Synthesis and Crystal Structure of *tert*-Butyl(((2R,3R,6R)-3-hydroxy-6-(nitromethyl)-3,6-dihydro-2H-pyran-2-yl)methyl)carbonate①

FENG Yong-Kui SU Hua-Lan MUKULA OTUKOL Billy Joel
ZHANG Xue-Qing YAO Hui② HUANG Nian-Yu②
(*Hubei Key Laboratory of Natural Products Research and Development, Key Laboratory of Functional Yeast* (*China National Light Industry*), *College of Biological and Pharmaceutical Sciences, China Three Gorges University, Yichang, Hubei* 443002, *China*)

ABSTRACT In this paper, a *β*-C-pyranogalactoside (**IX**) was synthesized from D-galactose through a nine-step reaction with a total yield of 32% under the palladium catalyst, and its structure was characterized by nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). The absolute configuration of this pyranogalactoside was confirmed with a Flack parameter of –0.01(6) by X-ray crystallography using a Cu radiation source. Compound (**IX**), $C_{12}H_{19}NO_7$, crystal data: monoclinic system, space group $P2_12_12_1$, a = 8.53480(10), b = 9.4207(2), c = 18.1308(3) Å, V = 1457.79(4) Å^3, Z = 4, $F(000)$ = 616, D_c = 1.318 g/cm^3, μ = 0.931 mm^{-1}, R = 0.0294 and wR = 0.0752 for 2875 independent reflections (R_{int} = 0.0163) and 2857 observed ones ($I > 2\sigma(I)$).

Keywords: X-ray diffraction, crystal structure, absolute configuration, *β*-C-pyranogalactoside, *α*-glucosidase inhibitory activity; DOI: 10.14102/j.cnki.0254–5861.2011–3120

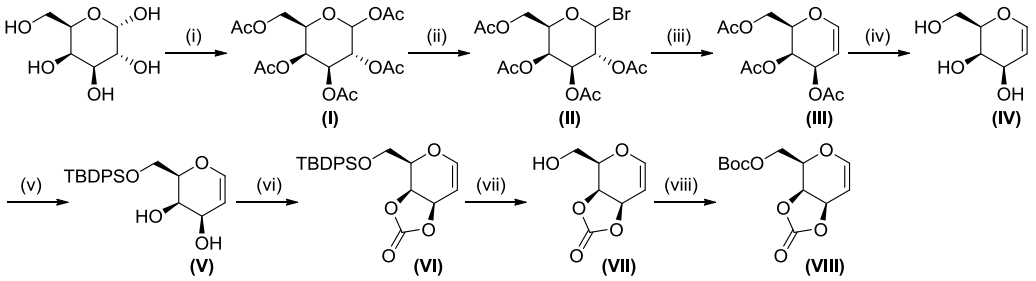
1 INTRODUCTION

C-glycosides are an important part of carbohydrate chemistry because they have been embedded in a variety of natural products with significant biological importance[1, 2]. Besides their potential bioactivity, C-glycosides are more stable than native N- or O-glycosylated residues, since N- or O-glycosidic bonds can be cleaved enzymatically under physiological conditions[3-5]. C-glycosides can behave like comparable conformations of O- and N-glycosides. Consequently, they can be beneficial as substantial mimics of biologically active natural O-glycosides and thus can be employed as potential therapeutic agents[6, 7]. For example, C-glycoside drugs could be used as the inhibitors of sodium glucose co-transporter 2 (SGLT2)[8], including dapagliflozin[9], canagliflozin[10] and empagliflozin[11], which have received considerable attention for the treatment of type II diabetes mellitus.

Chemical C-glycosylation has been well developed in recent years due to the extremely diverse structures of naturally existing C-glycosides. By activating the anomeric center with the exploitation of Lewis acid catalysts, C-nucleophiles have been utilized predominantly for the formulation of C-glycosidic linkages[12, 13]. Although Ferrier glycosylation[14], Heck reaction[15] and Tsuji-Trost reaction[16] have been applied successfully in C-glycosylation with a catalytic amount of transition-metal catalysts, most of these reactions need to be carried out at high temperature or aided by additives. Therefore, many efforts have been focused on developing milder, more efficient and highly stereoselective alternatives such as transition metal-catalyzed C-glycosylation of unsaturated glycoside donors. Transition metal-catalyzed cross-coupling reactions are being developed as a diverse approach for the synthesis of naturally abundant C-glycosides and have emerged as a powerful method for the construction of C-glycosides[17-19]. Inspired by the above researches, we

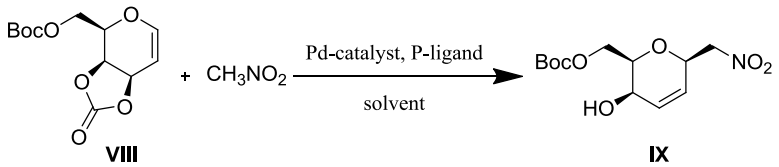
Received 26 January 2021; accepted 23 February 2021 (**CCDC 2054419**)
① Supported by the National Natural Science Foundation of China (No. 82003621) and Youth Talent Development Foundation of China Three Gorges University
② Corresponding authors. Prof. Yao Hui (1987-) and Huang Nian-Yu (1979-), both majoring in synthetic medicinal chemistry.
E-mails: yaohui@ctgu.edu.cn (Yao Hui) and huangny@ctgu.edu.cn (Huang Nian-Yu)

designed and synthesized the unsaturated Boc-protected 3,4-O-carbonate glycal donor (**VIII**, Scheme 1), then treated it to C-glycoside via transition metal-catalyzed cross-coupling reactions under mild conditions in this work (Scheme 2).



Reagents and conditions: (i) Ac_2O, pyridine, 0°C, 0.5 h; (ii) HBr, CH_3COOH, CH_2Cl_2, 0°C, 1.5 hrs; (iii) Zn, NaH_2PO_4, H_2O, $CH_3COOC_2H_5$, r.t., 12 hrs; (iv) KOH, CH_3OH, 0°C, 0.5 h; (v) TBDPSCl, imidazole, DMF, r.t., 6 hrs; (vi) N,N'-Carbonyldiimidazole, imidazole, THF, r.t., 8 hrs; (vii) TBAF, THF, 0°C, 1.5 hrs; (viii) Boc_2O, Et_3N, CH_2Cl_2, 0°C, 3 hrs.

Scheme 1. Synthetic routes for the title intermediates VIII



Scheme 2. Synthetic routes for the C-glycosides

2 EXPERIMENTAL

2. 1 Reagents and physical measurements

Most reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and were used without further purification. Solvents were dried and purified using standard techniques. D-Galactose was purchased from J&K Chemical Co., Ltd., China. Reactions were monitored by thin layer chromatography on silica gel GF_{254} pre-coated plates. 1D and 2D nuclear magnetic resonance (NMR) spectra were recorded at a Bruker Ultrashied^{TM} 400 MHz Plus spectrometer. Chemical shifts (δ) were reported in ppm, using residual solvent as an internal standard. Melting points were tested with an uncorrected X-4 digital melting point apparatus. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained using a Waters Q-TOF premier^{TM} mass spectrometer. Characterization data for known compounds were checked in comparison with literature for consistency and not presented in this report.

2. 2 Preparation of intermediates II-VIII

D-Galactose (10.0 g, 55.6 mmol) was dissolved in anhydrous pyridine (100 mL), and acetic anhydride (30 mL) was slowly added dropwise to the mixture at 0 °C. After stirring at room temperature for 30 minutes, the reaction was quenched with H_2O (200 mL) and extracted with ethyl acetate (40 mL × 3). The extracts were combined, dried with Na_2SO_4, filtered, and evaporated to dryness to give the crude beta-D-galactose pentaacetate (**I**), which could be used directly in the next step without further purification.

The crude beta-D-galactose pentaacetate (**I**, 10.0 g, 25.6 mmol) was dissolved in CH_2Cl_2 (200 mL), and the solution of HBr/AcOH was slowly added at 0 °C. The mixture was stirred at room temperature for 1.5 hours and thin-layer chromatography (TLC) was used to monitor the process until completion. The mixture was quenched with H_2O (200 mL), neutralized with saturated $NaHCO_3$ solution, and extracted with CH_2Cl_2 (50 mL × 3). The combined organic extracts were dried with Na_2SO_4 and filtered, and evaporated to dryness to give the oily product, which was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate = 15/1, v/v) to give the 2,3,4,6-tetra-O-acetyl-alpha-D-galactopyranosyl bromide (**II**) as colorless syrup[20] (9.5 g, yield 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.61 (d, J = 4.0 Hz, 1H), 5.56 (t, J = 9.7 Hz, 1H), 5.17 (t, J = 9.8 Hz, 1H), 4.84 (dd, J = 10.0, 4.1 Hz, 1H), 4.39～4.25 (m, 2H), 4.19～4.08 (m, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.4, 169.8, 169.7, 169.4, 86.5, 72.1, 70.5, 70.1, 67.1, 60.9, 20.6, 20.6, 20.5, 20.5.

The suspension mixture containing compound **II** (9.5 g, 24 mmol) and zinc powder (30.0 g, 461 mmol) in saturated NaH_2PO_4 aqueous solution (100 mL) and ethyl acetate (100

mL) was vigorously stirred at room temperature for 12 hours and TLC was used to monitor the reaction until completion. The solid was filtered and organic layer was separated. The aqueous phase was extracted with ethyl acetate (40 mL × 3). The organic extracts were combined, dried with Na_2SO_4 and filtered, and evaporated to dryness to give the crude product, which was purified by silicagel flash chromatography with a gradient solvent system (eluent:petroleum ether/ethyl acetate = 10/1, v/v) to obtain the (2*R*,3*R*,4*R*)-2-(acetoxymethyl)-3,4-dihydro-2*H*-pyran-3,4-diyl diacetate (**III**) as colorless syrup[21] (5.8 g, yield 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.48 (dd, *J* = 6.3, 1.4 Hz, 1H), 5.63～5.50 (m, 1H), 5.44 (d, *J* = 4.5 Hz, 1H), 4.73～4.76 (m, 1H), 4.36～4.19 (m, 3H), 2.15 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.6, 170.3, 170.1, 145.4, 98.8, 72.7, 63.8, 63.7, 61.9, 20.8, 20.8, 20.7.

Compound **III** (5.8 g, 21.3 mmol) and KOH (2.0 g, 36 mmol) were dissolved in methanol (50 mL) and stirred at 0 ^{o}C for 30 minutes. TLC was used to monitor the process until the reaction was complete. After removal of the solvent, the residue was purified by silica gel flash chromatography (eluent:ethyl acetate/methanol = 50/1, v/v) to get the D-galactal (**IV**) as white solids[21] (4.0 g, yield 83%, m.p. 99～100 ^{o}C). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.26 (dd, *J* = 6.2, 1.9 Hz, 1H), 4.74 (t, *J* = 5.6 Hz, 1H), 4.64 (d, *J* = 6.7 Hz, 1H), 4.48 (dt, *J* = 6.2, 2.0 Hz, 1H), 4.39 (d, *J* = 4.8 Hz, 1H), 4.22～4.17 (m, 1H), 3.79～3.67 (m, 2H), 3.63～3.52 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 143.5, 104.1, 77.9, 64.6, 63.8, 60.7.

To a mixture of compound **IV** (4.0 g, 27.4 mmol), *tert*-butyldiphenylchlorosilane (TBDPSCl, 8.46 g, 30.1 mmol) and imidazole (4.2 g, 61 mmol) were dissolved in N,N′-dimethylformamide (DMF, 60 mL), and stirred at room temperature for 6 hours until the completion of the reaction monitored by TLC. The reaction was quenched by water and crude product was extracted by ethyl acetate. After removing the solvent, pure (2*R*,3*R*,4*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3,4-dihydro-2*H*-pyran-3,4-diol (**V**) was obtained by silica gel flash chromatography (eluent:petroleum ether/ethyl acetate = 1/1, v/v) as colorless syrup[22] (9.2 g, yield 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.83～7.62 (m, 4H), 7.55～7.34 (m, 6H), 6.41 (dd, *J* = 6.2, 1.5 Hz, 1H), 4.75 (dt, *J* = 6.2, 1.9 Hz, 1H), 4.46～4.29 (m, 1H), 4.17 (t, *J* = 4.8 Hz, 1H), 4.01 (dd, *J* = 11.9, 6.9 Hz, 1H), 3.96～3.88 (m, 2H), 2.99 (d, *J* = 5.3 Hz, 1H), 2.60 (d, *J* = 10.2 Hz, 1H), 1.09 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 144.4, 135.6, 135.5, 132.6, 132.4, 130.0, 127.8, 127.8, 103.4, 65.8, 64.4, 63.8, 26.7, 19.1.

Compound **V** (8.7 g, 22.8 mmol), N,N′-carbonyldiimidazole (7.3 g, 45.6 mmol) and catalytic amount of imidazole (10 mg) were dissolved in anhydrous tetrahydrofuran (THF, 100 mL) and stirred at room temperature for 8 hours by TLC monitoring until all substrate was fully consumed. The mixture was quenched by ice water (100 mL), and crude product was extracted by ethyl acetate (60 mL × 3). After separating the organic layer and removal of the solvent, the oily crude was purified by silica gel flash chromatography (eluent:petroleum ether/ethyl acetate = 8/1, v/v) to give 1,5-anhydro-6-O-(*tert*-butyldiphenylsilyl)-3,4-O-carbonate-2-deoxy-D-lyxo-hex-1-enopyranose (**VI**) as colorless syrup[22] (4.5 g, yield 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.76～7.68 (m, 4H), 7.54～7.43 (m, 6H), 6.68 (d, *J* = 6.3 Hz, 1H), 5.25 (dd, *J* = 7.7, 3.1 Hz, 1H), 5.10 (d, *J* = 7.7 Hz, 1H), 4.99 (ddd, *J* = 6.2, 3.1, 1.1 Hz, 1H), 4.08～3.97 (m, 3H), 1.13 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 154.2, 149.1, 135.5, 135.5, 134.8, 132.7, 132.5, 130.1, 130.0, 127.9, 127.9, 127.7, 98.0, 73.8, 72.9, 68.9, 61.7, 26.8, 26.6, 19.2.

Compound **VI** (1.5 g, 3.6 mmol) and tetrabutylammonium fluoride hydrate (TBAF, 1.3 g, 3.9 mmol) were dissolved in anhydrous THF (30 mL). The reaction mixture was stirred at 0 ^{o}C for 1.5 hours. TLC was employed to monitor the reaction until it was complete. The subsequent mixture was thereafter concentrated to obtain a crude product which was purified by silica gel flash chromatography (eluent:petroleum ether/ethyl acetate = 4/1, v/v) to give the (3a*R*,4*R*,7a*R*)-4-(hydroxymethyl)-3a,7a-dihydro-4*H*-[1,3]dioxolo[4,5-c]-pyran-2-one (**VII**) as colorless syrup[23] (623 mg, yield 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.74 (d, *J* = 6.3 Hz, 1H), 5.24 (dd, *J* = 7.7, 3.2 Hz, 1H), 5.01 (ddd, *J* = 6.3, 3.2, 1.2 Hz, 1H), 4.98～4.91 (m, 1H), 4.10～3.99 (m, 2H), 3.93 (d, *J* = 9.6 Hz, 1H), 2.24 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 154.0, 149.1, 98.1, 73.9, 73.1, 69.0, 61.5, 29.7.

The mixture of **VII** (600 mg, 3.4 mmol), Et_3N (1.06 mL, 10.4 mmol) and di-*tert*-butyldicarbonate (912 mg, 4.2 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) and stirred at 0 ^{o}C for 3 hours. The reaction was monitored with TLC analysis until the completion. The solvent was removed under reduced pressure to afford a crude product, which was purified by silica gel flash chromatography (eluent: petroleum ether/ethyl acetate = 8/1, v/v) to get the *tert*-butyl(((3a*R*,4*R*,7a*R*)-2-oxo-3a,7a-dihydro-4*H*-[1,3]dioxolo-

[4,5-c]pyran-4-yl)methyl)carbonate (**VIII**) as yellow syrup[22] (706 mg, yield 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.71 (d, J = 6.3 Hz, 1H), 5.20 (dd, J = 7.7, 3.2 Hz, 1H), 5.00 (ddd, J = 6.3, 3.2, 1.1 Hz, 1H), 4.91 (d, J = 7.7 Hz, 1H), 4.42 (dd, J = 11.6, 7.2 Hz, 1H), 4.34 (dd, J = 11.6, 5.6 Hz, 1H), 4.21～4.12 (m, 1H), 1.50 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 149.1, 98.1, 83.3, 72.9, 71.5, 68.6, 64.6, 29.7, 27.7.

2. 3 Synthesis of *β*-C-pyranogalactoside

To the solution of CH_2Cl_2 (5 mL) of D-galactal carbonate (**VIII**, 272 mg, 1.0 mmol) and nitromethane (122 mg, 0.2 mmol) were added palladium acetylacetonate (2.3 mg, 0.0075 mmol) and 1,4-bis(diphenylphosphino)butane (DPPB, 2.2 mg, 0.005 mmol) under nitrogen atmosphere, and the reaction mixture was stirred at room temperature for 24 hours until the completion of reaction (TLC monitoring). The reaction was quenched by ice water (10 mL), and crude product was extracted by ethyl acetate (10 mL × 3). After removing the solvent, the crude product was purified by silica gel flash chromatography (eluent: petroleum ether/ethyl acetate = 10/1, v/v) to give the target product (**IX**) as white solids (257 mg, yield 91%. m.p. 111～112 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.28 (ddd, J = 10.1, 5.7, 2.2 Hz, 1H), 5.92 (dd, J = 10.4, 1.2 Hz, 1H), 4.91～4.84 (m, 1H), 4.59～4.55(m, 2H), 4.37 (dd, J = 11.6, 6.0 Hz, 1H), 4.29 (dd, J = 11.6, 7.2 Hz, 1H), 4.02～3.95 (m, 1H), 3.86～3.82 (m, 1H), 2.04 (d, J = 9.9 Hz, 1H), 1.53 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 153.4, 129.8, 128.2, 82.7, 77.9, 75.8, 71.9, 65.7, 61.7, 27.7. HRMS calcd. for $C_{12}H_{19}NO_7Na$ $[M+Na]^+$: 289.1162. Found 289.1169; $[\alpha]_D^{25}$ = –71.803. (c = 1.0, $CHCl_3$).

2. 4 Single-crystal structure determination

Compound **IX** crystallized after slow evaporation from a saturated chloroform solution as colorless blocks with dimensions of 0.14mm × 0.13mm × 0.11mm in the monoclinic system. Diffraction data of the single crystal were obtained at 100 K from a Bruker SMART APEX-II CCD diffractometer with graphite-monochromated Cu$K\alpha$ radiation (λ = 1.54184 Å) using the Bruker Collect software. A total of 5644 reflections were collected in the range of 2.39≤θ≤73.69 °by using an ω-scan mode, of which 2875 were unique with R_{int} = 0.0163 and 2857 were observed with $I > 2\sigma(I)$. After the initial corrections and data reduction, intensities of reflections were used to solve (by direct methods) and refine the structures (on F^2) using the WINGX program. A weighting scheme based upon $P = (F_o^2 + 2F_c^2)/3$ was employed. All the hydrogen atoms were located from difference maps and included in the refinements as riding. Empirical absorption corrections were applied. The structures were solved by direct methods using *SHELXL*-97 programs[23]. All of the non-hydrogen atoms were located from difference Fourier maps, and then refined anisotropically with *SHELXL*-97 via a full-matrix least-square procedure[23]. The final R = 0.0294, wR = 0.0752, $(\Delta/\sigma)_{max}$ = 0.000, S = 1.048, $(\Delta\rho)_{max}$ = 0.138 and $(\Delta\rho)_{min}$ = –0.253 e/Å^3. The Flack parameter was –0.01(6).

2. 5 *α*-Glucosidase inhibitory assay

The α-glucosidase inhibitory activity was performed according to a published method[24, 25]. 3 mM *p*-nitrophenyl-α-D-glucopyranoside (20 μL) and 0.2 U/mL α-glucosidase (20 μL) in 0.01 M phosphate buffer (pH = 7.0) were added to the sample solution and dissolved in dimethyl sulfoxide (DMSO, 10 μL) to start the reaction. Each reaction was carried out at 37 ℃ for 30 min and stopped by adding 0.1 M Na_2CO_3 (150 μL). The absorbance was recorded at 410 nm. All the samples were tested in triplicate, and the IC_{50} values were calculated from the dose-inhibition curve plotting using six different sample concentrations. 1-Deoxynojirimycin[26], a known α-glucosidase inhibitor, was used as a positive control.

3 RESULTS AND DISCUSSION

3. 1 Synthesis

The key intermediate D-galactal carbonate (**VIII**) was synthesized from D-galactose through an eight-step reaction with a total yield of 35% (Scheme 1). The glycosylation reaction was conducted by the palladium catalyst in the presence of phosphorus ligands (P-ligands) including 4,5-bis(diphnyl-phos-phino)-9,9-dimethylxan-thene (xantphos), 1,4-bis(diphenylphosphino)-butane (DPPB), 1,1′-bis(diphenylphosphino)ferrocene (DPPF), 2-di-*tert*-butylphosphino-2′,4′,6′-triiso-propylbiphenyl (*t*BuXPhos) and tricyclohexylphosphine ($P(Cy)_3$). By screening different palladium catalysts and P-ligands (Scheme 2), β-C-pyrano-galactoside (**IX**) was obtained with a satisfactory yield of 91% under optimal conditions using 2.5 mol% $Pd(acac)_2$ catalyst, and 5 mol% DPPB in dichloromethane at room temperature (Table 1).

Table 1. Optimization of Conditions for the C-glycosylation Reaction

Entry[a]	Catalyst	P-Ligand	Solvent	Yield[b]
1	$Pd_2(dba)_3$	Xantphos	THF	40%
2	$Pd(OAc)_2$	Xantphos	THF	10%
3	White catalyst	Xantphos	THF	-
4	$Pd(PPh_3)_4$	Xantphos	THF	-
5	$PdCl_2$	Xantphos	THF	-
6	$Pd(acac)_2$	Xantphos	THF	60%
7	$Pd(acac)_2$	DPPB	THF	86%
8	$Pd(acac)_2$	DPPF	THF	74%
9	$Pd(acac)_2$	*t*BuXPhos	THF	66%
10	$Pd(acac)_2$	$P(Cy)_3$	THF	57%
11	$Pd(acac)_2$	DPPB	CH_2Cl_2	91%
12	$Pd(acac)_2$	DPPB	CH_3CN	88%
13	$Pd(acac)_2$	DPPB	Toluene	76%

[a]Unless otherwise specified, all reactions were carried out with 0.1 mmol of **IX**, 0.2 mmol of nitromethane, 2.5 mol% Pd catalyst and 5 mol% P-ligand in 2 mL solvent and N_2 atmosphere at room temperature. [b]Isolated yield, N.R. = No reaction.

3. 2 Spectroscopy analysis

All the intermediates (**II**～**VIII**) were characterized by NMR, and their data obtained were found to be consistent with that of other literatures. In the ^{1}H NMR spectrum, the protons of the carbon-carbon double bond for the title compound (**IX**) were located at 6.28 and 5.92 ppm, and the hydrogen atoms of *tert*-butyl group were found as singlet at 1.53 ppm. Two sets of carbon signals for the methylene group appeared at 65.76 and 61.71 ppm in its ^{13}C NMR and DEPT-135 spectra. The adduction $[M + Na]^+$ in the HR-ESI-MS spectrum could be also observed for the title compound.

3. 3 Crystal structure description

The selected bond lengths, bond angles and torsion angles are listed in Table 2, and all the bond lengths of C–C, C=C and C=O were in accordance with the standard compilations and the literature[27]. The bond angles containing unsaturated bonds in C(3)–C(4)–C(5), O(4)–C(8)–O(3) and O(6)–N(1)–O(7) range from 112 ° to 126 °. The torsion angles of sugar chain O(2)–C(1)–C(2)–C(3) and O(2)–C(5)–C(4)–C(3) were equal to –49.34(16) °and 11.5(2) °, respectively. The aliphatic 6-membered ring O(2)–C(1)–C(2)–C(3)–C(4)–C(5) showed a twist-boat form (Φ = 217.6139 °, θ = 128.53 °, puckering amplitude (Q) = 0.4921 °).

Table 2. Selected Bond Lengths (Å) and Bond Angles (°) for Compound 3

Bond	Dist.	Bond	Dist.	Bond	Dist.
C(1)–O(2)	1.4383(17)	C(5)–C(6)	1.518(2)	O(3)–C(7)	1.4493(17)
O(2)–C(5)	1.4233(17)	N(1)–C(6)	1.493(2)	O(4)–C(8)	1.1994(19)
C(5)–C(4)	1.500(2)	O(6)–N(1)	1.215(2)	O(5)–C(8)	1.3280(19)
C(4)–C(3)	1.325(2)	O(7)–N(1)	1.2335(19)	O(1)–C(2)	1.4305(19)
Angle	(°)	Angle	(°)	Angle	(°)
C(5)–O(2)–C(1)	112.11(11)	O(6)–N(1)–O(7)	124.16(15)	O(2)–C(1)–C(7)–O(3)	83.57(13)
C(3)–C(4)–C(5)	122.03(14)	O(4)–C(8)–O(3)	125.22(15)	O(2)–C(1)–C(2)–O(1)	74.93(15)
C(3)–C(4)–C(5)	122.03(14)	C(8)–O(5)–C(9)	121.05(12)	O(2)–C(1)–C(2)–C(3)	–49.34(16)
C(7)–C(1)–C(2)	111.21(12)	C(11)–C(9)–C(10)	112.40(15)	O(2)–C(5)–C(4)–C(3)	11.5(2)

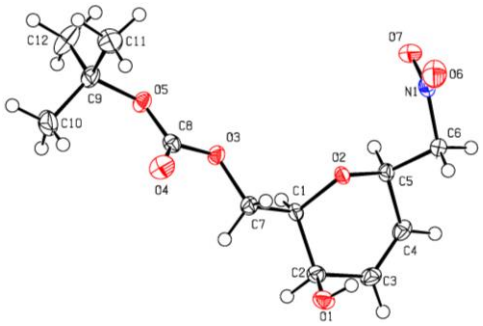


Fig. 1. ORTEP drawing of compound IX showing thermal ellipsoids at the 50% probability level

As listed in Table 3, intermolecular and intramolecular O–H···O and C–H···O interactions linked the molecules into a two-dimensional infinite plane running along the *c* axis, which helped to stabilize the crystal structure (Fig. 2).

Table 3. Hydrogen Bonds for Compound 3

D–H···A	d(D–H)	d(H···A)	d(D···A)	<(DHA)
O(1)–H(1)···O(7)[(a)]	0.82	2.08	2.8904(1)	172
C(4)–H(4)···O(4)[(b)]	0.93	2.37	3.2683(1)	163
C(10)–H(10C)···O(4)	0.96	2.37	2.9117(1)	115

Symmetry codes: (a) –1/2 + *x*, –1/2 – *y*, –*z*; (b) 1/2 – *x*, –*y*, –1/2 + *z*

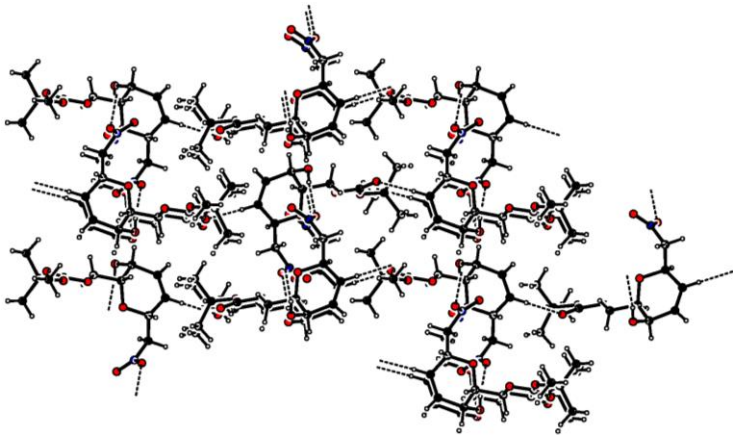


Fig. 2. Packing diagram of compound IX. The O–H ··O interactions are shown as dashed lines

3. 3 Evaluation of the bioactivity

C-glycoside derivatives have been reported to possess *α*-glucosidase inhibitory activity[9-11] that is a main target enzyme in the prevention and treatment of type 2 diabetes[28]. As our continuous interest in search of natural products-derived biological small molecules[29-32], the *β*-C-pyranogalactoside (**IX**) and its synthetic intermediates (**IV-VIII**) were evaluated for *α*-glucosidase inhibitory activity (Table 4). Compared with the positive control of 1-deoxynojirimycin (IC_{50}: 0.29 μg/mL), all the synthetic intermediates (**IV-VIII**) exhibited poor inhibitory effect against *α*-glucosidase (IC_{50} > 200 μg/mL), and the target C-glycoside (**IX**) just showed moderate inhibitory activity with the IC_{50} value of 89.86 μg/mL. Further exploration of the substrate scope for this reaction and structural modification of the C-glycosides were underway in our research group.

Table 4. *α*-Glucosidase Inhibitory Activity of Compounds IV-IX

Compound	IC_{50} (*μ*g/mL)	Compound	IC_{50} (*μ*g/mL)
IV	>200	VIII	>200
V	>200	IX	89.86
VI	>200	1-Deoxynojirimycin[a]	0.29
VII	>200	DMSO[b]	-

[a]Positive control, [b]blank test.

4 CONCLUSION

In summary, a *β*-C-pyranogalactoside (**IX**) was prepared by an efficient and highly stereoselective approach from D-galactose, and its absolute configuration was confirmed with a Flack parameter of –0.01(6) by X-ray crystallography. The *in vitro* *α*-glucosidase inhibitory activity evaluation indicated that C-pyranoside showed better inhibitory effect than the synthetic D-galactal intermediates, which encouraged us to further investigate the reaction with the aim of systematically assessing the biological activity for the C-glycosides.

REFERENCES

(1) Liao, H. Z.; Ma, J. M.; Yao, H.; Liu, X. W. Recent progress of C-glycosylation methods in the total synthesis of natural products and pharmaceuticals. *Org. Biomol. Chem.* **2018**, 16, 1791–1806.

(2) Yang, Y.; Yu, B. Recent advances in the chemical synthesis of C-glycosides. *Chem. Rev.* **2017**, 117, 12281–12356.

(3) Wei, B.; Wang, Y. K.; Qiu, W. H.; Wang, S. J.; Wu, Y. H.; Xu, X. W.; Wang, H. Discovery and mechanism of intestinal bacteria in enzymatic cleavage of C–C glycosidic bonds. *Appl. Microbiol. Biotechnol.* **2020**, 104, 1883–1890.

(4) Drohat, A. C.; Maiti, A. Mechanisms for enzymatic cleavage of the N-glycosidic bond in DNA. *Org. Biomol. Chem.* **2014**, 12, 8367–8378.

(5) Wolfenden, R.; Lu, X.; Young, G. Spontaneous hydrolysis of glycosides. *J. Am. Chem. Soc.* **1998**, 120, 6814–6815.

(6) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. The C-glycoside analogue of the immunostimulant *α*-galactosylceramide (KRN7000): synthesis and striking enhancement of activity. *Angew. Chem. Int. Ed.* **2004**, 43, 3818–3822.

(7) Kitamura, K.; Ando, Y.; Matsumoto, T.; Suzuki, K. Total synthesis of aryl C-glycoside natural products: strategies and tactics. *Chem. Rev.* **2018**, 118, 1495–1598.

(8) Rieg, T.; Vallon, V. Development of SGLT1 and SGLT2 inhibitors. *Diabetologia* **2018**, 61, 2079–2086.

(9) Dhillon, S. Dapagliflozin: a review in type 2 diabetes. *Drugs* **2019**, 79, 1135–1146.

(10) Deeks, E. D.; Scheen, A. J. Canagliflozin: a review in type 2 diabetes. *Drugs* **2017**, 77, 1577–1592.

(11) Levine, M. J. Empagliflozin for type 2 diabetes mellitus: an overview of phase 3 clinical trials. *Curr. Diabetes Rev.* **2017**, 13, 405–423.

(12) Sakamoto, K.; Nagai, M.; Ebe, Y.; Yorimitsu, H.; Nishimura, T. Iridium-promoted deoxyglycoside synthesis: stereoselectivity and mechanistic insight. *ACS Catal.* **2019**, 9, 1347–1352.

(13) Zhu, F.; Rourke, M. J.; Yang, T.; Rodriguez, J.; Walczak, M. A. Highly stereospecific cross-coupling reactions of anomeric stannanes for the synthesis of C-aryl glycosides. *J. Am. Chem. Soc.* **2016**, 138, 12049–12052.

(14) Wang, J.; Deng, C.; Zhang, Q.; Chai, Y. Tuning the chemoselectivity of silyl protected rhamnals by temperature and bronsted acidity: kinetically controlled 1,2-addition vs thermodynamically controlled Ferrier rearrangement. *Org. Lett.* **2019**, 21, 1103–1107.

(15) Mabit, T.; Siard, A.; Legros, F.; Guillarme, S.; Martel, A.; Lebreton, J.; Carreaux, F.; Dujardin, G.; Collet, S. Stereospecific C-glycosylation by Mizoroki-Heck reaction: a powerful and easy-to-set-up synthetic tool to access alpha- and beta-aryl-C-glycosides. *Chem. Eur. J.* **2018**, 24, 14069–14074.

(16) Leng, W. L.; Liao, H. Z.; Yao, H.; Ang, Z. E.; Xiang, S. H.; Liu, X. W. Palladium-catalyzed decarboxylative allylation/Wittig reaction: substrate-controlled synthesis of C-vinyl glycosides. *Org. Lett.* **2017**, 19, 416–419.

(17) Li, W.; Yu, B. Gold-catalyzed glycosylation in the synthesis of complex carbohydrate-containing natural products. *Chem. Soc. Rev.* **2018**, 47, 7954–7984.

(18) Ye, W.; Stevens, C. M.; Wen, P.; Simmons, C. J.; Tang, W. Mild $Cu(OTf)_2$-mediated C-glycosylation with chelation-assisted picolinate as a leaving group. *J. Org. Chem.* **2020**, 85, 16218–16225.

(19) Zhu, F.; Rodriguez, J.; Yang, T.; Kevlishvili, I.; Miller, E.; Yi, D.; O'Neill, S.; Rourke, M. J.; Liu, P.; Walczak, M. A. Glycosyl cross-coupling of anomeric nucleophiles: scope, mechanism, and applications in the synthesis of aryl C-glycosides. *J. Am. Chem. Soc.* **2017**, 139, 17908–17922.

(20) Liu, S. X.; Tsai, Y. T.; Lin, Y. T.; Li, J. Y.; Chang, C. C. Design and synthesis of trivalent Tn glycoconjugate polymers by nitroxide-mediated polymerization. *Tetrahedron* **2019**, 75, 1307–1311.

(21) Moore, P. W.; Schuster, J. K.; Hewitt, R. J.; Stone, M. R. L.; Harvey, J. E. Divergent synthesis of 2-C-branched pyranosides and oxepines from 1,2-gem-dibromocyclopropyl carbohydrates. *Tetrahedron* **2014**, 70, 7032–7043.

(22) Dai, Y. W.; Zheng, J. F.; Zhang, Q. General strategy for stereoselective synthesis of *β*-N-glycosyl sulfonamides viapalladium-catalyzed glycosylation. *Org. Lett.* **2018**, 20, 3923–3927.

(23) Sheldrick, G. M. *SHELXL* 97, *Program for the Refinement of Crystal Structure*. University of Göttingen, Germany **1997**.

(24) Ma, Y. Y.; Zhao, D. G.; Zhang, R.; He, X.; Li, B. Q.; Zhang, X. Z.; Wang, Z.; Zhang, K. Identification of bioactive compounds that contribute to the alpha-glucosidase inhibitory activity of rosemary. *Food Funct.* **2020**, 11, 1692–1701.

(25) Dan, W. J.; Zhang, Q.; Zhang, F.; Wang, W. W.; Gao, J. M. Benzonate derivatives of acetophenone as potent alpha-glucosidase inhibitors: synthesis, structure-activity relationship and mechanism. *J. Enzyme Inhib. Med. Chem.* **2019**, 34, 937–945.

(26) Tong, T. T.; Zhao, E. H.; Gao, H. L.; Xu, Y. H.; Zhao, Y. J.; Fu, G.; Cui, H. J. Recent research advances of 1-deoxynojirimycin and its derivatives. *China J. Chin. Mater. Med.* **2018**, 43, 1990–1997.

(27) Paolini, J. P. The bond order-bond length relationship. *J. Comput. Chem.* **1990**, 11, 1160–1163.

(28) Cai, W.; Jiang, L.; Xie, Y.; Liu, Y.; Liu, W.; Zhao, G. Design of SGLT2 inhibitors for the treatment of type 2 diabetes: a history driven by biology to chemistry. *Med. Chem.* **2015**, 11, 317–328.

(29) Wang, Y.; Yao, H.; Hua, M.; Jiao, Y.; He, H.; Liu, M.; Huang, N.; Zou, K. Direct N-glycosylation of amides/amines with glycal donors. *J. Org. Chem.* **2020**, 85, 7485–7493.

(30) Yao, Y.; Xiong, C. P.; Zhong, Y. L.; Bian, G. W.; Huang, N. Y.; Wang, L.; Zou, K. Intramolecular and Ferrier rearrangement strategy for the construction of C1-*β*-D-xylopyranosides: synthesis, mechanism and biological activity study. *Adv. Syn. Cat.* **2019**, 361, 1012–1017.

(31) Li, D. W.; Zuo, H. H.; Hu, M.; Zhang, J. Y.; Chen, L.; Huang, N. Y. Synthesis and absolute configuration of (2S,3S,3aS,6S,7aR)-2,3-dihydroxy-2-((R)-1-hydroxy-3-methylbutyl)-3,6-dimethylhexahydrobenzofuran-4(2H)-one. *Chin. J. Struct. Chem.* **2017**,36, 1276–1282.

(32) Huang, N. Y.; Wang, W. B.; Chen, L.; Luo, H. J.; Wang, J. Z.; Deng, W. Q.; Zou, K. Design, synthesis and biological evaluation of bisabolonalone oxime derivatives as potassium-competitive acid blockers (P-CABs). *Bioorg. Med. Chem. Lett.* **2016**, 26, 2268–2272.