Supporting Information for

Backbone Cleavage of Peptides and Proteins via Cysteine *S*-Fluoroacetylation

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Table S1. Summary of the predicted pK_a values of carboxylic acids.[a]

carboxylic acid	predicted pK_a
acetic acid	4.79
formic acid	3.74
fluoroacetic acid	2.62
chlorofluoroacetic acid	1.51
dichloroacetic acid	1.37
difluoroacetic acid	1.32
trifluoroacetic acid	0.046

Strong electron-withdrawing acyl group

[a]The predicted pK_a values were calculated using Advanced Chemistry Development (ACD/Labs) Software (© 1994-2025 ACD/Labs).

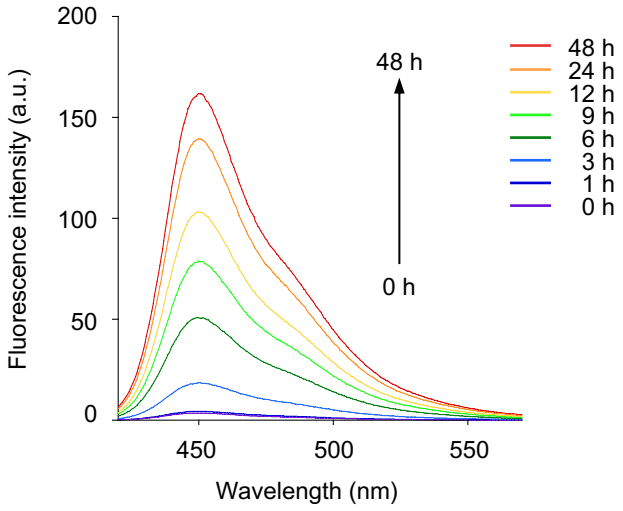


Figure S1. Fluorescence spectral change of peptide-a upon addition of *S*-fluoroacetylation reagent 1. Conditions: [peptide-**a**] = 2 μM, [**1**] = 1 mM, 100 mM sodium phosphate buffer (pH 7.4), 30% MeCN, 0.3 mM TCEP, 37 °C, λ_{ex} = 400 nm.

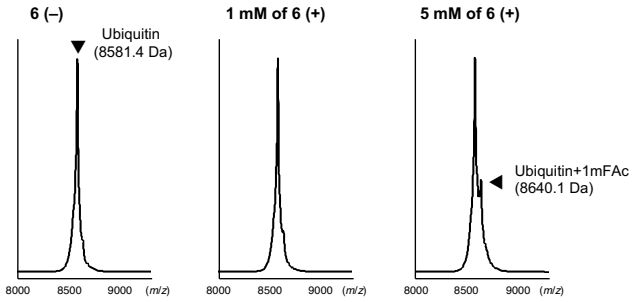


Figure S2. MALDI-TOF-MS analysis of ubiquitin in the presence or absence of 6. Ubiqutin (4 μM) was incubated with fluoroacetylation reagent **6** (1 or 5 mM) for 72 h in 100 mM sodium phosphate buffer (pH 7.4) containing 30% MeCN at 37 °C.

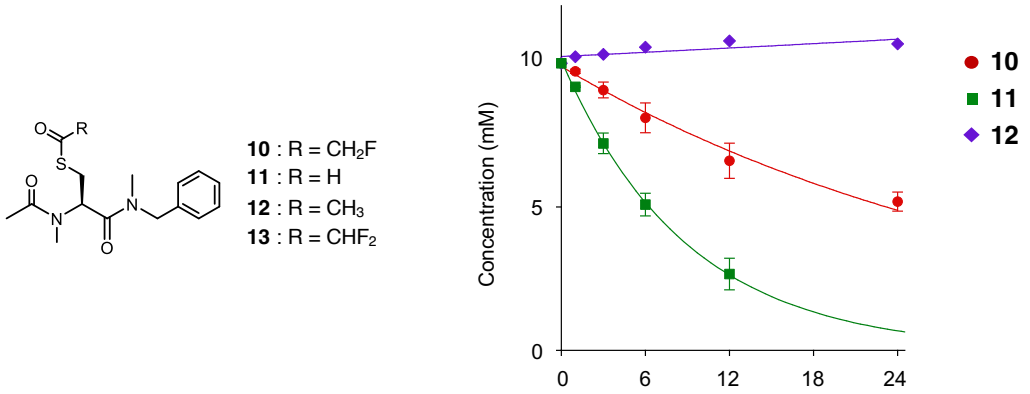


Figure S3. Time plot of the hydrolysis of *S*-acylated cysteine analyzed by ^{1}H-NMR. Conditions: [**10**-**13**] = 10 mM, 100 mM sodium deuterium phosphate buffer, 25% CD_3CN, pD 7.4, 37 °C, mean ± s.d.: three independent experiments. Hydrolysis of **13** cannot be not traced due to the rapid hydrolysis (< 1 h) under the measurement conditions (see Figure S4).

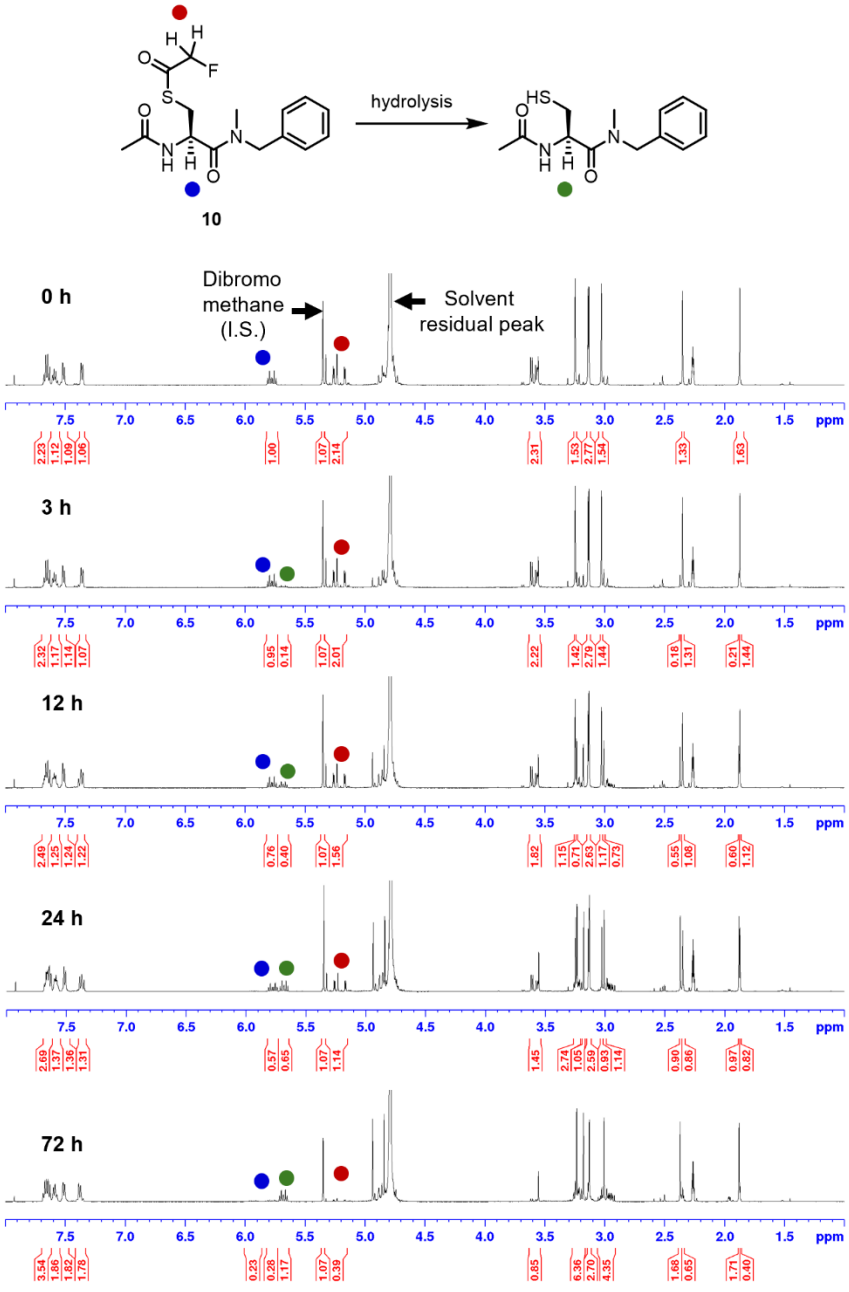


Figure S4. ^{1}H-NMR analysis of of aqueous stability of *S*-fluoroacetylated compound 10.

Time-course change of ^{1}H-NMR spectrum of **10** in 100 mM sodium deuterium phosphate buffer (25% CD_3CN, pD 7.4) at 37 °C. Dibromomethane was used as an internal standard (I. S.).

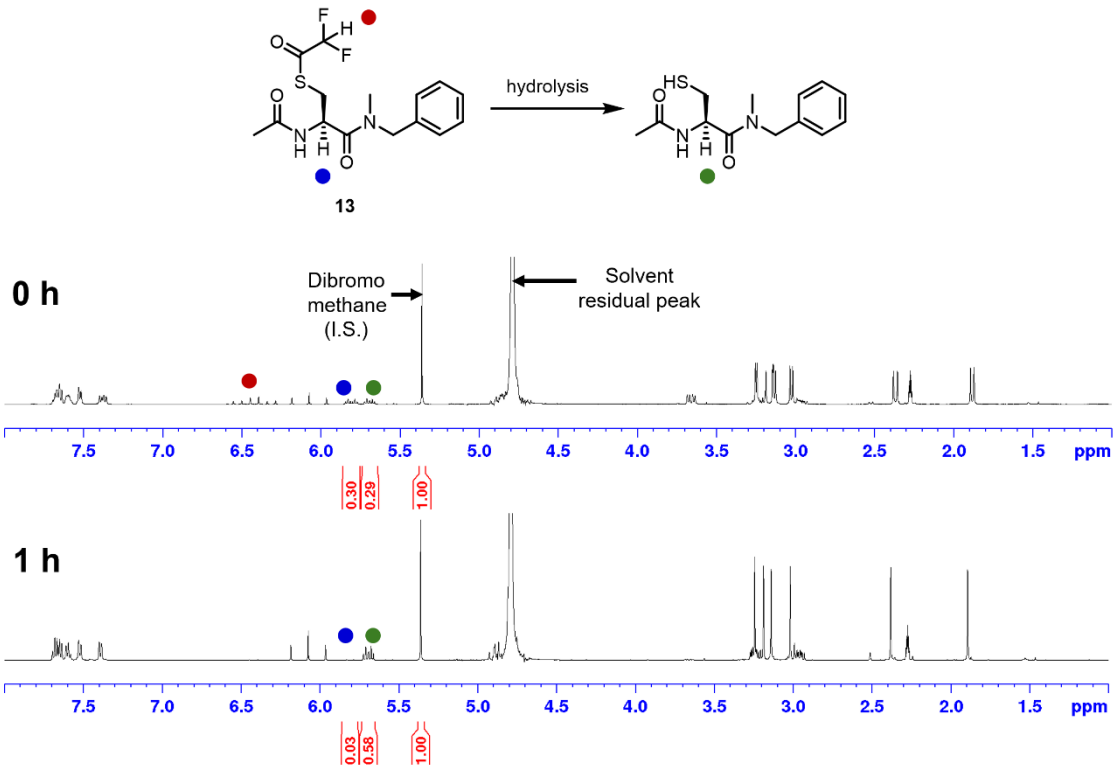


Figure S5. ^{1}H-NMR analysis of aqueous stability of *S*-difluoroacetylated compound 13. Time-course change of ^{1}H-NMR spectrum of **13** in 100 mM sodium deuterium phosphate buffer (25% CD_3CN, pD 7.4) at 37 °C. Dibromomethane was used as an internal standard (I. S.).

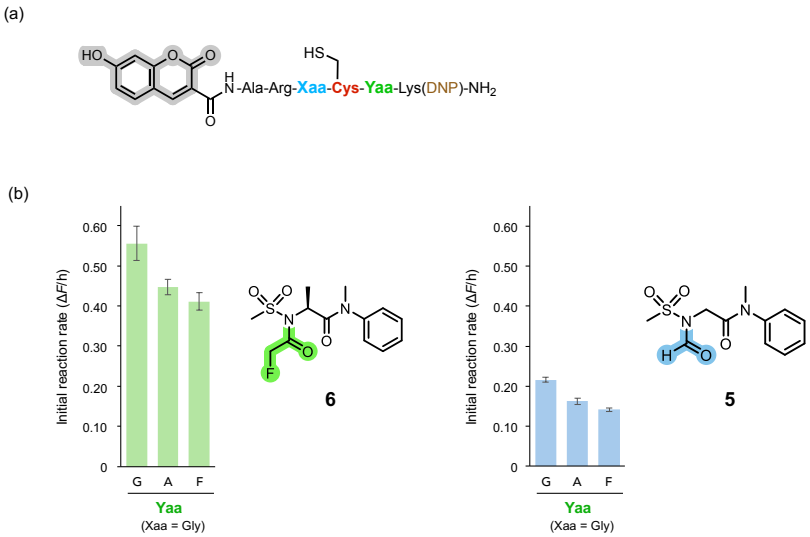


Figure S6. Sequence dependence of the peptide cleavage reaction induced by cysteine *S*-fluoroacetylation and *S*-formylation. (a) Peptide structure with different amino acids at Yaa position. (b, c) Initial reaction rates (0–3 h) of the peptide cleavage reaction (D*F*/h) upon treatment with reagents **6** (left) and **5** (right). Reactions were conducted with the peptide (15 μM) and the reagent (1 mM) in 100 mM sodium phosphate buffer (pH 7.4) containing 30% MeCN in the presence of 0.3 mM TCEP at 37 °C. l_{ex}/l_{em}: 400/450 nm. Data are expressed as the mean ± SD of three independent experiments.

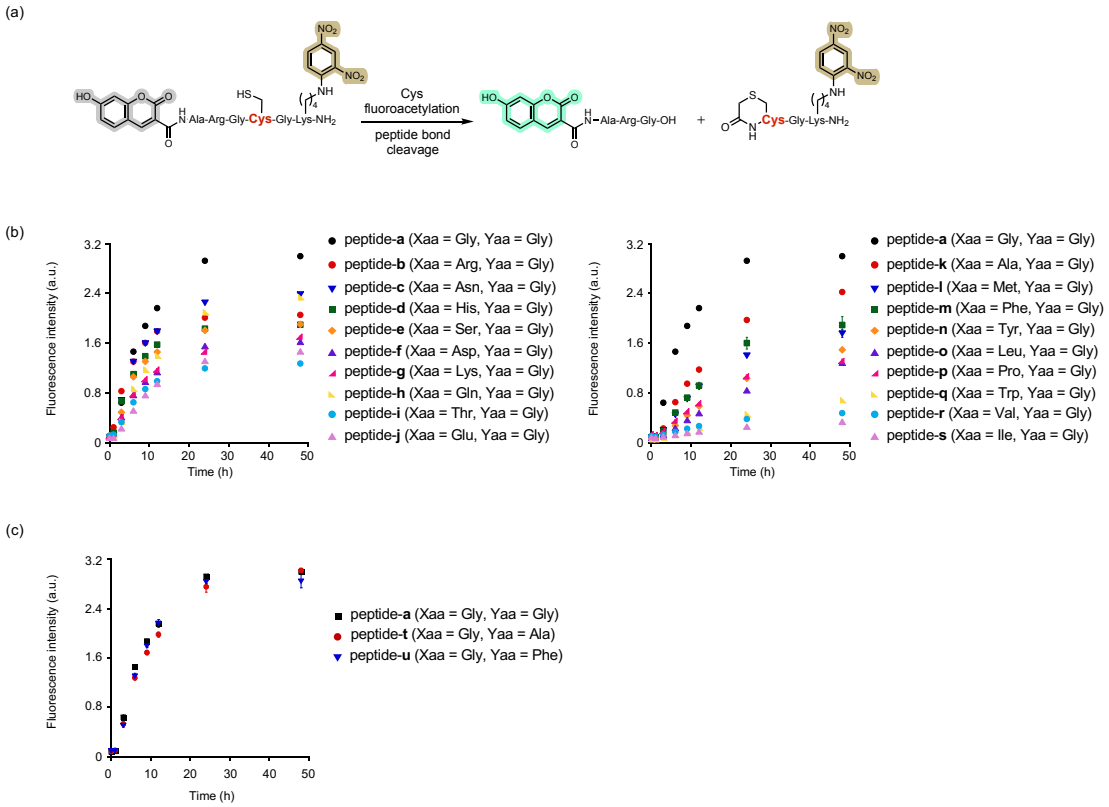


Figure S7. Fluorescence detection of the peptide cleavage induced by cysteine *S*-fluoroacetylation. (a) Reaction scheme of the peptide cleavage by cysteine fluoroacetylation. (b) Time plot of the cleavage reactions of peptide-**a**–**s** (Xaa-Cys-Gly) (15 μM) upon treatment with fluoroacetylation reagent **6** (1 mM). (c) Time-course plot of the cleavage reactions of peptide-**a**, **t** and **u** (Gly-Cys-Yaa, 15 μM) upon treatment with **6** (1 mM). Conditions: 100 mM sodium phosphate buffer (pH 7.4) containing 30% MeCN, 0.3 mM TCEP at 37 °C. $\lambda_{ex}/\lambda_{em}$ = 400/450 nm, mean ± s.d.: three independent experiments.

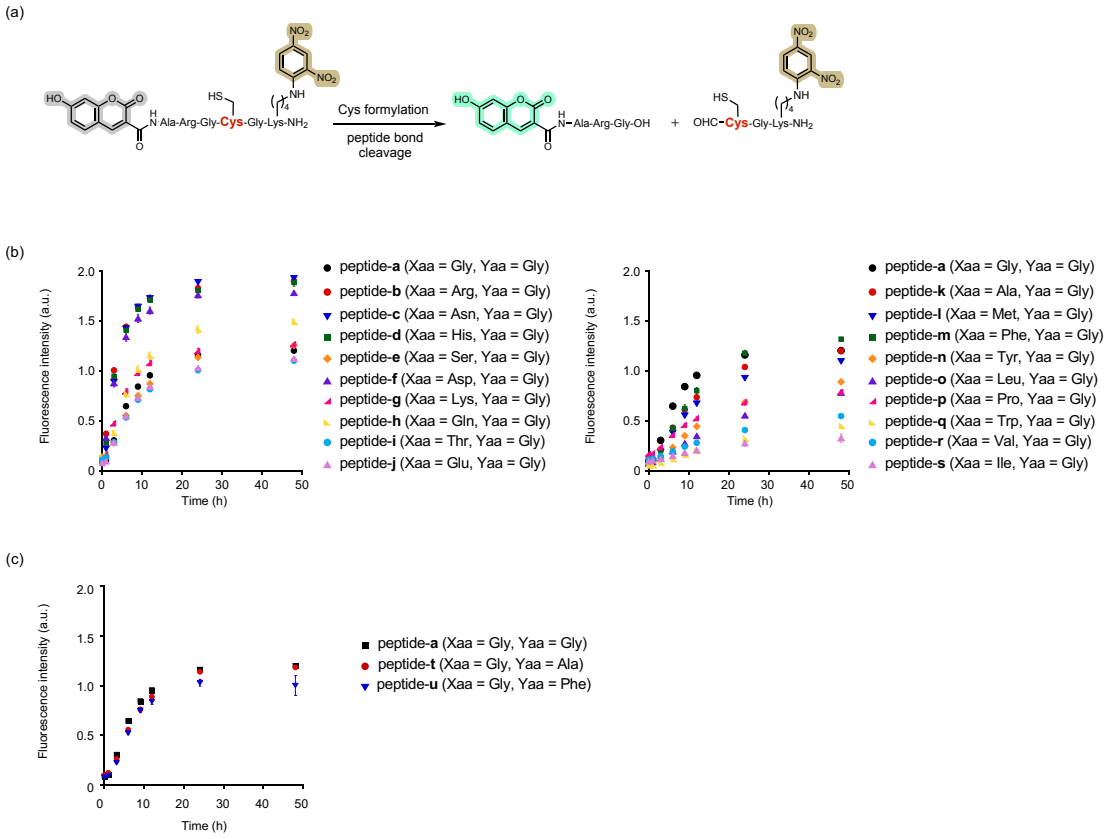


Figure S8. Fluorescence detection of the peptide cleavage reaction induced by cysteine *S*-formylation. (a) Reaction scheme of the peptide cleavage by cysteine formylation. (b) Time plot of the cleavage reactions of peptide-**a**–**s** (Xaa-Cys-Gly) (15 μM) upon treatment with formylation reagent **5** (1 mM). (c) Time-course plot of the cleavage reactions of peptide-**a**, **t** and **u** (Gly-Cys-Yaa, 15 μM) upon treatment with **5** (1 mM). Conditions: 100 mM sodium phosphate buffer (pH 7.4) containing 30% MeCN, 0.3 mM TCEP at 37 °C. $\lambda_{ex}/\lambda_{em}$ = 400/450 nm, mean ± s.d.: three independent experiments.

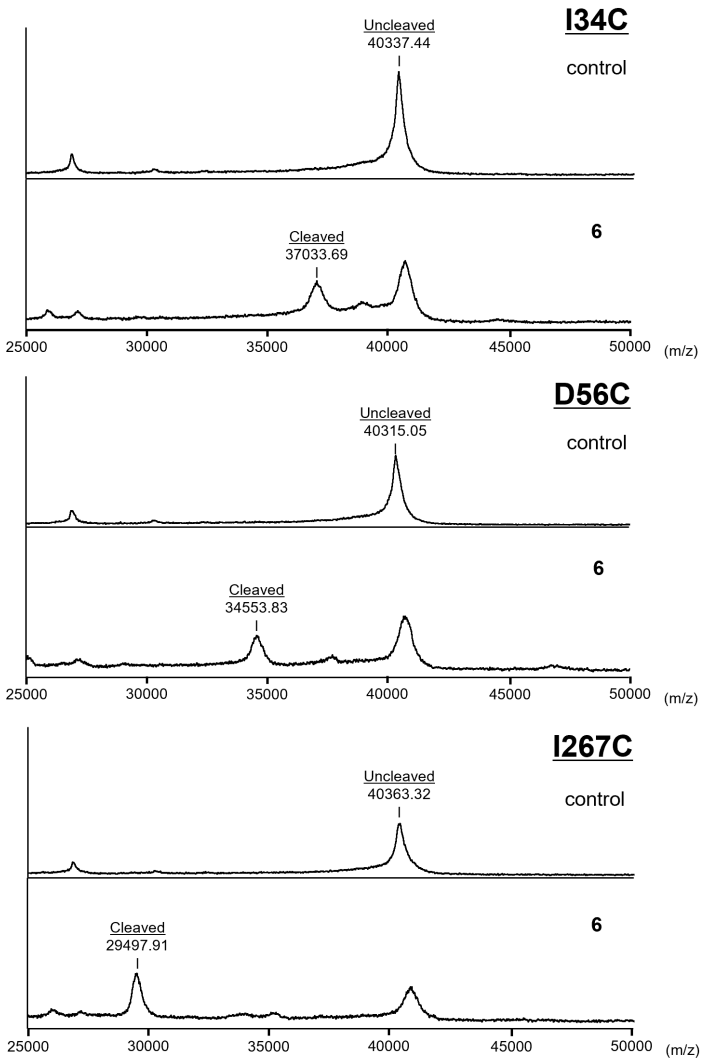


Figure S9. MALDI-TOF-MS analysis of the cleavage of the point mutated MBP by cysteine *S*-fluoroacetylation. The point mutated MBP I34C, D56C, and I267C (4 μM) was treated with **6** (5 mM) for 72 h in 100 mM sodium phosphate buffer (pH 7.4) containing 30% MeCN, 5 mM TCEP at 37 °C.

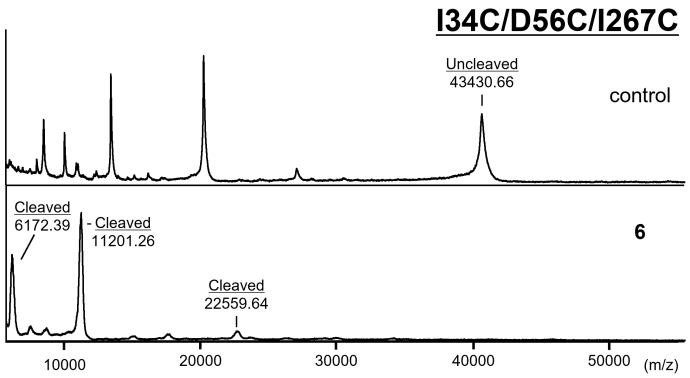


Figure S10. MALDI-TOF-MS analysis of the cleavage of the point mutated MBP by cysteine *S*-fluoroacetylation. The point mutated MBP I34C/D56C/I267C (4 μM) was treated with **6** (5 mM) for 72 h in 100 mM sodium phosphate buffer (pH 7.4) containing 30% MeCN, 5 mM TCEP at 37 °C.

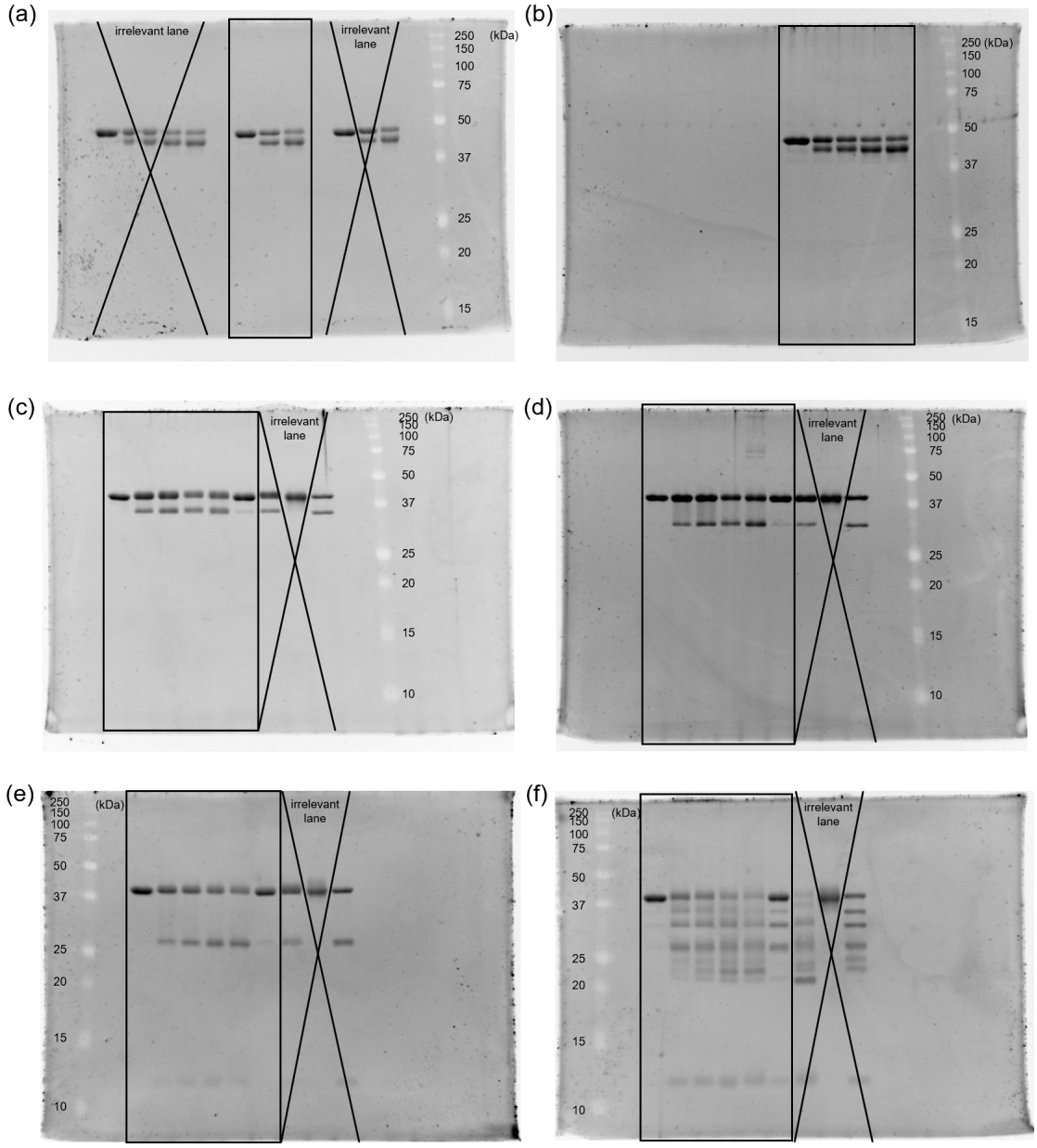


Figure S11. Raw gel electrophoresis images of protein cleavage by probes 5 and 6. Cropped gel images are shown in (a) Figure 4b, (b) Figure 4c, (c-e) Figure 4e, and (f) Figure 4f.

Experimental Method

Solid-Phase Peptide Synthesis

Peptides were synthesized by solid-phase peptide synthesis using standard Fmoc-based coupling chemistry. Coupling reactions (0.10 mmol scale) were performed using 3 equiv of amino acid, 4 equiv of HOBt and 4 equiv of HBTU on Rink Amide MBHA Resin (0.10 mmol, 0.76 mmol/g, Watanabe Chemical Industries, LTD.). *N*-terminal was acetylated by treatment with 2 equiv of 7-hydroxy coumarin *N*-succinimidyl ester and 1.5 equiv of DIEA in DMF. Peptide cleavage and deprotection were carried out by treatment of resin with TFA / H_2O / 1,2-ethanedithiol / triethylsilane (94 : 2.5 : 2.5 : 1) for 12 h at ambient temperature. The crude peptide was collected by precipitation in diethyl ether and purified by reverse-phase HPLC (column; YMC-Triant C18 250 x 10.0 mm) (YMC) using CH_3CN (0.1% TFA) / H_2O (0.1% TFA) solvent system with a linear gradient mode. Typical HPLC conditions are as follows; 5 / 95 (0 min) →5 / 95 (10 min) → 40 / 60 (50 min) →100 / 0 (60 min) →5 / 95 (70 min), flow rate 3.0 mL/min, UV detection at 220 nm. Purified peptide was lyophilized and stocked in a refrigerator (-30 °C).

[ESI-MS analysis]

peptide-**a**: $[M+H]^+$ calcd for $C_{38}H_{50}N_{13}O_{14}S^+$ 944.33; Found 944.30.

peptide-**b**: $[M+2H]^{2+}$ calcd for $C_{42}H_{60}N_{16}O_{14}S^{2+}$ 522.21; Found 522.21.

peptide-**c**: $[M+H]^+$ calcd for $C_{40}H_{53}N_{14}O_{15}S^+$ 1001.35; Found 1001.35

peptide-**d**: $[M+H]^+$ calcd for $C_{42}H_{54}N_{15}O_{14}S^+$ 1024.37; Found 1024.40.

peptide-**e**: $[M+H]^+$ calcd for $C_{39}H_{52}N_{13}O_{15}S^+$ 974.34; Found 974.34.

peptide-**f**: $[M+H]^+$ calcd for $C_{40}H_{52}N_{13}O_{16}S^+$ 1002.34; Found 1002.33.

peptide-**g**: $[M+H]^+$ calcd for $C_{42}H_{59}N_{14}O_{14}S^+$ 1015.41; Found 1015.40.

peptide-**h**: $[M+H]^+$ calcd for $C_{41}H_{55}N_{14}O_{15}S^+$ 1015.37; Found 1015.36.

peptide-**i**: $[M+H]^+$ calcd for $C_{40}H_{54}N_{13}O_{15}S^+$ 988.36; Found 988.40.

peptide-**j**: $[M+H]^+$ calcd for $C_{41}H_{54}N_{13}O_{16}S^+$ 1016.35; Found 1016.34.

peptide-**k**: $[M+H]^+$ calcd for $C_{39}H_{52}N_{13}O_{14}S^+$ 958.35; Found 958.33.

peptide-**l**: $[M+H]^+$ calcd for $C_{41}H_{56}N_{13}O_{14}S_2^+$ 1018.35; Found 1018.35.

peptide-**m**: $[M+H]^+$ calcd for $C_{45}H_{56}N_{13}O_{14}S^+$ 1034.38; Found 1034.36.

peptide-**n**: $[M+H]^+$ calcd for $C_{45}H_{56}N_{13}O_{15}S^+$ 1050.37; Found 1050.39.

peptide-**o**: $[M+H]^+$ calcd for $C_{42}H_{58}N_{13}O_{14}S^+$ 1000.39; Found 1000.42.

peptide-**p**: $[M+H]^+$ calcd for $C_{41}H_{54}N_{13}O_{14}S^+$ 984.36; Found 984.35.

peptide-**q**: $[M+H]^+$ calcd for $C_{47}H_{57}N_{14}O_{14}S^+$ 1073.39; Found 1073.43.

peptide-**r**: $[M+H]^+$ calcd for $C_{41}H_{56}N_{13}O_{14}S^+$ 986.38; Found 986.40.

peptide-**s**: $[M+H]^+$ calcd for $C_{42}H_{58}N_{13}O_{14}S^+$ 1000.39; Found 1000.42.

Fluorescence monitoring of peptide cleavage reaction.

In a 96-well plate, the stock solution of peptide (**a**–**u**) (2 mM) in DMF (1.5 μL) was diluted with 100 mM sodium phosphate buffer (139 μL, pH 7.4, supplemented with 0.3 mM TCEP, degassed) and MeCN (50 μL). A solution of acylation reagents (**1**–**6**) (20 mM) in MeCN (10 μL) was added to a peptide solution (200 μL). Fluorescence change was monitored by a multi plate reader (PerkinElmer, λ_{ex} /λ_{em} = 400/450 nm) at 37 °C.

HPLC analysis of peptide cleavage reaction by *S*-fluoroacetylation.

The peptide (10 μM) was incubated with fluoroacetylation reagent **1** or **6** (5 mM) in 100 mM sodium phosphate buffer containing 10% DMF and 0.3 mM TCEP (pH 7.4, degassed) at 37 °C. An aliquot of the reaction mixture (300 μL) was diluted with 400 μL 1% TFA aq. and 700 μL MeOH. The solution (200-400 μL) was injected into the reverse-phase HPLC using YMC-Triant C18 (YMC). Each peak was analyzed by ESI-MS (Bruker Daltonics) to identify the compound. HPLC conditions: mobile phase; MeCN (containing 0.1% TFA) / H_2O (containing 0.1% TFA) = 10/90 (0–10 min), 60/40 (50 min), 100/0 (60 min), 10/90 (75 min), flow rate = 1.0 mL/ min, λ_{abs} = 370 nm.

Evaluation of aqueous stability of *S*-acylated cysteine

A solution (600 μL) of *S*-acylated cysteine (**10**–**13**, 10 mM) in 100 mM sodium deuterium phosphate buffer (containing 25% CD_3CN, pD 7.4) was incubated at 37 °C and subjected to ^{1}H-NMR (500 Hz) measurement at the indicated times. For quantitative analysis, dibromomethane (20 mM) was used as an internal standard. The peak intensity plot was analyzed by first-order non-linear curve fitting to obtain the half-life time ($t_{1/2}$).

Evaluation of aqueous stability and electrophilic reactivity of fluoroacetylation reagent. A mixture of fluoroacetylation reagent (0.1 mM) was incubated with a nucleophile (1 mM, GSH or *N*-Ac-Lys) in 100 mM sodium phosphate buffer (pH7.4, degassed) containing 25% MeCN at 37 °C under nitrogen atmosphere. 1-Naphthoic acid or *m*-acetotoluidine (0.25 mM, were used as the internal standards. An aliquot of the reaction mixture (100 μL) was sampled at indicated times and diluted with 50 μL of 1 : 1 MeCN/H_2O (v/v) containing 0.1% TFA. The solution was analyzed by Nexera X2 ultra high-performance liquid chromatography (UHPLC) system (Shimadzu) using Kinetex® 2.6 μm EVO C18 100 Å Core-shell column. The peak intensity plot was analyzed by first-order non-linear curve fitting to obtain half-life time ($t_{1/2}$). UHPLC conditions: mobile phase; MeCN (containing 0.1% TFA) / H_2O (containing 0.1% TFA)

= 40/60 (0 min), 90/10 (15 min), 95/5 (16 min), 95/5 (18 min), 40/60 (20 min), 40/60 (23 min), flow rate = 0.5 mL/min, λ_{abs} = 230 nm.

Plasmid construction

The plasmid of Maltose Binding Protein (MBP) in pMal-c2x vector was purchased from addgene (#75286) and the three mutant vectors (I34C, D56C, I267C) were constructed using three pairs of primers (Table S2) to perform inverse polymerase chain reaction (PCR). After digestion of the template plasmid by the treatment with Dpn I, 5'-termini of the PCR product were phosphorylated by T4 polynucleotide kinase and then cyclized by DNA ligase to give the vector encoding cysteine point mutants of MBPs. DNA fragments of the triple mutant (I34C, D56C, I267C) was produced from each plasmid of the point mutants (Table S3) and assembled using NEBuilder HiFi DNA Assembly Tools (New England Biolabs) according to the manufacturer's protocol.

Table S2. Primer sequence used in inverse PCR.

primer	sequence
I34C forward	5'- TGCAAAGTCACCGTTGAGCATCCGGATAA -3'
I34C reverse	5'- TCCGGTATCTTTCTCGAATTTCTTACCGA -3'
D56C forward	5'- TGCGGACCTGACATTATCTTCTGGGCACA -3'
D56C reverse	5'- GCCAGTTGCGGCAACCTGTGGGAATTTCT -3'
I267C forward	5'- TGCAACGCCGCCAGTCCGAACAAAGAGCT -3'
I267C reverse	5'- TCCTGCGCTCAGCACGCCAACGAACGGTTT -3'

Table S3. Primer sequence used in NEBuilder DNA assembly.

primer	sequence
I34C fragment forward	5'- GCCCTGAAAGACGCGCAGACTTAATAG -3'
I34C fragment reverse	5'- GAATTTCTCTTCCAGTTTATCCGGATG -3'
D56C fragment forward	5'- GATAAACTGGAAGAGAAATTCCCACAG -3'
D56C fragment reverse	5'- GCCGTTGATGGTCATCGCTGTTTCGCC -3'
I267C fragment forward	5'- GAAACAGCGATGACCATCAACGGCCCG-3'
I267C fragment reverse	5'- CTGCGCGTCTTTCAGGGCTTCATC-3'

Protein expression and purification

The plasmids of MBPs were transformed into T7 Express *lysY/I*^{q} competent *E. coli* (New England Biolabs). Bacterial cultures were incubated in 2xYT containing 100 μg/mL ampicillin at 37 °C until OD600 reached 0.6 and were induced with 1 mM IPTG. Cultures were grown for 3 h at 37 °C while shaking at 150 rpm. The cultures were collected by centrifugation at 10000 rpm for 10 min, and the cell pellets were lysed using an ultra sonic homogenizer VP-300 in 50 mM HEPES (pH 7.4) containing 150 mM NaCl, 1 mM dithiothreitol (DTT), and 1 mM ethylenediaminetetraacetic acid (EDTA). Lysates were then clarified by centrifugation at 18,000 x g for 10 min at 4 °C. The supernatants were applied on MBPTrap HP column (Cytiva) and the column was washed with 5 column volumes wash buffer (50 mM HEPES, pH 7.4, 150 mM NaCl). Finally, MBPs were eluted in 0.5 column volume of elution buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 10 mM maltose) six times.

Protein cleavage induced by cysteine fluoroacetylation

A mixture of MBP (4 μM) and fluoroacetylation reagent **6** (1, 5 mM) in 50 mM HEPES, 150 mM NaCl buffer (pH7.4, degassed) containing 5 mM TCEP, and 30% MeCN was incubated at 37 °C. The reaction (20 μL) was quenched with 67 mM NEM in reaction buffer (12 μL) and diluted with SDS–PAGE loading buffer containing 100 mM TCEP (8 μL). After incubating for 1 h at 37 °C, the sample was resolved on 12 or 15% SDS–PAGE gel. The gel was subjected to ProteOrange Protein Gel Stain (Lumiprobe) and image analysis using a LAS-4000 lumino image analyzer (Fujifilm). For the MALDI-TOF-MS analysis, the cleavage reaction solution was quenched with 0.5% TFA aq. and desalinated with ZipTip C4 (Merck). The solution was mixed with matrix solution containing 10 mg/mL α-Cyano-4-hydroxycinnamic Acid (CHCA) and the mass spectrum was measured by MALDI-TOF-MS (JMS-S3000, JEOL).

Synthesis of compounds

General materials and methods for organic synthesis

Unless otherwise noted, chemical reagents were purchased from commercial suppliers (FUJIFILM Wako Pure Chemical Corporation, Tokyo Chemical Industry, Sigma-Aldrich, BLD Pharmatech Ltd.) and used without further purification. Reactions were carried out under a positive atmosphere of nitrogen, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on Merck TLC Silica gel 60 F254, using shortwave UV light as the visualizing agent. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker Advance III HD (500, 125 MHz for ^{1}H- and ^{13}C-NMR, respectively) spectrometer and chemical shifts (δ, ppm) were referenced to residual solvent peak ($CDCl_3$: 7.26 ppm; CD_3OD: 3.31 ppm in ^{1}H-NMR, $CDCl_3$: 77.16 ppm; CD_3OD: 49.00 ppm in ^{13}C-NMR). Mass spectrometry was recorded using a MicroTOF II (Bruker Daltonics) spectrometer and MALDI-TOF-MS (JMS-S3000, JEOL).

Preparation of fluoroacetyl chloride [S1]

Sodium fluoroacetate (1.0 eq.) and PCl_5 were stirred neat at 90 °C for 3 h. Fluoroacetyl chloride was obtained by distillation. The product was used without further purification.

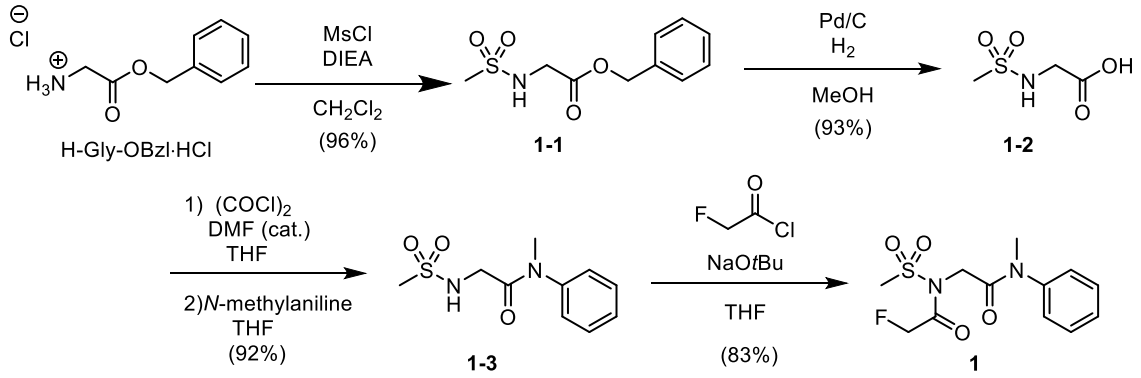
Preparation of fluoroacetyl acetic anhydride

To a stirred solution of sodium fluoroacetate (1.0 eq.) in dry Et_2O (10 mL) was added acetyl chloride (1.2 eq.) at 0 °C. After stirring for 10 min at 0 °C and 18 h at ambient temperature, NaCl was removed by filtration and the filtrate was concentrated in vacuo to give fluoroacetyl acetic anhydride (81% purity) as a colorless oil. The product was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.95 (d, $J_{(H\text{-}F)}$ = 46.5 Hz, 2H), 2.28 (s, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ 165.2, 163.9 (d, $^{2}J_{(C–F)}$ = 22.6 Hz), 77.5 (d, $^{1}J_{(C–F)}$ = 185.4 Hz), 22.3.

Preparation of chlorofluoroacetyl chloride

Chlorofluoroacetyl chloride was synthesized according to the procedure described in reference S2.



Synthesis of 1-1

To a stirred solution of H-Gly-OBzl HCl **(**1.50 g, 7.43 mmol) in dry CH_2Cl_2 (10 mL) was added DIEA (3.11 mL, 17.9 mmol) at 0 °C. After stirring for 10 min at the same temperature, to the mixture was added MsCl (864 μL, 11.2 mmol). After stirring for 3 h at ambient temperature, the reaction mixture was diluted with AcOEt and the organic phase was sequentially washed with 1N HCl aq., sat. $NaHCO_3$ aq., and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (Hexane : AcOEt = 2:1) to give **1-1** (1.74 g, 96%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.35 (m, 5H), 5.20 (s, 2H), 5.06 (brs, 1H), 3.99 (d, J = 5.8 Hz, 2H), 2.99 (s, 3H).**^{13}C NMR** (125 MHz, $CDCl_3$): δ 169.8, 134.9, 128.9, 128.8, 128.6, 67.8, 44.5, 41.5. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{10}H_{13}NO_4SNa^+$ 266.0457; Found 266.0478.

Synthesis of 1-2

A solution of **1-1** (1.74 g, 7.16 mmol) and 10 wt% Pd/C (174 mg) in MeOH (10 mL) was stirred overnight under H_2 atmosphere at ambient temperature. After removal of Pd/C by filtration, the filtrate was concentrated in vacuo to give **1-2** (1.02 g, 93%) as a white solid.

^{1}H NMR (500 MHz, CD_3OD): δ 3.87 (s, 2H), 3.00 (s, 3H).**^{13}C NMR** (125 MHz, CD_3OD): δ 173.1, 44.8, 41.3. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_3H_7NO_4SNa^+$ 175.9988; Found 175.9994. $[M-H]^-$ calcd for $C_3H_6NO_4S^-$ 152.0023; Found 152.0042.

Synthesis of 1-3

To a stirred solution of **1-2** (300 mg, 1.96 mmol) in dry THF (5 mL) was added oxalyl chloride (302 μL, 3.53 mmol) and DMF (four drops, cat.) at 0 °C. After stirring for 1 h at ambient temperature, to the solution was added another portion of oxalyl chloride (302 μL, 3.53 mmol).

The solution was azeotroped thrice with CH_2Cl_2. The residue was redissolved in dry THF (5 mL) and added *N*-methyl aniline (636 μL, 5.88 mmol). After stirring for 1 h at ambient temperature, to the solution was added another portion of *N*-methyl aniline (636 μL, 5.88 mmol). After stirring for 1 h at the same temperature, the reaction mixture was diluted with 1N HCl aq., and the aqueous phase was extracted thrice with $CHCl_3$/IPA (4/1). The combined organic layeres were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by Isolera One purification system (Rening 10 g cartridge, *n*-hexane/ AcOEt = 25/75 to 0/100) to give **1-3** (438 mg, 92%) as a yellow solid.

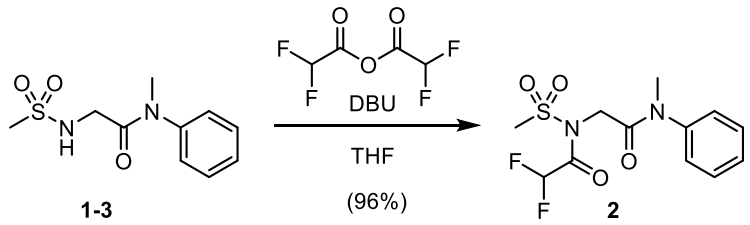
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48–7.45 (m, 2H), 7.43–7.40 (m, 1H), 7.21–7.19 (m, 2H), 5.22 (s, 1H), 3.63 (d, J = 5.0 Hz, 2H), 3.31 (s, 3H), 2.93 (s, 3H).**^{13}C NMR** (125 MHz, $CDCl_3$): δ 167.7, 141.6, 130.6, 129.1, 127.2, 45.0, 40.6, 37.9. **HRMS** (ESI) *m*/z $[M+Na]^+$ calcd for $C_{10}H_{14}N_2O_3SNa^+$ 265.0617; Found 265.0567.

Synthesis of 1

To a stirred solution of **1-3** (20 mg, 82.5 μmol) in dry THF (2 mL) was added NaO*t*Bu (9.52 mg, 99.1 μmol) at 0 °C. After stirring for 30 min at the same temperature, fluoroacetyl chrolide (8.65 μL, 124 μmol) was added and the mixture was stirred for 3 h at ambient temperature. After dilution with AcOEt, the organic phase was washed with 1N HCl aq. twice, sat. $NaHCO_3$ aq. twice and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (Hexane : AcOEt =3:1 to 3:2) to give **1** (20.6 mg, 83%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47 (t, J = 7.2 Hz, 2H), 7.41 (t, J = 7.4 Hz, 1H), 7.32 (d, J = 7.2 Hz, 2H), 5.22 (d, $J_{(H\text{-}F)}$ = 47.2 Hz, 2H), 4.27 (s, 2H), 3.44 (s, 3H) 3.28 (s, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 167.7 (d, $^2J_{(C–F)}$ = 20.0 Hz), 166.6, 142.1, 130.4, 129.0, 127.4, 80.1 (d, $^1J_{(C–F)}$ = 180.6 Hz), 48.0, 42.7, 37.8. **HRMS** (ESI) *m*/z $[M+Na]^+$ calcd for $C_{12}H_{15}FN_2O_4SNa^+$ 325.0629; Found 325.0637.

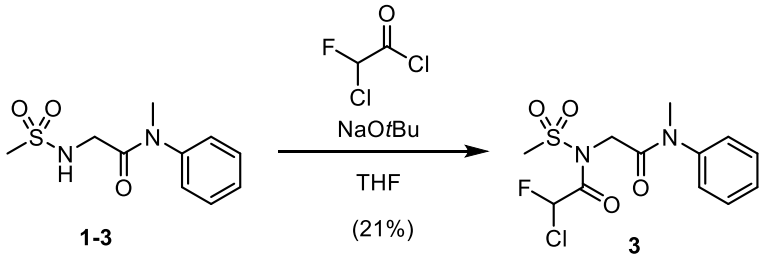
Synthesis of 2



To a stirred, ice-cooled solution of **1-3** (200 mg, 0.825 mmol) in dry THF (3 mL) was added DBU (185 μL, 1.24 mmol). The solution was stirred for 10 min at 0 °C. After adding difluoroacetic anhydride (180 μL, 1.65 mmol), the solution was stirred for 2 h at 0 °C to ambient temperature. After dilution with 1N HCl aq., the organic layer was extracted thrice with $CHCl_3$:IPA (4:1) followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane : AcOEt = 4:1) to give **2** (254 mg, 96%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46 (t, J = 7.2 Hz, 2H), 7.40 (t, J = 7.4 Hz, 1H), 7.29 (d, J = 7.4 Hz, 2H), 6.48 (t, $J_{(H–F)}$ = 52.9 Hz, 1H), 4.32 (s, 2H), 3.47 (s, 3H), 3.27 (s, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 166.0, 162.2 (t, $^2J_{(C–F)}$ = 27.8 Hz), 141.8, 130.4, 129.0, 127.2,107.4 (t, $^1J_{(C–F)}$ = 247.3 Hz), 48.0, 42.9, 37.8. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{12}H_{14}F_2N_2O_4SNa^+$ 343.0535; Found 343.0514.

Synthesis of 3

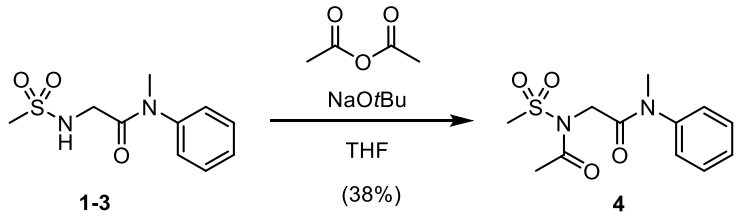


To a stirred, ice-cooled solution of **1-3** (70 mg, 0.289 mmol) in dry THF (1.5 mL) was added NaOtBu (33.3 mg, 0.347 mmol). The solution was stirred for 30 min at 0 °C. After adding chlorofluoroacetyl chloride (38.2 μL, 0.433 mmol), the solution was stirred for 6 h at 0 °C to ambient temperature. The reaction mixture was diluted with $CHCl_3$ and the organic layer was washed with 1N HCl aq. and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane : AcOEt =3:1 to 2:1) to give **3** (20.7 mg, 21%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48 (t, J = 7.4 Hz, 2H), 7.42 (t, J = 7.3 Hz, 1H), 7.30 (d, J =

7.4 Hz, 1H), 6.94 (br d, $J_{(H–F)}$ = 50.2 Hz, 1H), 4.35 (s, *2*H), 3.52 (s, 3H), 3.30 (s, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 166.1, 163.7 (d, $^2J_{(C–F)}$ = 25.6 Hz), 141.9, 130.5, 129.1, 127.3, 92.0 (d, $^1J_{(C–F)}$ = 250.4 Hz), 48.3, 42.6, 37.9. **HRMS** (ESI) *m*/z $[M+Na]^+$ calcd for $C_{12}H_{14}ClFN_2O_4SNa^+$ 359.0239; Found 359.0238.

Synthesis of 4

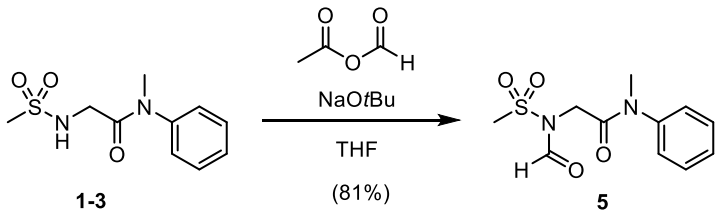


1-3 **4**

To a stirred, ice-cooled solution of **1-3** (25 mg, 0.103 mmol) in dry THF (2.5 mL) was added NaO*t*Bu (11.9 mg, 0.124 mmol). The solution was stirred for 30 min at 0 °C. After adding acetic anhydride (14.6 μL, 0.155 mmol), the solution was stirred for 3 h at 0 °C to ambient temperature. The reaction mixture was diluted with AcOEt and the organic layer was washed with 1N HCl aq. twice, sat. $NaHCO_3$ aq. and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane : AcOEt =2:1) to give **4** (11.8 mg, 38%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47 (t, *J* = 7.3 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 1H), 7.29 (d, *J* = 7.3 Hz, 1H), 4.29 (s, *2*H), 3.46 (s, 3H), 3.28 (s, 3H), 2.38 (s, 3H).**^{13}C NMR** (125 MHz, $CDCl_3$): δ 170.4, 167.2, 142.2, 130.4, 128.9, 127.3, 48.3, 42.3, 37.8, 24.2. **HRMS** (ESI) *m*/z $[M+Na]^+$ calcd for $C_{12}H_{16}N_2O_4SNa^+$ 307.0723; Found 307.0744.

Synthesis of 5

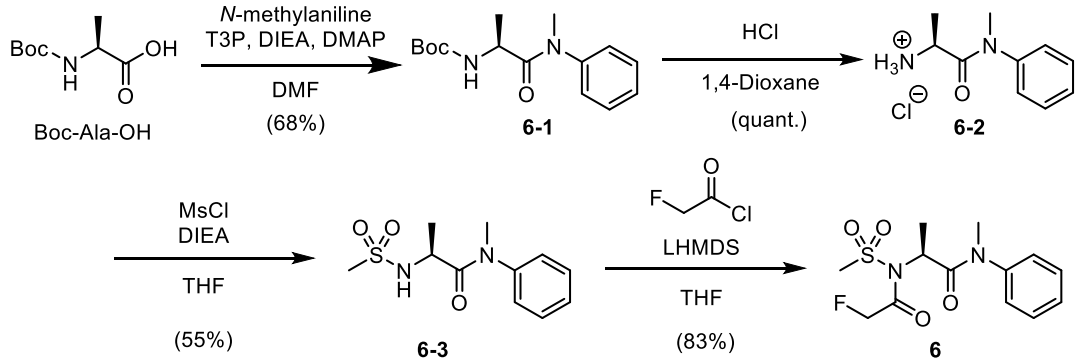


1-3 **5**

To a stirred, ice-cooled solution of **1-3** (25 mg, 0.103 mmol) in dry THF (2.5 mL) was added NaO*t*Bu (11.9 mg, 0.124 mmol). The solution was stirred for 30 min at 0 °C. After adding acetic formic anhydride (13.4 μL, 0.155 mmol), the solution was stirred for 3 h at 0 °C to ambient temperature. The reaction mixture was diluted and the organic layer was washed with 1N HCl aq.

twice, sat. $NaHCO_3$ aq. and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane : AcOEt =2:1) to give **5** (22.6 mg, 81%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (s, 1H), 7.47 (t, *J* = 7.3 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.32 (d, *J* = 7.3 Hz, 2H), 4.18 (s, *2*H), 3.38 (s, 3H), 3.28 (s, 3H).**^{13}C NMR** (125 MHz, $CDCl_3$): δ 165.9, 160.9, 142.1, 130.5, 129.0, 127.4, 44.6, 43.9, 37.8. **HRMS** (ESI) *m*/z $[M+Na]^+$ calcd for $C_{11}H_{14}N_2O_4SNa^+$ 293.0566; Found 293.0570.



Synthesis of 6-1

To a stirred, ice-cooled solution of Boc-L-Ala-OH (2.0 g, 10.6 mmol) in dry DMF (10 mL) was added *N*-methyl aniline (762.7 μL, 7.1 mmol) and DIEA (3.68 mL, 21.1 mmol). The solution was stirred for 10 min at 0 °C. After adding T3P (1.7 M in AcOEt, 6.22 mL, 10.6 mmol) and DMAP (344 mg, 2.8 mmol), the solution was stirred overnight at ambient temperature. The reaction mixture was diluted with AcOEt and the organic layer was washed with 1N HCl aq., sat. $NaHCO_3$ aq. and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt =2:1) to give **6-1** (1.34 g, 68%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46–7.26 (m, 5H), 5.27 (d, *J* = 5.5 Hz, 1H), 4.33 (brs, 1H), 3.27 (s, 3H), 1.41 (s, 9H), 1.10 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 173.4, 155.0, 142.9, 130.1, 128.4, 127.6, 79.4, 47.0, 38.0, 28.5, 19.3. **HRMS** (ESI) *m*/z $[M+Na]^+$ calcd for $C_{15}H_{22}N_2O_3Na^+$ 301.1523; Found 301.1517.

Synthesis of 6-2

6-1 (1.34 g, 4.80 mmol) was added 4N HCl/1,4-dioxane (12 mL, 48 mmol). After stirring overnight at ambient temperature, volatiles were removed in vacuo. The residue was redissolved

in CH_2Cl_2 and concentrated in vacuo twice to afford **6-2** (1.08 g, quant.) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.50 (brs, 3H), 7.47–7.38 (m, 5H), 4.16 (q, J = 6.8 Hz, 1H), 3.27 (s, 3H), 1.39 (d, J = 6.9 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 169.9, 141.8, 130.5, 128.9, 127.8, 47.6, 38.5, 16.9. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{10}H_{14}N_2ONa^+$ 201.0998; Found 201.0999.

Synthesis of 6-3

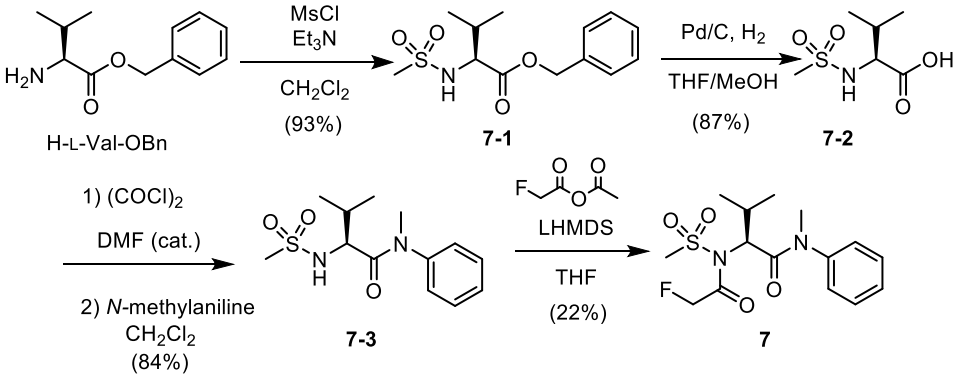
To a stirred, ice-cooled solution of **6-2** (201 mg, 938 μmol) in dry CH_2Cl_2 (4 mL) was added DIEA (196 μL, 1.13 mmol). The solution was stirred for 10 min at 0 °C. After adding MsCl (109 μL, 1.41 mmol), the solution was stirred overnight at ambient temperature. The reaction mixture was diluted with AcOEt and the organic layer was washed with 1N HCl aq., sat. $NaHCO_3$ aq. and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt =2:1 to 1:1) to give **6-3** (137.1 mg, 55%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.50–7.47 (t, J = 8.0 Hz, 2H), 7.43–7.40 (t, J = 8.0 Hz, 1H), 7.24 (d, J = 7.3 Hz, 2H), 5.84 (d, J = 9.1 Hz, 1H), 4.13–4.03 (m, 1H), 3.24 (s, 3H), 2.86 (s, 3H), 1.16 (d, J = 7.0 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 172.4, 142.2, 130.1, 128.6, 127.3, 49.4, 41.4, 37.9, 19.8. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{11}H_{16}N_2O_3SNa^+$ 279.0774; Found 279.0771.

Synthesis of 6

To a stirred, ice-cooled solution of **6-3** (179 mg, 0.630 mmol) in dry THF (2.5 mL) was added LHMDS (0.1M in THF, 1.13 mL, 1.13 mmol) dropwise. The solution was stirred for 30 min at 0 °C. After adding of fluoroacetyl chloride (65.9 μL, 0.940 mmol), the solution was stirred for 3 h at 0 °C to ambient temperature. The reaction mixture was diluted with AcOEt and the organic layer was washed with 1N HCl aq. twice, sat. $NaHCO_3$ aq. twice and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane : AcOEt =2:1) to give **6** (164.3 mg, 83%) as a brown amorphous material.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.45 (t, J = 7.5 Hz, 2H), 7.37 (t, J = 7.5 Hz, 1H), 7.30 (d, J = 7.5 Hz, 2H), 5.18 (q, J = 7.2 Hz, 1H), 5.09–4.99 (d, $J_{(H–F)}$ = 47.2 Hz, 2H), 3.28 (s, 3H), 3.27 (s, 3H), 1.39 (d, J = 7.3 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 169.5, 168.6 (d, $^2J_{(C–F)}$ = 19.5 Hz), 143.0, 130.2, 128.5, 127.4, 80.5 (d, $^1J_{(C–F)}$ = 179.8 Hz), 55.8, 43.6, 39.2, 17.3. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{13}H_{17}FN_2O_4SNa^+$ 339.0785; Found 339.0782.



Synthesis of 7-1

To a stirred, ice-cooled solution of H-L-Val-OBn (1.5 g, 6.15 mmol) in CH_2Cl_2 (30 mL) was added TEA (2.55 mL, 18.5 mmol) and MsCl (572 μL, 7.39 mmol). The solution was stirred for 0.75 h at ambient temperature. The reaction mixture was diluted with CH_2Cl_2 and the organic layer was washed with 1N HCl aq., sat. $NaHCO_3$ aq. and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo to give **7-1** (1.76 g, 93%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39–7.34 (m, 5H), 5.23 (d, J = 12.0 Hz, 1H), 5.19 (d, J = 12.0 Hz, 1H), 4.89 (brs, 1H), 3.97 (dd, J = 10.0, 4.7 Hz, 1H), 2.84 (s, 3H), 2.23–2.16 (m, 1H), 1.01 (d, J = 7.0 Hz, 3H), 0.84 (d, J = 7.0 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 172.1, 135.1, 128.92, 128.86, 128.7, 67.8, 61.4, 41.1, 31.5, 19.3, 17.1. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{13}H_{19}NO_4SNa^+$ 308.0927; Found 308.0908.

Synthesis of 7-2

A solution of **7-1** (1.63 g, 5.7 mmol) and 10 wt% Pd/C (162 mg) in MeOH (25 mL) was stirred for 1 h under H_2 atmosphere at ambient temperature. After removal of Pd/C by filtration, the filtrate was concentrated in vacuo to give **7-2** (972 mg, 87%) as a yellow oil. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 4.99 (d, J = 9.8 Hz, 1H), 4.03 (dd, J = 9.9, 4.4 Hz, 1H), 2.98 (s, 3H), 2.31–2.24 (m, 1H), 1.08 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 175.9, 61.1, 41.4, 31.3, 19.4, 17.1. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_6H_{13}NO_4SNa^+$ 218.0457; Found 218.0413. $[M-H]^-$ calcd for $C_6H_{12}NO_4S^-$ 194.0493; Found 194.0506.

Synthesis of 7-3

To a stirred, ice-cooled solution of **7-2** (218 mg, 1.1 mmol) in dry CH_2Cl_2 (5 mL) was added oxalyl chloride (172.4 μL, 2.0 mmol) and DMF (four drops). The solution was stirred for 1 h at ambient temperature. The solution was coevaporated with CH_2Cl_2 twice. The residue was added

dry CH_2Cl_2 (5 mL) and *N*-methylaniline (362.6 μL, 3.4 mmol). The solution was stirred overnight at ambient temperature. The reaction mixture was diluted with AcOEt and the organic layer was washed with 1N HCl aq., sat. $NaHCO_3$ aq. and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt = 3:2) to give **7-3** (268 mg, 84 %) as a white solid.

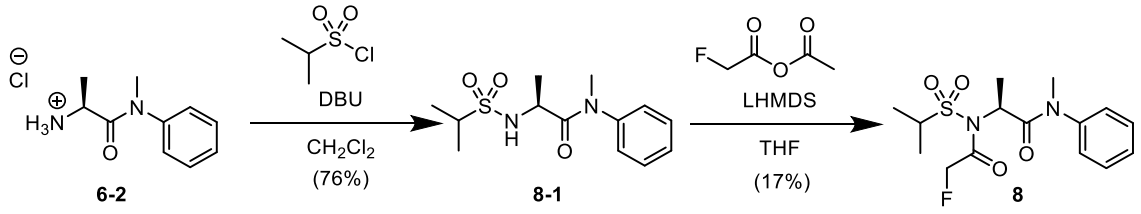
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49–7.46 (m, 2H), 7.41–7.38 (m, 1H), 7.26–7.25 (m, 2H), 5.20 (d, J = 9.7 Hz, 1H), 3.99 (dd, J = 6.0, 3.9 Hz, 1H), 3.32 (s, 3H), 2.91 (s, 3H), 1.80–1.74 (m, 1H), 0.80 (d, J = 6.9 Hz, 3H), 0.73 (d, J = 6.8 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 171.5, 142.4, 130.3, 128.6, 127.6, 58.6, 41.3, 38.3, 30.8, 20.0, 16.2.

HRMS (ESI) *m*/z $[M+Na]^+$ calcd for $C_{13}H_{20}N_2O_3SNa^+$ 307.1087; Found 307.1098.

Synthesis of 7

To a stirred, ice-cooled solution of **7-3** (59.6 mg, 0.21 mmol) in dry THF (1.8 mL) was added LHMDS (1M in THF, 0.23 mL, 0.23 mmol) dropwise. The solution was stirred for 10 min at 0 °C. After adding acetic fluoroacetic anhydride (30.2 μL, 0.31 mmol), the solution was stirred for 2 h at 0 °C to ambient temperature. The reaction mixture was diluted with AcOEt and the organic phase was washed with 1N HCl aq., sat. $NaHCO_3$ aq. × 2 and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt =2:1) to give **7** (16.0 mg, 22 %) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.45 (t, J = 7.2 Hz, 2H), 7.39 (t, J = 7.2 Hz, 1H), 7.18 (d, J = 7.2 Hz, 2H), 5.16 (dd, $J_{(H–F)}$ = 47.0, $J_{(H–H)}$ = 15.0 Hz, 1H), 4.95–4.83 (m, 2H), 3.31 (s, 6H), 2.72–2.64 (m, 1H), 1.02 (d, J = 6.4 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 169.1 (d, $^2J_{(C–F)}$ = 18.8 Hz), 167.9, 142.4, 130.3, 128.8, 127.6, 81.0 (d, $^1J_{(C–F)}$ = 181.0 Hz), 61.6, 43.24, 43.21, 38.7, 29.3, 20.7, 18.6. **HRMS** (ESI) *m*/z $[M+Na]^+$ calcd for $C_{15}H_{21}FN_2O_4SNa^+$ 367.1098; Found 367.1111.



Synthesis of 8-1

To a stirred, ice-cooled solution of **6-2** (178 mg, 0.83 mmol) in dry CH_2Cl_2 (7 mL) was added DBU (448 μL, 3.0 mmol). The solution was stirred for 10 min at 0 °C. After adding

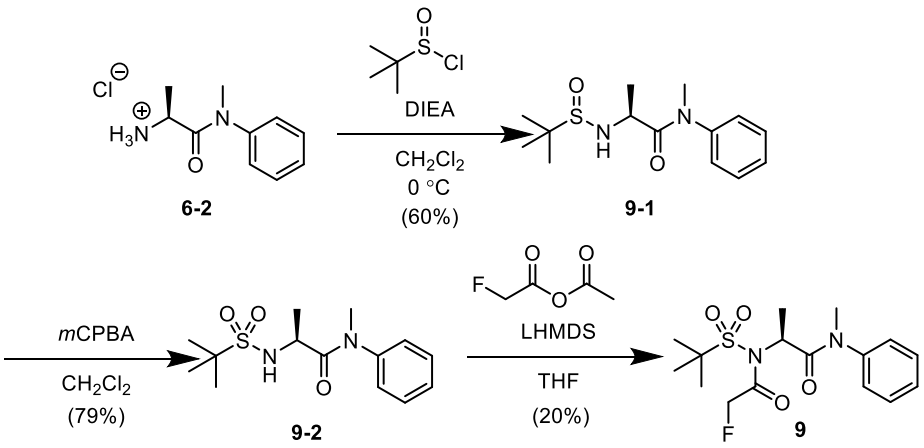
isopropylsulfonyl chloride (167 μL, 1.5 mmol), the solution was stirred overnight at 0 °C to ambient temperature. The reaction mixture was diluted with CH_2Cl_2 and the organic layer was washed with 1N HCl aq., sat. $NaHCO_3$ aq. and brine followed by drying over $MgSO_4$. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt =1:1) to give **8-1** (178 mg, 76 %) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48–7.45 (m, 2H), 7.41–7.38 (m, 1H), 7.26–7.25 (m, 2H), 5.02 (d, J = 9.2 Hz, 1H), 4.14–4.08 (m, 1H), 3.29 (s, 3H), 3.05–2.97 (m, 1H), 1.33 (dd, J = 6.8, 2.0 Hz, 6H), 1.18 (d, J = 6.9 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 172.8, 142.5, 130.3, 128.7, 127.5, 54.4, 50.0, 38.1, 20.7, 16.8, 16.7. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{13}H_{20}N_2O_3SNa^+$ 307.1087; Found 307.1086.

Synthesis of 8

To a stirred, ice-cooled solution of **8-1** (56.9 mg, 0.20 mmol) in dry THF (1.8 mL) was added LHMDS (1M in THF, 0.22 mL, 0.22 mmol). The solution was stirred for 10 min at 0 °C. After adding acetic fluoroacetic anhydride (28.8 μL, 0.30 mmol), the solution was stirred for 2 h at 0 °C to ambient temperature. The reaction mixture was diluted with AcOEt and the organic phase was washed with 1N HCl aq., sat. $NaHCO_3$ aq. × 2 and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt =2:1) to give **8** (11.8 mg, 17 %) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47–7.44 (m, 2H), 7.39–7.36 (m, 1H), 7.32–7.30 (m, 2H), 5.11 (d, $J_{(H–F)}$ = 47.6 Hz, 2H), 5.02 (q, J = 7.2 Hz, 1H), 3.70 (m, 1H), 3.29 (s, 3H), 1.44 (dd, J = 6.8, 5.3 Hz, 6H), 1.36 (d, J = 7.3 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 169.5 (d, $^2J_{(C–F)}$ = 19.9 Hz), 169.4, 143.1, 130.2, 128.5, 127.5, 80.6 (d, $^1J_{(C–F)}$ = 178.5 Hz), 57.2, 56.1, 39.2, 17.7, 16.5, 16.4. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{15}H_{21}FN_2O_4SNa^+$ 367.1098; Found 367.1095.



Synthesis of 9-1

To a stirred, ice-cooled solution of *tert*-butylsulfinyl chloride (104 µL, 0.84 mmol) was added **6-2** (271 mg, 1.3 mmol) in dry CH_2Cl_2 (9 mL) and DIEA (395 µL, 2.3 mmol) at 0 °C. After stirring for 2 h at the same temperature, the reaction mixture was diluted with AcOEt. The organic phase was washed with 1N HCl aq., sat. $NaHCO_3$ aq. and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo to give **9-1** (215 mg, 60%) as a yellow oil, which was used in the next step without further purification.

HRMS (ESI) *m*/z $[M+Na]^+$ calcd for $C_{14}H_{22}N_2O_2SNa^+$ 305.1294; Found 305.1282.

Synthesis of 9-2

To a stirred, ice-cooled solution of **9-1** (113 mg, 0.40 mmol) in dry CH_2Cl_2 (5 mL) was added *m*CPBA (98.3 mg, 0.40 mmol) at 0 °C. After stirring for 2 h at 0 °C to ambient temperature, the mixture was diluted with AcOEt. The organic phase was washed with 1N HCl aq., sat. $NaHCO_3$ aq. × 2 and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt = 2:1) to give **9-2** (94.5 mg, 79 %) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48–7.44 (m, 2H), 7.41–7.38 (m, 1H), 7.28–7.26 (m, 2H), 4.92 (d, *J* = 9.6 Hz,1H), 4.13–4.07 (m, 1H), 3.30 (s, 3H), 1.33 (s, 9H), 1.19 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 173.1, 142.5, 130.2, 128.7, 127.6, 59.7, 50.7, 38.1, 24.2, 20.9. **HRMS** (ESI) *m*/z $[M+Na]^+$ calcd for $C_{14}H_{22}N_2O_3SNa^+$ 321.1243; Found 321.1247.

Synthesis of 9

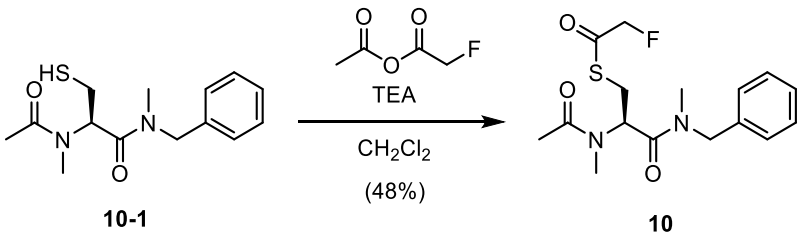
To a stirred, ice-cooled solution of **9-2** (93.7 mg, 0.31 mmol) in dry THF (3 mL) was added LHMDS (1M in THF, 0.35 mL, 0.35 mmol) at 0 °C. The solution was stirred for 10 min and was

added acetic fluoroacetic anhydride (45.3 μL, 0.47 mmol). After stirring for 2 h at 0 °C to ambient temperature, the reaction mixture was diluted with AcOEt. The organic phase was washed with 1N HCl aq., sat. $NaHCO_3$ aq. × 2 and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt =2:1) to give **9** (22.6 mg, 20%) as a white amorphous material.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46–7.43 (m, 2H), 7.38–7.35 (m, 1H), 7.26–7.24 (m, 2H), 5.38–5.20 (m, 2H), 4.86 (q, J = 7.0 Hz, 1H), 3.30 (s, 3H), 1.48 (s, 9H), 1.31 (d, J = 7.1 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 169.9 ($^2J_{(C–F)}$ = 19.6 Hz), 169.1, 143.5, 130.2, 128.4, 127.4, 80.7 ($^1J_{(C–F)}$ = 177.0 Hz), 64.7, 59.7, 39.4, 24.4, 18.6.

HRMS (ESI) m/z $[M+Na]^+$ calcd for $C_{16}H_{23}FN_2O_4SNa^+$ 381.1225; Found 381.1295.

Synthesis of **10**



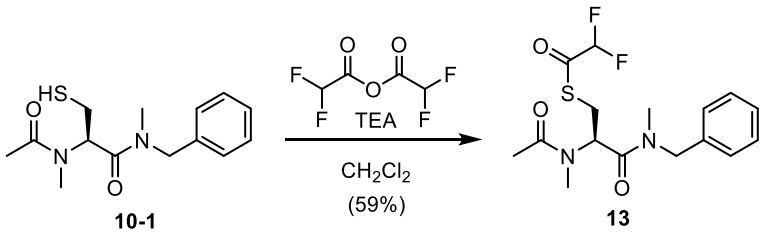
To a stirred, ice-cooled solution of **10-1**[S3] (42.3 mg, 0.15 mmol) in dry CH_2Cl_2 (1.5 mL) was added TEA (22.9 μL, 0.17 mmol) and acetic fluoroacetic anhydride (17.4 μL, 0.18 mmol) at 0 °C. After stirring for 1 h at 0 °C to ambient temperature, the reaction mixture was diluted with AcOEt. The orgainc phase was washed with 1N HCl aq., sat. $NaHCO_3$ aq. × 2 and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt =2:3) to give **10** (24.6 mg, 48%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers): δ 7.36–7.27 (m, 3H), 7.25–7.22 (m, 1H), 7.08 (d, J = 7.4 Hz, 1H), 5.76–5.68 (m, 1H), 4.96–4.74 (m, 2H), 4.70–4.46 (m, 2H), 3.45–3.29 (m, 2H), 3.01 (s, 1.4H), 2.914 (s, 1.6H), 2.906 (s, 1.6H), 2.80 (s, 1.4H), 2.10 (s, 1.6H), 1.66 (s, 1.4H).**^{13}C NMR** (125 MHz, $CDCl_3$): δ 196.8, 196.7, 196.6, 196.5, 171.1, 170.7, 169.8, 169.2, 136.9, 136.7, 128.9, 128.3, 127.8, 127.5, 126.0, 85.35, 85.31, 83.9, 83.8, 52.8, 51.9, 51.7, 51.4, 35.0, 34.7, 31.4, 31.3, 27.10, 27.07, 27.04, 27.01, 21.9, 21.2. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{16}H_{21}FN_2O_3SNa^+$ 363.1149; Found 363.1160.

Synthesis of 11,12

Reagents **11** and **12** were prepared according to the procedure reported in our previous publication.[S3]

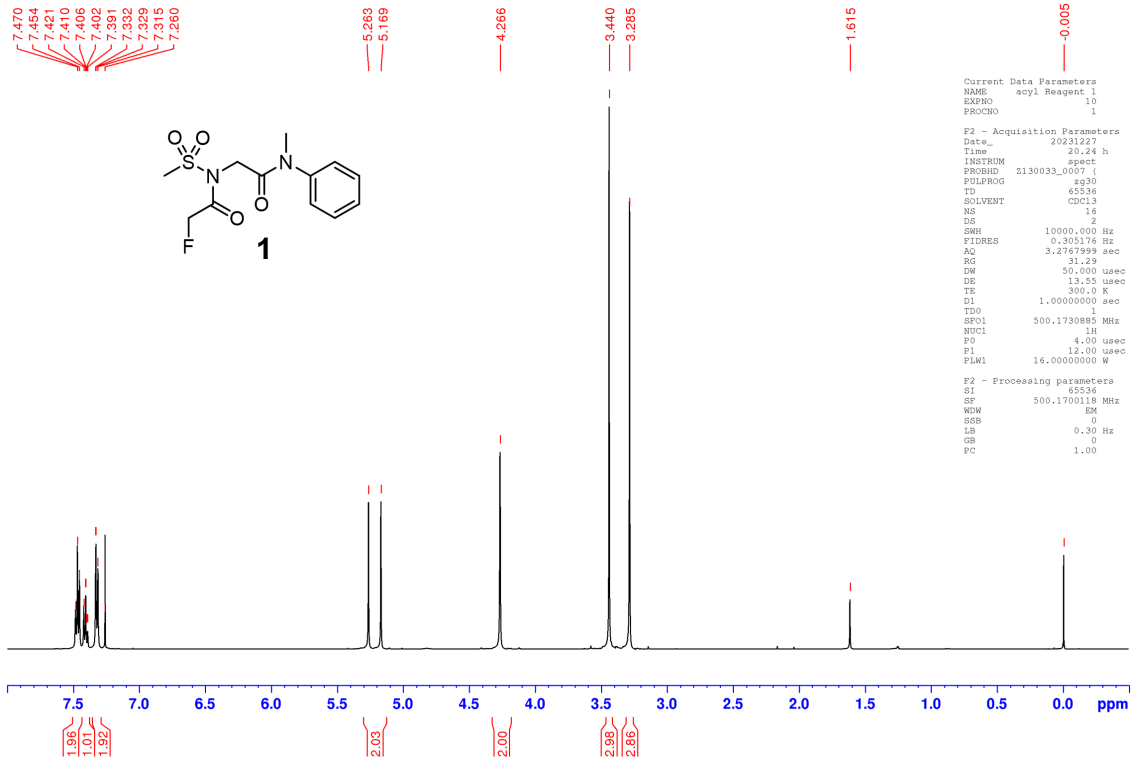
Synthesis of 13

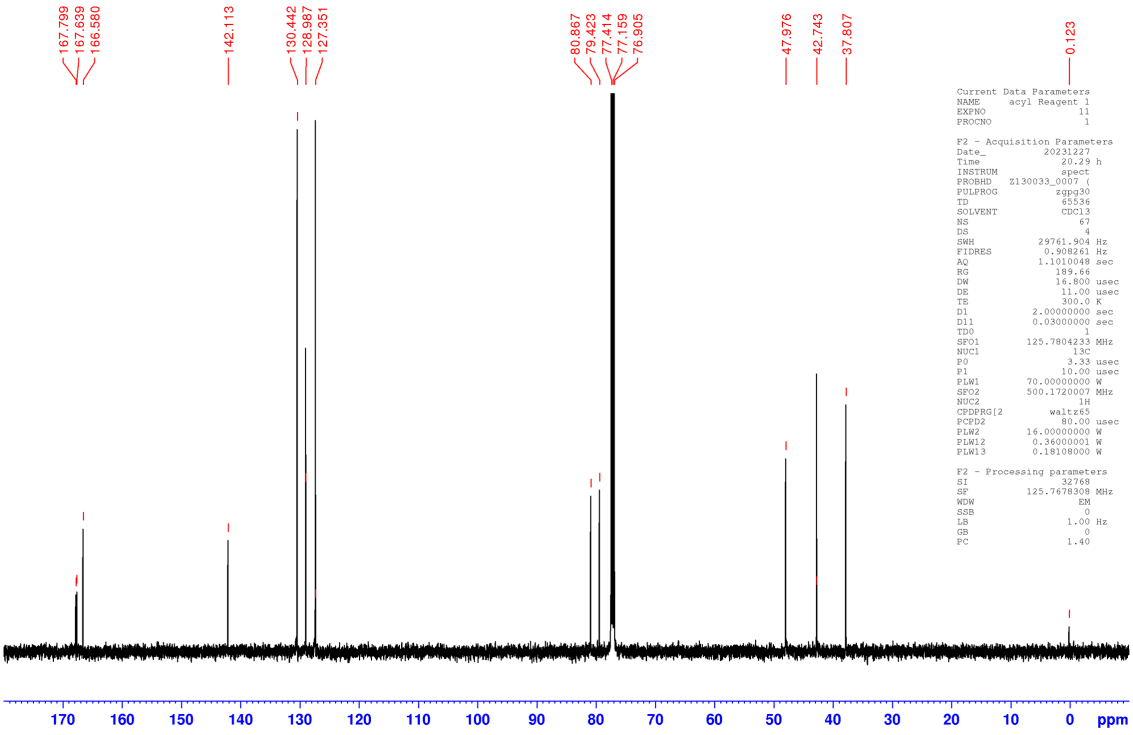


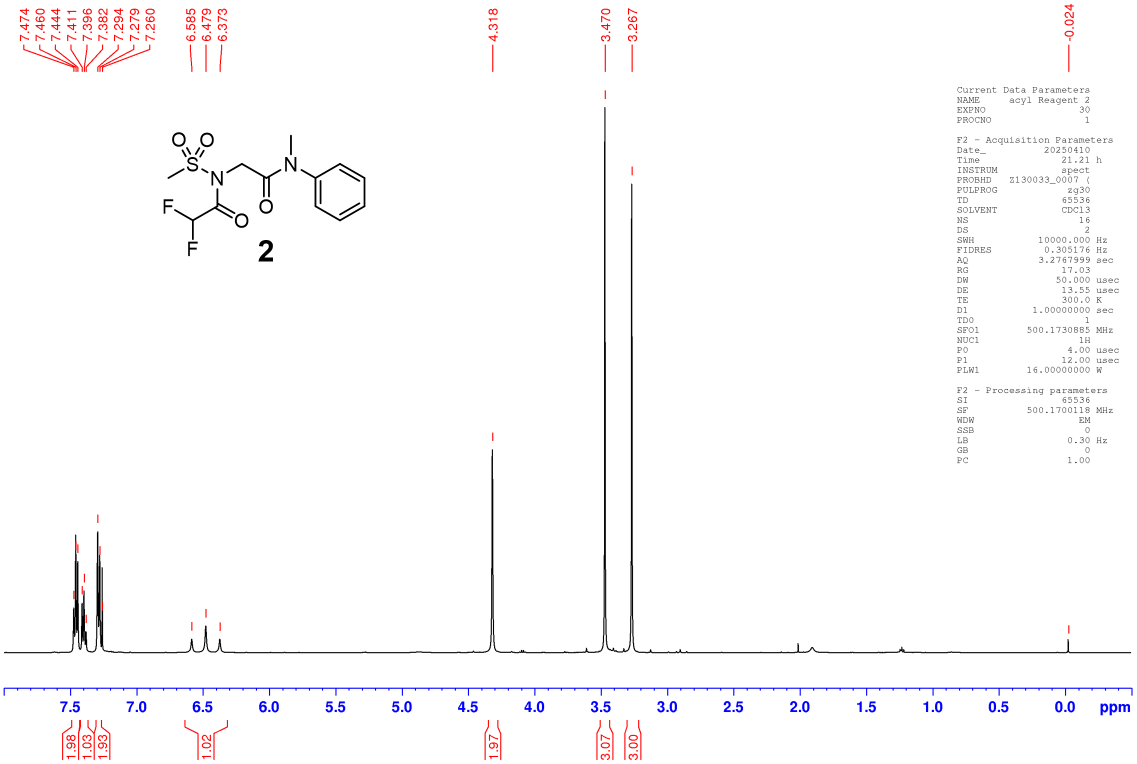
To a stirred, ice-cooled solution of **10-1**[S3] (25.6 mg, 91.3 μmol) in dry CH_2Cl_2 (1.5 mL) was added TEA (13.8 μL, 100 μmol) at 0 °C. After adding difluoroacetic anhydride (11.9 μL, 110 μmol), the solution was stirred for 4 h at 0 °C to ambient temperature. The mixture was diluted with AcOEt and the organic phase was washed with 1N HCl aq., sat. $NaHCO_3$ aq. and brine followed by drying over Na_2SO_4. Solvents were removed in vacuo and the residue was purified by column chromatography on SiO_2 (Hexane: AcOEt = 1:1) to give **13** (19.2 mg, 59%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers): δ 7.35–7.27 (m, 3H), 7.24–7.21 (m, 1H), 7.06 (d, J = 7.4 Hz, 1H), 5.96–5.68 (m, 2H), 4.71–4.44 (m, 2H), 3.44–3.38 (m, 2H), 3.02 (s, 1.4H), 2.89 (s, 1.6H), 2.87 (s, 1.6H), 2.78 (s, 1.4H), 2.09 (s, 1.6H), 1.63 (s, 1.4H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 191.4, 191.25, 191.17, 191.0, 190.9, 190.8, 170.9, 170.6, 169.5, 168.8, 136.7, 136.6, 128.89, 128.86, 128.3, 127.8, 127.5, 125.9, 111.04, 110.99, 109.02,108.97, 107.00, 106.94, 52.7, 51.8, 51.7, 51.3, 35.1, 34.6, 31.4, 31.3, 27.69, 27.66, 21.7, 21.1. **HRMS** (ESI) m/z $[M+Na]^+$ calcd for $C_{16}H_{20}F_2N_2O_3SNa^+$ 381.1055; Found 381.1060.

NMR Spectra of Compounds



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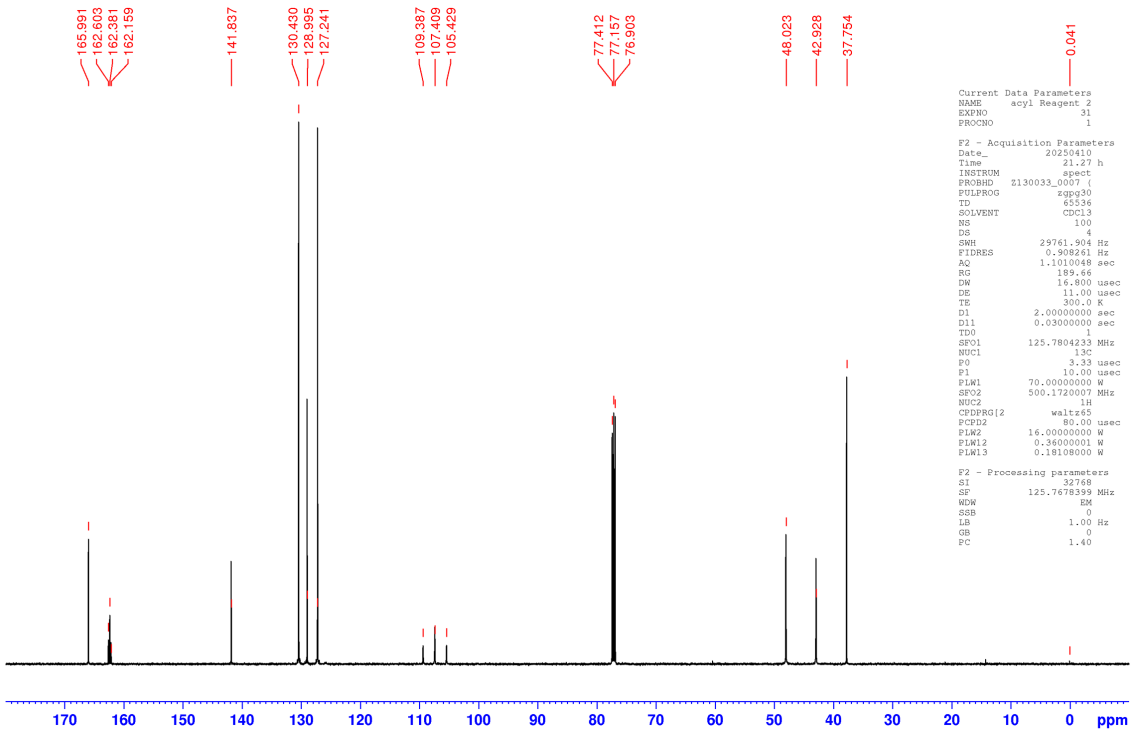
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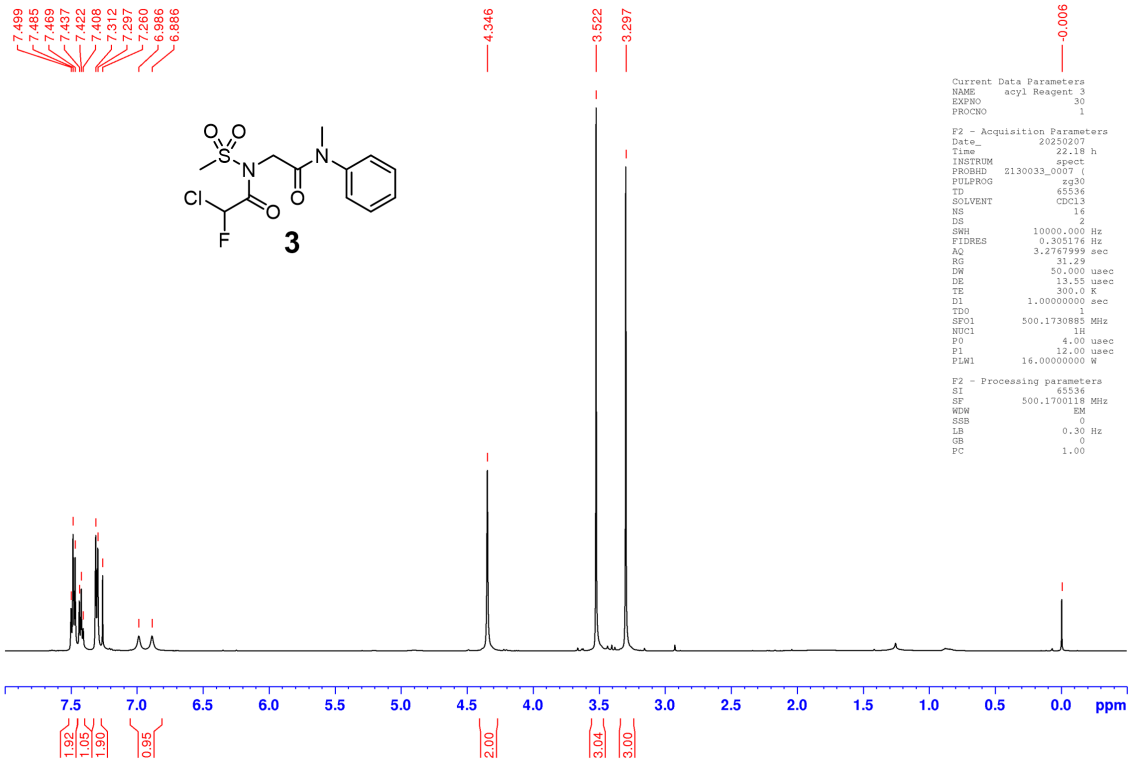
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TD0 1
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LB 0.30 Hz
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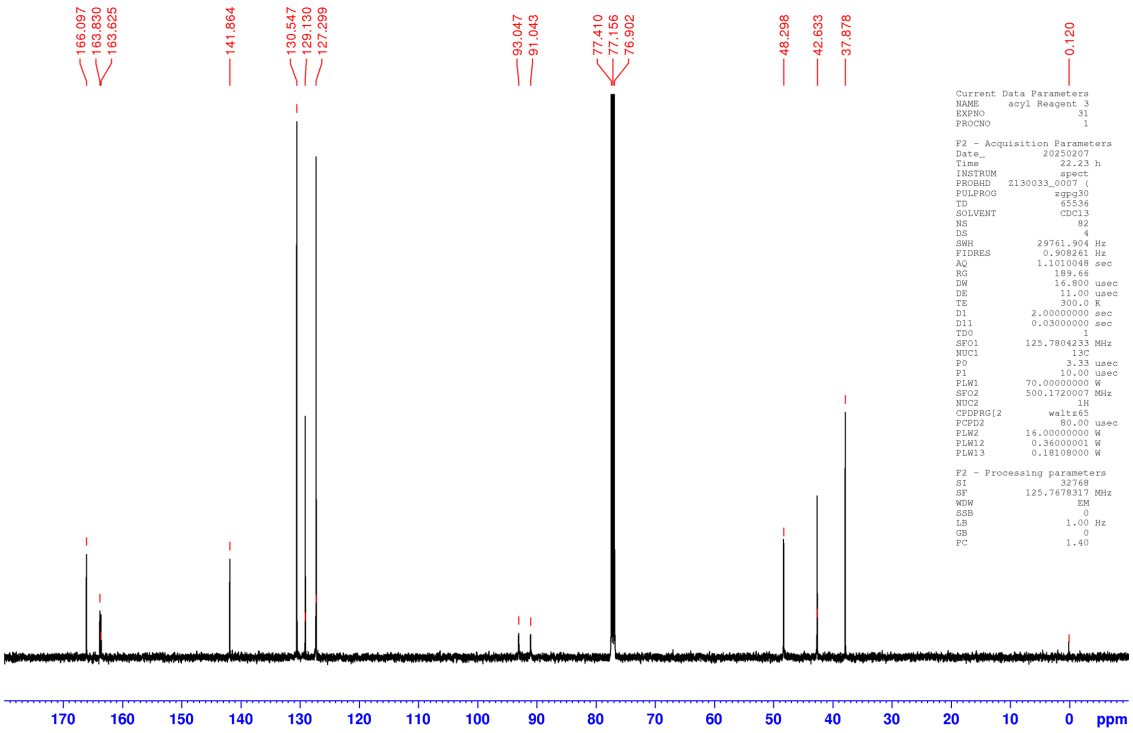
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NUC2 1H
CPDPRG[2 waltz65
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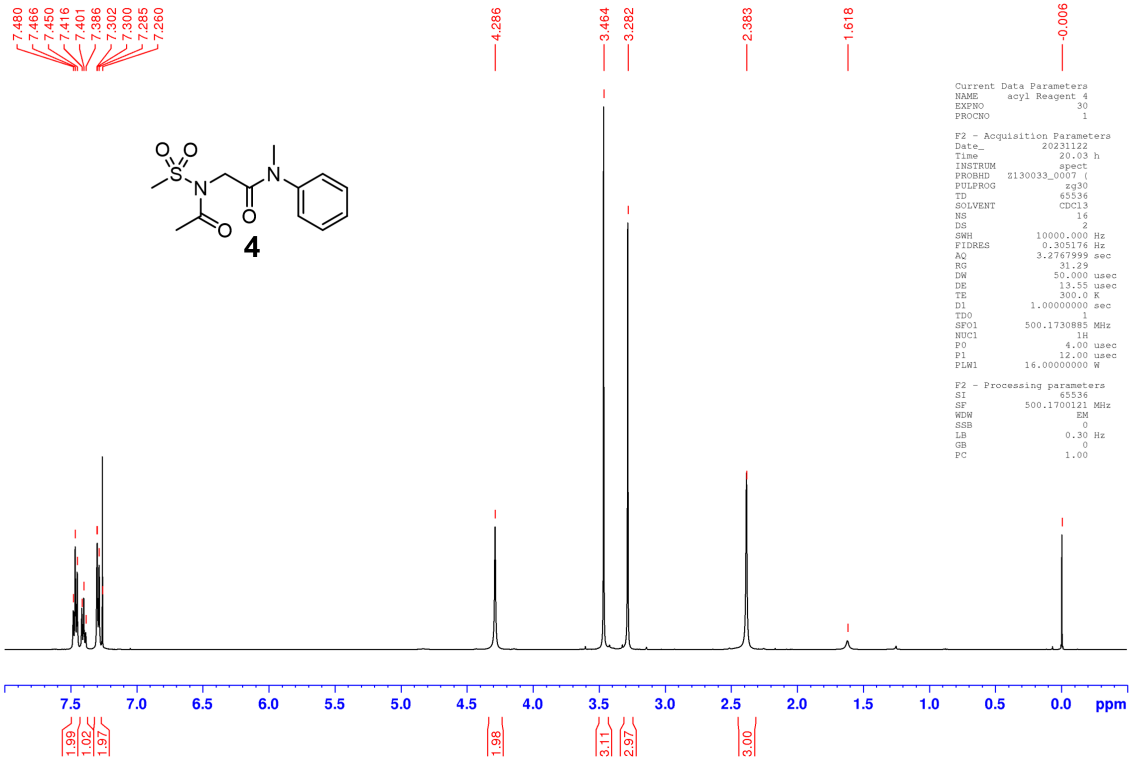
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LB 0.30 Hz
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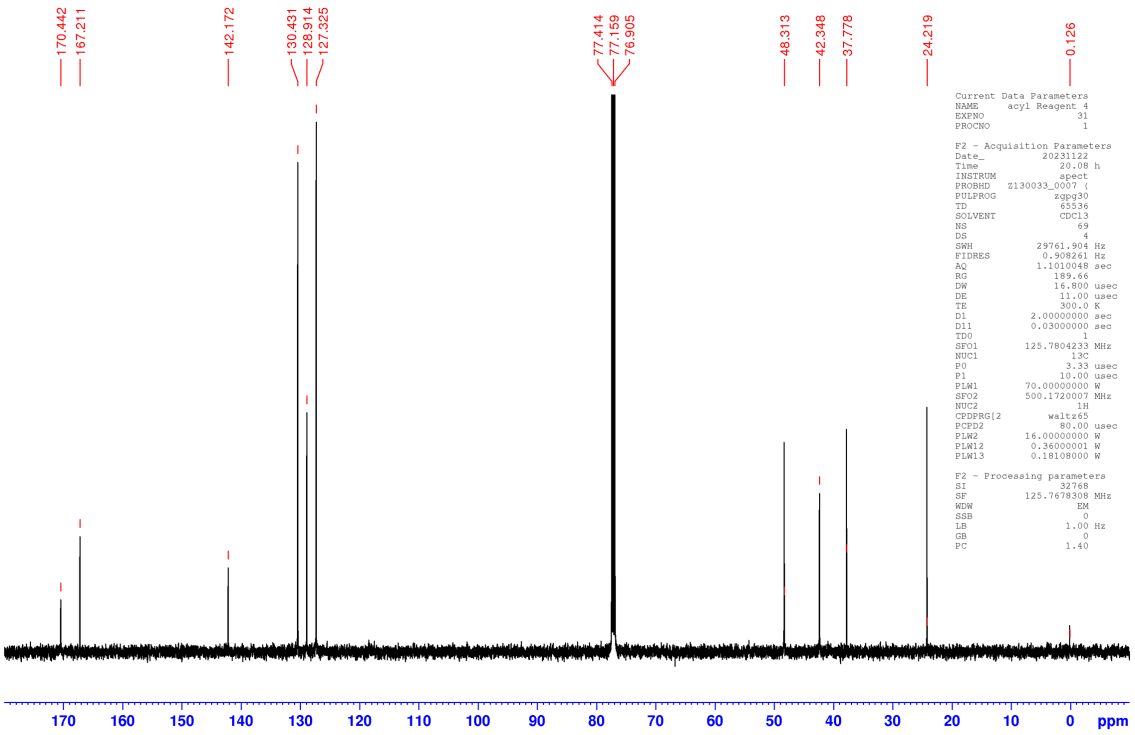
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NUC2 1H
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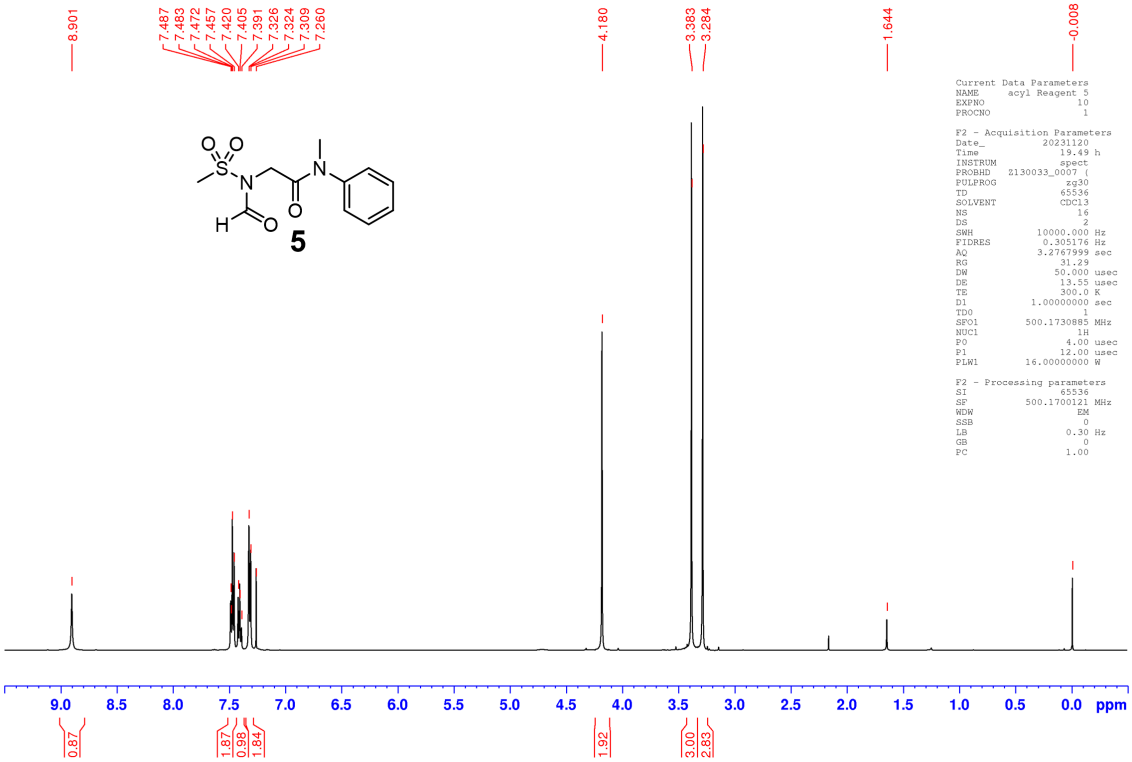
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RG 31.29
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DE 13.55 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
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P0 4.00 usec
P1 12.00 usec
PLW1 16.00000000 W
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WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
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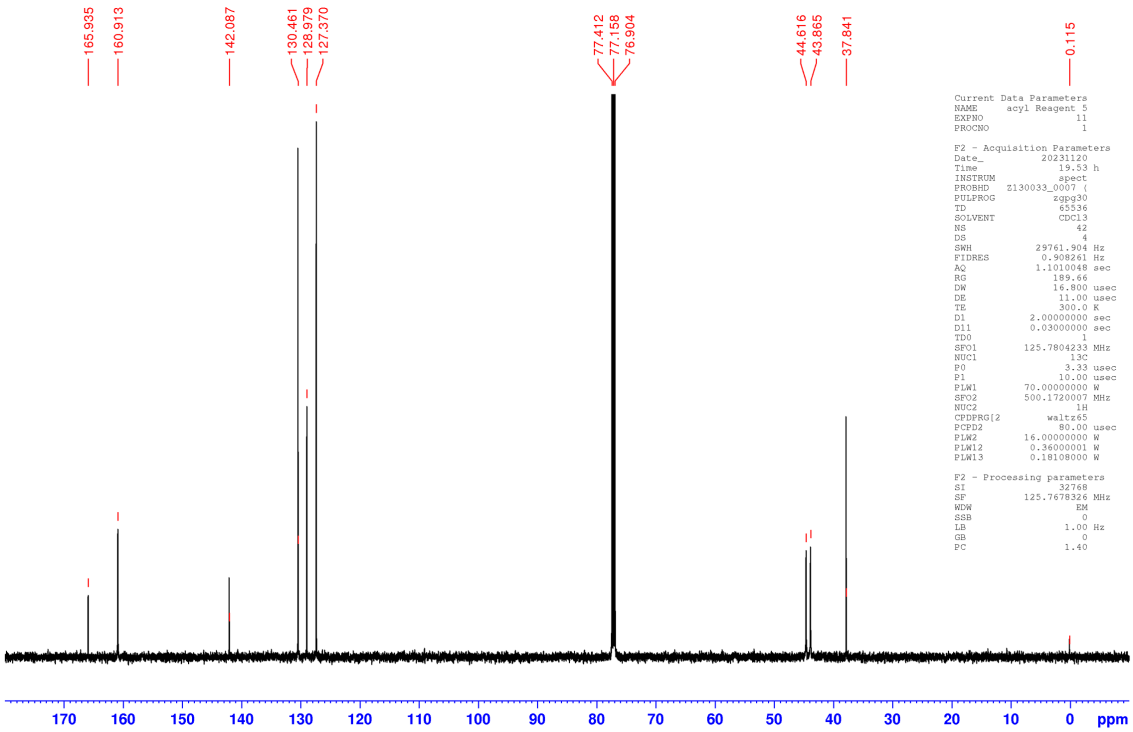
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PROCNO 1
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NUC1 13C
P0 3.33 usec
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PLW1 70.00000000 W
SFO2 500.1720007 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
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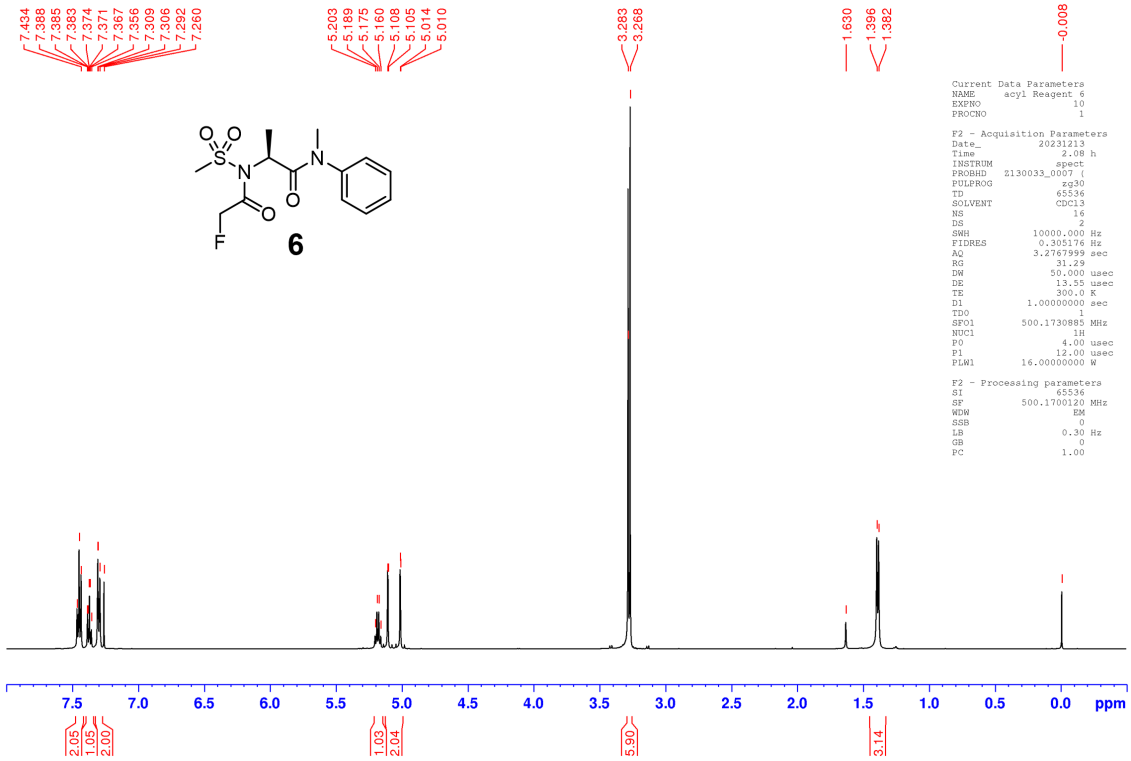
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PROCNO 1
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Time 2.08 h
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FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 31.29
DW 50.000 usec
DE 13.55 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
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P0 4.00 usec
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6.0
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4.0
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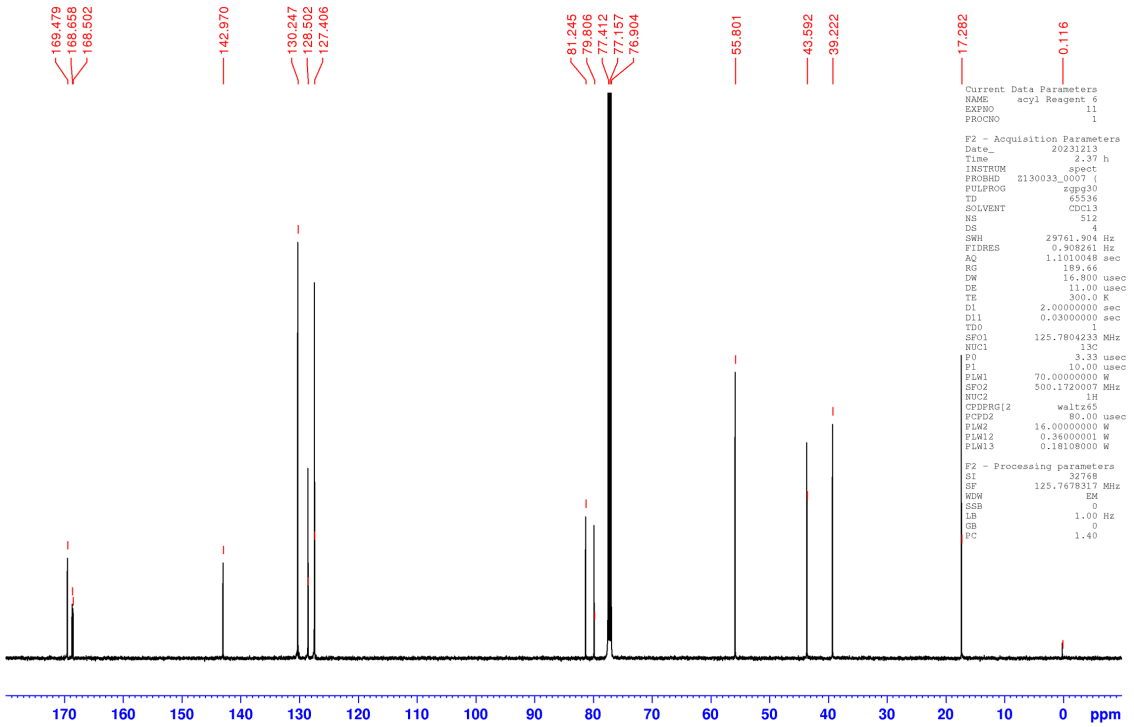
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Current Data Parameters
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PROCNO 1
F2 - Acquisition Parameters
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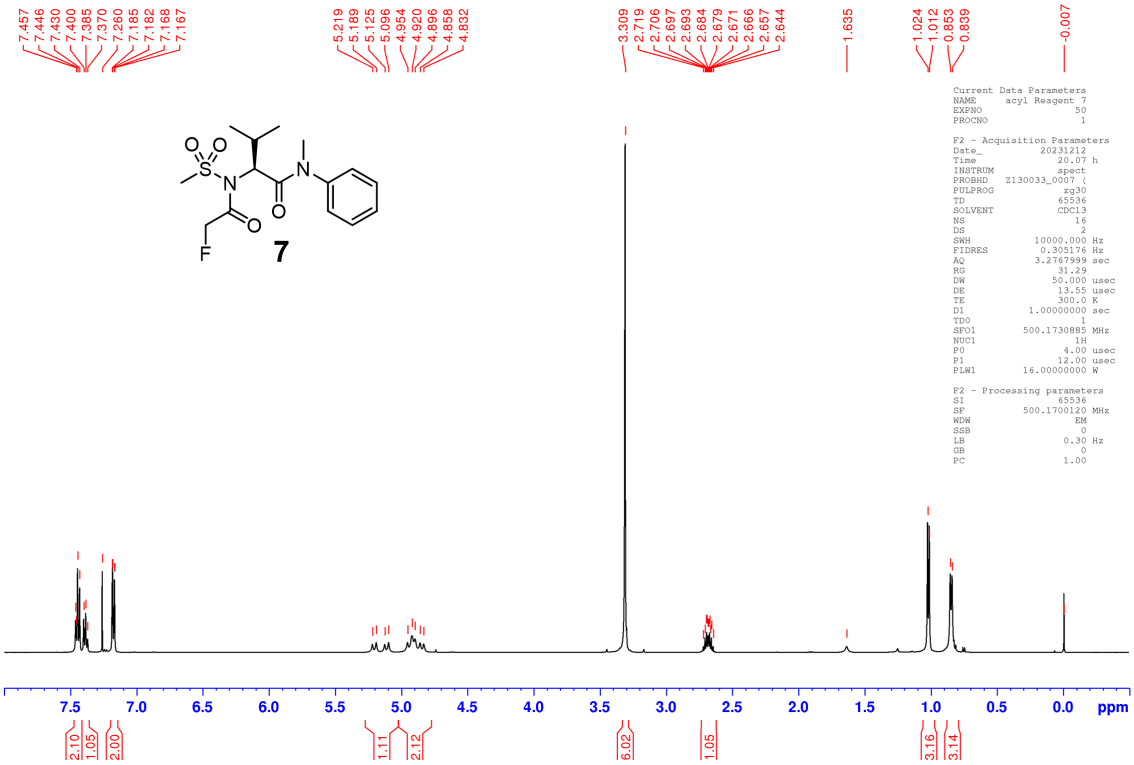
Time 2.37 h
INSTRUM spect
PROBHD Z130033_0007 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010048 sec
RG 189.66
DW 16.800 usec
DE 11.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7804233 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 70.00000000 W
SFO2 500.1720007 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 16.00000000 W
PLW12 0.36000001 W
PLW13 0.18108000 W
F2 - Processing parameters
SI 32768
SF 125.7678317 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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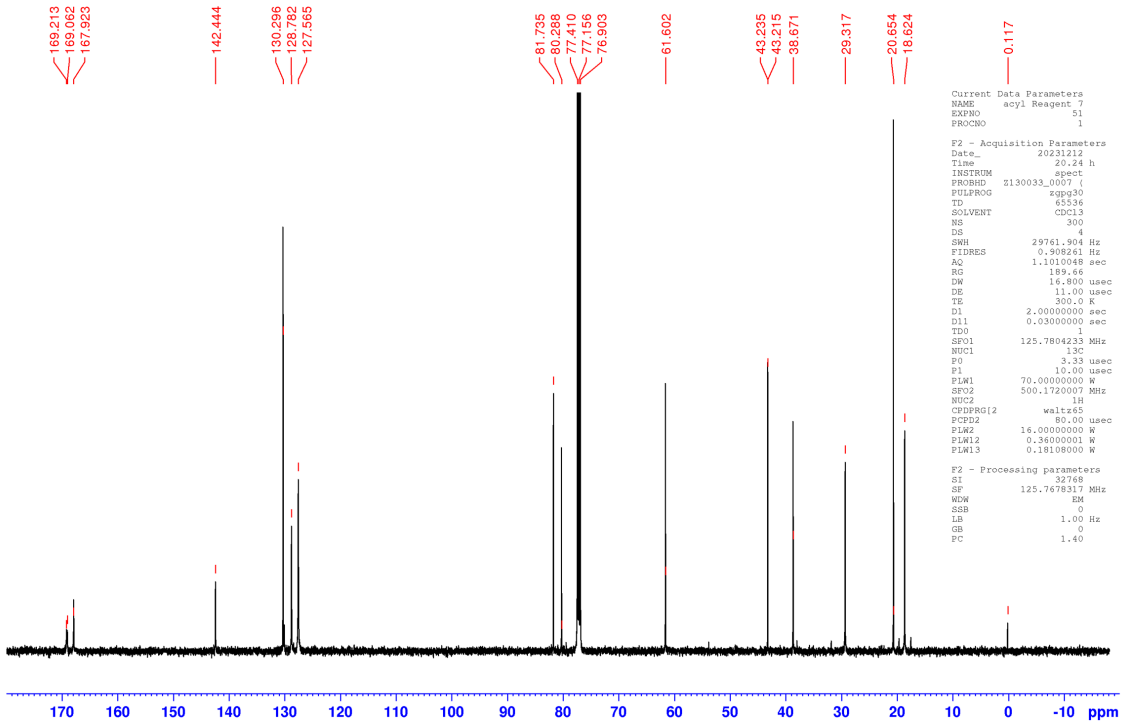
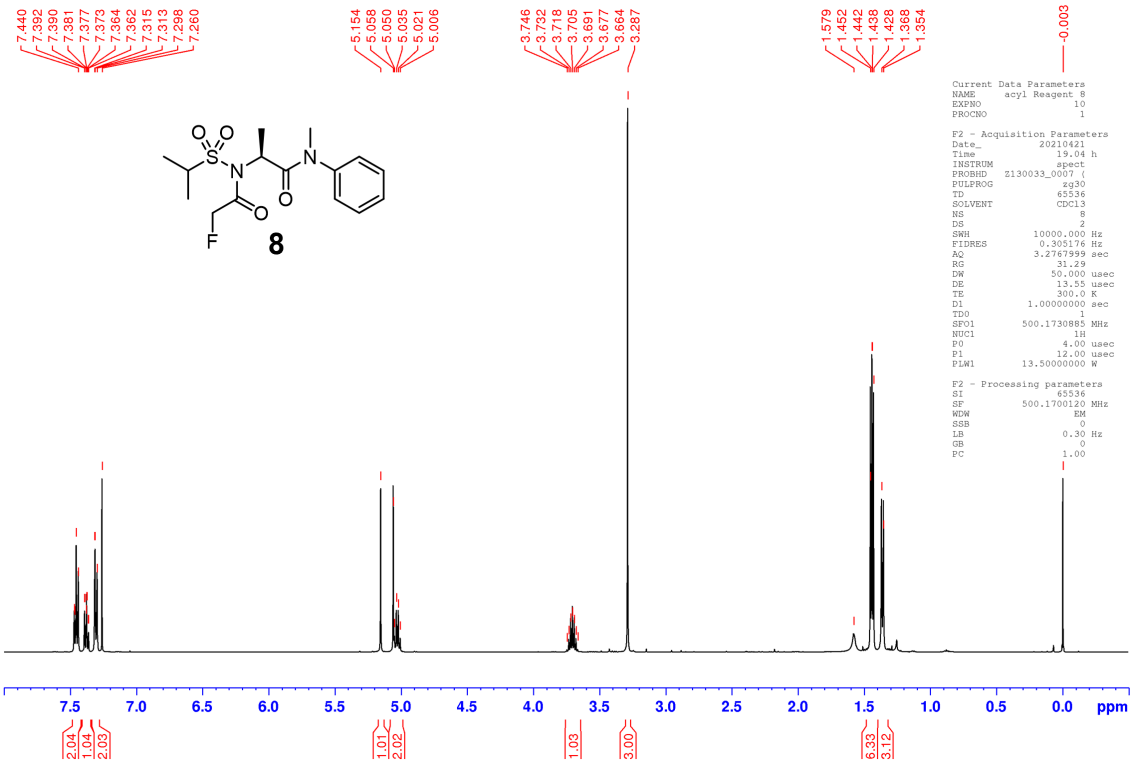
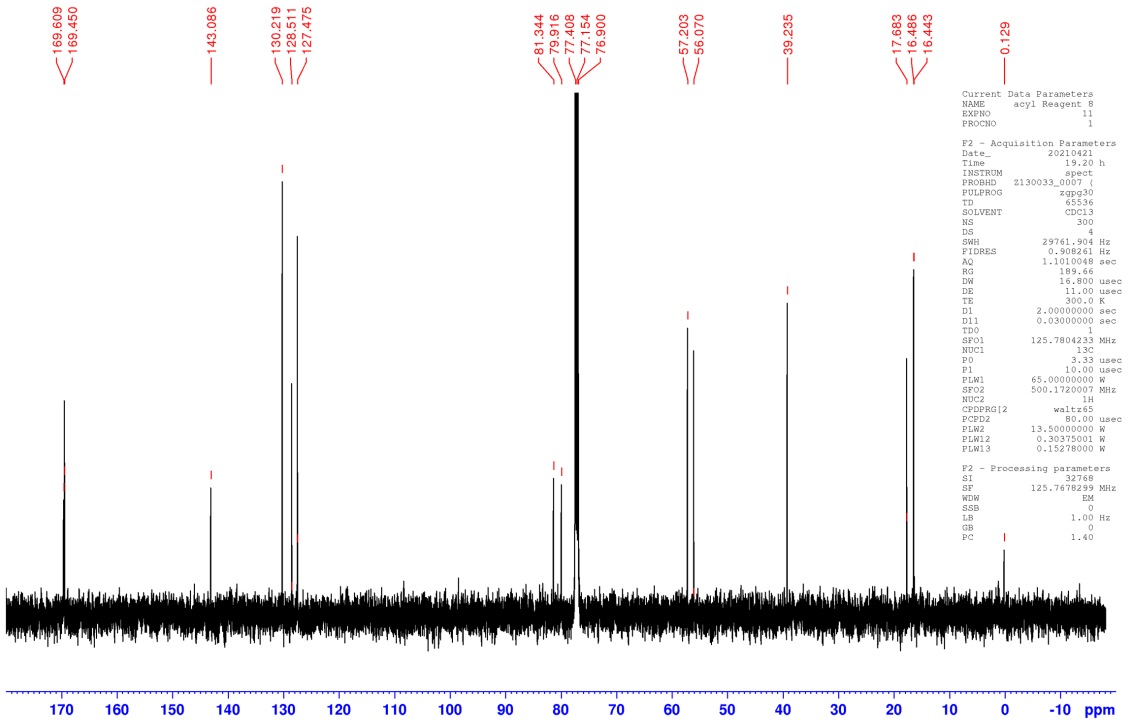
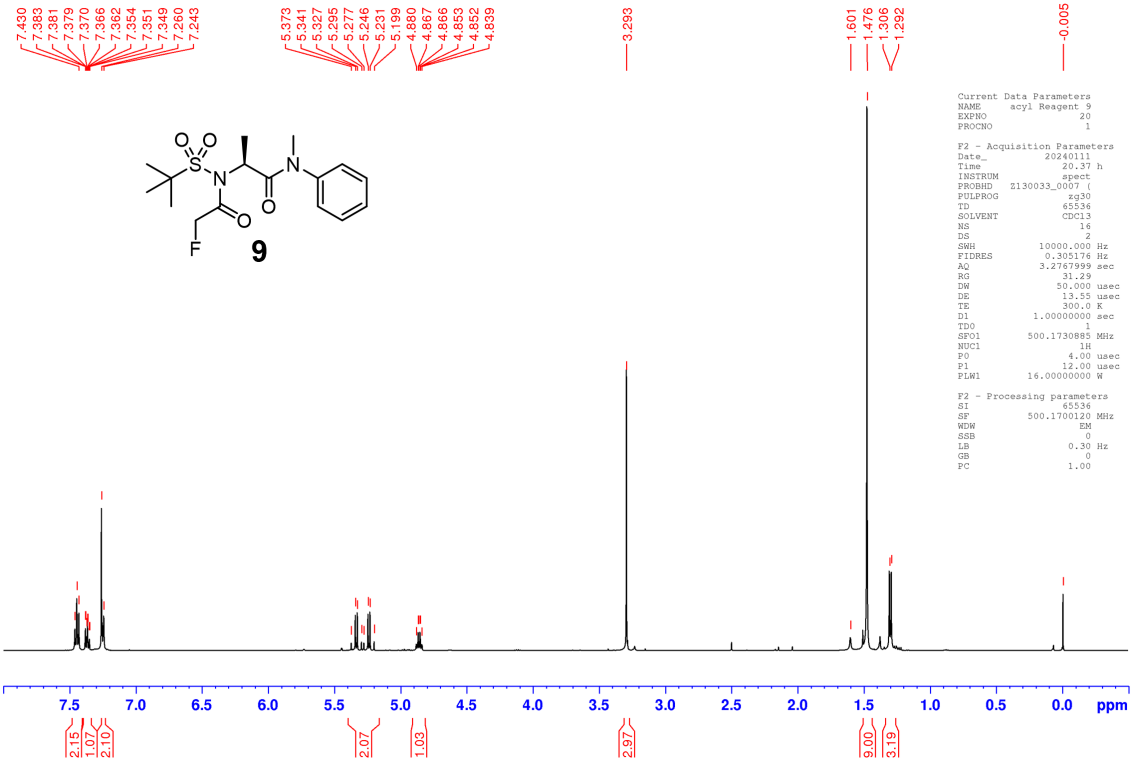
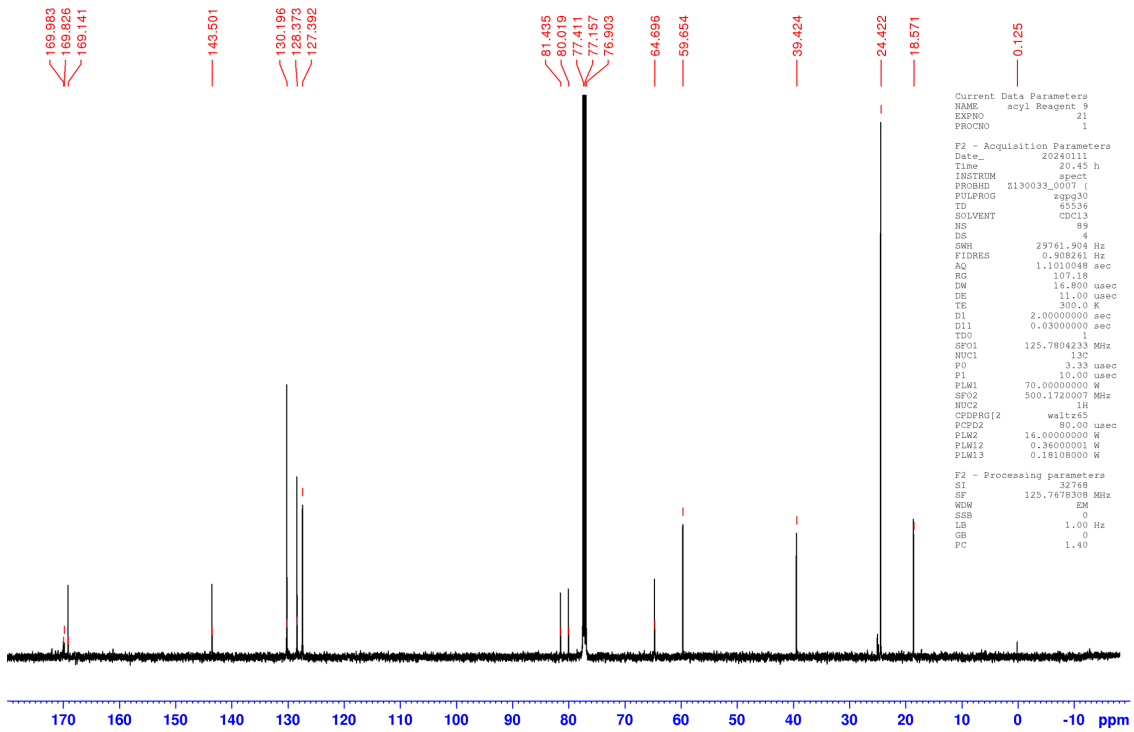
7.446
7.430
7.400
7.385
7.370
7.260
7.185
7.182
7.168
7.167
5.219
5.189
5.125
5.096
4.954
4.920
4.896
4.858
4.832
3.309
2.719
2.706
2.697
2.693
2.684
2.679
2.671
2.666
2.657
2.644
1.635
1.024
1.012
0.853
0.839
-0.007
7
Current Data Parameters
NAME acyl Reagent 7
EXPNO 50
PROCNO 1
F2 - Acquisition Parameters
Date_ 20231212
Time 20.07 h
INSTRUM spect
PROBHD Z130033_0007 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 31.29
DW 50.000 usec
DE 13.55 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 4.00 usec
P1 12.00 usec
PLW1 16.00000000 W
F2 - Processing parameters
SI 65536
SF 500.1700120 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
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
2.10
1.05
2.00
1.11
2.12
6.02
1.05
3.16
3.14

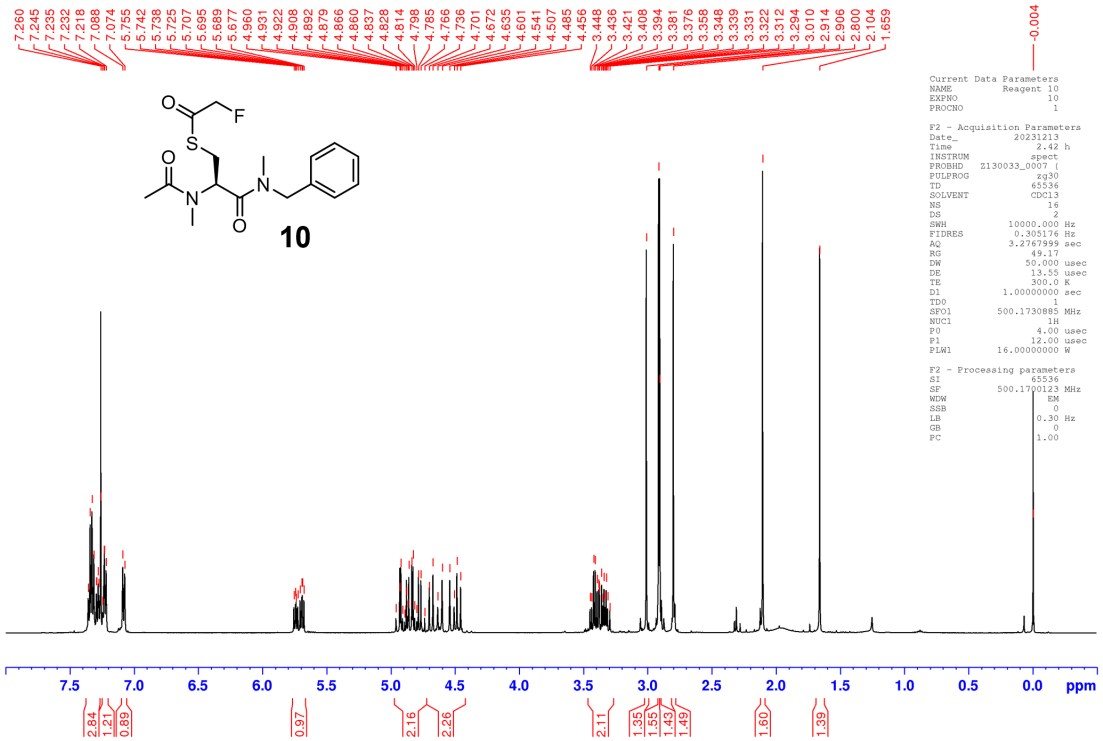

169.213
169.062
167.923
142.444
130.296
128.782
127.565
81.735
80.288
77.410
77.156
76.903
61.602
43.235
43.215
38.671
29.317
20.654
18.624
0.117
Current Data Parameters
NAME acyl Reagent 7
EXPNO 51
PROCNO 1
F2 - Acquisition Parameters
Date_ 20231212
Time 20.24 h
INSTRUM spect
PROBHD Z130033_0007 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 300
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010048 sec
RG 189.66
DW 16.800 usec
DE 11.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7804233 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 70.00000000 W
SFO2 500.1720007 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 16.00000000 W
PLW12 0.36000001 W
PLW13 0.18108000 W
F2 - Processing parameters
SI 32768
SF 125.7678317 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
ppm

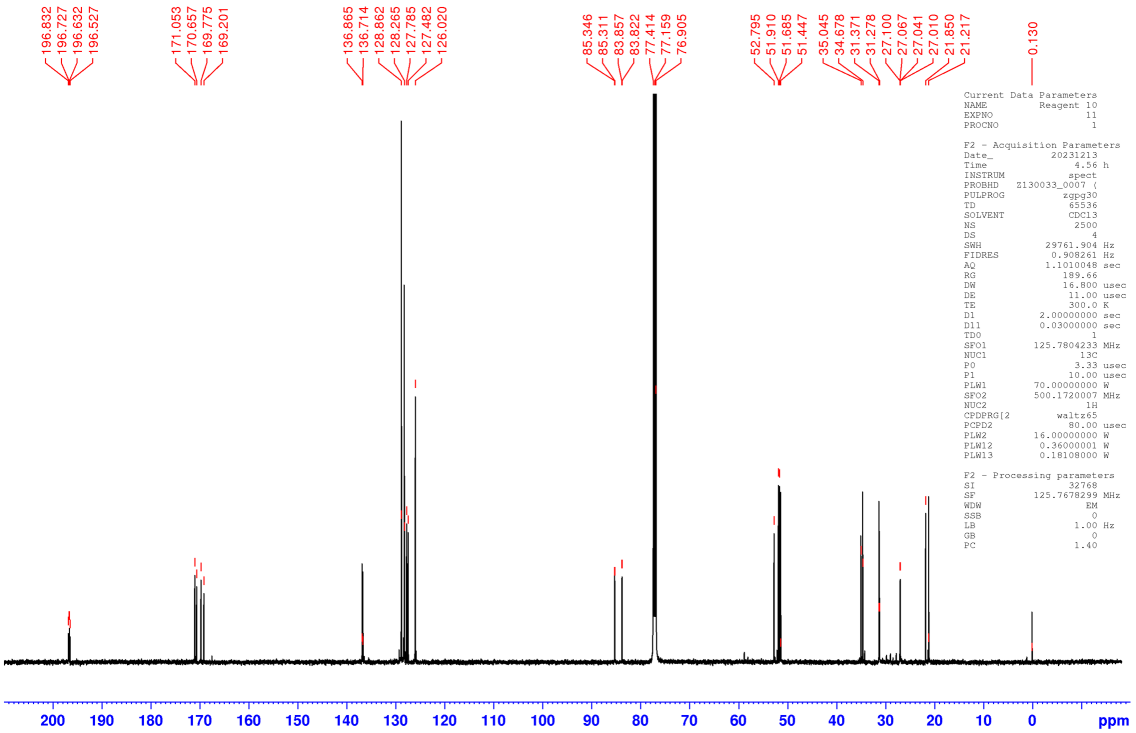

7.440
7.392
7.390
7.381
7.377
7.373
7.364
7.362
7.315
7.313
7.298
7.260
5.154
5.058
5.050
5.035
5.021
5.006
3.746
3.732
3.718
3.705
3.691
3.677
3.664
3.287
1.579
1.452
1.442
1.438
1.428
1.388
1.354
-0.003
8
Current Data Parameters
NAME acyl Reagent 8
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210421
Time 19.04 h
INSTRUM spect
PROBHD Z130033_0007 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 31.29
DW 50.000 usec
DE 13.55 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 4.00 usec
P1 12.00 usec
PLW1 13.50000000 W
F2 - Processing parameters
SI 65536
SF 500.1700120 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
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
2.04
1.04
2.03
1.01
2.02
1.03
3.00
6.33
3.12

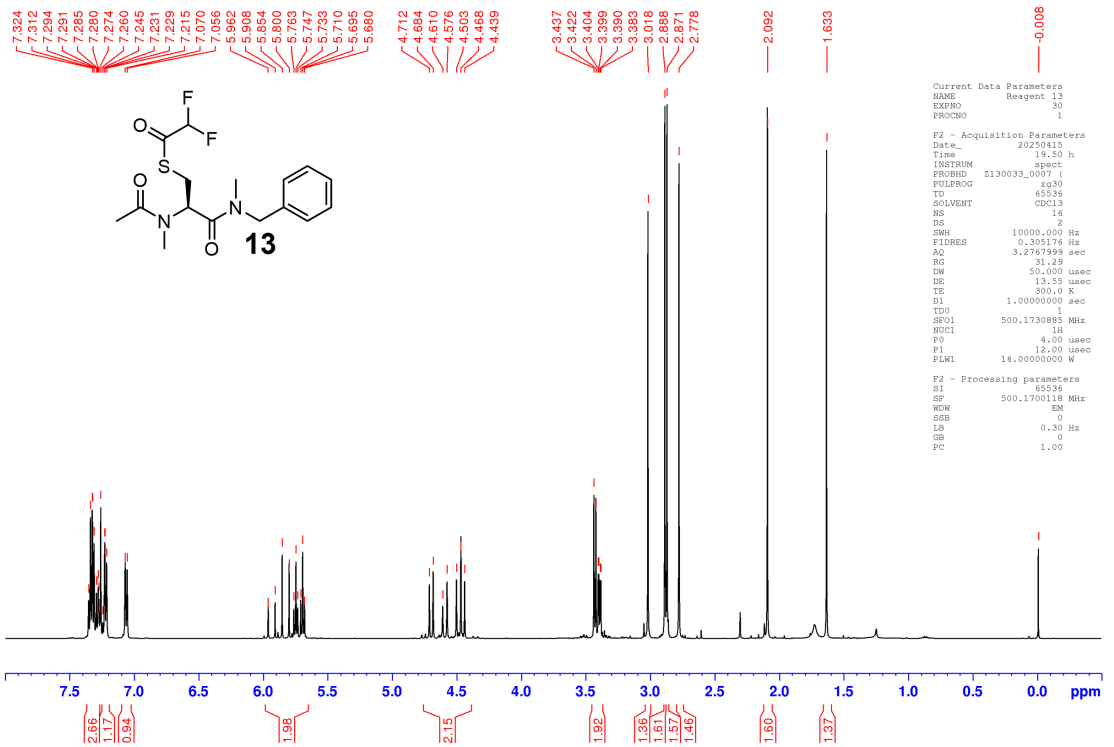

169.609
169.450
143.086
130.219
128.511
127.475
81.344
79.916
77.408
77.154
76.900
57.203
56.070
39.235
17.683
16.486
16.443
0.129
Current Data Parameters
NAME acyl Reagent 8
EXPNO 11
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210421
Time 19.20 h
INSTRUM spect
PROBHD Z130033_0007 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 300
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010048 sec
RG 189.66
DW 16.800 usec
DE 11.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7804233 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 65.00000000 W
SFO2 500.1720007 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 13.50000000 W
PLW12 0.30375001 W
PLW13 0.15278000 W
F2 - Processing parameters
SI 32768
SF 125.7678299 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
ppm

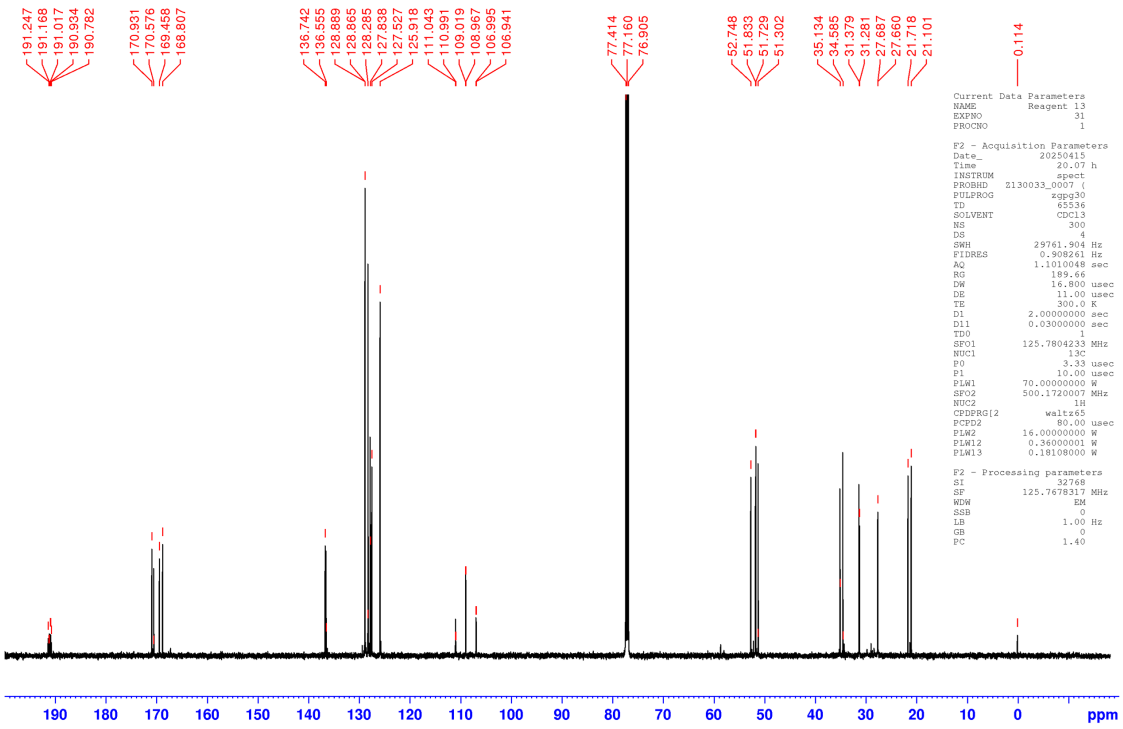

7.430
7.383
7.381
7.379
7.370
7.366
7.362
7.354
7.351
7.349
7.260
7.243
5.373
5.341
5.327
5.295
5.277
5.246
5.231
5.199
4.880
4.867
4.866
4.853
4.852
4.839
3.293
1.601
1.476
1.306
1.292
-0.005
9
Current Data Parameters
NAME acyl Reagent 9
EXPNO 20
PROCNO 1
F2 - Acquisition Parameters
Date_ 20240111
Time 20.37 h
INSTRUM spect
PROBHD Z130033_0007 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 31.29
DW 50.000 usec
DE 13.55 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 4.00 usec
P1 12.00 usec
PLW1 16.00000000 W
F2 - Processing parameters
SI 65536
SF 500.1700120 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
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
2.15
1.07
2.10
2.07
1.03
2.97
9.00
3.19


169.983
169.826
169.141
143.501
130.196
128.373
127.392
81.435
80.019
77.411
77.157
76.903
64.696
59.654
39.424
24.422
18.571
0.125
Current Data Parameters
NAME acyl Reagent 9
EXPNO 21
PROCNO 1
F2 - Acquisition Parameters
Date_ 20240111
Time 20.45 h
INSTRUM spect
PROBHD Z130033_0007 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 89
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010048 sec
RG 107.18
DW 16.800 usec
DE 11.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7804233 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 70.00000000 W
SFO2 500.1720007 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 16.00000000 W
PLW12 0.36000001 W
PLW13 0.18108000 W
F2 - Processing parameters
SI 32768
SF 125.7678308 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 ppm


10
ppm


ppm


7.324
7.312
7.294
7.291
7.285
7.280
7.274
7.260
7.245
7.231
7.229
7.215
7.070
7.056
5.962
5.908
5.854
5.800
5.763
5.747
5.733
5.710
5.695
5.680
4.712
4.684
4.610
4.576
4.503
4.468
4.439
3.437
3.422
3.404
3.399
3.390
3.383
3.018
2.888
2.871
2.778
2.092
1.633
-0.008
F
O
F
S
O
N
N
O
13
Current Data Parameters
NAME Reagent 13
EXPNO 30
PROCNO 1
F2 - Acquisition Parameters
Date_ 20250415
Time 19.50 h
INSTRUM spect
PROBHD Z130033_0007 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 31.29
DW 50.000 usec
DE 13.55 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 4.00 usec
P1 12.00 usec
PLW1 16.00000000 W
F2 - Processing parameters
SI 65536
SF 500.1700118 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
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
2.66
1.17
0.94
1.98
2.15
1.92
1.36
1.61
1.57
1.46
1.60
1.37


191.247
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191.017
190.934
190.782
170.931
170.576
169.458
168.807
136.742
136.555
128.889
128.865
128.285
127.838
127.527
125.918
111.043
110.991
109.019
108.967
106.995
106.941
77.414
77.160
76.905
52.748
51.833
51.729
51.302
35.134
34.585
31.379
31.281
27.687
27.660
21.718
21.101
0.114
Current Data Parameters
NAME Reagent 13
EXPNO 31
PROCNO 1
F2 - Acquisition Parameters
Date_ 20250415
Time 20.07 h
INSTRUM spect
PROBHD Z130033_0007 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 300
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010048 sec
RG 189.66
DW 16.800 usec
DE 11.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7804233 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 70.00000000 W
SFO2 500.1720007 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 16.00000000 W
PLW12 0.36000001 W
PLW13 0.18108000 W
F2 - Processing parameters
SI 32768
SF 125.7678317 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

References

S1. M. J. Kurth, C. B Green, M. E. Mount, D. Y. Jackson, Polypeptide fluoroacetate derivatives. *Int. J. Peptide Protein Res.* **31**, 388-395 (1988).

S2. W. J. Middleton, One-Step Method for Converting Esters to Acyl Chloride. *J. Org. Chem.,* **44**, 2291 (1979).

S3. N. Zenmyo, Y. Matsumoto, A. Yasuda, S. Uchinomiya, N. Shindo, K. Sasaki-Tabata, E. Mishiro-Sato, T. Tamura, I. Hamachi, A. Ojida, A Protein Cleavage Platform Based on Selective Formylation at Cysteine Residues. *J. Am. Chem. Soc.,* **147**, 3080-3091 (2025).