Synthesis and Biological Activity of Novel Deoxynojirimycin Derivatives as Potent α-Glucosidase Inhibitors

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Thirteen 1-deoxynojirimycin (DNJ) derivatives of five different skeletal structures were designed and synthesized. The newly synthesized compounds were evaluated using an *in vitro* α-glucosidase assay, and kinetic parameters (Ki, IC_{50}) were measured. Some DNJ derivatives showed weak α-glucosidase inhibitory activities, and the compounds 1-(3-benzyloxy-2-hydroxypropyl)-2-hydroxymethyl-piperidine-3,4,5-triol (**2a**) and 1-{3-[1-(4-fluorophenyl)-1*H*-[1,2,3]triazol-4-ylmethoxy]-2-hydroxypropyl}-2-hydroxymethyl-piperidine-3,4,5-triol (**13d**) showed activities comparable to that of DNJ. While **2a** was found to be a reversible, non-competitive inhibitor of α-glucosidase with a Ki value of 1.56×10^{-4} M and an IC_{50} value of 3.07×10^{-4} M, **13d** was a reversible, competitive inhibitor of α-glucosidase with a Ki value of 2.08×10^{-4} M and an IC_{50} value of 3.31×10^{-4} M.

Key words: 1-Deoxynojirimycin, α-Glucosidase Inhibitor, Diabetes Mellitus

Introduction

Type-II noninsulin-dependent diabetes mellitus (NIDDM), one of the most severe human metabolic disorder diseases which accounts for 90 – 95 % of all diabetes, has reached epidemic proportions globally, and its incidence is continuing to rise [1 – 3]. NIDDM is characterized by chronic hyperglycemia, insulin resistance and impaired insulin secretion [4]. One therapeutic approach to treat NIDDM is to retard the absorption of glucose *via* the inhibition of enzymes, such as α-glucosidase, in the digestive organs [5, 6].

α-Glucosidase, which is a membrane-bound enzyme located at the epithelium of small intestine, catalyzes the cleavage of a glucose molecule from disaccharides and plays an essential role in the proper digestion of carbohydrates [7]. It is beneficial for NIDDM patients to delay glucose release and its absorption after meals by inhibition of α-glucosidase in their therapy treatments [8].

1-Deoxynojirimycin (DNJ), a characteristic constituent of mulberry leaves, is a D-glucose analog with a secondary amine group instead of an oxygen atom in the pyranose ring. Resembling D-glucose in structure as well as in the transition state of glucosidase-catalyzed reactions, DNJ is able to inhibit α-glucosidase in a competitive manner [9, 10]. Given that DNJ showed good inhibitory activity against α-glucosidase [11], a number of DNJ derivatives have been synthesized in order to achieve significant potency of suppressing post-prandial elevation of the glucose level in blood. Successful results were first obtained with miglitol and emiglitate. The ED_{50} was evaluated respectively as 0.24 and 0.16 mg/kg body weight [12, 13].

The first marketed α-glucosidase inhibitor is acarbose, a pseudo-tetrasaccharide containing valienamine, developed by Bayers group in the early 1990s [14]. Valienamine shows structural similarity with DNJ except that the cyclohexane ring and the ring nitrogen atom are replaced with a cyclohexene ring and an exocyclic amino group, respectively. At present pseudosaccharide acarbose and azasugar miglitol are clinically used [15 – 17] for NIDDM patients to retard glucose absorption; however, these inhibitors are associated with severe side effects including adverse gastrointestinal effects and abdominal discomfort [18]. In addition, Kiyotaka *et al*. found that the absorption

ability of DNJ in rats was relatively low, and DNJ had a short half-life *in vivo* [19]. Hence, it is still of great interest to find new potent DNJ derivatives as α-glucosidase inhibitors.

In continuation of our work on the development of chemotherapeutic agents from DNJ, we designed several kinds of structural skeletons to introduce aryl, alkenyl, hydroxyl, and triazole groups into DNJ. We present here the synthesis and biological activities of thirteen DNJ derivatives, which are defined as **2a–c**, **6a–c**, **13a–e**, **8**, and **15**.

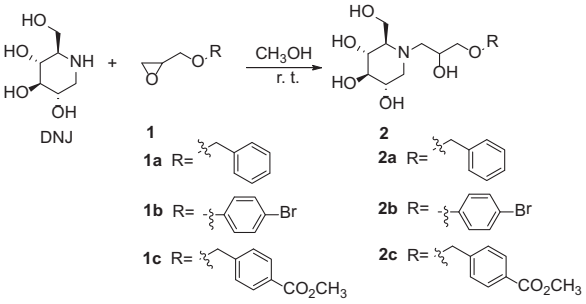
Results and Discussion

Synthesis of DNJ derivatives

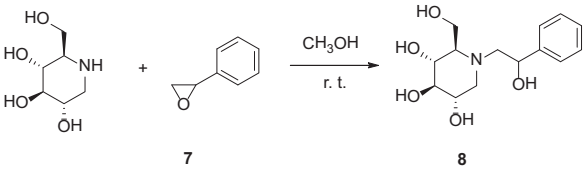
DNJ derivatives **2**, **6**, **8**, **13**, and **15** were synthesized as described in Schemes 1 – 5. Compounds **2**, **8** and **15** were prepared by the direct reactions between DNJ and epoxides **1a–c** (Scheme 1), 2-phenyloxirane **7** (Scheme 3) and the triazole compound **14** (Scheme 5), respectively.

Using cinnamic acid derivative **3** as a starting material, after esterification and reduction, cinnamyl alcohol derivative **4** was obtained, which subsequently reacted with (chloromethyl)oxirane to yield epoxy compound **5**. Condensation of DNJ and **5** afforded the designed compound **6** containing an alkene group (Scheme 2).

Click reaction between phenyl azide **9** and propargyl alcohol **10** gave triazole **11**, which subsequently reacted with 2-(chloromethyl)oxirane to yield epoxy compound **12**. Condensation of DNJ and **12** afforded the target compound **13** and resulted in the introduction of a triazole group (Scheme 4).



Scheme 1. General reaction pathway to compounds **2a–c**.

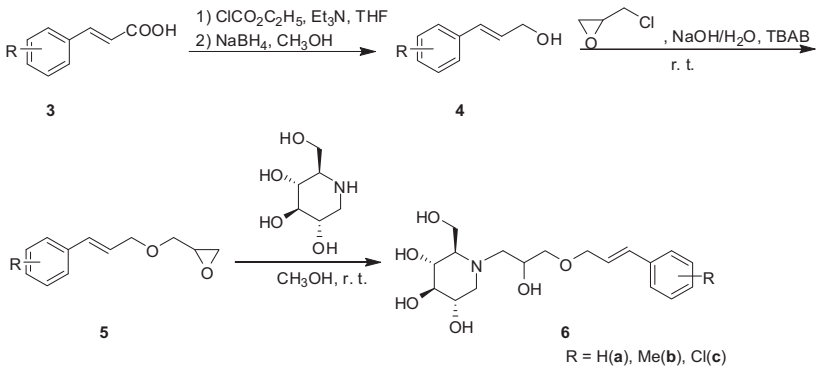


Scheme 3. General reaction pathway to compound **8**.

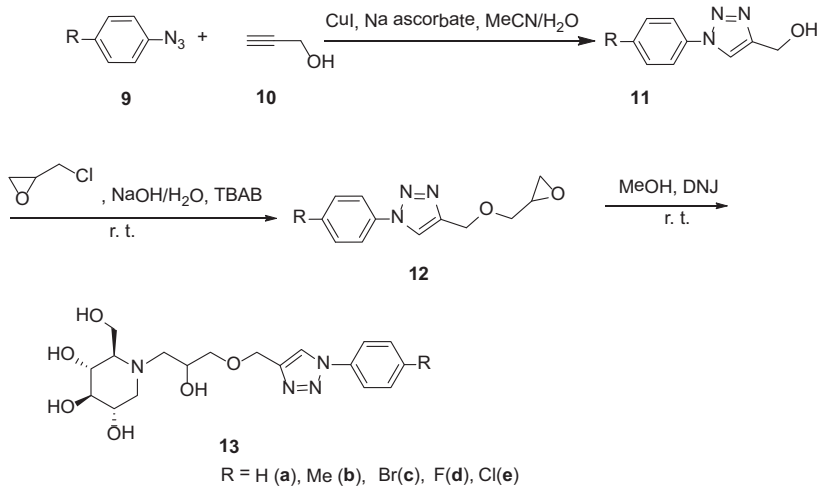
Inhibition study

As shown in Schemes 1 – 4, the synthesis of compounds **2**, **6**, **8**, and **13** introduced a new stereogenic center. In the present study, the biological tests were done on the mixtures of diastereomers.

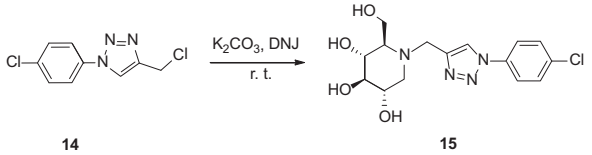
The inhibition assays were performed in a 96-well microtiter plate as described previously with a minor modification [20, 21]. The values of *K*i and inhibition types were determined by a Lineweaver-Burk plot. The concentration required to inhibit enzyme activity by 50 % (IC_{50}) was calculated by regression analysis. The α-glucosidase inhibition constants *K*i, inhibition types and IC_{50} values of DNJ, DNJ derivatives and acarbose are summarized in Table 1; the structure-activity rela-



Scheme 2. General reaction pathway to compounds **6a–c**. TBAB = tetrabutylammonium bromide.



Scheme 4. General reaction pathway to compounds **13a–e**.



Scheme 5. General reaction pathway to compound **15**.

tionship and the representative double-reciprocal plots of DNJ, **2a** and **13d** are shown in Figs. 1 and 2, respectively. Except **6a** and **13a**, the other DNJ derivatives displayed α-glucosidase inhibitory activities *in vitro* and were all found to be reversible inhibitors; particularly, **2a** and **13d** were the strongest inhibitors among them. The introduction of a $COOCH_3$ group in the benzene ring of **2a** caused the reduction of α-glucosidase inhibitory activity, as shown by compound **2c** in comparison with **2a**. Series **6** was designed by adding an ethylene group in the N-linked side chain of **2**, but **6a** showed no inhibitory effect on α-glucosidase, in spite of being an analog of **2a**. Therefore, the additional introduction of $COOCH_3$ and ethylene in **2a** did not increase its α-glucosidase inhibitory activity. Two other compounds, **2b** and **8**, which possess a shorter N-linked side chain than **2a**, showed less α-glucosidase inhibitory activities than **2a**. However, by replacing a hydrogen atom in the benzene ring of **6a** with a methyl or chloro substituent, moderate α-glucosidase inhibitory activities were achieved.

Compounds **13a–e** are another series of DNJ derivatives owing to their similar structures containing

Table 1. *K*i and IC_{50} values of DNJ derivatives obtained from yeast α-glucosidase enzymatic assays.

Compound	*K*i value (10^{-4} M)[a]	Inhibition type	IC_{50} (10^{-4} M)
2a	1.56	non-competitive	3.07
2b	3.53	competitive	39
2c	17	competitive	51
6a	n. i.[b]	n. i.[b]	n. i.[b]
6b	5.94	competitive	29
6c	8.55	competitive	36
8	7.41	competitive	17
13a	n. i.[b]	n. i.[b]	n. i.[b]
13b	9.17	competitive	26
13c	4.18	competitive	22
13d	2.08	competitive	3.31
13e	8.85	competitive	22
15	6.77	non-competitive	17
DNJ	4.40	competitive	4.51
Acarbose	4.25	competitive	3.44

[a] Substrate (PNP glycoside) concentration: 0, 62.75, 125, 250, 500, 1000×10^{-6} M, inhibitor concentration: 2×10^{-4} M and 5×10^{-4} M, α-glucosidase concentration 0.2 U mL^{-1}; [b] not inhibited.

a 1,2,3-1*H*-triazole structural moiety. **13a** does not contain any other substituent in the benzene ring, while **13b–e** in the same series contain a halogen or an alkyl group at the ring. Although **13a** did not show any potency of inhibiting α-glucosidase, the introduction of a halogen or an alkyl group at the benzene ring of **13a** resulted in significant inhibitory activities, in which the *K*i values were in the range of $2.08 - 9.17 \times 10^{-4}$ M for **13b–e**. In this series of DNJ derivatives, **13d** had potency similar to that of **2a**. Furthermore compounds **15**

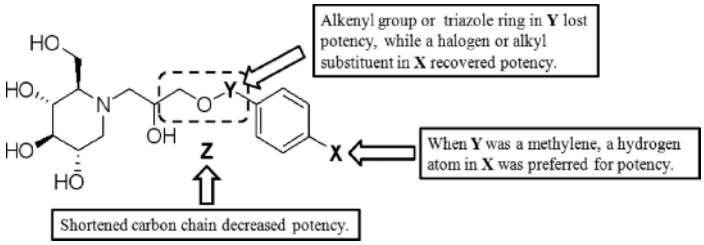


Fig. 1. Structure-activity relationships of DNJ derivatives as potent α-glucosidase inhibitors.

and **13e**, containing both a chloro substituent at their benzene rings, exhibited different activities owing to the shorter side chain of **15**.

Even though the studies of DNJ and acarbose have been carried out widely for many years, the sources reporting their IC_{50} and *K*i values are limited. The IC_{50} values of acarbose in the literature are quite different, ranging from 1.29×10^{-4} M to 4.025×10^{-3} M [22–26]. In our α-glucosidase inhibitory activity assay system, the observed IC_{50} of acarbose was 3.44×10^{-4} M, which was consistent with the reference data of 3.36×10^{-4} M and 2.90×10^{-4} M [24, 25]. However, under the present conditions, the IC_{50} of DNJ was determined to be 4.51×10^{-4} M, which was much higher than the sole published data of 1.0×10^{-5} M [27]. This phenomenon may be attributed to the complexity of the enzyme assay or the unidentified differences between the source and purity of DNJ.

Conclusion

In this study, we have synthesized thirteen new DNJ derivatives, evaluated their α-glucosidase inhibitory activities, investigated the structure-activity relationship and discovered two potent α-glucosidase inhibitors (compounds **2a** and **13d**) that showed α-glucosidase inhibitory activity comparable to that of DNJ. The inhibitor structures suggested that an N-linked side chain containing an aryl ring which increases the lipophility of DNJ would be beneficial for the α-glucosidase inhibition. The length and the component of the side chain as well as the substituent of the aryl ring varied the inhibitory activities. Compounds **2a** and **13d** would be a lead for designing new compounds, and further derivatives could be prepared with modification of these particular moieties. Therefore, our result provide very useful clues for the design and development of more potent α-glucosidase inhibitors.

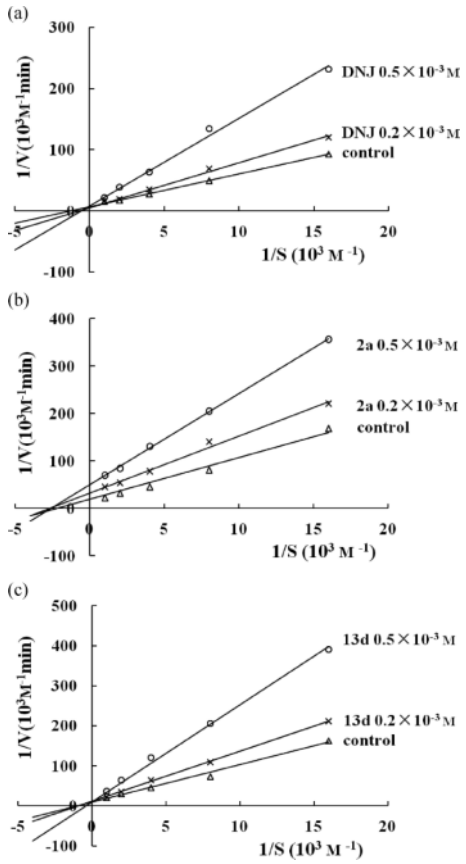


Fig. 2. Double-reciprocal plots of the inhibition kinetics of yeast α-glucosidase by DNJ (a), **2a** (b) and **13d** (c). Substrate (PNP glycoside) concentration: 0, 62.75, 125, 250, 500, 1000×10^{-6} M, inhibitor concentration 2×10^{-4} M and 5×10^{-4} M, α-glucosidase concentration 0.2 U mL^{-1}.

Experimental Section

General

Commercially obtained reagents were used without further purification. TLC: Huanghai GF_{254} silica gel-coated plates. Column chromatography (CC): SiO_2

(300 – 400 mesh), at medium pressure. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer in $CDCl_3$ with TMS as an internal standard. The chemical shifts are reported in parts per million (ppm) expressed in δ units; coupling constant (J) values are given in Hertz (Hz). MS data were measured with a Varian-310 spectrometer. High-resolution mass spectra were determined using a Finnigan-NAT GC/MS/DS 8430 spectrometer.

α-Glucosidase assay

p-Nitrophenyl-α-D-glucopyranoside (PNPG), yeast α-glucosidase (EC 3.2.1.20), sodium phosphate and sodium carbonate were purchased from Sigma-Aldrich (China). α-Glucosidase inhibitory activity was performed following the modified method of Pistia, Brueggeman and Hollingsworth [20, 21]. A reaction mixture containing 50 μL of phosphate buffer (5×10^{-2} M, pH = 6.8), 10 μL of yeast α-glucosidase (1 U mL^{-1}) and 20 μL of the DNJ derivative at varying concentrations was pre-incubated for 10 min at 37 °C, and then 20 μL of 10^{-3} M PNPG was added to the mixture as a substrate. After further incubation at 37 °C for 30 min, the reaction was terminated by adding one volume of Na_2CO_3 (1 M). All the enzyme, inhibitor and substrate solutions were made using the same buffer. Enzymatic activity was quantified by measuring the absorbance at 405 nm in a microtiter plate reader (Thermo Multiskan MK3, China). Each experiment was performed in triplicate. The concentration of an inhibitor required to inhibit 50 % of enzyme activity under the conditions is defined as the IC_{50} value. IC_{50} was calculated by regression analysis, using the following equation, where v is the percentage of inhibition, A_{100} is the maximum inhibition, I is the inhibitor concentration, IC_{50} is the concentration required to inhibit activity of the enzyme by 50 %, and s is the cooperative degree:

$$v = \frac{A_{100}}{1 + (I/\mathrm{IC}_{50})^{S}} \quad (1)$$

Kinetics of enzyme inhibition

The enzyme reaction was performed according to the above reaction conditions with inhibitors at various concentrations (2×10^{-4} to 2×10^{-3} M). Inhibition strength for the inhibitor was determined by a Dixon plot and its replot of slope *versus* the reciprocal of the substrate concentration.

*General procedure for the synthesis of DNJ derivatives **2***

DNJ (100 mg, 0.6 mmol) and oxirane **1** (0.7 mmol) were stirred in methanol-H_2O (5 mL : 1 mL) at r. t., for 24 h, and the reaction was monitored with TLC (EtOAc-MeOH = 4 : 1). After completion of the reaction, the methanol was removed *in vacuo,* and the residue was purified by column chromatography on silica gel to yield DNJ derivative **2**.

The starting chemicals 2-benzyloxymethyl-oxirane (**1a**) and 2-(4-bromo-phenoxymethyl)oxirane (**1b**) were used as obtained commercially.

*4-Oxiranylmethoxymethyl-benzoic acid methyl ester (**1c**)*

Epoxy chloropropane (0.462 g, 5 mmol), tetrabutylammonium bromide (TBAB, 16 mg, 0.05 mmol) and 50 % aqueous NaOH solution (0.6 mL) were added to a flask. The mixture was violently stirred at r. t. until dissolution, then it was chilled with an ice bath, and methyl 4-hydroxybenzoate (166 mg, 1 mmol) was slowly added. The solution was stirred for 18 h at 0 °C. After completion of the reaction, cold water was added to the mixture, and then EtOAc for extraction. The organic layer was washed with salt water to neutral, dried with anhydrous sodium sulfate, and EtOAc was removed *in vacuo*. The crude product was purified by column chromatography on silica gel to yield a pale-yellow liquid (**1c**); yield 158 mg (71 %). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.02 (d, 2H), 7.42 (d, 2H), 4.65 (m, 2H), 3.82 (m, 1H), 3.45 (m, 1H), 3.21 (m, 1H), 2.82 (t, 1H), 2.63 (m, 1H), 1.59 (s, 3H). – MS (ESI): m/z = 222.1 [M]$^+$. – HRMS ((+)-ESI): m/z = 222.0890 (calcd. 222.0892 for $C_{12}H_{14}O_4$, [M]$^+$).

*1-(3-Benzyloxy-2-hydroxypropyl)-2-hydroxymethyl-piperidine-3,4,5-triol (**2a**)*

Colorless solid. Yield 87 %. – ^{1}H NMR (500 MHz, D_2O): δ = 7.37 (s, 5H), 4.54 (t, 2H), 3.98 (m, 1H), 3.82 (m, 2H), 3.43 – 3.52 (m, 3H), 3.26 (m, 2H), 3.00 (m, 1H), 2.76 (m, 1H), 2.53 (m, 1H), 2.33 (m, 2H). – ^{13}C NMR (125 MHz, D_2O): δ = 54.1, 56.5, 57.9, 65.8, 67.4, 68.7, 70.1, 74.8, 78.3, 128.4, 128.6, 128.8, 137.5. – MS (ESI): m/z = 327.2 [M]$^+$. – HRMS ((+)-ESI): m/z = 327.1680 (calcd. 327.1682 for $C_{16}H_{25}NO_6$, [M]$^+$).

*1-[3-(4-Bromo-phenoxy)-2-hydroxypropyl]-2-hydroxymethyl-piperidine-3,4,5-triol (**2b**)*

Colorless solid. Yield 81 %. – ^{1}H NMR (500 MHz, D_2O): δ = 7.38 (t, 2H), 6.83 (t, 2H), 4.17 (m, 1H), 3.78 – 3.98 (m, 4H), 3.56 (m, 1H), 3.12 (m, 2H), 3.13 (m, 1H), 2.96 (d, 1H, J = 13.5 Hz), 2.67 (m, 1H), 2.37 (m, 2H). – ^{13}C NMR (125 MHz, D_2O): δ = 53.9, 57.2, 66.5, 67.5, 68.7, 70.0, 70.8, 78.3, 113.2, 116.9, 132.5, 157.6. – MS (ESI): m/z = 393.0 [M+2]$^+$, 391.0 [M]$^+$. – HRMS ((+)-ESI): m/z = 393.0608 (calcd. 393.0610 for $C_{15}H_{22}{}^{81}BrNO_6$, [M]$^+$).

*4-[2-Hydroxy-3-(3,4,5-trihydroxy-2-hydroxymethyl-piperidin-1-yl)propoxymethyl]benzoic acid methyl ester (**2c**)*

Colorless solid. Yield 82 %. – ^{1}H NMR (400 MHz, D_2O): δ = 7.96 (d, 2H), 7.49 (d, 2H), 4.65 (s, 1H), 4.34 (d, 1H), 4.07 (s, 1H), 3.68 – 3.97 (m, 5H), 3.31 – 3.57 (m, 5H), 3.09 (m, 1H), 2.85 (m, 1H), 2.65 (m, 1H), 2.37 (m, 2H). – MS (ESI): m/z = 385.2 [M]$^+$. – HRMS ((+)-ESI): m/z = 385.1734 (calcd. 385.1736 for $C_{18}H_{27}NO_8$, [M]$^+$).

General procedure for the synthesis of DNJ derivatives ***6***

The mixture of cinnamic acid derivative **3** (10 mmol), ethyl chloroformate (1.2 mL, 12 mmol), triethylamine (1.21 g, 12 mmol), and tetrahydrofuran (100 mL) was stirred at 0 °C for 30 min. Sodium borohydride (756.6 mg, 20 mmol) was added, and the temperature was raised to 10 °C, then methanol (1 mL) was added in drops into the mixture. TLC analysis showed that the reaction was completed in 24 h. The methanol was removed *in vacuo*, and the residue was partitioned between dichloromethane and water. The combined organic phase was dried with anhydrous sodium sulfate and then concentrated. Purification by column chromatography on silica gel afforded the desired cinnamyl alcohol derivative **4a–c** [28].

Compound **4** (1 mmol) was slowly added to the solution of TBAB (16 mg, 0.05 mmol), 2-(chloromethyl)oxirane (462 mg, 5 mmol) and 50% aqueous NaOH solution (0.6 mL) at 0 °C. TLC analysis showed that the reaction was completed in 18 h. To the reaction mixture cold water was added, and then EtOAc for extraction. The organic layer was washed to neutral with saturated NaCl solution, dried with anhydrous sodium sulfate, and concentrated *in vacuo*. Purification by column chromatography on silica gel afforded epoxy compounds **5a–c** [29, 30].

DNJ (10 mg, 0.06 mmol) and epoxy compound **5** (0.07 mmol) were stirred in methanol-H_2O (1 mL : 200 μL) at r. t. for 24 h, and the reaction was monitored with TLC (EtOAc-MeOH = 4 : 1). After removing of the solvent, the crude product was purified by chromatography on silica gel to yield DNJ derivatives **6a–c**.

*2-Hydroxymethyl-1-[2-hydroxy-3-(3-phenyl-allyloxy)propyl]piperidine-3,4,5-triol (**6a**)*

Colorless solid. Yield 89%. – ^{1}H NMR (500 MHz, D_2O): δ = 7.42 (t, 2H), 7.31 (m, 2H), 7.25 (t, 1H), 6.62 (m, 1H), 4.14 (t, 2H), 3.99 (m, 1H), 3.78 (m, 2H), 3.44 (m, 4H), 3.23 (t, 1H), 3.08 (m, 1H), 2.78 (m, 1H), 2.52 (m, 1H), 2.29 (m, 2H). – ^{13}C NMR (125 MHz, D_2O): δ = 53.75, 55.96, 57.01, 65.21, 66.27, 67.12, 67.90, 69.33, 70.86, 77.68, 124.8, 125.82, 125.91, 127.46, 128.24, 132.61, 135.79. – MS (ESI): m/z = 353.2 $[M]^+$. – HRMS ((+)-ESI): m/z = 353.4102 (calcd. 353.4100 for $C_{18}H_{27}NO_6$, $[M]^+$).

*2-Hydroxymethyl-1-[2-hydroxy-3-(3-p-tolyl-allyloxy)propyl]piperidine-3,4,5-triol (**6b**)*

Colorless solid. Yield 70%. – ^{1}H NMR (500 MHz, D_2O): δ = 7.37 (m, 2H), 7.19 (m, 2H), 6.64 (m, 1H), 6.30 (m, 1H), 4.20 (m, 2H), 4.08 (m, 1H), 3.85 (m, 2H), 3.59 (m, 2H), 3.49 (m, 2H), 3.40 (m, 1H), 3.32 (m, 1H), 3.13 (m, 1H), 2.86 (m, 1H), 2.40 (m, 2H), 2.29 (s, 3H). – MS (ESI): m/z = 367.2 $[M]^+$. – HRMS ((+)-ESI): m/z = 367.1992 (calcd. 367.1995 for $C_{19}H_{29}NO_6$, $[M]^+$).

*1-{3-[3-(4-Chlorophenyl)allyloxy]-2-hydroxypropyl}-2-hydroxymethyl-piperidine-3,4,5-triol (**6c**)*

Colorless solid. Yield 86%. – ^{1}H NMR (500 MHz, D_2O): δ = 7.43 (t, 2H), 7.37 (t, 2H), 6.66 (m, 1H), 6.36 (m, 1H), 4.21 (m, 2H), 4.07 (m, 1H), 3.82 (m, 2H), 3.65 (m, 2H), 3.38 (m, 2H), 3.28 (m, 1H), 3.12 (m, 1H), 2.86 (m, 1H), 3.12 (m, 1H), 2.40 (m, 2H). – MS (ESI): m/z = 387.1 $[M]^+$. – HRMS ((+)-ESI): m/z = 387.1446 (calcd. 387.1448 for $C_{18}H_{26}{}^{35}ClNO_6$, $[M]^+$).

General procedure for the synthesis of DNJ derivative ***8***

2-Phenyloxirane (**7**) reacts with DNJ in MeOH at r. t. to give 2-hydroxymethyl-1-(2-hydroxy-2-phenylethyl)piperidine-3,4,5-triol (**8**); detailed data were the same as that reported in the literature [31].

General procedure for the synthesis of DNJ derivatives ***13***

A flask containing a mixture of an aniline derivative (5 mmol) and an aqueous concentrated HCl-water (10 mL : 1 mL) solution was placed into an ice bath, and to the mixture an aqueous $NaNO_2$ solution was added in drops until KI-starch paper became blue. After stirring for 5 min, sodium azide (0.488 g, 7.5 mmol) in 4 mL water was slowly added, and the reaction was maintained for additional 1 – 2 h. After completion of the reaction, KOAc was added to adjust pH to neutral prior to extraction with EtOAc. The organic layer was washed with saturated NaCl solution, dried with anhydrous sodium sulfate, and then concentrated to give pale-yellow liquid phenyl azides **9a–e**.

The mixture of **9** (4.5 mmol), propargyl alcohol (0.280 g, 5.0 mmol), cuprous iodide (0.382 g, 2 mmol), sodium ascorbate (0.396 g, 2 mmol), and CH_3CN-H_2O (30 mL : 3 mL) was stirred at r. t. under nitrogen for 24 h. After completion of the reaction, the mixture was partitioned between EtOAc and water. The organic layer was dried with anhydrous sodium sulfate and concentrated. Purification by column chromatography on silica gel afforded the desired products **11a–e** [32].

Compound **11** (5 mmol) was slowly added to the mixture of TBAB (0.078 g, 0.2 mmol), 2-(chloromethyl)oxirane (2.312 g, 25 mmol) and a 50% aqueous NaOH solution (2 mL) at 0 °C. After completion of the reaction (18 h), cold water was added, and then ethyl ether for extraction. The organic layer was washed to neutral with saturated NaCl solution, dried with anhydrous sodium sulfate and concentrated. Purification by column chromatography on silica gel afforded the desired products **12a–e** [33].

The mixture of **12** (0.7 mmol) and DNJ (100 mg, 0.6 mmol) was stirred in methanol-H_2O (5 mL : 1 mL) at r. t. for 16 h. After the reaction was completed, the solvent was removed *in vacuo*. The residue was purified by column

chromatography on silica gel to afford the desired products **13a**–**e**.

*2-Hydroxymethyl-1-[2-hydroxy-3-(1-phenyl-1H-[1,2,3]-triazol-4-ylmethoxy)propyl]piperidine-3,4,5-triol (**13a**)*

Colorless solid. Yield 85 %. – ^{1}H NMR (500 MHz, D_2O): $\delta = 8.35$ (s, 1H), 7.61 (d, $J = 8.0$ Hz, 2H), 7.48 (m, 3H), 4.66 (s, 2H), 3.99 (m, 1H), 3.79 – 3.89 (m, 1H), 3.45 – 3.60 (m, 4H), 3.21 (t, 1H), 3.02 (m, 1H), 2.78 (m, 1H), 2.52 (m, 1H), 2.20 – 2.35 (m, 2H). – ^{13}C NMR (125 MHz, D_2O): $\delta = 53.4$, 56.3, 57.9, 63.3, 65.9, 67.7, 68.5, 70.0, 72.6, 78.3, 120.9, 123.5, 129.6, 129.9, 136.1, 144.5. – MS (ESI): $m/z = 394.1$ $[M]^+$. – HRMS ((+)-ESI): $m/z = 394.1850$ (calcd. 394.1852 for $C_{18}H_{26}N_4O_6$, $[M]^+$).

*2-Hydroxymethyl-1-[2-hydroxy-3-(1-p-tolyl-1H-[1,2,3]-triazol-4-ylmethoxy)propyl]piperidine-3,4,5-triol (**13b**)*

Colorless solid. Yield 89 %. – ^{1}H NMR(500 MHz, D_2O): $\delta = 8.30$ (s, 1H), 7.47 (d, $J = 8.1$ Hz, 2H), 7.29 (d, $J = 8.1$ Hz, 2H), 4.06 (m, 2H), 3.90 (m, 1H), 3.20 – 3.83 (m, 7H), 3.04 (m, 1H), 2.80 (m, 1H), 2.54 (m, 1H), 2.21 – 2.27 (m, 5H). – ^{13}C NMR (125 MHz, D_2O): $\delta = 20.3$, 53.5, 54.4, 56.5, 58.1, 66.0, 67.0, 67.8, 68.7, 70.1, 72.7, 78.3, 120.9, 123.5, 130.4, 133.9, 140.3, 144.6. – MS (ESI): $m/z = 408.2$ $[M]^+$. – HRMS ((+)-ESI): $m/z = 408.2006$ (calcd. 408.2008 for $C_{19}H_{28}N_4O_6$, $[M]^+$).

*1-{3-[1-(4-Bromophenyl)-1H-[1,2,3]triazol-4-ylmethoxy]-2-hydroxypropyl}-2-hydroxymethyl-piperidine-3,4,5-triol (**13c**)*

^{1}H NMR (500 MHz, D_2O): $\delta = 8.38$ (s, 1H), 7.65 (m, 2H), 7.56 (m, 2H), 4.65 (m, 2H), 4.00 (s, 1H), 3.76 (m, 2H), 3.43 (m, 4H), 3.04 (m, 2H), 2.78 (m, 1H), 2.57 (m, 1H), 2.35 (m, 2H). – MS (ESI): $m/z = 472.1$ $[M]^+$. – HRMS ((+)-ESI): $m/z = 472.0956$ (calcd. 472.0957 for $C_{18}H_{25}{}^{79}BrN_4O$, $[M]^+$).

*1-{3-[1-(4-Fluorophenyl)-1H-[1,2,3]triazol-4-ylmethoxy]-2-hydroxypropyl}-2-hydroxymethyl-piperidine-3,4,5-triol (**13d**)*

^{1}H NMR (500 MHz, D_2O): $\delta = 8.41$ (s, 1H), 7.70 (dd, $J = 4.2$ Hz, $J = 13.3$ Hz, 2H), 7.30 (t, 2H), 4.09 (m, 1H), 3.79 – 3.90 (m, 2H), 3.55 – 3.65 (m, 4H), 3.28 – 3.44 (m, 3H), 3.11 – 3.19 (m, 1H), 2.94 (m, 1H), 2.66 (m, 1H), 2.45 (m, 2H). – ^{13}C NMR (125 MHz, D_2O): $\delta = 54.0$, 56.5, 58.0, 66.1, 67.8, 68.6, 71.5, 76.1, 78.2, 116.9, 123.5, 132.7, 144.8, 163.7. – MS (ESI): $m/z = 412.1$ $[M]^+$. – HRMS ((+)-ESI): $m/z = 412.1756$ (calcd. 412.1758 for $C_{18}H_{25}FN_4O_6$, $[M]^+$).

*1-{3-[1-(4-Chlorophenyl)-1H-[1,2,3]triazol-4-ylmethoxy]-2-hydroxypropyl}-2-hydroxymethyl-piperidine-3,4,5-triol (**13e**)*

Pale-yellow solid. Yield 80 %. – ^{1}H NMR (500 MHz, D_2O): $\delta = 8.34$ (s, 1H), 7.56 (s, 2H), 7.44 (s, 2H), 4.66 (s, 2H), 3.99 (d, $J = 3.0$ Hz, 1H), 3.78 (m, 2H), 3.38 – 3.57 (m, 4H), 3.19 (m, 1H), 3.01 (m, 1H), 2.77 (m, 1H), 2.46 – 2.56 (m, 1H), 2.31 (m, 2H). – ^{13}C NMR (125 MHz, D_2O): $\delta =$ 52.7, 53.6, 55.9, 57.3, 59.7, 66.2, 67.0, 67.8, 69.3, 70.2, 70.8, 71.9, 77.6, 121.5, 122.6, 129.1, 133.8, 134.1, 144.0. – MS (ESI): $m/z = 428.1$ $[M]^+$. – HRMS ((+)-ESI): $m/z =$ 428.1460 (calcd. 428.1462 for $C_{18}H_{25}{}^{35}ClN_4O_6$, $[M]^+$).

*1-[1-(4-Chlorophenyl)-1H-[1,2,3]triazol-4-ylmethyl]-2-hydroxymethyl-piperidine-3,4,5-triol (**15**)*

4-Chloromethyl-1-(4-chlorophenyl)-1*H*-[1,2,3]triazole (**14**) was prepared according to the procedure reported in the literature [34]. Compound **14** (150 mg, 0.13 mmol) was added to a mixture of 1-DNJ (50 mg, 0.30 mmol), K_2CO_3 (35 mg, 0.25 mmol) and CH_3CN (5 mL), and the reaction was left to continue at 80 °C for 24 h. After the reaction was finished, the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel to afford the desired product **15**. Colorless solid, 75 mg (71 %). – ^{1}H NMR (500 MHz, D_2O): $\delta = 8.46$ (s, 1H), 7.75 (d, 2H), 7.57 (d, 2H), 4.14(d, 1H), 4.06 (m, 2H), 3.91(d, 1H), 3.50 (m, 1H), 3.40 (t, 1H), 3.08 (m, 1H), 2.98 (m, 1H), 2.14 (t, 1H), 2.07 (d, 1H). – MS (ESI): $m/z = 354.1$ $[M]^+$. – HRMS ((+)-ESI): $m/z = 354.1092$ (calcd. 354.1094 for $C_{15}H_{19}{}^{35}ClN_4O_4$, $[M]^+$).

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