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Synthesis and biological activity of glycosyl-1*H*-1,2,3-triazoles

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ABSTRACT

Glycosyl 1,2,3-triazoles with α-D-*gluco*, β-D-*gluco*, α-D-*galacto*, β-D-*galacto* and β-2-acetamido-2-deoxy-*gluco* (GlcNAc) stereochemistry were prepared by reaction of the corresponding azides with vinyl acetate under microwave irradiation. The deprotected glucosyl and galactosyl triazoles did not display inhibitory activity against the tested glycosidases at 1 mM. Of the four fungal glycosidases evaluated, GlcNAc-triazole was found to be hydrolyzed by *Talaromyces flavus* CCF 2686 β-*N*-acetylhexosaminidase. β-GlcNAc-triazole was furthermore established to act as a strong ligand of rat and human natural killer cell activating receptors.

The Cu(I)-catalyzed alkyne-azide cycloaddition has become widely used reaction for efficient conjugation of two reaction partners,[1,2] for example, a saccharide with an aglycone[3] or even a protein.[4] This has produced interesting, nitrogen-displaying carbohydrate mimics, which have been studied due to their potential use as medicinal agents (Fig. 1).[5,6] The effect of C-unsubstituted glycosyl triazoles, that is, the 1,2,3-triazole moiety itself, however, has not been studied with the exception of xylosyl triazoles.[7]

In this Letter, we report the first synthesis of five different C-unsubstituted glycosyl triazoles (**7**–**9**, **14**–**15**, Scheme 1), their deacetylated equivalents (**16**–**20**), and our investigations of these N-glycosides as potential inhibitors or substrates of glycosidases. We also report on the NK cell activation receptor interaction with the β-GlcNAc-1,2,3-triazole (**18**).

The convenient and direct synthesis of isomerically pure C-unsubstituted 1,2,3-triazoles by treatment of an azide with vinyl acetate under microwave (μw) irradiation was recently reported by us.[8] Although the established protocol was easy to carry out and typically provided high product yields, it was not clear whether potentially sensitive glycosyl azide would withstand the reaction conditions.

β-Glycosyl azides (**4**–**6**) were prepared as described by Roy and co-workers,[9] while α-glycosyl azides (**12** and **13**) were prepared by the treatment of the corresponding β-glycosyl chloride[10] following a protocol similar to that published by DeSong and co-workers.[11,12]

Each glycosyl azide (**4**–**6**, **12**–**13**) was dissolved in vinyl acetate and heated under microwave irradiation at 120 °C until full conversion resulted as judged by TLC analysis. Interestingly, β-configured azides **4** and **5** reacted significantly faster than their corresponding α-configured counterparts (**12** and **13**). An identical trend was recently observed and explained by Field and co-workers for the Cu(I)-catalyzed reaction of O-acetylated glycosyl azides with terminal alkynes.[13]

All glycosyl triazoles were treated under standard Zemplén conditions to give the desired deprotected compounds **16**–**20** in quantitative yield (Scheme 2).

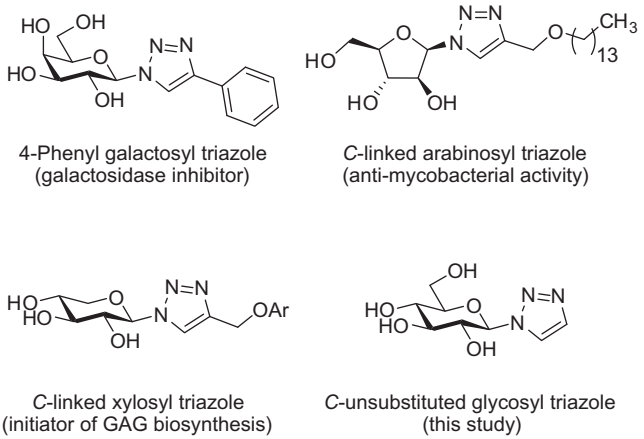

Figure 1. Examples of triazole-linked saccharides.[5–7]

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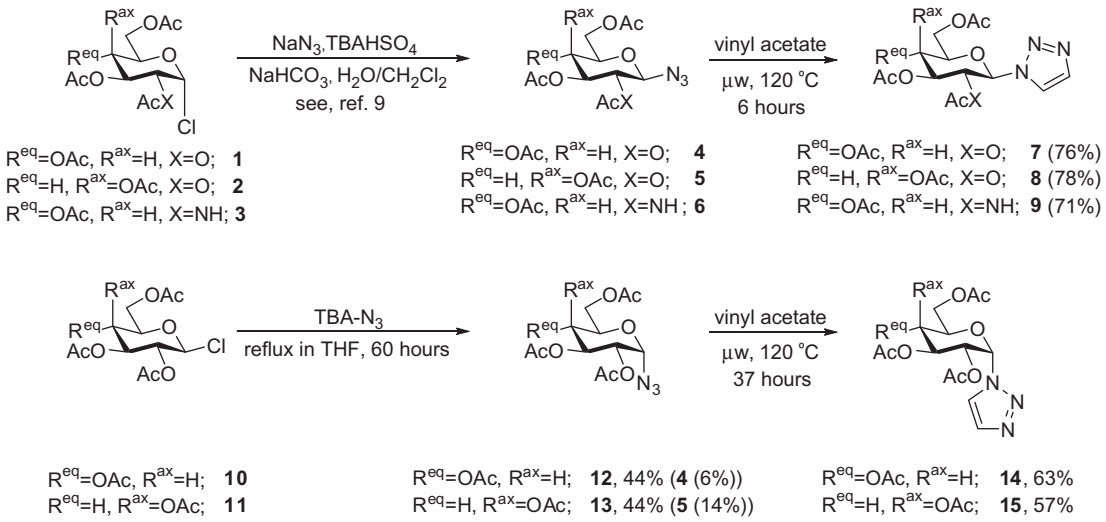


R^{eq}=OAc, R^{ax}=H, X=O; **1**
R^{eq}=H, R^{ax}=OAc, X=O; **2**
R^{eq}=OAc, R^{ax}=H, X=NH; **3**

R^{eq}=OAc, R^{ax}=H, X=O; **4**
R^{eq}=H, R^{ax}=OAc, X=O; **5**
R^{eq}=OAc, R^{ax}=H, X=NH; **6**

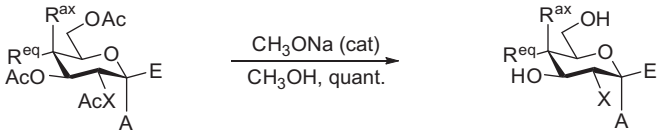
R^{eq}=OAc, R^{ax}=H, X=O; **7** (76%)
R^{eq}=H, R^{ax}=OAc, X=O; **8** (78%)
R^{eq}=OAc, R^{ax}=H, X=NH; **9** (71%)

R^{eq}=OAc, R^{ax}=H; **10**
R^{eq}=H, R^{ax}=OAc; **11**

R^{eq}=OAc, R^{ax}=H; **12**, 44% (**4** (6%))
R^{eq}=H, R^{ax}=OAc; **13**, 44% (**5** (14%))

R^{eq}=OAc, R^{ax}=H; **14**, 63%
R^{eq}=H, R^{ax}=OAc; **15**, 57%

Scheme 1. Synthesis of glycosyl 1,2,3-triazoles from the corresponding azides under microwave (μw) irradiation in vinyl acetate.



R^{eq}=OAc, R^{ax}=A=H, X=O; E=*N*-(1,2,3-triazolyl); **7**
R^{eq}=A=H, R^{ax}=OAc, X=O; E=*N*-(1,2,3-triazolyl); **8**
R^{eq}=OAc, R^{ax}=A=H, X=NH; E=*N*-(1,2,3-triazolyl); **9**
R^{eq}=OAc, R^{ax}=E=H, X=O; A=*N*-(1,2,3-triazolyl); **14**
R^{eq}=E=H, R^{ax}=OAc, X=O; A=*N*-(1,2,3-triazolyl); **15**

R^{eq}=OH, R^{ax}=A=H, X=OH; E=*N*-(1,2,3-triazolyl); **16**
R^{eq}=A=H, R^{ax}=OH, X=OH; E=*N*-(1,2,3-triazolyl); **17**
R^{eq}=OH, R^{ax}=A=H, X=AcNH; E=*N*-(1,2,3-triazolyl); **18**
R^{eq}=OH, R^{ax}=E=H, X=OH; A=*N*-(1,2,3-triazolyl); **19**
R^{eq}=E=H, R^{ax}=OH, X=OH; A=*N*-(1,2,3-triazolyl); **20**

Scheme 2. Zemplén deprotection of acetylated glycosyl 1,2,3-triazoles.

With the deprotected glycosyl triazoles (**16**–**20**) in hand, their inhibitory potential was evaluated against common glycosidases. No inhibition of sweet almond β-glucosidase was observed at 1 mM by β-glucosyl triazole (**16**).[14] Interestingly, β-galactosyl triazole (**17**) did not show any sign of inhibition of *Escherichia coli* β-galactosidase at 1 mM, although the analogous 4-phenyl substituted counterpart (Fig. 1) has been reported to inhibit this enzyme with K_i = 330 μM.[5] β-Galactosyl triazole (**17**) was furthermore tested against *Aspergillus oryzae* β-galactosidase without inhibition at 1 mM. α-Glycosyl triazoles **19** and **20** were in a similar way evaluated against yeast α-glucosidase and green coffee bean α-galactosidase, respectively, without inhibition at 1 mM.

β-GlcNAc-triazole (**18**) was evaluated as a potential inhibitor of extracellular fungal β-*N*-acetylhexosaminidase (*Aspergillus oryzae* CCF 1066, *Penicillium oxalicum* CCF 1959, *P. pittii* CCF 2277 and *Talaromyces flavus* CCF 2686), accordingly, no inhibition of these enzymes was observed at 2 mM.

Glycosyl triazoles with electron withdrawing substituents have previously been explored as donor substrates in chemical glycosyl transfer reactions to fluoride[15] or thiols/alcohols.[16,17] Therefore, we were interested to see whether glycosyl triazoles too could be enzymatically hydrolysed by glycosidases. No such cleavage was found in a previous study by Rossi and Basu, who studied the 4-phenyl galactosyl triazole (Fig. 1) and its *gluco*-isomer against a range of glycosidases.[5] To the best of our knowledge, no such enzyme able to catalyze the cleavage of a C–N glycosidic bond (where nitrogen is neutral)[18] has prior to this been reported with the exception of glycosyl azide hydrolysis.[19–21] It was decided to focus on GlcNAc-triazole **18** and investigate its potential hydrolysis by substrate-promiscuous fungal β-*N*-acetylhexosaminidases. The same four fungal β-*N*-acetylhexosaminidases as tested for inhibition by **18** were screened in this study (vide supra). Although the reaction appeared to be very slow, β-*N*-acetylhexosaminidase from *T. flavus* was indeed able to process GlcNAc-triazole **18**. No hydro-

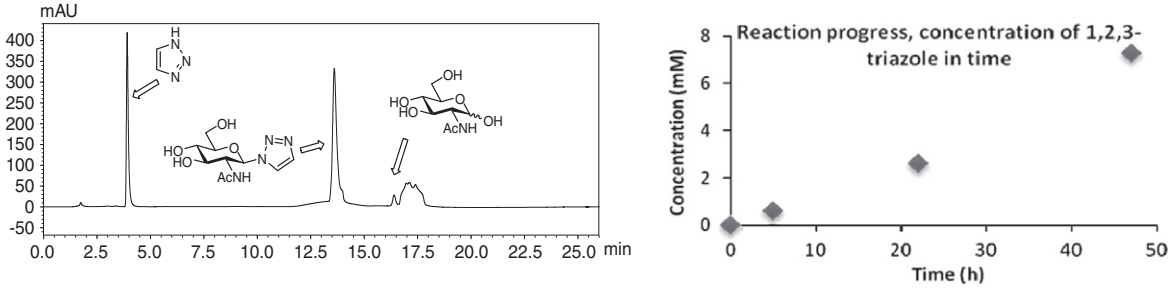
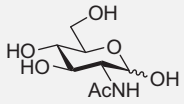
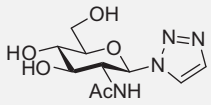


Figure 2. HILIC chromatogram after 22 h of reaction (left). Formation of 1,2,3-triazole by *T. flavus* β-*N*-acetylhexosaminidase catalyzed hydrolysis of **18** (right).

Table 1
Values for binding to lymphocyte activating receptors ($-\log IC_{50}$)

	NKR-P1A (rat)	CD69 (human)
 (GlcNAc)	5.7 ± 0.1	3.9 ± 0.1
 (**18**)	7.2 ± 0.2	5.4 ± 0.2

lysis was found in the absence of enzyme or among the remaining three β-*N*-acetylhexosaminidases.

To study the *T. flavus* catalyzed reaction in detail, a new HPLC (HILIC) method was developed to monitor the concentration of all reaction mixture components by determining the concentration of 1,2,3-triazole (Fig. 2, left). Hydrolysis of **18** was found to be linear in time (Fig. 2, right), but 2000 times slower compared to the standard substrate 4-nitrophenyl 2-acetamido-2-deoxy-glucopyranoside (*p*NP-GlcNAc) (2.7 mU vs 5 U, respectively). Despite the slow reaction rate, the hydrolysis of a C–N bond by an O-glycosidase is a rather unique finding. Upon this result, *T. flavus* β-*N*-acetylhexosaminidase was also tested as a potential catalyst for reaction with another C–N glycoside that is, the thioureido linkage of 1,2-bis[*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-thioureido]-ethane.[12,22] No reaction, however, was detected.

GlcNAc-triazole (**18**) was also evaluated as a potential ligand against both rat NKR-P1A and human CD69 receptors by measuring the ability of GlcNAc-triazole **18** to displace a high-affinity receptor ligand.[12,23] Triazole **18** showed an approximately 30-times increased affinity over *N*-acetyl-D-glucosamine (GlcNAc), whereas phenyl or nitrophenyl β-GlcNAc glycosides are usually associated with approximately a tenfold increase in affinity compared to GlcNAc alone. This significant increase in inhibitory potency of GlcNAc-triazole (**18**) could be a result of unspecific augmenting the interaction by the adjacent aromatic moiety.[24] This effect is often observed for interactions between aromatic glycosides and plant as well as animal lectins. From a structural point of view, this effect has been partially explained on the basis of chemical stacking interactions of aromatic amino acids often present in the carbohydrate binding site of these lectins with the hydrophobic upper or lower sites of the pyranose carbohydrate rings, and the adjacent aromatic group. However, very little systematic studies on these apparently 'nonspecific' effects have been performed so far. The current study is thus among the first to bring evidence that significant binding effects might be attained varying the exact chemical structure of the aromatic aglycon ring participating in these interactions, and thus the fine structure of the electron clouds involved in these interactions. GlcNAc-triazole (**18**) thus constitutes a suitable linkage moiety for the construction of multivalent neoglycoconjugates for immunological applications.

In conclusion, we have described the first synthesis of 5 different glycosyl-1,2,3-triazoles. The products displayed no inhibition activity towards a range of glycosidases, however, by an established HPLC protocol β-*N*-acetylhexosaminidase from *Talaromyces flavus* proved to be able to cleave the intact triazole. This C–N glycoside hydrolysis of GlcNAc-triazole **18** is an exceptional feature of this particular O-glycosidase.

We have furthermore demonstrated the significant binding affinity of GlcNAc-triazole (**18**) towards the NK cell activating receptors (Table 1) CD69 and NKRP-1. This activity could possibly be considerably enhanced by the construction of a dendrimeric GlcNAc-display by Cu(I)-catalyzed azide-alkyne cycloaddition.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.151.

References and notes

1. Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952.
2. Debola, S.; Nepogodiev, S. A.; Field, R. A. *Org. Biomol. Chem.* **2007**, *5*, 1006.
3. Dondoni, A. *Chem. Asian J.* **2007**, *2*, 700.
4. van Kasteren, S. I.; Kramer, H. B.; Jensen, H. H.; Campbell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; Davis, B. G. *Nature* **2007**, *446*, 1105.
5. Rossi, L. L.; Basu, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3596.
6. Wilkinson, B. L.; Long, H.; Sim, E.; Fairbanks, A. J. *Bioorg. Med. Chem. Lett.* **2008**, 6265.
7. C-Unsubstituted xylosyl triazoles were made by Cu(I)-catalysis between glycosyl azide and TMS-acetylene, see: Kuberan, B.; Ethirajan, M.; Victor, X. V.; Tran, V.; Nguyen, K.; Do, A. *ChemBioChem* **2008**, *9*, 198.
8. Hansen, S. G.; Jensen, H. H. *Synlett* **2009**, 3275.
9. Tropper, F. D.; Andersson, F. O.; Braun, S.; Roy, R. *Synthesis* **1992**, 618.
10. Korytnyk, W.; Mills, J. A. *J. Chem. Soc.* **1959**, 636.
11. Soli, E. D.; Manoso, A. S.; Patterson, M. C.; DeSong, P.; Favor, D. A.; Hirschmann, R.; Smith, A. B. *J. Org. Chem.* **1999**, *64*, 3171.
12. See Supplementary data for experimental details.
13. Dedola, S.; Hughes, D. L.; Nepogodiev, S. A.; Rejzek, M.; Field, R. A. *Carbohydr. Res.* **2010**, *345*, 1123.
14. Evaluation of inhibitory potential against glucosidases and galactosidases was performed according to: Rasmussen, T. S.; Jensen, H. H. *Org. Biomol. Chem.* **2010**, *8*, 433.
15. Bröder, W.; Kunz, H. *Carbohydr. Res.* **1993**, *249*, 221.
16. Bröder, W.; Kunz, H. *Synlett* **1990**, 252.
17. Watt, J. A.; Gannon, C. T.; Loft, K. J.; Dinev, Z.; Williams, S. J. *Aust. J. Chem.* **2008**, *61*, 837.
18. The hydrolysis of glycosyl pyridinium salts have been thoroughly studied, see Sinnott, M. L.; Withers, S. G. *Biochem. J.* **1974**, *143*, 751, and Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171.
19. Day, A. G.; Withers, S. G. *Biochem. Cell Biol.* **1986**, *64*, 914.
20. Fialová, P.; Carmona, A. T.; Robina, I.; Ettrich, R.; Sedmera, P.; Přikrylová, V.; Petrásková-Hušáková, L.; Křen, V. *Tetrahedron Lett.* **2005**, *46*, 8715.
21. Bojarová, P.; Petrásková, L.; Ferrandi, E. E.; Monti, D.; Pelantová, H.; Kuzma, M.; Simerská, P.; Křen, V. *Adv. Synth. Catal.* **2007**, *349*, 1514.
22. Bojarová, P.; Křenek, K.; Wetjen, K.; Adamiak, K.; Pelantova, H.; Bezouška, K.; Elling, L.; Křen, V. *Glycobiology* **2009**, *19*, 509.
23. Bezouška, K.; Vlahas, G.; Horváth, O.; Jinochová, G.; Fišerová, A.; Giorda, R.; Chambers, W. H.; Feizi, T.; Pospíšil, M. *J. Biol. Chem.* **1994**, *269*, 16945.
24. Krist, P.; Herkommerová-Rajnochová, E.; Rauvolfová, J.; Semeňuk, T.; Vavrušková, P.; Pavlíček, J.; Bezouška, K.; Petruš, L.; Křen, V. *Biochem. Biophys. Res. Commun.* **2001**, *287*, 11.