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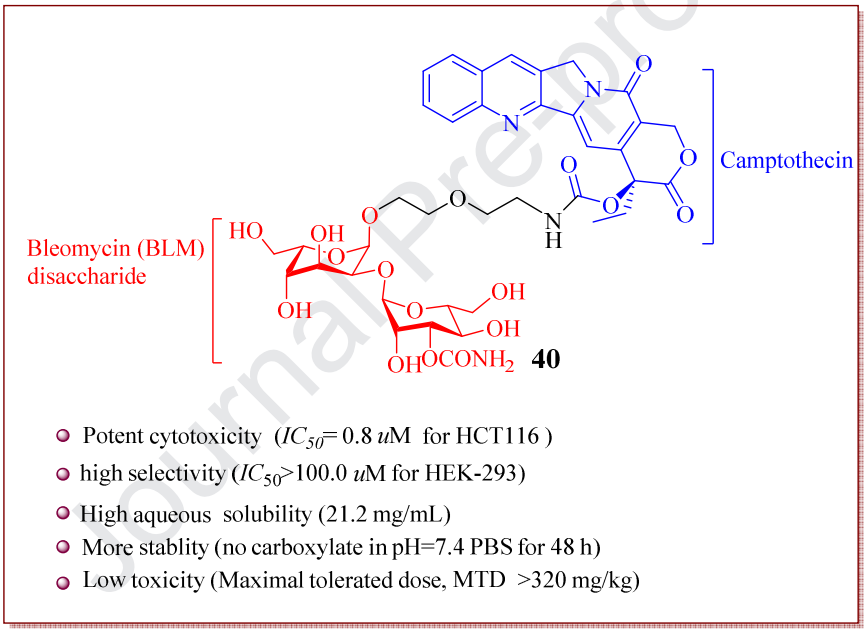

Thirty novel 20 (*S*)-*O*-linked camptothecin (CPT) glycoconjugates were synthesized. The SAR indicated that oligosaccharide types, length of a PEG linker and acetyl groups exerted obvious impacts on the antitumor activities and selectivity of the CPT glycoconjugates. Construct **40** demonstrated good druggablity profiles.

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Abstract:

Thirty novel 20 (S)-*O*-linked camptothecin (CPT) glycoconjugates were synthesized. They showed more potent *in vitro* cytotoxicities over irinotecan, but very weak direct topoisomerase I (Topo I) inhibition was observed at 100.0 μM. Oligosaccharide types, length of a PEG linker and acetyl groups exerted obvious effects on cytotoxicity, selectivity, water solubility and stability of the newly synthesized CPT glycoconjugates. Construct **40**, with a bleomycin (BLM) disaccharide linked to diethylene glycol in the introduced ester moiety, demonstrated a superior antitumor activity and a distinct selectivity compared to CPT. No toxicity was detectable in animal acute toxicity intravenously (160 mg/kg). Collectively, attachment of oligosaccharides with tumor targeting to 20 (*S*)-OH of CPT could offer a solution to the daunting problems posed by current Topo I poisons.

Key words: Camptothecin; Oligosaccharides; Aqueous solubility and stability; Anti-tumor activity; Toxicological assessment

1. Introduction

Camptothecin (Fig. 1, CPT, **1**), a plant-derived antitumor ingredient, exhibits a wide spectrum of inhibitory activity towards cancer cells as a unique DNA Topo I

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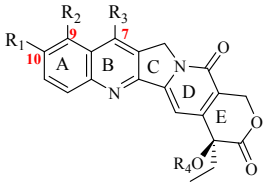
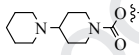
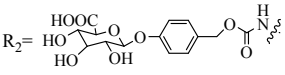
poison. [1-3] The molecule includes a planar pentacyclic ring system and one chiral center in a closed *α*-hydroxyl-*δ*-lactone (*E* ring). The planar ploycyclic geometry has been demonstrated as a guiding moiety that recognizes and stabilizes the Topo I/DNA cleavage complex.[2] *E* ring able to block reversal of the complex leads to cell apoptosis. Therefore, the planar geometry and the closed lactone are required for Topo I poisoning.[3] The approval of irinotecan (**2**) and topotecan (**3**) with an intact planar pentacyclic structure in the treatment for colorectal cancer,[3] liver cancer, [4] and small-cell lung cancer (SCLC)[3] highlights the privileged structure of CPT.

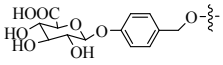


1, Camptothecin (CPT) $R_1=R_2=R_3=R_4=H$

2, Irinotecan $R_1=$  ; $R_2=R_4=H$; $R_3=CH_2CH_3$

3, Topotecan $R_1=OH$; $R_2=CH_2N(CH_3)_2$; $R_3=R_4=H$

4, $R_1=R_3=R_4=H$; $R_2=$ 

4a, $R_1=$  $R_2=R_3=R_4=H$;

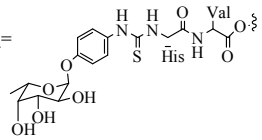
4b, $R_1=R_2=R_3=H$; $R_4=$ 

Fig.1. Structures of camptothecin (CPT) and CPT-derived agents.

CPT-derived Topo I poisons irinotecan (**2**) and topotecan (**3**) obviously suppress the growth of tumors, but the serious adverse effects increasingly compromise the clinical applications, including dose-limiting bone marrow suppression, grievous diarrhea. Meantime, the rapid diffusion of CPT and some CPT-based derivatives leads to the prolonged infusion times, and the poor aqueous solubility creates difficulty in formulation,[5] and the quick inactivation of *E*-ring-opening in human blood plasma generates the carboxylate form that readily binds to human blood proteins. [6,7] Accordingly, CPT derivatives that preserve the antitumor properties of the parent compound CPT, and improve the water solubility, *in vivo* stability and the safe profile, [5,8] are highly urgent to be developed. From CPT's structural analysis, the formation of intramolecular hydrogen bond of *E* ring with 20-OH favors the carboxylate form at physiological pH or above. Modification of 20-free hydroxyl group blocks the participation and stabilizes the closed-lactone moiety.[9] Coupling of oligosaccharides

with tumor targeting to 20-free hydroxyl group of CPT could offer access to solving the above issues.

Oligosaccharides such as mono- or disaccharides characteristic with little toxicity, high water solubility and tumor targeting are widely used in the drug design. [10-13] Glucuronide prodrugs **4** and **4a**, in which a glucuronic acid was coupled to positions 10 and 9 of CPT *via* a self-immolating linker, respectively, exhibited good water solubility, strong antitumor activity and differential toxicity [1,8]. These advantageous properties were ascribed to the hydrophilicity of a glucuronide moiety that was preferentially activated at tumor *β*-glucuronidase-overexpressing cells.[8,14]. But fast renal clearance of prodrugs **4** and **4a** caused by a highly polar glucuronide posed the clinical limitations. Additionally, 20 (*S*)-*O*-carbonate linked tripeptide conjugates with a fucoside derivative (**4b**) displayed good druggability properties,[15] whereas, a tripeptide spacer was difficultly manipulated and controlled due to instability towards enzymes, leading to no entry into clinical trials. Therefore, a saccharide type and a linker are very crucial for oligosaccharide-based CPT inhibitors. We envisage that an oligosaccharide with tumor targeting incorporated to 20 (*S*)-OH of CPT *via* an appropriate linker increases plasma stability and augments the antitumor activity.

Given the above considerations, intact CPT structure would maintain specificity of CPT derivatives to Topo I and exert the antitumor activities. The oligosaccharide scaffolds with tumor targeting as the hydrophilic moiety would increase water solubility, *in vivo* stability and selectivity of CPT derivatives. The suitable length of PEG (ethylene glycol)-based linkers, which were biocompatible in both hydrophobic and hydrophilic environments and hydrolytically stable towards various enzymes[16], would mediate a CPT moiety and a carbohydrate moiety as an integrity to enhance the antitumor activity and lower the untoward effect. In this study, four monosaccharides (glucose, galactose, mannose, 3-*O*-carbamyl mannose) and a bleomycin (BLM) disaccharide were selected to explore the potential influence on the physicochemical properties, Topo I inhibitions and antitumor activities of CPT glycoconjugates (**Fig. 2**). Construct **40** decorated with a BLM disaccharide, which displayed the potent

cytotoxicity and high selectivity, was further performed toxicological assessment.

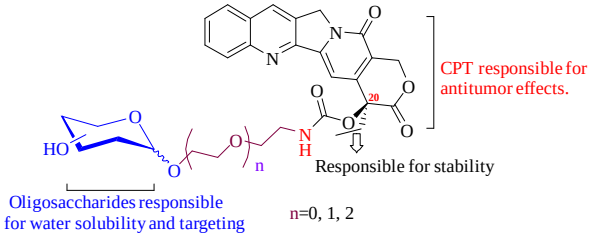
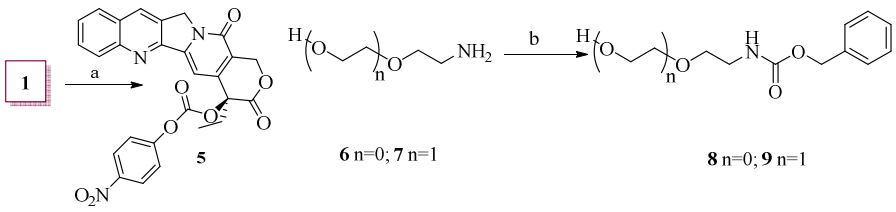


Fig. 2 Design of 20 (*S*)-O-linked CPT glycoconjugates.

2. Results and discussion.

2.1 Chemistry synthesis

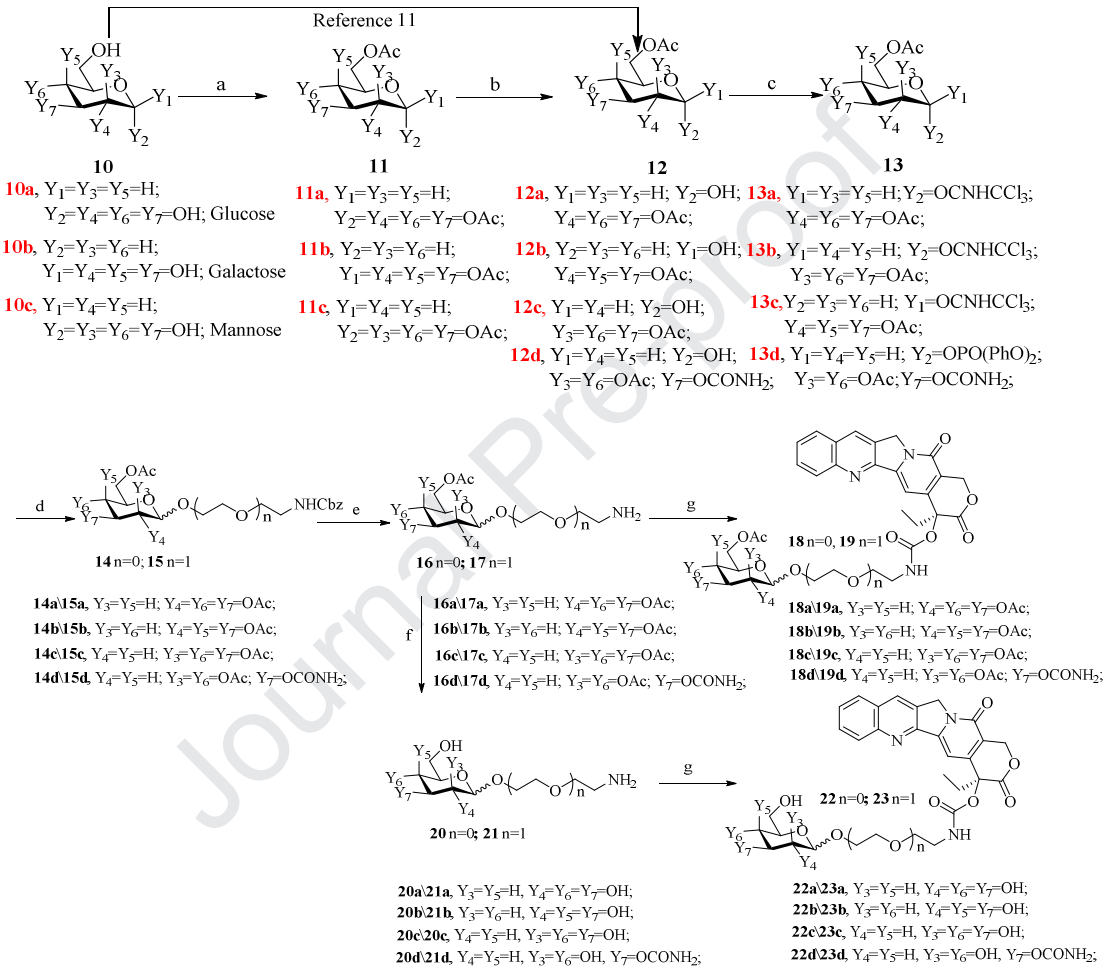
A series of CPT glycoconjugates were achieved by attaching five tumor-targeting simple sugars, such as glucose, galactose, mannose, 3-*O*-carbamyl mannose and BLM disaccharide, to the highly hydrophobic CPT scaffold. Prior to syntheses of CPT glycoconjugates, the general synthetic routes for activated CPT **5** and benzoxycarbonyl (Cbz)-protected linkers **8** and **9** were depicted in Scheme **1**. For the challenging acylation of the tertiary and unreactive 20 (*S*)-hydroxyl group, we used activated CPT **5** as a key intermediate, which was obtained by CPT with 4-nitrophenyl carbonochloridate in the presence of dimethylaminopyridine (DMAP) in an 86.3% yield[11]. In the syntheses of Cbz-protected linkers **8** and **9,** treatment of ethanolamine **6** and its derivative **7** with benzyl carbonochloridate easily afforded them in 85.2% and 87.5% yields, respectively[11].



Scheme 1. Reagents and conditions: a) 4-nitrophenyl carbonochloridate, DMPA, CH_2Cl_2, 0 ^oC-r.t., 3h, 86.3%; b) benzyl carbonochloridate, $N(Et)_3$, THF, r.t. 1h, 85.2% for **8**, 87.5% for **9**.

CPT glycoconjugates (**22a-d**, **23a-d**) and the corresponding peracetylated ones (**18a-d**, **19a-d**) were illustrated in scheme **2**. Peracetylated **11a-c** served as starting materials, which were prepared from the corresponding commercially available monosaccharides (**10a-c**).[17] The selective deacetylation of constructs **11a-c** and

subsequent nucleophilic addition reaction of compounds **12a-c** with trichloro-acetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the catalyst base yielded trichloroacetimidates **13a-c** in high yields, respectively[18]. Noticeably, synthesis of peracetylated unnatural 3-*O*-carbamyl mannose **12d** included a six-step reaction starting from mannose in an overall 69.1% yield[11], and the activation of acetylated **12d**

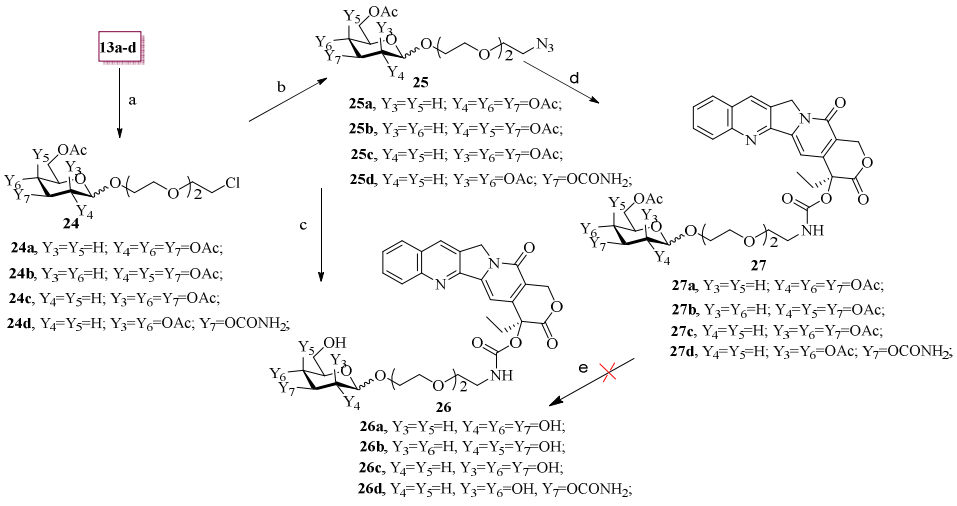


Scheme 2. Reagents and conditions: a) Ac_2O, 3% con. H_2SO_4,, r.t; b) $AcOHN_2H_4$, DMF, 50 °C; c) For **13a-c**, Cl_3CCN, 0.1 eq. DBU, CH_2Cl_2, 0 °C; For **13d,** $PO(OPh)_2Cl$, n-BuLi, THF, -78□; d) -5 °C-r.t., linker **8** or **9**, TMSOTf; e) H_2, 10% Pd/C, AcOEt, r.t.; f) CH_3ONa, CH_3OH, Dowex50 resin, r.t.; g) **5**, CH_2Cl_2, DIPEA, r.t..

with $PO(OPh)_2Cl$ and n-BuLi as a base afforded diphenyl phosphate **13d** in a high yield[19, 20]. Then treatment of compounds **13a-d** with Cbz-protected linkers **8** and **9** yielded linker-coupled derivatives **14a-d** and **15a-d** under the catalysis of TMSOTf, respectively. ^{1}H NMR analysis demonstrated coupling constants of H-H at the

reducing end of sugar residues in **14a-b** and **15a-b** were 8.0 Hz, indicating glucose and galactose were coupled to linkers **8** and **9** in a *β*-configured manner. By contrast, α-linkages for mannose and 3-*O*-carbamyl mannose connected to likers **8** and **9** were deduced in constructs **14c-d** and **15c-d** due to H-H coupling constants of 4 Hz at the reducing end. Nucleophilic substitution of activated **5** with amines **16a-d** and **17a-d**[20] favorably yielded protected CPT glycol-conjugates **18a-d** and **19a-d** in the presence of *N*,*N*-diisopropylethylamine (DIPEA), respectively. Deprotection of constructs **16a-d** and **17a-d** with CH_3ONa in anhydrous methanol followed by similar reactions with activated **5** afforded the corresponding CPT glycoconjugates **22a-d** and **23a-d**. No trace of **22a-d** and **23a-d** was detectable when directly deacetylated from the corresponding precursors **18a-d** and **19a-d**.

However, the synthesis of CPT conjugates (**26a-d**, **27a-d**) bearing longer-chain linkers (n=2) was accomplished by another strategy (Scheme **3**). Coupling of **13a-d** and 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol and subsequent azide substitution of chlorinated **24a-d** produced derivatives **25a-d**, which were reduced with 10% Pd/C and hydrogen followed by the similar reaction conditions as compounds **22a-d** and **18a-d** to yield target CPT conjugates **26a-d** and **27a-d** respectively. Analogously, deacetylation of compounds **27a-d** failed to generate the corresponding conjugates **26a-d**. The linkage modes of saccharides coupled to linkers in compounds **26a-d** and **27a-**

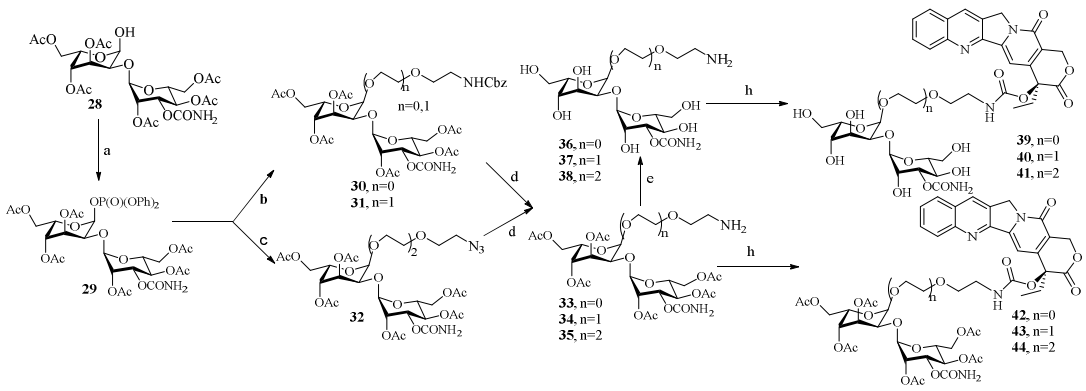


Scheme 3. Reagents and conditions: a) 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol, TMSOTf, anhydrous CH_2Cl_2, -5

^{o}C; b) NaN_3, DMF, 70 ^{o}C; c) i) CH_3ONa, anhydrous CH_3OH, Dowex50 resin, ii) H_2, 10% Pd/C, 1.0 atm, iii) **5**, DIPEA, 0 ^{o}C, overnight; d) i) H_2, 10% Pd/C, 1.0 atm, ii) **5**, DIPEA, 0 ^{o}C, overnight, e) $NaOCH_3$, anhydrous CH_3OH.

d were in complete accordance with those of compounds **24a-d**. The signals from ^{1}H NMR of **24a-d** clearly supported a *β* -glycosidic bond for glucose and galactose (*J*=8.0 Hz) but an α-linkage for mannose and 3-*O*-carbamyl mannose (*J*= 4.0 Hz) in the coupling reaction.

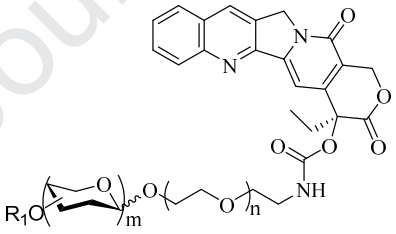
BLM disaccharide consisting of *L*-gulose and 3-*O*-carbamyl-*D*-mannose, a tumor-seeking moiety of BLM[12,13], was selected as a representative for exploring the effect of disaccharides on CPT. The appropriately designed route for the synthesis of BLM disaccharide-based CPT derivatives **39**-**44** was demonstrated in scheme **4**. Construct **28** was activated with $PO(OPh)_2Cl$ and n-BuLi, yielding the only α-glycosyl diphenyl phosphate **29** in 70.0% yield.[11] The nucleophilic substitution between construct **29** and Cbz-protected linkers **8** and **9** [19] yielded linker-coupled BLM disaccharides **30** and **31** respectively, which were subjected to a hydrogenolysis procedure followed by the *in situ* nucleophilic reaction with activated **5**[19] to afford the corresponding conjugates **42** and **43**. Similarly, treatment of construct **29** with 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol in the presence of TMSOTf followed by adize substitution yielded linker-coupled compound **32**. Then coupling reaction of amine **35**, which was derived from azide **32** under the catalysis of 10% Pd/C and H_2, and compound **5** afforded conjugate **44**. Linker-coupled disaccharides **33**-**35** were first deacylated with CH_3ONa in anhydrous CH_3OH followed by direct amidation with **5** to provide gylcoconjugates **39-41** in acceptable yields.



Scheme 4. Reagents and conditions: a) $PO(OPh)_2Cl$, n-BuLi, THF, -78□, 70.0%; b) linker **8** or **9**, TMSOTf, anhydrous CH_2Cl_2; c) i) 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol, TMSOTf, anhydrous CH_2Cl_2, ii)NaN_3, DMF, 70 ^oC; d) H_2, 10% Pd/C, r.t.; e) CH_3ONa, anhydrous CH_3OH; h) **5**, DIPEA, 0 ^oC, overnight.

All the newly synthesized CPT glycoconjugates were obtained according to the synthetic routes illustrated as Schemes **1-3**, respectively, and the yields were generally acceptable ranging from 23.7% to 73.3% (Table 1). Although the yields of these CPT derivatives lacked the comparative basis due to the different synthetic routes, some tendency was still observed. For instance, CPT modified with acetyl-capped oligosaccharides had higher yields than CPT with the corresponding oligosaccharides except for **18c** and **22c**, **19b** and **23b**, **19c** and **23c**, this was possibly ascribed to better separation and less reaction of CPT gylcoconjugates capped with acetyl groups. As for compounds **18a-d**, **19a-d**, **22a-d** and **23a-d** following the procedure listed in Scheme 1, the yields was largely dependent on sugar type and had little connection with length of a linker. However, the yields of compounds **39**-**44** prepared as the procedure shown in Scheme 3 were closely associated with length of a liner, and compounds **40** and **43** with diethylene glycol had the lowest yields.

Table 1. The yields of newly synthesized CPT glycoconjugates



Compds	R_1	m	n	Yield (%)	Compds	R1	m	n	Yield (%)
18a	Ac-	1	0	41.7[a]	**23d**	H-	1	1	24.5[a]
18b	Ac-	1	0	27.8[a]	**27a**	Ac-	1	2	73.3[b]
18c	Ac-	1	0	25.8[a]	**27b**	Ac-	1	2	45.3[b]
18d	Ac-	1	0	50.3[a]	**27c**	Ac-	1	2	48.4[b]
22a	H-	1	0	23.8[a]	**27d**	Ac-	1	2	41.3[b]
22b	H-	1	0	24.5[a]	**26a**	H-	1	2	27.0[b]
22c	H-	1	0	49.1[a]	**26b**	H-	1	2	32.3[b]

22d	H-	1	0	34.3 [a]	**26c**	H-	1	2	23.7 [b]
19a	Ac-	1	1	41.0 [a]	**26d**	H-	1	2	41.4 [b]
19b	Ac-	1	1	24.4 [a]	**39**	H-	2	0	38.5[c]
19c	Ac-	1	1	28.2 [a]	**40**	H-	2	1	25.7 [c]
19d	Ac-	1	1	44.4 [a]	**41**	H-	2	2	42.8 [c]
23a	H-	1	1	29.6 [a]	**42**	Ac-	2	0	60.5 [c]
23b	H-	1	1	34.3 [a]	**43**	Ac-	2	1	48.9 [c]
23c	H-	1	1	44.3 [a]	**44**	Ac-	2	2	60.3 [c]

[a] means the yield calculated from **13a-d** according to the synthetic route shown in Scheme **1**; [b] means the yield calculated from **13a-d** according to the synthetic route shown in Scheme **2**; [c] means the yield calculated from construct **29** according to the synthetic route shown in Scheme **3**

2.2 *In vitro* and *in vivo* evaluation

2.2.1 Cytotoxicity assay.

The *in vitro* antitumor activities of all the newly synthesized CPT gylcoconjugates were assessed in comparison with the marketed irinotecan and the parent compound CPT against three cancer cell lines including hepatocellular carcinoma cell line (HepG2), colorectal carcinoma cell line (HCT116) and pancreatic carcinomas cell line (SW1990) and one normal cell line HEK-293 (Table 2). Clinically, CPT derivatives treated the patients suffering from intestinal cancer or hepatocellular carcinoma [3,4]. Irinotecan was an approved anticancer agent by FDA, constituting the first-line therapy for colon cancer.[21] The *in vitro* cytotoxicity was expressed as a half-maximal inhibitory concentration (IC_{50}, the concentration of a compound needed to inhibit half growth of cells).

All the CPT gylcoconjugates (**18a-d**, **19a-d**, **22a-d**, **23a-d**, **26a-d**, **27a-d** and **39-44)** decorated with five oligosaccharides and the corresponding acetyl-capped ones exhibited potent or moderate anti-proliferative activities against three cancer cells with IC_{50} values ranging from 0.3 to 32.8 μM. Against three cancerous cell lines, the CPT derivatives tested were more sensitive towards HCT116 and HepG2 over SW1990. Compared to CPT, all the derivatives showed comparable or inferior antitumor activities and differential toxicities to normal cells HEK-293, implying that

acetyl groups, oligosaccharide type and length of ethylene glycol-based likers had considerable but complicated impacts on the cytotoxicity and selectivity. On the contrary, compared to the positive control irinotecan (>100 μM), the CPT glycoconjugates demonstrated more potent antitumor activity. This was probably ascribed to the action mechanisms different from that of prodrug irinotecan that was activated by the carboxylesterase to release free drug CPT[22].

Compounds **22a-b**, in which the carbohydrate moieties were GLUT-4-mediated glucose[23] and ASGPR-directed galactose[24], respectively, showed good cytotoxicities against tree cancer cells *(*10.3 μM >IC_{50} >3.8 μM) but displayed no selectivity to HEK-293 (IC_{50} =2.2 μM for **22b**, 3.5 μM for **22a**). When glucose (**22a**) was replaced with mannose receptor (MR)[25]-mediated mannose (**22c**) and 3-*O*-carbamyl mannose with unknown mechanism[13] (**22d**), respectively, the decreased tendency of the antitumor activities was observed, except that **22d** exhibited a slight increase in inhibiting HCT116 growth (IC_{50} =2.2 μM). The introduction of acetyl groups to sugar moieties (**18a-b** and **18d**) resulted in an obvious loss of the *in vitro* potency against cancer cells. **18b** with an acetyl-capped galactose (IC_{50} =60.2 μM) indicated 12 times less active than **22b** bearing a galactose (IC_{50} = 4.9 μM) in inhibiting ASGPR-overexpressing HepG2 cells, suggesting that galactose-mediated targeting outbalanced the lipophilicity of acetyl groups. By contrast, acetylation of a mannose scaffold (**18c**) doubled the antitumor activity towards HepG2 (IC_{50} =10.3 μM (**22c**) *vs* 4.3 μM (**18c**)) and SW1990 (IC_{50} =20.4 μM (**22c**) *vs* 9.7 μM (**18c**)). The selectivity of **18a-d** towards HEK-293 was not significantly affected.

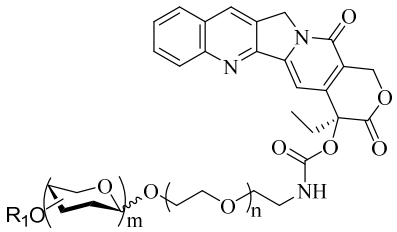
Compounds **23a-d** with diethylene glycol chains were more effective in the antiproliferative activities compared to compounds **22a-d** having monoethylene glycol chains, accompanying with less toxicity to HEK-293. **23a** carrying a glucose exhibited 8.6 times and 3.0 times more potent than the corresponding **22a** in the inhibition of HCT116 (IC_{50} =0.5 μM (**23a**) *vs* 4.3 μM (**22a**)) and HepG2 (IC_{50} = 1.2 μM (**23a**) *vs* 3.8 μM (**22a**)), respectively, however, no detectable changes for SW1990 was observed. Noticeably, **23c** with a mannose moiety displayed the most

obvious increase in the potency of antitumor activity against HepG2 (IC_{50} =0.3 μM (**23c**) *vs* 10.3 μM (**22c**)). Similar to constructs **22a-d**, attachment of the acetyl groups to a carbohydrate moiety (**19a-d**) led to a decrease with varying degrees, and acetylation had little impacts on the selectivity towards HEK-293. When diethylene glycol was elongated to triethylene glycol (**26a-d**), the antitumor activities (**26a-d,** 19.9 μM >IC_{50} >0.5 μM) decreased a little compared to **23a-d** (10.6 μM > IC_{50} >0.3μM). Surprisingly, the selectivity of **26a-d** completely disappeared. This hinted that length of a PEG linker was too long to mediate the selectivity of CPT *via* a carbohydrate moiety with tumor targeting. Therefore, the appropriate linker length turned to be of particular importance in the potency and selectivity of CPT glycoconjugates as antitumor agents.

The synthesis of glycoconjugates **39-41** was used to explore the effects of BLM disaccharide, which had been demonstrated to be a tumor-seeking scaffold[12,13] and to possess higher water-solubility, on the antitumor activity and selectivity of CPT. Compounds **42-44** were synthesized to investigate the effects of acetyl groups on the cytotoxicity and selectivity. With length of a PEG linker was increased from monomer to trimer, the antitumor activities of compounds **39-41** remained small change, whereas compound **40** with a diethylene glycol chain exhibited a high selectivity between HCT116 cells (IC_{50}=0.8 μM) and HEK-293 cells (IC_{50}>100 μM). Acetylation fail to lead to any significant effects on the antitumor activity (compounds **42-44)**, and no selectivity was observed. Taken together, oligosaccharide-CPT conjugates were sensitive to HepG2 cells and HCT116 cells. CPT modified with galactose and BLM disaccharide *via* exhibited higher selectivity, which were possibly associated with sugar moieties introduced. Diethylene glycol that was the optimal linker to assemble CPT and oligosaccharides improved the antitumor activity and selectivity of CPT glycoconjugates. Acetylation disfavored the antitumor activity, which possibly affected a cellular uptake of the CPT glycoconjugates mediated by oligosaccharides with tumor targeting [15].

Table 2. The cytotoxicities of CPT glycoconjugates against hepatocellular carcinoma cells (HepG2), colorectal carcinoma cells (HCT116), pancreatic carcinoma cells (SW1990) and kidney

cells (HET-293).



Compds	R_1	m	n	IC_{50} values[a] (μM)			
				HepG2	HCT-116	SW1990	HEK-293
18a	Ac-	1	0	29.9	15.0	24.8	29.5
18b	Ac-	1	0	60.2	29.5	37.5	8.6
18c	Ac-	1	0	4.3	7.5	9.7	2.3
18d	Ac-	1	0	18.5	10.8	20.6	3.9
22a	H-	1	0	3.8	4.3	10.3	3.5
22b	H-	1	0	4.9	4.1	9.3	2.2
22c	H-	1	0	10.3	8.2	20.4	5.8
22d	H-	1	0	11.6	2.3	32.8	3.2
19a	Ac-	1	1	2.1	4.2	10.3	12.3
19b	Ac-	1	1	5.6	3.5	15.7	>100
19c	Ac-	1	1	4.8	4.4	10.3	20.6
19d	Ac-	1	1	3.7	3.6	7.6	50.2
23a	H-	1	1	1.2	0.5	9.6	24.6
23b	H-	1	1	2.8	2.6	10.6	43.5
23c	H-	1	1	0.3	5.6	3.2	22.4
23d	H-	1	1	0.9	1.2	5.2	52.1
27a	Ac-	1	2	0.7	0.8	4.5	0.6
27b	Ac-	1	2	0.7	0.9	8.3	0.8
27c	Ac-	1	2	1.6	0.6	28.9	3.4
27d	Ac-	1	2	1.8	4.6	31.4	3.4
26a	H-	1	2	1.3	4.8	12.5	0.7
26b	H-	1	2	1.2	8.2	19.9	0.7

26c	H-	1	2	0.8	0.5	16.3	0.6
26d	H-	1	2	0.8	0.9	18.2	0.4
39	H-	2	0	2.5	13.2	30.1	6.2
40	H-	2	1	1.2	0.8	30.5	>100
41	H-	2	2	2.6	18.3	32.6	30.7
42	Ac-	2	0	4.2	6.7	25.1	1.6
43	Ac-	2	1	1.6	1.3	11.3	1.5
44	Ac-	2	2	1.9	23.3	28.6	2.3
CPT				0.4	0.4	0.2	0.6
Irinotecan				>100	>100	>100	>100

[a] indicates the average value of the three independent experiments.

2.2.2 DNA Topo I inhibition assays

To determine whether the antiproliferation activity against three cancer cell lines of the newly synthesized CPT glycoconjugates originated from the inhibition of DNA Topo I, a cell-free assay was conducted using purified recombinant human Topo I (**Fig**.**3**). In this assay, supercoiled plasmid DNA was relaxed and nicked by recombinant Topo I. With supercoiled plasmid DNA only plus recombinant human Topo I as the vehicle control, relaxed and nicked DNA were detectable. As the parent compound CPT was unable to reach a tested concentration of 100.0 μM due to poor aqueous solubility, 10-hyroxyl camptothecin (10-HCPT) was instead of CPT to act as a positive control. Irinotecan was another positive control. As demonstrated in **Fig 3A and Fig S153A-B**, 10-HCPT strongly inhibited Topo I activity at 100.0 μM in the cell free assay.[26] Whereas, the tested CPT derivatives showed very weaker inhibitory activity towards DNA Topo I, which were slightly superior or comparable to the positive control irinotecan. When a tested concentration was decreased to 50.0 μM, Topo I inhibition of the CPT glycoconjugates completely disappeared (**Fig**.**3B** and **Fig. S153C**). Unlike a sulfonylamidine group in the induced ester moiety desirable to better the inhibitory e□ect on Topo I,[27] modification with simple oligosaccharides had a devastating effect on the Topo I activity irrespective of sugar type and linker length, probably blocking recognition of *E*-ring to the interface of Top1/DNA

complex. Although the CPT glycoconjugates were similar to irinotecan in the inhibition of Topo I activity, the antiproliferation activity was entirely different (**Table 1**). Accordingly, we speculated that the antiproliferation activities of the CPT glycoconjugates were not due to direct anti-Topo I activity, hinting that new mechanisms of antitumor action were possibly associated with a carbohydrate moiety. [15]

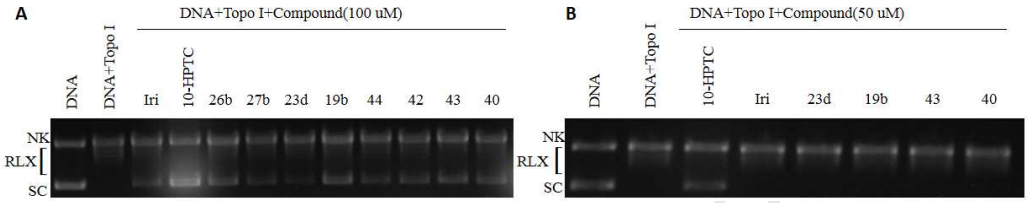


Fig. 3 The CPT gylcoconjugaes directly inhibit Topo I in a cell-free system. (**A**) Recombination human Topo I was incubated with the vehicle (DNA+Topo I), 100 μM Iri (Irinotecan), 100 μM 10-HCPT, or 100 μM the newly synthesized CPT glycoconjugates (**19b**, **23d**, **26b**, **27b**, **40**, **42-44**) followed by incubation with supercoiled plasmid DNA. (**B**) Recombination human Topo I was incubated with the vehicle (DNA+Topo I), 50 μM Iri (Irinotecan), 50 μM 10-HCPT, or 50 μM the CPT glycoconjugates (**19b**, **23d**, **40**, **43**) followed by incubation with supercoiled plasmid DNA. Plasmid DNA was separated by agarose gel and stained with ethidium bromide. NK means nicked, RLX means relaxed, SC means supercoiled.

2.2.3 *In vitro* evaluation of water solubility and stability

Water solubility and *in vivo* stability were major reasons for the side effects and the ineffectiveness of CPT and its derivatives. [5,7] To ascertain whether the introduction of oligosaccharides and acetyl-capped oligosaccharides to 20 (*S*)-OH increased the aqueous solubility and stability, **19b**, **23d** and **40** with the more potent cytotoxicity and higher selectivity were selected as model compounds for the assays. According to the procedures for aqueous solubility and *in vitro* stability reported previously [1,28], all the data were illustrated in Table 3. CPT was very poorly soluble in aqueous solution at pH=7.4 (0.06 mg/mL). The incorporation of an acetyl-capped monosaccharide residue (**19b**, 0.5 mg/mL**)** resulted in an 8-fold increase in aqueous solubility. In contrast, **40** with a BML disaccharide unit (21.2 mg/mL) and **23d** carrying a 3-*O*-carbamyl mannose residue (16.3 mg/mL) were 3533-fold and 2717-fold more soluble than the lead CPT, respectively. Incubation of CPT or **19b** or

23d or **40** in PBS (phosphate-buffered saline,

Table 3. Aqueous solubility and *in vitro* stability of CPT and compounds **19b**, **23d**, **40** at pH 7.4

Compds	Solubility [a,c] (mg/mL)	PBS stability [b,c] (carboxylate/lactone)
CPT	0.06	93/5
19b	0.5	3/91
23d	16.3	5/92
40	21.2	0/94

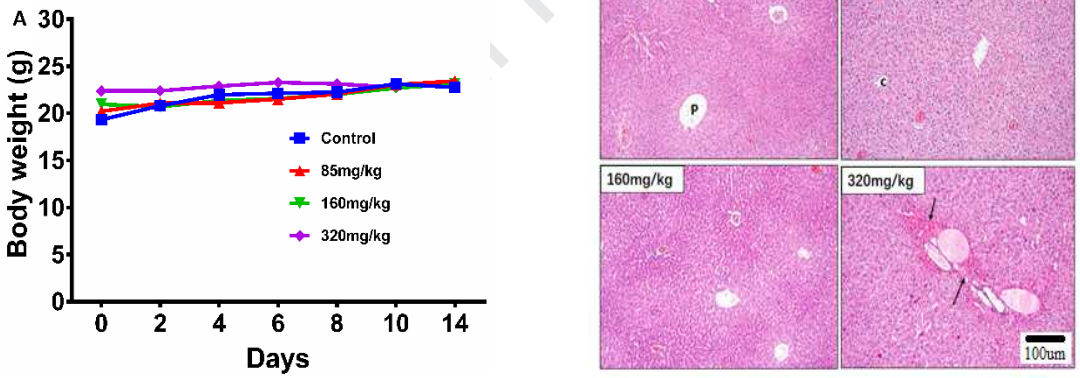
[a]Aqueous solubility was measured in mg/mL of a compound tested in 0.1M Na_2HPO_4 solution (pH=7.4, and 37 ^oC); [b]Stability was determined as the ratio of carboxylate and lactone of a compound tested in 10% phosphate buffer solution at pH=7.4 for 48 h at 37 ^oC. [c]The mean value of triplicate determinations was shown.

pH 7.4) revealed that the glycococonjugates were stable for 48 h but CPT due to its unstable lactone was absolutely converted into the carboxylate form in 50 min. **40** was found to be highly stable in PBS with 94% presence after 48 h without the generation of the carboxylate from. The prolonged stability of compounds **19b, 23d** and **40** in PBS at neutral pH indicated that the PEG-coupled carbamate linker was resistant to pH-mediated cleavage, confirming that oligosaccharides attached to 20 (*S*)-OH were conductive to existence of the lactone ring. Thus, such glycoconjugates with excellent water solubility, just like **23d** and **40**, should be sufficiently stable in culture medium and in serum.

2.2.4 *In vivo* toxicological evaluation.

Since **40** was unable to directly inhibit Topo I activity but possessed strong *in vitro* cytotoxicities against HCT116 and HepG2 and a higher selectivity towards HEK-293, suggesting that it was a novel antitumor agent with lower *in vivo* toxicity, we further performed the primary toxicological assessment of **40**. Acute toxicity of **40** in the ICR mouse was evaluated pathologically. Thirty-two 6-week-old female ICR mice were randomized into four groups (n = 8) to receive 0 (control), 85, 160, or 320 mg/kg of **40** intravenously on day 0. All the treated animals displayed no allergic responses, or significant body weight loss (Fig. **4A**) and were as healthy as the untreated animals, indicating significantly reduced toxicity compared to that of

irinotecan (LD_{50}=85.1 mg/kg, *i.v*)[29]. On day 14, animals were euthanized, and tissues from the liver, lung, kidney and spleen were evaluated histopathologically according to the guidelines[30], and symptomatic lesions, including inflammatory cell infiltration, and focal necrosis in the liver, kidney, spleen and lung, were graded. A few microscopic lesions were detectable in liver, lung and kidney from **40**-treated group. As demonstrated in Fig. **4B** and **4C**, the animals clearly tolerated treatment with 160 mg/kg of **40**, portending an acceptable safety profile. Noticeably, although inflammatory cell infiltration in lung was moderate at 320 mg/kg, no mice died during the experimental period, hinting maximum tolerated dose (MTD) was more than 320 mg/kg. We postulated that the toxicological improvement on normal tissues might be associated with introduction of a BLM disaccharide in the 20 (*S*) -OH of **40**. Further studies including metabolic and pharmacokinetic evaluations are currently underway to address this hypothesis.



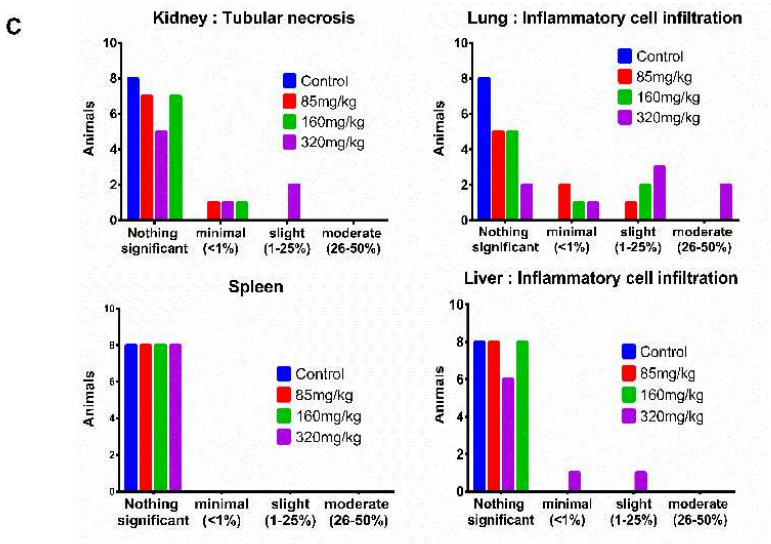


Fig. 4. Pathological evaluation of toxicity of **40** in mice. (A) No significant effects on body weight. Thirty-two ICR mice were randomly divided into four groups, and 8 animals in each group received an *i.v.* injection of **40** at 0 (Control), 85, 160, or 320 mg/kg. (B) Histopathological examination of hepatotoxicity. Formalin-fixed livers from animals treated with vehicle, 85 mg/kg, 160 mg/kg or 320 mg/kg, were embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (H&E). Stained sections were evaluated histopathologically for focal necrosis, and inflammatory cell infiltration. Central vein (C) and portal tract (P) were indicated (upper right panel). Inflammatory cell infiltration and focal necrosis were labeled as arrows. (C) Summary of histopathological evaluation of toxicity. Tissues from the kidney, spleen, lung, and liver were fixed with formalin and embedded in paraffin. Tissue sections were evaluated histopathologically as indicated after staining with H&E.

3. Conclusion

In summary, we have designed and synthesized thirty new CPT glycoconjugates for assessing the aqueous solubility, stability, *in vitro* antitumor activity, Topo I inhibition and *in vivo* toxicological assessment. Such 20 (*S*)-*O*-linked CPT glycoconjugates were conductive to the significant increase in aqueous solubility and sufficient hydrolytic stability of the lactone ring. Oligosaccharide type, length of a PEG linker and acetyl group had major impacts on the antitumor activity and selectivity. All the newly synthesized CPT glycoconjugates had no direct inhibition against Topo I. *In vivo* animal models demonstrated that MTD of **40** was more than 320 mg/kg, portending a more safety profile compared to irinotecan. Collectively, the compelling evidence advocated CPT glycoconjugates as potential targeted drugs.

4. Experimental section

4.1. Chemistry.

All chemicals were of reagent grade quality or better, which were obtained from commercial suppliers and used without further purification. Solvents were used as received or dried over molecular sieves. All the preparations were performed using standard Schlenk techniques. Column chromatography was conducted on silica gel (100-200 mesh) from Qingdao Ocean Chemical Factory. TLC (HSGF 254) from Yantai Jiangyou Silica Gel Development Co. LTD (Yantai, China) was monitored the reaction process. All the key intermediates and products were determined by ^{1}HNMR and ^{13}CNMR, recorded in a Bruker Avance 400 (^{1}H at 400 MHz, ^{13}C at 100 MHz), and chemical shifts were reported in parts per million using the residual solvent peaks as internal standards ($CDCl_3$=7.26 ppm for ^{1}HNMR and 77.16 ppm for ^{13}CNMR, CD_3SOCD_3=2.50 ppm for ^{1}HNMR and 39.6 ppm for ^{13}CNMR). Some key intermediates and products were also confirmed by electrospray ionization high resolution mass spectrometry (ESI-HRMS), recorded on AB Sciex triple TOF 5600+ system. The purity was more than 95%, which was determined with C_{18}-column (250 x 4.6 mm, 5 μm, Agilent Eclipse Plus) run on Agilent Technologies 1260 infinity II.

4.1.1 Synthesis of (*S*)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7] indolizino[1,2-b]quinolin-4-yl (4-nitrophenyl) carbonate (5)

To a solution of CPT (5.0 g, 14.3 mmoL) dissolved in anhydrous DCM (100 mL) 4-nitrophenyl chloroformate (28.6 mmoL) and 4-dimethylaminopyridine (85.8 mmoL) were added dropwisely at 0□, and the reaction mixture was heated to 40□ and proceeded for 4 h. After completion, the solution was diluted with 500 mL of ethyl acetate and was adjusted to be neutral with 0.1 M HCl, and the organic layer was washed 100 mL of brine and dried with anhydrous Na_2SO_4. The residue obtained by evaporating organic solvent was recrystallized with n-hexane to give 6.3 g of activated CPT **5** as a yellowish white solid.

Yield, 86.0 %; ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.26 (dd, *J* = 8.0 Hz, 1H), 8.22 (dd, *J* = 8.0 Hz, 2H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.89 (t, *J* = 8.0 Hz, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.44 (s, 1H), 7.4 (d, *J* = 9.0 Hz, 2H), 5.74 (d, *J* = 16.0 Hz, 1H), 5.44 (d, *J* = 16.0 Hz, 1H), 5.32 (d, *J* = 4.0 Hz, 2H), 2.39-2.34 (m, 1H), 2.28-2.21 (m, 1H), 1.07 (t, *J* = 8.0 Hz, 3H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 166.98, 157.37, 155.17,

152.21, 151.38, 149.01, 146.95, 145.71, 145.08, 131.55, 131.08, 129.67, 128.57, 128.43, 126.36, 125.41, 121.84, 120.48, 115.81, 95.78, 79.44, 67.32, 50.25, 32.02, 7.80.

4.1.2. Synthesis of benzyl (2-hydroxyethyl)carbamate (8) and benzyl (2-(2-hydroxyethoxy)ethyl)carbamate (9)

1.0 g of 2-aminoethan-1-ol (**6**, 16.39 mmoL) was dissolved in 15 mL of tetrahydrofuran and 16 mL of trimethylamine at 0 ^{o}C, and then 3.36 g of benzyl chloroformate (19.67 mmoL) was dropwisely added at the same temperature. The reaction was allowed to proceed for 1 h at r.t. The reaction progress was monitored with TLC. Ethyl acetate (50 mL) and water (20 mL) were poured into the reaction mixture，and the organic layer was washed with brine, and dried by anhydrous Na_2SO_4, and then concentrated under reduced pressure to obtain the residue, which was purified by silica gel column chromatography with ethyl acetate and petroleum ether (V/V=1:1) as an eluent to afford 2.88 g of compound **8** as a white solid.

Yield, 85.2%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.39-7.27 (m, 5H), 5.45 (brs, 1H), 5.08 (s, 2H), 3.65 (d, *J* = 8.0 Hz, 2H), 3.30 (d, *J* = 8.0 Hz, 2H), 2.87 (s, 1H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 157.18, 136.36, 128.55, 128.19, 128.09, 66.91, 61.96, 43.47.

Compound **9** was synthesized as the similar procedure of compound **8**, only differing in starting material 2-aminoethan-ol (**6**) replaced by 2-(2-aminoethoxy)ethan-1-ol (**7**).

Yield, 87.5%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.35-7.29 (m, 5H), 5.37 (brs, 1H), 5.10 (s, 2H), 3.76-3.69 (t, *J* = 4.0 Hz, 2H), 3.54 (t, *J* = 4.0 Hz, 4H), 3.39 (d, *J* = 4.0 Hz, 2H), 2.40 (s, 1H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 156.70, 136.52, 128.51, 128.12, 72.28, 70.08, 66.74, 61.60, 40.87.

4.1.3 General procedures of compounds 14a-d and 15a-d.

Compounds **13a-d** (1.0 mmoL) were dissolved in anhydrous DCM (7 mL), and then compound **8** (1.2 mmoL) and trimethylsilyl trifluoromethanesulfonate (1.0 mmoL) were added at -5℃. The mixture was stirred for 2 h under nitrogen atmosphere at the same temperature. After the completion of the reaction, the mixture was quenched with adequate trimethylamine. Ethyl acetate (30 mL) was added to the

resultant mixture. Then the organic layer was washed with brine (10 mL), dried by anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude residue, which was purified by silica gel column chromatography with ethyl acetate and petroleum ether (V/V=1:1) as an eluent to give the corresponding compounds **14a-d** as colorless transparent liquid. Similarly, when reagent **8** was replaced with compound **9**, compounds **15a-d** were obtained

(2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-(2-(((benzyloxy)carbonyl)amino)ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (**14a**): Yield, 62.4%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.34-7.31 (m, 5H), 5.20 (t, *J* = 8.0 Hz, 2H), 5.08 (d, *J* = 12.0 Hz, 3H), 4.96 (t, *J* = 8.0 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.22 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.14 (d, *J* = 12.0 Hz, 1H), 3.87-3.83 (m, 1H), 3.72-3.64 (m, 2H), 3.44-3.33 (m, 2H), 2.04 (s, 3H), 2.01 (s, 3H), 1.99 (s, 6H). ^{13}CNMR (100 MHz, $CDCl_3$) δ 170.67, 170.24, 169.49, 169.41, 156.36, 136.44, 128.53, 128.18, 128.14, 100.05, 72.64, 71.88, 71.25, 69.54, 68.26, 66.76, 61.83, 40.89, 20.70, 20.60.

(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(((benzyloxy)carbonyl)amino)ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (**14b**): Yield, 57.2%. ^{1}HNMR (400 MHz, $CDCl_3$): δ7.35-7.27 (m, 5H), 5.37 (d, *J* = 4.0 Hz, 1H), 5.22 (dd, *J* = 16.0, 8.0 Hz, 2H), 5.08 (s, 2H), 5.01 (m, 1H), 4.45 (d, *J* = 8.0 Hz, 1H), 4.13 (d, *J* = 8.0 Hz, 2H), 3.88 (t, *J* = 8.0 Hz, 2H), 3.70-3.65 (m, 1H), 3.44-3.33 (m, 2H), 2.14 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$) δ 170.50, 170.33, 170.22, 169.75, 156.45, 136.52, 128.62, 128.26, 101.61, 70.87, 70.85, 69.55, 68.91, 67.06, 66.86, 61.42, 40.97, 20.78, 20.67.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-6-(2-(((benzyloxy)carbonyl)amino)ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (**14c**)*:* Yield, 62.5%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.36-7.30 (m, 5H), 5.32-5.28 (m, 1H), 5.25-5.23 (m, 2H), 5.11 (s, 2H), 4.82 (d, *J* = 4.0 Hz, 1H), 4.27 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.09 (dd, *J* = 12.0, 8.0 Hz, 1H), 3.98-3.94 (m, 1H), 3.80-3.75 (m, 1H), 3.57-3.54 (m, 1H), 3.47-3.38 (m, 2H), 2.15 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 169.76, 169.10, 169.07, 168.80, 155.47, 135.47, 127.66, 127.30, 127.28, 96.86, 68.49, 68.06, 67.83, 66.84, 66.00, 65.19, 61.60, 39.78, 19.99, 19.80.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-6-(2-(((benzyloxy)carbonyl)amino)ethoxy)-4-(carbamoyloxy)tetrahydro-2H-pyran-3,5-diyl diacetate(**14d**): Yield, 62.3%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.37-7.27 (m, 5H), 5.35-5.33 (m, 1H), 5.28-5.20 (m, 3H), 5.10 (s, 2H), 4.84 (s, 2H), 4.82 (d, *J* = 4.0 Hz, 1H), 4.26 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.09-4.05 (m, 1H), 3.98-3.94 (m, 1H), 3.79-3.74 (m, 1H), 3.59-3.41(m, 1H), 3.41-3.35 (m, 2H), 2.13 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.76, 170.13, 170.08, 156.50, 155.46, 136.50, 128.63, 128.26, 128.24, 97.79, 70.02, 69.97, 68.81, 66.96, 66.31, 62.60, 40.79, 21.01, 20.84, 20.80.

(2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-(2-(2-(((benzyloxy)carbonyl)amino)ethoxy) ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**15a**): Yield, 71.6%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.34-7.28 (m, 5H), 5.34 (brs, 1H), 5.17 (t, *J* = 8.0 Hz, 1H), 5.07 (s, 2H), 5.03 (d, *J* = 8.0 Hz, 1H), 4.97 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.55 (d, *J* = 8.0 Hz, 1H), 4.21 (m, 1H), 4.09-4.07 (m, 1H), 3.87-385 (m, 1H), 3.66-3.83 (m, 2H), 3.56-3.51 (m, 2H), 3.49-3.48 (m, 2H), 3.33-3.32 (m, 2H), 2.03 (d, *J* = 4.0 Hz, 3H), 1.99-1.94 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.66, 170.23, 169.42, 156.49, 136.65, 128.50, 128.15, 128.08, 100.74, 72.75, 71.82, 71.29, 70.15, 69.96, 68.85, 68.39, 66.64, 61.92, 40.90, 20.72, 20.62, 20.59.

(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(2-(((benzyloxy)carbonyl)amino)ethoxy) ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (**15b**): Yield, 62.0%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35-7.29 (m, 5H), 5.35 (s, 1H), 5.31 (s, 1H), 5.19 (t, *J* = 8.0 Hz, 1H), 5.09 (s, 2H), 5.01 (t, *J* = 12.0 Hz, 1H), 4.52 (d, *J* = 8.0 Hz, 1H), 4.26-4.00 (m, 2H), 3.92-3.87 (m, 2H), 3.69 (d, *J* = 8.0 Hz, 1H), 3.59 (m, 2H), 3.54-3.45 (m, 2H), 3.36 (m, 2H), 2.11 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.49, 170.34, 170.21, 169.64, 156.56, 136.68, 128.59, 128.24, 128.19, 101.36, 70.92, 70.78, 70.21, 70.07, 68.90, 67.10, 66.76, 61.38, 40.98, 20.82, 20.78, 20.73, 20.68.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-6-(2-(2-(((benzyloxy)carbonyl)amino)ethoxy) ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**15c**): Yield, 61.0%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.33-7.27 (m, 5H), 5.40-5.39 (m, 1H), 5.33 (d, *J* = 8.0 Hz, 1H), 5.26-5.22 (m, 2H), 5.07 (s, 2H), 4.86 (s, 1H), 4.26-4.21 (m, 1H), 4.06 (dd, *J* = 16.0,

4.0 Hz, 2H), 3.76 (m, 1H), 3.62 (dd, *J* = 16.0, 4.0 Hz, 3H), 3.51 (d, *J* = 4.0 Hz, 2H), 3.35 (d, *J* = 8.0 Hz, 2H), 2.10 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.78, 170.22, 170.00, 169.87, 156.59, 136.67, 128.53, 128.10, 97.58, 70.33, 70.00, 69.68, 69.03, 68.47, 67.15, 66.69, 66.29, 62.64, 41.01, 20.93, 20.80, 20.74, 20.72.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-6-(2-(2-(((benzyloxy)carbonyl)amino)ethoxy) ethoxy)-4-(carbamoyloxy)tetrahydro-2H-pyran-3,5-diyl diacetate (**15d**): Yield, 58.0%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.34-7.27 (m, 5H), 5.45 (s, 1H), 5.24 (d, *J* = 4.0 Hz, 3H), 5.08 (s, 2H), 4.89 (d, *J* = 8.0 Hz, 3H), 4.30-4.23 (m, 1H), 4.13 (dd, *J* = 8.0, 4.0 Hz, 1H), 3.80-3.73 (m, 1H), 3.70-3.59 (m, 3H), 3.55 (d, *J* = 8.0 Hz, 3H), 3.38 (d, *J* = 8.0 Hz, 2H), 2.10 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.80, 170.22, 170.14, 156.63, 155.46, 136.73, 128.60, 128.55, 128.21, 128.15, 128.11, 97.58, 70.36, 70.19, 70.04, 69.91, 68.50, 67.20, 66.71, 66.41, 62.67, 41.04, 20.99, 20.82, 20.79.

4.1.4. General procedures for compounds 18a-d and 19a-d.

To a solution of compounds **14a-d** or **15a-d** (0.396 mmoL) dissolved in ethyl acetate (5 mL) catalytic amounts of 10 % Pd/C (80 mg) were added, and the mixture was stirred for 1 h at room temperature under hydrogen atmosphere. After completion, the reaction mixture was filtered with celite and concentrated under reduced pressure to provide the residues **16a-d** or **17a-d**, respectively, for next reaction without purification. To a solution of compound **5** (0.48 mmoL) dissolved in DCM (5 mL) crude residues **16a-d** or **17a-d** and diisopropylethylamine (0.1 mmoL) were added at 0□. The mixture was heated to r.t. and was stirred overnight. After completion of the reaction, DCM (100 mL) and statured ammonium chloride solution (20 mL) were added to dilute the reaction mixture and were shaken for several minutes. The organic layer was washed by brine, dried by anhydrous Na_2SO_4, and then concentrated under reduced pressure to give the crude residue, which was purified by silica gel column chromatography with ethyl acetate and DCM (V/V=1:1) as an eluent to afford the corresponding compounds **18a-d** or **19a-d** as white solids.

(2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tet

rahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl) oxy) carbonyl)amino) ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**18a**): Yield, 66.8%. ^{1}HNMR (400 MHz, DMSO): δ 8.70 (s, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.10 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.84 (t, *J* = 8.0 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 7.37 (s, 1H), 5.32 (s, 2H), 4.97 (t, *J* = 4.0 Hz, 1H), 4.77 (t, *J* = 4.0 Hz, 1H), 4.72-4.66 (m, 3H), 4.62 (d, *J*= 8.0 Hz, 1H), 4.27 (t, *J* = 8.0 Hz, 1H), 3.90 (t, *J* = 4.0 Hz, 2H), 3.85-3.58 (m, 5H), 2.65-2.56 (m, 1H), 2.37-2.28 (m, 1H), 2.02 (s, 3H), 1.90 (s, 3H), 1.64 (s, 3H), 1.42 (s, 3H), 0.93 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.58, 170.07, 169.28, 169.08, 168.75, 160.78, 153.71, 152.41, 148.19, 144.24, 143.92, 131.57, 130.34, 130.25, 130.22, 129.39, 128.53, 128.19, 127.68, 99.68, 96.93, 88.83, 71.75, 70.70, 70.44, 67.83, 64.90, 61.96, 55.18, 50.75, 31.08, 29.91, 20.79, 20.72, 20.56, 19.32, 7.84. TOF-MS, m/z: [M + Na^{+}], calcd for $C_{37}H_{39}N_3O_{15}$ Na^{+}, 788.2273, found, 788.2318.

(2R,3R,4S,5S,6R)-2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyran o[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy) carbonyl) amino) ethoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**18b)**: Yield, 48.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.75 (s, 1H), 7.69-7.65 (m, 1H), 5.40-5.33 (m, 3H), 5.11-5.02 (m, 3H), 4.62 (d, *J*=8.0 Hz, 1H), 4.44 (d, *J*=8.0 Hz, 1H), 4.17-4.02 (m, 3H), 3.95-3.76 (m, 4H), 2.49-2.40 (m, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.85 (s, 3H), 1.07 (t, *J* = 8.0, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.38, 170.48, 170.29, 170.05, 169.58, 162.30, 153.70, 152.37, 148.91, 144.65, 144.56, 131.42, 131.06, 130.83, 130.34, 129.74, 128.98, 128.39, 128.23, 101.10, 98.06, 89.17, 70.92, 70.75, 68.37, 67.01, 65.72, 64.22, 61.27, 50.47, 32.08, 29.91, 20.51, 20.30, 20.05, 19.63, 7.54. TOF-MS, m/z: [M + Na^{+}], calcd for $C_{37}H_{39}N_3O_{15}$ Na^{+}, 788.2273, found, 788.2263.

(2R,3R,4S,5S,6S)-2-(acetoxymethyl)-6-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tet rahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)amino) ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**18c**): Yield, 41.2%. ^{1}HNMR (400 MHz, DMSO): δ 8.69 (s, 1H), 8.18 (d, *J* = 12.0 Hz, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 8.07 (t, *J* = 8.0 Hz, 1H), 7.88 (t, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.04 (s, 1H),

5.44 (d, *J*= 4.0 Hz, 2H),5.29 (s, 2H), 5.18-5.09 (m, 2H), 5.09 (m, 1H), 4.85 (s, 1H), 4.15-4.10 (m, 1H), 4.03-3.99 (m, 2H), 3.61-3.43 (m, 2H), 3.23-3.09 (m, 2H), 2.15-2.12 (m, 2H), 2.08 (s, 3H), 2.00 (s, 3H), 1.93 (s, 3H), 1.89 (s, 3H), 0.90 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO): δ 170.07, 169.64, 169.58, 168.10, 156.72, 154.64, 152.48, 147.97, 146.22, 145.88, 131.70, 130.43, 129.88, 129.06, 128.62, 128.09, 127.77, 119.10, 97.01, 94.93, 74.87, 68.80, 68.72, 67.87, 66.40, 65.56, 61.92, 50.29, 30.86, 20.68, 20.53, 20.47, 7.62. TOF-MS, m/z: [M + H^+], calcd for $C_{37}H_{40}N_3O_{15}^+$, 766.2454, found, 766.2436.

(2R,3R,4S,5S,6S)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2b] quinolin-4-yl)oxy) carbonyl)amino)ethoxy)tetrahydro-2H-pyran-3,5-diyl diacetate (**18d**): Yield, 81.3%. ^{1}HNMR (400 MHz, DMSO): δ 8.67 (s, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.88 (t, *J* = 7.5 Hz, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.41 (s, 1H), 6.68 (brs, 1H), 6.54 (brs, 1H), 5.26 (s, 2H), 5.11-5.06 (m, 1H), 5.04 (d, *J* = 8.0 Hz, 1H), 4.98-4.93 (m, 1H), 4.91 (d, *J* = 8.0 Hz, 2H), 4.82 (t, *J* = 8.0 Hz, 2H), 4.09-3.96 (m, 3H), 3.87 (d, *J* = 8.0 Hz, 3H), 3.78-3.69 (m, 2H), 2.41-2.33 (m, 1H), 2.06 (s, 3H), 2.00-1.98 (m, 6 H), 0.99 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.63, 170.14, 169.65, 169.35, 160.63, 155.36, 153.85, 152.52, 148.02, 144.31, 144.10, 131.69, 130.44, 129.95, 129.74, 129.12, 128.59, 128.04, 127.77, 96.98, 96.53, 88.67, 69.17, 68.41, 68.05, 65.95, 62.98, 62.05, 54.98, 51.63, 31.25, 20.75, 20.59, 20.55, 7.67. TOF-MS, m/z: [M + Na^+], calcd for $C_{36}H_{38}N_4O_{15}Na^+$, 789.2226, found, 789.2257.

(2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12, 14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl) amino)ethoxy)ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**19a**)**:** Yield, 57.3%. ^{1}HNMR (400 MHz, DMSO): δ 8.69 (s, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.88 (t, *J* = 8.0 Hz, 1H), 7.81 (t, *J* = 8.0 Hz, 1H), 7.74-7.70 (m, 1H), 7.03 (s, 1H), 5.45 (d, *J* = 4.5 Hz, 2H), 5.30 (s, 2H), 5.25 (t, *J* = 12.0 Hz, 1H), 4.91 (t, *J* = 12.0 Hz, 1H), 4.77-4.69 (m, 2H), 4.15-4.13 (m, 1H), 3.99-3.88 (m, 2H), 3.74-3.71 (m, 1H), 3.61-3.54 (m, 1H), 3.62-3.56 (m, 1H), 3.53-3.03 (m, 3H), 3.09-3.04 (m, 2H), 2.15-2.10 (m, 2H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 0.89 (t, *J* =

8.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO): δ 170.14, 169.65, 169.39, 169.16, 168.15, 156.76, 154.54, 152.58, 147.92, 146.46, 145.77, 131.75, 130.53, 129.92, 129.06, 128.67, 128.12, 127.82, 119.00, 99.60, 94.97, 74.77, 72.16, 70.95, 70.61, 69.37, 69.05, 68.59, 68.24, 66.33, 61.76, 50.32, 31.26, 29.93, 20.58, 20.48, 20.45, 20.38, 7.65. TOF-MS, m/z: [M + Na^+], calcd for $C_{39}H_{43}N_3O_{16}Na^+$, 832.2536, found, 832.2548.

(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)amino) ethoxy)ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**19b)**: Yield, 39.4%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.88 (t, *J* = 8.0 Hz, 1H), 7.79 (s, 1H), 7.69 (t, *J* = 8.0 Hz, 1H), 5.35 (s, 1H), 5.31 (s, 2H), 5.18-5.02 (m, 3H), 4.97 (d, *J* = 12.0 Hz, 1H), 4.80 (d, *J* = 4.0 Hz, 1H), 4.47 (d, *J* = 4.0 Hz, 1H), 4.13 (t, *J* = 8.0 Hz, 2H), 3.92 (d, *J* = 4.0 Hz, 1H), 3.89-3.84 (m, 3H), 3.73-3.71 (m, 2H), 3.65-3.60 (m, 3H), 2.51-2.41 (m, 2H), 2.14 (s, 3H), 2.04 (s, 6H), 1.98 (s, 3H), 0.95 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 172.39, 170.55, 170.43, 170.31, 168.66, 162.27, 153.90, 152.37, 148.96, 144.82, 144.48, 131.39, 131.06, 130.88, 130.29, 129.86, 128.59, 128.29, 128.25, 101.37, 98.28, 89.35, 70.99, 70.70, 70.04, 69.09, 68.91, 67.17, 66.81, 65.72, 61.34, 50.33, 32.04, 29.45, 20.87, 20.84, 20.81, 20.73, 7.79. TOF-MS, m/z: [M + Na^+], calcd for $C_{39}H_{43}N_3O_{16}Na^+$, 832.2536, found, 832.2531.

(2R,3R,4S,5S,6S)-2-(acetoxymethyl)-6-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl) amino) ethoxy)ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**19c**): Yield, 46.4%. ^{1}HNMR (400 MHz, DMSO): δ 8.69 (s, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.87-7.82 (m, 2H), 7.73-7.69 (m, 1H), 7.04 (s, 1H), 5.44 (d, *J* = 2.2 Hz, 2H), 5.27 (s, 2H), 5.07 (m, 3H), 4.81 (s, 1H), 4.12-3.87 (m, 3H), 3.68-3.66 (m, 1H), 3.57-52 (m, 3H), 3.40-3.35 (m, 2H), 3.09-3.03 (m, 2H), 2.14-2.09 (m, 5H), 1.99 (s, 6H), 1.92 (s, 3H), 0.91 (t, *J* =8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 170.39, 170.10, 169.70, 169.50, 168.10, 156.73, 154.52, 152.47, 147.96, 146.47, 145.75, 131.71, 130.47, 129.88, 129.02, 129.86, 128.63, 128.08, 127.77, 118.93, 96.68, 94.92, 74.75, 69.05, 68.89, 68.72, 67.82, 66.52, 66.29, 65.41, 61.98, 50.29, 30.72, 20.67,

20.57, 20.50, 20.48, 7.62. TOF-MS, m/z: [M + Na^+], Calcd. for $C_{39}H_{43}N_3O_{16}Na^+$, 832.2536, found, 832.2561.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)amino)ethoxy)ethoxy)tetrahydro-2H-pyran-3,5-diyl diacetate (**19d**): Yield, 76.5%. ^{1}HNMR (400 MHz, DMSO): δ 8.66 (s, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.86-7.83 (m, 1H), 7.71-7.67 (m, 1H), 7.40 (s, 1H), 6.71 (brs, 1H), 6.60 (brs, 1H), 5.25 (s, 2H), 5.11-4.97 (m, 3H), 4.89 (t, *J* = 8.0 Hz, 1H), 4.85-4.75 (m, 3H), 4.13 (dd, *J* = 12.0, 4.0 Hz, 1H), 3.99-3.88 (m, 2H), 3.78-3.54 (m, 8 H), 2.50-2.43 (m, 1H), 2.40-2.31 (m, 1H), 2.09 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 0.98 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.59, 170.11, 169.61, 169.45, 160.64, 155.50, 153.90, 152.47, 147.98, 144.44, 144.09, 131.65, 130.47, 129.91, 129.78, 129.06, 128.57, 128.02, 127.75, 96.88, 96.80, 88.66, 69.31, 68.98, 66.41, 66.00, 62.19, 55.04, 55.01, 50.60, 31.45, 21.78, 20.61, 20.59, 7.45. TOF-MS, m/z: [M + Na^+], calcd for $C_{38}H_{42}N_4O_{16}Na^+$, 833.2488, found, 833.2510.

4.1.5 General procedures for compounds 22a-d and 23a-d.

To a solution of compounds **14a-d** or **15a-d** (1 mmoL) dissolved in ethyl acetate (5 mL) catalytic amounts of 10 % Pd/C (80 mg) were added, and then under hydrogen atmospheres, the reaction mixture was stirred for 1 h at room temperature. After the completion of the reduction reaction, the mixture was filtered and concentrated under reduced pressure to obtain crude residues **16a-d** or **17a-d**, respectively. The residues **16a-d** or **17a-d** were re-dissolved in anhydrous methanol (5 mL), and sodium methoxide (0.1 equiv) was added and the resultant solution was stirred for 1 h. Dowex H^+ resin (60 mg) was added to adjust pH, After shaken for another 10 min, the solution was filtered and concentrated under reduced pressure to obtain the corresponding compounds **20a-d** or **21a-d** as colorless oil for the next reaction without purification. To a solution of compound **5** (1.2 mmoL) dissolved in 5 mL of anhydrous DMF crude compounds **20a-d** or **21a-d** and diisopropylethylamine (0.1 mmoL) were added at 0□, and the reaction mixture was heated to room

temperature and stirred overnight. After consumption of compounds **20a-d** or **21a-d** monitored with TLC, DCM (100 mL) and saturated ammonium chloride solution (20 mL) were poured. The crude residues obtained were purified by silica gel column chromatography with methanol and DCM (V/V=1:20) as an eluent to afford the corresponding compounds **22a-d** and **23a-d** as white solids.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b] quinolin-4-yl(2-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethyl)carbamate (**22a**): Yield, 38.1%. ^{1}HNMR (400 MHz, DMSO): δ 9.2 (s, 1H), 8.69 (s, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.86 (t, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.40 (d, *J* = 4.0 Hz, 1 H), 5.45 (d, *J* = 4.0 Hz, 1H), 5.28 (d, *J* = 4.0 Hz, 2H), 4.96-4.84 (m, 1H), 4.81-4.66 (m, 2H), 4.56-4.45 (m, 1H), 4.39-4.33 (m, 1H), 4.11-4.06 (m, 1H), 3.87 (t, *J* = 4.0 Hz, 1H), 3.71 (s, 2H), 3.60 (d, *J* = 8.0 Hz, 2H), 3.51-3.14 (m, 3H), 3.12 (d, *J* = 8.0 Hz, 2H), 2.38-2.32 (m, 1H), 2.14-2.10 (m, 1H), 0.95-0.87 (m, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.64, 160.61, 154.61, 153.91, 152.56, 148.37, 148.00, 144.21, 131.74, 130.49, 129.97, 129.12, 128.60, 128.09, 127.72, 119.03, 97.32, 94.98, 88.58, 75.35, 73.53, 70.43, 67.99, 63.81, 60.42, 53.44, 50.62, 31.39, 7.65. TOF-MS, m/z: [M + Na^+], calcd for $C_{29}H_{31}N_3O_{11}Na^+$, 620.1851, found, 620.1855.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano [3',4':6,7] indolizino[1,2-b]quinolin-4-yl(2-(((2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxyl methyl)tetrahydro-2H-pyran-2-yl) oxy)ethyl)carbamate (**22b**): Yield, 42.6%. ^{1}HNMR (400 MHz, DMSO): δ 9.13 (s, 1H), 8.67 (d, *J* = 4.0 Hz, 1H), 8.20 (m, 1H), 8.11 (m, 1H), 7.85 (m, 1H), 7.71 (dd, *J* =8.0, 4.0 Hz, 1H), 7.38 (s, 1H), 5.45 (d, *J* = 4.0 Hz, 1H), 4.92-4.84 (m, 2H),4.79-4.67 (m, 2H), 4.56-4.53 (m, 1H), 4.38 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.13-4.05 (m, 1H), 3.91-3.84 (m, 1H), 3.68-3.65 (m, 2H), 3.62-3.57 (m, 2H), 3.50-3.41 (m, 2H), 3.33-3.24 (m, 2H), 3.15-3.09 (m, 2H), 2.38-1.88 (m, 2H), 0.96 (m, 3H). TOF-MS, m/z: [M + Na^+], calcd for $C_{29}H_{31}N_3O_{11}Na^+$, 620.1851, found, 620.1866.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7] indolizino[1,2-b]quinolin-4-yl(2-(((2S,3S,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethy

l)tetrahydro-2H-pyran-2-yl)oxy)ethyl)carbamate (**22 c**): Yield, 78.6%. ^{1}HNMR (400 MHz, DMSO) δ 8.69 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.86 (t, *J* = 8.0 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 7.40 (s, 1H), 5.25 (s, 2H), 4.91 (t, *J* = 4.0 Hz, 2H), 4.80 (t, *J* = 4.0 Hz, 2H), 4.70 (d, *J* = 4.0 Hz, 2H), 4.63 (s, 1H), 4.53 (d, *J* = 4.0 Hz, 1H), 4.42 (t, *J* = 4.0 Hz, 1H), 3.84-3.80 (m, 2H), 3.68-3.55 (m, 3H), 3.50 (d, *J* = 4.0 Hz, 1H), 3.43-3.30 (m, 2H), 3.22-3.18 (m, 1H), 2.53-2.33 (m, 2H), 0.96 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO) δ 172.62, 160.64, 153.85, 152.51, 147.99, 144.38, 144.13, 131.65, 130.45, 129.93, 129.67, 129.11, 128.56, 128.02, 127.74, 99.52, 96.78, 88.56, 74.17, 70.85, 70.18, 66.77, 61.88, 61.09, 55.02, 50.60, 31.51, 7.60. TOF-MS, m/z: [M + Na^{+}], calcd for $C_{29}H_{31}N_3O_{11}Na^{+}$, 620.1851, found, 620.1844.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7] indolizino[1,2-b]quinolin-4-yl(2-(((2R,3S,4S,5R,6R)-4-(carbamoyloxy)-3,5-dihydroxy -6-(hydroxyl-methyl)tetrahydro-2H-pyran-2-yl)oxy)ethyl)carbamate (**22d**): Yield, 55.1%. ^{1}HNMR (400 MHz, DMSO): δ 8.69 (s, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 4.0 Hz, 1H), 7.88 (t, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.05 (s, 1H), 6.42 (s, 2H), 5.45 (s, 2H), 5.29 (s, 2H), 4.68-4.60 (m, 2H), 3.93-3.31 (m, 10H), 3.24-3.07 (m, 2H), 2.14-2.09 (m, 2H), 0.89 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 168.14, 156.73, 154.61, 152.47, 147.96, 146.32, 145.79, 143.92, 131.73, 130.51, 129.90, 129.12, 128.60, 128.08, 126.55, 119.01, 100.32, 95.00, 74.82, 74.32, 73.79, 68.12, 66.38, 65.57, 64.35, 60.99, 50.31, 30.84, 7.63. TOF-MS, m/z: [M + Na^{+}], calcd for $C_{30}H_{32}N_4O_{12}Na^{+}$, 663.1909, found, 663.1903.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl(2-(2-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethyl)carbamate (**23a**): Yield, 41.3%. ^{1}HNMR (400 MHz, DMSO): δ 8.68 (s, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.88 (t, *J* = 8.0 Hz, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.40 (s, 1H), 5.27 (s, 2H), 4.98-4.86 (m, 3H), 4.81 (t, *J* = 8.0 Hz, 1H), 4.49 (t, *J* = 8.0 Hz, 1H), 4.12-4.03 (m, 1H), 3.86-3.47 (m, 8H), 3.46-3.41 (m, 2H), 3.17 (d, *J* = 4.0 Hz, 1H), 3.12-2.87 (m, 4H),

2.43-2.30 (m, 1H), 2.20-2.05 (m, 1H), 0.97 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.62, 160.65, 153.94, 152.50, 147.99, 144.40, 144.13, 131.69, 130.51, 129.96, 129.78, 129.10, 128.60, 128.05, 127.78, 103.09, 96.91, 88.67, 76.91, 76.77, 73.46, 70.07, 69.44, 67.84, 65.90, 61.12, 55.02, 50.63, 48.70, 31.41, 7.56. TOF-MS, m/z: [M + Na^+], calcd for $C_{31}H_{35}N_3O_{12}Na^+$, 664.2113, found, 664.2111.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl(2-(2-(((2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethyl)carbamate **(23b)**: Yield, 55.3%. ^{1}HNMR (400 MHz, DMSO): δ 8.64 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.37 (s, 1H), 5.23 (s, 2H), 4.94-4.84 (m, 1H), 4.81 (t, *J* = 8.0 Hz, 2H), 4.70 (t, *J* = 8.0 Hz, 1H), 4.37 (d, *J* = 4.0 Hz, 1H), 4.25 (d, *J* = 8.0 Hz, 1H), 4.03 (d, *J* = 4.0 Hz, 1H), 3.79-3.43 (m, 10H), 3.28-3.21 (m, 2H), 3.17 (d, *J* = 4.0 Hz, 2H), 3.31-3.02 (m, 1H), 2.51-2.49 (m, 1H), 2.40-2.33 (m, 1H), 0.97 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.62, 160.64, 153.95, 152.44, 147.96, 144.41, 144.09, 131.66, 130.48, 129.89, 129.77, 129.06, 128.55, 128.00, 127.75, 103.70, 96.90, 88.67, 75.24, 73.54, 70.62, 69.46, 68.14, 67.74, 65.92, 60.43, 55.04, 50.60, 48.70, 31.45, 7.55. TOF-MS, m/z: [M + Na^+], calcd for $C_{31}H_{35}N_3O_{12}Na^+$, 664.2113, found, 664.2090.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b] quinolin-4-yl (2-(2-(((2S,3S,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethyl)carbamate (**23c**): Yield, 72.6%. ^{1}HNMR (400 MHz, DMSO): δ 8.67 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 4.0 Hz, 1H), 7.88-7.83 (m, 2H), 7.71(d, *J* = 8.0 Hz, 1H), 7.04 (s, 1H), 5.44 (s, 2H), 5.27 (s, 2H), 4.72 (d, *J* = 4.0 Hz, 1H), 4.69 (*J* = 4.0 Hz, 1H), 4.57 (t, *J* = 4.0 Hz, 2H), 4.45 (t, *J* = 8.0 Hz, 1H), 3.68-3.58 (m, 3H), 3.57 (t, *J* = 4.0 Hz, 1H), 3.50 (t, *J* = 4.0 Hz, 1H), 3.46-3.36 (m, 6H), 3.31-3.25(m, 1H), 3.14-3.02 (m, 2H), 2.19-2.03 (m, 2H), 0.92 (t, *J* = 8.0 Hz, 3H).

^{13}CNMR (100 MHz, DMSO): δ 168.15, 156.73, 154.53, 152.43, 147.95, 146.49, 145.75, 131.72, 130.52, 129.84, 129.04, 128.61, 128.06, 127.77, 118.94, 100.03, 94.98, 74.76, 74.02, 70.99, 70.32, 69.43, 69.02, 67.04, 66.31, 65.73, 61.33, 53.46,

50.28, 30.74, 7.65. TOF-MS, m/z: [M + Na^+], calcd for $C_{31}H_{35}N_3O_{12}Na^+$, 664.2113, found, 664.2120.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b] quinolin-4-yl(2-(2-(((2R,3S,4S,5R,6R)-4-(carbamoyloxy)-3,5-dihydroxy-6-(hydroxyl -methyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethyl)carbamate (**23d**): Yield, 42.3%. ^{1}HNMR (400 MHz, DMSO): δ 8.66 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.88-7.85 (m, 2H), 7.69 (t, *J* = 8.0 Hz, 1H), 7.04 (s, 1H), 6.46 (s, 2H), 5.44 (s, 2H), 5.26 (s, 2H), 5.00 (d, *J* = 4.0 Hz, 1H), 4.88 (d, *J* = 8.0 Hz, 1H), 4.65-4.58 (m, 2H), 4.54 (t, *J* = 8.0 Hz, 1H), 3.75 (t, *J* = 8.0 Hz, 1H), 3.70-3.33 (m, 10H), 3.15-3.06 (m, 2H), 2.13-2.06 (m, 2H), 0.92 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 168.16, 156.80, 157.74, 154.55, 152.43, 147.95, 146.88, 146.51, 145.75, 131.71, 130.54, 129.84, 129.04, 128.60, 127.77, 118.94, 100.23, 94.99, 74.76, 74.26, 73.82, 69.39, 69.00, 68.15, 66.31, 65.73, 64.27, 61.07, 53.38, 50.28, 30.75, 7.66. TOF-MS, m/z: [M+Na^+], calcd for $C_{32}H_{36}N_4O_{13}Na^+$, 707.2171, found, 707.2158.

4.1.6. General procedures for compounds 24a-d

Compounds **13a-d** (0.86 mmoL) and 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (158.0 mg, 1.0 mmoL) were mixed in 7.0 mL of anhydrous DCM, and trimethylsilyl trifluoromethanesulfonate (0.9 mmoL) was added at -5□. The reaction solution was stirred for 2 h, and finally the reaction was quenched with suitable amounts of TEA. Ethyl acetate (30 mL) was added to dilute the reaction mixture. The organic layer was washed with water (20 mL) and brine (20 mL) in order, and dried by anhydrous Na_2SO_4 for 1 h, and then concentrated under reduced pressure to offer the crude residues, which were chromatographed on silica column with ethyl acetate and petroleum ether (V/V=1:1) as an eluent to afford the corresponding colorless transparent compounds **24a-d**.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(2-(2-chloroethoxy)ethoxy)ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate **(24a)**:*:*Yield, 67.5%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.20 (t, *J* = 12.0 Hz,1H), 5.05 (t, *J* = 8.0 Hz, 1H), 4.98 (t, *J* = 8.0 Hz, 1H), 4.60 (d, *J* = 8.0 Hz, 1H), 4.24 (d, *J* = 8.0 Hz, 1H), 4.12 (d, *J* = 12.0 Hz, 1H), 3.91 (d, *J* = 12.0 Hz, 1H), 3.78-3.57 (m, 12H), 2.05 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.97 (s,

3H). ^{13}CNMR (100 MHz, $CDCl_3$) δ 170.75, 170.34, 169.49, 169.45, 100.87, 72.88, 71.83, 71.42, 71.33, 70.72, 70.46, 69.12, 68.46, 62.02, 42.84, 20.82, 20.76, 20.69, 20.67.

(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(2-(2-chloroethoxy)ethoxy)ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (**24b**): Yield, 72.5%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.35 (t, *J* = 4.0 Hz, 1H), 5.18 (t, *J* = 8.0 Hz, 1H), 4.99 (d, *J* = 12.0 Hz, 1H), 4.55 (d, *J* = 8.0 Hz, 1H), 4.15-4.08 (m, 2H), 3.97-3.86 (m, 2H), 3.75-3.67 (m, 3H), 3.61 (s, 8H), 2.11 (s, 3H), 2.02 (d, *J* = 4.0 Hz, 6H), 1.94 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.44, 170.31, 170.20, 169.54, 101.34, 71.39, 70.94, 70.70, 70.67, 70.41, 69.08, 68.85, 67.12, 61.34, 42.83, 20.82, 20.72, 20.70, 20.62.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-6-(2-(2-(2-chloroethoxy)ethoxy)ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (**24c**): Yield, 66.5%; ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.34 (dd, *J* = 8.0, 4.0 Hz, 1H), 5.28 (d, *J* = 12.0 Hz, 1H), 5.24-5.22 (m, 1H), 4.84 (d, *J* = 2.6 Hz, 1H), 4.28 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.08-4.01 (m, 2H), 3.83-3.74 (m, 1H), 3.73 (d, *J* = 4.0 Hz, 2H), 3.68-3.58 (m, 9H), 2.12 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.94 (s, 3H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 169.74, 169.10, 168.96, 168.79, 96.75, 70.42, 69.76, 69.69, 69.09, 68.61, 68.12, 67.45, 66.43, 65.17, 61.46, 41.84, 19.95, 19.81, 19.77, 19.74.

(2R,3R,4S,5S,6S)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(2-(2-(2-chloroethoxy) ethoxy)ethoxy)tetrahydro-2H-pyran-3,5-diyl diacetate **(24d)**: Yield, 62.0%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.25 (dt, *J* =8.0, 1.8 Hz, 3H), 4.93 (d, *J* =4.0 Hz, 3H), 4.30 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.09 (d, *J* =4.0 Hz, 1H), 4.07-4.01 (m, 1H), 3.85-3.72 (m, 3H), 3.68-3.59 (m, 9 H), 2.13 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.86, 170.12, 170.10, 155.45, 97.75, 71.46, 70.80, 70.71, 70.12, 70.04, 68.48, 67.48, 66.33, 62.52, 42.89, 21.04, 20.86.

4.1.7 General procedures for compounds 25a-d.

Compounds **24a-d** (0.41mmoL) and sodium azide (260 mg) were dissolved in anhydrous DMF6 (mL), and the mixture was stirred at 70□ overnight. After the completion of the reaction, ethyl acetate (100 mL) and water (20 mL) were added in order. The organic layer was washed with brine, and dried with anhydrous Na_2SO_4,

and then concentrated under reduced pressure to the crude residue, which was chromatographed on silica column with ethyl acetate and petroleum ether (V/V=1:1) as an eluent to produce the colorless transparent compounds **25a-d,** respectively.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(2-(2-azidoethoxy)ethoxy)ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (**25a**): Yield, 94.0%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.21 (t, *J* = 8.0 Hz, 1H), 5.08 (t, *J* = 8.0 Hz, 1H), 4.99 (t, *J* = 8.0 Hz, 1H), 4.61 (d, *J* =8.0 Hz, 1H), 4.25 (t, *J* =8.0 Hz, 1H), 4.13 (d, *J* =12.0 Hz, 1H), 3.96-3.87 (m, 1H), 3.77-3.58 (m, 10H), 3.40 (t, *J* = 4.0 Hz, 2H), 2.07 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.80, 170.39, 169.54, 169.48, 100.92, 72.92, 71.87, 71.38, 70.81, 70.78, 70.51, 70.13, 69.16, 68.51, 62.06, 50.77, 20.85, 20.78, 20.73, 20.71.

(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(2-(2-azidoethoxy)ethoxy)ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (**25b**): Yield, 97.2%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.37 (t, *J* = 4.0 Hz, 1H), 5.20 (t, *J* = 4.0 Hz, 1H), 5.00 (d, *J* = 8.0 Hz, 1H), 4.56 (d, *J* = 8.0 Hz, 1H), 4.17(m, 2H), 3.97-3.84 (m, 2H), 3.78-3.68(m, 1H), 3.67-3.58 (m, 8H), 3.46 (t, *J* = 4.0 Hz, 2H), 2.12 (s, 3H), 2.02 (d, *J* = 4.0 Hz, 6H), 1.95 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$) δ 170.48, 170.34, 170.24, 169.56, 101.41, 70.98, 70.79, 70.76, 70.71, 70.47, 70.11, 69.13, 68.89, 67.15, 61.37, 50.74, 20.84, 20.75, 20.67.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-6-(2-(2-(2-azidoethoxy)ethoxy)ethoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (**25c**): Yield, 92.5%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.35 (dd, *J* = 8.0, 4.0 Hz, 1H), 5.29-5.24 (m, 2H), 4.85 (d, *J* = 1.8 Hz, 1H), 4.29 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.11-4.01 (m, 2H), 3.84-3.76 (m, 1H), 3.70-3.62 (m, 9H), 3.38 (t, *J* = 8.0 Hz, 2H), 2.13 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.78, 170.14, 170.00, 169.82, 97.80, 70.88, 70.77, 70.18, 70.14, 69.65, 69.16, 68.48, 67.49, 66.22, 62.49, 50.75, 20.99, 20.85, 20.79, 20.78.

(2R,3R,4S,5S,6S)-2-(acetoxymethyl)-6-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-(carba moyloxy)tetrahydro-2H-pyran-3,5-diyldiacetate (**25d**): Yield, 97.6%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.23 (d, *J* = 1.5 Hz, 1H), 5.21 (s, 1H), 4.97 (s, 2H), 4.84 (d, *J* = 1.6

Hz, 1H), 4.28 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.08-4.00 (m, 2H), 3.81-3.74 (m, 1H), 3.67-3.62 (m, 10H), 3.38 (t, *J* = 8.0 Hz, 2H), 2.11 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.76, 170.06, 155.52, 97.69, 70.79, 70.68, 70.10, 70.06, 69.94, 68.43, 67.42, 66.27, 62.46, 50.68, 20.96, 20.79.

4.1.8 General procedures for compounds 27a-d.

To a solution of compounds **25a-d (**0.38 mmoL) dissolved in methanol (5 mL) 10 % Pd/C (70 mg) was added. H_2 was bubbled into the reaction mixture at 1.0 atm. The reaction was stirred for 1 h at room temperature, and filtered, and concentrated to remove methanol. The obtained residue and compound **5** (190 mg, 0.37 mmoL) were dissolved in anhydrous DCM (8 mL), and diisopropylethylamine (DIPEA, 0.12 mL) was added at 0□ and the reaction was stirred for 3 h at room temperature. DCM (100 mL) and saturated NH_4Cl solution (20 mL) were poured to quench the reaction. The organic layer was washed with brine, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography with ethyl acetate and DCM (V/V=1:20) as an eluent to afford compounds **27a-d**.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)amino) ethoxy)ethoxy)ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**27a**): Yield, 73.3%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.40 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 7.95 (t, *J* = 8.0 Hz, 1H), 7.82 (t, *J* = 8.0 Hz,1H), 7.74 (s, 1H), 7.69 (t, *J* = 8.0 Hz, 1H), 5.28 (s, 2H), 5.21 (t, *J* = 8.0 Hz, 1H), 5.11 (t, *J* =8.0 Hz, 2H), 4.98 (t, *J* =12.0 Hz, 1H), 4.57 (d, *J* = 8.0 Hz, 1H), 4.31-4.27 (m, 1H), 4.18-4.06 (m, 2H), 3.92-3.47 (m, 13H), 2.44-2.39 (m, 2H), 2.07 (s, 3H), 2.01 (s, 6H), 1.98 (s, 3H), 1.07 (t, *J* = 7.5 Hz, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 172.38, 170.85, 170.42, 169.59, 169.52, 162.21, 153.95, 152.37, 148.99, 144.84, 144.51, 131.24, 130.78, 130.19, 129.88, 128.53, 128.25, 128.21, 128.16, 100.90, 98.27, 89.26, 72.93, 71.85, 71.37, 70.74, 70.36, 70.25, 69.15, 68.51, 66.70, 62.05, 59.02, 50.30, 32.21, 20.88, 20.78, 20.73, 7.78. TOF-MS, m/z: [M + Na^+], calcd for $C_{41}H_{47}N_3O_{17}Na^+$, 876.2798, found, 876.2806.

(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,

14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl) amino)ethoxy)ethoxy)ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**27b**): Yield, 71.4%. ^{1}HNMR (400 MHz, DMSO): δ 8.64 (d, *J* = 4.0 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.69 (t, *J* = 8.0 Hz, 1H), 7.38 (s, 1H), 5.75 (s, 1H), 5.26 (d, *J* = 4.0 Hz, 1H), 5.22 (s, 2H), 5.15 (d, *J* = 8.0 Hz, 1H), 4.97-4.86 (m, 2H), 4.80 (d, *J* = 4.0 Hz, 2H), 4.69 (d, *J* = 8.0 Hz, 1H), 4.18 (d, *J* = 8.0 Hz, 1H), 4.05 (d, *J* = 8.0 Hz, 2H), 3.78-3.34 (m, 11H), 2.40-2.31 (m, 2H), 2.11 (s, 3H), 1.99 (s, 6H), 1.91 (s, 3H), 0.97 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO): δ 172.59, 170.07, 170.00, 169.63, 169.25, 160.62, 153.90, 152.41, 147.96, 144.41, 144.06, 131.63, 130.44, 129.85, 129.78, 129.04, 128.53, 127.98, 127.73, 100.10, 96.82, 88.65, 70.34, 69.94, 69.82, 69.49, 68.71, 68.58, 67.43, 65.89, 61.39, 55.05, 55.01, 50.57, 31.44, 20.58, 20.55, 20.48, 20.44, 7.50. TOF-MS, m/z: [M + Na^{+}], calcd for $C_{41}H_{47}N_3O_{17}Na^{+}$, 876.2798, found, 876.2799.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-6-(2-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy) carbonyl) amino) ethoxy)ethoxy)ethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**27c**): Yield, 78.7%. ^{1}HNMR (400 MHz, DMSO): δ 8.69 (s, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.88-7.79 (m, 2H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.04 (s, 1H), 5.44 (d, *J* = 2.4 Hz, 2H), 5.30 (d, *J* = 2.4 Hz, 2H), 5.13-5.04 (m, 3H), 4.86 (s, 1H), 4.14 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.03 (dd, *J* = 12.0, 4.0 Hz, 1H), 3.97 (s, 1H), 3.70-3.36 (m, 10H), 3.06-3.04 (m, 2H), 2.13-2.08 (m, 2 H), 2.09 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.92 (s, 3H), 0.89 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 170.12, 169.73, 169.56, 168.12, 156.74, 154.50, 152.48, 147.98, 146.47, 145.75, 131.71, 130.48, 129.89, 129.05, 128.63, 128.10, 127.79, 118.96, 96.69, 94.95, 74.72, 69.69, 69.64, 69.13, 68.99, 68.74, 67.81, 66.58, 66.31, 65.43, 61.99, 50.30, 30.75, 20.67, 20.59, 20.49, 7.62. TOF-MS, m/z: [M + Na^{+}], calcd for $C_{41}H_{47}N_3O_{17}Na^{+}$, 876.2798, found, 876.2797.

(2R,3R,4S,5S,6S)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(2-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)amino)ethoxy)ethoxy)ethoxy)tetrahydro-2H-pyran-3,5-diyl diacetate (**27d**): Yield, 68.3%. ^{1}H NMR (400 MHz, DMSO): δ 8.67 (s, 1H), 8.21 (d, *J* = 8.0 Hz, 1H),

8.12 (d, *J* = 8.0 Hz, 1H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.69 (t, *J* = 8.0 Hz, 1H), 7.39 (s, 1H), 6.74 (s, 1H), 6.58 (s, 1H), 5.75 (s, 1H), 5.26 (s, 2H), 5.11-5.02 (m, 2H), 4.99 (m, 1H), 4.90 (t, *J* = 4.0 Hz, 1H), 4.80 (dd, *J* = 8.0, 4.0 Hz, 3H), 4.11 (m, 1H), 4.00-3.97 (m, 1H), 3.91-3.88 (m, 1H), 3.67-3.41 (m, 11H), 2.53-2.34 (m, 2H), 2.09 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 0.95 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.57, 170.11, 169.61, 169.50, 160.65, 155.51, 153.87, 152.47, 147.99, 144.43, 144.11, 131.66, 130.47, 129.92, 129.78, 129.06, 128.57, 128.02, 127.76, 96.80, 88.65, 69.76, 69.48, 69.40, 69.29, 68.16, 68.01, 66.56, 66.05, 65.95, 62.21, 55.05, 55.00, 50.60, 31.39, 20.79, 20.60, 20.58, 7.49. TOF-MS, m/z: [M + Na^+], calcd for $C_{40}H_{46}N_4NaO_{17}^+$, 877.2750, found, 877.2759.

4.1.9 General procedures for compounds 26a-d.

To a solution of compounds **25a-d** (0.38 mmoL) dissolved in 5 mL anhydrous methanol sodium methoxide (16 mg) was added. The mixture was stirred for half an hour at room temperature, and excessive Dowex H^+ resin was added to neutralize the resultant solution and the mixture was shaken for another 10 min. The mixture was filtered, and concentrated under reduced pressure to remove methanol. The obtained residue was re-dissolved in methanol (5 mL), and 10 % Pd/C (56 mg) was added. H_2 was bubbled at 1.0 atm into the reaction mixture, which was stirred for 1 h at room temperature. After completion, the solution was filtered and evaporated *in vacuum*. The above residue and compound **5** (210 mg, 0.41 mmoL) were dissolved in anhydrous DMSO (8 mL), and diisopropylethylamine (DIPEA, 0.15 mL) was added at 0□. The reaction was stirred overnight at r.t., and then concentrated under reduced pressure. The crude residue obtained was purified by silica gel-based column chromatography with methanol and DCM (V/V=1:20) as an eluent to afford compounds **26a-d**.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b] quinolin-4-yl-(2-(2-(2-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxylmethyl) tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)carbamate (**26a**): Yield, 42.6%. ^{1}HNMR (400 MHz, DMSO): δ 8.70 (s, 1H), 8.22 (t, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.90 (t, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 4.0 Hz, 1 H),

5.46 (t, *J* = 4.0 Hz, 1H), 5.30 (d, *J* = 4.0 Hz, 2H), 4.97-4.90 (m, 3H), 4.52 (t, *J* = 8.0 Hz, 1H), 4.12 (t, *J* = 8.0 Hz, 1H), 3.89-3.40 (m, 14H), 3.21-3.02 (m, 5H), 2.91 (m, *J* = 8.0 Hz, 1H), 2.18-2.02 (m, 2H), 0.98-0.85 (m, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.57, 156.73, 154.51, 152.45, 147.97, 146.46, 145.76, 131.74, 130.52, 129.87, 129.07, 128.63, 128.08, 127.79, 118.96, 103.03, 94.96, 88.65, 76.95, 76.81, 74.71, 73.43, 70.09, 69.73, 69.67, 67.81, 61.13, 53.50, 50.30, 31.38, 7.63. TOF-MS, m/z: [M + Na^+], calcd for $C_{33}H_{39}N_3O_{13}Na^+$, 708.2375, found, 708.2353.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl(2-(2-(2-(((2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)carbamate (**26b**): Yield, 45.8%. ^{1}HNMR (400 MHz, DMSO): δ 8.58 (s, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.83 (t, *J* = 8.0 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.40 (s, 1H), 5.18 (s, 2H), 4.99 (d, *J* = 5.8 Hz, 2H), 4.88 (s, 1H), 4.79-4.75 (m, 3H), 4.48 (d, *J* = 4.0 Hz, 1H), 3.63-3.56 (m, 7H), 3.47 (t, *J* = 4.0 Hz, 3H), 3.32-3.21 (m, 3H), 2.47-2.41 (m, 1H), 2.37-2.26 (m, 1H), 0.94 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.23, 161.48, 154.65, 152.68, 148.40, 145.45, 144.66, 132.46, 131.31, 130.22, 129.93, 129.43, 129.15, 128.55, 128.52, 103.93, 97.93, 89.30, 75.66, 73.93, 71.16, 70.29, 70.23, 69.98, 68.85, 68.31, 66.39, 61.17, 55.69, 51.19, 32.08, 8.02. TOF-MS, m/z: [M + Na^+], calcd for $C_{33}H_{39}N_3O_{13}Na^+$, 708.2375, found, 708.2344.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7] indolizino[1,2-b] quinolin-4-yl(2-(2-(2-(((2R,3S,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)carbamate (**26c**)**:** Yield, 38.6%. ^{1}HNMR (400 MHz, DMSO): δ 8.63 (s, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.83 (t, *J* = 8.0 Hz, 1H), 7.71-7.65 (m, 1H), 7.38 (s, 1H), 5.22 (s, 2H), 4.91 (t, *J* = 4.0 Hz, 1H), 4.79 (d, *J* = 4.0 Hz, 2H), 4.73 (dd, *J* = 8.0, 4.0 Hz, 2H), 4.60 (d, *J* =8.0 Hz, 2H), 4.46 (t, *J* = 8.0 Hz, 1H), 4.13 (d, *J* = 4.0 Hz, 1H), 3.78-3.41 (m, 15H), 3.29 (d, *J* = 8.0 Hz, 1H), 3.09 (m, 1H), 2.56-2.46 (m, 1H), 2.35-2.31 (m, 1H), 0.97 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.58, 160.64, 153.91, 152.39, 144.44, 144.07, 144.43, 131.68, 130.46, 129.46, 129.04, 128.76, 128.54, 127.98,

127.74, 100.05, 96.85, 88.65, 74.00, 72.38, 71.05, 70.35, 69.58, 67.48, 67.05, 65.73, 61.34, 60.36, 55.06, 50.58, 31.43, 7.51. TOF-MS, m/z: [M + Na^+], calcd for $C_{33}H_{39}N_3O_{13}Na^+$, 708.2375, found, 708.2365.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7] indolizino[1,2-b]quinolin-4-yl(2-(2-(2-(((2S,3S,4S,5R,6R)-4-(carbamoyloxy)-3,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)carbamate (**26d**): Yield, 68.4%. ^{1}HNMR (400 MHz, DMSO): δ 8.68 (s, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.41 (s, 1H), 6.44 (s, 2H), 5.28 (s, 2H), 5.00 (d, *J* =4.0 Hz, 1H), 4.93 (d, *J* = 8.0 Hz, 1H), 4.87-4.84 (m, 1H), 4.81 (d, *J* = 4.0 Hz, 1H), 4.62-4.56 (m, 2H), 4.51 (t, *J* = 4.0 Hz, 1H), 3.76-3.40 (m, 17H), 3.15 (s, 1H), 2.97-2.90 (m, 1H), 2.37-2.05 (m, 2H), 0.96 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.57, 160.67, 156.77, 153.88, 152.50, 148.00, 144.46, 144.14, 131.72, 130.51, 129.96, 129.77, 129.09, 128.61, 128.05, 127.79, 100.21, 96.85, 88.65, 74.22, 73.85, 69.71, 69.50, 69.46, 68.14, 65.90, 65.17, 64.29, 61.08, 55.04, 50.63, 31.39, 7.52. TOF-MS, m/z: [M + H^+], calcd for $C_{33}H_{41}N_4O_{14}^+$, 729.2614, found, 729.2602.

4.1.10 General procedures for compounds 30 and 31.

To a solution of 1.0 g of diphenyl phosphate (**29)** in anhydrous DCM (30 mL) compound **8** or **9** (1.1mmol) was added. The mixture was stirred for 20 min at the presence of 4Å molecule sieve. Prior to addition of TMSOTf (0.2 mL, 1.1mmoL), the reaction temperature was lowered to -5-0□ and stirred for an hour at the same temperature. After completion, the reaction was quenched with adequate TEA. Ethyl acetate (40 mL) was added to dilute the resultant mixture. The organic layer was washed with water (30 mL) and brine (15 mL) in order, dried by anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was chromatographed on silica gel-based column with petroleum and ethyl acetate (V/V=1:2) to afford 670 mg of conjugates **30** and **31** as white foams, respectively.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(((2R,3S,4S,5R,6S)-4,5-diacetoxy-6-(acetoxymethyl)-2-(2-(((benzyloxy)carbonyl)amino)ethoxy) tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,5-diyl diacetate **(30)**: Yield,

75.3%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.38-7.27 (m, 5H), 5.88 (s, 1H), 5.77 (s, 1H), 5.37 (t, *J* = 4.0 Hz, 1H), 5.31 (d, *J* = 8.0 Hz, 1H), 5.25 (d, *J* = 8.0 Hz, 1H), 5.19 (t, *J* = 4.0 Hz, 1H), 5.14 (s, 1H), 5.09-5.06 (d, *J* = 4.0 Hz, 1H), 5.03 (d, *J* = 8.0 Hz, 1H), 4.93-4.90 (m, 1H), 4.73-4.63 (m, 2H), 4.42 (t, *J* = 4.0 Hz, 1H), 4.31 (m, 1H), 4.10 (m, 5H), 3.98-3.86 (m, 1H), 3.74-3.53 (m, 1H), 3.51-3.33 (m, 2H), 2.19 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.07 (s, 6H), 2.03 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.69, 170.60, 170.30, 169.55, 169.51, 156.71, 155.24, 136.81, 128.58, 128.56, 128.16, 128.00, 100.88, 97.93, 72.28, 70.48, 69.65, 69.31, 68.27, 67.76, 66.59, 65.81, 62.72, 62.43, 62.14, 61.79, 41.11, 20.96, 20.93, 20.84, 20.82, 20.78.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(((2R,3S, 4S,5R,6S)-4,5-diacetoxy-6-(acetoxymethyl)-2-(2-(2-(((benzyloxy)carbonyl)amino)etho xy) ethoxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,5-diyl diacetate **(31)**: Yield, 67.3%. ^{1}HNMR (400 MHz, $CDCl_3$): δ7.34-7.30(m, 5H), 5.52-5.49 (m, 1H), 5.36 (t, *J*=4.0 Hz, 1H), 5.27-5.25 (m, 2H), 5.25-5.18 (m, 1H), 5.09-5.04 (m, 2H), 5.02(dd, *J*=8.0, 4.0 Hz, 1H), 5.00 (d, *J*=2.4 Hz, 1H), 4.97 (d, *J*=4.0 Hz, 1H), 4.81-4.71 (brs, 2H), 4.26-4.22 (dd, *J*=8.0, 4.0 Hz,1H), 4.15-4.01 (m, 5H), 3.98-3.90 (m, 1H), 3.85-3.79 (m, 1H), 3.78-3.70 (m, 1H), 3.69-3.45 (m, 4H), 3.40-3.31 (m, 2H), 2.16 (s, 3H), 2.09 (s, 6H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.68, 170.58, 170.26, 169.55, 169.50, 156.63, 155.40, 136.76, 128.59, 128.31, 128.24, 128.18, 100.92, 98.21, 72.00, 70.33, 70.10, 69.79, 69.44, 68.99, 68.81, 68.17, 66.70, 65.90, 62.72, 62.42, 62.12, 61.77, 40.91, 20.94, 20.83, 20.79, 20.76, 20.74. TOF-MS, m/z: [M + Na^+], calcd for $C_{37}H_{50}N_2Na\ O_{21}^+$, 881.2798, found, 881.2843.

4.1.11 Synthesis of (2R,3R,4S,5S,6R)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(((2R,3S,4S,5R,6S)-4,5-diacetoxy-6-(acetoxymethyl)-2-(2-(2-(2-azidoethoxy) ethoxy)ethoxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,5-diyl diacetate（32）

To a solution of compound **29** (1.1 g, 1.25 mmoL) and 2-(2-(2-chloroethoxy) ethoxy)ethan-1-ol (1.5 mmoL) dissolved in DCM (7 mL) containing 4Å molecule

sieve TMSOTf (1.25 mmoL) was added at -5□. The reaction mixture was stirred for 2 h. After completion, the reaction was quenched with adequate TEA. Ethyl acetate (80 mL) and water (20 mL) were added to dilute the resultant mixture. The organic layer was washed with brine (20 mL), dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The obtained residue was directly dissolved in anhydrous DMF (6 mL), and sodium azide (12.5 mmoL) was added. The reaction mixture was stirred at 70□ overnight. When completed, the mixture was quenched with ethyl acetate (50 mL) and water (20 mL). The organic layer was dried with anhydrous Na_2SO_4, and concentrated to offer the crude residue, which was purified by silica gel column chromatography with ethyl acetate and petroleum (V/V=2/1) as an eluent to offer 785.0 mg of compound **32** as a colorless oil.

Yield, 79.0%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.35 (t, *J* = 4.0 Hz, 1H), 5.26-5.18 (m, 4H), 5.00 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.90 (d, *J* = 4.0 Hz, 1H), 4.82 (s, 2H), 4.78 (d, *J* = 8.0 Hz, 1H), 4.21 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.14-3.91 (m, 5H), 3.82 (dd, *J* = 8.0, 4.0 Hz, 1H), 3.74-3.59 (m, 9H), 3.36 (t, *J* = 8.0 Hz, 2H), 2.18 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.05 (d, *J* = 1.8 Hz, 6H), 2.03 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.66, 170.58, 170.28, 169.84, 169.57, 155.36, 101.01, 97.96, 71.38, 70.66, 70.57, 70.42, 70.26, 70.05, 69.69, 69.37, 69.03, 68.73, 68.22, 65.96, 62.74, 61.76, 50.69, 20.96, 20.93, 20.91, 20.83, 20.80, 20.74.

4.1.12. General procedures for compounds 39-41.

To a solution of compounds **30-32** (0.37mmoL) dissolved in methanol (5 mL) 10% Pd/C (50 mg) was added. The reaction was initiated with the continuous inlet of hydrogen for 2 h. After finished, the mixture was filtered and concentrated to afford the corresponding compounds **33-35**, which were re-dissolved without further purification in anhydrous methanol (5mL). Sodium methoxide (15 mg) was added, and the reaction mixture was stirred for 1.5 h at room temperature, and Dowex H^+ resin was added to exchange sodium cation for another 10 min, and filtered, and then concentrated under reduced pressure. The residue obtained above (160 mg, 0.37mmol) and activated CPT **5** (200 mg, 0.4 mmoL) were taken up in anhydrous DMSO (3 mL), and addition of DIPEA (0.1 mL) was performed at 0□, and the reaction mixture was

stirred overnight and concentrated directly without work-up. The obtained residue was purified by silica gel column chromatography with methanol and DCM (V/V=1/6) to give conjugates **39-41,** respectively.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl (2-(((2R,3S,4S,5S,6S)-3-(((2R,3S,4S,5R,6R)-4-(carbamoyloxy)-3,5-dihydroxy-6-(hydr oxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-dihydroxy-6-(hydroxylmethyl)tetrahydr o-2H-pyran-2-yl)oxy)ethyl)carbamate **(39)**: Yield, 51.2%. ^{1}HNMR (400 MHz, DMSO): δ 8.69 (s, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.88 (t, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 6.48 (s, 1H), 6.41 (s, 1H), 5.27 (s, 2H), 5.04-4.67 (m, 8H), 4.62-4.53 (m, 1H), 4.52-4.41 (m, 1H), 4.14-4.08 (m, 1H), 3.92-3.43 (m, 16H), 2.52-2.28 (m, 2H), 0.97 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100MHz, DMSO): δ 172.65, 160.61, 156.53, 153.99, 152.57, 148.00, 144.18, 144.02, 131.71, 130.49, 129.98, 129.79, 129.09, 128.60, 128.05, 127.77, 97.24, 88.63, 88.54, 74.25, 73.74, 72.36, 72.22, 68.93, 68.38, 60.95, 60.82, 60.46, 60.34, 54.97, 50.62, 48.69, 31.69, 7.65. TOF-MS, m/z: [M + Na^{+}], calcd for $C_{36}H_{42}N_4O_{17}$ Na^{+}, 825.2437, found, 825.2423.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b] quinolin-4-yl (2-(2-(((2R,3S,4S,5S,6S)-3-(((2R,3S,4S,5R,6R)-4-(carbamoyloxy)-3,5-dihydroxy-6-(hy droxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-dihydroxy-6-(hydroxymethyl)tetrahyd ro-2H-pyran-2-yl)oxy)ethoxy)ethyl)carbamate (**40**): Yield, 38.2%. ^{1}HNMR (400 MHz, DMSO): δ 8.65 (s, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.86 (t, *J* = 8.0 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 7.39 (s, 1H), 6.44 (s, 2H), 5.24 (s, 2H), 5.04 (d, *J* = 4.0 Hz, 1H), 4.93-4.89 (m, 3H), 4.83-4.77 (m, 4H), 4.69 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.60-4.57 (m, 1H), 4.54-4.47 (s, 1H), 4.19-4.11 (m, 1H), 3.94-3.46 (m, 17H), 3.16 (s, 2H), 2.36-2.34 (m, 2H), 0.94 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100MHz, DMSO): δ 172.68, 160.70, 156.85, 154.02, 152.52, 148.01, 144.53, 144.16, 131.74, 130.56, 129.98, 129.80, 129.12, 128.63, 128.07, 127.83, 99.48, 96.99, 88.70, 74.42, 73.83, 71.03, 70.02, 69.49, 69.21, 68.44, 67.40, 66.92, 66.85, 66.17, 64.27, 60.86, 60.58,

55.05, 48.76, 29.96, 7.58. TOF-MS, m/z: [M+Na^+], calcd for $C_{38}H_{46}N_4O_{18}Na^+$, 869.2699, found, 869.2647.

(S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl (2-(2-(2-(((2R,3S,4S,5S,6S)-3-(((2R,3S,4S,5R,6R)-4-(carbamoyloxy)-3,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)carbamate **(41)**: Yield, 54.2%. ^{1}HNMR (400 MHz, DMSO): δ 8.69 (s, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.89 (t, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.41 (s, 1H), 6.40 (s, 2H), 5.29 (s, 2H), 4.97-4.67 (m, 8H), 4.59-4.50 (m, 2H), 4.16-4.07 (m, 1H), 3.94-3.87 (s, 1H), 3.82-3.65 (m, 4H), 3.66-3.44 (m, 17H), 3.18 (dd, *J* = 8.0, 4.0 Hz, 2H), 2.51-2.33 (m, 2H), 0.97 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100MHz, DMSO): δ 172.58, 160.67, 156.81, 153.90, 152.51, 148.01, 144.46, 144.14, 131.73, 130.53, 129.97, 129.76, 129.11, 128.61, 128.44, 128.06, 127.80, 101.79, 96.88, 88.64, 74.46, 73.86, 73.41, 72.37, 72.18, 71.15, 69.78, 69.47, 69.27, 68.36, 67.99, 65.83, 64.38, 60.86, 60.39, 55.04, 50.64, 48.69, 31.40, 7.51. TOF-MS, m/z: [M+Na^+], calcd for $C_{40}H_{50}N_4O_{19}Na^+$, 913.2961, found, 913.2946.

4.1.13. General procedures for compounds 42-44.

To a solution of compounds **30-32** (0.45 mmoL) dissolved in ethyl acetate (5 mL) 10% Pd/C (80 mg) was added. The reaction was initiated with the continuous inlet of hydrogen for 2 h. The mixture was filtered and concentrated to dryness. The reduced residue above (0.37 mmoL) and activated CPT **5** (0.4 mmoL) were dissolved in anhydrous DCM (5mL), and subsequent addition of DIPEA (0.1 mL) pushed the reaction at 0□. The reaction mixture was stirred for 4 h and then quenched with 0.1% HCl. DCM (100 mL) and water (30 mL) were added to dilute the reaction mixture. The organic layer was washed with brine (20 mL), and dried by anhydrous Na_2SO_4, and then concentrated to obtain the crude residue, which was purified by silica gel column chromatography with ethyl acetate and DCM (V/V=2/1) as an eluent to offer the corresponding conjugates **42-44**.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(((2R,3S, 4S,5R,6S)-4,5-diacetoxy-6-(acetoxymethyl)-2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)amino)ethoxy) tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,5-diyl diacetate (**42**): Yield, 80.4%. ^{1}HNMR (400 MHz, DMSO): δ 8.69 (s, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 7.87-7.85 (m, 1H), 7.70-7.71 (m, 2H), 7.05 (s, 1H), 6.64 (s, 1H), 6.56 (s, 1H), 5.43 (t, *J* = 4.0 Hz, 2H), 5.30 (s, 2H), 5.12-5.01 (m, 4H), 4.96 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.89-4.82 (m, 2H), 4.29 (t, *J* = 8.0 Hz, 1H), 4.15-3.86 (m, 6H), 3.74-3.58 (m, 1H),3.42-3.37 (m, 2H), 3.26-3.22 (m, 1H), 3.08 (q, *J* = 8.0 Hz, 3H), 2.15-1.96 (m, 18H), 0.87 (t, *J* = 8.0, 3H). ^{13}CNMR (100 MHz, DMSO): δ 170.08, 169.76, 169.71, 169.65, 169.53, 169.32, 168.08, 156.74, 155.41, 154.50, 152.47, 148.00, 146.25, 145.79, 131.73, 130.50, 129.83, 129.06, 128.62, 128.11, 127.79, 119.04, 95.90, 95.47, 94.87, 74.80, 69.25, 68.59, 67.84, 66.62, 66.42, 65.96, 62.88, 62.22, 61.99, 59.85, 50.62, 45.64, 31.23, 20.85, 20.72, 20.69, 20.64, 20.61, 20.51, 20.35, 7.57. TOF-MS, m/z: [M + Na^{+}], calcd for $C_{48}H_{54}N_4O_{23}$ Na^{+}, 1077.3071, found, 1077.3067.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(((2R,3S,4S,5R,6S)-4,5-diacetoxy-6-(acetoxymethyl)-2-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)amino)ethoxy) ethoxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,5-diyl diacetate (**43**): Yield, 72.6%. ^{1}HNMR (400 MHz, DMSO): δ 8.67 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.82 (t, *J* = 8.0 Hz, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 4.0 Hz, 1H), 6.71 (s, 1H), 6.55 (s, 1H), 5.34 (t, *J* = 4.0 Hz, 1H), 5.26 (s, 2H), 5.18-5.00 (m, 4H), 4.97-4.87 (m, 3H), 4.81-4.77 (m, 2H), 4.17-3.91 (m, 7H), 3.85-3.49 (m, 8H), 3.05-3.02 (m, 1H), 2.35-2.31 (m, 1H), 2.18-1.95 (m, 18H), 0.96 (dt, *J* = 8.0, 4.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.61, 170.45, 170.11, 169.69, 169.58, 169.48, 169.37, 160.66, 155.49, 153.91, 152.51, 148.00, 144.44, 144.16, 131.68, 130.48, 129.96, 129.79, 129.07, 128.69, 128.04, 127.77, 100.24, 96.91, 95.19, 88.63, 70.67, 69.99, 69.29, 69.02, 68.89, 67.89, 67.73, 66.30, 65.91, 62.89, 62.40, 61.91, 59.86, 55.05, 50.62, 45.76, 31.47, 20.86, 20.75, 20.70, 20.68, 20.62, 20.54, 7.48. TOF-MS, m/z: [M + Na^{+}], calcd for $C_{50}H_{58}N_4O_{24}$ Na^{+}, 1121.3333, found,

1121.3314.

(2R,3R,4S,5S,6R)-2-(acetoxymethyl)-4-(carbamoyloxy)-6-(((2R,3S,4S,5R,6S)-4,5-di acetoxy-6-(acetoxymethyl)-2-(2-(2-(2-(((((S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy) carbonyl) amino) ethoxy)ethoxy)ethoxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,5-diyl diacetate **(44)**: Yield, 76.3%. ^{1}HNMR (400 MHz, DMSO): δ 8.69 (s, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.89 (t, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.41 (s, 1H), 6.66 (s, 1H), 6.55 (s, 1H), 5.44 (s, 1H), 5.33-5.29 (m, 3H), 5.17-5.03 (m, 4H), 4.92-4.86 (m, 3H), 4.80-4.77 (m, 2H), 4.30 (d, *J* = 4.0 Hz, 1H), 4.16-3.90 (m, 4H), 3.78-3.37 (m, 11H), 3.10 (m, 3H), 2.35 (dd, *J* = 8.0, 4.0 Hz, 1H), 2.16-1.99 (m, 18H), 0.97 (t, *J* = 8.0 Hz, 3H). ^{13}CNMR (100 MHz, DMSO): δ 172.55, 170.43, 170.09, 169.66, 169.55, 169.38, 168.11, 160.66, 155.37, 153.86, 152.50, 148.01, 144.42, 144.14, 131.71, 130.49, 129.97, 129.78, 129.09, 128.62, 128.05, 127.78, 100.20, 96.84, 95.18, 88.63, 74.85, 70.47, 69.92, 69.69, 69.51, 69.40, 69.25, 68.99, 68.47, 68.23, 67.85, 67.72, 67.47, 65.83, 59.85, 55.04, 50.62, 45.61, 31.78, 20.85, 20.69, 20.65, 20.61, 20.58, 20.50, 7.47. TOF-MS, m/z: [M + Na^+], calcd for $C_{52}H_{62}N_4O_{25}Na^+$, 1165.3595, found, 1165.3603.

4.2 Cytotoxicity assay.

The *in vitro* cytotoxicities of irinotecan, CPT and CPT glycoconjugates (**18a-d**, **19a-d**, **22a-d**, **23a-d**, **26a-d**, **27a-d**, **39**-**44**) were assessed with the standard MTT assay.[31] HepG2 cells or HCT116 cells or SW1990 cells or HEK-293 cells were seeded into 96-well plates. The incubation period was extended to 48 h after the addition of freshly prepared concentrations of the tested compounds. Five concentrations of the appropriate drugs having four replicates at each concentration were performed, and each experiment was performed in triplicate. The supernatant was removed, and MTT solution (40 μL, 5 mg/mL) was added to each well. After re-incubation for another 4 h, DMSO (100 μL) was added to each well to dissolve the formazan crystals. The percentage of cell viability was determined by measuring the absorbance at a wavelength of 490 nm using a Multiskan MK3 microplate reader (BioTek Elx800, USA). IC_{50} values of the tested compounds were calculated by

nonlinear regression analysis using GraphPad Prism 5.0.

4.3 DNA relaxation assay.

According to the reported procedure[26], the reaction was incubated in a 20 μL solution containing 10 × DNA Topoismerase I Buffer, 350 mM Tris-HCl (pH 8.0), 720 mM KCl, 50 mM $MgCl_2$, 50 mM DTT, 0.1 % BSA, 0.5 μg supercoiled pBR322 DNA (Thermo Scientific, No. SD0041), glycoconjugates (**18a-d**, **19a-d**, **22a-d**, **23a-d**, **26a-d**, **27a-d**, **40-44**) and 1UTopoismerase I (TaKaRa, No. 2240A) at 37°C for 30 min. 10-HCPT and irinotecan were used as positive controls. The supercoiled, relaxed, or nicked DNA was separated by 0.8% agarose gel (Beyotime, ST004L) in the 1 x TAE (Tris-Acetate-EDTA) buffer (Beytotime, No. ST716) aided by electrophoresis with 90 V for 2 h. Agarose gel stained by ethidium bromide (EB) was observed and photographed using Gel Dox XR (Bio-Rad).

4.4. Water solubility assay.

Aqueous solubility was measured according to the modified HPLC method reported previously. [28] Specifically speaking, HPLC analysis run on Agilent Technologies 1260 infinity II with the following conditions: isocratic elution and CH_3CN:H_2O (V/V=40/60) as an eluent; Flow rate, 1.0 mL/min; Column temperature, 35 ^oC; Detection wavelength: 254 nm. Column (Agilent Eclipse Plus 250 x 4.6 mm, 5 μm, No. USUXA26257). Then the calibration curves of **19b**, **23d** and **40** were established. Accurately weighed 4 mg of **19b**, **23d**, **40** or CPT was dissolved in the exact 50 mL of DMSO. 5.0 mL of each solution was pipetted and diluted with DMSO to make exactly to 20 mL. 5.0 mL of each resulting solution were taken and exactly adjusted to chromatography. The peak area of **19b**, **23d**, **40** or CPT was applied to make the corresponding calibration curves. Finally, enough amount of **19b**, **23d**, **40**, or CPT was dissolved in 0.2 mL of 0.1 M phosphate buffer (pH 7.4), and the solution was gently shaken at 25 ^oC for 24 h. After filtered with 254 nm filter membrane, the saturated filter (100 μL) was diluted to 1.5 mL, and 10 μL was taken for HPLC analysis. The obtained peak was substituted into the corresponding calibration curve to calculate the value of aqueous solubility.

4.5. Stability assay.

1.0 mg of **19b**, **23d**, **40**, or CPT was dissolved in 50 mL of 0.1 M phosphate buffer (pH 7.4,), and the solution was gently shaken at 37 ^{o}C. A part of the solution was taken at 0, 30 min, 1.0 h, 2.5 h, 24 h and 48 h for HPLC analysis same to the conditions described in section **4.4**. The lactone ring opening was quantified by HPLC analysis using an area normalization method.

4.6. *In vivo* acute toxicity evaluation

Thirty-two 8-week-old ICR mice (Laboratory Animal Center for Chinese Medicine University of Guangzhou, Guangzhou, China, No. 20200401008) were used to evaluate single-dose toxicity. Mice were randomly divided into four groups (n = 8) and received a single *i.v* injection of **40** at 0 (vehicle), 85, 160, or 320 mg/kg on day 0. One group was the normal control without treatment. Body weight was measured every 2 days for 14 days. When the experiment ended, all animals were euthanized by CO_2, and tissues taken from the liver, lung, kidney, and spleen were weighed (data not offered). Tissues obtained were fixed with 10% formalin and embedded in paraffin. Sections 3-5 μm in thickness were prepared for histopathological assay. Hematoxylin and eosin (H&E) stained paraffin sections were histopathologically assessed and the symptomatic lesions were graded according to the reported guideline. [30] In terms of severity, the degree of lesions were graded from one to five: 1 = minimal (<1%), 2 = slight (1-25%), 3 =moderate (26-50%), 4 = moderately severe (51-75%), and 5 =severe/high (76-100%). Statistically significant results ($P < 0.05$) were shown.

Conflict of interest

None declared.

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Contributions

ML synthesized most compounds and analyzed the stability. WY measured water solubility of CPT glycoconjugates and performed Top I inhibition assays. KF conducted the *in vivo* animal evaluation. CZ prepared some key intermediates. YS screened the cytotoxicity assay. WH and WC synthesized some key intermediates. JH organized the biological data. ZJ designed some detailed experiments and gave critical reading the manuscript. WZ conceived the conception and design of this work, and wrote the manuscript. All the authors contributed to the manuscript revision and approved the submitted version.

Highlights:

♦Thirty novel 20 (S)-*O*-linked camptothecin (CPT) glycoconjugates were designed and synthesized.

♦Oligosaccharide types, length of a PEG linker and acetyl groups exerted obvious impacts on the anti-proliferative activity.

♦CPT glycoconjugates had no direct DNA Topo I inhibition similar to irinotecan.

♦ *In vitro* aqueous solubility and stability of CPT glycocojugates significantly increased.

♦ MTD of construct **40** was more than 320 mg/Kg for female mice intravenously.

Declaration of interests

☐ √ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: