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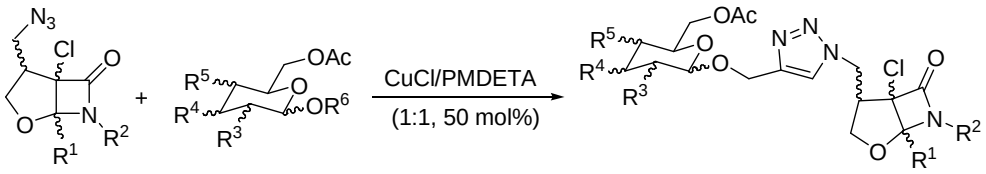

R^1 = Ph; R^2 = Ph, *p*-$MeOC_6H_4$

R^3 = OAc, H (ax/eq); R^4 = OAc (ax/eq); R^3- R^4 = Π-bond

R^5 = OAc (ax/eq); R^6 = H_2C—≡ (ax/eq)

ax : axial; eq : equatorial

An efficient method for the synthesis of some chlorinated and heteroatom rich triazole-linked β-lactam glycoconjugates

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Abstract: The synthetic utility of chlorinated bicyclic C-fused tetrahydrofuro[3,2-*c*] azetidin-2-ones synthesized in our laboratory by Cu(I)-catalyzed halogen atom transfer radical cyclization (ATRC) has been illustrated through the synthesis of some novel β-lactam glycoconjugates. The chlorine atom of the chloromethyl side chain of the bicyclic C-fused tetrahydrofuro[3,2-*c*] azetidin-2-ones was subjected to nucleophilic substitution with azide group followed by Cu(I)-catalyzed azide-alkyne click reaction (CuAAC) with propargyl glycosides to generate the desirous β-lactam glycoconjugates. A sequential optimization of the reaction procedure for CuAAC was carried out to obtain an efficient catalyst system to achieve β-lactam glycoconjugates in good yields. The β-lactam glycoconjugates are the compounds of interesting architecture and structurally suitable for bioactivity evaluation. Therefore, these β-lactam glycoconjugates were screened for in vitro antibacterial activity. Additionally, a representative β-lactam glycoconjugate was also tested for biocompatibility and cytotoxic activity against L929 cancer cell lines.

1. Introduction

Heteroatom rich organic compounds particularly containing N, O and Cl atoms are of immense importance both in chemistry and biology. The majority of the natural products, drugs and biologically active compounds are rich in heteroatoms. β-Lactams (azetidin-2-ones) occupy

an important place in chemistry and medicine.[1] The appeal of working with β-lactams stems from their ability to exhibit a diverse range of biological activities[1a,2-4] and interesting pharmacological activities.[1a,4,5] Furthermore, β-lactams are the useful kits in the tool-box of synthetic organic chemists for the synthesis of a variety of heterocyclic compounds of biological interest.[6] A chlorine substituent at the α-position of the β-lactam ring is well known to enhance the chemical reactivity and it also offers a great diversity regarding its biological activity.[7]

Conjugation of β-lactams has been emerging as a new strategy in drug discovery for increasing the effectiveness of β-lactam antibiotics and modification of their pharmacological potential.[8] Additionally, it provides an opportunity to design and synthesize diverse molecular architectures through modification of one or both the components.[8] Cu(I)-catalyzed azide-alkyne click reaction (CuAAC) has been widely used as the most powerful technique nowadays for the conjugation of β-lactams with some other bioactive molecules in pursuit of more potent bioactivity.[9] In addition to conjugation, mere the covalent attachment of a triazole ring to the β-lactam scaffold has been reported to make a significant enhancement in the biological properties of β-lactams.[10] Our laboratory has reported the synthesis of a triazolyl-β-lactam through CuAAC of an azido C-fused bicylic β-lactam with phenylacetylene.[11]

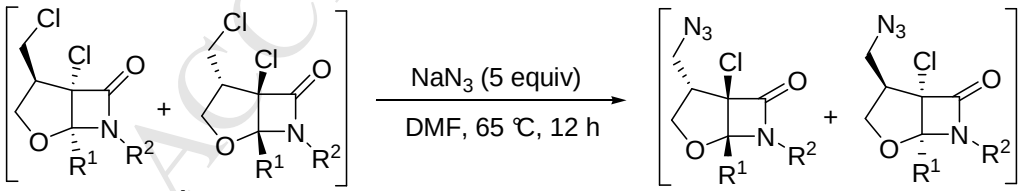
Sugars are the most important and most diverse class of biomolecules. Glycoconjugates are the promising scaffolds in drug discovery and development as they are involved in crucial life governing processes such as molecular recognition, inflammation, immunological response, pathogen defense, tumorigenesis and metastasis.[12] Furthermore, triazolyl glycoconjugates synthesized by CuAAC exhibit a broad spectrum of potential synthetic, pharmaceutical and biological applications.[13]

To the best of our knowledge, not many reports have appeared in the literature that reveals the presence of a sugar unit and a β-lactam scaffold in a single molecule.[14] However, some of the reported β-lactam sugar derivatives exhibit interesting biological and medicinal properties.[14] Furthermore, the β-lactam glycoconjugates are rarely reported in the literature. Curiously, the glycoconjugation of β-lactams through CuAAC which would bring together all the three medicinally significant components, the β-lactam, sugar and 1,2,3-triazole, together is scarcely reported in the literature. In fact we could locate only one such report in which the triazolyl-β-lactam glycoconjugate synthesized by CuAAC, acted as lectin inhibitor.[14d]

Enlightened by such innovative and promising work and in continuation of our interest to use the bicyclic azido β-lactam synthesized previously in our laboratory as a substrate, we envisioned to synthesize a series of chlorinated and heteroatom rich β-lactam glycoconjugates via click chemistry and explore their biological competences.

2. Results and Discussion

To begin with, the synthesis of azidotetrahydrofurano fused β-lactams **2** (**Scheme 1**) was carried out by the bimolecular substitution of chlorine atom of the chloromethyl side chain of chlorinated C-fused bicyclic β-lactams **1** with azido nucleophile as demonstrated earlier by this laboratory.[11]



1a,b → **2a,b** (69, 65%)

a: R^1 = Ph, R^2 = Ph; **b**: R^1 = Ph, R^2 = *p*-$MeOC_6H_4$

Scheme 1: Synthesis of azidotetrahydrofurano fused β-lactams 2

The acetylenic components needed for the glycoconjugation of azido β-lactams **2** via click reaction were easily prepared diastereoselectively from the appropriate sugar derivatives through glycosylation or Ferrier reaction with propargyl alcohol according to the reported literature procedures.[15] Thus, five different propargyl glycosides **3** (**Figure 1**) including both the saturated and unsaturated and the α as well as β-anomeric propargylic sugars were employed to prepare β-lactam glycoconjugates.

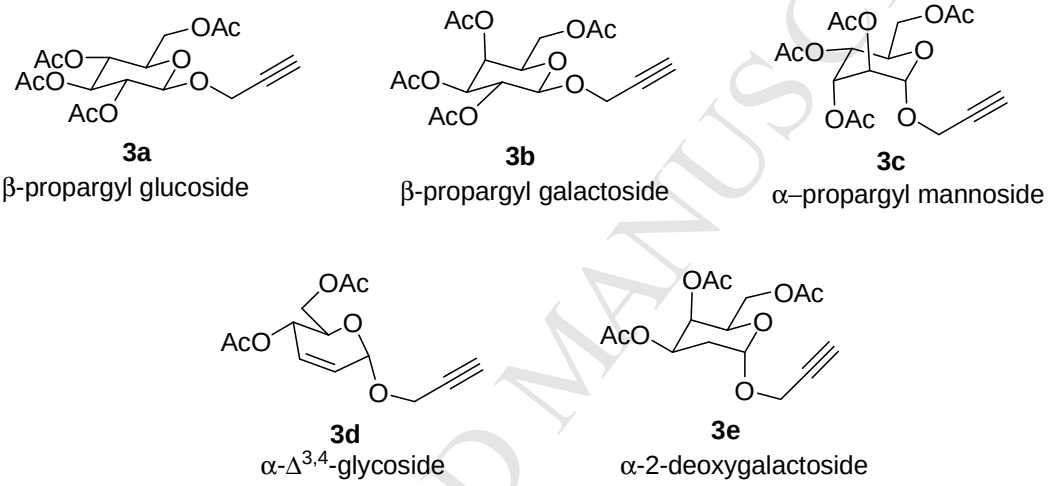
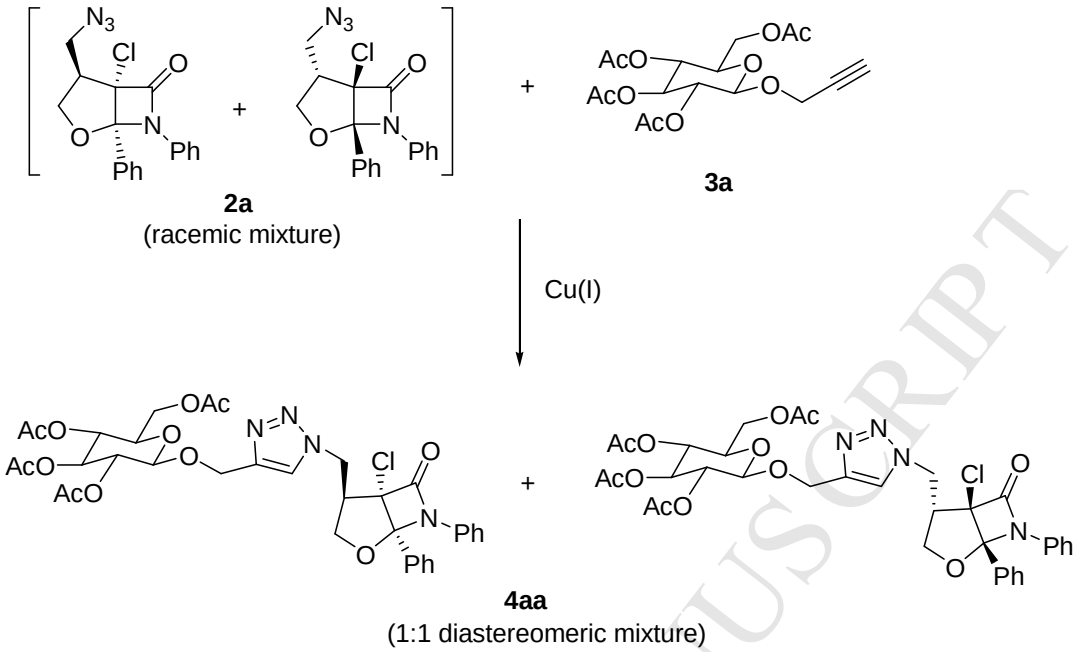


Figure 1. Different propargyl glycosides employed for glycoconjugation of azido β-lactam 2

2.1. Optimization of the reaction procedure for preparation of β-lactam glycoconjugate by CuAAC:

Next, the crucial Cu(I)-catalyzed azide-alkyne click reaction of the azidotetrahydrofurano fused bicyclic β-lactams with the sugar based alkynes was investigated. The reaction conditions for the glycoconjugation of the β-lactam through CuAAC were optimized by exploring the reaction of azido-β-lactam **2a** (**Scheme 2**) with the propargyl glucoside **3a** (Table 1).



Scheme 2: Optimization of reaction procedure for preparation of β-lactam glycoconjugate 4aa

Unfortunately, the reaction failed under the reaction conditions previously used in our laboratory for the synthesis of a triazolyl-β-lactam[11] (entry 1, Table 1). This might be ascribed to the poor solubility of the reactants in solvent system used and the insufficient catalyst loading. We then tended to optimize the reaction condition by changing the solvent and increasing the catalyst loading. To our delight, the partial formation of the β-lactam glycoconjugate **4aa** was observed in CH_2Cl_2/H_2O (1:1 v/v) system using $CuSO_4.5H_2O$ (2 mol equiv) and Na-Asc (4 mol equiv) at room temperature (entry 2, Table 1), as evidenced by ^1H NMR analysis of the crude reaction mixture. Presumably, the incomplete conversion of **2a** to **4aa** despite of using excess of catalyst and longer reaction times might be due to the deactivation of Cu(I) ions to Cu(0) particles and Cu(II) ions. This was evidenced by the change in the color of the reaction mixture from the original light yellow color to dark brown and the presence of some suspended brownish particles in the reaction mixture.

Table 1. Optimization of reaction procedure for glycoconjugation of β-lactam 2a[a]

Entry	Azide	Alkyne	Reaction Conditions	Time (h)	Yield (%)[b]	
					β-Lactam glycoconjugate 4aa	Recovered substrates 2a/3a
1.	**2a**	**3a**	$CuSO_4.5H_2O$ (10 mol%)/ Sodium ascorbate (Na-Asc) (20 mol%), *t*-BuOH/H_2O (5:1), 65 ˚C	24	-	76/75
2.	**2a**	**3a**	$CuSO_4.5H_2O$ (2 mol equiv)/Na-Asc (4 mol equiv), CH_2Cl_2/H_2O (1:1), rt (25-30 ˚C)	12	35	42/42
3.	**2a**	**3a**	$CuSO_4.5H_2O$ (1 mol equiv)/ *N,N'N'N''N''*-pentamethyldiethylene triamine (PMDETA) (1 mol equiv)/Na-Asc (2 mol equiv), CH_2Cl_2/H_2O (1:1), rt (25-30 ˚C)	1	64	-
4.	**2a**	**3a**	CuI (0.5 mol equiv)/ *N,N*-diisopropyl ethylamine (DIPEA) (0.75 mol equiv), CH_3CN, N_2 atm, rt (25-30 ˚C)	5	-	74/72
5.	**2a**	**3a**	CuCl (60 mol%)/ bipyridyl (bpy) (60 mol%), 1,2-dichloroethane (DCE), N_2 atm, reflux	12	-	79/72
6.	**2a**	**3a**	CuCl (20 mol%)/ *N,N,N',N'*-tetramethyl ethylenediamine (TMEDA) (40 mol%), DCE, N_2 atm, reflux	12	-	78/73
7.	**2a**	**3a**	CuCl (50 mol%)/ PMDETA (50 mol%), DCE, N_2 atm, rt (25-30 ˚C)	1	78	-
8.	**2a**	**3a**	CuCl (30 mol%)/ PMDETA (30 mol%), DCE, N_2 atm, rt (25-30 ˚C)	24	37	43/45

[a]All the reactions were performed by taking **2a** (1 mmol) and **3a** (1 mmol). [b]Isolated yield after purification by column chromatography.

The literature survey has shown that the nitrogen containing ligands have been the first and the most popular ligands for CuAAC and their affinity for copper is well-known.[16]

Alternatively, such additives increase the solubility of copper(I) species in the reaction media and also stabilize the copper(I) active species even in reactions carried out in water. Arguably, it was envisaged to use some nitrogen based ligand in the reaction medium of CuAAC to ascertain completion of the reaction. A triamine ligand, *N,N'N'N''N''*-pentamethyldiethylenetriamine (PMDETA) is probably the most commonly employed ligand in CuAAC, due to the well-established efficiency of CuCl/PMDETA in ATRC as previously demonstrated in our laboratory.[11]

Therefore, employing the use of $CuSO_4.5H_2O$/PMDETA/Na-Asc (1:1:2 mol equiv), in CH_2Cl_2/H_2O (1:1 v/v) as the solvent system at room temperature (25-30 ˚C), delightfully the reaction got completed in 1 h and resulted in isolation of the pure β-lactam glycoconjugate **4aa** in 64% yield (entry 3, Table 1). However, the requirement of high catalyst loading in the above biphasic reaction media seemed unsatisfactory, and therefore this method was not preceded subsequently.

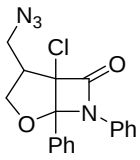
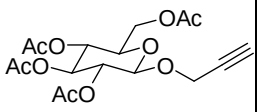
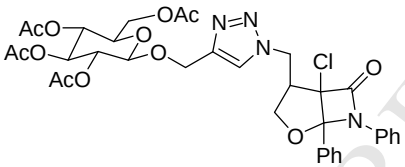
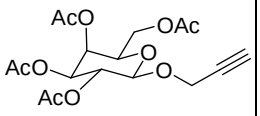
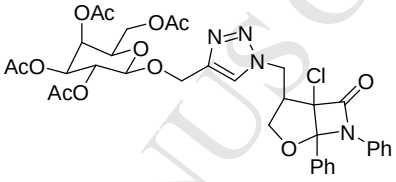
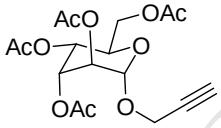
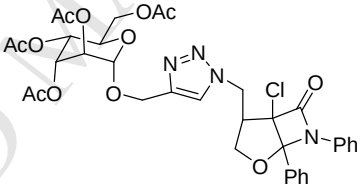
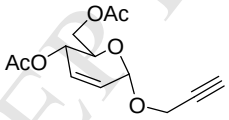
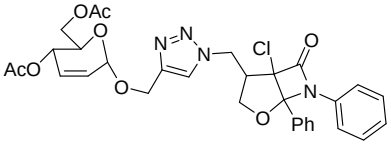
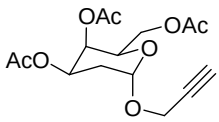
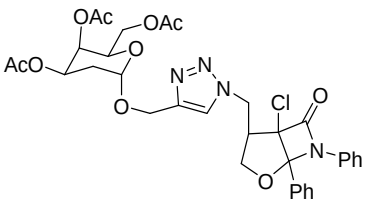
Consequently, it was thought to perform the click reaction in monophasic non-aqueous conditions using the pre-formed Cu(I) salt in the presence of a nitrogen based ligand. The screening of Cu(I)-salt and ligand showed that only CuCl/PMDETA catalyst system was found to be effective for the aforesaid click reaction. Gratifyingly, by using CuCl/PMDETA (1:1 mol ratio, 50 mol%) in DCE at room temperature (25-30 ˚C) (entry 7, Table 1), the remarkable increase in the product yield was observed as the desired β-lactam glycoconjugate **4aa** was obtained in 78% isolated yield. Thus, CuCl/PMDETA (1:1 mol ratio, 50 mol%), was found to be the most efficient catalyst system for the synthesis of β-lactam glycoconjugates in terms of reasonable improvement in product yield and an appreciable decrease in the amount of catalyst required for the completion of glycoconjugation reaction.

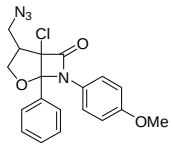
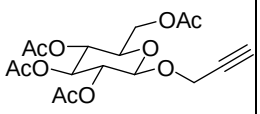
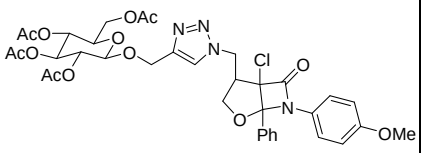
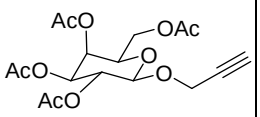
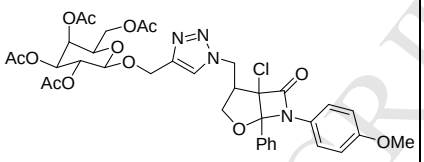
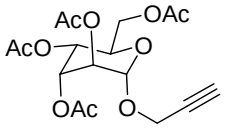
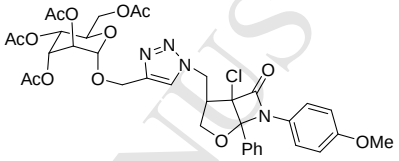
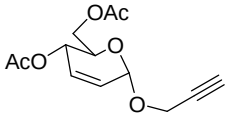
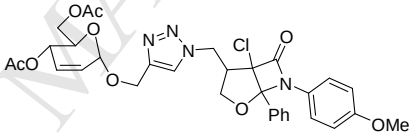
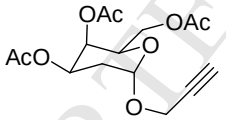
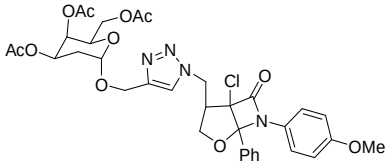
The click reaction proceeded smoothly with complete retention of the anomeric configuration and was highly regioselective as only 1,4-disubstituted-1,2,3-triazole was isolated. No 1,5-disubstituted-1,2,3-triazole could be detected. However, the reaction furnished a mixture of two diastereomers in 1:1 ratio because the azido β-lactam **2a** was racemic.

In the ^{1}H NMR spectrum of the compound **4aa**, the two most downfield singlets at δ 7.80 and 7.78 ppm, integrating together for one proton were assigned to the methine proton of the triazole ring of the two diastereomers with equal contribution to the total signal area from each of the diastereomer indicating that the two diastereomers were formed in 1:1 ratio. Other signals due to the diastereomeric protons were not well separated. Therefore, COSY NMR spectrum was used to help the assignments of the remaining protons. In the proton-decoupled ^{13}C NMR spectrum of **4aa**, few signals appeared as twins, further confirming the presence of two diastereomers of the β-lactam glycoconjugate **4aa**. The presence of a requisite number of carbons in the ^{13}C NMR spectrum further corroborates the assigned structure. IR spectrum and HRMS data also supports the assigned structure.

Having established the exact chemical procedure for synthesis of a β-lactam glycoconjugate **4aa**, we set out to synthesize a variety of β-lactam glycoconjugates with diversity in both β-lactam and sugar moieties. Nevertheless, the procedure was found to be more general, being equally applicable to the saturated and unsaturated and the α as well as β anomers of the propargylic sugars employed. Various β-lactam glycoconjugates synthesized under the optimized reaction conditions are shown in Table 2.

Table 2. **Generalization of the synthesis of β-lactam glycoconjugates 4**[a]

Entry	Azide 2	Alkyne 3	Product 4	Time (h)	Yield (%)[b]
1.	 **2a**	 **3a**	 **4aa**	2	78
2.	**2a**	 **3b**	 **4ab**	6	80
3.	**2a**	 **3c**	 **4ac**	2.5	82
4.	**2a**	 **3d**	 **4ad**	1.5	65
5.	**2a**	 **3e**	 **4ae**	0.5	75

6.	 **2b**	 **3a**	 **4ba**	3.5	86
7.	**2b**	 **3b**	 **4bb**	7	79
8.	**2b**	 **3c**	 **4bc**	4	92
9.	**2b**	 **3d**	 **4bd**	1	89
10.	**2b**	 **3e**	 **4be**	3.5	76

[a]All the reactions were performed with 2 mmol each of the azido-β-lactam **2** and the propargyl glycoside **3** using 50 mol% each of CuCl and PMDETA in DCE at 25-30 ˚C under a nitrogen atmosphere. [b]Isolated yield of **4** after purification by column chromatography.

The structures of all the newly synthesized β-lactam glycoconjugates **4ab-4be** were supported by IR, ^{1}H, and ^{13}C NMR spectroscopy and HRMS data. COSY NMR spectra were used for detailed assignment of all the protons in the β-lactam glycoconjugates. The scope of the synthesis of β-lactam glycoconjugates can be further expanded by installation of alkyne functionality on to

other positions of carbohydrate ring or on to different carbohydrate partners and or using other substituted azido β-lactams.

2.2. Biological studies

2.2.1. Evaluation of antibacterial activity

In order to study the preliminary biological activity of β-lactam glycoconjugates synthesized in the present work, a representative β-lactam glycoconjugate **4aa** was screened for antibacterial activity. Surprisingly, the β-lactam glycoconjugate **4aa** did not display any antibacterial activity against both Gram-positive (*Staphylococcus aureus and Bacillus subtilis*) and Gram-negative (*Escherichia coli and Pseudomonas aeruginosa*) bacterial strains even at much higher concentrations. The similar studies for antibacterial activity when extended for other derivatives of β-lactam glycoconjugates showed negative results. This observation revealed that the β-lactam glycoconjugates synthesized in the present work do not exhibit any antibacterial activity.

2.2.2. Evaluation of biocompatibility

Thereafter, a representative β-lactam glycoconjugate **4aa** was examined for in vitro hemolytic activity on human erythrocytes via hemolytic assay test. Human erythrocytes were treated with various concentrations of **4aa** and the cell viability was then determined by hemolytic assay and was expressed as mean of three separate experiments. This study showed that the β-lactam glycoconjugate **4aa** is biocompatible.

2.2.3. Evaluation of cytotoxicity

Further, the tumor cell growth inhibition activity of a representative β-lactam glycoconjugate **4aa** against L929 cancer cell line was studied in vitro via XTT assay. L929 cancer cells were exposed to different concentrations of **4aa** and the % cell viability was

observed by XTT assay and expressed as mean of three experiments. The study revealed the cytotoxic activity of β-lactam glycoconjugate **4aa** against L929 cancer cell lines.

However, the study in the present publication is mainly focused on the development of a facile chemical procedure and its utility that would enable the development of a relatively new small family of heteroatom rich β-lactam glycoconjugates for the potential bioanalytical applications. The biological competence of the synthesized β-lactam glycoconjugates was illustrated through antibacterial studies on all the compounds and hemolytic assay and XTT assay studies on a representative β-lactam glycoconjugate. The detailed biological studies of these β-lactam glycoconjugates are clearly outside the scope of the present publication.

3. Conclusion

In conclusion, we have developed a convenient route to synthesize a small ensemble of chlorinated and heteroatom rich β-lactam glycoconjugates using the well proven chemistry involved (Staudinger reaction, Cu(I)-catalyzed ATRC, glycosylation, Ferrier reaction and CuAAC). The appropriate optimization of the reaction procedure for CuAAC revealed that CuCl/PMDETA was the best catalyst to obtain maximum yield of the β-lactam glycoconjugates. The present method is mild, economical, wide in scope, and regioselective. The starting materials used are inexpensive and easily accessible. β-Lactam glycoconjugates synthesized in the present work represent a rare and interesting assembly of four different biologically active structural units, the tetrahydrofuran ring, the β-lactam ring, the triazole ring and a sugar moiety in a single molecular framework. The preliminary biological studies showed that the β-lactam glycoconjugates are non-antibacterial. The hemolytic assay and XTT assay studies on a representative β-lactam glycoconjugate revealed its good biocompatibility and cytotoxicity against L929 cancer cell lines. These results offer good opportunity to medicinal chemists for

their further study and biological evaluation. Thus, the versatility and potential of the synthesis has been realized in the present publication, providing an expedient access to an interesting and relatively new family of biologically relevant β-lactam glycoconjugates.

4. Experimental Section

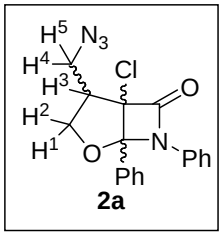
4.1. General Remarks. The entire data analysis of all the compounds synthesized in the present work was done in Chemistry Instrumentation Lab, IIT Delhi, New Delhi. Biological studies were done in Biomedical engineering Lab, IIT Delhi, New Delhi. IR spectra were recorded on an FT-IR spectrometer by taking solid samples as KBr pellets and liquids as thin films on KBr disks. NMR spectra were recorded on a 300 MHz FT NMR spectrometer in $CDCl_3$ with TMS as internal standard. Multiplicities are indicated by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet doublet). DEPT spectra were routinely recorded to identify different types of carbons. COSY NMR spectrum was also recorded to help the assignments of different protons. Mass spectra were recorded on a high-resolution mass spectrometer (ESI-TOF) in positive-ion mode. Melting points were determined on an electrically heated apparatus by taking the samples in a glass capillary sealed at one end and are uncorrected. The progress of the reaction was monitored by TLC using a glass plate coated with a TLC grade silica gel. Iodine was used for visualizing the spots. For column chromatography, silica gel (60-120 mesh) or neutral alumina (60-325 mesh) was used as the stationary phase, and *n*-hexane-ethyl acetate mixtures were used as the mobile phase. Solvents were evaporated on a rotary evaporator under reduced pressure using an aspirator. Dichloroacetyl chloride was commercially available and used as received. Et_3N was dried over KOH pellets overnight and distilled over CaH_2. CH_2Cl_2 and DCE were dried by distilling over anhydrous P_2O_5. Tetrahydrofuran (THF) and benzene were dried by distillation after a persistent

blue color was observed on treating them with enough amount of sodium in the presence of benzophenone.

4.2. Preparation of azidotetrahydrofurano fused bicyclic β-lactams 2a and 2b

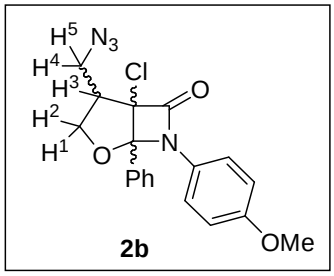
These were prepared according to a procedure previously used in our laboratory.[11] To a solution of the β-lactam **1a** or **1b** (0.004 mol) in DMF (25 mL) was added sodium azide (1.30 g, 0.020 mol) and the solution was stirred at 65 ˚C for 12 h. The solution was diluted with water (30 mL) and extracted with ethyl acetate (2×50 mL). The combined organic extract was washed with brine (2×30 mL), dried (Na_2SO_4) and evaporated under reduced pressure. The crude yellowish solid product was purified by flash column chromatography using a short band of silica gel as the stationary phase and a mixture of *n*-hexane-ethyl acetate (9:1 v/v) as the solvent for elution. The azido β-lactams thus obtained were further purified by recrystallization from *n*-hexane-diethyl ether to provide the crystalline products **2a** and **2b** in 69 and 65% yields, respectively.

*4.2.1. 3-Chloro-4-azidomethyl-1-(4-methoxyphenyl)-6-phenyltetrahydrofuro[3,2-c]-azetidin-2-one (**2a**).* Colorless needles, 0.979 g, 69%; mp 107-108 ˚C (*n*-hexane–diethyl ether); R_f (5% *n*-hexane/EtOAc, 5:1 v/v) 0.54; IR (KBr) ν_{max} 3066(m), 2925(w), 2103(s), 1768(s), 1595(w), 1501(m), 1451(w), 1383(m), 1290(m), 1241(w), 1186(w), 1133(m), 1057(m), 972(w), 897(w), 748(m), 689(m), 622(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.52-7.44 (m, 7H, aromatic), 7.31-7.26 (m, 2H, aromatic), 7.14 (t, J = 7.2 Hz, 1H, aromatic), 4.63 (t, J = 9.6 Hz, 1H, H^1), 3.98-3.84 (m, 2H, H^4, H^2), 3.60 (t, J = 10.2 Hz, 1H, H^5), 2.86-2.75 (m, 1H, H^3) ppm; δ_C (75.5 MHz, $CDCl_3$) 159.7, 134.8, 131.7, 129.9, 129.2, 128.7, 127.2, 125.4, 118.5, 101.2, 81.1, 69.6, 48.9, 48.8 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 377.0769. $C_{18}H_{15}ClN_4O_2Na$ requires 377.0776.



2a

*4.2.2. 3-Chloro-4-azidomethyl-1-(4-methoxyphenyl)-6-phenyltetrahydrofuro[3,2-c]-azetidin-2-one (**2b**).*[11] Colorless cubes, 1.0 g, 65%; mp 90-92 ˚C (*n*-hexane–diethyl ether) (Lit.[11] mp. 90-92 ˚C); IR (KBr) ν_{max} 3067(w), 3064(w), 2923(w), 2102(s), 1764(s), 1593(w), 1495(m), 1442(w), 1381(m), 1292(m), 1243(w), 1184(w), 1123(m), 1056(m), 974(w), 898(w), 746(m), 690(m), 647(w) cm^{-}1; δ_H (300 MHz, $CDCl_3$) 7.51-7.49 (m, 3H, aromatic), 7.42-7.36 (m, 4H, aromatic), 6.79 (dd, *J* = 7.2, 2.1 Hz, 2H, aromatic), 4.61 (dd, *J* = 9.9, 7.5 Hz, 1H, H^1), 3.93 (dd, *J* = 12.9, 4.8 Hz, 1H, H^4), 3.84 (t, *J* = 10.2 Hz, 1H, H^2), 3.73 (s, 3H, OMe), 3.60 (dd, *J* = 12.6, 9.9 Hz, 1H, H^5), 2.84-2.74 (m, 1H, H^3) ppm; δ_C (75.5 MHz, $CDCl_3$) 159.1, 157.1, 131.7, 129.8, 128.6, 128.0, 127.2, 119.9, 114.4, 101.0, 81.1, 69.5, 55.3, 48.9, 48.7 ppm.



2b

4.3. Preparation of propargyl glycosides 3

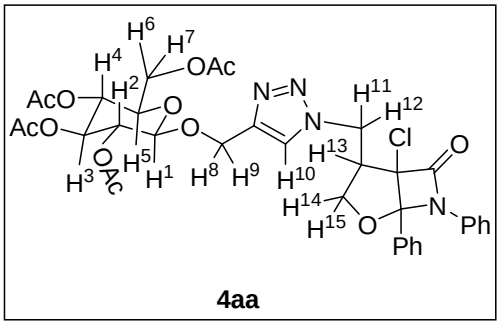
All the propargyl glycosides used are known in the literature and were prepared according to the reported procedures.[15a-d]

4.4. Typical procedure for preparation of β-lactam glycoconjugates 4

A two-neck round bottom flask fitted with a rubber septum was connected to a Schlenk tube through a condenser and was evacuated and filled with dry nitrogen. The flask was charged with the azido-β-lactam **2** (0.002 mol), propargyl glycoside **3** (0.002 mol), CuCl (0.01 g, 0.001 mol, 50 mol%) and degassed dichloroethane (20 mL) under a slow stream of the nitrogen gas. PMDETA (0.173 g, 0.208 mL, 0.001 mol, 50 mol%) was then injected into the mixture and the reaction mixture was stirred at room temperature (25-30 ˚C). The progress of the reaction was periodically monitored by TLC. After completion of the reaction in 0.5-7 h, as shown in Table 2, the solvent was evaporated. The residual material was taken up in ethyl acetate (30 mL), washed with brine (2×20 mL), dried (Na_2SO_4), filtered and evaporated. Purification of the crude

brownish solid products, thus obtained, by column chromatography using silica gel as the stationary phase and a mixture of *n*-hexane-ethyl acetate (1:1 v/v) as the eluting solvent afforded the pure β-lactam glycoconjugates **4** in 65-92% yields as a mixture of two diastereomers in 1:1 ratio.

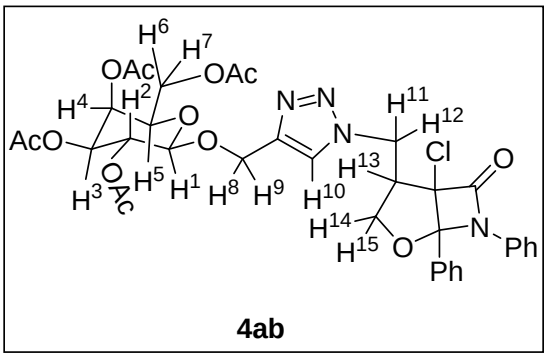
*4.4.1. β-Lactam glycoconjugate (**4aa**).* White solid, 1.156 g, 78%; mp 88-90 ˚C; R_f (50% EtOAc/*n*-hexane) 0.44; IR (KBr) ν_{max} 2953(w), 2361(w), 1757(s), 1634(w), 1498(w), 1377(m), 1229(w), 1045(m), 759(w), 694(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) [7.80 (s) (one diastereomer) + 7.78 (s) (other diastereomer), 1H, H^{10}], 7.48-7.43 (m, 7H, aromatic), 7.32-7.27 (m, 2H, aromatic), 7.15 (t, *J* = 7.5 Hz, 1H, aromatic), 5.20 (t, *J* = 9.3 Hz, 1H, H^3), 5.10 (t, *J* = 9.3 Hz, 1H, H^4), 5.02 (t, *J* = 9.0 Hz, 1H, H^2), 4.94 (d, *J* = 12.6 Hz, 1H, H^8), 4.84 (d, *J* = 13.2 Hz, 1H, H^9), 4.79-4.68 (m, 3H, H^1, H^{11}, H^{12}), 4.64-4.58 (m, 1H, H^{14}), 4.31-4.26 (m, 1H, H^6), 4.19-4.10 (m, 1H, H^7), 4.05-3.97 (m, 1H, H^{15}), 3.75 (d, *J* = 7.8 Hz, 1H, H^5), 3.29-3.18 (m, 1H, H^{13}), [2.09 (s) (one diastereomer) + 2.08 (s) (other diastereomer), 3H, COC*H*$_3$], 2.02 (s, 3H, COC*H*$_3$), 2.00 (s, 6H, COC*H*$_3$) ppm; δ_C (75.5 MHz, $CDCl_3$) 170.6, 170.1, 169.3 159.6, 144.0, 134.5, 131.2, 130.0, 129.2, 128.6, 127.1, 125.6, 124.2, 118.5, [101.31 (one diastereomer) + 101.26 (other diastereomer)], 99.6, 80.7, 72.7, 71.8, 71.1, 69.2, 68.2, 62.6, 61.6, 49.1, 47.6, 20.7, 20.6, 20.5 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 763.1955. $C_{35}H_{37}ClN_4O_{12}Na$ requires 763.1989.



4aa

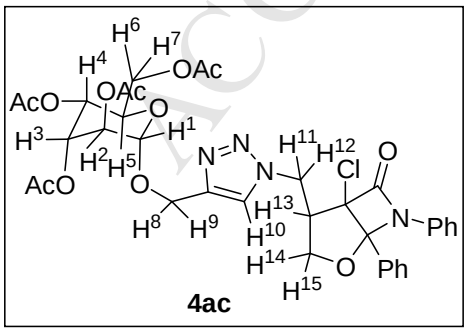
*4.4.2. β-Lactam glycoconjugate (**4ab**).* White solid, 1.185 g, 80%; mp 89-91 ˚C; R_f (50% EtOAc/*n*-hexane) 0.44; IR (KBr) ν_{max} 3145(w), 3069(w), 2924(w), 2852(w), 2360(w), 1754(s), 1637(w), 1513(m), 1451(w), 1372(m), 1297(w), 1248(s), 1228(s), 1126(w), 1177(w), 1135(w),

1048(m), 833(w), 739(w), 697(w), 671(w), 600(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) [7.78 (s) (one diastereomer) + 7.74 (s) (other diastereomer), 1H, H^{10}], 7.51-7.42 (m, 7H, aromatic), 7.33-7.30 (m, 2H, aromatic), 7.16 (t, J = 7.2 Hz, 1H, aromatic), [5.41 (s) (one diastereomer) + 5.40 (s) (other diastereomer), 1H, H^4], 5.25 (dd, J = 9.9, 8.1 Hz, 1H, H^2), 5.03-4.96 (m, 2H, H^3, H^1), 4.85-4.59 (m, 5H, H^8, H^9, H^{11}, H^{12}, H^{14}), 4.20-4.15 (m, 2H, H^6, H^7), 4.04-3.93 (m, 2H, H^{15}, H^5), 3.29-3.17 (m, 1H, H^{13}), 2.15 (s, 3H, COC*H*$_3$), 2.07 (s, 3H, COC*H*$_3$), [2.02 (s) (one diastereomer) + 2.01 (s) (other diastereomer), 3H, COC*H*$_3$], 1.98 (s, 3H, COC*H*$_3$) ppm; δ_C (75.5 MHz, $CDCl_3$) 170.4, 170.2, 170.0, [169.50 (one diastereomer) + 169.46 (other diastereomer)], 159.7, [144.2 (one diastereomer) + 144.1 (other diastereomer)], 134.5, 131.3, 130.1, 129.3, 128.7, 127.2, 125.7, [124.2 (one diastereomer) + 124.1 (other diastereomer)], 118.5, [101.4 (one diastereomer) + 101.3 (other diastereomer)], [100.13 (one diastereomer) + 100.06 (other diastereomer)], 80.8, 70.8, 69.3, 68.7, 67.0, [62.52 (one diastereomer) + 62.46 (other diastereomer)], [61.27 (one diastereomer) + 61.25 (other diastereomer)], 49.2, 47.7, 20.74, 20.66, 20.62, 20.5 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 763.1982. $C_{35}H_{37}ClN_4O_{12}Na$ requires 763.1989.



4ab

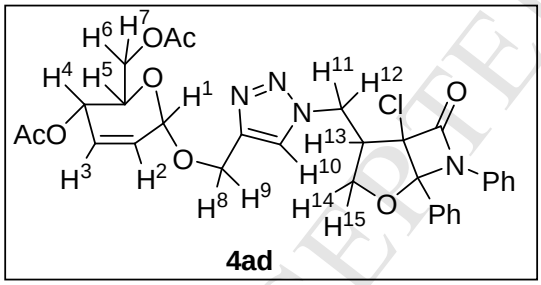
*4.4.3. β-Lactam glycoconjugate (**4ac**).* White solid, 1.215 g, 82%; mp 86-88 ˚C; R_f (50% EtOAc/*n*-hexane) 0.45; IR (KBr) ν_{max} 3144(w), 2931(w), 2365(w), 1749(s), 1647(w), 1598(w), 1497(m), 1447(w), 1375(s), 1225(s), 1135(m), 1052(s), 752(m), 694(w), 605(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$)



4ac

[7.82 (s) (one diastereomer) + 7.81 (s) (other diastereomer), 1H, H^{10}], 7.55-7.37 (m, 7H, aromatic), 7.32-7.28 (m, 2H, aromatic), 7.16 (t, *J* = 7.2 Hz, 1H, aromatic), 5.32-5.24 (m, 3H, H^2, H^3, H^4), 4.99 (s, 1H, H^1), 4.96-4.81 (m, 2H, H^8, H^9), 4.79-4.60 (m, 3H, H^{11}, H^{12}, H^{14}), 4.34-4.27 (m, 1H, H^6), 4.15-4.04 (m, 3H, H^7, H^5, H^{15}), 3.31-3.18 (m, 1H, H^{13}), 2.14 (s, 3H, COC*H*$_3$), 2.12 (s, 3H, COC*H*$_3$), [2.03 (s) (one diastereomer) + 2.02 (s) (other diastereomer), 3H, COC*H*$_3$], 1.98 (s, 3H, COC*H*$_3$) ppm; δ_C (75.5 MHz, $CDCl_3$) 170.6, [169.94 (one diastereomer) + 169.91 (other diastereomer)], 169.8, 169.6, 159.7, 143.5, 134.5, 131.3, 130.0, 129.2, 128.6, 127.2, 125.6, 124.2, 118.5, 101.3, [96.8 (one diastereomer) + 96.7 (other diastereomer)], 80.8, 69.4, 68.9, 68.7, 66.0, [62.4 (one diastereomer) + 62.3 (other diastereomer)], [60.8 (one diastereomer) + 60.7 (other diastereomer)], [49.21 (one diastereomer) + 49.18 (other diastereomer)], 47.7, 20.8, 20.7, 20.6 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 763.1997. $C_{35}H_{37}ClN_4O_{12}Na$ requires 763.1989.

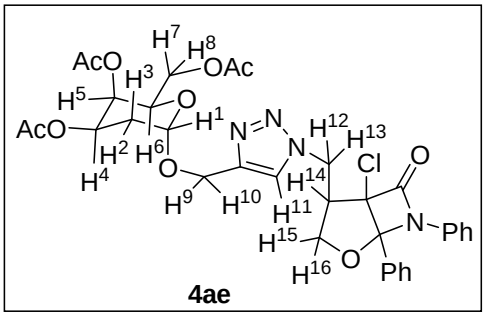
*4.4.4. β-Lactam glycoconjugate (**4ad**).* White solid, 0.810 g, 65%; mp 65-67 ˚C; R_f (50% EtOAc/*n*-hexane) 0.45; IR (KBr) ν_{max} 3144(w), 3065(w), 2924(w), 2360(w), 1768(s), 1743(s), 1636(w), 1598(w), 1498(m), 1451(w), 1376(s), 1236(s), 1103(w), 1038(s), 758(w), 694(w), 608(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.80 (s, 1H, H^{10}), 7.52-7.40 (m, 7H, aromatic), 7.33-7.27 (m, 2H, aromatic), 7.16 (t, *J* = 7.2 Hz, 1H, aromatic), 5.91 (d, *J* = 10.2 Hz, 1H, H^2), 5.83 (d, *J* = 10.2 Hz, 1H, H^3), 5.34 (d, *J* = 9.6 Hz, 1H, H^4), 5.18 (s, 1H, H^1), 4.93 (d, *J* = 12.3 Hz, 1H, H^8), 4.83-4.60 (m, 4H, H^9, H^{11}, H^{12}, H^{14}), 4.30-4.15 (m, 3H, H^6, H^7, H^5), 4.01 (t, *J* = 10.8 Hz, 1H, H^{15}), 3.28-3.17 (m, 1H, H^{13}), 2.11 (s, 3H, COC*H*$_3$), 2.08 (s, 3H, COC*H*$_3$) ppm; δ_C (75.5 MHz, $CDCl_3$) 170.7, 170.1, 159.6, [144.5 (one diastereomer) + 144.4 (other diastereomer)], 134.5, 131.2,



4ad

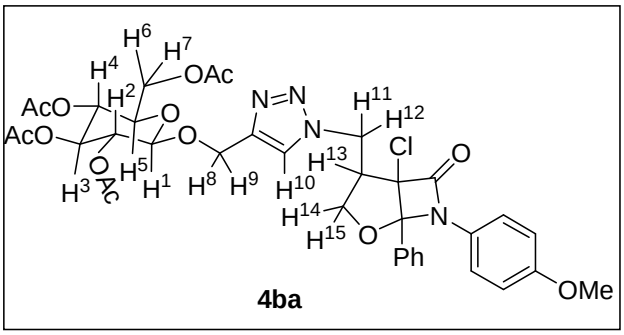
130.0, 129.4, 129.2, 128.6, 127.3, 127.1, 125.6, 124.0, 118.4, 101.3, [93.61 (one diastereomer) + 93.56 (other diastereomer)], 80.7, 69.3, 67.0, 65.2, 62.8, [61.24 (one diastereomer) + 61.20 (other diastereomer)], 49.1, 47.5, 20.8, 20.7 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 645.1696. $C_{31}H_{31}ClN_4O_8Na$ requires 645.1723.

*4.4.5. β-Lactam glycoconjugate (**4ae**).* White solid, 1.024 g, 75%; mp 73-75 ˚C; R_f (50% EtOAc/*n*-hexane) 0.46; IR (KBr): ν_{max} 3144(w), 2928(w), 1749(s), 1634(w), 1598(w), 1497(w), 1449(w), 1375(m), 1236(s), 1157(w), 1123(w), 1049(m), 752(w), 690(w), 613(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) [7.79 (s) (one diastereomer) + 7.77 (s) (other diastereomer), 1H, H^{11}], 7.52-7.40 (m, 7H, aromatic), 7.33-7.27 (m, 2H, aromatic), 7.17 (t, *J* = 7.2 Hz, 1H, aromatic), 5.34-5.26 (m, 2H, H^4, H^5), 5.17 (s, 1H, H^1), 4.85-4.61 (m, 5H, H^9, H^{10}, H^{12}, H^{13}, H^{15}), 4.22 (t, *J* = 6.0 Hz, 1H, H^6), 4.12 (d, *J* = 6.3 Hz, 2H, H^7, H^8), 4.00 (t, *J* = 12.0 Hz, 1H, H^{16}), 3.31-3.21 (m, 1H, H^{14}), 2.15 (s, 3H, COC*H*$_3$), 2.15-2.07 (m, 1H, H^2, overlapped between two singlets at 2.15 and 2.07), 2.07 (s, 3H, COC*H*$_3$), 1.98 (s, 3H, COC*H*$_3$), 1.91 (dd, *J* = 10.2, 5.1 Hz, 1H, H^3) ppm; δ_C NMR (75.5 MHz, $CDCl_3$) 170.6, 170.3, 170.0, 159.8, 144.3, 134.6, 131.3, 130.1, 129.3, 128.7, 127.2, 125.6, [124.0 (one diastereomer) + 123.9 (other diastereomer)], 118.5, 101.4, [96.9 (one diastereomer) + 96.8 (other diastereomer)], 80.9, 69.4, 66.9, 66.6, 66.0, 62.3, 60.5, 49.3, 47.7, 29.7, 20.84, 20.77, 20.73 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 705.1944. $C_{33}H_{35}ClN_4O_{10}Na$ requires 705.1934.



4ae

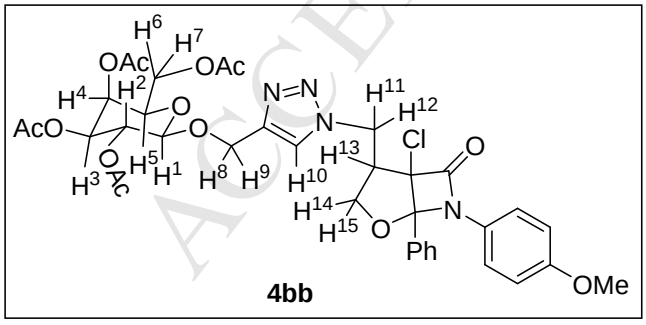
*4.4.6. β-Lactam glycoconjugate (**4ba**).* White solid, 1.326 g, 86%; mp 76-78 ˚C; R_f (50% EtOAc/*n*-hexane) 0.44; IR (KBr) ν_{max} 2934(w), 2340(w), 1757(s), 1622(w), 1513(w), 1450(w), 1377(w), 1235(s), 1129(w), 1047(m), 831(w), 692(w), 605(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) δ



4ba

[7.77 (s) (one diastereomer) + 7.74 (s) (other diastereomer), 1H, H^{10}], 7.49-7.37 (m, 7H, aromatic), 6.81 (d, J = 9.0 Hz, 2H, aromatic), 5.19 (t, J = 9.0 Hz, 1H, H^3), 5.09 (t, J = 9.3 Hz, 1H, H^4), 5.01 (t, J = 8.1 Hz, 1H, H^2), 4.94 (d, J = 13.2 Hz, 1H, H^8), 4.83 (d, J = 12.9 Hz, 1H, H^9), 4.81-4.65 (m, 3H, H^{11}, H^1, H^{12}), 4.62-4.56 (m, 1H, H^{14}), 4.30-4.24 (m, 1H, H^6), 4.18-4.13 (m, 1H, H^7), 3.98 (t, J = 10.2 Hz, 1H, H^{15}), 3.76 (s, 4H, multiplet for 1H (H^5), overlapped by singlet for 3H, OMe), 3.26-3.14 (m, 1H, H^{13}), 2.08 (s, 3H, COCH_3), 2.02 (s, 3H, COCH_3), 2.00 (s, 3H, COCH_3), 1.99 (s, 3H, COCH_3) ppm; δ_C (75.5 MHz, $CDCl_3$) 170.6, 170.1, 169.33, 169.29, 159.2, 157.2, 144.1, 131.3, 130.0, 128.6, 127.8, 127.2, 124.2, 120.0, 114.5, 101.3, 99.7, 80.8, 72.7, 71.9, 71.1, 69.2, 68.3, 62.6, 61.7, 55.4, 49.2, 47.7, 20.7, 20.6, 20.5 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 793.2101. $C_{36}H_{39}ClN_4O_{13}Na$ requires 793.2094.

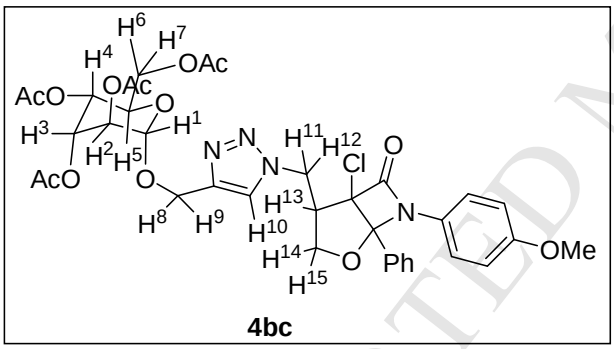
*4.4.7. β-Lactam glycoconjugate (**4bb**).* White solid, 1.218 g, 79%; mp 89-91 ˚C; R_f (50% EtOAc/*n*-hexane) 0.44; IR (KBr) ν_{max} 3145(w), 2940(m), 1768(s), 1743(s), 1619(w), 1513(m), 1450(m), 1376(s), 1221(s), 1133(m), 1047(s), 910(w), 830(m), 742(w), 701(w), 598(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) [7.79 (s) (one diastereomer) + 7.76 (s) (other diastereomer), 1H, H^{10}], 7.51-7.38 (m, 7H, aromatic), 6.82 (d, J = 9.0 Hz, 2H, aromatic), 5.40 (s, 1H, H^4), 5.27-5.21 (m, 1H, H^2), 5.01 (dd, J = 9.6, 3.3 Hz, 1H, H^3), 4.96 (s, 1H, H^1), 4.85-4.58 (m, 5H, H^8, H^9, H^{11}, H^{12}, H^{14}), 4.19-4.09 (m, 2H, H^6, H^7), 4.03-3.95 (m, 2H, H^{15}, H^5), 3.76 (s, 3H, OMe), 3.24-3.18 (m,



4bb

1H, H^{13}), 2.15 (s, 3H, COC*H*$_3$), [2.02 (s) (one diastereomer) + 2.01 (s) (other diastereomer), 3H, COC*H*$_3$], [2.06 (s) (one diastereomer) + 2.05 (s) (other diastereomer), 3H, COC*H*$_3$], 1.98 (s, 3H, COC*H*$_3$) ppm; δ_C (75.5 MHz, $CDCl_3$) 170.3, 170.2, 170.0, [169.48 (one diastereomer) + 169.45 (other diastereomer)], 159.1, 157.2, [144.09 (one diastereomer) + 144.03 (other diastereomer)], 131.3, 130.0, 128.6, 127.7, 127.2, [124.2 (one diastereomer) + 124.1 (other diastereomer)], 120.0, 114.5, [101.3 (one diastereomer) + 101.2 (other diastereomer)], [100.1 (one diastereomer) + 100.0 (other diastereomer)], 80.8, 70.8, 69.2, 68.7, 67.0, [62.5 (one diastereomer) + 62.4 (other diastereomer)], 61.2, 55.3, 49.2, 47.7, 20.7, 20.62, 20.57, 20.5 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 793.2110. $C_{36}H_{39}ClN_4O_{13}Na$ requires 793.2094.

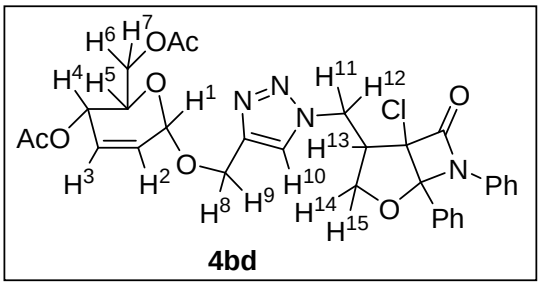
*4.4.8. β-Lactam glycoconjugate (**4bc**).* White solid, 1.419 g, 92%; mp 90-92 ˚C; R_f (50% EtOAc/*n*-hexane) 0.45; IR (KBr) ν_{max} 3146(w), 2930(w), 1752(s), 1634(w), 1498(m), 1451(w), 1373(m), 1301(w), 1227(s), 1126(w), 1057(m), 823(w), 759(w), 694(w), 606(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) [7.814 (s) (one diastereomer) + 7.805 (s) (other diastereomer), 1H, H^{10}], 7.50-7.38 (m, 7H, aromatic), 6.82 (d, *J* = 9.0 Hz, 2H, aromatic), 5.31-5.24 (m, 3H, H^2, H^3, H^4), 4.95 (s, 1H, H^1), 4.87-4.59 (m, 5H, H^8, H^9, H^{11}, H^{12}, H^{14}), 4.33-4.27 (m, 1H, H^6), 4.14-3.98 (m, 3H, H^7, H^5, H^{15}), 3.76 (s, 3H, OMe), 3.28-3.16 (m, 1H, H^{13}), 2.14 (s, 3H, COC*H*$_3$), 2.12 (s, 3H, COC*H*$_3$), [2.02 (s) (one diastereomer) + 2.01 (s) (other diastereomer), 3H, COC*H*$_3$], 1.98 (s, 3H, COC*H*$_3$) ppm; δ_C (75.5 MHz, $CDCl_3$) 170.6, [169.90 (one diastereomer) + 169.87 (other diastereomer)], 169.8, 169.6, 159.2, 157.2, 143.5, 131.4, 129.9, 128.6, 127.8, 127.3, 124.2, 120.0, 114.5, 101.3, [96.84 (one diastereomer) + 96.77 (other diastereomer)], 80.8, 69.4, 69.0, 68.7, 66.1, 62.4,



4bc

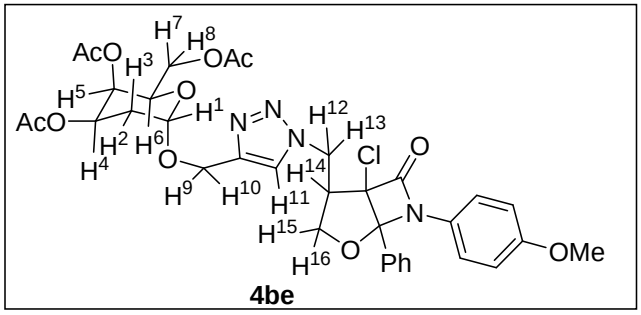
[60.81 (one diastereomer) + 60.78 (other diastereomer)], 55.4, 49.2, 47.8, 20.8, 20.7, 20.6 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 793.2112. $C_{36}H_{39}ClN_4O_{13}Na$ requires 793.2094.

*4.4.9. β-Lactam glycoconjugate (**4bd**).* White solid, 1.162 g, 89%; mp 76-78 ˚C; R_f (50% EtOAc/*n*-hexane) 0.45; IR (KBr) ν_{max} 3143(w), 3068(w), 2933(m), 1765(s), 1736(s), 1616(w), 1513(s), 1451(m), 1377(m), 1298(m), 1246(s), 1184(w), 1135(m), 1042(s), 873(w), 830(m), 740(m), 699(w), 592(w), 548(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.74 (s, 1H, H^{10}), 7.42-7.30 (m, 7H, aromatic), 6.74 (d, *J* = 9.0 Hz, 2H, aromatic), 5.83 (d, *J* = 10.2 Hz, 1H, H^2), 5.75 (d, *J* = 10.2 Hz, 1H, H^3), 5.26 (d, *J* = 9.3 Hz, 1H, H^4), 5.10 (s, 1H, H^1), 4.85 (d, *J* = 12.0 Hz, 1H, H^8), 4.75-4.63 (m, 3H, H^9, H^{11}, H^{12}), 4.58-4.51 (m, 1H, H^{14}), 4.22-4.07 (m, 3H, H^6, H^7, H^5), 3.92 (t, *J* = 10.5 Hz, 1H, H^{15}), 3.69 (s, 3H, OMe), 3.19-3.07 (m, 1H, H^{13}), [2.04 (s) (one diastereomer) + 2.03 (s) (other diastereomer), 3H, COC*H*$_3$], 2.00 (s, 3H, COC*H*$_3$) ppm; δ_C (75.5 MHz, $CDCl_3$) 170.7, 170.2, 159.2, 157.2, [144.6 (one diastereomer) + 144.5 (other diastereomer)], 131.3, 130.0, 129.5, 128.6, 127.7, 127.4, 127.2, 124.0, 120.0, 114.5, 101.3, [93.68 (one diastereomer) + 93.63 (other diastereomer)], 80.8, 69.3, 67.0, 65.2, 62.8, [61.32 (one diastereomer) + 61.28 (other diastereomer)], 55.3, 49.2, 47.6, 20.9, 20.8 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 675.1812. $C_{32}H_{33}ClN_4O_9Na$ requires 675.1828.



4bd

*4.4.10. β-Lactam glycoconjugate (**4be**).* White solid, 1.083 g, 76%; mp 83-85 ˚C; R_f (50% EtOAc/*n*-hexane) 0.46; IR (KBr) ν_{max} 3144(w), 3069(w), 2926(w), 2361(w), 1748(s), 1637(w), 1512(s), 1450(w), 1374(m), 1298(w), 1248(s), 1122(w), 1035(m), 833(w), 739(m), 697(w), 671(w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) [7.71 (s) (one diastereomer) + 7.70 (s) (other diastereomer), 1H, H^{10}], 7.43-7.29 (m, 7H, aromatic), 6.76-6.71 (m, 2H, aromatic), 5.25-5.17 (m, 2H, H^4, H^5),



4be

[5.08 (s) (one diastereomer) + 5.07 (s) (other diastereomer), 1H, H^1], 4.76-4.50 (m, 5H, H^9, H^{10}, H^{12}, H^{13}, H^{15}), 4.15-4.11 (m, 1H, H^6), 4.03 (d, J = 6.0 Hz, 2H, H^7, H^8), 3.97-3.90 (m, 1H, H^{16}), 3.68 (s, 3H, OMe), 3.17-3.11 (m, 1H, H^{14}), 2.06 (s, 3H, COCH_3), 2.06-2.00 (m, 1H, H^2, overlapped by singlet at 2.06), 1.98 (s, 3H, COCH_3), 1.89 (s, 3H, COCH_3), 1.84-1.78 (m, 1H, H^2) ppm; δ_C (75.5 MHz, $CDCl_3$) 170.5, 170.2, 170.0, 159.2, 157.2, 144.2, 131.4, 130.0, 128.6, 127.7, 127.2, [124.0 (one diastereomer) + 123.9 (other diastereomer)], 120.0, 114.5, [101.24 (one diastereomer) + 101.21 (other diastereomer)], [96.84 (one diastereomer) + 96.76 (other diastereomer)], 80.8, [69.4 (one diastereomer) + 69.3 (other diastereomer)], 66.8, 66.5, 66.0, 62.2, 60.4, 55.3, 49.2, 47.7, 29.9, 20.8, 20.70, 20.65 ppm; HRMS (ESI-TOF): m/z $[M + Na]^+$, found 735.2035. $C_{34}H_{37}ClN_4O_{11}Na$ requires 735.2040.

4.5. Antibacterial activity assay

The in vitro antibacterial activities of test compounds were determined by the well diffusion method. Muller Hinton Agar (MHA) medium was used for preparation of plates. The medium was poured to the sterile petriplates of 90 mm diameter. Fresh human pathogenic bacterial cultures of two Gram-positive bacteria, (*B. subtilis* and *S. aureus)* and two Gram-negative bacteria, (*E. coli* and *P. aeruginosa)* were spread on surface of the MHA plate. After incubation, the MHA plates were allowed for the pre-incubation for 10 min using a sterile cork borer, the well was cut from the agar in the plate. Freshly prepared solutions of β-lactam glycoconjugates **4** with different concentrations were poured into wells. The inoculated plates were initially

incubated for 15 min at room temperature and then at 37 ˚C for 24 h. Thereafter, the plates were examined for any zone of growth inhibition.

4.6. Hemolytic assay

Freshly prepared solutions of β-lactam glycoconjugate **4aa** with different concentrations were added to freshly prepared human RBC suspension in a 96-well culture plate. The resulting mixture was kept at 37℃ for 30 min under rotary agitation. Afterwards, the plate was centrifuged and the supernatant in each well was transferred to a new plate. Hemolysis was monitored by measuring the absorbance of the released hemoglobin at 414 nm.[17] All experiments were done in triplicate.

4.7. XTT assay

L929 cancer cells cultured into 96-well microculture plates were exposed to β-lactam glycoconjugate **4aa** at different concentrations. Absorbance was measured after 4 h of incubation at 37℃. XTT assay was quantitatively used to evaluate % cell proliferation.[18] Each experiment was performed three times.

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Supplementary data

Supplementary data related to this article comprised of copies of ^{1}H and ^{13}C NMR spectra of compounds **2a**, **2b** and **4aa**-**4be** and COSY NMR spectrum of **4aa** and data of hemolytic assay and XTT assay of **4aa**.

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