NJC

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. Li, W. Huang, Z. Jiang, Y. Shi, S. Yuan, K. Fu, Y. Chen, L. Zhou and W. Zhou, *New J. Chem.*, 2019, DOI: 10.1039/C8NJ06191B.

View Article Online
DOI: 10.1039/C8NJ06191B

Multi-gram scale synthesis of a bleomycin (BLM) carbohydrate moiety: exploring the antitumor beneficial effect of BLM disaccharide attached to 10-hydroxycamptothecine (10-HCPT)

MaoLin Li[1#], Weiping Huang[1#], Zhilin Jiang[2#], Yonghui Shi[3], Sisi Yuan[1], Kaishuo Fu[1], YongJun Chen[4], Li Zhou[5*] and Wen Zhou[1*]

[1] *School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, University town, Waihuan Rd, Panyu, Guangzhou, 510006, China*

[2]*Puer University, Puer, 665000, Yunan, China*

[3]*Department of Pharmacy, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, Guangdong, China*

[4]*South China Research Centre for Acupuncture and Moxibustion, Medical College of Acu-Moxi and Rehabilitation, Guangzhou University of Chinese Medicine, University town, Waihuan Rd, Panyu, Guangzhou, 510006, China*

[5]*College of Science, Hunan Agricultural University, Changsha, Hunan, 410128, China.*

[#] These authors contributed equally to the work.

[*] Correspondent. E-mail: zhoulizss@163.com; zhouwen60@126.com

Abstract: The "tumor-seeking" role of bleomycin (BLM) disaccharide has been demonstrated to be severed as a promising tool for cancer diagnosis and a potential ligand for targeted therapy, however, these practical applications are often hampered by lack of BLM disaccharide. Herein, an efficient multi-gram synthesis of peracetylated BLM disaccharide **20** is achieved by a TMSOTF-mediated glycosidation coupling manner in 43.6% overall yield in terms of benzyl galactoside. The critical innovation of the synthetic strategy is that inexpensive benzyl galactoside was first adopted to prepare *L*-gulose subunit **3** as a glycosyl acceptor, with a much shorter route in 73.0% yield, and 3-*O*-carbamoyl-mannosely donor **4** was achieved in 47.2 % yield by lowering the amount of dibutyltin oxide, and merging aminolysis and selective deacetylation into one-pot reaction. Next, incorporation of BLM disaccharide into 10-hydroxycamptothecin (10-HCPT), a non-specific model compound, to form conjugate **1** could significantly improve the antitumor activity and display obvious selectivity toward cancerous and normal cells in comparison with 10-HCPT, moreover, BLM disaccharide itself was non-cytotoxic, clearly indicating the importance and potential of BLM disaccharide in solving the targeted antitumor therapy of cytotoxic drugs.

Key works: BLM disaccharides, multi-gram synthesis, 10-hydroxycamptothecin, targeting property, antitumor activity.

View Article Online
DOI: 10.1039/C8NJ06191B

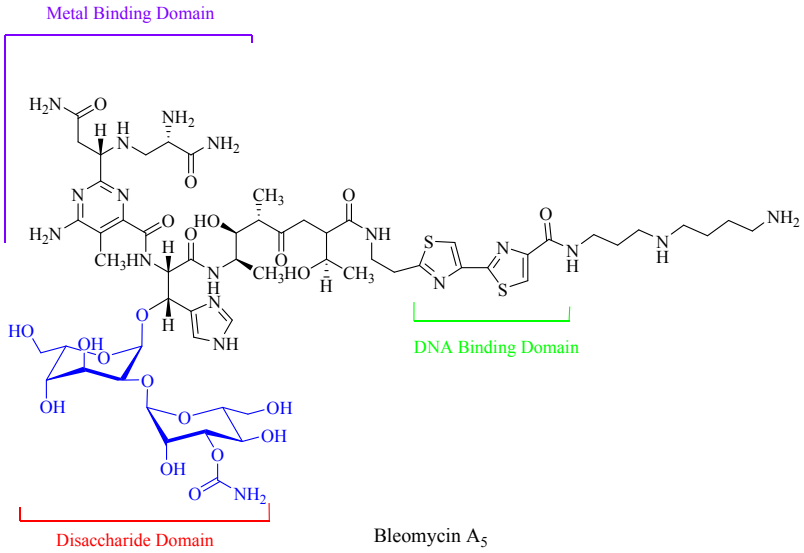
1. Introduction

"Aerobic glycolysis" is an emerging hallmark that cancer cells, even in the presence of oxygen, are capable of reprogramming their glucose metabolism to confer the requirements of uncontrollable cell proliferation and growth [1]. Significant alterations of activated oncogenes and mutant tumor suppressors in tumor cells give rise to glycolytic fueling, for the purpose of avoidance of cytostatic controls and attenuation of apoptosis. Selectively blocking the pathway of glycolytic fueling even the glycolysis switch is a therapeutic approach to killing cancer cells with high specificity. Glyco-conjugates, just like clinically used anticancer drugs coupled with various mono- or disaccharides, [2] pronouncedly improve their targeting, selectivity and efficacy towards cancer cells.

Bleomycins (BLMs) such as Bleomycin A5 (Fig.**1**) are a family of glycopeptide-derived antitumor antibiotics for treating several types of cancers. Their antitumor activity originates from the ability to effect DNA cleavage in cancer cells. [3] Apart from this, the beneficial effects of BLM are largely dependent on the ability of carbohydrate moiety to recognize tumor cells [4-7]. As demonstrated by the *in vitro* cell experiments that micro-bubbles [4, 5] and dyes [6, 7] were used to explore the bio-functions of BLM disaccharide, the tumor-seeking property of BLM was driven by its unique carbohydrate moiety. Structurally, BLM is regarded as a modular system, the aglycon is a tumoricidal domain while the BLM disaccharide composed of two unusual sugars, *L*-gulose and 3-*O*-carbamoyl-*D*-mannose, is responsible for the targeting property. Deglyco BLM is much less cytotoxic than BLM toward cultured cancer cells, [4] further emphasizing the importance of the disaccharide moiety to effect efficiently on tumor cells. Again, exploring the effect of adding the BLM disaccharide to different positions of deglycoBLM, the potential role of the carbohydrate moiety in mediating the targeting property of BLM is underscored.[4]

The unique targeting of BLM disaccharide fragment, holding great potential in targeted antitumor therapy and drug delivery, arouse our keen interest to develop a practicable multi-gram scale synthetic route, in hope to solve its source limitation and extend the clinical applications. Although a large amount of synthetic methods of BLM disaccharide have been reported for decades, summarized in Fig. **2**, the scale of the vast majority is still on milligram range due to the unreasonable synthetic strategies. Dissecting its structure from retro-synthetic analysis,

theoretically, the BLM carbohydrate motif can be derived from the globe deprotection of the appropriately protected disaccharides, which are achieved by a diastereospecific glycosidation coupling of *L*-gulopyranoside and 3-*O*-carbamonylmannose derivatives. Accordingly, BLM disaccharide precursor was first synthesized using the bromide-mediated coupling of suitably protected *L*-



Bleomycin A_5

Fig. 1 Structure of the representative member of BLM family (Bleomycin A5)

gulopyranoside or 1,6-dehydrated gulopyranoside as an acceptor and appropriately protected 3-*O*-carbamonyl-mannose as a donor, unfortunately, acetyl located in 3-*O*-carbamonyl group cause big trouble in the following deacetylation.[9] Synthesis of 3-*O*-carbamonyl-mannose derivatives as donors is relatively simple, and mannose as a starting material is attractive due to inexpensive cost and no chiral inversion, while gulose is a rare naturally occurring monosaccharide that becomes inaccessible to direct preparation, incurring the great challenges in constructing the gulose motif. Numerous continued efforts have been made to develop more efficient approaches to gaining the BLM disaccharide and its derivatives,[10-15] however, the construction of gulose subunit is a major bottleneck constraint. Generally, selective inversion of one or several hydroxyl configurations is preferred from cheap and easy to obtain monosaccharide including mannose, glucose, galactose and so on. The most popular strategy is that epimerization of *D*-mannose at C5 is carried out to prepare different properly protected *L*-gulose derivatives, in combination with accomplishing the selectivity between C2 and C3 hydroxyl groups. [10] Also, using an oxidation-reduction sequence to fulfill inversion of stereochemistry at C5, a novel

stereoselective build-up of *L*-gulose subunit from D-mannose was developed.[11] Similarly, a synthetic route emerge that a linear allylic alcohol derived from a mannose analogue was envisaged as an equivalent of *L*-gulose.[12] Additionally, utilizing the contrary stereochemistry at C2 and C5 of glucose and gulose, 1,6-hydrated *L*-gulose was explored with an oxidation-reduction sequence.[13] However, the yields of these multistep synthetic routes are extremely low and only reach milligram scales. Although the conversion of *L*-xylose derivative to *L*-gulose one was reported in a preparative scale using a thiazole-based homologation strategy, use of mercuric chloride and inseparable isomers produced in the extension process of *L*-xylose to *L*-gulcose imposed more restrictions on its larger scale preparation. [14] Recently, a novel access to the gulose subunit was proposed that starting material *D*-sorbitol applied to prepare gulose moiety is suitable to the gram synthesis of BLM disaccharide derivatives. However, the crucial factor was neglected, that the poor selectivity of aldehyde to protect *D*-sorbitol sharply decrease the total yield, in considering the large-scale synthesis. [15]

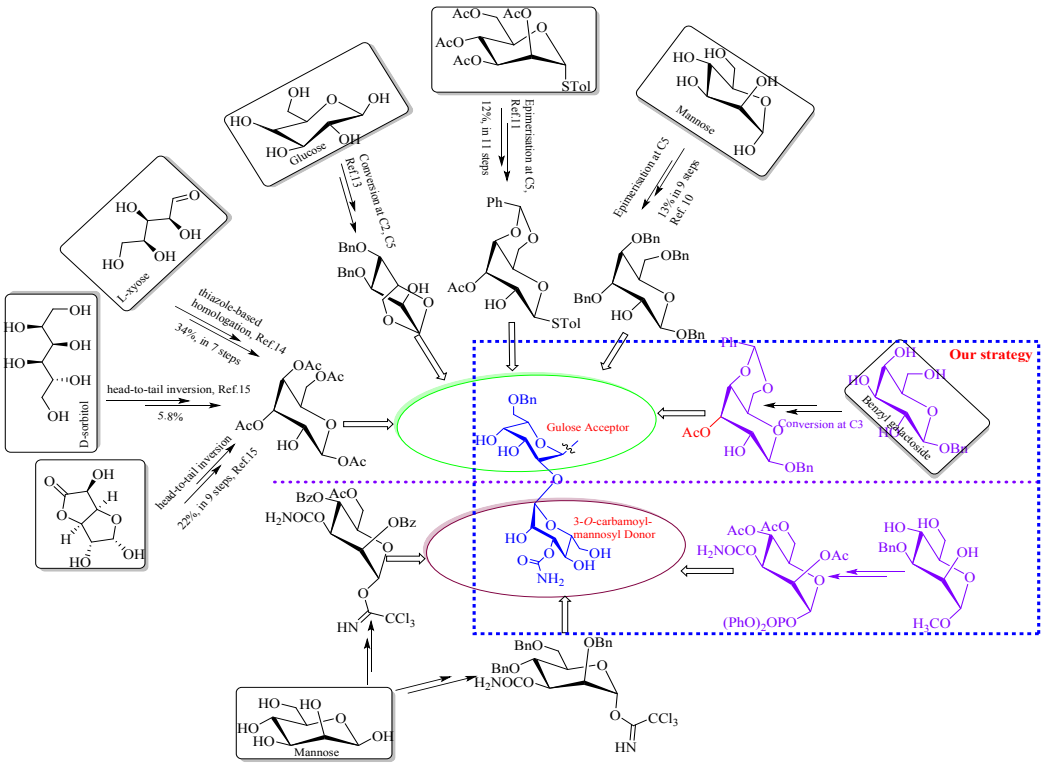


Fig. 2 Summary of the synthetic routes of two subunits, gulose and 3-*O*- carbamoyl-mannose, contained in BLM disaccharide

Taking these reasons mentioned above into accounts, we herein described a practical multi-gram synthesis of BLM disaccharide (**2**), derived from the trimethylsilyl

trifluoromethanesulfonate (TMSOTF)-mediated glycosidation coupling of *L*-glucose and 3-*O*-carbamonyl-mannose derivatives **3** and **4** followed by the globe deprotection. Afterwards, attachment of BLM disaccharide (**2**) to 10-hydroxyl of naturally occurring 10-HPTC, which is characteristic of poor water solubility and low selectivity towards cancerous and normal cells, [16] generated conjugate **1** (Fig. **3**), and then the antitumor activity was evaluated to explore the bio-function of BLM disaccharide, in hope to extend the underlying and practicable application scope, especially its improvement on the targeting and efficacy of non-targeted drugs.

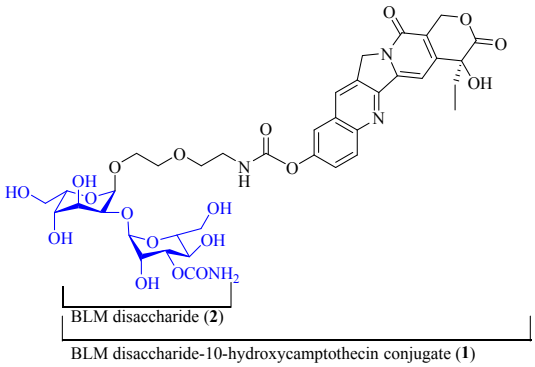
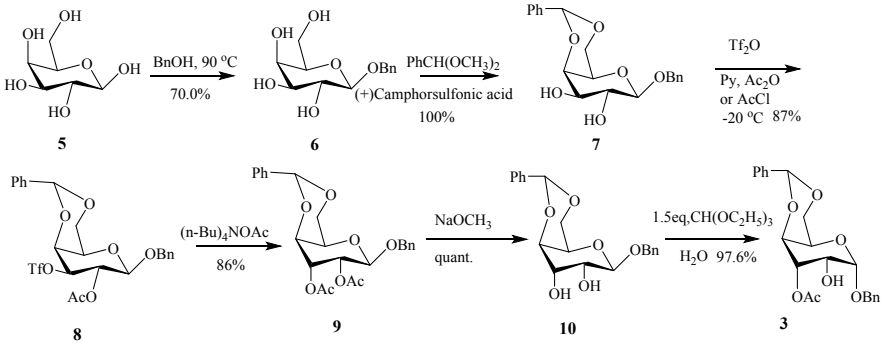


Fig. 3, Structure of BLM disaccharide-10-hydroxycamptochecin conjugate (**1**)

2. Results and discussion

In terms of retro-synthetic analysis and reported procedures [9-14], the synthesis of BLM disaccharide is generally accomplished by the assembly of appropriately protected *L*-gulose and *D*-mannose derivatives with 1, 2-*O*-*a*-glycosidic linkage followed by globe deprotection. The overall yield and preparation scale are overwhelmingly dependent on the choice of protecting groups and starting materials. Although staring materials are diversified (Fig.**2**), among all the reported protecting groups for synthesizing BLM disaccharide acetyl group was preferred, mainly originating from its good compatibility with other protecting groups and easy deprotection. [10,17] Therefore, peracetyated BLM disaccharide (**20**) was viewed as a crucial intermediate to synthesize target compounds **1** and **2**. *L*-gulose residue **3** with unmasked 2-OH for incorporation into precursor BLM disaccharide derivatives as an acceptor was prepared by selective inversion of 3-OH of benzyl galactoside **6** through a series of protection and deprotection operations (**Scheme 1**). Selective etherification of galactoside **5** with benzyl alcohol directly produced galactoside **6** in an acceptable yield (70%) at 90 ºC, significantly simplifying the previous procedures consisted of acetylation, selective benzylation and deacetylation.[18, 19] When *p*-toluene sulfonic acid (TsOH)

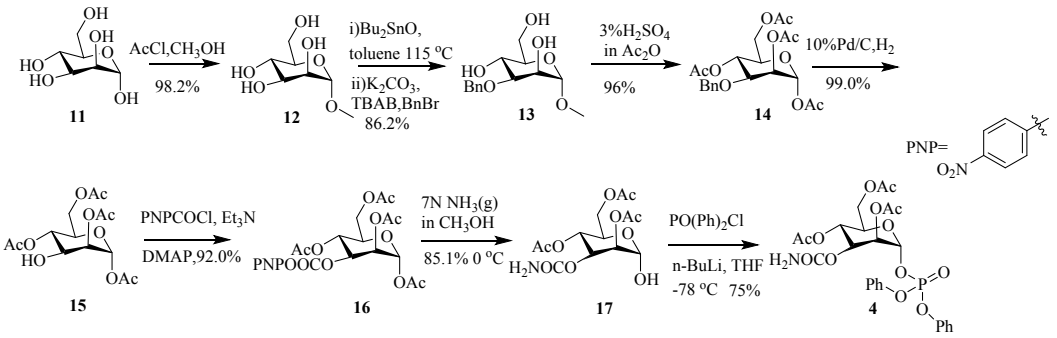
was replaced by camphorsulfonic acid (CSA), reaction of galactoside **6** with benzaldehyde dimethyl acetal easily produced acetal **7** in a quantitative yield[10]. Treatment of **7** with trifluoromethanesulfonic anhydride (Tf_2O) and pyridine at -20 °C selectively esterified 3-OH followed by *in situ* addition of Ac_2O or AcCl, generating acetylated **8** in one-pot reaction with the yield of 87%. It was noted that temperature and mole ratio of Tf_2O and Ac_2O or AcCl were determinant factors. Since trifluoromethylsulfonyloxyl group (TfO-) is an excellent leaving group, tetrabutylammonium acetoxide ($(n\text{-}Bu)_4NOAc$) was selected to substitute TfO- for acetoxyl (AcO-), [21] achieving net inversion of configuration to afford gulose derivative **9**, which was quantitatively deacetylated to diol **10** by treatment with sodium methoxide and methanol. The selective acetylation at 3-OH was mediated by triethylorthoacetate to offer *L*-gulose subunit **3** [22] in an exceptional yield, which functioned first as a glycosyl acceptor for coupling of BLM disaccharide **2**, and ultimately as a glycosyl donor for the following synthesis of conjugate **1**.



Scheme 1. Synthesis of *L*-gulose unit **3**

Mannose was widely applied to synthesize precursor *D*-mannose derivatives as starting material.[4,10,23] Our modified synthetic route to donor *D*-mannose subunit, which was based on the method previously proposed by Boger D.H. et al., [10] was illustrated in Scheme **2**. Key strategic elements of the approach included selective benzylation of 3-OH of methylmannoside **12**, along with simultaneous formation of carbamoyl at C3 and release of acetyl at anomeric carbon of *p*-nitrophenylcarbonate **16** using ammonolysis, leading to significant increase in the overall yield. Starting material methylmannoside **12**, which was prepared according to the previous procedure,[24] was refluxing with a catalytic amount of dibutyltin oxide (Bu_2SnO), differing in the amount that 1.1 equivalence of Bu_2SnO was required for the formation of intermediate 2,3-*O*-dibutylstannylene in the previous report,[10, 25] to regulate the selectivity of the following

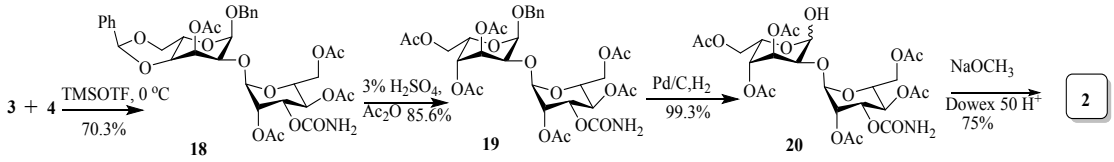
reaction, and then *in situ* addition of tetrabutylammonium bromide and benzyl bromide promoted the generation of methyl 3-*O*-benzyl mannoside **13** in 86.2% yield, which was further acetylated to produce acetylated **14** with 3% H_2SO_4 in Ac_2O. Applying 10% Pd/C not Pt $(OH)_2$/C [10]as a catalyst, benzyl group of **14** under the atmosphere of hydrogen was removed to provide tetraacetyl **15** in a quantitative yield, followed by reaction with 4-nitrophenyl carbonochloridate (*p*NPCOCl) in the presence of triethylamine (Et_3N) and dimethylaminopyridine (DMAP) to generate precursor carbamyl **16**. Next, compound **16** was subjected to aminolysis and selective deacetylation with 7N ammonia (gas) in methanol to yield carbamyl 3-*O*-carbamoyl-2, 4, 6-tri-*O*-acetyl-α-D-mannose (**17**) in one-pot reaction, differing in ammonolysis of PNPCO- and selective release of Ac- at anomeric carbon with aniline [10] or ammonia [15] separately. With extension of the reaction time, other acetyls were also released from carbamoyl **17**. The α-glycosyl diphenylphosphate of compound **17** proceed smoothly to produce donor suitably protected mannose derivative **4**[15]for next coupling.



Scheme 2. Synthesis of *D*-mannose derivative **4**

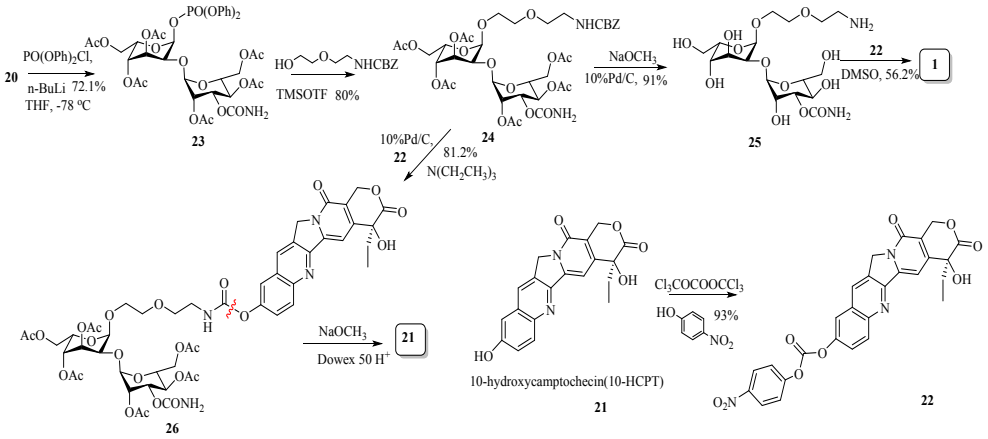
With two building blocks appropriately protected *L*-gulose and *D*-mannose subunits **3** and **4** in hand, we embarked on the assembly of naturally occurring BLM disaccharide **2**. As described in scheme **3**, the only α-1, 2-linkage assembly of compounds **3** and **4** was mediated by TMSOTF at 0 °C to afford disaccharide **18**. Considering the stability of benzyl group exposed to various conditions, acetal hydrolysis with acetic acid and *in situ* acetylation of **18** in the presence of Ac_2O and pyridine smoothly generated hexaacetylate **19**, which was further debenzylated under hydrogen atmosphere to produce the crucial intermediate **20** in 99.3% yield. When debenzylation of **18** was first applied, no trance of debenzylated product was observed, possibly influenced by the presence of acetal group.

View Article Online
DOI: 10.1039/C8NJ06191B



Scheme 3. Synthesis of BLM disaccharide **2**

To confirm whether the steric configuration of carbohydrate moiety we prepared was in accordance with naturally occurring the BLM disaccharide, target disaccharide **2** from the entire deacetylation of **20** was used to analyze its specific rotation. As we expected, **2** had the similar optical property, bearing the value of $[\alpha]_D^{25}$ 64.9º (c, 0.5, H_2O), to BLM disaccharide ever reported ($[\alpha]_D^{25}$ 65.8º (c, 0.5, H_2O). [9]



Scheme **4**. Synthesis of BLM disaccharide-10-HCPT conjugate **1**

To confirm the beneficial property of the BLM disaccharide, non-specific drug 10-hydroxycamptochecin (10-HCPT, **21**), a DNA topoisomerase I (Topo I) inhibitor, [17] was selected as a model compound to conjugate with BLM disaccharide. As illustrated in scheme **4**, construct **20** was activated with $PO(OPh)_2Cl$ and n-Butyllithium (n-BuLi), yielding the only α-glycosyl diphenyl phosphate **23** in 72.1% yield. The latter as a glycosyl donor was then coupled with CBz (benzoxycarbonyl) protected 2-(2-aminoethoxy) ethan-1-ol, which was prepared according to the previous procedure,[7] to yield linker coupled disaccharide **24**. A hydrogenolysis procedure was applied to remove CBz- of compound **24** and *in situ* treatment with activated 10-HCPT **22**[26] to afford conjugate **26**. Disappointingly, when **26**

View Article Online
DOI: 10.1039/C8NJ06191B

was treated with sodium methoxide and Dowex 50 H^+, no conjugate **1** was found. An alternative approach proved successful that disaccharide **24** was first deacylated to remove all the protecting groups with NaOMe followed by direct amidation with compound **22** in the absence of alkali to yield BLM disaccharide-10-HCPT conjugate **1** in an acceptable yield.

Next, the cell-based anticancer activity of compound **1** was investigated to understand the effect of BLM disaccharide on the non-specific drug. The lead compound 10-HPCT (**21**) was selected as a positive control. Two cancerous cell lines including HCT-116 (human colorectal cell), HepG2 (Human liver cell) and one normal cell HEK-293T (human embryonic kidney) were used as *in vitro* cell models to screen all the tested compounds. As illustrated in **Table 1**, the IC_{50} values of target conjugate **1** against HCT-116 and HepG2 were 0.5, 0.2 μ M respectively, displaying approximately 10-fold more active than the lead compound **21**. Replacing BLM disaccharide of compound **1** with peracetyated BLM disaccharide to obtain compound **26** caused a sharp drop in inhibiting the activity of cancerous cells, which was comparative to 10-HPCT, implying that BLM disaccharide moiety was beneficial to an increase in antitumor effects. The observation was in accordance with the previous report regarding the antitumor activity of BLM. [4] Interestingly, BLM disaccharide (**2**) and linker coupled BLM disaccharide (**25**) themselves displayed no cytotoxicity toward the cancerous and normal cells (IC_{50}>100 μ M), and no synergistic effect on the anticancer activity was observed when co-administrated with10-HPCT (**21**). The results emphasized that attachment of BLM disaccharide to cytotoxic drug **21** was capable of enhancing the anticancer activity, possibly ascribing to the recognition of BLM disaccharide to some targets on the surface of

Table 1. The antitumor activity of all the compounds tested against human tumor cell lines HCT-116 and HepG2 and normal cell line HEK-293T.[a]

Compounds	*In vitro* antitumor activity (IC_{50}, μmolL^{-1})		
	HCT-116	HepG2	HEK-293T
21 (10-HPCT)	4.1	2.1	7.6
2	>100	>100	>100
25	>100	>100	>100
26	2.7	1.7	9.2

View Article Online
DOI: 10.1039/C8NJ06191B

1	0.5	0.2	22.3	
25+**21**[b]	3.5	3.1	5.7	

[a] The *in vitro* antitumor activity of all the tested compound against two cancer cells and one normal cell was measured by the MTT assay after 48 h of incubation and indicated as the half maximal inhibitory concentration (IC_{50}, μ molL^{-1}), and all the data were obtained from three independent experiments; [b]**25**+**21** indicated that compounds **25** and **21** were co-administrated.

tumor cells, not the antitumor activity of BLM disaccharide itself. Although there were no direct evidences to be found and clear mechanisms to be proposed, one or more cell surface receptors associated with glucose transports in tumor cells may be regarded as one of the most reasonable explanations.[4] Selectivity of conjugate **1** toward cancerous cells (IC_{50}=0.5 μM against HCT-116; IC_{50}=0.2 μM against HepG2) and normal cell HEK-293T (IC_{50}=22.3 μM) gave a clear hint that the markedly different expression of the action targets of BLM disaccharide existed, and this phenomenon was testified by the previous experiments that the different uptake ability of cancer and normal cells for BLM disaccharide was measured with micro-bubbles [4, 5] and dyes. [6, 7] Taken together, it could be concluded that BLM disaccharide was a targeting molecule as a vehicle or a ligand to selectively deliver cytotoxic drugs to tumor cells.

3. Conclusion

In summary, an economic and practical multi-gram synthesis of a BLM disaccharide derivative has been developed to explore the role of BLM carbohydrate moiety in improving the antitumor ability to 10-HCPT. The light spots of the synthetic strategy are that inexpensive benzyl galactoside was first applied to prepare *L*-gulose subunit **3** as a glycosyl acceptor, with a much shorter route in 73.0% overall yield, and 3-*O*-carbamoyl-mannosely donor was achieved in 47.2% overall yield by lowering the amount of dibutyltin oxide and combining aminolysis and selective deacetylation into one-pot reaction. Afterwards, BLM disaccharide was incorporated into non-specific antitumor drug 10-HCPT to form conjugate **1**, which can selectively target to cancer cells, clearly indicating that BLM disaccharide indeed holds great potential in improving the targeting effect on non-specific drugs.

4. Experimental section

General procedure. All chemicals were of reagent grade quality or better, obtained from commercial suppliers, and used without further purification. Solvents were used as received or dried over molecular sieves. All the preparations were carried out using standard Schlenk techniques. All the key intermediates and products were confirmed by electrospray ionization mass spectrometry (ESI-MS), recorded on AB Sciex triple TOF 5600+ system, and all the structures of products were also confirmed by ^{1}H NMR and ^{13}C NMR, 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 peak as internal standard ($CDCl_3$=7.26 ppm for ^{1}H NMR and 77.16 ppm for ^{13}C NMR, CD_3OD=3.31 ppm for ^{1}H NMR and 48.8 ppm for ^{13}C NMR, CD_3SOCD_3=2.50 ppm for ^{1}H NMR and 39.6 ppm for ^{13}C NMR). The optical rotation was recorded in an Autopol VI (Rudolph research analytical). The purity, analyzed with C_{18}-column run on Agilent Technologies 1260 infinity II, was more than 95%,

4.1. *Synthesis* of *benzyl 4, 6-O-benzylidene -β-D-galactopyranoside* **(7)**

10 g (37.0 mmol) of 1-*O*-benzyl-*β*-galactopyranoside and 0.86 mg (3.7 mmol) of camphorsulfonic acid (CSA) were dissolved in 40 mL of dry $CHCl_3$, and 6.75 mL (44.43 mmol) of benzaldehyde dimethyl aetal was added dropwise. The reaction mixture was stirred and refluxed for 2 h and poured into a mixture of 500 mL of CH_2Cl_2 and 100 mL of saturated aq $NaHCO_3$. The organic layer was washed twice with water (100 mL) and once with brine (100 mL), and then concentrated under reduced pressure to afford white residue, which was further recrystallized with 50 mL of AcOEt to give 13.1g of compound **7** as a white solid. Yield 99.0 % ; TLC R_f=0.30 (DCM: MeOH (V/V) =50:1); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.55-7.30 (m, 10H), 5.56 (s, 1H), 5.01 (d, *J* = 12.0 Hz, 1H), 4.63 (d, *J* = 12.0 Hz, 1H), 4.39 (s, 1H), 4.36 (d, *J* = 5.6 Hz, 1H), 4.20 (t, *J* = 2.1 Hz, 1H), 4.10 (d, *J* = 12.4 Hz, 1H), 3.82 (t, *J* = 8.3 Hz, 1H), 3.71-3.66 (m, 1H), 3.47 (s, 1H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 137.49, 137.07, 129.28, 128.53, 128.28, 128.23, 128.03, 126.42, 101.65, 101.48, 75.33, 72.69, 71.83, 70.92, 69.18, 66.78

4.2. *Synthesis* of *benzyl 2-O-acetyl-3-O-tfyl-4, 6-O-benzylidene -β-D-galactopyranoside* **(8)**

To a solution containing 12 g of compound **7** (33.5 mmol) dissolved in dry CH_2Cl_2 (100 mL) and dry pyridine (6 mL) was added slowly 6.5 mL of Tf_2O (38.5 mmol) at -20℃. Prior to addition of acetyl chloride (4.76 mL, 67.0 mmoL), the reaction continued to gently stirred for 2 h at -20℃.

The reaction mixture was stirred for another 1 h at the same temperature and then poured into a mixture of 1L of AcOEt and 80 mL of 1N HCl. The organic layer was washed with 400 mL and 150 mL of brine, and dried over anhydrous Na_2SO_4 and filtered, and then concentrated under diminished pressure to give crude residue as yellow oil, which was further purified by column chromatography over silica gel with petroleum ether (PE) and ethyl acetate (EA) (V/V=3/1) to afford 15.5 g of compound **8** as a white solid. Yield, 87.0% ; TLC R_f=0.34 (PE:EA=3:1); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.55 -7.27 (m, 10H), 5.60 (s, 1H), 5.54 (t, *J* = 8.8 Hz, 1H), 4.96 (d, *J* = 10.0 Hz, 1H), 4.89 (m, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.67 (d, *J* = 12.0 Hz, 1H), 4.56 (d, *J* = 8.0 Hz, 1H), 4.45 (t, *J* = 3.6, 5.2 Hz, 1H), 4.41 (s, 1H), 4.10 (d, *J*=12.0 Hz, 1H), 3.51 (s, 1H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ 168.82, 136.75, 136.72, 129.25, 128.49, 128.27, 128.03, 127.81, 126.14, 101.00, 99.16, 83.73, 74.10, 70.32, 68.59, 67.68, 65.75, 20.49.

4.3. *Synthesis* of *benzyl 2, 3-O-diacetyl-4,6-O-benzylidene -β-L-gulopyranoside* **(9)**

10 g of compound **8** (18.8 mmoL) and 11.4 g of tetrabutylammonium aceate (37.6 mmol) were taken up in 70 mL of dry DMF. The reaction was heated up to 50 ℃ and stirred for 4 hours. After compound **8** was completely consumed by TLC analysis, the resulting result was diluted with a mixture of 500 mL of AcOEt and 100 mL of water. The organic layer was concentrated under reduced pressure to afford a reddish-yellow residue, which was further purified by column chromatography over silica gel with a mixture PE and EA (V/V=2:1) as an eluent to produce 7.2 g of compound **9** as a white solid. Yield, 86.0%; TLC R_f=0.40 (PE:EA=2:1); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.53-7.14 (m, 10H), 5.55(s, 1H), 5.39 (t, *J*=2.1Hz, 1H), 5.27 (t, *J*=5.2, 3.2Hz, 1H) 4.99-4.93 (m, 2H), 4.68 (d, *J* =12.8 Hz, 1H), 4.39 (d, *J* =12.8 Hz, 1H), 4.11-4.08 (m, 1H), 4.01 (s, 1H), 3.77 (s, 1H), 2.12 (s, 3H), 2.02 (s, 3H); ^{13}H NMR (100 MHz, Chloroform-*d*) δ 169.33, 137.48, 137.28, 129.13, 128.29, 128.19, 127.60, 127.47, 126.27, 101.21, 97.44, 83.73, 74.29, 70.11, 69.43, 69.01, 68.00, 66.02, 20.80.

4.4. *Synthesis* of *benzyl 4, 6-O-benzylidene -β-L-gulopyranoside* **(10)**

To a solution containing compound **9** (5 g, 11.31mmol) dissolved in 20 mL of dry methanol was added sodium methoxide (61.0 mg, 1.1 mmol), and the reaction mixture was stirred at room temperature for 1h until compound **9** was completely consumed under the monitor of TLC. After completion, 500 mg of resin Dowex 50 was added and gently stirred for another 15 mins, and filtered through a pad of celite, and then concentrated under diminished pressure to afford 4.1 g of

compound **9** as a white solid in quantitative yield. TLC R_f=0.34 (PE:EA=1:1, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.52 (d, *J* = 6.5 Hz, 2H), 7.42-7.29 (m, 8H) , 5.55 (t, *J* = 3.9 Hz, 1H), 5.01 (dt, *J* = 11.7, 3.6 Hz, 1H), 4.79 (dd, *J* = 8.0, 3.3 Hz, 1H), 4.59 (dd, *J* = 11.7, 3.1 Hz, 1H), 4.38 (dd, *J* = 13.2, 4.9 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.14-4.04 (m, 2H), 3.88 (m, 1H), 3.80 (d, *J* = 6.8 Hz, 1H), 2.51 (m, 1H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 137.69, 137.25, 129.11, 128.53, 128.23, 128.02, 126.27, 101.25, 99.25, 99.23, 75.90, 70.91, 69.87, 69.36, 68.88, 65.96.

4.5. *Synthesis* of *benzyl 3-O-acetyl-4, 6-O-benzylidene -β-L-gulopyranoside* **(3)**

To a solution containing 4 g of compound **10** (11.2 mmoL) dissolved in 30 mL of dry DMF was added 3.06 mL of ethyl orthoacetate (1.5 equiv, 16.8 mmol) and a catalytic amount of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 1h before addition of 2 mL of water. After completion, the resulting solution was poured into a mixture of 200 mL of AcOEt and 60 mL of water. The organic layer was washed with 100 mL of brine, and dried over anhydrous Na_2SO_4, and then filtered, and ultimately concentrated under diminished pressure to produce colorless oil, which was further dried *in vacuum* for 6 h to offer 4.1 g of donor **3** as a white solid. Yield, 97.6%. TLC R_f=0.25 (PE:EA=2:1, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.53-7.51 m, 2H), 7.42-7.28 (m, 8H), 5.55 (s, 1H), 5.42 (d, *J* = 3.7 Hz, 1H) 5.03 (d, *J* = 12.4 Hz, 1H), 4.84 (d, *J* = 8.0 Hz, 1H), 4.63 (d, *J* = 11.6 Hz, 1H), 4.38 (d, *J* = 12.4 Hz, 1H), 4.11-4.01 (m, 3H), 3.72 (s, 1H), 2.14 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 169.92, 137.41, 137.22, 129.15, 128.49, 128.23, 128.13, 127.96, 126.23, 101.19, 99.68, 74.22, 70.97, 70.88, 69.17, 67.44, 66.09, 20.96; TOF-MS, m/z: [M+ Na^+] , Calcd for $C_{22}H_{26}NaO_7^+$, 423.1414, Found, 423.1405.

4.6. *Synthesis* of *methyl 3-benzyl-α-D-mannopyranoside* **(13)**

20 g of methyl α-D-mannopyranoside (103.1mmol) and 0.1 equiv. of dibutyltin oxide (2.6g, 10.3 mmoL) were taken up in 100 mL of toluene, and refluxed for 1h. After evaporation of the solvent under diminished pressure, the residue was dried *in vacuum* for 2 h and re-dissolved in a mixture of acetonitrile (150 mL) and dimethyl formamide (12 mL) in the presence of K_2CO_3 (21.3g, 154.6 mmol) and tetrabutylammonium bromide of (3.3 g, 10.3mmol). To the mixture was added slowly 24.5 mL of BnBr (206.2 mmol), and the resulting mixture was stirred at 80 ℃ for 3 h and poured into 500 mL of AcOEt and 100 mL of water. The organic layer was washed with 100 mL of brine, and dried over anhydrous Na_2SO_4, and the filter was concentrated under diminished pressure to afford the crude residue, which was further purified by flash column chromatography

over silica gel with a mixture of PE and EA (V/V=1:2) to give 25.0 g of mannopyranoside **13** as a colorless oil. Yield, 86.2%. TLC R_f=0.16 (PE: EA=1:2, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39-7.27 (m, 5H), 4.72 (s, 1H), 4.68-4.65 (d, *J* =12.0 Hz, 1H), 4.59-4.56 (d, *J* = 12.0 Hz, 1H), 4.03 (t, *J* = 9.6 Hz,1H), 3.91 (s, 1H), 3.81 (d, *J* = 8.0 Hz, 1H), 3.72 (d, *J* = 12.8 Hz, 1H), 3.66 (d, *J* = 9.2Hz, 1H), 3.53 (d, *J* = 8.0 Hz, 1H), 3.32 (bs, 3H), 3.30 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ137.68, 128.47, 128.25, 128.01, 100.73, 79.60, 72.27, 71.97, 67.94, 65.02, 61.08, 54.84.

4.7. *Synthesis* of *1, 2, 4, 6-tetra-O-acetyl-3-benzyl- α-D-mannopyranoside* **(14)**

14.2 g of compound **13** (50 mmol) was dissolved in 50 mL of 3% H_2SO_4-Ac_2O, and the solution was stirred at room temperature for 3h. When the reaction ended, and the mixture was quenched by addition of 100 mL of saturated aq $NaHCO_3$, and then poured into the mixture of 1 L of AcOEt and 800 mL of saturated $NaHCO_3$. The organic layer was washed with 200 mL of brine, and dried over anhydrous Na_2SO_4. The filter was concentrated under reduced pressure to give the residue, which was dried *in vacuum* for 3h to afford 20.8 g of compound **14** as a colorless oil. Yield, 96.0%

TLC R_f=0.28 (PE: EA=3:1, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.32-7.25 (m, 5H), 6.10 (d, *J* = 2.0 Hz, 1H), 5.36 (dd, *J* =2.4, 2.0 Hz, 1H), 5.29 (t, *J*=10.0 Hz, 1H), 4.68 (d, *J* =12.4 Hz, 1H), 4.45 (d, *J* = 12.4 Hz, 1H), 4.23 (m, 1H), 4.09 (dd, *J* = 12.3, 2.4 Hz, 1H), 3.94 (m, 1H), 3.86 (d, *J* = 8.4 Hz, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.77, 170.02, 169.55, 168.09, 137.41, 128.45, 127.97, 127.78, 91.06, 74.12, 71.54,70.80, 67.05, 20.91, 20.87, 20.81, 20.76.

4.8 *Synthesis* of *1, 2, 4, 6-tetra-O-acetyl-α-D-mannopyranoside* **(15)**

To a solution containing 20 g of compound **14** (22.8 mmoL) in 100 mL of ethyl acetate was added 9.0 g of 10% Pd/C. The reaction mixture was stirred at room temperature overnight under the atmosphere of H_2. The whole process was monitored by TLC. When finished, the resulting mixture was filtrated to get rid of Pd/C and concentrated under diminished pressure to give 15.7g of compound **15** as a colorless oil. Yield, 99.0%. TLC, R_f=0.18 (PE:EA=2:1, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.12 (d, *J* = 2.0 Hz, 1H), 5.14 (t, *J*=10.0 Hz, 1H), 5.098 (m, 1H), 4.30 (m, 1H), 4.12 (d, *J*=2.0 Hz, 1H), 4.09 (m, 1H), 3.98 (m, 1H), 2.41 (brs, 1H), 2.19 (s, 3H), 2.13 (d, *J* = 2.4 Hz, 6H), 2.08 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.15, 170.74, 170.23, 168.07,

View Article Online
DOI: 10.1039/C8NJ06191B

90.43, 71.09, 70.25, 68.77, 68.50, 62.18, 20.90, 20.88, 20.84, 20.74.

4.9. *Synthesis* of *1,2,4,6-tetra-O-acetyl-3-O-(p-nitrophenylcarbamoyl) -α-D-mannopyranoside* **(16)**

To a solution of compound **15** (15 g, 43.1 mmol) dissolved in 90 mL of THF was added 24.4 mg of DMAP (0.2equiv, 8.6mmol), 12.7 mL of triethylamine (92.0 mmol) and 23.9 g of *p*-nitrophenyl chloroformate (172.4 mmol). The reaction mixture was stirred at 40℃ for 4h. When done, the resulting mixture was diluted with 1000 mL of AcOEt and 100 mL of 1N HCl, and then washed carefully with 100 mL of saturated $NaHCO_3$ and 150 mL of brine in order. The organic layer was concentrated under reduced pressure to afford yellow residue, which was further purified by column chromatography over silica gel with a mixture of PE and EA (V/V, 3:1→2:1) as an eluent to afford 19.9 g of a white solid. Yield, 92.0%. TLC R_f=0.30 (PE:EA=3:1, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.29 (d, *J*=9.2 Hz, 2H), 7.41 (d, *J*=9.2 Hz, 2H), 6.15 (d, *J* =2.0 Hz, 1H), 5.48 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.44 (t, *J* =10.0 Hz, 1H), 5.21 (dd, *J* = 10.0, 3.6 Hz, 1H), 4.32 (dd, *J* = 12.4, 4.8 Hz, 1H), 4.15 (dd, *J* = 12.4, 2.4 Hz, 1H), 4.12-4.06 (m, 1H), 2.21 (s, 3H), 2.17 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.66, 170.03, 169.45, 167.92, 155.23, 151.75, 145.71, 125.38, 121.93, 90.69, 74.27, 70.67, 67.57, 65.06, 61.95, 20.83, 20.79, 20.76, 20.74.

4.10. *Synthesis* of *2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranose* **(17)**

To a solution containing 15.0 g of compound **16** (29.3 mmol) dissolved in 80 mL of dry THF was added 26 mL of 7 M NH_3 in methanol (184.4 mmol), and stirred for 6 h at room temperature until compound **16** was completely consumed. The reaction mixture was directly concentrated under diminished pressure to produce yellow oil, which was purified by flash column chromatography over silica gel with the mixture solvent (PE: EA: Et_3N =1:2:0.01,V/V/V) to afford 8.3 g of compound **17** as a white foam. Yield, 85.1%. TLC R_f=0.10 (PE:EA=1:2,V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.36-5.22 (m, 4H), 4.73 (brs, 2H), 4.29-4.22 (m, 2H), 4.18-4.07 (m, 2H), 2.16 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.87, 170.26, 170.14, 155.50, 92.15, 70.53, 69.80, 68.49, 66.27, 62.58, 20.98, 20.80.

4.11. *Synthesis* of *2, 4, 6-tri-O-acetyl-3-O-carbamoyl -α-D-mannopyranosyl diphenyl phosphate* **(4)**

5 g of compound **17** (14.3 mmol) was dissolved in 20 mL of dry THF containing 4Å molecular

sieve. The temperature was cooled to -78℃, and 13.4 mL of n-BuLi (1.6 M solution in hexane, 21.5 mmol) was added and stirred for 30 mins, and finally diphenyl chlorophosphate (5.9 mL, 28.7 mmol) was added and proceed for another 1h. The resulting reaction mixture was diluted with 500 mL of AcOEt, and the organic layer was washed with 200 mL of brine, and dried over Na_2SO_4, and then filtered and concentrated under reduced pressure to produce the crude residue, which was further purified by flash column chromatography with the mixture of PE and EA (V/V=1:1) to afford 6.3 g of compound **4** as a white solid. Yield, 75%. TLC R_f=0.36 (PE:EA=1:1, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40-7.13 (m, 10H), 5.89 (d, *J* = 6.4 Hz, 1H), 5.39-5.24 (m, 3H), 4.92 (brs, 2H), 4.20 (dd, *J* = 12.4, 4.8 Hz, 1H), 4.12-3.86 (m, 1H), 3.92 (d, *J* = 12.4 Hz, 1H), 2.21 (s, 3H), 2.12 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.55, 169.77, 169.50, 155.12, 129.97, 125.88, 125.77, 120.26, 120.21, 120.11, 120.06, 96.01, 70.72, 69.06, 65.26, 61.69, 20.77, 20.72, 20.52; TOF-MS, m/z: (M+ Na^+), Cacld for $C_{25}H_{28}NLiO_{13}P^+$, 588.4062, Found, 588.4077 , (M+ Na^+), Cacld for $C_{25}H_{28}NNaO_{13}P^+$, 604.1190, Found, 604.1174.

4.12 *Synthesis* of *benzyl 3-O-acetyl-4,6-O-benzylidene-2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D- mannopyranosyl)-β-L-gulopyranoside* **(18)**

To a solution of donor **4** (6 g, 10.4 mmol) and acceptor **3** (5.0 g, 12.4 mmol) dissolved in 30 mL of dry CH_2Cl_2 in the presence of 4Å molecular sieve was added 2.6 mL of TMSOTf (15.4 mmol) at -5℃, and the reaction mixture was stirred for 1 h at the same temperature. After completion, it was poured into a mixture of 100 mL of AcOEt and 20 mL of saturated ammonium chloride. The organic layer was washed with 100 mL of brine and dried over anhydrous Na_2SO_4. The filter was concentrated under diminished pressure to afford a crude residue, which was further purified with column chromatography over silica gel with a mixture of PE and EA (V/V=1:2) as an eluent to give 5.5 g of compound **18** as a white solid. Yield, 70.3%; TLC R_f=0.36 (PE:EA=1:2, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.51-7.44 (m, 2H), 7.37-7.26 (m, 8H), 5.52 (s, 1H), 5.45 (t, *J* = 3.5 Hz, 1H), 5.36 (dd, *J* =3.4, 1.7 Hz, 1H), 5.26 (d, *J* =1.7 Hz, 1H), 5.16 (*t*, *J* =10.1 Hz, 1H), 5.04 (dd, *J* = 10.2, 3.4 Hz, 1H), 4.96 (d, *J* = 11.4 Hz, 1H), 4.87 (d, *J* = 8.3 Hz, 1H), 4.82 (s, 2H), 4.60 (d, *J* = 11.4 Hz, 1H), 4.37 (dd, *J* = 12.6, 1.5 Hz, 1H), 4.20 (dd, *J* = 11.8, 6.9 Hz, 1H), 4.13 (dd, *J* = 9.8, 2.1 Hz, 1H), 4.09 (d, *J* = 2.0 Hz, 1H), 4.07-4.04 (m, 1H), 4.01 (dd, *J* = 6.0, 1.6 Hz, 1H), 3.99 (dd, *J* = 2.9, 1.6 Hz, 1H), 3.73 (d, *J* = 1.5 Hz, 1H), 2.18 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.69 (s, 3H);

^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.83, 170.18, 169.94, 169.61, 155.30, 137.33, 137.05, 129.24, 128.35, 128.19, 128.09, 127.78, 126.37, 101.51, 99.59, 97.76, 74.69, 71.09, 70.45, 70.23, 69.79, 69.38, 69.22, 68.99, 65.97, 65.86, 62.53, 20.81, 20.79, 20.71, 20.16; TOF-MS, m/z: [M + Na^+], Calcd. for $C_{35}H_{41}NNaO_{16}^+$ 754.2318, Found, 754.2305.

4.13 *Synthesis* of *benzyl 3, 4, 6-tri-O-acetyl-2-O-(2, 4, 6-tri-O-acetyl-3-O-carbamoyl-α-D-manno- pyranosyl)- β-L-gulopyranoside* **(19)**

5 g of compound **18** (6.8 mmol) was dissolved in 20 mL of 3% H_2SO_4-Ac_2O, and the reaction mixture was stirred for 2 h at room temperature and was poured into a mixture of 60 mL of AcOEt and 2 mL of triethylamine and 60 mL of water. The organic layer was washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL) in order, and then dried over anhydrous Na_2SO_4 for several hours. The filter was concentrated under diminished pressure to afford crude residue, which was further purified with column chromatography over silica gel with a mixture PE and EA (V/V=1/2) to give 4.3 g of compound **19** as a white solid. Yield 85.6%; TLC R_f=0.38 (PE:EA=1:2, V/V); ^{1}H NMR (400 MHz, Chloroform-d) δ 7.35-7.27 (m, 5H), 5.38 (t, *J* = 3.7 Hz, 1H), 5.34 (dd, *J* = 3.4, 1.7 Hz, 1H), 5.25 (d, *J* = 5.5 Hz, 1H), 5.22 (d, *J* = 10.1 Hz, 1H), 5.02 (dd, *J* = 10.2, 3.3 Hz, 1H), 4.94 (d, *J* = 3.5 Hz, 1H), 4.88 (d, *J* = 11.3 Hz, 1H), 4.80 (d, *J* = 8.2 Hz, 1H), 4.72 (s, 2H), 4.61 (d, *J* = 11.3 Hz, 1H), 4.27 (dd, *J* = 12.0, 4.6 Hz, 1H), 4.16 (s, 3H), 4.10-4.05 (m, 1H), 4.04 -4.00 (m, 1H), 3.91 (dd, *J* = 8.2, 3.8 Hz, 1H), 2.18 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.07 (s, 6H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ 170.60, 170.51, 170.19, 169.65, 169.55, 169.52, 155.20, 136.59, 128.48, 128.31, 128.06, 99.78, 98.03, 71.47, 71.09, 70.28, 69.75, 69.36, 69.34, 68.67, 68.16, 65.83, 62.63, 61.70, 20.87, 20.77, 20.70, 20.69; TOF-MS, m/z: [M + Na^+], Calcd for $C_{35}H_{41}NNaO_{16}^+$ 750.2216, Found, 750.2198.

4.14 *Synthesis* of *3,4,6-tri-O-acetyl-2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranosyl) -β-L- gulopyranose* **(20)**

To a solution of compound **19** (5.0 g, 6.9 mmol) in 30 mL of ethyl acetate was added a catalytic amount of Pd/C, and H_2 was bubbled into the reaction mixture and stirred overnight. The reaction mixture was filtered through a pad of celite. The filtrate was concentrated under reduced pressure to afford 4.4 g of product **20** as colorless oil without purification. Yield, 99.3%; TLC R_f=0.15 (PE:EA=1:3, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.37 (t, *J* = 3.6 Hz, 1H), 5.30 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.25 (d, *J* = 1.8 Hz, 1H), 5.22 (d, *J* = 10.2 Hz, 1H), 5.05 (d, *J* = 8.1 Hz, 1H), 4.99

(dd, J = 10.6, 3.5 Hz, 2H), 4.96 (s, 2H), 4.93 (dd, J = 3.7, 1.3 Hz, 1H), 4.28 -4.21 (m, 1H), 4.21-4.18 (m, 1H), 4.14 (dd, J = 11.3, 5.8 Hz, 1H), 4.11-4.04 (m, 3H), 3.78 (dd, J = 8.1, 3.6 Hz, 1H), 2.18 (s, 3H), 2.12 (s, 3H), 2.12 (s, 3H), 2.07 (s, 6H), 2.04 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.72, 170.67, 170.25, 169.53, 155.71, 98.23, 94.36, 73.14, 70.35, 69.93, 69.63, 69.37, 68.78, 68.19, 65.71, 62.61, 62.00, 20.92, 20.87, 20.77, 20.74, 20.69; TOF-MS, m/z: [M + Na^+], Calcd for $C_{25}H_{35}NaO_{18}^+$ 660.1746, Found: 660.1726.

4.15. *Synthesis of BLM disaccharide* (**2)**

To a solution containing 100 mg of compound **20** (0.16 mmol) was dissolved in 2 mL of methanol was added a catalytic amount of sodium methoxide. The reaction mixture was stirred at room temperature for 20min before addition of Dowex 50 (H^+ form), and filtered, and then concentrated under diminished pressure to afford 60 mg of BLM disaccharide as a colorless foams. Yield, 100%. $[\alpha]_D^{25}$ 41.3° (c, 0.9, H_2O); ^{1}H NMR (400 MHz, Methanol-d_4) δ 5.21 (d, J = 1.9 Hz, 1H), 4.92 (dd, J = 8.2, 3.7 Hz, 2H), 4.86 (s, 2H), 4.05-4.03 (m, 2H), 3.89 (t, J =6.0 Hz, 1H), 3.84-3.77 (m, 2H), 3.75-3.64 (m, 7H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 159.52, 102.29, 95.37, 76.15, 75.91, 75.16, 74.94, 72.99, 71.18, 70.20, 66.04, 62.72, 62.61. TOF-MS, m/z: [M+Na^+], Calcd. for $C_{13}H_{23}NNaO_{12}^+$, 408.1112, Found: 408.1101.

4.16. *Synthesis of activated 10-hydroxycamptochecin* (**22)**

5 g of 10-hydroxycamptochecin (13.8 mmol) in tetrahydrofuran (THF, 200 mL) was treated with 10.8 g of 4-nitrophenyl chloroformate (54.0 mmol), 20 mL of triethylamine , and the reaction mixture was allowed to proceed at room temperature for 1 h. After completion, the product was extracted with 1L of ethyl acetate, and the organic layer was washed with 500 mL of water and 150 mL of brine, and then dried with anhydrous Na_2SO_4, and the solvent was removed *in vacuo* to offer a crude residue, which was recrystallized with ethyl acetate to afford 6.7 g of activated 10-hydroxycamptochecin as a yellow solid. Yield 93%; ^{1}H NMR (400 MHz, Dimethyl sulfoxide-d_6) δ 8.72 (s, 1H), 8.39 (d, J = 12.0 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.18 (s, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 12.0 Hz, 1H), 7.35 (s, 1H), 6.55 (s, 1H), 5.43 (s, 2H), 5.30 (s, 2H), 1.87 (t, J = 8.0 Hz, 2H), 0.89 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, Dimethyl sulfoxide-d_6)) δ172.92, 157.25, 155.51, 153.51, 151.03, 150.46, 149.29, 146.60, 145.98, 145.69, 131.95, 131.31, 131.16, 128.64, 126.66, 126.01, 125.57, 123.21, 119.78, 119.47, 116.26, 97.33, 72.84, 65.72, 50.73, 30.76, 8.25.

View Article Online
DOI: 10.1039/C8NJ06191B

4.17. *Synthesis of 3,4,6-Tri-O-acetyl-2-O-(3,4,6-tri-O-acetyl-2-O-carbamoyl-α-D manno-pyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate* (**23**)

To a solution of compound **20** (6 g, 9.3 mmol) containing 4Å molecular sieve in 40 mL of dry THF at -78℃ was added 8.8 mL of 1.6 M n-BuLi solution in hexane (14.0 mmol). After the mixture was stirred for 10min, 3.9 mL of diphenyl chlorophosphate (18.8 mmol) was added and proceeded for another 40 min. The resulting mixture was poured into a mixture of 200 mL of AcOEt and 100 mL of saturated NH_4Cl. The organic layer was washed with 100 mL of brine, and dried over anhydrous Na_2SO_4, and filtered, and then concentrated under reduced pressure to yield the crude residue, which was further purified by flash column chromatography over silica gel with a mixture PE and EA (V/V=1:1) to afford 6.2 g of compound **23** as a white solid. Yield 72.1 %; TLC R_f=0.26 (PE:EA=1:2, V/V); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.31 (m, 4H), 7.19 (m, 6H), 5.74-5.62 (m, 1H), 5.42 (d, *J* = 3.7 Hz, 1H), 5.28 (s, 1H), 5.25-5.19 (m, 1H), 5.13 (s, 1H), 5.04 (d, *J* = 9.9 Hz, 1H), 4.98 (d, *J* = 3.7 Hz, 1H), 4.69 (s, 2H), 4.29 (m, 2H), 4.15-4.02 (m, 4H), 4.01-3.95 (m, 1H), 2.22 (s, 3H), 2.15 (s, 3H), 2.08 (s, 6H), 2.01 (s,3H), 1.98 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 169.58, 169.40, 169.17, 168.44, 168.27, 168.20, 128.83, 128.65, 124.57, 124.53, 119.26, 119.21, 119.04, 118.99, 97.63, 96.38, 96.32, 70.91, 70.81, 70.49, 68.69, 68.55, 68.23, 67.69, 66.55, 64.88, 61.60, 60.15, 19.82, 19.71, 19.63; TOF-MS, m/z: [M+Na^+], Calcd for $C_{37}H_{44}NNaO_{21}P^+$, 892.2036, Found, 892.1998.

4.18 *Synthesis* of *2-[2-(Benzyloxycarbonylamino)ethyloxy]ethyl 3,4,6-Tri-Oacetyl-2-O-(3,4,6-tri-O-acetyl-2-O-(carbamoyl)-α-D-mannopyranosyl)-α,β-L-gulopyranose* (**24)**

To a stirred solution containing 6.0g of compound **23** (6.8 mmol) and 4Å molecular sieve mixed in 30 mL of dry CH_2Cl_2 was added 2.6 g of CBz linker of (11.2 mmol). The reaction mixture was stirred at 0℃ for 30 min before addition of the activated agent TMSOTf (1.3 mL, 9.4 mmol). At the same temperature the reaction mixture was stirred for another 1h and quenched with adequate Et_3N. The resulting solvent was extracted with 250 mL of AcOEt, and the organic layer was washed with water (300 mL) and brine (150 mL) in order, and dried over anhydrous Na_2SO_4. The filter was concentrated under reduced pressure to give a residue, which was purified by flash column chromatography over silica gel with a mixture of PE and EA (V/V=1/2) to afford

View Article Online
DOI: 10.1039/C8NJ06191B

4.6 g of compound **24** as white foams. Yield, 80%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ7.34-7.30(m, 5H), 5.52-5.49 (m, 1H), 5.36-5.34 (t, *J*=4.0 Hz, 1H), 5.27-5.25 (m, 2H), 5.36-5.34 (d, *J*=8.0 Hz, 1H), 5.12-5.04 (m, 2H), 5.02-4.89 (m, 3H), 4.80 (m, 1H), 4.78-4.70 (brs, 2H), 4.26-4.22 (m, 1H), 4.15-4.02 (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, 5H), 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}C NMR (100 MHz, Chloroform-*d*) δ 170.60,170.49,170.18, 170.04,169.47,169.42,156.55, 155.31, 136.66, 128.51, 128.16, 128.10, 100.84, 98.13, 71.92, 70.25, 70.19, 70.02, 69.71, 69.68, 69.31, 68.92, 68.71, 68.09, 67.6, 66.62, 65.82, 62.64, 62.01, 61.69, 40.83, 20.86, 20.75, 20.71, 20.68, 20.66;TOF-MS, m/z: [M + Na^+], Calcd for $C_{37}H_{50}N_2$ Na O_{21}^+, 881.2798, Found, 881.2843.

4.19 *Synthesis of conjugate* ***26***

To a stirred solution containing 500 mg of compound **24** (1.06 mmol) were dissolved in a mixture of DMF (10 mL) and methanol (1 mL) was added 50 mg of 10% Pd/C, and the reaction was stirred for 4 h under atmosphere of H_2. After filtered to remove Pd/C, 0.67 g of compound **22** (1.27mmol), triethyamine (0.5 mL) and DMAP (100 mg) were added to the reaction mixture, and stirred at room temperature for 24 h, and the consumption of compound **24** was analyzed by TLC. The reactive mixture was diluted with 100 mL of AcOEt and 100 mL of saturated NH_4Cl. The organic phase was washed with 200 mL of water and 100 mL of brine in order, and dried with anhydrous Na_2SO_4, and filtered, and then evaporated under diminished pressure to afford yellow residue, which was purified by flash column chromatography over silica gel to afford 741.9 mg of conjugate **1** as yellow solid. Yield 81.2%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.73 (s, 1H), 7.66 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 6.09 (t, *J* = 8.0 Hz, 1H), 5.74 (d, *J* = 16.0 Hz, 1H), 5.40 (t, *J* = 4.0 Hz, 1H), 5.36 -5.23 (m, 5H), 5.09-5.02 (m, 1H), 4.95 (d, *J* = 4.0 Hz, 1H), 4.84 (d, *J* = 12.0 Hz, 1H), 4.75 (s, 2H), 4.27 (m, 1H), 4.17-3.97 (m, 6H), 3.97-3.87 (m, 2H), 3.77-3.60 (m, 5H), 3.54-3.45 (m, 2H), 2.21 (s, 3H), 2.16 (s, 3H), 2.10 (s, 3H), 2.08 (s, 9H), 1.91 (m, *J* = 2H), 1.04 (t, *J* = 8.0 Hz, 3H). TOF-MS, m/z: [M + H^+], Calcd for $C_{50}H_{59}N_4O_{25}$, 1115.3458, Found, 1115.3377.

4.20 *Synthesis of 2-[2-(amino)ethyloxy]ethyl 2-O-(3-O-Carbamoyl- α -D-mannopyranosyl)-α,β-L-gulopyranose* **(25)**

To a solution containing 1.0 g of **24** (1.16mmol) dissolved in 10 mL of anhydrous methanol was added a freshly prepared solution of 5.0 mL of 0.4 M sodium methoxide in methanol. The reaction

mixture was allowed to stir at room temperature for 30min, and the complete consumption of starting material was confirmed by TLC. After completion, 800 mg of Dowex 50X resin was added, and shaken for 10min, and then filtered. To the solution of the crude product in methanol was added a catalytic amount of Pd/C, and then H_2 was bubbled continuously for 1h. The reaction was monitored with HPLC, when done, the reaction mixture was filtered with Celite and concentrated under diminished pressure to afford 500 mg of compound **25** as a white foams for next reaction without purification. Yield, 91.0%; TOF-MS, m/z: [M+Na$^+$], Calcd for $C_{17}H_{32}N_2O_{13}Na$, 495.1797, Found, 495.1785.

4.21. *Synthesis of BLM disaccharide-10-HCPT conjugate* **(1)**

To a stirred solution containing 500 mg of compound **25** (1.06 mmol) were dissolved in 10 mL of DMSO, was added 0.67 g of compound **22** (1.2equiv, 1.27mmol). The reaction mixture was stirred at room temperature for 24h, and the consumption of compound **25** was monitored by TLC. The solvent DMSO was removed under diminished pressure to afford yellow residue, which was purified by flash column chromatography to afford conjugate **1** as yellow solid. Yield 56.2%; ^{1}H NMR (400 MHz, Dimethyl sulfoxide-d_6) δ 8.64-8.40 (m, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.80-7.96 (m, 1H), 7.87 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.33 (s, 1H), 7.25 (s, 1H), 6.54 (s,1H), 5.42 (s, 1H), 5.26 (d, J=20.0 Hz, 2H), 4.98-4.36(m,10H), 4.01-3.11 (m, 20H), 1.92 (m, J = 2H), 0.98 (m, 3H). ^{1}H NMR (100 MHz, Dimethyl sulfoxide-d_6) δ 173.04, 157.28, 154.69, 152.58, 150.56, 150.12, 149.81,146.36, 145.89, 143.65, 131.53, 131.06, 130.66, 130.32, 129.71, 128.75, 126.78, 123.52, 119.17, 109.26, 97.14, 74.63, 74.19, 73.86, 72.86, 71.54, 70.08, 69.40, 68.47, 67.34, 66.98, 65.71, 64.72, 60.82, 49.07, 41.00, 30.75, 8.25. TOF-MS, m/z: [M+Na$^+$], Calcd for $C_{38}H_{46}N_4O_{19}Na$, 885.2654, Found, 885.2608.

4.22. *In vitro antitumor activity test*

4.22.1 *Cells culture*

HCT-116 cells, HepG2 cells and HEK-293 T cells were grown in DEME (Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS, Gibco, Life technologies) and 1% penicillin-streptomycin mixed antibiotic supplement (Gibco, Life technologies). Cell lines were maintained at 37 °C under a humidified atmosphere of 5% CO_2 and 95% air.

4.22.2 *Assessment of antitumor activity by MTT assay*

The tested compounds were dissolved in appropriate amount of DMSO before the experiment

to obtain the known concentration of solution, from which a definite amount was taken, and diluted to the various concentrations with DEME. HCT-116, HepG2 and HEK-293T, bearing period of logarithmic growth, were adjusted to $5*10^4$ cells/mL, and then seeded into 96-well plates. After cultured for 12 h in 37 °C under a humidified incubator (5% CO_2 and 95% air). Cells were incubated in complete mediums in the absence (negative control) and presence of various concentrations of compounds tested, respectively, for 48 h. Each compound was arranged three parallel wells. The supernatant was removed, and then 40 μL MTT solution (5 mg/mL) was added to each well. After re-incubated for another 4 h, 100 μL of DMSO was added to each well for dissolving the formazan crystals. The percentage of cell viability was determined by measuring the absorbance (Abs) at wavelength of 490 nm using a Multiskan MK3 microplate reader (BioTek Elx800, USA). The determination of IC_{50} values was acquired in accordance with linear regression analysis of the concentration-response curves plotted for each compound tested.

Conflict of interest

None declared.

Acknowledgments

The work is supported in part by financial support from National Natural Science Foundation of China (No.21772028, 31360456, 3106052), Start-up Foundation of Guangzhou University of Chinese Medicine for Young scholar (A1-AFD018171Z) and General Scientific Research project of Guangzhou (201804010325), and Science and Technology plan of puer city (No. 2014-38). We also thank professor Tian Xu very much for critical reading our manuscript.

Reference

[1] H. Douglas and A. W. Robert A, *cell*, 2011, **144**, 646.

[2] S. S. Yuan, M. L. Li, J. Chen, L. Zhou and W. Zhou, *ChemMedChem*., 2018, **23**,13 (8), 764.

[3] C. J. Thomas, A. O. Chizhov, C. J. Leitheiser, M. J. Rishel, K. Konishi, Z.Tao and S. M. Hecht, *J. Am. Chem. Soc*., 2002, **124**, 12926.

[4] B. R. Schroeder, M. I. Ghare, C. Bhattcharya, R. Paul, Z. Yu, P. A. Zaleski, T. C. Bozeman, M. J. Rishel and S. M. Hecht, *J. Am. Chem. Soc*., 2014, **136**, 13641.

[5] J. C. Chapuis, R. M. Schmaltz, K. S. Tsosie, T. M. Bozeman and S. M. Hecht, *J. Am. Chem. Soc.,* 2009, **131**, 2438.

[6] Z. Yu, R. M. Schmaltz, T. C. Bozeman, R. Paul, M. J. Rishel, K. S. Tsosie and S. M. Hecht, *J.*

Am. Chem. Soc. **2013**, 135, 2883.

[7] C. Bhattacharya, Z. Yu, M. J. Rishel and S. M. Hecht, *Biochemistry*, 2014, **53** (43), 6800.

[8] C. Bhattacharya, Z. Yu, M.J. Rishel and S. M. Hecht, *Biochemistry*, 2014, **53** (20), 3264.

[9] V. Pozsgay, T. Ohgi and S. M. Hecht, *J. Org. Chem.* 1981, **46**, 3761.

[10] D. L. Boger and H. Honda, *J. Am. Chem. Soc.,* 1994, **116**, 5647.

[11] D. L. Boger and H.Cai, *Angew. Chem. Int. Ed.* 1999, **388**, 448.

[12] T. Oshiari, M. Shibasaki, T. Yoshizawa, M. Tomita, K. Takao and S. Kobayashi, *Tetrahedron,* 1997, **53**, 10993.

[13] K .Katano, P. I. Chang, A. Milliar, V. Possgay, D. K. Minster, T. Ohgi and S. M. Hecht, *J. Org. Chem.* 1985, **50**, 5807.

[14] A. Dondoni and A. Marra, *J. Org. Chem.*, 1997, **62**, 6261.

[15] W. Lu, R. Che, Q. Zhu, J. Yu, J. Li, J. Yu and W. Lu, *Tetrahedron*, 2017, **73** (43), 6172.

[16] P. Xu, D. Chen, J. Xi and Z. Yao, *Chem. Asian. J.,* 2015, **10** (4), 976.

[17] T. Tsuchiya, T. Miyake, S. Kageyama and S. Umezawa, *Tetrahedron Lett.,* 1981, **22** (20), 1413.

[18] X. M. van Wijk, R. Lawrence, V. L. Thijssen, B. A. van den Broek, R. Troost, M. van Scherpenzeel, N. Naidu, A. Oosterhof, A. W. Griffioen, D. J. Lefeber, D. van Floris and T. H. van Kuppvelt, *FASSEB J*. 2015, **29** (7), 2993.

[19] A. Malleron, A. Benjdia, O. Alhosna and C. Le Narvor, *Carbonhyd. Res.* 2012, **353**, 96.

[21] C. T. Oberg, A. L. Noresson, T. Delaine, A. Larumber, J. Tejler, H. von Wacjemfedt and U. Nilsson, *Carbohyd. Res.*2009, **344**, 1282.

[22] I. Bhaumik and A. K. Misra, *RSC Adv.* 2014, **4**, 61589.

[23] O. Tetsuto and K. Susumu, *Tetrahedron Lett.* 1995, **36** (7), 1089.

[24] S. S. Mandal, L. Duncombe, N. V. Ganesh, S. Sarkar, L. Howells, P. J. Hogarth, D. R. Bundle and J. McGiven, *ACS Central Sci.* 2017, **3**(3), 224.

[25] B. Wu, J. T. Ge, B. Ren, Z. C. Pei and H. Dong, *Tetrahedron*, 2015, **71**(23), 4023.

[26] Y. Q. Wang, L. F. Li, W. Jiang and W. L. James, *Bioorg. Med. Chem. Lett*. 2005, **13**, 5592.

Multi-gram scale synthesis of a bleomycin (BLM)

carbohydrate moiety: exploring the antitumor beneficial effect of BLM disaccharide attached to 10-hydroxycamptothecine (10-HCPT)

MaoLin Li[1#], Weiping Huang[1#], Zhilin Jiang[2#], Yonghui Shi[3], Sisi Yuan[1], Kaishuo Fu[1], YongJun Chen[4], Li Zhou[5*] and Wen Zhou[1*]

[1] *School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, University town, Waihuan Rd, Panyu, Guangzhou, 510006, China*

[2]*Puer University, Puer, 665000, Yunan, China*

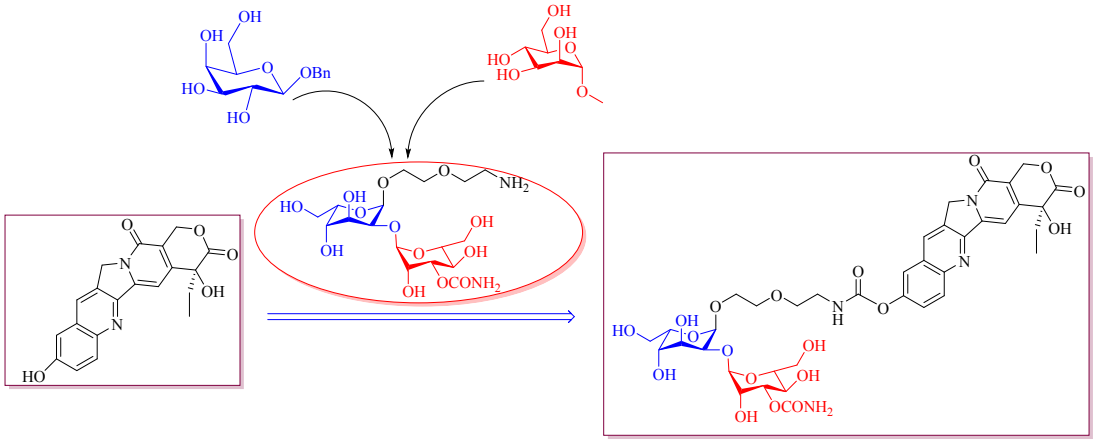
[3]*Department of Pharmacy, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, Guangdong, China*

[4] *South China Research Centre for Acupuncture and Moxibustion, Medical College of Acu-Moxi and Rehabilitation, Guangzhou University of Chinese Medicine, University town, Waihuan Rd, Panyu, Guangzhou, 510006, China*

[5]*College of Science, Hunan Agricultural University, Changsha, Hunan, 410128, China.*

[#] These authors contributed equally to the work.

[*] Correspondent. E-mail: zhoulizss@163.com; zhouwen60@126.com



10-hydroxycamptothecin (Non-specific drug)

Conjugate (**1**) (Specific drug)