Supporting Information

New Negamycin-Based Potent Readthrough Derivative Effective against TGA-Type Nonsense Mutations

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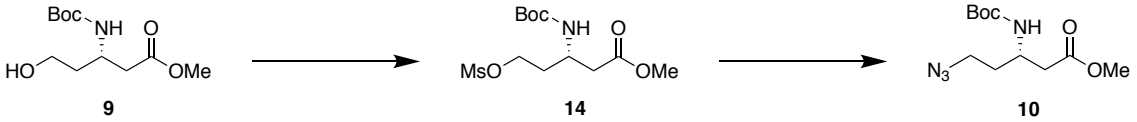
1. General information

All reaction mixtures were magnetically stirred. ^{1}H NMR spectra were measured in $CDCl_3$, D_2O and DMSO-d_6 solutions, and referenced to TMS (0.00 ppm), D_2O (4.79 ppm) and 3-(trimethylsilyl)propionic-2, 2, 3, 3-d_4 acid, s odium salt (TSP-d_4, 0.00 ppm) using Bruker AVANCE-III (400 MHz) and Bruker DPX-400 NMR spectrometers (400 MHz). ^{13}C NMR spectra were measured in $CDCl_3$, D_2O and DMSO-d_6 solutions, and referenced to $CDCl_3$ (77.05 ppm) and TSP-d_4 (0.00 ppm) using Bruker AVANCE-III (400 MHz) and Bruker DPX-400 NMR (400 MHz) spectrophotometers. When peak multiplicities are reported, the following abbreviations are used: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; br s, broad singlet; br d, broad doublet. Melting points were measured with a Yanaco MP-500D melting point apparatuses. Mass spectra were obtained on Waters MICRO MASS LCT-premier. Optical rotations were measured with a JASCO Polarimeter P-1030 at the sodium-D line (589 nm) at the concentrations (*c*, in g 100 mL^{-1}). The measurements were carried out between at 24-25 ºC in a cell with path length (*l*) of 1 dm. Specific rotations $[\alpha]_D$ are given in 10^{-1} deg cm^2 g^{-1}. Column chromatography was performed on silicagel 60N (spherical, neutral) (4-50 μm or 63-210 μm), thin layer chromatography (TLC) was performed on precoated plates (0.25 mm, silica gel Merk Kieselgel $60F_{254}$), and compounds were visualized with UV light, phosphomolybdic acid stain, and ninhydrin stain. Preparative HPLC was performed using a C18 reversed-phase column (250 x 20 mm; YMC-Pack ODS-AM) with a binary solvent system. Solvents and reagents were purchased from Kanto Chemical Co., Inc., Tokyo Chemical Industry Co., Ltd., Kokusan Chemical Co., Ltd., Wako Pure Chemical Industries, Ltd., and Watanabe Chemical Industries, Ltd..

2. Synthesis of derivative 10

(*S*)-Methyl 5-azido-3-(*tert*-butoxycarbonylamino)pentanoate (10)

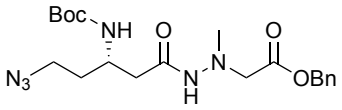


Triethylamine (42.1 μL, 0.303 mmol) and methanesulfonyl chloride (31.1 μL, 0.404 mmol) were added to a solution of **9** (50.0 mg, 0.202 mmol) in CH_2Cl_2 (1mL) at 0 °C. The mixture was then stirred at RT. After stirring for 2 hours at RT, H_2O was added to the mixture at 0 °C and it was extracted with $CHCl_3$. The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with hexane/EtOAc (1:1) to give the mesylate (**14)** (59.7 mg, 0.183 mmol, 90%) as a white solid. $[\alpha]_D^{25} = -16.1$ (*c* = 2.00, $CHCl_3$); mp 52.7-53.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.20 (br d, 1H), 4.35-4.22 (m, 2H), 4.17-4.02 (m, 1H), 3.70 (s, 3H), 3.05 (s, 3H), 2.69-2.53 (m, 2H), 2.07-1.96 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 155.3, 79.5, 66.9, 51.7, 44.2, 38.7, 37.2, 33.6, 28.2 (3 carbons); HRMS (ES+) calcd for $C_{12}H_{24}NO_7S$ $[M+H]^+$ 326.1273, found 326.1267.

Sodium azide (219 mg, 3.36 mmol) was added to a solution of **14** (365 mg, 1.12 mmol) in DMF at RT. The reaction mixture was then stirred for overnight at 50 °C. After the reaction, the temperature was changed to RT and the DMF was removed under reduced pressure. The residue was purified by silica gel flash column chromatography with hexane/EtOAc (1:1) to give compound **10** (278 mg, 1.02 mmol, 91%) as a colorless oil; $[\alpha]_D^{25} = -20.7$ (*c* = 1.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.06 (br d, 1H), 4.10-3.92 (m, 1H), 3.70 (s, 3H), 3.45-3.30 (m, 2H), 2.64-2.50 (m, 2H), 1.83-1.69 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 155.3, 79.6, 51.8, 48.6, 45.3, 38.8, 33.5, 28.3 (3 carbons); HRMS (ES+) calcd for $C_{11}H_{20}N_4O_4Na$ $[M+Na]^+$ 295.1382, found 295.1383.

3. Synthesis of derivatives 11, 12a-y

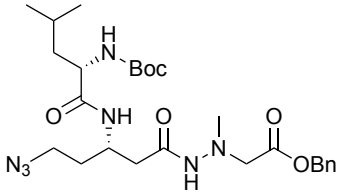
(*S*)-Benzyl 2-{2-[5-azido-3-(*tert*-butoxycarbonylamino)pentanoyl]-1-methylhydrazinyl}acetate (11)



KOH (473 mg, 8.43 mmol) was added to a solution of **10** (765 mg, 2.81 mmol) in MeOH/H_2O (2: 1, 45 mL) at 0 °C. After stirring for 5 h at RT, the solvent was removed under reduced pressure. The residue was acidified to pH 1 by addition of 1M HCl at 0 °C and extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used in the next step without further purification.

$H_2NN(Me)CH_2CO_2Bn$ (920 mg, 4.74 mmol) and HOBt·H_2O (860 mg, 5.62 mmol) were added to a solution containing the above residue in DMF (45 mL) at RT. Et_3N (779 μL, 5.62 mmol) and EDC·HCl (1.08 g, 5.62 mmol) were added to the mixture at 0 ºC. After stirring for overnight at RT, the mixture was poured into 10% citric acid aqueous solution and extracted with EtOAc. The extracts were washed with saturated aqueous $NaHCO_3$ solution, H_2O, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with $CHCl_3$: MeOH =100:1 to give **11** (984 mg, 2.26 mmol, 81%) which was obtained as a colorless oil; $[\alpha]_D^{25}$ = –12.4 (*c* = 0.38, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s) and 7.52 (br d, total 1H), 7.40-7.21 (m, 5H), 5.54 (br d, 1H), 5.17 (s, 2H), 4.11-3.91 (m, 1H), 3.81-3.53 (m, 2H), 3.44-3.30 (m, 2H), 3.00-2.47 (m, 4H), 2.41-2.23 (m, 1H), 2.00-1.68 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 168.9, 155.1, 134.9, 128.2 (2 carbons), 128.0, 127.9 (2 carbons), 78.6, 66.1, 57.3, 48.2, 45.6, 43.5, 33.4, 27.9 (3 carbons); HRMS (ES+) calcd for $C_{20}H_{30}N_6O_5Na$ $[M+Na]^+$ 457.2175, found 457.2175.

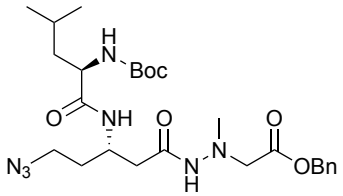
(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-isobutyl-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12a)



4 M HCl/dioxane (5 mL) was added to **11** (35.9 mg, 0.108 mmol) at 0 °C. After stirring for 1.5h

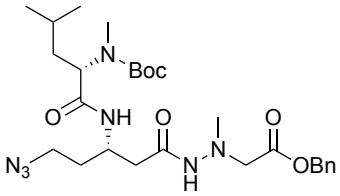
at RT, the solvent was evaporated under reduced pressure. The residue was used in the next step without further purification. Boc-Leu-OH·H_2O (35.9 mg, 0.215 mmol), and HOBt·H_2O (32.9 mg, 0.215 mmol) were added to a solution containing the above residue in DMF (2 mL) at RT. Et_3N (30.2 μL, 0.215 mmol) and EDC·HCl (41.2 mg, 0.215 mmol) were added to the mixture at 0 °C. After stirring for overnight at RT, the mixture was poured into 10% citric acid aqueous solution and extracted with EtOAc. The extracts were washed with saturated aqueous $NaHCO_3$ solution, H_2O, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with $CHCl_3$: MeOH =100:1 to give **12a** (49.5 mg, 82.9 μmol, 2 steps 84%) which was obtained as a yellow oil; $[\alpha]_D^{25}$ = –12.4 (*c* = 0.38, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s) and 7.15-7.04 (m, total 1H), 7.40-7.28 (m, 5H), 5.18 (s, 2H), 4.87 (br s, 1H), 4.40-4.20 (m, 1H), 4.09-3.97 (m, 1H), 3.80-3.50 (m, 2H), 3.40-3.29 (m, 2H), 2.99-2.48 (m, 4H), 2.41-2.25 (m, 1H), 2.00-1.58 (m, 7H), 1.44 (s, 9H), 0.98-0.88 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 170.6, 168.9, 155.6, 135.1, 128.7, 128.5, 128.4, 80.0, 66.8, 57.7, 53.5, 48.6, 45.3, 44.9, 41.4, 38.3, 33.1, 29.7, 28.3, 24.8 (3 carbons), 22.9; HRMS (ES+) calcd for $C_{26}H_{42}N_7O_6$ $[M+H]^+$ 548.3197, found 548.3201.

(7*S*,10*R*)-Benzyl 7-(2-azidoethyl)-10-isobutyl-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12b)



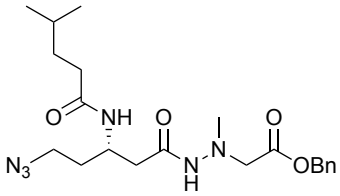
12b was prepared in the same manner described for compound **12a** using **11** (53.4 mg, 0.123 mmol) and Boc-$_D$-Leu-OH·H_2O (61.3 mg, 0.246 mmol). Compound **12b** (56.7 mg, 10.4 μmol, 2 steps 84%) was obtained as a colorless oil; $[\alpha]_D^{25}$ = –7.28 (*c* = 1.61, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s) and 7.23 (br d, total 1H), 7.42-7.30 (m, 5H), 7.14 (br s, 1H), 5.22-5.13 (m, 2H), 5.12-4.93 (m, 1H), 4.43-4.20 (m, 1H), 4.19-3.98 (m, 1H), 3.86-3.50 (m, 2H), 3.43-3.36 (m, 2H), 3.06-2.52 (m, 4H), 2.39 (dd, *J* = 14 and 4.8 Hz) and 2.49 (dd, *J* = 14 and 4.9 Hz, total 1H), 2.00-1.89 (m, 1H), 1.88-1.77 (m, 2H), 1.76-1.57 (m, 2H), 1.43 (s, 9H), 1.02-0.88 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 172.6, 170.8, 155.9, 135.1, 128.7, 128.5, 128.4, 80.2, 66.9, 57.8, 53.8, 48.6, 44.6, 41.7, 38.8, 33.5, 32.3, 28.3 (3 carbons), 24.8, 23.0; HRMS (ES+) calcd for $C_{26}H_{41}N_7O_6$ $[M+Na]^+$ 570.3016, found 570.3032.

(7S,10S)-benzyl 7-(2-azidoethyl)-10-isobutyl-3,11,14,14-tetramethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12c)



12c was prepared in the same manner described for compound **12a** using **11** (52.6 mg, 0.121 mmol) and (S)-2-((*tert*-butoxycarbonyl)(methyl)-amino)-4-methylpentanoic acid (75.5 mg, 0.308 mmol). Compound **12c** (58.0 mg, 10.3 μmol, 2 steps 85%) was obtained as a colorless oil; $[\alpha]_D^{25}$ = –240.7 (*c* = 0.66, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s) and 7.40-7.36 (m, total 6H), 7.05-6.99 (m, 1H), 5.18-5.16 (m, 2H), 4.62-4.52 (m, 1H), 4.34-4.24 (m, 1H), 3.74-3.56 (m, 2H), 3.32-3.31 (m, 2H), 2.78-2.72 (m, 6H), 2.60-2.57 (m, 1H), 2.36-2.32 (m, 1H), 1.80-1.66 (m, 4H), 1.48 (s, 10H), 0.95-0.90 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.5, 168.6, 156.6, 135.1, 128.7 (2 carbons), 128.6, 128.5, 128.4, 80.3, 66.8, 66.7, 58.9, 57.7, 48.7, 45.5, 44.1, 38.5, 36.5, 33.5, 30.3, 28.3 (3 carbons), 23.3, 21.7; HRMS (ES+) calcd for $C_{27}H_{43}N_7O_6$ $[M+Na]^+$584.3173, found 584.3173.

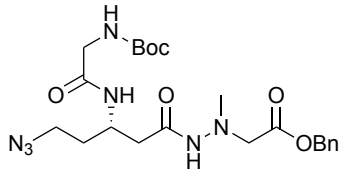
(S)-Benzyl 2-{2-(5-azido-3-(4-methylpentanamido)pentanoyl)-1-methylhydrazinyl}acetate (12d)



4 M HCl/dioxane (2 mL) was added to **11** (54.0 mg, 0.124 mmol) at 0 °C. After stirring for 1.5 h at RT, the solvent was evaporated under reduced pressure. The residue was used in the next step without further purification. 4-methylpentanoic acid (31.4 μL, 0.249 mmol) were added to a solution containing above residue in DMF (2 mL) at RT. Et_3N (34.5 μL, 0.2 mmol) and EDC·HCl (47.7 mg, 0.249 mmol) were added to the mixture at 0 °C. After stirring for overnight at RT, the mixture was poured into 10% citric acid aqueous solution and extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$ solution, H_2O, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with $CHCl_3$:MeOH =100:1 to give **12d** (38.1 mg, 9.07 μmol, 2 steps

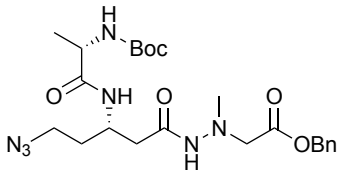
78%) which was obtained as a white solid; $[\alpha]_D^{25}$ = –8.53 (*c* = 1.05, $CHCl_3$); mp. 82.9-83.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s) and 7.46-7.31 (m, total 6H), 6.85-6.67 (m, 1H), 7.20-7.14 (m, 2H), 4.41-4.18 (m 1H), 3.76-3.55 (m, 1H), 3.40-3.28 (m, 2H), 3.04-2.51 (m, 4H), 2.45-2.41 (m, 1H), 2.24-2.11 (m, 2H), 2.00-1.65 (m, 1H), 1.63-1.44 (m, 3H), 1.34-1.23 (m, 1H), 0.94-0.83 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 170.5, 169.3, 135.0, 128.7 (3 carbons), 128.5 (2 carbons), 66.8, 57.7, 48.8, 44.7, 44.2, 43.7, 37.8, 34.9, 34.5, 27.8, 22.3 (2 carbons); HRMS (ES+) calcd for $C_{21}H_{32}N_6O_4$ $[M+H]^+$ 433.2563, found 433.2558.

(*S*)-Benzyl 7-(2-azidoethyl)-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraaza pentadecan-1-oate (12e)



12e was prepared in the same manner as described for compound **12a** using **11** (46.2 mg, 0.106 mmol) and Boc-Gly-OH (37.3 mg, 0.213 mmol). Compound **12e** (42.7 mg, 89.7 μmol, 2 steps 84%) was obtained as a yellow oil; $[\alpha]_D^{25}$ = –7.64 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s) and 7.47-7.25 (m, total 6H), 7.24 (br d, 1H), 5.29 (m, 1H), 5.18 (s, 2H), 4.45-4.20 (m, 1H), 3.85-3.46 (m, 4H), 3.43-3.23 (m, 2H), 3.09-2.56 (m, 4H), 2.40 (dd, *J* = 15 and 4.8 Hz) and 2.11 (dd, *J* = 15 and 5.6 Hz, total 1H), 2.00-1.69 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5,170.6, 169.4, 156.2, 135.1, 128.7 (4 carbons), 128.5, 80.4, 66.8, 58.8, 48.7, 45.0, 44.3, 44.0, 38.3, 33.5, 28.3; HRMS (ES+) calcd for $C_{22}H_{34}N_7O_6$ $[M+H]^+$ 492.2571, found 492.2576.

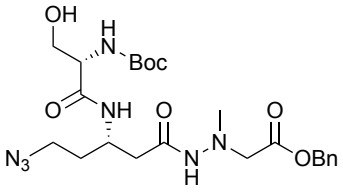
(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-3,10,14,14-tetramethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12f)



12f was prepared in the same manner as described for compound **12a** using **11** (41.7 mg, 96.0 μmol) and Boc-Ala-OH (36.2 mg, 0.192 mmol). Compound **12f** (37.8 mg, 74.8 μmol, 2 steps 78%) was obtained as a yellow oil; $[\alpha]_D^{25}$ = –17.5 (*c* = 1.09, $CHCl_3$); ^{1}H NMR (400

MHz, $CDCl_3$) δ 7.96 (s) and 7.47-7.25 (m, total 6H), 7.24 (br d, 1H), 5.40-5.10 (m, 3H), 4.40-4.18 (m, 1H), 4.09 (br s, 1H), 3.81-3.50 (m, 2H), 3.34 (t, J = 6.9 Hz, 2H), 3.04-2.50 (m, 4H), 2.42-2.28 (m, 1H), 2.00-1.69 (m, 2H), 1.44 (s, 9H), 1.33 (t, J = 5.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 170.5, 168.9, 135.0, 128.7, 128.6, 128.4 (2 carbons) 80.0, 66.7, 57.7, 50.4, 45.0, 44.1, 43.8, 33.5, 33.0, 28.9; HRMS (ES+) calcd for $C_{23}H_{36}N_7O_6$ $[M+H]^+$ 506.2727, found 506.2720.

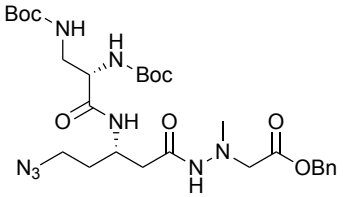
(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-(hydroxymethyl)-3,15,15-trimethyl5,9,13-trioxo-14-oxa-3,4,8,12-tetraazahexadecan-1-oate (12g)



12g was prepared in the same manner as described for compound **12a** using **11** (55.7 mg, 0.108 mmol) and Boc-Ser-OH·H_2O (52.6 mg, 0.256 mmol). Compound **12g** (32.3 mg, 63.4 μmol, 2 steps 49%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –38.3 (c = 0.31, $CHCl_3$); mp 52.7-53.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s) and 7.64-7.43 (m, total 1H), 7.36-7.27 (m, 5H), 7.25-7.16 (m, 1H), 5.61 (br d 1H) 5.17 (s, 2H), 4.47-4.37 (m, 1H), 4.32-3.24 (m, 1H), 4.20-4.06 (m, 2H), 3.92-3.75 (m, 2H), 3.66-3.62 (m, 2H), 3.38-3.30 (m, 2H), 2.85-2.71 (m, 3H), 2.43-2.25 (m, 1H), 2.09-1.69 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 171.0, 170.8, 155.8, 135.1, 128.7, 128.6, 128.5 (2 carbons), 128.4, 80.1, 66.8, 63.2, 63.0, 57.5, 48.5, 48.3, 45.2, 44.3, 44.0, 28.3 (3 carbons); HRMS (ES+) calcd for $C_{23}H_{35}N_7O_7Na$ $[M+Na]^+$ 544.2496, found 544.2496.

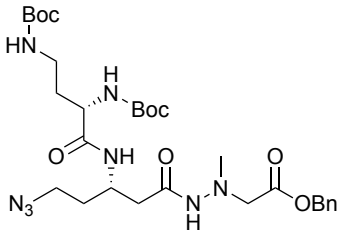
(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-(*tert*-butoxycarbonylamino)-3,15,15-trimethyl-5,9,13-trioxo-14-oxa-3,4,8,12-tetraazahexadecan-1-oate (12h)



12h was prepared in the same manner as described for compound **12a** using **11** (52.7 mg, 0.121 mmol) and Boc-Dap(Boc)-OH (73.7 mg, 0.242 mmol). Compound **12h** (55.1 mg, 88.8 μmol, 2 steps 73%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –25.1 (*c* = 1.93, $CHCl_3$); mp 103.1-103.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s) and 7.49-7.27 (m, total 7H), 5.91 (br s, 1H), 5.34 (br s, 1H), 5.17 (s, 2H), 4.40-4.23 (m, 1H), 4.22-4.11 (m, 1H), 3.91-3.40 (m, 4H), 3.39-3.23 (m, 2H), 3.05-2.55 (m, 4H), 2.42-2.26 (m, 1H), 1.87-1.76 (m, 2H), 1.44 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.1, 168.5, 156.9, 155.8, 135.1, 128.6, 128.5, 128.4 (2 carbons), 80.0 (2 carbons), 66.7, 59.1, 57.6, 48.6, 44.3, 43.9, 42.4, 38.6, 32.8, 28.3 (6 carbons); HRMS (ES+) calcd for $C_{28}H_{45}N_8O_8$ $[M+H]^+$ 621.3360, found 621.3353.

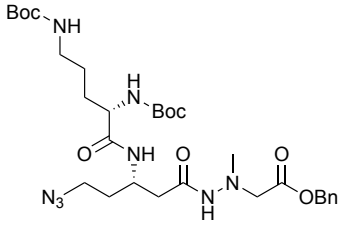
(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-(*tert*-butoxycarbonylamino)-3,16,16-trimethyl-5,9,14-trioxo-15-oxa-3,4,8,13-tetraazahepadecan-1-oate (12i)



12i was prepared in the same manner as described for compound **12a** using **11** (61.2 mg, 0.141 mmol) and Boc-Dab(Boc)-OH (89.7 mg, 0.282 mmol). Compound **12i** (79.3 mg, 0.125 mmol, 2 steps 89%) was obtained as a colorless oil.

$[\alpha]_D^{25}$ = –19.2 (*c* = 1.99, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s) and 7.42-7.27 (m, total 6H), 7.69-7.50 (m, 1H), 5.54 (br s, 1H), 5.26 (br s, 1H), 5.18 (s, 2H), 4.47-4.23 (m, 1H), 4.10 (br s, 1H), 3.83-3.52 (m, 2H), 3.46-3.24 (m, 3H), 3.08 (br s, 1H), 2.88-2.55 (m, 4H), 2.37 (d, *J* = 5.7 Hz, 1H), 1.99-1.72 (m, 4H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 170.5, 168.7, 155.6, 135.0, 128.6 (2 carbons), 128.4 (2 carbons), 128.3, 79.7 (2 carbons), 66.6, 59.1, 57.6, 52.1, 48.5, 44.9, 44.0, 38.7, 37.0, 33.5, 28.2 (6 carbons); HRMS (ES+) calcd for $C_{29}H_{47}N_8O_8$ $[M+H]^+$ 635.3517, found 635.3534.

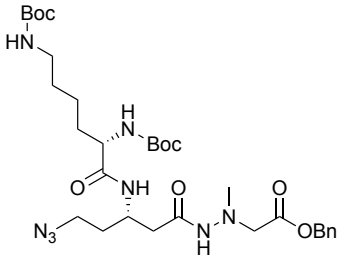
(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-(*tert*-butoxycarbonylamino)-3,17,17-trimethyl-5,9,15-trioxo-16-oxa-3,4,8,14-tetraazaoctadecan-1-oate (12j)



12j was prepared in the same manner as described for compound **12a** using **11** (51.4 mg, 0.118 mmol) and Boc-Orn(Boc)-OH (78.6 mg, 0.237 mmol). Compound **12j** (74.3 mg, 0.115 mmol, 2 steps 97%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –8.63 (*c* = 1.02, $CHCl_3$); mp 82.6-83.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s) and 7.60-7.27 (m, total 6H), 7.25-7.09 (m, 1H), 5.39-5.06 (m, 3H), 5.03-4.71 (m, 1H), 4.53-4.19 (m, 1H), 4.09 (br s, 1H), 3.80-3.43 (m, 2H), 3.42-3.25 (m, 2H), 3.24-2.89 (m, 2H), 2.87-2.55 (m, 4H), 2.46-2.10 (m, 2H), 1.97-1.74 (m, 2H), 1.69-1.52 (m, 3H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 170.5, 168.8, 156.3, 135.1, 128.7 (2 carbons), 128.4 (2 carbons), 128.3, 80.0, 79.2, 66.8, 57.7, 54.1, 48.5, 45.1, 44.1, 39.9, 38.7, 33.1, 29.9, 28.4 (6 carbons), 26.2; HRMS (ES+) calcd for $C_{30}H_{49}N_8O_8$ $[M+H]^+$ 649.3673, found 649.3679.

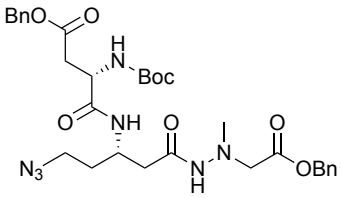
(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-(*tert*-butoxycarbonylamino)-3,18,18-trimethyl-5,9,16-trioxo-16-oxa-3,4,8,15-tetraazanonadecan-1-oate (12k)



12k was prepared in the same manner as described for compound **12a** using **11** (50.9 mg, 0.117 mmol) and Boc-Lys(Boc)-OH (35.9 mg, 0.234 mmol). Compound **12k** (72.3 mg, 0.109 mmol, 2 steps 93%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = –10.9 (*c* = 1.56, $CHCl_3$); mp 70.0-70.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s) and 7.29-7.08 (m, total 1H), 7.42-7.30 (m, 5H), 5.31 (br d, 1H), 5.18 (s, 2H), 4.97-4.71 (m, 1H), 4.48-4.20 (m, 1H), 4.10-3.90 (m, 1H), 3.81-3.54 (m, 2H), 3.43-3.28 (m, 2H), 3.20-3.00 (m, 3H), 2.91-2.55 (m, 4H), 2.42-2.47 (m, 1H), 1.98-1.37 (m, 26H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 170.4, 169.0, 156.1, 135.1, 128.6 (2 carbons), 128.56, 128.4, 128.3, 79.9, 79.0, 66.7, 57.7, 54.6, 48.5, 44.9, 44.1, 39.9, 29.6, 28.3 (6 carbons), 22.6, 14.0; HRMS (ES+) calcd for $C_{31}H_{51}N_8O_8$ $[M+H]^+$ 663.3830, found 663.3823.

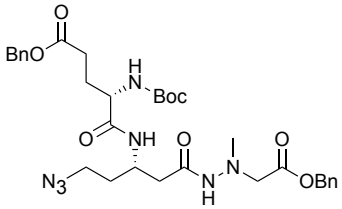
(7*S*,10*S*)-Benzyl-7-(2-azidoethyl)-10-[2-(benzyloxy)-2-oxoethyl]-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12l)



12l was prepared in the same manner as described for compound **12a** using **11** (52.0 mg, 0.120 mmol) and Boc-Asp(OBn)-OH (74.7 mg, 0.231 mmol). Compound **12l** (59.5 mg, 93.1 μmol, 2 steps 78%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –4.57 (*c* = 1.91, $CHCl_3$); mp 62.2-62.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s) and 7.57-7.25 (m, total 11H), 5.65-5.57 (m, 1H), 5.17-5.06 (m, 5H), 4.51-4.44 (m, 1H), 4.40-4.20 (m, 1H), 3.83-3.49 (m, 2H), 3.38-3.27 (m, 2H), 3.14-2.99 (m, 1H), 2.97-2.50 (m, 5H), 2.38-2.25 (m, 1H), 1.99-1.70 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 171.6, 170.4, 168.6, 155.4, 135.4, 135.0, 128.5 (4 carbons), 128.5 (4 carbons), 128.4, 128.2, 80.4, 66.7, 58.9, 57.5, 50.8, 48.4, 44.1, 38.2, 36.1, 32.9, 28.2; HRMS (ES+) calcd for $C_{31}H_{42}N_7O_8$ $[M+H]^+$ 640.3095, found 640.3099.

(7*S*,10*S*)-Benzyl-7-(2-azidoethyl)-10-[3-(benzyloxy)-3-oxopropyl]-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12m)

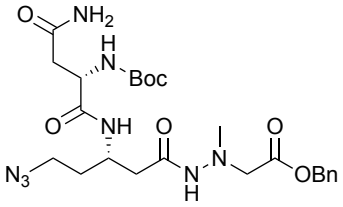


12m was prepared in the same manner as described for compound **12a** using **11** (55.7 mg, 0.129 mmol) and Boc-Glu(OBn)-OH (81.8 mg, 0.242 mmol). Compound **12m** (78.6 mg, 0.124 mmol, 2 steps 96%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –14.6 (*c* = 1.00, $CHCl_3$); mp 107.5-108.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s) and 7.40-7.27 (m, total 11H), 7.25 (br s, 1H), 5.40 (br s, 1H), 5.17 (s, 2H), 5.09 (s, 2H), 4.40-4.19 (m, 1H), 4.18-4.02 (m, 1H), 3.80-3.49 (m, 2H), 3.38-3.19 (m, 2H), 3.07-2.83 (m, 4H), 2.58- 2.29 (m, 3H), 2.10-2.04 (m, 1H), 2.00-1.70 (m, 3H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 171.3, 170.5, 168.7, 155.4, 135.6, 135.1, 128.6 (2 carbons), 128.53, 128.50, 128.4, 128.3, 128.2 (2 carbons), 128.1, 79.9, 66.5, 66.4, 57.6, 54.0, 48.6, 48.2, 44.1, 33.4 33.0, 30.4, 28.2 (3 carbons), 27.8; HRMS (ES+) calcd for $C_{32}H_{44}N_7O_8$ $[M+H]^+$ 654.3251, found 654.3262.

(7*S*,10*S*)-Benzyl 10-(2-amino-2-oxoethyl)-7-(2-azidoethyl)-3,14,14-trimethyl-5,9,12-

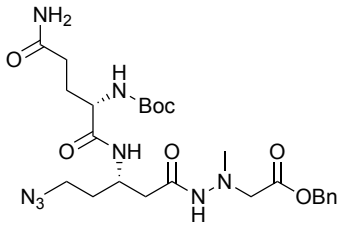
trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12n)



12n was prepared in the same manner as described for compound **12a** using **11** (50.0 mg, 0.115 mmol) and Boc-Asn-OH (53.5 mg, 0.230 mmol). Compound **12n** (41.0 mg, 74.8 μmol, 2 steps 65%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –1.41 (*c* = 0.80, $CHCl_3$); mp 157.9-158.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s) and 7.54-7.42 (m, total 1H), 7.39-7.30 (m, 5H), 6.22 (br s, 1H), 6.05 (br s, 1H), 5.65 (s, 1H), 4.47-4.32 (m, 1H), 4.31-4.27 (m, 1H), 3.82-3.47 (m, 2H), 3.46-3.24 (m, 2H), 2.97-2.82 (m, 2H), 2.82-2.68 (m, 3H), 2.67-2.60 (m, 2H), 2.40-2.22 (m, 2H), 2.00-1.68 (m, 3H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 171.4, 169.3, 168.0, 155.0, 135.9, 128.4, 128.3, 128.1, 128.0, 127.9, 78.1, 65.6, 65.4, 59.6, 57.9, 51.6, 47.7, 47.5, 43.7, 42.9, 36.9, 32.8, 28.0; HRMS (ES+) calcd for $C_{24}H_{37}N_8O_7$ $[M+H]^+$ 549.2785, found 549.2785.

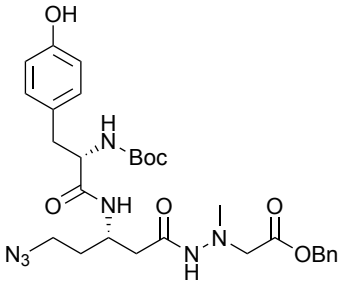
(7*S*,10*S*)-Benzyl 10-(3-amino-2-oxoethyl)-7-(2-azidoethyl)-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12o)



12o was prepared in the same manner as described for compound **12a** using **11** (50.9 mg, 0.117 mmol) and Boc-Gln-OH (57.7 mg, 0.234 mmol). Compound **12o** (54.9 mg, 97.6 μmol, 2 steps 83%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –6.98 (*c* = 1.97, $CHCl_3$); mp 147.5-148.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s) and 7.84-7.78 (m, total 1H), 7.72-7.43 (m, 1H), 7.42-7.25 (m, 5H), 7.02-6.86 (m, 1H), 6.43-6.31 (m, 1H), 5.60 (br d, 1H), 5.18 (s, 2H), 4.43-4.24 (m, 1H), 4.12-4.02 (m, 1H), 3.80-3.53 (m, 2H), 3.42-3.29 (m, 2H), 2.99-2.49 (m, 4H), 2.39-2.18 (m, 3H), 2.12-1.70 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 171.6, 170.6, 169.0, 156.0, 135.2, 128.7 (2 carbons), 128.6, 128.5 (2 carbons), 128.4, 80.0, 66.7, 57.7, 53.7, 48.3, 45.1, 44.2, 33.7, 31.7, 31.6, 28.3 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{39}N_8O_7$ $[M+H]^+$ 563.2942, found 563.2930.

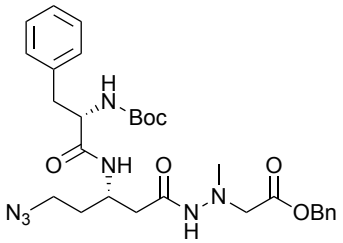
(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-(4-hydroxybenzyl)-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12p)



12p was prepared in the same manner as described for compound **12a** using **11** (42.8 mg, 98.6 μmol) and Boc-Tyr-OH (55.4 mg, 0.197 mmol). Compound **12p** (49.5 mg, 82.9 μmol, 2 steps 84%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –7.00 (*c* = 1.11, $CHCl_3$); mp 68.6-69.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s) and 7.42-7.25 (m, total 6H), 7.09-6.91 (m, 3H), 6.76 (d, *J* = 9.6 Hz, 2H), 5.22-5.09 (m, 3H), 4.32-4.11 (m, 2H), 3.83-3.43 (m, 2H), 3.33-3.20 (m, 2H), 3.02-2.82 (m, 2H), 2.79-2.61 (m, 4H), 2.42-2.00 (m, 1H), 1.90-1.58 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.7, 169.1, 155.5, 135.1, 130.4 (2 carbons) 128.7 (3 carbons), 128.4, 115.7 (2 carbons), 80.2, 66.9, 66.8, 57.7, 48.6, 48.4, 44.7, 44.1, 37.8, 32.8, 28.3 (3 carbons); HRMS (ES+) calcd for $C_{29}H_{39}N_7O_7Na$ $[M+Na]^+$ 620.2809, found 620.2811.

(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-benzyl-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12q)

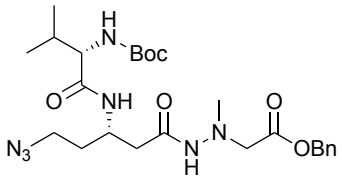


12q was prepared in the same manner as described for compound **12a** using **11** (53.6 mg, 0.123 mmol) and Boc-Phe-OH (65.4 mg, 0.246 mmol). Compound **12q** (68.5 mg, 0.118 mmol, 2 steps 96%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –10.0 (*c* = 1.00, $CHCl_3$); mp 84.8-85.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (s) and 7.42-7.13 (m, total 11H), 7.09-6.94 (m, 1H), 5.23-4.96 (m, 3H), 4.37-4.13 (m, 2H), 3.82-3.48 (m, 2H), 3.34-3.20 (m, 2H), 3.17-2.97 (m, 2H), 2.96-2.44 (m, 3H), 2.39-2.02 (m, 2H), 1.94-1.58 (m,

2H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 170.6, 168.7, 155.4, 136.7, 129.4 (2 carbons), 128.7 (4 carbons), 128.6, 128.5, 128.4 (2 carbons), 80.0, 66.8, 58.8, 57.6, 48.6, 45.2, 44.7, 43.7, 33.2, 32.8, 29.7, 28.3 (3 carbons); HRMS (ES+) calcd for $C_{29}H_{40}N_7O_6$ $[M+H]^+$ 582.3040, found 582.3030.

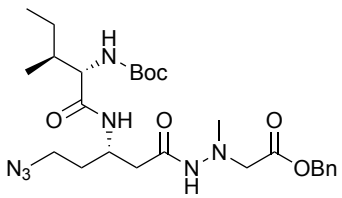
(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-isopropyl-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12r)



12r was prepared in the same manner as described for compound **12a** using **11** (44.3 mg, 0.102 mmol) and Boc-Val-OH (44.3 mg, 0.204 mmol). Compound **12r** (53.2 mg, 99.8 μmol, 2 steps 98%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –19.3 (*c* = 1.00, $CHCl_3$); mp 74.5-75.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s) and 7.47-7.25 (m, total 6H), 7.25-7.09 (m, 1H), 5.22-5.08 (m, 3H), 4.43-4.23 (m, 1H), 3.95-3.83 (m, 1H), 3.82-3.71 (m, 2H), 3.40-3.29 (m, 2H), 3.10-2.50 (m, 4H), 2.45-2.29 (m, 1H), 2.19-2.02 (m, 1H), 2.01-1.84 (m, 1H), 1.83-1.69 (m, 1H), 1.44 (s, 9H), 1.02-0.82 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 170.6, 169.0, 135.1, 128.7 (3 carbons), 128.5, 128.4, 79.7, 66.8, 60.1, 57.7, 48.7, 44.9, 44.1, 43.8, 38.0, 35.6, 30.9, 28.3, 19.3; HRMS (ES+) calcd for $C_{25}H_{40}N_7O_6$ $[M+H]^+$ 534.3040, found 534.3029.

(7*S*,10*S*)-Benzyl 7-(2-azidoethyl)-10-*sec*-butyl-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12s)

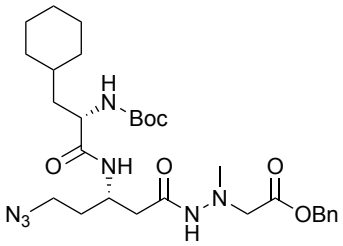


12s was prepared in the same manner as described for compound **12a** using **11** (52.8 mg, 0.122 mmol) and Boc-Ile-OH (56.2 mg, 0.243 mmol). Compound **12s** (58.5 mg, 10.7 μmol, 2 steps 88%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –16.4 (*c* = 1.00, $CHCl_3$); mp 147.1-147.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s) and 7.44-7.27 (m, total 6H), 7.25-7.12 (m, 1H), 5.22-5.09 (m, 3H), 4.43-4.23 (m, 1H), 3.93-3.82 (m,

1H), 3.78-3.50 (m, 2H), 3.40-3.30 (m, 2H), 3.05-2.52 (m, 4H), 2.42-2.26 (m, 1H), 2.20-1.70 (m, 4H), 1.44 (s, 9H), 1.18-1.05 (m, 1H), 0.99-0.83 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 171.4, 170.6, 155.9, 135.1, 128.6, 128.5, 128.4, 127.7 (2 carbons), 79.8, 66.7, 59.5, 57.7, 48.7, 44.9, 44.1, 38.2, 37.1, 37.0, 28.3 (3 carbons), 15.6, 11.4; HRMS (ES+) calcd for $C_{26}H_{42}N_7O_6$ $[M+H]^+$ 548.3197, found 548.3201.

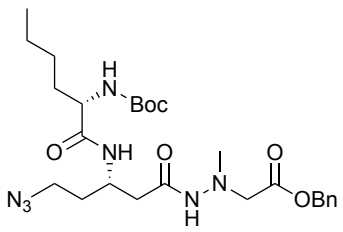
(7S,10S)-Benzyl 7-(2-azidoethyl)-10-(cyclohexylmethyl)-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12t)



12t was prepared in the same manner as described for compound **12a** using **11** (77.1 mg, 0.177 mmol) and (S)-2-((*tert*-butoxycarbonyl)amino)-3-cyclohexylpropanoic acid (96.2 mg, 0.355 mmol). Compound **12t** (89.1 mg, 15.1 μmol, 2 steps 86%) was obtained as a clear oil.

$[\alpha]_D^{25}$ = –19.3 (*c* = 1.67, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s) and 7.55-7.43 (m, total 1H), 7.42-7.31 (m, 5H), 7.31-7.15 (m, 1H), 5.20-5.17 (m, 2H), 5.00-4.97 (m, 1H), 4.41-4.18 (m, 1H), 4.12-4.00 (m, 1H), 3.83-3.49 (m, 3H), 3.40-3.28 (m, 2H), 3.05-2.91 (m, 1H), 2.81-2.77 (s, 2H), 2.68-2.65 (m, 1H) 2.43-2.28 (br d, 1H) 1.99-1.57 (m, 9H), 1.44 (s, 9H), 1.35-1.11 (m, 4H), 1.03-0.79 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 172.7, 170.5, 155.7, 135.1, 128.7 (2 carbons), 128.6 (2 carbons), 128.5, 79.9, 66.7, 57.7, 52.8, 48.6, 48.5, 45.7, 44.9, 44.1, 40.0, 33.6, 33.5, 32.5, 28.3 (3 carbons), 26.4, 26.2, 26.1; HRMS (ES+) calcd for $C_{29}H_{46}N_7O_6$ $[M+H]^+$ 588.3510, found 588.3488.

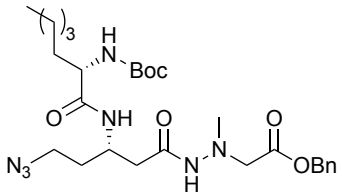
(7S,10S)-Benzyl 7-(2-azidoethyl)-10-butyl-3,14,14-trimethyl-5,9,12-trioxo-13-oxa-3,4,8,11-tetraazapentadecan-1-oate (12u)



12u was prepared in the same manner as described for compound **12a** using **11** (54.9 mg, 0.126 mmol) and Boc-Nle-OH (65.1 mg, 0.252 mmol). Compound **12u** (58.2 mg, 10.6 μmol, 2 steps 84%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –17.2 (*c* = 3.56, $CHCl_3$); mp 84.7-85.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s) and 7.44-7.29 (m, total 6H), 7.21-7.06 (m, 1H), 5.23-5.11 (m, 2H), 5.10-4.96 (m, 1H), 4.40-4.19 (m, 1H), 4.06-3.89 (m, 1H), 3.80-3.49 (m, 1H), 3.40-3.20 (m, 2H), 3.04-2.74 (m, 2H), 2.73-2.48 (m, 2H), 2.43-2.41 (m, 1H), 2.00-1.88 (m, 1H), 1.83-1.67 (m, 2H), 1.60-1.50 (m, 1H), 1.38 (s, 9H), 1.38-1.16 (m, 5H), 0.93-0.82 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 172.0, 170.6, 168.9, 135.1, 128.7 (3 carbons), 128.5, 128.4, 66.8, 57.7, 55.0, 48.7, 48.6, 44.9, 44.2, 43.8, 33.5, 33.0, 28.3, 27.7 (2 carbons), 22.4 (2 carbons), 13.9; HRMS (ES+) calcd for $C_{26}H_{42}N_7O_6$ $[M+H]^+$ 548.3197, found 548.3207.

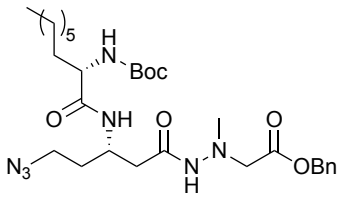
Benzyl N-((S)-5-azido-3-((S)-2-((*tert*-butoxycarbonyl)amino)heptanamido)pentanamido)-N-methylglycinate (12v)



12v was prepared in the same manner as described for compound **12a** using **11** (120 mg, 0.276 mmol) and (S)-2-((*tert*-butoxycarbonyl)amino)heptanoic acid (176 mg, 0.717 mmol). Compound **12v** (169 mg, 30.0 μmol, 2 steps 84%) was obtained as a white solid.

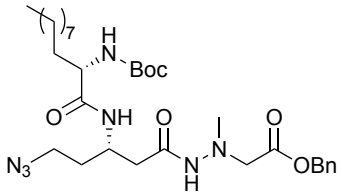
$[\alpha]_D^{25}$ = –20.3 (*c* = 1.28, $CHCl_3$); mp 78.9-79.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s) and 7.69 (m, total 2H), 7.32-7.40 (m, 5H), 5.16-5.30 (m, 2H), 4.25-4.37 (m, 1H), 4.00 (t, *J* = 7.1 Hz, 1H), 3.58-3.75 (m, 2H), 3.32-3.35 (m, 2H), 2.78 (d, *J* = 5.6 Hz, 1H), 2.71 (s, 1H), 2.36 (d, *J* = 5.6 Hz, 1H), 1.72-1.93 (m, 2H), 1.53-1.59 (m, 1H), 1.44 (d, *J* = 3.9 Hz, 9H), 1.27-1.34 (m, 6H), 0.85-0.88 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 172.4, 169.1, 155.7, 135.2, 128.7 (2 carbons), 128.6, 128.4 (2 carbons), 79.7, 66.7, 59.2, 57.7, 48.6, 45.0, 44.1, 38.4, 35.8, 31.4 (2 carbons), 28.3 (3 carbons), 22.5, 22.4, 14.0; HRMS (ES+) calcd for $C_{27}H_{44}N_7O_6$ $[M+H]^+$ 562.3353, found 562.3358.

Benzyl N-((S)-5-azido-3-((S)-2-((*tert*-butoxycarbonyl)amino)nonanamido)pentanamido)-N-methylglycinate (12w)



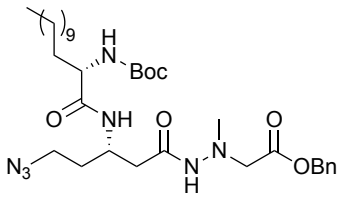
12w was prepared in the same manner as described for compound **12a** using **11** (500 mg, 1.15 mmol) and (S)-2-((*tert*-butoxycarbonyl)amino)nonanoic acid (629 mg, 2.36 mmol). Compound **12w** (529 mg, 898 μmol, 2 steps 78%) was obtained as a white solid. $[\alpha]_D^{25}$ = –19.9 (*c* = 0.82, $CHCl_3$); mp 68.9-71.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s) and 7.36-7.40 (m, total 6H), 7.11-7.21 (m, 1H), 5.19 (dd, *J* = 16.9 and 7.2 Hz, 2H), 4.96 (d, *J* = 7.3 Hz, 1H), 4.20-4.38 (m, 1H), 3.92-3.98 (m, 1H), 3.51-3.75 (m, 2H), 3.29-3.40 (m, 2H), 2.55-3.02 (m, 4H), 2.33 (m, 1H), 1.84-2.00 (m, 1H), 1.67-1.82 (m, 3H), 1.58 (d, *J* = 13.6 Hz, 1H), 1.43 (d, *J* = 5.8 Hz, 9H), 1.27 (d, *J* = 10.8 Hz, 10H), 0.86 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 170.6, 168.9, 155.6, 135.1, 128.7 (2 carbons), 128.6, 128.5, 128.4, 79.9, 66.7, 58.9, 57.7, 55.0, 48.7, 44.9, 44.1, 38.2, 33.5, 31.7, 29.3, 29.0, 28.3 (3 carbons), 25.6, 22.6, 14.1; HRMS (ES+) calcd for $C_{29}H_{48}N_7O_6$ $[M+H]^+$ 590.3666, found 590.3676.

Benzyl N-((S)-5-azido-3-((S)-2-((*tert*-butoxycarbonyl)amino)undecanamido)pentanamido)-N-methylglycinate (12x)



12x was prepared in the same manner as described for compound **12a** using **11** (303 mg, 0.698 mmol) and (S)-2-((*tert*-butoxycarbonyl)amino)heptanoic acid (437 mg, 1.45 mmol). Compound **12x** (391 mg, 633 μmol, 2 steps 91%) was obtained as a white solid. $[\alpha]_D^{25}$ = –15.9 (*c* = 0.97, $CHCl_3$); mp 66.4-67.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s) and 7.89 (s, total 1H), 7.36-7.38 (m, 5H), 5.52-5.59 (m, 1H), 5.28 (dd, *J* = 18.1 and 11.0 Hz, 1H), 5.16-5.18 (m, 2H), 5.02 (s, 1H), 4.23-4.35 (m, 1H), 4.02-4.08 (m, 1H), 3.52-3.78 (m, 2H), 3.32-3.35 (m, 2H), 2.71-2.96 (m, 3H), 2.27-2.54 (m, 3H), 1.70-2.03 (m, 3H), 1.42-1.59 (m, 8H), 1.27-1.30 (m, 8H), 0.89 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 170.6, 168.9, 155.5, 135.1, 128.7 (3 carbons), 128.5, 128.4, 80.1, 66.8, 57.7, 54.4, 48.6, 45.0, 44.2, 38.1, 35.3, 32.9, 31.7, 29.7, 29.5, 29.2, 29.0, 28.3, 27.4, 22.6, 14.1; HRMS (ES+) calcd for $C_{31}H_{50}N_7O_6$ $[M+H]^+$ 616.3813, found 616.3823.

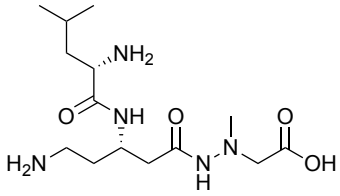
Benzyl *N*-((*S*)-5-azido-3-((*S*)-2-((*tert*-butoxycarbonyl)amino)tridecanamido)pentanamido)-*N*-methylglycinate (12y)



12y was prepared in the same manner as described for compound **12a** using **11** (84.0 mg, 0.251 mmol) and (*S*)-2-((*tert*-butoxycarbonyl)amino)tridecanoic acid (99.1mg, 0.301 mmol). Compound **12y** (108 mg, 168 μmol, 2 steps 67 %) was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s) and 7.92 (s, total 1H), 7.30-7.58 (m, 5H), 7.10-7.20 (m, 1H), 5.02-5.22 (m, 3H), 4.20-4.35 (m, 1H), 3.91-3.99 (m, 1H), 3.52-3.79 (m, 2H), 3.29-3.40 (m, 2H), 2.71-2.77 (m, 3H), 2.26-2.42 (m, 1H), 1.69-1.99 (m, 3H), 1.55-1.59 (m, 1H), 1.44 (s, 8H), 1.24 (s, 15H), 0.72-0.89 (m, 3H); HRMS (ES+) calcd for $C_{33}H_{55}N_7O_6Na$ $[M+Na]^+$ 668.4112, found 668.4113

4. Synthesis of derivatives 13a-y

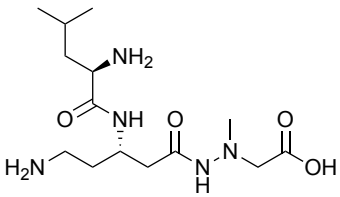
2-{2-[(*S*)-5-Amino-3-((*S*)-2-amino-4-methylpentanamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13a)



10 % Pd/C (2.5 mg) was added to a solution of **12a** (25.4 mg, 46.4 μmol) in MeOH (2 mL) at RT. The resulting reaction mixture was subjected to three cycles of vacuum followed by flushing with H_2 before stirring for 1 h under an atmosphere of H_2. The mixture was filtered through a pad of Celite® with MeOH and concentrated removed under reduced pressure. The residue was used in the next step without further purification. 4 M HCl/dioxane (2 mL) was added to the residue at 0 °C. After stirring for 1 h at RT, the solvent was evaporated under reduced pressure.The residue was purified by preparative HPLC to give compound **13a** (8.09 mg, 14.5 μmol, 2 steps 31%) which was obtained as a yellow solid.

$[\alpha]_D^{25}$ = 0.78 (*c* = 0.78, H_2O); mp 160.6-161.5 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.34-4.32 (m, 1H), 3.98-3.96 (m, 1H), 3.65-3.56 (m, 2H), 3.05-3.01 (m, 2H), 2.67 (s, 3H), 2.55-2.37 (m, 2H), 1.96-1.65 (m, 5H), 0.96-0.95 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 176.7, 173.4, 172.8, 61.8, 54.8, 47.6, 47.0, 43.1, 41.5, 39.4, 34.6, 26.8, 24.8, 23.7; HRMS (ES+) calcd for $C_{14}H_{30}N_5O_4$ $[M+H]^+$ 332.2298, found 332.2293.

2-{2-[(*S*)-5-Amino-3-((*R*)-2-amino-4-methylpentanamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13b)

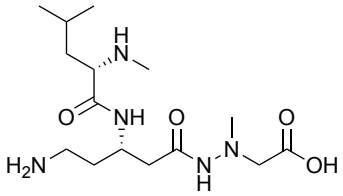


13b was prepared in the same manner as described for compound **13a** using **12b** (24.1 mg, 44.1 μmol). Compound **13b** (9.40 mg, 16.8 μmol, 2 steps 38%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = –23.0 (*c* = 0.48, H_2O); mp 130.9-131.2 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.36-4.22 (m,

1H), 3.94 (t, *J* = 6.1 Hz, 1H), 3.67 (s, 2H), 3.06-2.89 (m, 2H), 2.69 (s, 3H), 2.51 (dd, *J* = 15 and 5.5 Hz, 1H), 2.42 (dd, *J* = 15 and 8.5 Hz, 1H), 2.02-1.82 (m, 2H), 1.79-1.58 (m, 3H), 1.02-0.86 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 175.7, 173.4, 173.0, 61.5, 54.9, 48.0, 47.3, 42.9, 41.7, 41.3, 39.5, 26.9, 24.8, 23.7; HRMS (ES+) calcd for $C_{14}H_{30}N_5O_4$ $[M+H]^+$ 332.2298, found 332.2298.

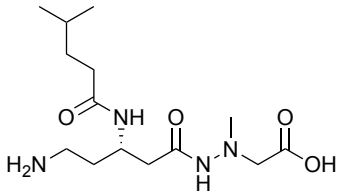
2-{2-((S)-5-Amino-3-((S)-4-methyl-2-(methylamino)pentanamido)pentanoyl)-1-methylhydrazinyl}acetic acid (13c)



13c was prepared in the same manner as described for compound **13a** using **12c** (44.8 mg, 10.3 μmol). Compound **13c** (9.40 mg, 16.8 μmol, 2 steps 38%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = –0.84 (*c* = 0.13, H_2O); mp 131.2-131.7 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.40-4.29 (m, 1H), 3.80 (t, *J* = 7.1 Hz, 1H), 3.59 (s, 2H), 3.01 (t, *J* = 8.0 Hz, 2H), 2.70-2.63 (m, 3H), 2.52 (dd, *J* = 15.3 and 5.0 Hz, 1H), 2.40 (dd, *J* = 15.3 and 8.8 Hz, 1H), 2.03-1.86 (m, 2H), 1.77-1.66 (m, 2H), 1.65-1.51 (m, 1H), 0.93 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, D_2O) δ 176.0, 172.7, 171.8, 63.5, 61.4, 47.8, 47.1, 42.1, 41.2, 39.5, 34.5 (2 carbons), 26.9, 24.7, 24.2; HRMS (ES+) calcd for $C_{15}H_{32}N_5O_4$ $[M+H]^+$ 346.2454, found 346.2454.

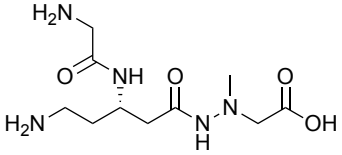
(S)-2-{2-(5-Amino-3-(4-methylpentanamido)pentanoyl)-1-methylhydrazinyl}acetic acid (13d)



10 % Pd/C (3.7 mg) was added to a solution of **12d** (36.9 mg, 87.8 μmol) in MeOH (2 mL) at RT. The resulting reaction mixture was subjected to three cycles of vacuum followed by flush with H_2 before stirring for 1 h under an atmosphere of H_2. The mixture was filtered through a pad of Celite® with MeOH and concentrated removed under reduced pressure. The residue was purified by preparative HPLC to give **13d** (10.2 mg, 23.8 μmol, 2 steps 27%) as a white solid.

$[\alpha]_D^{25}$ = –18.8 (*c* = 0.49, H_2O); mp 102.7-103.3 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.32-4.22 (m, 1H), 3.61 (s, 1H), 3.07-2.93 (m, 2H), 2.67 (s, 3H), 2.49 (dd, *J* = 14.5 and 5.2 Hz, 1H), 2.34 (dd, *J* = 14.5 and 8.9 Hz, 1H), 2.26 (t, J = 7.7 Hz, 2H), 2.00-1.78 (m, 2H), 1.58-1.42 (m, 3H), 0.88 (d, *J* = 6.3 Hz, 6H); ^{13}C NMR (100 MHz, D_2O) δ 180.4, 177.5, 173.1, 62.2, 47.3, 46.9, 42.0, 39.5, 37.4, 36.8, 34.7, 30.1, 24.5, 24.4; HRMS (ES+) calcd for $C_{14}H_{29}N_4O_4$ $[M+H]^+$ 317.2189, found 317.2188.

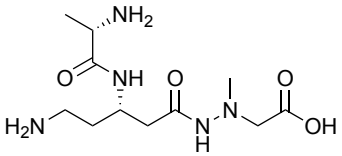
(*S*)-2-{2-[5-Amino-3-(2-aminoacetamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13e)



13e was prepared in the same manner as described for compound **13a** using **12e** (24.7 mg, 51.9 μmol). Compound **13e** (7.50 mg, 14.9 μmol, 2 steps 29%) was obtained as a green solid.

$[\alpha]_D^{25}$ = –3.49 (*c* = 0.30, H_2O); mp 160.0-160.8 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.45-4.26 (m, 1H), 3.80 (d, *J* = 4.1 Hz, 2H), 3.64 (s, 2H), 3.11-2.97 (m, 2H), 2.68 (s, 3H), 2.50 (dd, *J* = 15 and 5.3 Hz, 1H), 2.38 (dd, *J* = 15 and 8.7 Hz, 1H), 2.04-1.78 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 172.3, 170.0, 166.8, 58.4, 44.8, 44.2, 40.3, 38.6, 36.4, 31.6; HRMS (ES+) calcd for $C_{10}H_{22}N_5O_4$ $[M+H]^+$ 276.1672, found 276.1675.

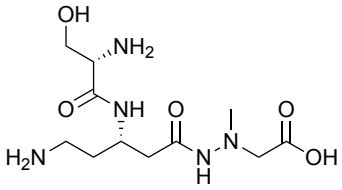
2-{2-[(*S*)-5-Amino-3-((*S*)-2-aminopropanamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13f)



13f was prepared in the same manner as described for compound **13a** using **12f** (29.8 mg, 58.9 μmol). Compound **13f** (11.2 mg, 21.6 μmol, 2 steps 38%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = –2.02 (*c* = 1.01, H_2O); mp 145.6-155.6 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.39-4.21 (m, 1H), 4.10-3.97 (m, 1H), 3.65 (br s 2H), 3.12-2.91 (m, 2H), 2.75-2.62 (m, 3H), 2.52 (dd, *J* = 4.9 Hz and 18.9 Hz, 1H), (dd, *J* = 9.1 Hz and 14.9 Hz, 1H), 2.02-1.77 (m, 2H), 1.60-1.36 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 175.6, 173.6, 172.9, 61.3, 52.0, 47.7, 47.2, 41.6, 39.4, 34.6, 19.7; HRMS (ES+) calcd for $C_{11}H_{24}N_5O_4$ $[M+H]^+$ 290.1828, found 290.1819.

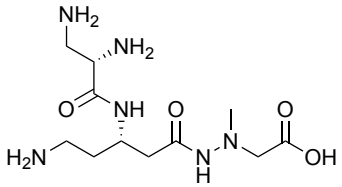
2-{(S)-5-Amino-3-((S)-2-amino-3-hydroxypropanamido)pentanoyl}-1-methylhydrazinecarboxylicacid (13g)



13g was prepared in the same manner as described for compound **13a** using **12g** (31.3 mg, 63.4 μmol). Compound **13g** (15.0 mg, 28.0 μmol, 2 steps 44%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = –4.27 (*c* = 0.65, H_2O); mp 137.5-138.3 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.33-4.20 (m, 1H), 4.11-4.13 (m, 1H), 3.97-3.80 (m, 2H), 3.67 (s, 2H), 3.05-2.95 (m, 2H), 2.69 (s, 3H), 2.49 (dd, *J* = 15 and 5.4 Hz, 1H), 2.38 (dd, *J* = 15 and 8.5 Hz, 1H), 1.98-1.78 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 176.0, 173.0, 170.6, 63.2, 61.4, 57.6, 48.0, 47.1, 41.5, 39.4, 34.5; HRMS (ES+) calcd for $C_{11}H_{24}N_5O_5$ $[M+H]^+$ 306.1777, found 306.1776.

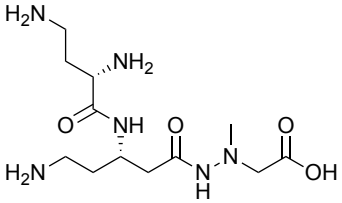
2-{2-[(*S*)-5-Amino-3-((*S*)-2,3-diaminopropanamido)pentanoyl]-1-methylhydrazinyl}acetic-acid (13h)



13h was prepared in the same manner as described for compound **13a** using **12h** (30.0 mg, 48.4 μmol). Compound **13h** (9.20 mg, 14.2 μmol, 2 steps 29%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = 0.76 (*c* = 4.32, H_2O); mp 150.9-151.3 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.36 (t, *J* = 5.8 Hz, 1H), 4.33-4.20 (m, 1H), 3.62 (s, 2H), 3.62-3.48 (m, 2H), 3.15-2.97 (m, 2H), 2.66 (s, 3H), 2.56 (dd, *J* = 15 and 4.3 Hz, 1H), 2.38 (dd, *J* = 15 and 9.5 Hz, 1H), 2.05-1.80 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 165.9 (2 carbons), 58.4, 50.7, 45.3, 44.3, 39.5, 38.0, 37.9, 36.3, 31.6; HRMS (ES+) calcd for $C_{11}H_{25}N_6O_4$ $[M+H]^+$ 305.1937, found 305.1930.

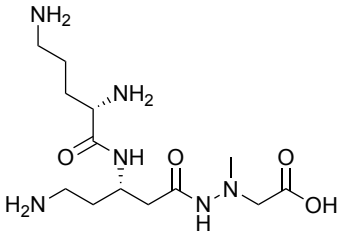
2-{2-[(*S*)-5-Amino-3-((*S*)-2,4-diaminobutanamido)pentanoyl]-1-methylhydrazinyl}acetic-acid (13i)



13i was prepared in the same manner as described for compound **13a** using **12i** (25.9 mg, 40.8 μmol). Compound **13i** (4.96 mg, 7.51 μmol, 2 steps 18%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = –0.72 (*c* = 3.78, H_2O); mp 136.5-137.3 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.43-4.20 (m, 1H), 4.10-4.00 (m, 1H), 3.51 (m, 2H), 3.17-2.97 (m, 4H), 2.70-2.52 (m, 4H), 2.45-2.32 (m, 1H), 2.31-2.15 (m, 2H), 2.02-1.79 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 174.8, 172.6, 171.3, 61.4, 53.8, 47.7, 47.6, 41.0, 39.4, 38.1, 34.7, 31.4; HRMS (ES+) calcd for $C_{12}H_{27}N_6O_4$ $[M+H]^+$ 319.2094, found 319.2085.

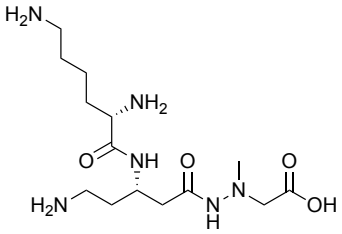
2-{2-[(*S*)-5-Amino-3-((*S*)-2,4-diaminohexamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13j)



13j was prepared in the same manner as described for compound **13a** using **12j** (44.6 mg, 68.8 μmol). Compound **13j** (3.35 mg, 4.97 μmol, 2 steps 7.2%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = 4.26 (*c* = 1.03, H_2O); mp 82.6-83.6 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.43-4.20 (m, 1H), 4.03-3.93 (m, 1H), 3.65 (s, 2H), 3.10-2.92 (m, 4H), 2.72-2.48 (m, 4H), 2.37 (dd, *J* = 15 and 9.5 Hz, 1H), 2.02-1.58 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 176.0, 174.9, 172.7, 172.1, 61.4, 55.6, 47.7, 47.5, 41.7, 41.2, 39.4, 34.7, 30.9, 25.2; HRMS (ES+) calcd for $C_{13}H_{29}N_6O_4$ $[M+H]^+$ 333.2250, found 333.2238.

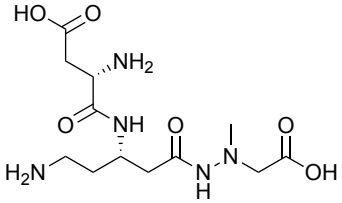
2-{2-[(*S*)-5-Amino-3-((*S*)-2,5-diaminoheptamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13k)



13k was prepared in the same manner as described for compound **13a** using **12k** (57.8 mg, 87.3 μmol). Compound **13k** (11.9 mg, 17.3 μmol, 2 steps 20%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = 7.69 (*c* = 0.57, H_2O); mp 143.2-144.0 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.46-4.26 (m, 1H), 3.98 (t, *J* = 6.4 Hz, 1H), 3.62 (s, 2H), 3.12-2.97 (m, 4H), 2.65 (s, 3H), 2.56 (dd, *J* = 15 and 4.5 Hz, 1H), 3.36 (dd, *J* = 15 and 9.9 Hz, 1H), 2.04-1.79 (m, 4H), 1.78-1.61 (m, 2H), 1.52-1.32 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 176.5, 172.9, 172.5, 62.1, 56.0, 47.8, 47.2, 41.9, 41.5, 39.4, 34.9, 33.4, 29.3, 23.9; HRMS (ES+) calcd for $C_{14}H_{31}N_6O_4$ $[M+H]^+$ 347.2407, found 347.2408.

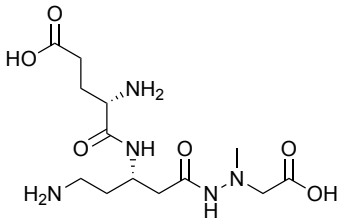
(*S*)-3-Amino-4-{(*S*)-5-amino-1-[2-(carboxymethyl)-2-methylhydrazinyl]-1-oxopentan-3-ylamino}-4-oxobutanoic acid (13l)



13l was prepared in the same manner as described for compound **13a** using **12l** (34.4 mg, 53.8 μmol). Compound **13l** (9.56 mg, 17.0 μmol, 2 steps 32%) was obtained as a green solid.

$[\alpha]_D^{25}$ = 0.46 (*c* = 0.43, H_2O); mp 128.1-129.0 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.35-4.22 (m, 2H), 3.64 (s, 2H), 3.10-2.92 (m, 4H), 2.67 (s, 3H), 2.49 (dd, *J* = 15 and 5.3 Hz, 1H), 2.40 (dd, *J* = 15 and 8.5 Hz, 1H), 2.05-1.82 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 172.8, 172.6, 169.8, 168.4, 58.3, 49.5, 45.0, 44.1, 38.3, 36.4, 34.9, 31.4; HRMS (ES+) calcd for $C_{12}H_{24}N_5O_6$ $[M+H]^+$ 334.1727, found 334.1724.

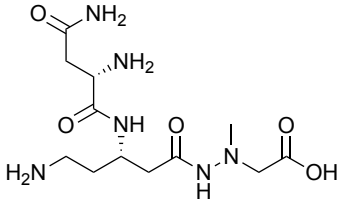
(*S*)-4-Amino-5-{(*S*)-5-amino-1-[2-(carboxymethyl)-2-methylhydrazinyl]-1-oxopentan-3-ylamino}-5-oxobutanoic acid (13m)



13m was prepared in the same manner as described for compound **13a** using **12m** (44.8 mg, 70.5 μmol). Compound **13m** (18.6 mg, 32.3 μmol, 2 steps 46%) was obtained as a green solid.

$[\alpha]_D^{25}$ = –5.10 (*c* = 0.80, H_2O); mp 152.9-153.2 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.45-4.22 (m, 1H), 4.03 (t, *J* = 6.6 Hz, 1H), 3.65 (s, 2H), 3.12-2.98 (m, 2H), 2.74-2.48 (m, 6H), 2.42 (dd, *J* = 15 and 8.7 Hz, 1H), 2.20-2.07 (m, 2H), 2.02-1.79 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 176.1, 172.8, 170.0, 169.3, 58.4, 52.6, 44.7, 44.3, 38.3, 36.5, 31.7, 29.1, 26.1; HRMS (ES+) calcd for $C_{13}H_{26}N_5O_6$ $[M+H]^+$ 348.1883, found 348.1873.

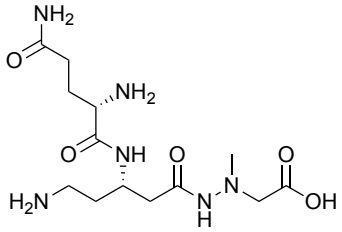
2-{2-[(*S*)-5-Amino-3-((*S*)-2,4-diamino-4-oxobutamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13n)



13n was prepared in the same manner as described for compound **13a** using **12n** (34.6 mg, 63.1 μmol). Compound **13n** (10.6 mg, 19.0 μmol, 2 steps 30%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = –2.15 (*c* = 0.43, H_2O); mp 157.6-158.3 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.28-4.15 (m, 2H), 3.56 (s, 2H), 3.04-2.76 (m, 4H), 2.59 (s, 3H), 2.42 (dd, *J* = 15 and 5.3 Hz, 1H), 2.31 (dd, *J* = 15 and 8.4 Hz, 1H), 1.96-1.73 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 175.7, 175.6, 172.9, 171.6, 61.4, 52.8, 47.9, 47.3, 41.3, 39.4, 38.0, 34.4; HRMS (ES+) calcd for $C_{12}H_{25}N_6O_5$ $[M+H]^+$ 333.1886, found 333.1880.

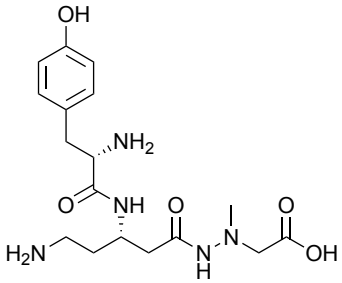
2-{2-[(*S*)-5-Amino-3-((*S*)-2,5-diamino-5-oxopentamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13o)



13o was prepared in the same manner as described for compound **13a** using **12o** (48.2 mg, 85.7 μmol). Compound **13o** (20.5 mg, 35.7 μmol, 2 steps 42%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = 1.02 (*c* = 0.89, H_2O); mp 129.8-130.7 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.43-4.18 (m, 1H), 4.02 (t, *J* = 6.4 Hz, 1H), 3.66 (s, 2H), 3.14-2.93 (m, 2H), 2.68 (s, 3H), 2.52 (dd, *J* = 15 and 5.1 Hz) and 2.46-2.37 (m, total 4H), 2.23-2.05 (m, 2H), 2.03-1.80 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 176.9, 172.6, 169.9, 169.3, 58.4, 52.7, 44.8, 44.4, 38.4, 36.5, 31.8, 30.2, 26.7; HRMS(ES+) calcd for $C_{13}H_{27}N_6O_5$ $[M+H]^+$ 347.2043, found 347.2040.

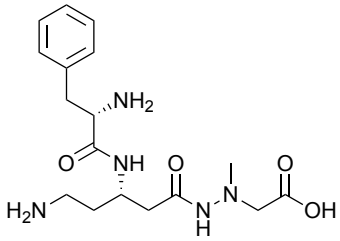
2-{2-[(*S*)-5-Amino-3-((*S*)-2-amino-3-(4-hydroxyphenyl)propanamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13p)



13p was prepared in the same manner as described for compound **13a** using **12p** (30.0 mg, 50.2 μmol). Compound **13p** (5.12 mg, 8.95 μmol, 2 steps 16%) was obtained as a white solid.

$[\alpha]_D^{25}$ = 0.28 (*c* = 0.24, H_2O); mp 162.7-163.4 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.15 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 4.27-4.08 (m, 1H), 4.12 (t, *J* = 7.5 Hz, 1H), 3.49 (s, 2H), 3.07 (d, *J* = 7.5 Hz, 2H), 3.05-2.90 (m, 2H), 2.62 (s, 3H), 2.30-2.15 (m, 2H), 1.98-1.69 (m, 2H); HRMS (ES+) calcd for $C_{17}H_{28}N_5O_5$ $[M+H]^+$ 382.2090, found 382.2089.

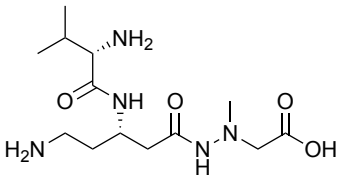
2-{2-[(*S*)-5-Amino-3-((*S*)-2-amino-3-phenylpropanamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13q)



13q was prepared in the same manner as described for compound **13a** using **12q** (42.4 mg, 72.9 μmol). Compound **13q** (10.4 mg, 17.5 μmol, 2 steps 24%) was obtained as a white solid.

$[\alpha]_D^{25}$ = 12.0 (*c* = 0.50, H_2O); mp 134.5-135.5 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.50-7.37 (m, 3H), 7.36-7.23 (m, 2H), 4.32-4.12 (m, 2H), 3.56 (s, 2H), 3.22-3.08 (m, 2H), 3.04-2.88 (m, 2H), 2.64 (s, 3H), 2.24 (dd, *J* = 14 and 6.8 Hz, 1H), 2.16 (dd, *J* = 16 and 7.2 Hz, 1H), 1.98-1.70 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.6, 169.7, 169.0, 133.7, 129.4, 129.2 (2 carbons), 128.1 (2 carbons), 58.7, 54.4, 44.5, 44.0, 38.1, 37.0, 36.4, 31.2; HRMS (ES+) calcd for $C_{17}H_{28}N_5O_4$ $[M+H]^+$ 366.2141, found 366.2141.

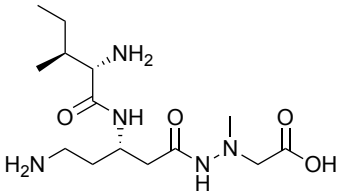
2-{2-[(*S*)-5-Amino-3-((*S*)-2-amino-3-methylbutanamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13r)



13r was prepared in the same manner as described for compound **13a** using **12r** (33.4 mg, 62.6 μmol). Compound **13r** (4.07 mg, 7.47 μmol, 2 steps 12%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = 0.29 (*c* = 0.38, H_2O); mp 171.2-171.4 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.48-4.27 (m, 1H), 3.84-3.74 (m, 1H), 3.62 (s, 2H), 3.14-2.97 (m, 2H), 2.67 (s, 3H), 2.59-2.38 (m, 2H), 2.25-2.10 (m, 1H), 2.03-1.80 (m, 2H), 1.09-0.94 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 188.6, 169.8, 169.0, 58.6, 58.3, 44.5, 43.9, 38.3, 36.4, 31.7, 29.9, 17.6, 16.8; HRMS (ES+) calcd for $C_{13}H_{28}N_5O_4$ $[M+H]^+$ 318.2141, found 318.2142.

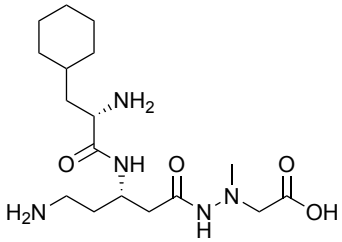
2-{2-[(*S*)-5-Amino-3-((2*S*,3*S*)-2-amino-4-methylpentanamido)pentanoyl]-1-methylhydrazinyl}acetic acid (13s)



13s was prepared in the same manner as described for compound **13a** using **12s** (32.0 mg, 58.5 μmol). Compound **13s** (13.4 mg, 23.9 μmol, 2 steps 41%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = 7.09 (*c* = 0.56, H_2O); mp 159.9-160.5 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.36 (br s, 1H), 3.86-3.78 (m, 1H), 3.66 (s, 2H), 3.11-2.94 (m, 2H), 2.69 (s, 3H), 2.52 (dd, *J* = 15 and 4.5 Hz, 1H), 2.43 (dd, *J* = 16 and 8.7 Hz, 1H), 2.03-1.80 (m, 3H), 1.54-1.38 (m, 1H), 1.26-1.10 (m, 1H), 0.98-0.83 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 172.6, 169.7, 169.0, 58.3, 57.8, 44.4, 44.1, 38.2, 36.5, 36.4, 31.7, 24.3, 14.2, 10.6; HRMS (ES+) calcd for $C_{14}H_{30}N_5O_4$ $[M+H]^+$ 332.2298, found 332.2291.

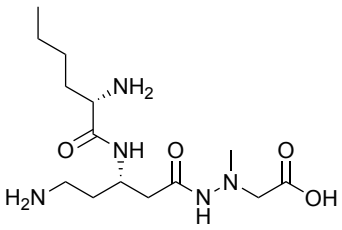
2-(2-((S)-5-Amino-3-{(S)-2-amino-3-cyclohexylpropanamido)pentanoyl)-1-methylhydrazinyl}acetic acid (13t)



13t was prepared in the same manner as described for compound **13a** using **12t** (46.9 mg, 81.5 μmol). Compound **13t** (13.7 mg, 22.9 μmol, 2 steps 28%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –3.16 (*c* = 0.41, H_2O); mp 159.5-159.9 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.35-4.23 (m, 1H), 4.03-3.93 (m, 1H), 3.06-2.96 (m, 2H), 2.65 (s, 3H), 2.49 (dd, *J* = 15.1 and 5.1 Hz, 1H), 2.36 (dd, *J* = 15.2 and 8.8 Hz, 1H), 2.00-1.78 (m, 2H), 1.76-1.53 (m, 7H), 1.37-1.25 (m, 1H), 1.25-1.04 (m, 3H) 1.02-0.78 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 175.9, 173.6, 172.9, 61.4, 54.1, 47.6, 47.1, 41.8, 41.4, 39.4, 36.0, 35.9, 34.6, 34.5, 28.7, 28.5, 28.3; HRMS (ES+) calcd for $C_{17}H_{34}N_5O_4$ $[M+H]^+$ 372.2611, found 372.2600.

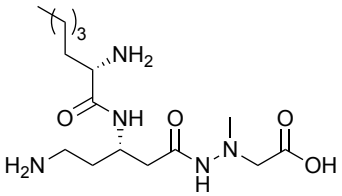
2-{2-((S)-5-Amino-3-((S)-2-aminohexanamido)pentanoyl)-1-methylhydrazinyl}acetic acid (13u)



13u was prepared in the same manner as described for compound **13a** using **12u** (33.0 mg, 60.3 μmol). Compound **13u** (9.37 mg, 16.7 μmol, 2 steps 28%) was obtained as a yellow solid.

$[\alpha]_D^{25}$ = –2.27 (*c* = 0.62, H_2O); mp 124.6-125.5 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.39-4.28 (m, 1H), 3.67 (d, *J* = 3.7, 2H), 3.11-2.97 (m, 2H), 2.73-2.67 (m, 3H), 2.56 (dd, *J* = 15.1 and 5.1 Hz, 1H), 2.44 (dd, *J* = 15.1 and 8.8 Hz, 1H), 2.04-1.93 (m, 1H), 1.92-1.78 (m, 3H), 1.41-1.28 (m, 4H), 0.90 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 172.8, 170.0, 169.8, 58.3, 53.3, 44.6, 44.1, 38.4, 36.5, 31.7, 30.7, 26.2, 21.6, 12.9; HRMS (ES+) calcd for $C_{14}H_{30}N_5O_4$ $[M+H]^+$332.2298, found 332.2285.

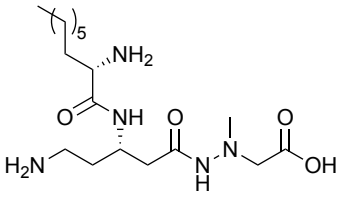
2-{2-((S)-5-Amino-3-((S)-2-aminoheptanamido)pentanoyl)-1-methylhydrazinyl}acetic acid (13v)



13v was prepared in the same manner as described for compound **13a** using **12v** (149 mg, 266 μmol). Compound **13v** (58.8 mg, 170 μmol, 2 steps 64%) was obtained as a white solid.

$[\alpha]_D^{25}$ = 1.59 (*c* = 1.96, H_2O); mp 152.2-152.9 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.31 (s, 1H), 3.91-3.95 (m, 1H), 3.37 (s, 2H), 3.00-3.06 (m, 2H), 2.38-2.60 (m, 5H), 1.81-2.01 (m, 4H), 1.31 (s, 6H), 0.86 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 175.6, 173.0, 172.7, 61.4, 56.3, 47.5, 47.2, 41.4, 39.4, 34.6, 33.9, 33.4, 26.7, 24.5, 16.1; HRMS (ES+) calcd for $C_{15}H_{31}N_5O_4$ $[M+H]^+$346.2454, found 346.2459.

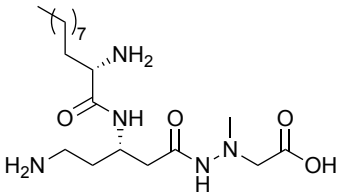
2-{2-((S)-5-Amino-3-((S)-2-aminononanamido)pentanoyl)-1-methylhydrazinyl}acetic acid (13w)



13w was prepared in the same manner as described for compound **13a** using **12w** (19.5 mg, 412 μmol). Compound **13w** (5.82 mg, 156 μmol, 2 steps 39%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –3.71 (*c* = 0.19, H_2O); mp 131.8-132.8 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.32 (s, 1H), 3.93 (s, 1H), 3.37 (s, 2H), 3.03 (d, *J* = 7.5 Hz, 2H), 2.72-2.38 (m, 5H), 1.98-1.83 (m, 4H), 1.30 (m, 10H), 0.86 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 178.4, 173.0, 172.6, 62.8, 56.3, 47.7, 46.9, 41.5, 39.4, 34.6, 34.1, 34.0 (2 carbons), 31.2, 31.0, 27.1, 25.0, 16.4; HRMS (ES+) calcd for $C_{17}H_{36}N_5O_4$ $[M+H]^+$374.2767, found 374.2763.

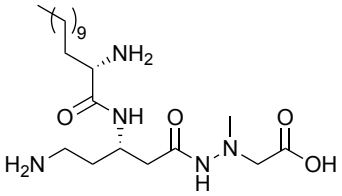
2-{2-((S)-5-Amino-3-((S)-2-aminoundecanamido)pentanoyl)-1-methylhydrazinyl}acetic acid (13x)



13x was prepared in the same manner as described for compound **13a** using **12x** (130 mg, 252 μmol). Compound **13x** (27.0 mg, 65.0 μmol, 2 steps 26%) was obtained as a white solid.

$[\alpha]_D^{25}$ = –8.30 (*c* = 0.62, H_2O); mp 134.2-135.3 °C; ^{1}H NMR (400 MHz, D_2O) δ 4.19-4.37 (m, 1H), 3.92-3.97 (m, 1H), 3.59-3.74 (m, 3H), 3.00-3.09 (m, 2H), 2.64-2.68 (m, 3H), 2.38-2.53 (m, 2H), 1.83-2.01 (m, 4H), 1.26-1.33 (m, 14H), 0.85 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 176.2, 174.5, 172.8, 56.2, 47.4, 47.0, 46.9, 41.4, 39.3, 34.4, 34.0, 33.9, 31.5, 31.3, 31.2 (2 carbons), 26.9, 24.9, 16.3; HRMS (ES+) calcd for $C_{19}H_{40}N_5O_4$ $[M+H]^+$402.3080, found 402.3098.

***N*-((*S*)-5-Amino-3-((*S*)-2-aminotridecanamido)pentanamido)-*N*-methylglycine (13y)**



13y was prepared in the same manner as described for compound **13a** using **12y** (53.2

mg, 84.3 μmol). Compound **13y** (6.83 mg, 15.9 μmol, 2 steps 19%) was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.33 (d, *J* = 3.0 Hz, 1H), 3.95 (s, 1H), 3.56 (m, 2H), 3.04 (d, *J* = 8.3 Hz, 2H), 2.64 (s, 3H), 2.41-2.48 (m, 2H), 1.84-1.99 (m, 4H), 1.34 (m, 18H), 0.84 (s, 3H); HRMS (ES+) calcd for $C_{21}H_{44}N_5O_4$ $[M+H]^+$ 430.3393, found 430.3386.

6. Biological evaluation

6-1. Chemical

Geneticin (G418) solution was purchased from Roche Diagnostics K.K., Switzerland

6-2. Plasmid

The previously reported plasmids[1] were used in Table 1, Table 2, and Figure 2.
The dual reporter plasmid has the premature termination codon (PTC), a 27-mer stretch of DNA that contains the sequence surrounding the PTC in exon 23 of the *mdx* gene for mouse dystrophin: 5'-TTGAAAGAGCAATAAAATGGCTTCAAC-3', between the β-galactosidase and luciferase encoding genes. The PTC was originally TAA only, but we used TGA and TAG in addition to the TAA sequence in this study. The readthrough activity was determined as the ratio of luciferase activity to β-galactosidase activity. The activity of compounds was expressed as a ratio relative to the control (=1).
The plasmid containing TGG sequence instead of PTC was used for the measurement of readthrough efficiency of the compounds. The readthrough efficiency was determined as the percentage of the ratio of luciferase activity to β-galactosidase activity against the PTC sequence divided by that against the TGG sequence.

In Figure 3, we constructed twelve new plasmids which consist of a dual reporter encoding renilla and firefly luciferase genes with or without PTCs. The linear vector of the pGL4.75 backbone was generated by inverse PCR with one set of primers (shown in 6-2, primers section). After agarose gel electrophoresis, the linear vector was purified using the Qiagen gel extraction kit (Qiagen., USA). Firefly luciferase gene containing PTCs with peripheral nucleotide sequences, which were selected from several typical nonsense mutations instead of the original PTC of firefly luciferase, were amplified from pGL4.38 using KOD -Plus- (TOYOBO Inc., Japan) and using the primer set in Table S1. After purification using the Qiagen gel extraction kit, we cloned the desired luciferase fragments into linearized pGL4.75 vector using the In-Fusion HD cloning kit (Takara Bio USA, Inc., USA) according to the manufacturer's protocol. All plasmids were purified using Qiagen Midiprep Kit (Qiagen, USA). The sequences of all these constructs were confirmed by Sanger sequencing (Europhin, Japan).

6-3. Cell-based readthrough activity evaluation.

The cell-based readthrough activity in Table 1, Table 2, and Figure 2 was evaluated as described previously[1]. COS-7 cells were maintained in DMEM (high glucose, Wako Pure Chemical Industries, Ltd., Japan) containing 10% fetal bovine serum (FBS, Nichirei Biosciences Inc., Japan) at 37 ˚C in a humidified 5% CO_2 atmosphere. Cells were plated in 96-well plates at 8000 cells/well. After incubation at 37 °C for 12 h, cells were transfected with one plasmid with a PTC using the FuGene® HD transfect reagent (Promega, USA). The medium was removed from the well, and the medium containing compounds at a concentration of 12.5-200 μM was added to wells. As a control, medium without the compounds was also added. The cells were incubated at 37 °C for 48 h, cells were collected, and β-galactosidase activity in the cell lysates was measured according to the manufacturer's protocol for the β-galactosidase enzyme assay with reporter lysis buffer (Promega, USA). The β-galactosidase activity was measured by Multiskan (Thermofisher Scientific Inc., Japan) at 420 nm. The luciferase activity in the cell lysates was measured according to the manufacturer's protocol for using the PicaGene® BrillianStar-LT (Toyo Ink Co., Ltd., Japan). The luciferase activity was measured using Luminoskan (Thermofisher Scientific Inc., Japan). The readthrough activity was determined as the ratio of luciferase activity to β-galactosidase activity. The activities of compounds were expressed as a ratio relative to the control (=1).

In Figure 3, after seeding using the same conditions as a previously described, the dual luciferase plasmid was transfected using the FuGene®HD transfect reagent (Promega, USA) following the manufacturer's protocol. The cells were incubated at 37 °C for 48 h, then the cells were collected and lysed with passive lysis buffer (Promega, USA). Firefly luciferase and renilla luciferase activity were measured using the dual luciferase reporter assay system (Promega, USA) according to the manufacturer's protocol. The readthrough efficiency (%) of each compound at 200 μM was calculated by dividing the readthrough activity obtained in the use of each plasmid with PTC by the activity obtained without PTC as 100% efficiency.

6-4. Primer sequences

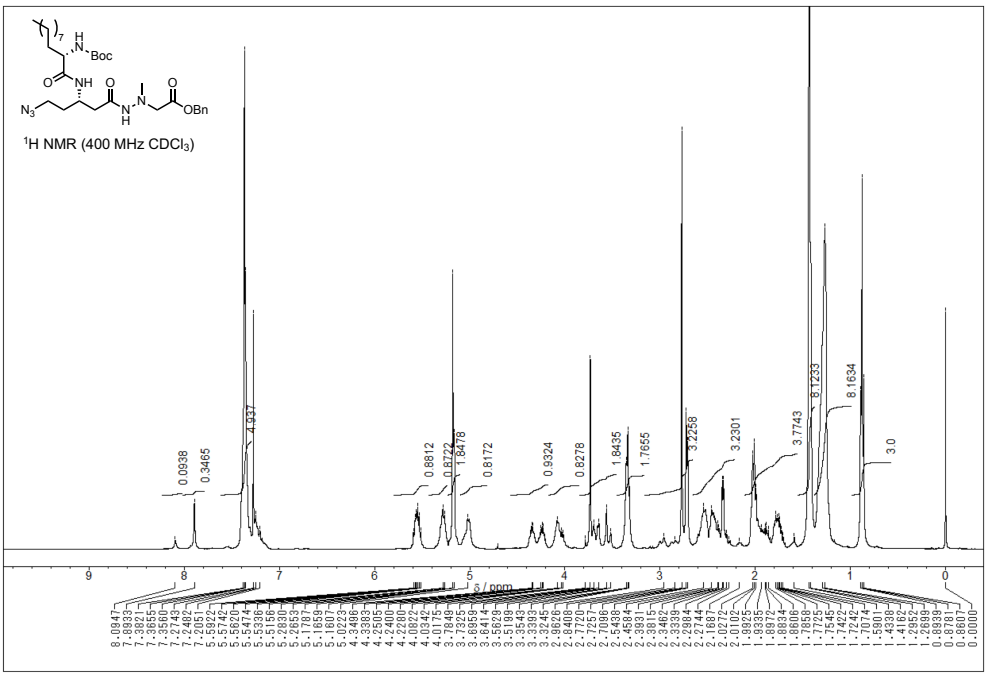
hRluc_CMV_deltaTAA_F	5’-TTCTAGAGTCGGGGCGGCCGGCCGCTTCGAGCAGAC-3’
hRluc_CMV_deltaTAA_R	5’-CTGCTCGTTCTTCAGCACGCG-3’
hRluc_deltaPESTTAA_infusion_R	5’-CGCCCCGACTCTAGAAttaCACGGCGATCTTGCCGCCCTTCTTG-3’
hRluc_DMD_R3381TGA_infusion_F	5’-GCTGAAGAACGAGCAGaaaaacaaatttTGAaccaaaaggtatATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_DMD_R3381CGA_infusion_F	5’-GCTGAAGAACGAGCAGaaaaacaaatttCGAaccaaaaggtatATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_p53_Q192TAG_infusion_F	5’-GCTGAAGAACGAGCAGctggcccctcctTAGcatcttatccgaATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_p53_Q192CAG_infusion_F	5’-GCTGAAGAACGAGCAGctggcccctcctCAGcatcttatccgaATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_p53_R213TGA_infusion_F	5’-GCTGAAGAACGAGCAGagaaacactttTGAcatagtgtggtgATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_p53_R213CGA_infusion_F	5’-GCTGAAGAACGAGCAGagaaacactttCGAcatagtgtggtgATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_CF_G542TGA_infusion_F	5’-GCTGAAGAACGAGCAGactttgcaacagTGAaggaaagcctttATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_CF_G542TGG_infusion_F	5’-GCTGAAGAACGAGCAGactttgcaacagTGGaggaaagcctttATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_CF_W1282TGA_infusion_F	5’-GCTGAAGAACGAGCAGaatatagttcttTGAgaaggtggaatcATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_CF_W1282GGA_infusion_F	5’-GCTGAAGAACGAGCAGaatatagttcttGGAgaaggtggaatcATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_LAMA_C1546TGA_infusion_F	5’-GCTGAAGAACGAGCAGgtcacaggattcTGAacgtgccgacctATGGAAGATGCCAAAAACATTAAGAAG-3’
hRluc_LAMA2_C1546TGC_infusion_F	5’-GCTGAAGAACGAGCAGgtcacaggattcTGCacgtgccgacctATGGAAGATGCCAAAAACATTAAGAAG-3’

6-5. Automated capillary electrophoresis western analysis.

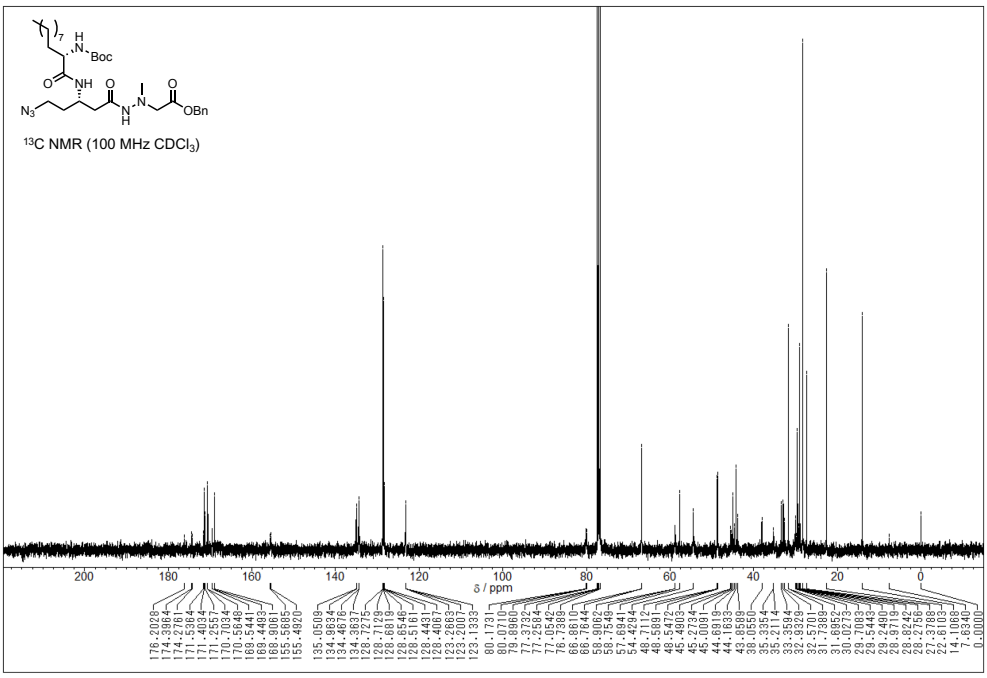
The p53 readthrough activity was evaluated in HDQ-P1 cells homozygous for the *TP53* R213X nonsense mutation using a ProteinSimple Wes automated capillary electrophoresis western analysis system exactly as described previously[2].

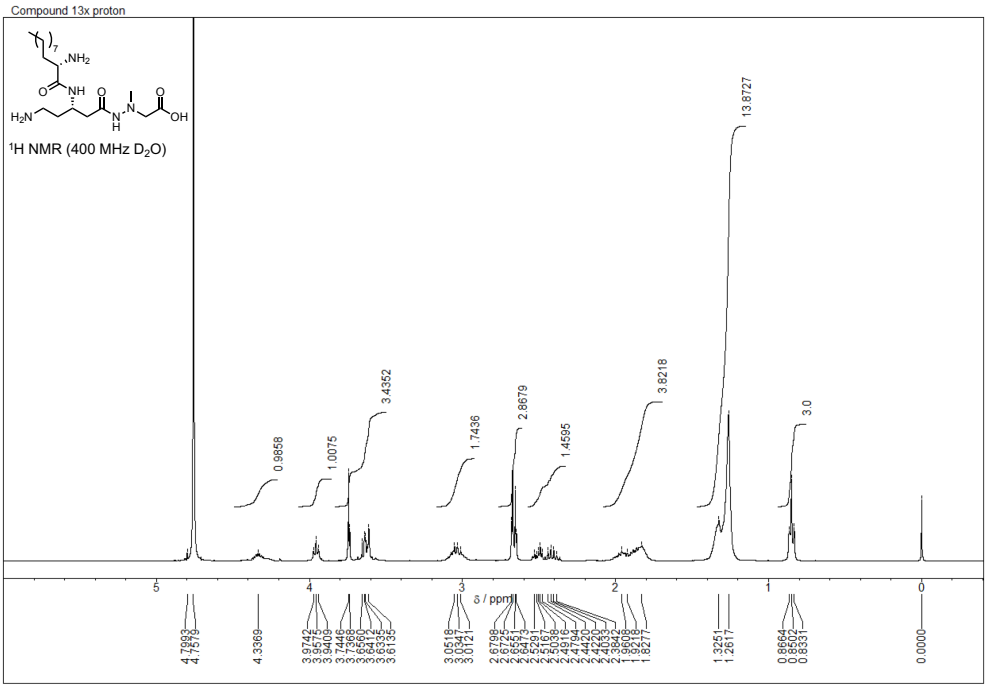
6. ^{1}H and ^{13}C NMR spectra

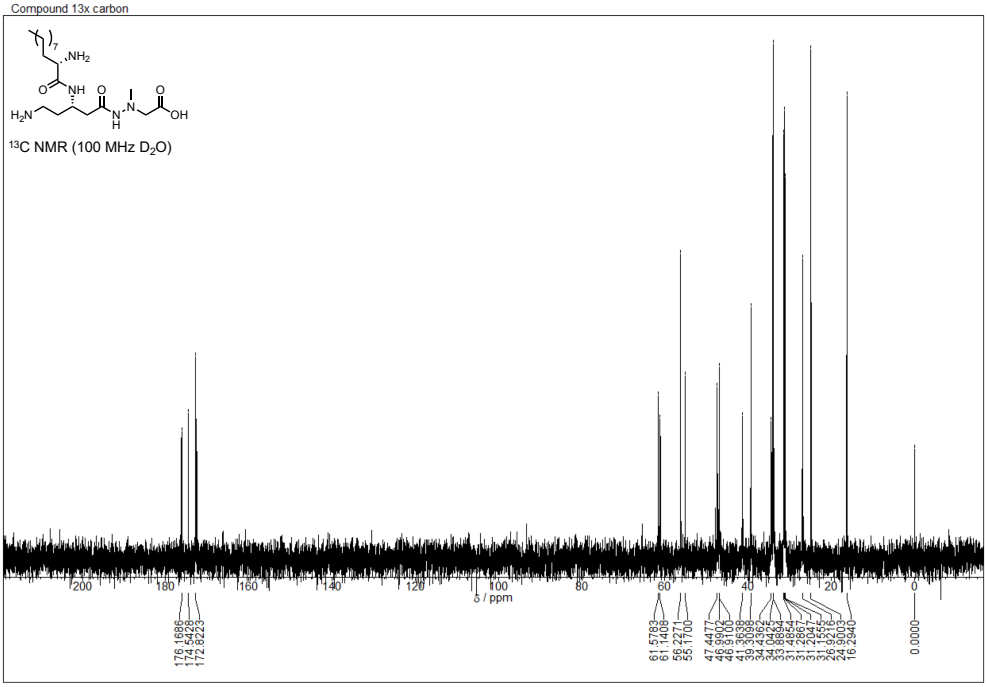
Compound 12x proton



Compound 12x carbon




Compound 13x proton
^{1}H NMR (400 MHz D_2O)
0.9858
1.0075
3.4352
1.7436
2.8679
1.4595
3.8218
13.8727
3.0
δ / ppm


Compound 13x carbon
^{13}C NMR (100 MHz D_2O)
δ / ppm

7. References

(1) Taguchi, A.; Hamada, K.; Kotake, M.; Shiozuka, M.; Nakaminami, H.; Pillaiyar, T.; Takayama, K.; Yakushiji, F.; Noguchi, N.; Usui, T.; Matsuda, R.; Hayashi, Y. Discovery of natural products possessing selective eukaryotic readthrough activity: 3-*epi*-deoxynegamycin and its leucine adduct. *ChemMedChem*. **2014**, *9*, 2233-2237.

(2) Baradaran-Heravi, A.; Balgi, A. D.; Zimmerman, C.; Choi, K.; Shidmoosavee, F. S.; Tan, J. S.; Bergeaud, C.; Krause, A.; Flibotte, S.; Shimizu, Y.; Anderson, H. J.; Jan, E.; Pfeifer, T.; Jaquith, J. B.; Roberge, M. Novel small molecules potentiate premature termination codon readthrough by aminoglycosides. *Nucleic Acids Res.* **2016**, *44*, 6583-6598.