Supplemental Methods

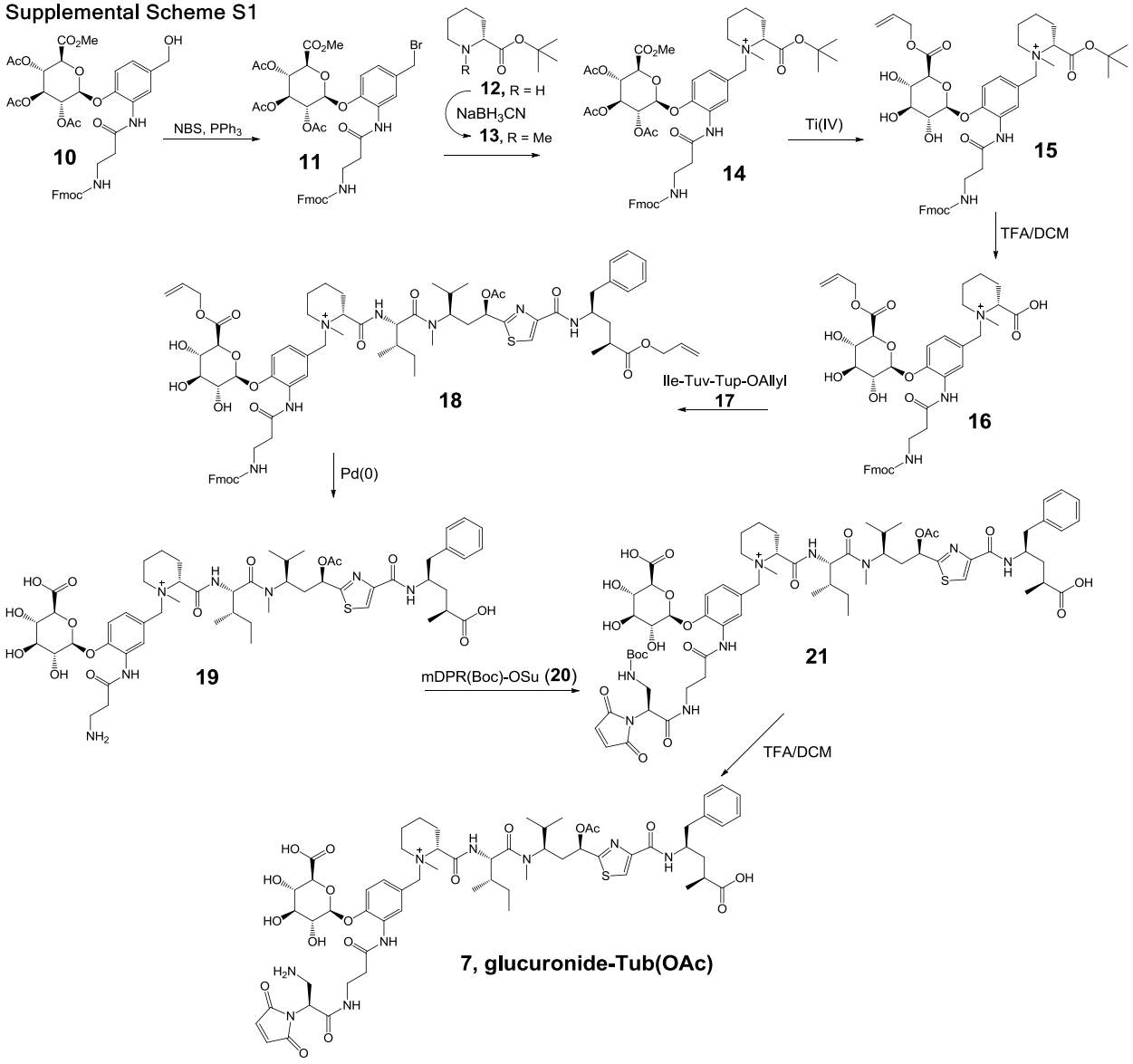
Tubulysin acetate ADC stability in SCID mice. Drug stability of drug-linker **7** was assessed in SCID mice, the strain used in the xenograft models. Anti-CD30 conjugates were loaded at 4-drugs/mAb on interchain disulfides. SCID mice were administered conjugate as a single ip dose of 3 mg/kg and then subjected to terminal bleeds at 4 and 10 days post-dose. Blood samples from each animal were processed to plasma using centrifugation into EDTA coated Eppendorf tubes. The plasma was batch purified using anti-human capture affinity resin (IgSelect, GE Healthcare) for two hours at 2-8 °C. The bound samples were washed using 0.5 M NaCl and eluted using 50 mM glycine, pH 3. Eluted samples were deglycosylated using PNGase F (New England BioLabs Inc) then reduced using 5 mM DTT. Each sample was analyzed using reversed-phased UPLC (PLRP xum, Agilent) coupled with mass spectrometric detection (Waters Xevo G2-S QTOF). The drug-antibody ratio (DAR) of each sample was calculated using the relative ratios of total ion counts from the deconvoluted masses of the non-loaded and drug-loaded (acetylated and deacetylated) antibody peaks. Intact drug (%acetylation) was calculated using total ion counts of the drug loaded light chain and heavy chain species, assessed by a loss of 42 daltons.

Rat tolerability. All in vivo experiments were conducted in concordance with the Institutional Animal Care and Use Committee in a facility fully accredited by the Association for the Assessment of Laboratory Animal Care. Humanized IgG antibody was loaded with lead drug-linkers glucuronide-Tub(OAc) **7** and glucuronide-Tub(OEt) **8** at 4-drugs/Ab on interchain disulfides as described in the Materials and Methods. Sprague-Dawley rats (n=3) were administered a single intravenous 10 mg/kg dose of ADC on day one. The animals were then carefully monitored for clinical observations and weight loss for 28 days following dosing.

Chemistry experimental - General Information. All commercially available anhydrous solvents were used without further purification. Analytical thin layer chromatography was performed on silica gel 60 F254 aluminum sheets (EMD Chemicals, Gibbstown, NJ). Column chromatography was performed

on a Biotage Isolera One flash purification system (Charlotte, NC). Analytical HPLC was performed on a Varian ProStar 210 solvent delivery system configured with a Varian ProStar 330 PDA detector. Samples were eluted over a C12 Phenomenex Synergi 2.0 x 150 mm, 4 µm, 80 Å reverse-phase column. The acidic mobile phase consisted of acetonitrile and water both containing either 0.05% trifluoroacetic acid or 0.1% formic acid. Compounds were eluted with a linear gradient of acidic acetonitrile from 5% at 1 min post injection, to 95% at 11 min, followed by isocratic 95% acetonitrile to 15 min (flow rate = 1.0 mL/min). UPLC-MS was performed on two different systems. UPLC-MS system 1 consisted of a Waters SQ mass detector interfaced to an Acquity Ultra Performance LC equipped with an Acquity UPLC BEH C18 2.1 x 50 mm, 1.7µm reverse phase column. The acidic mobile phase (0.1% formic acid) consisted of a gradient of 3% acetonitrile/97% water to 100% acetonitrile (flow rate = 0.5 mL/min). UPLC-MS system 2 consisted of a Waters Xevo G2 ToF mass spectrometer interfaced to a Waters Acquity H-Class Ultra Performance LC equipped with an Acquity UPLC BEH C18 2.1 x 50 mm, 1.7µm reverse phase column. The acidic mobile phase (0.1% formic acid) consisted of a gradient of 3% acetonitrile/97% water to 100% acetonitrile (flow rate = 0.7 mL/min). Preparative HPLC was carried out on a Varian ProStar 210 solvent delivery system configured with a Varian ProStar 330 PDA detector. Products were purified over a C12 Phenomenex Synergi 10.0 x 250 mm, 4 µm, 80 Å reverse phase column eluting with 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in acetonitrile (solvent B). The purification methods generally consisted of linear gradients of solvent A to solvent B, ramping from 90% aqueous solvent A to 10% solvent A. The flow rate was 4.6 mL/min with monitoring at 254 nm. Glucuronide-tubulysin drug-linkers **7**-**9** were prepared as described in Supplemental Schemes S1-S3.

Supplemental Scheme S1



(2S,3R,4S,5S,6S)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(bromomethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (11): A flame dried flask was charged with known (*Bioconjugate Chem*. **2006**, *17*, 831-840) glucuronide linker fragment (**10**, 210 mg, 281 µmol) in 4.5 mL anhydrous THF. The solution was stirred at room temperature under nitrogen. Triphenylphosphine (111 mg, 421.5 µmol) and N-bromosuccinimide (75 mg, 421.5 µmol) were added sequentially and the solution was stirred for 2 hours. The reaction was condensed under reduced pressure and purified over silica via a Biotage column (Hexanes/EtOAc, 30%-

50%-70%) to provide **11** (222 mg, 97%). Analytical UPLC-MS (system 1): t_r = 2.36 min, *m/z* (ES+) calculated 811.17 $(M+H)^+$, found 811.34.

(R)-tert-butyl 1-methylpiperidine-2-carboxylate (13). Commercially available H-Pip-OtBu (**12**, 500 mg, 2.70 mmol) was taken up in MeOH (4.50 mL), AcOH (4.50 mL) and 37% CH_2O in H_2O (4.50 mL) and stirred for 20 minutes. $NaBH_3CN$ (509 mg, 8.10 mmol) added slowly as a solid to vigorous bubbling, the reaction was stirred for 30 minutes. The reaction was then poured into 200 mL saturated $NaHCO_3$ solution and extracted 3x with 200 mL DCM. The organic layers were washed with brine, dried over $NaSO_4$, and condensed under reduced pressure to provide **20** (516 mg, 96%) to be carried forward without further purification. Analytical UPLC-MS (system 2): t_r = 0.53 min, *m/z* (ES+) calculated 200.17 $(M+H)^+$, found 200.21.

(2R)-1-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(tert-butoxycarbonyl)-1-methylpiperidin-1-ium (14). A pressure vessel was charged with brominated glucuronide linker fragment (**11**, 104 mg, 128 µmol) and Mep-OtBu (**13**, 34 mg, 171 µmol) in anhydrous 2-butanone (1.71 mL). The reaction vessel was flushed with nitrogen and sealed. The reaction was then stirred and heated to 60 °C for 12 hours. The resulting mixture was cooled, condensed under reduced pressure, taken up in minimal DMSO and purified by preparative HPLC to provide **14** (97 mg, 82%). Analytical UPLC-MS (system 2): t_r = 1.32 min, *m/z* (ES+) calculated 930.40 $(M)^+$, found 930.49.

(2R)-1-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(tert-butoxycarbonyl)-1-methylpiperidin-1-ium (15). A flame dried flask was charged with Fmoc-glucQ-Mep-OtBu (**14**, 97 mg, 104 µmol) in anhydrous allyl alcohol (2.09 mL) under nitrogen. $Ti(OC_2H_5)_4$ (87 µL, 417 µmol) was added and the reaction was heated to 80 °C with stirring for 2 hours. The reaction was then cooled to room temperature and poured into 50 mL 1M HCl. After resting for 45 minutes, the HCl was extracted 3x with

50 mL DCM. Resulting organics were washed with brine, dried over $NaSO_4$, condensed, and purified by preparative HPLC to provide **15** (42 mg, 48%). Analytical UPLC-MS (system 2): t_r = 1.18 min, *m/z* (ES+) calculated 830.39 $(M)^+$, found 830.49.

(2R)-1-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-carboxy-1-methylpiperidin-1-ium (16). A flask containing Fmoc-gluc(Allyl)Q-Mep-OtBu (**15**, 42 mg, 50 µmol) was cooled to 0 ^oC under nitrogen. A solution of 30% TFA in CH_2Cl_2 (2.5 mL) was added dropwise and stirred for 18 hours. The reaction was concentrated under reduced pressure, taken up in minimal DMSO and purified by preparative HPLC to provide **16** (25 mg, 64%). Analytical UPLC-MS (system 2): t_r = 1.05 min, *m/z* (ES+) calculated 774.32 $(M)^+$, found 774.42.

(2R)-1-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(((2S,3S)-1-(((1R,3R)-1-acetoxy-1-(4-(((2R,4S)-5-(allyloxy)-4-methyl-5-oxo-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-4-methylpentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-methylpiperidin-1-ium (18). To a flask charged with H-Ile-Tuv(OAc)-Tup-OAllyl (**17**, 23 mg, 36 µmol) was added Fmoc-gluc(Allyl)Q-Mep-OH (**16**, 28 mg, 36 µmol) and HATU (27 mg, 72 µmol) as solids followed by DMF (0.714 mL). N,N-Diisopropylethylamine (25 uL, 143 µmol) was added and the reaction was stirred at room temperature for 1 hour. The reaction was then taken up in DMSO and purified by preparative HPLC to provide **18** (23 mg, 46%). Analytical UPLC-MS (system 2): t_r = 1.39 min, *m/z* (ES+) calculated 1398.66 $(M)^+$, found 1398.81.

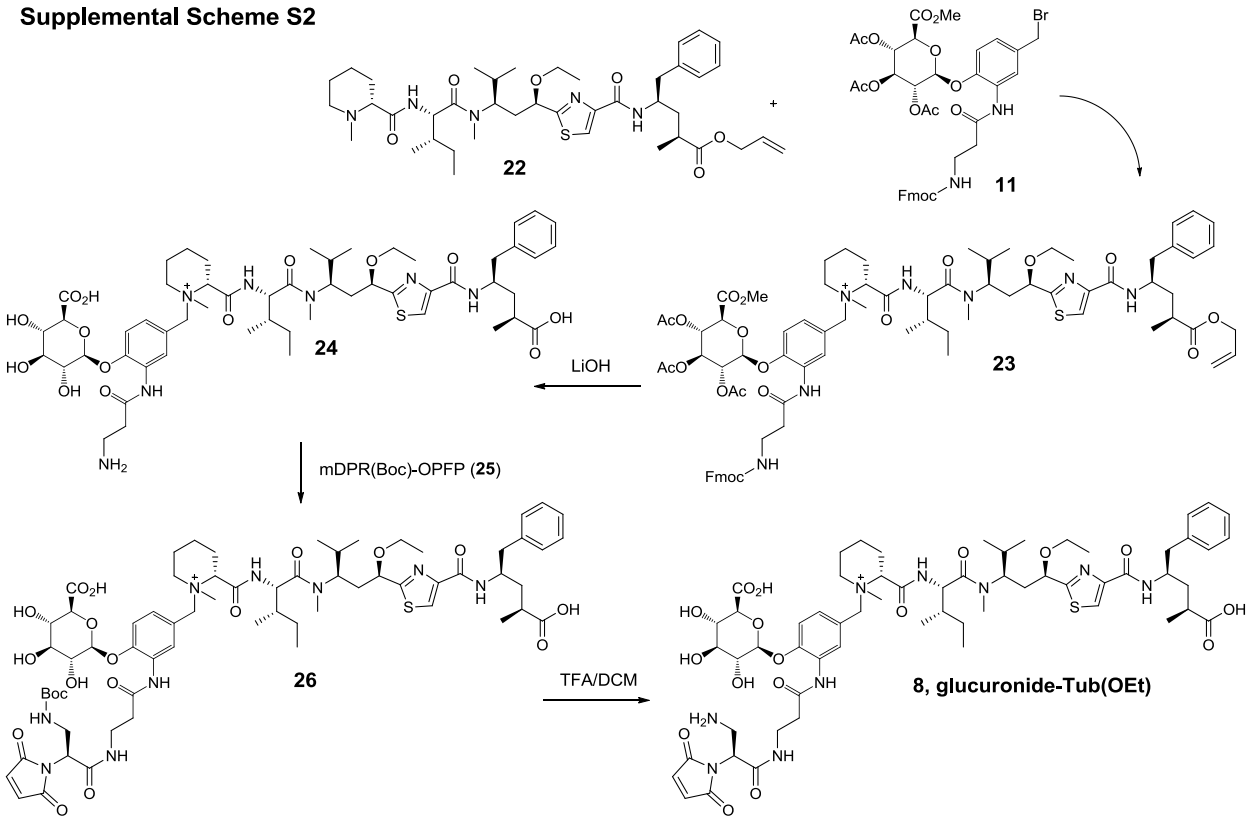
(2R)-2-(((2S,3S)-1-(((1R,3R)-1-acetoxy-1-(4-(((2R,4S)-4-carboxy-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-4-methylpentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-1-methylpiperidin-1-ium (19). Fmoc-gluc(Allyl)Q-Tub-OAllyl (**18**, 21 mg, 15

µmol) was taken up in DCM (1.5 mL) stirring under nitrogen. $Pd(PPh_3)_4$ (3.5 mg, 3.1 µmol) and PPh_3 (1.6 mg, 6.1 µmol) were added as solids followed by pyrrolidine (20.1 µL, 245 µmol). The reaction was stirred to 2 hours at room temperature then taken up in 1 mL DMSO, condensed under reduced pressure, and purified by preparative HPLC to provide **19** (13 mg, 79%). Analytical UPLC-MS (system 2): t_r = 0.94 min, *m/z* (ES+) calculated 1096.53 $(M)^+$, found 1096.65.

(2R)-2-(((2S,3S)-1-(((1R,3R)-1-acetoxy-1-(4-(((2R,4S)-4-carboxy-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-4-methylpentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-(3-(3-((S)-3-((tert-butoxycarbonyl)amino)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-1-methylpiperidin-1-ium (21). A flask was charged with H-glucQ-Tub (**19**, 13.1 mg, 11.9 µmol) in anhydrous DMF (0.595 mL) to which mDPR(Boc)-OSu (**20**, 4.6 mg, 11.9 µmol) was added under nitrogen. N,N-Diisopropylethylamine (8.3 uL, 47.8 µmol) was added and the reaction was stirred at room temperature for 3 hours. The reaction was then quenched with acetic acid (8.3 uL) and purified by preparative HPLC to provide **21** (5.2 mg, 33%). Analytical UPLC-MS (system 2): t_r = 1.20 min, *m/z* (ES+) calculated 1362.62 $(M)^+$, found 1362.75.

(2R)-2-(((2S,3S)-1-(((1R,3R)-1-acetoxy-1-(4-(((2R,4S)-4-carboxy-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-4-methylpentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-(3-(3-((S)-3-amino-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-1-methylpiperidin-1-ium (7). A flask charged with mDPR(Boc)-glucQ-Tub (**21**, 5.2 mg, 3.8 µmol) was cooled to 0 °C under nitrogen. A solution of 10% TFA in CH_2Cl_2 (0.84 mL) was added dropwise and stirred for 4 hours. The reaction was then taken up in DMSO, condensed under reduced pressure, and purified by preparative HPLC to provide **7** (4.8 mg, 81%). Analytical UPLC-MS (system 2): t_r = 0.95 min, *m/z* (ES+) calculated 1262.56 $(M)^+$, found 1262.68.

Supplemental Scheme S2



(2R)-1-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(((2S,3S)-1-(((1R,3R)-1-(4-(((2R,4S)-5-(allyloxy)-4-methyl-5-oxo-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-1-ethoxy-4-methylpentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-methylpiperidin-1-ium (23). A pressure vessel was charged with Tub(OEt)-OAllyl (**22**, 1 equivalent, 148 mg, 196 µmol) and brominated glucuronide linker fragment (**11,** 1.5 equivalents, 175 mg, 216 µmol) in anhydrous 2-butanone (50 mM). The reaction vessel was flushed with nitrogen and sealed. The reaction was then stirred and heated to 60 °C for 18 hours. The resulting mixture was cooled, condensed to residue under reduced pressure, then carried forward as the crude residue. Analytical UPLC-MS (system 2): t_r = 1.49 min, *m/z* (ES+) calculated 1484.69 $(M)^+$, found 1484.84.

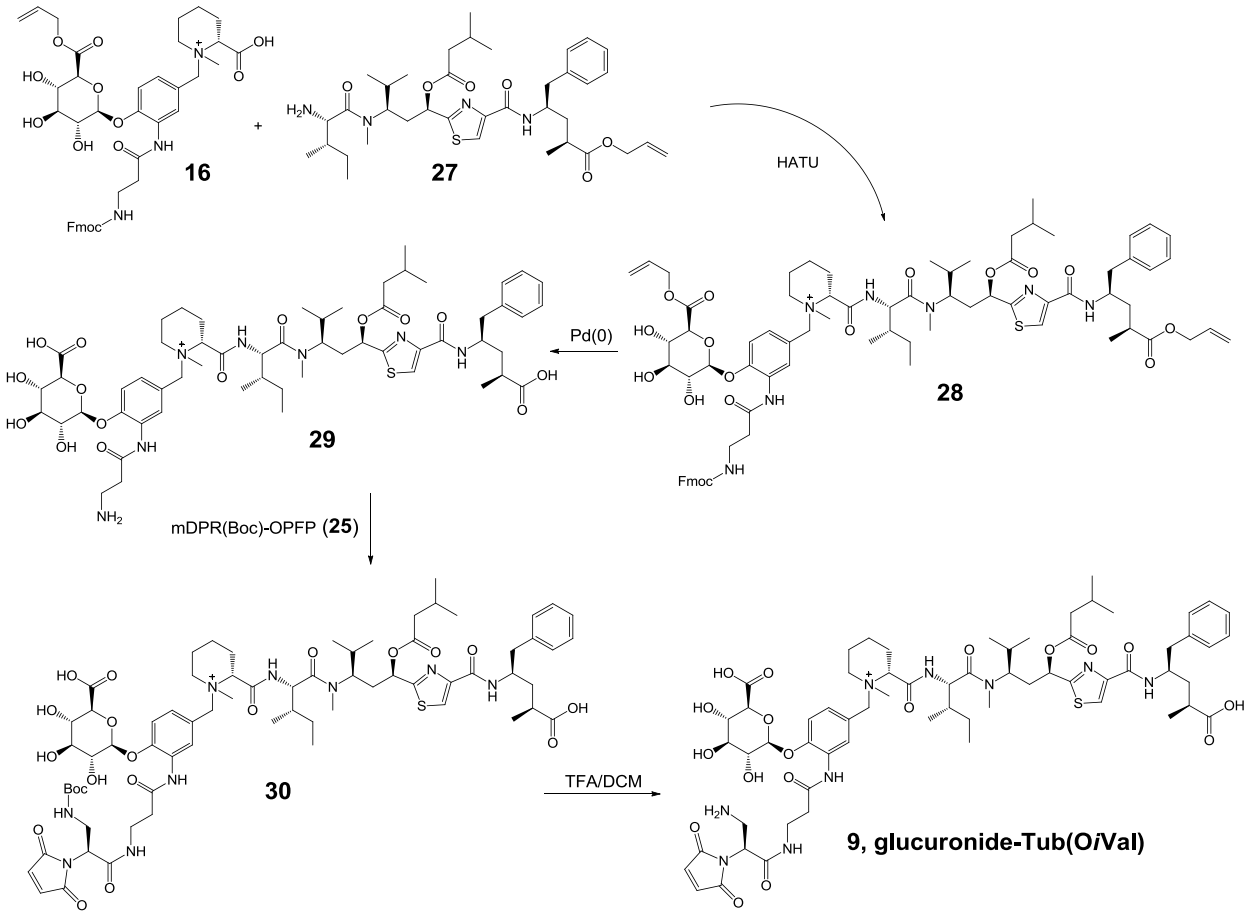
(2R)-1-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(((2S,3S)-1-(((1R,3R)-1-(4-(((2R,4S)-4-carboxy-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-1-ethoxy-4-methylpentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-methylpiperidin-1-ium (24). A flask was charged with Fmoc-GlucQ-Tub(OEt)-OAllyl (**23**, 92 mg, 197 µmol) in THF and MeOH and cooled to 0 ^{O}C. LiOH·H_2O (6.0 equivalents) in H_2O was added dropwise (1:1:1 THF:MeOH:H_2O, 50 mM end concentration) and the reaction was allowed to warm to room temperature and stir overnight. THF and MeOH were removed under reduced pressure, the resulting precipitate was resolubilized using minimal DMSO and the mixture was purified by preparative HPLC to provide **24** (116 mg, 54%). Analytical UPLC-MS (system 2): t_r = 0.95 min, *m/z* (ES+) calculated 1082.55 $(M)^+$, found 1082.68.

(2R)-1-(3-(3-((S)-3-((tert-butoxycarbonyl)amino)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(((2S,3S)-1-(((1R,3R)-1-(4-(((2R,4S)-4-carboxy-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-1-ethoxy-4-methylpentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-methylpiperidin-1-ium (26). A flask was charged with H-Gluc-Tub(OEt) (**24,** 29 mg, 27 µmol) to which mDPR(Boc)-OPFP (**25**, 1.2 equivalents, 14 mg, 32 µmol) was added as a solution in DMF (10 mM). N,N-Diisopropylethylamine (4.0 equivalents) was added and the reaction was stirred at room temperature for 3 hours. The reaction was quenched with AcOH (4.0 equivalents) then diluted in DMSO (1 volume) and purified by preparative HPLC to provide **26** (26 mg, 72%). Analytical UPLC-MS (system 2): t_r = 1.19 min, *m/z* (ES+) calculated 1348.64 $(M)^+$, found 1348.79.

(2R)-1-(3-(3-((S)-3-amino-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(((2S,3S)-1-(((1R,3R)-1-(4-(((2R,4S)-4-carboxy-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-1-ethoxy-4-methylpentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-methylpiperidin-1-ium

(8). A flask was charged with mDPR(Boc)-GlucQ-Tub(OEt) (**26,** 26 mg, 19 µmol) and cooled to 0 ^{O}C. A 10% solution of TFA in DCM (50 mM) was added and the reaction was allowed to warm to room temperature while stirring for 1 hour. The reaction was then diluted with DMSO (1 volume), DCM removed via reduced pressure, then purified by preparative HPLC to provide **8** (24 mg, 99%). Analytical UPLC-MS (system 2): t_r = 0.95 min, *m/z* (ES+) calculated 1248.59 $(M)^+$, found 1248.72.

Supplemental Scheme S3



(2R)-1-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(((2S,3S)-1-(((1R,3R)-1-(4-(((2R,4S)-5-(allyloxy)-4-methyl-5-oxo-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-4-methyl-1-((3-methylbutanoyl)oxy)pentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-methylpiperidin-1-ium (28). To a flask charged with H-Ile-Tuv(O*i*Val)-Tup-OAllyl (**27**, 34 mg, 50 µmol)

was added Fmoc-Gluc(Allyl)Q-Mep-OH (**16**, 38 mg, 50 µmol) and HATU (38 mg, 99 µmol) as solids followed by DMF (0.50 mL). N,N-Diisopropylethylamine (34 uL, 199 µmol) was added and the reaction was stirred at room temperature for 1 hour. The reaction was then taken up in DMSO and purified by preparative HPLC to provide **28** (28 mg, 39%). Analytical UPLC-MS (system 2): t_r = 1.45 min, m/z (ES+) calculated 1440.70 $(M)^+$, found 1440.85.

(2R)-1-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(((2S,3S)-1-(((1R,3R)-1-(4-(((2R,4S)-4-carboxy-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-4-methyl-1-((3-methylbutanoyl)oxy)pentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-methylpiperidin-1-ium (29). Fmoc-Gluc(Allyl)Q-Tub(O*i*Val)-OAllyl (**28**, 28 mg, 19 µmol) was taken up in DCM (0.97 mL) stirring under nitrogen. $Pd(PPh3)_4$ (6.7 mg, 5.8 µmol) and PPh_3 (3.0 mg, 11.7 µmol) were added as solids followed by pyrrolidine (38 µL, 466 µmol). The reaction was stirred for 2 hours at room temperature then taken up in 1 mL DMSO, condensed under reduced pressure, and purified by preparative HPLC to provide **29** (15 mg, 68%). Analytical UPLC-MS (system 2): t_r = 1.02 min, m/z (ES+) calculated 1138.57 $(M)^+$, found 1138.70.

(2R)-1-(3-(3-((S)-3-((tert-butoxycarbonyl)amino)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(((2S,3S)-1-(((1R,3R)-1-(4-(((2R,4S)-4-carboxy-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-4-methyl-1-((3-methylbutanoyl)oxy)pentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-methylpiperidin-1-ium (30). A flask was charged with H-GlucQ-Tub(O*i*Val) (**29**, 9 mg, 8 µmol) in anhydrous DMF (0.395 mL) to which mDPR(Boc)-OPFP (**25**, 4.3 mg, 9.5 µmol) was added under nitrogen. N,N-Diisopropylethylamine (4.1 uL, 24 µmol) was added and the reaction was stirred at room temperature for 3 hours. The reaction was then quenched with acetic acid (4.1 uL) and purified by preparative HPLC to provide **30** (5.5 mg, 67%). Analytical UPLC-MS (system 2): t_r = 1.30 min, m/z (ES+) calculated 1404.66 $(M)^+$, found 1404.97.

(2R)-1-(3-(3-((S)-3-amino-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)-2-(((2S,3S)-1-(((1R,3R)-1-(4-(((2R,4S)-4-carboxy-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-4-methyl-1-((3-methylbutanoyl)oxy)pentan-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-1-methylpiperidin-1-ium (9). A flask charged with mDPR(Boc)- GlucQ-Tub(O*i*Val) (**30**, 5.5 mg, 3.9 μmol) was cooled to 0 ^{o}C under nitrogen. A solution of 10% TFA in CH_2Cl_2 (0.39 mL) was added dropwise and stirred for 4 hours. The reaction was then taken up in DMSO, condensed under reduced pressure, and purified by preparative HPLC to provide **9** (4.2 mg, 82%). Analytical UPLC-MS (system 2): t_r = 1.06 min, m/z (ES+) calculated 1304.61 $(M)^+$, found 1304.89.