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Synthesis of aromatic glycoconjugates as anti-fungal agents against *Candida* spp. and assessment of their covalent crosslinking capabilities

Kyle Doherty [a,c], Keela Kessie [a,c], Harlei Martin [a,1], Jordan Loughlin [a,c], Oliwier Dulawa [a,c], Kaja Kasemets [b,*], Trinidad Velasco-Torrijos [a,c,**]

[a] Department of Chemistry, Maynooth University, Maynooth, Co. Kildare, Ireland
[b] Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia
[c] The Kathleen Lonsdale Institute for Human Health Research, Maynooth University, Maynooth, Co. Kildare, Ireland

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ABSTRACT

Covalent drugs are becoming increasingly attractive in drug discovery, as they can enhance potency and selectivity for their molecular targets. Covalent inhibitors have been investigated for several therapeutic applications, including anti-cancer and anti-infection agents. However, there are only a few examples of covalent inhibitors targeting fungal pathogens. We have previously reported aromatic glycoconjugates (AGCs) capable of inhibiting the adhesion of *Candida albicans* to buccal epithelial cells. In this work, we synthesize novel derivatives of the AGCs to which we have added reactive functional groups, such as acryloyl and vinyl sulfones, and investigated their antifungal efficacy against *Candida* spp. Although the compounds were ineffective at clinically relevant concentrations, we found that some of the galactose derivatives featuring reactive groups were amongst the most active, so their ability to crosslink nucleophilic side chains was assessed in model reactions.

1. Introduction

In recent years, there has been a renewed interest in covalent inhibitors in drug discovery.[1,2] As the name implies, these molecules can form a covalent bond with their target proteins, which usually results in their irreversible modification. Covalent inhibitors have been traditionally disfavoured due to disadvantages such as off-target reactivity, leading to undesired side effects. However, the need for new antimicrobials to combat rapidly emerging antibiotic resistance requires that research into this class of inhibitors is conducted.[3] A good example of successful antimicrobials which act as covalent inhibitors are penicillins and their clinically used derivatives, cephalosporins and carbapenems. In this class of compounds, the β-lactam moiety contained in these drugs reacts with a serine residue in transpeptidase enzymes which are essential for the integrity of the bacterial cell wall.[4] Despite these successes, the threat of immunogenicity due to non-specific covalent drug-protein adducts is a drawback and covalent inhibitors must be designed with a suitable electrophilic group to ensure selectivity.[5] This strategy has been applied for the identification of new antimicrobial agents, typically as inhibitors of bacterial biosynthesis,[6,7] or as agents targeting mechanisms of resistance such as β-lactamases.[8] Some covalent inhibitors have also been developed against fungal enzymes, like fructose-1,6-bisphosphate aldolase (FBA)[9] or aspartate semialdehyde dehydrogenase (ASADH)[10] in *Candida albicans* or Prp8 intein in *Cryptococcus neoformans*[11] (Fig. 1a).

Carbohydrates play key roles in infectious processes and hence, offer the possibility for the development of antimicrobial agents with new mechanisms of action.[12,13] Interestingly, there are very few examples of carbohydrate-based covalent inhibitors investigated as antimicrobial agents. Titz and co-workers reported the synthesis and evaluation of epoxide-containing galactosides targeting LecA (Fig. 1b), a key virulence factor involved in the adherence of *Pseudomonas aeruginosa* to epithelial host cells.[14] The authors showed that a covalent linkage was formed between a cysteine residue in the carbohydrate-binding site of LecA and the galactoside through a ring-opening reaction of the strained epoxide. Multivalent mannosides designed as covalent inhibitors of

* Corresponding author.
** Corresponding author at: Department of Chemistry, Maynooth University, Maynooth, Co. Kildare, Ireland.
E-mail addresses: kaja.kasemets@kbfi.ee (K. Kasemets), trinidad.velascotorrijos@mu.ie (T. Velasco-Torrijos).
[1] Present address: Department of Chemistry, University of British Columbia, Faculty of Science Vancouver Campus, 2036 Vancouver, British Columbia V6T 1Z1, Canada.

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FimH adhesion protein in *E. coli* were prepared by Fischer and co-workers.[15] These compounds featured catechol moieties (Fig. 1b), which oxidize to benzoquinones under physiological conditions and subsequently react with nucleophilic side chains in FimH via a Michael addition-type reaction. To our knowledge, these are the only two examples of carbohydrate derivatives targeting bacterial lectins featuring reactive groups designed for covalent cross-linking.

C. albicans has been categorized as a critical priority fungal pathogen by the World Health Organization (WHO).[16] The threat presented by fungal pathogens to public health is significantly on the rise, with increasing resistance in the four main classes of antifungal agents available and a lack of new therapeutic alternatives with novel mechanisms of action.[17] Host cell glycans, such as blood group antigens and mucin glycans, have been shown to mediate interactions with *C. albicans,* hence carbohydrate-containing molecules offer potential opportunities to manage fungal infections.[18,19] Unfortunately, structural information on the fungal proteins that these glycans may bind to is very limited. We have previously reported the synthesis of a small library of aromatic glycoconjugates (AGCs) containing simple carbohydrates which were evaluated as inhibitors of the adhesion of *C. albicans* to buccal epithelial cells (BECs).[20,21] Interestingly, Als1 adhesin from *C. albicans* has been shown to specifically bind fucose-containing glycans,[22] but we found that the most active compounds in our library featured galactose moieties, with compound **1a** displacing up to 50 % of yeast cells adhered to BECs. Although EPA adhesins in *C. glabrata* bind oligosaccharides that contain terminal galactose residues,[23] so far there are no reports for any adhesin in *C. albicans* known to bind galactosides.[24] In our previous study,[20] we found that modification of the aniline nitrogen of the AGCs with fluorescein isothiocyanate (FITC) did not appear to disrupt the interactions of the lead compound **1a** with a putative membrane target, which suggest that substitution at this position should not be detrimental for binding. In this work, we sought to study the activity of several galacto-, manno- and fucosylated AGCs (Fig. 2) in pathogenic *Candida* species (*C. albicans, C. parapsilosis, C. glabrata C. lusitaniae, C. guilliermondii* and *C. auris*). Since we lack structural information of the potential targets for these compounds, we introduced different substituents in the aromatic ring to expand the chemical diversity of the library. Some of them have electrophilic character (i.e. acrylamides, vinyl sulfones), which represents the first examples of glycoconjugates featuring covalent "warheads" targeting fungal pathogens.

2. Results and discussion

2.1. Chemistry

The synthesis of the glycoconjugates relied on the assembly of the sugar azides and aromatic cores with alkyne functional groups using the CuAAC (Copper-catalyzed Azide-Alkyne Cycloaddition) methodology. While all the compounds share a common aromatic core, a range of substituents (including two electrophilic groups) with different electronic and steric properties are also featured. Compound **1a** has previously been described by us.[20] Compounds **2a-c**, **3a** and **4a,b** were prepared from *N*-Boc protected intermediate **6**,[20] while compounds **5a,b** were prepared from the nitro-diamide **7**. A CuAAC reaction was used to conjugate the corresponding sugar azide with the alkynes present in scaffolds **6** and **7** through the formation of a 1,2,3-triazolyl linkage. These reactions gave compounds **8a-c** and **9a,b**. Further functionalization was achieved after *N*-Boc deprotection of **8a-c** and reaction with acryloyl chloride (to give **10a-c**), ethanesulfonyl chloride (to give **11a**), or 2-chloroethanesulfonyl chloride (to give **12a,b**). Optimization of the conditions for reaction with 2-chloroethanesulfonyl chloride was necessary, as slow addition of the reagent was required to minimize the formation of the di-sulfonamide product. Mild basic hydrolysis using triethyl amine afforded the deacetylation of the protected compounds, yielding the corresponding acryloyl (**2a-c**), ethanesulfonyl (**3a**), vinylsulfonyl (**4a,b**) and nitro-substituted (**5a,b**) AGCs (Scheme 1).

2.2. Evaluation of antifungal efficacy of target compounds

The antifungal efficacy of compounds **1a, 2a-c, 3a, 4a,b** and **5a,b** was evaluated on different clinically relevant *Candida* species (Table 1). *C. albicans* and *C. auris* are considered fungal pathogens of critical priority by the World Health Organization, while *C. glabrata and C.*

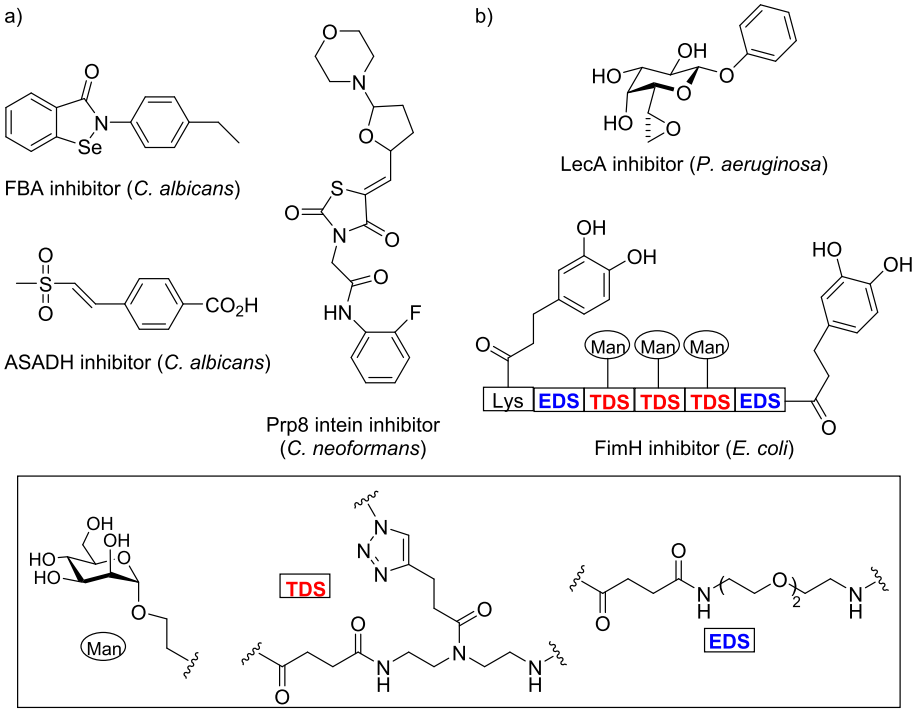


Fig. 1. A) Chemical structure of covalent inhibitors of fungal enzymes[9–11]; b) carbohydrate-based covalent inhibitors of bacterial lectins.

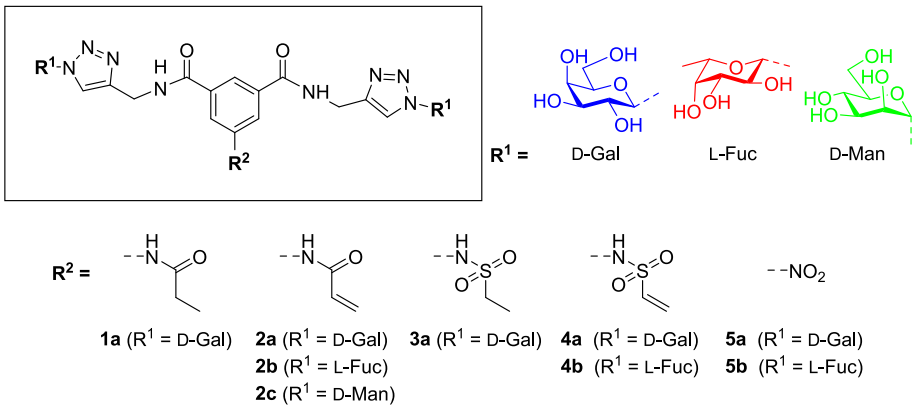
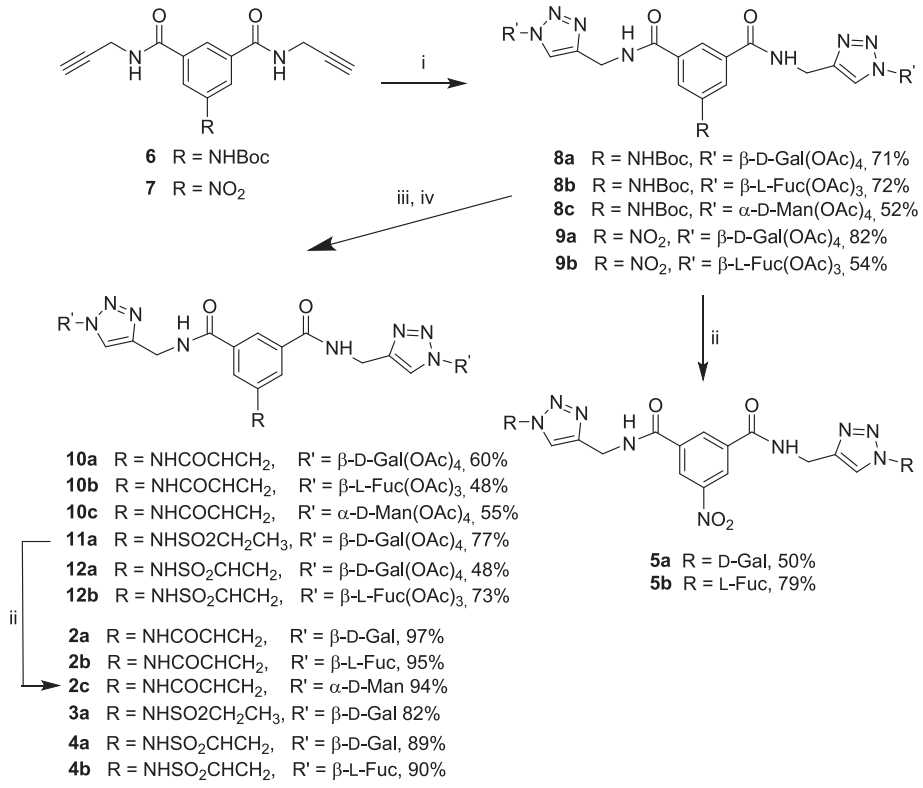


Fig. 2. Chemical structure of the aromatic glycoconjugates (ACGs) evaluated against *Candida* spp.



Scheme 1. *Reagents and conditions*: i) tetra-*O*-acetyl-1-azido glycoside (β-Gal for **8a, 9a**, β-Fuc for **8b**, **9b**, α-Man for **8c**), $CuSO_4{\cdot}5H_2O$/sodium ascorbate, CH_3CN/H_2O, 100 °C in MW, 30 min-2 h; ii) CH_3OH, H_2O, NEt_3, 45 °C, 6–18 h; (iii) DCM, TFA, RT, 2–5 h; iv) Acryloyl chloride, ethanesulfonyl chloride or 2-chloroethanesulfonyl chloride, DCM, NEt_3, RT, 18 h.

parapsilosis are within the high-priority fungal pathogens classification.[16] The fungal cells were incubated with the target compounds at concentrations ranging from 0.01 to 100 mg/mL in deionized water in 96-well microplates for 24 h at 30 °C. The highest concentration tested for **2b, 2c, 4a**, and **4b** was 10 mg/L and for **1a**, **2a**, **3a**, **5a** and **5b** was 100 mg/ml. After 24 h of exposure, the cell culture was pipetted onto YPD (yeast extract peptone dextrose) agar plates and then incubated at 30 °C for 48 h. The growth of cells (colony formation) was visually evaluated, and the minimum fungicidal concentration (MFC) was determined. MFC was defined as the lowest tested concentration of the compound, which completely prevented the subsequent growth of cells (colony formation) on the agar medium.

The 24-h MFC values are shown in Table 1. While all the compounds tested were found to be ineffective at clinically relevant concentrations, some fungicidal efficacy was observed at higher concentrations. The lead compound, divalent galactoside **1a**, had an MFC of 10 mg/mL for *C. glabrata* and *C. guilliermondii*. As mentioned earlier, *C. glabrata* is known to express EPA adhesins, which recognize and bind terminal galactosides in host cells' surface glycans.[23] Notably, while compound **1a** was previously shown to effectively inhibit the adhesion of *C. albicans* to buccal epithelial cells at concentrations of 1–10 mg/mL, in this study we found that **1a** has a minimum fungicidal concentration (MFC) of 100 mg/mL against *C. albicans*. This supports that the compound's anti-adhesion activity at concentrations of 1–10 mg/mL was not due to its fungicidal properties.[20] Divalent galactoside-acryloyl derivative **2a** was fungicidal at 100 mg/mL concentrations across all the species tested. The corresponding vinyl sulfonamide derivative **4a** had a 24-h MFC of 10 mg/mL for *C. glabrata* and *C. guilliermondii*. Importantly, **4a** was also fungicidal at this concentration for *C. auris*, an emerging fungal pathogen of the highest concern due to its multi-drug resistance.[25] On the

Table 1
Minimum fungicidal concentrations (MFC) for compounds **1a, 2a, 2b, 2c, 3a, 4a, 4b, 5a, and 5b** to *Candida* spp. after 24 h exposure in deionized water at 30 °C. (n.d. not determined).

Compounds	24-h MFC (mg/mL)					
	C. albicans 10231	*C. parapsilosis* 22019	*C. glabrata* 90030	*C. guilliermondii* C94	*C. lusitaniae* C18	*C. auris CMYA-5001*
1a	100	100	10	10	100	n.d
2a	100	100	100	100	100	n.d
2b	>10	>10	>10	>10	>10	>10
2c	>10	>10	>10	>10	>10	n.d
3a	>100	>100	>100	>100	>100	n.d
4a	>10	>10	10	10	>10	10
4b	>10	>10	>10	>10	>10	>10
5a	>100	100	100	100	100	n.d
5b	100	100	100	100	100	100

10 mg/mL	>10 mg/mL	100 mg/mL	>100 mg/mL

other hand, ethyl sulfonamide derivative **3a**, which lacks cross-linking ability, showed no fungicidal activity for any tested *Candida* spp., even at the highest tested concentration (24-h MFC > 100 mg/mL). The presence of the electrophilic groups in the fucoside derivatives (acryloyl **2b** and vinyl sulfone **4b**) did not result in a fungicidal effect at 10 mg/mL for any of the *Candida* spp. evaluated, however, a fungicidal activity may be observed at higher concentrations. The only fucoside found to exhibit fungicidal activity at high concentrations (100 mg/mL) against all *Candida* species was compound **5b,** which features a nitro-substituted aromatic scaffold. The corresponding galactoside compound **5a** showed similar fungicidal efficacy, except for *C. albicans*, for which no adverse effect was observed at 100 mg/mL.

2.3. Model cross-linking experiments

To assess the reactivity of the glycoconjugates featuring electrophilic groups that had shown some toxicity against *Candida* spp., model cross-linking experiments were designed. In these experiments, three amino acids with nucleophilic groups were investigated (*N*-α-acetyl-L-lysine, *N*-acetyl-L-histidine and *N*-acetyl-L-cysteine), as they are considered models of the common reactive sidechains of amino acids found in lectin binding sites. These compounds were reacted with vinyl compounds **2a** or **4a** in either pH 7.4 or 10.2 phosphate buffer.[26] The extent, if any, of the crosslinking between the glycoconjugates and each of these nucleophiles was assessed by HPLC and HRMS. After 24 h, of reaction of both **2a** and **4a** with *N*-acetyl-L-cysteine, still a significant amount of glycoconjugate was unreacted, while no reaction was observed between **2a** or

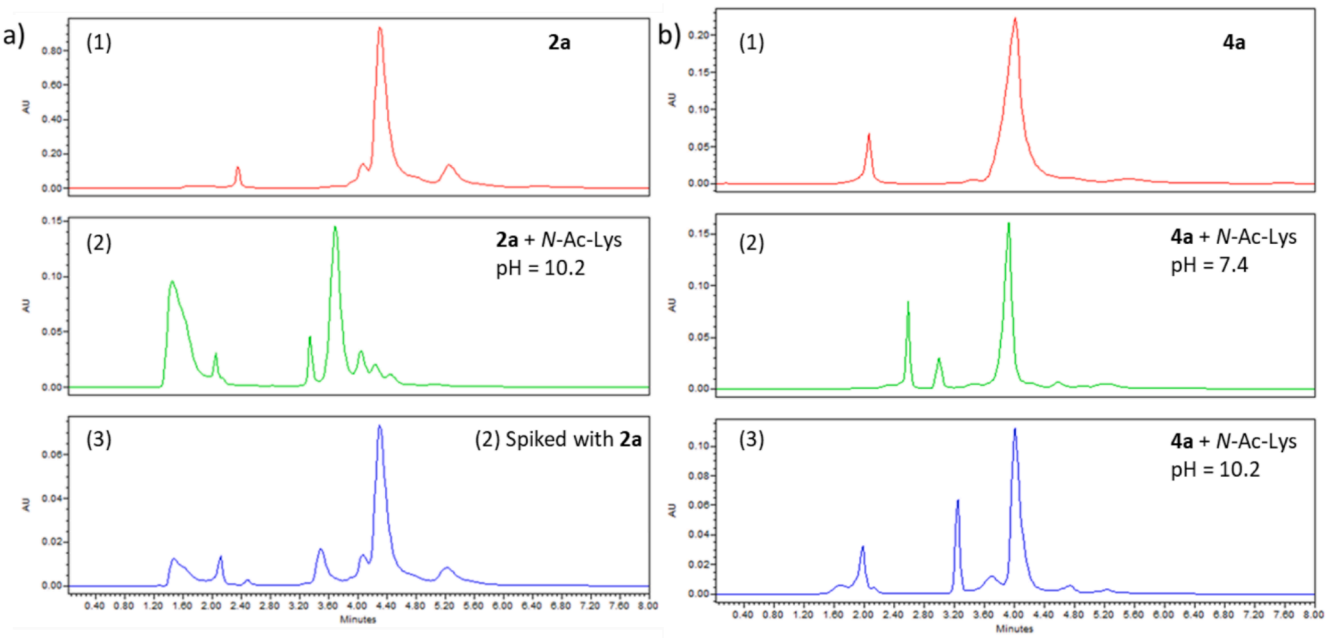


Fig. 3. HPLC chromatograms for the reaction of **2a** and **4a** at RT with *N*-α-acetyl-L-lysine after 24 h: a) Chromatograms (top to bottom); 1) **2a**; 2) **2a** with *N*-α-acetyl-L-lysine at pH 10.2; 3) **2a** with *N*-α-acetyl-L-lysine at pH 10.2 spiked with 1 mg/mL **2a**; b) Chromatograms (top to bottom); 1) **4a;** 2) **4a** with *N*-α-acetyl-L-lysine at pH 7.4; 3) **4a** with *N*-α-acetyl-L-lysine at pH 10.2. (All chromatograms were run at 90:10 H_2O:ACN isocratic gradient with a Phenomenex Luna CN column 5 μm (detector set at 254 nm).

4a with *N*-acetyl-L-histidine, at either pH 7.4 or 10.2 (**Figs. SI1, SI2,** respectively). No reaction between **2a** and *N*-α-acetyl-L-lysine took place at pH 7.4 (**Fig. S3**), however the reaction appeared to come to completion when it was carried out at pH 10.2. Since the peaks observed after HPLC analysis of the reaction mixture had very similar retention time (R_t) to the starting material, an aliquot of the reaction mixture was spiked with a sample of **2a** (Fig. 3**a**), which confirmed the presence of a new peak attributed to the product between the reaction of acryloyl derivative **2a** and *N*-α-acetyl-L-lysine. Regarding the reaction between vinyl sulfone **4a** with *N*-α-acetyl-L-lysine at pH 7.4, the appearance of a new peak was observed, although a significant amount of starting material **4a** remained even after 24 h. The intensity of the new peak increased when the reaction was performed at pH 10.2, although it did not appear to come to completion even after 24 h (Fig. 3**b**). The fact that acryloyl derivative **2a** was not reactive at neutral pH may account for its decreased toxicity against some of the *Candida* spp. evaluated, compared to vinyl sulfone **4a.**

The presence of the cross-linking products between **2a** and **4a** and *N*-α-acetyl-L-lysine was confirmed by HRMS analysis of the corresponding reaction mixtures (Fig. 4).

3. Conclusion

The need for new approaches to tackle the emergence of fungal pathogens resistant to conventional antifungal treatments has prompted us to explore the fungicidal activity of glycoconjugates against various fungal pathogens of the *Candida* genus. Building on our previous work on inhibitors of adhesion of *C. albicans* to buccal epithelial cells, in this study, we describe the synthesis of a small library of fucose, mannose and galactoside derivatives, some of which feature cross-linking moieties capable of forming covalent bonds with fungal proteins. The evaluation of the fungicidal properties of the glycoconjugates found that the most active compounds featured galactose moieties: lead compound, galactoside **1a**, showed activity at higher concentrations (100 mg/mL) against *C. albicans, C. parapsilosis* and *C. lusitaniae,* but had a 24-h minimum fungicidal concentration of 10 mg/mL for *C. glabrata* and *C. guilliermondii*. On the other hand, the acryloyl galactosides **2a** showed fungicidal activity across all the species tested at 100 mg/mL, while vinyl sulfonamide **4a** was active at 10 mg/mL to *C. glabrata*, *C. guilliermondii* and *C. auris*. Model cross-linking reactions showed that these compounds reacted more favourably with *N*-α-acetyl-L-lysine than other amino acids with nucleophilic side chains. Our results provide the first example of covalent glycoconjugates evaluated for fungicidal activity against high-priority fungal pathogens such as *C. albicans* and *C. auris*. Further optimization of the anti-fungal activity of this class of compounds is under way.

4. Experimental

4.1. Chemistry

4.1.1. General methods

All reagents for synthesis were bought commercially and used without further purification unless otherwise specified. Dichloromethane (DCM) was freshly distilled over CaH_2 prior to use. Reactions were monitored with thin layer chromatography (TLC) on Merck Silica Gel F254 plates. Detection was affected by UV (λ = 254 nm) or charring in a mixture of 5 % sulfuric acid–ethanol. NMR spectra were recorded using Bruker Ascend 500 spectrometer at 293 K. All chemical shifts were referenced relative to the relevant deuterated solvent residual peaks. Assignments of the NMR spectra were deduced using ^{1}H NMR and ^{13}C 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} cm^2 g^{-1}. High performance liquid chromatography (HPLC, Waters Alliance 2695) was performed in final compounds and indicated purity of ca. 95 %. High resolution mass spectrometry (HRMS) was performed on an Agilent-LC 1200 Series coupled to a 6210 Agilent Time-Of-Flight (TOF) or mass spectrometer equipped with an electrospray source in both positive and negative (ESI+/−) modes. Infrared spectra were obtained as a film on NaCl plates, as KBr disks or via ATR as a solid on a zinc selenide crystal in the region 4000–400 cm^{-1} on a Perkin Elmer Spectrum 100 FT-IR spectrophotometer.

4.1.2. Typical procedure for Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction

Copper sulphate pentahydrate (60 mg) and sodium ascorbate (100 mg) were added to a solution of the acetylated sugar azide (1.1 equiv. per propargyl group) and the corresponding propargyl amide scaffold in acetonitrile/water (2:1 ratio, approx. 2 mL per 100 mg of

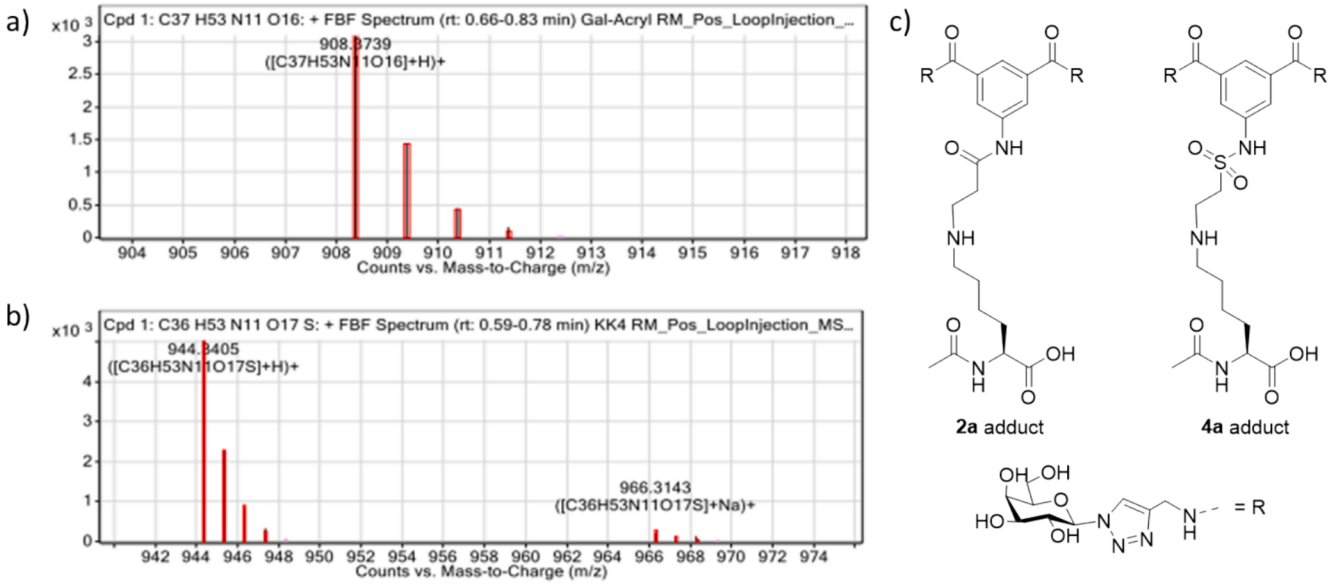


Fig. 4. HRMS spectra corresponding to the cross-linking adducts of *N*-α-acetyl-L-lysine and **2a** (expected mass 908.3705, found 908.3739); b) **4a** (expected mass 944.3375, found 944.3405)**;** c) chemical structure of these adducts.

glycoconjugate). The reaction was allowed to stir in the MW at 100 °C until deemed complete by TLC analysis (typically 30 mins). The solvent was removed in vacuo. The residue was dissolved in DCM, washed with brine (x3) and the organic phase was dried ($MgSO_4$). 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 99:1–90:10) to give the corresponding product.

4.1.3. Typical procedure for reaction of glycoconjugate with acryloyl chloride or ethanesulfonyl chloride

The corresponding amino glycoconjugate was placed under N_2, dissolved in dry DCM (approx. 1 mL per 100 mg of glycoconjugate) and anhydrous NEt_3 (1.2 equiv.) was added, Acryloyl chloride (4 equiv.) was then added, and the reaction mixture was stirred overnight at RT. The reaction was deemed complete following TLC analysis (90:10 DCM: MeOH) and an extraction was carried out using 1 M HCl (x1), sat. $NaHCO_3$ solution (x1) and brine (x1), the organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo to yield the crude product which was purified via flash column chromatography (99:1–90:10 DCM: MeOH).

4.1.4. Typical procedure for reaction of glycoconjugate with 2-chloroethansulfonyl chloride

The corresponding amino glycoconjugate was placed under N_2, dissolved in dry DCM (approx. 5 mL per 100 mg of glycoconjugate) and anhydrous NEt_3 (2.5 equiv.) was added. Another solution of 2-chloroethansulfonyl chloride (1 equiv.) in dry DCM (approx. 5 mL per equiv.) was then added over 30 min and the reaction mixture was stirred overnight at RT. The reaction was deemed complete following TLC analysis (90:10 DCM:MeOH) and an extraction was carried out using 1 M HCl (x1), sat. $NaHCO_3$ solution (x1) and brine (x1), the organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo to yield the crude product which was purified via flash column chromatography (99:1–90:10 DCM:MeOH).

4.1.5. Typical procedure for reaction of acetyl ester hydrolysis

The acetylated glycoconjugate was dissolved in methanol/water (2:1 ratio). NEt_3 (0.1 mL) was added, and the reaction mixture was allowed to stir at 45 °C until completion (typically 5–8 h). The solvent was removed in vacuo, the residue was dissolved in deionised water, Amberlite H^+ was added and the mixture was allowed to stir for 40 mins. The solution was filtered, and the filtrate was removed in vacuo and the residue was lyophilized to give the deprotected glycoconjugate.

4.1.6 N,N′-di-(prop-2-yn-1-yl)-5-nitroisophthalamide 7: 5-Nitroisophthalic acid (0.50 g, 2.4 mmol), TBTU (1.62 g, 5.04 mmol) were added to a round bottomed flask and dissolved in anhydrous DMF (10 mL) under N_2. NEt_3 (0.75 mL, 5.04 mmol) was added, and reaction mixture was stirred for 10 min. Propargylamine (0.3 mL, 5.04 mmol) was added and the reaction was stirred overnight at RT. The solvent was removed in vacuo and the crude product was dissolved in EtOAc (20 mL) and washed with water (2 x 20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica gel column chromatography (EtOAc: Petroleum ether 1:1–2:1) to give the pure product 7 as a white solid (1.44 g, 65 %). R_f = 0.27 (EtOAc: Petroleum ether 1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.64 (t, J = 5.5 Hz, 2H, N-H), 8.83 (s, 2H, Ar-H), 8.78 (s, 1H, Ar-H), 4.12 (dd, J = 5.5, 2.6 Hz, 4H, $NHCH_2$), 3.19 (t, J = 2.5 Hz, 2H, $NHCH_2CCH$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.9 ($CONHCH_2$), 148.4 (Ar-C), 135.9 (Ar-C), 132.9 (Ar-CH), 125.1 (Ar-CH), 81.2 (CH_2CCH) 73.9 (CH_2CCH), 29.4 (CH_2CCH). IR (ATR): 3342, 3283, 3080, 1640, 1584, 1534 cm^{-1}.

4.1.6. N,N′-di-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-1,2,3-triazol-4-ylmethylamide)-N″-tert-butoxycarbonyl-5-aminobenzene-1,3-dicarboxamide **8a**

Prepared from 6[20] and 2,3,4,6-tetra-O-acetyl-1- β-azido-D-galactopyranoside according to the typical procedure for Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction; the pure product 8a was obtained as a white solid (1.25 g, 71 %). R_f = 0.38 (DCM:MeOH 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (s, 2H, Ar-H), 7.98–7.84 (m, 4H, triaz-H and $NHCH_2$-triaz), 7.81 (bs, 2H, Ar-H and NHBoc), 5.95 (d, J = 9.2 Hz, 2H, H-1), 5.48 (t, J = 9.7 Hz, 4H, H-2 and H-4), 5.27 (dd, J = 10.3, 3.1 Hz, 2H, H-3), 4.63 (dd, J = 15.0, 5.1 Hz, 4H, CH_2-triaz), 4.31 (t, J = 6.4 Hz, 2H, H-5), 4.09 (dd, J = 11.5, 6.4 Hz, 4H, H-6 and H-6′), 2.14 (s, 6H, OAc), 1.92 (overlapping of 2 s, 12H, OAc x2), 1.72 (s, 6H, OAc), 1.41 (s, 9H, Boc). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3 (CO of OAc), 170.1 (CO of OAc), 169.8 (CO of OAc), 169.0 (CO of OAc), 166.7 ($CONHCH_2$-triaz), 152.8 (CO of Boc), 145.5 (C-triaz), 139.9 (Ar-C), 134.9 (Ar-C), 121.8 (CH_2-triaz), 120.5 (Ar-CH), 119.3 (Ar-CH), 86.0 (C-1), 73.8 (C-5), 70.8 (C-3), 68.1 (C-2), 67.0 (C-4), 61.2 (C-6), 35.3 (CH_2-triaz), 28.2 (CH_3 of Boc), 20.6 (CH_3 of OAc), 20.8 (CH_3 of OAc), 20.5 (CH_3 of OAc), 20.1 (CH_3 of OAc). IR (film on NaCl): 3434, 2106, 1752, 1648, 1558 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{47}H_{59}N_9O_{22}$ + H^+ $[M+H]^+$ 1102.3853, found 1102.3847.

4.1.7. N,N′-di-(2,3,4-tri-O-acetyl- β-L-fucopyranosyl-1,2,3-triazol-4-ylmethylamide)-N″-tert-butoxycarbonyl-5-aminobenzene-1,3-dicarboxamide **8b**

Prepared from 6[20] and 2,3,4-tri-O-acetyl-1- β-azido-L-fucopyranoside according to the typical procedure for Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction; the pure product 8b was obtained as a white solid (1.26 g, 72 %). R_f = 0.42 (DCM:MeOH 9:1). 1H NMR (500 MHz, CDCl3) δ 8.02 (s, 2H, ArH), 7.96 (s, 2H, triaz-H), 7.85 (s, 1H, ArH), 7.67 (s, 2H, NH), 7.16 (s, 1H, NHBoc), 5.86 (d, J = 9.2 Hz, 2H, H-1), 5.52–5.44 (m, 2H, H-2), 5.38 (dd, J = 3.4, 0.8 Hz, 2H, H-4), 5.25 (dd, J = 10.3, 3.4 Hz, 2H, H-3), 4.76 (dd, J = 15.2, 5.8 Hz, 2H, CH2), 4.63 (dd, J = 15.1, 5.6 Hz, 2H, CH2), 4.16 (q, J = 6.4 Hz, 2H, H-5), 2.23 (s, 6H, CH3 of OAc), 1.99 (s, 6H, CH3 of OAc), 1.82 (s, 6H, CH3 of OAc), 1.50 (s, 9H, Boc CH3), 1.24 (d, J = 6.4 Hz, 6H, C-6 CH3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5 (s, CO of OAc), 169.9 (s, CO of OAc), 169.2 (s, CO of OAc), 166.5 (s, CONH), 145.3 (s, qC-triaz), 139.6 (s, qCAr), 135.0 (s, qCAr), 121.5 (s, C-triaz), 120.4 (s, CAr), 119.4 (s, CAr), 86.2 (s, C-1), 72.6 (s, C-5), 71.1 (s, C-3), 69.8 (s, C-4), 68.2 (s, C-2), 35.3 (s, CH_2), 28.2 (s, Boc CH_3), 20.7 (s, CH_3 of OAc), 20.5 (s, CH_3 of OAc), 20.2 (s, CH_3 of OAc), 16.0 (s, C-6). IR (ATR): 1747, 1367, 1213, 1156, 1092, 1061 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{43}H_{56}N_9O_{18}$ + H^+ $[M+H]^+$: 986.3743, found 986.3767.

4.1.8. N,N′-di-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-1,2,3-triazol-4-ylmethylamide)-N″-tert-tbutoxycarbonyl-5-aminobenzene-1,3-dicarboxamide **8c**

Prepared from 6[20] and 2,3,4,6-tetra-O-acetyl-1-α-azido-D-mannopyranoside according to the typical procedure for Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction; the pure product 8c was obtained as a white solid (154 mg, 52 %). R_f = 0.38 (DCM:MeOH 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (s, 4H, triaz-H (x2) & $NHCH_2$ (x2)), 7.71 (s, 2H, ArH), 7.39 (s, 2H, ArH & NHBoc), 6.10 (d, J = 12.5 Hz, 2H, H-1), 6.05 (d, J = 9.6 Hz, 2H, H-2), 5.92–5.85 (m, 2H, H-3), 5.37 (td, J = 9.6, 3.4 Hz, 2H, H-4), 4.59 (td, J = 14.8, 4.6 Hz, 4H, CH_2-triaz), 4.23 (m, 2H, H-6/7), 4.01 (ddd, J = 14.2, 7.2, 4.7 Hz, 2H, H-6/7), 3.98–3.92 (m, 2H, H-5), 2.18–2.12 (m, 6H, CH_3 of OAc), 2.03 (m, 6H, CH_3 of OAc), 2.02–1.99 (m, 6H, CH_3 of OAc), 1.99–1.94 (m, 6H, CH_3 of OAc), 1.49–1.42 (m, 9H, CH_3 of Boc). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5 (s, CO of OAc), 170.0 (s, CO of OAc), 169.7 (s, CO of OAc), 169.6 (s, CO of OAc), 166.2 (s, CO), 145.5 (s, qCtriaz), 139.1 (s, qCAr), 134.5 (s, qCAr), 123.5 (s, C-triaz), 120.2 (s, CAr), 118.7 (s, CAr), 84.2 (s, C-1), 71.6 (s, C-5), 69.1 (s, C-3), 68.3 (s, C-2), 65.7 (s, C-4), 61.7 (s, C-6), 35.1 (s, CH_2NH), 28.2 (s, Boc CH_3 (x3)), 20.9 (s, CH_3 of OAc), 20.7 (s, CH_3 of OAc), 20.6 (s, CH_3 of OAc). IR (ATR): 1743, 1367, 1214, 1155, 1122, 1039 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{47}H_{59}N_9O_{22}$ + H^+ $[M+H]^+$ 1102.3853, found 1102.3842.

4.1.9. N,N′-di-(2,3,4,6-tetra-O-acetyl-1- β-D-galactopyranosol-1,2,3-triazol-4-ylmethylamide)-N″-propyl-5-nitrobenzene-1,3-dicarboxamide **9a**

Prepared from 7 and 2,3,4,6-tetra-O-acetyl-1- β-azido-D-galactopyranoside according to the typical procedure for Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction; the pure product 9a was obtained as a white solid (0.401 g, 82 %). $R_f = 0.56$ (DCM:MeOH 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 2H, Ar-H), 8.58 (s, 1H, Ar-H), 8.36 (t, $J = 5.5$ Hz, 2H, NH), 8.03 (s, 2H, triaz-H,), 6.00 (d, $J = 9$ Hz, 2H, H-1) 5.54–5.48 (m, 4H, H-2 and H-4), 5.31 (dd, $J = 10.3$, 3.3 Hz, 2H, H-3), 4.76 (dd, $J = 15$, 5.5 Hz, 2H, $NHCH_2$), 4.60 (dd, $J = 15$, 5.5 Hz, 2H, $NHCH_2'$), 4.35 (t, $J = 6.5$ Hz, 2H, H-5), 4.19–4.11 (m, 4H, H-6), 2.20 (s, 6H, OAc), 1.99–1.97 (m, 12H, OAc), 1.78 (s, 6H, OAc). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9 (CO of OAc), 169.6 (CO of OAc), 169.3 (CO of OAc), 168.6 (CO of OAc), 164.1 ($CONHCH_2$-triaz), 148.1 (Ar-C), 144.5 (C-triaz), 135.6 (Ar-C), 130.3 (Ar-CH), 124.9 (Ar-CH), 121.6 (CH-triaz), 85.7 (C-1), 73.5 (C-5), 70.2 (C-3), 67.7 (C-2), 66.4 (C-4), 60.7 (C-6), 34.8 ($NHCH_2$-triaz), 20.2 (CH_3 of OAc), 20.1 (CH_3 of OAc), 20.0 (CH_3 of OAc), 19.7 (CH_3 of OAc). IR (KBR): 3378, 3086, 2917, 1753, 1666, 1533 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{42}H_{49}N_9O_{22} + H^+$ $[M+H]^+$ 1032.3070, found 1032.3046.

4.1.10. N,N′-di-(2,3,4-tri-O-acetyl-1- β-L-fucopyranosol-1,2,3-triazol-4-ylmethylamide)-N″-propyl-5-nitrobenzene-1,3-dicarboxamide **9b**

Prepared from 7 and 2,3,4-tri-O-acetyl-1- β-azido-L-fucopyranoside according to the typical procedure for Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction; the pure product 9b was obtained as a white solid (0.112 g, 54 %). $R_f = 0.59$ (DCM:MeOH 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.83 (s, 2H, Ar-H), 8.59 (s, 1H, Ar-H), 8.31 (t, $J = 5.8$ Hz, 2H, NH), 8.04 (s, 2H, triaz-H), 5.95 (d, $J = 9$ Hz, 2H, H-1), 5.47–5.39 (m, 4H, H-2 and H-4), 5.28 (dd, $J = 10$, 3.5 Hz, 2H, H-3), 4.78 (dd, $J = 15$, 6 Hz, 2H, $NHCH_2$), 4.62 (dd, $J = 15.3$, 5.8 Hz, 2H, $NHCH_2'$), 4.21 (dd, $J = 12.8$, 6.3 Hz, 2H, H-5), 2.24 (s, 6H, OAc), 1.99 (s, 6H, OAc), 1.81 (s, 6H, OAc), 1.25 (d, $J = 6$ Hz, 6H, fuc-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5 (CO of OAc), 169.9 (CO of OAc), 169.2 (CO of OAc), 164.6 ($CONHCH_2$-triaz), 148.7 (Ar-C), 144.9 (C-triaz), 136.1 (Ar-C), 130.5 (Ar-CH), 125.5 (Ar-CH), 121.9 (CH-triaz), 86.4 (C-1), 72.8 (C-5), 71.0 (C-3), 69.8 (C-4 or C-2), 68.4 (C-4 or C-2), 35.3 ($NHCH_2$-triaz), 20.7 (CH_3 of OAc), 20.6 (CH_3 of OAc), 20.3 (CH_3 of OAc), 16.1 (C-6). IR (ATR): 3357, 2984, 1747 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{38}H_{45}N_9O_{22} + H^+$ $[M+H]^+$ 980.2757, found 980.2723.

4.1.11. N, N′-di-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-1,2,3-triazol-4-ylmethylamide)-N″-(1-oxo-2-propen-1-yl)-5-aminobenzene-1,3-dicarboxamide **10a**

Compound 8a (725 mg, 0.66 mmol) was dissolved in DCM (5 mL) and was cooled to 0 °C in an ice-bath. TFA (1.5 mL) was added, and the reaction mixture was stirred at RT. When the reaction was deemed complete by TLC analysis (2 h), DCM (40 mL) was added to the reaction mixture, it was washed with sat. $NaHCO_3$ (40 mL) and brine (40 mL), and dried ($MgSO_4$). The mixture was filtered, and the solvent was removed in vacuo to yield the *N*-Boc deprotected product which was used without further purification: pale yellow solid (689 mg, 99 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (s, 4H, triaz-H, $NHCH_2$-triaz), 7.43 (s, 1H, Ar-H), 7.17 (s, 2H, Ar-H), 5.96 (d, $J = 9.2$ Hz, 2H, H-1), 5.50 (m, 4H, H-2 and H-4), 5.29 (dd, $J = 10.3$, 3.3 Hz, 2H, H-3), 4.59 (dd, $J = 15.1$, 5.6 Hz, 4H, CH_2-triaz), 4.32 (t, $J = 6.5$ Hz, 2H, H-5), 4.23–4.00 (m, 4H, H-6 and H-6′), 2.16 (s, 6H, OAc), 1.94 (s, 12H, OAc), 1.73 (s, 6H, OAc). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.5 (CO of OAc), 170.4 (CO of OAc), 169.9 (CO of OAc), 169.0 (CO of OAc), 167.1 ($CONHCH_2$-triaz), 146.1 (C-triaz), 135.6 (Ar-C), 122.8 (CH-triaz), 115.7 (Ar-CH) 113.8 (Ar-CH) 84.7 (C-1), 73.4 (C-5), 71.0 (C-3), 68.1 (C-2), 67.8 (C-4), 62.0 (C-6), 35.1 (CH_2NH), 21.0 (CH_3 of OAc), 20.9 (CH_3 of OAc), 20.8 (CH_3 of OAc), 20.5 (CH_3 of OAc). IR (film on NaCl): 3434, 2103, 1751, 1642, 1534 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{42}H_{51}N_9O_{20} + H^+$ $[M+H]^+$ 1002.3329, found 1002.3323. Compound 10a was prepared from the *N*-Boc deprotected product according to the typical procedure for reaction of glycoconjugate with acryloyl chloride; the pure product 10a was obtained as a white solid (246 mg, 60 %). $R_f = 0.53$ (DCM:MeOH 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.08 (s, 1H, $NHCOCHCH_2$), 8.13–7.89 (m, 6H, HAr, CH-triaz & NH), 7.77 (s, 1H, HAr), 6.31 (q, $J = 16.8$ Hz, 2H, $COCHCH_2$ & $COCHCH_2$), 5.92 (d, $J = 9.2$ Hz, 2H, H-1), 5.65 (d, $J = 10.0$ Hz, 1H, $COCHCH_2$), 5.60 (t, $J = 9.7$ Hz, 2H, H-2), 5.53 (d, $J = 2.6$ Hz, 2H, H-4), 4.65 (dd, $J = 36.0$, 10.8 Hz, 4H, CH_2-triaz), 4.30 (t, $J = 6.0$ Hz, 2H, H-5), 4.15 (qd, $J = 11.5$, 6.6 Hz, 4H, H-6/7), 2.16 (d, $J = 17.1$ Hz, 6H, CH_3 of OAc), 1.98 (s, 12H, CH_3 of OAc), 1.79 (s, 6H, CH_3 of OAc). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3 (s, CO of OAc), 170.1 (s, CO of OAc), 169.8 (s, CO of OAc), 169.2 (s, CO of OAc), 166.7 (s, CO), 164.1 (s, CO-acryloyl), 145.5 (s, qC-triaz), 139.0 (s, qCAr), 134.8 (s, qCAr), 131.0 (s, Ce), 128.1 (s, Cf), 121.6 (s, CAr & C-triaz), 121.4 (s, CAr & C-triaz), 121.0 (s, CAr), 86.1 (s, C-1), 73.9 (s, C-5), 70.8 (s, C-3), 68.0 (s, C-2), 66.9 (s, C-4), 61.1 (s, C-6), 35.4 (s, CH_2), 20.6 (s, CH_3 of OAc), 20.6 (s, CH_3 of OAc), 20.4 (s, CH_3 of OAc), 20.2 (s, CH_3 of OAc). IR (ATR): 1744, 1367, 1208, 1091, 1044 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{45}H_{53}N_9O_{21} + Na^+$ $[M+Na]^+$ 1078.3254, found 1078.3253.

4.1.12. N, N′-di-(2,3,4-tri-O-acetyl-β-L-fucopyranosyl-1,2,3-triazol-4-ylmethylamide)-N″-(1-oxo-2-propen-1-yl)-5-aminobenzene-1,3-dicarboxamide **10b**

Compound 8b (1.26 g, 1.28 mmol) was dissolved in DCM (12 mL) and placed on ice, TFA (2.6 mL) was then gradually added and the reaction mixture was stirred at RT until deemed complete via TLC analysis (90:10 DCM:MeOH) (5 h). Sat. $NaHCO_3$ (40 mL) was then gradually added to the reaction mixture and stirred for 2-3mins, an extraction was then carried out using DCM and brine. The organic phase was dried ($MgSO_4$), filtered and the solvent was removed in vacuo to yield the *N*-Boc deprotected product which was used without further purification: white solid (715 mg, 63 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.82 (t, $J = 5.7$ Hz, 2H, NH), 8.06 (s, 2H, triaz-H), 7.44 (s, 1H, ArH), 7.16 (s, 2H, ArH), 6.16 (d, $J = 9.3$ Hz, 2H, H-1), 5.57 (t, $J = 9.7$ Hz, 2H, H-2), 5.45 (s, 2H, NH_2), 5.39 (dd, $J = 10.2$, 3.5 Hz, 2H, H-3), 5.25 (d, $J = 3.2$ Hz, 2H, H-4), 4.48 (d, $J = 5.6$ Hz, 4H, CH_2), 4.37 (q, $J = 6.2$ Hz, 2H, H-5), 2.19 (s, 6H, CH_3 of OAc), 1.95 (s, 6H, CH_3 ¬of OAc), 1.80 (s, 6H, CH_3 ¬of OAc), 1.10 (d, $J = 6.4$ Hz, 6H, C-6 CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.7 (s, CO of OAc), 169.9 (s, CO of OAc), 169.0 (s, CO of OAc), 167.0 (s, CO), 149.2 (s, qCAr), 145.9 (s, qC-triaz), 135.6 (s, qCAr), 122.6 (s, C-triaz), 115.7 (s, CAr), 113.7 (s, CAr), 84.7 (s, C-1), 71.8 (s, C-5), 71.3 (s, C-3), 70.3 (s, C-4), 68.1 (s, C-2), 35.1 (s, CH_2), 20.9 (s, CH_3 of OAc), 20.8 (s, CH_3 of OAc), 20.5 (s, CH_3 of OAc), 16.0 (s, C-6). IR (ATR): 1745, 1367, 1212, 1092, 1061, 1042, 1020 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{38}H_{48}N_9O_{16} + H^+$ $[M+H]^+$ 886.3219, found 886.3210. Compound 10b was prepared from the *N*-Boc deprotected product according to the typical procedure for reaction of glycoconjugate with acryloyl chloride; the pure product 10b was obtained as a white solid (41 mg, 48 %). $R_f = 0.6$ (DCM:MeOH 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.32 (s, 1H, NH), 8.19 (t, $J = 5.4$ Hz, 2H, NH), 8.00 (s, 2H, ArH), 7.97 (s, 2H, triaz-H), 7.78 (s, 1H), 6.38–6.24 (m, 2H, $COCHCH_2$ & $COCHCH_2$), 5.82 (d, $J = 9.2$ Hz, 2H, H-1), 5.65 (dd, $J = 9.1$, 2.5 Hz, 1H, $COCHCH_2$), 5.54 (dd, $J = 10.1$, 9.4 Hz, 2H, H-2), 5.39–5.34 (m, 2H, H-4), 5.27–5.21 (m, 2H, H-3), 4.64 (qd, $J = 15.4$, 5.6 Hz, 4H, CH_2), 4.17–4.10 (m, 2H, H-5), 2.20 (s, 6H, CH_3 of OAc), 1.98 (s, 6H, CH_3 of OAc), 1.83 (s, 6H, CH_3 of OAc), 1.22 (d, $J = 6.4$ Hz, 6H, C-6 CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2 (s, CO of OAc), 168.4 (s, CO of OAc), 168.2 (s, CO of OAc), 165.5 (s, $CONHCH_2$triaz), 163.0 (s, $COCHCH_2$), 144.0 (s, qC-triaz), 137.5 (s, qCAr), 133.4 (s, qCAr), 129.6 (s, Ce), 126.8 (s, Cf), 120.1 (s, CAr & C-triaz), 119.9 (s, CAr), 84.9 (s, C-1), 71.4 (s, C-5), 69.8 (s, C-3), 68.5 (s, C-4), 66.8 (s, C-2), 34.0 (s, CH_2), 19.3 (s, CH_3 of OAc), 19.2 (s, CH_3 of OAc), 19.0 (s, CH_3 of OAc), 14.7 (s, C-6). IR (ATR): 1746, 1367, 1212, 1092, 1061, 1042 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{41}H_{49}N_9NaO_{17} + Na^+$ $[M+Na]^+$ 962.3144, found 962.3124.

4.1.13. N, N′-di-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-1,2,3-triazol-4-ylmethylamide)-N″-(1-oxo-2-propen-1-yl)-5-aminobenzene-1,3-dicarboxamide **10c**

Compound 8c (255 mg, 0.23 mmol) was dissolved in DCM (4 mL) and was cooled to 0 °C in an ice-bath. TFA (0.6 mL) was added, and the reaction mixture was stirred at RT. When the reaction was deemed complete by TLC analysis (2 h), DCM (40 mL) was added to the reaction mixture, it was washed with sat. $NaHCO_3$ (40 mL) and brine (40 mL), and dried ($MgSO_4$). The mixture was filtered, and the solvent was removed in vacuo to yield the *N*-Boc deprotected product which was used without further purification: pale yellow solid (218 mg, 95 %). Compound 10c was prepared from the *N*-Boc deprotected product according to the typical procedure for reaction of glycoconjugate with acryloyl chloride; the pure product 10c was obtained as a white solid (127 mg, 55 %). $R_f = 0.55$ (DCM:MeOH 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (d, $J = 23.8$ Hz, 1H, NH), 8.21 (s, 2H, $NHCH_2$), 7.97 (s, 2H, triaz-H), 7.86 (s, 2H, ArH), 7.59 (s, 1H, ArH), 6.33 (dd, $J = 15.2$, 10.1 Hz, 2H, $COCHCH_2$), 6.12 (d, $J = 1.3$ Hz, 2H, H-1), 6.02 (s, 2H, H-2), 5.91 (dd, $J = 9.6$, 3.6 Hz, 2H, H-3), 5.68 (s, 1H, $COCHCH_2$), 5.42 (t, $J = 9.7$ Hz, 2H, H-4), 4.62 (qd, $J = 15.6$, 5.7 Hz, 4H, CH_2-triaz), 4.28 (dd, $J = 12.5$, 4.7 Hz, 2H, H-6/7), 4.11–4.02 (m, 2H, H-6/7), 3.99 (dd, $J = 5.2$, 2.6 Hz, 2H, H-5), 2.18 (s, 6H, CH_3 of OAc), 2.07 (s, 6H, CH_3 of OAc), 2.01 (s, 6H, CH_3 of OAc), 2.00 (s, 6H, CH_3 of OAc). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (s, CO of OAc), 170.0 (s, CO of OAc), 169.9 (s, CO of OAc), 169.7 (s, CO of OAc), 166.4 (s, CO), 164.3 (s, $COCHCH_2$), 145.5 (s, qCtriaz), 134.4 (s, qCAr), 131.0 (s, $COCHCH_2$), 128.0 (s, $COCHCH_2$), 123.5 (s, C-triaz), 121.6 (s, CAr), 120.4 (s, CAr), 84.1 (s, C-1), 71.8 (s, C-5), 69.3 (s, C-3), 68.3 (s, C-2), 65.5 (s, C-4), 61.7 (s, C-6), 35.1 (s, CH_2NH), 20.8 (s, CH_3 of OAc), 20.7 (s, CH_3 of OAc), 20.6 (s, CH_3 of OAc), 20.6 (s, CH_3 of OAc). IR (ATR): 1479, 1370, 1219, 1041 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{45}H_{53}N_9O_{21} + H^+$ $[M+H]^+$ 1056.3434, found 1056.3423.

4.1.14. N, N′-di-(2,3,4,6-tetra-O-acetyl- β-D-galactopyranosyl-1,2,3-triazol-4-ylmethylamide)-N″-(1-sulfoxo-2-propyl)-5-aminobenzene-1,3-dicarboxamide **11a**

Compound **11a** was prepared from the *N*-Boc deprotected product resulting from **8a** according to the typical procedure for reaction of glycoconjugate with ethanesulfonyl chloride; the pure product **11a** was obtained as a brown solid (169 mg, 77 %). $R_f = 0.2$ (DCM: MeOH 98:2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (bs, 1H, NH-S), 8.03 (s, 2H, triaz-CH), 7.99 (s, 2H, Amide-H), 7.95 (s, 1H, Ar-H), 7.95 (s, 2H, Ar-H), 5.97 (d, $J = 9.2$ Hz, 2H, H-1), 5.56 (m, 4H, H-2 and H-4), 5.32–5.29 (m, 2H, H-3), 4.79–4.61 (m, 4H, CH_2-triaz), 4.34 (t, $J = 6.8$ Hz, 2H, H-5), 4.19–4.15 (m, 4H, H-6 and H-6′), 3.14 (m, 2H, S-CH_2), 2.23 (s, 6H, OAc), 2.02 (s, 12H, OAc), 1.84 (s, 6H, OAc), 1.32 (t, $J = 7.4$ Hz, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.4 (CO of OAc), 170.1 (CO of OAc), 169.9 (CO of OAc), 169.3 (CO of OAc), 165.1 ($CONHCH_2$-triaz), 145.5 (Triaz-C), 135.4 (Ar-C), 134.7 (Ar-C), 133.2 (Amide CO), 126.7 (triaz-CH), 121.6 (Ar-CH), 86.2 (C-1), 74.09 (C-5), 70.7 (C-3), 68.2 (C-2), 66.9 (C-4), 61.2 (C-6), 50.38 (S-CH_2), 35.3 (CH_2NH), 20.7 (CH_3 of OAc), 20.6 (CH_3 of OAc), 20.5 (CH_3 of OAc), 20.3 (CH_3 of OAc), 7.7 (SCH_2CH_3). HRMS (ESI+): *m/z* calcd. for $C_{44}H_{55}N_9O_{22}S + H^+$ $[M+H]^+$ 1094.3260, found 1094.3270.

4.1.15. N′,N″-di-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-1H-1,2,3-triazol-4-yl)methyl))-5-(vinylsulfonamido)isophthalamide **12a**

Compound 12a was prepared from the *N*-Boc deprotected product resulting from 8a according to the typical procedure for reaction of glycoconjugate with 2-chloroethansulfonyl chloride; the pure product 12a was obtained as a brown solid (158 mg, 48 %). $R_f = 0.28$ (Ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 2H, triaz-CH), 7.96 (s, 1H, Ar-H), 7.94 (s, 2H, Ar-H), 7.87 (s, 2H, NHCO), 6.59 (dd, $J = 16.5$, 9.9 Hz, 1H, HC = CH_2), 6.28 (d, $J = 16.5$ Hz, 1H, C = CH_2), 5.97 (d, $J = 9.2$ Hz, 2H, H-1), 5.94 (d, $J = 9.9$ Hz, 1H, C = CH_2), 5.58–5.54 (m, 4H, H-2 & H-4), 5.32–5.29 (m, 2H, H-3), 4.81–4.77 (m, 4H, $NHCH_2$-triaz), 4.33 (t, $J = 6.6$ Hz, 2H, H-5), 4.21–4.13 (m, 4H, H-6 and H-6′), 2.23 (s, 6H, OAc), 2.02 (d, $J = 4.6$ Hz, 12H OAc x 2), 1.84 (s, 6H, OAc). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4 (s, CO of OAc), 170.1 (s, CO of OAc), 169.8 (s, CO of OAc), 169.2 (s, CO of OAc), 166.1 (s, $CONHCH_2$), 145.1 (s, qCtriaz), 135.4 (s, qCAr), 135.1 (s, SO_2CHCH_2), 128.8 (s, SO_2CHCH_2), 122.1 (s, C-triaz), 121.7 (s, CAr), 121.2 (s, CAr), 86.1 (s, C-1), 73.9 (s, C-5), 70.7 (s, C-3), 68.1 (s, C-2), 66.8 (s, C-4), 61.1 (s, C-6), 35.2 (s, $NHCH_2$-triaz), 20.7 (s, CH_3 of OAc), 20.6 (s CH_3 of OAc), 20.5 (s CH_3 of OAc), 20.2 (s, CH_3 of OAc). IR (ATR): 2965, 2918, 1746, 1655, 1533, 1432, 1368, 1329, 1210, 1157, 1045 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{44}H_{53}N_9O_{22}S + H^+$ $[M+H]^+$ 1092.3104, found 1092.3089.

4.1.16. N′,N″-di-(2,3,4-tri-O-acetyl- β-L-fucopyranosyl-1,2,3-triazol-4-ylmethylamide)-1H-1,2,3-triazol-4-yl)methyl))-5-(vinylsulfonamido) isophthalamide **12b**

Compound **12b** was prepared from the *N*-Boc deprotected product resulting from **8b** according to the typical procedure for reaction of glycoconjugate with 2-chloroethansulfonyl chloride; the pure product **12b** was obtained as a white solid (107 mg, 73 %). $R_f = 0.32$ (Ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (s, 1H, $NHSO_2CHCH_2$), 8.00 (s, 2H, triaz-H), 7.94 (s, 2H, ArH), 7.88 (s, 3H, ArH & $NHCH_2$), 6.56 (dd, $J = 16.5$, 9.9 Hz, 1H, SO_2CHCH_2), 6.27 (d, $J = 16.5$ Hz, 1H, SO_2CHCH_2), 5.91 (m, 3H, SO_2CHCH_2 & H-1), 5.50 (dd, $J = 18.9$, 9.6 Hz, 2H, H-2), 5.39 (d, $J = 3.1$ Hz, 2H, H-4), 5.28 (dd, $J = 10.3$, 3.4 Hz, 2H, H-3), 4.79 (dd, $J = 15.1$, 5.7 Hz, 2H, CH_2-triaz), 4.64 (dd, $J = 15.2$, 5.4 Hz, 2H, CH_2-triaz), 4.23–4.14 (m, 2H, H-5), 2.27–2.20 (m, 6H, CH_3 of OAc), 2.03–1.96 (m, 6H, CH_3 of OAc), 1.83 (s, 6H, CH_3 of OAc), 1.26 (dd, $J = 13.2$, 4.1 Hz, 6H, C-6 CH3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5 (s, CO), 169.9 (s, CO), 169.3 (s, CO), 166.1 (s, CONHCH2), 145.1 (s, qCAr), 135.4 (s, qCAr), 135.2 (s, SO_2CHCH_2), 128.8 (s, SO_2CHCH_2), 122.0 (s, CAr), 121.6 (s, Ctriaz), 121.1 (s, CAr), 86.3 (s, C-1), 72.7 (s, C-5), 71.1 (s, C-3), 69.8 (s, C-4), 68.2 (s, C-2), 35.2 (s, CH_2-triaz), 20.7 (s, CH_3 of OAc), 20.5 (s, CH_3 of OAc), 20.3 (s, CH_3 of OAc), 16.0 (s, C-6). HRMS (ESI+): *m/z* calcd. for $C_{40}H_{49}N_9O_{18}S + H^+$ $[M+H]^+$ 976.2995, found 976.3018.

4.1.17. N, N′-di-(β-D-galactopyranosyl-1,2,3-triazol-4- ylmethylamide)-N″-(1-oxo-2-propen-1-yl)-5-aminobenzene-1,3-dicarboxamide **2a**

Compound 2a was prepared from 10a according to the typical procedure for acetyl ester hydrolysis. Compound 2a was obtained as an off-white fluffy solid (114 mg, 97 %). ^{1}H NMR (500 MHz, D_2O) δ 8.26 (s, 2H, triaz-H), 8.07 (s, 2H, Ar-H), 7.94 (s, 1H, Ar-H), 6.47–6.33 (m, 2H, He & Hf $COCHCH_2$ & $COCHCH_2$), 5.91 (d, $J = 10.2$ Hz, 1H, $COCHCH_2$), 5.70 (d, $J = 9.1$ Hz, 2H, H-1), 4.72 (s, 4H, CH_2), 4.23 (t, $J = 9.5$ Hz, 2H, H-2), 4.10 (d, $J = 2.9$ Hz, 2H, H-4), 4.01 (t, $J = 6.1$ Hz, 2H, H-5), 3.89 (dd, $J = 9.8$, 3.3 Hz, 2H, H-3), 3.79 (d, $J = 6.0$ Hz, 4H, H-6/7). ^{13}C NMR (125 MHz, D_2O) δ 168.1 (s, CO), 166.2 (s, CO), 138.0 (s, qCAr), 134.0 (s, qCtriaz), 129.9 (s, Calkene), 129.0 (s, Calkene), 123.1 (s, C-triaz), 122.0 (s, CAr), 121.8 (s, CAr), 88.0 (s, C-1), 78.2 (s, C-5), 72.8 (s, C-3), 69.7 (s, C-2), 68.5 (s, C-4), 60.8 (s, C-6), 34.9 (s, CH_2). IR (ATR): 3266, 1644, 1538, 1287, 1211, 1090, 1052, 1015 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{29}H_{37}N_9O_{13} + Na^+$ $[M+Na]^+$ 742.2409, found 742.2399.

4.1.18. N, N′-di-(β-L-fucopyranosyl-1,2,3-triazol-4- ylmethylamide)-N″-(1-oxo-2-propen-1-yl)-5-aminobenzene-1,3-dicarboxamide **2b**

Compound 2b was prepared from 10b according to the typical procedure for acetyl ester hydrolysis. Compound 2b was obtained as an off-white fluffy solid (76 mg, 95 %). ^{1}H NMR (500 MHz, D_2O) δ 8.23 (s, 2H, triaz-H), 7.84 (s, 2H, ArH), 7.74 (s, 1H, ArH), 6.24 (d, $J = 5.2$ Hz, 2H, He & Hf), 5.78 (m, 1H, Hf), 5.63 (d, $J = 9.1$ Hz, 2H, H-1), 4.65–4.55 (m, 4H, CH_2), 4.18 (t, $J = 9.3$ Hz, 2H, H-2), 4.04 (d, $J = 6.5$ Hz, 2H, H-5), 3.91–3.81 (m, 4H, H-3 & H-4), 1.21 (dd, $J = 19.7$, 6.0 Hz, 6H, C-6 CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.1 (s, CO), 165.1 (s, CO-acryloyl), 137.1 (s, qCAr), 133.1 (s, qCAr), 129.0 (s, Ce), 128.1 (s, Cf), 122.2 (s, C-triaz), 120.9 (s, Cf), 87.1 (s, C-1), 73.4 (s, C-5), 72.2 (s, C-3/4), 70.2 (s, C-3/4), 68.5 (s, C-2), 34.1 (s, CH_2), 14.6 (s, C-6). IR (ATR): 3271, 1651, 1596, 1538, 1445, 1423, 1339, 1285, 1211, 1158, 1091 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{29}H_{37}N_9NaO_{11} + Na^+$ $[M+Na]^+$ 710.2510,

found 710.2488.

4.1.19. N, N′-di-(α-D-mannopyranosyl-1,2,3-triazol-4- ylmethylamide)-N″-(1-oxo-2-propen-1-yl)-5-aminobenzene-1,3-dicarboxamide **2c**

Compound 2c was prepared from 10c according to the typical procedure for acetyl ester hydrolysis. Compound 2c was obtained as a white fluffy solid (47 mg, 94 %). ^{1}H NMR (500 MHz, D_2O) δ 8.10 (s, 2H, triaz-H), 7.83 (d, J = 1.4 Hz, 2H, ArH), 7.73 (s, 1H, ArH), 6.27–6.22 (m, 2H, $COCHCH_2$ & $COCHCH_2$), 6.04 (d, J = 2.2 Hz, 2H, H-1), 5.81–5.74 (m, 1H, $COCHCH_2$), 4.72 (dd, J = 3.4, 2.4 Hz, 2H, H-2), 4.57 (s, 4H, CH_2-triaz), 4.10 (dd, J = 9.1, 3.5 Hz, 2H, H-3), 3.81–3.68 (m, 6H, H-4, H-6 & H-7), 3.30–3.23 (m, 2H, H-5). 13C NMR (125 MHz, D_2O) δ 168.3 (s, $CONHCH_2$), 166.3 (s, $COCHCH_2$), 144.5 (s, qCtriaz), 138.0 (s, qCAr), 134.2 (s, qCAr), 130.0 (s, $COCHCH_2$), 129.0 (s, $COCHCH_2$), 123.6 (s, C-triaz), 122.1 (s, ArH), 121.8 (s, ArH), 86.6 (s, C-1), 76.1 (s, C-5), 70.4 (s, C-3), 68.2 (s, C-2), 66.4 (s, C-4), 60.4 (s, C-6), 34.9 (s, CH_2-triaz). IR (ATR): 3272, 1648, 1541, 1445, 1424, 1340, 1266, 1207, 1046 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{29}H_{37}N_9O_{13}$ + Na^+ $[M+Na]^+$ 742.2409, found 742.2401.

4.1.20. N, N′-di-(β-D-galactopyranosyl-1,2,3-triazol-4- ylmethylamide)-N″-(1-sulfoxo-2-propyl)-5-aminobenzene-1,3-dicarboxamide **3a**

Compound 3a was prepared from 11a according to the typical procedure for acetyl ester hydrolysis. Compound 3a was obtained as a white fluffy solid (95 mg, 82 %). ^{1}H NMR: (500 MHz, D_2O) δ 8.25 (s, 2H, Ar-H), 7.93 (s, 1H, Ar-H), 7.75 (s, 2H, Triaz-CH), 5.69 (d, J = 9.1 Hz, 2H, H-1), 4.71–4.70 (m, 4H, CH_2-Triaz), 4.22 (t, J = 9.4 Hz, 2H, H-2), 4.10–4.09 (m, 2H, H-4), 4.00 (t, J = 5.9 Hz, 2H, H-3), 3.90–3.87 (dd, J = 9.8, 3.1 Hz, 2H, H-5), 3.79 (d, J = 6.0 Hz, 4H, H-6 & H-6′), 3.31–3.27 (m, 3H CH_3), 2.94–2.89 (q, J = 7.4 Hz, 2H, CH_2). ^{13}C NMR: (126 MHz, D_2O) δ 168.65 (s, CONH), 137.84 (s Triaz-C), 135.29 (s qCAr), 122.36 (s Ar-CH), 122.20 (s Ar-CH), 88.05 (s C-1), 78.28 (s C-3), 72.92 (s, C-5), 69.75 (s, C-2), 68.56 (s, C-4), 60.84 (s C-6), 46.00 (s, S-CH_2), 45.38 (s, CH_3), 35.08 (s, CH_2-triaz). IR: 3295, 2927, 2884 1641, 1539, 1437, 1402, 1321, 1285, 1235, 1138, 1091, 1051 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{28}H_{39}N_9O_{14}$ + Na^+ $[M+Na]^+$ 757.2337, found 780.2227.

4.1.21. N′,N″-di-((β-D-galactopyranosyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(vinylsulfonamido)isophthalamide 4a

Compound 4a was prepared from 12a according to the typical procedure for acetyl ester hydrolysis. Compound 4a was obtained as a white fluffy solid (75 mg, 89 %). ^{1}H NMR (500 MHz, D_2O) δ 8.22 (s, 2H, Triaz-H), 7.86 (s, 1H, Ar-H), 7.61 (s, 2H, Ar-H), 6.71 (ddd, J = 16.5, 10.0, 1.0 Hz, 1H, SOCH), 6.21 (d, J = 16.5 Hz, 1H, HC = CH_2), 6.06–6.04 (m, 1H, HC = CH_2), 5.66 (d, J = 9.2 Hz, 2H, H-1), 4.64 (s, 4H, $NHCH_2$), 4.22 (t, J = 9.5 Hz, 2H, H-2), 4.08 (d, J = 3.2 Hz, 2H, H-4), 3.98 (t, J = 6.0 Hz, 2H, H-5), 3.88–3.85 (m, 2H, H-3), 3.76 (d, J = 5.9 Hz, 4H, H-6 &H-6′). ^{13}C NMR (125 MHz, D_2O) δ 168.4 (s, CONH), 144.8 (s, qCtriaz), 137.3 (s, qCAr), 136.1 (s, qCAr), 133.7 (s,SO_2CHCH_2), 130.1 (s,SO_2CHCH_2), 123.5 (s, Ar-CH), 123.4 (s, Ar-CH), 122.9 (s, Triaz-CH), 122.3 (s, Ar-CH), 88.0 (s, C-1), 78.2 (s, C-5), 72.9 (s, C-3), 69.7 (s, C-2), 68.5 (s, C-4), 60.8 (s, C-6), 35.0 (s, CH_2NH.) IR: 2962, 1720, 1645, 1596, 1541, 1405, 1331, 1288, 1203, 1135, 1092, 1051 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{28}H_{37}N_9O_{14}S$ + H^+ $(M+H)^+$ 755.21, found 756.2264.

4.1.22. N′,N″-di-(β-L-fucopyranosyl-1,2,3-triazol-4-ylmethylamide)-1H-1,2,3-triazol-4-yl)methyl))-5-(vinylsulfonamido)isophthalamide **4b**

Compound **4b** was prepared from **12b** according to the typical procedure for acetyl ester hydrolysis. Compound **4b** was obtained as a white fluffy solid (74 mg, 90 %). ^{1}H NMR (500 MHz, D_2O) δ 8.14 (s, 2H, triaz-H), 7.75 (s, 1H, ArH), 7.53 (s, 2H, ArH), 6.59 (dt, J = 24.3, 12.1 Hz, 1H, SO_2CHCH_2), 6.11 (d, J = 16.5 Hz, 1H, SO_2CHCH_2), 5.94 (t, J = 9.3 Hz, 1H, SO_2CHCH_2), 5.55 (d, J = 9.1 Hz, 2H, H-1), 4.57 (s, 4H, CH_2-triaz), 4.09 (t, J = 9.4 Hz, 2H, H-2), 3.96 (d, J = 6.3 Hz, 2H, H-5), 3.78 (dd, J = 14.8, 5.1 Hz, 4H, H-3 & H-4), 1.23–1.09 (m, 6H, C-6 CH_3). 13C NMR (125 MHz, D2O) δ 168.2 (s, CO), 145.2 (s, qCtriaz), 137.9 (s, qCAr), 134.9 (s, qCAr), 133.9 (s, SO2CHCH2), 129.7 (s, SO2CHCH2), 122.6 (s, CAr & C-triaz), 121.9 (s, CAr), 87.9 (s, C-1), 74.3 (s, C-5), 73.0 (s, C-3/4), 71.1 (s, C-3/4), 69.4 (s, C-2), 35.0 (s, CH2-triaz), 15.5 (s, C-6). HRMS (ESI+): *m/z* calcd. for $C_{28}H_{37}N_9O_{12}$ + Na^+ $[M+Na]^+$ 714.2459, found 714.2425.

4.1.23. N,N′-di-(β-D-galactopyranosol-1,2,3-triazol-4-ylmethylamide)-N″-propyl-5-nitrobenzene-1,3-dicarboxamide **5a**

Compound 5a was prepared from the 9a according to the typical procedure for acetyl ester hydrolysis. Compound 5a was obtained as a pale yellow solid (77 mg, 50 %). ^{1}H NMR (500 MHz, D_2O) δ 8.65 (s, 2H, Ar-H), 8.43 (s, 1H, Ar-H), 8.25 (s, 2H, triaz-H), 5.65 (d, J = 9 Hz, 2H, H-1), 4.67 (s, 4H, $NHCH_2$), 4.17 (t, J = 9.5 Hz, 2H, H-2), 4.04 (d, J = 3 Hz, 2H, H-4), 3.96 (s, 2H, H-5), 3.84 (dd, J = 10, 3.5 Hz, 2H, H-3), 3.73 (d, J = 6 Hz, 4H, H-6). ^{13}C NMR (125 MHz, D_2O) δ 166.5 ($CONHCH_2$-triaz), 147.6 (Ar-C), 134.9 (Ar-C), 131.5 (Ar-CH), 124.8 (Ar-CH), 122.9 (CH-triaz), 122.9 (C-triaz), 87.7 (C-1), 77.9 (C-5), 72.5 (C-3), 69.4 (C-2), 68.2 (C-4), 60.5 (C-6), 34.7 ($NHCH_2$-triaz). IR (ATR): 3269, 1651, 1531 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{26}H_{33}N_9O_{14}$ + H^+ $[M+H]^+$ 695.2147, found 696.2206.

4.1.24. N,N′-di-(β-L-fucopyranosol-1,2,3-triazol-4-ylmethylamide)-N″-propyl-5-nitrobenzene-1,3-dicarboxamide **5b**

Compound 5b was prepared from the 9b according to the typical procedure for acetyl ester hydrolysis. Compound 5b was obtained as a pale yellow solid (64 mg, 79 %). ^{1}H NMR (500 MHz, D_2O) δ 8.63 (s, 2H, Ar-H), 8.42 (s, 1H, Ar-H), 8.23 (s, 2H, triaz-H), 5.63 (d, J = 9 Hz, 2H, H-1), 4.66 (s, 4H, $NHCH_2$), 4.15 (t, J = 9.5 Hz, 2H, H-2), 4.05 (d, J = 6.5 Hz, 2H, H-5), 3.88–3.83 (m, 4H, H-3 and H-4), 1.23 (d, 6.5 Hz, 6H, H-6). ^{13}C NMR (125 MHz,D_2O) δ 169.32 ($CONHCH_2$-triaz), 150.45 (Ar-C), 147.21 (C-triaz), 137.87 (Ar-C), 134.45 (Ar-CH), 127.67 (Ar-CH), 125.52 (CH-triaz), 90.54 (C-1), 76.89 (C-5), 75.61 (C-3), 73.71 (C-4 or C-2), 72.01 (C-4 or C-2), 37.63 ($NHCH_2$-triaz), 18.04 (C-6). IR (ATR): 3357, 2984, 1747 cm^{-1}. HRMS (ESI+): *m/z* calcd. for $C_{26}H_{33}N_9O_{12}$ + Na^+ $[M+Na]^+$ 686.2146, found 686.2175.

4.2. Determination of minimum fungicidal concentration

The fungicidal efficacy of the compounds was evaluated against *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC 90030, *C. auris* (ATCC CMYA-5001), *C. lusitaniae* C18 and *C. guilliermondii* C94. The tested *Candida* species were obtained from the American Type Culture Collection (ATCC), except for *C. lusitaniae* and *C. guilliermondii*, sourced from Vilnius University and described in Lastauskienė et al.[27]

The antifungal efficacy of the compounds was evaluated using a Spot assay described by Suppi *et al.*[28] and Kasemets *et al.*[29] Briefly, the relevant *Candida* species were incubated with the studied compounds at concentrations ranging from 0–10 mg/mL (for compounds **2b**, **2c**, **4a** and **4b**) or 0–100 mg/L (for compounds **1a**, **2a**, **3a**, **5a** and **5b**) in deionized (DI) water (Milli-Q, Millipore) in a 96-well microplate (BD Falcon 351172, USA) for 24 h at 30 °C without shaking. After 24-h incubation, 5 μl of the cell cultures from each well were transferred (spotted) onto yeast extract peptone dextrose (YPD) agar plates (2 % peptone, 1 % yeast extract, 2 % glucose and 2 % agar) and incubated at 30 °C for 48 h. Then, the growth of cells, indicated by colony formation, was visually evaluated, and the minimum fungicidal concentration (MFC) was determined. MFC was defined as the lowest tested concentration of the compound, which completely inhibited the subsequent growth of cells (i.e., no formation of colonies) on agar medium after 24-h exposure in DI water. The experiments were conducted twice, each with two replicates per test.

4.3. Model cross-linking experiments

250 μL of a stock 20 mM solution of the electrophile (compounds **2a**

or **4a**, 1 equiv.) was placed under N_2; 4.5 mL of the nucleophile (*N*-α-acetyl-L-lysine, *N*-acetyl-L-histidine or *N*-acetyl-L-cysteine, 50 equiv.) dissolved in either pH 7.4 or pH 10.2 phosphate buffer (55.5 mM stock solution) was then added. Aliquots were taken after 3 h, 6 h and 24 h, freeze dried and redissolved to be analysed by HPLC using a 90:10 H_2O:ACN isocratic mobile phase with a Phenomenex Luna CN column 5 μm (detector set at 254 nm), along with standard samples of the electrophile (**2a** or **4a**) and the nucleophiles (*N*-α-acetyl-L-lysine, *N*-acetyl-L-histidine or *N*-acetyl-L-cysteine).

CRediT authorship contribution statement

Kyle Doherty: Methodology, Investigation, Formal analysis, Conceptualization. **Keela Kessie:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Harlei Martin:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Jordan Loughlin:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Oliwier Dulawa:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Kaja Kasemets:** Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Trinidad Velasco-Torrijos:** Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supporting information includes Supplementary Figures and copies of NMR spectra of new compounds. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2024.118020.

Data availability

Data will be made available on request.

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