1. Supplementary Materials and methods

1.1 General
Solvents were purified and dried by standard procedures prior to use; petroleum ether of boiling range 60 – 80 °C was used. All reactions were performed under an argon atmosphere. Flash chromatography was carried out using Merck Kieselgel (230 – 400 mesh). Thin layer chromatography was performed on silica gel and was visualized by staining with KMnO₄. NMR spectra were recorded on Varian Mercury spectrometer (600, 400 and 200 MHz) with chemical shifts values (δ) in ppm relative to TMS as internal standard. LC/MS were performed on Waters 2695 Alliance instrument, column: phenomenex, Gemini 5u C18 11OA, 50x2 mm, 5 µm; mobile phase: acetonitrile –0.1 % aq. HCOOH. Reagents and starting materials were obtained from commercial sources and used as received. (S)-3-Amino-N-cyclopropyl-2-hydroxypentanamide hydrochloride was prepared according to the literature method (Han et al., 2003). Tert-butyl 3-(3-amino-2-hydroxybutanamido) propanoate was prepared by analogy.
1.2. Synthetic schemes for peptidic ketoamide KS-182, KS-466 and peptidic aminoalcohol KS-378

Scheme 1. Reagents and conditions: (i) Fmoc-Thr(tBu)-OH, EDCI, HOBT, DIEA, DCM, r.t., 8 h; (ii) 4-(aminomethyl) piperidine, DCM, r.t., 1 h; (iii) Fmoc-Ile-OH, EDCI, HOBT, DIEA, DCM, r.t., 8 h; (iv) 4-(aminomethyl) piperidine, (v) Fmoc-Lys(Boc)-OH, EDCI, HOBT, DIEA, DCM, (vi) 4-(aminomethyl) piperidine, r.t., 8 h; (vii) acetic anhydride, pyridine, DMF, r.t., 8 h; (viii) LiOH, THF : H₂O (20:1), r.t., 5 h; (ix) (S)-3-amino-N-cyclopropyl-2-hydroxypentanamide hydrochloride, EDCI, HOBT, DIEA, DCM, r.t., 8 h; (x) Dess-Martin periodinane, NMP, r.t., 23 h, (xi) TFA : DCM (1:1), r.t., 2 h, then HCl in Et₂O.
Scheme 2. **Reagents and conditions:** (i) tert-butyl 3-(3-amino-2-hydroxybutanamido)-propanoate, EDCI, HOBT, DIEA, DCM, r.t., 8 h; (ii) Dess-Martin periodinane, NMP, r.t., 23 h, (iii) TFA : DCM (1:1), r.t., 2 h, then HCl in Et₂O.

Scheme 3. **Reagents and conditions:** (i) TFA : DCM (1:1), r.t., 2 h, then HCl in Et₂O.

1.3. **Description of experiments**

**Synthesis of** (10S,13S,16S,19S)-methyl-10-acetamido-16-((R)-1-tert-butoxylethyl)-13-sec-butyl-2,2,19-trimethyl-4,11,14,17-tetraoxo-3-oxa-5,12,15,18-tetraazaicosan-20-oate (2)

A mixture of Fmoc-Thr(tBu)-OH (1.00 g, 2.5 mmol), AlaOMe*HCl (0.38 g, 2.7 mmol), EDCI (0.43 g, 2.7 mmol) and HOBT (0.37 g, 2.7 mmol) and DIEA (0.88 mL, 5.0 mmol) in DCM (20 ml) was stirred for 8 h at room temperature (TLC control –
hexane : ethyl acetate, 4:1). The reaction mixture was washed with H₂O (5x10 ml) and then with brine (10 ml). Organic phase was dried over Na₂SO₄, filtrated and evaporated in vacuo. The residue was purified by flash chromatography on silica gel eluting with (hexane: ethyl acetate, 4:1) to provide Fmoc-Thr(tBu)-Ala-OMe (0.95 g, 95%) as a colourless crystalline compound. LC/MS: 484 (M+1).

Intermediate (0.95 g, 2.0 mmol) was dissolved in DCM (20 mL) and 4-aminomethyl piperidine (0.89 g, 7.8 mmol) was added to the solution. The resulting mixture was stirred for 1 h at room temperature, during this time the suspension was formed. The mixture was filtered and the solution obtained was washed with 10% (w/v) aqueous phosphate buffer (pH 5.5, 3x 5 mL). Organic phase was separated, dried over Na₂SO₄, filtered and evaporated in vacuo to obtain crude Thr-Ala-OMe (0.42 g, 82%) as a yellowish solid.

To the solution of Thr(tBu)-Ala-OMe (0.42 g, 1.6 mmol) in DCM (20 mL), Fmoc-Ile-OH (0.50 g, 1.5 mmol), EDCI (0.25 g, 1.6 mmol), HOBt (0.21 g, 1.6 mmol) and DIEA (0.38 mL, 2.1 mmol) were added in the given order and the resulting mixture was stirred for 8 h at room temperature (TLC control- hexane : ethyl acetate, 4:1). The reaction mixture was washed with H₂O (5x10 ml) and then with brine (10 ml). Organic phase was dried over Na₂SO₄, filtrated and evaporated in vacuo. The residue was purified by flash chromatography on silica gel eluting with hexane : ethyl acetate (4:1) to provide Fmoc-Ile-Thr-Ala-OMe (0.52 g, 58%) as a colourless crystalline compound. LC/MS: 597 (M+1).

Intermediate (0.52 g, 0.87 mmol) was dissolved in DCM (20 ml) and 4-aminomethyl piperidine (0.39 g, 3.4 mmol) was added to the solution. The resulting mixture was stirred for 1 h at room temperature, during this time the suspension was formed. The mixture was filtered and the solution obtained was washed with 10% (w/v) aqueous phosphate buffer (pH 5.5, 3x5 mL). Organic phase was separated, dried over Na₂SO₄, filtered and evaporated in vacuo to obtain crude Ile-Thr(tBu)-Ala-OMe (0.31 g, 60 %) as a yellowish solid.

To the solution of Ile-Thr(tBu)-Ala-OMe (0.31 g, 0.83 mmol) in DCM (20 mL), Fmoc-Lys(Boc)-OH (0.22 g, 0.46 mmol), EDCI (0.080 g, 0.51 mmol), HOBt (0.069 g, 0.51 mmol) and DIEA (0.09 mL, 0.56 mmol) were added in the given order and the resulting mixture was stirred for 8 h at room temperature (TLC control - hexane: ethyl acetate, 4:1). The reaction mixture was washed with H₂O (5x10 ml) and then with brine (10 ml). Organic phase was dried over Na₂SO₄, filtrated and evaporated in vacuo. The residue was purified by flash chromatography on silica gel
eluting with hexane: ethyl acetate, 1:1 to provide Fmoc-Lys(Boc)-Ile-Thr(tBu)-Ala-OMe (0.26 g, 61%) as a colourless crystalline solid. LC/MS: 825 (M+1)

Intermediate (0.26 g, 0.31 mmol) was dissolved in DCM (20 ml) and 4-aminomethyl piperidine (0.14 g, 1.26 mmol) was added to the solution. The resulting mixture was stirred for 1 h at room temperature, during this time the suspension was formed. The mixture was filtered through and the solution obtained was washed with 10% (w/v) aqueous phosphate buffer (pH 5.5, 3x5 mL). Organic phase was separated, dried over Na₂SO₄ filtered and evaporated in vacuo to obtain a crude Lys(Boc)-Ile-Thr(tBu)-Ala-OMe (0.18 g, 96%) as a yellowish solid.

Pyridine (0.026 mL, 0.32 mmol) and Ac₂O (0.031 mL, 0.32 mmol) were added to the solution of Lys(Boc)-Ile-Thr(tBu)-Ala-OMe (0.18 g, 0.30 mmol) in DCM (20 mL) and the mixture stirred for 1 h at room temperature (TLC control- hexane: ethyl acetate, 4:1). The reaction mixture was washed with H₂O (5x10 ml) and then with brine (10 ml). Organic phase was dried over Na₂SO₄, filtrated and evaporated in vacuo. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate to provide with 2 (0.14 g, 73%) as a colourless crystalline compound.

1H NMR : (400 MHz; DMSO-d₆,TMS) δ: 0.78-0.80 (8H, m); 0.96 (3H, d, 6.0 Hz); 1.07 (9H, s); 1.10-1.40 (18H, m); 1.52 (1H, m); 1.71 (3H; s); 2.81 (2H, m); 3.56(3H, s); 3.98-3.99 (1H, m); 4.19-4.26 (5H, m); 6.68 (1H, m),7.58 (1H, d, 8.8 Hz); 7.84-7.93 ppm (3H, m). LC/MS: 645 (M+1)

**Synthesis of (10S,13S,16S,19S)-10-acetamido-16-((R)-1-tert-butoxyethyl)-13-sec-butyl-2,2,19-trimethyl-4,11,14,17-tetraoxo-3-oxa-5,12,15,18-tetraazaicosan-20-oic acid (3)**

A mixture of 2 (0.14 g, 0.21 mmol), LiOH (0.051 g, 2.1 mmol) in THF : H₂O (20:1, 10 mL) was stirred at room temperature for 3 h and then acidified to pH 2 by addition of 1 M HCl. Water (10 mL) was added and the mixture extracted with ethyl acetate (10x3 mL). Organic phase was washed with brine, dried over Na₂SO₄ and evaporated in vacuo to provide compound 3 as a colourless solid (0.12 g, 88%).
\(^1\)H NMR: (400 MHz; DMSO-\(d_6\); TMS) \(\delta\): 0.76-0.80 (6H, m); 0.95 (3H, dd, 6.4 and 2.4 Hz); 1.07 (9H, s); 1.12-1.23 (1H, m); 1.24 (1H, m); 1.71 (3H; d; 6.8 Hz); 1.32 (9H, s); 1.40-1.50 (1H, m); 1.50-1.60 (1H, m); 1.70-1.77 (2H, m); 1.78 (3H, d, 5.2 Hz), 1.95 (1H, s); 2.81 (2H, q); 3.13 (1H, d, 4.8Hz); 3.58-3.54 (2H, t, 6.8 Hz); 3.90-3.92 (1H, m); 4.14-4.22 (4H, m); 6.68 (1H, t, 4.6 Hz); 7.58 (1H, d, 8.8 Hz); 7.68-7.73 (1H, m); 7.89-7.94 (1H, m); 8.02 (1H, d, 8.8 Hz); 12.5 ppm (1H, s). LC/MS: 631(M+1); 653 (M+Na).

Synthesis of tert-butyl (3\(S\),6\(S\),9\(S\),12\(S\),15\(S\))-15-acetamido-9-(\(R\))-1-tert-butoxyethyl)-12-sec-butyl-1-(cyclopropylamino)-3-ethyl-2-hydroxy-6-methyl-1,5,8,11,14-pentaoxo-4,7,10,13-tetraazanonadecan-19-ylcarbamate (4)

\[
\begin{align*}
\text{AcNH} & \quad \text{NHBoc} \\
\text{OH} & \quad \text{NHBoc} \\
\text{Me} & \quad \text{Me} \\
\text{NH} & \quad \text{Me} \\
\text{Me} & \quad \text{Me} \\
\end{align*}
\]

A mixture of 3 (50 mg, 0.08 mmol), (\(S\))-3-amino-\(N\)-cyclopropyl-2-hydroxypentanamide hydrochloride (19 mg,0.09 mmol), EDCI (14 mg, 0.09 mmol), HOBt (12 mg, 0.09 mmol), DIEA (20 \(\mu\)L, 0.1 mmol) and DCM (20 mL) was stirred at room temperature for 8 h. Organic phase was washed with brine, dried over Na\(_2\)SO\(_4\) and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluting with ethyl acetate followed by a mixture of ethyl acetate and methanol (10 : 1) to provide compound 4 as a yellowish solid (59 mg, 94%).

\(^1\)H NMR: (400 MHz ; DMSO-\(d_6\) ; TMS), \(\delta\): 0.47 -0.50 (2H, m); 0.52-0.61 (2H, m); 0.70-0.90 (10H, m); 0.95-1.10 (3H, d, 6.4 Hz); 1.15-1.30 (14H, m); 1.35 (9H, s); 1.51 (2H, m); 1.75 (1H, m); 1.81 (3H, s); 2.85 (2H, m); 3.78-4.00 (2H, m); 4.20-4.90 (4H, m); 6.71 (1H, t, 6.6 Hz); 7.22 (1H, m); 7.62-7.63 (3H, m); 7.95 ppm (2H, m). LC/MS: 784 (M+1), 802 (M+1+H\(_2\)O)

Synthesis of tert-butyl (3\(S\),6\(S\),9\(S\),12\(S\),15\(S\))-15-acetamido-9-(\(R\))-1-tert-butoxyethyl)-12-sec-butyl-1-(cyclopropylamino)-3-ethyl-6-methyl-1,5,8,11,14-pentaoxo-4,7,10,13-tetraazanonadecan-19-yl carbamate (5)
A stirred solution of compound 4 (25 mg, 0.02 mmol) in anhydrous NMP (10 ml) was cooled to 0-5°C under N₂ and to this was added DMP (28 mg, 0.06 mmol). The reaction mixture stirred for 23 h at room temperature. Organic phase was washed with Na₂S₂O₃ (3x5 mL), NaHCO₃ (3x5 mL), H₂O (3x5 mL), dried over Na₂SO₄ and evaporated in vacuo. The residue was purified by column chromatography on silica gel ethyl acetate followed by a mixture of ethyl acetate and methanol (10 : 1) to provide 5 as a yellowish solid (17 mg, 68%).

1H NMR: (400 MHz ; DMSO-d₆ ; TMS), δ: 0.57 -0.66 (2H, m); 0.80-0.90 (8H, m); 0.97-0.99 (3H, d, 6.4 Hz); 1.12-1.24( 13H, m); 1.37-1.60 ( 13H, m ); 1.76-1.87 (5H, m); 2.73-2.75 (1H, m); 2.85-2.87 (2H, m); 3.94-3.95 (1H, m); 4.21-4.38 (4H, m); 4.86-4.87 (1H, m); 6.71-6.74 (1H, m); 7.60-7.61 (1H, m); 7.62-7.63 (1H, m); 7.95 (1H, d, 8 Hz); 8.05-8.10 (1H, m); 8.14 (1H, t, 6.6 Hz); 8.72 ppm (1H, d, 5.2 Hz). LC/MS = 783 (M+1), 805 (M+Na), 683 (M+1 - Boc).

**Synthesis of (S)-2-acetamido-6-amino-N-((2S,3S)-1-((2S,3R)-1-(S)-1-(S)-1-(cyclopropylamino)-1,2-dioxopentan-3-ylamino)-1-oxopropan-2-ylamino)-3-hydroxy-1-oxobutan-2-ylamino)-3-methyl-1-oxopentan-2-yl)hexanamide hydrochloride (6) (KS-182).**

A mixture of 5 (10 mg, 0.01 mmol) in TFA : DCM (1:1, 10 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and treated with HCl (gass) in diethyl ether (3x2 mL, evaporation after each addition). The residue was treated with CH₃CN (3x5 mL, the precipitate was separated by centrifugation after each addition) and then with treated with Et₂O (3x5 mL, the precipitate was separated by centrifugation after each addition). The product was dried over NaOH in vacuo to provide with compound 6 as a yellowish crystalline material (8.1 mg, 95%).
$^1$H NMR: (400 MHz; DMSO-$d_6$; TMS), δ: 0.56 - 1.06 (9H, m); 1.09-1.48 (7H, m); 1.82 (3H, s); 2.11-2.14 (2H, m); 2.73-2.82 (3H, m); 3.95-4.00 (4H, m); 4.21-4.23 (2H, m); 4.88 (1H, m); 7.29 (2H, m); 7.70-7.83 (3H, m); 8.05-8.08 (1H, m); 8.71-8.76 ppm (1H, m). LC/MS: 626 (M+1)


![Chemical Structure](7)

A mixture of 3 (100 mg, 0.15 mmol), tert-butyl 3-(3-amino-2-hydroxybutanamido)propanoate (19 mg, 0.18 mmol), EDCI (14 mg, 0.18 mmol), HOBt (12 mg, 0.18 mmol), DIEA (33 μL, 0.18 mmol) and DMF (20 mL) was stirred at room temperature for 8 h. Organic phase was washed with brine, dried over Na$_2$SO$_4$ and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluting with ethyl acetate followed by a mixture of ethyl acetate and methanol (10 : 1) to provide compound 7 (48 mg, 35%).

$^1$H NMR: (400 MHz ; DMSO-$d_6$ ; TMS), δ: 0.70 - 0.90 (9H, m); 0.92-1.00 (3H, m); 1.01-1.25 (12H, m); 1.40-1.50 (19H, m); 1.51-1.56 (1H, m); 1.57-1.64 (1H, m); 1.80 (3H, s); 2.40-2.45 (2H, t, 7 Hz); 2.80-2.90 (2H, m); 3.25 (2H, t, 7 Hz); 3.84-3.93 (2H, m); 4.06-4.23 (5H, m); 5.71 (1H, d, 5.6 Hz); 6.68 (1H, t, 5.2 Hz); 7.50-7.76 (4H, m); 7.90-7.93(1H, m) ppm. LC/MS: 859 (M+1), 881 (M+Na), 759 (M-Boc+1), 645 (M-Boc-2tBu).

A stirred solution of compound 7 (30 mg, 0.03 mmol) in anhydrous NMP (10 ml) was cooled to 0-5°C under N₂ and to this was added DMP (31 mg, 0.06 mmol). The reaction mixture stirred for 23 h at room temperature. Organic phase was washed with Na₂S₂O₃ (3x5 mL), NaHCO₃ (3x5 mL), H₂O (3x5 mL), dried over Na₂SO₄ and evaporated in vacuo. The residue was purified by column chromatography on silica gel ethyl acetate followed by a mixture of ethyl acetate and methanol (10 : 1) to provide 8 as a yellowish solid (20 mg, 66%).

\[ \text{1H NMR} = (400 \text{ MHz; DMSO- d₆; TMS}), \delta: 0.70-0.85 \text{ (6H, m); 1.82 \text{ (3H, d); 1.11 \text{ (9H, s); 1.12-1.20 \text{ (6H, m); 1.22-1.49 \text{ (20H, m); 1.50-1.53 \text{ (2H, m); 1.60-1.63 \text{ (2H, m); 1.80 \text{ (3H, s); 1.81-1.95 \text{ (2H, m); 2.10 \text{ (1H, s); 2.11-2.15 \text{ (2H, t, 8 Hz); 2.36-2.40 \text{ (2H, t, 7 Hz); 2.65 \text{ (3H, s); 2.76-2.82 \text{ (2H, m); 3.83-3.98 \text{ (1H, m); 4.15-4.31 \text{ (5H, m); 4.87-4.96 \text{ (1H, d, m); 6.68 \text{ (1H, s); 7.56-7.58 \text{ (1H, d, 7.6 Hz); 7.65-7.81 \text{ (1H, m); 7.90-8.03 \text{ (2H, m); 8.16-8.18 \text{ (1H, d, 6.4 Hz); 8.59-8.64 \text{ (1H, q, 5.6 and 11.2 Hz) ppm. LC/MS} = 857 \text{ (M+1), 757 (M+1-Boc)}}]}

Synthesis of (4S,7S,10S,13S,16SR)-4-(4-aminobutyl)-7-sec-butyl-10-((R)-1-hydroxyethyl)-13,16-dimethyl-2,5,8,11,14,17,18-heptaoxo-3,6,9,12,15,19-hexaazadocosan-22-oic acid (9) (KS-466)

A mixture of 8 (18 mg, 0.021 mmol) in TFA : DCM (1:1, 10 mL) was stirred for 2 h at room temperature and the reaction mixture was concentrated in vacuo. The residue was treated with CH₃CN (3x5 mL, the precipitate was separated by centrifugation after each addition) and then with treated with Et₂O (3x5 mL, the precipitate was separated by centrifugation after each addition). The product was dried over NaOH in vacuo to provide with compound 9 (10 mg, 70 %) as a yellowish crystalline solid.
\[^{1}\text{H NMR} = (400 \text{ MHz}; \text{DMSO-d}_6; \text{TMS}), \delta: 0.70-0.91 (8\text{H, m}); 1.82 (3\text{H, d}); 1.00-1.10 (6\text{H, m}); 1.11-1.35 (9\text{H, m}); 1.40-1.65 (3\text{H, m}); 1.70-2.00 (5\text{H, m}); 2.15 (2\text{H, t, 7.8 Hz}); 2.44 (2\text{H, t, 7 Hz}); 2.72-2.79 (4\text{H, m}); 3.27-3.39 (5\text{H, m}); 4.17-4.29 (4\text{H, m}); 4.94 (1\text{H, m}); 7.68-7.77 (3\text{H, m}); 7.92-8.04 (1\text{H, m}); 8.60-8.68 (1\text{H, m}) \text{ ppm. LC/MS} = 644 (M+1), 662 (M+1+H}_2\text{O).}

Synthesis of (2S)-2-acetamido-6-amino-N-((2S,3S)-1-((2S,3R)-1-((2S)-1-((3S)-1-(cyclopropylamino)-2-hydroxy-1-oxopentan-3-ylamino)-1-oxopropan-2-ylamino)-3-hydroxy-1-oxobutan-2-ylamino)-3-methyl-1-oxopentan-2-yl)hexanamide hydrochloride 10 (KS-378)

A mixture of 4 (10 mg, 0.012 mmol) in TFA : DCM (1:1, 10 mL) was stirred for 2 h at room temperature and the reaction mixture was concentrated in vacuo. The residue was treated with CH\textsubscript{3}CN (3x5 mL, the precipitate was separated by centrifugation after each addition) and then with treated with Et\textsubscript{2}O (3x5 mL, the precipitate was separated by centrifugation after each addition). The product was dried over NaOH in vacuo to provide with compound 10 (8 mg, 94.4 %) as a yellowish crystalline solid.

\[^{1}\text{H NMR:} (400 \text{ MHz;} \text{DMSO-d}_6; \text{TMS}), \delta: 0.40-0.62 (2\text{H, m}); 0.70-0.90 (6\text{H, m}); 0.95-1.02 (2\text{H, m}); 1.10-1.35 (12\text{H, m}); 1.40-1.60 (2\text{H, m}); 1.81 (3\text{H, s}); 2.72 (1\text{H, d, 4.8 Hz}); 2.80-3.10 (2\text{H, m}); 3.80-4.10 (2\text{H, m}); 4.15-4.32 (3\text{H, m}); 7.63-7.80 (3\text{H, m}); 7.85-8.00 (2\text{H, m}); 8.01 ppm (1\text{H, d, 10 Hz}). \text{LC/MS:} 628 (M+1).}

References