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ARTICLE TYPE

Synthesis of L-Rhamnose derived chiral bicyclic triazoles as novel sodium-glucose transporter (SGLT) inhibitors

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Herein we described synthesis of a series of novel fused bicyclic 1,2,3-triazoles from commercially available, natural deoxy sugar, L-rhamnose. The key reactions involved are i) $Zn(OTf)_2$ catalyzed enantioselective alkynylation of L-rhamnose derived azidoaldehyde and ii) deprotection of acid sensitive 1,2-isopropylidene group followed by *in situ* intramolecular click-cycloaddition of azidoalkynols. Some compounds exhibit excellent sodium-glucose transporter (SGLT1 and SGLT2) inhibition activity.

Introduction

Compounds containing fused triazoles have become increasingly important in recent years as chemotherapeutic and cardiovascular agents.[1] In particular, sugar derived fused 1,2,3-triazoles have gained interest as candidates to treat variety of carbohydrate-mediated diseases, such as diabetes, viral infections including HIV, and cancer metastasis (Compounds **1-6**; Figure 1).[2] Moreover these fused triazole based bioactive heterocycles have proved to be valuable as genuine amide surrogates due to their physiochemical properties (peptide isosters) and remarkable metabolic stability.[3]

Although several efforts were made for synthesis of aromatic fused bicyclic triazoles[1a,4] their application for the synthesis of carbohydrate derived triazole-fused bicyclic heterocycles was scarce.[2] Most of the early reported methods used D-glucose as the starting material.[5] L-Rhamnose, a naturally occurring and easily available 6-deoxysugar[6] without any mammalian toxicity, was used as the starting material replacing glucose. For example, George *et al*[7] reported total synthesis of bioactive (-)-salicylihalamides using L-rhamnose as the starting substrate replacing glucose. As a part of our ongoing research[8] on development of rhamnose derived heterocycles, we herein describe synthesis of novel fused chiral bicyclic triazoles from readily available 6-deoxymannose (L-rhamnose) and their evaluation as potent SGLT inhibitors.

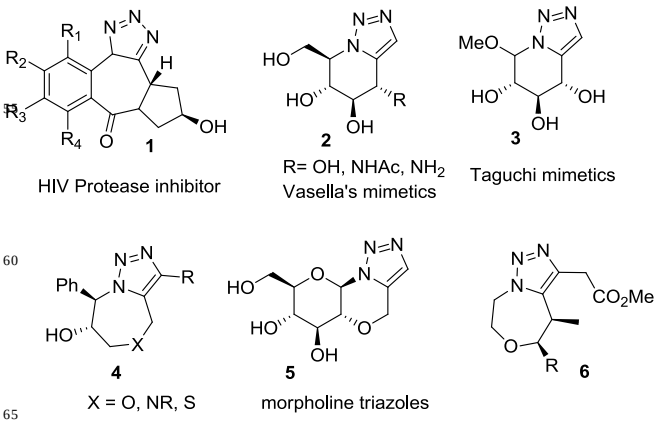
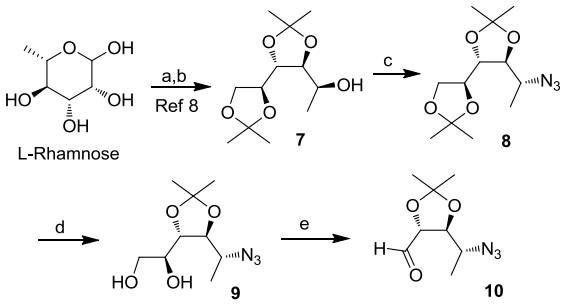


Figure 1. Examples of Fused bicyclic 1,2,3-triazoles as potential drug candidates

Scheme 1. Synthesis of azidoaldehyde **10.**



Reagents and conditions: a) $NaBH_4$, MeOH; b) 2,2-dimethoxypropane, acetone, Conc.H_2SO_4; c) TPP, DIAD, DPPA, THF, 0°-RT; d) 50% *aq*. AcOH; e) CH_3OH-H_2O, $NaIO_4$.

Results and Discussions

Initiating the study, the required azidoaldehyde **10** [(4*R*,5*S*)-5-((*R*)-1-azidoethyl)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde] was synthesized from commercially available L-rhamnose

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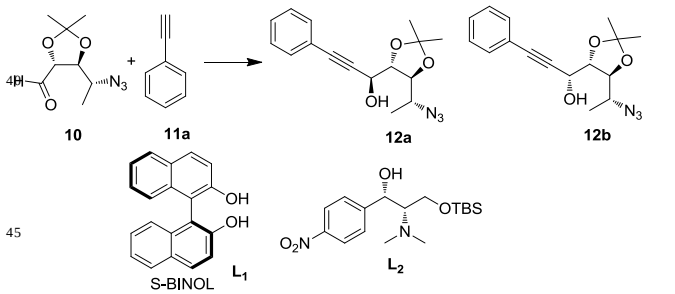
† Electronic Supplementary Information (ESI) available: Experimental data for **12c-q**, **13c-q**, and copies of ^1H & ^{13}C NMR, HRMS spectra of **8, 9, 10, 12a-r**, and **13a-r**, Cif files of single crystal X-ray diffraction data CCDC 944748 (for **13a**) and CCDC 944749 (for **13b**). for crystallographic data. See DOI: 10.1039/ b000000x/

(Scheme 1). Alcohol **7** synthesized previously in our lab,[8] was converted to the respective azido analogue **8** in 90% yield under Mitsunobu reaction conditions. Selective deprotection of primary acetonide in **8** was achieved in 85% yield by stirring the mixture in 50% aqueous acetic acid. The required building block **10** was obtained after oxidative cleavage of 1,2-diol **9** with $NaIO_4$ in methanol: water (4:1) at room temperature. All the compounds described in scheme 1 were fully characterized by their ^1H, ^{13}C NMR and mass spectral analysis.

Having azidoaldehyde **10** in hand, the selective addition of phenyl acetylene (**11a)** was examined under various reaction conditions. The diastereomeric ratio of products **12a** & **12b** was assessed using ^1H NMR spectra of reaction mixture. Initially, addition of **11a** to **10** using *n*-butyl lithium (n-BuLi) as the base in dry THF at -78 °C gave both diastereomeric products **12a** & **12b** in equal ratio (entry 1, Table 1). Use of hexamethyl phosphoramide (HMPA) as an additive in combination with n-BuLi increased ratio of diastereoselectivity to 79:21 respectively (entry 2, Table 1). Later experiments revealed that addition of phenyl acetylene in presence of S-Binol ligand $\mathbf{L_1}$, diethyl zinc and HMPA as additive to **10** yielded **12a** and **12b** in 83:17 diastereomeric ratio. Use of titanium isopropoxide in the place of HMPA further increased the ratio to 93:7. After series of experiments varying reaction conditions, catalysts and additives, **12a** was obtained in >97 diastereomeric ratio, after enantioselective addition of phenyl acetylene to **10** in the presence of ligand $\mathbf{L_2}$ in combination with zinc triflate and triethylamine in dry toluene at room temperature. The ligand $\mathbf{L_2}$ required here was synthesized from the (1*S*,2*S*)-(+)-2-Amino-1-(4-nitrophenyl)-1,3-propanediol in two steps using literature procedure.[9] The general scope and versatility of the reaction was further investigated using a series of alkynes **11b-q**. All these reactions proceed smoothly to give diastereoselective azidoalkynols **12b-q** in excellent yields (Scheme 2). The products **12a-q** was fully characterized by their IR, ^1H & ^{13}C NMR and Mass spectral data.

Table 1. Optimization of reaction conditions for addition of **11a** to **10**[a]

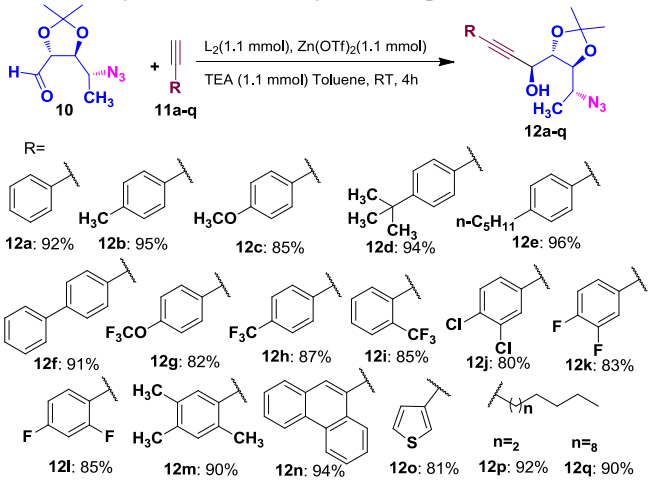


S.No	Reagent/ additive	Ligand/ catalyst	Temp (°C)	Yield (%)[b]	Dr (**12a**: **12b**)[c]
1	BuLi	-	-78	75	50: 50
2	BuLi/ HMPA	-	-78	78	79: 21
3	HMPA	L_1	RT	73	83: 17
4	-	L_1/Ti(*i*-OPr)$_4$	RT	80	93: 7
5	-	L_2/Zn(OTf)$_2$	RT	92	>97: 3

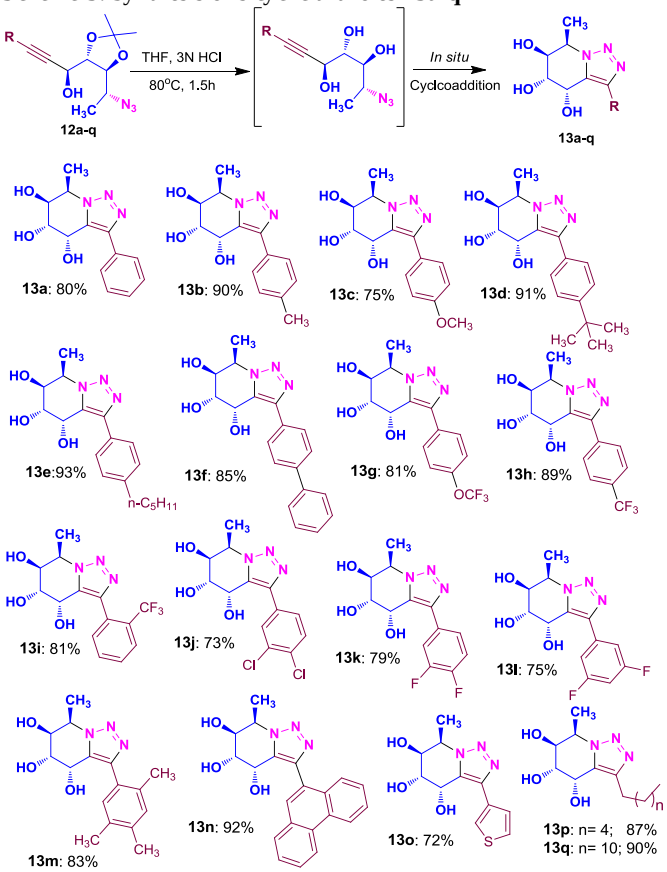
[a]*Reaction conditions*: **10** (1 mmol), **11a** (1.2 mmol), BuLi (1.1 mmol), HMPA (1.1 mmol), L_1 (30 mol%), Ti(i-OPr)$_4$ (30 mol%), L_2 (1.1 mmol), and Zn(OTf)$_2$ (1.1 mmol). [b]Isolated yields. [c]The diastereomeric ratio was determined using ^1H NMR spectra of diasteromeric products **12a** and **12b.** Dr = Diastereomeric ratio.

Now with series of azidoalkynols **12a-q** in hand, we next examined various reaction conditions for the preparation of fused chiral bicyclic triazoles. Initial attempts using standard click reaction conditions [Na-ascorbate, $CuSO_4.5H_2O$, ^{t}BuOH:H_2O (1:1), RT] did not give fruitful results. Further increase in temperature and time did not help in the progress of reaction; instead, it led to decomposition of reaction mixture. In next set of experiments, deprotection of acid sensitive 1,2-isopropylidene group was planned before azide-alkyne cycloaddition reaction.

Scheme 2. Synthesis of azidoalkynols **12a-q**

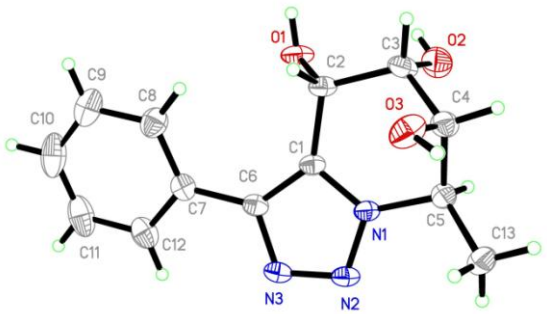


Scheme 3: Synthesis of bicyclic triazoles **13a-q**



Compound **12a** when treated with 3N HCl in THF at 80°C, surprisingly resulted fused chiral bicyclic triazole **13a** in 80% yield, in addition to the deprotection of acid sensitive 1,2-isopropylidene group (Scheme 3). The product **13a** was fully characterized by its IR, ^{1}H & ^{13}C NMR and Mass (ESI and HRMS) spectral data. Single crystal X-ray analysis unambiguously confirmed the structure for **13a** (Figure 2).

Figure 2: ORTEP diagram of **13a.** Displacement ellipsoids are drawn at 30% probability level.



Scheme 4: Synthesis of bis-fused bicyclic triazole **13r**

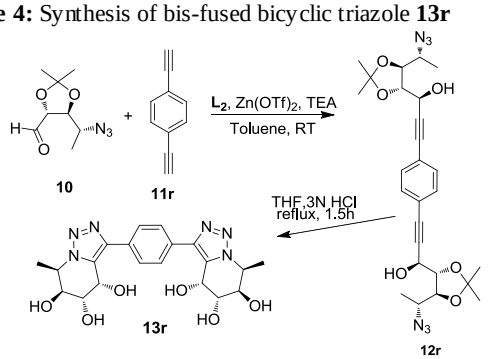


Table 2: Evaluation of SGLT1 and SGLT2 activity[16] for **13a-r**

Entry	Product	SGLT1 (IC_{50} nM)	SGLT2 (IC_{50} nM)	SGLT1/ SGLT2
1.	13a	135.3	381.4	0.3549
2.	13b	174.8	114.5	1.5269
3.	13c	187.3	554.3	0.3380
4.	13d	100.3	204.1	0.4914
5.	13e	230.8	96.9	2.3818
6.	13f	290.6	102.0	2.8481
7.	13g	102.1	134.2	0.7614
8.	13h	218.5	169.1	1.2916
9.	13i	256.0	260.4	0.9832
10.	13j	68.3	132.1	0.4494
11.	13k	229.8	110.5	2.0796
12.	13l	265.5	185.2	1.4335
13.	13m	344.4	186.6	1.8459
14.	13n	136.2	157.6	0.8645
15.	13o	82.9	143.5	0.5774
16.	13p	125.7	409.5	0.3069
17.	13q	118.3	545.03	0.2170
18.	13r	154.8	223.4	0.6926
19.	Phlorizin	65.5	77.9	0.8408

To generalize the protocol, all other azidoalkynols, **12b-q** were reacted with 3N HCl in THF for 1.5 h at 80°C. Fused bicyclic triazoles **13b-q**, were indeed formed in 72-93% yields. All the products **13b-q** was fully characterized through ^{1}H & ^{13}C NMR and Mass spectral analysis. The product **13b** was also confirmed by single crystal X-ray analysis (See ESI). Further, reaction of **10** with 1,4-diethynyl benzene **11r** in presence of ligand $\mathbf{L_2}$, zinc triflate, and triethylamine in dry toluene at room temperature gave bis-azidoalkynol **12r** in 79% yield (Scheme 4). Compound **12r** when treated with 3N HCl in THF at 80°C gave bis-fused derivative **13r** in 70% yield.

Mechanistically, soon after deprotection of acid sensitive 1,2-isopropylidene group, the transition intermediate may be attaining conformational flexibility in its architecture, and triggering *in situ* intramolecular Huisgen [3+2] cycloaddition to yield required fused chiral bicyclic triazoles **13a-r** (Scheme 3 & 4).

Pharmacology

Inhibition of sodium-glucose co-transporters (SGLTs) was considered as one of the therapeutic options to reduce blood glucose level independent of insulin.[10] Over the past 10 years, a series of O-glucosides and C-glucosides has been reported as SGLT2 inhibitors.[11] T-1095 is the first structural derivative of Phlorizin, a natural non selective SGLT inhibitor.[12] Sergliflozin and remogliflozin are representatives of the O-glucoside class of SGLT2 inhibitors.[13] Meanwhile, dapagliflozin, followed by canagliflozin and empagliflozin have emerged as C-aryl glucoside class of SGLT2 inhibitors.[14] Besides this, C-glucosides with indole, benzisothiazole, thiophene and triazole aglycon were also investigated as inhibitors of SGLT2 (Figure 3).[15]

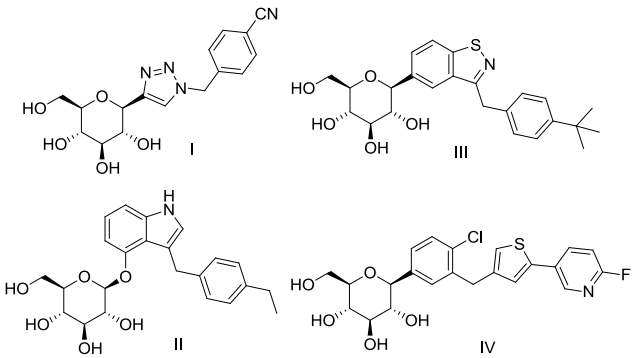


Figure 3: Examples of SGLT2 inhibitors having triazole(I), indole(II) benzisothiazole(III), and thiophene(IV) aglycons.

In our efforts to find new and potent acyclic C-nucleosides[8] as novel SGLT inhibitors, new chiral fused triazoles **13a-r** was screened for sodium glucose co-transporters SGLT1 and SGLT2 inhibitory activity using cell-based nonradioactive fluorescent glucose uptake assay.[16] The IC_{50} values (concentration to inhibit 50% D-glucose uptake in cells) of **13a-r** were determined from the glucose uptake inhibition curves with reference to phlorizin. The determined IC_{50} values of **13a-r** and the reference drug phlorizin (for comparison purpose) are depicted in Table 2. All eighteen compounds **13a-r** showed SGLT1 and SGLT2 inhibition activity with IC_{50} ranging from 68.3-545.0 nM. Among all these derivatives, **13e**, **13f**, and **13k** are found to be the most potent SGLT2 inhibitors with IC_{50}: 96.9, 102.0, and 110.5 nM,

respectively. Other analogues **13d** (100.3 nM), **13g** (102.1 nM), and **13j** (68.3 nM) are found to be the most potent SGLT1 inhibitors. Alkyl chain residue analogs **13p** and **13q** are selective SGLT1 inhibitors with IC_{50}: 125.7, and 118.3 nM respectively.

Conclusions

In conclusion, we have synthesized a series of novel fused bicyclic heterocycles **13a-r** from L-rhamnose and evaluated them as SGLT inhibitors. The key reaction of azidoalkynols **12a-r** with 3N HCl in THF at 80 °C resulted 3+2 cycloaddition along with deprotection of 1,2-isopropylidene to give fused chiral bicyclic 1,2,3-triazoles **13a-r** in high yields. All the compounds synthesized and screened, exhibited potent sodium-glucose co-transporter (SGLT1 and SGLT2) inhibitory activity.

Acknowledgements

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Experimental

General Remarks

All the solvents were dried according to standard procedures. The reactions were carried out under nitrogen atmosphere. All the compounds were purified by column chromatography on 60-120 mesh silica gel using hexanes-ethyl acetate as eluent. All the reactions were monitored by TLC analysis. ^{1}H NMR spectra were recorded on 500 MHz or 300 MHz instruments using $CDCl_3$ or DMSO-d_6 as solvent and TMS as an internal standard. ^{13}C NMR spectra were recorded at 75 MHz or 125 MHz using $CDCl_3$ or DMSO-d_6 as solvent and reference. Optical rotation was recorded on DIGIPOL DP 786-M6U Polarimeter. Absolute configuration of the product was determined by single crystal X-ray analysis. Based on the stereochemistry of **13a** and **13b**, the relative configurations of all the products were determined.

(4*R*,4'*S*,5*S*)-5-((*R*)-1-Azidoethyl)-2,2,2',2'-tetramethyl-4,4'-bi(1,3-dioxolane) (8). To a solution of alcohol **7** (4.0 g, 16.2 mmol) and triphenylphospine (5.1g, 19.5 mmol) in dry THF was added diisopropylazadicarboxylate (4 mL, 20 mmol) slowly at 0°C. After 10 minutes diphenylphosponic azide (4.2 mL, 19.5 mmol) was added at 0°C and stirred at room temperature over night. After completion, THF was evaporated under vacuo and the crude product was thus obtained purified by silica gel column chromatography eluted with hexane: ethyl acetate (98:2) to give azido compound **8** (4.1 g, 90%) as light yellow liquid. $[\alpha]_D^{29}$ 1.7(c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13-4.03(m, 1H), 3.98-3.80(m, 4H), 3.38(dq, J=6.7 Hz, 1.7, 1H), 1.48(d, J=7.0 Hz, 3H), 1.44(s, 3H), 1.37(s, 3H), 1.35(s, 3H), 1.30(s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 109.9, 109.7, 96.1, 83.7, 77.7, 77.4, 67.9, 55.9, 27.3, 26.7, 25.3, 16.3. IR (neat) 2986, 2935, 2101, 1456, 1378, 1247, 1055, 875 cm^{-1}. MS (ESI) *m/z* 272[M+H]$^+$, HRMS (ESI) Calcd for $C_{12}H_{21}N_3O_4Na[M+Na]^+$: 294.1429, found: 294.1440.

(*S*)-1-((4*S*,5*S*)-5-((*R*)-1-Azidoethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol (9).

The azide **8** (4.0 g,14.7 mmol) in 50% acetic acid (30 mL) was stirred at room temperature overnight, poured in water (30 mL), extracted with ethyl acetate (2 x 70 mL) and washed with *aq.* $NaHCO_3$. The organic extract was washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The crude residue thus obtained was purified over silica gel column chromatography (ethyl acetate/hexane, 1:1) to give **9** (2.9 g, 85%) as a thick syrup. $[\alpha]_D^{29}$ 14.7 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.03-3.96(m, 2H), 3.88-3.80(m, 1H), 3.76-3.68(m, 2H), 3.50(dq, J=6.7 Hz, 1.5, 1H), 1.47(s, 3H), 1.45(d, J=6.7 Hz, 3H), 1.40(s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 109.9, 82.7, 77.0, 73.0, 63.8, 56.4, 27.1, 26.7, 16.0. IR (neat) 3416, 2988, 2937, 2108, 1456, 1379, 1246, 1057, 874 cm^{-1}. MS (ESI) *m/z* 232[M+H]$^+$, HRMS (ESI) Calcd for $C_9H_{17}N_3O_4Na[M+Na]^+$: 254.1116, found: 254.1130.

(4*R*,5*S*)-5-((*R*)-1-Azidoethyl)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (10).

To a solution of diol **9** (1.0 g, 4.3 mmol) in methanol: water (8:2, 10 mL), $NaIO_4$ (1.1 g, 5.1 mmol) was added and stirred at RT for 1.5 h. Water (40 mL) was added to reaction mixture and extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The crude residue of **10** (0.86 g, 100 %) thus obtained was used as such for further reaction. For analytical purpose small amount of crude residue was purified over silica gel flash chromatography (ethyl acetate /hexane, 1:1) to result **10** as thick syrup. $[\alpha]_D^{29}$ -6.2 (c 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.81(d, J=1.2 Hz, 1H), 4.32(dd, J=1.2, 6.2 Hz, 1H), 4.10(dd, J=4.7, 6.1 Hz, 1H), 3.80(dq, J=1.6, 6.5 Hz, 1H), 1.55(s, 3H), 1.37(s, 3H), 1.26(d, J=6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.4, 111.7, 81.5, 79.9, 58.2, 26.4, 25.7, 15.7. IR (neat) 2987, 2936, 2121, 1733, 1456, 1379, 1254, 1069, 871 cm^{-1}. MS (ESI) *m/z* 200[M+H]$^+$, HRMS (ESI) Calcd for $C_8H_{14}N_3O_3[M+H]^+$: 200.1035, found: 200.1040.

General procedure for the enantioselective alkynylation of 10.

To a solution of $Zn(OTf)_2$ (399 mg, 1.1 mmol) and chiral ligand L_2 (389 mg, 1.1 mmol) in dry toluene was added triethylamine (0.15 mL, 1.1 mmol) and alkyne **11a-r** (1.2 mmol) under N_2 atmosphere. After 15 min, the azidoaldehyde **10** (200 mg, 1mmol) was introduced by syringe. The reaction mixture was stirred for 4h at 25°C. After the reaction was completed, the propargylic alcohol was separated from the ligand by washing with aq. HCl. The crude product was purified through a short flash chromatography column to give the propargylic alcohols **12a-r** as syrupy liquids.

(*S*)-1-((4*S*,5*S*)-5-((*R*)-1-Azidoethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-3-phenylprop-2-yn-1-ol (12a). Yield: 92%; $[\alpha]_D^{27}$ -27.4 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44-7.41(m, 2H), 7.36-7.30(m, 3H), 4.80(d, J=3.6 Hz, 1H), 4.27(dd, J=3.6 Hz, 7.7 Hz, 1H), 4.17(dd, J=2.7, 7.7 Hz, 1H), 3.58(dq, J=2.8, 7.0 Hz, 1H), 1.52 (s, 3H), 1.49(s, 3H), 1.47(d, J=6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.0, 131.5, 129.1, 118.7, 110.3, 87.2, 84.7, 80.0, 79.3, 62.3, 56.7, 27.0, 21.4, 16.1. IR (neat) 3429, 2986, 2927, 2112, 1378, 1245, 1067, 767, 694 cm^{-1}. MS (ESI) *m/z* 302[M+H]$^+$, HRMS (ESI) Calcd for $C_{16}H_{20}N_3O_3[M+H]^+$: 302.14992, found: 302.14838.

(*S*)-1-((4*S*,5*S*)-5-((*R*)-1-Azidoethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-3-(p-tolyl)prop-2-yn-1-ol (12b). Yield: 95%; $[\alpha]_D^{27}$ -117.8 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32(d, J=8.3 Hz, 2H), 7.13(d, J=7.5 Hz, 2H), 4.80(d, J=3.7 Hz, 1H), 4.26(dd, J=3.7, 8.3 Hz, 1H), 4.17(dd, J=3.0, 7.5 Hz, 1H), 3.57(dq, J=2.2, 6.7 Hz, 1H), 2.35(s, 3H), 1.52(s, 3H), 1.48(s, 3H), 1.46(d, J=7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.0, 131.5, 129.1, 118.7, 110.3, 87.2, 84.7, 80.0, 79.3, 62.3, 56.7, 27.1, 27.0, 21.4, 16.1. IR (neat) 3429, 2986, 2927, 2112, 1378, 1245, 1067, 767, 694 cm^{-1}. MS (ESI) *m/z* 316[M+H]$^+$**,** HRMS (ESI) Calcd for $C_{17}H_{22}N_3O_3$[M+H]$^+$: 316.16557, found: 316.16373**.**

(*S*,*S*,*R*,1*S*,1'*S*)-3,3'-(1,4-Phenylene)bis(1-((4*S*,5*S*)-5-((*R*)-1-azidoethyl)-2,2-dimethyl-1,3-dioxolan-4-yl) prop-2-yn-1-ol) (12r). Yield: 79%; $[\alpha]_D^{27}$ -72.0 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19(d, J=8.8 Hz, 2H), 7.59(d, J=8.6 Hz, 2H), 4.78(d, J=3.9 Hz, 2H), 4.26(dd, J= 3.9, 7.7 Hz, 2H), 4.14(dd, J= 2.8, 7.7 Hz, 2H), 3.54(dq, J= 2.8, 6.8 Hz, 2H), 1.52(s, 6H), 1.48(s, 6H), 1.46(d, J=6.8 Hz, 6H). ^{13}C NMR (75 MHz,$CDCl_3$) δ 131.6, 128.0, 123.3, 122.2, 110.3, 87.8, 86.2, 80.2, 79.2, 71.2, 69.0, 62.5, 56.6, 57.0, 41.6, 27.1, 26.9, 25.6, 16.0. IR (neat) 3436, 2987, 2932, 2112, 1502, 1384, 1250, 1088, 840 cm^{-1}. MS (ESI) *m/z* 525[M+H]$^+$**,** HRMS (ESI) Calcd for $C_{26}H_{33}N_6O_6$[M+H]$^+$: 525.24561, found: 525.24315**.**

A typical procedure for the synthesis of triazolo pyridines (13a-r):

To a solution of 12 (100 mg) in 2 mL of THF, 3N HCl (3mL) was added and refluxed for 1.5h. After the reaction was completed, the reaction mixture cool to RT, and neutralized with aq $NaHCO_3$ and extracted with ethyl acetate (2 x 15 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by silica gel column chromatography (ethyl acetate/hexane, 8:2) to afford 13 as a white solid.

(4*S*,5*R*,6*S*,7*R*)-7-Methyl-3-phenyl-4,5,6,7-tetrahydro-[1,2,3] triazolo[1,5-a]pyridine-4,5,6-triol (13a). Yield: 80%; MP= 243°C; $[\alpha]_D^{27}$ -8.1 (c 1.0, CH_3OH). ^{1}H NMR (300 MHz, CD_3OD+$CDCl_3$) δ 7.86(d, J=7.1 Hz, 2H), 7.46-7.29(m, 3H), 5.21(d, J=4.1 Hz, 1H), 4.88-4.81(m, 2H), 4.27(dd, J=3.7, 6.6 Hz,1H), 4.14(dd, J=4.1, 6.6 Hz ,1H), 1.70(d, J=6.7 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD+$CDCl_3$) δ 146.0, 131.9, 131.7, 129.0, 128.6, 128.3, 70.1, 69.4, 63.1, 55.0, 15.6. IR (neat) 3549, 3447, 3201, 2979, 1495, 1368, 1267, 1226, 1098, 1038, 994, 695, 648 cm^{-1}. MS (ESI) *m/z* 262[M+H]$^+$; HR-MS (ESI) Calcd for $C_{13}H_{16}N_3O_3$[M+H]$^+$: 262.1191, found: 262.1179.

(4*S*,5*R*,6*S*,7*R*)-7-Methyl-3-(p-tolyl)-4,5,6,7-tetrahydro-[1,2,3] triazolo[1,5-a]pyridine-4,5,6-triol (13b). Yield: 90%; MP= 236°C; $[\alpha]_D^{27}$ -60.9 (c 1.0, CH_3OH). ^{1}H NMR (300 MHz, DMSO+$CDCl_3$) δ 7.77(d, J=8.1 Hz, 2H), 7.14(d, J=7.9 Hz, 2H), 5.49(brs, 1H), 5.22 (brs, 1H), 5.05 (brs, 2H), 4.71-4.62(m, 1H), 4.15-3.99(m, 2H), 2.31(s, 3H), 1.60(d, J=6.6 Hz, 3H). ^{13}C NMR (75 MHz, DMSO+$CDCl_3$) δ 143.8, 136.1, 130.7, 128.5, 128.4, 126.9, 69.1, 68.2, 62.0, 52.9, 20.7, 15.0 . IR (neat) 3549, 3447, 3201, 2979, 1495, 1368, 1267, 1226, 1098, 1038, 994, 695, 648 cm^{-1}. MS (ESI) *m/z* 276[M+H]$^+$; HR-MS (ESI) Calcd for $C_{14}H_{18}N_3O_3$M+H]$^+$: 276.1348, found: 276.1360.

(4*S*,5*R*,6*S*,7*R*)-7-Methyl-3-(4-((4R,5S,6R,7S)-4,5,6-tri hydroxy-7-methyl-4,5,6,7-tetrahydro-[1,2,3]triazolo[1,5-a] pyridin-3-yl)phenyl)-4,5,6,7-tetrahydro-[1,2,3]triazolo[1,5-a] pyridine-4,5,6-triol (13r). Yield: 70%; $[\alpha]_D^{27}$ 24.2 (c 1.0, CH_3OH). ^{1}H NMR (500 MHz, DMSO) δ 8.22(d, J=8.8 Hz, 2H), 7.67(d, J=8.6 Hz, 2H), 5.65(d, J=4.5 Hz, 2H), 5.49(d, J=4.1 Hz, 2H), 5.40(d, J=8.6 Hz, 2H), 5.09(dd, J=3.5, 8.3 Hz, 4H), 4.66(dq, J=3.2, 6.7 Hz, 4H), 4.12-3.97(m, 6H), 1.60(d, J=6.7 Hz, 6H). ^{13}C NMR (75 MHz, DMSO) δ 143.5, 137.5, 131.7, 130.4, 128.2, 126.9, 69.5, 68.4, 62.1, 52.8, 15.1. IR (neat) 3539, 3282, 2926, 2852, 2696, 1463, 1098, 1061, 994 cm^{-1}. MS (ESI) *m/z* 445 [M+H]$^+$; HR-MS (ESI) Calcd for $C_{20}H_{25}N_6O_6$ [M+H]$^+$: 445.1835, found: 445.1850.

X-ray single crystal data for 13a : $C_{13}H_{15}N_3O_3$, *M* = 261.28, colorless block, 0.22 × 0.19 × 0.14 mm^3, orthorhombic, space group $P2_12_12_1$ (No. 19), *a* = 7.0280(11), *b* = 12.5922(19), *c* = 14.037(2) Å, *V* = 1242.2(3) Å^3, *Z* = 4, D_c = 1.397 g/cm^3, F_{000} = 552, CCD Area Detector, MoKα radiation, λ = 0.71073 Å, *T* = 294(2)K, $2\theta_{max}$ = 50.0º, 11307 reflections collected, 1285 unique (R_{int} = 0.0228). Final *GooF* = 1.060, *R1* = 0.0277, *wR2* = 0.0720, *R* indices based on 1239 reflections with I>2σ(I) (refinement on F^2), 185 parameters, 0 restraints, μ = 0.101 mm^{-1}. CCDC 944748 contains supplementary Crystallographic data for the structure.

X-ray single crystal data for 13b : $C_{14}H_{17}N_3O_3$, *M* = 275.31, colorless needle, 0.18 × 0.09 × 0.07 mm^3, orthorhombic, space group $P2_12_12_1$ (No. 19), *a* = 7.5550(5), *b* = 11.9870(8), *c* = 15.0918(11) Å, *V* = 1366.74(16) Å^3, *Z* = 4, D_c = 1.338 g/cm^3, F_{000} = 584, CCD Area Detector, MoKα radiation, λ = 0.71073 Å, *T* = 294(2)K, $2\theta_{max}$ = 50.0º, 13098 reflections collected, 1402 unique (R_{int} = 0.0220). Final *GooF* = 1.083, *R1* = 0.0282, *wR2* = 0.0717, *R* indices based on 1355 reflections with I>2σ(I) (refinement on F^2), 195 parameters, 0 restraints, μ = 0.096 mm^{-1}. CCDC 944749 contains supplementary Crystallographic data for the structure.

Evaluation of 13a-r for SGLT Inhibition:[8a,16]

Cell culture: Human embryonic kidney (HEK293) cells were purchased from ATCC, USA and made two stable cell lines after expressing SGLT1 & SGLT2, respectively. Previously, we searched the selectivity of these cell lines for SGLT1 and SGLT2 inhibition study. Glucose uptake by these cell lines was only inhibited by SGLT inhibitors but not by any other GLUTs inhibitors. SGLT1 & SGLT2 transfected HEK cell lines were propagated at 37 °C in 5% CO_2 in Dulbecco's minimal essential medium (DMEM) supplemented with 1.0% of penicillin-streptomycin and 10% heat inactivated fetal bovine serum (FBS). The cells were cultured in a 90mm dish in DMEM with 10% FBS until 70-80 % confluency was obtained for further use for SGLT1 and SGLT2 inhibition activity.

SGLT inhibition assay: SGLT1/ SGLT2 transfected stable HEK cells were plated at 1X10^4/well in 96-well plate and used at sub confluence after 24h pre-incubation. For measuring SGLT1-mediated glucose uptake, all culture media was removed from each well and replaced with 100 µl of culture medium with newly synthesized molecules **13a-r** at different concentrations (50nm-1000nm). After half an hour, fluorescent 2-deoxy-glucose (2-NBDG) was added to the plates and incubated at 37°C with 5% CO_2 for a period of 30 min (Kanwal et al, 2012). Cells were lysed with 50 µL of 0.1 N NaOH and fluorescence of aliquots from the lysate was measured at excitation/emission maxima of ~465/540 nm. Phlorizin (non-Specific SGLT1 inhibitor) was used as a standard for this study.

Notes and References

1. (a) K.C. Majumdar, K. Ray, *Synthesis*, 2011, 3767-3783; (b) Agalave, S. G.; Maujan, S. R.; Pore, V. S. *Chem. Asian J.* 2011, **6**, 2696-2718.
2. (a) N. D. Adhikary, P. Chattopadhyay, *J. Org. Chem.* 2012, **77**, 5399-5405; (b) S. Miriam, R-E. Carles, A.P. Miquel, *Org. Lett.*, 2011, **13**, 5044-5047; (c) R A. Brawn, M. Welzel, J.T. Lowe, J.S. Panek, *Org. Lett.*, 2010, **12**, 336-339; (d) Y. S. Reddy, A. P. J. Pal, P. Gupta, A. A. Ansari, Y. D. Vankar. *J. Org. Chem*. 2011, **76**, 5972–5984; (e) M. L H. Yanai, S. Obara, T. Taguchi, *Org. Biomol. Chem*., 2008, **6**, 2679-2685; (f) Mitchell, F. Tian L. V. Lee, C- H. Wong, *Angew. Chem. Int. Ed*. 2002, **41,** 3041-3044.
3. J. M. Holub, K. Kirshenbaum, *Chem. Soc. Rev*., 2010, **39**, 1325–1337.
4. (a) B. Abarca, R. Ballesteros-Garrido, *Top. Heterocycl. Chem*. 2014, DOI: 10.1007/7081_2014_121; (b) J. M. Schulman, A. A. Friedman, J. Panteleev, M. Lautens *Chem. Commun.*, 2012, **48**, 55–57; (c) R. Li, D.J. Jansen, A. Datta *Org. Biomol. Chem*., 2009, 7, 1921-1930. (d) F. Zhou, C. Tan, J. Tang, Y-Y. Zhang, W-M. Gao, H-H. Wu, Y-H. Yu, and J. Zhou, *J. Am. Chem. Soc.*, 2013, **135**, 10994–10997.
5. (a) V. S. Sudhir, N. Y. P. Kumar, R. B N. Baig, S. Chandrasekaran, *J. Org. Chem.* 2009, **74**, 7588–7590; (b) R.I. Anegundi, V.G. Puranik, S. Hotha, *Org. Biomol. Chem.*, 2008, **6,** 779-786.
6. (a) S. Singh, A. Chang, K. E. Helmich, C. A. Bingman, R. L. Wrobel, E. T. Beebe, S-i. Makino, D. J. Aceti, K. Dyer, G. L. Hura, M. Sunkara, A. J. Morris, G. N. Phillips, Jr., J. S. Thorson, *ACS Chem. Biol*. 2013, **8**, 1632-1639; (b) A.A. Ghoneim, Chemistry Central J. 2011, **5**, 1-7. doi:10.1186/1752-153X-5-7; (c) B. G. Davis, T. W. Brandstetter, L. Hackett, B. G. Winchester, R. J. Nash, A. A. Watson, R. C. Griffiths, C. Smith, G. W.J. Fleet, *Tetrahedron*, 1999, **55**, 4489-4500.
7. H. Torsten, H. Kyounglang, E. Wibke, B. Blackman, S. Roy, P. Srinivas, G. George, *J. Org. Chem*. 2005, **70**, 7592-7604.
8. (a) S. R. Putapatri, A. Kanwal, S. K. Banerjee, S. Kantevari, *Bioorg. Med. Chem. Lett*., 2014, **24**, 1528-1531; (b) S. Kantevari, S.R. Putapatri, *Synlett*, 2010, 2251-2256.
9. B. Jiang, Z. Chen, W. Xiong, *Chem. Commun.*, 2002, **48**, 1524–1525.
10. (a) A. Kanwal, S. K. Banerjee, *Pharm. Pat. Analyst*, 2013, **2**, 77-91. (b) D. Hinnen, *J. Cardiovas. Nurs*. 2013, **28(2)**, 157-165. (c) A. Mather, C. Pollock, *Nat. Rev. Neph*. 2010, **6(5),** 307-311. (d) E. M. Wright, D. D. F. Loo, B. A. Hirayama, *Physiol. Rev*. 2011, **91(2),** 733-794.
11. (a) D. Vasilakou, T. Karagiannis, E. Athanasiadou, M. Mainou, A. Liakos, E. Bekiari, M. Sarigianni, D. R. Matthews, A. Tsapas, *Ann. Intern. Med.* 2013, **159**, 262-274. (b) J. Liu, T. W. Lee, *SGLT2 Inhibitors for Type 2 Diabetes*, J.E. Macor, (ed.), Book Series: *Annual Reports in Medicinal Chemistry*, 2011, **46**, 103-115. (c) M. Pfister, J. M. Whaley, L. Zhang, J. F. List, *Clin Pharmacol. Ther*. 2011, **89**, 621-625. (d) L-T Ho, S. S. Kulkarni, J-C. Lee, *Curr. Top. Med. Chem.*, 2011, **11**, 1476-1512.
12. (a) E. C. Chao, R. R. Henry, *Nat. Rev. Drug Discov*. 2010, **9**, 551-559. (b) H-Y. Hung, K. Qian, S. L. Morris-Natschke, C-S. Hsu, K-H. Lee, *Nat. Prod. Rep.*, 2012, **29**, 580-606.
13. (a) K. Katsuno, Y. Fujimori, Y. Takemura, M. Hiratochi, F. Itoh, Y. Komatsu, H. Fujikura, M. Isaji, *J Pharmacol Exp Ther*. 2007, **320(1),** 323-330. (b) R. L. Dobbins, R. O'Connor-Semmes, A. Kapur, C. Kapitza, G. Golor, I. Mikoshiba, W. Tao, E.K. Hussey, *Diabetes Obes Metab*. 2012, **14**, 15-22. (c) Y. Fujimori, K. Katsuno, I. Nakashima, Y. Ishikawa-Takemura, H. Fujikura, M.J. Isaji, *Pharmacol. Exp. Ther*. 2008, **327**, 268–276.
14. (a) Y. Ohtake, T. Sato, T. Kobayashi, M. Nishimoto, N. Taka, K. Takano, k. Yamamoto, M. Ohmori, M. Yamaguchi, K. Takami, S.Y. Yeu, K.H. Ahn, H. Matsuoka, K. Morikawa, M. Suzuki, H. Hagita, K. Ozawa, K. Yamaguchi, M. Kato, S. Ikeda, *J. Med. Chem*., 2012, **55,** 7828–7840. (b) E.M. Lamos, L.M. Younk, S.N Davis, *Expert Opin. Drug Metab. Toxicol.* 2013, **9**, 763-775. (c) R. Grempler, L. Thomas, M. Eckhardt, F. Himmelsbach, A. Sauer, D.E Sharp, R.A. Bakker, M. Mark, T. Klein, P. Eickelmann, *Diabetes Obes. Metab*. 2012, **14**, 83–90.
15. (a) Y. Yamamoto, E. Kawanishi, Y. Koga, S. Sakamaki, T. Sakamoto, K. Ueta, Y. Matsushita, C. Kuriyama, M. Tsuda-Tsukimoto, S. Nomura, *Bioorg. Med, Chem. Lett.* 2013, **23**, 5641–5645. (b) Y. Koga, S. Sakamaki, M. Hongu, E. Kawanishi, T. Sakamoto, Y. Yamamoto, H. Kimata, K. Nakayama, C. Kuriyama, Y. Matsushita, K. Ueta, M. Tsuda-Tsukimoto, S. Nomura, *Bioorg. Med. Chem*. 2013, **21**, 5561-5572. (c) L.T. Li, L.F. Zhou, Y.J. Li, J. Huang, R.H. Liu, B. Wang, P. Wang, *Bioorg. Med. Chem. Lett.* 2012, **22**, 642-644. (d) H. Zhou, D.P. Danger, S.T. Dock, L. Hawley, S.G. Roller, C.D. Smith, A.L. Handlon, *ACS Med. Chem. Lett.*, 2010, **1**, 19–23.
16. (a) H.C. Chang, S.F. Yang, C. C. Huang, T. Lin, P.H. Liang, C.J. Lin, L.C. Hsu, *Mol Biosyst.* 2013, **9**, 2010-2020; (b) A. Kanwal, S. P. Singh, P. Grover, S. K. Banerjee, *Anal. Biochem*., 2012, **429,** 70-75.