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Design and synthesis of O-GlcNAcase inhibitors via 'click chemistry' and biological evaluations

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ABSTRACT

Protein O-GlcNAcylation has been shown to play an important role in a number of biological processes, including regulation of the cell cycle, DNA transcription and translation, signal transduction, and protein degradation. O-GlcNAcase (OGA) is responsible for the removal of O-linked β-*N*-acetylglucosamine (O-GlcNAc) from serine or threonine residues, and thus plays a key role in O-GlcNAc metabolism. Potent OGA inhibitors are useful tools for studying the cellular processes of O-GlcNAc, and may be developed as drugs for the treatment neurodegenerative diseases. In this study, Cu(I)-catalyzed 'Click' cycloaddition reactions between glycosyl azides and alkynes were exploited to generate inhibitory candidates of OGA. Enzymatic kinetic screening revealed that compound **7** was a potent competitive inhibitor of human O-GlcNAcase (K_i = 185.6 μM). Molecular docking simulations of compound **7** into *Cp*OGA (*Clostridium perfringens* OGA) suggested that strong π–π stacking interaction between the compound and W490 considerably contributed to improving the inhibitory activity.

1. Introduction

The covalent attachment of a single *N*-acetylglucosamine moiety onto serine or threonine residues of proteins via a β-O-glycosidic linkage is termed the O-GlcNAc modification (O-GlcNAcylation). O-GlcNAcylation was discovered 26 years ago by Torres and Hart,[1] and it is one of the most common post-translational modifications related to cellular regulation and signal transduction.[1–3] So far, more than 1000 proteins involved in cellular processes (transporting, transcription, cell shaping, cell signaling and apoptosis[4–6]) have been identified to be O-GlcNAcylated. These proteins include nuclear pore proteins, cytoskeletal proteins, transcription factors, oncogenic proteins, tumor suppressor proteins and so on. It has been demonstrated that O-GlcNAcylation plays a significant role in many fundamental cellular processes, and its dysregulation contributes to the etiology of cardiovascular diseases, type-2 diabetes, cancer, and neurological disorders.[7–10] Especially, O-GlcNAcylation is closely related to Alzheimer's disease (AD). Many AD-associated proteins are modified by O-GlcNAc and phosphorylation, including tau, neurofilaments, and beta-amyloid precursor protein (APP).[11] Hyperphosphorylated tau accumulates into insoluble paired helical filaments, which is a major characteristic of AD. In the diseased brain, tau is hyperphosphorylated and underglycosylated at same Ser/Thr site as Thr231 and Ser396.[12]

The hydrolytic cleavage of O-GlcNAc from O-GlcNAcylated proteins is catalyzed by O-GlcNAcase (OGA). OGA contains two discrete domains: an N-terminal glycoside hydrolase domain and a C-terminal domain with histone acetyltransferase activity (Fig. 1).[13] According to primary sequence similarity, the N-terminal domain is classified into the glycoside hydrolase family 84 (http://www.cazy.org/),[14] OGA uses a catalytic mechanism involving substrate-assisted catalysis that relies on the involvement of the 2-acetamido group of the substrate to form a transient oxazoline intermediate (Fig. 2).[15–17] Asp^{174} and Asp^{175} have been identified as two key residues of human OGA involved in the de-GlcNAcylation.[16,18] In the first step, Asp^{174} directs and polarizes the 2-acetamido group to act as a nucleophile and form the oxazoline intermediate. Meanwhile, Asp^{175} acts as a general acid to facilitate departure of the aglycone leaving group. The following step is nearly the microscopic reverse of the first step. Asp^{174} facilitates departure of the 2-acetamido group, while Asp^{175} acts as a general base, promoting the attack of one molecule of water to yield the β-hemiacetal product.[16] Since O-GlcNAcylation is directly regulated by OGA, modulation of O-GlcNAc levels with small molecule inhibitors of OGA is a useful strategy to probe the role of this O-GlcNAcylation in a range of cellular processes. PUGNAc (Fig. 3) a nanomolar inhibitor of OGA (K_i = 46 nM), was recently reported by Vocadlo and co-workers.[15] However, PUGNAc lacked selectivity

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Figure 1. Sketch map of OGA.

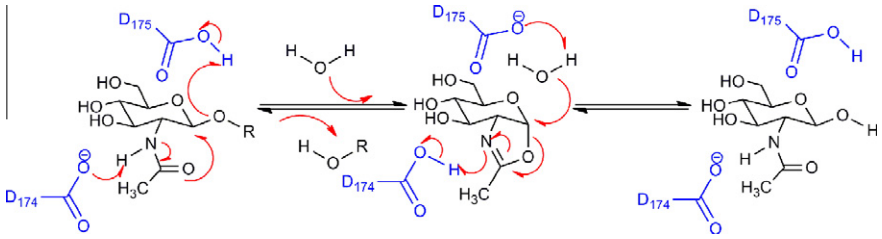


Figure 2. The proposed catalytic mechanism of OGA uses substrate-assisted catalysis involving a two-step double-displacement mechanism via forming a transient oxazoline intermediate.

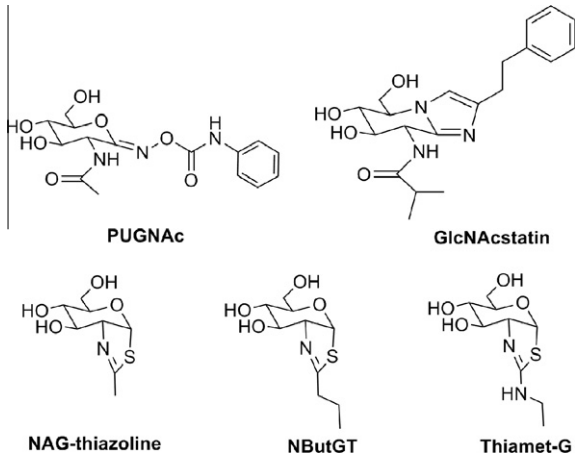


Figure 3. Some previously reported inhibitors of O-GlcNAcase.

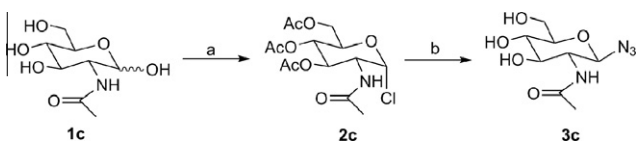
and did not cross the blood brain barrier.[15,19] GlcNAcstatin (Fig. 3) has been found to be an extremely potent inhibitor of a bacterial homolog of OGA from *Clostridium perfringens* (K_i = 4.6 pM).[20] Subsequently, Dorfmueller found that GlcNAcstatin showed a K_i value of 4.4 nM toward human OGA, and it also displayed 125-fold selectivity for human OGA over β-hexosaminidase A and β-hexosaminidase B (Hex A and B, K_i = 550 nM). In addition, GlcNAcstatin was shown to be a cell-permeant compound that modulated O-GlcNAcylation levels within the cells by inhibiting human OGA.[21] NAG-thiazoline (Fig. 3), which has an obvious geometric resemblance to the oxazoline intermediate, has been found to be a potent inhibitor of OGA (K_i = 70 nM) by virtue of its geometric mimicry of the transition state.[15,22] Unfortunately, NAG-thiazoline lacked selectivity and therefore perturbed multiple cellular processes. In order to improve the selectivity of NAG-thiazoline, NButGT (Fig. 3), which was generated by varying the bulk of the thiazoline substituent, displayed 800-fold selectivity for human OGA (K_i = 600 nM) over Hex A and B.[15,23] Although this inhibitor had reasonable potency and good selectivity, it had limited chemical stability in the solution over extended periods of a few days to weeks.[15,23] Thiamet-G (Fig. 3) has been identified as a potent inhibitor of OGA (K_i = 21 nM), which displayed exquisite 37,000-fold selectivity for OGA over Hex A and B.[23] Moreover, Thiamet-G was highly stable and effective in cell culture with an EC_{50} of 30 nM for increasing O-GlcNAc levels in PC12 rat pheochromocytoma cells.[23]

Huisgen 1,3-dipolar cycloadditions[24] are exergonic fusion processes that connect two unsaturated reactants and afford fast access to an enormous variety of five-membered heterocycles.[25] The Cu(I)-catalyzed [3+2] azide–alkyne cycloaddition reaction to generate triazole is the most useful member of this family. It provides an expedient method to connect azides and alkynes in high yields under mild conditions.[26,27] Moreover, the triazole moiety usually has favorable physicochemical properties, which are capable of interacting with the biological targets through hydrogen bonding, dipole–dipole, and π-stacking interactions. This reaction has become a powerful tool in generating combinatorial libraries,[28,29] and increasing the application of bioconjugation, discovery of the lead compound and optimization of the lead compound.[30,31] For example, recently, a click-based library of protein tyrosine phosphatase (PTP) inhibitor candidates was prepared via copper(I)-assisted 'click chemistry', in which a compound with an IC_{50} value of 4.7 μM against PTP1B was screened out.[32] Rossi and Basu prepared 1-glycosyl-4-phenyl triazoles via this reaction, some of which can inhibit the activity of certain glycosidases.[33] Poulsen and co-workers generated a novel class of carbonic anhydrase inhibitors that were glycoconjugate benzene sulfonamides prepared by 'click-tailing'.[34] Lee identified a potent and highly selective inhibitor of human α-1,3-fucosyltransferase by screening a GDP-triazole library synthesized via 'click chemistry'.[35] Herein, we described a rapid parallel synthesis of a small triazole-linked carbohydrate library via 'click chemistry'. Subsequent screening and evaluation revealed a potent inhibitor of human OGA.

2. Results and discussion

2.1. Synthesis of inhibitory candidates via 'click chemistry'

Glycosyl azide (Scheme 1, compound **3c**) was a key intermediate for the synthesis of glycosyl triazoles through the Cu(I)-catalyzed [3+2] azide–alkyne cycloaddition reaction. 2-Acetamido-2-deoxy-D-glucose (**1c**) was chosen as the starting material, which was reacted with acetyl chloride to afford 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl chloride (**2c**) in a yield of 72%.[36] Subsequently, the desired glycosyl azide (**3c**) was prepared by nucleophilic substitution of compound **2c** with sodium

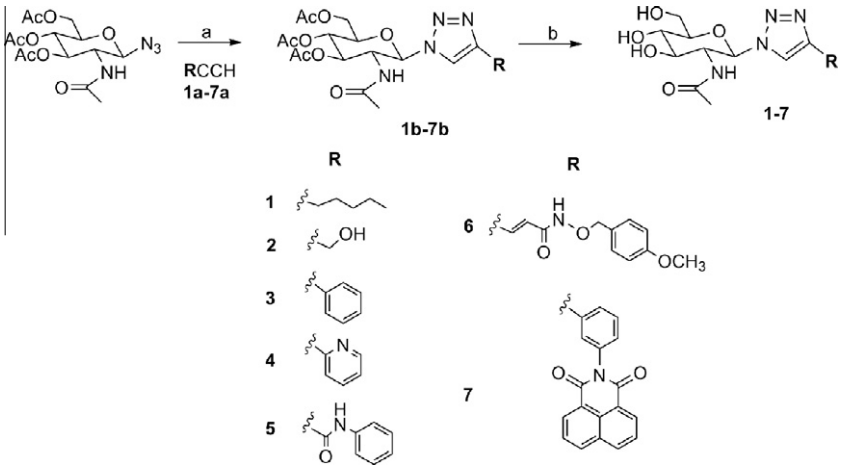


Scheme 1. Reagents and conditions: (a) AcCl (12 equiv), rt, 24 h, 72%; (b) Bu_4NHSO_4 (1 equiv), $NaHCO_3$ (satd), CH_2Cl_2, NaN_3 (3 equiv), rt, 1 h, 59%.

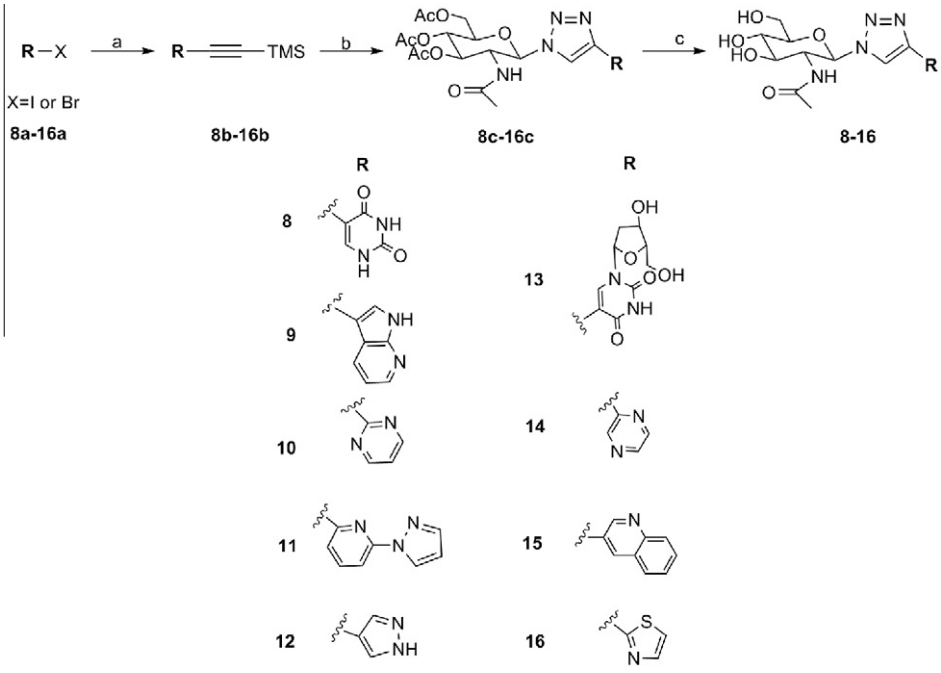
azide in the presence of tetrabutylammonium hydrogen sulfate, CH_2Cl_2 and a saturated aqueous solution of $NaHCO_3$.[37]

Triazole-linked carbohydrates were then synthesized as outlined in Scheme 2. When using catalytic conditions described by Sharpless and co-workers,[30] the triazole-forming reaction was found to be sluggish, even at elevated temperatures. However, when using 20 mol % of $CuSO_4$ and 40 mol % of sodium ascorbate in the presence of 1:1 *tert*-butyl alcohol–water, the triazole formation proceeded smoothly under very mild conditions in high yields (72–92%).[38] Reaction of glycosyl azide (**3c**) and alkyne (**1a**–**7a**, **1a**–**5a** were commercially available, **6a** and **7a** were synthesized in our group[39,40]) in the ratio of 1:1.2 was complete with approximate 8 h of vigorous stirring (detected by TLC) to form triazoles (**1b**–**7b**). Finally, compounds **1b**–**7b** were deprotected employing sodium methoxide in dry methanol to give triazole-linked carbohydrates (**1**–**7**).

Compounds **8**–**16** connected with different heterocyclic groups were prepared as outlined in Scheme 3. The synthesis began with commercially available mono-iodo substituted heterocyclic compounds (**8a**, **9a**, **12a**, **13a**, and **14a**) or mono-bromo substituted heterocyclic compounds (**10a**, **11a**, **15a**, and **16a**). TMS-protected alkynes (**8b**–**16b**) were obtained in high yields (83–89%) via a Sonogashira coupling reaction using trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI and Et_3N.[41] Compounds **8b**–**16b** were treated with tetrabutylammonium fluoride to give deprotected alkynes,[42] to which the glycosyl azide (**3c**) was connected via Cu(I)-catalyzed cycloaddition reaction (20 mol % of $CuSO_4$ and 40 mol % of sodium ascorbate, 1:1 *tert*-butyl alcohol–water). The resulting triazoles (**8c**–**16c**)[38] were finally deprotected to generate triazole-linked carbohydrates with various heterocyclic groups (**8**–**16**).



Scheme 2. Reagents and conditions: (a) RCCH (1.2 equiv), 1:1 H_2O/*t*-BuOH, sodium ascorbate (0.4 equiv), $CuSO_4$ (0.2 equiv), rt, overnight, 72–92%; (b) CH_3OH, CH_3ONa, 30 min, Dowex-50 (H^+), 90–96%.



Scheme 3. Reagents and conditions: (a) $Pd(PPh_3)_4$ (0.05 equiv), CuI (0.1 equiv), trimethylsilylacetylene (3 equiv), Et_3N, DMF, rt, 18 h, 83–89%; (b) (i) TBFA (1.2 equiv), THF, rt, 30 min; (ii) 1:1 H_2O/*t*-BuOH, glycosyl azide (1 equiv), sodium ascorbate (0.4 equiv), $CuSO_4$ (0.2 equiv), rt, overnight, 72–92%; (c) CH_3OH, CH_3ONa, 30 min, Dowex-50 (H^+), 92–96%.

2.2. Evaluation of the inhibitory activity

Percent inhibition assays of human OGA toward triazoles **1**–**16** were performed as preliminary screenings (Table 1). The results showed that triazole **1** bearing a completely hydrophobic pentyl group and triazole **2** bearing completely hydrophilic hydroxymethyl group showed very weak inhibitory potency; triazoles **3**, **5**, **6**, and **7** with benzene rings displayed strong inhibitory potencies, which suggested that π–π stacking interaction contributing to improving the inhibitory activity; triazoles **4**, **8**–**16** bearing heterocyclic groups showed general inhibition activities, which may result from heteroatoms of heterocyclic groups forming unfavorable H-bonds with certain important residues to have an effect on π–π stacking interaction. Obviously, compound **7** was the strongest inhibitor against human OGA among all of the synthesized triazoles. Subsequently, a Lineweaver–Burk plot of the velocity of 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside (4MU-GlcNAc) hydrolysis by OGA in the presence of various concentrations of compound **7** indicated the mode of enzyme inhibition (Fig. 4). It was obvious that compound **7** acted as a potent and competitive inhibitor of human OGA, with K_i = 185.6 μM. In addition, inhibition of hexosaminidase A was also evaluated, giving a K_i of 232.0 μM. Unfortunately, this compound did not show selectivity for human OGA over hexosaminidase A.

Table 1
Inhibition activities of 1*H*-1,2,3-triazole derivatives (1 mM) over OGA

Compound	Inhibition[a] (%)
1	0.5
2	3.5
3	57.8
4	12.4
5	55.0
6	37.4
7	81.3
8	50.1
9	57.4
10	37.3
11	42.1
12	48.1
13	46.9
14	44.4
15	44.5
16	39.1

[a] Values were the averages of at least three experiments. Errors were in the range of ±0.2–3% of the reported value.

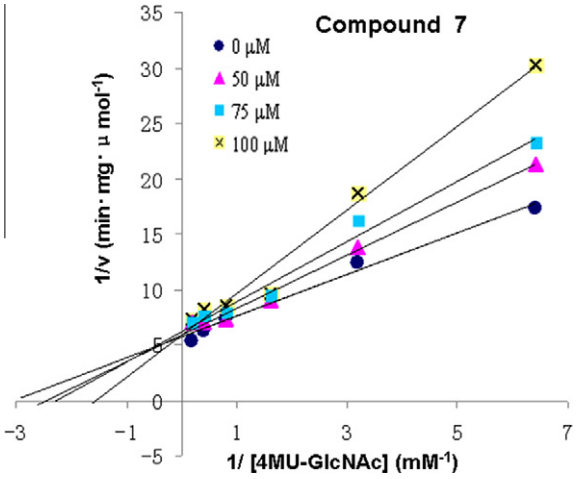


Figure 4. Inhibition Lineweaver–Burk plot of OGA toward compound **7** at concentrations of 0, 50, 75, and 100 μM.

Molecular docking simulations were applied to investigate the binding mode of the most potent inhibitor (compound **7**) into the enzyme-active sites. A bacterial enzyme (*C. perfringens* OGA, *Cp*OGA) bearing the highest sequence homology with human OGA was selected for the docking study since there is no human OGA crystal structure available.[43] Figure 5(A) showed the best docked pose obtained for compound **7** using C*p*OGA structure 2J62. The sugar moiety of the inhibitor was shown to occupy the active site of the enzyme, establishing hydrogen-bond interactions mainly with residues D401, N396, D297, and D298. The pyranose ring adopted a ^4E envelope conformation which was similar to the PUGNAc complex.[43] D298, the catalytic acid, formed a strong hydrogen bond with a nitrogen atom of the triazole ring. The substituted group of the triazole ring with a big conjugated π-system stretched away from the active site, forming a strong π–π stacking interaction with the solvent-exposed W490, which made the inhibitor productively contact with the enzyme. Figure 5(B–D) showed the best docked pose obtained for compounds **3**, **4,** and **10** using C*p*OGA structure 2J62. D298, the catalytic acid, did not form a strong hydrogen bond with a nitrogen atom of the triazole ring in compounds **3**, **4,** and **10** against *Cp*OGA structure 2J62. Although compounds **3**, **4,** and **10** formed a π–π stacking interaction with W490, the π–π stacking interaction was weaker than that of compound **7** with W490 by virtue of the large conjugated π-system of compound **7**. In addition, the binding energies of **7**, **3**, **4,** and **10** with *Cp*OGA calculated by Autodock 4.2 indicated that compound **3** showed 2.55 kcal/mol higher binding energy than that of compound **7**, compound **10** showed 0.57 kcal/mol higher than that of compound **3**, compound **4** showed 0.38 kcal/mol higher than that of compound **10**. The computational binding energies were consistent with the order of inhibitory activity (compound **7** > compound **3** > compound **10** > compound **4**).

3. Conclusions

In summary, the synthesis of glycosyl triazoles by a Cu(I)-catalyzed 'click' cycloaddition reaction between glycosyl azides and alkynes was well-suited for rapid generation of combinatorial libraries. Herein we have described the synthesis and screening of compounds generated by this method. It was noteworthy that compound **7** was identified as a potent and competitive inhibitor of human OGA, with K_i = 185.6 μM. Moreover, the molecular docking results suggested that strong π–π stacking interaction with W490 considerably contributed to improving the inhibitory activity. Compound **7** may be used as a valuable tool to investigate the role of O-GlcNAcylation in cellular biology.

4. Experimental

4.1. Chemistry

All reagents were purchased from commercial sources and were used without further purification. All solvents were available commercially dried or were freshly dried and distilled prior to use. Reactions were monitored by thin-layer chromatography (TLC) using silica gel GF_{254} plates under detection of short-wave UV fluorescence (λ 254 nm) or by staining with 10% phosphomolybdic acid in EtOH and heating. Column chromatography was conducted using silica gel (200–300 mesh) with EtOAc and petroleum ether (PE, 60–90 °C) or CH_2Cl_2 and MeOH as eluent. ^{1}H NMR spectra were recorded with a Bruker AV 400 spectrometer at 400 MHz, and ^{13}C NMR spectra were recorded with either a Bruker AV 400 spectrometer at 100 MHz or a Bruker AV 300 spectrometer at 75 MHz. Solvents were either $CDCl_3$, DMSO-d_6 or CD_3OD, and chemical shifts

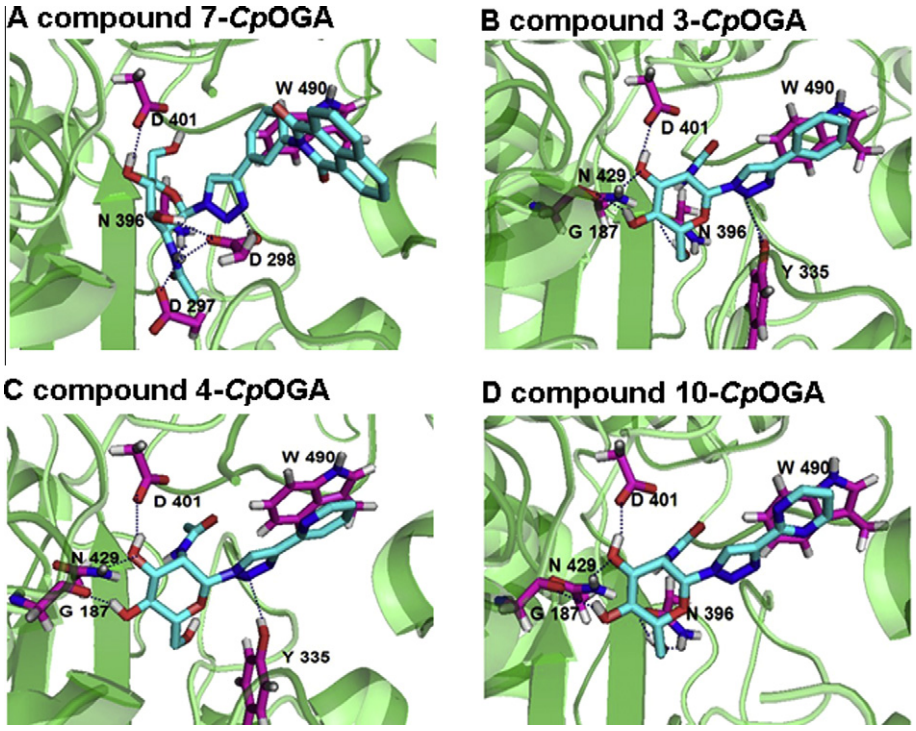


Figure 5. The best docked pose of compounds **7**, **3**, **4,** and **10** against *Cp*OGA structure 2J62.

were reported in δ (ppm) from an internal standard of TMS (δ 0.00). Coupling constants are reported in hertz. High-resolution electrospray-ionization mass spectra (HRESIMS) were obtained on a Varian QFT-ESI mass spectrometer.

4.1.1. 3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl azide (3c)

3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl chloride[36] (4.0 g, 10.9 mmol) was dissolved in CH_2Cl_2 (40 mL), and a solution of sodium azide (2.2 g, 32.7 mmol) in satd aq $NaHCO_3$ (40 mL) was added. Tetrabutylammonium hydrogen sulfate (3.7 g, 10.9 mmol) was added, and the reaction mixture was stirred at room temperature for l h. TLC (2:1 EtOAc–PE) indicated the formation of a product (R_f 0.34) with complete consumption of the starting material (R_f 0.50). The organic layer was washed with water (100 mL), dried over Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by column chromatography (1:1 EtOAc–PE) to yield **3c** (2.4 g, 59%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.99 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.11 (s, 3H), 3.84 (ddd, *J* 2.0 Hz, *J* 4.8 Hz, *J* 10.0 Hz, 1H), 3.91–3.98 (m, 1H), 4.18 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.28 (dd, *J* 4.8 Hz, *J* 12.4 Hz, 1H), 4.83 (d, *J* 9.2 Hz, 1H), 5.11 (t, *J* 9.6 Hz, 1H), 5.30 (t, *J* 9.6 Hz, 1H), 6.24 (d, *J* 9.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.53, 20.58, 20.67, 23.12, 53.95, 61.96, 68.31, 72.16, 73.79, 88.31, 169.33, 170.70, 170.74, 170.80; HRESIMS: calcd for $[C_{14}H_{20}N_4O_8+Na]^+$ *m*/*z* 395.1173, found, *m*/*z* 395.1176.

4.1.2. General procedure for the synthesis of compounds 1b–7b

A mixture of the glycosyl azide (1.0 equiv) and alkyne (1.2 equiv) was suspended in 1:1 *tert*-butyl alcohol–water. A solution of sodium ascorbate (0.4 equiv) in water and $CuSO_4$ (0.2 equiv) in water were successively added. The bright-yellow suspension was stirred vigorously at room temperature until the reaction was completed (detected by TLC). The mixture was evaporated under reduced pressure, and the resultant residue was purified by flash chromatography to yield pure material.

4.1.2.1. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-pentyl-1,2,3-triazole (1b). A white solid (92%, R_f 0.63, 5:1 EtOAc–PE). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.86 (t, *J* 6.8 Hz, 3H), 1.24–1.32 (m, 4H), 1.53–1.60 (m, 5H), 1.95 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 2.58–2.61 (m, 2H), 4.05 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.15 (dd, *J* 4.8 Hz, *J* 12.4 Hz, 1H), 4.22 (ddd, *J* 2.0 Hz, *J* 4.8 Hz, *J* 10.0 Hz, 1H), 4.55–4.62 (m, 1H), 5.08 (t, *J* 10.0 Hz, 1H), 5.33 (t, *J* 10.0 Hz, 1H), 6.03 (d, *J* 10.0 Hz, 1H), 8.01 (s, 1H), 8.05 (d, *J* 9.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.78, 20.21, 20.35, 20.45, 21.80, 22.22, 24.76, 28.34, 30.52, 52.03, 61.78, 68.08, 72.42, 73.31, 84.60, 120.47, 147.03, 169.24, 169.29, 169.53, 169.97; HRESIMS: calcd for $[C_{21}H_{32}N_4O_8+Na]^+$ *m*/*z* 491.2112, found, *m*/*z* 491.2117.

4.1.2.2. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-hydroxymethyl-1,2,3-triazole (2b). A white solid (72%, R_f 0.14, 5:1 EtOAc–PE). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.66 (s, 3H), 2.01 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 4.11 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.20 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.29 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.56 (s, 2H), 4.63–4.70 (m, 1H), 5.16 (t, *J* 9.6 Hz, 1H), 5.41 (t, *J* 10.0 Hz, 1H), 6.15 (d, *J* 10.0 Hz, 1H), 8.15 (d, *J* 9.2 Hz, 1H), 8.19 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.23, 20.36, 20.46, 22.31, 52.03, 54.82, 61.80, 68.03, 72.45, 73.32, 84.59, 121.38, 148.14, 169.30, 169.39, 169.56, 169.99; HRESIMS: calcd for $[C_{17}H_{24}N_4O_9+Na]^+$ *m*/*z* 451.1435, found, *m*/*z* 451.1439.

4.1.2.3. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-phenyl-1,2,3-triazole (3b). A white solid (90%, R_f 0.61, 5:1 EtOAc–PE). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.60 (s, 3H), 1.97 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 4.09 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.18 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.29 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.63–4.71 (m, 1H), 5.12 (t, *J* 10.0 Hz, 1H), 5.40 (t, *J* 10.0 Hz, 1H), 6.15 (d, *J* 10.0 Hz, 1H), 7.37 (t, *J* 7.2 Hz, 1H), 7.46–7.49 (m, 2H), 7.83–7.85 (m, 2H), 8.13 (d, *J* 9.2 Hz, 1H), 8.86 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.73, 20.87, 20.98,

22.77, 52.74, 62.21, 68.47, 72.75, 73.82, 85.36, 120.68, 125.64, 128.65, 129.46, 130.66, 146.95, 169.85, 169.96, 170.06, 170.51; HRESIMS: calcd for $[C_{22}H_{26}N_4O_8+Na]^+$ *m*/*z* 497.1643, found, *m*/*z* 497.1648.

4.1.2.4. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-pyridyl-1,2,3-triazole (4b). A pale-yellow solid (88%, R_f 0.35, 5:1 EtOAc–PE). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.61 (s, 3H), 1.97 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 4.09 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.17 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.27 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.71–4.78 (m, 1H), 5.17 (t, *J* 9.6 Hz, 1H), 5.39 (t, *J* 10.0 Hz, 1H), 6.18 (d, *J* 10.0 Hz, 1H), 7.38 (dd, *J* 4.8 Hz, *J* 6.4 Hz, 1H), 7.90–7.94 (m, 1H), 8.04 (d, *J* 8.0 Hz, 1H), 8.14 (d, *J* 9.2 Hz, 1H), 8.63 (d, *J* 8.0 Hz, 1H), 8.84 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.23, 20.37, 20.46, 22.26, 52.11, 61.83, 68.05, 72.30, 73.46, 84.97, 119.60, 122.08, 123.25, 137.26, 147.34, 149.35, 149.68, 169.33, 169.49, 169.59, 170.02; HRESIMS: calcd for $[C_{21}H_{25}N_5O_8+Na]^+$ *m*/*z* 498.1595, found, *m*/*z* 498.1599.

4.1.2.5. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(phenylcarbamoyl)-1,2,3-triazole (5b). A pale-yellow solid (86%, R_f 0.43, 5:1 CH_2Cl_2–MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.61 (s, 3H), 1.96 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 4.09 (dd, *J* 2.4 Hz, *J* 12.4 Hz, 1H), 4.16 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.27 (ddd, *J* 2.4 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.67–4.74 (m, 1H), 5.16 (t, *J* 10.0 Hz, 1H), 5.38 (t, *J* 10.0 Hz, 1H), 6.19 (d, *J* 10.0 Hz, 1H), 7.11 (t, *J* 7.6 Hz, 1H), 7.32–7.36 (m, 2H), 7.81–7.83 (m, 2H), 8.11 (d, *J* 9.2 Hz, 1H), 8.99 (s, 1H), 10.49 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.26, 20.42, 20.52, 22.28, 52.19, 61.78, 67.95, 72.13, 73.48, 85.12, 120.45, 123.88, 126.44, 128.59, 138.36, 143.08, 157.88, 169.35, 169.52, 169.58, 170.04; HRESIMS: calcd for $[C_{23}H_{27}N_5O_9+H]^+$ *m*/*z* 518.1882, found, *m*/*z* 518.1889.

4.1.2.6. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-((*E*)-3-(4-methoxybenzyloxyamino)-3-oxoprop-1-enyl)-1,2,3-triazole (6b). A white solid (83%, R_f 0.46, EtOAc). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.59 (s, 3H), 1.95 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 3.76 (s, 3H), 4.07 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.16 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.26 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.56–4.64 (m, 1H), 4.80 (s, 2H), 5.10 (t, *J* 10.0 Hz, 1H), 5.35 (t, *J* 10.0 Hz, 1H), 6.11 (d, *J* 9.6 Hz, 1H), 6.57 (d, *J* 16.0 Hz, 1H), 6.93–6.96 (m, 2H), 7.33–7.35 (m, 2H), 7.43 (d, *J* 16.0 Hz, 1H), 8.09 (d, *J* 9.2 Hz, 1H), 8.61 (s, 1H), 11.31 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.25, 20.39, 20.49, 22.25, 52.13, 55.07, 61.68, 67.95, 72.22, 73.38, 76.62, 84.84, 113.70, 119.78, 123.77, 127.73, 127.79, 130.69, 142.94, 159.38, 162.29, 169.33, 169.44, 169.57, 170.01; HRESIMS: calcd for $[C_{27}H_{33}N_5O_{11}+Na]^+$ *m*/*z* 626.2069, found, *m*/*z* 626.2060.

4.1.2.7. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(3-*N*-(1,3-dioxo-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)phenyl)-1,2,3-triazole (7b). A pale-yellow solid (87%, R_f 0.16, PE–EtOAc 1:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.75 (s, 3H), 1.95 (s, 3H), 2.01 (s, 3H), 2.05 (s, 3H), 4.01–4.13 (m, 2H), 4.27 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.49–4.57 (m, 1H), 5.21 (t, *J* 9.6 Hz, 1H), 5.57 (t, *J* 9.6 Hz, 1H), 6.16 (d, *J* 10.0 Hz, 1H), 6.82 (d, *J* 9.2 Hz, 1H), 7.30 (d, *J* 7.6 Hz, 1H), 7.60 (t, *J* 7.6 Hz, 1H), 7.76–7.80 (m, 2H), 7.85–7.89 (m, 2H), 8.13 (s, 1H), 8.24–8.26 (m, 2H), 8.61–8.63 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.56, 20.60, 20.74, 22.86, 53.68, 61.71, 68.04, 72.24, 74.73, 85.62, 119.34, 122.67, 126.13, 126.33, 127.09, 128.50, 128.80, 130.02, 131.31, 131.69, 134.45, 136.17, 147.10, 164.37, 169.40, 170.61, 170.71, 171.10; HRESIMS: calcd for $[C_{34}H_{31}N_5O_{10}+H]^+$ *m*/*z* 670.2144, found, *m*/*z* 670.2138.

4.1.3. General procedure for the synthesis of compounds 8b–16b

A mixture of **8a**–**16a** (1.0 equiv), CuI (0.1 equiv) and $Pd(PPh_3)_4$ (0.05 equiv) in Et_3N and DMF was degassed with Ar. Trimethylsilylacetylene (2.0 equiv) was then added and the solution was stirred for 18 h at room temperature under an Ar atmosphere. TLC analysis showed complete conversion of starting material to a major product. The reaction mixture was then cooled to room temperature, diluted with Et_2O, washed twice with satd aq NH_4Cl, dried over Na_2SO_4, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography to yield pure material.

4.1.3.1. 5-((Trimethylsilyl)ethynyl)uracil (8b). A pale-yellow solid (86%, R_f 0.42, 10:1 CH_2Cl_2–MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.18 (s, 9H), 7.80 (s, 1H), 11.36 (br s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 0.01, 96.56, 96.89, 98.30, 146.77, 150.40, 162.54; HRESIMS: calcd for $[C_9H_{12}N_2O_2Si+H]^+$ *m*/*z* 209.0741, found, *m*/*z* 209.0741.

4.1.3.2. 3-((Trimethylsilyl)ethynyl)-7-azaindole (9b). A pale-yellow solid (85%, R_f 0.33, 2:1 PE–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.29 (s, 9H), 7.19 (dd, *J* 4.8 Hz, *J* 7.6 Hz, 1H), 7.62 (s, 1H), 8.09 (dd, *J* 1.2 Hz, *J* 7.6 Hz, 1H), 8.37 (dd, *J* 1.2 Hz, *J* 4.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 0.38, 95.90, 97.20, 98.03, 116.70, 121.78, 129.10, 129.90, 143.37, 148.03; HRESIMS: calcd for $[C_{12}H_{14}N_2Si+H]^+$ *m*/*z* 215.0999, found, *m*/*z* 215.1001.

4.1.3.3. 2-((Trimethylsilyl)ethynyl)pyrimidine (10b). A pale-yellow solid (89%, R_f 0.54, 3:1 PE–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.28 (s, 9H), 7.23 (t, *J* 5.2 Hz, 1H), 8.71 (d, *J* 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −0.38, 94.60, 102.36, 120.18, 152.67, 157.36; HRESIMS: calcd for $[C_9H_{12}N_2Si+H]^+$ *m*/*z* 177.0843, found, *m*/*z* 177.0843.

4.1.3.4. 2-(1*H*-Pyrazol-1-yl)-6-((trimethylsilyl)ethynyl)pyridine (11b). A brown liquid (87%, R_f 0.38, 15:1 PE–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.30 (s, 9H), 6.45 (s, 1H), 7.35 (d, *J* 7.6 Hz, 1H), 7.73–7.78 (m, 2H), 7.94 (d, *J* 8.4 Hz, 1H), 8.62 (d, *J* 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −0.14, 95.56, 103.32, 107.96, 112.36, 125.36, 127.65, 138.88, 141.30, 142.36, 151.52; HRESIMS: calcd for $[C_{13}H_{15}N_3Si+H]^+$ *m*/*z* 242.1108, found, *m*/*z* 242.1106.

4.1.3.5. 4-((Trimethylsilyl)ethynyl)pyrazole (12b). A white solid (83%, R_f 0.16, 5:1 PE–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.23 (s, 9H), 7.72 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 0.11, 95.70, 96.04, 103.39, 137.12; HRESIMS: calcd for $[C_8H_{12}N_2Si+H]^+$ 165.0843, found, *m*/*z* 165.0842.

4.1.3.6. 3′,5′-Di-*O*-acetyl-2′-deoxy-5-((trimethylsilyl)ethynyl)-uridine (13b). A white solid (86%, R_f 0.56, 1:2 PE–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.21 (s, 9H), 2.11 (s, 3H), 2.16–2.23 (m, 4H), 2.52 (ddd, *J* 2.4 Hz, *J* 5.6 Hz, *J* 14.4 Hz, 1H), 4.28 (dd, *J* 2.4 Hz, *J* 5.6 Hz, 1H), 4.36–4.37 (m, 2H), 5.23–5.25 (m, 1H), 6.32 (dd, *J* 6.0 Hz, *J* 8.0 Hz, 1H), 7.83 (s, 1H), 8.86 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −0.07, 21.02, 21.07, 38.38, 63.97, 74.14, 82.71, 85.43, 95.10, 100.07, 101.10, 142.27, 149.18, 160.84, 170.18, 170.53; HRESIMS: calcd for $[C_{18}H_{24}N_2O_7Si+H]^+$ *m*/*z* 409.1426, found, *m*/*z* 409.1428.

4.1.3.7. 2-((Trimethylsilyl)ethynyl)pyrazine (14b). A brown liquid (85%, R_f 0.26, 20:1 PE–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.27 (s, 9H), 8.46 (d, *J* 2.4 Hz, 1H), 8.52 (dd, *J* 1.6 Hz, *J* 2.4 Hz, 1H), 8.66 (d, *J* 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −0.33, 99.99, 100.72, 140.03, 143.16, 144.44, 148.01; HRESIMS: calcd for $[C_9H_{12}N_2Si+H]^+$ *m*/*z* 177.0843, found, *m*/*z* 177.0840.

4.1.3.8. 3-((Trimethylsilyl)ethynyl)quinoline (15b). A brown liquid (87%, R_f 0.33, 30:1 PE–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.30 (s, 9H), 7.53–7.57 (m, 1H), 7.68–7.76 (m, 2H), 8.07 (d, *J* 8.4 Hz, 1H), 8.25 (d, *J* 1.6 Hz, 1H), 8.91 (d, *J* 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −0.16, 98.22, 102.00, 117.25, 127.05, 127.25, 127.57, 129.30, 130.15, 138.92, 146.76, 152.22; HRESIMS: calcd for $[C_{14}H_{15}NSi+H]^+$ *m*/*z* 226.1047, found, *m*/*z* 226.1046.

4.1.3.9. 2-((Trimethylsilyl)ethynyl)thiazole (16b). A brown liquid (87%, R_f 0.38, 30:1 PE–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.27 (s, 9H), 7.32 (d, *J* 3.2 Hz, 1H), 7.79 (d, *J* 3.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −0.38, 96.54, 100.93, 120.83, 143.50, 148.68; HRESIMS: calcd for $[C_8H_{11}NSSi+H]^+$ *m*/*z* 182.0454, found, *m*/*z* 182.0453.

4.1.4. General procedure for the synthesis of compounds 8c–16c

To a stirred solution of alkyne precursor **8b–16b** (1.2 equiv) in THF was added dropwise a 1.0 M solution of TBAF in THF (1.44 equiv). The reaction mixture was stirred at room temperature for about 15 min. TLC analysis showed complete conversion of starting material to a major product (the de-silylation intermediate was slightly more polar than the alkyne precursor). The glycosyl azide (1.0 equiv), and 1:1 *tert*-butyl alcohol–water were then added to the above solution. A solution of sodium ascorbate (0.4 equiv) in water, followed by $CuSO_4$ (0.2 equiv) in water, was successively added. The bright-yellow suspension was stirred vigorously at room temperature overnight. TLC analysis showed complete conversion of the starting material to a major product. The mixture was evaporated under reduced pressure, and the resultant residue was purified by flash chromatography to yield pure material.

4.1.4.1. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(5-uracilyl)-1,2,3-triazole (8c). A white solid (89%, R_f 0.31, 10:1 CH_2Cl_2–MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.59 (s, 3H), 1.95 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 4.06 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.17 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.23 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 9.6 Hz, 1H), 4.60–4.67 (m, 1H), 5.16 (t, *J* 9.6 Hz, 1H), 5.35 (t, *J* 9.6 Hz, 1H), 6.14 (d, *J* 10.0 Hz, 1H), 8.06 (s, 1H), 8.11 (d, *J* 9.2 Hz, 1H), 8.49 (s, 1H), 11.25 (br s, 1H), 11.46 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.27, 20.41, 20.50, 22.30, 51.93, 61.88, 67.97, 72.42, 73.38, 84.69, 103.33, 120.53, 138.01, 139.31, 150.52, 162.02, 169.30, 169.36, 169.61, 170.05; HRESIMS: calcd for $[C_{20}H_{24}N_6O_{10}+H]^+$ *m*/*z* 509.1627, found, *m*/*z* 509.1616.

4.1.4.2. 4-(3-(7-Azaindolyl))-1-(2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-1,2,3-triazole (9c). A pale-yellow solid (92%, R_f 0.13, 1:5 PE–EtOAc). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.60 (s, 3H), 1.97 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 4.08–4.24 (m, 2H), 4.28 (dd, *J* 3.2 Hz, *J* 9.6 Hz, 1H), 4.69–4.77 (m, 1H), 5.14 (t, *J* 9.6 Hz, 1H), 5.41 (t, *J* 9.6 Hz, 1H), 6.17 (d, *J* 10.0 Hz, 1H), 7.18 (dd, *J* 4.4 Hz, *J* 7.6 Hz, 1H), 7.93 (s, 1H), 8.12 (d, *J* 9.2 Hz, 1H), 8.30 (d, *J* 4.4 Hz, 1H), 8.41 (d, *J* 7.6 Hz, 1H), 8.74 (s, 1H), 11.94 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.27, 20.42, 20.52, 22.34, 52.22, 61.79, 68.02, 72.35, 73.27, 84.77, 104.63, 116.01, 116.83, 118.29, 123.42, 128.08, 142.02, 143.27, 148.55, 169.39, 169.48, 169.58, 170.04; HRESIMS: calcd for $[C_{23}H_{26}N_6O_8+H]^+$ *m*/*z* 515.1885, found, *m*/*z* 515.1892.

4.1.4.3. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(2-pyrimidyl)-1,2,3-triazole (10c). A pale-yellow solid (89%, R_f 0.37, 20:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.60 (s, 3H), 1.97 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 4.09 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.17 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.27 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.73–4.80 (m, 1H), 5.18 (t, *J* 10.0 Hz, 1H), 5.38 (t, *J* 10.0 Hz, 1H), 6.18 (d, *J* 10.0 Hz, 1H), 7.48 (t, *J* 4.8 Hz, 1H), 8.13 (d, *J* 9.2 Hz, 1H), 8.91 (d, *J* 4.8 Hz, 2H), 9.01 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.27, 20.42, 20.51, 22.27, 52.02, 61.86, 67.97, 72.22, 73.41, 84.99, 120.36, 125.30, 146.55, 157.82, 158.17, 169.36, 169.46, 169.60, 170.05; HRESIMS: calcd for $[C_{20}H_{24}N_6O_8+H]^+$ *m*/*z* 477.1728, found, *m*/*z* 477.1720.

4.1.4.4. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(2-(1*H*-pyrazol-1-yl)-6-pyridyl)-1,2,3-triazole (11c). A pale-yellow solid (84%, R_f 0.46, 1:4 PE–EtOAc). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.61 (s, 3H), 1.98 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 4.10 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.20 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.32 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.67–4.75 (m, 1H), 5.17 (t, *J* 10.0 Hz, 1H), 5.44 (t, *J* 10.0 Hz, 1H), 6.23 (d, *J* 9.6 Hz, 1H), 6.66 (t, *J* 2.0 Hz, 1H), 7.88–7.90 (m, 2H), 7.95 (d, *J* 7.6 Hz, 1H), 8.10 (d, *J* 7.6 Hz, 1H), 8.17 (d, *J* 9.2 Hz, 1H), 8.87 (d, *J* 2.8 Hz, 1H), 9.20 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.26, 20.42, 20.53, 22.31, 52.47, 61.80, 67.98, 72.17, 73.37, 84.93, 108.16, 110.86, 116.96, 123.08, 127.21, 140.73, 142.45, 146.84, 148.06, 150.57, 169.40, 169.57, 170.04; HRESIMS: calcd for $[C_{24}H_{27}N_7O_8+H]^+$ *m*/*z* 542.1994, found, *m*/*z* 542.1994.

4.1.4.5. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(4-pyrazolyl)-1,2,3-triazole (12c). A white solid (91%, R_f 0.47, 10:1 CH_2Cl_2–MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.60 (s, 3H), 1.96 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 4.07 (dd, *J* 1.6 Hz, *J* 12.4 Hz, 1H), 4.17 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.27 (ddd, *J* 1.6 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.55–4.62 (m, 1H), 5.10 (t, *J* 9.6 Hz, 1H), 5.38 (t, *J* 10.0 Hz, 1H), 6.12 (d, *J* 10.0 Hz, 1H), 7.83 (s, 1H), 8.11–8.13 (m, 2H), 8.46 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.27, 20.40, 20.51, 22.31, 52.25, 61.77, 68.00, 72.33, 73.32, 84.75, 111.47, 118.64, 125.75, 140.50, 169.34, 169.42, 169.57, 170.02; HRESIMS: calcd for $[C_{19}H_{24}N_6O_8+H]^+$ *m*/*z* 465.1728, found, *m*/*z* 465.1727.

4.1.4.6. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(5-(2′-deoxy-3′,5′-di-*O*-acetyl-uridyl))-1,2,3-triazole (13c). A white solid (92%, R_f 0.36, 25:1 CH_2Cl_2–MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.58 (s, 3H), 1.95 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 2.38–2.47 (m, 2H), 4.06 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.15–4.31 (m, 5H), 4.61–4.69 (m, 1H), 5.17 (t, *J* 10.0 Hz, 1H), 5.24–5.25 (m, 1H), 5.34 (t, *J* 10.0 Hz, 1H), 6.15 (d, *J* 9.6 Hz, 1H), 6.25 (t, *J* 6.8 Hz, 1H), 8.09 (d, *J* 9.2 Hz, 1H), 8.43 (s, 1H), 8.55 (br s, 1H), 11.81 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.26, 20.40, 20.50, 20.53, 20.75, 22.23, 36.87, 51.95, 61.90, 63.70, 68.00, 72.41, 73.43, 74.16, 81.72, 84.82, 85.00, 105.06, 120.97, 135.55, 138.88, 149.48, 160.93, 169.33, 169.59, 170.04, 170.32; HRESIMS: calcd for $[C_{29}H_{36}N_6O_{15}+H]^+$ *m*/*z* 709.2311, found, *m*/*z* 709.2312.

4.1.4.7. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(2-pyrazyl)-1,2,3-triazole (14c). A white solid (88%, R_f 0.32, 1:4 PE–EtOAc). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.61 (s, 3H), 1.97 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 4.09 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.17 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.29 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.73–4.80 (m, 1H), 5.18 (t, *J* 10.0 Hz, 1H), 5.39 (t, *J* 10.0 Hz, 1H), 6.21 (d, *J* 10.0 Hz, 1H), 8.14 (d, *J* 9.2 Hz, 1H), 8.65 (d, *J* 2.4 Hz, 1H), 8.72 (dd, *J* 1.6 Hz, *J* 2.4 Hz, 1H), 9.03 (s, 1H), 9.25 (d, *J* 1.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.26, 20.42, 20.50, 22.28, 52.05, 61.81, 67.95, 72.21, 73.41, 85.04, 123.34, 141.13, 144.13, 144.65, 144.90, 145.08, 169.36, 169.50, 169.59, 170.04; HRESIMS: calcd for $[C_{20}H_{24}N_6O_8+H]^+$ *m*/*z* 477.1728, found, *m*/*z* 477.1718.

4.1.4.8. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(3-quinolyl)-1,2,3-triazole (15c). A white solid (86%, R_f 0.41, 20:1 CH_2Cl_2–MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.62 (s, 3H), 1.98 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 4.11 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.21 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.35 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.64–4.71 (m, 1H), 5.14 (t, *J* 10.0 Hz, 1H), 5.44 (t, *J* 10.0 Hz, 1H), 6.23 (d, *J* 10.0 Hz, 1H), 7.65–7.69 (m, 1H), 7.77–7.82 (m, 1H), 8.06–8.10 (m, 2H), 8.17 (d, *J* 9.2 Hz, 1H), 8.85 (d, *J* 2.0 Hz, 1H), 9.16 (s, 1H), 9.37 (d, *J* 2.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.28, 20.41, 20.52, 22.30, 52.46, 61.73, 68.00, 72.19, 73.41, 85.00, 121.25, 123.47, 127.32, 127.48, 128.37, 128.81, 129.77, 131.12, 143.95, 147.16, 148.02, 169.37, 169.55, 169.58, 170.03; HRESIMS: calcd for $[C_{25}H_{27}N_5O_8+H]^+$ *m*/*z* 526.1932, found, *m*/*z* 526.1936.

4.1.4.9. 1-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-4-(2-thiazolyl)-1,2,3-triazole (16c). A white solid (88%, R_f 0.52, 20:1 CH_2Cl_2–MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.61 (s, 3H), 1.97 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 4.09 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.15 (dd, *J* 5.2 Hz, *J* 12.4 Hz, 1H), 4.27 (ddd, *J* 2.0 Hz, *J* 5.2 Hz, *J* 10.0 Hz, 1H), 4.73–4.80 (m, 1H), 5.17 (t, *J* 10.0 Hz, 1H), 5.38 (t, *J* 10.0 Hz, 1H), 6.18 (d, *J* 10.0 Hz, 1H), 7.81 (d, *J* 3.2 Hz, 1H), 7.96 (d, *J* 3.2 Hz, 1H), 8.13 (d, *J* 9.2 Hz, 1H), 8.97 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.26, 20.42, 20.50, 22.28, 52.03, 61.78, 67.92, 72.17, 73.41, 85.11, 120.29, 121.31, 142.34, 143.68, 157.91, 169.36, 169.51, 169.59, 170.03; HRESIMS: calcd for $[C_{19}H_{23}N_5O_8S+H]^+$ *m*/*z* 482.1340, found, *m*/*z* 482.1336.

4.1.5. General procedure for synthesis of compounds 1–16

Compounds **1**–**16** were prepared by treating the precursor **1b**–**7b** and **8c**–**16c** (final concentration 0.1–0.2 M) with NaOMe in dry MeOH or dry MeOH and CH_2Cl_2 (final pH 9–10). Reactions were complete within 30 min as detected by TLC. The solution was neutralized by Dowex-50 (H^+), followed by filtration and evaporation to afford compounds **1**–**16**.

4.1.5.1. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-pentyl-1,2,3-triazole (1). A white solid (96%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.86 (t, *J* 6.8 Hz, 3H), 1.21–1.33 (m, 4H), 1.52–1.57 (m, 2H), 1.61 (s, 3H), 2.56–2.60 (m, 2H), 3.25 (t, *J* 9.2 Hz, 1H), 3.40–3.55 (m, 3H), 3.70 (dd, *J* 4.8 Hz, *J* 10.8 Hz, 1H), 4.04 (t, *J* 10.0 Hz, 1H), 5.63 (d, *J* 9.6 Hz, 1H), 7.83 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.34, 22.31, 23.08, 25.28, 28.96, 31.04, 54.72, 61.08, 70.34, 74.35, 80.44, 86.31, 120.69, 147.07, 169.40; HRESIMS: calcd for $[C_{15}H_{26}N_4O_5+Na]^+$ *m*/*z* 365.1795, found, *m*/*z* 365.1790.

4.1.5.2. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-hydroxymethyl-1,2,3-triazole (2). A white solid (92%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.64 (s, 3H), 3.27 (t, *J* 9.2 Hz, 1H), 3.52–3.58 (m, 1H), 3.70 (dd, *J* 4.8 Hz, *J* 11.2 Hz, 1H), 4.06 (t, *J* 10.0 Hz, 1H), 4.89 (s, 2H), 5.22–5.29 (m, 2H), 5.69 (d, *J* 10.0 Hz, 1H), 7.97 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.70, 54.41, 54.89, 60.69, 69.93, 74.01, 79.95, 85.87, 121.11, 147.68, 169.16; HRESIMS: calcd for $[C_{11}H_{18}N_4O_6+Na]^+$ *m*/*z* 325.1119, found, *m*/*z* 325.1111.

4.1.5.3. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-phenyl-1,2,3-triazole (3). A white solid (94%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.62 (s, 3H), 3.30 (t, *J* 9.2 Hz, 1H), 3.46–3.52 (m, 2H), 3.60 (t, *J* 9.2 Hz, 1H), 3.73 (dd, *J* 4.8 Hz, *J* 10.4 Hz, 1H), 4.09–4.16 (m, 1H), 5.74 (d, *J* 10.0 Hz, 1H), 7.34 (t, *J* 7.2 Hz, 1H), 7.43–7.47 (m, 2H), 7.84–7.86 (m, 2H), 8.67 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.15, 55.21, 61.15, 70.36, 74.20, 80.51, 86.67, 120.50, 125.63, 128.45, 129.38, 130.96, 146.56, 169.69; HRESIMS: calcd for $[C_{16}H_{20}N_4O_5+Na]^+$ *m*/*z* 371.1326, found, *m*/*z* 371.1318.

4.1.5.4. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-pyridyl-1,2,3-triazole (4). A pale-yellow solid (93%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.77 (s, 3H), 3.58–3.68 (m, 2H), 3.74–3.82 (m, 2H), 3.94 (dd, *J* 2.0 Hz, *J* 12.0 Hz, 1H), 4.25 (t, *J* 10.0 Hz, 1H), 5.88 (d, *J* 9.6 Hz, 1H), 7.50 (t, *J* 6.0 Hz, 1H), 8.04–8.13 (m, 2H), 8.58 (s, 1H), 8.88 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 22.62, 54.71, 60.64, 69.73, 73.69, 79.98, 86.30, 119.89, 122.10, 123.33, 138.06, 146.25, 148.97, 169.28; HRESIMS: calcd for $[C_{15}H_{19}N_5O_5+Na]^+$ *m*/*z* 372.1278, found, *m*/*z* 372.1273.

4.1.5.5. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(phenylcarbamoyl)-1,2,3-triazole (5). A white solid (92%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.70 (s, 3H), 3.47–3.56 (m, 2H), 3.62–3.71 (m, 2H), 3.82 (dd, *J* 1.6 Hz, *J* 12.0 Hz, 1H), 4.15 (t, *J* 9.6 Hz, 1H), 5.77 (d, *J* 9.6 Hz, 1H), 7.05 (t, *J* 7.6 Hz, 1H), 7.24–7.28 (m, 2H), 7.60–7.62 (m, 2H), 8.63 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 22.51, 57.03, 62.30, 71.30, 75.46, 81.39, 88.50, 121.96, 125.78, 126.72, 129.90, 139.11, 144.28, 160.31, 170.25, 173.66; HRESIMS: calcd for $[C_{17}H_{21}N_5O_6-H]^-$ *m*/*z* 390.1419, found, *m*/*z* 390.1424.

4.1.5.6. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-((*E*)-3-(4-methoxybenzyloxyamino)-3-oxoprop-1-enyl)-1,2,3-triazole (6). A white solid (94%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.68 (s, 3H), 3.43–3.52 (m, 2H), 3.58–3.67 (m, 2H), 3.70 (s, 3H), 3.80 (dd, *J* 1.2 Hz, *J* 12.0 Hz, 1H), 4.11 (t, *J* 10.0 Hz, 1H), 4.74 (s, 2H), 5.69 (d, *J* 10.0 Hz, 1H), 6.47 (d, *J* 15.6 Hz, 1H), 6.82–6.84 (m, 2H), 7.26–7.28 (m, 2H), 7.45 (d, *J* 15.6 Hz, 1H), 8.30 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 22.50, 55.74, 56.89, 62.30, 71.33, 75.58, 78.88, 81.32, 88.29, 114.88, 120.26, 124.52, 128.90, 130.05, 132.16, 144.74, 161.65, 165.49, 173.56; HRESIMS: calcd for $[C_{21}H_{27}N_5O_8+Na]^+$ *m*/*z* 500.1752, found, *m*/*z* 500.1745.

4.1.5.7. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(3-*N*-(1,3-dioxo-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)phenyl)-1,2,3-triazole (7). A pale-yellow solid (92%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.63 (s, 3H), 3.30 (t, *J* 8.8 Hz, 1H), 3.47–3.50 (m, 2H), 3.55–3.66 (m, 2H), 4.09 (t, *J* 10.0 Hz, 1H), 5.73 (d, *J* 10.0 Hz, 1H), 7.35 (d, *J* 7.6 Hz, 1H), 7.61 (t, *J* 7.6 Hz, 1H), 7.86–7.94 (m, 4H), 8.48–8.52 (m, 4H), 8.69 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.45, 54.70, 60.48, 69.62, 73.47, 79.72, 86.16, 120.33, 122.31, 125.00, 125.79, 127.28, 127.75, 128.72, 129.72, 130.91, 131.20, 131.38, 131.56, 134.62, 136.58, 145.55, 163.79, 169.69; HRESIMS: calcd for $[C_{28}H_{25}N_5O_7+H]^+$ *m*/*z* 544.1827, found, *m*/*z* 544.1824.

4.1.5.8. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(5-uracilyl)-1,2,3-triazole (8). A white solid (91%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.68 (s, 3H), 3.46–3.53 (m, 2H), 3.60–3.69 (m, 2H), 3.82 (dd, *J* 2.0 Hz, *J* 12.4 Hz, 1H), 4.13 (t, *J* 10.0 Hz, 1H), 5.72 (d, *J* 10.0 Hz, 1H), 8.00 (s, 1H), 8.41 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 22.53, 57.02, 62.47, 71.44, 75.67, 81.37, 88.20, 105.78, 122.43, 139.19, 140.79, 152.80, 163.87, 173.54; HRESIMS: calcd for $[C_{14}H_{18}N_6O_7+H]^+$ *m*/*z* 383.1310, found, *m*/*z* 383.1305.

4.1.5.9. 4-(3-(7-Azaindolyl))-1-(2′-acetamido-2′-deoxy-β-D-glucopyranosyl)-1,2,3-triazole (9). A white solid (90%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.70 (s, 3H), 3.50–3.56 (m, 2H), 3.62–3.73 (m, 2H), 3.83 (dd, *J* 2.0 Hz, *J* 11.6 Hz, 1H), 4.26 (t, *J* 9.6 Hz, 1H), 5.76 (d, *J* 9.6 Hz, 1H), 7.13 (dd, *J* 4.8 Hz, *J* 8.0 Hz, 1H), 7.75 (s, 1H), 8.16 (d, *J* 4.4 Hz, 1H), 8.34 (d, *J* 7.6 Hz, 1H), 8.41 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 22.60, 56.76, 62.29, 71.33, 75.81, 81.25, 88.30, 106.62, 117.43, 119.73, 119.82, 124.90, 130.63, 143.47, 143.64, 149.03, 173.63; HRESIMS: calcd for $[C_{17}H_{20}N_6O_5+H]^+$ *m*/*z* 389.1568, found, *m*/*z* 389.1570.

4.1.5.10. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(2-pyrimidyl)-1,2,3-triazole (10). A white solid (95%). ^{1}H NMR

(400 MHz, CD_3OD): δ 1.69 (s, 3H), 3.48–3.56 (m, 2H), 3.62–3.71 (m, 2H), 3.83 (dd, *J* 2.0 Hz, *J* 12.0 Hz, 1H), 4.19 (t, *J* 9.6 Hz, 1H), 5.78 (d, *J* 9.6 Hz, 1H), 7.30 (t, *J* 4.8 Hz, 1H), 8.73–8.75 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 22.50, 57.00, 62.36, 71.35, 75.61, 81.42, 88.42, 121.52, 125.99, 147.87, 158.95, 159.79, 173.60; HRESIMS: calcd for $[C_{14}H_{18}N_6O_5+H]^+$ *m*/*z* 351.1411, found, *m*/*z* 351.1409.

4.1.5.11. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(2-(1*H*-pyrazol-1-yl)-6-pyridyl)-1,2,3-triazole (11). A white solid (93%). ^1H NMR (400 MHz, DMSO-d_6): δ 1.63 (s, 3H), 3.34 (t, *J* 8.8 Hz, 1H), 3.53–3.79 (m, 3H), 4.10–4.22 (m, 2H), 5.80 (d, *J* 9.6 Hz, 1H), 6.62 (s, 1H), 7.85–7.87 (m, 2H), 7.92–7.98 (m, 2H), 8.07 (t, *J* 7.6 Hz, 1H), 8.88 (s, 1H), 8.96 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.66, 55.01, 60.77, 69.94, 73.64, 80.10, 86.31, 108.13, 110.69, 116.95, 122.81, 127.44, 140.59, 142.37, 146.39, 148.38, 150.60, 169.24; HRESIMS: calcd for $[C_{18}H_{21}N_7O_5+H]^+$ *m*/*z* 416.1677, found, *m*/*z* 416.1677.

4.1.5.12. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(4-pyrazolyl)-1,2,3-triazole (12). A white solid (96%). ^1H NMR (400 MHz, CD_3OD): δ 1.69 (s, 3H), 3.45–3.53 (m, 2H), 3.60–3.70 (m, 2H), 3.82 (dd, *J* 2.0 Hz, *J* 12.0 Hz, 1H), 4.16 (t, *J* 10.0 Hz, 1H), 5.71 (d, *J* 9.6 Hz, 1H), 7.88 (s, 2H), 8.23 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 22.55, 56.89, 62.38, 71.41, 75.72, 81.31, 88.29, 120.17, 132.71, 141.94, 173.60; HRESIMS: calcd for $[C_{13}H_{18}N_6O_5+H]^+$ *m*/*z* 339.1411, found, *m*/*z* 339.1407.

4.1.5.13. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(5-(2′-deoxyuridyl))-1,2,3-triazole (13). A white solid (90%). ^1H NMR (400 MHz, CD_3OD): δ 1.68 (s, 3H), 2.14–2.27 (m, 2H), 3.46–3.52 (m, 2H), 3.60–3.68 (m, 3H), 3.74 (dd, *J* 3.2 Hz, *J* 12.0 Hz, 1H), 3.80–3.87 (m, 2H), 4.12 (t, *J* 10.0 Hz, 1H), 4.32–4.35 (m, 1H), 5.72 (d, *J* 9.6 Hz, 1H), 6.25 (t, *J* 6.8 Hz, 1H), 8.42 (s, 1H), 8.53 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 22.54, 41.55, 57.00, 62.46, 62.96, 71.43, 72.34, 75.69, 81.38, 86.91, 88.24, 89.12, 106.57, 122.72, 138.05, 140.78, 151.54, 162.94, 173.51; HRESIMS: calcd for $[C_{19}H_{26}N_6O_{10}+H]^+$ *m*/*z* 499.1783, found, *m*/*z* 499.1780.

4.1.5.14. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(2-pyrazyl)-1,2,3-triazole (14). A white solid (95%). ^1H NMR (400 MHz, DMSO-d_6): δ 1.63 (s, 3H), 3.48–3.63 (m, 3H), 3.73 (dd, *J* 4.8 Hz, *J* 10.4 Hz, 1H), 4.10–4.17 (m, 1H), 4.66 (t, *J* 9.6 Hz, 1H), 5.79 (d, *J* 9.6 Hz, 1H), 7.93 (d, *J* 8.8 Hz, 1H), 8.63 (d, *J* 2.0 Hz, 1H), 8.70 (s, 1H), 8.83 (s, 1H), 9.25 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 22.63, 54.70, 60.65, 69.71, 73.67, 80.03, 86.35, 123.05, 141.11, 143.96, 144.44, 144.60, 145.31, 169.26; HRESIMS: calcd for $[C_{14}H_{18}N_6O_5+H]^+$ *m*/*z* 351.1411, found, *m*/*z* 351.1414.

4.1.5.15. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(3-quinolyl)-1,2,3-triazole (15). A white solid (94%). ^1H NMR (400 MHz, CD_3OD): δ 1.71 (s, 3H), 3.50–3.57 (m, 2H), 3.63–3.74 (m, 2H), 3.74 (dd, *J* 1.2 Hz, *J* 12.4 Hz, 1H), 4.22 (t, *J* 10.0 Hz, 1H), 5.78 (d, *J* 10.0 Hz, 1H), 7.56 (t, *J* 7.2 Hz, 1H), 7.69 (t, *J* 7.2 Hz, 1H), 7.90–7.96 (m, 2H), 8.66 (d, *J* 1.6 Hz, 1H), 8.75 (s, 1H), 9.23 (d, *J* 1.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 22.55, 57.05, 62.36, 71.38, 75.63, 81.35, 88.51, 122.13, 125.41, 128.89, 128.97, 129.53, 129.55, 131.52, 134.03, 145.61, 148.06, 148.96, 173.64; HRESIMS: calcd for $[C_{19}H_{21}N_5O_5+H]^+$ *m*/*z* 400.1615, found, *m*/*z* 400.1622.

4.1.5.16. 1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)-4-(2-thiazolyl)-1,2,3-triazole (16). A white solid (96%). ^1H NMR (400 MHz, CD_3OD): δ 1.70 (s, 3H), 3.47–3.53 (m, 2H), 3.61–3.71 (m, 2H), 3.84 (dd, *J* 1.6 Hz, *J* 12.4 Hz, 1H), 4.15 (t, *J* 10.0 Hz, 1H), 5.77 (d, *J* 10.0 Hz, 1H), 7.54 (d, *J* 2.4 Hz, 1H), 7.78 (d, *J* 2.4 Hz, 1H), 8.56 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 22.48, 57.09, 62.36, 71.32, 75.53, 81.43, 88.49, 120.87, 122.12, 143.83, 144.29, 160.37, 173.63; HRESIMS: calcd for $[C_{13}H_{17}N_5O_5S+Na]^+$ *m*/*z* 378.0843, found, *m*/*z* 378.0837.

4.2. Enzymatic assays

4.2.1. Cloning, expression, and purification of OGA[44,45]

The OGA gene was amplified using human OGA cDNA (GenBank accession number AB014579, provided by Dr. Hart) as template. The following primers were used for the amplification: 5′CGCGCGGCCGCGTGCAGAAGGAGAGTCAA3′ (*NcoI* underlined) and 5′GCGCTCGAGTTAATCTTCACT GTCAGTCATC3′ (*XhoI* underlined). The PCR product was digested with *NcoI* and *XhoI*, and then inserted into vector pET28a digested with corresponding restriction sites. The resultant construction was checked by DNA sequencing and transformed into *Escherichia coli* BL21 (DE3) for protein expression. *E. coli* BL21 (DE3) carrying the plasmid was grown at 37 °C in LB medium containing kanamycin. When the optical density (OD_{600}) of the culture reached 0.6–0.7, isopropyl 1-thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.015 mM, and the culture was allowed to grow for another 10 h at 13 °C, 110 rpm. Cells were harvested and resuspended with lysis buffer (50 mM phosphate buffer, pH 8.0, 300 mM NaCl, 0.5 mg/mL lysozyme, 1 mM PMSF) to 5% of their original volume. After brief sonication, the lysate was centrifuged (12,000*g*, 45 min, 4 °C) and passed through a 0.45 mm filter. The filtrate was then loaded onto Ni^{2+}–NTA (nickel–nitrilotriacetic acid) column (Qiagen), and the resin was washed with washing buffer (phosphate buffer containing 30 mM imidazole). Target proteins were eluted with Elute buffer (phosphate buffer containing 100 mM imidazole). The proteins were concentrated and desalted against 50 mM Tris–HCl buffer (pH 7.0) containing 1 mM dithiothreitol (DTT), 10% glycerol and 150 mM NaCl. The protein concentration was determined by the Bradford method,[46] and the purity was determined by SDS–PAGE.[47]

4.2.2. Activity assays of OGA

The reaction system (25 μL, pH 6.5) contained 50 mM NaH_2PO_4, 100 mM NaCl, 0.1% BSA, fluorescent substrate 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside (concentrations used: 0.15625, 0.3125, 0.625, 1.25, 2.5, 5.0 mM), compound **7** (concentrations used: 0, 50, 75, 100 μM) and 5 μL of purified OGA. Reactions were incubated at 37 °C for 4 min and terminated by the addition of 150 μL of quenching buffer (200 mM glycine–NaOH, pH 10.8). The assay solution (150 μL) was then transferred into a 96-well plate and the fluorescence was measured on a microplate reader (excitation, 368 nm; emission, 450 nm). Inhibition constants (K_i) were determined as previously reported.[48] Hexosaminidases A from human placenta (Sigma, A56152) assay and percent inhibition assays of OGA toward compounds **1**–**16** were measured as described above.

4.3. Docking

Computational docking of compounds **3**, **4**, **7,** and **10** against C*p*OGA structure 2J62 was performed by Autodock 4.2.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.03.026.

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