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Synthesis of furanosyl α-C-glycosides derived from 4-chloro-4-deoxy-α-D-galactose and their cytotoxic activities

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Abstract—Condensation of a new unnatural sugar **1** with 1,3-dicarbonyl compounds in the presence of anhydrous zinc chloride gave the polyhydroxyalkyl-furans in excellent yields. Further modification afforded the corresponding furanosyl α-C-glycoside derivatives. The absolute configuration of 3-acetyl-2-methyl-5-(2′-chloro-D-galacto-tetritol-1-yl)-furan was confirmed by single-crystal X-ray analysis. The in vitro cytotoxic activities of these furanosyl C-glycosides were also investigated.

Due to their unique properties and applications, C-glycosides are becoming useful building blocks[1] for the total synthesis of various types of natural products such as palytoxin,[2] brevetoxin[3], and polyether antibiotics,[4] and used as a model in enzymatic and metabolic studies as well. A number of recent reviews have been devoted to synthetic methodology for C-glycosides.[5] In general, introduction of halogen atom may significantly modify the chemical, physical, and biological activities of the natural substances.[6,7] At the same time, structural modifications via substitution with nitrogen, sulfur, phosphate, and other groups[8] could endow compounds with new and attractive characteristics. Therefore, in recent years many efforts have been directed toward the introduction of azido and halogen atom into carbohydrate analogues.

In our previous paper, we reported the preparation of 1′,4′:3′,6′-dianhydro-4-chloro-4-deoxy-galacto-sucrose by using sucralose as the starting material[9] and further hydrolysis to afford 4-chloro-4-deoxy-α-D-galactose (**1**),[10] in which 4-hydroxyl group was substituted by a chlorine atom. Some new bio-based quinoxaline derivatives were synthesized by using this building block.[11] Thus, we decided to apply this unnatural sugar as starting material into preparation of desired new C-glycosides with potential biological activities. Several attempts have been made to prepare furanosyl C-glycosides from the free sugar, but in most cases β-linked derivatives are obtained in the literature.[12–15] Herein, we report the synthesis of new polyhydroxyalkyl furan derivatives and further cyclization to furnish corresponding α-C-glycosides. The mechanism of this reaction and the determination of configuration were detailedly expatiated as well in this article.

Aiming to prepare polyhydroxyalkyl furan scaffolds through the Knoevenagel condensation of the new unnatural sugar and 1,3-dicarbonyl compounds, 4-chloro-4-deoxy-α-D-galactose was treated with acetylacetone and ethyl acetoacetate, respectively, in acidic medium ($ZnCl_2$, MeOH). The reaction afforded high yields of corresponding polyhydroxyalkyl furan derivatives (**2a** and **2b**), which have a chlorine atom in the polyhydroxylic chain as confirmed by spectral analysis. The absolute configuration of the polyhydroxyalkyl furan derivatives was established by X-ray crystallographic analysis of a suitable crystal of **2a** (as shown in Fig. 1) after recrystallization from ethanol. It can be seen from the structure that C(6), C(7), and C(8) remained *R*, *S*, and *R* configurations, respectively, as those in the starting material.

Cyclization of the polyhydroxyalkyl furan derivatives **2a** and **2b** in the presence of $POCl_3$ in anhydrous acetonitrile at room temperature afforded furanosyl C-glycoside derivatives **3a** and **3b** (Scheme 1), whose structures were confirmed by spectral analysis. The correlations of H-4′a (δ 4.19) and H-4′b (δ 4.01) to C-1′ (δ 80.8)

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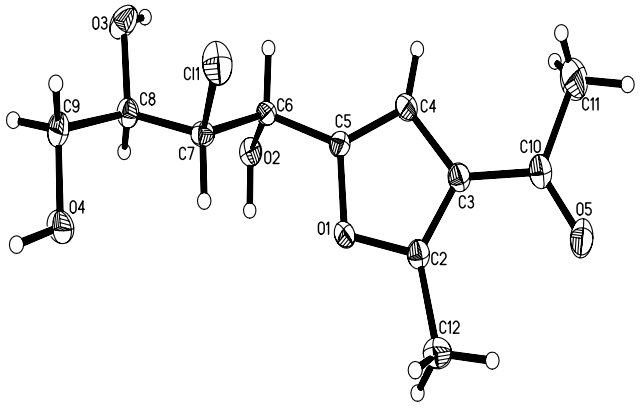
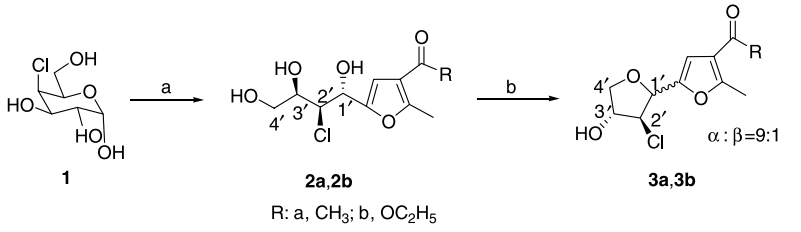


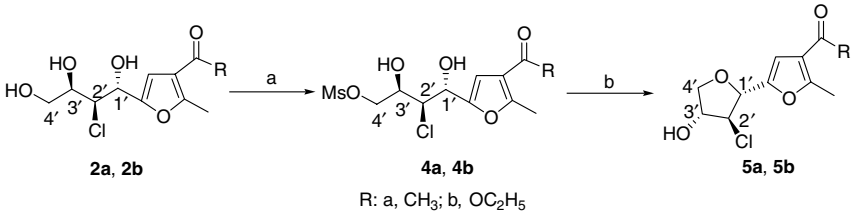
Figure 1. X-ray crystal structure for compound **2a**.

observed in the HMBC spectrum of **3a** indicated the formation of tetrahydrofuran ring. The HRMS spectrum revealed the molecular formula of **3a** to be $C_{11}H_{13}ClO_4$ from its $[M+Na]^+$ peak at *m/z* 267.0396 and the $[M+Na+2]^+$ peak at *m/z* 269.0375, and the intensity ratio of the two peaks was 3:1, corresponding to Cl-35 and Cl-37 isotopes, respectively. All of these indicated that **3a** is a dehydrated product of **2a**. It is worth pointing out that the product **3a** is a mixture of two stereoisomers in the ratio of 9:1 based on their ^1H NMR integrals. This fact indicates that the cyclodehydration predominantly follows the S_N1 mechanism. That is to say, the easily formed carbocation at C(1′) undergoes nucleophilic attack of the hydroxyl group on C(4′) in a stereoselective process to give the mixture of α-isomer (retention of the configuration of C-1′) and β-isomer (inversion of the configuration of C-1′). However, it is not easy to confirm the absolute configuration of the main isomer.

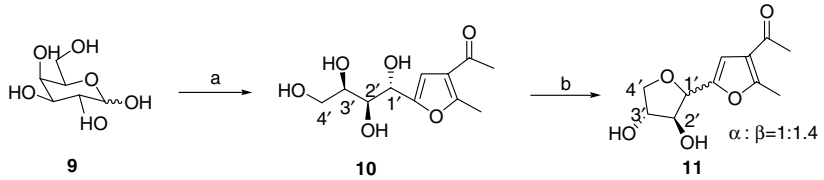
In order to further clarify the configurations of **3a**, we designed and conducted a stereospecific S_N2 substitution as follows. First, selective monomesylation of primary hydroxyl group at C-4′ of **2a** afforded a mesylate **4a**. Then treatment of **4a** with alkali allowed a ring closure of the polyhydroxyalkyl chain to form a sole furanosyl C-glycoside **5a**. This reaction, not involving the cleavage of C(1′)–O bond, should give a stereospecifically product with retention of the configuration of C-1′, that is, **5a** is an α-C-glycoside. Compound **5b** was stereospecifically synthesized by following above procedure (Scheme 2). Comparing the NMR spectrum of **3a** with that of **5a**, we concluded the main product of **3a** has α-configuration. Compound **3b** have a same anomeric ratio as **3a** due to its similar NMR spectrum. It should be noted that the anomeric ratio of **3a** was α:β = 9:1, which was higher than those of the normal S_N1 reactions, probably due to the participation of the neighboring chlorine atom. This proposition was confirmed by the fact that under the same conditions described above for preparation of **3a** and **3b**, the D-galactose (**9**) gave furanosyl C-glycoside (**11**) with a 1:1.4 ratio of α and β isomers (Scheme 3), in a lower stereoselectivity.



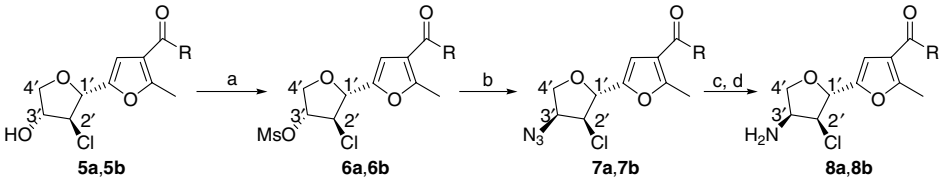
Scheme 1. Reagents and conditions: (a) 1,3-dicarbonyl compounds, $ZnCl_2$, CH_3OH, reflux, 86–91%; (b) $POCl_3$, CH_3CN, rt, 89–93%.



Scheme 2. Reagents and conditions: (a) MsCl, pyridine, rt, 92–94%; (b) Na_2CO_3, CH_3OH, reflux, 86–91%.



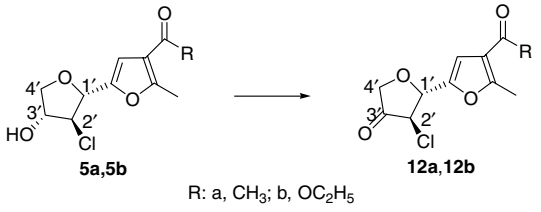
Scheme 3. Reagents and conditions: (a) acetylacetone, $ZnCl_2$, CH_3OH, reflux, 88%; (b) $POCl_3$, CH_3CN, rt, 91%.



Scheme 4. Reagents and conditions: (a) MsCl, pyridine, DMAP, rt, 88–92%; (b) 10 equiv NaN_3, DMF, 80 °C, 67–75%; (c) 1.5 equiv PPh_3, THF, rt, overnight; (d) H_2O, reflux, 3 h, 76–85%.

Glycosyl azides are frequently applied as carbohydrate intermediates, especially as precursors to glycosylamines and 1,2,3-triazole nucleosides.[16] Herein conventional mesylation of **5a** and **5b** gave compounds **6a** and **6b**, respectively (Scheme 4), and subsequent treatment with NaN_3 (10 equiv) in *N*,*N*-dimethylformamide at 80 °C overnight afforded compounds **7a** and **7b**, respectively. Both of the compounds showed IR absorption at ~2100 cm^{-1} corresponding to the azido group. The HRMS spectra showed the molecular formula of **7a** and **7b** to be $C_{11}H_{12}ClN_3O_3$ and $C_{12}H_{14}ClN_3O_4$, respectively, from the $[M_{\mathbf{7a}}+H]^+$ peak at *m*/*z* 270.0646 and $[M_{\mathbf{7b}}+H]^+$ peak at *m*/*z* 300.0753. All the spectral data elucidated the products **7a** and **7b** to be the corresponding azide derivatives. The azidoglycosides **7a** and **7b** were further treated with Ph_3P overnight and followed by H_2O offering the corresponding aminoglycosides **8a** and **8b** (Scheme 4). Resonances of H-3′ and C-3′ in **8a** and **8b** were upfield shifted comparing to the corresponding signals in **7a** and **7b**, indicating the conversion of the azido groups to the amino groups.

Moreover, oxo derivatives of glycosides have biological importance,[17] thus we attempted to convert 3′-OH to carbonyl group. In organic synthesis, PDC is a common reagent used to oxidize hydroxyl groups of cyclic compound to the corresponding ketones. Therefore, treatment of compounds **5a** and **5b** with PDC in CH_2Cl_2 provided the ketones **12a** and **12b** (Scheme 5). The disappearance of the signal of H-3′ in ^1H NMR spectrum and the presence of two carbonyl groups at δ205.2 and 193.5, respectively, in ^{13}C NMR spectrum of **12a** revealed that the new carbonyl group was generated. In consideration of the α-configurations of the compounds **5a** and **5b**, and basic conditions being unfavorable to anomerization,[18] we could deduce the α-configuration of the compounds **6a** and **6b**, **7a** and **7b**, **8a** and **8b**, and **12a** and **12b**.



Scheme 5. Reagents and condition: PDC, CH_2Cl_2, reflux, 76–81%.

We are interested in the influence on the bioactivity with introduction of chlorine at C-2′ and the effect of other substituents at C-3′ in furanosyl α-C-glycosides. The cytotoxic activities of the above furanosyl C-glycoside derivatives were evaluated against A549, human lung adenocarcinoma cells, as described.[19] The results are summarized in Table 1. All the compounds exhibited moderate cytotoxic activity in the evaluation. Comparison of the data for the chlorinated compound (**5a**) with their hydroxylated counterpart (**11**) showed that compound **5a** has higher cytotoxicity than compound **11**. The introduction of 3′-OMs, 3′-N_3, 3′-NH_2, and 3′-carbonyl substituents, respectively, caused the changes of the inhibition to some extent. The slightly poor inhibitions of the compounds **8a** and **8b** showed that 3′-NH_2 substituent is unfavorable for the cytotoxic activity. The lower inhibitions of the compounds **5b**, **6b**, **7b**, and **8b**, than those of the corresponding compounds **5a**, **6a**, **7a**, and **8a**, suggested that replacement of the methyl group with the ethoxyl group is not well tolerated in most cases. An exception was that in oxo derivatives the inhibitory activity of **12b** is higher than that of **12a**.

In conclusion, the reaction of 4-chloro-4-deoxy-α-D-galactose **1** with 1,3-dicarbonyl compounds led to polyhydroxyalkyl furan derivatives bearing a chlorine atom in the polyhydroxylic chain. Further modification afforded the corresponding α-C-glycoside derivatives. The preliminary study on structure–activity relationships revealed that introduction of chlorine and other substituents at sugar ring has an important influence on the cytotoxicity of the furanosyl α-C-glycoside derivatives.

Table 1. Cytotoxic activities of the compounds determined at different concentrations

Compound	Percentage of inhibition (%)		
	50 μmol mL^{-1}	100 μmol mL^{-1}	200 μmol mL^{-1}
5a	24.36	31.92	39.36
5b	15.15	18.51	25.72
6a	26.42	35.65	46.80
6b	15.30	18.55	27.87
7a	32.73	48.60	56.70
7b	18.28	19.79	36.89
8a	13.04	24.86	34.57
8b	10.09	16.29	24.82
12a	21.49	28.55	37.67
12b	28.81	31.36	45.68
11	23.14	27.40	33.09

Supplementary data

CCDC-628469 (derivative **2a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033.

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19. In vitro cytotoxicity study: human lung adenocarcinoma cell line A549 (purchased from Institute of Biochemistry and Cell Biology, SIBS, CAS) was cultured in RPMI-1640 medium (GIBCO Co., Grand Island, NY) supplemented with 10% FBS, 100 IU/mL of penicillin, and 100 μg/mL of streptomycin (Sigma Chemical Co., St. Louis, MO) at 37 °C in humidified air atmosphere of 5% CO_2 (Binder, CB150, Germany). Cell cytotoxicity was assessed by MTT assay. Briefly, cells were plated into 96-well-plate (5000 cells/well). The next day compound at various concentrations diluted in culture medium was added (200 μL/well) to the wells. Forty-eight hours later 20 μL of MTT (Sigma Chemical Co., St. Louis, MO) (0.5 mg/mL MTT in PBS) was added and cells were incubated for a further 4 h. Two hundred microliters of DMSO was added to each culture to dissolve the reduced MTT crystals. The MTT-formazan product dissolved in DMSO was estimated by measuring absorbance at 570 nm with a microplate reader (Biotech, Power Wave, CA). Then the inhibitory percentage of each compound at various concentrations was calculated.