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Expanding the application scope of glycosidases using click chemistry

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ABSTRACT

Glycosidase-mediated glycosylation of alkynyl alcohols and azide-containing alcohols was followed by a click reaction, affording various types of triazole glycosides. The activities of triazole glycosides detected in subsequent bioassays show that this procedure is a feasible approach to the development of anti-fungal drugs.

1. Introduction

The enzymatic approach, by virtue of its mildness, high selectivity, and acceptance of unprotected sugars as substrates, is of increasing importance in the synthesis of glycosides. The glycosyltransferase,[1] and recent glycosynthases[2] show remarkable application potential. The glycosidase, mediating the cleavage of glycosidic bonds in vivo, can be used for glycoside synthesis via reverse hydrolysis (Fig. 1A, thermodynamic control) or transglycosylation (Fig. 1B, kinetic control), is also attractive due to its ability of synthesis of glycosides from unprotected and unactivated sugars in one step.[3]

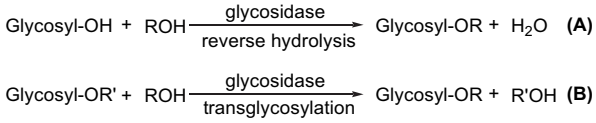

Figure 1. Enzymatic synthesis of glycosides based on reverse hydrolysis and transglycosylation.

In our previous reports, we demonstrated that crude meal of some cyanogenic plant seeds, such as almond, peach kernel, apple seed, and apricot kernel, are robust and cheap alternatives to purified or immobilized β-glucosidases for glycoside synthesis via reverse hydrolysis.[4,5] This procedure was successfully applied to synthesize some naturally occurring glycosides, such as salidroside and rosvin (Fig. 2).[5] Furthermore, a reactor integrating with product adsorption column was developed for salidroside production. This process shows a higher efficiency than the chemical or glycosyltransferase approach.[6] Recently, the synthesis of salidroside was scaled up to kilogram in a pilot reactor.

Reversed hydrolysis, though elegant in its simplicity, has its inherited problems, the yield of product will decrease with the increase of molecular weight of alcohols. To overcome this problem, glucosidation of allylic alcohol followed by Mizoroki–Heck (MH) type reaction approach was developed for the synthesis of Rosvin and its analogs.[7] However, the Mizoroki–Heck (MH) type reaction between the aryl boron reagents and unprotected allyl β-glucopyranoside afforded only a 20% yield and protection–deprotection

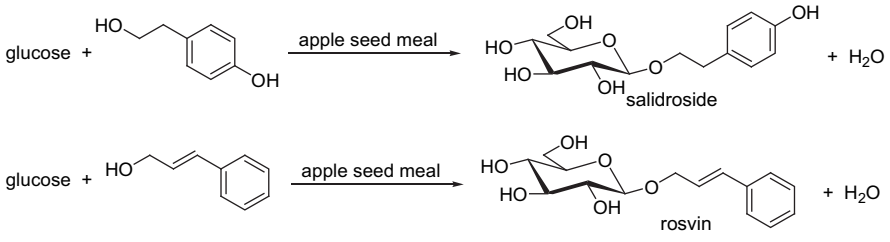

Figure 2. Enzymatic synthesis of salidroside and rosvin.

steps are still required. In the meanwhile, to develop an efficient and seamless chemoenzymatic approach to various glycosides, the copper(I) catalyzed azide–alkyne cycloaddition (CuAAC), or 'click

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reaction'[8] was introduced for the ligation of simple glycosides and other molecules.

CuAAC has gained great popularity because of its unique 'click' nature: namely, the reaction proceeds with high yields and no byproducts and exhibits functional group orthogonality. CuAAC not only is an effective ligation tool but also creates a 1,2,3-triazole structure, which provides a facile route to mimic triazole fungalcides.[9] Interestingly, it was also reported that alkyl glycosides containing C8 to C12 alkyl chains showed a broad spectrum of antimicrobial activity.[10] Therefore, we envisaged that the glycoside with a triazole moiety could be a good candidate for fungalcide.

2. Results and discussion

Propargyl, a highly reactive functional group, is sensitive to heat and light. Unsurprisingly, there is no product isolated from the system and the glucosidase was deactivated when propargyl alcohol was applied both as substrate and organic phase (Fig. 1A). Therefore, a co-solvent was introduced to reduce the concentration of propargyl alcohol. Based on our early result,[11] seven water-miscible solvents, including *tert*-butyl alcohol, *tert*-amyl alcohol, acetonitrile, acetone, 1,4-dioxane, 1,2-dimethoxyethane, and 1,2-diacetoxyethane were employed as media for the enzymatic glycosidation of propargyl alcohol. The results were summarized in Figure 3. The content of propargyl alcohol should not be higher than 50% (v/v) to maintain the activity of the enzyme (Fig. 3A). When 50% (v/v) of solvent was added, acetonitrile can achieve the best result (yield, 35%, Fig. 3B).

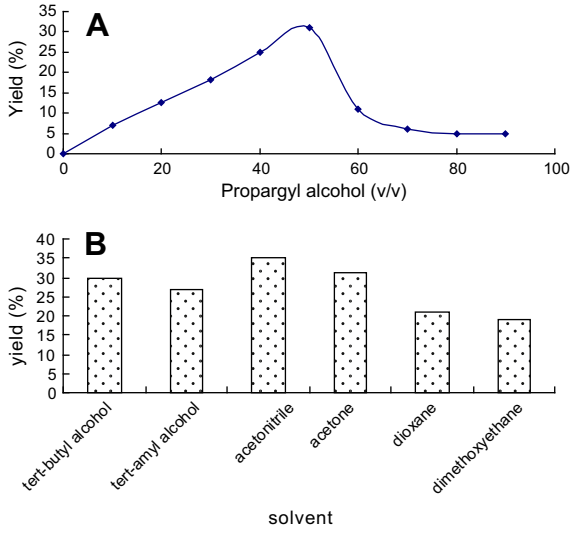
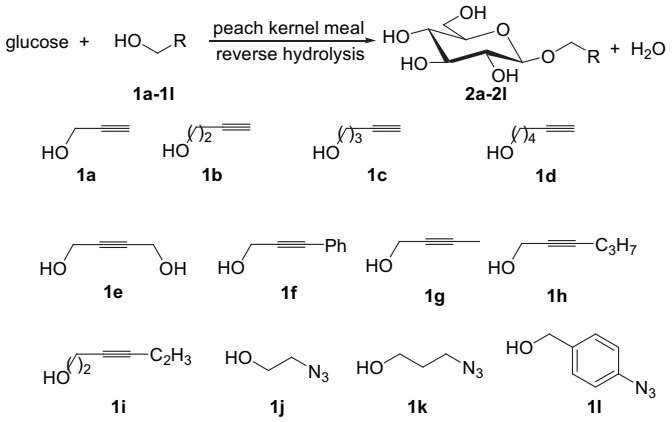


Figure 3. (A) Dependence of propargyl β-glucosides synthesis on volume fraction of the propargyl alcohol in *tert*-butyl alcohol/water system; The reaction was carried out by shaking at 50 °C a mixture of 0.30 g *P. persica* kernel meal, 0.5 ml water containing 0.25 mmol glucose, 4.5 mL propargyl alcohol/*tert*-butyl alcohol with various ratio. (B) Screen of solvents for the synthesis of propargyl β-glucosides. The reaction was carried out by shaking at 50 °C a mixture of 0.30 g *P. persica* kernel meal, 0.5 ml water containing 0.25 mmol glucose, 4.5 mL 50% (v/v) propargyl alcohol/mentioned solvent.

With the optimized conditions established, we began to evaluate the reaction scope. Four terminal alkynyl alcohols (**1a**–**1d**) and three N_3-contained alcohols (**1j**–**1l**) were employed by applying two different types of enzyme preparation, from peach kernel meal and apple seed meal. Additionally, to investigate the substrate scope of the enzyme and the potential activity of alkynyl glycosides[12] several internal alkynyl alcohols (**1e**–**1i**) were also included as substrates.

As indicated in Table 1, the peach and apple glucosidase exhibited relatively broad substrate scope. They were capable of accepting terminal alkynyl alcohols, internal alkynes alcohols, and N_3-containing alcohols. The glycosylation of 2-azidoethanol (**1j**) and 3-azidopropan-1-ol (**1k**) achieved a higher yield (Table 1, entries 10 and 11) than the alkynes containing alcohols. It seems that N_3-containing alkyl alcohols have no toxicity to apple/peach β-glucosidase. However, due to the instability of aromatic azide, a yield for 4-azidobenzyl β-glucoside (**2l**) cannot be obtained (Table 1, entry 12).

Table 1
Synthesis of β-glucosides using fruit kernel meal



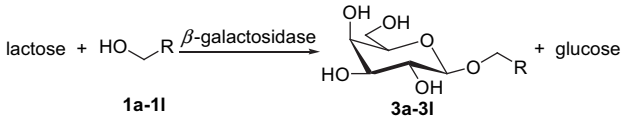
Entry	Substrate	Solvent	**2** Yield (%)	
			Peach kernel[a]	Apple seed[b]
1	**1a**	CH_3CN	32	35
2	**1b**	CH_3CN	30	31
3	**1c**	CH_3CN	17	18
4	**1d**	CH_3CN	15	16
5	**1e**	*tert*-Butyl alcohol	31	27
6	**1f**	CH_3CN	12	12
7	**1g**	CH_3CN	5	12
8	**1h**	CH_3CN	9	14
9	**1i**	CH_3CN	15	16
10	**1j**	CH_3CN	39	45
11	**1k**	CH_3CN	53	51
12	**1l**	Various	N.R.	N.R.

[a] Isolated yield, using 60 mg (1.8 U) of home-made acetone powder of peach kernel meal per mL of reaction mixture.
[b] Isolated yield, using 60 mg (1.9 U) of home-made acetone powder of apple seed meal per mL of reaction mixture.

Generally, reversed hydrolysis (Fig. 1A), a reaction of a monosaccharide with an alcohol, is simpler than transglycosylation (Fig. 1B), a reaction of a reactive glycosyl donor with an alcohol (acceptor). In contrast to the synthesis of glucosides, transglycosylation is preferred for the synthesis of β-galactosides, because lactose, glycosyl donor, is abundant and cheap. The synthesis of β-galactosides catalyzed by β-galactosidase has been documented.[13] However, little investigation had been focused on the galactosidation of alkynes alcohols or N_3-containing alcohols.[14] The enzymatic galactosidation were then carried out as transglycosylation with lactose as the glycosyl donor. Gratifyingly, all the substrates (**1a**–**1l**) were accepted by β-galactosidase from *Aspergillus oryzae* and afforded corresponding β-galactosides in an acceptable yield (Table 2). However, attempts to enhance the yield by adding a water-miscible solvent, such as DMF, *tert*-butyl alcohol, acetonitrile, or acetone, were not hopeful.

The [3+2] cycloaddition of an alkynl between an azide achieved near complete conversion and high isolated yield (>85%) regardless of which functional group the glycoside was linked to (Table 3). An increased reaction rate could be achieved using sodium ascorbate[8b] to reduce Cu^{II} to Cu^{I} (Table 3, entries 1 and

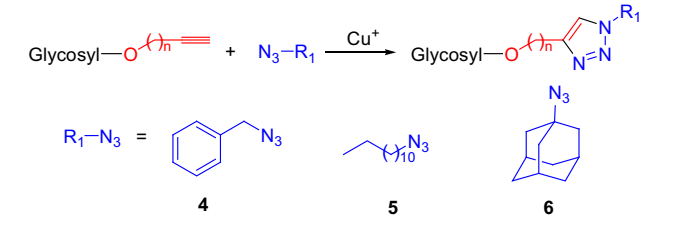
Table 2
Synthesis of β-galactosides based on transglycosidation



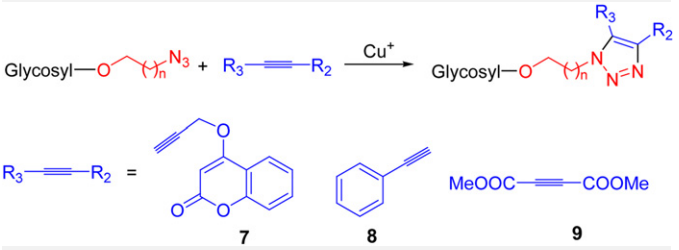
Entry	Substrate	**3** Yield[a] (%)
1	**1a**	36
2	**1b**	18
3	**1c**	17
4	**1d**	11
5	**1e**	21
6	**1f**	5
7	**1g**	14
8	**1h**	16
9	**1i**	15
10	**1j**	12
11	**1k**	16
12	**1l**	6

[a] Isolated yield. The reaction was conducted under 25 °C for 4–24 h.

Table 3
Synthesis of triazole-containing glycosides



Entry	Glycoside	Azide	Time (h)	Product	Yield (%)
1[a]	**2a**	**4**	4	**11**	96
2[b]	**2a**	**6**	24	**12**	89
3[a]	**3a**	**5**	6	**13**	90
4[b]	**3a**	**6**	24	**14**	85



Entry	Glycoside	Alkyne	Time (h)	Product	Yield (%)
5[b]	**2j**	**7**	8	**15**	95
6[b]	**3j**	**7**	8	**16**	92
7[b]	**3k**	**8**	12	**17**	95
8[c]	**3l**	**9**	12	**18**	86

[a] $CuSO_4$, sodium ascorbate.
[b] $CuSO_4$, copper powder.
[c] Methanol, reflux.

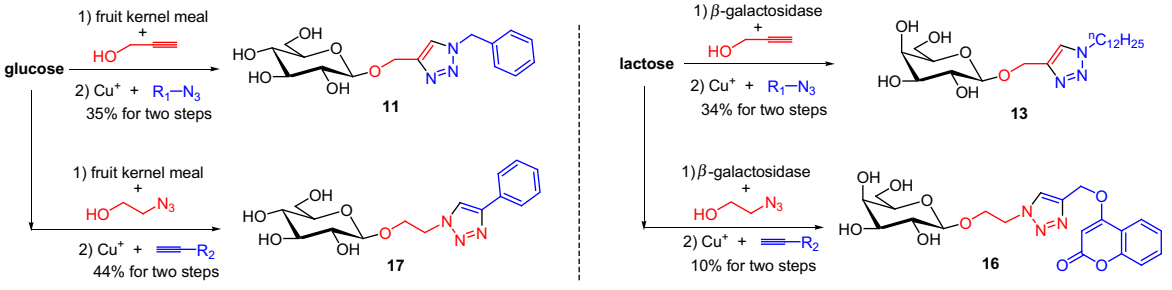


Figure 4. Sequential one-pot-like reaction.

3). When a bulky organic azide (**6**) was used, copper powder[8d] was found to be a better reductant (Table 3, entries 2 and 4). Additionally, it is worth to note that the standard thermal cycloaddition of and alkyne can also conduct smoothly (Table 3, entry 8).

The obtained alkynl and triazole glycosides were tested for their antifungal activities. The alkynl glycosides (**2d**, **2f**) and triazole-containing glycosides (**13**, **15**, and **17**) showed inhibitory activities against *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, and *Cryptococcus neoformans* with minimum inhibitory concentration (MIC) of 4 μg/mL. Despite the anti-candida activity of triazole glycoside is only one quarter of that of fluconazole, these results indicate the potential and feasibility of this approach to the development of antifungal drugs.

We next investigated the possibility of performing the glycosidation and cycloaddition as a sequential one-pot-like reaction. Because of high molar excess of the alcohols, filtration and evaporation are needed to remove the enzyme and the excess alcohol. The click reaction was then accomplished by dissolving the resultant syrup and adding other reactants. Mostly, this sequential one-pot-like reaction could achieve a similar yield to enzymatic glycosylation (Fig. 4).

3. Conclusion

In conclusion, a series of sugar modules bearing two kinds of reactive handles (alkynl and azide) were enzymatically synthesized from unprotected sugars in one step. The efficient synthesis of alkynl and azide containing glycosides provide a series of modules, which can be directly installed to other molecules. Furthermore, sequential one-pot procedures for enzymatic glycosylation and azide–alkyne cycloaddition have been developed giving access to triazole-containing glycosides commencing with alkynl alcohols being commercially available in a great variety.

4. Experimental section

4.1. Material and methods

NMR spectra were recorded on a Varian Mercury 300 (300 MHz) or Bruker AM 300 spectrometer (300 MHz). The ^1H NMR chemical shifts are reported relative to δ=4.79 (D_2O) and δ=4.87 (CD_3OD), respectively. The ^{13}C NMR chemical shifts are reported as δ values relative to δ=0.00 (D_2O, TMS as external standard) and δ=49.0 (CD_3OD, the center of the solvent resonance), respectively. The following abbreviations are used to indicate the multiplicity: s-singlet; d-doublet; t-triplet; q-quartet; p-pentlet; h-sixtet; m-multiplet. Mass spectra were recorded on a Bruker APEXIII 7.0 TESLA FTMS using ESI mode. Optical rotations were measured using Jasco P-1030 polarimeter. Analytical TLC was performed with silica gel 60 F254 plates, and the products were visualized with H_2SO_4 (10%) solution in MeOH followed by

heating. Column chromatography was carried out using silica gel (300–400 mesh).

Prunus persica L. var. *scleropersica* (Reich) Yü et Lu (Peach) and *Malus pumila* Mill (apple) was purchased from the supermarket in Shanghai as fresh fruits. All other chemicals were of the highest purity commercially available and used without further purification if not mentioned. β-Galactosidase from *A. oryzae* was purchased from Sigma (Lot 91K1450). Standardized with dextrin 1 U will hydrolyze 1 μmol of indicated substrate per min at pH 4.5 at 30 °C. Activity with lactose 9.0 U/mg solid; activity with *o*-nitrophenyl-β-galactoside 10.4 U/mg solid.

The β-glucosidase activity was determined by measuring the release of *p*-nitrophenol from *p*NPG, and 1 U of β-glucosidase activity (U) is defined as the amount of enzyme that release 1 μmol of *p*-nitrophenol per min. All samples were assayed in potassium phosphate buffer (50 mM, pH 7.0) at 50 °C under conditions that activity was proportional to enzyme concentration. A control test without enzyme was included.

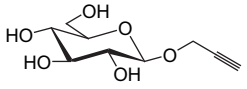
P. persica L. (Peach) stones were cracked with a hammer to release soft kernels inside. The kernels were collected, peeled, and then powdered in cold ethyl acetate (0 °C) with a homogenizer. The powder was defatted by further three washes with ethyl acetate, two washes with acetone, and dried in vacuum and then stored at 4 °C. The apple seeds were treated in the same manner. *P. persica* kernel meal (28.7 U/g) and *M. pumila* Mill seed meal (32.8 U/g) were obtained as a white powder and stored at 4 °C.

4.2. Synthesis of β-glucosides using peach kernel meal

4.2.1. General procedure. To a solution of glucose (0.27 g, 1.5 mmol) in water (0.5 mL) was added a solution (4.5 mL) of substrate (10–38 mmol) in mentioned solvent, and *P. persica* kernel meal/apple seed meal (0.30 g) was then added. The mixture was stirred for 72 h at 50 °C, then filtered and concentrated under vacuum. The resultant syrup was applied to flash column chromatography (eluent EtOAc/MeOH=15–10/1 or CH_2Cl_2/MeOH=5/1). The corresponding β-D-glucopyranosides were collected as white solid or clear syrup.

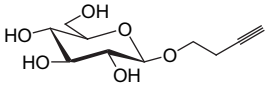
4.2.2. Spectral and analytical data for all β-glucosides.

*4.2.2.1. Prop-2-ynyl β-glucoside (**2a**).*



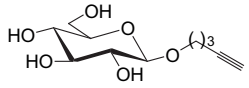
White solid; $[\alpha]_D^{26}$ −91.9 (*c* 0.66, CH_3OH); ^1H NMR (300 MHz, D_2O) *δ*: 2.92(t, *J*=2.4 Hz, 1H), 3.29 (dd, J_1=8.1 Hz, J_2=9.0 Hz, 1H), 3.34–3.53 (m, 4H), 3.71 (dd, *J*=12.3, 5.7 Hz, 1H), 3.91 (dd, *J*=12.3, 1.8 Hz, 1H), 4.47 (t, *J*=1.8 Hz, 2H), 4.50 (d, *J*=8.1 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) *δ* 56.6, 60.8, 69.6, 72.9, 75.7, 76.0, 76.4, 78.9, 100.6; MS(ESI) *m*/*z*: 241.0 [M+Na^+].

*4.2.2.2. But-3-ynyl β-glucoside (**2b**).*



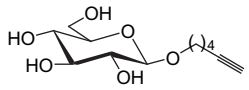
White solid; $[\alpha]_D^{26}$ −30.0 (*c* 1.35, CH_3OH); ^1H NMR (300 MHz, D_2O) *δ* 2.39 (t, *J*=2.4 Hz, 1H), 2.56 (dt, *J*=6.1, 2.7 Hz, 2H), 3.29 (t, *J*=8.4 Hz, 1H), 3.38–3.52 (m, 3H), 3.69–3.85 (m, 2H), 3.90–4.04 (m, 2H), 4.51 (d, *J*=7.8 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) *δ* 19.3, 60.9, 68.3, 69.8, 70.6, 73.2, 75.9, 76.1, 82.5, 102.4; HRMS (ESI): calcd for $C_{10}H_{16}O_6Na^+$ (M+Na^+) 255.0839, found 255.0839.

*4.2.2.3. Pent-4-ynyl β-glucoside (**2c**).*



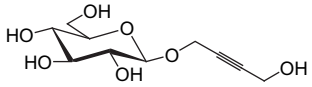
White solid; $[\alpha]_D^{23}$ −32.6 (*c* 0.75, CH_3OH); ^1H NMR (300 MHz, CD_3OD) *δ* 1.75 (p, *J*=6.9 Hz, 2H), 2.16 (t, *J*=2.4 Hz, 1H), 2.25 (dt, *J*=9.3, 2.4 Hz, 2H), 3.11 (t, *J*=8.4 Hz, 1H), 3.22–3.28 (m, 3H), 3.55–3.64 (m, 2H), 3.81 (dd, *J*=18.0, 1.5 Hz, 1H), 3.88–3.95 (m, 1H), 4.20 (*J*=8.1 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) *δ* 16.1, 30.3, 63.0, 69.6, 69.9, 71.9, 75.4, 78.1, 75.4, 78.1, 78.3, 85.0, 104.7; HRMS (ESI): calcd for $C_{11}H_{18}O_6Na^+$ (M+Na^+) 269.0996, found 269.0998.

*4.2.2.4. Hex-5-ynyl β-glucoside (**2d**).*



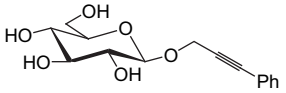
White solid; $[\alpha]_D^{26}$ −32.9 (*c* 0.68, CH_3OH); ^1H NMR (300 MHz, D_2O) *δ* 1.57–1.66 (m, 2H), 1.70–1.76 (m, 2H), 2.20–2.24 (m, 3H), 3.17 (t, *J*=8.1 Hz, 1H), 3.27–3.38 (m, 3H), 3.53–3.60 (m, 1H), 3.64–3.70 (m, 1H), 3.85–3.97 (m, 2H), 4.25 (d, *J*=8.1 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) *δ* 18.8, 26.2, 29.7, 62.7, 69.2, 70.1, 70.6, 75.0, 77.8, 78.0, 84.9, 104.3; HRMS (ESI): calcd for $C_{12}H_{20}O_6Na^+$ (M+Na^+) 283.1152, found 283.1146.

*4.2.2.5. 4-Hydroxybut-2-ynyl β-glucoside (**2e**).*



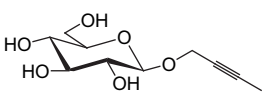
White solid; $[\alpha]_D^{24}$ −71.5 (*c* 1.55, CH_3OH); ^1H NMR (300 MHz, D_2O) *δ* 3.29 (t, *J*=8.1 Hz, 1H), 3.39 (t, *J*=9.3 Hz, 1H), 3.45–3.54 (m, 2H), 3.72 (dd, *J*=12.3, 6.0 Hz, 1H), 3.92 (dd, *J*=12.3, 0.9 Hz, 1H), 4.29 (s, 2H), 4.52 (s, 2H), 4.64 (d, *J*=8.1 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) *δ* 49.4, 56.6, 60.6, 69.5, 72.8, 75.6, 75.9, 80.2, 85.2, 100.4; HRMS (ESI): calcd for $C_{10}H_{16}O_7Na^+$ (M+Na^+) 271.0788, found 271.0785.

*4.2.2.6. 3-Phenylprop-2-ynyl β-glucoside (**2f**).*



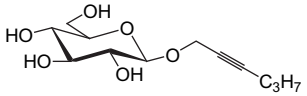
White solid; $[\alpha]_D^{23}$ −91.5 (*c* 0.90, CH_3OH); ^1H NMR (300 MHz, CD_3OD) *δ* (dd, *J*=9.0, 7.5 Hz, 1H), 3.62–3.67 (m, 1H), 3.85 (d, *J*=9.3 Hz, 1H), 4.49 (d, *J*=7.8 Hz, 1H), 4.61 (dd, *J*=16.9, 15.6 Hz, 1H), 7.26–7.31 (m, 3H), 7.36–7.40 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) *δ* 57.7, 63.0, 71.9, 75.2, 78.3, 85.8, 87.6, 102.7, 124.2, 129.7, 129.9, 132.9; HRMS (ESI): calcd for $C_{15}H_{18}O_6Na^+$ (M+Na^+) 317.0091, found 317.0096.

*4.2.2.7. But-2-ynyl β-glucoside (**2g**).*



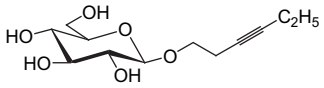
White solid; $[\alpha]_D^{23}$ −30.1 (*c* 0.73, CH_3OH); ^1H NMR (300 MHz, D_2O) *δ* 1.78 (s, 3H), 3.21 (t, *J*=2.7 Hz, 1H), 3.28–3.46 (m, 3H), 3.64 (dd, *J*=16.9, 15.6 Hz, 1H), 3.85 (d, *J*=12.0 Hz, 1H), 4.35 (s, 2H), 4.56 (d, t, *J*=8.1 Hz, 1H); ^1H NMR (300 MHz, D_2O) *δ* 2.5, 57.1, 60.6, 69.5, 72.9, 73.8, 75.7, 75.9, 84.9, 102.2; HRMS (ESI): calcd for $C_{10}H_{16}O_6Na^+$ (M+Na^+) 255.0839, found 255.0839.

*4.2.2.8. Hex-2-ynyl β-glucoside (**2h**).*



White solid; $[\alpha]_D^{23}$ −26.7 (*c* 0.95, CH_3OH); ^1H NMR (300 MHz, CD_3OD) δ 1.00 (t, *J*=7.2 Hz, 3H), 1.47 (m, 2H), 2.18–1.24 (m, 2H), 3.20 (dd, *J*=9.0, 8.1 Hz, 1H), 3.30–3.40 (m, 3H), 3.64–3.70 (m, 1H), 3.87 (dd, *J*=12.3, 1.8 Hz, 1H), 4.41 (dd, *J*=4.8, 2.1 Hz, 2H), 4.47 (d, *J*=7.5 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 13.8, 21.4, 23.1, 57.2, 62.7, 71.6, 74.9, 78.00, 78.02, 87.9, 101.9; HRMS (ESI): calcd for $C_{12}H_{20}O_6Na^+$ (M+Na^+) 283.1152, found 283.1148.

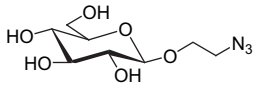
*4.2.2.9. Hex-3-ynyl β-glucoside (**2i**).*



White solid; $[\alpha]_D^{23}$ −21.2 (*c* 0.83, CH_3OH); ^1H NMR (300 MHz, CD_3OD) δ 1.13 (t, *J*=9.0 Hz, 3H), 2.14–2.20 (m, 2H), 2.48–2.54 (m, 2H), 3.23 (t, *J*=8.4 Hz, 1H), 3.32–3.41 (m, 3H), 3.67–3.75 (m, 2H), 3.89–3.97 (m, 2H), 3.35 (d, *J*=8.1 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 13.0, 14.6, 20.8, 62.6, 69.5, 71.4, 74.9. 76.6, 77.80, 77.85, 83.5, 104.2; HRMS (ESI): calcd for $C_{12}H_{20}O_6Na^+$ (M+Na^+) 283.1152, found 283.1144.

*4.2.2.10. 2-Azidoethanol (**1j**)*[15]*.* 2-Chloroethanol (25.2 mL, 375 mmol) was added to a solution of NaN_3 (30 g, 461 mmol) and NaOH (1.5 g, 37.5 mmol) in water (115 mL). The mixture was stirred at rt for 3 days, and sodium sulfate (35 g) was added. After 10 min, the mixture was extracted with CH_2Cl_2 (3×70 mL). The combined extracts were dried in Na_2SO_4 and concentrated. The residue was distilled (*Caution*! Explosion hazards) to give clear liquid (bp, 30 °C, 20 Pa). Yield: 86%. ^1H NMR (300 MHz, $CDCl_3$) δ 3.79–3.76 (t, *J*=5.1 Hz, 2H), 3.47–3.44 (t, *J*=5.1 Hz, 2H), 2.02 (s, 1H).

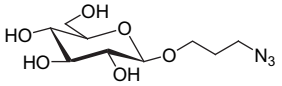
*4.2.2.11. 2-Azidoethyl β-glucoside (**2j**).*



Clear syrup; $[\alpha]_D^{23}$ −38.9 (*c* 0.91, CH_3OH); ^1H NMR (300 MHz, D_2O) δ 3.30 (dd, *J*=9.0, 8.1 Hz, 1H), 3.39–3.53 (m, 3H), 3.56 (t, *J*=5.1 Hz, 2H), 3.73 (dd, *J*=12.3, 5.4 Hz, 1H), 3.84 (dt, *J*=11.4, 5.1 Hz, 1H), 3.92 (dd, *J*=12.3, 2.1 Hz, 1H), 4.06 (dt, *J*=11.4, 5.1 Hz, 1H), 4.50 (d, *J*=8.1 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) 50.5, 60.7, 68.5, 69.6, 73.1, 75.7, 75.9, 102.3; HRMS (ESI): calcd for $C_8H_{15}N_3O_6Na^+$ (M+Na^+) 272.0853, found 272.0852.

*4.2.2.12. 3-Azidopropanol (**1k**)*[16]*.* A solution of sodium azide (29.3 g, 0.461 mol, in 113 mL of H_2O) was added during 30 min to a solution of acrolein (17.2 g, 0.307 mol, in 45 mL of HOAc) with intermittent cooling (dry ice/acetone) to keep the temperature below 5 °C. Stirring was then continued for 30 min without cooling. The solution was extracted with ether (2×250 mL), the extract was washed with 100 mL of saturated Na_2CO_3 solution (*Caution*! Foaming), dried ($MgSO_4$), and concentrated at rt to ca. 250 mL. The ether solution was added during 30 min to a solution of $NaBH_4$ (5 g in 30 mL of H_2O) with cooling to keep the temperature below 20 °C. The mixture was stirred for 15 min and saturated with solid NaC1. The organic layer was dried ($MgSO_4$) and concentrated. The residue was distilled (*Caution*! Explosion hazards) to give clear liquid (bp, 35 °C, 20 Pa). Yield: 86%. ^1H NMR (300 MHz, $CDCl_3$) δ 1.84 (p, *J*=6.3 Hz, 2H), 2.12 (t, *J*=1.5 Hz, 2H, 1H), 3.45 (t, *J*=6.3 Hz, 2H), 3.75 (dd, *J*=10.5, 5.4 Hz, 2H).

*4.2.2.13. 3-Azidopropyl β-glucoside (**2k**).*



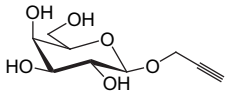
Clear syrup; $[\alpha]_D^{23}$ −26.1 (*c* 1.20, CH_3OH); ^1H NMR (300 MHz, D_2O) δ 1.89 (p, *J*=6.6 Hz, 2H), 3.25 (dd, *J*=8.4, 8.4 Hz, 1H), 3.33–3.50 (m, 6H), 3.66–3.77 (m, 2H), 3.88–4.00 (m, 2H), 4.43 (d, *J*=8.1 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 28.3, 47.9, 60.8, 67.3, 69.7, 73.2, 75.8, 75.9, 102.3; HRMS (ESI): calcd for $C_9H_{17}N_3O_6Na^+$ (M+Na^+) 286.1010, found 286.1008.

4.3. Synthesis of β-galactosides based on transglycosidation

4.3.1. General procedure. A solution of 2.5 g lactose in 5 mL sodium acetate buffer (20 mM, pH 4.5) was prepared and 0.5 mL propargyl alcohol was added. The reaction was initiated by the addition of 0.1 g (900 U) galactosidase from *A. oryzae* and the mixture stirred at 100 rpm and 25 °C. The reaction was stopped by filtering off the residual solid enzyme particles and the filtrate was dried under rotary vacuum to give yellow syrup. The resultant syrup was applied to flash column chromatography (eluent EtOAc/MeOH=15–10/1 or CH_2Cl_2/MeOH=5/1). The corresponding β-D-glucopyranosides were collected as white solid or clear syrup.

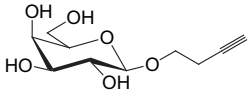
4.3.2. Spectral and analytical data for all β-galactosides.

*4.3.2.1. Prop-2-ynyl β-galactoside (**3a**).*



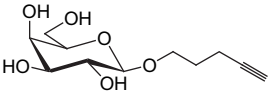
White solid; $[\alpha]_D^{26}$ −30.2 (*c* 0.71, CH_3OH); ^1H NMR (300 MHz, D_2O) δ 2.91 (s, 1H), 3.53 (dd, *J*=9.9, 8.1 Hz, 1H), 3.64–3.79 (m, 4H), 3.93 (d, *J*=2.7 Hz, 1H), 4.48 (s, 2H), 4.57 (d, *J*=7.8 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 56.4, 60.8, 68.5, 70.4, 72.6, 75.2, 76.2, 78.9, 101.0; MS(ESI) *m*/*z*: 241.0 [M+Na^+].

*4.3.2.2. But-3-ynyl β-galactoside (**3b**).*



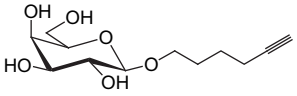
White solid; $[\alpha]_D^{26}$ −24.6 (*c* 0.85, CH_3OH); ^1H NMR (300 MHz, D_2O) δ 2.35 (t, *J*=2.1 Hz, 1H), 2.53 (dt, *J*=6.3, 2.1 Hz, 2H), 3.50 (dd, *J*=9.6, 7.8 Hz, 1H), 3.60–3.82 (m, 5H), 3.90 (d, *J*=3.0 Hz, 1H), 3.95–4.02 (m, 1H), 4.42 (d, *J*=7.8 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 21.79, 21.82, 63.6, 70.7, 71.3, 73.0, 75.4, 77.8, 105.5; HRMS (ESI): calcd for $C_{10}H_{16}O_6Na^+$ (M+Na^+) 255.0839, found 255.0834.

*4.3.2.3. Pent-4-ynyl β-galactoside (**3c**).*



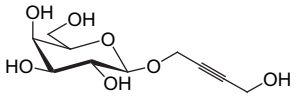
White solid; $[\alpha]_D^{26}$ −24.6 (*c* 0.85, CH_3OH); ^1H NMR (300 MHz, D_2O) δ 1.84 (p, *J*=6.6 Hz, 2H), 2.31–2.37 (m, 3H), 3.50 (dd, *J*=9.6, 7.8 Hz, 1H), 3.62–3.70 (m, 2H), 3.73–3.81 (m, 3H), 3.92 (d, *J*=3.3 Hz, 1H), 3.97–4.05 (m, 1H), 4.39 (d, *J*=7.8 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 14.2, 27.7, 60.9, 68.6, 68.8, 69.6, 70.8, 72.8, 75.1, 85.1, 102.8; HRMS (ESI): calcd for $C_{11}H_{18}O_6Na^+$ (M+Na^+) 269.0982, found 269.0996.

*4.3.2.4. Hex-5-ynyl β-galactoside (**3d**).*



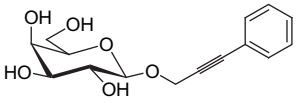
White solid; $[\alpha]_D^{26}$ −26.3 (*c* 0.59, CH_3OH); ^1H NMR (300 MHz, CD_3OD) δ 1.54–1.63 (m, 2H), 1.67–1.73 (m, 2H), 2.16–2.21 (m, 3H), 3.41–3.58 (m, 4H), 3.70 (d, *J*=1.5 Hz, 1H), 3.76 (dd, *J*=24.9, 3.0 Hz, 2H), 3.86–3,94 (m, 1H), 4.18 (d, *J*=7.2 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 19.0, 26.5, 30.0, 62.7, 69.9, 70.4, 70.5, 72.8, 75.3, 76.8, 105.2; HRMS (ESI): calcd for $C_{12}H_{20}O_6Na^+$ ($M+Na^+$) 283.1152, found 283.1142.

*4.3.2.5. 4-Hydroxybut-2-ynyl β-galactoside (**3e**).*



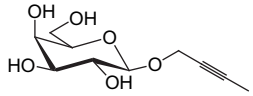
White solid; $[\alpha]_D^{22}$ −78.6 (*c* 0.63, CH_3OH);^1H NMR (300 MHz, D_2O) δ 3.53 (dd, J_1=7.8 Hz, J_2=9.6 Hz, 1H), 3.65–3.80 (m, 4H), 3.93 (d, *J*=3.3 Hz, 1H), 4.30 (s, 2H), 4.53 (s, 2H), 4.58 (d, *J*=7.5 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 49.5, 56.7, 60.9, 68.6, 70.6, 72.7, 75.3, 80.4, 85.2, 101.0; HRMS (ESI): calcd for $C_{10}H_{16}O_7Na^+$ ($M+Na^+$) 271.0788, found 271.0786.

*4.3.2.6. 3-Phenylprop-2-ynyl β-galactoside (**3f**).*



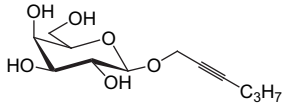
White solid; $[\alpha]_D^{23}$ −104.3 (*c* 0.31, CH_3OH);^1H NMR (300 MHz, D_2O) δ 3.55 (dd, *J*=9.6, 7.8 Hz, 1H), 3.63–3.80 (m, 4H), 3.92 (d, *J*=3.3 Hz, 1H), 4.63 (d, *J*=7.2 Hz, 1H), 4.68 (s, 2H), 7.39–7.43 (m, 3H), 7.51–7.54 (m, 2H); ^{13}C NMR (75 MHz, D_2O) δ 57.3, 60.9, 68.6, 70.6, 72.8, 75.3, 84.2, 86.9, 101.3, 121.6, 128.6, 129.1, 131.7; HRMS (ESI): calcd for $C_{15}H_{18}O_6Na^+$ ($M+Na^+$) 317.0096, found 317.1010.

*4.3.2.7. But-2-ynyl β-galactoside (**3g**).*



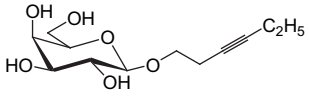
White solid; $[\alpha]_D^{23}$ −68.3 (*c* 0.57, CH_3OH); ^1H NMR (300 MHz, D_2O) δ 1.79 (t, *J*=2.4 Hz, 3H), 3.45–3.49 (m, 3H), 3.68–3.79 (m, 3H), 4.32–4.35 (m, 2H), 4.37 (d, *J*=7.5 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 3.6, 57.9, 62.3, 70.2, 74.7, 75.5, 76.7, 85.0, 102.6; HRMS (ESI): calcd for $C_{10}H_{16}O_6Na^+$ ($M+Na^+$) 255.0839, found 255.0839.

*4.3.2.8. Hex-2-ynyl β-galactoside (**3h**).*



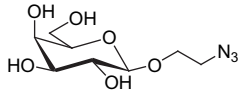
White solid; $[\alpha]_D^{23}$ −43.5 (*c* 0.63, CH_3OH); ^1H NMR (300 MHz, CD_3OD) δ 0.98 (t, *J*=7.2 Hz, 3H), 1.51 (h, *J*=7.2 Hz, 2H), 2.18 (tt, *J*=9.0, 2.1 Hz, 2H), 3.43–3.54 (m, 3H), 3.67–3.78 (m, 2H), 3.81 (d, *J*=7.2 Hz, 1H), 4.38 (dd, *J*=4.8, 2.7 Hz, 2H), 4.40 (d, *J*=7.2 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 14.0, 21.7, 23.4, 57.4, 62.8, 70.6, 72.6, 75.3, 76.9, 77.1, 88.1, 102.9; HRMS (ESI): calcd for $C_{12}H_{20}O_6Na^+$ ($M+Na^+$) 283.1152, found 283.1143.

*4.3.2.9. Hex-3-ynyl β-galactoside (**3i**).*



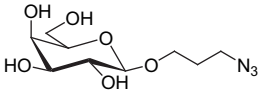
White solid; $[\alpha]_D^{23}$ −18.1 (*c* 0.98, CH_3OH); ^1H NMR (300 MHz, CD_3OD) δ 1.11 (t, *J*=7.5 Hz, 3H), 2.12–2.20 (m, 2H), 2.46–2.52 (m, 2H), 3.51–3.57 (m, 3H), 3.63–3.71 (m, 1H), 3.76–3.79 (m, 2H), 3.86 (d, *J*=2.4 Hz, 1H), 3.89–3.95 (m, 1H), 4.28 (d, *J*=6.6 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) 13.0, 14.6, 20.8, 62.4, 69.5, 71.2, 72.4, 74.8, 76.59, 75.65, 83.5, 104.9; HRMS (ESI): calcd for $C_{12}H_{20}O_6Na^+$ ($M+Na^+$) 283.1152, found 283.1142.

*4.3.2.10. 2-Azidoethyl β-galactoside (**3j**).*



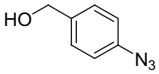
Clear syrup; $[\alpha]_D^{23}$ −24.6 (*c* 0.14, CH_3OH); ^1H NMR (300 MHz, D_2O) δ 3.49–3.57 (m, 3H), 3.62–3.72 (m, 2H), 3.76 (d, 3.81–3.86 (m, 2H)), 3.92 (d, *J*=3.0 Hz, 1H), 4.02–4.09 (m, 1H), 4.42 (d, *J*=7.8 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 50.5, 60.8, 68.3, 68.5, 70.6, 72.6, 75.1, 102.8; HRMS (ESI): calcd for $C_8H_{15}N_3O_6Na^+$ ($M+Na^+$) 272.0853, found 272.0858.

*4.3.2.11. 3-Azidopropyl β-galactoside (**3k**).*



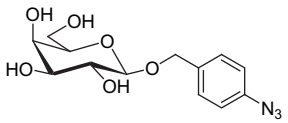
Clear syrup; $[\alpha]_D^{23}$ −15.9 (*c* 0.90, CH_3OH); ^1H NMR (300 MHz, D_2O) δ: 1.93 (p, *J*=6.6 Hz, 2H), 3.48 (t, *J*=6.9 Hz, 2H), 3.52–3.55 (m, 1H), 3.63–3.80 (m, 5H), 3.94 (d, *J*=3.3 Hz, 1H), 3.98–4.06 (m, 1H), 4.40 (d, *J*=7.8 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ: 28.3, 47.9, 60.9, 67.2, 68.6, 70.7, 72.8, 75.1, 102.9; HRMS (ESI): calcd for $C_9H_{17}N_3O_6Na^+$ ($M+Na^+$) 286.1010, found 286.1005.

*4.3.2.12. (4-Azidophenyl)methanol (**1l**).*



(4-Nitrophenyl)methanol (7.6 g, 5 mmol) in methanol. To a mixture of aniline derivative (107 mmol) in AcOH (92 mL) and concd H_2SO_4 (43 mL) was added sodium nitrite (7.70 g, 111 mmol) in water (50 mL) dropwise under vigorous stirring at 0–5 °C. After 10 min aqueous urea was added to the reaction mixture to remove excess sodium nitrite. Then sodium azide (7.70 g, 118 mmol) in water (50 mL) was added to the reaction mixture at 0–5 °C for 3 h. The reaction mixture was poured into ice water and the resulting mixture was basified with sodium hydroxide and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous sodium sulfate, and evaporated to give (4-azidophenyl)-methanol (**1l**). ^1H NMR (300 MHz, $CDCl_3$) δ 1.99 (s, 1H), 4.65 (s, 2H), 7.01 (d, *J*=8.1 Hz, 2H), 7.33 (d, *J*=8.1 Hz, 2H).

*4.3.2.13. 4-Azidobenzyl β-galactoside (**3l**).*



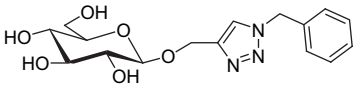
Yellow solid; $[\alpha]_D^{23}$ −30.0 (*c* 1.30, CH_3OH); ^1H NMR (300 MHz, CD_3OD) δ 3.43–3.58 (m, 3H), 3.74–3.83 (m, 3H), 4.29 (d, *J*=7.5 Hz,

1H), 4.76 (dd, *J*=77.1, 12.0 Hz, 1H), 7.02 (d, *J*=8.4 Hz, 2H), 7.44 (d, *J*=8.4 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 62.2, 70.1, 71.1, 72.3, 74.6, 76.5, 103.6, 119.8, 131.0, 135.7, 140.7; HRMS (ESI): calcd for $C_{13}H_{17}N_3O_6Na^+$ ($M+Na^+$) 334.1010, found 334.1006.

4.4. Procedure for copper(I) catalyzed azide-alkyne cycloaddition

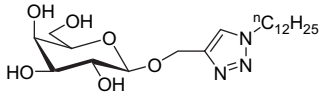
Glycoside (0.5 mmol), substrate (0.6 mmol), copper (II) acetate (9 mg, 0.05 mmol), and sodium ascorbate (20 mg, 0.1 mmol) or copper powder (190 mg) were mixed in *tert*-butyl alcohol/water (1:1; 4.0 mL) and stirred at rt overnight. The solution was directly subjected to flash column chromatography (eluent EtOAc/MeOH/H_2O=12–8/1/0.2).

4.4.1. Compound **11**.



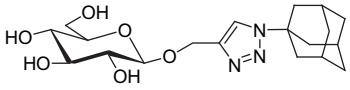
Clear syrup; $[\alpha]_D^{25}$ −29.4 (*c* 2.30, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 3.15 (t, *J*=7.8 Hz, 1H), 3.23–3.28 (m, 4H), 3.62 (dd, *J*=11.7, 4.8 Hz, 1H), 3.83 (d, *J*=11.7, 1H), 4.32 (d, *J*=8.1 Hz, 1H), 4.72 (d, *J*=12.3 Hz, 1H), 4.92 (d, *J*=12.3 Hz, 1H), 7.26–7.37 (m, 5H), 7.97 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 54.9, 62.7, 63.0, 71.6, 75.0, 77.8, 78.0, 103.6, 125.3, 129.1, 129.6, 130.0, 136.7, 146.1; HRMS (ESI): calcd for $C_{16}H_{21}N_3O_6Na^+$ ($M+Na^+$) 374.1323, found 374.1325.

4.4.2. Compound **12**.



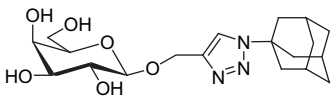
White solid; $[\alpha]_D^{25}$ −24.6 (*c* 1.20, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.85 (t, *J*=6.6 Hz, 3H), 1.23–1.27 (m, 18H), 1.84 (p, *J*=6.6 Hz, 3H), 3.26 (t, *J*=6.6 Hz, 2H), 3.41 (dd, *J*=9.6, 3.0 Hz, 1H), 3.47–3.53 (m, 2H), 3.65–3.75 (m, 2H), 3.78 (d, *J*=3.0 Hz, 1H), 4.28 (d, *J*=7.5 Hz, 1H), 4.34 (t, *J*=7.2 Hz, 2H), 4.82 (dd, *J*=27.6, 12.6 Hz, 2H), 7.96 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 14.7, 24.0, 27.7, 30.4, 30.7, 30.8, 30.9, 31.0, 31.5, 33.3, 51.6, 62.8, 63.3, 70.6, 72.7, 75.1, 77.1, 104.5, 125.5, 146.0; HRMS (ESI): calcd for $C_{21}H_{39}N_3O_6Na^+$ ($M+Na^+$) 452.2731, found 452.2728.

4.4.3. Compound **13**.



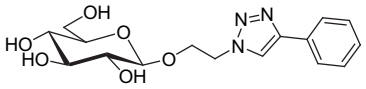
Clear syrup; $[\alpha]_D^{25}$ −35.6 (*c* 1.05, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.83 (s, 6H), 2.25 (s, 9H), 3.19–3.25 (m, 1H), 3.28–3.38 (m, 3H), 3.67 (dd, *J*=12.0, 5.4 Hz, 1H), 3.90 (d, *J*=11.4 Hz, 1H), 4.38 (d, *J*=7.8 Hz, 1H), 4.77 (d, *J*=12.3 Hz, 1H), 4.96 (d, *J*=12.3 Hz, 1H), 8.12 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 30.9, 36.9, 43.9, 61.1, 62.8, 63.2, 71.6, 75.0, 77.9, 78.0, 103.6, 122.0, 144.9; HRMS (ESI): calcd for $C_{19}H_{29}N_3O_6Na^+$ ($M+Na^+$) 418.1949, found 418.1947.

4.4.4. Compound **14**.



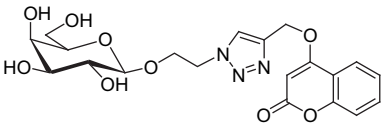
White solid; $[\alpha]_D^{25}$ −18.6 (*c* 0.95, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.84 (s, 6H), 2.25 (s, 9H), 3.47 (dd, *J*=9.6, 3.3 Hz, 1H), 3.52–3.58 (m, 2H), 3.73–3.80 (m, 2H), 3.83 (d, *J*=3.0 Hz, 1H), 4.33 (d, *J*=8.1 Hz, 1H), 4.78 (d, *J*=12.0 Hz, 1H), 4.96 (d, *J*=12.0 Hz, 1H), 8.12 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 29.6, 35.5, 42.5, 59.7, 61.2, 61.8, 68.9, 71.0, 73.4, 75.4, 102.9, 120.6; HRMS (ESI): calcd for $C_{19}H_{29}N_3O_6Na^+$ ($M+Na^+$) 418.1949, found 418.1945.

4.4.5. Compound **15**.



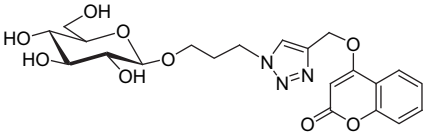
Clear syrup; $[\alpha]_D^{25}$ −33.2 (*c* 1.10, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 3.20 (t, 1H), 3.28–3.35 (m, 3H), 3.65 (dd, *J*=12.0, 5.1 Hz, 1H), 3.86 (dd, *J*=12.0, 1.2 Hz, 1H), 4.02–4.08 (m, 1H), 4.26–4.32 (m, 1H), 4.33 (d, *J*=7.5 Hz, 1H), 4.69 (t, *J*=5.1 Hz, 2H), 7.30–7.35 (m, 1H), 7.29–7.44 (m, 2H), 7.80–7.83 (m, 2H), 8.45 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 50.6, 61.5, 67.9, 70.3, 73.8, 76.8, 76.9, 103.4, 122.3, 125.5, 128.2, 128.8, 130.6, 147.5; HRMS (ESI): calcd for $C_{16}H_{21}N_3O_6Na^+$ ($M+Na^+$) 374.1323, found 374.1318.

4.4.6. Compound **16**.



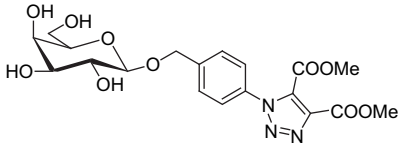
Clear syrup; $[\alpha]_D^{25}$ −26.3 (*c* 1.20, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 3.47–3.59 (m, 3H), 3.70–3.82 (m, 2H), 3.85 (d, *J*=3.0 Hz, 1H), 4.03–4.11 (m, 1H), 4.26–4.33 (m, 2H), 4.74 (t, *J*=5.4 Hz, 2H), 5.44 (s, 2H), 6.07–6.08 (m, 1H), 7.35–7.38 (m, 2H), 7.61–7.67 (m, 1H), 7.83–7,88 (m, 1H), 8.42 (s, 1H); ^{13}C NMR (75 MHz, DMSO) δ 55.1, 65.6, 68.0, 72.4, 73.2, 73.2, 75.5, 78.3, 80.5, 96.4, 108.7, 120.2, 121.5, 128.1, 129.4, 131.2, 137.9, 145.9, 157.9, 166.8, 169.6; HRMS (ESI): calcd for $C_{20}H_{23}N_3O_9Na^+$ ($M+Na^+$) 472.1327, found 472.1331.

4.4.7. Compound **17**.



Clear syrup; $[\alpha]_D^{25}$ −30.8 (*c* 1.25, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 2.24 (p, *J*=6.3 Hz, 2H), 3.24–3.34 (m, 3H), 3.43 (t, *J*=9.0 Hz, 1H), 3.54–3.62 (m, 1H), 3.66–3.74 (m, 1H), 3.88–3.97 (m, 2H), 4.31 (d, *J*=8.1 Hz, 1H), 4.65 (t, *J*=6.6 Hz, 2H), 5.42 (s, 2H), 6.02 (s, 1H), 7.25–7.30 (m, 2H), 7.55–7.61 (m, 1H), 7.75 (dd, *J*=7.8 1.5 Hz, 1H), 8.36 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 31.4, 62.7, 63.7, 66.8, 71.5, 75.0, 77.90, 77.95, 91.8, 104.3, 116.6, 117.5, 124.2, 125.5, 126.9, 133.9, 142.5, 154.4, 164.8, 166.8; HRMS (ESI): calcd for $C_{21}H_{25}N_3O_9Na^+$ ($M+Na^+$) 486.1483, found 486.1479.

4.4.8. Compound **18**.



White solid; $[\alpha]_D^{25}$ −41.3 (*c* 1.10, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 3.46–3.65 (m, 3H), 3.75–3.81 (m, 2H), 3.85 (d, *J*=3.3 Hz, 1H), 3.87 (s, 3H), 3.95 (s, 3H), 4.36 (d, *J*=8.1 Hz, 1H), 4.79 (d, *J*=12.6 Hz, 1H), 5.04 (d, *J*=12.6 Hz, 1H), 7.54 (d, *J*=8.7 Hz, 2H), 7.67 (d, *J*=8.7 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 53.2, 54.4, 62.5,

70.3, 70.6, 72.5, 74.9, 76.8, 104.3, 125.3, 130.0, 133.8, 135.9, 139.5, 142.7, 160.5, 161.4; HRMS (ESI): calcd for $C_{21}H_{39}N_3O_6H^+$ ($M+H^+$) 454.1456, found 454.1462.

4.5. Procedure for sequential one-pot-like reaction

4.5.1. Reverse hydrolysis+click. The reaction mixture was filtered and concentrated under vacuum after the enzymatic glycosidation was conducted as general procedure. To the resultant syrup was then added substrate (1.5 equiv, calculated based on glycosidation yield), copper (II) acetate (9 mg, 0.05 mmol) and copper powder (190 mg, 3 mmol), and *tert*-butyl alcohol/water (1:1; 4.0 mL) and stirred at rt for 4–24 h. The solution was filtered and concentrated under vacuum. The residue was subjected to flash column chromatography (eluent EtOAc/MeOH/H_2O=12–8/1/0.2) to give the product.

4.5.2. Transglycosidation+click. After the enzymatic glycosidation was conducted as general procedure, to the reaction mixture was added 10 mL ethanol, and then filtered through Celite and concentrated under vacuum. The resultant syrup was then treated as 4.5.1.

4.6. Antifungal susceptibility test

The antifungal activity was measured based on the recommendations of NCCLS.[17] The compounds were dissolved in water and diluted in a twofold manner in RPMI 1640 (pH 7.0) in 96-microwell plates. The MIC was the minimum concentration of the agent that shows a full inhibition of the fungal growth in the well, examined by naked eyes.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2009.11.044.

References and notes

1. For reviews on glycosyltransferase and references therein see: (a) Brik, A.; Ficht, S.; Wong, C.-H. *Curr. Opin. Chem. Biol.* **2006**, *10*, 638–644; (b) Koeller, K. M.; Wong, C.-H. *Chem. Rev.* **2000**, *100*, 4465–4493; (c) De Luca, C.; Lansing, M.; Martini, I.; Crescenzi, F.; Shen, G.-J.; O'Regan, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 5869–5870.
2. For reviews on glycosynthases and references therein see: (a) Mackenzie, L. F.; Wang, Q.-P.; Warren, R. A. J.; Withers, S. G. *J. Am. Chem. Soc.* **1998**, *120*, 5583–5584; (b) Ducros, V. M. A.; Tarling, C. A.; Zechel, D. L.; Brzozowski, A. M.; Frandsen, T. P.; von Ossowski, I.; Schulein, M.; Withers, S. G.; Davies, G. J. *Chem. Biol.* **2003**, *10*, 619–628; (c) Jahn, M.; Chen, H. M.; Mullegger, J.; Marles, J.; Warren, R. A. J.; Withers, S. G. *Chem. Commun.* **2004**, 274–275; (d) Hancock, S. M.; Vaughan, M. D.; Withers, S. G. *Curr. Opin. Chem. Biol.* **2006**, *10*, 509–519.
3. For reviews on glycosidase see: (a) Crout, D. H. G.; Vic, G. *Curr. Opin. Chem. Biol.* **1998**, *2*, 96–111; (b) van Rantwijk, F.; Woudenberg-van Oosterom, M.; Sheldon, R. A. *J. Mol. Catal. B: Enzym.* **1999**, *6*, 511–532; (c) Ruiz, J. M. J.; Oßwald, G.; Peterson, M.; Fessner, W. D. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 189–197; (d) Faber, K. *Biotransformations in Organic Chemistry: A Textbook*, 4th ed.; Springer: Berlin, 2000; pp 307–321; (e) de Roode, B. M.; Franssen, M. C. R.; van der Padt, A.; Boom, R. M. *Biotechnol. Prog.* **2003**, *19*, 1391–1402.
4. (a) Tong, A.-M.; Xu, J.-H.; Lu, W.-Y.; Lin, G.-Q. *J. Mol. Catal. B: Enzym.* **2005**, *32*, 83–88; (b) Tong, A.-M.; Xu, J.-H.; Lu, W.-Y.; Lin, G.-Q. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2095–2097; (c) Yu, H.-L.; Xu, J.-H.; Lu, W.-Y.; Lin, G.-Q. *Enzyme Microb. Technol.* **2007**, *40*, 354–361; (d) Yu, H.-L.; Xu, J.-H.; Wang, Y.-X.; Lu, W.-Y.; Lin, G.-Q. *J. Comb. Chem.* **2008**, *10*, 79–87.
5. (a) Lu, W.-Y.; Lin, G.-Q.; Yu, H.-L.; Tong, A.-M.; Xu, J.-H. *J. Mol. Catal. B: Enzym.* **2007**, *44*, 72–77; (b) Linh, P. T.; Kim, Y. H.; Hong, S. P.; Jian, J. J.; Kang, J. S. *Arch. Pharm. Res.* **2000**, *23*, 349–352; (c) Ming, H. Q.; Xia, G. C.; Zhang, R. D. *Chin. Trad. Herb. Drugs.* **1988**, *19*, 229–234.
6. Yu, H.-L.; Xu, J.-H.; Lu, W.-Y.; Lin, G.-Q. *J. Biotechnol.* **2008**, *133*, 469–477.
7. (a) Kishida, M.; Akita, H. *Tetrahedron Lett.* **2005**, *46*, 4123–4125; (b) Kishida, M.; Akita, H. *Tetrahedron: Asymmetry* **2005**, *16*, 2625–2630.
8. (a) Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 565–598; (b) Kolb, H. C.; Finn, N. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021; (c) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064; (d) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599; (e) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210–216.
9. For a review and some recent examples on 1,2,3- and 1,2,4-triazoles, see: (a) Como, J. A.; Dismukes, W. E. N. *Engl. J. Med.* **1994**, *330*, 263–272; (b) Dandia, A.; Singh, R.; Khaturia, S.; Mérienne, C.; Morgant, G.; Loupy, A. *Bioorg. Med. Chem.* **2006**, *14*, 2409–2417; (c) Lin, R.; Connolly, P. J.; Huang, S.; Wetter, S. K.; Lu, Y.; Murray, W. V.; Emanuel, S. L.; Gruninger, R. H. *J. Med. Chem.* **2005**, *48*, 4208–4211; (d) Tehranchian, S.; Akbarzadeh, T.; Fazeli, M. R.; Jamalifar, H.; Shafiee, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1023–1025; (e) Alam, M. S.; Kajiki, R.; Hanatani, H.; Kong, X.; Ozoe, F.; Matsui, Y.; Matsumara, F.; Ozoe, Y. *J. Agric. Food Chem.* **2006**, *54*, 1361–1372; (f) Wroblewski, A. E.; Glowacka, I. E. *Tetrahedron: Asymmetry* **2005**, *16*, 4056–4064; (g) Tornøe, C. W.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Meldal, M. *J. Comb. Chem.* **2004**, *6*, 312–324; (h) Aufort, M.; Herscovici, J.; Bouhours, P.; Moreau, N.; Girard, C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1195–1198.
10. Matsumura, S.; Imai, K.; Yoshikawa, S.; Kawada, K.; Uchibori, T. *J. Am. Oil Chem. Soc.* **1990**, *67*, 996–1001.
11. Lu, W.-Y.; Lin, G.-Q.; Yu, H.-L.; Su, J.-H.; Xu, J.-H., unpublished result.
12. Wang, N. L.; Yao, X. S.; Ishii, R.; Kitanaka, S. *Chem. Pharm. Bull.* **2001**, *49*, 938–942.
13. (a) Nakkharat, P.; Haltrich, D. *Appl. Biochem. Biotechnol.* **2006**, *129–132*, 215–225; (b) Hinz, S. W. A.; Doeswijk-Voragen, C. H. L.; Schipperus, R.; van den Broek, L. A. M.; Vincken, J.-P.; Voragen, A. G. J. *Biotechnol. Bioeng.* **2006**, *93*, 122–131; (c) Das-Bradoo, S.; Svensson, I.; Santos, J.; Plieva, F.; Mattiasson, B.; Hatti-Kaul, R. *J. Biotechnol.* **2004**, *110*, 273–286; (d) Chen, C. W.; Ou-Yang, C.-C.; Yeh, C.-W. *Enzyme Microb. Technol.* **2003**, *33*, 497–507.
14. Blinkovsky, A. M.; Dordick, J. S. *Tetrahedron: Asymmetry* **1993**, *4*, 1221–1228.
15. Cheng, H.; Cao, X.-H.; Xian, M.; Fang, L.-Y.; Cai, T. B.; Ji, J. J.; Tunac, J. B.; Sun, D.-X.; Wang, P. G. *J. Med. Chem.* **2005**, *48*, 645–652.
16. Szmuszkovicz, J.; Kane, M. P.; Laurian, L. G.; Chidester, C. G.; Scahill, T. A. *J. Org. Chem.* **1981**, *46*, 3562–3564.
17. National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing for Yeasts, Proposed Standards, Document M27-P; National Committee for Clinical Laboratory Standards: Villanova, PA, 1992.