Supporting Information

Linker Substitution Influences Succinimide Ring Hydrolysis Equilibrium Impacting Stability of Attachment to the Antibody-Drug Conjugate

Lu Wang, [a*] Adrian D. Hobson,[a] Paulin Salomon, [a] Julia Fitzgibbons,[a] Jianwen Xu,[a] Sean McCarthy,[a] Kan Wu, [a] Ying Jia,[a] Axel Hernandez Jr,[a] Xiang Li[b], Zhou Xu[b], Zhongyuan Wang[b], Yajie Yu[b], Junxian Li[b], Lin Tao.[b]

[a] AbbVie Bioresearch Center, 381 Plantation Street, Worcester, Massachusetts 01605, United States

[b] WuXi AppTec, 168 Nanhai Road, Tianjin Economic-Technological Development Area TEDA, TJS 300457 China

Email: l.wang@abbvie.com

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MS analysis protocol

ADC samples were first diluted to 10 ug/mL with 10% MeOH in water. Reduced MS samples were obtained by addition of 5 mM of TCEP and incubated at 37 ˚C for 30 min. Approximately 100 ng of MS sample was injected onto an Agilent 1290 Infinity II UHPLC equipped with an Agilent PLRP-S column (4000 Å, 8 um, 2.1 x 50 mm) and coupled to an Agilent 6545XT QTOF MS system. The gradient was 10 % B for 0.5 min, 10-90 % B in 0.1 min and held at 90 % B for 1.1 min, 90-10% B in 0.1 min, and then held at 10% B for 0.2 min. The mobile phases were A: 0.1% formic acid in water, and B: 0.1% formic acid in MeCN. Flow rate was 0.6 mL/min, and the column compartment was maintained at 60 °C. Agilent DAR Calculator was used to calculate DAR of the ADC samples.

Protocol for size exclusion chromatography (SEC) analysis of ADCs.

SEC was performed using an Ultimate 3000 Dual LC system (Thermo Scientific) equipped with a 7.8 X 300 mm TSK-gel 3000 SWXL column (Tosoh Bioscience, cat. 08541). Approximately 20 µg of ADC was loaded onto the column and eluted over 17 min using an isocratic gradient of 100 mM sodium sulfate, 100 mM sodium phosphate, pH 6.8 at a flow rate of 1.0 mL/min.

Thiosuccinimide hydrolysis pH 9 protocol.

Arginine buffer (0.7 M), pH 9.0 solution was prepared and added to the ADC in PBS buffer to bring the total arginine concentration to 50 mM (pH ~ 8.9). The material was then incubated at RT for 72 hours. Hydrolysis of the succinimide ring was confirmed by reduced mass spectrometry, after which the ADC was buffer exchanged to UBC buffer (pH ~ 6.0).

Thiosuccinimide hydrolysis pH 8 protocol.

Borate buffer (1.0 M), pH 8.0 solution was prepared and added to the ADC in PBS buffer to bring the total borate concentration to 100 mM (pH ~ 7.9). The material was then incubated at RT for 72 hours. Hydrolysis of the succinimide ring was confirmed by reduced mass spectrometry, after which the ADC was buffer exchanged to UBC buffer (pH ~ 6.0).

ADC hydrolysis kinetic study protocol

200 ug of each ADC at about 2 mg/mL in PBS buffer pH 7.4 was hydrolysed using 100 mM borate buffer pH 8.0. The extent of hydrolysis for each ADC was measured at 1h, 2h, 4h, 8h and 24h timepoints using MS.

K562 Human FL-TNF-α (delta1-12) GRE Luciferase Reporter Assay

K562/human FL-TNF-α delta 1-12 GRE (pGL4.36[luc2)/MMTV/Hygro) (Abbvie Inc.) cells were plated onto 96-well tissue culture treated white plates (Costar: #3917) at 50,000 cells per well in 50 µL of assay medium (1x RPMI+ L-Glutamine, 1% Charcoal FBS (Thermo Fisher Scientific Inc., # 12676-029), 1% Na Pyruvate and 1% MEM Non-Essential Amino Acids). The cells were treated with 25 µL of 3x serial diluted antibody drug conjugate in assay medium, small molecule or media alone and incubated for 48 hours at 37˚C and 5% CO_2 . After 48 hours incubation, cells were treated with 75 µL of Dual-Glo Luciferase Assay System (Promega, #E2920) for 10 minutes and analyzed for luminescence using the Microbeta (PerkinElmer). The full response was determined using 100 nM dexamethasone. The dose response data were fitted to a sigmoidal curve using nonlinear regression and the EC_{50} values calculated with the aid of GraphPad 6.0 (GraphPad Software, Inc.).

Thermal stress test protocol

To assess long-term stability, all purified ADCs were subjected to a stress test at 40 °C at pH 6.0. A subset of ADCs was selected for additional test with different buffer pH (5.5, 6.0 and 6.5). Small aliquots of each ADC (2 mg/mL) in 15 mM histidine at predetermined pH were incubated at 40 °C for 0, 14 and 28 days. An aliquot of some ADCs stored at 4 °C for 6 months was also included in the study.

Peptide Mapping Analysis via Mass Spectrometry protocol

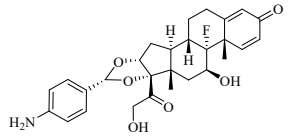
Forced degraded ADC samples were subjected to an automated digest protocol using a Hamilton STARlet. Briefly, a 50 µl aliquot of each sample was placed in a 96 well plate with 175 µl of denaturing buffer (8M Guanidine HCl, 400 mM Tris, 20 mM L-methionine, pH 8.0) and 40 µl of 0.5 M Dithiothreitol. After 1 hour incubation, 10 µl of 1 M Iodoacetic acid was added. The reaction was quenched by addition of 15 µl of 0.5 M dithiothreitol. Samples were desalted using 10 K MWCO desalting tips (Phynexus) prepared according to the manufacturer recommendations and eluted using 200 µl of hydrolysis buffer (50 mM Tris-HCl, 1 M Urea, 20 mM L-methionine, pH 7.5). A 100 µl aliquot of the desalted sample was subjected to proteolytic digestion using 40 µl of Trypsin/Lys-C (0.075 µg/µl) and incubated for 2 hours at room temperature. The digested was quenched by addition of 10 µl of 5 % formic acid. LCMS Peptide mapping analysis of digest samples was completed using a QExactive EMR Orbitrap mass spectrometer coupled with a Vanquish liquid chromatograph. Mobile phase A was water and mobile phase B was acetonitrile with each containing 0.08% formic acid and 0.02% trifluoroacetic acid. The column used was a Waters BEH C18 packed with 1.7 µm particles with 130 Å pore size, 2.1 mm x 150 mm. The column temperature was 55°C. 10 µl of the digested sample was injected for analysis and separated with a flow rate of 0.55 ml/min using standard gradient. All data were processed using Protein Metrics Byos Software.

All the reagents and solvents were purchased from TCI China, Titan (Adamas), Yinuokai, Accela ChemBio, Bide Pharmatech Ltd and Sigma Aldrich and used without further purification. ^{1}H NMR, ^{13}C NMR ^{19}F NMR spectra were recorded on a Bruker AVANCE III 400MHz spectrometer or a Bruker AVANCE III 1 BAY 400 MHz spectrometer or a Bruker 800 MHz spectrometer with HCN CryoProbe. The data were processed with MestReNova software, measuring proton shifts in parts per million (ppm) downfield from an internal standard tetramethyl silane. Whenever possible reactions were monitored by LCMS. All purified compounds were ≥95% purity based on analytical HPLC. HPLC conditions are detailed in Table 4 in the Supporting Information.

Chemical Synthesis

All the reagents and solvents were purchased from TCI China and Sigma Aldrich and used without further purification. ^{1}H NMR spectra were recorded on a Bruker AVANCE III 400MHz spectrometer. The data were processed with MestReNova software, measuring proton shifts in parts per million (ppm) downfield from an internal standard tetramethyl silane. Whenever possible reactions were monitored by LCMS. All purified compounds were ≥95% purity based on analytical HPLC. HPLC conditions are detailed in Table S1. Compound names were generated with Perkin Elmer ChemDraw 20.1.

(6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one.



To a solution of (8S,9R,10S,11S,13S,14S,16R,17S)-9-fluoro-11,16,17-trihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-3-one (25.4 g, 64.4 mmol) and tert-butyl (4-formylphenyl)carbamate (15 g, 67.8 mmol) in acetonitrile (500 mL) was added perchloric acid (20.39 mL, 339 mmol) at 20°C. The resulting mixture was stirred at 20°C for 2 h. The reaction was quenched with saturated $NaHCO_3$ and extracted with DCM (2 × 1 L). The combined organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a residue. The residue was purified by Prep-HPLC (Method n, Table S1 or 5 if no supporting material) to afford the title compound (7 g, 21%) as a yellow solid. LCMS (Method f, Table S1) R_t = 2.098 min; MS m/z: 498.0 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.31 – 7.29 (m, 1H), 7.05 – 7.03 (m, 2H), 6.51 – 6.49 (m, 2H), 5.26 – 5.05 (m, 4H), 4.87 – 4.86 (m, 1H), 4.50 – 4.44 (m,1H), 4.20 – 4.14(m, 2H), 2.52 –2.50 (m, 2H), 2.22 – 2.17 (m, 1H), 2.02 – 1.65 (m, 6H), 1.62 – 1.41 (m, 4H), 0.85 (s, 3H). ^{13}C NMR (101MHz, DMSO-d6) δ ppm 209.54, 185.70, 167.16, 153.08, 150.66, 129.51, 128.34, 124.80, 122.95, 118.54, 113.57, 104.27, 101.96, 101.09, 97.30, 81.30, 70.90, 70.72, 66.58, 48.35, 48.23, 45.42, 43.68, 36.65, 33.15, 30.59, 27.95, 23.36, 17.18. HRMS: found 498.2286 $C_{28}H_{32}FNO_6$ requires 498.2292.

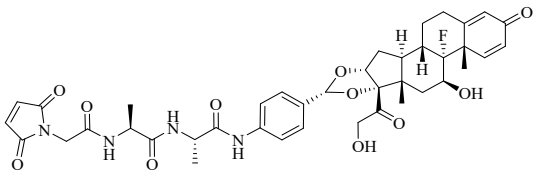
tert-Butyl ((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate.

To a mixture of (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) (115 mg, 0.30 mmol), (S)-2-((S)-2-((tert-butoxycarbonyl)amino)propanamido)propanoic acid (63 mg, 0.24 mmol) and (6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-6a,6b,7,8,8a,8b,11a,12,12a,12b-decahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4(2H)-one (0.100 g, 0.201 mmol) in DMF (3 mL) was added 2,6-dimethylpyridine (65 mg, 0.60 mmol), stirred for 2 h at RT then subjected to purification by prep-HPLC to afford the tile compound (60 mg, 40%) as a white solid. LCMS (Method g, Table S1) R_t = 1.430 min; MS m/z: 740.4 $(M+H)^+$.

(S)-2-Amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide.

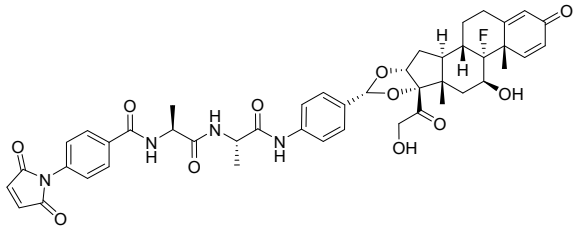
To a solution of tert-butyl ((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (400 mg, 0.541 mmol) in EtOAc (10 mL) was added HCl (0.14 mL, 0.54 mmol) at 0 °C. The mixture was stirred for 3 h at RT, evaporated to dryness and subjected to purification by prep-HPLC to afford the title compound (200 mg, 58%) as yellow solid. LCMS (Method b, Table S1) R_t = 0.705 min; MS m/z: 640.4 $(M+H)^+$.

(S)-2-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido)-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (DL15).



To a solution of (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (120 mg, 0.188 mmol) in DMF (5 mL) was added DIEA (0.1 mL, 0.56 mmol) and 2,5-dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate (52.0 mg, 0.206 mmol). The reaction was stirred at RT for 1 h then subjected to purification by prep-HPLC (Method o, Table S6) to afford the title compound (20 mg, 14%) as a white powder. LCMS (Method a, Table S1) Rt = 2.710 min; MS m/z: 777.4 $(M+H)^+$. ^{1}H NMR (400MHz, MeOD) δ ppm 7.64 - 7.57 (m, 2H), 7.38 (br dd, J = 9.4, 18.4 Hz, 3H), 6.75 (d, J = 5.1 Hz, 2H), 6.31 (br d, J = 10.1 Hz, 1H), 6.14 (br d, J = 6.0 Hz, 1H), 5.45 (d, J = 3.1 Hz, 1H), 5.04 (d, J = 5.1 Hz, 1H), 4.67 - 4.60 (m, 1H), 4.47 - 4.39 (m, 1H), 4.38 - 4.13 (m, 6H), 2.80 - 2.58 (m, 3H), 2.47 - 2.27 (m, 3H), 1.97 (br s, 1H), 1.84 - 1.69 (m, 2H), 1.61 (s, 3H), 1.49 - 1.36 (m, 6H), 1.00 (s, 3H). HRMS: found 776.3070 $C_{40}H_{45}FN_4O_{11}$ requires: 776.8150.

4-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)benzamide (DL11).



To a solution of 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzoic acid (53.5 mg, 0.246 mmol), (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (150 mg, 0.234 mmol), 1-hydroxybenzotriazole hydrate (39.5 mg, 0.26 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (49.4 mg, 0.26 mmol) in DMF (5 mL) was added N,N-diisopropylethylamine (0.12 mL, 0.70 mmol) at RT. The mixture was stirred for 1 hr at RT then subjected to purification by prep-HPLC to afford the title compound (41 mg, 20%) as a white solid. LCMS (Method b, Table S1) Rt = 1.086 min; MS m/z: 839.2 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.95 (s, 1H), 8.60 (d, *J* = 7.1 Hz, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 8.01 - 7.96 (m, 2H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.46 - 7.42 (m, 2H), 7.35 (d, *J* = 8.6 Hz, 2H), 7.28 (d, *J* = 10.0 Hz, 1H), 7.20 (s, 2H), 6.21 (dd, *J* = 1.8, 10.1 Hz, 1H), 6.02 (s, 1H), 5.42 (s, 2H), 5.08 (t, *J* = 6.0 Hz, 1H), 4.92 (d, *J* = 4.6 Hz, 1H), 4.55 - 4.47 (m, 2H), 4.41 (t, *J* = 7.2 Hz, 1H), 4.23 - 4.15 (m, 2H), 2.36 (br d, *J* = 3.5 Hz, 1H), 2.16 (dt, *J* = 7.1, 11.9 Hz, 1H), 2.04 (br d, *J* = 14.1 Hz, 1H), 1.88 - 1.80 (m, 1H), 1.71 - 1.62 (m, 3H), 1.49 (s, 3H), 1.46 - 1.38 (m, 1H), 1.35 (d, *J* = 7.2 Hz, 3H), 1.31 (d, *J* = 7.2 Hz, 3H), 0.86 (s, 3H).

1-(tert-Butyl) 3-(2,5-dioxopyrrolidin-1-yl) (3S,4R)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)pyrrolidine-1,3-dicarboxylate.

To a solution of(3S,4R)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-1-(tert-butoxycarbonyl)pyrrolidine-3-carboxylic acid (35 mg, 0.077 mmol) in dioxane (1 mL) was added N-hydroxysuccinimide (10.7 mg, 0.09 mmol) at 10 °C. The mixture was stirred at 0 °C for 30 min and then N,N'-methanediylidenedicyclohexanamine (16.0 mg, 0.08 mmol) was added and the mixture then stirred at 10-25 °C for 90 min. The mixture was filtered and evaporated to dryness to afford the title compound (43 mg, 100%) as a white solid. LCMS (Method b, Table S1) Rt = 1.251 min; MS m/z: 449.9 $(M\text{-}Boc)^+$.

tert-butyl (3R,4S)-3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-4-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate.

To a solution of (3S,4R)-1-tert-butyl 3-(2,5-dioxopyrrolidin-1-yl) 4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)pyrrolidine-1,3-dicarboxylate (60.1 mg, 0.109 mmol) in DMF (1 mL) was added N,N-diisopropylethylamine (0.06 mL, 0.33 mmol) at 10 °C. The reaction was stirred at 10 °C for 0.5h then (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (70 mg, 0.109 mmol) was added. The reaction was stirred at RT for 3h then subjected to purification by prpe-HPLC to afford the title compound (30mg, 25%) as a white solid. LCMS (Method b, Table S1) Rt = 1.276 min; MS m/z: 1074 (M+H)+.

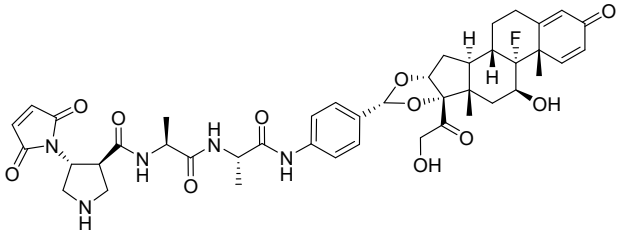
tert-Butyl (3R,4S)-3-Amino-4-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate.

To a solution of (3R,4S)-tert-butyl 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (30mg, 0.03 mmol) in MeCN (1 mL) was added piperidine (2.4 mg, 0.03 mmol) at RT. The reaction was stirred at for 4 h at RT then subjected to purification by prep-HPLC to afford the title compound (15 mg, 60%) as a white solid. LCMS (Method b, Table S1) Rt = 0.999 min; MS m/z: 852.2 $(M+H)^+$.

tert-Butyl (3R,4S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate.

To a solution of (3R,4S)-tert-butyl 3-amino-4-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (50mg, 0.06 mmol) in dioxane (1 mL) was added methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate (63.7 mg, 0.411 mmol) and aqueous $NaHCO_3$ 0.2mL) at RT and stirred at RT for 16 h. The reaction mixture was adjusted to pH = 7 with aqueous $NaHCO_3$ and stirred at RT for 30 min, combined with 10 identical reactions and subjected to purification by prep-HPLC to afford the title compound (110 mg, 12%) as a white solid. LCMS (Method b, Table S1) Rt = 1.099 min; MS m/z: 933 $(M+H)^+$.

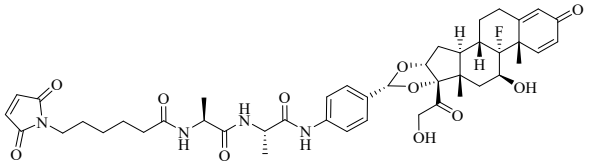
(3S,4R)-4-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)pyrrolidine-3-carboxamide (DL16).



To a solution of (3R,4S)-tert-butyl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (100 mg, 0.11 mmol) in DCM (4 mL) was added TFA (1 mL) at RT and the mixture was stirred at RT for 30 min. Purification by prep-HPLC afforded the title compound (100 mg, xx%) as a white solid. LCMS (Method b, Table S1) Rt = 0.943 min; MS m/z: 832.3 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 10.04 (s, 1H), 9.18 - 8.95 (m, 2H), 8.47 (d, *J* = 7.5 Hz, 1H), 8.14 (d, *J* = 7.0 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.36 (d, *J* = 8.7 Hz, 2H), 7.29 (d, *J* = 10.1 Hz, 1H), 7.07 (s, 2H), 6.23 (dd, *J* = 1.7, 10.1 Hz, 1H), 6.03 (s, 1H), 5.52 - 5.41 (m, 2H), 5.24 - 5.00 (m, 1H), 4.93 (d, *J* = 4.3 Hz, 1H), 4.85 - 4.77 (m, 1H), 4.52 (br d, *J* = 19.3 Hz, 1H), 4.41 - 4.29 (m, 2H), 4.20 (br d, *J* = 19.4 Hz, 2H), 3.70 - 3.58 (m, 1H), 3.47 (br d, *J* = 7.2 Hz,

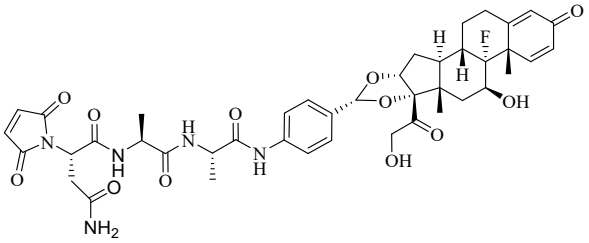
4H), 2.58 (br d, *J* = 2.2 Hz, 1H), 2.43 - 2.32 (m, 2H), 2.21 - 2.09 (m, 1H), 2.03 (br d, *J* = 13.7 Hz, 1H), 1.89 - 1.81 (m, 1H), 1.73 - 1.63 (m, 3H), 1.50 (s, 3H), 1.43 - 1.35 (m, 1H), 1.29 (d, *J* = 7.1 Hz, 3H), 1.18 (d, *J* = 7.0 Hz, 3H), 0.87 (s, 3H).

6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)hexanamide (DL20).



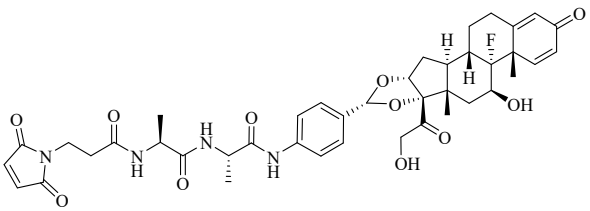
To a solution of (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (100 mg, 0.16 mmol) in DMF (1 mL) was added N-ethyl-N-isopropylpropan-2-amine (121 mg, 0.94 mmol) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (48.2 mg, 0.16 mmol) at RT and the mixture stirred at RT for 2 h. Purification by prep-HPLC afforded the title compound (40 mg, 30%) as a white solid. LCMS (Method b, Table S1) Rt = 1.088 min; MS m/z: 833.2 $(M+H)^+$. ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 9.94 (s, 1H), 8.07 (br d, *J*=7.2 Hz, 1H), 7.99 (br d, *J*=7.1 Hz, 1H), 7.61 (d, *J*=8.4 Hz, 2H), 7.35 (br d, *J*=8.6 Hz, 2H), 7.29 (d, *J*=10.1 Hz, 1H), 6.99 (s, 2H), 6.23 (dd, *J*=1.7, 10.1 Hz, 1H), 6.03 (s, 1H), 5.43 (s, 2H), 4.93 (d, *J*=4.5 Hz, 1H), 4.52 (d, *J*=19.4 Hz, 1H), 4.37 (quin, *J*=7.1 Hz, 1H), 4.30 - 4.13 (m, 3H), 3.35 (br t, *J*=7.0 Hz, 2H), 2.71 - 2.57 (m, 1H), 2.56 - 2.52 (m, 1H), 2.46 - 2.41 (m, 1H), 2.36 (br s, 1H), 2.22 - 2.12 (m, 1H), 2.12 - 1.98 (m, 3H), 1.91 - 1.78 (m, 1H), 1.73 - 1.58 (m, 3H), 1.55 - 1.34 (m, 8H), 1.29 (br d, *J*=7.1 Hz, 3H), 1.18 (br d, *J*=7.1 Hz, 5H), 0.87 (s, 3H).

(S)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)succinamide (DL10).



To a solution of (S)-2-amino-N1-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)succinamide (60 mg, 0.080 mmol) in dioxane (2mL) was added methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate (86 mg, 0.56 mmol) and $NaHCO_3$ (0.200 mL) at RT. The mixture stirred at RT for 16 h, adjusted to pH 7 with aqueous $NaHCO_3$, then subjected to purification by prep-HPLC to afford the title compound (3 mg, 4%) as a white solid. LCMS (Method d, Table S1) Rt = 1.009 min; MS m/z: 834.2 $(M+H)^+$.

3-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide (DL18).



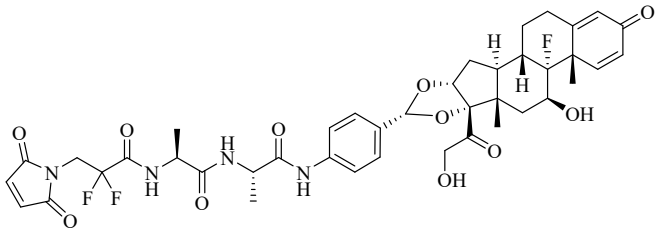
Hunig's Base (0.15 mL, 0.86 mmol) was added to a solution of (S)-2-amino-N-((S)-1-((4-

((2S,6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-2,6b-difluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)-2-methoxyphenyl)amino)-1-oxopropan-2-yl)propanamide (164 mg, 0.24 mmol) and N-succinimidyl 3-maleimidopropionate (70 mg, 0.26 mmol) in DMF (2.4 mL) at RT. Purification by prep-HPLC afforded the title compound (74 mg, 37%) as an amorphous white solid. LCMS (Method b, Table S1) Rt = 2.333 min; MS m/z: 791.3 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d6) d 8.96 (s, 1H), 8.25 (d, J = 7.1 Hz, 1H), 8.14 (d, J = 7.5 Hz, 1H), 8.01 (d, J = 8.2 Hz, 1H), 7.23 (d, J = 10.2 Hz, 1H), 7.07 (d, J = 1.8 Hz, 1H), 7.01 6.94 (m, 1H), 6.96 (s, 2H), 6.26 (dd, J = 10.2, 1.9 Hz, 1H), 6.09 (s, 1H), 5.73 5.51 (m, 1H), 5.51 5.46 (m, 1H), 5.45 (s, 1H), 5.06 (t, J = 5.9 Hz, 1H), 4.92 (d, J = 5.1 Hz, 1H), 4.51 (dd, J = 19.5, 6.4 Hz, 1H), 4.42 (p, J = 7.1 Hz, 1H), 4.27 (p, J = 7.2 Hz, 1H), 4.21 4.11 (m, 1H), 3.71 (s, 3H), 3.56 (t, J = 7.3 Hz, 2H), 2.73 2.54 (m, 1H), 2.39 2.31 (m, 2H), 2.32 2.19 (m, 3H), 2.03 (d, J = 13.6 Hz, 1H), 1.79 1.61 (m, 3H), 1.47 (s, 4H), 1.26 (d, J = 7.1 Hz, 3H), 1.16 (d, J = 7.1 Hz, 3H), 0.84 (s, 3H).

3-Amino-2,2-difluoro-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide.

To a solution of tert-butyl (2,2-difluoro-3-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-3-oxopropyl)carbamate (240 mg, 0.28 mmol) in DCM (1 mL) was added TFA (1 mL) dropwise at RT and the mixture stirred for 1 h at RT. Purification by prep-HPLC afforded the title compound (15 mg, 7%) as a white solid. LCMS (Method b, Table S1) Rt = 0.942 min; MS m/z: 747.2 $(M+H)^+$.

3-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,2-difluoro-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide (DL14).



To a solution of 3-amino-2,2-difluoro-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide (35.0 mg, 0.047 mmol)and methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate (50.9 mg, 0.33 mmol) in water (0.1 mL) and dioxane (1 mL) was added $NaHCO_3$ (3.9 mg, 0.05 mmol). The mixture was stirred for 24 h at RT then subjected to purification by prep-HPLC to afford the title compound (28 mg, 23%) as a white solid. LCMS (Method b, Table S1) Rt = 1.072 min; MS m/z: 827.2 $(M+H)^+$. ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.06 (s, 1H), 8.89 (d, *J*=7.3 Hz, 1H), 8.25 (d, *J*=7.2 Hz, 1H), 7.58 (d, *J*=8.7 Hz, 2H), 7.36 (d, *J*=8.7 Hz, 2H), 7.29 (d, *J*=10.1 Hz, 1H), 7.12 (s, 2H), 6.23 (dd, *J*=1.8, 10.0 Hz, 1H), 6.03 (s, 1H), 5.43 (s, 2H), 4.93 (d, *J*=4.5 Hz, 1H), 4.52 (d, *J*=19.4 Hz, 1H), 4.43 - 4.31 (m, 2H), 4.20 (br d, *J*=19.3 Hz, 2H), 4.02 (t, *J*=15.0 Hz, 2H), 2.37 (br s, 1H), 2.16 (br dd, *J*=11.6, 19.1 Hz, 2H), 2.09 - 1.99 (m, 2H), 1.91 - 1.79 (m, 2H), 1.73 - 1.60 (m, 4H), 1.50 (s, 3H), 1.41 (br d, *J*=7.5 Hz, 1H), 1.28 (dd, *J*=7.1, 16.3 Hz, 6H), 0.87 (s, 3H).

(9H-Fluoren-9-yl)methyl (2-(3-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-3-oxopropoxy)ethyl)carbamate.

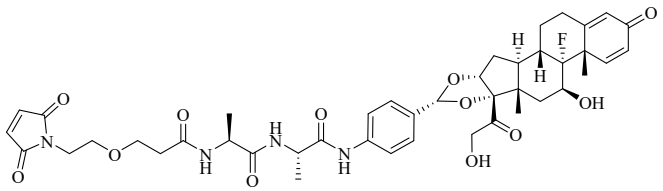
To a solution of (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (100 mg, 0.16 mmol) and 3-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)propanoic acid (55.6 mg, 0.156 mmol) in DMF (2 mL) was added ((1H-[1,2,3]triazolo[4,5-b]pyridin-1-yl)oxy)tri(pyrrolidin-1-yl)phosphonium hexafluorophosphate(V) (82 mg, 0.16 mmol) and N,N-diisopropylethylamine (0.08 mL, 0.47 mmol) at 0 °C and the

mixture stirred at RT for 2 h. Water was added and the mixture filtered to afford the title compound (110 mg, 72%) as a white solid. LCMS (Method b, Table S1) Rt = 1.198 min; MS m/z: 977.6 $(M+H)^+$.

3-(2-Aminoethoxy)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide.

To a solution of (9H-fluoren-9-yl)methyl (2-(3-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-3-oxopropoxy)ethyl)carbamate (420 mg, 0.43 mmol) in acetone (4 mL) was added piperidine (0.8 mL, 0.43 mmol) at -20 °C. The mixture stirred at -20°C for 2 h then subjected to purification by prep-HPLC to afford the title compound (150 mg, 46%) as a white solid. LCMS (Method b, Table S1) Rt = 0.932 min; MS m/z: 755 $(M+H)^+$.

3-(2-(2,5-Sioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide (DL19).



To a solution of 3-(2-aminoethoxy)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide (40 mg, 0.05 mmol) in dioxane (1 mL) was added methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate (57.5 mg, 0.37 mmol) and aqueous $NaHCO_3$ (0.1ml) at RT. The mixture was stirred for 16 h at RT then subjected to purification by prep-HPLC to afford the title compound (39 mg, 87%) as a white solid. LCMS (Method b, Table S1) Rt = 1.088 min; MS m/z: 835 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (s, 3 H) 1.18 (d, *J*=7.09 Hz, 3 H) 1.29 (d, *J*=7.09 Hz, 3 H) 1.41 (br dd, *J*=12.65, 4.22 Hz, 1 H) 1.50 (s, 3 H) 1.59 - 1.72 (m, 3 H) 1.79 - 1.91 (m, 1 H) 2.05 (br d, *J*=13.69 Hz, 1 H) 2.10 - 2.26 (m, 2 H) 2.28 - 2.40 (m, 3 H) 2.52 (d, *J*=1.96 Hz, 2 H) 2.63 - 2.70 (m, 1 H) 3.41 - 3.48 (m, 3 H) 3.51 - 3.54 (m, 3 H) 4.16 - 4.30 (m, 3 H) 4.37 (quin, *J*=7.12 Hz, 1 H) 4.52 (d, *J*=19.44 Hz, 1 H) 4.93 (d, *J*=4.65 Hz, 1 H) 5.43 (s, 1 H) 6.03 (s, 1 H) 6.23 (dd, *J*=10.15, 1.83 Hz, 1 H) 7.00 (s, 2 H) 7.29 (d, *J*=10.15 Hz, 1 H) 7.36 (d, *J*=8.56 Hz, 2 H) 7.61 (d, *J*=8.56 Hz, 2 H) 8.05 (dd, *J*=9.17, 7.34 Hz, 2 H) 9.91 (s, 1 H).

(S)-2-((tert-Butoxycarbonyl)amino)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid.

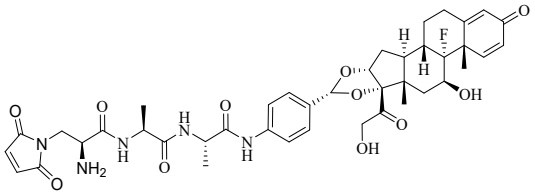
To the mixture of (R)-3-amino-2-((tert-butoxycarbonyl)amino)propanoic acid (1.0 g, 4.9 mmol) and methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate (1.14 g, 7.3 mmol) in 1,4-dioxane (10 mL) was added aqueous $NaHCO_3$ (1 mL). The mixture was stirred for 16 h at RT, adjusted to pH = 5 with aqueous HCl (1 M) and subjected to purification by prep-HPLC to afford the title compound (650 mg, 45%) as a white solid. LCMS (Method a, Table S1) Rt = xxxx min; MS m/z: xxxx $(M+H)^+$.

tert-Butyl ((S)-3-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate.

To a solution of (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (150 mg, 0.23 mmol) and (R)-2-((tert-butoxycarbonyl)amino)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid (133 mg, 0.47 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (0.12 mL, 0.70 mmol) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-4-ium tetrafluoroborate (85 mg, 0.26 mmol). The reaction mixture was stirred for 2 h at RT then subjected to purification by prep-HPLC to afford the title compound (90 mg, 37%) as a white solid. LCMS (Method h, Table S1) Rt = xxxx min; MS m/z: xxxx $(M+H)^+$. ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 9.76 (s, 1H), 8.37 (br d, *J*=7.2 Hz, 1H), 7.99 (br d, *J*=7.5 Hz, 1H), 7.63 (br d, *J*=8.4 Hz, 2H), 7.36 (d, *J*=8.7 Hz, 2H), 7.30 (d, *J*=10.0 Hz, 1H), 7.03 (s, 1H), 7.07 - 6.94 (m, 1H), 6.99 (d, *J*=7.8 Hz, 1H), 6.24 (dd, *J*=1.8, 10.1 Hz, 1H), 6.03 (s, 1H), 5.46 (br d, *J*=2.6

Hz, 1H), 5.43 (s, 1H), 5.41 - 5.40 (m, 1H), 5.11 (t, *J*=5.9 Hz, 1H), 4.94 (d, *J*=4.4 Hz, 1H), 4.53 (dd, *J*=6.2, 19.7 Hz, 1H), 4.37 (br t, *J*=7.2 Hz, 1H), 4.27 - 4.09 (m, 4H), 3.68 (br d, *J*=6.4 Hz, 1H), 3.59 (br dd, *J*=7.7, 14.1 Hz, 1H), 2.23 - 2.11 (m, 2H), 2.05 (br d, *J*=12.3 Hz, 1H), 1.85 (br s, 1H), 1.74 - 1.61 (m, 3H), 1.51 (s, 3H), 1.31 (s, 12H), 1.19 (br d, *J*=7.1 Hz, 3H), 0.87 (s, 3H).

(S)-2-Amino-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide (DL6).



To a solution of tert-butyl ((S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (10 mg, 0.011 mmol) in DCM (0.5 mL) was added TFA (8.50 µL, 0.11 mmol). The mixture was stirred for 30 min at RT and then subjected to purification by prep-HPLC to afford the title compound (5 mg, 53%) as a white solid. LCMS (Method h, Table S1) Rt = xxxx min; MS m/z: xxxxx $(M+H)^+$. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm 10.07 (s, 1H), 8.83 (d, *J*=7.8 Hz, 1H), 8.34 (br d, *J*=7.3 Hz, 1H), 8.28 (br s, 2H), 7.58 (d, *J*=8.6 Hz, 2H), 7.36 (d, *J*=8.7 Hz, 2H), 7.29 (d, *J*=10.1 Hz, 1H), 7.10 (s, 2H), 6.23 (dd, *J*=1.9, 10.1 Hz, 1H), 6.03 (s, 1H), 5.45 (br s, 1H), 5.44 (s, 1H), 5.44 - 5.42 (m, 1H), 5.11 (br s, 1H), 4.94 (d, *J*=3.9 Hz, 1H), 4.52 (br d, *J*=18.8 Hz, 1H), 4.39 (br dd, *J*=5.1, 7.0 Hz, 2H), 4.20 (br d, *J*=19.6 Hz, 2H), 3.86 (br s, 1H), 3.76 (br d, *J*=4.0 Hz, 2H), 2.37 (br s, 1H), 2.22 - 2.11 (m, 1H), 2.10 - 1.97 (m, 1H), 1.86 (br d, *J*=6.2 Hz, 1H), 1.68 (br s, 3H), 1.51 (s, 3H), 1.40 (br d, *J*=7.5 Hz, 1H), 1.29 (d, *J*=7.2 Hz, 3H), 1.21 - 1.11 (m, 1H), 1.17 (d, *J*=7.0 Hz, 2H), 0.87 (s, 3H).

tert-Butyl 1-(2-hydroxyethyl)piperidine-4-carboxylate.

To a solution of tert-butyl piperidine-4-carboxylate (3.0 g, 16.2 mmol) in toluene (30 mL) was added 2-bromoethanol (2.4 g, 19.4 mmol), sodium carbonate (3.4 g, 32.4 mmol) and sodium iodide (0.24 g, 1.6 mmol) at 120 °C. The reaction mixture was stirred at 120 °C for 12 h then subjected to purification by prep-HPLC to afford the title compound (2.8 g, 75%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.598 min; MS m/z: 230.2 $(M+H)^+$.

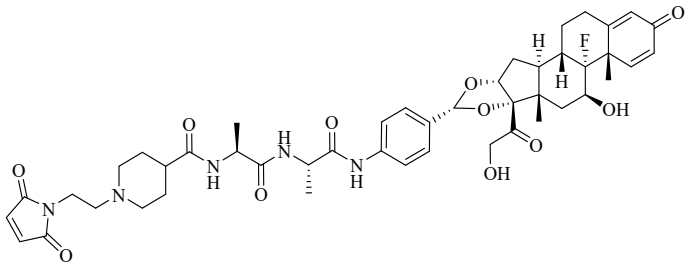
tert-Butyl 1-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)piperidine-4-carboxylate.

To a solution of 1H-pyrrole-2, 5-dione (0.93 g, 9.6 mmol), triphenylphosphine (3.15 g, 12.0 mmol) in THF (10 mL) was added tert-butyl 1-(2-hydroxyethyl)piperidine-4-carboxylate (1.1 g, 4.8 mmol) at 0 °C and the reaction stirred at 0 °C for 30 min. Then (E)-diisopropyl diazene-1, 2-dicarboxylate (2.2 mL, 12.0 mmol) was added at 0 °C. The mixture stirred at RT for 12 h then subjected to purification by prep-HPLC to afford the title compound (300 mg, 10%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.889 min; MS m/z: 309.4 $(M+H)^+$.

1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)piperidine-4-carboxylic acid.

To a solution of tert-butyl 1-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl) piperidine-4-carboxylate (300 mg, 0.97 mmol) in DCM (3 mL) was added TFA (3 mL) at RT. The mixture was stirred at RT for 1 h, combined with an identical reaction and dried by lyophilization to afford the title compound (270 mg, 85%) as yellow oil. LCMS (Method b, Table S1) Rt = 0.176 min; MS m/z: 253.4 $(M+H)^+$.

1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)piperidine-4-carboxamide (DL5).



To a solution of (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (197 mg, 0.78 mmol) in DMF (3 mL) was added PYAOP (245 mg, 0.47 mmol), 1-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)piperidine-4-carboxylic acid (200 mg, 0.31 mmol) and N,N-diisopropylethylamine (0.16 mL, 0.94 mmol) at RT. The mixture was stirred at RT for 2 h then subjected to purification by prep-HPLC to afford the title compound (100 mg, 41%) as a white solid. LCMS (Method e, Table S1) Rt = 1.916 min; MS m/z: 874.2 $(M+H)^+$. ^{1}H NMR 15042494-1506-p1k (400 MHz, DMSO-d_6) δ ppm 0.89 (s, 3 H) 1.18 - 1.26 (m, 3 H) 1.31 (br d, J=7.00 Hz, 3 H) 1.38 - 1.54 (m, 4 H) 1.64 - 1.80 (m, 5 H) 1.81 - 1.89 (m, 1 H) 1.95 (br d, J=13.76 Hz, 2 H) 2.00 - 2.10 (m, 2 H) 2.12 - 2.23 (m, 1 H) 2.38 (br s, 1 H) 2.56 - 2.67 (m, 1 H) 2.91 - 3.04 (m, 2 H) 3.29 (br d, J=4.13 Hz, 2 H) 3.64 (br d, J=11.13 Hz, 2 H) 3.81 (br t, J=5.69 Hz, 2 H) 4.16 - 4.26 (m, 2 H) 4.27 - 4.43 (m, 2 H) 4.53 (br d, J=19.39 Hz, 1 H) 4.95 (br d, J=3.88 Hz, 1 H) 5.03 - 5.24 (m, 1 H) 5.39 - 5.50 (m, 2 H) 6.05 (s, 1 H) 6.25 (dd, J=10.07, 1.44 Hz, 1 H) 7.11 - 7.16 (m, 2 H) 7.31 (d, J=10.13 Hz, 1 H) 7.38 (d, J=8.63 Hz, 2 H) 7.55 - 7.67 (m, 2 H) 8.07 - 8.15 (m, 1 H) 8.20 (br d, J=7.50 Hz, 1 H) 8.92 - 9.06 (m, 1 H) 10.02 - 10.08 (m, 1 H) 11.34 - 11.37 (m, 1 H).

Ethyl 1-(2-((tert-butoxycarbonyl)amino)ethyl)azetidine-3-carboxylate.

To a solution of ethyl azetidine-3-carboxylate hydrochloride (714 mg, 5.53 mmol) in methanol (10 mL) was added acetic acid (0.58 mL, 10.05 mmol), sodium acetate (825 mg, 10.05 mmol) and tert-butyl (2-oxoethyl)carbamate (800 mg, 5.03 mmol) at RT, the mixture was stirred for 30 min at RT. Sodium triacetoxyborohydride (5.3 g, 25.1 mmol) was added at RT and the mixture was stirred for 2 h at RT. The mixture was filtered the filtrate evaporated to dryness and partitioned between EtOAc (3 X 80 mL) and water (3 X 80 mL). The combined organic layers were washed with brine (80 mL), dried (Na_2SO_4), filtered, evaporated to dryness and subjected to purification by prep-HPLC to afford the title compound (640 mg, 47%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.629 min; MS m/z: 273.2 (M+H)+.

1-(2-((tert-Butoxycarbonyl)amino)ethyl)azetidine-3-carboxylic acid.

To a solution of ethyl 1-(2-((tert-butoxycarbonyl)amino)ethyl)azetidine-3-carboxylate (640 mg, 2.35 mmol) in methanol (2 mL), THF (2 mL) and water (2 mL) was added LiOH (338 mg, 14.10 mmol) at RT. The mixture was stirred for 2 h at RT, concentrated under reduced pressure, adjusted to pH = 4 with aqueous HCl (1 M) and subjected to purification by prep-HPLC to afford the title compound (390 mg, 68%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.311 min; MS m/z: 245.2 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.40 (s, 9 H) 3.11 - 3.17 (m, 2 H) 3.18 - 3.23 (m, 2 H) 3.55 - 3.65 (m, 1 H) 4.12 - 4.30 (m, 4 H).

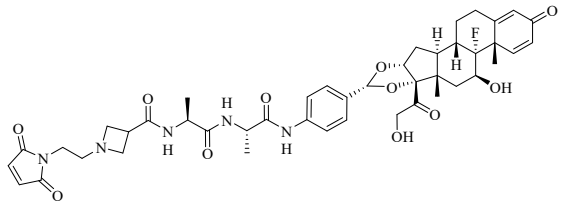
1-(2-Aminoethyl)azetidine-3-carboxylic acid.

To a solution of 1-(2-((tert-butoxycarbonyl)amino)ethyl)azetidine-3-carboxylic acid (380 mg, 1.56 mmol) in DCM (2 mL) was added TFA (2 mL) at RT. The mixture was stirred for 1 h at RT and then dried by lyophilization to afford the title compound (360 mg, 161%) as yellow oil. LCMS (Method b, Table S1) Rt = 0.139 min; MS m/z: 145.2 $(M+H)^+$.

1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)azetidine-3-carboxylic acid.

To a solution of 1-(2-aminoethyl)azetidine-3-carboxylic acid (300 mg, 2.08 mmol) in toluene (3 mL) was added furan-2, 5-dione (224 mg, 2.29 mmol) and the mixture stirred for 12 h at 110°C. The mixture was concentrated under reduced pressure and subjected to purification by prep-HPLC to afford the title compound (110 mg, 24%) as a white solid. LCMS (Method b, Table S1) Rt = 0.208 min; MS m/z: 224.9 $(M+H)^+$.

1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)azetidine-3-carboxamide (DL8).



To a solution of 1-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)azetidine-3-carboxylic acid (42.1 mg, 0.19 mmol) in DMF (3 mL) was added (6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one (60 mg, 0.09 mmol) and 2-bromo-1-ethylpyridinium tetrafluoroborate (38.5 mg, 0.14 mmol), N,N-diisopropylethylamine (0.05 mL, 0.28 mmol) at RT. The mixture was stirred for 2 h at RT, combined with an identical reaction and subjected to purification by prep-HPLC to afford the title compound (33.7 mg, 19%) as a white solid. LCMS (Method e, Table S1) Rt = 1.930 min; MS m/z: 846.2 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (s, 3 H) 1.22 (br d, *J*=6.50 Hz, 3 H) 1.29 (br d, *J*=7.13 Hz, 3 H) 1.36 - 1.53 (m, 4 H) 1.61 - 1.73 (m, 3 H) 1.79 - 1.90 (m, 1 H) 1.98 - 2.08 (m, 1 H) 2.09 - 2.21 (m, 1 H) 2.36 (br d, *J*=1.13 Hz, 2 H) 2.54 - 2.65 (m, 2 H) 3.44 - 3.67 (m, 4 H) 3.98 - 4.13 (m, 2 H) 4.20 (br d, *J*=19.26 Hz, 4 H) 4.31 - 4.43 (m, 2 H) 4.52 (br d, *J*=19.26 Hz, 1 H) 4.93 (br d, *J*=4.00 Hz, 1 H) 5.04 - 5.20 (m, 1 H) 5.40 - 5.48 (m, 2 H) 6.02 (s, 1 H) 6.23 (br d, *J*=10.01 Hz, 1 H) 7.10 (s, 2 H) 7.29 (d, *J*=10.26 Hz, 1 H) 7.36 (br d, *J*=8.63 Hz, 2 H) 7.59 (br d, *J*=8.00 Hz, 2 H) 8.26 (br t, *J*=5.75 Hz, 1 H) 8.31 - 8.48 (m, 1 H) 9.65 - 9.97 (m, 1 H) 10.04 (br d, *J*=6.13 Hz, 1 H).

tert-Butyl 3-((2-hydroxyethyl)amino)propanoate.

To a solution of 2-aminoethan-1-ol (5 g, 66.6 mmol) in methanol (50 mL) was added tert-butyl acrylate (11.02 g, 86 mmol) at RT. The mixture was stirred at for 12 h at RT, evaporated under reduced pressure and subjected to purification by column chromatography using hexane and ethyl acetate (3:1) to afford the title compound (6.7 g, 53%,) as a thick oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ=0.02 - 0.14 (m, 3 H) 1.46 (s, 8 H) 2.43 (t, *J*=6.36 Hz, 2 H) 2.73 - 2.91 (m, 4 H) 3.63 (t, *J*=5.38 Hz, 2 H).

tert-Butyl 3-((tert-butoxycarbonyl)(2-hydroxyethyl)amino)propanoate.

To a solution of tert-butyl 3-((2-hydroxyethyl)amino)propanoate (5.0 g, 26.4 mmol) in THF (50 mL) was added triethylamine (3.68 mL, 26.4 mmol), 4-dimethylaminopyridine) (0.16 g, 1.32 mmol) and di-tert-butyl dicarbonate(6.13 ml, 26.4 mmol) at RT. The mixture was stirred for 4 h at RT then partitioned between EtOAc (50 mL) and water (50 mL). The aqueous phase was extracted with DCM (3 X 40 mL) and the combined organic layers washed with brine (2 X 20 mL), dried (Na_2SO_4), filtered and evaporated to dryness to give a residue that was subjected to purification by column chromatography using DCM/MeOH (30:1) to afford the title compound (5 g, 59%) as a white solid. LCMS (Method b, Table S1) Rt = 0.688 min; MS m/z: 290.3 $(M+H)^+$.

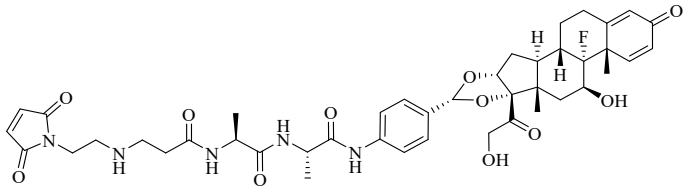
tert-Butyl 3-((tert-butoxycarbonyl) (2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) ethyl)amino)propanoate.

To a solution of 1H-pyrrole-2,5-dione (2.2 g, 22.5 mmol), triphenylphosphine (5.89 g, 22.46 mmol) in tetrahydrofuran (50 mL) was added tert-butyl 3-((tert-butoxycarbonyl)(2-hydroxyethyl)amino)propanoate (5.0 g, 17.3 mmol). The reaction mixture was cooled to -70 °C and added (E)-diisopropyl diazene-1,2-dicarboxylate (4.1 mL, 22.5 mmol) added. The mixture was stirred for 3 h at RT, partitioned between DCM (100 mL) and water (200 mL) and the aqueous layer extracted with DCM (3 X100 mL). The combined organic layers were washed with brine (2 X 40 mL), dried (Na_2SO_4), filtered, evaporated to dryness and subjected to purification by column chromatography using DCM/MeOH (30:1) to afford the title compound (5 g, 59%) Check, same as previous reaction as a white solid. LCMS (Method b, Table S1) Rt = 0.763 min; MS m/z: 369.3 $(M+H)^+$.

3-((2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)propanoic acid.

To a solution of tert-butyl 3-((tert-butoxycarbonyl) (2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) ethyl)amino)propanoate (2 g, 5.4 mmol) in dichloromethane (5 mL) and TFA (5 mL) at RT. The mixture was stirred for 1 h at RT then subjected to purification by prep-HPLC to afford the title compound (1 g, 78%) as a white solid. LCMS (Method a, Table S1) Rt = 0.273 min; MS m/z: 213.1 $(M+H)^+$.

3-((2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide (DL2).



To a solution of 3-((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)propanoic acid (199 mg, 0.94 mmol) in DMF (3 mL) was added N, N-diisopropylethylamine) (0.16 mL, 0.94 mmol), PYAOP (245 mg, 0.47 mmol), and (6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one (200 mg, 0.31 mmol) at RT. The mixture was stirred for 2 h at RT then subjected to purification by prep-HPLC to afford the title compound (87 mg, 26%) as a white solid. LCMS (Method e, Table S1) Rt = 1.975 min; MS m/z: 834.2 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 0.87 (s, 3 H) 1.21 (d, *J*=7.00 Hz, 3 H) 1.26 - 1.34 (m, 3 H) 1.35 - 1.53 (m, 4 H) 1.61 - 1.73 (m, 3 H) 1.80 - 1.90 (m, 1 H) 2.03 (br d, *J*=13.63 Hz, 1 H) 2.10 - 2.20 (m, 1 H) 2.36 - 2.47 (m, 1 H) 2.54 (br s, 3 H) 3.13 (br d, *J*=3.88 Hz, 4 H) 3.70 (br t, *J*=5.69 Hz, 2 H) 4.20 (br d, *J*=19.39 Hz, 2 H) 4.30 - 4.45 (m, 2 H) 4.52 (d, *J*=19.51 Hz, 1 H) 4.93 (d, *J*=4.25 Hz, 1 H) 5.39 - 5.50 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, *J*=10.07, 1.69 Hz, 1 H) 7.06 - 7.15 (m, 2 H) 7.25 - 7.42 (m, 3 H) 7.54 - 7.65 (m, 2 H) 8.24 (d, *J*=7.00 Hz, 1 H) 8.32 - 8.40 (m, 1 H) 8.46 (br s, 2 H) 10.03 - 10.08 (m, 1 H).

tert-Butyl 3-((2-hydroxyethyl)(methyl)amino)propanoate.

To a solution of tert-butyl acrylate (5.0 g , 66.6 mmol) in methanol (50 mL), 2-(methylamino)ethan-1-ol (9.0 g, 69.9 mmol) was added at RT. The mixture was stirres for 24 h at RT, evaporated to dryness and subjected to purifaction by prep-HPLC to afford the title compound (6.7 g, 53%) as a thick oil that was used directly in the next step.

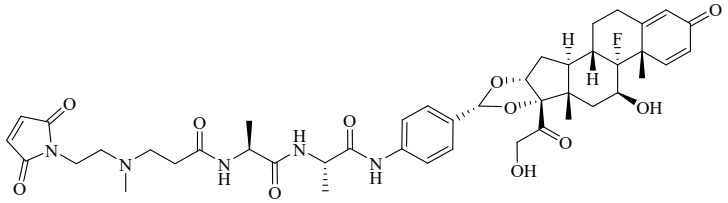
tert-Butyl 3-((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)(methyl)amino)propanoate.

To a stirred solution of DIAD (1.17 mL, 6.40 mmol) and triphenylphosphine (1.7 g, 6.4 mmol) in 10 mL of THF at -78 °C was added 1H-pyrrole-2,5-dione (621 mg, 6.4 mmol) and tert-butyl 3-((2-hydroxyethyl)(methyl)amino)propanoate (1.0 g, 4.9 mmol) in THF (10 mL). The mixture was allowed to warm to RT and stirred overnight. The mixture was diluted with DCM (20 mL), washed with brine (2 X 50 mL), dried (Na_2S0_4), filtered and subjected to purification by column chromatography using WtOAC/heptane to afford the title compound (903 mg, 43%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.139 min; MS m/z: 204.2 $(M+H)^+$.

3-((2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)(methyl)amino)propanoic acid.

A solution of tert-butyl 3-((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)(methyl)amino)propanoate (100 mg, 0.35 mmol) and TFA (0.5 mL) in DCM (0.5 mL) was stirred for 2 h at RT. The mixture was lyophilized to afford the title compound (50 mg, 50%) as a brown solid. LCMS (Method h, Table S1) Rt = 0.264 min; MS m/z: 227.1 $(M+H)^+$.

3-((2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)(methyl)amino)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide (DL3).



To a solution of (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (118 mg, 0.18 mmol) in DMF (2 mL) was added EEDQ (137 mg, 0.55 mmol) at RT and the mixture stirred at for 1 h at RT. 3-((2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)(methyl)amino)propanoic acid (50 mg, 0.22 mmol) was added, the mixture stirred for 2 h at RT then subjected to purification by prep-HPLC to afford the title compound (31 mg, 19%) as a yellow solid. LCMS (Method i, Table S1) Rt = 1.937 min; MS m/z: 848.4 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (s, 3 H) 1.22 (br d, *J*=7.00 Hz, 3 H) 1.29 (br d, *J*=7.00 Hz, 3 H) 1.40 (m, 1 H) 1.50 (s, 3 H) 1.63 - 1.73 (m, 3 H) 1.80 - 1.90 (m, 1 H) 2.00 - 2.08 (m, 1 H) 2.10 - 2.20 (m, 1 H) 2.31 - 2.39 (m, 1 H) 2.57 - 2.72 (m, 4 H) 2.76 - 2.82 (m, 3 H) 3.22 - 3.39 (m, 4 H) 3.76 (br d, *J*=5.50 Hz, 3 H) 4.15 - 4.23 (m, 2 H) 4.32 - 4.42 (m, 2 H) 4.52 (d, *J*=19.51 Hz, 1 H) 4.93

(d, *J*=4.00 Hz, 1 H) 5.40 - 5.52 (m, 2 H) 6.03 (s, 1 H) 6.23 (m, 1 H) 7.10 (s, 2 H) 7.26 - 7.42 (m, 3 H) 7.55 - 7.65 (m, 2 H) 8.25 (br d, *J*=7.50 Hz, 1 H) 8.37 (br d, *J*=7.00 Hz, 1 H) 9.25 - 9.42 (m, 1 H) 9.97 - 10.10 (m, 1 H).

Ethyl (R)-1-(2-((tert-butoxycarbonyl)amino)ethyl)piperidine-3-carboxylate.

To a solution of ethyl (R)-piperidine-3-carboxylate (494 mg, 3.1 mmol) in methanol (15 mL) was added acetic acid (0.36 mL, 6.28 mmol), sodium acetate (515 mg, 6.28 mmol) and tert-butyl (2-oxoethyl)carbamate (500 mg, 3.14 mmol) at RT and the mixture stirred for 30 min at RT. Sodium triacetoxyborohydride (3.3 g, 15.7 mmol) was added the mixture stirred for 2 h at RT. The mixture was combined with two identical reactions and partitioned between EtOAc (3 X 100 mL) and water (3 X 100 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), filtered, evaporated to dryness and subjected to purification by prep-HPLC to afford the title compound (900 mg, 40%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.732 min; MS m/z: 301.2 $(M+H)^+$.

(R)-1-(2-((tert-Butoxycarbonyl)amino)ethyl)piperidine-3-carboxylic acid.

To a solution of ethyl (R)-1-(2-((tert-butoxycarbonyl)amino)ethyl)piperidine-3-carboxylate (800 mg, 2.7 mmol) in THF (3 mL), methanol (3 mL) and water (3 mL) was added LiOH (383 mg, 16.0 mmol) at RT and the mixture stirred for 1 h at RT. The mixture was adjusted to pH = 4 with aqueous HCl (1 M) then subjected to purification by prep-HPLC to afford the title compound (560 mg, 69%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.302 min; MS m/z: 273.2 $(M+H)^+$.

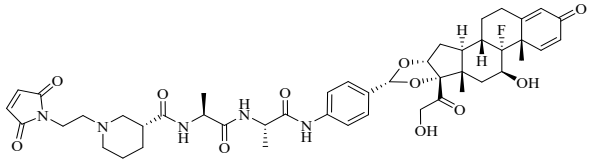
(R)-1-(2-Aminoethyl)piperidine-3-carboxylic acid.

To a solution of (R)-1-(2-((tert-butoxycarbonyl)amino)ethyl)piperidine-3-carboxylic acid (560 mg, 2.05 mmol) in DCM (2 mL) and TFA (2 mL) at RT. The mixture was stirred for 1 h at RT then dried by lyophilization to afford the title compound (350 mg, 99%) as a colourless oil which was used directly in the next step. LCMS (Method b, Table S1) Rt = 0.146 min; MS m/z: 173.2 $(M+H)^+$.

(R)-1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)piperidine-3-carboxylic acid.

To a solution of (R)-1-(2-aminoethyl)piperidine-3-carboxylic acid (170 mg, 0.99 mmol) in acetic acid (3 mL) was added furan-2,5-dione (106 mg, 1.08 mmol) at RT and the mixture then stirred for 36 h at 60°C. The mixture was combined with an identical reaction and subjected to purification by prep-HPLC to afford the title compound (210 mg, 42%) as a white solid. LCMS (Method b, Table S1) Rt = 0.150 min; MS m/z: 253.2 $(M+H)^+$.

(R)-1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)piperidine-3-carboxamide (DL4).



To a solution of (R)-1-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)piperidine-3-carboxylic acid (104 mg, 0.41 mmol) in DMF (3 mL) was added (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (105 mg, 0.16 mmol), 2-bromo-1-ethylpyridin-1-ium tetrafluoroborate (53.9 mg, 0.20 mmol), and N, N-diisopropylethylamine (0.09 mL, 0.49 mmol) at RT. The mixture stirred for 2 h at RT then subjected to purification by prep-HPLC to afford the title compound (105 mg, 36%) as a white solid. LCMS (Method e, Table S1) Rt = 1.956 min; MS m/z: 874.1 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (s, 3 H) 1.18 - 1.25 (m, 3 H) 1.25 - 1.31 (m, 3 H) 1.35 - 1.47 (m, 2 H) 1.50 (s, 3 H) 1.57 - 1.73 (m, 4 H) 1.81 - 1.96 (m, 3 H) 2.03 (br d, *J*=13.51 Hz, 1 H) 2.11 - 2.21 (m, 1 H) 2.35 - 2.46 (m, 1 H) 2.63 (br s, 1 H) 2.83 - 3.12 (m, 3 H) 3.22 - 3.33 (m, 3 H) 3.51 - 3.62 (m, 3 H) 3.68 - 3.91 (m, 2 H) 4.14 - 4.24 (m, 2 H) 4.28 - 4.42 (m, 2 H) 4.52 (d, *J*=19.39 Hz, 1 H) 4.93 (d, *J*=3.88 Hz, 1 H) 5.41 - 5.47 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, *J*=10.07, 1.69 Hz, 1 H) 7.08 - 7.14 (m, 2 H) 7.29 (d, *J*=10.13 Hz, 1 H) 7.36 (d, *J*=8.63 Hz, 2 H) 7.58 (d, *J*=8.63 Hz, 2 H) 8.13 (d, *J*=7.13 Hz, 1 H) 8.27 - 8.46 (m, 1 H) 8.74 - 9.24 (m, 1 H) 10.02 - 10.11 (m, 1 H).

Ethyl (S)-1-(2-((tert-butoxycarbonyl)amino)ethyl)piperidine-3-carboxylate.

To a solution of ethyl (S)-piperidine-3-carboxylate (1.28 g, 8.2 mmol) in methanol (20 mL) was added acetic acid (0.93 mL, 16.3 mmol), sodium acetate (1.34 g, 16.3 mmol) and tert-butyl (2-oxoethyl)carbamate (1.3 g, 8.2 mmol)

at RT and the mixture stirred for 30 min at RT. Sodium triacetoxyborohydride (8.65 g, 40.8 mmol) was added and the mixture stirred for 2 h at RT. LCMS showed the product with desired Ms was generated. One additional vial was set up as described above. The mixture was combined with an identical reaction and partitioned between EtOAc (3 X 100 mL) and water (3 X 100 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), filtered, evaporated to dryness and subjected to purification by prep-HPLC to afford the title compound (1.3 g, 47%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.706 min; MS m/z: 301.2 $(M+H)^+$.

(S)-1-(2-((tert-Butoxycarbonyl)amino)ethyl)piperidine-3-carboxylic acid.

To a solution of ethyl (S)-1-(2-((tert-butoxycarbonyl)amino)ethyl)piperidine-3-carboxylate (1.2 g, 4.0 mmol) in methanol (5 mL), THF (5 mL) and water (5 mL) was added LiOH (0.574 g, 24.0 mmol) at RT and the mixture stirred for 1 h at RT. The mixture was combined with an identical reaction, adjusted to pH = 4 with aqueous HCl (1 M), and subjected to purification by prep-HPLC to afford the title compound (780 mg, 39%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.613 min; MS m/z: 273.2 $(M+H)^+$.
Check retention time

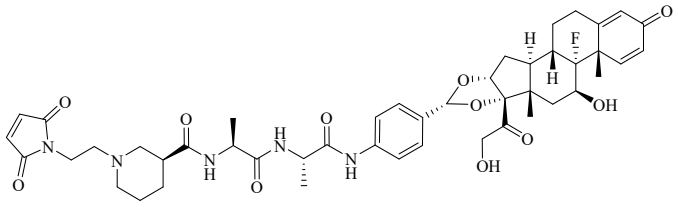
(S)-1-(2-Aminoethyl)piperidine-3-carboxylic acid.

To a solution of (S)-1-(2-((tert-butoxycarbonyl)amino)ethyl)piperidine-3-carboxylic acid (680 mg, 2.5 mmol) in DCM (2 mL) was added TFA (2 mL) at RT. The mixture was stirred for 1 h at RT then dried by lyophilization to afford the title compound (430 mg, 100%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.143 min; MS m/z: 173.2 $(M+H)^+$.

(S)-1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)piperidine-3-carboxylic acid.

To a solution of (S)-1-(2-aminoethyl)piperidine-3-carboxylic acid (215 mg, 1.24 mmol) in acetic acid (2 mL) was added furan-2, 5-dione (135 mg, 1.37 mmol) at RT and the mixture then stirred for 36 h at 60 °C. The mixture was combined with an identical reaction and subjected to purification by prep-HPLC to afford the title compound (260 mg, 41%) as a white solid. LCMS (Method b, Table S1) Rt = 0.154 min; MS m/z: 253.2 $(M+H)^+$.

(S)-1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)piperidine-3-carboxamide (DL7).



To a solution of (S)-1-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)piperidine-3-carboxylic acid (128 mg, 0.51 mmol) in DMF (4 mL) was added 2-bromo-1-ethylpyridinium tetrafluoroborate (66.8 mg, 0.24 mmol), (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (130 mg, 0.203 mmol) and N, N -diisopropylethylamine (0.11 mL, 0.61 mmol) at RT and the mixture stirred for 2 h at RT. The mixture was combined with an identical reaction and subjected to purification by prep-HPLC to afford the title compound (55 mg, 15%) as a white solid. LCMS (Method e, Table S1) Rt = 1.960 min; MS m/z: 874.1 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.89 (s, 3 H) 1.19 - 1.27 (m, 3 H) 1.28 - 1.35 (m, 3 H) 1.37 - 1.49 (m, 2 H) 1.52 (s, 3 H) 1.58 - 1.75 (m, 4 H) 1.83 - 1.98 (m, 3 H) 2.05 (br d, *J*=13.01 Hz, 1 H) 2.12 - 2.23 (m, 1 H) 2.38 - 2.49 (m, 1 H) 2.56 - 2.67 (m, 2 H) 2.87 - 3.12 (m, 3 H) 3.26 - 3.34 (m, 3 H) 3.52 - 3.61 (m, 3 H) 3.79 (br t, *J*=5.82 Hz, 2 H) 4.15 - 4.27 (m, 2 H) 4.29 - 4.45 (m, 2 H) 4.54 (br d, *J*=19.51 Hz, 1 H) 4.95 (d, *J*=4.13 Hz, 1 H) 5.42 - 5.49 (m, 2 H) 6.05 (s, 1 H) 6.25 (dd, *J*=10.19, 1.56 Hz, 1 H) 7.10 - 7.16 (m, 2 H) 7.31 (d, *J*=10.13 Hz, 1 H) 7.38 (d, *J*=8.50 Hz, 2 H) 7.60 (br d, *J*=8.51 Hz, 2 H) 8.11 - 8.49 (m, 2 H) 8.76 - 9.22 (m, 1 H) 10.04 - 10.13 (m, 1 H).

Ethyl (R)-1-(2-((tert-butoxycarbonyl)amino)ethyl)pyrrolidine-3-carboxylate.

To a solution of ethyl (R)-pyrrolidine-3-carboxylate hydrochloride (720 mg, 5.03 mmol) in methanol (20 mL) was added acetic acid (0.575 mL, 10.05 mmol), sodium acetate (825 mg, 10.05 mmol) and tert-butyl (2-oxoethyl)carbamate (800 mg, 5.03 mmol) at RT and the mixture stirred for 30 min at RT. Sodium triacetoxyborohydride (5.3 g, 25.1 mmol) was added and the mixture stirred for 2 h at RT. The mixture was combined with an identical reaction and partitioned between EtOAc (3 X 200 mL) and water (3 X 200 mL). The

combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), filtered, evaporated to dryness and subjected to purification by prep-HPLC to afford the title compound (720 mg, 40%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.635 min; MS m/z: 287.0 $(M+H)^+$.

(R)-1-(2-((tert-butoxycarbonyl)amino)ethyl)pyrrolidine-3-carboxylic acid.

To a solution of ethyl (R)-1-(2-((tert-butoxycarbonyl)amino)ethyl)pyrrolidine-3-carboxylate (600 mg, 2.1 mmol) in tetrahydrofuran (3 mL), methanol (3 mL) and water (3 mL) was added LiOH (301 mg, 12.6 mmol) at RT and the mixture stirred for 2 h at RT. The mixture was combined with an identical reaction, adjusted to pH = 4 with aqueous HCl (1 M), and subjected to purification by prep-HPLC to afford the title compound (530 mg, 82%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.461 min; MS m/z: 259.0 $(M+H)^+$.

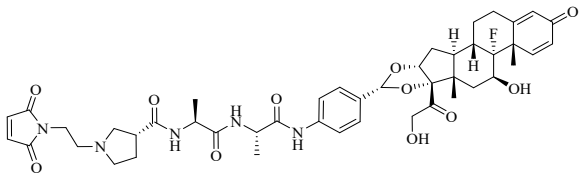
(R)-1-(2-Aminoethyl)pyrrolidine-3-carboxylic acid.

To a solution of (R)-1-(2-((tert-butoxycarbonyl)amino)ethyl)pyrrolidine-3-carboxylic acid (530 mg, 2.05 mmol) in DCM (4 mL) and TFA (1 mL) at RT. The mixture was stirred for 2 h at RT then dried by lyophilization to afford the title compound (300 mg, 92%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.140 min; MS m/z: 159.3 $(M+H)^+$.

(R)-1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)pyrrolidine-3-carboxylic acid.

To a solution of (R)-1-(2-aminoethyl)pyrrolidine-3-carboxylic acid (250 mg, 1.58 mmol) in acetic acid (3 mL) was added furan-2,5-dione (170 mg, 1.74 mmol) at RT. The mixture was stirred for 36 h at 60 °C then subjected to purification by prep-HPLC to afford the title compound (170 mg, 45%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.144 min; MS m/z: 239.2 $(M+H)^+$.

(R)-1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)pyrrolidine-3-carboxamide (DL1).



To a solution of (R)-1-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)pyrrolidine-3-carboxylic acid (158 mg, 0.66 mmol) in DMF (3 mL) was added (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (170 mg, 0.27 mmol), 2-bromo-1-ethylpyridinium tetrafluoroborate (87 mg, 0.32 mmol), and N, N-diisopropylethylamine (0.14 mL, 0.80 mmol) at RT. The mixture was stirred at for 2 h at RT then subjected to purification by prep-HPLC to afford the title compound (18 mg, 8%) as a white solid. LCMS (Method e, Table S1) Rt = 1.941 min; MS m/z: 860.3 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.86 (s, 3 H) 1.23 (br d, *J*=7.00 Hz, 3 H) 1.28 (br d, *J*=6.75 Hz, 3 H) 1.35 - 1.52 (m, 4 H) 1.60 - 1.73 (m, 3 H) 1.78 - 1.97 (m, 2 H) 1.98 - 2.20 (m, 3 H) 2.36 - 2.47 (m, 1 H) 2.53 - 2.64 (m, 1 H) 3.06 - 3.22 (m, 3 H) 3.54 - 3.61 (m, 1 H) 3.65 - 3.78 (m, 3 H) 4.13 - 4.25 (m, 2 H) 4.26 - 4.42 (m, 2 H) 4.51 (br d, *J*=19.51 Hz, 1 H) 4.93 (br d, *J*=3.75 Hz, 1 H) 5.01 - 5.24 (m, 1 H) 5.38 - 5.51 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, *J*=10.07, 1.56 Hz, 1 H) 7.10 (s, 2 H) 7.29 (d, *J*=10.13 Hz, 1 H) 7.36 (br d, *J*=8.50 Hz, 2 H) 7.57 (br d, *J*=8.50 Hz, 2 H) 7.99 - 8.22 (m, 1 H) 8.34 - 8.42 (m, 1 H) 9.58 - 9.84 (m, 1 H) 10.07 (br s, 1 H)**.**

Ethyl (S)-1-(2-((tert-butoxycarbonyl)amino)ethyl)pyrrolidine-3-carboxylate.

To a solution of ethyl (S)-pyrrolidine-3-carboxylate hydrochloride (360 mg, 5.03 mmol) in methanol (20 mL) was added acetic acid (0.29 mL, 5.03 mmol), sodium acetate (412 mg, 5.03 mmol) and tert-butyl (2-oxoethyl)carbamate (400 mg, 2.51 mmol) at RT and the mixture stirred for 30 min at RT. Sodium triacetoxyborohydride (2.7 g, 12.6 mmol) was added and the mixture stirred for 2 h at RT. The mixture was combined with two identical reactions and partitioned between EtOAc (3 X 200 mL) and water (3 X 200 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), filtered, evaporated to dryness and subjected to purification by prep-HPLC to afford the title compound (700 mg, 39%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.645 min; MS m/z: 287.0 $(M+H)^+$.

(S)-1-(2-((tert-Butoxycarbonyl)amino)ethyl)pyrrolidine-3-carboxylic acid.

To a solution of ethyl (S)-1-(2-((tert-butoxycarbonyl)amino)ethyl)pyrrolidine-3-carboxylate (600 mg, 2.1 mmol) in THF (2 mL), methanol (2 mL) and water (2 mL) was added LiOH (301 mg, 12.6 mmol) at RT and the mixture stirred for 2 h at RT. The mixture was combined with an identical reaction, adjusted to pH = 4 with aqueous HCl (1 M), and subjected to purification by prep-HPLC to afford the title compound (490 mg, 78%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.623 min; MS m/z: 259.2 $(M+H)^+$.

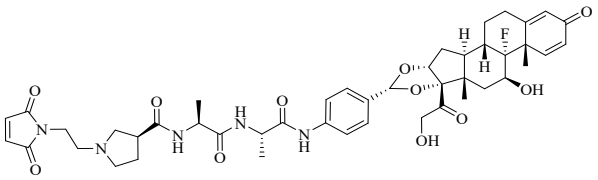
(S)-1-(2-Aminoethyl)pyrrolidine-3-carboxylic acid.

To a solution of (S)-1-(2-((tert-butoxycarbonyl)amino)ethyl)pyrrolidine-3-carboxylic acid (490 mg, 1.9 mmol) in DCM (4 mL) and TFA (2 mL) at RT. The mixture was stirred for 2 h at RT then dried by lyophilization to afford the title compound (300 mg, 100%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.144 min; MS m/z: 159.2.

(S)-1-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)pyrrolidine-3-carboxylic acid.

To a solution of (S)-1-(2-aminoethyl)pyrrolidine-3-carboxylic acid (150 mg, 0.95 mmol) in acetic acid (4 mL) was added furan-2,5-dione (102 mg, 1.04 mmol) at RT. The mixture was stirred at 60 °C for 36 h then subjected to purification by prep-HPLC to afford the title compound (160 mg, 35%) as a colourless oil. LCMS (Method b, Table S1) Rt = 0.142 min; MS m/z: 239.2

(S)-1-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)pyrrolidine-3-carboxamide (DL9).



To a solution of (S)-1-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)pyrrolidine-3-carboxylic acid (93 mg, 0.39 mmol) in DMF (3 mL) was added (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (100 mg, 0.16 mmol), 2-bromo-1-ethylpyridinium tetrafluoroborate (51.4 mg, 0.188 mmol) and N, N-diisopropylethylamine) (0.082 mL, 0.469 mmol) at RT and stirred for 2 h at RT. The mixture was combined with an identical reaction then subjected to purification by prep-HPLC to afford the title compound (35 mg, 16%) as a white solid. LCMS (Method e, Table S1) Rt = 1.938 min; MS m/z: 860.3 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (s, 3 H) 1.18 - 1.25 (m, 3 H) 1.30 (d, J=7.13 Hz, 3 H) 1.36 - 1.53 (m, 4 H) 1.61 - 1.73 (m, 3 H) 1.79 - 1.93 (m, 2 H) 1.98 - 2.22 (m, 3 H) 2.46 (br s, 1 H) 2.54 - 2.65 (m, 1 H) 3.08 - 3.24 (m, 3 H) 3.51 - 3.64 (m, 1 H) 3.65 - 3.79 (m, 3 H) 4.14 - 4.25 (m, 2 H) 4.28 - 4.44 (m, 2 H) 4.52 (d, J=19.51 Hz, 1 H) 4.93 (d, J=4.00 Hz, 1 H) 5.00 - 5.22 (m, 1 H) 5.40 - 5.49 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, J=10.13, 1.50 Hz, 1 H) 7.11 (s, 2 H) 7.29 (d, J=10.13 Hz, 1 H) 7.36 (d, J=8.63 Hz, 2 H) 7.59 (d, J=8.38 Hz, 2 H) 8.22 (br t, J=7.19 Hz, 1 H) 8.40 (br dd, J=10.13, 7.75 Hz, 1 H) 9.67 - 9.83 (m, 1 H) 10.03 (br d, J=12.13 Hz, 1 H).

tert-Butyl ((S)-5-amino-1-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1,5-dioxopentan-2-yl)carbamate.

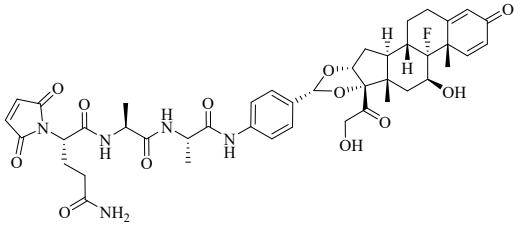
A solution of (S)-5-amino-2-((tert-butoxycarbonyl)amino)-5-oxopentanoic acid (231 mg, 0.94 mmol) in DMF (3 mL) was added DMTMM (308 mg, 0.94 mmol), N,N-diisopropylethylamine (0.33 mL, 1.88 mmol) was stirred at 0°C for 30 min then (S)-2-Amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (400 mg, 0.63 mmol) was added. The mixture stirred for 90 min at RT, added to water and filtered to afford the title compound (400 mg, 74%) as a white solid. LCMS (Method b, Table S1) Rt = 1.058 min; MS m/z: 868.1 $(M+H)^+$.

(S)-2-Amino-N1-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)pentanediamide.

To a solution of tert-butyl ((S)-5-amino-1-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-

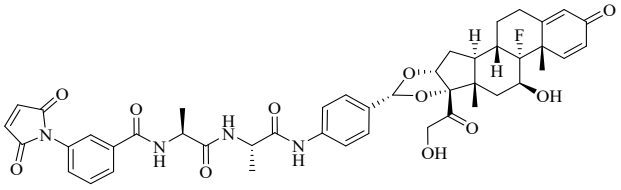
naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1,5-dioxopentan-2-yl)carbamate (400 mg, 0.46 mmol) in DCM (4 mL) was added TFA (1.3 mL) at RT. The mixture was stirred for 30 min at RT, combined with two identical reactions, MeCN and water added then lyophilized to afford the title compound (350 mg, 99%) as a white solid. LCMS (Method b, Table S1) Rt = 0.929 min; MS m/z: 768.2 $(M+H)^+$.

(S)-2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)pentanediamide (DL13).



To a solution of (S)-2-amino-N1-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)pentanediamide (50 mg, 0.06 mmol) in dioxane(2 mL) was added methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate (71 mg, 0.46 mmol) and aqueous $NaHCO_3$ (0.2 mL) at RT. The mixture was stirred for 16 h at RT, adjusted to pH = 7 with aqueous $NaHCO_3$ then subjected to purification by prep-HPLC to afford the title compound (11 mg, 19%) as a white solid. LCMS (Method d, Table S1) Rt = 1.007 min; MS m/z: 848.3 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.97 (s, 1H), 8.20 (d, *J* = 7.3 Hz, 1H), 8.11 (d, *J* = 7.2 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 10.1 Hz, 1H), 7.20 (br s, 1H), 7.02 (s, 2H), 6.71 (br s, 1H), 6.23 (dd, *J* = 1.5, 10.0 Hz, 1H), 6.03 (s, 1H), 5.43 (s, 2H), 4.93 (d, *J* = 4.6 Hz, 1H), 4.56 - 4.44 (m, 2H), 4.36 (br t, *J* = 7.0 Hz, 1H), 4.29 (br t, *J* = 7.2 Hz, 1H), 4.20 (br d, *J* = 19.3 Hz, 2H), 2.37 (br d, *J* = 3.5 Hz, 2H), 2.20 - 2.09 (m, 3H), 2.07 (br d, *J* = 4.9 Hz, 1H), 2.04 - 1.95 (m, 3H), 1.90 - 1.79 (m, 2H), 1.72 - 1.62 (m, 3H), 1.50 (s, 3H), 1.45 - 1.37 (m, 1H), 1.30 (d, *J* = 7.1 Hz, 3H), 1.16 (d, *J* = 7.1 Hz, 3H), 0.87 (s, 3H).

3-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)benzamide (DL12).



Prepared in a similar manner to compound **DL17** A-1802117 (90 mg, 34%) as a white solid. LCMS (Method j, Table S1) Rt = 2.770 min; MS m/z: 839.3 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.97 (s, 1H), 8.61 (d, *J* = 7.0 Hz, 1H), 8.24 (d, *J* = 7.1 Hz, 1H), 7.98 - 7.92 (m, 1H), 7.88 (t, *J* = 1.7 Hz, 1H), 7.65 - 7.56 (m, 3H), 7.54 - 7.48 (m, 1H), 7.37 (d, *J* = 8.7 Hz, 2H), 7.30 (d, *J* = 10.0 Hz, 1H), 7.22 (s, 2H), 6.24 (dd, *J* = 1.8, 10.1 Hz, 1H), 6.04 (s, 1H), 5.44 (s, 2H), 4.94 (d, *J* = 4.5 Hz, 1H), 4.58 - 4.38 (m, 3H), 4.25 - 4.16 (m, 2H), 2.23 - 2.12 (m, 1H), 2.09 - 2.01 (m, 1H), 1.90 - 1.82 (m, 1H), 1.74 - 1.64 (m, 3H), 1.51 (s, 3H), 1.42 (br dd, *J* = 5.0, 13.1 Hz, 1H), 1.34 (dd, *J* = 7.1, 15.5 Hz, 6H), 0.88 (s, 3H).

(9H-Fluoren-9-yl)methyl tert-butyl ((S)-5-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-5-oxopentane-1,4-diyl)dicarbamate.

To a solution of (S)-2,5-dioxopyrrolidin-1-yl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((tert-butoxycarbonyl)amino)pentanoate (259 mg, 0.47 mmol) and (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (300 mg, 0.47 mmol) in DMF (5 mL) was added N,N-

diisopropylethylamine 0.25 mL, 1.41 mmol) at RT. The mixture was stirred for 3 h at RT, added to water and filtered to afford the title compound (400 mg, 79%) as a yellow solid. LCMS (Method i, Table S1) Rt = 1.246 min; MS m/z: 976.4 $(M+H-100)^+$.

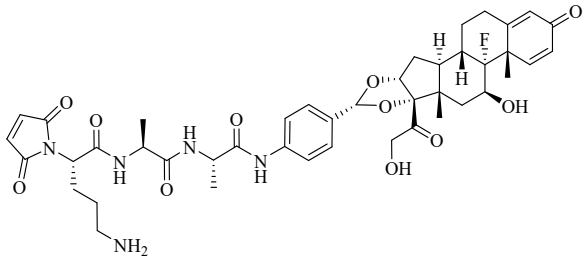
tert-Butyl ((4S)-4-amino-5-(((2S)-1-(((2S)-1-((4-((6aS,6bR,7S,8aS,8bS,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-5-oxopentyl)carbamate.

To a solution of (9H-fluoren-9-yl)methyl tert-butyl ((4S)-5-(((2S)-1-(((2S)-1-((4-((6aS,6bR,7S,8aS,8bS,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-5-oxopentane-1,4-diyl)dicarbamate (400 mg, 0.37 mmol) in MeCN (1 mL) was added piperidine (32 mg, 0.37 mmol) at RT. The mixture was stirred for 30 min at RT then subjected to purification by prep-HPLC to afford the title compound (170 mg, 0.119 mmol, 32.1%) as a white solid. LCMS (Method i, Table S1) Rt = 0.964 min; MS m/z: 854.3 $(M+H)^+$.

tert-Butyl ((S)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-5-oxopentyl)carbamate.

To a solution of tert-butyl ((S)-4-amino-5-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-5-oxopentyl)carbamate (55 mg, 0.06 mmol)in dioxane (1 mL) was added methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate (70 mg, 0.45 mmol) and aqueous $NaHCO_3$ (0.1mL) at RT. The mixture was stirred for 16 h at RT then subjected to purification by prep-HPLC to afford the title compound (23mg, 18%) as a white solid. LCMS (Method b, Table S1) Rt = 1.098 min; MS m/z: 834.2 $(M+H-100)^+$.

(S)-5-amino-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)pentanamide (DL17).



To a solution of tert-butyl ((S)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-(((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-5-oxopentyl)carbamate (45 mg, 0.048 mmol) in DCM (1 mL) was added DCM (1 mL) and TFA (0.34 mL) at RT. The mixture was stirred for 30 min at RT then subjected to purification by prep-HPLC to afford the title compound (17 mg, 42% yield) as a white solid. LCMS (Method b, Table S1) Rt = 0.932 min; MS m/z: 834.2 $(M+H-100)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (s, 3 H) 1.16 (d, *J*=7.09 Hz, 3 H) 1.29 (d, *J*=7.09 Hz, 3 H) 1.43 (br d, *J*=7.34 Hz, 3 H) 1.50 (s, 3 H) 1.63 - 1.74 (m, 3 H) 1.80 - 1.90 (m, 1 H) 1.97 - 2.09 (m, 4 H) 2.12 - 2.21 (m, 2 H) 2.36 (br s, 2 H) 2.76 (br d, *J*=6.48 Hz, 1 H) 4.20 (br d, *J*=19.44 Hz, 2 H) 4.28 - 4.41 (m, 2 H) 4.45 - 4.56 (m, 2 H) 4.94 (d, *J*=4.03 Hz, 1 H) 5.08 (br s, 1 H) 5.43 (s, 2 H) 6.03 (s, 1 H) 6.23 (dd, *J*=10.15, 1.71 Hz, 1 H) 7.08 (s, 2 H) 7.29 (d, *J*=10.15 Hz, 1 H) 7.36 (br d, *J*=8.68 Hz, 2 H) 7.58 (br d, *J*=8.44 Hz, 5 H) 8.08 - 8.22 (m, 2 H) 10.00 (s, 1 H).

LCMS Conditions

Table S1

Method	Description

a	Gradient: 10-80% B in 2.0 min, 80-80% B in 0.5 min, 80-10% B in 0.01 min, and then hold at 10% B for 0.5 min. Mobile phase A was 10 mM NH4HCO3, mobile phase B was MeCN. Column: 2.1 x 50 mm Xbridge C18 column, 5 μm particles. Flow rate: 1 mL/min.
b	Gradient: 5-95% B in 2.00 min. 5% B in 0.01 min, 5-95% B (0.01-1.00 min), 95-100% B (1.00 -1.80 min), 5% B in 1.81 min with a hold at 5% B for 0.19 min. Mobile phase A was 0.037% TFA in water, and mobile phase B was 0.018% TFA in MeCN. Column: Luna-C18 2.0×30mm, 3 um particles. Flow rate: 1.0 mL/min (0.00-1.80 min) and 1.2 mL/min (1.81 -2.00 min).
c	Gradient: 0-60% B in 2.1 min, 60-60% B in 0.4 min, 60-0% B in 0.01 min, and then hold at 0% B for 0.5 min. Mobile phase A was 10 mM NH4HCO3, mobile phase B was HPLC grade MeCN. Column: 2.1 x 50 mm Xbridge C18 column (5 μm particles). Flow rate: 0.8 mL/min.
d	Gradient: 5-90% B in 3 min, 90-100% B to 0.85 min, 100-5% B in 0.01 min, and then held at 5% B for 0.65 min. Mobile phase A was 10 mM NH4HCO3, mobile phase B was MeCN. Column: 2.0 x50 mm Gemini-C18 column (3 μm particles). Flow rate: 0.6 mL/min.
e	Gradient: 5% B in 0.40 min and 5-95% B at 0.40-3.00 min, hold on 95% B for 1.00min, and then 95-5%B in 0.01min. Mobile phase A was 0.037% TFA in water, mobile phase B was 0.018% TFA in MeCN. Column: Kinetex C18 50*2.1mm, 5um particles. Flow rate 1.0 ml/min.
f	Gradient: 25-100% B in 3.4 min with a hold at 100% B for 0.45 min, 100-25% B in 0.01 min, and then held at 25% B for 0.65 min. Mobile phase A was 0.0375% TFA in water, mobile phase B was 0.018% TFA in MeCN. Column 2.1x 30 mm SunFire C18 3.5 μm. Column: Kinetex C18 50*2.1mm, 5um particles. Flow rate: 0.8 mL/min flow rate.
g	Gradient: 10-90% B in 1.15 with a hold at 90% B for 0.4 min, 90-10% B in 0.01 min, and then hold at 10% B for 0.54 min. Mobile phase A was 0.0375% TFA in water, mobile phase B was 0.018% TFA in MeCN. Column:2.1 x 30 mm Halo C18, 2.7 μm particles. Flow rate:1.0 mL/min.
h	Gradient: 5-95% B in 0.7 min, 95-95% B in 0.45 min, 95-5% B in 0.01 min, and then held at 0% B for 0.44 min). Mobile phase A was 0.0375% TFA in water, mobile phase B was 0.018% TFA in MeCN. Column: Chromolith Flash RP-18e 25-2mm. Flow rate: 1.5 mL/min.
i	Gradient: 5-95% B in 2.05 min .5% B in 0.01 min, 5-95% B (0.01-1.00 min), 95-100% B (1.00 -1.80 min), 5% B in 1.81 min with a hold at 5% B for 0.24 min. Mobile phase A was 10 mM NH4HCO3 in water, and mobile phase B was MeCN. Column: Xbridge Shield RP18 2.1*50mm, (5 um particles). Flow rate: 1.0 mL/min.
j	Gradient: 1-90% B in 3.4 min, 90-100% B in 0.45 min, 100-1% B in 0.01 min, and then held at 1% B for 0.65 min. Mobile phase A was 0.0375% TFA in water, mobile phase B was 0.018% TFA in MeCN. Column: 2.0 x 50 mm Phenomenex Luna-C18 column, 5 μm particles. Flow rate: 0.8 mL/min.