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An efficient synthesis of quinoxaline derivatives from 4-chloro-4-deoxy-α-D-galactose and their cytotoxic activities

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Abstract—A novel and efficient method for the synthesis of quinoxaline derivatives has been developed. Isopropylidenation of 4-chloro-4-deoxy-α-D-galactose with 2,2-dimethoxypropane, followed by selective hydrolysis, afforded 2,3-*O*-isopropylidene-4-chloro-4-deoxy-D-galactose di-methyl acetal (**3**) as a sole product. Oxidation of compound **3** with $(Bu_3Sn)_2O$–Br_2 gave corresponding hex-5-ulose derivative in high yields. The hex-5-ulose derivative reacted with *o*-phenylenediamines under neutral conditions to afford quinoxaline derivatives in reasonable yields. The in vitro cytotoxic activities of these quinoxaline derivatives were investigated.

Quinoxalines have shown a broad spectrum of biological activities such as antibacterial and anti-inflammatory activity, hence they are an important class of nitrogen-containing heterocycles and useful intermediates in organic synthesis.[1,2] Moreover, they have been evaluated as antitumor and anthelmintic agents.[3] Therefore, a variety of conventional methods have been developed for the preparation of quinoxaline derivatives including the Bi-catalyzed oxidative coupling of epoxides and ene-1,2-diamines,[4] a solid-phase synthesis on Synphase™ Lanterns,[5] cyclization of α-arylimino oximes of α-dicarbonyl compounds in refluxing acetic anhydride,[6] and the most common method: condensation of an aryl 1,2-diamine with 1,2-dicarbonyl compounds in refluxing ethanol or acetic acid.[7] An improved synthesis by generating the carbonyl in situ from α-hydroxy ketones via a tandem oxidation process using $Pd(OAc)_2$ or $RuCl_2$–$(PPh_3)_3$–TEMPO[8] and MnO_2[9,10] was reported also, in which the specific oxidation agents are necessary.

On the other hand, modified carbohydrate monomers are of both synthetic and pharmaceutical interest. Incorporation of carbohydrates into new bio-based materials is likely to entail structural modifications to change their properties. 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[11] and further hydrolysis to afford two monosaccharides. One of them is 1,4:3,6-dianhydro-D-fructose. Some novel bio-based chiral spiro-tetrahydroquinolines were stereoselectively synthesized by using this building block in one pot under mild conditions.[12] Another is unnatural 4-chloro-4-deoxy-α-D-galactose,[13] which is a new chiral building block having five chiral centers, four hydroxyl groups, and a chlorine atom. Thus, we attempted to incorporate this building block into new bio-based organic molecule. We present here a new strategy to prepare hex-5-ulose (**4**) from 4-chloro-4-deoxy-α-D-galactose and further synthesize quinoxalines in excellent yields from the α-hydroxy ketone without any oxygenant.

Acetonation is one of the most widely used reactions for carbohydrate derivatization and several methods have been developed to accomplish this reaction.[14–16] The use of 2,2-dimethoxypropane (DMP) in the presence of a catalytic amount of *p*-TsOH in combination with an appropriate solvent such as DMF has been employed for the conversion of 1,2-diequatorial hydroxyl groups of carbohydrates into isopropylidene derivatives.[17–20] But many syntheses failed because the reaction gave a mixture of products or a low yield of the target molecule. However, when 4-chloro-4-deoxy-α-D-galactose (**1**) was treated with DMP and a small amount of *p*-TsOH in dry DMF, a single product was obtained in high yield. The ^1H NMR spectrum of the product revealed the presence of two methoxy groups (δ 3.47, 3.48) and two isopropylidene groups (δ 1.39, 1.43,

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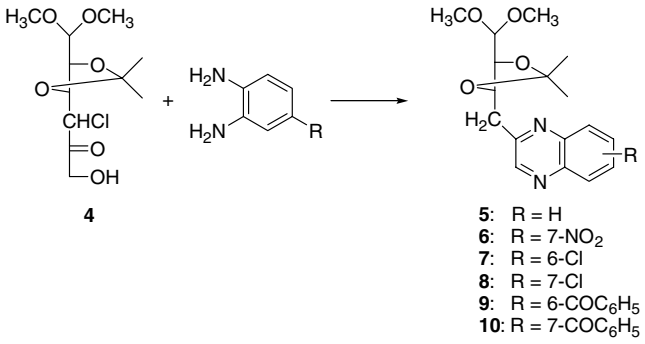
1.45, 1.47). In the HRMS spectrum of **2**, the M + Na^+ peak at *m*/*z* 347.1230 and the M + Na^+ + 2 peak at *m*/*z* 349.1221, as well as the 3:1 ratio of the heights of the two peaks (corresponding to Cl-35 and Cl-37 isotopes, respectively) indicated that the formula of **2** should be $C_{14}H_{25}ClO_6$. A two-dimensional NMR study finally confirmed the compound as 4-chloro-4-deoxy-2,3:5,6-di-*O*-isopropylidene-D-galactose di-methyl acetal (**2**).[21]

Then we employed a mild and efficient procedure to selectively deprotect acetal **2**. When **2** was dissolved in 40% acetic acid and stirred at room temperature for 2 days, interestingly, the 5,6-*O*-isopropylidene group was hydrolyzed but the two methoxy groups and the 2,3-*O*-isopropylidene group remained. 2,3-*O*-isopropylidene-4-chloro-4-deoxy-D-galactose di-methyl acetal (**3**) was obtained in almost quantitative yield.

Based on our previous study and knowledge on characteristics of carbohydrates,[22] we attempted to employ bistributyltin oxide together with bromine as an oxidant to afford the oxo derivative of sugar. Oxidation of **3** with $(Bu_3Sn)_2O$–Br_2 in boiling toluene gave product **4** in 94% yield. The absorption at 1729 cm^{-1} in the IR spectrum and the chemical shift at δ 204.8 in ^{13}C NMR spectrum of **4** indicated that the desired carbonyl had been formed. The absence of an aldehydic proton in ^1H NMR spectrum revealed the product to be a ketone derivative. Finally the position of the carbonyl group was established by 2D NMR spectral study. It was deduced from all the spectral data that compound **4** is 2,3-*O*-isopropylidene-4-chloro-4-deoxy-hex-5-ulose di-methyl acetal[23] (Scheme 1).

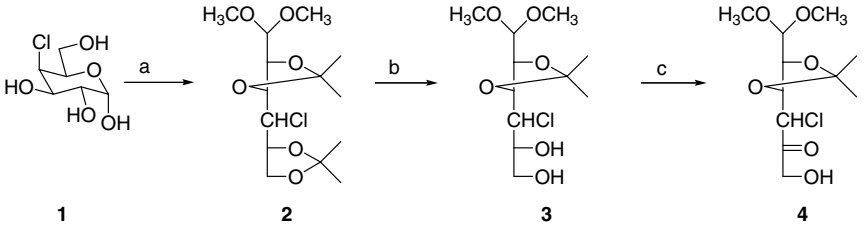
Treatment of hexulose **4** with *o*-phenylenediamine in dichloromethane gave product **5** in excellent yields. The ^1H NMR spectrum of **5** showed a singlet at δ 8.84 (1H) corresponding to a alkylene proton. The two multiplets at δ 7.75 (2H) and 8.08 (2H) were attributed to aromatic protons. In order to further identify the structure of **5**, the extensive NMR study (HSQC, DEPT-135, and HMBC) was performed. The DEPT-135 spectrum of the product revealed the presence of methylene absorption (δ_C 40.0). The HRMS spectrum confirmed the compound **5** having no chlorine atom in the molecule and its formula being $C_{17}H_{22}N_2O_4$, which implies an elimination of chlorine from intermediate **4** during the formation of product. Therefore, we concluded that compound **5** would be the quinoxaline derivative of compound **4**. The correlations of methylene proton (δ 3.49, 3.27) to C-3′ (δ 79.1), C-2 (δ 153.9), and C-3 (δ 146.7) were observed from the HMBC spectrum. Consequently, the structure of compound **5** was the 2-((5-dimethoxymethyl-2,2-dimethyl-[1,3]dioxolan-4-yl)methyl)-quinoxaline[24] as shown in Scheme 2.



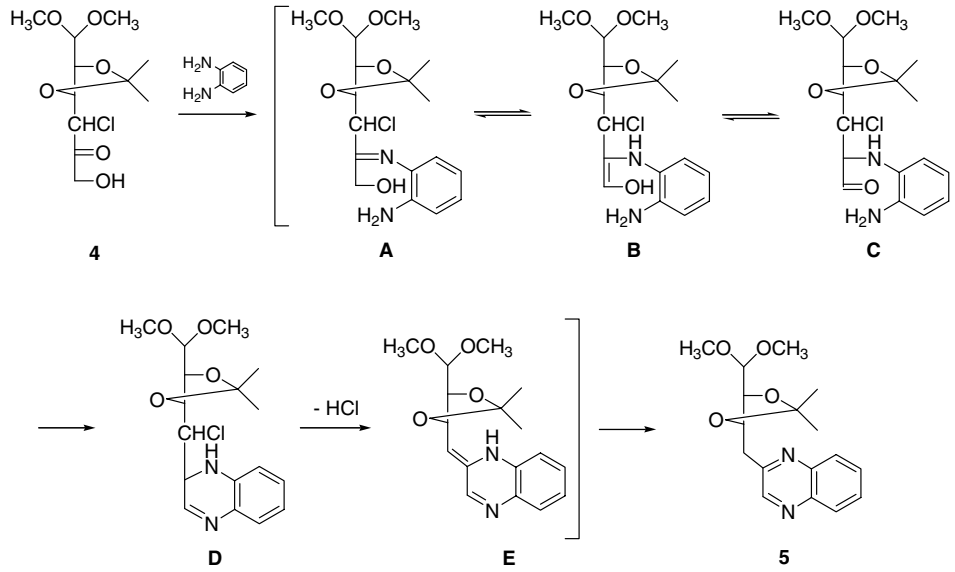
Scheme 2. Synthesis of quinoxaline derivatives.

The generality of this method was investigated by utilization of three other 4-substituted *o*-phenylenediamines in the reaction to furnish corresponding quinoxaline derivatives as well. When 4-nitro-*o*-phenylenediamine was employed, corresponding 2-((5-(dimethoxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-7-nitroquinoxaline (**6**) was obtained. But condensation of 2,3-*O*-isopropylidene-4-chloro-4-deoxy-hex-5-ulose di-methyl acetal (**4**) with 4-chloro-*o*-phenylenediamine in dry THF gave an isomeric mixture of 6-chloro-2-((5-dimethoxymethyl-2,2-dimethyl-[1,3]dioxolan-4-yl)methyl)-quinoxaline (**7**) and 7-chloro-2-((5-dimethoxymethyl-2,2-dimethyl-[1,3]dioxolan-4-yl)methyl)-quinoxaline (**8**). The two isomers **7** and **8** showed the same R_f value on TLC; however, the ^1H NMR spectrum of the mixture showed, downfield, the H-3 signal, characteristic of quinoxalines, as two close singlets at δ 8.81 and 8.83 in the ratio of 1:1 as calculated from their ^1H NMR integrals. Similarly, refluxing 2,3-*O*-isopropylidene-4-chloro-4-deoxy-hex-5-ulose di-methyl acetal (**4**) and 3,4-diaminobenzophenone in dry CH_2Cl_2 afforded the 6-benzoyl and 7-benzoyl-2-((5-dimethoxymethyl-2,2-dimethyl-[1,3]dioxolan-4-yl)methyl)-quinoxaline (**9** and **10**). The ^1H NMR spectrum showed the ratio of two isomers is 1.4:1, in favor of the 6-benzoyl isomer **9**.[25]

The possible pathway for the transformation is proposed as shown in Scheme 3. First, one amino group of *o*-phenylenediamine attacked the carbonyl of hex-5-ulose **4** to generate a condensation product **A** (Schiff



Scheme 1. Reagents and conditions: (a) $Me_2C(OMe)_2$, TsOH, DMF, 80 °C, 2 h; (b) 40% AcOH, rt, 2 days; (c) $(Bu_3Sn)_2O$, Br_2, toluene, reflux, 5 h.



Scheme 3. Proposed pathway for the formation of compound **5**.

Table 1. Cytotoxic activity of the compounds assayed expressed as the IC_{50} (μg mL^{-1})

	5	6	7 and 8 (dia)	9 and 10 (dia)
Ec9706	>100 μg/mL	69.2	30.5	80.0

base). Schiff base **A** can isomerize to enamine **B** and then to aldehyde **C** via the resonance transfer of double bond. Second, the aldehyde carbonyl of **C** was further condensed with another amino group from *o*-phenylenediamine to form an aza-heterocycle compound **D**. Then **D** eliminated a molecule of HCl to form an exocyclic double bond derivative **E**. Finally, **E** tautomerized to the more stable endocyclic double bond to give quinoxaline (**5**). In the transformation, the nature of substitution on *o*-phenylenediamines affects the structures of resulting quinoxalines. Generally an isomeric mixture of 6- and 7-substituted products was obtained by using 4-substituted *o*-phenylenediamines except for that with strong electron-withdrawing group such as 4-nitro-*o*-phenylenediamine in the reaction. The strong electron-withdrawing group greatly decreases the nucleophilicity of *p*-amino group, leading to the much higher reactivity of *m*-amino group than *p*-amino group, which results in a sole condensation product.

The cytotoxic activities of these compounds were then evaluated against Ec9706 cell lines as described.[26] When no substitution group exists at benzene, the IC_{50} value of quinoxaline is more than 100 μg/mL. But those 6- or 7-substituted quinoxaline derivatives have higher cytotoxicities, whose IC_{50} values are distributed over the range of 30.5–80.0 μg/mL, and chloro substituted *o*-phenylenediamine is most favorable among the tested compounds. The results are presented in Table 1. Hazeldine et al.[27] reported that changes in the nature and location of substituents in the benzene ring effected significant differences in both the vitro and in vivo activities of quinoxaline analogues. So we concluded that change of substitution groups at benzene ring is an effective way to enhance the activity of the quinoxaline derivatives.

In summary, we have developed a new simple synthesis of chiral building block-substituted hex-5-ulose and its quinoxaline derivatives from 4-chloro-4-deoxy-α-D-galactose. This multi-step approach gave hex-5-ulose intermediate (**4**) in 88% overall yield from compound **1** and quinoxaline derivatives in 62–78% yields from **4**. The quinoxaline possessing two blocking hydroxyls and one blocking carbonyl group is a potential intermediate for synthesis of other bioactive molecules. The preliminary study on structure–activity relationships revealed that the different groups at benzene ring give a great influence on the cytotoxicity of the quinoxaline derivatives.

Acknowledgment

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References and notes

1. Cheeseman, G. W. H.; Werstiuk, E. S. G. *Adv. Heterocycl. Chem.* **1978**, *22*, 367.
2. Sato, N. In *Comprehensive Heterocyclic Chemistry II*; Katritzky, A. R., Rees, C. W., Scriven, E. F. V., Eds.; Pergamon: Oxford, 1996; Vol. 6, p 233.
3. Sakata, G.; Makino, K.; Kurasawa, Y. *Heterocycles* **1988**, *27*, 2481, and references cited therein.
4. Antoniotti, S.; Dunach, E. *Tetrahedron Lett.* **2002**, *43*, 3971.
5. Wu, Z.; Ede, N. J. *Tetrahedron Lett.* **2001**, *42*, 8115.
6. Xekoukoulotakis, N. P.; Hadjiantonious-Maroulis, C. P.; Maroulis, A. J. *Tetrahedron Lett.* **2000**, *41*, 10299.
7. More, S. V.; Sastry, M. N. V.; Wang, C.-C.; Yao, C.-F. *Tetrahedron Lett.* **2005**, *46*, 6345, and references cited therein.
8. Robinson, R. S.; Taylor, R. J. K. *Synlett* **2005**, *6*, 1003.

9. Raw, S. A.; Wilfred, C. D.; Taylor, R. J. K. *Org. Biomol. Chem.* **2004**, *2*, 788.
10. Raw, S. A.; Wilfred, C. D.; Taylor, R. J. K. *Chem. Commun.* **2003**, *18*, 2286.
11. Liu, F.-W.; Liu, H.-M.; Ke, Y.; Zhang, J.-Y. *Carbohydr. Res.* **2004**, *339*, 2651.
12. Liu, H.-M.; Liu, F.-W.; Zou, D.-P.; Dai, G.-F. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1821.
13. Liu, F.-W.; Zhang, Y.-B.; Liu, H.-M.; Song, X.-P. *Carbohydr. Res.* **2005**, *340*, 489.
14. de Belder, A. N. *Adv. Carbohydr. Chem.* **1965**, *20*, 219.
15. de Belder, A. N. *Adv. Carbohydr. Chem. Biochem.* **1977**, *34*, 179.
16. Clode, D. M. *Chem. Rev.* **1979**, *79*, 491.
17. He, D. Y.; Li, Z. J.; Liu, Y. Q.; Qiu, D. X.; Cai, M. S. *Synth. Commun.* **1992**, *22*, 2653.
18. Evans, M. E.; Parrish, F. W.; Long, L., Jr. *Carbohydr. Res.* **1967**, *23*, 453.
19. Hasegawa, A.; Fletcher, H. G., Jr. *Carbohydr. Res.* **1973**, *29*, 209.
20. Hasegawa, A.; Nakajima, M. *Carbohydr. Res.* **1973**, *29*, 239.
21. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 4.46 (d, 1H, *J* 5.0 Hz, H-5), 4.45 (d, 1H, $J_{1,2}$ 4.6 Hz, H-1), 4.26 (d, 1H, *J* 4.8 Hz, H-2), 4.24 (d, 1H, $J_{3,4}$ 7.7 Hz, H-3), 4.11 (dd, 1H, $J_{5,6a}$ 6.8, $J_{6a,6b}$ 8.4 Hz, H-6a), 3.91 (dd, 1H, $J_{5,6b}$ 6.8, $J_{6a,6b}$ 8.4 Hz, H-6b), 3.89 (m, 1H, H-4), 3.48 (s, 3H, CH_3O–),3.47 (s, 3H, CH_3O–), 1.47 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.39 (s, 3H, CH_3) ppm; ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 111.2 (Me_2C), 109.6 (Me_2C), 103.9 (C-1), 79.7 (C-2), 78.1 (C-3), 75.4 (C-5), 66.8 (C-6), 62.9 (C-4), 55.8(CH_3O), 54.7 (CH_3O), 28.0 (CH_3), 27.6 (CH_3), 26.1 (CH_3), 25.4 (CH_3) ppm; HRMS(ESI): Calcd for $C_{14}H_{25}ClO_6Na$: 347.1237. Found: 347.1230 $[M+Na]^+$.
22. (a) Liu, H. M.; Tsuda, Y. *Chem. Pharm. Bull* **1996**, *44*, 80; (b) Liu, H. M.; Xu, W.; Liu, Z. Z. *Carbohydr. Res.* **2001**, *331*, 229; (c) Liu, H. M.; Zhang, F. Y.; Zhang, J. Y.; Li, S. *Carbohydr. Res.* **2003**, *338*, 1737; (d) Liu, H. M.; Zhang, F. Y.; Zhang, J. Y. *Carbohydr. Res.* **2001**, *334*, 323.
23. ^{1}H NMR ($CDCl_3$, 400.1 MHz) δ = 4.68 (d, 1H, $J_{4,3}$ 3.5 Hz, H-4), 4.59 (d, 1H, *J* 2.5 Hz, H-6), 4.47 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{3,2}$7.1 Hz, H-3), 4.36 (d, 1H, $J_{1,2}$ 6.0 Hz, H-1), 4.22 (dd, 1H, $J_{2,1}$ 6.0 Hz, $J_{2,3}$ 7.1 Hz, H-2), 3.46 (s, 3H, CH_3O–), 3.43 (s, 3H, CH_3O–), 1.49 (s, 3H, CH_3), 1.43 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ = 204.8 (C═O), 111.2 (Me_2C), 104.6 (C-1), 79.6 (C-3), 76.9 (C-2), 68.2 (C-6), 62.4 (C-4), 56.8 (CH_3O), 54.3 (CH_3O), 27.5 (CH_3), 27.4 (CH_3) ppm; HRMS(ESI): Calcd for $C_{11}H_{19}ClO_6Na$: 305.0768. Found: 305.0771 $[M+Na]^+$.
24. ^{1}H NMR ($CDCl_3$, 400.1 MHz) δ = 8.84 (s, 1H, H-3), 8.08 (m, 2H, Ar-H), 7.74 (m, 2H, Ar-H), 4.50 (m, 1H, H-2′), 4.42 (d, 1H, $J_{4',3'}$6.4 Hz, H-4′), 3.97 (dd, 1H, $J_{3',4'}$ 6.4 Hz, $J_{3',2'}$ 7.7 Hz, H-3′), 3.49 (dd, 1H, $J_{1'a,2'}$ 3.7 Hz, $J_{1'a,1'b}$14.1 Hz, H-1′a), 3.45 (s, 3H, CH_3O–), 3.44 (s, 3H, CH_3O–), 3.27 (dd, 1H, $J_{1'b,2'}$ 7.8 Hz, $J_{1'b,1'a}$ 14.1 Hz, H-1′b), 1.37 (s, 3H, CH_3), 1.24 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ = 153.9 (C-2), 146.7 (C-3), 142.1 (C-Ar), 141.2 (C-Ar), 129.8 (C-Ar), 129.1 (C-Ar), 129.1 (C- Ar), 129.0 (C-Ar), 109.6 (Me_2C), 104.9 (C-4′), 79.1 (C-3′), 76.8 (C-2′), 55.9 (CH_3O), 53.8 (CH_3O), 40.1 (C-1′), 27.1 (CH_3), 26.7 (CH_3) ppm; HRMS(ESI): Calcd for $C_{17}H_{22}N_2O_4Na$: 341.1477. Found: 341.1467 $[M+Na]^+$.
25. Sallam, M. A. E.; Louis, F. F.; Anthonsen, T.; Quartey, E. G. K. *Carbohydr. Res.* **2000**, *324*, 1.
26. In vitro cytotoxicity study: Ec9706, human esophageal cancer cell line (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 penicillin, and 100 μg/mL 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 (1×10^4 cells/well). The next day compound at various concentrations diluted in culture medium was added (200 μL/well) to the wells. 48 h later 20 μl 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, and the IC_{50} value was determined.
27. Hazeldine, S. T.; Polin, L.; Kushner, J.; White, K.; Corbett, T. H.; Biehl, J.; Horwitz, J. P. *Bioorg. Med. Chem.* **2005**, *13*, 1069.