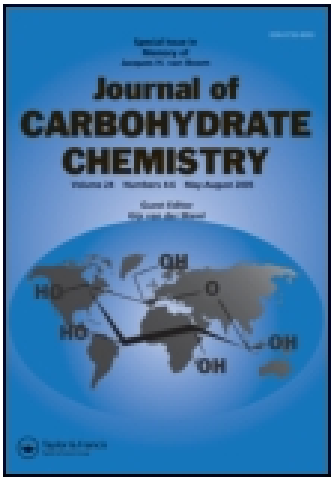

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Mikio Fujii [a] , Shinji Sudo [a] , Yasuyuki Kitagawa [b] & Keisuke Kato [a]
[a] Faculty of Pharmaceutical Sciences , Toho University , Funabashi , Chiba , 274-8510 , Japan
[b] School of Pharmacy , Showa University , Shinagawa-ku , Tokyo , 142-8555 , Japan
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Efficient Synthesis of a Water-Soluble Glucoamide Inhibitor Against Human Aldose Reductase by Click Chemistry

Mikio Fujii,[1] Shinji Sudo,[1] Yasuyuki Kitagawa,[2] and Keisuke Kato[1]

[1]Faculty of Pharmaceutical Sciences, Toho University, Funabashi, Chiba 274-8510, Japan
[2]School of Pharmacy, Showa University, Shinagawa-ku, Tokyo 142-8555, Japan

While D-glucose is the natural substrate of aldose reductase (AR) in the polyol pathway, the K_m value of D-glucose against AR is large. A glucoamide **1** was designed as a tool to investigate whether AR has a strong affinity for the open form of D-glucose. Glucoamide **1** was synthesized in high yield by modification of the reaction condition for click chemistry. It was found that our modified condition was applicable for highly polar alkynes and gave coupling products in excellent yield (90% to 100%). Although weak inhibitory activity against AR was observed, kinetic studies showed that AR does not accept glucoamide **1** in its active site.

Keywords Click chemistry; Aldose reductase; Glycoside

INTRODUCTION

Aldose reductase (AR, EC 1.1.1.21) catalyzes the conversion of D-glucose to D-sorbitol, which is the first step in the polyol pathway (Sch. 1) of glucose metabolism and is implicated in the pathogenesis of a variety of diabetic complications such as neuropathy.[1–3] Many AR inhibitors, such as epalrestat, sorbinil, and fidarestat, have been developed to prevent diabetic complications.[4–7]

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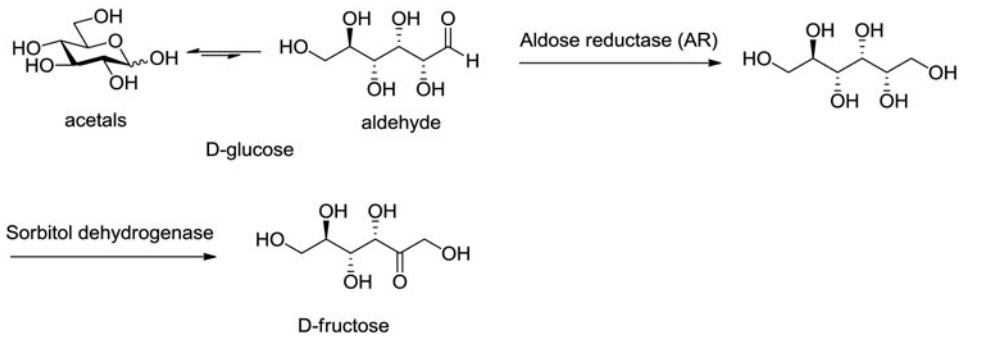
Address correspondence to Mikio Fujii, Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan. E-mail: mfujii@phar.toho-u.ac.jp



Scheme 1: Polyol pathway.

There are many reports investigating substrate specificities and characteristics of AR.[8–10] It is an NADP-dependent enzyme and formally promotes a hydride shift from NADPH to the aldehyde group of D-glucose (Fig. 1). AR possesses broad substrate specificity. The apparent K_m value of D-glucose (55.0 mM) for AR was much larger than that of D,L-glyceraldehyde (0.1 mM).[9] This phenomenon has also been reported by Grimshow.[10] We questioned whether the large K_m of D-glucose against AR arises from the nature of its cyclic structure or its poor binding ability. D-Glucose, being in equilibrium between the aldehyde and the cyclic hemiacetal forms (Sch. 1), mainly forms

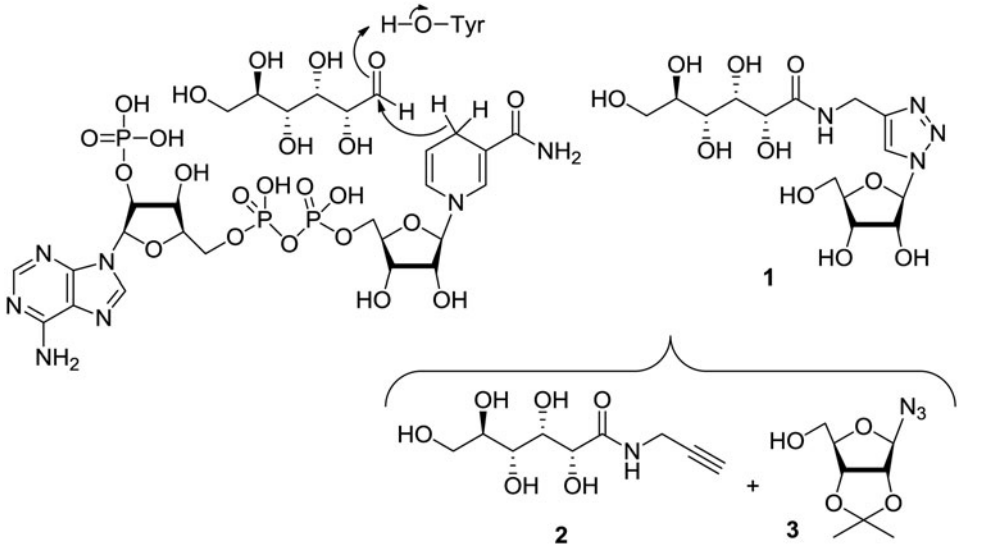


Figure 1: Reaction mechanism of AR and structure of inhibitor **1**.

cyclic hemiacetals in water. AR accepts the aldehydic form of D-glucose to reduce its aldehyde group, but hemiacetals were not reduced. Normally, the apparent K_m value involves the equilibrium constant between hemiacetal and aldehyde forms and is estimated to be larger than expected with the intrinsic binding affinity against the aldehyde form. Thus, a glucoamide inhibitor **1** was designed as a model of the open-chain structure of D-glucose (Fig. 1). If a glucoamide inhibitor **1** is a strong inhibitor against AR, it becomes a highly water-soluble inhibitor that differs from hydrophobic known inhibitors.[4–7]

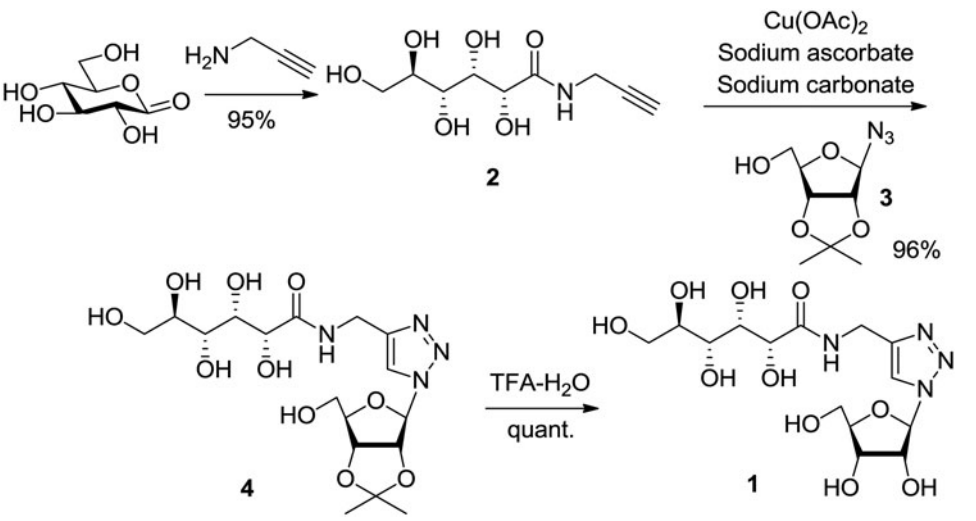
Compound **1** was a coenzyme–substrate complex-type inhibitor possessing ribofuranoside for the NADPH and glucoamide moieties and could be synthesized by conjugation of glucoamide **2** and azide **3** with a ribosyl moiety added by the Cu (I)-catalyzed Huisgen reaction (click chemistry).[11]

In this paper we report the synthesis of **1** and its inhibitory activity against AR.

RESULTS AND DISCUSSION

Synthesis of 1

Compound **1** was synthesized as shown in Scheme 2. Glucoamide **2** was synthesized by a reported method.[12] Azide **3** was synthesized by a modified reported method.[13,14] Coupling of **2** and **3** under conditions using $CuSO_4$ and sodium ascorbate was tested,[11] but the reaction was not completed within 2 days, and separation of **4** (68% conversion on ^{1}H NMR) from the remaining



Scheme 2: Synthesis of **1**.

2 and **3** by column chromatography and precipitation failed. While these coupling conditions have commonly been used for water-soluble compounds, there were several reports that gave products in low to moderate yields and required long reaction times and heating.[15–17] Our compounds **1** and **4** were difficult to separate from starting materials **2** and **3** and reagents such as sodium ascorbate. We tested several conditions and found that basic conditions using an excess amount of **3** (1.2 eq. against **2**) were required to complete the reaction. Coupling of **2** and **3** in the presence of $Cu(OAc)_2$, Na_2CO_3, and sodium ascorbate proceeded smoothly, and **2** disappeared on TLC after 1 h of reaction to give **4**. Inorganic and organic salts were separated by ODS column chromatography and the obtained **4** was washed with ethyl acetate to afford pure **4** in 96% isolated yield. Treatment of **4** with aqueous TFA (30%) gave **1** quantitatively.

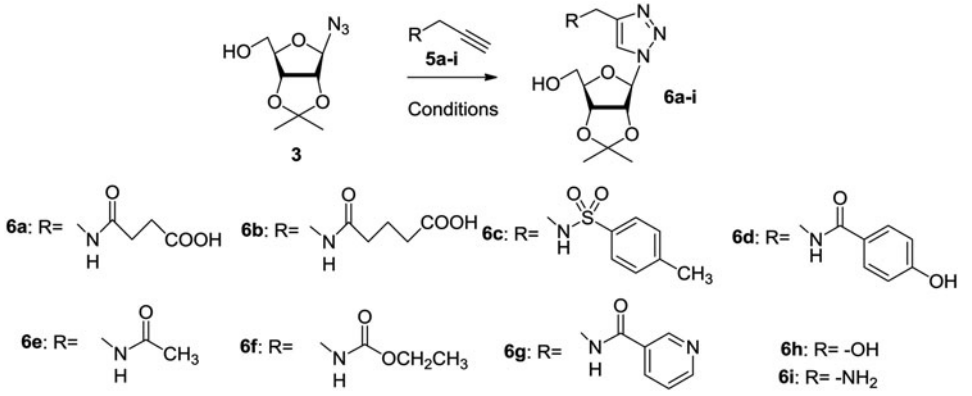
Modified Click Reaction of the Other Compounds

Table 1 shows the results of application of our modified condition (condition A) to other alkynes (**5a**–**f**). The standard condition[11] (condition B) was also tested with the alkynes and the conversion is listed in Table 1 as well. The modified condition was applied to acidic compounds (**5a**–**c**), neutral compounds (**5e**, **5f,** and **5h**), phenolic compounds (**5d**), and basic compounds (**5g** and **5i**). The reactions proceeded smoothly in all cases and the starting alkynes disappeared within 1 h shown by TLC analysis, while the reactions under condition B using copper sulfate were not completed within 1 h. Although the conversion under condition B in Table 1 might have been increased when water was removed by evaporation during the workup procedure, the starting materials **5** and **3** still remained after the evaporation in the case of neutral compounds (**5e** and **5h**) and basic compounds (**5g** and **5i**). Improvement of the conversions on the reaction was observed under condition A. The separation of the product and the reactants became easier and the products **6a**–**i** were obtained in excellent isolated yield (90%–100%) by the basic conditions.

Inhibitory Activities of Glucoamides 1 and 2

Inhibitory activities of **1** and **2** against AR were tested. When epalrestat was used as a control, 1 μM of epalrestat reduced the activity to 38%. While 10 mM of glucoamide **2** did not inhibit the activity of AR, 10 mM of glucoamide **1** reduced the activity to 82% (Fig. 2). Since weak inhibitory activity of **1** was observed, the inhibitory manner of **1** was further investigated by Cornish-Bowden's method as shown in Figures 3 and 4. The K_m for D,L-glyceraldehyde was estimated to be 0.14 mM, which is similar to the reported value.[9] As shown in Figures 3 and 4, glucoamide **1** exhibited uncompetitive inhibition against both glyceraldehyde and NADPH, and the K_i value was 14 mM. The binding affinity of **1** against AR was two orders lower than that

Table 1: The click reactions of **3** with various alkynes



	Condition A[a]		Condition B[b]
Product	Isolated yield (%)	(Conv.[c])	Conv.[c]
6a	98%	(>98%)	>98%
6b	99%	(>98%)	>98%
6c	95%	(>98%)	98%
6d	100%	(>98%)	>98%
6e	90%	(>98%)	86%
6f	100%	(>98%)	>98%
6g	100%	(>98%)	85%
6h	100%	(>98%)	91%
6i	90%	(≥98%)	91%

[a]**3**: 1.0 mmol; alkyne: 0.9 mmol; $Cu(OAc)_2$: 0.25 mmol; Na_2CO_3: 0.2 mmol; sodium ascorbate: 1.0 mmol; water: 2 mL, rt, 2 h.
[b]**3**: 1.0 mmol; alkyne: 0.9 mmol; $CuSO_4$: 0.25 mmol; sodium ascorbate: 1.0 mmol; water: 2 mL, rt, 2 h.
[c]Conversion was calculated based on ^{1}H NMR spectra of reaction mixture.

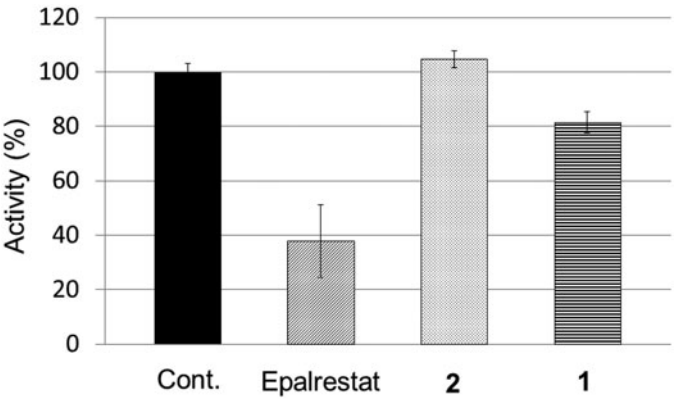


Figure 2: Inhibitory activity of **1** and **2.** Conditions: aldose reductase 15 mg/mL, NADPH 0.25 mM, glyceraldehyde 0.2 mM, 0.1% BSA, sodium phosphate buffer (0.1 M, pH 7.0). Inhibitors: epalrestat: 1 μM, **1** and **2**: 10 mM.

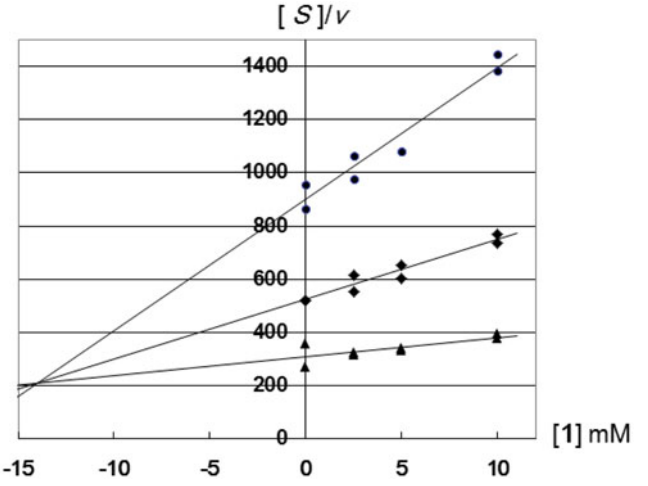


Figure 3: Cornish-Bowden's plot for inhibition by **1** against AR at various concentrations of glyceraldehyde. Conditions: 15 μg/mL aldose reductase, 0.25 mM NADPH, 0.1% BSA, sodium phosphate buffer (0.1 M, pH 7.0), (**1**) = 0, 2.5, 5, and 10 mM, (S: glyceraldehyde) = 0.2 (•), 0.1 (♦), and 0.05(▲) mM (color figure available online).

of D,L-glyceraldehyde. Thus, AR was not found to accept our glucoamide inhibitors **1** and **2** for its substrate binding site.

In conclusion, synthesis of glucoamide **1** was achieved by modifying the reaction conditions of click chemistry. Although there are reports to activate the click reaction by using organic base,[18] our method is useful for the synthesis

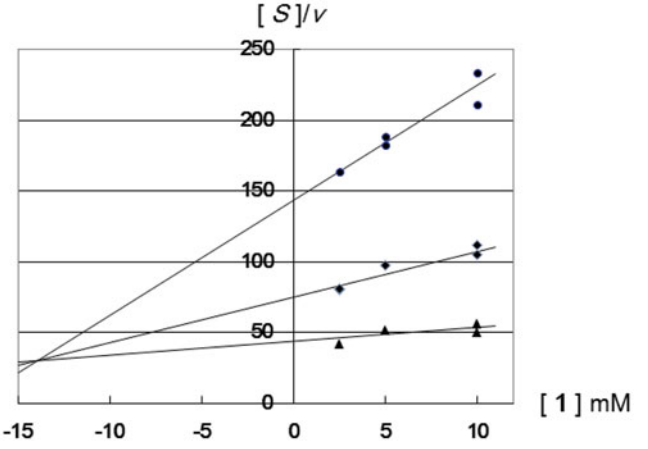


Figure 4: Cornish-Bowden's plot for inhibition by **1** against AR at various concentrations of NADPH. Conditions: 15 μg/mL aldose reductase, 0.1 mM glyceraldehyde, 0.1% BSA, sodium phosphate buffer (0.1 M, pH 7.0), (**1**) = 2.5, 5, and 10 mM. (S: NADPH) = 0.25 (•), 0.13 (♦), and 0.063 (▲) mM (color figure available online).

of water-soluble nucleotides and nucleosides since inorganic salts were utilized as activators of the reaction. Use of copper acetate and sodium carbonate are considered to activate the generation of the acetylide ion in order to coordinate to copper (I) under basic conditions. Inhibitory activities of glucoamides **1** and **2** against AR were investigated to determine whether the aldehyde form of D-glucose bound to AR strongly. However, glucoamides **1** and **2** containing the structure of the open form of D-glucose were not found to bind to the active site of AR. Thus, D-glucose, known as the natural substrate on the polyol pathway, is a poor substrate due to its low binding affinity against AR, but not due to its cyclic hemiacetal structures.

EXPERIMENTAL

General

The NMR (^{1}H, ^{13}C, H-H COSY, HMQC, and HMBC) spectra were recorded on JEOL-ECP-500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) and JEOL-ECS-400 spectrometers (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). Mass spectra were measured by a JEOL JMS-T100LP. IR spectra were obtained on a JASCO FT/IR 4100 using the ATR method (neat). Specific rotation was measured on a JASCO P-2200. Chemicals were purchased from Tokyo Kasei Industry Co., Ltd., Wako Chemicals, or Aldrich Inc. unless otherwise indicated. ODS column chromatography was performed on Chromatorex ODS DM1020T (Fuji-Silysia Chemical Co. Ltd.). Inhibition studies were performed on GeneQuant 1300 spectrophotometers. Human AR (1.0 U/μg, ALR2, AKR 1B1, recombinant from *E. coli*) was purchased from AT Gen Co., Ltd.

Synthesis of 1

*2,3-*O*-Isopropylidene-1β-azido-D-ribofuranose (3)*

To a solution of tetra-*O*-acetyl-β-D-ribofuranose (20.0 g, 63.0 mmol) and $TMSN_3$ (13.0 mL, 98.8 mmol) in CH_2Cl_2 (100 mL) was added boron trifluoride-diethyl etherate (6.0 mL, 94.4 mmol) under Ar atmosphere at 0°C. The resulting mixture was stirred for 6 h, then poured into aq. $NaHCO_3$ and extracted by $CHCl_3$ twice. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in methanolic NH_3 (2 M, 300 mL). The solution was stirred for 12 h and then evaporated under reduced pressure. The residue was dissolved in acetone (100 mL) containing 2,2-dimethoxypropane (12 mL, 97.9 mmol) and $HClO_4$ (1.0 mL). The mixture was stirred for 10 min and methanolic NH_3 (2 M, 3 mL) was added to the mixture. The residue was purified by silica gel column chromatography (200 g

of silica gel) eluted with hexane/ethyl acetate (20/1 to 8/1) to afford **3** (12.1 g, 56.1 mmol, 89%).

N-*(1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-triazol-4-ylmethyl)-D-glucoamide (4)*

To a solution of **2** (212 mg, 0.907 mmol) and $Cu(OAc)_2$ monohydrate (49.9 mg, 0.300 mmol) in water (2 mL) was added a solution of sodium L-ascorbate (198.3 mg, 1.00 mmol), sodium carbonate (20 mg, 0.20 mmol), and **3** (219 mg, 1.01 mmol) in water (1 mL) at rt. The resulting mixture was stirred for 2 h and then evaporated under reduced pressure. The residue was purified by ODS column chromatography (30 g ODS gel) eluted with water to afford crude **4** with unreacted **3**. Removal of **3** from the crude **4** by washing with ethyl acetate afforded **4** (390 mg, 0.871 mmol, 96%). $[\alpha]_D^{22} = -23.0$ (c 0.51, H_2O). ^{1}H NMR (D_2O, 500 MHz): δ 1.32 (3H, s, CH_3), 1.50 (3H, s, CH_3), 3.36 (1H, dd, J = 12.1, 6.6 Hz, rib-5″), 3.48 (1H, dd, J = 12.4, 4.6 Hz, rib-5″), 3.53 (1H, dd, J = 11.7, 4.8 Hz, glc-6), 3.62 (2H, s, glc-4, glc-5), 3.69 (1H, d, J = 11.7 Hz, glc-6), 3.98 (1H, d, J = 2.3 Hz, glc-3), 4.24 (1H, d, J = 2.3 Hz, glc-2), 4.37 (1H, s, rib-4″), 4.44 (2H, s, CH_2), 4.92 (1H, d, J = 5.0 Hz, rib-3″), 5.44 (1H, d, J = 5.0 Hz, rib-2″), 6.23 (1H, s, rib-1″), 8.03 (1H, s, triazole-5'). ^{13}C NMR (D_2O, 125 MHz): δ 24.2 (CH_3), 25.8 (CH_3), 34.3 (CH_3), 61.4 (5″), 62.7 (6), 70.5 (3), 71.1 (5), 72.1 (4), 73.5 (2), 81.3 (3″), 84.0 (2″), 88.1 (4″), 93.3 (1″), 114.5 (isopropylidene-C), 123 (br, 5'), 174.7(1). ESI-MS: Calcd. for $C_{17}H_{28}N_4O_{10}Na$ [M+Na^+]: 471.1703; Found: 471.1710. IR: ν 3324, 1649, 1587, 1535, 1428, 1383 cm^{-1}.

N-*(1-(β-D-Ribofuranosyl)-triazol-4-ylmethyl)-D-glucoamide (1)*

Compound **4** (491 mg, 0.955 mmol) was dissolved in 30% aq. trifluoroacetic acid (TFA) (2 mL). The mixture was stirred for 3 h and then evaporated under reduced pressure to afford **1** (500 mg, partially TFA complex). To remove TFA from the obtained **1**, the TFA complex of **1** was dissolved in a small portion of aq. $NaHCO_3$ and subjected to ODS column chromatography (50 g ODS gel) eluted with water to afford **1** (324 mg, 0.793 mmol, 83%) and recovered TFA complex of **1** (**1**:**1-TFA** = 4:3 by ^{1}H NMR analysis, 75.0 mg, 0.163 mmol, 17%). $[\alpha]_D^{22} = -17.2$ (c = 1.04, H_2O). ^{1}H NMR (D_2O, 500 MHz): δ 3.51 (1H, dd, J = 12.3, 5.6 Hz, glc-6), 3.60 (3H, m, glc-4, glc-5, rib-5″), 3.68 (1H, dd, J = 12.3, 2.3 Hz, glc-6), 3.72 (1H, dd, J = 12.6, 3.2 Hz, rib-5″), 3.97 (1H, t, J = 3.1 Hz, glc-3), 4.10 (1H, dd, J = 8.3, 5.0 Hz, rib-4″), 4.23 (1H, d, J = 3.1 Hz, glc-2), 4.29 (1H, t, J = 5.0 Hz, rib-3″), 4.42 (2H, s, NCH_2), 4.54 (1H, dd, J = 5.0, 4.5 Hz, rib-2″), 5.99 (1H, d, J = 4.5 Hz, rib-1″), 7.99 (1H, s, triazole-5'). ^{13}C NMR (D_2O, 125 MHz): δ 34.3 (CH_2), 61.2 (5″), 62.7 (6), 70.2 (3″), 70.5 (3), 71.2 (5), 72.1 (4), 73.5 (2), 74.9 (2″), 85.3 (4″), 92.1 (1″), 122.8 (5'), 145.2 (br, 4'), 174.7 (1). ESI-MS m/z: Calcd. for $C_{14}H_{24}N_4O_{10}Na$ [M + Na^+]: 431.1390; Found: 431.1361. IR: ν 3314, 1687, 1650, 1642, 1535 cm^{-1}.

General Procedure (Condition A) of Click Reactions for Table 1

To a solution of alkyne (0.90 mmol) and $Cu(OAc)_2$ monohydrate (40 mg, 0.25 mmol) in water (2 mL) was added a solution of sodium L-ascorbate (198 mg, 1.00 mmol), sodium carbonate (20 mg, 0.20 mmol), and **3** (215 mg, 1.0 mmol) in water (1 mL) at rt. In the case of condition B, copper (II) sulfate monohydrate (50 mg, 0.25 mmol) was used instead of $Cu(OAc)_2$ monohydrate and sodium carbonate. The resulting mixture was stirred for 2 h and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (10 g silica gel) eluted with CH_3Cl/CH_3OH (30/1∼10/1) to afford **5**.

N-*(1-(2,3-*O-*Isopropylidene-β-D-ribofuranosyl)-triazol-4-ylmethyl)succinamide (6a)*

6a (329 mg, 0.889 mmol, 98%) from **5a**[18] (140 mg, 0.903 mmol) and **3** (215 mg, 1.00 mmol). $[\alpha]_D^{21} = -50.5$ (c = 0.88, CH_3OH). ^1H NMR (CD_3OD, 500 MHz): δ 1.38 (3H, s, CH_3), 1.56 (3H, s, CH_3), 2.51 (2H, t, $J = 6.9$ Hz, CH_2), 2.62 (2H, t, $J = 6.9$ Hz, CH_2), 3.49 (1H, dd, $J = 12.0$, 4.4 Hz, rib-5'), 3.52 (1H, dd, $J = 12.0$, 4.0 Hz, rib-5′), 4.33–4.37 (1H, m, rib-4′), 4.45 (2H, d, $NHCH_2$), 4.95 (1H, d, $J = 4.8$ Hz, rib-3′), 5.35 (1H, d, $J = 4.8$ Hz, rib-2′), 6.21 (1H, s, rib-1′), 8.08 (1H, s, triazole-5′); ^{13}C NMR (125 MHz, CD_3OD): δ 24.2 (CH_3), 26.0 (CH_3), 28.9 (CH_2), 30.1 (CH_2), 34.5 (CH_2), 61.8 (5′), 82.0 (3′), 84.8 (2′), 88.6 (4′), 94.3 (1′), 113.5 (isopropylidene-C), 122.5 (5), 145.6 (4), 173.3 (CO), 175.0 (CO); ESI- MS: Calcd. for $C_{15}H_{22}N_4O_7Na$ ($M+Na^+$): 393.1386; Found: 393.1297; IR: ν 3335, 1716, 1652,1541 cm^{-1}.

N-*(1-(2,3-*O-*Isopropylidene-β-D-ribofuranosyl)-triazol-4-ylmethyl)glutaramide (6b)*

6b (380 mg, 0.959 mmol, 99%) from **5b**[19] (163 mg, 0.964 mmol) and **3** (230 mg, 1.07 mmol). $[\alpha]_D^{25} = -37.1$ (c = 1.26, CH_3OH). ^1H NMR (CD_3OD, 500 MHz): δ 1.39 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.90 (2H, quintet, $J = 6.0$ Hz, CH_2), 2.29 (2H, t, $J = 6.0$ Hz, CH_2), 2.31 (2H, t, $J = 6.0$ Hz, CH_2), 3.50 (1H, dd, $J = 12.0$, 4.2 Hz, rib-5′), 3.53 (1H, dd, $J = 12.0$, 4.4 Hz, rib-5′), 4.34–4.38 (1H, m, rib-4′), 4.45 (2H, s, $NHCH_2$), 4.90–4.96 (1H, m, rib-3′), 5.34 (1H, d, $J =$ 1.3 Hz, rib-2′), 6.21 (1H, d, $J = 1.3$ Hz rib-1′), 8.11 (1H, s, triazole-5′); ^{13}C NMR (125 MHz, CD_3OD): δ 20.9 (CH_2), 24.1 (CH_3), 26.0 (CH_3), 33.5 (CH_2), 34.3 (CH_2), 34.7 (CH_2), 61.8 (5′), 82.0 (3′), 84.8 (2′), 88.6 (4′), 94.8 (1′), 113.6 (isopropylidene-C), 122.5 (5), 145.1 (4), 174.2 (CO), 175.9 (CO); ESI- MS: Calcd. for $C_{16}H_{24}N_4O_7Na$ ($M+Na^+$): 407.1543; Found: 407.1533; IR: ν 3396, 1646, 1558, 1541 cm^{-1}.

N-*(1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-triazol-4-ylmethyl)-4-methylbenzene sulfonamide (6c)*

6e (369 mg, 0.870 mmol, 95%) from **5e** (191 mg, 0.918 mmol) and **3** (215 mg, 1.00 mmol). $[\alpha]_D^{26} = -43.2$ (c 1.02, CH_3OH). ^1H NMR ($CDCl_3$, 500 MHz): δ 1.34 (3H, s, CH_3), 1.55 (3H, s, CH_3), 2.38, (3H, s, CH_3), 3.52 (1H, dd, $J = 12.0$, 2.9 Hz, rib-5′), 3.65 (1H, dd, $J = 12.0$, 3.3 Hz, rib-5′), 4.15 (2H, d, $NHCH_2$), 4.41–4.43 (1H, m, rib-4′), 4.90 (1H, d, $J = 6.0$, 1.1 Hz, rib-3′), 5.15 (1H, d, $J = 6.0$, 2.0 Hz, rib-2′), 6.01 (1H, d, $J = 2.0$ Hz, rib-1′), 7.25 (2H, d, $J = 8.3$ Hz, Ts-3,5), 7.67 (2H, d, $J = 8.3$ Hz, Ts-2,6), 7.77 (1H, s, triazole-5′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 21.5 (CH_3), 25.1 (CH_3), 27.0 (CH_3), 28.2 (CH_2), 62.6 (5′), 81.8 (3′), 85.3 (2′), 88.7 (4′), 95.1 (1′), 113.9 (isopropylidene-C), 122.6 (5), 127.1 (2C,Ts-3,5), 129.8 (2C,Ts-2,6), 136.7 (Ts-4), 143.7 (Ts-1), 145.5 (4); ESI- MS: Calcd. for $C_{18}H_{22\backslash 4}N_4O_6SNa$ ($M + Na^+$): 447.1314; Found: 393.1268; IR: ν 3258, 1457, 1319 cm^{-1}.

*4-Hydroxy-*N*-(1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-triazol-4-ylmethyl)benzamide (6d)*

6d (361 mg, 0.926 mmol, 100%) from **5d**[20] (162 mg, 0.926 mmol) and **3** (215 mg, 1.00 mmol). $[\alpha]_D^{26} = -59.5$ (c = 1.11, CH_3OH). ^1H NMR (CD_3OD, 500 MHz): δ 1.37 (3H, s, CH_3), 1.56 (3H, s, CH_3), 3.49 (1H, dd, $J = 12.4$, 5.5 Hz, rib-5′), 3.54 (1H, dd, $J = 12.4$, 5.5 Hz, rib-5′), 4.35 (1H, dt, $J = 1.6$, 5.5 Hz, rib-4′), 4.63 (2H, s, $NHCH_2$), 4.94 (1H, dd, $J = 1.6$, 6.1 Hz, rib-3′), 5.34 (1H, dd, $J = 1.9$, 6.1 Hz, rib-2′), 6.20 (1H, d, $J = 1.9$ Hz, rib-1′), 6.82 (2H, d, $J = 8.6$ Hz, PhOH-H-3,5), 7.73 (2H, d, $J = 8.6$ Hz, PhOH-H-2,6), 8.11 (1H, s, triazole-5′). ^{13}C NMR (125 MHz, CD_3OD): δ 24.2 (CH_3), 26.0 (CH_3), 34.8 (CH_2), 61.8 (5′), 82.0 (3′), 84.8 (2′), 88.6 (4′), 94.3 (1′), 113.5 (isopropylidene-C), 114.8 (2C, PhOH-3,5), 122.6 (5), 124.7 (PhOH-1), 129.1 (2C, PhOH-2,6), 145.6 (4), 160.9 (C-O), 168.7 (C = O); ESI- MS: Calcd. for $C_{18}H_{22}N_4O_6Na$ ($M + Na^+$): 413.1437; Found: 417.1372; IR: ν 3336, 1352, 1607, 1507 cm^{-1}.

N-*(1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-triazol-4-ylmethyl)acetamide (6e)*

6e (253 mg, 0.811 mmol, 90%) from **5e** (87.1 mg, 0.896 mmol) and **3** (215 mg, 1.00 mmol). $[\alpha]_D^{21} = -72.1$ (c = 1.03, CH_3OH). ^1H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.98 (3H, s, CH_3), 2.03 (1H, s, OH), 3.64 (1H, dd, $J = 12.4$, 4.4 Hz, rib-5′), 3.77 (1H, dd, $J = 12.4$, 3.6 Hz, rib-5′), 4.44 (2H, d, $NHCH_2$), 4.49–4.52 (1H, m, rib-4′), 4.98 (1H, dd, $J = 5.8$, 1.8 Hz, rib-3′), 5.23 (1H, dd, $J = 5.8$, 2.0 Hz, rib-2′), 6.11 (1H, d, $J = 2.0$ Hz, rib-1′), 7.19 (1H, s, NH), 7.91 (1H, s, triazole-5′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.8 (CH_3), 25.0 (CH_3), 26.9 (CH_3), 34.6 (CH_2), 62.8 (5′), 81.6 (3′), 85.3 (2′), 88.6 (4′), 95.1 (1′), 113.7 (isopropylidene-C), 122.6 (5), 144.7 (4), 170.9 (CO).

ESI- MS: Calcd. for $C_{13}H_{20}N_4O_5Na$ ($M + Na^+$): 335.1331; Found: 335.1193; IR: ν 3297, 1658, 1550 cm^{-1}.

*4-(Ethoxycarbamoylmethyl)-triazol-1-yl 2,3-*O*-isopropylidene-β-D-ribofuranoside (6f)*

6f (351 mg, 1.02 mmol, 100%) from **5f**[21] (141 mg, 1.11 mmol) and **3** (219 mg, 1.02 mmol). $[\alpha]_D^{26} = -57.8$ (c = 0.52, CH_3OH). ^1H NMR ($CDCl_3$, 500 MHz): δ 1.10 (3H, t, $J = 7.0$ Hz, CH_3), 1.26 (3H, s, CH_3), 1.48 (3H, s, CH_3), 3.40–3.70 (2H, m, rib-5′), 3.98 (3H, t, OCH_2), 4.29 (2H, s, NCH_2), 4.34 (1H, s, rib-4′), 4.90 (1H, d, $J = 5.0$ Hz, rib-3′), 5.20 (1H, d, $J = 5.0$ Hz, rib-2′), 6.03 (1H, s, rib-1′), 6.16 (1H, brs, OH), 7.68 (1H, brs, NH), 7.81 (1H, s, triazole-5′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.6 (CH_3), 25.1 (CH_3), 27.0 (CH_3), 36.2 (CH_2), 61.0 (CH_2), 62.5, (5′), 81.9 (3′), 85.2 (2′), 88.8 (4′), 94.7 (1′), 113.6 (isopropylidene-C), 122.4 (5), 148.7 (4), 156.9 (CO); ESI- MS: Calcd. for $C_{14}H_{22}N_4O_6Na$ ($M+Na^+$): 365.1437; Found: 365.1320; IR: ν 3340, 1698, 1540 cm^{-1}.

N-*(1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-triazol-4-ylmethyl)-pyridin-3-ylcarbamide (6e)*

6e (270 mg, 0.750 mmol, 100%) from **5e**[22] (120 mg, 0.750 mmol) and **3** (192 mg, 0.89 mmol). $[\alpha]_D^{27} = -62.6$ (c = 1.41, CH_3OH). ^1H NMR ($CDCl_3$, 500 MHz): δ 1.35 (3H, s, CH_3), 1.50 (3H, s, CH_3), 3.50 (1H, dd, $J = 12.2$, 4.9 Hz, rib-5′), 3.62 (1H, dd, $J = 12.2$, 3.9 Hz, rib-5′), 4.39 (1H, ddd, $J = 4.9$, 3.9, 1.8 Hz, rib-4′), 4.54 (1H, d, $J = 12.0$ Hz, $NHCH_2$), 4.57 (1H, d, $J = 12.0$ Hz, $NHCH_2$), 4.87 (1H, dd, $J = 6.0$, 1.8 Hz, rib-3′), 5.17 (1H, dd, $J = 2.0$, 6.0 Hz, rib-2′), 6.21 (1H, d, $J = 2.0$ Hz, rib-1′), 7.34 (1H, br, pyr-5), 8.00 (1H, s, triazole-5′), 8.12 (1H, br, pyr-4), 8.58 (1H, br, pyr-6), 8.95 (1H, br, pyr-2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 25.1 (CH_3), 26.9 (CH_3), 35.1 (CH_2), 62.4 (5′), 81.7 (3′), 85.3 (2′), 88.5 (4′), 95.0 (1′), 113.8 (isopropylidene-C), 122.9 (5), 123.8 (pyr-5), 130.0 (pyr-4), 144.7 (pyr-6) 148.1 (4), 151.7 (pyr-2), 166.1 (CO); ESI- MS: Calcd. for $C_{17}H_{21}N_5O_5Na$ ($M+Na^+$): 398.1440; Found: 398.1401; IR: ν 3309, 1646, 1541 cm^{-1}.

*4-Hydroxymethyltriazol-1-yl 2,3-*O*-isopropylidene-β-D-ribofuranoside (6h)*

6h (278 mg, 1.02 mmol, 100%) from **5h** (63.0 mg, 1.13 mmol) and **3** (221 mg, 1.02 mmol). $[\alpha]_D^{21} = -72.7$ (c = 0.99, CH_3OH); ^1H NMR (CD_3OD, 400 MHz): δ 1.28 (3H, s, CH_3), 1.48 (3H, s, CH_3), 3.42 (1H, dd, $J = 12.4$, 4.4 Hz, rib-5′), 3.51 (1H, dd, $J = 12.4$, 3.6 Hz, rib-5′), 4.29–4.33 (1H, m, rib-4′), 4.60 (2H, s, OCH_2), 4.84 (1H, d, $J = 5.8$ Hz, rib-3′), 5.23 (1H, d, $J = 5.8$ Hz, rib-2′), 6.08 (1H, s, rib-1′), 7.89 (1H, s, triazole-5′); ^{13}C NMR (100 MHz, CD_3OD): δ 24.0 (CH_3), 25.8 (CH_3), 54.7 (CH_2), 61.3 (5′), 81.2 (3′), 84.2 (2′), 87.8 (4′), 93.8 (1′), 113.1 (isopropylidene-C), 121.5 (5), 147.5 (4); ESI-MS: Calcd. for $C_{13}H_{20}N_4O_5Na$ ($M+Na^+$): 294.1066; Found: 194.0752; IR: ν 3352, 1682 cm^{-1}.

*4-Aminomethyltriazol-1-yl 2,3-*O*-isopropylidene-β-D-ribofuranoside (6i)*

6i (242 mg, 0.898 mmol, 90%) from **5i** (61.0 mg, 1.11 mmol) and **3** (215 mg, 1.00 mmol) (purified by silica gel chromatography using Cromatorex NH purchased from Fuji Silisia Chemical Ltd.) $[\alpha]_D^{21} = -0.4$ (c = 0.15, CH_3OH); ^1H NMR (CD_3OD, 500 MHz): δ 1.38 (3H, s, CH_3), 1.57 (3H, s, CH_3), 3.42–3.60 (2H, m, rib-5′), 3.78 (2H, s, NCH_2), 4.30–4.35 (1H, m, rib-4′), 4.94 (1H, d, J = 5.0 Hz, rib-3′), 5.34 (1H, d, J = 5.0 Hz, rib-2′), 6.22 (1H, s, rib-1′), 8.10 (1H, s, triazole-5′); ^{13}C NMR (100 MHz, CD_3OD): δ 25.2 (CH_3), 27.0 (CH_3), 52.7 (CH_2), 62.3 (5′), 81.6 (3′), 85.2 (2′), 88.7 (4′), 94.8 (1′), 113.2 (isopropylidene-C), 125.7 (5), 147.4 (4); ESI-MS: Calcd. for $C_{11}H_{18}N_4O_4Na$ ($M+Na^+$): 293.1226; Found: 293.1485; IR: ν 3287, 1652, 1285 cm^{-1}.

Kinetics and Inhibitory Activity of Glucoamide

Enzyme activity was determined by measuring the change in absorbance due to NADPH at 340 nm (ε = 6300 $M^{-1}cm^{-1}$). The activity was recorded for 4 min after 30 s from mixing an enzyme solution and a substrate solution. A standard reaction mixture contained 15 μg/mL AR, 0.25 mM NADPH, 0.2 mM glyceraldehyde, and 0.1% BSA in sodium phosphate buffer (0.1 M, pH 7.0). Initial velocities were fitted to appropriate equations (1/v vs. [I] plot for competitive inhibition or [S]/v vs. [I] plot for uncompetitive inhibition) with graphical methods to determine the inhibitory manner.[23] The K_m value for glyceraldehyde against AR was calculated from the data in the absence of inhibitor in Figure 3.

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