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Supporting Information

Amide-to-Chloroalkene Substitution for Overcoming Intramolecular Acyl Transfer Challenges in Hexapeptidic Neuromedin U Receptor 2 Agonists

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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. (*S*)-*tert*-butylsulfinamide was purchased from Combi-Blocks. For reactions that required heating, an oil bath was used as the heat source. 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 PSQ60B (Fuji Silysia Chemical, Ltd.). Structural assignments were made with additional information from COSY, HSQC, and HMBC experiments.

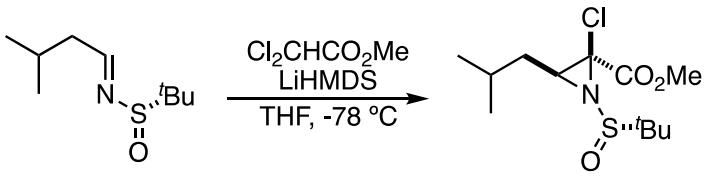
Characterization Data. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using a Bruker Biospin AVANCE III HD. Chemical shifts are reported in δ (ppm) relative to Me_4Si (in $CDCl_3$) as an internal standard. Infrared (IR) spectra were recorded on a JASCO FT/IR 6300 with the ATR method and are reported as wavenumbers (cm^{-1}). Low- and high-resolution mass spectra were recorded on a Bruker Daltonics compact (ESI-Q-TOF) spectrometer in the positive and negative detection mode. Optical rotations were measured on a JASCO DIP-370 polarimeter operating at the sodium D line with a 100 mm path length cell and were reported as follows: $[\alpha]_D$ (concentration (g/100 mL), solvent).

HPLC condition. For HPLC separations, a Cosmosil $5C_{18}$-AR-II analytical column (Nacalai Tesque, 3.0 × 50 mm, flow rate 0.3 mL min^{-1}), Cosmosil Cholester analytical column (Nacalai Tesque, 4.6 × 150 mm, flow rate 1.0 mL min^{-1}), Cosmosil $5C_{18}$-AR-II preparative column (Nacalai Tesque, 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.

UHPLC condition. For UHPLC separations, Unifinepak C_{18} 03050-1.9M analytical column (JASCO, 4.6 × 250 mm, flow rate 1.0 mL min^{-1}), 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 UHPLC elution.

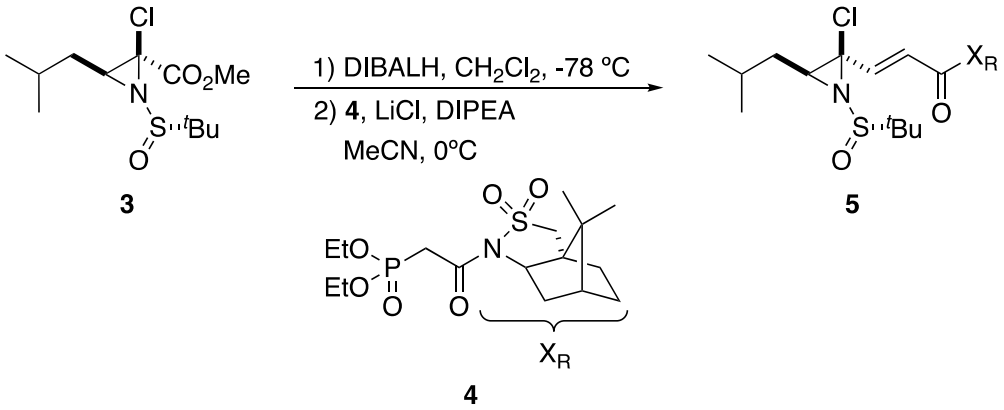
Calcium mobilization assay. As previously reported[S1], Chinese hamster ovary (CHO) cells stably expressing human NMUR1 or NMUR2 were used for evaluating the agonistic activity of (*Z*)-chloroalkene type CPN-116 analogue. NMUR1 or NMUR2-expressing CHO cells were established in National Cerebral and Cardiovascular Center Research Institute. CPN-116 and hNMU were similarly used as positive controls. Curve fitting on a five-parameter logistic model and calculation of EC_{50} values were performed using GraphPad Prism 9 ver. 9.5.1.

II. Experimental procedures of Leu-Dap-type CADI containing peptidomimetic



2 **3**

Methyl (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-2-chloro-3-isobutylaziridine-2-carboxylate (3): To a solution of Cl_2CHCO_2Me (3.64 mL, 35.1 mmol) in THF (117 mL) was added LiHMDS in THF (1.3 M, 27.0 mL, 35.1 mmol) at -78 °C, and the mixture was stirred at -78 °C for 30 min. To the solution was added *N*-sulfinyl imine **2** (2.21 g, 11.7 mmol) in THF (23.4 mL), and the mixture was stirred at -78 °C for 1 h. The reaction was quenched with anhydrous MeOH at -78 ºC for 30 min. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (5:1) gave the 2-chloroaziridine **3** as a colorless oil (3.39 g, 98%): $[\alpha]_D^{28.1}$ = +2.79 (*c* 0.87, $CHCl_3$); IR (ATR) ν 1726 (CO), 1137 (NSO); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.84 (s, 3H), 3.34–3.43 (m, 1H), 1.78–1.90 (m, 1H), 1.56–1.71 (m, 2H), 1.30 (s, 9H), 0.97–1.02 (m, 6H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 165.4, 63.0, 58.2, 54.1, 46.4, 37.3, 26.5, 22.6, 22.4 (2C); HRMS (ESI), m/z calcd for $C_{12}H_{23}ClNO_3S$ $[M+H]^+$ 296.1082, found 296.1079.

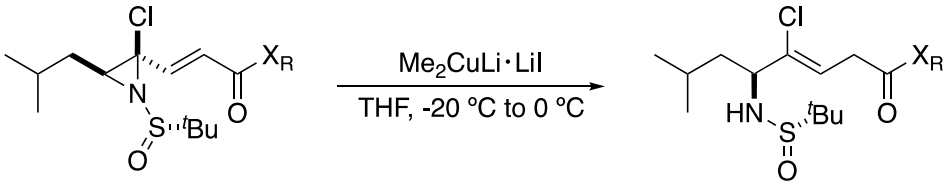


3 **4** **5**

(*E*)-3-((2*R*,3*S*)-1-((*S*)-*tert*-Butylsulfinyl)-2-chloro-3-isobutylaziridin-2-yl)-1-((3a*S*,6*R*)-8,8-dimethyl-2,2-dioxidotetrahydro-3*H*-3a,6-methanobenzo[*c*]isothiazol-1(4*H*)-yl)prop-2-en-1-one (5): To a solution of ester **3** (1.60 g, 5.42 mmol) in CH_2Cl_2 (54.2 mL) was added dropwise a solution of DIBAL-H in toluene (1.00 M, 10.8 mL, 10.8 mmol) at -78 °C, and the mixture was stirred at -78 °C for 5 min. The reaction was quenched by a saturated aqueous solution of Rochelle salt. The reaction mixture was extracted with diethyl ether, washed with brine, and dried over Na_2SO_4. Concentration under reduced pressure gave an oily aldehyde, which was used immediately in the next step without purification.

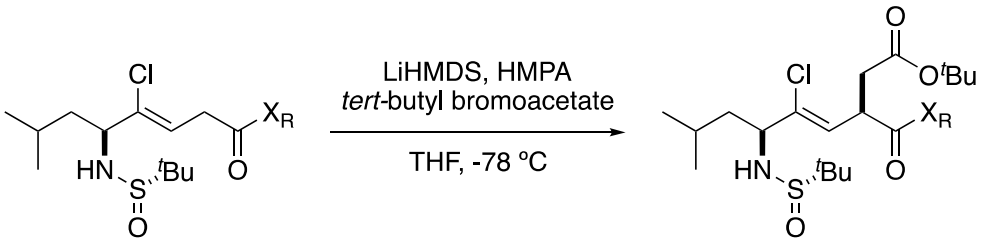
To a stirred solution of diethyl-2-oxo-2-((2*R*)-*N*-camphor-10,2-sultam)-ethylphosphonate **4**[S2] (2.77 g, 7.05 mmol) in MeCN (16.0 mL) were added LiCl (574.1 mg, 13.6 mmol) and DIPEA (2.37 mL, 13.6 mmol) at 0 °C. After stirring for 30 min, a solution of the above aldehyde in MeCN (11.0 mL) was added to the mixture, and the mixture was stirred at 0 ºC for 1 h. The reaction mixture was quenched by saturated aqueous NH_4Cl and extracted with EtOAc. The extract was washed with brine and dried over Na_2SO_4. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (3:1) gave the title compound **5** as a white solid (902.1 mg, 33% in two steps, *E:Z* =16:1): $[\alpha]_D^{23.8}$ = +1.49 (*c* 0.47, $CHCl_3$); IR (ATR) ν 1682 (CO), 1325 (NSO),

1132 (NSO); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 14.4 Hz, 1H), 7.14 (d, *J* = 14.4 Hz, 1H), 3.88–3.97 (m, 1H), 3.52 (d, *J* = 13.6 Hz, 1H), 3.45 (d, *J* = 13.6 Hz, 1H), 2.86–2.92 (m, 1H), 2.04–2.23 (m, 2H), 1.73–1.99 (m, 5H), 1.59–1.70 (m, 1H), 1.32–1.48 (m, 2H), 1.28 (s, 9H), 1.17 (s, 3H), 0.96–1.02 (s, 3H/d, 3H/d, 3H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 162.6, 145.3, 123.8, 67.8, 65.2, 58.1, 53.1, 48.5, 47.8, 46.8, 44.7, 38.5, 37.6, 32.8, 26.6, 26.5, 23.3 (3C), 22.6, 22.5, 20.8, 19.9; HRMS (ESI), m/z calcd for $C_{23}H_{38}ClN_2O_4S_2$ $[M+H]^+$ 505.1956, found 505.1960.



5 **6**

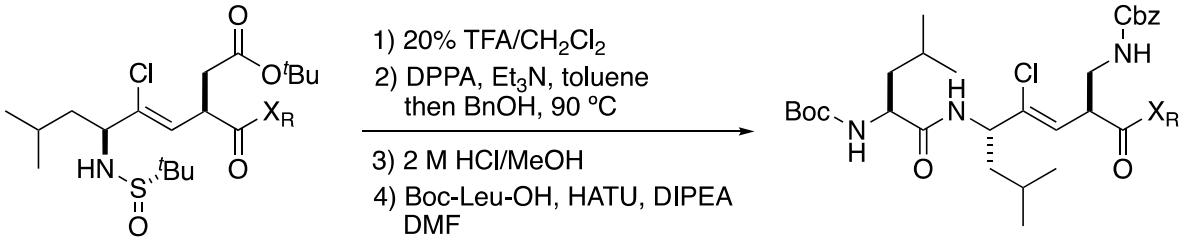
(*S*)-*N*-((4*S*,*Z*)-5-Chloro-8-((3a*S*,6*R*)-8,8-dimethyl-2,2-dioxidotetrahydro-3*H*-3a,6-methanobenzo[*c*]isothiazol-1(4*H*)-yl)-2-methyl-8-oxooct-5-en-4-yl)-2-methylpropane-2-sulfinamide (6): To a suspension of CuI (2.02 g, 10.6 mmol) in THF (52.8 mL) was added dropwise a solution of MeLi·LiBr complex in diethyl ether (1.18 M, 21.4 mL, 25.3 mmol) at -78 ºC, and the mixture was stirred at 0 ºC for 10 min. To the solution of the above organocuprate was added dropwise a solution of the *N*-enoyl sultam **5** (1.33 g, 2.64 mmol) in THF (13.2 mL) at -20 ºC. After stirring at 0 ºC for 5.0 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 diethyl ether and the extract was washed with brine and dried over Na_2SO_4. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (1:1) gave the title compound **6** as a white solid (1.07 g, 44% yield, *Z*:*E* =12:1): $[\alpha]_D^{24.9}$ = -7.65 (*c* 0.51, $CHCl_3$); IR (ATR) ν 1697 (CO), 1329 (NSO), 1136 (NSO); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.15 (t, *J* = 6.1 Hz, 1H), 3.95–4.03 (m, 1H), 3.83–3.91 (m, 1H), 3.72 (ddd, *J* = 15.6, 9.2, 6.4 Hz, 2H), 3.41–3.57 (m, 3H), 2.00–2.21 (m, 2H), 1.82–1.98 (m, 3H), 1.57–1.71 (m, 1H), 1.47–1.57 (m, 2H), 1.29–1.46 (m, 2H), 1.21 (s, 9H), 1.15 (s, 3H), 0.97 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 168.4, 139.1, 119.1, 65.2, 60.8, 56.2, 52.8, 48.6, 47.8, 44.6, 42.9, 38.4, 35.0, 32.8, 26.4, 24.4, 22.5 (3C), 22.2, 20.8, 19.9; HRMS (ESI), m/z calcd for $C_{23}H_{40}ClN_2O_4S_2$ $[M+H]^+$ 507.2113, found 507.2115.



6 **7**

***tert*-Butyl (3*R*,6*S*,*Z*)-6-(((*S*)-*tert*-butylsulfinyl)amino)-5-chloro-3-((3a*S*,6*S*)-8,8-dimethyl-2,2-dioxidohexahydro-3*H*-3a,6-methanobenzo[*c*]isothiazole-1-carbonyl)-8-methylnon-4-enoate (7):** To a solution of **6** (1.08 g, 2.12 mmol) in THF (21.2 mL) was added dropwise LiHMDS in THF (1.30 M, 2.45 mL, 3.18 mmol) at -78 °C. After stirring at -78 °C for 5 min, HMPA (2.21 mL, 12.7 mmol) was added dropwise. After stirring at -78 ºC for 30 min, *tert*-butyl bromoacetate (1.87 mL, 12.7 mmol) was added dropwise, and the mixture was stirred at -78 ºC for 17 h. The reaction mixture was quenched by saturated aqueous NH_4Cl and extracted with EtOAc. The extract was washed with brine and dried over Na_2SO_4. Concentration under reduced pressure followed by flash

chromatography over silica gel with *n*-hexane-EtOAc (2:1) gave the title compound **7** as a white solid (867.1 mg, 66 %, 7:1 dr): $[\alpha]_D^{27.1}$ = -20.0 (*c* 0.98, $CHCl_3$); IR (ATR) ν 1729 (CO), 1642 (CO), 1365 (NSO), 1162 (NSO); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74–5.80 (m, 1H), 4.28–4.40 (m, 1H), 3.84–3.91 (m, 1H), 3.75–3.84 (m, 1H), 3.40 (d, *J* = 13.6 Hz, 1H), 3.36–3.41 (m, 1H), 3.34 (d, *J* = 13.6 Hz, 1H), 2.77–2.89 (m, 1H), 2.56–2.67 (m, 1H), 1.91–2.12 (m, 2H), 1.74–1.89 (m, 3H), 1.53–1.68 (m, 1H), 1.40–1.52 (m, 2H), 1.32 (s, 9H), 1.22–1.40 (m, 2H), 1.17 (s, 3H), 1.13 (s, 9H), 0.90 (s, 3H), 0.78–0.85 (d, 3H/d, 3H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 171.8, 170.3, 139.4, 123.4, 80.8, 65.0, 61.3, 56.2, 53.0, 48.6, 47.8, 44.7, 42.6, 42.0, 37.8, 37.4, 32.7, 28.0 (3C), 26.5, 24.0, 22.5 (3C), 22.4, 22.3, 20.5, 20.0; HRMS (ESI), m/z calcd for $C_{29}H_{50}ClN_2O_6S_2$ $[M+H]^+$ 621.2793, found 621.2794.

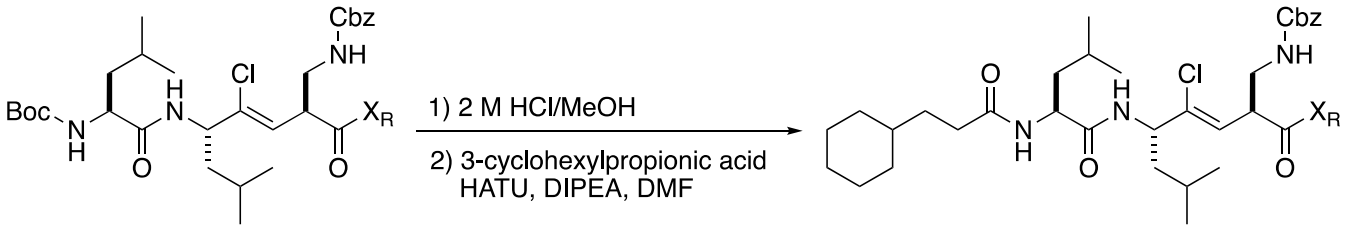


Benzyl ((2*S*,5*S*,*Z*)-5-(2-((*tert*-butoxycarbonyl)amino)-4-methylpentanamido)-4-chloro-2-((3a*S*,6*S*)-8,8-dimethyl-2,2-dioxidohexahydro-3*H*-3a,6-methanobenzo[*c*]isothiazole-1-carbonyl)-7-methyloct-3-en-1-yl)carbamate (9): To a solution of **7** (660.0 mg, 1.10 mmol) in CH_2Cl_2 (8.8 mL) was added TFA (2.2 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure to give the carboxylic acid as a yellow oil, which was used immediately in the next step without purification.

To a solution of the above carboxylic acid in toluene (2.20 mL), DPPA (239 μL, 1.11 mmol) and Et_3N (549 μL, 3.96 mmol) were added at room temperature. The mixture was stirred at room temperature for 23 h, then BnOH (915 μL, 8.80 mmol) was added and the mixture was refluxed for 20 h. After cooling to room temperature, H_2O (44.8 mL) was added. The mixture was extracted with EtOAc. The extract was washed with brine and dried over Na_2SO_4. Concentration under reduced pressure gave an oily amine, which was used immediately in the next step without purification.

A mixture of the above amine (1.04 g, 1.55 mmol) and 2 M HCl/MeOH (15.5 mL) was stirred at room temperature for 23 h. The reaction mixture was concentrated under reduced pressure to give the amine as a brown oil, which was used immediately in the next step without purification.

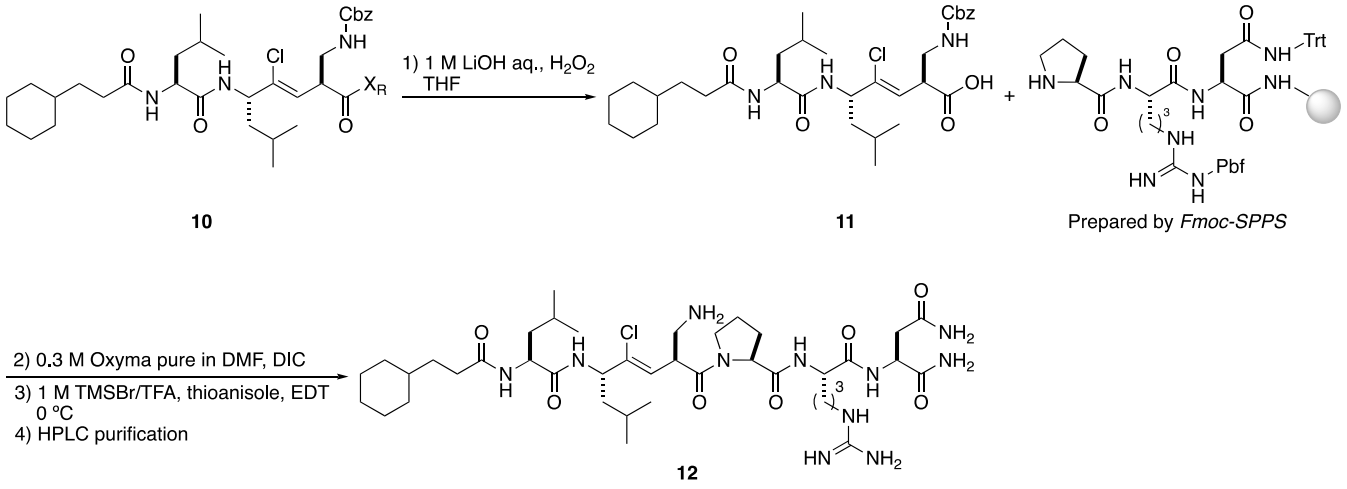
To a solution of HATU (1.14 g, 3.00 mmol) and Boc-Leu-OH (747 mg, 3.00 mmol) in DMF (15.0 mL) was added DIPEA (2.60 m, 15.0 mmol), the mixture was added above amine (903 mg, 1.50 mmol). The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was quenched by saturated aqueous NH_4Cl and extracted with EtOAc. The extract was washed with brine and dried over Na_2SO_4. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (3:1) gave the title compound **9** as a white solid (69.8 mg, 6% in four steps): $[\alpha]_D^{26.2}$ = -57.3 (*c* 0.58, $CHCl_3$); IR (ATR) ν 1697 (CO), 1660 (CO), 1336 (NSO), 1161 (NSO); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.39 (m, 4H), 6.13–6.26 (m, 1H), 6.02–6.09 (m, 1H), 5.34–5.44 (m, 1H), 5.10 (d, *J* = 12.4 Hz, 1H), 5.05 (d, *J* = 12.4 Hz, 1H), 4.76–4.85 (m, 1H), 4.54–4.66 (m, 1H), 4.31–4.42 (m, 1H), 3.94–4.03 (m, 1H), 3.84–3.91 (m, 1H), 3.54–3.72 (m, 1H), 3.51 (d, *J* = 13.8 Hz, 1H), 3.42 (d, *J* = 13.8 Hz, 1H), 2.07–2.19 (m, 1H), 1.94–2.07 (m, 1H), 1.81–1.93 (m, 3H), 1.43 (s, 9H), 1.33–1.61 (m, 6H), 1.16 (s, 3H), 0.95 (s, 3H), 0.82–0.93 (m, 12H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 172.2, 170.3, 156.3, 137.1, 136.8, 128.4, 128.4, 127.9, 127.9, 123.1, 77.2, 66.6, 65.3, 54.2, 53.1, 48.5, 47.8, 46.0, 44.6, 43.3, 41.3, 40.7, 38.0, 32.9, 28.3, 26.5, 24.7, 24.4, 22.8, 22.5, 22.4, 20.7, 19.9; HRMS (ESI), m/z calcd for $C_{43}H_{66}ClN_4O_7S$ $[M+H]^+$ 779.3815, found 779.3816.



9 **10**

Benzyl ((2*S*,5*S*,*Z*)-4-chloro-5-(2-(3-cyclohexylpropanamido)-4-methylpentanamido)-2-((3a*S*,6*S*)-8,8-dimethyl-2,2-dioxidohexahydro-3*H*-3a,6-methanobenzo[*c*]isothiazole-1-carbonyl)-7-methyloct-3-en-1-yl)carbamate (10): A mixture of **9** (72.5 mg, 0.090 mmol) and 2 M HCl/MeOH (900 μL) was stirred at room temperature for 22 h. The reaction mixture was concentrated under reduced pressure to give the amine as a brown oil, which was used immediately in the next step without purification.

To a solution of HATU (68.4 mg, 0.18 mmol) and cyclohexanepropionic acid (28.0 μL, 0.18 mmol) in DMF (900 μL) was added DIPEA (157.0 μL, 0.90 mmol), the mixture was added above amine (64.4 mg, 0.090 mmol). The reaction mixture was stirred at room temperature for 4.5 h. The reaction mixture was quenched by 0.1 M HCl aqueous and extracted with EtOAc. The extract was washed with brine and dried over Na_2SO_4. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (2:1) gave the title compound **10** as a white solid (68.4 mg, 93% in two steps): $[\alpha]_D^{27.8}$ = -74.4 (*c* 0.59, $CHCl_3$); IR (ATR) ν 1715 (CO), 1642 (CO), 1336 (NSO), 1129 (NSO); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.37 (m, 5H), 6.15–6.21 (m, 1H), 6.05 (d, *J* = 8.9 Hz, 1H), 5.69–5.77 (m, 1H), 5.36 (t, *J* = 6.8 Hz, 1H), 5.09 (d, *J* = 12.4 Hz, 1H), 5.07 (d, 12.4 Hz, 1H), 4.51–4.60 (m, 1H), 4.36 (dd, *J* = 8.8, 6.4 Hz, 2H), 4.24–4.44 (m, 1H), 3.85–3.90 (m, 1H), 3.61 (dd, *J* = 6.4 Hz, 1H), 3.51 (dd, *J* = 6.4 Hz, 2H), 3.51 (d, *J* = 13.6 Hz, 2H), 3.42 (d, *J* = 13.6 Hz, 1H), 2.09–2.23 (m, 3H), 1.96–2.08 (m, 1H), 1.82–1.94 (m, 4H), 1.33–1.74 (m, 12H), 1.17–1.29 (m, 3H), 1.16 (s, 3H), 1.07–1.51 (m, 10H), 0.95 (s, 3H), 0.84–0.92 (m, 14H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 173.6, 171.8, 170.2, 156.2, 137.0, 136.8, 128.8, 128.4, 127.9, 123.3, 77.2, 66.6 65.3, 54.4, 53.1, 51.6, 48.5, 47.8, 46.0, 44.6, 43.3, 41.1, 40.7, 38.1, 37.3, 33.1, 33.0, 32.9, 29.7, 26.5, 26.2, 24.8, 24.4, 22.8, 22.5, 22.3, 22.2, 20.7, 19.9; HRMS (ESI), m/z calcd for $C_{43}H_{66}ClN_4O_7S$ [M+H]+ 817.4335, found 817.4331.

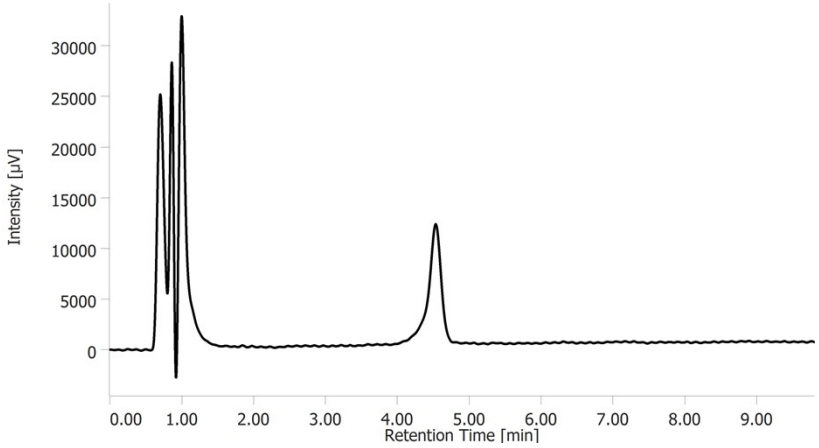


3-Cyclohexylpropionyl-Leu-Leu– Ψ [(*Z*)-CCl=CH]-Dap-Pro-Arg-Asn-NH$_2$ (12): To a solution of the sultam **10** (63.2 mg, 0.080 mmol) in THF–H_2O (5:1, 2.70 mL) was added dropwise 30% H_2O_2 aq. (24.0 μL, 0.24 mmol) and 1 M LiOH aq. (240 μL, 0.24 mmol) at 0 °C. After stirring at 0 °C for 1.5 h and at room temperature for 17 h, the

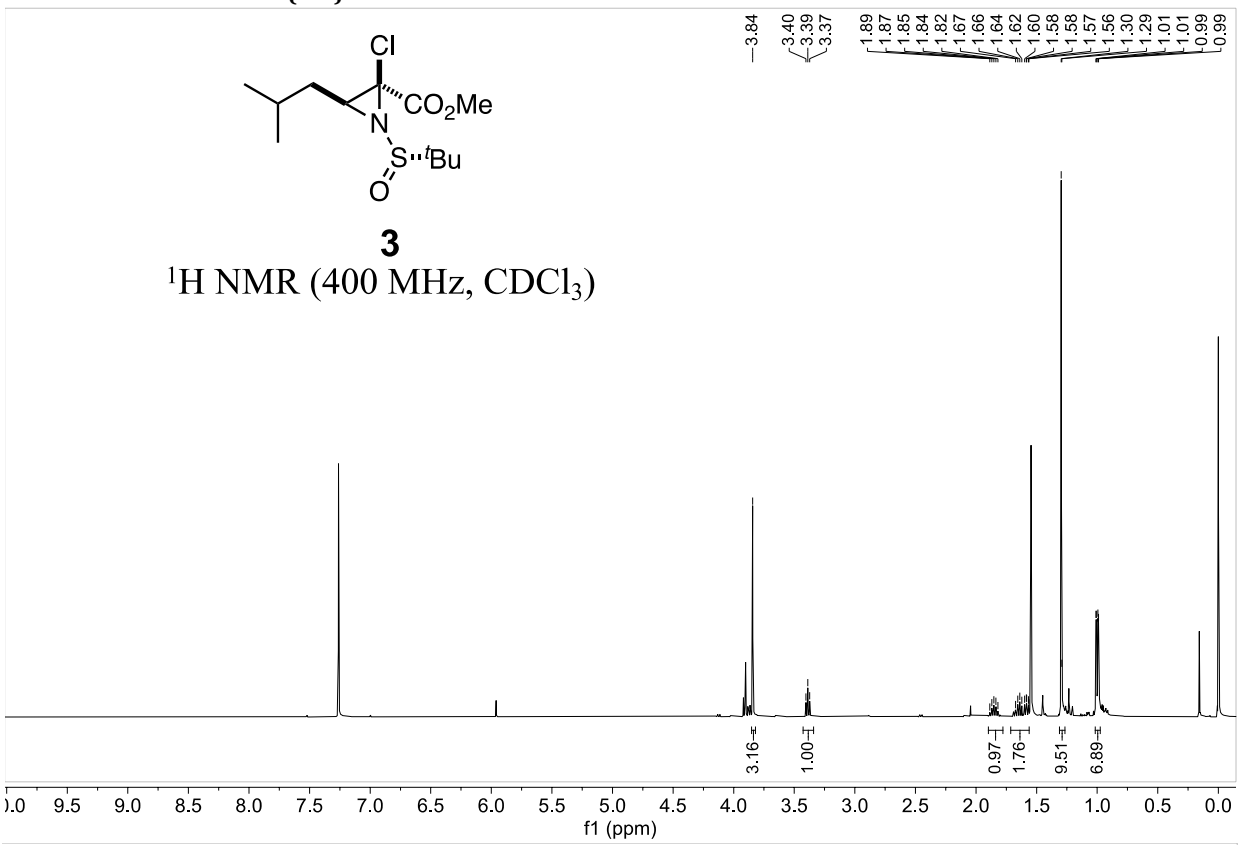
reaction mixture was diluted with EtOAc and washed with saturated NH_4Cl aq. After dried over Na_2SO_4, concentration under reduced pressure gave an oily carboxylic acid **11**, which was used in the next step without further purification.

On Rink Amide AM Resin was used. On this resin (0.7 mmol amine/g, 71.0 mg, 0.050 mmol) was coupled Fmoc-Asn(Trt)-OH (3.0 equiv), Fmoc-Arg(Pbf)-OH (3.0 equiv), Fmoc-Pro-OH (3.0 equiv) with the aid of DIC (3.0 equiv) and Oxyma Pure® (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 Pro-Arg(Pbf)-Asn(Trt)- incorporated resin. The quarter of resulting resin was treated with an 3-cyclohexylpropionic-Leu-Leu-Ψ[(*Z*)-ClC=CH]-Dap(Cbz)-X_R (2.8 equiv), DIC (3.0 equiv) and Oxyma Pure® (3.0 equiv) to yield the resin containing the Leu-Dap-type CADI. The resulting completed resin was treated with 1 M TMSBr/TFA-thioanisole-EDT (1:0.05:0.025 (v/v), 133 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 **12**. The crude pseudopeptide **12** was purified by preparative HPLC gave the title pseudopeptide **12** as colorless freeze-dried powder.

12 Analytical UHPLC conditions: Unifinepak C_{18} 03050-1.9M analytical column analytical column with a isocratic of 0.1% TFA-MeCN in 0.1% TFA aq., 38% over 10 min, detected at 220 nm, retention time = 4.5 min, HRMS (ESI) *m*/*z* calcd ($[M+H]^+$) 852, found 852.

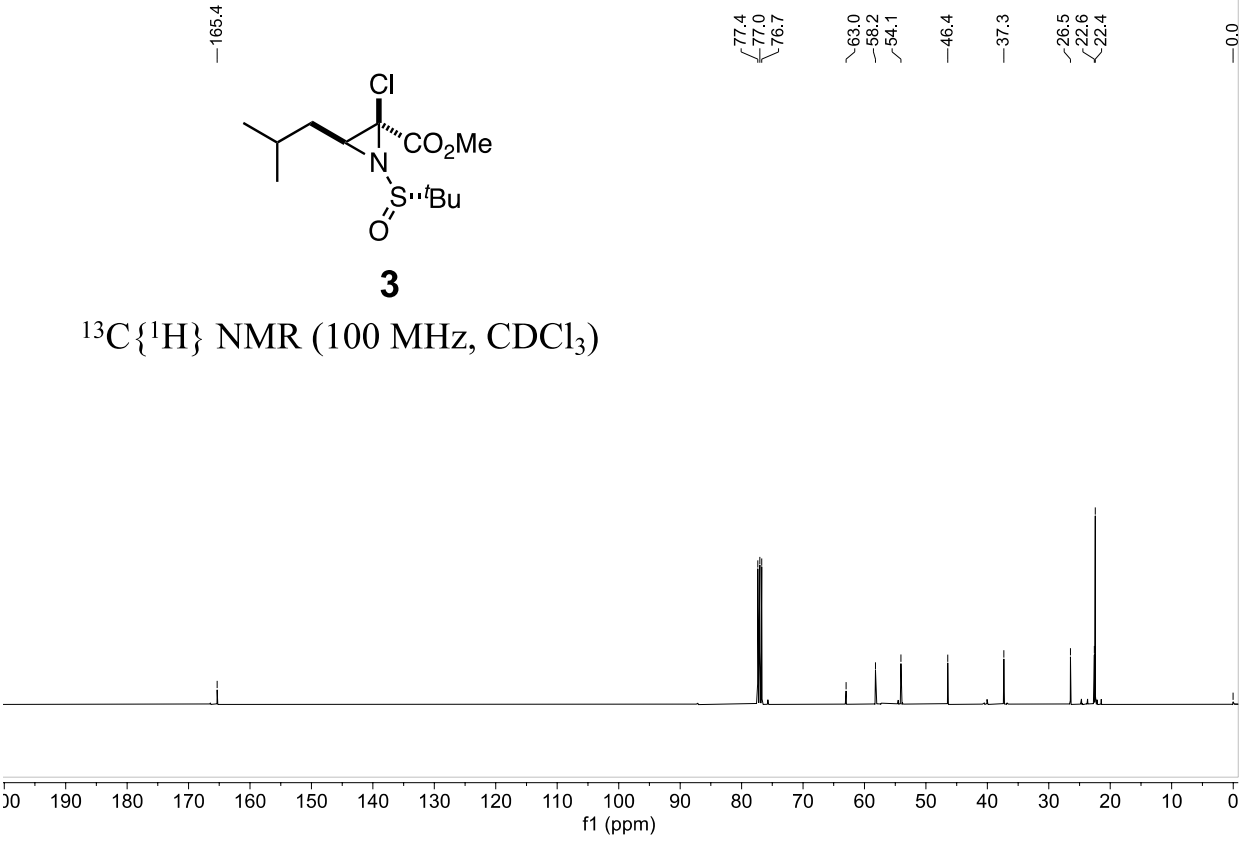


III. ^{1}H NMR and ^{13}C{^{1}H} NMR charts



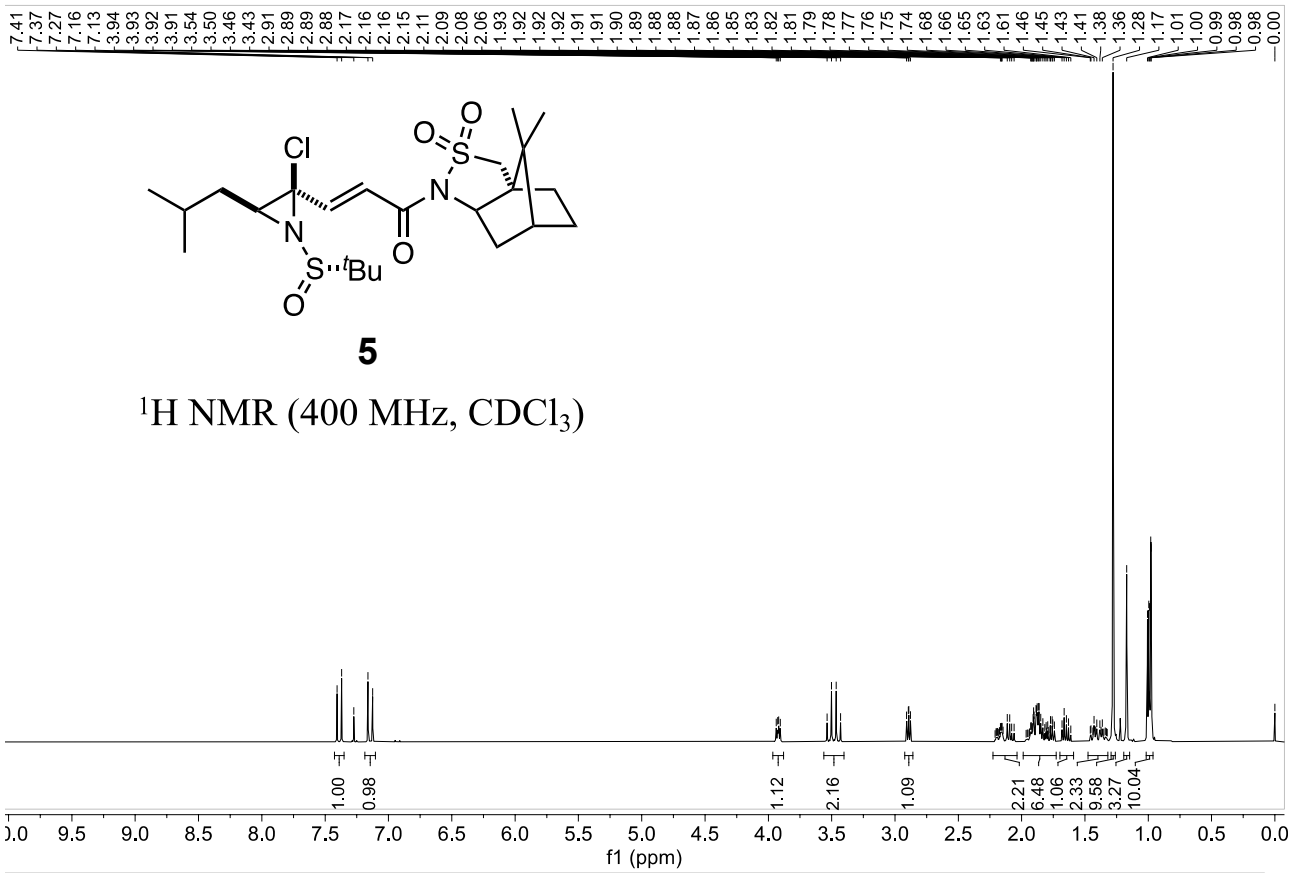
3

^{1}H NMR (400 MHz, $CDCl_3$)



3

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$)



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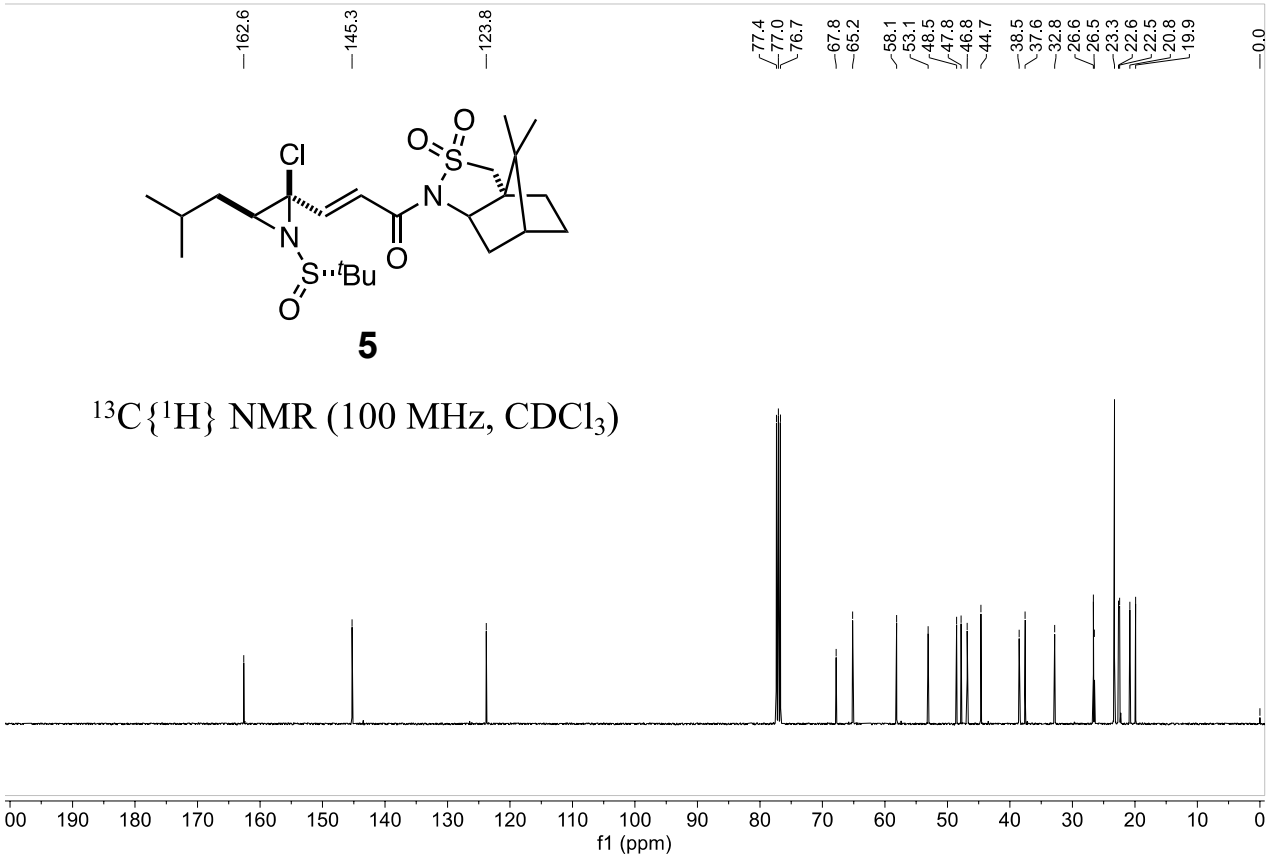
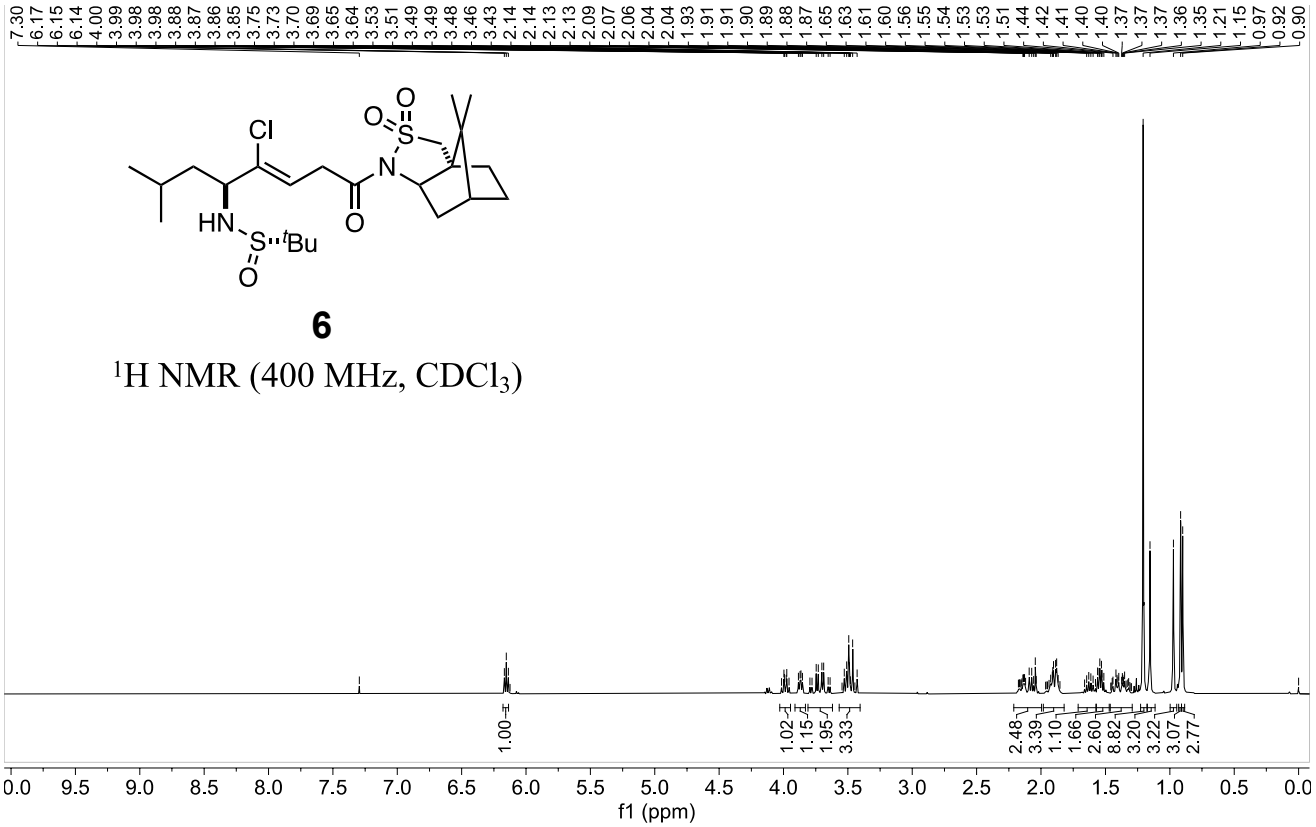
^{1}H NMR (400 MHz, $CDCl_3$)



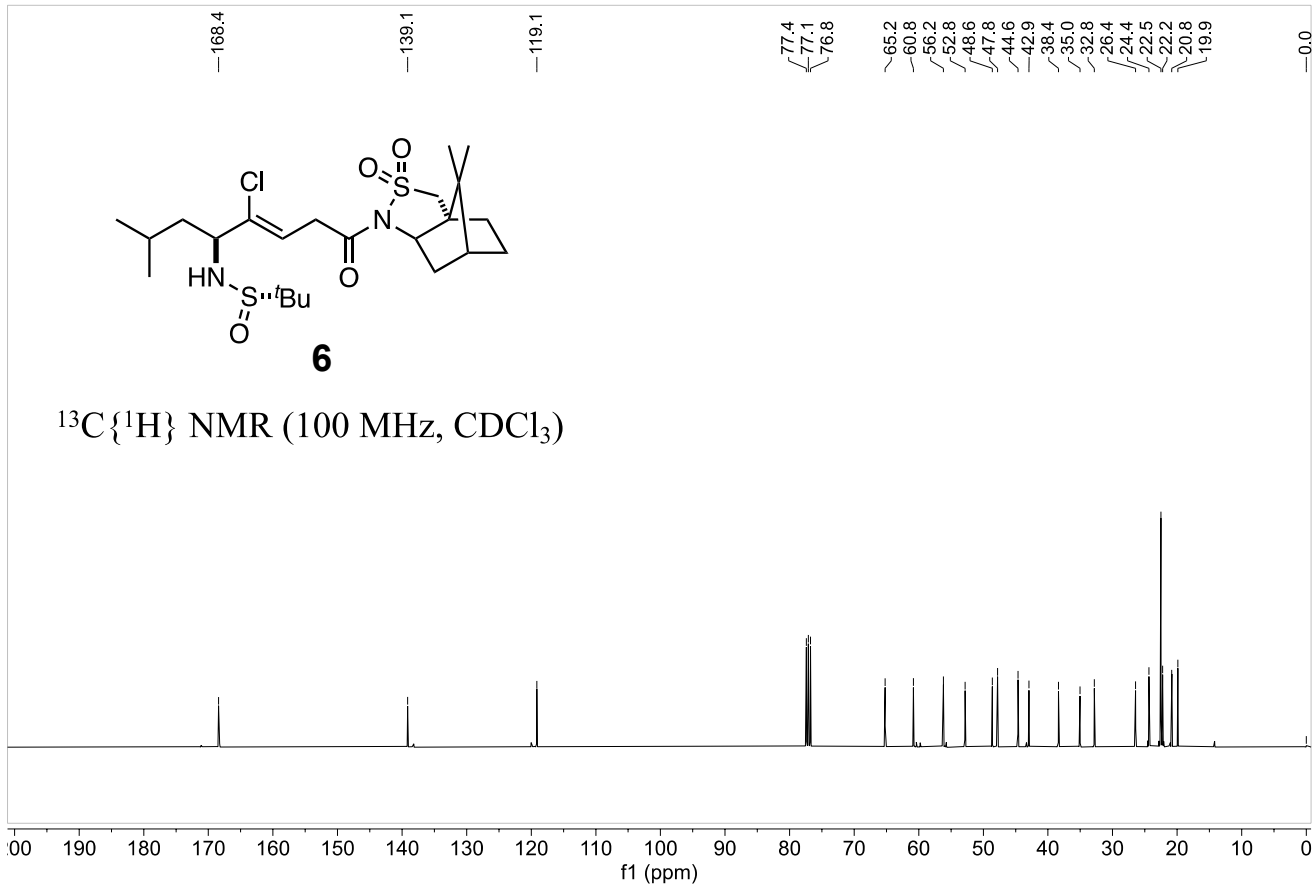
5

$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$)



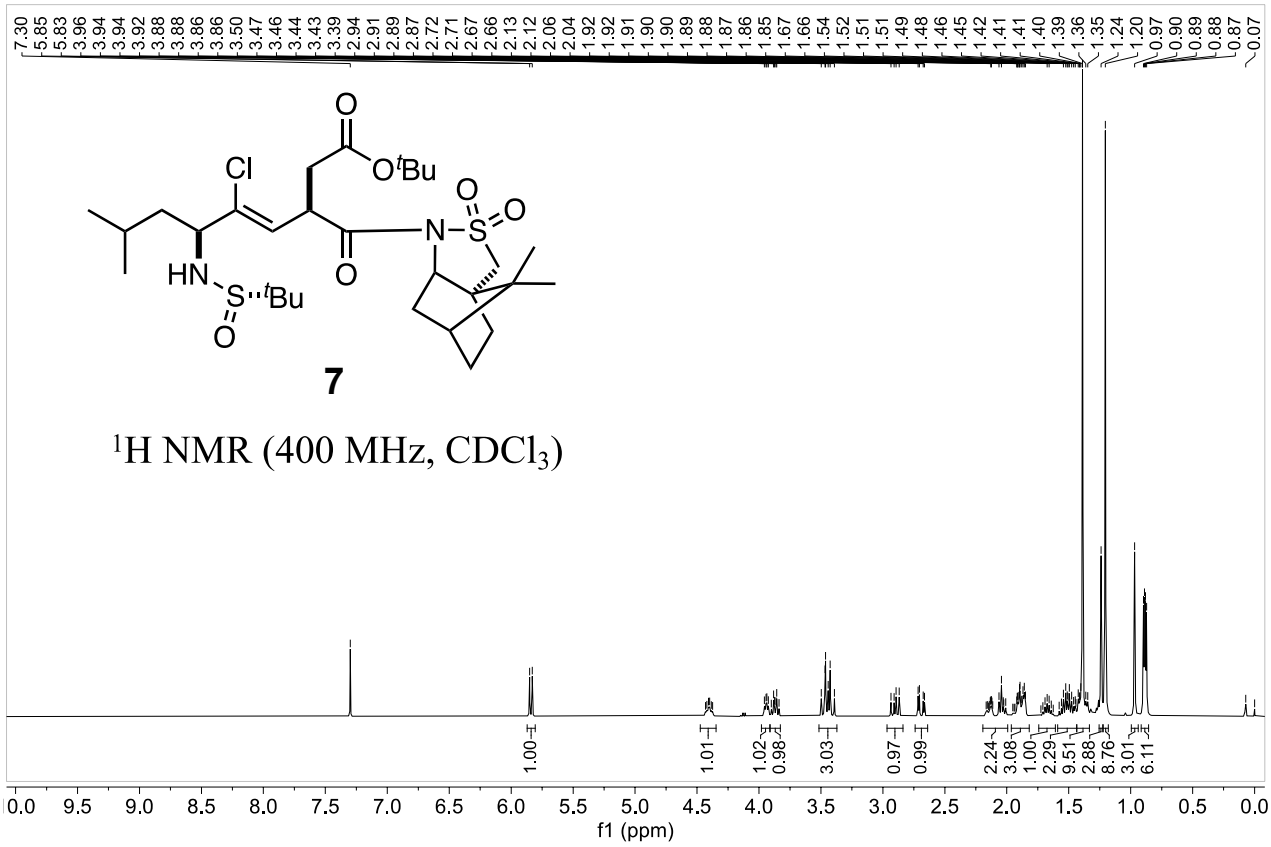
6

^{1}H NMR (400 MHz, $CDCl_3$)



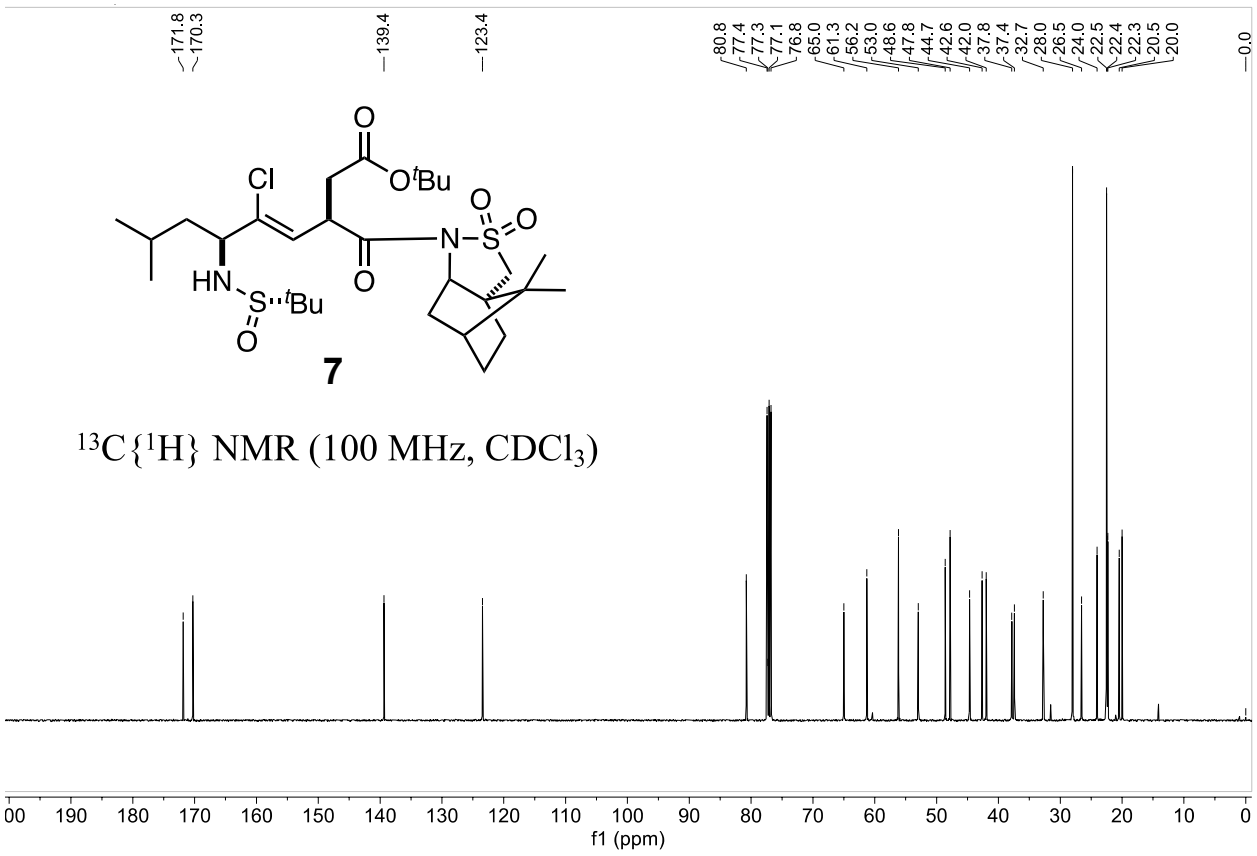
6

$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$)



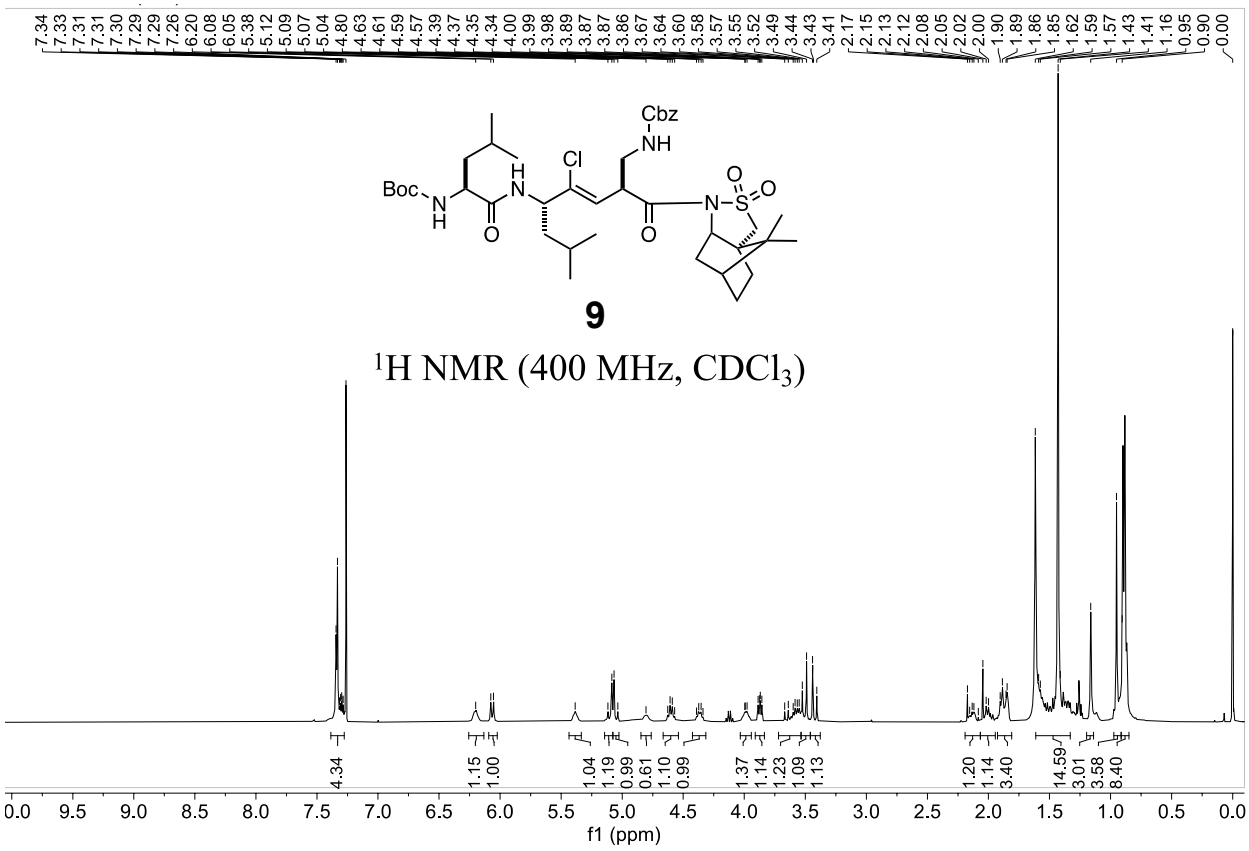
7

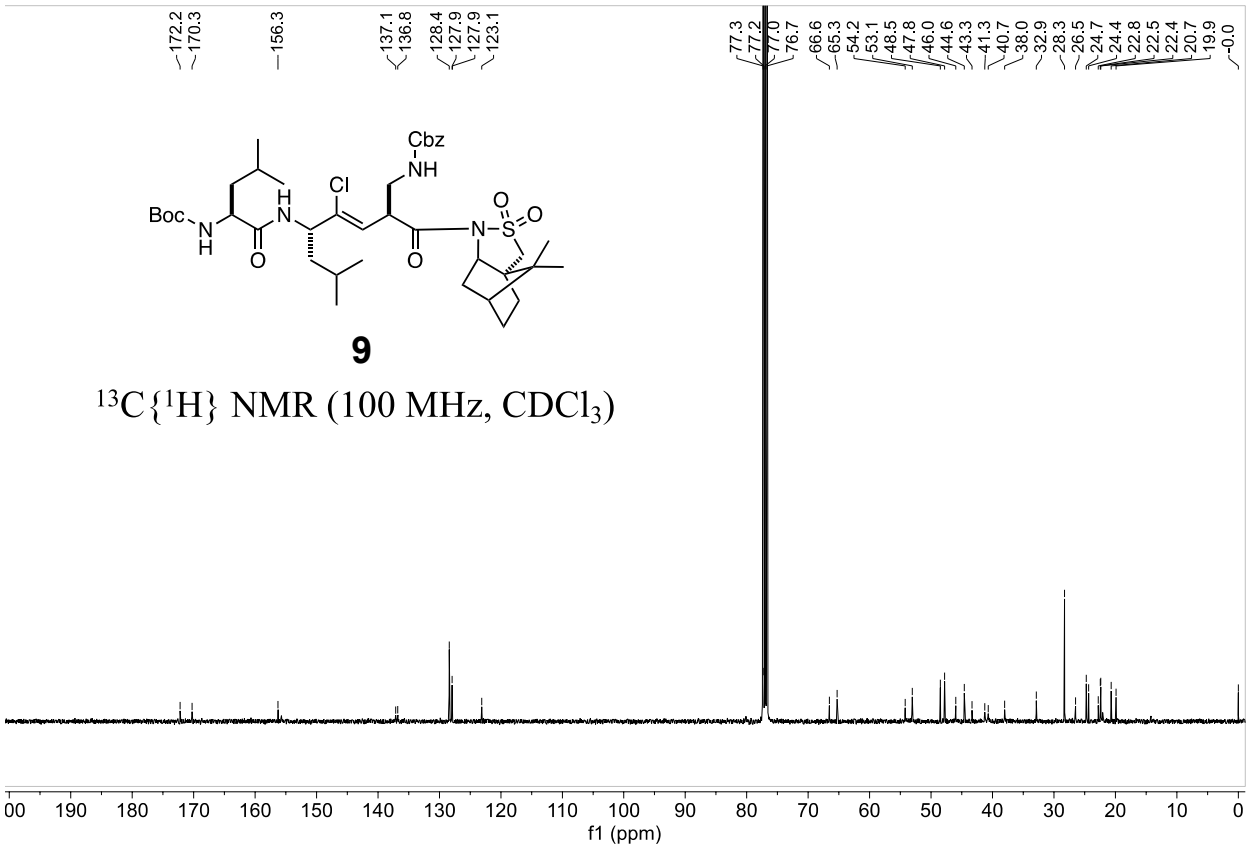
^{1}H NMR (400 MHz, $CDCl_3$)

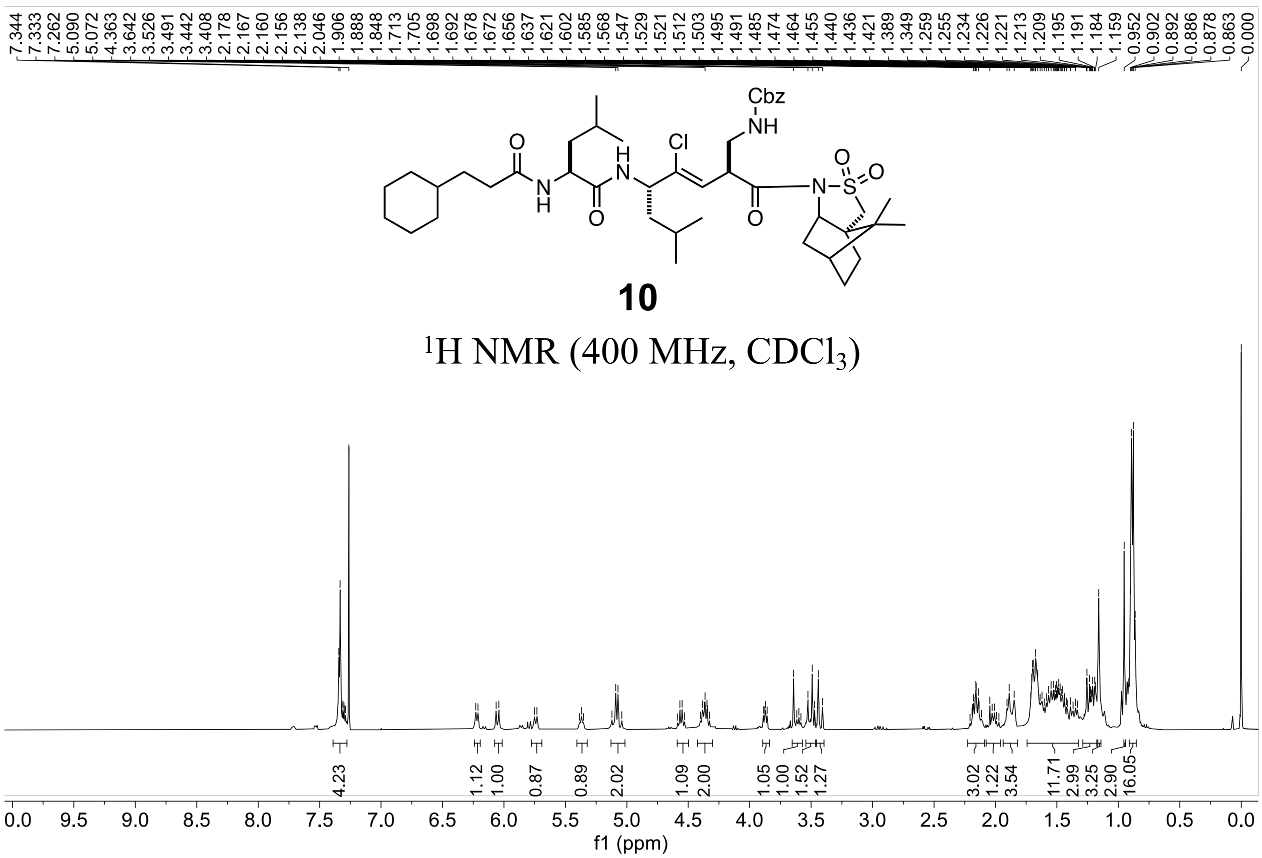


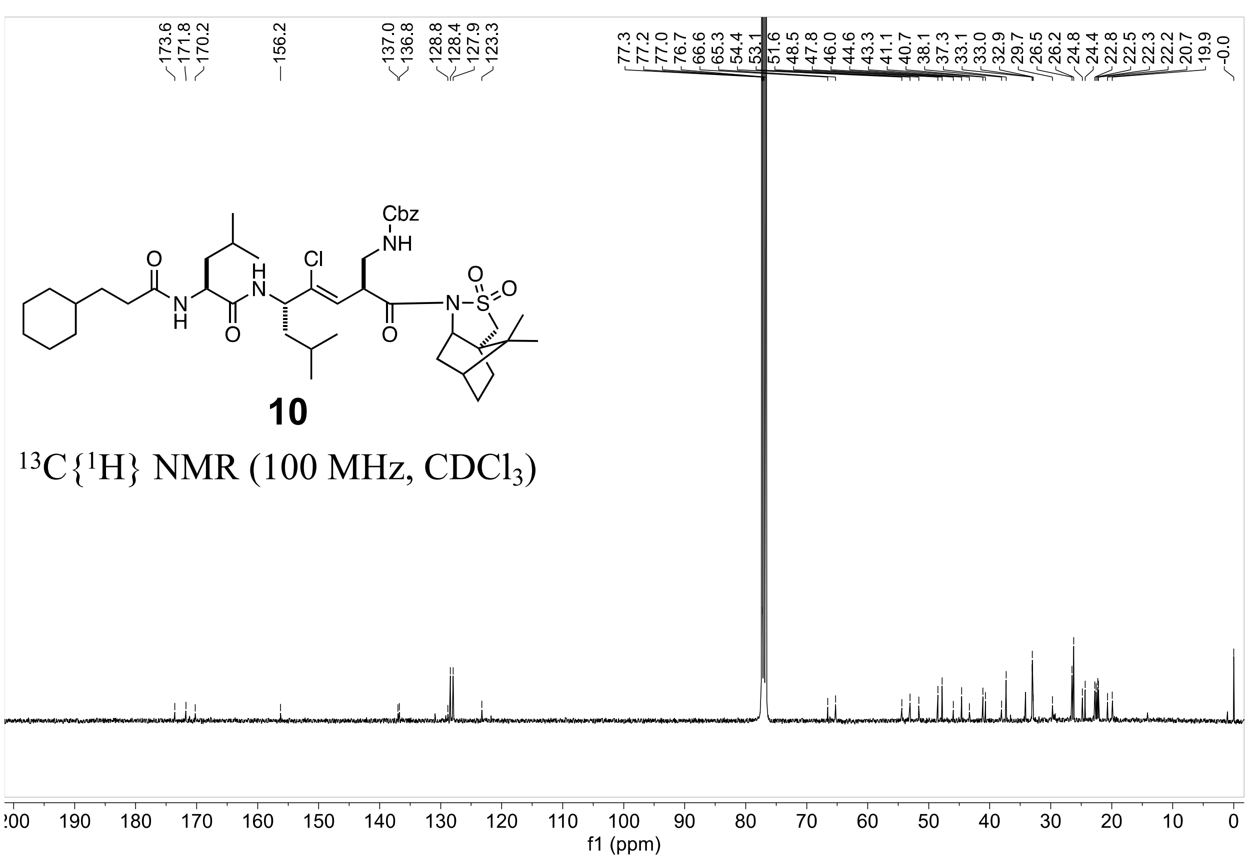
7

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$)


Cbz
NH
Cl
Boc
N
H
H
N
O
O
O
N
S
O
O
9
1H NMR (400 MHz, CDCl3)
f1 (ppm)


Cbz
NH
Cl
Boc
N
H
H
N
O
O
O
N
S
O
O
9
13C{1H} NMR (100 MHz, CDCl3)
f1 (ppm)


7.344 7.333 7.262 5.090 5.072 4.363 3.642 3.526 3.491 3.442 3.408 2.178 2.167 2.160 2.156 2.138 2.046 1.906 1.888 1.848 1.713 1.705 1.698 1.692 1.678 1.672 1.656 1.637 1.621 1.602 1.585 1.568 1.547 1.529 1.521 1.512 1.503 1.495 1.491 1.485 1.474 1.464 1.455 1.440 1.436 1.421 1.389 1.349 1.259 1.255 1.234 1.226 1.221 1.213 1.209 1.195 1.191 1.184 1.159 0.952 0.902 0.892 0.886 0.878 0.863 0.000
Cbz
NH
Cl
O
N
H
O
O
N-S
O
O
10
^{1}H NMR (400 MHz, $CDCl_3$)
4.23 1.12 1.00 0.87 0.89 2.02 1.09 2.00 1.05 1.00 1.52 1.27 3.02 1.22 3.54 11.71 2.99 3.25 2.90 16.05
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


173.6 171.8 170.2 156.2 137.0 136.8 128.8 128.4 127.9 123.3 77.3 77.2 77.0 76.7 66.6 65.3 54.4 53.1 51.6 48.5 47.8 46.0 44.6 43.3 41.1 40.7 38.1 37.3 33.1 33.0 32.9 29.7 26.5 26.2 24.8 24.4 22.8 22.5 22.3 22.2 20.7 19.9 -0.0
Cbz
NH
Cl
10
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

IV. Supplementary Figure

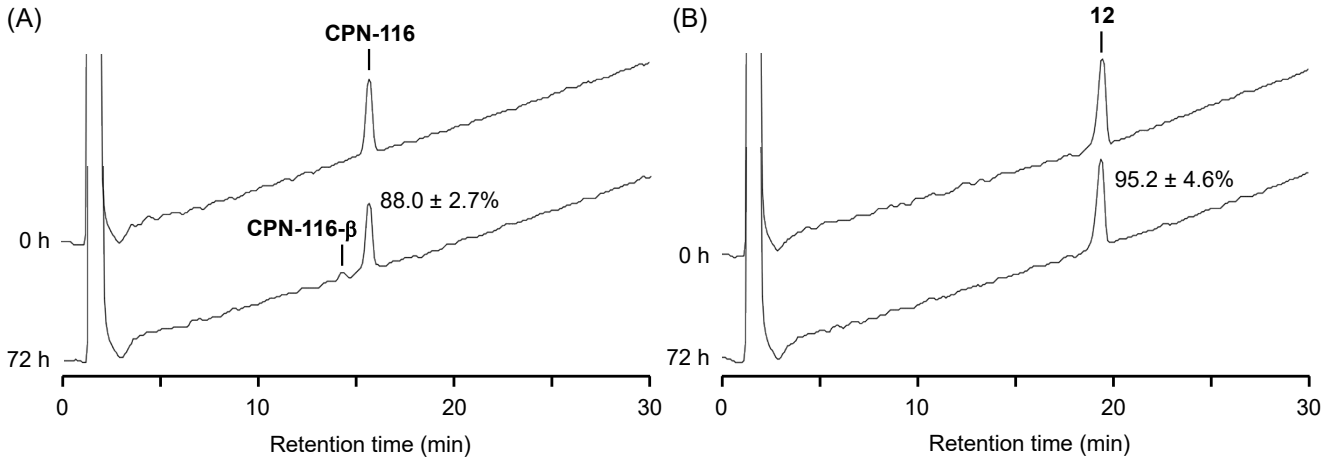


Figure S1. Analytical RP-HPLC chromatograms showing the chemical stabilities of CPN-116 (A) and **12** (B) incubated in artificial cerebrospinal fluid (CSF) at 37 °C for 72 h. An aliquot of each sample was analyzed using a cholesterol-bound reverse-phase column with a binary solvent system: a linear gradient of MeCN containing 0.1% TFA (25–40%, 30 min) in 0.1% aqueous TFA. Data were determined in triplicate. All values resent means ± SD. Ion concentrations in solution (in mM): Na 150; K 3.0; Ca 1.4; Mg 0.8; P 1.0; Cl 155 (manufacturer information). As similar to the case (87% recovery) incubated in 1 mM phosphate buffer (pH 7.4) for 72 h,[S1] CPN-116 is relatively stable under the ion composition of CSF although the peak of CPN-116-β was detected after the incubation.

IV. References

S1. (a) Mori, K., Miyazato, M., Ida, T., Murakami, N., Serino, R., Ueta, Y., Kojima, M., Kangawa, K. (2005). *EMBO J.*, 2005, 24, 325. (b) Takayama, K. Mori, A. Tanaka, Y. Sasaki, Y. Sohma, A. Taguchi, A. Taniguchi, T. Sakane, A. Yamamoto, M. Miyazato, N. Minamino, K. Kangawa and Y. Hayashi, *Bioorg. Med. Chem*. 2020, **28**, 115454.

S2. A. R. L. Cecil, Y. Hu, M. J. Vicent, R. Duncan and R. C. D. Brown, *J. Org. Chem.* 2004, **69**, 3368.