Supplementary Information

Preparation of sterically hindered peptides using trifluoroacetyl protection: Incorporation of *N*-alkyl-α,α-dialkyl amino acids into *N*-alkyl amino acids

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Table of Contents

1. General remarks

Unless otherwise noted, all materials, solvents and reagents were obtained from commercial suppliers and used without further purification. All reactions involving reagents or intermediates sensitive to air or moisture were performed under an inert atmosphere of nitrogen.

Nuclear magnetic resonance (NMR) spectra were determined with an AVANCE NEO 400 iProbe (400 MHz, Bruker), and an AVANCE III 600 Cryo-TCI (600 MHz, Bruker). Chemical shifts are given in parts per million (p.p.m., δ units) relative to tetramethylsilane or solvent peaks and coupling constants (*J*) are reported in Hz. The following NMR abbreviations are used: s = singlet, d = doublet, q = quartet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublet, ddt = doublet of doublet of triplet, td = triplet of doublet, bs = broad singlet. High-resolution mass spectrometry (HRMS) analyses were performed with a Vanquish Orbitrap Exploris 120 (Thermo Fisher Scientific), and a Xevo G2-S Tof (Waters). Solid phase peptide synthesis (SPPS) was performed on an automated peptide synthesizer Intavis Multipep RSi. Optical rotations ($[\alpha]_D$) were measured with a polarimeter SEPA-500 (HORIBA). Liquid chromatography mass spectrometry (LCMS) analyses were performed with an ACQUITY UPLC, an ACQUITY UPLC I-Class/SQ Detector, a SQ Detector 2 (Waters). High performance liquid chromatography (HPLC) analyses were performed with an ACQUITY UPLC H-Class PLUS system (Waters). High-performance flash chromatography for purification was performed with an Isolera one (Biotage).

Abbreviations

AA: ammonium acetate
Al: allyl
DBF: dibenzofulvene
DBU: 1,8-diazabicyclo[5.4.0]-7-undecene
DCE/$(CH_2Cl)_2$: 1,2-dichloroethane
DIAD: diisopropyl azodicarboxylate
DIC: *N,N'*-diisopropylcarbodiimide
DIPEA: *N,N*-diisopropylethylamine
DMF: *N*,*N*-dimethylformamide
DMSO: dimethyl sulfoxide
EDCI·HCl: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride
FA: formic acid
Fmoc: (9*H*-Fluoren-9-ylmethoxy)carbonyl
NMP: 1-methyl-2-pyrrolidone

HATU: 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate

HFIP: 1,1,1,3,3,3-hexafluoro-2-propanol

HOAt: 1-hydroxy-7-azabenzotriazole

HOOBt: 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine

oxyma: ethyl cyano(hydroxyimino)acetate

pip: piperidine amide

pyrro: pyrrolidine amide

TFA: trifluoroacetic acid

TFE:2,2,2-trifluoroethanol

THP: tetrahydropyranyl

TIPS: triisopropylsilane

Trt: triphenylmethyl (trityl)

2. General procedure for the syntheses of *N*-Me-rich cyclic peptides

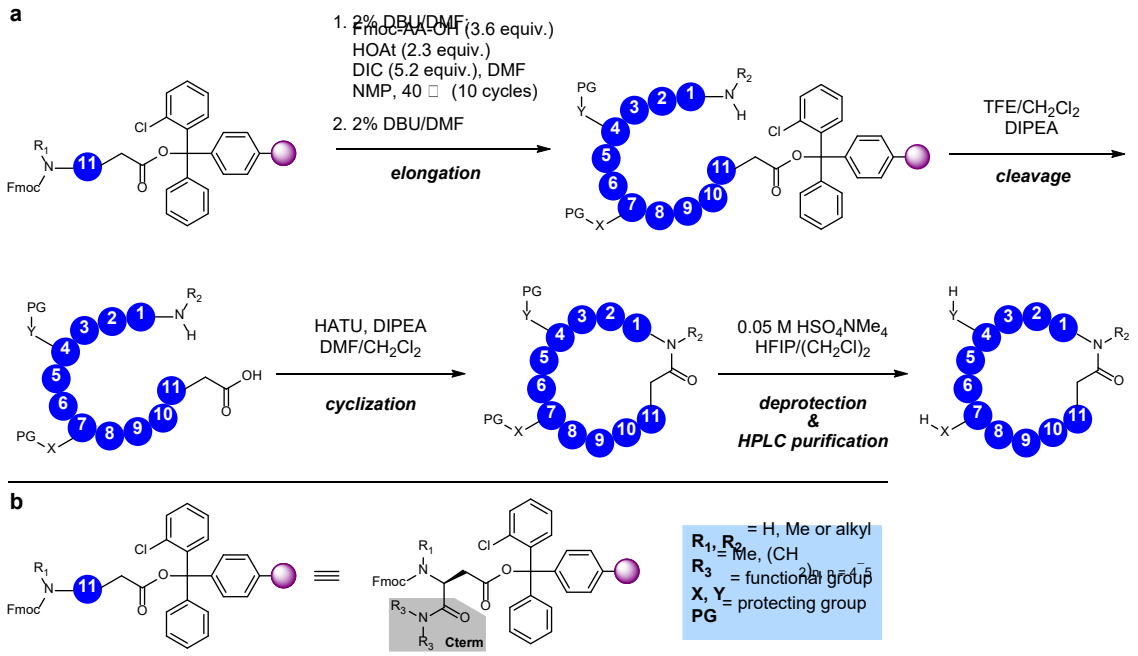


Figure S1. The overview for the synthesis of drug-like cyclic peptides. **a**, Synthetic route for *N*-Me-rich cyclic peptides. **b**, Representative structure of the resin-bound *C*-terminus Asp-amide and meanings of abbreviated characters.

General procedure for Fmoc SPPS. Peptides were synthesized in a parallel manner using an automated Intavis Multipep RSi peptide synthesizer (Intavis Inc., currently CEM corporation, up to 72 sequences can be synthesized simultaneously). The reaction column (2.0 mL, CEM corporation) was used as a reaction vessel. MS4A was added to the solvent (DMF, CH_2Cl_2, 2.0% DBU/DMF, and 0.71 M DIC in DMF). The standard Fmoc SPPS protocol was shown as follows:

Step 1: The solid supported N_α-Fmoc amino acid (100 mg in each reaction vessel, 0.40–0.60 mmol/g) was swelled with CH_2Cl_2 (1.0 mL, room temperature, 45 min).

Step 2: The solid supported N_α-Fmoc amino acid was washed with DMF (0.70 mL x 2 or 3) and deprotected with 2.0% DBU/DMF (0.70 mL, 35 ºC, 10 min).

Step 3: The resin in the reaction vessel was washed with DMF (0.70 mL x 4).

Step 4: A solution of 0.60 M N_α-Fmoc-amino acid/0.38 M HOAt in NMP and a solution of 0.71 M DIC in DMF were mixed at a ratio of 1:1.2. Then the resultant mixture (0.66 mL) was transferred to the reaction vessel.

Step 5: The activated N_α-Fmoc-amino acid was coupled with the peptide on the resin (40 ºC, 2.5 h) and the resin in the reaction vessel was washed with DMF (0.70 mL x 3).

Step 2–5 were repeated and amino acids were condensed on the solid support to give the N_α-Fmoc resin-bound peptide. Step 2 of the first cycle was carried out in 4 min 30 sec at 30 ºC.

Removal of N_α-Fmoc group on the elongated resin-bound linear peptide. The elongated N_α-Fmoc resin-bound peptide in the reaction vessel was swelled with CH_2Cl_2 (1.0 mL, 45 min), and washed with DMF (0.70 mL x 2). The N_α-Fmoc group of the above resin-bound peptide was removed by adding 2.0% DBU/DMF (0.70 mL) at room temperature for 15 min. The solution was filtered, and the resultant resin was washed with DMF (0.70 mL x 5) and CH_2Cl_2 (0.70 mL x 5).

General procedure for the peptide cleavage. To the above resin was added a solution of TFE/CH_2Cl_2 (1:1, 2.0 mL) with DIPEA (1.8 equiv.) and shaken at room temperature for 2 h. After completion of the cleavage, the reaction mixture was filtered to remove the resin. The resultant resin was washed with TFE/CH_2Cl_2 (1:1, 1.0 mL x 2) and the filtrates were collected. The combined filtrates were added DMF (4.0 mL) and concentrated by a centrifugal evaporator (Genevac HT-12) to give crude linear peptide.

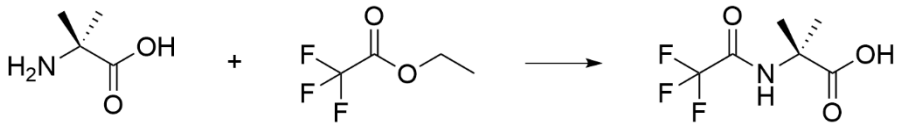
General procedure for the peptide cyclization. To a solution of the above crude linear peptide in DMF/CH_2Cl_2 (1:1, 8.0 mL) was added a solution of HATU (1.5 equiv.) in DMF (0.15 mL) and a solution of DIPEA (1.8 equiv.) in DMF (0.10 mL) successively at room temperature in a test tube. The resultant mixture was stirred at room temperature for 2 h. Then the reaction mixture was concentrated by a centrifugal evaporator (Genevac HT-12) to give crude cyclic peptide.

General procedure for deprotection of the cyclic peptides. The above crude cyclic peptide was dissolved in 0.050 M HSO_4NMe_4 in HFIP/TIPS/$(CH_2Cl)_2$ (117/2.4/1, 4.0 mL). The reaction mixture was stirred at room temperature for 1–2 h until the reaction completed. Then DIPEA (70 μL) was added to the reaction mixture and the resultant mixture was concentrated by a centrifugal evaporator (Genevac HT-12) to give crude of deprotected cyclic peptide. This step was skipped when peptides did not have protecting groups.

Purification method. The obtained crude peptide was dissolved in DMSO (0.80 mL) and purified by preparative HPLC.

3. Material preparations

Preparation of Tfa-Aib-OH (S1, 8 in manuscript**)**



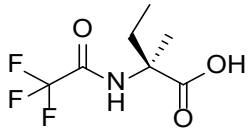
S1

To a solution of 2-amino-2-methylpropionic acid (25.0 g) in MeOH (242 mL), DIPEA (63.5 mL, 1.5 equiv.) and ethyl trifluoroacetate (37.6 mL, 1.3 equiv.) were added. The mixture was stirred at 50 °C for 18 h. The volatiles were removed under reduced pressure. To the obtained residue, 1N HClaq. and EtOAc were added, and the aqueous layer was removed. The obtained organic layer was dried over Na_2SO_4, and evaporated to give 18.2 g of the crude product of Tfa-Aib-OH.

The obtained crude product (16.0 g) was dissolved in tert-butyl methyl ether (TBME, 80 mL). Heptane (320 mL) was added dropwise over 1 h. The mixture was cooled with an ice-water bath, and stirred for an additional 1 h. The mixture was filtered, and washed with TBME/heptane (1/4, 32 mL). The obtained powder was dried under reduced pressure to give Tfa-Aib-OH (13.5 g, 28%).

white solid; ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K) δ 12.67 (1H, s), 9.48 (1H, s), 1.42 (6H, s); ^{13}C-NMR (101 MHz, DMSO-d_6, 300 K) δ 173.9, 155.5 (q, J = 36.9 Hz), 115.5 (q, J = 290.2 Hz), 56.1, 24.2; ^{19}F-NMR (376 MHz, DMSO-d_6, 300 K) δ -74.1; HRMS (ESI-TOF) *m/z* calcd for $C_6H_7F_3NO_3$ $[M-H]^-$ 198.0378, found 198.0378.

Tfa-(Me)Abu-OH (S2)

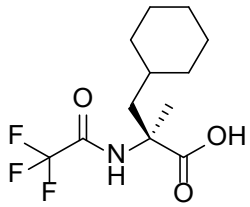


To a solution of (S)-2-amino-2-methylbutanoic acid (H-(Me)Abu-OH, 15.0 g, 128 mmol) in MeOH (150 mL), DIPEA (82.7 g, 640 mmol) and ethyl trifluoroacetate (54.6 g, 384 mmol) were added. The mixture was stirred at 50 °C for 16 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in TBME and washed twice with 1N hydrochloric acid solution. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the crude product. Recrystallization of the crude product from TBME/hexane (1:7) yielded (S)-2-methyl-2-(2,2,2-trifluoroacetamido)butanoic acid) (Acid **S2**, 12 g, 44%).

white solid; $[\alpha]_D^{25}$ -1.00 (*c* 1.00, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K) δ 12.72 (1H, s), 9.25 (1H, s), 1.92 (1H, dq, J = 15.2, 7.6 Hz), 1.77 (1H, dq, J = 14.0, 7.6 Hz), 1.37 (3H, s), 0.80 (3H, t, J = 7.6 Hz); ^{13}C-NMR (101 MHz, DMSO-d_6, 300 K) δ 173.4, 155.4 (q, J = 36.8 Hz), 115.5 (q, J =

290.2 Hz), 59.6, 28.7, 20.9, 7.8; ^{19}F-NMR (376 MHz, DMSO-d_6, 300 K) δ -74.0; HRMS (ESI-TOF) *m/z* calcd for $C_7H_9F_3NO_3$ $[M-H]^-$ 212.0535, found 212.0536.

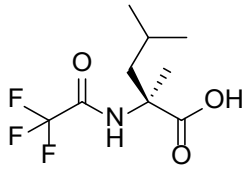
Tfa-(Me)Cha-OH (S3)



To a solution of (S)-2-amino-3-cyclohexyl-2-methylpropanoic acid (H-(Me)Cha-OH, 1.1 g, 6.0 mmol) in MeOH (20 mL), DIPEA (3.1 mL, 18 mmol) and ethyl trifluoroacetate (2.1 mL) were added. The mixture was stirred at 50 °C for 2 h. After cooling to room temperature, additional DIPEA (3.1 mL, 18 mmol) and ethyl trifluoroacetate (2.1 mL) were added, and the mixture was stirred at 50 °C for 20 h. The reaction mixture was concentrated under reduced pressure, and the resulting residue was dissolved in TBME (30 mL). The solution was washed twice with 1N hydrochloric acid solution (30 mL) and once with saturated NaCl aq. (40 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the crude product. Purification of the crude product by reverse-phase column chromatography (0.1% formic acid-water/0.1% formic acid-acetonitrile) yielded (S)-3-cyclohexyl-2-methyl-2-(2,2,2-trifluoroacetamido)propanoic acid (Acid **S3**, 1.22 g, 72%).

white solid; $[\alpha]_D^{25}$ +23.12 (*c* 1.00, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K) δ 12.82 (1H, s), 9.16 (1H, s), 1.83 (1H, dd, *J* = 14.4, 6.0 Hz), 1.73 (1H, dd, *J* = 14.4, 6.0 Hz), 1.68-1.51 (5H, m), 1.41 (3H, s), 1.37-1.25 (1H, m), 1.25-1.04 (3H, m), 0.99-0.84 (2H, m); ^{13}C-NMR (101 MHz, DMSO-d_6, 300 K) δ 173.8, 155.2 (q, *J* = 36.8 Hz), 115.5 (q, *J* = 290.2 Hz), 59.1, 42.1, 34.0, 33.7, 32.7, 25.72, 25.68, 25.6, 22.2 ; ^{19}F-NMR (376 MHz, DMSO-d_6, 300 K) δ -74.2; HRMS (ESI-TOF) *m/z* calcd for $C_{12}H_{17}F_3NO_3$ $[M-H]^-$ 280.1161, found 280.1165.

Tfa-(Me)Leu-OH (S4)

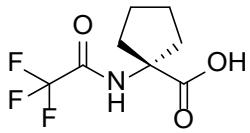


To a solution of (S)-2-amino-2,4-dimethylpentanoic acid (H-(Me)Leu-OH, 15.0 g, 103 mmol) in MeOH (50 mL), DIPEA (40.1 g, 310 mmol) and ethyl trifluoroacetate (44.0 g, 310 mmol) were added. The mixture was stirred at 50 °C for 16 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in TBME and washed twice

with 1N hydrochloric acid solution. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the crude product. Recrystallization of the crude product from TBME/hexane (1:7) yielded (S)-2,4-dimethyl-2-(2,2,2-trifluoroacetamido)pentanoic acid (Acid **S4**, 10 g, 40%).

white solid; $[\alpha]_D^{25}$ +14.95 (*c* 1.00, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K) δ 12.86 (1H, s), 9.15 (1H, s), 1.85 (1H, dd, *J* = 14.0, 6.8 Hz), 1.78 (1H, dd, J = 14.0, 5.6 Hz), 1.63 (1H, qq, *J* = 6.8, 5.6 Hz), 1.43 (1H, s), 0.89 (3H, d, *J* = 6.4 Hz), 0.86 (3H, d, *J* = 6.4 Hz); ^{13}C-NMR (101 MHz, DMSO-d_6, 300 K) δ 173.8, 155.2 (q, *J* = 36.2 Hz), 115.5 (q, *J* = 290.2 Hz), 59.2, 43.3, 24.0, 23.5, 23.4, 22.3; ^{19}F-NMR (376 MHz, DMSO-d_6, 300 K) δ -74.2; HRMS (ESI-TOF) *m/z* calcd for $C_9H_{13}F_3NO_3$ $[M-H]^-$ 240.0848, found 240.0852.

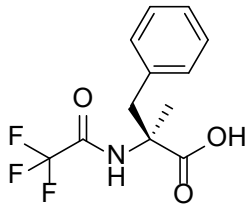
Tfa-cLeu-OH (S5)



To a solution of 1-aminocyclopentanecarboxylic acid (H-cLeu-OH, 25 g, 194 mmol) in MeOH (100 mL), DIPEA (37.5 g, 290 mmol) and ethyl trifluoroacetate (41.3 g, 290 mmol) were added. The mixture was stirred at 50 °C for 2 days. After cooling to room temperature, additional DIPEA (4.0 mL, 23 mmol) and ethyl trifluoroacetate (2.7 mL) were added, and the mixture was stirred at 50 °C for 16 h. The reaction mixture was concentrated under reduced pressure, and the resulting residue was dissolved in TBME. The solution was washed sequentially with 1N hydrochloric acid solution and saturated NaCl aq. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the crude product. Recrystallization of the crude product from TBME/hexane (3:20) yielded 1-(2,2,2-trifluoroacetamido)cyclopentane-1-carboxylic acid (Acid **S5,** 20 g, 46%).

white solid; ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K) δ 12.62 (1H, s), 9.52 (1H, s), 2.14-1.98 (4H, m), 1.72-1.62 (4H, m); ^{13}C-NMR (101 MHz, DMSO-d_6, 300 K) δ 173.8, 156.0 (q, *J* = 36.8 Hz), 115.6 (q, *J* = 289.5 Hz), 65.8, 35.9, 24.1; ^{19}F-NMR (376 MHz, DMSO-d_6, 300 K) δ -74.0; HRMS (ESI-TOF) *m/z* calcd for $C_8H_9F_3NO_3$ $[M-H]^-$ 224.0535, found 224.0536.

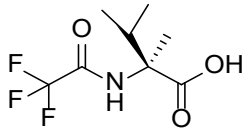
Tfa-(Me)Phe-OH (S6)



To a solution of (2S)-2-amino-2-methyl-3-phenylpropanoic acid (H-(Me)Phe-OH, 10.0 g, 55.8 mmol) in MeOH (500 mL), DIPEA (21.63 g, 167.4 mmol) and ethyl trifluoroacetate (23.78 g, 167.4 mmol) were added. The mixture was stirred at 50 °C for 16 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in TBME and washed twice with 1N hydrochloric acid solution and once with saturated NaCl aq. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the crude product. Recrystallization of the crude product from TBME/hexane (1:15) yielded (S)-2-methyl-3-phenyl-2-(2,2,2-trifluoroacetamido)propanoic acid (Acid **S6**, 8 g, 52%).

white solid; $[\alpha]_D^{25}$ -26.91 (*c* 1.00, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K) δ 12.94 (1H, s), 9.23 (1H, s), 7.33-7.23 (3H, m), 7.10-7.07 (2H, m), 3.35 (1H, d, *J* = 14.0 Hz), 3.03 (1H, d, *J* = 14.0 Hz), 1.32 (3H, s); ^{13}C-NMR (101 MHz, DMSO-d_6, 300 K) δ 173.3, 155.6 (q, *J* = 36.8 Hz), 135.8, 130.3, 127.9, 126.7, 115.5 (q, *J* = 290.2 Hz), 59.4, 39.4, 21.8; ^{19}F-NMR (376 MHz, DMSO-d_6, 300 K) δ -74.1; HRMS (ESI-TOF) *m/z* calcd for $C_{12}H_{11}F_3NO_3$ $[M-H]^-$ 274.0691, found 274.0694.

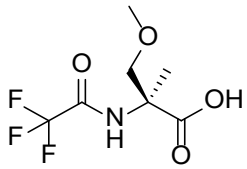
Tfa-(Me)Val-OH (S7)



To a solution of (S)-2-amino-2,3-dimethylbutanoic acid (H-(Me)Val-OH, 2.0 g, 15 mmol) in MeOH (25 mL), DIPEA (8.0 mL, 46 mmol) and ethyl trifluoroacetate (5.5 mL) were added. The mixture was stirred at 50 °C for 3 h. After cooling to room temperature, additional DIPEA (4.0 mL, 23 mmol) and ethyl trifluoroacetate (2.7 mL) were added, and the mixture was stirred at 50 °C for 16 h. The reaction mixture was concentrated under reduced pressure, and the resulting residue was dissolved in TBME (40 mL). The solution was washed sequentially with 1N hydrochloric acid solution (40 mL) and saturated NaCl aq (40 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the crude product. The crude product was purified by reverse-phase column chromatography (0.1% formic acid-water / 0.1% formic acid-acetonitrile) to yield (S)-2,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoic acid (Acid **S7**, 1.17 g, 34%).

white solid; $[\alpha]_D^{25}$ +0.17 (*c* 1.00, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K) δ 12.68 (1H, s), 9.10 (1H, s), 2.18 (1H, sep, *J* = 6.8 Hz), 1.35 (3H, s), 0.92 (3H, d, *J* = 6.8 Hz), 0.89 (3H, d, *J* = 6.8 Hz); ^{13}C-NMR (101 MHz, DMSO-d_6, 300 K) δ 172.6, 155.8 (q, *J* = 36.1 Hz), 115.6 (q, *J* = 289.5 Hz), 62.8, 33.1, 17.3, 17.2, 17.1; ^{19}F-NMR (376 MHz, DMSO-d_6, 300 K) δ -73.8; HRMS (ESI-TOF) *m/z* calcd for $C_8H_{11}F_3NO_3$ $[M-H]^-$ 226.0691, found 226.0693.

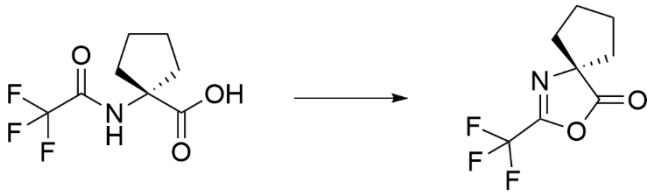
Tfa-(Me)Ser(Me)-OH (S8)



To a solution of (S)-2-amino-3-methoxy-2-methylpropanoic acid (H-(Me)Ser(Me)-OH, 1.5 g, 11 mmol) in MeOH (19 mL), DIPEA (5.9 mL, 34 mmol) and ethyl trifluoroacetate (4.0 mL) were added. The mixture was stirred at 50 °C for 21 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in TBME (45 mL) and washed twice with 1N hydrochloric acid solution (45 mL) and once with saturated NaCl aq (45 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the crude product. The crude product was purified by reverse-phase column chromatography (0.1% formic acid-water / 0.1% formic acid-acetonitrile) to yield (S)-3-methoxy-2-methyl-2-(2,2,2-trifluoroacetamido)propanoic acid (Acid **S8**, 2.07 g, 72%).

white solid; $[\alpha]_D^{25}$ -4.80 (*c* 1.00, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K) δ 12.96 (1H, s), 9.31 (1H, s), 3.72 (1H, d, *J* = 9.2 Hz), 3.55 (1H, d, *J* = 9.2 Hz), 3.27 (3H, s), 1.43 (3H, s); ^{13}C-NMR (101 MHz, DMSO-d_6, 300 K) δ 172.0, 155.5 (q, *J* = 36.9 Hz), 115.5 (q, *J* = 289.4 Hz), 73.4, 59.6, 58.7, 19.9 ; ^{19}F-NMR (376 MHz, DMSO-d_6, 300 K) δ -74.0; HRMS (ESI-TOF) *m/z* calcd for $C_7H_9F_3NO_4$ $[M-H]^-$ 228.0484, found 228.0482.

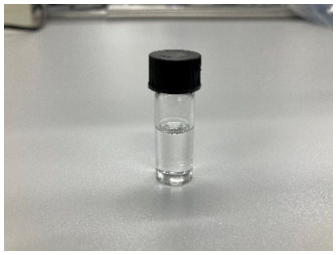
<u>Oxazolone of Tfa-cLeu-OH (S9, 21</u> <u>in manuscript)</u>



S9

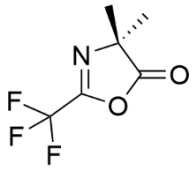
To a solution of Tfa-cLeu-OH (25 g, 111 mmol) in dichloromethane (225 mL), WSCI・HCl (27.7 g, 144 mmol) was added, and the resulting mixture was stirred at room temperature for 2 days. Then the volatiles were evaporated. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether) to give the titled compound (11.9 g, 52%). A part of the obtained product (10.0 g) was distilled by Kugelrohr (150 ºC, 80 hPa) to give oxazolone **S9** (7.5 g, 75% yield by distillation) and used for the reactions conducted in the manuscript.

colorless oil; ^{1}H-NMR (400 MHz, $CDCl_3$, 300 K) δ 2.20-2.09 (2H, m), 2.08-1.95 (6H, m); ^{13}C-NMR (101 MHz, $CDCl_3$, 300 K) δ 178.2, 151.3 (q, *J* = 44.1 Hz), 115.5 (q, *J* = 275.4 Hz), 74.9, 38.1, 25.7 ; ^{19}F-NMR (376 MHz, $CDCl_3$, 300 K) δ -72.0; HRMS (ESI-TOF) *m/z* calcd for $C_8H_9F_3NO_2$ $[M+H]^+$ 208.0585, found 208.0587.



The image of oxazolone of Tfa-cLeu-OH (**S9**, **21** in manuscript)

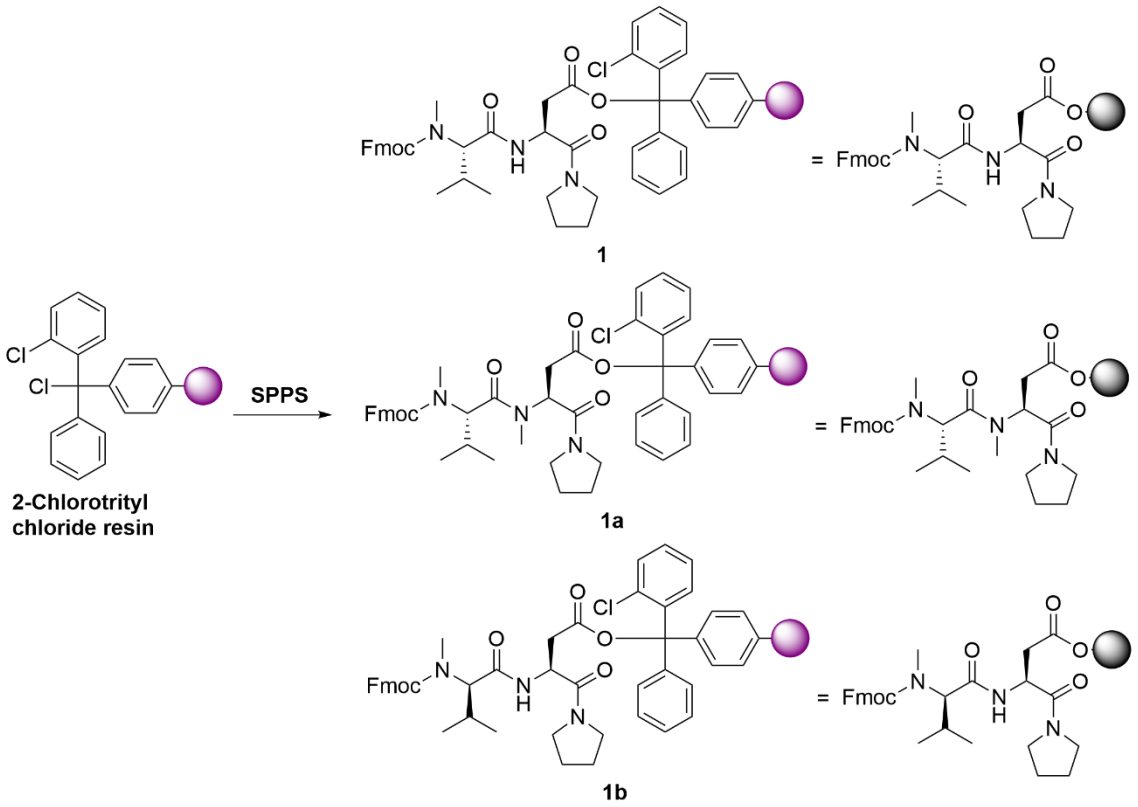
Oxazolone of Tfa-Aib-OH (S10)



To a stirred solution of $SOCl_2$ (96.00 mL, 1.32 mol, 3.3 equiv.) at 0 °C was added in portions 2-methyl-2-(2,2,2-trifluoroacetamido) propanoic acid (**S1**, 80.00 g, 402 mmol, 1 equiv.). The resulting mixture was stirred at 60 °C for 1 h and then concentrated under vacuum. The mixture of the residue and quinoline (64.00 g, 496 mmol, 1.23 equiv.) was stirred at 100 °C for 1 h. The crude product was purified by distillation at 60 °C under vacuum to afford 4,4-dimethyl-2-(trifluoromethyl)-1,3-oxazol-5-one (oxazolone **S10**, 50 g, 69%).

colorless oil; ^{1}H-NMR (400 MHz, $CDCl_3$, 300 K) δ 1.54 (6H, s); ^{13}C-NMR (101 MHz, $CDCl_3$, 300 K) δ 177.3, 151.4 (q, J = 44.2 Hz), 115.5 (q, J = 275.4 Hz), 66.4, 23.7; ^{19}F-NMR (376 MHz, $CDCl_3$, 300 K) δ -72.1; HRMS (ESI-TOF) *m/z* calcd for $C_6H_7F_3NO_2$ $[M+H]^+$ 182.0429, found 182.0432.

Preparation of Fmoc-MeVal-Asp(OTrt(2-Cl) resin)-pyrro (1), Fmoc-MeVal-MeAsp(OTrt(2-Cl) resin)-pyrro (1a), and Fmoc-D-MeVal-Asp(OTrt(2-Cl) resin)-pyrro (1b)



SPPS was conducted using 2-chlorotrityl chloride resin (1% DVB, 100–200 mesh, 6 mmol/g) according to the reported procedure (ref. K. Nomura, S. Hashimoto, R. Takeyama, M. Tamiya, T. Kato, T. Muraoka, M. Kage, K. Nii, K. Kotake, S. Iida, T. Emura, M. Tanada and H. Iikura, *J. Med. Chem.* 2022, **65**, 13401-13412, and its supporting information) for the syntheses of *N*-Me-rich cyclic peptides to obtain **1** (15 g, 0.375 mmol/g), **1a** (200 mg, 0.457 mmol/g), and **1b** (1.0 g, 0.425 mmol/g).

4. General procedures of elongation of Tfa-AA-OH and on-resin *N*-alkylation, and Results

General procedure of elongation of Tfa-AA-OH

The solid supported resin (**1**, 200 mg, 0.375 mmol/g) [a] was swelled with CH_2Cl_2 (10 v/w, room temperature, 5 min). Then the resin bounded peptide was washed with DMF (7.0 v/w x 3) and deprotected with 2.0% DBU/DMF (7.0 v/w, rt, 10 min). The resin in the reaction vessel was washed with DMF (7.0 v/w x 3), and 1 mM BHT/DMF (7.0 v/w x 2). A solution of Tfa-(Me)Abu-OH (7.0 equiv.) in 1 mM BHT/DMF (3.0 v/w) and a solution of DIC (8.0 equiv.) in 1.0 mM BHT/DMF (3.6 v/w) were mixed for 10 min [b]. Then the resultant mixture (6.6 v/w) was transferred to the reaction vessel. The reaction vessel was shaken at 40 ºC for 20 h twice, and at 60 ºC for 20 h [c]. Then, the resin in the reaction vessel was washed with DMF (7.0 v/w x 5). The obtained resin was washed with CH_2Cl_2, (7.0 v/w x 4) and dried under reduced pressure. To confirm the conversion, a part of the obtained resin was picked and treated with TFE/CH_2Cl_2 (1:1), and the resulting solution was analyzed by LCMS.

[a] The resin (**1**, 4.0 g, 0.375 mmol/g) was used in **11aa-11ae**. The resin (**1**, 200 mg, 0.375 mmol/g) was used in **11ba-11fa**, **11ha**. The resin (**1**, 1.0 g, 0.375 mmol/g) was used in **11ga**. The resin (**1a**, 200 mg, 0.457 mmol/g) was used in **27**. The resin (**1b**, 1.0 g, 0.425 mmol/g) was used in **28**. [b] BHT was added to suppress byproducts in all the cases other than **11aa-11ae**. [c]**11aa-11ae**, **27**, **28**, 40 °C, 20 h**, 11ba**, 40 °C, 20 h x 2, 60 °C, 20 h; **11ca**, 60 °C, 20 h x 2; **11da-11fa, 11ha**, 60 °C, 48 h; **11ga**, 60 °C, 48 h, 30 h.

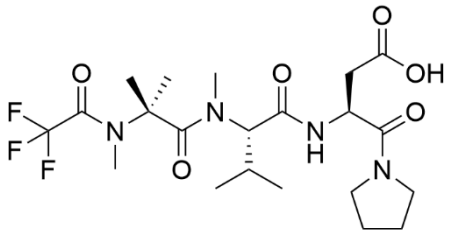
General procedure of on-resin *N*-alkylation

To the obtained resin [a] was swelled with CH_2Cl_2 (10 v/w, room temperature, 15 min). Then the resin bounded peptide was washed with DMF (7.0 v/w x 4). To the resin, a solution of TMGN (21 equiv.)/DMF (14 v/w) and a solution of MeI (84 equiv.)/DMF (14 v/w) [b] were added, then shaken at 40 ºC for 2 h [c]. The resin was washed with DMF (7.0 v/w x 4). The same alkylation protocol was repeated twice additionally. After the protocol, the obtained resin was washed with DMF (7.0 v/w x 4) and CH_2Cl_2 (7.0 v/w x 4), then dried under reduced pressure.

To confirm the conversion, a part of the obtained resin was picked and treated with TFE/CH_2Cl_2 (1:1), and the resulting solution was analyzed by LCMS. The resin was treated with 10 v/w of TFE/ CH_2Cl_2 (1:1) with DIPEA (1.8 equiv.) solution for 2 h. The eluted solution was collected to the test tube, and resin was washed with 5.0 v/w of CH_2Cl_2 twice. The solution was evaporated under reduced pressure, purified by a reversed-phase column (C18, 30 g, 0.10%FA MeCN / 0.10%FA H_2O), and lyophilized to obtain the compound.

[a] **11ba-11ha, 27, 28,** All of the resins obtained in the previous step were used as is. **11aa-11ae,** 200 mg, 0.381 mmol/g. [b] **11ba-11ha**, MeI (84 equiv.), **27**, MeI (69 equiv.), **28**, MeI (80 equiv.), **11ab,** EtI (82 equiv.), **11ac,** nPrI, (82 equiv.), **11ad,** allyl bromide (82 equiv.), **11ae,** BnBr (82 equiv.). [c] **11aa, 11ha, 27, 28**, 40 °C, 2 h, **11ba-11da**, 40 °C, 2 h x 3, **11ea-11fa**, 40 °C, 2 h x 2, **11ga**, 60 °C, 4 h, 15 h, 3 h, **11ab**, 40 °C, 2 h, 60 °C, 2 h x 4, **11ac, 11ae**, 40 °C, 2 h x 3, 60 °C, 5 h, 4 h, 15 h, 3 h, **11ad**, 60 °C, 2 h x 5.

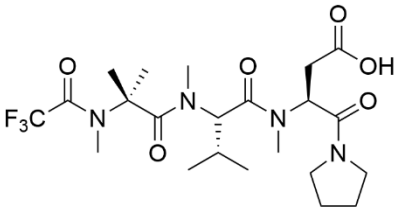
Tfa-MeAib-MeVal-Asp-pyrro (The peptide cleavaged from 11aa)

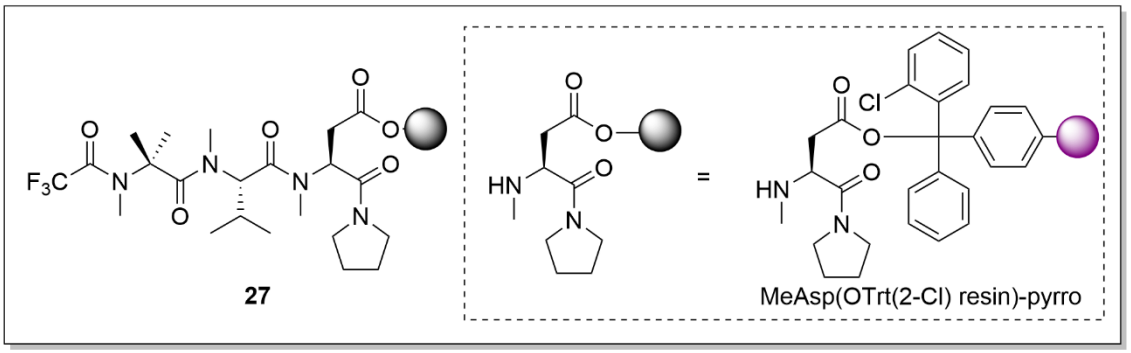


Elongation of Tfa-Aib-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-MeAib-MeVal-Asp-pyrro (19.0 mg, 50% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.24 (1H, bs), 7.77 (1H, d, J = 8.2 Hz), 4.75 (1H, td, J = 8.4, 5.3 Hz), 4.60 (1H, d, J = 10.8 Hz), 3.47-3.40 (2H, m), 3.26 (2H, t, J = 6.9 Hz), 3.11 (3H, s), 2.72 (3H, s), 2.68 (1H, dd, J = 16.5, 8.6 Hz), 2.30 (1H, dd, J = 16.4, 5.1 Hz), 2.13-2.05 (1H, m), 1.89-1.72 (4H, m), 1.42 (6H, d, J = 14.1 Hz), 0.84 (3H, d, J = 6.4 Hz), 0.70 (3H, d, J = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 171.9, 171.1, 168.7, 167.6, 154.7 (q, J = 34.5 Hz), 116.0 (q, J = 288.3 Hz), 63.3, 61.9, 47.2, 45.5, 35.9, 30.6, 29.6, 25.5, 25.4, 23.5, 22.5, 22.4, 19.6, 18.4; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.37; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{21}H_{34}F_3N_4O_6$ $[M+H]^+$ 495.2425, found 495.2418.

Tfa-MeAib-MeVal-MeAsp-pyrro (The peptide cleavaged from 27, Table1, entry 4: over-methylation product)

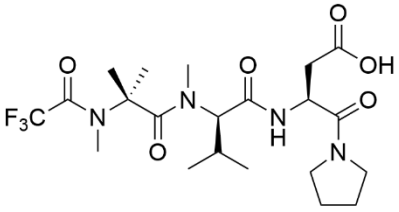


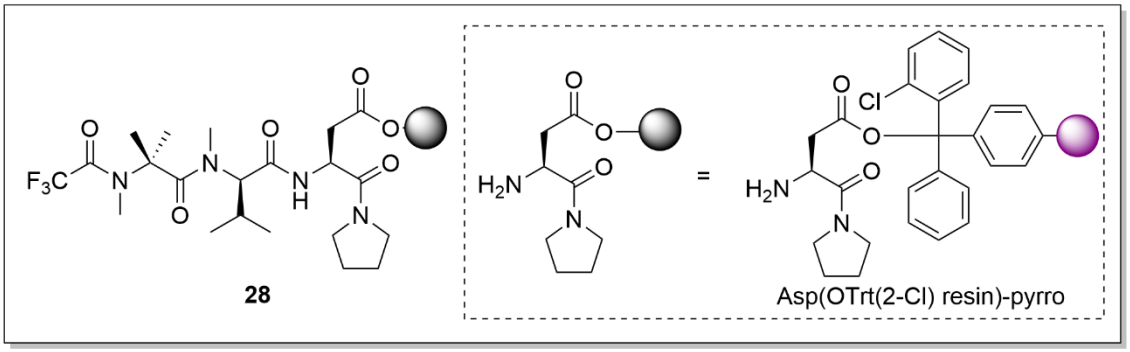


Elongation of Tfa-Aib-OH and on-resin *N*-alkylation were conducted using **1a** according to the general procedures (supplementary information p14-15). The elongation of Tfa-Aib-OH was conducted with oxazolone **S10** (10 equiv.) at 40 ºC for 20 h, instead of the general procedure of elongation of Tfa-AA. The authentic sample of over-methylated product Tfa-MeAib-MeVal-MeAsp-pyrro was obtained (23.2 mg, 50% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.24 (1H, bs), 5.41 (1H, dd, J = 10.5, 3.9 Hz), 5.10 (1H, d, J = 10.6 Hz), 3.32-3.29 (3H, m), 3.15-3.12 (1H, m), 3.11 (3H, s), 2.82 (1H, dd, J = 16.8, 10.8 Hz), 2.80 (3H, s), 2.63 (3H, s), 2.28-2.19 (1H, m), 1.95 (1H, dd, J = 16.3, 3.8 Hz), 1.87-1.71 (4H, m), 1.47 (3H, s), 1.40 (3H, s), 0.80 (3H, d, J = 6.4 Hz), 0.74 (3H, d, J = 6.8 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 171.8, 170.7, 168.4, 166.4, 154.7 (q, J = 34.3 Hz), 116.0 (q, J = 288.5 Hz), 63.5, 57.8, 52.0, 45.6, 45.4, 33.0, 30.4, 30.2, 29.6, 26.1, 25.5, 23.4, 22.8, 22.2, 19.3, 17.6; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.41; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{22}H_{36}F_3N_4O_6$ $[M+H]^+$ 509.2582, found 509.2573.

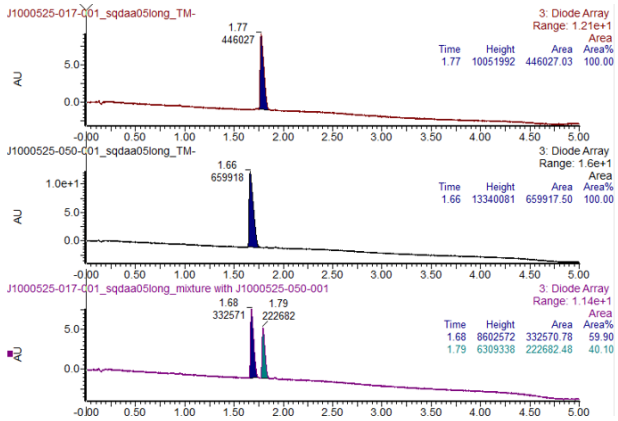
Tfa-MeAib-D-MeVal-Asp-pyrro (The peptide cleavaged from 28) (The authentic sample to confrim racemization risk during the on-resin *N*-alkylation)





Elongation of Tfa-Aib-OH and on-resin *N*-alkylation were conducted using **1b** according to the general procedures (supplementary information p14-15) to obtain Tfa-MeAib-D-MeVal-Asp-pyrro (140.7 mg, 75% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.23 (1H, bs), 8.06 (1H, d, J = 8.6 Hz), 4.79 (1H, td, J = 8.5, 5.9 Hz), 4.58 (1H, d, J = 10.8 Hz), 3.43-3.36 (2H, m), 3.29-3.20 (2H, m), 3.12 (3H, s), 2.80 (3H, s), 2.73 (1H, dd, J = 16.5, 8.4 Hz), 2.30 (1H, dd, J = 16.4, 5.7 Hz), 2.11-2.02 (1H, m), 1.87-1.67 (4H, m), 1.42 (3H, s), 1.38 (3H, s), 0.85 (3H, d, J = 6.4 Hz), 0.69 (3H, d, J = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 171.7, 171.1, 169.3, 167.8, 154.8 (q, J = 34.3 Hz), 116.0 (q, J = 288.8 Hz), 63.4, 61.8, 47.0, 45.6, 45.4, 35.8, 30.8, 29.7, 25.8, 25.5, 23.5, 22.48, 22.46, 19.5, 18.7; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.39; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{21}H_{34}F_3N_4O_6$ $[M+H]^+$ 495.2425, found 495.2415.



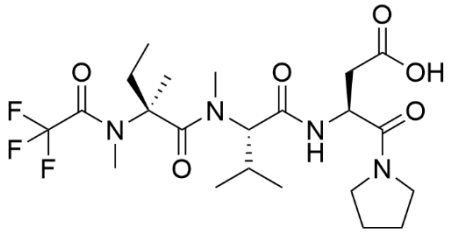
Tfa-MeAib-MeVal-Asp-pyrro

Tfa-MeAib-D-MeVal-Asp-pyrro

Mixture of Tfa-MeAib-MeVal-Asp-pyrro and Tfa-MeAib-D-MeVal-Asp-pyrro

According to the NMR and LCMS data of Tfa-MeAib-MeVal-Asp-pyrro and Tfa-MeAib-D-MeVal-Asp-pyrro, no epimerization was observed during the on-resin *N*-alkylation.

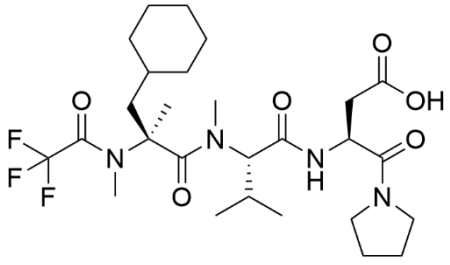
Tfa-Me(Me)Abu-MeVal-Asp-pyrro (The peptide cleavaged from 11ba)



Elongation of Tfa-(Me)Abu-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-Me(Me)Abu-MeVal-Asp-pyrro (13.7 mg, 36% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.20 (1H, bs), 7.85 (1H, d, J = 8.0 Hz), 4.74 (1H, td, J = 8.3, 5.2 Hz), 4.57 (1H, d, J = 11.0 Hz), 3.47-3.40 (2H, m), 3.26 (2H, t, J = 6.9 Hz), 3.11 (3H, s), 2.75 (3H, s), 2.69 (1H, dd, J = 16.5, 8.8 Hz), 2.35 (1H, dd, J = 16.5, 5.0 Hz), 2.13-2.05 (2H, m), 1.89-1.72 (5H, m), 1.40 (3H, s), 0.83 (3H, d, J = 6.4 Hz), 0.80 (3H, t, J = 7.4 Hz), 0.70 (3H, d, J = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 172.0, 170.8, 168.8, 167.6, 154.8 (q, J = 34.4 Hz), 116.1 (q, J = 289.0 Hz), 66.3, 62.1, 47.2, 45.6, 35.8, 30.6, 30.5, 27.4, 25.5, 25.3, 23.5, 19.5, 19.1, 18.7, 7.9; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.34; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{22}H_{36}F_3N_4O_6$ $[M+H]^+$ 509.2582, found 509.2573.

Tfa-Me(Me)Cha-MeVal-Asp-pyrro (The peptide cleavaged from 11ca)

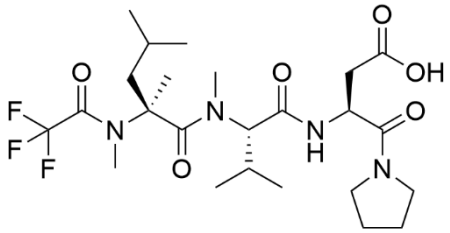


Elongation of Tfa-(Me)Cha-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-Me(Me)Cha-MeVal-Asp-pyrro (13.4 mg, 31% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.22 (1H, bs), 7.91 (1H, d, J = 7.8 Hz), 4.72 (1H, td, J = 8.4, 5.2 Hz), 4.54 (1H, d, J = 11.0 Hz), 3.48-3.40 (2H, m), 3.26 (2H, t, J = 6.9 Hz), 3.13 (3H, s), 2.76 (3H, s), 2.68 (1H, dd, J = 16.5, 8.6 Hz), 2.37 (1H, dd, J = 16.5, 5.0 Hz), 2.11-1.97 (2H, m), 1.88-1.70 (5H, m), 1.63-1.57 (4H, m), 1.56-1.52 (1H, m), 1.45 (3H, s), 1.42-1.34 (1H, m), 1.23-1.07 (3H, m), 11.03-0.87 (2H, m), 0.82 (3H, d, J = 6.4 Hz), 0.71 (3H, d, J = 6.6 Hz); ^{13}C-NMR (151

MHz, DMSO-d_6, 300 K) δ 171.9, 171.1, 169.0, 167.6, 154.8 (q, J = 34.2 Hz), 116.0 (q, J = 289.9 Hz), 66.3, 62.3, 47.3, 45.6, 41.7, 35.8, 35.4, 34.5, 32.2, 30.8, 30.5, 25.8, 25.7, 25.52, 25.46, 23.6, 20.7, 19.4, 18.8; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.58; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{27}H_{44}F_3N_4O_6$ $[M+H]^+$ 577.3208, found 577.3200.

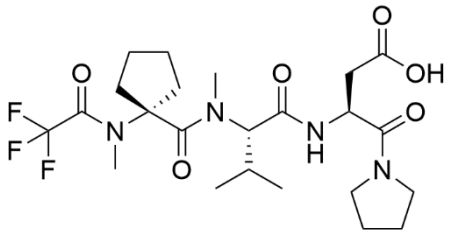
Tfa-Me(Me)Leu-MeVal-Asp-pyrro (The peptide cleavaged from 11da)



Elongation of Tfa-(Me)Leu-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-Me(Me)Leu-MeVal-Asp-pyrro (10.8 mg, 37% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.23 (1H, bs), 7.88 (1H, d, J = 7.8 Hz), 4.72 (1H, td, J = 8.3, 5.3 Hz), 4.53 (1H, d, J = 11.0 Hz), 3.49-3.41 (2H, m), 3.26 (2H, t, J = 6.9 Hz), 3.14 (3H, s), 2.76 (3H, s), 2.65 (1H, dd, J = 16.4, 8.5 Hz), 2.35 (1H, dd, J = 16.4, 5.1 Hz), 2.09-2.00 (2H, m), 1.87-1.68 (6H, m), 1.46 (3H, s), 0.92 (3H, d, J = 6.6 Hz), 0.85 (3H, d, J = 6.6 Hz), 0.82 (3H, d, J = 6.4 Hz), 0.70 (3H, d, J = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 172.0, 171.2, 169.0, 167.7, 154.8 (q, J = 34.3 Hz), 116.0 (q, J = 289.2 Hz), 66.3, 62.3, 47.3, 45.57, 45.54, 43.2, 36.1, 30.9, 30.5, 25.53, 25.46, 25.4, 24.2, 23.6, 22.8, 20.4, 19.5, 18.8; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.55; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{24}H_{40}F_3N_4O_6$ $[M+H]^+$ 537.2895, found 537.2885.

Tfa-MecLeu-MeVal-Asp-pyrro (The peptide cleavaged from 11ea)

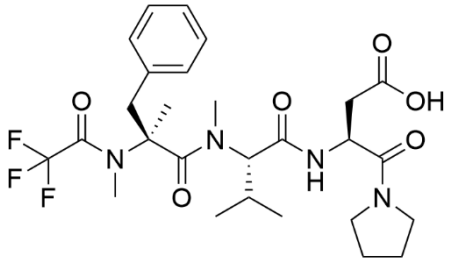


Elongation of Tfa-cLeu-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-MecLeu-MeVal-Asp-pyrro (12.1 mg, 27% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.21 (1H, bs), 7.92 (1H, d, J = 8.2 Hz), 4.72 (1H, td, J = 8.2, 5.4 Hz), 4.49 (1H, d, J = 11.0 Hz), 3.47-3.39 (2H, m), 3.26 (2H, t, J = 6.9 Hz), 3.10 (3H, s), 2.72 (3H, s), 2.68 (1H, dd, J = 16.3, 8.6 Hz), 2.48-2.41 (1H, m), 2.30 (1H, dd, J = 16.5, 5.2 Hz), 2.16-2.02 (3H, m), 1.88-1.71 (5H, m), 1.68-1.55 (4H, m), 0.83 (3H, d, J = 6.4 Hz), 0.67 (3H, d,

J = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 171.8, 171.2, 169.0, 167.5, 155.2 (q, J = 34.5 Hz), 116.1 (q, J = 288.8 Hz), 72.9, 62.0, 47.2, 45.6, 35.8, 34.9, 33.9, 30.6, 30.52, 30.49, 25.51, 25.46, 24.8, 24.6, 23.5, 19.4, 18.5; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.38; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{23}H_{36}F_3N_4O_6$ $[M+H]^+$ 521.2582, found 521.2576.

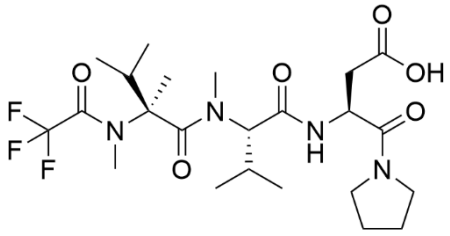
Tfa-Me(Me)Phe-MeVal-Asp-pyrro (The peptide cleavaged from 11fa)



Elongation of Tfa-(Me)Phe-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-Me(Me)Phe-MeVal-Asp-pyrro (11.0 mg, 31% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.44 (1H, bs), 7.98 (1H, d, J = 8.0 Hz), 7.35-7.28 (3H, m), 7.10-7.08 (2H, m), 4.75 (1H, td, J = 7.9, 6.0 Hz), 4.54 (1H, d, J = 11.0 Hz), 3.60 (1H, d, J = 13.9 Hz), 3.52-3.43 (2H, m), 3.27 (2H, t, J = 6.9 Hz), 2.98 (1H, d, J = 13.9 Hz), 2.77 (3H, s), 2.68 (1H, dd, J = 16.3, 8.0 Hz), 2.37 (3H, s), 2.35 (1H, dd, J = 16.2, 5.4 Hz), 2.11-2.02 (1H, m), 1.89-1.72 (4H, m), 1.48 (3H, s), 0.84 (3H, d, J = 6.4 Hz), 0.72 (3H, d, J = 6.4 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 171.8, 171.3, 169.3, 167.8, 155.1 (q, J = 34.5 Hz), 136.0, 130.5, 128.2, 126.9, 116.0 (q, J = 288.8 Hz), 65.4, 62.6, 47.4, 45.63, 45.58, 36.2, 31.3, 31.2, 30.6, 25.5, 23.6, 19.5, 19.4, 19.0; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.51; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{27}H_{38}F_3N_4O_6$ $[M+H]^+$ 571.2738, found 571.2729.

Tfa-Me(Me)Val-MeVal-Asp-pyrro (The peptide cleavaged from 11ga)

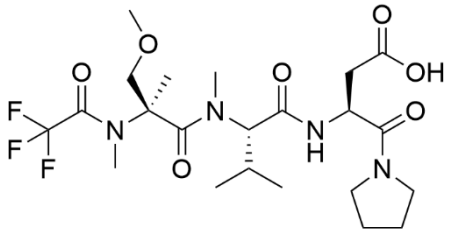


Elongation of Tfa-(Me)Val-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-Me(Me)Val-MeVal-Asp-pyrro (17.3 mg, 26% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.22 (1H, bs), 7.97 (1H, d, J = 8.0 Hz), 4.72 (1H, dt, J = 7.8, 6.0 Hz), 4.50 (1H, d, J = 11.0 Hz), 3.48-3.41 (2H, m), 3.26 (2H, t, J = 6.9 Hz), 3.10

(3H, s), 2.94-2.87 (1H, m), 2.77 (3H, s), 2.65 (1H, dd, J = 16.4, 7.9 Hz), 2.34 (1H, dd, J = 16.3, 5.8 Hz), 2.09-2.03 (1H, m), 1.88-1.71 (4H, m), 1.36 (3H, s), 1.04 (3H, d, J = 6.6 Hz), 0.82 (3H, d, J = 6.4 Hz), 0.78 (3H, d, J = 7.0 Hz), 0.67 (3H, d, J = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 171.8, 171.2, 169.2, 167.7, 155.0 (q, J = 34.2 Hz), 116.1 (q, J = 289.0 Hz), 68.1, 62.4, 47.4, 45.6, 36.0, 31.6, 31.5, 30.7, 25.5, 25.4, 23.5, 19.5, 19.3, 18.9, 17.1, 14.6; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ –68.61; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{23}H_{38}F_3N_4O_6$ $[M+H]^+$ 523.2738, found 523.2725.

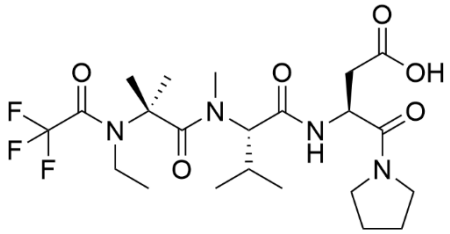
Tfa-Me(Me)Ser(Me)-MeVal-Asp-pyrro (The peptide cleavaged from 11ha)



Elongation of Tfa-(Me)Ser(Me)-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-Me(Me)Ser(Me)-MeVal-Asp-pyrro (14.4 mg, 9% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.26 (1H, bs), 7.94 (1H, d, J = 8.0 Hz), 4.73 (1H, td, J = 7.9, 5.9 Hz), 4.49 (1H, d, J = 11.0 Hz), 3.97 (1H, d, J = 10.0 Hz), 3.55 (1H, d, J = 10.0 Hz), 3.49-3.41 (2H, m), 3.27 (3H, s), 3.26 (2H, t, J = 6.9 Hz), 3.13 (3H, s), 2.77 (3H, s), 2.66 (1H, dd, J = 16.3, 8.0 Hz), 2.33 (1H, dd, J = 16.4, 5.7 Hz), 2.10-2.01 (1H, m), 1.89-1.70 (4H, m), 1.52 (3H, s), 0.82 (3H, d, J = 6.4 Hz), 0.68 (3H, d, J = 6.4 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 171.8, 170.0, 169.0, 167.7, 155.0 (q, J = 34.7 Hz), 116.0 (q, J = 288.8 Hz), 74.5, 65.0, 61.9, 58.9, 47.3, 45.59, 45.58, 36.0, 31.6, 30.2, 25.5, 25.4, 23.6, 19.4, 18.7, 18.5; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.59; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{22}H_{36}F_3N_4O_7$ $[M+H]^+$ 525.2531, found 525.2528.

Tfa-EtAib-MeVal-Asp-pyrro (The peptide cleavaged from 11ab)

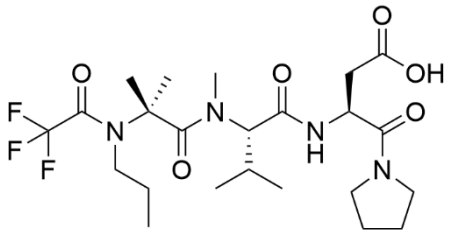


Elongation of Tfa-Aib-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-EtAib-MeVal-Asp-pyrro (7.0 mg, 18% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 7.70 (1H, d, J = 7.4 Hz), 4.74 (1H, td, J = 8.3, 5.2 Hz), 4.57 (1H, d, J = 10.8 Hz), 3.61 (2H, q, J = 7.0 Hz), 3.46-3.44 (2H, m), 3.25 (2H, t, J = 6.9

Hz), 2.71 (3H, s), 2.63 (1H, dd, *J* = 16.3, 8.6 Hz), 2.25 (1H, dd, *J* = 16.3, 5.0 Hz), 2.10-2.04 (1H, m), 1.87-1.71 (4H, m), 1.49 (6H, d, *J* = 10.6 Hz), 1.28 (3H, t, *J* = 7.0 Hz), 0.83 (3H, d, *J* = 6.4 Hz), 0.68 (3H, d, *J* = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 172.1, 171.2, 168.7, 167.9, 154.9 (q, *J* = 34.9 Hz), 116.2 (q, *J* = 288.6 Hz), 63.6, 62.1, 47.4, 45.6, 45.5, 36.5, 30.7, 25.45, 25.36, 23.6, 23.1, 19.6, 18.4, 16.1; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -67.91; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{22}H_{36}F_3N_4O_6$ $[M+H]^+$ 509.2582, found 509.2576.

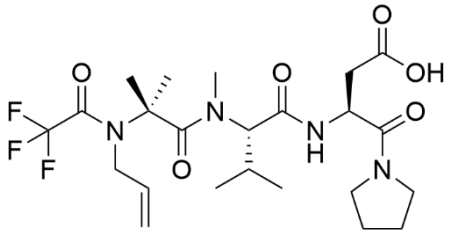
Tfa-nPrAib-MeVal-Asp-pyrro (The peptide cleavaged from 11ac)



Elongation of Tfa-Aib-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-nPrAib-MeVal-Asp-pyrro (3.5 mg, 9% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 7.60 (1H, d, *J* = 7.4 Hz), 4.69-4.66 (1H, m), 4.48 (1H, d, *J* = 10.4 Hz), 3.19 (2H, t, *J* = 6.6 Hz), 2.64 (3H, s), 2.54 (1H, dd, *J* = 16.2, 8.3 Hz), 2.44 (3H, s), 2.17 (1H, dd, *J* = 16.2, 4.3 Hz), 2.02-1.98 (1H, m), 1.78-1.75 (2H, m), 1.71-1.69 (2H, m), 1.63-1.58 (2H, m), 1.42 (6H, d, *J* = 8.6 Hz), 0.82 (3H, t, *J* = 7.1 Hz), 0.77 (3H, d, *J* = 5.8 Hz), 0.61 (3H, d, *J* = 6.2 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 172.5, 171.6, 169.0, 168.3, 155.2 (q, *J* = 34.9 Hz), 116.5 (q, *J* = 288.8 Hz), 63.9, 62.4, 47.7, 45.9, 45.8, 45.7, 37.1, 30.9, 25.7, 25.6, 24.2, 23.8, 19.8, 18.7, 11.1; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -67.91; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{23}H_{38}F_3N_4O_6$ $[M+H]^+$ 523.2738, found 523.2727.

Tfa-allylAib-MeVal-Asp-pyrro (The peptide cleavaged from 11ad)

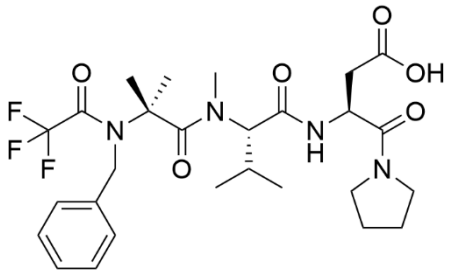


Elongation of Tfa-Aib-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-allylAib-MeVal-Asp-pyrro (8.6 mg, 22% yield).

white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.28 (1H, bs), 7.77 (1H, d, *J* = 7.6 Hz), 5.95-5.88 (1H, m), 5.41 (1H, d, *J* = 17.1 Hz), 5.30 (1H, d, *J* = 10.4 Hz), 4.74 (1H, td, *J* = 8.4, 5.2 Hz), 4.57

(1H, d, *J* = 10.8 Hz), 4.25 (2H, d, *J* = 5.8 Hz), 3.44 (2H, t, *J* = 6.7 Hz), 3.26 (2H, t, *J* = 6.9 Hz), 2.74 (3H, s), 2.66 (1H, dd, *J* = 16.3, 8.6 Hz), 2.27 (1H, dd, *J* = 16.4, 5.1 Hz), 2.11-2.05 (1H, m), 1.88-1.70 (4H, m), 1.47 (6H, d, *J* = 9.8 Hz), 0.83 (3H, d, *J* = 6.6 Hz), 0.69 (3H, d, *J* = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 171.9, 171.0, 168.8, 167.7, 155.0 (q, *J* = 35.1 Hz), 134.4, 119.2, 116.0 (q, *J* = 289.0 Hz), 64.3, 62.1, 47.3, 46.3, 45.55, 45.53, 36.2, 30.7, 25.5, 25.4, 23.5, 23.4, 23.3, 19.5, 18.4; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -67.44; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{23}H_{36}F_3N_4O_6$ $[M+H]^+$ 521.2582, found 521.2577.

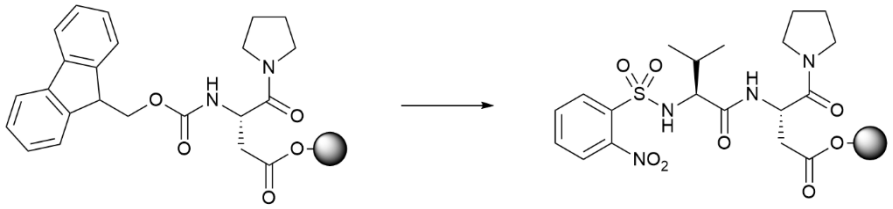
Tfa-BnAib-MeVal-Asp-pyrro (The peptide cleavaged from 11ae)



Elongation of Tfa-Aib-OH and on-resin *N*-alkylation were conducted using **1** according to the general procedures (supplementary information p14-15) to obtain Tfa-BnAib-MeVal-Asp-pyrro (6.3 mg, 14% yield).

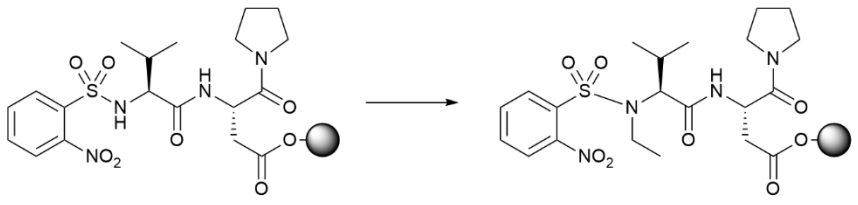
white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 7.75 (1H, d, *J* = 6.2 Hz), 7.43-7.41 (2H, m), 7.36-7.33 (3H, m), 4.90 (2H, s), 4.73 (1H, td, *J* = 8.1, 5.4 Hz), 4.56 (1H, d, *J* = 10.6 Hz), 3.51-3.44 (2H, m), 3.25 (2H, t, *J* = 6.9 Hz), 2.85 (3H, s), 2.59 (1H, dd, *J* = 16.3, 8.6 Hz), 2.23 (1H, dd, *J* = 16.0, 4.7 Hz), 2.14-2.07 (1H, m), 1.88-1.70 (4H, m), 1.33 (6H, s), 0.85 (3H, d, *J* = 6.4 Hz), 0.70 (3H, d, *J* = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 172.3, 171.3, 168.9, 168.3, 155.6 (q, *J* = 32.4 Hz), 136.0, 128.7, 127.9, 127.5, 116.4 (q, *J* = 289.4 Hz), 65.1, 62.5, 47.7, 45.7, 45.6, 37.0, 30.9, 25.6, 24.1, 23.7, 23.5, 19.7, 18.7; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -66.54; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{27}H_{38}F_3N_4O_6$ $[M+H]^+$ 571.2738, found 571.2728.

5. Installation of Tfa-MecLeu to bulky EtVal and nPrVal at *N*-term



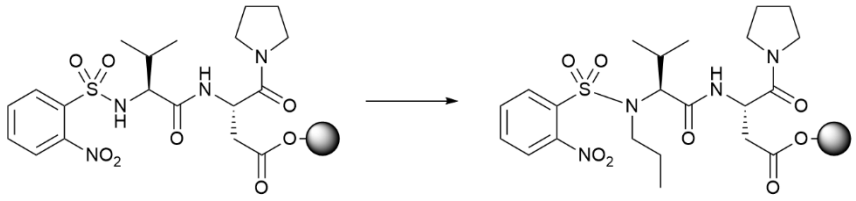
To a Fmoc-Asp-pyrro bounded resin (0.392 mmol/g, 3.0 g, 1.176 mmol) in a filter reaction vial, CH_2Cl_2 (30 mL) was added for swelling at rt for 45 min, then removed. The resin was washed with DMF (21 mL, 3 times). Then, 2.0% DBU/DMF (21 mL) was added, and the mixture was shaken at rt for 10 min. The resulting resin was washed with DMF (21 mL), a solution of DIPEA/HOAt/DMF (0.51 mL/0.41 g/21 mL), and DMF (21 mL, 4 times). To the resulting resin, an elongation cocktail (premixed of 0.60 M Fmoc-Val-OH/0.375 M HOAt/DMF (9.0 mL) and 0.71 M DIC/DMF (10.8 mL)) was added, and the mixture was shaked at 40 ºC. for 4 h. The resin was washed with DMF (21 mL, 4 times) and CH_2Cl_2 (21 mL, 6 times). The obtained wet resin was dried under reduced pressure at rt for 5 days.

To the resulting resin, CH_2Cl_2 (30 mL) was added for swelling at rt for 15 min, then removed. The resin was washed with DMF (21 mL, 4 times). Then, 2.0% DBU/DMF (21 mL) was added, and the mixture was shaken at rt for 10 min. The resulting resin was washed with DMF (21 mL), a solution of DIPEA/HOAt/DMF (0.51 mL/0.41 g/21 mL), DMF (21 mL, 2 times), and THF (21 mL, 6 times). To the resulting resin, a solution of NsCl (1.04 g, 4 equiv.)/ 2,4,6-collidine (1.5 mL, 10 equiv.)/THF (21 mL) was added. The mixture was shaken at 40 ºC. for 2 h. Then the mixture was washed with THF (21 mL, 4 times) and CH_2Cl_2 (21 mL, 6 times). The obtained wet resin was dried under reduced pressure at rt for 24 h.

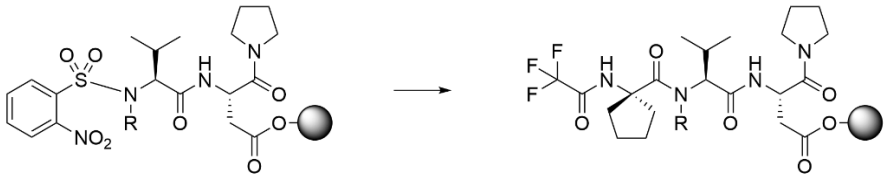


To the prepared Ns-Val-Asp(OTrt(2-Cl) resin)-pyrro (0.392 mmol/g, 1.5 g, 0.588 mmol) in a filter reaction vial, THF (10 mL) was added for swelling at rt for 15 min, then removed. To the resin, a solution of triphenylphosphine/THF (771 mg, 5.0 equiv./10 mL), ethanol (0.34 mL, 10 equiv.), and DIAD (0.63 mL, 5.0 equiv.) were added. The resulting resin was shaken at 40 ºC. for 2 h. Then the resin was washed with THF (10 mL, twice). Additionally, the same procedure was repeated once.

Then the resin was washed with THF (10 mL, 4 times), and CH_2Cl_2 (10 mL, 6 times). The obtained wet resin was dried under reduced pressure at rt for 24 h.

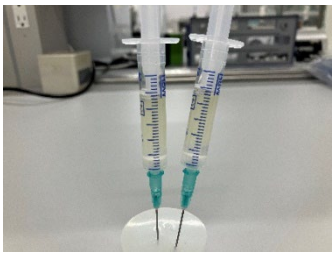


To the prepared Ns-Val-Asp(OTrt(2-Cl) resin)-pyrro (0.392 mmol/g, 1.5 g, 0.588 mmol) in a filter reaction vial, THF (10 mL) was added for swelling at rt for 15 min, then removed. To the resin, a solution of triphenylphosphine/THF (771 mg, 5.0 equiv./10 mL), 1-propanol (0.44 mL, 10 equiv.), and DIAD (0.63 mL, 5.0 equiv.) were added. The resulting resin was shaken at 40 ºC. for 2 h. Then the resin was washed with THF (10 mL, twice). Additionally, the same procedure was repeated once. Then the resin was washed with THF (10 mL, 4 times), and CH_2Cl_2 (10 mL, 6 times). The obtained wet resin was dried under reduced pressure at rt for 24 h.

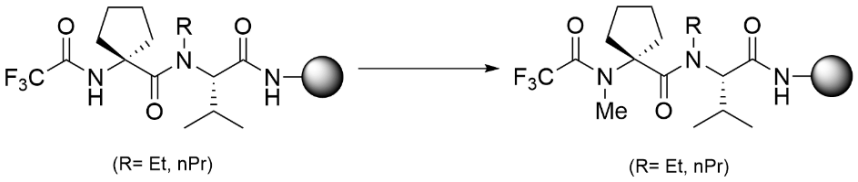


(R = Et, nPr) (R = Et, nPr)

To the prepared Ns-EtVal-Asp(OTrt(2-Cl) resin)-pyrro (0.388 mmol/g, 200 mg, 0.0776 mmol) or Ns-nPrVal-Asp(OTrt(2-Cl) resin)-pyrro (0.386 mmol/g, 200 mg, 0.0772 mmol) in a filter reaction vial, THF (1.4 mL) was added for swelling at rt for 15 min, then removed. The resin was washed with NMP (1.4 mL, 3 times). To the resin, a solution of DBU (0.075 mL, 6.5 equiv.)/NMP (0.70 mL) and a solution of 1-dodecanethiol (0.24 mL, 13 equiv.)/NMP (0.70 mL) were added. The mixture was shaken at 40 ºC. for 2 h. The resin was washed with DMF (1.4 mL, 6 times). To the resulting resin, the prepared oxazolone of Tfa-cLeu-OH (1.4 mL) was added. The mixture was shaken at 50 ºC. for 72 h. The resin was washed with DMF (1.4 mL, 6 times) and CH_2Cl_2 (1.4 mL, 6 times).



Solid phase elongation with the obtained oxazolone (**S9**, **21** in manuscript) without solvents.

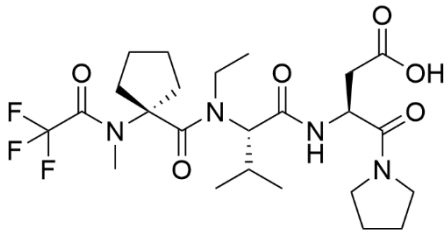


To the obtained Tfa-cLeu-EtVal-Asp(OTrt(2-Cl) resin)-pyrro (0.0776 mmol) or Tfa-cLeu-nPrVal-Asp(OTrt(2-Cl) resin)-pyrro (0.0772 mmol) was swelled with CH_2Cl_2 (2.0 mL, room temperature, 15 min). Then the resin bounded peptide was washed with DMF (1.4 mL x 4). To the resin, a solution of TMGN (20 equiv.)/DMF (2.8 mL) and a solution of MeI (81 equiv.)/DMF (2.8 mL) were added, then shaken at 40 ºC for 2 h. The resin was washed with DMF (0.70 mL x 4). After the protocol, the obtained resin was washed with DMF (1.4 mL x 4) and CH_2Cl_2 (1.4 mL x 4), then dried under reduced pressure.

To confirm the conversion, a part of the obtained resin was picked and treated with TFE/CH_2Cl_2 (1:1), and the resulting solution was analyzed by LCMS.

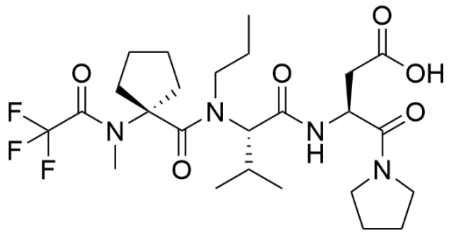
The resin was treated with 2.0 mL of TFE/ CH_2Cl_2 (1:1) with DIPEA (24 uL) solution for 2 h. The eluted solution was collected in the test tube, and resin was washed with 1.0 mL of CH_2Cl_2 twice and DMF (4.0 mL). The solution was evaporated under reduced pressure, purified by a reversed-phase column (C18, 30 g, 0.10%FA MeCN / 0.10%FA H_2O), and lyophilized to obtain the compound.

Tfa-MecLeu-EtVal-Asp-pyrro (The peptide cleavaged from 17a)



11.0 mg, 24% yield; white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.34 (1H, bs), 7.80 (1H, d, J = 7.6 Hz), 4.81 (1H, dd, J = 14.1, 7.8 Hz), 3.85 (1H, s), 3.50-3.43 (2H, m), 3.28-3.21 (2H, m), 3.11 (3H, s), 2.65 (1H, dd, J = 16.2, 8.1 Hz), 2.47-2.26 (4H, m), 2.00-1.92 (1H, m), 1.89-1.70 (5H, m), 1.67-1.55 (4H, m), 1.59 (2H, d, J = 8.6 Hz), 0.96 (3H, t, J = 7.0 Hz), 0.87 (3H, d, J = 6.0 Hz), 0.75 (3H, d, J = 6.2 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 172.0, 171.8, 169.8, 168.0, 155.8 (q, J = 34.9 Hz), 116.1 (q, J = 288.8 Hz), 73.5, 66.9, 47.1, 45.6, 45.5, 36.4, 34.7, 30.5, 26.3, 25.5, 24.7, 23.6, 20.5, 19.0, 13.1; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.70; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{24}H_{38}F_3N_4O_6$ $[M+H]^+$ 535.2738, found 535.2732.

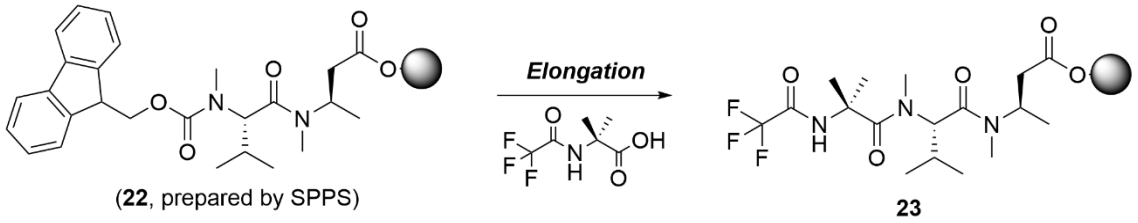
Tfa-MecLeu-nPrVal-Asp-pyrro (The peptide cleavaged from 17b)



10.6 mg, 27% yield; white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) δ 12.46 (1H, bs), 7.77 (1H, d, J = 6.8 Hz), 4.81 (1H, dd, J = 14.2, 7.9 Hz), 3.84 (1H, bs), 3.51-3.43 (2H, m), 3.28-3.21 (2H, m), 3.12 (3H, s), 3.03-2.93 (2H, m), 2.65 (1H, dd, J = 16.1, 8.2 Hz), 2.44-2.25 (4H, m), 2.01-1.92 (1H, m), 1.89-1.70 (5H, m), 1.68-1.53 (4H, m), 1.47-1.36 (1H, m), 1.33-1.23 (1H, m), 0.87 (3H, d, J = 6.2 Hz), 0.77 (3H, t, J = 7.2 Hz), 0.75 (3H, d, J = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) δ 171.9, 171.8, 169.7, 168.0, 155.9 (q, J = 33.8 Hz), 116.0 (q, J = 287.9 Hz), 73.6, 48.8, 47.1, 45.6, 45.5, 36.5, 34.6, 30.62, 30.59, 26.3, 25.4, 24.7, 23.6, 20.9, 20.5, 19.0, 10.9; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) δ -68.83; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{25}H_{40}F_3N_4O_6$ $[M+H]^+$ 549.2895, found 549.2890.

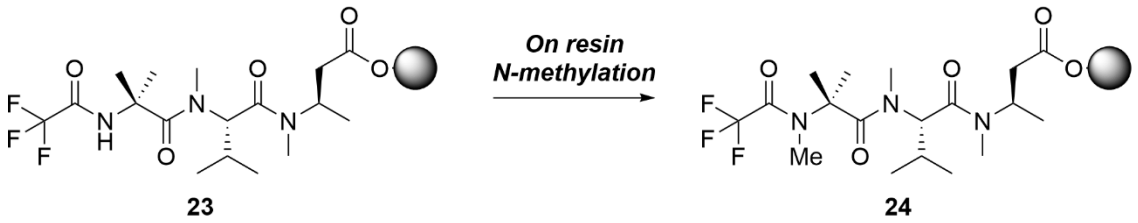
6. Preparation of LUNA18 analogue containing MeAib-MeVal motif

Elongation of Tfa-Aib-OH



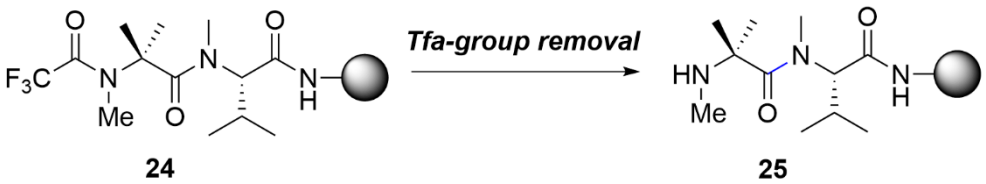
The solid supported Fmoc-MeVal-D-3-MeAbu(OTrt(2-Cl) resin) (0.0343 mmol x 12, already swollen by SPPS for MeVal elongation) was washed with DMF (0.70 mL x 3) and deprotected with 2.0% DBU/DMF (0.70 mL, rt) using an automated Intavis Multipep RSi peptide synthesizer. The resin in the reaction vessel was washed with DMF (0.70 mL x 4). A solution of Tfa-Aib-OH (5.0 equiv.) in DMF (0.30 mL) and a solution of 10% DIC in DMF (0.36 mL) were mixed and transferred to the reaction vessel. The reaction vessel was kept at 40 ºC for 20 h and 2.2 h. Then, the resin in the reaction vessel was washed with DMF (1.2 mL x 3). The obtained resin was washed with CH_2Cl_2, (1.25 mL x 2) and dried under reduced pressure.

On-resin *N*-methylation



To the obtained Tfa-Aib-MeVal-D-3-MeAbu(OTrt(2-Cl) resin) (0.0343 mmol x 12) was swelled with CH_2Cl_2 (1.0 mL, room temperature). Then the resin bounded peptide was washed with DMF (1.0 mL x 4). To the resin, a solution of P_1-tBu (3.0 equiv.)/DMF (0.18 mL) and a solution of MeI (20 equiv.)/DMF (0.18 mL) were added, then shaken at 40 ºC for 40 min. After the protocol, the obtained resin was washed with DMF (1.0 mL x 4) and CH_2Cl_2 (1.0 mL x 4), then dried under reduced pressure. To confirm the conversion, a part of the obtained resin was picked and treated with TFE/CH_2Cl_2 (1:1), and the resulting solution was analyzed by LCMS.

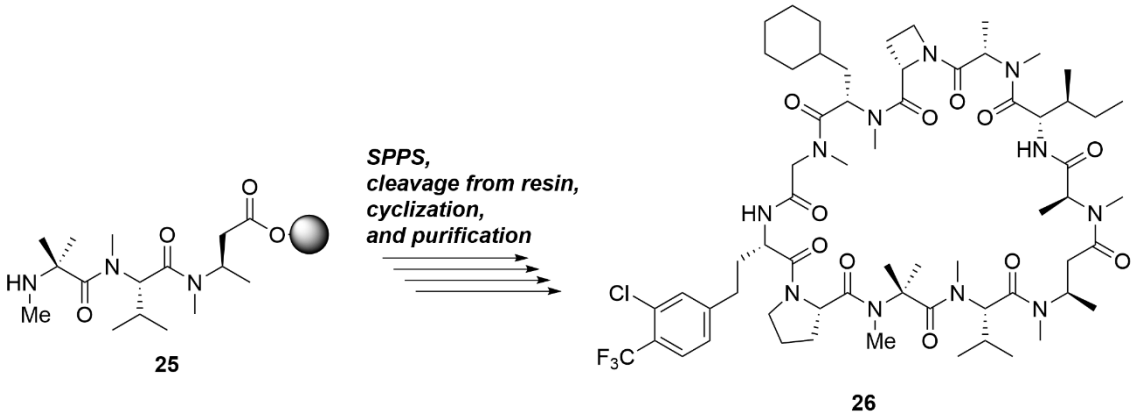
Tfa-group removal



To the obtained Tfa-MeAib-MeVal-D-3-MeAbu(OTrt(2-Cl) resin) (0.0343 mmol x 12) was swelled with CH_2Cl_2 (1.0 mL, room temperature, 30 min). Then the resin bounded peptide was washed with THF (0.70 mL x 4). To the resin, THF (0.50 mL), MeOH (0.25 mL), and 2.0 M NaBH4/triglyme (0.25 mL) were added, then shaken at rt for 40 min. The resin was washed with MeOH (0.70 mL, 1 min x 4). After the protocol, the obtained resin was washed with CH_2Cl_2 (0.70 mL x 4), then dried under reduced pressure.

To confirm the conversion, a part of the obtained resin was picked and treated with TFE/CH_2Cl_2 (1:1), and the resulting solution was analyzed by LCMS.

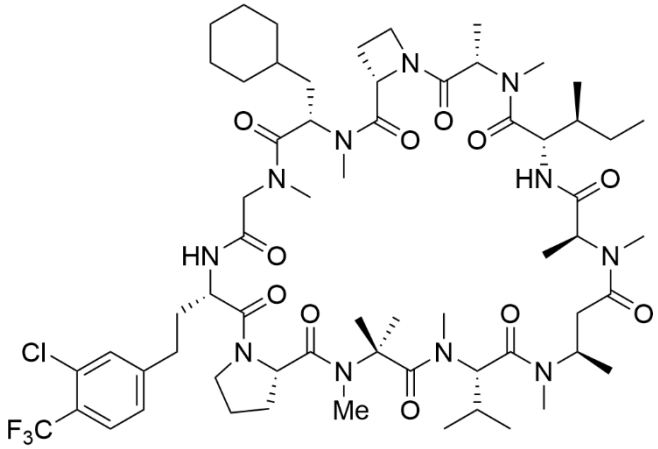
SPPS, cleavage from resin, cyclization, and purification



25 **26**

SPPS, cleavage from resin, cyclization and purification were conducted using **25** according to the general procedure (ref. *J. Med. Chem.* **2022**, *65*, 13401-13412, and its supporting information) for the syntheses of *N*-Me-rich cyclic peptides to obtain **26** (67.1 mg, 13% yield).

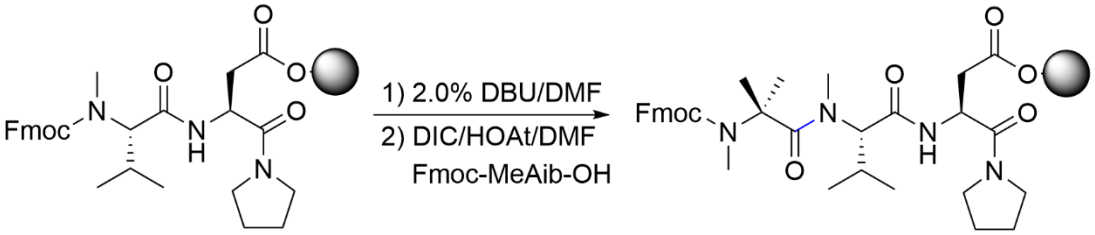
LUNA18 analogue containing MeAib-MeVal motif (26)



white solid; HRMS (ESI-Orbitrap) *m/z* calcd for $C_{63}H_{98}ClF_3N_{11}O_{11}$ $[M+H]^+$ 1276.7082, found 1276.7068.

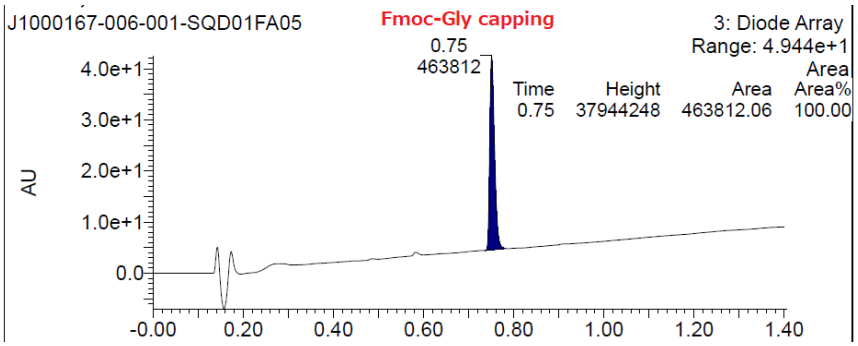
7. Comparison examples

7-1. Attempt of Fmoc-MeAib elongation by the general method



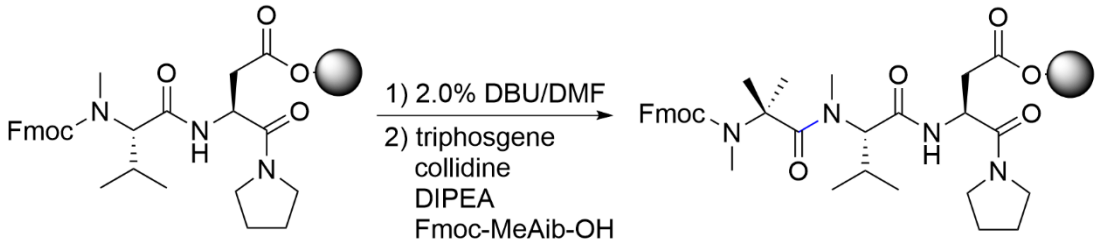
The solid supported Fmoc-MeVal-Asp(OTrt(2-Cl) resin)-pyrro (100 mg, 0.478 mmol/g) was swelled with CH_2Cl_2 (1.0 mL, room temperature, 45 min). Then the resin bounded peptide was washed with DMF (0.70 mL x 3) and deprotected with 2.0% DBU/DMF (0.70 mL, rt, 5 min). The resin in the reaction vessel was washed with DMF (0.70 mL x 4). A solution of 0.60 M Fmoc-MeAib-OH/0.38 HOAt in NMP and a solution of 0.71 M DIC in DMF were mixed at a ratio of 1:1.2. Then the resultant mixture (0.66 mL) was transferred to the reaction vessel. The reaction vessel was shaken at 40 ºC for 2.5 h. Then the resin in the reaction vessel was washed with DMF (0.70 mL x 4). To confirm the conversion of the reaction, Fmoc-Gly-OH capping was conducted. A solution of 0.60 M Fmoc-Gly-OH/0.38 HOAt in NMP and a solution of 0.71 M DIC in DMF were mixed at a ratio of 1:1.2. Then the resultant mixture (0.66 mL) was transferred to the reaction vessel. The reaction vessel was shaken at 40 ºC for 2.5 h. The obtained resin was washed with CH_2Cl_2, and dried under reduced pressure.

A part of the obtained resin was picked and treated with TFE/CH_2Cl_2 /DIPEA (1:1:0.015), and the resulting solution was analyzed by LCMS. The desired compound (Fmoc-MeAib-MeVal-Asp-pyrro) was not observed.



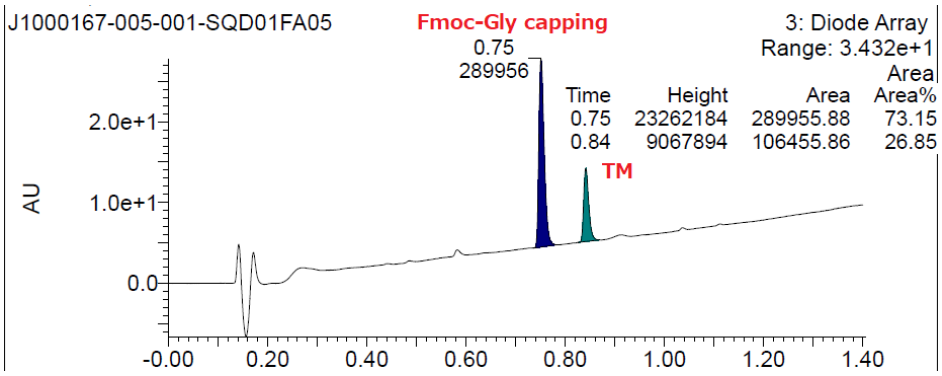
7-2. Attempt of Fmoc-MeAib elongation by triphosgene-mediated method

(ref. *Angew. Chem. Int. Ed.* **2002**, *41*, 2307-2309)



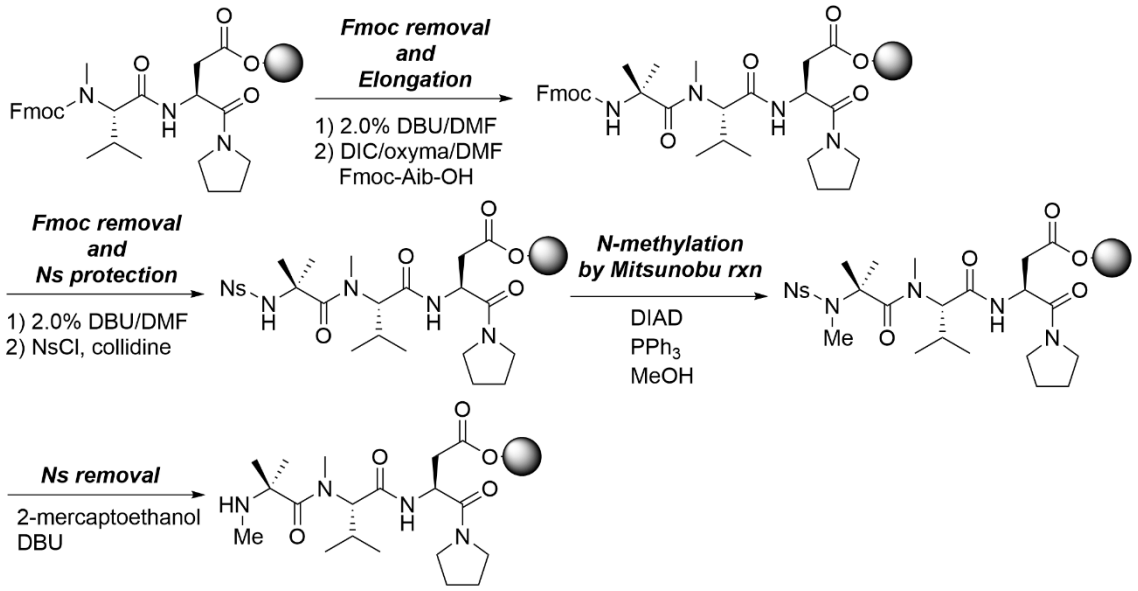
The solid supported Fmoc-MeVal-Asp(OTrt(2-Cl) resin)-pyrro (100 mg, 0.478 mmol/g) was swelled with CH_2Cl_2 (1.0 mL, room temperature, 45 min). Then the resin bounded peptide was washed with DMF (0.70 mL x 3) and deprotected with 2.0% DBU/DMF (0.70 mL, rt, 5 min). The resin in the reaction vessel was washed with DMF (0.70 mL x 4) and THF (0.70 mL x 4). Fmoc-MeAib-OH (3.5 equiv.) was added to a triphosgene/THF solution (0.40 mL; 1.15 equiv. triphosgene). 2,4,6-collidine (0.063 mL; 10 equiv.) was added to the solution (collidinium chloride was precipitated). A solution of DIPEA (0.065 mL, 8.0 equiv.) in THF (0.40 mL) was added to the resin, immediately followed by the addition of the suspension. The mixture was shaken at rt for 3 h. The resulting resin was washed with THF (0.70 mL x 4) and then DMF (0.70 mL x 4). To confirm the conversion of the reaction, Fmoc-Gly-OH capping was conducted. A solution of 0.60 M Fmoc-Gly-OH/0.38 HOAt in NMP and a solution of 0.71 M DIC in DMF were mixed at a ratio of 1:1.2. Then the resultant mixture (0.66 mL) was transferred to the reaction vessel. The reaction vessel was shaken at 40 ºC for 2.5 h. The obtained resin was washed with CH_2Cl_2, and dried under reduced pressure.

A part of the obtained resin was picked and treated with TFE/CH_2Cl_2 /DIPEA (1:1:0.015) at rt for 15 min., and the resulting solution was analyzed by LCMS. The desired compound (Fmoc-MeAib-MeVal-Asp-pyrro) was observed in only 27%.



7-3. Attempt of installation of MeAib to MeVal at *N*-term by 4 step processes

(Fmoc-Aib elongation, replacement of Fmoc into Ns, *N*-methylation by Mitsunobu reaction, and Ns removal)



The solid supported Fmoc-MeVal-Asp(OTrt(2-Cl) resin)-pyrro (0.464 mmol/g, 100 mg) was swelled with CH_2Cl_2 (1.0 mL) at room temperature for 30 min. The resin was washed with DMF (1.0 mL x 2). To the resin, 2.0% DBU/DMF (0.70 mL) was added, and the mixture was shaken at room temperature for 10 min. Then the resulting resin was washed with DMF (0.70 mL x 4). A solution of 0.60 M Fmoc-Aib-OH/0.38 oxyma in NMP and a solution of 0.71 M DIC in DMF were mixed at a ratio of 1:1.2. Then the resultant mixture (0.66 mL) was transferred to the reaction vessel. The reaction vessel was shaken at 50 ºC for 15 h. The same elongation protocol was repeated twice (2nd: 50 ºC for 24 h, 3rd: 50 ºC for 20 h). The unreacted *N*-term was capped with Z-Gly-OH. Then the resin in the reaction vessel was washed with DMF (0.70 mL x 4) and CH_2Cl_2 (0.70 mL x 4).

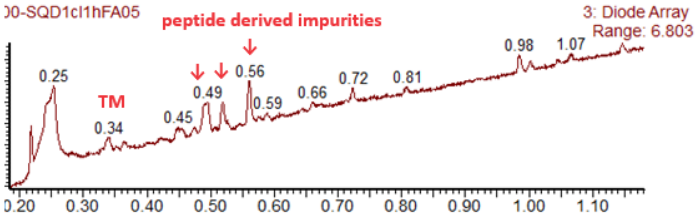
The obtained resin was swelled with CH_2Cl_2 (1.0 mL) at room temperature for 30 min. The resin was washed with DMF (1.0 mL x 2). To the resin, 2.0% DBU/DMF (0.70 mL) was added, and the mixture was shaken at room temperature for 10 min. Then the resulting resin was washed with DMF (1.0 mL x 3) and THF (1.0 mL x 4). To the resin, a solution of 2,4,6-collidine (0.062 mL, 10 equiv.)/THF (0.35 mL) and a solution of 2-nitrobenzenesulfonyl chloride (41 mg, 4.0 equiv.)/THF (0.35 mL) were added, and the resulting mixture was shaken at 40 ºC for 2 h. The resin was washed with THF (1.0 mL x 3) and CH_2Cl_2 (1.0 mL x 4). The same nosylation process was repeated twice (2nd: 40 ºC for 16 h, 3rd: 40 ºC for 21 h). The unreacted *N*-term was capped with Z-Gly-OH.

The obtained resin was swelled with CH_2Cl_2 (1.0 mL) at room temperature for 20 min. The resin was washed with THF (1.0 mL x 4). To the resin, a solution of triphenylphosphine (61 mg, 5 equiv.)/methanol (0.019 mL, 10 equiv.)/THF (0.70 mL) and DIAD (0.045 mL, 5.0 equiv.) was added,

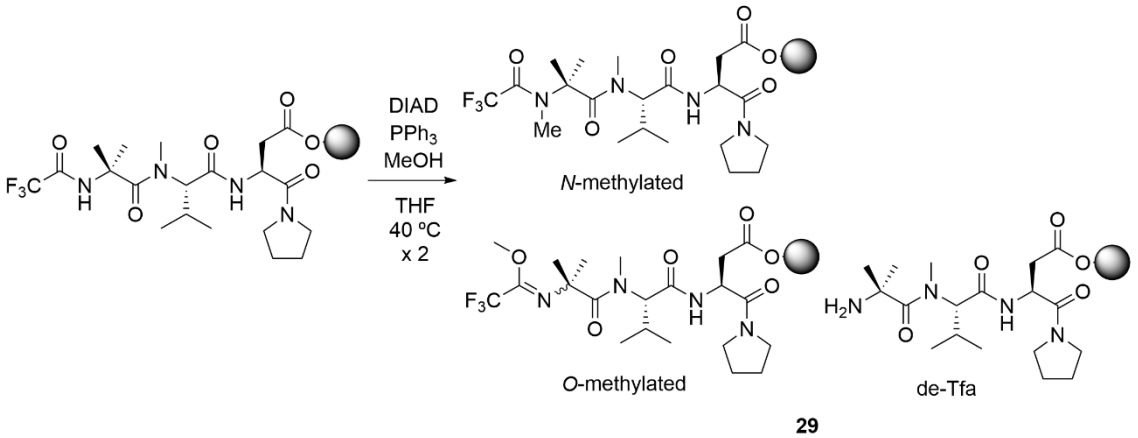
and the resulting mixture was shaken at 40 ºC for 30 min. The resin was washed with THF (1.0 mL x 4) and CH_2Cl_2 (1.0 mL x 4), and dried under reduced pressure.

The obtained resin (Ns-MeAib-MeVal-Asp(OTrt(2-Cl) resin)-pyrro, 50 mg) was swelled with CH_2Cl_2 (0.50 mL) at room temperature for 20 min. The resin was washed with NMP (0.50 mL x 4). To the resin, a solution of DBU (0.017 mL, 5.0 equiv.)/NMP (0.35 mL) and a solution of 2-mercaptoethanol (0.016 mL, 10 equiv.) were added, and the resulting mixture was shaken at room temperature for 1 h. Then the resin was washed with NMP (0.50 mL x 4) and CH_2Cl_2 (0.50 mL x 4), and dried under reduced pressure.

A part of the obtained resin (~5 mg) was picked and treated with TFE/CH_2Cl_2 /DIPEA (1:1:0.015) at rt for 15 min., and the resulting solution was analyzed by LCMS. Although the desired compound (H-MeAib-MeVal-Asp-pyrro, TM) was observed, the purity of the obtained crude product was low as shown below.



7-4. Attempt of on-resin *N*-methylation of Tfa-Aib at *N*-term by Mitsunobu reaction



29

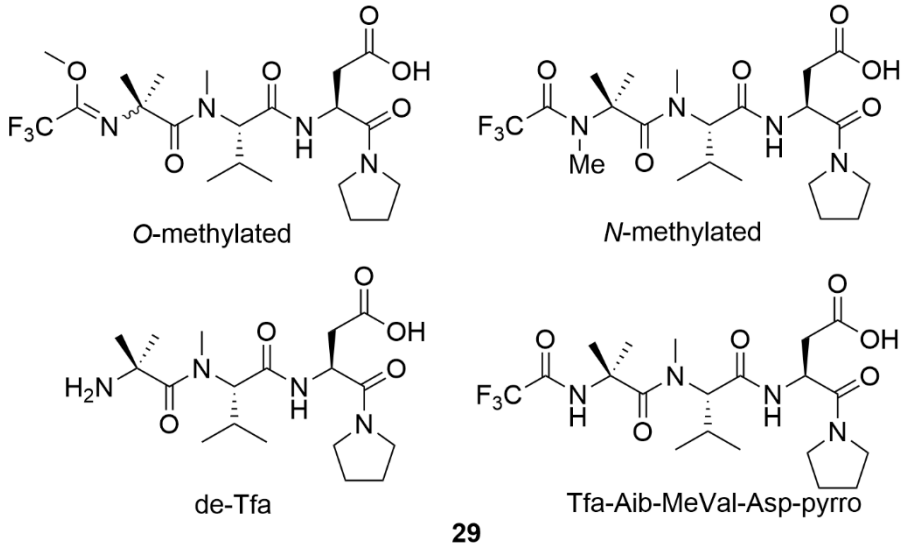
The solid supported Tfa-Aib-MeVal-Asp(OTrt(2-Cl) resin)-pyrro (3 g, 0.381 mmol/g) was swelled with THF (21.0 mL, room temperature, 15 min). To the resin, a solution of triphenylphosphine (1.50 g, 5.0 equiv.)/THF (21.0 mL), methanol (0.462 mL, 10 equiv.) and DIAD (1.13 mL, 5.0 equiv.) were added. The mixture was shaken at 40 ºC. for 2 h. The same procedure was repeated twice. The resulting resin was washed with THF (21.0 mL x 4) and CH_2Cl_2 (21.0 mL x6). The obtained resin was dried under reduced pressure.

A part of the obtained resin was picked and treated with TFE/CH_2Cl_2 (1:1) at rt for 15 min., and the resulting solution was analyzed by LCMS.

The resin (1 g) was treated with 10.0 mL of TFE/ CH_2Cl_2 (1:1) for 15 min. The eluted solution was collected in the flask, and the resin was washed with 5.0 mL of CH_2Cl_2 twice. The volatiles were evaporated under reduced pressure to obtain the crude product (43.0 mg).

The *O*-methylated product formed in the reaction was unstable and therefore impossible to isolate by column chromatography. Consequently, detailed NMR studies (^{13}C, ^{13}C-dept135, COSY, HSQC, HMBC, NH-HMBC, ROESY, NOESY, 19F, CF-HMQC, CF-HMBC) were conducted on the mixture of crude reaction products to determine the structure. The structure of *O*-methylated product was confirmed by 2D-NMR analysis. In particular, it was verified by the ^{13}C chemical shift of introduced Me group at 66.5 ppm and the ^{15}N chemical shift of nitrogen of generated imidate moiety at 271 ppm. NMR data of the crude corroborated the major product was an *O*-methylated compound, and the minor product was an *N*-methylated product. The target mass ($[M-H]^-$ 493) was observed from 2 peaks (0.68 min and 0.77 min). Compared to the authentic sample of *N*-methylated target compound, the peak at 0.68 min was the desired *N*-methylated compound. Therefore, another peak at 0.77 min was supposed to be *O*-methylated product. Meanwhile, the peaks with less than 1% UV area derived from de-Tfa product and Tfa-Aib-MeVal-Asp-pyrro were also observed at 0.50 min and 0.64 min, respectively.

The peptides cleavaged from 29



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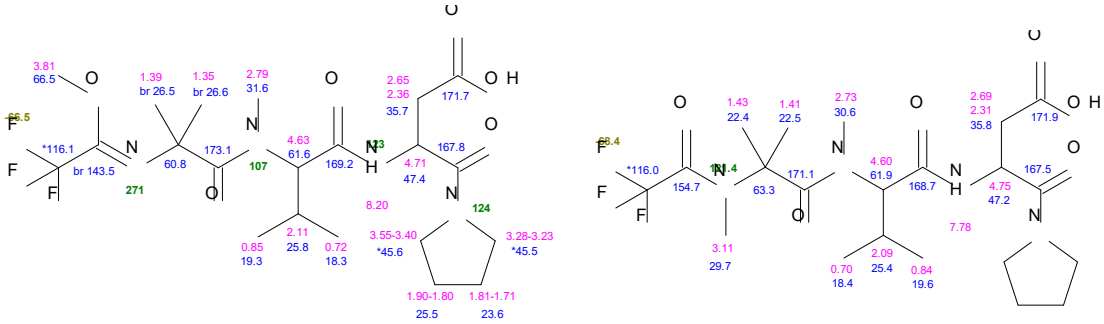
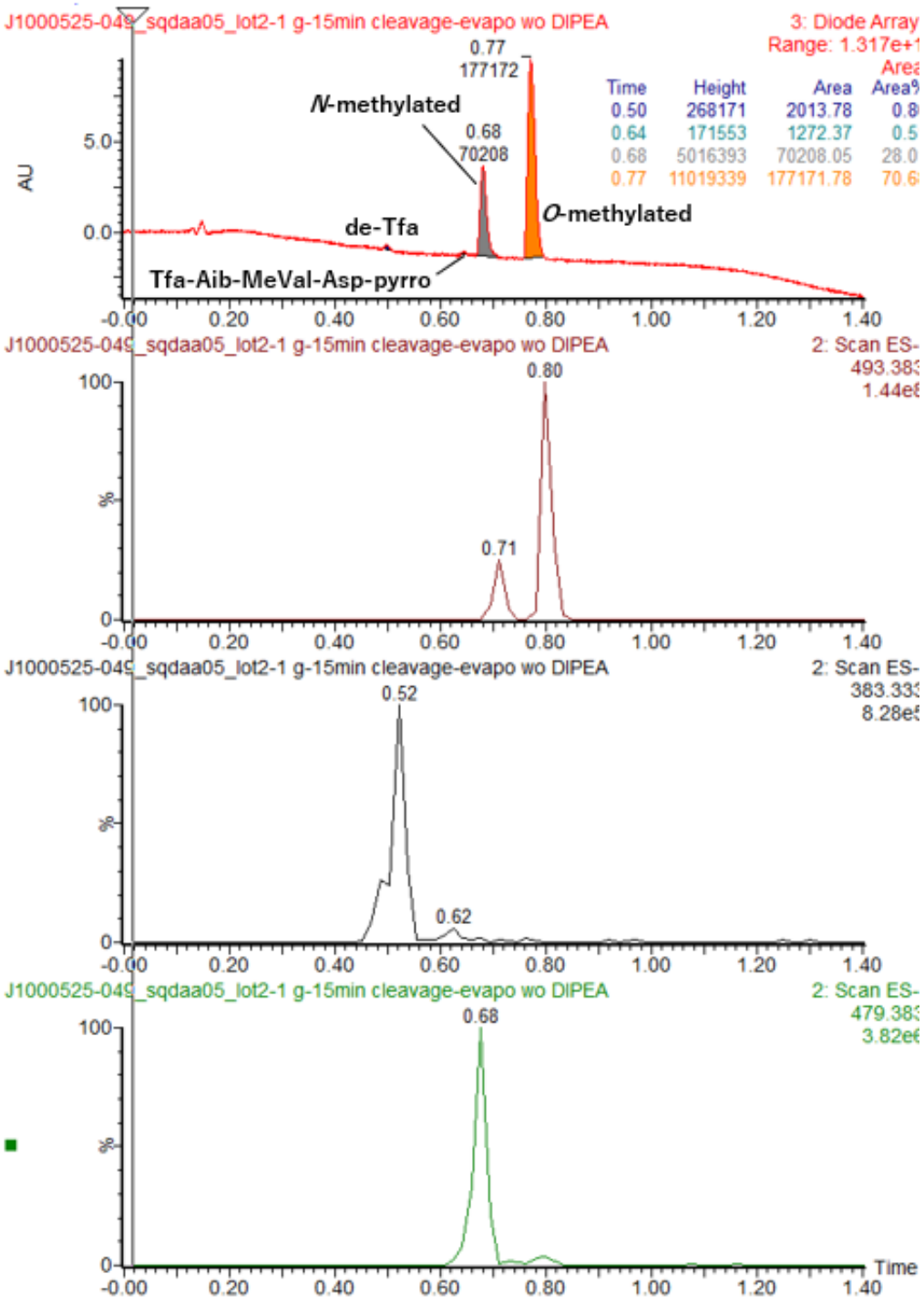
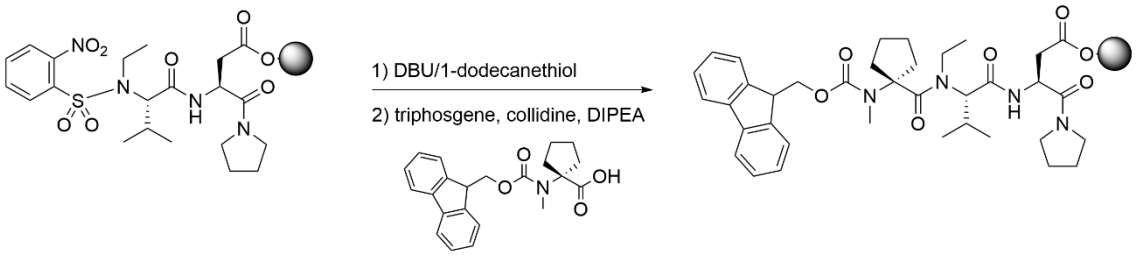
white solid; ^{1}H-NMR (600 MHz, DMSO-d_6, 300 K) *O*-methylated compound: δ 8.20 (1H, d, J = 7.6 Hz), 4.73-4.69 (1H, m), 4.63 (1H, d, J = 11.2 Hz), 3.81 (3H, s), 3.55-3.40 (2H, m), 3.28-3.23 (2H, m), 2.79 (3H, s), 2.65 (1H, dd, J = 16.8, 7.7 Hz), 2.36 (1H, dd, J = 16.4, 6.3 Hz), 2.15-2.05 (1H, m), 1.90-1.71 (4H, m), 1.39 (3H, s), 1.35 (3H, s), 0.89-0.84 (3H, m), 0.72 (3H, d, J = 6.6 Hz); *N*-methylated compound: δ 7.78 (1H, d, J = 8.2 Hz), 4.75 (1H, td, J = 8.4, 5.2 Hz), 4.61 (1H, d, J = 13.5 Hz), 3.55-3.40 (2H, m), 3.27-3.24 (2H, m), 3.11 (3H, s), 2.73 (3H, s), 2.69 (1H, dd, J = 16.6, 8.9 Hz), 2.31 (1H, dd, J = 16.6, 5.1 Hz), 2.15-2.05 (1H, m), 1.90-1.71 (4H, m), 1.43 (3H, s), 1.41 (3H, s), 0.89-0.84 (3H, m), 0.70 (3H, d, J = 6.6 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6, 300 K) *O*-methylated compound: δ 173.1, 171.7, 169.2, 167.8, 143.5, 116.1*, 66.5, 61.6, 60.8, 47.4, 45.6*, 45.5*, 35.7, 31.6, 26.6, 26.5, 25.8, 25.5, 23.6, 19.3, 18.3; *N*-methylated compound: δ 171.9, 171.1, 168.7, 167.5, 154.7 (q, J = 35.5 Hz), 116.0*, 63.3, 61.9, 47.2, 45.6*, 45.5*, 35.8, 30.6, 29.7, 25.5, 25.4, 23.5, 22.5, 22.4, 19.6, 18.4; ^{19}F-NMR (565 MHz, DMSO-d_6, 300 K) *O*-methylated compound: δ -66.49; *N*-methylated compound: δ -68.37; HRMS (ESI-Orbitrap) *O*-methylated compound: *m/z* calcd for $C_{21}H_{34}F_3N_4O_6$ $[M+H]^+$ 495.2425, found 495.2422; *N*-methylated compound: *m/z* calcd for $C_{21}H_{34}F_3N_4O_6$ $[M+H]^+$ 495.2425, found 495.2418.

*assigned by CF-HMQC



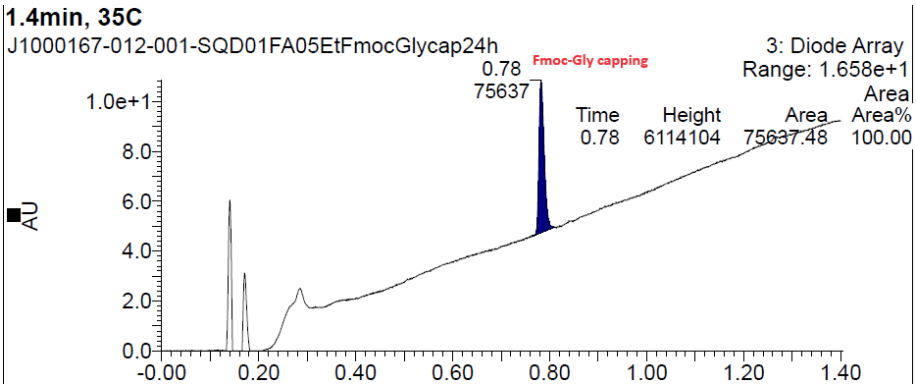

J1000525-049_sqdaa05_lot2-1 g-15min cleavage-evapo wo DIPEA
3: Diode Array
Range: 1.317e+1
Time Height Area Area%
0.50 268171 2013.78 0.8
0.64 171553 1272.37 0.5
0.68 5016393 70208.05 28.0
0.77 11019339 177171.78 70.6
0.77
177172
N-methylated
0.68
70208
O-methylated
de-Tfa
Tfa-Aib-MeVal-Asp-pyrro
AU
5.0
0.0
-0.00 0.20 0.40 0.60 0.80 1.00 1.20 1.40
J1000525-049_sqdaa05_lot2-1 g-15min cleavage-evapo wo DIPEA
2: Scan ES-
493.383
1.44e8
0.80
0.71
100
%
0
-0.00 0.20 0.40 0.60 0.80 1.00 1.20 1.40
J1000525-049_sqdaa05_lot2-1 g-15min cleavage-evapo wo DIPEA
2: Scan ES-
383.333
8.28e5
0.52
0.62
100
%
0
-0.00 0.20 0.40 0.60 0.80 1.00 1.20 1.40
J1000525-049_sqdaa05_lot2-1 g-15min cleavage-evapo wo DIPEA
2: Scan ES-
479.383
3.82e6
0.68
100
%
0
Time
-0.00 0.20 0.40 0.60 0.80 1.00 1.20 1.40

7-5. Attempt of Fmoc-MecLeu elongation to EtVal at *N*-term by triphosgene-mediated method



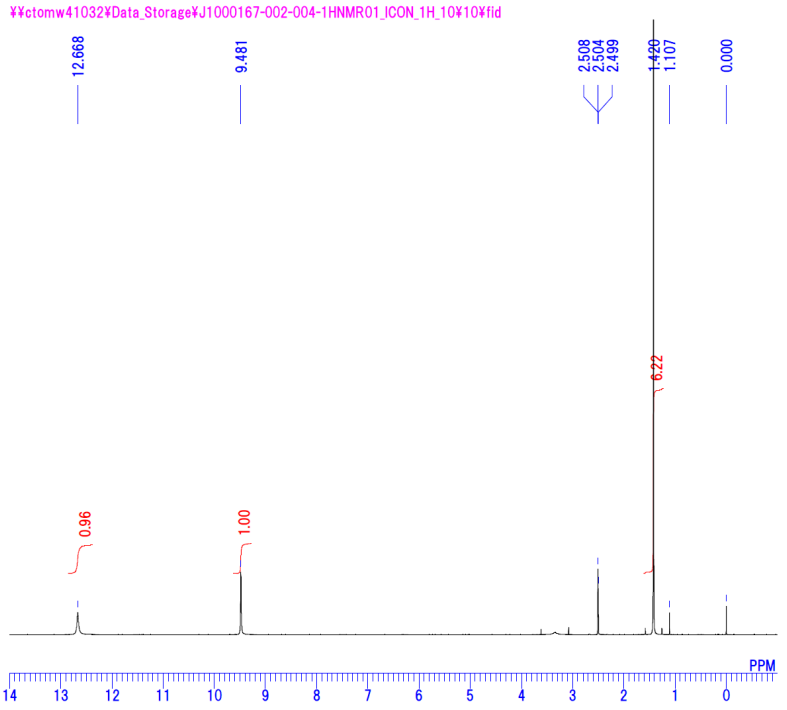
The solid supported Ns-EtVal-Asp(OTrt(2-Cl) resin)-pyrro (100 mg, 0.392 mmol/g) was swelled with THF (1.0 mL, room temperature, 15 min). Then the resin bounded peptide was washed with NMP (0.70 mL x 3) and deprotected with a solution of DBU (0.029 mL, 5.0 equiv.)/1-dodecanethiol (0.093 mL, 10 equiv.)/NMP (0.70 mL). The mixture was shaken at 40 ºC. for 2 h. The resin in the reaction vessel was washed with NMP (0.70 mL x 4) and THF (0.70 mL x 6). Fmoc-MecLeu-OH (50.1 mg, 3.5 equiv.) was added to a triphosgene/THF solution (0.40 mL; 1.15 equiv. triphosgene). 2,4,6-collidine (0.052 mL; 10 equiv.) was added to the solution (collidinium chloride was precipitated). A solution of DIPEA (0.053 mL, 8.0 equiv.) in THF (0.40 mL) was added to the resin, immediately followed by the addition of the suspension. The mixture was shaken at rt for 3 h. The resulting resin was washed with THF (0.70 mL x 4) then DMF (0.70 mL x 4). To confirm the conversion of the reaction, Fmoc-Gly-OH capping was conducted. A solution of 0.60 M Fmoc-Gly-OH/0.38 HOAt in NMP and a solution of 0.71 M DIC in DMF were mixed at a ratio of 1:1.2. Then the resultant mixture (0.66 mL) was transferred to the reaction vessel. The reaction vessel was shaken at 50 ºC for 24 h. The obtained resin was washed with CH_2Cl_2, and dried under reduced pressure.

A part of the obtained resin was picked and treated with TFE/CH_2Cl_2 /DIPEA (1:1:0.015) at rt for 15 min., and the resulting solution was analyzed by LCMS. The desired compound (Fmoc-MecLeu-EtVal-Asp-pyrro) was not observed at all.



8. NMR spectra

Tfa-Aib-OH (S1)

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FREQU    8802.82 Hz
SCANS          8
ACQTM     3.7224 sec
PD        3.4000 sec
PW1        10.00 usec
IRNUC
CTEMP       24.9 c
SLVNT   DMSO
EXREF       0.00 ppm
BF          0.12 Hz
RGAIN        101
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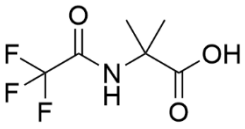

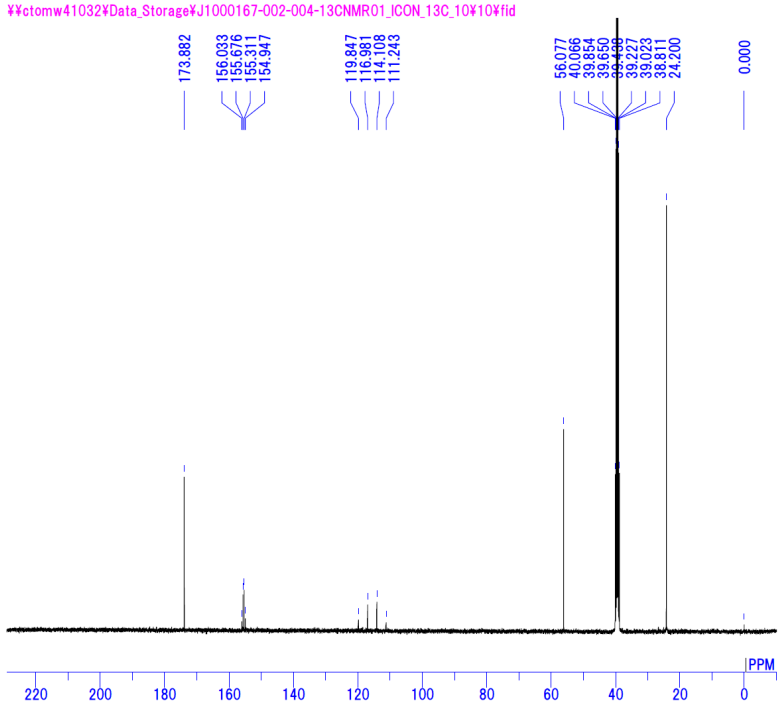


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POINT      32768
FREQU   24038.46 Hz
SCANS       1024
ACQTM     1.3631 sec
PD        2.5000 sec
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IRNUC
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SLVNT   DMSO
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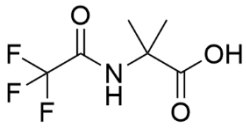

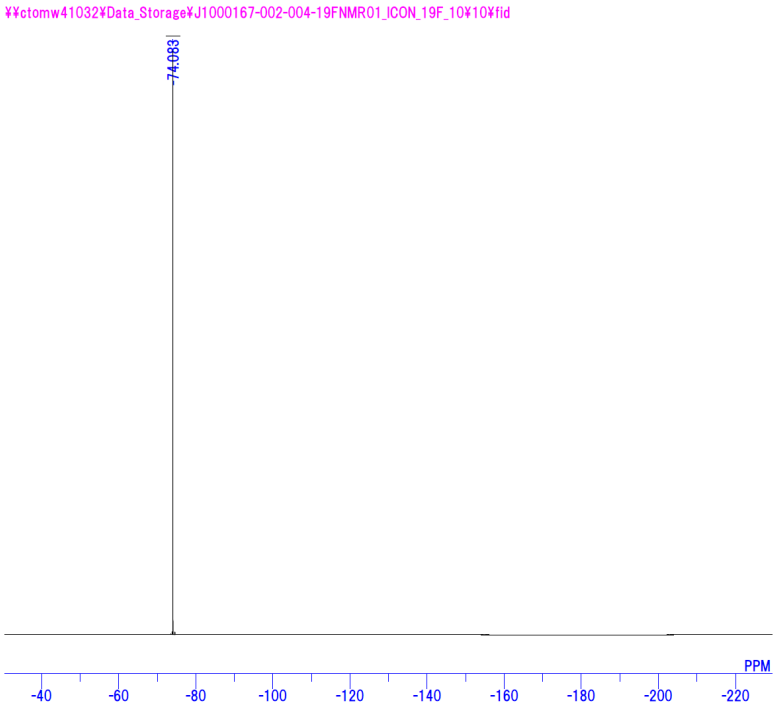

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CTEMP	26.8 c
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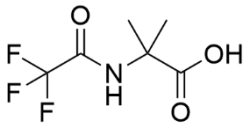
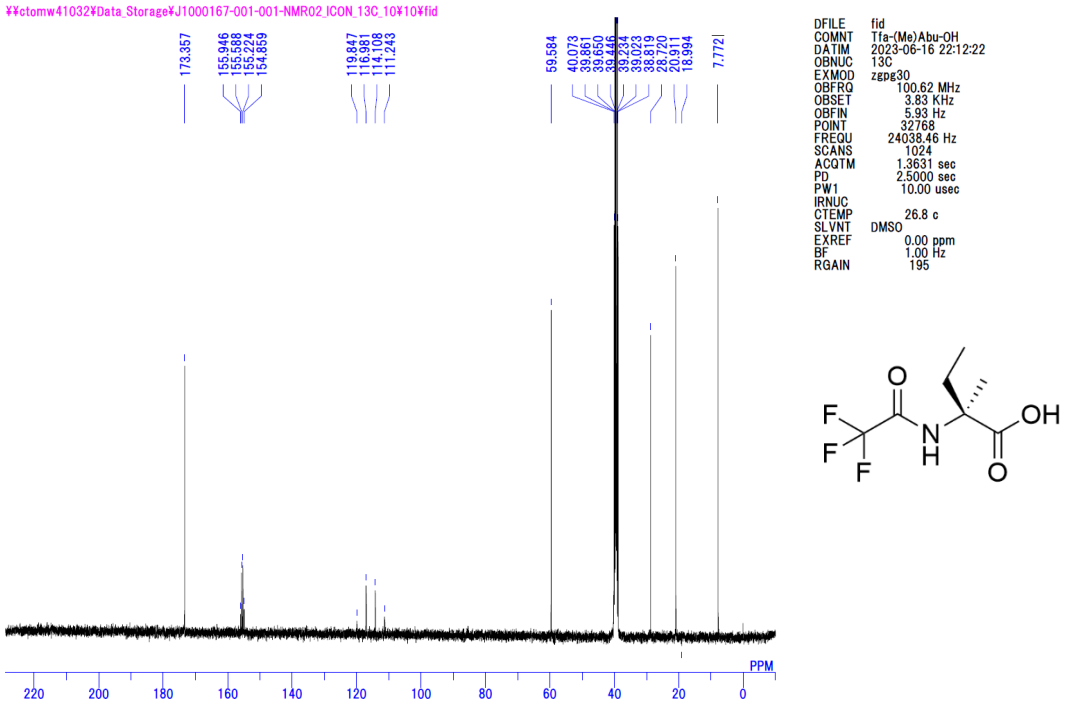
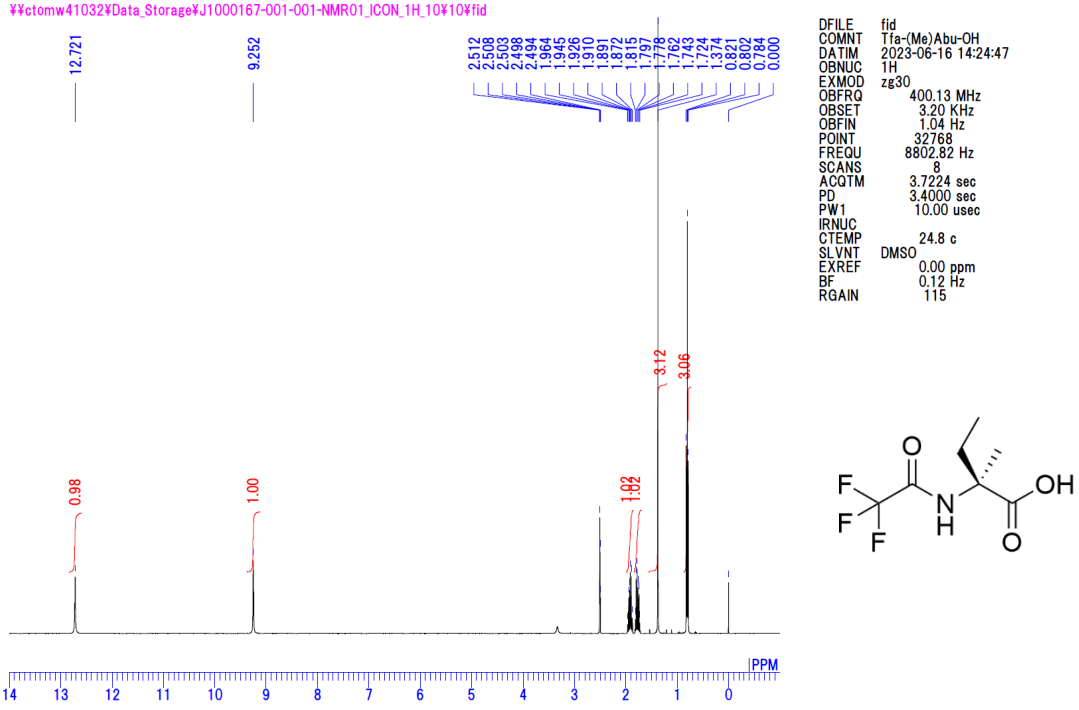
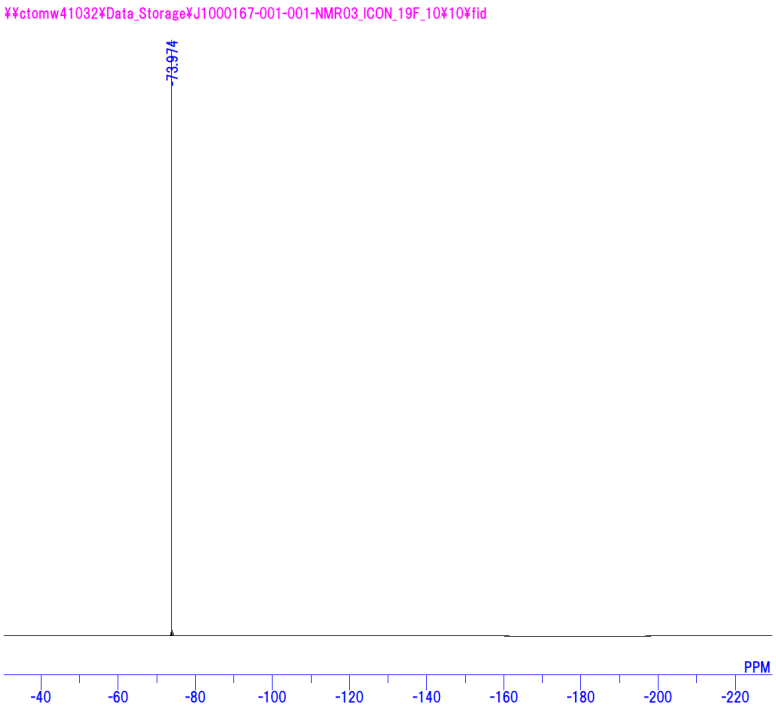
RGAIN	195



Tfa-(Me)Abu-OH (S2)







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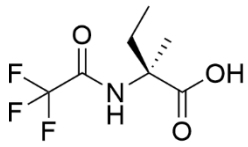
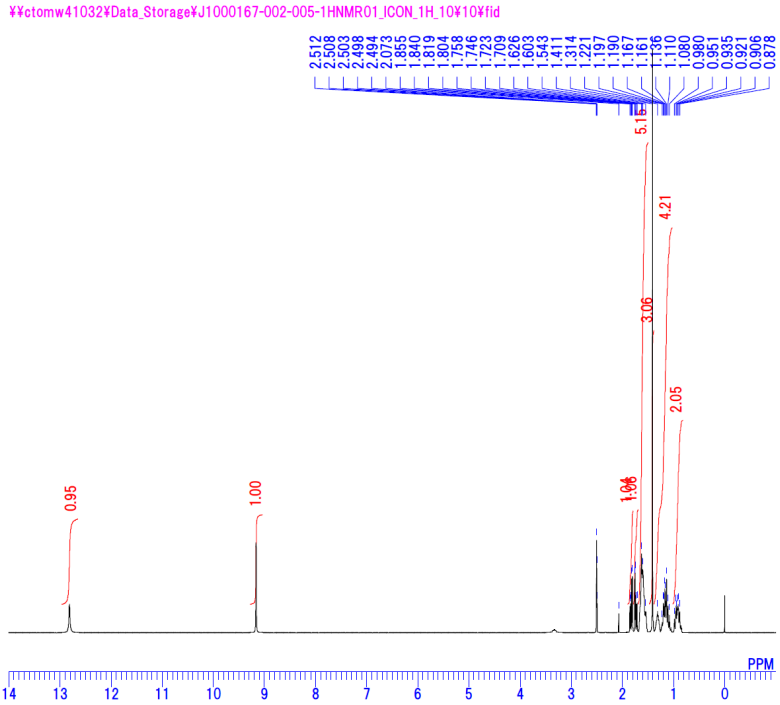
OBFIN	1.43 Hz
POINT	262144
FREQU	75000.00 Hz
SCANS	128
ACQTM	3.4953 sec
PD	3.0000 sec
PW1	18.00 usec
IRNUC	
CTEMP	26.9 c
SLVNT	DMSO
EXREF	-30.40 ppm
BF	0.30 Hz
RGAIN	195



Tfa-(Me)Cha-OH (S3)



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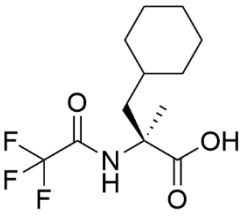
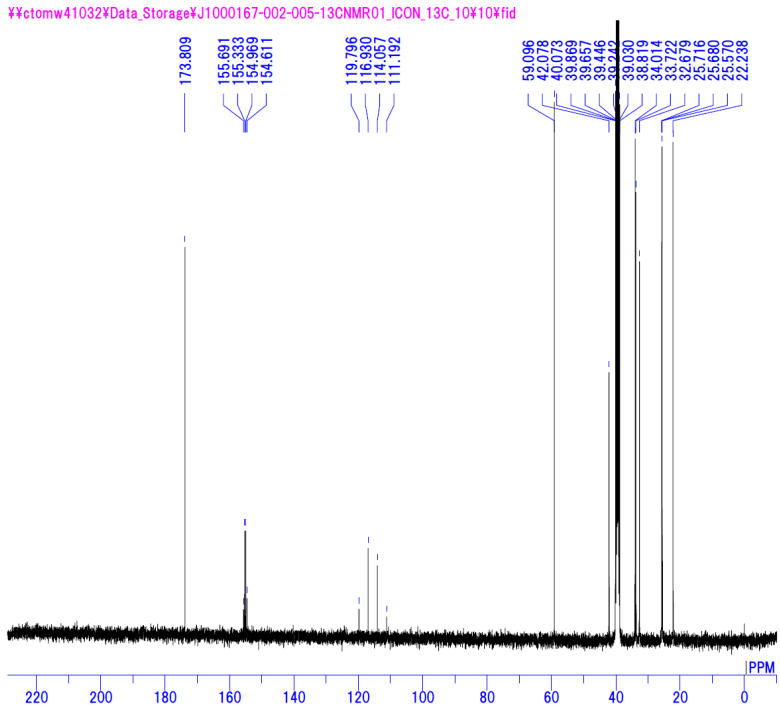
EXMOD zg30
OBFRQ 400.13 MHz
OBSET 3.20 KHz
OBFIN 1.04 Hz
POINT 32768
FREQU 8802.82 Hz
SCANS 8
ACQTM 3.7224 sec
PD 3.4000 sec
PW1 10.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 76





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EXMOD zgpg30
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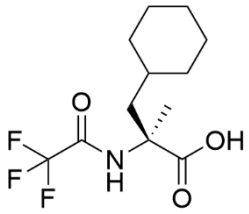
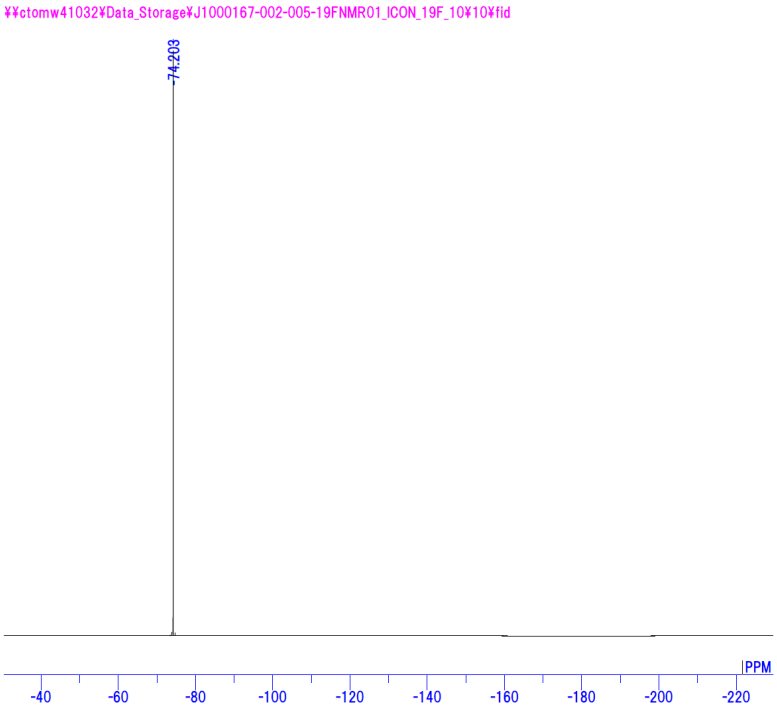
OBFIN 5.93 Hz
POINT 32768
FREQU 24038.46 Hz
SCANS 1024
ACQTM 1.3631 sec
PD 2.5000 sec
PW1 10.00 usec
IRNUC
CTEMP 26.9 c
SLVNT DMSO
EXREF 0.00 ppm
BF 1.00 Hz
RGAIN 195





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EXMOD zg
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OBSET 9.42 KHz
OBFIN 1.43 Hz
POINT 262144
FREQU 75000.00 Hz
SCANS 128
ACQTM 3.4953 sec
PD 3.0000 sec
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IRNUC
CTEMP 26.8 c
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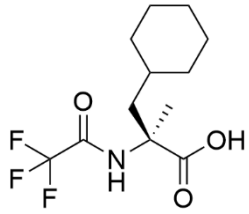
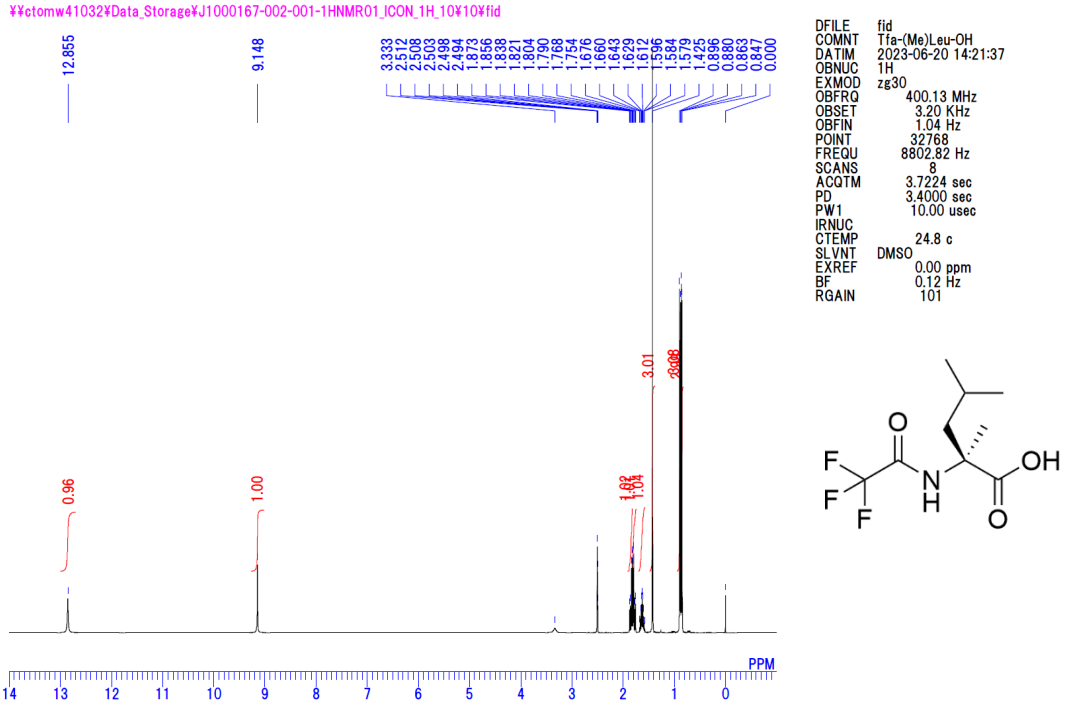
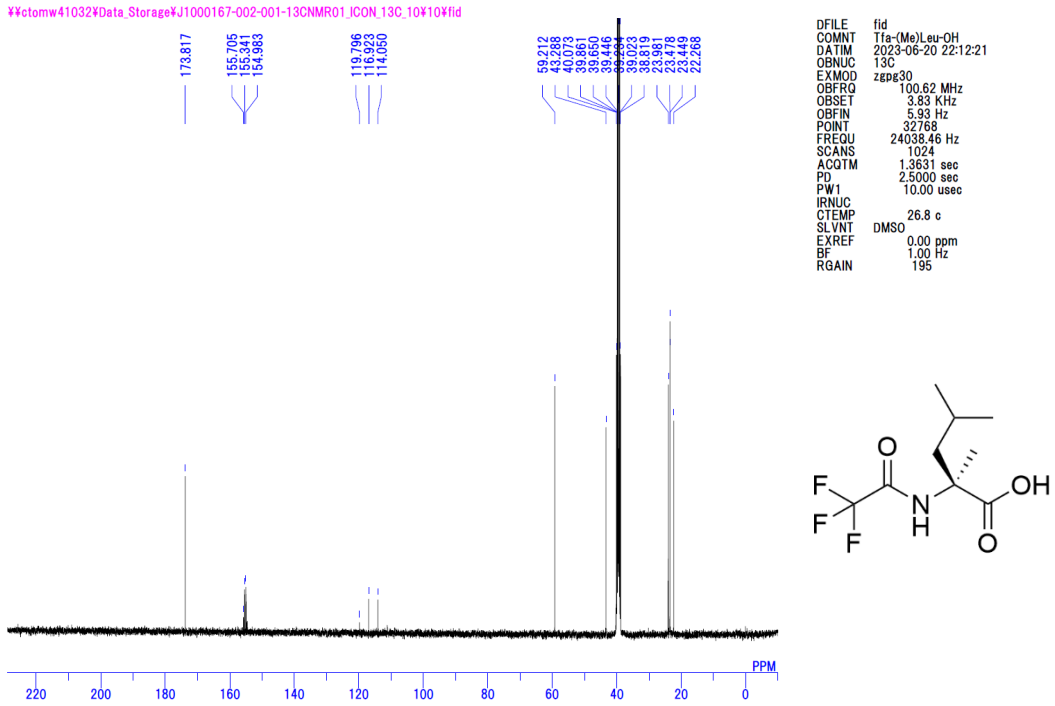
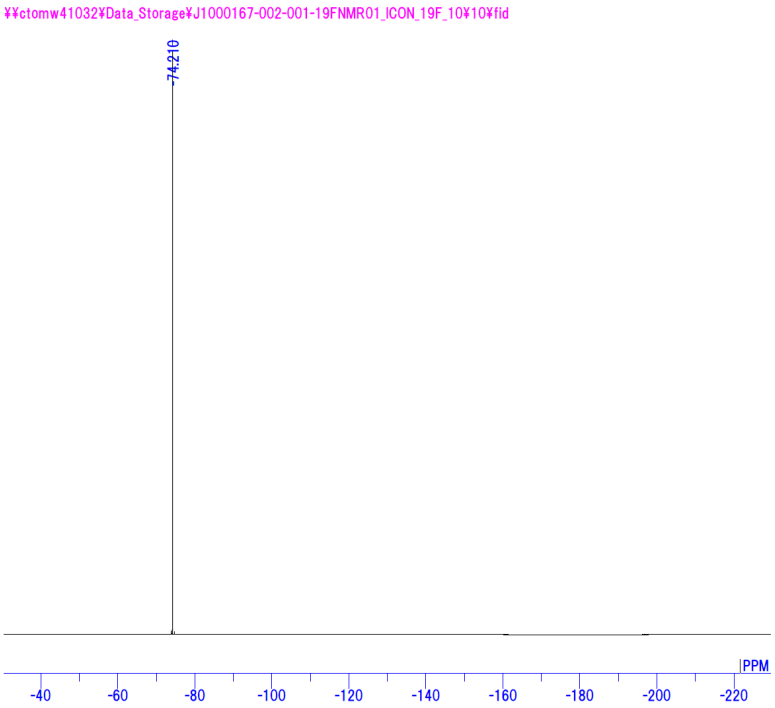


Tfa-(Me)Leu-OH (S4)







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FREQU 75000.00 Hz
SCANS 128
ACQTM 3.4953 sec
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CTEMP 26.9 c
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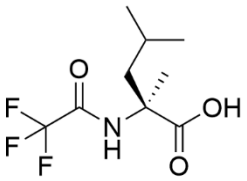
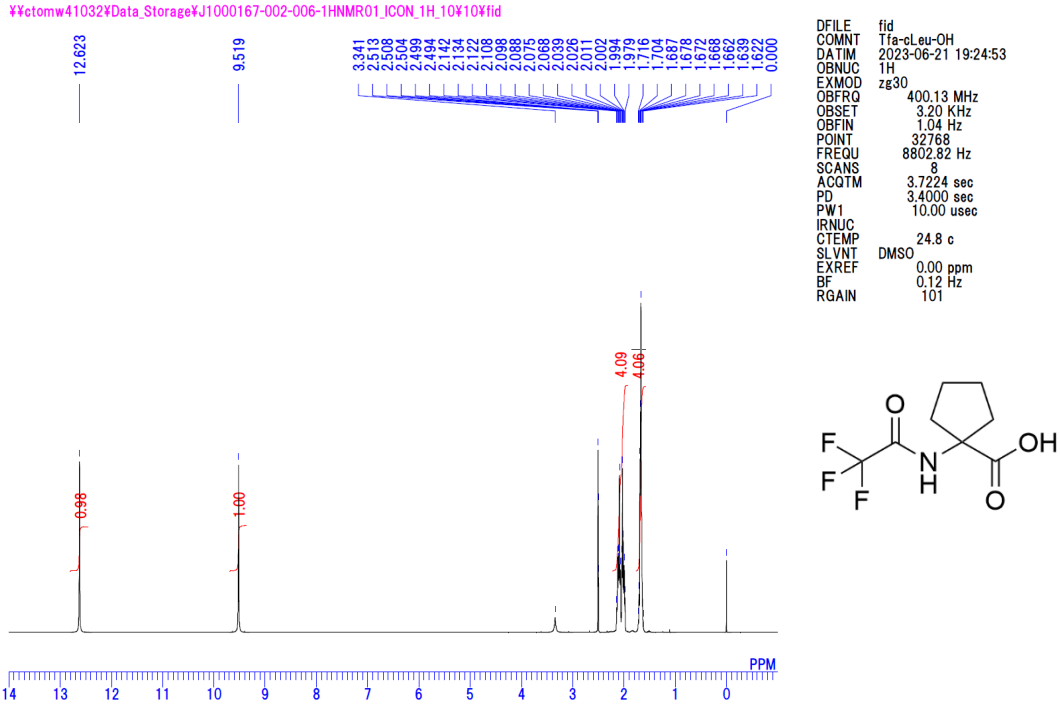
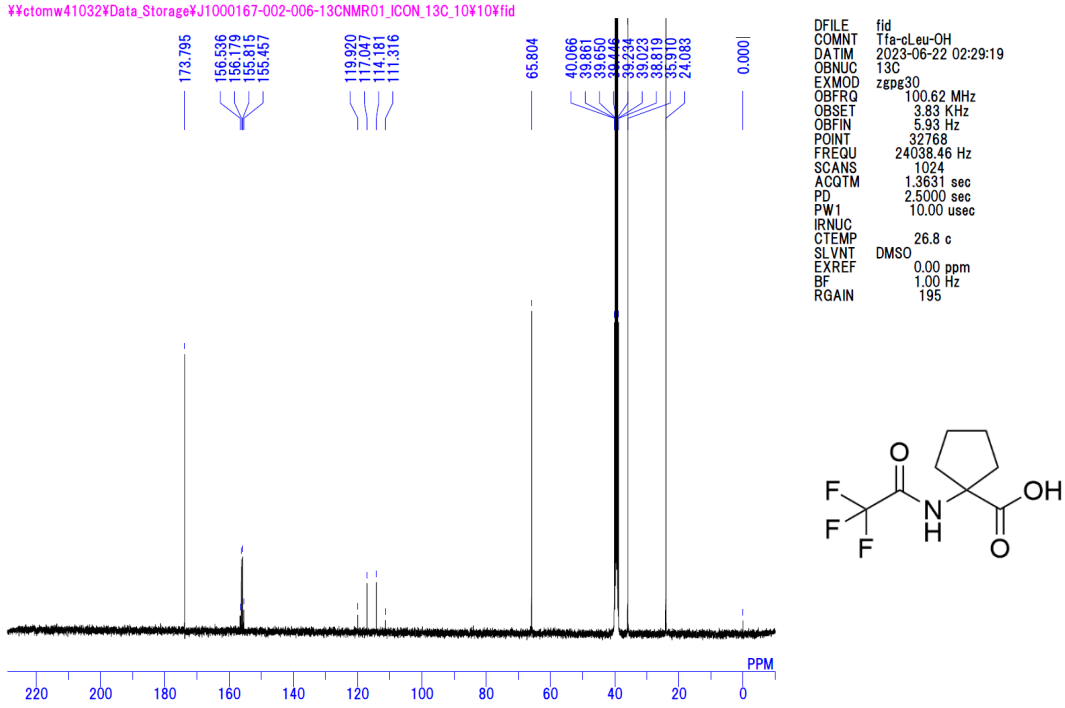
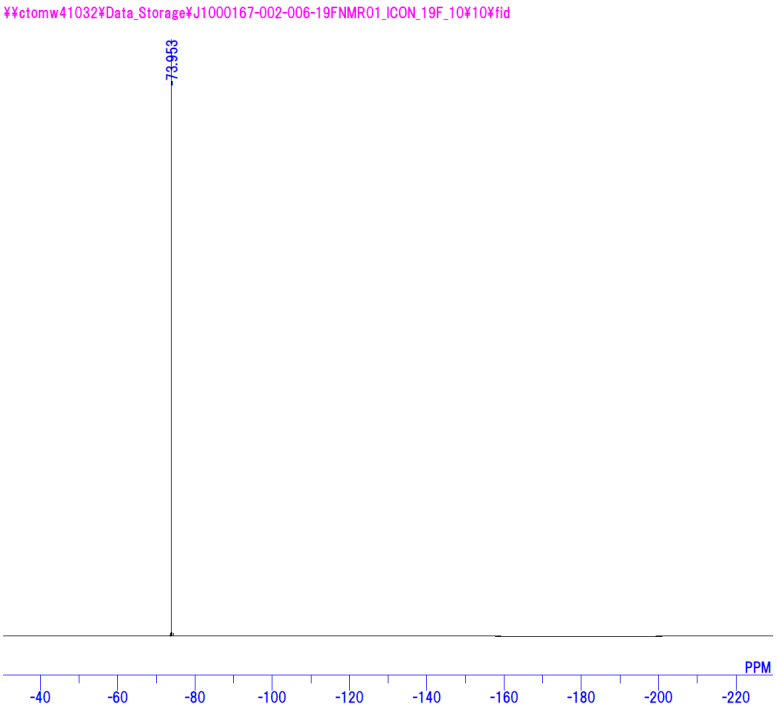
RGAIN 195



Tfa-cLeu-OH (S5)







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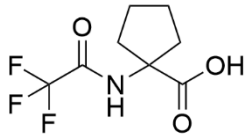
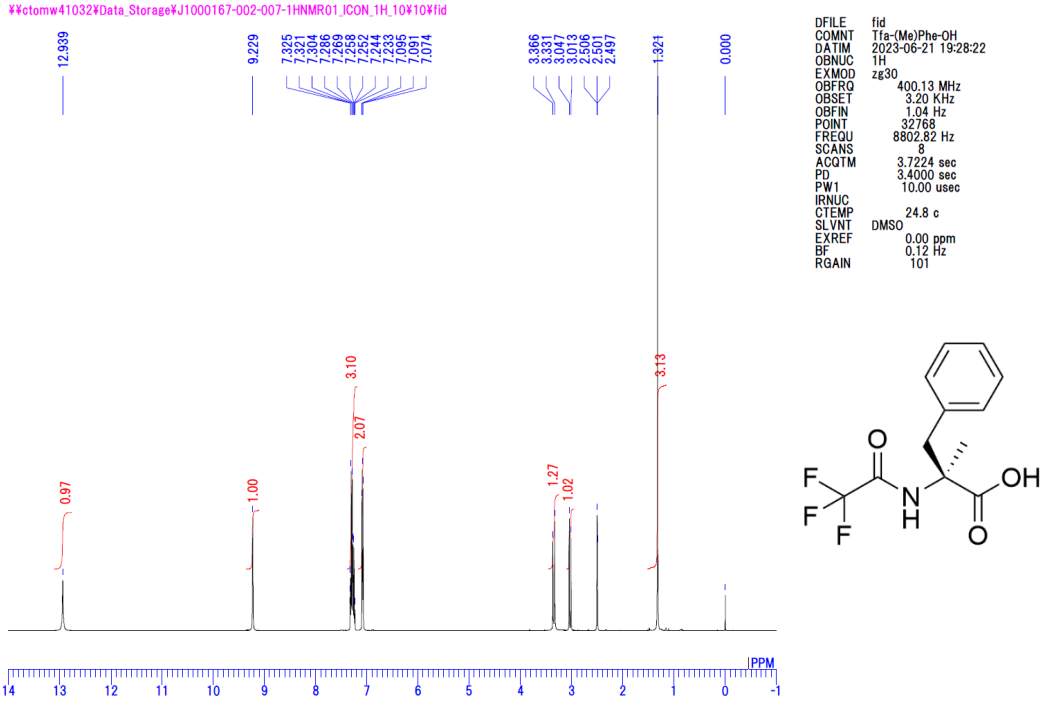
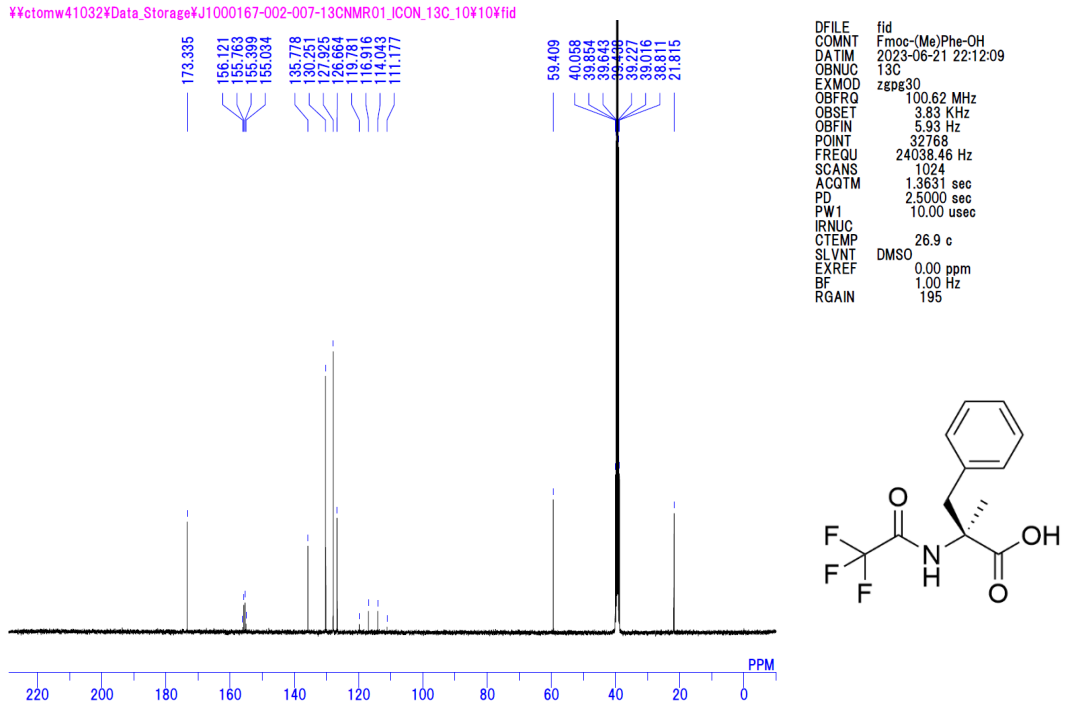
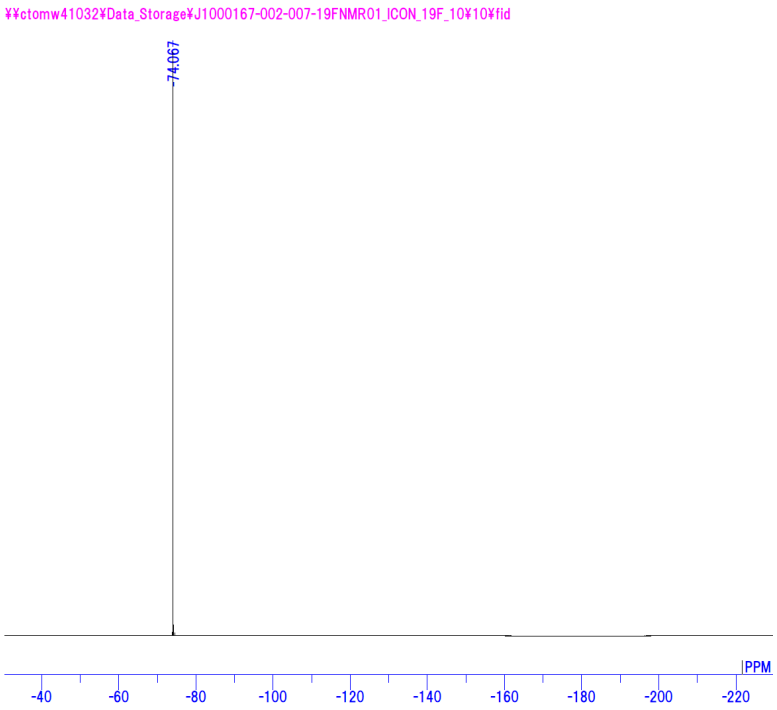
SCANS 128
ACQTM 3.4953 sec
PD 3.0000 sec
PW1 18.00 usec
IRNUC
CTEMP 26.9 c
SLVNT DMSO
EXREF -30.40 ppm
BF 0.30 Hz
RGAIN 195



Tfa-(Me)Phe-OH (S6)







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SCANS 128
ACQTM 3.4953 sec
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IRNUC
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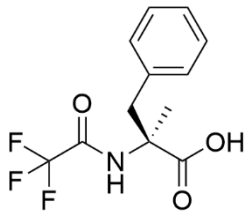
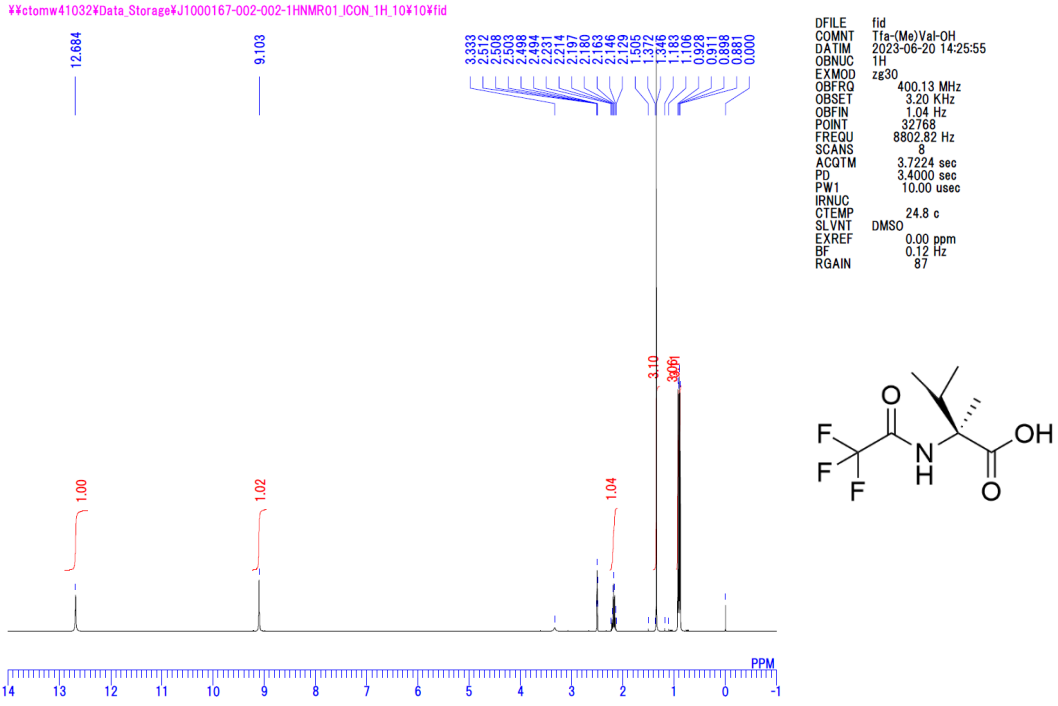
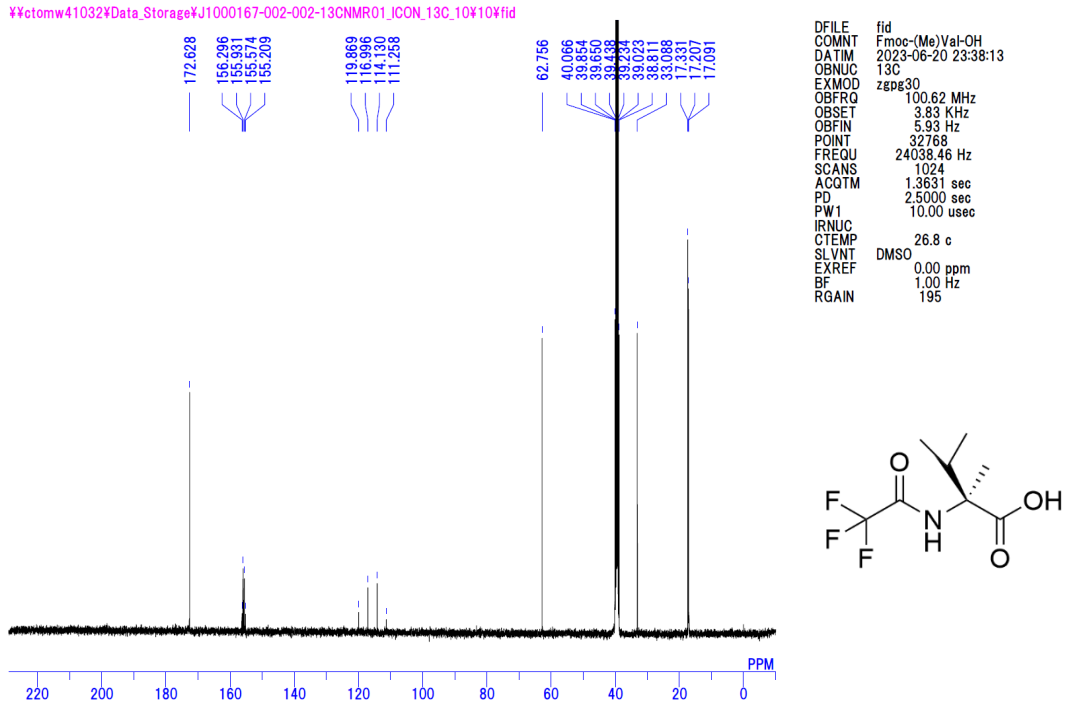
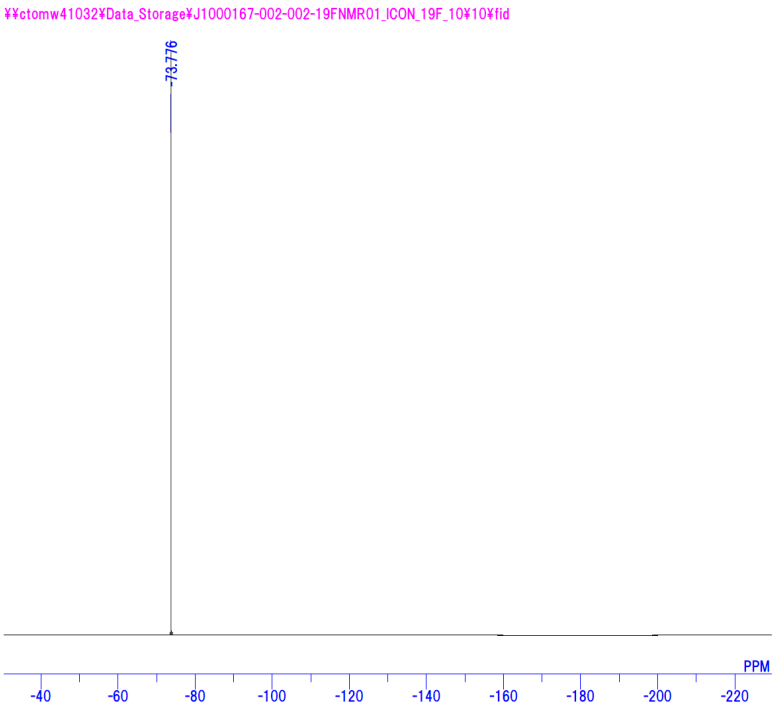
BF 0.30 Hz
RGAIN 195



Tfa-(Me)Val-OH (S7)







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ACQTM	3.4953 sec
PD	3.0000 sec
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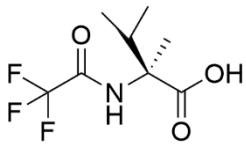
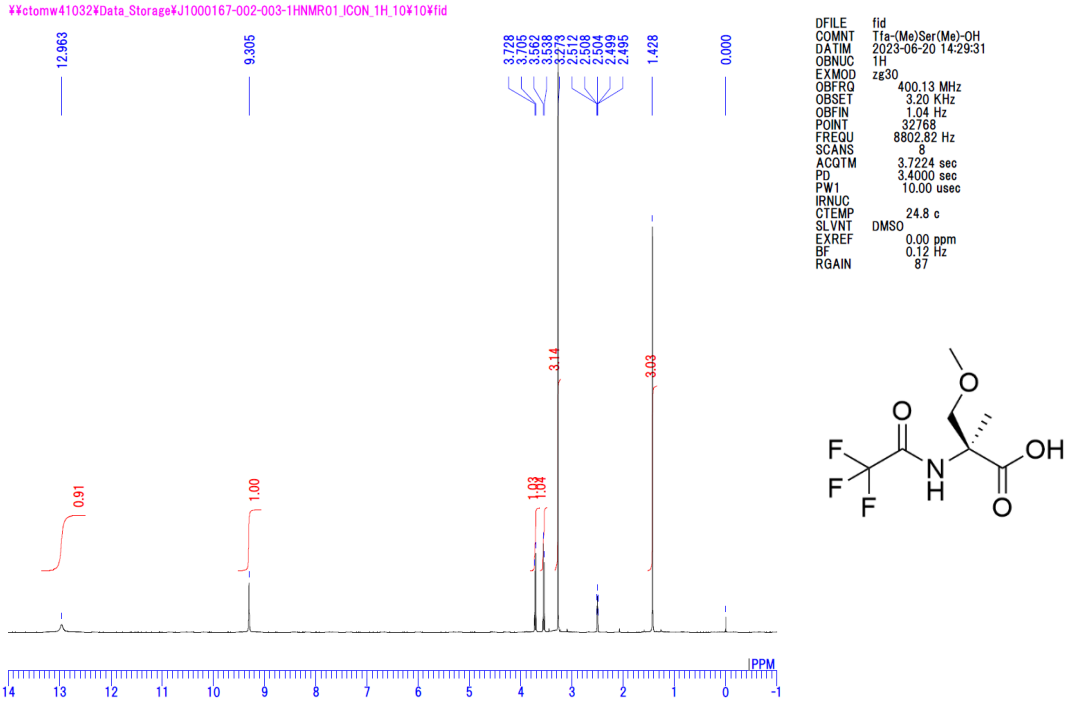
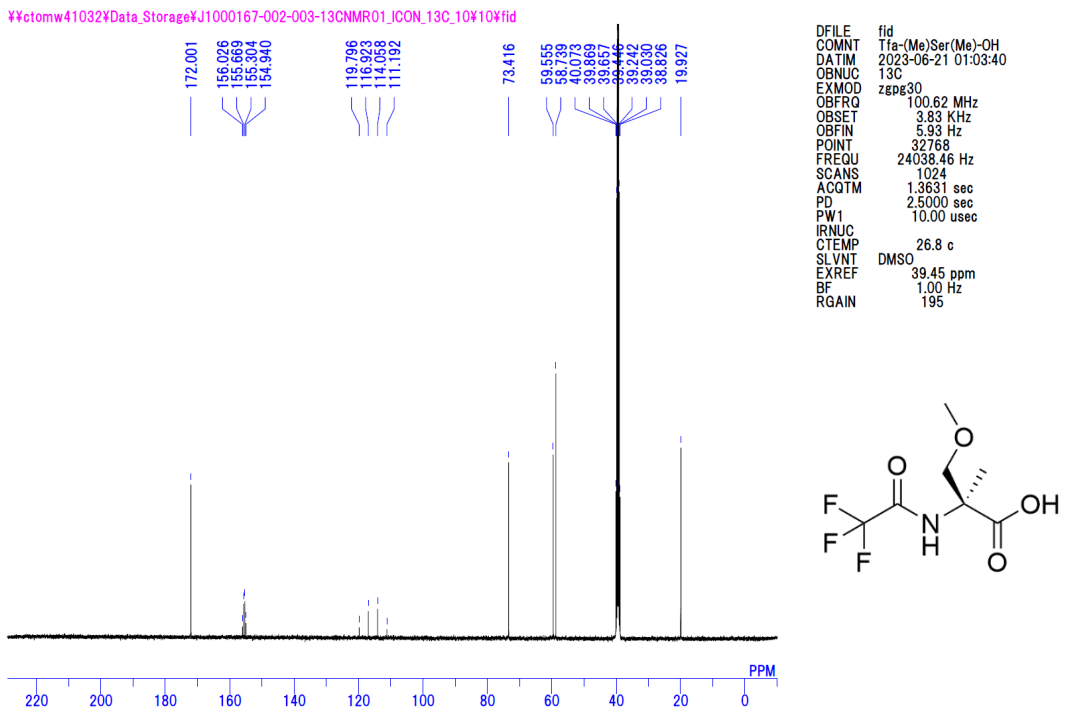
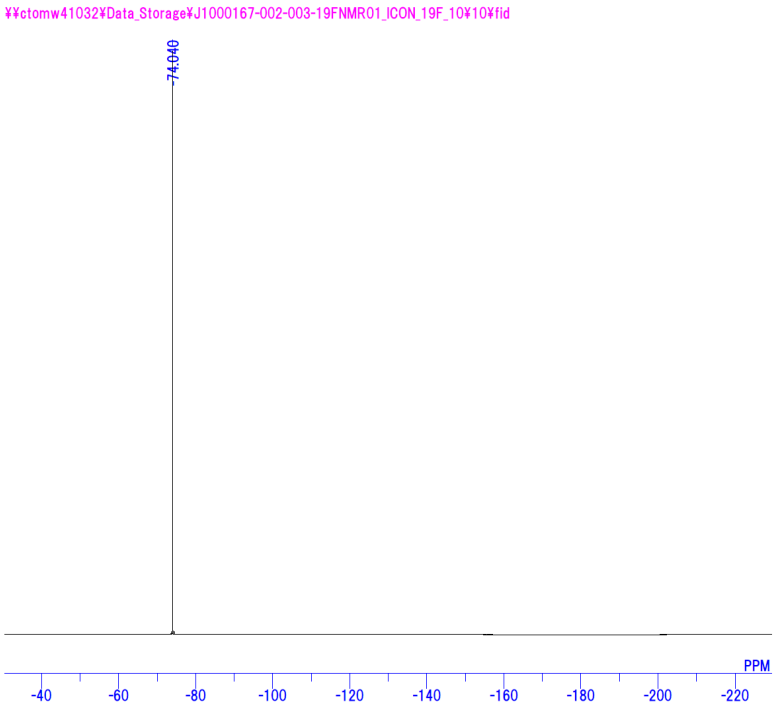
RGAIN	195



Tfa-(Me)Ser(Me)-OH (S8)







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OBFIN	1.43 Hz
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SCANS	128
ACQTM	3.4953 sec
PD	3.0000 sec
PW1	18.00 usec
IRNUC	
CTEMP	26.9 c
SLVNT	DMSO
EXREF	-30.40 ppm
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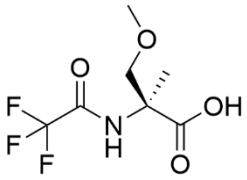
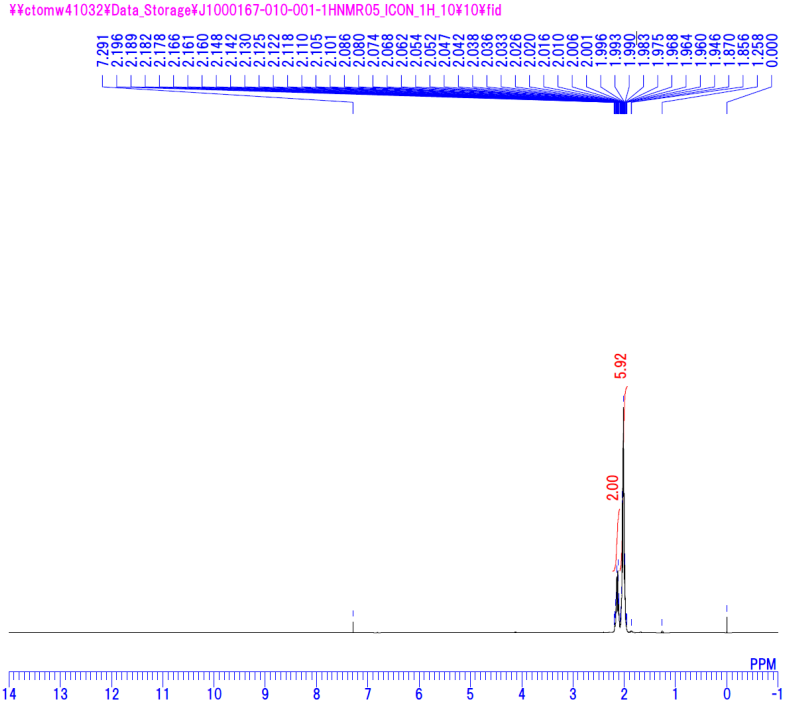
RGAIN	195



Oxazolone of Tfa-cLeu-OH (S9)



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EXMOD zg30
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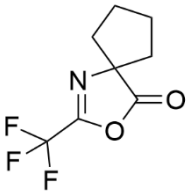
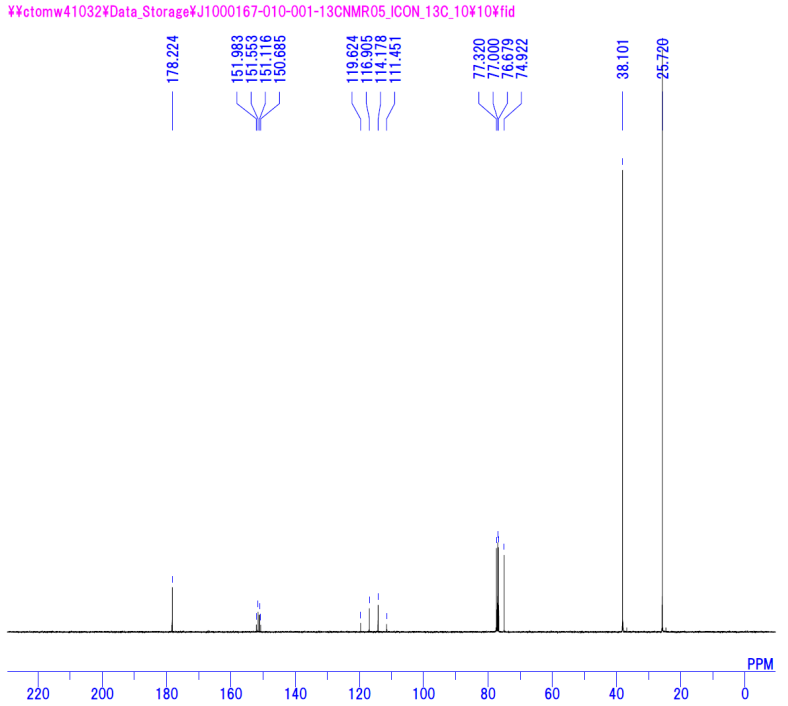
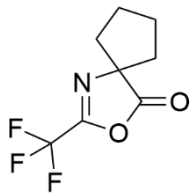
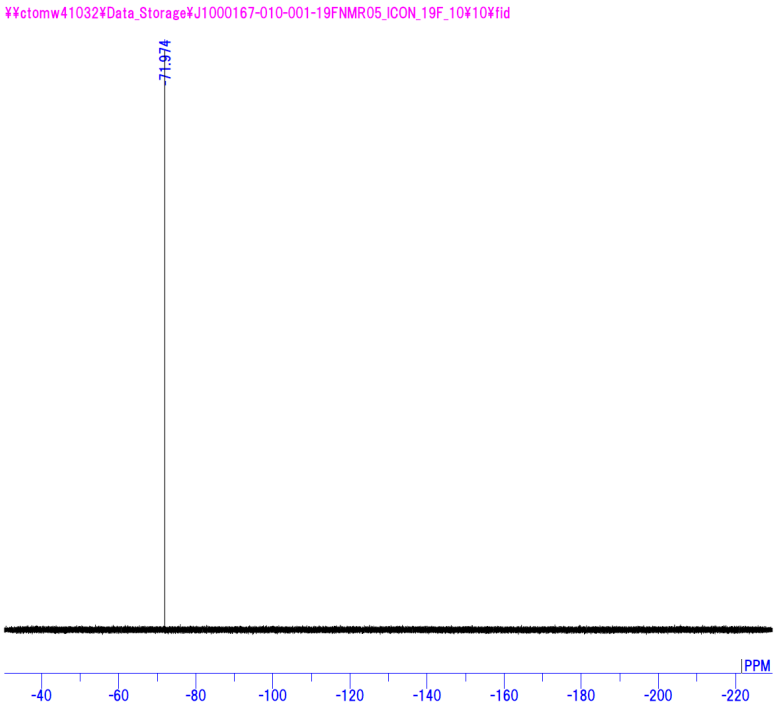
OBSET 3.20 KHz
OBFIN 1.04 Hz
POINT 32768
FREQU 8802.82 Hz
SCANS 8
ACQTM 3.7224 sec
PD 3.4000 sec
PW1 10.00 usec
IRNUC
CTEMP 24.8 c
SLVNT CDCl3
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 31



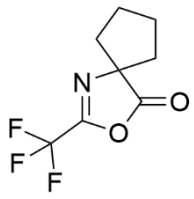


DFILE fid
COMNT oxazolone of Tfa-cLeu-OH
DATIM 2024-07-03 19:05:01
OBNUC 13C
EXMOD zgpg30
OBFRQ 100.62 MHz
OBSET 3.83 KHz
OBFIN 5.93 Hz
POINT 32768
FREQU 24038.46 Hz
SCANS 256
ACQTM 1.3631 sec
PD 2.5000 sec
PW1 10.00 usec
IRNUC
CTEMP 26.8 c
SLVNT CDCl3
EXREF 229.46 ppm
BF 1.00 Hz
RGAIN 195

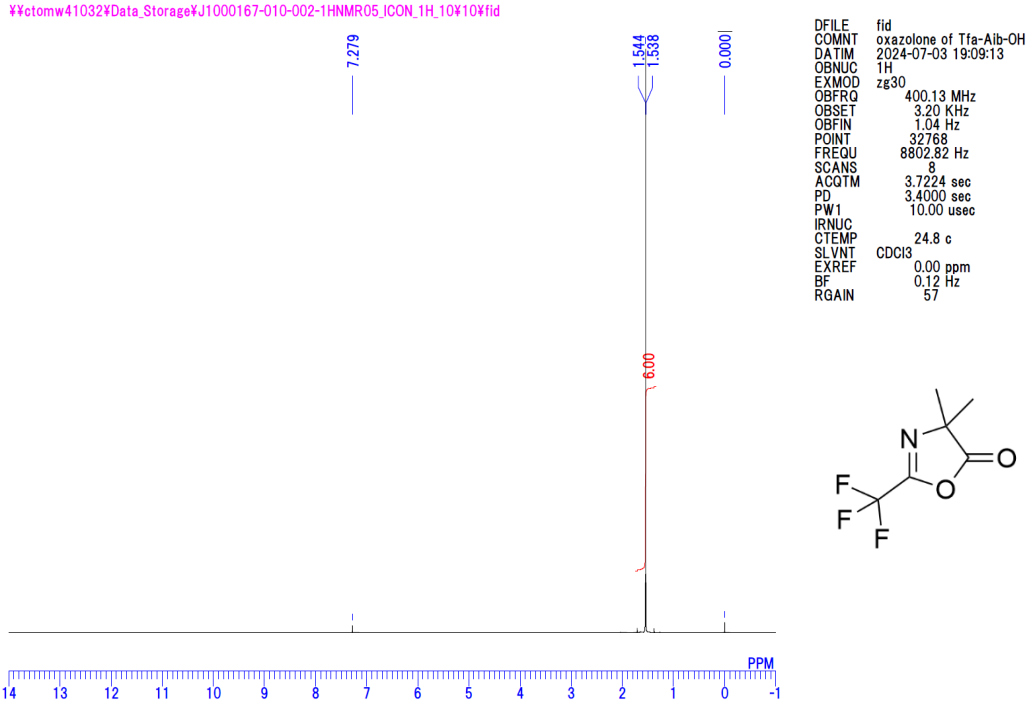


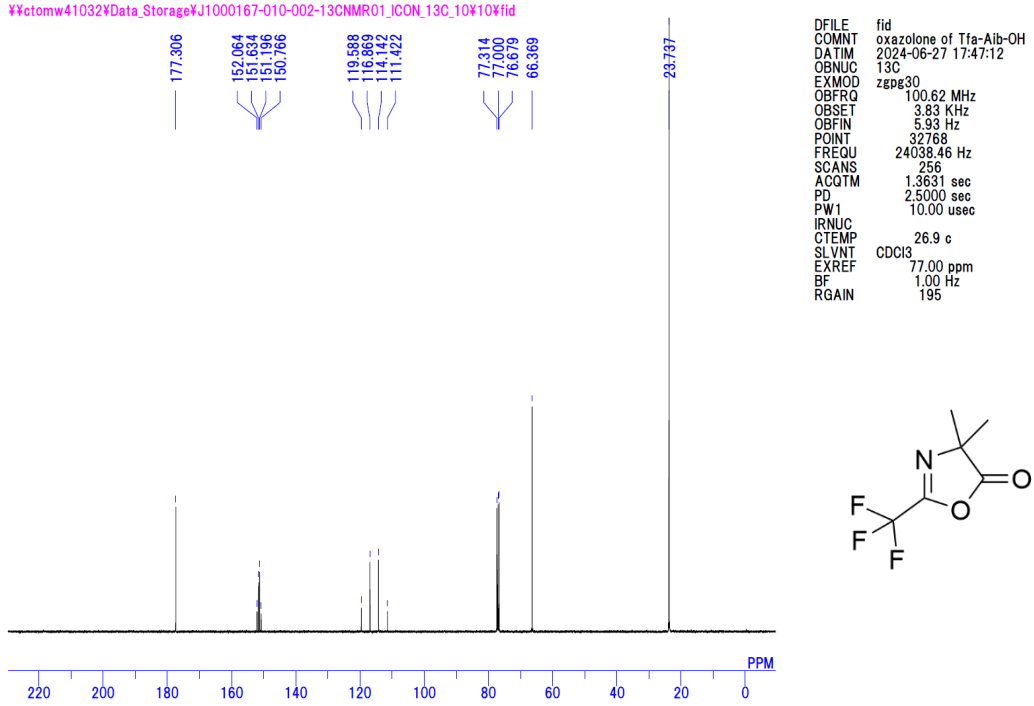


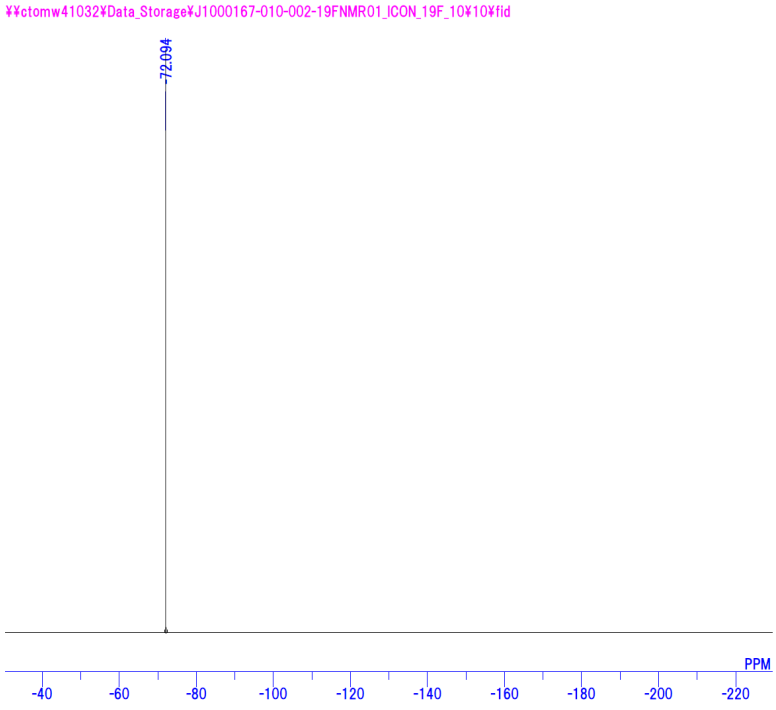
DFILE	fid
COMNT	oxazolone of Tfa-cLeu-OH
DATIM	2024-07-03 18:46:47
OBNUC	19F
EXMOD	zg
OBFRQ	376.44 MHz
OBSET	9.42 KHz
OBFIN	1.43 Hz
POINT	262144
FREQU	75000.00 Hz
SCANS	64
ACQTM	3.4953 sec
PD	3.0000 sec
PW1	18.00 usec
IRNUC	
CTEMP	26.9 c
SLVNT	CDCl3
EXREF	-30.40 ppm
BF	0.30 Hz
RGAIN	195



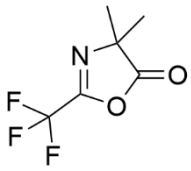
Oxazolone of Tfa-Aib-OH (S10)



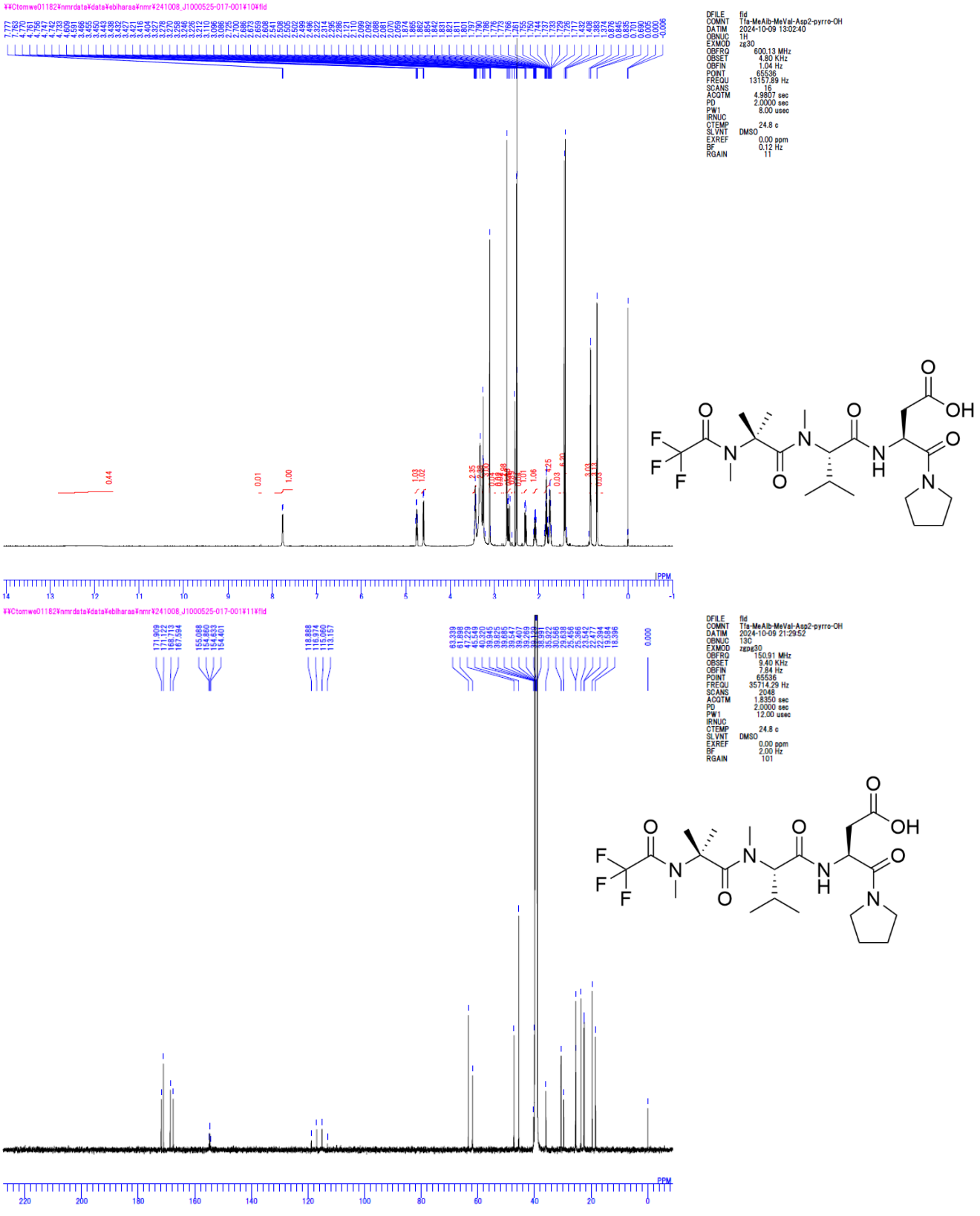


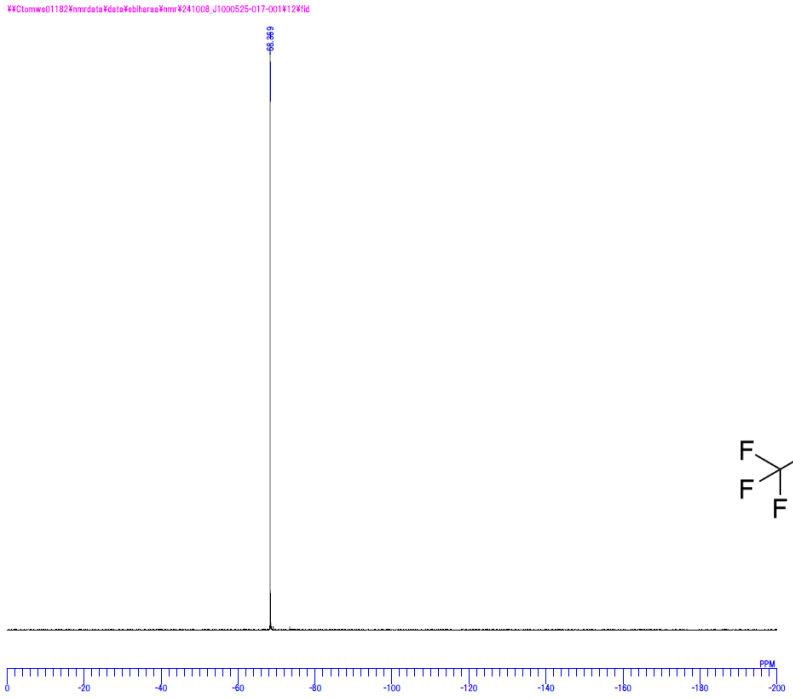


DFILE	fid
COMNT	oxazolone of Tfa-Aib-OH
DATIM	2024-07-03 19:19:46
OBNUC	19F
EXMOD	zg
OBFRQ	376.44 MHz
OBSET	9.42 KHz
OBFIN	1.43 Hz
POINT	262144
FREQU	75000.00 Hz
SCANS	64
ACQTM	3.4953 sec
PD	3.0000 sec
PW1	18.00 usec
IRNUC	
CTEMP	26.9 c
SLVNT	CDCl3
EXREF	-30.40 ppm
BF	0.30 Hz
RGAIN	125

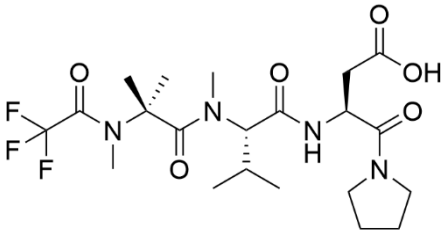


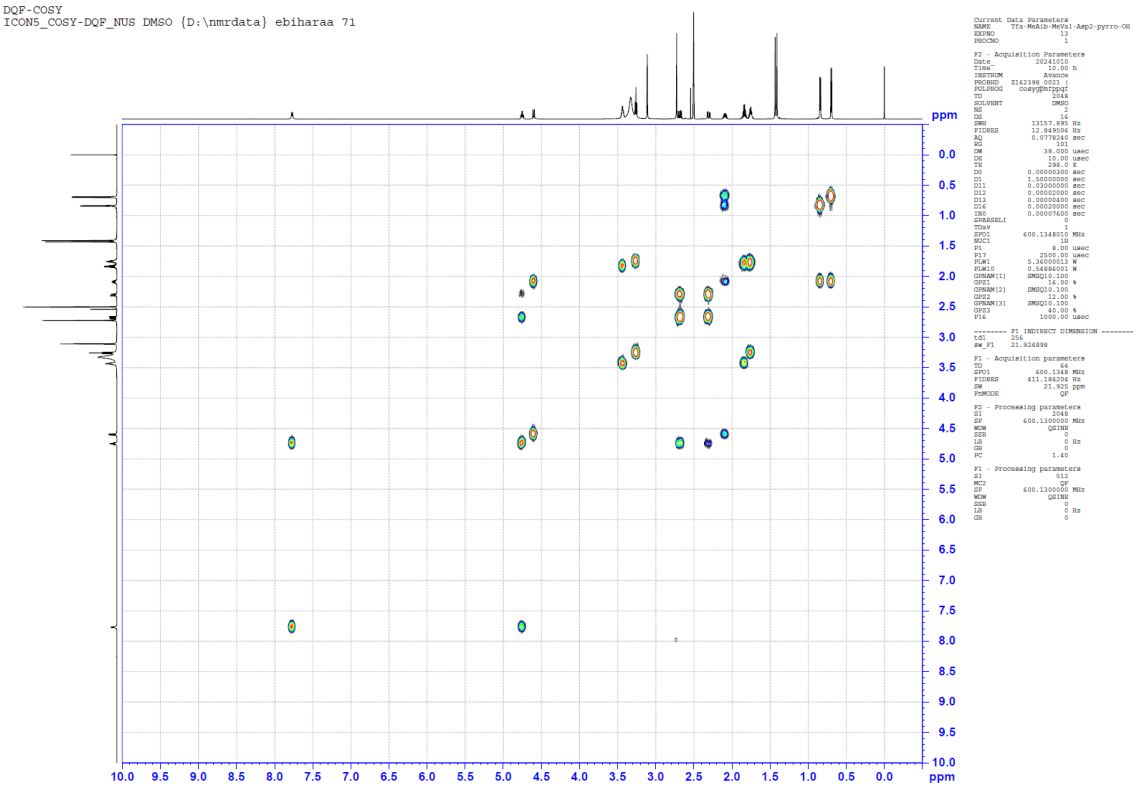
Tfa-MeAib-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11aa)





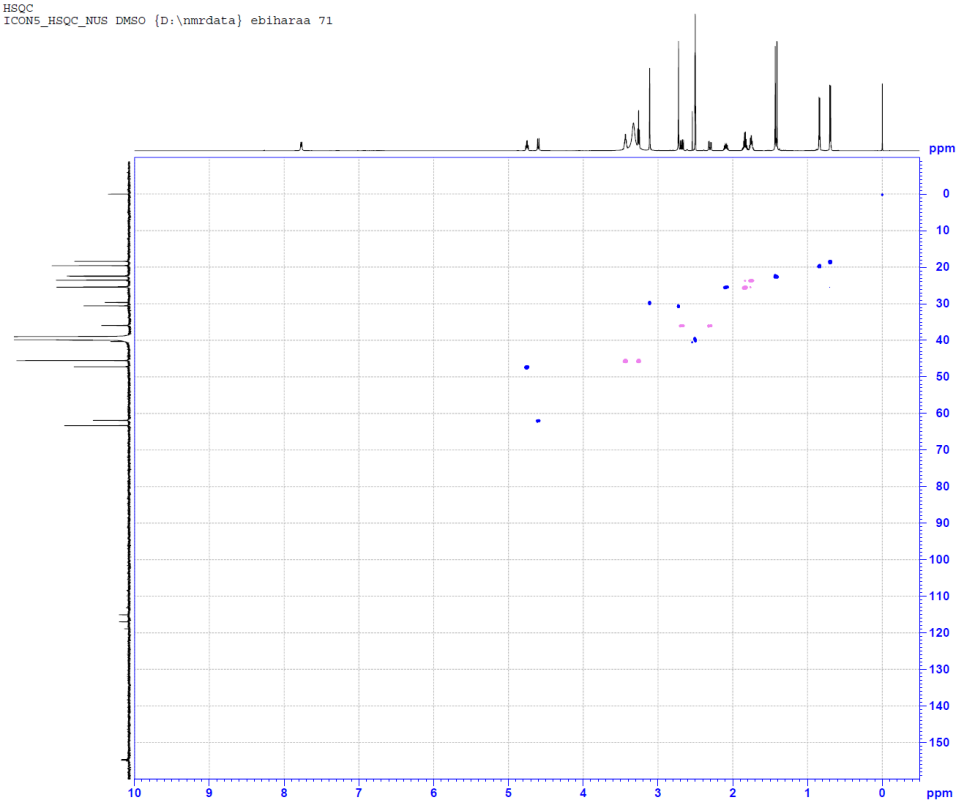

```
DFILE     fid
COMNT     Tfa-MeAib-MeVal-Asp-pyrro
DATIM     2024-10-09 13:06:32
OBNUC     19F
EXMOD     zg
OBFRQ       564.62 MHz
OBSET         9.91 KHz
OBFIN         9.36 Hz
POINT       131072
FREQU    131578.95 Hz
SCANS           16
ACQTM       0.9961 sec
PD          5.0000 sec
PW1           11.00 usec
IRNUC
CTEMP         24.8 c
SLVNT       DMSO
EXREF        16.51 ppm
BF            1.00 Hz
RGAIN          101
```





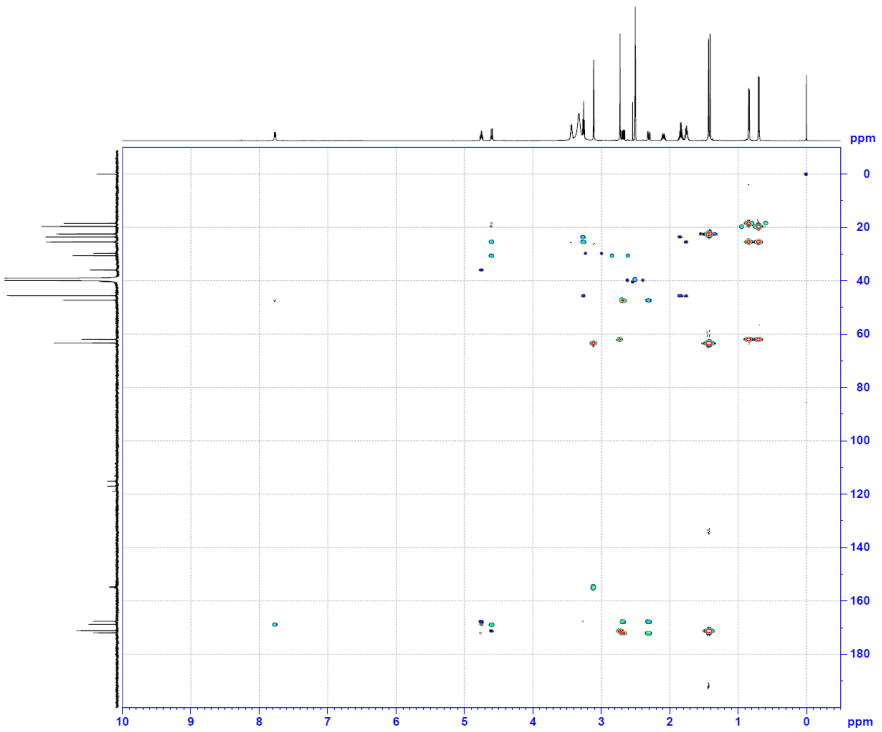

```
Current Data Parameters
NAME      Tfa-MeAib-MeVal-Asp2-pyrro-OH
EXPNO              13
PROCNO              1

F2 - Acquisition Parameters
Date_        20241010
Time              10.00 h
INSTRUM          Avance
PROBHD    Z142398 0021 (
PULPROG    coasygpmfppqf
TD                 2048
SOLVENT            DMSO
NS                    2
DS                   16
SWH           13157.895 Hz
FIDRES        12.849506 Hz
AQ             0.0778240 sec
RG                  101
DW                38.000 usec
DE                10.00 usec
TE                298.0 K
D0             0.00000300 sec
D1             1.50000000 sec
D11            0.03000000 sec
D12            0.00002000 sec
D13            0.00000400 sec
D16            0.00020000 sec
IN0            0.00007600 sec
SPARSELI             no
TDAV                  1
SFO1          600.1348010 MHz
NUC1                1H
P1                 8.00 usec
P17             2500.00 usec
PLW1          5.36000013 W
PLW10         0.54886001 W
GPNAM[1]     SMSQ10.100
GPZ1              16.00 %
GPNAM[2]     SMSQ10.100
GPZ2              12.00 %
GPNAM[3]     SMSQ10.100
GPZ3              40.00 %
P16             1000.00 usec

-------- F1 INDIRECT DIMENSION --------
td1               256
sw_F1       21.926898

F1 - Acquisition parameters
TD                 64
SFO1          600.1348 MHz
FIDRES        411.184204 Hz
SW             21.925 ppm
FnMODE              QF

F2 - Processing parameters
SI                 2048
SF          600.1300000 MHz
WDW                QSINE
SSB                   0
LB                  0 Hz
GB                    0
PC                 1.40

F1 - Processing parameters
SI                  512
MC2                  QF
SF          600.1300000 MHz
WDW                QSINE
SSB                   0
LB                  0 Hz
GB                    0
```

HSQC
ICON5_HSQC_NUS DMSO {D:\nmrdata} ebiharaa 71



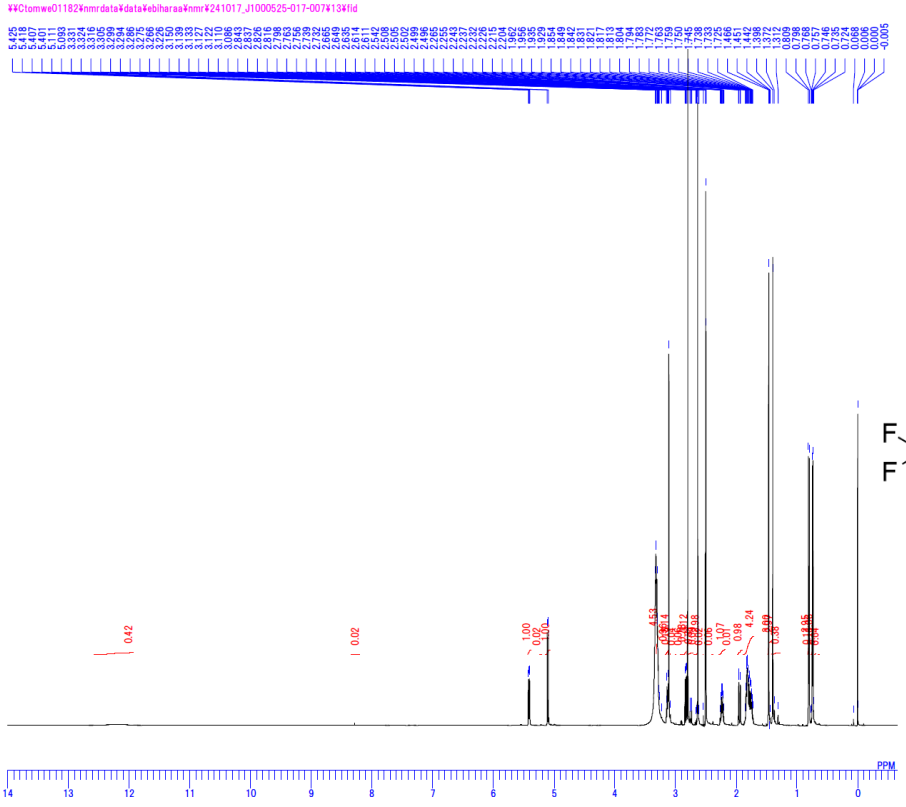

```
Current Data Parameters
NAME      241008_J1000525-017-001
EXPNO             14
PROCNO             1

F2 - Acquisition Parameters
Date_        20241010
Time              10.09 h
INSTRUM         Avance
PROBHD    Z142398_0021
PULPROG   hsqcedetgpsisp2.3
TD                2048
SOLVENT           DMSO
NS                   2
DS                  16
SWH           8196.721 Hz
FIDRES        8.004411 Hz
AQ            0.1249280 sec
RG                 101
DW              61.000 usec
DE              10.00 usec
TE              298.0 K
CNST2       145.0000000
CNST17       -0.5000000
D0           0.00000300 sec
D1           1.50000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D21          0.00360000 sec
D24          0.00089000 sec
IN0          0.00001841 sec
SPARSELI             0
TDav                 1
SFO1        600.1330006 MHz
NUC1               1H
P1                8.00 usec
P2               16.00 usec
PLW1         5.36000013 W
SFO2       150.9148808 MHz
NUC2              13C
CPDPRG[2 bi_p5m4sp_4sp.2
P3               12.50 usec
P14             500.00 usec
P24            2000.00 usec
P31            1720.00 usec
PH3            1500.00 usec
PLW0                 0 W
PLW2       107.09999847 W
PLW12        5.09829998 W
SPNAM[3] Crp40,0.5,20.1
SPOAL3             0.500
SPOFFS3              0 Hz
SPW3        23.56399918 W
SPNAM[7]  Crp60comp.4
SPOAL7             0.500
SPOFFS7              0 Hz
SPW7        23.56399918 W
SPNAM[14 Crp42,0.75,20.2
SPOAL14            0.500
SPOFFS14             0 Hz
SPW14        6.59779978 W
SPNAM[18  Crp60_xfil5.2
SPOAL18            0.500
SPOFFS18             0 Hz
SPW18        6.81029987 W
SPNAM[31 Crp42,1.5,20.2
SPOAL31            0.500
SPOFFS31             0 Hz
SPW31        3.29889989 W
GPNAM[1]       SMSQ10.50
GPZ1                80.00 %
GPNAM[2]       SMSQ10.50
GPZ2                20.10 %
GPNAM[3]       SMSQ10.50
GPZ3                11.00 %
GPNAM[4]       SMSQ10.50
GPZ4                 5.00 %
P16              1000.00 usec
P19               600.00 usec

-------- F1 INDIRECT DIMENSION --------
td1          512
sw_F1    180.000000

F1 - Acquisition parameters
TD               128
SFO1       150.9149 MHz
FIDRES     424.448090 Hz
SW         180.000 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI              2048
SF       600.1300029 MHz
WDW             QSINE
SSB                 2
LB                0 Hz
GB                  0
PC               1.40

F1 - Processing parameters
SI               512
MC2      echo-antiecho
SF       150.9028870 MHz
WDW             QSINE
SSB                 2
LB                0 Hz
GB                  0
```

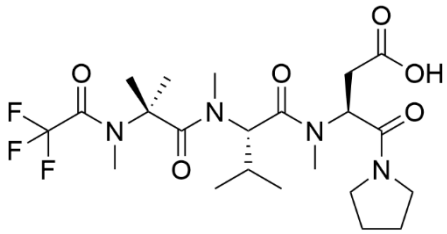
HMBC
ICON5_HMBC_NUS DMSO {D:\nmrdata} ebiharaa 71

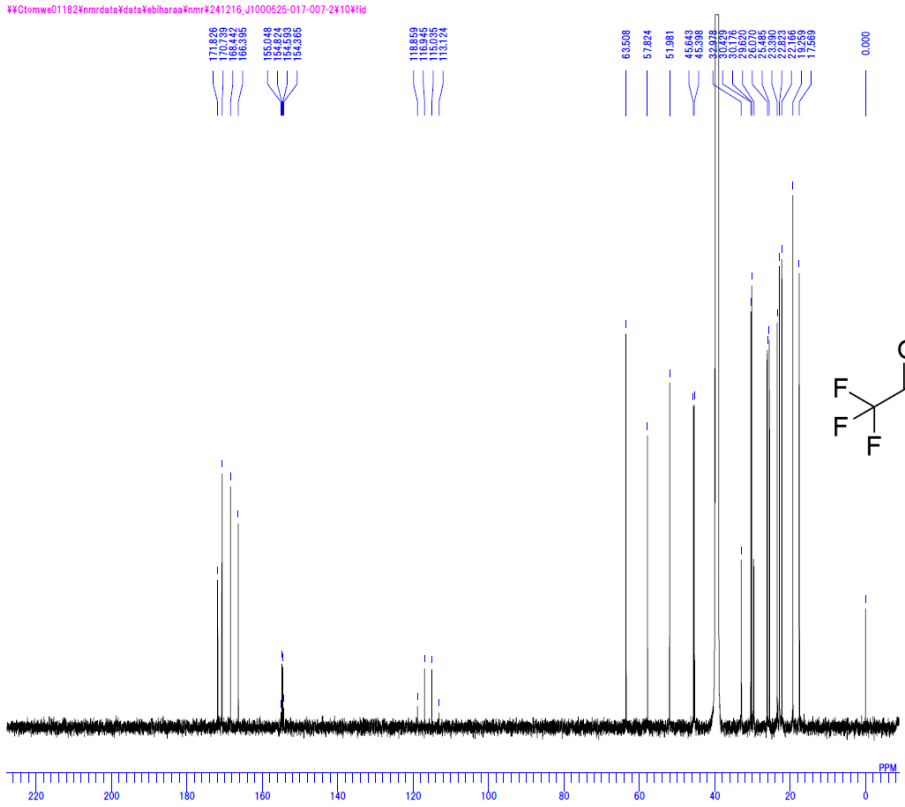


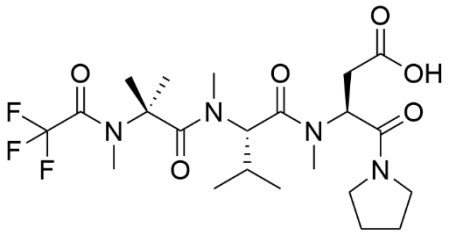

```
Current Data Parameters
NAME      Tfa-MeAib-MeVal-Asp2-pyrro-OH
EXPNO             15
PROCNO             1

F2 - Acquisition Parameters
Date_        20241010
Time              10.22 h
INSTRUM         Avance
PROBHD    Z162398_0021 (
PULPROG   hmbcgplpndqf
TD                2048
SOLVENT           DMSO
NS                   4
DS                  16
SWH          13157.895 Hz
FIDRES       12.849506 Hz
AQ            0.0778240 sec
RG                 101
DW              38.000 usec
DE              10.00 usec
TE              298.0 K
CNST2       145.0000000
CNST13        8.3000002
D0           0.00000300 sec
D1           1.10000002 sec
D2           0.00344828 sec
D6           0.06024096 sec
D16          0.00020000 sec
IN0          0.00001381 sec
SPARSELI             0
TDav                 1
SFO1        600.1348010 MHz
NUC1               1H
P1                7.21 usec
P2               14.42 usec
PLW1         5.36000013 W
SFO2       150.9194078 MHz
NUC2              13C
P3               12.00 usec
PLW2       107.09999847 W
GPNAM[1]       SINE.100
GPZ1                50.00 %
GPNAM[2]       SINE.100
GPZ2                30.00 %
GPNAM[3]       SINE.100
GPZ3                40.10 %
P16              1000.00 usec

======== F1 INDIRECT DIMENSION ========
td1          512
sw_F1    240.000000

F1 - Acquisition parameters
TD               128
SFO1       150.9194 MHz
FIDRES     565.947754 Hz
SW         240.000 ppm
FnMODE             QF

F2 - Processing parameters
SI              2048
SF       600.1300029 MHz
WDW             SINE
SSB                 0
LB                0 Hz
GB                  0
PC               1.40

F1 - Processing parameters
SI               512
MC2               QF
SF       150.9028870 MHz
WDW             SINE
SSB                 0
LB                0 Hz
GB                  0
```

Tfa-MeAib-MeVal-MeAsp-pyrro (<u>The peptide cleavaged from</u> 12)







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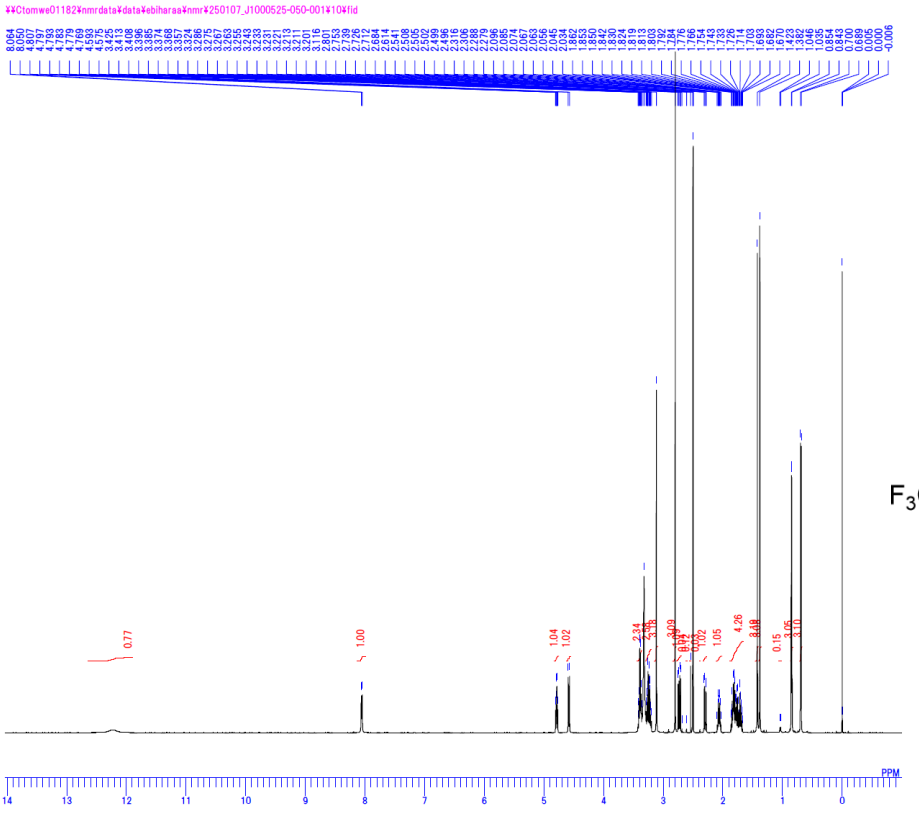
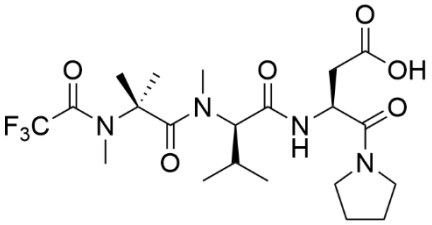
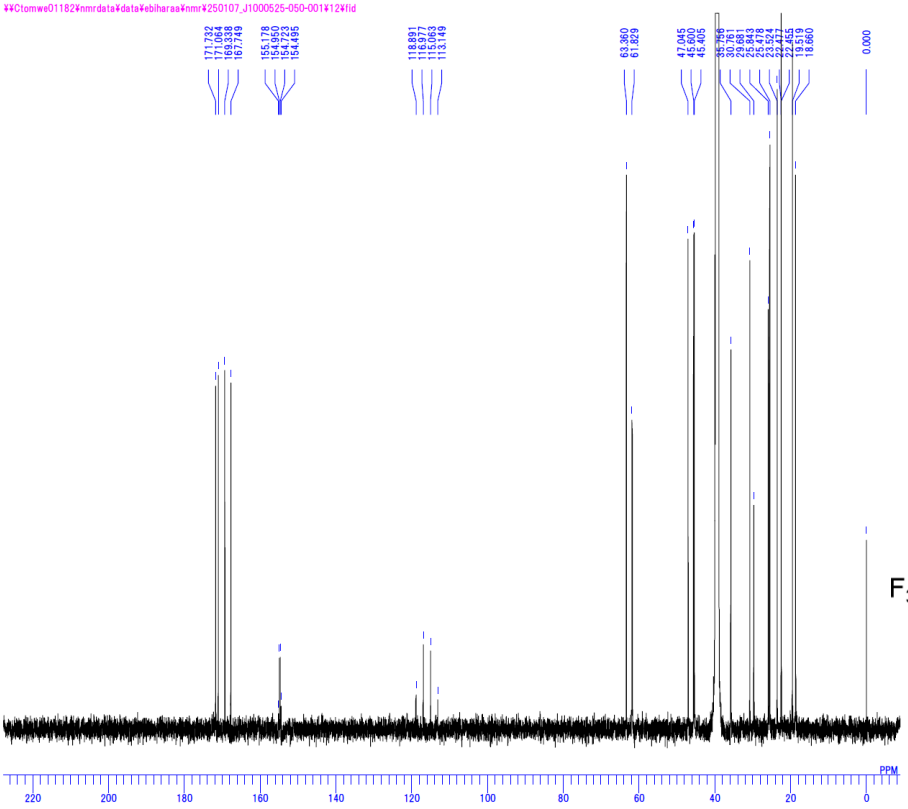
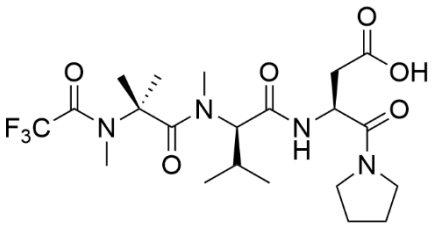
-66.413
DFILE fid
COMNT Tfa-MeAib-MeVal-MeAsp-pyrro
DATIM 2024-10-17 21:12:18
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
F
F
F
O
N
O
N
O
N
O
OH
O
N
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180

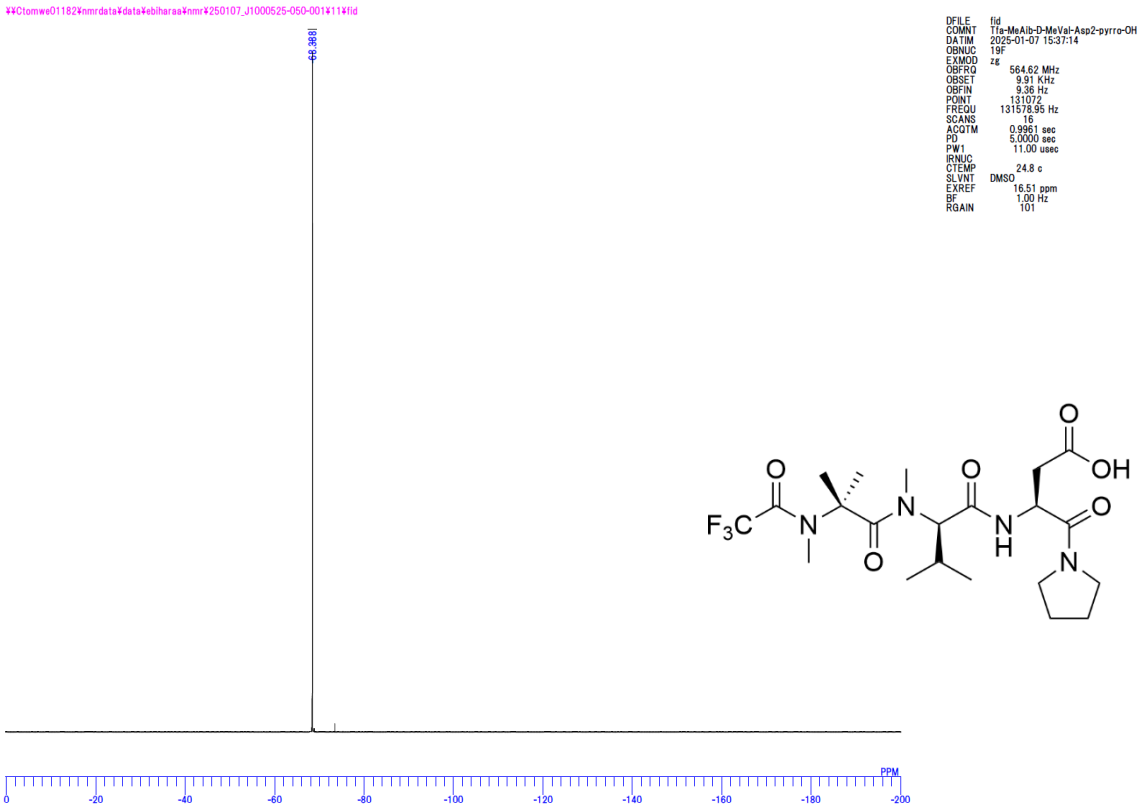
Tfa-MeAib-D-MeVal-Asp-pyrro



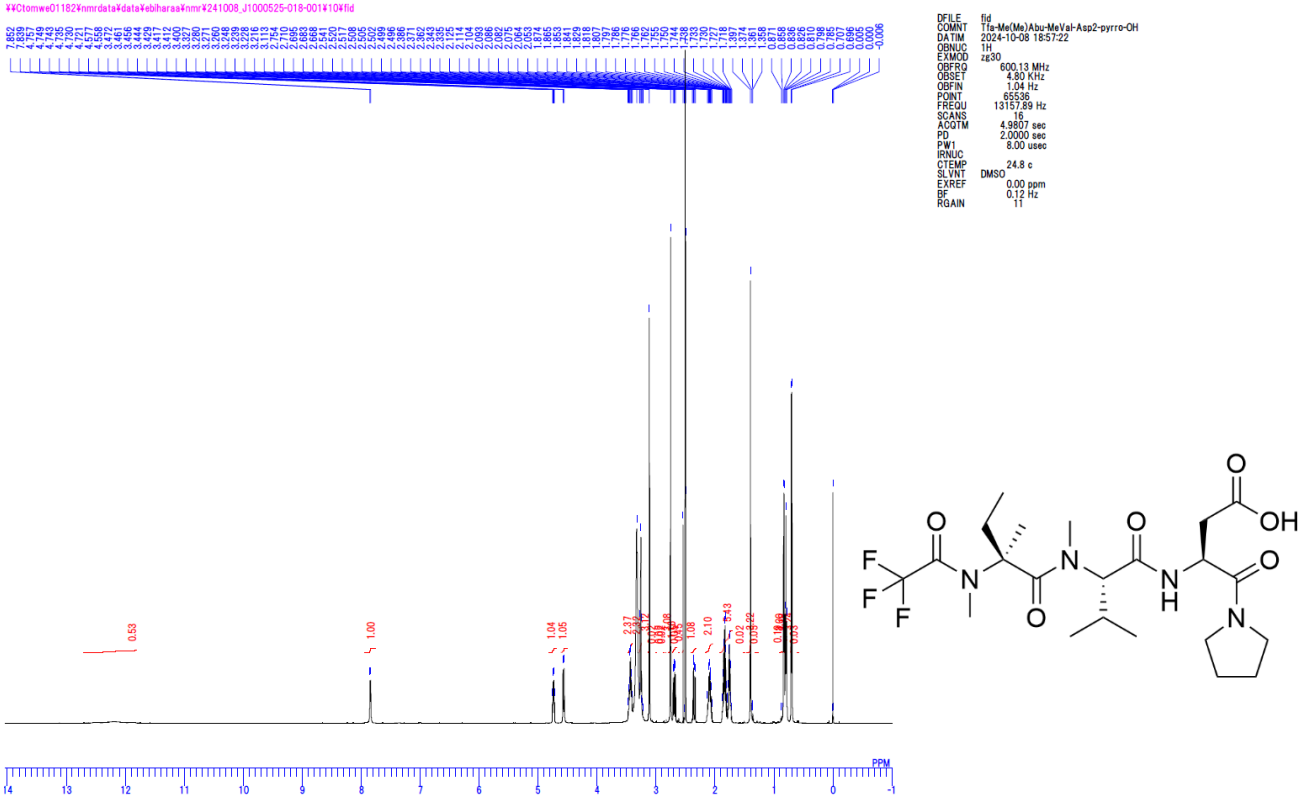


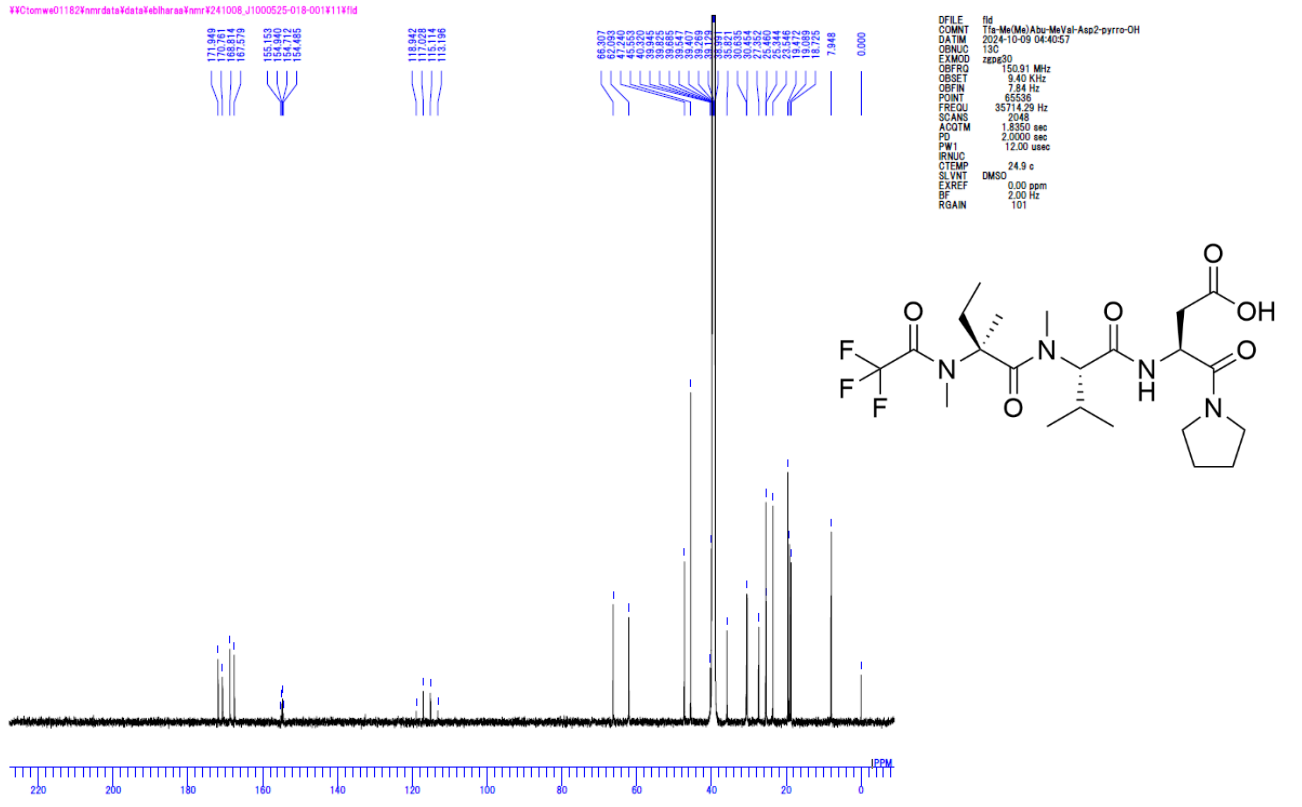





¥¥Ctomwe01182¥nmrdata¥data¥ebiharaa¥nmr¥250107_J1000525-050-001¥11¥fid
-68.388
DFILE fid
COMNT Tfa-MeAib-D-MeVal-Asp2-pyrro-OH
DATIM 2025-01-07 15:37:14
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

Tfa-Me(Me)Abu-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11ba)



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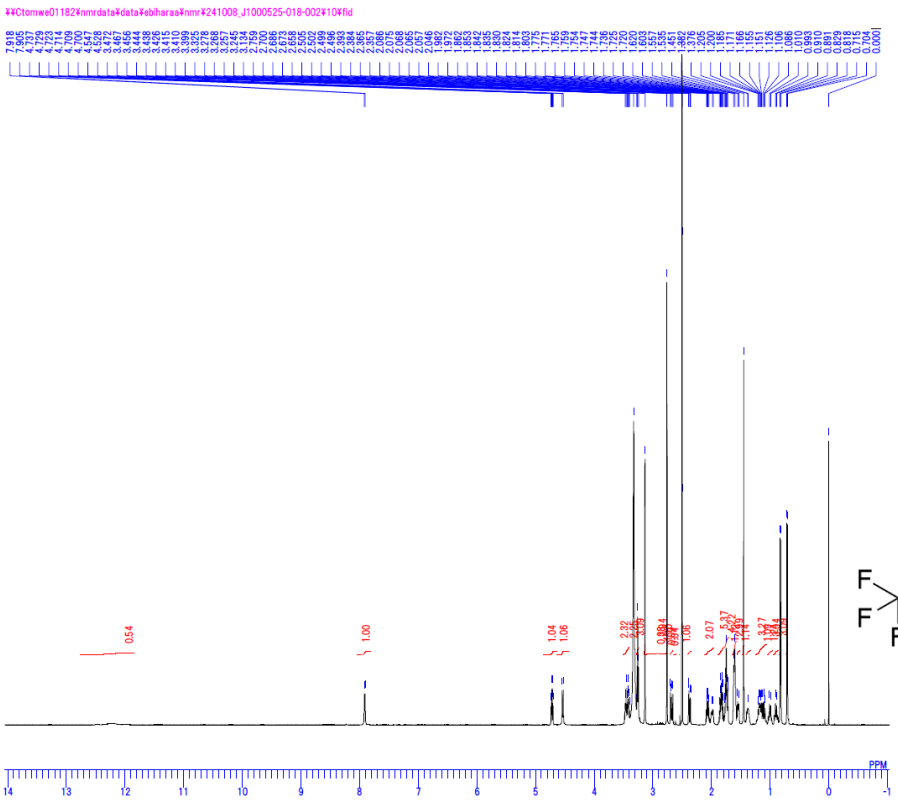
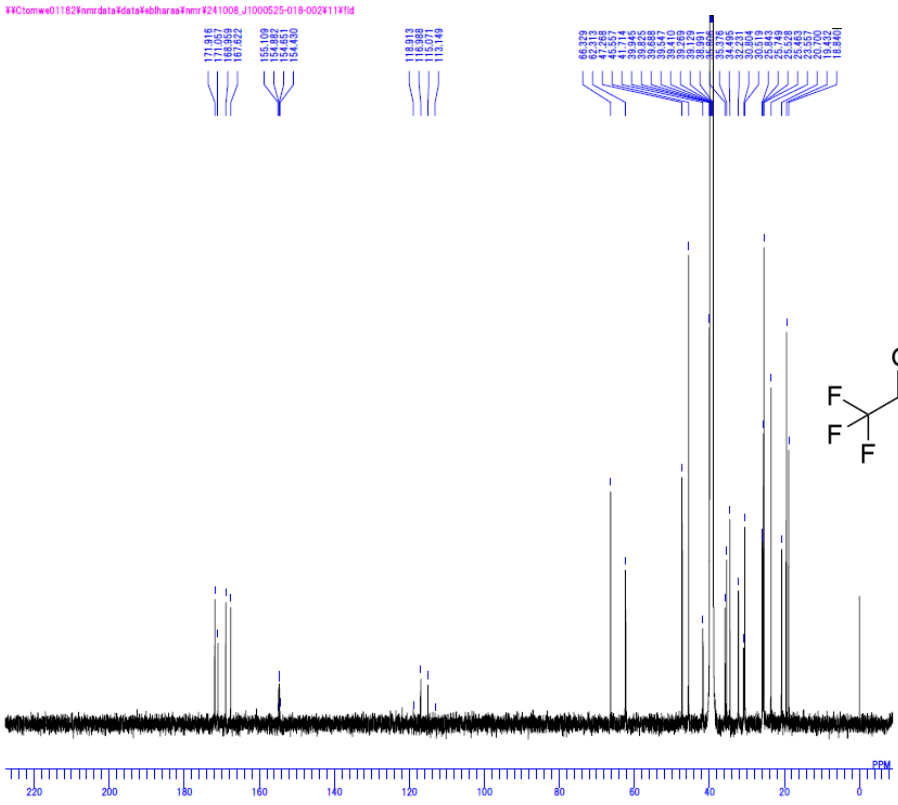
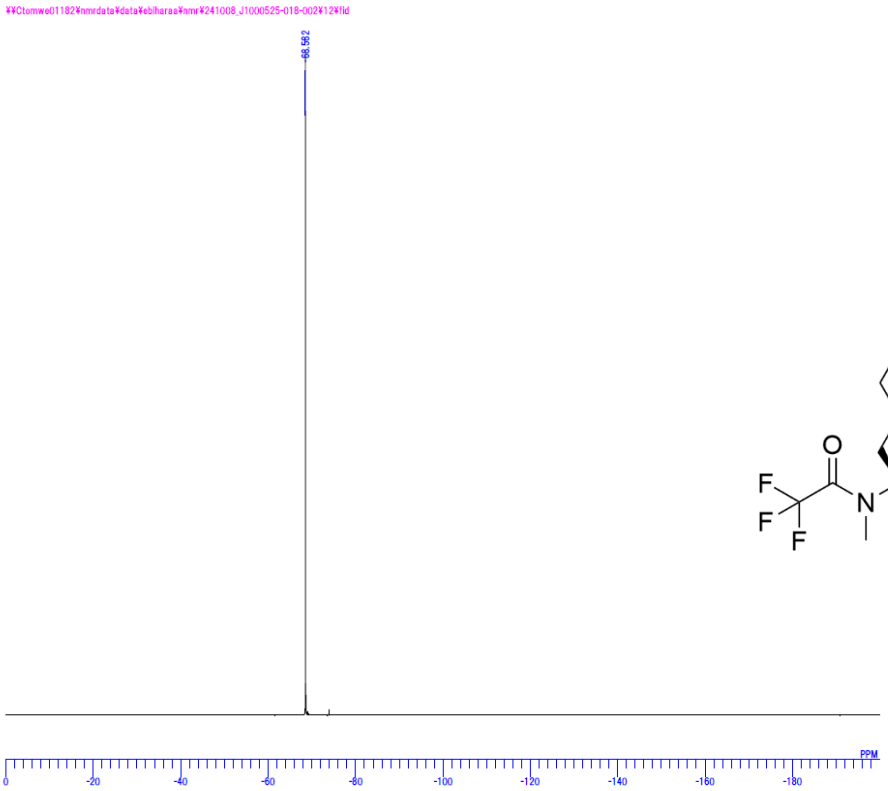
-68.865
DFILE fid
COMNT Tfa-Me(Me)Abu-MeVal-Asp-pyrro
DATIM 2024-10-09 13:46:14
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
F
F
F
O
N
O
N
O
N
H
O
OH
O
N

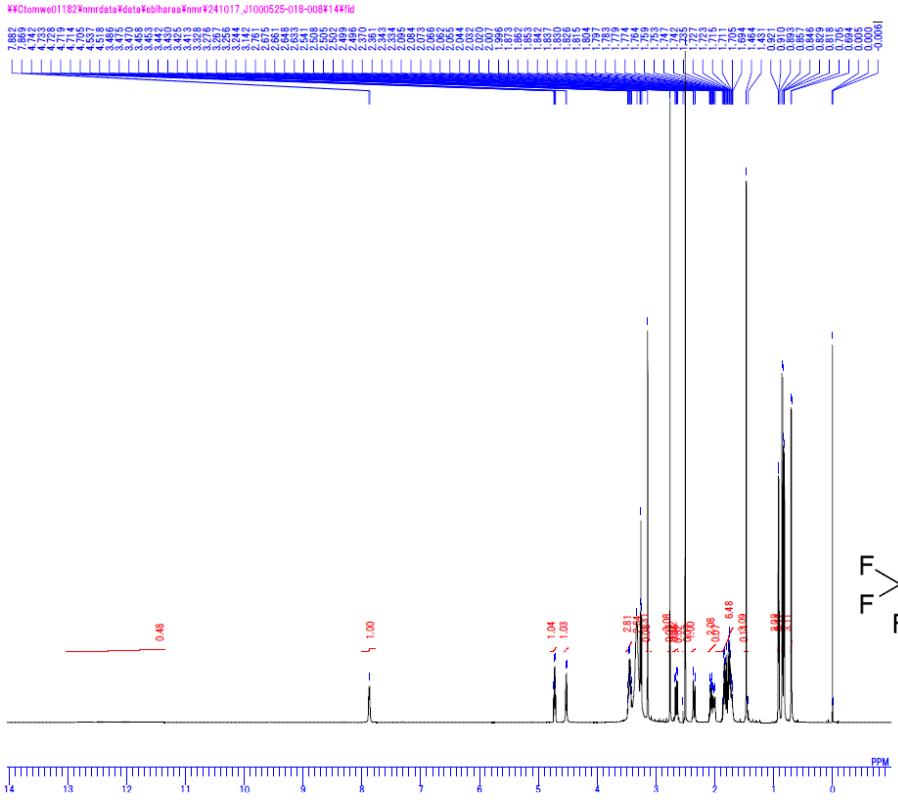
Tfa-Me(Me)Cha-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11ca)

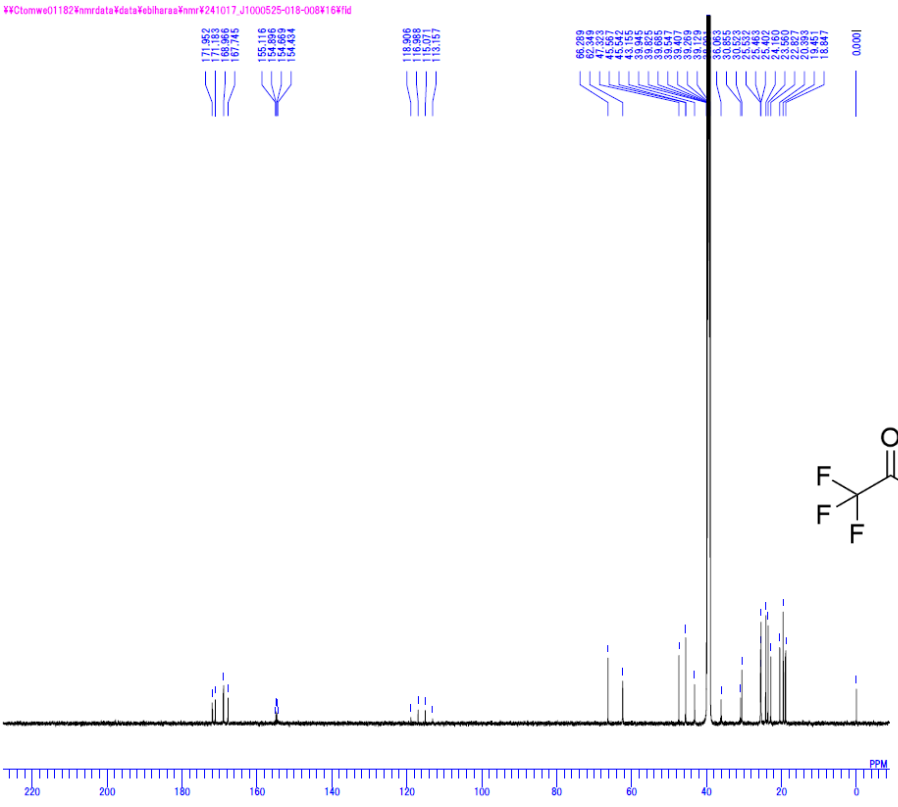


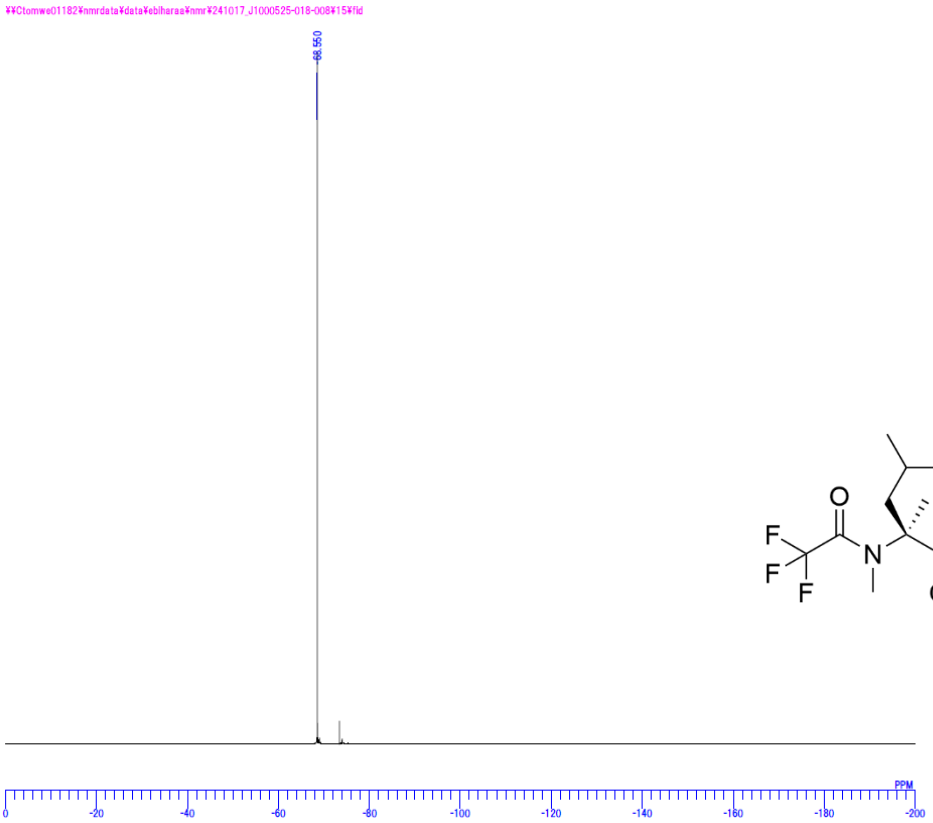



¥¥Ctomwe011824¥nmrdata¥data¥ebihara¤¥nmr¥241008_J1000525-018-002¥12¥fid
-68.562
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
DFILE fid
COMNT Tfa-Me(Me)Cha-MeVal-Asp-pyrro
DATIM 2024-10-09 13:57:09
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
F
F
F
O
N
O
N
O
N
H
O
OH
O
N

Tfa-Me(Me)Leu-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11da)



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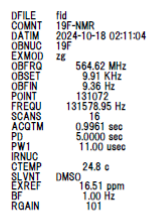
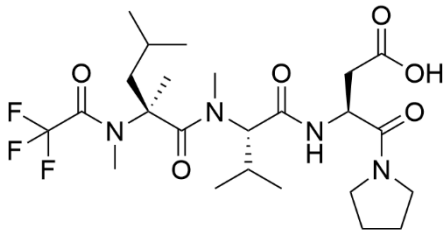
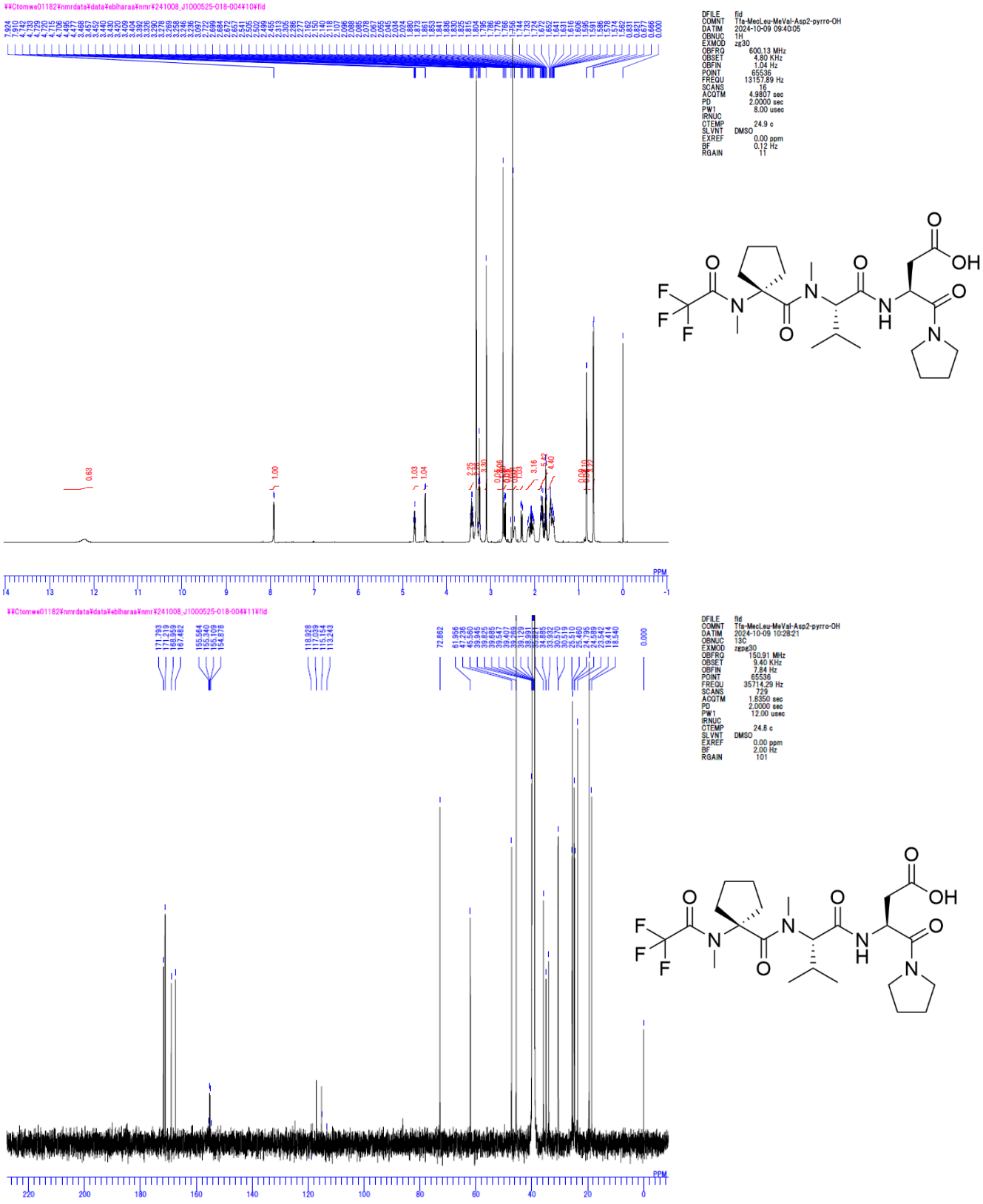
-140
-160
-180
-200


DFILE fid
COMNT 19F-NMR
DATIM 2024-10-18 02:11:04
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101


F
F
F
O
N
O
N
O
N
H
O
OH
O
N

Tfa-MecLeu-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11ea)

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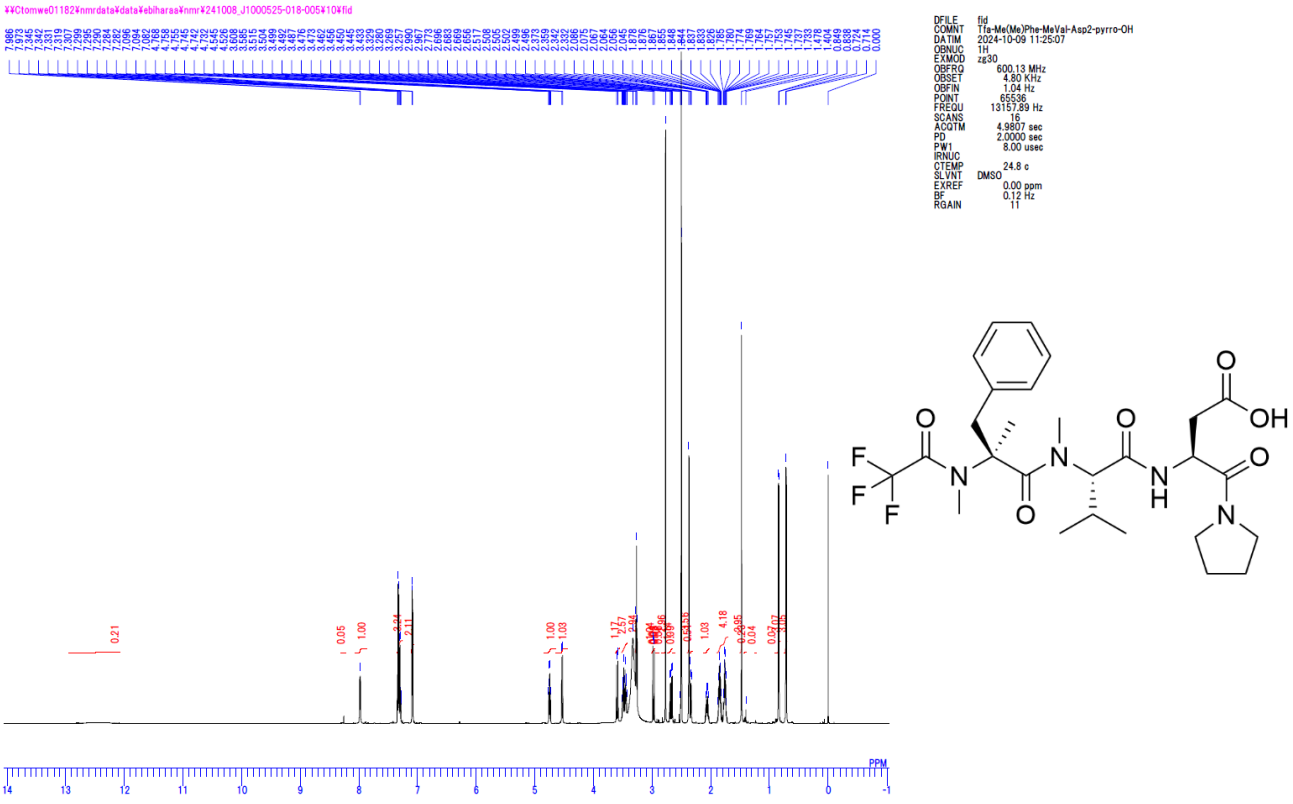
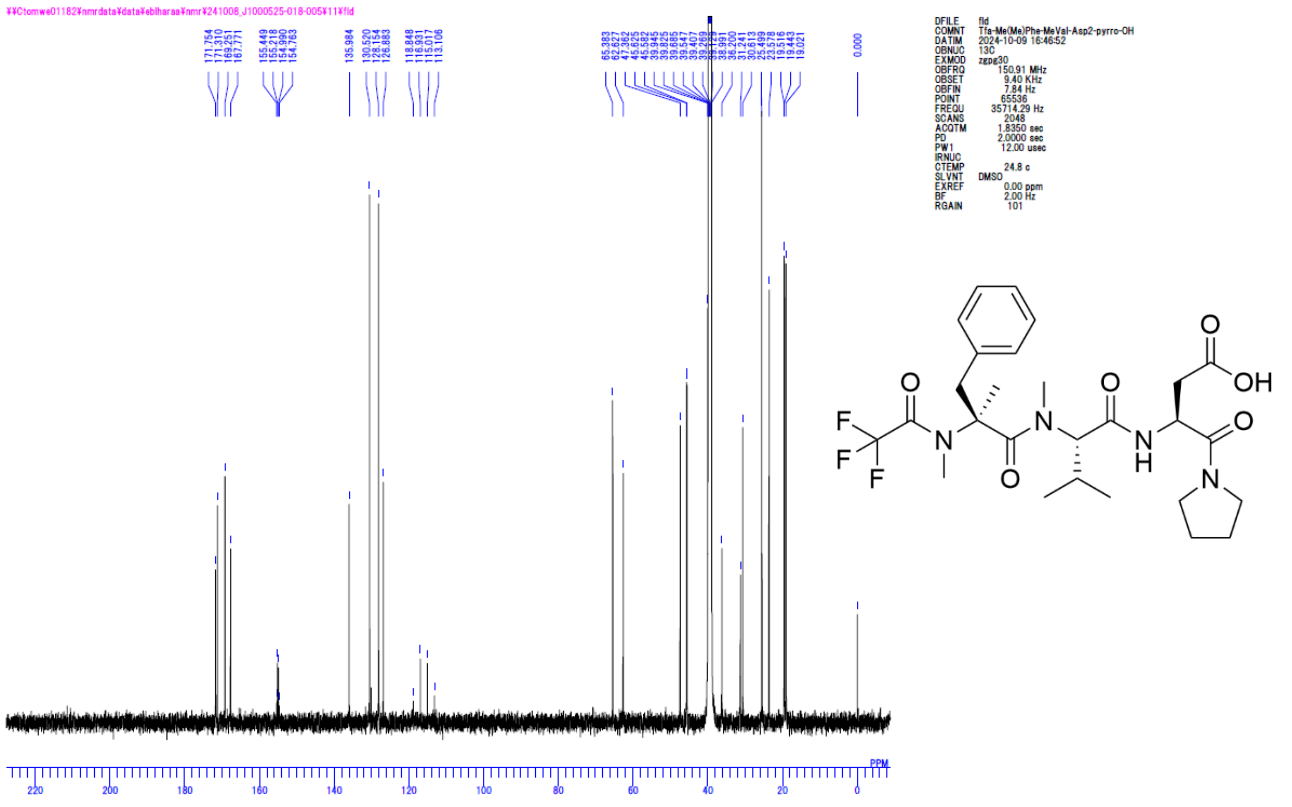
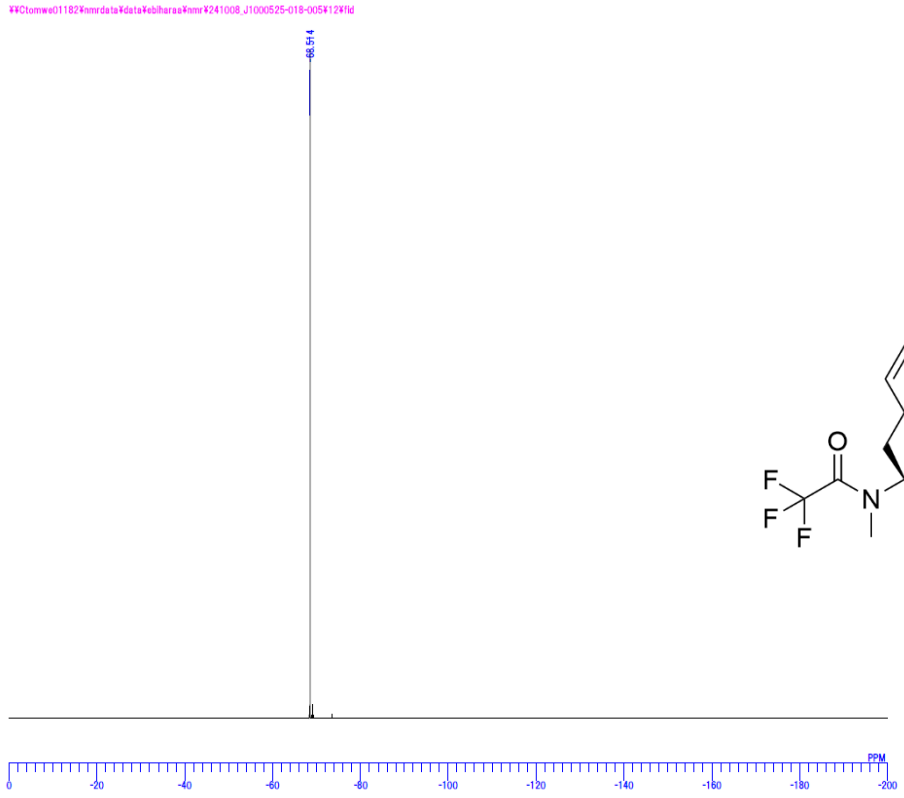
COMNT Tfa-MeoLeu-MeVal-Asp-pyrro
DATIM 2024-10-09 11:46:21
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
F
F
F
O
N
O
N
O
N
H
O
OH
O
N
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

Tfa-Me(Me)Phe-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11fa)





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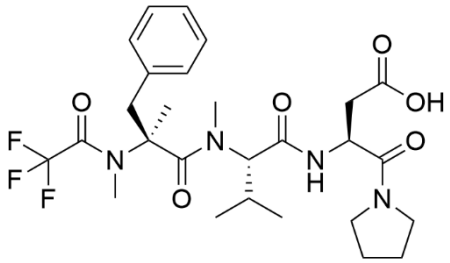
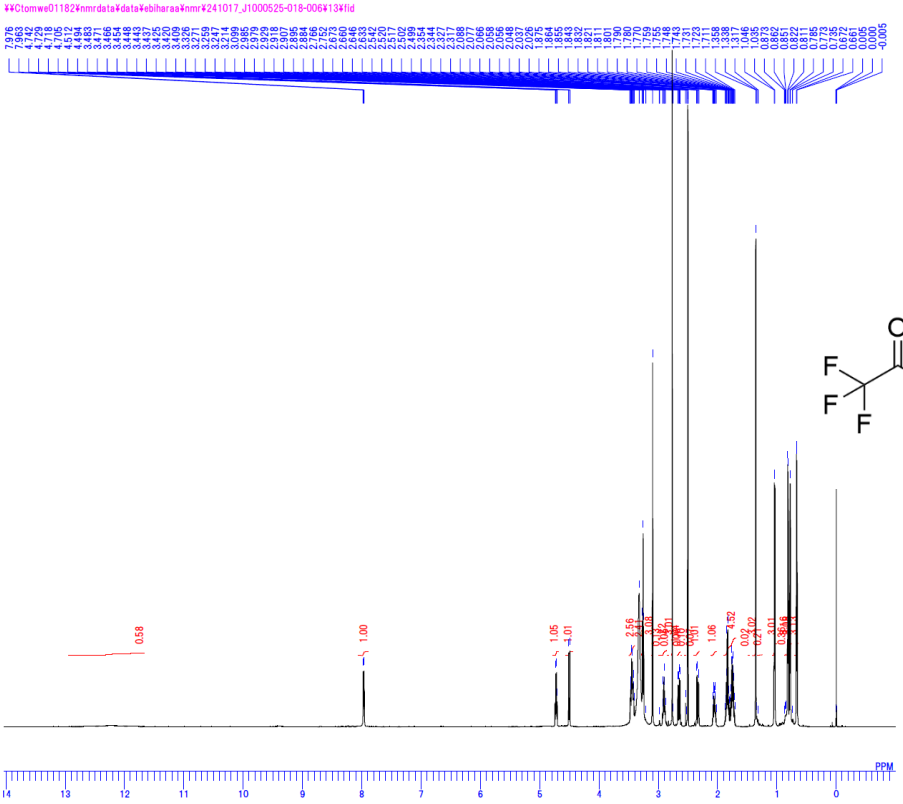
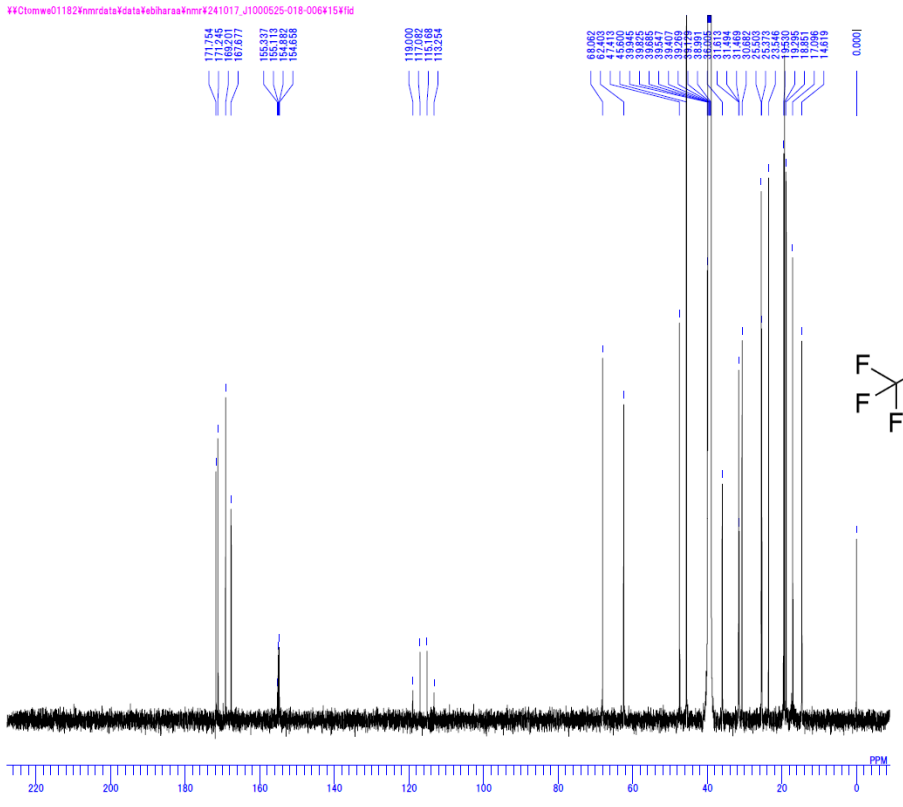
COMNT     Tfa-Me(Me)Phe-MeVal-Asp-pyrro
DATIM     2024-10-10 09:48:39
OBNUC     19F
EXMOD     zg
OBSFRQ      564.62 MHz
OBSET         9.91 KHz
OBFIN         9.36 Hz
POINT       131072
FREQU    131578.95 Hz
SCANS           16
ACQTM       0.9961 sec
PD          5.0000 sec
PW1        11.00 usec
IRNUC
CTEMP        24.9 c
SLVNT     DMSO
EXREF       16.51 ppm
BF           1.00 Hz
RGAIN         101
```



Tfa-Me(Me)Val-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11ga)



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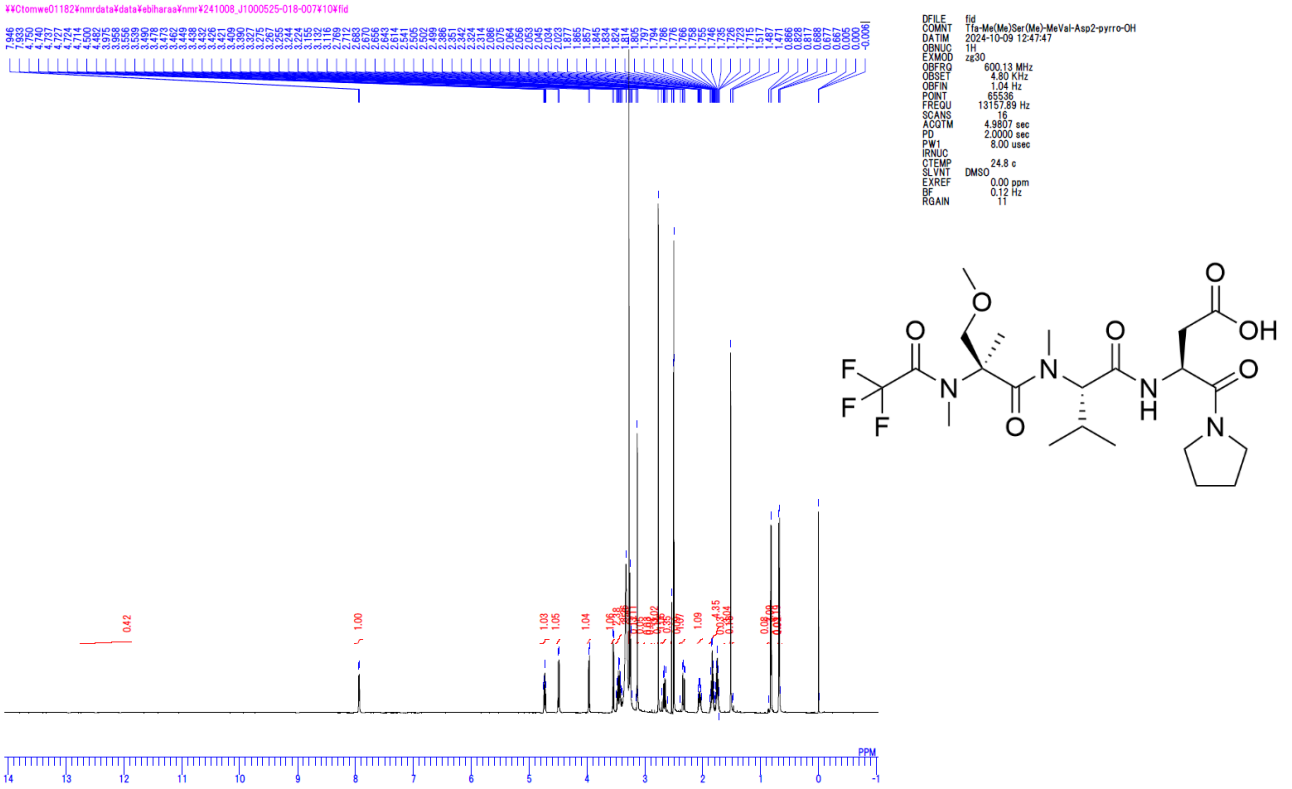
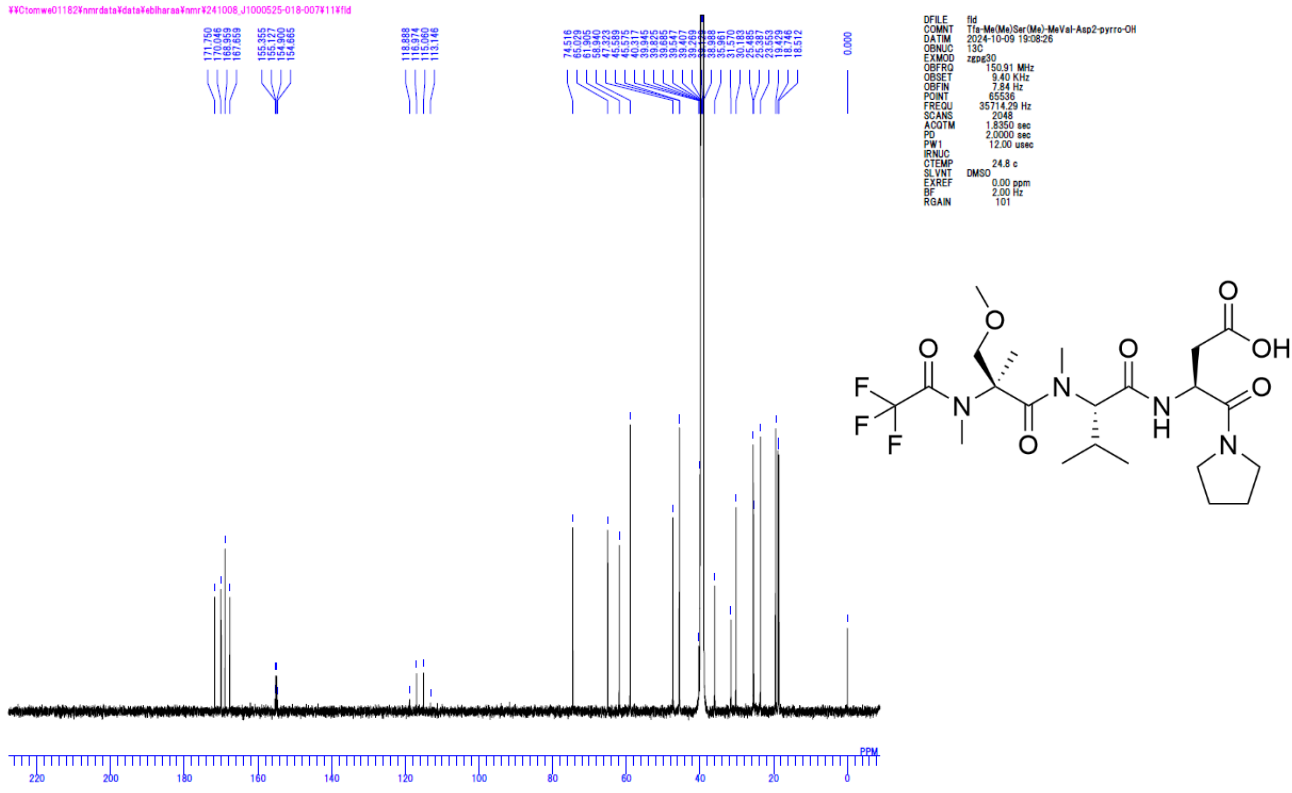
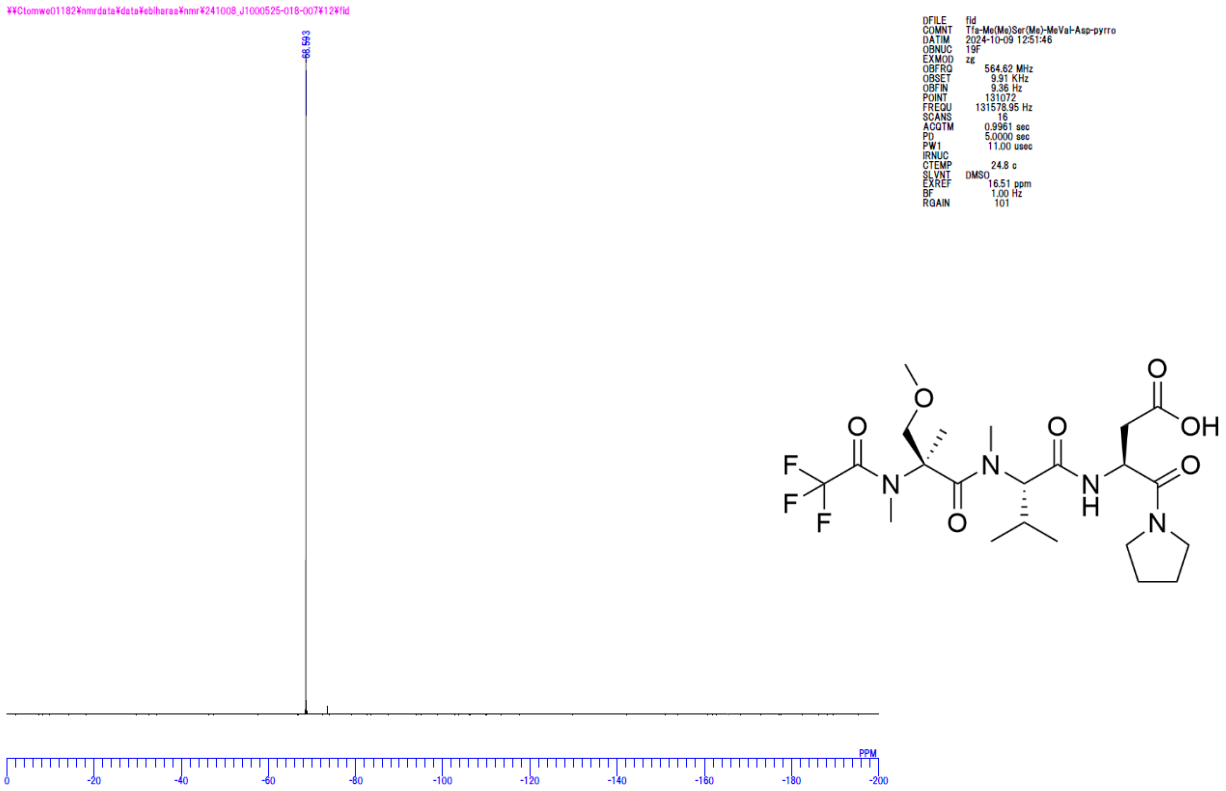
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
DFILE fid
COMNT Tfa-Me(Me)Val-MeVal-Asp-pyrro
DATIM 2024-10-17 23:42:20
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
F
F
F
O
N
O
N
O
N
H
O
OH
O
N

Tfa-Me(Me)Ser(Me)-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11ha)



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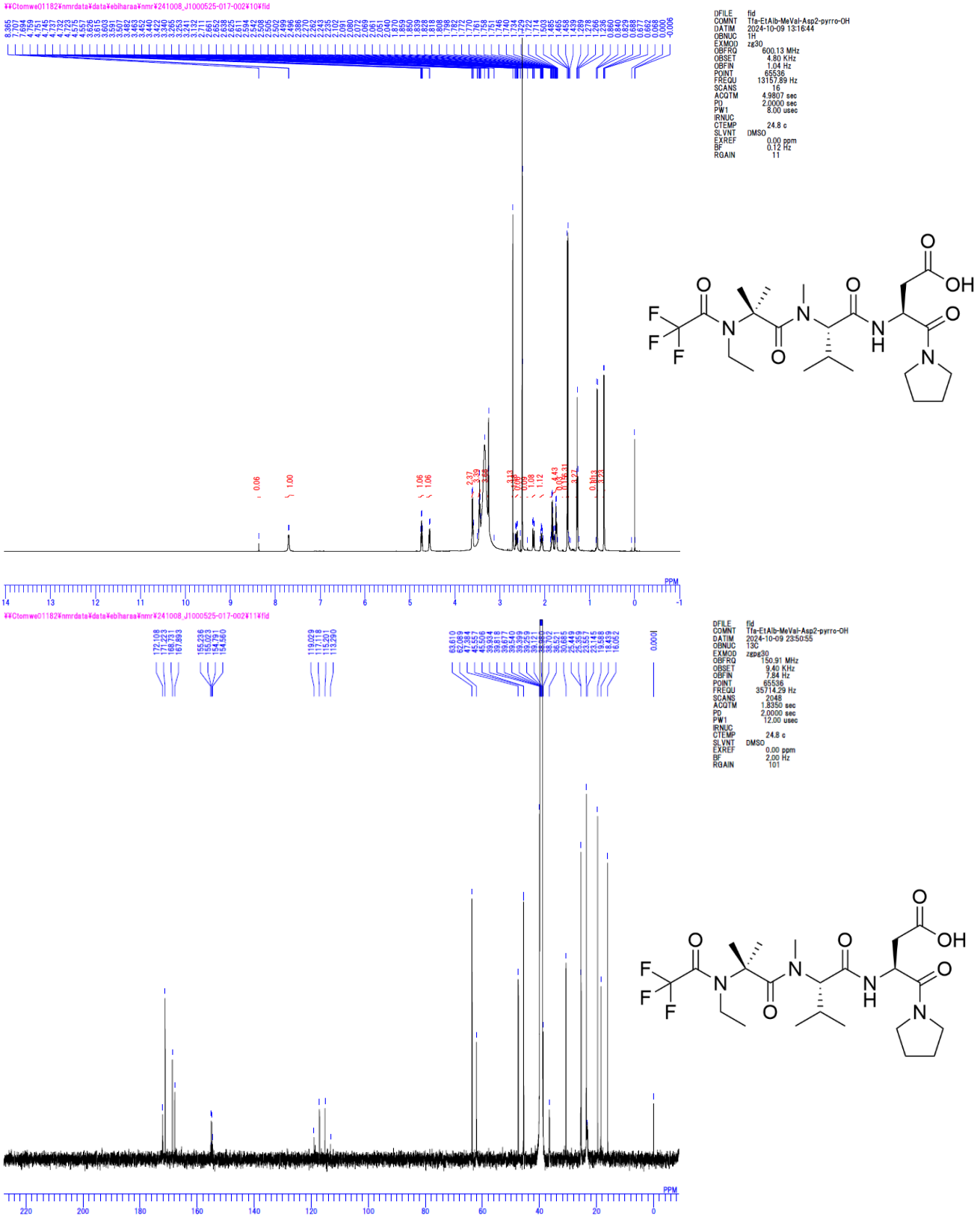
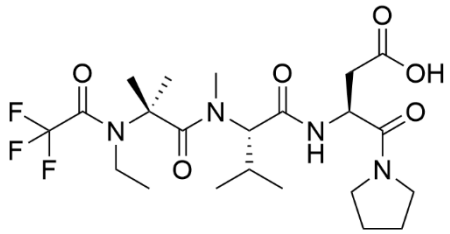
-68.593
DFILE fid
COMNT Tfa-Me(Me)Ser(Me)-MeVal-Asp-pyrro
DATIM 2024-10-09 12:51:46
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
F
F
F
O
N
O
O
N
O
N
H
O
OH
O
N

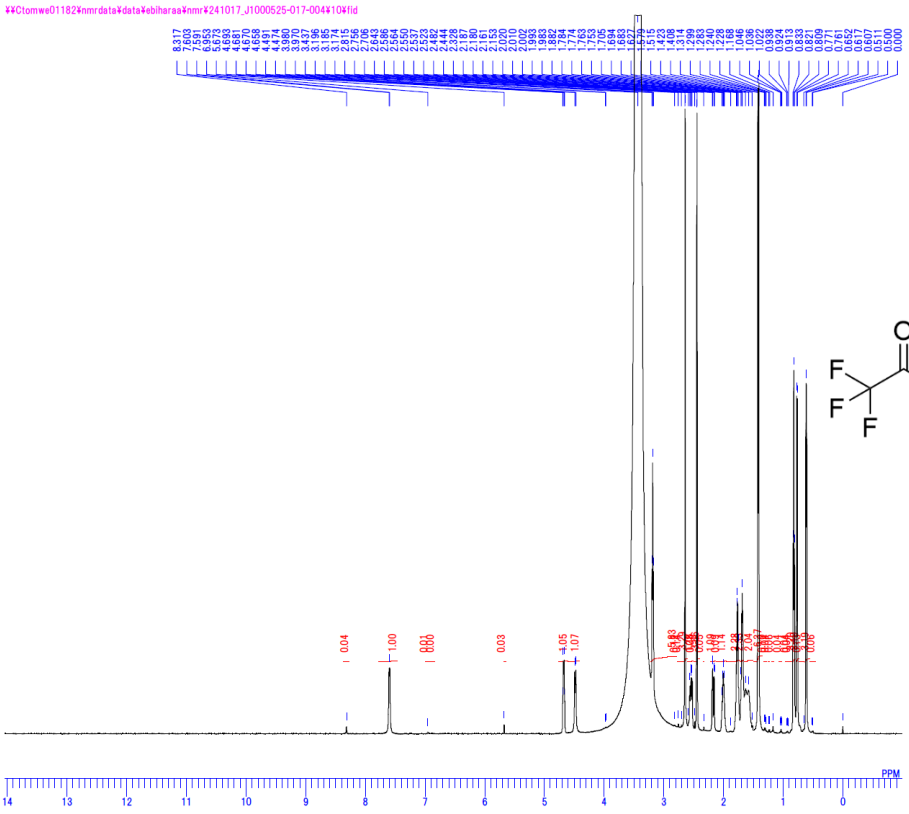
Tfa-EtAib-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11ab)

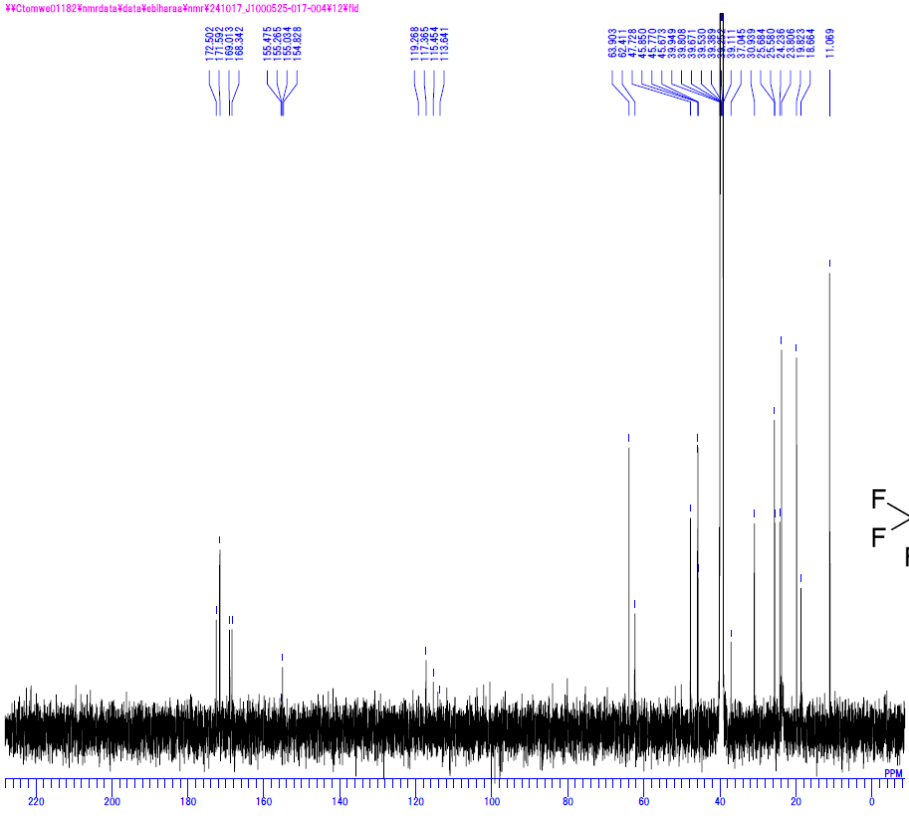


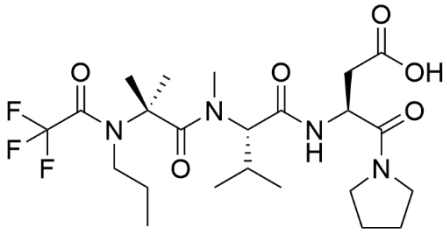
DFILE	fid
COMNT	Tfa-EtAib-MeVal-Asp-pyrro
DATIM	2024-10-09 13:20:50
OBNUC	19F
EXMOD	zg
OBFRQ	564.62 MHz
OBSET	9.91 KHz
OBFIN	9.36 Hz
POINT	131072
FREQU	131578.95 Hz
SCANS	16
ACQTM	0.9961 sec
PD	5.0000 sec
PW1	11.00 usec
IRNUC	
CTEMP	24.8 c
SLVNT	DMSO
EXREF	16.51 ppm
BF	1.00 Hz
RGAIN	101



Tfa-nPrAib-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11ac)





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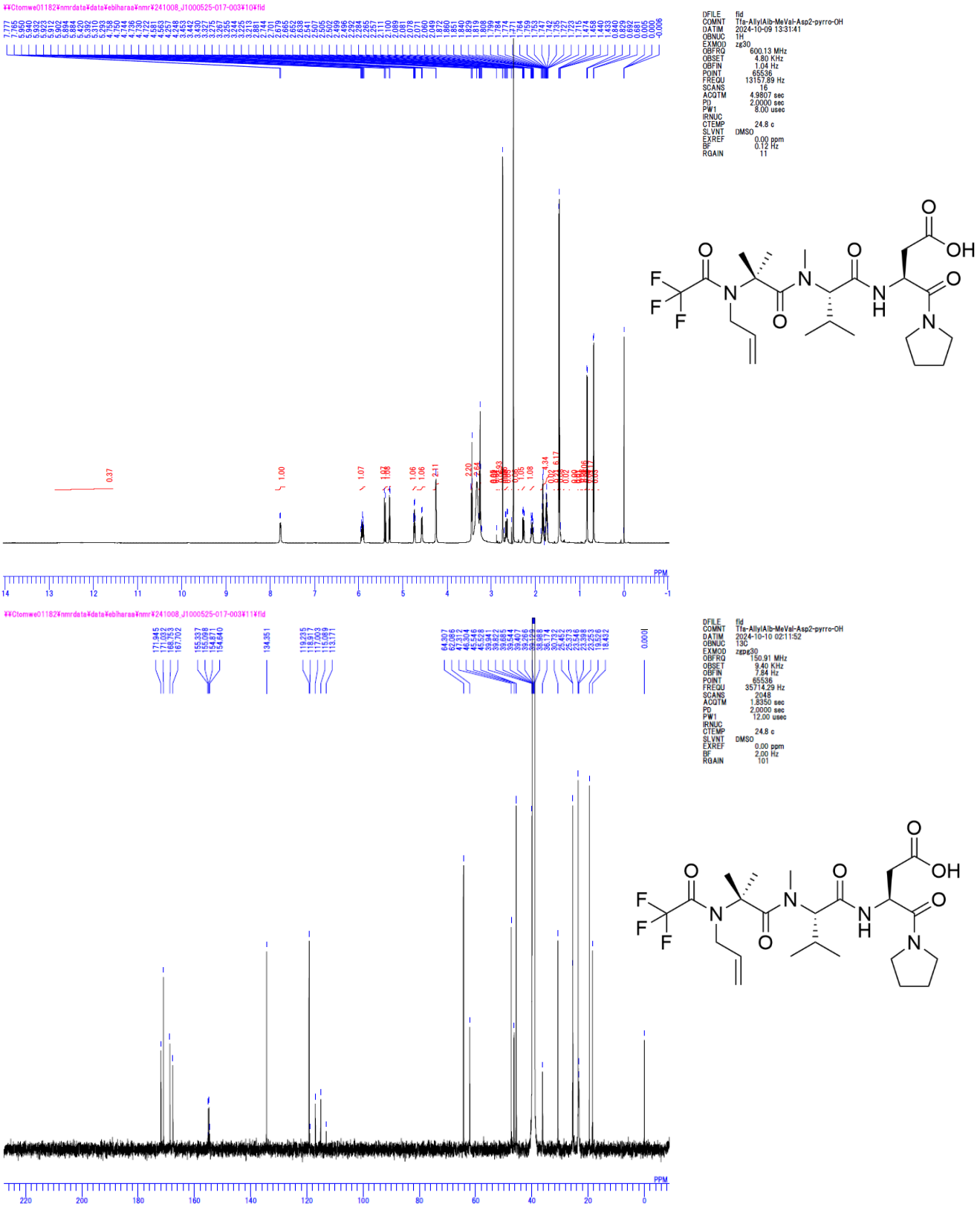
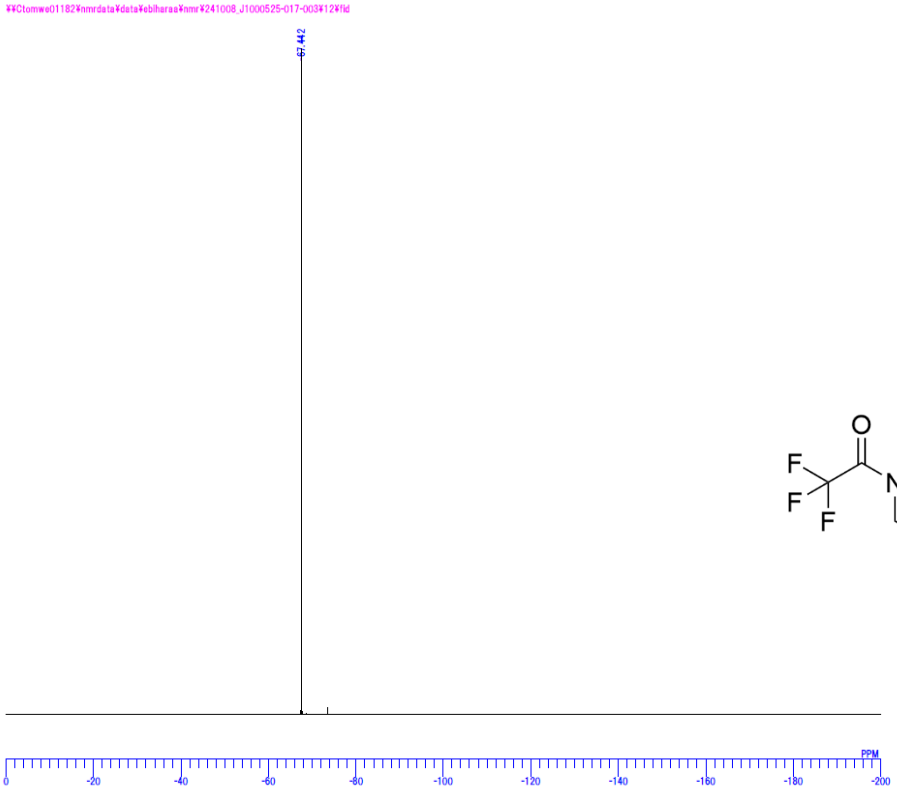
DFILE fid
COMNT TFa-nPrAib-MeVal-Asp-pyrro
DATIM 2024-10-17 15:35:34
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

O
OH
F
F
F
N
O
N
O
N
H
O
N

Tfa-allylAib-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11ad)

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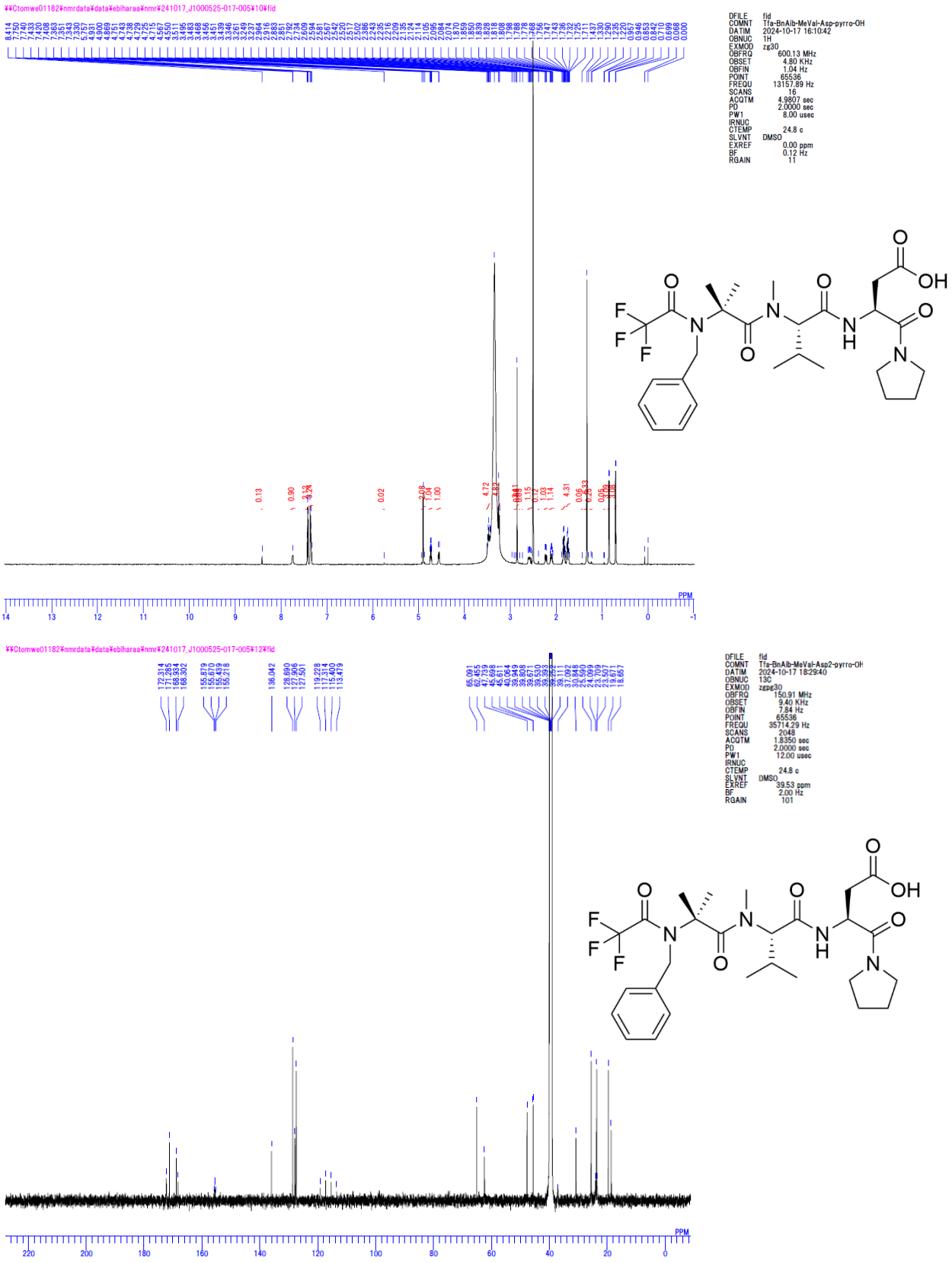
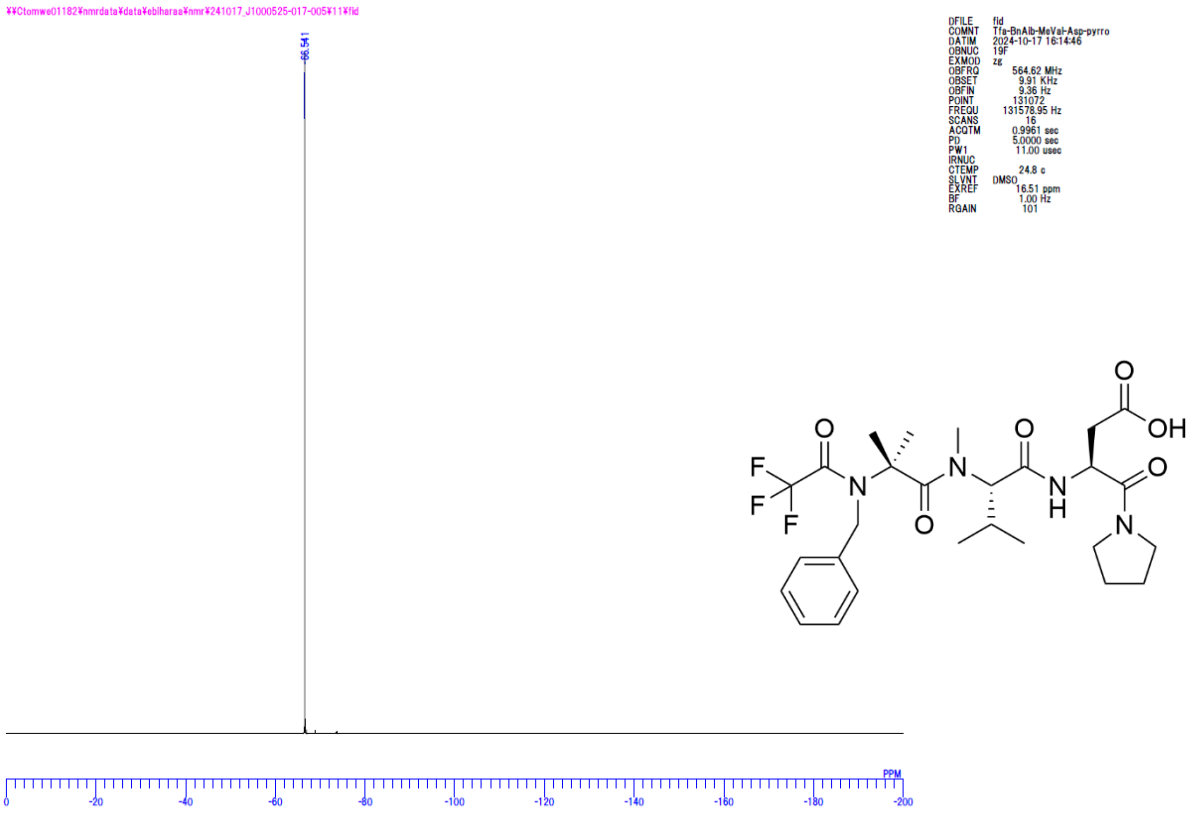
DFILE fid
COMNT Tfa-Allyl(Alb-MeVal-Asp-pyrro
DATIM 2024-10-09 13:35:34
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
O
OH
F
F
F
N
O
N
O
N
H
O
N
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

Tfa-BnAib-MeVal-Asp-pyrro (<u>The peptide cleavaged from</u> 11ae)

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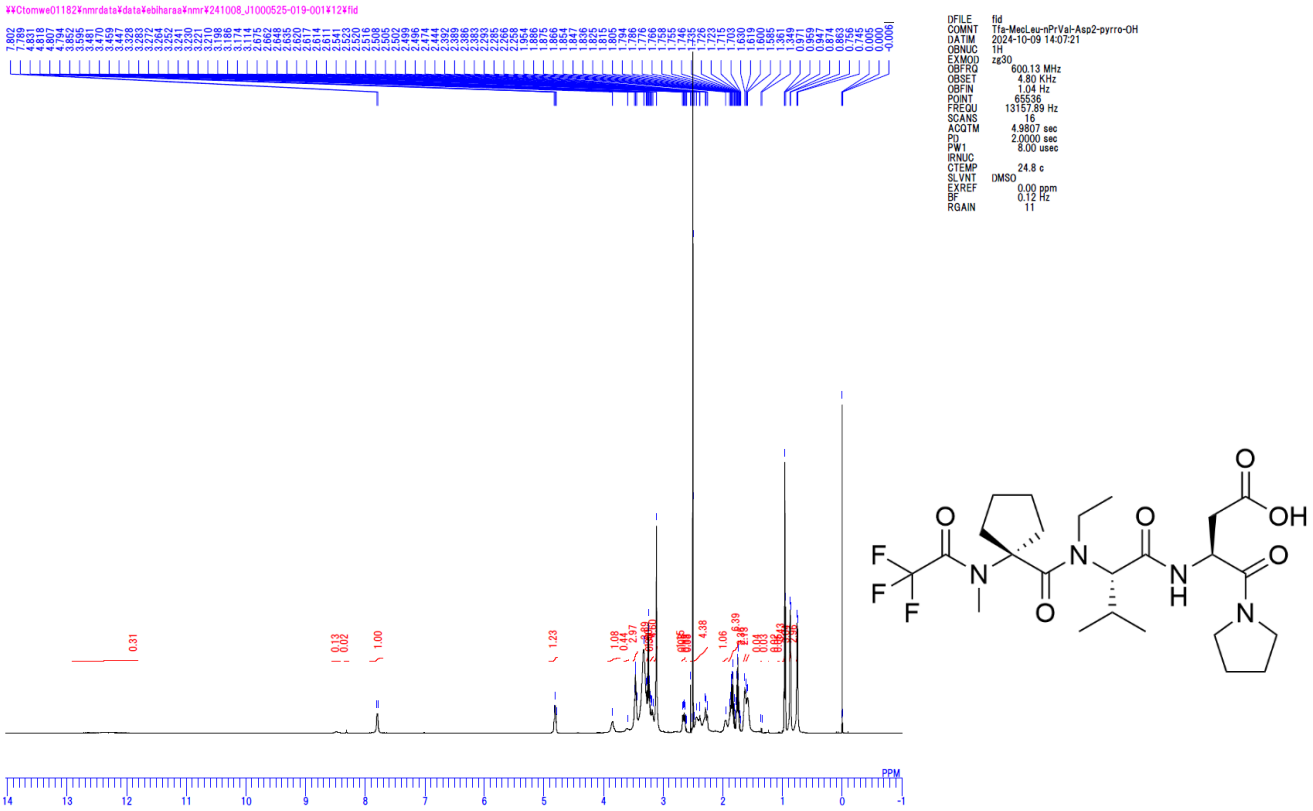
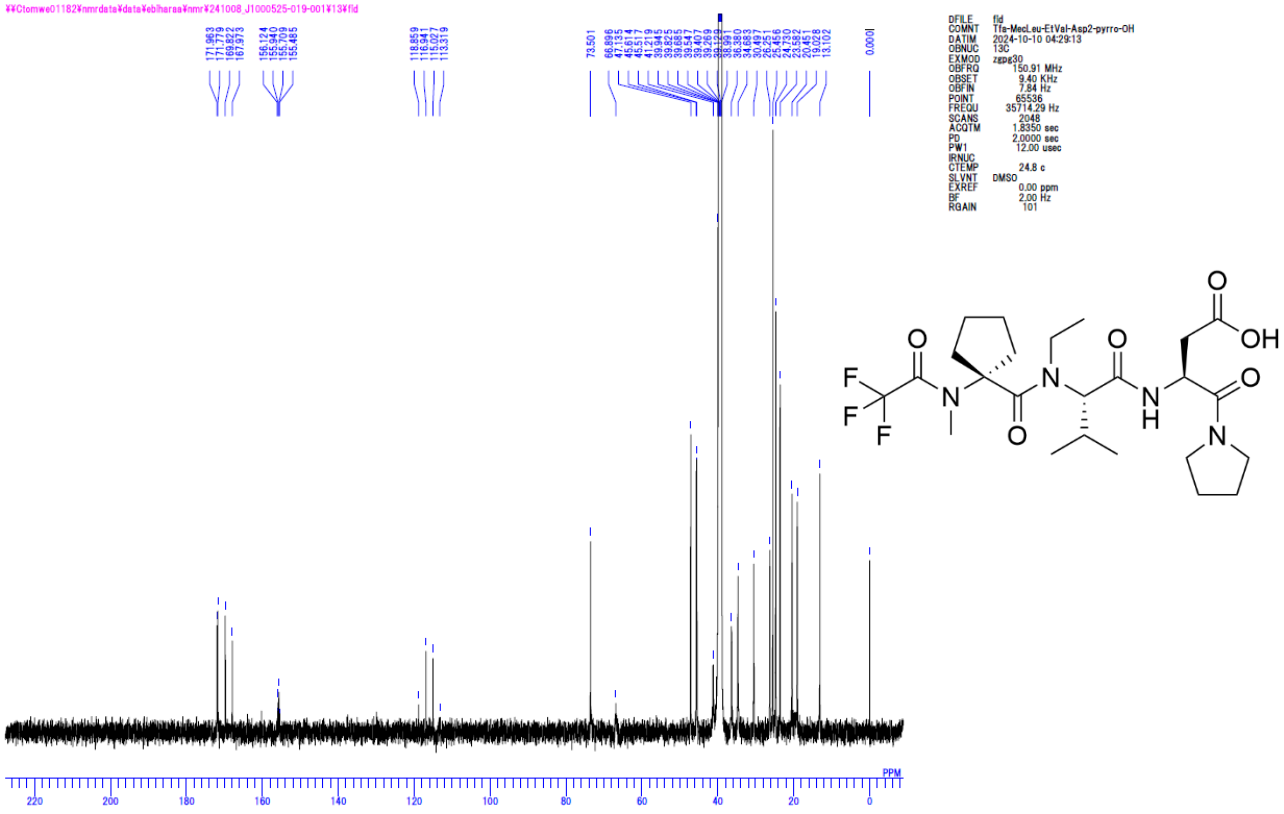
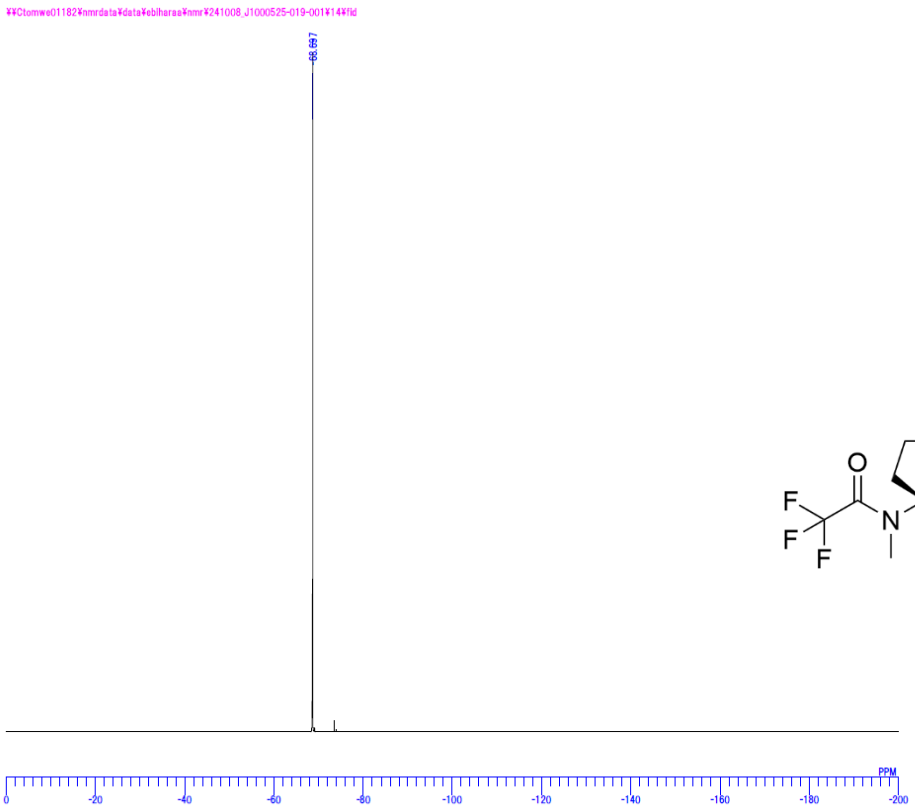
-66.541
DFILE fid
COMNT Tfa-BnAib-MeVal-Asp-pyrro
DATIM 2024-10-17 16:14:46
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
O
OH
F
F
F
O
N
O
N
O
N
H
O
N
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

Tfa-MecLeu-EtVal-Asp-pyrro (<u>The peptide cleavaged from</u> 17a)





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DFILE     fid
COMNT     Tfa-MecLeu-EtVal-Asp-pyrro
DATIM     2024-10-09 14:11:14
OBNUC     19F
EXMOD     ze
OBFRQ       564.62 MHz
OBSET         9.91 KHz
OBFIN         9.36 Hz
POINT       131072
FREQU     131578.95 Hz
SCANS           16
ACQTM       0.9961 sec
PD          5.0000 sec
PW1          11.00 usec
IRNUC
CTEMP         24.8 c
SLVNT     DMSO
EXREF       16.51 ppm
BF            1.00 Hz
RGAIN          101
```
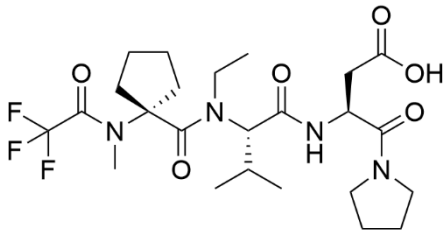
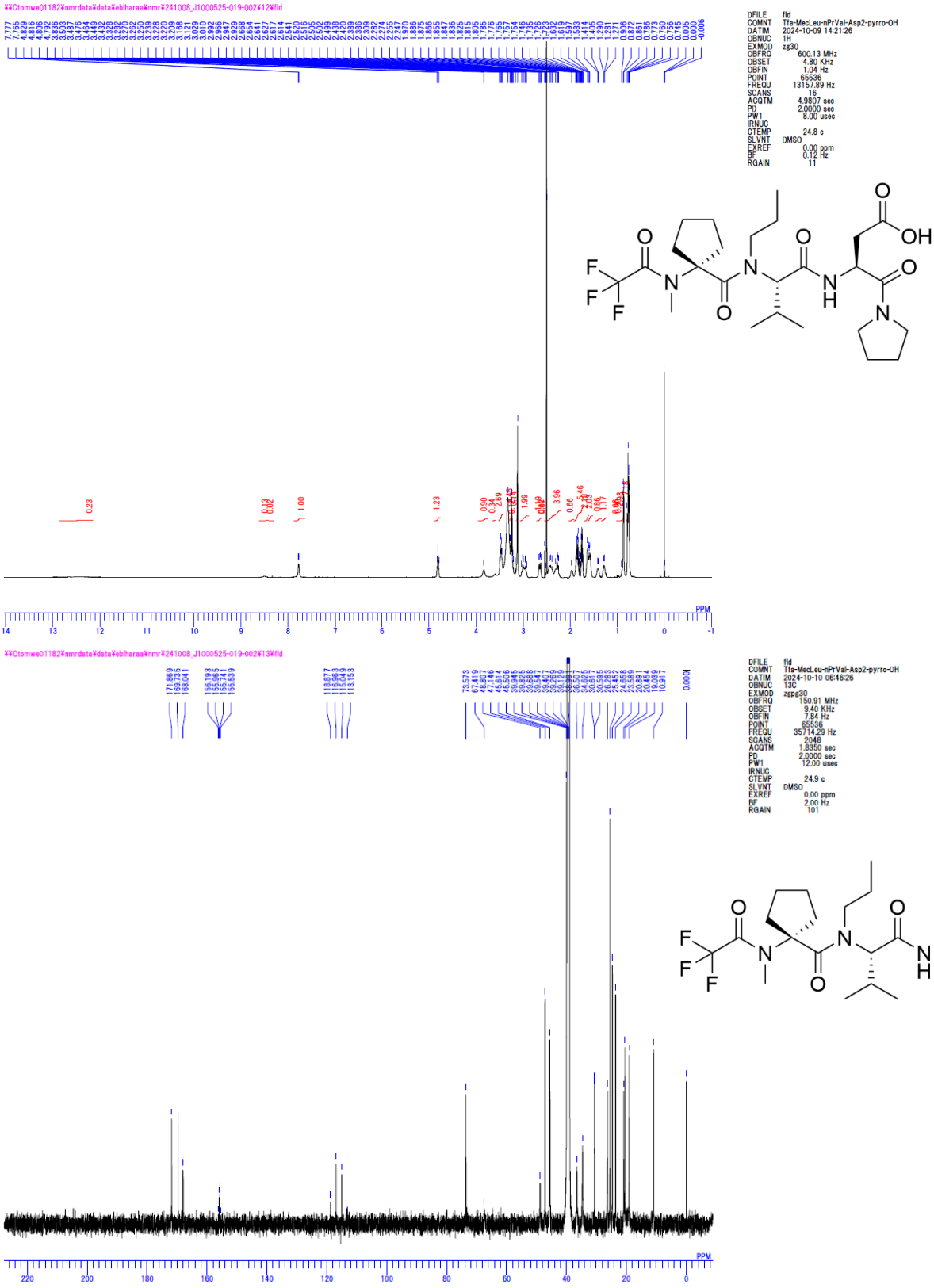
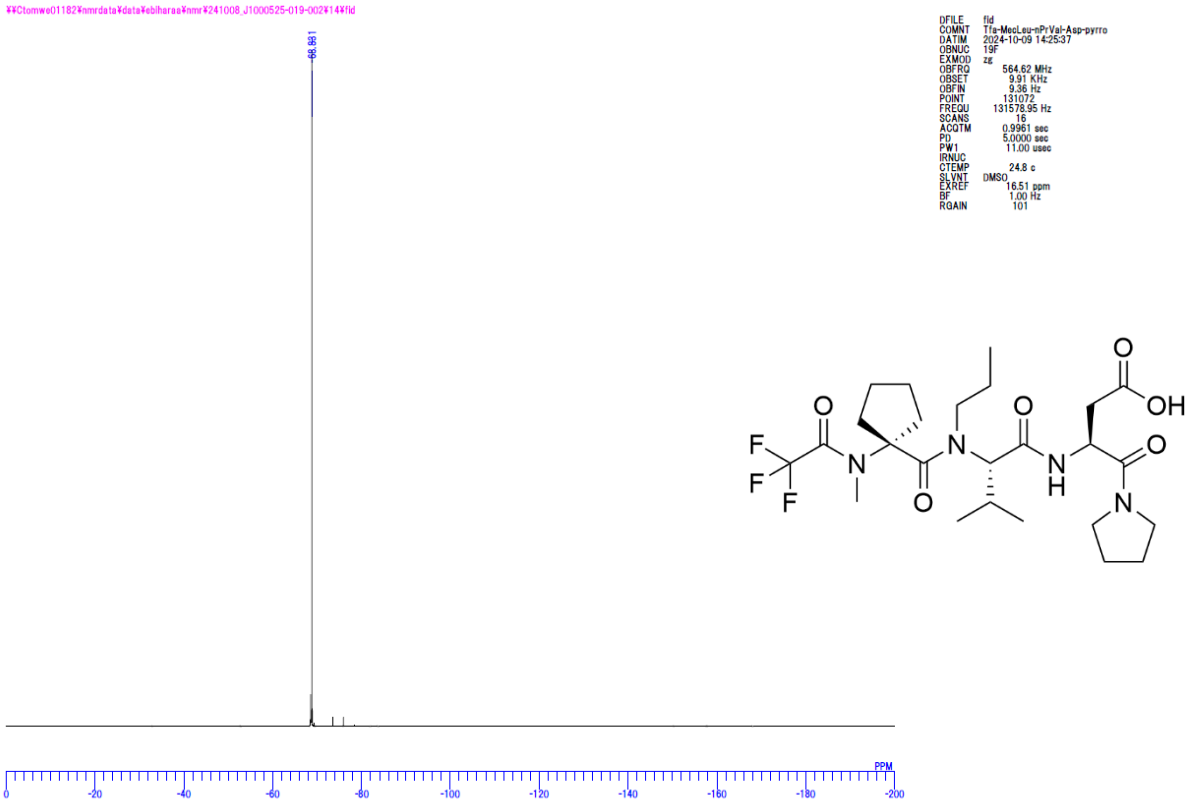



Tfa-MecLeu-nPrVal-Asp-pyrro (<u>The peptide cleavaged from</u> 17b)

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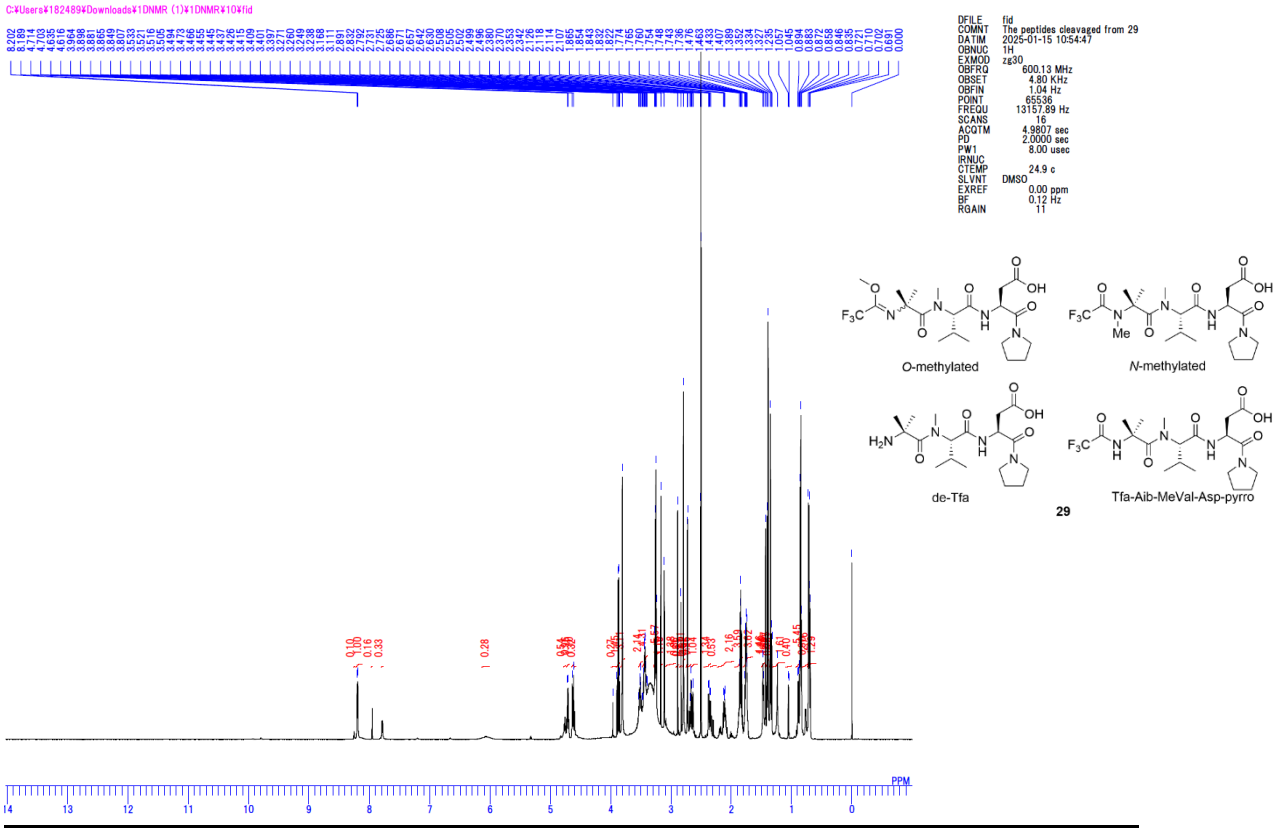
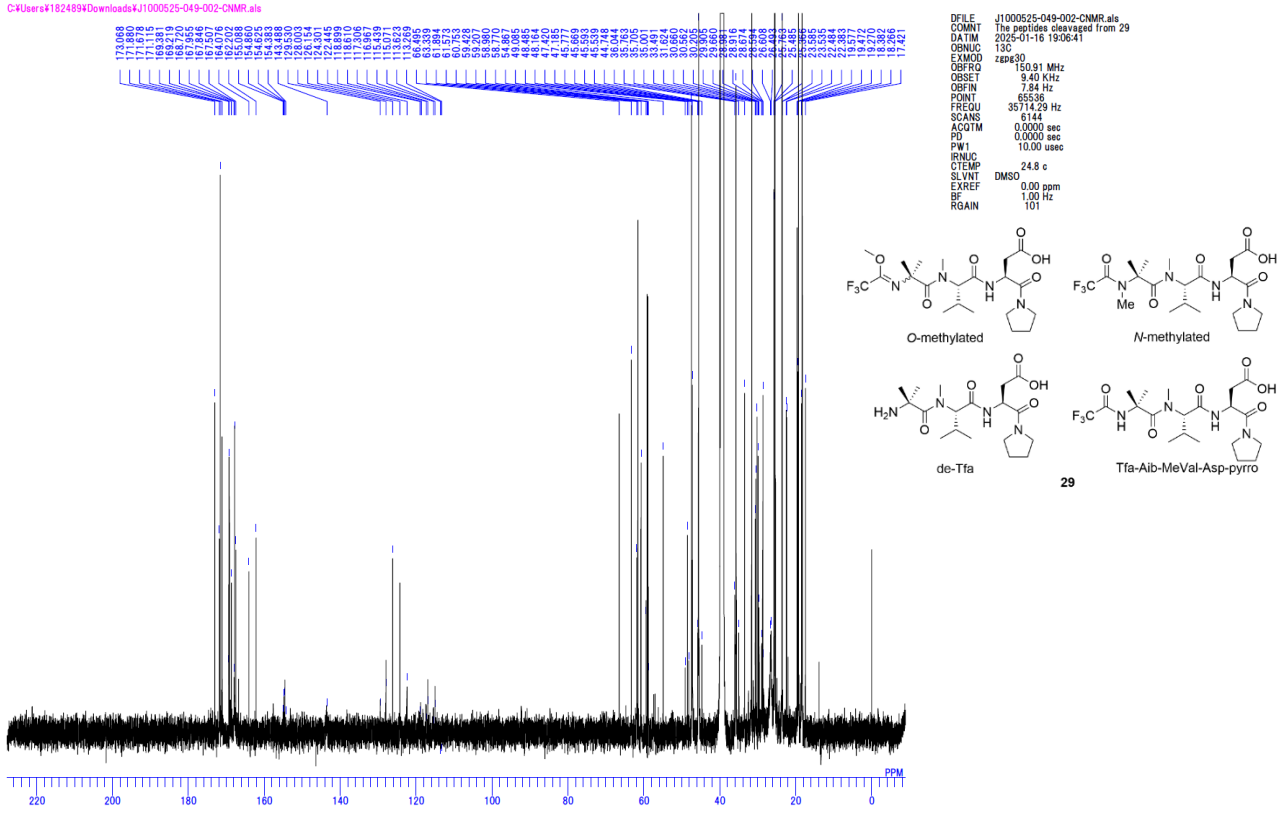
DFILE fid
COMNT Tfa-MecLeu-nPrVal-Asp-pyrro
DATIM 2024-10-09 14:25:37
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
F
F
F
O
N
O
N
O
N
H
O
OH
O
N
PPM
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

The peptides cleavaged from 29

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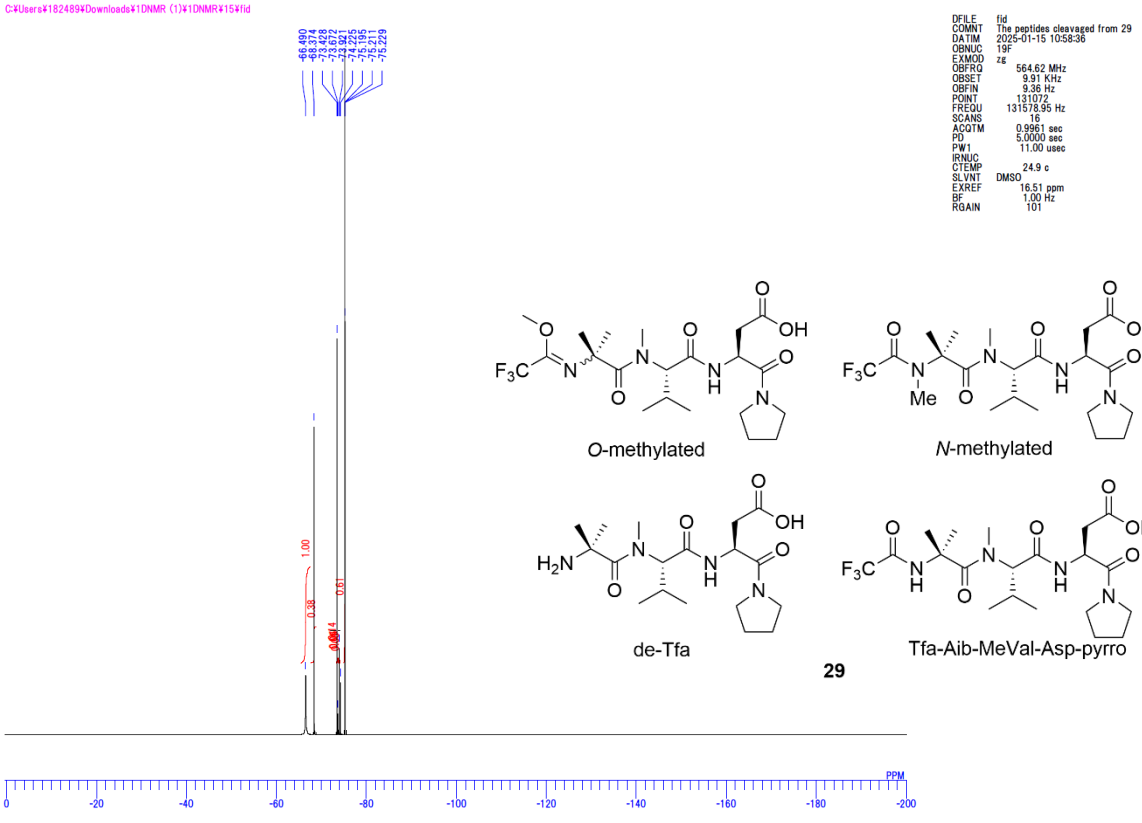
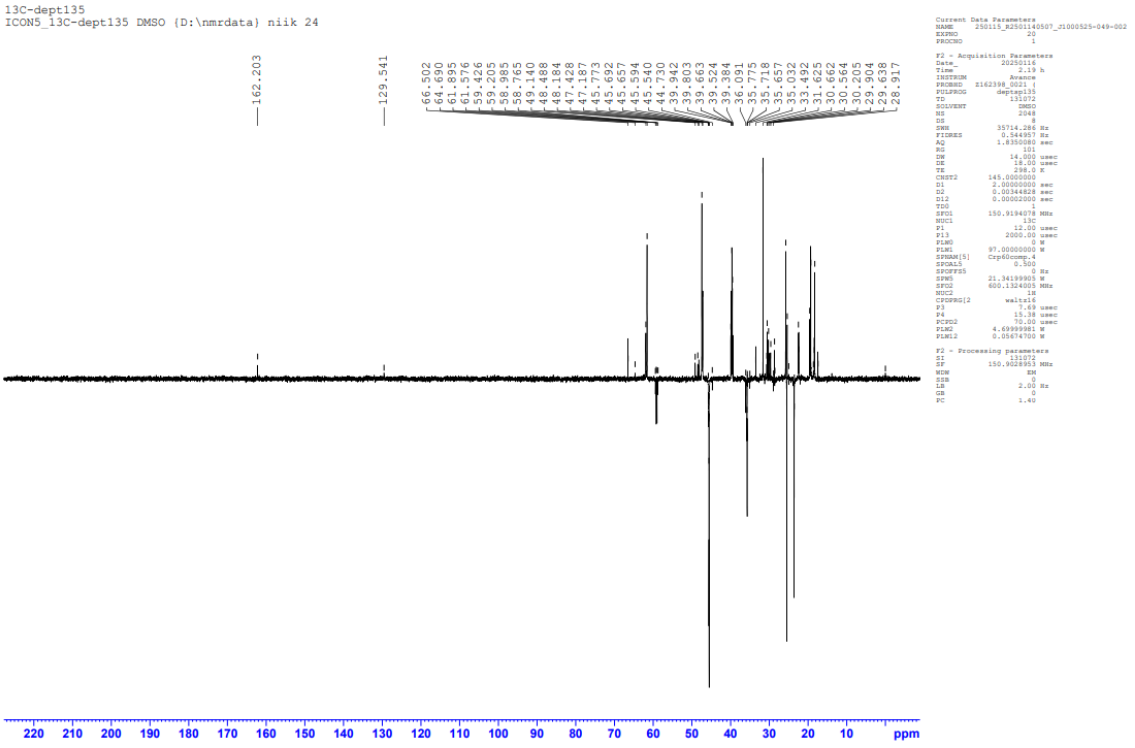
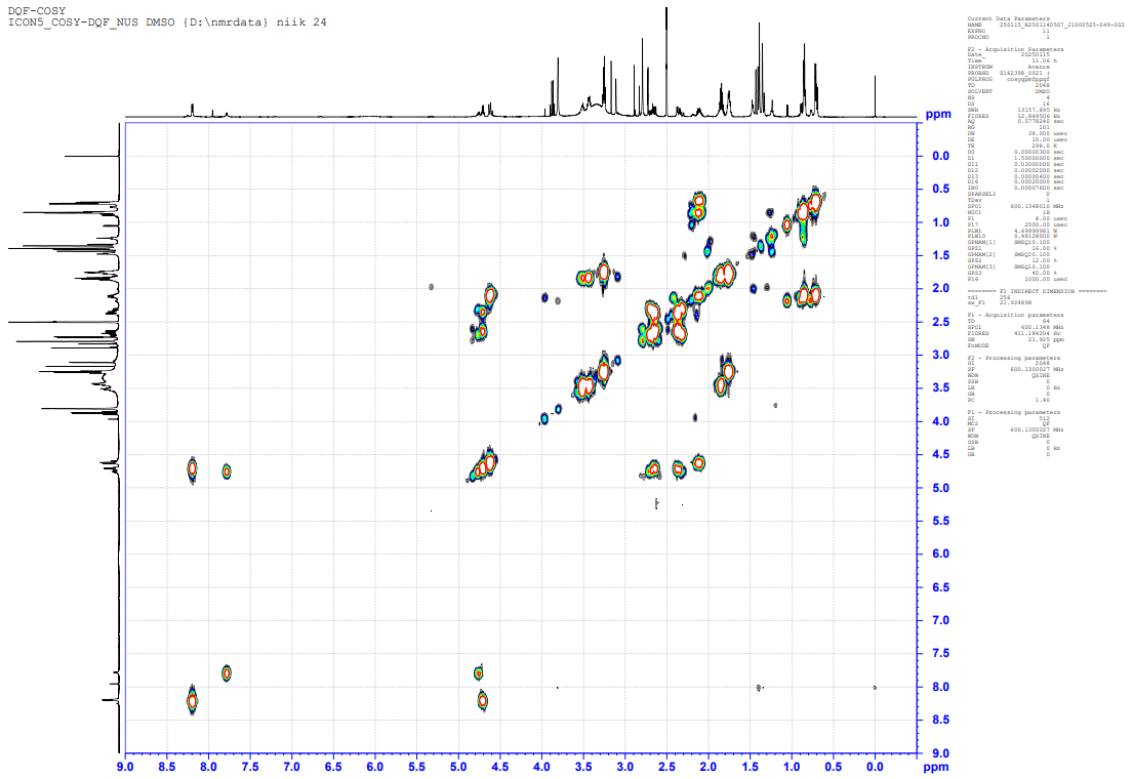
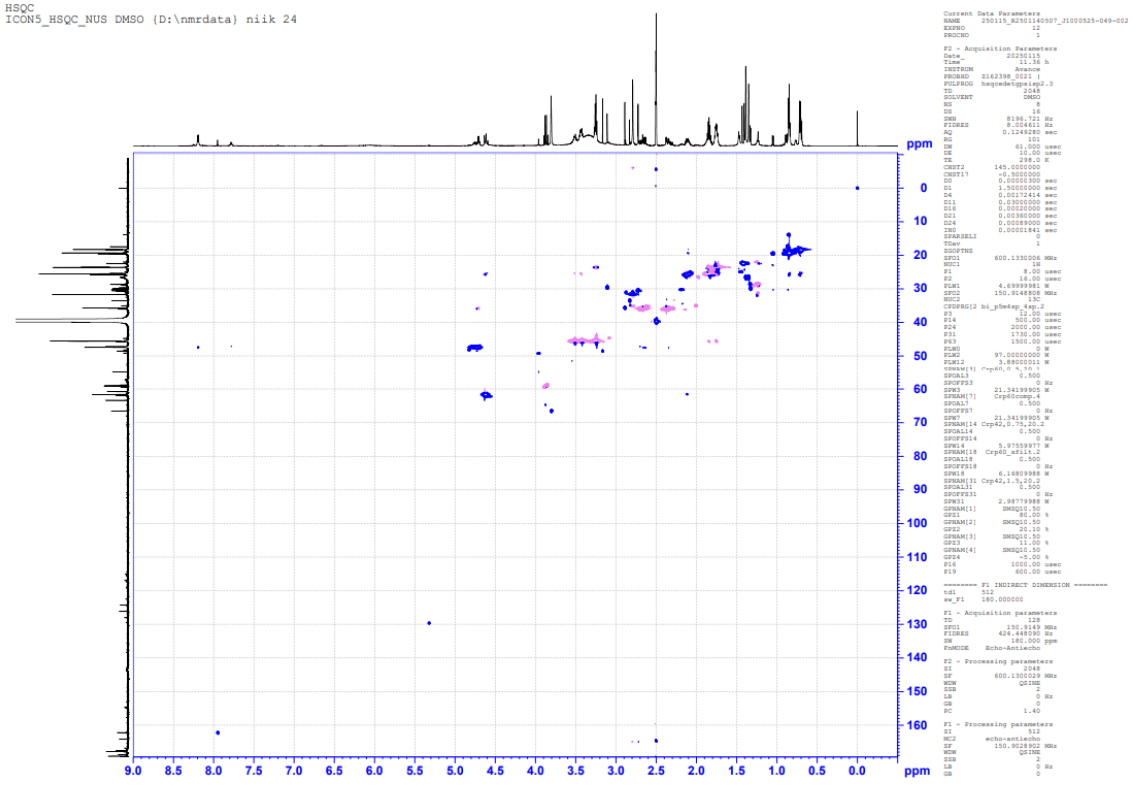
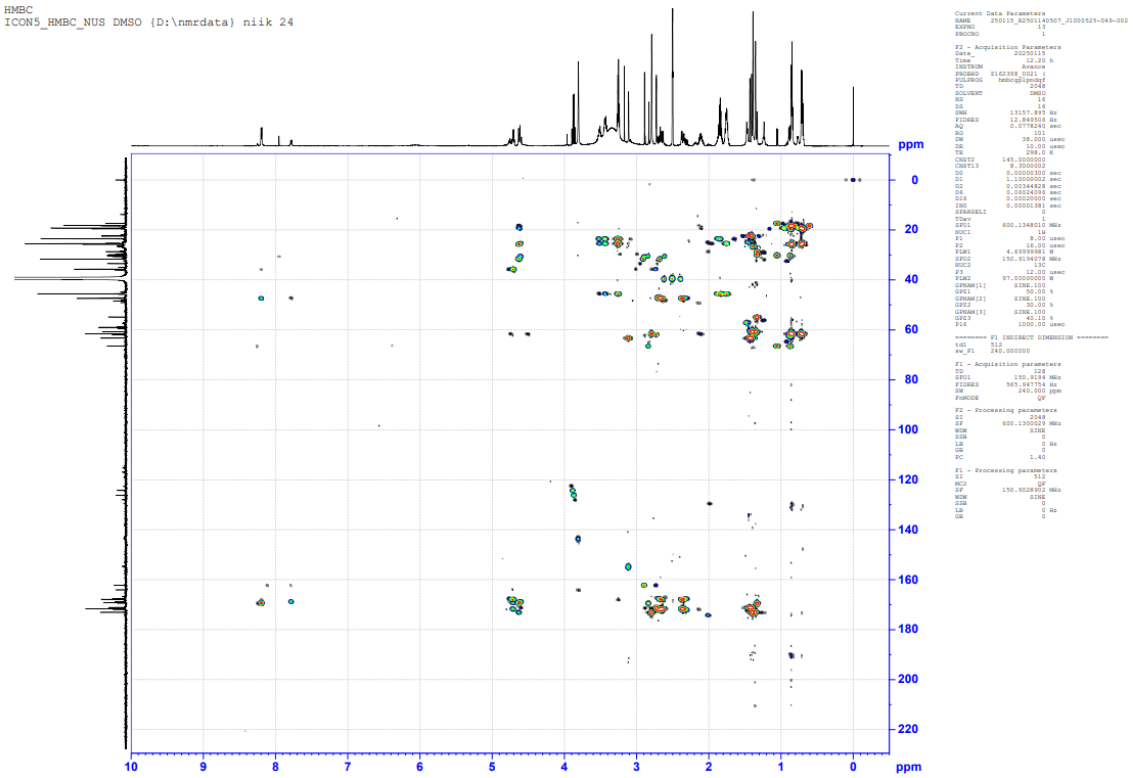
DFILE J1000525-049-002-CNMR.als
COMNT The peptides cleavaged from 29
DATIM 2025-01-16 19:06:41
OBNUC 13C
EXMOD zgpg30
OBFRQ 150.91 MHz
OBSET 9.40 KHz
OBFIN 7.84 Hz
POINT 65536
FREQU 35714.29 Hz
SCANS 6144
ACQTM 0.0000 sec
PD 0.0000 sec
PW1 10.00 usec
IRNUC
CTEMP 24.8 c
SLVNT DMSO
EXREF 0.00 ppm
BF 1.00 Hz
RGAIN 101
O-methylated
N-methylated
de-Tfa
Tfa-Aib-MeVal-Asp-pyrro
29
PPM

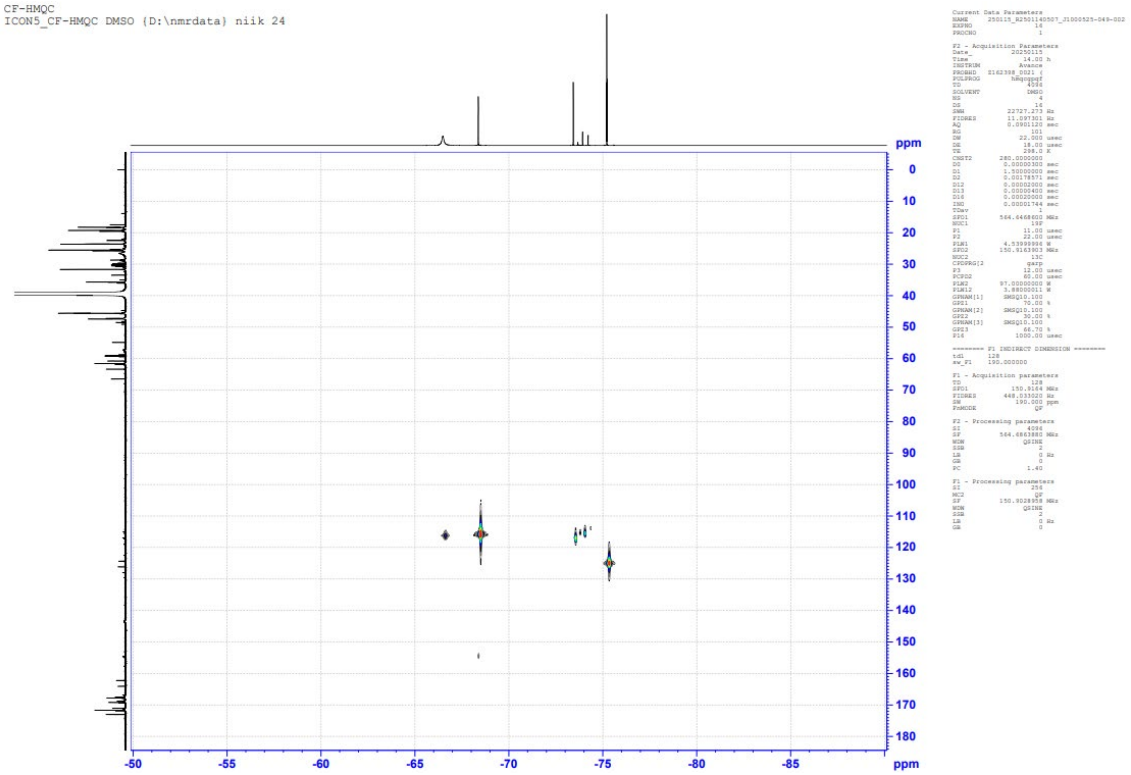

C:¥Users¥182489¥Downloads¥1DNMR (1)¥1DNMR¥15¥fid
DFILE fid
COMNT The peptides cleavaged from 29
DATIM 2025-01-15 10:58:36
OBNUC 19F
EXMOD zg
OBFRQ 564.62 MHz
OBSET 9.91 KHz
OBFIN 9.36 Hz
POINT 131072
FREQU 131578.95 Hz
SCANS 16
ACQTM 0.9961 sec
PD 5.0000 sec
PW1 11.00 usec
IRNUC
CTEMP 24.9 c
SLVNT DMSO
EXREF 16.51 ppm
BF 1.00 Hz
RGAIN 101
O-methylated
N-methylated
de-Tfa
Tfa-Aib-MeVal-Asp-pyrro
29
PPM

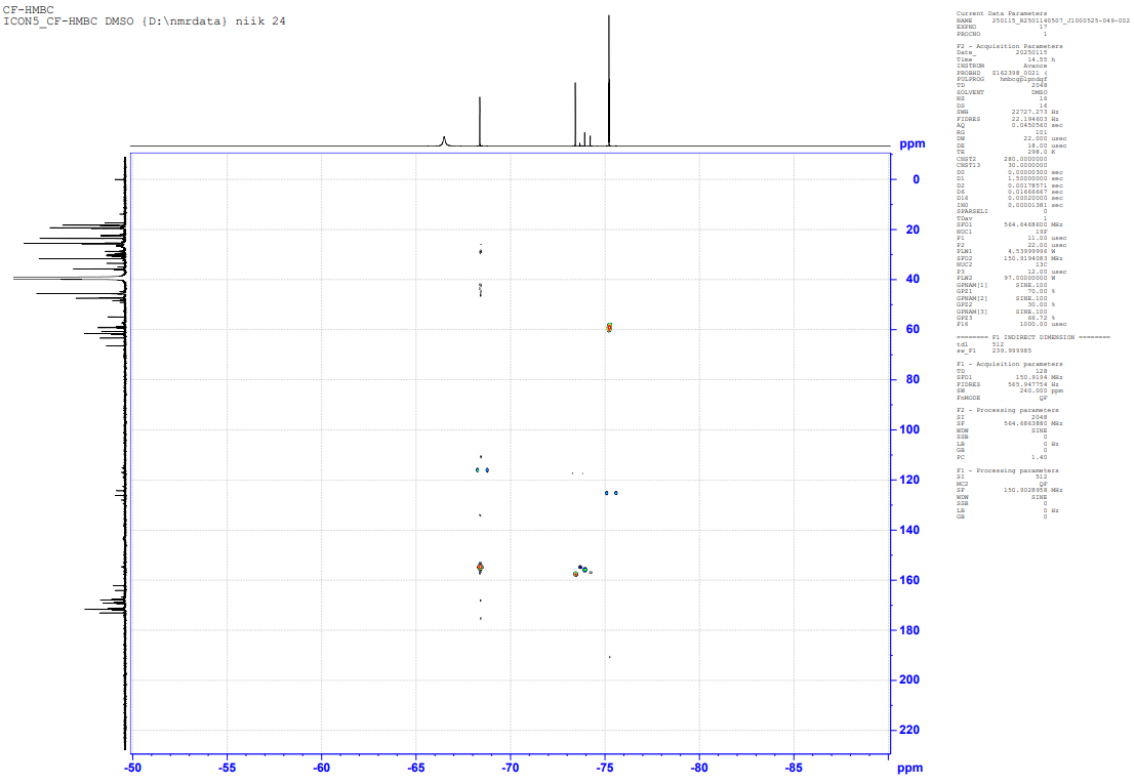

13C-dept135
ICON5_13C-dept135 DMSO {D:\nmrdata} niik 24
162.203
129.541
66.502
64.690
61.895
61.576
59.426
59.205
58.985
58.765
49.140
48.488
48.184
47.987
47.187
45.773
45.692
45.657
45.594
45.540
44.730
39.942
39.803
39.663
39.524
39.384
36.091
35.778
35.657
35.032
33.492
31.625
30.662
30.564
30.205
29.904
29.638
28.917
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

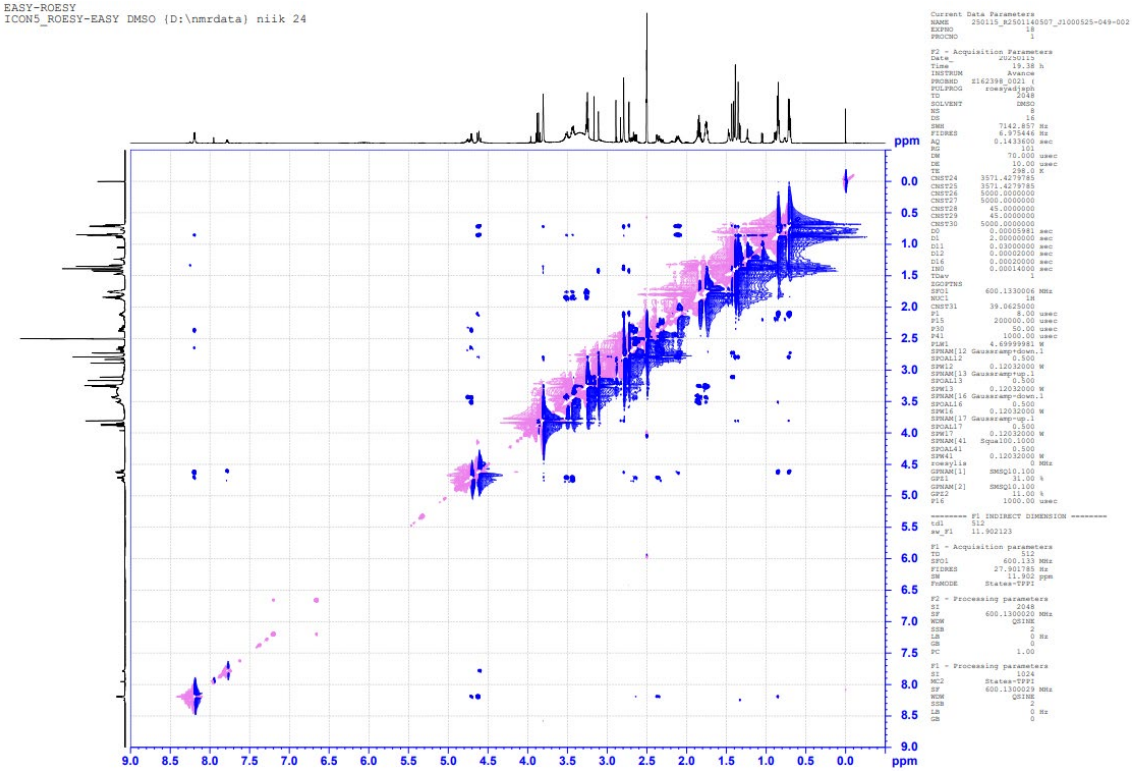

DQF-COSY
ICON5_COSY-DQF_NUS DMSO {D:\nmrdata} niik 24
ppm
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 ppm
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

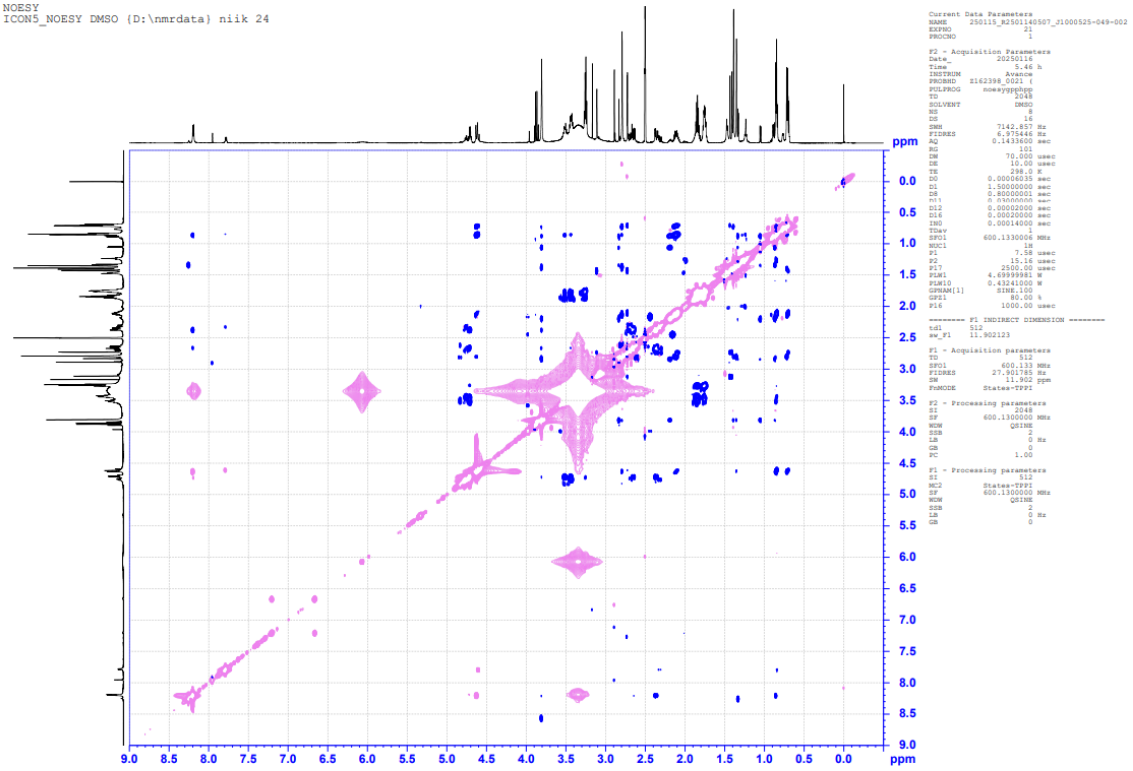

HSQC
ICON5_HSQC_NUS DMSO {D:\nmrdata} niik 24
ppm
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160

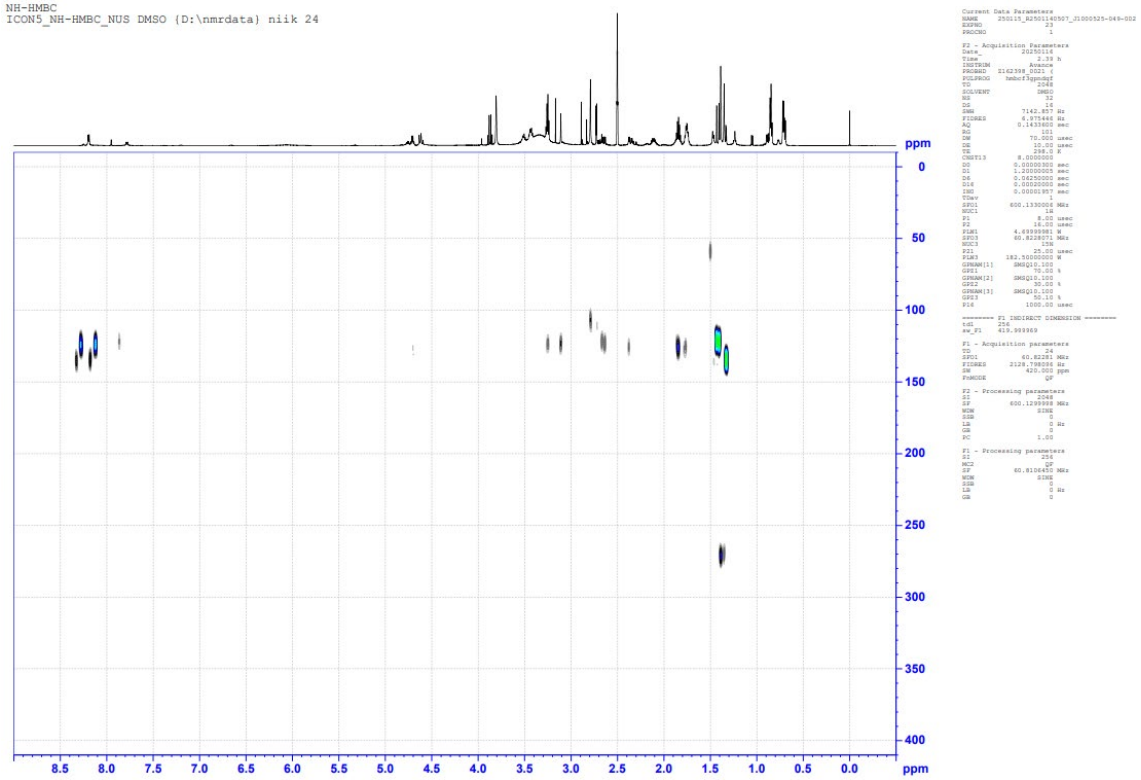

HMBC
ICON5_HMBC_NUS DMSO {D:\nmrdata} niik 24
ppm
10 9 8 7 6 5 4 3 2 1 0
ppm
0 20 40 60 80 100 120 140 160 180 200 220


CF-HMQC
ICON5_CF-HMQC DMSO (D:\nmrdata) niik 24
ppm
-50
-55
-60
-65
-70
-75
-80
-85
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180


CF-HMBC
ICON5_CF-HMBC DMSO (D:\nmrdata) niik 24
ppm
-50
-55
-60
-65
-70
-75
-80
-85
0
20
40
60
80
100
120
140
160
180
200
220


EASY-ROESY
ICON5_ROESY-EASY DMSO (D:\nmrdata) niik 24
ppm
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm


NOESY
ICON5_NOESY DMSO (D:\nmrdata) niik 24
ppm
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm


NH-HMBC
ICON5_NH-HMBC_NUS DMSO (D:\nmrdata) niik 24
ppm
0
50
100
150
200
250
300
350
400
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm