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Synthesis of α- and β-D-glucopyranosyl triazoles by CuAAC 'click chemistry': reactant tolerance, reaction rate, product structure and glucosidase inhibitory properties

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ABSTRACT

Cu^{I}-catalysed azide alkyne 1,3-dipolar cycloaddition (CuAAC) 'click chemistry' was used to assemble a library of 21 α-D- and β-D-glucopyranosyl triazoles, which were assessed as potential glycosidase inhibitors. In the course of this work, different reactivities of isomeric α- and β-glucopyranosyl azides under CuAAC conditions were noted. This difference was further investigated using competition reactions and rationalised on the basis of X-ray crystallographic data, which revealed significant differences in bond lengths within the azido groups of the α- and β-anomers. Structural studies also revealed a preference for perpendicular orientation of the sugar and triazole rings in both the α- and β-glucosyl triazoles in the solid state. The triazole library was assayed for inhibition of sweet almond β-glucosidase (GH1) and yeast α-glucosidase (GH13), which led to the identification of a set of glucosidase inhibitors effective in the 100 μM range. The preference for inhibition of one enzyme over the other proved to be dependent on the anomeric configuration of the inhibitor, as expected.

1. Introduction

Glycoside hydrolases are involved in a large number of natural processes related to carbohydrate metabolism. Detailed knowledge of the mechanisms[1] that these enzyme use to hydrolyse glycosidic bonds of complex carbohydrates will facilitate the search for new inhibitors,[2,3] which is directly relevant to the discovery of new therapeutics.[4] During the last few years there has been increasing interest in the use of the Cu^{I}-catalysed azide-alkyne cycloaddition reaction (CuAAC), an example of so-called 'click chemistry',[5,6] in the field of carbohydrate research.[7,8] This reaction has been used to assemble mimics of oligosaccharides and glycopeptides,[7–12] as well as glycoclusters and dendrimers;[13,14] it has also found applications in in vivo imaging of glycoconjugates.[15,16] Several attempts have been made to utilise click chemistry for the synthesis of potential glycosidase inhibitors.[17–19] For instance, β-linked 1-glycosyl-4-phenyltriazoles **1** and **2** (Fig. 1), prepared from β-glycosyl azides and phenylacetylene, were assessed for inhibitory activity against three different glycosidases.[18] Compound **1** displayed 48% inhibition of the bovine liver galactosidase activity at 0.24 mM concentration, but in general the inhibitory activity of compounds **1** and **2** was weak. A series of acarbose-like *pseudo*-oligosaccharides, for example triazole derivative **3**, was assayed against α- and β-glycosidases from various sources and showed only weak inhibitory activity.[17] Compounds **4a**–**4c** were synthesised[19] using 'click chemistry' in order to combine two distinct classes of

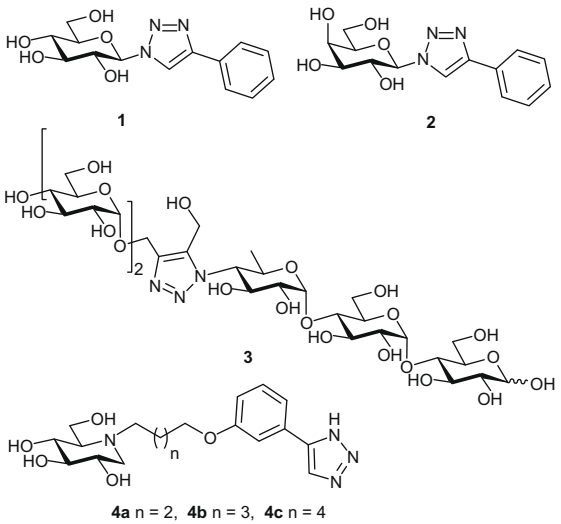

Figure 1. Potential glycosidase inhibitors synthesised using 'click chemistry': glucoside **1**, galactoside **2**, acarbose mimic **3** and MetAP2 II inhibitors **4**.

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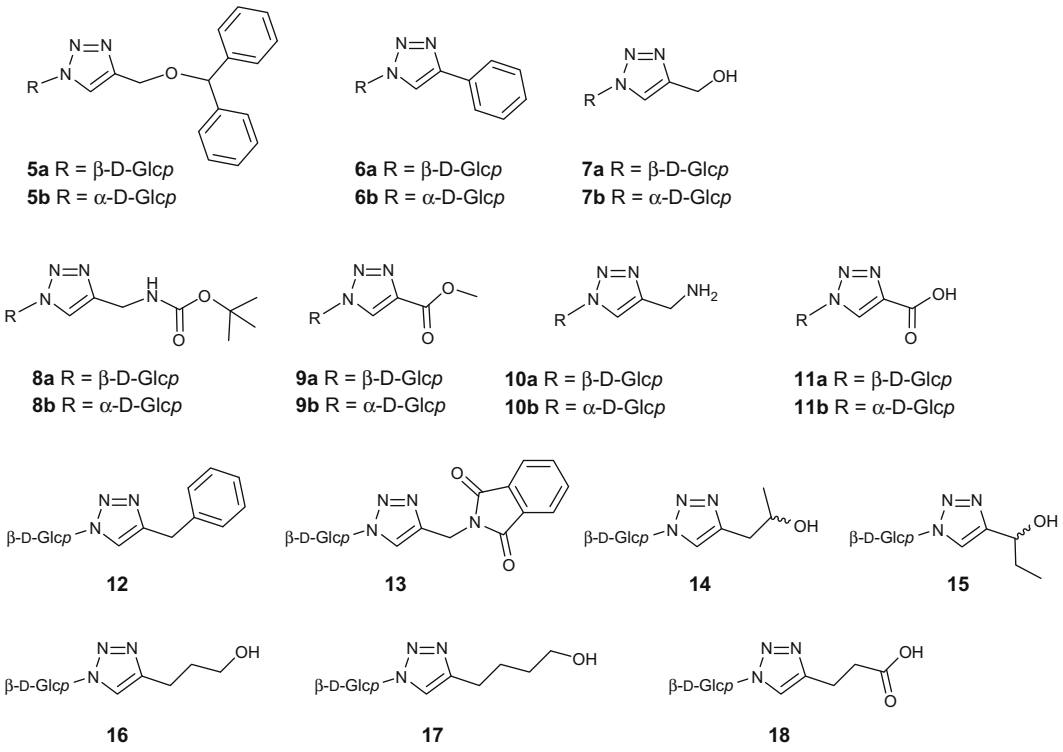


Figure 2. Structures of target glucopyranosyl triazoles.

therapeutic agents—iminosugars and aryl-triazoles—which can interfere with both glycosidases and methionine aminopeptidase involved in angiogenesis.[20,21]

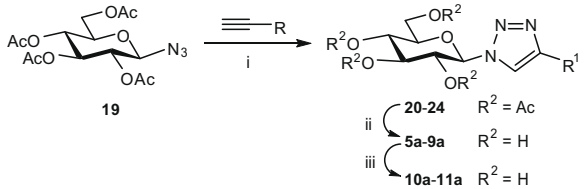
The approach adopted in the present work was based on a simple assumption that new glucosidase inhibitors can be identified by screening libraries of glucosides having variable aglycones.[22–27] Utilisation of 'click chemistry' for building libraries of this type should allow one to overcome common synthetic challenges associated with the preparation of O- or C-glycosides and make it possible to incorporate a wide variety of functional groups into the aglycone portion of the glucopyranoside analogues. In this study we have used the CuAAC reaction of glucopyranosyl azide building blocks, both acetylated and deprotected, with assorted commercially available alkynes possessing lipophilic, hydrophilic, acidic or basic functionalities. The presence of these functional groups provides an opportunity to probe interactions of putative inhibitors with the active site while the relevance of the anomeric configuration can be explored using compounds synthesised by CuAAC of the same set of alkynes and either α- or β-glucopyranosyl azide. A small library of prospective glucopyranosyl triazole-based glycosidase inhibitors assembled using the CuAAC reaction (Fig. 2) was screened against the cognate and non-cognate GH1 sweet almond β-glucosidase and GH13 yeast α-glucosidase, which are readily available and well-characterised model glucosidases. The different reactivities of α- and β-glucopyranosyl azides towards the CuAAC reaction, which was noted in recent work from this group,[28] are also discussed in more detail.

2. Results and discussion

2.1. Synthesis of glucopyranosyl triazoles

Since the discovery of the CuAAC reaction[29,30] a large number of different reaction conditions and various forms of Cu(I) catalyst have been reported.[6] For our purpose, we adopted a method based on the procedure first described by Sharpless[30] that involves 0.25–0.5 M reactants, 0.01 mol equiv of $CuSO_4$ plus 0.1 mol equiv of sodium ascorbate (NaAsc) as a catalyst, in 1:1 *t*-BuOH/H_2O solvent mixture at room temperature. Testing these conditions for coupling between peracetylated β-D-glucopyranosyl azide **19**[31] and propargyl alcohol revealed a low conversion to the desired product, apparently as a result of the limited solubility of acetylated glucopyranosyl azides in the chosen reaction medium. Therefore two variations of these conditions were used (referred to as Methods A and B), which allowed significant improvement of the cycloaddition yields of the target products. In Method A the reactions were conducted at 70 °C (cf. Ref. 32) and the conversion of **19** into 1,4-substituted triazoles was complete in 10 min, affording single regioisomers **21**, **22** and **24** (as judged by ^{1}H NMR spectroscopy)

Table 1
Synthesis of β-D-glucopyranosyl triazoles **20**–**24** and **5a**–**11a**



R^1	Compound (yield, %)	
$-CH_2OCHPh_2$	**20**^{B} (98)	**5a** (88)
–Ph	**21**^{A} (97)	**6a** (94)
$-CH_2OH$	**22**^{A} (99)	**7a** (92)
$-CH_2NHCO_2CMe_3$	**23**^{B} (95)	**8a** (91)
$-CO_2Me$	**24**^{A} (70)	**9a** (95)
$-CH_2NH_2$		**10a** (91)
$-CO_2H$		**11a** (92)

(i) CuAAC conditions: ^{A}Method A; ^{B}Method B; (ii) NaOMe–MeOH; (iii) CH_2Cl_2–TFA 9:1 (for **10a**) or aq 1 M NaOH for (**11a**).

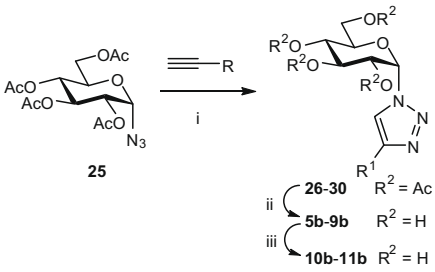
in high yield (>90%) and purity. In Method B, the same concentrations of reagents were used, and the same catalytic couple of $CuSO_4$/NaAsc, but a microwave reactor operating at 70 °C was employed and DMF was used as a solvent, as first suggested by Khanetskyy.[33] This method has the advantage of short reaction times and it has the potential for automation[34]—key features for the rapid assembly of compound libraries. Some adjustment of reaction time was required for individual reactants to avoid product decomposition, which was easily monitored by TLC. Generally CuAAC reactions are characterised by good tolerance of functional groups, but for some functionalities application of protecting groups proved necessary. For example, unprotected amines inhibit CuAAC reactions, apparently by destabilising the oxidation state of catalytic Cu^I by ligation.[35] Thus in the synthesis of aminomethylene-triazole derivative **10a** it was necessary to use Boc-protected propargyl amine. Incompatibility with CuAAC reaction conditions was also noticed in synthesis of carboxylic acid derivative **11a** from propiolic acid; the problem was overcome by using the methyl ester as a temporary protecting group. The preparation of the first small library of 1,4-substituted β-glucopyranosyl triazoles (Table 1) was completed by removal of protecting groups which included NaOMe-catalysed de-O-acetylation for all compounds, with additional acid-catalysed cleavage of the Boc group in **23** and base-catalysed hydrolysis of methyl ester **24**.

The same set of alkynes as used in preparation of β-glucopyranosyl triazoles **5a–11a** was applied for syntheses of the isomeric α-glucopyranosyl triazoles **5b–11b** (Table 2), starting from peracetylated α-glucopyranosyl azide **25**. This building block was obtained in 60% yield by reacting 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride[36] with Me_3SiN_3 in the presence of Bu_4NF in THF.[28,37,38] CuAAC reactions were carried out in a microwave reactor at 70 °C with $CuSO_4$/NaAsc as a catalyst and DMF as a solvent (Method B as above). All the target compounds were synthesised in high yield and purity (Table 2), but completion of the cycloaddition required longer reaction time than in similar reactions with β-glucopyranosyl azide (vide infra). All acetylated products were deprotected using standard conditions similar to those described for preparation of β-glucopyranosyl isomers **5a–11a**.

The structure and purity of acetylated (**20–24** and **26–30**) and deprotected glucopyranosyl triazoles (**5a**,**b–11a**,**b** and **12–18**) were unequivocally confirmed by NMR spectroscopy and ES mass spectrometry data. In particular, the 1,4 regioselectivity of the cycloaddition reaction was confirmed in all the cases by characteristic large positive $\Delta(\delta_{C4}-\delta_{C5})$ values (ca. 26 ppm) for triazole carbon atoms in ^{13}C NMR spectra.[39] ^1H NMR spectra of the majority of triazole derivatives showed signals with a chemical shift at δ 8.00–8.50 ppm, which is typical for the 1,4-substituted triazole ring. In some cases triazole proton signals were less useful diagnostically, falling in the range reported for 1,5-substituted triazoles (δ ~7.70 ppm).[39,40]

A more direct approach to carbohydrate-containing triazoles can be achieved by the direct reaction of unprotected sugar azides. Clearly the synthetic procedure should be highly efficient in order to generate products which do not require the laborious purification that is often associated with unprotected carbohydrate derivatives. CuAAC reactions satisfy these criteria, as demonstrated in parallel synthesis with unprotected starting materials coupled, without purification, with direct screening of products for biological activity.[41–44] Typical protocols for CuAAC coupling of water soluble reagents[30] (0.01 mol equiv $CuSO_4$ and 0.1 mol equiv NaAsc in 1:1 *t*-BuOH–H_2O) were found to be suitable for the rapid preparation of glucopyranosyl triazoles, such as **12** and **13**, in a microtiter plate format at 40 °C. However, the utility of this procedure was hampered by the necessity for NaAsc/$CuSO_4$ removal from the reaction mixture. An alternative catalytic system which generates Cu^I by the in situ reaction of $CuSO_4$ with copper turnings[30] was found to be advantageous as the inorganic catalyst components are easily separated from products by filtration through a short plug of silica gel. In this case, reactions between β-D-glucopyranosyl azide **31** and selected alkynes were carried out in a 3:2:5 mixture of H_2O–EtOH–*t*-BuOH[42] for 48 h at room temperature, resulting in triazoles **12–18** (Table 3). Relatively low yields in some of these reactions can be attributed to loss of the material on silica gel purification. The purity of each product was confirmed by ^1H NMR spectroscopy and mass spectrometry, and glucopyranosyl triazole derivatives **12–18** were used directly for evaluation of their glycosidase inhibitory activity.

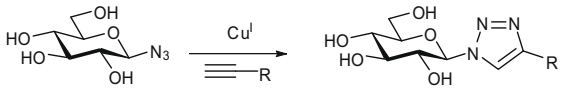
Table 2
Synthesis of α-D-glucopyranosyl triazoles **5b–11b**



R^1	Compound (yield, %)	
$-CH_2OCHPh_2$	**26** (99)	**5b** (90)
–Ph	**27** (63)	**6b** (99)
$-CH_2OH$	**28** (60)	**7b** (94)
$-CH_2NHCO_2CMe_3$	**29** (94)	**8b** (99)
$-CO_2Me$	**30** (96)	**9b** (99)
$-CH_2NH_2$		**10b** (99)
$-CO_2H$		**11b** (94)

(i) CuAAC conditions: Method B; (ii) NaOMe–MeOH; (iii) CH_2Cl_2–TFA 9:1 (for **10b**) or aq 1 M NaOH for (**11b**).

Table 3
β-Glucopyranosyl triazoles **12–18**



31 **12-18**

R	Compound (yield, %)
$-CH_2Ph$	**12** (32)
$-CH_2NPhth$	**13** (80)
$-CH_2CH(Me)OH$	**14** (28)
$-CH(OH)CH_2Me$	**15** (82)
$-CH_2CH_2CH_2OH$	**16** (68)
$-CH_2CH_2CH_2CH_2OH$	**17** (92)
$-CH_2CH_2CO_2H$	**18** (50)

2.2. Relative reactivity of α- and β-glucopyranosyl azides under CuAAC reaction conditions

The above-mentioned experiments highlighted the impact of glycosyl azide anomeric configuration on the rate of CuACC reactions, though final yields were high for both anomeric series. While reactions with β-glucopyranosyl azide were typically complete in 10–45 min, similar reactions with α-glucopyranosyl azide often required 45–120 min. The lower reactivity of α-glycopyranosyl azides has been observed previously by Wilkinson et al.,[45] although the comparison was indirect since the glucopyranosyl azides studied had different protecting groups (acetates vs benzyl ethers). In order to rationalise the difference in reactivity of β- and α-glucopyranosyl azides towards the CuAAC reaction, we

Table 4
Interatomic distances (Å) for CN_3 fragment in glycosyl azides **19** and **25**[a]

	β-Azide **19**	α-Azide **25**
C(1)–N(11)	1.460 (2)	1.510 (3)
N(11)–N(12)	1.243 (2)	1.165 (3)
N(12)–N(13)	1.119 (3)	1.195 (4)

[a] Atom numbering for C(1)–N(11)–N(12)–N(13) fragment is adapted from the original publications.[28,46]

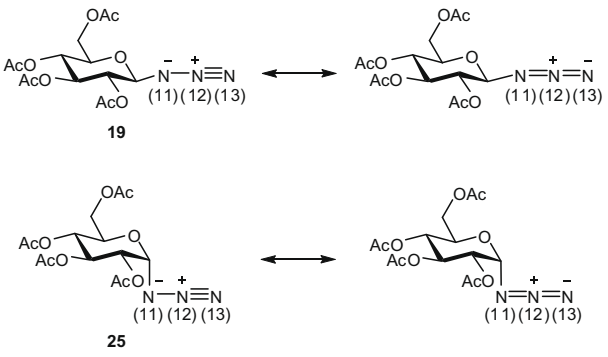
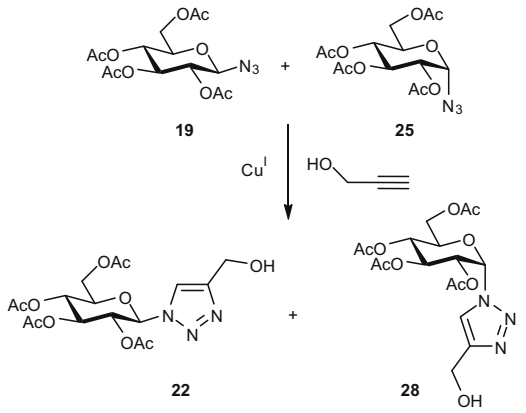


Figure 3. Resonance structures for α- and β-glucopyranosyl azides.



Scheme 1. Competition experiment between β- and α-glucopyranosyl azides **19** and **25** in the presence of propargyl alcohol. Reagents and conditions: 1 equiv **19**, 1 equiv **25**, 0.05 equiv $CuSO_4$; 0.1 equiv NaAsc, MW 70 °C; 15 min. The ratio between β- and α-glucopyranosyl triazoles in the products mixture **22**:**28** = 3:1.

elucidated the X-ray crystal structure of the starting α-azide **25**[28] and compared it to the known crystal structure of the corresponding β-azide **19**.[46] This comparison revealed that acetylated glucopyranose rings of **19** and **25** are very similar, except for the anomeric azido groups having different configurations (axial vs equatorial). In addition, the interatomic distances within the C(1)–N(11)–N(12)–N(13) fragment (Table 4 and Fig. 3) are in keeping with the expected influence of the anomeric effect.[47,48] The C(1)–N(11) distance in **19** is longer than the corresponding distance in **25**; the terminal N(12)–N(13) bond is shorter than the inner N(11)–N(12) bond in β-isomer **19**, whereas in α-isomer **25** these bonds are more similar in value. This particular difference between the azido group in **19** and **25** indicates a significant difference in electron distribution within this group for the two isomeric compounds, which manifests itself as a difference in dipolar character and hence different reaction rates under CuAAC reaction conditions. The higher reactivity of the β-azide **19** can be interpreted as a result of a partial negative charge on atom N(11), which plays a crucial role in the mechanism of the CuAAC reactions, consistent with results from DFT calculations.[49]

Direct evidence for the lower reactivity of the α- compared to the β-glucopyranosyl azide was obtained from a competition reaction between equimolar **19**, **25** and propargyl alcohol (Scheme 1). The reaction was run in the microwave under conditions discussed earlier (Method B) and the composition of the reaction mixture was analysed after 15 min. Characteristic $J_{1,2}$ coupling constants for the anomeric H-1 signal in glucopyranosyl triazoles (6.0 and 9.0 Hz for α- and β-anomer, respectively) allowed straightforward identification of these signals (δ 6.36 ppm for α-anomer **28** and δ 5.89 ppm for β-anomer **22**) and complete assignment of the ^{1}H NMR spectrum of the mixture. Integration of sugar H-1 signals and triazole H-5 signals (δ 7.66 and 7.89 for α- and β-anomers, respectively) indicated that α- and β-glucopyranosyl triazoles were formed in an approximately 1:3 ratio, confirming the greater reactivity of the β-configured azide.

2.3. X-ray crystal structure of glucopyranosyl triazoles 24 and 27

Ahead of investigating the glycosidase inhibitory properties of our library of glycosyl triazoles we sought to gain some insight into their structure, in particular the relative orientation of the sugar and triazole rings. X-ray crystallographic studies were therefore conducted on two representative glucopyranosyl triazoles having β- and α-anomeric configurations, namely compounds **24** and **27** (Fig. 4A and B, respectively). The planes of triazole and glucopyranose rings of these molecules in the solid state were found to be nearly perpendicular to each other, with the triazoles occupying the C(1),C(4),O(4) plane bisecting the pyranose ring. The torsion angle H(1)–C(1)–N(11)–N(12) is close to 180° for both β-isomer **24** and α-isomer **27** resulting in orientation of the triazole nitrogen atoms as shown in Figure 5. Comparison of the crystallographic parameters of **24** and **27** with X-ray data of known β-glucopyranosyl triazole derivative **22**[50] revealed similarity of the geometrical parameters for the glucopyranosyl fragments of these molecules, all of which exist in the usual $^4C_1$ chair conformation with minimal distortion. The triazole ring of **22** occupies the same C(1),C(4),O(4) plane of the molecule as in **24** but the triazole nitrogens in **22** are facing in the opposite direction to that in β-glucopyranosyl triazole **24**, as shown in Figure 4. In these examples, the perpendicular orientation of the sugar and triazole rings is to be expected on steric grounds. The difference between C(1)–N(11) bond lengths in glucopyranosyl triazoles **24** (1.449(2) Å) and **27** (1.463(3) Å) is small, suggesting a limited influence of the anomeric effect on the structure of these molecules. The lengths of the analogous bonds in precursors β-glucopyranosyl azide **19** (1.460(2) Å)[46] and α-glucopyranosyl azide **25**[28] (1.510 (3) Å) suggest that the influence of the anomeric effect is much stronger for the electronegative azide group than for the triazoles.

2.4. Enzyme inhibition assays

The library of triazoles shown in Figure 2 was assayed against two glycosidases: sweet almond β-glucosidase (GH1) and yeast α-glucosidase (GH13). In addition to the synthetic CuAAC-derived products, 4-phenyl-triazole **32**, benzhydrol **33** and two known low micromolar β-glucosidase inhibitors, deoxynojirimycin (DNJ) **34**[51] and 4-phenylimidazole **35**,[52] were used as positive controls (Fig. 6). Under the conditions of the enzyme assay the triazole nitrogen (pK_a 1.1–1.3)[53] is not protonated, excluding any electrostatic interactions with charged amino acid residues within the active site. Therefore the contribution of the triazole group per se to glycosidase inhibition was expected to be limited.

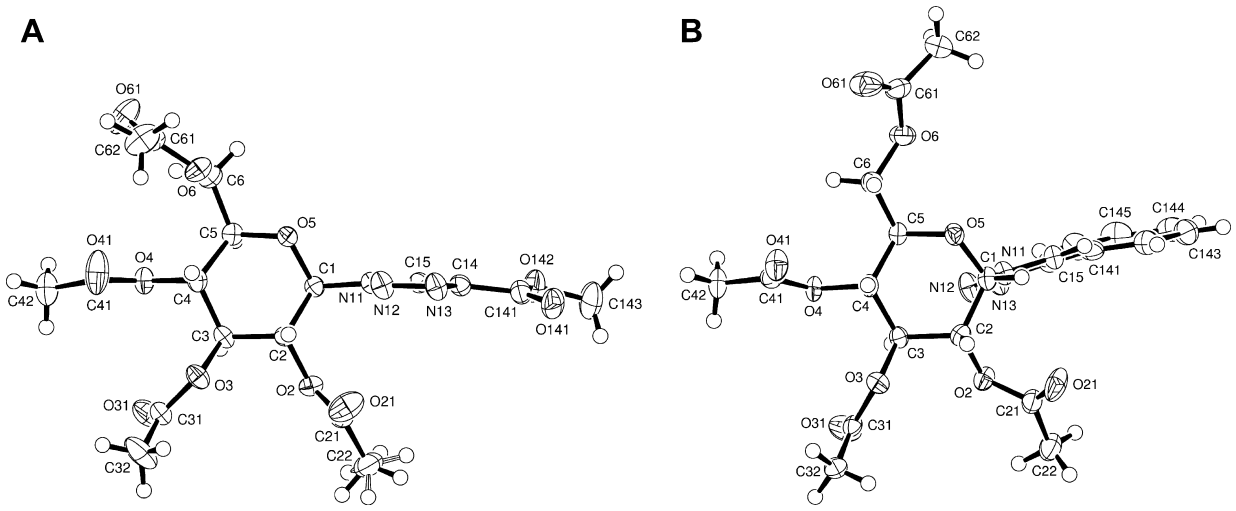


Figure 4. ORTEP representation of the solid state structure of fully acetylated glucopyranosyl triazoles **24** (A) and **27** (B) viewed from the β-face of D-glucopyranose ring.

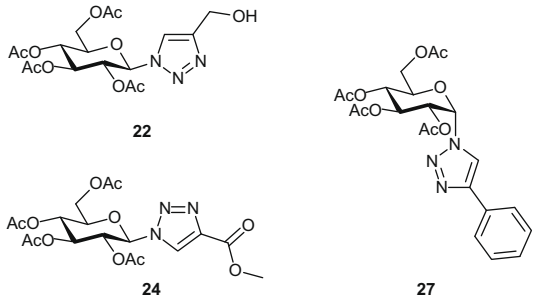


Figure 5. Structure of glucopyranosyl triazoles **22**,[50] **24** and **27** showing the relative orientation of the sugar and triazole rings as determined by X-ray crystallography.

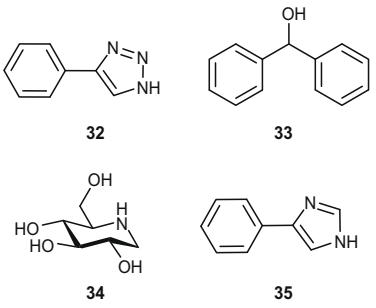


Figure 6. Compounds **32** and **33** (positive controls), **34** and **35** screened against glycosidases in parallel with synthetic glycosyl triazoles.

2.4.1. β-Linked glucosyl triazoles and sweet almond β-glucosidase

Screening of β-glucopyranosyl triazoles **5a**–**11a** and **12**–**18** (Fig. 2) against their cognate partner (based on stereospecificity) sweet almond β-glucosidase revealed that only triazoles **5a** (benzhydryloxymethylene), **6a** (phenyl), **11a** (carboxy) and **14** (phthalimido-methylene) possessed inhibitory activity, with the remaining compounds exhibiting no measurable inhibition at 1 mM concentration (Fig. 7a). As expected, DNJ **34** and 4-phenyl-imidazole **35** showed inhibitory activity in the 10s of μM range.[51,52] Figure 7a shows enhancement of inhibition on β-glucosylation of benzhydrol **33**, giving triazole **5a**. Overall, these data show only very limited β-glucosidase inhibition by β-glucopyranosyl triazoles, which is perhaps surprising given the wide range of hydrophobic compounds, including β-glycosides, that are known to inhibit the almond enzyme.[23,24,51,52]

2.4.2. β-Linked glucosyl triazoles and yeast α-glucosidase

The same set of β-linked triazoles **5a**–**11a**, **12**–**18** and associated controls **32**–**35** were also assayed against their non-cognate partner yeast α-glucosidase (Fig. 7b). Once again, the DNJ **34** positive control demonstrated activity in the 10s of μM range; whilst benzhydrol **33** and 4-phenyl-imidazole **35** did not display significant inhibition at 1 mM concentration, 4-phenyl-triazole **32** displayed some activity in the mM range. Of the library of β-glucosyl triazoles assessed, only acid- and ester-substituted β-D-glucopyranosides **9a** and **11a**, respectively, showed activity, giving rise to weak inhibition (60% and 40%) at 1 mM concentration. Poor inhibition of an α-glucosidase by non-cognate β-glucopyranosides is to be expected, given the stereochemical preference of the enzyme.

2.4.3. α-Linked glucosyl triazoles and sweet almond β-glucosidase

Screening of α-glucopyranosyl triazoles **5b**–**11b** (Fig. 2) against their non-cognate partner (based on stereospecificity) sweet almond β-glucosidase revealed only one compound displaying any inhibitory activity—aminomethylene-triazole **10b**—but even then the activity was very weak compared to DNJ **34** and 4-phenyl-imidazole **35** (Fig. 8a). Given that sweet almond β-glucosidase hydrolyses β-linkages, while the compounds screened are all α-configured, limited inhibition was to be expected.

2.4.4. α-Linked glucosyl triazoles and yeast α-glucosidase

The set of α-linked glucopyranosyl triazoles **5b**–**11b** (Fig. 2) was also assayed against a cognate binding partner, yeast α-glucosidase (Fig. 8b). In contrast to all the other inhibition data obtained, all the α-glucosyl triazoles **5b**–**11b** showed an inhibitory activity towards α-glucosidase in the 100 μM–1 mM range. Benzhydryloxymethylene derivative **5b** proved to be significantly more active than benzhydrol **33** itself, as was the case for α-glucosyl phenyltriazole **6b** compared to the parent 4-phenyl-triazole **32**, indicating positive contribution to binding from both the glycone and aglycone portions of the inhibitor. The Boc-protected amine derivative **8b** and the methyl ester **9b** were also reasonable inhibitors of the yeast enzyme, whilst their deprotected counterparts, amine **10b** and carboxylic acid **11b**, were somewhat less active.

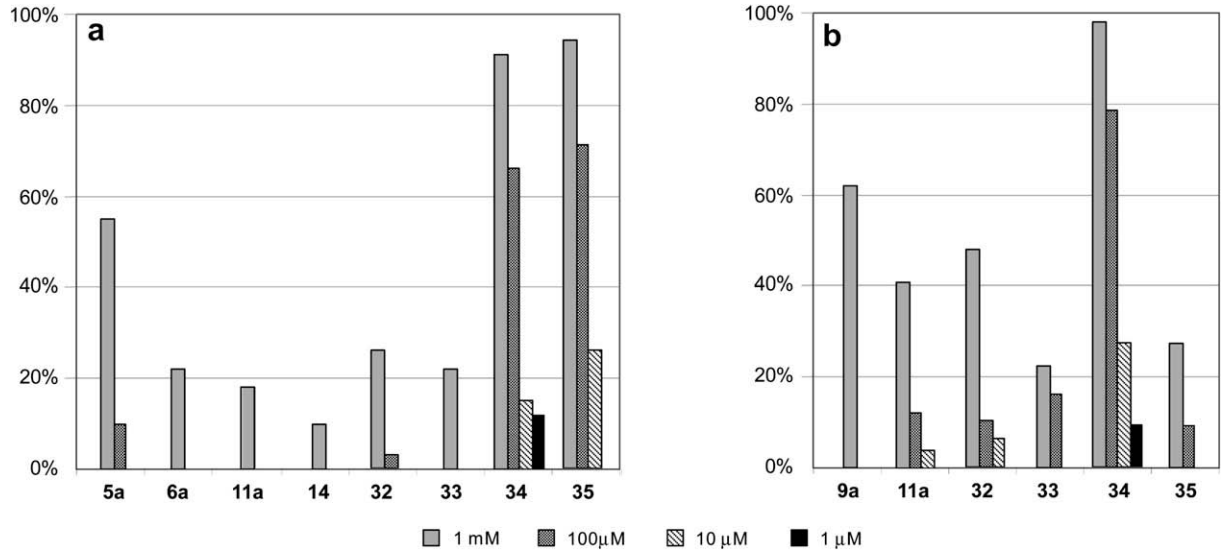


Figure 7. Inhibition assay results for the library of β-linked glucopyranosyl triazoles **5a**–**11a**, **12**–**18** and control compounds **32**–**35**. Only positive responses are shown; the error in the data is typically 5–10% or less. (a) Activity against sweet almond β-glucosidase [37 °C, pH 6.2 in AcONa 20 mM, PIPES 10 mM, EDTA 0.1 mM, DMSO 2%, NaN_3 0.002%, monitoring the rate of release of *p*-nitrophenolate from *p*-nitrophenyl β-D-glucopyranoside (625 μM) over 30 min]. (b) Activity against yeast α-glucosidase [37 °C, pH 6.8 in AcONa 20 mM, PIPES 10 mM, EDTA 0.1 mM, DMSO 2%, NaN_3 0.002%, monitoring the rate of release of *p*-nitrophenol from *p*-nitrophenyl α-D-glucopyranoside (200 μM) over 30 min].

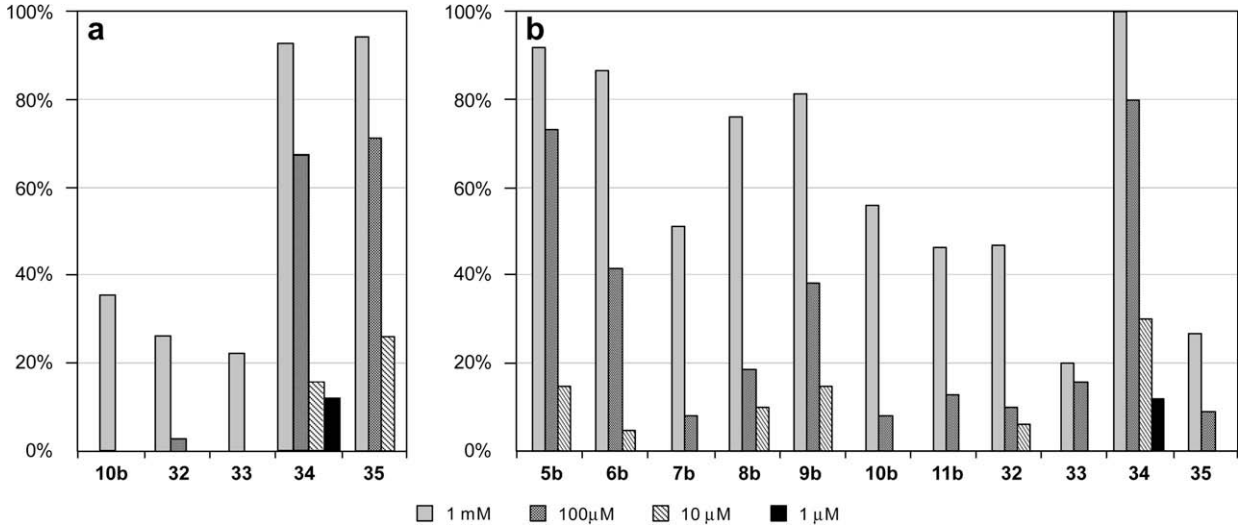


Figure 8. Inhibition assay results for the library of α-linked glucopyranosyl triazoles **5b**–**11b** and control compounds **32**–**35**. Only positive responses are shown; the error in the data is typically 5–10% or less. (a) Activity against sweet almond β-glucosidase [37 °C, pH 6.2 in AcONa 20 mM, PIPES 10 mM, EDTA 0.1 mM, DMSO 2%, NaN_3 0.002%, monitoring the rate of release of *p*-nitrophenolate from *p*-nitrophenyl β-D-glucopyranoside (625 μM) over 30 min]. (b) Activity against yeast α-glucosidase [37 °C, pH 6.8 in AcONa 20 mM, PIPES 10 mM, EDTA 0.1 mM, DMSO 2%, NaN_3 0.002%, monitoring the rate of release of *p*-nitrophenol from *p*-nitrophenyl α-D-glucopyranoside (200 μM) over 30 min].

2.5. Conclusions

In the present study an expeditious synthesis of a library of variously substituted α- and β-linked glucopyranosyl triazoles **5a**,**b**–**11a**,**b** and **12**–**18** has been achieved in a parallel manner, using the flexibility of the CuAAC 'click chemistry' approach. These syntheses gave rise to a number of observations. Firstly, for ease of parallel synthesis, a catalytic couple of $CuSO_4$/Cu proved practical for microtitre plate-based triazole syntheses, while $CuSO_4$/NaAsc was effective for microwave-mediated reactions with either acetylated or unprotected sugar azides as starting materials. In exploring a range of alkynes possessing diverse functional groups, the tolerance of CuACC reaction towards various functionalities became an issue, although incompatibility of propargyl amine and propiolic acid with CuAAC conditions was overcome by application of temporary protecting groups.

A significant difference in the reactivity of α- and β-D-glucopyranosyl azides towards alkynes in the CuAAC reaction was noted, with the β-configured azide being at least threefold more reactive than the α-azide. Comparison of X-ray crystallographic data for both the α- and β-linked glucopyranosyl azides highlighted the impact of the anomeric effect on the dipolar character of the anomeric azides, which likely accounts for the observed reactivity differences.

The library of α- and β-linked glucosyl triazoles was assessed for glucosidase inhibitory activity. Given the remarkably relaxed

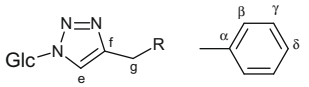
donor and aglycone site specificities of sweet almond β-glucosidase,[51] the very weak inhibition observed in this study is perhaps surprising. In contrast, the yeast α-glucosidase showed a strong preference for α-linked glucosyl triazoles over their β-anomers, regardless of the substitution on the triazole ring. However, at best the inhibition observed was modest (50% inhibition in the 100s of μM range).

In summary, these studies have highlighted points relating to reactant compatibility and reactivity under CuAAC 'click chemistry' conditions, as well as providing rapid access to series of glucosyl triazoles for biological evaluation.

3. Experimental

3.1. General methods

Microwave-assisted syntheses were conducted in Biotage Initiator system. Thin-layer chromatography (TLC) was performed on aluminium-backed, pre-coated silica gel plates (Silica Gel 60 F254, Merck). Spots were detected by immersion in a 5% ethanolic H_2SO_4, followed by heating to 200 °C. Column chromatography was performed on a Biotage Horizon purification system using pre-packed silica gel cartridges and gradient elution. Evaporation of solvents was performed under reduced pressure at 25–40 °C. Optical rotations were measured at 20 °C using a Perkin–Elmer 341 polarimeter. NMR spectra were recorded at 24 °C with a Varian Unity Plus spectrometer at 400 MHz (^{1}H) or 100.6 MHz (^{13}C) or with a Varian Gemini 2000 spectrometer at 75 MHz (^{13}C). ^{1}H NMR and ^{13}C NMR spectra were referenced to residual solvent peaks. ^{1}H NMR spectra were assigned with the help of gCOSY experiments and ^{13}C NMR spectra with the help of gHMBC and gHSQC. Accurate electrospray ionisation mass spectra (ESI-MS) were obtained using Thermofisher LTQ Orbitrap XL in positive mode. Enzymatic assays were performed using a Dynex multiwell plate reader. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide (**19**) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl azide (**25**) were prepared according to the known procedure.[31] Ring atom labelling of triazole, triazole side chains and benzene used throughout this work is exemplified in the structures below.



3.2. General procedure for the Cu(I) catalysed 1,3-dipolar cycloaddition

3.2.1. Method A (applied for synthesis of 21, 22 and 24)

A solution of the sugar azide (1 equiv), the alkyne (1.1 equiv), 1 M aq $CuSO_4{\cdot}5H_2O$ (0.01 equiv) and 1 M aq sodium ascorbate (0.1 equiv) in a *t*-BuOH–H_2O 1:1 mixture (2.8 mL for 1 mmol of the sugar azide) was heated at 70 °C at reflux until TLC showed no traces of the starting sugar azide. The solvent was removed under reduced pressure, the residue was dispersed in EtOAc (50 mL) and the solution was washed with H_2O (3 × 15 mL), dried over $MgSO_4$ and concentrated to give a target adduct. In most cases, materials prepared in this manner did not require further purification.

3.2.2. Method B (applied for synthesis of 20, 23 and 26–30)

A solution of β-azide **19** or α-azide **5** (1 equiv), the alkyne (5 equiv), 1 M aq $CuSO_4{\cdot}5H_2O$ (0.01 equiv) and 1 M sodium ascorbate (0.1 equiv) in DMF (0.35 mL for 1 mmol of the sugar azide) was irradiated in a microwave reactor until the reaction was complete (TLC). The target adduct was isolated as described in Method A and purified by column chromatography on silica gel using an appropriate gradient elution with hexane–EtOAc as a mobile phase.

3.2.3. Method C (applied for synthesis of 12–18)

To a solution of azide **31** (30 mg, 0.146 mmol) in H_2O (130 μL) were added the alkyne (1.1 equiv), 1 M aq $CuSO_4{\cdot}5H_2O$ (14.6 μL), a piece of copper turning (~5 mg) and 5:3:2 mixture of *t*-BuOH–H_2O–EtOH (500 μL). The reaction was carried out in an HPLC vial (2 mL) with stirring at 45 °C until reaction was complete (~24 h) as judged by TLC (CH_2Cl_2–CH_3OH 8:2). Solvents were removed under reduced pressure to give a blue-green residue which was suspended in CH_3OH (3 mL) and filtered through a plug of silica gel (1 cm in a Pasteur pipette), silica gel was washed with additional CH_3OH (3 mL) and the combined filtrates were concentrated under reduced pressure to give a white amorphous solid. The product was used in biological assays without further purification.

3.3. General protecting group removal procedures

3.3.1. Deacetylation

To a solution of the acetylated compound in dry CH_3OH a piece of metallic sodium was added, the mixture was stirred at room temperature until completion of the reaction (TLC), the solution was neutralised with Dowex 50WX8-200 (H^+) resin, the resin was filtered off and the solvent was removed under reduced pressure.

3.3.2. Removal of *N*-Boc protecting group

The *N*-Boc protected derivative was suspended in a mixture of CH_2Cl_2–TFA 1:1 (5 mL) and stirred at room temperature until completion of the reaction (TLC). The mixture was concentrated with toluene (20 mL) at reduced pressure and the operation was repeated several times until complete removal of TFA.

3.3.3. Deesterification of methyl esters

To a solution of methyl ester in H_2O or 9:1 H_2O–CH_3OH mixture 1 M aq NaOH solution (10 equiv) was added and the mixture was stirred at room temperature until reaction is complete (TLC). The reaction mixture was neutralised with Dowex 50WX8-200 (H^+) resin, the resin was filtered off and solvents were removed under reduced pressure to give the free carboxylic acid.

3.4. Synthesis of acetylated glucopyranosides

3.4.1. Prop-2-ynyloxydiphenylmethane

A 60% suspension of NaH mineral oil (200 mg 5.08 mmol) was slowly added to a stirred soln of Ph_2CHOH (696 mg, 3.76 mmol) and $CH{\equiv}CCH_2Br$ (20 μL, 4.70 mmol) in DMF (20 mL) at 0 °C under nitrogen. After stirring for 1 h at 22 °C the reaction was quenched by slow addition of CH_3OH at 0 °C with continuous stirring. The solvents were removed under reduced pressure, the solid residue was suspended in EtOAc (50 mL) and washed with H_2O (15 mL × 2), the organic soln was dried over $MgSO_4$, filtered and concentrated. The title compound was obtained as a yellow oil, yield 820 mg, (97%), R_f 0.67 (Hex–EtOAc 9:1) and used without further purification in synthesis of **20** and **26**.

3.4.2. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-benzhydryloxymethyl-[1,2,3]-triazole (20)

Prepared from azide **19** and prop-2-ynyloxydiphenylmethane following Method B (10 min) as a colourless solid (238 mg 98%); $[\alpha]_D$ −26.1 (*c* 1.0, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 7.79 (1H, s, H-e), 7.32 (10H, m, Ar), 5.88 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 5.49 (1H, s, CH-h), 5.46 (1H, t, $J_{1,2} = J_{2,3}$ 9.2 Hz, H-2), 5.42 (1H, t, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 4.66 (2H, s, CH_2-g), 4.30 (1H, dd, $J_{5,6}$ 4.8 Hz, $J_{6a,6b}$ 12.7 Hz,

H-6$_a$), 4.16 (1H, d, $J_{5,6b}$ 4.0 Hz, $J_{6a,6b}$ 12.7 Hz, H-6$_b$), 4.00 (1H, m, H-5), 2.08, 2.07, 2.03, 1.85 (12H, s, CH_3CO); δ_C (150 MHz, $CDCl_3$): 170.8, 170.2, 169.7, 169.2 (CH_3CO), 146.4 (C-f), 141.9 (C-Ar), 128.8 (C-Ar), 128.7 (C-Ar), 127.9 (C-Ar × 2), 120.5 (C-e), 86.0 (C-1), 83.1 (C-h), 75.4 (C-5), 73.0 (C-3), 70.6 (C-2), 68.0 (C-4), 62.4 (C-g), 61.7 (C-6), 21.0, 20.9, 20.8, 20.5 (CH_3CO); HR ESI-MS found *m/z* 618.2061 $[M+Na]^+$; calcd for $C_{30}H_{33}N_3O_{10}$·Na 618.2058.

3.4.3. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-phenyl-[1,2,3]-triazole (21)

Prepared from azide **19** and ethynylbenzene following Method A (45 min) as a colourless solid (248 mg, 97%); R_f 0.17 (Tol–EtOAc 8:2); $[\alpha]_D$ −56.2 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 8.00 (1H, s, H-e), 7.84 (2H, d, $J_{\beta,\gamma}$ 8.4 Hz, H-β), 7.42 (3H, m, H-γ, H-δ), 5.93 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 5.53 (1H, t, $J_{1,2} = J_{2,3}$ 9.2 Hz, H-2), 5.44 (1H, t, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 5.25 (1H, t, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4), 4.33 (1H, dd, $J_{5,6}$ 5.2 Hz, $J_{6a,6b}$ 12.6 Hz, H-6$_a$), 4.15 (1H, d, $J_{6a,6b}$ 12.6 Hz, H-6$_b$), 4.04 (1H, m, H-5), 2.15, 2.10, 2.07 (9H, s, CH_3CO), 1.88 (3H, s, CH_3CO); δ_C (100 MHz, $CDCl_3$): 170.9, 170.4, 169.8, 169.4 (CH_3CO), 148.9 (C-f), 130.3 (C-α) 129.3 (2 × C-γ), 128.9 (C-δ), 126.3 (2 × C-β), 118.1 (C-e), 86.1 (C-1), 75.4 (C-5) 73.0 (C-3), 70.5 (C-2), 69.0 (C-4), 61.9 (C-6), 20.7 (×3) and 20.4 (CH_3CO); HR ESI-MS found *m/z* 498.1484 $[M+Na]^+$; calcd for $C_{22}H_{25}N_3O_9Na$ 498.1483.

3.4.4. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-hydroxymethyl-[1,2,3]-triazole (22)

Prepared from azide **19** and prop-2-yn-1-ol following Method A (10 min) as a colourless solid (232 mg, 99%); R_f 0.26 (Tol–EtOAc 1:9); $[\alpha]_D$ −6.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.82 (1H, s, H-e), 5.89 (1H, d, $J_{1,2}$ 9.1 Hz, H-1), 5.45 (1H, t, $J_{1,2} = J_{2,3}$ 9.1 Hz, H-2), 5.43 (1H, t, $J_{2,3} = J_{3,4}$ 9.1 Hz, H-3), 5.24 (1H, t, $J_{3,4} = J_{4,5}$ 9.1 Hz, H-4), 4.76 (2H, s, CH_2-g), 4.26 (1H, dd, $J_{5,6}$ 4.8 Hz, $J_{6a,6b}$ 12.4 Hz, H-6$_a$), 4.12 (1H, d, $J_{6a,6b}$ 12.4 Hz, H-6$_b$), 4.00 (1H, m, H-5), 3.15 (1H, s, CH_2OH), 2.04 (6H, 2 × s, CH_3CO), 2.01 (3H, s, CH_3CO), 1.84 (3H, s, CH_3CO); δ_C (100 MHz, $CDCl_3$): 171.0, 170.5, 169.9, 169.6, (CH_3CO), 149.0 (C-f), 120.7 (C-e), 81.1 (C-1), 75.5 (C-5), 73.1 (C-3), 70.8 (C-2), 68.1 (C-4), 62.0 (C-6), 56.8 (CH_2-g), 20.9, 20.8 (×2) and 20.6 (CH_3CO); HR ESI-MS found *m/z* 452.1272 $[M+Na]^+$; calcd for $C_{17}H_{23}N_3O_{10}$·Na·452.1276.

3.4.5. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-(*tert*-butoxycarbonyl-aminomethyl)-[1,2,3]-triazole (23)

Prepared from azide **19** and *tert*-butyl prop-2-ynylcarbamate following Method B (2 h) as a colourless solid (85 mg, 95%); R_f 0.40 (Hex–EtOAc 4:6); $[\alpha]_D$ −20.4 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.74 (1H, s, H-e), 5.85 (1H, d, $J_{1,2}$ 8.2 Hz, H-1), 5.21 (2H, m, H-2, H-3), 5.22 (1H, t, $J_{3,4} = J_{4,5}$ 8.2 Hz, H-4), 5.10 (1H, s, NH), 4.40 (1H, d, *J* 4.8 Hz, CH_2-g), 4.3 (1H, dd, $J_{5,6}$ 4.9 Hz, $J_{6a,6b}$ 12.7 Hz, H-6$_a$), 4.11 (1H, d, 4.0 $J_{6a,6b}$ 12.7 Hz, H-6$_b$), 3.98 (1H, m, H-5), 2.07, 2.05, 2.26 (9H, s, CH_3CO), 1.86 (3H, s, CH_3CO), 1.43 [9H, s, $(CH_3)_3CNH$]; δ_C (100 MHz, $CDCl_3$): 170.8, 170.2, 169.6, 169.0 (CH_3CO), 156.3 [$C(CH_3)_3$], 155.9 (NHCO), 146.4 (C-f), 120.5 (C-e), 86.0 (C-1), 75.4 (C-5), 72.9 (C-3), 70.5 (C-2), 67.9 (C-4), 61.7 (C-6), 36.4 (CH_2-g), 29.9 [$C(CH_3)_3$]; 20.9, 20.8, 20.8, 20.4 (CH_3CO); HR ESI-MS found *m/z* 551.1962 $[M+Na]^+$; calcd for $C_{22}H_{32}N_4O_{11}Na$ 551.1960.

3.4.6. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-methoxycarbonyl-[1,2,3]-triazole (24)

Prepared from azide **19** and methyl propiolate were reacted following Method A (45 min) as a colourless solid (88 mg 70%); R_f 0.20 (PET–EtOAc 5:5); mp 205–207.5 (DMF); $[\alpha]_D$ −28.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 8.36 (1H, s, H-e), 5.92 (1H, d, $J_{1,2}$ 9.1 Hz, H-1), 5.43 (1H, t, $J_{2,3} = J_{3,4}$ 9.1 Hz, H-3), 5.39 (1H, t, $J_{1,2} = J_{2,3}$ 9.1 Hz, H-2), 5.24 (1H, t, $J_{3,4} = J_{4,5}$ 9.1 Hz, H-4), 4.31 (1H, dd, $J_{5,6}$ 5.2 Hz, $J_{6a,6b}$ 12.6 Hz, H-6$_a$), 4.16 (1H, d, $J_{6a,6b}$ 12.6 Hz, H-6$_b$), 4.0 (1H, m, H-5), 3.95 (3H, s, CH_3CO_2-g), 2.07, 2.06, 2.02 (9H, s, CH_3CO), 1.88 (3H, s, CH_3CO); δ_C (100 MHz, $CDCl_3$): 170.8, 170.2, 169.7, 169.3 (CH_3CO), 160.9 (CH_3OCO), 140.9 (C-f), 126.4 (C-e), 86.2 (C-1), 75.6 (C-5), 72.5 (C-3), 70.7 (C-2), 67.82 (C-4), 61.6 (C-6), 52.51 (triazole-CH_3CO), 20.7, 20.6, 20.57, 20.2 (CH_3CO); HR ESI-MS found *m/z* 480.1228 $[M+Na]^+$; calcd for $C_{18}H_{23}N_3O_{11}Na$ 480.1225.

3.4.7. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-4-benzhydryloxymethyl-[1,2,3]-triazole (26)

Prepared from azide **25** (105 mg 0.281 mmol) and prop-2-ynyloxydiphenylmethane (100 μL) following Method B (30 min) as a colourless solid (167 mg 99%); $[\alpha]_D$ +78.9 (*c* 1.0, CH_3Cl); δ_H (600 MHz, $CDCl_3$): 7.65 (1H, s, H-e), 7.37 (4H, m, Ar), 7.34 (4H, m, Ar), 7.26 (2H, m, Ar), 6.34 (1H, d, $J_{1,2}$ 6.0 Hz, H-1), 6.28 (1H, t, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3), 5.54 (1H, s, CH-h), 5.31 (1H, dd, $J_{1,2}$ 6.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.27 (1H, t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 4.66 (2H, s, CH_2-g), 4.36 (1H, m, H-5), 4.25 (1H, dd, $J_{5,6}$ = 2.7 Hz, $J_{6a,6b}$ = 12.6 Hz, H-6$_a$), 4.02 (1H, dd, $J_{5,6}$ 2.7 Hz, $J_{6a,6b}$ 12.6 Hz, H-6$_b$), 2.06, 2.05, 2.03, 1.85 (12H, s, CH_3CO); δ_C (150 MHz, $CDCl_3$): 170.8, 170.5, 170.0, 169.9 (CH_3CO), 145.5 (C-Ar), 141.8 (C-f), 128.8 (C-Ar), 128.7 (C-Ar), 127.4 (C-Ar), 125.0 (C-e), 83.1 (C-h), 81.6 (C-1), 71.4 (C-5), 70.7 (C-3), 70.1 (C-2), 68.3 (C-4), 62.5 (C-g), 61.5 (C-6), 20.9, 20.8, (CH_3CO); HR ESI-MS found *m/z* 596.2237 $[M+H]^+$; calcd for $C_{30}H_{33}N_3O_{10}$·H 596.2239.

3.4.8. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-4-phenyl-[1,2,3]-triazole (27)

Azide **25** (168 mg, 0.451 mmol) and phenyl acetylene (247 μL, 0.451 mmol) were allowed to react following Method B (120 min) and the product was purified by re-crystallisation from EtOAc–Hex to give **27** (134 mg, 63%); R_f 0.17 (Tol–EtOAc 8:2); mp 196–197 °C; $[\alpha]_D$ +85.5 (*c* 1.0, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 7.86 (1H, s, H-e), 7.45 (2H, d, $J_{\beta,\gamma}$ = 8.4 Hz, H-β), 7.42 (3H, m, H-γ, H-δ), 6.42 (1H, d, $J_{1,2}$ 6.0 Hz, H-1), 6.35 (1H, t, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3) 5.34 (1H, dd, $J_{1,2}$ 6.0, Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.81 (1H, t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 4.39 (1H, m, H-5), 4.27 (1H, dd, $J_{5,6}$ 4.2 Hz, $J_{6a,6b}$ 12.6 Hz, H-6$_a$), 4.04 (1H, d, $J_{6a,6b}$ 12.6 Hz, H-6$_b$), 2.07, 2.06, 2.04, (9H, s, CH_3CO), 1.88 (3H, s, CH_3CO); δ_C (150 MHz, $CDCl_3$): 170.6, 170.5, 169.9, 169.8 (CH_3CO), 147.5 (C-e), 130.3 (C-α) 129.3 (×2) (C-γ), 128.9 (C-δ), 126.3 (×2 C-β), 118.1 (C-f), 86.1 (C-1), 75.4 (C-5) 73.0 (C-3), 70.5 (C-2), 69.0 (C-4), 61.9 (C-6), 20.7 (×3), 20.4 (CH_3CO); HR ESI-MS found *m/z* 476.1662 $[M+H]^+$; calcd for $C_{22}H_{25}N_3O_9$·H 476.1664.

3.4.9. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-4-hydroxymethyl-[1,2,3]-triazole (28)

Prepared from azide **25** (205 mg, 0.549 mmol) and prop-2-yn-1-ol (0.160 μL, 2.745 mmol) following Method B (60 min) to give a colourless solid (165 mg, 70%); R_f 0.26 (Tol–EtOAc 1:9); $[\alpha]_D$ +99.4 (*c* 1.0, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 7.66 (1H, s, H-e), 6.36 (1H, d, $J_{1,2}$ 6.0 Hz, H-1), 6.25 (1H, t, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3), 5.32 (1H, dd, $J_{1,2}$ 6.0, $J_{2,3}$ 9.6 Hz, H-2), 5.25 (1H t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 4.83 (2H, s, CH_2-g), 4.32 (1H, m, H-5), 4.30 (1H, dd, $J_{5,6}$ 4.2 Hz, $J_{6a,6b}$ 12.6 Hz, H-6$_a$), 4.00 (1H, dd, $J_{5,6}$ 4.2 Hz, $J_{6a,6b}$ 12.6 Hz, H-6$_b$), 2.81 (1H, s, CH_2OH), 2.06, 2.05, 2.02 (9H, CH_3CO), 1.87 (3H, s, CH_3CO); δ_C (150 MHz, $CDCl_3$): 170.6, 170.3, 169.8, 169.7, (CH_3CO), 147.4 (C-f), 124.1 (C-e), 81.5 (C-1), 71.2 (C-5), 70.5 (C-3), 69.89 (C-2), 68.1 (C-4), 61.4 (C-6), 56.4 (CH_2-g), 20.8 (×2), 20.7, 20.5 (CH_3CO); HR ESI-MS found *m/z* 430.1446 $[M+H]^+$; calcd for $C_{17}H_{23}N_3O_{10}$·H 430.1456.

3.4.10. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-4-(*tert*-butyl-oxycarbonylaminomethyl)-[1,2,3]-triazole (29)

Prepared from azide **25** (157 mg, 0.421 mmol) and *tert*-butyl prop-2-ynylcarbamate (176 mg, 1.052 mmol) following Method B

to give a colourless solid (209 mg, 94%); R_f 0.47 (Hex–EtOAc 4:6); $[\alpha]_D$ +3.6 (*c* 0.5, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 7.59 (1H, s, H-e), 6.30 (1H, d, $J_{1,2}$ = 6.0 Hz, H-1), 6.25 (1H, t, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3), 5.30 (1H, dd, $J_{1,2}$ 6.0, $J_{2,3}$ 9.6 Hz, H-2), 5.26 (1H, t, $J_{3,4} = J_{4,3}$ 9.6 Hz, H-4), 5.12 (1H, s, NH), 4.42 (1H, br d, CH_2-g), 4.35 (1H, m, H-5), 4.25 (1H, dd, $J_{5,6}$ 4.22 Hz, $J_{6a,6b}$ 12.6 Hz, H-6_a), 4.00 (1H, d, $J_{5,6}$ 4.22 Hz, $J_{6a,6b}$ 12.7 Hz, H-6_b), 2.07, 2.06, 2.03 (9H, s, CH_3CO), 1.87 (3H, s, CH_3CO), 1.44 [9H, s, (CH_3)$_3$C]; δ_C (150 MHz, $CDCl_3$): 158.3 (NH*C*O), 147.2 (C-f), 123.2 (C-e), 89.6 (C-1), 81.1 (C-5), 80.4 [*C*(CH$_3$)$_3$], 78.4 (C-3), 74.0 (C-2), 70.9 (C-4), 62.4 (C-6), 36.7 (CH_2-g), 28.7 [C(*CH$_3$*)$_3$]; HR ESI-MS found *m/z* 529.2139 $[M+H]^+$; calcd for $C_{22}H_{32}N_4O_{11}$·H 529.1956.

3.4.11. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-4-methyloxycarbonyl-[1,2,3]-triazole (30)

Prepared from azide **25** (168 mg, 0.451 mmol) and methyl propiolate (200 μL, 2.255 mmol) following Method B (45 min) and purified by crystallisation from EtOAc–Hex to give a white powder (198 mg 96%); R_f 0.17 (Hex–EtOAc 5:5); $[\alpha]_D$ +100.1 (*c* 1.0, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 8.22 (1H, s, H-e), 6.41 (1H, d, $J_{1,2}$ 6.0 Hz, H-1), 6.17 (1H, t, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3), 5.33 (1H, dd, $J_{1,2}$ 6.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.24 (1H, t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 4.35 (1H, m, H-5), 4.25 (1H, dd, $J_{5,6}$ 4.2 Hz, $J_{6a,6b}$ 12.6 Hz, H-6_a), 4.02 (1H, dd, $J_{5,6}$ 4.2 Hz, $J_{6a,6b}$ 12.6 Hz, H-6_b), 3.95 (3H, s, CH_3OCO), 2.05, 2.04, 2.01 (9H, s, *CH$_3$*CO), 1.86 (3H, s, *CH$_3$*COC-2); δ_C (150 MHz, $CDCl_3$): 170.5, 170.1, 169.7, 169.6 (CH$_3$*C*O), 160.7 (triazole-CH$_3$O*C*O), 139.7 (C-f), 129.8 (C-e), 82.1 (C-1), 71.7 (C-5), 70.2 (C-3), 69.6 (C-2), 67.9 (C-4), 61.3 (C-6), 52.5 (*C*H$_3$CO), 20.7, 20.6, 20.5, 20.4 (*CH$_3$*CO); HR ESI-MS found *m/z* 480.1225 $[M+Na]^+$; calcd for $C_{18}H_{23}N_3O_{11}$·Na 480.1225.

3.5. Unprotected glucopyranosides

3.5.1. 1-(β-D-Glucopyranosyl)-4-methyl-benzhydryloxymethyl-[1,2,3]-triazole (5a)

Compound **20** (25 mg, 40.5 μmol) was deacetylated to give a white solid (16 mg, 88%); $[\alpha]_D$ −26.1 (*c* 1.0, CH_3OH); δ_H (600 MHz, CD_3OD): 8.19 (1H, s, H-e), 7.37 (3H, m, Ar), 7.31 (3H, m, Ar), 7.24 (2H, m, Ar), 5.61 (1H, d, $J_{1,2}$ 9.1 Hz, H-1), 5.55 (1H, s, CH-h), 4.06 (2H, s, CH_2-g), 3.90 (1H, t, $J_{1,2} = J_{2,3}$ 9.1 Hz, H-2), 3.88 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 10.9 Hz, H-6_a), 3.72 (1H, dd, $J_{5,6}$ 5.4 Hz $J_{6a,6b}$ 10.9 Hz, H-6_b), 3.57 (2H, m, H-3, H-5), 3.51 (1H, t, $J_{3,4} = J_{4,5}$ 9.1 Hz, H-4); δ_C (150 MHz, CD_3OD): 146.1 (C-f), 143.2 (C-Ar), 130.9 (C-Ar), 129.4 (C-Ar), 128.6 (C-Ar), 128.1 (C-Ar), 124.4 (C-e), 89.6 (C-1), 84.2 (C-h), 81.0 (C-3 or C-5), 78.4 (C-3 or C-5), 73.9 (C-2), 70.8 (C-4), 62.7 (C-6), 62.3 (C-g); HR ESI-MS found *m/z* 428.1816 $[M+Na]^+$; calcd for $C_{25}H_{25}N_3O_6$·H 428.1816.

3.5.2. 1-(β-D-Glucopyranosyl)-4-phenyl-[1,2,3]-triazole (6a)

Compound **21** (110 mg, 0.235 mmol) was deacetylated to give a white solid (67 mg, 94%); R_f 0.78 (CH_2Cl_2–MeOH–H_2O 6:3:1); $[\alpha]_D$ −7.6 (*c* 0.6, CH_3OH); δ_H (400 MHz, CD_3OD): 8.55 (1H, s, H-e), 7.84 (2H, d, $J_{\beta,\gamma}$ 4.8 Hz, H-β), 7.44 (2H, t, $J_{\beta,\gamma} = J_{\gamma,\delta}$ 4.8 Hz, H-γ), 7.35 (1H, t, $J_{\gamma,\delta}$ 4.8 Hz, H-δ), 5.66 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 3.95 (1H, t, $J_{1,2} = J_{2,3}$ 9.2 Hz, H-2) 3.90 (1H, br d, $J_{5,6}$ 3.6 Hz, H-6_a) 3.74 (1H, dd, $J_{5,6}$ 3.6 Hz, $J_{6a,6b}$ 8.0 Hz, H-6_b), 3.61 (2H, m, H-3, H-5), 3.54 (1H, t, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4); δ_C (100 MHz, CD_3OD): 148.9 (C-f), 131.5 (C-α), 130.0 (C-γ × 2), 129.5 (C-δ), 126.7 (C-β × 2), 121.4 (C-e), 89.7 (C-1), 81.1 (C-5), 78.4 (C-3), 74.1 (C-2), 70.9 (C-4), 62.4 (C-6); HR ESI-MS found *m/z* 308.1242 $[M+H]^+$; calcd for $C_{14}H_{17}N_3O_5$·H 308.1241.

3.5.3. 1-(β-D-Glucopyranosyl)-4-hydroxymethyl-[1,2,3]-triazole (7a)

Compound **22** (123 mg, 0.287 mmol) was deacetylated to give a white solid (70 mg, 92%); R_f 0.38 (CH_2Cl_2–MeOH–H_2O 6:3:1); $[\alpha]_D$ +85.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, D_2O) 8.21 (1H, s, H-e), 5.76 (1H, d, $J_{1,2}$ 9.1 Hz, H-1), 4.76 (2H, s, CH_2-g), 4.01 (1H, t, $J_{1,2} = J_{2,3}$ 9.1 Hz, H-2), 3.91 (1H, br d, $J_{6a,6b}$ 10.9 Hz, H-6_a), 3.75 (3H, m, H-3, H-5, H-6_b), 3.63 (1H, t, $J_{3,4} = J_{4,5}$ 9.1 Hz, H-4); δ_C (100 MHz, D_2O): 148.0 (C-f), 124.1 (C-e), 88.1 (C-1), 79.6 (C-5), 76.7 (C-3), 73.0 (C-2), 69.7 (C-4), 61.1 (C-6), 55.3 (C-g); HR ESI-MS found *m/z* 284.0852 $[M+Na]^+$; calcd for $C_9H_{15}N_3O_6$·Na 284.08653.

3.5.4. 1-(β-D-Glucopyranosyl)-4-(*tert*-butyl-oxycarbonylaminomethyl)-[1,2,3]-triazole (8a)

Compound **23** (83 mg, 0.158 mmol) was deacetylated to give a white solid (53.5 mg, 91%); R_f 0.80 (CH_2Cl_2–MeOH–H_2O 7:2:1); $[\alpha]_D$ +1.0 (*c* 0.5, CD_3OD); δ_H (400 MHz, CD_3OD) 8.02 (1H, s, H-e), 5.57 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 4.32 (2H, s, CH_2-g), 3.87 (2H, m, H-2, H-6_a), 3.71 (1H, dd, $J_{5,6}$ 3.0 Hz, $J_{6a,6b}$ 8.0 Hz, H-6_b), 3.55 (2H, m, H-3, H-5), 3.49 (1H, t, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4), 1.44 [9H, s, *(CH$_3$)$_3$*]; δ_C (100 MHz, CD_3OD): 158.3 (NH*C*O), 147.2 (C-f), 123.2 (C-e), 89.6 (C-1), 81.1 (C-5), 80.4 [*C*(CH$_3$)$_3$], 78.4 (C-3), 74.0 (C-2), 70.9 (C-4), 62.4 (C-6), 36.7 (CH_2-g), 28.7 [C(*C*H$_3$)$_3$]; HR ESI-MS found *m/z* 383.1539 $[M+Na]^+$; calcd for $C_{14}H_{24}N_4O_7$·Na 383.1537.

3.5.5. 1-(β-D-Glucopyranosyl)-4-methoxycarbonyl-[1,2,3]-triazole (9a)

Compound **24** (97 mg, 0.213 mmol) was deacetylated to give a white solid (58 mg, 95%); R_f 0.60 (CH_2Cl_2–MeOH–H_2O 6:3:1); $[\alpha]_D$ +0.5 (*c* 0.5, CH_3OH); δ_H (400 MHz, CD_3OD): 8.81 (1H, s, H-e), 5.68 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 3.89 (5H, m, H-2, H-6_a, CH_3OCO-g), 3.72 (1H, dd, $J_{5,6}$ 5.8 Hz, $J_{6a,6b}$ 12.4 Hz, H-6_b), 3.58 (2H, m, H-3, H-5), 3.52 (1H, t, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4); δ_C (100 MHz, CD_3OD): 141.9 (C-f), 124.6 (C-e), 89.5 (C-1), 81.1 (C-5), 78.5 (C-3), 74.0 (C-2), 70.9 (C-4), 62.3 (C-6), 35.6 (CH_2-g); HR ESI-MS found *m/z* 290.0982 $[M+H]^+$; calcd for $C_{10}H_{16}N_3O_7$·H 290.0983.

3.5.6. 1-(β-D-Glucopyranosyl)-4-aminomethyl-[1,2,3]-triazole (10a)

Compound **8a** (28 mg, 0.079 mmol) was treated as described in Section 3.3.2 to give **10a** as a yellow solid (27 mg, 91%); $[\alpha]_D$ −0.5 (*c* 0.5, CH_3OH); δ_H (400 MHz, CD_3OD): 8.25 (1H, s, H-e), 5.64 (1H, d, $J_{1,2}$ 6.2 Hz, H-1), 4.24 (2H, s, CH_2-g), 3.89 (2H, m, H-2, H-6_a), 3.71 (1H, dd, $J_{5,6}$ 3.6 Hz, $J_{6a,6b}$ 8.0 Hz, H-6_b), 3.55 (2H, m, H-3, H-5), 3.49 (1H, t, $J_{3,4} = J_{4,5}$ 6.2 Hz, H-4); δ_C (100 MHz, CD_3OD): 141.9 (C-f), 124.6 (C-e), 89.5 (C-1), 81.1 (C-5), 78.5 (C-3), 74.0 (C-2), 70.9 (C-4), 62.3 (C-6), 35.6 (CH_2-g); HR ESI-MS found *m/z* 261.1189 $[M+H]^+$; calcd for $C_{14}H_{24}N_4O_7$?H 261.1193.

3.5.7. 1-(β-D-Glucopyranosyl)-4-carboxy-[1,2,3]-triazole (11a)

Compound **9a** (159 mg, 0.552 mmol) was deprotected according method Section 3.3.3 to give a white solid (139 mg, 92%); δ_H (400 MHz, CD_3OD): 8.48 (1H, s, H-e), 5.66 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 3.89 (1H, t, $J_{1,2} = J_{2,3}$ 9.2 Hz, H-2), 3.78 (1H, br d, $J_{6a,6b}$ 12.0 Hz H-6_a), 3.62 (3H, m, H-3, H-5, H-6_b) 3.50 (1H, t, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4); δ_C (100 MHz, CD_3OD): 164.9 (CO), 142.2 (C-f), 127.5 (C-e), 87.4 (C-1), 78.7 (C-5), 75.6 (C-3), 72.1 (C-2), 68.7 (C-4), 60.2 (C-6); HR ESI-MS found *m/z* 290.0982 $[M+H]^+$; calcd for for $C_{10}H_{16}N_3O_7$·H 290.0983.

3.5.8. 1-(α-D-Glucopyranosyl)-4-methyl-benzhydryloxymethyl-[1,2,3]-triazole (5b)

Compound **26** (70 mg, 0.118 mmol) was deacetylated to give **5b** as a white solid (45 mg, 90%); R_f 0.38 (CH_2Cl_2–CH_3OH); $[\alpha]_D$ +50.1 (*c* 1.0, CH_3OH); δ_H (600 MHz, CD_3OD): 8.12 (1H, s, H-e), 7.39 (4H, m, Ar), 7.31 (4H, m, Ar), 7.24 (2H, m, Ar), 6.20 (1H, d, $J_{1,2}$ 6.0 Hz, H-1), 5.57 (1H, s, CH_2-h), 4.64 (2H, s, CH_2-g), 4.38 (1H, t, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3), 3.98 (1H, dd, $J_{1,2}$ 6.0 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.81 (1H, m, H-5), 3.78 (1H, dd, $J_{5,6}$ 3.9 Hz, $J_{6a,6b}$ 12.0 Hz, H-6_a), 3.69 (1H, dd, $J_{5,6}$ 3.9 Hz $J_{6a,6b}$ 12.0 Hz, H-6_b), 3.51 (1H, t, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4); δ_C (150 MHz, CD_3OD): 145.2 (C-Ar), 143.3 (C-f), 128.6

(C-Ar), 128.1 (C-Ar), 128.6 (C-Ar), 128.1 (C-Ar), 127.4 (C-e), 87.2 (C-1), 84.2 (C-h), 77.5 (C-5), 74.8 (C-3), 72.4 (C-2), 71.4 (C-4), 62.7 (C-g), 62.4 (C-6); HR ESI-MS found *m/z* 428.1818 $[M+H]^+$; calcd for $C_{25}H_{25}N_3O_6$·H 428.1816.

3.5.9. 1-(α-D-Glucopyranosyl)-4-phenyl-[1,2,3]-triazole (6b)

Compound **27** (65 mg, 0.137 mmol) was deacetylated to give **6b** as a white solid (49 mg, 99%); R_f 0.55 (CH_2Cl_2–MeOH–H_2O 8:2); δ_H (600 MHz, CD_3OD): 8.44 (1H, s, H-e), 7.84 (2H, d, $J_{\beta,\gamma}$ = 7.5 Hz, H-β), 7.42 (2H, t, $J_{\beta,\gamma} = J_{\gamma,\delta}$ 7.5 Hz, H-γ), 7.35 (1H, t, $J_{\gamma,\delta}$ 7.5 Hz, H-δ), 6.23 (1H, d, $J_{1,2}$ 5.4 Hz, H-1), 4.21 (1H, t, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3), 4.00 (1H, dd, $J_{1,2}$ 5.4 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.84 (1H, m, H-5), 3.79 (1H, dd, $J_{5,6}$ 4.2 Hz, $J_{6a,6b}$ 12.3, H-6_a), 3.69 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.3 Hz, H-6_b), 3.52 (1H, t, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4); δ_C (150 MHz, CD_3OD): 148.4 (C-f), 132.0 (C-α), 130.5 (C-γ × 2), 129.9 (C-δ), 127.3 (C-β × 2), 125.0 (C-e), 87.8 (C-1), 78.1 (C-5), 75.4 (C-3), 72.9 (C-2), 71.9 (C-4), 62.9 (C-6); HR ESI-MS found *m/z* 308.1240 $[M+H]^+$; calcd for $C_{14}H_{17}N_3O_5$·H 308.12410.

3.5.10. 1-(α-D-Glucopyranosyl)-4-hydroxymethyl-[1,2,3]-triazole (7b)

Compound **28** (50 mg, 0.137 mmol) was deacetylated to give **7b** as a white solid (28 mg, 94%); δ_H (600 MHz, CD_3OD): 8.04 (1H, s, H-e), 6.17 (1H, d, $J_{1,2}$ 6.0 Hz, H-1), 4.70 (2H, s, CH_2-g), 4.35 (1H, t, $J_{2,3} = J_{3,4}$ 9.6 Hz, Hz, H-3), 3.97 (1H, dd, $J_{1,2}$ 6.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 3.75 (2H, m, H-5, H-6_a), 3.68 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.3 Hz, H-6_b), 3.49 (1H, t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4); δ_C (150 MHz, CD_3OD): 148.7 (C-f), 126.9 (C-e), 87.6 (C-1), 77.9 (C-5), 75.4 (C-3), 72.9 (C-2), 71.9 (C-4), 62.8 (C-6), 56.9 (CH_2-g); HR ESI-MS found *m/z* 284.0852 $[M+Na]^+$; calcd for $C_9H_{15}N_3O_6$·Na 284.08653.

3.5.11. 1-(α-D-Glucopyranosyl)-4-(*tert*-butyl-oxycarbonylaminomethyl)-[1,2,3]-triazole (8b)

Compound **29** (141 mg, 0.266 mmol) was deacetylated to give **8b** as a white solid (92 mg, 99%); R_f 0.46 (CH_2Cl_2–MeOH 8:2); δ_C (600 MHz, CD_3OD): 7.96 (1H, s, H-e), 6.16 (1H, d, $J_{1,2}$ 6.0 Hz, H-1), 4.34 (1H, t, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3), 4.00 (1H, dd, $J_{1,2}$ 6.0 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.75 (2H, m, H-5, H-6_a), 3.66 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.3 Hz, H-6_b), 3.52 (1H, t, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4), 1.44 [9H, s, (CH_3)$_3$C]; δ_C (150 MHz, CD_3OD): 158.3 (NH*C*O), 146.7 (C-f), 126.8 (C-e), 87.6 (C-1), 80.9 [*C*(CH_3)$_3$], 77.9 (C-5), 75.4 (C-3), 72.9 (C-2), 71.9 (C-4), 62.8 (C-6), 36.8 (CH_2-g), 28.9 [C(*C*H_3)$_3$]; HR ESI-MS found *m/z* 361.17178 $[M+H]^+$; calcd for $C_{14}H_{24}N_4O_6$·H 361.17178.

3.5.12. 1-(α-D-Glucopyranosyl)-4-carboxymethyl-[1,2,3]-triazole (9b)

Compound **30** (158 mg, 0.346 mmol) was deacetylated to give **9b** as a white solid (113 mg, 99%); R_f 0.41 (CH_2Cl_2–MeOH–H_2O 8:2); δ_H (600 MHz, CD_3OD): 8.68 (1H, s, H-e), 6.25 (1H, d, $J_{1,2}$ 6.0 Hz, H-1), 4.30 (1H, t, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3), 3.98 (1H, dd, $J_{1,2}$ 6.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 3.98 (3H, s, C*H*$_3$OCO), 3.87 (1H, m, H-5), 3.78 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.3 Hz, H-6_a), 3.69 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.3 Hz, H-6_b), 3.50 (1H, t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4); δ_C (150 MHz, CD_3OD): 162.9 (C*H*$_3$O*C*O), 140.1 (C-f), 132.5 (C-e), 88.2 (C-1), 78.5 (C-5), 75.1 (C-3), 72.6 (C-2), 71.7 (C-4), 62.8 (C-6), 53.1 (*C*H$_3$OCO); HR ESI-MS found *m/z* 312.0801 $[M+Na]^+$; calcd for $C_{10}H_{15}N_3O_7$·Na 312.0802.

3.5.13. 1-(β-D-Glucopyranosyl)-4-aminomethyl-[1,2,3]-triazole (10b)

Compound **8b** was treated as described in Section 3.3.2 to give the title compound as a yellow solid (27 mg, 99%); δ_H (600 MHz, CD_3OD): 8.57 (1H, s, H-e), 6.29 (1H, d, $J_{1,2}$ 5.8 Hz, H-1), 4.32 (1H, t, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3), 3.98 (1H, dd, $J_{1,2}$ 5.8 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.84 (1H, m, H-5), 3.77 (1H, br d, $J_{6a,6b}$ 12.3 Hz, H-6_a), 3.69 (1H, dd, $J_{5,6}$ 4,8 Hz, $J_{6a,6b}$ 12.3 Hz, H-6_b), 3.51 (1H, t, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4); δ_C (150 MHz, CD_3OD): 140.3 (C-f), 127.8 (C-e), 87.2 (C-1), 77.6 (C-5), 74.7 (C-3), 72,2 (C-2), 71.3 (C-4), 62.3 (C-6), 35.3 (C-g); HR ESI-MS found *m/z* 261.1189 $[M+H]^+$; calcd for $C_{14}H_{24}N_4O_7$·H 261.1193.

3.5.14. 1-(α-D-Glucopyranosyl)-4-carboxy-[1,2,3]-triazole (11b)

Compound **9b** (37 mg, 0.128 mmol) was deprotected following the general method Section 3.3.3 to give **11b** as a white solid (33 mg, 94%); δ_H (600 MHz, CD_3OD): 8.05 (1H, s, H-e), 6.24 (1H, d, $J_{1,2}$ 6.0 Hz, H-1), 4.35 (1H, t, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3), 3.99 (1H, dd, $J_{1,2}$ 6.0 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.85 (1H, m, H-5), 3.77 (1H, dd, $J_{5,6}$ 3.6 Hz, $J_{6a,6b}$ 12.0 Hz, H-6_a), 3.70 (1H, dd, $J_{5,6}$ 3.6 Hz, $J_{6a,6b}$ 12.0 Hz, H-6_b), 3.50 (1H, t, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4); δ_C (150 MHz, CD_3OD): 142.8 (C-f), 131.0 (C-e), 87.5 (C-1), 77.8 (C-5), 74.7 (C-3), 72.3 (C-2), 71.3 (C-4), 62.3 (C-6); HR ESI-MS found *m/z* 261.1189 $[M+H]^+$; calcd for $C_{14}H_{24}N_4O_7$·H 261.1193.

3.5.15. 1-(β-D-Glucopyranosyl)-4-benzyl-[1,2,3]-triazole (12)

Prepared from azide **31** and prop-2-ynylbenzene according to Method C as a white solid, (15 mg, 33%); δ_H (600 MHz, CD_3OD): 7.90 (1H, s, H-e), 7.28 (4H, m, H-β, H-γ), 7.20 (1H, t, $J_{\gamma,\delta}$ = 6.6 Hz, H-δ), 5.56 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 4.06 (2H, s, CH_2-g) 3.87 (2H, m, H-2, H-6_a) 3.69 (1H, dd, $J_{5,6}$ 5.6 Hz, $J_{6a,6b}$ 12.3 Hz, H-6_b) 3.54 (2H, m, H-3, H-5), 3.47 (1H, t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4); δ_C (150 MHz, CD_3OD): 140.2 (C-f), 129.6 (C-α, C-γ × 2, C-β × 2), 129.5 (C-δ), 127.5 (C-e), 89.6 (C-1), 81.1 (C3 or C-5), 78.5 (C-3 or C5), 73.9 (C-2), 70.9 (C-4), 62.4 (C-6), 32.6 (CH_2-g); HR ESI-MS found *m/z* 322.1399 $[M+H]^+$; calcd for $C_{15}H_{19}N_3O_5$·H 322.1397.

3.5.16. 1-(β-D-Glucopyranosyl)-4-phthalimidomethyl-[1,2,3]-triazole (13)

Prepared from azide **31** and 3-phthalimidoprop-1-yne according to Method C as a white solid (57 mg, 80%); δ_H (600 MHz, CD_3OD): 8.19 (1H, s, H-e), 7.89, (2H, m, CH-Ar), 7.83 (2H, m, CH-Ar), 5.58 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 4.98 (2H, s, CH_2-g), 3.89 (2H, m, H-2, H-6_a), 3.70 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.0 Hz, H-6_b), 3.57 (2H, m, H-3, H-5), 3.49 (1H, t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4); δ_C (150 MHz, CD_3OD): 144.5 (C-f), 169.05 (CO × 2), 135.5 (C-Ar × 2), 133.4 (C-Ar × 2), 124.4 (C-Ar × 2), 124.1 (C-e), 89.6 (C-1), 81.1 (C-3 or C5), 78.4 (C-3 or C5), 74.0 (C-2), 70.9 (C-4), 62.4 (C-6), 33.7 (CH_2-g); HR ESI-MS found *m/z* 391.1250 $[M+H]^+$; calcd for $C_{17}H_{18}N_4O_7$·H 391.1248

3.5.17. 1-(β-D-Glucopyranosyl)-4-(2-hydroxypropyl)-[1,2,3]-triazole (14)

Prepared from azide **31** and pent-4-yn-2-ol according to Method C as a white solid (12 mg, 28%) δ_H (600 MHz, CD_3OD): 7.99 (1H, s, H-e), 5.57 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 4.06 (1H, br s, CH-h), 3.89 (2H, m, H-2, H-6_a), 3.70 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.0 Hz, H-6_b), 3.57 (2H, m, H-3, H-5), 2.40 (2H, m, CH_2-g), 3.49 (1H, t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4) 1.21 (3H, m, CH_3-i); δ_C (150 MHz, CD_3OD): 141.9 (C-f), 123.8 (C-e), 89.5 (C-1), 81.1 (C-3 or C5), 78.5 (C-3 or C5), 74.1 (C-2), 70.9 (C-4), 68.0 (C-h), 62.3 (C-6), 35.9 (C-g). 23.1 (CH_3-i); HR ESI-MS found *m/z* 290.1345 $[M+H]^+$; calcd for $C_{11}H_{19}N_3O_6$·H 290.1347.

3.5.18. 1-(β-D-Glucopyranosyl)-4-(1-hydroxypropyl)-[1,2,3]-triazole (15)

Prepared from azide **31** and pent-1-yn-3-ol according to Method C as a white solid (24 mg, 82%); δ_H (600 MHz, CD_3OD): 8.76 (1H, s, H-e), 5.60 (1H, d, $J_{1,2}$ 9.4 Hz, H-1), 4.74 (1H, br s, CH-g), 3.89 (2H, m, H-2, H-6_a), 3.70 (1H, dd, $J_{5,6}$ 4.8 Hz, $J_{6a,6b}$ 12.3 Hz, H-6_b), 3.57 (2H, m, H-3, H-5), 3.49 (1H, t, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4), 1.86 (2H, m, CH_2-h), 0.97 (3H, m, CH_3-i), δ_C (150 MHz, CD_3OD): 153.0 (C-f), 122.5 (C-e), 89.6 (C-1), 81.1 (C-3 or C5), 78.5 (C-3 or C5), 74.0 (C-2), 70.9 (C-4), 69.0 (CH-g), 62.4 (C-6), 31.3 (CH_2-h). 10.1

(CH_3-i); HR ESI-MS found *m/z* 290.1349 $[M+H]^+$; calcd for $C_{11}H_{19}N_3O_6{\cdot}H$ 290.1347

3.5.19. 1-(β-D-Glucopyranosyl)-4-(3-hydroxypropyl)-[1,2,3]-triazole (16)

Prepared from azide **31** and pent-4-yn-1-ol according to Method C as a white solid (27 mg, 68%) δ_H (600 MHz, CD_3OD): 7.96 (1H, s, H-e), 5.60 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 3.89 (2H, m, H-2, H-6_a), 3.70 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.0 Hz, H-6_b), 3.60 (2H, t, $J_{h,i}$ 6.6 Hz, CH_2-i), 3.56 (2H, m, H-3, H-5) 3.49 (1H, t, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4), 2.79 (2H, $J_{g,h}$ 7.8 Hz, CH_2-g), 1.89 (2H, m, CH_2-h); δ_C (150 MHz, CD_3OD): 148.8 (C-f), 122.5 (C-e), 89.5 (C-1), 81.1 (C-3 or C-5), 78.5 (C-3 or C-5), 74.0 (C-2), 70.9 (C-4), 62.3 (C-6), 62.0 (C-i) 33.2 (C-g). 22.7 (C-h); HR ESI-MS found *m/z* 290.1348 $[M+H]^+$; calcd for $C_{11}H_{19}N_3O_6{\cdot}H$ 290.1347.

3.5.20. 1-(β-D-Glucopyranosyl)-4-(4-hydroxybutyl)-[1,2,3]-triazole (17)

Prepared from azide **31** and hex-5-yn-1-ol according to Method C as a white solid (58 mg, 90%) δ_H (600 MHz, CD_3OD): 7.97 (1H, s, H-e), 5.57 (1H, d, $J_{1,2}$ 9.0 Hz, H-1), 3.87 (2H, m, H-2, H-6_a), 3.70 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.0 Hz, H-6_b), 3.58 (3H, m, H-3, H-5, CH_2-l), 3.49 (1H, t, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4), 2.74 (2H, $J_{g,h}$ 7.8 Hz, CH_2-g), 1.74 (2H, m, CH_2-h), 1.58 (2H, m, CH_2-i); δ_C (150 MHz, CD_3OD): 149.2 (C-f), 122.5 (C-e), 89.5 (C-1), 81.0 (C5), 78.5 (C-3), 73.9 (C-2), 70.8 (C-4), 64.4 (C-l), 62.5 (C-6), 62.0 (C-i) 33.9 (C-g). 26.7 (C-h); HR ESI-MS found *m/z* 304.1504 $[M+H]^+$; calcd for $C_{12}H_{21}N_3O_6{\cdot}H$ 304.1503.

3.5.21. 1-(β-D-Glucopyranosyl)-4-(2-carboxyethyl)-[1,2,3]-triazole (18)

Prepared from azide **31** and pent-4-ynoic acid according to Method C as a white solid (16 mg, 38%); δ_H (600 MHz, CD_3OD): 7.99 (1H, s, H-e), 5.60 (1H, d, $J_{1,2}$ 9.4 Hz, H-1), 3.89 (2H, m, H-2, H-6_a), 3.70 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6a,6b}$ 12.0 Hz, H-6_b), 3.57 (2H, m, H-3, H-5), 3.49 (1H, t, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4), 2.71 (2H, m, CH_2-g), 2.38 (2H, m, CH_2-h), 1.98 (2H, m, CH_2-i); δ_C (150 MHz, CD_3OD): 148.7 (C-f), 122.7 (C-e), 89.6 (C-1), 81.1 (C-3 or C5), 78.5 (C-3 or C5), 74.0 (C-2), 70.9 (C-4), 64.4 (CH_2-i) (CH-g), 62.4 (C-6), 26.0 (CH_2-h), 25.7 (CH_3-g); HR ESI-MS found *m/z* 304.1140 $[M+H]^+$; calcd for $C_{11}H_{17}N_3O_7{\cdot}H$ 304.1139.

3.6. Enzymatic assays

All the enzymes used in this study were purchased from Sigma Chemical Co. Ltd, and were the purest forms available. The enzyme concentrations were chosen so that the 20% of hydrolysis was reached in 20 min and the assays were routinely run at 37 °C. The library of compounds was screened at three different concentrations (1 mM, 100 μM and 10 μM), except DNJ which was also screened at 1 μM concentration. In order to avoid solubility issues with the more lipophilic triazoles and of the controls the assays also contained 2% DMSO. The assay was performed in duplicate and the two readings were averaged; replicates were typically within 5–10% of each other.

3.6.1. Yeast α-glucosidase inhibition assay[54]

Yeast α-glucosidase activity was routinely assessed by a continuous spectrophotometric assays at 400 nm performed in 96-well plates at 37 °C in pH 6.8 buffer (20 mM AcONa, 10 mM PIPES, 0.1 mM EDTA, 2% DMSO, 0.002% w/v NaN_3). The incubation mixtures included α-glucosidase (5 μg/mL), *p*-nitrophenyl α-D-glucopyranoside (200 μM) and inhibitor.

3.6.2. Sweet almond β-glucosidase inhibition assay[51]

Sweet almond β-glucosidase activity was assessed by continuous spectrophotometric assays at 400 nm performed in 96-well plates at 37 °C in pH 6.5 buffer (20 mM AcONa, 10 mM PIPES, 0.1 mM EDTA, 2% DMSO, 0.002% w/v NaN_3). The incubation mixtures included β-glucosidase (5 μg/mL), *p*-nitrophenyl β-D-glucopyranoside (625 μM) and inhibitor.

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Supplementary data

Crystallographic information and structural diagrams for **24** and **27** have been provided as supplementary data with this paper. Full crystallographic details, excluding structure features, have been deposited (Deposition Nos. CCDC 767414–767415) with the Cambridge Crystallographic Data Centre. These data may be obtained, on request, from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (tel.: +44 1223 336408; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.03.041.

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