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Article

Pseudoenantiomeric glycoclusters: Synthesis and testing of heterobivalency in carbohydrate-protein interactions

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Multivalent carbohydrate-protein interactions are key events in cell recognition processes and have been extensively studied by the means of synthetic glycomimetics. To date, frequently the valency, i.e. the multiplicity of the ligand attached to a polyvalent scaffold, has been considered in the design of multivalent structures but these studies have not led to a conclusive understanding of glycan recognition at the molecular level. In this work, we add a new aspect to carbohydrate-lectin recognition studies by designing the first heterobivalent diastereomeric glycoclusters in order to investigate the influence of both, heteromultivalency and of relative ligand orientation. Two enantiomeric scaffolds, derived from D- and L-serine, respectively, were glycosylated with two distinct carbohydrate ligands to obtain a library of pseudoenantiomeric glycoclusters. They all have an α-D-mannosyl residue in common as a specific ligand for the lectins FimH and ConA, while they differ in the second carbohydrate portion, consisting of a β-D-glucosyl, a β-D-galactosyl or a β-D-glucosaminyl residue as unspecific ligands. The synthesised heteroclusters were tested in standard binding-inhibition assays investigating FimH-mediated bacterial adhesion and ConA binding to mannosylated surfaces. A striking difference was observed between the potencies of the two pseudoenantiomeric glucose-containing glycoclusters as inhibitors of FimH-mediated bacterial adhesion. For the other diastereomeric glycocluster pairs smaller inhibitory potency differences were detected in the bacterial adhesion assay. In contrast, the assays with ConA showed no significant variation for all tested clusters pairs. The results obtained with the diastereomeric glucose-mannose glycocluster pair were rationalised by molecular docking. Binding energies for the FimH carbohydrate recognition domain were calculated for both diastereomers and are in agreement with experimental data obtained in the bacterial adhesion assays.

Introduction

Cell surface carbohydrates play an essential role in key cellular communication processes, which are, i.a., mediated by carbohydrate-lectin interactions. These include signalling, cell trafficking and cell adhesion.[1] In particular, the adhesion of pathogens to tissues, an initial step in infection, is often mediated by the specific recognition of host cell glycoconjugates by pathogen adhesins (lectins).[2] For instance, the adhesion of uropathogenic *E. coli* bacteria (UPEC) to the endothelial cells of the host organism occurs through binding of α-mannosyl residues by the bacterial lectin FimH, which is located at the tips of so-called type 1 fimbriae.[3] Fimbriae are adhesive organelles, multiple projected from the bacterial cell surface.[4] Hence, carbohydrate-specific bacterial adhesion clearly relies on multivalent interactions between cell surfaces.

As manifold copies of a variety of carbohydrate epitopes are expressed on cell surfaces, multivalent interactions with lectins, which often possess several recognition domains, leads to strong binding as a result of a proper combination of single low-affinity interactions in a cooperative fashion. These avidity or cluster effects, respectively, have long been known[5] and have been exploited extensively, in particular employing synthetic polyvalent glycomimetics. These studies have advanced our understanding of carbohydrate-protein interactions and also have opened the door to the application of carbohydrates as antiadhesives to treat infectious diseases.[6]

However, multivalency effects in carbohydrate recognition are not conclusively understood as yet. While some of the synthetic multivalent glycomimetics were able to bind lectins with high avidity (up to the picomolar range),[7] others unexpectedly exhibited poor binding ability despite of a large number of displayed ligands.[3b,6d] In light of these contrasting results, it was important to investigate multivalency effects in an extended and advanced way than only taking into account the multiplicity of the sugar epitope.[8] For instance, proper distances between ligands, their spatial arrangement as well as the flexibility of the linker moieties are important factors and have been investigated in several studies.[9] In order to test more complex glycoconjugates which are structurally closer to the heterogeneous sugar coat of eukaryotic cells (glycocalyx), García-Fernández and co-workers prepared heteroglycoclusters and evaluated their glycobiology. Strikingly, these compounds showed a remarkable "heterocluster effect" towards binding to lectins such as Concanavalin A (ConA) and peanut butter agglutinin (PNA), in comparison with their counterparts displaying only the specific lectin ligand.[10] With these findings,

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a new thinking about multivalency effects in carbohydrate recognition has begun.[11]

Further, some reports showed that the presentation mode of carbohydrate ligands on a surface is also critical for proper recognition by lectins.[12] Among others, our group showed that the specific recognition of α-D-mannosyl residues by the lectin FimH is clearly impacted by the orientation of the sugar epitopes. This was demonstrated by using photoswitchable azobenzene mannosides attached onto gold surfaces (glycoSAMs) or the surface of human cells to systematically re-orient sugar ligands.[12b,c]

Based on the reported findings, we believe that multivalency effects in carbohydrate recognition strongly depend on the three-dimensional organization of carbohydrate ligands and glycoepitopes, respectively.[13] In order to further investigate how the relative positioning of ligands governs sugar recognition, we approached a new design of heterobivalency in which two different sugars are arranged on enantiomeric scaffold molecules. This leads to a pair of diastereomeric glycoclusters, which can be regarded as pseudoenantiomers with respect to the configuration at the focal point of the scaffold moiety (Figure 1A). Hence, the sugar ligands conjugated in a particular bivalent heterocluster are displayed in different relative orientation. We targeted three principal heterobivalent pseudoenantiomeric glycocluster pairs, combining β-D-glucopyranosyl (Glc), β-D-galactopyranosyl (Gal) and β-D-glucosaminyl (GlcNHR) moieties, respectively, with an α-D-mannopyranoside (Figure 1B).

The diastereomeric pairs of heterobivalent glycoclusters are based on scaffold molecules derived from D-and L-serine, respectively. These enantiomeric α-amino acids were converted into the respective mono-protected 2-azido-propanediol derivatives (Figure 1C) and submitted to two sequential glycosylation reactions, employing suitable glycosyl donors to achieve the target glycoclusters βGlc-αMan, βGal-αMan, and βGlcNAc-αMan. In order to have a bivalent reference compound in hand, we also prepared the respective homobivalent mannose glycocluster $(\alpha Man)_2$ from the symmetrical diol 2-azido-propanediol (Scheme 1). As our synthetic approach is based on enantiomeric bivalent scaffold molecules from the chiral pool, it is highly flexible and allows for the rapid preparation of libraries of "mirror image" glycoclusters, without the need of separation of diastereomers during the synthesis. The employed carbohydrates, –mannose, glucose, galactose, and GlcNAc–, were selected for their biological relevance as lectin ligands. All cluster glycosides prepared herein were thus tested in binding studies, on the one hand with the plant lectin ConA[14] and on the other hand in bacterial adhesion studies with live type 1-fimbriated *E. coli* bacteria, where carbohydrate binding is mediated by the adhesin FimH.[3]

Results and Discussion

Synthesis

At first, we required the homobivalent mannoside cluster **6** (Scheme 1) as a reference compound for later testing of heterobivalency effects. The synthesis of **6** was accomplished in three steps starting from serinol (**1**), which was converted into the respective azido-functionalised diol **3**[15] with imidazole sulfonyl azide (**2**)[16] in 72 % yield. The bivalent scaffold **3** was then glycosylated using the known thiomannoside **4** as glycosyl donor.[17] The mannosyl donor **4** was activated at -77 °C with *N*-iodosuccinimide (NIS) and a catalytic amount of triflic acid.[18] Then, the reaction mixture was allowed to warm to room temperature over three hours to provide the benzoyl-protected bivalent cluster mannoside **5** in 60 % yield after work up and purification. Deprotection of **5** with sodium methoxide in methanol gave the pure target molecule **6**.

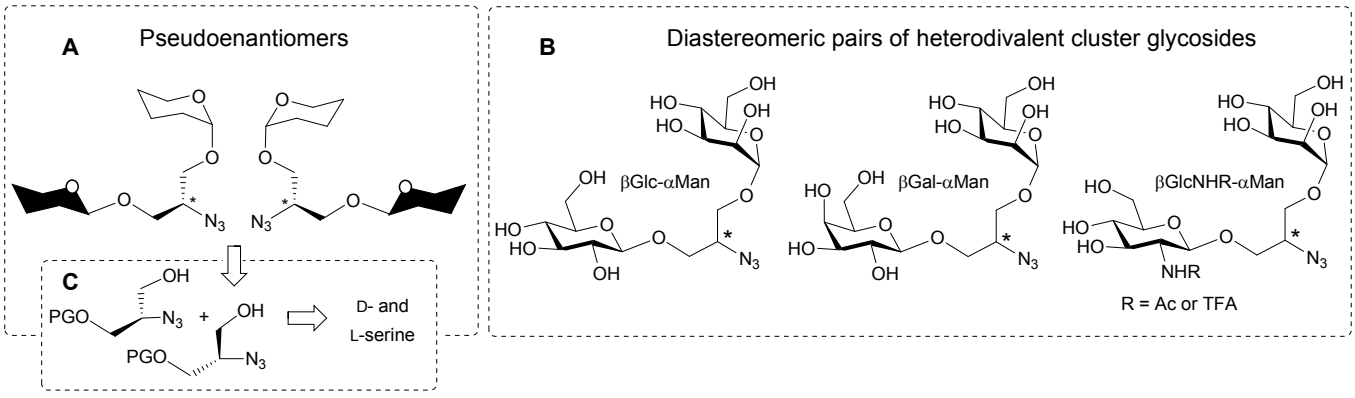
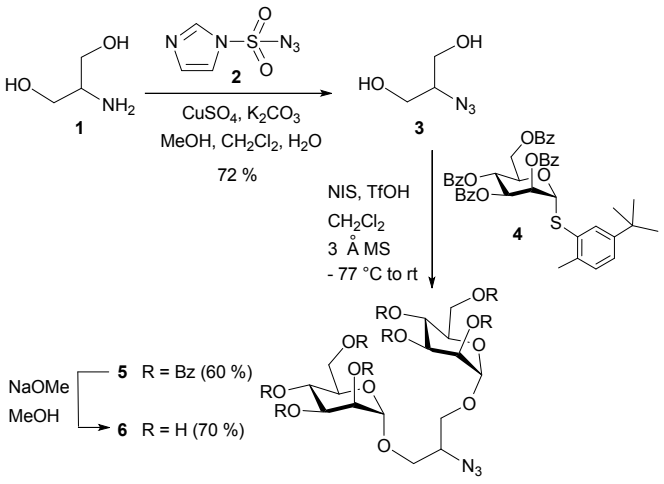


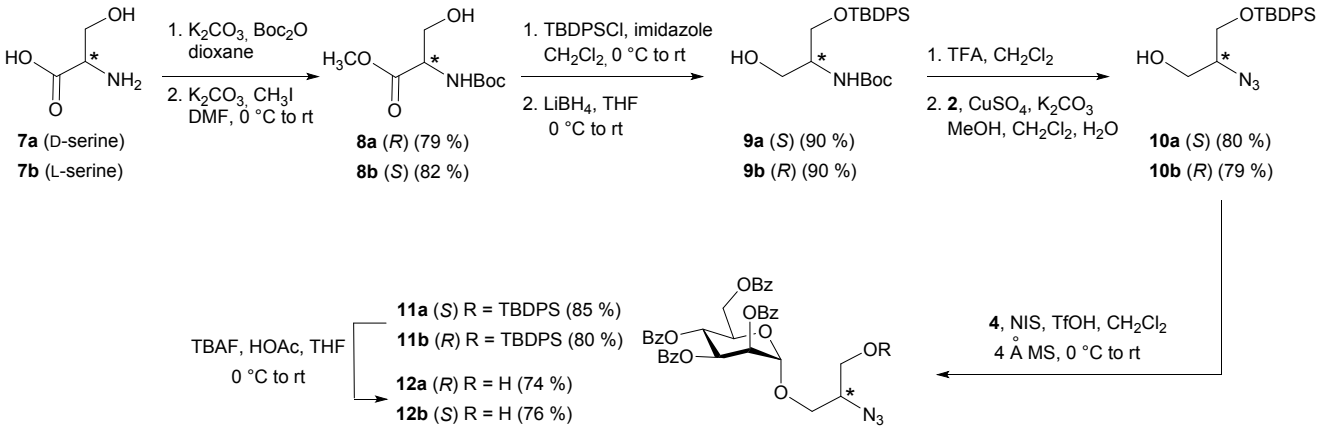
Figure 1 Pseudoenantiomeric cluster glycosides (A) were targeted, comprising two different carbohydrate moieties (black and white chairs), and differing in the configuration at the focal point stereocenter (*) of the scaffold moiety. Four diastereomeric pairs of hetereobivalent glycoclusters were synthesised (B), βGlc-αMan, βGal-αMan, βGlcNHR-αMan, together with the analogous homobivalent mannoside cluster $(\alpha Man)_2$ for comparison (not shown, cf. Scheme 1). The diastereomeric glycoclusters were built on mono-protected 2-azido-propanediol enantiomers (C), which were derived from D- and L-serine as enantiopure chiral pool scaffold molecules. TFA = trifluoroacetyl; PG = protecting group.



Scheme 1 Synthesis of the homobivalent cluster mannoside **6**.

The synthesis of the targeted diastereomeric pairs of heterobivalent cluster glycosides (Figure 1B) was based on D- and L-serine, **7a** and **7b**, respectively, as enantiopure chiral pool compounds (Scheme 2). First of all, the α-amino function of each enantiomeric amino acid was Boc-protected under standard conditions,[19] and the resulting crude material directly converted into the respective methyl ester in the presence of iodomethane and potassium carbonate. The enantiomeric serine derivatives **8a** and **8b** were thus received in 79 and 82 % respective yield over two steps.[20] In order to block the primary alcohol of the serine scaffold, the *tert*-butyldiphenylsilyl (TBDPS) ether was used and introduced with *tert*-butyldiphenylsilyl chloride and imidazole.[21] The resulting crude silyl ethers were carried on in the next reduction step with lithium borohydride and after purification, the alcohols **9a** and **9b** were both obtained in 90 % yield over two steps. We also tried to reduce the methyl esters with lithium aluminium hydride, but remarkably, this gave only poor yields. The primary alcohols **9a** and **9b** were further used as acceptor molecules in the first glycosylation step. However, we found that the *N*-Boc protecting group was labile under the acidic conditions required for the glycosylation reaction, whereas under the same conditions the TBDPS group was stable. Therefore, compounds **9a** and **9b** were first treated with trifluoroacetic acid in order to cleave the *N*-Boc group and the resulting free amines were converted into the respective azides in a diazo transfer reaction employing **2** under similar conditions as described above.[16] The two enantiomeric scaffold molecules **10a** and **10b** were isolated in 80 % and 79 %, respectively, over two steps. Notably, no racemization was observed in any of the described steps.

With the enantiomeric building blocks **10a** and **10b** in hand, the first glycosylation reaction was carried out to obtain the pivotal mono-mannosylated acceptor molecules as precursors for the synthesis of all bivalent heteroclusters. As expected, the glycosylation of **10a** and **10b** proceeded much better than when the carbamates **9a** and **9b** were employed. In first mannosylation attempts, the well-known tetraacetylated α-D-mannosyl trichloroacetimidate[22] was used as the glycosyl donor. However, the resulting glycosides were isolated in only unsatisfying yields (36 to 56 %) which we could not further optimise. This was due to acetyl migration resulting in acetylation of the acceptor alcohol.[23] Thus, we used the benzoylated thiophenyl mannoside **4**,[17] as benzoyl groups are less prone to acyl migration under acidic conditions.[24] The thioglycoside **4** was again activated under standard conditions employing NIS and triflic acid to provide the mannosides **11a** and **11b** in very satisfying yields around 80 %. Then, the subsequent desilylation with TBAF (*n*-tetrabutylammonium fluoride) afforded compounds **12a** and **12b** in good yields, hence setting a further alcohol group available for the second glycosylation step.

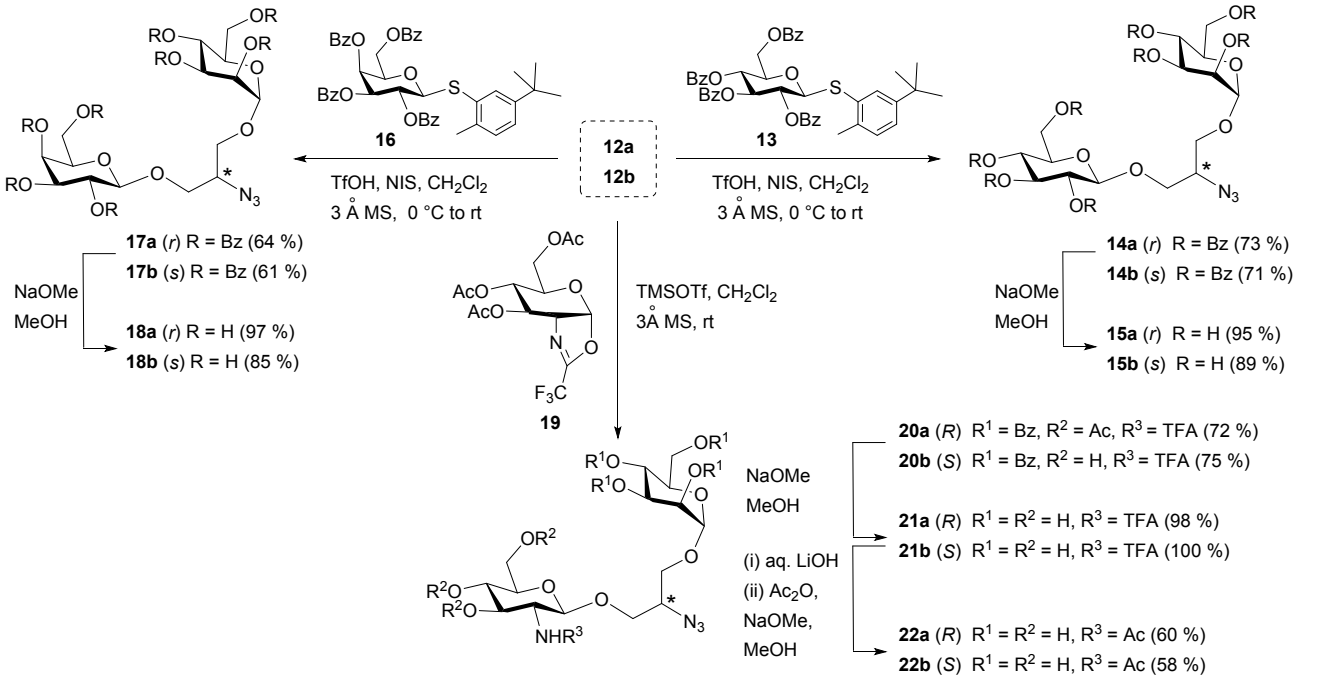


Scheme 2 Synthesis of a diastereomeric pair of mannosides, derived from D- and L-serine as enantiopure scaffold molecules from the chiral pool. The obtained mannosides **12a** and **12b** were needed as acceptor alcohols for all following glycosylation reactions (cf. Scheme 3).

We targeted three principal diastereomeric pairs of heterobivalent cluster glycosides (Figure 1B). For this, we used glucose, galactose and GlcNAc donors for the glycosylation of the mannosides **12a** and **12b**. The protected diastereomeric pairs βGlc-αMan and βGal-αMan were obtained employing the benzoyl-protected thioglucoside (**13**)[17] and the respective thiogalactoside donor (**16**) (Scheme 3). The glycosylation reactions proceeded efficiently, furnishing **14a**/**b** and **17a**/**b** in

yields from 61 to 71 %. Deprotection under Zemplén conditions[25] provided the final diastereomeric cluster pairs **15a**/**b** and **18a**/**b**, respectively, in very good to excellent yields after purification by size exclusion chromatography.

For the synthesis of the βGlcNAc-αMan glycocluster, the acetylated trifluoromethyl oxazoline **19**,[26] derived from D-glucosamine, was used as the donor in a glycosylation reaction promoted by a catalytic amount of trimethylsilyl trifluoromethanesulfonate at room temperature.[27] The β-configured glycosides **20a** and **20b** were isolated in good yields and no α-anomer was obtained. The following standard Zemplén deprotection delivered the diastereomeric βGlcNTFA-αMan pair **21a**/**21b**. In order to achieve the respective βGlcNAc-αMan pair, the *N*-trifluoroacetyl (TFA) derivatives were cleaved with aqueous lithium hydroxide[28] and subsequent *N*-acetylation in methanol under basic conditions afforded compounds **22a** and **22b** (Scheme 3). Both heterobivalent diastereomeric pairs involving glucosamine, **21a**/**21b** (βGlcNTFA-αMan) and **22a**/**22b** (βGlcNAc-αMan), were used in lectin binding studies (see below).



Scheme 3 **Scheme 3** Synthesis of four diastereomeric pairs of heterobivalent glycoclusters. In the diastereomeric pairs βGlc-αMan and βGal-αMan, the focal point represents a pseudoasymmetric centre as the two attached sugar moieties only differ in their configuration (Glc, Man, and Gal have the same constitution). Thus, the configuration at the focal point is assigned by using small letters, *r* and *s*. Otherwise, the two diastereomeric pairs βGlcNHAc-αMan and βGlcNHTFA-αMan comprise sugars of different mass and hence capitalised descriptors *R* and *S* are used to assign the configuration at the focal point. (Note, that according to IUPAC, the diastereomeric pairs comprising mannosyl and glucosaminyl residues cannot be called "pseudoenantiomeric".) Please note further that for assigning the CIP priorities of the sugar moieties, their anomeric configuration is decisive, with a sugar with anomeric (*R*)-configuration having a higher priority than its isomer with anomeric (*S*)-configuration. CIP = Cahn-Ingold-Prelog.

Biological Testing

The synthesised bivalent glycoclusters were tested in solution as inhibitors of the adhesion of type 1 fimbriae-mediated *E. coli* bacteria and of binding of ConA, respectively, to a mannan-coated microtiter plate surface. The type 1-fimbrial lectin is FimH, exhibiting a pronounced specificity for α-D-mannosyl residues.[3] ConA on the other hand, recognises α-D-mannosides as well as α-D-glucosides.[29] Depending on the pH, ConA exists in two forms, as a dimer (at pH ≤ 5.6) or as a tetramer (at pH 5.8 to 7).[14] Accordingly, in the assay performed here, ConA is tested as a tetramer.

In the employed assays, a soluble inhibitor has to compete with the bacterial lectin FimH or ConA, respectively, for binding to a mannosylated (mannan-coated) surface. Using 96-well plates, serial dilutions of each tested inhibitor were applied in order to measure dose-response inhibition curves (cf. ESI) from which IC_{50} values can be determined. The IC_{50} value of a specific compound reflects the concentration at which bacterial or ConA binding is reduced by 50% and thus correlates with its inhibitory potency. Bacterial binding (adhesion) and ConA binding to the surface were measured by fluorescence read-out. For this, we used the *E. coli* strain PKL1162, expressing the green fluorescent protein (GFP),[30] and fluorescein-labelled ConA. In all assays, methyl α-D-mannopyranoside (MeMan) was tested as reference inhibitor on the same plate in order to allow the quantitative comparison of all tested cluster glycosides, even when tested in different experiments. Hence, we report relative inhibition potency (RIP) values which are all related to the inhibitory potency of MeMan, tested in the same experiment. We also compared the various heteroclusters with the homobivalent mannoside **6**, tested on the same microplate, to observe whether a heteromultivalency effect occurs.[10] In this respect, we also report valency-corrected RIP values (RIP_{vc}), where the determined RIP value is divided by the number of clustered mannose ligands (two in the case of **6**).

The so determined IC_{50} values are summarised in Table 1 for the inhibition of both, adhesion of type 1-fimbriated *E. coli* and ConA binding. The corresponding RIP values are compared in Figure 2. Overall, the homobivalent cluster mannoside **6** and every tested heterobivalent glycocluster show more or less pronounced multivalency effects with RIP values higher than 1, with the exception of the *N*-GlcNAc-containing glycoclusters **22a**/**b** as inhibitors of ConA binding (Fig. 2, top chart). As inhibitors of FimH-mediated bacterial adhesion (Fig. 2, bottom chart), some of the heterobivalent glycoclusters, **15b**, **18b**, **21a** and **21b**, also exhibit a heterocluster effect with RIP values 2- to 6-fold higher than RIP_{vc} of **6** (always tested in parallel). On the other hand, in the ConA assay, no significant heterocluster effects were observed. This is not surprising according to the results reported by García Fernández et al. with ConA, that evidenced the heteromultivalency effect occurring only at a high density of ligands on the scaffold.[10]

Table 1. IC_{50} values of synthetic inhibitors of type 1 fimbriae-mediated adhesion of *E. coli* cells and of ConA binding, respectively, both to mannan-coated microtiter plates, employing the *E. coli* strain GFP-PKL1162 and FITC-labelled ConA.

	FITC-ConA	GFP-PKL1162
Inhibitor	IC_{50} (SD)[a] [mmol]	IC_{50} (SD)[a] [mmol]
MeMan	5.55 (±0.48)	11.87 (±0.96)
βGlc-αMan **15a**	4.37 (±0.33)	8.17 (±0.9)
βGlc-αMan **15b**	2.71 (±0.76)	0.43 (±0.06)
$(\alpha Man)_2$ **6**	1.62 (±0.12)	0.60 (±0.10)
MeMan	4.95 (±1.29)	13.31 (±4.00)
βGal-αMan **18a**	3.43 (±0.97)	3.63 (±0.10)
βGal-αMan **18b**	3.88 (±0.92)	1.68 (±0.19)
$(\alpha Man)_2$ **6**	1.27 (±0.33)	1.54 (±0.33)
MeMan	5.78 (±1.06)	6.47 (±1.13)
βGlcTFA-αMan **21a**	3.60 (±0.21)	0.44 (±0.24)
βGlcTFA-αMan **21b**	3.60 (±0.18)	0.57 (±0.19)
$(\alpha Man)_2$ **6**	1.78 (±0.17)	1.00 (±0.09)
MeMan	4.76 (±0.36)	6.67 (±0.67)
βGlcNAc-αMan **22a**	6.48 (±0.15)	2.31 (±0.88)
βGlcNAc-αMan **22b**	6.56 (±0.41)	2.66 (±0.29)
$(\alpha Man)_2$ **6**	1.77 (±0.10)	0.37 (±0.04)

[a] IC_{50} values are averaged from the mean values obtained in at least two independent adhesion experiments (cf. ESI). Note, that the IC_{50} values can vary significantly in independent experiments as live bacteria are investigated. SD: standard deviation; GFP: green fluorescent protein; FITC: fluorescein isothiocyanate.

We were especially excited to test, if the pseudoenantiomeric pairs of heterobivalent glycoclusters would show any significant difference in their inhibitory potencies. In the ConA-based assay, the pseudoenantiomeric glycoclusters performed very similar or equal. Only some RIP difference was observed with the βGlc-αMan pair (1.27 and 2.05, respectively, for **15a** and **15b**). However, a striking difference was indeed observed for the same βGlc-αMan pair **15a** and **15b** when tested as inhibitors of bacterial adhesion. Hence, **15b**, based on the L-serine-derived scaffold, is nearly 20-fold more potent (RIP = 27.44), than its isomer **15a** (RIP = 1.45). We confirmed this exciting result in several independent assays (cf. ESI).[31] A similar trend was observed for the galactose-containing clusters **18a** and **18b**, although their RIP values differ only by a factor of approx. 2 in favour of **18b** (RIP = 7.97). The *N*-TFA-β-GlcNAc-containing glycoclusters **21a** and **21b**, exhibited a small RIP difference, in this case in favour of the D-serine-derived cluster **21a** (RIP = 14.89). The GlcNAc-containing clusters **22a/b** only displayed weak inhibitory potencies with almost no difference between the diastereomers. It might be concluded at this point that the *N*-trifluoroacetyl group seems to have a beneficial effect on inhibition of bacterial adhesion.

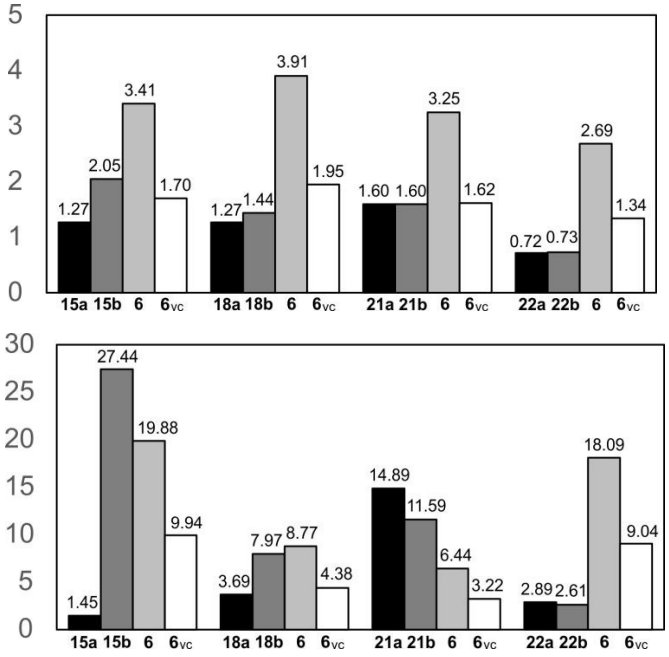


Figure 2. Relative inhibitory potencies (RIP values) of the tested compounds deduced from the measured IC_{50} values as listed in Table 1. Top chart: Inhibition of ConA (FITC-labelled) binding to mannan; bottom chart: Inhibition of *E. coli* (GFP-PKL1162) adhesion to mannan. RIP values are based on the inhibitory potency of methyl α-D-mannopyranoside (MeMan) tested on the same microplate (MeMan, IP ≡ 1); RIP = IC_{50}(MeMan)/IC_{50}(tested compound). For the homobivalent cluster mannoside $(\alpha Man)_2$ (**6**) also the valency-corrected value RIP_{vc} (**6**$_{vc}$) is depicted for comparison.

To rationalise the remarkable difference in inhibitory power, which was seen in the bacterial adhesion assay with the pseudoenantiomeric heterobivalent glycoclusters **15a** and **15b**, we studied their interactions with the bacterial adhesin FimH by molecular modelling. At this point we concentrated on **15a/b** and did not consider the molecular interactions of the other pseudoenantiomeric pairs which showed less pronounced effects.

Molecular modelling

We figured that the spatial orientation of the glucosyl residue in **15a** versus **15b** might have a significant influence on the recognition of the bivalent structure by the bacterial adhesin FimH. To support this assumption, we examined the interaction of both cluster glycosides with the lectin by molecular modelling based on force-field methods. We first performed a molecular docking study involving **15a** and **15b** and the homobivalent cluster mannoside **6** for comparison. For docking, Glide was used, a specific software implemented in the Schrödinger

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suite.[32] FimH has been crystallised in two conformations, a "closed" form[33] and an "open" one,[34] depending on the orientation of two tyrosine residues (Tyr 48 and Tyr 137) which flank the carbohydrate recognition domain (CRD) and form the so-called "tyrosine gate". Thus, we used both, the closed and the open crystal structures of the lectin (pdb codes 1UWF and 1KLF, respectively) for docking. Because docking scores do not generally reflect the affinity of ligands, each docking output was next submitted to a MM-GBSA calculation (molecular mechanics energies combined with the generalised Born and surface area continuum solvation)[35] to provide binding energies.[36] These values are more reliable than docking scores to estimate and compare protein-ligand interactions.[36]

Table 2 depicts the docking score for the clusters **6**, **15a** and **15b** along with the corresponding lowest binding energy for both the open and the closed gate conformation of the lectin FimH. The obtained ranking of the glycoclusters is consistent with the RIP values deduced from the adhesion inhibition assays, **15b** forming a more stable complex with FimH than **6** and **15a**.

As the difference in energy between **15b** and **15a** is more important in the closed gate conformation (about 9.5 kJ·mol^{-1}), we selected the corresponding docking conformations for comparing the position of the different ligands bound to FimH (Figure 3). While the α-D-mannosyl residue of each cluster equally fits into the binding pocket (Figure 3A), there is a clear difference in the orientation of the glucoside moiety. In case of **15b**, a stacking of the glucosyl residue with a polar domain of the CRD is visible and the unpolar azido group points towards the tyrosine gate. In case of **15a** on the other hand, the azido function is further away from the tyrosine gate while the glucoside portion is shifted closer to a hydrophobic domain of the CRD.

Table 2. Docking scores[a] and binding energies[b] of the clusters **6**, **15a**, and **15b** in complex with the open and closed gate conformation of FimH.

Ligand	Docking score[c] Open gate	Docking score[c] Closed gate	Binding energy (kJ mol^{-1}) Open gate	Binding energy (kJ mol^{-1}) Closed gate
6	-9.911	-8.231	-74.892	-68.407
15a	-9.561	-8.552	-72.526	-60.940
15b	-9.340	-8.858	-78.104	-70.465

[a] Calculated with Glide; [b] calculated using the MM-GBSA method based on the docking output; [c] the lower the docking score, the better the predicted binding.

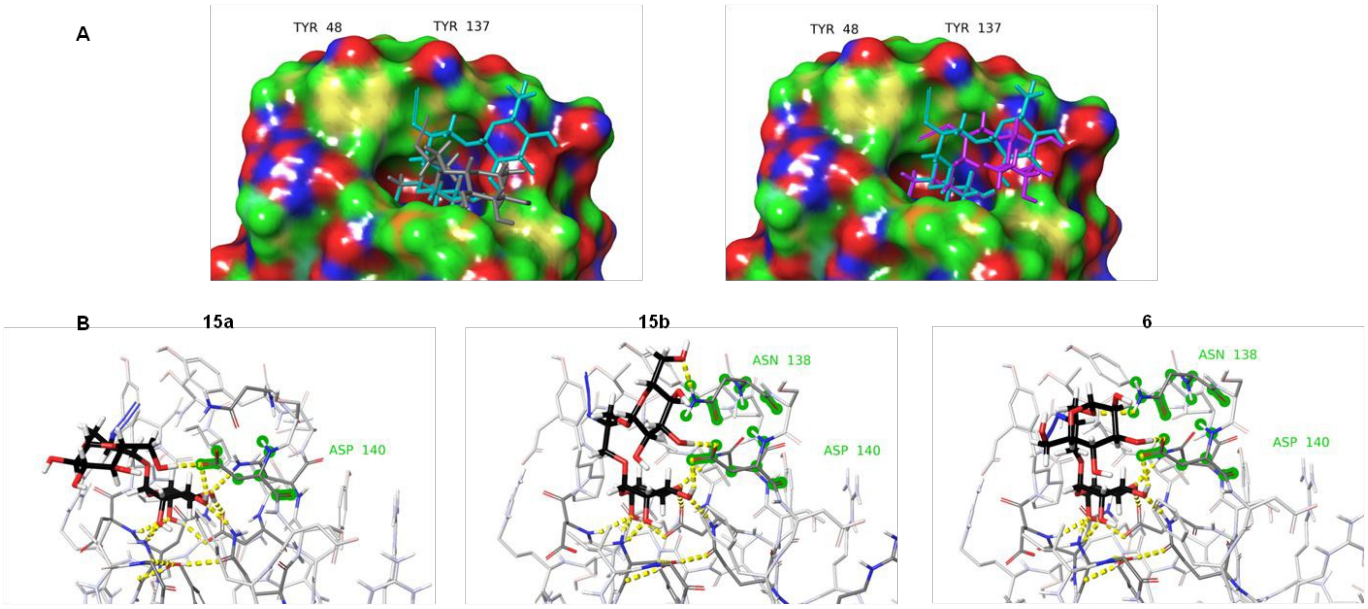

Figure 3. Interaction of FimH (closed gate conformation, pdb code 1UWF) with clusters **15a**, **15b** (diastereomeric βGlc-αMan pair) and **6** $(\alpha Man)_2$. A: Partial charge coloured Connolly description[38] (negative charges in red, positive in blue); left image: overlay of **15a** (grey) and **15b** (blue) in the carbohydrate recognition domain (CRD) of FimH; right image: overlay of **6** (purple) and **15b** (blue) in the FimH CRD. B: H-bond network between **15a** (left), **15b** (middle) and **6** (right) and the CRD of FimH: the amino acid residues interacting with the cluster outside of the binding pocket are highlighted in green, suggesting that **15b** and **6** are better FimH ligands that **15a**.

The hydrogen bond network was also examined (Figure 3B). Hence, the mannoside ligand in both diastereomers, **15a** and **15b**, forms exactly the same H-bond pattern with the amino acids of the FimH binding pocket. The difference between **15a** and **15b** resides in the non-covalent interactions of the glucoside portion. In **15b**, two hydrogen bonds are provided by the two protein residues forming the aforementioned polar domain (Asn 138 and Asp 140). In contrast, only a single H-bond with the same asparagine residue Asn 138 is observed for **15a**. Hence, the results of our modelling study provide a good basis for a possible explanation of the differences between the heterobivalent diastereomeric glycoclusters **15a** and **15b** (the pseudoenantiomeric βGlc-αMan pair) which was observed in the bacterial adhesion-inhibition assay. It might also give hints for the interpretation of data obtained earlier and likewise for

the future design of inhibitors and the understanding of carbohydrate recognition in a complex environment.

Conclusions

In conclusion, we introduced the first pseudoenantiomeric cluster glycosides as a new tool for investigating the influence of the relative epitope orientation on lectin binding. A small library of heterobivalent glycoclusters was readily prepared from two enantiomeric scaffolds derived from L- and D-serine, leading to pairs of diastereomers according to the configuration at the focal point of the molecules. All synthesised heterobivalent glycoclusters contain one α-mannosyl residue and vary in the nature of the second sugar epitope. They were tested as inhibitors of bacterial adhesion, mediated by the lectin FimH, and as ligands of the lectin ConA. Both lectins, FimH and ConA, specifically recognise α-mannosyl epitopes. The results reveal a sharp difference in the inhibitor potency of the diastereomeric βGlc-αMan pair **15a**/**b** when tested in bacterial adhesion. Much smaller potency gaps were detected with the other pseudoenantiomeric glycocluster pairs. In the ConA-based assays on the other hand, almost no significant variations were seen. In addition to the effect of the focal point stereochemistry, a marked heterocluster effect was observed with several derivatives, but again only in the context of inhibition of bacterial adhesion.

Molecular docking with FimH provided means for rationalizing the experimental data found with the βGlc-αMan clusters. In fact, the models of the cluster-lectin complexes showed a clear difference in the orientation of the glucosyl residue and the resulting stabilization of the sugar-FimH complex by H-bonds in the periphery of the CRD depending on sugar scaffolding. We believe that our study opens new prospects in the design of multivalent glycomimetics and the understanding of carbohydrate recognition. Further analytical methods, more complex glycoconjugates as well as other lectins shall be employed to deepen the approach we have introduced herein.

Experimental Section

General information

Air- or moisture-sensitive reactions were carried out under nitrogen in dry glassware unless otherwise stated. All reactions were monitored by thin layer chromatography (TLC) on silica gel plates (F 254, Merck). Detection of spots was effected by UV light and/or subsequent charring with 10% sulphuric acid in ethanol, vanillin, or ninhydrin followed by heat treatment at ~150 °C. Flash chromatography was performed on silica gel 60 (0.040-0.063 mm) using distilled solvents. Optical rotations were measured with a Perkin-Elmer 241 polarimetry (sodium D-line: 589 nm) in the solvents indicated. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer at 300 K. Chemical shifts (in ppm) are relative to residual non-deuterated solvent as an internal reference. Full assignment of the peaks was achieved with the aid of 2D NMR techniques (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H NOESY). ESI mass spectra were recorded on a Mariner 5280 instrument (Applied Biosystems).

For diastereomeric compounds, the one based on D-serine is specified as "a", while the other one, based on L-serine, is called "b". Molecule names are according to IUPAC nomenclature. To facilitate assignment of NMR peaks, the glycocluster skeleton was numbered as shown in Fig. 4. The nature of the carbohydrate portion is indicated by “Gal” (D-galactose), “Glc” (D-glucose), “GlcNAc” (*N*-acetyl-D-glucosamine), “GlcNTFA” (*N*-trifluoroacetyl-D-glucosamine), and “Man” (D-mannose) subscripts, respectively.

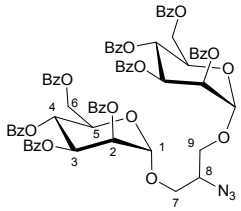

Figure 4. Numbering of the synthesized molecules for assignment of NMR data.

General procedure for glycosylation (general procedure A).

To a round bottom flask containing the acceptor, the donor and 3 Å molecular sieves, dry dichloromethane (c = 0.1 M) was added. After stirring at room temperature for 15 min, the mixture was cooled to 0 °C then *N*-iodosuccinimide (1.5 eq.) and trifluoromethanesulfonic acid (0.15 eq.) were sequentially added. After stirring at 0 ˚C for 30 min, the mixture was allowed to warm to room temperature and stirred until completion then diluted with dichloromethane and filtered over celite. After washing with satd. aq. sodium bicarbonate and satd. aq. sodium thiosulfate, the aqueous layer was extracted with dichloromethane then the combined organic layers were dried over magnesium sulfate, filtered and concentrated. The crude residue was purified by flash chromatography to afford the expected compound.

General procedure for glycosylation (general procedure B).

To a round bottom flask containing the acceptor, the donor and 3 Å molecular sieves, dry dichloromethane (c = 0.1 M) was added. After stirring at room temperature for 15 min, trimethylsilyl trifluoromethanesulfonate (0.1 eq.) was added and the mixture was stirred until completion. The reaction mixture was neutralised with triethylamine then diluted with dichloromethane, filtered over celite and concentrated. The crude residue was purified by flash chromatography to afford the expected compound.

General procedure for ester cleavage (general procedure C).

To a solution of the ester-protected glycocluster in dry methanol (c = 0.03 M), sodium methoxide (c = 5.4 M in methanol, two drops) was added and the mixture was stirred at room temperature until completion then neutralised with Amberlite IR120-H^{+}, diluted with methanol, filtered and concentrated. The residue was taken up into a 1:1 mixture of water and methanol then washed with diethyl ether and the aqueous layer was concentrated to dryness. The residue was purified by size exclusion chromatography on Sephadex G10 gel and eluting with deionised water.

General procedure for silyl cleavage (general procedure D).

The silylated starting material was dissolved in anhydrous tetrahydrofuran (*c* = 0.1 M) then the mixture was buffered with acetic acid (6 eq.) before dropwise adding 1 M *n*-tetrabutylammonium fluoride in tetrahydrofuran (3 eq.). The mixture was stirred until completion then diluted with ethyl acetate. After washing with satd. aq. sodium bicarbonate then 1 N hydrochloric acid, the aqueous phases were extracted with ethyl acetate then the combined organic layers were dried over magnesium sulfate, filtered and concentrated. The crude residue was purified by flash chromatography to afford the expected compound.

General procedure for cleavage of the *N*-trifluoroacetyl group and subsequent *N*-acetylation (general procedure E).

To a solution of the *N*-trifuoroacetyl derivative in methanol (*c* = 0.03 M), 2 M aq. lithium hydroxide (40 eq.) was added at room temperature. The solution was sonicated at 40 °C until completion then neutralised with Amberlite IR120-H^+, diluted with methanol, filtered and concentrated to dryness. The residue was dissolved in dry methanol (*c* = 0.03 M) then sodium methoxide (*c* = 5.4 M in methanol, two drops) and acetic anhydride (5 eq.) were sequentially added under nitrogen. After stirring overnight at room temperature, sodium methoxide (*c* = 5.4 M in methanol, two drops) was added and the mixture was stirred for a further 30 min then neutralised with Amberlite IR120-H^+, diluted with methanol, filtered and concentrated.

2–Azido–1,3–di–*O*–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–1,3–propanediol (5). General procedure A was applied to acceptor **3** (27.0 mg, 227 µmol) and donor **4** (207 mg, 273 µmol, 1.2 eq.). Reagents and conditions: *N*- iodosuccinimide (76.8 mg, 340 µmol, 1.5 eq.), trifluoromethanesulfonic acid (2.3 µl, 20.0 µmol, 0.15 eq.), dichloromethane (*c* = 0.1 M, 2.8 mL), T = -77 °C to room temperature after three hours. Flash chromatography with ethyl acetate/cyclohexane 2.5/7.5 afforded compound **5** as a white foam (174 mg, 60 %); R_f = 0.3 (ethyl acetate/cyclohexane 3/7); $[\alpha]_{20}^{D}$ = -46.1 (*c* 0.7, dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13-8.10 (m, 4H, 4 H-Ar), 8.06-8.04 (m, 4H, 4 H-Ar), 7.98-7.96 (m, 4H, 4 H-Ar), 7.84-7.82 (m, 4H, 4 H-Ar),7.61-7.55 (m, 4H, 4 H-Ar), 7.50-7.45 (m, 3H, 3 H-Ar), 7.44-7.37 (m, 10H, 10 H-Ar), 7.35-7.31 (m, 4H, 4 H-Ar) 7.28-7.26 (m, 1H, H-Ar), 7.25-7.23 (m, 2H, 2 H-Ar), 6.20-6.14 (m, 2H, 2 H-4), 5.97-5.92 (m, 2H, 2 H-3), 5.77 (dd, $^3J_{2,3}$ = 3.3 Hz, $^3J_{1,2}$ = 1.8 Hz, 2H, 2 H-2), 5.21 (d, $^3J_{1,2}$ = 1.8 Hz, 1H, H-1), 5.19 (d, $^3J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.79-4.75 (m, 2H, 2 H-6a), 4.58-4.47 (m, 4H, 2 H-6b, 2 H-5), 4.07-4.03 (m, 3H, H-8, H-7a, H-9a), 3.84-3.74 (m, 2H, H-7b,H-9b) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.1, 165.5, 165.4, 165.3(8C, 8 PhC=O), 133.5, 133.4, 133.1, 129.9, 129.8, 129.2, 129.0, 128.9, 128.6, 128.5, 128.4, 128.3 (48C, 48 C-Ar), 98.2, 97.8 (2C, 2 C-1), 70.2 (2C, 2 C-2), 69.9, 69.8 (2C, 2 C-3), 69.4, 69.4 (2C, 2 C-5), 67.6, 67.5 (2C, C-7, C-9), 66.7, 66.6 (2C, 2 C-4), 62.7 (2C, 2 C-6), 59.9 (C-8) ppm; IR (ATR) ν_{max}/cm^{-1} 2097, 1721, 1258, 1092, 705; ESI-HRMS: m/z calcd. for $C_{71}H_{59}N_3O_{20}$ + Na^+: 1296.35590 $[M+Na]^+$; found 1296.35841.

2–Azido–1,3–di–*O*–(α–D–mannopyranosyl)–1,3–propanediol (6). General procedure C was applied to compound **5** (500 mg, 392 µmol). Reagents and conditions: sodium methoxide (*c* = 5.4 M in methanol, two drops), methanol (*c* = 0.03 M, 7.84 mL). Compound **6** (100 mg, 70 %) was obtained as a white foam after lyophilisation; $[\alpha]_{20}^{D}$ = +53.5 (*c* 0.7, water); ^{1}H NMR (500 MHz, D_2O) δ 4.89-4.86 (m, 2H, 2 H-1), 3.98-3.87 (m, 6H, 2 H-2, 2 H-4, 2 H-6a), 3.85-3.80 (m, 3H, 2 H-3, H-8), 3.76-3.70 (m, 3H, H-9a, H-9b, H-6b), 3.66-3.59 (m, 5H, H-7a, H-7b, 2 H-5, H-6b) ppm; ^{13}C NMR (126 MHz, D_2O) δ 100.3, 99.8 (2C, 2 C-1), 73.1, 73.1 (2C, 2 C-4), 70.5 (2C, 2 C-3), 69.9 (2C, 2 C-2), 67.2 (C-6), 66.7 (2C, 2 C-5), 66.7 (C-6), 61.0 (2C, C-7, C-9), 60.1 (C-8) ppm; IR (ATR) ν_{max}/cm^{-1} 3332, 2927, 2097, 1048; ESI-HRMS: m/z calcd. for $C_{15}H_{27}N_3O_{12}$ + Na^+: 464.14869; $[M+Na]^+$; found 464.14822.

(*S*)–2–Azido–1–*O*–(*tert*–butyldiphenylsilyl)–3–*O*–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–1,3–propanediol (11a). General procedure A was applied to acceptor **10a** (169 mg, 476 µmol) and donor **4** (434 mg, 572 µmol, 1.2 eq.). Reagents and conditions: *N*-iodosuccinimide (160.7 mg, 715 µmol, 1.5 eq.), trifluoromethane sulfonic acid (4.8 µl, 47.0 µmol, 0.1 eq.), dichloromethane (*c* = 0.1 M, 4.76 mL). Flash chromatography with ethyl acetate/cyclohexane 1/9 yielded compound **11a** (374 mg, 85 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 1/9); $[\alpha]_{20}^{D}$ = -26.9 (*c* 0.4, dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11-8.09 (m, 2H, 2 H-Ar), 8.06-8.04 (m, 2H, 2 H-Ar), 7.96-7.94 (m, 2H, 2 H-Ar), 7.85-7.84 (m, 2H, 2 H-Ar), 7.70-7.67 (m, 4H, 4 H-Ar), 7.62-7.48 (m, 3H, 3 H-Ar), 7.46-7.33 (m, 15H, 15 H-Ar), 6.14 (dd, $^3J_{3,4}$ = $^3J_{4,5}$ = 9.9 Hz, 1H, H-4), 5.91 (dd, $^3J_{3,4}$ = 10.1 Hz, $^3J_{2,3}$ = 3.3 Hz, 1H, H-3), 5.71 (dd, $^3J_{2,3}$ = 3.3 Hz, $^3J_{1,2}$ = 1.8 Hz, 1H, H-2), 5.10 (d, $^3J_{1,2}$ = 1.8 Hz, 1H, H-1), 4.70 (dd, $^2J_{6a,\ 6b}$ = 13.4 Hz, $^3J_{5,6a}$ = 3.6 Hz, 1H, H-6a), 4.50-4.43 (m, 2H, H-5, H-6b), 3.93 (dd, $^2J_{7a,7b}$ = 9.9 Hz, $^3J_{7a,8}$ = 7.0 Hz, 1H, H-7a), 3.84-3.73 (m, 4H, H-8, H-9a, H-9b, H-7b), 1.08 (s, 9H, Si-C(CH_3)$_3$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.1, 165.4, 165.3(4C, 4 PhC=O) , 135.54, 133.4, 133.2, 133.0, 132.7, 130.0, 129.9, 129.8, 129.7, 129.2, 129.0, 128.9, 128.6, 128.4, 128.3, 127.9 (36C, 36 C-Ar) 97.7 (C-1), 70.1 (C-2), 69.9 (C-3), 69.2 (C-5), 67.7 (C-7), 66.6 (C-4), 63.5 (C-9), 62.7 (C-6), 62.0 (C-8), 26.6 (Si-C(CH_3)$_3$), 19.2 (Si-C(CH_3)$_3$) ppm; IR (ATR) ν_{max}/cm^{-1} 2103, 1724, 1451, 1260, 1093, 705, 503; ESI-HRMS: m/z calcd. for $C_{53}H_{51}N_3O_{11}Si$ + Na^+: 956.31851 $[M+Na]^+$; found 956.31719.

(*R*)–2–Azido–1–*O*–(*tert*–butyldiphenylsilyl)–3–O–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–1,3–propanediol (11b). General procedure A was applied to acceptor **10b** (300 mg, 843 µmol) and donor **4** (768 mg, 1.01 mmol, 1.2 eq.). Reagents and conditions: *N*-iodosuccinimide (284 mg, 1.26 mmol, 1.5 eq.), trifluoromethane sulfonic acid (8.4 µl, 50.0 µmol, 0.1 eq.), dichloromethane (*c* = 0.1 M, 8.4 mL). Flash chromatography with ethyl acetate/cyclohexane 1/9 afforded compound **11b** (665 mg, 85 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 1/9); $[\alpha]_{20}^{D}$ = -33.8 (*c* 0.6, dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12-8.02 (m, 4H, 4 H-Ar), 7.91-7.89 (m, 2H, 2 H-Ar), 7.85-7.83 (m, 2H, 2 H-Ar), 7.65 (m, 4H, 4 H-Ar), 7.64-7.47 (m, 4H, 4 H-Ar), 7.47-7.33 (m, 14H, 14 H-Ar), 6.18-6.06 (dd, $^3J_{3,4}$ = $^3J_{4,5}$ = 10.0 Hz, 1H, H-4), 5.89 (dd, $^3J_{3,4}$ = 10.1 Hz, $^3J_{2,3}$= 3.3 Hz, 1H, H-3), 5.71 (dd, $^3J_{2,3}$ = 3.3 Hz, $^3J_{1,2}$ = 1.8 Hz, 1H, H-2), 5.12 (d, $^3J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.71-4.61 (dd, $^2J_{6a,6b}$ = 11.8 Hz, $^3J_{5,6a}$ = 2.1 Hz, 1H, H-6a), 4.43 (m, 2H, H-5, H-6b), 3.99 (dd, $^2J_{7a,7b}$ = 8.4 Hz, $^3J_{7a,8}$ = 2.4 Hz, 1H, H-7a), 3.87-3.82 (m, 2H, H-9a, H-9b), 3.77-3.70 (m, 2H, H-8, H-7b), 1.09 (s, 9H, Si-C(CH_3)$_3$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2, 165.5, 165.4, 165.3 (4C, 4 PhC=O), 135.6, 133.5, 133.2, 133.1, 132.8, 132.7, 130.0, 129.8, 129.9, 129.8, 129.7, 129.8, 129.7, 129.2, 129.0, 128.9, 128.6, 128.5, 128.3, 127.9, (36C, 36 C-Ar),

98.2 (C-1), 70.2 (C-2), 69.8 (C-3), 69.2 (C-5), 67.7 (C-7), 66.7 (C-4), 63.5 (C-9), 62.6 (C-6), 62.0 (C-8), 26.9 (Si-C(CH_3)$_3$), 19.2 (Si-C(CH_3)$_3$); IR (ATR) ν_{max}/cm^{-1} 2101, 1724, 1451, 1259, 1067, 704, 504; ESI-HRMS: m/z calcd. for $C_{19}H_{25}N_3O_2Si$ + Na^+: 956.31851 $[M+Na]^+$; found 956.31800.

(*R*)–2–Azido–1–*O*–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–1,3–propanediol (12a). General procedure D was applied to compound **11a** (593 mg, 634 µmol). Reagents and conditions: tetrabutylammonium fluoride (*c* = 1 M, 1.91 mL, 1.91 mmol, 3 eq.), acetic acid (0.22 mL, 3.81 mmol, 6 eq.), tetrahydrofuran (*c* = 0.1 M, 6.35 mL). Flash chromatography with ethyl acetate/cyclohexane 1/9 to 2/8 afforded compound **12a** (348 mg, 78 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 2/3); $[\alpha]_{20}^{D}$ = -57.8 (*c* 0.5, dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09-8.07 (m, 2H, 2 H-Ar), 8.05-8.03 (m, 2H, 2 H-Ar), 7.96-7.94 (m, 2H, 2 H-Ar), 7.84-7.82 (m, 2H, 2 H-Ar), 7.63-7.48 (m, 3H, 3 H-Ar), 7.45-7.34 (m, 8H, 8 H-Ar), 7.29-7.26 (m, 1H, H-Ar), 6.12 (dd, $^3J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, 1H, H-4), 5.92 (dd, $^3J_{3,4}$ = 10.1 Hz, $^3J_{3,2}$ = 3.4 Hz, 1H, H-3), 5.73 (dd, $^3J_{2,3}$ = 3.3 Hz, $^3J_{1,2}$ = 1.8 Hz, 1H, H-2), 5.15 (d, $^3J_{1,2}$ = 1.8 Hz, 1H, H-1), 4.74-4.69 (dd, $^2J_{6a,6b}$ = 11.9 Hz, $^3J_{5,6a}$ = 2.3 Hz, 1H, H-6a), 4.54-4.45 (m, 2H, H-5, 6b), 4.02 (dd, $^2J_{7a,7b}$ = 10.0 Hz, $^3J_{7a,8}$ = 7.5 Hz, 1H, H-7a), 3.94-3.88 (m, 1H, H-8), 3.83 (dd, $^2J_{9a,9b}$ = 11.5 Hz, $^3J_{8,9a}$ = 4.4 Hz, 1H, H-9a), 3.78 (dd, $^2J_{7a,7b}$ = 10.0 Hz, $^3J_{7b,8}$ = 4.3 Hz, 1H, H-7b), 3.71 (dd, $^2J_{9a,9b}$ = 11.5 Hz, $^3J_{8,9b}$ = 5.9 Hz, 1H, H-9b) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2 165.5, 165.4, (4C, 4 PhC=O), 129.9, 129.8, 129.7, 128.6, 128.5, 128.3 (24C, 24 C-Ar), 97.7 (C-1), 70.2 (C-2), 69.8 (C-3), 69.3 (C-5), 67.9 (C-7), 66.7 (C-4), 62.4 (C-6), 62.1 (C-8), 62.0 (C-9) ppm; IR (ATR) ν_{max}/cm^{-1} 2933, 2095, 1723, 1451, 1259, 1093, 705, 503; ESI-HRMS: m/z calcd. for $C_{37}H_{33}N_3O_{11}$+ Na^+: 718.20073 $[M+Na]^+$; found 718.19969.

(*S*)–2–Azido–1–O–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–1,3–propanediol (12b). General procedure D was applied to compound **11b** (393 mg, 427 µmol). Reagents and conditions: tetrabutylammonium fluoride (*c* = 1 M, 1.28 mL, 3eq.), acetic acid (150 µl, 2.56 mmol, 6eq.), tetrahydrofuran (*c* = 0.1 M, 4.3 mL). Flash chromatography with ethyl acetate/cyclohexane 1/9 to 2/8 afforded compound **12b** (586 mg, 76 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 2/3); $[\alpha]_{20}^{D}$ = -45.6 (*c* 0.42, dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10-8.08 (m, 2H, 2 H-Ar), 8.06-8.04 (m, 2H, 2 H-Ar), 7.98-7.96 (m, 2H, 2 H-Ar), 7.85-7.83 (m, 2H, 2 H-Ar), 7.64-7.34 (m, 10H, 10 H-Ar), 7.29-7.27 (m, 2H, 2 H-Ar), 6.13 (dd, $^3J_{4,5}$ = $J_{3,4}$ = 10.0 Hz, 1H, H-4), 5.90 (dd, $^3J_{3,4}$ = 10.1 Hz, $^3J_{2,3}$ = 3.3 Hz, 1H, H-3), 5.73 (dd, $^3J_{2,3}$ = 3.3 Hz,$^3J_{1,2}$ = 1.8 Hz, 1H, H-2), 5.16 (d, $^3J_{1,2}$ = 1.8 Hz, 1H, H-1), 4.74-4.69 (dd, $^2J_{6a,6b}$ = 11.9 Hz, $^3J_{5,6a}$ = 2.3 Hz, 1H, H-6a), 4.53-4.45 (m, 2H, H-5, H-6b), 4.07 (dd, $^2J_{7a,7b}$ = 10.1 Hz, $^3J_{7a,8}$ = 4.1 Hz, 1H, H-7a), 3.89-3.72 (m, 4H, H-8, H-9a, H-9b, H-7b), 1.88 (br s, 1H, OH) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2, 165.5, 165.4 (4C, 4 PhC=O), 133.6, 133.5, 133.3, 133.2, 129.9, 129.8, 129.2, 129.0, 128.9, 128.6, 128.5, 128.4 (24C, 24 C-Ar), 98.0 (C-1), 70.2 (C-2), 69.8 (C-3), 69.2 (C-5), 67.8 (C-7), 66.7 (C-4), 62.8 (C-6), 62.1 (2C, C-8, C-9) ppm IR (ATR) ν_{max}/cm^{-1} 2932, 2094, 1722, 1451, 1258, 1093, 705, 504; ESI-HRMS: m/z calcd. for $C_{37}H_{33}N_3O_{11}$ + Na^+: 718.20073 $[M+Na]^+$; found 718.19968.

(*r*)–2–Azido–1–*O*–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–3–*O*–(2,3,4,6–tetra–*O*–benzoyl–β–D–glucopyranosyl)–1,3–propanediol (14a). General procedure A was applied to acceptor **12a** (325 mg, 468 µmol) and donor **13** (426 mg, 561 µmol, 1.5 eq.). Reagents and conditions: *N*-iodosuccinimide (157 mg, 702 µmol, 1.5 eq.), trifluoromethane sulfonic acid (4.7 µl, 50.0 µmol, 0.1 eq.), dichloromethane (*c* = 0.1 M, 4.68 mL). Flash chromatography with ethyl acetate/cyclohexane 1/4 yielded compound **14a** (434 mg, 73 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 3/7); $[\alpha]_{20}^{D}$ = -15.8 (*c* 1.0, dichloromethane); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11-8.07 (m, 2H, 2 H-Ar), 8.06-8.01 (m, 4H, 4 H-Ar), 7.98-7.96 (m, 4H, 4 H-Ar), 7.92-7.90 (m, 2H, 2 H-Ar), 7.86-7.81 (m, 4H, 4 H-Ar), 7.60-7.58 (m, 2H, 2 H-Ar), 7.53-7.44 (m, 4H, 4 H-Ar) 7.45-7.33 (m, 15H, 15 H-Ar), 7.29-7.27 (m, 3H, 3 H-Ar), 6.10 (dd, $^3J_{4,5}$ = $^3J_{3,4}$ = 10.1 Hz, 1H, H-4$_{Man}$), 5.94 (dd, $^3J_{3,4}$ = $^3J_{3,2}$ = 9.7 Hz, 1H, H-3$_{Glc}$), 5.85 (dd, $^3J_{3,4}$ = 10.1 Hz, $^3J_{3,2}$ = 3.3 Hz, 1H, H-3$_{Man}$), 5.72 (dd, $^3J_{3,4}$ = $^3J_{4,5}$ = 9.9 Hz,1H, H-4$_{Glc}$), 5.70-5.68 (dd, $^3J_{2,3}$ = 3.3 Hz, $^3J_{1,2}$ = 1.8 Hz,, 1H, H-2$_{Man}$), 5.57 (dd, $^3J_{2,3}$ = 9.8 Hz, $^3J_{1,2}$ = 7.8 Hz, 1H, H-2$_{Glc}$), 5.00 (d, $^3J_{1,2}$ = 1.6 Hz, 1H, H-1$_{Man}$), 4.98 (d, $^3J_{1,2}$ = 7.9 Hz, 1H, H-1$_{Glc}$), 4.70 (dd, $^2J_{6a,6b}$ = 12.2 Hz, $^3J_{5,6a}$ = 3.1 Hz, 1H, H-6a$_{Glc}$), 4.65 (dd, $^2J_{6a,6b}$ = 12.2 Hz, $^3J_{5,6a}$ = 2.5 Hz, 1H, H-6a$_{Man}$), 4.54 (dd, $^2J_{6a,6b}$ = 12.2 Hz, $^3J_{5,6a}$ = 4.9 Hz, 1H, H-6b$_{Glc}$), 4.46 (dd, $^2J_{6a,6b}$ = 12.2 Hz, $^3J_{5,6a}$ = 4.2 Hz, 1H, H-6b$_{Man}$), 4.43-4.38 (m, 1H, H-5$_{Man}$), 4.25-4.20 (m, 1H, H-5$_{Glc}$), 4.11 (dd, $^2J_{9a,9b}$ = 10.1 Hz, $^3J_{8,9a}$ = 3.9 Hz, 1H, H-9a), 3.90 (dd, $^2J_{7a,7b}$ = 10.4 Hz, $^3J_{7a,8}$ = 3.6 Hz, 1H, H-7a), 3.88-3.80 (m, 2H, H-8, H-9b), 3.59-3.57 (dd, $^2J_{7a,7b}$ = 10.5 Hz, $^3J_{7a,8}$ = 5.6 Hz, 1H, H-7b) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.1, 165.8, 165.5, 165.3, 165.2, 165.1 (8C, 8 PhC=O), 133.5, 133.3, 133.2, 133.1, 130.0, 129.9, 129.8, 129.8, 129.7, 129.5, 129.2, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, (48C, 48 C-Ar) 101.5 (C-1$_{Glc}$), 97.9 (C-1$_{Man}$), 72.8 (C-3$_{Glc}$), 72.4 (C-5$_{Glc}$), 71.7 (C-2$_{Glc}$), 70.1 (C-2$_{Man}$), 69.9 (C-3$_{Man}$), 69.5 (C-4$_{Glc}$), 69.4 (C-9), 69.2 (C-5$_{Man}$), 67.9 (C-7), 66.6 (C-4$_{Man}$), 62.8 (C-6$_{Glc}$), 62.7 (C-6$_{Man}$), 60.2 (C-8) ppm; IR (ATR) ν_{max}/cm^{-1} 2933, 2095, 1722, 1451, 1258, 1093, 704; ESI-HRMS; m/z calcd. for $C_{71}H_{59}N_3O_{20}$ +H^+: 1274.37647 $[M+H]^+$; found 1274.37451.

(*s*)–2–Azido–1–*O*–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–3–*O*–(2,3,4,6–tetra–*O*–benzoyl–β–D–glucopyranosyl)–1,3–propanediol (14b). General procedure A was applied to acceptor **12b** (102 mg, 147 µmol) and donor **13** (134 mg, 176 µmol, 1.5 eq.). Reagents and conditions: *N*-iodosuccinimide (49.6 mg, 220 µmol, 1.5 eq.), trifluoromethane sulfonic acid (1.5 µl, 15.0 µmol, 0.1 eq.), dichloromethane (*c* = 0.1 M, 1.47 mL). Flash chromatography with ethyl acetate/cyclohexane 2.5/7.5 afforded compound **14b** (210 mg, 71 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 3/7); $[\alpha]_{20}^{D}$ = -11.9 (*c* 1.0, dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13-8.11 (m, 2H, 2 H-Ar), 8.07-8.01 (m, 4H, 4 H-Ar), 7.99-7.89 (m, 6H, 6 H-Ar), 7.86-7.78 (m, 4H, 4 H-Ar), 7.59 (m, 2H, 2 H-Ar), 7.54-7.47 (m, 3H, 3 H-Ar), 7.46-7.22 (m, 19H, 19 H-Ar), 6.11 (dd, $^3J_{4,5}$ = $^3J_{3,4}$ = 10.1 Hz, 1H, H-4$_{Man}$), 5.93 (dd, $^3J_{2,3}$ = $^3J_{3,4}$ = 9.7 Hz, 1H, H-3$_{Glc}$), 5.87 (dd, $^2J_{3,4}$ = 10.1 Hz, $^3J_{2,3}$ = 3.3 Hz, 1H, H-3$_{Man}$), 5.73-5.66 (m, 2H, H-2$_{Man}$, H-4$_{Glc}$), 5.54 (dd, $^2J_{2,3}$ = 9.8 Hz, $^3J_{1,2}$ = 7.8 Hz, 1H, H-2$_{Glc}$), 4.96-4.90 (m, 2H, H-1$_{Man}$, H-1$_{Glc}$), 4.69 (dd, $^2J_{6a,6b}$ = 12.2 Hz, $^3J_{5,6a}$ =3.1 Hz, 1H, H-6a$_{Glc}$), 4.63 (dd, $^2J_{6a,6b}$ = 12.2 Hz, $^3J_{5,6a}$ = 2.5 Hz, 1H, H-6a$_{Man}$), 4.53 (dd, $^2J_{6a,6b}$ = 12.2 Hz, $^3J_{5,6b}$ =5.1 Hz, 1H, H-6b$_{Glc}$), 4.44 (dd, $^2J_{6a,6b}$ = 12.3 Hz, $^3J_{5,6b}$ = 4.0 Hz, 1H, H-6b$_{Man}$), 4.37-

4.31 (m, 1H, H-5_{Man}), 4.21 (m, 1H, H-5_{Glc}), 4.04 (dd, $^2J_{9a,9b}$ = 10.6 Hz, $^3J_{8,9a}$ = 5.0 Hz, 1H, H-9a), 3.96-3.89 (m, 1H, H-8), 3.86 (dd, $^2J_{7a,7b}$ = 10.1 Hz, $^3J_{7a,8}$ = 7.7 Hz, 1H, H-7a), 3.75-3.65 (m, 2H, H-9b, H-7b) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.1, 165.8, 165.4, 165.2, 165.0 (8C, 8 PhC=O), 133.5, 133.4, 133.3, 133.2, 133.1, 130.0, 129.9, 129.8, 129.7, 129.5, 129.3, 129.1, 129.0, 128.8, 128.60, 128.5, 128.4, 128.3, (48C, 48 C-Ar) 101.5 (C-1_{Glc}), 97.7 (C-1_{Man}), 72.7 (C-3_{Glc}), 72.5 (C-5_{Glc}), 71.7 (C-2_{Glc}), 70.1, 69.8, 69.6 (3C, C-4_{Glc}, C-2_{Man}, C-3_{Man}), 69.2 (C-5_{Man}), 68.6 (C-9), 67.9 (C-7), 66.6 (C-4_{Man}), 62.9 (C-6_{Glc}), 62.6 (C-6_{Man}), 60.4 (C-8); IR (ATR) ν_{max}/cm^{-1} 2933, 2095, 1722, 1451, 1258, 1092, 705; m/z calcd. for $C_{71}H_{59}N_3O_{20}$ +H$^+$: 1274.37647 [M+H]$^+$; found 1274.37450.

(*r*)–2–Azido–1–*O*–(α–D–mannopyranosyl)–3–*O*–(β–D–glucopyranosyl)–1,3–propanediol (15a). General procedure C was applied to compound **14a** (410 mg, 322 µmol). Reagents and conditions: sodium methoxide (*c* = 5.4 M in methanol, two drops), methanol (*c* = 0.03 M, 10.7 mL). Compound **15a** (135 mg, 96 %) was obtained as a white foam after lyophilisation; $[\alpha]_{20}^{D}$ = +27.0 (*c* 0.8, water); ^{1}H NMR (600 MHz, D_2O) δ 4.87 (d, $^3J_{1,2}$ = 1.6 Hz, 1H, H-1_{Man}), 4.47 (d, $^3J_{1,2}$= 8.0 Hz, 1H, H-1_{Glc}), 4.05 (dd, $^2J_{9a,9b}$= 11.1 Hz, $^3J_{8,9a}$ = 4.0 Hz, 1H, H-9a), 4.00-3.85 (m, 5H, H-2_{Man}, H-5_{Man}, H-$6a_{Man}$, H-$6a_{Glc}$, H-7a), 3.82 (dd, $^3J_{2,3}$ = 9.1 Hz, $^3J_{3,4}$= 3.4 Hz, 1H, H-3_{Man}), 3.79-3.58 (m, 6H, H-8, H-4_{Man}, H-$6b_{Glc}$, H-9b, H-7b, H-$6b_{Man}$), 3.52-3.41 (m, 2H, H-3_{Glc}, H-5_{Glc}), 3.40-3.34 (m, 1H, H-4_{Glc}), 3.28 (dd, $^3J_{2,3}$ = 9.4 Hz, $^3J_{2,1}$ = 8.0 Hz, 1H, H-2_{Glc}) ppm; ^{13}C NMR (151 MHz, D_2O) δ 102.8 (C-1_{Glc}), 100.3 (C-1_{Man}), 75.9 (C-5_{Glc}), 75.7 (C-3_{Glc}), 73.1 (C-2_{Glc}), 73.0 (C-4_{Man}), 70.4 (C-3_{Man}), 69.9 (C-5_{Man}), 69.6 (C-4_{Glc}), 69.0, 67.2 (C-6_{Man} or C-6_{Glc}), 66.7 (C-7), 61.0 (C-6_{Glc} or C-6_{Man}), 60.7 (C-9), 60.7 (C-8) ppm; IR (ATR) ν_{max}/cm^{-1} 3325, 2932, 2127, 1672, 1021; ESI-HRMS: m/z calcd. for $C_{15}H_{27}N_3O_{12}$ + Na$^+$ = 464.14869 [M+Na]$^+$; found 464.14853.

(*s*)–2–Azido–1–*O*–(α–D–mannopyranosyl)–3–*O*–(β–D–glucopyranosyl)–1,3–propanediol (15b). General procedure C was applied to compound **14b** (123 mg, 96.0 µmol). Reagents and conditions: sodium methoxide (*c* = 5.4 M in methanol, two drops), methanol (*c* = 0.03 M, 3.2 mL). Compound **15b** (37.2 mg, 89 %) was obtained as a white foam after lyophilisation; $[\alpha]_{20}^{D}$ = +51.9 (*c* 0.8, water); ^{1}H NMR (600 MHz, D_2O) δ 4.89 (d, $^3J_{1,2}$ = 1.5 Hz, 1H, H-1_{Man}), 4.47 (d, $^3J_{1,2}$ = 8.0 Hz, 1H, H-1_{Glc}), 4.00-3.95 (m, 3H, H-2_{Man}, H-3_{Man}, H-9a), 3.92-3.79 (m, 5H, H-4_{Man}, H-9b, H-$6a_{Man}$, H-$6a_{Glc}$, H-7a), 3.77-3.68 (m, 3H, H-7b, H-$6b_{Man}$, H-$6b_{Glc}$), 3.66-3.62 (m, 2H, H-5_{Man}, H-8), 3.47 (dd, $^3J_{2,3}$ = $^3J_{3,4}$ = 9.2 Hz, 1H, H-3_{Glc})3.45-3.41 (m, 1H, H-5_{Glc}), 3.39-3.34 (dd, $^3J_{3,4}$ = $^3J_{4,5}$ = 9.4 Hz,, 1H, H-4_{Glc}), 3.28 (dd, $^3J_{2,3}$ = 9.3 Hz, $^3J_{1,2}$ = 8.0 Hz, 1H, H-2_{Glc}) ppm; ^{13}C NMR (151 MHz, D_2O) δ 102.4 (C-1_{Glc}), 99.7 (C-1_{Man}), 76.0 (C-3_{Glc}), 75.7 (C-5_{Glc}), 73.1 (C-4_{Man}), 73.0 (C-2_{Glc}), 70.4 (C-2_{Man}), 69.9 (C-3_{Man}), 69.72 (C-4_{Glc}), 69.6 (C-5_{Man}), 68.7 (C-6_{Man} or C-6_{Glc}), 66.9 (C-6_{Man} or C-6_{Glc}), 61.0 (C-7), 60.7 (C-9), 60.2 (C-8) ppm; IR (ATR) ν_{max}/cm^{-1} 3338, 2932, 2115, 1259, 1033; ESI-HRMS: m/z [M+Na]$^+$calcd. for $C_{15}H_{27}N_3O_{12}$ + Na$^+$ = 464.14869 [M+Na]$^+$; found 464.14847.

(*r*)–2–Azido–1–*O*–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–3–*O*–(2,3,4,6–tetra–*O*–benzoyl–β–D–galactopyranosyl)–1,3–propanediol (17a). General procedure A was applied to acceptor **12a** (167 mg, 241 µmol) and donor **16** (274 mg, 361 µmol, 1.5 eq.). Reagents and conditions: *N*-iodosuccinimide (109 mg, 482 µmol, 2 eq.), trifluoromethane sulfonic acid (4.8 µl, 48.0 µmol, 0.1 eq.), dichloromethane (*c* = 0.1 M, 2.05 mL). Flash chromatography with ethyl acetate/cyclohexane 2.5/7.5 afforded compound **17a** (196 mg, 64 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 3/7); $[\alpha]_{20}^{D}$ = -16.3 (*c* 1, dichloromethane);^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 – 8.07 (m, 4H, 4 H-Ar), 8.03-8.01 (m, 4H, 4 H-Ar), 7.98-7.92 (m, 4H, 4 H-Ar), 7.83-7.77 (m, 4H,4 H-Ar), 7.62-7.28 (m, 22H, 22 H-Ar), 7.24-7.22 (m, 2H, 2 H-Ar) 6.11 (dd, $^3J_{4,5}$ = $^3J_{3,4}$ = 10.1 Hz, 1H, H-4_{Man}), 6.01 (dd, $^3J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.0 Hz, 1H, H-4_{Gal}), 5.86 (dd, $^3J_{3,4}$ = 10.1 Hz, $^3J_{2,3}$ = 3.3 Hz, 1H, H-3_{Man}), 5.80 (dd, $^3J_{2,3}$ = 10.4 Hz, $^3J_{1,2}$ = 7.9 Hz, 1H, H-2_{Gal}), 5.67 (dd, $^3J_{2,3}$ = 3.3 Hz, $^3J_{2,1}$ = 1.8 Hz, 1H, H-2_{Man}), 5.63 (dd, $^3J_{2,3}$ = 10.4 Hz, $^3J_{3,4}$ = 3.5 Hz, 1H, H-3_{Gal}), 4.90-4.87 (m, 2H, H-1_{Man}, H-1_{Gal}), 4.71 (dd, $^2J_{6a,6b}$ = 11.4 Hz, $^3J_{5,6a}$ = 6.5 Hz, 1H, H-$6a_{Man}$), 4.63 (dd, $^2J_{6a,6b}$ = 12.2 Hz, $^3J_{5.6a}$ =2.5 Hz, 1H, H-$6a_{Gal}$), 4.48-4.41 (m, 2H, H-$6b_{Man}$, H-$6b_{Gal}$), 4.40-4.35 (1H, H-5_{Gal}), 4.35-4.30 (m, 1H, H-5_{Man}), 4.11 (dd, $J_{9a,9b}$ = 10.6 Hz, $J_{8,9a}$ = 5.1 Hz, 1H, H-9a), 3.99-3.91 (m, 1H, H-8), 3.85 (dd, $J_{7a,7b}$ = 10.2 Hz, $J_{7a,8}$ = 7.9 Hz, 1H, H-7a), 3.74-3.67 (m, 2H, H-7b, H-9b) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.1, 166.0, 165.6, 165.54, 165.4, 165.4, 165.3, 165.2 (8C, 8 PhC=O), 133.6, 133.5, 133.5, 133.4, 133.3, 133.2, 133.1, 130.0, 129.9, 129.8, 129.8, 129.8, 129.8, 129.7, 129.7, 129.4, 129.2, 129.2, 129.0, 128.9, 128.7, 128.6, 128.5, 128.3, 128.2 (48C, 48 C-Ar), 101.9 (C-1_{Gal}), 97.6 (C-1_{Man}), 71.6 (C-3_{Gal} or C-5_{Gal}), 71.5 (C-3_{Gal} or C-5_{Gal}), 70.0 (C-2_{Man}), 69.8 (C-3_{Man}), 69.6 (C-2_{Gal}), 69.2 (C-5_{Man}), 68.7 (C-9), 68.1 (C-4_{Gal} or C-7), 68.0 (C-4_{Gal} or C-7), 66.5 (C-4_{Man}), 62.5 (C-6_{Gal}), 61.9 (C-6_{Man}), 59.9 (C-8) ppm; IR (ATR) ν_{max}/cm^{-1} 2096, 1722, 1451, 1257, 1091, 705; ESI-HRMS: m/z calcd. for $C_{71}H_{59}N_3O_{20}$ + Na$^+$: 1296.35841 [M+Na]$^+$; found 1296.35802.

(*s*)–2–Azido–1–*O*–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–3–*O*–(2,3,4,6–tetra–*O*–benzoyl–β–D–galactopyranosyl)–1,3–propanediol (17b). General procedure A was applied to acceptor **12a** (226 mg, 324 µmol) and donor **16** (369 mg, 486 µmol, 1.5 eq.). Reagents and conditions: *N*-iodosuccinimide (195 mg, 648 µmol, 2 eq.), trifluoromethane sulfonic acid (6.5 µl, 64.0 µmol, 0.1 eq.) dichloromethane (*c* = 0.1 M, 3.24 mL). Flash chromatography with ethyl acetate/cyclohexane 2.5/7.5 afforded compound **17b** (251 mg, 61 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 3/7); $[\alpha]_{20}^{D}$ = -21.1 (*c* 1, dichloromethane); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11-8.08 (m, 4H, 4 H-Ar), 8.03-8.01 (m, 6H, 6 H-Ar), 7.96-7.95 (m, 2H, 2 H-Ar), 7.84-7.78 (m, 4H, 4 H-Ar), 7.63-7.33 (m, 22H, 22 H-Ar), 7.27-7.23 (m, 2H, 2 H-Ar), 6.11 (dd, $^3J_{4,5}$ = $^3J_{3,4}$ =10.1 Hz, 1H, H-4_{Man}), 6.02 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ =0.7 Hz, 1H, H-4_{Gal}), 5.87-5.82 (m, 2H, H-3_{Man}, H-2_{Gal}), 5.68 (dd, $^3J_{2,3}$ = 3.3 Hz, $^3J_{1,2}$ = 1.8 Hz, 1H, H-2_{Man}), 5.64 (dd, $^3J_{3,4}$ = 10.4 Hz, $^3J_{2,3}$ = 3.5 Hz, 1H, H-3_{Gal}), 4.99 (d, $^3J_{1,2}$ = 1.6 Hz, 1H, H-1_{Man}), 4.96 (d, $^3J_{1,2}$ = 7.9 Hz, 1H, H-1_{Gal}), 4.73 (dd, $^2J_{6a,6b}$ = 11.2 Hz, $^3J_{5,6a}$ = 6.3 Hz, 1H, H-$6a_{Man}$), 4.65 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5.6a}$ =2.5 Hz, 1H, H-$6a_{Gal}$), 4.48-4.43 (m, 2H, H-$6b_{Gal}$, H-$6b_{Man}$), 4.42-4.38 (m, 2H, H-5_{Man}, H-5_{Gal}), 4.17 (dd, $^2J_{9a,9b}$ = 10.4 Hz, $J_{8,9a}$ = 3.8 Hz, 1H, H-9a), 3.92-3.87 (m, 2H, H-8, H-7a), 3.85 (dd, $^2J_{9a,9b}$ = 10.3 Hz, $^3J_{8,9b}$ = 7.7 Hz, 1H, H-9b), 3.61 (dd, $^2J_{7a,7b}$ = 11.6 Hz, $^3J_{7b,8}$ = 6.7 Hz, 1H, H-7b) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.1, 166.0, 165.6, 165.5, 165.3, (8C, 8 PhC=O), 133.6, 133.5, 133.3, 133.2, 133.1, 132.9, 130.1, 129.9, 129.8, 129.8, 129.6, 129.5, 129.3, 129.2, 129.0, 129.0, 128.9, 128.7, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, (48C, 48 C-Ar), 102.0 (C-1_{Gal}), 97.9 (C-1_{Man}), 71.6 (C-3_{Gal} or C-5_{Gal}), 71.5 (C-3_{Gal} or C-5_{Gal}),

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70.1 (C-2_{Man}), 69.8 (C-3_{Man} or C-2_{Gal}), 69.7 (C-9), 69.6 (C-3_{Man} or C-2_{Gal}), 69.2 (C-5_{Man}), 68.0 (C-7), 67.9 (C-4_{Gal}), 62.6 (C-6_{Gal}), 61.9 (C-6_{Man}), 60.3 (C-8) ppm; IR (ATR) ν_{max}/cm^{-1} 2933, 2094, 1721, 1451, 1258, 1066, 705; ESI-HRMS: m/z calcd. for $C_{71}H_{59}N_3O_{20}$ + Na^+: 1296.35841 $[M+Na]^+$; found 1296.35793.

(*r*)–2–Azido–1–*O*–(α–D–mannopyranosyl)–3–*O*–(β–D–galactopyranosyl)–1,3–propanediol (18a). General procedure C was applied to compound **17a** (196 mg, 153 µmol). Reagents and conditions: sodium methoxide (*c* = 5.4 M in methanol, two drops), methanol (*c* = 0.03 M, 4.02 mL). Compound **18a** (71.3 mg, 98 %) was obtained as a white foam after lyophilisation; $[\alpha]_{20}^{D}$ = +37.1 (*c* 0.8, water); ^{1}H NMR (600 MHz, D_2O) δ 4.89 (d, $^3J_{1,2}$ = 1.6 Hz, 1H, H-1_{Man}), 4.40 (d, $^3J_{1,2}$ = 7.9 Hz, 1H, H-1_{Gal}), 4.01-3.95 (m, 3H, H-2_{Man}, H-3_{Man}, H-9a), 3.92-3.70 (m, 9H, H-4_{Man}, H-5_{Man}, H-6_{Man}, H-6_{Gal}, H-9b, H-7), 3.67 (dd, $^3J_{3,4}$ = 8.0 Hz, $^3J_{4,5}$ = 4.1 Hz, 1H, H-4_{Gal}), 3.65-3.61 (m, 3H, H-3_{Gal}, H-8, H-5_{Gal}), 3.51 (dd, $^3J_{2,3}$ = 9.9 Hz, $^3J_{2,1}$ = 7.9 Hz, 1H, H-2_{Gal}) ppm; ^{13}C NMR (151 MHz, D_2O) δ 102.9 (C-1_{Gal}), 99.7 (C-1_{Man}), 75.2 (C-4_{Gal}), 73.0 (C-3_{Gal} or C-5_{Gal}), 72.7 (C-3_{Gal} or C-5_{Gal}), 70.7 (C-2_{Gal}), 70.4 (C-4_{Man} or C-5_{Man}), 69.9 (C-3_{Man}), 68.6 (C-4_{Man} or C-5_{Man}), 68.6 (C-9), 66.9 (2C, C-7, C-8), 61.0 (2C, C-6_{Man}, C-6_{Gal}), 60.2 (C-2_{Man}) ppm; IR (ATR) ν_{max}/cm^{-1} 3338, 2933, 2124, 1640, 1032; ESI-HRMS: m/z calcd. for $C_{15}H_{27}N_3O_{12}$ + Na^+ = 464.14869 $[M+Na]^+$; found 464.14847.

(*s*)–2–Azido–1–*O*–(α–D–mannopyranosyl)–3–*O*–(β–D–galactopyranosyl)–1,3–propanediol (18b). General procedure C was applied to compound **17b** (210 mg, 165 µmol). Reagents and conditions: sodium methoxide (*c* = 5.4 M in methanol, two drops), methanol (*c* = 0.03 M, 5.5 mL). Compound **18b** (61.8 mg, 85 %) was obtained as a white foam after lyophilisation; $[\alpha]_{20}^{D}$ = +22.9 (*c* 0.8, water); ^{1}H NMR (600 MHz, D_2O) δ 4.88 (d, $^3J_{1,2}$ = 1.6 Hz, 1H, H-1_{Man}), 4.40 (d, $^3J_{1,2}$ = 7.9 Hz, 1H, H-1_{Gal}), 4.06 (dd, $^2J_{9a,9b}$ = 11.0 Hz, $^3J_{8,9a}$ = 4.0 Hz, 1H, H-9a), 3.99-3.86 (m, 5H, H-2_{Man}, H-5_{Gal}, H-$6a_{Man}$, H-3_{Man}, H-7a), 3.82 (dd, $^3J_{3,4}$ = 9.0 Hz, $^3J_{4,5}$ = 3.4 Hz, 1H, H-4_{Gal}), 3.79-3.71 (m, 4H, H-$6a_{Gal}$, H-7b, H-9b, H-$6b_{Man}$), 3.70-3.61 (m, 5H, H-8, H-3_{Gal}, H-5_{Man}, H-4_{Man}, H-$6b_{Gal}$), 3.52 (dd, $^3J_{2,3}$ = 9.9 Hz, $^3J_{1,2}$ = 7.9 Hz, 1H, H-2_{Gal}) ppm; ^{13}C NMR (151 MHz, D_2O) δ 103.4 (C-1_{Gal}), 100.4 (C-1_{Man}), 75.2 (C-5_{Man}), 73.0 (C-4_{Man} or C-3_{Gal}), 72.7 (C-4_{Man} or C-3_{Gal}), 70.7 (C-2_{Gal}), 70.4 (C-4_{Gal}), 69.9 (C-5_{Gal}), 69.0 (C-7), 68.7 (C-3_{Man}), 67.2 (C-9), 66.7 (C-2_{Man}), 61.0 (2C, C-6_{Gal}, C-6_{Man}), 60.7 (C-8) ppm; IR (ATR) ν_{max}/cm^{-1} 3337, 2933, 2103, 1639, 1038; ESI-HRMS: m/z calcd. for $C_{15}H_{27}N_3O_{12}$ + Na^+ = 464.14869 $[M+Na]^+$; found 464.14846.

(*R*)–2–Azido–1–*O*–(2–deoxy–2–trifluoroacetamido–3,4,6–tri–*O*–acetyl–β–D–glucopyranosyl)–3–*O*–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–1,3–propanediol (20a). General procedure B was applied to acceptor **12a** (188 mg, 271 µmol) and donor **19** (125 mg, 325 µmol, 1.2 eq.). Reagents and conditions: trimethylsilyl trifluoromethanesulfonate (5.0 µl, 27.0 µmol, 0.1 eq.), dichloromethane (*c* = 0.1 M, 2.7 mL). Flash chromatography with ethyl acetate/cyclohexane 2/3 afforded compound **20a** (215 mg, 73 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 2/3); $[\alpha]_{20}^{D}$ = -37.10 (*c* 0.8, dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09-8.07 (m, 2H, 2 H-Ar), 8.03-8.01 (m, 2H, 2 H-Ar), 7.97-7.95 (m, 2H, 2 H-Ar), 7.84-7.81 (m, 2H, 2 H-Ar), 7.61-7.56 (m, 2H, 2 H-Ar), 7.53-7.49 (m, 1H, H-Ar), 7.47-7.35 (m, 7H, 7 H-Ar), 7.32-7.27 (m, 3H, 2 H-Ar, $NHCOCF_3$), 6.17 (dd, $^3J_{4,5}$ = $J_{3,4}$ = 10.1 Hz, 1H, H-4_{Man}), 5.89 (dd, $^3J_{3,4}$ = 10.2 Hz, $^3J_{2,3}$ = 3.3 Hz, 1H, H-3_{Man}), 5.72 (dd, $^3J_{2,3}$ = 3.2 Hz, $^3J_{2,1}$ = 1.9 Hz, 1H, H-2_{Man}), 5.43 (dd, $^3J_{3,4}$ = 10.7 Hz, $^3J_{2,3}$ = 9.3 Hz, 1H, H-$3_{GlcNTFA}$), 5.16-5.10 (m, 2H, H-1_{Man}, H-$4_{GlcNTFA}$), 4.84 (d, $^3J_{1,2}$ = 8.3 Hz, 1H, H-$1_{GlcNTFA}$), 4.79 (dd, $^2J_{6a,6b}$ = 12.2 Hz, $^3J_{5,6a}$ = 2.9 Hz, 1H, H-$6a_{Man}$), 4.54-4.41 (m, 2H, H-5_{Man}, H-$6b_{Man}$), 4.31 (dd, $^2J_{6a,6b}$ = 12.4 Hz, $^3J_{5,6a}$ = 4.7 Hz, 1H, H-$6a_{GlcNTFA}$), 4.19 (dd, $^2J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-$6b_{GlcNTFA}$), 4.15-3.98 (m, 3H, H-$2_{GlcNTFA}$, H-7a, H-9a), 3.87-3.78 (m, 3H, H-8, H-$5_{GlcNTFA}$, H-7b), 3.75 (dd, $^2J_{9a,9b}$ = 10.4 Hz, $^3J_{8.9a}$ = 5.7 Hz, 1H, H-9b), 2.08, 2.05, 2.03 (each s, each 3H, 3 $\underline{CH_3}$C=O) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 170.7, 169.3, 166.4, 165.8, 165.5, 165.4 (7C, 4 Ph$\underline{C}$=O, 3 $CH_3\underline{C}$=O), 157.6, (q, *J* = 37.4 Hz, $CF_3\underline{C}$=O) 133.6, 133.5, 133.4, 133.2, 129.8, 129.8, 129.7, 129.1, 128.9, 128.6, 128.5, 128.4 (24C, 24 C-Ar), 100.7 (C-1_{GlcTFA}), 98.0 (C-1_{Man}), 72.2 (C-$5_{GlcNTFA}$), 71.5 (C-$3_{GlcNTFA}$), 70.1 (C-2_{Man}), 70.0 (C-3_{Man}), 69.2 (C-5_{Man}), 68.8 (C-9), 68.2 (C-$4_{GlcNTFA}$), 67.7 (C-7), 66.6 (C-4_{Man}), 62.7 (C-6_{Man}), 61.7 (C-$6_{GlcNTFA}$), 59.8 (C-8), 55.0 (C-$2_{GlcNTFA}$), 20.7, 21.0, 20.4 (3C, 3 $\underline{C}H_3$C=O) ppm; IR (ATR) ν_{max}/cm^{-1} 3327, 2933, 2308, 2103, 1723, 1219, 1027, 708; ESI-HRMS: m/z calcd. for $C_{51}H_{49}F_3N_4O_{19}$ +H^+ : 1079.30159 $[M+H]^+$; found 1079.29950.

(*S*)–2–Azido–1–*O*–(2–deoxy–2–trifluoroacetamido–3,4,6–tri–*O*–acetyl–β–(D)–glucopyranosyl)–3–*O*–(2,3,4,6–tetra–*O*–benzoyl–α–D–mannopyranosyl)–1,3–propanediol (20b). General procedure B was applied to acceptor **12b** (150 mg, 216 µmol) and donor **19** (99.2 mg, 258 µmol, 1.2 eq.). Reagents and conditions: trimethylsilyl trifluoromethanesulfonate (3.7 µl, 22.0 µmol, 0.1 eq.), dichloromethane (*c* = 0.1 M, 2.87 mL). Flash chromatography with ethyl acetate/cyclohexane 2/3 afforded compound **20b** (175 mg, 75 %) as a white foam; R_f = 0.3 (ethyl acetate/cyclohexane 2/3); $[\alpha]_{20}^{D}$ = -14.4 (*c* 0.8, dichloromethane); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11-8.09 (m, 2H, 2 H-Ar), 8.03-8.01 (m, 2H, 2 H-Ar), 7.98-7.96 (m, 2H, 2 H-Ar), 7.85-7.83 (m, 2H, 2 H-Ar), 7.61-7.56 (m, 2H, 2 H-Ar), 7.53-7.51 (m, 1H, H-Ar), 7.47-7.36 (m, 7H, 7 H-Ar), 7.29-7.27 (m, 2H, 2 H-Ar), 6.94 (d, *J* = 8.9 Hz, 1H, N$\underline{H}$COCF$_3$), 6.14 (dd, $J_{4,5}$ = $J_{3,4}$ = 10.0 Hz, 1H, H-4_{Man}), 5.86 (dd, $^3J_{3,4}$ = 10.2 Hz, $^3J_{2,3}$ = 3.3 Hz, 1H, H-3_{Man}), 5.70 (dd, $^3J_{2,3}$ = 3.2 Hz, $^3J_{2,1}$ = 1.8 Hz, 1H, H-2_{Man}), 5.38-5.32 (m, 1H, H-$3_{GlcNTFA}$), 5.16 (dd, $J_{4,5}$ = $J_{3,4}$ = 9.6 Hz, 1H, H-$4_{GlcNTFA}$), 5.09 (d, $^3J_{1,2}$ = 1.6 Hz, 1H, H-1_{Man}), 4.83 (d, $^3J_{1,2}$ = 8.3 Hz, 1H, H-$1_{GlcNTFA}$), 4.75 (dd, $^2J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 2.3 Hz, 1H, H-$6a_{Man}$), 4.48 (m, 2H, H-5_{Man}, H-$6b_{Man}$), 4.32 (dd, $^2J_{6a,6b}$ = 12.4 Hz, $^3J_{5,6a}$ = 4.5 Hz, 1H, H-$6a_{GlcNTFA}$), 4.22 (dd, $^2J_{6a,6b}$ = 12.4 Hz, $^3J_{5,6a}$ = 2.3 Hz, 1H, H-$6b_{GlcNTFA}$), 4.15 (dd, $^2J_{9a,9b}$ = 10.0 Hz, $^3J_{8,9a}$ = 3.9 Hz, 1H, H-9a), 4.12-4.06 (m, 1H, H-$2_{GlcNTFA}$), 3.98 (dd, $^2J_{7a,7b}$ = 10.5 Hz, $^3J_{7a,8}$ = 4.2 Hz, 1H, H-7a), 3.86-3.78 (m, 3H, H-$5_{GlcNTFA}$, H-8, H-9b), 3.70 (dd, $^2J_{7a,7b}$ = 10.5 Hz, $^3J_{7b,8}$ = 4.8 Hz, 1H, H-7b), 2.09, 2.06, 2.05 (each s, each 3H, 3 $\underline{CH_3}$C=O) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9, 170.7, 169.3, 166.3, 165.8, 165.5, 165.4 (7C, 4 Ph$\underline{C}$=O, 3 $CH_3\underline{C}$=O), 133.6, 133.4, 133.2, 129.9, 129.8, 129.8, 129.7, 129.1, 128.9, 128.6, 128.5, 128.4 (24C, 24 C-Ar), 100.5 (C-$1_{GlcNTFA}$), 97.8 (C-1_{Man}), 72.3 (C-$5_{GlcNTFA}$), 71.6 (C-$3_{GlcNTFA}$), 70.0 (2C, C-2_{Man}, C-3_{Man}), 69.5 (C-9), 69.3 (C-5_{Man}), 68.0 (C-$4_{GlcNTFA}$), 67.7 (C-7), 66.6 (C-4_{Man}), 62.7 (C-6_{Man}), 61.7 (C-$6_{GlcNTFA}$), 59.7 (C-8), 55.0 (C-$2_{GlcNTFA}$), 21.8, 20.6, 20.4 (3 CH_3C=O) ppm; IR (ATR) ν_{max}/cm^{-1} 2933, 2308, 2105, 1724, 1260, 1067, 708; ESI-HRMS: m/z calcd. for $C_{51}H_{49}F_3N_4O_{19}$ +H^+: 1079.30159 $[M+H]^+$; found 1079.29953.

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(*R*)–2–Azido–1–*O*–(2–deoxy–2–trifluoroacetamido–β–D–glucopyranosyl)–3–*O*–(α–D–mannopyranosyl)–1,3–propanediol (21a). General procedure C was applied to compound **20a** (169 mg, 157 µmol). Reagents and conditions: sodium methoxide (*c* = 5.4 M in methanol, two drops), methanol (*c* = 0.03 M, 5.23 mL). Compound **21a** (74.2 mg, 98 %) was obtained as a white foam after lyophilisation; $[\alpha]_{20}^{D}$ = +14.6 (*c* 0.8, water); ^{1}H NMR (600 MHz, D_2O) δ 4.86 (d, $^3J_{1,2}$ = 1.5 Hz, 1H, H-1_{Man}), 4.65 (d, $^3J_{1,2}$ = 8.5 Hz, 1H, H-$1_{GlcNTFA}$), 4.00-3.84 (m, 5H, H-2_{Man}, H-$5_{GlcNTFA}$, H-9a, H-$6a_{GlcNTFA}$, H-$6a_{Man}$), 3.82-3.73 (m, 6H, H-$6b_{Man}$, H-$6b_{GlcNTFA}$, H-9b, H-7a, H-$2_{GlcNTFA}$, H-3_{Man}), 3.69-3.58 (m, 4H, H-7b, H-4_{Man}, H-$3_{GlcNTFA}$, H-$4_{GlcNTFA}$), 3.48-3.45 (m, 2H, H-8, H-5_{Man}) ppm; ^{13}C NMR (151 MHz, D_2O) δ 100.6 (C-$1_{GlcNTFA}$), 99.9 (C-1_{Man}), 76.1 (C-8), 73.0 (2C, C-$3_{GlcNTFA}$, C-$4_{GlcNTFA}$), 70.4 (C-3_{Man}), 69.9 (C-2_{Man} or C-$5_{GlcNTFA}$), 69.8 (C-2_{Man} or C-$5_{GlcNTFA}$), 68.9 (C-9), 66.8 (C-7), 66.6 (C-4_{Man}), 60.8 (C-$6_{GlcNTFA}$), 60.6 (C-6_{Man}), 59.9 (C-5_{Man}), 56.0 (C-$2_{GlcNTFA}$) ppm; IR (ATR) ν_{max}/cm^{-1} 3306, 2933, 2102, 1707, 1023; ESI-HRMS: m/z calcd. for $C_{17}H_{27}F_3N_4O_{12}$ + Na^+: 559.14776 $[M+Na]^+$; found 559.14681.

(*S*)–2–Azido–1–*O*–(2–deoxy–2–trifluoroacetamido–β–D–glucopyranosyl)–3–*O*–(α–D–mannopyranosyl)–1,3–propanediol (21b). General procedure C was applied to compound **20b** (159 mg, 147 µmol). Reagents and conditions: sodium methoxide (*c* = 5.4 M in methanol, two drops), methanol (*c* = 0.03 M, 5.0 mL). Compound **21b** (74.3 mg, quantitative) was obtained as a white foam after lyophilisation; $[\alpha]_{20}^{D}$ = +11.8 (*c* 0.8, water); ^{1}H NMR (600 MHz, D_2O) δ 4.83 (d, $^3J_{1,2}$ = 1.7 Hz, 1H, H-1_{Man}), 4.64 (d, $^3J_{1,2}$ = 8.5 Hz, 1H, H-$1_{GlcNTFA}$), 4.10 (dd, $^2J_{9a,9b}$ = 10.9 Hz, $^3J_{8,9a}$ = 3.5 Hz, 1H, H-9a), 3.95 (dd, $^3J_{2,3}$ = 3.3 Hz, $^3J_{1,2}$ = 1.6 Hz, 1H, H-$2_{GlcNTFA}$), 3.94-3.91 (m, 1H, H-$6a_{Man}$), 3.90-3.85 (m, 3H, H-7a, H-$4_{GlcNTFA}$, H-$6a_{GlcNTFA}$), 3.82-3.72 (m, 4H, H-3_{Man}, H-$2_{GlcNTFA}$, H-$5_{GlcNTFA}$, H6b_{Man}), 3.69-3.60 (m, 4H, H-$6b_{GlcNTFA}$, H-9b, H-4_{Man}, H-$3_{GlcNTFA}$), 3.57-3.52 (m, 1H, H-7b), 3.50-3.45 (m, 2H, H-5_{Man}, H-8) ppm; ^{13}C NMR (151 MHz, D_2O) δ 100.9 (C-1_{GlcTFA}), 100.3 (C-1_{Man}), 76.0 (C-8), 73.0 (C-$3_{GlcNTFA}$ or C-4_{Man}), 70.4 (C-3_{Man}), 69.9 (C-2_{Man}), 69.4 (C-9), 67.0 (C-7), 66.7 (C-$3_{GlcNTFA}$ or C-4_{Man}), 60.6 (C-$6_{GlcNTFA}$), 60.5 (2C, C-$5_{Man,}$ C-6_{Man}), 56.1 (C-$2_{GlcNTFA}$) ppm; IR (ATR) ν_{max}/cm^{-1} 3288, 2933, 2108, 1707, 1022; ESI-HRMS: m/z calcd. for $C_{17}H_{27}F_3N_4O_{12}$ + Na^+: 559.14776 $[M+Na]^+$; found 559.14623.

(*R*)–2–Azido–1–*O*–(2–deoxy–2–acetamido–β–D–glucopyranosyl)–3–*O*–(α–D–mannopyranosyl)–1,3–propanediol (22a). General procedure E was applied to compound **21a** (76.8 mg, 141 µmol). Reagents and conditions: (i) aq. lithium hydroxide (*c* = 2 M, 2.80 mL, 5.66 mmol, 40 eq.), methanol (*c* = 0.03 M, 4.70 mL); (ii) sodium methoxide (*c* = 5.4 M in methanol, two drops), acetic anhydride (44 µl, 465 µmol, 5 eq.), methanol (*c* = 0.03 M, 3.1 mL). Compound **22a** (28.0 mg, 60 %) was obtained as a white foam after lyophilisation; $[\alpha]_{20}^{D}$ = +3.1 (*c* 0.8, water); ^{1}H NMR (500 MHz, D_2O) δ 4.84 (d, $^3J_{1,2}$ = 1.7 Hz, 1H, H-1_{Man}), 4.53 (d, $^3J_{1,2}$ = 8.5 Hz, 1H, H-1_{GlcNAc}), 4.09 (dd, $^2J_{9a,9b}$ = 10.9 Hz, $^3J_{8,9a}$ = 3.5 Hz, 1H, H-9a), 3.96 (dd, $^3J_{2,3}$ = 3.4 Hz, $^3J_{2,1}$ = 1.8 Hz, 1H, H-2_{Man}), 3.93-3.83 (m, 4H, H-$6a_{GlcNAc}$, H-7a, H-5_{GlcNAc}, H-$6a_{Man}$), 3.81 dd, $^3J_{3,4}$ = 9.4 Hz, $^3J_{2,3}$ = 3.4 Hz, 1H, H-3_{Man}), 3.78-3.62 (m, 6H, H-$6b_{GlcNAc}$, H-4_{Man}, H-8, H-3_{GlcNAc}, H-7b, H-9b), 3.58-3.50 (m, 2H, H-5_{Man}, H-$6b_{Man}$), 3.44 (m,2H, H-4_{GlcNAc}, H-2_{GlcNAc}), 2.04 (s, 3H, C$\underline{H}_3$C=O) ppm; ^{13}C NMR (126 MHz, D_2O) δ 177.4 (CH$_3\underline{C}$=O), 104.4 (C-1_{GlcNAc}), 103.1 (C-1_{Man}), 78.7 (C-4_{GlcNAc} or C-2_{GlcNAc}), 76.5 (C-5_{GlcNAc}), 75.7 (C-4_{Man}), 73.2 (C-3_{Man}), 72.7 (C-2_{Man}), 72.6 (C-4_{GlcNAc} or C-2_{GlcNAc}), 72.3 (C-9), 69.8 (C-6_{Man}), 69.4 (C-3_{GlcNAc}), 63.7 (C-7 or C-6_{GlcNAc}), 63.5 (C-7 or C-6_{GlcNAc}), 63.4 (C-5_{Man}), 58.3 (C-8), 25.0 (C$\underline{H}_3$C=O) ppm IR (ATR) ν_{max}/cm^{-1} 3279, 2103, 1557, 1410, 1054; ESI-HRMS: m/z calcd. for $C_{17}H_{30}N_4O_{12}$ + Na^+: 505.17524 $[M+Na]^+$; found 505.17514.

(*S*)–2–Azido–1–*O*–(2–deoxy–2–acetamido–β–D–glucopyranosyl)–3–*O*–(–α–D–mannopyranosyl)–1,3–propanediol (22b). General procedure E was applied to compound **21b** (74.0 mg, 137 µmol). Reagents and conditions: (i) aq. lithium hydroxide (*c* = 2 M, 2.8 mL, 5.51 mmol, 40 eq.), methanol (*c* = 0.03 M, 4.59 mL);(ii) sodium methoxide (*c* = 5.4 M in methanol, two drops.), acetic anhydride (34 µl, 363 µmol, 5 eq.), methanol (*c* = 0.03 M, 2.4 mL). Compound **22b** (23.0 mg, 58 %) was obtained as a white foam after lyophilisation; $[\alpha]_{20}^{D}$ = +3.4 (*c* 0.8, water); ^{1}H NMR (500 MHz, D_2O) δ 4.88 (d, $^3J_{1,2}$ = 1.7 Hz, 1H, H-1_{Man}), 4.56 (d, $^3J_{1,2}$ = 8.4 Hz, 1H, H-1_{GlcNAc}), 3.99-3.95 (m, 2H, H-2_{Man}, H-$6a_{Man}$), 3.94-3.85 (m, 3H, H-5_{Man}, H-5_{GlcNAc}, H-$6b_{Man}$), 3.83-3.60 (m, 9H, H-3_{Man}, H-2_{GlcNAc}, H-7a, H-7b, H-9a, H-9b, H-4_{Man}, H-6_{GlcNAc}), 3.57-3.50 (m, 1H, H-3_{GlcNAc}), 3.46-3.40 (m, 2H, H-4_{GlcNAc}, H-8), 2.04 (s, 3H, C$\underline{H}_3$C=O) δ ppm; ^{13}C NMR (151 MHz, D_2O) δ 174.7 (CH$_3\underline{C}$=O), 101.2 (C-1_{GlcNAc}), 99.8 (C-1_{Man}), 76.0 (C-4_{GlcNAc}), 73.8 (C-3_{GlcNAc}), 73.0 (C-4_{Man}), 70.4 (C-3_{Man} or C-5_{Man}), 68.5 (C-8), 69.9 (C-5_{GlcNAc}), 68.7 (C-3_{Man} or C-5_{Man}), 66.7 (C-2_{Man}), 60.9 (C-6_{Man} or C-6_{GlcNAC}), 60.7 (C-6_{Man} or C-6_{GlcNAC}), 59.9 (C-2_{GlcNAc}), 55.5 (C-7 or C-9), 48.9 (C-7 or C-9), 22.3 (C$\underline{H}_3$C=O) ppm; IR (ATR) ν_{max}/cm^{-1} 3266, 2114, 1557, 1410, 1054; ESI-HRMS: m/z calcd. for $C_{17}H_{30}N_4O_{12}$ + Na^+: 505.17524 $[M+Na]^+$; found 505.17505.

Conflicts of interest

There are no conflicts to declare.

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