SUPPORTING INFORMATION

The Consequence of Hapten Stereochemistry: An Efficacious Methamphetamine Vaccine

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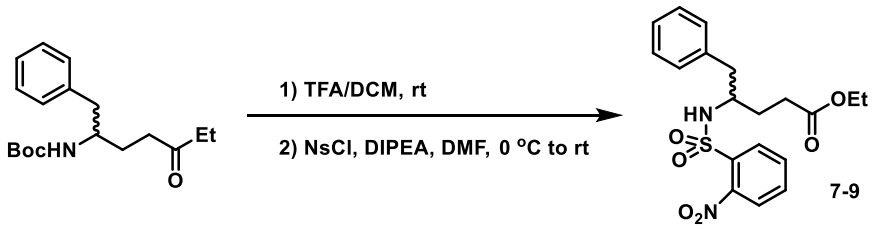
I. Experimental Procedures

Materials and Methods

^{1}H and ^{13}C NMR spectra were obtained on Bruker spectrometers. Multiplicities are quoted as singlet (s), doublet (d), triplet (t), unresolved multiplet (m), or broad singlet (br). All chemical shifts are given on the δ–scale in parts per million ((ppm) relative to internal CDCl3(δ7.26, 1H NMR; δ77.0, 13CNMR). ^{1}H coupling constants (J values) are given in Hz. The concentration of the NMR samples was in the range of 2-5 mg/mL. Analytical LCMS was performed on an Agilent ESI-ToF (LC/MSD ToF) with an Agilent Zorbax 300SB-C8 (4.6 x50 mm), 5 µm column using a flow rate of 0.5 mL/min. The LCMS was run using the following solvents: Solvent A: 0.1% formic acid in H_2O, Solvent B: 0.1% formic acid in acetonitrile (MeCN) and each run was ten minutes (0-7 min: 5-95% Solvent B, 7-10 min: 95% Solvent B) with detection at wavelength 254 nm. Matrix-assisted laser desorption/ionization (MALDI) mass spectra were obtained using an Applied Biosystems Voyager DE. All chemicals were purchased from commercial sources and used without further purification. Tetanus toxoid (TT) was purchased from UMass. All reactions were run under inert gas and with dry, distilled solvents unless otherwise noted. LCMS and TLC visualized with UV light were routinely used to monitor reactions.



Ethyl (*S*)-4-((2-nitrophenyl)sulfonamido)-5-phenylpentanoate (7)

To a solution of ethyl (*S*)-4-((tert-butoxycarbonyl)amino)-5-phenylpentanoate (**4**, 3.1 g, 9.7 mmol) in dichloromethane (9.0 mL) was added trifluoroacetic acid (6.0 mL), and the resultant mixture was stirred overnight at room temperature. After, the mixture was concentrated under reduced pressure and dissolved in dichloromethane (60 mL). To this mixture was added *N*, *N*-diisopropylethylamine (4.1 mL, 24 mmol) and 2-nitrobenzenesulfonyl chloride (2.6 g, 12 mmol), and the mixture was stirred overnight at room temperature. After, the mixture was partitioned between dichloromethane and water. The organic layer was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 1/0-3/1) to give **7** (2.1 g, 54%) as a pale-yellow oil. $[\alpha]_D^{25}$ -62.8 (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 – 7.84 (m, 1H), 7.78 – 7.72 (m, 1H), 7.65 – 7.56 (m, 2H), 7.05 – 6.95 (m, 5H), 5.33 (d, *J* = 8.3 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.88 – 3.77 (m, 1H), 2.81 (dd, *J* = 14.0, 5.8 Hz, 1H), 2.65 (dd, *J* = 13.9, 8.1 Hz, 1H), 2.53 – 2.41 (m, 2H), 2.04 – 1.95 (m, 1H), 1.83 – 1.72 (m, 1H), 1.25 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.28, 147.35, 136.82, 135.06,

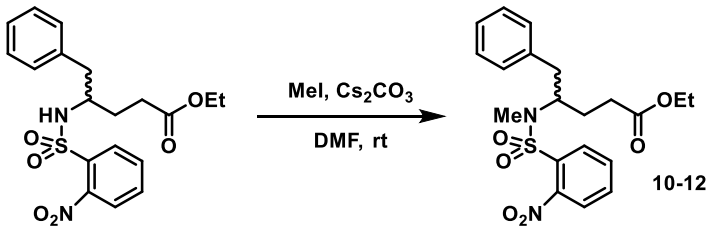
133.12, 133.06, 130.33, 129.27, 128.46, 126.94, 125.54, 60.69, 56.82, 42.12, 30.89, 30.51, 14.31; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 407.1271, obsd 407.1272.

Ethyl (*R*)-4-((2-nitrophenyl)sulfonamido)-5-phenylpentanoate (8)

The title compound was prepared from ethyl (*R*)-4-((tert-butoxycarbonyl)amino)-5-phenylpentanoate (**5**, 1.1 g, 3.5 mmol) via the same procedure reported for the synthesis of **7**. The product was obtained as a colorless oil (0.75 g, 52%). $[\alpha]_D^{25}$ +59.6 (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 – 7.84 (m, 1H), 7.77 – 7.73 (m, 1H), 7.64 – 7.56 (m, 2H), 7.05 – 6.96 (m, 5H), 5.37 (d, *J* = 8.3 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.88 – 3.79 (m, 1H), 2.81 (dd, *J* = 13.9, 5.7 Hz, 1H), 2.65 (dd, *J* = 14.0, 8.1 Hz, 1H), 2.53 – 2.42 (m, 2H), 2.05 – 1.96 (m, 1H), 1.85 – 1.73 (m, 1H), 1.25 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.31, 147.32, 136.83, 135.02, 133.11, 133.06, 130.31, 129.25, 128.43, 126.91, 125.51, 60.68, 56.82, 42.08, 30.87, 30.50, 14.29; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 407.1271, obsd 407.1282.

Ethyl (*RS*)-4-((2-nitrophenyl)sulfonamido)-5-phenylpentanoate (9)

The title compound was prepared from ethyl (*RS*)-4-((tert-butoxycarbonyl)amino)-5-phenylpentanoate (**6**, 1.4 g, 4.3 mmol) via the same procedure reported for the synthesis of **7**. The product was obtained as a pale yellow oil (1.5 g, 86%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 – 7.85 (m, 1H), 7.78 – 7.73 (m, 1H), 7.65 – 7.56 (m, 2H), 7.06 – 6.95 (m, 5H), 5.33 (d, *J* = 8.3 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.89 – 3.78 (m, 1H), 2.81 (dd, *J* = 14.0, 5.8 Hz, 1H), 2.65 (dd, *J* = 14.0, 8.0 Hz, 1H), 2.54 – 2.40 (m, 2H), 2.05 – 1.95 (m, 1H), 1.81 – 1.73 (m, 1H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.28, 147.35, 136.81, 135.07, 133.12, 133.06, 130.33, 129.27, 128.46, 126.94, 125.55, 60.69, 56.81, 42.13, 30.89, 30.51, 14.32; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 407.1271, obsd 407.1279.



Ethyl (*S*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoate (10)

To a suspension of ethyl (*S*)-4-((2-nitrophenyl)sulfonamido)-5-phenylpentanoate (**7**, 2.1 g, 5.2 mmol) and cesium carbonate (2.5 g, 7.6 mmol) in *N*, *N*-dimethylformamide (30 mL) was added iodomethane (0.57 mL, 9.2 mmol), and the resultant mixture was stirred overnight at room temperature. The mixture was then partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel (hexane/EtOAc = 1/0-3/7) to give **10** (2.0 g, 91%) as a pale-yellow oil; $[\alpha]_D^{25}$ +17.7 (*c* = 1.0, MeOH);

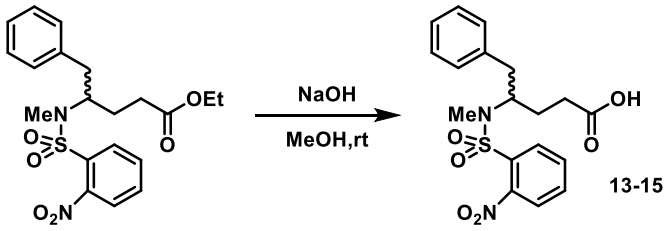
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (dd, J = 7.9, 1.4 Hz, 1H), 7.65 – 7.59 (m, 1H), 7.59 – 7.50 (m, 2H), 7.23 – 7.08 (m, 5H), 4.22 – 4.15 (m, 1H), 4.11 – 4.03 (m, 2H), 2.94 (s, 3H), 2.81 (dd, J = 13.5, 6.3 Hz, 1H), 2.71 (dd, J = 13.6, 8.4 Hz, 1H), 2.39 – 2.29 (m, 1H), 2.28 – 2.18 (m, 1H), 1.88 – 1.76 (m, 2H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.04, 147.95, 137.61, 133.54, 133.34, 131.76, 131.03, 129.29, 128.68, 126.87, 124.19, 60.58, 59.04, 39.51, 30.83, 28.38, 26.22, 14.27; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 421.1428, obsd 421.1427.

Ethyl (*R*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoate (11)

The title compound was prepared from ethyl (*R*)-4-((2-nitrophenyl)sulfonamido)-5-phenylpentanoate (**8**, 0.71 g, 1.8 mmol) via the same procedure reported for the synthesis of **10**. The product was obtained as a pale yellow oil (581 mg, 79%); $[\alpha]_D^{25}$ -23.1 (c = 1.0, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74 (dd, J = 8.0, 1.4 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.58 – 7.51 (m, 2H), 7.22 – 7.16 (m, 2H), 7.16 – 7.10 (m, 3H), 4.23 – 4.15 (m, 1H), 4.11 – 4.01 (m, 2H), 2.93 (s, 3H), 2.80 (dd, J = 13.6, 6.3 Hz, 1H), 2.71 (dd, J = 13.6, 8.5 Hz, 1H), 2.38 – 2.30 (m, 1H), 2.28 – 2.19 (m, 1H), 1.87 – 1.77 (m, 2H), 1.21 (t, J = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.05, 147.94, 137.60, 133.54, 133.34, 131.76, 131.03, 129.29, 128.69, 126.87, 124.19, 60.59, 59.04, 39.51, 30.83, 28.38, 26.22, 14.27; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 421.1428, obsd 421.1433.

Ethyl (*RS*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoate (12)

The title compound was prepared from ethyl (*RS*)-4-((2-nitrophenyl)sulfonamido)-5-phenylpentanoate (**9**, 1.5 g, 3.6 mmol) via the same procedure reported for the synthesis of **10**. The product was obtained as a pale yellow oil (1.2 g, 81%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (dd, J = 7.9, 1.4 Hz, 1H), 7.62 (td, J = 7.7, 1.4 Hz, 1H), 7.58 – 7.51 (m, 2H), 7.22 – 7.16 (m, 2H), 7.16 – 7.10 (m, 3H), 4.23 – 4.16 (m, 1H), 4.11 – 4.03 (m, 2H), 2.94 (s, 3H), 2.81 (dd, J = 13.5, 6.2 Hz, 1H), 2.71 (dd, J = 13.6, 8.5 Hz, 1H), 2.38 – 2.30 (m, 1H), 2.28 – 2.19 (m, 1H), 1.87 – 1.77 (m, 2H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.06, 147.96, 137.62, 133.57, 133.33, 131.76, 131.05, 131.05, 128.70, 126.89, 124.21, 60.60, 59.05, 39.54, 30.85, 28.39, 26.24, 14.28; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 421.1428, obsd 421.1436.



(*S*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoic acid (13)

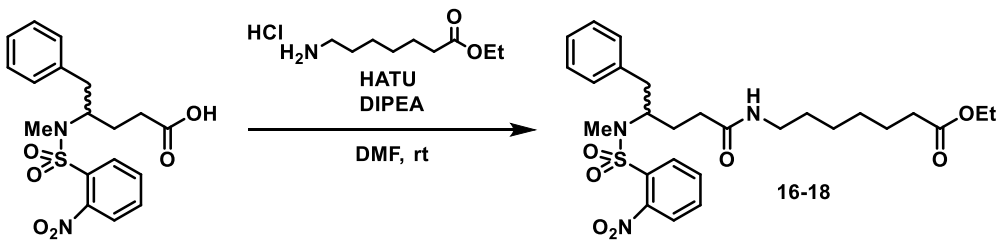
To a mixture of ethyl (*S*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoate (**10**, 2.0 g, 4.7 mmol) in methanol (20 mL) was added 2 M aq. NaOH (5.0 mL, 10 mmol), and the mixture was stirred overnight at room temperature. To the mixture was added 2 M aq. HCl (5.0 mL) to quench the reaction, and the resulting aq. mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to give **13** (1.8 g, 100%) as a colorless amorphous solid; $[\alpha]_D^{25}$ + 17.5 (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.65 – 7.59 (m, 1H), 7.59 – 7.51 (m, 2H), 7.24 – 7.11 (m, 5H), 4.22 – 4.13 (m, 1H), 2.94 (s, 3H), 2.84 (dd, *J* = 13.5, 6.1 Hz, 1H), 2.71 (dd, *J* = 13.6, 8.6 Hz, 1H), 2.42 – 2.33 (m, 1H), 2.33 – 2.23 (m, 1H), 1.87 – 1.74 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.43, 147.89, 137.49, 133.50, 133.41, 131.86, 131.11, 129.29, 128.73, 126.94, 124.28, 58.84, 39.61, 30.40, 28.39, 25.82; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 393.1115, obsd 393.1119.

(*R*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoic acid (14)

The title compound was prepared from ethyl (*R*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoate (**11**, 0.55 g, 1.3 mmol) via the same procedure reported for the synthesis of **13**. The product was obtained as a pale yellow oil (0.49 mg, 94%); $[\alpha]_D^{25}$ -17.3 (*c* = 1.0, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.65 – 7.59 (m, 1H), 7.58 – 7.51 (m, 2H), 7.22 – 7.11 (m, 5H), 4.21 – 4.13 (m, 1H), 2.94 (s, 3H), 2.84 (dd, *J* = 13.5, 6.1 Hz, 1H), 2.71 (dd, *J* = 13.5, 8.6 Hz, 1H), 2.42 – 2.19 (m, 2H), 1.86 – 1.74 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.48, 147.88, 137.50, 133.50, 131.87, 131.10, 129.29, 128.72, 126.93, 124.27, 58.85, 39.59, 30.46, 28.38, 25.86; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 393.1115, obsd 393.1125.

(*RS*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoic acid (15)

The title compound was prepared from ethyl (*RS*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoate (**12**, 1.2 g, 2.9 mmol) the same procedure reported for the synthesis of **13**. The product was obtained as a colorless oil (1.15 g, 100%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.65 – 7.59 (m, 1H), 7.59 – 7.52 (m, 2H), 7.24 – 7.11 (m, 5H), 4.22 – 4.15 (m, 1H), 2.94 (s, 3H), 2.84 (dd, *J* = 13.5, 6.1 Hz, 1H), 2.71 (dd, *J* = 13.5, 8.6 Hz, 1H), 2.42 – 2.34 (m, 1H), 2.32 – 2.23 (m, 1H), 1.87 – 1.76 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.92, 147.92, 137.50, 133.49, 133.46, 131.85, 131.12, 129.31, 128.74, 126.96, 124.30, 58.87, 39.62, 30.34, 28.40, 25.87; ESI-TOF-MS (*m/z*): $[M-H]^-$ calcd 391.0969, obsd 391.0971.



Ethyl (*S*)-7-(4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanamido)heptanoate (16)

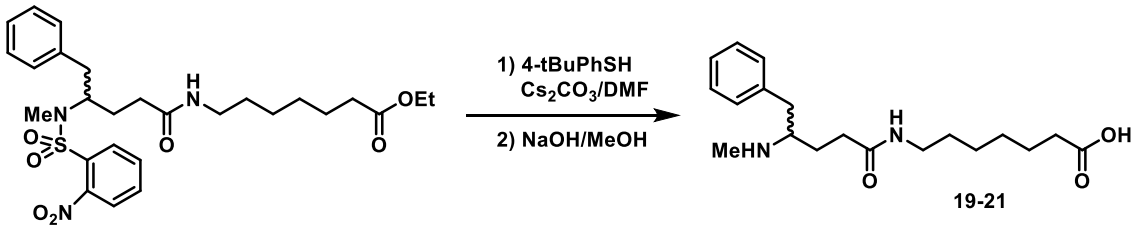
To a mixture of (*S*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoic acid (**13**, 0.15 g, 0.39 mmol) and ethyl 7-aminoheptanoate (0.12 g, 0.58 mmol) in *N*, *N*-dimethylformamide (3.0 mL) were added *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (0.18 g, 0.58 mmol) and *N*, *N*-diisopropylethylamine (0.20 mL, 1.2 mmol), and the mixture was stirred overnight at room temperature. The mixture was then partitioned between ethyl acetate and sat. aq. $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 1/0-0/1) to give **16** (0.20 g, 94%) as a colorless oil; $[\alpha]_D^{25}$ +13.6 (*c* = 1, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 – 7.57 (m, 2H), 7.54 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.51 (ddd, *J* = 8.3, 7.3, 1.3 Hz, 1H), 7.20 – 7.06 (m, 5H), 5.67 (t, *J* = 5.6 Hz, 1H), 4.23 – 4.15 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.27 – 3.14 (m, 2H), 2.91 (s, 3H), 2.75 – 2.66 (m, 2H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.27 – 2.12 (m, 2H), 1.93 – 1.84 (m, 2H), 1.66 – 1.58 (m, 2H), 1.48 (q, *J* = 7.1 Hz, 2H), 1.38 – 1.29 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (151MHz, $CDCl_3$) δ 173.90, 172.13, 148.10, 137.60, 133.37, 133.31, 131.79, 130.83, 129.30, 128.71, 126.89, 124.05, 60.33, 59.45, 39.63, 39.43, 34.38, 33.09, 29.41, 28.87, 28.45, 26.90, 26.68, 24.93, 14.38; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 548.2425, obsd 548.2441.

Ethyl (*R*)-7-(4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanamido)heptanoate (17)

The title compound was prepared from (*R*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoic acid (**14**, 0.15 g, 0.37 mmol) via the same procedure reported for the synthesis of **16**. The product was obtained as a colorless oil (0.18 g, 89%); $[\alpha]_D^{25}$ -14.8 (*c* = 1.0, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 – 7.57 (m, 2H), 7.54 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.53 – 7.48 (m, 1H), 7.19 – 7.07 (m, 5H), 5.65 (d, *J* = 5.8 Hz, 1H), 4.23 – 4.15 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.27 – 3.14 (m, 2H), 2.91 (s, 3H), 2.75 – 2.66 (m, 2H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.27 – 2.12 (m, 2H), 1.95 – 1.83 (m, 2H), 1.66 – 1.57 (m, 2H), 1.51 – 1.44 (m, 2H), 1.38 – 1.29 (m, 4H), 1.25 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.91, 172.13, 148.10, 137.60, 133.37, 133.32, 131.79, 130.84, 129.31, 128.72, 126.90, 124.06, 60.34, 59.45, 39.65, 39.44, 34.39, 33.10, 29.42, 28.88, 28.45, 26.89, 26.69, 24.95, 14.39; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 548.2425, obsd 548.2433.

Ethyl (*RS*)-7-(4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanamido)heptanoate (18)

The title compound was prepared from (*RS*)-4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanoic acid (**15**, 0.15 g, 0.38 mmol) via the same procedure reported for the synthesis of **16**. The product was obtained as a colorless oil (0.19 g, 93%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 – 7.57 (m, 2H), 7.54 (dd, J = 7.9, 1.3 Hz, 1H), 7.51 (ddd, J = 8.2, 7.3, 1.3 Hz, 1H), 7.20 – 7.07 (m, 5H), 5.67 (t, J = 5.8 Hz, 1H), 4.23 – 4.15 (m, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.27 – 3.14 (m, 2H), 2.91 (s, 3H), 2.75 – 2.66 (m, 2H), 2.28 (t, J = 7.5 Hz, 2H), 2.27 – 2.12 (m, 2H), 1.95 – 1.83 (m, 2H), 1.66 – 1.58 (m, 2H), 1.48 (p, J = 7.2 Hz, 2H), 1.36 – 1.29 (m, 4H), 1.25 (t, J = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.91, 172.14, 148.10, 137.60, 133.37, 133.31, 131.79, 130.84, 129.31, 128.71, 126.90, 124.05, 60.33, 59.45, 39.64, 39.43, 34.38, 33.09, 29.41, 28.87, 28.45, 26.90, 26.68, 24.94, 14.38; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 548.2425, obsd 548.2434.



(*S*)-7-(4-(methylamino)-5-phenylpentanamido)heptanoic acid (19)

To a mixture of ethyl (*S*)-7-(4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanamido)heptanoate (**16**, 0.17 g, 0.31 mmol), cesium carbonate (0.20 g, 0.62 mmol), and *N*, *N*-dimethylformamide (2.0 mL) was added 4-*tert*-butylthiophenol (0.11 mL, 0.62 mmol), and the mixture was stirred overnight at room temperature. The mixture was then concentrated *in vacuo*. To the resulting residue were added methanol (3.0 mL) and 2 M aq. NaOH (1.0 mL), and the mixture was stirred overnight at room temperature. The reaction was quenched by adding trifluoroacetic acid (0.5 mL), and the mixture was partitioned between water and dichloromethane. The water layer was separated, washed twice with dichloromethane, and evaporated *in vacuo*. The resultant residue was purified on a reverse phase ODS C_{18} column (0.1% aq. TFA: acetonitrile = 1:0 - 0:1) to give the crude product. The crude product was then purified with column chromatography on silica gel (CH_2Cl_2/MeOH = 1/0-1/1), followed by purification on a reverse phase ODS C_{18} column (0.1% aq. TFA: acetonitrile = 1:0 - 0:1) to give **19** (27 mg, 26%) as a colorless oil; $[\alpha]_D^{25}$ +2.4 (*c* = 0.5, MeOH); ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.40 – 7.34 (m, 2H), 7.32 – 7.27 (m, 3H), 3.48 – 3.41 (m, 1H), 3.16 (t, J = 7.1 Hz, 2H), 3.11 (dd, J = 14.1, 5.8 Hz, 1H), 2.90 (dd, J = 14.1, 8.5 Hz, 1H), 2.72 (s, 3H), 2.45 – 2.37 (m, 1H), 2.38 – 2.31 (m, 1H), 2.28 (t, J = 7.4 Hz, 2H), 1.96 – 1.84 (m, 2H), 1.64 – 1.56 (m, 2H), 1.53 – 1.45 (m, 2H), 1.40 – 1.29 (m, 4H); ^{13}C NMR (151 MHz, Methanol-d_4) δ 177.76, 174.77,

136.93, 130.36, 130.15, 128.55, 61.88, 40.48, 37.57, 34.83, 32.75, 31.13, 30.01, 29.75, 27.57, 26.72, 25.90; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 335.2329, obsd 335.2334.

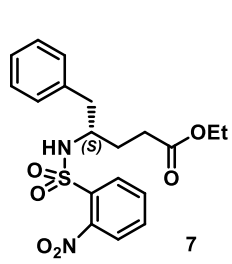
(*R*)-7-(4-(methylamino)-5-phenylpentanamido)heptanoic acid (20)

The title compound was prepared from ethyl (*R*)-7-(4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanamido)heptanoate (**17**, 0.15 g, 0.28 mmol) via the same procedure reported for the synthesis of **19**. The product was obtained as a colorless oil (32 mg, 34%); $[\alpha]_D^{25}$ -2.4 (*c* = 0.5, MeOH); ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.40 – 7.34 (m, 2H), 7.33 – 7.28 (m, 3H), 3.48 – 3.41 (m, 1H), 3.16 (t, *J* = 7.1 Hz, 2H), 3.11 (dd, *J* = 14.1, 5.8 Hz, 1H), 2.90 (dd, *J* = 14.1, 8.5 Hz, 1H), 2.72 (s, 3H), 2.41 (dt, *J* = 15.8, 6.7 Hz, 1H), 2.37 – 2.31 (m, 1H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.95 – 1.84 (m, 2H), 1.64 – 1.56 (m, 2H), 1.53 – 1.44 (m, 2H), 1.40 – 1.27 (m, 4H); ^{13}C NMR (151 MHz, Methanol-d_4) δ 177.76, 174.78, 136.92, 130.36, 130.16, 128.55, 61.89, 40.48, 37.58, 34.83, 32.75, 31.13, 30.02, 29.75, 27.57, 26.72, 25.90.; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 335.2329, obsd 335.2335.

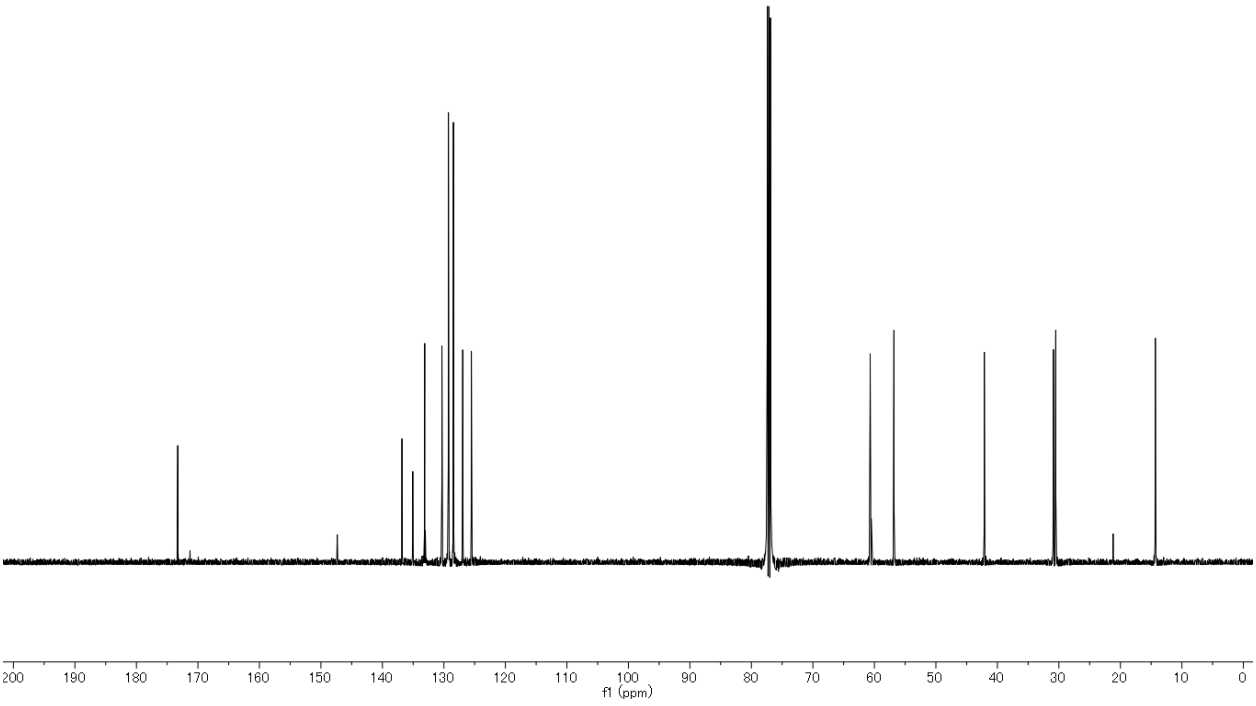
(*RS*)-7-(4-(methylamino)-5-phenylpentanamido)heptanoic acid (21)

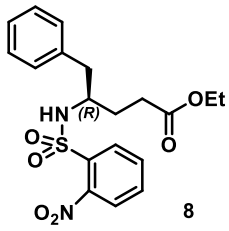
The title compound was prepared from ethyl (*RS*)-7-(4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanamido)heptanoate (**18**, 0.18 g, 0.32 mmol) via the same procedure reported for the synthesis of **19**. The product was obtained as a colorless oil (20 mg, 19%); ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.40 – 7.34 (m, 2H), 7.33 – 7.28 (m, 3H), 3.48 – 3.42 (m, 1H), 3.16 (t, *J* = 7.1 Hz, 2H), 3.11 (dd, *J* = 14.1, 5.8 Hz, 1H), 2.90 (dd, *J* = 14.1, 8.5 Hz, 1H), 2.72 (s, 3H), 2.45 – 2.38 (m, 1H), 2.37 – 2.31 (m, 1H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.95 – 1.84 (m, 2H), 1.64 – 1.56 (m, 2H), 1.53 – 1.45 (m, 2H), 1.40 – 1.29 (m, 4H); ^{13}C NMR (151 MHz, Methanol-d_4) δ 177.77, 174.78, 136.92, 130.16, 128.55, 61.89, 40.48, 37.57, 34.82, 32.75, 31.13, 30.02, 29.75, 27.57, 26.72, 25.90; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 335.2329, obsd 335.2339.

II. NMR Spectra



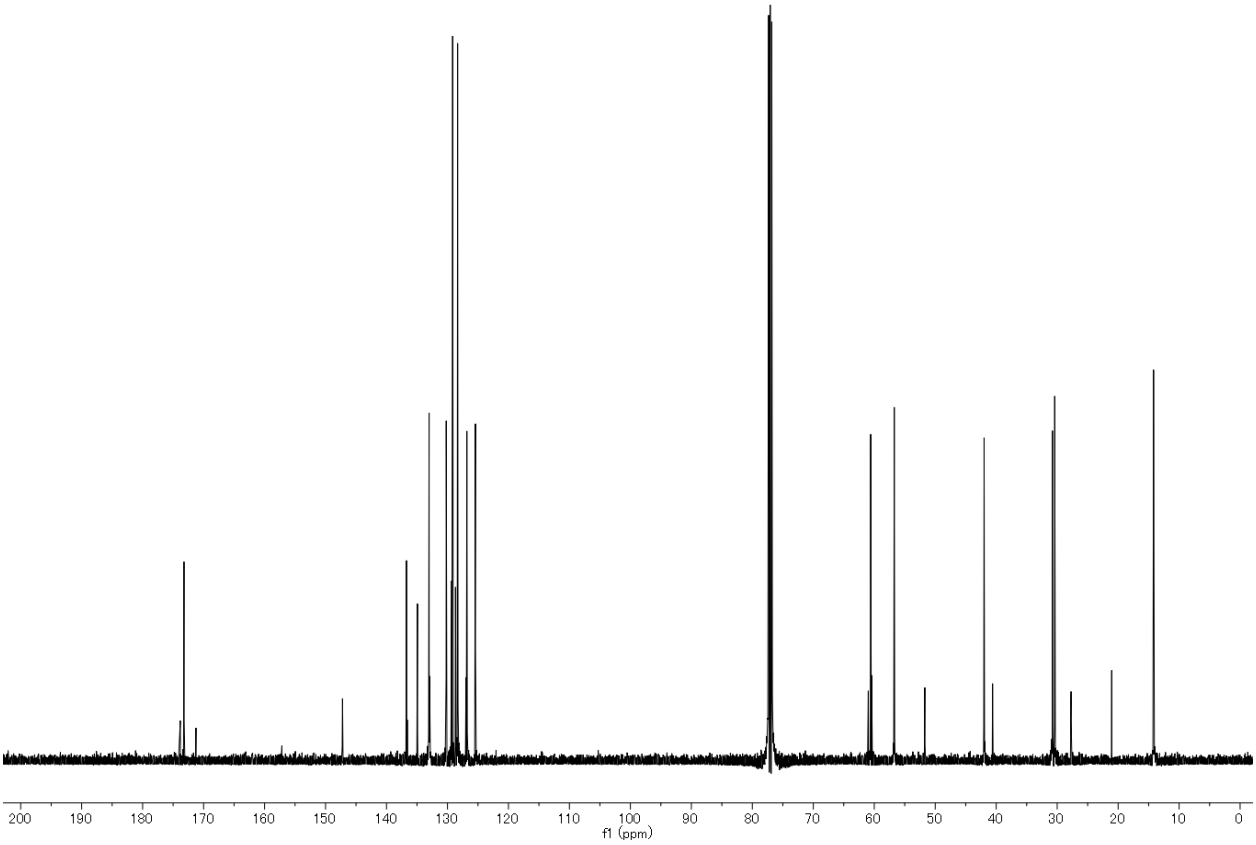
7




HN
(R)
OEt
O
O
S
O
O2N

8

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


200
190
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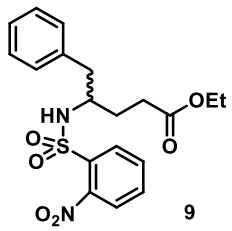
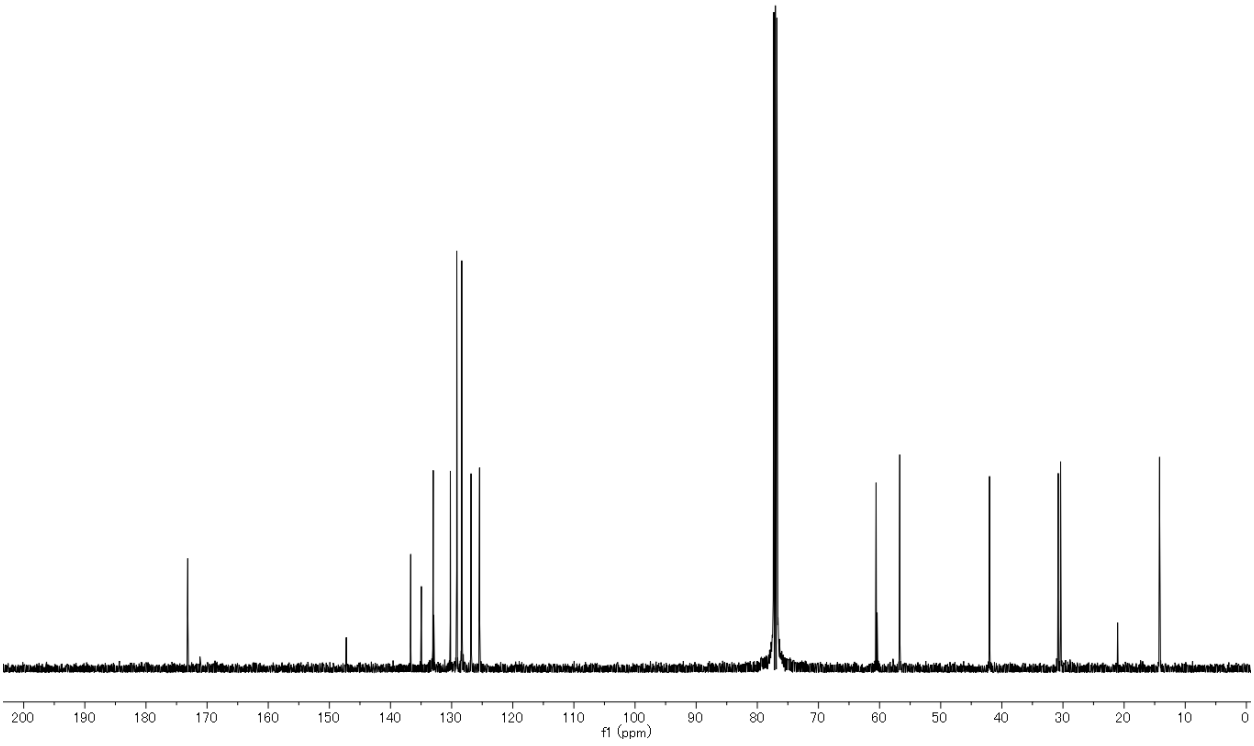
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


HN
OEt
O
O
S
O
O_2N

9

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)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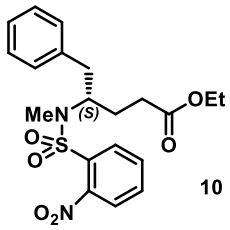
f1 (ppm)


MeN
(S)
OEt
O
O
S
O
O2N

10

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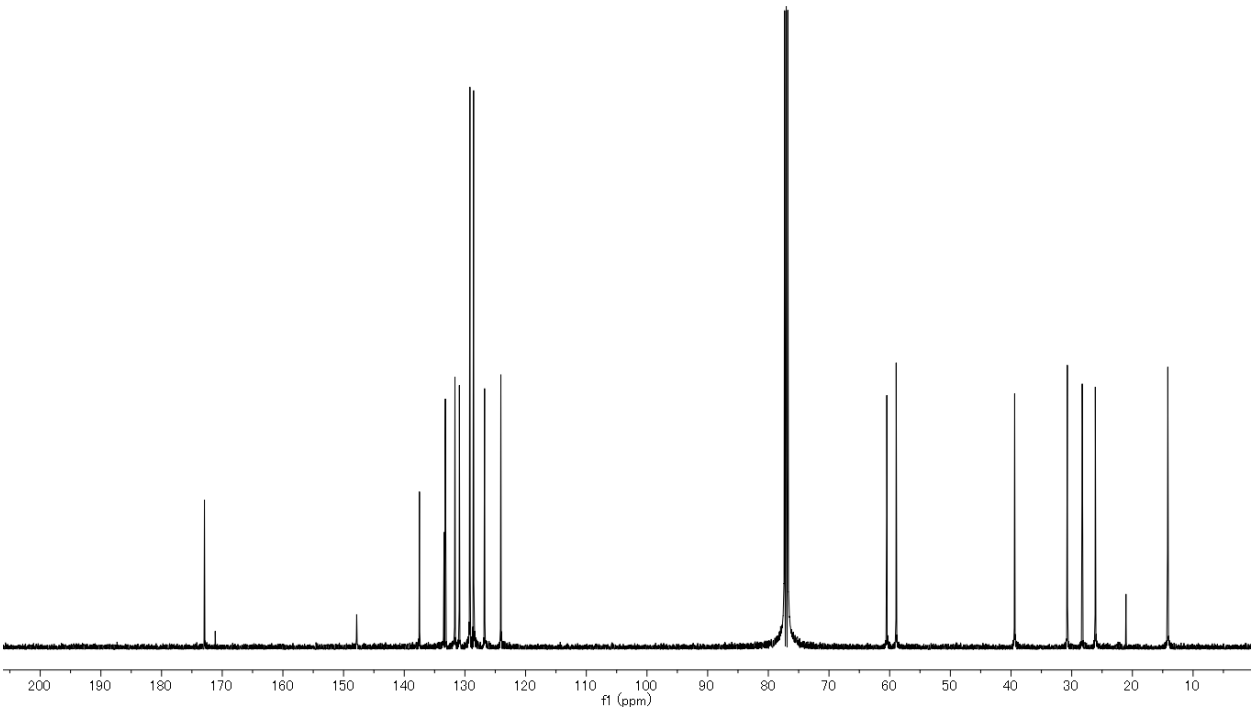
f1 (ppm)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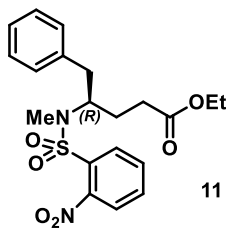
f1 (ppm)


MeN
(R)
OEt
O
O
S
O
O2N

11

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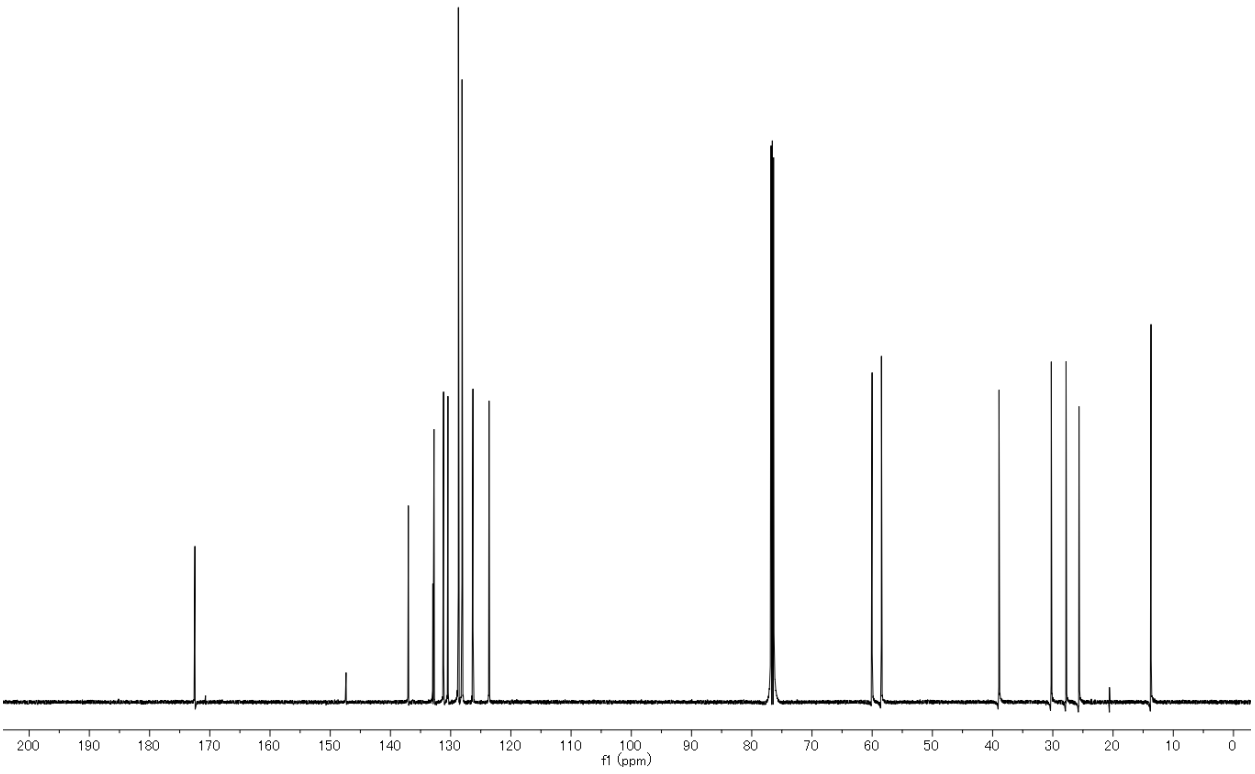
f1 (ppm)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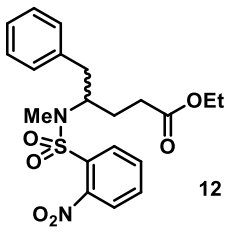
f1 (ppm)


MeN
OEt
O
O
S
O
O2N

12

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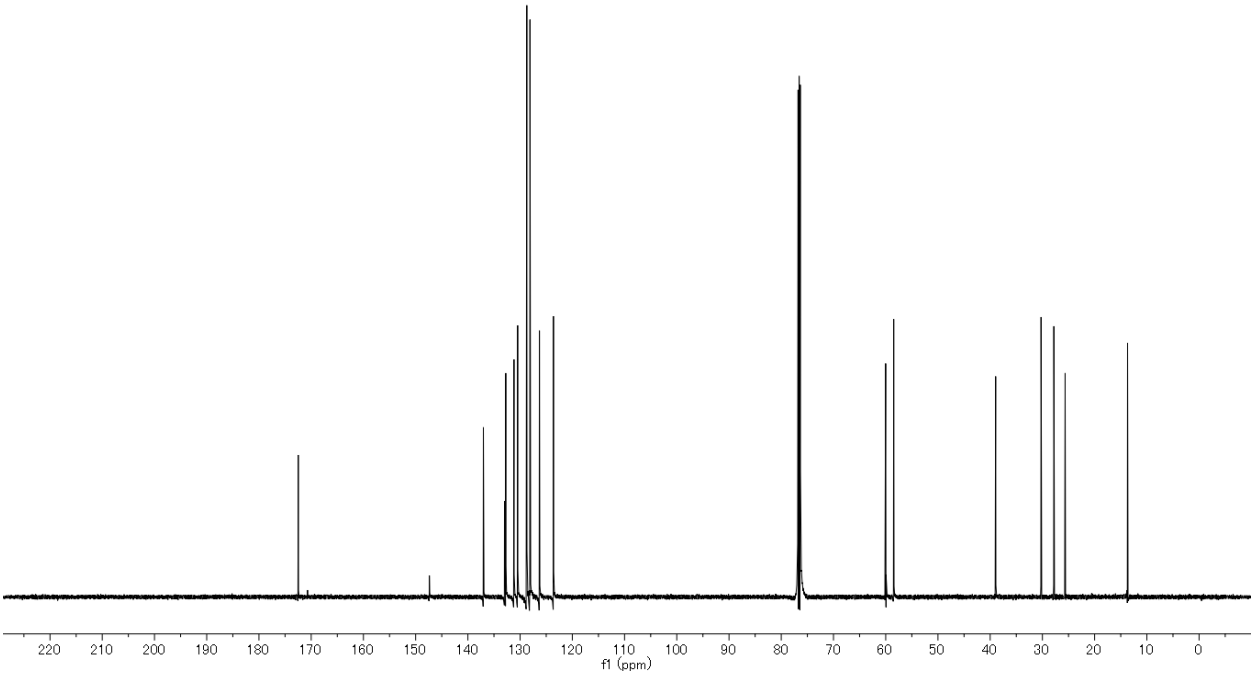
f1 (ppm)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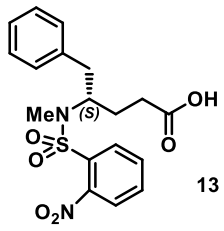
f1 (ppm)


MeN
(S)
OH
O
O
S
O
O_2N

13

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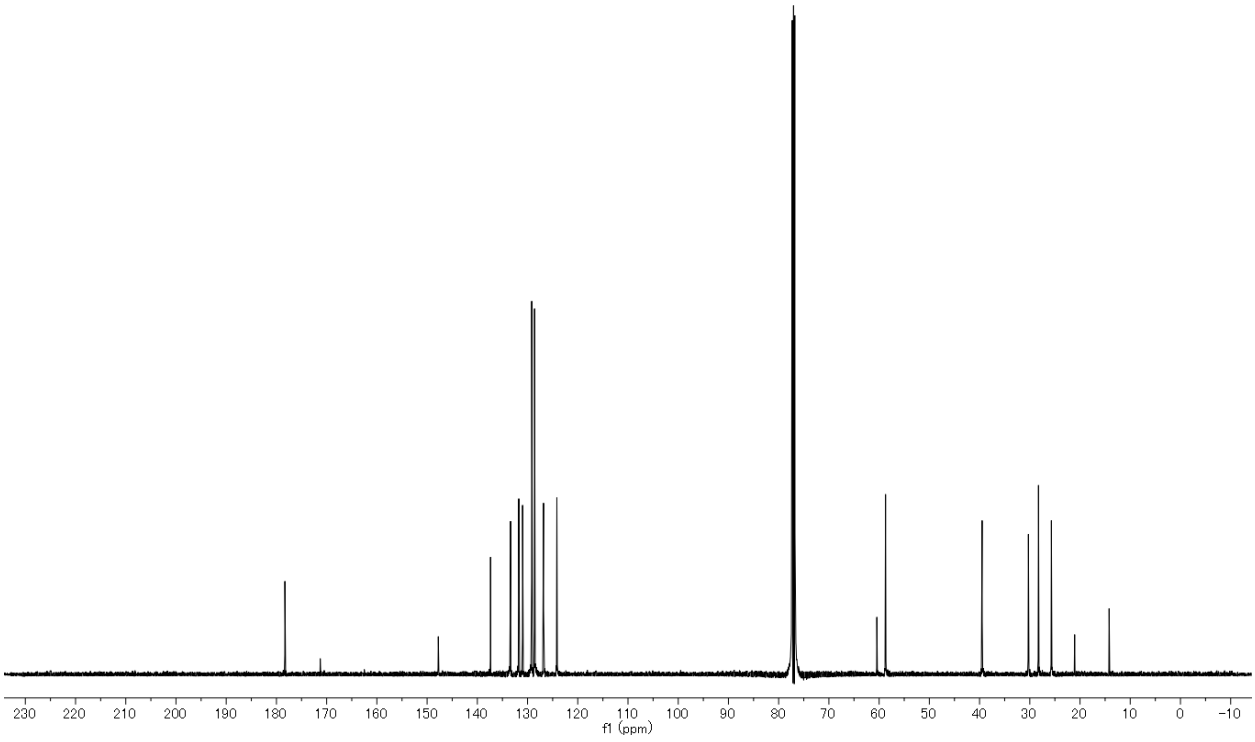
f1 (ppm)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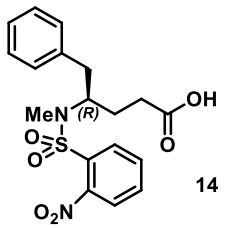
f1 (ppm)


MeN
(R)
OH
O
O
S
O
O2N

14

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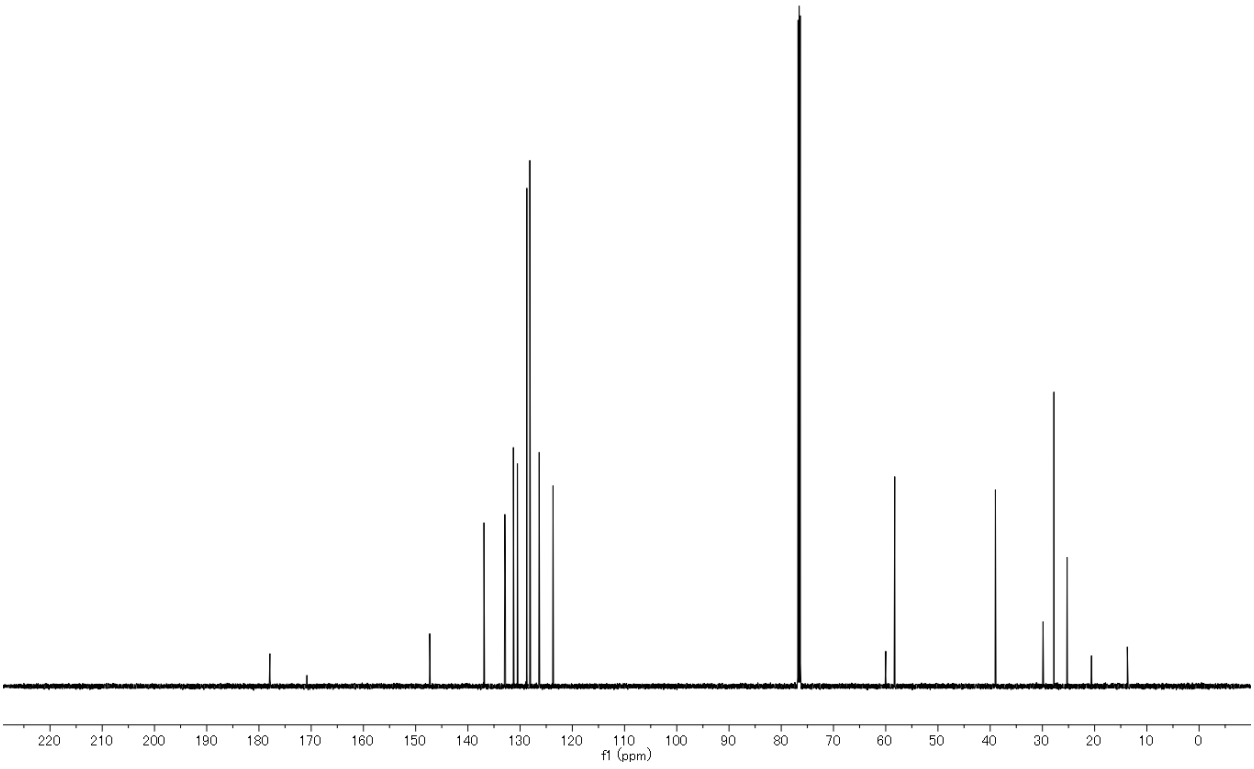
f1 (ppm)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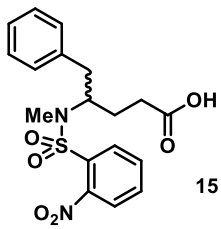
f1 (ppm)


MeN
OH
O
O
S
O
O2N

15

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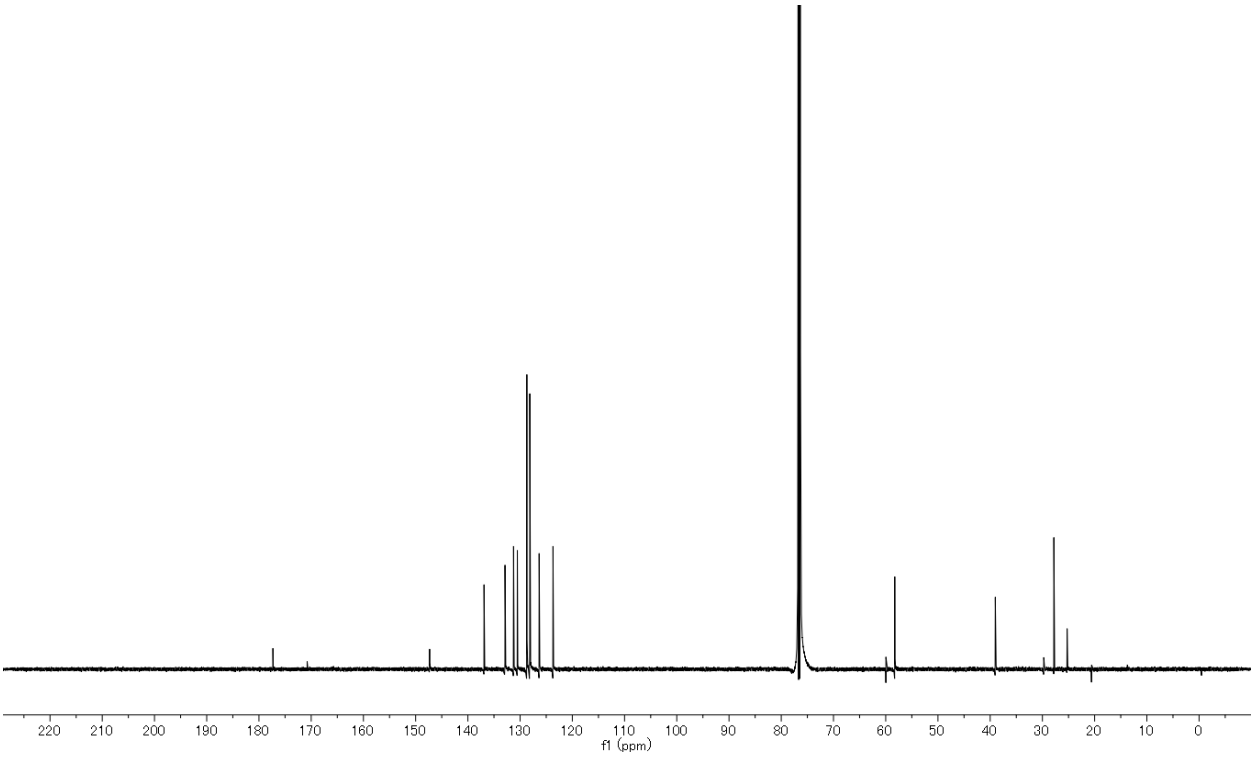
f1 (ppm)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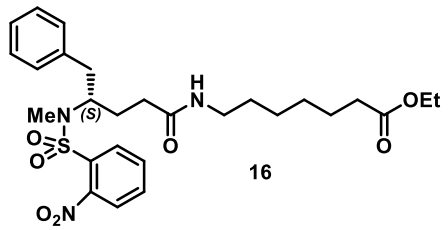
f1 (ppm)


MeN
(S)
H
N
O
OEt
O
O
S
O
O2N

16

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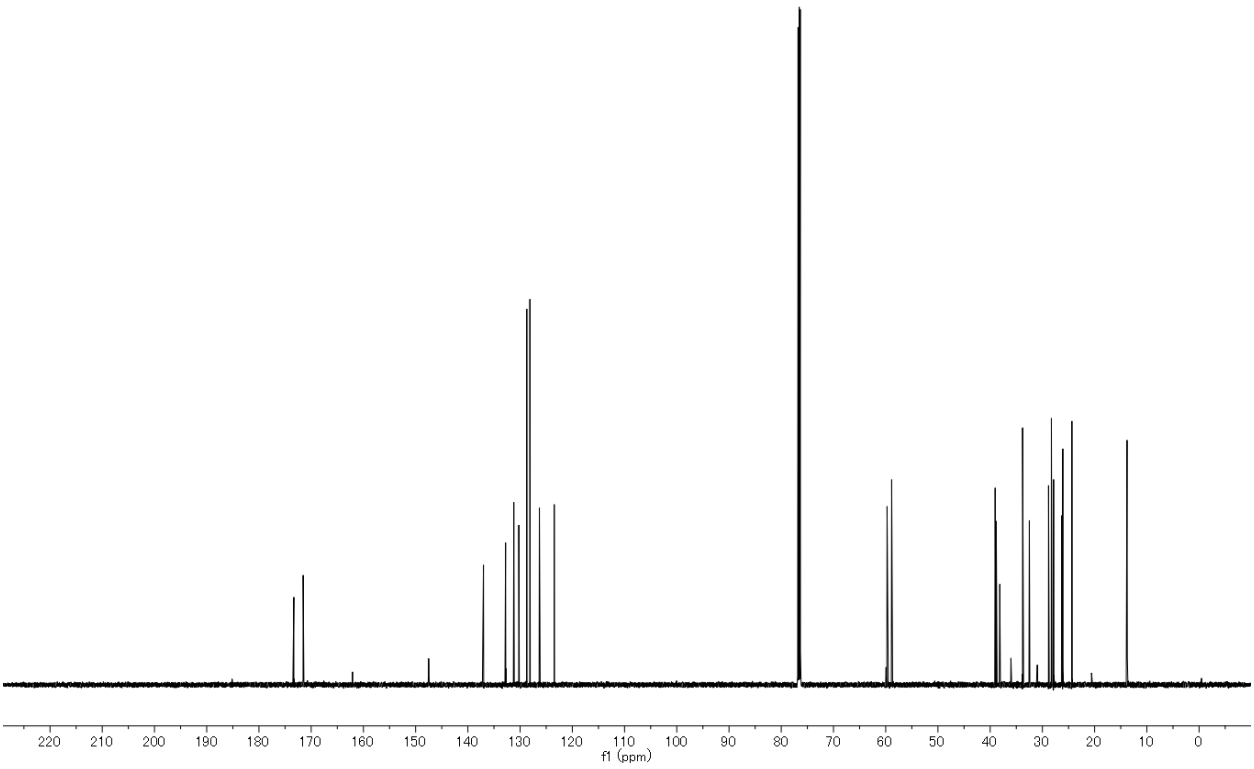
f1 (ppm)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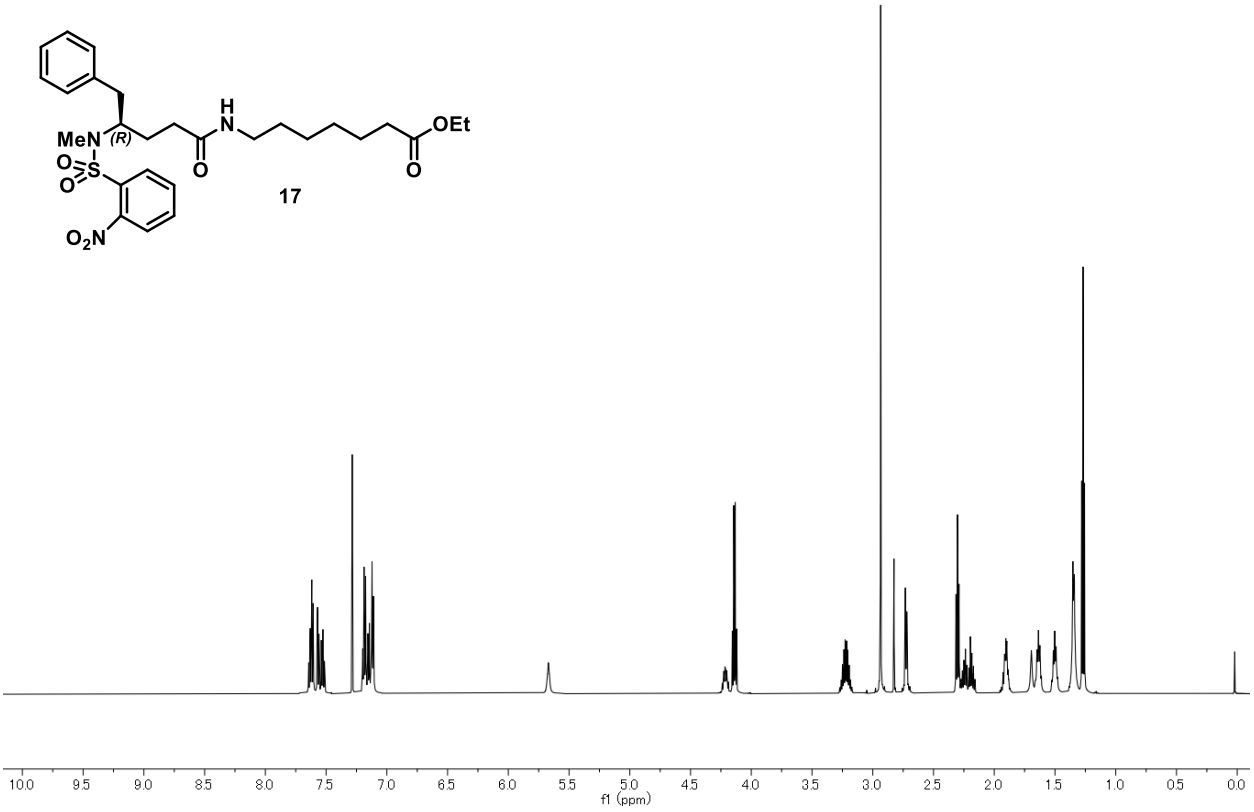
f1 (ppm)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(R)
H
N
O
O
OEt
O
O
S
O
O2N
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9.5
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8.5
8.0
7.5
7.0
6.5
6.0
5.5
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3.5
3.0
2.5
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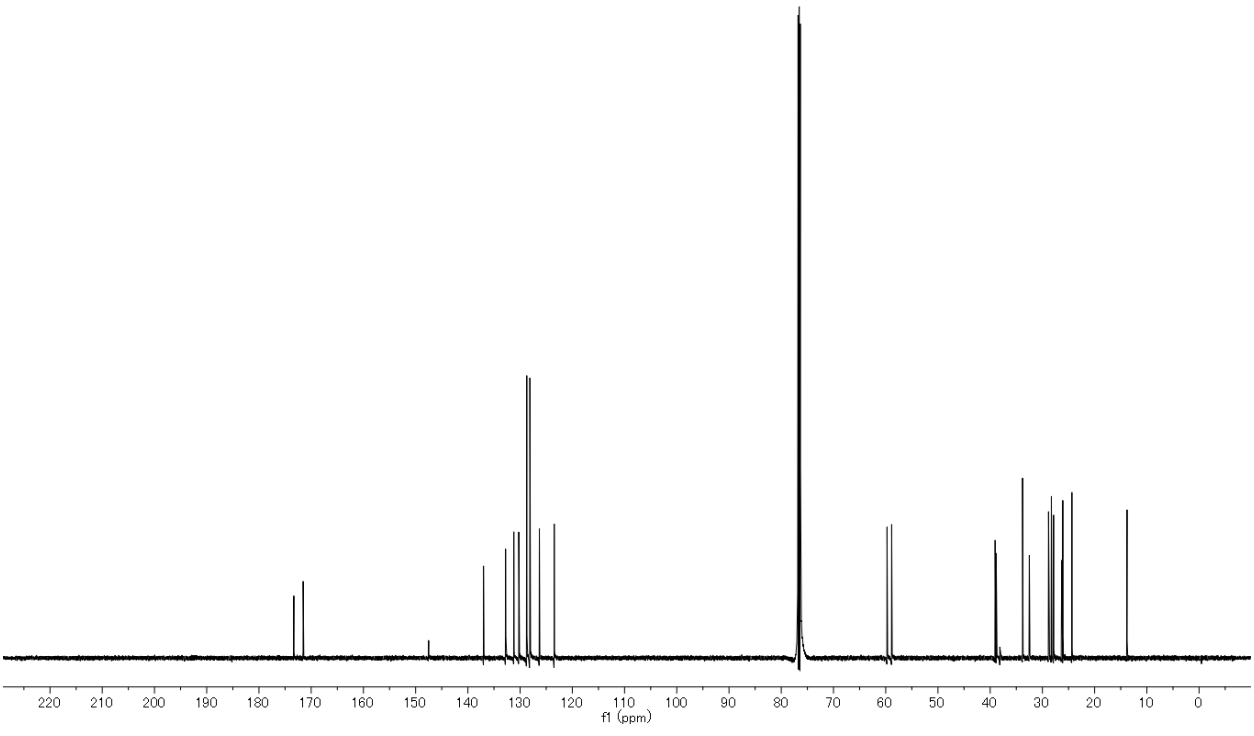
1.0
0.5
0.0
f1 (ppm)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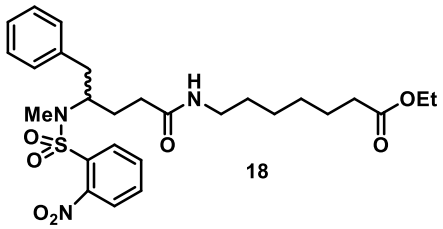
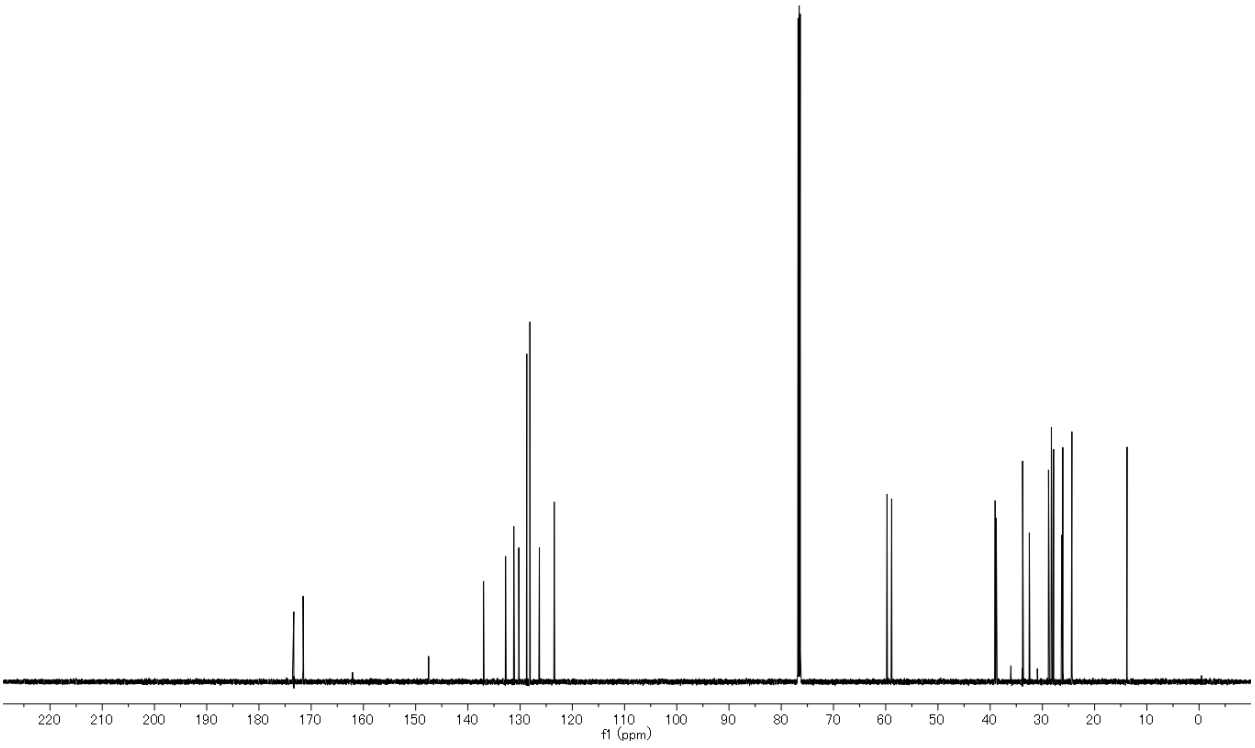
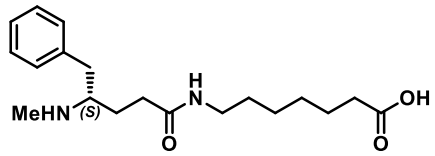
f1 (ppm)



18




MeHN
(S)
H
N
O
OH
O

19

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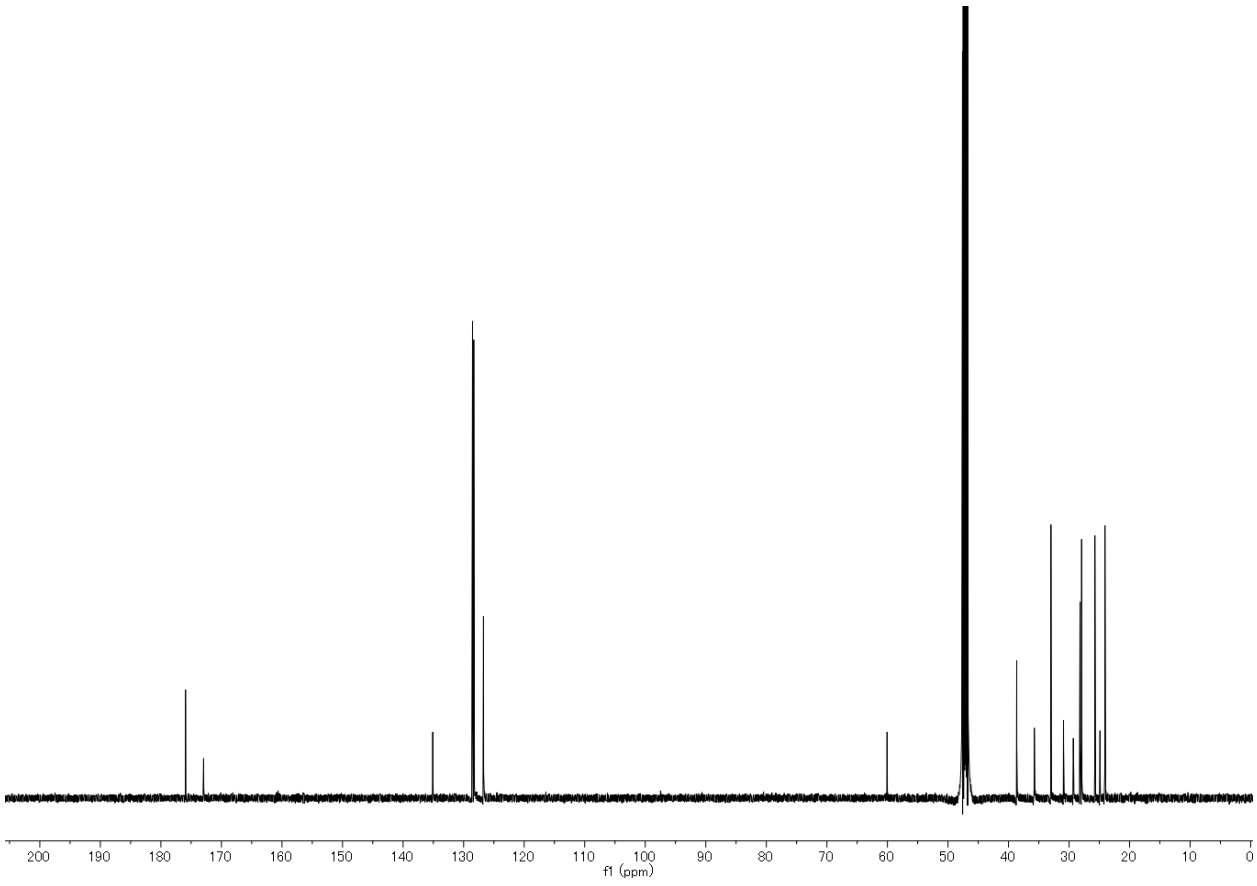
f1 (ppm)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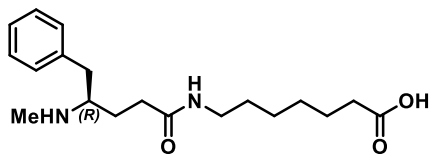
0
f1 (ppm)


MeHN
(R)
H
N
O
O
OH
O

20

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8.5
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6.5
6.0
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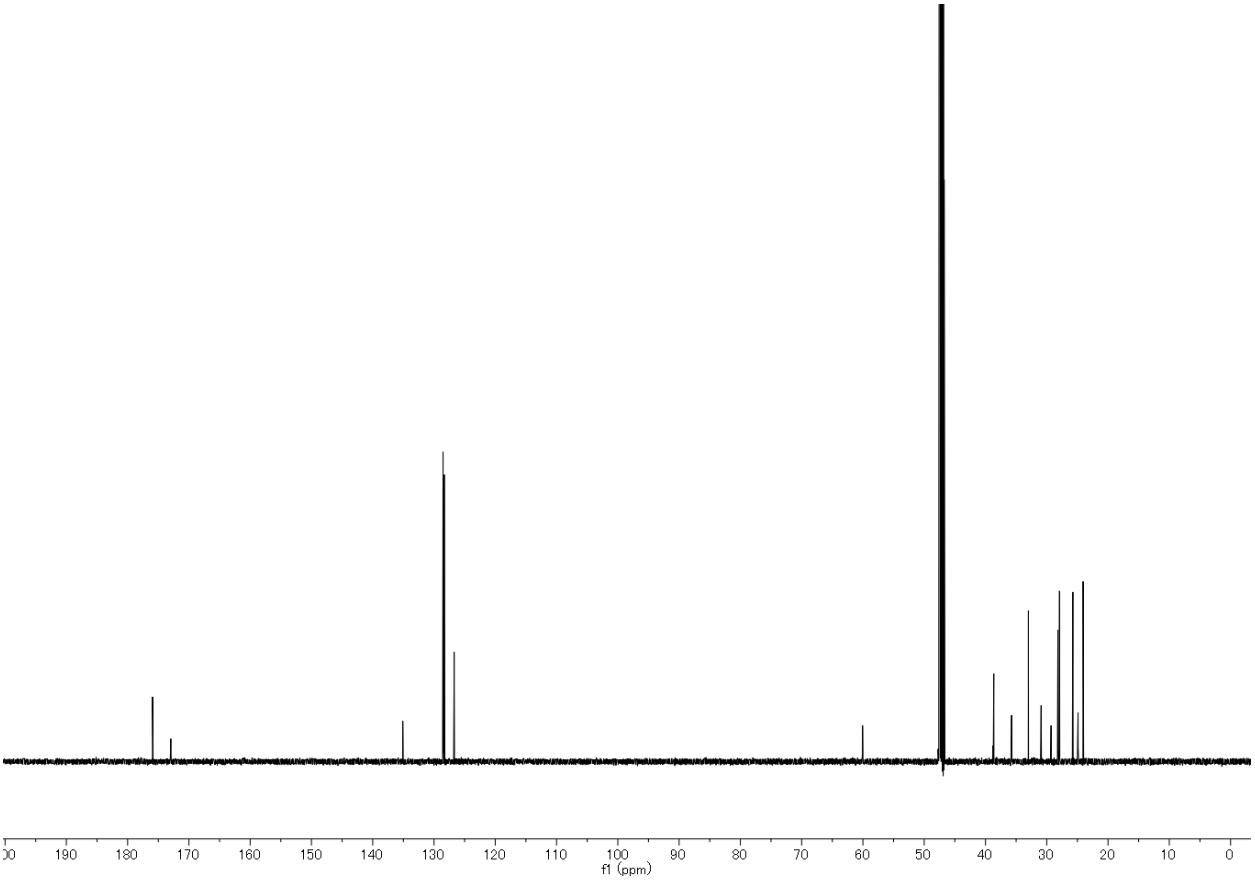
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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180
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160
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80
70
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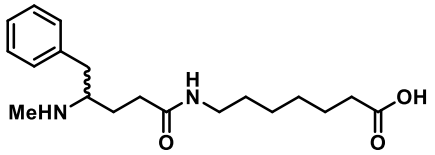
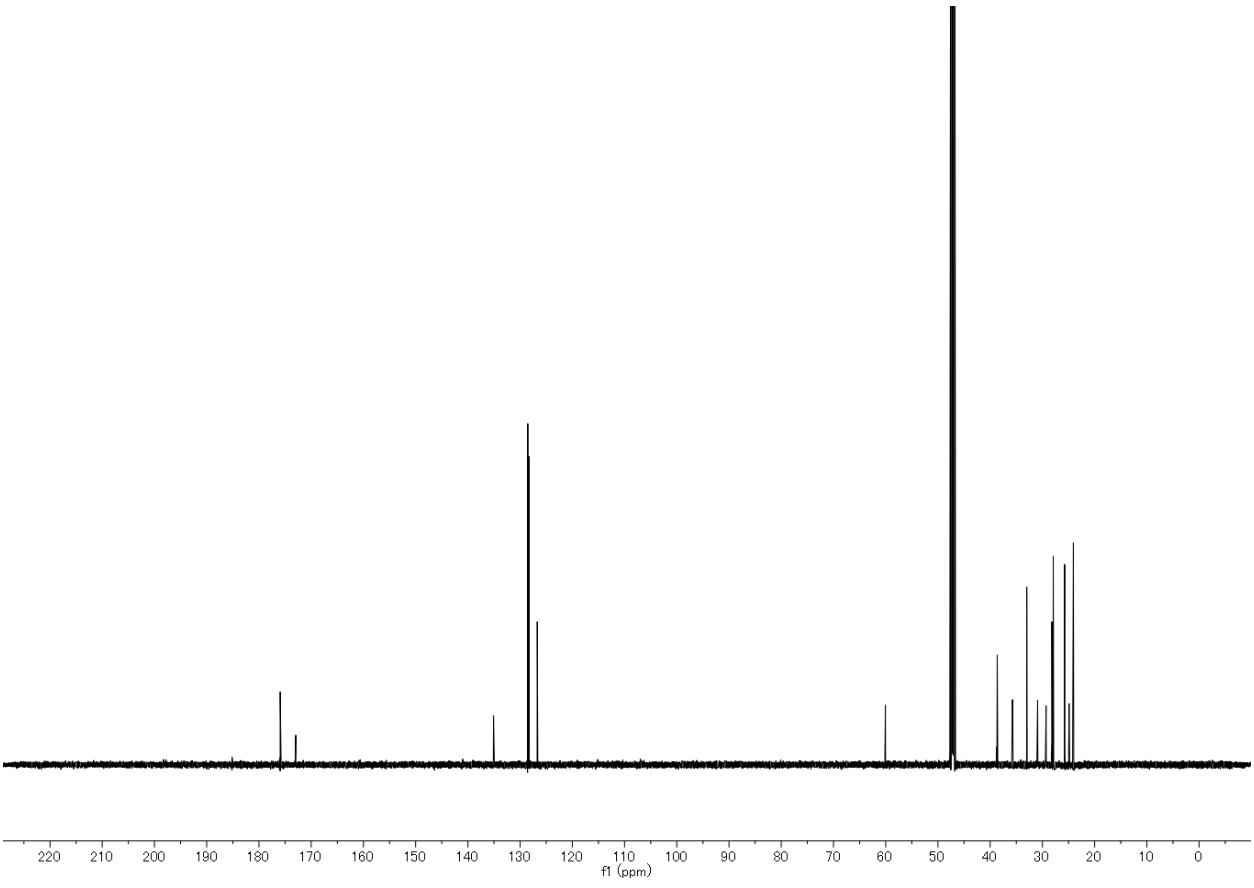
40
30
20
10
0
f1 (ppm)


MeHN
H
N
O
O
OH

21

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)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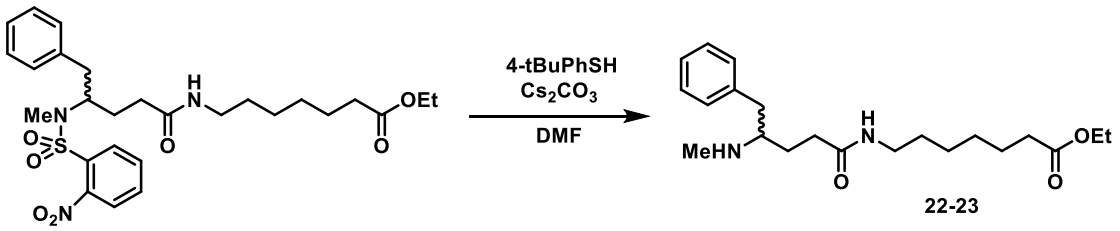
f1 (ppm)

III. Synthesis of Mosher's Amides/Confirmation of Optical Purity

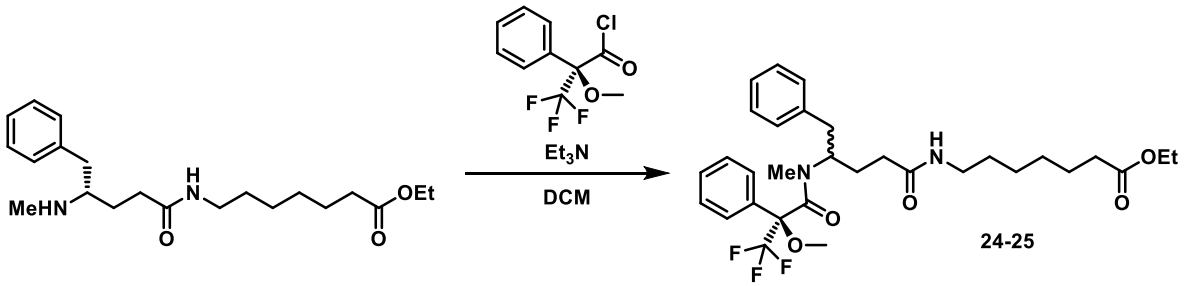


Ethyl (*S*)-7-(4-(methylamino)-5-phenylpentanamido)heptanoate (22)

To a mixture of ethyl (*S*)-7-(4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanamido)heptanoate (**16**, 0.23 g, 0.42 mmol), cesium carbonate (0.33 g, 1.0 mmol), and *N*, *N*-dimethylformamide (2.0 mL) was added 4-*tert*-butylthiophenol (0.17 mL, 1.0 mmol), and the mixture was stirred overnight at room temperature. After, the mixture was partitioned between ethyl acetate and brine. The organic layer was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The resultant residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 1/0-7:3) to give **22** (81 mg, 53%) as a colorless oil; $[\alpha]_D^{25}$ -2.2 (*c* = 0.5, MeOH); ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.29 (t, *J* = 7.6 Hz, 2H), 7.23 – 7.17 (m, 3H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.14 (t, *J* = 7.0 Hz, 2H), 2.77 – 2.66 (m, 3H), 2.36 (s, 3H), 2.30 (t, *J* = 7.4 Hz, 2H), 2.28 – 2.18 (m, 2H), 1.80 – 1.71 (m, 1H), 1.73 – 1.64 (m, 1H), 1.64 – 1.56 (m, 2H), 1.52 – 1.45 (m, 2H), 1.39 – 1.29 (m, 4H), 1.24 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, Methanol-d_4) δ 175.73, 175.49, 140.20, 140.20, 129.63, 127.46, 61.69, 61.38, 40.67, 40.29, 35.01, 33.47, 33.33, 30.23, 29.82, 29.80, 27.62, 14.54; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 363.2642, obsd 363.2651.

Ethyl (*R*)-7-(4-(methylamino)-5-phenylpentanamido)heptanoate (23)

The title compound was prepared from ethyl (*R*)-7-(4-((*N*-methyl-2-nitrophenyl)sulfonamido)-5-phenylpentanamido)heptanoate (**17**, 0.15 g, 0.27 mmol) via the same procedure reported for the synthesis of **22**. The product was obtained as a colorless oil (64 mg, 65%); $[\alpha]_D^{25}$ +4.0 (*c* = 0.5, MeOH); ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.29 (t, *J* = 7.6 Hz, 2H), 7.23 – 7.17 (m, 3H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.14 (t, *J* = 7.0 Hz, 2H), 2.71 (hept, *J* = 5.3 Hz, 3H), 2.35 (s, 3H), 2.32 – 2.18 (m, 4H), 1.80 – 1.72 (m, 1H), 1.72 – 1.65 (m, 1H), 1.64 – 1.56 (m, 2H), 1.52 – 1.45 (m, 2H), 1.38 – 1.29 (m, 4H), 1.24 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, Methanol-d_4) δ 175.73, 175.48, 140.24, 130.31, 130.31, 129.62, 127.45, 61.69, 61.37, 40.71, 40.28, 35.00, 33.50, 33.33, 30.23, 29.86, 29.81, 27.62, 25.95, 14.55; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 363.2642, obsd 363.2651.



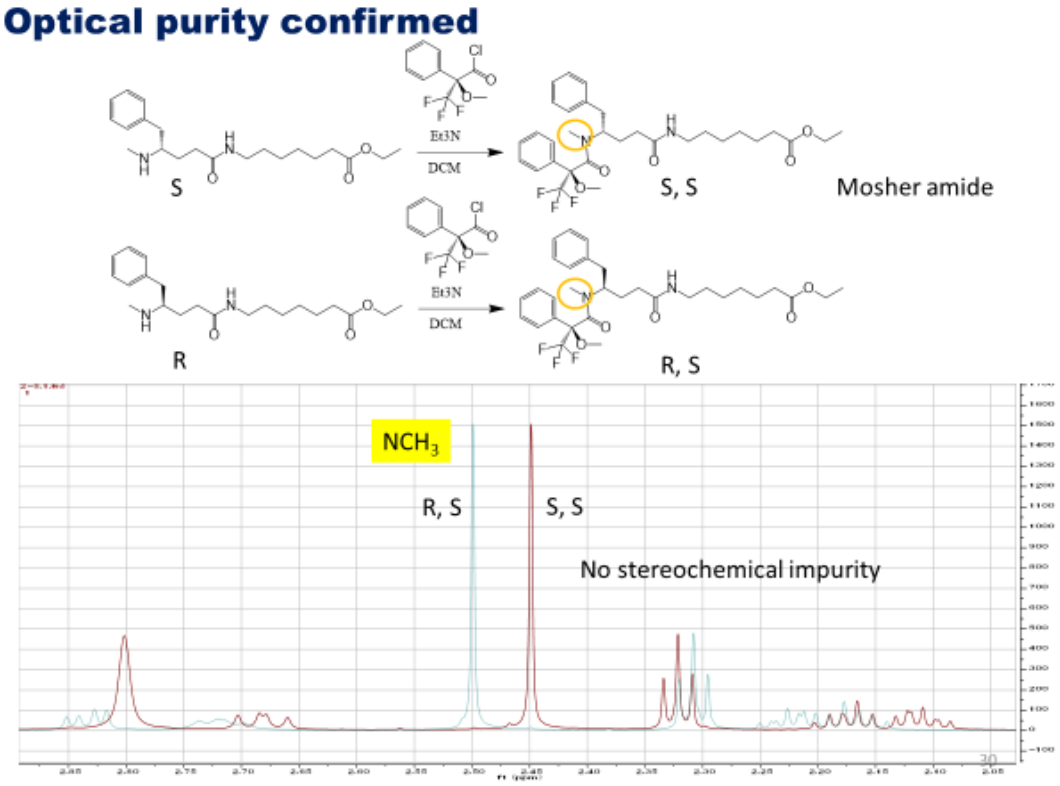
Ethyl 7-((*S*)-5-phenyl-4-((*S*)-3,3,3-trifluoro-2-methoxy-*N*-methyl-2-phenylpropanamido)pentanamido)heptanoate (24)

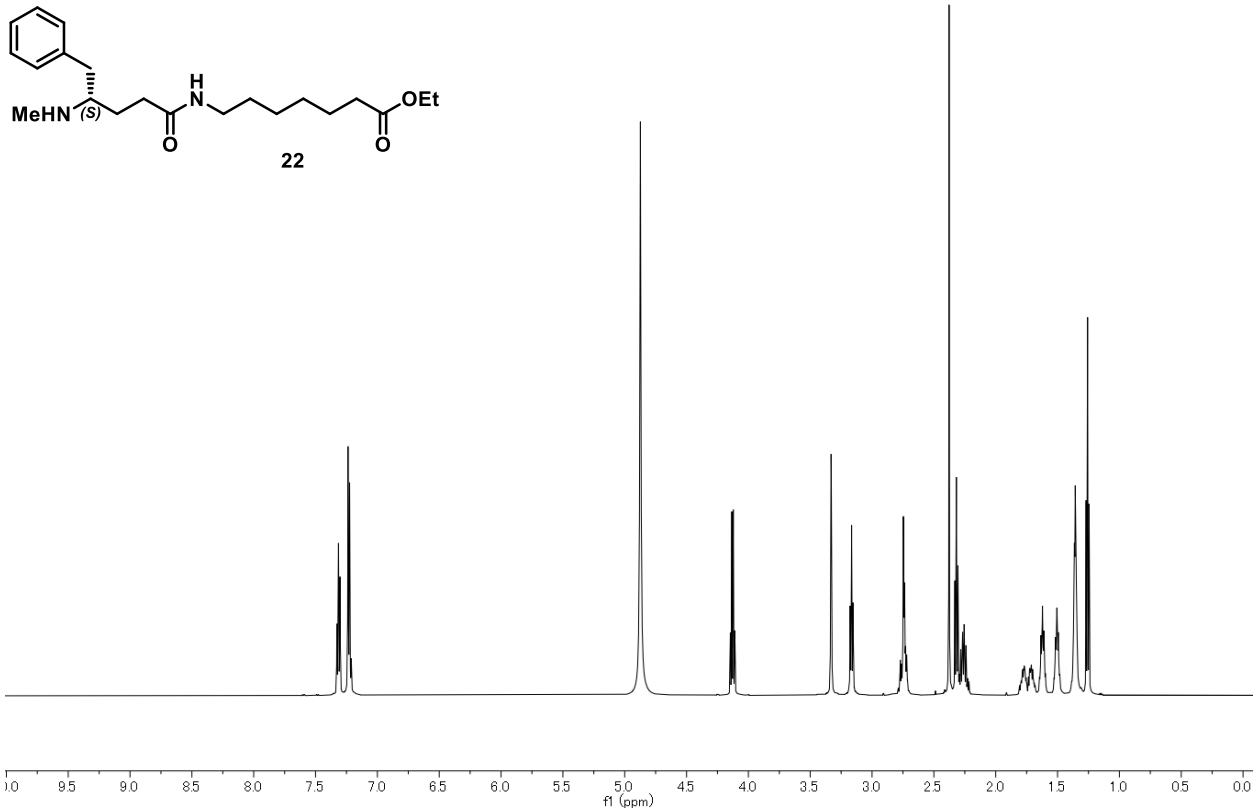
To a mixture of ethyl (*S*)-7-(4-(methylamino)-5-phenylpentanamido)heptanoate (**22**, 33 mg, 0.09 mmol) and triethylamine (0.02 mL, 0.14 mmol) in dichloromethane (1.0 mL) was added (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride (0.02 mL, 0.12 mmol), and the mixture was stirred overnight at room temperature. After, the mixture was partitioned between dichloromethane and sat. aq. $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The resultant residue was purified by column chromatography on silica gel (hexane/EtOAc = 1/0-0/1) to give **24** (34 mg, 65%) as a colorless oil; $[\alpha]_D^{25}$ -42.0 (*c* = 1.0, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 – 7.46 (m, 2H), 7.40 – 7.32 (m, 3H), 7.30 – 7.25 (m, 2H), 7.22 (d, *J* = 7.5 Hz, 2H), 7.17 (t, *J* = 7.3 Hz, 1H), 6.20 (s, 1H), 5.22 (s, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.28 (q, *J* = 6.7 Hz, 2H), 2.95 (dd, *J* = 14.8, 5.5 Hz, 1H), 2.78 (s, 3H), 2.66 (dd, *J* = 14.8, 11.0 Hz, 1H), 2.43 (s, 3H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.21 – 2.12 (m, 1H), 2.12 – 2.05 (m, 1H), 1.91 – 1.83 (m, 1H), 1.83 – 1.75 (m, 1H), 1.75 – 1.69 (m, 2H), 1.69 – 1.61 (m, 2H), 1.60 – 1.54 (m, 2H), 1.44 – 1.34 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.91, 172.26, 167.07, 137.63, 133.80, 129.48, 129.04, 128.84, 128.42, 126.93, 126.66, 85.00 (q, *J* = 24.9 Hz), 77.37, 77.16, 76.95, 60.35, 54.33, 53.63, 39.66, 38.69, 34.38, 33.44, 29.53, 28.90, 27.99, 26.72, 24.97, 14.37; ESI-TOF-MS (*m/z*): $[M+H]^+$ calcd 579.3040, obsd 579.3042.

Ethyl 7-((*R*)-5-phenyl-4-((*S*)-3,3,3-trifluoro-2-methoxy-*N*-methyl-2-phenylpropanamido)pentanamido)heptanoate (25)

The title compound was prepared from ethyl (*R*)-7-(4-(methylamino)-5-phenylpentanamido)heptanoate (**23**, 25 mg, 0.07 mmol) via the same procedure reported for the synthesis of **24**. The product was obtained as a colorless oil (25 mg, 63%); $[\alpha]_D^{25}$ -57.1 (*c* = 1.0, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33 – 7.27 (m, 2H), 7.28 – 7.21 (m, 1H), 7.21 – 7.16 (m, 2H), 7.14 (t, *J* = 7.7 Hz, 2H), 7.06 (d, *J* = 7.8 Hz, 2H), 5.88 (s, 1H), 5.12 (s, 1H), 4.12 (q, *J* = 7.2 Hz, 2H), 3.64 (s, 3H), 3.29 – 3.18 (m, 2H), 2.81 (dd, *J* = 14.4, 6.4 Hz, 1H), 2.74 – 2.66 (m, 1H), 2.48 (s, 3H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.25 – 2.11 (m, 2H), 1.95 – 1.85 (m, 2H), 1.66 – 1.58 (m, 2H), 1.51 (p, *J* = 7.5 Hz, 2H), 1.39 – 1.30 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (151 MHz,, $CDCl_3$) δ173.90, 172.02, 166.40, 137.51, 133.52, 129.15, 128.93, 128.83, 128.33, 126.84, 126.51,

85.23 (q, J = 24.9 Hz), 77.37, 77.32, 77.16, 76.95, 60.36, 55.50, 54.59, 39.66, 38.31, 34.35, 34.02, 29.49, 28.84, 27.75, 26.66, 24.92, 14.37; ESI-TOF-MS (m/z): $[M+H]^+$ calcd 579.3040, obsd 579.3043.

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OEt
O
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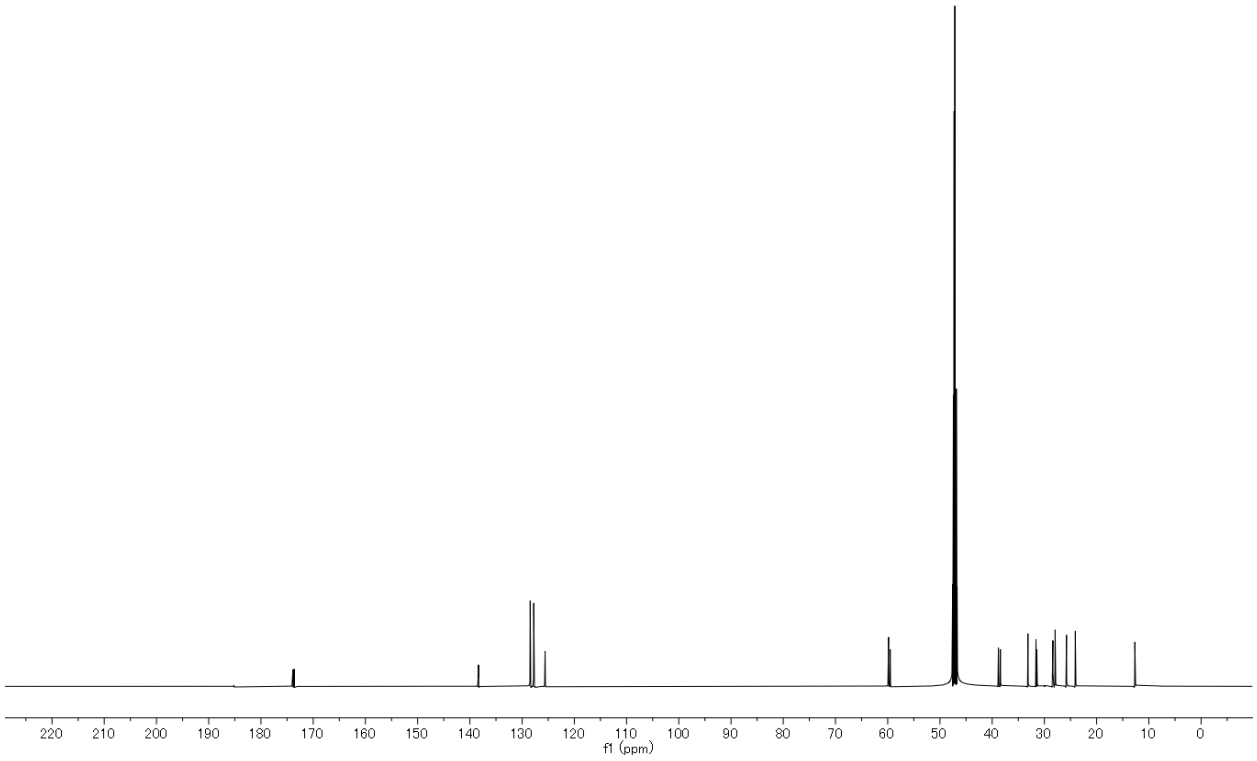
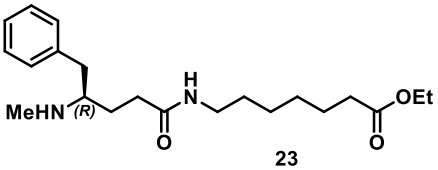
f1 (ppm)


f1 (ppm)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(R)
H
N
O
O
OEt
O

23

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6.5
6.0
5.5
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3.0
2.5
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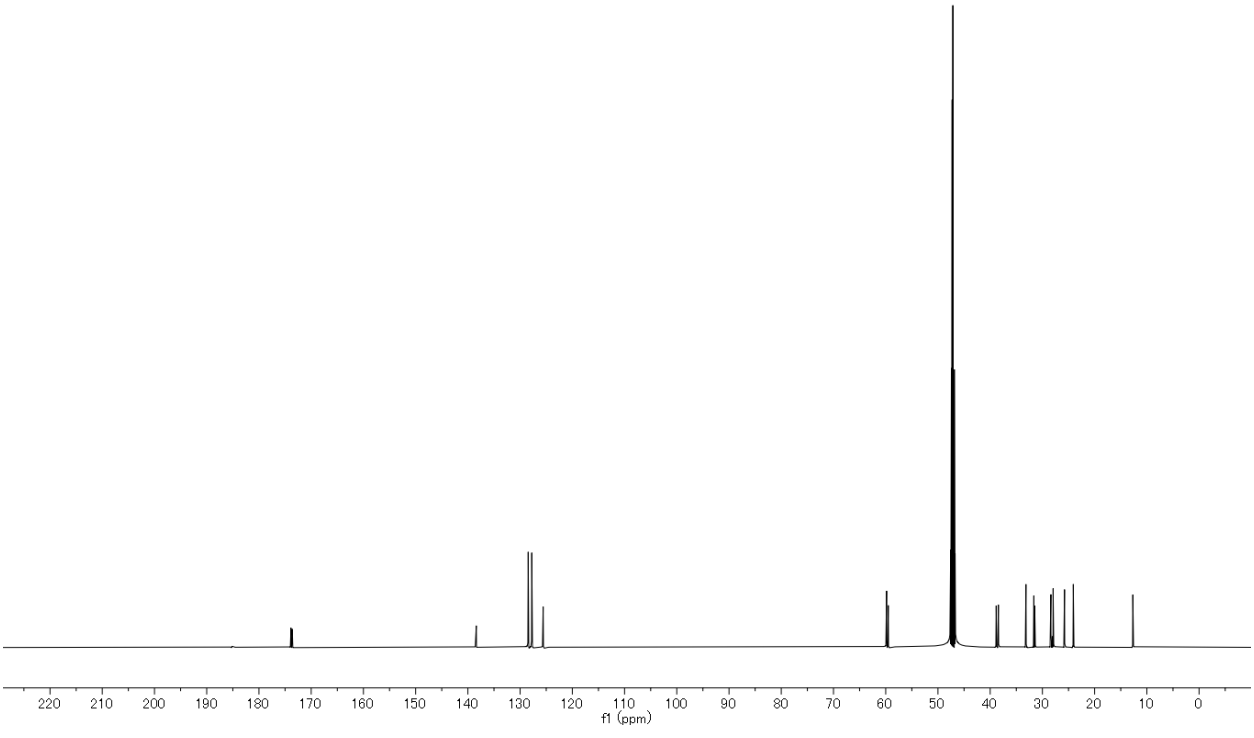
f1 (ppm)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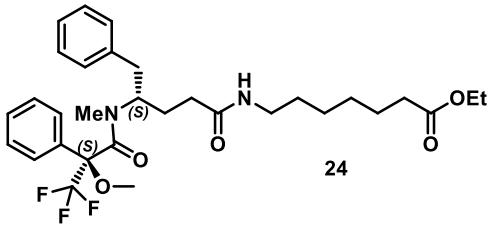
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(S)
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O
OEt
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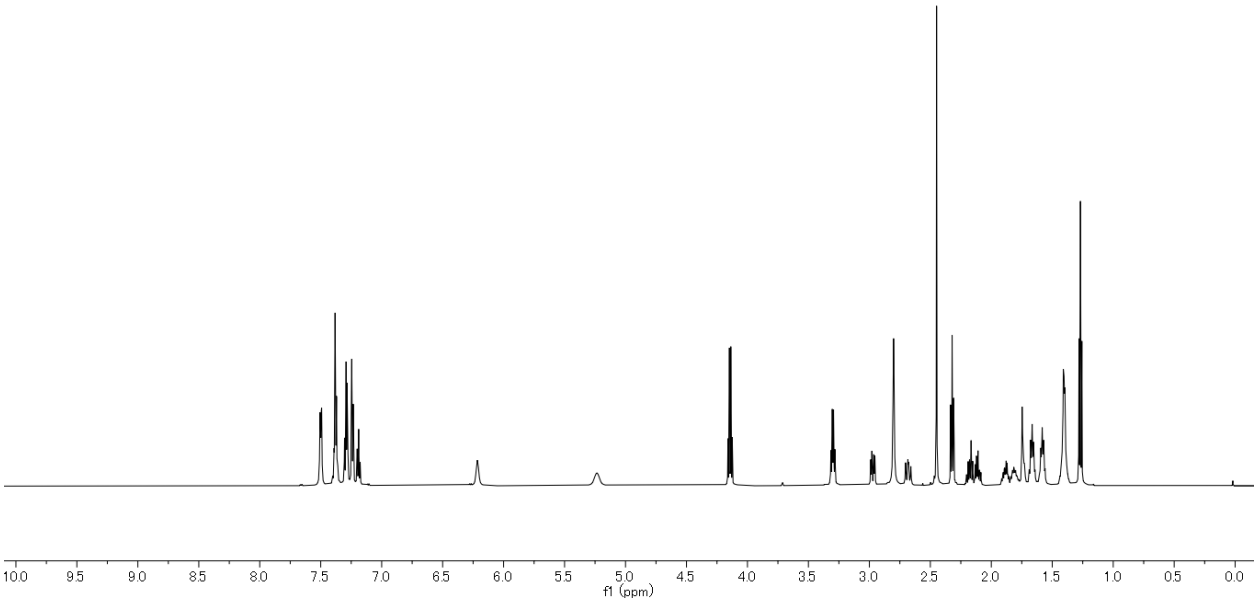
F
F
F
O

24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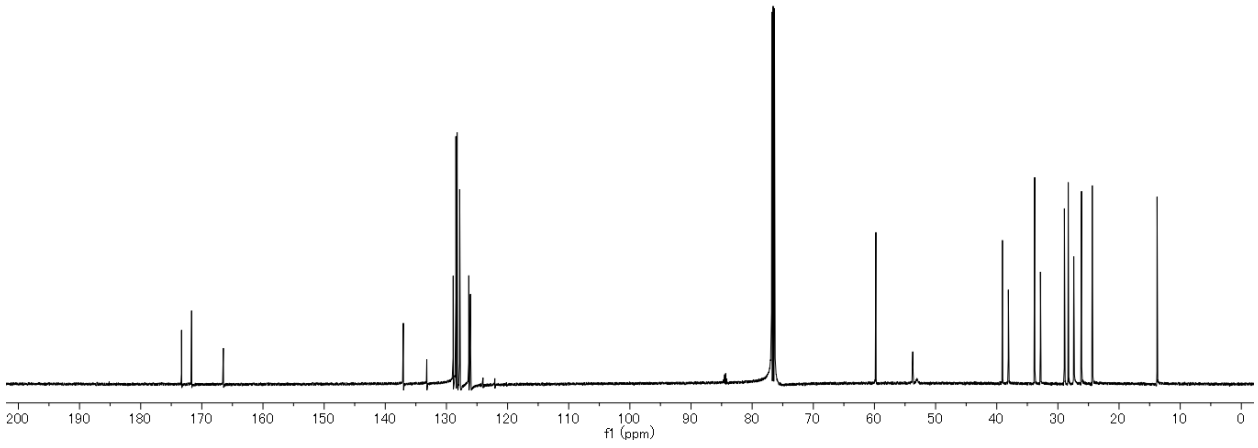
f1 (ppm)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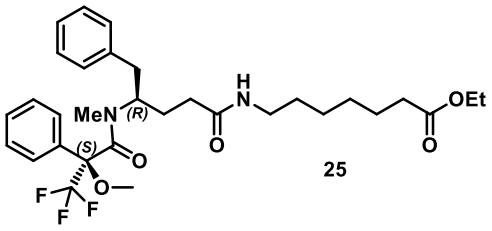
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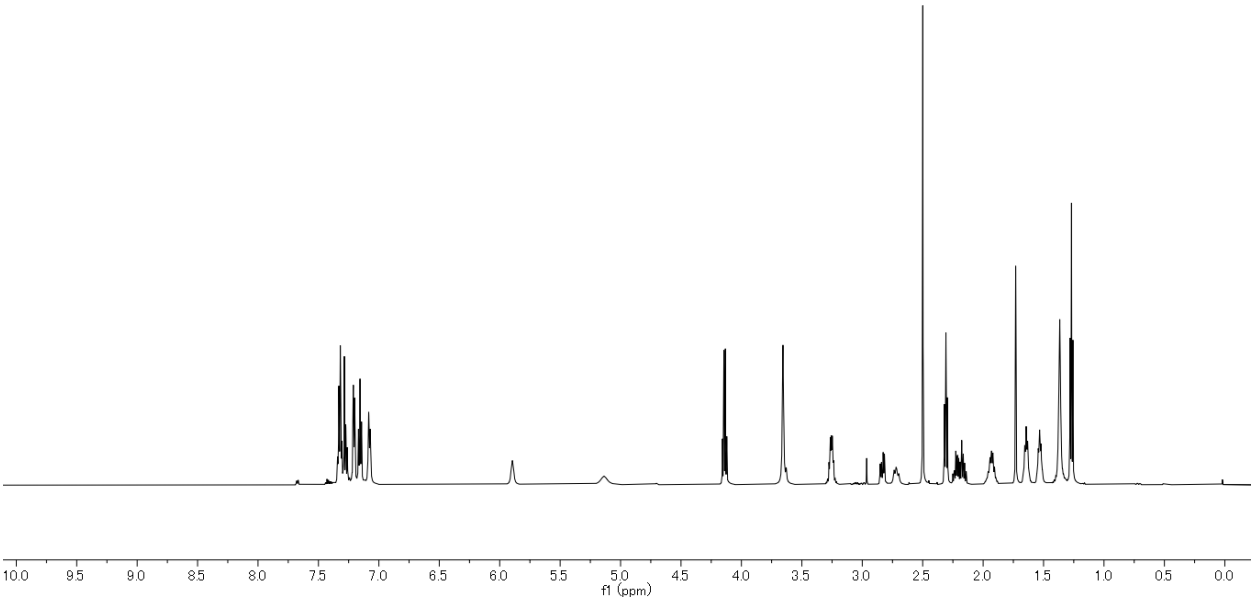
(R)
(S)
H
N
O
O
O
OEt
O
F
F
F

25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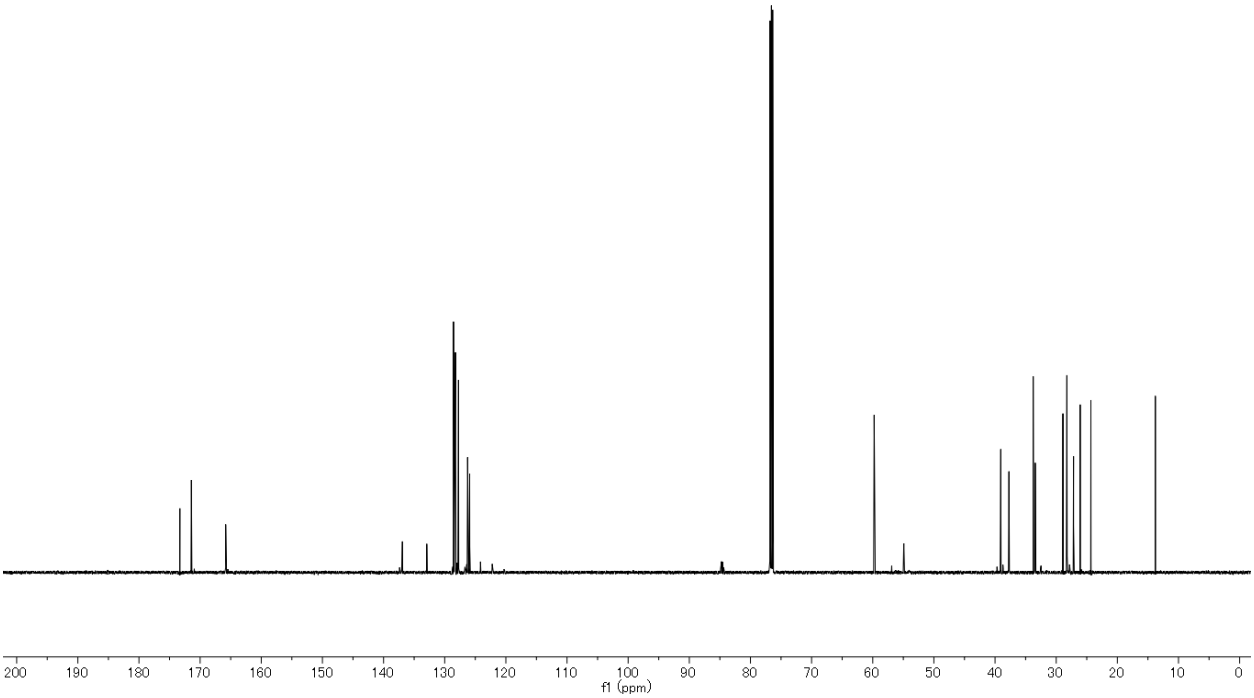
f1 (ppm)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100
90
80
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60
50
40
30
20
10
0
f1 (ppm)

IV. Bioconjugation Chemistry

TT-conjugates

A solution of hapten (**19**, **20**, or **21**, 6.0 mg, 18 μM), EDC·HCl (10 mg, 54 μM), and sulfo-NHS (12 mg, 54 μM) in 10% aq. DMF (300 μL) was agitated at room temperature for 4.5 h. Complete activation of the carboxylic acid was observed by LCMS, then a solution of TT in PBS (2.5 mL, 1.0 mg/mL, pH 7.4) was added. This solution was agitated for 24 h at 4 °C. The resulting protein conjugates were dialyzed into PBS (pH 7.4) 2x at rt for 2 h each, then 1x at 4 °C for 24 h to give a final concentration of 0.8 - 1.0 mg/mL protein (BCA Assay). TT conjugates were used for immunization.

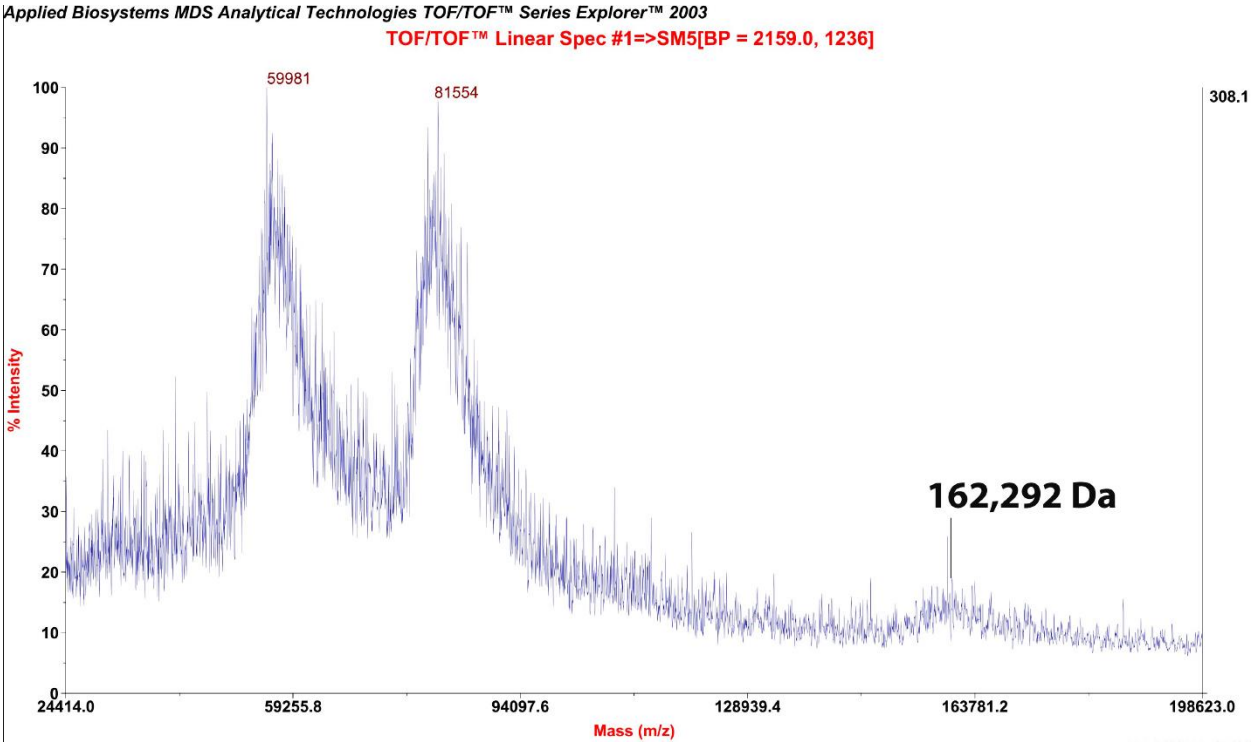
BSA-conjugates

A solution of hapten (**19**, **20**, or **21**, 3.0 mg, 9.0 μM), EDC·HCl (5.2 mg, 27 μM), and sulfo-NHS (5.9 mg, 27 μM) in 10% aq. DMF (150 μL) was agitated at room temperature for 4.5 h. Complete activation of the carboxylic acid was observed by LCMS, then a solution of BSA in PBS (0.12 mL, 10 mg/mL, pH 7.4) was added. This solution was further agitated for 24 h at 4 °C. The resulting protein conjugates were dialyzed into PBS (pH 7.4) 2x at rt for 2 h each, then 1x at 4 °C for 24 h to give a final concentration of 0.9-1.3 mg/mL protein (BCA Assay). BSA conjugates were used to coat ELISA plates.

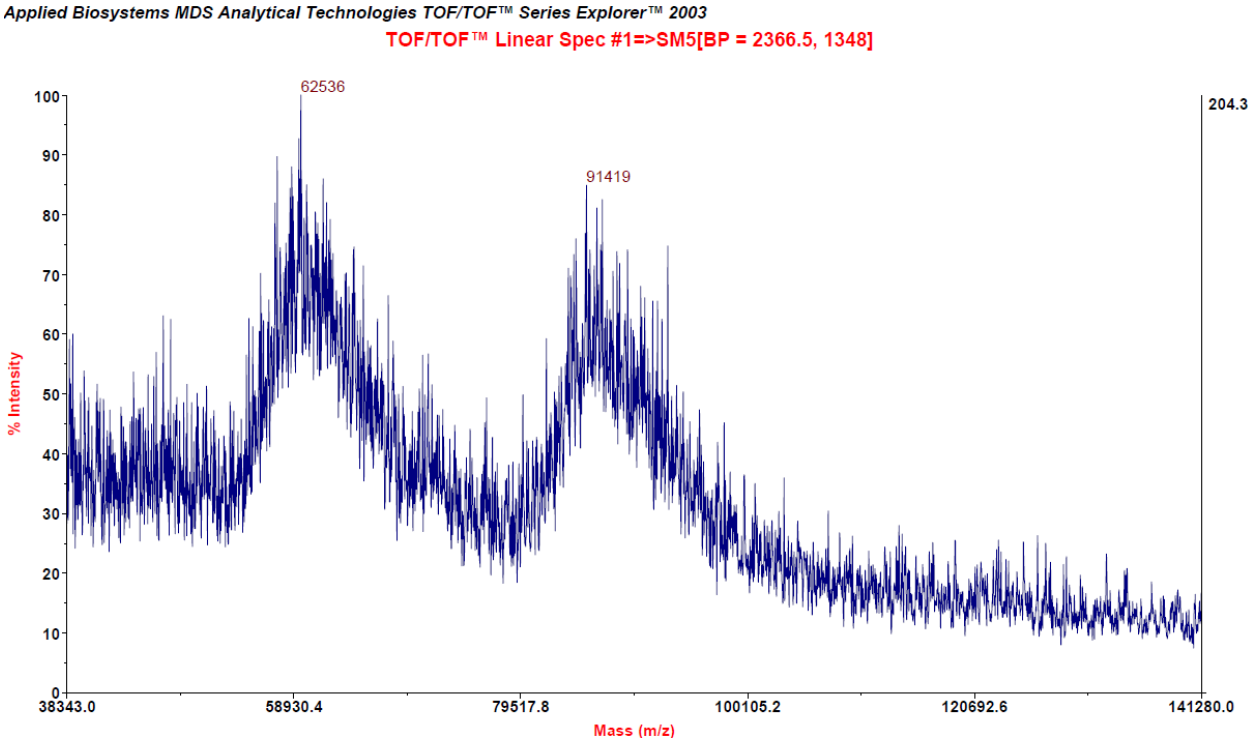
Mass Spectrometry Analysis. In order to quantify the hapten copy number (hapten density) for (*S*)MLMH-TT, (*R*)MLMH-TT and (*RS*)MLMH-TT, samples were submitted for MALDI-ToF MS (Applied Biosystems Voyager DE) analysis and compared to unconjugated protein. Copy numbers were calculated using the following formula: Copy # = (Conjugated MW – Unconjugated MW) / Hapten MW.

VACCINE	CONCENTRATION MLMH-TT	MALDI MS MLMH-TT	COPY NUMBER
TT	-	59981; 81554	-
(*S*)MLMH-TT	1.1 mg/mL	62536; 91419	29
(*R*)MLMH-TT	1.3 mg/mL	70400; N/D	31
(*RS*)MLMH-TT	0.9 mg/mL	63417; 91416	29

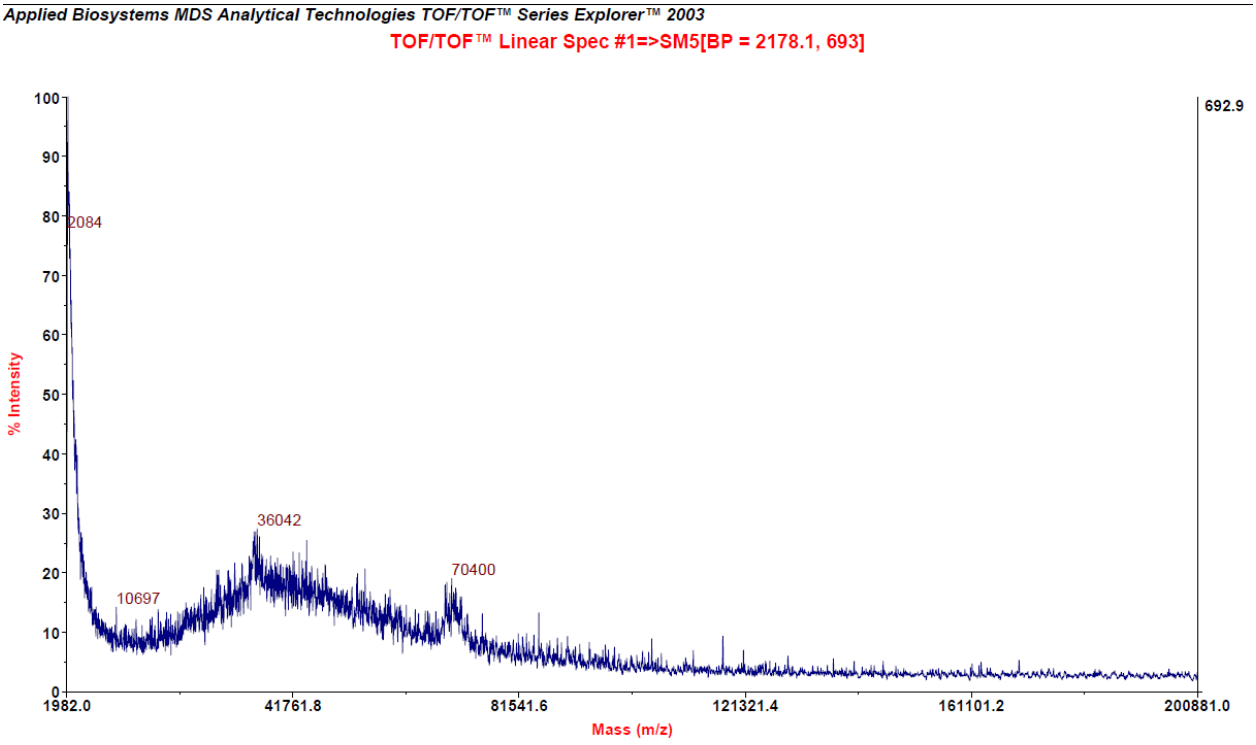
MALDI Mass Spectrum of TT



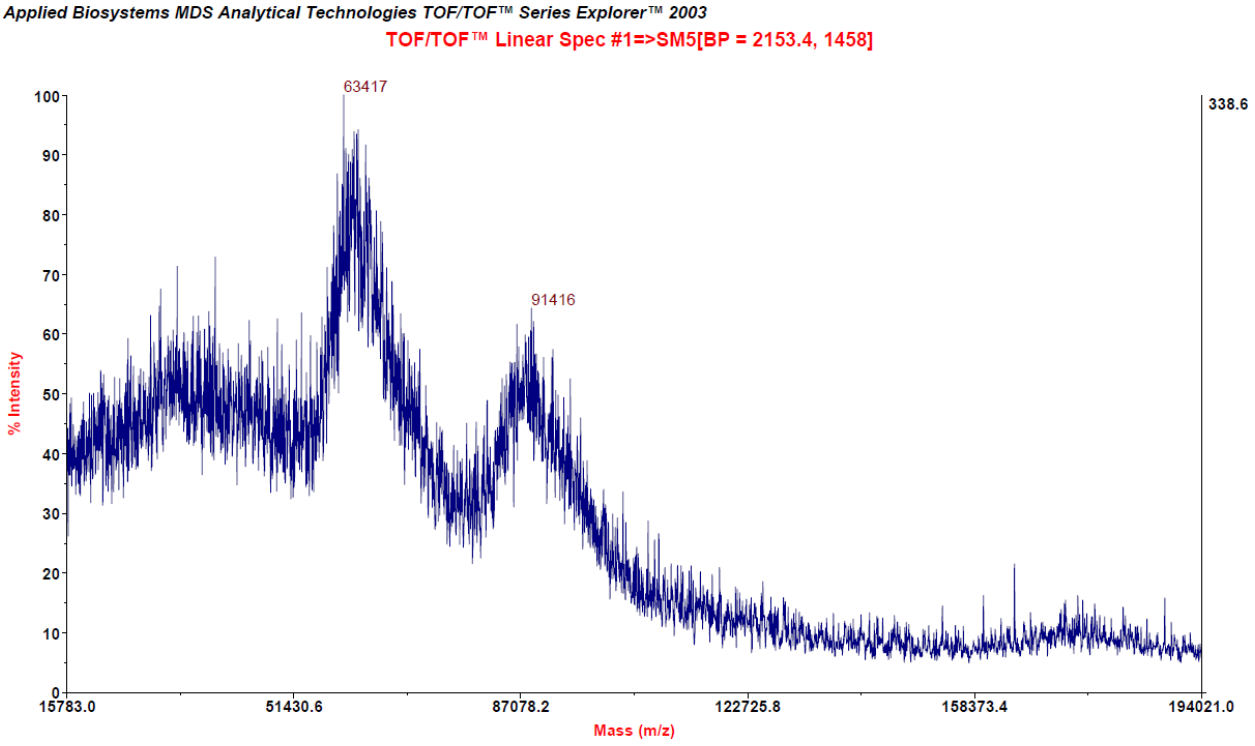
MALDI Mass Spectrum of (*S*)MLMH-TT



MALDI Mass Spectrum of (*R*)MLMH-BSA

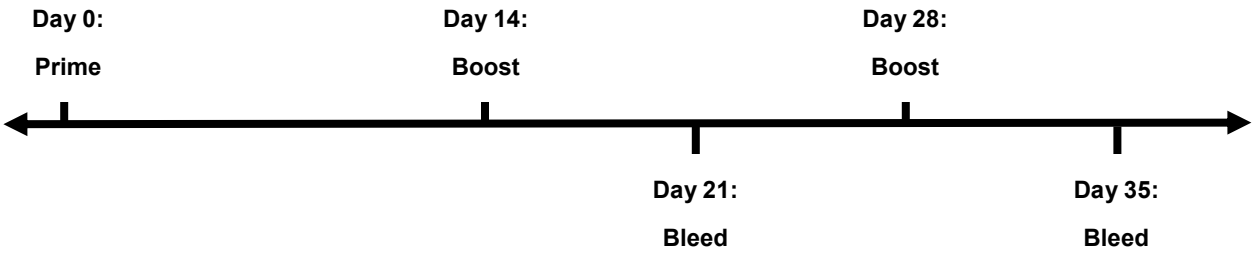


MALDI Mass Spectrum of (*RS*)MLMH-BSA



V. Biochemical and *In Vivo* Procedures

Animals and Vaccinations. 6-8 week old male Swiss Webster mice (n = 6/group) were obtained from Taconic Farms (Germantown, NY). Mice were group-housed in an AAALAC-accredited vivarium containing temperature- and humidity-controlled rooms, with mice kept on a reverse light cycle (lights on: 9PM-9AM). All experiments were performed during the dark phase, generally between 1PM-4PM. General health was monitored by both the scientists and veterinary staff of The Scripps Research Institute, and all studies were performed in compliance with the Scripps Institutional Animal Care and Use Committee (Protocol #08-0127) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. (*S*)-MLMH-TT, (*R*)-MLMH-TT, and (*R/S*)-MLMH-TT immunoconjugates in PBS pH 7.4 (75 μg) were formulated with alum (Alhydrogel®, Invivogen, 75 μL, 10 mg/mL) and CpG ODN 1826 (Eurofins MWG Operon, 2.5 μL, 20 mg/mL). All vaccine injections were conducted intraperitoneally (100-200 μL) on days 0, 14 and 28. No adverse reactions were observed, and all mice maintained a healthy weight throughout the vaccine trial. Blood sampling was performed via retro-orbital bleed in order to collect 50 μL whole blood on days 21 and 35. Whole blood samples were centrifuged at 10,000 rpm for 10 min to isolate serum. The vaccination schedule can be extended with alternating boosting injections and blood sampling.



Enzyme-Linked Immunosorbent Assay (ELISA). PBS pH 7.4 was prepared from 10x powder (Fisher Scientific) and used throughout the assay, except for the washing steps which used ddH_2O. First, half-area, high-binding 96-well microtiter plates (Costar 3690) were coated with 25 ng of (*S*)-MLMH-BSA, (*R*)-MLMH-BSA, or (*RS*)-MLMH-BSA per well and incubated at 37 °C overnight, allowing the liquid to evaporate. Titers were determined by analyzing the serum of a vaccinated mouse versus its corresponding BSA conjugate, e.g. (*S*)MLMH-TT serum antibodies were evaluated against (*S*)MLMH-BSA, (*R*)MLMH-TT serum antibodies against (*R*)MLMH-BSA, and (*R/S*)MLMH-TT serum antibodies against (R/*S*)MLMH-BSA. Serum from mice immunized with TT and CpG ODN 1826 + alum only, the control group, were evaluated against TT. Following blocking with 5% skim milk in PBS pH 7.4 at rt for 1 h, mouse serum was serially diluted 1:2 in 5% skim milk in PBS pH 7.4 across the 12 columns starting at 1:200. After a 2 h incubation at rt, the plates were washed 10x with ddH_2O, then donkey anti-mouse IgG horseradish peroxidase (HRP) secondary (Jackson ImmunoResearch) was added at a 1:10,000 dilution in 5% skim milk

in PBS pH 7.4 and incubated at rt for 2 h. After incubation, 10x washing with ddH_2O was performed and 3,3′,5,5′-tetramethylbenzidine (TMB) substrate (ThermoPierce) was added. TMB was incubated for 10 min at rt, then the mixture was quenched with 2 M aq. H_2SO_4. Plates were incubated at rt for 15 min before their absorbance was read at 450 nm. Midpoint titers were determined using GraphPad PRISM 6. Raw absorbance values were normalized to the highest absorbance value per dilution, and a curve was fit using the log(inhibitor) vs. normalized response – variable slope equation to determine the EC_{50}. The mean and standard error of two EC_{50} values per mouse for a total of n = 6 mice per vaccine were reported as "midpoint titer".

Surface Plasmon Resonance (SPR). The binding IC_{50} for mouse IGs and free methamphetamine (+-METH) was determined by competitive binding assay via surface plasmon resonance using a Biacore 3000 (GE Healthcare) equipped with a research-grade CM3 sensor chip. The ligands, (*S*)MLMH-BSA, (*R*)MLMH-BSA and *(RS)*MLMH-BSA, were immobilized using NHS/EDC coupling. The surface of both flow cells (flow cells 1 and 2) were activated for 7 min with a 1:1 mixture of 0.1 M NHS and 0.1 M EDC at a flow rate of 5 μL/min. The ligands resuspended in 10 mM sodium acetate (pH 4.0) were immobilized at a density of 2,000 RU on flow cell 2; whereas flow cell 2 was immobilized with BSA at the same density to serve as a reference surface. All the surfaces were blocked with a 7 min injection of 1.0 M ethanolamine-HCl (pH 8.5). The mouse IGs were diluted in running buffer (HBS-EP+ buffer) and titrated on both coated flow cells to give a response of ~ 60 RU with 3 min of injection and 2.5 min dissociation at a flow rate of 30 μL/min. The mouse IGs prepared in HBS-EP+ buffer at a determined concentration were incubated with a series of concentrations of (+)-METH for 1 h at room temperature before conducting the competitive binding assay. The concentrations of (+)-METH analyzed were 0.01 – 24300 nM. To collect binding data, the analyte, comprised of the mouse IGs and (+)-METH, was injected over the two flow cells at a flow rate of 30 μL/min at 25 °C for 3 min and was dissociated in buffer for 2.5 min before regeneration. The chip surface was regenerated by injection of 10 mM Gly-HCl (pH 1.5) for 30 seconds before the next round of assay. The response at the end of dissociation phase for each cycle of binding was used to calculate the IC_{50} value for the *(S)*MLMH, (*R*)MLMH and *(RS)*MLMH IGs by GraphPad Prism 6 software. The IC_{50}'s are reported in **Table S1**.

Hyperlocomotion Test. Mice were acclimated for one hour in a plastic cage (10.5 × 19 × 8 inch) with a clear ventilated acrylic top, then quickly removed and injected with either saline, 1, 2, or 4 mg/kg methamphetamine; cages were wiped down with dry paper towels to remove excess debris while mice were being injected. The mice were then returned to the cage for 1.5 h to be recorded and tracked by an overhead camera using ANY-Maze video tracking software (Stoelting Co). Sessions were run during the middle of the dark cycle in a 4.6 × 4.6 m room with a single 60 W upward-directed light source (35-45 lux) and

repeated after a two-day washout period until all mice received all drug doses. Distance travelled (m) (s; 80%-pixel consistency for at least 5 s threshold) was measured.

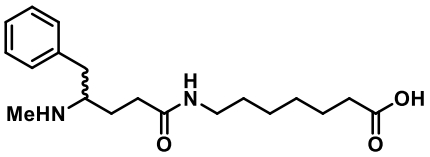
Biodistribution Study. Male Swiss Webster mice (n = 6/group) were injected intraperitoneally with methamphetamine in PBS at a dose of 4 mg/kg then returned to their home cage. Fifteen minutes following the injection, the mice were fully anesthetized with isoflurane. The animals were then rapidly decapitated using a guillotine, the brains were extracted with rongeurs, and trunk blood was collected. The blood was placed on ice for 0.5-2 h, then centrifuged at 10,000 RPM for 10 min to collect the serum. The brain was weighed and added to an equal volume of ice-cold acetate buffer in 1.5 mL conical sample tubes. Brains were then and homogenized using a Tissue Tearor (Biospec; Bartlesville, OK) through mechanical disruption with aluminum beads. Serum and brain samples were stored at –80 °C until extraction and LCMS analysis was performed. On the day of analysis, serum samples were thawed on ice and prepared by spiking 40 µL of serum from each sample into 40 µL of acetonitrile. The mixture was spun at 13,000 rpm for 10 min to remove the precipitated protein, and the supernatant was collected for analysis. 100 µL of the brain homogenate was diluted with 200 µL of MeCN. The mixture was spun at 13,000 rpm for 10 min to remove the precipitated protein, and the supernatant was collected for analysis. Standard curves for serum and brain samples (0, 10, 100, 1000, and 10000 nM) were prepared by spiking known concentrations of methamphetamine into blank tissue samples and a determined concentration of METH-d_5 internal standard. All samples were analyzed using LCMS with an Agilent Poroshell 120 SB-C8 column using H_2O + 0.1% formic acid and MeCN + 0.1% formic acid as the mobile phases. The percentage of MeCN + 0.1% formic acid was linearly increased from 10-95% over a ten-minute run (150 µL/minflow rate), followed by a 10-minute wash phase at 10% MeCN + 0.1% formic acid. Ions with mass corresponding to methamphetamine (m/z 149.7-150.7, ESI+) were extracted and the resulting peaks were integrated. The peak area was plotted against the known methamphetamine concentration for the standards to generate a standard curve, and the concentrations of the experimental samples were interpolated onto this curve. To analyze experimental samples, an aliquot of sample (20 µL blood or homogenized brain supernatant) was added to METH-d_5 (20 µL, 50 ng/mL) and MeCN (20 µL), and then the mixture was vortex mixed and allowed to equilibrate. 1 20 µL of this mixture was analyzed by LCMS. Deuterated and non-deuterated masses were extracted in MassHunter and resulting peaks were integrated. Using the ratio of non-deuterated to deuterated integration values, methamphetamine concentrations were determined via a six-point standard curve.

Lethality Study. Male Swiss Webster mice (n = 6/group) were injected intraperitoneally with 20 mg/kg methamphetamine in pH 7.4 PBS. The mice were visually monitored over a period of 48 hours for lethal events.

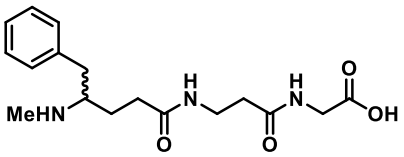
Computational Analyses. Computational and statistical analysis was performed in GraphPad Prism 6 (La Jolla, CA). All values are reported as means ± SEM. For the ELISA assays, absorbance values were normalized to highest absorbance value per sample, and a curve was fit using the log (inhibitor) vs. normalized response – variable slope equation to determine the midpoint titer and standard errors.

VI. Figure S1 Preliminary Linker Designs

A.



B.



C.

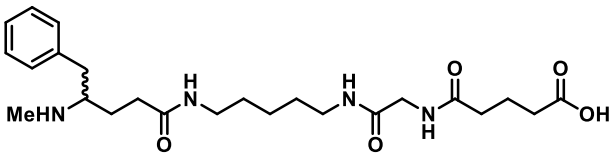
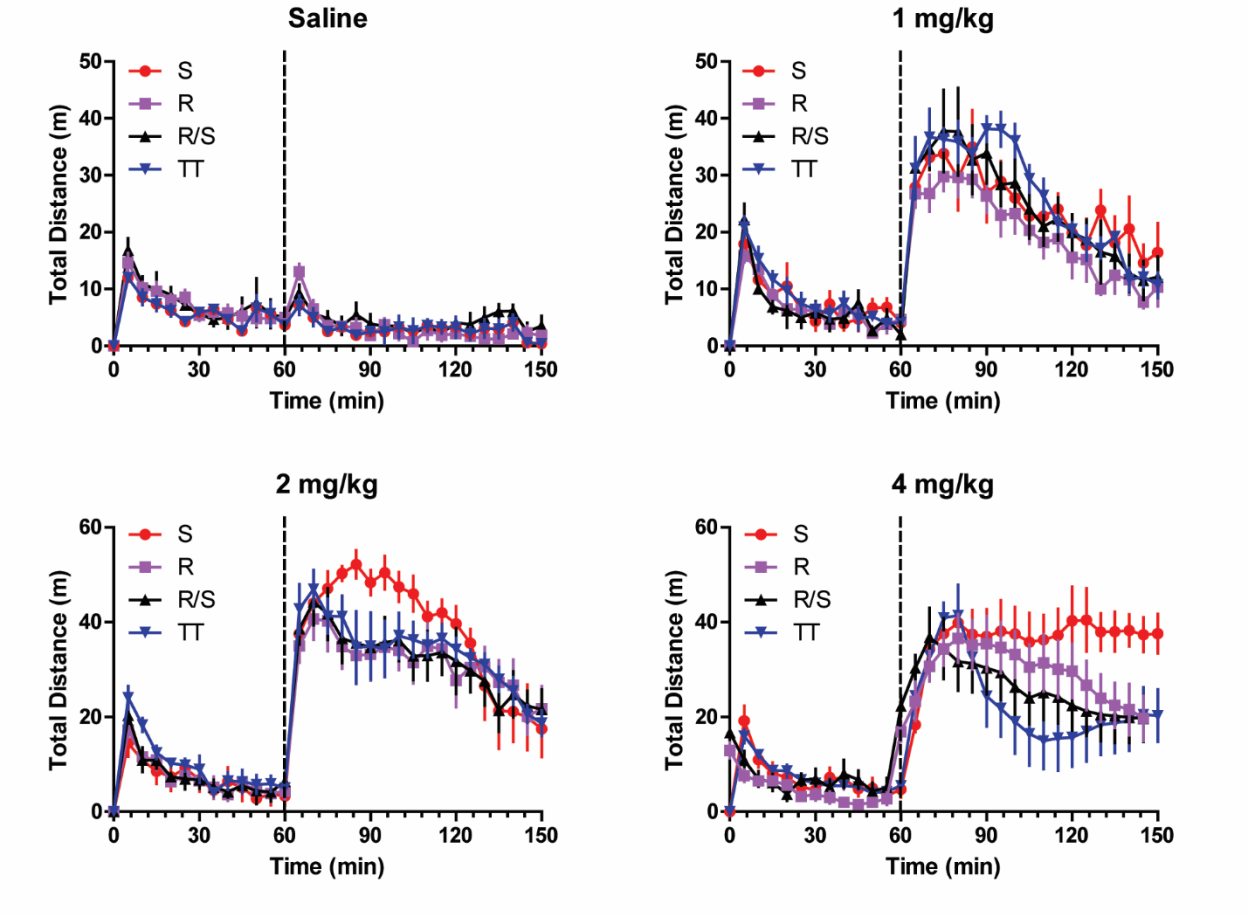


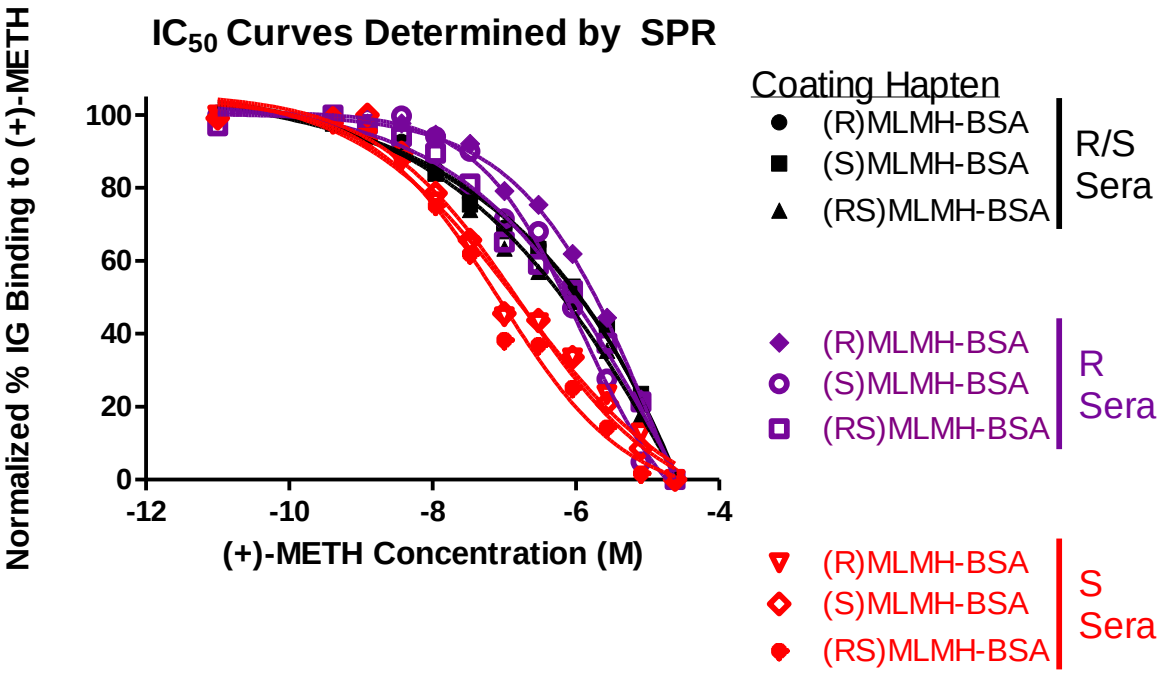
Figure S1 Preliminary Linker Design. Three linker identities were considered in the initial design of the methyl-linked METH hapten (MLMH) series, 7-propionamidoheptanoic acid (**A**), (3-propionamidopropanoyl)glycine] (**B**), and 5-oxo-5-((2-oxo-2-((5-propionamidopentyl)amino)ethyl)amino)pentanoic acid (**C**). Ultimately, 7-propionamidoheptanoic acid (**A**) yielded the optimal series of MLMHs by generating the highest anti-METH antibody titers.

VII. Figure S2 Complete Hyperlocomotion Data (0, 1, 2 and 4 mg/kg METH)

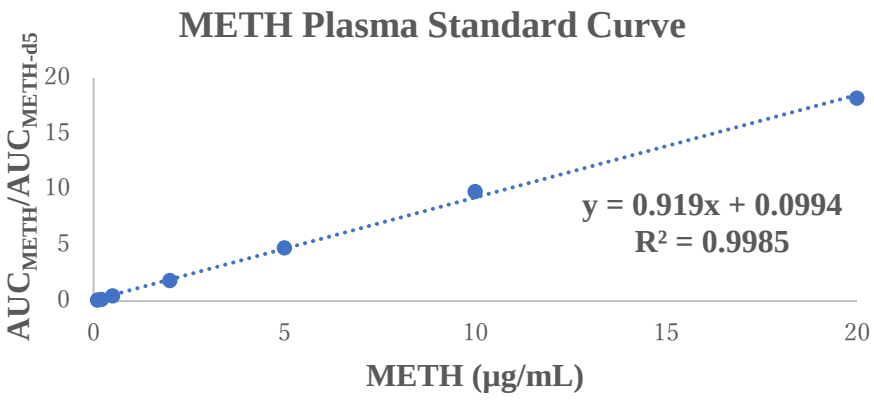


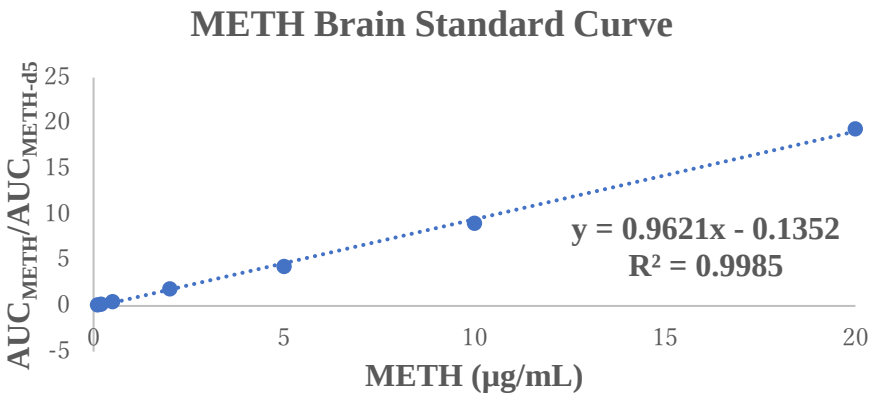
VIII. Anti-METH Antibody IC_{50} Values and Binding Curves by SPR

	(*R/S*)METH-BSA	(*R*)METH-BSA	(*S*)METH-BSA
(*R/S*)METH Sera	1.26 µM	1.89 µM	1.70 µM
(*R*)METH Sera	2.47 µM	5.96 µM	1.96 µM
(*S*)METH Sera	0.09 µM	0.18 µM	0.17 µM



IX. Blood-Brain Distribution Standard Curves





X. Complete Set of MH6 vs. (*S*)MLMH Data