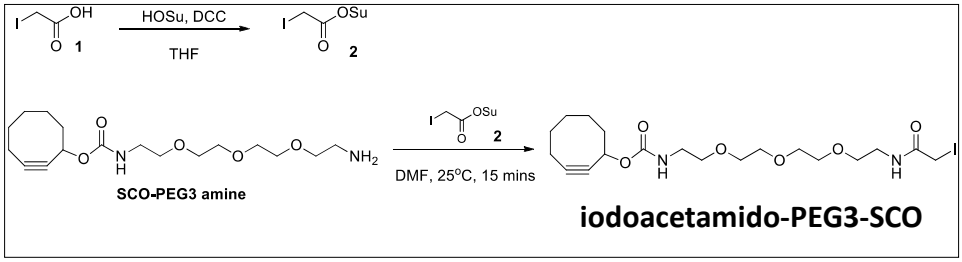
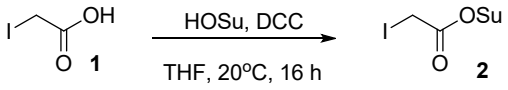
Supporting Information

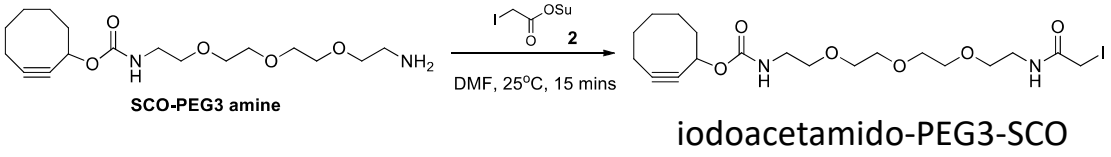
Synthesis of linker-payloads

Synthesis of iodoacetamido-PEG3-SCO:





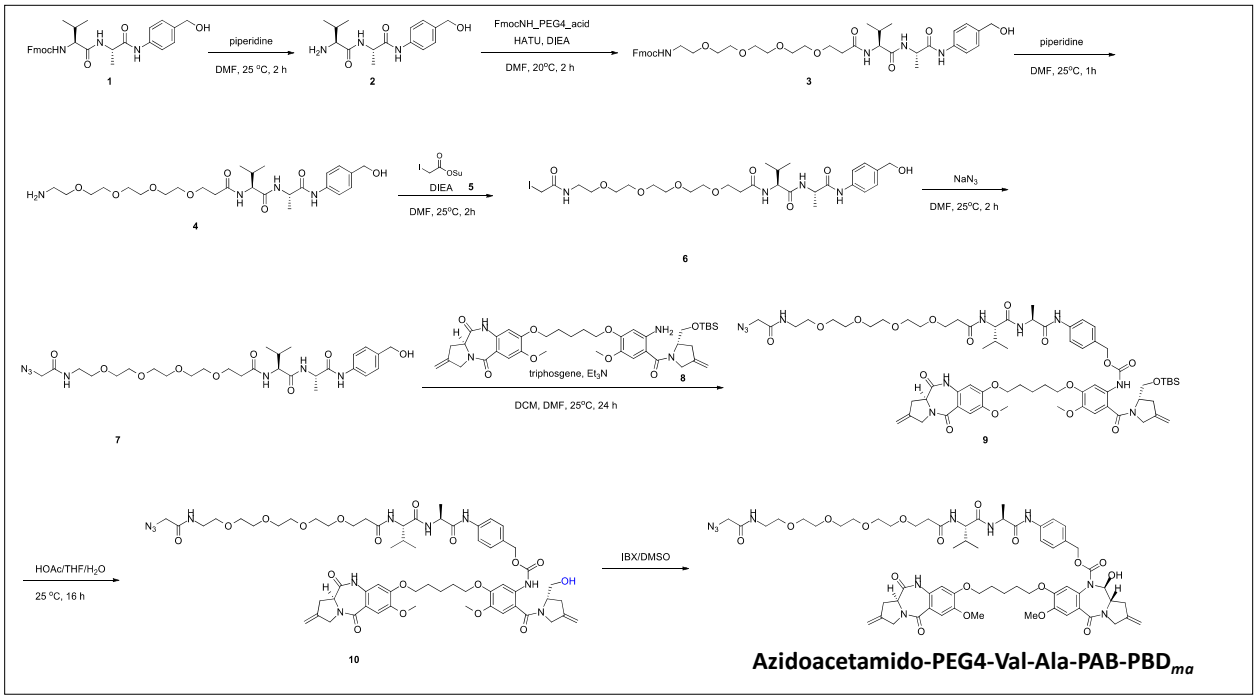
To a mixture of *2-iodoacetic acid* (1.0 g, 5.38 mmol) in anhydrous THF (25 mL) was added *N-hydroxysuccinimide* (HOSu, 0.68 g, 5.92 mmol), followed by *N,N-dicyclohexylcarbodiimide* (DCC, 1.22 g, 5.92 mmol). The reaction mixture was stirred at 20 °C for 16 h. The mixture was filtered, and the filtrate was concentrated to afford *2,5-dioxopyrrolidin-1-yl 2-iodoacetate* as yellow solid (**2**, 1.52 g, 100%). ^{1}HNMR (400MHz, $CDCl_3$) δ 3.95 (s, 2H), 2.86 (s, 4H).

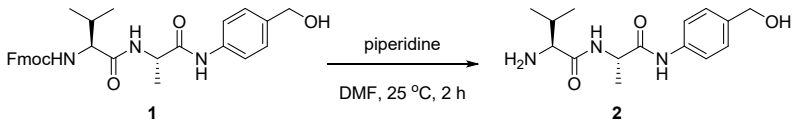


To a solution of **SCO-PEG3 amine** (SiChem, 70.0 mg, 0.20 mmol) in DMF (3 mL) was added *2,5-dioxopyrrolidin-1-yl 2-iodoacetate* (**2**, 87 mg, 0.31mmol). The mixture was stirred at 25 °C for 15min. The mixture was purified by reverse phase chromatography (acetonitrile 25-55/0.225% FA in water) to afford **iodoacetamido-PEG3-SCO** as a pale yellow oil (35.2 mg, 33.3%). ^{1}H NMR (400MHz, $CDCl_3$) δ 6.82 (brs, 1H), 5.31 (brs, 2H), 3.73 - 3.71 (m, 2H), 3.66-

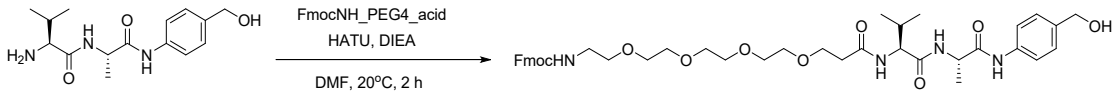
3.64 (m, 8H), 3.60 - 3.55 (m, 4H), 3.49-3.66 (m, 2H), 3.38 - 3.36 (m, 2H), 2.25 - 2.16 (m, 3H), 1.92 - 1.87 (m, 4H), 1.68 - 1.65 (m, 2H), 1.57 - 1.48 (m, 1H). LCMS (5-95, AB, 1.5min): R_T (220/254nm) = 0.880 min, m/z = 511.1$[M+H]^+$.

Synthesis of azidoacetamido-PEG4-Val-Ala-PAB-PBD$_{ma}$:

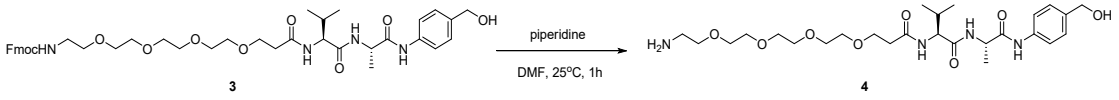




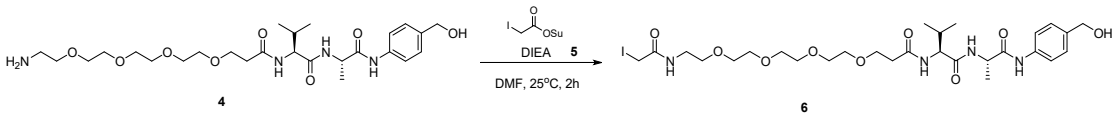
To a solution of *(9H-fluoren-9-yl)methyl((S)-1-(((S)-1-((4-(hydroxymethyl) phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate* (**1**, 750.0 mg, 1.45 mmol) in DMF (20 mL) was added piperidine (0.2 mL, 1.45 mmol), the mixture was stirred at 25°C for 2h. The mixture was concentrated and washed with methyl tertiary butyl ether (20 mL x 3), the precipitate was collected to afford *(S)-2-amino-N-((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)-3-methylbutanamide* as a white solid (**2**, 400 mg, 93.7%), which was used in next step directly.



To a mixture of *1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16-pentaoxa-4-azanonadecan-19-oic acid* (**FmocNH-PEG4-acid**, Broadpharm, 797.7 mg, 1.64 mmol) in anhydrous N,N-dimethylformamide (20 mL) was added *2-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate* (HATU, 622.2 mg, 1.64 mmol), followed by N,N-Diisopropylethylamine (DIEA, 528.7 mg, 4.09 mmol). The reaction solution was stirred at 20 °C for 10 min. Then *(S)-2-amino-N-((S)-1-((4-(hydroxymethyl) phenyl)amino)-1-oxopropan-2-yl)-3-methylbutanamide* (**2**, 400.0 mg, 1.36 mmol) was added. The reaction mixture was stirred at 20°C for 2h. TLC (10% MeOH in DCM, Rf = 0.4) showed the reaction had gone completion. The mixture was concentrated to afford crude product which was purified by flash chromatography eluting with 0-10% MeOH in DCM to afford *(9H-fluoren-9-yl)methyl ((17S,20S)-21-((4-(hydroxymethyl)phenyl)amino)-17-isopropyl-20-methyl-15,18,21-trioxo-3,6,9,12-tetraoxa-16,19-diazahenicosyl)carbamate* as a yellow solid (**3**, 500 mg, 35.5%). LCMS (5-95, AB, 1.5min): R_T = 0.794 min, m/z = 785.3[M+Na]$^+$.

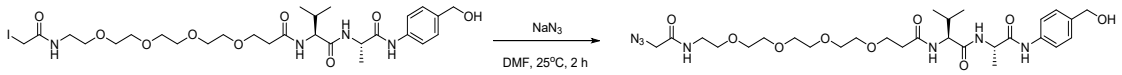


A solution of *(9H-fluoren-9-yl)methyl ((17S,20S)-21-((4-(hydroxymethyl)phenyl)amino)-17-isopropyl-20-methyl-15,18,21-trioxo-3,6,9,12-tetraoxa-16,19-diazahenicosyl)carbamate* (**3**, 500.0 mg, 0.66 mmol) and piperidine (1.0 mL, 10.92mmol) in DMF (5 mL) was stirred at 25°C for 1h. The solvent were concentrated in vacuo, the residue was washed with methyl tertiary-butyl ether (10 mL x 3), and the filtered cake was dried in *vacuo* to afford *1-amino-N-((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3,6,9,12-tetraoxapentadecan-15-amide* (**4**, 350 mg, 98.8%) as a white solid. LCMS (5-95, AB, 1.5min): RT = 0.567 min, m/z = 541.3[M+H]$^+$.



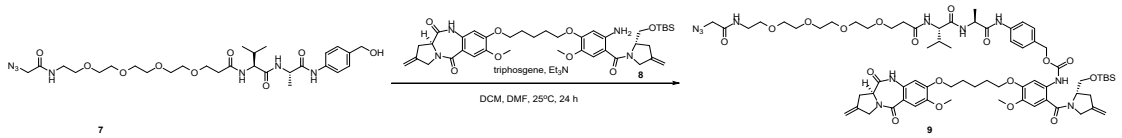
To a mixture of *2,5-dioxopyrrolidin-1-yl 2-iodoacetate* (**5**, 267 mg, 0.94 mmol) in anhydrous N,N-dimethylformamide (15 mL) was added *1-amino-N-((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-*

yl)amino)-3-methyl-1-oxobutan-2-yl)-3,6,9,12-tetraoxapentadecan-15-amide (**4**, 340.0 mg, 0.63 mmol). The reaction mixture was stirred at 25°C for 2h. The mixture was concentrated to afford crude *N-((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-1-(2-iodoacetamido)-3,6,9,12-tetraoxapentadecan-15-amide* (**6**, 445 mg, theoretical yield) as a yellow solid which was used in next step directly. LCMS (5-95, AB, 1.5min): RT = 0.642 min, m/z = 731.2[M+Na]$^+$.



6 7

To a solution of *N-((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl) amino)-3-methyl-1-oxobutan-2-yl)-1-(2-iodoacetamido)-3,6,9,12-tetraoxapentadecan-15-amide* (**6**, 445.0 mg, 0.63 mmol) in N,N-Dimethylformamide (15 mL) was added sodium azide (120.0 mg, 1.85 mmol). The formed mixture was stirred at 25°C for 2h. TLC (10% MeOH in DCM, Rf = 0.4) showed the reaction had gone to completion. Then most of N,N-dimethylformamide was removed and methyl tertiary butyl ether (15 mL) was added to the mixture, and the precipitate was collected and washed with methyl tertiary butyl ether (5 mL x 2), the filter cake was purified by flash chromatography eluting with 0-10% MeOH in DCM to afford *1-(2-azidoacetamido)-N-((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3,6,9,12-tetraoxapentadecan-15-amide* (**7**, 360 mg, 89.2%) as a yellow solid. LCMS (5-95, AB, 1.5min): R_T = 0.773 min, m/z = 646.1 [M+Na]$^+$.

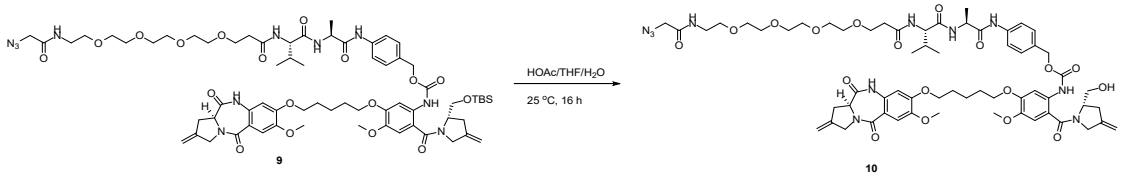


7 8 9

To a mixture of triphosgene (20.2 mg, 0.07 mmol) and 4 Å MS (100 mg) in anhydrous dichloromethane (4 mL) was added a solution of *(6aS)-3-[5-[5-amino-4-[(2S)-2-[[tert-butyl(dimethyl)silyl]oxymethyl]-4-methylene-pyrrolidine-1-carbonyl]-2-methoxy-phenoxy]pentoxy]-2-methoxy-8-methylene-5,6a,7,9-tetrahydropyrrolo[2,1-c][1,4]benzodiazepine-6,11-dione* (**8**, 50.0 mg, 0.07 mmol) and trimethylamine (21 mg, 0.20 mmol) in anhydrous dichloromethane (4 mL). The reaction mixture was stirred at 25 °C for 0.5h. The mixture was concentrated in vacuo to give the crude product which was used for the next step directly.

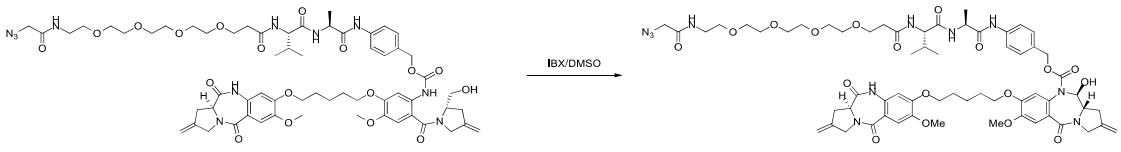
To a solution of *(2S)-2-[3-[2-[2-[2-[2-[(2-azidoacetyl)amino]ethoxy]ethoxy] ethoxy]ethoxy]propanoylamino]-N-[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]-3-methyl-butanamide* (**7**, 41.8 mg, 0.07 mmol) and 4A molecular sieves in DCM (5 mL) was added a solution of triethylamine (20.4 mg, 0.20 mmol) and the above product

(51.0 mg, 0.07 mmol) in DMF (5 mL). The mixture was stirred at 25 °C for 24h. The mixture was filtered, concentrated and purified by preparative TLC (10% MeOH in DCM, Rf = 0.5) to give *[4-[[(2S)-2-[[(2S)-2-[3-[2-[2-[2-[2-[(2-azidoacetyl)amino]ethoxy]ethoxy]ethoxy]ethoxy] propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl N-[5-[5-[[(6aS)-2-methoxy-8-methylene-6,11-dioxo-5,6a,7,9-tetrahydropyrrolo[2,1-c][1,4]benzodiazepin-3-yl]oxy]pentoxy]-2-[(2S)-2-[[tert-butyl(dimethyl)silyl]oxymethyl]-4-methylene-pyrrolidine-1-carbonyl]-4-methoxy-phenyl]carbamate* (**9**, 50 mg, 40.9%) as a pale yellow solid. LCMS (5-95, AB, 1.5min): RT (220/254nm) = 1.090 min, m/z = 693.0 [M/2+H]+.



9 10

A solution of *4-((20S,23S)-1-azido-20-isopropyl-23-methyl-2,18,21-trioxo-6,9,12,15-tetraoxa-3,19,22-triazatetracosanamido)benzyl(2-((S)-2-(((tert-butyldimethylsilyl) oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxy-5-((5-(((S)-7-methoxy-2-methylene-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4] diazepin-8-yl)oxy)pentyl)oxy)phenyl)carbamate* (**9**, 50.0 mg, 0.04 mmol) in THF (1.0 mL), water (1.0 mL) and acetic acid (HOAc, 1.5mL) was stirred at 25 °C for 16h. The mixture was concentrated to give the product *4-((20S,23S)-1-azido-20-isopropyl-23-methyl-2,18,21-trioxo-6,9,12,15-tetraoxa-3,19,22-triazatetracosanamido)benzyl (2-((S)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxy-5-((5-(((S)-7-methoxy-2-methylene-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)phenyl)carbamate* (**10**, 45 mg, 98.1%) as a pale yellow solid. LCMS (5-95, AB, 1.5min): RT (220/254nm) = 0.898 min, m/z = 636.0 [M/2+H]+.



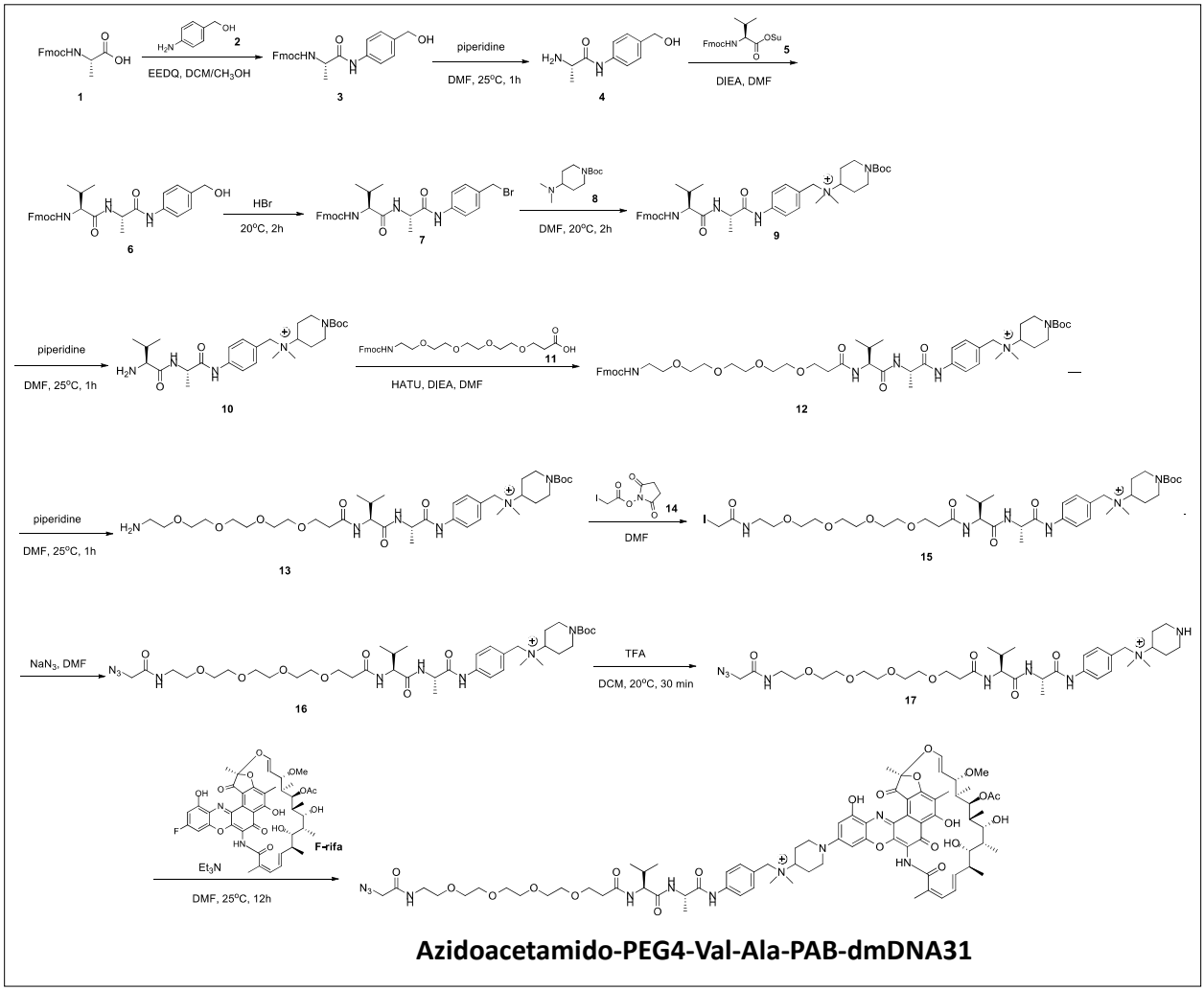
10

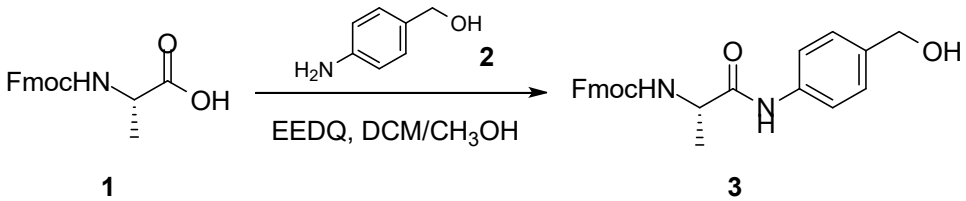
Azidoacetamido-PEG4-Val-Ala-PAB-PBD*ma*

To a solution of *4-((20S,23S)-1-azido-20-isopropyl-23-methyl-2,18,21-trioxo-6,9,12,15-tetraoxa-3,19,22-triazatetracosanamido)benzyl (2-((S)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxy-5-((5-(((S)-7-methoxy-2-methylene-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)phenyl)carbamate* (**10**, 45.0 mg, 0.04 mmol) in dimethyl sulfoxide (DMSO, 2 mL) was added IBX (49.6 mg, 0.18 mmol). The reaction mixture was stirred at 38 °C for 24h. The mixture was purified by reverse

phase chromatography (acetonitrile 46-70% / 0.225% FA in water) to afford **azidoacetamido-PEG4-Val-Ala-PAB-PBD$_{ma}$** (6.9 mg, 15.1%) as a white solid. LCMS (10-80, AB, 7.0min): RT =3.409 min, m/z = 1290.9 [M+Na]+.

Synthesis of azidoacetamido-PEG4-Val-Ala-PAB-dmDNA31:

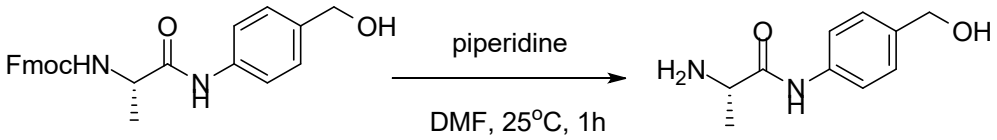




1 **3**

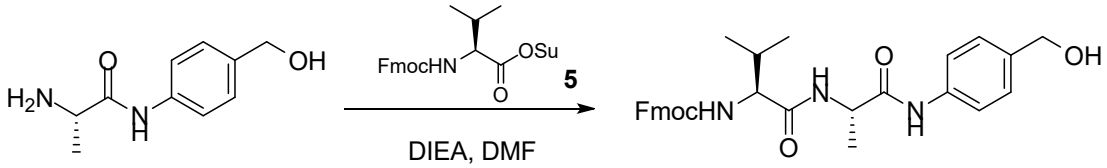
To a stirred solution of *(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid* (**1**, 2100.0 mg, 6.75mmol) in dichloromethane (DCM, 20 mL) and MeOH (5 mL) was added *2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline* (EEDQ, 3336.0 mg, 13.49 mmol), after ten minutes, (4-aminophenyl)methanol (**2**, 1246.5 mg, 10.12 mmol) was

added. The mixture was stirred at 25 °C for 12h. The reaction mixture was concentrated to dryness and the residue was washed by methyl tertiary butyl ether (20 mL x 3) and dried in vacuo to afford (S)-(9H-fluoren-9-yl)methyl(1-((4-(hydroxymethyl) phenyl)amino)-1-oxopropan-2-yl) carbamate (2400 mg, 84.6%) as a white solid. LCMS (5-95, AB, 1.5min): RT = 0.871 min, m/z = 439.1[M+Na]+. 1H NMR (400MHz, Methanol-d4): δ 7.77 (d, J = 7.6 Hz, 2H), 7.66 (t, J = 8.0 Hz, 2H), 7.51 (d, J = 8.0 Hz, 2H), 7.34 (t, J = 7.6 Hz, 2H), 7.28 (d, J = 7.6 Hz, 4H), 4.36 (d, J = 6.4 Hz, 2H), 4.27 – 4.19 (m, 4H), 1.39 (d, J = 7.2 Hz, 3H).



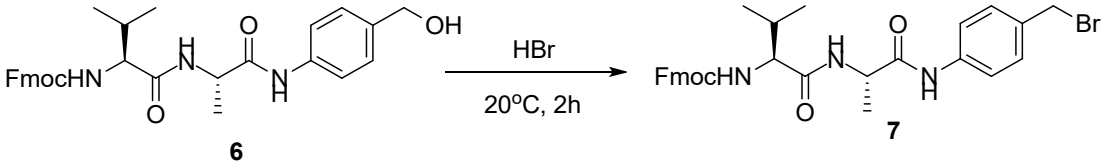
3 **4**

To a solution of *(S)-(9H-fluoren-9-yl)methyl(1-((4-(hydroxymethyl)phenyl)amino)-1 -oxopropan -2-yl)carbamate* (**3**, 1000.0 mg, 2.4 mmol) in DMF (10 mL) was added piperidine (2.2 mL, 24.01 mmol). The mixture was stirred at 25 °C for 1h. The solvent was removed in vacuum. The residue was washed by methyl tertiary butyl ether (20 mL x 3), the precipitate was collected to afford *(S)-2-amino-N-(4-(hydroxymethyl)phenyl)propanamide* (**4**, 370 mg, 79.3%) as white solid.



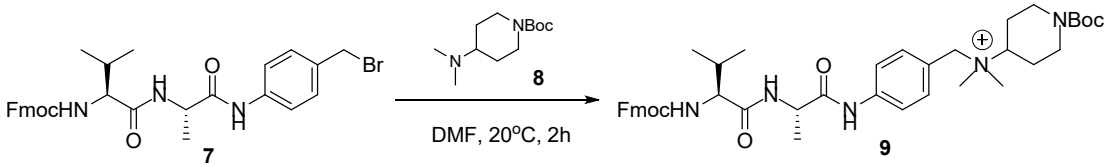
4 **6**

To a mixture of *(S)-2,5-dioxopyrrolidin-1-yl-2-((((9H-fluoren-9-yl)methoxy) carbonyl)amino)-3-methylbutanoate* (**5**, 1164.0 mg, 2.67 mmol) in anhydrous DMF (20 mL) was added *(S)-2-amino-N-(4-(hydroxymethyl)phenyl)propanamide* (**4**, 370.0 mg, 1.9 mmol), followed by DIEA (738.6 mg, 5.71 mmol). The reaction mixture was stirred at 20 °C for 2 h. TLC (10 % MeOH in DCM, Rf = 0.4) showed the reaction had gone to completion. The mixture was concentrated to give the residue which was washed with methyl tertiary butyl ether (25 mL×3) to afford *(9H-fluoren-9-yl)methyl((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1 -oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate* (**5**, 982 mg, 93.6%) as a white solid. LCMS (5-95, AB, 1.5min): RT = 0.885 min, m/z = 538.1[M+Na]+.



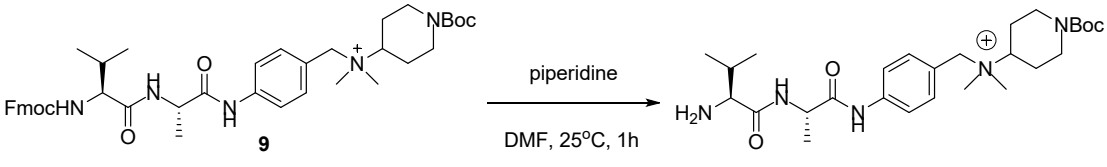
6 **7**

A mixture *(9H-fluoren-9-yl)methyl((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate* (**6**, 500.0 mg, 0.97 mmol) in HBr/HOAc (8mL) was stirred at 20 °C for 2h. The mixture was pour into ice water (30 mL), the precipitate was collected and dried in vacuum to afford *(9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-(bromomethyl)phenyl)amino)-1- oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate* (**7**, 560 mg, 66.9%) as a whiter solid. LCMS (5-95, AB, 1.5min): RT = 0.857 min, m/z = 579.9 [M+2+H]+.



7 **9**

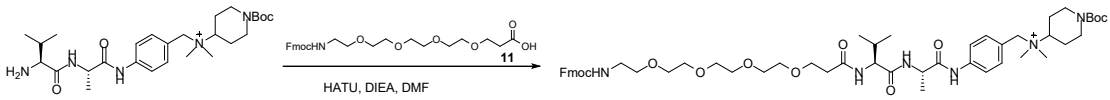
To a solution of *(9H-fluoren-9-yl)methyl((S)-1-(((S)-1 -((4-(bromomethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate* (**7**, 560.0 mg, 0.97 mmol) in DMF (5 mL) was added *tert-butyl 4-(dimethylamino)piperidine-1-carboxylate* (**8**, 221.0 mg, 0.97 mmol). The mixture was stirred at 20 °C for 2h. The mixture was concentrated to give the crude N-*(4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)-1-(tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**9**, 700 mg, 96.5%) as a white solid, which was used for the next step directly. LCMS (5-95, AB, 1.5min): RT = 0.917 min, m/z = 726.3 [M]+.



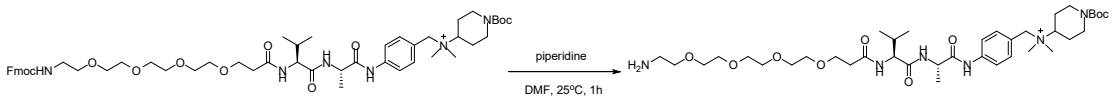
9 **10**

To a solution of *N-(4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino) -3-methylbutanamido)propanamido)benzyl)-1-(tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**9**, 600.0 mg, 0.83 mmol) in DMF (5 mL) was added piperidine (0.76 mL, 8.25mmol). The mixture was stirred at 20 °C for 1h. The solvent was removed in vacuum. Then more DMF (10 mL×2) was added and concentrated. The formed

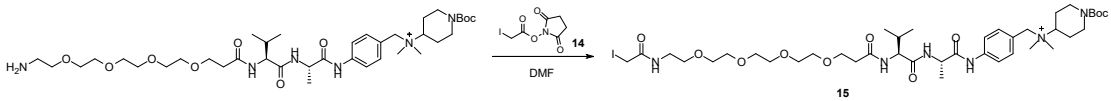
residue was washed by methyl tertiary butyl ether (20 mL x 3) and dried in vacuo to afford *N-(4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl)-1- (tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**10**, 315 mg, 75.6%) as a white solid.



To a solution of *1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16-pentaoxa-4-azanonadecan -19-oic acid* (**11**, Broadpharm, 365.2 mg, 0.75 mmol) in anhydrous DMF (5 mL) was added HATU (284.8 mg, 0.75 mmol), followed by DIEA (242 mg, 1.87 mmol). The reaction solution was stirred at 20 °C for 10 min. Then *N-(4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl)-1-(tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**10**, 315.0 mg, 0.62 mmol) was added. The reaction mixture was stirred at 20 °C for 2h. The mixture was concentrated in vacuo to afford crude product which was purified by reverse HPLC (acetonitrile 42 - 52%/0.225% FA in water) to afford *N-(4-((21S,24S)-1-(9H-fluoren-9-yl)-21- isopropyl-24-methyl-3,19,22-trioxo-2,7,10,13,16-pentaoxa-4,20,23-triazapentacosanamido)benzyl)-1-(tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**12**, 315 mg, 44%) as a white solid. LCMS (5-95, AB, 1.5min): RT =0.740 min, m/z = 973.4[M+H]+.

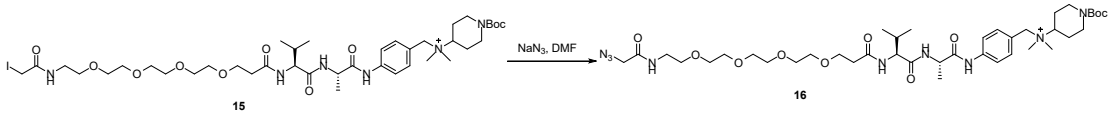


To a solution of *N-(4-((21S,24S)-1-(9H-fluoren-9-yl)-21- isopropyl-24-methyl-3,19,22-trioxo-2,7,10,13,16-pentaoxa-4,20,23-triazapentacosanamido)benzyl)-1-(tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**12**, 315.0 mg, 0.32 mmol) in DMF (5mL) was added piperidine (0.3 mL, 3.23 mmol). The mixture was stirred at 25 °C for 1h. The solvent was removed in vacuum, and more DMF (5 mL × 2) was added and concentrated. The residue was washed by methyl tertiary butyl ether (10 mL x 3) and dried in vacuo to afford *N-(4-((17S,20S)-1-amino-17-isopropyl-20-methyl-15,18-dioxo-3,6,9,12-tetraoxa-16,19-diazahenicosanamido)benzyl)-1-(tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**13**, 175 mg, 72%) as a white solid.

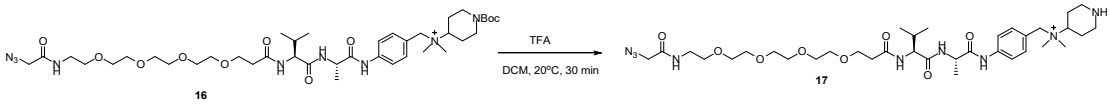


To a solution of *N-(4-((17S,20S)-1-amino-17-isopropyl-20-methyl-15,18-dioxo- 3,6,9,12-tetraoxa-16,19-diazahenicosanamido)benzyl)-1-(tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**13**, 165.0 mg, 0.22

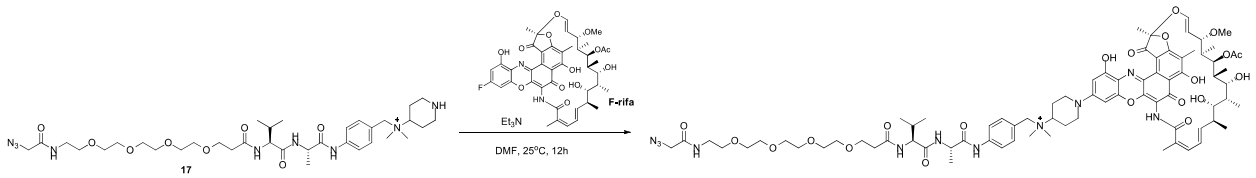
mmol) in anhydrous DMF (4 mL) was added *2,5-dioxopyrrolidin-1-yl 2-iodoacetate* (**14**, 93.2 mg, 0.33 mmol). The reaction mixture was stirred at 20 °C for 2h. The mixture was filtered, and the filtrate was concentrated to afford the crude *1-(tert-butoxycarbonyl)-N-(4-((20S,23S)-1-iodo-20-isopropyl-23-methyl-2,18,21 -trioxo-6,9,12,15-tetraoxa-3,19,22-triazatetracosanamido)benzyl)-N,N-dimethylpiperidin-4-aminium* salt (**15**, 200 mg, 64.2%) as a yellow solid, which was used for the next step directly. LCMS (5-95, AB, 1.5min): RT = 0.798 min, m/z = 919.6 [M]+.



To a solution of *1-(tert-butoxycarbonyl)-N-(4-((20S,23S)-1-iodo-20-isopropyl-23- methyl-2,18,21-trioxo-6,9,12,15-tetraoxa-3,19,22-triazatetracosanamido)benzyl)-N,N-dimethylpiperidin-4-aminium* salt (**15**, 200.0 mg, 0.22 mmol) in DMF (4 mL) was added sodium azide (NaN_3, 8.1 mg, 0.12 mmol). Then the mixture was stirred at 25 °C for 2h. The mixture was purified by HPLC (acetonitrile 20 - 50% / 0.1% TFA in water) to give *N-(4-((20S,23S)-1-azido-20-isopropyl-23-methyl-2,18,21-trioxo-6,9,12,15 -tetraoxa-3,19,22-triazatetracosanamido)benzyl)-1-(tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**16**, 50 mg, 27.5%) as a white solid. LCMS (5-95, AB, 1.5min): RT = 0.635 min, m/z = 834.4 [M]+.



To a mixture of *N-(4-((20S,23S)-1-azido-20-isopropyl-23-methyl-2,18,21-trioxo- 6,9,12,15-tetraoxa-3,19,22-triazatetracosanamido)benzyl)-1-(tert-butoxycarbonyl)-N,N-dimethylpiperidin-4-aminium* salt (**16**, 50.0 mg, 0.06 mmol) in anhydrous dichloromethane (2 mL) was added 50% trifluoroacetic acid (TFA) in DCM (2 mL, v/v). The reaction mixture was stirred at 20 °C for 30 min. The mixture was concentrated in vacuo and washed with methyl tertiary butyl ether (2 mL x 3) to afford *N-(4-((20S,23S)-1-azido-20-isopropyl -23-methyl-2,18,21-trioxo-6,9,12,15-tetraoxa-3,19,22-triazatetracosanamido)benzyl)-N,N-dimethylpiperidin-4-aminium* salt (**17**, 40 mg, 90.9%) as a yellow solid.



Azidoacetamido-PEG4-Val-Ala-PAB-dmDNA31

To a mixture of **F-rifa** (53.55 mg, 0.07 mmol) and triethylamine (16.5 mg, 0.16 mmol) in DMF (2 mL) was added *N-(4-((20S,23S)-1-azido-20-isopropyl -23-methyl-2,18,21-trioxo-6,9,12,15-tetraoxa-3,19,22-triazatetracosanamido)benzyl)-N,N-dimethylpiperidin-4-aminium* salt (**17**, 40.0 mg, 0.05 mmol). The mixture was stirred for 12 hours at 25 ^{o}C. Then the mixture was filtered and the filtrate was purified by reverse HPLC (acetonitrile 33-63/0.225%FA in water) to afford **azido-PEG4-Val-Ala-PAB-dmDNA31** (37mg, 44.3%) as a blue solid. LCMS (5-95, AB, 1.5min): RT = 0.923 min, m/z = 750.9 [(M-MeOH)/2+H]+. HRMS (5-95, AB, 4 Min): m/z = 1532.7647 [M]+.