Supporting Information

Stereoselective synthesis of Gly-Gly-type (*E*)-methylalkene and (*Z*)-chloroalkene dipeptide isosteres and their application to 14-mer RGG peptidomimetics

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I. General information

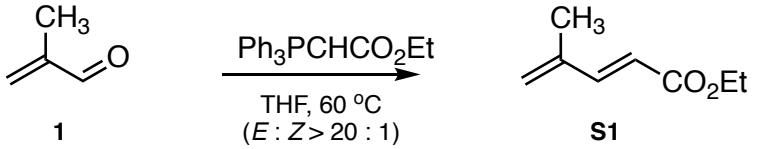
General methods. All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of nitrogen, using commercially supplied solvents and reagents unless otherwise noted. Thin-layer chromatography (TLC) was performed on Merck $60F_{254}$ precoated silica gel plates and was visualized by fluorescence quenching under UV light and by staining with phosphomolybdic acid, *p*-anisaldehyde, or ninhydrin, respectively. Flash column chromatography was carried out silica gel 60 N (Kanto Chemical Co., Inc.).

Characterization data. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded using a JNM-AL300 spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using Bruker Biospin AVANCE III HD. Chemical shifts are reported in δ (ppm) relative to Me_4Si (in $CDCl_3$) as internal standard. Infrared (IR) spectra were recorded on a JASCO FT/IR 6300, and are reported as wavenumber (cm^{-1}). Low- and high-resolution mass spectra were recorded on a Bruker Daltonics compact (ESI-MS) spectrometers in the positive and negative detection mode.

HPLC condition. For HPLC separations, a COSMOSIL $5C_{18}$-AR-II analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL min-1), YMC-Actus Triart C18 preparative column (YMC, 20 × 250 mm, flow rate 10 mL min-1) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA aqueous solution (v/v, solvent A) and 0.1% TFA in MeCN (v/v, solvent B) was used for HPLC elution.

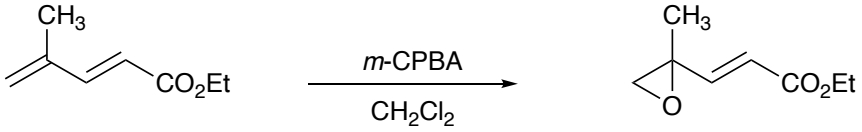
Circular dichroism spectroscopy. CD spectra were recorded from 190 to 260 nm at 25 °C using a Jasco J-1500 spectropolarimeter (JASCO, Tokyo, Japan). The peptide solutions in 50 mM Tris buffer (pH 7.4) with 100 mM KCl were subjected to CD measurements in a 2 mm quartz cuvette, and the results were corrected by subtracting the buffer baseline.

II. Experimental procedures of Gly-Gly-type (*E*)-methylalkene dipeptide isostere



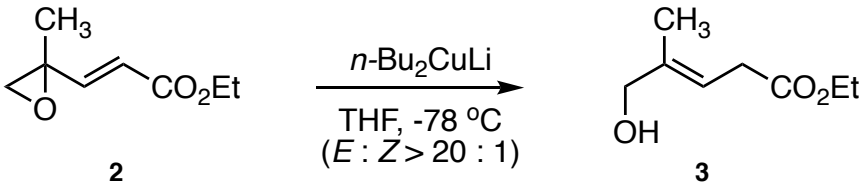
1 S1

Ethyl (*E*)-4-methylpenta-2,4-dienoate (S1): To a stirred solution of ethyl (triphenylphosphoranylidene)acetate (10.5 g, 30 mmol) in THF (80.0 mL) was added methacrolein (1.67 mL, 20.0 mmol). After being stirred at 60 °C for 20 h, concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (98:2) gave the title compound **S1** as a colorless oil (2.18 g, 77%): IR (ATR) ν 1706 (CO), cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 1.31 (t, *J* = 7.1 Hz, 3H), 1.89 (s, 3H), 4.22 (q, *J* = 7.1 Hz, 2H), 5.35 (d, *J* = 8.1 Hz, 2H), 5.87 (d, *J* = 15.8 Hz, 1H), 7.36 (d, *J* = 15.8 Hz, 1H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.2, 18.0, 60.3, 118.7, 124.2, 140.5, 146.9, 167.1.; HRMS (ESI), *m/z* calcd for $C_8H_{13}O_2$ $[M+H]^+$ 140.0910, found 140.0915.



S1 2

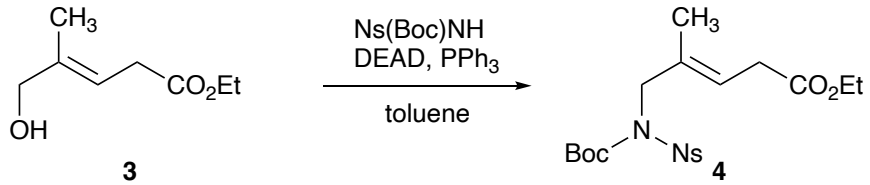
Ethyl (*E*)-3-(2-methyloxiran-2-yl)acrylate (2): To a stirred solution of *m*-CPBA (70%, 9.57 g, 38.8 mmol) in CH_2Cl_2 (32.1 mL) was added **S1** (2.72 g, 19.4 mmol) in CH_2Cl_2 (42.5 mL) at room temperature. After being stirred at room temperature for 16 h, the reaction was quenched with saturated aqueous solution of $Na_2S_2O_3$. The reaction mixture was extracted with CH_2Cl_2, and the extract was washed with saturated aqueous solution of $NaHCO_3$ and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (9:1) gave the title compound **2** as a colorless oil (2.83 g, 94%): IR (ATR) ν 1717 (CO) cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 1.29 (t, *J* = 7.1 Hz, 3H), 1.51 (s, 3H), 2.81 (d, *J* = 5.3 Hz, 1H), 2.91 (d, *J* = 5.3 Hz, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 6.06 (d, *J* = 15.8 Hz, 1H), 6.71 (d, *J* = 15.8 Hz, 1H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.2, 19.1, 54.8, 56.0, 60.6, 122.7, 148.3, 165.9; HRMS (ESI), *m/z* calcd for $C_8H_{12}NaO_3$ $[M+Na]^+$ 179.0677, found 179.0679.



2 3

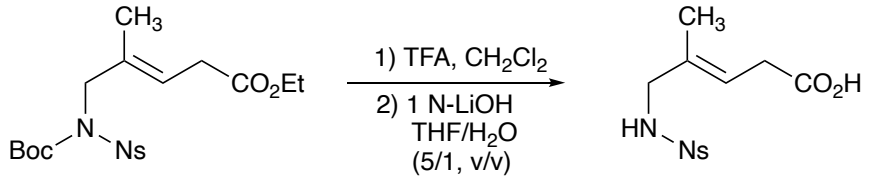
Ethyl (*E*)-5-hydroxy-4-methylpent-3-enoate (3): To a stirred solution of CuI (12.19 g, 64.0 mmol) in THF (96 mL) was added dropwise *n*-BuLi in hexane (1.64 M, 93.7 mL, 153.6 mmol) at -78 ℃ under nitrogen, and the mixture was stirred at 0 ℃ for 10 min. To the solution of the above organocuprate was added dropwise a solution of the enoate **2** (2.50 g, 16.0 mmol) in THF (64.0 mL) at -78 ºC. After being stirred at -78 ºC for 30 min, the reaction was quenched by addition of a 3:2 saturated NH_4Cl-28% NH_3 aqueous solution with additional stirring at room temperature for 2 h. the mixture was extracted with EtOAc and the extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (2:1) gave the title

compound **10-Z** (365.5 mg, 37%) as colorless oil: IR (ATR) ν 3421(OH), 1731 (CO) cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 1.27 (t, *J* = 7.1 Hz, 3H), 1.69 (s, 3H), 3.10 (d, *J* = 7.1 Hz, 2H), 4.05 (s, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 5.63 (td, *J* = 7.1, 1.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 13.8, 14.1, 33.3, 60.7, 68.1, 116.9, 138.5, 172.1.; HRMS (ESI), *m/z* calcd for $C_8H_{15}O_3$ $[M+H]^+$ 159.1016, found 159.1018.



3 **4**

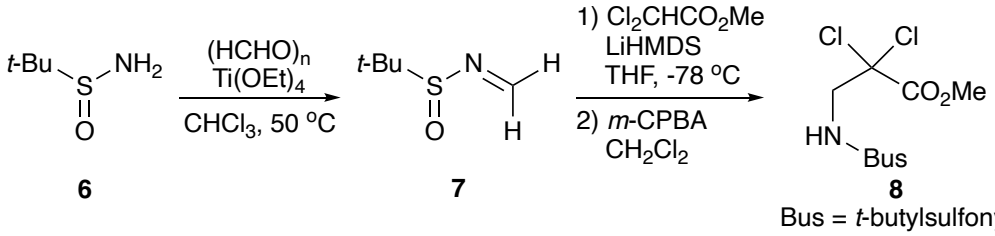
Ethyl (*E*)-5-((*N*-(*tert*-butoxycarbonyl)-2-nitrophenyl)sulfonamido)-4-methylpent-3-enoate (4): To a stirred solution of ethyl (E)-5-hydroxy-4-methylpent-3-enoate **3** (1281.3 mg, 8.10 mmol), Ns(Boc)NH (2938.4 mg, 9.72 mmol) and PPh_3 (2974.4 mg, 11.4 mmol) in toluene (81.0 mL) was added a solution of DEAD in toluene (2.2 M, 5.15 mL, 11.4 mmol) at 0 °C under nitrogen. After being stirred at room temperature for 18 h, concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (3:1) gave the title compound **4** as a white solid (3543.3 mg, 99%): IR (ATR) ν 1730 (CO), 1545 (NO_2), 1363 ($NHSO_2$) 1149 ($NHSO_2$) cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 1.26 (t, *J* = 7.1 Hz, 3H), 1.36 (s, 9H), 1.75 (s, 3H), 3.12 (d, *J* = 6.9 Hz, 2H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.33 (s, 2H), 5.67 (t, *J* = 6.9 Hz, 1H), 7.76 (s, 4H), 8.36 (s, 1H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 14.2, 14.5, 27.8, 33.7, 54.1, 60.6, 84.9, 118.8, 124.4, 131.8, 133.3, 133.9, 134.2, 147.6, 150.4, 171.6.; HRMS (ESI), *m/z* calcd for $C_{19}H_{26}N_2NaO_8S$ $[M+Na]^+$ 465.1308, found 465.1302.



4 **5**

(*E*)-4-Methyl-5-((4-nitrophenyl)sulfonamido)pent-3-enoic acid (Ns-Gly-Ψ^{Me}-Gly-OH, 5): To a solution of ester **4** (2794.3 mg, 6.32 mmol) in CH_2Cl_2 (12.6 mL) was added TFA (12.6 mL). The mixture was stirred for 1 h at room temperature. The mixture was concentrated in vacuo and the residue was further azeotropically concentrated with toluene and used without further purification. To a stirred solution of the above ester in THF/H_2O (63.2 mL, 5/1, v/v) was added 1 N-LiOH aq. at 0 °C. After being stirred at room temperature for 13 h, concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (1:1) followd by EtOAc gave the title compound **5** as a white solid (1867.9 mg, 94%): IR (ATR) ν 3276 (NH), 1748 (CO), 3336 (OH) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.51 (s, 3H), 2.92 (d, *J* = 7.2 Hz, 2H), 3.65 (s, 2H), 5.51 (t, *J* = 7.2 Hz, 1H), 7.72 – 7.86 (m, 3H), 8.01-8.05 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 12.9, 32.6, 50.3, 120.0, 124.4, 130.4, 132.0, 133.4, 133.9, 133.9, 148.1; HRMS (ESI), *m/z* calcd for $C_{12}H_{15}N_2O_6S$ $[M+H]^+$ 315.0645, found 315.0646.

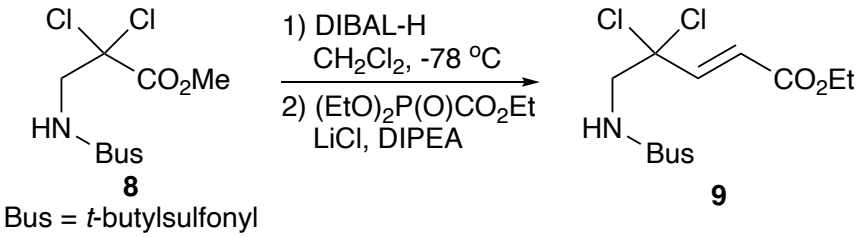
III. Experimental procedures of Gly-Gly-type (*Z*)-chloroalkene dipeptide isostere



Methyl 2,2-dichloro-3-((1,1-dimethylethyl)sulfonamido)propanoate (8): To a stirred solution of *tert*-butylsulfinamide **6** (2427.1 mg, 20.0 mmol) and paraformaldehyde (961.6 mg, 32.0 mmol) in anhydrous CH_3Cl (100.0 mL) was added $Ti(OEt)_4$ (8.40 mL, 40.0 mmol) at room temperature under N_2 atmosphere. After being stirred at 50 °C for 20 h, the reaction was quenched with saturated aqueous solution of $NaHCO_3$. The mixture was filtrated with celite to remove the titanium salt and washed with Et_2O. The organic layer was extracted with CH_2Cl_2, washed with brine, and dried over Na_2SO_4. Concentration under reduced pressure followed by flash chromatography over silica gel with CHCl3-MeOH (15:1) gave the imine **7** as a yellow oil (2448.2 mg, 92%).

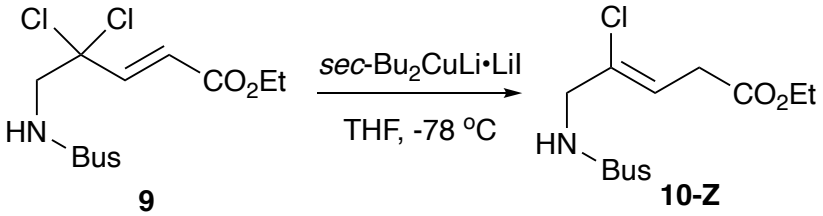
To a stirred solution of Cl_2CHCO_2Me (3.40 mL, 33.2 mmol) in THF (180 mL) was added LiHMDS in THF (1.30 M, 25.5 mL, 33.2 mmol) at -78 ºC under nitrogen, and the mixture was stirred at -78 ºC for 30 min. To the solution was added the imine (2.84 g, 21.3 mmol) in THF (20.5 mL), and the mixture was stirred at -78 ºC for 1 h. The reaction was quenched with saturated aqueous solution of NH_4Cl. The reaction mixture was extracted with Et_2O, and the extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure gave the crude ester as a pale yellow oil, which was used immediately in next step without purification.

To a stirred solution of the above ester (*ca.* 21.3 mmol) in CH_2Cl_2 (213 mL) was added *m*-CPBA (70%, 6.34 g, 25.6 mmol) was added, and the mixture was stirred at room temperature for 13 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$. The reaction mixture was extracted with Et_2O, and the extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (2:1) gave the title compound **8** as a white solid (4.87 g, 79%); IR (ATR) ν 1743 (CO), 1309 ($NHSO_2$), 1123 ($NHSO_2$) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 3.93 (s, 3H), 3.98 (d, J = 6.6 Hz, 2H), 4.74 (t, J = 6.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 24.3, 55.1, 60.6, 80.9, 165.6; HRMS (ESI), *m/z* calcd for $C_8H_{16}Cl_2NO_4S$ $[M+H]^+$ 293.0172, found 293.0171.



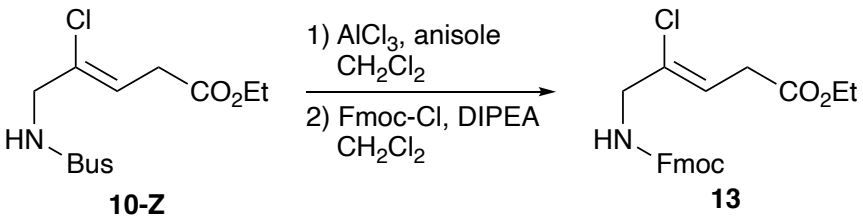
Ethyl (*E*)-4,4-dichloro-5-((1,1-dimethylethyl)sulfonamido)pent-2-enoate (9): To a stirred solution of ester **8** (1963.0 mg, 6.76 mmol) in CH_2Cl_2 (77.0 mL) was added dropwise DIBAH in toluene (1.01 M,

22.9 mL, 23.1 mmol) at -78 ºC under nitrogen, and the mixture was stirred at -78 ºC for 1.0 h. The reaction was quenched by slowly addition of MeOH (10 mL) followed by saturated aqueous Rochelle salt. The reaction mixture was extracted with Et_2O, washed with brine and dried over $MgSO_4$. Concentration under reduced pressure gave a white solid, which was used immediately in next step without purification. To a stirred solution of ethyl diethylphosphonoacetate (3.50 mL, 15.4 mmol) in MeCN (23.1 mL) were added LiCl (1.66 g, 38.5 mmol) and DIPEA (6.50 mL, 38.5 mmol) at 0 ºC under nitrogen. After being stirred for 30 min, a solution of the above aldehyde in MeCN (15.4 mL) was added to the mixture, and the mixture was stirred at room temperature for 17 h. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (2:1) gave the title compound **9** as off white powder (1.69 g, 76% in 2 steps); IR (ATR) ν 1722 (CO), 1310 ($NHSO_2$), 1127 ($NHSO_2$) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (t, J = 7.2 Hz, 3H), 1.42 (s, 9H), 3.87 (d, J = 6.6 Hz, 2H), 4.25 (q, J = 7.2 Hz, 2H), 4.64 (t, J = 6.6 Hz, 2H), 6.41 (d, J = 15.0 Hz, 1H), 7.06 (d, J = 15.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.2, 24.3, 57.3, 60.7, 61.3, 85.7, 125.2, 143.5, 164.8; HRMS (ESI), *m/z* calcd for $C_{11}H_{19}Cl_2NNaO_4S$ $[M+Na]^+$ 354.0304, found 332.0315.



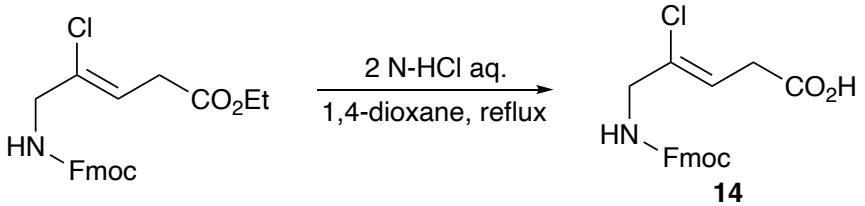
9 **10-Z**

Ethyl (*Z*)-4-chloro-5-((1,1-dimethylethyl)sulfonamido)pent-3-enoate (10-Z): To a stirred solution of CuI (3.81 g, 20.0 mmol) in THF (133 mL) was added dropwise *sec*-BuLi in Et_2O (1.05 M, 41.0 mL, 48.0 mmol) at -78 ℃ under nitrogen, and the mixture was stirred at 0 ℃ for 10 min. To the solution of the above organocuprate was added dropwise a solution of the enoate **9** (1.67 g, 5.01 mmol) in THF (66.7 mL) at -78 ºC. After being stirred at -78 ºC for 30 min, the reaction was quenched by addition of a 3:2 saturated NH_4Cl-28% NH_3 aqueous solution with additional stirring at room temperature for 30 min. the mixture was extracted with Et_2O and the extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (3:1) gave the title compound **10-Z** (889.5 mg, 60%) as white solid; IR (ATR) ν 1781 (CO), 1308 ($NHSO_2$), 1124 ($NHSO_2$) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.27 (t, J = 7.1 Hz, 3H), 1.41 (s, 9H), 3.27 (d, J = 6.6 Hz, 2H), 3.99 (d, J = 6.4 Hz, 2H), 4.17 (q, J = 7.1 Hz, 2H), 4.50 (t, J = 6.4 Hz, 1H), 6.08 (t, J = 6.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.2, 24.2, 33.9, 50.5, 60.1, 61.1, 120.8, 133.4, 170.3; HRMS (ESI), *m/z* calcd for $C_{11}H_{20}ClNNaO_4S$ $[M+Na]^+$ 320.0694, found 320.0702.



10-Z **13**

Fmoc-Gly-Ψ[(*Z*)-CCl=CH]-Gly-OEt (13): To a stirred solution of the ester **10-Z** (889.5 mg, 2.99 mmol) in CH_2Cl_2 (29.9 mL) were added anisole (648 μL, 5.97 mmol) and $AlCl_3$ (2.40 g, 17.9 mmol) under nitrogen. After stirring at room temperature for 1 h, the reaction was quenched with saturated aqueous solution of $NaHCO_3$ followed by celite filtration. The filtrated solution was extracted with CH_2Cl_2 and dried over $MgSO_4$. The reaction mixture was concentrated under reduced pressure to give the deprotected amine as colorless oil, which was used immediately in the next step without purification. To a stirred solution of the above amine (*ca.* 2.99 mmol) in CH_2Cl_2 (29.9 mL) were added DIPEA (587.3 μL, 3.29 mmol) and Fmoc-Cl (1.30 g, 3.84 mmol). After being stirrred for 3 h at room temperature, the reaction was quenched with aqueous 1 M solution of HCl. The mixture was extracted with CH_2Cl_2, washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (3:1) gave the title compound **13** (451.2 mg, 38%) as pale yellow powder; IR (ATR) ν 3291 (NH), 1781 (CO) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, J = 7.2 Hz, 5H), 3.25 (d, J = 6.6 Hz, 2H), 4.01 (d, J = 6.4 Hz, 2H), 4.09 – 4.27 (m, 4H), 4.43 (d, J = 7.0 Hz, 2H), 5.14 (d, J = 6.6 Hz, 1H), 5.97 (t, J = 6.8 Hz, 1H), 7.31 (t, J = 7.5 Hz, 3H), 7.39 (t, J = 7.5 Hz, 3H), 7.58 (d, J = 7.5 Hz, 3H), 7.76 (d, J = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.2, 33.9, 47.2, 47.5, 61.0, 67.0, 119.8, 120.0, 125.0, 127.1, 127.7, 133.6, 141.3, 143.8, 156.1, 170.5; HRMS (ESI), *m/z* Calcd for $C_{22}H_{22}ClNNaO_4$ $[M+Na]^+$ 422.1130, found 422.1148.

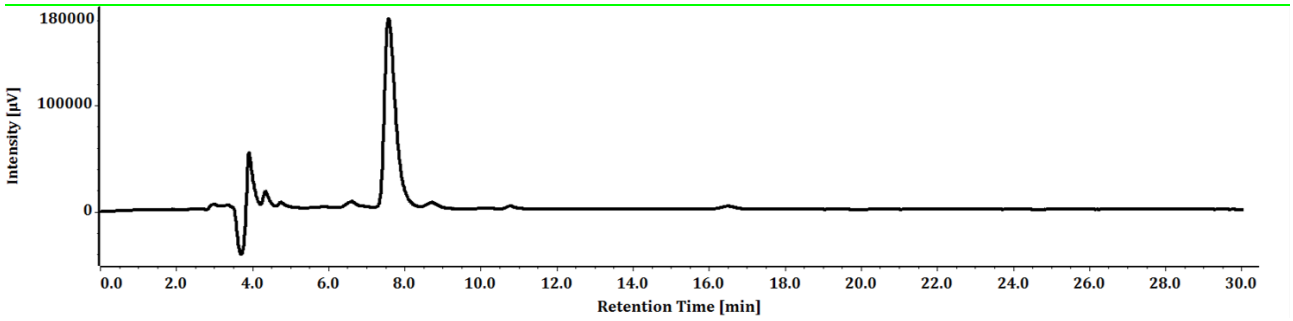


13 **14**

Fmoc-Gly-Ψ[(*Z*)-CCl=CH]-Gly-OH (14): To a stirred solution of the ester **13** (445.6 mg, 1.11 mmol) in 1,4-dioxane (8.1 mL) was added 2 N HCl aq (8.1 mL). After being stirred at reflux for 3.5 h, the mixture was concentrated under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (2:1) followed by EtOAc gave the title compound **13** (335.1 mg, 81% yield) as off white powder; IR (ATR) ν 3330 (NH), 3063 (OH), 1691 (CO) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 3.13 (d, J = 6.8 Hz, 2H), 3.8 (s, 2H), 4.11 (d, J = 6.8 Hz, 1H), 4.28 (d, J = 6.8 Hz, 2H), 5.83 (t, J = 6.8 Hz, 1H), 7.21 (t, J = 7.5 Hz, 2H), 7.29 (t, J = 7.5 Hz, 2H), 7.55 (s, 1H), 7.69 (d, J = 7.5 Hz, 2H), 7.7 (d, J = 7.5 Hz, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 33.1, 46.60, 46.97, 66.5, 119.5, 124.5, 124.8, 126.8, 127.4, 133.5, 141.2, 143.9, 157.3, 172.8; HRMS (ESI), *m/z* Calcd for $C_{20}H_{18}ClNaNO_4$ $[M+Na]^+$ 394.0817, found 394.0836.

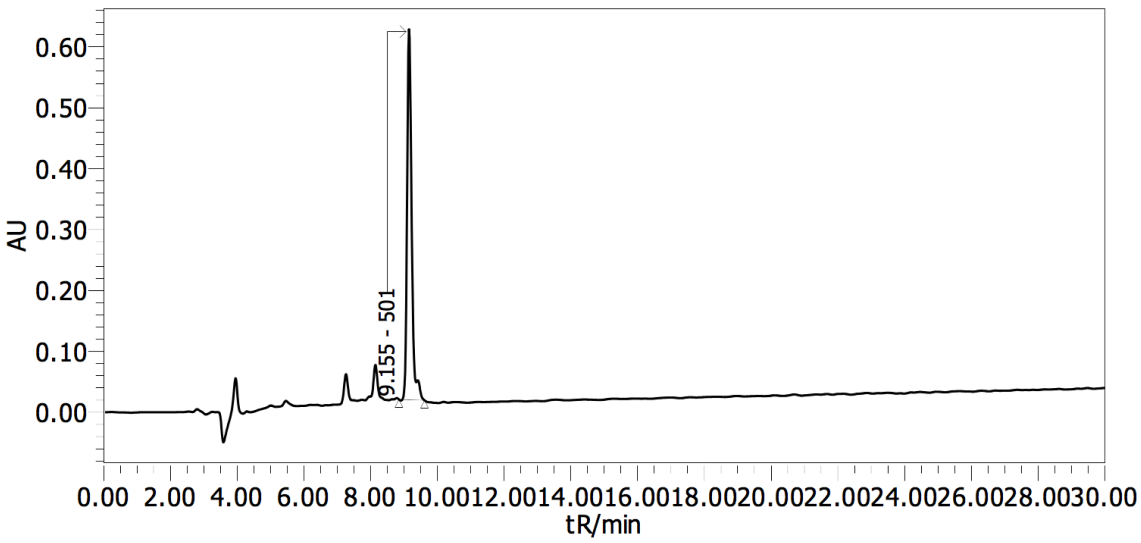
IV. Experimental procedures of (*E*)-methylalkene-type peptidomimetics 17

H-Arg-Gly-Ψ [(*E*)-CMe=CH]-Gly-Tyr-Asp-Arg-Gly-Gly-Tyr-Arg-Gly-Arg-Gly-Gly-NH_2 (17):
Rink Amide ChemMatrix Resin was used. On this resin (0.49 mmol amine/g, 102.0 mg, 0.05 mmol) was coupled Fmoc-Gly-OH (5.0 equiv), Fmoc-Gly-OH (5.0 equiv), Fmoc-Arg(Pbf)-OH (5.0 equiv), Fmoc-Gly-OH (5.0 equiv), Fmoc-Arg(Pbf)-OH (5.0 equiv), Fmoc-Tyr(^{t}Bu)-OH (5.0 equiv) Fmoc-Gly-OH (5.0 equiv), Fmoc-Gly-OH (5.0 equiv), Fmoc-Arg(Pbf)-OH (5.0 equiv), Fmoc-Asp(O^{t}Bu)-OH (5.0 equiv), and Fmoc-Tyr(^{t}Bu)-OH with the aid of DIC (5.0 equiv) and Oxyma Pure® (5.0 equiv) in DMF at room temperature for 1 h. Then Fmoc removal was performed with 20% (v/v) piperidine/DMF to give a Tyr(^{t}Bu)-Asp(O^{t}Bu)-Arg(Pbf)-Gly-Gly-Tyr(^{t}Bu)-Arg(Pbf)-Gly-Arg(Pbf)-Gly-Gly- incorporated resin. The resulting resin was treated with an Ns-Gly-Ψ[(*E*)-MeC=CH]-Gly-OH **5** (2.0 equiv), HATU (1.96 equiv), and DIPEA (2.0 equiv) to yield the resin containing the Gly-Gly-type MADI. Then, Ns group removal was performed with K_2CO_3 (2.0 equiv) in 5% (v/v) thiophenol/DMF to give a Gly-Ψ[(*E*)-MeC=CH]-Tyr(^{t}Bu)-Asp(O^{t}Bu)-Arg(Pbf)-Gly-Gly-Tyr(^{t}Bu)-Arg(Pbf)-Gly-Arg(Pbf)-Gly-Gly-incorporated resin. On these resins, standard Fmoc-based SPPS was performed for the chain elongation to give a protected peptide resin for the pseudopeptide **17**. The resulting completed resin was treated with TFA-*m*-cresol-thioanisole-H_2O (87.5:5:5:2.5 (v/v), 50 μL/1 mg resin) at room temperature for 2 h. The resin in the reaction mixture was filtrated off. To the resulting filtrate was added cooled Et_2O to give a precipitate. The formed precipitate was collected by centrifugation and thoroughly washed with Et_2O to afford crude pseudopeptide **17**. The crude pseudopeptide **17** was purified by preparative HPLC gave the title pseudopeptide **17** as colorless freeze-dried powder.
17 Analytical HPLC conditions: COSMOSIL 5C_{18}-AR-Ⅱ analytical column with a isocratic of 0.1% TFA-MeCN in 0.1% TFA aq., 12% over 30 min, detected at 220 nm, retention time = 7.5 min, HRMS (ESI) *m*/*z* calcd ([M + 2H]$^+$) 740, found 740.



V. Experimental procedures of (*Z*)-chloroalkene-type peptidomimetics 19

H-Arg-Gly-Ψ [(*Z*)-CCl=CH]-Gly-Tyr-Asp-Arg-Gly-Gly-Tyr-Arg-Gly-Arg-Gly-Gly-NH_2 (19):
Rink Amide ChemMatrix resin was used. On this resin (0.54 mmol amine/g, 93.0 mg, 0.05 mmol) was coupled Fmoc-Gly-OH (3.0 equiv), Fmoc-Gly-OH (3.0 equiv), Fmoc-Arg(Pbf)-OH (3.0 equiv), Fmoc-Gly-OH (3.0 equiv), Fmoc-Arg(Pbf)-OH (3.0 equiv), and Fmoc-Tyr(^{t}Bu)-OH (3.0 equiv) with the aid of DIC (3.0 equiv) and HOBt·H_2O (3.0 equiv) in DMF at room temperature for 1 h. Then Fmoc removal was performed with 20% (v/v) piperidine/DMF to give a Tyr(^{t}Bu)-Arg(Pbf)-Gly-Arg(Pbf)-Gly-Gly-incorporated resin. The resulting resin was treated with an Fmoc-Gly-Ψ[(*Z*)-ClC=CH]-Gly-OH **14** (2.0 equiv), HATU (1.96 equiv), and DIPEA (2.0 equiv) to yield the resin containing the Gly-Gly-type CADI. On these resins, standard Fmoc-based SPPS was performed for the chain elongation to give a protected peptide resin for the puedopeptide **19**. The resulting completed resin was treated with TFA-*m*-cresol-thioanisole-H_2O (87.5:5:5:2.5 (v/v), 50 μL/1 mg resin) at room temperature for 2 h. The resin in the reaction mixture was filtrated off. To the resulting filtrate was added cooled Et_2O to give a precipitate. The formed precipitate was collected by centrifugation and thoroughly washed with Et_2O to afford crude puedopeptide **19**. The crude pseudopeptide **19** was purified by preparative HPLC gave the title pseudopeptide **19** as colorless freeze-dried powder.
19 analytical HPLC conditions: YMC-Actus Triart C18 analytical column with a linear gradient of 0.1% TFA-MeCN / 0.1% TFA aq. (10:90-40:60 over 30 min), detected at 220 nm, retention time = 9.15 min, LRMS (ESI) *m*/*z* calcd ([M + 3H]$^+$) 501, found 501.



VI. Comparison of vinyl protons of peptidomimetics and control compounds

In order to check the potential olefin isomerization, the purified peptidomimetics were analyzed by ^{1}H-NMR in DMSO-d_6 with the corresponding control compounds.

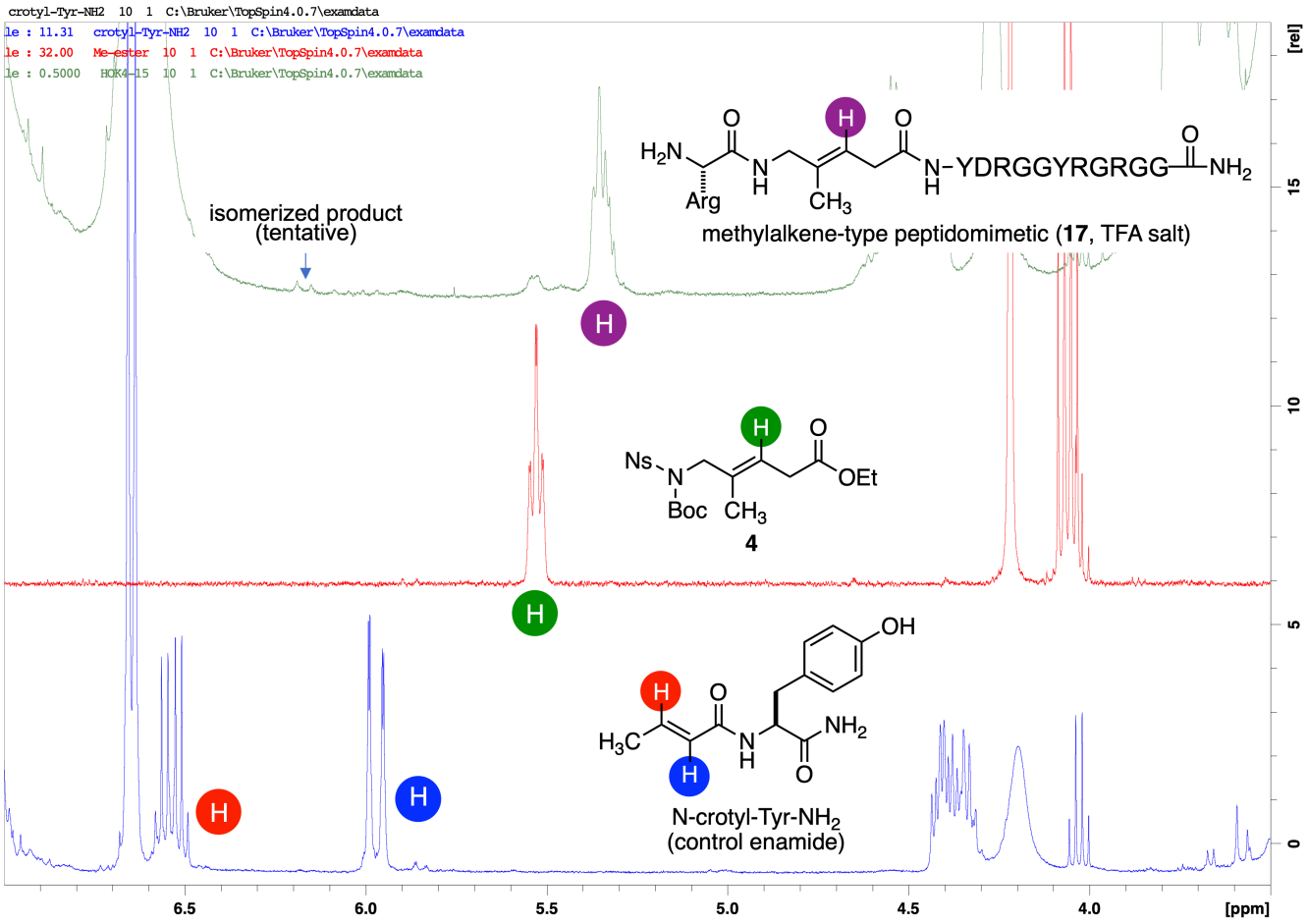


Figure S1. ^{1}H NMR spectra of (*E*)-methylalkene-type peptidomimetic and control compounds (ester (4) and N-crotyl-Tyr-NH_2). A trace amount of potential olefin isomerized compounds of 17 is observed as a side product, which is possibly generated under the coupling condition (HATU/DIPEA in DMF).

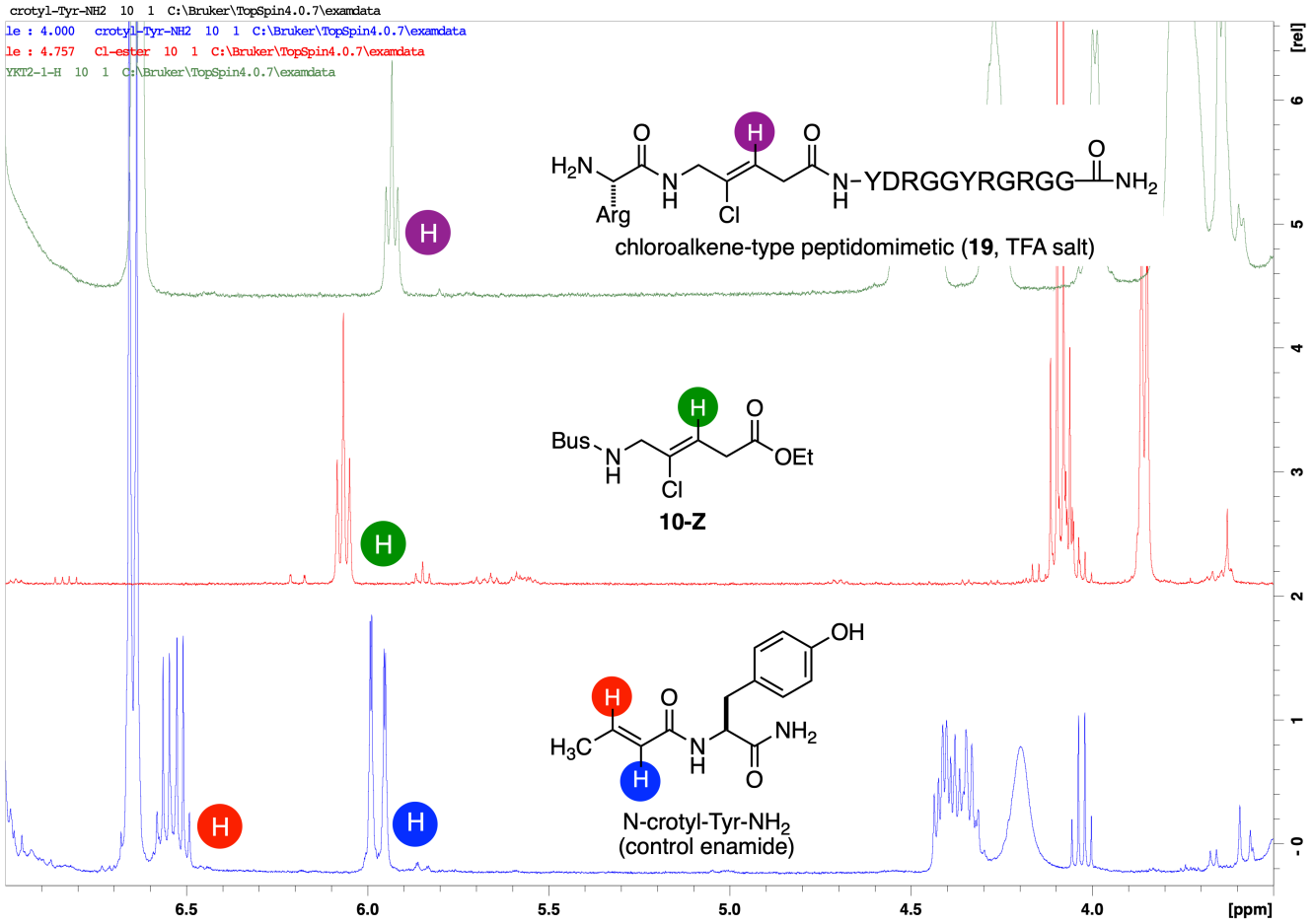
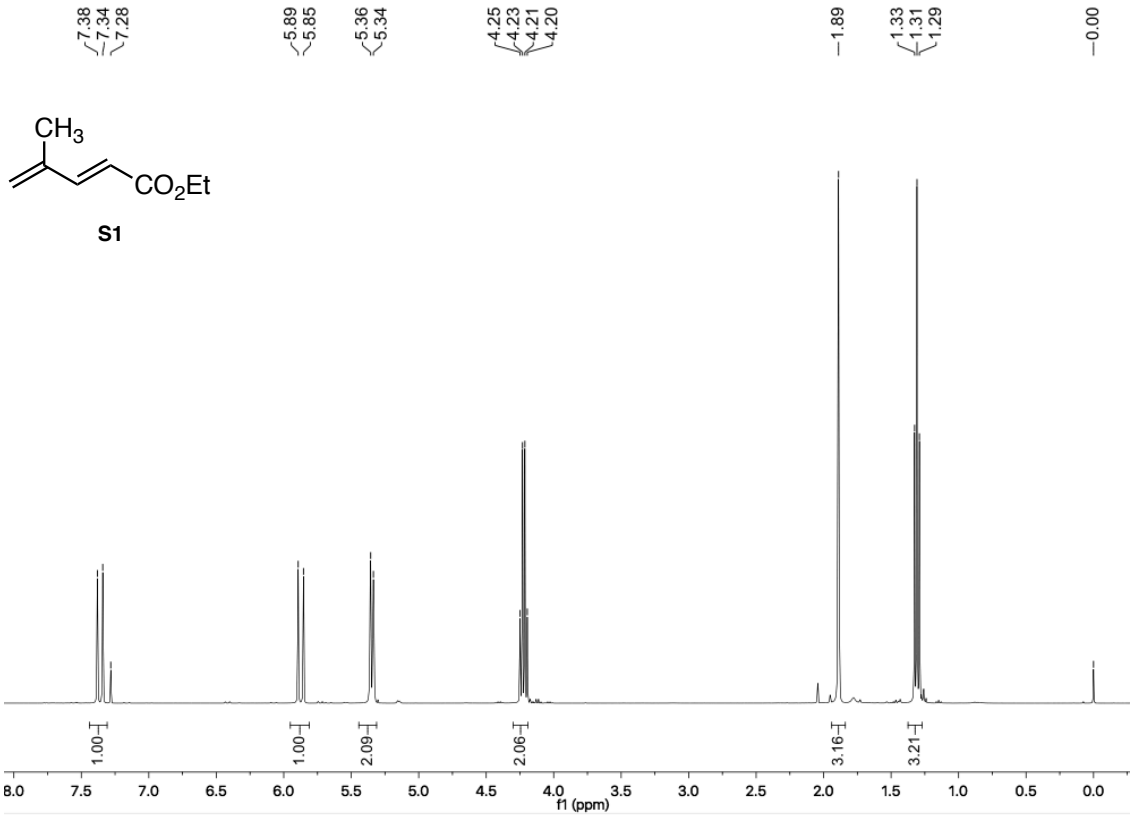
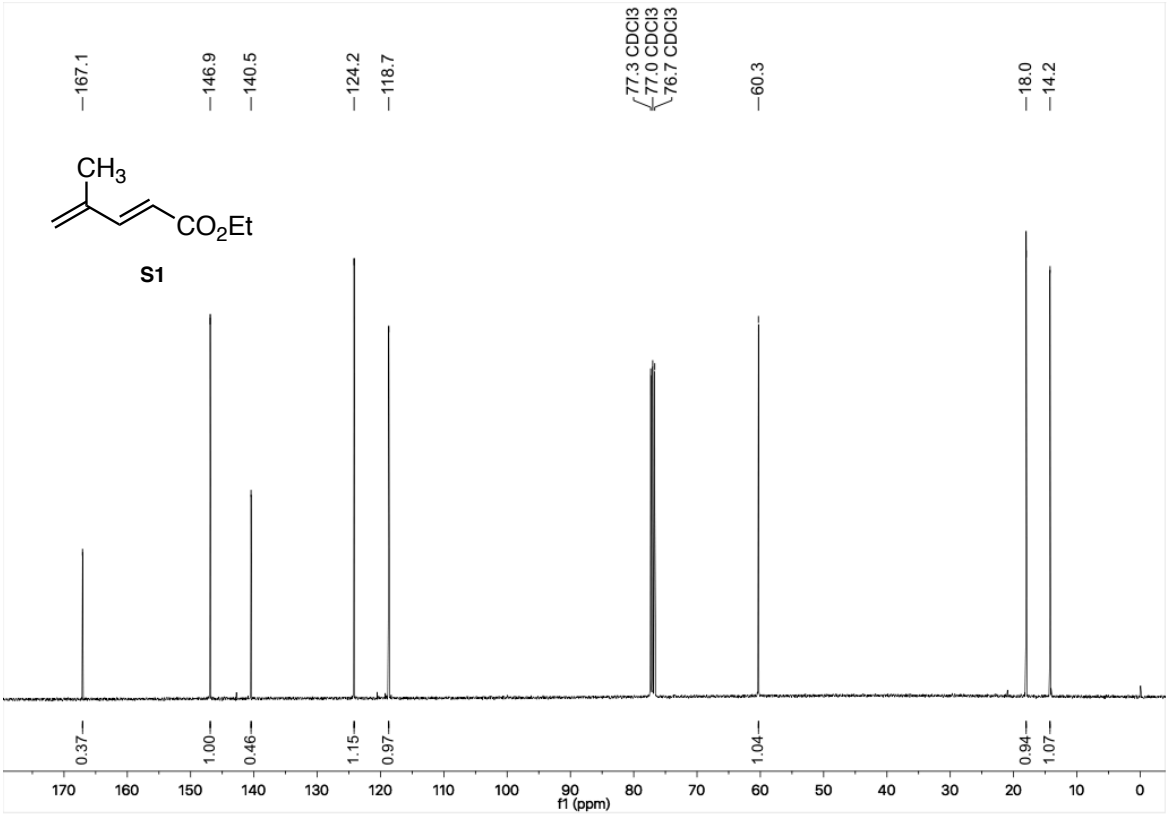
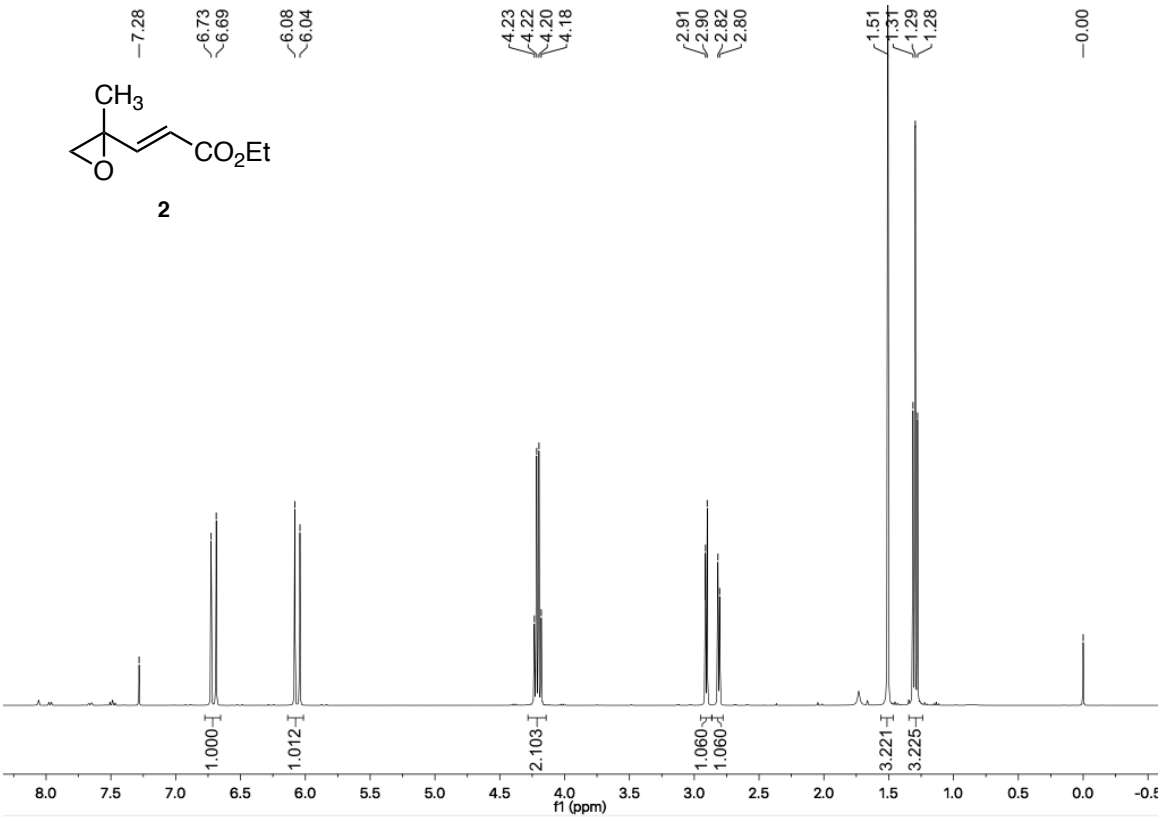


Figure S2. ^{1}H NMR spectra of (*Z*)-chloroalkene-type peptidomimetic and control compounds (ester (10-Z) and N-crotyl-Tyr-NH_2).

VII. ^{1}H NMR and ^{13}C NMR charts



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6.04
4.23
4.22
4.20
4.18
2.91
2.90
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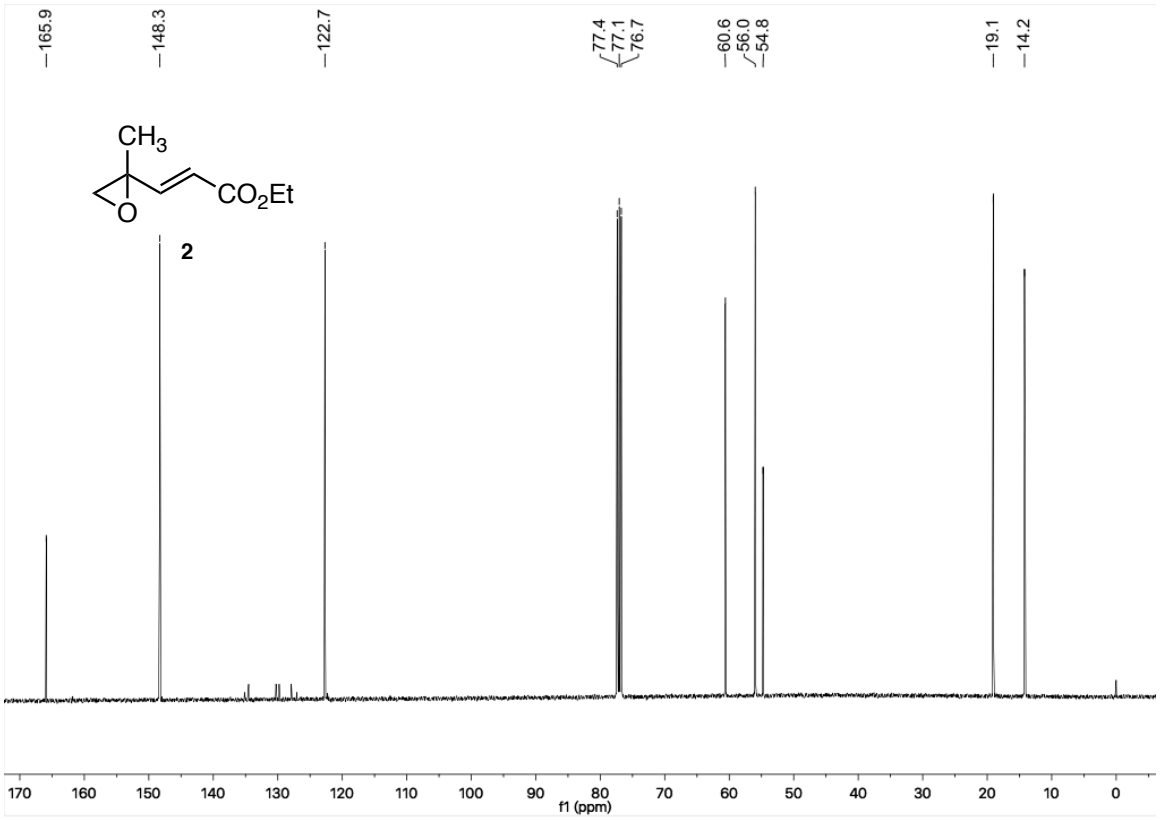
2.80
1.51
1.31
1.29
1.28
0.00
CH3
CO2Et
O
2
1.000
1.012
2.103
1.060
1.060
3.221
3.225
f1 (ppm)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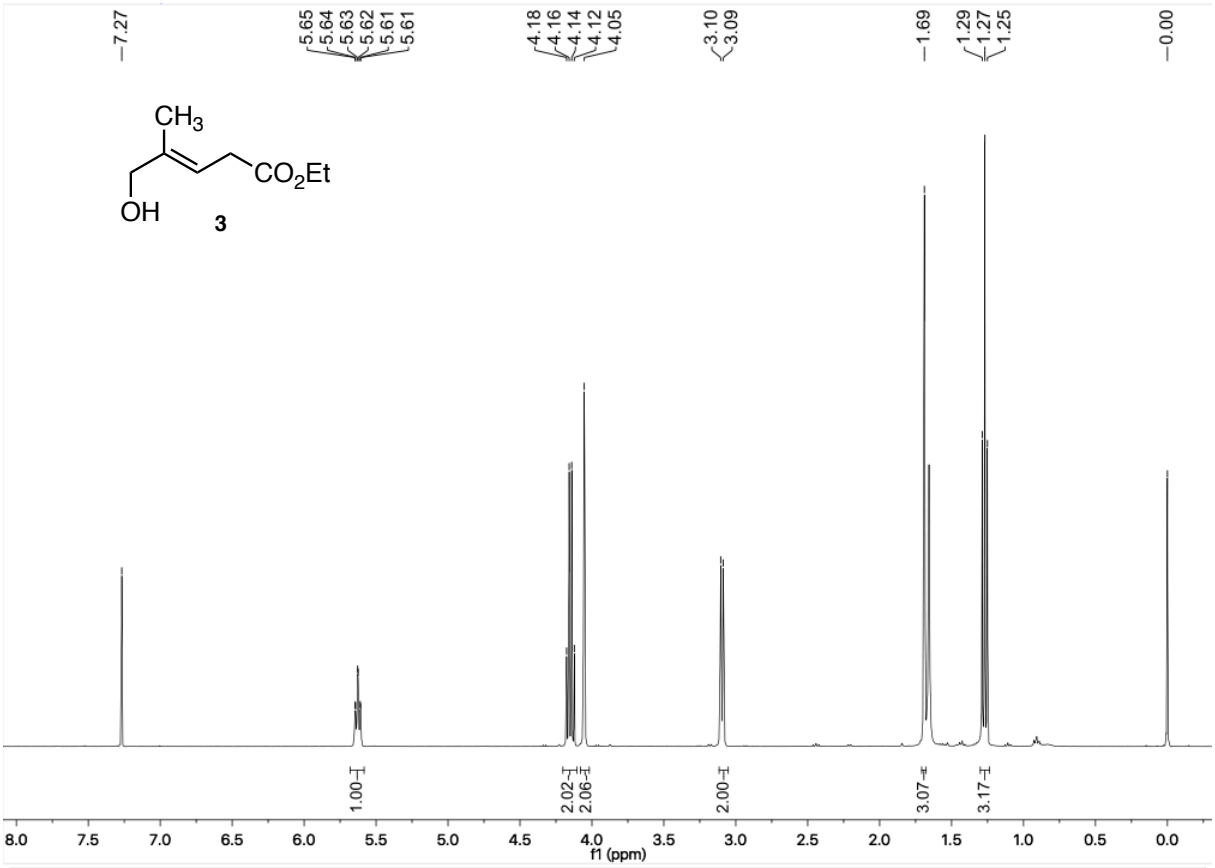
122.7
77.4
77.1
76.7
60.6
56.0
54.8
19.1
14.2
CH3
CO2Et
O
2
f1 (ppm)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5.63
5.62
5.61
5.61
4.18
4.16
4.14
4.12
4.05
3.10
3.09
1.69
1.29
1.27
1.25
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CO2Et
OH
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1.00
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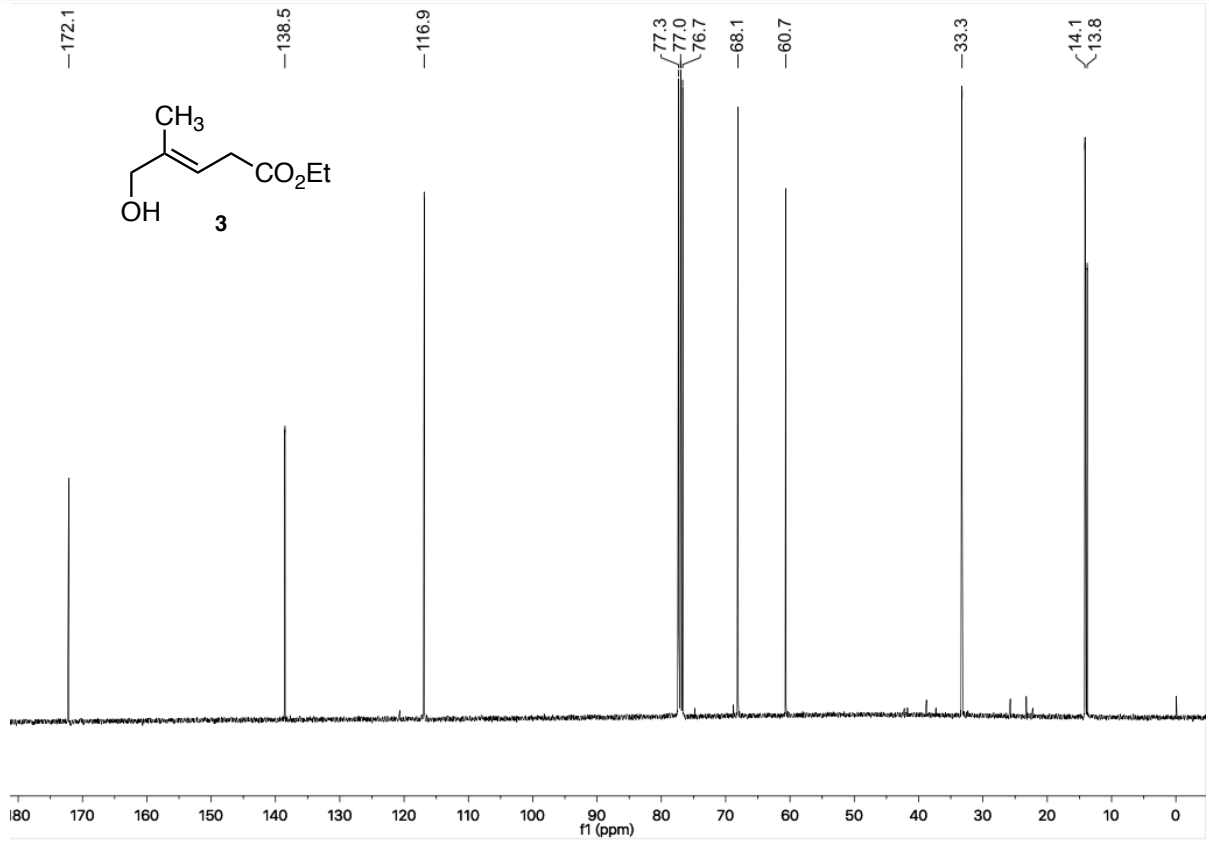
2.06
2.00
3.07
3.17
f1 (ppm)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60.7
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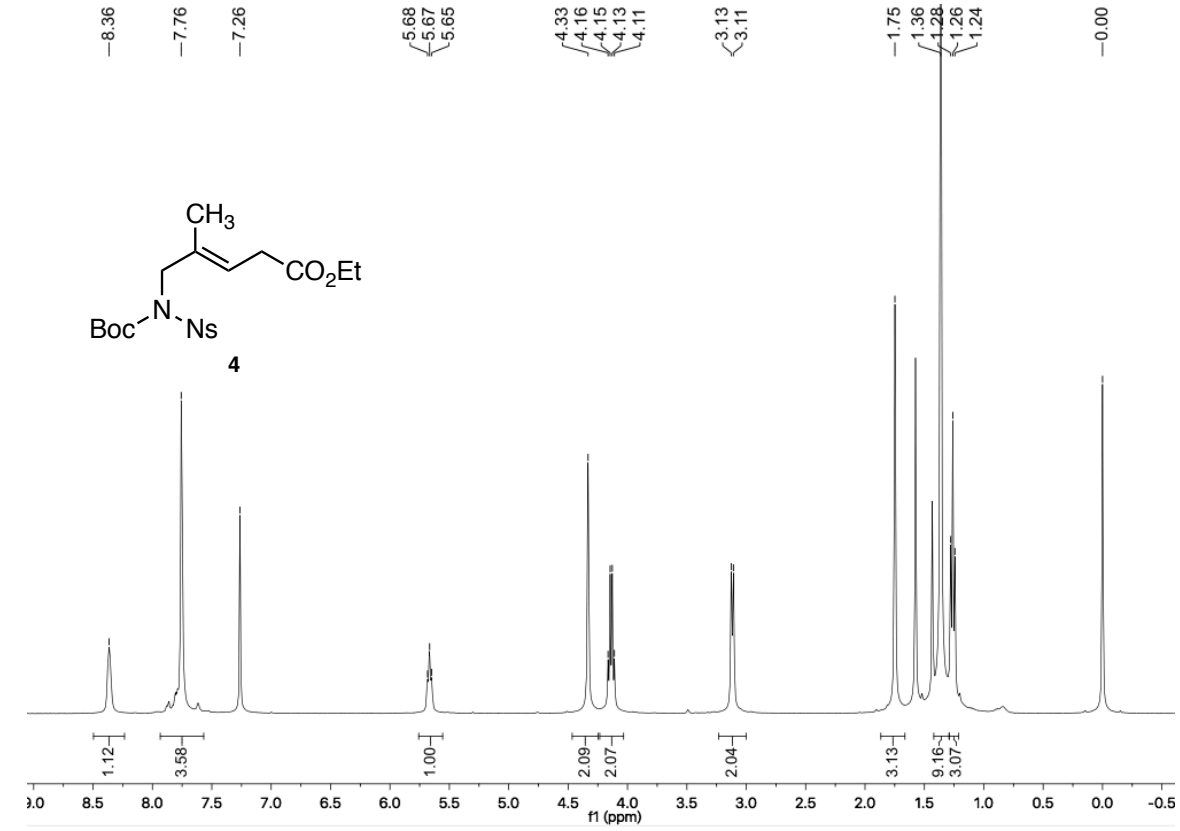
CO2Et
OH
3
f1 (ppm)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7.76
7.26
5.68
5.67
5.65
4.33
4.16
4.15
4.13
4.11
3.13
3.11
1.75
1.36
1.28
1.26
1.24
0.00
CH3
CO2Et
Boc
N
Ns
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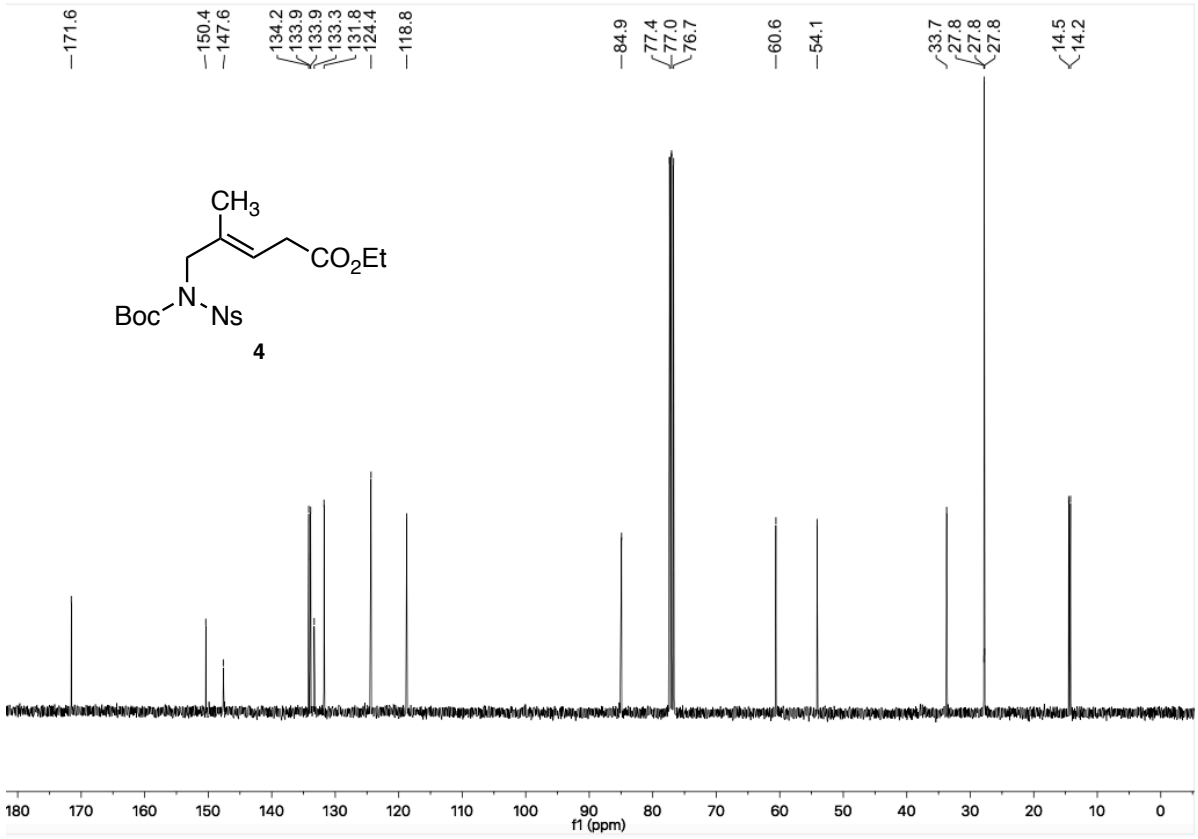
3.58
1.00
2.09
2.07
2.04
3.13
9.16
3.07
f1 (ppm)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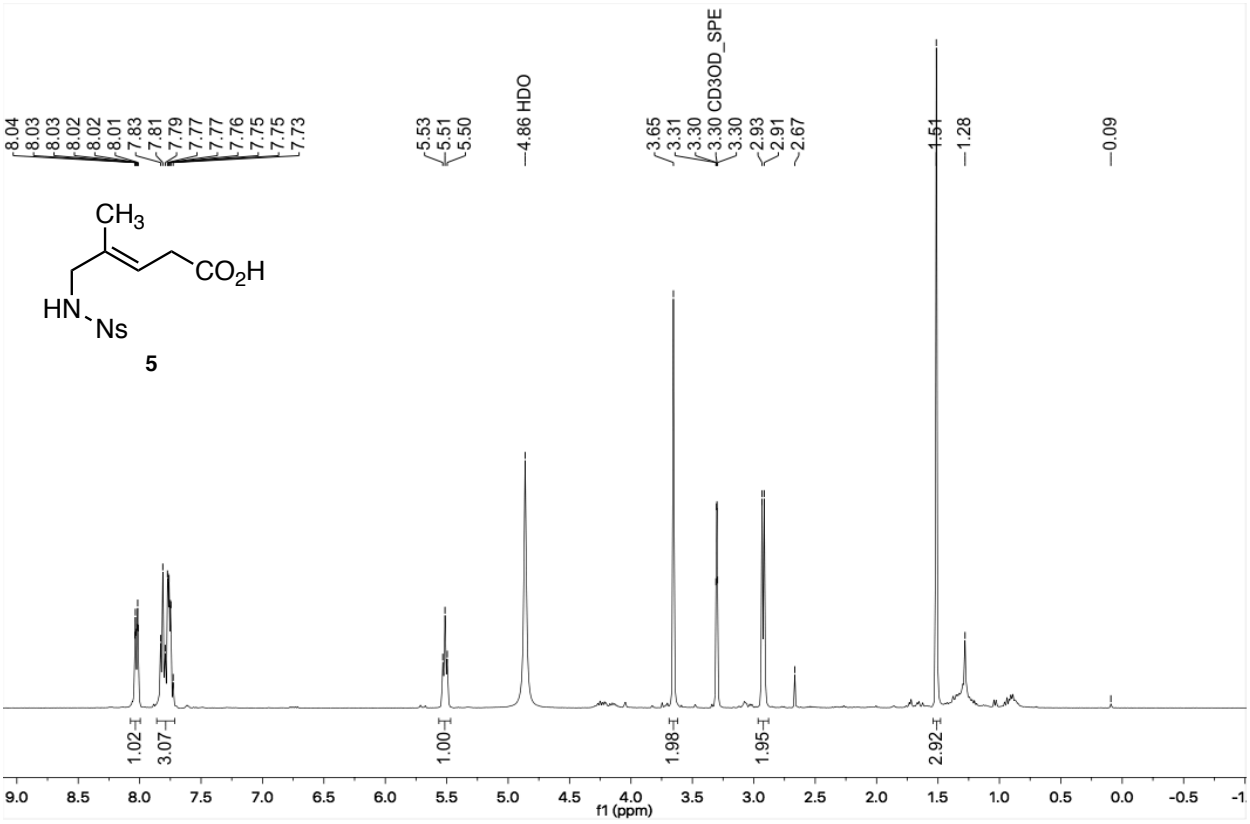
150.4
147.6
134.2
133.9
133.9
133.3
131.8
124.4
118.8
84.9
77.4
77.0
76.7
60.6
54.1
33.7
27.8
27.8
27.8
14.5
14.2
CH3
CO2Et
Boc
N
Ns
4
f1 (ppm)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8.03
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8.02
8.01
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7.75
7.75
7.73
5.53
5.51
5.50
4.86 HDO
3.65
3.31
3.30
3.30 CD3OD_SPE
3.30
2.93
2.91
2.67
1.51
1.28
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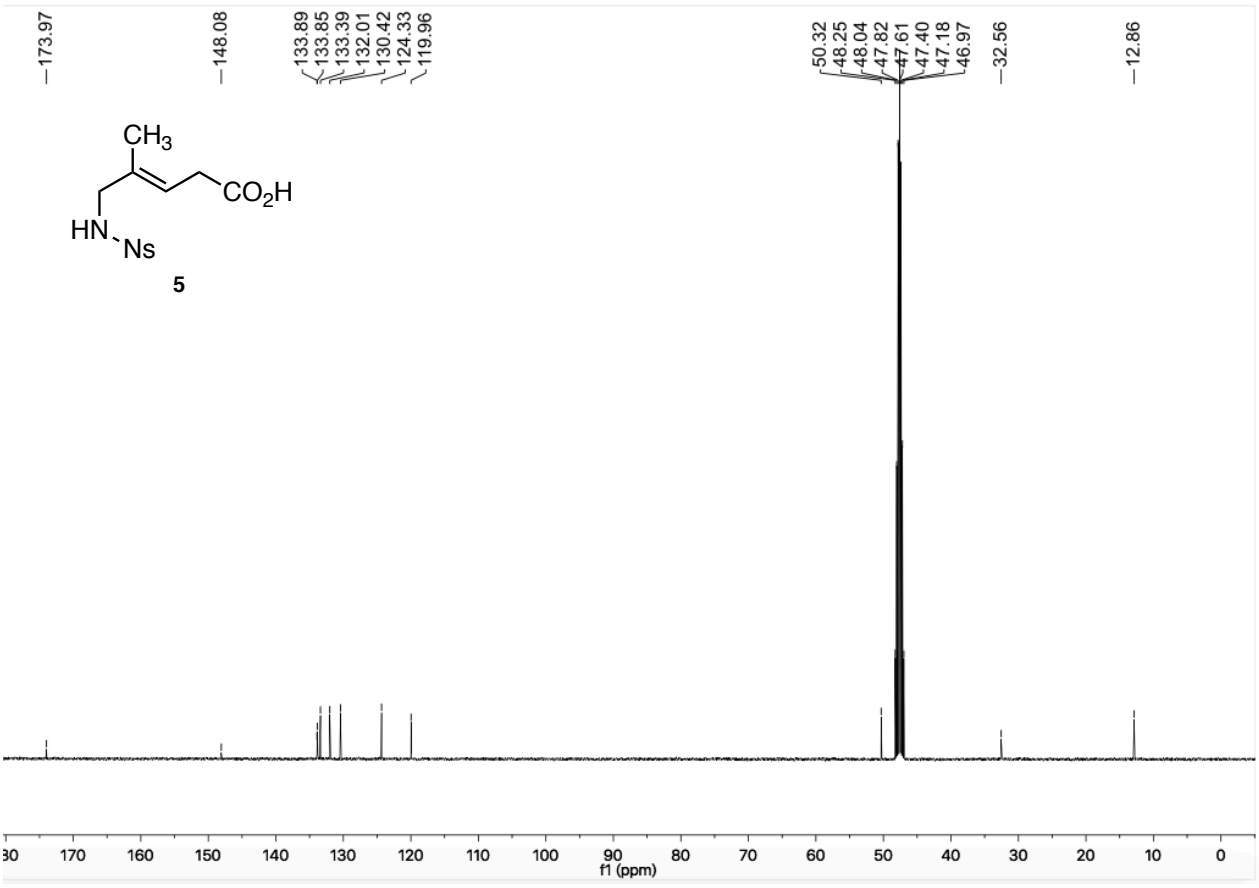
HN
Ns
5
1.02
3.07
1.00
1.98
1.95
2.92
f1 (ppm)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50.32
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46.97
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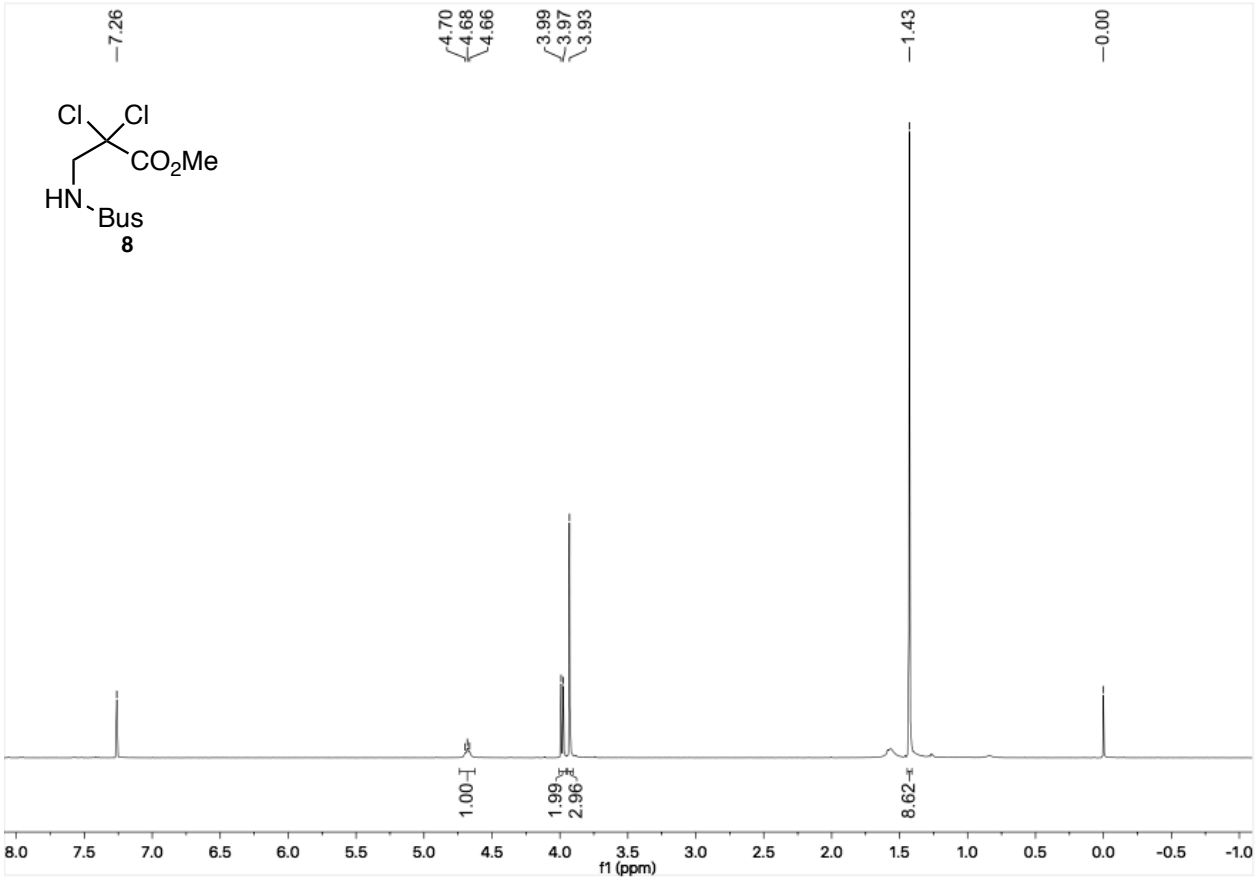
12.86
CH3
CO2H
HN
Ns
5
f1 (ppm)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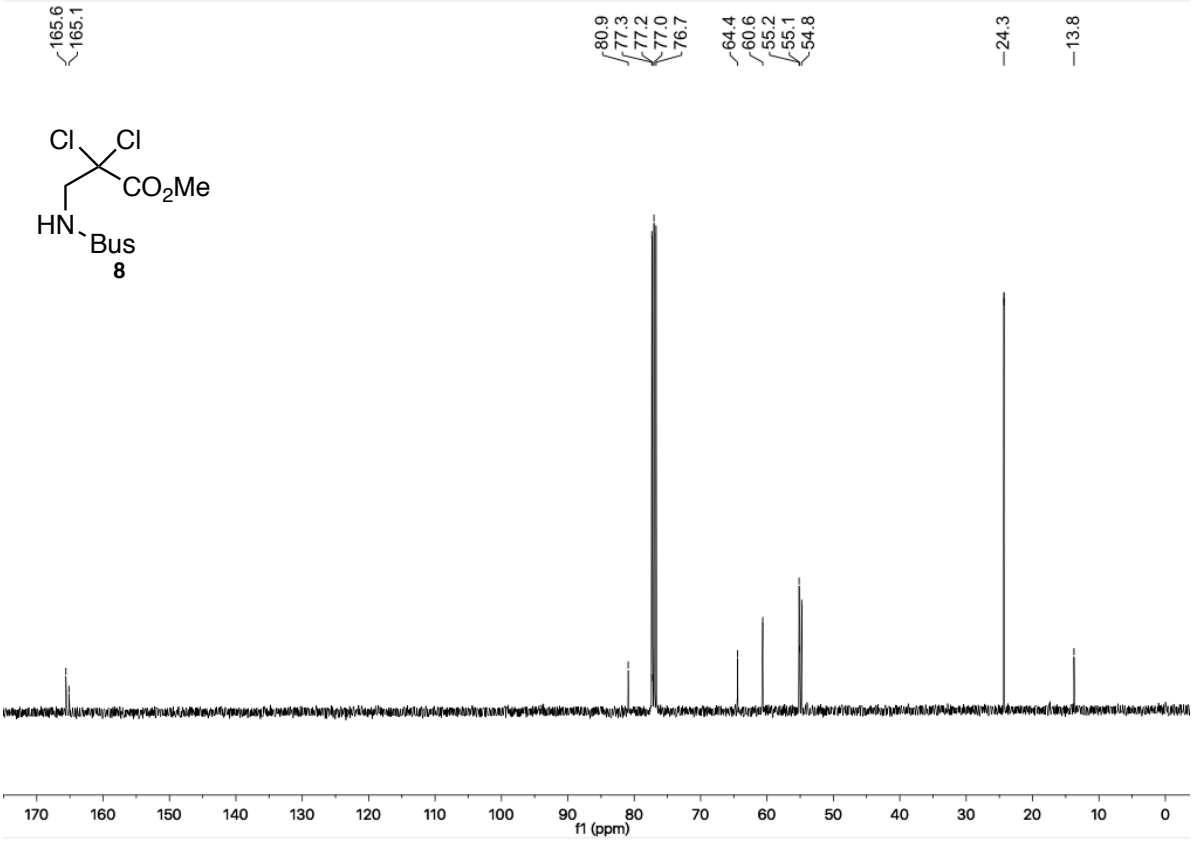
8.62
f1 (ppm)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77.0
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55.1
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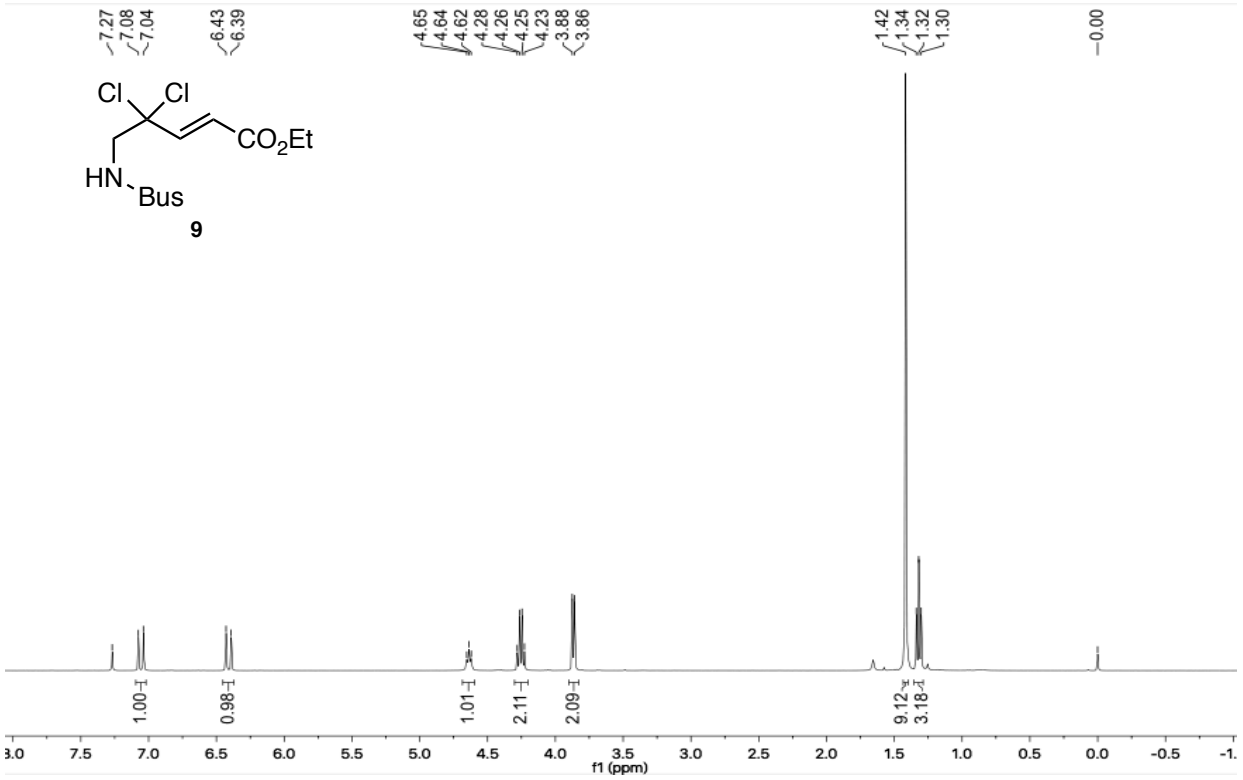
24.3
13.8
Cl
Cl
CO2Me
HN
Bus
8
f1 (ppm)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3.86
1.42
1.34
1.32
1.30
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Bus
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0.98
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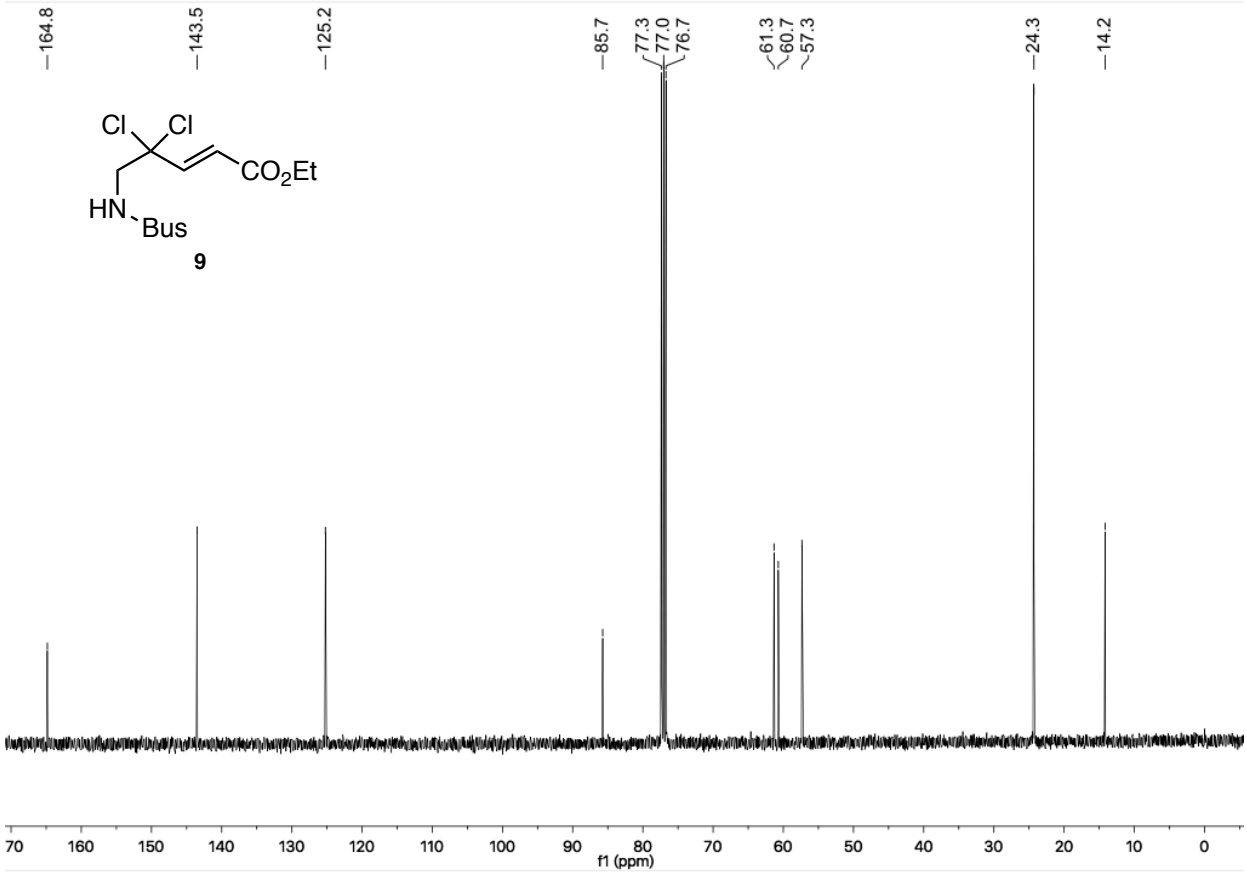
2.09
9.12
3.18
f1 (ppm)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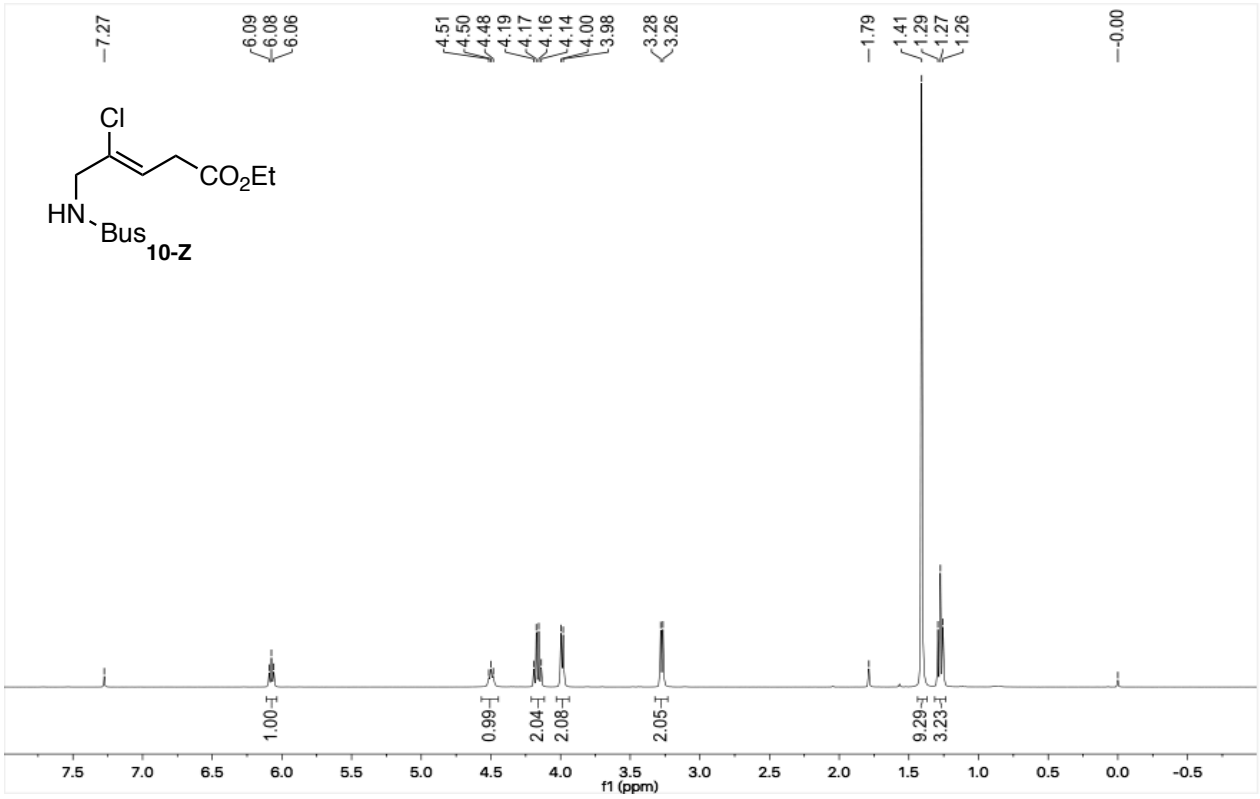
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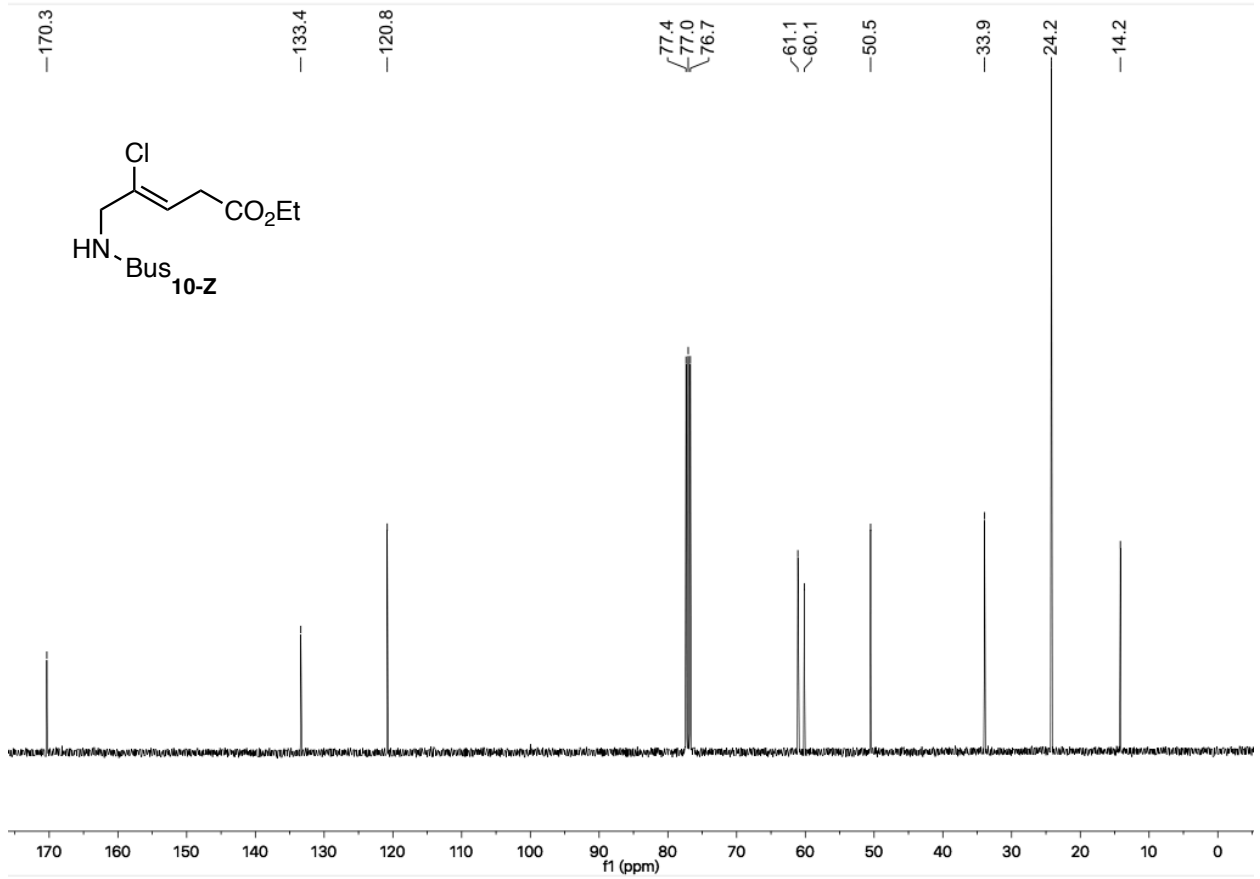
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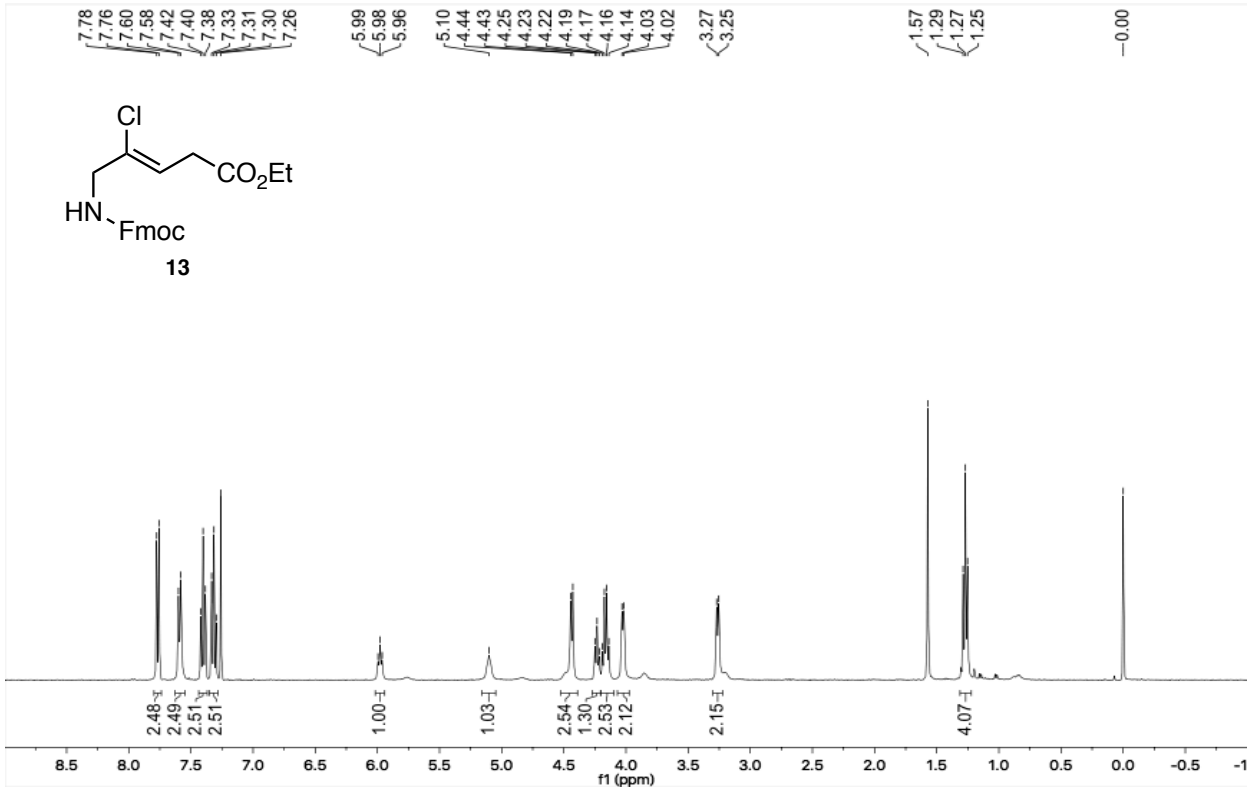
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4.03
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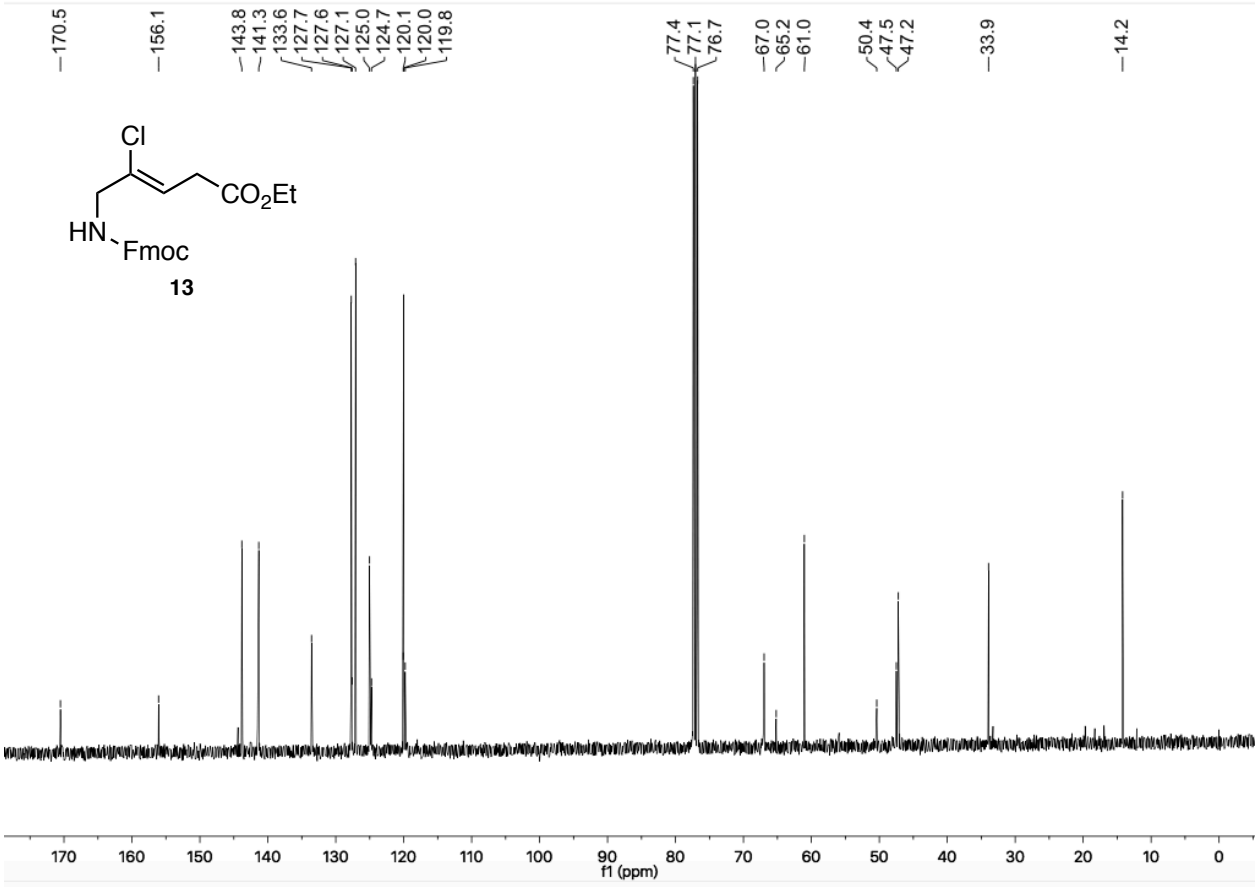
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Fmoc
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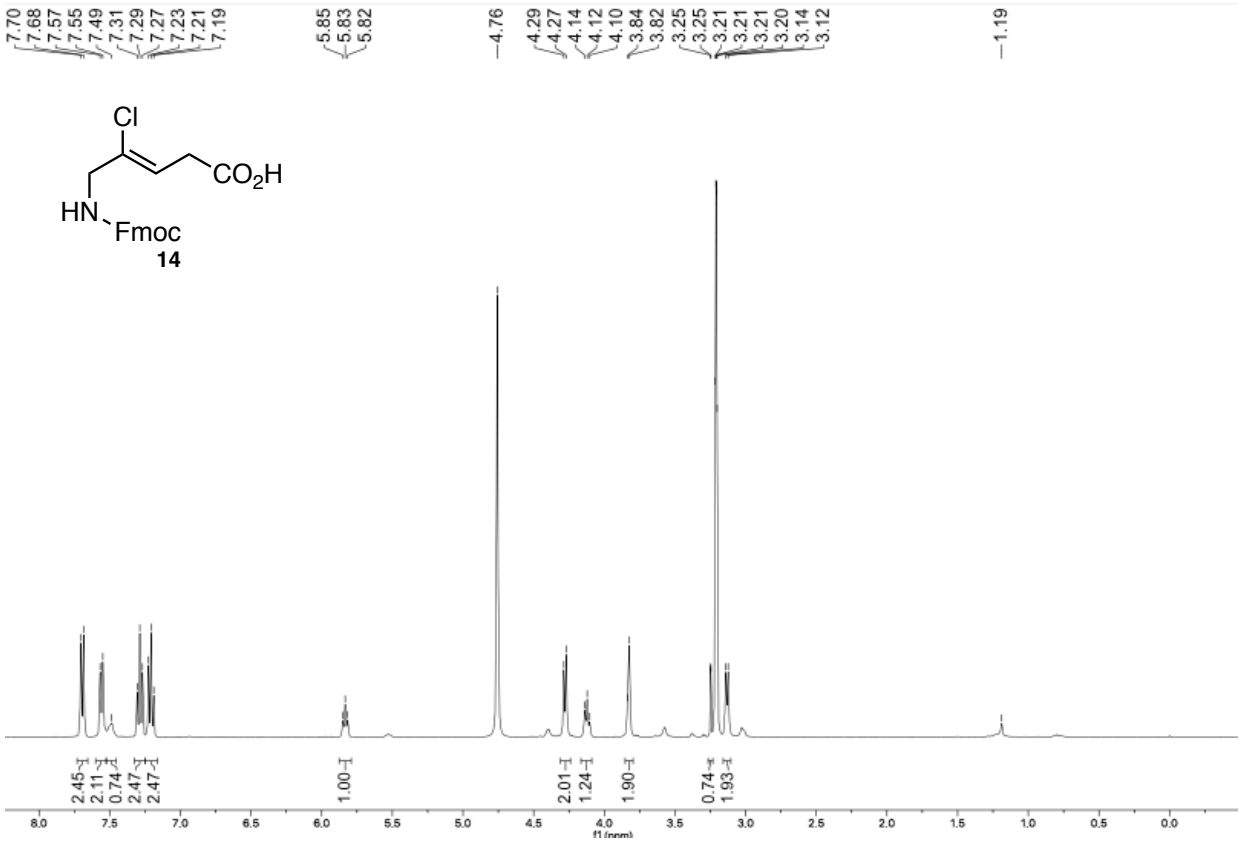
f1 (ppm)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4.27
4.14
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3.84
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3.21
3.20
3.14
3.12
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Cl
CO2H
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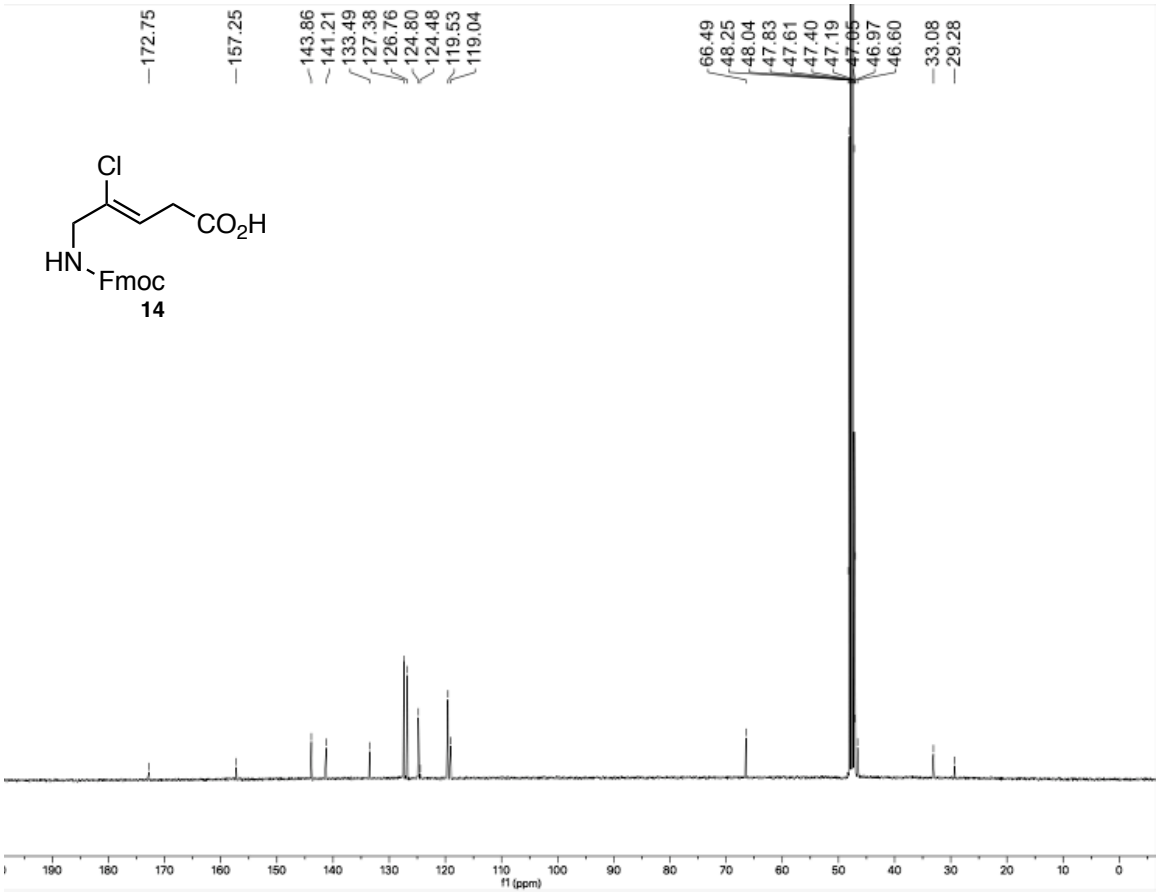
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47.83
47.61
47.40
47.19
47.05
46.97
46.60
33.08
29.28
Cl
CO2H
HN
Fmoc
14
f1 (ppm)