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The synthesis and biological activity of novel spiro-isoxazoline *C*-disaccharides based on 1,3-dipolar cycloaddition of *exo*-glycals and sugar nitrile oxides

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Abstract—A series of novel spiro-isoxazoline *C*-disaccharides were synthesized by the key step of 1,3-dipolar cycloaddition reactions of *exo*-glycals and sugar nitrile oxides, and followed by catalytic debenzylation in the presence of $Pd(OH)_2/C$. The cycloaddition reactions were carried out stereoselectively and afforded α-isomers exclusively except in the case of galactose. The biological activities of the novel disaccharides against the glycosidases (α-amylase, α-glucosidase, and β-glucosidase) and HIV and BVDV were evaluated.

Spiro-glycosides, such as spiro-nucleosides, spiro-orthoesters, and spiro-ketals are very important substructures found in many biologically active natural products.[1–3] For instance, the first isolated natural spironucleoside (+)-hydantocidin (**A**) exhibited non-toxically herbicidal and regulatory plant growth activities,[1] glucopyranosyl spirooxathiazole (**B**)[4] and spiro-(thio)hydantoins (**C**)[5] showed a good glycogen phosphorylase inhibition (see Fig. 1). These results indicated that the spiro-glycosides may often have potential bioactivities and have triggered great interest in synthesizing such spiro-furanoid and pyranoid derivatives and analogues for bioactive study.[6] However, the study on spiro-isoxazoline glycoside derivatives is very scarce.[7] Considering the strong biological activities of isoxazoline and spiro-isoxazoline derivatives including anticancer, antibiotic, or antiviral and anti-HIV activities,[8] we turned our interest to the synthesis of spiro-isoxazoline saccharides as the continuation of the synthesis of functionalized *C*-glycosides using *exo*-glycal as the precursor,[7e,9] and would like to report herein the synthesis of novel spiro-isoxazoline *C*-disaccharides by the key step of the stereoselective 1,3-dipolar cycloaddition of *exo*-glycals to sugar nitrile oxides and the studies on the glycosidase inhibitory and antiviral activities of the *C*-disaccharides.

exo-Glycals (**3**–**5**) were prepared by the methylenation[10] from their corresponding sugar lactones with Petasis's reagent.[11] The sugar nitrile oxides (**2a**–**c**) would be synthesized by the dehydrochlorination of oximinoyl halides (Huisgen procedure) generated in situ by the chlorination reaction[7b,c] of oximes (**1a**–**c**), which were prepared from the corresponding sugar aldehyde derivatives[12] as shown in Scheme 1. However, the pure nitrile oxides (**2**) were not isolated due to their easily dimerization[13–15] during separation, and they were directly used to the next step reaction of 1,3-dipolar cycloaddition without further purification. It should be mentioned that the other method for preparing the nitrile oxides were also examined with **1a**, for instance, by a direct oxidation of oxime with hypochlorite[14] and by oxime halogenation and then dehydrohalogenation using NBS[15]

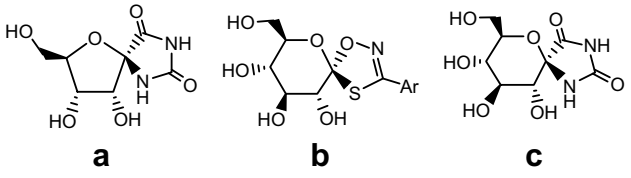

Figure 1.

Keywords: Spiro-isoxazoline; *C*-Disaccharide; 1,3-Dipolar cycloaddition; Nitrile oxide; Glycosidase inhibition.
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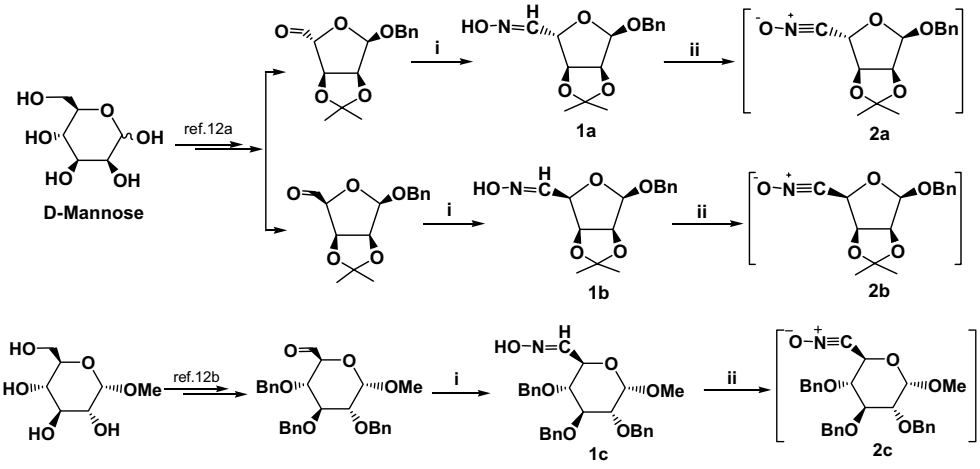
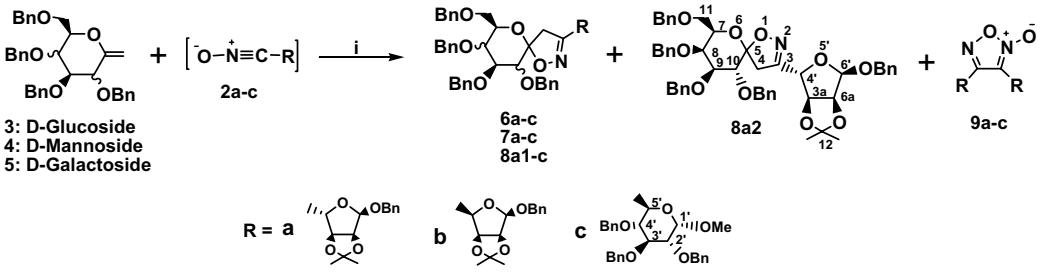


Scheme 1. Synthesis of sugar nitrile oxides (**2**). Reagents and conditions: (i) $HONH_2HCl$ (1.2 equiv), THF, Na_2CO_3, rt, 1 h; (ii) (a) NCS, DCE, reflux, 20 min; (b) Et_3N, rt.



Scheme 2. The 1,3-dipolar cycloaddition of *exo*-glycals and sugar nitrile oxides. Reagents and conditions: (i) DCE, reflux 24 h.

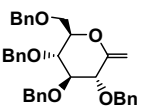
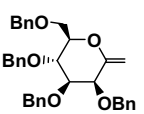
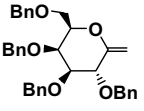
and chloramines-T[16] as the halogenation reagent, respectively.

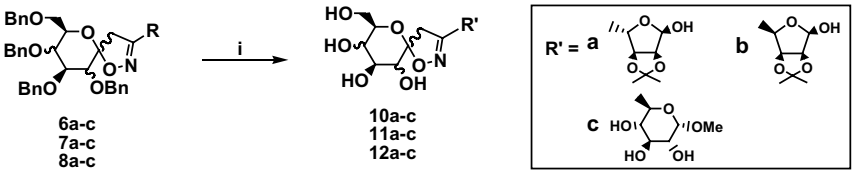
The 1,3-dipolar cycloaddition reactions were firstly carried out with *exo*-glycal (**3**) and the in situ generated nitrile oxide (**2a**).[17] Thus, a solution of the L-ribose oxime (**1a**) and NCS in 1,2-dichloroethane (DCE) was refluxed for 20 min, and after cooling to room temperature the solution of the benzylated *exo*-glycal **3** and Et_3N in DCE was added dropwise. The reaction mixture was refluxed for 24 h to afford spiro-isoxazoline disaccharide **6a** as a single diastereoisomer in good yield and a small amount of dimer (**9a**) of nitrile oxide (**2a**) as the by-product (Scheme 2). It should be mentioned that although the 1,3-dipolar cycloadditions of the *exo*-glycals with the nitrile oxide should be supposed to afford two diastereomers, that is, the α- and β-anomeric isomers, the 1,3-dipolar cycloaddition of **3** with **2a** underwent stereospecifically and gave anomeric isomer (**6a**) exclusively, which was consistent with the RajanBabu's report[7a] and our previous observation.[7e] Under the same conditions the cycloaddition of the mannose derivative **4** to nitrile oxide **2a** was carried out and afforded the corresponding cycloadduct **7a** and the nitrile oxide dimer (**9a**). However, the cycloaddition of *exo*-galactal (**5**) and **2a** provided an inseparable mixture of two anomeric isomers (diastereoisomers) **8a1** and **8a2** in the ratio of 3:2 which was determined by ^1H NMR spectra, and dimer (**9a**) of the nitrile oxide (**2a**) (Scheme 2).

Similarly, the 1,3-dipolar cycloadditions of *exo*-glycals (**3**–**5**) with the other sugar nitrile oxides **2b**, and **2c** were investigated and afforded the corresponding cycloadducts **6b**, **6c**, **7b**, **7c** and **8b**, **8c**, and the dimers (**9b** and **9c**) of the nitrile oxides **2b** and **2c**, respectively. The results are shown in Scheme 2 and Table 1.

Subsequently, the debenzylation of cycloadducts (**6**–**8**) was carried out by catalytic hydrogenation with

Table 1. The 1,3-dipolar cycloaddition of *exo*-glycals (**3**–**5**) with nitrile oxides (**2**) and the chemical shifts of the spiro carbons of adducts (**6**–**8**)

Entry	*exo*-Glycal	Nitrone	Products	Yields (%)	$\delta_{C\text{-spiro}}$ (ppm)
1		**2a**	**6a**	50	109.23
2		**2b**	**6b**	71	109.08
3		**2c**	**6c**	65	108.79
	3				
4		**2a**	**7a**	50	109.61
5		**2b**	**7b**	83	109.77
6		**2c**	**7c**	81	108.79
	4				
7		**2a**	**8a1**	52	109.82
			8a2		116.45
8		**2b**	**8b**	53	109.78
9		**2c**	**8c**	55	109.47
	5				



Scheme 3. Debenzylation of adducts (**6**–**8**) by catalytic hydrogenation. Reagents and conditions: (i) MeOH, $Pd(OH)_2/C$, H_2, 2 h.

$Pd(OH)_2/C$ in MeOH solution at room temperature and produced the corresponding debenzylated spiro-isoxazoline *C*-disaccharides (**10**–**12**), respectively (Scheme 3).[17] Although the mixture of cycloadducts **8a1** and **8a2** was difficult to separation, the corresponding debenzylated products **12a1** and **12a2** could be readily isolated by flash column chromatography.

The structures of cycloadducts (**6**–**8**) and the debenzylated products (**10**–**12**) were determined by the analyses of their spectral data of ^{1}H NMR, ^{13}C NMR, 2D-COSY and NOESY, and mass spectra. It has been reported that in the ^{13}C NMR spectra of *C*-glycosides[9a] and spiro-*C*,*O*-ketosides[9b] the signals of the anomeric carbon in the α-isomers appeared in higher field than those in the β-isomers. From this point of view, we have previously assigned the anomeric configurations of spiro-isoxazolindine *C*-glycosides by the comparison of the anomeric signals of the ^{13}C NMR spectra between each two anomeric isomers.[7e,9c] Accordingly, with the isolated two anomeric isomers of **12a1** and **12a2** in hand and considering the structural similarity of the cycloadducts, the configuration of cycloadducts **6**–**8** and their debenzylated products **10**–**12** could be confirmed to be α-form, and **8a2** and **12a2** to be β-isomers by comparison of the anomeric signals of their ^{13}C NMR spectra (see Tables 1 and 2). The stereochemistry of the cycloaddition was identical to the reports.[7a,e,f]

The glycosidase inhibitory activities of the compounds were examined on hydrolytic reactions of α-amylase, α-glucosidase, and β-glucosidase by comparison with acarbose, respectively.[17] As shown in Table 3, although the compounds gave low inhibitory effects on the glycosidases contrasted to the positive control, a certain inhibitory selectivity to the three glycosidases was observed, and the inhibitory activities of the compounds against the enzymes are in the order of α-amylase > β-glucosidase > α-glucosidase. The antiviral activities of the spiro-isoxazoline disaccharides on HIV-1 (replication in MT-4 cells) and BVDV (nose strain, replication in MDBK cells, a model system of HCV) were also evaluated.[17] It was found that the compounds had little inhibitory effects on both the viruses. Further synthesis and biological study of new *C*-disaccharides are under way in this lab.

Table 2. Debenzylation of cycloadducts (**6**–**8**) and the chemical shifts of the spiro carbons of products (**10**–**12**)

Entry	Adducts	Products	Yields (%)	$\delta_{C\text{-spiro}}$ (ppm)
1	**6a**	**10a**	63	109.56
2	**6b**	**10b**	54	108.64
3	**6c**	**10c**	72	109.54
4	**7a**	**11a**	60	109.78
5	**7b**	**11b**	53	109.48
6	**7c**	**11c**	85	109.75
7	**8a1** + **8a2**	**12a1**	45	109.92
		12a2	30	116.91
8	**8b**	**12b**	55	109.71
9	**8c**	**12c**	73	109.45

Table 3. Inhibitory activities of the compounds against glycosidases

Compounds	Inhibition % (in vitro at 2.6 mmol/mL)		
	α-Amylase	β-Glucosidase	α-Glucosidase
Glucoside			
10a	23.07	7.35	—[a]
10b	—[a]	18.20	—
10c	16.85	8.71	1.87
Mannoside			
11a	10.60	10.45	—
11b	17.51	8.07	1.43
11c	39.97	12.33	1.76
Galactoside			
12a1	24.95	5.58	—
12b	24.01	6.90	6.93
12c	26.56	12.28	—
Acarbose	54.57[b]		

[a] No inhibition.
[b] In the concentration of 22.46 μmol/mL.

In summary, we have synthesized a series of novel spiro-isoxazoline *C*-disaccharides via the key step of 1,3-dipolar cycloaddition of *exo*-glycals and carbohydrate nitrile oxides, which were in situ generated from the corresponding oximes. The cycloaddition was carried out stereospecifically and provided the α-anomeric isomer exclusively in the cases of D-glucose and D-mannose. Hydrogenation of the cycloadducts under the catalysis of $Pd(OH)_2/C$ gave the debenzylated products with the spiro-isoxazoline ring remaining. The spiro-isoxazoline *C*-disaccharides exhibited certain selective inhibitions against α-amylase, β-glucosidase, and α-glucosidase, but no anti-HIV and anti-BVDV activities, providing useful information on the structure design for the future study.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.09.007.

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17. The experimental procedure and the physical and spectral data of the new compounds (**6**–**12**) are compiled in Supplementary data.