 (d, J = 8.2 Hz, 2H), 7.73 (d, J = 9.2 Hz, 1H), 7.68 (s, 1H), 7.36 (t, J = 7.9 Hz, 1H), 7.17 (d, J = 7.7 Hz, 1H), 4.59 (d, J = 11.4 Hz, 1H), 4.45 (d, J = 11.4 Hz, 1H), 4.12 (d, J = 1.5 Hz, 1H), 4.07 (d, J = 1.3 Hz, 1H); ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.33 (s). HRMS: $[M+H]^+$ calcd for: $C_{19}H_{18}F_3N_2O_6$ 427,1112, found 427,1108.

6.8 **(DL-*threo*)-3-[3-[2-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (DL-*threo*-S12b)**

Compound (DL-*threo*)-**S12b** was prepared from (DL-*threo*)-**S10b** (130 mg, 0.22 mmol) following a procedure similar to that used for (DL-*threo*)-**S12a**. The pure title product (DL-*threo*)-**S12b** was obtained as a white solid (24 mg, two steps yield 38%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.59 (s, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.72 – 7.67 (m, 2H), 7.63 (d, J = 10.4 Hz, 2H), 7.31 (t, J = 7.7 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 4.76 (d, J = 10.8 Hz, 1H), 4.45 (d, J = 10.8 Hz, 1H), 4.13 (d, J = 8.2 Hz, 1H), 3.73 (d, J = 8.2 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.89, 168.39, 165.59, 138.60, 138.54, 136.22, 132.60, 130.01, 128.55, 128.49, 126.32 (q, J = 5.0 Hz), 125.80 (q, J = 31.5 Hz), 124.01, 123.81 (q, J = 274.7 Hz), 119.41, 118.93, 75.35, 72.45, 53.51; ^{19}F NMR (376 MHz, DMSO-d_6) δ -57.91(s). HRMS: calcd. for $C_{19}H_{18}F_3N_2O_6$ $[M+H]^+$: 427.1112, found 427.1112. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (D-*threo*-**S12b)** = 2.3 min, t_R (L-*threo*-**S12b**) = 2.6 min.

6.9 **(DL-*threo*)-3-[3-[3-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (DL-*threo*-S12c)**

Compound (DL-*threo*)-**S12c** was prepared from (DL-*threo*)-**S10c** (144 mg, 0.24 mmol) following a procedure similar to that used for (DL-*threo*)-**S12a**. The pure title product (DL-*threo*)-**S12c** was obtained as a white solid (25 mg, two steps yield 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 8.31 (d, J = 10.7 Hz, 2H), 7.96 (d, J = 7.7 Hz, 1H), 7.80 – 7.73 (m, 3H), 7.34 (t, J = 7.7 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 4.79 (d, J = 10.8 Hz, 1H), 4.47 (d, J = 10.8 Hz, 1H), 4.13 (d, J = 8.3 Hz, 1H), 3.74 (d, J = 8.3 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.05, 168.41, 163.94, 138.57, 138.41, 135.66, 131.96, 129.69, 129.15 (q, J = 32.8 Hz), 128.31, 128.12 (q, J = 3.8 Hz), 124.40 (q, J = 32.8 Hz), 124.02 (q, J = 273.4 Hz), 123.96, 120.45, 119.93, 75.54, 72.30, 53.72; ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.08 (s). HRMS: calcd. for $C_{19}H_{18}F_3N_2O_6$ $[M+H]^+$: 427.1112, found 427.1110. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (D-*threo*-**S12c**) = 2.3 min, t_R (L-*threo*- **S12c**) = 2.7 min.

6.10 **(DL-*threo*)-3-[3-[2-[4-(trifluoromethyl)phenyl]acetamido]benzyloxy]aspartate (DL-*threo*-S12d)**

Compound (DL-*threo*)-**S12d** was prepared from (DL-*threo*)-**S10d** (60 mg, 0.10 mmol) following a procedure similar to that used for (DL-*threo*)-**S12a**. The title product (DL-*threo*)-**S12d** was obtained as a white solid (7 mg, two steps yield 28%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.69 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 7.6 Hz, 4H), 7.26 (t, J = 8.1 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1H), 4.76 (d, J = 10.6 Hz, 1H), 4.43 (d, J = 10.6 Hz, 1H), 4.09 (d, J = 9.4 Hz, 1H), 3.80 (d, J = 9.5 Hz, 1H), 3.77 (s, 2H); ^{19}F NMR (376 MHz, DMSO-d_6) δ -60.84 (s). HRMS: calcd. for $C_{20}H_{20}F_3NO_6$ $[M+H]^+$: 441.1268, found 441.1268. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (D-*threo*-**S12d)** = 2.3 min, t_R (L-*threo*-**S12d**) = 2.6 min.

6.11 **(DL-*erythro*)-3-[3-[2-[4-(trifluoromethyl)phenyl]acetamido]benzyloxy]aspartate (DL-*erythro*- S12d)**

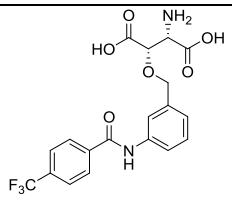
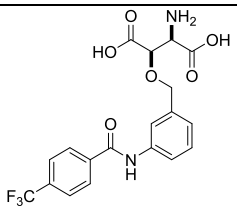
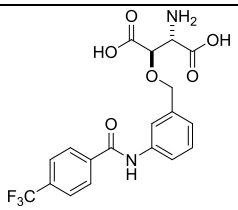
Compound (DL-*erythro*)-**S12d** was prepared from the mixture of (DL-*threo*)-**S10d** and (DL-*erythro*)-**S10d** (20 mg, 14%, *threo/erythro* = 1:2) following a procedure similar to that used for (DL-*threo*)-**S12a**. The title product (DL-*erythro*)-**S12d** was obtained as a white solid (2 mg, two steps yield 35%, *erythro/threo* = 88:12). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.28 (s, 1H), 7.69 (d, J = 8.1 Hz, 2H), 7.56 (t, J = 8.4 Hz, 3H), 7.46 (s, 1H), 7.27 (t, J = 7.9

Hz, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 4.54 (d, *J* = 11.4 Hz, 1H), 4.40 (d, *J* = 11.4 Hz, 1H), 4.10 (s, 1H), 4.06 (d, *J* = 1.3 Hz, 1H), 3.77 (s, 2H); ^{19}F NMR (376 MHz, DMSO-d_6) δ -60.84(s). HRMS: calcd. for $C_{20}H_{20}F_3NO_6$ $[M+H]^+$: 441.1268, found 441.1268.

6.12 **(DL-*threo*)-3-[3-[3-[4-(trifluoromethyl)phenyl]propanamido]benzyloxy]aspartate (DL-*threo*-S12e)**

Compound (DL-*threo*)-**S12e** was prepared from (DL-*threo*)-**S10e** (54 mg, 0.087 mmol) following a procedure similar to that used for (DL-*threo*)-**S12a**. The pure title product (DL-*threo*)-**S12e** was obtained as white solid (8 mg, two steps yield 31%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 7.65 (d, *J* = 8.1 Hz, 2H), 7.54 – 7.47 (m, 4H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 7.7 Hz, 1H), 4.73 (d, *J* = 10.7 Hz, 1H), 4.41 (d, *J* = 10.7 Hz, 1H), 4.08 (d, *J* = 8.8 Hz, 1H), 3.74 (d, *J* = 8.8 Hz, 1H), 3.00 (t, *J* = 7.6 Hz, 2H), 2.67 (t, *J* = 7.6 Hz, 2H); ^{19}F NMR (376 MHz, DMSO-d_6) δ -60.72; HRMS: calcd. for $C_{21}H_{22}F_3N_2O_6$ $[M+H]^+$: 455,1424, found 455,1423. Chiral HPLC analysis: CROWNPAK CR-I (+) 150 x 3 mm. Phase A: ACN+0.5%TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C, t_R (D-*threo*-**S12e)** = 2.2 min, t_R (L-*threo*-**S12e**) = 2.6 min.

Table S1. Comparison of the ^{1}H-NMR data of **TFB-TBOA**[a] and **TFB-EBOA**[b].

Chemoenzymatic product **2a**	Authentic reference[c]	(DL-*threo*)-**S12a**	(DL-*erythro*)-**S12a**
L-TFB-TBOA	L-TFB-TBOA	DL-TFB-TBOA	DL-TFB-EBOA
			
10.49 (s, 1H)	10.48 (s, 1H)	10.48 (s, 1H)	10.50 (s, 1H)
8.16 (d, *J* = 8.1 Hz, 2H)	8.16 (d, *J* = 8.1 Hz, 2H)	8.16 (d, *J* = 8.1 Hz, 2H)	8.15 (d, *J* = 8.1 Hz, 2H)
7.92 (d, *J* = 8.2 Hz, 2H)	7.92 (d, *J* = 8.2 Hz, 2H)	7.92 (d, *J* = 8.2 Hz, 2H)	7.92 (d, *J* = 8.2 Hz, 2H)
7.76 (s, 1H)	7.75 (s, 1H)	7.75 (s, 1H)	7.73 (d, *J* = 9.2 Hz, 1H)
7.71 (d, *J* = 8.1 Hz, 1H)	7.71 (d, *J* = 7.9 Hz, 1H)	7.71 (d, *J* = 8.5 Hz, 1H)	7.68 (s, 1H)
7.35 (t, *J* = 7.8 Hz, 1H)	7.35 (t, *J* = 7.8 Hz, 1H)	7.35 (t, *J* = 7.8 Hz, 1H)	7.36 (t, *J* = 7.9 Hz, 1H)
7.28 (t, *J* = 7.7 Hz, 1H)	7.28 (d, *J* = 7.6 Hz, 1H)	7.28 (d, *J* = 7.6 Hz, 1H)	7.17 (d, *J* = 7.7 Hz, 1H)
4.83 (d, *J* = 10.6 Hz, 1H)	4.83 (d, *J* = 10.6 Hz, 1H)	4.83 (d, *J* = 10.6 Hz, 1H)	4.59 (d, *J* = 11.4 Hz, 1H)
4.48 (d, *J* = 10.6 Hz, 1H)	4.48 (d, *J* = 10.6 Hz, 1H)	4.48 (d, *J* = 10.6 Hz, 1H)	4.45 (d, *J* = 11.4 Hz, 1H)
4.13 (d, *J* = 9.5 Hz, 1H)	4.12 (d, *J* = 9.6 Hz, 1H)	4.12 (d, *J* = 9.5 Hz, 1H)	4.12 (d, *J* = 1.5 Hz, 1H)
3.83 (d, *J* = 9.5 Hz, 1H)	3.82 (d, *J* = 9.6 Hz, 1H)	3.82 (d, *J* = 9.5 Hz, 1H)	4.07 (d, *J* = 1.3 Hz, 1H)

[a] **TFB-TBOA,** (*threo*)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate.

[b] **TFB-EBOA,** (*erythro*)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate.

[c] Authentic sample of L-TFB-TBOA was purchased from Tocris Bioscience.

Figure S1. Comparison of the ^{1}H-NMR spectra of **TFB-TBOA** and **TFB-EBOA**.

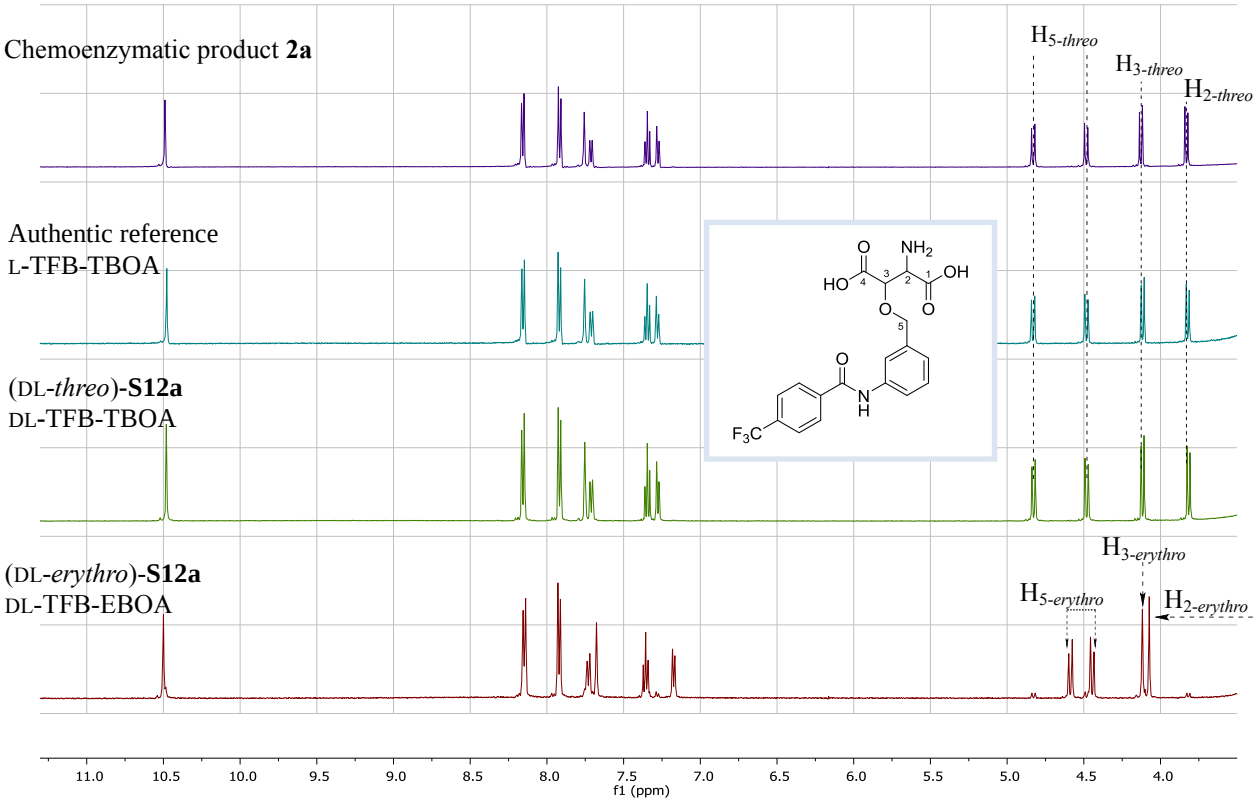
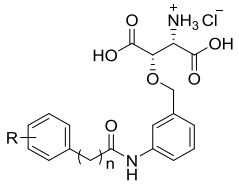


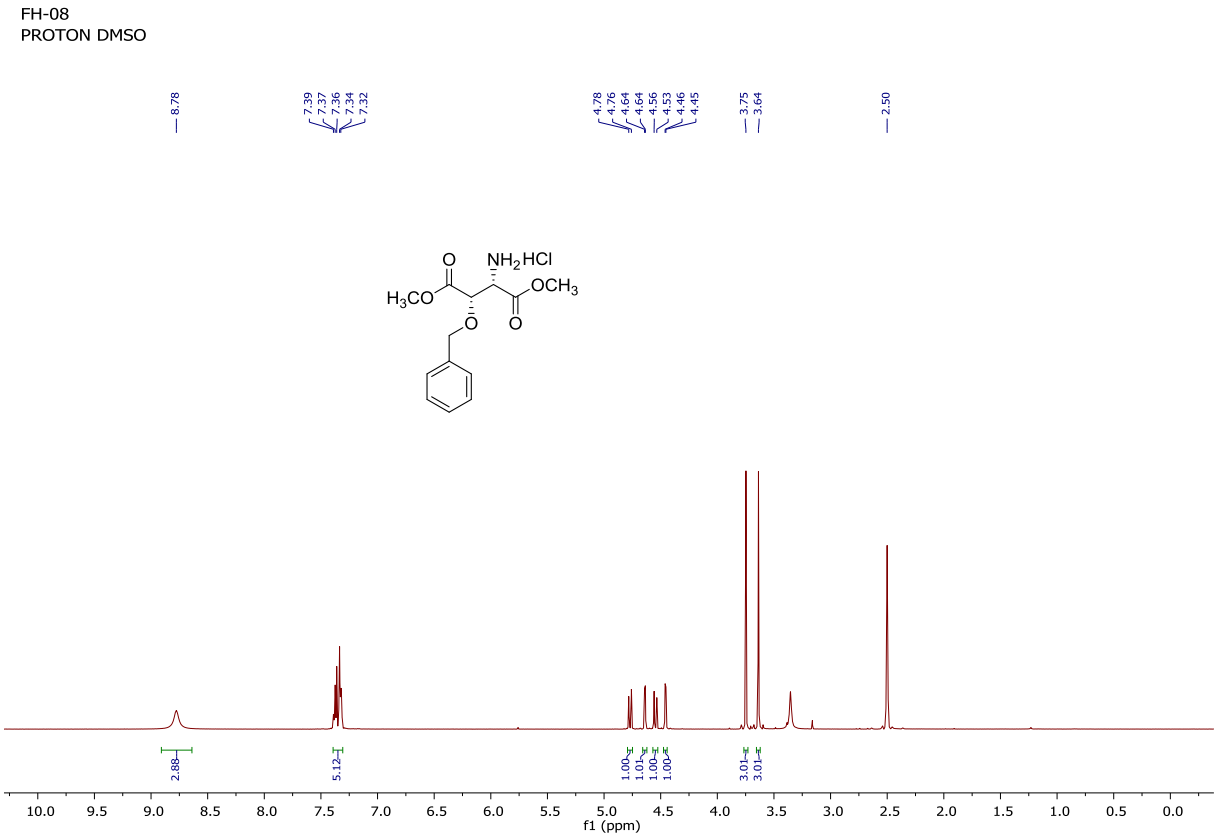
Table S2. Optical rotation of L-TFB-TBOA and its derivatives.

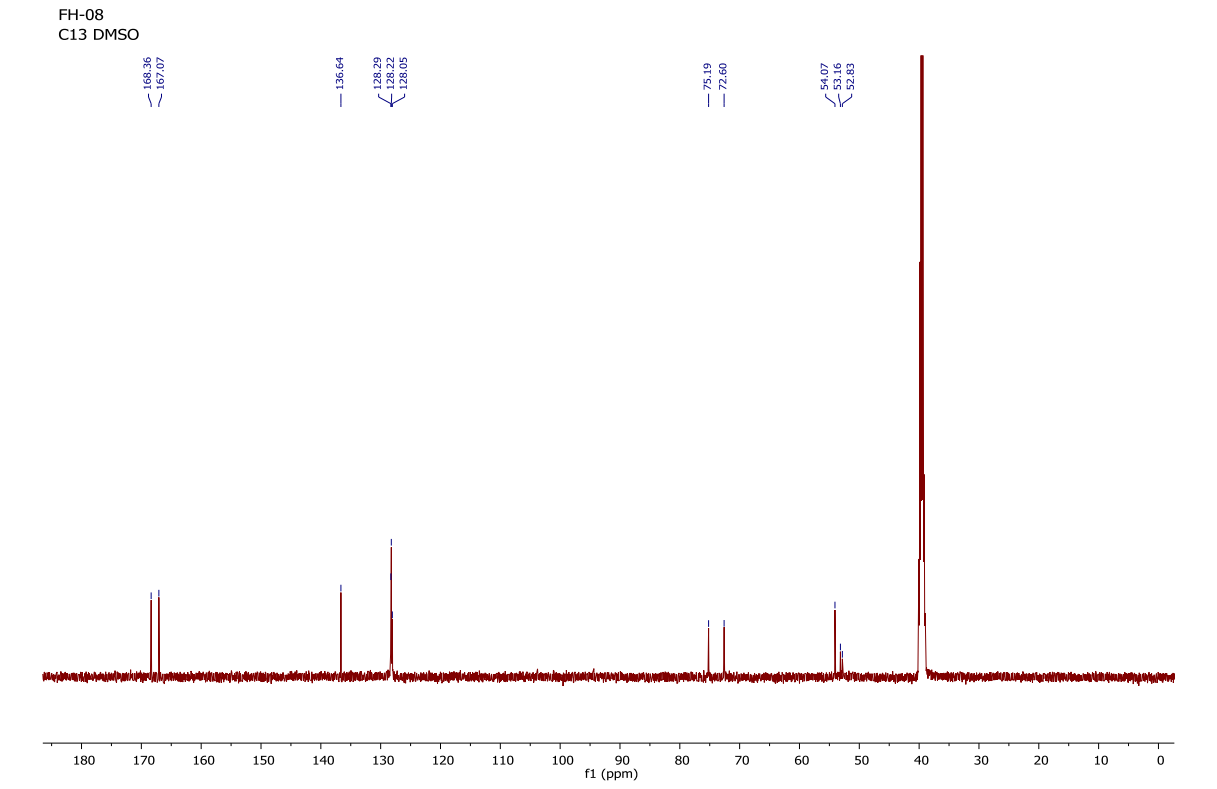


Entry	No.	R	n	Optical rotation $[\alpha]_D^{25}$
1	**Authentic sample** L-TFB-TBOA	*p*-CF_3	0	− 52.6 (*c* 0.60, DMSO)
2	**2a**	*p*-CF_3	0	− 55.0 (*c* 0.60, DMSO)
3	**2b**	*o*-CF_3	0	− 53.1 (*c* 0.43, DMSO)
4	**2c**	*m*-CF_3	0	− 50.7 (*c* 0.43, DMSO)
5	**16a**	*p*-CF_3	1	− 46.7 (*c* 0.45, DMSO)
6	**16b**	*p*-CF_3	2	− 34.3 (*c* 0.42, DMSO)

III) NMR spectra

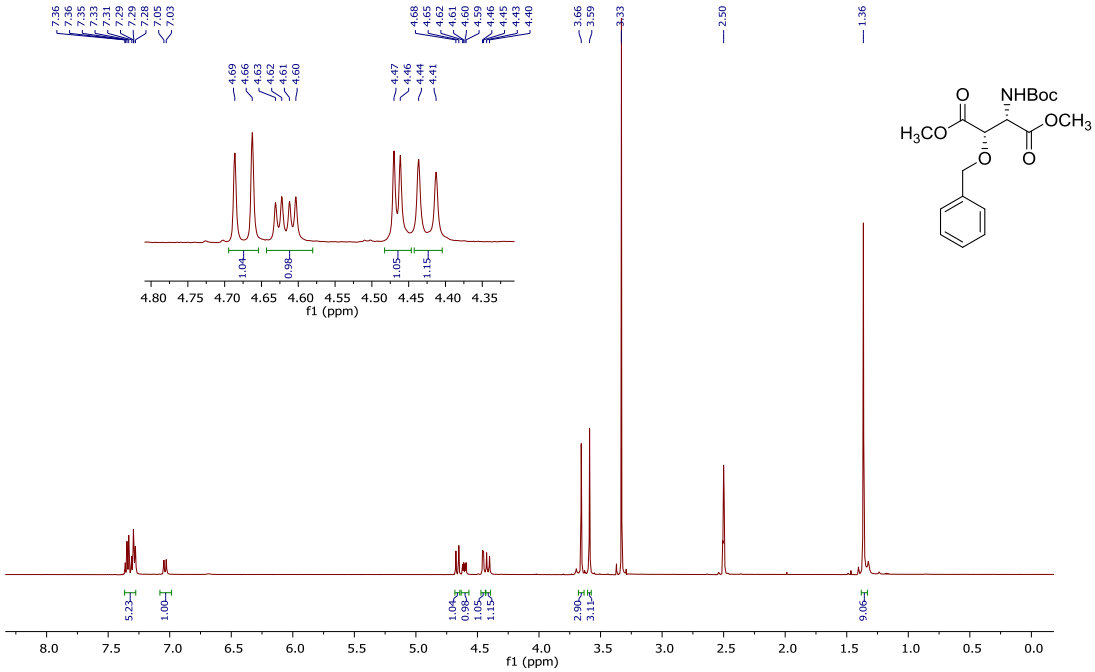
^{1}H NMR and ^{13}C NMR of compound **7**



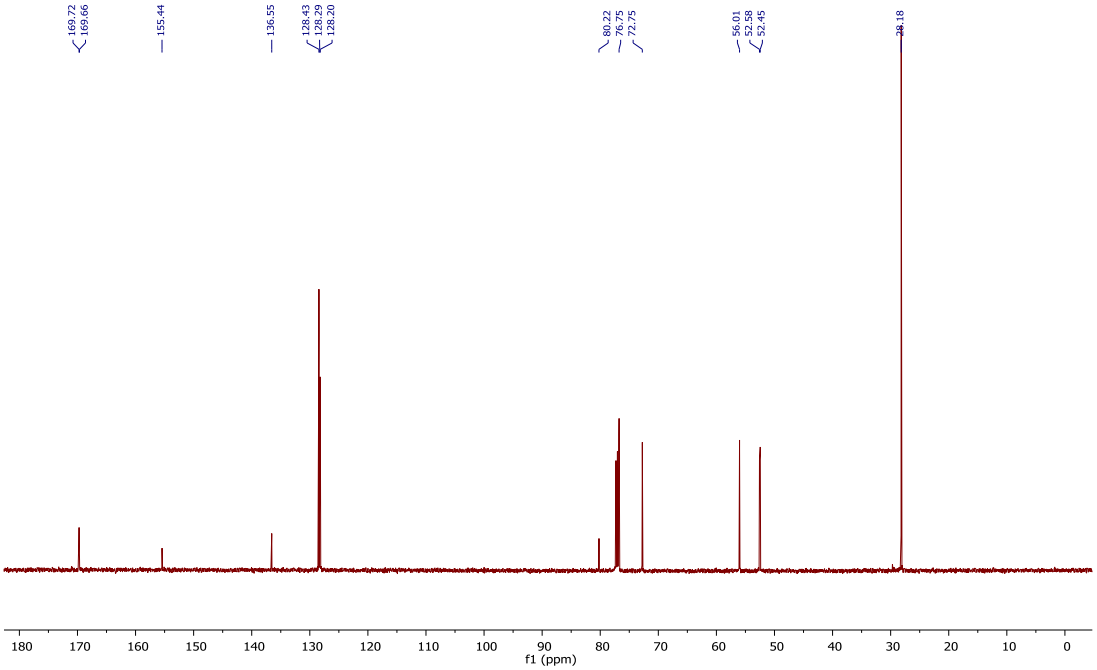


^{1}H NMR and ^{13}C NMR of compound **8**

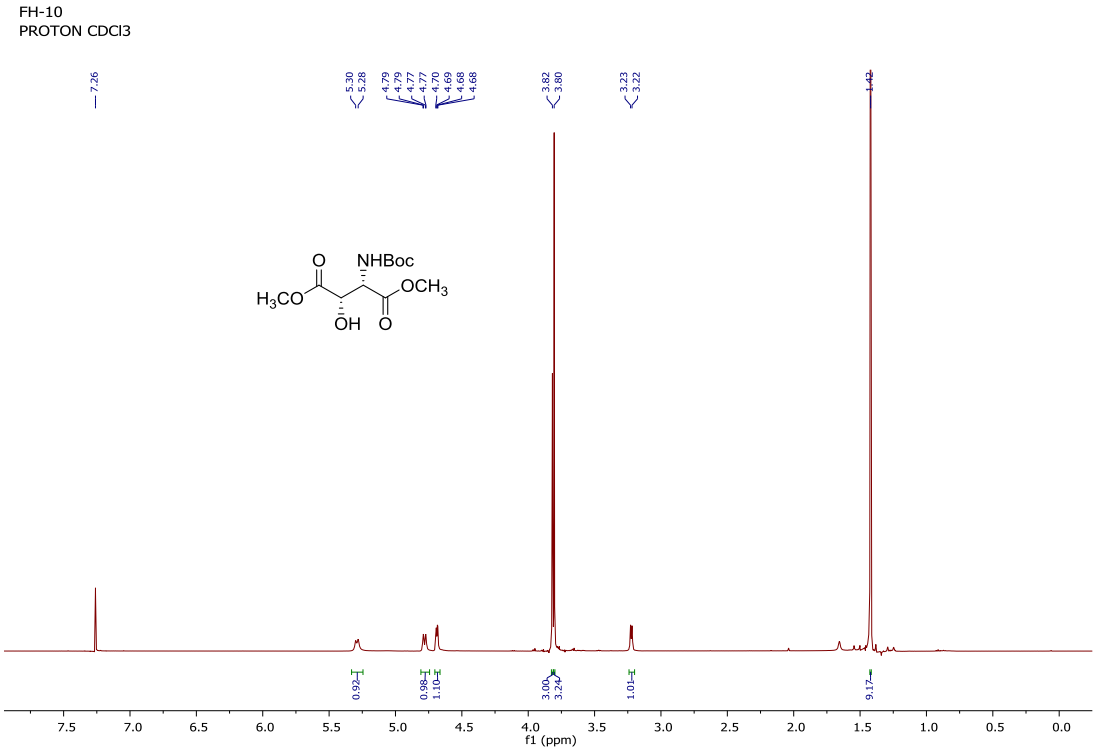
FH-09-peak-1
PROTON DMSO

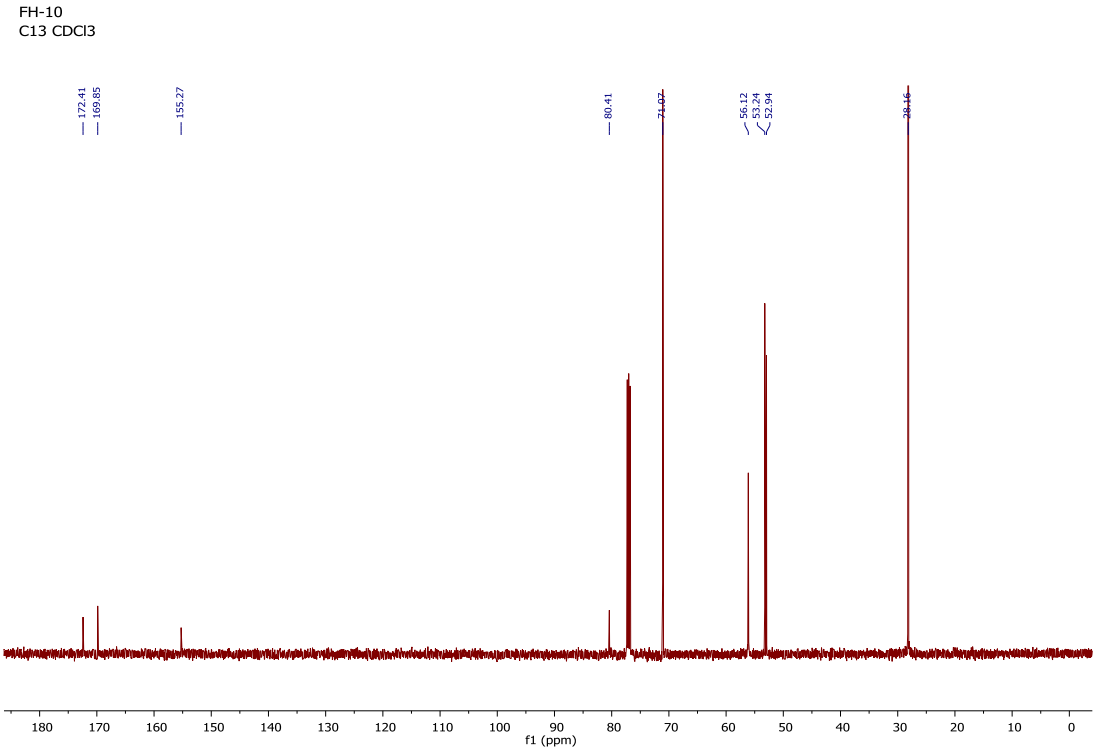


FH-09
C13 CDCl3

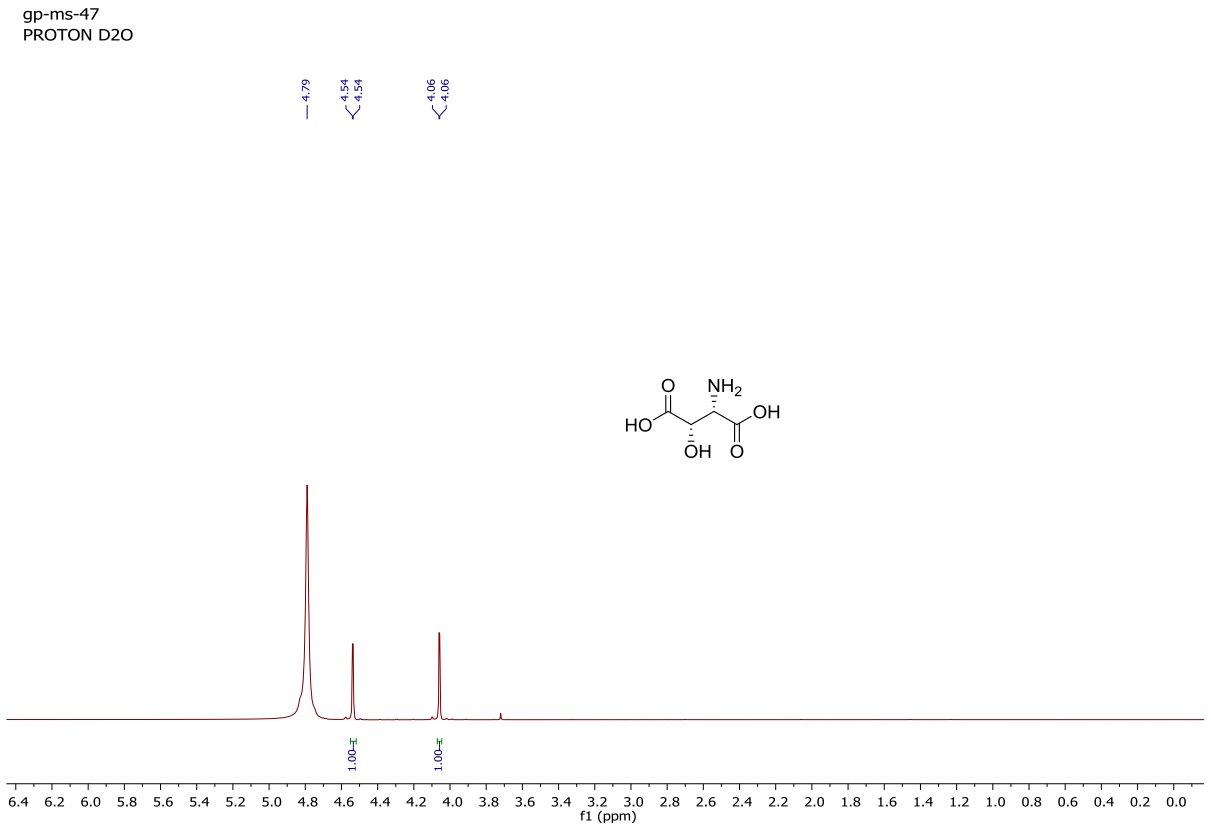


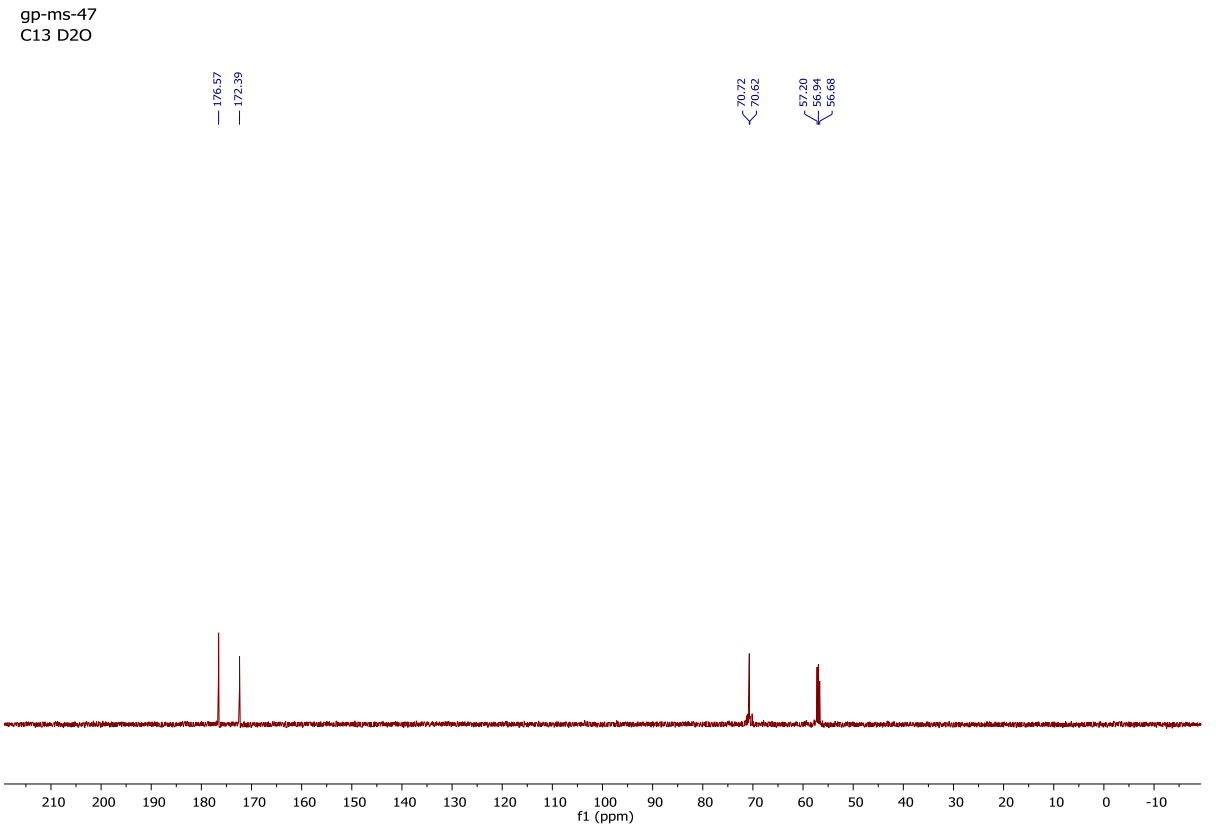
^{1}H NMR and ^{13}C NMR of compound **4**



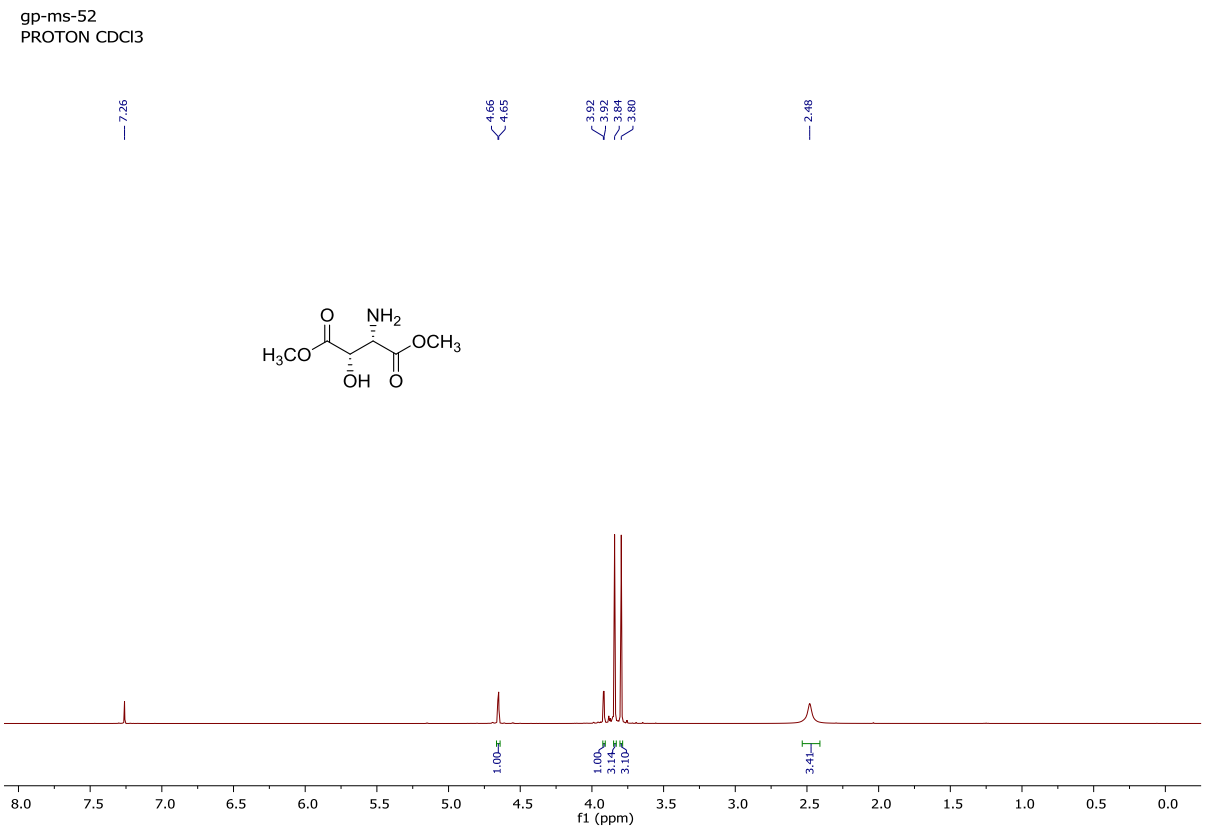


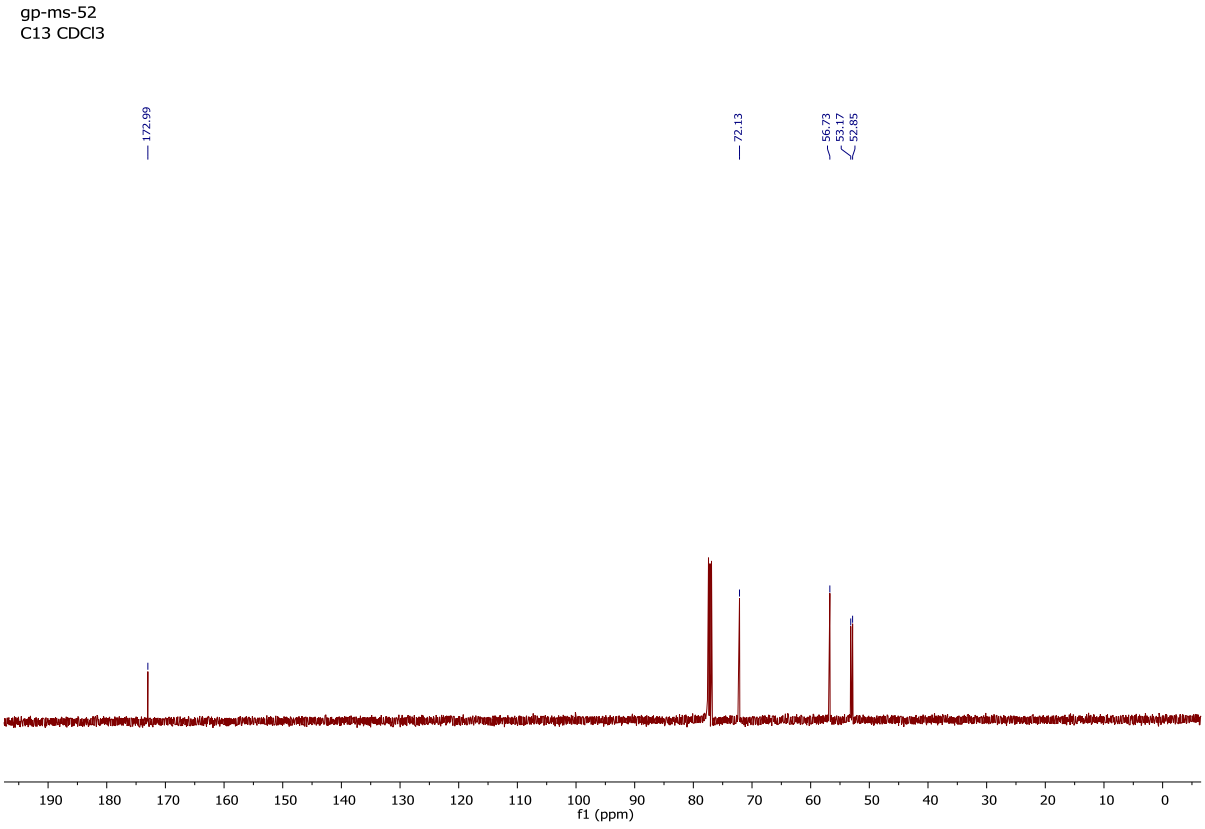
^{1}H NMR and ^{13}C NMR of compound **9**



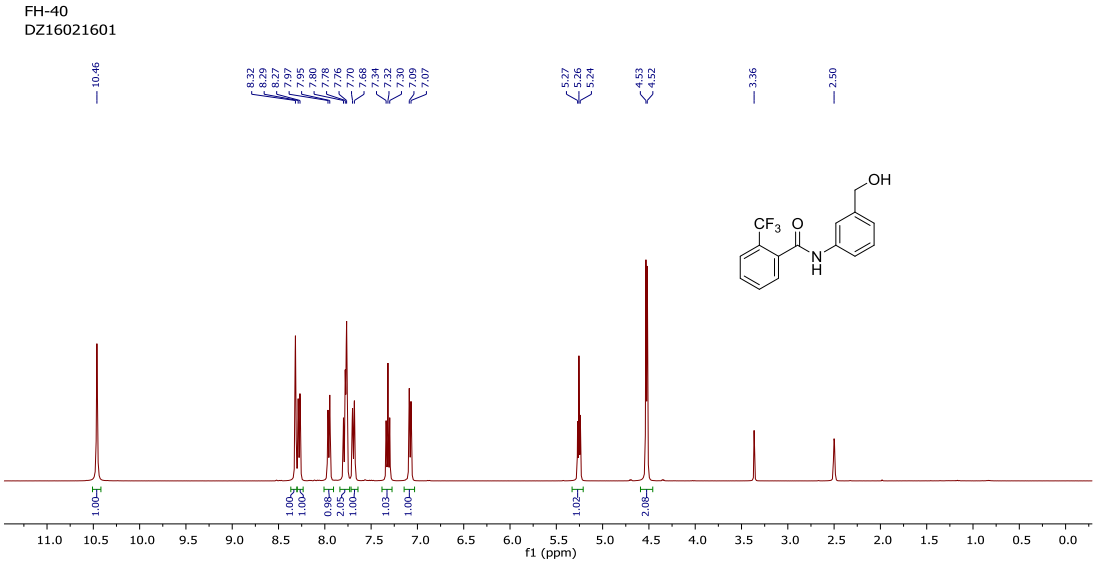


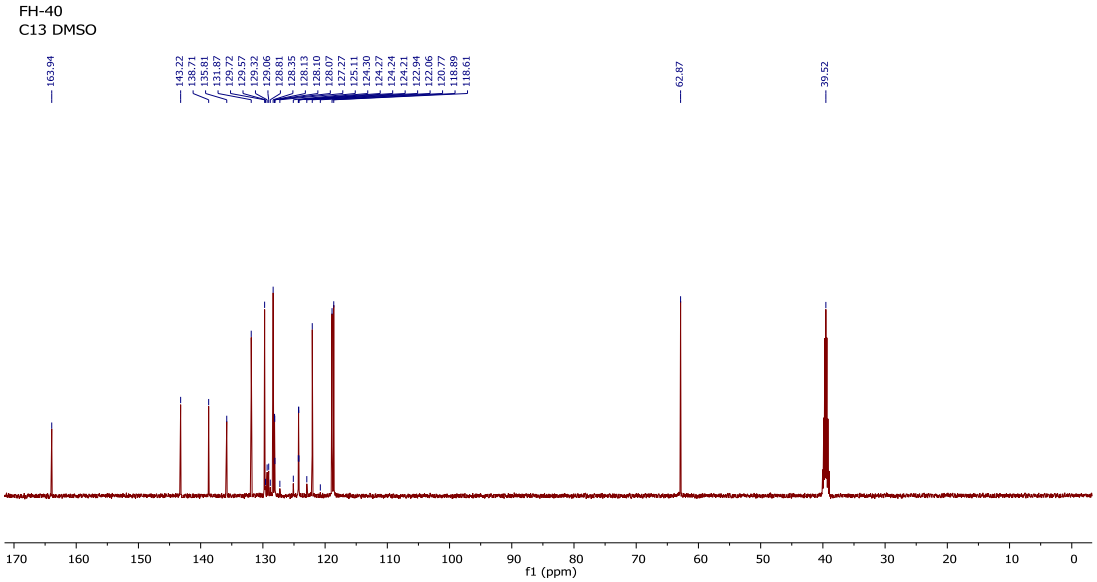
^{1}H NMR and ^{13}C NMR of compound **10**

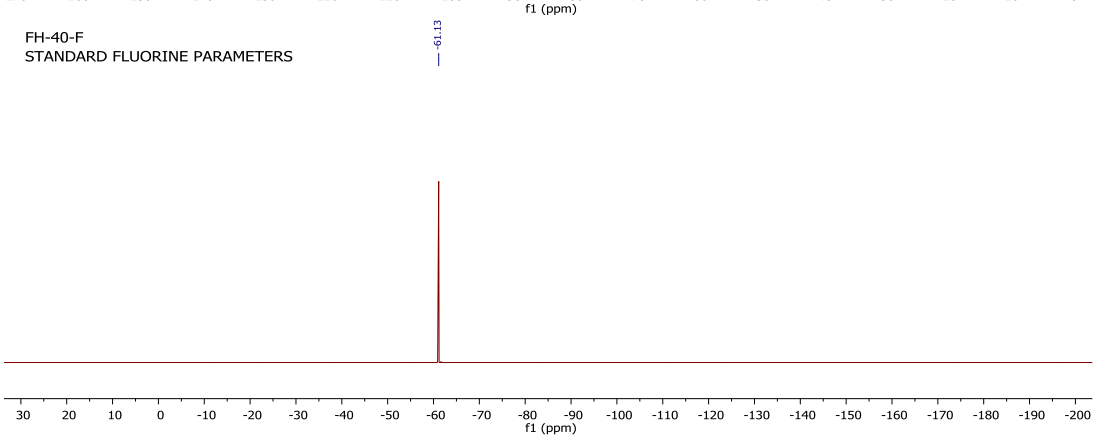




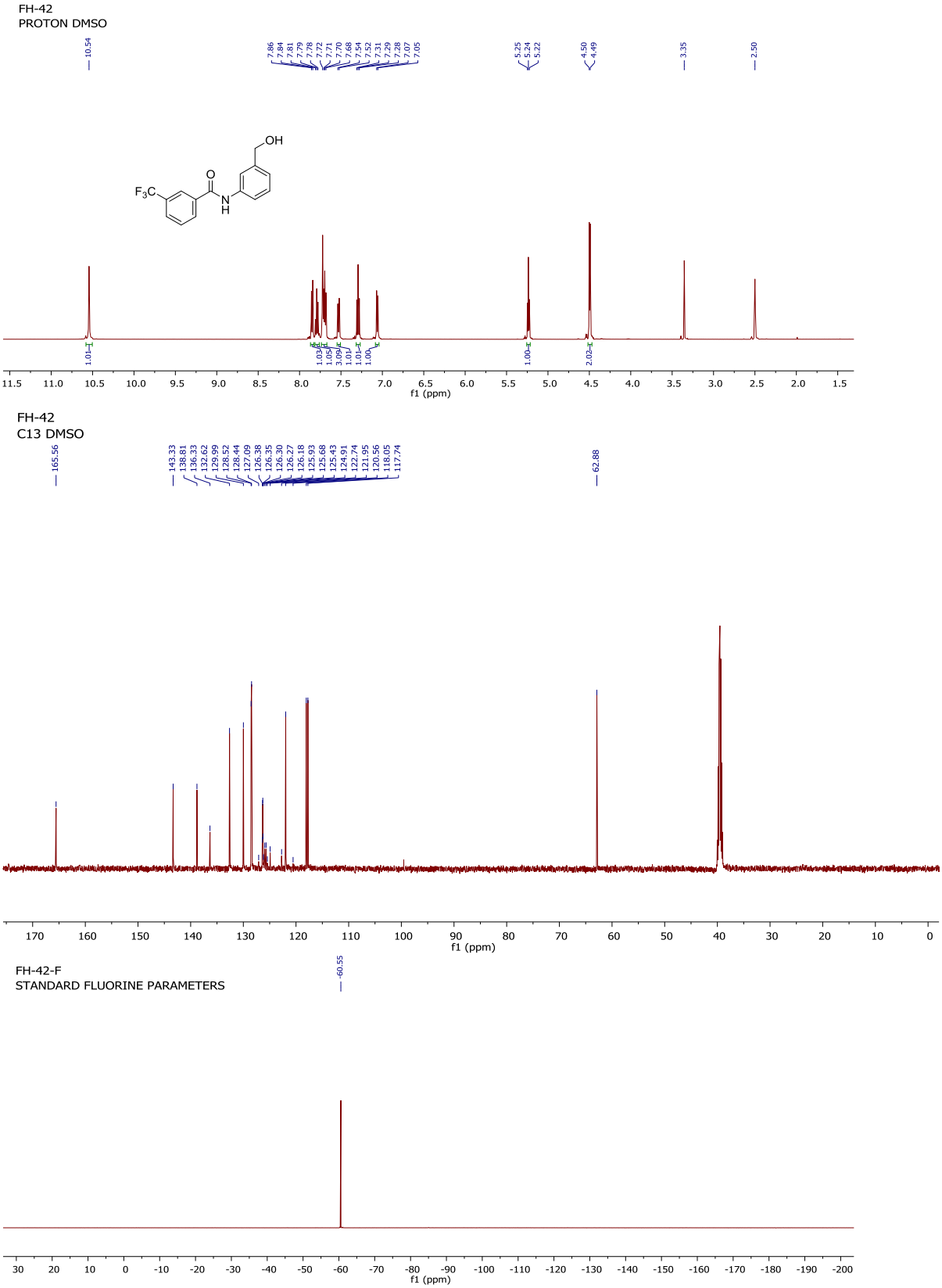
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **S4b**



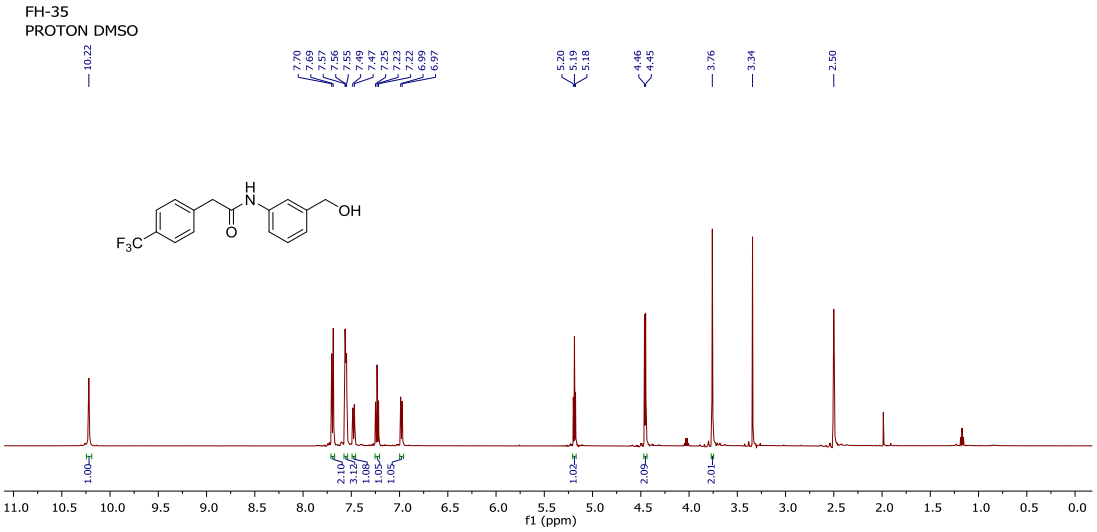


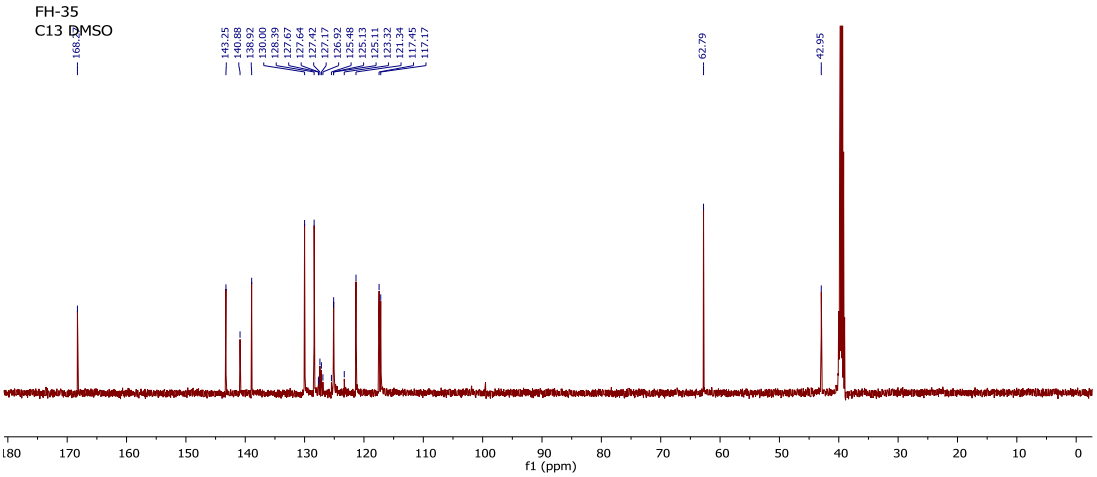


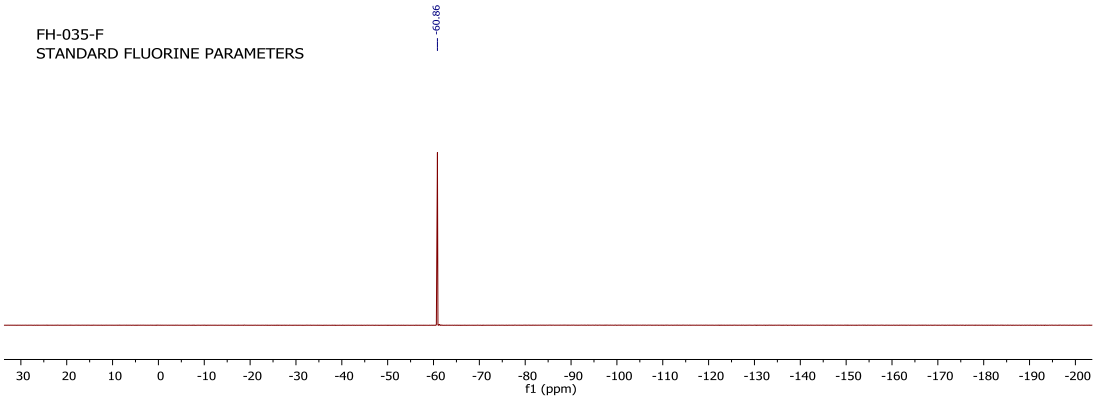
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **S4c**



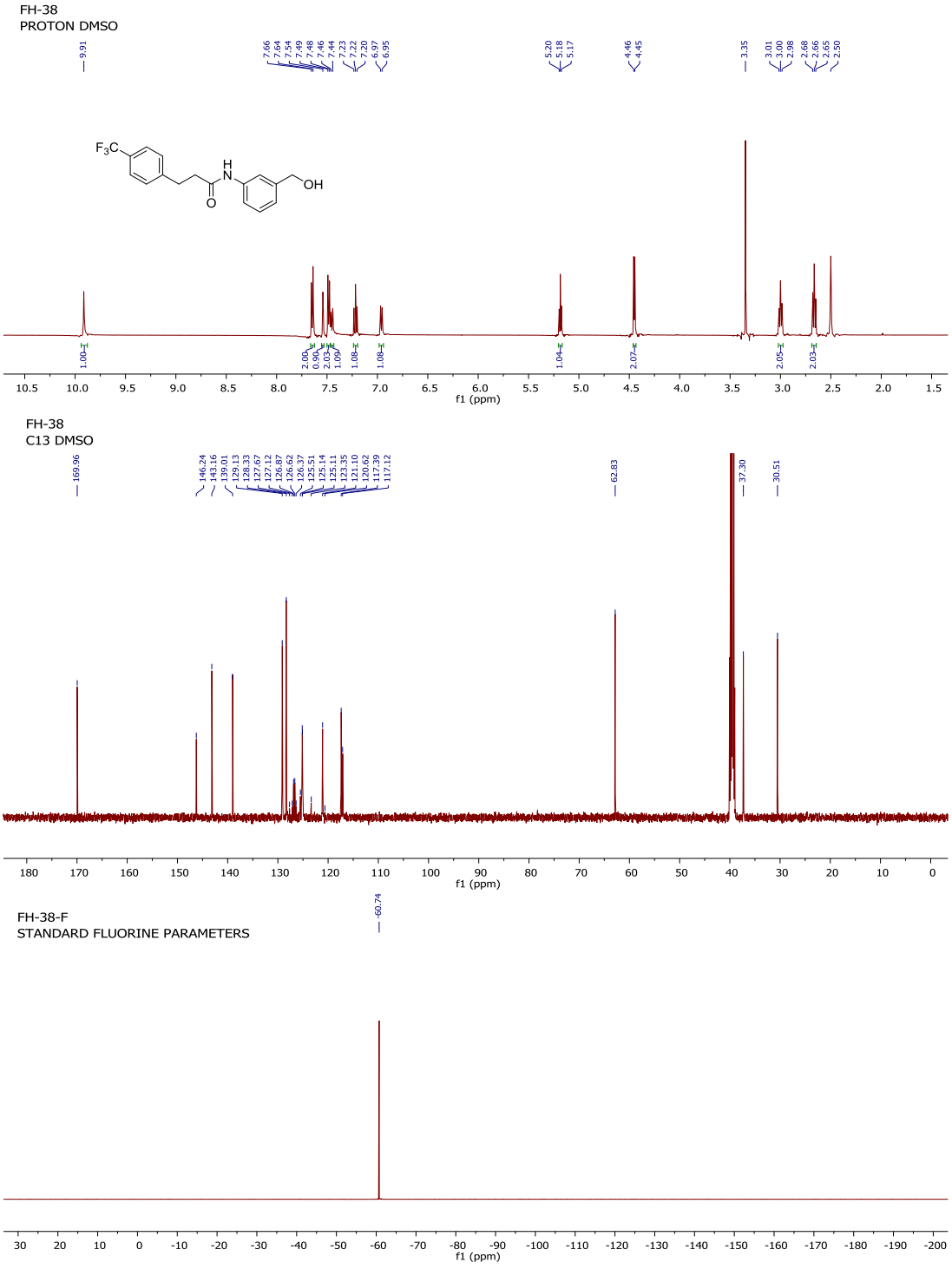
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **S4d**



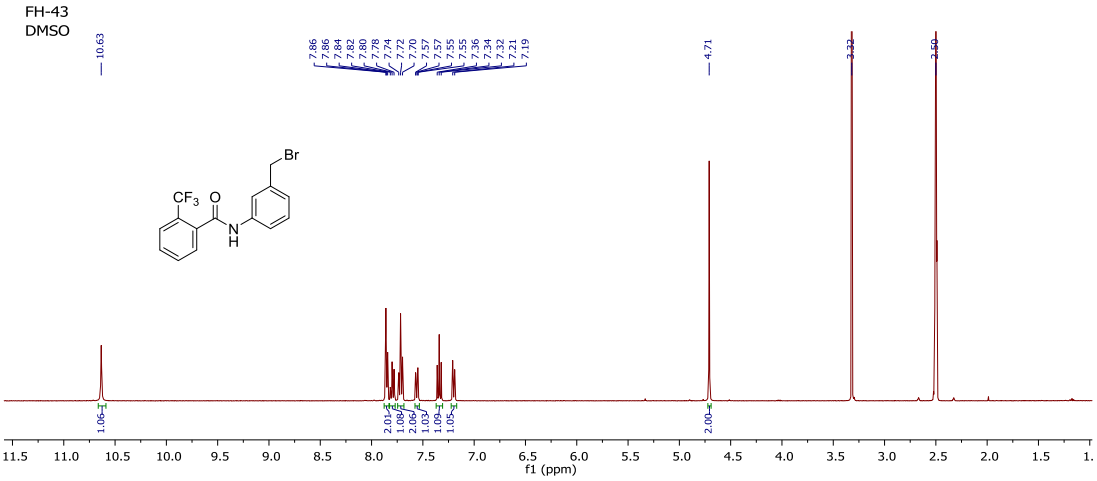


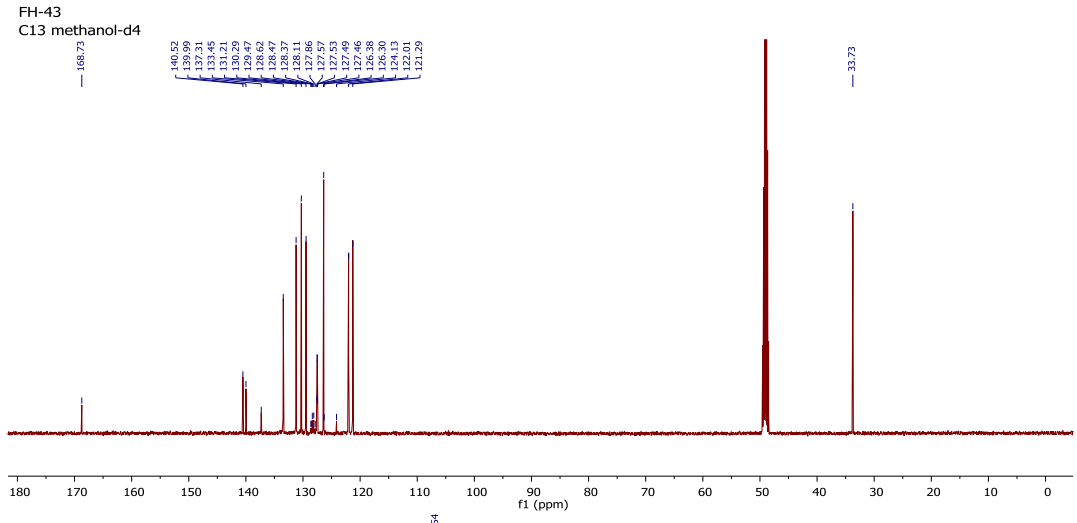


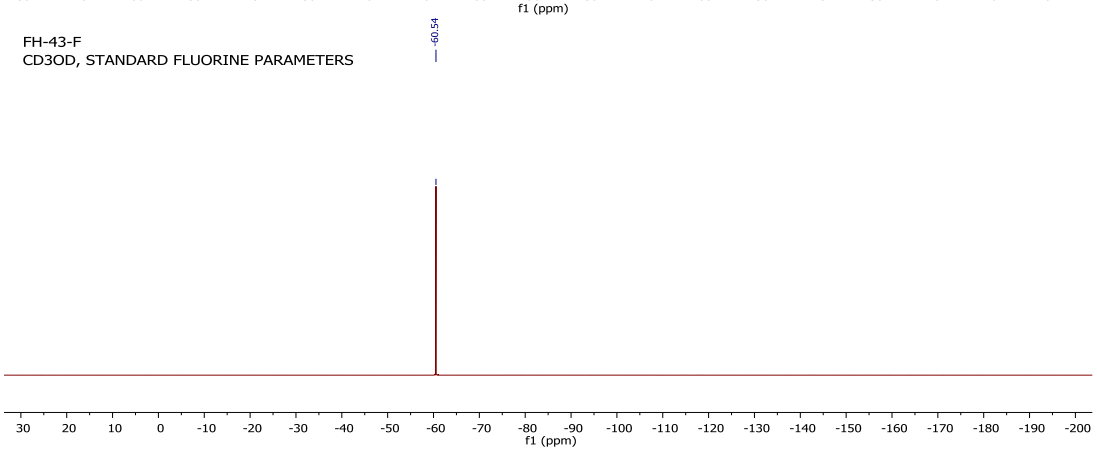
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **S4e**



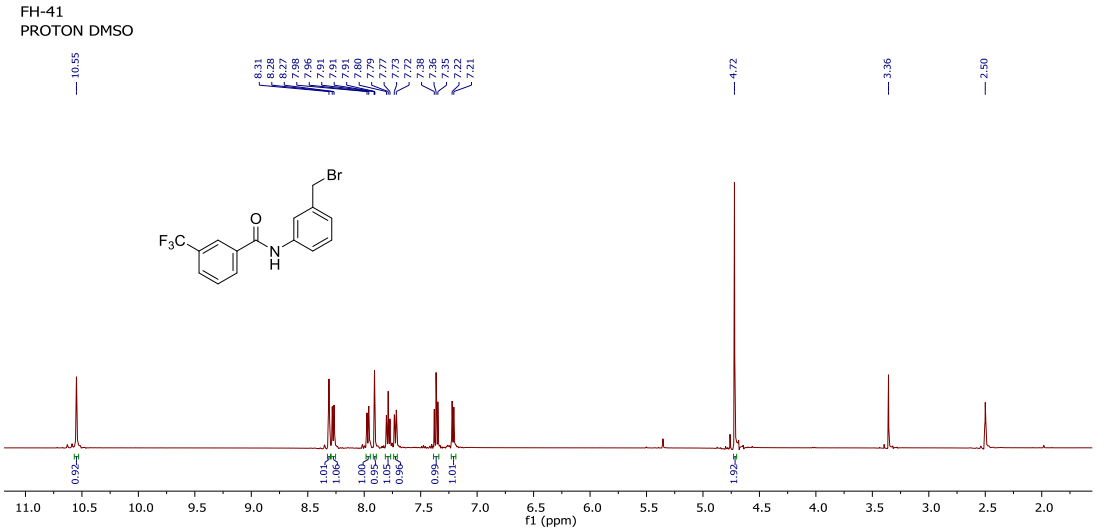
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **5b**

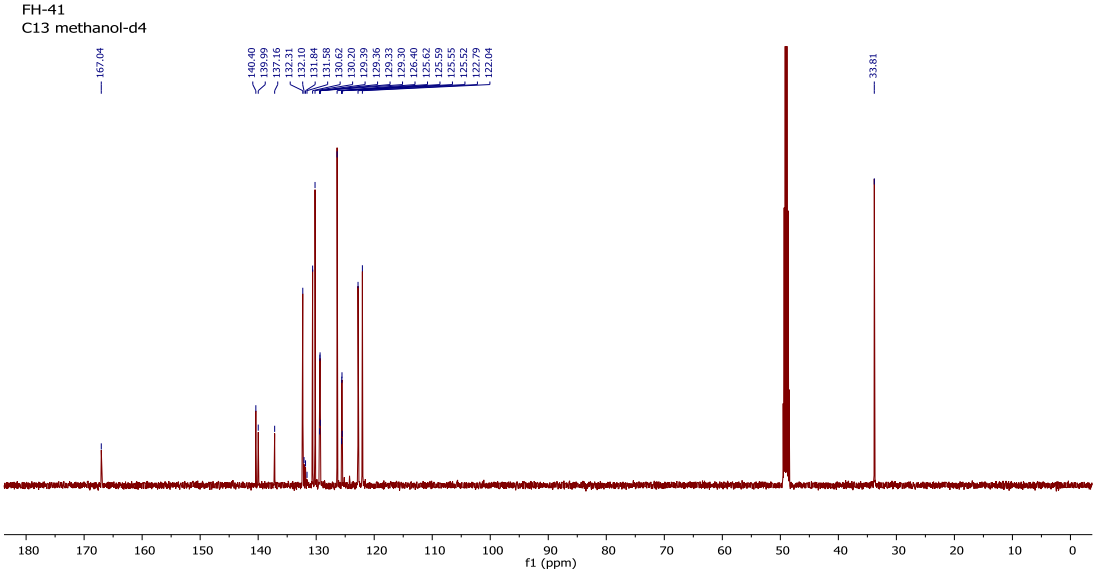


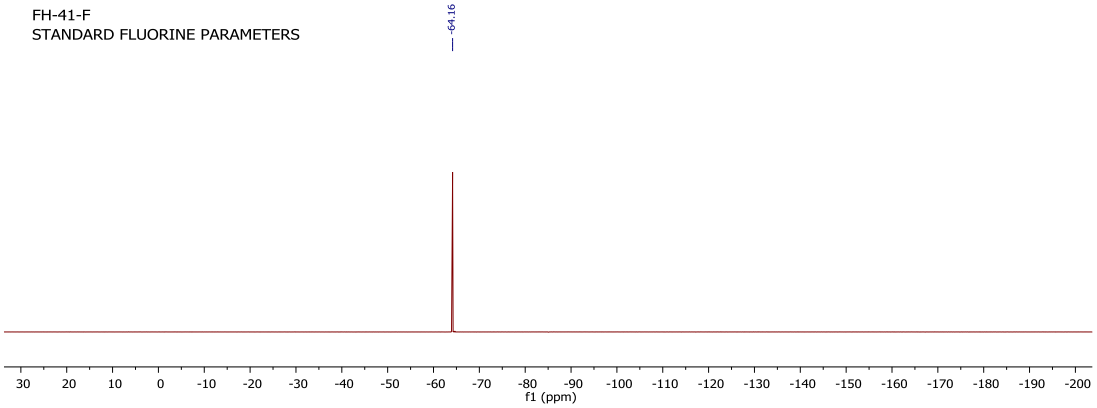




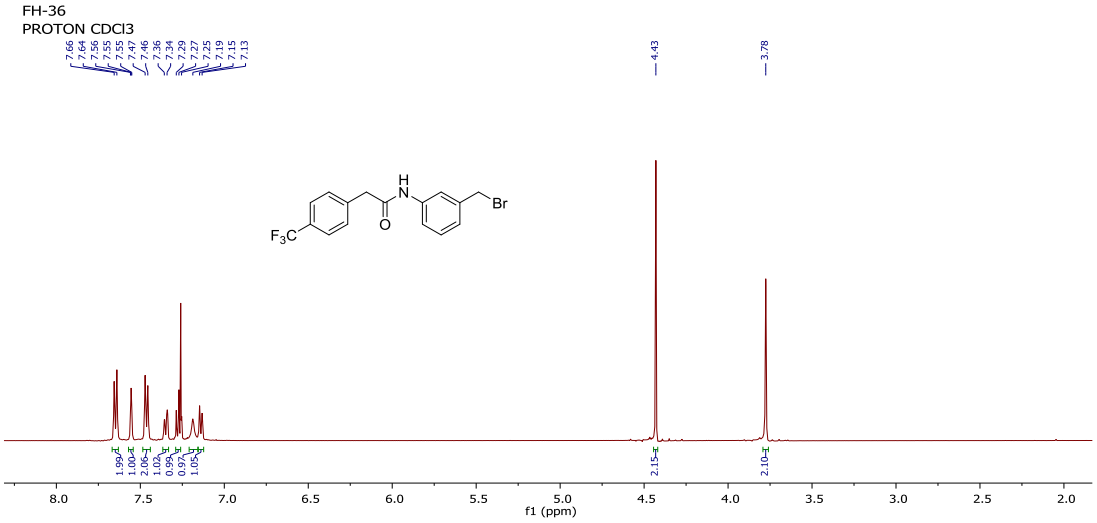
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **5c**

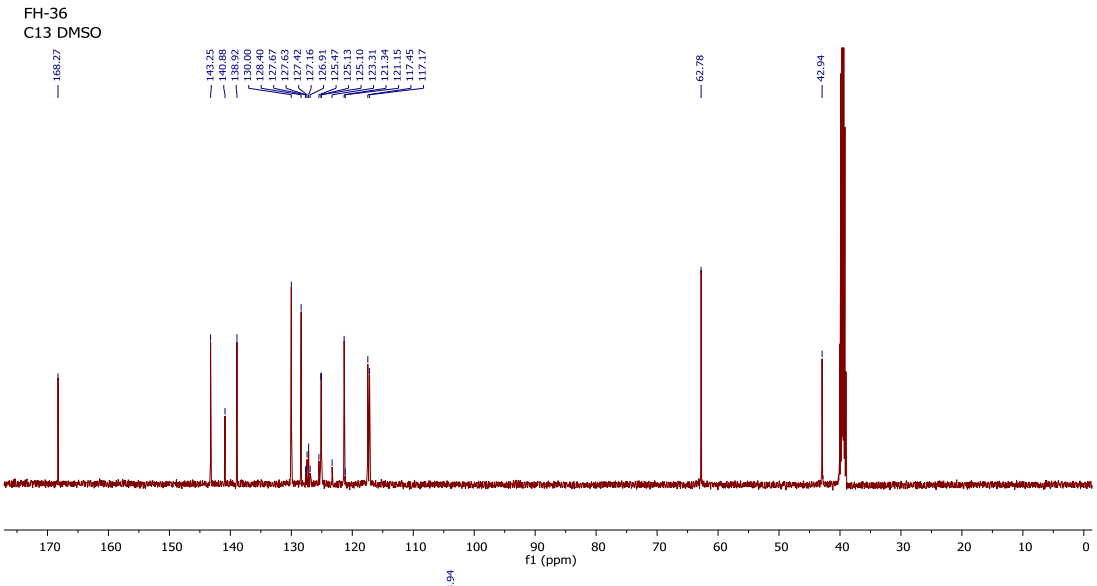


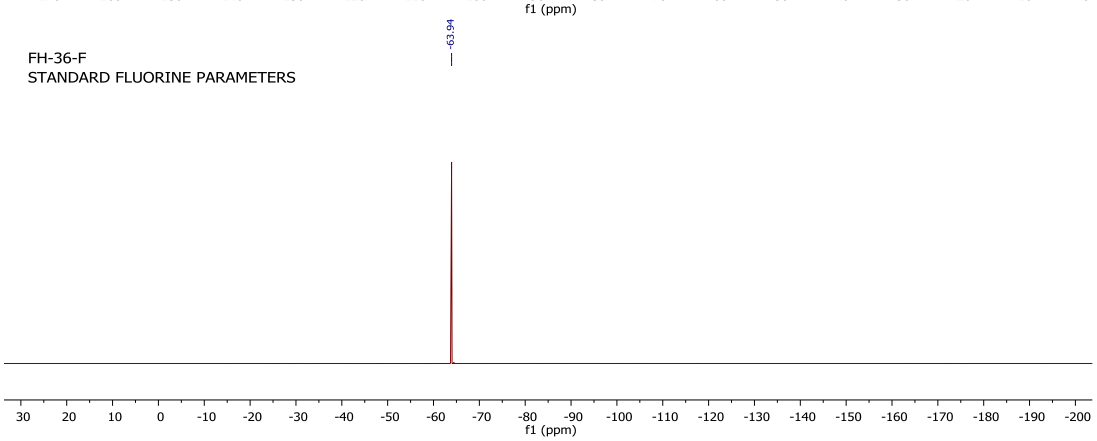




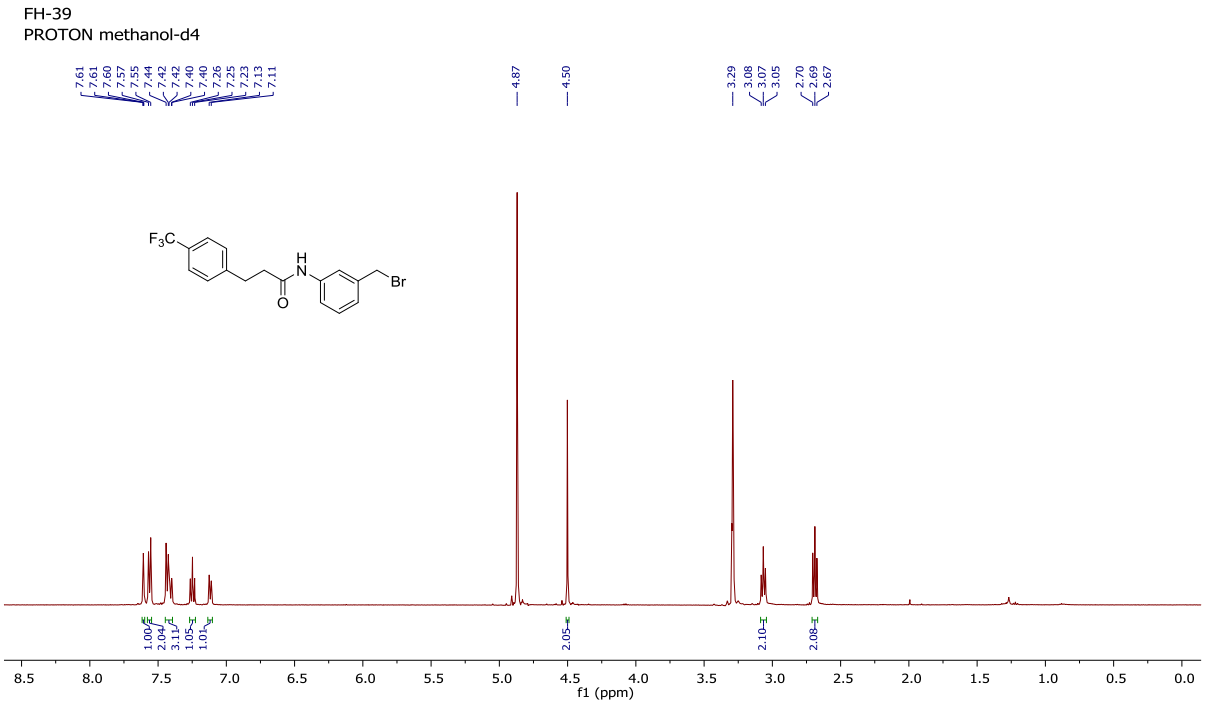
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **5d**

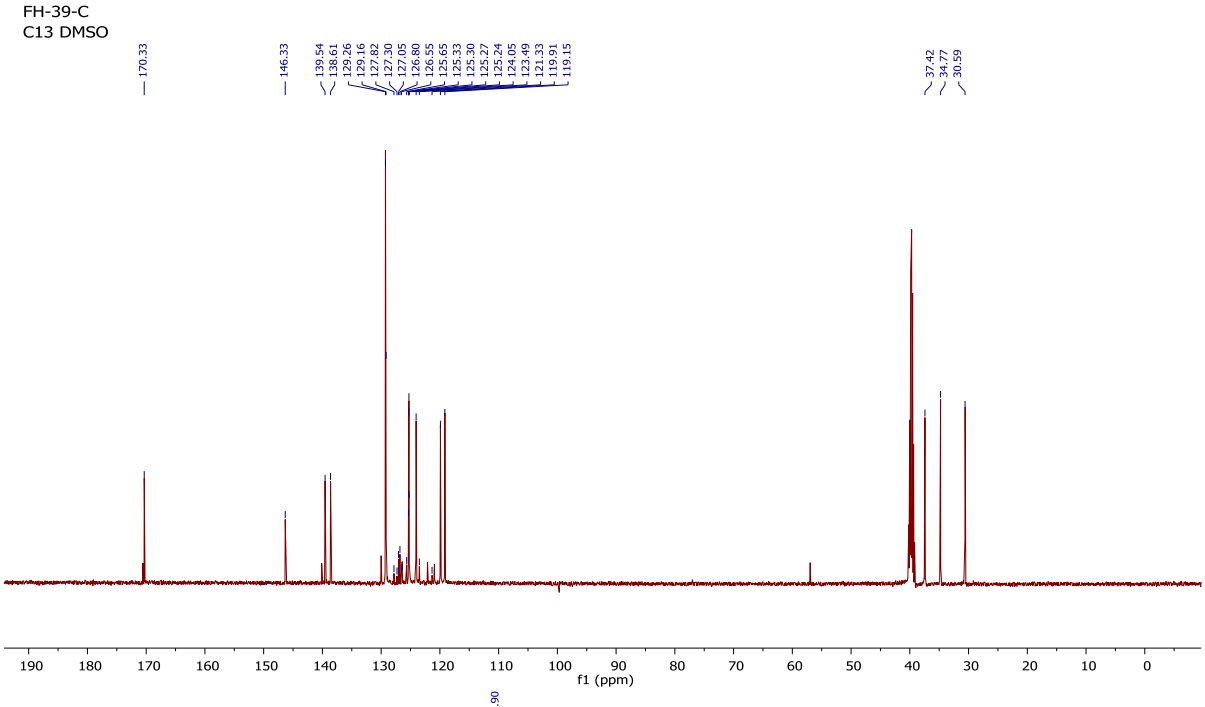


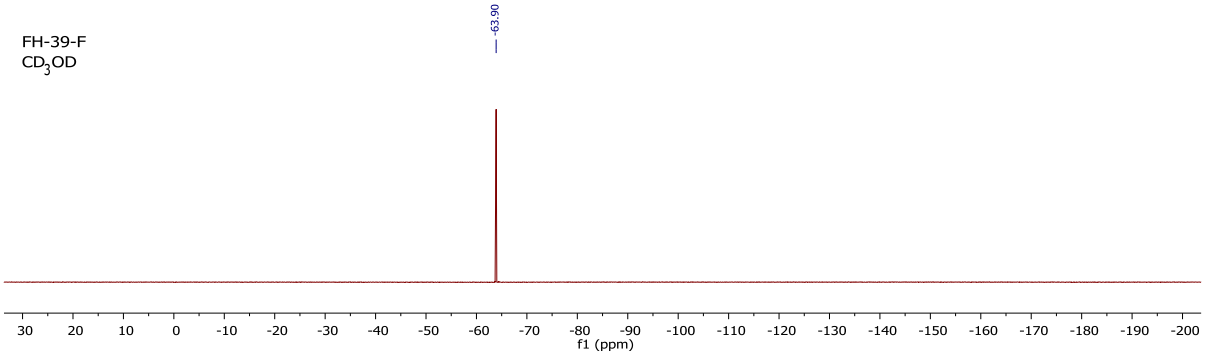




^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **5e**

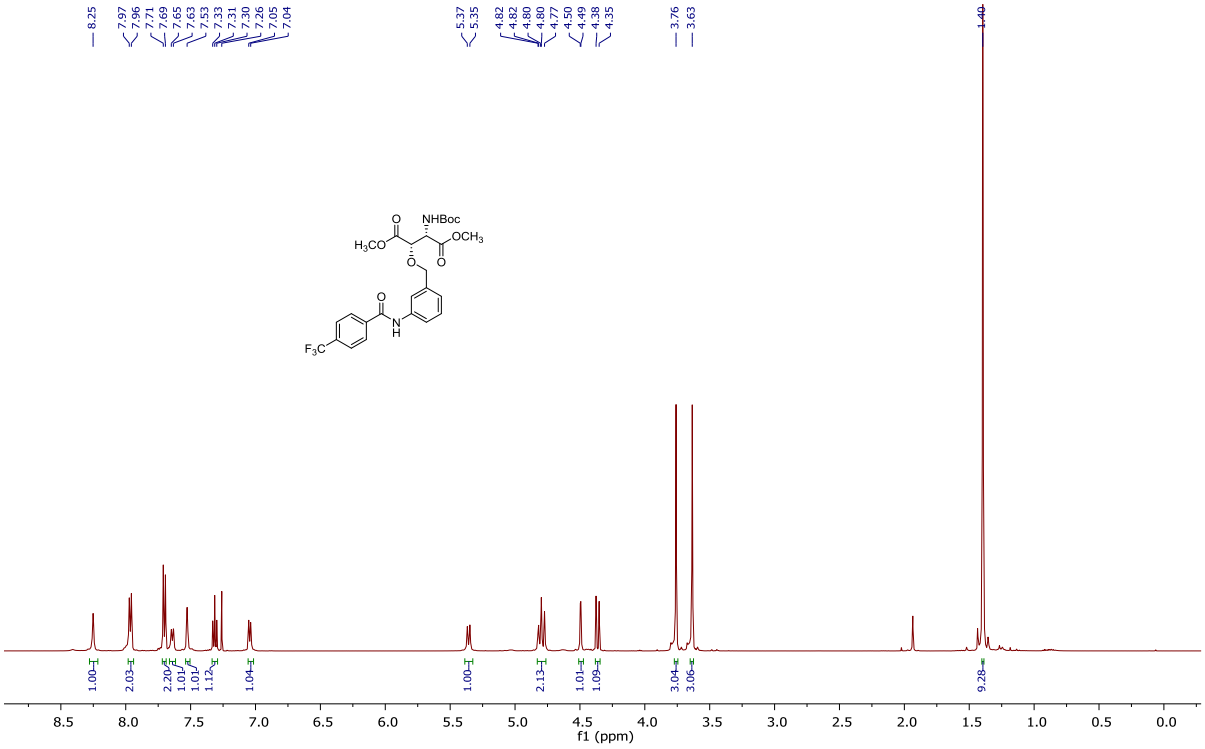




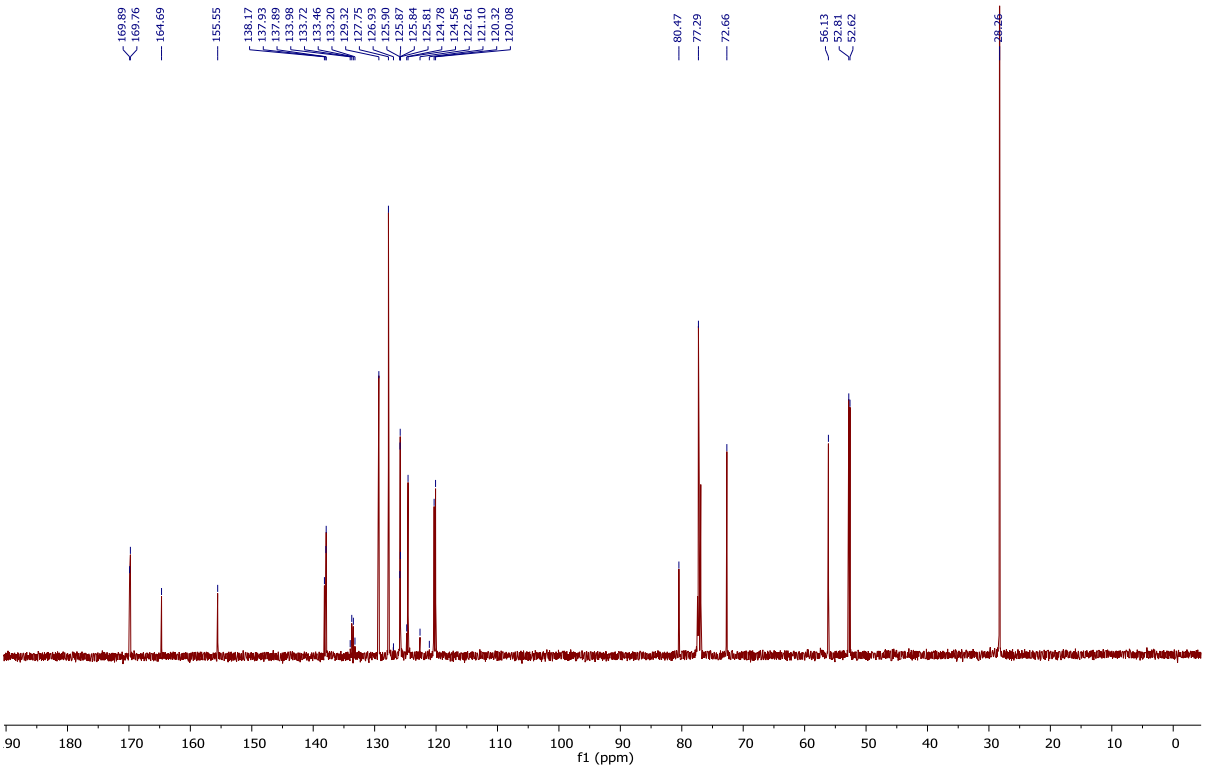


^{1}H NMR and ^{13}C NMR of compound **11a**

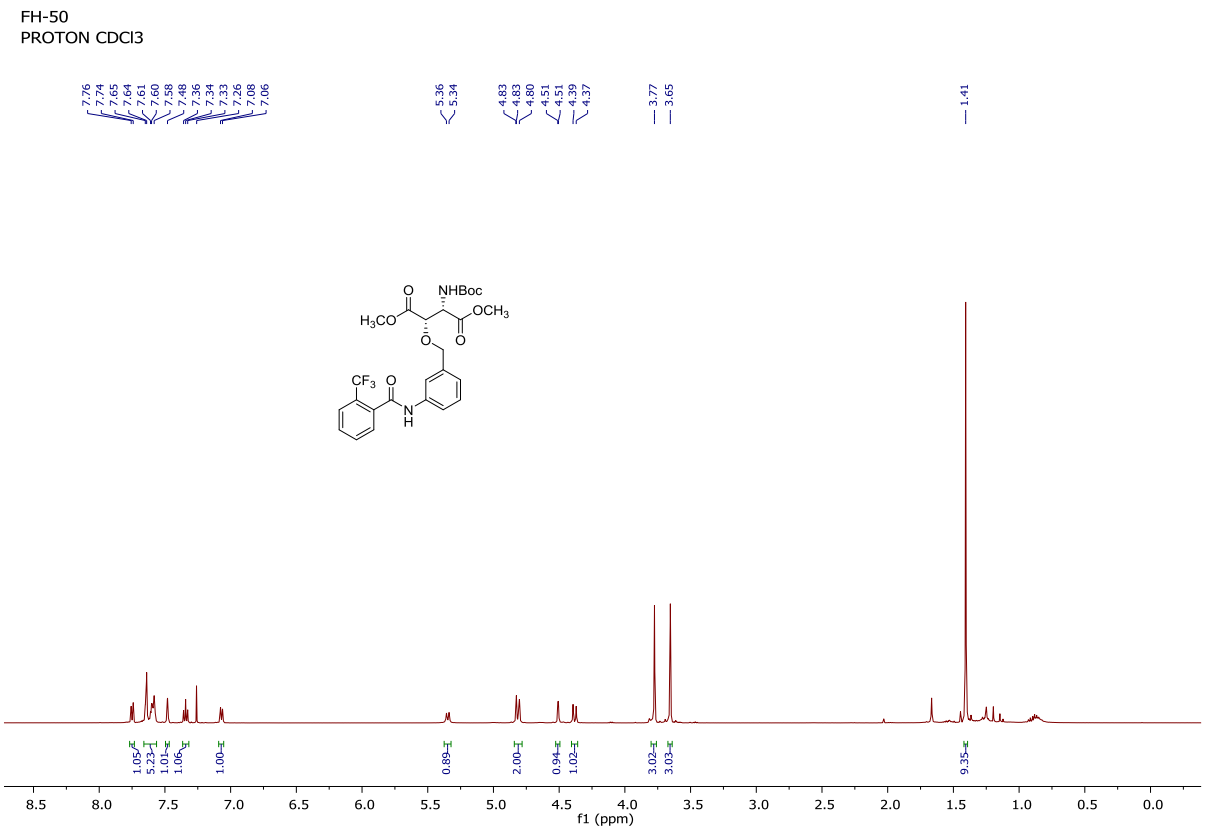
FH-11
PROTON CDCl3

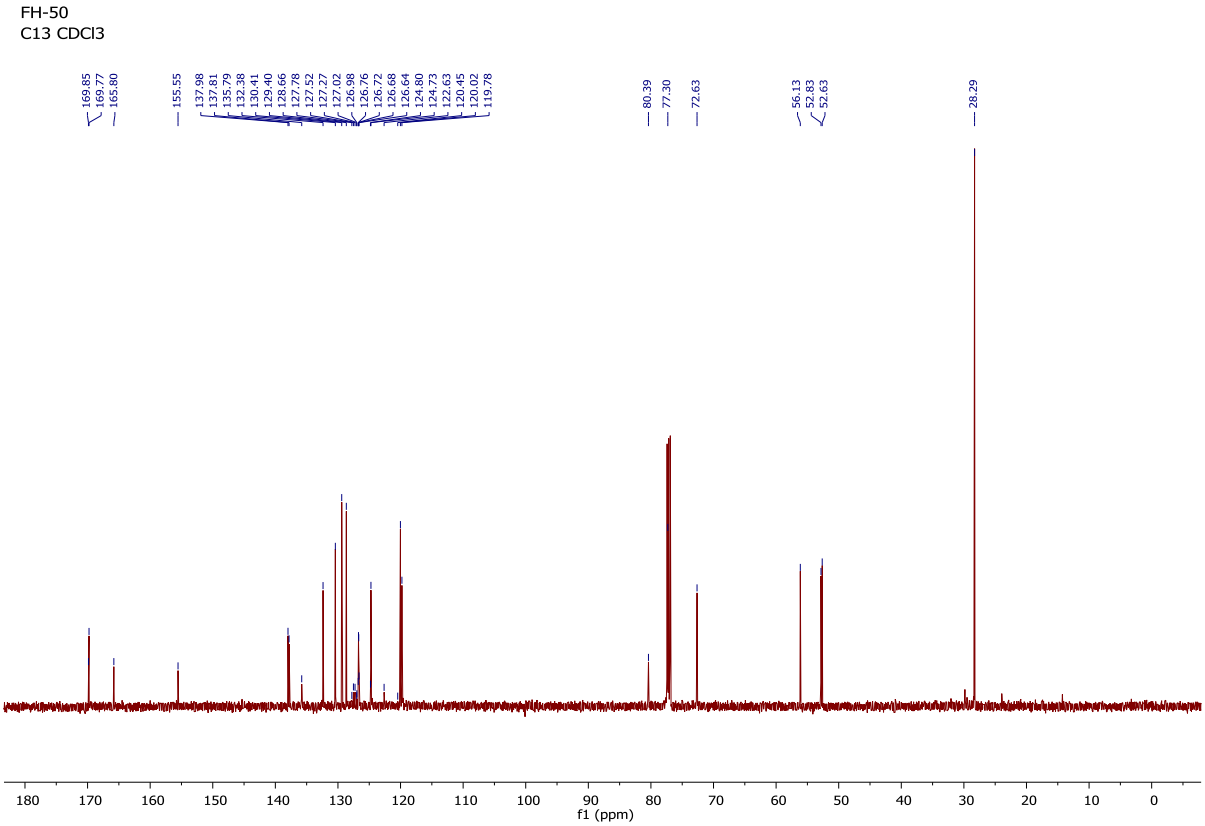


HF-11
C13 CDCl3

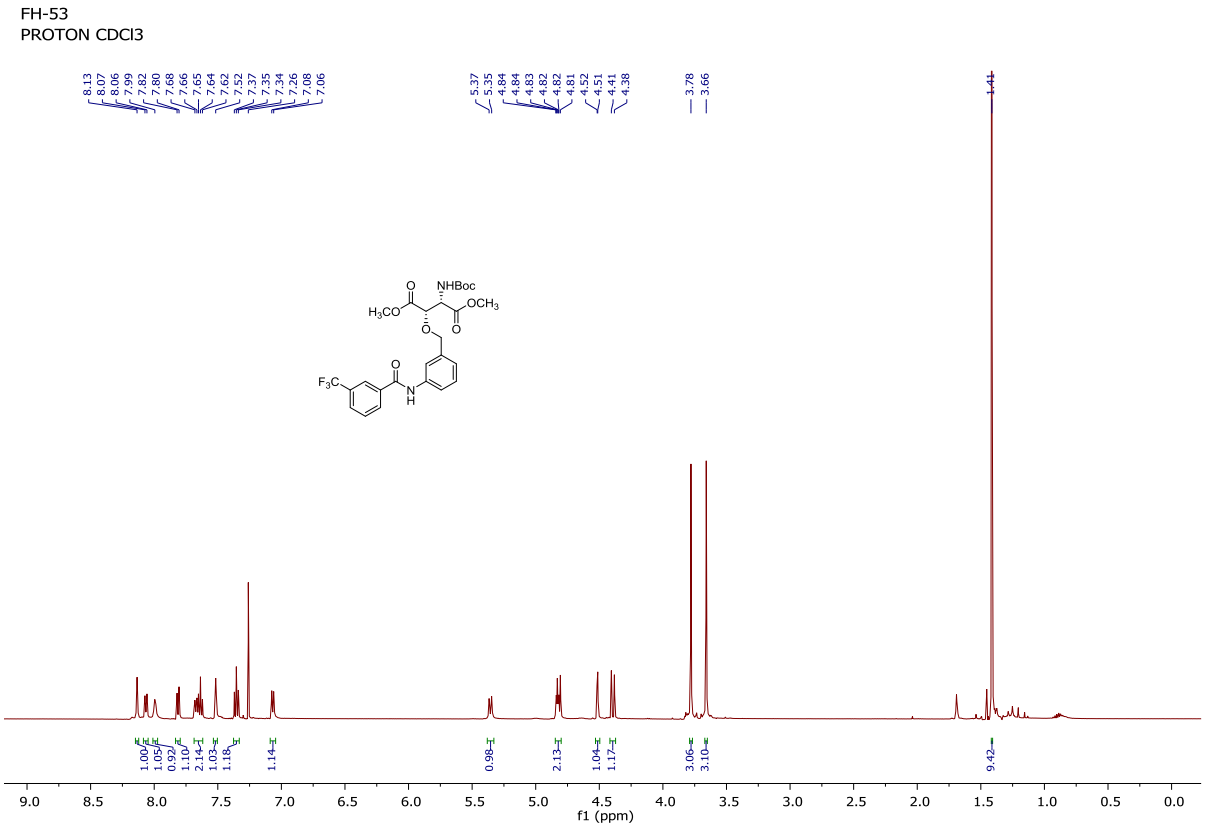


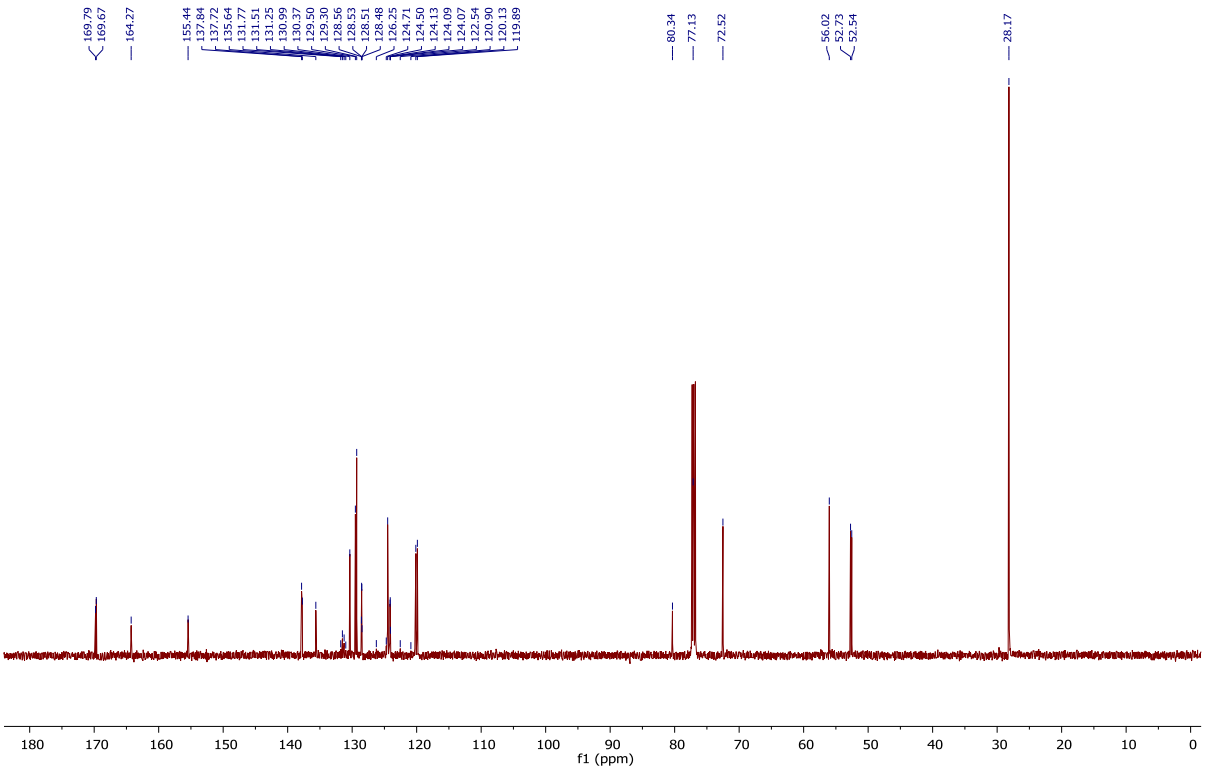
^{1}H NMR and ^{13}C NMR of compound **11b**



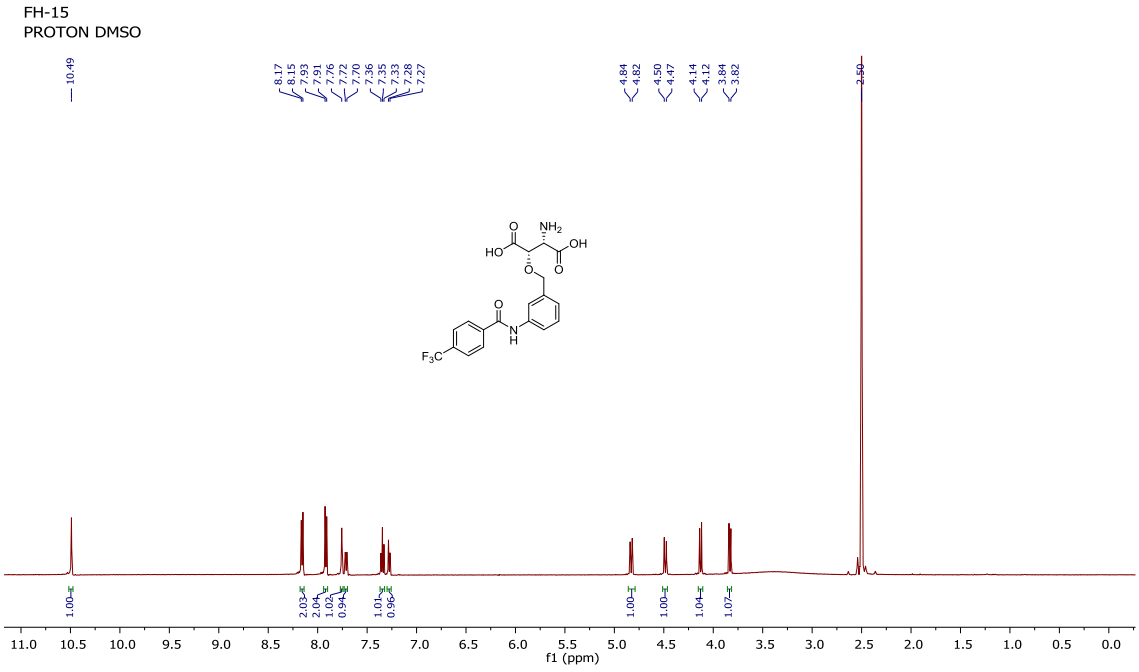


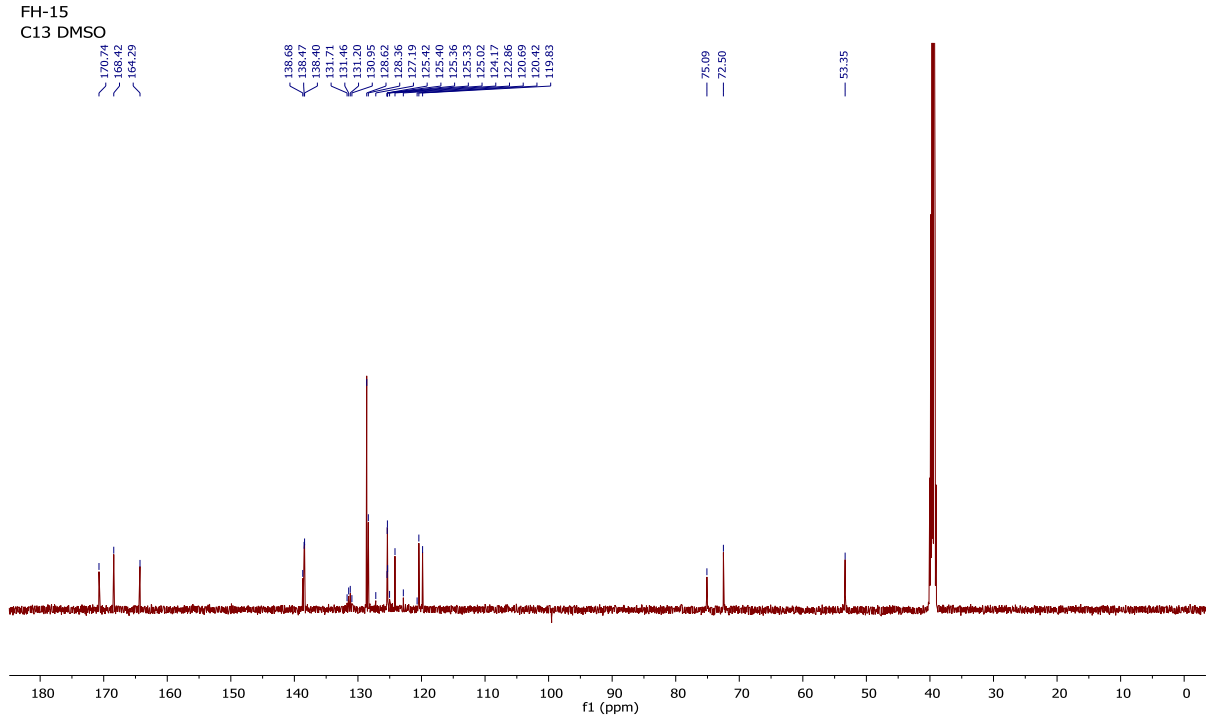
^{1}H NMR and ^{13}C NMR of compound **11c**

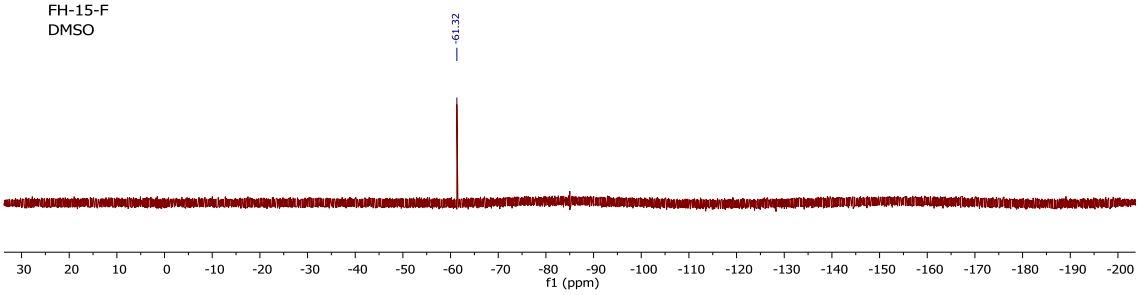




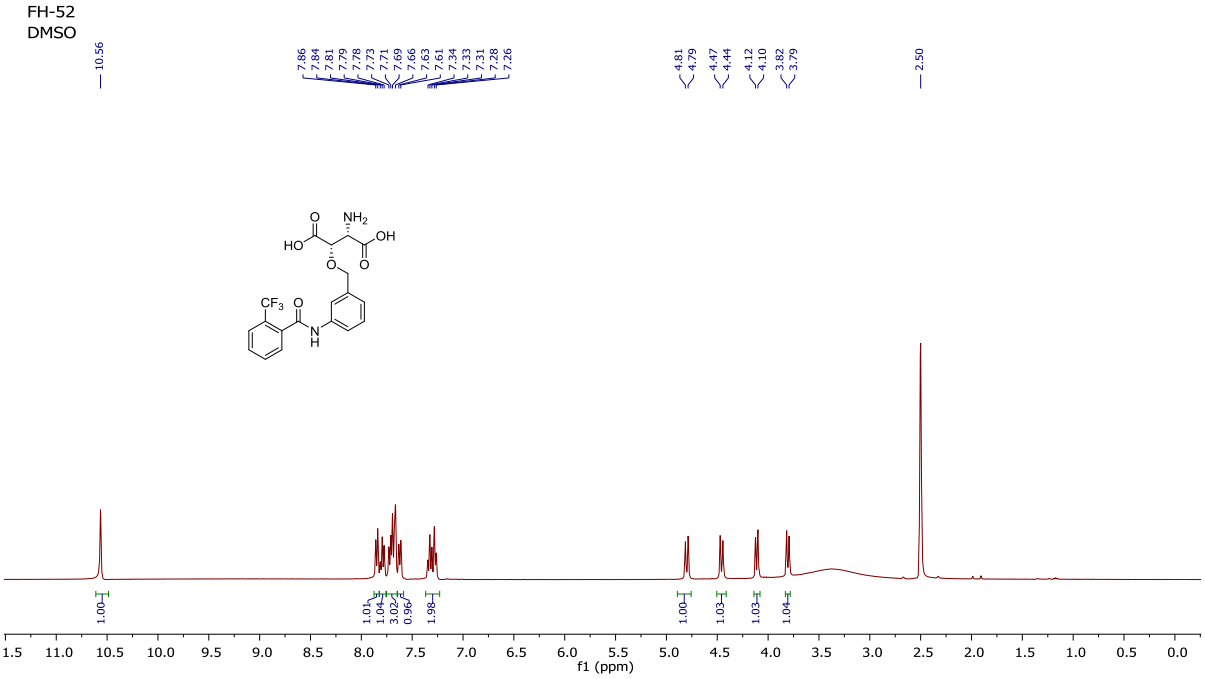
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **2a**

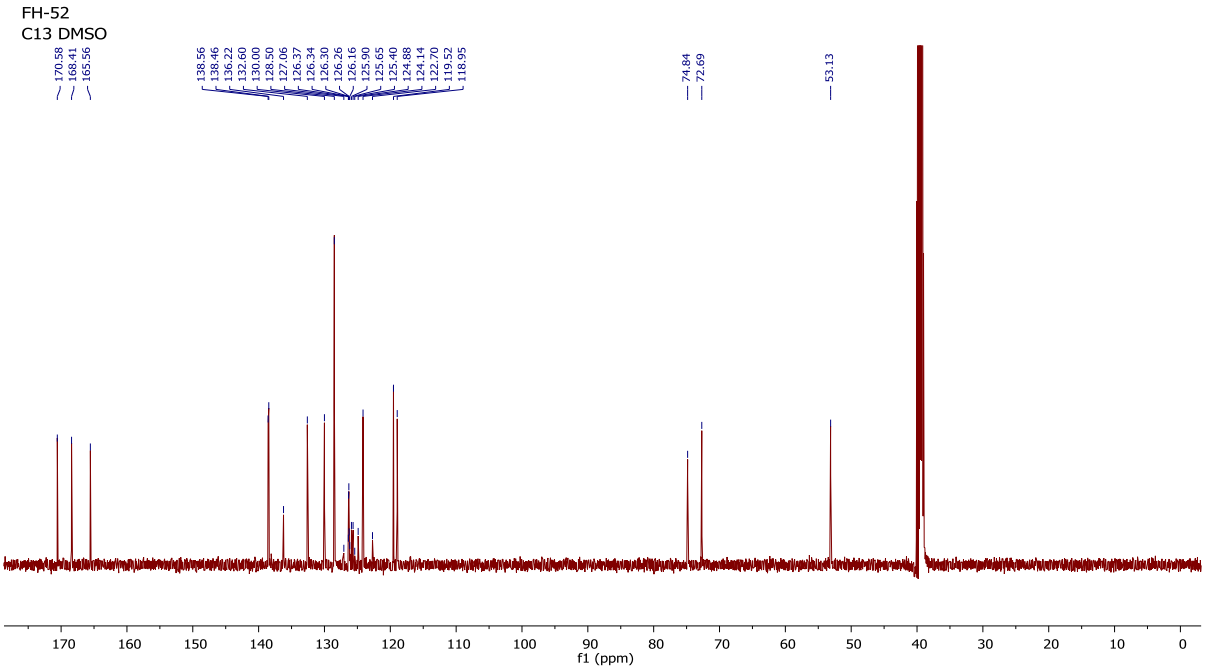


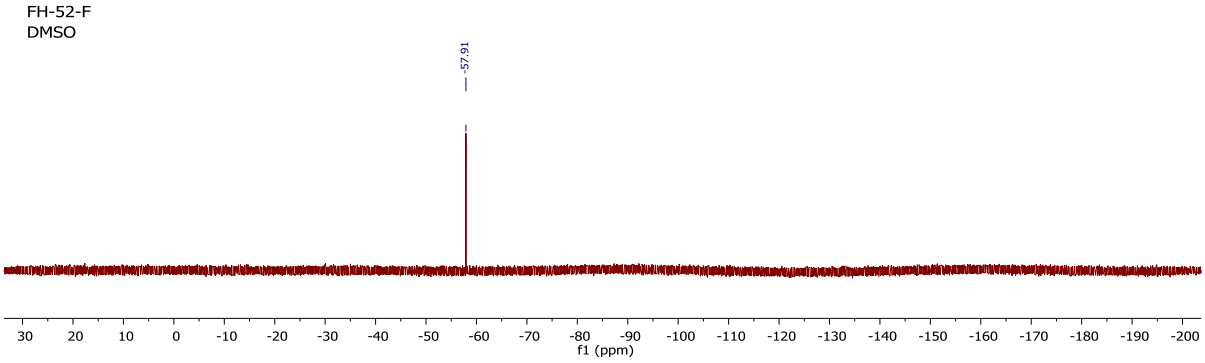




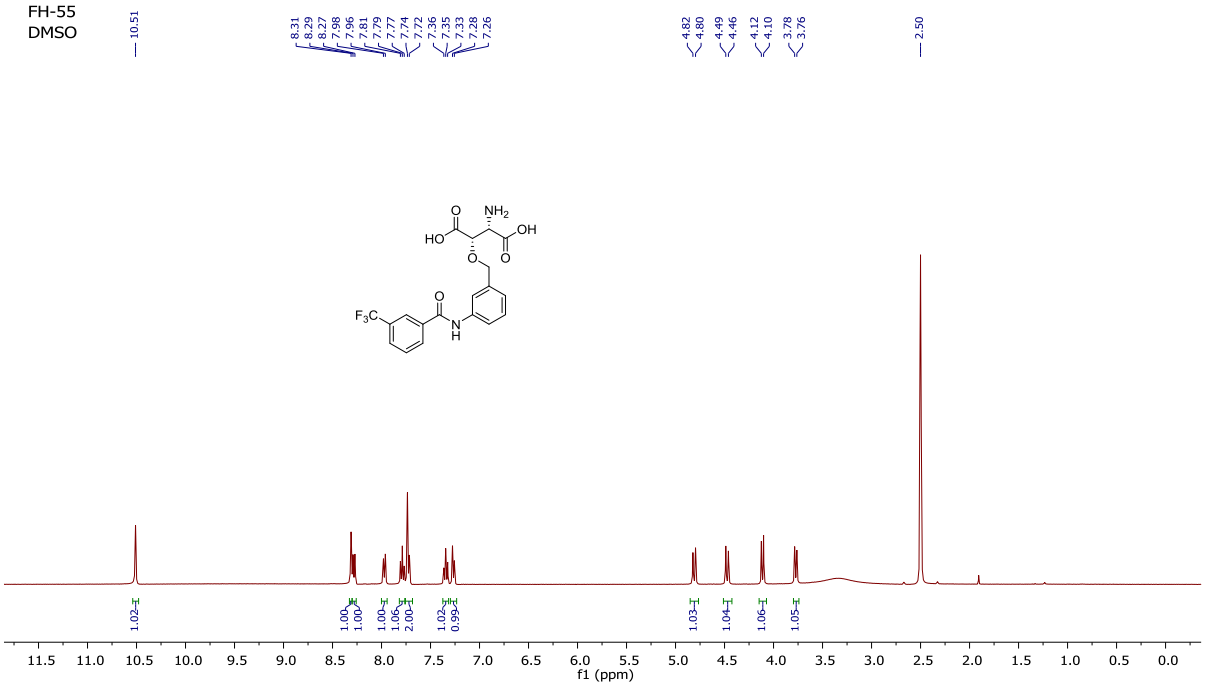
^{1}H NMR, ^{13}C-NMR and ^{19}F NMR of compound **2b**

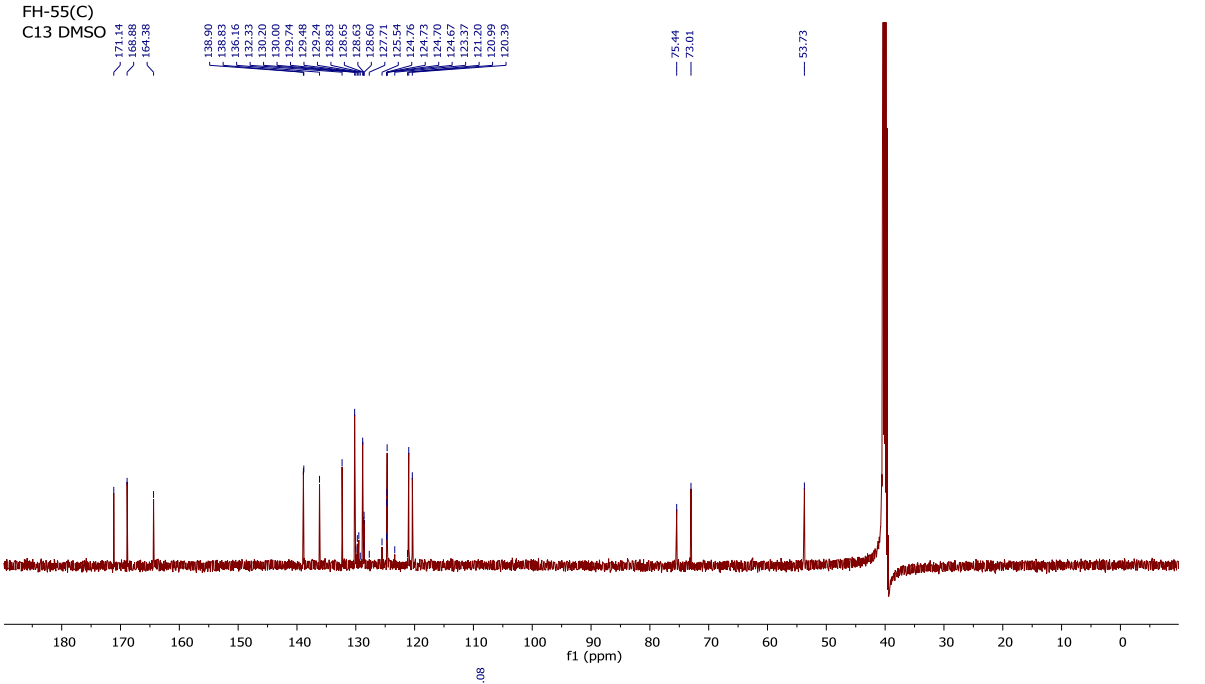


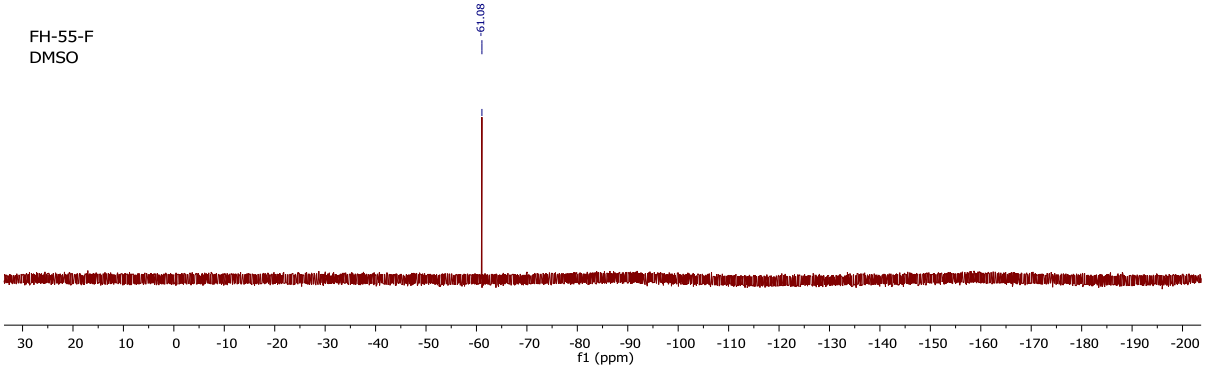




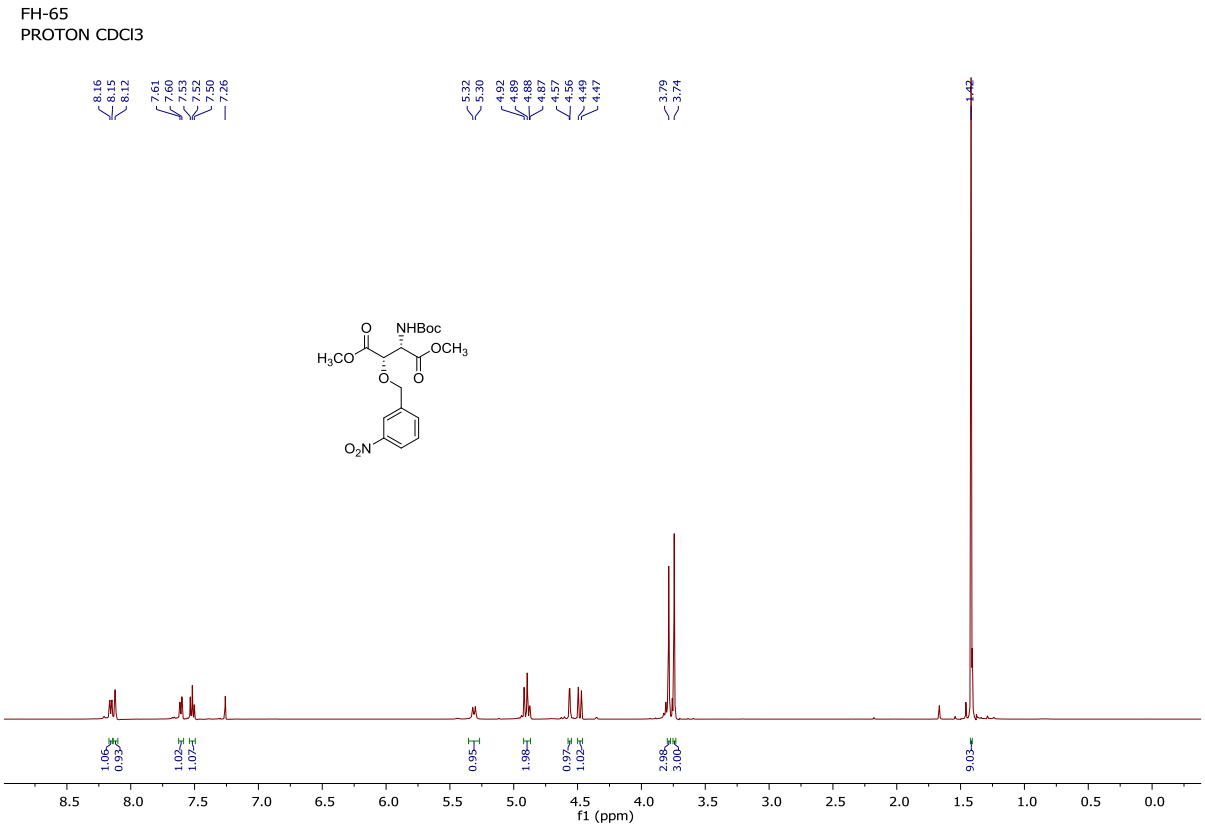
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **2c**

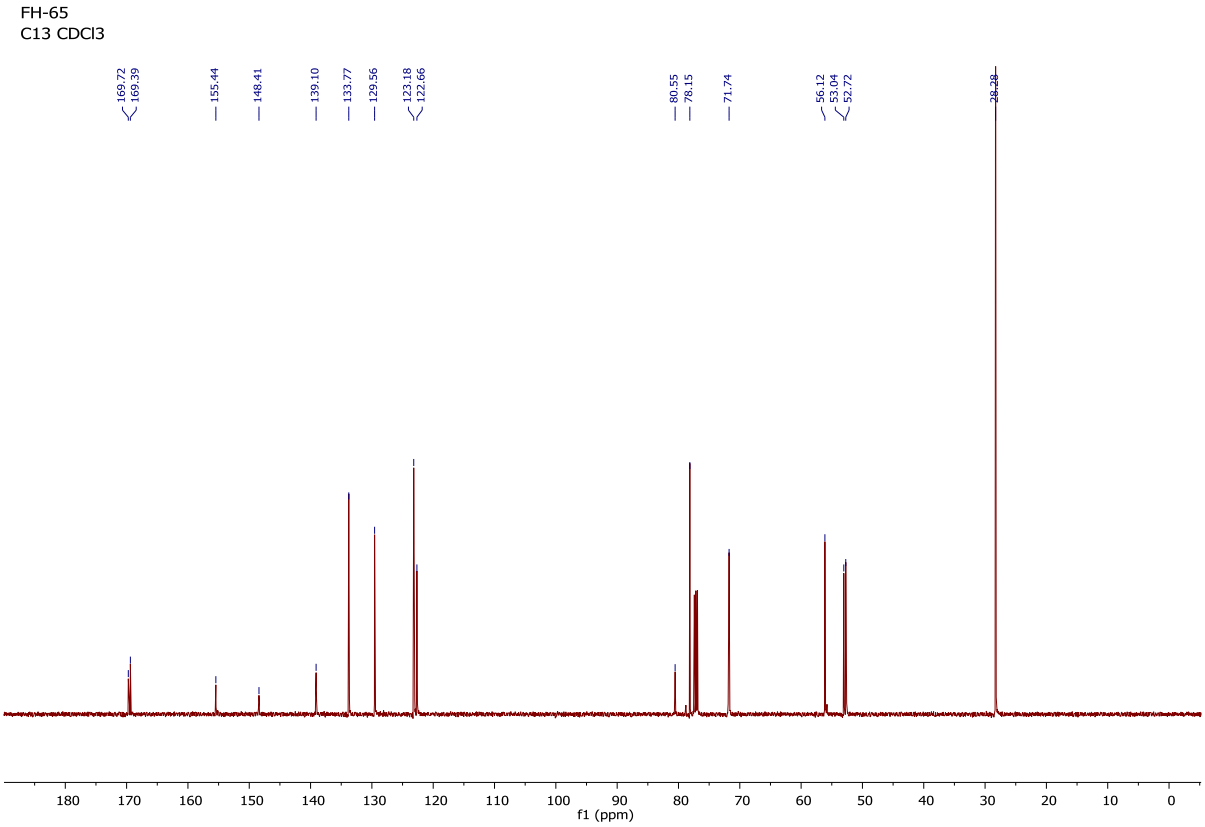




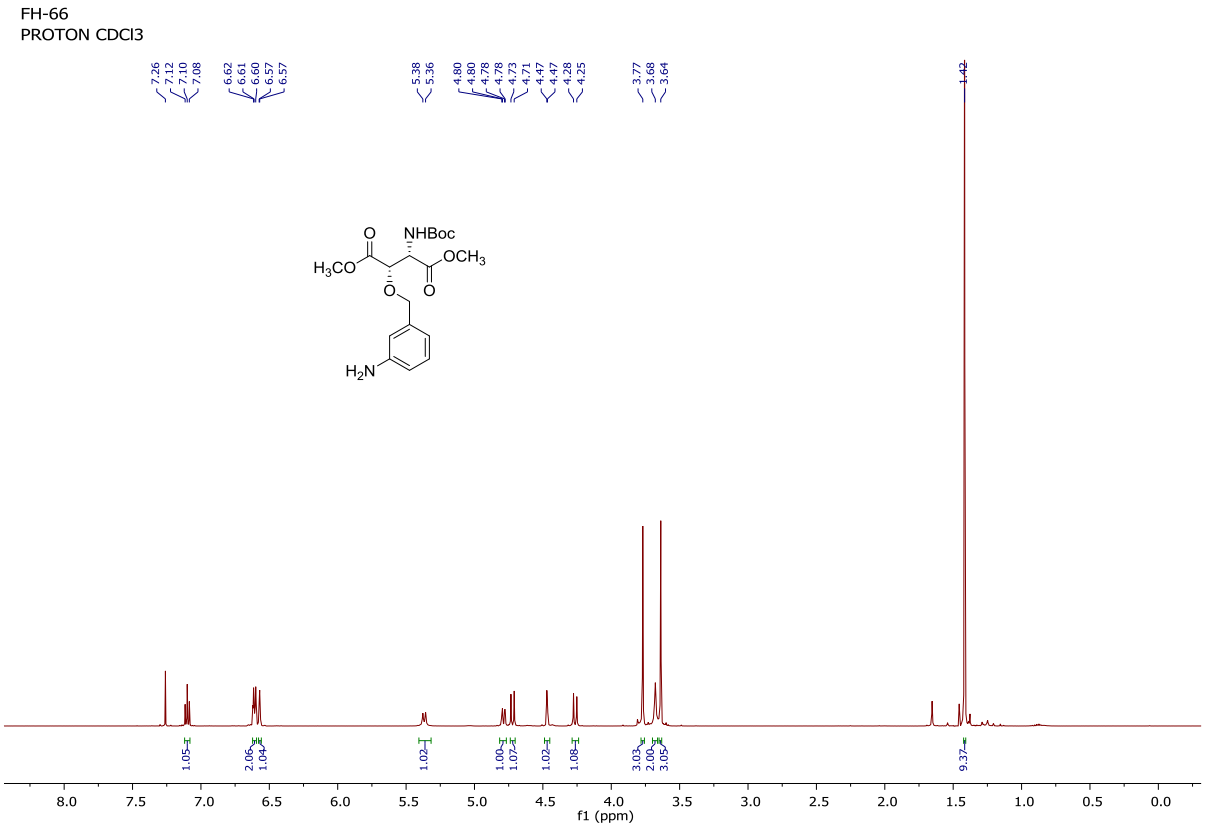


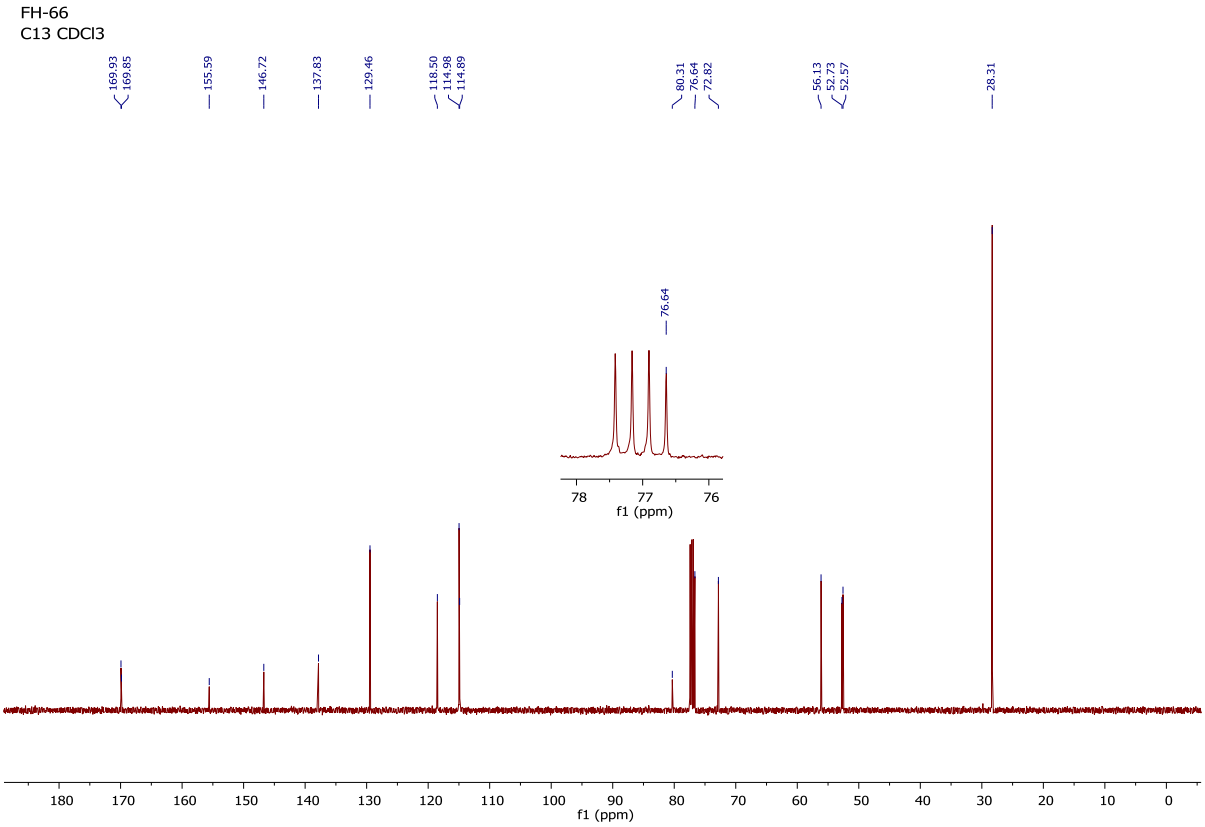
^{1}H NMR and ^{13}C NMR of compound **12**



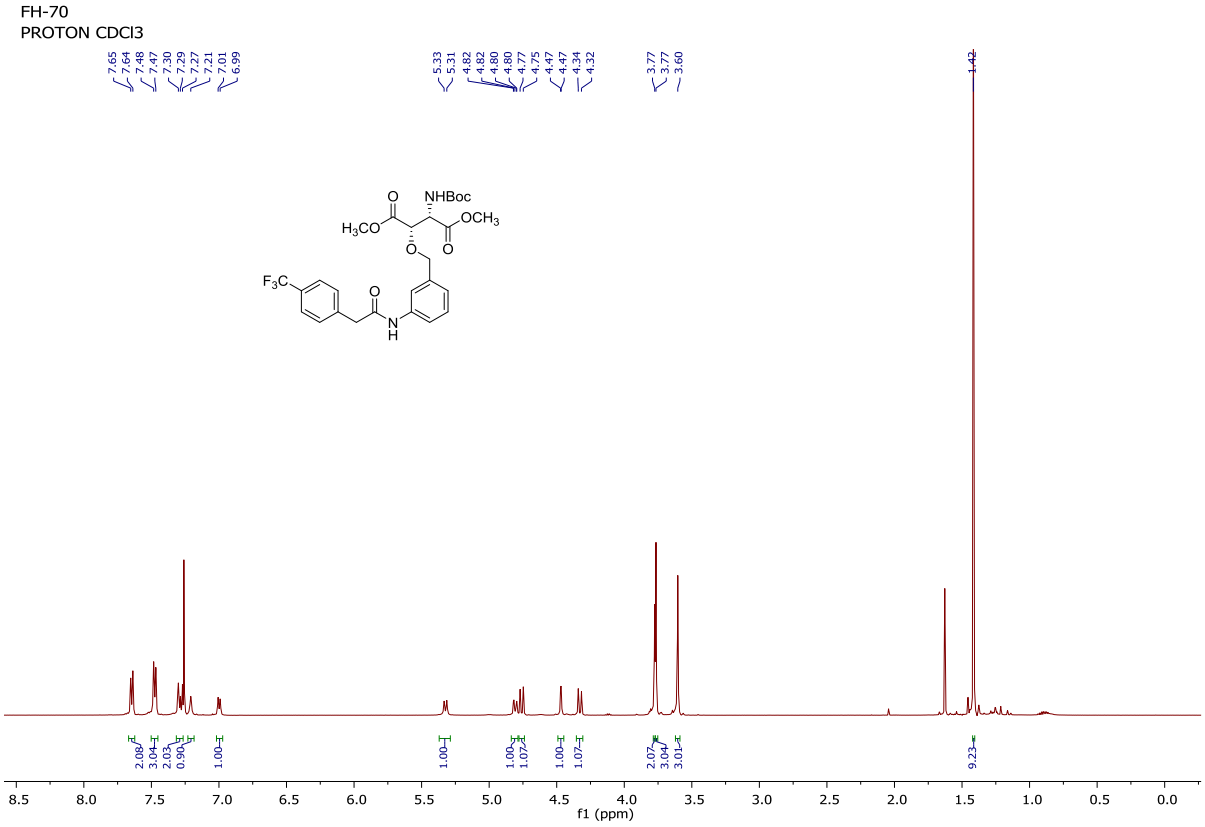


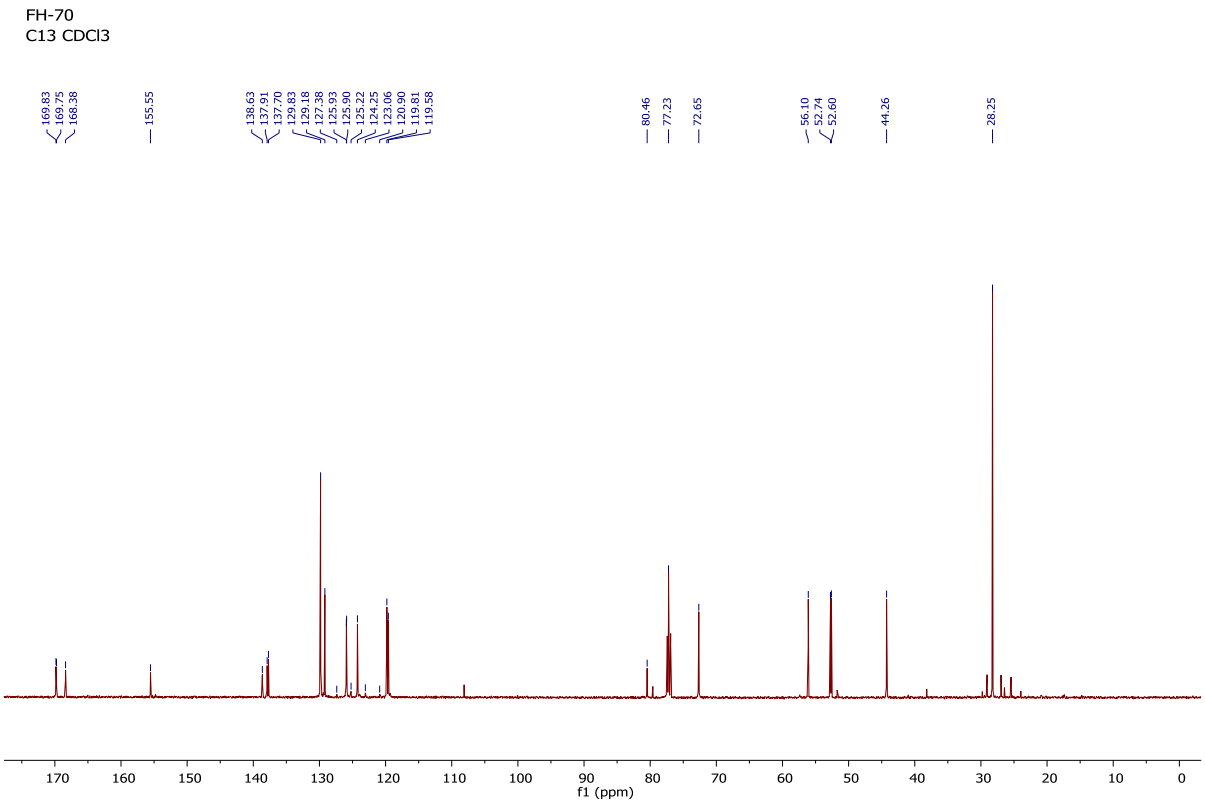
^{1}H NMR and ^{13}C NMR of compound **13**



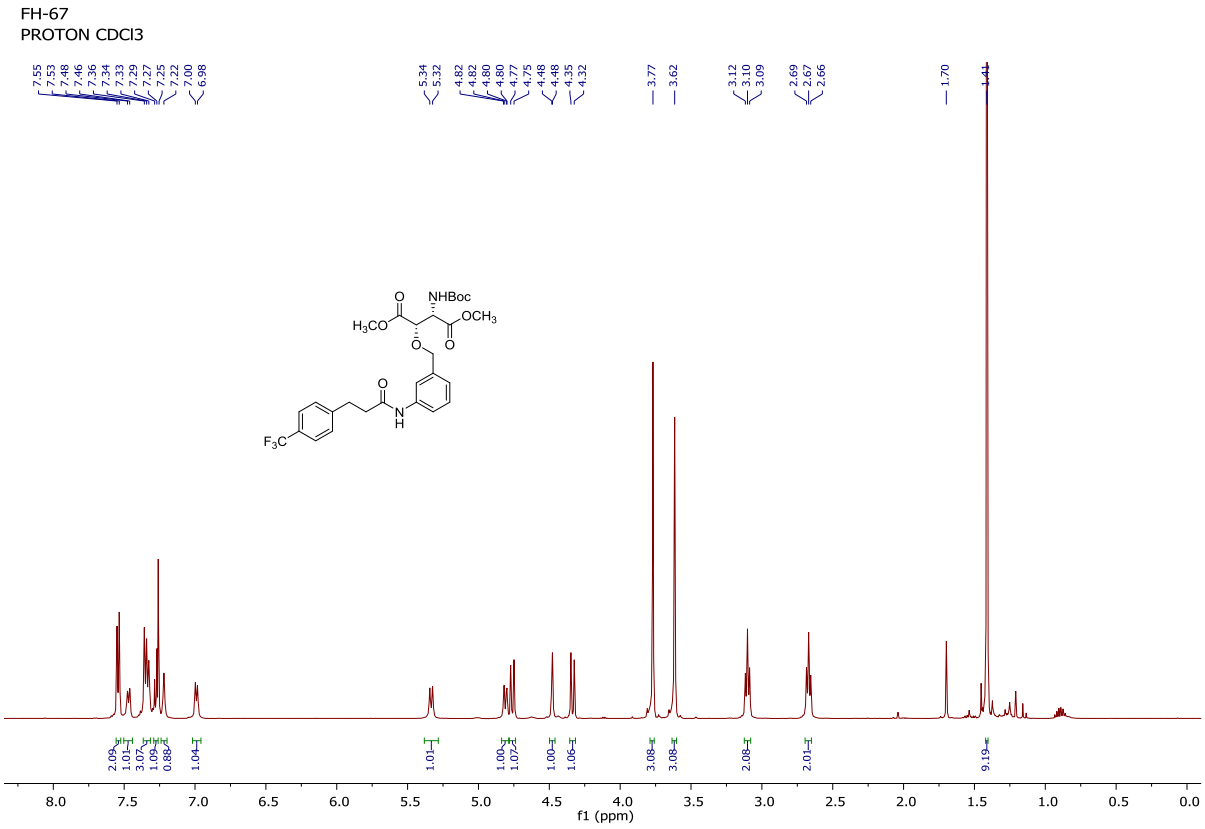


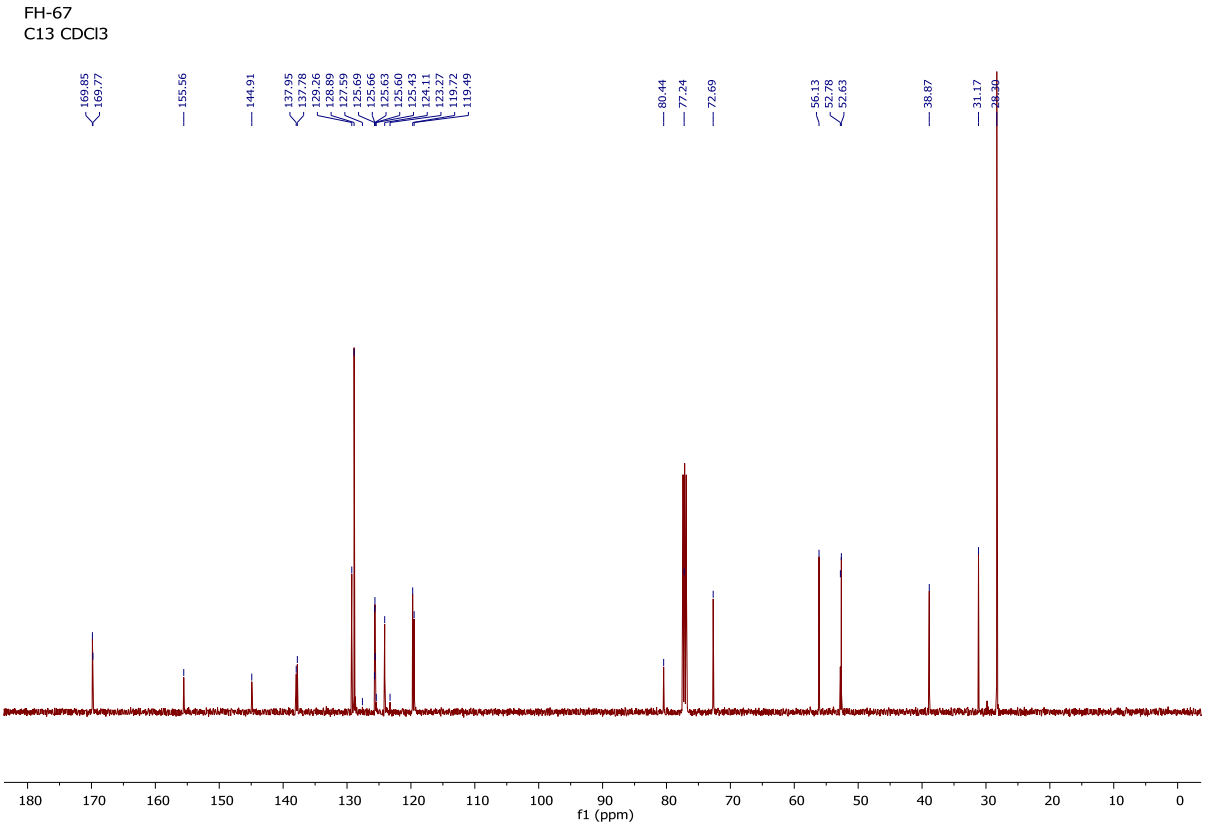
^{1}H NMR and ^{13}C NMR of compound **15a**



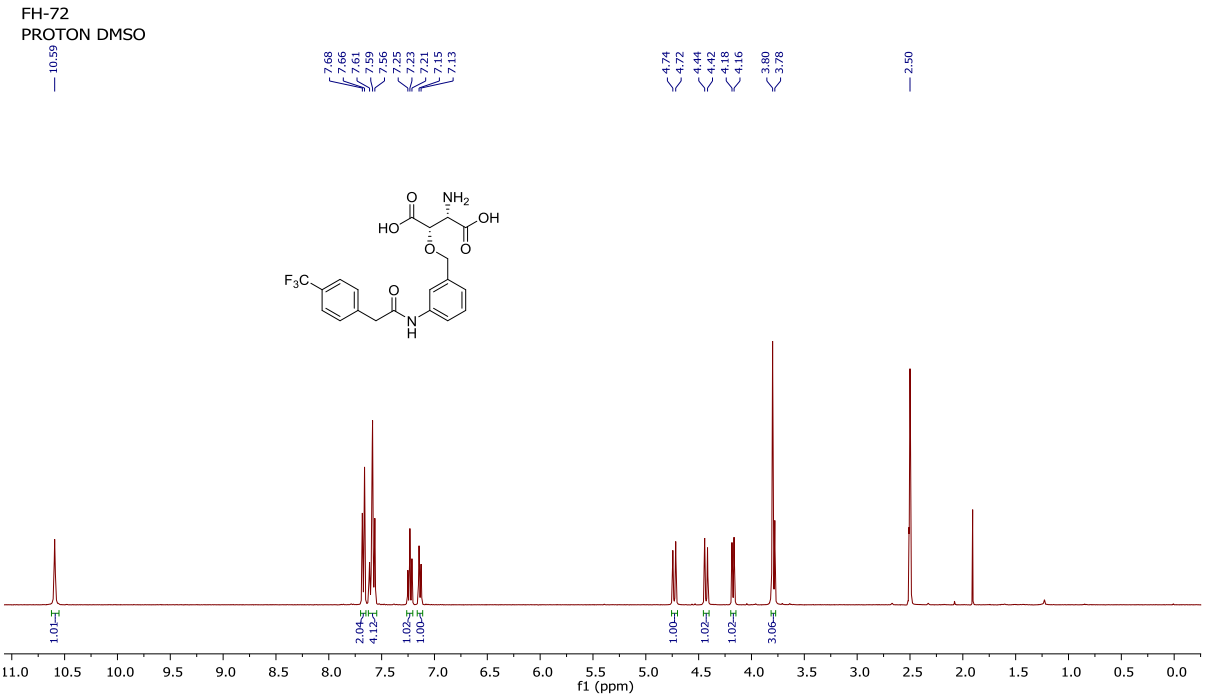


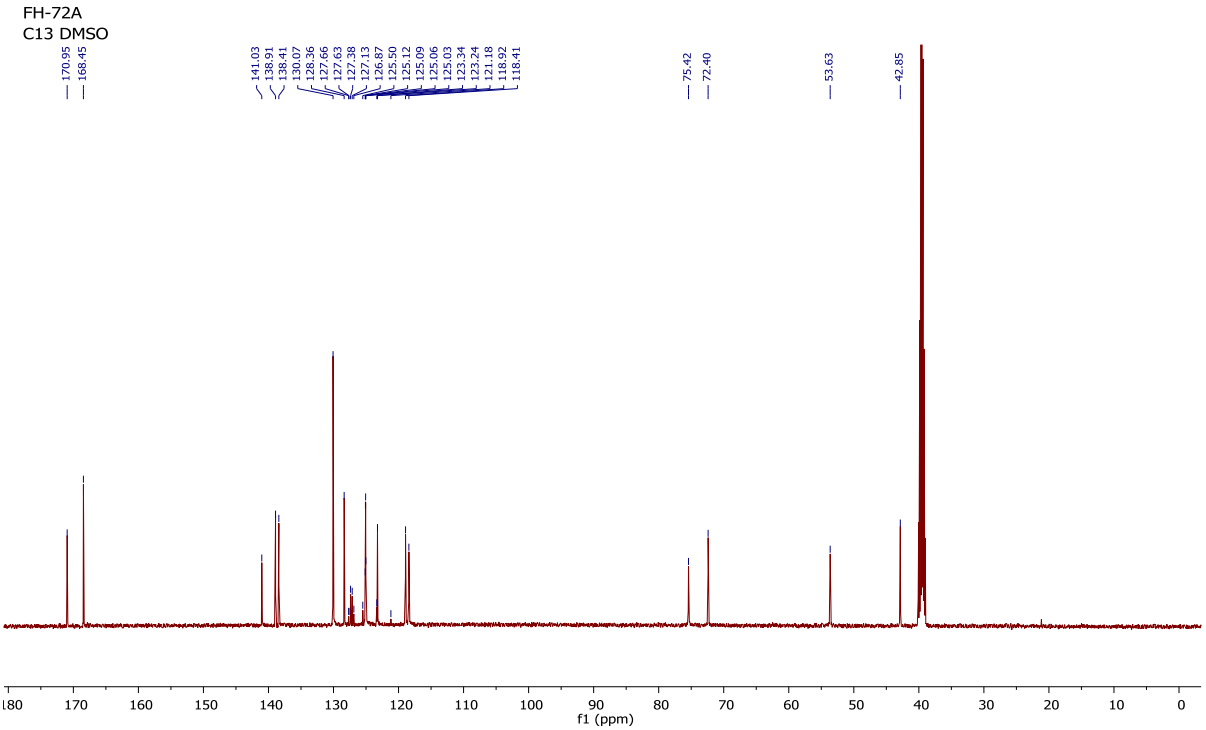
^{1}H NMR and ^{13}C NMR of compound **15b**

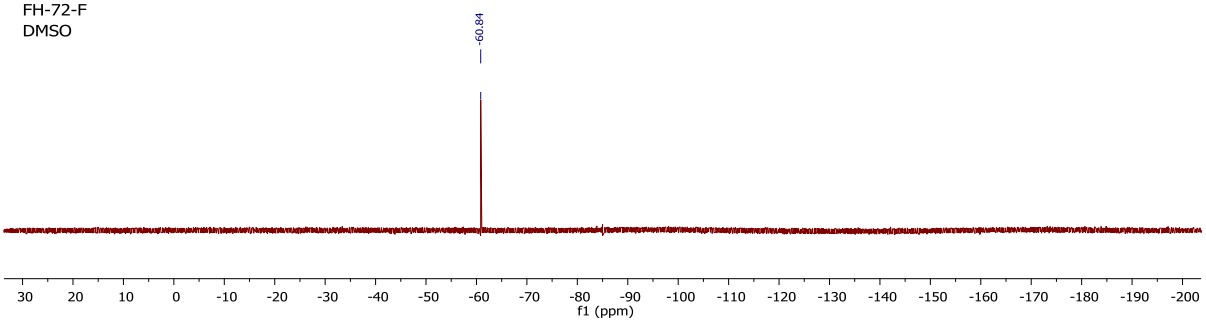




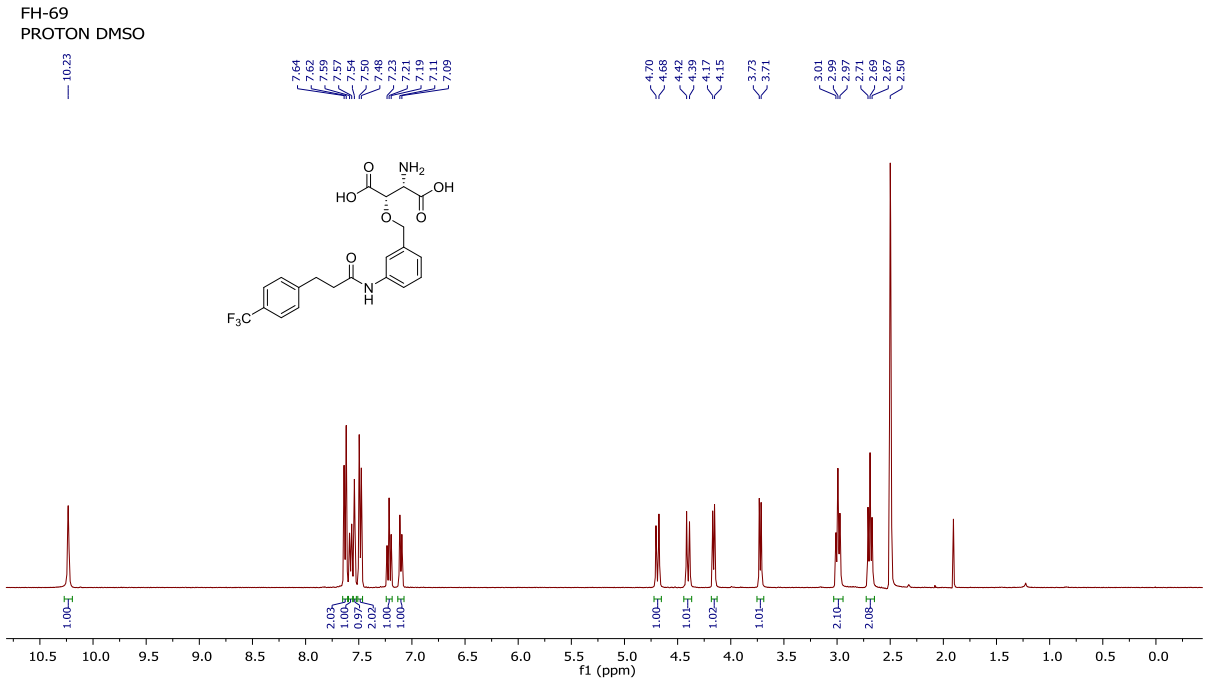
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **16a**

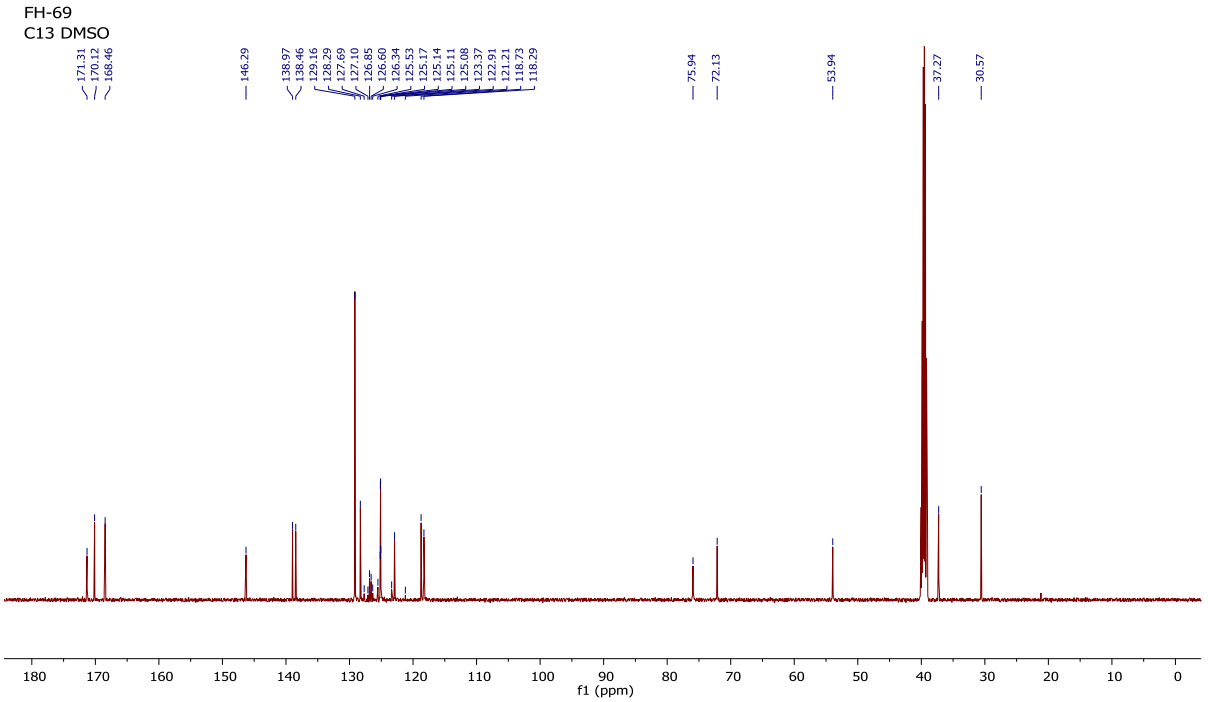


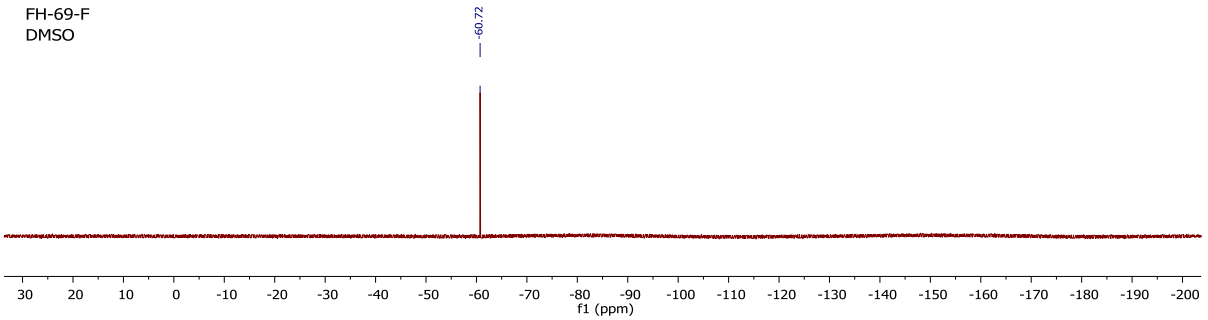




^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound **16b**

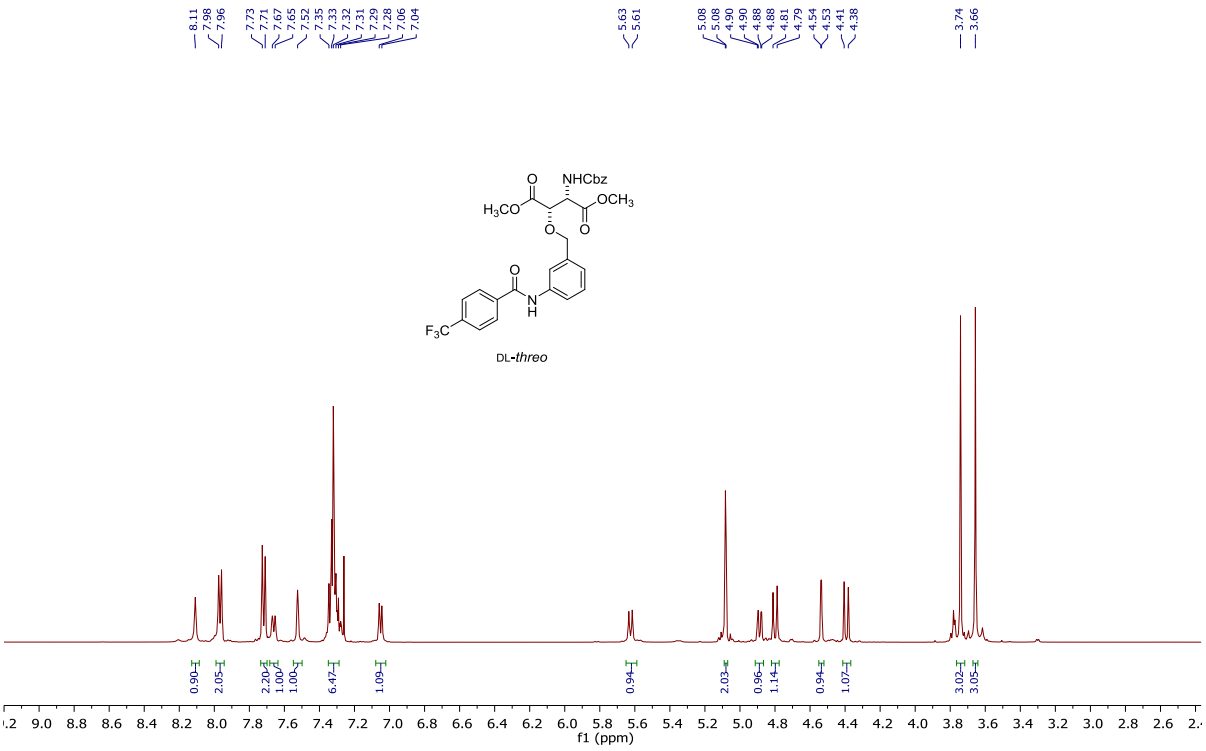




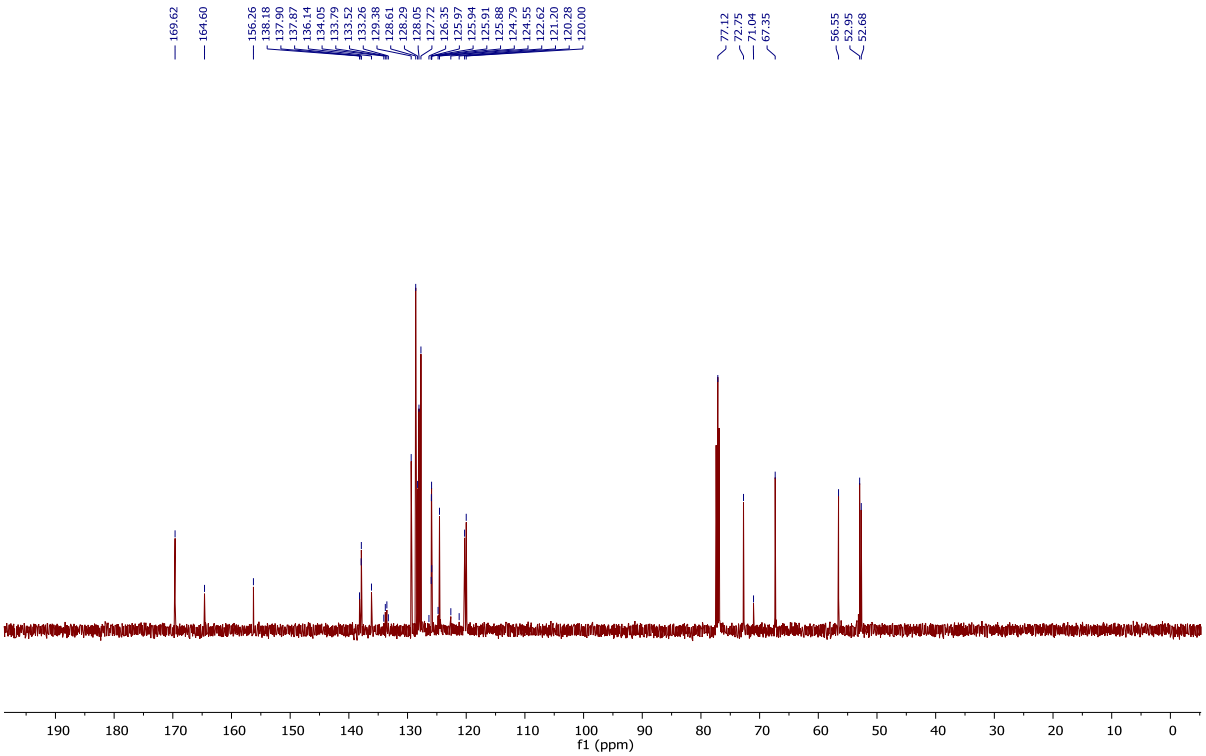


^{1}H NMR and ^{13}C NMR of compound **S10a**

FH-31
PROTON CDCl3

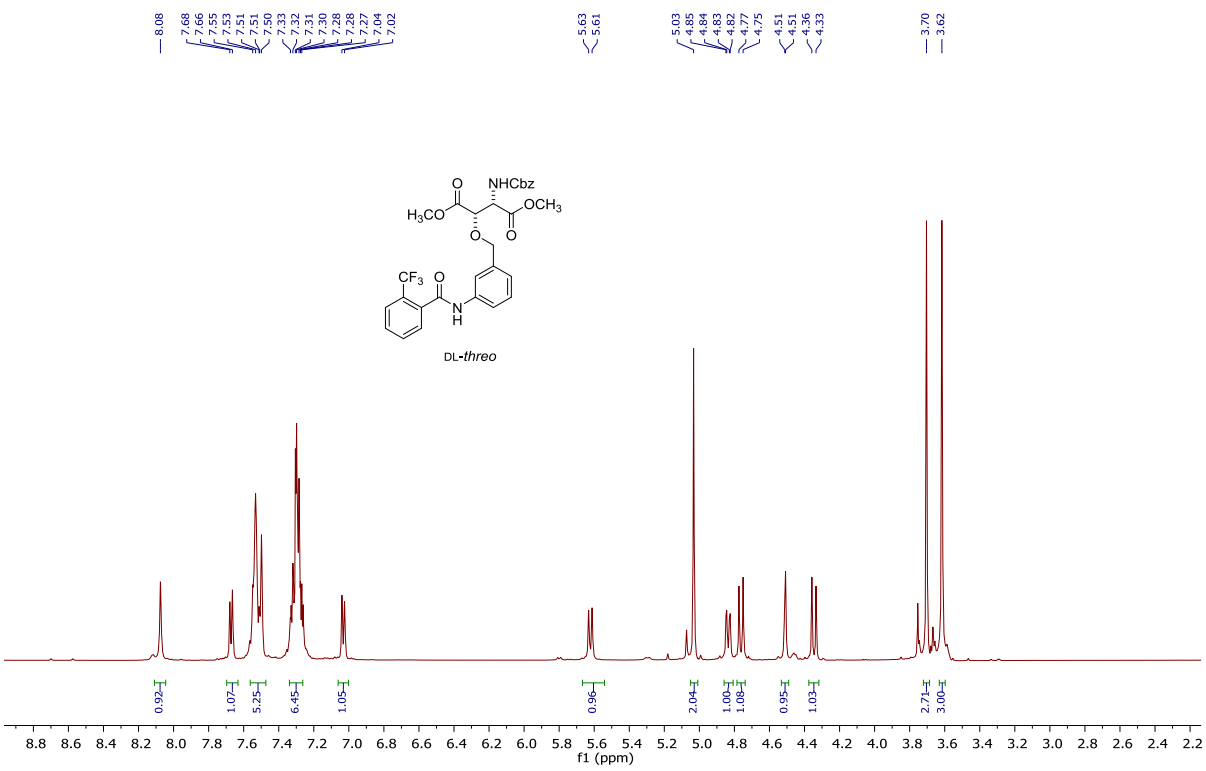


FH-31
C13 CDCl3

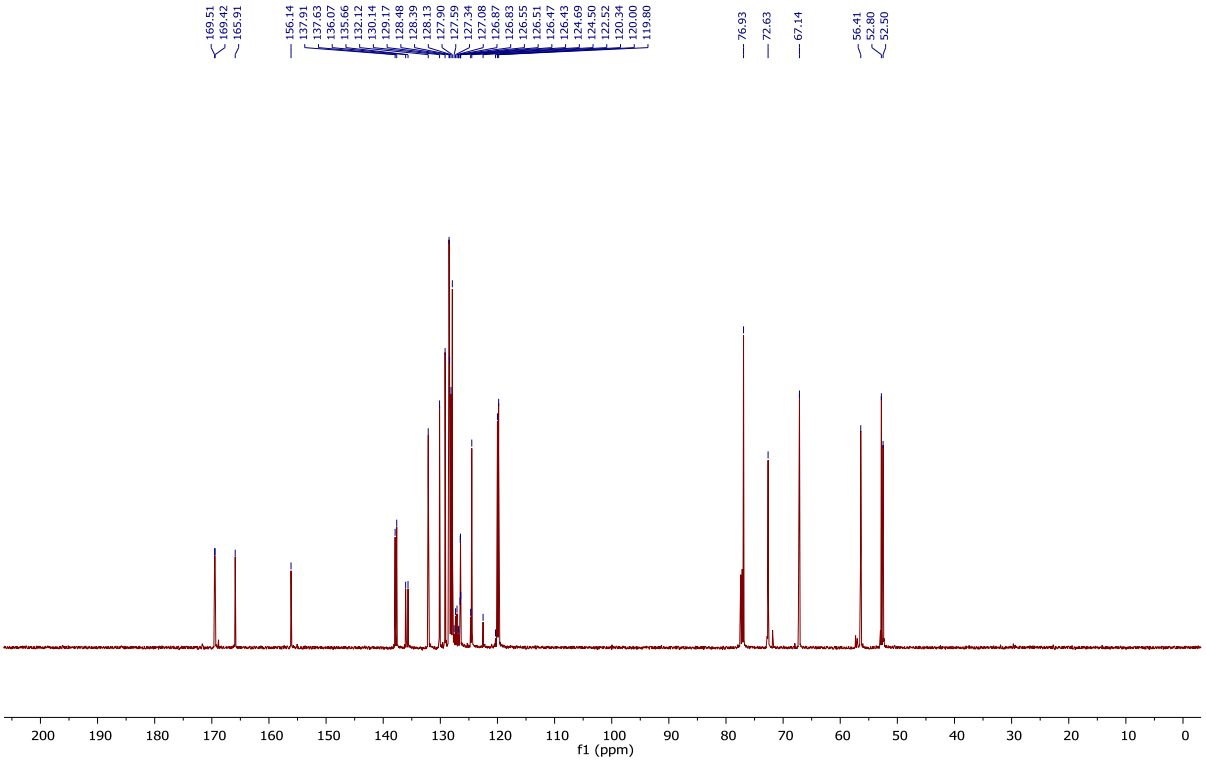


^{1}H NMR and ^{13}C NMR of compound **S10b**

FH-59
PROTON CDCl3

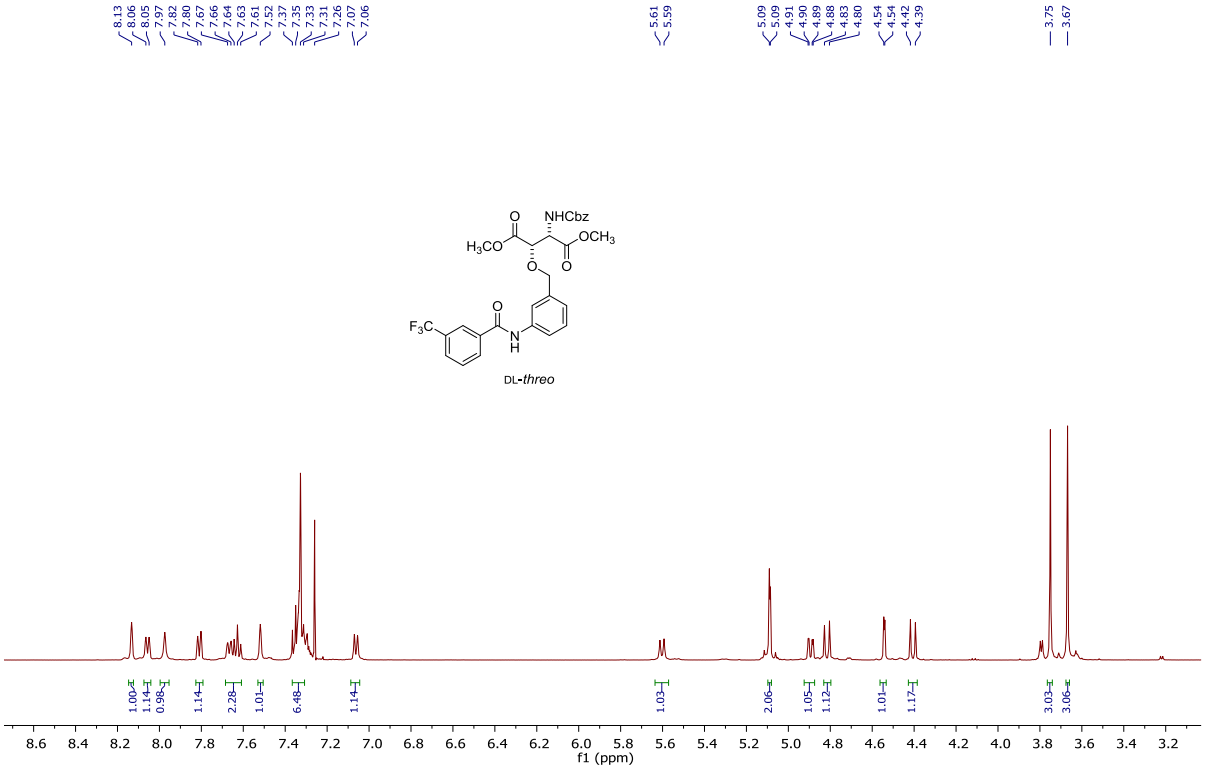


FH-59
C13 CDCl3

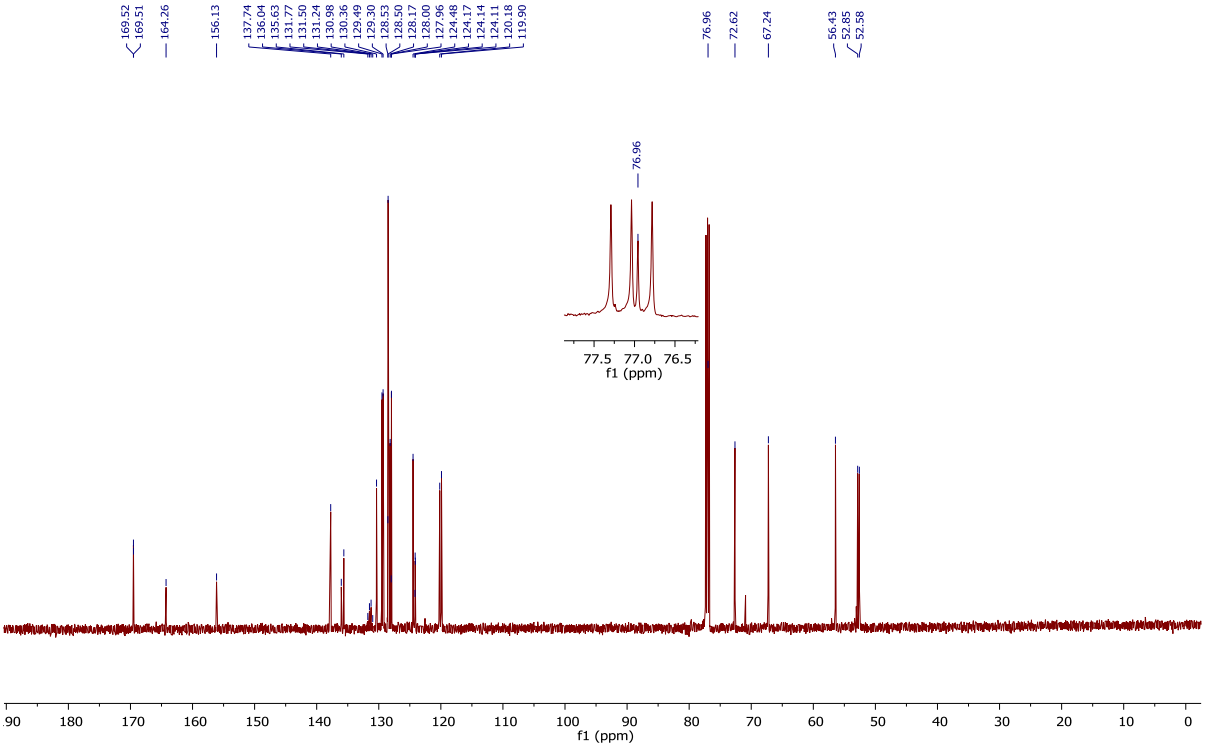


^{1}H NMR and ^{13}C NMR of compound **S10c**

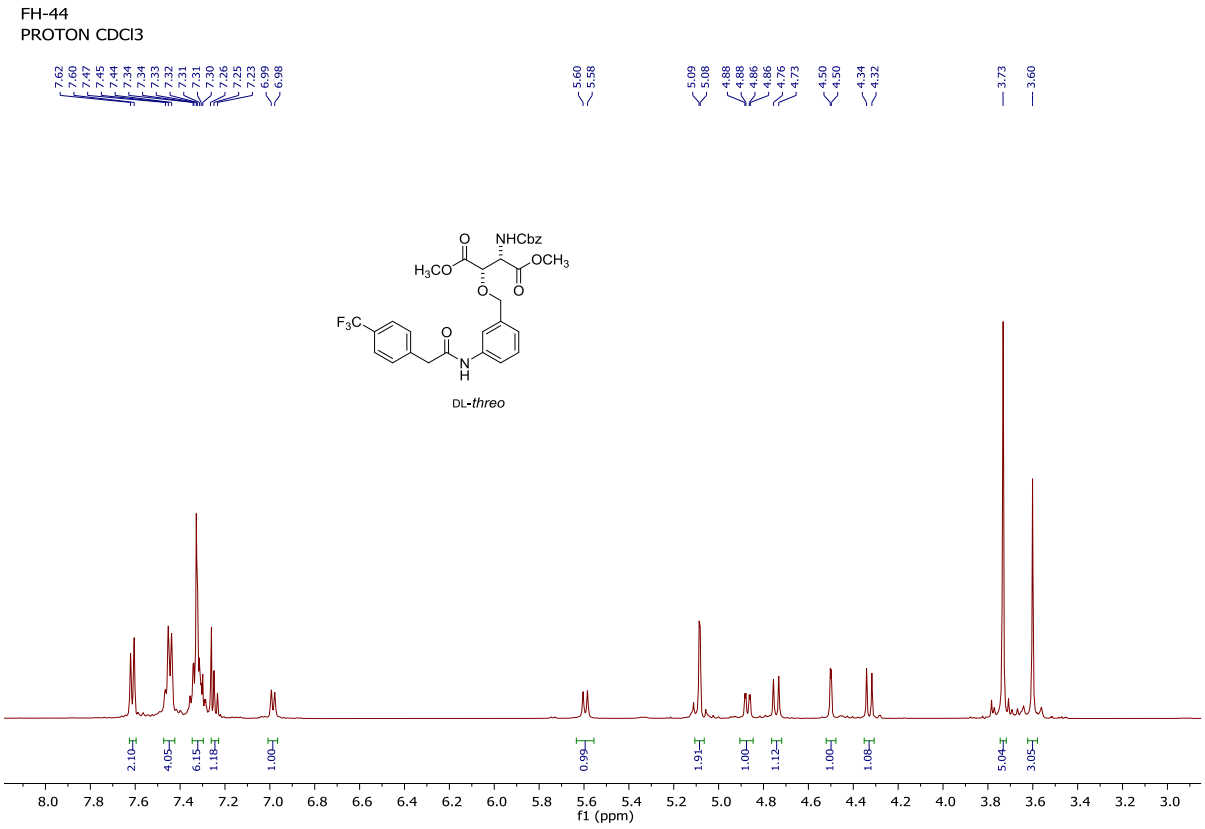
FH-56
PROTON CDCl3

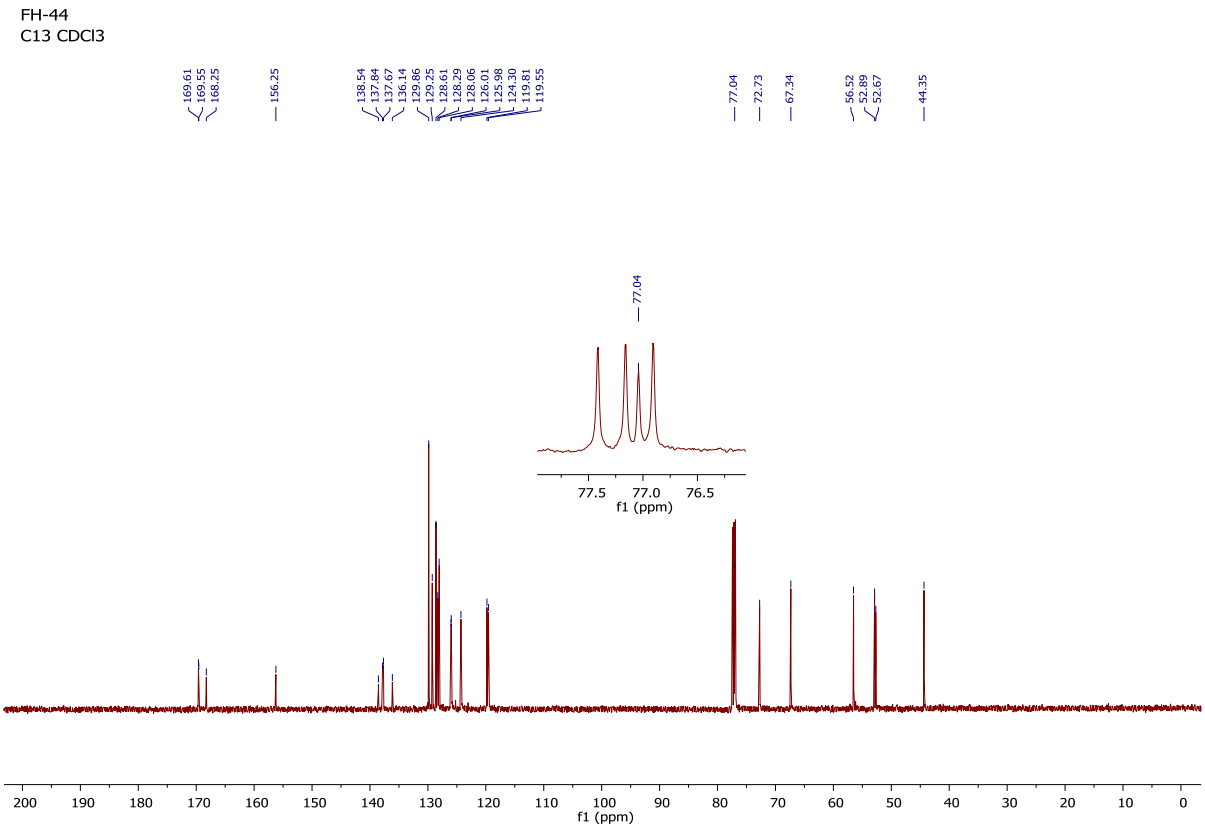


FH-56
C13 CDCl3

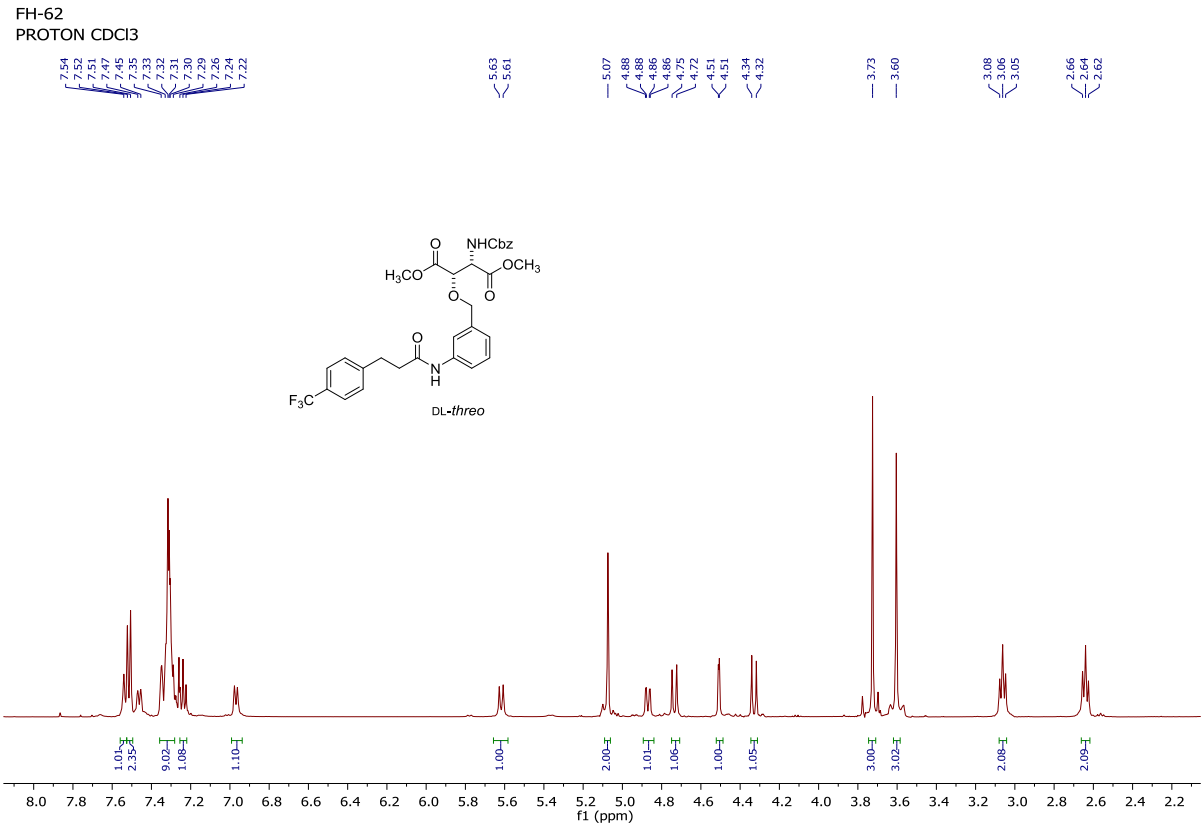


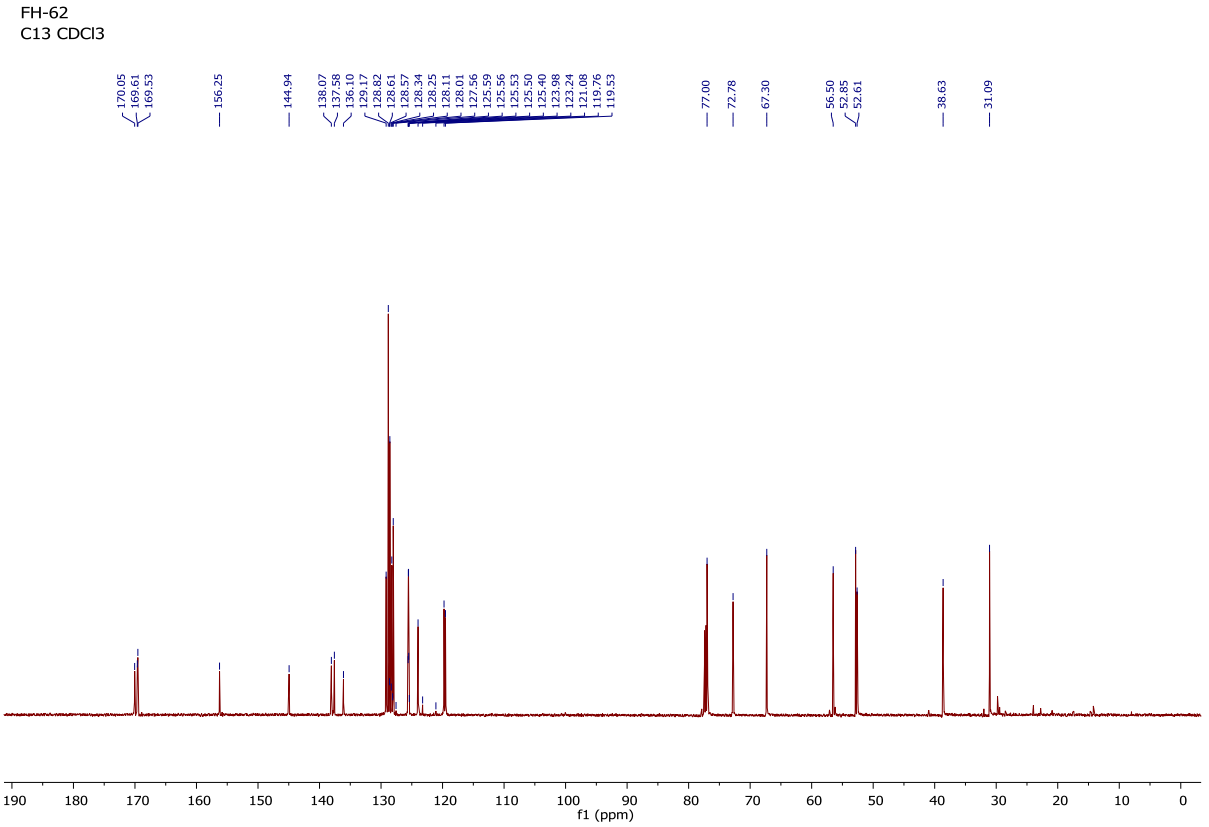
^{1}H NMR and ^{13}C NMR of compound **S10d**



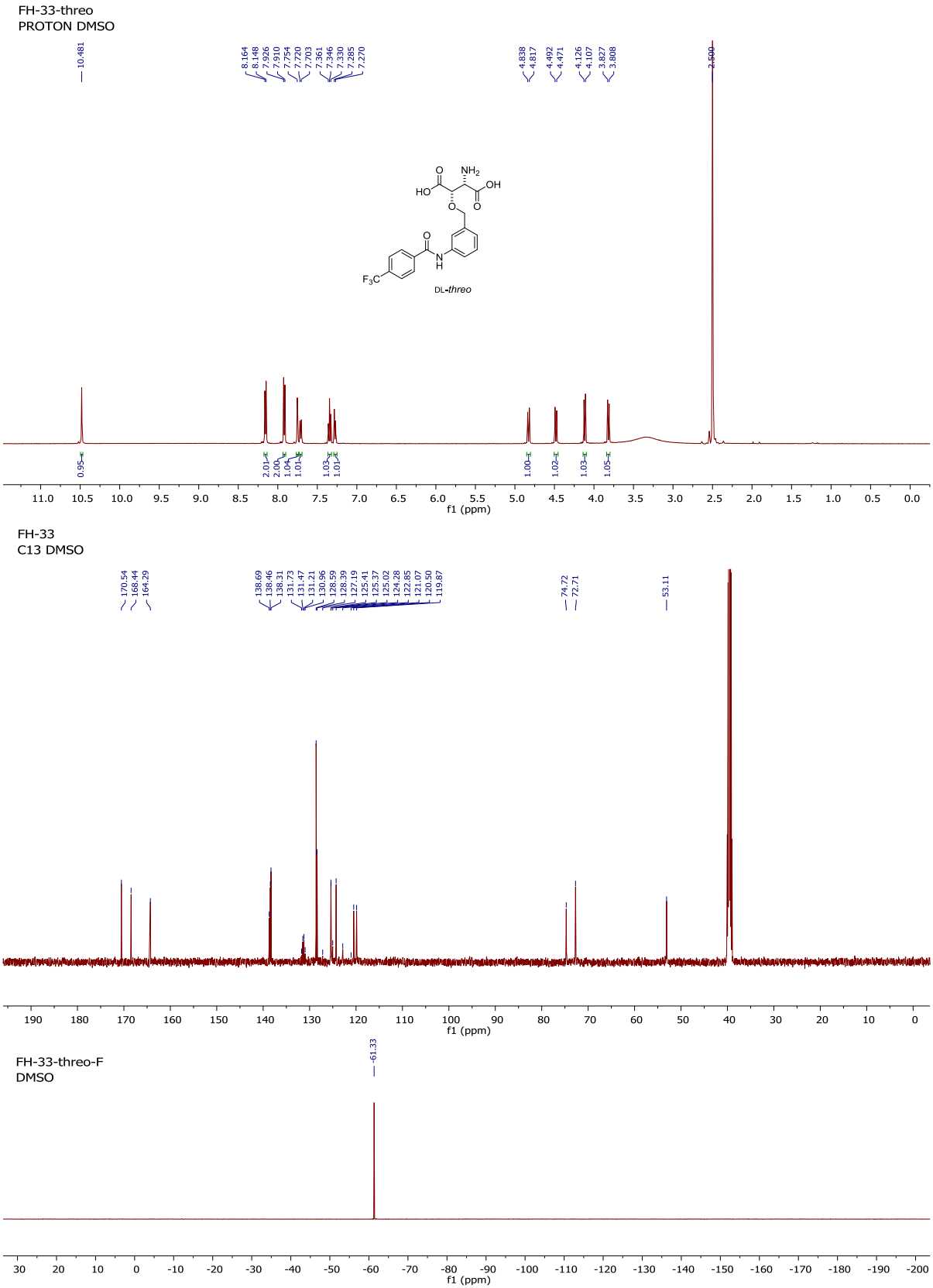


^{1}H NMR and ^{13}C NMR of compound **S10e**

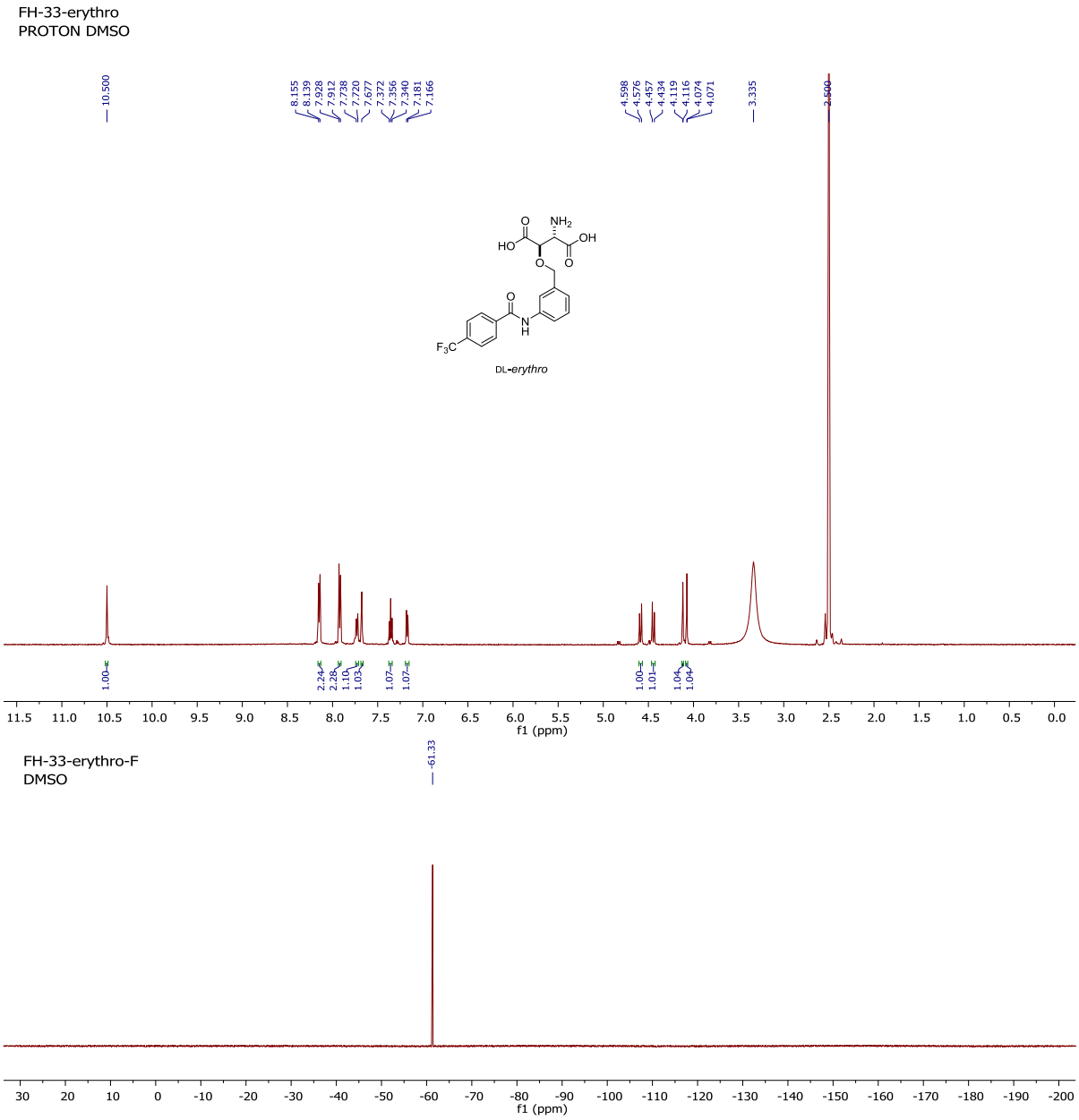




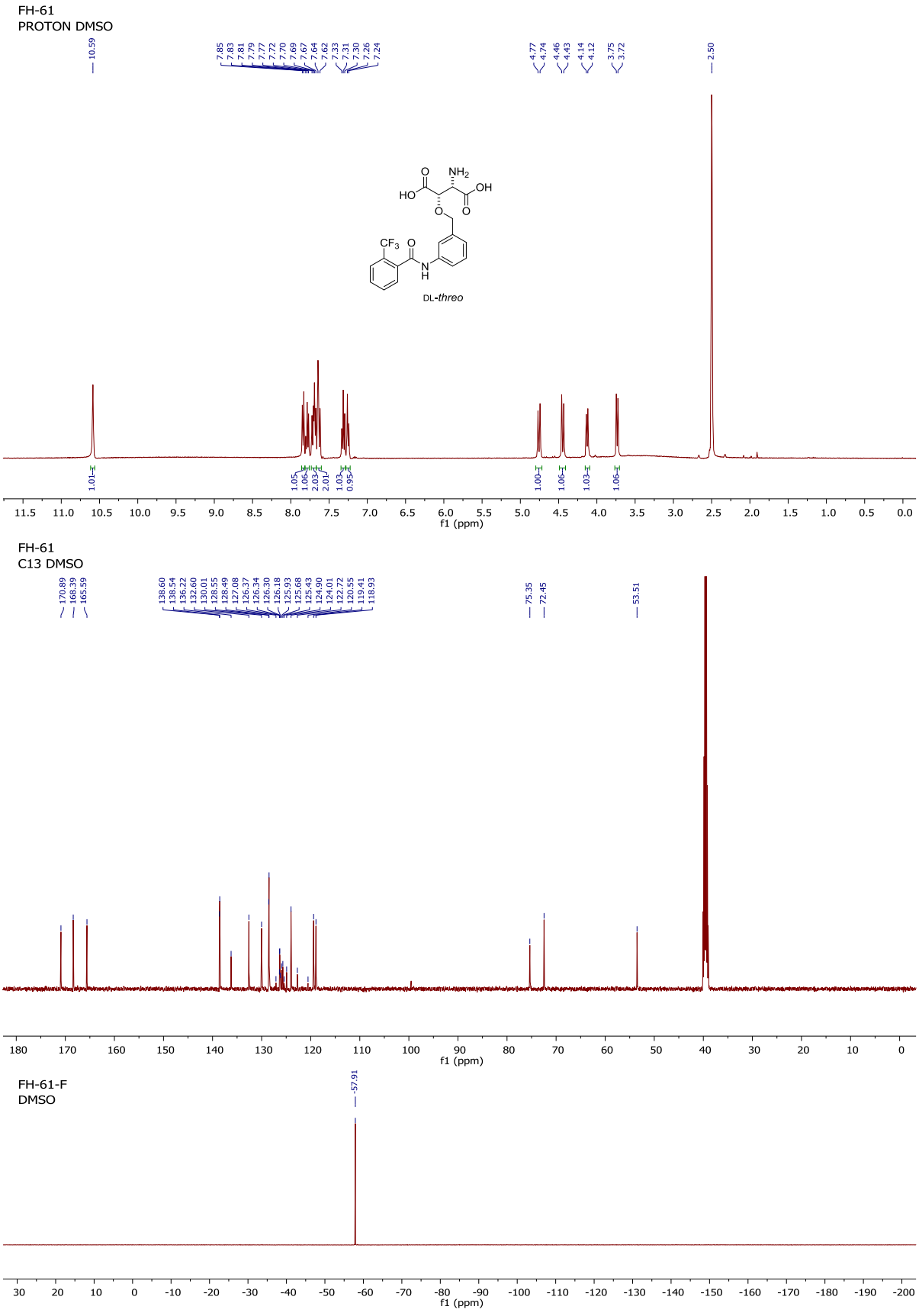
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound (DL-*threo*)-**S12a**



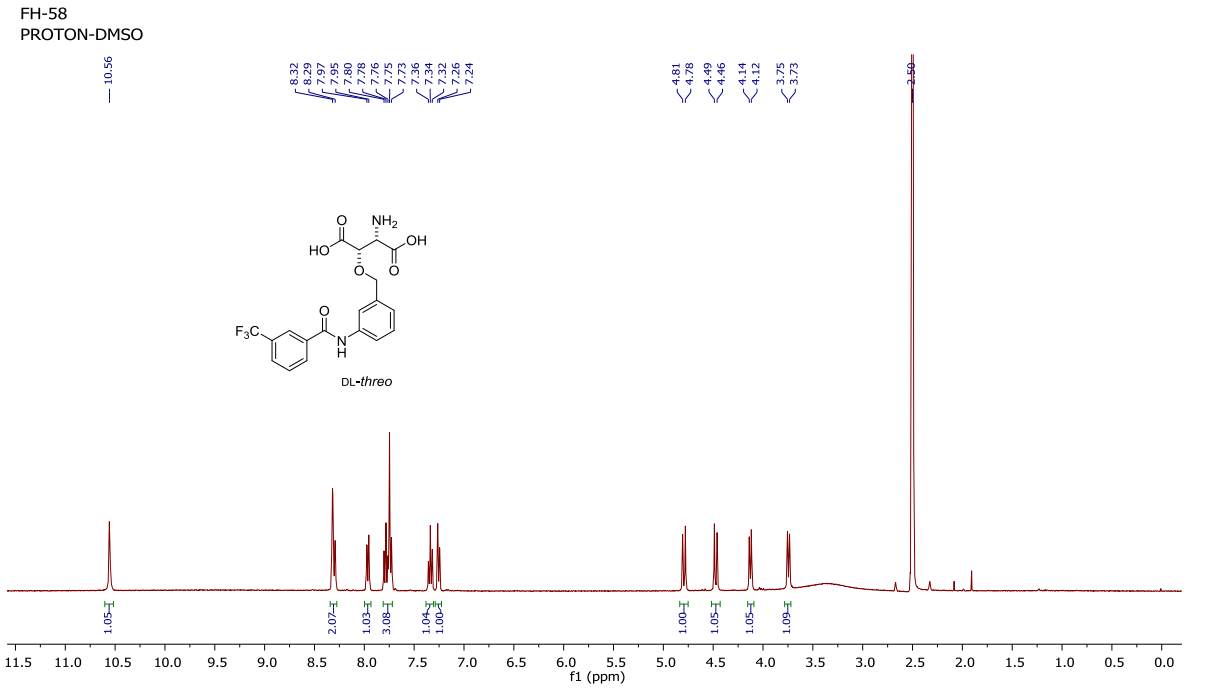
^{1}H NMR and ^{19}F NMR of compound (DL-*erythro*)-**S12a**

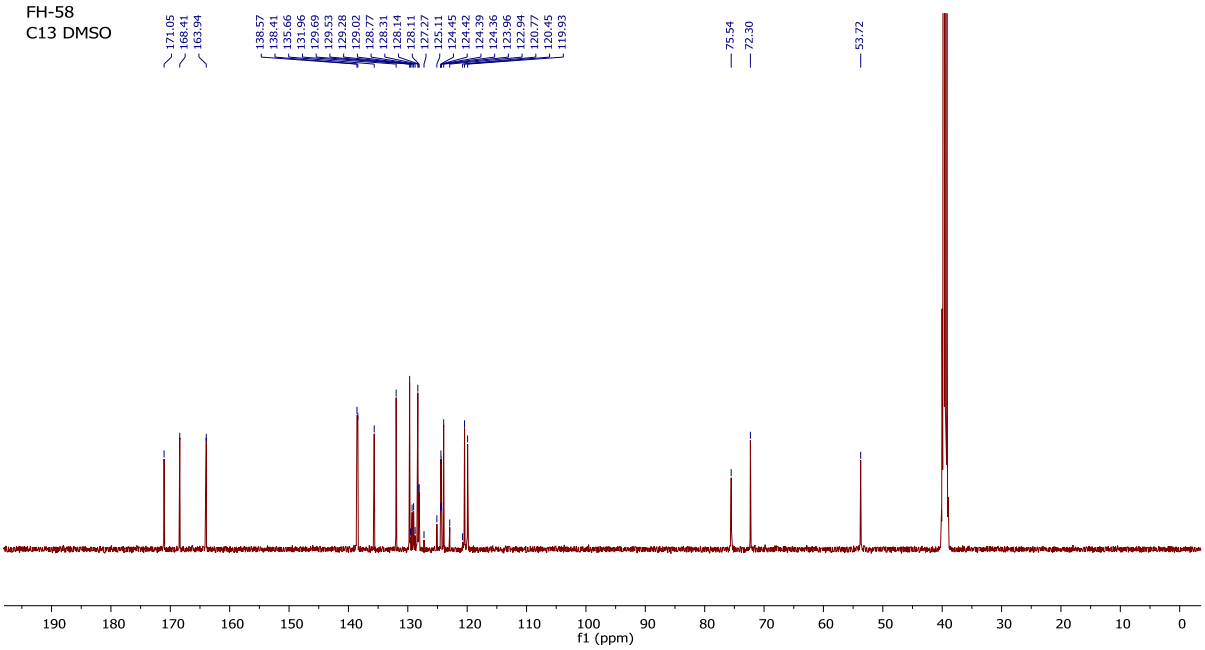


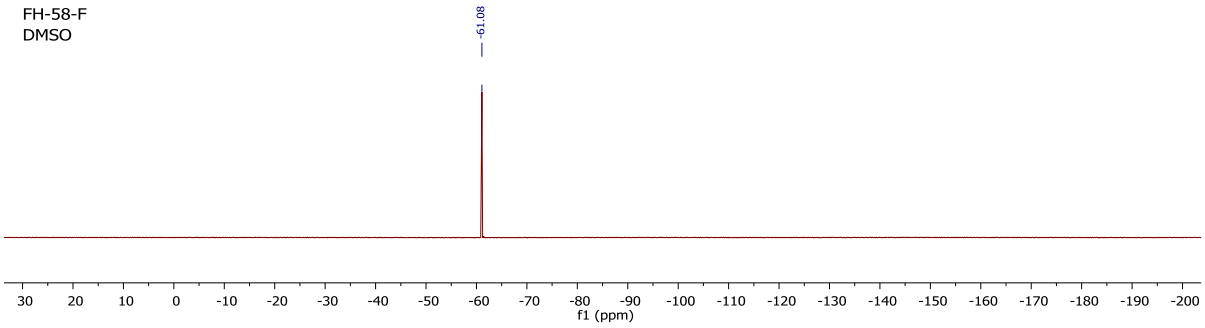
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound (DL-*threo*)-**S12b**



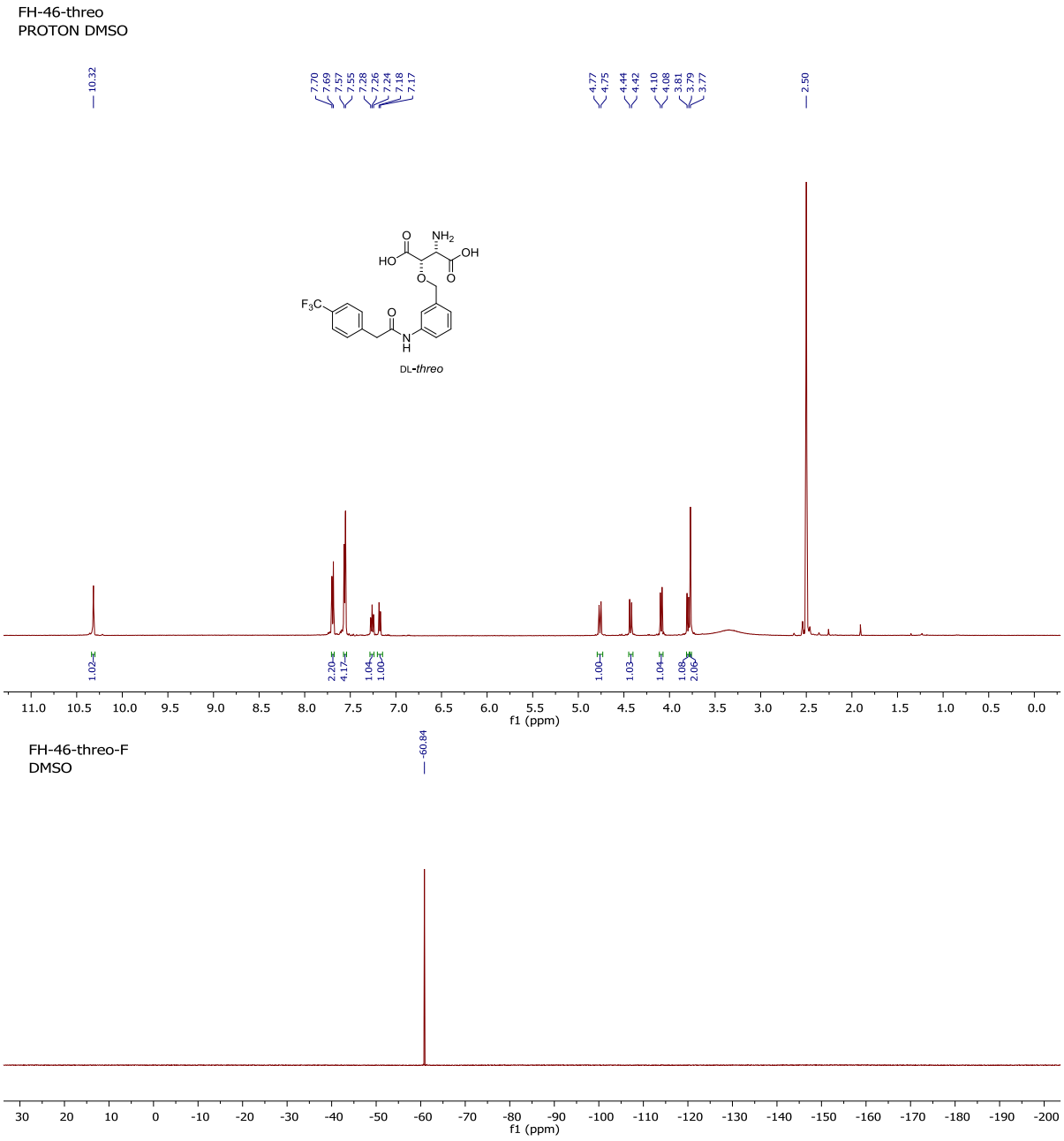
^{1}H NMR, ^{13}C NMR and ^{19}F NMR of compound (DL-*threo*)-**S12c**



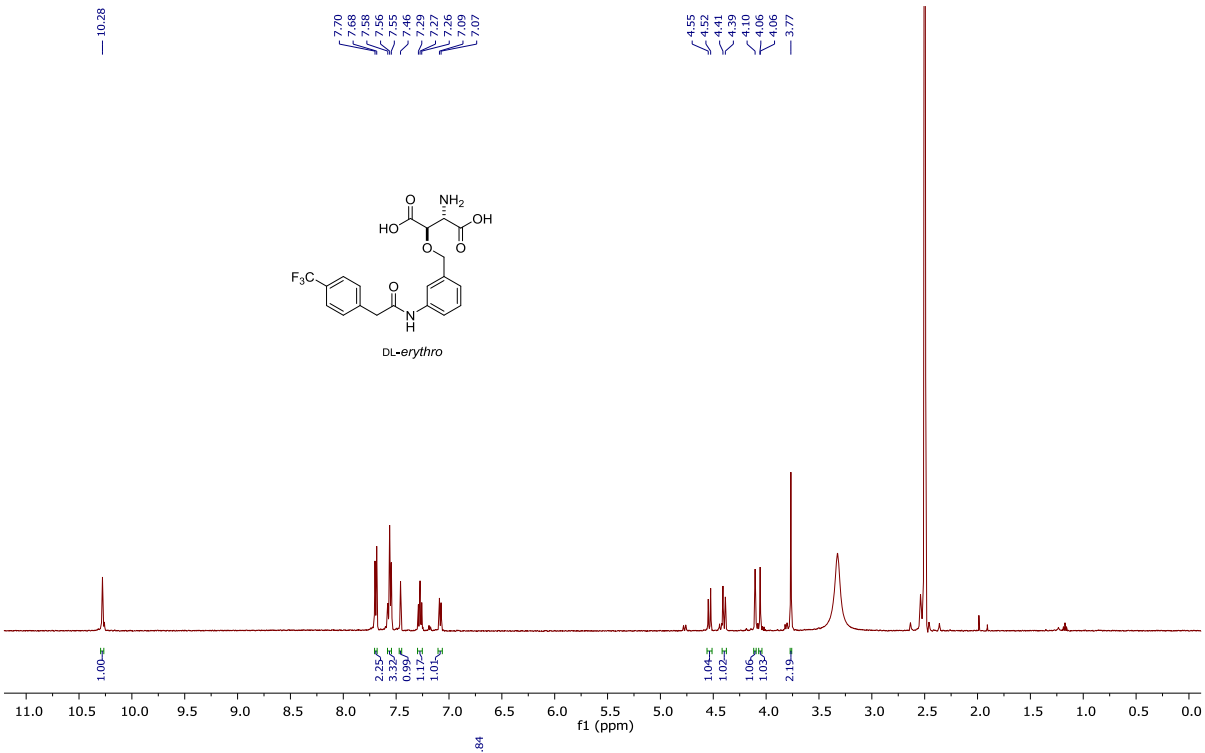


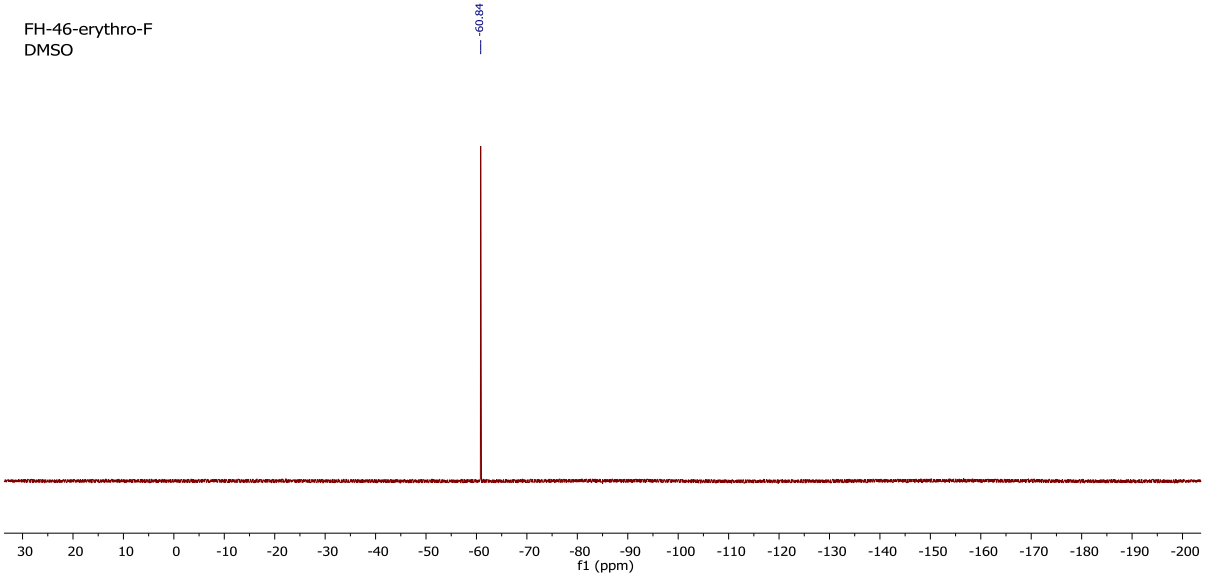


[1]H NMR and F NMR of compound (DL-*threo*)-**S12d**

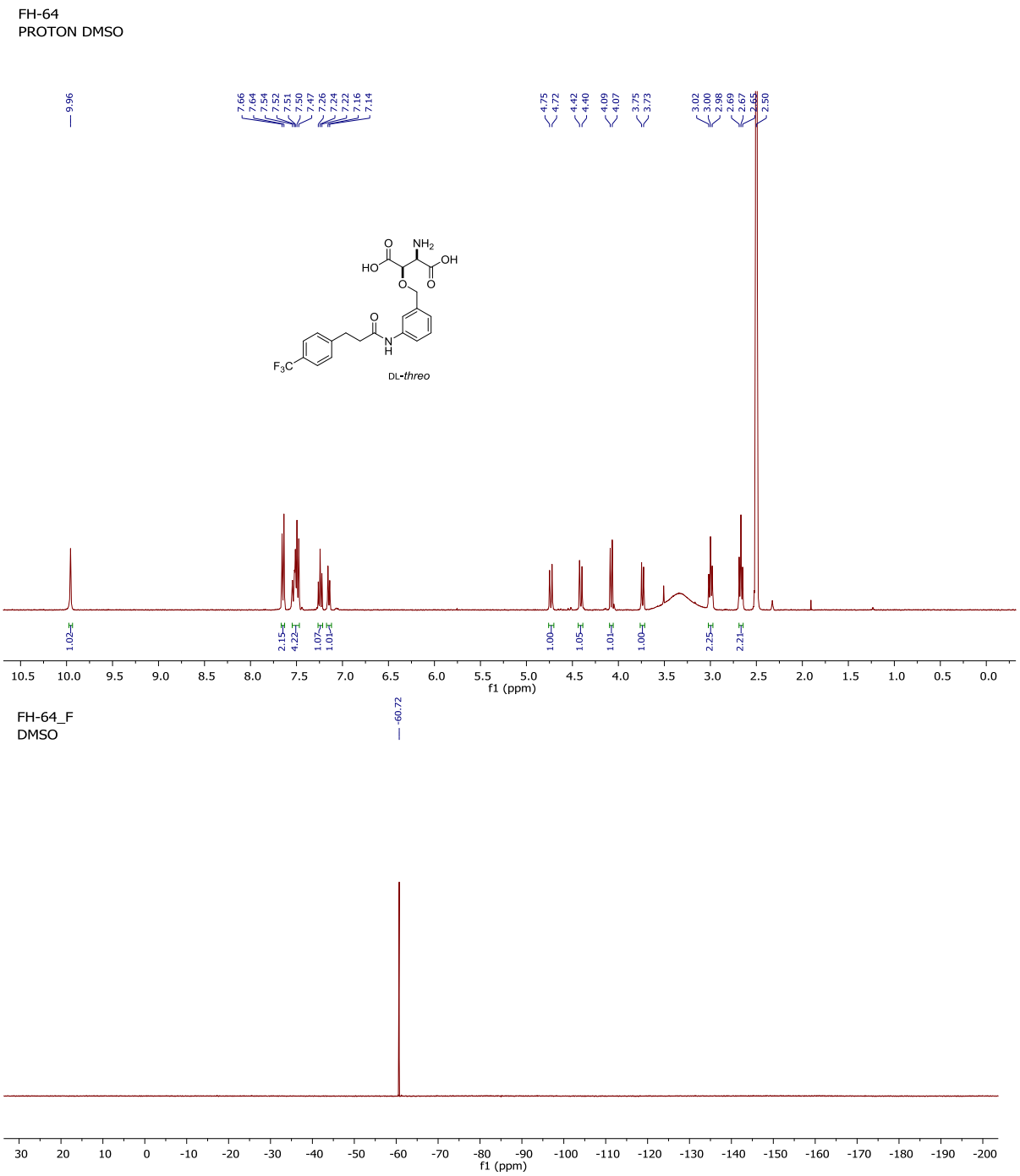


^{1}H NMR and ^{19}F NMR of compound (DL-*erythro*)-**S12d**





^{1}H NMR and ^{19}F NMR of compound (DL-*threo*)-**S12e**



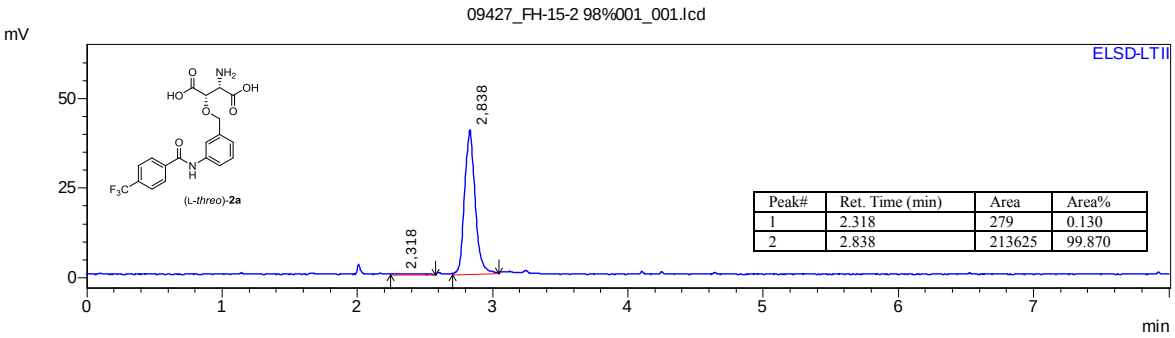
IV) Chiral HPLC analysis

General chiral HPLC conditions: CROWNPAK CR-I (+) 150 x 3 mm, 5 um. Phase A: ACN+0.5% TFA, phase B: H_2O+0.5% TFA, A/B = 98:2. Flow rate 0.4 mL/min, column temperature 25 °C, detected by ELSD at 35 °C.

1. **(L-*threo*)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (2a)**

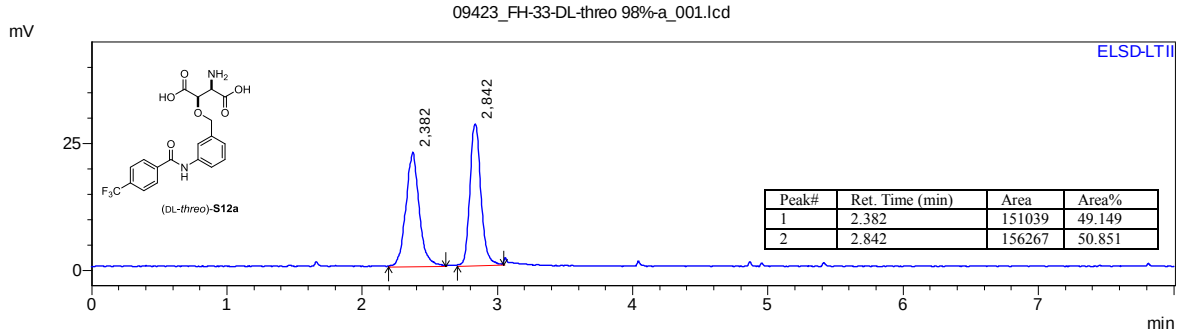
Chiral HPLC analysis indicated *ee* >99%, t_R (D-*threo*) = 2.4 min, t_R (L-*threo*) = 2.8 min.

1.1 HPLC analysis of (L-*threo*)-**2a**



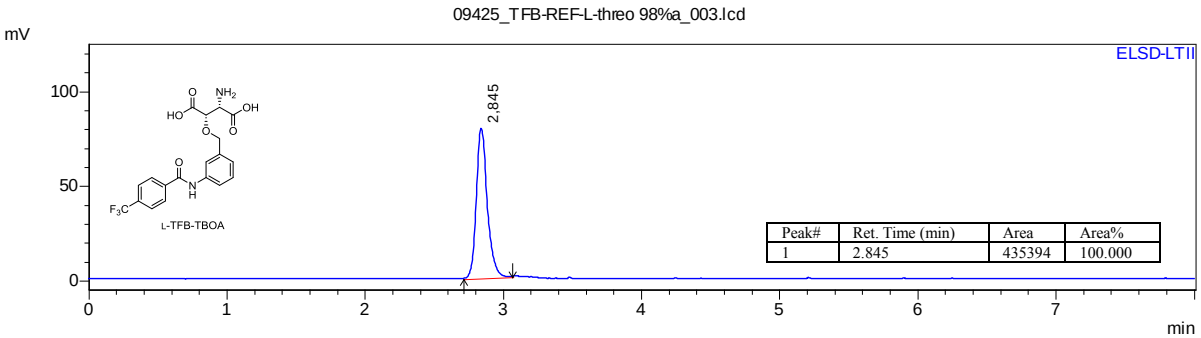
Peak#	Ret. Time (min)	Area	Area%
1	2.318	279	0.130
2	2.838	213625	99.870

1.2 HPLC analysis of (DL-*threo*)-**S12a**



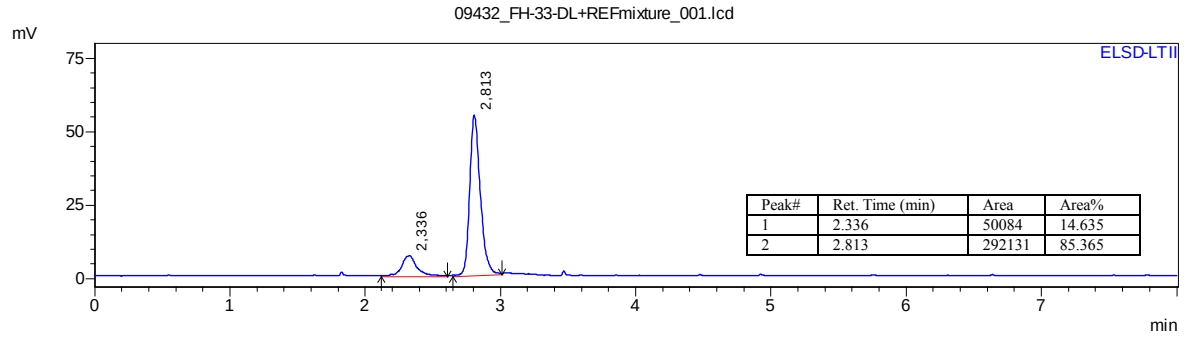
Peak#	Ret. Time (min)	Area	Area%
1	2.382	151039	49.149
2	2.842	156267	50.851

1.3 HPLC analysis of authentic reference of L-TFB-TBOA



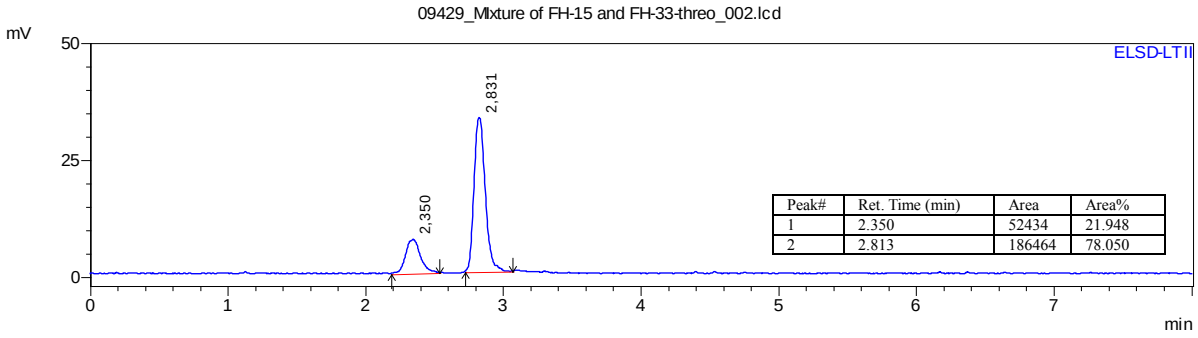
Peak#	Ret. Time (min)	Area	Area%
1	2.845	435394	100.000

1.4 Spiking of (DL-*threo*)-**S12a** with authentic reference (L-TFB-TBOA)



Peak#	Ret. Time (min)	Area	Area%
1	2.336	50084	14.635
2	2.813	292131	85.365

1.5 Spiking of (L-*threo*)-**2a** with (DL-*threo*)-**S12a**

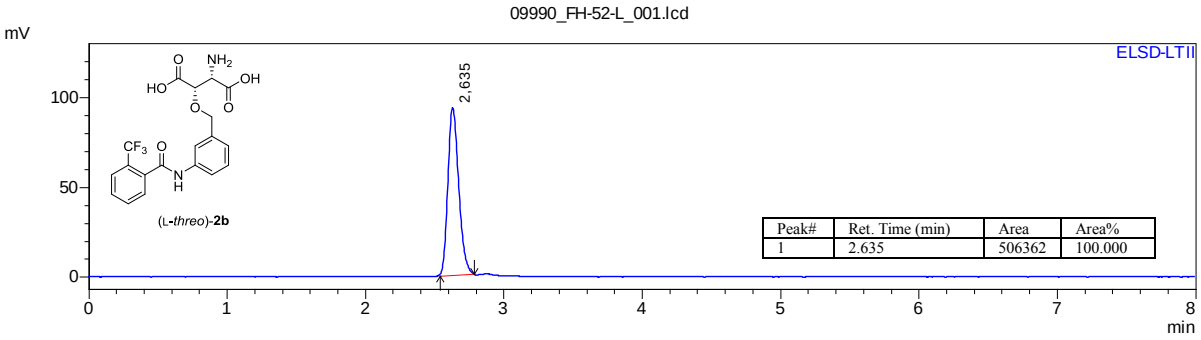


Peak#	Ret. Time (min)	Area	Area%
1	2.350	52434	21.948
2	2.813	186464	78.050

2. (L-*threo*)-3-[3-[2-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (2b)

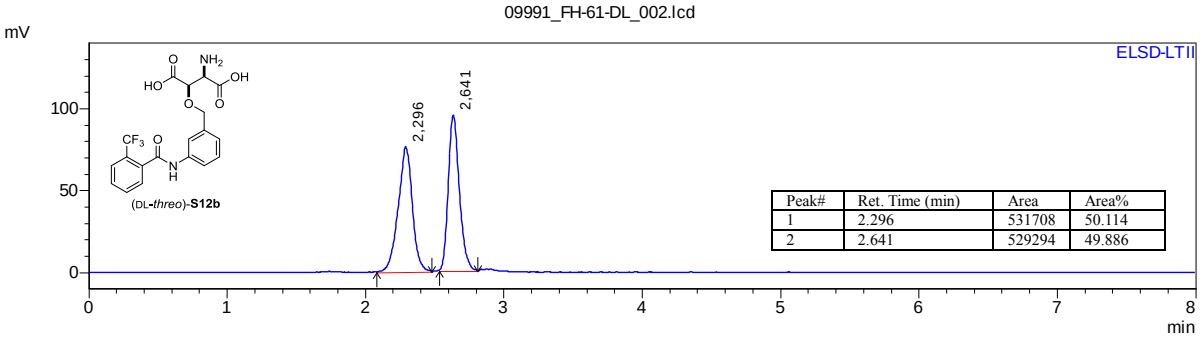
HPLC analysis indicated *ee* >99%, t_R (D-*threo*) = 2.3 min, t_R (L-*threo*) = 2.6 min.

2.1 HPLC analysis of (L-*threo*)-**2b**



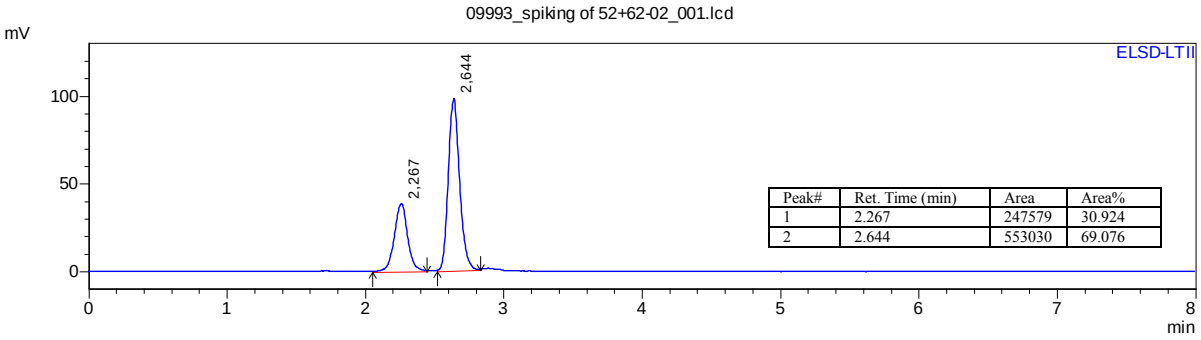
Peak#	Ret. Time (min)	Area	Area%
1	2.635	506362	100.000

2.2 HPLC analysis of (DL-*threo*)-**S12b**



Peak#	Ret. Time (min)	Area	Area%
1	2.296	531708	50.114
2	2.641	529294	49.886

2.3 Spiking of (L-*threo*)-**2b** with (DL-*threo*)-**S12b**

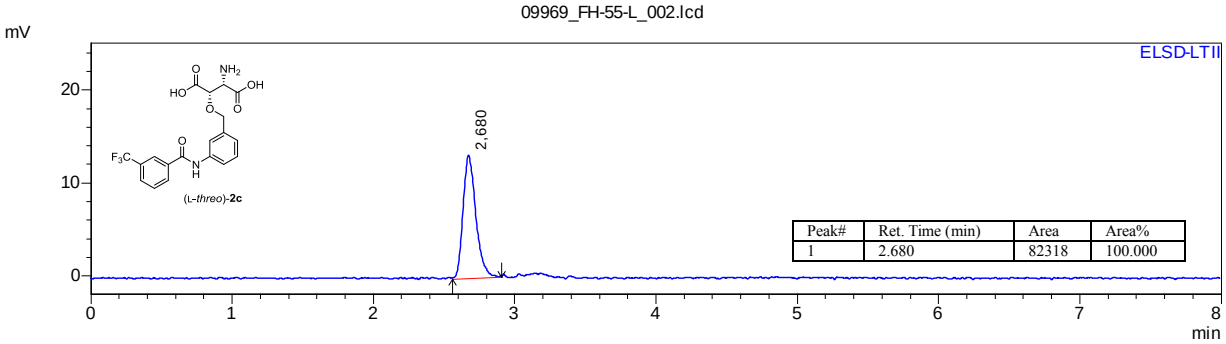


Peak#	Ret. Time (min)	Area	Area%
1	2.267	247579	30.924
2	2.644	553030	69.076

3. (L-*threo*)-3-[3-[3-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (2c)

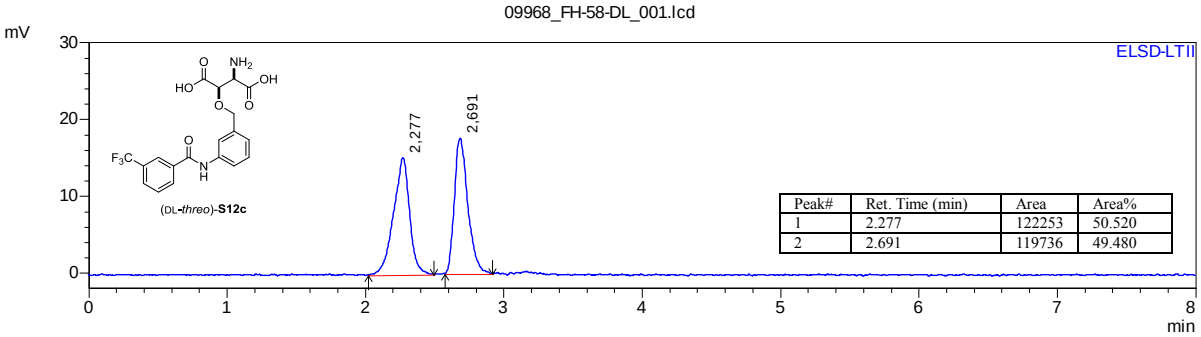
HPLC analysis indicated e.e. >99%, t_R (D-*threo*) = 2.3 min, t_R (L-*threo*) = 2.7 min.

3.1 HPLC analysis of (L-*threo*)-**2c**



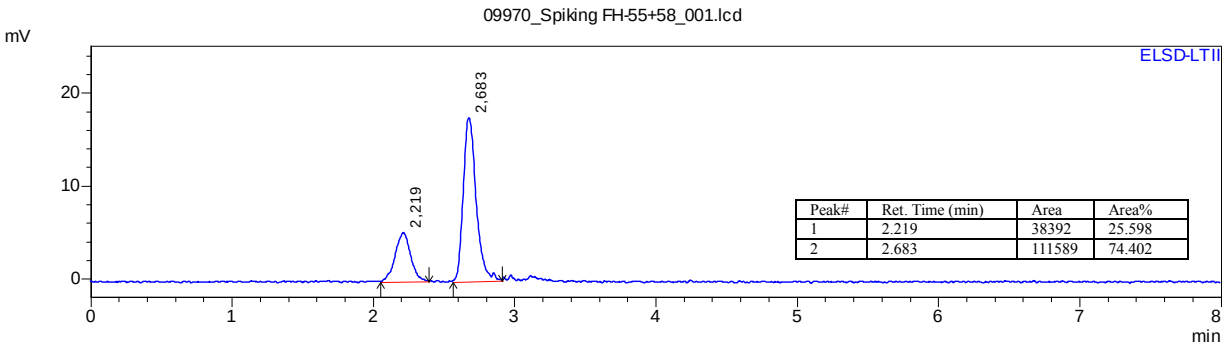
Peak#	Ret. Time (min)	Area	Area%
1	2.680	82318	100.000

3.2 HPLC analysis of (DL-*threo*)-**S12c**



Peak#	Ret. Time (min)	Area	Area%
1	2.277	122253	50.520
2	2.691	119736	49.480

3.3 Spiking of (L-*threo*)-**2c** with (DL-*threo*)-**S12c**

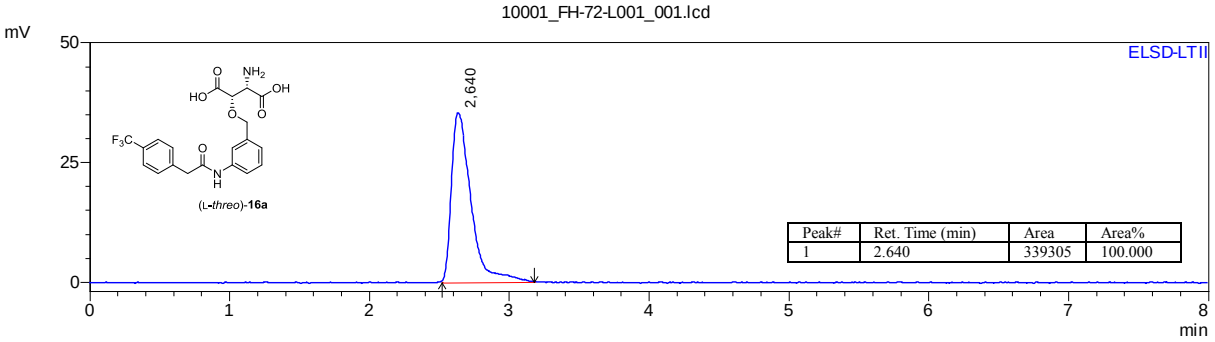


Peak#	Ret. Time (min)	Area	Area%
1	2.219	38392	25.598
2	2.683	111589	74.402

4. (L-*threo*)-3-[3-[2-[4-(trifluoromethyl)phenyl]acetamido]benzyloxy]aspartate (16a)

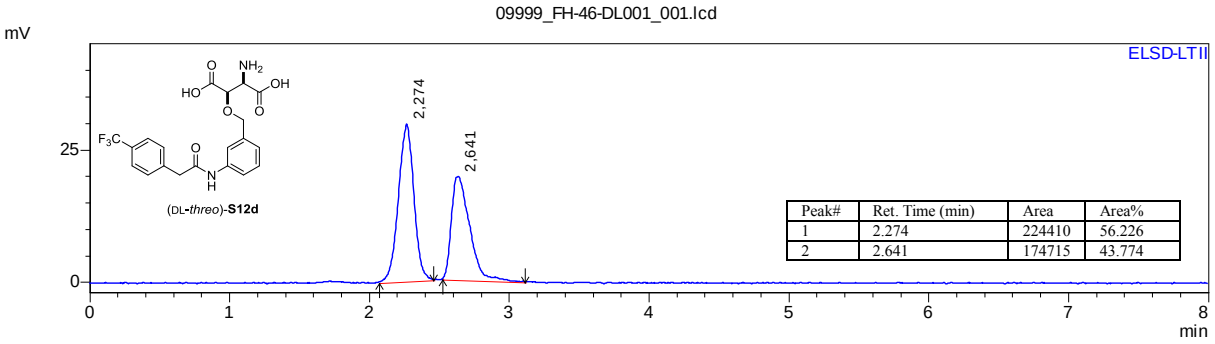
HPLC analysis indicated *ee* >99%, t_R (D-*threo*) = 2.3 min, t_R (L-*threo*) = 2.6 min.

4.1 HPLC analysis of (L-*threo*)-**16a**



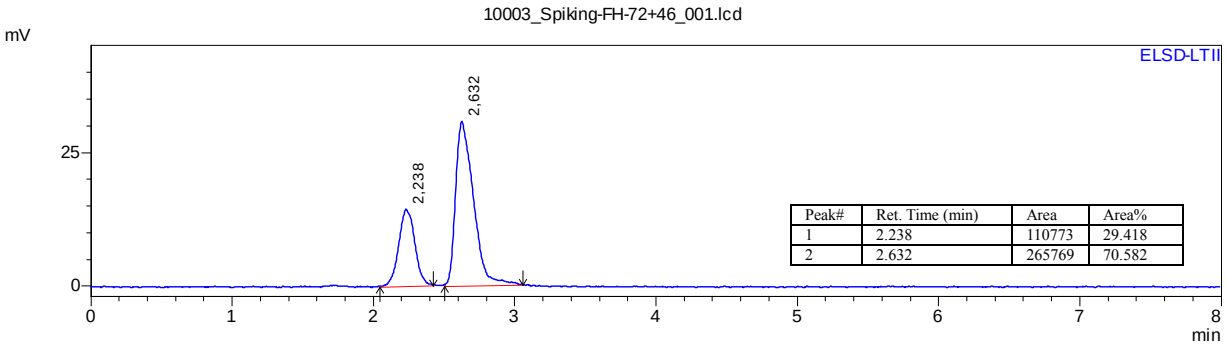
Peak#	Ret. Time (min)	Area	Area%
1	2.640	339305	100.000

4.2 HPLC analysis of (DL-*threo*)-**S12d**



Peak#	Ret. Time (min)	Area	Area%
1	2.274	224410	56.226
2	2.641	174715	43.774

4.3 Spiking of (L-*threo*)-**16a** with (DL-*threo*) **S12d**

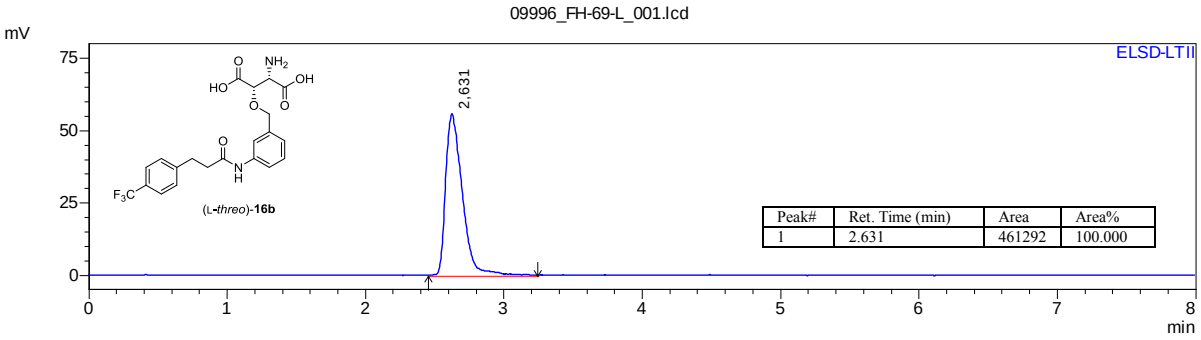


Peak#	Ret. Time (min)	Area	Area%
1	2.238	110773	29.418
2	2.632	265769	70.582

5. (L-*threo*)-3-[3-[3-[4-(trifluoromethyl)phenyl]propanamido]benzyloxy]aspartate (16b)

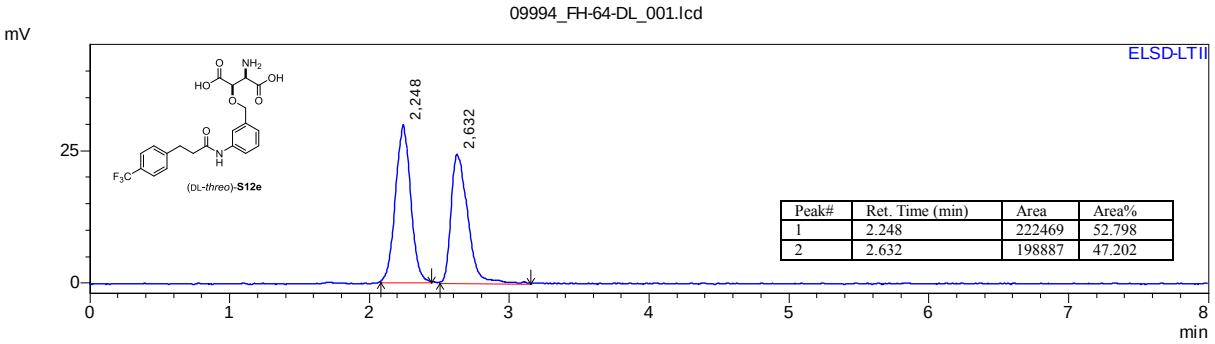
HPLC analysis indicated *ee* >99%, t_R (D-*threo*) = 2.2 min, t_R (L-*threo*) = 2.6 min.

5.1 HPLC analysis of (L-*threo*)-**16b**



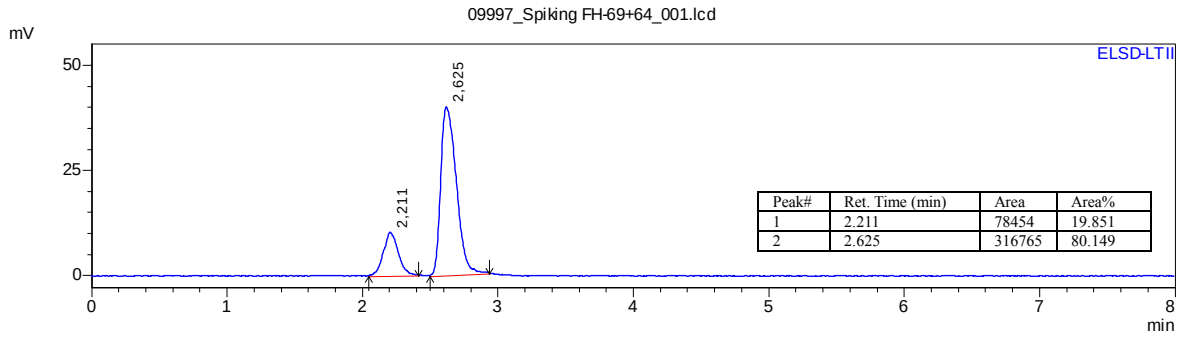
Peak#	Ret. Time (min)	Area	Area%
1	2.631	461292	100.000

5.2 HPLC analysis of (DL-*threo*)-**S12e**



Peak#	Ret. Time (min)	Area	Area%
1	2.248	222469	52.798
2	2.632	198887	47.202

5.3 Spiking of (L-*threo*)-**16b** with (DL-*threo*)-**S12e**



Peak#	Ret. Time (min)	Area	Area%
1	2.211	78454	19.851
2	2.625	316765	80.149

V) References

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