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Design, synthesis and biological evaluation of mannosyl triazoles as FimH antagonists

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ABSTRACT

Urinary tract infection (UTI) caused by uropathogenic *Escherichia coli* (UPEC) is one of the most prevalent infectious diseases. Particularly affected are women, who have a 40–50% risk to experience at least one symptomatic UTI episode at some time during their life. In the initial step of the infection, the lectin FimH, located at the tip of bacterial pili, interacts with the high-mannosylated uroplakin Ia glycoprotein on the urinary bladder mucosa. This interaction is critical for the ability of UPEC to colonize and invade the bladder epithelium. X-ray structures of FimH co-crystallized with two different ligands, the physiological binding epitope oligomannose-3 and the antagonist biphenyl α-D-mannoside **4a** revealed different binding modes, an *in-docking-mode* and an *out-docking-mode*, respectively. To accomplish the *in-docking-mode*, that is the docking mode where the ligand is hosted by the so-called tyrosine gate, FimH antagonists with increased flexibility were designed and synthesized. All derivatives **5**–**8** showed nanomolar affinities, but only one representative, the 4-pyridiyl derivative **5j**, was as potent as the reference compound *n*-heptyl α-D-mannoside (**1b**). Furthermore, a loss of affinity was observed for *C*-glycosides and derivatives where the triazole aglycone is directly *N*-linked to the anomeric center. A conformational analysis by NMR revealed that the triazolyl-methyl-*C*-mannosides **8** adopt an unusual $^1C_4$ chair conformation, explaining the comparably lower affinity of these compounds. Furthermore, to address the drug-likeness of this new class of FimH antagonists, selected pharmacokinetic parameters, which are critical for oral bioavailability (lipophilicity, solubility, and membrane permeation), were determined.

1. Introduction

Urinary tract infections (UTIs) are among the most common infections, affecting millions of people each year. Although UTIs rarely cause severe diseases such as pyelonephritis or urosepsis, they are associated with extensive morbidity and generate considerable medical expenses.[1] Uropathogenic *Escherichia coli* (UPEC) are the primary cause of UTIs accounting for 70–95% of the reported cases. Particularly affected are women, who have a 40–50% risk to suffer from a symptomatic UTI episode at some time during their life.[2,3] Symptomatic UTIs require antimicrobial treatment, resulting in selection and development of bacterial resistance. Consequently, treatment of consecutive infections becomes increasingly difficult. Especially patients with diabetes, urinary tract anomaly, paraplegia and those with permanent urinary catheter experience repeated UTIs with resistant strains. Therefore, a new approach for the treatment and prevention of UTI with non-antibiotic and orally applicable therapeutics with a low potential for resistance would have a great impact on patient care, public healthcare, and medical expenses.

UPEC express a number of well-studied virulence factors for successful colonization of and survival within the host.[1,4,5] One important virulence factor, the mannose-specific FimH adhesin, is located at the tip of bacterial type 1 pili.[6] Type 1 pili are the most prevalent fimbriae encoded by UPEC, consisting of the four subunits FimA, FimF, FimG and FimH. The FimH lectin enables UPEC to attach to high-mannosylated uroplakin Ia glycoproteins on the urinary bladder mucosa, thus enabling adherence and invasion of host cells and at the same time preventing the rapid clearance of

Abbreviations: ABTS, 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid); AUC, area under the curve; BSA, bovine serum albumin; CRD, carbohydrate recognition domain; D, distribution coefficient; DCM, dichloromethane; DMSO, dimethyl sulfoxide; GIT, gastrointestinal tract; GPE, guinea pig erythrocytes; HEPES, 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid; IC_{50}, half maximal inhibitory concentration; iv, intravenous; Man, D-mannose; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser enhancement spectroscopy; PAA, polyacrylamide; PAMPA, parallel artificial membrane permeation assay; P_{app}, apparent permeability; P_e, effective permeation; po, peroral; rIC_{50}, relative inhibitory concentration; SAR, structure–activity relationship; THF, tetrahydrofurane; TM-PAA, Manα(1-3)-[Manα(1-6)]-Manβ(1-4)-Glc*N*Acβ(1-4)-Glc*N*Acβ-PAA; UPEC, uropathogenic *E. coli*; UTI, urinary tract infection.

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E. coli from the UTI by the bulk flow of urine.[1,7] As a part of the FimH subunit, a carbohydrate-recognition domain (CRD) is responsible for bacterial interactions with the host cells within the urinary tract.[7] The crystal structure of methyl α-D-mannopyranoside bound to the FimH-CRD was solved[8] and the structures of the corresponding complexes with *n*-butyl α-D-mannopyranoside,[9] Manα(1-3)-[Manα(1-6)]-Manβ(1-4)-Glc*N*Acβ-(1-4)Glc*N*Ac (oligomannose-3)[10] and biphenyl α-D-mannopyranoside[11] recently became available.

Previous studies showed that colonization and subsequent *E. coli* infection of the human urothelium can be prevented by vaccination with FimH adhesin.[12,13] Furthermore, adherence and invasion of host cells by *E. coli* can also be inhibited by oligomannosides representing the glycosylation of uroplakin **1a**.[14] For some α-D-mannosides it was shown that they prevent type 1 pili mediated adhesion, that is, they do not act by killing or arresting the growth of the pathogen as antibiotics do. Therefore, the spread of strains resistant to such agents are expected to be significantly delayed as compared to that of strains resistant to antibiotics.[15] In addition, environmental contamination is less problematic compared to antibiotics.[15a]

More than two decades ago, various oligomannosides and aromatic α-D-mannosides that antagonize type 1 fimbriae-mediated bacterial adhesion were identified.[15,16] However, for these mannosides only weak interactions in the milli- to micromolar range were observed. To improve their affinity, the multivalent presentation of the α-mannoside epitope,[17] and the rational design of ligands guided by structural information were explored.[9–11] Recently, various reports on high affinity monovalent FimH antagonists were published.[11,18,19]

The CRD of the FimH protein consists of amino acids with hydrophilic side chains and can therefore establish a perfect network of hydrogen bonds with the hydroxyl groups at the 2-, 3-, 4- and 6-positions of D-mannose. The entrance to this mannose-binding pocket, the so-called 'tyrosine gate', is shaped by two tyrosines (Tyr48 and Tyr137), and one isoleucine (Ile52) which support hydrophobic contacts.[20] Generally, long chain alkyl and aryl mannosides (for selected examples see Fig. 1) displayed the highest affinities.[8,9,11,16–21]

Recently, we reported the synthesis, the critical pharmacokinetic properties and affinity data of low molecular weight α-D-mannosides with the ability to block the FimH-mediated bacterial adhesion in a mouse infection model.[19] The orally available, nanomolar FimH antagonist **4b** (Fig. 1) exhibited the potential to reduce the colony forming units (CFU) in the urine and in the bladder by two and four orders of magnitude, respectively, demonstrating the therapeutic potential of this new class of anti-infectives for the effective treatment of urinary tract infections.

However, a potential drawback of FimH antagonists with aglycons consisting of biphenyls directly linked to the carbohydrate moiety is their limited conformational flexibility, which could hamper an optimal fit with the tyrosine gate.[11] To increase the conformational flexibility, the spacers between the mannose moiety and the first aromatic ring of the biphenyl moiety in **i** (Fig. 2) as well as between the aromatic rings was extended. Furthermore, the rotational barrier of the biphenyl[25] was reduced by replacing one of the rings by a triazole (for the torsion profile see Fig. 2). Overall, these modifications should lead to a reduction of the conformational restraints and therefore an optimized spatial arrangement of the aglycone in the tyrosine gate.

Oligomannose-3 is present on the high-mannosylated uroplakin Ia located on urothelial cells and is supposed to interact with UPEC. The crystal structure of the FimH-CRD[10] complexed with oligomannose-3 (PDB code 2VCO, Fig. 3A) clearly shows the important role of the tyrosine gate hosting this physiological ligand in the so-called *in-docking-mode*. Interestingly, for **4a** complexed with FimH-CRD a different binding mode outside of the tyrosine gate was reported (*out-docking-mode*, see Fig. 3B).[11] In analogy to oligomannose-3, docking of triazole derivative **5b** to the crystal structure of the FimH lectin domain (PDB code 3MCY)[11] led - as a result of the increased flexibility of the aglycone - to the *in-docking-mode*. Thus, in contrast to the biphenyl aglycone present in **4a**, the phenyl-triazole **5b** is expected to be hosted by the tyrosine gate. The three-dimensional structure **5b** was generated using Glide 5.5[26] and the kinetic stability of the protein–ligand complex was then assessed with a 2 ns molecular-dynamics simulation using Desmond.[27]

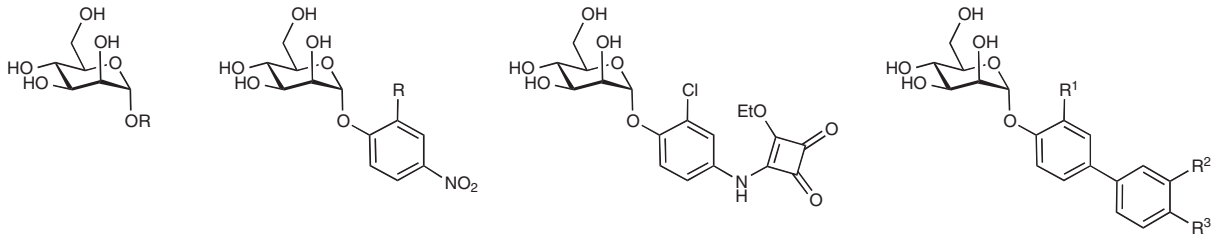
A comparison of the docking modes of oligomannose-3, **4a** and **5b** reveals that the interaction of the mannose moiety is highly conserved for all three compounds. However, in contrast to oligomannose-3 and **5b**, the biphenyl moiety in **4a** is not able to reach the tyrosine gate due to its rigid structure. Instead, a π-π-stacking interaction of the second aromatic ring of the biphenyl aglycone with Tyr48 outside of the tyrosine gate[11] (*out-docking-mode*, Fig. 3B) is achieved by induced fit, that is, a substantial move of Tyr48. In addition, a further stabilization of the protein–ligand complex by a hydrogen bond between the ester in the *meta*-position of **4a** and the side-chain of Arg98 was assumed.[11]

Based on these evidences, a library of derivatives according to the criteria summarized in Figure 2 was designed. Here, we describe synthesis, biological evaluation, and determination of pharmacokinetic parameters of triazole derivatives.

2. Results and discussion

2.1. Synthesis of triazolyl-methyl and -ethyl α-D-mannopyranosides

In a first approach, the phenyl ring adjacent to the anomeric center (see Fig. 2) was replaced by a triazolyl-methyl moiety to increase the conformational flexibility. To avoid solubility problems as well as to take advantage of additional polar interactions, for



1a (R: Me)
1b (R: *n*-heptyl)[9]

2a (R: H)[18a,b]
2b (R: Cl)[18c]

3[18c]

4a (R^1: H, R^2: CO_2Me, R^3: H)[11]
4b (R^1: Cl, R^2: H, R^3: CO_2Me)[19]
4c (R^1: Cl, R^2: H, R^3: CO_2Na)[19]

Figure 1. Known alkyl (**1**) and aryl (**2**–**4**) α-D-mannosides exhibiting micro- to nanomolar affinities.

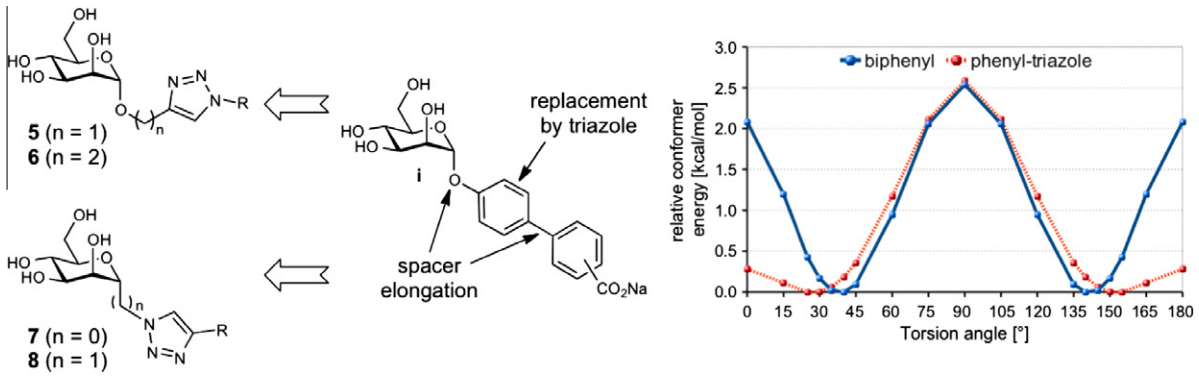


Figure 2. Design of FimH antagonists with aglycons of increased flexibility. Spacer elongations and replacement of one phenyl ring by a triazole should reduce the conformational restraints and lead to an improved fit in the tyrosine gate. The torsion profiles for biphenyl and 1-phenyl-1,2,3-triazole were calculated at the B3LYP level of theory[22,23] with 6-31G(d,p) basis set in the gas phase using Gaussian 03.[24]

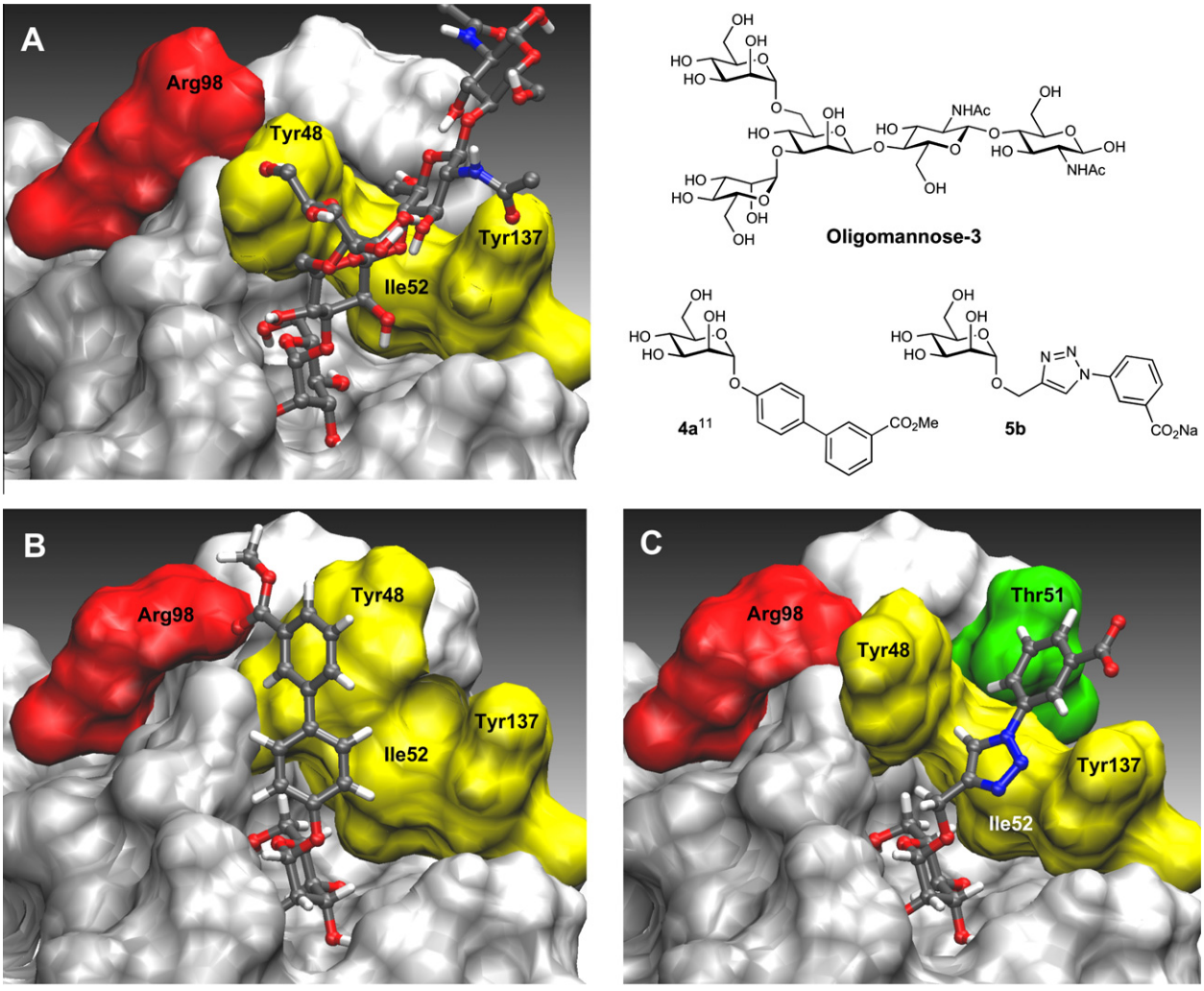
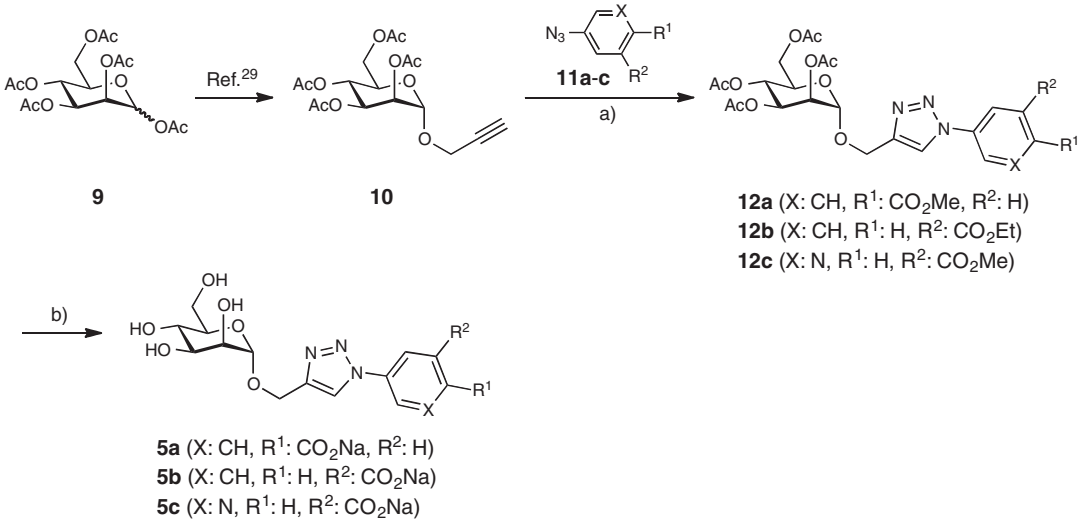


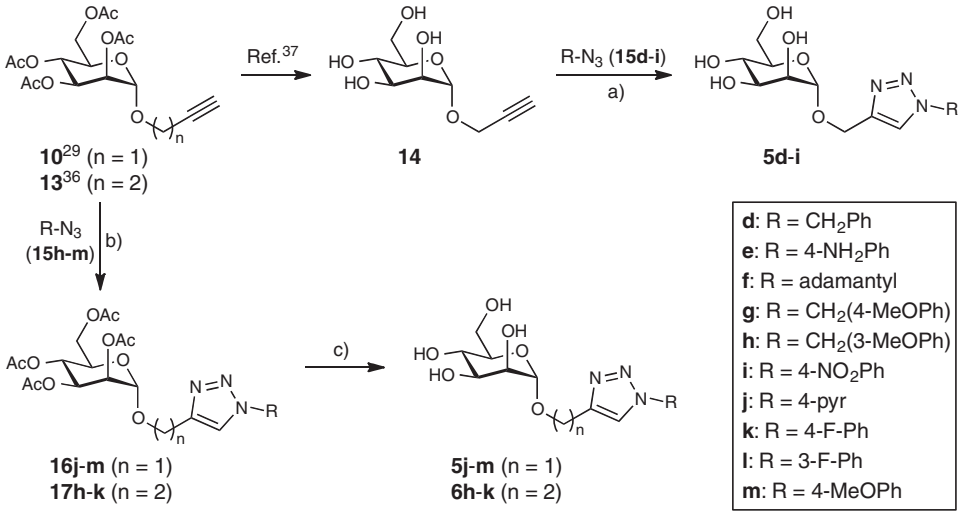
Figure 3. (A) & (B) Crystal structures of oligomannose-3 (A, PDB code 2VCO)[10] and biphenyl **4a** (B, PDB code 3MCY)[11] bound to the FimH-CRD. C) Automated docking of triazole **5b** into the lectin domain of FimH (PDB code 3MCY).[11] The images have been generated using VMD.[28] The ligands are depicted colored by atom (C: dark grey, H: white, O: red, N: blue); the tyrosine gate (residues Tyr48, Tyr137 and Ile52) is shown in yellow, residue Thr51 in green and residue Arg98 in red. While **4a** binds in the *out-docking-mode*, compound **5b**, like oligomannose-3, is inserted into the tyrosine gate (*in-docking-mode*).

example, H-bonds with the hydroxyl-groups of Thr51 or Tyr137 (Fig. 3C), the second aromatic ring was substituted with a carboxylate in *para*- or *meta*-position (→**5a–c**, Scheme 1).

For the synthesis of mannosyl triazoles **5a–c**, alkyne **10**[29] readily available from peracetylated D-mannose (**9**) was reacted with the known aryl azides **11a**,[30] **11b**,[31] and **11c**[32] in a copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition[33,34] using *tert*-butanol/water/THF (1:1:1) as solvent.[35] The saponification of the *anti*-substituted triazoles **12a–c** yielded the test compounds **5a–c** (Table 1).



Scheme 1. Reagents and conditions: (a) $CuSO_4 \cdot 5H_2O$, Na-ascorbate, *t*-BuOH/H_2O/THF (1:1:1), rt, 24 h, 73–97%; (b) (i) NaOMe, MeOH, rt, 3 h; (ii) 1 M NaOH, H_2O/dioxane (1:1), 16 h, 78–91%.



Scheme 2. Reagents and conditions: (a) $CuSO_4 \cdot 5H_2O$, Na-ascorbate, *t*-BuOH/H_2O (1:1), rt, 24 h, (**5d–i**: 27–77%); (b) $CuSO_4 \cdot 5H_2O$, Na-ascorbate, *t*-BuOH/H_2O/THF (1:1:1), rt, 24 h (**16j–m**: 85–94%, **17h–k**: 83–96%); (c) NaOMe, MeOH, rt, 2–6 h, (**5j–m**: 75–85%, **6h–k**: 73–90%).

In a second approach, the terminal aromatic ring was replaced by various substituents like (hetero)aryl, benzyl, and adamantyl groups (→**5d–i** & **5j–m**). Furthermore, in compounds **6h–k** the spacer between the carbohydrate moiety and the triazole ring was elongated from methyl to ethyl allowing for a higher conformational flexibility (Scheme 2).

The mannosyl triazoles **5d–m** and **6h–k** were obtained by reacting the known mannosyl alkynes **10**,[29] **13**[36] and **14**[37] with the azides **15d–m**. Whereas the azides **15d–f** are commercially available, **15g**,[38] **15h**,[39] **15i**,[40] **15j–l**,[41] and **15m**[40] were obtained by known procedures.

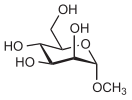
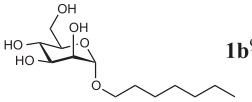
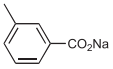
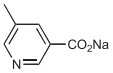
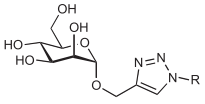
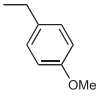
The cycloaddition of alkyne **14** and azides **15d–i** under Cu(I)-catalyzed click conditions[33,34] yielded directly the *anti*-substituted triazoles **5d–i** in 27–77% (Table 1). However, due to the cumbersome purification of the unprotected mannosyl triazoles, test compounds **5j–m** were obtained by an alternative sequence starting from the protected alkyne **10** and azides **15j–m** followed by saponification of the intermediates **16j–m**. The analogous cycloaddition of butinyl mannoside **13** with azides **15h–k** yielded the protected triazoles **17h–k** in 83–96%. Final deacetylation under Zemplén conditions gave the test compounds **6h–k**, which contain a linker extended by an additional carbon between mannose and aglycone (Table 1).

2.2. Synthesis of FimH antagonists modified at the anomeric center

To avoid the low metabolic stability of *O*-mannosides like compounds **5** and **6** due to potential cleavage by mannosidases, the corresponding *N*-linked mannosyl triazoles **7** and *C*-mannosides **8** were prepared (Scheme 3). Mannosyl azide **18** was obtained according to published procedures.[42] The Cu(I)-catalyzed click reaction of **18** with the commercially available acetylenes **19n–s** gave exclusively the anomerically pure *anti*-substituted α-D-mannosyl-triazoles **20n–s** in 84–98% yield and after deacetylation the test compounds **7n–s** (Table 1).

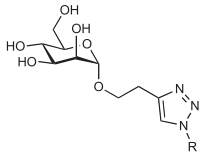
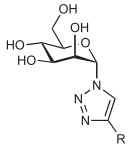
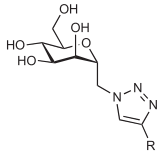
Finally, the synthesis of triazolyl-methyl-*C*-mannosides **8n–s** (Scheme 3) started from mannosyl cyanide **21**, which was obtained

Table 1
Pharmacodynamic and pharmacokinetic parameters of mannosylated triazoles **5–8**

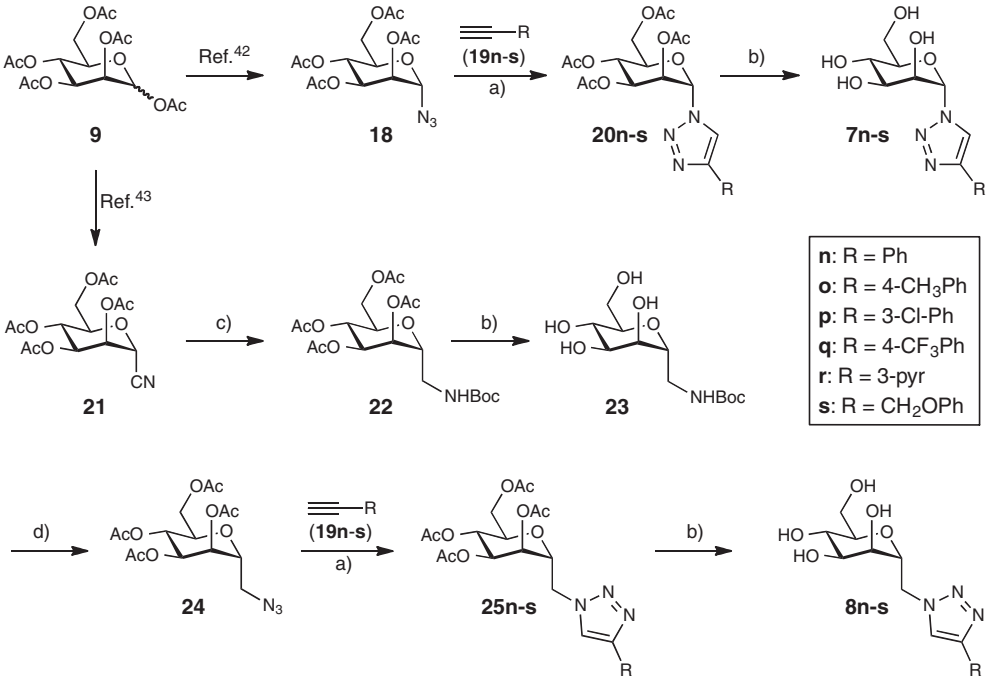
Entry	Ligand	R	Competitive binding assay		$logD_{7.4}$	PAMPA $logP_e$	Solubility
			IC_{50} [μM]	rIC_{50}		[$log10^{-6}$ cm/s]/%Mm	[μg/mL]
1		**1a**[45]	1.9	29	n.d.	n.d./n.d.	n.d.
2		**1b**[9]	0.0656	**1.0**	1.65	-4.89/21.0	> 3000
3		**5a** 	0.207	3.2	n.d.	n.d./n.d.	> 3000
4		**5b** 	0.296	4.5	n.d.	n.d./n.d.	> 3000
5		**5c** 	0.169	2.6	n.d.	n.d./n.d.	> 3000
6		**5d** 	0.667	10.2	-0.33	-8.60/3.0	> 3000
7		**5e** 	0.420	6.4	0.20	-10/4.0	> 2000
8		**5f** 	0.135	2.1	0.07	-7.42/9.8	> 3000
9		**5g** 	0.511	7.8	-0.40	-8.21/3.1	> 3000
10		**5h** 	0.452	6.9	-0.38	-8.18/4.0	> 3000
11		**5i** 	0.397	6.1	-0.16	-9.40/4.9	2600
12		**5j** 	0.070	1.1	0.24	-8.70/9.2	> 3000
13		**5k** 	0.778	11.9	-0.15	-10/4.6	> 3000
14		**5l** 	0.348	5.3	0.18	-8.80/5.4	> 3000
15		**5m** 	0.161	2.5	-0.05	-8.90/7.9	2600

(*continued on next page*)

Table 1. (*continued*)

Entry	Ligand	R	Competitive binding assay		$logD_{7.4}$	PAMPA $logP_e$	Solubility
			IC_{50} [μM]	rIC_{50}		[$log10^{-6}$ cm/s]/%Mm	[μg/mL]
16		**6h**	0.229	3.5	< -1	-8.70/11.8	> 3000
17		**6i**	0.112	1.7	-0.30	-9.20/2.9	> 3000
18		**6j**	0.153	2.3	< -1.5	-9.10/4.3	> 3000
19		**6k**	0.196	3.0	-0.21	-10/4.0	> 3000
20		**7n**	0.250	3.8	0.21	-8.6/6.4	> 3000
21		**7o**	0.248	3.8	0.91	-8.1/11.5	445
22		**7p**	0.331	5.1	1.22	-7.8/13.2	705
23		**7q**	0.144	2.2	1.45	-7.7/17.2	159
24		**7r**	0.216	3.3	-1.04	-10/10.3	1378
25		**7s**	0.493	7.5	-0.18	-9.0/5.5	> 3000
26		**8n**	0.560	8.5	-0.36	-9.1/7.8	> 3000
27		**8o**	0.565	8.6	0.23	-9.3/6.3	1489
28		**8p**	0.639	9.7	0.68	-9.7/n.p.	> 3000
29		**8q**	0.194	3.0	1.07	-9.3/n.p.	525
30		**8r**	0.333	5.1	< -1.5	-10/n.p.	1877
31		**8s**	0.327	5.0	-0.83	-10/n.p.	n.d.

The IC_{50}s were determined with a cell-free competitive binding assay.[45] Relative IC_{50}s (rIC_{50}) were calculated by dividing the IC_{50} of the substance of interest by the IC_{50} of the reference compound **1b** (entry 2). Passive permeation through an artificial membrane and retention therein was determined by PAMPA (parallel artificial membrane permeation assay).[50] Distribution coefficients ($logD_{7.4}$ values) were measured by a miniaturized shake flask procedure.[51] Thermodynamic solubility was measured by an equilibrium shake flask approach.[52] P_e effective permeation; n.p. not permeable; n.d. not determined.



Scheme 3. Reagents and conditions: (a) $CuSO_4{\cdot}5H_2O$, Na-ascorbate, *t*-BuOH/H_2O/THF (1:1:1), rt, 1–2 d (**20n**–**s**: 84–98%, **25n**–**s**: 93–98%); (b) NaOMe, MeOH, rt, 3–6 h, (**7n**–**s**: 65–92%, **8n**–**s**: 83–87%, **23**: 95%); (c) H_2 (4 bar), cat. Pd/C, Boc_2O, EtOAc, 1 d (72%); d) (i) concd HCl, dioxane/H_2O (2:1), 4 h; (ii) TfN_3, $NaHCO_3$, cat. $CuSO_4{\cdot}5H_2O$, PhMe/H_2O/MeOH, rt, 20 h; (iii) Ac_2O, pyridine, rt, 4 h (81%).

from **9** as reported earlier.[43] Catalytic hydrogenation in the presence of Boc_2O (→**22**) followed by deacetylation led to the Boc-protected amine **23**. Cleavage of the Boc-group, amine-azide exchange[44] and subsequent re-acetylation yielded azide **24** in 81% over three steps. The cycloaddition of **24** and the acetylenes **19n**–**s** gave the *anti*-substituted triazoles **25n**–**s** in excellent yields. Final deprotection afforded the test compounds **8n**–**s** (Table 1).

2.3. Biological evaluation

For an initial biological in vitro characterization, a cell-free competitive binding assay[45] and later on, a cell-based aggregation assay[46] were applied. Whereas for the cell-free competitive binding assay only the CRD of the pili was expressed, the complete pili are present in the cell-based assay format. Furthermore, both formats are competitive assays, that is, the analyzed antagonists compete with mannosides for the binding site. In the cell-free competitive binding assay, the competitors are polymer-bound trimannosides, whereas in the aggregation assay the antagonist competes with more potent oligo- and polysaccharide chains[14] present on the surface of erythrocytes.[47] The interaction is further complicated by the existence of a high- and a low-affinity state of the CRD of FimH. Aprikian et al. experimentally demonstrated that in full-length fimbriae the pilin domain stabilizes the CRD domain in the low-affinity state, whereas the CRD domain alone adopts the high-affinity state.[48] Furthermore, it was recently shown that shear stress can induce a conformational switch (twist in the β-sandwich fold of the CRD domain) resulting in improved affinity.[49] Therefore, different affinities are expected when - as in the cell-based aggregometry assay - full-length fimbriae are present, when compared to the CRD domain alone.

2.4. Cell-free competitive binding assay

The cell-free inhibition assay is based on the interaction of a biotinylated polyacrylamide glycopolymer with the carbohydrate recognition domain (CRD) of FimH as previously reported.[45] A soluble recombinant protein consisting of the FimH-CRD (amino acid residues 1–156), a C-terminal thrombin cleavage site and a 6His-tag (FimH-CRD-Th-6His) was expressed in *E. coli* strain HM125 and purified by affinity chromatography on a Ni-NTA column. For the determination of IC_{50} values, a microtiter plate coated with FimH-CRD-Th-6His was incubated with biotinylated Manα(1-3)-[Manα(1-6)]-Manβ(1-4)-Glc*N*Acβ(1-4)-Glc*N*Acβ-polyacrylamide (TM-PAA) polymer conjugated to streptavidin-horseradish peroxidase and the FimH antagonist in fourfold serial dilution (Fig. 4). The assay was performed in duplicates and repeated twice for each compound. To ensure comparability of different antagonists, the reference compound *n*-heptyl α-D-mannopyranoside (**1b**)[9,46] was tested in parallel on each individual microtiter plate. The affinities are reported relative to **1b** as rIC_{50} in Table 1. The relative IC_{50} (rIC_{50}) is the ratio of the IC_{50} of the test compound to the IC_{50} of **1b** (entry 2).

Interestingly, all antagonists in Table 1 except methyl α-D-mannoside (**1a**) exhibit nanomolar affinities. When compared to **1a**, an up to 30-fold improvement was obtained. In the first series, containing a triazolyl-methyl moiety (**5a–m**, entries 3–15), **5j** (entry 12) exhibits the highest affinity with an IC_{50} of 70 nM. This is in the range of *n*-heptyl α-D-mannoside (**1b**), however, compared to the biphenyl derivative **4b**[19] (Fig. 1), this in fact represents a 18-fold reduction of affinity (rIC_{50} 0.06[19] for **4b** vs. rIC_{50} 1.1 for **5j**). At this point, we should recollect that **4b** and **5j** address different docking modes (*out*- and *in-docking-mode*) and therefore also different structural environments.

Antagonists where the linker between the anomeric center and the triazole is extended by an additional carbon (→**6h**–**k**, entries 16–19) show affinities in the range of 200 nM and therefore - with the exception of 4-pyridyl derivative **6j** (entry 18) - two- to fourfold higher affinity compared to their counterparts with the shorter linker. When the triazole is directly linked to the anomeric center (→**7n**–**s**, entries 20–25) affinities are 2- to 8-fold reduced, probably as a consequence of the reduced flexibility preventing an optimal

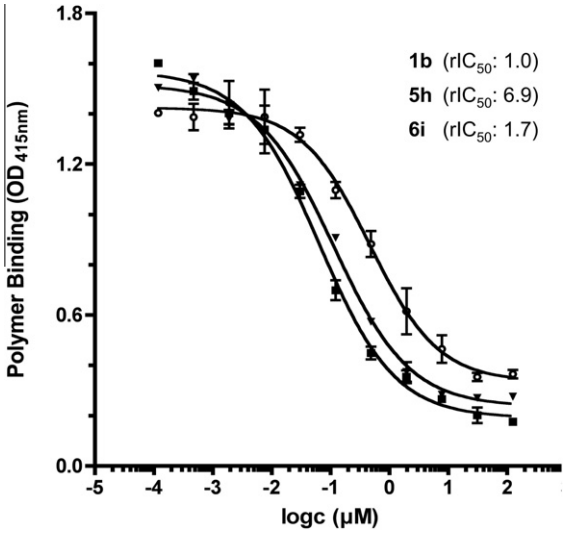


Figure 4. Examples of inhibition curves obtained from the cell-free competitive binding assay.[45] Each assay was run in duplicate and was repeated at least twice. For antagonists **5h**, **6i** and the reference compound **1b** IC_{50} values in the nM range were obtained.

interaction of the aglycone with the tyrosine gate. Finally, the *C*-mannosides **8n–s** (entries 26–31), which do not exhibit the exo-anomeric effect of the *O*-mannosides and therefore can more easily adopt the optimal orientation within the tyrosine gate, surprisingly show a twofold reduction in affinity.

2.5. Aggregometry assay

The potential to disaggregate *E. coli* from guinea pig erythrocytes (GPE) was determined for a variety of the mannosylated triazoles in a function-based aggregometry assay.[46] The measurements were performed in triplicates and the corresponding IC_{50} values were calculated by plotting the area under the curve (AUC) of disaggregation against the concentration of the antagonists. *n*-Heptyl α-D-mannopyranoside (**1b**) was again used as reference compound with an IC_{50} of 77.1 μM (Table 2, entry 1). While the antagonists **5e**, **6j**, **6k**, **7o** and **7q** showed IC_{50} values in the range of 200–300 μM, surprisingly no activities could be determined for compounds **5j**, **8q** and **8r** up to a concentration of 700 μM. As earlier observed,[46] the activities obtained from the aggregometry assay are approximately 1000-fold lower than the affinities determined in the target-based competitive assay.

2.6. Conformational analysis of mannosyl triazoles

Compared to their counterparts **7**, where the triazole is directly linked to the anomeric center, most of the *C*-mannosides **8** exhibit a lower affinity. By applying NMR techniques, it was investigated whether this loss of affinity originates from distorted ring conformations. Due to signal overlap, the unprotected mannosides **7** and **8** were not suited for the conformational analysis. However, for the peracetylated derivatives **20n** and **25n** the ring conformation could be assigned based on coupling constants and NOESY experiments. First, the observed ^3J coupling constants for their ring protons were strikingly different. In **20n**, they were in agreement with those expected for a regular $^4C_1$ chair conformation of an α-D-mannopyranosyl ring, with small $J_{1,2}$ and $J_{2,3}$ couplings and large values for $J_{3,4}$ and $J_{4,5}$ (Fig. 5A). In contrast, the large $J_{1,2}$ coupling constant (8.4 Hz) and small to medium values for $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ found for **25n**, are in agreement with a ring flip of the α-D-mannopyranosyl chair from the common $^4C_1$ to the unusual $^1C_4$ conformation (Fig. 5B). A similar conformational switch has also been observed for α-CF_2-mannosides.[53] As a consequence, the triazolyl-methyl group now is oriented equatorially in *C*-mannoside **25n**, while in **20n** the triazole moiety adopts an axial position.

Subsequent 2D-NOESY measurements (Fig. 5C and D) confirmed this analysis. For both compounds a sequence of seven 2D-NOESY experiments with increasing mixing times from 0.5 s to 2.0 s in steps of 0.25 s was recorded. The intensity of the positive signals grows with increasing mixing time and indicates the relative spatial proximity of a particular proton to that of the source proton. The NOEs of the proton of interest (int_{cross}) were normalized to the intensity of the diagonal peak of the source proton (int_{diag}). Plotting these normalized intensities against the mixing time results in a straight line for each pair of protons. The distances r_{ij} were then calculated from the slopes σ of the linear regression according to $r_{ij} = r_{ref}\,(\sigma_{ref}/\sigma_{ij})^{1/6}$, were r_{ref} is the average distance of the geminal protons H-6a and H-6b, which was chosen as reference (r_{ref} = 1.78 Å).[54,55]

Typically, in the chair conformation of carbohydrates the vicinal proton–proton distances are approx. 2.95 ± 0.15 Å for a diaxial, 2.45 ± 0.15 Å for an axial-equatorial and 2.50 ± 0.20 Å for a diequatorial orientation.[56] As shown in Figure 5A and B, the distances of the ring protons in **20n** and **25n** determined from NOE experiments correlate well with the theoretical values and support the results obtained from the analysis of the coupling constants. In summary, NMR spectroscopic data indicate that the mannopyranosyl chairs in these compounds adopt different conformations, depending on the substituent at C-1.

This conformational analysis offers an explanation for the twofold reduction of affinity found for most of the *C*-mannosides **8** compared to the corresponding *N*-linked triazoles **7**. Due to the inversion of the ring conformation in **8** ($^1C_4$ vs $^4C_1$), the optimal fit into the hydrophilic mannose-binding pocket of FimH is disturbed.

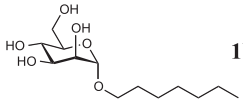
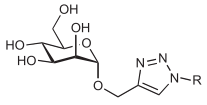
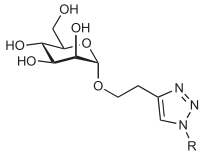
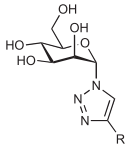
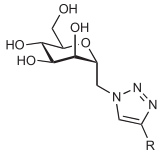
2.7. Pharmacokinetic properties of mannosyl triazoles

Finally, the druglikeness of this new class of FimH antagonists was addressed. For a successful po application in our UTI mouse model,[19] FimH antagonists have to exhibit oral bioavailability, metabolic stability and fast renal elimination to the urinary tract, their place of action. For the evaluation of oral absorption and renal excretion of the triazoles **5–8** physicochemical parameters such as solubility, lipophilicity (distribution coefficients, log $D_{7.4}$) and permeability were determined (Table 1). The mannosides of all four compound families (**5–8**) are all highly soluble (159 μg/mL to > 3 mg/mL) and therefore fulfill a first prerequisite for absorption in the gastrointestinal tract (GIT). All compounds showed low to moderate log $D_{7.4}$ values in the range of < -1.5 to 1.45. While these parameters are beneficial for renal excretion,[57] oral absorption by passive diffusion can only be expected to a minor extent. Indeed, for none of the tested compounds a significant permeation through an artificial membrane (PAMPA,[50] log P_e, P_e: effective permeation) nor membrane retention could be detected. Whereas for a successful oral absorption a log P_e >−5.7 and/or a membrane retention %Mm > 80 % are required,[58] the corresponding values for all triazoles are far from being in this range. Overall, only poor absorption from the GIT can be therefore expected.

3. Conclusions

Crystal structures indicate that the natural ligand oligomannose-3[10] inserts into the tyrosine gate formed by Tyr48, Tyr137 and Ile52 of the carbohydrate recognition domain of FimH (*in-docking-mode*). In contrast, the recently reported high-affinity

Table 2
IC_{50} values of mannosylated triazoles determined in the aggregometry assay[46]

Entry	Ligand		R	Aggregometry assay	
				IC_{50} [μM]	rIC_{50}
1		**1b**[9]		77.1	1.0
2		**5e**		299	3.9
3		**5j**		n.a.	-
5		**6j**		277	3.6
6		**6k**		216	2.8
8		**7o**		289	3.7
9		**7q**		249	3.2
10		**8q**		n.a.	-
11		**8r**		n.a.	-

The relative IC_{50} (rIC_{50}) was calculated by dividing the IC_{50} of the substance of interest by the IC_{50} of the reference compound **1b**. n.a., not active.

biphenyl mannoside **4c** was shown to bind in the *out-docking-mode*, that is, it establishes a π-π-stacking interaction with Tyr48 from the outside of the tyrosine gate.[11] Based on docking studies, we designed a series of low molecular weight mannosyl triazoles, which exhibit an increased conformational flexibility of the aglycone and therefore should allow for binding to the tyrosine gate in the *in-docking-mode*. For their pharmacodynamical evaluation two assay formats, a target-based binding assay[45] and a function-based aggregation assay,[46] were applied. In general, all triazoles **5–8** showed nanomolar affinities, but only one representative, the 4-pyridyl derivative **5j**, was as potent as the reference compound *n*-heptyl mannoside (**1b**). Obviously, the high flexibility of the *n*-heptyl aglycone in **1b** optimally fulfills the spatial requirements of the tyrosine gate. In addition, the hydrophobic contacts established by the substituted triazole aglycone within the tyrosine gate in the *in-docking-mode* are less favorable than the π-π-stacking interaction of biphenyl derivatives[11,19] with Tyr48 in the *out-docking-mode*.

Furthermore, the reduced affinities of the triazolyl-methyl-*C*-mannosides **8** can be rationalized by a disturbed interaction of the mannose moiety. A conformational analysis by ^{1}H NMR and NOESY NMR revealed that in contrast to the other three classes of mannosyl triazoles (compounds **5**, **6** and **7**), the *C*-mannosides **8** do not adopt the common $^4C_1$ but an unusual $^1C_4$ chair conformation, thus preventing an optimal fit of the mannosyl moiety into the hydrophilic mannose-binding pocket of FimH.

Finally, for a successful therapeutic application, FimH antagonists have to exhibit appropriate pharmacokinetic properties, that is, oral bioavailability and fast renal elimination to the urinary tract, their place of action. One prerequisite for absorption in the GIT is sufficient solubility, a property, which is fulfilled by all synthesized antagonists. However, according to their lipophilicity and membrane permeation properties, the mannosyl triazoles are not expected to be orally absorbed. Possible improvements of the pharmacokinetic profiles of mannosyl triazoles are currently studied.

4. Experimental part

4.1. Chemistry

General. NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC). Chemical shifts are expressed in ppm using residual $CHCl_3$ and CD_2HOD as references. Optical rotations were measured using a Perkin-Elmer Polarimeter 341. Electron spray ionization mass

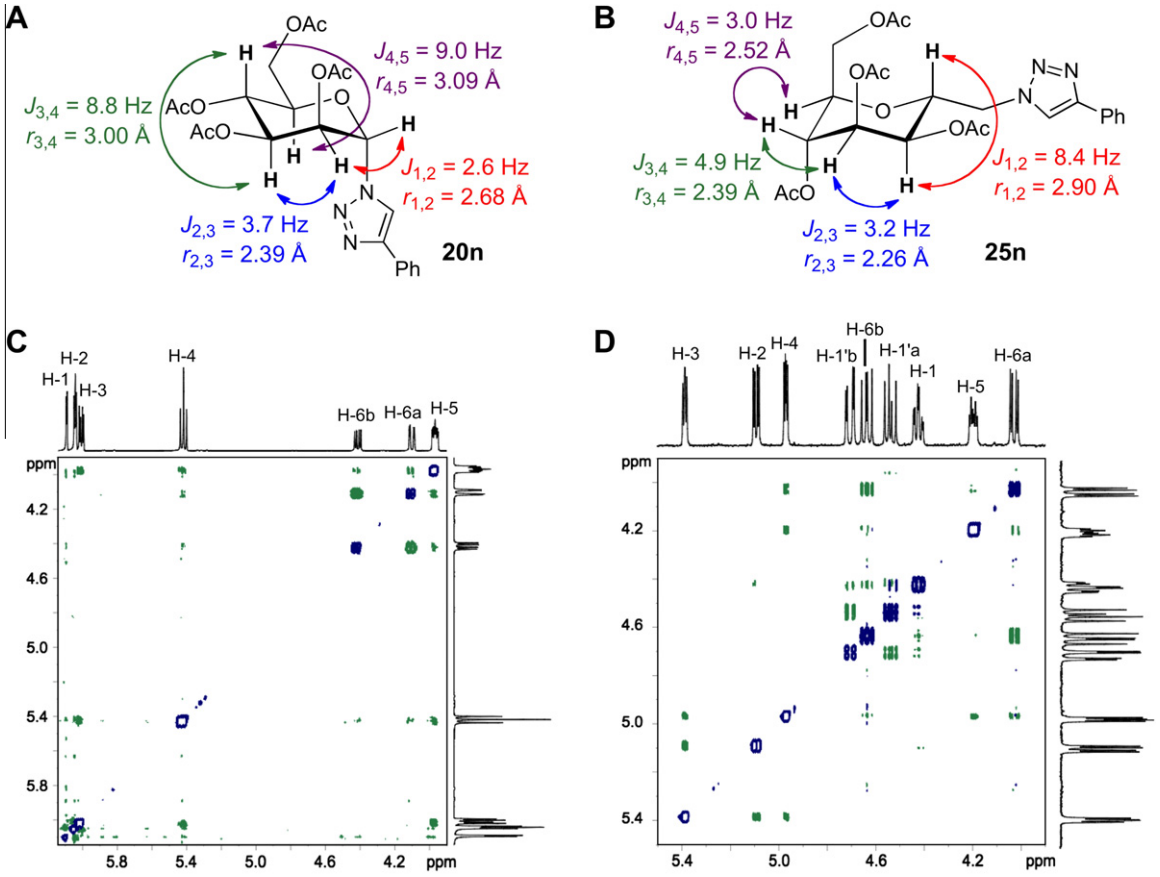


Figure 5. Coupling constants and proton-proton distances for peracetylated triazoles **20n** (A) and **25n** (B) determined by ^{1}H NMR and 2D-NOESY experiments; 2D-NOESY spectra of **20n** (C) and **25n** (D) in $CDCl_3$ with mixing times of 1.5 s (C) and 750 ms (D).

spectra (ESI-MS) were obtained on a Waters micromass ZQ. The HRMS analyses were carried out using a Bruker QTOF. Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). MPLC separations were carried out on a Combi-Flash Companion from Teledyne Isco equipped with RediSep normal-phase or C_{18} reversed-phase flash columns. Tetrahydrofurane (THF) was freshly distilled under argon over sodium and benzophenone. Methanol (MeOH) was dried by refluxing with sodium methoxide and distilled immediately before use. Dichloromethane (DCM), ethyl acetate (EtOAc), and toluene were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic).

4.1.1. General procedure A for the synthesis of mannosyl triazoles 5d–i

A mixture of acetylene **14**[37] (1.0 eq), azide **15d–i** (1.5 eq), $CuSO_4{\cdot}5H_2O$ (0.25 eq) and sodium ascorbate (0.5 eq) was dissolved in degassed *tert*-BuOH/H_2O (1:1, 2 mL/0.1 mmol **14**) under argon. After stirring for 1 d the solvents were removed in vacuo and the crude product was first purified by MPLC on silica (DCM/MeOH) and then by reversed-phase chromatography (RP-18, H_2O/MeOH) to yield **5d–i** as colorless solids.

4.1.2. General procedure B for the synthesis of mannosyl triazoles 12a–c, 16j–m and 17h–k

Acetylene **10**[29] or **13**[36] (1.0 eq) and azide **11a–c** or **15h–m** (1.5–2 eq) were dissolved in THF/*tert*-BuOH/H_2O (1:1:1, 1.5 mL/0.1 mmol **10** or **11**). The mixture was degassed in an ultrasound bath under a flow of argon for 20 min. Then 0.5 M aq $CuSO_4{\cdot}5H_2O$ (0.25 eq) and 1 M aq sodium ascorbate (0.5 eq) were added under argon at rt. After stirring overnight the solvents were removed in vacuo and the crude product was purified by MPLC on silica (petrol ether/EtOAc) to yield **12a–c**, **16j–m** and **17h–k** as colorless oils.

4.1.3. General procedure C for the synthesis of mannosyl triazoles 20n–s and 25n–s

Azide **18**[42] or **24** (1.0 eq) and acetylene **19n–s** (2.0 eq) were dissolved in THF/*tert*-BuOH/H_2O (1:1:1, 3 mL/0.1 mmol **18** or **24**). The mixture was degassed in an ultrasound bath under a flow of argon for 20 min. Then 0.2 M aq $CuSO_4{\cdot}5H_2O$ (0.2 eq) and 1 M aq sodium ascorbate (0.4 eq) were added under argon at rt. After stirring for 1–2 d the solvents were removed in vacuo and the crude product was purified by MPLC on silica (petrol ether/EtOAc) to yield **20n–s** and **25n–s** as colorless oils.

4.1.4. General procedure D for deacetylation

To a solution of the acetylated compound (38–50 mg) in MeOH (3 mL) was added 1 M NaOMe/MeOH (0.3 mL). The mixture was stirred at rt for 3–6 h. The solution was concentrated and the residue was purified by MPLC on reversed phase (RP-18 column, H_2O/MeOH) and P2 size-exclusion chromatography to afford the target molecule as a colorless solid after a final lyophilization from water/dioxane.

4.1.5. Synthesis of azide 24

4.1.5.1. (2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-*N*-*tert*-butoxycarbonyl-methylamine (22). Cyanide **21**[43] (1.63 g, 4.57 mmol), Boc_2O (1.49 g, 6.86 mmol) and Pd/C (10%, 250 mg) were suspended in EtOAc (25 mL) and hydrogenated (4 bar H_2) at rt for 4 h. After filtration over Celite, fresh Pd/C (10%, 750 mg)

was added and the mixture was hydrogenated (4 bar H_2) for additional 17 h. The suspension was filtered over Celite and concentrated. The residue was purified by MPLC on silica (petrol ether/EtOAc) to give **22** (1.51 g, 72%) as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.44 (s, 9H, C(CH_3)$_3$), 2.07, 2.10, 2.10 (3 s, 12H, 4 $COCH_3$), 3.38 (m, 2H, H-1′), 3.99–4.05 (m, 2H, H-1, H-5), 4.07 (dd, J = 3.8, 11.8 Hz, 1H, H-6a), 4.54 (dd, J = 6.9, 11.7 Hz, 1H, H-6b), 4.79 (m, 1H, NH), 5.07 (dd, J = 5.3, 6.4 Hz, 1H, H-4), 5.10 (dd, J = 3.3, 6.0 Hz, 1H, H-2), 5.26 (dd, J = 3.3, 6.5 Hz, 1H, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.70, 20.73, 20.76, 20.81 (4 $COCH_3$), 28.3 (C(CH_3)$_3$), 39.7 (C-1′), 61.2 (C-6), 67.50, 67.51 (C-2, C-4), 68.0 (C-3), 71.1 (C-1), 72.2 (C-5), 79.7 (C(CH_3)$_3$), 155.8 (NCO), 169.5, 169.6, 169.9, 170.7 (4 $COCH_3$); ESI-MS Calcd for $C_{20}H_{31}NNaO_{11}$ $[M+Na]^+$: 484.18, Found: 484.11.

4.1.5.2. *N-tert*-Butoxycarbonyl-(α-D-mannopyranosyl)methylamine (23).

A solution of **22** (1.47 g, 3.19 mmol) in MeOH (20 mL) was treated with 1 M methanolic NaOMe (2 mL) under argon at rt for 3 h. The reaction mixture was neutralized with acetic acid and concentrated. The residue was purified by MPLC on silica (DCM/MeOH) to give **23** (925 mg, 99%) as a colorless solid.

^{1}H NMR (500 MHz, CD_3OD): δ 1.44 (s, 9H, C(CH_3)$_3$), 3.32 (m, 2H, H-1′), 3.54 (m, 1H, H-5), 3.63 (t, J = 7.6 Hz, 1H, H-4), 3.68 (dd, J = 3.1, 7.8 Hz, 1H, H-3), 3.74 (dd, J = 2.8, 11.8 Hz, 1H, H-6a), 3.77 (m, 1H, H-2), 3.79 (dd, J = 6.4, 11.8 Hz, 1H, H-6b), 3.86 (m, 1H, H-1), 6.72 (m, 1H, NH); ^{13}C NMR (125 MHz, CD_3OD): δ 28.8 (C(CH_3)$_3$), 40.6 (C-1′), 62.7 (C-6), 69.6 (C-4), 70.2 (C-2), 72.7 (C-3), 76.9 (C-1), 77.4 (C-5), 80.2 (C(CH_3)$_3$), 158.6 (NCO); ESI-MS Calcd for $C_{12}H_{23}NNaO_7$ $[M+Na]^+$: 316.14, Found: 316.03.

4.1.5.3. (2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)methylazide (24).

Triflyl azide stock solution preparation:[44] Sodium azide (796 mg, 12.2 mmol) was dissolved in water (2 mL). Toluene (2 mL) was added, and the mixture was cooled to 0 °C with stirring. Then triflic anhydride (1.31 mL, 6.12 mmol) was added dropwise. The biphasic reaction mixture was stirred vigorously at 0 °C for 30 min and at 10 °C for another 2 h. The reaction mixture was neutralized with satd aq $NaHCO_3$. The phases were separated, and the aqueous phase extracted with toluene (2 × 2 mL). The organic layers were combined to give the triflyl azide stock solution. *Amine-azide exchange*: A solution of **23** (430 mg, 1.47 mmol) in dioxane/water (2:1, 15 mL) was treated with concentrated HCl (5 mL) under argon at rt for 4 h. The mixture was concentrated and the residue was dried in high vacuo. Then, the crude amine hydrochloride (341 mg), $NaHCO_3$ (492 mg, 5.86 mmol) and $CuSO_4 \cdot 5H_2O$ (14.1 mg, 61 μmol) were dissolved in water (1.91 mL). The triflyl azide stock solution (3.25 mL, 3.3 mmol) was added and the biphasic reaction mixture was made homogenous by the addition of MeOH (12.6 mL). The mixture was stirred at rt for 20 h. The solvents were removed in vacuo and the residue was taken up in dry pyridine (10 mL), and acetic anhydride (4 mL) was added. The reaction mixture was stirred at rt under argon for 4 h. The solvents were removed in vacuo and the crude product was purified by MPLC on silica (petrol ether/EtOAc) to yield **24** (459 mg, 81%) as a colorless oil.

IR (film) 2102 (vs, N_3), 1747 (vs, CO) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.04, 2.07, 2.08, 2.10 (4 s, 12H, 4 $COCH_3$), 3.29 (dd, J = 3.3, 13.4 Hz, 1H, H-1′a), 3.49 (dd, J = 7.3, 13.4 Hz, 1H, H-1′b), 4.05–4.09 (m, 2H, H-5, H-6a), 4.15 (dt, J = 3.2, 7.1 Hz, 1H, H-1), 4.60 (m, 1H, H-6b), 5.01 (dd, J = 4.4, 6.0 Hz, 1H, H-4), 5.14 (dd, J = 3.4, 6.9 Hz, 1H, H-2), 5.27 (dd, J = 3.4, 6.0 Hz, 1H, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.61, 20.63, 20.65, 20.74 (4 $COCH_3$), 50.1 (C-1′), 60.8 (C-6), 67.0 (C-2), 67.5 (C-3), 67.6 (C-4), 70.5 (C-1), 72.9 (C-5), 169.3, 169.5, 169.6, 170.6 (4 $COCH_3$); ESI-MS Calcd for $C_{15}H_{21}N_3NaO_9$ $[M+Na]^+$: 410.12, Found: 410.04.

4.1.6. Synthesis of peracetylated mannosyl triazoles

4.1.6.1. Methyl 4-[4-((2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)methyl)-1*H*-1,2,3-triazol-1-yl]-benzoate (12a).

Following general procedure B, **10** (40.0 mg, 0.103 mmol) was reacted with methyl 4-azidobenzoate (**11a**,[30] 36.5 mg, 0.206 mmol), 0.5 M $CuSO_4$ (52 μL, 26 μmol) and 1 M sodium ascorbate (52 μL, 52 μmol) to yield **12a** (55.8 mg, 96%).

$[\alpha]_D$ +45.0 (c 1.03, $CHCl_3$); IR (film) 1747 (vs, CO) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.99, 2.04, 2.12, 2.16 (4 s, 12H, 4 $COCH_3$), 3.97 (s, 3H, OMe), 4.10 (ddd, J = 2.2, 5.1, 9.5 Hz, 1H, H-5), 4.14 (dd, J = 2.3, 12.2 Hz, 1H, H-6a), 4.32 (dd, J = 5.2, 12.2 Hz, 1H, H-6b), 4.79, 4.95 (A, B of AB, J = 12.6 Hz, 2H, H-1′), 5.01 (d, J = 1.1 Hz, 1H, H-1), 5.28 (dd, J = 1.7, 3.1 Hz, 1H, H-2), 5.32 (t, J = 9.8 Hz, 1H, H-4), 5.36 (dd, J = 3.2, 10.0 Hz, 1H, H-3), 7.88 (AA′ of AA′BB′, J = 8.7 Hz, 2H, C_6H_4), 8.11 (s, 1H, C_2N_3H), 8.23 (BB′ of AA′BB′, J = 8.7 Hz, 2H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.7, 20.8, 20.9 (4C, 4 $COCH_3$), 52.5 (OMe), 61.0 (C-1′), 62.4 (C-6), 66.0 (C-4), 68.8, 69.0, 69.4 (C-2, C-3, C-5), 97.0 (C-1), 120.0 (2C, C_6H_4), 121.0 (C_2N_3H–C5), 130.4 (C_6H_4–C1), 131.4 (2C, C_6H_4), 139.9 (C_6H_4–C4), 144.7 (C_2N_3H–C4), 169.7, 170.0, 170.1, 170.7 (5C, 5 CO); ESI-MS Calcd for $C_{25}H_{29}N_3NaO_{12}$ $[M+Na]^+$: 586.02, Found: 586.16.

4.1.6.2. Ethyl 3-[4-((2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)methyl)-1*H*-1,2,3-triazol-1-yl]-benzoate (12b).

Following general procedure B, **10** (50.0 mg, 0.129 mmol) was reacted with ethyl 3-azidobenzoate (**11b**,[31] 49.3 mg, 0.258 mmol), 0.5 M $CuSO_4$ (64 μL, 32 μmol) and 1 M sodium ascorbate (64 μL, 64 μmol) to yield **12b** (72.7 mg, 97%).

$[\alpha]_D$ +40.2 (c 1.04, $CHCl_3$); IR (film) 1749 (vs, CO) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (t, J = 7.2 Hz, 1H, CH_3), 1.99, 2.04, 2.12, 2.16 (4 s, 12H, 4 $COCH_3$), 4.09–4.15 (m, 2H, H-5, H-6a), 4.32 (dd, J = 5.0, 12.1 Hz, 1H, H-6b), 4.44 (q, J = 7.2 Hz, 2H, OCH_2), 4.79, 4.95 (A, B of AB, J = 12.4 Hz, 2H, H-1′), 5.02 (d, J = 1.4 Hz, 1H, H-1), 5.28 (dd, J = 1.7, 3.2 Hz, 1H, H-2), 5.31 (m, 1H, H-4), 5.36 (dd, J = 3.3, 9.9 Hz, 1H, H-3), 7.64 (t, J = 8.0 Hz, 1H, C_6H_4–H5), 8.02 (ddd, J = 0.9, 2.1, 8.0 Hz, 1H, C_6H_4–H4), 8.11 (s, 1H, C_2N_3H), 8.14 (d, J = 7.9 Hz, 1H, C_6H_4-H6), 8.38 (t, J = 1.7 Hz, 1H, C_6H_4-H2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.3 (CH_3), 20.67, 20.69, 20.78, 20.88 (4 $COCH_3$), 60.9 (C-1′), 61.7 (OCH_2), 62.4 (C-6), 66.0 (C-4), 68.8 (C-3), 69.0 (C-5), 69.4 (C-2), 96.9 (C-1), 121.2 (C_6H_4), 121.3 (C_2N_3H–C5), 124.8, 129.9, 130.0, 132.3, 137.0 (C_6H_4), 144.5 (C_2N_3H–C4), 165.2, 169.7, 169.9, 170.1, 170.7 (5 CO); ESI-MS Calcd for $C_{26}H_{32}N_3O_{12}$ $[M+H]^+$: 578.20, Found: 578.19.

4.1.6.3. Methyl 5-[4-((2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)methyl)-1*H*-1,2,3-triazol-1-yl]-nicotinate (12c).

Following general procedure B, **10** (40.0 mg, 0.103 mmol) was reacted with methyl 5-azidonicotinate (**11c**,[32] 32.5 mg, 0.182 mmol), 0.5 M $CuSO_4$ (52 μL, 26 μmol) and 1 M sodium ascorbate (52 μL, 52 μmol). The crude product was dissolved in DCM (10 mL) and washed with 0.1 M aq EDTA (5 mL). The aqueous phase was extracted with DCM (2 × 10 mL) and the combined organic layers were dried with Na_2SO_4 and evaporated to dryness. The residue was purified by MPLC on silica (petrol ether/EtOAc) to give **12c** (42.4 mg, 73%).

$[\alpha]_D$ +39.7 (c 1.06, $CHCl_3$); IR (film) 1733 (vs, CO) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.98, 2.03, 2.11, 2.15 (4 s, 12H, 4 $COCH_3$), 4.01 (s, 3H, OCH_3), 4.09 (m, 1H, H-5), 4.14 (dd, J = 2.4, 12.2 Hz, 1H, H-6a), 4.31 (dd, J = 5.2, 12.3 Hz, 1H, H-6b), 4.79, 4.96 (A, B of AB, J = 12.5 Hz, 2H, H-1′), 5.01 (d, J = 1.4 Hz, 1H, H-1), 5.27 (dd, J = 1.7, 3.0 Hz, 1H, H-2), 5.30 (t, J = 9.8 Hz, 1H, H-4), 5.34 (dd, J = 3.3, 9.9 Hz, 1H, H-3), 8.17 (s, 1H, C_2N_3H), 8.69 (t, J = 2.0 Hz, 1H, C_5H_3N-H2), 9.27, 9.30 (2 s, 2H, C_5H_3N-H4, H6); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.63, 20.65, 20.75, 20.84 (4 $COCH_3$), 52.9 (OMe), 60.8 (C-1′), 62.4 (C-6), 66.0 (C-4), 68.8, 68.9 (C-3, C-5), 69.3 (C-2), 97.0 (C-1), 121.2 (C_2N_3H–C5), 126.9, 128.6, 133.3

(C_5H_3N), 145.1 (2C, C_5H_3N, (C_2N_3H–C4), 150.7 (C_5H_3N), 164.4, 169.7, 169.9, 170.1, 170.7 (5 CO); ESI-MS Calcd for $C_{24}H_{29}N_4O_{12}$ $[M+H]^+$: 565.18, Found: 565.15.

4.1.6.4. [1-(Pyridin-4-yl)-1,2,3-triazol-4-yl]methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (16j). Following general procedure B, **10** (100 mg, 0.26 mmol) was reacted with 4-azidopyridine (**15j**,[41] 47 mg, 0.39 mmol), 0.5 M $CuSO_4$ (130 μL, 65 μmol) and 1 M sodium ascorbate (130 μL, 130 μmol). The crude product was dissolved in DCM (20 mL) and washed with 0.1 M aq EDTA (10 mL). The aqueous phase was extracted with DCM (2 × 20 mL) and the combined organic layers were dried with Na_2SO_4 and evaporated to dryness. The residue was purified by MPLC on silica (DCM/MeOH) to give **16j** (114 mg, 87%).

$[\alpha]_D$ +52.1 (*c* 2.26, DCM); IR (film) 1748 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.92, 1.97, 2.05, 2.09 (4s, 12H, 4 $COCH_3$), 3.96–4.14 (m, 2H, H-5, H-6a), 4.24 (dd, *J* = 4.8, 12.1 Hz, 1H, H-6b), 4.73, 4.89 (A, B of AB, *J* = 12.5 Hz, 2H, H-1′), 4.94 (s, 1H, H-1), 5.14–5.33 (m, 3H, H-2, H-3, H-4), 7.70 (m, 2H, C_5H_4N), 8.15 (s, 1H, C_2N_3H), 8.73 (m, 2H, C_5H_4N); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.77, 20.78, 20.88, 20.96 (4 $COCH_3$), 61.0 (C-1′), 62.5 (C-6), 66.1, 68.9, 69.0, 69.5 (C-2, C-3, C-4, C-5), 97.1 (C-1), 113.9 (2C, C_5H_4N), 120.7 (C_2N_3H–C5), 143.0 (C_5H_4N–C1), 145.2 (C_2N_3H–C4), 151.9 (2C, C_5H_4N), 169.8, 170.1, 170.2, 170.8 (4 $COCH_3$); ESI-MS Calcd for $C_{22}H_{26}N_4NaO_{10}$ $[M+Na]^+$: 529.16, Found: 529.07.

4.1.6.5. [1-(4′-Fluorophenyl)-1,2,3-triazol-4-yl]methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (16k). Following general procedure B, **10** (100 mg, 0.26 mmol) was reacted with 1-azido-4-fluorobenzene (**15k**,[41] 53 mg, 0.39 mmol), 0.5 M $CuSO_4$ (130 μL, 65 μmol) and 1 M sodium ascorbate (130 μL, 130 μmol) to yield **16k** (127 mg, 94%).

$[\alpha]_D$ +42.0 (*c* 1.00, DCM); IR (film) 1749 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.96, 2.01, 2.09, 2.13 (4s, 12H, 4 $COCH_3$), 4.07 (m, 1H, H-5), 4.11 (dd, *J* = 2.4, 12.2 Hz, 1H, H-6a), 4.28 (dd, *J* = 5.2, 12.2 Hz, 1H, H-6b), 4.75, 4.91 (A, B of AB, *J* = 12.4 Hz, 2H, H-1′), 4.98 (d, *J* = 1.6 Hz, 1H, H-1), 5.24–5.31 (m, 2H, H-2, H-4), 5.33 (dd, *J* = 3.3, 10.0 Hz, 1H, H-3), 7.21 (m, 2H, C_6H_4), 7.71 (m, 2H, C_6H_4), 7.97 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.87, 20.88, 20.98, 21.07 (4 $COCH_3$), 61.3 (C-1′), 62.6 (C-6), 66.3, 69.0, 69.2, 69.7 (C-2, C-3, C-4, C-5), 97.2 (C-1), 117.0 (d, *J* = 22.5 Hz, 2C, C_6H_4), 121.6 (C_2N_3H–C5), 122.9 (d, *J* = 8.8 Hz, 2C, C_6H_4), 133.4 (d, *J* = 3.8 Hz, C_6H_4–C1), 145.0 (C_2N_3H–C4), 163.8 (d, *J* = 247.5 Hz, C_6H_4–C4), 169.9, 170.2, 170.3, 170.9 (4 $COCH_3$); ESI-MS Calcd for $C_{23}H_{26}FN_3NaO_{10}$ $[M+Na]^+$: 546.16, Found: 546.15.

4.1.6.6. [1-(3′-Fluorophenyl)-1,2,3-triazol-4-yl]methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (16l). Following general procedure B, **10** (100 mg, 0.26 mmol) was reacted with 1-azido-3-fluorobenzene (**15l**,[41] 53 mg, 0.39 mmol), 0.5 M $CuSO_4$ (130 μL, 65 μmol) and 1 M sodium ascorbate (130 μL, 130 μmol) to yield **16l** (115 mg, 85%).

$[\alpha]_D$ +47.5 (*c* 2.14, DCM); IR (film) 1748 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.96, 2.01, 2.09, 2.13 (4s, 12H, 4 $COCH_3$), 4.02–4.15 (m, 2H, H-5, H-6a), 4.29 (dd, *J* = 5.1, 12.2 Hz, 1H, H-6b), 4.75, 4.91 (A, B of AB, *J* = 12.5 Hz, 2H, H-1′), 4.98 (s, 1H, H-1), 5.22–5.36 (m, 3H, H-2, H-3, H-4), 7.14 (m, 1H, C_6H_4), 7.44–7.59 (m, 3H, C_6H_4), 8.02 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.86, 20.88, 20.97, 21.07 (4 $COCH_3$), 61.2 (C-1′), 62.6 (C-6), 66.2, 69.0, 69.2, 69.6 (C-2, C-3, C-4, C-5), 97.2 (C-1), 108.6 (d, *J* = 26.3 Hz, C_6H_4), 116.0 (d, *J* = 17.5 Hz, C_6H_4), 116.1 (m, C_6H_4), 121.3 (C_2N_3H–C5), 131.5 (d, *J* = 8.8 Hz, C_6H_4), 138.2 (d, *J* = 10.0 Hz, C_6H_4–C1), 144.7 (C_2N_3H–C4), 163.3 (d, *J* = 247.5 Hz, C_6H_4–C4), 169.9, 170.1, 170.3, 170.9 (4 $COCH_3$); ESI-MS Calcd for $C_{23}H_{26}FN_3NaO_{10}$ $[M+Na]^+$: 546.16, Found: 546.15.

4.1.6.7. [1-(4′-Methoxyphenyl)-1,2,3-triazol-4-yl]methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (16m). Following general procedure B, **10** (100 mg, 0.26 mmol) was reacted with 1-azido-4-methoxybenzene (**15m**,[40] 58 mg, 0.39 mmol), 0.5 M $CuSO_4$ (130 μL, 65 μmol) and 1 M sodium ascorbate (130 μL, 130 μmol) to yield **16m** (128 mg, 92%).

$[\alpha]_D$ +45.0 (*c* 2.26, DCM); IR (film) 1749 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.93, 1.98, 2.06, 2.10 (4s, 12H, 4 $COCH_3$), 3.81 (s, 3H, OCH_3), 3.99–4.12 (m, 2H, H-5, H-6a), 4.26 (dd, *J* = 5.3, 12.4 Hz, 1H, H-6b), 4.71, 4.87 (A, B of AB, *J* = 12.4 Hz, 2H, H-1′), 4.95 (d, *J* = 1.4 Hz, 1H, H-1), 5.20–5.28 (m, 2H, H-2, H-4), 5.30 (dd, *J* = 3.2, 10.0 Hz, 1H, H-3), 6.97 (m, 2H, C_6H_4), 7.58 (m, 2H, C_6H_4), 7.91 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.78, 20.80, 20.89, 20.98 (4 $COCH_3$), 55.7 (OCH_3), 61.2 (C-1′), 62.5 (C-6), 66.1, 68.9, 69.1, 69.5 (C-2, C-3, C-4, C-5), 97.0 (C-1), 114.9 (2C, C_6H_4), 121.5 (C_2N_3H–C5), 122.4 (2C, C_6H_4), 130.4 (C_6H_4–C1), 144.1 (C_2N_3H–C4), 160.0 (C_6H_4–C4), 169.8, 170.0, 170.2, 170.8 (4 $COCH_3$); ESI-MS Calcd for $C_{24}H_{29}N_3NaO_{11}$ $[M+Na]^+$: 558.18, Found: 558.18.

4.1.6.8. [1-(3′-Methoxybenzyl)-1,2,3-triazol-4-yl]ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (17h). Following general procedure B, **13** (55 mg, 0.14 mmol) was reacted with 3-methoxybenzylazide (**15h**,[39] 34 mg, 0.21 mmol), 0.5 M $CuSO_4$ (70 μL, 35 μmol) and 1 M sodium ascorbate (70 μL, 70 μmol) to yield **17h** (65 mg, 83%).

$[\alpha]_D$ +36.5 (*c* 1.00, DCM); IR (film) 1748 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.85, 1.88, 1.93, 1.99 (4s, 12H, 4 $COCH_3$), 2.86 (tt, *J* = 4.8, 9.9 Hz, 2H, H-2′), 3.56 (dt, *J* = 6.6, 9.5 Hz, 1H, H-1′a), 3.63 (s, 3H, OCH_3), 3.69 (m, 1H, H-5), 3.83 (dt, *J* = 6.6, 9.5 Hz, 1H, H-1′b), 3.91 (dd, *J* = 2.4, 12.3 Hz, 1H, H-6a), 4.09 (dd, *J* = 5.2, 12.3 Hz, 1H, H-6b), 4.65 (d, *J* = 1.7 Hz, 1H, H-1), 5.03 (dd, *J* = 1.8, 3.0 Hz, 1H, H-2), 5.07–5.16 (m, 2H, H-3, H-4), 5.33 (s, 2H, CH_2Ar), 6.68, 6.72, 7.13 (m, 4H, C_6H_4), 7.68 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.85, 20.88, 21.02 (4C, 4 $COCH_3$), 26.4 (C-2′), 54.1 (CH_2Ar), 55.4 (OCH_3), 62.5 (C-6), 66.2 (C-4), 67.2 (C-1′), 68.9, 69.2, 69.7 (C-2, C-3, C-5), 97.6 (C-1), 113.9, 114.2, 120.4, 130.3 (C_6H_4, C_2N_3H–C5), 136.5 (C_6H_4–C1), 144.8 (C_2N_3H–C4), 160.2 (C_6H_4–C3), 169.8, 170.1, 170.2, 170.8 (4 $COCH_3$); ESI-MS Calcd for $C_{26}H_{33}N_3NaO_{11}$ $[M+Na]^+$: 586.21, Found 586.29.

4.1.6.9. [1-(4′-Nitrophenyl)-1,2,3-triazol-4-yl]ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (17i). Following general procedure B, **13** (55 mg, 0.14 mmol) was reacted with 1-azido-4-nitrobenzene (**15i**,[40] 34 mg, 0.21 mmol), 0.5 M $CuSO_4$ (70 μL, 35 μmol) and 1 M sodium ascorbate (70 μL, 70 μmol) to yield **17i** (74 mg, 94%).

$[\alpha]_D$ +28.1 (*c* 1.00, DCM); IR (film) 1748 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.92, 1.99, 2.06, 2.11 (4s, 12H, 4 $COCH_3$), 3.12 (m, 2H, H-2′), 3.69–3.82 (m, 2H, H-5, H-1′a), 3.99–4.09 (m, 2H, H-6a, H-1′b), 4.19 (dd, *J* = 5.3, 12.3 Hz, 1H, H-6b), 4.84 (d, *J* = 1.6 Hz, 1H, H-1), 5.17–5.27 (m, 2H, H-2, H-4), 5.30 (dd, *J* = 3.3, 10.2 Hz, 1H, H-3), 8.03 (s, 1H, C_2N_3H), 8.06, 8.37 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.75, 20.89, 20.91, 21.02 (4 $COCH_3$), 26.3 (C-2′), 62.5 (C-6), 66.1 (C-4), 66.6 (C-1′), 69.1, 69.2, 69.6 (C-2, C-3, C-5), 97.5 (C-1), 120.6 (C_2N_3H–C5), 120.6 (2C, C_6H_4), 125.6 (2C, C_6H_4), 141.5 (C_6H_4–C1), 146.2 (C_2N_3H–C4), 147.2 (C_6H_4–C4), 169.7, 170.3, 170.4, 170.8 (4 $COCH_3$); ESI-MS Calcd for $C_{26}H_{33}N_3NaO_{11}$ $[M+Na]^+$: 587.17, Found 587.25.

4.1.6.10. [1-(Pyridin-4′-yl)-1,2,3-triazol-4-yl]ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (17j). Following general procedure B, **13** (60 mg, 0.15 mmol) was reacted with 4-azidopyridine (**15j**,[41] 28 mg, 0.23 mmol), 0.5 M $CuSO_4$ (75 μL, 38 μmol) and 1 M sodium ascorbate (75 μL, 75 μmol). The crude product was dissolved in DCM (20 mL) and washed with 0.1 M aq EDTA

(10 mL). The aqueous phase was extracted with DCM (2 × 20 mL) and the combined organic layers were dried with Na_2SO_4 and evaporated to dryness. The residue was purified by MPLC on silica (petrol ether/EtOAc) to give **17j** (74 mg, 94%).

$[\alpha]_D$ +32.6 (*c* 0.99, DCM); IR (film) 1748 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.88, 1.97, 2.05, 2.10 (4s, 12H, 4 $COCH_3$), 3.10 (m, 2H, H-2′), 3.64–3.79 (m, 2H, H-5, H-1′a), 3.96–4.06 (m, 2H, H-6a, H-1′b), 4.17 (dd, *J* = 5.3, 12.3 Hz, 1H, H-6b), 4.83 (d, *J* = 1.6 Hz, 1H, H-1), 5.16–5.26 (m, 2H, H-2, H-4), 5.28 (dd, *J* = 3.4, 10.1 Hz, 1H, H-3), 7.78 (dd, *J* = 1.6, 4.7 Hz, 2H, C_5H_4N), 8.04 (s, 1H, C_2N_3H), 8.72 (dd, *J* = 1.4, 4.9 Hz, 2H, C_5H_4N); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.68, 20.88, 21.0 (4C, 4 $COCH_3$), 26.3 (C-2′), 62.5 (C-6), 66.0 (C-4), 66.5 (C-1′), 69.0, 69.2, 69.7 (C-2, C-3, C-5), 97.4 (C-1), 113.9 (2C, C_5H_4N), 120.0 (C_2N_3H–C5), 143.3 (C_5H_4N–C1), 146.1 (C_2N_3H–C4), 151.8 (2C, C_5H_4N), 169.7, 170.3, 170.4, 170.8 (4 $COCH_3$); ESI-MS Calcd for $C_{26}H_{33}N_3NaO_{11}$ $[M+Na]^+$: 543.18, Found: 543.14.

4.1.6.11. [1-(4′-Fluorophenyl)-1,2,3-triazol-4-yl]ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (17k). Following general procedure B, **13** (60 mg, 0.15 mmol) was reacted with 1-azido-4-fluorobenzene (**15k**,[41] 32 mg, 0.23 mmol), 0.5 M $CuSO_4$ (77 μL, 38 μmol) and 1 M sodium ascorbate (75 μL, 75 μmol) to yield **17k** (77 mg, 96%).

$[\alpha]_D$ +32.0 (*c* 1.01, DCM); IR (film) 1751 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.90, 1.96, 2.05, 2.10 (4s, 12H, 4 $COCH_3$), 3.07 (m, 2H, H-2′), 3.67–3.79 (m, 2H, H-5, H-1′a), 3.96–4.06 (m, 2H, H-6a, H-1′b), 4.18 (dd, *J* = 5.2, 12.3 Hz, 1H, H-6b), 4.82 (d, *J* = 1.6 Hz, 1H, H-1), 5.16–5.24 (m, 2H, H-2, H-4), 5.28 (dd, *J* = 3.4, 10.1 Hz, 1H, H-3), 7.16, 7.73 (m, 4H, C_6H_4), 7.85 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.71, 20.85, 20.88, 21.01 (4 $COCH_3$), 26.3 (C-2′), 62.5 (C-6), 66.1 (C-4), 66.8 (C-1′), 68.9, 69.2, 69.7 (C-2, C-3, C-5), 97.4 (C-1), 116.8 (d, *J* = 22.5 Hz, 2C, C_6H_4), 120.7 (C_2N_3H–C5), 122.6 (d, *J* = 8.8 Hz, 2C, C_6H_4), 133.6 (d, *J* = 2.5 Hz, C_6H_4–C1), 145.4 (C_2N_3H–C4), 162.5 (d, *J* = 247.5 Hz, C_6H_4–C4), 169.7, 170.2, 170.3, 170.8 (4 $COCH_3$); ESI-MS Calcd for $C_{26}H_{33}N_3NaO_{11}$ $[M+Na]^+$: 560.18, Found 560.17.

4.1.6.12. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-4-phenyl-1,2,3-triazole (20n). Following general procedure C, **18** (102 mg, 0.273 mmol) was reacted with phenylacetylene (**19n**, 60 μL, 0.55 mmol), 0.2 M $CuSO_4$ (273 μL, 54.6 μmol) and 1 M sodium ascorbate (109 μL, 109 μmol) to yield **20n** (110 mg, 84%).

$[\alpha]_D$ +65.5 (*c* 1.01, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.07, 2.07, 2.09, 2.20 (4 s, 12H, 4 $COCH_3$), 3.94 (ddd, *J* = 2.5, 5.4, 9.0 Hz, 1H, H-5), 4.08 (dd, *J* = 2.5, 12.5 Hz, 1H, H-6a), 4.39 (dd, *J* = 5.4, 12.5 Hz, 1H, H-6b), 5.39 (t, *J* = 8.9 Hz, 1H, H-4), 5.98 (dd, *J* = 3.7, 8.8 Hz, 1H, H-3), 6.02 (dd, *J* = 2.7, 3.7 Hz, 1H, H-2), 6.07 (d, *J* = 2.6 Hz, 1H, H-1), 7.32–7.39, 7.44–7.47, 7.85–7.87 (m, 5H, C_6H_5), 7.96 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.5, 20.6, 20.6, 20.7 (4 $COCH_3$), 61.5 (C-6), 66.0 (C-4), 68.2 (C-2), 68.7 (C-3), 72.1 (C-5), 83.5 (C-1), 119.7 (C_2N_3H–C5), 125.8, 128.6, 128.9, 129.6 (6C, C_6H_5), 148.2 (C_2N_3H–C4), 169.2, 169.6, 169.6, 170.4 (4 $COCH_3$); ESI-MS Calcd for $C_{22}H_{25}N_3NaO_9$ $[M+Na]^+$: 498.15, Found: 498.20.

4.1.6.13. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-4-(4-methylphenyl)-1,2,3-triazole (20o). Following general procedure C, **18** (50 mg, 0.13 mmol) was reacted with *p*-tolylacetylene (**19o**, 34 μL, 0.27 mmol), 0.2 M $CuSO_4$ (134 μL, 27 μmol) and 1 M sodium ascorbate (54 μL, 54 μmol) to yield **20o** (64 mg, 98%).

$[\alpha]_D$ +62.4 (*c* 1.09, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.06, 2.07, 2.09, 2.19 (4 s, 12H, 4 $COCH_3$), 2.39 (s, 3H, PhCH_3), 3.94 (ddd, *J* = 2.4, 5.3, 9.0 Hz, 1H, H-5), 4.07 (dd, *J* = 2.4, 12.5 Hz, 1H, H-6a), 4.38 (dd, *J* = 5.4, 12.5 Hz, 1H, H-6b), 5.39 (t, *J* = 8.9 Hz, 1H, H-4), 5.98 (dd, *J* = 3.7, 8.7 Hz, 1H, H-3), 6.01 (dd, *J* = 2.6, 3.7 Hz, 1H, H-2), 6.05 (d, *J* = 2.4 Hz, 1H, H-1), 7.26, 7.74 (AA′, BB′ of AA′BB′, *J* = 7.9 Hz, 4H, C_6H_4), 7.90 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 20.7, 20.8 (4C, 4 $COCH_3$), 21.3 (PhCH_3), 61.6 (C-6), 66.1 (C-4), 68.3 (C-2), 68.8 (C-3), 72.1 (C-5), 83.6 (C-1), 119.3 (C_2N_3H–C5), 125.8, 126.8, 129.6, 138.6 (6C, C_6H_4), 148.4 (C_2N_3H–C4), 169.3, 169.7, 169.7, 170.5 (4 $COCH_3$); ESI-MS Calcd for $C_{23}H_{27}N_3NaO_9$ $[M+Na]^+$: 512.16, Found: 512.15.

4.1.6.14. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-4-(3-chlorophenyl)-1,2,3-triazole (20p). Following general procedure C, **18** (50 mg, 0.13 mmol) was reacted with 3-chloro-1-ethinylbenzene (**19p**, 33 μL, 0.27 mmol), 0.2 M $CuSO_4$ (134 μL, 27 μmol) and 1 M sodium ascorbate (54 μL, 54 μmol) to yield **20p** (59 mg, 86%).

$[\alpha]_D$ +56.3 (*c* 1.03, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.07, 2.08, 2.09, 2.19 (4 s, 12H, 4 $COCH_3$), 3.95 (m, 1H, H-5), 4.08 (dd, *J* = 2.1, 12.5 Hz, 1H, H-6a), 4.41 (dd, *J* = 5.4, 12.5 Hz, 1H, H-6b), 5.38 (t, *J* = 8.7 Hz, 1H, H-4), 5.95 (dd, *J* = 3.6, 8.6 Hz, 1H, H-3), 6.00 (m, 1H, H-2), 6.07 (d, *J* = 2.4 Hz, 1H, H-1), 7.34–7.40 (m, 2H, C_6H_4–H5, H6), 7.75 (d, *J* = 7.4 Hz, 1H, C_6H_4–H4), 7.85 (s, 1H, C_6H_4-H2), 7.98 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 20.7, 20.7, 20.8 (4 $COCH_3$), 61.5 (C-6), 66.1 (C-4), 68.2 (C-2), 68.7 (C-3), 72.4 (C-5), 83.5 (C-1), 120.1 (C_2N_3H–C5), 124.0 (C_6H_4–C4), 126.0 (C_6H_4–C2), 128.7, 130.3 (C_6H_4–C5, C6), 131.5 (C_6H_4–C3), 134.9 (C_6H_4–C1), 147.2 (C_2N_3H–C4), 169.3, 169.6, 169.7, 170.5 (4 $COCH_3$); ESI-MS Calcd for $C_{22}H_{24}ClN_3NaO_9$ $[M+Na]^+$: 532.11, Found: 532.13.

4.1.6.15. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-4-(4-trifluoromethylphenyl)-1,2,3-triazole (20q). Following general procedure C, **18** (50 mg, 0.13 mmol) was reacted with 1-ethinyl-4-trifluoromethylbenzene (**19q**, 44 μL, 0.27 mmol), 0.2 M $CuSO_4$ (134 μL, 27 μmol) and 1 M sodium ascorbate (54 μL, 54 μmol) to yield **20q** (62 mg, 85%).

$[\alpha]_D$ +54.5 (*c* 0.95, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.08, 2.09, 2.19 (3 s, 12H, 4 $COCH_3$), 3.97 (ddd, *J* = 2.5, 5.4, 8.7 Hz, 1H, H-5), 4.09 (dd, *J* = 2.5, 12.5 Hz, 1H, H-6a), 4.41 (dd, *J* = 5.5, 12.5 Hz, 1H, H-6b), 5.39 (t, *J* = 8.8 Hz, 1H, H-4), 5.95 (dd, *J* = 3.7, 8.7 Hz, 1H, H-3), 6.00 (dd, *J* = 3.1, 3.4 Hz, 1H, H-2), 6.09 (d, *J* = 2.8 Hz, 1H, H-1), 7.71, 7.98 (AA′, BB′ of AA′BB′, *J* = 8.1 Hz, 4H, C_6H_4), 8.05 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.60, 20.69, 20.70, 20.73 (4 $COCH_3$), 61.4 (C-6), 66.0 (C-4), 68.2 (C-2), 68.7 (C-3), 72.5 (C-5), 83.5 (C-1), 120.6 (C_2N_3H–C5), 124.0 (q, *J* = 272 Hz, CF_3), 126.0 (q, *J* = 3.8 Hz, 2C, C_6H_4–C3, C5), 126.1 (2C, C_6H_4–C2, C6), 130.5 (d, *J* = 32.6 Hz, C_6H_4–C4), 133.1 (C_6H_4–C1), 147.0 (C_2N_3H–C4), 169.3, 169.6, 169.7, 170.5 (4 $COCH_3$); ESI-MS Calcd for $C_{23}H_{24}F_3N_3NaO_9$ $[M+Na]^+$: 566.14, Found: 566.10.

4.1.6.16. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-4-(3-pyridyl)-1,2,3-triazole (20r). Following general procedure C, **18** (50 mg, 0.13 mmol) was reacted with 3-ethinylpyridine (**19r**, 27.6 mg, 0.27 mmol), 0.2 M $CuSO_4$ (134 μL, 27 μmol) and 1 M sodium ascorbate (54 μL, 54 μmol). Then the reaction mixture was diluted with DCM (20 mL) and extracted with 25 mM aq EDTA (10 mL). The organic layer was dried (Na_2SO_4), concentrated and the residue was purified by MPLC on silica (petrol ether/EtOAc) to yield **20r** (61 mg, 96%).

$[\alpha]_D$ +56.0 (*c* 0.70, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.06, 2.06, 2.07, 2.17 (4 s, 12H, 4 $COCH_3$), 3.95 (ddd, *J* = 2.6, 5.5, 8.8 Hz, 1H, H-5), 4.07 (dd, *J* = 2.6, 12.5 Hz, 1H, H-6a), 4.40 (dd, *J* = 5.6, 12.5 Hz, 1H, H-6b), 5.38 (t, *J* = 8.8 Hz, 1H, H-4), 5.92 (dd, *J* = 3.7, 8.8 Hz, 1H, H-3), 6.00 (dd, *J* = 3.2, 3.5 Hz, 1H, H-2), 6.11 (d, *J* = 2.9 Hz, 1H, H-1), 7.45 (dd, *J* = 4.9, 7.2 Hz, 1H, C_5H_4N-H5), 8.11 (s, 1H, C_2N_3H), 8.28 (d, *J* = 7.9 Hz, 1H, C_5H_4N-H6), 8.62 (m, 1H, C_5H_4N-H4), 9.06 (br s, 1H, C_5H_4N–H2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.56, 20.66, 20.68, 20.70 (4 $COCH_3$), 61.4 (C-6), 66.0 (C-4), 68.1 (C-2), 68.6 (C-3), 72.5 (C-5), 83.6 (C-1), 120.4 (C_2N_3H–C5), 124.1

(C_5H_4N–C5), 126.3 (C_5H_4N–C1), 133.7 (C_5H_4N–C6), 145.0 (C_2N_3H–C4), 146.5 (C_5H_4N–C2), 148.9 (C_5H_4N–C4), 169.3, 169.6, 169.6, 170.5 (4 *C*OCH$_3$); ESI-MS Calcd for $C_{21}H_{24}N_4NaO_9$ $[M+Na]^+$: 477.16, Found: 477.08.

4.1.6.17. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-4-phenoxymethyl-1,2,3-triazole (20s). Following general procedure C, **18** (50 mg, 0.13 mmol) was reacted with phenylpropargyl ether (**19s**, 34 μL, 0.27 mmol), 0.2 M $CuSO_4$ (134 μL, 27 μmol) and 1 M sodium ascorbate (54 μL, 54 μmol) to yield **20s** (58 mg, 85%).

$[\alpha]_D$ +38.8 (*c* 0.61, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.06, 2.07, 2.09, 2.18 (4 s, 12H, 4 COCH$_3$), 3.90 (m, 1H, H-5), 4.05 (dd, *J* = 2.0, 12.5 Hz, 1H, H-6a), 4.37 (dd, *J* = 5.4, 12.5 Hz, 1H, H-6b), 5.26 (s, 2H, C*H*$_2$OPh), 5.37 (t, *J* = 8.9 Hz, 1H, H-4), 5.93 (dd, *J* = 3.6, 8.8 Hz, 1H, H-3), 5.97 (dd, *J* = 2.7, 3.3 Hz, 1H, H-2), 6.00 (d, *J* = 2.3 Hz, 1H, H-1), 6.98–7.00, 7.30–7.32 (m, 5H, C_6H_5), 7.81 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.59, 20.67, 20.69, 20.73 (4 CO*C*H$_3$), 61.5 (C-6), 61.8 (*C*H$_2$OPh), 66.0 (C-4), 68.2 (C-2), 68.7 (C-3), 72.2 (C-5), 83.6 (C-1), 114.7 (2C, C_6H_5–C2, C6), 121.4 (C_6H_5–C4), 123.0 (C_2N_3H–C5), 129.6 (2C, C_6H_5–C3, C5), 145.2 (C_2N_3H–C4), 158.0 (C_6H_5–C1), 169.3, 169.6, 169.7, 170.5 (4 *C*OCH$_3$); ESI-MS Calcd for $C_{23}H_{27}N_3NaO_{10}$ $[M+Na]^+$: 528.16, Found: 528.14.

4.1.6.18. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)methyl-4-phenyl-1,2,3-triazole (25n). Following general procedure C, **24** (40 mg, 0.10 mmol) was reacted with phenylacetylene (**19n**, 23 μL, 0.21 mmol), 0.2 M $CuSO_4$ (103 μL, 21 μmol) and 1 M sodium ascorbate (41 μL, 41 μmol) to yield **25n** (47 mg, 93%).

$[\alpha]_D$ -1.76 (*c* 1.50, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.83, 2.08, 2.11, 2.12 (4 s, 12H, 4 COCH$_3$), 4.00 (dd, *J* = 3.9, 12.2 Hz, 1H, H-6a), 4.16 (dt, *J* = 3.5, 9.0 Hz, 1H, H-5), 4.39 (dt, *J* = 2.6, 8.6 Hz, 1H, H-1), 4.50 (dd, *J* = 8.8, 14.3 Hz, 1H, H-1′a), 4.59 (dd, *J* = 9.2, 12.1 Hz, 1H, H-6b), 4.66 (dd, *J* = 2.4, 14.3 Hz, 1H, H-1′b), 4.94 (dd, *J* = 3.0, 4.9 Hz, 1H, H-4), 5.06 (dd, *J* = 3.2, 8.4 Hz, 1H, H-2), 5.35 (t, *J* = 4.0 Hz, 1H, H-3), 7.32 (t, *J* = 7.4 Hz, 1H, C_6H_5-H4), 7.41 (t, *J* = 7.4 Hz, 2H, C_6H_5-H3, H5), 7.83 (d, *J* = 8.0 Hz, 2H, C_6H_5-H2, H6), 7.92 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.4, 20.6, 20.7, 20.8 (4 CO*C*H$_3$), 50.3 (C-1′), 60.0 (C-6), 66.8 (C-2), 66.9 (C-3), 68.0 (C-4), 68.4 (C-1), 73.4 (C-5), 120.9 (C_2N_3H–C5), 125.6, 128.2, 128.8, 130.5 (6C, C_6H_5), 148.0 (C_2N_3H–C4), 169.1, 169.4, 169.6, 170.4 (4 *C*OCH$_3$); ESI-MS Calcd for $C_{23}H_{28}N_3O_9$ $[M+H]^+$: 490.18, Found: 490.17.

4.1.6.19. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)methyl-4-(4-methylphenyl)-1,2,3-triazole (25o). Following general procedure C, **24** (40 mg, 0.10 mmol) was reacted with *p*-tolylacetylene (**19o**, 26 μL, 0.21 mmol), 0.2 M $CuSO_4$ (103 μL, 21 μmol) and 1 M sodium ascorbate (41 μL, 41 μmol) to yield **25o** (50 mg, 97%).

$[\alpha]_D$ -1.57 (*c* 1.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.83, 2.07, 2.10, 2.11 (4 s, 12H, 4 COCH$_3$), 2.36 (s, 3H, PhC*H*$_3$), 3.99 (dd, *J* = 4.0, 12.2 Hz, 1H, H-6a), 4.15 (dt, *J* = 3.5, 9.0 Hz, 1H, H-5), 4.38 (dt, *J* = 2.7, 8.6 Hz, 1H, H-1), 4.50 (dd, *J* = 8.8, 14.4 Hz, 1H, H-1′a), 4.58 (dd, *J* = 9.1, 12.2 Hz, 1H, H-6b), 4.64 (dd, *J* = 2.8, 14.4 Hz, 1H, H-1′b), 4.94 (dd, *J* = 3.1, 5.0 Hz, 1H, H-4), 5.06 (dd, *J* = 3.3, 8.3 Hz, 1H, H-2), 5.34 (dd, *J* = 3.5, 4.8 Hz, 1H, H-3), 7.21, 7.70 (AA′, BB′ of AA′BB′, *J* = 8.0 Hz, 4H, C_6H_4), 7.87 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.41, 20.62, 20.64, 20.74 (4 CO*C*H$_3$), 21.2 (PhC*H*$_3$), 50.2 (C-1′), 60.0 (C-6), 66.8 (C-2), 66.9 (C-3), 68.0 (C-4), 68.5 (C-1), 73.3 (C-5), 120.5 (C_2N_3H–C5), 125.5, 127.6, 129.5, 138.0 (6C, C_6H_4), 147.8 (C_2N_3H–C4), 169.1, 169.4, 169.6, 170.4 (4 *C*OCH$_3$); ESI-MS Calcd for $C_{24}H_{30}N_3O_9$ $[M+H]^+$: 504.20, Found: 504.20.

4.1.6.20. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)methyl-4-(3-chlorophenyl)-1,2,3-triazole (25p). Following general procedure C, **24** (40 mg, 0.10 mmol) was reacted with 3-chloro-1-ethinylbenzene (**19p**, 25 μL, 0.21 mmol), 0.2 M $CuSO_4$ (103 μL, 21 μmol) and 1 M sodium ascorbate (41 μL, 41 μmol) to yield **25p** (51 mg, 94%).

$[\alpha]_D$ -2.55 (*c* 1.27, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.84, 2.07, 2.10, 2.11 (4 s, 12H, 4 COCH$_3$), 3.99 (dd, *J* = 4.0, 12.2 Hz, 1H, H-6a), 4.15 (dt, *J* = 3.4, 9.0 Hz, 1H, H-5), 4.38 (dt, *J* = 2.7, 8.6 Hz, 1H, H-1), 4.51 (dd, *J* = 8.7, 14.4 Hz, 1H, H-1′a), 4.60 (dd, *J* = 9.2, 12.2 Hz, 1H, H-6b), 4.65 (dd, *J* = 2.7, 14.4 Hz, 1H, H-1′b), 4.93 (dd, *J* = 2.9, 4.9 Hz, 1H, H-4), 5.03 (dd, *J* = 3.3, 8.5 Hz, 1H, H-2), 5.34 (dd, *J* = 3.6, 4.6 Hz, 1H, H-3), 7.27 (m, 1H, C_6H_4-H6), 7.33 (t, *J* = 7.8 Hz, 1H, C_6H_4–H5), 7.72 (d, *J* = 7.7 Hz, 1H, C_6H_4–H4), 7.81 (t, *J* = 1.7 Hz, 1H, C_6H_4-H2), 7.94 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.41, 20.60, 20.64, 20.73 (4 CO*C*H$_3$), 50.4 (C-1′), 59.9 (C-6), 66.7 (C-2), 66.8 (C-3), 68.0 (C-4), 68.3 (C-1), 73.5 (C-5), 121.3 (C_2N_3H–C5), 123.6, 125.6, 128.1, 130.1, 132.3, 134.8 (C_6H_4), 146.5 (C_2N_3H–C4), 169.1, 169.3, 169.5, 170.3 (4 *C*OCH$_3$); ESI-MS Calcd for $C_{23}H_{27}ClN_3O_9$ $[M+H]^+$: 524.14, Found: 524.04.

4.1.6.21. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)methyl-4-(4-trifluoromethylphenyl)-1,2,3-triazole (25q). Following general procedure C, **24** (40 mg, 0.10 mmol) was reacted with 1-ethinyl-4-trifluoromethylbenzene (**19q**, 34 μL, 0.21 mmol), 0.2 M $CuSO_4$ (103 μL, 21 μmol) and 1 M sodium ascorbate (41 μL, 41 μmol) to yield **25q** (56 mg, 98%).

$[\alpha]_D$ +0.47 (*c* 1.19, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82, 2.08, 2.10, 2.12 (4 s, 12H, 4 COCH$_3$), 3.97 (dd, *J* = 4.0, 12.2 Hz, 1H, H-6a), 4.15 (dt, *J* = 3.4, 9.0 Hz, 1H, H-5), 4.40 (dt, *J* = 2.5, 8.6 Hz, 1H, H-1), 4.53 (dd, *J* = 8.7, 14.4 Hz, 1H, H-1′a), 4.63 (dd, *J* = 9.2, 12.2 Hz, 1H, H-6b), 4.67 (dd, *J* = 2.7, 14.4 Hz, 1H, H-1′b), 4.93 (dd, *J* = 2.9, 4.7 Hz, 1H, H-4), 5.04 (dd, *J* = 3.2, 8.6 Hz, 1H, H-2), 5.34 (t, *J* = 4.0 Hz, 1H, H-3), 7.66, 7.95 (AA′, BB′ of AA′BB′, *J* = 8.2 Hz, 4H, C_6H_4), 8.00 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.42, 20.63, 20.67, 20.76 (4 CO*C*H$_3$), 50.4 (C-1′), 59.9 (C-6), 66.7 (C-2), 66.8 (C-3), 68.0 (C-4), 68.2 (C-1), 73.5 (C-5), 121.7 (C_2N_3H–C5), 124.0 (q, *J* = 272 Hz, CF_3), 125.7 (2C, C_6H_4–C2, C6), 125.8 (q, *J* = 3.8 Hz, 2C, C_6H_4–C3, C5), 129.9 (q, *J* = 32.5 Hz, C_6H_4–C4), 133.9 (C_6H_4–C1), 146.4 (C_2N_3H–C4), 169.1, 169.4, 169.6, 170.3 (4 *C*OCH$_3$); ESI-MS Calcd for $C_{24}H_{27}F_3N_3O_9$ $[M+H]^+$: 558.17, Found: 558.22.

4.1.6.22. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)methyl-4-(3-pyridyl)-1,2,3-triazole (25r). Following general procedure C, **24** (40 mg, 0.10 mmol) was reacted with 3-ethinylpyridine (**19r**, 21.2 mg, 0.206 mmol), 0.2 M $CuSO_4$ (103 μL, 21 μmol) and 1 M sodium ascorbate (41 μL, 41 μmol) to yield **25r** (50 mg, 98%).

$[\alpha]_D$ -0.08 (*c* 1.04, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.83, 2.07, 2.09, 2.10 (4 s, 12H, 4 COCH$_3$), 3.97 (dd, *J* = 4.0, 12.2 Hz, 1H, H-6a), 4.14 (dt, *J* = 3.4, 8.9 Hz, 1H, H-5), 4.39 (dt, *J* = 2.6, 8.6 Hz, 1H, H-1), 4.54 (dd, *J* = 8.6, 14.4 Hz, 1H, H-1′a), 4.60 (dd, *J* = 9.1, 12.2 Hz, 1H, H-6b), 4.66 (dd, *J* = 2.7, 14.4 Hz, 1H, H-1′b), 4.92 (dd, *J* = 2.9, 4.8 Hz, 1H, H-4), 5.02 (dd, *J* = 3.3, 8.5 Hz, 1H, H-2), 5.33 (m, 1H, H-3), 7.37 (m, 1H, C_5H_4N-H5), 8.02 (s, 1H, C_2N_3H), 8.21 (d, *J* = 7.9 Hz, 1H, C_5H_4N-H6), 8.56 (s, 1H, C_5H_4N–H2), 9.00 (m, 1H, C_5H_4N-H4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.44, 20.61, 20.65, 20.74 (4 CO*C*H$_3$), 50.4 (C-1′), 59.9 (C-6), 66.6 (C-2), 66.8 (C-3), 67.9 (C-4), 68.3 (C-1), 73.5 (C-5), 121.3 (C_2N_3H–C5), 123.8 (C_5H_4N–C5), 126.8 (C_5H_4N–C1), 133.0 (C_5H_4N–C6), 144.6 (C_2N_3H–C4), 146.7 (C_5H_4N–C2), 149.0 (C_5H_4N–C4), 169.1, 169.3, 169.5, 170.3 (4 *C*OCH$_3$); ESI-MS Calcd for $C_{22}H_{27}N_4O_9$ $[M+H]^+$: 491.18, Found: 491.17.

4.1.6.23. 1-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)methyl-4-phenoxymethyl-1,2,3-triazole (25s). Following general procedure C, **24** (40 mg, 0.10 mmol) was reacted with phenylpropargyl ether (**19s**, 26 μL, 0.21 mmol), 0.2 M $CuSO_4$ (103 μL, 21 μmol) and 1 M sodium ascorbate (41 μL, 41 μmol) to yield **25s** (51 mg, 96%).

$[\alpha]_D$ +2.34 (*c* 1.03, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.00, 2.08, 2.12, 2.13 (4 s, 12H, 4 $COCH_3$), 4.04 (dd, *J* = 4.1, 12.1 Hz, 1H, H-6a), 4.15 (m, 1H, H-5), 4.37 (dt, *J* = 2.4, 8.6 Hz, 1H, H-1), 4.50 (dd, *J* = 8.7, 14.4 Hz, 1H, H-1′a), 4.55 (dd, *J* = 9.0, 12.1 Hz, 1H, H-6b), 4.63 (dd, *J* = 2.5, 14.4 Hz, 1H, H-1′b), 4.96 (dd, *J* = 3.2, 4.9 Hz, 1H, H-4), 5.04 (dd, *J* = 3.3, 8.3 Hz, 1H, H-2), 5.34 (m, 1H, H-3), 6.96–6.99, 7.29–7.31 (m, 5H, C_6H_5), 7.80 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.49, 20.62, 20.66, 20.74 (4 $COCH_3$), 50.2 (C-1′), 60.0 (C-6), 61.9 (CH_2OPh), 66.7 (C-2), 66.9 (C-3), 67.8 (C-4), 68.5 (C-1), 73.3 (C-5), 114.6, 121.2 (3C, C_6H_5), 123.8 (C_2N_3H–C5), 129.5 (2C, C_6H_5), 144.3 (C_2N_3H–C4), 158.1 (C_6H_4–C1), 169.1, 169.4, 169.6, 170.4 (4 $COCH_3$); ESI-MS Calcd for $C_{24}H_{30}N_3O_9$ $[M+H]^+$: 520.19, Found: 520.16.

4.1.7. Synthesis of mannosyl triazoles

4.1.7.1. Sodium 4-[4-((α-D-mannopyranosyloxy)methyl)-1*H*-1,2,3-triazol-1-yl]-benzoate (5a). To a solution of **12a** (48.0 mg, 85.2 μmol) in MeOH (4 mL) was added freshly prepared 1 M NaOMe in MeOH (0.4 mL) under argon. The mixture was stirred at rt for 3 h and then evaporated to dryness. The remains were dissolved in H_2O/dioxane (1:1, 5 mL) and treated with 1 M aq. NaOH (0.5 mL) for 16 h. The solution was concentrated and the residue purified by MPLC on RP-18 (H_2O/MeOH) and P2 size exclusion chromatography to give **5a** (31.2 mg, 91%) as white powder after a final lyophilization from water.

$[\alpha]_D$ +41.7 (*c* 0.60, H_2O); IR (KBr) 3413 (vs b, OH), 1607 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 3.60–3.66 (m, 2H, H-4, H-5), 3.72 (dd, *J* = 5.5, 12.1 Hz, 1H, H-6a), 3.78 (dd, *J* = 3.4, 9.4 Hz, 1H, H-3), 3.82 (dd, *J* = 1.4, 12.2 Hz, 1H, H-6b), 3.93 (dd, *J* = 1.7, 3.4 Hz, 1H, H-2), 4.71 (m, 1H, H-1′a), 4.82 (m, 1H, H-1′b), 4.97 (s, 1H, H-1), 7.68 (m, 2H, C_6H_4), 7.94 (m, 2H, C_6H_4), 8.41 (m, 1H, C_2N_3H); ^{13}C NMR (125 MHz, D_2O): δ 59.7 (C-1′), 60.8 (C-6), 66.6 (C-4), 69.9 (C-2), 70.4 (C-3), 72.9 (C-5), 99.5 (C-1), 120.2 (2C, C_6H_4), 123.3 (C_2N_3H–C5), 130.3, 137.0, 137.7 (4C, C_6H_4), 144.2 (C_2N_3H–C4), 174.1 (CO); HR-MS Calcd for $C_{16}H_{19}N_3NaO_8$ $[M+H]^+$: 404.1070, Found: 404.1071.

4.1.7.2. Sodium 3-[4-((α-D-mannopyranosyloxy)methyl)-1*H*-1,2,3-triazol-1-yl]-benzoate (5b). According to the procedure described for **5a**, **12b** (67.0 mg, 0.116 mmol) was subsequently treated with 1 M methanolic NaOMe (0.5 mL) in MeOH (5 mL) and 1 M aq. NaOH (0.5 mL) in H_2O/dioxane (1:1, 6 mL) to yield **5b** (37.7 mg, 81%).

$[\alpha]_D$ +44.4 (*c* 0.89, H_2O); IR (KBr) 3401 (vs b, OH), 1610 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 3.61–3.66 (m, 2H, H-4, H-5), 3.72 (dd, *J* = 5.4, 12.2 Hz, 1H, H-6a), 3.78 (dd, *J* = 3.5, 9.5 Hz, 1H, H-3), 3.82 (dd, *J* = 1.5, 12.2 Hz, 1H, H-6b), 3.92 (dd, *J* = 1.7, 3.3 Hz, 1H, H-2), 4.69 (A of AB, *J* = 12.8 Hz, 1H, H-1′a), 4.80 (m, 1H, H-1′b), 4.96 (s, 1H, H-1), 7.51 (m, 1H, C_6H_4–H5), 7.69 (d, *J* = 7.0 Hz, 1H, C_6H_4–H4), 7.87 (d, *J* = 7.7 Hz, 1H, C_6H_4-H6), 8.01 (s, 1H, C_6H_4-H2), 8.36 (m, 1H, C_2N_3H); ^{13}C NMR (125 MHz, D_2O): δ 59.7 (C-1′), 60.8 (C-6), 66.7 (C-4), 69.9 (C-2), 70.4 (C-3), 72.9 (C-5), 99.5 (C-1), 120.8 (C_6H_4), 123.0 (C_2N_3H–C5), 123.3, 129.5, 129.8, 135.9, 138.1 (C_6H_4), 144.1 (C_2N_3H–C4), 173.6 (CO); HR-MS Calcd for $C_{16}H_{19}N_3NaO_8$ $[M+H]^+$: 404.1070, Found: 404.1068.

4.1.7.3. Sodium 5-[4-((α-D-mannopyranosyloxy)methyl)-1*H*-1,2,3-triazol-1-yl]-nicotinate (5c). According to the procedure described for **5a**, **12c** (41.0 mg, 72.6 μmol) was subsequently treated with 1 M methanolic NaOMe (0.4 mL) in MeOH (4 mL) and 1 M aq. NaOH (0.4 mL) in H_2O/dioxane (1:1, 4 mL) to yield **5c** (23.0 mg, 78%).

$[\alpha]_D$ +36.0 (*c* 0.69, H_2O); IR (KBr) 3413 (vs b, OH), 1616 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 3.61–3.66 (m, 2H, H-4, H-5), 3.73 (dd, *J* = 5.4, 12.1 Hz, 1H, H-6a), 3.79 (dd, *J* = 3.5, 9.4 Hz, 1H, H-3), 3.83 (d, *J* = 12.0 Hz, 1H, H-6b), 3.94 (dd, *J* = 1.7, 3.2 Hz, 1H, H-2), 4.76, 4.87 (A, B of AB, *J* = 12.6 Hz, 1H, H-1′), 4.99 (d, *J* = 0.7 Hz, 1H, H-1), 8.47 (t, *J* = 2.0 Hz, 1H, C_5H_3N-H2), 8.56 (m, 1H, C_2N_3H), 8.96 (d, *J* = 2.1 Hz, 1H, C_5H_3N-H6), 8.97 (d, *J* = 1.4 Hz, 1H, C_5H_3N-H4); ^{13}C NMR (125 MHz, D_2O): δ 59.7 (C-1′), 60.8 (C-6), 66.7 (C-4), 69.9 (C-2), 70.4 (C-3), 73.0 (C-5), 99.5 (C-1), 123.6 (C_2N_3H–C5), 129.5, 133.1, 133.4, 142.6 (C_5H_3N), 144.6 (C_2N_3H–C4), 149.7 (C_5H_3N), 171.1 (CO); HR-MS Calcd for $C_{15}H_{17}N_4Na_2O_8$ $[M+Na]^+$: 427.0842, Found: 427.0844.

4.1.7.4. (1-Benzyl-1,2,3-triazol-4-yl)methyl α-D-mannopyranoside (5d). Following general procedure A, **14** (40 mg, 0.18 mmol) was reacted with benzyl azide (**15d**, 34 μL, 0.27 mmol), $CuSO_4$ (11 mg, 45 μmol) and sodium ascorbate (18 mg, 90 μmol) to yield **5d** (57 mg, 71%).

$[\alpha]_D$ +53.3 (*c* 1.03, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 3.50 (m, 1H, H-5), 3.56 (t, *J* = 9.4 Hz, 1H, H-4), 3.60–3.68 (m, 2H, H-3, H-6a), 3.73 (m, 1H, H-2), 3.79 (dd, *J* = 1.7, 11.7 Hz, 1H, H-6b), 4.60, 4.75 (A, B of AB, *J* = 12.4 Hz, 2H, H-1′), 4.80 (d, *J* = 1.6 Hz, 1H, H-1), 5.56 (s, 2H, CH_2Ph), 7.24–7.41 (m, 5H, C_6H_5), 7.97 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 55.1 (CH_2Ph), 60.8 (C-1′), 63.1 (C-6), 68.7 (C-4), 72.1 (C-2), 72.6 (C-3), 75.1 (C-5), 100.9 (C-1), 125.5 (C_2N_3H–C5), 129.3, 129.8, 130.2, 136.9 (6C, C_6H_5), 145.9 (C_2N_3H–C4); HR-MS Calcd for $C_{16}H_{21}N_3NaO_6$ $[M+Na]^+$: 374.1328, Found: 374.1334.

4.1.7.5. [1-(4′-Aminophenyl)-1,2,3-triazol-4-yl]methyl α-D-mannopyranoside hydrochloride (5e). Following general procedure A, **14** (40 mg, 0.18 mmol) was reacted with 4-azidoaniline hydrochloride (**15e**, 46 mg, 0.27 mmol), $CuSO_4$ (11 mg, 45 μmol) and sodium ascorbate (18 mg, 90 μmol) to yield **5e** (19 mg, 27%).

$[\alpha]_D$ +55.2 (*c* 1.00, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 3.55–3.60 (m, 2H, H-4, H-5), 3.64–3.72 (m, 2H, H-3, H-6a), 3.78 (m, 1H, H-2), 3.83 (m, 1H, H-6b), 4.68, 4.82 (A, B of AB, *J* = 12.4 Hz, 2H, H-1′), 4.86 (m, 1H, H-1), 6.78, 7.45 (AA′, BB′ of AA′BB′, *J* = 8.7 Hz, 4H, C_6H_4), 8.33 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 60.8 (C-1′), 63.2 (C-6), 68.8 (C-4), 72.2 (C-2), 72.6 (C-3), 75.2 (C-5), 100.9 (C-1), 116.2 (2C, C_6H_4), 123.3 (2C, C_6H_4), 123.7 (C_2N_3H–C5), 128.8 (C_6H_4–C1), 145.8 (C_2N_3H–C4), 150.8 (C_6H_4–C4); HR-MS Calcd for $C_{15}H_{21}N_4O_6$ $[M+H]^+$: 353.1461, Found: 353.1463.

4.1.7.6. (1-Adamantyl-1,2,3-triazol-4-yl)methyl α-D-mannopyranoside (5f). Following general procedure A, **14** (40 mg, 0.18 mmol) was reacted with 1-azidoadamantane (**15f**, 48 mg, 0.27 mmol), $CuSO_4$ (11 mg, 45 μmol) and sodium ascorbate (18 mg, 90 μmol) to yield **5f** (20 mg, 28%).

$[\alpha]_D$ +50.5 (*c* 1.04, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 1.76–1.88 (m, 6H, Ad), 2.24 (s, 9H, Ad), 3.50–3.60 (m, 2H, H-4, H-5), 3.61–3.70 (m, 2H, H-3, H-6a), 3.75 (dd, *J* = 1.7, 3.3 Hz, 1H, H-2), 3.82 (m, 1H, H-6b), 4.60, 4.76 (A, B of AB, *J* = 12.3 Hz, 2H, H-1′), 4.80 (d, *J* = 1.4 Hz, 1H, H-1), 8.09 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 31.1, 37.1, 44.0 (10 C, Ad), 60.9 (C-1′), 63.2 (C-6), 68.8 (C-4), 72.2 (C-2), 72.6 (C-3), 75.1 (C-5), 100.9 (C-1), 122.2 (C_2N_3H–C5), 144.6 (C_2N_3H–C4); HR-MS Calcd for $C_{19}H_{29}N_3NaO_6$ $[M+Na]^+$: 418.1954, Found: 418.1951.

4.1.7.7. [1-(4′-Methoxybenzyl)-1,2,3-triazol-4-yl]methyl α-D-mannopyranoside (5g). Following general procedure A, **14** (50 mg, 0.23 mmol) was reacted with 4-methoxybenzylazide (**15g**,[38] 57 mg, 0.35 mmol), $CuSO_4$ (15 mg, 60 μmol) and sodium ascorbate (24 mg, 120 μmol) to yield **5g** (64 mg, 73%).

$[\alpha]_D$ +66.6 (*c* 1.01, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 3.50 (m, 1H, H-5), 3.57 (t, *J* = 9.4 Hz, 1H, H-4), 3.61–3.69 (m, 2H, H-3, H-6a), 3.74 (m, 4H, H-2, OCH_3), 3.78 (dd, *J* = 1.7, 11.7 Hz, 1H, H-6b), 4.58, 4.73 (A, B of AB, *J* = 12.4 Hz, 2H, H-1′), 4.79 (m, 1H, H-1), 5.46 (s, 2H, CH_2Ar), 6.88, 7.25 (AA′, BB′ of AA′BB′, *J* = 8.6 Hz, 4H, C_6H_4), 7.91 (s, 1H, C_2N_3H);

^{13}C NMR (125 MHz, CD_3OD): δ 54.6 (CH_2Ar), 55.8 (OCH_3), 60.7 (C-1′), 63.0 (C-6), 68.6 (C-4), 72.0 (C-2), 72.5 (C-3), 75.0 (C-5), 100.8 (C-1), 115.4 (2C, C_6H_4), 125.1 (C_2N_3H–C5), 128.6 (2C, C_6H_4), 130.8 (C_6H_4–C1), 145.6 (C_2N_3H–C4), 161.4 (C_6H_4–C4); HR-MS Calcd for $C_{17}H_{23}N_3NaO_7$ $[M+Na]^+$: 404.1434, Found: 404.1431.

4.1.7.8. [1-(3′-Methoxybenzyl)-1,2,3-triazol-4-yl]methyl α-D-mannopyranoside (5h). Following general procedure A, **14** (50 mg, 0.23 mmol) was reacted with 3-methoxybenzylazide (**15h**,[39] 57 mg, 0.35 mmol), $CuSO_4$ (15 mg, 60 μmol) and sodium ascorbate (24 mg, 120 μmol) to yield **5h** (68 mg, 77%).

$[\alpha]_D$ +62.2 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.52 (m, 1H, H-5), 3.58 (t, *J* = 9.4 Hz, 1H, H-4), 3.61–3.70 (m, 2H, H-3, H-6a), 3.70–3.84 (m, 5H, H-2, H-6b, OCH_3), 4.60, 4.75 (A, B of AB, *J* = 12.3 Hz, 2H, H-1′), 4.81 (m, 1H, H-1), 5.52 (s, 2H, CH_2Ar), 6.85 (s, 3H, C_6H_4), 7.24 (t, *J* = 7.9 Hz, 1H, C_6H_4), 7.98 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 55.5 (CH_2Ar), 55.9 (OCH_3), 60.8 (C-1′), 63.0 (C-6), 68.7 (C-4), 72.1 (C-2), 72.6 (C-3), 75.1 (C-5), 100.9 (C-1), 114.9, 115.2, 121.4 (3C, C_6H_4), 125.6 (C_2N_3H–C5), 131.3 (C_6H_4), 138.2 (C_6H_4–C1), 145.8 (C_2N_3H–C4), 161.7 (C_6H_4–C3); HR-MS Calcd for $C_{17}H_{23}N_3NaO_7$ $[M+Na]^+$: 404.1434, Found: 404.1435.

4.1.7.9. [1-(4′-Nitrophenyl)-1,2,3-triazol-4-yl]methyl α-D-mannopyranoside (5i). Following general procedure A, **14** (40 mg, 0.18 mmol) was reacted with 1-azido-4-nitrobenzene (**15i**,[40] 44 mg, 0.27 mmol), $CuSO_4$ (11 mg, 45 μmol) and sodium ascorbate (18 mg, 90 μmol) to yield **5i** (31 mg, 44%).

$[\alpha]_D$ +50.4 (*c* 1.02, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.56–3.60 (m, 2H, H-4, H-5), 3.64–3.72 (m, 2H, H-3, H-6a), 3.80 (dd, *J* = 1.7, 3.3 Hz, 1H, H-2), 3.84 (dd, *J* = 1.0, 11.7 Hz, 1H, H-66), 4.75 (A of AB, *J* = 12.5 Hz, 1H, H-1′a), 4.88–4.91 (m, 2H, H-1, H-1′b), 8.16 (m, 2H, C_6H_4), 8.44 (m, 2H, C_6H_4), 8.75 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 60.9 (C-1′), 63.2 (C-6), 68.8 (C-4), 72.1 (C-2), 72.6 (C-3), 75.3 (C-5), 101.2 (C-1), 122.0 (2C, C_6H_4), 123.9 (C_2N_3H–C5), 126.7 (2C, C_6H_4), 142.7 (C_6H_4–C1), 147.1 (C_2N_3H–C4), 148.9 (C_6H_4–C4); HR-MS Calcd for $C_{15}H_{18}N_4NaO_8$ $[M+Na]^+$: 405.1022, Found: 405.1020.

4.1.7.10. [1-(Pyridin-4′-yl)-1,2,3-triazol-4-yl]methyl α-D-mannopyranoside (5j). Prepared from **16j** (102 mg, 0.20 mmol) according to general procedure D. Yield: 58 mg, 85%.

$[\alpha]_D$ +70.3 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.53–3.63 (m, 2H, H-4, H-5), 3.64–3.74 (m, 2H, H-3, H-6a), 3.77–3.87 (m, 2H, H-2, H-6b), 4.73 (A of AB, *J* = 12.6 Hz, 1H, H-1′a), 4.84–4.92 (m, 2H, H-1, H-1′b), 7.96, 8.67 (m, 4H, C_5H_4N), 8.77 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 60.9 (C-1′), 63.1 (C-6), 68.7 (C-4), 72.1 (C-2), 72.6 (C-3), 75.2 (C-5), 101.2 (C-1), 115.5 (2C, C_5H_4N), 123.5 (C_2N_3H–C5), 145.2 (C_2N_3H–C4), 147.2 (C_5H_4N–C1), 152.5 (2C, C_5H_4N); HR-MS Calcd for $C_{14}H_{19}N_4O_6$ $[M+H]^+$: 339.1305, Found: 339.1302.

4.1.7.11. [1-(4′-Fluorophenyl)-1,2,3-triazol-4-yl]methyl α-D-mannopyranoside (5k). Prepared from **16k** (106 mg, 0.20 mmol) according to general procedure D. Yield: 56 mg, 78%.

$[\alpha]_D$ +78.5 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.60–3.69 (m, 2H, H-4, H-5), 3.71–3.78 (m, 2H, H-3, H-6a), 3.86 (dd, *J* = 1.7, 3.4 Hz, 1H, H-2), 3.89 (dd, *J* = 1.8, 11.8 Hz, 1H, H-6b), 4.77, 4.91 (A, B of AB, *J* = 12.5 Hz, 2H, H-1′), 4.94 (d, *J* = 1.6 Hz, 1H, H-1), 7.34, 7.89 (m, 4H, C_6H_4), 8.56 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 60.9 (C-1′), 63.2 (C-6), 68.8 (C-4), 72.2 (C-2), 72.6 (C-3), 75.2 (C-5), 101.1 (C-1), 117.8 (d, *J* = 23.8 Hz, 2C, C_6H_4), 124.0 (2C, C_6H_4), 124.0 (C_2N_3H–C5), 134.9 (d, *J* = 3.8 Hz, C_6H_4–C1), 146.5 (C_2N_3H–C4), 164.1 (d, *J* = 246.3 Hz, C_6H_4–C4); HR-MS Calcd for $C_{15}H_{18}FN_3NaO_6$ $[M+Na]^+$: 378.1077, Found: 378.1079.

4.1.7.12. [1-(3′-Fluorophenyl)-1,2,3-triazol-4-yl]methyl α-D-mannopyranoside (5l). Prepared from **16l** (105 mg, 0.20 mmol) according to general procedure D. Yield: 58 mg, 81%.

$[\alpha]_D$ +73.8 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.51–3.62 (m, 2H, H-4, H-5), 3.62–3.73 (m, 2H, H-3, H-6a), 3.78 (dd, *J* = 1.6, 3.1 Hz, 1H, H-2), 3.84 (m, 1H, H-6b), 4.71 (A of AB, *J* = 12.4 Hz, 1H, H-1′a), 4.82–4.89 (m, 2H, H-1, H-1′b), 7.23 (m, 1H, C_6H_4), 7.57 (m, 1H, C_6H_4), 7.64–7.74 (m, 2H, C_6H_4), 8.61 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 60.8 (C-1′), 63.1 (C-6), 68.8 (C-4), 72.1 (C-2), 72.6 (C-3), 75.2 (C-5), 101.1 (C-1), 109.1 (d, *J* = 26.3 Hz, C_6H_4), 116.9 (d, *J* = 21.3 Hz, C_6H_4), 117.3 (d, *J* = 3.8 Hz, C_6H_4), 123.8 (C_2N_3H–C5), 132.9 (d, *J* = 10.0 Hz, C_6H_4), 139.7 (d, *J* = 10.0 Hz, C_6H_4–C1), 146.6 (C_2N_3H–C4), 161.7 (d, *J* = 245.0 Hz, C_6H_4–C3); HR-MS Calcd for $C_{15}H_{18}FN_3NaO_6$ $[M+Na]^+$: 378.1077, Found: 378.1081.

4.1.7.13. [1-(4′-Methoxyphenyl)-1,2,3-triazol-4-yl]methyl α-D-mannopyranoside (5m). Prepared from **16m** (113 mg, 0.21 mmol) according to general procedure D. Yield: 58 mg, 75%.

$[\alpha]_D$ +37.4 (*c* 1.01, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.54–3.64 (m, 2H, H-4, H-5), 3.65–3.74 (m, 2H, H-3, H-6a), 3.80 (dd, *J* = 1.6, 3.2 Hz, 1H, H-2), 3.81–3.87 (m, 4H, H-6b, OCH_3), 4.69, 4.83 (A, B of AB, *J* = 12.4 Hz, 2H, H-1′), 4.87 (m, 1H, H-1), 7.05, 7.68 (AA′, BB′ of AA′BB′, *J* = 9.0 Hz, 4H, C_6H_4), 8.42 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 56.3 (OCH_3), 60.8 (C-1′), 63.1 (C-6), 68.8 (C-4), 72.2 (C-2), 72.6 (C-3), 75.2 (C-5), 101.0 (C-1), 116.0 (2C, C_6H_4), 123.4 (2C, C_6H_4), 123.8 (C_2N_3H–C5), 131.7 (C_6H_4–C1), 146.1 (C_2N_3H–C4), 161.7 (C_6H_4–C4); HR-MS Calcd for $C_{16}H_{21}N_3NaO_7$ $[M+Na]^+$: 390.1277, Found: 390.1279.

4.1.7.14. [1-(3′-Methoxybenzyl)-1,2,3-triazol-4-yl]ethyl α-D-mannopyranoside (6h). Prepared from **17h** (53 mg, 94 μmol) according to general procedure D. Yield: 30 mg, 81%.

$[\alpha]_D$ +45.9 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 2.99 (t, *J* = 6.6 Hz, 2H, H-2′), 3.41 (m, 1H, H-5), 3.57–3.75 (m, 4H, H-3, H-4, H-6a, H-1′a), 3.71 (dd, *J* = 1.7, 3.1 Hz, 1H, H-2), 3.78–3.83 (m, 4H, H-6b, OCH_3), 3.97 (dt, *J* = 6.7, 9.7 Hz, 1H, H-1′b), 4.77 (d, *J* = 1.6 Hz, 1H, H-1), 5.54 (s, 2H, CH_2Ar), 6.80–6.96, 7.29 (m, 4H, C_6H_4), 7.79 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 27.2 (C-2′), 54.9 (CH_2Ar), 55.9 (OCH_3), 63.0 (C-6), 67.6 (C-1′), 68.7 (C-4), 72.2 (C-2), 72.7 (C-3), 74.9 (C-5), 101.7 (C-1), 114.8, 115.1, 121.2 (3C, C_6H_4), 124.2 (C_2N_3H–C5), 131.3 (C_6H_4), 138.4 (C_6H_4–C1), 146.7 (C_2N_3H–C4), 161.7 (C_6H_4–C3); HR-MS Calcd for $C_{18}H_{25}N_3NaO_7$ $[M+Na]^+$: 418.1590, Found: 418.1591.

4.1.7.15. [1-(4′-Nitrophenyl)-1,2,3-triazol-4-yl]ethyl α-D-mannopyranoside (6i). Prepared from **17i** (61 mg, 0.11 mmol) according to general procedure D. Yield: 37 mg, 86%.

$[\alpha]_D$ +44.4 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.13 (t, *J* = 6.5 Hz, 2H, H-2′), 3.42 (m, 1H, H-5), 3.60 (t, *J* = 9.5 Hz, 1H, H-4), 3.64–3.73 (m, 2H, H-3, H-6a), 3.78–3.88 (m, 3H, H-2, H-6b, H-1′a), 4.09 (dt, *J* = 6.6, 9.8 Hz, 1H, H-1′b), 4.83 (d, *J* = 1.5 Hz, 1H, H-1), 8.18, 8.48 (m, 4H, C_6H_4), 8.55 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 27.3 (C-2′), 63.1 (C-6), 67.4 (C-1′), 68.7 (C-4), 72.2 (C-2), 72.8 (C-3), 75.0 (C-5), 101.8 (C-1), 122.0 (2C, C_6H_4), 122.5 (C_2N_3H–C5), 126.6 (2C, C_6H_4), 142.8 (C_6H_4–C1), 148.1 (C_2N_3H–C4), 148.9 (C_6H_4–C4); HR-MS Calcd for $C_{16}H_{20}N_4NaO_8$ $[M+Na]^+$: 419.1179, Found: 419.1177.

4.1.7.16. [1-(Pyridin-4′-yl)-1,2,3-triazol-4-yl]ethyl α-D-mannopyranoside (6j). Prepared from **17j** (63 mg, 0.12 mmol) according to general procedure D. Yield: 31 mg, 73%.

$[\alpha]_D$ +48.3 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.12 (t, *J* = 6.5 Hz, 2H, H-2′), 3.43 (m, 1H, H-5), 3.61 (t, *J* = 9.5 Hz, 1H, H-4),

3.64–3.73 (m, 2H, H-3, H-6a), 3.77–3.87 (m, 3H, H-2, H-6b, H-1′a), 4.08 (dt, J = 6.6, 9.8 Hz, 1H, H-1′b), 4.83 (d, J = 1.5 Hz, 1H, H-1), 7.99 (dd, J = 1.6, 4.8 Hz, 2H, C_5H_4N), 8.58 (s, 1H, C_2N_3H), 8.74 (m, 2H, C_5H_4N); ^{13}C NMR (125 MHz, CD_3OD): δ 27.2 (C-2′), 63.1 (C-6), 67.3 (C-1′), 68.7 (C-4), 72.2 (C-2), 72.7 (C-3), 75.0 (C-5), 101.8 (C-1), 115.5 (2C, C_5H_4N), 122.0 (C_2N_3H–C5), 145.3 (C_5H_4N–C1), 148.1 (C_2N_3H–C4), 152.4 (2C, C_5H_4N); HR-MS Calcd for $C_{15}H_{21}N_4O_6$ $[M+H]^+$: 353.1461, Found: 353.1460.

4.1.7.17. [1-(4′-Fluorophenyl)-1,2,3-triazol-4-yl]ethyl α-D-mannopyranoside (6k). Prepared from **17k** (65 mg, 0.12 mmol) according to general procedure D. Yield: 40 mg, 90%.

$[\alpha]_D$ +50.7 (c 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.10 (t, J = 6.5 Hz, 2H, H-2′), 3.43 (m, 1H, H-5), 3.61 (t, J = 9.5 Hz, 1H, H-4), 3.65–3.74 (m, 2H, H-3, H-6a), 3.77–3.87 (m, 3H, H-2, H-6b, H-1′a), 4.07 (dt, J = 6.6, 9.8 Hz, 1H, H-1′b), 4.82 (d, J = 1.6 Hz, 1H, H-1), 7.34, 7.88 (m, 4H, C_6H_4), 8.33 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 27.2 (C-2′), 63.1 (C-6), 67.5 (C-1′), 68.7 (C-4), 72.2 (C-2), 72.8 (C-3), 75.0 (C-5), 101.8 (C-1), 117.8 (d, J = 23.8 Hz, 2C, C_6H_4), 122.6 (C_2N_3H–C5), 124.0 (d, J = 8.8 Hz, 2C, C_6H_4), 135.0 (d, J = 2.5 Hz, C_6H_4–C1), 147.4 (C_2N_3H–C4), 164.1 (d, J = 246.3 Hz, C_6H_4–C4); HR-MS Calcd for $C_{16}H_{20}FN_3NaO_6$ $[M+Na]^+$: 392.1234, Found: 392.1238.

4.1.7.18. 1-(α-D-Mannopyranosyl)-4-phenyl-1,2,3-triazole (7n). Prepared from **20n** (50 mg, 0.11 mmol) according to general procedure D. Yield: 29 mg, 89%.

$[\alpha]_D$ +98.0 (c 1.34, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.38 (ddd, J = 2.5, 6.6, 8.9 Hz, 1H, H-5), 3.76–3.80 (m, 2H, H-4, H-6a), 3.85 (dd, J = 2.5, 12.1 Hz, 1H, H-6b), 4.12 (dd, J = 3.5, 8.5 Hz, 1H, H-3), 4.76 (t, J = 3.1 Hz, 1H, H-2), 6.08 (d, J = 2.7 Hz, 1H, H-1), 7.34–7.38, 7.43–7.46, 7.84–7.85 (m, 5H, C_6H_5), 8.51 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 62.6 (C-6), 68.7 (C-4), 70.1 (C-2), 72.6 (C-3), 78.7 (C-5), 88.5 (C-1), 122.1 (C_2N_3H–C5), 126.8, 129.5, 130.0, 131.4 (6C, C_6H_5), 149.0 (C_2N_3H–C4); HR-MS Calcd for $C_{14}H_{17}N_3NaO_5$ $[M+Na]^+$: 330.1066, Found: 330.1060.

4.1.7.19. 1-(α-D-Mannopyranosyl)-4-(4-methylphenyl)-1,2,3-triazole (7o). Prepared from **20o** (46 mg, 94 μmol) according to general procedure D. Yield: 20 mg, 65%.

$[\alpha]_D$ +84.6 (c 0.63, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 2.36 (s, 3H, PhCH_3), 3.36 (ddd, J = 2.3, 6.7, 8.9 Hz, 1H, H-5), 3.74–3.78 (m, 2H, H-4, H-6a), 3.84 (dd, J = 2.4, 12.1 Hz, 1H, H-6b), 4.10 (dd, J = 3.5, 8.5 Hz, 1H, H-3), 4.74 (t, J = 3.0 Hz, 1H, H-2), 6.05 (d, J = 2.6 Hz, 1H, H-1), 7.25, 7.72 (AA′, BB′ of AA′BB′, J = 8.0 Hz, 4H, C_6H_4), 8.45 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 21.3 (PhCH_3), 62.6 (C-6), 68.7 (C-4), 70.1 (C-2), 72.6 (C-3), 78.7 (C-5), 88.5 (C-1), 121.7 (C_2N_3H–C5), 126.7, 128.6, 130.6, 139.6 (6C, C_6H_5), 149.2 (C_2N_3H–C4); HR-MS Calcd for $C_{15}H_{18}N_3NaO_5$ $[M+Na]^+$: 344.1222, Found: 344.1215.

4.1.7.20. 4-(3-Chlorophenyl)-1-(α-D-mannopyranosyl)-1,2,3-triazole (7p). Prepared from **20p** (43 mg, 84 μmol) according to general procedure D. Yield: 25 mg, 87%.

$[\alpha]_D$ +89.2 (c 0.50, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.37 (ddd, J = 2.4, 6.7, 8.7 Hz, 1H, H-5), 3.77 (dd, J = 6.6, 12.2 Hz, 1H, H-6a), 3.78 (t, J = 8.6 Hz, 1H, H-4), 3.87 (dd, J = 2.4, 12.1 Hz, 1H, H-6b), 4.11 (dd, J = 3.5, 8.3 Hz, 1H, H-3), 4.75 (t, J = 3.1 Hz, 1H, H-2), 6.07 (d, J = 2.6 Hz, 1H, H-1), 7.37 (d, J = 8.1 Hz, 1H, C_6H_4-H6), 7.44 (t, J = 7.9 Hz, 1H, C_6H_4–H5), 7.79 (d, J = 7.7 Hz, 1H, C_6H_4–H4), 7.90 (s, 1H, C_6H_4-H2), 8.58 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 62.6 (C-6), 68.7 (C-4), 70.1 (C-2), 72.6 (C-3), 78.7 (C-5), 88.6 (C-1), 122.7 (C_2N_3H–C5), 125.0, 126.6, 129.4, 131.6, 133.5, 136.0 (C_6H_4), 147.7 (C_2N_3H–C4); HR-MS Calcd for $C_{14}H_{16}ClN_3NaO_5$ $[M+Na]^+$: 364.0676, Found: 364.0676.

4.1.7.21. 4-(4-Trifluoromethylphenyl)-1-(α-D-mannopyranosyl)-1,2,3-triazole (7q). Prepared from **20q** (46 mg, 85 μmol) according to general procedure D. Yield: 27 mg, 86%.

$[\alpha]_D$ +83.4 (c 0.34, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.38 (m, 1H, H-5), 3.77–3.81 (m, 2H, H-4, H-6a), 3.85 (dd, J = 1.9, 12.0 Hz, 1H, H-6b), 4.11 (dd, J = 3.3, 8.4 Hz, 1H, H-3), 4.76 (t, J = 2.7 Hz, 1H, H-2), 6.10 (d, J = 2.3 Hz, 1H, H-1), 7.76, 8.06 (AA′, BB′ of AA′BB′, J = 8.0 Hz, 4H, C_6H_4), 8.66 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 62.6 (C-6), 68.7 (C-4), 70.1 (C-2), 72.6 (C-3), 78.8 (C-5), 88.6 (C-1), 123.2 (C_2N_3H–C5), 125.6 (q, J = 272 Hz, CF_3), 127.0 (q, J = 3.7 Hz, 2C, C_6H_4–C3, C5), 127.2 (2C, C_6H_4–C2, C6), 131.2 (d, J = 32.4 Hz, C_6H_4–C4), 135.5 (C_6H_4–C1), 147.6 (C_2N_3H–C4); HR-MS Calcd for $C_{15}H_{16}F_3N_3NaO_5$ $[M+Na]^+$: 398.0940, Found: 398.0942.

4.1.7.22. 1-(α-D-Mannopyranosyl)-4-(3-pyridyl)-1,2,3-triazole (7r). Prepared from **20r** (47 mg, 98 μmol) according to general procedure D. Yield: 28 mg, 92%.

$[\alpha]_D$ +86.7 (c 0.93, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.40 (ddd, J = 2.3, 6.6, 8.6 Hz, 1H, H-5), 3.77–3.82 (m, 2H, H-4, H-6a), 3.86 (dd, J = 2.4, 12.1 Hz, 1H, H-6b), 4.11 (dd, J = 3.5, 8.4 Hz, 1H, H-3), 4.76 (t, J = 3.1 Hz, 1H, H-2), 6.11 (d, J = 2.7 Hz, 1H, H-1), 7.54 (dd, J = 5.0, 7.8 Hz, 1H, C_5H_4N-H5), 8.31 (m, 1H, C_5H_4N-H6), 8.53 (dd, J = 1.4, 4.9 Hz, 1H, C_5H_4N-H4), 8.68 (s, 1H, C_2N_3H), 9.04 (d, J = 1.5, 1H, C_5H_4N–H2); ^{13}C NMR (125 MHz, CD_3OD): δ 62.6 (C-6), 68.7 (C-4), 70.1 (C-2), 72.6 (C-3), 78.8 (C-5), 88.6 (C-1), 123.1 (C_2N_3H–C5), 125.6 (C_5H_4N–C5), 128.5 (C_5H_4N–C1), 135.0 (C_5H_4N–C6), 145.6 (C_2N_3H–C4), 147.3 (C_5H_4N–C2), 149.7 (C_5H_4N–C4); HR-MS Calcd for $C_{13}H_{16}N_4NaO_5$ $[M+Na]^+$: 331.1018, Found: 331.1013.

4.1.7.23. 1-(α-D-Mannopyranosyl)-4-phenoxymethyl-1,2,3-triazole (7s). Prepared from **20s** (46 mg, 90 μmol) according to general procedure D. Yield: 27 mg, 89%.

$[\alpha]_D$ +57.0 (c 0.90, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.30 (m, 1H, H-5), 3.70–3.74 (m, 2H, H-4, H-6a), 3.78 (dd, J = 1.8, 12.1 Hz, 1H, H-6b), 4.04 (dd, J = 3.2, 8.4 Hz, 1H, H-3), 4.67 (m, 1H, H-2), 5.14 (s, 2H, CH_2OPh), 6.00 (d, J = 1.7 Hz, 1H, H-1), 6.91 (t, J = 7.3 Hz, 1H, C_6H_5-H4), 6.96 (d, J = 8.1 Hz, 2H, C_6H_5-H2, H6), 7.24 (t, J = 7.8 Hz, 2H, C_6H_5-H3, H5), 8.20 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 62.2 (CH_2OPh), 62.5 (C-6), 68.6 (C-4), 70.1 (C-2), 72.5 (C-3), 78.6 (C-5), 88.4 (C-1), 115.8 (2C, C_6H_5–C2, C6), 122.3 (C_6H_5–C4), 125.3 (C_2N_3H–C5), 130.5 (2C, C_6H_5–C3, C5), 145.4 (C_2N_3H–C4), 159.7 (C_6H_5–C1); HR-MS Calcd for $C_{15}H_{19}N_3NaO_5$ $[M+Na]^+$: 360.1172, Found: 360.1171.

4.1.7.24. 1-(α-D-Mannopyranosyl)methyl-4-phenyl-1,2,3-triazole (8n). Prepared from **25n** (38 mg, 78 μmol) according to general procedure D. Yield: 22 mg, 87%.

$[\alpha]_D$ +30.6 (c 0.91, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.73–3.75 (m, 2H, H-4, H-6a), 3.79–3.85 (m, 3H, H-2, H-3, H-5), 3.88 (dd, J = 7.2, 11.5 Hz, 1H, H-6b), 4.25 (dt, J = 4.8, 7.9 Hz, 1H, H-1), 4.73 (dd, J = 8.0, 14.4 Hz, 1H, H-1′a), 4.76 (dd, J = 4.5, 14.3 Hz, 1H, H-1′b), 7.33 (t, J = 7.7 Hz, 1H, C_6H_5-H4), 7.42 (t, J = 7.8 Hz, 2H, C_6H_5-H3, H5), 7.81 (d, J = 8.0 Hz, 2H, C_6H_5-H2, H6), 8.45 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 50.9 (C-1′), 62.1 (C-6), 69.1 (C-2), 70.0 (C-4), 72.5 (C-3), 74.9 (C-1), 78.5 (C-5), 123.4 (C_2N_3H–C5), 126.7, 129.3, 129.9, 131.8 (6C, C_6H_5), 148.8 (C_2N_3H–C4); HR-MS Calcd for $C_{15}H_{19}NaN_3O_5$ $[M+Na]^+$: 344.1222, Found: 344.1222.

4.1.7.25. 1-(α-D-Mannopyranosyl)methyl-4-(4-methylphenyl)-1,2,3-triazole (8o). Prepared from **25o** (42 mg, 84 μmol) according to general procedure D. Yield: 25 mg, 87%.

$[\alpha]_D$ +33.8 (c 1.12, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 2.35 (PhCH_3), 3.72–3.76 (m, 2H, H-4, H-6a), 3.80 (dt, J = 3.2, 7.1 Hz, 1H, H-5), 3.81–3.85 (m, 2H, H-2, H-3), 3.87 (dd, J = 7.1, 11.5 Hz,

1H, H-6b), 4.24 (dt, J = 5.1, 7.4 Hz, 1H, H-1), 4.72–4.75 (m, 2H, H-1′), 7.24, 7.69 (AA′, BB′ of AA′BB′, J = 8.0 Hz, 4H, C_6H_4), 8.40 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 21.3 (Ph*C*H$_3$), 50.8 (C-1′), 62.0 (C-6), 69.1 (C-2), 69.9 (C-4), 72.4 (C-3), 75.0 (C-1), 78.4 (C-5), 123.0 (C_2N_3H–C5), 126.6, 128.9, 130.5, 139.3 (6C, C_6H_5), 148.9 (C_2N_3H–C4); HR-MS Calcd for $C_{16}H_{21}NaN_3O_5$ [M+Na]$^+$: 358.1379, Found: 358.1380.

4.1.7.26. 4-(3-Chlorophenyl)-1-(α-D-mannopyranosyl)methyl-1,2,3-triazole (8p). Prepared from **25p** (40 mg, 77 μmol) according to general procedure D. Yield: 23 mg, 83%.

[α]$_D$ +31.5 (*c* 1.05, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.73 (m, 1H, H-4), 3.74 (dd, J = 3.0, 11.5 Hz, 1H, H-6a), 3.79–3.82 (m, 2H, H-3, H-5), 3.83 (dd, J = 3.4, 8.7 Hz, 1H, H-2), 3.89 (dd, J = 7.4, 11.6 Hz, 1H, H-6b), 4.24 (dt, J = 4.7, 7.9 Hz, 1H, H-1), 4.73 (dd, J = 8.0, 14.4 Hz, 1H, H-1′a), 4.77 (dd, J = 4.4, 14.5 Hz, 1H, H-1′b), 7.33 (dd, J = 0.9, 8.1 Hz, 1H, C_6H_4-H6), 7.41 (t, J = 7.9 Hz, 1H, C_6H_4–H5), 7.74 (d, J = 7.8 Hz, 1H, C_6H_4–H4), 7.90 (t, J = 1.6 Hz, 1H, C_6H_4-H2), 8.51 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 50.9 (C-1′), 62.0 (C-6), 69.0 (C-2), 70.0 (C-4), 72.4 (C-3), 74.7 (C-1), 78.5 (C-5), 124.0 (C_2N_3H–C5), 124.9, 126.5, 129.1, 131.5, 133.9, 135.9 (6C, C_6H_4), 147.4 (C_2N_3H–C4); HR-MS Calcd for $C_{15}H_{18}ClNaN_3O_5$ [M+Na]$^+$: 378.0833, Found: 378.0833.

4.1.7.27. 4-(4-Trifluoromethylphenyl)-1-(α-D-mannopyranosyl)methyl-1,2,3-triazole (8q). Prepared from **25q** (47 mg, 84 μmol) according to general procedure D. Yield: 28 mg, 86%.

[α]$_D$ +32.6 (*c* 1.03, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.73–3.76 (m, 2H, H-4, H-6a), 3.81–3.86 (m, 3H, H-2, H-3, H-5), 3.89 (dd, J = 7.5, 11.5 Hz, 1H, H-6b), 4.25 (dt, J = 4.8, 7.9 Hz, 1H, H-1), 4.76 (dd, J = 8.0, 14.5 Hz, 1H, H-1′a), 4.79 (dd, J = 4.4, 14.5 Hz, 1H, H-1′b), 7.72, 8.01 (AA′, BB′ of AA′BB′, J = 8.2 Hz, 4H, C_6H_4), 8.60 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 51.0 (C-1′), 62.0 (C-6), 69.0 (C-2), 70.0 (C-4), 72.4 (C-3), 74.7 (C-1), 78.6 (C-5), 124.5 (C_2N_3H–C5), 125.6 (q, J = 271 Hz, CF_3), 126.9 (q, J = 3.7 Hz, 2C, C_6H_4–C3, C5), 127.0 (2C, C_6H_4–C2, C6), 130.9 (q, J = 32.4 Hz, C_6H_4–C4), 135.7 (C_6H_4–C1), 147.2 (C_2N_3H–C4); HR-MS Calcd for $C_{16}H_{18}F_3NaN_3O_5$ [M+Na]$^+$: 412.1096, Found: 412.1095.

4.1.7.28. 1-(α-D-Mannopyranosyl)methyl-4-(3-pyridyl)-1,2,3-triazole (8r). Prepared from **25r** (44 mg, 90 μmol) according to general procedure D. Yield: 24 mg, 83%.

[α]$_D$ +31.2 (*c* 0.99, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.71–3.74 (m, 2H, H-4, H-6a), 3.80–3.83 (m, 3H, H-2, H-3, H-5), 3.89 (dd, J = 7.7, 11.6 Hz, 1H, H-6b), 4.23 (dt, J = 4.6, 8.6 Hz, 1H, H-1), 4.76 (dd, J = 8.4, 14.4 Hz, 1H, H-1′a), 4.80 (dd, J = 4.2, 14.4 Hz, 1H, H-1′b), 7.53 (dd, J = 5.0, 7.9 Hz, 1H, C_5H_4N-H5), 8.28 (d, J = 8.0 Hz, 1H, C_5H_4N-H6), 8.51 (d, J = 4.8 Hz, 1H, C_5H_4N-H4), 8.63 (s, 1H, C_2N_3H), 9.02 (s, 1H, C_5H_4N–H2); ^{13}C NMR (125 MHz, CD_3OD): δ 48.1 (C-1′), 60.6 (C-6), 67.2 (C-2), 68.5 (C-4), 70.6 (C-3), 75.1 (C-1), 76.2 (C-5), 123.2 (C_2N_3H–C5), 124.5 (C_5H_4N–C5), 126.82 (C_5H_4N–C1), 134.3 (C_5H_4N–C6), 144.4 (C_2N_3H–C4), 145.7 (C_5H_4N–C2), 148.4 (C_5H_4N–C4); HR-MS Calcd for $C_{14}H_{18}NaN_4O_5$ [M+Na]$^+$: 345.1175, Found: 345.1175.

4.1.7.29. 1-(α-D-Mannopyranosyl)methyl-4-phenoxymethyl-1,2,3-triazole (8s). Prepared from **25s** (41 mg, 79 μmol) according to general procedure D. Yield: 23 mg, 83%.

[α]$_D$ +22.8 (*c* 1.01, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.69–3.76 (m, 3H, H-4, H-5, H-6a), 3.79–3.82 (m, 2H, H-2, H-3), 3.83 (dd, J = 6.5, 11.5 Hz, 1H, H-6b), 4.19 (dt, J = 5.0, 7.0 Hz, 1H, H-1), 4.69 (dd, J = 7.5, 14.5 Hz, 1H, H-1′a), 4.72 (dd, J = 5.0, 14.5 Hz, 1H, H-1′b), 5.15 (s, 2H, C*H*$_2$OPh), 6.94 (t, J = 7.4 Hz, 1H, C_6H_5-H4), 7.00 (d, J = 8.1 Hz, 2H, C_6H_5-H2, H6), 7.27 (m, 2H, C_6H_5-H3, H5), 8.17 (s, 1H, C_2N_3H); ^{13}C NMR (125 MHz, CD_3OD): δ 50.9 (C-1′), 62.0 (C-6), 62.3 (*C*H$_2$OPh), 69.0 (C-2), 69.8 (C-4), 72.4 (C-3), 74.9 (C-1), 78.4 (C-5), 115.9 (2C, C_6H_5–C2, C6), 122.2 (C_6H_5–C4), 126.4 (C_2N_3H–C5), 130.5 (2C, C_6H_5–C3, C5), 145.0 (C_2N_3H–C4), 159.8 (C_6H_5–C1); HR-MS Calcd for $C_{16}H_{21}N_3NaO_5$ [M+Na]$^+$: 374.1328, Found: 374.1328.

4.2. Biological evaluation

4.2.1. Competitive binding assay

A recombinant protein consisting of the CRD of FimH linked with a thrombin cleavage site to a 6His-tag (FimH-CRD-Th-6His) was expressed in *E. coli* strain HM125 and purified by affinity chromatography.[45] To determine the affinity of the various FimH antagonists, an competitive binding assay described previously[45] was applied. Microtiter plates (F96 MaxiSorp, Nunc) were coated with 100 μL/well of a 10 μg/mL solution of FimH-CRD-Th-6His in 20 mM HEPES, 150 mM NaCl and 1 mM $CaCl_2$, pH 7.4 (assay buffer) overnight at 4 °C. The coating solution was discarded and the wells were blocked with 150 μL/well of 3% BSA in assay buffer for 2 h at 4 °C. After three washing steps with assay buffer (150 μL/well), a four-fold serial dilution of the test compound (50 μL/well) in assay buffer containing 5% DMSO and streptavidin-peroxidase coupled TM-PAA polymer (50 μL/well of a 0.5 μg/mL solution) were added. On each individual microtiter plate *n*-heptyl α-D-mannopyranoside (**1b**) was tested in parallel. The plates were incubated for 3 h at 25 °C and 350 rpm and then carefully washed four times with 150 μL/well assay buffer. After the addition of 100 μL/well of ABTS-substrate, the colorimetric reaction was allowed to develop for 4 min, then stopped by the addition of 2% aqueous oxalic acid before the optical density (OD) was measured at 415 nm on a microplate-reader (Spectramax 190, Molecular Devices, California, USA). The IC_{50} values of the compounds tested in duplicates were calculated with prism software (GraphPad Software, Inc., La Jolla, USA). The IC_{50} defines the molar concentration of the test compound that reduces the maximal specific binding of TM-PAA polymer to FimH-CRD by 50%. The relative IC_{50} (rIC_{50}) is the ratio of the IC_{50} of the test compound to the IC_{50} of **1b**.

4.2.2. Aggregometry assay

The aggregometry assay was carried out as previously described.[46] In short, the percentage of aggregation of *E. coli* UTI89[59] (UTI89wt) with guinea pig erythrocytes (GPE) was quantitatively determined by measuring the optical density at 740 nm and 37 °C under stirring at 1000 rpm using an APACT 4004 aggregometer (Endotell AG, Allschwil, Switzerland). GPE were separated from guinea pig blood (Charles River Laboratories, Sulzfeld, Germany) using Histopaque (density of 1.077 g/mL at 24 °C, Sigma-Aldrich, Buchs, Switzerland). Prior to the measurements, the cell densities of *E. coli* and GPE were adjusted to an OD_{600} of 4, corresponding to 1.9×10^8 CFU/mL and 2.2×10^6 cells/mL respectively. For the calibration of the instrument, the aggregation of protein poor plasma (PPP) using PBS alone was set as 100% and the aggregation of protein rich plasma (PRP) using GPE as 0%. After calibration, measurements were performed with 250 μL GPE and 50 μL bacterial suspension and the aggregation monitored over 600 s. After the aggregation phase of 600 s, 25 μL of antagonist in PBS were added to each cuvette and disaggregation was monitored for 1400 s. UTI89 Δ*fimA-H* was used as negative control.

4.3. Determination of the pharmacokinetic parameters

4.3.1. Materials

Dimethyl sulfoxide (DMSO) and 1-octanol were purchased from Sigma-Aldrich (St. Louis MI, USA). PAMPA System Solution, GIT-0 Lipid Solution, and Acceptor Sink Buffer were ordered from pIon (Woburn MA, USA). Acetonitrile (MeCN) was bought from Acros Organics (Geel, Belgium).

4.3.2. LC–MS measurements

Analyses were performed using an Agilent 1100/1200 Series HPLC System coupled to a 6410 Triple Quadrupole mass detector (Agilent Technologies, Inc., Santa Clara CA, USA) equipped with electrospray ionization. The system was controlled with the Agilent MassHunter Workstation Data Acquisition software (version B.01.04). The column used was an Atlantis® T3 C18 column (2.1 x 50 m) with a 3 μm particle size (Waters Corp., Milford MA, USA). The mobile phase consisted of two eluents: solvent A (H_2O, containing 0.1% formic acid, v/v) and solvent B (MeCN, containing 0.1% formic acid, v/v), both delivered at 0.6 mL/min. The gradient was ramped from 95% A/5% B to 5% A/95% B over 1 min, and then hold at 5% A/95% B for 0.1 min. The system was then brought back to 95% A/5% B, resulting in a total duration of 4 min. MS parameters such as fragmentor voltage, collision energy and polarity were optimized individually for each compound, and the molecular ion was followed for each compound in the multiple reaction monitoring mode. The concentrations of the analytes were quantified by the Agilent Mass Hunter Quantitative Analysis software (version B.01.04).

4.3.3. log $D_{7.4}$ determination

The *in silico* prediction tool ALOGPS[60] was used to estimate the log *P* values. Depending on these values, the compounds were classified into three categories: hydrophilic compounds (log *P* below zero), moderately lipophilic compounds (log *P* between zero and one) and lipophilic compounds (log *P* above one). For each category, two different ratios (volume of 1-octanol to volume of buffer) were defined as experimental parameters:

Compound type	log *P*	Ratios (1-octanol:buffer)
Hydrophilic	<0	30:140, 40:130
Moderately lipophilic	0–1	70:110, 110:70
Lipophilic	>1	3:180, 4:180

Equal amounts of phosphate buffer (0.1 M, pH 7.4) and 1-octanol were mixed and shaken vigorously for 5 min to saturate the phases. The mixture was left until separation of the two phases occurred, and the buffer was retrieved. Stock solutions of the test compounds were diluted with buffer to a concentration of 1 μM. For each compound, six determinations, that is, three determinations per 1-octanol : buffer ratio, were performed in different wells of a 96-well plate. The respective volumes of buffer containing analyte (1 μM) were pipetted to the wells and covered by saturated 1-octanol according to the chosen volume ratio. The plate was sealed with aluminium foil, shaken (1350 rpm, 25 °C, 2 h) on a Heidoph Titramax 1000 plate-shaker (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) and centrifuged (2000 rpm, 25 °C, 5 min, 5804 R Eppendorf centrifuge, Hamburg, Germany). The aqueous phase was transferred to a 96-well plate for analysis by liquid chromatography-mass spectrometry (LC-MS).

log $D_{7.4}$ was calculated from the 1-octanol : buffer ratio (o:b), the initial concentration of the analyte in buffer (1 μM), and the concentration of the analyte in buffer (c_B) with equilibration:

$$\log D_{7.4} = \left(\frac{1\mu M - c_B}{c_B} \times \frac{1}{o:b}\right)$$

The average of the three log $D_{7.4}$ values per 1-octanol:buffer ratio was calculated. If the two mean values obtained for a compound did not differ by more than 0.1 unit, the results were accepted.

4.3.4. Parallel artificial membrane permeation assay (PAMPA)

log P_e was determined in a 96-well format with the PAMPA[50] permeation assay. For each compound, measurements were performed at three pH values (5.0, 6.2 and 7.4) in quadruplicates. For this purpose, 12 wells of a deep well plate, that is, four wells per pH-value, were filled with 650 μL PAMPA System Solution. Samples (150 μL) were withdrawn from each well to determine the blank spectra by UV-spectroscopy (SpectraMax 190, Molecular Devices, Silicon Valley Ca, USA). Then, analyte dissolved in DMSO was added to the remaining PAMPA System Solution to yield 50 μM solutions. To exclude precipitation, the optical density was measured at 650 nm, with 0.01 being the threshold value. Solutions exceeding this threshold were filtrated. Afterwards, samples (150 μL) were withdrawn to determine the reference spectra. Further 200 μL were transferred to each well of the donor plate of the PAMPA sandwich (pIon, Woburn MA, USA, P/N 110 163). The filter membranes at the bottom of the acceptor plate were impregnated with 5 μL of GIT-0 Lipid Solution and 200 μL of Acceptor Sink Buffer were filled into each acceptor well. The sandwich was assembled, placed in the GutBox™, and left undisturbed for 16 h. Then, it was disassembled and samples (150 μL) were transferred from each donor and acceptor well to UV-plates. Quantification was performed by both UV-spectroscopy and LC-MS. log P_e-values were calculated with the aid of the PAMPA Explorer Software (pIon, version 3.5).

4.3.5. Thermodynamic solubility

Microanalysis tubes (Labo-Tech J. Stofer LTS AG, Muttenz, Switzerland) were charged with 1 mg of solid substance and 250 μL of phosphate buffer (50 mM, pH 6.5). The samples were briefly shaken by hand, then sonicated for 15 min and vigorously shaken (600 rpm, 25 °C, 2 h) on a Eppendorf Thermomixer comfort. Afterwards, the samples were left undisturbed for 24 h. After measuring the pH, the saturated solutions were filtered through a filtration plate (MultiScreen® HTS, Millipore, Billerica MA, USA) by centrifugation (1500 rpm, 25 °C, 3 min). Prior to concentration determination by LC-MS, the filtrates were diluted (1:1, 1:10 and 1:100 or, if the results were outside of the calibration range, 1:1000 and 1:10000). The calibration was based on six values ranging from 0.1 to 10 μg/mL.

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Supplementary data

Supplementary data (HRMS and HPLC data of target compounds **5**–**8**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.08.057.

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