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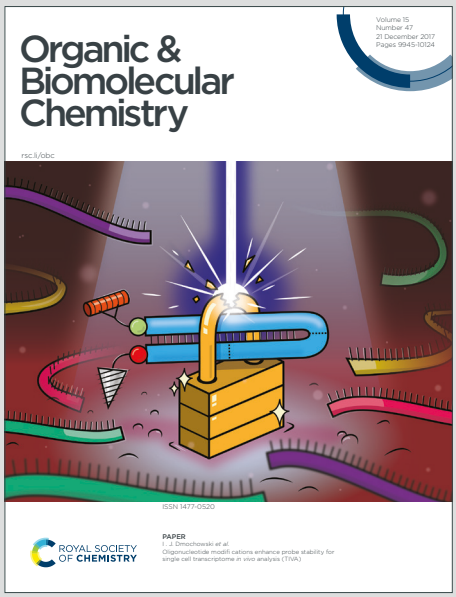

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Synthesis of disaccharide modified berberine derivatives and their anti-diabetic investigation in zebrafish using a fluorescence-based technology

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Abstract

Berberine is a naturally occurring isoquinoline alkaloid and has been used as important functional food additive in China due to its various pharmacological activities. Berberine exhibits great potential for developing anti-diabetic agents against type 2 diabetes mellitus (T2DM), as it can reduce the blood glucose level in many animal models. However, the low anti-diabetic activity and poor bioavailability of berberine (below 5%) by oral administration significantly limit its practical applications. To solve these problems, this article focuses on the structural modification of berberine using some disaccharide groups, because the carbohydrate moiety has been proved to improve the bioavailability and enhance the receptor-binding affinity of drugs. Anti-diabetic investigation of the synthesized compounds was performed in zebrafish model using a fluorescently labeled glucose analog 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) as glucose tracker. The results indicated that modification of berberine with carbohydrate groups could give derivatives with improved anti-diabetic activity, in particular the diglucose modified berberine derivative **1** which could dramatically promote the uptake of 2-NBDG both in zebrafish larvae and their eyes even at very low concentrations. Furthermore, the fluorescence-based anti-diabetic investigation method in zebrafish shows great potential for anti-diabetic drug screening.

Keywords: Berberine, Disaccharide, Anti-diabatic, Zebrafish, 2-NBDG

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Introduction

Diabetes is a chronic metabolic disorder disease that threatens human health all over the world. The number of people having diabetes is increasing every year, and it is estimated that over 422 million people worldwide have diabetes in 2019. Type 2 diabetes mellitus (T2DM), which occupies 90-95% of diabetes, is characterized as abnormal hyperglycaemic state caused by insulin resistance and impaired insulin secretion.[1] Treatment of T2DM can be achieved by oral hypoglycemic drugs including metformin, pioglitazone, exenatide, pramlintide, thiazolidinediones, and sulphonylureas.[2, 3] However, currently used drugs always suffer from many adverse effects, such as hypoglycemia, lactic acidosis, peripheral edema, and serious hepatotoxicity and gastrointestinal reaction caused by chronic administration.[4, 5] Therefore, it is of great importance to develop novel anti-diabetic agents with good hypoglycemic activity but low side effects.

Berberine is a naturally occurring isoquinoline alkaloid isolated from the well-known Chinese herb *Coptischinensis*.[6-9] In China and India, berberine is widely used for treating gastrointestinal diseases caused by bacteria infection, such as gastroenteritis and diarrhea. Recent studies have revealed that berberine exhibits various pharmacological activities, such as anti-diabetic,[10, 11] anti-leishmanial,[12] anti-inflammation,[13-16] anti-oxidative,[17, 18] anti-cancer,[19-23] anti-cardiovascular[24] and anti-alzheimer's diseases.[15, 25, 26] Due to the positive effect on human health described above, berberine has been employed as important functional food added to tea and beverages in China.[27] The anti-diabetic effect of berberine attracts increasing interests recently, because it can obviously increase the glucose consumption in cells and reduce the blood glucose level in animal models.[11, 28-30] Molecular mechanism investigation reveals that berberine shows good inhibition activity on α-glucosidase, thereby restraining the hydrolyzation of polysaccharides to result in low glucose level *in vivo*.[31] Berberine also affects glucose level through some other signal pathways, such as activating AMP-activated protein kinase (AMPK), protecting the function of pancreatic β-cells, down-regulating the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) gene, and inhibiting the function of mitochondria.[32-37] Human clinical study has proved that berberine can reduce the fasting and post-load plasma glucose level in T2DM patients.[38, 39] All these data indicate that berberine should be a promising lead compound for developing high efficient anti-diabetic drugs with low side effects.

However, the major problem for developing berberine-based drugs against T2DM is the poor bioavailability (below 5% by oral administration).[30] The poor water solubility of berberine caused by the rigid planer structure and quaternary ammonium unit (Scheme 1) always leads to very low absorption efficiency in gastrointestinal tract.[40] In previous research, the pharmacokinetic study of berberine in human was carried out on a liquid chromatography–electrospray ionization–mass

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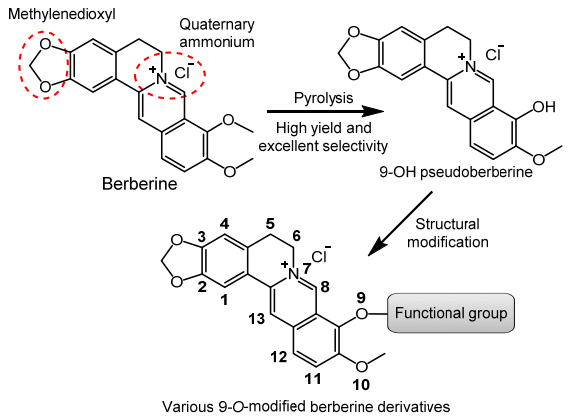
spectrometry (LC–ESI–MS), which revealed that the C_{max} of berberine in human plasma was as low as 0.4 ng/mL after oral administration of 400 mg of berberine.[41, 42] Another problem is the moderate anti-diabetic activity of berberine compared with the clinically used anti-diabetic drugs.[27] To overcome these problems, many structurally modified berberine derivatives have been prepared, and some of them exhibit improved anti-diabetic effect.[43-51] For example, the 8,8-dimethyldihydroberberine[45] and 9/10-OH pseudoberberines[51] exhibit higher anti-diabetic activity than berberine. Structure-activity relationship investigation reveals that the methylenedioxyl group and quaternary ammonium unit (Scheme 1) in berberine are two key structural components related to its anti-diabetic activity.[50] Removal of the methylenedioxyl group or reduction of the quaternary ammonium to a tertiary amine will result in significant decrease of the anti-diabetic effect of berberine. Among all the references reported previously, modification of berberine on its 9-*O*-position is the most attracting work, as it can obviously enhance its anti-diabetic activity.[43, 44, 46] Furthermore, the 9-*O*-modified berberine derivatives can be easily synthesized by constructing a key intermediate 9-OH pseudoberberine (Scheme 1), obtained by pyrolysis of berberine with high yield and excellent selectivity.[52, 53] In contrast, the 8-*C*-, 13-*C*- and 10-*O*-modified berberine derivatives are always achieved with very low overall yields, due to the poor leaving activity of hydrogen atom and the low selectivity of 10-*O*-demethylation reaction.

Scheme 1. Construction of 9-OH pseudoberberine and 9-*O*-modified berberine derivatives



Glycosylation has been proved as an efficient strategy for drug modification, because it can improve the bioavailability and enhance the receptor-binding affinity of drugs.[54-57] Therefore, many glycosylated drugs or natural products have been developed in the past several years.[58-61] In 2012, Chen and coworkers[43] synthesized a 9-*O*-glucosylated berberine derivative, a major metabolite of berberine *in vivo*.[62, 63] This derivative exhibited improved bioavailability and anti-diabetic effect, but

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could be easily degraded into 9-OH pseudoberberine under mild conditions due to the presence of a *O*-glycosidic bond.[43] Later, Han and coworkers[48] synthesized some 9-*O*-monosaccharide modified berberine derivatives with good water solubility and high stability under acidic condition, and some of them exhibited improved but moderate anti-diabetic activity compared with berberine. Compared with monosaccharide moiety, disaccharide should have excellent water solubility due to the presence of more hydroxyl groups. Furthermore, previous reports have revealed that conjugation of drugs with disaccharide moiety can improve their biological activity, because carbohydrate plays critical role in mediating the cellular uptake of disaccharide-drug conjugates.[64-68] For example, bleomycin bearing a disaccharide group exhibits higher tumor cell targetability than monosaccharide modified bleomycin.[64] Therefore, this article focuses on the synthesis and biological investigation of a set of 9-*O*-disaccharide modified berberine derivatives. The disaccharide building blocks are prepared by a highly efficient procedure from different types of water-soluble monosaccharides, such as glucose, mannose and ribose. Conjugation of disaccharides with berberine is accomplished using a stable triazole group as linker, which can avoid the degradation reaction occurred in 9-*O*-glycosylated berberine derivative. All the synthesized compounds were subjected to anti-diabetic investigation in zebrafish model using a fluorescence-based technology. In order to conveniently visualize the glucose uptake in zebrafish, a fluorescently labeled glucose mimetic 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose[69] (2-NBDG, Figure 1) with low cytotoxicity is used as bioprobe for zebrafish maintenance. 2-NBDG has been proved to exhibit higher binding ability towards glucose transporters in cells than glucose, and can be taken in by zebrafish as glucose.[70, 71]

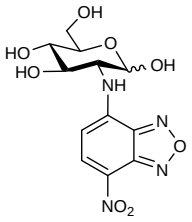

Figure 1. Structure of 2-NBDG

Results and discussion

Synthesis of disaccharide modified berberine derivatives

Disaccharide building blocks **6**–**10** were synthesized from previously reported monosaccharide β-D-glucopyranosyl azide **11**[48] as shown in Scheme 2. Selective protection of the 6-OH group in **11** with a *tert*-butyldimethylsilyl (TBS) group followed by benzoylation of the remaining hydroxyl

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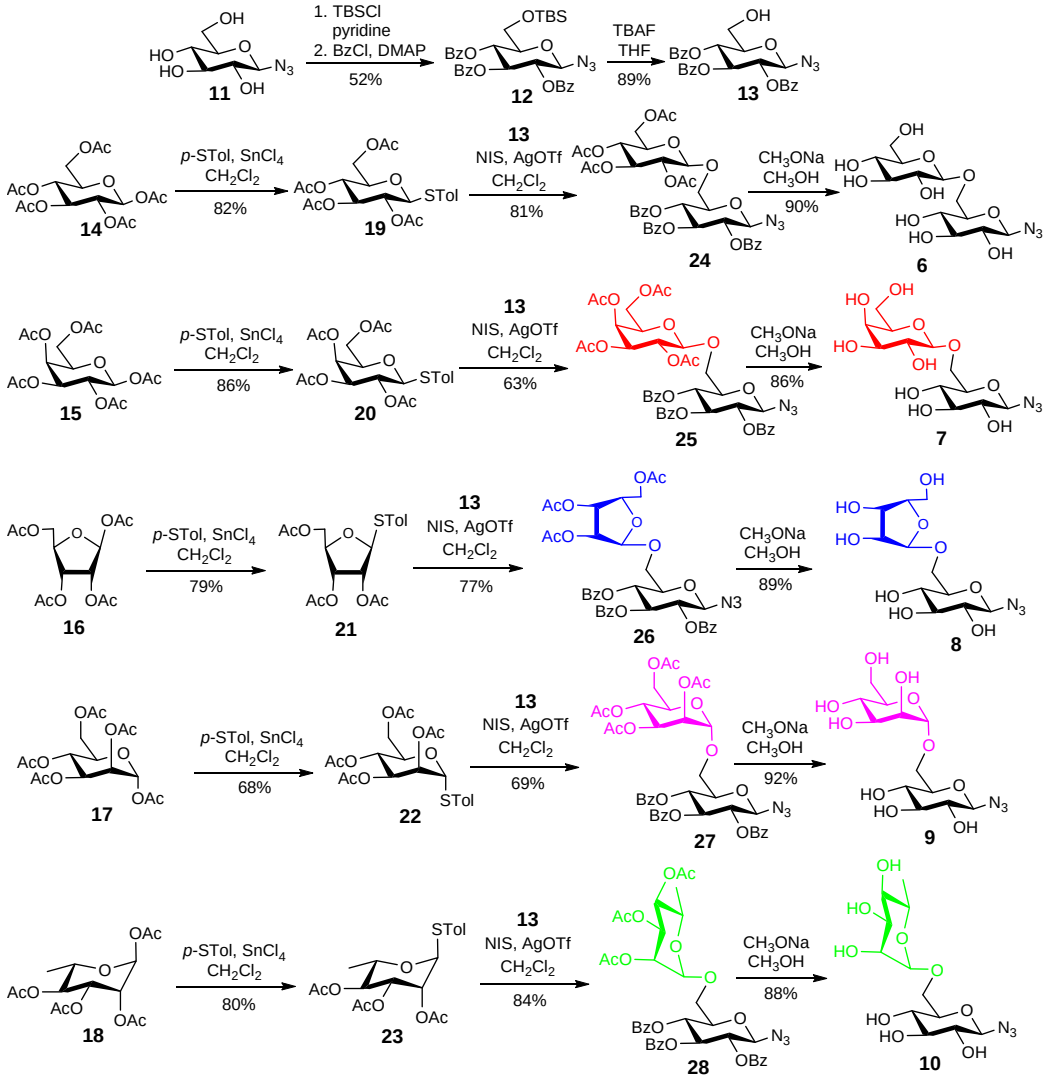
groups (OH) afforded compound **12** with 52% total yield. Subsequent selective removal of the TBS group in **12** promoted by tetrabutylammonium fluoride (TBAF) provided glycosyl acceptor **13** with 89% isolated yield. On the other hand, reaction of fully acetylated sugars **14**–**18** with *p*-thiocresol (*p*-STol) under the influence of stannic chloride ($SnCl_4$) gave glycosyl donors **19**–**23**, which were subjected to standard glycosylation procedure described previously[72, 73] with acceptor **13** in the presence of *N*-iodosuccinimide (NIS) and silver trifluoromethanesulfonate (AgOTf) to provide fully protected disaccharides **24**–**28**. Cleavage of all the acetyl (Ac) and benzoyl (Bz) groups in **24**–**28** using sodium methoxide (CH_3ONa) in methanol smoothly furnished the disaccharide building blocks **6**–**10** with high total yields.

Scheme 2. Synthesis of carbohydrate building blocks **6**–**10**



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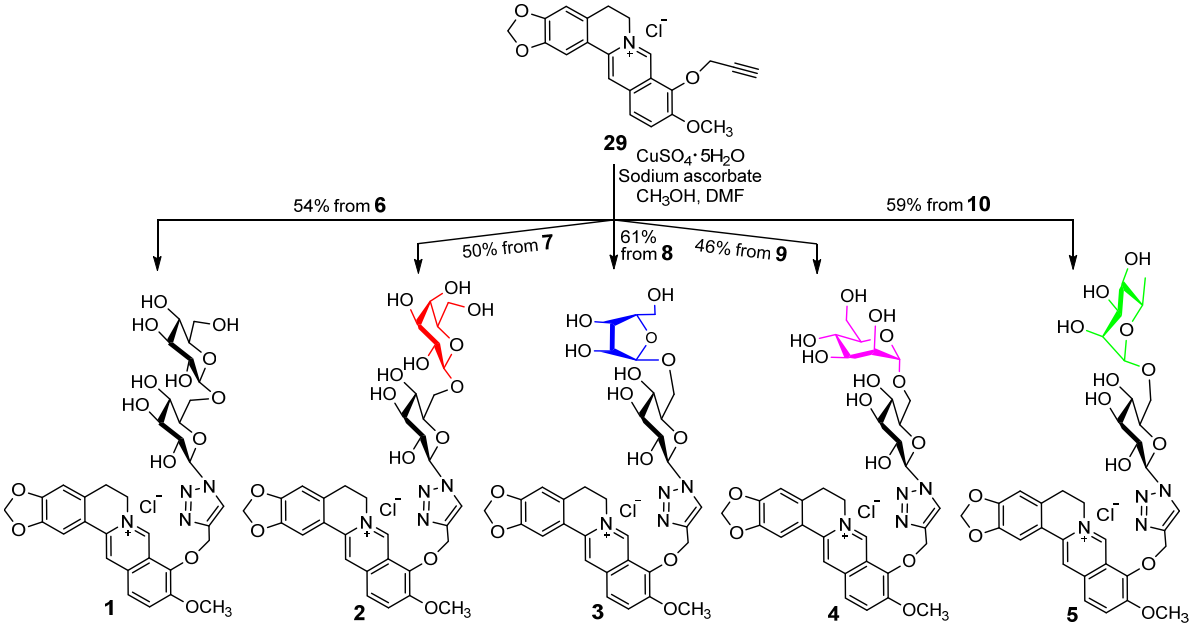
Conjugation of alkynylated berberine derivative **29**[52] with disaccharide building blocks **6**–**10** was achieved by the classcial "click" chemistry[74] in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate (Scheme 3), which afforded the desired disaccharide modified berberine derivatives **1**–**5**. Purification of the target compounds on silica gel column chromatography resulted in very low isolated yields (<10%), possibly due to the high polarity of disaccharide modified berberine derivatives. To solve this problem, all the compounds were subjected to a neutral alumina column chromatography, which gave derivatives **1**–**5** with moderate isolated yields (46%–61%).

Scheme 3. Synthesis of disaccharide modified berberine derivatives **1**–**5**



Uptake of 2-NBDG in zebrafish

Biological investigations are performed in zebrafish model, because drug screening can be conveniently carried out using 24/48-well plate format compared with the currently used animal models.[75] More importantly, zebrafish owns highly similar genome (~80%) and glucose transportation mechanisms as humans,[76-79] which indicates its great potential for anti-diabetic investigation *in vivo*. Therefore, the zebrafish model had been used by Williams group for studying the anti-diabetic activity of various isolated natural products, and 2-NBDG with green fluorescent emission was employed for conveniently visualizing the glucose uptake in zebrafish.[70]

In our work, the 3-day old zebrafish larvae are employed for drug screening because of the well-worked glucose transportation and metabolism at this stage,[79, 80] and the transparent body of larvae makes 2-NBDG uptake being easily visualized on a fluorescent microscopy.[70] Therefore, we

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firstly investigated the 2-NBDG uptake in zebrafish by treating 3-day old larvae with different concentrations (0, 0.1 0.2, 0.6, 1, and 2 mM) of 2-NBDG for 3 h. The results were depicted in Figure 2A, zebrafish larvae without any treatment gave very weak background interference in abdominal cavity, due to the presence of interfering biological species, such as ascorbic acid (AA) and dehydroascorbic acid (DHA).[81] In contrast, addition of 2-NBDG led to obvious fluorescence enhancement with a dose-dependent dependence on the 2-NBDG concentration (Figure 2B), thus indicating the uptake of 2-NBDG in zebrafish. This result was also proved by the enhanced fluorescent emission in zebrafish eyes (Figure 2C), an organ that could express a large amount of glucose transporters[80] for 2-NBDG transportation. To achieve the anti-diabetic drug screening, the optimized concentration for zebrafish incubation was 0.6 mM, as low or high concentration gave too weak or strong fluorescence which was not suitable for discriminating the fluorescence improvement. In parallel, the time-dependent uptake of 2-NBDG in zebrafish was carried out by treating zebrafish larvae with 0.6 mM of 2-NBDG for 0, 1, 2, 3, 6, and 12 h as shown in Figure 2D, 2E and 2F. The results indicated that 2-NBDG could be taken in zebrafish after 0.5 h of incubation, and the fluorescent signal became steady in 3 h. Consequently, the optimized incubation time was 3 h. To confirm the uptake of 2-NBDG was promoted by glucose transporter, zebrasfish larvae were further competitively coincubated with glucose (0.6 mM) and 2-NBDG (0.6 mM) for 3 h. As a result, the fluorescent signal in zebrafish obviously decreased (Figure S1), which revealed that the uptake of 2-NBDG was significantly reduced due to the competitive uptake of glucose. These results gave solid evidence that uptake of 2-NBDG was mainly caused by glucose transporter, which could promote the uptake of glucose. In our experiments, the Image J software was used for calculating the integrated optical density (IOD). The 2-NBDG in abdominal cavity that was not transported to tissues of zebrafish might give interfering fluorescent signals, therefore, the fluorescence in abdominal cavity was deducted.

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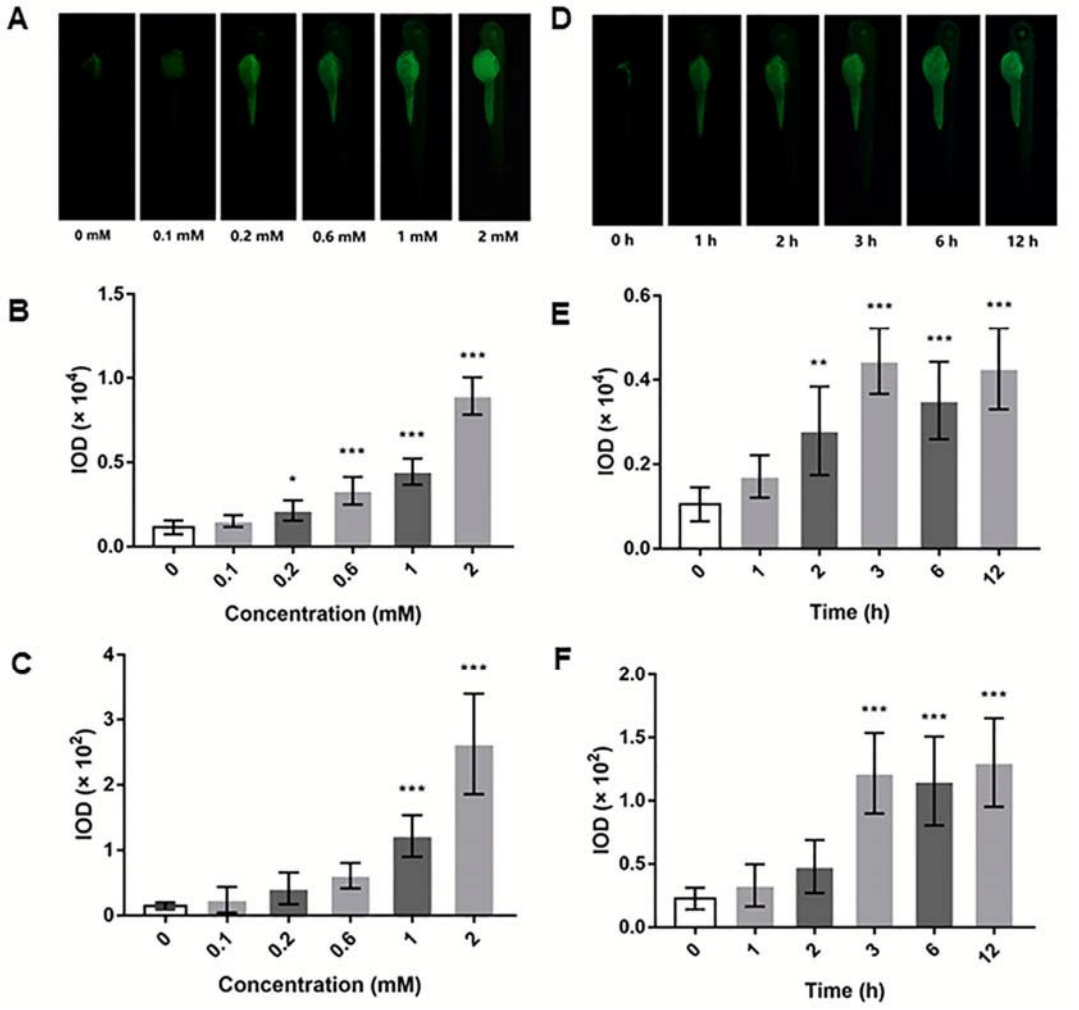

Figure 2. Time and dose-dependent uptake of 2-NBDG in zebrafish. (A) Treatment of 3-day old zebrafish with different concentrations (0, 0.1, 0.2, 0.6, 1, and 2 mM) of 2-NBDG for 3 h could induce increased fluorescent emission; (B) Integrated optical density (IOD) in zebrafish after incubating with different concentrations of 2-NBDG; (C) IOD in zebrafish eyes after incubating with different concentrations of 2-NBDG; (D) Treatment of 3-day old zebrafish with 0.6 mM of 2-NBDG for 0, 1, 2, 3, 6 and 12 h could induce increased fluorescent emission; (E) IOD in zebrafish after incubating with 0.6 mM of 2-NBDG for 0, 1, 2, 3, 6 and 12 h; (F) IOD in zebrafish eyes after incubating with 0.6 mM of 2-NBDG for 0, 1, 2, 3, 6 and 12 h. **P* <0.05, ***P* <0.01, ****P* <0.001 vs zebrafish treated with 0 mM 2-NBDG. The fluorescence in abdominal cavity was deducted to remove the interfering fluorescent signals.

Anti-diabetic investigations

To study the anti-diabetic activity of the synthesized compounds, 3-day old zebrafish larvae were treated with compounds **1**–**5** and berberine (10 μM) for 1 h, which was followed by incubating with 2-NBDG (0.6 mM) for 3 h. According to the literature, treating larvae with tested compounds for 1 h could induce 2-NBDG uptake accompanied with dramatic fluorescent emission.[70] Images of larvae were captured on a fluorescent microscopy, and the results were depicted in Figure 3A and 3B.

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Compared with larvae treated with 2-NBDG only, incubation of larvae with berberine and 2-NBDG could result in obvious fluorescent enhancement, thereby proving the improvement of 2-NBDG uptake in zebrafish and the moderate anti-diabetic activity of berberine. In contrast, treatment of larvae with 2-NBDG and berberine derivative **1** could cause significant fluorescence improvement and high 2-NBDG uptake, and other berberine derivatives **2**–**5** also induced detectable fluorescence enhancement. These results indicated that introducing disaccharide group to berberine could enhance its anti-diabetic activity, in particular the diglucose modified berberine derivative **1** which exhibited the highest anti-diabetic effect. This result was confirmed by the dramatic fluorescence improvement in zebrafish eyes (Figure 3C), because the overexpression of glucose transporters promoted by compounds **1**–**5** could enhance the uptake of 2-NBDG. As a comparison, the anti-diabetic activity of emodin (a natural product with high anti-diabetic activity) and monoglucosylated berberine **30** (Figure 3D) was investigated in zebrafish through the procedure described above. The results indicated that diglucose modified berberine **1** could induce significant fluorescence improvement as emodin, thereby proving again the good anti-diabetic activity of compound **1**. In addition, incubation of zebrafish larvae with glucose and diglucose **6** could induce no detectable fluorescence improvement (Figure S1), which indicated that the fluorescence signal was not induced by glucose hydrolysis.

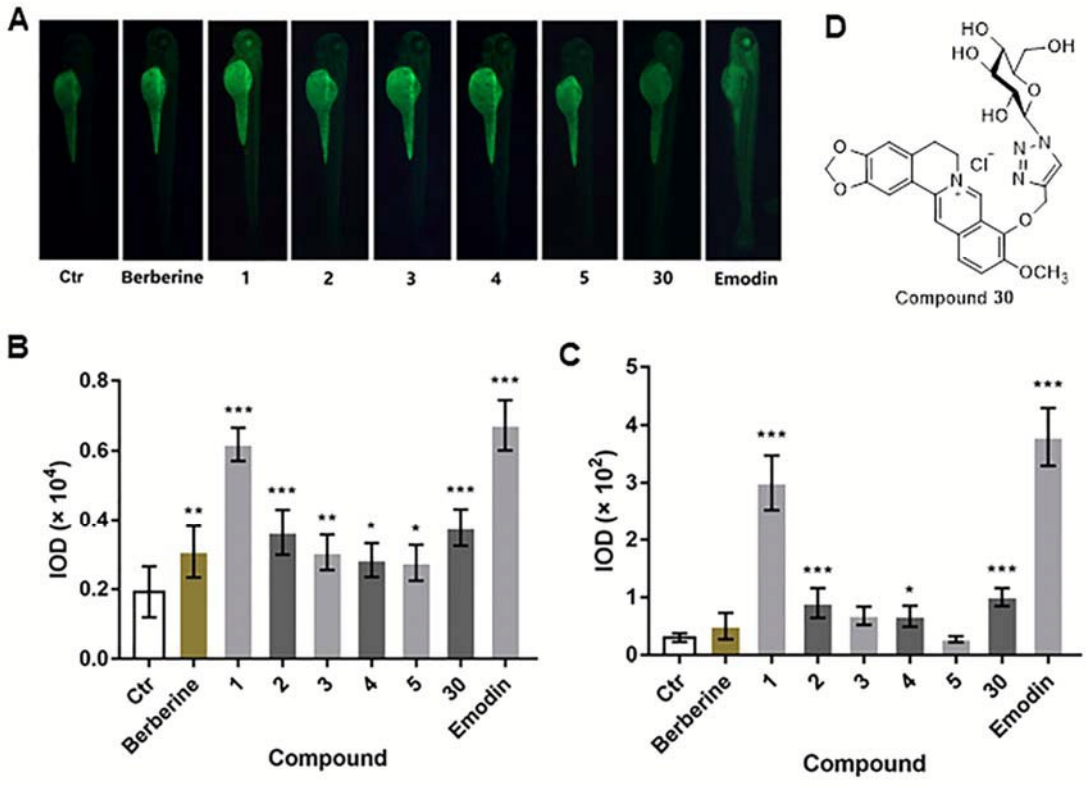

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Figure 3. Berberine derivative induced 2-NBDG uptake in zebrafish. (A) Treatment of 3-day old zebrafish with 10 μM of tested compounds for 1 h and 0.6 mM of 2-NBDG for 3 h could induce obvious fluorescent emission; (B) IOD in zebrafish after incubating with tested compounds and 2-NBDG; (C) IOD in zebrafish eyes after incubating with tested compounds and 2-NBDG. Control group (Ctr) was incubated with E3 water for 1 h, and then treated with 0.6 mM of 2-NBDG for 3 h. **P* <0.05, ***P* <0.01, ****P* <0.001 vs Ctr. The fluorescence in abdominal cavity was deducted to remove the interfering fluorescent signals.

Diglucose modified berberine derivative **1** with high anti-diabetic activity was further subjected to a dose-dependent investigation in zebrafish. The 3-day old larvae were incubated with different concentrations (0, 2.5, 5, 10, 20, and 40 μM) of **1** for 1 h, and then treated with 2-NBDG (0.6 mM) for 3 h. As shown in Figure 4, fluorescent images of larvae revealed that 10 μM of compound **1** could significantly promote the uptake of 2-NBDG in body and eyes of zebrafish, while increasing the concentration induced inapparent fluorescence improvement. These results indicated that compound **1** showed excellent anti-diabetic effect even at low concentrations.

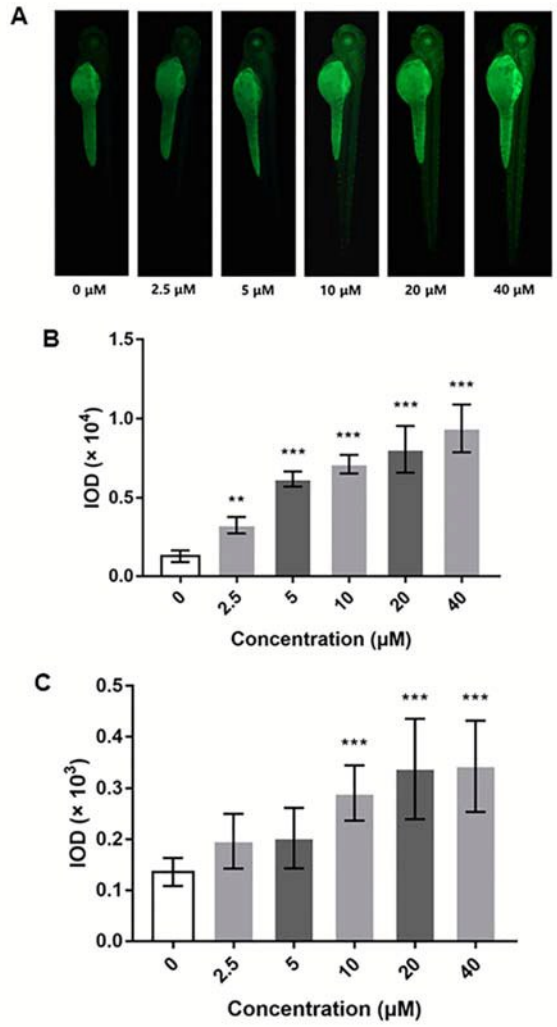

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Figure 4. (A) 2-NBDG uptake in zebrafish incubated with different concentrations (0, 2.5, 5, 10, 20, and 40 μM) of **1** for 1 h, and then treated with 2-NBDG (0.6 mM) for 3 h; (B) IOD in zebrafish after incubating with **1** and 2-NBDG; (C) IOD in zebrafish eyes after incubating with **1** and 2-NBDG. ***P* <0.01, ****P* <0.001 vs zebrafish treated with 0 mM 2-NBDG. The fluorescence in abdominal cavity was deducted to remove the interfering fluorescent signals.

Conclusions

In this paper, we synthesized some novel disaccharide modified berberine derivatives **1**–**5**. Attachment of disaccharide moiety with berberine was accomplished by the classical "click" chemistry, which afforded the target compounds with high total yields. Anti-diabetic investigation of the synthesized derivatives was performed in 3-day old zebrafish larvae, and 2-NBDG with strong fluorescent emission was used for tracking the glucose transportation on a fluorescent microscope. As a result, zebrafish larvae incubated with berberine derivative **1** and 2-NBDG could result in significant fluorescence improvement, which indicated the huge uptake of 2-NBDG in zebrafish. Furthermore, the zebrafish eyes also emitted strong fluorescent signals, thereby proving the overexpression of various glucose transporters that could promote the uptake of 2-NBDG. Dose-dependent investigation revealed that treatment of zebrafish with low concentration (10 μM) of compound **1** could promote the uptake of 2-NBDG both in zebrafish larvae and their eyes. These results indicated that the diglucose modified berberine derivative **1** should be an interesting lead compound for developing novel anti-diabetic drugs, and the 2-NBDG based fluorescent technology could be widely applied for anti-diabetic drug screening in zebrafish.

Experimental section

Compounds and reagents

Chemicals and reagents were obtained commercially and used without any further purification. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV 400 spectrometer using $CDCl_3$, D_2O, or DMSO as solvent. HRMS spectra were recorded on an Agilent QTOF 6520 mass spectrometer. Thin layer chromatography (TLC) was carried out on silica gel HF_{254} plates with detection using a UV detector or by charring with a solution of H_2SO_4 in MeOH (1/5, v/v).

*2,3,4-Tri-O-benzoyl-β-D-glucopyranosyl azide **13***

To a solution of azido sugar **11** (2.00 g, 9.76 mmol) in 50 mL of pyridine was slowly added *tert*-butyldimethylsilyl chloride (TBSCl, 1.77 g, 11.71 mmol). The mixture was stirred at room temperature overnight, and TLC (dichloromethane/methanol, 4/1, v/v) showed the complete conversion of the starting material. Then, to the mixture was slowly added benzoyl chloride (BzCl, 6.83 g, 48.80 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP, 122 mg, 1.00

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mmol). The reaction was stirred at room temperature for 6 h, diluted with 150 mL of ethyl acetate and washed with water (50 mL×3). The organic layer was evaporated, and the resulting residue was subjected to column chromatography (petroleum ether/ethyl acetate, 2/1, v/v) to give **12** as a white solid. To the mixture of compound **12** (3.20 g, 5.08 mmol) in 50 mL of tetrahydrofuran was added tetrabutylammonium fluoride (TBAF, 1.0 M solution in THF, 6.1 mL, 6.10 mmol), and the reaction was stirred at room temperature for 8 h. After TLC (petroleum ether/ethyl acetate 2/1) showed the complete conversion of the starting material, the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography (petroleum ether/ethyl acetate, 1/1, v/v) to give **13** as a white solid (2.34 g, 46% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (t, *J* = 6.8 Hz, 4 H, Ar*H*), 7.82 (d, *J* = 7.6 Hz, 2 H, Ar*H*), 7.56–7.51 (m, 2 H, Ar*H*), 7.45–7.35 (m, 5 H, Ar*H*), 7.28 (t, *J* = 8.0 Hz, 2 H, Ar*H*), 5.96 (t, *J* = 9.6 Hz, 1 H), 5.54 (t, *J* = 10.0 Hz, 1 H), 5.47 (t, *J* = 9.6 Hz, 1 H), 4.96 (d, *J* = 8.8 Hz, 1 H), 3.93–3.87 (m, 2 H), 3.79 (dd, *J* = 4.4, 12.8 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.01, 165.73, 164.98, 133.82, 133.53, 133.40, 129.97, 129.91, 129.74, 128.75, 128.63, 128.56, 128.46, 128.39, 128.36, 88.40, 72.62, 71.27, 69.04, 61.18; ESI-TOF HRMS (*m/z*): calcd for $C_{27}H_{24}N_3O_8^+$, $[M + H]^+$, 518.1558; found, 518.1560.

*General procedure for the synthesis of fully protected disaccharide **24**–**28***

To a solution of acetylated sugar **14**–**18** (3.08 mmol) and *p*-thiocresol (*p*-STol, 764 mg, 6.16 mmol) in 100 mL of dry dichloromethane was added stannic chloride ($SnCl_4$, 801 mg, 3.08 mmol) at 0 °C. The reaction was slowly warmed to room temperature and stirred for 4 h. Thereafter, the reaction was neutralized with saturated $NaHCO_3$ aqueous solution and extracted with dichloromethane (60 mL×3). The organic layer was evaporated and the resulting residue was purified by column chromatography (petroleum ether/ethyl acetate, 1/1, v/v) to give glycosyl donor **19**–**23** as colorless oil. To the mixture of donor **19**–**23** (1.10 mmol) and acceptor **13** (569 mg, 1.10 mmol) in 10 mL of dry dichloromethane was added *N*-iodosuccinimide (NIS, 297 mg, 1.32 mmol) followed by a catalytic amount of silver trifluoromethanesulfonate (AgOTf, 26 mg, 0.10 mmol) at –20 °C. The reaction was slowly warmed to room temperature and stirred for 2 h. Then, the reaction was neutralized with three drops of triethylamine, and the solvent was evaporated. The resulting residue was purified by column chromatography (hexane/ethyl acetate, 1/1, v/v) to give fully protected disaccharide **24**–**28** as colorless oil.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranosyl azide **24**

Compound **24** was obtained as colorless oil (755 mg, 66% for 2 steps) from **14** by the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96–7.90 (m, 4 H, Ar*H*), 7.80 (d, *J* = 7.6 Hz, 2 H, Ar*H*), 7.53 (t, *J* = 7.6 Hz, 2 H, Ar*H*), 7.45–7.37 (m, 5 H, Ar*H*), 7.29–7.26 (m, 2 H, Ar*H*),

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5.87 (t, *J* = 9.6 Hz, 1 H), 5.41 (dt, *J* = 3.6, 10.0 Hz, 2 H), 5.20 (t, *J* = 9.6 Hz, 1 H), 5.08–4.98 (m, 2 H), 4.91 (d, *J* = 8.8 Hz, 1 H), 4.62 (d, *J* = 8.0 Hz, 1 H), 4.23 (dd, *J* = 4.8, 12.0 Hz, 1 H), 4.15–4.04 (m, 3 H), 3.78 (dd, *J* = 7.6, 11.2 Hz, 1 H), 3.70–3.66 (m, 1 H), 2.10 (s, 3 H), 2.01 (s, 6 H), 2.00 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.58, 170.19, 169.43, 169.38, 165.62, 165.24, 164.98, 133.71, 133.54, 133.36, 129.90, 129.84, 129.72, 128.71, 128.59, 128.53, 128.50, 128.45, 128.32, 101.04, 88.16, 76.04, 72.75, 71.92, 71.24, 71.11, 69.19, 68.42, 68.29, 61.80, 20.65, 20.61, 20.60, 20.58; ESI-TOF HRMS (m/z): calcd for $C_{41}H_{42}N_3O_{17}^+$, $[M + H]^+$, 848.2509; found, 848.2505.

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranosyl azide **25**

Compound **25** was obtained as colorless oil (587 mg, 54% for 2 steps) from **15** by the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97–7.90 (m, 4 H, Ar*H*), 7.81 (d, *J* = 7.2 Hz, 2 H, Ar*H*), 7.53 (t, *J* = 7.2 Hz, 2 H, Ar*H*), 7.45–7.37 (m, 5 H, Ar*H*), 7.30–7.26 (m, 2 H, Ar*H*), 5.87 (t, *J* = 9.6 Hz, 1 H), 5.45–5.40 (m, 2 H), 5.38 (d, *J* = 3.2 Hz, 1 H), 5.35 (s, 1 H), 5.24 (dd, *J* = 8.0, 10.4 Hz, 1 H), 5.00 (dd, *J* = 3.6, 10.8 Hz, 1 H), 4.93 (d, *J* = 8.8 Hz, 1 H), 4.58 (d, *J* = 8.0 Hz, 1 H), 4.15–4.04 (m, 4 H), 3.89 (t, *J* = 6.4 Hz, 1 H), 3.76 (dd, *J* = 7.2, 11.2 Hz, 1 H), 2.16 (s, 3 H), 2.12 (s, 3 H), 2.11 (s, 3 H), 1.98 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.33, 170.30, 170.17, 170.10, 170.08, 169.85, 169.54, 165.61, 165.24, 164.98, 133.71, 133.53, 133.36, 129.89, 129.82, 129.70, 128.71, 128.59, 128.53, 128.45, 128.32, 101.35, 89.73, 88.16, 76.06, 72.77, 71.24, 70.86, 70.80, 69.15, 68.77, 68.62, 68.42, 67.44, 67.38, 66.99, 66.46, 61.25, 61.19, 20.69, 20.61, 20.56, 20.53; ESI-TOF HRMS (m/z): calcd for $C_{41}H_{42}N_3O_{17}^+$, $[M + H]^+$, 848.2509; found, 848.2503.

2,3,5-Tri-O-acetyl-β-D-ribofuranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranosyl azide **26**

Compound **26** was obtained as colorless oil (656 mg, 61% for 2 steps) from **16** by the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97–7.91 (m, 4 H, Ar*H*), 7.81 (d, *J* = 7.6 Hz, 2 H, Ar*H*), 7.53 (t, *J* = 7.2 Hz, 2 H, Ar*H*), 7.45–7.36 (m, 5 H, Ar*H*), 7.30–7.26 (m, 2 H, Ar*H*), 5.88 (t, *J* = 9.6 Hz, 1 H), 5.50 (t, *J* = 10.0 Hz, 1 H), 5.44 (t, *J* = 9.6 Hz, 1 H), 5.34–5.29 (m, 2 H), 5.08 (s, 1 H), 4.94 (d, *J* = 8.8 Hz, 1 H), 4.34–4.26 (m, 2 H), 4.13–4.05 (m, 2 H), 3.92 (dd, *J* = 1.6, 11.6 Hz, 1 H), 3.73 (dd, *J* = 6.8, 12.0 Hz, 1 H), 2.10 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.53, 169.57, 169.47, 165.67, 165.09, 164.96, 133.58, 133.48, 133.32, 129.91, 129.83, 129.75, 128.78, 128.64, 128.49, 128.43, 128.31, 106.10, 88.14, 78.94, 76.08, 74.66, 72.81, 71.40, 71.27, 69.11, 67.01, 64.43, 20.68, 20.53, 20.50; ESI-TOF HRMS (*m/z*): calcd for $C_{38}H_{38}N_3O_{15}^+$, $[M + H]^+$, 776.2297; found, 776.2290.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranosyl azide **27**

Compound **27** was obtained as colorless oil (643 mg, 47% for 2 steps) from **17** by the general

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procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98–7.93 (m, 4 H, Ar*H*), 7.81 (d, *J* = 7.2 Hz, 2 H, Ar*H*), 7.54 (t, *J* = 7.6 Hz, 2 H, Ar*H*), 7.45–7.38 (m, 5 H, Ar*H*), 7.30–7.26 (m, 2 H, Ar*H*), 5.90 (t, *J* = 9.6 Hz, 1 H), 5.53 (t, *J* = 9.6 Hz, 1 H), 5.46 (t, *J* = 9.6 Hz, 1 H), 5.37 (dd, *J* = 3.6, 10.0 Hz, 1 H), 5.30–5.25 (m, 2 H), 5.02 (d, *J* = 8.8 Hz, 1 H), 4.84 (s, 1 H), 4.24 (dd, *J* = 5.2, 12.4 Hz, 1 H), 4.13 (t, *J* = 8.4 Hz, 1 H), 4.07–4.02 (m, 2 H), 3.94 (dd, *J* = 6.8, 11.2 Hz, 1 H), 3.73 (d, *J* = 8.8 Hz, 1 H), 2.14 (s, 3 H), 2.08 (s, 3 H), 2.03 (s, 3 H), 2.00 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.59, 169.94, 169.81, 169.77, 165.67, 165.16, 164.93, 133.73, 133.52, 133.37, 129.91, 129.88, 129.76, 128.74, 128.55, 128.45, 128.34, 97.44, 88.14, 75.33, 72.72, 71.22, 69.26, 69.10, 69.01, 68.68, 66.53, 65.92, 62.23, 20.84, 20.69; ESI-TOF HRMS (*m/z*): calcd for $C_{41}H_{42}N_3O_{17}^+$, $[M + H]^+$, 848.2509; found, 848.2504.

2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranosyl azide **28**

Compound **28** was obtained as colorless oil (729 mg, 67% for 2 steps) from **18** by the general procedure described above. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90–7.85 (m, 4 H, Ar*H*), 7.75 (d, *J* = 7.6 Hz, 2 H, Ar*H*), 7.46 (t, *J* = 7.2 Hz, 2 H, Ar*H*), 7.37–7.29 (m, 5 H, Ar*H*), 7.21 (t, *J* = 7.0 Hz, 2 H, Ar*H*), 5.82 (t, *J* = 9.6 Hz, 1 H), 5.45–5.35 (m, 2 H), 5.25–5.24 (m, 1 H), 5.21 (dd, *J* = 3.6, 10.0 Hz, 1 H), 4.97 (t, *J* = 10.0 Hz, 1 H), 4.87 (d, *J* = 8.8 Hz, 1 H), 4.76 (s, 1 H), 4.07–4.03 (m, 1 H), 3.83–3.78 (m, 2 H), 3.73 (dd, *J* = 6.4, 12.0 Hz, 1 H), 2.06 (s, 3 H), 1.96 (s, 3 H), 1.92 (s, 3 H), 1.08 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.97, 168.90, 168.85, 164.65, 164.06, 163.94, 132.59, 132.84, 132.32, 128.90, 128.85, 128.75, 127.74, 127.57, 127.53, 127.48, 127.42, 127.30, 97.22, 87.15, 75.28, 71.70, 70.23, 69.96, 68.47, 68.16, 67.97, 65.65, 65.58, 19.83, 19.77, 19.69, 16.31; ESI-TOF HRMS (*m/z*): calcd for $C_{39}H_{40}N_3O_{15}^+$, $[M + H]^+$, 790.2454; found, 790.2457.

General procedure for the synthesis of disaccharide ***6***–***10***

Disaccharide **24**–**28** (0.70 mmol) was dissolved in 15 mL of a dichloromethane/methanol solution (1:2, v/v). To the mixture was added sodium methoxide (CH_3ONa, 38 mg, 0.70 mmol), and the reaction was stirred at room temperature for 2 h. Then, the reaction was neutralized with acetic acid, and the solvent was evaporated. The resulting residue was purified by column chromatography using methanol as eluent to give disaccharide **6**–**10** as colorless oil.

β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl azide **6**

Compound **6** was obtained as white solid (231 mg, 90%) from **24** (593 mg, 0.70 mmol) by the general procedure described above. ^{1}H NMR (400 MHz, D_2O): δ 4.69 (s, 1 H), 4.67 (s, 1 H), 4.40 (d, *J* = 8.0 Hz, 1 H), 4.11 (d, *J* = 10.8 Hz, 2 H), 3.84–3.76 (m, 4 H), 3.65–3.59 (m, 4 H), 3.45–3.15 (m, 14 H); ^{13}C NMR (100 MHz, D_2O): δ 102.76, 90.24, 76.87, 75.93, 75.65, 73.09, 72.76, 69.63, 69.05, 68.58, 60.74; ESI-TOF HRMS (m/z): calcd for $C_{12}H_{22}N_3O_{10}^+$, $[M + H]^+$, 368.1300; found, 368.1304.

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β-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl azide **7**

Compound **7** was obtained as white solid (221 mg, 86%) from **25** (593 mg, 0.70 mmol) by the general procedure described above. ^{1}H NMR (400 MHz, D_2O): δ 4.66 (d, *J* = 8.8 Hz, 1 H), 4.34 (d, *J* = 8.0 Hz, 1 H), 4.11 (d, *J* = 11.6 Hz, 1 H), 3.80 (d, *J* = 2.8 Hz, 1 H), 3.76 (dd, *J* = 6.4, 11.6 Hz, 1 H), 3.69–3.52 (m, 6 H), 3.46–3.36 (m, 3 H), 3.17–3.13 (m, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 103.36, 90.27, 76.89, 75.66, 75.19, 72.77, 70.77, 69.05, 68.67, 68.47, 61.01; ESI-TOF HRMS (m/z): calcd for $C_{12}H_{22}N_3O_{10}^+$, $[M + H]^+$, 368.1300; found, 368.1306.

β-D-ribofuranosyl-(1→6)-β-D-glucopyranosyl azide **8**

Compound **8** was obtained as white solid (210 mg, 89%) from **26** (543 mg, 0.70 mmol) by the general procedure described above. ^{1}H NMR (400 MHz, D_2O): δ 4.93 (s, 1 H), 4.63 (d, *J* = 8.8 Hz, 1 H), 4.13 (dd, *J* = 4.8, 6.4 Hz, 1 H), 4.01–3.91 (m, 3 H), 3.71 (dd, *J* = 2.8, 12.4 Hz, 1 H), 3.62–3.52 (m, 3 H), 3.41 (t, *J* = 9.2 Hz, 1 H), 3.32 (t, *J* = 9.6 Hz, 1 H), 3.15 (t, *J* = 9.6 Hz, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 107.47, 90.16, 82.97, 76.60, 75.65, 74.27, 72.76, 70.72, 69.22, 66.83, 62.78; ESI-TOF HRMS (m/z): calcd for $C_{11}H_{20}N_3O_9^+$, $[M + H]^+$, 338.1194; found, 338.1198.

α-D-mannopyranosyl-(1→6)-β-D-glucopyranosyl azide **9**

Compound **9** was obtained as white solid (236 mg, 92%) from **27** (593 mg, 0.70 mmol) by the general procedure described above. ^{1}H NMR (400 MHz, D_2O): δ 4.80 (s, 1 H), 4.68 (s, 1 H), 4.66 (s, 1 H), 3.89–3.84 (m, 2 H), 3.77 (d, *J* = 12.0 Hz, 1 H), 3.74–3.64 (m, 3 H), 3.61–3.53 (m, 3 H), 3.43–3.38 (m, 2 H), 3.17–3.13 (m, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 99.69, 90.26, 76.14, 75.93, 72.77, 70.57, 69.89, 60.70, 66.69, 65.38, 60.89; ESI-TOF HRMS (m/z): calcd for $C_{12}H_{22}N_3O_{10}^+$, $[M + H]^+$, 368.1300; found, 368.1305.

α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl azide **10**

Compound **10** was obtained as white solid (216 mg, 88%) from **28** (552 mg, 0.70 mmol) by the general procedure described above. ^{1}H NMR (400 MHz, D_2O): δ 4.72 (s, 1 H), 4.65 (d, *J* = 8.8 Hz, 1 H), 3.93–3.88 (m, 2 H), 3.70–3.55 (m, 4 H), 3.41 (t, *J* = 8.8 Hz, 1 H), 3.33 (t, *J* = 9.2 Hz, 1 H), 3.17 (t, *J* = 9.2 Hz, 1 H), 1.20 (d, *J* = 6.0 Hz, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 100.57, 90.19, 76.77, 75.67, 72.75, 72.04, 70.20, 70.00, 69.31, 68.76, 66.73, 16.65; ESI-TOF HRMS (m/z): calcd for $C_{12}H_{22}N_3O_9^+$, $[M + H]^+$, 352.1351; found, 352.1357.

*General procedure for the synthesis of disaccharide modified berberine derivative **1**–**5***

Disaccharide **6**–**10** (0.447 mmol) and 9-*O*-(propargyl) berberine chloride (177 mg, 0.447 mmol) were dissolved in 23 mL of a methanol/acetonitrile/H2O solution (10:10:3, v/v) at room temperature. To the mixture was added $CuSO_4 \cdot 5H_2O$ (67 mg, 0.268 mmol) and sodium ascorbate (53 mg, 0.268 mmol), the reaction was heated to 60 °C and stirred for 6 h. After TLC

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(dichloromethane/methanol/water, 5/5/1, v/v) showed the conversion of the starting materials, the solvent was evaporated and the resulting residue was subjected to neutral alumina column chromatography (acetonitrile/methanol/water, 3/3/1, v/v) to give the desired berberine derivative **1**–**5** as yellow solid.

*9-O-(1-(β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)-4-methylene-1H-1,2,3-triazole)berberine chloride **1***

Compound **1** was synthesized from **6** (164 mg, 0.447 mmol) and **29** (177 mg, 0.447 mmol) by the general procedure described above (184 mg, 54%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.62 (s, 1 H, C*H*=N), 8.93 (s, 1 H, Ar*H*), 8.57 (s, 1 H, Ar*H*), 8.22 (d, *J* = 9.2 Hz, 1 H, Ar*H*), 8.01 (d, *J* = 8.8 Hz, 1 H, Ar*H*), 7.79 (s, 1 H, Ar*H*), 7.09 (s, 1 H, Ar*H*), 6.17 (s, 2 H), 5.54 (d, *J* = 9.2 Hz, 1 H), 5.46 (s, 2 H, CH_2), 5.39 (d, *J* = 4.4 Hz, 1 H), 5.35 (d, *J* = 5.6 Hz, 1 H), 5.28 (d, *J* = 5.6 Hz, 1 H), 4.93 (d, *J* = 4.8 Hz, 1 H), 4.90–4.84 (m, 3 H), 4.48 (t, *J* = 5.6 Hz, 1 H), 4.11–4.04 (m, 4 H), 3.76 (q, *J* = 6.4 Hz, 1 H), 3.68–3.64 (m, 2 H), 3.50 (q, *J* = 4.4 Hz, 1 H), 3.45–3.37 (m, 2 H), 3.29–3.25 (m, 1 H), 3.24–3.16 (m, 2 H), 3.12–2.99 (m, 3 H), 2.95–2.90 (m, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.43, 150.33, 148.16, 145.60, 142.07, 137.94, 133.41, 131.16, 127.04, 124.55, 122.40, 120.87, 120.78, 87.84, 78.70, 77.37, 77.25, 77.22, 73.82, 72.57, 70.42, 70.04, 68.96, 67.08, 61.44, 57.58, 55.90, 26.82. ESI-TOF HRMS (*m/z*): calcd for $C_{34}H_{39}N_4O_{14}^+$, $[M - Cl]^+$, 727.2457; found, 727.2460.

*9-O-(1-(β-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl)-4-methylene-1H-1,2,3-triazole)berberine chloride **2***

Compound **2** was synthesized from **7** (164 mg, 0.447 mmol) and **29** (177 mg, 0.447 mmol) by the general procedure described above (171 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.66 (s, 1 H, C*H*=N), 8.98 (s, 1 H, Ar*H*), 8.63 (s, 1 H, Ar*H*), 8.22 (d, *J* = 9.6 Hz, 1 H, Ar*H*), 8.03 (d, *J* = 8.8 Hz, 1 H, Ar*H*), 7.81 (s, 1 H, Ar*H*), 7.09 (s, 1 H, Ar*H*), 6.18 (s, 2 H), 5.53 (d, *J* = 9.2 Hz, 1 H), 5.46 (s, 2 H, CH_2), 4.92 (t, *J* = 5.6 Hz, 2 H), 4.83–4.81 (m, 1 H), 4.68–4.63 (m, 1 H), 4.52–4.46 (m, 1 H), 4.09 (s, 3 H), 4.06–4.01 (m, 2 H), 3.75 (t, *J* = 8.8 Hz, 1 H), 3.66–3.60 (m, 3 H), 3.54–3.40 (m, 8 H), 3.32–3.16 (m, 8 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.66, 151.43, 150.25, 148.09, 145.58, 142.85, 141.95, 137.83, 133.38, 131.15, 126.93, 125.04, 124.60, 122.39, 120.86, 120.77, 108.88, 105.95, 104.21, 102.52, 87.90, 78.77, 77.19, 75.68, 74.01, 72.67, 71.05, 69.91, 68.85, 68.32, 66.97, 60.66, 57.56, 55.87, 26.81, 25.21. ESI-TOF HRMS (*m/z*): calcd for $C_{34}H_{39}N_4O_{14}^+$, $[M - Cl]^+$, 727.2457; found, 727.2462.

*9-O-(1-(β-D-ribofuranosyl-(1→6)-β-D-glucopyranosyl)-4-methylene-1H-1,2,3-triazole)berberine chloride **3***

Compound **3** was synthesized from **8** (151 mg, 0.447 mmol) and **29** (177 mg, 0.447 mmol) by the general procedure described above (200 mg, 61%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.67 (s, 1 H,

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C*H*=N), 8.98 (s, 1 H, Ar*H*), 8.62 (s, 1 H, Ar*H*), 8.22 (d, *J* = 9.2 Hz, 1 H, Ar*H*), 8.03 (d, *J* = 9.2 Hz, 1 H, Ar*H*), 7.80 (s, 1 H, Ar*H*), 7.09 (s, 1 H, Ar*H*), 6.17 (s, 2 H), 5.56–5.41 (m, 6 H), 5.05 (s, 1 H), 4.92 (s, 3 H), 4.68–4.63 (m, 2 H), 4.09 (s, 3 H), 3.90 (d, *J* = 10.8 Hz, 1 H), 3.79–3.63 (m, 5 H), 3.49–3.28 (m, 6 H), 3.24–3.13 (m, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.42, 150.30, 148.14, 145.64, 142.86, 142.06, 137.91, 133.43, 131.15, 127.00, 125.05, 124.56, 122.40, 120.89, 120.79, 108.89, 108.12, 105.97, 102.54, 87.69, 84.11, 78.63, 77.22, 74.70, 72.59, 71.46, 70.44, 67.79, 67.02, 63.67, 57.56, 55.86, 26.83. ESI-TOF HRMS (*m/z*): calcd for $C_{33}H_{37}N_4O_{13}^+$, [M – Cl]$^+$, 697.2352; found, 697.2356.

9-O-(1-(α-D-mannopyranosyl-(1→6)-β-D-glucopyranosyl)-4-methylene-1H-1,2,3-triazole)berberine chloride ***4***

Compound **4** was synthesized from **9** (164 mg, 0.447 mmol) and **29** (177 mg, 0.447 mmol) by the general procedure described above (157 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.67 (s, 1 H, C*H*=N), 8.98 (s, 1 H, Ar*H*), 8.66 (s, 1 H, Ar*H*), 8.21 (d, *J* = 9.2 Hz, 1 H, Ar*H*), 8.03 (d, *J* = 9.2 Hz, 1 H, Ar*H*), 7.80 (s, 1 H, Ar*H*), 7.09 (s, 1 H, Ar*H*), 6.17 (s, 2 H), 5.62–5.39 (m, 6 H), 4.93–4.71 (m, 5 H), 4.60 (s, 1 H), 4.49 (s, 1 H), 4.09 (s, 3 H), 3.77–3.55 (m, 6 H), 3.45–3.29 (m, 6 H), 3.21–3.16 (m, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.41, 150.28, 148.12, 145.64, 142.92, 142.04, 137.88, 133.43, 131.17, 126.98, 124.93, 124.57, 122.37, 120.88, 120.78, 108.89, 105.97, 102.53, 100.31, 87.84, 78.25, 77.36, 74.22, 72.66, 71.37, 70.62, 69.84, 67.29, 67.00, 65.89, 61.48, 57.57, 55.88, 26.83. ESI-TOF HRMS (*m/z*): calcd for $C_{34}H_{39}N_4O_{14}^+$, [M – Cl]$^+$, 727.2457; found, 727.2454.

9-O-(1-(α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl)-4-methylene-1H-1,2,3-triazole)berberine chloride ***5***

Compound **5** was synthesized from **10** (157 mg, 0.447 mmol) and **29** (177 mg, 0.447 mmol) by the general procedure described above (197 mg, 59%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.68 (s, 1 H, C*H*=N), 8.98 (s, 1 H, Ar*H*), 8.63 (s, 1 H, Ar*H*), 8.21 (d, *J* = 9.2 Hz, 1 H, Ar*H*), 8.03 (d, *J* = 8.8 Hz, 1 H, Ar*H*), 7.80 (s, 1 H, Ar*H*), 7.09 (s, 1 H, Ar*H*), 6.17 (s, 2 H), 5.58 (d, *J* = 9.2 Hz, 1 H), 5.49–5.42 (m, 5 H), 4.93 (t, *J* = 5.2 Hz, 2 H), 4.81–4.77 (m, 2 H), 4.69–4.66 (m, 1 H), 4.50 (s, 1 H), 4.09 (s, 3 H), 3.83 (d, *J* = 8.8 Hz, 1 H), 3.78–3.72 (m, 1 H), 3.63 (t, *J* = 7.2 Hz, 1 H), 3.56 (s, 1 H), 3.45–3.23 (m, 6 H), 3.24–3.13 (m, 4 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.41, 150.29, 148.13, 145.65, 142.91, 142.05, 137.90, 133.44, 131.15, 126.98, 124.92, 124.56, 122.37, 120.89, 120.78, 108.89, 105.97, 102.54, 100.94, 87.77, 78.72, 77.22, 72.61, 72.44, 71.14, 70.73, 70.18, 68.84, 67.02, 66.96, 57.56, 55.87, 26.83, 18.33. ESI-TOF HRMS (*m/z*): calcd for $C_{34}H_{39}N_4O_{13}^+$, [M – Cl]$^+$, 711.2508; found, 711.2504.

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Zebrafish maintenance

The light (14 h) and dark (10 h) alternate culture condition was adopted for zebrafish maintenance , and the cycle temperature was controlled at 28 ± 0.5 °C by the air conditioner system to ensure normal spawning. Before experiment, female and male zebrafishes were placed in the breeding tank in proportion to 1/1 or 1/2, and were separated by the partition board. In the next day, the female and male zebrafishes chased each other after contacting and spawning. Then, zebrafish eggs were maintained with E3 water containing 5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, and 0.33 mM $MgSO_4$). 2-Phenylthiourea (0.2 mM) was added to the water for increasing transparency of zebrafish larvae. Finally, the larvae were incubated in light incubator at 28 ± 0.5 °C.

Anti-diabetic investigation

The 3-day old zebrafish larvae were placed into a 24-well plate with 10 larvae and 2 mL of E3 water in each well. Larvae were treated with tested compounds (10 μM) for 1 h, and the E3 water was removed. Then, larvae were incubated with E3 water containing 2-NBDG (0.6 mM) for 3 h, and the E3 water removed. The resultant larvae were washed with E3 water for 3 times, and anesthetized using E3 water containing 0.02% tricaine. Fluorescent images of 2-NBDG uptake in zebrafish larvae were captured on a fluorescent microscopy, and the 2-NBDG uptake was quantified using an Image J software.

Conflict of Interest

The authors declare no competing financial interests.

Acknowledgments

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Graphical abstract

Synthesis of disaccharide modified berberine derivatives and their anti-diabetic investigation in zebrafish using a fluorescence-based technology

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Diglucose modified berberine derivative can dramatically promote the uptake of 2-NBDG both in zebrafish larvae and their eyes.