Scaffold Diversity for Enhanced Activity of Glycosylated Inhibitors of Fungal Adhesion

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ABSTRACT: Candida albicans is one of the most prevalent fungal pathogens involved in hospital acquired infections. It uses adhesins to bind to glycans at the cell surface of epithelial cells and thus initiate infection. These interactions can be blocked by synthetic carbohydrates (such as compound 1) that mimics the structure of cell surface glycans. Herein we report the synthesis of a new series of divalent galactosides featuring aromatic (benzene, squaramides) and aliphatic (norbornenes) central scaffolds, with the latter being the first examples of their kind as small molecule anti-adhesion glycoconjugates. The evaluation of these compounds as inhibitors of adhesion of C. albicans to exfoliated buccal epithelial cells (BECs) revealed that galactosides 1 and 6, built on an aromatic core, were the most efficient inhibitors of adhesion, displacing up to 36% and 48%, respectively, of yeast cells already attached to the BECs at 0.138 μM. Conformational analysis of compound 1 identified the preference for a folded
presentation in the lowest energy conformers. Remarkably, cis-endo-norbornene \textbf{21} performed comparably to the benzene-core derivatives, highlighting the potential of norbornenes as a new class of aliphatic scaffolds for the synthesis of anti-adhesion compounds.

\textbf{1-Introduction}

The yeast \textit{Candida albicans} is a body commensal which is capable of causing a variety of superficial and systemic diseases in immunocompromised patients. \textsuperscript{1,2} \textit{C. albicans} infections have become a major threat to hospitalized individuals, particularly those who are immunocompromised (such as patients undergoing cancer chemotherapy, immunosuppression therapies or AIDS). \textit{C. albicans} needs to bind to host cells in order to colonize mucosal epithelia and subsequently cause infection. \textsuperscript{3,4} \textit{C. albicans} is also able to adhere to abiotic surfaces, such as plastics and metals, which increases the risk of healthy people with implanted medical devices developing fungal infections. \textsuperscript{5} The adhesion processes in \textit{C. albicans} are complex and involve a combination of non-selective hydrophobic binding together with specific interactions mediated by proteins such as adhesins. \textsuperscript{6,7,8,9} Of these, some are known to be lectin-like and are suspected to mediate fungal adhesion through binding of epithelial host cell surface oligosaccharides and glycolipids. \textsuperscript{10} The carbohydrate-binding specificities for several yeast and fungal adhesins have been described. \textsuperscript{11} \textit{Candida} species seem to display a preference to bind glycans with terminal galactosides, \textsuperscript{12,13} but affinities for fucose \textsuperscript{14} and $N$-acetyl glucosamine moieties (GlcNAc) \textsuperscript{15} have also been reported. However, there are very few X-ray crystallographic studies that can provide detailed structural information on the mode of binding between fungal adhesins and their carbohydrate ligands\textsuperscript{12,13,16} and to the best of our knowledge, none refers to \textit{C. albicans} carbohydrate-binding adhesins. Recently, we have reported the synthesis and evaluation of a small library of aromatic glycoconjugates as inhibitors of adherence of \textit{C. albicans} to buccal epithelial cells (BECs). \textsuperscript{17} In this study we...
screened several glycoconjugates featuring terminal galactosides (Figure 1a). The results highlighted the influence of the structural elements connecting the galactosyl motifs to the aromatic core in the observed biological activity. For example, compound 1, with a triazolyl group directly linked to the anomeric position, was found to be a very effective inhibitor of fungal adhesion, capable of displacing over 50% of *C. albicans* cells already attached to BECs. Addition of an O-ethylene linker in compound 2 slightly decreased anti-adhesion ability. On the other hand, the replacement of galactosides by lactosides in compound 3 lead to increased adhesion between fungal and epithelial cells. X-ray crystallographic studies of lectins from microbial pathogens have enabled the design of high affinity glycoconjugates and glycomimetics. 18, 19, 20, 21, 22 Since the putative target to which lead compound 1 binds to exert its anti-adhesion activity is unknown, structure-based strategies to enhance affinity are not possible. Instead, we approached its optimization by exploring molecular scaffolds onto which the recognition epitopes necessary for anti-adhesion activity could be installed. Since a divalent presentation of terminal galactosides appears to be important for anti-adhesion activity, 17 we decided to generate a second generation of compounds (analogue of compound 1) by changing the central framework. The new core scaffolds can potentially orient both galactosyl moieties in an improved three-dimensional arrangement for the receptor mediating the adhesion of *C. albicans* to the BECs. In this work, we compare two aromatic (benzene, squaramides) and two aliphatic (norbornenes) molecular scaffolds (Figure 1b), with the latter being used for the first time in the synthesis of small molecule anti-adhesion glycoconjugates.
Figure 1: a) Chemical structures of selected aromatic-core glycoconjugates (AGCs) evaluated as inhibitors of adhesion of opportunistic yeast C. albicans. \(^{17}\); b) Structures of the core scaffolds used in the synthesis of a second-generation anti-adhesion ligands.

2-Results and Discussion

2.1-Synthesis

2.1.1- 1,4 disubstituted benzene scaffolds: Our original study focused on aromatic core glycoconjugates built around scaffolds with either a 1,3 or 1,3,5 substitution pattern. We decided to first explore 1,4 substituted analogues of lead compound 1. Thus, terephthalic acid was reacted with propargyl amine using freshly prepared 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, \(^{23}\)) to give diamide 4 in 81% yield (Scheme 1). The resulting scaffold was reacted with 2,3,4,6-tetra-O-acetyl-1-β-azido-D-galactopyranoside \(^{24}\) using microwave mediated copper-catalyzed Azide-Alkyne Cycloaddition (CuAAC) methodology, resulting in 73% yield of the protected compound 5. The deacetylation of this
compound was accomplished under mild basic conditions to give compound 6 in excellent yield (94%).

\[ \text{Scheme 1: Synthesis of 1,4-benzene core divalent galactosyl 6. Reagents and conditions: i) DMTMM, propargylamine, DMF, N}_2, 16 \text{ h, 81 \%}; \text{ ii) 2,3,4,6-tetra-O-acetyl-1-\beta-azido-D-galactopyranoside, CuSO}_4\cdot H_2O/Na Asc, CH}_3CN/H_2O, 100 ^\circ \text{C, \mu w, 10 min, 73 \%}; \text{ iii) methanol, NEt}_3, H_2O, 45 ^\circ \text{C, 6 h, 94 \%.} \]

2.1.2- Squaramide scaffolds: Squaramides have been extensively investigated in supramolecular chemistry as ion receptors and, more recently, in the organocatalysis field due to their ability to act as effective hydrogen bond donors. Interestingly, squaramides have also been used in chemical biology, primarily in bioconjugation applications. Carbohydrate conjugations mediated by squaramide tethers are often used for the grafting of carbohydrate epitopes onto peptides and proteins. Some examples have been reported by Lindhorst and co-workers where heteromeric mannosides, monoamides and dendrimers, designed as inhibitors of FimH adhesion, have been constructed through couplings with diethyl squarate. With these, there are very limited examples in which squaramides have been used as scaffolds to display carbohydrates in a multivalent fashion. Given the planar, aromatic character of squaramide derivatives, we proceeded to synthesize a series of analogues of lead compound 1
featuring this core as a relevant comparison to the benzene glycoconjugates described in our earlier work. Diethyl squarate was reacted with propargylamine to give N,N-dipropargyl squaramide 7 in 81% yield. CuAAC reaction with (a) 2,3,4,6-tetra-O-acetyl-1-β-azido-D-galactopyranoside 24, (b) tetra-O-acetyl-1-β-2-azidoethyl-D-galactopyranoside 34 and (c) 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-1-β-azido-D-glucopyranoside 35 produced divalent compounds 8, 10 and 12, respectively. The acetyl protecting groups were removed under mild basic conditions to give the corresponding deprotected derivatives 9, 11 and 13, all of which display terminal galactosides (Scheme 2).

**Scheme 2:** Synthesis of divalent galactosyl squaramides 9, 11 and 13. Reagents and conditions: i) propargylamine, DMF, N₂, 16 h, 81%; ii) CuSO₄·5H₂O/Na Asc, CH₃CN/H₂O, 100 °C, µv, 10 min, and a) 2,3,4,6-tetra-O-acetyl-1-β-azido-D-galactopyranoside, 24 81% for 8; (b) tetra-
O-acetyl-1-β-O-2-azidoethyl-D-galactopyranoside, 34 80 % for 9; and (c) 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-1-β-azido-D-glucopyranoside, 35 75 % for 10; iii) methanol, NEt₃, H₂O, 45 °C, 6 h, 77-99 %.

2.1.3- 5-Norbornene scaffolds: While the 1,4-disubstituted and N,N-dipropargyl squaramide scaffolds described above are readily prepared, no further functionalization is possible once the grafting of the carbohydrate moieties takes place. To overcome this drawback, we sought for a suitable molecular scaffold which would still afford the formation of the N,N-di-propargyl amides required for CuAAC reaction with the sugar azides while allowing for the introduction of reporter tags, such as fluorescent labels, or other chemically reactive groups to continue derivatisation of the analogues. Hence, we decided to investigate 5-norbornene dicarboxylic acids as starting materials to synthesise the next family of analogues of lead compound 1. In addition, the use of (±) 2-endo-3-exo-dicarboxylic acid 14 (trans) or endo-2,3-dicarboxylic acid (cis) 18 allows for a different spatial presentation of the galactosyl moieties. Norbornenes derivatives have been commonly used as monomers in block copolymerization reactions 36,37 and to provide molecular frameworks for self-assembled constructs 38 and ion receptors. 39

Recently, a series of norbornane-based cationic peptidomimetics with potent antibacterial activity have been reported. 40 Therefore, dicarboxylic acids 14 and 18 appeared to be suitable molecular scaffolds which have largely been unexplored in the design of multivalent glycomimetics. The synthesis of the analogues of lead compound 1 based on 5-norbornene scaffolds is shown in Scheme 3. Both 5-norbornene-2-endo-3-exo-dicarboxylic acid (trans) 14 and the cis-5-norbornene-endo-2,3-dicarboxylic acid 18 were reacted with propargylamine and TBTU to give diamides 15 and 19. CuAAC reaction of 15 and 19 with 2,3,4,6-tetra-O-acetyl-1-β-azido-D-galactopyranoside, 24 produced the peracetylated divalent galactosides 16 (trans-product) and 20 (cis-product), respectively. Removal of the acetyl protecting groups to give final products 17 and 21 was attempted by reaction of compounds 16 and 20 under mild basic
conditions. As part of the purification of the deprotected glycoconjugates, the reaction crude is generally treated with Amberlite H\textsuperscript{+} resin. Interestingly, when this procedure was applied in the deprotection of the acetylated \textit{cis}-norbornene 20, treatment with the acidic resin resulted in cyclisation to the imide, loss of one of the galactosyl-triazolyl moieties and ultimately formation of the monovalent glycoconjugate 22 in quantitative yield. This unexpected reaction was not observed for the deprotection of the \textit{trans}-compound 16. In order to circumvent this problem, the reaction was repeated without using the resin and the desired product was isolated in 96\% yield.

Scheme 3: Synthesis of divalent and monovalent galactosyl norbornenes 17, 21 and 22.

Reagents and conditions: i) propargylamine, TBTU, NE\textsubscript{t}\textsubscript{3}, DMF, N\textsubscript{2}, 48 h, 93\% for 15, 79\% for 19; ii) 2,3,4,6-tetra-\textit{O}-acetyl-1-\textit{β}-azido-\textit{D}-galactopyranoside, \textsuperscript{24} CuSO\textsubscript{4}.5H\textsubscript{2}O/Na Asc,
CH₃CN/H₂O, 100 °C, µw, 20 min, 54% for 16, 74% for 20; iii) methanol, NEt₃, H₂O, 45°C, 16 h, Amberlite H⁺ resin, 30 min, 97% for 17, quantitative yield for 22; iv) methanol, NEt₃, H₂O, 45°C, 16 h, 96%.

2.2-Biological Evaluation

Adherence assays were carried out at a range of concentrations of the alternative scaffold glycoconjugates, using original lead compound 1 as a positive control. Toxicity assays confirmed that all compounds tested are non-toxic to C. albicans at the concentrations used in the adherence assays (Figure SI.1). Exclusion, competition and displacement assays (see experimental section) were carried out using the second generation glycoconjugates.

2.2.1 1,4-Aromatic Scaffolds: Firstly, an exclusion assay where the yeast cells were pre-treated with the glycoconjugates 1 and 6, was carried out. At 13.8 μM, compound 1 (at 10 mg/mL) induced a 42% reduction in adherence, while compound 6 (at 9 mg/mL) decreased adherence by 33.5% reduction (Figure 2a). This assay was then performed at lower concentrations of compound 6 (1.38 μM and 0.138 μM). Interestingly, it was found that at lower concentrations of compound 6 there was a 61% reduction in adherence (Figure 2b). The competition assay, where yeast, BECs and glycoconjugates were co-incubated, showed a similar trend as the previous assay: compound 6 was not as efficient at reducing yeast adherence as original lead compound 1. The competition assay was carried out again at decreasing glycoconjugate concentrations of 13.8 μM, 1.38 μM and 0.138 μM. The average percentage decrease in adhesion is shown in Figure 2c. As observed in the exclusion assay discussed above, the greatest anti-adhesive properties were observed at 1.38 μM. The displacement assay, in which the yeast and BECs are co-incubated first to allow for adherence to occur, followed by subsequent addition of the glycoconjugates, provides a closer resemblance to the initial steps of C. albicans infection and can give useful insights into a possible therapeutic application of
these compounds. Two control measurements are carried out in this experiment: control 1 involved the assessment of the binding of *C. albicans* to BECs prior to exposure to the glycoconjugates, with DMSO as the control. Control 2 shows the average number of yeast attached per BEC after the second filtration of the procedure and provides an indication of physical detachment of the yeast cells, rather than inhibition of adhesion induced by the glycoconjugates. In this assay compound 6 performed better than original compound 1: the data identified a 56 % reduction in adherence for 1 at 0.138 μM (compared to control 1) and a 36 % reduction (compared to control 2, Figure SI.2) while compound 6 effected a 63 % reduction in yeast adherence (compared to control 1) and a 48 % reduction (compared to control 2, Figure 2d).
**Figure 2:** Anti-adherence evaluation of glycoconjugate 6 with a 1,4-aromatic core: a) exclusion assay showing average number of yeast attached per BEC ([glycoconjugates] = 13.8 μM; b) exclusion assay showing average number of yeast attached per BEC [6]: 13.8 μM, 1.38 μM and 0.138 μM; c) competitive assay showing the percentage decrease in adhesion induced by glycoconjugates 1 and 6; d) displacement assay showing the average number of yeast attached per BEC for glycoconjugate 6 at [0.138 μM].

2.2.2 **Squaramide scaffolds:** The squaramide glycoconjugates 9, 11 and 13 did not display better anti-adhesive properties than the original lead compound 1 in exclusion assays. While compound 1 was capable of reducing yeast adherence by 45 %, squaramides 11 and 13 showed similar results, reducing adherence by 33-34 %. Compound 9, which structurally only differs to lead compound 1 in the 4-membered cyclic core, did not perform as well as the other squaramides, producing only a 27 % reduction in adhesion (Figure 3a). If the exclusion assay is performed in reverse (with BECs pre-treated with the glycoconjugates prior to exposure to the yeast), the percentage reduction decreases for all compounds tested (Figure 3b). This may indicate that the glycoconjugates interact more favourably with structural elements in *C. albicans* than in the BECs. In the competitive assay, compound 1 again showed the best results, inhibiting adhesion by 41%. Compounds 11 and 13 showed similar performance, inhibiting adhesion by 31-36 %. Compound 9 again produced the lowest decrease in adhesion, only 17 %. (Figure 3c). The displacement assay was performed on the two best-performing squaramides 11 and 13. Compounds 11 and 13 at [glycoconjugates] = 13.8 μM produced a reduction of yeast adhesion of 35 % and 39 % respectively (compared to control 1) and 21 and 25 % reduction, respectively (compared to control 2, Figure 3d). The displacement assay of original lead compound 1 at 13.8 μM showed slightly higher anti-adhesive properties, with a reduction of yeast adherence of 42% (compared to control 1) and 31% (compared to control 2, Figure SI.3). These results indicate that divalent terminal galactosides with a benzene-aromatic
core seem to be more efficient inhibitors of *C. albicans* adhesion to BECs than their counterparts built on aromatic-squaramide scaffolds.

**Figure 3**: Anti-adherence evaluation of glycoconjugates 9, 11 and 13 with squaramide core: exclusion assay where a) the yeast are pre-treated and b) the BEC are pre-treated; c) competitive assay showing the percentage decrease in adhesion induced by the
glycoconjugates; d) displacement assay showing the average number of yeast attached per BEC for glycoconjugates 11 and 13. All compounds were tested at 13.8 μM.

2.2.3 Norbornene scaffold: the norbornene derivatives 17, 21 and 22 were evaluated in a range of anti-adhesion assays at [13.8 μM]. In the exclusion assay (Figure 4a) where the yeast were pre-treated, the glycoconjugates were compared to lead compound 1, which reduced adherence by 51% in this particular assay. The *trans*-norbornene compound 17 and the monovalent derivative 22 showed similar results, reducing yeast adherence by 46% and 43%, respectively. The *cis*-norbornene compound 21 showed very promising results in this assay with a 65% inhibition of adherence of the yeast to the BECs, performing better than lead compound 1. In the competition assay (Figure 4b), the two divalent galactosyl norbornenes 17 and 21 showed similar results, causing a greater reduction of yeast adherence than the lead compound 1. The monovalent derivative 22 did not have a significant effect in yeast adhesion (17% inhibition). Finally, the displacement assay (Figure 4c) showed that the divalent *trans*-norbornene 17 and the monovalent compound 22 show again very similar results, with a 23% reduction in adherence compared to control 1, and only 7-8% reduction compared to control 2. On the other hand, the divalent *cis*-norbornene compound 21 presented the best results with 45% reduction in adherence compared to control 1, and 34% reduction compared to control 2, again outperforming lead compound 1 (42% and 31% inhibition at [1] = 13.8 μM, compared to control 1 and 2, respectively, as outlined earlier, Figure SI3). These results highlight the important role of the preorganised configurations that are enabled by the norbornene scaffolds, with a remarkable difference in activity between the *trans* and *cis* glycoconjugates 17 and 21.
Figure 4: Anti-adherence evaluation of glycoconjugates 17, 21 and 22 with a norbornene core: a) exclusion assay showing average number of yeast attached per BEC; b) competition assay showing the percentage decrease in adhesion of *C. albicans* to BECs; c) displacement assay showing the average number of yeast attached per BEC. All compounds were tested at [13.8 μM].

2.3-Computational analysis

In order to globally explore the conformational space of lead compound 1, metadynamics (MTD) simulations were performed using the GFN2-xTB method. This method is designed
around a semiempirical tight-binding quantum model to facilitate efficient and robust screening of the conformational space of large molecular systems. From a chemically reasonable starting geometry of 1, a default conformer search algorithm was utilised to automatically generate an ensemble of low-energy conformers and rotamers. This procedure runs through iterative metadynamics sampling and subsequent pre-optimizations steps of selected MTD snapshots. By this technique, the initially >2000 structures generated by the independent MTD runs were narrowed down to 150 unique conformers of compound 1 (Figure SI.4). These final structures were subsequently subjected to full DFT geometry optimisations, which allowed for their ranking in terms of their relative enthalpies. The results indicate that 1 has a relatively large number of conformers within a 10 kcal mol\(^{-1}\) bracket of the lowest energy conformer. The latter corresponds to structure 99 (Figure 5) and by far dominates the Boltzmann population (~96%). However, only 2 conformers are energetically within a ~2 kcal mol\(^{-1}\) relative to structure 99, corresponding to Boltzmann populations around 2% of these states (Table 1 S1, Figure SI.5). The highest energy conformer lies about 20 kcal mol\(^{-1}\) above the most stable conformer. The geometry of the lowest-energy conformers 99 is stabilised by several hydrogen bonding interactions (Figure 5). One of the two side-arms is slightly folded due to the presence of a hydrogen bond between a galactosyl OH group and the amide carbonyl group attached to the central benzene ring. This “semi-open” structural motif is also featured in the other two low-energy conformers. Considering all three low energy conformers, the distances between the anomeric carbons centres are in the range 15 – 18 Å. There are no obvious hydrophobic interactions involving apolar C-H from the galactoses and the aromatic ring in the molecule.
Figure 5: Optimized geometry (B3PW91/6-31G**) corrected for water solvent) of the lowest-energy conformer (structure 99) of lead compound 1 along with selected hydrogen-bond distances (given in Å). Oxygens red, nitrogen blue, carbon cyan, hydrogen white.

Whilst the conformational sampling yields these conformers as lowest in energy, it is important to note that more extended conformers may still be important when the ligand binds to the target protein. It has been demonstrated in many cases that flexible drug molecules adopt more compact forms in the homogenous bulk solvent environment \(^{41}\), but unfold to open conformer forms when bound to the anisotropic receptor binding sites. In the absence of structural information of the target binding site of the protein, structural proposals of the binding conformer are unattainable.

3. Conclusion

Adherence to host tissue is essential to the ability of \textit{C. albicans} to colonise and disseminate. The ability to inhibit adherence or to reverse it offers a novel therapeutic approach for the treatment of \textit{Candida} infections of the mucosal surfaces. The molecular scaffold is a critical structural element in the design of anti-adherence glycomimetics and glycoconjugates. A series of divalent galactosides built on aromatic (benzene, squaramides) and aliphatic (norbornene) molecular scaffolds have been synthesised and their activities as inhibitors of the adhesion of fungal pathogen \textit{C. albicans} have been evaluated. The results show that the glycoconjugates
featuring a benzene core (i.e. lead compound 1 and 1,4-disubstitued aromatic compound 6) performed significantly better than the squaramide analogues. On the other hand, divalent galactosides based on norbornene scaffolds display anti-adhesive properties comparable to lead compound 1, with cis-norbornene derivative 21 slightly outperforming it. The compounds reported in this study are, to the best of our knowledge, the first example of small molecule anti-adhesion glycoconjugates built on norbornene scaffolds. Conformational analysis of the original lead compound 1 reveals a preference for folded conformations, which leaves anomic carbons 15 – 18 Å apart in the lowest energy conformers. Thus, it is possible that molecular scaffolds that display binding epitopes in a comparable presentation are suitable for construction of anti-adhesion glycomimetics against C. albicans. The results from this study highlight the potential of underexplored molecular scaffolds, such as norbornenes, in the design and synthesis of glycomimetics and multivalent glycoconjugates.

4. Experimental Section

Chemistry. Synthesis: General Methods: All reagents for synthesis were bought commercially and used without further purification. Tetrahydrofuran (THF) was freshly distilled over sodium wire and benzophenone. Dichloromethane (DCM) was freshly distilled over CaH₂ prior to use. Reactions were monitored with thin layer chromatography (TLC) on Merck Silica Gel F₂₅₄ plates. Detection was effected by UV (λ=254nm) or charring in a mixture of 5% sulfuric acid-ethanol. NMR spectra were recorded using Bruker Ascend 500 spectrometer at 293K. All chemical shifts were referenced relative to the relevant deuterated solvent residual peaks. Assignments of the NMR spectra were deduced using ^1H NMR and ^13C NMR, along with 2D experiments (COSY, HSQC and HMBC). Chemical shifts are reported in ppm. Flash chromatography was performed with Merck Silica Gel 60. Microwave reactions were carried out using a CEM Discover Microwave Synthesizer. Optical rotations were obtained from an AA-100 polarimeter and [α]D values are given in 10⁻¹ cm²·g⁻¹. High performance liquid
chromatography analysis (HPLC, Waters Alliance 2695) was performed in final compounds and indicated purity of 95% based on integrations without the use of an internal standard. High resolution mass spectrometry (HRMS) was performed on an Agilent-LC 1200 Series coupled to a 6210 Agilent Time-Of-Flight (TOF) mass spectrometer equipped with an electrospray source in both positive and negative (ESI+/−) modes. Infrared spectra were obtained via ATR as a solid on a zinc selenide crystal or as a film on NaCl plates in the region 4000–400 cm−1 on a Perkin Elmer Spectrum 100 FT-IR spectrophotometer. Synthetic schemes and spectroscopic data for all members of the ACG library are provided in the SI.

*General Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction procedures:*

*Method A*: Copper sulphate pentahydrate (20 mg) and sodium ascorbate (40 mg) were added to a solution of the acetylated sugar azide (1,25 equiv per propargyl group) and the corresponding propargyl amide scaffold in acetone/water (2:1 ratio). The reaction was allowed to stir at rt until deemed complete by TLC analysis (typically 16-24 h). The solvent was removed in vacuo. The residue was dissolved in DCM, washed with water (x3) and dried (MgSO₄). The mixture was filtered and the solvent was removed *in vacuo* to yield the crude product, which was purified by silica gel column chromatography (DCM:MeOH 98:2-93:7) to give the corresponding product.

*Method B*: Copper sulphate pentahydrate (20 mg) and sodium ascorbate (40 mg) were added to a solution of the acetylated sugar azide (1,25 equiv per propargyl group) and the corresponding propargyl amide scaffold in acetonitrile/water (2:1 ratio). The reaction was heated (µw at 100 ºC) with stirring until deemed complete by TLC analysis (typically 5-15 min). The solvent was removed *in vacuo*. The residue was dissolved in DCM, washed with water (x3) and dried (MgSO₄). The mixture was filtered and the solvent was removed *in vacuo*
to yield the crude product, which was purified by silica gel column chromatography (DCM:MeOH 98:2-93:7) to give the corresponding product.

**General acetyl ester hydrolysis procedure:** The acetylated glycoconjugate was dissolved in methanol/water (2:1 ratio). NEt₃ (0.1 mL) was added and the reaction mixture was allowed to stir at 45 °C until completion (typically 6-18 h). The solution was cooled to rt, Amberlite H⁺ was added and the mixture was allowed to stir for 30 min. The solution was filtered and the solvent was removed in the rotatory evaporator and the residue was dried under high vacuum or lyophilized to give the deprotected glycoconjugate.

**N’-di(prop-2-yn-1-yl)terephthalamide (4).** Terephthalic acid (200 mg, 1.204 mmol) and DMTMM (733 mg, 2.649 mmol) were suspended in anhydrous DMF (15 mL) under N₂. Propargylamine (0.169 mL, 2.649 mmol) was added to the reaction mixture, which went clear upon addition. The reaction was allowed to stir for 16 hours. The reaction mixture was poured into ice/water (20 mL). The resulting precipitate was filtered and washed with cold water. The pure product was allowed to dry overnight in the fumehood to give the pure product 4: white amorphous solid (234 mg, 81 %). ¹H NMR (500 MHz, DMSO) δ 9.05 (t, J = 5.5 Hz, 2H, NH), 7.93 (s, 4H, Ar-H), 4.07 (dd, J = 5.5, 2.5 Hz, 4H, CH₂CCH), 3.13 (t, J = 2.5 Hz, 2H, CH₂CCH). ¹³C NMR (126 MHz, DMSO) δ 165.8 (CO), 136.7 (Ar-C), 127.8 (Ar-CH), 81.6 (CH₂CCH), 73.5 (CH₂CCH), 29.0 (CH₂CCH). IR (ATR): 3278, 3237, 1619, 1534, 1493, 1439, 1352, 1309, 1279, 1224, 1160, 1063, 991, 710 cm⁻¹. HRMS (ESI+): m/z calculated for C₁₄H₁₁₂N₂O₂ Na⁺: [M+Na⁺]: 263.0796. Found 263.0795.

**N’-di(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-1,2,3-triazol-4-ylmethylamide)-terephthalamide (5).** Prepared from 4 and 2,3,4,6-tetra-O-acetyl-1-β-azido-D-galactopyranoside, according to Method B: off-white amorphous solid (164 mg, 73 %). Rf = 0.36 (DCM:MeOH 9:1). [α]D²⁴: -10.9° (c 1.1, DCM). ¹H NMR (500 MHz, CDCl₃) δ 7.95 (s, 2H, triaz-H), 7.83 (s, 4H, Ar-H), 7.34 – 7.27 (m, 2H, NHCH₂-triaz), 5.85 (d, J = 9.3 Hz, 2H,
H-1), 5.58 – 5.51 (m, 4H, H-2 and H-4), 5.27 (dd, J = 10.3, 3.4 Hz, 2H, H-3), 4.82 – 4.64 (m, 4H, CH₂-triaz), 4.28 – 4.23 (m, 2H, H-5), 4.17 (ddd, J = 18.3, 11.4, 6.4 Hz, 4H, H-6 and H-6'), 2.22 (s, 6H, OAc), 2.03 (s, 6H, OAc), 2.01 (s, 6H, OAc), 1.86 (s, 6H, OAc). ¹³C NMR (126 MHz, CDCl₃) δ 170.3 (CO of OAc), 170.0 (CO of OAc), 169.8 (CO of OAc), 169.0 (CO of OAc), 166.6 (CONH), 145.1 (CH₂CCH), 136.7 (Ar-C), 127.4 (Ar-CH), 121.3 (CH₂CCH), 86.3 (C-1), 74.1 (C-2), 70.7 (C-3), 68.1 (C-2/C-4), 66.8 (C-2/C-4), 61.2 (C-6), 35.4 (CH₃ of OAc), 20.7 (CH₃ of OAc), 20.6 (CH₃ of OAc), 20.5 (CH₃ of OAc), 20.2 (CH₃ of OAc). IR (ATR): 3380, 1743, 1644, 1533, 1495, 1431, 1368, 1212, 1046, 923 cm⁻¹. HRMS (ESI+): m/z calculated for C₄₂H₅₀N₈O₂⁺ Na⁺: [M+Na⁺]: 1009.3039. Found 1009.3032.

**N, N′-di-(β-D-galactopyranosyl-1,2,3-triazol-4-ylmethylamide)-terephthalamide (6).**

White amorphous solid (59 mg, 90 %). [α]D²³: 13.75° (c= 0.8, H₂O). ¹H NMR (500 MHz, D₂O) δ 8.15 (s, 2H, triaz-H), 7.54 (s, 4H, Ar-H), 5.56 (d, J = 9.2 Hz, 2H, H-1), 4.47 (s, 4H, CH₂-triaz), 4.10 (t, J = 9.5 Hz, 2H, H-2), 3.96 (d, J = 3.2 Hz, 2H, H-3), 3.76 (dd, J = 9.8, 3.3 Hz, 2H, H-4), 3.64 (d, J = 9.6 Hz, 4H, H-6 and H-6'). ¹³C NMR (125 MHz, D₂O) δ 168.9 (CO), 144.6 (CH₂CCH), 135.9 (Ar-C), 127.4 (Ar-CH), 123.2 (CH₂CCH), 88.2 (C-1), 78.3 (C-2), 72.9 (C-3), 69.8 (C-2), 68.6 (C-4), 60.9 (C-6), 34.8 (CH₂CCH). IR (NaCl disc): 3290, 1636, 1542, 1498, 1293, 1091, 1053, 890 cm⁻¹. HRMS (ESI+): m/z calculated for C₂₆H₃₄N₈O₁₂ + Na⁺ [M+Na⁺]: 673.2194. Found 673.2206.

**3,4-dii(2-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-ethyl-1,2,3-triazol-4-ylmethylamino)cyclobut-3-ene-1,2-dione (10).** Prepared from 7 and 2-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)ethyl azide according to Method B: yellow amorphous solid (197 mg, 80 %). R_f= 0.5 (DCM:MeOH 9:1). [α]D²²: -3.8° (c= 1.05, DCM). ¹H NMR (500 MHz, CDCl₃) δ 8.06 (s, 2H, NHCH₂-triaz), 7.78 (s, 2H, triaz-H), 5.38 (d, J = 3.4 Hz, 2H, H-4), 5.15 (dd, J = 10.4, 8.0 Hz, 2H, H-2), 5.04 – 4.86 (m, 6H, H-3 and CH₂CCH), 4.68 – 4.53 (m, 4H, CH₂CH₂O), 4.51 (d, J = 7.9 Hz, 2H, H-1), 4.25 – 4.18 (m, 2H, CHO-Gal), 4.11 (ddd, J = 30.2,
11.3, 6.7 Hz, 4H, H-6 and H-6'), 4.02 – 3.94 (m, 2H, CHO-Gal), 3.92 (t, J = 6.6 Hz, 2H, H-5), 2.17 (s, 6H, OAc), 2.04 (s, 6H, OAc), 1.98 (s, 6H, OAc), 1.96 (s, 6H, OAc). 13C NMR (126 MHz, CDCl3) δ 183.6 (CO), 170.4 (CO of OAc), 170.2 (CO of OAc), 170.1 (CO of OAc), 169.4 (CO of OAc), 167.7 (NHCCO), 144.7 (CH2CCH), 124.4 (CH2CCH), 109.0 (C-1), 70.9 (C-5), 70.6 (C-3), 68.5 (C-2), 67.3 (CH2CH2O), 66.9 (C-4), 61.1 (C-6), 50.3 (CH2CH2O), 38.6 (CH2CCH), 20.7 (CH3 of OAc), 20.7 (CH3 of OAc), 20.5 (CH3 of OAc). IR (NaCl disc): 3261, 2964, 1750, 1677, 1602, 1535, 1432, 1370, 1227, 1175, 1139, 1059 cm⁻¹. HRMS (ESI+): m/z calculated for C42H54N8O22+: [M+Na⁺]: 1045.3250. Found 1045.3249.

3,4-di(2-O-(β-D-galactopyranosyl)-ethyl-1,2,3-triazol-4-ylmethylamino)cyclobut-3-ene-1,2-dione (11). White amorphous solid (95 mg, 94 %). [α]D²²: 12.0° (c = 1, H2O). 1H NMR (500 MHz, D2O) δ 8.01 (s, 2H, triaz-H), 4.85 (s, 4H, CH2CCH), 4.62 (t, J = 5.0 Hz, 4H, O-CH2CH2), 4.27 (d, J = 7.9 Hz, 2H, H-1), 4.25 – 4.20 (m, 2H, O-CH-CH2), 4.09 – 4.02 (m, 2H, O-C-H-CH2), 3.84 (d, J = 3.4 Hz, 2H, H-4), 3.70 – 3.63 (m, 4H, H-6 and H-6'), 3.59 (dd, J = 7.4, 4.8 Hz, 2H, H-5), 3.54 (dd, J = 9.9, 3.5 Hz, 2H, H-3), 3.41 (dd, J = 9.9, 7.9 Hz, 2H, H-2).

13C NMR (125 MHz, D2O) δ 182.22 (CO), 168.0 (COCO), 144.5 (CH2CCH), 124.70 (CH2CCH), 102.95 (C-1), 75.07 (C-5), 72.54 (C-3), 70.51 (C-2), 68.48 (C-4), 67.95 (O-CH2CH2), 60.85 (C-6), 50.38 (O-CH2CH2), 38.82 (CH2CCH). IR (ATR): 3269, 2924, 1800, 1662, 1591, 1531, 1427, 1338, 1224, 1140, 1042, 889, 826, 775 cm⁻¹. HRMS (ESI+): m/z calculated for C26H38N8O14 + H⁺ [M+H⁺]: 686.2586. Found 687.2576.

3,4-di-[(4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-1,2,3-triazol-4-ylmethylamino]cyclobut-3-ene-1,2-dione (12). Prepared from 7 and 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-1-β-azido-D-glucopyranoside according to Method B: white amorphous solid (529 mg, 75 %). Rf = 0.27 (DCM:MeOH 9:1). [α]D²²: 7.0° (c = 1.0, DCM). 1H NMR (500 MHz, CDCl3) δ 8.23 (s, 2H, NHCH2-triaz), 8.09 (s, 2H, triaz-H), 6.07 (d, J = 6.4 Hz, 2H, H-1 Gal), 5.49 – 5.38 (m, 4H, H-
2 Gal and H-3 Gal), 5.36 (d, \( J = 3.3 \) Hz, 2H, H-4 Glc), 5.11 (dd, \( J = 10.2, 8.0 \) Hz, 2H, H-2 Glc), 4.99 (dd, \( J = 10.4, 3.3 \) Hz, 2H, H-3 Glc), 4.94 (apps, 4H, \( CH_2 \)-triaz), 4.58 (d, \( J = 7.9 \) Hz, 2H, H-1 Glc), 4.51 (d, \( J = 11.6 \) Hz, 2H, H-6 Glc), 4.28 – 4.05 (m, 10H, H-6' Glc, H-5 Gal, H-4 Gal, H-6 and H-6' Gal), 4.03 – 3.92 (m, 2H, H-5 Glc), 2.14 (s, 6H, OAc), 2.06 (s, 6H, OAc), 2.04 (appd, 18H, 3 x OAc), 1.95 (s, 6H, OAc), 1.76 (s, 6H, OAc). 13C NMR (126 MHz, CDCl3) δ 183.6 (CO), 170.4 (CO of OAc), 170.2 (CO of OAc), 170.0 (CO of OAc), 169.9 (CO of OAc), 169.7 (CO of OAc), 168.9 (CO of OAc), 167.6 (NHCCO), 145.1 (CH2CCH), 123.5 (CH2CCH), 101.2 (C-1 Glc), 85.4 (C-1 Gal), 75.9 (C-4/5 Gal), 75.6 (C-4/5 Gal), 72.6 (C-3 Gal), 71.0 (C-3 Glc), 70.8 (C-2 Gal), 70.7 (C-5 Glc), 69.1 (C-2 Glc), 66.8 (C-4 Glc), 61.9 (C-6 Glc), 60.8 (C-6 Gal), 38.4 (CH2CCH), 20.9 (CH3 of OAc), 20.8 (CH3 of OAc), 20.7 (CH3 of OAc), 20.6 (CH3 of OAc), 20.5 (CH3 of OAc), 20.3 (CH3 of OAc), 20.1 (CH3 of OAc). IR (NaCl disc): 3478, 3263, 2964, 1753, 1597, 1536, 1370, 1227, 1048 cm⁻¹. HRMS (ESI+): m/z calculated for C62H78N8O36 + Na⁺ [M+Na⁺]: 1533.4416. Found 1533.3743.

3,4-di-\{4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl\}-1,2,3-triazol-4-ylmethylamino)cyclobut-3-ene-1,2-dione (13). White amorphous solid (172 mg, 99 %). \([\alpha]_{D}^{32} : 10° \) (c= 1, H2O). 1H NMR (500 MHz, D2O) δ 8.11 (appd, \( J = 3.0 \) Hz, 2H, triaz-H), 5.70 – 5.59 (m, 2H, H-1), 4.81 (s, 4H, \( CH_2 \)-CCH), 4.47 (d, \( J = 7.9 \) Hz, 1H, H-1 Glc), 4.40 (d, \( J = 7.8 \) Hz, 1H, H-1 Glc), 3.99 – 3.42 (m, 24H). 13C NMR (126 MHz, D2O) δ 182.3 (CO), 168.2 (CCO), 144.9 (CH2CCH), 123.3 (CH2CCH), 102.9 (C-1 Glc), 96.4 (C-1 Glc), 87.5 (C-1 Gal), 78.9, 77.7, 77.4, 75.9, 75.4, 75.1, 74.5, 72.8, 72.5, 72.2, 71.9, 71.9, 70.9, 70.5, 69.2, 68.9, 68.7, 68.6, 68.3, 61.2, 61.1, 61.0, 60.4, 59.7, 38.8 (CH2CCH). IR (ATR): 3300, 2939, 2452, 1803, 1670, 1585, 1516, 1379, 1015 cm⁻¹. HRMS (ESI+): m/z calculated for C34H30N8O22 + Na⁺ [M+Na⁺]: 945.2937. Found 945.2967.

Bicyclo[2.2.1]hept-5-ene-2-endo-3-exo-2,3-dicarboxamide, \( N-\) (prop-2-yn-1-yl) (15). 5-Norbornene-2-endo-3-exo-dicarboxylic acid 14 (200 mg, 1.098 mmol) and TBTU (881 mg, 2.7
mmol) were dissolved in anhydrous DMF (15 mL) under N₂. Triethylamine (0.38 mL, 2.7 mmol) and propargylamine (0.15 mL, 2.3 mmol) were added after 10 mins. The reaction was allowed to stir for 48 hrs. The DMF was removed in vacuo, the resulting residue was dissolved in DCM (20 mL) and washed with brine (3 × 20 mL), dried over MgSO₄, filtered and concentrated in vacuo to yield the crude product. This was then purified by silica gel column chromatography (1:1-1.5:1 EtOAc:Pet. Ether) to give 15: white solid (260 mg, 93 %). \( R_f = 0.25 \) (1:1 EtOAc:Pet. Ether). \(^1\)H NMR (500 MHz, MeOD) δ 6.29 (dd, \( J = 5.6, 3.1 \) Hz, 1H, H\(_{e/f}\)), 6.05 (dd, \( J = 5.6, 2.8 \) Hz, 1H, H\(_{e/f}\)), 4.04–3.87 (m, 4H, C\(_2\)H\(_2\)CC), 3.26 (dd, \( J = 4.6, 3.7 \) Hz, 1H, H\(_{b/c}\)), 3.21 (d, \( J = 0.6 \) Hz, 1H, H\(_{a/d}\)), 2.95 (dd, \( J = 1.9, 1.1 \) Hz, 1H, H\(_{a/d}\)), 2.59–2.55 (m, 3H, CH\(_2\)CCH and H\(_{b/c}\)), 1.82 (d, \( J = 8.4 \) Hz, 1H, H\(_{g}\)), 1.41 (dq, \( J = 8.4, 1.7 \) Hz, 1H, H\(_{g'}\)). \(^{13}\)C NMR (125 MHz, MeOD) δ 174.8 (CO), 173.7 (CO), 137.4 (C\(_{e/f}\)), 134.1 (C\(_{e/f}\)), 70.6 (CH\(_2\)CCH), 70.4 (CH\(_2\)CCH), 48.5 (C\(_{a/d}\)), 48.1 (C\(_{b/c}\)), 47.1 (C\(_{b/c}\)), 47.0 (C\(_{g}\)), 46.1 (C\(_{a/d}\)), 28.3 (CH\(_2\)CCH), 28.1 (CH\(_2\)CCH), 13.1. IR (ATR): 3284, 1635, 1531, 1447, 1333, 1276, 1215, 1031, 862 cm\(^{-1}\). HRMS (ESI+): m/z calculated for C\(_{15}\)H\(_{16}\)N\(_2\)O\(_2\) + Na\(^+\) [M+Na\(^+\)]: 279.1109. Found 279.1119.

**Bicyclo[2.2.1]hept-5-ene-2-endo-3-exo-2,3-dicarboxamide, N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-1,2,3-triazol-4-ylmethylamino) (16).** Prepared from 15 and 2,3,4,6-tetra-O-acetyl-1-β-azido-D-galactopyranoside according to Method B: off-white solid (488 mg, 54 %). \( R_f = 0.58 \) (DCM:MeOH 9:1). \([\alpha]^\text{D}_{23}\) = -6.0° (c = 1, DCM). \(^1\)H NMR (500 MHz, CDCl\(_3\)) δ 7.79 (d, \( J = 6.7 \) Hz, 1H, triaz-H), 7.75 (s, 1H, triaz-H’), 7.17 (dt, \( J = 9.0, 5.8 \) Hz, 1H, NH\(_{CH2\text{-triaz}}\), 6.93 (dt, \( J = 24.9, 5.7 \) Hz, 1H, NH’\(_{CH2\text{-triaz}}\)), 6.16 (td, \( J = 5.7, 3.2 \) Hz, 1H, H\(_{e/f}\)), 6.08 – 6.02 (m, 1H, H\(_{e/f}\)), 5.82 (d, \( J = 9.0, 2\)H, H-1), 5.52 – 5.42 (m, 4H, H-2 and H-4), 5.24 (dd, \( J = 10.3, 3.2 \) Hz, 2H, H-3), 4.49 – 4.37 (m, 4H, C\(_2\)H\(_2\)-triaz x2), 4.29 – 4.18 (m, 2H, H-5), 4.18 – 4.04 (m, 4H, H-6 and H-6’), 3.11 – 3.00 (m, 2H, H\(_{a/d}\) and H\(_{b/c}\)), 2.97 (s, 1H, H\(_{a/d}\)), 2.40 (dd, \( J = 12.7, 3.7 \) Hz, 1H, H\(_{b/c}\)), 2.18 – 2.13 (m, 6H, OAc), 1.98 – 1.92 (m, 12H, OAc), 1.86 – 1.75 (m, 6H, OAc), 1.75 – 1.70 (m, 1H, H\(_{g}\)), 1.41 (d, \( J = 8.5 \) Hz, 1H, H\(_{g'}\)). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) δ 173.7
and 173.6 (CO-NHCH₂), 172.6 and 172.5 (C’O-NHCH₂), 169.3 (CO of OAc), 169.1 (CO of OAc), 169.0 (CO of OAc), 168.8 (CO of OAc), 168.1 (CO of OAc), 168.0 (CO of OAc), 144.7 and 144.6 (CH₂CCH), 136.6 and 136.5 (Cₜ), 134.0 and 133.9 (Cₜ), 119.9 and 119.7 (CH₂CCH), 85.2 (C-1), 73.0 (C-5), 69.8 and 69.7 (C-3), 67.1 and 67.0 (C-2/4), 66.0 (C-2/4), 60.3 and 60.2 (C-6), 49.4 and 49.2 (Cₘ), 47.6 (Cₘ), 47.2 (Cₘ), 45.5 and 45.3 (Cₐ), 44.1 and 44.0 (Cₐ), 34.0 and 33.9 (CH₂-triaz), 19.7 (CH₃ of OAc), 19.6 (CH₃ of OAc), 19.5 (CH₃ of OAc), 19.3 (CH₃ of OAc), 19.2 (CH₃ of OAc). IR (ATR): 3387, 2972, 1745, 1651, 1526, 1368, 1210, 1044, 923, 733 cm⁻¹. HRMS (ESI+): m/z calculated for C₄₃H₅₄N₈O₂₀ + H⁺ [M+H⁺]: 1003.3533. Found 1003.3555.

**Bicyclo[2.2.1]hept-5-ene-2-endo-3-exo-2,3-dicarboxamide, N-(β-D-galactopyranosyl-1,2,3-triazol-4-ylmethylamino)** (17). Prepared from 16 using general acetyl ester hydrolysis procedure. White amorphous solid (242 mg, 97 %). [α]D¹⁹ : 11.0° (c= 1, H₂O). ¹H NMR (500 MHz, D₂O) δ 8.16 (appd, J = 15.9 Hz, 2H, triaz-H), 6.33 – 6.28 (m, 1H, H₂/H₃), 6.05 – 5.99 (m, 1H, H₃/H₄), 5.68 (dd, J = 9.2, 2.3 Hz, 2H, H-1), 4.53 – 4.43 (m, 4H, CH₂-triaz), 4.19 (appt, J = 9.5 Hz, 2H, H-2), 4.08 (appd, J = 3.3 Hz, 2H, H-4), 3.99 (appt, J = 6.1 Hz, 2H, H-5), 3.87 (dd, J = 9.8, 3.3 Hz, 2H, H-3), 3.78 (appd, J = 6.0 Hz, 4H, H-6 and H-6'), 3.25 – 3.19 (m, 2H, H₅/H₆ and H₆/H₇), 2.81 (s, 1H, H₇/H₈), 2.53 (d, J = 4.1 Hz, 1H, H₈/H₉), 1.66 (d, J = 8.6 Hz, 1H, H₉), 1.42 (d, J = 7.6 Hz, 1H, H₁₀). ¹³C NMR (126 MHz, D₂O) δ 176.8 (CO), 175.8 (C’O), 145.2 (C-triaz), 138.2 (C=C), 134.6 (C=C), 123.1 (CH₂CCH), 88.2 (C-1), 78.4 (C-5), 73.0 (C-3), 69.8 (C-2), 68.7 (C-4), 60.9 (C-6), 48.6 (Cₐ/Cₙ), 48.2 (Cₐ/Cₙ), 47.8 (Cₚ/Cₚ), 47.5 (Cₚ), 46.4 (Cₚ/Cₚ), 34.6 (CH₂-triaz), 34.5 (C’H₂-triaz).. IR (ATR): 3282, 2929, 1760, 1642, 1535, 1355, 1300, 1243, 1089, 1052, 986, 889 cm⁻¹. HRMS (ESI+): m/z calculated for C₇₁H₅₈N₈O₁₂ + Na⁺ [M+Na⁺]: 689.2507. Found 689.2490.

**Bicyclo[2.2.1]hept-5-ene-2-endo-3-dicarboxamid, N-(prop-2-yn-1-yl)** (19). Cis-5-Norbornene-2,3-endo-dicarboxylic acid 18 (300 mg, 1.65 mmol) and TBTU (1.323 g, 4.12
mmol) were dissolved in anhydrous DMF (15 mL) under N₂. Triethylamine (0.57 mL, 4.12 mmol) and propargylamine (0.22 mL, 3.46 mmol) were added after 10 mins. The reaction was allowed to stir for 48 hrs. The DMF was removed \textit{in vacuo}, the resulting residue was dissolved in DCM (20 mL) and washed with brine (3 x 20 mL), and NaHCO₃ (2 x 20 mL), dried of MgSO₄, filtered and concentrated \textit{in vacuo} to yield the crude product. This was then purified by silica gel column chromatography (1:1-1.5:1 EtOAc:Pet. Ether) to give 19: white solid (334 mg, 79 %). Rᵣ=0.08 (1:1 EtOAc:Pet. Ether). ¹H NMR (500 MHz, DMSO) δ 7.70 (t, \( J = 5.3 \) Hz, 2H, NH), 6.09 (t, \( J = 1.8 \) Hz, 2H, Hₑ and Hᵣ), 3.82 – 3.64 (m, 4H, CH₂CCH), 3.12 – 3.09 (m, 2H, Hᵢ and Hᵣ), 3.04 (t, \( J = 2.5 \) Hz, 2H, CH₂CCH), 2.96 – 2.94 (m, 2H, Hᵣ and Hₐ), 2.08 (s, 1H), 1.25 – 1.19 (m, 1H). ¹³C NMR (125 MHz, DMSO) δ 171.5 (CO), 134.9 (Cₑ and Cᵣ), 81.9 (CH₂CCH), 73.2 (CH₂CCH), 50.1 (Cₐ and Cᵣ), 48.9 (Cᵣ), 46.7 (Cₐ and Cᵢ), 81.9 (CH₂CCH). IR (ATR): 3286, 1654, 1525, 1415, 1333, 1278, 1256, 1226, 1098, 1029, 908, 846 cm⁻¹. HRMS (ESI+): m/z calculated for C₁₅H₁₆N₂O₂⁺ Na⁺: 279.1109. Found 279.1105.

\textit{Bicyclo[2.2.1]cis-hept-5-ene-2,3-endo-2,3-dicarboxamide, N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-1,2,3-triazol-4-ylmethylamino) (20).} Prepared from 18 and 2,3,4,6-tetra-\textit{O}-acetyl-1-β-azido-D-galactopyranoside according to Method B: off-white solid (200 mg, 74 %). Rᵣ=0.41 (DCM:MeOH 9:1). [\( \alpha \)]D²₀ = -6.36 ° (c= 1.1, DCM). ¹H NMR (500 MHz, CDCl₃) δ 7.83 (s, 1H, triaz-H), 7.79 (s, 1H, triaz-H’), 6.97 (t, \( J = 5.5 \) Hz, 1H, NHCH₂-triaz), 6.83 (t, \( J = 5.6 \) Hz, 1H, NHCH₂-triaz), 6.27 (ddd, \( J = 35.6, 5.3, 3.0 \) Hz, 2H, Hₑ and Hᵣ), 5.83 – 5.77 (m, 2H, H-1 and H-1’), 5.52 – 5.46 (m, 4H, H-2, H-2’, H-4 and H-4’), 5.24 – 5.18 (m, 2H, H-3 and H-3’), 4.42 – 4.01 (m, 10H, CH₂-triaz x2, H-5, H-5’, H-6, H-6’, H-6” and H-6’’), 3.25 – 3.17 (m, 2H, H₅ and H₂₅), 3.06 (app s, 2H, Hₐ and Hᵣ), 2.16 (s, 6H, OAc), 2.01 – 1.88 (m, 12H, OAc x2), 1.80 (app d, 6H, OAc), 1.43 – 1.23 (m, 2H, Hₕ and Hₕ). ¹³C NMR (126 MHz, CDCl₃) δ 171.7 (CO), 171.7 (CO), 169.3 (CO of OAc), 169.3 (CO of OAc), 169.1 (CO of OAc), 168.9 (CO of OAc), 168.8 (CO of OAc), 167.9 (CO of OAc), 167.9 (CO of OAc), 144.6 (C-triaz), 25
144.5 (C-triaz), 134.6 (C=C), 134.2 (C=C), 120.4 (CH₂CCH), 120.2 (CH₂CCH), 85.1 (C-1), 72.9 (C-5), 69.9 (C-3), 67.0 (C-2/4), 65.9 (C-2/4), 60.2 (C-6), 60.1 (C-6'), 50.5 (C₅/C₆), 50.3 (C₅/C₆), 48.7 (C₆), 46.1 (C₃ and C₄), 33.7 (H₃), 72.9 (C-5), 69.9 (C-3), 67.0 (C-2/4), 65.9 (C-2/4), 60.2 (C-6), 60.1 (C-6'), 50.5 (Cb/c), 50.3 (C₅/C₆), 48.7 (C₆), 46.1 (C₃ and C₄), 33.7 (H₃), 72.9 (C-5), 69.9 (C-3), 67.0 (C-2/4), 65.9 (C-2/4), 60.2 (C-6), 60.1 (C-6'), 50.5 (C₅/C₆), 50.3 (C₅/C₆), 48.7 (C₆), 46.1 (C₃ and C₄), 33.7 (H₃).


Bicyclo[2.2.1]cyclopentane-2,3-dicarboxamide, N-(β-D-galactopyranosyl-1,2,3-triazol-4-ylmethylamino) (21). Prepared from 20 using general acetyl ester hydrolysis procedure without Amberlite treatment. White amorphous solid (169 mg, 96%). [α]D²₀: 14.0° (c= 1, H₂O). ¹H NMR (500 MHz, D₂O) δ 8.32 (s, 1H, triaz-H), 8.18 (s, 1H, triaz-H'), 5.89 (qd, J = 5.5, 3.0 Hz, 2H, Hₑ and Hₑ'), 5.74 (d, J = 9.2 Hz, 1H, H-1), 5.68 (d, J = 9.2 Hz, 1H, H-1'), 4.68 (s, 4H, C₂H₂-triaz), 4.29 – 4.18 (m, 4H, H-2 and H-2'), 4.13 (dd, J = 3.3, 0.6 Hz, 1H, H-4), 4.11 (dd, J = 3.3, 0.6 Hz, 1H, H-4'), 4.06 – 3.99 (m, 2H, H-5 and H-5'), 3.92 (dd, J = 9.8, 3.3 Hz, 1H, H-3), 3.89 (dd, J = 9.8, 3.3 Hz, 1H, H-3'), 3.81 (appdd, J = 7.4, 6.2 Hz, 4H, H-6, H-6'', H-6''' and H-6'''), 3.50 (dd, J = 3.0, 1.5 Hz, 2H, H₅ and H₅'), 3.35 (dd, J = 2.5, 1.2 Hz, 2H, H₆ and H₆'), 1.67 (dt, J = 8.9, 1.6 Hz, 1H, H₁), 1.60 (d, J = 8.9 Hz, 1H, H₁'). ¹³C NMR (126 MHz, D₂O) δ 180.8 (CO), 143.4 (C-triaz), 142.3 (C'-triaz), 134.3 (C=C), 124.0 (CH-triaz), 123.6 (C'H-triaz), 88.0 (C-1), 87.9 (C'-1), 78.4 (C-5), 78.3 (C'-5), 73.1 (C-3), 73.0 (C'-3), 69.7 (C-2), 69.7 (C'-2), 68.6 (C-4), 68.6 (C'-4), 60.9 (C-6), 60.8 (C'-6), 51.9 (C₆), 45.7 (C₇ and C₈), 44.9 (C₉ and C₁₀), 32.8 (CH₂CCH). IR (ATR): 3293, 2932, 1764, 1688, 1560, 1401, 1336, 1232, 1091, 1051, 886, 815, 728 cm⁻¹. HRMS (ESI+): m/z calculated for C₂₇H₃₈N₈O₂₀ + Na⁺ [M+Na⁺]: 689.2507. Found 689.2501.

N-(β-D-galactopyranosyl-1,2,3-triazol-4-ylmethyl)bicyclo[2.2.1]cyclopentane-2,3-dicarboximide (22). As per general acetyl ester hydrolysis procedure: Cis-norbornene compound 20 (155 mg, 0.155 mmol) was dissolved in methanol/H₂O (4 mL, 2 mL). NEt₃ (0.1
mL) was added, and the reaction mixture was allowed to stir at 45 °C for 6 hours. The solution was cooled, Amberlite H+ was added and the mixture was allowed to stir for 30 mins. The solution was filtered and the solvent was removed in vacuo. Monovalent-imide analogue 22 was formed: white amorphous solid (63 mg, 100 %). [α]26°: 5 ° (c= 1.2, MeOH). 1H NMR (500 MHz, D2O) δ 8.10 (s, 1H, triaz-H), 5.82 (qd, J = 5.5, 3.0 Hz, 2H, H_e and H_f), 5.60 (d, J = 9.2 Hz, 1H, H-1), 4.61 (s, 2H, CH2-triaz), 4.13 (t, J = 9.5 Hz, 1H, H-2), 4.03 (dd, J = 3.3, 0.7 Hz, 1H, H-3), 3.93 (td, J = 6.0, 0.8 Hz, 1H, H-5), 3.81 (dd, J = 9.8, 3.3 Hz, 1H, H-3), 3.72 (d, J = 6.1 Hz, 2H, H-6 and H-6'), 3.44 – 3.41 (m, 2H, H_b and H_c), 3.28 – 3.26 (m, 2H, H_a and H_d), 1.62 – 1.49 (m, 2H, H_g and H_g'). 13C NMR (125 MHz, D2O) δ 180.8 (CO), 142.4 (CH2CCH), 134.3 (C_e and C_d), 124.1 (CH2CCH), 88.0 (C-1), 78.4 (C-5), 73.1 (C-3), 69.8 (C-2), 68.7 (C-4), 60.9 (C-6), 52.0 (C_g), 45.8 (C_b and C_c), 45.0 (C_a and C_d), 32.9 (CH2CCH). IR (ATR): 3346, 2943, 1765, 1686, 1399, 1336, 1168, 1091, 1050, 883, 727 cm⁻¹. HRMS (ESI+): m/z calculated for C_{18}H_{22}N_{4}O_{7} + Na+: 429.1386. Found 429.1362.

Chemistry. Computational Methods: Initial conformational searching of lead compound 1 was carried out with the Conformer-Rotamer Ensemble Sampling Tool utility (crest, version 2.7) based on the GFN2-xtb method, as implemented in the xtb (version 6.1) code. The default iterative version of the MTD-GC routine (iMTD-GC) was utilized to generate a complete conformer ensemble. This workflow performs several independent metadynamics (MTD) simulations at T = 300 K utilizing a history-dependent biasing potential with different parameters for the potential. The collective variables are defined as previous minima in the conformational space, expressed as root-mean-square-deviation (RMSD) between the structures. Snapshots are then geometry optimized in a multi-level filtering procedure applying energy windows of 15, 10, and 6 kcal mol⁻¹, respectively. Regular molecular dynamics (MD) simulations are carried out to sample rotameric structures, and in the final step a genetic z-matrix crossing (GC) procedure is applied in order to filter out
identical geometries. For the complete sampling a generalized Born model with solvent accessible surface area (GBSA) was invoked to account for the effect of water solvent and prevent electrostatic collapse of the molecules. Subsequent full geometry optimizations on the final conformer ensemble were performed with the Gaussian 09 (revision E.01) program. All geometries were fully optimized with the B3PW91 functional in conjunction with the 6-31G(d,p) basis set on all atoms. Subsequent vibrational frequency calculations on optimized geometries were utilized to confirm that each structure represents a true minimum on the potential energy surface. Dispersion effects were not explicitly taken into account, since initial optimized geometries of selected isomers invoking Grimme’s empirical D3 dispersion correction appeared to be very compact due to hydrophobic collapse. Solvent effects were approximated by performing all geometry optimizations in the presence of a reaction field using the using the integral equation formalism model (IEFPCM) in combination with the radii and non-electrostatic terms for the SMD solvation model. The employed dielectric constant (\(\varepsilon = 78.35\)) and related solvent parameters correspond to those of water.

**Biology.** *Fungal Strain: C. albicans* was maintained on sabouraud dextrose agar and cultures were grow to the stationary phase (1 – 2 x 10^8/ml) overnight in YEPD broth (1% (w/v) yeast extract, 2% (w/v) bacteriological peptone, 2% (w/v) glucose) at 30°C and 200rpm. Stationary phase yeast cells were harvested, washed with PBS and resuspended at a density of 1 x 10^8/mL in PBS.

*Buccal epithelial cells:* Buccal epithelial cells (BECs) were harvested from healthy volunteers by gently scraping the inside of the cheek with a sterile tongue depressor. Cells were washed in PBS and resuspended at a density of 5 x 10^5/ml.

*Adherence assays:* Yeast cells were mixed with BECs in a ratio of 50:1 in a final volume of 2 mL and incubated at 30°C and 200 rpm for 90 min. The BEC/yeast cell mixture was harvested by passing through a polycarbonate membrane containing 30 µm pores which trapped the
BECs but allowed unattached yeast cells to pass through. This was washed x 2 with 10 mL PBS and cells remaining on the membrane were collected and placed on glass slides which were left to air dry overnight. The cells were heat fixed and stained using 0.5% (w/v) crystal violet, rinsed using cold water to remove any surplus stain and left to air dry for 30 min. The number of *C. albicans* cells adhering to a sample of 200 BECs per treatment was assessed microscopically. In the *exclusion assay* the yeast cells were incubated for 90 min in the presence of each compound at the given concentration. After this time the cells were harvested and washed twice with PBS before being resuspended in 1 mL PBS before being mixed with BECs (as described). In the *competition assay* format yeast cells, BECs and compound (at the given concentration) were co-incubated for 90 min prior to harvesting. In the *displacement assay* adherence was allowed to occur by mixing the yeast cells and BECs together. BECs and adherent yeast cells were harvested and re-incubated with the compound (at the given concentration) for a further 90 min after which time the level of adherence was measured.

*Statistics:* All experiments were performed on three independent occasions. In each assay the number of yeast cells adhering to 200 randomly chosen BECs was determined. Results are mean ± SEM.

**ASSOCIATED CONTENT**

Electronic Supporting Information (SI) is available free of charge. It includes supplementary Figures and spectroscopic data.

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Graphical abstract