Supplementary Information

Methyl to trifluoromethyl substitution as a strategy to increase membrane permeability of short peptides

Takahiro Ono,[a] Kohsuke Aikawa,[a] Takashi Okazoe[a,b] Jumpei Morimoto*[a] and Shinsuke Sando*[a,c]

[a] *Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8656, Japan*

[b] *Material Integration Laboratories, Yokohama Technical Center, AGC Inc., Yokohama 230-0045, Japan.*

[c] *Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8656, Japan*

**e-mail: jmorimoto@chembio.t.u-tokyo.ac.jp, ssando@chembio.t.u-tokyo.ac.jp*

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Materials and Methods

Abbreviations

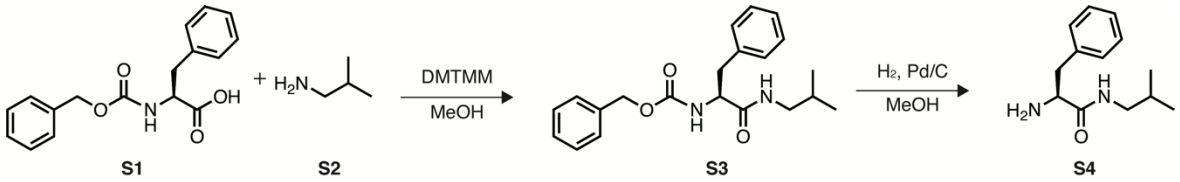
ACN, acetonitrile; Ac_2O, acetic anhydride; Boc_2O, di-*tert*-butyl dicarbonate; Cbz, carbobenzoxy; Cbz-OSu, *N*-(benzyloxycarbonyloxy)succinimide; CTC resin, 2-chlorotrityl chloride resin; DCM, dichloromethane; DIC, *N*,*N*'-diisopropylcarbodiimide; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; DMTMM, 4-(4,6-dimehoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; EtOAc, ethyl acetate; Fmoc, 9-fluorenylmethyloxycarbonyl; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; HOAt, *3H*-[1,2,3]Triazolo[4,5-*b*]pyridin-3-ol; HPLC, high performance liquid chromatography; UPLC, ultra performance liquid chromatography; MeOH, methanol; NMP, *N*-methylpyrrolidone; Oxyma, ethyl cyano(hydroxyimino)acetate; PBS, phosphate-buffer saline; PyAOP, (7-Azabenzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIPS, triisopropylsilane.

General

Chemicals and solvents used in this study were purchased from commercial suppliers and used without further purification. Preparative HPLC was performed on a Prominence HPLC system (Shimadzu) with a $5C_{18}$-AR-II column (Nacalai tesque, 10 mm I.D.×150 mm, 34350-41). All the HPLC were performed using two solvents (solvent A: H_2O containing 0.1% TFA; Solvent B: acetonitrile containing 0.1% TFA). NMR spectra were recorded using ECS-400 (JEOL). NMR spectrum data are reported as follows: chemical shift, multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration and coupling constant (Hz). LC-MS was performed on a ACQUITYUPLC H-Class/SQD2 (Waters) using InertSustain AQ-C18 (GL Science, 2.1 I.D. x 50 mm) for quantitative analysis and ACQUITY UPLC® Peptide CSH™ C18 Column (Waters, 2.1 I.D. x 100 mm) for purity check of peptides. To separate racemic amino acids, preparative HPLC was performed on a Prominence HPLC System (Shimadzu) with a CHIRALPAK AD-H (DAICEL, 4.6 mmI.D. x250 mm, 19325) using two solvents (Solvent A: Hexane containing 0.1% acetic acid; Solvent B: isopropanol containing 0.1% acetic acid). Optical rotations were measured on a P-1010 polarimeter (JASCO). HRMS data was obtained using micrOTOF II (Bruker Daltonics).

Synthesis

1. H_2N-Phe-iBu (**S4**)

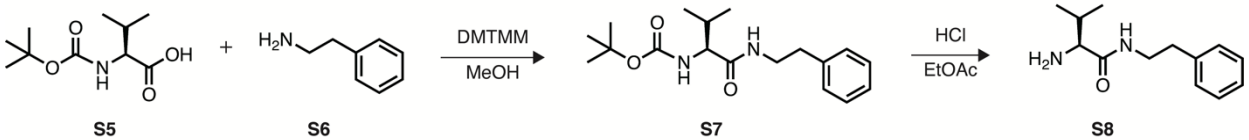


S1 S2 S3 S4

Cbz-L-Phe-OH (**S1**) (700 mg, 2.34 mmol) and isobutyl amine (**S2**) (231 μL, 2.31 mmol, 1.0 equiv.) were dissolved in 2 mL MeOH and the solution was stirred at room temperature. DMTMM·1.3H_2O (838 mg, 2.8 mmol, 1.2 equiv.) was added to the solution. The reaction mixture was stirred at room temperature for 5 h and evaporated *in vacuo*. DCM was added to the residue and the solution was washed with 1 M Na_2CO_3 aq., water, 1 M HCl aq., water and brine. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography to give **S3** (523 mg, 1.48 mmol, 64%).

To a recovery flask, **S3** (523 mg, 1.48 mmol), palladium 10% on carbon (55 mg) and 7.4 mL MeOH were added. The flask was charged with H_2 with a balloon and the mixture was stirred for 15 h at room temperature. The reaction mixture was filtered through celite. The solvent was removed under reduced pressure to give **S4** (319 mg, 1.45 mmol, 98%).

2. H_2N-Val-Phenethylamine (**S8**)

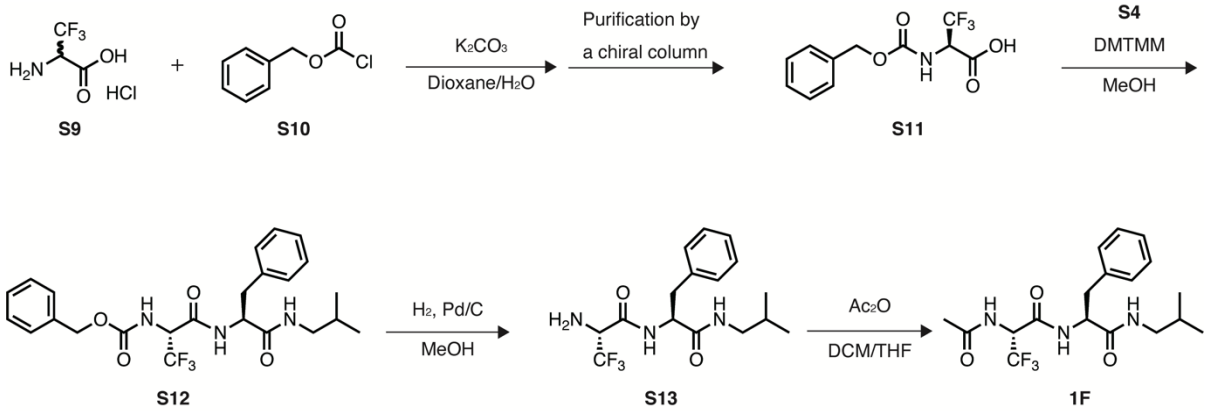


S5 S6 S7 S8

Boc-L-Val-OH (**S5**) (401 mg, 1.84 mmol) and phenethylamine (**S6**) (278 μL, 2.21 mmol, 1.2 equiv.) were dissolved in 18.4 mL MeOH and the solution was stirred at room temperature. DMTMM·1.3H_2O (665 mg, 2.21 mmol, 1.2 equiv.) was added to the solution. The reaction mixture was stirred at room temperature for 5 h and evaporated *in vacuo*. Water was added to the residue and the solution was extracted with DCM three times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo* to give **S7** (539 mg, 1.68 mmol, 91%).

To **S7** (534 mg, 1.67 mmol), 4 M HCl in EtOAc was added and the reaction mixture was stirred for 1 h. The solvent was evaporated *in vacuo*. To the residue, $NaHCO_3$ aq. was added and the solution was extracted with DCM four times and EtOAc twice. The solvent was evaporated to give **S8** (372 mg, 1.69 mmol, quant.).

3. Ac-Ala(F_3)-Phe-iBu (**1F**)

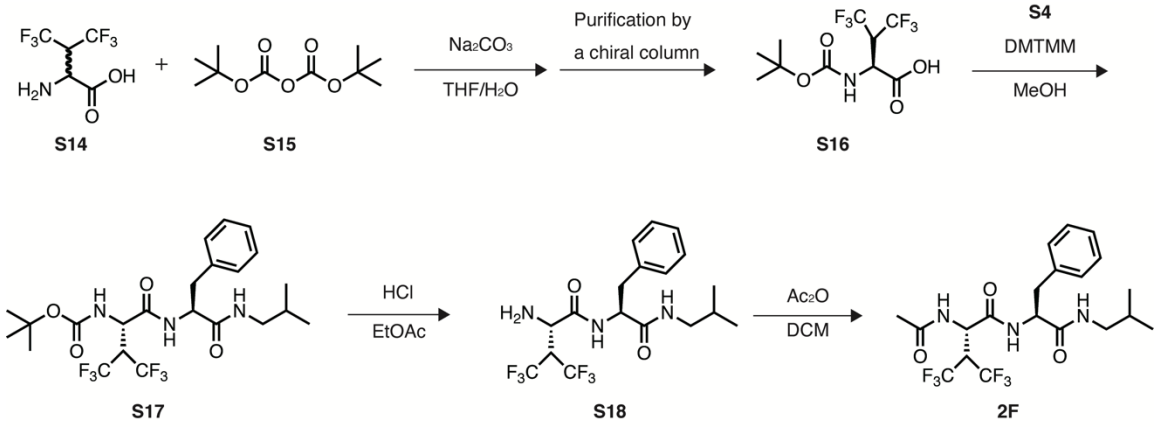


D, L-Trifluoroalanine hydrochloride (**S9**) (120 mg, 0.67 mmol) and potassium carbonate (368 mg, 2.67 mmol, 4.0 equiv.) were dissolved in 5 mL water and 3 mL dioxane. To the solution, benzyl chloroformate (**S10**) (236 µL, 1.67 mmol, 2.5 equiv.) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 10 min and warmed up to room temperature for 14 h. To the solution, benzyl chloroformate (236 µL, 1.67 mmol, 2.5 equiv.) was added dropwise and the reaction mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure. To the residue, water was added and washed with DCM three times. The aqueous phase was acidified using 1 M HCl aq. and extracted with DCM three times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by a reversed phase column on HPLC. The racemic Cbz-Ala(F_3)-OH was dissolved in 0.1% acetic acid in hexane/isopropanol (=3/1) and purified using a chiral column on HPLC to give Cbz-L-Ala(F_3)-OH and Cbz-D-Ala(F_3)-OH as white solid. To determine which isomer is the L-isomer, optical rotation was measured. The specific rotation of an isomer was $[\alpha]_{589}^{20} = -1.66$ (c 0.83, MeOH) and that of the other isomer was $[\alpha]_{589}^{20} = +1.69$ (c 0.83, MeOH). From these values of specific rotation, the former isomer was determined as Cbz-L-Ala(F_3)-OH (**S11**) (47 mg, 0.32 mmol, 25%).[1] ^{1}H NMR ($(CD_3)_2CO$, 400 MHz): 7.42–7.32 (m, 6H), 5.16–5.10 (m, 3H); ^{19}F NMR ($(CD_3)_2SO$, 376 MHz): δ–70.5 (d, 3F, J = 8.5 Hz); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz): δ166.1, 156.7, 137.0, 128.9, 128.5, 128.4, 123.9 (q, J = 281.8 Hz), 66.8, 56.0 (q, J = 27.8 Hz).

Cbz-L-Ala(F_3)-OH (**S11**) (25 mg, 90 µmol) and **S4** (22 mg, 99 µmol, 1.1 equiv.) were dissolved in 2 mL MeOH and the solution was stirred at room temperature. DMTMM·$3H_2O$ (51 mg, 154 µmol, 1.7 equiv.) was added to the solution. The reaction mixture was stirred at room temperature for 1 day and evaporated *in vacuo*. Water was added to the residue and the solution was extracted using DCM three times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH = 19/1) to give **S12**. To a recovery flask, **S12**, palladium 10% on carbon (3.6 mg) and 3 mL MeOH were added. The flask was charged with H_2 and the mixture was stirred for 3 h at room temperature. The reaction mixture was filtered through celite. The solvent was removed under reduced pressure and the residue was purified using a reversed phase column on HPLC to give **S13**. To **S13** in 2 mL THF, acetic anhydride (84 µL, 892 µmol, 50 equiv.) and DIPEA (6 µL, 36 µmol, 2 equiv.) were added. The reaction mixture was stirred at room temperature for 2 h and then evaporated *in vacuo*. The residue was purified using a reversed phase column on HPLC to give **1F** as a white solid (0.9 mg, 2.3 µmol,

4 %). ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ8.83 (d, 1H, *J* = 8.2 Hz), 8.65 (d, 1H, *J* = 9.2 Hz), 7.96 (t, 1H, *J* = 5.5 Hz), 7.25–7.16 (m, 5H), 5.47–5.38 (m, 1H), 4.56–4.51 (m, 1H), 2.97–2.77 (m, 4H), 1.90 (s, 3H), 1.63–1.56 (m, 1H), 0.77–0.74 (m, 6H); ^{19}F NMR ((CD$_3$)$_2$SO, 376 MHz): δ–71.1 (d, 3F, *J* = 8.6 Hz); HRMS (ESI) for $C_{18}H_{24}F_3N_3O_3Na$ [M+Na]$^+$: calcd. 410.1662, found 410.1667.

4. Ac-Val(F$_6$)-Phe-iBu (**2F**)



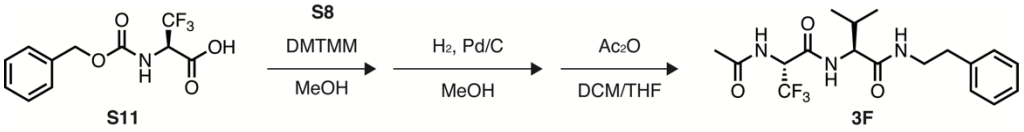
To a recovery flask, D,L-Val(F$_6$) (**S14**) (120 mg, 0.53 mmol), sodium carbonate (57 mg, 0.53 mmol, 1.0 equiv.), 4 mL H_2O and 1 mL THF were added and the solution was stirred at 0 °C. To the solution, Boc_2O (**S15**) (245 μL, 1.07 mmol, 2.0 equiv.) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 5 min and warmed up to room temperature for 15 h. Boc_2O (245 μL, 1.07 mmol, 2.0 equiv.) was added to the solution and the reaction mixture was stirred for 4 h. Water was added to the reaction mixture and the solution was washed with DCM three times. The aqueous phase was acidified using 1 M HCl aq. and the solution was extracted with DCM three times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by a reversed phase column on HPLC. The racemic Boc-Val(F$_6$)-OH was dissolved in 0.1% acetic acid in hexane/isopropanol (=3/1) and purified using a chiral column on a HPLC system. To determine which isomer is the L-isomer, a small fraction of Boc-Val(F$_6$)-OH after the purification on the chiral column was deprotected by treating with 3 M HCl in EtOAc and optimal rotation was measured for the resulting L-Val(F$_6$)·HCl and D-Val(F$_6$) ·HCl. The specific rotation of an isomer was $[\alpha]_{589}^{20} = +8.67$ (c 1.67, H_2O) and, from the value of specific rotation, we determined this isomer is L-isomer.[2] Boc-L-Val(F$_6$)-OH (**S16**) was obtained as white solid (22 mg, 67 μmol, 13%).[3] ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ7.34 (d, 1H, *J* = 10.0 Hz), 4.94–4.91 (m, 1H), 4.44–4.39 (m, 1H), 1.39 (s, 9H); ^{19}F NMR ((CD3)$_2$SO, 376 MHz): δ−61.25–−61.32 (m, 3F), −64.88–−64.97 (m, 3F); ^{13}C NMR ((CD$_3$)$_2$SO, 100 MHz): δ169.7, 155.6, 123.3 (q, *J* = 281.8 Hz), 123.0 (q, *J* = 282.8), 79.3, 48.9, 48.3–47.7 (m), 28.1.

S16 (7 mg, 22 μmol) and **S4** (5.2 mg, 24 μmol, 1.1 equiv.) were dissolved in 1 mL MeOH and the solution was stirred at room temperature. DMTMM·1.3H_2O (7.1 mg, 24 μmol, 1.1 equiv.) was added to the solution. The reaction mixture was stirred at room temperature for 2 h and evaporated *in vacuo*. Water was added to the residue and the solution was extracted with DCM three times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH = 19/1) to give **S17** (4.5 mg, 8.5 μmol, 40%). ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ8.17 (d, 1H, *J* = 8.4 Hz), 7.98 (t, 1H, *J* = 5.6 Hz), 7.28–7.16 (m, 6H), 4.89 (d, 1H, *J* = 9.9 Hz), 4.60–4.54 (m, 1H), 4.45–4.28 (m,

1H), 2.96–2.69 (m, 4H), 1.61–1.51 (m, 1H), 1.41 (s, 9H), 0.74–0.70 (m, 6H); ^{19}F NMR ((CD$_3$)$_2$SO, 376 MHz): δ−60.80–−60.85 (m, 3F), −63.93–−64.00 (m, 3F); ^{13}C NMR ((CD$_3$)$_2$SO, 100 MHz): δ169.8, 166.5, 154.8, 137.1, 129.1, 128.0, 126.3, 123.2 (q, *J* = 281.8 Hz), 122.9 (q, *J* = 281.8 Hz), 79.6, 54.5, 49.8, 48.3–47.2 (m), 46.1, 38.0, 28.0, 27.8, 19.9, 19.9; HRMS (ESI) for $C_{23}H_{31}F_6N_3O_4Na$ [M+Na]$^+$: calcd. 550.2111, found 550.2091.

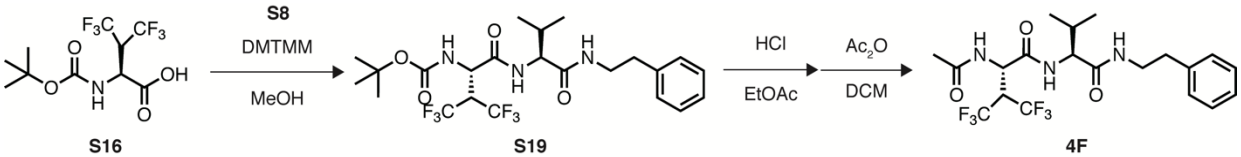
To **S17** (4.5 mg, 8.5 μmol), 4 M HCl in EtOAc was added at 0 °C. The reaction mixture was stirred at 0 °C for 20 min and warmed up to room temperature for 10 min. To the solution, water was added and the solution was basified with sodium carbonate. The solution was extracted with DCM nine times and EtOAc six times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo* to give **S18**. To **S18** (2.7 mg, 6.3 μmol) in 2 mL DCM, Ac_2O (30 μL, 32 μmol, 5.0 equiv.) was added. The reaction mixture was stirred for 20 h and the solvent was evaporated in vacuo. Water was added to the residue and the solution was extracted with DCM twice. The residue was purified by a reversed phase column on HPLC to give **2F** (0.2 mg, 0.32 μmol, 5%). ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ8.27 (brs, 1H), 8.25 (brs, 1H), 7.89 (t, 1H, *J* = 5.6 Hz), 7.27–7.18 (m, 5H), 5.24 (dd, 1H, *J* = 3.8, 10.0 Hz), 4.53–4.48 (m, 1H), 4.44–4.35 (m, 1H), 2.99–2.75 (m, 4H), 1.92 (s, 3H), 1.65–1.53 (m, 1H), 0.76–0.73 (m, 6H); ^{19}F NMR ((CD$_3$)$_2$SO, 376 MHz): δ－61.06–－61.15 (m, 3F), －64.15–－64.24 (m, 3F); ^{13}C NMR ((CD$_3$)$_2$SO, 100 MHz): δ170.0, 169.6, 166.7, 137.3, 129.1, 128.1, 126.3, 54.7, 47.8–47.3 (m), 46.9, 46.1, 37.8, 27.9, 22.4, 19.9; HRMS (ESI) for $C_{20}H_{25}F_6N_3O_3Na$ [M+Na]$^+$: calcd. 492.1692, found 492.1695.

5. Ac-Ala(F$_3$)-Val-Phenethylamine (**3F**)



Preparation of **3F** was conducted by following the same synthetic procedure as **1F** using **S8** instead of **S4**. ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ8.76 (d, 1H, *J* = 9.4 Hz), 8.57 (d, 1H, *J* = 8.8 Hz), 8.11 (t, 1H, *J* = 6.1 Hz), 7.28–7.17 (m, 5H), 5.54–5.50 (m, 1H), 4.15–4.11 (m, 1H), 3.55–3.32 (m, 2H), 2.73–2.65 (m, 2H), 2.01–1.85 (m, 4H), 0.77 (d, 6H, *J* = 6.6 Hz); ^{19}F NMR ((CD$_3$)$_2$SO, 376 MHz): δ−70.09 (d, 3F, *J* = 8.0 Hz); HRMS (ESI) for $C_{18}H_{24}F_3N_3O_3Na$ [M+Na]$^+$: calcd. 410.1662, found 410.1677.

6. Ac-Val(F$_6$)-Val-Phenethylamine (**4F**)



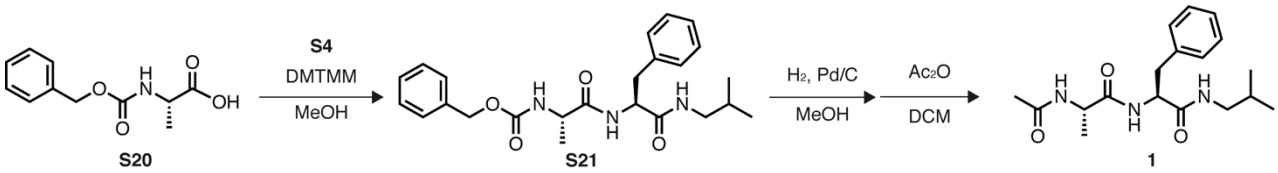
Preparation of Boc-Val(F$_6$)-Val-Phenetnylamine (**S19**) and **4F** was conducted by following the same synthetic procedure as **2F** using **S8** instead of **S4**.

Compound **S19**: ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ8.19 (t, 1H, *J* = 4.8 Hz), 7.75 (d, 1H, *J* = 8.8 Hz), 7.65 (d, 1H, *J* = 10.4 Hz), 7.35–7.16 (m, 5H), 4.91 (d, 1H, *J* = 10.3 Hz), 4.48–4.38 (m, 1H), 4.16 (dd, 1H, *J* = 7.1, 8.6 Hz), 3.40–3.21 (m, 2H), 2.71 (t, 2H, *J* = 7.3 Hz), 1.94–1.85 (m, 1H), 1.42 (s, 9H), 0.79–0.77 (m,

6H); ^{19}F NMR ($(CD_3)_2SO$, 376 MHz): δ−60.8–−60.9 (m, 3F), −63.9–−64.1 (m, 3F); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz): δ170.0, 166.6, 155.1, 139.2, 128.6, 128.2, 126.1, 123.2 (q, *J* = 279.9 Hz), 123.0 (q, *J* = 280.8 Hz), 79.7, 58.2, 50.0, 48.2–47.2 (m), 34.9, 31.0, 28.0, 19.0, 17.9; HRMS (ESI) for $C_{23}H_{31}F_6N_3O_4Na$ $[M+Na]^+$: calcd. 550.2111, found 550.2090.

Compound **4F**: ^{1}H NMR ($(CD_3)_2SO$, 400 MHz): δ8.46 (d, 1H, *J* = 10.2 Hz), 8.13 (t, 1H, *J* = 4.9 Hz), 7.79 (d, 1H, *J* = 8.7 Hz), 7.29–7.18 (m, 5H), 5.30 (d, 1H, *J* = 8.7 Hz), 4.53–4.44 (m, 1H), 4.13–4.10 (m, 1H), 3.27–3.16 (m, 2H), 2.70 (t, 2H, *J* = 7.2 Hz), 1.97–1.88 (m, 4H), 0.79 (d, 6H, *J* = 5.4 Hz); ^{19}F NMR ($(CD_3)_2SO$, 376 MHz): δ−61.07–−61.16 (m, 3F), −64.28–−64.37 (m, 3F); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz): δ170.0, 169.9, 166.7, 139.3, 128.6, 128.3, 126.1, 123.2 (q, *J* = 280.8 Hz), 123.1 (q, *J* = 280.8 Hz), 58.4, 47.8–47.2 (m), 47.1, 34.9, 30.7, 22.4, 19.1, 18.0; HRMS (ESI) for $C_{20}H_{25}F_6N_3O_3Na$ $[M+Na]^+$: calcd. 492.1692, found 492.1701.

7. Ac-Ala-Phe-iBu (**1**)

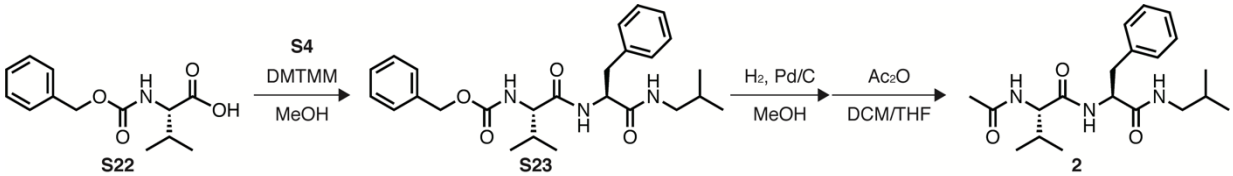


Preparation of Cbz-Ala-Phe-iBu (**S21**) and **1** was conducted by following the same synthetic procedure as **1F** using **S20** instead of **S11**.

Compounds **S21**: ^{1}H NMR ($(CD_3)_2SO$, 400 MHz): δ7.91 (d, 1H, *J* = 8.2 Hz), 7.84 (t, 1H, *J* = 5.6 Hz), 7.44 (d, 1H, *J* = 7.2 Hz), 7.39–7.17 (m, 10H), 5.02 (dd, 2H, *J* = 12.6, 18.6 Hz), 4.50–4.44 (m, 1H), 4.04–3.97 (m, 1H), 3.00–2.77 (m, 4H), 1.69–1.57 (m, 1H), 1.12 (d, 3H, *J* = 7.2 Hz), 0.79–0.76 (m, 6H); ^{13}C NMR ($(CD_3)_2SO$. 100 MHz): δ172.1, 170.5, 155.7, 137.7, 136.9, 129.2, 128.3, 128.0, 127.8, 127.7, 126.2, 65.4, 53.9, 50.3, 46.1, 37.8, 27.9, 20.0, 18.0; HRMS (ESI) for $C_{24}H_{31}N_3O_4Na$ $[M+Na]^+$: calcd. 448.2207, found 448.2191.

Compounds **1**: ^{1}H NMR (CD_3OD, 400 MHz): δ7.30–7.17 (m, 5H), 4.54 (dd, 1H, *J* = 6.6, 8.1 Hz), 4.22 (q, 1H, *J* = 7.2 Hz), 3.12 (dd, 1H, *J* = 6.5, 13.7 Hz), 3.02–2.86 (m, 3 H), 1.94 (s, 3H), 1.74–1.64 (m, 1H), 1.23 (d, 3 H, *J* = 7.2 Hz), 0.83–0.80 (m, 6H); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz): δ172.1, 170.5, 169.4, 137.8, 129.1, 128.0, 126.2, 53.9, 48.5, 46.1, 37.5, 27.9, 22.5, 20.0, 17.8; HRMS (ESI) for $C_{18}H_{27}N_3O_3Na$ $[M+Na]^+$: calcd. 356.1945, found 356.1947.

8. Ac-Val-Phe-iBu (**2**)

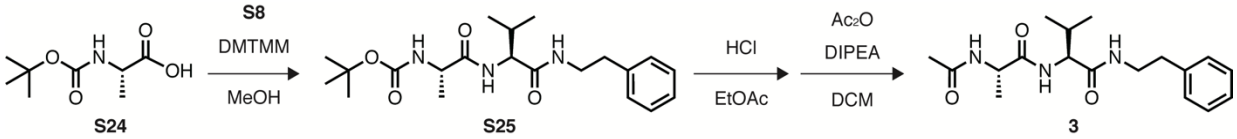


Preparation of Cbz-Val-Phe-iBu (**S23**) and **2** was conducted by following the same synthetic procedure as **1F** using **S22** instead of **S11**.

Compound **S23**: ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ7.97 (d, 1H, *J* = 8.4 Hz), 7.87 (t, 1H, *J* = 5.8 Hz), 7.39–7.13 (m, 11H), 5.06–4.99 (m, 2H), 4.56–4.50 (m, 1H), 3.82 (dd, 1H, *J* = 7.0, 8.7 Hz), 2.97–2.77 (m, 4H), 1.93–1.83 (m, 1H), 1.65–1.55 (m, 1H), 0.77–0.72 (m, 12H); ^{13}C NMR ((CD$_3$)$_2$SO, 100 MHz): δ170.7, 170.6, 156.1, 137.6, 137.0, 129.1, 128.3, 128.0, 127.8, 127.6, 126.2, 65.4, 60.4, 53.9, 46.0, 37.9, 30.3, 27.9, 20.0, 20.0, 19.0, 18.0; HRMS (ESI) for $C_{26}H_{35}N_3O_4Na$ [M+Na]$^+$: calcd. 476.2520, found 476.2519.

Compounds **2**: ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ7.95 (d, 1H, *J* = 8.4 Hz), 7.81–7.77 (m, 2H), 7.27–7.15 (m, 5H), 4.51–4.45 (m, 1H), 4.06 (dd, 1H, *J* = 6.8, 8.3 Hz), 2.97 (dd, 1H, *J* = 5.6, 13.7 Hz), 2.89 –2.78 (m, 3H), 1.92–1.81 (m, 4H), 1.69–1.57 (m, 1H), 0.78–0.73 (m, 12H); ^{13}C NMR ((CD$_3$)$_2$SO, 100 MHz): δ170.8, 170.6, 169.5, 137.8, 129.1. 128.0, 126.2, 58.0, 53.9, 46.0, 37.6, 30.2, 27.9, 22.5, 20.0, 19.1, 18.0; HRMS (ESI) for $C_{20}H_{31}N_3O_3Na$ [M+Na]$^+$: calcd. 384.2258, found 384.2263.

9. Ac-Ala-Val-Phenethylamine (**3**)

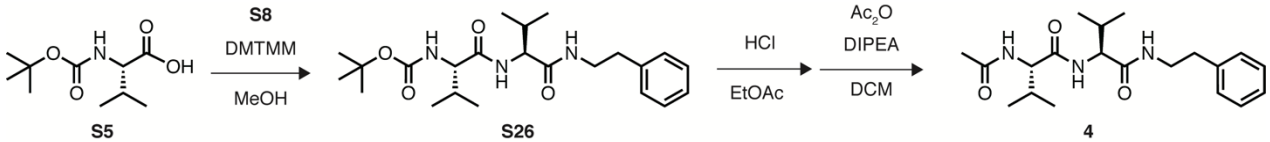


Preparation of Boc-Ala-Val-Phenethylamine (**S25**) and **3** was conducted by following the same synthetic procedure as **2F** using **S24** instead of **S16**.

Compound **S25**: ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ8.07 (s, 1H), 7.47 (d, 1H, *J* = 8.6 Hz), 7.29–7.18 (m, 5H), 7.08 (d, 1H, *J* = 7.4 Hz), 4.11–4.07 (m, 1H), 4.01–3.97 (m, 1H), 3.33–3.19 (m, 2H), 2.72–2.68 (m, 2H), 1.91–1.83 (m, 1H), 1.38 (s, 9H), 1.16 (d, 3H, *J* = 7.0 Hz), 0.78–0.76 (m, 6H); ^{13}C NMR ((CD$_3$)$_2$SO, 100 MHz): δ172.4, 170.6, 155.1, 139.3, 128.6, 128.3, 126.1, 78.2, 57.3, 49.9, 35.0, 31.0, 28.2, 19.1, 17.8, 17.8; HRMS (ESI) for $C_{21}H_{33}N_3O_4Na$ [M+Na]$^+$: calcd. 414.2363, found 414.2348.

Compound **3**: ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ8.04 (d, 1H, *J* = 7.6 Hz), 8.00 (t, 1H, *J* = 5.7 Hz), 7.60 (d, 1H, J = 9.0 Hz), 7.30–7.16 (m, 5H), 4.35–4.28 (m, 1H), 4.06 (dd, 1H, *J* = 6.8, 9.0 Hz), 3.37–3.20 (m, 2H), 2.71 (t, 2H, *J* = 7.4 Hz), 1.92–1.83 (m, 4H), 1.16 (d, 3H, *J* = 7.0 Hz), 0.78–0.76 (m, 6H); ^{13}C NMR ((CD$_3$)$_2$SO, 100 MHz): δ172.1, 170.6, 169.0, 139.3, 128.6, 128.2, 126.0, 57.5, 48.1, 35.0, 30.7, 22.4, 19.2, 17.9, 17.9; HRMS (ESI) for $C_{18}H_{27}N_3O_3Na$ [M+Na]$^+$: calcd. 356.1945, found 356.1958.

10. Ac-Val-Val-Phenethylamine (**4**)



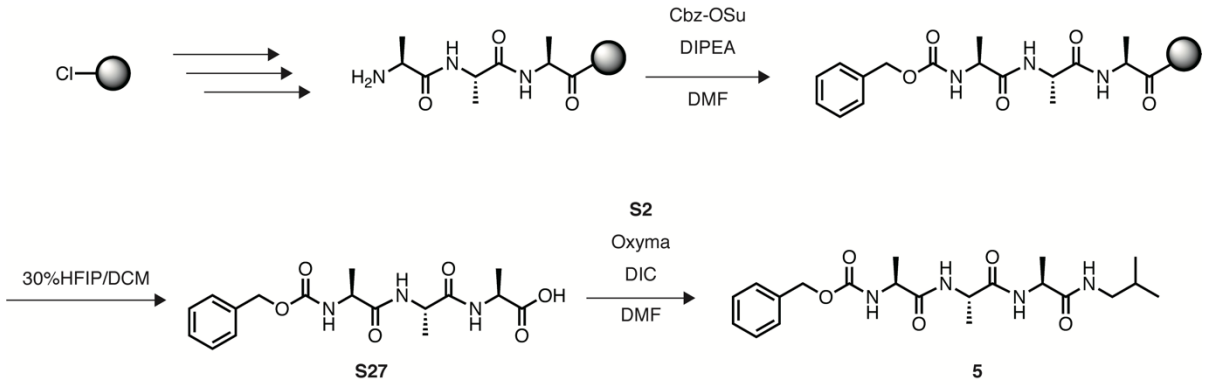
Preparation of Boc-Val-Val-Phenethylamine (**S26**) and **4** was conducted by following the same synthetic procedure as **2F** using **S5** instead of **S16**.

Compound **S26**: ^{1}H NMR ((CD$_3$)$_2$SO, 400 MHz): δ8.05 (t, 1H, *J* = 5.0 Hz), 7.55 (d, 1H, *J* = 8.9 Hz), 7.29–7.25 (m, 2H), 7.20–7.16 (m, 3H), 6.88 (d, 1H, *J* = 9.0 Hz), 4.11 (dd, 1H, *J* = 7.1, 8.8 Hz), 3.81–3.77 (m, 1H), 3.37–3.20 (m, 2H), 2.70 (t, 2H, *J* = 7.3 Hz), 1.99–1.80 (m, 2H), 1.38 (s, 9H), 0.84–0.75 (m, 12H); ^{13}C NMR ((CD$_3$)$_2$SO, 100 MHz): δ171.1, 170.6, 155.5, 139.3, 128.6, 128.3, 126.1, 78.1, 60.2, 57.5, 35.0, 30.8,

30.1, 28.2, 19.3, 19.2, 18.2, 18.1; HRMS (ESI) for $C_{23}H_{37}N_3O_4Na$ $[M+Na]^+$: calcd. 442.2676, found 442.2658.

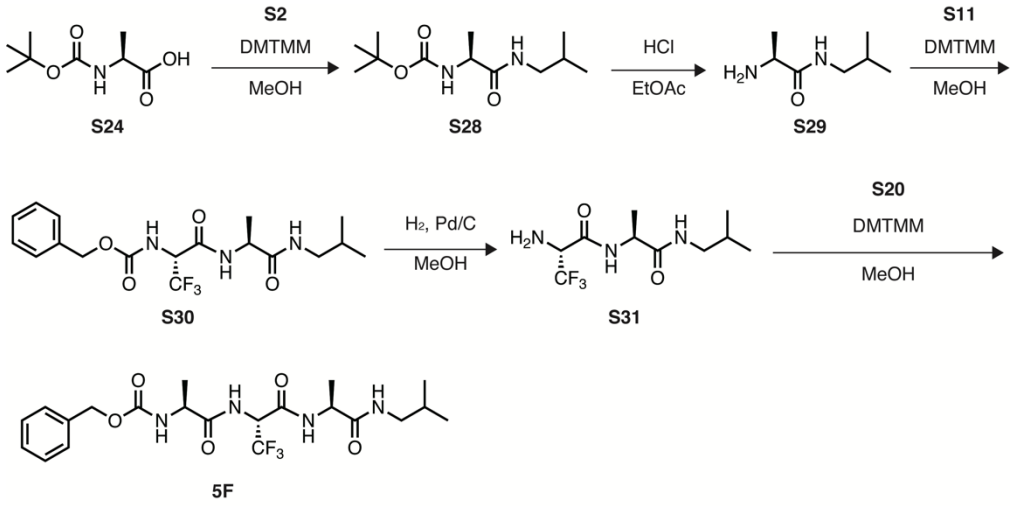
Compound **4**; ^1H NMR ($(CD_3)_2SO$, 400 MHz): δ7.97 (t, 1H, J = 5.4 Hz), 7.88 (d, 1H, J = 8.8 Hz), 7.63 (d, 1H, J = 8.9 Hz), 7.29–7.16 (m, 5H), 4.19–4.16 (m, 1H), 4.09–4.05 (m, 1H), 3.27–3.21 (m, 2H), 2.70 (t, 2H, J = 7.2 Hz), 2.02–1.83 (m, 5H), 0.83–0.76 (m, 12H); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz): δ170.9, 170.6, 169.2, 139.3, 128.6, 128.2, 126.0, 57.8, 57.7, 35.0, 30.5, 30.1, 22.5, 19.2, 19.2, 18.1; HRMS (ESI) for $C_{20}H_{31}N_3O_3Na$ $[M+Na]^+$: calcd. 384.2258, found 384.2248.

11. Cbz-Ala-Ala-Ala-iBu (**5**)



S27 was synthesized on 2-chlorotrityl chloride resin (1.80 mmol/g). Resin (237 mg, 0.43 mmol) was first swelled in DCM in a 6 mL fritted syringe with continuous shaking. DIPEA (297 μL, 1.72 mmol, 4.0 equiv.) and Fmoc-Ala-OH (264 mg, 0.86 mmol, 2.0 equiv.) were dissolved in 4.3 mL DCM and the solution was applied to the resin. The resin was incubated for 2 h at room temperature. After the reaction, the resin was washed with DCM/MeOH/DIPEA = 17/2/1 and DCM, three times each. The resin was applied to further peptide synthesis. Fmoc deprotection was performed by incubating the resin with 20% piperidine in DMF for 15 min. After the reaction, the resin was washed with DMF three times. Coupling reaction of Fmoc-Ala-OH was performed using Fmoc-Ala-OH (530 mg, 1.72 mmol, 4 equiv.), oxyma (242 mg, 1.72 mmol, 4 equiv.) and DIC (264 μL, 1.72 mmol, 4 equiv.) in DMF (4 mL) over 1 h. Before the coupling reaction, pre-activation of the reaction mixture was conducted at room temperature for 10 min. After the reaction, the resin was washed with DMF three times. The coupling and deprotection were repeated until trialanine was synthesized. Only a fraction of the resin (0.11 mmol) was used for further synthesis. Cbz protection on N-terminus was performed by incubating the resin in a reaction mixture of Cbz-OSu (133 mg, 0.53 mmol, 5 equiv.) and DIPEA (185 μL, 4.3 mmol, 10 equiv.) in DMF (1.1 mL) for 2 h at room temperature. The synthesized peptide was cleaved from the resin by incubating the resin with 30% HFIP in DCM for 15 min three times. The filtrate was collected in a recovery flask. The resin was washed with DCM and MeOH. All the filtrates were combined, and the solution was evaporated under a reduced pressure to give **S27** (37 mg, 101 μmol). To **S27** (11 mg, 30 μmol) in DMF (0.3 mL), oxyma (6.4 mg, 45 μmol, 1.5 equiv.) and DIC (7 μL, 45 μmol, 1.5 equiv.) were added and the solution was stirred for 10 min at room temperature. To the solution, isobutyl amine (4.4 μL, 45 μmol, 1.5 equiv.) was added and the reaction mixture was stirred overnight. The solvent was evaporated in vacuo. The residue was dissolved in 40%ACN in H_2O (4 mL) and purified by a reversed phase column on HPLC to give **5** (3.6 mg, 8.6 μmol, 29%). HRMS (ESI) for $C_{21}H_{32}N_4O_5Na$ $[M+Na]^+$: calcd. 443.2265, found 443.2265.

12. Cbz-Ala-Ala(F_3)-Ala-iBu (**5F**)

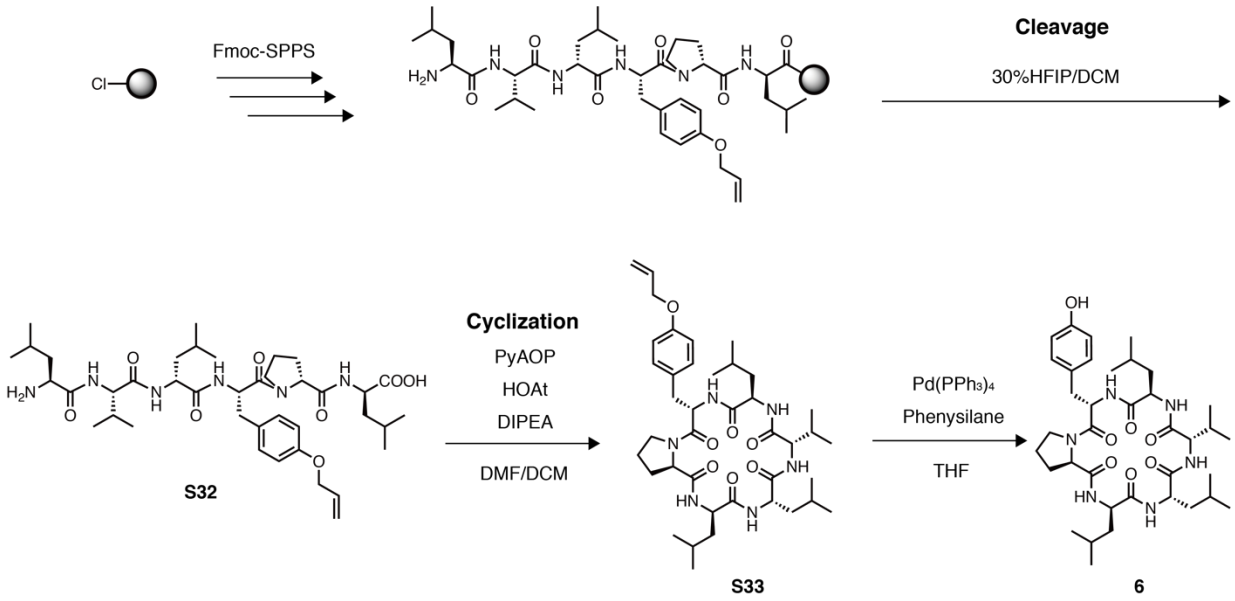


Cbz-L-Ala-OH (**S24**) (201 mg, 1.06 mmol) and isobutyl amine (**S2**) (117 µL, 1.16 mmol, 1.1 equiv.) were dissolved in 2 mL MeOH and the solution was stirred at room temperature. DMTMM·1.3H_2O (348 mg, 1.16 mmol, 1.1 equiv.) was added to the solution. The reaction mixture was stirred at room temperature for 1 h and evaporated *in vacuo*. Water was added to the residue and the solution was extracted with DCM three times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH = 19/1) to give **S28** (233 mg, 0.95 mmol, 90%).

To **S28** (233 mg, 0.95 mmol), 4 M HCl in EtOAc was added. The reaction mixture was stirred at room temperature for 2 h. The solution was extracted with water twice. The solution was basified with sodium carbonate and sodium hydroxide. The solution was extracted with EtOAc once and DCM 7 times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo* to give **S29** (135 mg, 0.75 mmol, 78%).

Cbz-L-Ala(F_3)-OH (**S11**) (15 mg, 54 µmol) and **S29** (9.4 mg, 65 µmol, 1.2 equiv.) were dissolved in 3 mL MeOH and the solution was stirred at 0 °C for 30 min. DMTMM·1.3H_2O (21 mg, 70 µmmol, 1.3 equiv.) was added to the solution. The reaction mixture was stirred at 0 °C for 20 h and evaporated *in vacuo*. Water was added to the residue and the solution was extracted using DCM three times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH = 19/1) and by a reversed phase column on HPLC to give **S30**. To a recovery flask, **S30**, palladium 10% on carbon (1 mg) and 2 mL MeOH were added. The flask was charged with H_2 and the mixture was stirred for 1 day at room temperature. The reaction mixture was filtered through celite. The solvent was removed under reduced pressure. The residue was purified by a reversed phase column on HPLC to give **S31**. Cbz-L-Ala-OH (**S20**) (6.6 mg, 29 µmol, 2.4 equiv.) and **S31** were dissolved in 1 mL MeOH and the solution was stirred at room temperature. DMTMM·1.3H_2O (8.6 mg, 28 µmmol, 2.2 equiv.) was added to the solution. The reaction mixture was stirred at room temperature for 1 day. The solution was evaporated *in vacuo*. Water was added to the residue and the solution was extracted using DCM three times. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by a reversed phase column on HPLC to give **5F** (0.4 mg, 0.8 µmol, 1%). HRMS (ESI) for $C_{21}H_{29}F_3N_4O_5Na$ $[M+Na]^+$: calcd. 497.1982, found 497.1959.

13. cyclo[L-Val-D-Leu-L-Tyr-D-Pro-D-Leu-L-Leu] (**6**)

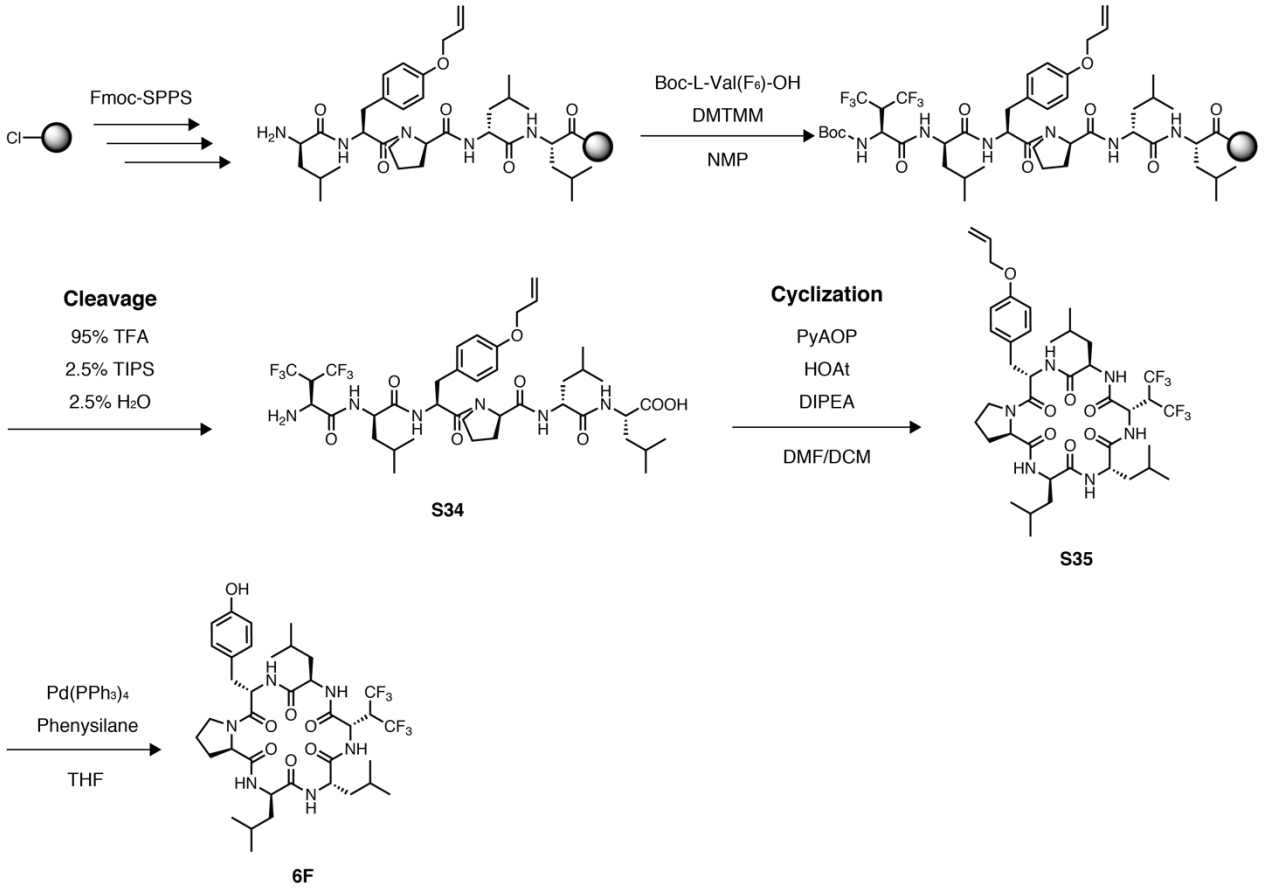


S32 was synthesized on 2-chlorotrityl polystyrene resin (1.34 mmol/g). Resin (87 mg, 0.12 mmol) was first swelled in DCM in a 6 mL fritted syringe with continuous shaking. DIPEA (82 μL, 0.47 mmol) and Fmoc-D-Leu-OH (84 mg, 0.23 mmol) were dissolved in 1.2 mL DCM and the solution was applied to the resin. The resin was incubated for 2 h at room temperature with continuous shaking. After the reaction, resin was washed with DCM, DCM/methanol/DIPEA = 17/2/1 and DCM, three times each. A half of the resin was applied to further peptide synthesis. Fmoc deprotection was performed by incubating the resin with 20% piperidine in DMF for 15 min. After the reaction, the resin was washed with DMF three times. Coupling reaction of amino acids was performed using Fmoc-protected amino acid (4 equiv.), Oxyma (4 equiv.) and DIC (4 equiv.) in DMF (0.2 M with respect to Fmoc-protected amino acid) for 1–3 h. Before the coupling reaction, pre-activation of the reaction mixture was conducted at room temperature for 10 min. After the reaction, the resin was washed with DMF, three times each. The coupling and deprotection were repeated until the 6th L-Leu residue. The synthesized peptides were cleaved from the resin by incubating the resin with 30% HFIP in DCM for 15 min three times. The resin was washed with DCM and MeOH three times each. The filtrate was collected in a recovery flask. All the filtrates were combined, and the solution was evaporated under a reduced pressure and the residue was purified by a reversed phase column on HPLC to give **S32** (30.5 mg, 40 μmol, 93 %).

To a vial, **S32** (15 mg, 20 μmol), PyAOP (30 mg, 3 equiv.), HOAt (8 mg, 3 equiv.), DIPEA (10.2 μL, 3 equiv.) and anhydrous DMF/DCM (=1/1, 4 mL) were added and the reaction mixture was shaken for 1.5 days. The solvent was removed under reduced pressure and the residue was purified by a reversed phase column on HPLC to give **S33** (5.4 mg, 7.3 μmol, 37%).

Under nitrogen atmosphere, to **S33** (5.4 mg, 7.3 μmol) and $Pd(PPh_3)_4$ (8.4 mg, 7.3 μmol, 1.0 equiv.), phenylsilane (3.6 μL, 29 μmol, 4 equiv.) and anhydrous THF (2.0 mL) were added. The reaction mixture was stirred for 1 day. The solvent was removed under reduced pressure and the residue was purified by a reversed phase column on HPLC to give **6** (2.4 mg, 3.4 μmol, 47%). HRMS (ESI) for $C_{37}H_{58}N_6O_7Na$ $[M+Na]^+$: calcd. 721.4259, found 721.4286.

14. cyclo[L-Val(F_6)-D-Leu-L-Tyr-D-Pro-D-Leu-L-Leu] (**6F**)



S34 was synthesized on 2-chlorotrityl polystyrene resin (1.34 mmol/g). Resin (34 mg, 46 μmol) was first swelled in DCM in a 6 mL fritted syringe with continuous shaking. DIPEA (32 μL, 0.18 mmol) and Fmoc-L-Leu-OH (32 mg, 92 μmol) were dissolved in 0.9 mL DCM and the solution was applied to the resin. The resin was incubated for 2 h at room temperature with continuous shaking. After the reaction, resin was washed with DCM, DCM/methanol/DIPEA = 17/2/1 and DCM, three times each. The resin was applied to further peptide synthesis. Fmoc deprotection was performed by incubating the resin with 20% piperidine in DMF for 15 min. After the reaction, the resin was washed with DMF three times. Coupling reaction other than Boc-L-Val(F_6)-OH was performed using Fmoc-protected amino acid (4 equiv.), Oxyma (4 equiv.) and DIC (4 equiv.) in DMF (0.2 M with respect to Fmoc-protected amino acid) for 1–3 h. Before the coupling reaction, pre-activation of the reaction mixture was conducted at room temperature for 10 min. Coupling reaction of Boc-L-Val(F_6)-OH was performed using Boc-L-Val(F_6)-OH (23 mg, 70 μmol, 2 equiv.) and DMTMM·3H_2O (23 mg, 70 μmol, 2 equiv.) in anhydrous NMP (0.7 mL) for 4 h. After the reaction, the resin was washed with DMF, three times each. The coupling and deprotection were repeated until the 6th L-Val(F_6) residue. The synthesized peptide was cleaved from the resin by incubating the resin with 95% TFA, 2.5% TIPS and 2.5% H_2O for 15 min three times. The resin was washed with DCM and MeOH three times each. The filtrate was collected in a recovery flask. All the filtrates were combined, and the solution was evaporated under reduced pressure and the residue was purified by a reversed phase column on HPLC to give **S34** (19 mg, 22 μmol, 63 %).

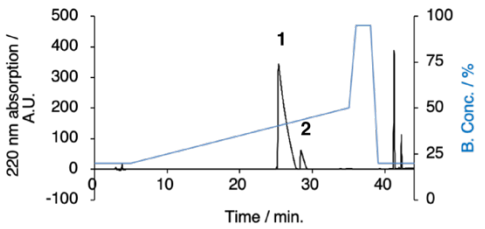
To a vial, **S34** (17 mg, 19 μmol), PyAOP (15 mg, 28.8 μmol, 1.5 equiv.), HOAt (3.9 mg, 28.8 μmol, 1.5

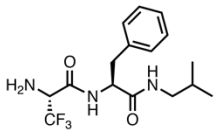
equiv.), DIPEA (14.7 μL, 86.4 μmol, 4.5 equiv.) and anhydrous DMF/DCM (=1/1, 4 mL) were added and the reaction mixture was shaken for 3 h. The solvent was removed under reduced pressure and the residue was purified by a reversed phase column on HPLC to give **S35** (7.8 mg, 9.2 μmol, 48%).

Under nitrogen atmosphere, to **S35** (7.8 mg, 9.2 μmol) and $Pd(PPh_3)_4$ (5.3 mg, 4.6 μmol, 0.5 equiv.), phenylsilane (2.3 μL, 18.4 μmol, 2 equiv.) and anhydrous THF (1 mL) were added. The reaction mixture was stirred for 3 h. The solvent was removed under reduced pressure and the residue was purified by a reversed phase column on HPLC to give **6F** (5.0 mg, 6.2 μmol, 67%). HRMS (ESI) for $C_{37}H_{52}F_6N_6O_7Na$ $[M+Na]^+$: calcd. 829.3694, found 829.3681.

The degree of epimerization during synthesis of 1F and 3F

(a)

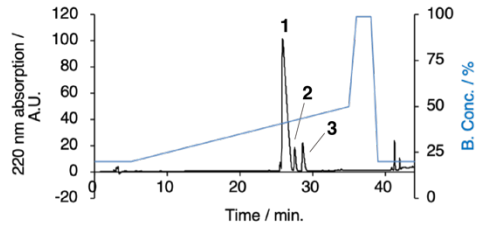


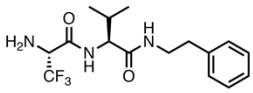


S13
Exact Mass : 345.17

Peak	Obsd	Ratio
1	346.55 ([M+H]⁺)	93
2	346.60 ([M+H]⁺)	7

(b)





S36
Exact Mass : 345.17

Peak	Obsd	Ratio
1	346.55 ([M+H]⁺)	92
2	245.46	-
3	346.60 ([M+H]⁺)	8

Fig. S1 The degree of epimerization observed during purification of **S13** (a synthetic intermediate of **1F**) and **S36** (a synthetic intermediate of **3F**). **(a)** UV chromatogram of **S13**. **(b)** UV chromatogram of **S36**. Purification of each peptide was conducted by running HPLC at a flow rate of 3 mL/min using mobile phase A (0.1% TFA in water) and B (0.1% TFA in acetonitrile) as indicated by the blue line in each chromatogram.

Aqueous solubility of 2-/3-mer peptides

Aqueous solubility of 2-/3-mer peptides (**1**, **2**, **3**, **4**, **5**, **1F**, **2F**, **3F**, **4F**, **5F**) was analyzed. 2 μL of 10 mM peptide DMSO stock was dissolved in 198 μL PBS. The solution was incubated at 37 °C for 4 h. The solution was filtered using 0.45 μm PVDF membrane and concentration of the peptide in the filtered solution was determined using LC/MS.

Table S1 Aqueous solubility of 2-/3-mer peptides.

Name	Solubility in 1% DMSO/PBS / μM
1	93.8 ± 2.8
1F	15.4 ± 4.4
2	29.6 ± 12.9
2F	2.8 ± 0.3
3	84.6 ± 4.3
3F	62.0 ± 2.7
4	42.4 ± 1.3
4F	19.7 ± 5.5
5	80.0 ± 2.3
5F	13.8 ± 1.8

The effect of CH_3 to CF_3 substitution on the surface area of 2-mer peptides

Molecular mechanics and quantum mechanical calculations of 2-mer peptides containing Ala(F_3) and Val(F_6) (peptide **1**, **1F**, **2**, **2F**) were conducted in order to assess the solvent shielding effect of -CF_3 groups on amide hydrogens. We summarized the surface area, polar surface area, accessible surface area and accessible polar surface area of the most stable conformations of peptides from the calculations as the Table S2 shown below. Note that the most stable conformations were not identical with each other between **1** and **1F** and between **2** and **2F**.

Table S2 Surface area of the most stable conformer of peptides **1**, **1F**, **2** and **2F**.

Peptide	Surface area (Å^2)	Polar surface area (Å^2)	Accessible surface area (Å^2)	Accessible polar surface area (Å^2)
1 Ac-Ala-Phe-iBu	386.84	138.35	219.92	76.45
1F Ac-Ala(F_3)-Phe-iBu	405.79	103.88	243.76	54.25
2 Ac-Val-Phe-iBu	420.9	135.26	236.34	66.44
2F Ac-Val(F_6)-Phe-iBu	442.37	110.78	252.04	54.01

After systematic search of conformers were conducted by molecular mechanics using MMFF *in vacuo* as a force field, DFT calculations (B3LYP/6-31+G*) in water were conducted for most stable 10 conformers to calculate more accurate energies of each conformer. The most stable conformation was selected for the evaluation of surface area. All the calculations were conducted by Spartan'18 (Wavefunction Inc.).

Analysis of purified peptides by UPLC

All the peptides after purification were analyzed using UPLC. Purity of each peptide was checked by running UPLC at a flow rate of 0.4 mL/min using mobile phase A (0.01% formic acid in water) and B (0.01% formic acid in acetonitrile) over 20 min gradient: 5% B in 2 min, 5 to 95% B in 15 min, held at 95% B for 1 min and held at 5% B in 2 min.

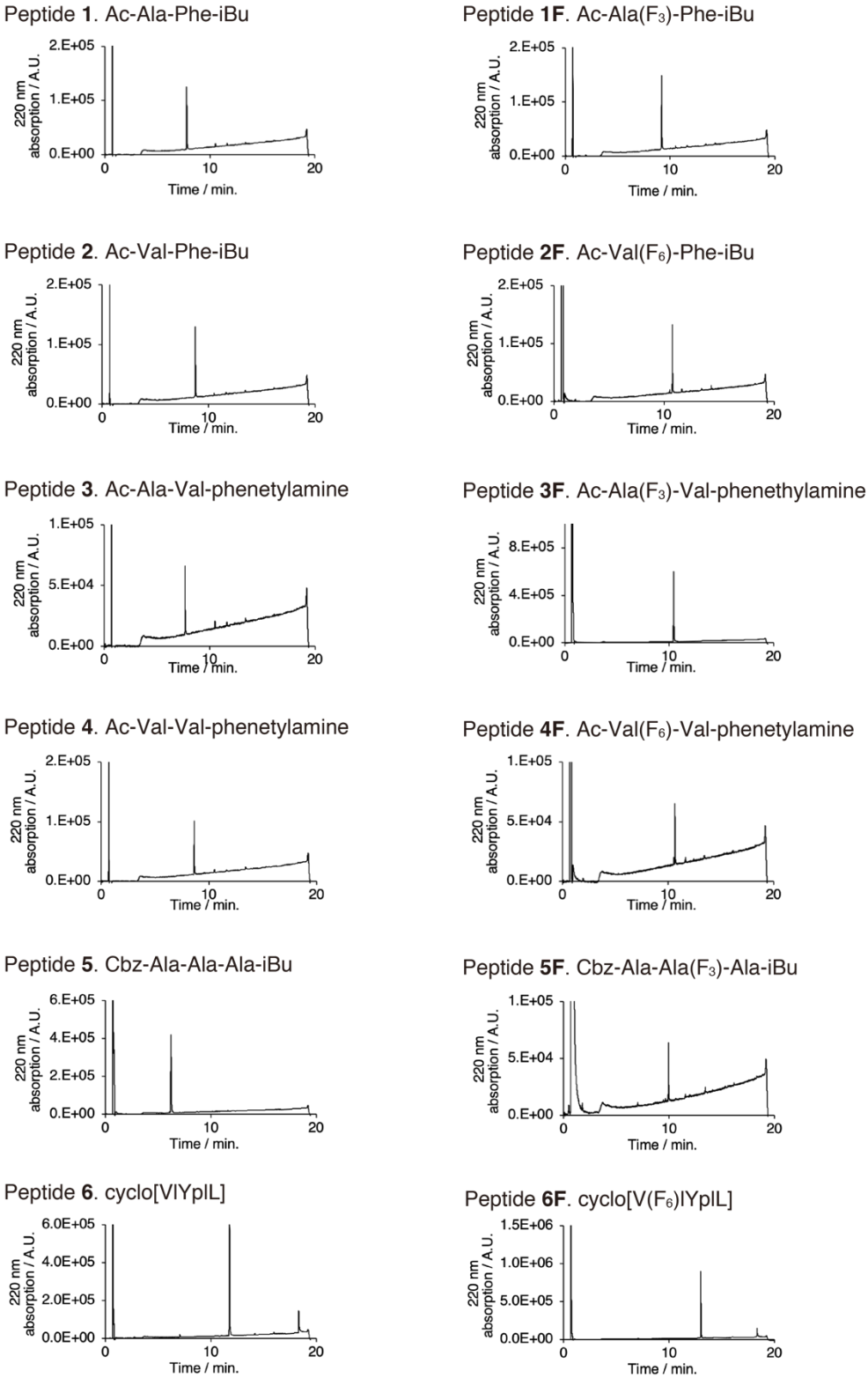


Fig. S2 UV chromatograms of all the peptides analyzed in this study.

Shake flask Log$P_{octanol/water}$ measurements[4]

Before the assay, octanol was staruteted with H_2O by shaking with the equal volume of PBS (pH = 7.4) overnight. To the octanol (200 μL for **1**, **1F**, **2**, **2F**, **5** and **5F** or 180 μL for others) and PBS (400 μL for **1**, **1F**, **2**, **2F**, **5** and **5F** or 180 μL for others) in a spin column, 2 mM or 200 μM peptide (6 μL for **1**, **1F**, **2**, **2F**, **5** and **5F** or 3.6 μL for others) in DMSO was added and the spin column was shaked for 2 h at room temperature. Each phase was collected carefully and quantified using LC/MS.

PAMPA[5]

Peptide permeability was measured by PAMPA. On PAMPA, 300 μL of 5% DMSO in PBS was added to each well of the acceptor plate (MultiScreen 96-well Transport Receiver Plate, Merck). 150 μL of peptide solution (10 μM for peptide **1**,**1F**, **5**, **5F** and 4 μM for peptide **2**, **2F**, **3**, **3F**, **4**, **4F, 6, 6F**) in 5 % DMSO/PBS was added to each well of the donor plate (MultiScreen-IP Filter Plate, 0.45 μm, Merck). 1% lecithin (from soybean) in dodecane was sonicated for 30 min before use and 5 μL of the solution was applied to the membrane support (PVDF) on each well of the donor plate. The donor plate was put on the acceptor plate. The plate was left for 18 h for peptide **1** and **1F** and 16 h for others at 25 °C in an incubator. Concentration of peptides was determined using LC/MS. The experiment was performed in triplicate. The permeability value (P_e) was calculated using the following equations:

$$P_e = -\frac{\ln\left[1 - C_A(t)/C_{equilibrium}\right]}{A \times (1/V_D + 1/V_A) \times t}$$

$$C_{equilibrium} = \frac{C_D(t) \times V_D + C_A(t) \times V_A}{V_D + V_A}$$

Where:

A = filter area (0.3 cm^2)

V_D = donor well volume (0.15 cm^3)

V_A = acceptor well volume (0.3 cm^3)

t = incubation time (s)

$C_D(t)$ = compound concentration in donor well at time t

$C_A(t)$ = compound concentration in acceptor well at time t

NMR spectrums

^{1}H NMR

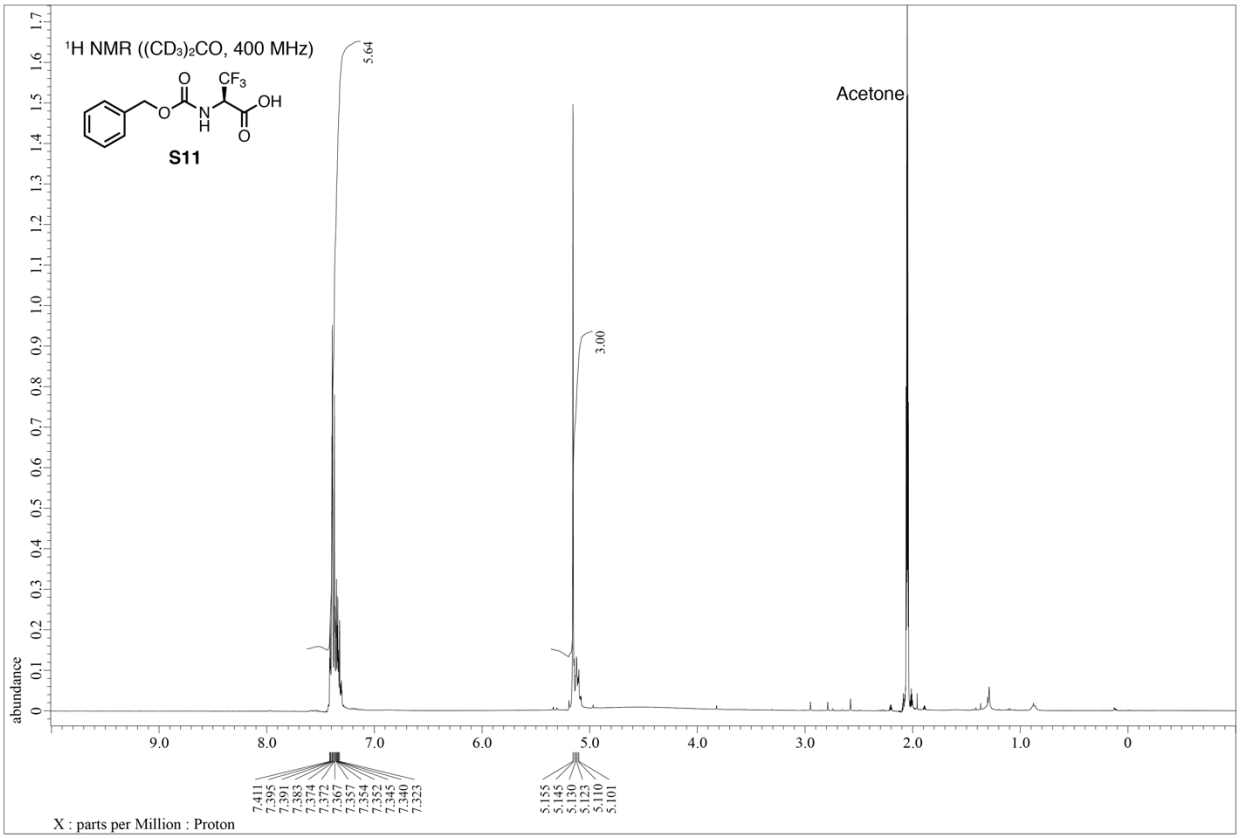


Fig. S3 ^{1}H NMR spectrum of Cbz-L-Ala(F_3)-OH (**S11**). Spectrum was recorded in $(CD_3)_2CO$ at 25 °C.

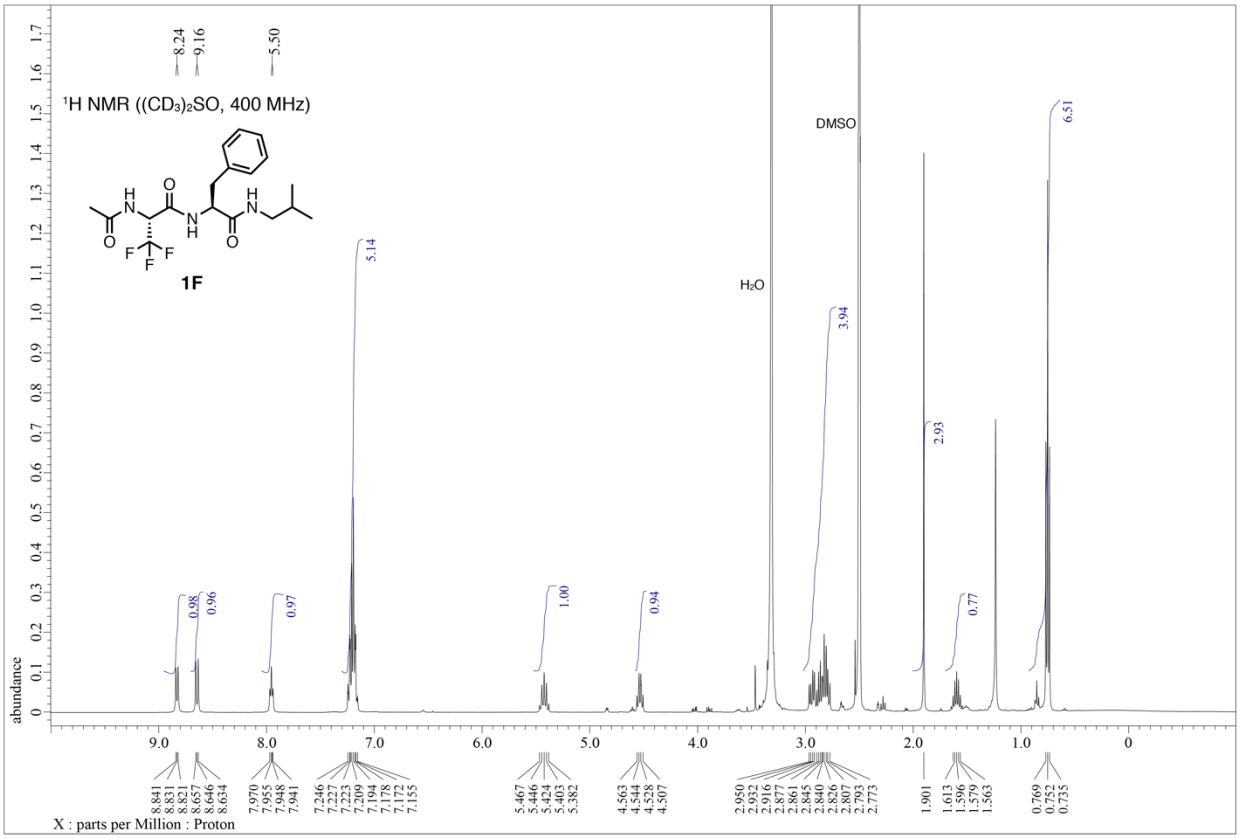


Fig. S4 ^{1}H NMR spectrum of **1F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

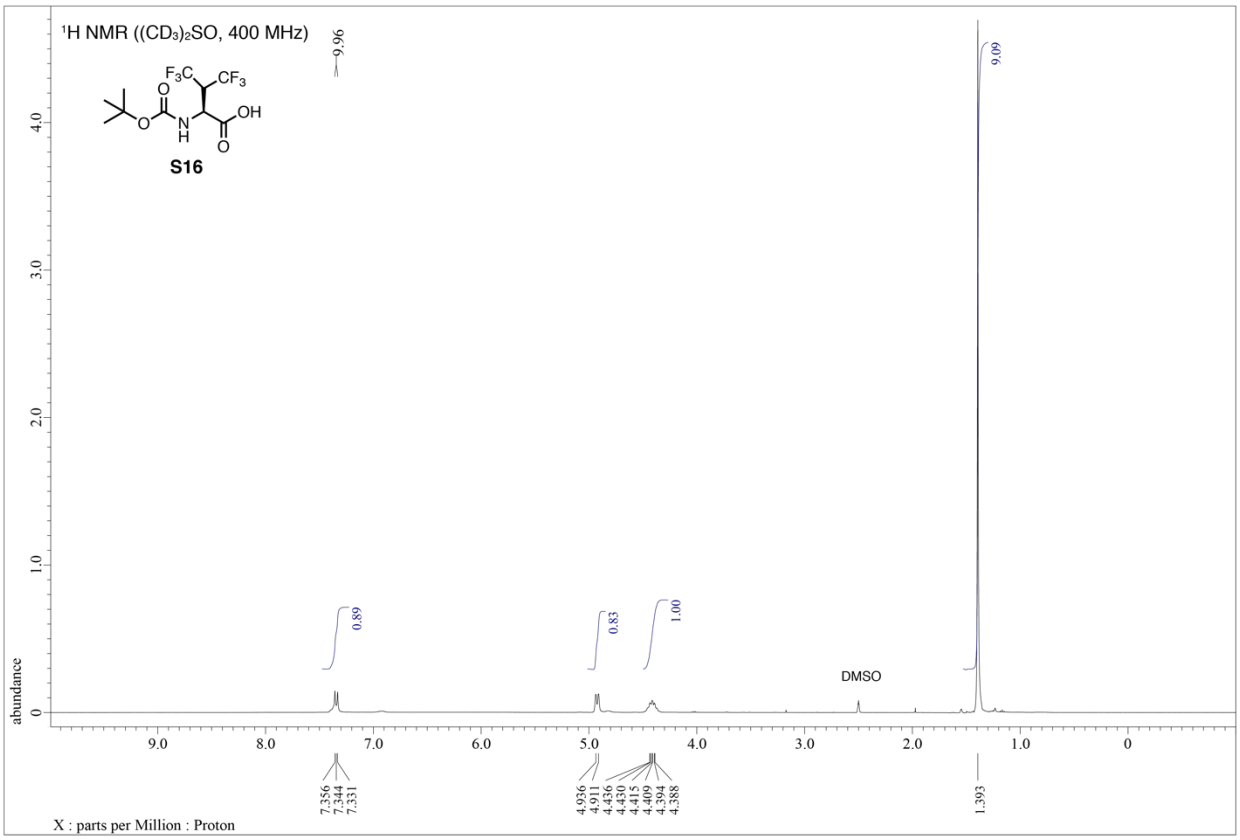


Fig. S5 ^{1}H NMR spectrum of **S16**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

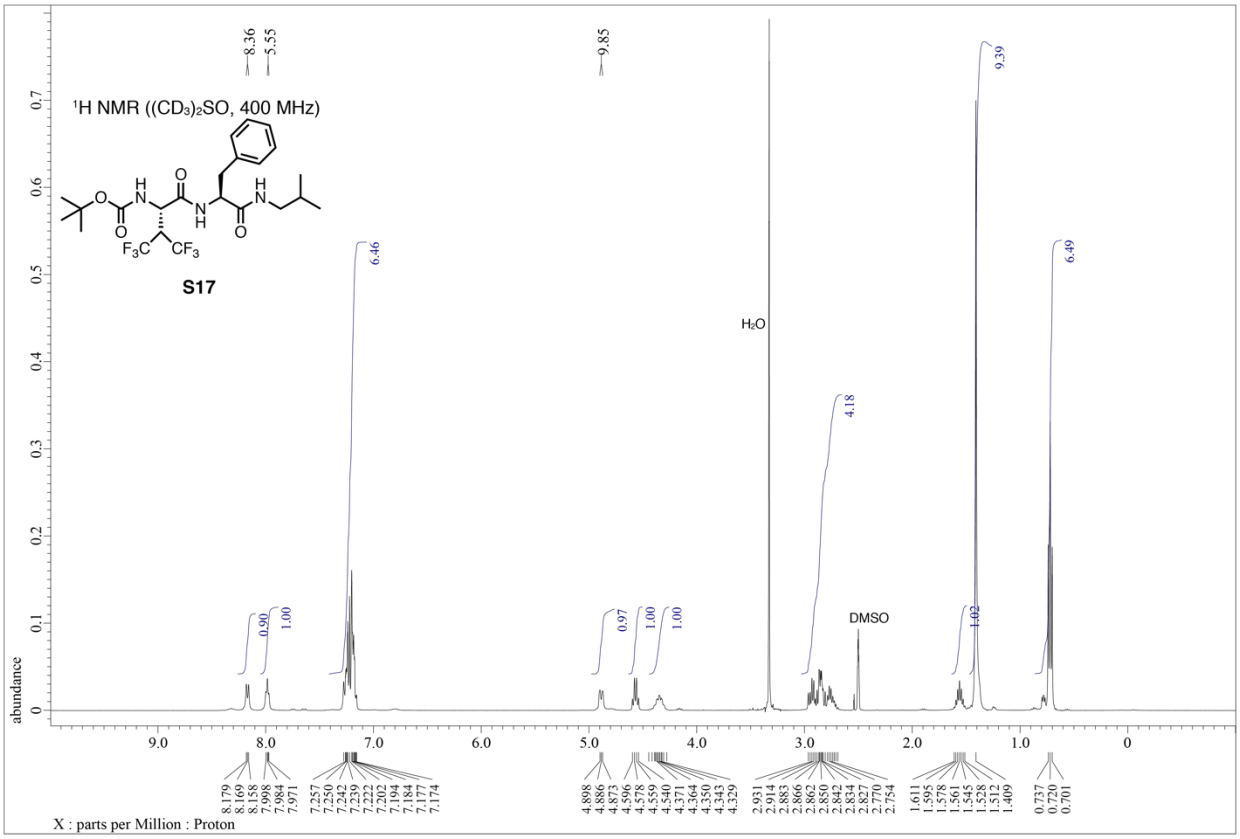


Fig. S6 ^{1}H NMR spectrum of **S17**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

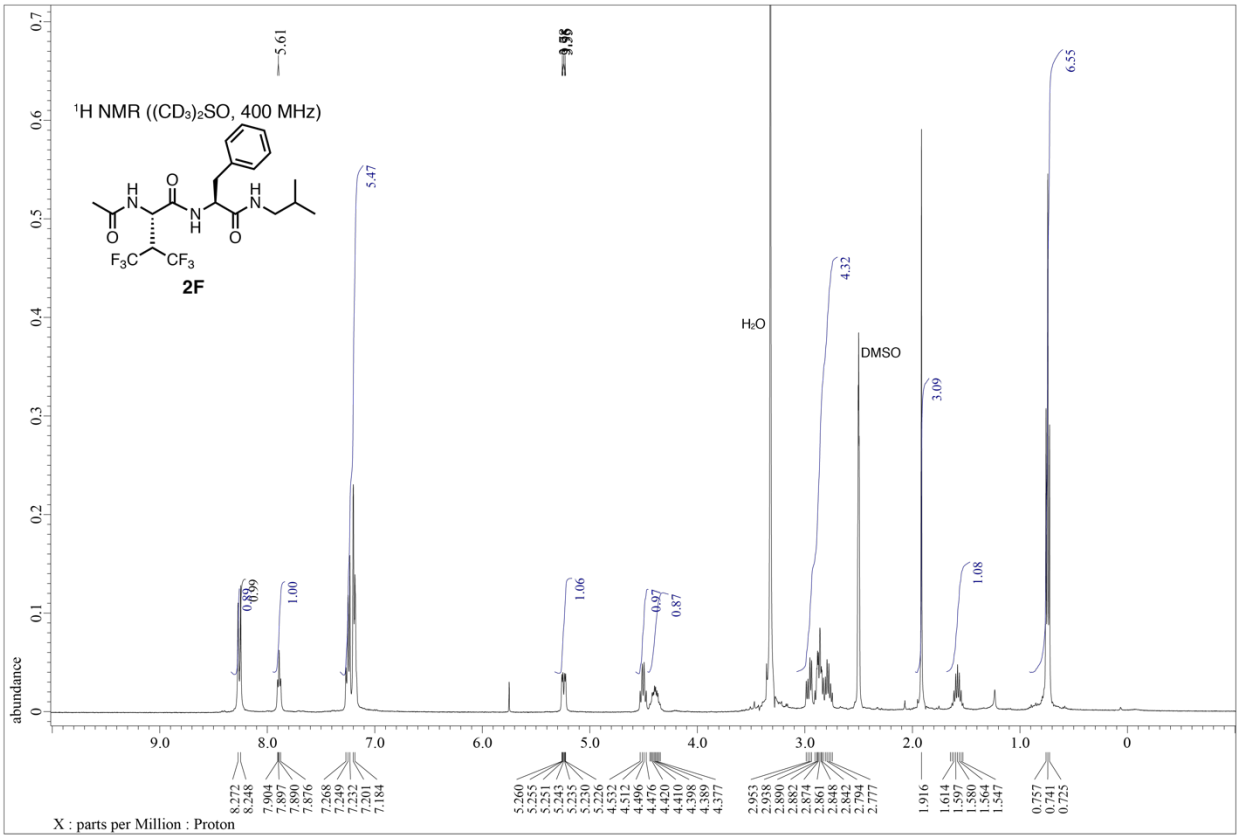


Fig. S7 ^{1}H NMR spectrum of **2F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

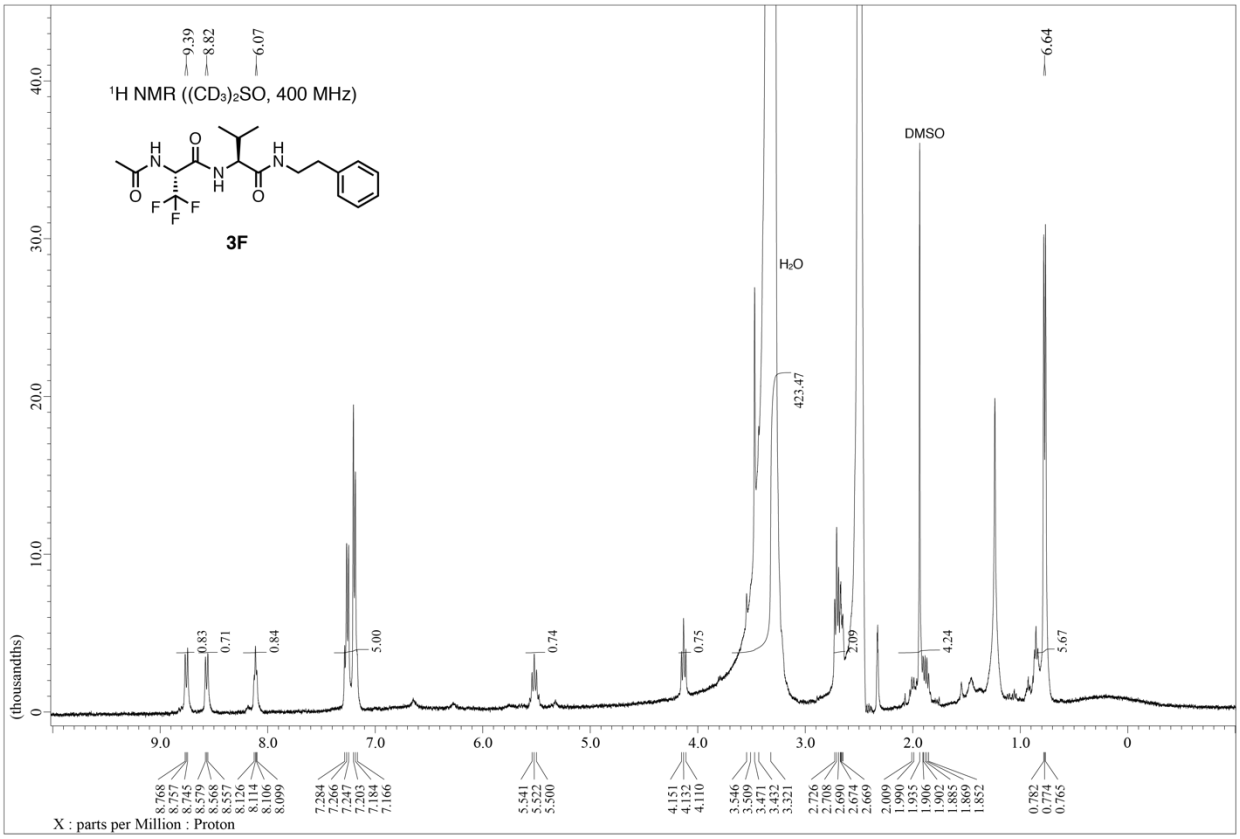


Fig. S8 ^{1}H NMR spectrum of **3F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

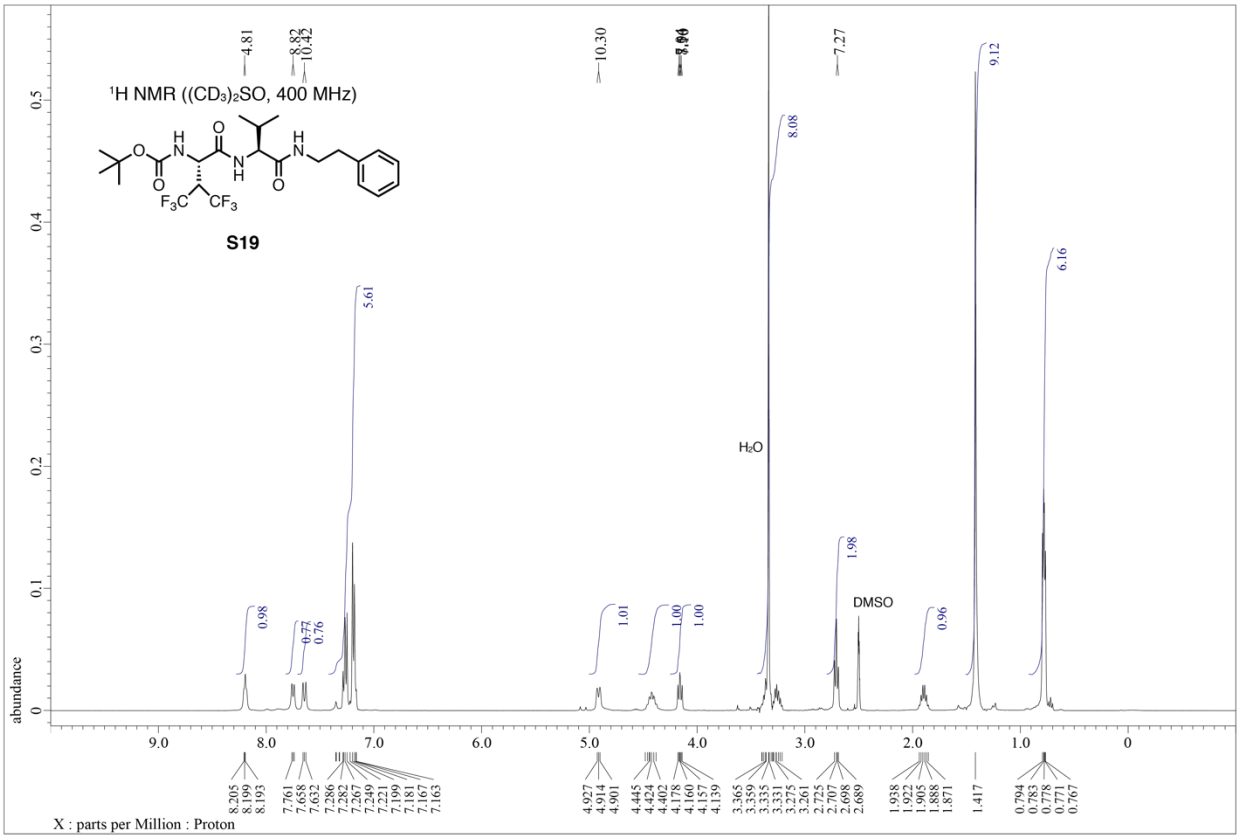


Fig. S9 ^{1}H NMR spectrum of **S19**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

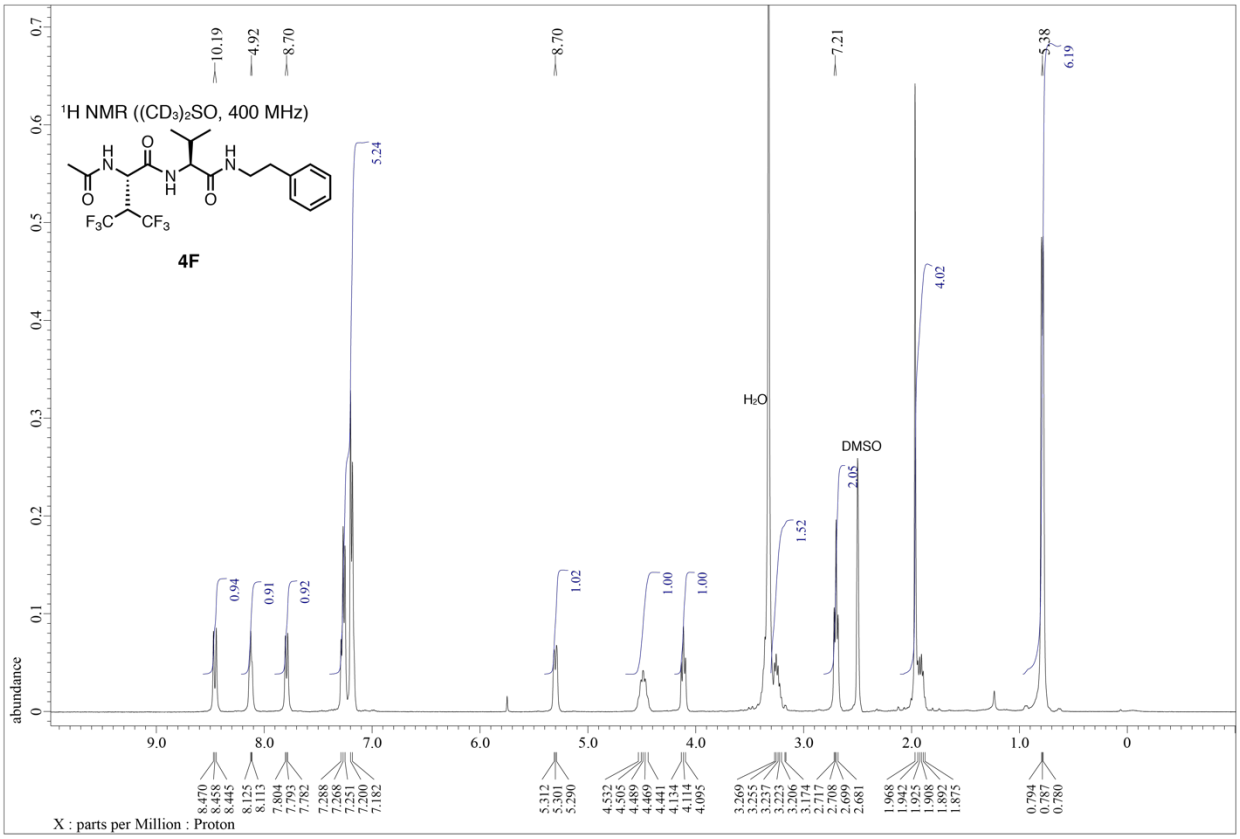


Fig. S10 ^{1}H NMR spectrum of **4F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

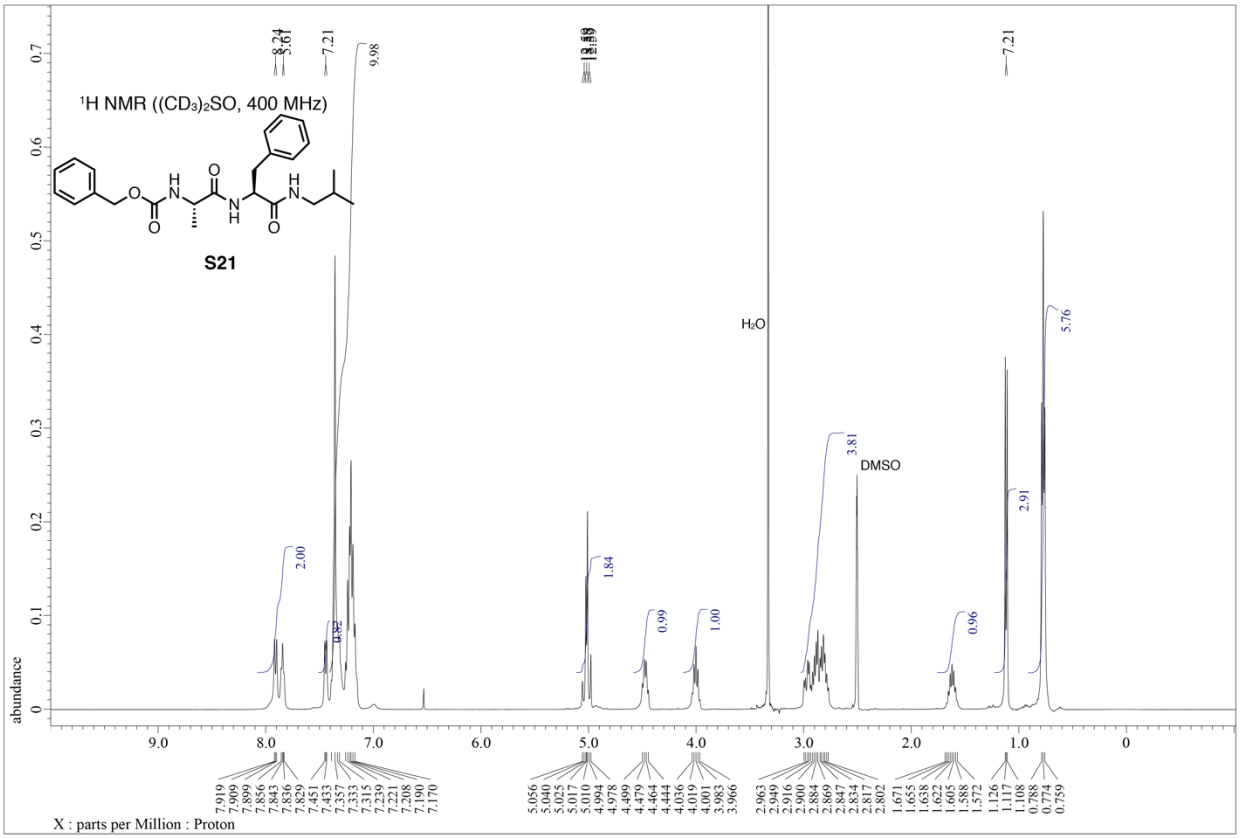


Fig. S11 ^{1}H NMR spectrum of **S21**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

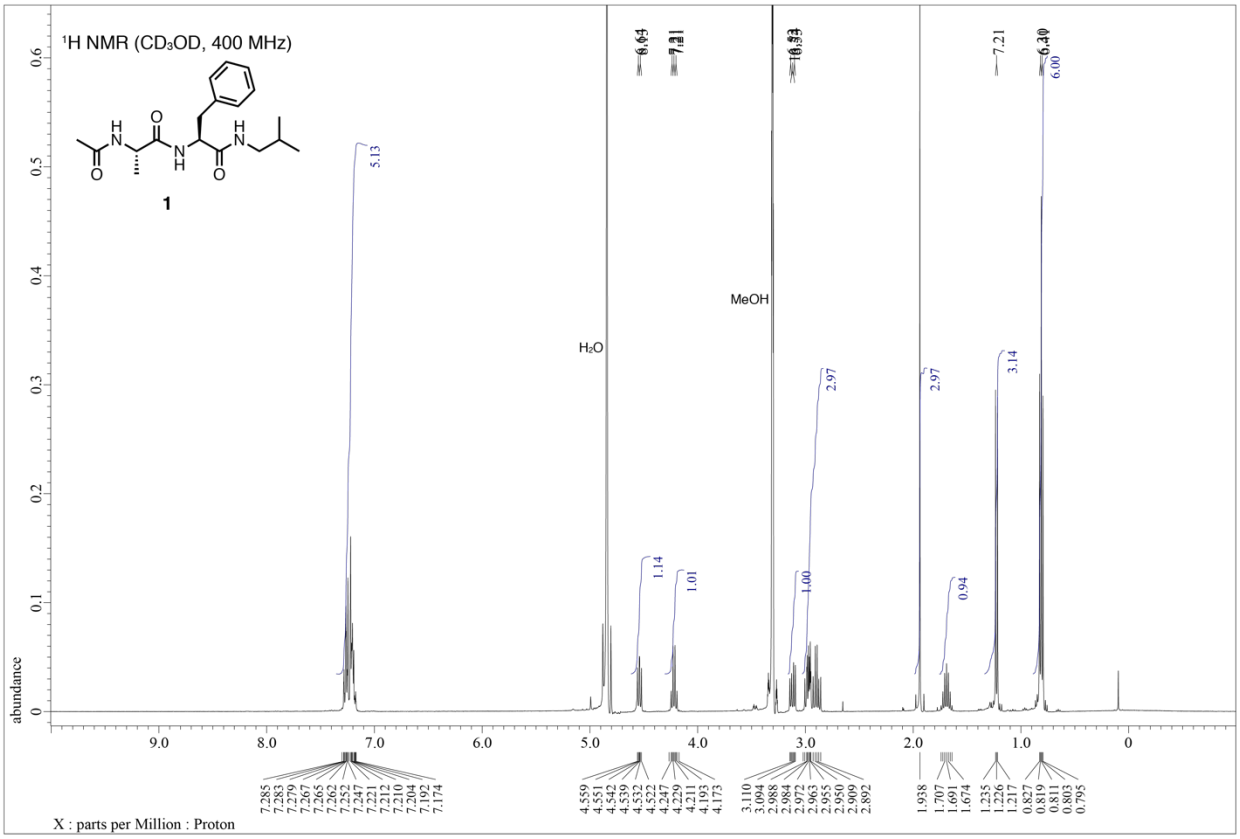


Fig. S12 ^{1}H NMR spectrum of **1**. Spectrum was recorded in CD_3OD at 25 °C.

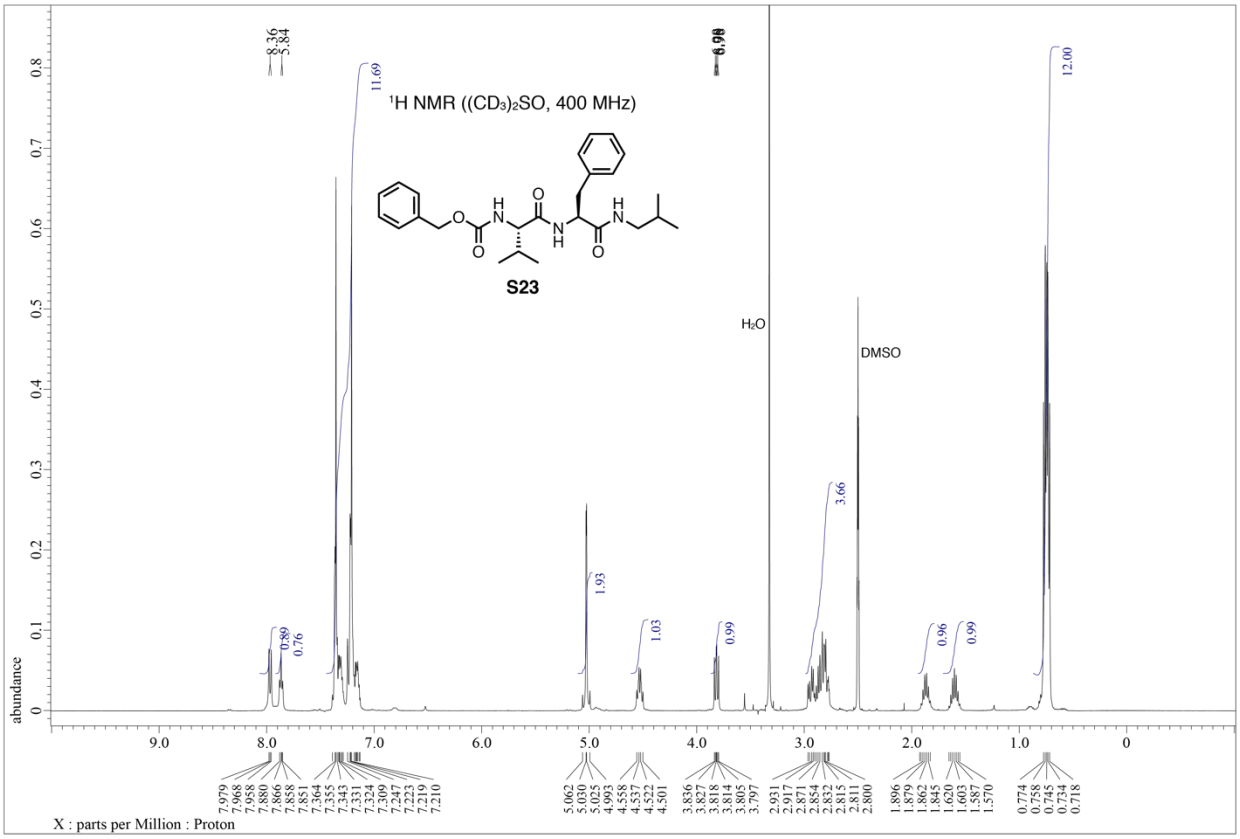


Fig. S13 ^{1}H NMR spectrum of **S23**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

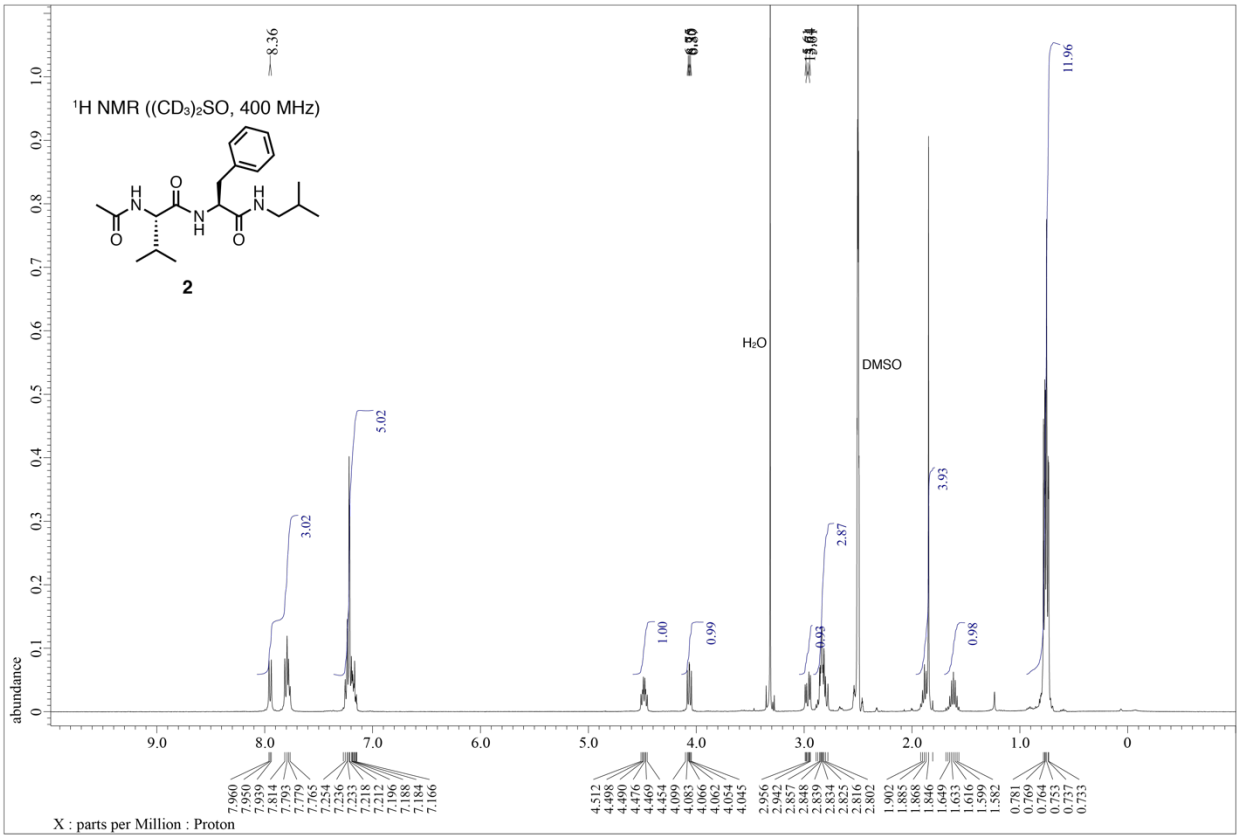


Fig. S14 ^{1}H NMR spectrum of **2**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

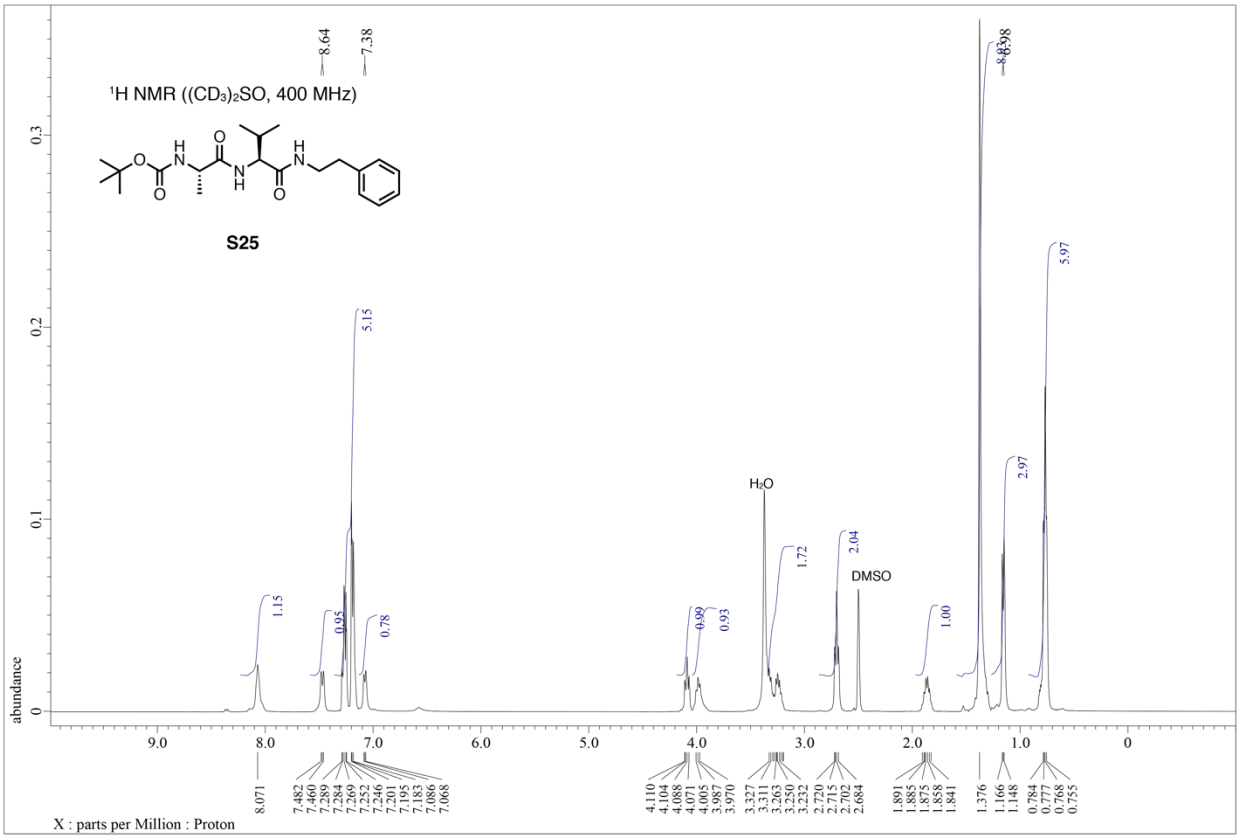


Fig. S15 ^{1}H NMR spectrum of **S25**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

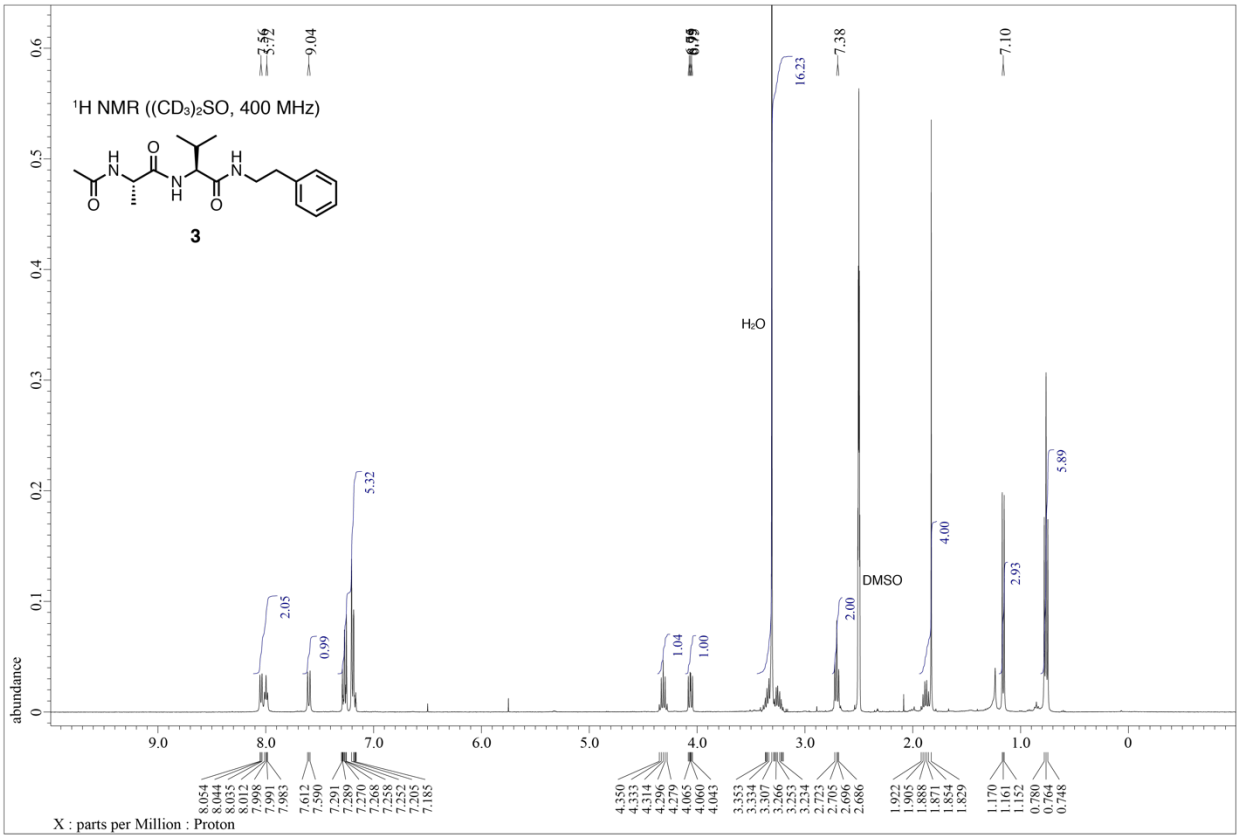


Fig. S16 ^{1}H NMR spectrum of **3**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

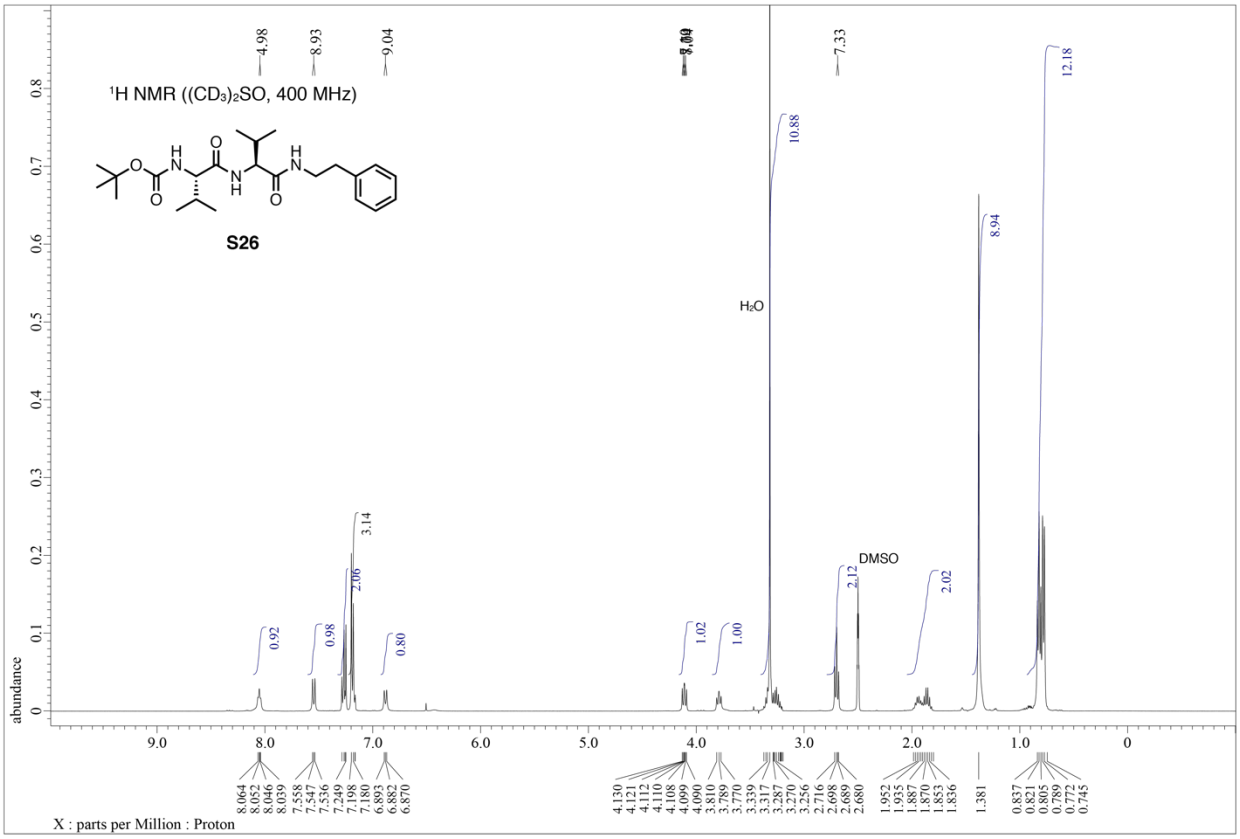


Fig. S17 ^{1}H NMR spectrum of **S26**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

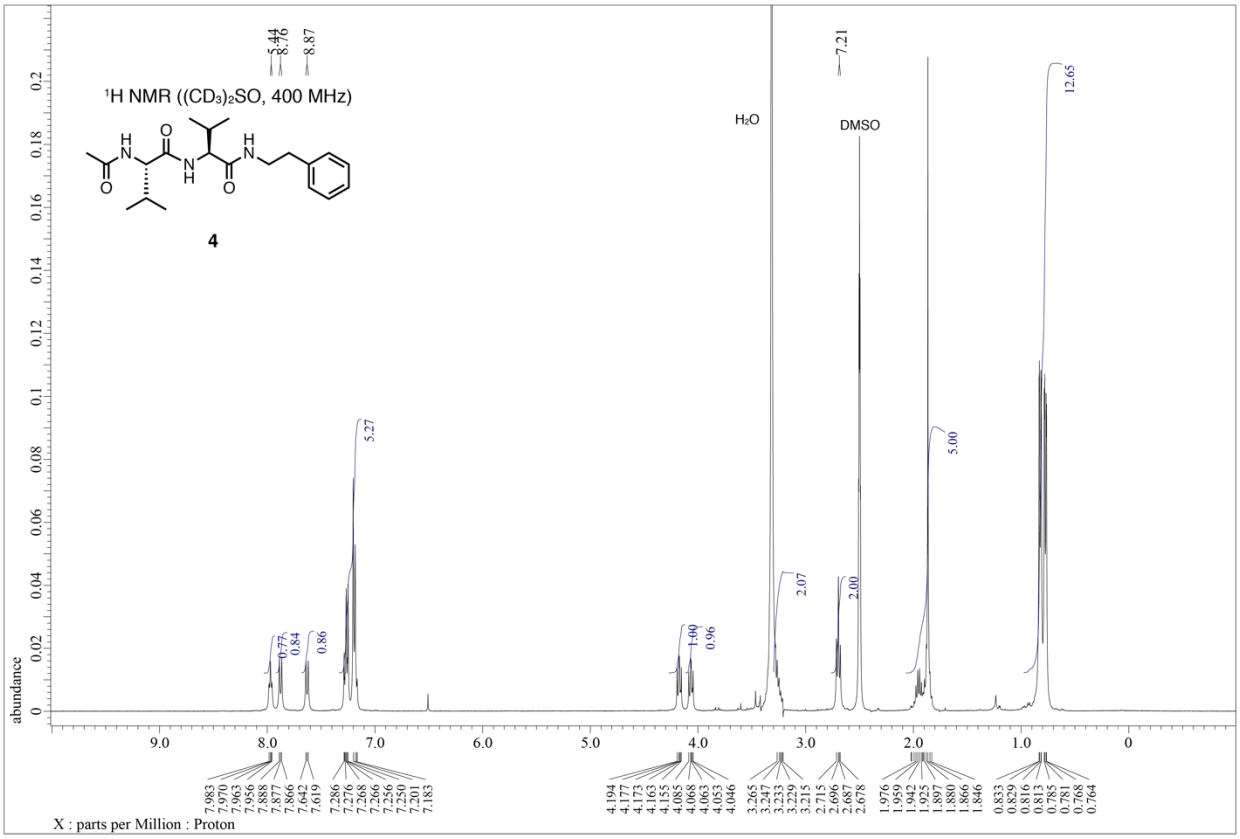


Fig. S18 ^{1}H NMR spectrum of **4**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

^{19}F NMR

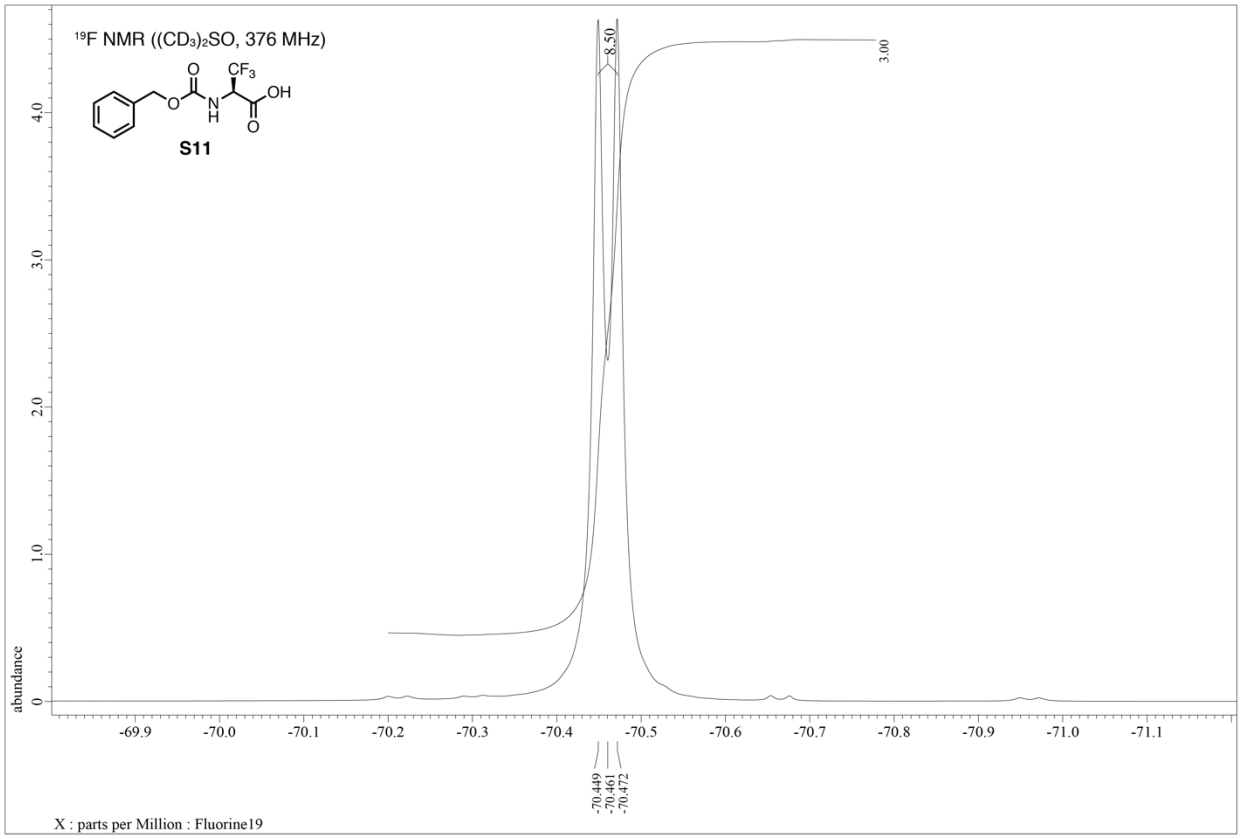


Fig. S19 ^{19}F NMR spectrum of **S11**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

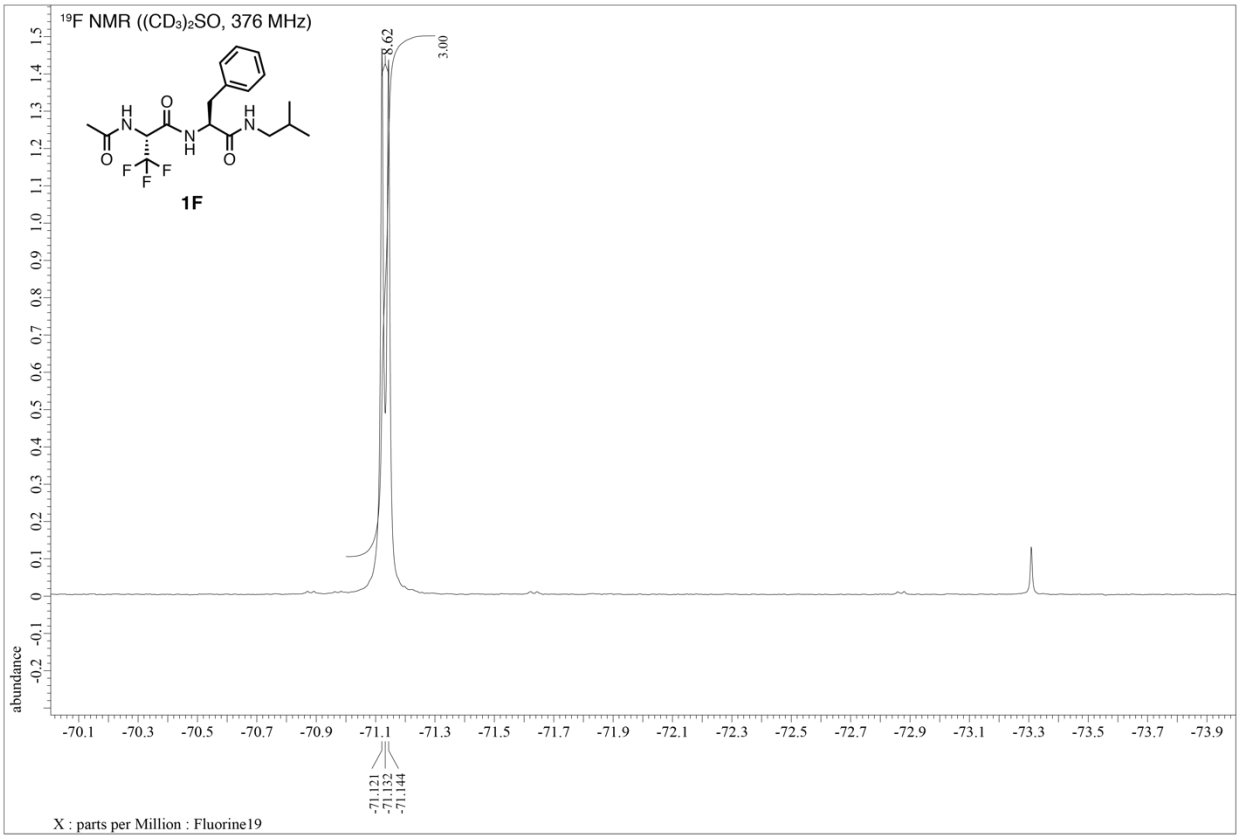


Fig. S20 ^{19}F NMR spectrum of **1F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

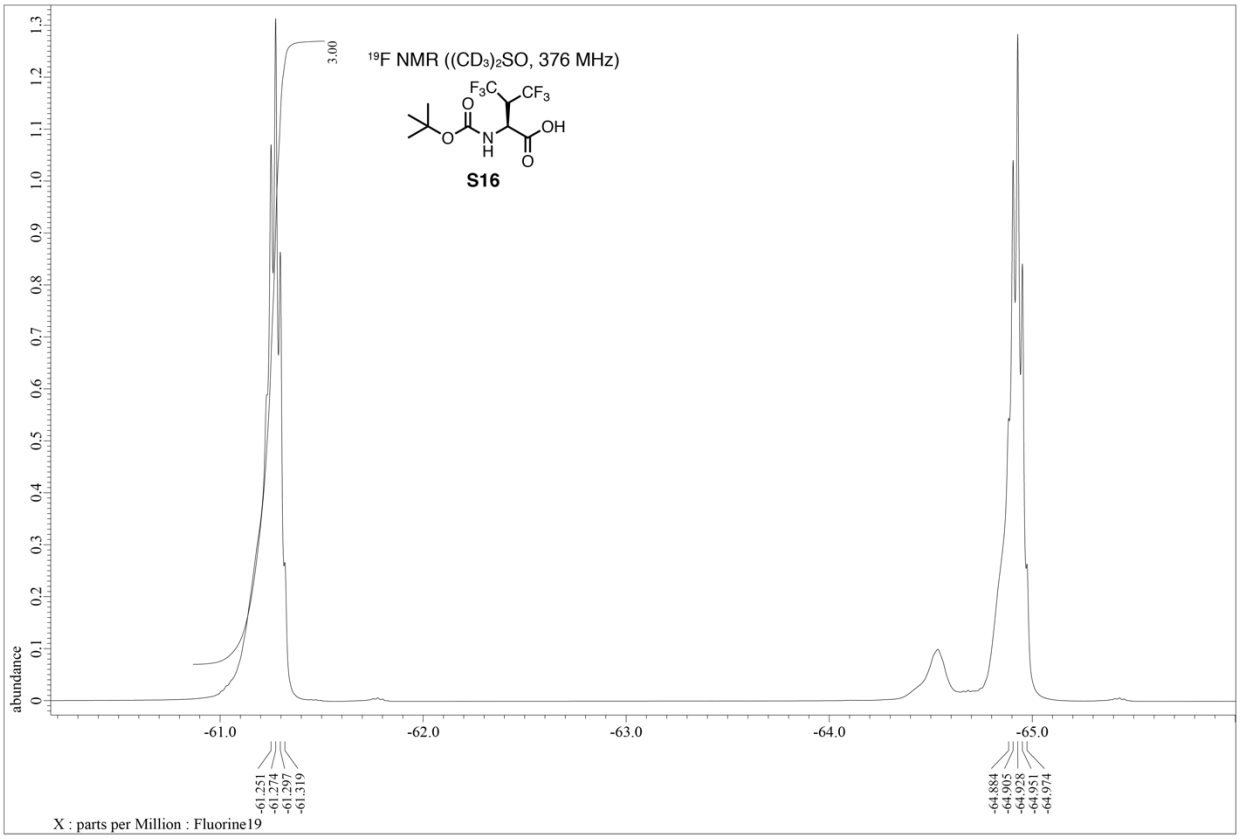


Fig. S21 ^{19}F NMR spectrum of **S16**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

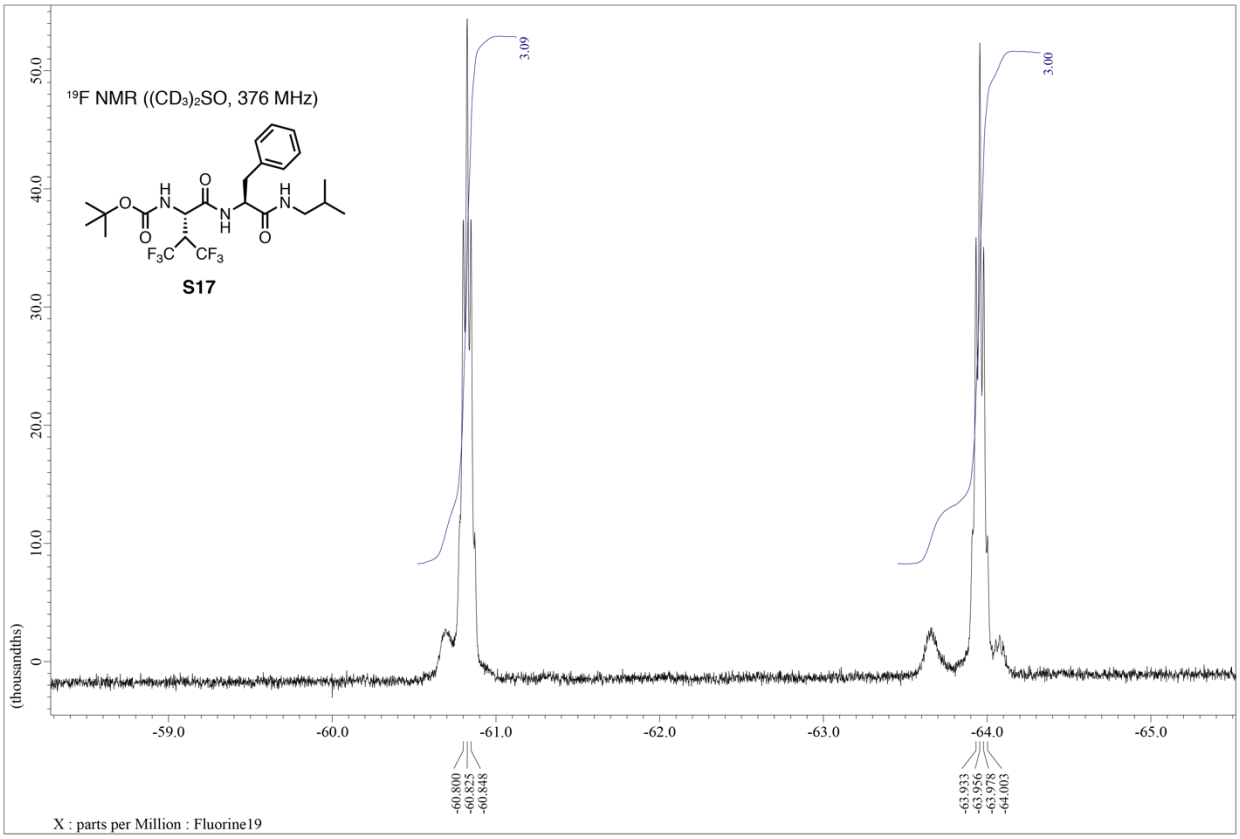


Fig. S22 ^{19}F NMR spectrum of **S17**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

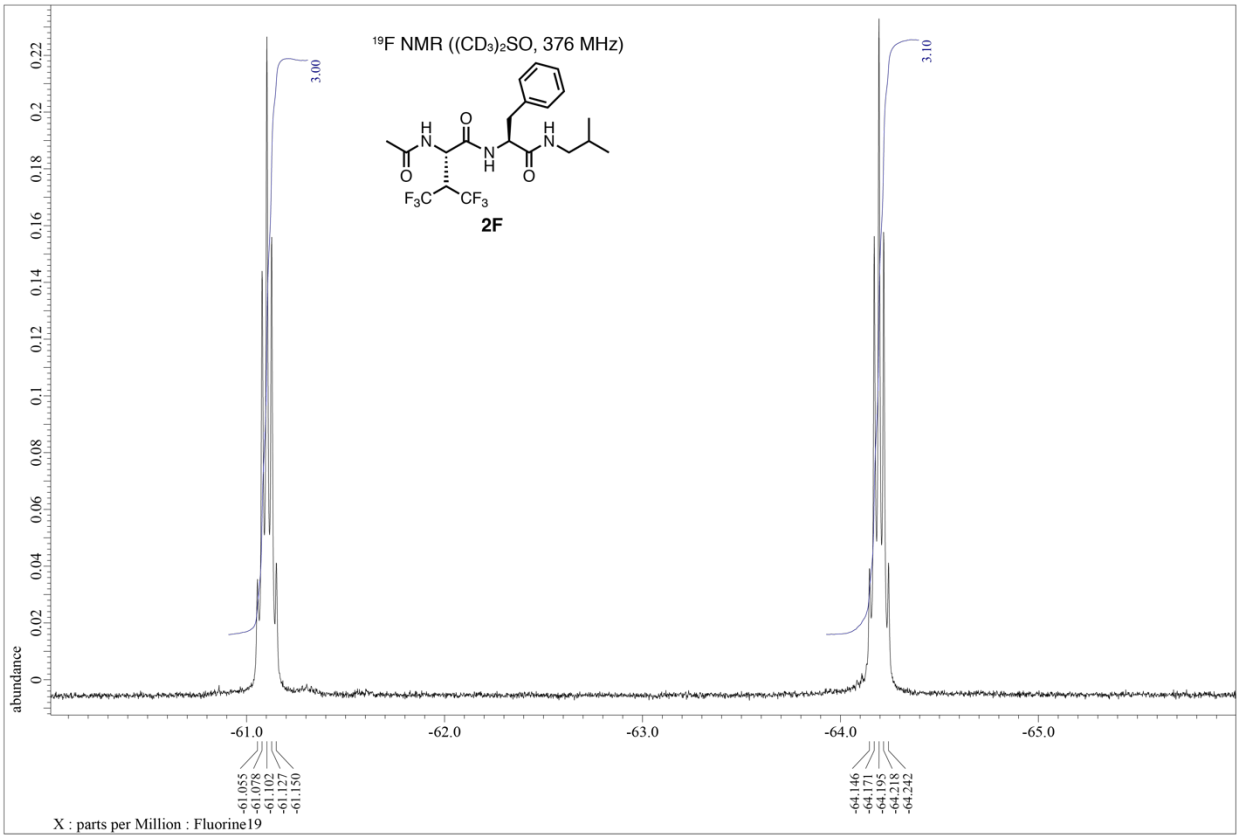


Fig. S23 ^{19}F NMR spectrum of **2F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

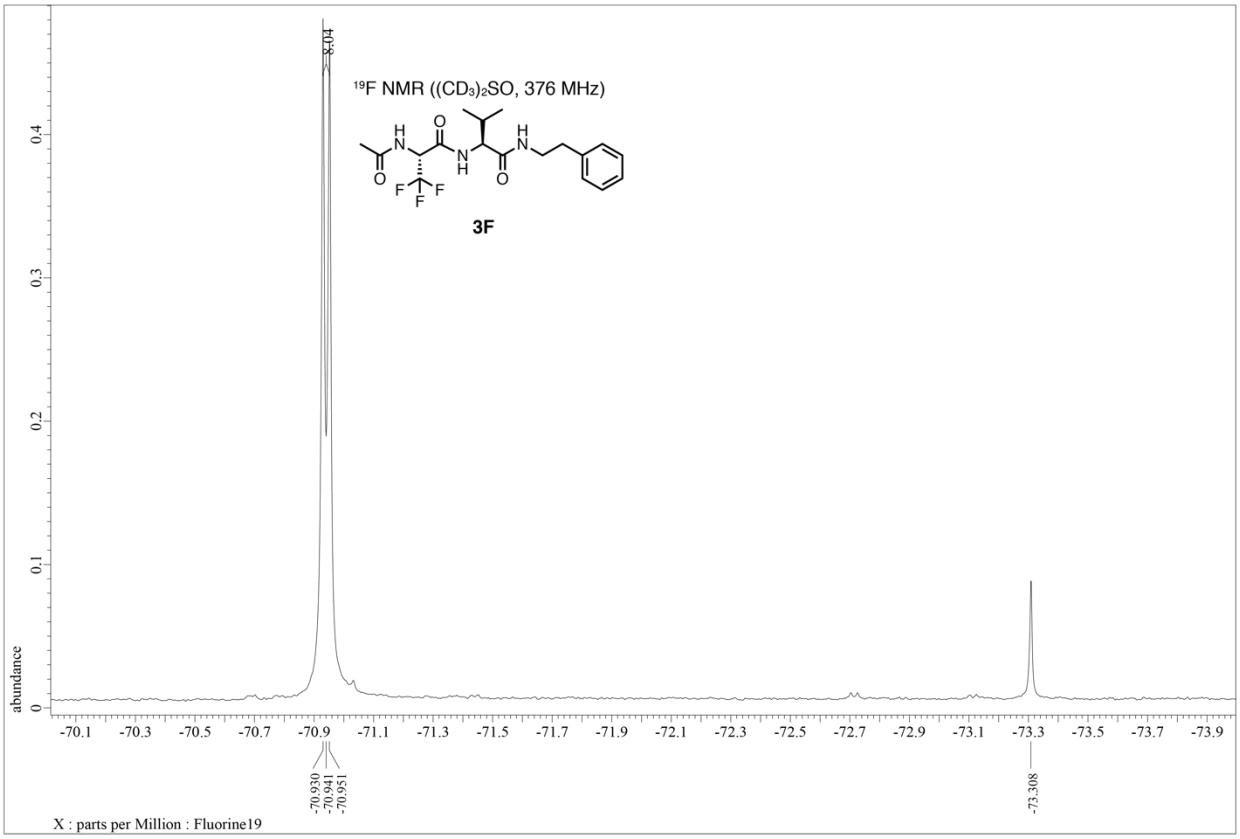


Fig. S24 ^{19}F NMR spectrum of **3F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

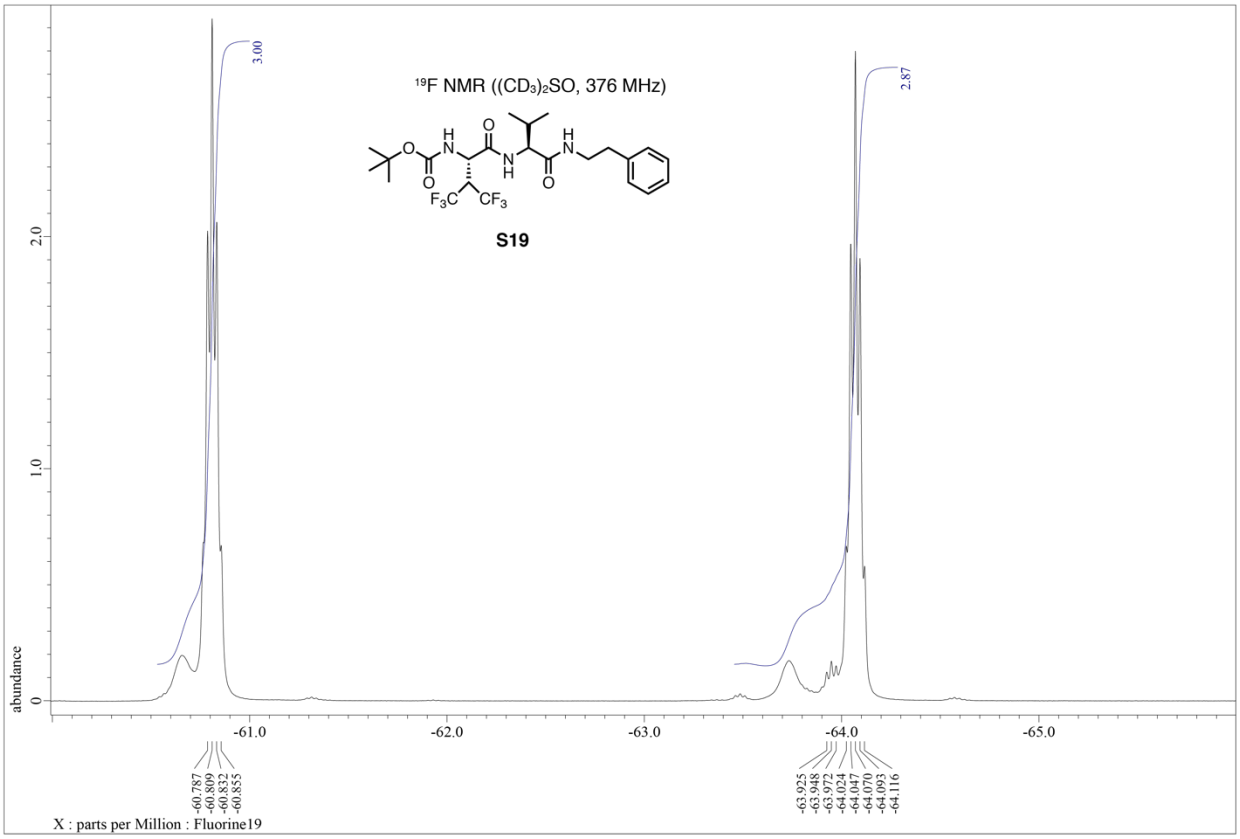


Fig. S25 ^{19}F NMR spectrum of **S19**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

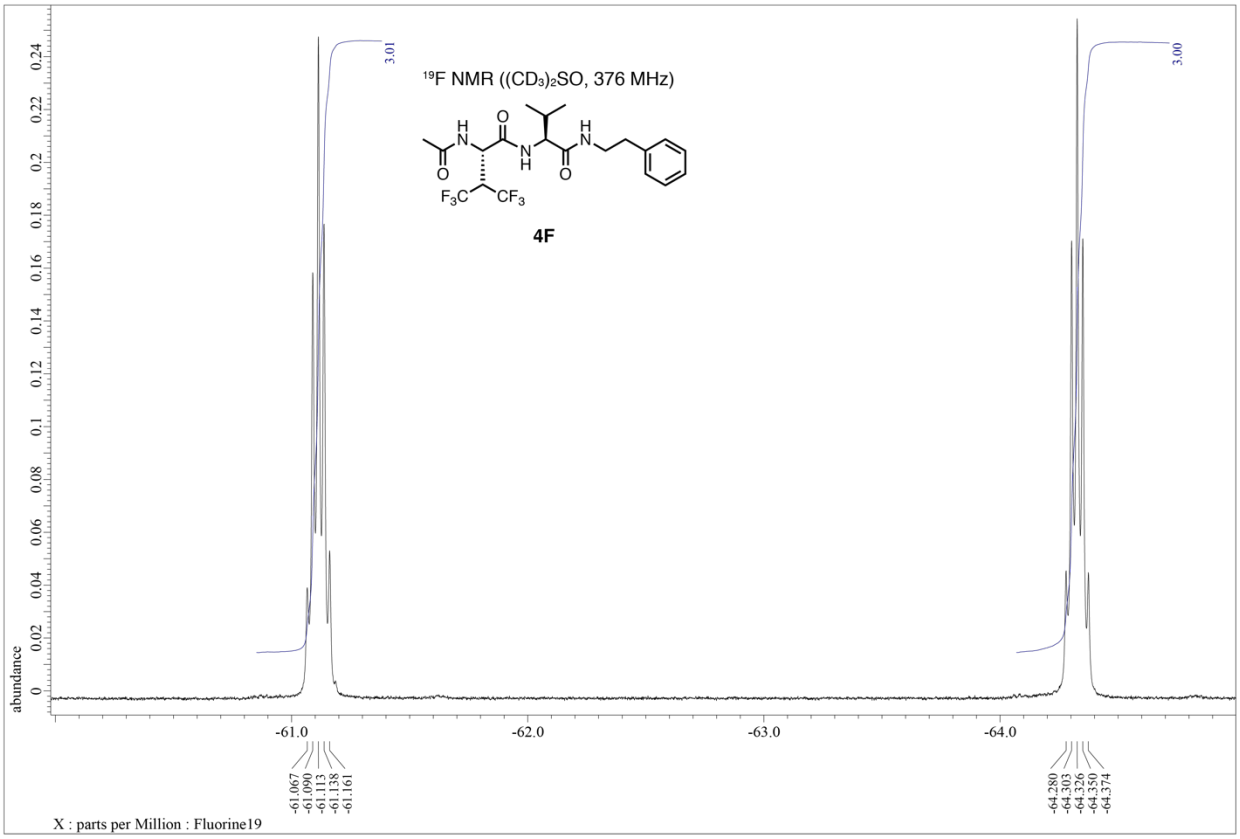


Fig. S26 ^{19}F NMR spectrum of **4F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

^{13}C NMR

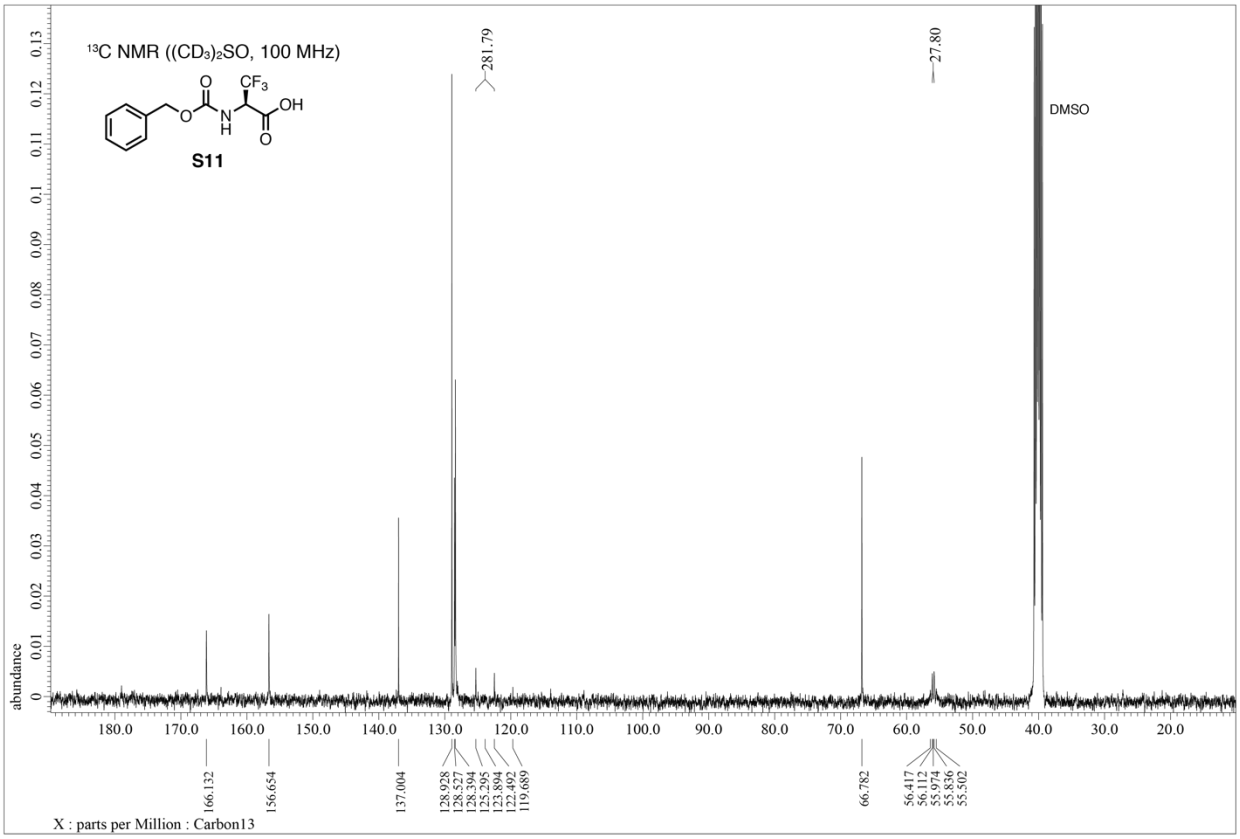


Fig. S27 ^{13}C NMR spectrum of **S11**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

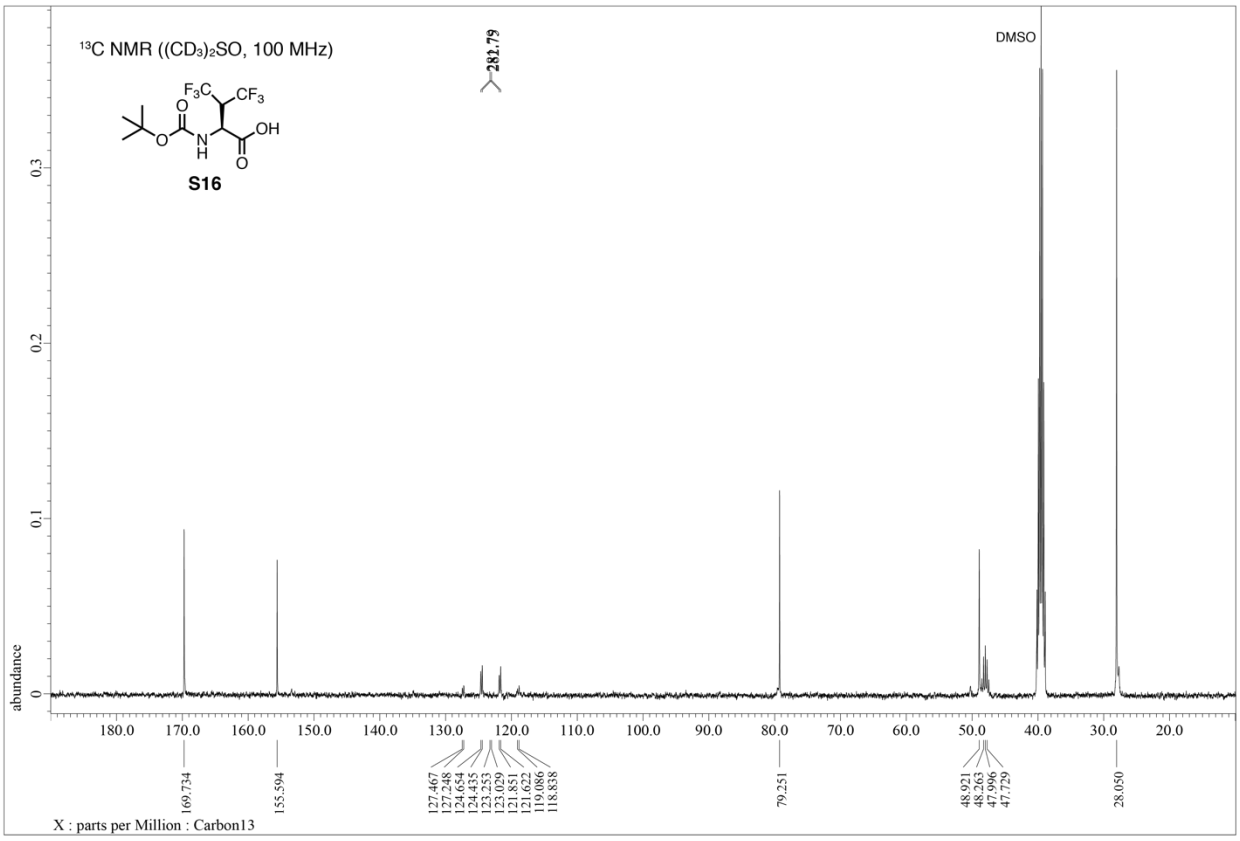


Fig. S28 ^{13}C NMR spectrum of **S16**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

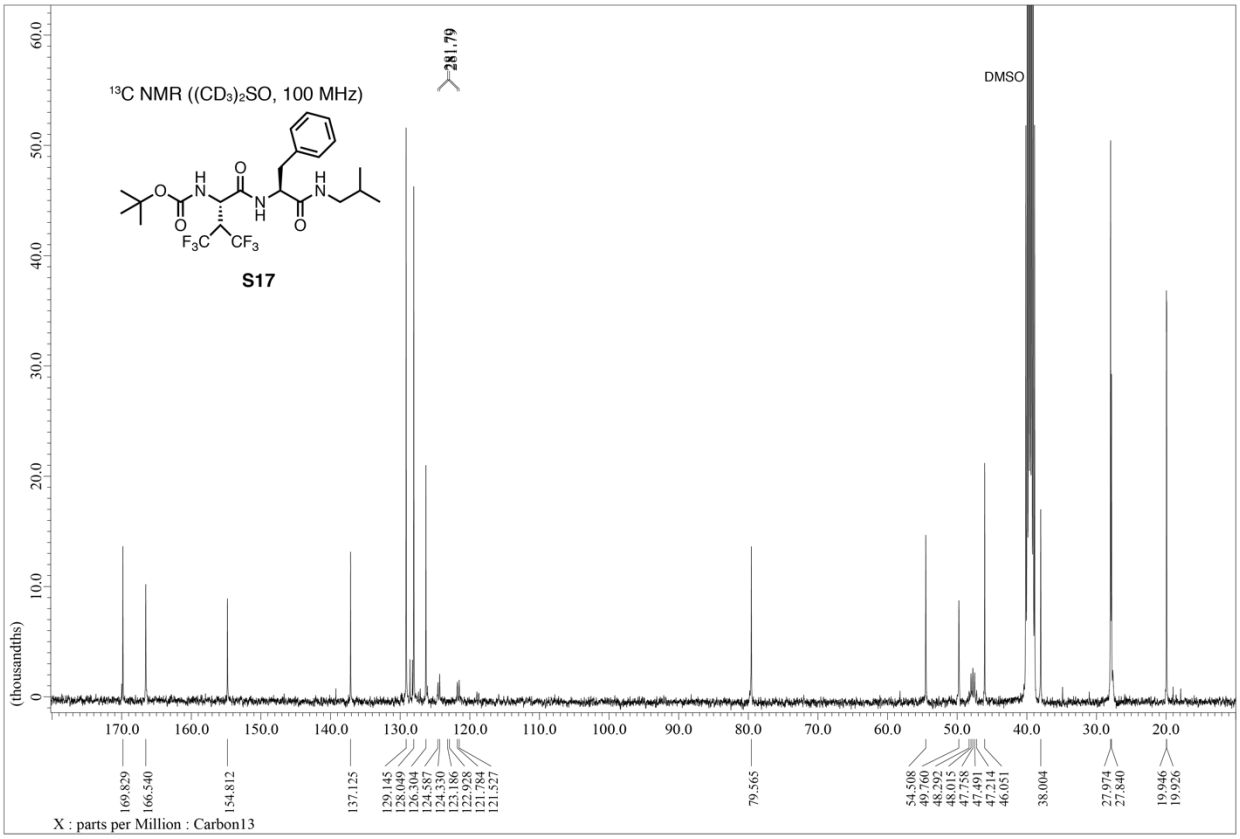


Fig. S29 ^{13}C NMR spectrum of **S17**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

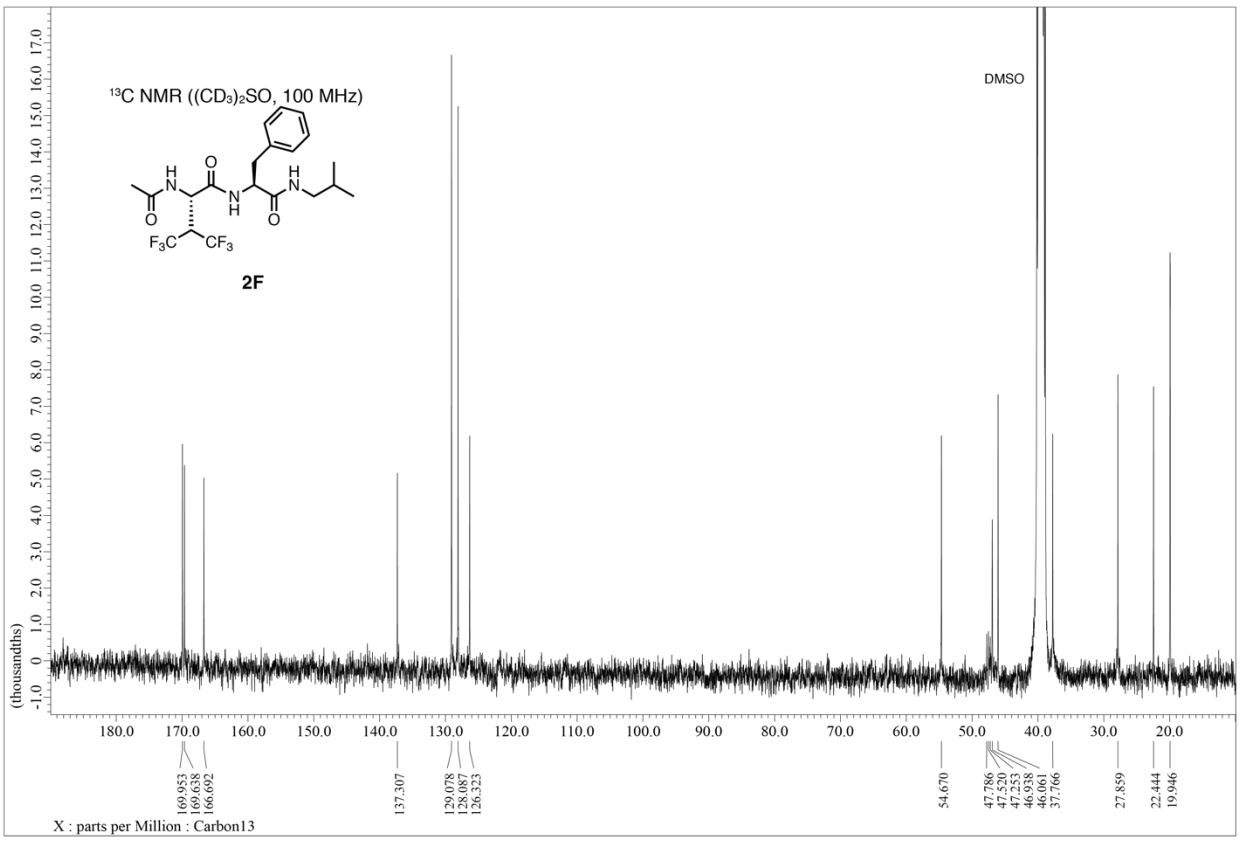


Fig. S30 ^{13}C NMR spectrum of **2F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

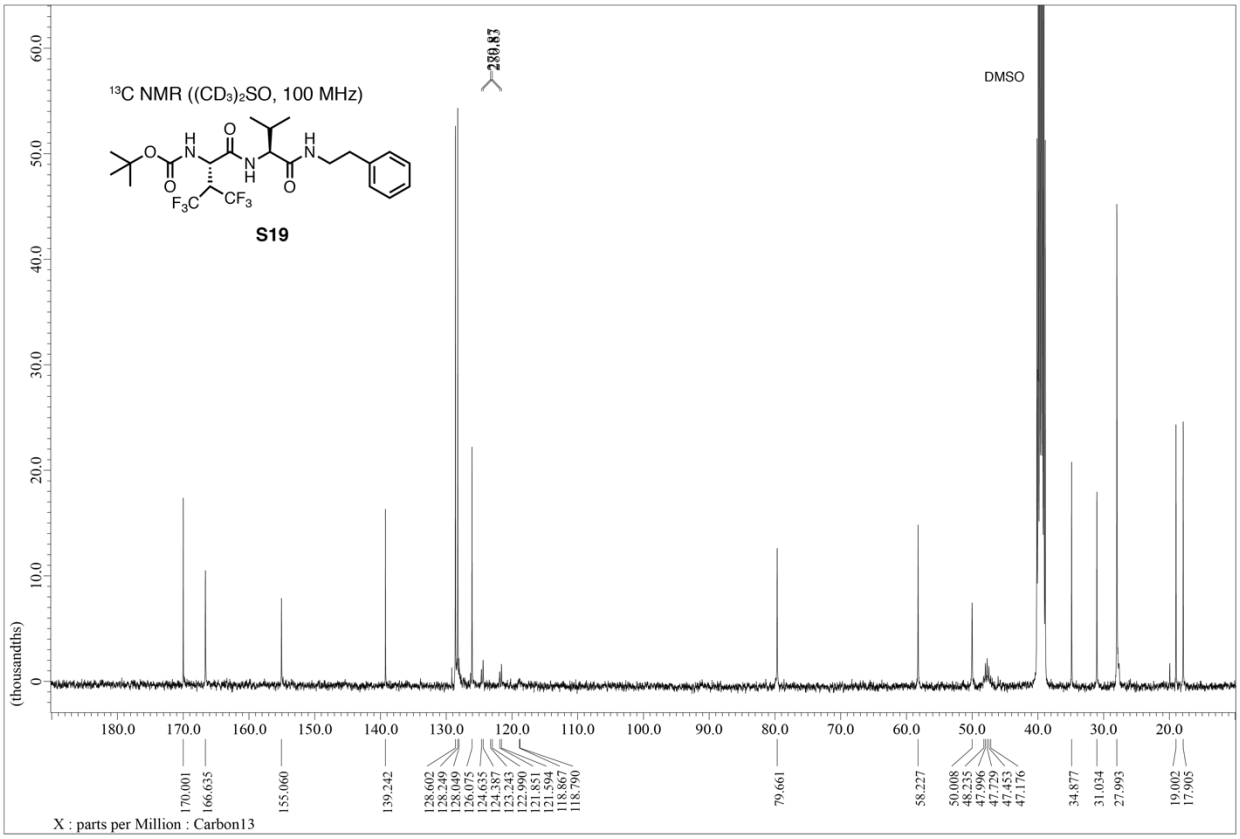


Fig. S31 ^{13}C NMR spectrum of **S19**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

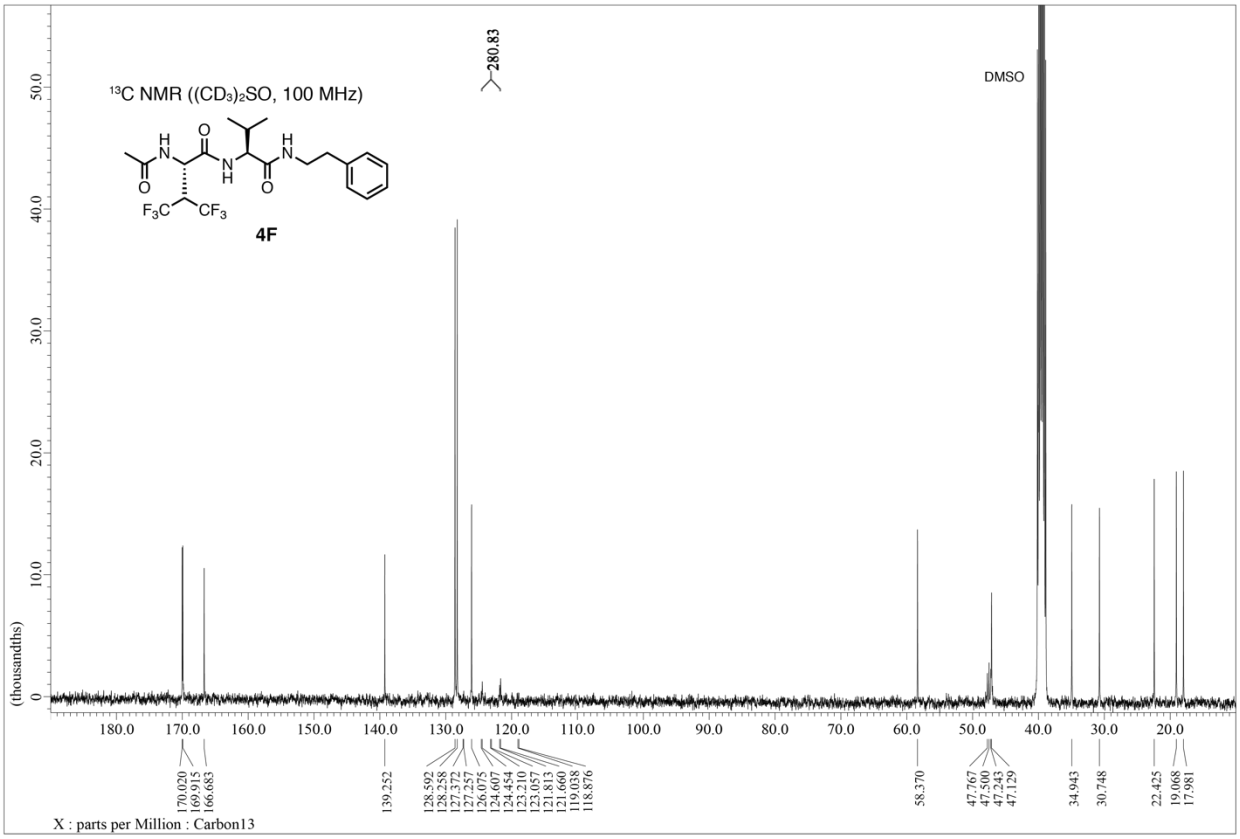


Fig. S32 ^{13}C NMR spectrum of **4F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

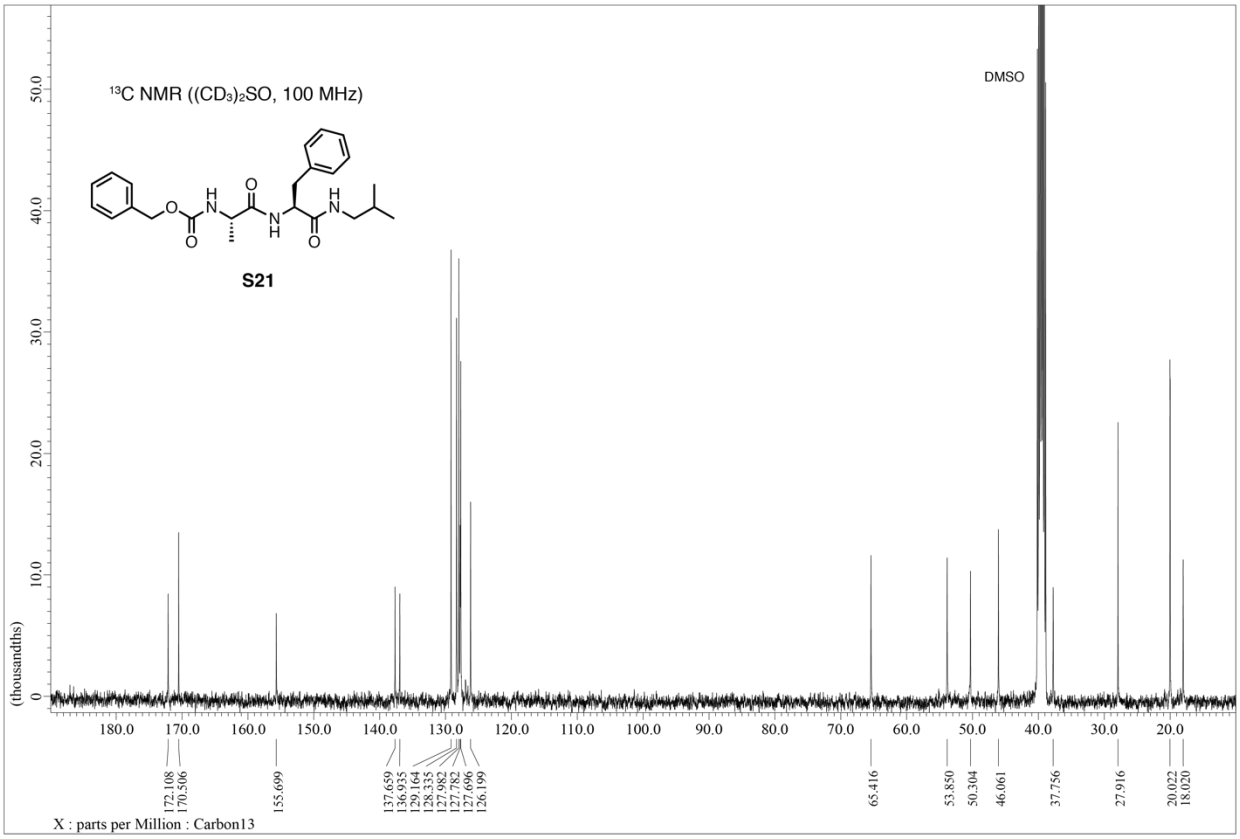


Fig. S33 ^{13}C NMR spectrum of **S21**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

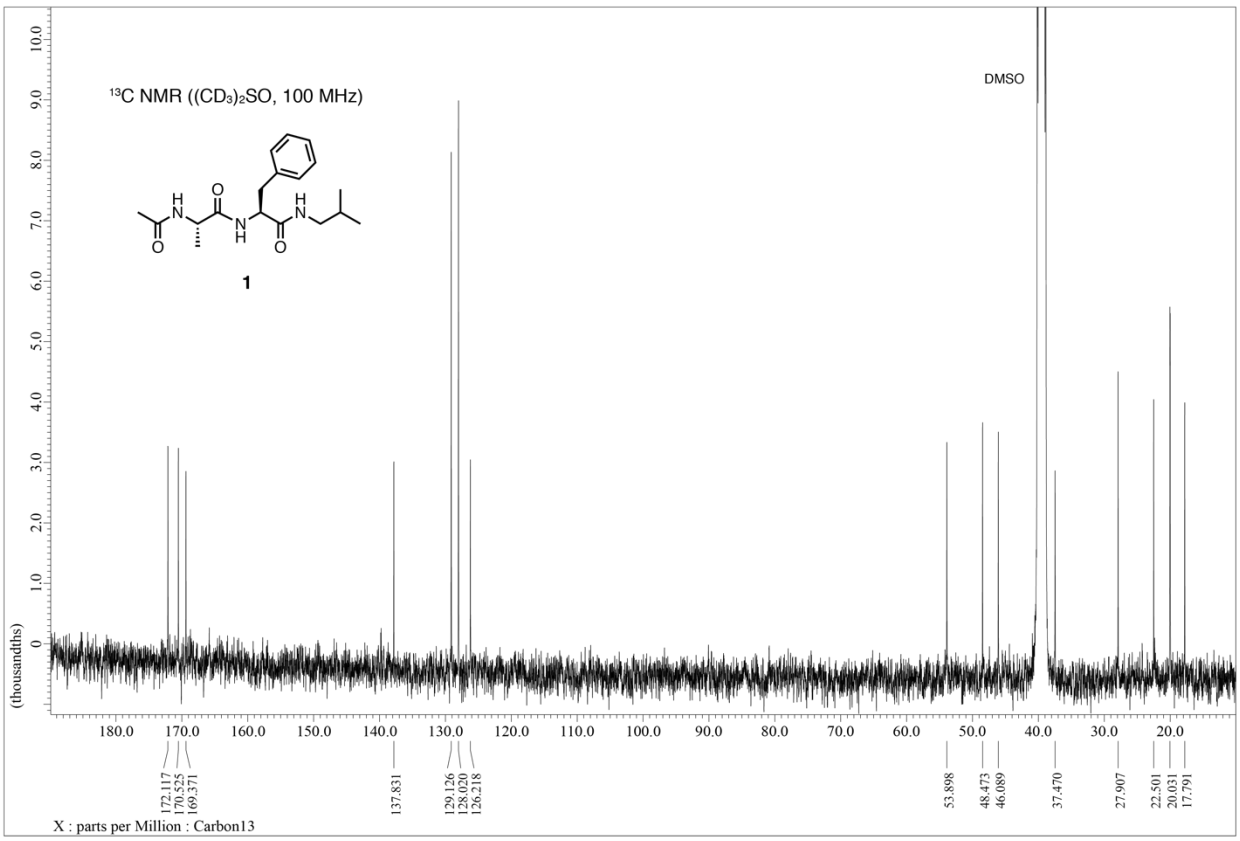


Fig. S34 ^{13}C NMR spectrum of **1**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

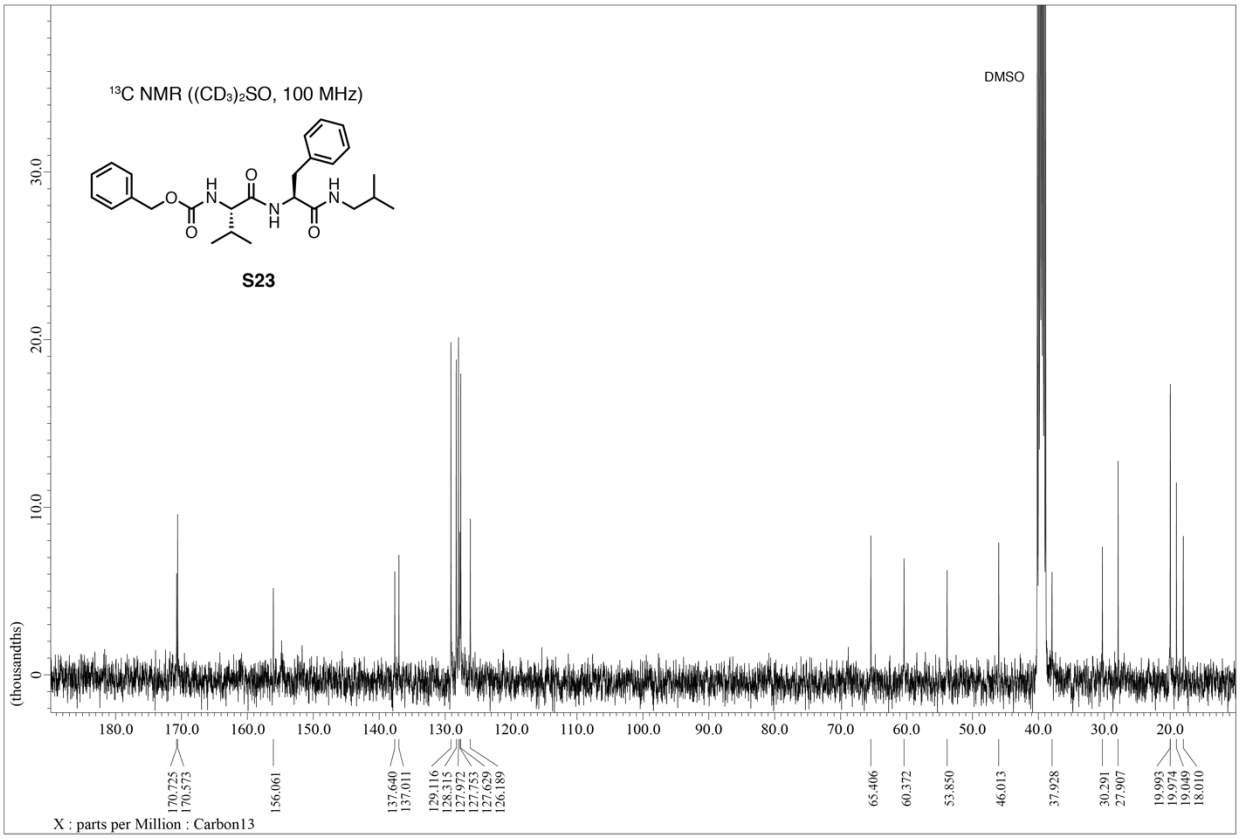


Fig. S35 ^{13}C NMR spectrum of **S23**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

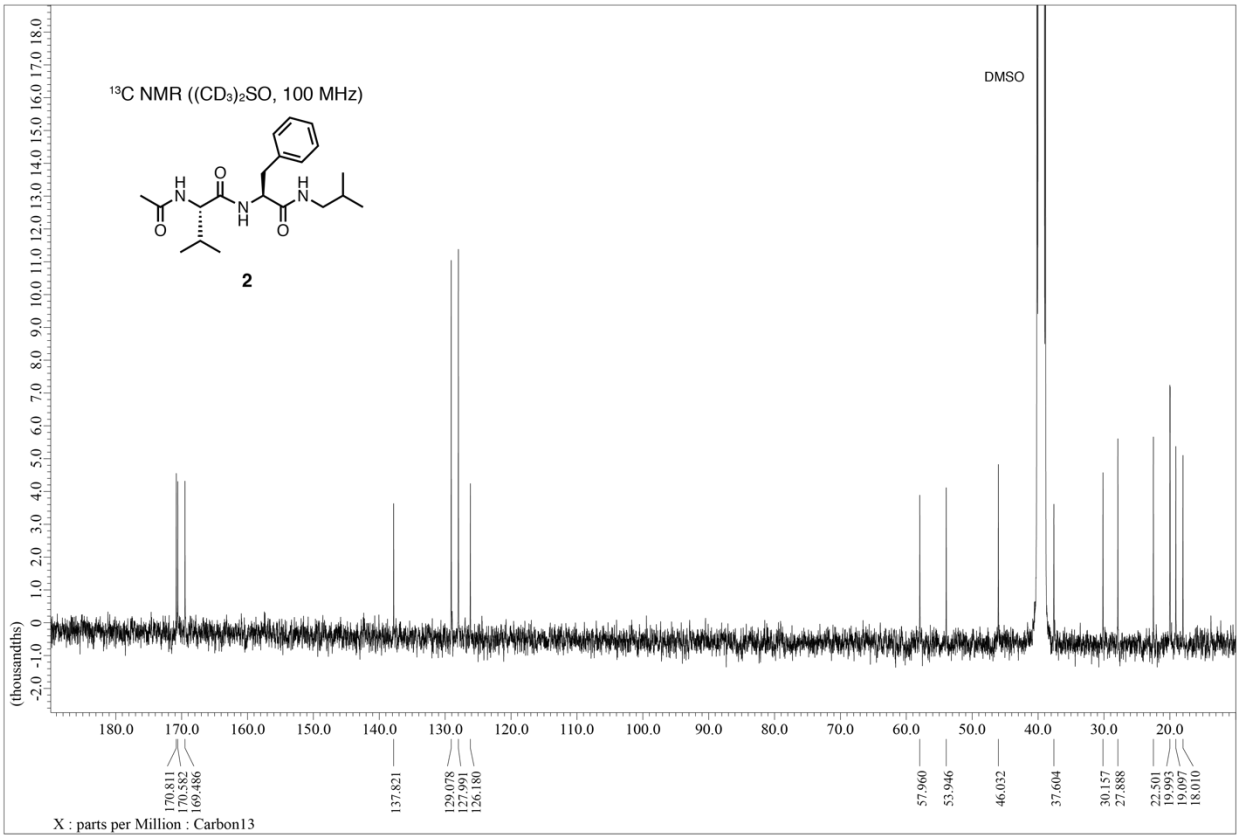


Fig. S36 ^{13}C NMR spectrum of **2**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

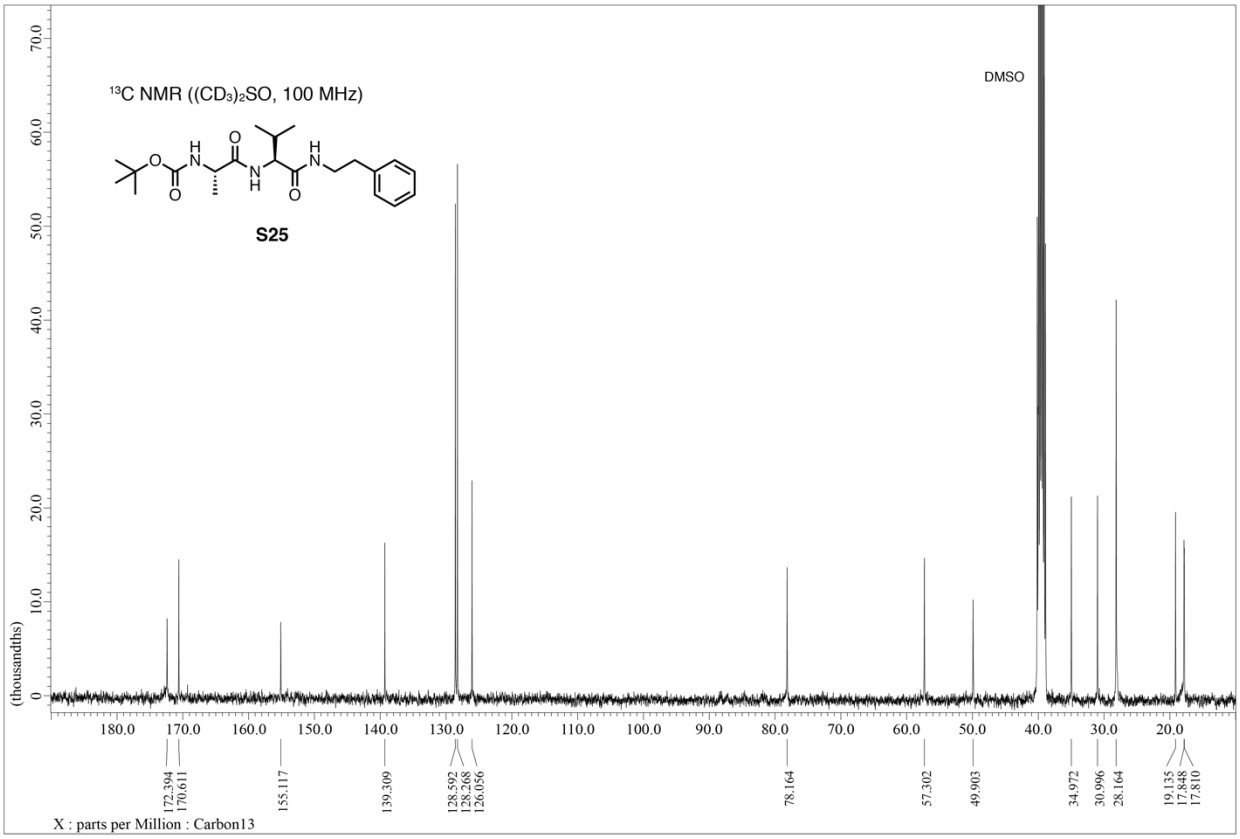


Fig. S37 ^{13}C NMR spectrum of **S25**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

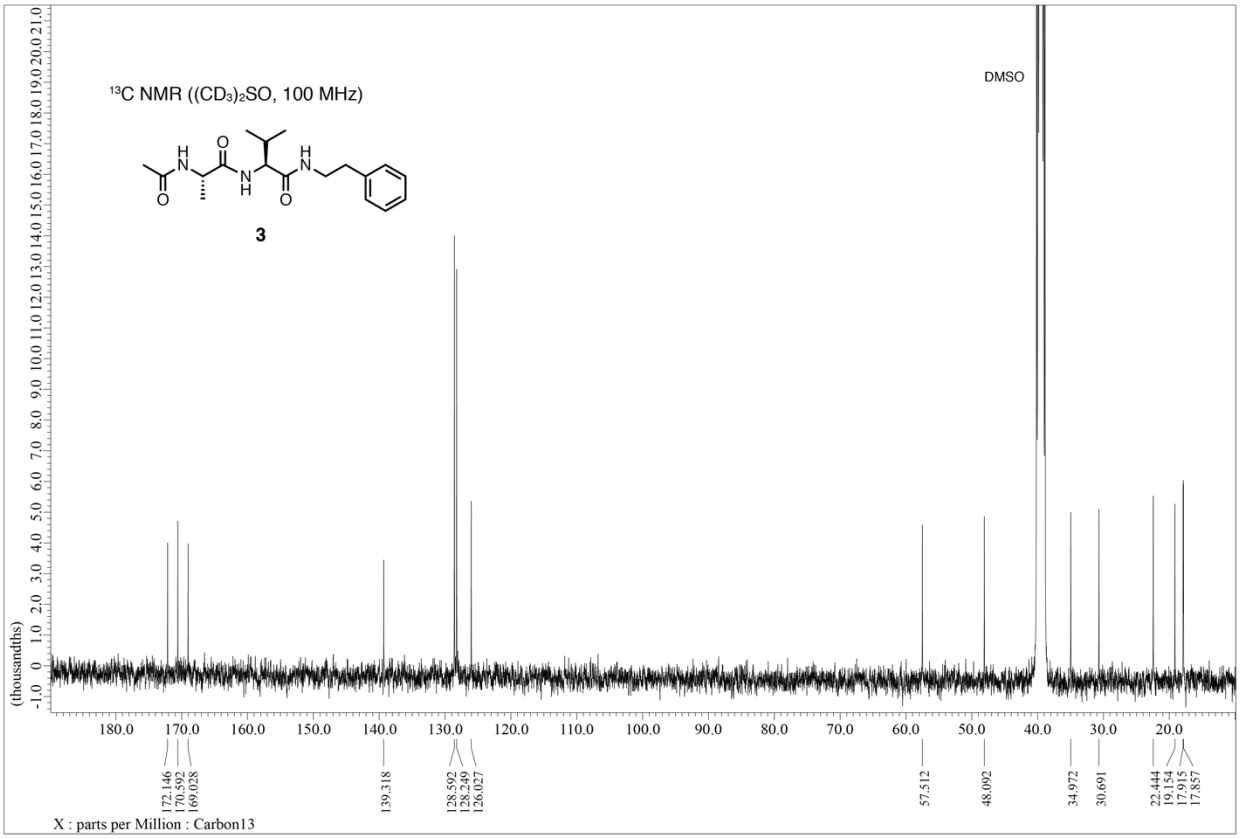


Fig. S38 ^{13}C NMR spectrum of **3**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

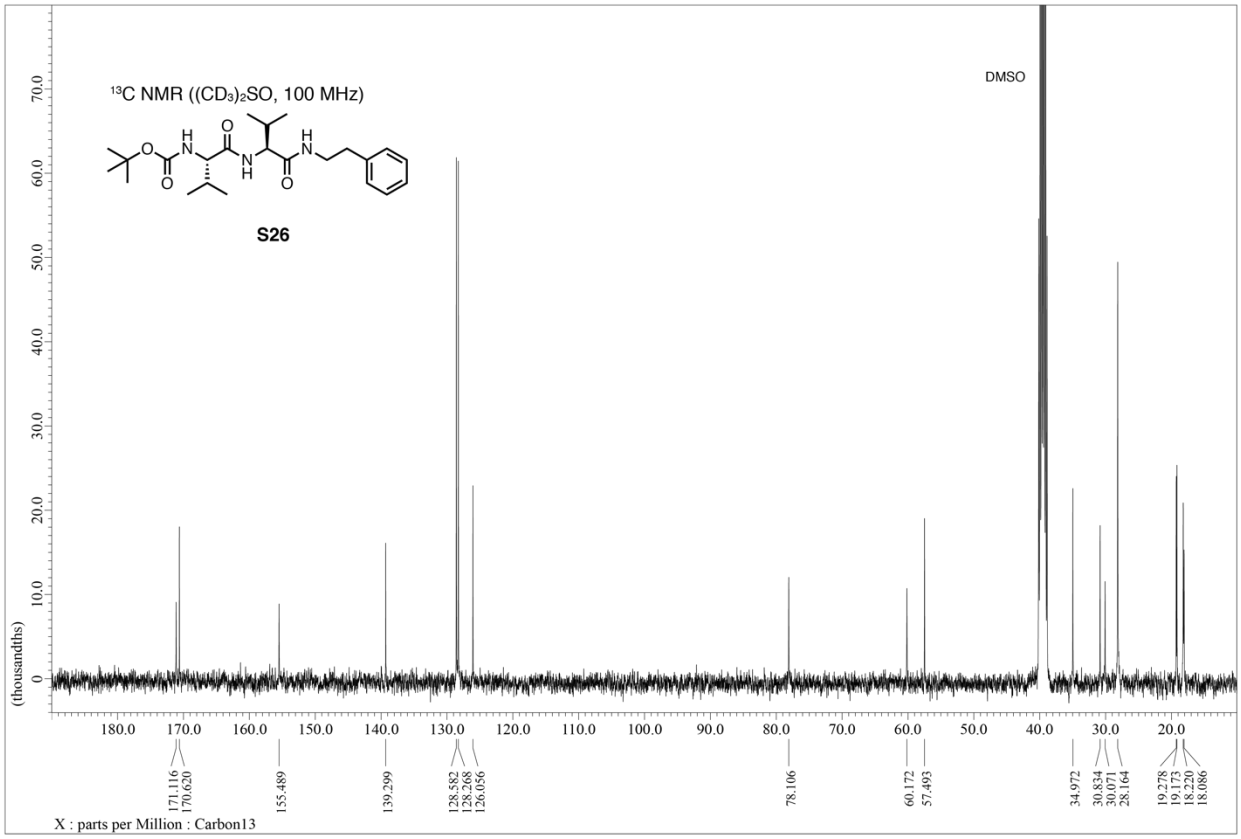


Fig. S39 ^{13}C NMR spectrum of **S26**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

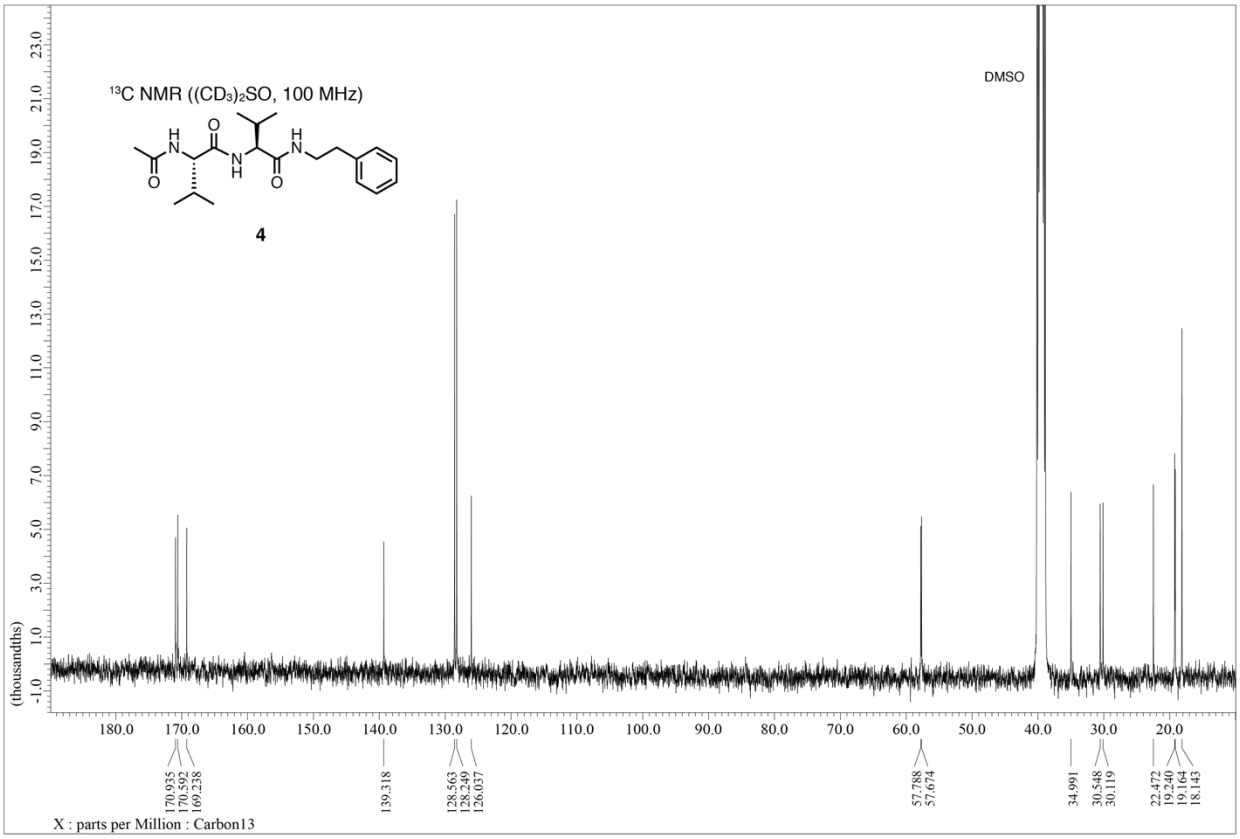


Fig. S40 ^{13}C NMR spectrum of **4**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

COSY

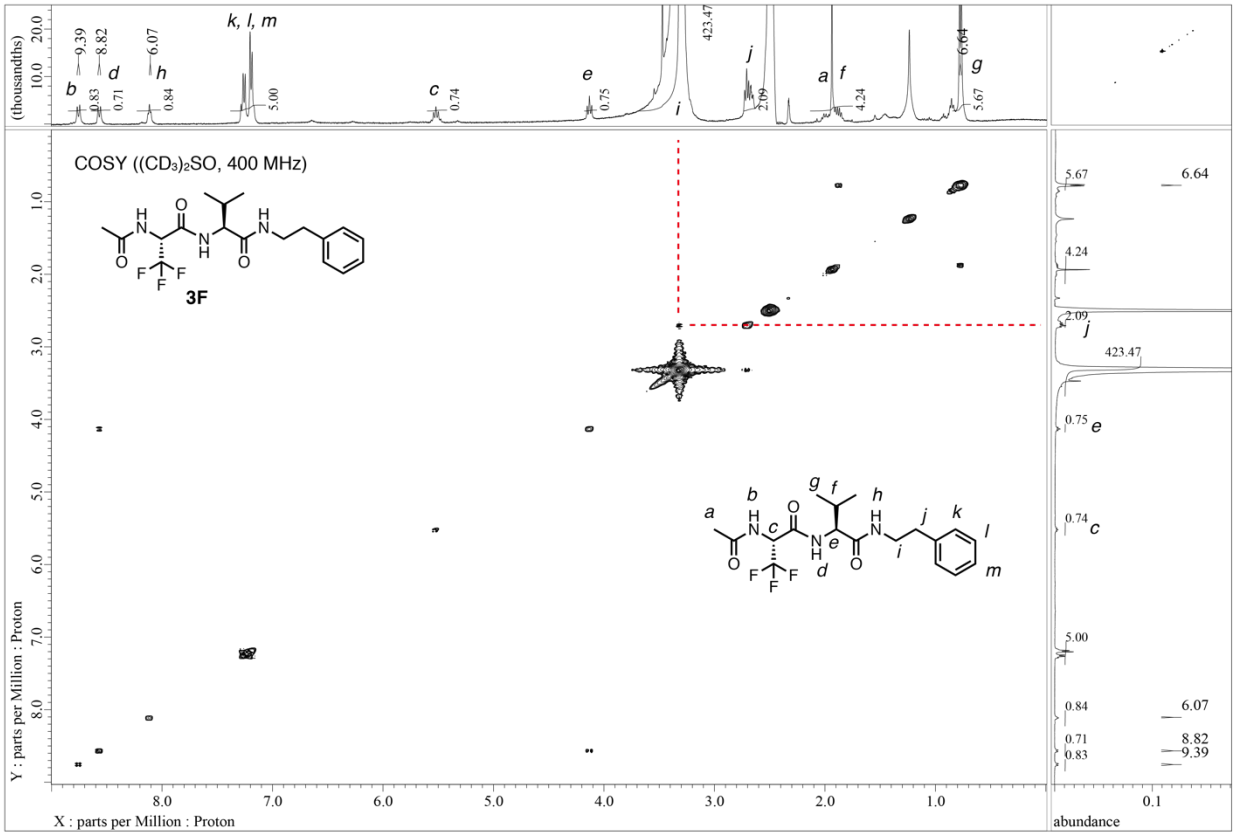


Fig. S41 COSY spectrum of **3F**. Spectrum was recorded in $(CD_3)_2SO$ at 25 °C.

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2 M. K. Eberle and R. Keese, *Helv. Chim. Acta*, 2010, **93**, 1583–1601.

3 K. N. Dack, X. Liang, M. Larsen, M. Andrews, A. S. Jessiman, M. N. Burhardt, P. S. Johnson, P. Andersen and L. Jørgensen, European Patent Office, WO/2020/127685, 2020.

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