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Asymmetric synthesis of novel tetrahydroquinoline derivatives with a sugar building block and their bioactivities

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Abstract—Some novel spiro-tetrahydroquinolines were stereoselectively synthesized by using keto-sugar derived from sucrose as a building block in one pot under mild conditions. The in vitro immunobiological activity and cytotoxicity of these novel tetrahydroquinolines were investigated. The results implied that these spiro-compounds have obvious bioactivity and may be structurally modified to improve bioactivity further.

Tetrahydroquinoline (THQ) moiety is an essential structural unit of various natural products and pharmaceutical agents having a wide spectrum of biological activities,[1,2] hence many approaches have been developed for the construction of THQ skeleton.[3–6] Among these approaches, the inverse electron demand hetero-Diels–Alder reaction is regarded as one of the most powerful approaches to asymmetric six-membered heterocyclic compounds due to its high regioselectivity and diastereoselectivity.[7,8] Much previous work was accordingly focused on [4+2] cycloaddition reactions of electron-deficient Schiff bases with electron-rich dienophiles.[9,10] The study on the structure–activity relationships of THQs showed that the main attention should be paid to the following aspects: the stereochemistry of the THQ, the substitution on the THQ and the ring fused to the 3,4-position of the THQ.[11] Many efforts directed the electron-donating group toward the 4-position of THQ ring but the introduction of a substituent at the 3-position proved to be difficult.[12] Also, limited work has been devoted to asymmetric THQ derivatives.[13–15]

On the other hand, carbohydrates are attracting increasingly wide attention as their inherent biological activities and physicochemical properties are being better understood,[16] and have long been used as chiral auxiliaries or chiral building blocks and used in asymmetric transformations due to their known absolute stereochemistry, ready availability and often their low cost.[17,18] Therefore, the study related to carbohydrates will be the most attractive work in the future. Incorporation of carbohydrates into new biobased materials is likely to entail structural modifications to change their properties. On the basis of our experience and knowledge of stereochemistry of carbohydrates,[19,20] we attempted to search and employ a chiral sugar in stereoselective assembly of THQ derivatives to alter the stereochemistry and realize the combination of carbohydrates with bioactive products. We herein report a new and convenient method for asymmetric synthesis of THQ derivatives by using 1,4:3,6-dianhydro-D-fructose derived from sucrose as a chiral building block and their bioactivities.

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[21] and the further hydrolysis to afford 4-chloro-4-deoxy-α-D-galactose.[22] In the fractionation of the hydrolysis product with chromatography, we can also get 1,4:3,6-dianhydro-D-fructose (**1**) as a thick liquid, which is a chiral building block having three chiral centers and a highly reactive prochiral carbonyl group. Thus we attempted to incorporate the building block into asymmetric organic molecule bearing expected biological activity.

We planned to react Schiff base derived from amine and **1** with dienophile to prepare THQ derivatives and then

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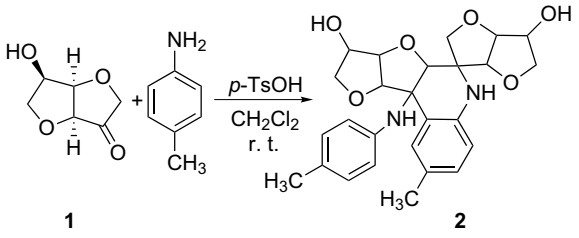
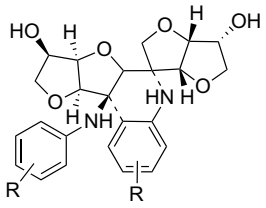
evaluate the bioactivity of the product obtained. Our first attempt was to treat **1** with equal equivalents of *p*-toluidine in dichloromethane (CH_2Cl_2) in the presence of catalytic toluene-*p*-sulfonic acid (*p*-TsOH) at ambient temperature. Unexpectedly, before the dienophile was added, a white precipitate was observed. Filtration of the mixture gave a white product. The HRMS, ^1H NMR, ^{13}C NMR, DEPT-135, and 2D NMR spectra[23] showed the new product to be THQ **2** (as shown in Scheme 1). The stereochemistry of **2** was finally established through single-crystal X-ray structure determination (Fig. 1)[24] after recrystallization from absolute ethanol. From the structure of **2** we noted that a arylamino group was introduced at 4-position and a furano ring was fused to 3,4-position of the THQ in the reaction. The product is structurally similar to 4-phenylamino-tetrahydroquinoline derivatives that have some potent bioactivities.[25] There are three chiral centers in the newly formed THQ ring (2*S*,3*S*,4*R*). To our knowledge, this is first example for utilization of keto-sugars in asymmetric synthesis of THQs despite their known chirality.

Under similar conditions, keto-sugar **1** was treated with several other anilines in CH_2Cl_2 and acetonitrile (MeCN), respectively, to illustrate the novelty of the present strategy and the results are presented in Table 1. When anilines bearing electron-donating substituents (entries 1–5) were used, the reaction afforded the expected corresponding THQ derivatives (**2**–**6**). But with



Scheme 1. Synthesis of THQ derivative **2**.

Table 1. Reactions of various anilines with keto-sugar **1**



2: R = *p*-CH_3
3: R = *p*-OCH_3
4: R = H
5: R = *p*-OH
6: R = *m*-CH_3
7: R = *p*-NO_2
8: R = *p*-Cl

Entry	Anilines	Solvents	Reaction time (h)	Products	Yields (%)
1	*p*-Toluidine	CH_2Cl_2	24	**2**	72
2	*p*-Aminoanisole	CH_2Cl_2	24	**3**	82
3	Aniline	CH_2Cl_2	24	**4**	65
4	*p*-Aminophenol	CH_2Cl_2	48	**5**	16
5	*m*-Toluidine	CH_2Cl_2	48	**6**	36
6	*p*-Nitroaniline	CH_2Cl_2	48	**7**	0
7	*p*-Chloroaniline	CH_2Cl_2	48	**8**	0
8	*p*-Toluidine	MeCN	24	**2**	81
9	*p*-Aminoanisole	MeCN	24	**3**	83
10	Aniline	MeCN	24	**4**	71
11	*p*-Aminophenol	MeCN	48	**5**	55
12	*m*-Toluidine	MeCN	48	**6**	49
13	*p*-Nitroaniline	MeCN	48	**7**	0
14	*p*-Chloroaniline	MeCN	48	**8**	0

p-aminophenol (entry 4) the reaction gave product **5** in poor yield due to the poor solubility of *p*-aminophenol in CH_2Cl_2. In order to improve the efficiency of entry 4, we used MeCN to replace CH_2Cl_2 as the solvent in the reaction. Product **5** was successfully obtained in a better yield (55%, entry 11). When MeCN was used in all the other reactions, we found that the yields were all improved in different degrees (entries 8–10, and 12). When aniline was *m*-toluidine (Table 1, entry 5), we found that not C-2 but C-6 on benzene ring was incorporated into the heterocyclic core (product **6**) for the hindrance of methyl group at C-3. The configuration of **6** was established by single crystal X-ray analysis (Fig. 1)[26] also. However, anilines bearing electron-withdrawing groups (entries 6, 7, 13, and 14) used in the

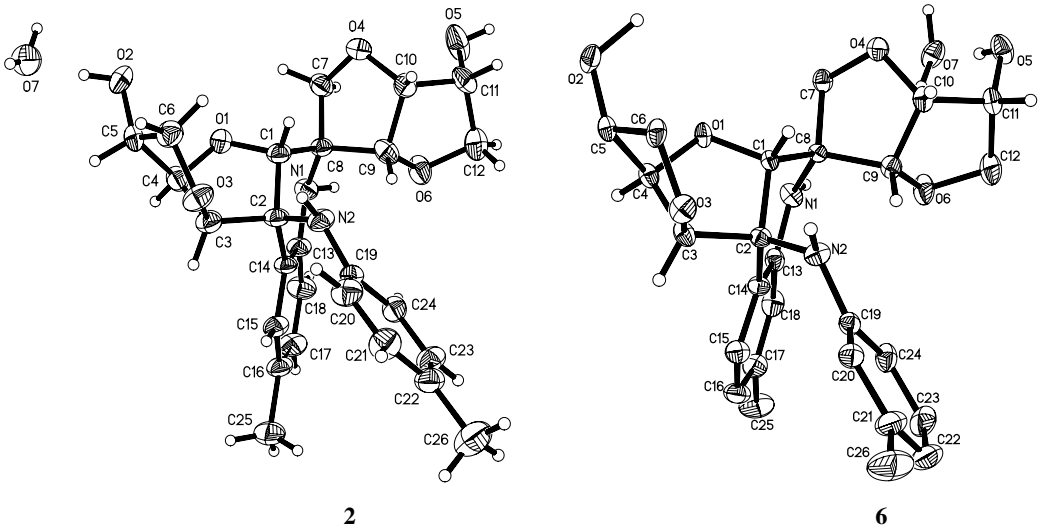
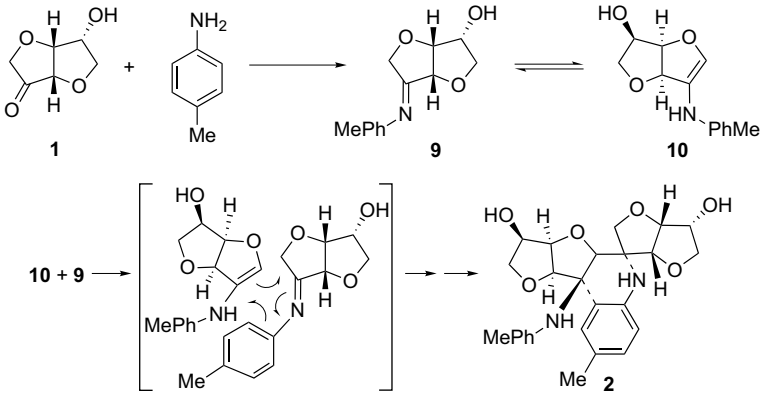


Figure 1. ORTEP of THQ derivative **2** and **6**.



Scheme 2. Proposed pathway for the formation of THQ.

reaction, either in CH_2Cl_2 or in MeCN, gave no product **7** and **8**. All the products obtained were spectroscopically characterized.

The pathway for the formation of THQ derivative is proposed in Scheme 2. Treatment of keto-sugar **1** with aniline in the presence of *p*-TsOH gave first Schiff base **9**. Then **9** was competitively isomerized to enamine **10** and a dynamic equilibrium between the two isomers was founded. Finally, the self-cycloaddition of **9** with **10** led to the formation of Doebner–von Miller intermediate type THQ derivative.[27] Only *exo–exo* combination product was obtained in the cycloaddition process as shown in Figure 1, which resulted not only from the regio-control of the inverse electron demand Diels–Alder reaction but also from the stereochemistry of substrates for the existence of three chiral centers at both azadiene **9** and dienophile **10**.

The electron donating group (EDG) on the aromatic ring increases the HOMO energy of the electron-rich enamine dienophile, subsequently decreases the energy gap between the azadiene LUMO and dienophile HOMO, although it enhanced the LUMO energy of azadiene to some extent. Therefore, the anilines bearing EDG are favorable to the cycloaddition reaction.

The in vitro immunobiological activities of these THQs were tested using the lymphocyte proliferation assay.[28] We found that compound **6** has an obviously stimulatory effect on T lymphocyte proliferation. The OD values of compound **6** were 0.316 ± 0.019 (25 μg/mL) and 0.356 ± 0.015 (50 μg/mL), much higher than that of the control with concanavalin A (0.259 ± 0.016).

The in vitro cytotoxicity[29] of these novel THQ derivatives **2**–**6** and **2**-diacetate (**2′**) against human cancer cell lines were evaluated in two assay systems, that is, Eca-109 and Hela cell lines. The results were presented in Table 2, which implied that the cytotoxicity was sensitive to a variety of functional groups and position of the substitution at benzene ring. Conversion of two hydroxyl groups at the sugar ring into two *O*-acetyl groups (from THQ **2** to **2′**) allowed significantly diminished IC_{50} values (from >1000 diminished to 150 for Hela and from 240 to 135 for Eca-109), which was indicative that transformation of hydroxyl group at the sugar ring is another effective way to improve the cytotoxicity.

Table 2. IC_{50} (μg mL^{-1}) of THQ derivatives **2**–**6**, and **2′** against human cancer cell lines

	2	**2′**	**3**	**4**	**5**	**6**
Hela[a]	—[c]	150	648	—	—	276
Eca-109[b]	240	135	—	—	78	95

[a] Human cervical carcinoma cell lines, time 48 h.
[b] Human esophagus cancer cell lines, time 72 h.
[c] Symbol '—' represents a IC_{50} value of >1000.

In summary, we have synthesized asymmetric polycyclic spiro-tetrahydroquinoline derivatives by using the chiral building block derived from sucrose. The formation of THQs was stereocontrolled by the stereochemistry of sugar, which is the first example for incorporation of keto-sugar building block into THQs. The methodology undoubtedly extends the utilization of carbohydrates in asymmetric synthesis of biobased heterocyclic compound. The investigation of bioactivity of the THQ derivatives showed the obvious in vitro immunocompetence and cytotoxicity. The results indicated that incorporation of carbohydrates into new biobased materials could entail structural modifications to endow them with new properties. The further structural modification of these compounds to improve the bioactivity is in progress.

Supplementary data

CCDC-243889 (derivative **2**) and CCDC-243890 (derivative **6**) 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.

Acknowledgements

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23. ^{1}H NMR (400.1 MHz, DMSO-d_6, 25 °C, TMS): δ = 1.97 (s, 3H, PhCH_3), 2.09 (s, 3H, PhCH_3), 3.45 [t, $^2J_{H,H}$ = 8.4 Hz, 1H, C(12)–H$_b$], 3.49 [d, $^2J_{H,H}$ = 8.4 Hz, 1H, C(7)–H$_b$], 3.66 [t, $^2J_{H,H}$ = 8.4 Hz, 1H, C(6)–H$_b$], 3.70 [dd, $^3J_{H,H}$ = 7.2 Hz, $^2J_{H,H}$ = 8.4 Hz, 1H, C(12)–H$_a$], 3.93 [s, 1H, C(1)–H], 3.98 [m, 2H, C(6)–H$_a$ and C(11)–H], 4.01 [t, $^3J_{H,H}$ = 7.2 Hz, 1H, C(4)–H], 4.15 [dd, $^3J_{H,H}$ = 7.2, 11.6 Hz, 1H, C(5)–H], 4.19 [d, $^2J_{H,H}$ = 8.4 Hz, 1H, C(7)–H$_a$], 4.29 [t, $^3J_{H,H}$ = 4.8 Hz, 1H, C(9)–H], 4.44 [d, $^3J_{H,H}$ = 4.8 Hz, 1H, C(10)–H], 4.45 [d, $^3J_{H,H}$ = 7.2 Hz, 1H, C(3)–H], 6.25 [d, $^3J_{H,H}$ = 8.0 Hz, 2H, C(20)–H and C(24)–H], 6.49 [s, 1H, C(15)–H], 6.66 [d, $^3J_{H,H}$ = 8.0 Hz, 1H, C(18)–H], 6.72 [d, $^3J_{H,H}$ = 8.0 Hz, 2H, C(21)–H and C(23)–H], 6.79 [dd, $^3J_{H,H}$ = 8.0 Hz, $^4J_{H,H}$ = 1.6 Hz, 1H, C(17)–H] ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C, TMS): δ = 20.3, 20.5, 65.2, 65.3, 72.1, 72.3, 72.5, 72.6, 73.4, 80.2, 81.1, 81.2, 82.1, 90.1, 115.0, 117.4, 119.2, 124.6, 127.1, 128.8, 129.1, 130.1, 142.3, 142.9 ppm; HRMS: Calcd for $C_{26}H_{30}N_2O_6$: 466.2104. Found: 466.2106 $[M]^+$, 489.2004 $[M+Na]^+$.
24. Crystal data for **2**: $C_{26}H_{32}N_2O_7$ ($C_{26}H_{30}N_2O_6{\cdot}H_2O$), M = 484.54, monoclinic, a = 8.5849 (17), b = 7.4415 (15), c = 18.326 (4) Å, α = 90°, β = 91.45 (3)°, γ = 90°, V = 1170.4 (4) Å^3, T = 291 (2) K, space group $P2(1)$, Z = 2, D = 1.375 Mg m^{-3}, μ (Mo Kα) = 0.100 mm^{-1}, θ range 1.11–27.56, F(0 0 0) = 516, 2518 reflection collected, 2518 unique [R(int) = 0.0000], final R indices [$I > 2\sigma(I)$]: R_1 = 0.0654, wR_2 = 0.1301, R indices (all data): R_1 = 0.1062, wR_2 = 0.1448.
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26. Crystal data for **6**: $C_{26}H_{32}N_2O_7$ ($C_{26}H_{30}N_2O_6{\cdot}H_2O$), M = 484.54, monoclinic, a = 8.3153 (17), b = 7.6786 (15), c = 19.259 (4) Å, α = 90°, β = 98.29 (3)°, γ = 90°, V = 1216.8 (4) Å^3, T = 293 (2) K, space group $P2(1)$, Z = 2, D = 1.322 Mg m^{-3}, μ(Mo Kα) = 0.096 mm^{-1}, θ range 2.48–26.49, F(0 0 0) = 516, 3592 reflection collected, 3496 unique [R(int) = 0.0897], final R indices [$I > 2\sigma(I)$]: R_1 = 0.0677, wR_2 = 0.1453, R indices (all data): R_1 = 0.1194, wR_2 = 0.1668.
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28. T lymphocyte proliferation assay: Spleen was taken from 4–6 week old Kunming mice under sterile conditions and gently crushed to make single cell suspension. Erythrocytes in the cell pellet were eliminated by using Tris–ammonium chloride–lysis buffer (pH 7.2), followed by washing three times with fresh RPMI 1640 media. The cell-suspension was adjusted to 4×10^6 cells/mL in complete RPMI 1640 media supplemented with 10% of fetal bovine serum (FBS), 100 IU/mL penicillin and 100 IU/mL streptomycin.
    To each well in a 96-well microplate were added 50 μL of splenic cell suspension and 50 μL of RPMI 1640 medium containing 8 μg/mL of concanavalin A. Then 100 μL of RPMI 1640 containing THQ was added to each well to make a final cell count of 2×10^5 cells/well. The final concentrations of THQ in the wells were 25, 50, 100 μg/mL. Control wells were prepared similarly without Con-A or drugs. Plates were incubated for 48 h in the incubator (37 °C, humidified 5% CO_2 atmosphere). Media (150 μL) was gently removed and 150 μL of fresh FBS-free media containing 66.7 μg/mL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium (MTT) was added to each well. Following another 4 h incubation, the media was removed, and 150 μL of DMSO was added. After the plate was shaken for 10 min, absorbance was read at 570 and 630 nm on a spectrophotometric plate reader (PowerWave$_x$, BIO-TEK INSTRUMENT, INC), respectively. OD values were achieved by subtracting the absorbance at 630 nm from the 570 nm absorbance.
29. In vitro cytotoxicity study: Hela and Eca-109 cell lines were pursed from the Institute of Biochemistry and Cell Biology, SIBS, CAS. Culture medium was RPMI-1640 supplemented with 10% (v/v) fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin.
    Sensitivity test: Cancer cells, growing in monolayer cultures at 37 °C under 5% CO_2, were trypsinized, rinsed with PBS (w/o Ca^{2+} and Mg^{2+}) and plated into 96-well-plates (6×10^3 cells/well). The next day test substances were freshly dissolved in DMSO resulting in 10 mg/mL stock solutions. Stock solutions were diluted in culture medium and added (200 μL/well) at various concentrations to the wells, resulting in seven final concentrations between 1 and 100 μg/mL. 48 or 72 h later the MTT-test was performed.